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Synthesis and *In Vitro* Evaluation of 5-[¹⁸F]Fluoroalkyl Pyrimidine Nucleosides for Molecular Imaging of Herpes Simplex Virus Type-1 Thymidine Kinase Reporter Gene Expression

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Abstract

Two novel series of 5-fluoroalkyl-2'-deoxyuridines (FPrDU, FBuDU, FPeDU) and 2'-fluoro-2'-deoxy-5-fluoroalkylarabinouridines (FFPrAU, FFBuAU, FFPeAU), having three, four or five methylene units (propyl, butyl, or pentyl) at C-5, were prepared and tested as reporter probes for imaging HSV1-*tk* gene expression. The Negishi coupling methodology was employed to efficiently synthesize the radiolabeling precursors. All six 5-[¹⁸F]fluoroalkyl pyrimidines were prepared readily from 3-*N*-benzoyl-3',5'-di-*O*-benzoyl-protected 5-*O*-mesylate precursors in 17–35% radiochemical yield (decay-corrected). *In vitro* studies highlighted that all six [¹⁸F]labeled nucleosides selectively accumulated in cells expressing the HSV1-TK protein, with negligible uptake in control cells. [¹⁸F]FPrDU, [¹⁸F]FBuDU, [¹⁸F]FPeDU, and [¹⁸F]FFBuAU had the best uptake profiles. Despite selective accumulation in HSV1-*tk* expressing cells, all 5-fluoroalkyl pyrimidine nucleosides had low to negligible cytotoxic activity (CC₅₀>1000–209 μM). Ultimately, results demonstrated that 5-[¹⁸F]fluoropropyl, [¹⁸F]fluorobutyl, and [¹⁸F]fluoropentyl pyrimidine nucleosides have potential as *in vivo* HSV1-TK PET reporter probes over a dynamic range of reporter gene expression levels.

Introduction

The herpes simplex virus type-1 thymidine kinase (HSV1-*tk*) gene is currently the most actively investigated reporter gene for the non-invasive molecular imaging of gene expression and regulation by such modalities as positron emission tomography (PET).¹ The HSV1-*tk* gene encodes a cytosolic thymidine kinase enzyme, HSV1-TK, which phosphorylates the pyrimidine nucleoside thymidine (TdR) independently of the ubiquitous cell-cycle regulated cellular thymidine kinase 1, TK1. The high degree of flexibility of the HSV1-TK active site allows it to phosphorylate not only TdR but a wide variety of

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Supporting Information Available. Procedures for synthesizing some intermediates, ¹H and ¹³C NMR spectra of all bioassayed compounds, HPLC purity analysis for all bioassayed compounds in two different HPLC systems, typical radio-HPLC chromatogram, procedure for cell uptake inhibition, cell growth inhibition profile. This material is available free of charge via the Internet at <http://pubs.acs.org>.

pyrimidine and purine nucleoside derivatives.^{2,3} The more restricted substrate specificity of mammalian TK1 (cytosolic)⁴ and TK2 (mitochondrial),⁵ limits the binding and phosphorylation of non-TdR nucleosides. Both antiviral therapy and suicide gene therapy have taken advantage of the promiscuity of HSV1-TK when it is selectively expressed in herpes virus-infected cells or cells transduced/transfected with the HSV1-*tk* gene. Only in those cells expressing the HSV1-TK protein can prodrugs like the acyclic purine derivative ganciclovir (GCV) be activated by phosphorylation. Following initial conversion to the monophosphate by HSV1-TK, cellular kinases further phosphorylate the GCV-monophosphate to the di- and triphosphate metabolites. The triphosphate form of the nucleoside analog can inhibit DNA polymerase resulting in chain termination and inhibition of DNA synthesis and cell replication. This and other interactions with key cellular DNA machinery leads to cytotoxicity and cell death.^{6,7} Cells not expressing HSV1-TK would remain unaffected by prodrug treatment.

Just as HSV1-*tk* gene expression is a requisite for prodrug activation *via* its gene product, HSV1-TK, analogously, the accumulation of the imaging reporter probe is dependent on the expression of HSV1-*tk* as a reporter gene. To date, a variety of different HSV1-TK PET reporter probes have been reported, with their structures derived from successful antiviral agents for HSV infection. These include the pyrimidine nucleoside analog of 5-iodo-2'-deoxyuridine⁸ (IDU), [¹²⁴I]FIAU (2'-fluoro-2'-deoxy-5-[¹²⁴I]iodo-1-β-D-arabinofuranosyluracil),⁹ and the purine analog of GCV, [¹⁸F]FHBG (9-(4-[¹⁸F]fluoro-3-hydroxymethyl)butyl)guanine).^{10,11} Each reporter probe has a unique set of advantages and disadvantages regarding, for example, their routine radiosynthesis, degree of cellular accumulation (sensitivity), selective phosphorylation by HSV1-TK *versus* mammalian TKs, rate of cellular uptake, and metabolic stability. Recent studies have highlighted [¹⁸F]FEAU (2'-[¹⁸F]fluoro-2'-deoxy-5-ethyl-1-β-D-arabinofuranosyluracil)¹²⁻¹⁵ and [¹⁸F]FFEAU (2'-fluoro-2'-deoxy-5-(2-[¹⁸F]fluoroethyl)-1-β-D-arabinofuranosyluracil)^{12,16} as promising imaging agents for assessing HSV1-*tk* gene expression. Both [¹⁸F]FEAU and [¹⁸F]FFEAU, with their two carbon substituent at C-5, have been shown to rapidly accumulate in HSV1-*tk* expressing cells *in vitro* and *in vivo*, with minimal uptake in nontransduced cells. The accumulation and sensitivity characteristics of [¹⁸F]FEAU and [¹⁸F]FFEAU are similar to that previously reported for FIAU, but they have greater selectivity than FIAU due to lower uptake and retention in nontransduced cells and tissues.²²

Crystal structure data of HSV1-TK complexed with TdR and 5-bromovinyl-2'-deoxyuridine (BVDU) emphasizes a considerable volume of the hydrophobic P1 subpocket remaining about the C-5 substituent for ligand binding to the enzyme.¹⁷⁻¹⁹ We rationalize that pyrimidine nucleosides with nonpolar alkane chains with more than two carbons at C-5 will more closely fit the HSV1-TK enzyme pocket to improve substrate binding affinity and subsequent phosphorylation; binding to mammalian TKs would be negligible since these kinases do not possess the P1 subpocket.

In this paper we describe our efforts to elucidate the structural requirements for enhanced HSV1-TK reporter probe efficacy using *in vitro* screening assays. We have synthesized and evaluated two novel series of 5-fluoroalkyl pyrimidine nucleosides having three (propyl), four (butyl), or five (pentyl) carbon units at C-5, and a C-2' hydrogen atom or fluorine atom

in the arabino, or “up” position, as depicted in Figure 1. The 5-[^{18}F]fluoroalkyl-2'-deoxyuridines [^{18}F]FPrDU, [^{18}F]FBuDU, and [^{18}F]FPeDU (**[^{18}F]1a–c**, respectively) and 2'-fluoro-2'-deoxy-5-[^{18}F]fluoroalkylarabinouridines [^{18}F]FFPrAU, [^{18}F]FFBuAU, and [^{18}F]FFPeAU (**[^{18}F]1d–f**, respectively) have been designed for: (i) rapid and efficient preparation of **[^{18}F]1a–f** by convenient radiochemistry at the last stages of radiopharmaceutical synthesis to increase radiochemical yields and shorten preparation times; (ii) improved affinity and selectivity for HSV1-TK over mammalian TKs, and (iii) assessment of resistance to defluorination and glycosidic bond cleavage *in vivo* of the 2'-hydrogen *versus* 2'-fluorine pyrimidine nucleosides.

Results and Discussion

Chemistry

We have recently developed a methodology to conveniently access two series of 5-fluoroalkylated pyrimidine nucleosides **3a–c** and **3d–f** from core nucleoside precursors that are locked in the β -configuration about C-1'.²⁰ Negishi cross-coupling reactions of the key tribenzoyl-protected intermediates, 3-*N*-benzoyl-3',5'-di-*O*-benzoyl-5-iodo-2'-deoxyuridine (**2a**) or 3-*N*-benzoyl-3',5'-di-*O*-benzoyl-2'-fluoro-2'-deoxy-5-iodo-1- β -D-arabinofuranosyluracil (**2b**) with fluoroalkylzinc reagents in the presence of bis(*tri-tert*-butylphosphine)palladium ($\text{Pd}(\text{P}(t\text{-Bu})_3)_2$) afforded the two series of nucleosides **3a–f** in one step (Scheme 1). Subsequent treatment of **3a–f** with 0.5 N NaOMe in MeOH selectively cleaved the benzoate groups without affecting the 5-fluoroalkyl moiety to give the fully deprotected 2'-deoxyuridine analogs 5-(3-fluoropropyl)-2'-deoxyuridine (FPrDU, **1a**), 5-(4-fluorobutyl)-2'-deoxyuridine (FBuDU, **1b**), and 5-(5-fluoropentyl)-2'-deoxyuridine (FPeDU, **1c**), as well as the 2'-fluoro-2'-deoxyarabinouridine analogs 2'-fluoro-2'-deoxy-5-(3-fluoropropyl)-1- β -D-arabinofuranosyluracil (FFPrAU, **1d**), 2'-fluoro-2'-deoxy-5-(4-fluorobutyl)-1- β -D-arabinofuranosyluracil (FFBuAU, **1e**), and 2'-fluoro-2'-deoxy-5-(5-fluoropentyl)-1- β -D-arabinofuranosyluracil (FFPeAU, **1f**) in 85% to quantitative yield.

