Synthesis and Cellular Uptake of 2′-Substituted Analogues of (*E*)-5-(2-[^{125}I]Iodovinyl)-2′-deoxyuridine in Tumor Cells Transduced with the Herpes Simplex Type-1 Thymidine Kinase Gene. Evaluation as Probes for Monitoring Gene Therapy

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A useful synthetic methodology was developed to synthesize and radiolabel a series of (*E*)-5-(2-[^{125}I]iodovinyl)uracil nucleoside substrates for herpes simplex virus type-1 thymidine kinase (HSV-1 TK). (*E*)-5-(2-[^{125}I]Iodovinyl)-2′-deoxyuridine ([^{125}I]IVDU, **10**), (*E*)-5-(2-[^{125}I]iodovinyl)-2′-fluoro-2′-deoxyuridine ([^{125}I]IVFRU, **11**), (*E*)-5-(2-[^{125}I]iodovinyl)-2′-fluoro-2′-deoxyarabinouridine ([^{125}I]IVFAU, **12**), and (*E*)-5-(2-[^{125}I]iodovinyl)arabinouridine ([^{125}I]IVAU, **13**) were synthesized in 63−83% radiochemical yield by reaction of the unprotected (*E*)-5-(2-(trimethylsilyl)vinyl) precursors (**6**−**9**) with [^{125}I]ICl. Cellular uptake of these labeled compounds (**10**−**13**) was evaluated *in vitro.* All compounds showed minimal uptake in the KBALB cell line. However, increased uptake was observed for all compounds in KBALB-STK cells which are transduced with a replication incompetent Moloney murine leukemia virus vector encoding the HSV-1 TK gene. The results indicate that uptake of these compounds in KBALB-STK cells is variable and highly dependent on the nature of the sugar 2′-substituent. When a fluoro (**12**) or a hydroxy (**13**) substituent is present in the arabinofuranosyl (up) configuration at the 2′-position, there is diminished cellular uptake in KBALB-STK cells relative to hydrogen (**10**) or fluorine (**11**) in the ribofuranosyl (down) configuration at the 2′-position. Our results indicate that radiolabeled IVFRU (**11**) is most promising for further *in vivo* studies.

The potential of gene therapy as a clinical treatment modality for a variety of disorders has attracted considerable attention in recent years. Clinical trials investigating the benefits of gene transfer have proliferated rapidly. In particular, gene therapy protocols for the treatment of cancer have occupied a central role in this rapidly evolving area of research. A gene therapy strategy for cancer that draws heavily on advances in medicinal chemistry is directed enzyme prodrug therapy (DEPT), where expression of metabolic suicide genes convert relatively nontoxic prodrugs into highly cytotoxic agents.[1] The suicide gene that has been studied most extensively in the laboratory and clinic for treatment of cancer is the herpes simplex type-1 thymidine kinase (HSV-1 TK) gene.[2,3] This virus-specific kinase has been exploited successfully as a target for activation of antiviral nucleoside prodrugs.[4] The high degree of selectivity observed in HSV-1-infected cells is attributed to selective phosphorylation of nucleoside substrates by HSV-1 TK.[5]

Transfer of the HSV-1 TK gene into proliferating tissue and expression of the gene product can result in sensitization to the cytotoxic effects of various purine and uracil nucleoside analogs.[6] Selective phosphorylation of these analogs by HSV-1 TK and further phosphorylation of these prodrugs by cellular kinases results in generation of highly toxic triphosphate derivatives. The acyclic guanosine analog ganciclovir has been shown to be particularly effective as a cytotoxic agent in gene therapy protocols involving HSV-1 TK gene transfer.[7] However, several 5-substituted uracil nucleoside derivatives have been shown to have more potent and selective cytotoxic activity in cells transfected with the HSV-1 TK gene.[8,9] (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVDU) and related compounds are particularly potent cytotoxic agents against FM3A murine mammary carcinoma expressing HSV-1 TK.[10,11] The observation that (*E*)-5-(2-[^{125}I]iodovinyl)-2′-deoxyuridine accumulates by metabolic trapping in tumor cells expressing HSV-1 TK implicates phosphorylation as a requisite for cytotoxic activity.[12]

Previous studies with radiolabeled IVDU have demonstrated that, despite rapid accumulation in cells expressing HSV-1 TK *in vitro*, it is an unsuitable agent for *in vivo* detection of HSV-1 TK expressing tissue due to rapid phosphorylase-mediated deglycosylation.[13] Consequently, the catabolic instability of IVDU precluded its development as an imaging agent for herpes simplex encephalitis. However, several structural analogs of IVDU have enhanced stability toward phosphorylases. Incorporation of a fluorine (ribo or arabino configuration) or hydroxyl (arabino configuration) at the 2′-position results in resistance to phosphorylase-mediated degradation, retention of antiviral activity, and selective metabolic trapping in herpes-infected (TK$^+$) cells.[14−16] These are desirable properties for a putative radiopharmaceutical for imaging HSV-1 TK expression during gene therapy.

Noninvasive imaging of gene expression during gene therapy could provide potentially useful information. The extent of gene delivery and expression may be assessed with a radiopharmaceutical selectively localizing in HSV-1 TK expressing tissue. This is particularly important since treatment failures of gene therapy with HSV-1 TK in animal models is associated with inefficient gene delivery and expression prior to ganci-

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clovir administration.[17] Recently, the feasibility of such an approach has been demonstrated using quantitative autoradiography and single photon emission computed tomography (SPECT) with radiolabeled 5-iodo-2′-fluoro-2′-deoxyarabinouridine (FIAU) in tumors transduced with the HSV-1 TK gene.[18,19] In this report, we describe new radiosyntheses of (*E*)-5-(2-[^{125}I]iodovinyl)-2′-deoxyuridine ([^{125}I]IVDU), (*E*)-5-(2-[^{125}I]iodovinyl)-2′-fluoro-2′-deoxyuridine ([^{125}I]IVFRU), (*E*)-5-(2-[^{125}I]iodovinyl)-2′-fluoro-2′-deoxyarabinouridine ([^{125}I]IVFAU), and (*E*)-5-(2-[^{125}I]iodovinyl)arabinouridine ([^{125}I]IVAU) as potential noninvasive radiopharmaceuticals for imaging HSV-1 TK expression. In order to select a radiopharmaceutical with high sensitivity for scintigraphic imaging of HSV-1 TK expression, we have compared the uptake of each compound in cells expressing HSV-1 TK.

