Oxime and Dithiolane Derivatives of 5-Formyl-2'-deoxyuridine and Their 5'-Phosphates: Antiviral Effects and Thymidylate Synthetase Inhibition

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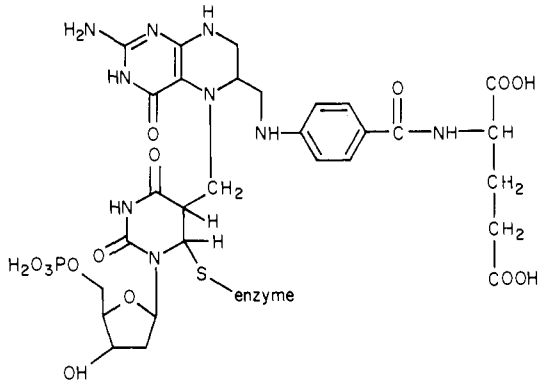
5-Formyl-2'-deoxyuridine (**2a**), an effective inhibitor of herpes simplex virus type 1 or 2 (HSV-1, HSV-2) and vaccinia virus, was converted to the oxime (**3a**) and dithiolane (**4a**) derivatives. The oxime (**3a**) was equally as potent as the formyl compound against HSV-1, but one-fifth as active against HSV-2, 100 times less effective against vaccinia, and 25 times less toxic for the host cells. In addition, compound **3a** was about 10 times less active than **2a** in inhibiting thymidylate synthetase in vivo (as reflected by a differential inhibition of dThd and dUrd incorporation into host cell DNA). The dithiolane (**4a**) did not exert an appreciable effect on either virus multiplication or dThd or dUrd incorporation, nor was it cytotoxic. All these compounds as their 5'-phosphate derivatives were potent in vitro inhibitors of thymidylate synthetase (*Lactobacillus casei*). The inhibition was competitive with substrate with K_i/K_m ratios of 0.05 for the formyl **2b**, 0.5 for the oxime **3b**, and 0.2 for the dithiolane **4b**. Thus, **3b** was 10 times less active than **2b** as an in vitro inhibitor of thymidylate synthetase, which appears to corroborate the in vivo findings.

The reductive methylation of 2'-deoxyuridine 5'-phosphate (dUMP) to 2'-deoxythymidine 5'-phosphate (dTMP) is catalyzed by thymidylate synthetase. This reaction currently is postulated to occur through an enzyme-bound bridged intermediate, 1, formed from the enzyme, substrate, formaldehyde as the carbon donor, and the reducing agent tetrahydrofolic acid.[1,2] The nature of this intermediate and consideration of the known reactivity of tetrahydrofolic acid with carbonyl compounds such as formaldehyde prompted the synthesis[3] of 5-formyl-2'-deoxyuridine (**2a**) which, as its nucleotide, was designed to bind to the substrate site of thymidylate synthetase and interact with the enzyme-bound cofactor to give what may be called a multisubstrate inhibitor.



Subsequent biological studies have shown that the formyl nucleotide **2b** is a potent in vitro inhibitor of thymidylate synthetase from several sources.[4] Moreover, this inhibition is irreversible when the assay is performed in the presence of cofactor.[5] The K_i values for reversible inhibition range from 0.013 to 0.1 μM, and the inhibition is competitive with substrate. The K_i/K_m ratios for the enzyme purified from four sources were 0.05 or less, which indicates that the inhibitor has a much greater affinity than substrate for this enzyme.

Although the antitumor effects of **2a** against L-1210 leukemia were marginal (T/C = 129% at 100 mg/kg), the compound is an effective antiviral agent. Langen and co-workers[6] noted a significant reduction in pseudorabies growth in cell culture at 10^{-6} M concentration. They also reported cytotoxic effects when baby hamster kidney cells 21/C13 or Ehrlich ascites tumor cells in culture were exposed to **2a** (ID_{50} = 4×10^{-6} and 6×10^{-6} M, respectively). More recently, 5-formyl-2'-deoxyuridine (**2a**) has been found to inhibit the replication of herpes simplex virus (HSV) and vaccinia virus.[7] The concentrations required for 50% inhibition approximated that of the standard drug 5-iodo-2'-deoxyuridine. The minimum concentration of **2a**, giving rise to microscopic cytotoxicity in primary rabbit kidney (PRK) cell cultures, is two to four times the ID_{50} for HSV-1 replication.

