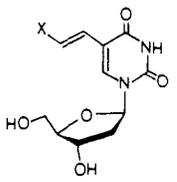
# Synthesis of (*E*)-5-(3,3,3-Trifluoro-1-propenyl)-2′-deoxyuridine and Related Analogues: Potent and Unusually Selective Antiviral Activity of (*E*)-5-(3,3,3-Trifluoro-1-propenyl)-2′-deoxyuridine against Herpes Simplex Virus Type 1

Donald E. Bergstrom,*,† Jerry L. Ruth,‡ P. Anantha Reddy,† and Erik De Clercq§

*Department of Chemistry, University of North Dakota, Grand Forks, North Dakota 58202, Department of Chemistry, University of California, Davis, California 95616, and Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium.* *Received May 9, 1983*

Syntheses of (*E*)-5-(3,3,3-trifluoro-1-propenyl)-2′-deoxyuridine (TFPe-dUrd) (1), 5-(3,3,3-trifluoro-1-propyl)-2′-deoxyuridine (11), 5-(3,3,3-trifluoro-1-methoxy-1-propyl)-2′-deoxyuridine (8), and 5-(3,3,3-trifluoro-1-hydroxy-1-propyl)-2′-deoxyuridine (10) from 5-chloromercuri-2′-deoxyuridine are described. The antiviral activity of TFPe-dUrd was determined in cell culture against herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), and vaccinia virus and compared concurrently with 5-(1-propenyl)-2′-deoxyuridine, 5-(2-bromovinyl)-2′-deoxyuridine, 5-iodo-2′-deoxyuridine, and 5-(trifluoromethyl)-2′-deoxyuridine. TFPe-dUrd demonstrated a potent and unusually selective activity against HSV-1, with a 2-log reduction in virus yield at 0.03 μg/mL (0.09 μM); L-1210 cell growth was inhibited by 50% only at 290 μg/mL. Isopycnic centrifugation of $^{32}$P-labeled DNA indicated that if 0.5 or 2 μM TFPe-dUrd was present for 0–6 h postinfection, viral DNA synthesis was reduced by ca. 50 and 85%, respectively; concomitantly, a new DNA band appeared at lower density than normal cellular or viral DNA.

Several C-5 substituted pyrimidine nucleosides are known to inhibit the growth of herpes viruses in cell culture. The most potent and selective inhibitors of this class, particularly against herpes simplex virus type 1 (HSV-1), are 2′-deoxyuridine analogues, which contain a vinyl or trans-substituted vinyl moiety at C-5 of the uracil ring.[1-4] Within this class, halovinyl analogues **4–6** are the



1, X = $CF_3$
2, X = $CH_3$
3, X = H
4, X = Cl
5, X = Br
6, X = I

most active, with (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (**5**) exhibiting the highest reported inhibitory activity against HSV-1 of any antiviral compound.[1,2]

From results obtained in several laboratories,[3,5] the selective antiviral action of these compounds correlates well with the ability of the infected cell to activate the analogue by phosphorylation mediated through the viral-induced thymidine kinase (TK). HSV-1 strains that do not express viral TK are much less sensitive to the compounds. Cis stereoisomers, such as (*Z*)-5-(2-bromovinyl)-2′-deoxyuridine,[6] are much less active against the virus and are phosphorylated much more poorly by the viral TK.[5]

In addition, and without exception, each of nucleosides **1–6** are more potent against type 1 than type 2 HSV infections. This increased potency against HSV-1 relative to HSV-2 may also be a direct result of the relative phosphorylation rates by the viral-specific TK.

Although the basis of antiviral *selectivity* may be an activation by the viral TK, the actual antiviral *target* has been presumed to be the viral-induced DNA polymerase. However, the nucleosides appear to be incorporated into DNA,[7] and when tested against the isolated viral DNA polymerases as their 5′-triphosphates, nucleosides **2** and **5** support DNA synthesis very efficiently and have no apparent effect on subsequent primer elongation after incorporation.[8]

(*E*)-5-(3,3,3-Trifluoro-1-propenyl)-2′-deoxyuridine (TFPe-dUrd, 1) as its 5′-monophosphate is a mechanism-based inhibitor of thymidylate (dTMP) synthetase.[9] However, on the basis of data obtained on similar structures,[23] there is not necessarily a direct correlation between the inhibition of dTMP synthetase and inhibition of HSV replication.

Despite its significant biological activity, details of the preparation of TFPe-dUrd (1) have not been reported, and the antiviral data have been incomplete. In this study, the syntheses of TFPe-dUrd and related analogues are discussed, and results are presented pertaining to the potency and selectivity of TFPe-dUrd as an antiviral agent.

## Results and Discussion

**Synthesis.** 5-Chloromercuri-2′-deoxyuridine (**7**) is known to react with a variety of terminal olefins in the presence of $Pd^{2+}$ to give adducts in which C-5 of the uracil ring is coupled to the terminal olefinic carbon.[9-14] When

† University of North Dakota.
‡ University of California.
§ Rega Institute for Medical Research.