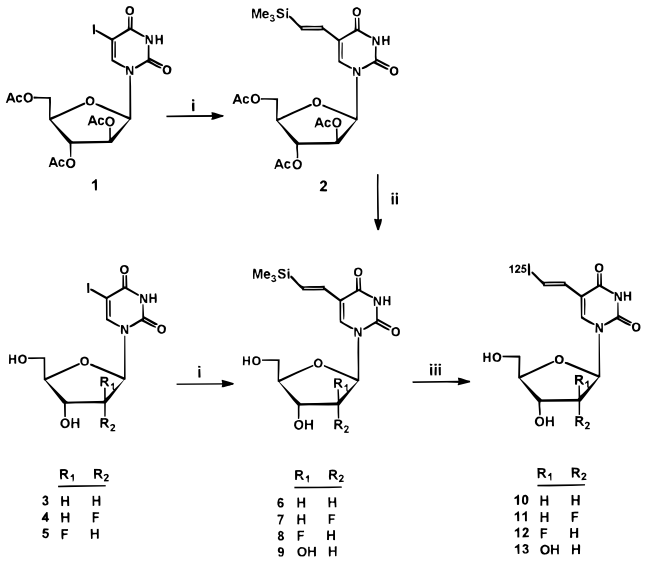
Chemistry

Several strategies have been reported for the synthesis of radiolabeled IVDU. Radiohalogen exchange has been shown to be effective for the synthesis of lower specific activity product.[20] Similarly, carrier-added and high specific activity products have been isolated in respectable radiochemical yields using modified Hunsdiecker reactions.[21] Recent reports for the highly efficient radiosyntheses of labeled IVAU from vinylstannane and vinylsilane precursors under mild conditions are particularly appealing.[22,23] However, radioiodination of these precursors has only been attempted using protected nucleosides. Deprotection after radiohalogenation requires additional undesired laboratory handling of radioactive materials, which results in some loss of radiolabeled product. A general radiosynthetic strategy where the final radiohalogenation is carried out using an unprotected (*E*)-5-(2-(trimethylsilyl)vinyl) precursor is now reported.

The introduction of an (*E*)-5-(2-(trimethylsilyl)vinyl) functionality at the 5-position of 5-iodoarabinouridine has been reported using a reaction sequence which involved palladium-catalyzed coupling of (trimethylsilyl)acetylene followed by reduction over a Lindlar catalyst.[23] However, this procedure produces mixtures of reduced compounds that require HPLC separation. Direct coupling of (*E*)-2-(tributylstannyl)-1-(trimethylsilyl)ethene with protected 5-iodo-2′-deoxyuridine in the presence of a palladium catalyst gives the desired protected (*E*)-5-(2-(trimethylsilyl)vinyl)-2′-deoxyuridine in good yield.[24] It was therefore of interest to investigate whether a similar coupling reaction could be performed using unprotected nucleosides to simplify the subsequent radioiodination procedure.

The palladium-catalyst coupling reaction proceeded smoothly for 5-iodo-2′-deoxyuridine (**3**) and the 2′-fluorinated derivatives (**4**, **5**). Although the nucleoside precursors had a low solubility in acetonitrile or THF, the reactions proceeded to completion, providing yields ranging from 64 to 80%. The purification of the (*E*)-5-(2-(trimethylsilyl)vinyl) products (**6**−**8**) was troublesome since trace impurities tended to co-elute with the desired products during silica gel column chromatography, and these impurities impeded subsequent crystallization attempts. Therefore, it was necessary to perform additional column chromatography purification of fractions containing small amounts of impurities prior to crystallization. The choice of recrystallization solvent was important since isomerization of vinylsilane nucleosides from the *E* to *Z* stereochemistry has been observed in polar solvents.[23] Recrystallization from ethyl acetate did not appear to induce this isomerization.

Scheme 1[a]



[a] Reagents: (i) $Bu_3SnCH{=}CHSiMe_3$, $(Ph_3P)_2Pd(II)Cl_2$; (ii) LiOH, benzene; (iii) ^{125}ICl, acetonitrile.

Although the coupling reaction could be performed using the unprotected 5-iodo-2′-deoxy nucleosides (**3**−**5**), the extremely low solubility of 5-iodoarabinouridine in the reaction solvents necessitated the introduction of protecting groups. The reaction of (*E*)-2-(tributylstannyl)-1-(trimethylsilyl)ethene with 2′,3′,5′-tri-*O*-acetyl-5-iodoarabinouridine (**1**) in the presence of the palladium catalyst in dry acetonitrile gave the coupled product (**2**) in 64% yield (Scheme 1). Several methods to remove the acetyl protecting groups, while retaining the reactive (*E*)-5-(2-(trimethylsilyl)vinyl) moiety, were investigated. Selective deprotection of **2** was achieved after 80 h at 25 °C by treatment with a suspension of 2% lithium hydroxide in dry benzene to give (*E*)-5-(2-(trimethylsilyl)vinyl)arabinouridine (**9**, 39%).