As elaborated in Scheme 2, the 5-silyloxy pyrimidine nucleosides, *via* the *O*-TBS moiety, offered an easily accessible handle to which we introduced an *O*-mesylate group for subsequent ^{18}F -radiolabeling to achieve the desired HSV1-TK molecular imaging probes **[^{18}F]1a–f**. In our previous work, the Negishi coupling reaction of **2a** with (3-*tert*-butyldimethylsiloxypropyl)zinc **5a** successfully afforded the *O*-TBS-protected nucleoside **6a** in 45% yield.²⁰ It was therefore of interest to investigate whether we could extend our optimized coupling methodology to synthesize the two series of *O*-TBS-protected nucleosides **6a–f** for later derivatization.

The bromoalkyl alcohols, having the alcohol functionality protected as a TBS-ether (**4a–c**), were used to prepare the respective alkylzinc bromide reagents **5a–c** by heating at 85 °C with Zn powder and catalytic amounts of I_2 in *N,N*-dimethylacetamide (DMA) for 1 h.^{20,21} The Negishi coupling reaction of **2a** and **2b** with the zinc reagents **5a–c** and catalytic $\text{Pd}(\text{P}(t\text{-Bu})_3)_2$ in DMA at room temperature afforded the silylated coupling products **6a–f** in 29–45% yield. Desilylation of the TBS protecting groups of **6a–f** under mild conditions using 1% HCl/EtOH for 2 h at room temperature gave the hydroxylated nucleosides **7a–f** in very

high yields. The nucleosides **7a–f**, with a free hydroxyl moiety, were next converted to 5-*O*-mesylates **8a–f** *via* reaction with methanesulfonyl chloride and Et₃N in DCM in very high yields (81–99% yield). Interestingly, these mesylates could not afford the “cold” 5-fluoroalkyl pyrimidines **3a–f** by refluxing **8a–f** in anhydrous TBAF/THF.²²

This three-step synthetic approach to the 5-*O*-mesylate radiofluorination precursors that was developed in our research is considerably shorter than reports on the eleven-step synthesis of labeling precursors for [¹⁸F]FEDU²³ and the more recent five-step approach to labeling precursors for [¹⁸F]FFEAU.¹⁶ Both these approaches involve the low-yielding glycosylation of a sugar derivative with appropriately protected 5-(2-hydroxyethyl)uracil derivatives, followed by a variety of protection and deprotection steps to afford tosylate or tresylate labeling precursors.^{16,23}

The [¹⁸F]labeled nucleosides [¹⁸F]**1a–f** were prepared in two rapid steps from a one-pot reaction from the tribenzoyl-protected *O*-mesylate nucleosides **8a–f** as shown in Scheme 3. Briefly, **8a–f** were reacted with [¹⁸F]fluoride, in the presence of Kryptofix 222 (K[2,2,2]) and potassium carbonate in DMF at 135 °C for 5 min. Radio-HPLC analysis of this crude reaction mixture highlighted multiple [¹⁸F]labeled products, including that of the desired [¹⁸F]labeled tribenzoyl-protected nucleosides [¹⁸F]**3a–f**. Interestingly, separate treatment of the isolated [¹⁸F]**3a–f** fraction, and the fraction with all the other unidentified [¹⁸F]labeled products, with 0.5 N NaOMe in MeOH at 135 °C for 5 min, resulted in only one major radioactive peak with the same retention time. HPLC co-elution with a non-radioactive “cold” standard confirmed the identity of the peak as that of the fully-debenzoylated 5-[¹⁸F]fluoroalkyl pyrimidine nucleoside. This would suggest that the conditions of the labeling step produced multiple combinations of *N*, 3', and 5'-debenzoylated nucleosides that all had the ¹⁸F-radiolabel incorporated at the terminus of the 5-alkyl chain. As a result of this observation, unlike previous reports for the synthesis of 5-[¹⁸F]fluoroalkyl pyrimidine nucleosides,^{16,24} we did not attempt to isolate the [¹⁸F]labeled tribenzoyl-protected nucleoside [¹⁸F]**3a–f** from the crude reaction mixture *via* solid-phase extraction or HPLC. Instead, we immediately heated the reaction mixture at 135 °C for 5 min with 0.5 M NaOMe in MeOH to remove all remaining benzoyl-protecting groups. [¹⁸F]**1a–f** were isolated by solid-phase extraction followed by HPLC purification with an appropriate HPLC solvent system. The purified product had a HPLC retention time consistent with the co-eluted “cold” carrier (an example of a typical HPLC chromatogram is shown in the Supporting Information).

Following our labeling method we were able to obtain purified [¹⁸F]**1a–f** with decay corrected yields of 17–35% (Table 1). These yields are higher than the 9.5% and <0.2% (decay-corrected) yields reported for [¹⁸F]FEDU and [¹⁸F]FFEAU, respectively. The radiochemical purity was >99 % and the specific activity, as estimated by HPLC, ranged from 18.5–925 GBq/μmol (500–25000 mCi/μmol) at end of synthesis (EOS). The total procedure time was 60–70 min, and is a considerable improvement over the synthesis time of 180 min reported for all other [¹⁸F]sugar labeled nucleosides targeting HSV1-TK.^{25–27} It is also an improvement over the 90–120 min synthesis time reported for [¹⁸F]FFEAU.^{12,16} It is likely that our two-step, one-pot synthesis can be easily adapted for automation to obtain higher radioactive preparations in even shorter time.

Partition Coefficient

The lipophilicity ($\log P$) of the labeled tracers [^{18}F]FPrDU, [^{18}F]FBuDU, [^{18}F]FPeDU, [^{18}F]FFPrAU, [^{18}F]FFBuAU, and [^{18}F]FFPeAU ([^{18}F]1a–f, respectively) at pH 7.4 were -0.57 ± 0.09 , -0.24 ± 0.02 , 0.23 ± 0.02 , -0.12 ± 0.01 , 0.27 ± 0.002 , and 0.74 ± 0.01 , respectively (Table 1). As expected, in both the 2'-deoxyuridine series ([^{18}F]1a–c) and the 2'-fluoro-2'-deoxyarabinouridine series ([^{18}F]1d–f), the $\log P$ values increased with increasing fluoroalkyl chain length, indicating increased lipophilicity attributed to additional methylene units. The $\log P$ values of the 2'-deoxyuridines [^{18}F]1a–c were all lower than their corresponding 2'-fluoro-2'-deoxyarabinouridines [^{18}F]1d–f. For comparison, the $\log P$ of [^{125}I]FIAU at pH 7.4 in our system was 0.05 ± 0.01 . $\log P$ values can often indicate the ability of compounds to passively diffuse across intact cell membranes, including the blood-brain barrier (BBB). In recent years, there has been a drive to find reporter probes that are selectively phosphorylated by HSV1-TK whilst being able to permeate the BBB to allow for potential imaging of HSV1-*tk* gene expression in gliomas.^{28,29} The moderately lipophilic FIAU does not diffuse across the intact BBB.³⁰ By reason of $\log P$ values alone, [^{18}F]FFPeAU ([^{18}F]1f) would be expected to cross the BBB more readily by passive diffusion than FIAU, or our other synthesized nucleosides. However, very recent studies with the fairly lipophilic 5-iodovinyl pyrimidine nucleosides [^{123}I]IVFRU ($\log P = 1.22$) and [^{123}I]IVFAU ($\log P = 1.24$) demonstrated very low brain uptake in normal mice.²⁹ These results demonstrate that the usual relationship between BBB permeability and drug lipophilicity does not apply to these compounds.²⁹ In addition to $\log P$ 1–4, the optimal requirements for crossing the BBB include molecular weight <400, and polar surface area (which correlates highly with hydrogen bonding ability) <90 Å².³¹ The higher polar surface area of pyrimidine nucleosides (~100 Å²)³² would suggest their capacity for passive BBB diffusion is inherently low. However, brain uptake is not necessarily limited to passive diffusion. It is well established that nucleosides targeting HSV1-TK are substrates for a variety of nucleoside transporters^{33–36} found on many cellular membranes, including the BBB. The presence of these transporters on the BBB, and in neurons, astrocytes, and glia, may also play an important role in reporter probe accumulation in the brain. As alluded to in recent studies, a driving force, such as a glioma with HSV1-*tk* gene expression, may be required to increase the brain uptake of the radiolabeled pyrimidine nucleosides.²⁹ However, further *in vivo* studies are required to illustrate this point.

Biological Evaluations. Cellular Uptake of [^{18}F]1a–f

The cellular uptake of [^{18}F]FPrDU, [^{18}F]FBuDU, [^{18}F]FPeDU, [^{18}F]FFPrAU, [^{18}F]FFBuAU, and [^{18}F]FFPeAU, was studied in two glioma cell lines of murine origin. The first cell line is stably transduced with HSV1-*tk* (RG2TK+) and thus features cytosolic HSV1-TK enzyme activity.^{37,38} The second cell line is the non-transduced wild-type glioma cell line (RG2) with no HSV1-TK activity but with native TK1 activity. The cellular accumulation of each compound over a period of 2 h in RG2TK+ cells and in RG2 cells is illustrated in Figure 2. All of the [^{18}F]tracers accumulated in RG2TK+ cells in a time dependent manner, and the uptake was significantly higher than the minimal uptake observed in RG2 cells ($P < 0.006$). [^{18}F]FPrDU and [^{18}F]FBuDU showed the greatest accumulation in RG2TK+ cells ($P < 0.0003$) at 8.4% and 8.3% of the total radioactivity per

10^6 cells, respectively. While [^{18}F]FPrDU uptake looked to plateau at 2 h post-treatment, [^{18}F]FBuDU uptake was steadily increasing even at 2 h. In comparison, the uptake of [^{125}I]FIAU at 2 h is 29.4% per 10^6 cells. The degree of uptake of the [^{18}F]tracers in RG2TK+ cells at 2 h followed the order [^{18}F]FBuDU $\approx$ [^{18}F]FPrDU $\gg$ [^{18}F]FPeDU $\approx$ [^{18}F]FFBuAU $>$ [^{18}F]FFPrAU $>$ [^{18}F]FFPeAU.