Since it is recognized that 5-substituted derivatives of 2'-deoxyuridine, such as the fluoro, trifluoromethyl, cyano, and nitro, are potent in vitro inhibitors of thymidylate synthetase, a series of experiments was devised to establish the in vivo action of these antiviral agents.[8] The assay measures the effects of inhibitors on 2'-deoxyuridine and thymidine incorporation into cellular DNA. Agents which block 2'-deoxyuridine but not thymidine incorporation can be considered to act at the level of thymidylate synthetase. 5-Formyl-2'-deoxyuridine (**2a**) suppressed 2'-deoxy[2-^{14}C]uridine incorporation into DNA at a concentration of 0.5 μg/mL 100 times less than the concentration required for inhibition of [*methyl*-^{3}H]thymidine incorporation. Thus, **2a** is an in vivo inhibitor of thymidylate synthetase.

In an effort to achieve greater antiviral selectivity, to reduce the cytotoxic effects of 5-formyl-2'-deoxyuridine, and to evaluate the relationship of the in vivo effects with the in vitro potency for the inhibition of thymidylate synthetase, two derivatives of the nucleoside **2a** and the nucleotide **2b** were synthesized. The oxime, while having

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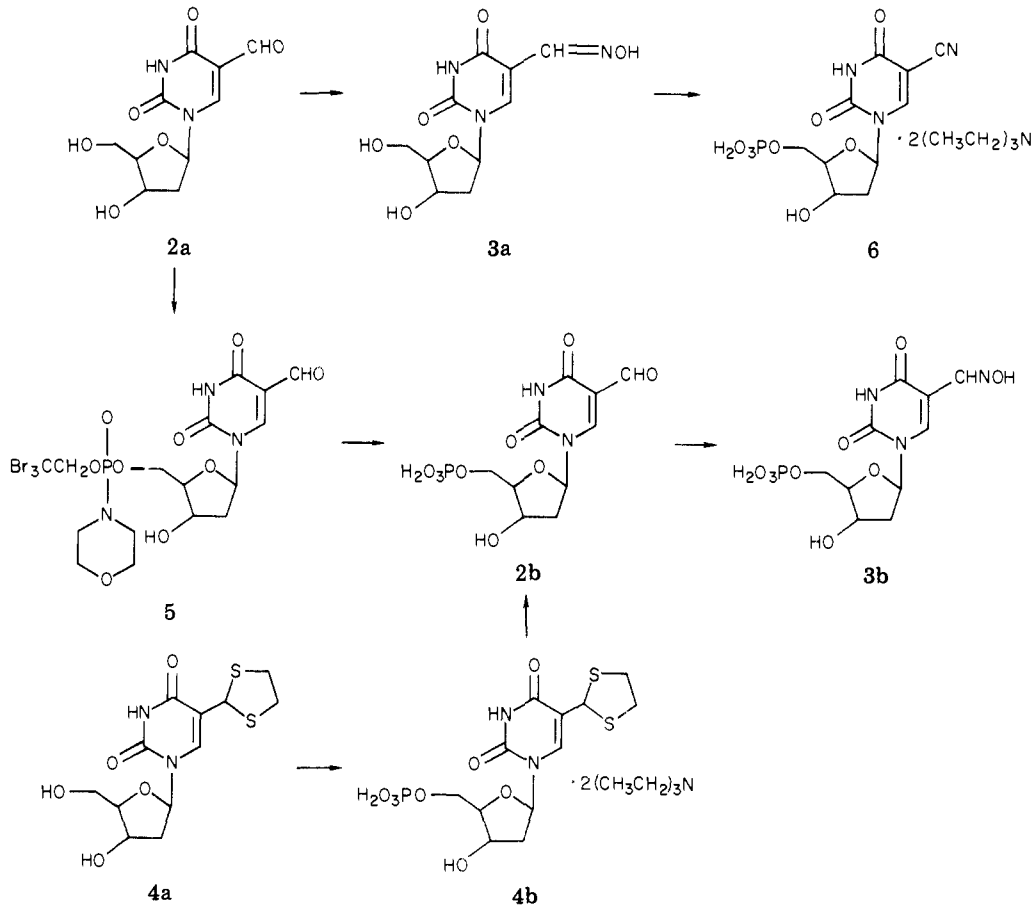
Scheme I