Unlabeled iodination and carrier-added radioiodination reactions were performed using the unprotected (*E*)-5-(2-(trimethylsilyl)vinyl) precursors (**6**−**9**). Thus, treatment with 1 equiv of iodine monochloride in dry acetonitrile resulted in rapid formation of the desired (*E*)-5-(2-iodovinyl) products (**10**−**13**). It was noted that when the vinylsilane compounds were added to the iodine monochloride solution immediately after dissolution, only formation of the *E* isomer was observed. Carrier-added radiolabeling was carried out by simply adding Na^{125}I to small-scale reactions using iodine monochloride. However, radiochemical yields were improved when acetic acid (20%) was included in the reaction mixture. Radiochemical yields, after reverse-phase HPLC purification, were about 85% for all reactions except for [^{125}I]IVAU (**13**), which was obtained in 63% radiochemical yield. The lower solubility of (*E*)-5-(2-(trimethylsilyl)vinyl)arabinouridine (**9**) in acetonitrile may account for the lower radiochemical yield obtained in this reaction. The specific activity of products obtained in carrier-added reactions, which ranged between 30 and 41 GBq/mmol (0.81−1.1 Ci/mmol), was acceptable for cellular uptake experiments.

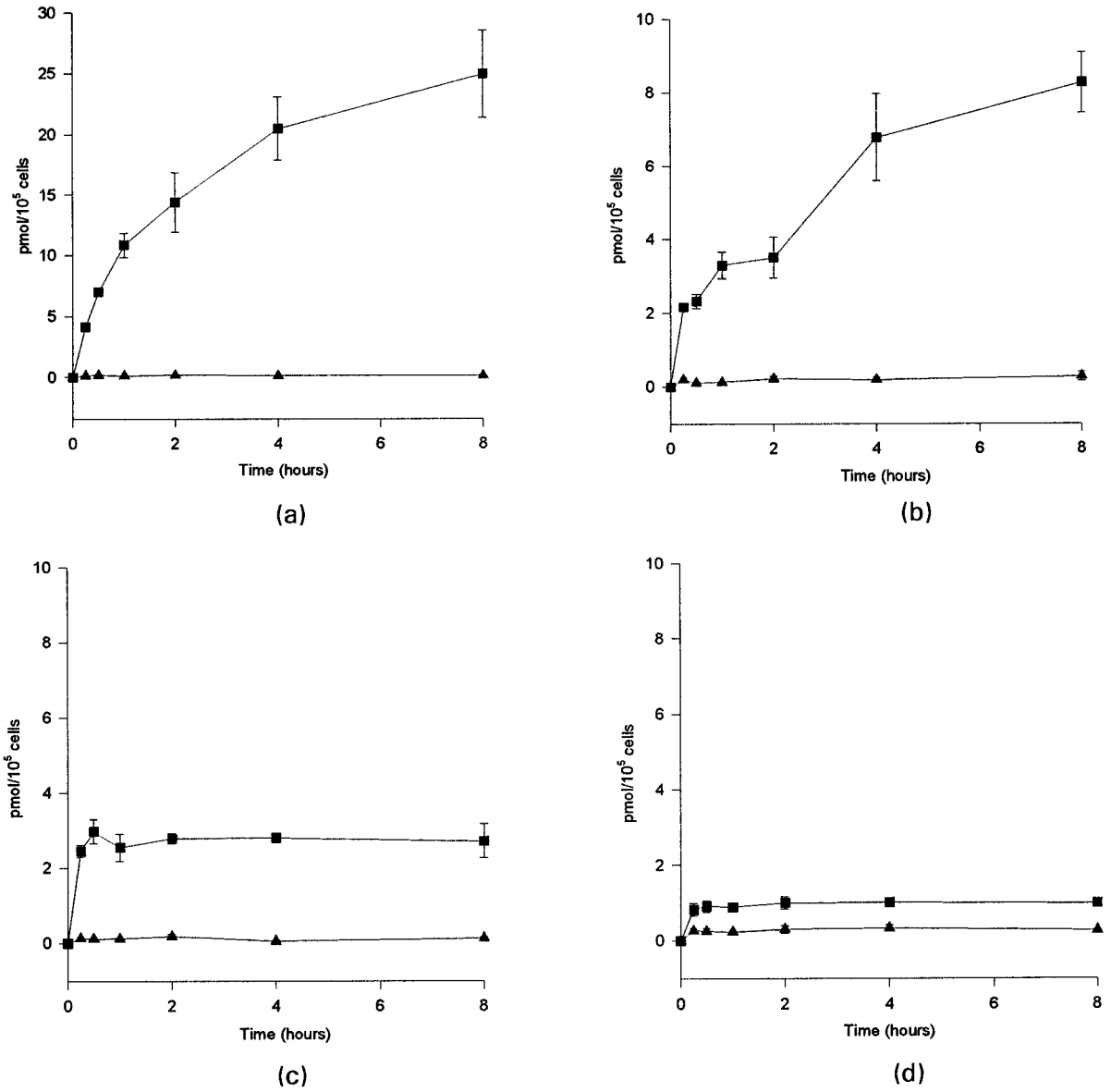


Figure 1. *In vitro* uptake of [^{125}I]IVDU (a), [^{125}I]IVFRU (b), [^{125}I]IVFAU (c), and [^{125}I]IVAU (d) in KBALB-STK (■) and KBALB (▲) cells.