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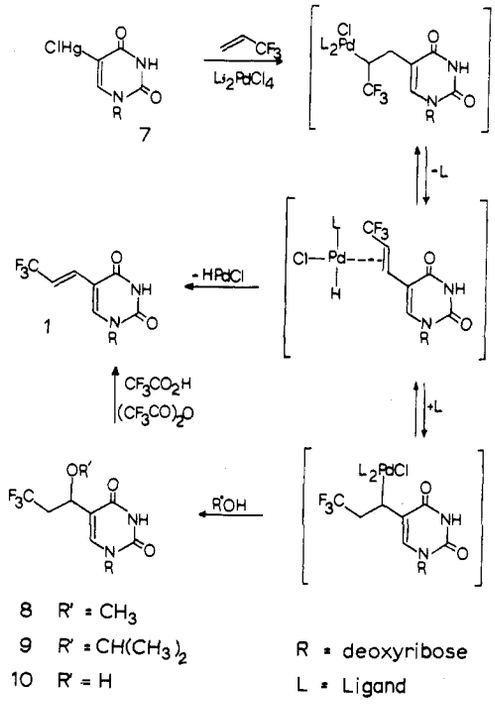
Scheme I. Reaction of 3,3,3-Trifluoropropene with 5-Chloromercuri-2′-deoxyuridine



8 R′ = $CH_3$
9 R′ = $CH(CH_3)_2$
10 R′ = H

R = deoxyribose
L = Ligand

appropriate moieties that can serve as leaving groups at allylic positions are present (e.g., allylic chlorides,[11,13] acetate,[13] some alcohols,[13] and ethers[15]), eliminations of the palladium-leaving group can give 5-(2-propenyl)-2′-deoxyuridine adducts (e.g., 5-allyl-2′-deoxyuridine). When no active allylic groups are present, the major products formed in methanol are the α,β-unsaturated C-5 adduct and the α-methoxy saturated C-5 adduct.[9,12,14]

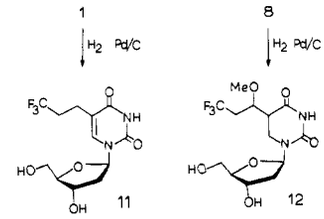
During our studies on the addition of fluoroolefins to mercuri nucleosides, we found that olefins substituted by one fluorine, such as fluoroethene and 3-fluoropropene (allyl fluoride), tended to eliminate fluorine during the course of the coupling reaction with palladium. For example, the reaction of 3-fluoropropene with 5-chloromercuri-2′-deoxyuridine (7) and $Li_2PdCl_4$ in methanol gave almost exclusively 5-allyl-2′-deoxyuridine, presumably by a pathway similar to that described for the reaction of 7 with allyl chloride.[11,13] In contrast 3,3,3-trifluoropropene does not eliminate fluorine in the palladium-mediated reaction with 7. When a saturated solution of 3,3,3-trifluoropropene in methanol is combined with 5-chloromercuri-2′-deoxyuridine (7) and $Li_2PdCl_4$, two major products, (*E*)-5-(3,3,3-trifluoro-1-propenyl)-2′-deoxyuridine (1) and 5-(3,3,3-trifluoro-1-methoxypropyl)-2′-deoxyuridine (8), are formed in approximately 1:2 ratio (see Scheme I). This product distribution is similar to that observed for purely hydrocarbon olefins,[12] although no isomeric products resulting from coupling at the internal carbon of the trifluoropropene are observed.

Other solvents, including *N*,*N*-dimethylformamide (DMF), 2-propanol, and acetonitrile, led to significantly lower yields of C-5 substituted product. For example, in 2-propanol, 1 was isolated in 8% yield, while 5-[3,3,3-trifluoro-1-(2-propoxy)prop-1-yl]-2′-deoxyuridine (9) was obtained in 12% yield.

Since preliminary screening of the α-methoxy adduct 8 showed it to be devoid of significant biological activity,[9] reaction conditions were developed for conversion of 8 to the more desirable α,β-unsaturated TFPe-dUrd (1). The

(15) Ruth, J. L.; Bergstrom, D. E. unpublished results.

Scheme II



elimination of methanol from 8 did not occur under basic conditions [1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) and molecular sieves in refluxing tetrahydrofuran]. However, 8 could be converted to 1 in 65% yield by treatment with a refluxing mixture of trifluoroacetic acid (TFA) and trifluoroacetic acid anhydride (TFAA).

The α-hydroxy adduct 10 was the major isolated product of the reaction between 7 and trifluoropropene in methanol under certain workup conditions (cf. Experimental Section). Although 10 would be expected in aqueous solutions, it is not normally observed in methanol, and exact reasons for the formation of 10 are unclear. Experiments in which the alternate product, 5-(3,3,3-trifluoro-1-methoxypropyl)-2′-deoxyuridine (8), was subjected to the same isolation procedure did not lead to nucleoside 10. Direct treatment of 10 with TFA/TFAA also gives good yields of TFPe-dUrd (1).

Hydrogenolysis of the methoxy group at the α-carbon of C-5 substituted uridine derivatives is a routine procedure.[10,12] However, extended reaction of nucleoside 8 with $H_2$ over Pd/C gave a compound identified as 5-(3,3,3-trifluoro-1-methoxyprop-1-yl)-5,6-dihydro-2′-deoxyuridine (12), (Scheme II) by UV and $^1H$ NMR, rather than 11, indicating that ring reduction occurs before hydrogenolysis in this particular case. Reasons for the failure of 8 to undergo hydrogenolysis to 11 are not clear. 5-(3,3,3-Trifluoropropyl)-2′-deoxyuridine (11) was obtained in good yield by hydrogenation of 1.