similar polar characteristics, would be expected to enhance the nucleophilic addition of the enzyme thiol at carbon-6 of the pyrimidine ring in the substrate. In addition, unlike the formyl group, the oxime must be hydrolyzed to the formyl if any Schiff base interactions with the enzyme[5] or cofactor[3,4b] are to occur. The dithiolane derivative addresses the enzyme mechanism and also the effect of the 5-substituent on enzyme affinity. In the latter point, it has been proposed that the electronic effect of the 5-substituent is one of the primary features that contributes to enzyme affinity.[4c] If this is the primary determinant for enzyme affinity, the dithiolane substituent being nonpolar should be a poor inhibitor. In addressing the mechanism, analysis of mechanism-based inhibitors of thymidylate synthetase has suggested that Michael addition of the enzyme thiol anion to carbon-6 of the pyrimidine ring of the substrate is greatly enhanced when the 5-substituent can either delocalize negative charge at the 5 position (5-nitro)[9] or polarize the pyrimidine 5,6 double bond (5-fluoro). The dithiolane derivative should not polarize the 5,6 double bond nor should it enhance addition by the enzyme thiol. This would require the unlikely elimination of a mercaptan anion and ring opening of the dithiolane substituent.

Results

The oxime (**3a**) derivative of 5-formyl-2′-deoxyuridine[3] (**2a**) could be prepared conveniently from the formyl compound **2a** (Scheme I). Treatment of **2a** with hydroxylamine afforded the oxime **3a**; the proton NMR resonance pattern showed four singlets at δ 7.3, 7.85, 8.2, and 9.1, each integrating for 0.5 proton. These are assigned to the carbon-bonded protons of the aldoxime and the ring carbon-6 and confirm that the product **3a** is a 50:50 mixture of the syn and anti isomers. The dithiolane **4a** was prepared by a reported procedure.[10]

Two of the most popular routes for the synthesis of pyrimidine nucleoside 5′-phosphates are those of Yoshikawa et al. and Sowa and Ouchi.[11] While the primary reagent in both is phosphorus oxychloride, the solvents, trialkyl phosphates in the former and pyridine–acetonitrile–water in the latter, provide, with some exceptions,[12] a highly selective route to 5′-phosphates. Since prior attempts[4b] to phosphorylate the aldehyde **2a** were unsuccessful using the trialkyl phosphate method,[11a] an enzymatic method was used. This procedure, however, was not convenient for the large-scale preparation of **2b**.

Application of the Sowa and Ouchi procedure[11b] to **2a** yielded two unidentified nucleotides with ultraviolet maxima at 266 and 269 nm compared to the expected maxima of 278 nm reported for 5-formyl-2′-deoxyuridine 5′-phosphate[4b] (**2b**). Another reagent, 2,2,2-tribromoethyl phosphoromorpholinochloridate,[13] was utilized in a two-reaction sequence leading to **2b**. The intermediate **5** (formed in 52% yield) was converted to the nucleotide **2b**

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Table I. Antiviral Activity of 5-Formyl-2'-deoxyuridine (2a) and Its Oxime (3a) and Dithiolane (4a) Derivatives in Primary Rabbit Kidney Cell Cultures

	ID_{50},[a] μg/mL						
compd	HSV-1 KOS	HSV-1 F	HSV-1 McIntyre	HSV-2 Lyons	HSV-2 196	HSV-2 G	vaccinia
2a	1-2	1-2	0.7	2-4	1-4	1-2	0.1-0.2
3a	0.7	1	0.7	20	20	7	10
4a	300	150	300	>400	300	200	200
5-I-dUrd	0.1	0.1	0.1	0.2	0.2	0.2-0.4	0.1-0.2

[a] Concentration required to reduce virus-induced cytopathogenicity by 50%.

Table II. Antimetabolic Activity of 5-Formyl-2'-deoxyuridine (2a) and Its Oxime (3a) and Dithiolane (4a) Derivatives in Primary Rabbit Kidney Cell Cultures

	ID_{50}[a] μg/mL		ratio of ID_{50} for [*Me*-^{3}H]dThd incorp to ID_{50} for [1',2'-^{3}H]dUrd incorp[b]	min cytotoxic concn,[c] μg/mL
compd	[*Me*-^{3}H]dThd incorp	[1',2'-^{3}H]dUrd incorp		
2a	75	0.2	375	4
3a	300	7.5	40	100
4a	100	100	1	>400
5-I-dUrd	1	0.25	4	>200

[a] Concentration required to reduce [*Me*-^{3}H]dThd or [1',2'-^{3}H]dUrd incorporation into DNA by 50%. [b] As postulated previously,[8] the ratio of ID_{50} for dThd incorporation to ID_{50} for dUrd incorporation can be considered as a parameter for selective inhibition of thymidylate synthetase in vivo. [c] Causing a microscopically visible alteration of cell morphology.