Results and Discussion

Cellular uptake of each radiolabeled compound (**10**−**13**) was determined in two cell lines of murine origin. The KBALB cell line is a Kirsten virus-transformed sarcoma tumor cell line.[25] KBALB-STK cells are derived from the KBALB cell line, express HSV-1 TK, and are sensitive to the cytotoxic effects of ganciclovir *in vitro* and *in vivo*.[26] The KBALB-STK cells have been transduced with the STK vector which is a replication-incompetent Moloney murine leukemia retrovirus vector containing the HSV-1 TK gene promoted by the SV40 early promoter−enhancer and the neomycin resistance gene driven by the viral long terminal repeat.[27] Continuous *in vitro* selection by culturing in the presence of the neomycin analogue G-418 ensures that resistant cells are propagated. In order to accurately compare cellular uptake, each radiolabeled compound was assayed under identical molar concentration (0.076 μM) and cellular density (1×10^5 cells) in cell cultures of KBALB and KBALB-STK cells. The cellular uptake of each compound (**10**−**13**) in both cell lines over a period of 8 h is illustrated in Figure 1. Minimal uptake of all four compounds (**10**−**13**) occurred in the KBALB cell line since less than 0.3 pmol compound/10^5 cells was present at all time periods in KBALB cells.

In contrast to the KBALB cells which lack HSV-1 TK expression, the KBALB-STK cell line was capable of accumulating the nucleoside analogues to varying degrees. [^{125}I]IVDU was metabolically trapped to the greatest extent, with cellular radioactivity increasing up to 8 h after exposure (see Figure 1). Rapid influx of compound was evident at 15 min, when approximately 11% of the total radioactivity or 4.1 pmol/10^5 cells was present. At 8 h after exposure, 66% of the radioactivity (25 pmol/10^5 cells) was intracellularly trapped. The kinetic profile of the uptake process is qualitatively similar to that observed previously with [^{131}I]IVDU in TK$^+$ HSV-1-infected rabbit kidney cells.[28] [^{125}I]IVFRU also accumulated rapidly in KBALB-STK cells, with increasing cellular radioactivity over time (see Figure 1b). However, the presence of a 2′-fluorine substituent in the ribofuranosyl configuration appears to affect cellular nucleoside uptake, since there was substantially lower cellular uptake of [^{125}I]IVFRU relative to [^{125}I]-

IVDU. Influx of [^{125}I]IVFRU after 15 min was 2.2 pmol/10^5 cells or 6% of the total radioactivity. After 8 h exposure to [^{125}I]IVFRU, KBALB-STK cells accumulated 8.2 pmol/10^5 cells or 21.6% of the total activity added to the culture.

Unexpected uptake kinetics were observed for both [^{125}I]IVFAU (see Figure 1c) and [^{125}I]IVAU (see Figure 1d) which possess 2′-fluoro and 2′-hydroxy substituents in the arabinofuranosyl configuration, respectively. In both cases, rapid uptake of radioactivity was evident at the earliest time intervals. However, the influx of labeled compounds was minimal after 30 min exposure. In the case of [^{125}I]IVFAU, initial uptake at 15 min was 2.4 pmol/10^5 cells, which is comparable to [^{125}I]IVFRU at this early time point. However, after 8 h exposure to [^{125}I]IVFAU, only 2.7 pmol/10^5 cells had accumulated in the KBALB-STK cells. This represents a 3-fold decrease in uptake relative to [^{125}I]IVFRU, which differs structurally from [^{125}I]IVFAU only in that [^{125}I]IVFRU has a 2′-fluoro substituent in the ribofuranosyl configuration. A similar time−response profile with substantially lower uptake of activity was observed for [^{125}I]-IVAU. Preferential uptake in HSV-1 TK expressing cells was evident at 15 min in KBALB-STK cells relative to KBALB cells lacking HSV-1 TK. However, less than 1 pmol/10^5 cells had accumulated at the earliest time point in the KBALB-STK cells. Exposure to [^{125}I]-IVAU for up to 8 h did not substantially increase its influx into KBALB-STK cells. After 8 h, cellular uptake of [^{125}I]IVAU was only 0.9 pmol/10^5 cells, representing a 3-fold decrease in uptake relative to [^{125}I]IVFAU where the 2′-substituent in the arabino configuration is fluorine.

Structure−activity correlations for a variety of 2′-fluoro-2′-deoxyuridine analogs have indicated that the sugar 2′-configuration results in differential antiviral effects.[15] Moreover, 5-substituted 2′-fluoro-2′-deoxyuridine analogs possessing the arabinofuranosyl configuration are generally more potent against HSV-1 than similar compounds possessing the ribofuranosyl configuration.[29] Similarly, we observed that there was a correlation between nucleoside uptake in rabbit kidney cells infected with HSV-1 (TK$^+$) and antiviral effect, since radiolabeled 5-iodo-2′-fluoro-2′-deoxyarabinouridine (FIAU) accumulated to a greater extent than 5-iodo-2′-fluoro-2′-deoxyuridine (FIRU) in HSV-1-infected cells over a 24 h period.[30] However, a comparative uptake investigation revealed that [^{125}I]IVDU and [^{125}I]IVFRU had significantly higher cellular uptake than [^{125}I]IVFRU in HSV-1 (TK$^+$)-infected rabbit kidney cells despite the comparable antiviral activity of IVFAU.[14] Interestingly, this latter study indicated that the change in cellular uptake of [^{125}I]IVFAU between 4 and 24 h was quite low compared to that of [^{125}I]IVDU and [^{125}I]IVFRU. The present results for HSV-1 TK expressing tumor cells is in agreement with the previous observations since [^{125}I]IVDU and [^{125}I]IVFRU accumulated to a greater degree than [^{125}I]IVFAU and [^{125}I]-IVAU, with the latter two compounds showing very little change in uptake between 15 min and 8 h. In addition, the extent of [^{125}I]IVAU uptake in KBALB-STK cells is similar to that previously observed in HSV-1 (TK$^+$)-infected rabbit kidney cells.[23]