A similar series of reactions was attempted with 3,3,3-trifluoropropene and 5-chloromercuri-2′-deoxycytidine. However, subsequent separation and purification of the products from the coupling reaction with 3,3,3-trifluoropropene proved more difficult. Impure 5-(3,3,3-trifluoro-1-methoxypropyl)-2′-deoxycytidine could not be converted to either 5-(3,3,3-trifluoro-1-propenyl)-2′-deoxycytidine by refluxing TFA/TFAA or to 5-(3,3,3-trifluoropropyl)-2′-deoxycytidine by catalytic hydrogenolysis.

The reactions of 3-fluoropropene and 3,3,3-trifluoropropene with 5-mercurated pyrimidine nucleosides were part of an overall effort to construct a variety of C-5 fluorinated carbon substituents. Other fluorinated olefins, such as hexafluoropropene, vinyl fluoride, 1,1-difluoroethene, and 1,1,2-trifluoro-2-chloroethene, gave complex mixtures, from which no products could be isolated.

**Antiviral Activity.** The activities of five C-5 substituted pyrimidine nucleosides, TFPe-dUrd (1), Pe-dUrd (2), BV-dUrd (5), 5-I-dUrd, and 5-$CF_3$-dUrd, against HSV-1, HSV-1 ($TR^-$), HSV-2, and vaccinia virus are summarized in Table I. The nucleoside concentrations necessary for a 2-log reduction in virus yield ($ID_{99}$) are given. Striking differences in antiviral activity are apparent. The three vinylic analogues 1, 2, and 5 are very potent against HSV-1 but are much less effective against HSV-1 ($TK^-$). All three analogues, and in particular TFPe-dUrd (1), are also significantly less effective against HSV-2 and vaccinia viruses. TFPe-dUrd (1) is more effective than Pe-dUrd (2) against HSV-1 but exerts little, if any, activity against HSV-1 ($TK^-$), HSV-2, or vaccinia virus, whereas 2, although inactive against HSV-1 ($TK^-$) and vaccinia, is still active

Table I. Differential Susceptibility of the Replication of HSV-1 (Strain KOS), HSV-1 (TK⁻, Strain B2006), and HSV-2 (Strain G) to the Inhibitory Effects of (E)-5-(1-Propenyl)-2'-deoxyuridine (2), (E)-5-(3,3,3-Trifluoro-1-propenyl)-2'-deoxyuridine (1), and (E)-5-(2-Bromovinyl)-2'-deoxyuridine (5) in Primary Rabbit Kidney Cell Cultures

| | $ID_{99}$,[a] μg/mL | | | | | | |
|---|---|---|---|---|---|---|---|
| compd | HSV-1 (KOS) | HSV-1 (TK⁻, B2006) | HSV-2 (G) | vaccinia | HSV-1 (TK⁻)/HSV-1 | HSV-2/ HSV-1 | vaccinia/ HSV-1 |
| 1 | 0.03 | >100 | 100 | >100 | >3333 | 3300 | >3333 |
| 2 | 0.15 | >100 | 18 | >100 | >667 | 120 | >667 |
| 5 | 0.008 | ⩾100 | 3 | 22 | ⩾12500 | 375 | 2750 |
| 5-$CF_3$-dUrd | 0.7 | 0.4 | 1.2 | 0.12 | 0.6 | 1.7 | 0.17 |
| 5-I-dUrd | 0.1 | >100 | 0.8 | 0.25 | >1000 | 8 | 2.5 |

[a] Concentration of compound required to inhibit virus yield by 99%.

Table II. Antimetabolic Activities of (E)-5-(1-Propenyl)-2'-deoxyuridine (2), (E)-5-(3,3,3-Trifluoro-1-propenyl)-2'-deoxyuridine (1), and 5-(2-Bromovinyl)-2'-deoxyuridine (5) in Primary Rabbit Kidney Cell Cultures

| | $ID_{50}$,[a] μg/mL | |
|---|---|---|
| compd | [Me-³H]dThd | [1',2'-³H]dUrd |
| 1 | 300 | 150 |
| 2 | 25 | 40 |
| 5 | 70 | 20 |
| 5-$CF_3$-dUrd | 20 | 0.01 |
| 5-I-dUrd | 1 | 0.25 |

[a] Concentration of compound required to inhibit [*methyl*-³H]dThd or [1',2'-³H]dUrd incorporation into host cell DNA by 50%.

against HSV-2. Presumably, these antiviral effects reflect the ability of the viral-mediated TK (if present) to phosphorylate/activate the analogues, and the three fluorines in 1 (relative to 2) exert subtle steric or electronic effects on the interaction of the compound with the TK.

With a 3000-fold greater inhibitory effect on HSV-1 than on HSV-2 replication, TFPe-dUrd shows a greater difference in intertypic potency than any other nucleoside analogue. Although other nucleosides have been suggested for this purpose,[1,2,16] TFPe-dUrd (1) appears to be the most ideal candidate for the rapid and specific identification of HSV-1 and HSV-2 isolates by either differential inhibition of virus replication in cell culture or differential TK affinity in cell lysates. The susceptibility of 12 different isolates of HSV-1 and HSV-2 to 1 has already been investigated by a viral cytopathogenicity assay,[2] and these studies indicated a difference of three orders of magnitude in the susceptibility of HSV-1 and HSV-2 with surprisingly little variation between the isolates.

