Nucleic Acid Related Compounds. 65. New Syntheses of 1-(β-D-Arabinofuranosyl)-5(E)-(2-iodovinyl)uracil (IVAraU) from Vinylsilane Precursors. Radioiodine Uptake as a Marker for Thymidine Kinase Positive Herpes Viral Infections[1]

Morris J. Robins,*,† Stefano Manfredini,†,‡ Steven G. Wood,† R. James Wanklin,§ Bruce A. Rennie,§ and Stephen L. Sacks§,⊥

Department of Chemistry, Brigham Young University, Provo, Utah 84602, and Departments of Medicine and Pharmacology and Therapeutics, The University of British Columbia, Vancouver, BC V6T 1W5, Canada. *Received July 16, 1990*

(Trimethylsilyl)acetylene was coupled with 1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)-5-iodouracil (3) to give 1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)-5-[2-(trimethylsilyl)ethynyl]uracil (4). Lindlar hydrogenation of 4 gave 1-(2,3,4-tri-*O*-acetyl-β-D-arabinofuranosyl)-5(*Z*)-[2-(trimethylsilyl)vinyl]uracil (5). Treatment of 5 with iodine monochloride (or sodium iodide/phenyliodine(III) dichloride) in benzene gave 1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)-5(*E*)-(2-iodovinyl)uracil (7), whereas polar solvents favored the (*Z*)-iodovinyl isomer 8. Deacetylation of 7 gave 1-(β-D-arabinofuranosyl)-5(*E*)-(2-iodovinyl)uracil (IVAraU, 9). A microscale in situ synthesis with Na*I gave [*I]IVAraU. Treatment of HSV-infected cells with [^{125}I]IVAraU resulted in virus-dependent uptake associated with nucleoside phosphorylation by wild type or acyclovir-resistant DNA polymerase mutants (but not with TK$^-$ HSV-1 mutants). Uptake was virus-inoculum dependent and was detectable within 4 h postinfection. The process was not completely reversible. Virus-specified uptake of [^{125}I]IVAraU may allow automated in vitro detection of HSV isolates.

Introduction

Herpes simplex virus (HSV) and varicella zoster virus (VZV) are ubiquitous viruses which usually cause mild, self-limited infections in immunocompetent hosts.[2,3] A small proportion of patients, however, develop deep visceral encephalitic (HSE) or ophthalmic infections.[4] Because of difficult access to the involved tissue, diagnoses may be delayed or missed. Delays have become more problematic with the advent of effective antiviral chemotherapy since reduced drug efficacy or neglected opportunities for treatment could result. Available invasive diagnostic procedures may not be used because of disagreements over rationales for their use.[5] Without treatment, the mortality of HSE approaches 70%, and fewer than 10% of the afflicted patients regain fully normal lives.[6,7] The use of brain biopsy, the only definitive diagnostic method, is controversial.[8] Traditional noninvasive methods for diagnosis including measuements of serum and CSF HSV-specific antibodies have become more promising with the advent of the polymerase chain reaction.[9] However, even this sensitive method may be hampered by an inherent delay between the onset of symptoms and a positive test result.

Another diagnostic concept, originally suggested by Saito et al.,[10] would exploit increased concentrations of antiviral nucleoside phosphates in cases where HSV infection results in expression of viral-specified thymidine kinases (TK's). A radionuclide tagged to the antiviral agent or incorporated into its structure could be used to trace the infection by imaging with single photon emission computed tomography (SPECT). However, the clinical development of this idea has been hampered by nonspecific catabolism of certain agents[11] and the lack of an appropriate agent with a clinically safe, proven radionuclide incorporated into its structure.[10] The simplicity of a radiochemical synthesis and the time required for its execution also are crucial factors.[12]

HSV-specified TK's effect monophosphorylation of the anti-HSV agents 5(*E*)-(2-bromovinyl)-2′-deoxyuridine (BVDU) and 1-(β-D-arabinofuranosyl)-5(*E*)-(2-bromovinyl)uracil (BVAraU).[13] The HSV-1 TK also has thymidylate kinase activity.[13] This accounts for the selective activity of these agents only in HSV-1 or VZV infected cells in which they are anabolized beyond the 5′-monophosphate level. The related iodo (IVAraU) and chloro (CVAraU) analogues also have demonstrated high therapeutic selectivities in TK$^+$ HSV-1 and VZV infections.[14]

That highly selective viral-TK phosphorylation could be useful for diagnostic as well as therapeutic applications has been considered previously.[10] Radioiodinated IVDU and IVAraU represent potential scanning agents for non-

† Brigham Young University.
‡ On leave from the Department of Pharmaceutical Science, University of Ferrara, Ferrara, Italy: summers of 1987 and 1988.
§ The University of British Columbia.
⊥ To whom inquiries regarding antiviral effects should be addressed.