The reasons for the different time−response profiles are not directly apparent in this study. Uncompromised cytoplasmic membrane integrity due to an absence of cytopathic viral infection imply that facilitated nucleoside transport mechanisms may be of increased importance relative to HSV-1 cell infection. It is known that NBMPR-sensitive, equilibrative nucleoside transporters in both human and murine cells are capable of accepting a variety of nucleoside substrates which are relatively insensitive to modifications at the 2′-position of uracil nucleosides.[31,32] Thus, it is likely that other biochemical processes are affecting metabolic trapping of [^{125}I]-IVFAU and [^{125}I]IVAU. IVDU, IVFRU, and IVFAU are effective substrates for HSV-1 TK, display comparable cytostatic activity against HSV−1 TK gene-transfected murine mammary carcinoma, and appear to evoke this cytostatic effect primarily through inhibition of thymidylate synthase.[33] However, the observation that the structurally related (*E*)-5-(2-bromovinyl)-2′-arabinouridine completely lacks cytostatic and thymidylate synthase inhibitory activity in HSV-1 TK expressing tumor cells whereas (*E*)-5-(2-bromovinyl)-2′-deoxyuridine is one of the most potent cytostatic agents in these cells demonstrates that a small modification in structure can have dramatic biochemical consequences even though both compounds exhibit potent antiviral activity against HSV-1.[34]

In conclusion, the objective of this study was to identify a suitable radiolabeled probe which could prove useful as a marker for cells transduced or transfected with the HSV-1 TK gene during *in vivo* gene therapy. All of the compounds evaluated (**10**−**13**) displayed very low uptake in KBALB cells that lack the HSV-1 TK gene. In HSV-1 TK gene-transduced tumor cells (KBALB-STK), the order of cellular uptake is [^{125}I]IVDU > [^{125}I]IVFRU > [^{125}I]IVFAU > [^{125}I]IVAU. Although [^{125}I]IVDU displayed the highest uptake in KBALB-STK cells at all time periods examined, it is considered unsuitable as an imaging agent due to its susceptibility to rapid, phosphorylase-mediated deglycosylation.[13] The structure−uptake relationship observed in this study indicates that for these 2′-substituted 2′-deoxyuridine derivatives possessing an (*E*)-5-(2-iodovinyl) substituent, the presence of a 2′-fluoro substituent in the ribo configuration confers a superior ability to accumulate in tumor cells expressing HSV-1 TK. The combination of avid cellular accumulation of radiolabeled IVFRU in HSV-1 TK expressing cells and resistance to phosphorylase-mediated deglycosylation after *in vivo* administration to rodents are desirable characteristics for a putative HSV-1 TK imaging agent.[14] However, it should be noted that cellular accumulation of IVFRU in human cells expressing HSV-1 TK may differ from that observed in murine cells. A potential concern regarding the predictive value of the murine models should be addressed by performing uptake studies in human cells. Therefore, IVFRU radiolabeled with radionuclides that emit tissue-penetrating photons (^{131}I, ^{123}I, or ^{124}I) warrant further investigation as a noninvasive *in vivo* probe to detect HSV-1 TK gene expression during gene therapy.

Experimental Section

Melting points were determined on a Buchi capillary apparatus and are uncorrected. NMR spectra were acquired on a Bruker AM-300 spectrometer. The assignment of exchangeable protons (NH, OH) were confirmed by addition of D_2O. ^{13}C NMR spectra were acquired using the *J*-modulated spin echo

technique where methyl and methine carbon resonances appear as positive peaks and methylene and quaternary carbon resonances appear as negative peaks. High-resolution mass spectrometry was performed on a AEI MS-50 mass spectrometer. Solvents and reagents used were of reagent quality. Solvents were dried using standard methods and were freshly distilled prior to use. TLC analysis was performed using Whatman MK6F silica gel microslides. Column chromatography was performed on Merck 7734 silica gel (60−200 μm particle size). HPLC separations of radiolabeled compounds were performed on a preparative Whatman Partisil M9 10/25 ODS reverse phase column using a Waters HPLC system consisting of Model 510 solvent pumps, Model 860 gradient controller, Model U6K injector unit, and a Hewlett-Packard Model 1040A diode array ultraviolet detector. Radioactivity was determined by gamma counting using a Beckman Gamma 8000 scintillation counter. 5-Iodo-2′-deoxyuridine was purchased from the Aldrich Chemical Co. 5-Iodo-2′-fluoro-2′-deoxyuridine (**4**)[35] and 2′,3′,5′-tri-*O*-acetyl-5-iodoarabinouridine (**1**)[23] were prepared using literature procedures. 5-Iodo-2′-fluoro-2′-deoxyarabinouridine (**5**) was obtained as a generous gift from Bristol Meyers Squibb. (*E*)-2-(Tributylstannyl)-1-(trimethylsilyl)ethene was prepared as previously described.[36] KBALB cells were obtained from the American Type Culture Collection (ATCC), and KBALB-STK cells were a kind gift from Dr. Scott Freeman.

2′,3′,5′-Tri-*O*-acetyl-(*E*)-5-(2-(trimethylsilyl)vinyl)arabinouridine (2). (*E*)-$Bu_3SnCH{=}CHSiMe_3$ (10.3 g, 26.2 mmol) was added to a stirred solution of **1** (5.2 g, 9.9 mmol) and $(Ph_3P)_2Pd(II)Cl_2$ (0.99 g, 1.41 mmol) in dry acetonitrile (300 mL) under an argon atmosphere, and the reaction was allowed to proceed for 16 h at 50 °C with stirring. Removal of the solvent *in vacuo* gave a residue that was purified by silica gel column chromatography using hexane−ethyl acetate (1.5:1, v/v) as eluent to yield **2** (3.32 g, 64%) as a white crystalline product, mp 159−160 °C. The ^{1}H and ^{13}C NMR spectra were identical to those reported previously.[23]