The sensitivity of the tracers for HSV1-*tk* expressing cells should depend on the binding affinity of the nucleosides for HSV1-TK, and their subsequent phosphorylation rate by the enzyme. Despite the considerable flexibility of the HSV1-TK active site towards C-5 pyrimidine nucleosides, our data suggested a significant decrease in sensitivity of the viral enzyme towards 2'-fluoro-2'-deoxyarabinouridine nucleosides. This is evidenced by the substantially lower cellular uptake of the 2'-fluoro-2'-deoxyarabinouridines [^{18}F]FFPrAU, [^{18}F]FFBuAU, and [^{18}F]FFPeAU as compared to their 2'-deoxyribose counterparts. Similar observations were also reported for studies comparing 5-iodovinyl [^{125}I]IVDU and [^{125}I]IVFAU uptake in KBALB-STK cells expressing HSV1-*tk*, where the 2'-fluorine substituent in the arabino configuration of [^{125}I]IVFAU lowered cellular accumulation in comparison to [^{125}I]IVDU.^{39,40}

Results also suggested that the five-carbon chain of the pyrimidine ring in [^{18}F]FPeDU and [^{18}F]FFPeAU may be too long for the HSV1-TK enzyme P1 subpocket, leading to diminished binding capacity, and thus significantly decreased accumulations of phosphorylated products relative to the shorter-chained nucleosides ($0.0007 < P < 0.05$). Uptake was maximal when the carbon chain length was three or four in the 2'-deoxyuridine series, and four carbons in the 2'-fluoro-2'-deoxyarabinouridine series. For longer time points, [^{18}F]FBuDU and [^{18}F]FFBuAU, with their four-carbon chain lengths, would apparently lead to greatest cellular accumulation in HSV1-TK-positive cells. These results were consistent with antiviral-SAR relationships that establish optimal inhibition occurring when the 5-substituent is no longer than four carbons.⁴¹

An initial screen for the assessing the binding potential of the nonradioactive nucleoside **1a-f** for HSV1-TK was performed in RG2TK+ cells. The *in vitro* accumulation of [^{125}I]FIAU was monitored in the presence of increasing concentrations of nucleoside **1a-f** as inhibitors (Figure 3) to determine the relative strength of these compounds towards inhibiting [^{125}I]FIAU binding to HSV1-TK (for experimental details see Supporting Information). [^{125}I]FIAU accumulation in RG2TK+ cells was inhibited by **1a-f** with IC_{50} values that ranged from $7.6 \pm 0.5 \mu\text{M}$ for FPrDU to $36.8\text{--}8.3 \mu\text{M}$ for FPeDU (Table 2). The 5-fluoroalkyl pyrimidines **1a-f** were at least 10-fold less sensitive than FIAU ($\text{IC}_{50} 0.7 \pm 0.09 \mu\text{M}$) towards inhibiting [^{125}I]FIAU accumulation. In RG2 cells, where there is only mammalian TK activity, [^{125}I]FIAU accumulation is 10-fold lower than in RG2TK+ cells. As such the relative potencies of **1a-f** as compared to FIAU could not be accurately gauged (data not shown). Nonetheless, no correlation could be found between the results from the uptake inhibition assay using [^{125}I]FIAU with **1a-f**, and the cellular uptake assays with [^{18}F]**1a-f**. Upon further consideration, it is apparent that the net uptake of [^{125}I]FIAU inside RG2TK+ cells reflects not only the enzyme activity of HSV1-TK but also the transport of [^{125}I]FIAU across the cell membrane. Since these new nucleosides have not been evaluated as substrates for nucleoside transporters we are unsure of which mechanism is predominant in inhibiting

the cellular accumulation of [125 I]FIAU. Consequently, this screening assay may not truly be reflective of binding potential to HSV1-TK, but rather the inhibition of nucleoside transport.

Using wild-type RG2 cells as a control for non-specific accumulation, the cellular uptake at 2 h ranged from 0.66% for [18 F]FFBuAU to 0.23% for [18 F]FPeDU, and were all significantly lower than the uptake in RG2TK+ cells ($P<0.006$) (Table 3). Comparable to previous reports, there was considerable uptake of [125 I]FIAU in RG2 cells, that has been attributed to phosphorylation by endogenous mammalian TK1. Furthermore, the [125 I]FIAU uptake at 3.44% was significantly higher than [18 F]1a-f. The very low uptake of [18 F]1a-f in the control cells would suggest that our tracers are not phosphorylated by endogenous mammalian thymidine kinase. As a result, background activity of [18 F]1a-f in rapidly proliferating tissue, such as tumor cells, would be very low in comparison to FIAU. This is especially critical when trying to detect lower levels of HSV1-*tk* gene expression.

In developing and comparing tracers for *in vivo* HSV1-*tk* reporter gene imaging, the contrast, or selectivity, between radiotracer uptake in HSV1-*tk* expressing cells, and HSV1-*tk*-negative cells has to be considered. The uptake selectivity indices among the six different nucleosides, as determined by taking the average ratio of tracer accumulation between RG2TK+ and RG2 cells, are shown in Figure 4. In the RG2TK+/RG2 cellular system, the uptake selectivity index of [18 F]FPrDU and [18 F]FPeDU were both significantly higher than that of [18 F]FBuDU ($P<0.0007$ and $P<0.05$, respectively) but not significantly different from each other ($P>0.05$) (Figure 4). The selectivity indices of the 2'-fluoro-2'-deoxyarabinouridines [18 F]FFPrAU, [18 F]FFBuAU, and [18 F]FFPeAU were not statistically different from one another ($P>0.05$). The uptake selectivity indices were in the order of [18 F]FPrDU $\approx$ [18 F]FPeDU $>$ [18 F]FBuDU $\gg$ [18 F]FFBuAU $\approx$ [18 F]FFPrAU $\approx$ [18 F]FFPeAU. The selectivity of [125 I]FIAU fell between that of the 2'-deoxyuridines and the 2'-fluoro-2'-deoxyarabinouridines (Table 3).

In our experiments, the affinity of the reference tracer [125 I]FIAU was relatively high as compared to our new [18 F]tracers. However, FIAU was actively phosphorylated by cellular mammalian thymidine kinase, which significantly lowered the selectivity index of FIAU. Although our tracers apparently have a lower affinity for HSV1-TK as compared to FIAU, they are minimally phosphorylated by mammalian thymidine kinase. As such, the two series of [18 F]labeled nucleosides, in particular [18 F]FPrDU, [18 F]FBuDU, [18 F]FPeDU, and [18 F]FFBuAU, exhibited favorable HSV1-TK sensitivity and uptake selectivity that would allow for the *in vivo* imaging of a dynamic range of HSV1-TK expression levels. This would include the detection of lower levels of HSV1-*tk* gene expression in highly proliferating tissues, a considerable challenge that has been raised for successful *in vivo* HSV1-TK imaging using [123 I]/[124 I]/[18 F]FIAU. Hence, this study shows the potential of [18 F]FPrDU, [18 F]FBuDU, [18 F]FPeDU, and [18 F]FFBuAU as novel tracers for HSV1-TK imaging and merits additional studies to assess the *in vivo* potential of these HSV1-*tk* imaging probes.

Cytotoxic Activity

In view of the fact that the tracers [18 F]1a-f accumulated selectively in RG2TK+ cells and not in RG2 cells, the nonradioactive nucleosides FPrDU, FBuDU, FPeDU, FFPrAU,

FFBuAU, and FFPeAU (**1a–f**, respectively) were evaluated for their antiproliferative activity towards the RG2TK+ and RG2 glioma cells using the MTT assay. The RG2 cell line was used as a negative control to assess the intrinsic cytotoxicity of the nucleosides. GCV and FIAU both exerted a significant cytotoxic effect to RG2TK+ cells with a CC₅₀ of 5.86 and 0.79 μ M, respectively (Table 4). This was in stark contrast to **1a–f**, which all exhibited negligible to low cytotoxicity to RG2TK+ cells (CC₅₀>1000–209 μ M). Interestingly enough, the 2'-fluoro-2'-deoxyarabinouridines **1d–f** were generally more cytotoxic to RG2TK+ cells than the analogous 2'-deoxyuridines **1a–c**, an observation made often when comparing the two classes of pyrimidine nucleosides for antiviral activity.⁴² This is contrary to what was observed in the *in vitro* uptake assays where the 2'-deoxyuridines [**¹⁸F**]**1a–c** were more active for HSV1-TK directed phosphorylation. As expected, GCV was not active against RG2 cells. However, FIAU was found to be moderately toxic to RG2 cells. This is indicative of prodrug activation by TK1, and is in agreement with the significant *in vitro* accumulation of [¹²⁵I]FIAU in RG2 cells due to phosphorylation by TK1. Nucleosides **1a–f** had the same or slightly higher toxicity to RG2 cells than to RG2TK+ and a broad correlation between cytotoxicity and 5-fluoroalkyl chain length was revealed only in RG2 cells.

Nucleosides are almost always prodrugs of metabolically activated phosphate derivatives. Once inside the cell, prodrug activation relies exclusively on the presence of HSV1-TK activity. However, the monophosphate must be further phosphorylated by cellular kinases, and only then is the “active drug” available for binding and inhibition of other cellular enzymes. Our results emphasized that although HSV1-TK phosphorylated the 5-fluoroalkyl pyrimidine nucleosides, as evidenced by intracellular accumulation of [¹⁸F]labeled products, the nucleoside prodrugs were not efficiently processed to the cytotoxic triphosphate species. Hence, the lower toxicity toward both HSV1-*tk* expressing cells and non-transduced cells as compared to GCV or FIAU.