(1) (a) Presented in part at the XXVIIIth Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, CA, October 1988, Abstract 97; the XXIXth Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, TX, September 1989, Abstract 737; and the International Symposium on Antiviral Chemotheraphy, Porto Cervo, Italy, October 1989. (b) The previous paper in this series is: Chen, Y.-C. J.; Hansske, F.; Janda, K. D.; Robins, M. J. *J. Org. Chem.* **1991**, *56*, 3410.
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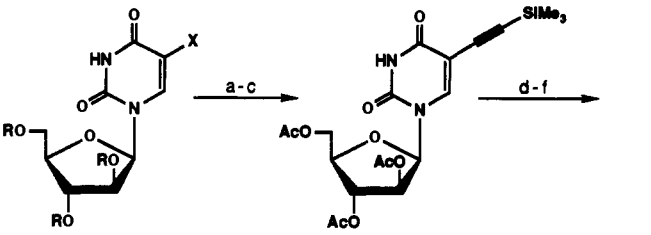
0022-2623/91/1834-2275$02.50/0

invasive diagnosis since clinically-proven radioisotopes of iodine are available. However, BVDU and its iodo analogue, IVDU, are subject to rapid catabolism by pyrimidine nucleoside phosphorylases and possibly other pathways.[13,15,16] Stabilization of 2′-deoxyuridine nucleosides against phosphorolysis in vivo has been achieved by incorporation of fluorine (ribo configuration)[17] or a hydroxyl group (arabino configuration)[18] at C2′ of the furanosyl ring. The chemical/biological stability of the readily accessible arabinosyl analogues is attractive for minimizing the limiting factors of spontaneous glycosyl cleavage and nonspecific catabolism in the development of radiodiagnostic agents. Although the synthesis of ^{14}C-labeled BVAraU was reported recently[18] for monitoring metabolic studies, ^{14}C is not a suitable radionuclide for in vivo diagnosis.[10] Accordingly, 1-(β-D-arabinofuranosyl)-5(*E*)-(2-[*I]iodovinyl)uracil ([*I]IVAraU, **9**) was targeted as having the desired characteristics for a viral radiodiagnostic agent. In the present studies, a general method for the preparation of 5(*E*)-(2-iodovinyl)uracil nucleosides from a precursor vinylsilane is illustrated with a laboratory-scale (100-mg) synthesis of 1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)-5-(*E*)-(2-iodovinyl)uracil (**7**) and then applied on a modified microscale for the preparation of [^{125}I]IVAraU ([^{125}I]-**9**).

Results and Discussion

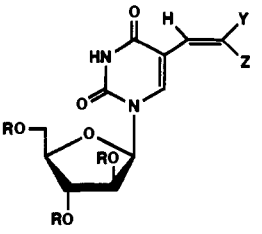
Chemistry. Prior syntheses of BVAraU and its halogen analogues[19] have utilized halogenation of parent vinyl derivatives[19,20a] and modified Hunsdiecker reactions of 5(*E*)-(2-carboxyvinyl)uracil nucleosides with halosuccinimides[20b] or other halogen species.[20c,21] Radiohalogenation to give 5(*E*)-(2-halovinyl)uracil deoxynucleosides has been effected by radiohalogen exchange[21,22] and neutron activation.[21] Harsh reaction conditions, moderate yields with significant levels of byproduct formation, and the use of specialized equipment decrease the appeal of certain prior methods. Radioiodination of vinylstannanes is a mild method, but few halogenations of vinylsilanes have been examined. Indeed, a recent review of the synthesis of radiolabeled compounds via organometallic intermediates[23] did not report procedures readily applicable to our needs. We now describe a straightforward new route to XVAraU compounds (XV = 2-halovinyl) beginning with uridine and employing mild and high-yield reactions. Conversions of the penultimate precursor vinylsilane to the protected 5-(2-halovinyl)AraU derivatives proceed smoothly at ambient temperature with routine equipment, and are directly applicable to radiochemical syntheses. The use of an alternative oxidant (xenon difluoride) for the preparation of iodo, bromo, and chloro analogues has been communicated.[24]

Scheme I[a]



	R	X
1	H	H
2	Ac	H
3	Ac	I

4



	R	Y	Z
5	Ac	H	$SiMe_3$
6	Ac	$SiMe_3$	H
7	Ac	I	H
8	Ac	H	I
9	H	I	H

[a] (a) Ac_2O/DMAP; (b) $ICl/CH_2Cl_2/\Delta$; (c) (trimethylsilyl)-acetylene/$(Ph_3P)_2PdCl_2$/CuI/Et_3N/50 °C; (d) H_2/Lindlar catalyst/quinoline/EtOAc; (e) (i) ICl/benzene or NaI/$PhICl_2$/benzene (for **7**); or (ii) ICl/(MeOH/H_2O, 4:1) (for **8**); (f) NH_3/MeOH.

Uridine was converted into 2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil (90%) as described.[25] This product was treated with aqueous base and the resulting AraU (**1**) (see Scheme I) was acylated with 4-(dimethylamino)pyridine (DMAP)/acetic anhydride[26] to give 1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)uracil[27] (**2**) (90%). Treatment of **2** with iodine monochloride in dichloromethane[28] gave 1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)-5-iodouracil[29] (**3**) (88%). Coupling of **3** and (trimethylsilyl)acetylene with $(Ph_3P)_2PdCl_2$/CuI in triethylamine[30] gave crystalline 1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)-5-[2-(trimethylsilyl)ethynyl]uracil (**4**) (82%). A mixture containing **4** had been prepared previously[31] by a modification of our procedure.[30a,b] Careful hydrogenation of **4** over a Lindlar catalyst[30a] and HPLC purification of the alkyne/alkene/alkane mixture gave

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1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)-5(*Z*)-[2-(trimethylsilyl)vinyl]uracil (**5**) (70%). Success with this reduction and yields of **5** are sensitive to the purity of **4**. Double chromatographic purification (silica gel flash[32] and C_{18} reversed-phase HPLC) of **4** prior to crystallization removed trace contaminants that gave spurious results.