(36% yield) by reduction and hydrolysis using a zinc–copper couple in 90% acetic acid. This reaction did not give the monoester **2b** when the literature solvent (dimethylformamide) was used.[13]

The method of Sowa and Ouchi[11b] was successful for the conversion of the dithiolane nucleoside **4a** to the 5'-phosphate **4b**; however, the multicomponent mixture afforded only 8% of **4b**, which was isolated after elution from DEAE-cellulose. Treatment of **4b** with mercuric oxide and mercuric chloride provided yet another route to the formyl nucleotide **2b** in 50% yield.

The synthesis of the oxime nucleotide **3b** was attempted from the nucleoside **3a** using phosphorus oxychloride in pyridine–acetonitrile–water; however, the dehydrating conditions of the reaction resulted in the conversion of the oxime to the nitrile in addition to phosphorylation of the sugar. A clue to the structure of the product, 5-cyano-2'-deoxyuridine 5'-phosphate (**6**), was the absence of the expected aldoxime proton NMR resonance signals; an infrared absorption at 2220 cm^{-1} (C≡N) coupled with an ultraviolet absorption that was identical with 5-cyano-2'-deoxyuridine 5'-phosphate[14] confirmed the structure. The desired oxime nucleotide **3b** was prepared in low yield (21%) from the formyl nucleotide **2b** by treatment with hydroxylamine.

The oxime (**3a**) derivative was equally effective as the formyl compound (**2a**) in inhibiting the replication of HSV-1 in primary rabbit kidney (PRK) cells (Table I). However, **3a** was about 5–10 times less effective than **2a** in inhibiting HSV-2 replication and about 50–100 times less effective than **2a** against vaccinia. Whereas **2a** caused a microscopically visible alteration of cell morphology from a concentration of 4 μg/mL onward, **3a** did not prove cytotoxic unless the concentration was raised up to 100 μg/mL (Table I). Addition of deoxythymidine (dThd) completely reversed the antiviral activity of **2a** and **3a**; i.e., the ID_{50} values of **2a** and **3a** for HSV-1 and vaccinia were ≥400 μg/mL in the presence of 100 μg/mL dThd. One may postulate, therefore, that **2a** and **3a** owe their antiviral activity to inhibition of one or another step of deoxythymidine metabolism.

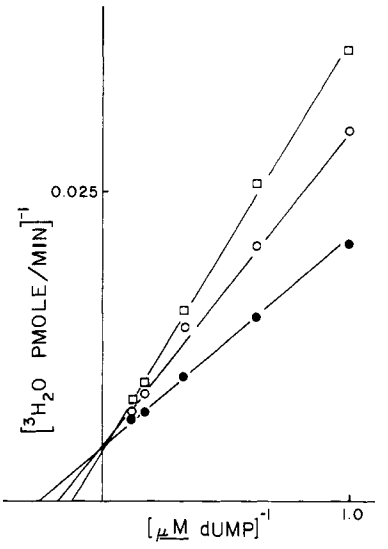

Figure 1. Double-reciprocal plot of the velocity of formation of ^3H_2O vs. concentration of substrate, 2'-deoxyuridine 5'-phosphate (dUMP), with and without added inhibitor, 1-(2'-deoxy-β-D-ribofuranosyl)-1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinecarboxaldehyde oxime 5'-phosphate (**3b**): no inhibitor (●); 1 μM inhibitor (○), 2 μM inhibitor (□).

Both **2a** and **3a** inhibited the incorporation of [1',2'-^{3}H]dUrd into PRK cell DNA to a significantly greater extent than the incorporation of [*methyl*-^{3}H]dThd (Table II), suggesting that they inhibited thymidylate synthetase activity in these cells.[8] However, as reflected by the ratio of the ID_{50} values required to inhibit dThd and dUrd incorporation (Table II), **3a** proved about 10 times less active than **2a**. The oxime (**3a**) was also less toxic for the cells (Table II).