Summary and Conclusions

In employing the Negishi coupling method developed for the synthesis of six 5-fluoroalkylated pyrimidine nucleosides (**1a–f**), we achieved a very short and efficient three-step synthesis of respective 5-*O*-mesylate precursors for ¹⁸F-labeling. The radiolabeling method described herein afforded three new 5-[¹⁸F]fluoroalkyl-2'-deoxyuridines ([**¹⁸F**]**1a–c**) and three new 2'-fluoro-2'-deoxy-5-[¹⁸F]fluoroalkyl arabinouridines ([**¹⁸F**]**1d–f**) in good yields and very short preparation times. This is favorably compared to previously reported approaches to synthesizing [¹⁸F]labeled pyrimidine nucleosides targeting HSV1-TK. Maximal cellular accumulation of the radiotracers occurs when the 5-alkyl chain length is three to four carbons in both nucleoside series, although 5-(5-[¹⁸F]fluoropentyl)-2'-deoxyuridines still accumulates to an appreciable degree in HSV1-TK+ cells. Our results allude that 5-alkyl substituent length does not necessarily dictate the sensitivity of radiotracers as PET imaging probes of HSV1-TK. Indeed the pentose sugar moiety, in our case a deoxyribose or an arabinose, also plays a critical role in directing the binding potential and subsequent phosphorylation of these nucleosides by HSV1-TK. Despite the significant accumulation of phosphorylated products of [**¹⁸F**]**1a–f** in HSV1-*tk* expressing cells, there was negligible cytotoxicity of these compounds to these same cells. Taken

together, 5-[^{18}F]fluoropropyl, [^{18}F]fluorobutyl, and [^{18}F]fluoropentyl pyrimidine nucleosides [^{18}F]FPrDU, [^{18}F]FBuDU, [^{18}F]FFBuAU, and [^{18}F]FPeDU, exhibit promising *in vitro* sensitivity and selectivity that make them candidates for further *in vivo* evaluations as HSV1-TK PET probes for HSV1-*tk* reporter gene expression.

Experimental Section

I. General Information

All commercially available materials were used without further purification unless otherwise indicated. Radioactive [^{125}I]Iodide was purchased from Perkin Elmer Life and Analytical Sciences, Inc. (Boston, MA) as [^{125}I]NaI (185 MBq (5 mCi) in 0.1 M NaOH, pH 12–14) in no-carrier-added form. [^{18}F]Fluoride was purchased from IBA Molecular (Somerset, NJ) as an [^{18}O]-enriched aqueous solution of [^{18}F]fluoride. Tetrahydrofuran (THF), dichloromethane (DCM), and ether (Et_2O) were dried by solvent tower (PURE SOLVE systems, Innovative Technologies, Inc., Newburyport, MA). All reactions described were carried out in flame-dried glassware under a dry argon atmosphere, unless otherwise indicated. All alkylzinc reagents were prepared following published procedure.^{20,21} 3-*N*-benzoyl-3',5'-di-*O*-benzoyl-5-iodo-2'-deoxyuridine^{43,44} (**2a**) and 3-*N*-benzoyl-3',5'-di-*O*-benzoyl-2'-fluoro-2'-deoxy-5-iodo-1- β -D-arabinouridine⁴⁵ (**2b**) were prepared following modified literature protocols.^{20,25,46} UV Spectra were recorded on a Beckman DU 640 Spectrophotometer. ^1H spectra were recorded at 200 MHz and ^{13}C NMR spectra were recorded at 50 MHz on a Bruker DPX spectrometer. ^1H and ^{13}C NMR spectra for the 2'-deoxyuridine derivatives were referenced to CDCl_3 or CD_3OD , whereas ^1H and ^{13}C NMR data for the 2'-deoxy-2'-fluoroarabinouridine derivatives were referenced to CD_3CN . Chemical shifts (δ) are given in parts per million (ppm) and coupling constants are in hertz (Hz). ^1H NMR splitting patterns are designated as singlet (s), doublet (d), doublet of doublets (dd), doublet of triplets (dt), doublet of doublet of doublets (ddd), triplet (t), quartet (q), pentet (p), multiplet (m) and broad (br). ^{13}C NMR data are recorded as singlet(s) unless otherwise indicated. High-resolution mass spectrometry (HRMS) was performed using an Agilent G3250AA instrument (LC/MSD time-of-flight (TOF)). HPLC was performed on an Agilent 1100 series system. Two systems were used to confirm the purity of the bioactive compounds listed in this section: System A conditions: Phenomenex Gemini C_{18} reverse-phase analytical column (4.6×250 mm, 5 μm), MeOH/ H_2O (70:30), flow rate 1 mL/min, 268 nm; and System B conditions: Phenomenex Silica normal-phase column (4.6×250 mm, 10 μm), MeOH/ CH_2Cl_2 (10:90), flow rate 1 mL/min, 268 nm. All compounds used for further biological evaluation in this paper showed >95% purity in both HPLC systems. Radioactivity was determined by gamma counting using a Cobra II AutoGamma Counter D5003 Spectrometer (Canberra-Packard). ^{125}I counts were measured in the 15–75 keV range and ^{18}F counts were measured in the 400–1600 keV energy range. Solid-phase extraction cartridges, Sep-Pak C_{18} , Sep-Pak QMA Light, and OASIS HLB (6cc) were purchased from Waters (Milford, MA). RG2 cells were obtained from the American Tissue Culture Collection (ATCC) and RG2TK+ cells were a kind gift from Dr. Juri Gelovani. Cell proliferation assays using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, Milwaukee, WI) were analyzed using a Bio-RAD Model 550 microplate reader.

II. Chemistry

General Procedure A for Deprotection of Bz-Moieties—3-*N*-Benzoyl-3',5'-di-*O*-benzoyl-5-(5-fluoroalkyl) pyrimidine nucleosides **3a–f** were prepared according to an optimized Negishi coupling method developed in our laboratory.²⁰ To **3a–f** in anhydrous MeOH (2 mL) was added 0.5 N NaOMe/MeOH solution (0.5 mL) and the reaction mixture heated at 80 °C for 15 min with stirring. After cooling to room temperature, 1 N HCl (0.25 mL) was added and the solvent removed *in vacuo*. The residue was purified *via* preparative silica gel TLC (10% MeOH in DCM).

5-(3-Fluoropropyl)-2'-deoxyuridine (FPrDU) (1a): Compound **1a** was prepared from **3a** (14 mg, 0.023 mmol) as a white solid (6 mg, 97%) following general procedure A: ¹H NMR (CD₃OD) δ 7.87 (s, 1 H), 6.28 (t, 1 H, *J* = 6.7 Hz), 4.45 (dt, 2 H, *J*₁ = 5.9 Hz, *J*₂ = 47.4 Hz), 4.39–4.36 (m, 1 H), 3.92 (q, 1 H, *J* = 3.3 Hz), 3.78 (dd, 1 H, *J*₁ = 12.0 Hz, *J*₂ = 17.2 Hz), 3.76 (dd, 1 H, *J*₁ = 12.0 Hz, *J*₂ = 17.6 Hz), 2.46–2.39 (m, 2 H), 2.28–2.21 (m, 4 H), 2.02–1.79 (m, 2 H); ¹³C NMR (CD₃OD) δ 166.0, 152.4, 138.8, 114.9, 89.1, 86.6, 84.3 (d, *J* = 163.1 Hz), 72.3, 62.9, 41.5, 30.5 (d, *J* = 19.7 Hz), 24.1 (d, *J* = 5.7 Hz); λ_{max} (MeOH) = 266 nm (ε = 8400 M⁻¹cm⁻¹); HRMS calcd for C₁₂H₁₇FN₂O₅Na ([M + Na]⁺) 311.1019, found 311.1028; HRMS calcd for C₁₂H₁₇FN₂O₅K ([M + K]⁺) 327.0759, found 327.0765.

5-(4-Fluorobutyl)-2'-deoxyuridine (FBuDU)²⁰ (1b): Compound **1b** was prepared from **3b** (22 mg, 0.036 mmol) as a white solid (11 mg, >99%) following general procedure A: ¹H NMR (CD₃OD) δ 8.06 (s, 1 H), 6.49 (t, 1 H, *J*₁ = 6.7 Hz), 4.64 (dt, 2 H, *J*₁ = 5.6 Hz, *J*₂ = 47.6 Hz), 4.64–4.57 (m, 1 H), 4.12 (t, 1 H, *J*₁ = 3.1 Hz), 3.98 (dd, 1 H, *J*₁ = 12.1 Hz, *J*₂ = 16.8 Hz), 3.96 (dd, 1 H, *J*₁ = 12.1 Hz, *J*₂ = 17.3 Hz), 2.59–2.37 (m, 4 H), 2.00–1.81 (m, 4 H); ¹³C NMR (CD₃OD) δ 166.1, 152.4, 138.6, 115.6, 89.1, 86.6, 84.8 (d, *J* = 162.8 Hz), 72.4, 62.9, 41.5, 31.2 (d, *J* = 19.6 Hz), 27.5, 25.6 (d, *J* = 5.1 Hz); λ_{max} (MeOH) = 266 nm (ε = 10,400 M⁻¹cm⁻¹); HRMS calcd for C₁₃H₂₀FN₂O₅ ([M + H]⁺) 303.1356, found 303.1349.