Structurally, TFPe-dUrd (1), Pe-dUrd (2), and (E)-5-(2-iodovinyl)-2'-deoxyuridine (6) can be considered as C-5 vinyl analogues of 5-(trifluoromethyl)-2'-deoxyuridine, thymidine, and 5-iodo-2'-deoxyuridine, respectively. The presence of the ethenyl bridge in the three former compounds drastically alters the antiviral activity. For example, 5-(trifluoromethyl)-2'-deoxyuridine is equally effective against HSV-1, HSV-1 (TK⁻), and HSV-2 and most effective against vaccinia; its vinylic counterpart, TFPe-dUrd, is ca. 25-fold *more* potent against HSV-1, ca. 80-fold *less* active against HSV-2, and essentially inactive against vaccinia.

TFPe-dUrd (1) and Pe-dUrd (2) did not affect normal cell metabolism, as monitored by dUrd or dThd incorporation into host cell DNA, unless concentrations were employed that were about 1000-fold greater than the 50% inhibitory dose for HSV-1 (Table II). Unlike 5-(trifluoromethyl)-2'-deoxyuridine, which inhibited (1',2'-³H)dUrd incorporation at a 2000-fold lower concentration than (*methyl*-³H)dThd incorporation, both 1 and 2 inhibited dUrd and dThd incorporation to approximately the same extent. A significantly greater inhibition of dUrd than of dThd incorporation has been interpreted as evidence for a specific inhibition of thymidylate synthetase in intact cells.[17] Indeed, the conversion of dUMP to dTMP is the only metabolic step that distinguishes the incorporation of dUrd and dThd into DNA. The finding that 1 and 2 did not preferentially inhibit dUrd incorporation indicates that neither analogue interferes with dTMP synthetase in intact cells. The cytotoxicity of TFPe-dUrd, like that of Pe-dUrd,[3] is very low. Proliferation of murine leukemia L-1210 cells is not inhibited except at high concentrations; i.e., at 292 μg/mL, TFPe-dUrd causes 50% inhibition of cell growth. By comparison, 5-(trifluoromethyl)-2'-deoxyuridine has an $ID_{50}$ of 0.007 μg/mL.

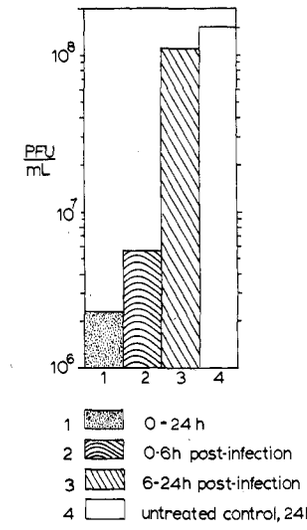



**Figure 1.** Time-dependent effect of TFPe-dUrd (1 μM) on HSV-1 (KOS) infections in HeLa cells. The number of plague-forming units (pfu) per milliliter of cells was determined after 24 h. The infected cells were exposed to TFPe-dUrd for 24 (1), 6 (2), and 18 h (3, compound added 6 h after cells were infected).

**Effect of TFPe-dUrd on Viral DNA Synthesis.** When nucleoside analogues are evaluated for their anti-HSV activity in cell culture, they are generally added to the cells immediately after these have been inoculated with the virus. There is evidence,[18-20] however, that the antiviral

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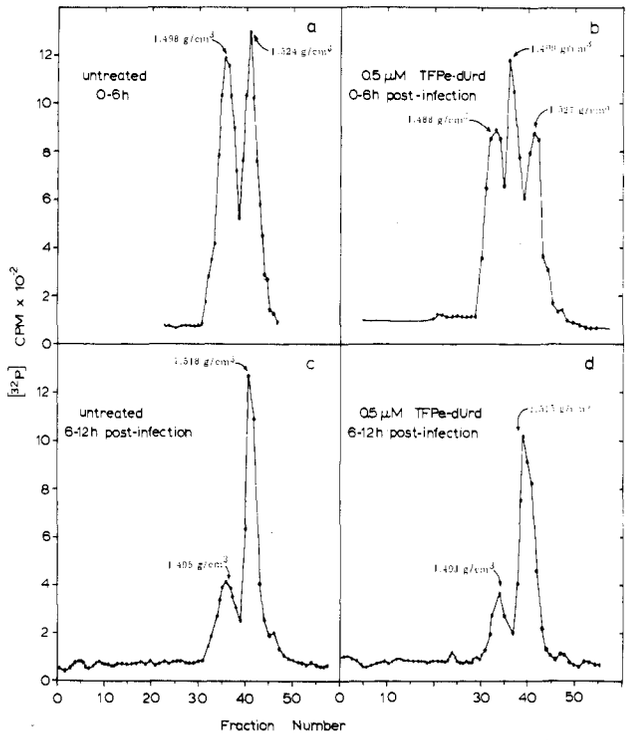


**Figure 2.** Isopycnic centrifugation in NaI gradient of DNA from HSV-1 (KOS) infected HeLa cells. HSV-1 Infected cells were grown in the absence (a, c) or presence of 0.5μM TFPe-dUrd (b, d).