In marked contrast with **2a** and **3a**, the dithiolane (**4a**) derivative did not exert an appreciable inhibitory effect on the replication of either HSV or vaccinia virus (Table I). Neither did it affect normal cell morphology or cell metabolism (as monitored by either dThd or dUrd incorporation into DNA) (Table II).

The nucleotides **2b**, **3b**, and **4b** were examined as inhibitors of thymidylate synthetase purified from *Lactobacillus casei* at a specific activity of 3.0 μmol of product

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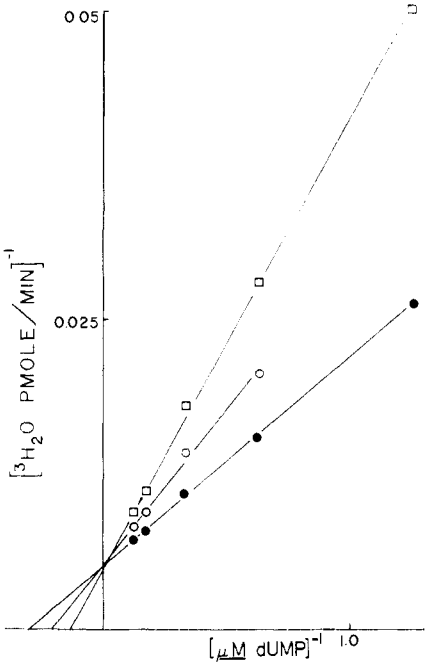

Figure 2. Double-reciprocal plot of the velocity of formation of ^3H_2O vs. concentration of substrate, 2′-deoxyuridine 5′-phosphate (dUMP), with and without added inhibitor, 2′-deoxy-5-(1,3-dithiolan-2-yl)uridine 5′-phosphate (**4b**): no inhibitor (●); 0.36 μM inhibitor (○); 0.72 μM inhibitor (□).

formed min^{-1} (mg of protein)$^{-1}$. The assay conditions have been described[15] and, for this particular study, the substrate K_m values were 4.0 μM in the analysis of the oxime **3b** and 3.2 μM in the assay using the dithiolane derivative **4b**. Figures 1 and 2 are double-reciprocal plots of the velocity of the reaction in pmol of ^3H_2O formed min^{-1} vs. the substrate concentration in the presence and absence of the inhibitors. Under the conditions of this assay, the aldehyde **2b** had a K_i of 0.17 μM, 10 times higher than that reported by Wataya and co-workers.[4c] Values for the K_i of **2b** for the enzyme from other sources have been reported: *Escherichia coli*, 0.1,[4b] 0.013 μM;[4a,5] calf thymus, 0.09 μM;[4b] Ehrlich ascites tumor, 0.08 μM.[4b]

The oxime **3b** was a competitive inhibitor (Figure 1) with a calculated K_i of 2.1 μM, approximately 10 times less potent than the aldehyde **2b**. The dithiolane also was a competitive inhibitor with an intermediate value (0.67 μM) for the K_i. Neither the oxime (**3b**) nor the dithiolane nucleotide (**4b**) showed time-dependent inactivation of the enzyme upon incubation in the absence of cofactor.

Discussion

The pattern of in vitro thymidylate synthetase inhibition did not correlate with the antiviral effects. While the aldehyde (**2a**) and the oxime (**3a**) nucleosides were effective and equally potent inhibitors of HSV-1, the latter nucleotide (**3b**) was 10 times less effective against the enzyme. Equally unexpected is the fact that the dithiolane derivative **4b**, while a potent enzyme inhibitor, was inactive [as nucleoside (**4a**)] when tested against HSV and vaccinia virus. One explanation for the high activity of the dithiolane (**4b**) as an enzyme inhibitor is that **4b**, under the conditions of the enzyme assay, is converted by hydrolysis to the aldehyde **2b**, which is a potent inhibitor. This possibility was examined; incubation of **4b** for 24 h in the assay medium lacking cofactor, substrate, and enzyme failed to give the change in the ultraviolet spectrum from a λ_{max} at 270 nm (**4b**)[10] to a λ_{max} at 281 nm (**2b**).[3] Therefore, **4b** is chemically stable in the assay media; however, we have not examined the possibility that the enzyme may catalyze the conversion of the dithiolane to the aldehyde **2b**.