5-(5-Fluoropentyl)-2'-deoxyuridine (FPeDU) (1c): Compound **1c** was prepared from **3c** (14 mg, 0.022 mmol) as a white solid (6 mg, 85%) following general procedure A: ¹H NMR (CD₃OD) δ 7.85 (s, 1 H), 6.29 (t, 1 H, *J*₁ = 6.7 Hz), 4.42 (dt, 2 H, *J*₁ = 6.0 Hz, *J*₂ = 47.5 Hz), 4.44–4.37 (m, 1 H), 4.12 (q, 1 H, *J*₁ = 3.2 Hz), 3.77 (dd, 1 H, *J*₁ = 12.1 Hz, *J*₂ = 16.8 Hz), 3.76 (dd, 1 H, *J*₁ = 12.0 Hz, *J*₂ = 17.3 Hz), 2.36–2.20 (m, 4 H), 1.84–1.34 (m, 6 H); ¹³C NMR (CD₃OD) δ 166.2, 152.4, 138.5, 115.8, 89.1, 86.5, 84.9 (d, *J* = 162.4 Hz), 72.4, 63.0, 41.5, 31.4 (d, *J* = 19.6 Hz), 29.3, 27.8, 26.0 (d, *J* = 5.4 Hz); λ_{max} (MeOH) = 267 nm (ε = 10,100 M⁻¹cm⁻¹); HRMS calcd for C₁₄H₂₂FN₂O₅ ([M + H]⁺) 315.1513, found 317.1502.

2'-Fluoro-2'-deoxy-5-(3-fluoropropyl)-1-β-D-arabinofuranosyluracil (FFPrAU) (1d): Compound **1d** was prepared from **3d** (14 mg, 0.023 mmol) as a white solid (7 mg, >99%) following general procedure A: ¹H NMR (CD₃CN) δ 9.08 (bs, 1 H), 7.53 (s, 1 H), 6.14 (dd, 1 H, *J*₁ = 4.2 Hz, *J*₂ = 15.7 Hz), 5.02 (dt, 1 H, *J*₁ = 3.9 Hz, *J*₂ = 52.5 Hz), 4.44 (dt, 2 H, *J*₁ = 5.9 Hz, *J*₂ = 47.4 Hz), 4.40–4.23 (m, 1 H), 4.12 (t, 1 H, *J*₁ = 3.1 Hz), 3.88–3.70 (m, 3 H), 2.41–2.33 (m, 2 H), 1.91–1.71 (m, 2 H); ¹³C NMR (CD₃CN) δ 138.9, 96.8 (d, *J* = 190.5 Hz), 84.4 (d, *J* = 161.1 Hz), 84.6 (d, *J* = 4.8 Hz), 84.1 (d, *J* = 16.7 Hz), 74.4 (d, *J* = 24.6 Hz), 61.5, 30.1 (d, *J* = 19.5 Hz), 23.6 (d, *J* = 5.6 Hz); λ_{max} (MeOH) = 266 nm (ε = 2000).

$\text{M}^{-1}\text{cm}^{-1}$); HRMS calcd for $\text{C}_{12}\text{H}_{16}\text{F}_2\text{N}_2\text{O}_5\text{Na}$ ($[\text{M} + \text{Na}]^+$) 329.0925, found 329.0925; HRMS calcd for $\text{C}_{12}\text{H}_{16}\text{F}_2\text{N}_2\text{O}_5\text{K}$ ($[\text{M} + \text{K}]^+$) 345.0664, found 345.0664.

2'-Fluoro-2'-deoxy-5-(4-fluorobutyl)-1- β -D-arabinofuranosyluracil (FFBuAU)²⁰ (1e):

Compound **1e** was prepared from **3e** (13 mg, 0.021 mmol) as a white solid (6 mg, 95%) following general procedure A: ^1H NMR (CD_3CN with D_2O) δ 7.53 (s, 1 H), 6.13 (dd, 1 H, $J_1 = 4.1$ Hz, $J_2 = 16.4$ Hz), 5.02 (dt, 2 H, $J_1 = 3.9$ Hz, $J_2 = 52.2$ Hz), 4.43 (dt, 2 H, $J_1 = 5.9$ Hz, $J_2 = 47.3$ Hz), 4.24–4.20 (m, 1 H), 3.90–3.70 (m, 3 H), 2.32–2.25 (m, 2 H), 1.78–1.45 (m, 4 H); ^{13}C NMR (CD_3CN) δ 164.2, 151.2, 138.0 (d, $J = 3.0$ Hz), 114.2, 96.8 (d, $J = 190.4$ Hz), 85.0 (d, $J = 160.7$ Hz), 84.5 (d, $J = 4.6$ Hz), 84.0 (d, $J = 16.6$ Hz), 74.4 (d, $J = 24.5$ Hz), 61.5, 30.6 (d, $J = 19.4$ Hz), 27.1, 25.0 (d, $J = 5.4$ Hz); λ_{max} (MeOH) = 265 nm ($\epsilon = 8800$ $\text{M}^{-1}\text{cm}^{-1}$); HRMS calcd for $\text{C}_{13}\text{H}_{19}\text{F}_2\text{N}_2\text{O}_5$ ($[\text{M} + \text{H}]^+$) 321.1262, found 321.1253.

2'-Fluoro-2'-deoxy-5-(5-fluoropentyl)-1- β -D-arabinofuranosyluracil (FFPeAU) (1f):

Compound **1f** was prepared from **3f** (30 mg, 0.046 mmol) as a white solid (15 mg, 99 %) following general procedure A: ^1H NMR (CD_3CN with D_2O) δ 7.91 (s, 1 H), 6.13 (dd, 1 H, $J_1 = 4.1$ Hz, $J_2 = 16.5$ Hz), 5.03 (dt, 2 H, $J_1 = 3.5$ Hz, $J_2 = 52.2$ Hz), 4.41 (dt, 2 H, $J_1 = 6.1$ Hz, $J_2 = 47.4$ Hz), 4.24–4.20 (m, 1 H), 3.91–3.63 (m, 3 H), 2.29–2.22 (m, 2 H), 1.79–1.30 (m, 6 H); ^{13}C NMR (CD_3CN) δ 164.3, 151.2, 137.9 (d, $J = 2.9$ Hz), 114.5, 96.8 (d, $J = 190.4$ Hz), 85.2 (d, $J = 160.6$ Hz), 84.5 (d, $J = 4.7$ Hz), 84.0 (d, $J = 16.7$ Hz), 74.4 (d, $J = 24.5$ Hz), 61.6, 30.9 (d, $J = 19.2$ Hz), 28.9, 27.4, 25.4 (d, $J = 5.7$ Hz); λ_{max} (MeOH) = 265 nm ($\epsilon = 10,200$ $\text{M}^{-1}\text{cm}^{-1}$); HRMS calcd for $\text{C}_{14}\text{H}_{21}\text{F}_2\text{N}_2\text{O}_5$ ($[\text{M} + \text{H}]^+$) 335.1419, found 335.1419.

General Procedure B for the Preparation of 5-O-TBS-Protected Nucleosides via Negishi Cross-Coupling—

In a flame-dried 5-mL two-neck flask equipped with a stir bar was added **2a** (300 mg, 0.45 mmol) or **2b** (308 mg, 0.45 mmol), $\text{Pd}(\text{P}(t\text{-Bu})_3)_2$ (11.5 mg, 0.023 mmol) and anhydrous *N,N*-dimethylacetamide (DMA) (5 mL) under argon. To the reaction mixture, alkylzinc reagent **5a–c**^{20,21} (0.7–0.8 M solution in DMA, 1.35 mmol) was added dropwise and the reaction stirred at room temperature for 30 min. The reaction mixture was quenched with saturated NH_4Cl (10 mL) and extracted with EtOAc (3×5 mL). The combined organic phases were washed with brine, dried over MgSO_4 , filtered and concentrated. The crude product mixture was purified by silica gel chromatography using of 20%–30% EtOAc in hexanes to yield a white solid.

3-N-Benzoyl-3',5'-di-O-benzoyl-5-(3-tert-butyldimethylsilyloxypropyl)-2'-deoxyuridine²⁰ (6a):

Compound **6a** was prepared from **2a** and (3-tert-butyldimethylsilyloxypropyl)zinc bromide **5a** (1.93 mL of 0.7 M solution in DMA) as a white solid in 45% yield according to general procedure B: ^1H NMR (CDCl_3) δ 8.09–7.90 (m, 6 H), 7.69–7.42 (m, 9 H), 7.40 (s, 1 H), 6.43 (dd, 1 H, $J_1 = 5.4$ Hz, $J_2 = 8.7$ Hz), 5.67–5.64 (m, 1 H), 4.77 (dd, 1 H, $J_1 = 12.2$ Hz, $J_2 = 14.4$ Hz), 4.75 (dd, 1 H, $J_1 = 12.2$ Hz, $J_2 = 14.6$ Hz), 4.59–4.55 (m, 1 H), 3.51 (t, 2 H, $J = 6.2$ Hz), 2.77 (ddd, 1 H, $J_1 = 1.4$ Hz, $J_2 = 5.4$ Hz, $J_3 = 14.2$ Hz), 2.40 (ddd, 1 H, $J_1 = 6.6$ Hz, $J_2 = 8.6$ Hz, $J_3 = 14.4$ Hz), 2.30–2.19 (m, 2 H), 1.66–1.52 (m, 2 H), 0.88 (s, 9 H), 0.03 (s, 6 H); ^{13}C NMR (CDCl_3) δ 169.0, 166.2, 162.4, 149.4, 135.2, 134.4, 134.0, 133.9, 131.9, 130.7, 130.0, 129.8, 129.6, 129.4, 129.2, 129.0, 128.8, 115.9, 85.8, 83.1, 75.2, 64.5, 62.6, 38.3, 31.6, 26.2, 24.1, 18.5, –5.1; HRMS

calcd for $C_{39}H_{44}N_2O_9SiNa$ ($[M + Na]^+$) 735.2174, found 735.2688; HRMS calcd for $C_{39}H_{45}N_2O_9Si$ ($[M + H]^+$) 713.2894, found 713.2881.