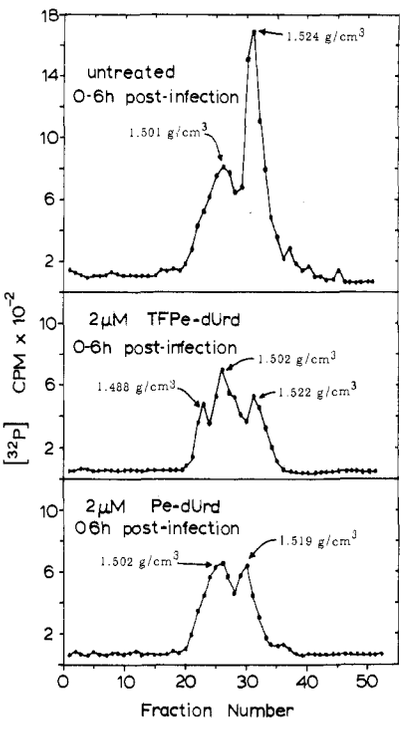


**Figure 3.** Comparison of the effects of TFPe-dUrd (2 μM) and Pe-dUrd (2 μM) on DNA from HSV-1 infected cells as measured by isopycnic centrifugation in a NaI gradient.

activity of some analogues may be drastically reduced if the drug is added only during an early-intermediate (4–10 h) period postinfection. To investigate this possibility with TFPe-dUrd (1), we first infected HeLa BU cells with HSV-1 at 10 pfu/cell and then incubated them with 1 during the periods of 0–24, 1–6, or 6–24 h postinfection. Virus was harvested at 24 h and quantitated by plaque yield. The results clearly indicated (Figure 1) that (1) the antiviral activity of TFPe-dUrd is determined very early in virus infection, that (2) the antiviral effect is essentially irreversible if 1 is present immediately after infection, and that (3) 1 added after the infection is established has little apparent effect on the ultimate course of infection.

To further explore the antiviral effects of TFPe-dUrd at the subcellular level, we performed similar incubations in the presence of $^{32}P$-labeled orthophosphate, followed by isopycnic centrifugation in sodium iodide to resolve viral from cellular DNA. In uninfected HeLa $S_3$ cells, 10 μM TFPe-dUrd did not reduce DNA synthesis and did not affect the buoyant density (1.497 ± 0.0004 g/cm$^3$ of NaI) of the HeLa cell DNA (results not shown). When [$^{32}P$]-orthophosphate and 0.5 μM TFPe-dUrd were added from 3–6 h following infection and DNA was harvested at 6-h postinfection, isopynic centrifugation showed a reduction of about 30% for viral DNA (1.520 ± 0.005 g/cm$^3$ of NaI) (Figure 2b) relative to untreated control cells (Figure 2a), while cellular DNA showed little, if any, decrease. However, a new [$^{32}P$]DNA band was consistently evident at a density (1.488 g/cm$^3$) that was lighter than that of either cellular or viral DNA derived from untreated cells. When TFPe-dUrd was added at 6 h postinfection and the harvest at 12 h was submitted to isopynic centrifugation, the [$^{32}P$]DNA profile of treated samples (Figure 2d) was consistently similar to the untreated control samples (Figure 2c).

Based on whole-cell data, the marked decrease in viral DNA for the 0–6-h postinfection samples was expected. However, the appearance of the third band at lesser density than either untreated cellular or viral DNA was unexpected. No attempts have been made to discern if the [$^{32}P$]DNA bands at normal cellular or viral densities in the TFPe-dUrd-treated infected cells were in fact of cellular or viral origin or if the extra band was of cellular or viral origin. Similar experiments with 2 μM TFPe-dUrd present at 0–6 h postinfection were run concurrently with 2 μM Pe-dUrd (2). The results (Figure 3a–c) show that both TFPe-dUrd and Pe-dUrd do not markedly decrease cellular [$^{32}P$]DNA synthesis relative to untreated control, whereas viral DNA is significantly (>75%) reduced. At this concentration (2 μM), the lower density band again appeared at 1.488 g/cm$^3$ for TFPe-dUrd (1) (Figure 3b), whereas Pe-dUrd (2) treated viral infected cells showed no lower density DNA band (Figure 3c). For both Pe-Urd (2) and BrV-dUrd (5), the shift in viral DNA density is proportional to the extent of incorporation of the analogues into the viral DNA.[4,7] If, as the evidence suggests, these compounds (1, 2, and 5) are incorporated into DNA during the early stages of replication, then TFPe-dUrd must effect DNA structure in a more complex way than do Pe-Urd and BrV-dUrd. The occurrence of the DNA band at lower density suggests an effect at the macromolecular level, such as might occur if a critical step in DNA processing were inhibited or if a polypeptide (or similar lower density molecule) were attached to the DNA. This latter aberration may be uniquely possible for DNA substituted with TFPe-dUrd, since an important chemical difference between TFPe-dUrd and the other vinyl analogues is the reactivity of TFPe-dUrd toward nucleophiles, a consequence of the strong electron-withdrawing $CF_3$ group. Clearly, more work will be required to establish both the basis for the low-density DNA band and the antiviral activity of TFPe-dUrd.

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## Conclusion

TFPe-dUrd exhibits potent and selective inhibitory activity on HSV-1 replication with an $ID_{99}$ of 0.03 μg/mL. It is much less active against HSV-2 and is inactive against vaccinia virus. The significantly decreased activity (>$10^3$ less) against HSV-2 suggests that TFPe-dUrd may be used as a diagnostic tool in the typing of HSV; this premise is supported by the results obtained for the clinical isolates. Finally, it should be noted that the data shown in Figures 1 and 2 show a direct correspondence between morphological results (amount of viral replication) and a biochemical effect (amount of labeled viral DNA synthesized). Isopycnic centrifugation indicates that TFPe-dUrd at 10 μM does not affect DNA synthesis in uninfected cells but causes a ~75% decrease in viral DNA synthesis at 2.0 μM. At both 0.5 and 2 μM, TFPe-dUrd produces a new DNA band with a density lighter than that for either cellular or viral DNA.