Spontaneous geometric isomerization[33] of **5**(*Z*) ⇌ **6**(*E*) occurred at ambient temperature in benzene solution. In one experiment, the ^{1}H NMR spectral changes after 24 h indicated **5**/**6** in a 1:19 ratio. However, attempted crystallization of the residual solid, after flash evaporation, from ethanol resulted in rapid reversion to **5**. Difference NOE spectra of **5** and **6** in C_6D_6 solutions were consistent with the proposed geometric isomers. Irradiation at the trimethylsilyl proton frequency resulted in enhancements of the geminal (vinyl H2) proton signals in both isomers. Significant enhancement of the uracil H6 proton resonance was observed with the *Z* isomer (**5**), whereas the vicinal (vinyl H1) proton peak was enhanced similarly in the *E* isomer (**6**). The isomerization of **5** ⇌ **6** was accompanied by the formation of unknown byproducts (HPLC) upon standing in solution. This solvent-dependent isomerization of an alkene at ambient temperature without irradiation[33] is remarkable, and precluded our evaluation of the stereochemistry of the vinylsilane halogenations. Vinylsilanes are known to be very reactive, but stereospecific formation of 2-bromostyrenes from the corresponding 2-(trimethylsilyl)styrenes has been noted.[34] Our previous attempt to execute stereospecific bromination with a 5(*Z*)-[2-(trimethylsilyl)vinyl]uracil nucleoside resulted in formation of the more thermodynamically stable *E* isomer as the highly predominant product.[30a]

In the present study, we observed the formation of *E* and *Z* iodovinyl products upon treatment of **5** and **6** with iodine monochloride in several solvents. It appeared that the isomeric ratios were dependent on reaction solvent polarity. Treatment of **5** with ICl in methanol/water (4:1) gave the lowest ^{1}H NMR integrated ratio (~1:6) of **7**-(*E*)/**8**(*Z*). Increased proportions of **7** were observed with reactions in methanol and acetonitrile, and highly predominant formation of **7** occurred in dichloromethane and carbon tetrachloride. In benzene, the integrated ratio of **7**/**8** was >49:1. Some isomerization of **8** to **7** occurred during heating/crystallization of **8**, which further complicated evaluations of purified samples. Thus, reasonable stereoselectivity for the *Z* isomer (**8**) can be achieved with methanol/water solutions, and very high stereoselectivity for the desired *E* isomer (**7**) can be obtained with benzene. Iodinations of **5** and/or **6** in benzene gave **7** in high yields, but **5** was converted much more rapidly than the *E* isomer (**6**). Deprotection of **7** with methanolic ammonia or methanolic sodium methoxide proceded without incident to give the target 1-(β-D-arabinofuranosyl)-5(*E*)-(2-iodovinyl)uracil (IVAraU, **9**).

A semimicro adaptation with an in situ procedure utilizing sodium iodide and the convenient oxidant phenyliodine(III) dichloride (iodobenzene dichloride)[35] with a two-phase benzene/water system gave high-yield conversions of **5** to **7**. This product was purified on a small column (silica gel in a Pasteur pipet) and then subjected to deprotection with methanolic sodium methoxide. The resulting **9** was purified by HPLC (see the Experimental Section). This sequence was further adapted for the preparation of carrier-added [^{125}I]-**9** by reducing the scale approximately a thousandfold. The identities of [^{125}I]-**9** and **9** were confirmed by comigration on TLC and identical HPLC retention times.

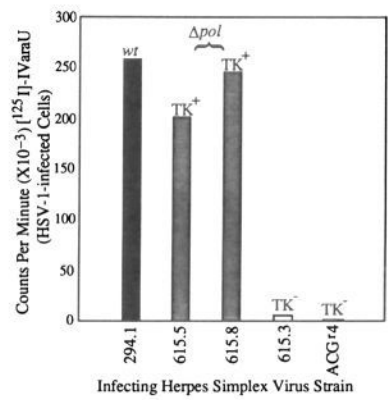

Figure 1. HSV-1 TK-dependence of [^{125}I]IVAraU uptake. PRK cells were infected with wild-type HSV-1 (294.1), Δ*pol* mutants (615.5; 615.8), or TK$^-$ mutants (615.3; ACG'4). *Y* axis: total counts per minute (^{125}I) per tube. *X* axis: infecting HSV-1 strains.

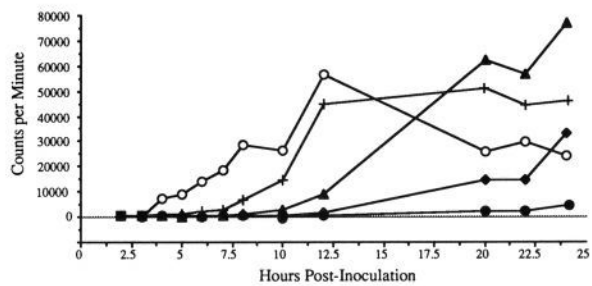

Figure 2. Inoculum and duration of exposure-dependency of [^{125}I]IVAraU uptake. PRK cells were infected with HSV-1 and treated with [^{125}I]IVAraU for 1 h. *Y* axis: total counts per minute (^{125}I) per tube. *X* axis: times postinoculation with virus. (●) 0.0001 pfu per cell; (◆) 0.001 pfu per cell; (▲) 0.01 pfu per cell; (+) 0.1 pfu per cell; (○) 1.0 pfu per cell.