3-N-Benzoyl-3',5'-di-O-benzoyl-5-(4-tert-butyldimethylsilyloxybutyl)-2'-deoxyuridine

(6b): Compound **6b** was prepared from **2a** and (4-tert-butyldimethylsilyloxybutyl)zinc bromide **5b** (1.69 mL of 0.8 M solution in DMA) as a white solid (122 mg, 38% yield) according to general procedure B: 1H NMR ($CDCl_3$) δ 8.09–7.89 (m, 6 H), 7.69–7.43 (m, 9 H), 7.35 (s, 1 H), 6.45 (dd, 1 H, $J_1 = 5.4$ Hz, $J_2 = 8.6$ Hz), 5.69–5.66 (m, 1 H), 4.77 (dd, 1 H, $J_1 = 12.2$ Hz, $J_2 = 23.0$ Hz), 4.76 (dd, 1 H, $J_1 = 12.2$ Hz, $J_2 = 23.5$ Hz), 4.59–4.56 (m, 1 H), 3.55–3.49 (m, 2 H), 2.77 (ddd, 1 H, $J_1 = 1.4$ Hz, $J_2 = 5.5$ Hz, $J_3 = 14.1$ Hz), 2.40 (ddd, 1 H, $J_1 = 6.6$ Hz, $J_2 = 8.6$ Hz, $J_3 = 14.3$ Hz), 2.13–2.06 (m, 2 H), 1.42–1.40 (m, 4 H), 0.89 (s, 9 H), 0.04 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 168.9, 166.2, 162.4, 149.4, 135.2, 134.4, 134.0, 131.9, 130.6, 130.0, 129.8, 129.4, 129.1, 128.8, 116.2, 85.6, 83.1, 75.2, 64.5, 63.0, 38.3, 32.7, 27.2, 26.2, 25.2, 23.2, –5.0; HRMS calcd for $C_{40}H_{46}N_2O_9SiNa$ ($[M + Na]^+$) 749.2870, found 749.2901; HRMS calcd for $C_{40}H_{47}N_2O_9Si$ ($[M + H]^+$) 727.3051, found 727.3128.

3-N-Benzoyl-3',5'-di-O-benzoyl-5-(5-tert-butyldimethylsilyloxypropyl)-2'-deoxyuridine

(6c): Compound **6c** was prepared from **2a** and (5-tert-butyldimethylsilyloxypropyl)zinc bromide **5c** (1.90 mL of 0.72 M solution in DMA) as a white solid (144 mg, 43% yield) according to general procedure B: 1H NMR ($CDCl_3$) δ 8.10–7.89 (m, 6 H), 7.69–7.42 (m, 9 H), 7.34 (s, 1 H), 6.46 (dd, 1 H, $J_1 = 5.4$ Hz, $J_2 = 8.8$ Hz), 5.70–5.67 (m, 1 H), 4.79 (dd, 1 H, $J_1 = 12.2$ Hz, $J_2 = 28.1$ Hz), 4.76 (dd, 1 H, $J_1 = 12.2$ Hz, $J_2 = 28.7$ Hz), 4.59–4.55 (m, 1 H), 3.55 (t, 2 H, $J = 6.4$ Hz), 2.77 (ddd, 1 H, $J_1 = 1.2$ Hz, $J_2 = 5.5$ Hz, $J_3 = 14.3$ Hz), 2.40 (ddd, 1 H, $J_1 = 6.3$ Hz, $J_2 = 8.5$ Hz, $J_3 = 14.5$ Hz), 2.17–1.96 (m, 2 H), 1.49–1.15 (m, 6 H), 0.90 (s, 9 H), 0.05 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 169.0, 166.2, 162.4, 149.4, 135.2, 134.2, 134.0, 131.9, 130.6, 130.0, 129.8, 129.6, 129.4, 129.2, 129.1, 128.8, 116.4, 85.5, 83.1, 75.2, 64.5, 63.3, 38.3, 32.7, 28.7, 27.4, 26.2, 25.9, 26.2, 18.6, –5.0; HRMS calcd for $C_{41}H_{48}N_2O_9SiNa$ ($[M + Na]^+$) 763.3027, found 763.3014; HRMS calcd for $C_{41}H_{49}N_2O_9Si$ ($[M + H]^+$) 741.3207, found 741.3200.

3-N-Benzoyl-3',5'-di-O-benzoyl-2'-fluoro-5-(3-tert-

butyldimethylsilyloxypropyl)-1- β -D-arabinofuranosyluracil (6d): Compound **6d** was prepared from **2b** and (3-tert-butyldimethylsilyloxypropyl)zinc bromide **5a** (1.93 mL of 0.7 M solution in DMA) as a white solid in 38% yield according to general procedure B: 1H NMR (CD_3CN) δ 8.11–7.93 (m, 6 H), 7.75–7.47 (m, 10 H), 6.30 (dd, 1 H, $J_1 = 3.2$ Hz, $J_2 = 20.7$ Hz), 5.67 (ddd, 1 H, $J_1 = 1.0$ Hz, $J_2 = 3.6$ Hz, $J_3 = 19.2$ Hz), 5.44 (ddd, 1 H, $J_1 = 1.0$ Hz, $J_2 = 3.1$ Hz, $J_3 = 50.3$ Hz), 4.78 (dd, 1 H, $J_1 = 12.1$ Hz and $J_2 = 21.4$ Hz), 4.76 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 22.3$ Hz), 4.61 (q, 1 H, $J = 3.8$ Hz), 3.53 (t, 2 H, $J = 6.2$ Hz), 2.29–2.22 (m, 2 H), 1.63–1.50 (m, 2 H), 0.86 (s, 9 H), 0.01 (s, 6 H); ^{13}C NMR (CD_3CN) δ 170.4, 167.1, 166.4, 163.5, 150.1, 137.9, 137.8, 136.5, 135.0, 134.6, 132.7, 131.4, 130.8, 130.6, 130.5, 130.1, 129.8, 114.9, 94.4 (d, $J = 189.8$ Hz), 85.7 (d, $J = 16.4$ Hz), 81.4, 77.8 (d, $J = 30.3$ Hz), 64.3, 63.1, 32.3, 26.4, 24.3, 19.0, –5.0; HRMS exact mass calculated for $C_{39}H_{43}FN_2O_9SiNa$ ($[M + Na]^+$) 753.2620, found 753.2639; HRMS calcd for $C_{39}H_{44}FN_2O_9Si$ ($[M + H]^+$) 731.2800, found 731.2865.

3-*N*-Benzoyl-3',5'-di-*O*-benzoyl-2'-fluoro-2'-deoxy-5-(4-*tert*-butyldimethylsilyloxybutyl)-1- β -D-arabinofuranosyluracil (6e): Compound **6e** was prepared from **2b** and (4-*tert*-butyldimethylsilyloxybutyl)zinc bromide **5b** (1.69 mL of 0.8 M solution in DMA) as a white solid (95 mg, 29% yield) according to general procedure B: ^1H NMR (CD_3CN) δ 8.12–7.90 (m, 6 H), 7.79–7.48 (m, 10 H), 6.36 (dd, 1 H, $J_1 = 3.2$ Hz, $J_2 = 20.8$ Hz), 5.68 (ddd, 1 H, $J_1 = 1.0$ Hz, $J_2 = 3.8$ Hz, $J_3 = 19.0$ Hz), 5.44 (ddd, 1 H, $J_1 = 1.0$ Hz, $J_2 = 3.3$ Hz, $J_3 = 50.4$ Hz), 4.78 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 25.7$ Hz), 4.76 (dd, 1 H, $J_1 = 12.5$ Hz, $J_2 = 27.0$ Hz), 4.64–4.59 (m, 1 H), 3.54 (t, 2 H, $J = 6.0$ Hz), 2.18–2.05 (m, 2 H), 1.42–1.32 (m, 4 H), 0.86 (s, 9 H), 0.01 (s, 6 H); ^{13}C NMR (CD_3CN) δ 170.4, 167.1, 166.4, 163.5, 150.1, 138.0, 137.9, 136.5, 135.0, 134.6, 133.8, 132.6, 131.3, 130.8, 130.5, 129.85, 129.82, 129.8, 115.1, 94.5 (d, $J = 189.7$ Hz), 85.6 (d, $J = 16.5$ Hz), 81.4, 77.8 (d, $J = 30.2$ Hz), 64.3, 63.6, 33.1, 27.4, 26.4, 25.8, 19.0, -5.0; HRMS calcd for $\text{C}_{40}\text{H}_{45}\text{FN}_2\text{O}_9\text{SiNa}$ ($[\text{M} + \text{Na}]^+$) 767.2776, found 767.2796; HRMS calcd for $\text{C}_{40}\text{H}_{46}\text{FN}_2\text{O}_9\text{Si}$ ($[\text{M} + \text{H}]^+$) 745.2957, found 745.3015.

3-*N*-Benzoyl-3',5'-di-*O*-benzoyl-2'-fluoro-2'-deoxy-5-(5-*tert*-butyldimethylsilyloxypropyl)-1- β -D-arabinofuranosyluracil (6f): Compound **6f** was prepared from **2b** and (5-*tert*-butyldimethylsilyloxypropyl)zinc bromide **5c** (1.90 mL of 0.72 M solution in DMA) as a white solid (131 mg, 38% yield) according to general procedure B: ^1H NMR (CD_3CN) δ 8.11–7.92 (m, 6 H), 7.77–7.48 (m, 10 H), 6.30 (dd, 1 H, $J_1 = 3.2$ Hz, $J_2 = 20.7$ Hz), 5.68 (dd, 1 H, $J_1 = 3.5$ Hz, $J_2 = 18.9$ Hz), 5.43 (dd, 1 H, $J_1 = 3.2$ Hz, $J_2 = 50.3$ Hz), 4.77 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 28.1$ Hz), 4.75 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 29.5$ Hz), 4.63–4.57 (m, 1 H), 3.54 (t, 2 H, $J = 6.1$ Hz), 2.21–2.11 (m, 2 H), 1.46–1.18 (m, 6 H), 0.87 (s, 9 H), 0.02 (s, 6 H); ^{13}C NMR (CD_3CN) δ 170.4, 167.1, 166.4, 163.5, 150.1, 137.9, 137.86, 136.5, 135.0, 134.6, 132.6, 131.1, 130.8, 130.58, 130.55, 130.53, 129.84, 129.81, 129.80, 115.2, 94.4 (d, $J = 189.7$ Hz), 85.6 (d, $J = 16.4$ Hz), 81.4, 77.8 (d, $J = 30.3$ Hz), 64.2, 63.8, 33.4, 29.2, 27.6, 26.4, 26.1, 19.0, -5.0; HRMS calcd for $\text{C}_{41}\text{H}_{47}\text{FN}_2\text{O}_9\text{SiNa}$ ($[\text{M} + \text{Na}]^+$) 781.2933, found 781.2977; HRMS calcd for $\text{C}_{41}\text{H}_{48}\text{FN}_2\text{O}_9\text{Si}$ ($[\text{M} + \text{H}]^+$) 759.3113, found 759.3170.