## Experimental Section

**Synthesis.** 5-Chloromercuri-2'-deoxyuridine (7) was prepared[21] from 2'-deoxyuridine (Sigma Chemical Co.) and mercuric acetate (Mallinkcrodt). 3,3,3-Trifluoropropene was from PCR chemicals, and palladium(II) chloride was from Matthey Bishop. Melting points were determined on a Büchi 510 programmable melting point apparatus and are uncorrected. $^1H$ NMR spectra were recorded on a Varian EM-360 60-MHz spectrometer in $D_2O$; the $^{13}C$ spectra were obtained on a JOEL FX 60-MHz FT NMR spectrometer; δ values reported are in parts per million downfield from sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate as an internal standard. UV spectra were recorded to ±0.5 nm in $H_2O$ with a Cary 17 spectrophotometer, and the pH extremes were obtained by diluting the stock neutral solution with 15 vol % 1.0 N HCl or 1.0 N NaOH (final pH approximately 1.2 or 12.6, respectively); wavelengths are reported in nanometers. TLC was carried out on 3.5 × 10 cm × 0.25 mm E. Merck 60F-254 chromatogram sheets in 5 × 5 × 12 cm lined chambers. The following TLC solvent systems were used (relative proportions are v/v): system A, $CH_3OH/CHCl_3$ (1:3); system B, $CH_3OH$/EtOAc (3:2); system C, $CH_3CN$/*n*-BuOH/0.10 M $NH_4OAc$/concentrated $NH_4OH$ (10:60:20:10). IR spectra were recorded on a Beckman IR-8 in solid KBr using the 1601-$cm^{-1}$ absorption of polystyrene for calibration. Low-resolution mass spectroscopy was done at 70 eV by the instrumentation center at the University of California, Berkeley. The mass to charge fragmentation peaks listed for the mass spectroscopy of compounds are the molecular ion peak (M) and the peak corresponding to the 5-alkylpyrimidine formed after loss of the sugar (which is usually 30–40% of the base peak). Elemental analyses were determined by Chemalytics, Inc. (Tempe, AZ). Silica gel 60 (70–230 mesh ASTM) from E. Merck was used for column chromatography. Bio-Gel P-2 spherical polyacrylamide gel (200–400 mesh, exclusion limit 1800 daltons) was from Bio-Rad Laboratories, and Sephadex G-10 (medium) was from Pharmacia.

**(*E*)-5-(3,3,3-Trifluoro-1-propenyl)-2'-deoxyuridine (1). In Methanol.** A 4.23 g (9.14 mmol) sample of 5-chloromercuri-2'-deoxyuridine (7) in 20 mL of methanol was stirred in a 500-mL Parr flask, and 110 mL (11 mmol, 1.2 equiv) of 0.10 M $Li_2PdCl_4$ in methanol was added. The system was evacuated by an aspirator, pressurized to 20 psig with 3,3,3-trifluoropropene, and repressurized as necessary to keep the pressure above 10 psig. After the mixture was stirred at room temperature for 6.5 h, the flask was evacuated, and the black suspension was filtered. The yellow filtrate was treated with hydrogen sulfide for ca. 30 s and then filtered through Celite, and the filtrate was concentrated in vacuo. Chromatography on a column of 250 g of silica gel (2.4 × 118 cm), eluting with increasing vol % methanol in chloroform (5–18%), gave one major UV-absorbing peak. TLC analysis of this peak indicated some separation of two products (with some overlap), which were individually rechromatographed on a column of Sephadex G-10 (2.0 × 65 cm) eluting with water to yield 5-(3,3,3-trifluoro-1-methoxyprop-1-yl)-2'-deoxyuridine (8; 1.9 g, 59%) and TFPe-dUrd (1; 786 mg, 26%) as white fluffy solids.

The 5-(3,3,3-trifluoro-1-propenyl)-2'-deoxyuridine (1) was recrystallized from water to yield white crystals, which soften with evolution of gas at 139–141 °C and melt with decomposition at 176–177 °C: $^1H$ NMR δ 8.26 (s, 1), 6.78 (narrow m, 2), 6.23 (t, 1, $J$ = 6.5 Hz), 4.45 (m, 1), 4.0 (m, 1), 3.82 (narrow m, 2), 2.37 (dd, 2, $J_1$ = 5.5 Hz, $J_2$ = 6.5 Hz); $^{13}C$ NMR ($D_2O$) δ 61.9 (C-2'), 63.30 (C-5'), 72.9 (C-3'), 88.68 (C-4'), 89.66 (C-1'), 111.49 (C-5), 120.07 (C-2 of side chain), 124.98 (q, $J_{C-F}$ = 216 Hz, $CF_3$), 132.67 (C-1 of side chain), 145.34 (C-6), 153.27 (C-4), 166.65 (C-2); UV $\lambda_{max}$ 243 nm (ε 6970), 285 (6500), $\lambda_{min}$ 221 (4280), 263 (4050); UV $\lambda_{max}$ (pH 6.6) 243 nm (ε 7010), 285 (6490), $\lambda_{min}$ 221 (4280), 263 (4060); UV $\lambda_{max}$ (base) 247 nm (ε 8300), 283 (5780), $\lambda_{min}$ 230 (6970), 269 (5250); TLC $R_f$ (A) 0.53, (B) 0.73, (C) 0.44; IR 3440, 3340, 1710, 1680, 1480, 1295, 1170, 1120, 1080, 1050, 970 (indicating trans) $cm^{-1}$; mass spectrum $m/e$ 322 (M), 206 [5-(3,3,3-trifluoropropen-1-yl)uracil]. Anal. ($C_{12}H_{13}N_2O_5F_3$) C, H, N.