Biological. HSV TK-Dependence of [^{125}I]IVAraU Uptake. Primary rabbit kidney (PRK) cell monolayers that were infected with wild-type (*wt*) HSV-1 strain (294.1) or acyclovir-resistant/TK$^+$/DNA polymerase-altered/HSV-1 (615.5, 615.8) mutants and then incubated with carrier-added [^{125}I]IVAraU (4.8×10^6 cpm) for 1 h took up $\geq 2 \times 10^5$ cpm of labeled drug in excess of those that were uninfected (Figure 1). PRK monolayers that were mock-infected or infected with acyclovir-resistant TK$^-$ HSV-1 mutants (ACG'4, 615.3) showed the absence of, or negligible, drug uptake. The latter TK$^-$ mutants, however, cause the complete cytopathic effects usually associated with "membrane leakiness".

Sensitivity of [^{125}I]IVAraU Uptake. Serial dilutions of *wt* HSV-1 (strain 294.1) to 1.5 plaque forming unit (pfu) per tube were tested in PRK cells to determine the effects of virus inoculum and duration of drug exposure on the HSV-specific uptake of [^{125}I]IVAraU. Uptake was arbitrarily defined as virus-specific when intracellular levels of [^{125}I]IVAraU reached and were sustained at levels of at least twice the background. These results are depicted graphically in Figure 2. HSV-1 infection with a multiplicity (moi) of 1.0 pfu per cell was detected by virus-specific radioiodine uptake by 4 h with 0.1–1.0 moi; by 8 h with 0.01 moi; by 12 h with 0.001 moi; and by 20 h with 0.0001 moi. TK$^-$ strains were negative for virus-directed uptake by this method also. Therefore, [^{125}I]IVAraU provides a novel method for early detection of the presence

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Table I. ^{1}H NMR Chemical Shift Data[a]

compd	H1′[b]	H2′[c]	H3′[d]	H4′[e]	H5′,5″[e]	vinyl (H2, H1)		H6[f]	NH[g]	OAc[h]
4[i]	6.26	5.43	5.15	4.18	4.40			7.73	8.45	2.03–2.15
5[j]	6.20	5.34	4.98	4.06–4.35	4.06–4.35	5.90[k]	6.92[l]	7.45[m]	8.45	1.98–2.12
6[j]	6.29	5.41	5.11	4.20	4.42	6.59[n]	6.62[n]	7.60	8.70	1.98–2.13
7	6.25	5.40	5.06	4.20	4.54[o], 4.32[p]	7.04[q]	7.45[q]	7.52	8.00	2.00–2.13
8	6.34	5.44	5.17	4.25	4.39	6.52[r]	7.24[s]	8.66[t]	8.84	2.05, 2.13
9[u]	5.99	4.03	3.92	3.75	3.64	7.15[v]	7.16[v]	7.59	11.50	

[a] 199.98 MHz; $CDCl_3$. [b] d, $J_{1'-2'}$ = 3.8–4.4 Hz. [c] dd, $J_{2'-3'}$ = 1.6–2.6 Hz. [d] dd, $J_{3'-4'}$ = 2.8–4.2 Hz. [e] m. [f] s. [g] br s. [h] 3-s (2-s for 8). [i] 0.2, s, $SiMe_3$. [j] 0.1, s, $SiMe_3$. [k] d, J = 14.8 Hz. [l] dd, $J_{(H1\ vinyl)-H6}$ = 1.2 Hz. [m] d, J = 1.2 Hz. [n] d (collapsed), J = 19.8 Hz. [o] dd, $J_{5'-4'}$ = 7 Hz, $J_{5'-5''}$ = 12 Hz, H5′. [p] dd, $J_{5''-4'}$ = 4 Hz, H5″. [q] d, J = 14.8 Hz. [r] d, J = 9 Hz. [s] dd, J = 0.8 Hz. [t] d, J = 0.8 Hz. [u] 400.14 MHz; Me_2SO-d_6. [v] d (collapsed), J = 14.5 Hz.

Table II. ^{13}C NMR Chemical Shift Data[a,b]

compd	C2	C4	C5	C6	vinyl[c] (C1 and C2)		C1′	C2′	C3′	C4′	C5′
4	149.61	161.77	100.40	143.90	100.13[d]	95.49[e]	84.65	76.33	74.88	80.64	62.85
5	150.10	162.77	114.47	136.75	136.25	135.92	84.69	76.64	74.86	80.62	63.32
6	149.60	162.11	113.28	136.98	133.86	132.04	84.82	76.63	74.87	81.04	62.82
7	149.06	161.37	112.79	138.34	135.62	81.47	85.07	76.48	74.66	81.58	62.84
8	149.47	162.22	111.23	137.56	128.98	79.89	85.12	76.46	74.75	80.77	63.50
9[f]	149.17	161.81	110.02	140.96	137.06	84.70	85.29	75.23	74.93	77.54	60.44

[a] 50.29 MHz; $CDCl_3$. [b] Acetyl's: 168.20–171.22 (CO); 20.57–21.10 (CH_3). [c] Ethynyl for 4. [d] Cl. [e] C2. [f] 75.47 MHz; Me_2SO-d_6.

of HSV-1 in vitro that does not require interpretation of microscopic cytopathic effects. Furthermore, this method is amenable to automation. The sensitivity of this assay suggests that selective in vivo uptake might be detected with alternative radionuclide derivatives such as [^{123}I]-9 that are more amenable to external γ imaging.