General Procedure C for Deprotection of *O*-TBS-Moiety—A solution of 1% concentrated HCl in EtOH (5 mL) was added to the *O*-TBS-protected nucleosides **6a–f** and stirred for 10 min at room temperature.⁴⁷ To the reaction mixture was added 0.25 M NaOH in EtOH (5 mL). The solvent was removed *in vacuo* and the residue purified by silica gel chromatography (10% MeOH in DCM) to afford a white solid.

3-*N*-Benzoyl-3',5'-di-*O*-benzoyl-5-(3-hydroxypropyl)-2'-deoxyuridine (7a): Compound **7a** was prepared from **6a** (144 mg, 0.2 mmol) as a white solid (121 mg, >99%) following general procedure C: ^1H NMR (CDCl_3) δ 8.10–7.89 (m, 6 H), 7.69–7.44 (m, 10 H), 6.45 (dd, 1 H, $J_1 = 5.4$ Hz, $J_2 = 8.7$ Hz), 5.69–5.66 (m, 1 H), 4.79 (dd, 1 H, $J_1 = 12.2$ Hz, $J_2 = 25.0$ Hz), 4.77 (dd, 1 H, $J_1 = 12.2$ Hz, $J_2 = 26.0$ Hz), 4.60–4.57 (m, 1 H), 3.49 (t, 2 H, $J = 5.9$ Hz), 2.77 (dd, 1 H, $J_1 = 5.1$ Hz, $J_2 = 13.9$ Hz), 2.39 (ddd, 1 H, $J_1 = 6.6$ Hz, $J_2 = 8.5$ Hz, $J_3 = 14.5$ Hz), 2.30–2.13 (m, 2 H), 1.64–1.51 (m, 2 H); ^{13}C NMR (CDCl_3) δ 168.8, 166.4, 166.3, 163.1, 149.4, 135.3, 133.9, 131.6, 130.6, 129.91, 129.90, 129.7, 129.5, 129.4, 129.1, 129.0, 128.8, 128.7, 115.4, 85.7, 83.2, 75.1, 64.5, 61.2, 38.4, 32.0, 23.2; HRMS calcd for

$C_{33}H_{30}N_2O_9Na$ ($[M + Na]^+$) 621.1849, found 621.1878; HRMS calcd for $C_{33}H_{31}N_2O_9$ ($[M + H]^+$) 599.2030, found 599.2036.

3-*N*-Benzoyl-3',5'-di-*O*-benzoyl-5-(4-hydroxybutyl)-2'-deoxyuridine (7b): Compound **7b** was prepared from **6b** (119 mg, 0.16 mmol) as a white solid (100 mg, >99%) following general procedure C: 1H NMR ($CDCl_3$) δ 8.10–7.83 (m, 6 H), 7.69–7.43 (m, 9 H), 7.39 (s, 1 H), 6.46 (dd, 1 H, $J_1 = 5.4$ Hz, $J_2 = 8.7$ Hz), 5.69–5.66 (m, 1 H), 4.78 (dd, 1 H, $J_1 = 12.2$ Hz, $J_2 = 26.5$ Hz), 4.77 (dd, 1 H, $J_1 = 12.2$ Hz, $J_2 = 27.4$ Hz), 4.60–4.57 (m, 1 H), 3.61–3.55 (m, 2 H), 2.52 (ddd, 1 H, $J_1 = 1.2$ Hz, $J_2 = 5.5$ Hz, $J_3 = 14.1$ Hz), 2.47–2.33 (m, 1 H), 2.16–2.10 (m, 2 H), 1.48–1.45 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 168.9, 166.3, 166.2, 162.5, 149.4, 135.3, 134.5, 134.0, 132.3, 131.8, 130.6, 130.0, 129.8, 129.6, 129.4, 129.2, 129.1, 129.8, 115.9, 85.6, 83.1, 75.1, 64.6, 62.6, 38.3, 32.1, 26.8, 24.9; HRMS calcd for $C_{34}H_{32}N_2O_9Na$ ($[M + Na]^+$) 635.2006, found 635.2038; HRMS calcd for $C_{34}H_{33}N_2O_9$ ($[M + H]^+$) 613.2186, found 613.2211.

3-*N*-Benzoyl-3',5'-di-*O*-benzoyl-5-(5-hydroxypentyl)-2'-deoxyuridine (7c): Compound **7c** was prepared from **6c** (139 mg, 0.19 mmol) as a white solid (115 mg, 98%) following general procedure C: 1H NMR ($CDCl_3$) δ 8.10–7.90 (m, 6 H), 7.70–7.43 (m, 9 H), 7.35 (s, 1 H), 6.47 (dd, 1 H, $J_1 = 5.5$ Hz, $J_2 = 8.8$ Hz), 5.70–5.67 (m, 1 H), 4.78 (dd, 1 H, $J_1 = 12.2$ Hz, $J_2 = 31.5$ Hz), 4.76 (dd, 1 H, $J_1 = 12.2$ Hz, $J_2 = 32.3$ Hz), 4.59–4.55 (m, 1 H), 3.60 (t, 2 H, $J = 6.3$ Hz), 2.78 (ddd, 1 H, $J_1 = 1.4$ Hz, $J_2 = 5.5$ Hz, $J_3 = 14.2$ Hz), 2.49–2.34 (m, 1 H), 2.23–1.97 (m, 2 H), 1.55–1.16 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 169.0, 166.2, 162.4, 149.4, 135.2, 134.4, 134.0, 131.9, 130.6, 130.0, 129.9, 129.5, 129.4, 129.2, 129.1, 128.8, 116.1, 85.5, 83.0, 75.2, 64.6, 62.9, 38.3, 32.4, 28.4, 27.1, 25.5; HRMS calcd for $C_{35}H_{34}N_2O_9Na$ ($[M + Na]^+$) 649.2162, found 649.2167; HRMS calcd for $C_{35}H_{35}N_2O_9$ ($[M + H]^+$) 627.2343, found 627.2383.

3-*N*-Benzoyl-3',5'-di-*O*-benzoyl-2'-fluoro-2'-deoxy-5-(3-hydroxypropyl)-1- β -D-arabinofuranosyluracil (7d): Compound **7d** was prepared from **6d** (117 mg, 0.16 mmol) as a white solid (89 mg, 90%) following general procedure C: 1H NMR (CD_3CN) δ 8.12–7.94 (m, 6 H), 7.79–7.48 (m, 10 H), 6.30 (dd, 1 H, $J_1 = 3.2$ Hz, $J_2 = 20.7$ Hz), 5.68 (ddd, 1 H, $J_1 = 0.8$ Hz, $J_2 = 3.6$ Hz, $J_3 = 19.0$ Hz), 5.44 (ddd, 1 H, $J_1 = 0.8$ Hz, $J_2 = 3.1$ Hz, $J_3 = 50.4$ Hz), 4.79 (dd, 1 H, $J_1 = 12.2$ Hz, $J_2 = 23.9$ Hz), 4.77 (dd, 1 H, $J_1 = 12.2$ Hz, $J_2 = 25.2$ Hz), 4.64–4.58 (m, 1 H), 3.46–3.37 (m, 2 H), 2.54 (t, 1 H, $J = 5.4$ Hz), 2.29–2.22 (m, 2 H), 1.62–1.48 (m, 2 H); ^{13}C NMR (CD_3CN) δ 170.4, 167.2, 166.4, 163.7, 150.1, 138.2, 138.1, 136.5, 135.0, 134.6, 132.6, 131.4, 130.8, 130.6, 130.5, 130.1, 129.84, 129.82, 114.9, 94.9 (d, $J = 189.8$ Hz), 85.6 (d, $J = 16.4$ Hz), 81.4, 77.6 (d, $J = 30.2$ Hz), 64.3, 61.7, 32.4, 24.1; HRMS calcd for $C_{33}H_{29}FN_2O_9Na$ ($[M + Na]^+$) 639.1755, found 639.1757; HRMS calcd for $C_{33}H_{30}FN_2O_9$ ($[M + H]^+$) 617.1935, found 617.1944.

3-*N*-Benzoyl-3',5'-di-*O*-benzoyl-2'-fluoro-2'-deoxy-5-(4-hydroxybutyl)-1- β -D-arabinofuranosyluracil (7e): Compound **7e** was prepared from **6e** (183 mg, 0.25 mmol) as a white solid (137 mg, 87%) following general procedure C: 1H NMR (CD_3CN) δ 8.13–7.91 (m, 6 H), 7.79–7.48 (m, 10 H), 6.31 (dd, 1 H, $J_1 = 3.2$ Hz, $J_2 = 20.8$ Hz), 5.69 (ddd, 1 H, $J_1 = 0.9$ Hz, $J_2 = 3.6$ Hz, $J_3 = 19.0$ Hz), 5.44 (ddd, 1 H, $J_1 = 1.0$ Hz, $J_2 = 3.2$ Hz, $J_3 = 50.2$ Hz),

4.79 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 27.7$ Hz), 4.77 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 29.0$ Hz), 4.64–4.58 (m, 1 H), 3.46–3.34 (m, 2 H), 2.43 (t, 1 H, $J = 5.4$ Hz), 2.25–2.15 (m, 2 H), 1.43–1.32 (m, 4 H); ^{13}C NMR (CD_3CN) δ 170.5, 167.1, 166.4, 163.5, 150.1, 138.1, 138.0, 136.5, 135.0, 134.6, 133.9, 132.6, 131.4, 130.8, 130.5, 129.9, 129.8, 115.1, 94.5 (d, $J = 189.8$ Hz), 85.6 (d, $J = 16.3$ Hz), 81.4, 77.9 (d, $J = 30.2$ Hz), 64.2, 62.3, 33.0, 27.4, 25.8; HRMS calcd for $\text{C}_{34}\text{H}_{31}\text{FN}_2\text{O}_9\text{Na}$ ($[\text{M} + \text{Na}]^+$) 653.1911, found 653.1924; HRMS calcd for $\text{C}_{34}\text{H}_{32}\text{FN}_2\text{O}_9$ ($[\text{M} + \text{H}]^+$) 631.2092, found 631.2122.