5-(3,3,3-Trifluoro-1-methoxyprop-1-yl)-2'-deoxyuridine (8): mp, decomposes below 70 °C; $^1H$ NMR δ 8.07 (s, 1), 6.33 (t, 1, $J$ = 6.5 Hz), 4.58 (m, 1), 4.1 (m, 1), 3.89 (narrow m, 2), 3.36 (s, 3), 2.9-2.3 (br m, 4); $^{13}C$ NMR δ 40.28 (q, $J_{C-F}$ = 27.4 Hz, C-2 of side chain) 41.90 (C-2'), 58.99 (OMe), 63.60 (C-5'), 72.90 (C-3'), 74.52 (C-1 of side chain), 88.36 (C-4'), 89.53 (C-1'), 114.28 (C-5), 128.48 (q, $J$ = 276.3 Hz, $CF_3$), 142.29 (C-6), 153.79 (C-4), 166.65 (C-2); UV $\lambda_{max}$ (acid) 264 nm (ε 9140); UV $\lambda_{max}$ (pH 6.6) 264 nm (ε 9460), $\lambda_{min}$ 232; UV $\lambda_{max}$ (base) 263 nm (ε 7050); TLC $R_f$ (A) 0.54, (B) 0.72, (C) 0.46; IR 3440, 3080, 2960, 2850, 1680, 1470, 1280, 1260, 1205, 1100, 1050, 840, 790 $cm^{-1}$; mass spectrum, $m/e$ 354 (M). Anal. ($C_{13}H_{17}N_2O_6F_3$) C, H, N.

Two similar reactions of 7 with 3,3,3-trifluoropropene in methanol with excess $Pd^{2+}$ gave much smaller amounts of 8 (12–15%), and both gave the same amounts of 1 (17–21%), with a third compound, 5-(3,3,3-trifluoro-1-hydroxy)-2'-deoxyuridine (10), isolated in 38–40% yield. In these trials, the Pd was not filtered from the reaction before $H_2S$ treatment; no other changes were noted. 5-(3,3,3-Trifluoro-1-hydroxypropyl)-2'-deoxyuridine (10) was recrystallized from acetonitrile to give white crystals: mp 92.0–92.5 °C; NMR δ 8.01 (s, 1), 6.32 (t, 1, $J$ = 6.5 Hz), 5.01 (t, 1, $J$ = 6 Hz), 4.5 (m, 1), 4.16 (m, 1), 3.91 (narrow m, 2), 2.6 (broad unsymmetrical m, 4); UV $\lambda_{max}$ 264 nm, $\lambda_{min}$ 232 nm; TLC $R_f$ (A) 0.43, (B) 0.73, (C) 0.38. Anal. ($C_{12}H_{15}N_2O_6F_3$) C, H, N.

**In 2-Propanol.** Reaction of 1.18 g (2.55 mmol) of 7 and 1.1 equiv of $Li_2PdCl_4$ with 3,3,3-trifluoropropene in 2-propanol for 3 h, followed by hydrogen sulfide treatment and chromatography on Bio-Gel P-2 (2.0 × 135 cm) eluting with water, gave 52 mg (6%) of TFPe-dUrd (1), as identified by $^1H$ NMR and TLC in systems A–C. A second product was identified by $^1H$ NMR as 5-[3,3,3-trifluoro-1-(2-propoxy)propyl]-2'-deoxyuridine (100 mg, 10%): $^1H$ NMR δ 8.02 (s, 1), 6.39 (dt, 1, $J_1$ = 2 Hz, $J_2$ = 6.5 Hz), 4.54 (m, 1), 4.11 (m, 1), 3.88 (narrow m, 2), 2.8–2.2 (broad m, 4), 1.19 (dd, 6, $J_1$ = 3 Hz, $J_2$ = 6 Hz); UV $\lambda_{max}$ 266 nm, $\lambda_{min}$ 233 nm; TLC $R_f$ (A) 0.59, (B) 0.72, (C) 0.47.

**Dehydration of 5-(3,3,3-Trifluoro-1-hydroxypropyl)-2'-deoxyuridine (10) to TFPe-dUrd (1).** A 308 mg (0.905 mmol) sample of 10 was refluxed in a solution of 20 mL of trifluoroacetic acid and 7 mL of trifluoroacetic anhydride. After a 23-h reflux, $\lambda_{max}$ ($H_2O$) had shifted from 266 to 272 nm, 245 nm. The solution was cooled, concentrated by rotary evaporation to a thick brown oil, and neutralized with $KHCO_3$. Chromatography on a column of Bio-Gel P-2 (1.2 × 66 cm), followed by a column of 50 g of silica gel (2.0 × 42 cm) eluting with increasing vol % methanol in chloroform (5–18%), gave one major product. Evaporation gave 188 mg (65%) of white solid, identified as TFPe-dUrd (1) by UV, $^1H$ NMR, and TLC in systems A and C.