The 5-[(methylthio)methyl] derivative of 2′-deoxyuridine and the corresponding 5-[(methylsulfinyl)methyl] and 5-[(methylsulfonyl)methyl] derivatives offer an interesting contrast. While all three are reasonable inhibitors of thymidylate synthetase, only the thioether and the sulfone are active anti-herpes agents, whereas the sulfoxide is not.[16] A similar situation also occurs in the present study. The dithiolane **4b** is three times more potent as an inhibitor of thymidylate synthetase than the oxime **3b**, yet the dithiolane nucleoside **4a** is totally ineffective as an antiviral agent.

Previous studies[4b] comparing the sensitivity of the enzyme from different sources found little difference in inhibition of *E. coli*, calf thymus, and Ehrlich ascites tumor enzymes by the aldehyde **2b**. In the current studies, the aldehyde **2a** is a potent antiviral agent and is cytotoxic; it would appear again that no discrimination was observed for selectivity based on the enzyme source.

While 5-formyl-2′-deoxyuridine (**2a**) was not selective in its antiviral activity, inhibiting HSV-1, HSV-2, and vaccinia virus replication at doses that corresponded closely to the cytotoxic doses,[6,7] the oxime (**3a**) derivative proved rather selective in its activity against HSV-1: it inhibited HSV-2 and vaccinia at a tenfold higher concentration than HSV-1, and was not cytotoxic up to 100 μg/mL, that is 100 times the dose required to inhibit HSV-1 (Tables I and II). Thus, substitution of the aldehyde by an aldoxime group did not affect the inhibitory activity of **2a** toward HSV-1, while reducing its inhibitory effects on HSV-2, vaccinia virus, and normal cell metabolism. Concomitantly to an increase in specificity toward HSV-1, substitution of aldoxime for aldehyde (**2a** → **3a**) resulted in about a tenfold decrease in inhibitory potency toward thymidylate synthetase both in vitro (Figure 1) and in vivo (Table II).

From these data one may infer that inhibition of thymidylate synthetase contributes to the anti-vaccinia activity of 5-substituted 2′-deoxyuridines (as has been suggested for 5-NO_2-dUrd[8]) and to the cytotoxic properties of 5-substituted 2′-deoxyuridines (as has also been suggested for 5-NO_2-dUrd[17]), but not to their anti-herpes activity. On the contrary, the selectivity of the 5-substituted dUrd as an anti-herpes agent appears to be inversely correlated to its potency as a dTMP synthetase inhibitor.

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Proton magentic resonance spectra were obtained with a Varian EM 360, and chemical shifts are reported relative to tetramethylsilane as an internal standard for the nucleosides or as an external standard for the nucleotides. Ultraviolet spectra were measured with a Cary 219 spectrophotometer. Thin-layer chromatography was run on precoated silica gel 60 F-254, 0.25-mm thick plates supplied by E. M. Laboratories, Inc. Column chromatography was run on silica gel 60 (70–230 mesh), supplied by E. M. Laboratories, Inc., and on DE-32 DEAE-cellulose, supplied by Whatman, Ltd. All solvent proportions are given by volume.

Thymidylate synthetase purified from methotrexate-resistant *Lactobacillus casei* was purchased from the New England Enzyme

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Center, Tufts University, at a specific activity of 1.1 μmol of TMP formed min^{-1} (mg of protein)$^{-1}$ using the radioisotope assay. The crude preparation was purified according to the method of Maley[18] to give enzyme with a specific activity in excess of 3 μmol of ^3H_2O formed min^{-1} (mg of protein)$^{-1}$. The enzyme was activated by dialysis for 4 days at 4 °C against 0.1 M potassium phosphate (pH 6.8) containing 50 mM mercaptoethanol and assayed by reported procedures.[16] The substrate 2'-deoxy[5-^{3}H]uridine 5'-phosphate at a specific activity above 15 Ci/mmol was purchased from Moravek Biochemicals, Industry, Calif., and diluted with cold substrate purchased from Sigma Chemical Co., St. Louis, to give a specific activity of 500 μCi/μmol. The cofactor, *dl*-tetrahydrofolic acid, also was purchased from Sigma Chemical Co.