Reversibility of [^{125}I]IVAraU Uptake. Parallel series of PRK cells were infected with 10 moi of the *wt* isolate (294.1) and the TK$^-$ strain (ACG^{r}4). Following removal of [^{125}I]IVAraU, a biphasic reversibility of drug uptake was observed with the wild-type cells. Roughly 77% of the intracellular [^{125}I]IVAraU was lost within the first 3 h during the initial rapid phase. Over the next 21 h, a much slower rate of drug loss was observed resulting in retention of about 33% of the remaining intracellular [^{125}I]IVAraU at 24 h postdrug removal in the TK$^+$ relative to TK$^-$ HSV-1 infected cells (Figure 3). Thus, the uptake of [^{125}I]IVAraU and its intracellular retention via phosphorylation is sufficiently rapid and efficient to allow radiochemical diagnosis, but the reversal of this overall process (presumed dephosphorylation and transport/diffusion of the nucleoside across the cell membrane) is also quite rapid after exposure to the drug is terminated. This sequence of uptake and entrapment for diagnosis followed by cellular excretion of the labeled agent further illustrates the desirable radiopharmaceutical properties of [*I]IVAraU's.

In summary, the rapid and efficient synthesis of IVAraU (9) and [^{125}I]-9 from a crystalline vinylsilane precursor (5) has been demonstrated on laboratory and radiochemical scales. [^{125}I]IVAraU uptake was shown to be a highly sensitive and specific marker of herpes viral TK activity. This agent was resistant to phosphorolytic cleavage in vitro and did not undergo passive accumulation in cells in the absence of viral TK, even when cell membrane "leakiness" had advanced secondarily to viral infection or when incubation was prolonged. This virus-specific uptake was dependent upon virus inoculum with an apparent maximum. The overall uptake of [^{125}I]IVAraU by infected cells was reversible in vitro, although intracellular concentration differences between cells infected with TK$^+$ and TK$^-$ virus strains persisted for at least 24 h. Pharmacokinetic and tissue distribution studies are currently underway to explore the role of in vitro reversibility on the uptake process in vivo. Automated determination of [^{125}I]IVAraU uptake in HSV-infected cells might obviate present microscopic or immunostaining procedures for viral diagnosis in tissue culture. Since characteristics of this compound fit criteria for noninvasive radiodiagnostic agents, in vivo studies with clinically relevant radionuclides including [^{123}I]IVAraU are in progress and will be reported separately.

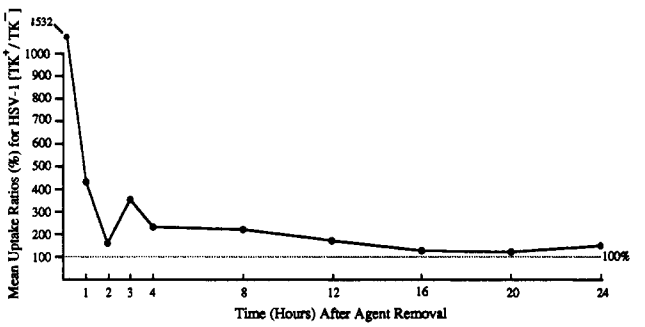

Figure 3. Reversibility of [^{125}I]IVAraU uptake. *X* axis: time. *Y* axis: concentration ratios (%) for HSV-1 [(TK$^+$/TK$^-$) × 100]. Infected cells were exposed to [^{125}I]IVAraU followed by unlabeled medium for the designated periods.

Experimental Section

Melting points are uncorrected. Ultraviolet (UV) spectra of solutions in methanol were determined with a Hewlett-Packard 8451A diode array spectrophotometer. ^{1}H (199.98 or 400.14 MHz) and ^{13}C (50.29 or 75.47 MHz) NMR spectra were determined in $CDCl_3$ or Me_2SO-d_6 with Me_4Si as internal standard. NMR data are reported in Tables I and II. Mass spectra were obtained with a Finnigan-MAT instrument at 20 eV with direct sample introduction. Evaporations were conducted at <35 °C with a rotary evaporator under water aspirator or mechanical oil pump vacuum. Room temperature was 17–19 °C. All solvents were reagent grade and were distilled before use. DMF, Et_3N, and benzene were heated at reflux over CaH_2 for several hours and then distilled. Reactions were monitored by TLC with E. Merck 60 F_{254} precoated silica gel sheets. For enhancement of resolution (required for monitoring the coupling, hydrogenation, and ICl reactions with the vinylsilanes) the sheets were predeveloped with Et_3N/MeOH (1:9) and then dried at ambient temperature before use. Products on TLC plates were detected under a 254 nm UV lamp and/or by spraying with 5% H_2SO_4/MeOH and heating. Open column chromatography and flash chromatography were performed on Merck Kieselgel 60 (9385, 230–400 mesh). Analytical and semipreparative HPLC were performed with a Waters instrument on Rainin (Dynamax 12μ) C_{18} columns. Preparative HPLC was performed on a Waters 500A PREP LC system. Elemental analyses agreed within ±0.3% of theory.

1-(β-D-Arabinofuranosyl)uracil (1). The intermediate 2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil was prepared[25] in 90%

yield (after recrystallization). A sample of this material (12 g, 53.1 mmol) was dissolved in 0.1 M NaOH/H_2O (600 mL) and stirred overnight at ambient temperature. The solution was neutralized with 6 M HCl/H_2O and evaporated. The residual I[27] was coevaporated with CH_3CN (3 × 300 mL) and acetylated without further purification.