**5-(3,3,3-Trifluoro-1-propyl)-2'-deoxyuridine (11).** A 181 mg (0.56 mmol) sample of TFPe-dUrd (1) and 30 mg of 10% palladium on charcoal in 15 mL of methanol in a 250-mL Parr flask was stirred for 1.3 h under a 30 psig hydrogen atmosphere and then gravity filtered, and the solvent was removed under vacuum to leave 137 mg (75%) of a white solid. Recrystallization from EtOAc gave white crystals identified as 5-(3,3,3-trifluoro-1-propyl)-2'-deoxyuridine (11): mp 164.0–165.0 °C (slow dec); $^1H$ NMR δ 7.89 (s, 1), 6.32 (t, 1, $J$ = 6.5 Hz), 4.52 (m, 1), 4.09 (m, 1), 3.87 (narrow m, 2), 2.58 (m, 4), 2.41 (dd, 2, $J_1$ = 5.5 Hz, $J_2$ =

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6.5 Hz); UV ($CH_3OH$) $\lambda_{max}$ 265 nm, $\lambda_{min}$ 232; mass spectrum, *m/e* 324 (M), 208 [5-(3,3,3-trifluoro-1-propyl)uracil]. Anal. ($C_{12}H_{15}N_2O_5F_3$) C, H, N.

**Antiviral Assays.** The origin of the herpes viruses was as follows: HSV-1 (strain KOS), Dr. W. E. Rawls (Baylor University College of Medicine, Houston, TX); HSV-2 (strain G), American Type Culture Collection (Rockville, MD); HSV-1 ($TK^-$, B2006), Dr. Y.-C. Cheng (University of North Carolina School of Medicine, Chapel Hill, NC). All HSV stocks were prepared in primary rabbit kidney (PRK) cell cultures. Vaccinia virus stock was prepared in the chorio-allantoic membrane cells of embryonated eggs.

The antiviral assays were based on the inhibition of virus multiplication in primary rabbit kidney cell cultures. For the antiviral assays described in Table I, the cell cultures were inoculated at a multiplicity of infection of 0.03 pfu (plaque forming units) per cell. The compounds were added immediately after virus infection, and virus yield was measured at either 24 [HSV-1 (KOS), HSV-2 (G), vaccinia virus] or 48 h [HSV-1 ($TK^-$, B2006)] after infection, by plague formation in Vero cells [HSV-1 (KOS), HSV-2 (G), HSV-1 ($TK^-$, B2006)] or primary rabbit kidney cells (vaccinia virus). The antiviral activity is expressed as $ID_{99}$ (inhibitory dose), i.e., the concentration of compound required to reduce viral yield by 99%.

**Antimetabolic Assays.** The procedure for measuring [*methyl*-$^3$H]dThd or [1′,2′-$^3$H]dUrd incorporation into DNA of PRK cell cultures has been described previously.[17] The input of the radiolabeled precursors was 10 pmol (0.38 μCi) of [*methyl*-$^3$H]dThd or 6 pmol (0.25 μCi) of [1′,2′-$^3$H]dUrd per $10^5$ PRK cells.

**Determination of Cellular and Viral DNA Synthesis.** HeLa BU and HeLa $S_3$ cells were grown in monolayer to 70–80% confluency in 25-$cm^2$ flasks. Media were removed and replaced with Joklik-modified (low phosphate) media. Virus was added at 5–10 pfu/cell. After 1 h virus adsorption, the media and excess virus were removed, and 5.0 mL of fresh low phosphate media was added. At defined times (0 or 6 h postinfection) were added 50–100 μCi of [$^{31}$P]orthophosphate per flask and/or TFPe-dUrd (1) solution, as appropriate. At the designated times, the cells were harvested, centrifuged for 8 min at 2000 rpm, washed in phosphate-buffered saline, and recentrifuged. The cells were resuspended in 500 μL of lysing buffer containing 10 mM Tris-HCl (pH 7.9), 1 mM EDTA, 100 mM NaCl, 0.5% sarkosyl, and 1% sodium dodecyl sulfate and mixed thoroughly. After 30 min, 100 μL of 6 mg/mL of proteinase K in lysing buffer was added, and the suspension was mixed well and then incubated at 37 °C for 18–24 h with agitation. Isopycnic ultracentrifugation using sodium iodide was a modification of the procedure described by Walboomers and Ter Schegget;[22] 40 mL of sodium iodide solution contained 10 mM Tris-HCl (pH 7.9), 10 mM EDTA, 20 mg of $NaHSO_3$, and ~29.5 sodium iodide to a final apparent density of 1.53–1.54 g/$cm^3$ by refractive index.[19] In 5-mL cellulose nitrate tubes, 500 μL of the cell lysate mixture was layered onto 4.5 mL of the sodium iodide solution and centrifuged at 44 000 rpm for 38–46 h at 20 °C in a Beckman L5-50B ultracentrifuge using a Beckman SW 50.1 rotor. Samples were immediately dripped from the top of the tubes using a Buchler Auto Densi-Flow IIC pump dripper, and fractions of 3 drops (70–80 μL average fraction volume) was collected. The refractive index ($n^{20}_D$) of 20-μL samples from eight to ten representative fractions were taken at 20 °C (±0.5) for calculation of gradient densities. Ten microliters of 6 N KOH was added to 50 μL of each fraction. The fractions were incubated for 2 h with agitation at 37 °C to degrade RNA, and then each was spotted (50 μL) onto Whatman 3MM paper disks. Acid-insoluble material was precipitated onto the disks by three washes with cold 5% trichloroacetic acid containing 10 mM pyrophosphate (10 mL/disk) and two washes with 95% ethanol. The disks were thoroughly dried and then radiocounted for 5 min in liquid scintillant. In more than 19 trials, cellular DNA banded at 1.497 ± 0.004 g/$cm^3$ of NaI and viral DNA at 1.520 ± 0.005 g/$cm^3$ of NaI.

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