Antiviral and Antimetabolic Assays. The methodology for measuring the inhibition of virus-induced cytopathogenicity in primary rabbit kidney (PRK) cell cultures and for measuring the inhibition of incorporation of 2'-deoxythymidine or 2'-deoxyuridine into DNA of these cells has been described previously.[7,8] However, in the present experiments, 2'-deoxy[1',2'-^{3}H]uridine (specific activity 42 Ci/mmol), and not 2'-deoxy[2-^{14}C]uridine, served as the radiolabeled 2'-deoxyuridine: it was added at 0.25 μCi or 6 pmol per 10^5 PRK cells per well (Linbro microplates).

1-(2-Deoxy-β-D-ribofuranosyl)-1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinecarboxaldehyde 5-Oxime (3a). A cold solution of hydroxylamine (38.5 mg, 0.55 mmol) in 1 mL of absolute ethanol was added to a solution of **2a** (128 mg, 0.5 mmol) in 10 mL of absolute ethanol. The reaction mixture was refluxed for 2 h and concentrated in vacuo, to leave a glassy solid material which was crystallized from ethanol in 81% yield (110 mg): mp 198 °C; UV λ_{max} (H_2O) 288 nm; NMR (Me_2SO-d_6) δ 9.1 (s, 0.5), 8.2 (s, 0.5), 7.85 (s, 0.5), 7.3 (s, 0.5), 6.2 (t, 1, H-1'). Anal. ($C_{10}H_{13}N_3O_6$; M_r 271.2) C, H, N.

5-Formyl-2'-deoxyuridine 5'-(2,2,2-Tribromoethyl Phosphoromorphoridate) (5). A mixture of 250 mg (1 mmol) of **2a** and 450 mg (1 mmol) of 2,2,2-tribromoethyl phosphoromorpholinochloridate[13] in 10 mL of dry pyridine was stirred at room temperature for 48 h. The solvent was removed under high vacuum, and the residue was applied on a silica gel column with 5% methanol in chloroform as eluent. The corresponding fractions were collected and concentrated in vacuo to leave 350 mg (52%) of a yellowish compound: UV λ_{max} (ethanol) 279 nm, λ_{min} 250 nm. Anal. ($C_{16}H_{21}Br_3N_3O_9P$; M_r 670.0) H, N; C: calcd, 28.68; found, 30.09.

5-Formyl-2'-deoxyuridine 5'-Phosphate (2b). Method A. Compound **5** (100 mg, 0.15 mmol) was treated with 80 mg of Zn/Cu couple in 10 mL of 90% acetic acid overnight at room temperature. The filtrate was concentrated in vacuo to leave a yellowish viscous material, which was applied on a DEAE-cellulose column (2.5 × 40 cm) with 0.01–0.3 M triethylammonium bicarbonate as a gradient eluent. The corresponding fractions (900 mL) were collected, lyophilized, and dried. The dried material was applied on Dowex 50 (H^+), collected, lyophilized, and dried, to leave 20 mg (36%) of **2b** which was identical (UV, TLC) with the compound prepared by method B.

Method B. Compound **4b** (100 mg, 0.15 mmol) in 6 mL of water–ethanol (1:1) was stirred with mercuric oxide (40 mg, 0.18 mmol) and mercuric chloride (100 mg, 0.37 mmol) at room temperature. Within a few minutes, a yellow precipitate was formed. The reaction mixture was stirred at room temperature for 2 h and filtered. The cooled filtrate was treated with H_2S gas and the suspension filtered with the aid of Celite 545. The filtrate was applied on a DEAE-cellulose column (2.5 × 40 cm). Gradient elution was performed using 0.01–0.3 M triethylammonium bicarbonate buffer (pH 7.5). The corresponding fractions (retention volume 900 mL) were collected and lyophilized to give a white solid, which was dried at 35 °C to yield 30 mg (50.8%) of the bis(triethylammonium) salt of **2b** as a hygroscopic powder: UV λ_{max} (H_2O and 0.1 N HCl) 278 nm, λ_{min} 250 nm; NMR (D_2O) δ 9.6 (s, 1, CHO), 8.7 (s, 1, H-6), 6.1 (t, 1, H-1'), 4.6 (m, 1, H-3'), 4.0 (m, 3, H-4' and H-5'), 3.1 (q, 12, NCH_2), 2.4 (m, 2, H-2'), 1.1 (t, 18, NCH_2CH_3). This compound was dissolved in 2 mL of water and applied on a Dowex 50 (H^+) column, using water as the eluent. The eluate was lyophilized and dried to give 20 mg (33.9%) of **2b** as a white fluffy powder. Anal. ($C_{10}H_{13}N_2O_9P \cdot 1.5H_2O$; M_r 363.2) C, H, N.