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)uracil (2). The solid (1) obtained in the previous step was suspended in Ac_2O (233 mL), DMAP (0.3 g, 2.45 mmol) was added, and the suspension was stirred for 5 h at ambient temperature (TLC, MeOH/$CHCl_3$, 1:9; indicated formation of primarily one faster migrating compound). The mixture was evaporated, and the residue was coevaporated with 95% EtOH (3 × 100 mL). The solid mass was dissolved in CH_2Cl_2 (100 mL), washed with H_2O (3 × 50 mL), dried (Na_2SO_4), and evaporated. The resulting white foam was crystallized (EtOH/H_2O) and recrystallized (95% EtOH) to give white crystalline 2 [18.2 g, 90% from 2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil] with mp 128–130 °C (lit.[27] mp 129–130 °C).

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-5-iodouracil (3). A sample of 2 (10 g, 27.0 mmol) was dissolved in CH_2Cl_2 (350 mL), and ICl (6.5 g, 40 mmol) was added. The solution was heated at reflux for 5 h (TLC, acetone/hexane, 7:13; indicated disappearance of starting material) and cooled, and the organic phase was carefully decolorized with the minimum amount of 2% $NaHSO_3/H_2O$. The pale yellow organic phase was washed with H_2O (2 × 100 mL), dried (Na_2SO_4), and evaporated. The resulting yellow solid was crystallized (95% EtOH) to give pale yellow needles of 3 (11.8 g, 88%): mp 185–187 °C (lit.[29] mp 184–186 °C); UV max 282 nm (ε 8900), min 244 nm (ε 3400).

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-5-[2-(trimethylsilyl)ethynyl]uracil (4). Freshly distilled dry Et_3N (200 mL) was vigorously purged with argon for 30 min, and 3 (3.0 g, 6.0 mmol) was added followed by (trimethylsilyl)acetylene (3.41 mL, 2.37 g, 24 mmol), $(Ph_3P)_2PdCl_2$ (90 mg, 0.128 mmol), and CuI (60 mg, 0.31 mmol). The suspension was stirred under argon at 50 °C for 3 h (TLC, acetone/hexane, 7:13, indicated conversion of starting 3 to 4 plus traces of 2) and evaporated. The brown residue was dissolved in CH_2Cl_2 (250 mL), washed with 2% disodium EDTA/H_2O (2 × 100 mL) and H_2O (100 mL), dried (Na_2SO_4), and evaporated. The resulting brown foam was dissolved in acetone and purified by flash chromatography (acetone/hexane, 7:13). Appropriate fractions were evaporated to give a yellow foam (2.5 g) that was further purified by preparative HPLC (Waters 500A, C_{18} column, MeOH/H_2O, 7:3, 100 mL/min). The product (4) precipitated as a white solid during evaporation of appropriately pooled fractions. Recrystallization (EtOH/H_2O) gave 4 (2.3 g, 82%): mp 135–137 °C; UV max 234, 294 nm (ε 13900, 14600), min 209, 256 nm (ε 8700, 4300).

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-5(*Z*)-[2-(trimethylsilyl)vinyl]uracil (5). To a solution of 4 (150 mg, 0.32 mmol) in freshly distilled EtOAc (20 mL) were added freshly distilled quinoline (0.8 mL) and Lindlar catalyst (110 mg, Aldrich Chemical Co.). The mixture was hydrogenated (1 atm) at ambient temperature, and disappearance of starting material was monitored by TLC (acetone/hexane, 7:13, on sheets predeveloped in MeOH/Et_3N, 9:1). Three compounds [the fully saturated most rapidly migrating, the desired vinyl compound (5), and the slowest migrating starting material (4)] were present. Hydrogenation was terminated when consumption of 4 was almost complete, and the formation of the fully saturated compound was minimal. The mixture was filtered through a Celite pad, and the filter cake washed with EtOAc (50 mL). The combined filtrate was washed with 1 M HCl/H_2O (3 × 30 mL) and H_2O (2 × 30 mL) and evaporated to give a white solid. This material was purified by semipreparative HPLC (MeOH/H_2O, 3:2, 9.9 mL/min). Evaporation of appropriate fractions gave an amorphous product (106 mg, 70%; the yield is very sensitive to the purity of starting 4). Crystallization (EtOH/H_2O) gave 5: mp 129–130 °C; UV max 232, 280 nm (ε 11900, 8800), min 215, 263 nm (ε 11100, 7700); MS m/z 467 (M − 1). Anal. ($C_{20}H_{28}N_2O_9Si$) C, H, N.

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-5(*E*)-[2-(trimethylsilyl)vinyl]uracil (6). A sample of 5 (60 mg, 0.128 mmol) was dissolved in anhydrous benzene and allowed to stand at ambient temperature. The formation of a second major compound (6) was monitored by ^1H NMR. The solution was allowed to stand until no significant change in the ratio of the two isomers was observed (24 h: starting 5/product 6, 1:19) and then was evaporated. Attempted crystallization of the residue from EtOH resulted in reversion to 5 as indicated by its ^1H NMR spectrum.