(*E*)-5-(2-(Trimethylsilyl)vinyl)arabinouridine (9). Compound **2** (0.18 g, 0.34 mmol) was added to a 2% suspension of LiOH in dry benzene (200 mL), and the reaction was allowed to proceed for 80 h at 25 °C with stirring. After filteration to remove solid LiOH, and removal of the solvent *in vacuo*, the residue obtained was purified by silica gel column chromatography using methanol−chloroform (12:88, v/v) as eluent. Recrystallization of the product **9** from methanol gave white crystals (51 mg, 39%): mp 103−105 °C; UV (CH_3OH) λ_{max} 246, 296 nm; ^{1}H NMR (CD_3OD) δ 0.10 (s, 9H, Si*Me*$_3$), 3.79−3.92 (m, 3H, H-5′, H-4), 4.10 (dd, 1H, $J_{3',4'}$ = 4.2, $J_{2',3'}$ = 3.9 Hz, H-3′), 4.19 (dd, 1H, $J_{1',2'}$ = 4.5, $J_{2',3'}$ = 3.9 Hz, H-2′), 6.16 (d, 1H, $J_{1',2'}$ = 4.5 Hz, H-1′), 6.69 and 6.53 (two d, 1H each, J_{trans} = 19.4 Hz, C*H*=C*H*SiMe$_3$), 8.07 (s, 1H, uracil H-6); ^{13}C NMR (CD_3OD) δ 164.70 (C-4, *C*=O), 151.60 (C-2, *C*=O), 140.56 (C-6), 135.71 and 130.19 (vinyl, C-1 and C-2), 112.78 (C-5), 87.12 (C-1′), 85.82 (C-4′), 77.19 (C-2′), 76.93 (C-3′), 61.99 (C-5′), −1.20 (Si*Me*$_3$); exact mass calculated for $C_{14}H_{22}N_2O_6Si$ 342.1247, found (HRMS), 342.1243 (M^+, 1.74).

(*E*)-5-(2-(Trimethylsilyl)vinyl)-2′-deoxyuridine (6). (*E*)-$Bu_3SnCH{=}CHSiMe_3$ (220 mg, 0.56 mmol) and $(Ph_3P)_2Pd(II)Cl_2$ (20 mg, 0.028 mmol) were added to a solution of **3** (100 mg, 0.28 mmol) in dry acetonitrile (10 mL), and the reaction was allowed to proceed at 60 °C for 16 h under an argon atmosphere with stirring. Removal of the solvent *in vacuo* gave a residue that was purified by silica gel column chromatography. Elution with methanol−chloroform (3:20, v/v) afforded **6**, which was recrystallized from ethyl acetate as a colorless solid (67 mg, 73% yield): mp 108−110 °C; ^{1}H NMR (DMSO-d_6) δ 0.09 (s, 9H, Si*Me*$_3$), 2.08−2.22 (m, 2H, H-2′), 3.56−3.64 (m, 2H, H-5′), 3.78−3.79 (m, 1H, H-4′), 4.22−4.30 (m, 1H, H-3′), 5.17 (t, 1H, $J_{OH,5'}$ = 5.0 Hz, C-5′ OH), 5.27 (d, 1H, $J_{OH,3'}$ = 4.4 Hz, C-3′ OH), 6.16 (t, 1H, $J_{1',2'}$ = 6.1 Hz, H-1′), 6.59 (s (A_2), 2H, C*H*=C*H*SiMe$_3$), 8.18 (s, 1H, H-6), 11.42 (s, 1H, NH); exact mass calculated for $C_{14}H_{22}N_2O_5Si$ 326.1298, found (HRMS) 326.1301 (M^+, 2.39). Anal. ($C_{14}H_{22}N_2O_5Si$) C, H, N.

(*E*)-5-(2-(Trimethylsilyl)vinyl)-2′-fluoro-2′-deoxyuridine (7). $(Ph_3P)_2PdCl_2$ (37 mg, 0.05 mmol) and (*E*)-$Bu_3SnCH{=}CHSiMe_3$ (412 mg, 1.06 mmol) were added with stirring to a solution of **4** (197 mg, 0.53 mmol) in dry THF (5 mL) at 50 °C, and the reaction mixture was stirred for 16 h at 50 °C under an atmosphere of argon at which time TLC analysis indicated the reaction was completed. Removal of the solvent *in vacuo* and purification of the residue by silica gel column chromatography with methanol−dichloromethane (1:25, v/v) as eluent afforded **7** as a white solid (146 mg, 80% yield) after recrystallization from ethyl acetate: mp 164−165 °C; ^{1}H NMR (DMSO-d_6) δ 0.08 (s, 9H, Si*Me*$_3$), 3.62−3.65 (m, 1H, J_{gem} = 9.9 Hz, H-5′), 3.84−3.88 (m, 1H, H-5″), 3.89 (d, 1H, $J_{3',4'}$ = 8.8 Hz, H-4′), 4.14−4.25 (m, 1H, $J_{3',F}$ = 24.2, $J_{2',3'}$ = 3.8 Hz, H-3′), 5.04 (dd, 1H, $J_{2',F}$ = 53.3, $J_{2',3'}$ = 3.8 Hz, H-2′), 5.43 (t, 1H, $J_{OH,5'}$ = 4.8 Hz, C-5′ OH), 5.63 (d, 1H, $J_{OH,3'}$ = 6.6 Hz, C-3′ OH), 5.92 (d, 1H, $J_{1',F}$ = 17.0 Hz, H-1′), 6.52 and 6.59 (two d, 1H each, J_{trans} = 19.8 Hz, C*H*=C*H*SiMe$_3$), 8.33 (s, 1H, uracil H-6), 11.51 (s, 1H, NH); exact mass calculated for $C_{14}H_{21}FN_2O_5Si$ 344.1204, found (HRMS) 344.1208 (M^+, 2.2). Anal. ($C_{14}H_{21}FN_2O_5Si$) C, H, N.