Bis(triethylammonium) Salt of 2'-Deoxy-5-(1,3-dithiolan-2-yl)uridine 5'-Phosphate (4b). Using the method of Sowa and Ouchi,[11b] 166 mg (0.5 mmol) of **4a** was added to a cold (0 °C) solution of acetonitrile (500 μL), water (25 μL), phosphorus oxychloride (200 μL), and pyridine (190 μL) prepared in the above sequence. The reaction mixture was stirred for 3 h at 0 °C under nitrogen. Cold water (6 mL) was added and the mixture stirred for 1 h at that temperature. The entire mixture after neutralization with triethylamine was applied to a DEAE-cellulose column (2.5 × 40 cm). Gradient elution was performed using 0.01 and 0.3 M triethylammonium bicarbonate buffer (pH 7.5). The corresponding fractions (retention volume 580 mL) were collected and lyophilized, to leave a white material which was dried at 35 °C for 24 h to give 25 mg (7.7%) of **4b** as a hygroscopic powder: UV λ_{max} (H_2O) 269 nm, λ_{min} 238 nm; NMR (D_2O) δ 7.65 (s, 1, H-6), 5.95 (t, 1, H-1'), 5.25 (s, 1, CHS_2), 3.8 (br, 3), 3.15 (s, 4, SCH_2CH_2S), 2.7 (q, 12, NCH_2), 2.2 (2 sets of d, 2, H-2'), 1.1 (t, 18, NCH_2CH_3). Anal. ($C_{24}H_{47}N_4O_8PS_2 \cdot 2H_2O$; M_r 650.8) C, H, N.

Bis(triethylammonium) Salt of 5-Cyano-2'-deoxyuridine 5'-Phosphate (6). Using the same method as described in the synthesis of compound **4b**, **3a** (136 mg, 0.5 mmol) was reacted with an ice-cold solution of acetonitrile, water, phosphoryl chloride, and pyridine. The reaction mixture was stirred for 5 h at 0 °C under a nitrogen stream. Cold water (6 mL) was added and the mixture stirred for 1 h at 0 °C. The neutralized solution was applied on a DEAE-cellulose column (2.5 × 40 cm) with 0.01–0.3 M triethylammonium bicarbonate buffer as a gradient eluent. The eluate was lyophilized and rechromatographed on a longer DEAE-cellulose column (2.5 × 70 cm) with the same gradient elution. The corresponding fractions (750 mL) were collected, lyophilized, and dried at 35 °C to give 10 mg of the product as a very hygroscopic powder:[14] UV λ_{max} (H_2O) 276 nm; IR (KBr) 2220 cm^{-1}; NMR (D_2O) δ 8.4 (s, 1, H-6), 5.95 (t, 1, H-1'), 4.3 (m, 1, H-3'), 3.9 (m, 3, H-4' and H-5'), 2.9 (q, 12, NCH_2), 2.2 (m, 2, H-2'), 1.1 (t, 18, NCH_2CH_3). Anal. ($C_{22}H_{42}N_5O_8P \cdot 7H_2O$; M_r 661.7) C, N; H: calcd, 8.53; found, 7.80.

Bis(triethylammonium) Salt of 1-(2-Deoxy-β-D-ribofuranosyl)-1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinecarboxaldehyde 5-Oxime 5'-Phosphate (3b). Compound **2b** (100 mg, 0.28 mmol) in 3 mL of water was titrated with aqueous hydroxylamine (generated from hydroxylamine hydrochloride and sodium carbonate) at 3 °C and stirred at that temperature for 30 min. The reaction mixture was applied to a DEAE-cellulose column (2.5 × 40 cm) with 0.01–0.3 M triethylammonium bicarbonate buffer as a gradient eluent. The column was monitored at 280 nm. The corresponding fractions (retention volume 750 mL) were collected and lyophilized. The material was dried at 35 °C for 48 h to give 35 mg (21.2%) of compound **3b**: UV λ_{max} (H_2O and 0.1 N HCl) 288 nm (ε 11 300), λ_{min} 264 nm (ε 8000), 243 (ε 11 400); UV (0.1 N NaOH) λ_{max} 250 nm (ε 11 410), 282 (sh). Anal. ($C_{22}H_{44}N_5O_9P \cdot 2.5H_2O$; M_r 598.6) C, H, N.

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