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-5(*E*)-(2-iodovinyl)uracil (7). A solution of 5 (120 mg, 0.25 mmol) in dry benzene (5 mL) was treated with ICl (60 mg, 0.37 mmol) in benzene (1 mL) at ambient temperature. A ruby color appeared, and after 15 min, TLC (acetone/hexane, 7:13) indicated complete reaction of 5. The solution was evaporated and the residue was purified by semipreparative HPLC (MeOH/H_2O, 13:7, 9.9 mL/min). Evaporation of appropriate fractions gave 7 (118 mg, 90%). Crystallization of this material (EtOH/H_2O) gave white crystalline 7 (100 mg, 76%): mp 149–151 °C; UV max 252, 298 nm (ε 18800, 15400), min 224, 274 nm (ε 12000, 12400); MS m/z 522 (M^+). Anal. ($C_{17}H_{19}IN_2O_9$) C, H, N.

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-5(*Z*)-(2-iodovinyl)uracil (8). A solution of 5 (50 mg, 0.106 mmol) in MeOH/H_2O (4:1, 5 mL) was cooled at 0 °C, and ICl (17.85 mg, 0.110 mmol) in MeOH (0.41 mL) was added in one portion. After 15 min, TLC (acetone/hexane, 7:13) indicated essentially complete reaction (traces of unreacted 5 were observed by HPLC, MeOH/H_2O, 13:7, 1 mL/min). The solution was evaporated, and the resulting solid mass was purified by semipreparative HPLC (MeOH/H_2O, 13:7, 9.9 mL/min). Evaporation of appropriate fractions gave the *Z* isomer 8 (40.4 mg, 73%) plus the *E* isomer 7 (6.6 mg, 12%). Crystallization of 8 (MeOH/H_2O) gave pale yellow crystals (35 mg, 63%).

During crystallization, isomerization of 8 to give some *E* isomer (7) was observed. Crystalline 8 had the following characteristic: mp 162–164 °C; UV max 240, 300 nm (ε 16200, 7900), min 215, 271 nm (ε 10500, 5600).

1-(β-D-Arabinofuranosyl)-5(*E*)-(2-iodovinyl)uracil (9). A sample of 7 (50 mg, 0.095 mmol) was dissolved in MeOH (10 mL) and treated with NH_3/MeOH (40 mL, saturated at 0 °C). The flask was firmly capped and stirred overnight at ambient temperature. TLC ($CHCl_3$/MeOH, 4:1) indicated a major product and traces of fluorescent material. After evaporation, the residue was triturated with Et_2O (3 × 10 mL) and purified by semipreparative HPLC (MeOH/H_2O, 1:1, 9.9 mL/min). Evaporation of appropriate fractions gave a white solid (34 mg, 90%) that was crystallized (MeOH) and recrystallized (95% EtOH with diffusion of Et_2O[36]) to give pale yellow needles of 9 (26 mg, 70%): mp 175 °C dec [lit.[19b] mp 170–175 °C dec]; UV max 256, 300 nm (ε 16600, 14100) min 224, 276 nm (ε 6600, 10000); MS m/z 396 (M^+). Anal. ($C_{11}H_{13}IN_2O_6$) C, H, N.

In Situ Semimicroiodination Procedure for Preparation of 9. Iodination. A sample of NaI (12 mg, 0.08 mmol) was placed in a 3-mL reaction vial equipped with a stir bar. Water (0.15 mL) and benzene (1 mL, HPLC or spectral grade) were added followed by iodobenzene dichloride[35] (20 mg, 0.074 mmol). The mixture was stirred for 5 min at ambient temperature, and 5 (28 mg, 0.06 mmol) was added. Stirring was continued for 1 h, after which TLC (acetone/hexane, 7:13) and HPLC (MeOH/H_2O, 13:7, 1 mL/min) indicated complete consumption of 5. A 5% solution of $NaHSO_3/H_2O$ (1 mL) was added, and the mixture was stirred for 30 min. The organic phase was applied to a small dry column of silica (6 × 50 mm). The aqueous bisulfite layer was extracted with additional benzene (1 mL), and the organic phase was applied to the column. The column was washed with CH_2Cl_2 (2 column volumes, ~2 mL, to remove iodobenzene) followed by elution with CH_2Cl_2/EtOAc, 2:1 (3 column volumes, ~3 mL). The product (7) was recovered in the first two fractions (2 × 1 mL) containing EtOAc. These fractions were placed in a reaction vial and evaporated under a stream of argon.

Deprotection. The solid residual 7 (23 mg) was treated with anhydrous 0.1 M NaOMe/MeOH (2.4 mL, 0.24 mmol) and stirred for 40 min at ambient temperature. TLC ($CHCl_3$/MeOH, 4:1; R_f ~0.75) indicated that deprotection was complete. MeOH was evaporated under a stream of argon, and the residual IVAraU (9) was dissolved in 0.5 M Na_2HPO_4/H_2O buffer (pH 7.4, 1 mL) and purified by semipreparative HPLC (MeOH/H_2O, 1:1, 9.9 mL/

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min) followed by MeCN/H_2O (1:4) under isocratic conditions.