(*E*)-5-(2-(Trimethylsilyl)vinyl)-2′-fluoro-2′-deoxyarabinouridine (8). (*E*)-$Bu_3SnCH{=}CHSiMe_3$ (220 mg, 0.56 mmol) was added to a solution of **5** (100 mg, 0.27 mmol) and $(Ph_3P)_2Pd(II)Cl_2$ (20 mg, 0.028 mmol) in dry acetonitrile (10 mL), and the reaction was allowed to proceed at 60 °C for 16 h under an argon atmosphere with stirring. Removal of the solvent *in vacuo*, purification of the residue by silica gel column chromatography with methanol−chloroform (1:10, v/v) as eluent, and recrystallization of the product from EtOAc gave **8** as a white solid (59 mg, 64% yield): mp 182−183 °C; ^{1}H NMR (DMSO-d_6) δ 0.09 (s, 9H, $SiMe_3$), 3.60−3.70 (m, 2H, H-5′), 3.78−3.80 (m, 1H, H-4′), 4.24−4.31 (m, 1H, $J_{3',F}$ = 20.9 Hz, H-3′), 5.11 (dt, 1H, $J_{2',F}$ = 52.8, $J_{1',2'}$ = 4.4, $J_{2',3'}$ = 4.4 Hz, H-2′), 5.27 (t, 1H, $J_{OH,5'}$ = 5.5 Hz, C-5′ OH), 5.90 (d, 1H, $J_{OH,3'}$ = 5.0 Hz, C-3′ OH), 6.15 (dd, 1H, $J_{1',F}$ = 13.2, $J_{1',2'}$ = 4.4 Hz, H-1′), 6.59 (two d, 1H each, J_{trans} = 19.2 Hz, C*H*=C*H*SiMe$_3$), 8.04 (s, 1H, uracil H-6), 11.58 (s, 1H, NH); exact mass calculated for $C_{14}H_{21}FN_2O_5Si$ 344.1204, found (HRMS) 344.1202 (M^+, 3.53). Anal. ($C_{14}H_{21}FN_2O_5Si$) C, H, N.

General Procedure for Iodination of (*E*)-5-(2-(Trimethylsilyl)vinyl) Precursors (6−9) (Method A). After dissolution of **6**−**9** (0.116 mmol) in dry acetonitrile (2 mL), iodine monochloride (19 mg, 0.116 mmol) was added immediately, and the reaction was allowed to proceed at 25 °C for 30 min with stirring. The solvent was removed *in vacuo*, and the residue was purified by silica gel column chromatography using methanol (7−10%) in chloroform as eluent. The solvent from pooled fractions was removed *in vacuo*, and the respective product (**10**, **11**, **12**, or **13**) was recrystallized from methanol.

General Procedure for Carrier-Added Radioiodination of (*E*)-5-(2-(Trimethylsilyl)vinyl) Precursors (6−9) (Method B). Na^{125}I (6.5 MBq) in 0.1 N NaOH solution (5 μL) was placed in a Wheaton vial, and a solution of ICl (25 μg, 0.154 μmol) in 20% acetic acid in acetonitrile (10 μL) was added. A solution of **6**, **7**, **8**, or **9** (0.73 μmol) in 20% acetic acid in acetonitrile (10 μL) was added to the contents in the Wheaton vial, and the reaction was allowed to proceed for 15 min at 25 °C. The desired product (**10**, **11**, **12**, or **13**) was purified by preparative reverse phase HPLC on a Whatman Partisil M9 10/25 C8 column using isocratic elution with acetonitrile−H_2O (3:7, v/v) at a flow rate of 1.5 mL/min and UV detection at 254 nm. Radiolabeled compounds (**10**−**13**) displayed identical HPLC chromatographic retention times as observed for authentic unlabeled reference samples under a variety of chromatographic conditions.

(*E*)-5-(2-Iodovinyl)-2′-deoxyuridine (10, Method A) and (*E*)-5-(2-[^{125}I]Iodovinyl)-2′-deoxyuridine (10, Method B). (*E*)-5-(2-Iodovinyl)-2′-deoxyuridine (**10**) was prepared in 77% yield (method A) after recrystallization from methanol: mp 167−169 °C; ^{1}H NMR (DMSO-d_6) δ 2.10−2.16 (m, 2H, H-2′), 3.52−3.68 (m, 2H, H-5′), 3.76−3.80 (m, 1H, H-4′), 4.20−4.26 (m, 1H, H-3′), 5.10 (t, 1H, $J_{OH,5'}$ = 4.0 Hz, C-5′ OH), 5.27 (d, 1H, $J_{OH,3'}$ = 3.0 Hz, C-3′ OH), 6.13 (t, 1H, $J_{1',2'}$ = 4.0 Hz, H-1′), 7.12 (d, J_{trans} = 14.3 Hz, 1H, C*H*=CHI), 7.20 (d, J_{trans} = 14.3 Hz, 1H, CH=C*H*I), 8.07 (s, 1H, H-6), 11.55 (s, 1H, NH).

(*E*)-5-(2-[^{125}I]Iodovinyl)-2′-deoxyuridine ([^{125}I]IVDU) (**10**, 5.6 MBq, 86% radiochemical yield, >98% radiochemical purity, specific activity 38 GBq/mmol), prepared according to method B, had a HPLC retention time of 11.49 min whereas the unreacted (trimethylsilyl)vinyl precursor (**6**) had a retention time of 22.51 min.