Microiodination Procedure for the Preparation of 1-(β-D-Arabinofuranosyl)-5(*E*)-(2-[^{125}I]iodovinyl)uracil ([^{125}I]-9). Iodination. $Na^{125}I$/NaOH [17.4 Ci per mg, 5 mCi] was purchased from Dupont/New England Nuclear (Wilmington, DE) and was prepared on the day of the synthesis in a 42 MeVH-cyclotron. The basic $Na^{125}I$ solutions were evaporated to dryness under a stream of N_2 and neutralized (1 M H_3PO_4). Stock solutions of **5** (0.01 M) and phenyliodine(III) dichloride (0.018 M) were prepared in HPLC-grade benzene, and carrier NaI (0.0067 M) was prepared in double-distilled water. Aliquots of the carrier NaI (5 μL, 0.034 μmol) and **5** (10 μL, 0.1 μmol) solutions were added to the neutralized solutions of $Na^{125}I$ in 0.5-mL microcentrifuge vials, followed by the addition of phenyliodine(III) dichloride (6 μL, 0.11 μmol). The reaction was allowed to proceed for 1 h in the dark at ambient temperature with periodic agitation. Benzene (5 μL) then was added to facilitate removal of the organic phase. (Addition of bisulfite was omitted from microscale experiments.) The organic phase was applied to a dry silica column prepared in a micropipet tip. The column was washed with CH_2Cl_2 (5 μL × 2) to remove iodobenzene, and the product was eluted with CH_2Cl_2/EtOAc (2:1, v/v, 5 μL × 3 volumes). The first 2 fractions containing [^{125}I]-**7** were pooled and evaporated to dryness under a stream of N_2.

Deprotection. Anhydrous NaOMe/MeOH (0.1 M, 15 μL, 1.5 μmol) was added to the dry residue of [^{125}I]-**7** and the suspension agitated for 30 min. TLC ($CHCl_3$/MeOH, 4:1, R_f ~0.75) indicated that deprotection was complete. The solution was evaporated to dryness under a stream of N_2. The residual [^{125}I]-**9** was dissolved in H_2O (20 μL) and purified by semipreparative HPLC (MeCN/H_2O, 1:5, 1 mL/min) under isocratic conditions. This product comigrated with unlabeled **9** on TLC and had an identical HPLC retention time.

In Vitro Uptake Assays. (a) Virus Isolates. HSV-1 strains 294.1, 615.3, 615.5, and 615.8 were obtained from an immunocompromized patient with herpes esophagitis and have been previously characterized.[37] Strain 294.1 is wild type (*wt*) prior to antiviral chemotherapy. Strains 615.3, 615.5, and 615.8 are acyclovir resistant and were found during therapy; 615.5 and 615.8 are DNA polymerase (*pol*) mutants without apparent alteration in TK activities; 615.3 is TK-deficient. ACG^{r}4 is a previously described TK$^-$ strain.[38,39]

(b) HSV TK-Dependence of [^{125}I]IVAraU Uptake. Primary rabbit kidney (PRK) cells were grown to confluence overnight in tissue culture grade roller tubes (rotated at 1 rpm at 37 °C) that contained approximately 1.4 × 10^6 cells/tube. Monolayers of 1 × 10^5 PRK cells were cultivated in 6-mL tissue culture tubes in α Eagle's minimal essential medium supplemented with 5% fetal bovine serum (FBS), L-glutamine (2 mM), and 0.2% sodium bicarbonate. They then were infected with HSV-1 at an moi of 10 in 1.0 mL of Medium 199 with Hank's salts, 2% inactivated FBS, and antibiotics (M199) and then were incubated for 6 h at 37 °C. Control cells were mock-infected. Approximately 4.8 × 10^6 cpm (carrier added; 37 Ci/mmol) of [^{125}I]IVAraU in 100 μL of media were added to each tube after 6 h, and incubation was continued for 0.75 h at 37 °C. This was followed by cold phosphate buffered saline (PBS) wash (×3). Cells were transferred into 12 × 75 mm plastic tubes using trypsinization, centrifuged at 800*g*, washed three times in PBS, pH 7.4 at 4 °C, and counted in a Beckman γ counter. Uptake levels are shown in Figure 1.

(c) Sensitivity of [^{125}I]IVAraU Uptake. Five duplicate 10-fold serial dilutions (from 1.0 to 0.0001 moi, 1.5 pfu per tube) of HSV-1 (strain 294.1) were adsorbed onto human embryonic lung (HEL) fibroblasts in 12 × 75 mm polystyrene tubes at 5000*g* for 30 min at 37 °C with 2.84 × 10^6 cpm of [^{125}I]IVAraU (carrier-added; 37 Ci/mmol) in 1 mL of Medium 199. Incubations at 37 °C were stopped every 2 h until 24 h. The monolayers were washed four times with cold PBS and counted in situ in the γ counter. Results are shown in Figure 2.

(d) Reversibility of [^{125}I]IVAraU Uptake. PRK cells were infected under identical conditions as above. After 6 h at 37 °C, 4 × 10^4 cpm of [^{125}I]IVaraU were added and the incubation continued for 0.75 h. The media were then removed, the monolayers washed with PBS, pH 7.4, 37 °C, and the media (without [^{125}I]IVaraU) were replaced. Incubation was resumed in the roller apparatus at 37 °C. At 0, 1, 2, 3, 4, 8, 12, 16, 20, and 24 h the monolayer was removed with trypsin and washed as described above in section (b). Cell pellets were counted in the γ counter. Results are shown in Figure 3.

Acknowledgment. This work was supported in part by Brigham Young University Development Funds (M. J.R.) and by grants from the British Columbia Health Care Research Foundation, the U.S. National Institutes of Health (RO1 AI 27209), and The Medical Research Council of Canada (S.L.S.).

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