(*E*)-5-(2-Iodovinyl)-2′-fluoro-2′-deoxyuridine (11, Method A) and (*E*)-5-(2-[^{125}I]Iodovinyl)-2′-fluoro-2′-deoxyuridine (11, Method B). (*E*)-5-(2-Iodovinyl)-2′-fluoro-2′-deoxyuridine (IVFRU) was prepared in 78% yield (method A) after recrystallization from methanol as a white solid: mp 107−109 °C (lit.[14] mp 108−110 °C); ^{1}H NMR (DMSO-d_6) δ 3.65 (d, 1H, J_{gem} = 12 Hz, H-5′), 3.82−3.96 (m, 2H, H-4′, H-5″), 4.20 (dd, 1H, $J_{3',F}$ = 23, $J_{2',3'}$ = 6 Hz, H-3′), 5.06 (dd, 1H, $J_{2',F}$ = 54, $J_{2',3'}$ = 6 Hz, H-2′), 5.47 (br s, 1H, C-5′ OH), 5.72 (br s, 1H, C-3′ OH), 5.92 (d, 1H, $J_{1',F}$ = 18 Hz, H-1′), 7.09 (d, 1H, J_{trans} = 16 Hz, C*H*═CHI), 7.20 (d, 1H, J_{trans} = 16 Hz, CH═C*H*I), 8.23 (s, 1H, uracil H-6), 11.60 (br s, 1H, NH).

(*E*)-5-(2-[^{125}I]Iodovinyl)-2′-fluoro-2′-deoxyuridine ([^{125}I]IVFRU) (**11**, 5.5 MBq, 85% radiochemical yield, >98% radiochemical purity, specific activity 41 GBq/mmol), prepared according to method B, had a HPLC retention time of 11.76 min, whereas the unreacted (trimethylsilyl)vinyl precursor (**7**) had a retention time of 24.19 min.

(*E*)-5-(2-Iodovinyl)-2′-fluoro-2′-deoxyarabinouridine (12, Method A) and (*E*)-5-(2-[^{125}I]Iodovinyl)-2′-fluoro-2′-deoxyarabinouridine (12, Method B). (*E*)-5-(2-Iodovinyl)-2′-fluoro-2′-deoxyarabinouridine (IVFAU) was prepared in 64% yield (method A) after recrystallization from methanol: mp 178 °C (lit.[37] mp 178−179 °C); ^{1}H NMR (DMSO-d_6) δ 3.61−3.71 (m, 2H, H-5′), 3.79−3.80 (m, 1H, H-4′), 4.17−4.27 (m, 1H, $J_{3',F}$ = 20 Hz, H-3′), 5.06 (dt, 1H, $J_{2',F}$ = 54, $J_{1',2'}$ = 3.0 Hz, H-2′), 5.20 (t, 1H, $J_{OH,5'}$ = 6.0 Hz, C-5′ OH), 5.94 (d, 1H, $J_{OH,3'}$ = 4.9 Hz, C-3′ OH), 6.10 (dd, 1H, $J_{1',F}$ = 10, $J_{1',2'}$ = 4.9 Hz, H-1′), 7.15 (d, 1H, J_{trans} = 14.8 Hz, C*H*═CHI), 7.23 (d, 1H, J_{trans} = 14.8 Hz, CH═C*H*I), 7.98 (s, 1H, uracil H-6), 11.73 (s, 1H, NH).

(*E*)-5-(2-[^{125}I]Iodovinyl)-2′-fluoro-2′-deoxyarabinouridine([^{125}I]-IVFAU) (**12**, 5.6 MBq, 86% radiochemical yield, >98% radiochemical purity, specific activity 38 GBq/mmol), prepared according to method B, had a HPLC retention time of 13.21 min whereas the unreacted (trimethylsilyl)vinyl precursor (**8**) had a retention time of 27.59 min.

(*E*)-5-(2-Iodovinyl)arabinouridine (13, Method A) and (*E*)-5-(2-[^{125}I]Iodovinyl)arabinouridine (13, Method B). (*E*)-5-(2-Iodovinyl)arabinouridine was isolated in 75% yield (method A) after recrystallization from methanol: mp 171−175 °C (lit.[23] mp 170−175 °C); ^{1}H NMR (DMSO-d_6) δ 3.62−3.65 (m, 2H, H-5′), 3.72−3.76 (m, 1H, H-4′), 3.90−3.93 (m, 1H, H-3′), 4.00−4.05 (m, 1H, H-2′), 5.13 (t, 1H, $J_{OH,5'}$ = 5.5 Hz, C-5′ OH), 5.46 (d, 1H, $J_{OH,3'}$ = 4.4 Hz, C-3′ OH), 5.56 (d, 1H, $J_{OH,2'}$ = 5.3 Hz, C-2′ OH), 5.98 (d, 1H, $J_{1',2'}$ = 4.7 Hz, H-1′), 7.13 (d, 1H, J_{trans} = 14.6 Hz, C*H*═CHI), 7.19 (d, 1H, J_{trans} = 14.6 Hz, CH═C*H*I), 7.88 (s, 1H, uracil H-6), 11.54 (s, 1H, NH).

(*E*)-5-(2-[^{125}I]Iodovinyl)arabinouridine ([^{125}I]IVAU) (**13**, 4.1 MBq, 63% radiochemical yield, >98% radiochemical purity, specific activity 30 GBq/mmol), prepared using Method B had a HPLC retention time of 10.24 min, whereas the unreacted trimethylsilylvinyl precursor (**9**) had a retention time of 17.69 min.

***In Vitro* Uptake Studies.** Cells (1×10^5) of each cell line (KBALB, KBALB-STK) were grown in 24-well culture plates. Radiolabeled compound (**10**, **11**, **12**, or **13**; 38 pmol; specific activity = 30−41 GB1/mmol) was added to each well and incubated at 37 °C in Dulbecco's Modified Eagles Medium (0.5 mL). At varying times after exposure to the radiolabeled compounds, the supernatants were removed, the cells were rinsed with phosphate-buffered saline, and the adherant cells were then trypsinized and removed. Cellular uptake of radioactivity was determined by gamma counting in a Beckmann 8000 gamma counter.

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