3-*N*-Benzoyl-3',5'-di-*O*-benzoyl-2'-fluoro-2'-deoxy-5-(5-hydroxypentyl)-1- β -D-

arabinofuranosyluracil (7f): Compound **7f** was prepared from **6f** (86 mg, 0.11 mmol) as a white solid (71 mg, >99%) following general procedure C: ^1H NMR (CD_3CN) δ 8.13–7.93 (m, 6 H), 7.79–7.48 (m, 10 H), 6.31 (dd, 1 H, $J_1 = 3.2$ Hz, $J_2 = 20.8$ Hz), 5.69 (ddd, 1 H, $J_1 = 1.0$ Hz, $J_2 = 3.4$ Hz, $J_3 = 19.1$ Hz), 5.44 (ddd, 1 H, $J_1 = 1.0$ Hz, $J_2 = 3.4$ Hz, $J_3 = 50.4$ Hz), 4.79 (dd, 1 H, $J_1 = 12.0$ Hz, $J_2 = 30.8$ Hz), 4.77 (dd, 1 H, $J_1 = 12.0$ Hz, $J_2 = 32.3$ Hz), 4.64–4.59 (m, 1 H), 3.47–3.38 (m, 2 H), 2.41 (t, 1 H, $J = 5.4$ Hz), 2.22–2.15 (m, 2 H), 1.46–1.14 (m, 6 H); ^{13}C NMR (CD_3CN) δ 170.5, 168.2, 166.4, 163.5, 150.1, 138.0, 137.8, 136.5, 135.0, 134.6, 132.7, 131.6, 130.8, 130.5, 129.9, 129.8, 115.2, 94.5 (d, $J = 190.1$ Hz), 85.6 (d, $J = 16.7$ Hz), 81.4, 77.8 (d, $J = 30.2$ Hz), 64.2, 62.6, 33.3, 29.2, 27.6, 26.2; HRMS calcd for $\text{C}_{35}\text{H}_{33}\text{FN}_2\text{O}_9\text{Na}$ ($[\text{M} + \text{Na}]^+$) 667.2068, found 667.2072; HRMS calcd for $\text{C}_{35}\text{H}_{34}\text{FN}_2\text{O}_9$ ($[\text{M} + \text{H}]^+$) 645.2248, found 645.2255.

General Procedure D for Mesylation of Alcohols. 3-*N*-Benzoyl-3',5'-di-*O*-benzoyl-5-(3-methanesulfonyloxypropyl)-2'-deoxyuridine (8a).²⁰

—To a stirred solution of **7a** (36 mg, 0.06 mmol) and Et_3N (9 μL , 0.07 mmol) in DCM (5 mL) was added methanesulfonyl chloride (5 μL , 0.7 mmol) at 0 °C under argon. The temperature was maintained at 0 °C for 10 min, the ice bath removed, and stirring was continued at room temperature for 2.5 h. The reaction mixture was diluted with saturated NH_4Cl and Et_2O , and the organics washed with NH_4Cl and brine. The organic phase was dried with Na_2SO_4 , and concentrated *in vacuo*. Silica gel chromatography (10% MeOH in DCM) afforded **8a** as a white solid (33 mg, 81%): ^1H NMR (200 MHz, CDCl_3) δ 8.11–7.91 (m, 6 H), 7.67–7.43 (m, 10 H), 6.46 (dd, 1 H, $J_1 = 5.5$ Hz, $J_2 = 8.7$ Hz), 5.68–5.65 (m, 1 H), 4.79 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 17.1$ Hz), 4.78 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 17.9$ Hz), 4.60–4.57 (m, 1 H), 4.14 (t, 2 H, $J = 5.8$ Hz), 3.00 (s, 3 H), 2.76 (ddd, 1 H, $J_1 = 1.4$ Hz, $J_2 = 5.4$ Hz, $J_3 = 14.4$ Hz), 2.47 (ddd, 1 H, $J_1 = 6.7$ Hz, $J_2 = 8.6$ Hz, $J_3 = 14.3$ Hz), 2.31 (t, 2 H, $J = 7.2$ Hz), 1.94–1.81 (m, 2 H); ^{13}C NMR (50 MHz, CDCl_3) δ 168.8, 166.3, 166.2, 162.4, 149.4, 136.1, 135.3, 133.9, 131.7, 130.7, 130.0, 129.8, 129.7, 129.4, 129.2, 129.1, 128.8, 113.7, 85.7, 83.2, 75.1, 68.8, 64.4, 38.1, 37.7, 27.5, 23.9; HRMS calcd for $\text{C}_{34}\text{H}_{32}\text{N}_2\text{O}_{11}\text{SNa}$ ($[\text{M} + \text{Na}]^+$) 699.1625, found 699.1604.

3-*N*-Benzoyl-3',5'-di-*O*-benzoyl-5-(4-methanesulfonyloxybutyl)-2'-deoxyuridine (8b):

Compound **8b** was prepared from alcohol **7b** (91 mg, 0.15 mmol) as a white solid (85 mg, 83%) following general procedure D: ^1H NMR (CDCl_3) δ 8.10–7.89 (m, 6 H), 7.71–7.40 (m, 10 H), 6.46 (dd, 1 H, $J_1 = 5.4$ Hz, $J_2 = 8.7$ Hz), 5.70–5.67 (m, 1 H), 4.79 (dd, 1 H, $J_1 = 12.2$ Hz, $J_2 = 31.5$ Hz), 4.77 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 32.4$ Hz), 4.60–4.55 (m, 1 H), 4.15 (t, 2 H, $J = 6.2$ Hz), 3.00 (s, 3 H), 2.78 (ddd, 1 H, $J_1 = 1.2$ Hz, $J_2 = 5.5$ Hz, $J_3 = 14.2$ Hz),

2.49–2.34 (m, 1 H), 2.16–2.09 (m, 2 H), 1.65–1.44 (m, 4 H); ^{13}C NMR (CDCl_3) δ 168.9, 166.2, 162.4, 149.4, 135.3, 135.0, 134.1, 134.0, 131.8, 130.6, 130.0, 129.8, 129.6, 129.4, 129.2, 129.1, 129.8, 115.3, 85.7, 83.2, 75.2, 69.6, 64.5, 38.3, 37.6, 28.8, 26.7, 24.8; HRMS calcd for $\text{C}_{35}\text{H}_{34}\text{N}_2\text{O}_{11}\text{SNa}$ ($[\text{M} + \text{Na}]^+$) 713.1781, found 713.1772; HRMS calcd for $\text{C}_{35}\text{H}_{35}\text{N}_2\text{O}_{11}\text{S}$ ($[\text{M} + \text{H}]^+$) 691.1962, found 691.1952.

3-N-Benzoyl-3',5'-di-O-benzoyl-5-(5-methanesulfonyloxypentyl)-2'-deoxyuridine (8c):

Compound **8c** was prepared from alcohol **7c** (89 mg, 0.14 mmol) as a white solid (91 mg, 91%) following general procedure D: ^1H NMR (CDCl_3) δ 8.10–7.90 (m, 6 H), 7.70–7.43 (m, 9 H), 7.37 (s, 1 H), 6.47 (dd, 1 H, $J_1 = 5.5$ Hz, $J_2 = 8.5$ Hz), 5.70–5.67 (m, 1 H), 4.78 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 33.2$ Hz), 4.77 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 34.3$ Hz), 4.62–4.52 (m, 1 H), 4.17 (t, 2 H, $J = 6.3$ Hz), 3.00 (s, 3 H), 2.78 (dd, 1 H, $J_1 = 5.0$ Hz, $J_2 = 14.3$ Hz), 2.49–2.34 (m, 1 H), 2.16–2.05 (m, 2 H), 1.69–1.59 (m, 2 H), 1.32–1.27 (m, 4 H); ^{13}C NMR (CDCl_3) δ 168.9, 166.2, 162.4, 149.4, 135.3, 134.6, 134.1, 134.0, 131.8, 130.6, 130.0, 129.8, 129.4, 129.1, 128.8, 115.7, 85.6, 83.1, 75.2, 70.1, 64.6, 38.3, 37.6, 28.8, 28.1, 27.1, 25.2; HRMS calcd for $\text{C}_{36}\text{H}_{36}\text{N}_2\text{O}_{11}\text{SNa}$ ($[\text{M} + \text{Na}]^+$) 727.1938, found 727.1921; HRMS calcd for $\text{C}_{36}\text{H}_{37}\text{N}_2\text{O}_{11}\text{S}$ ($[\text{M} + \text{H}]^+$) 705.2118, found 705.2107.

3-N-Benzoyl-3',5'-di-O-benzoyl-2'-fluoro-2'-deoxy-5-(3-methanesulfonyloxy-propyl)-1- β -D-arabinofuranosyl uracil (8d):

Compound **8d** was prepared from alcohol **7d** (42 mg, 0.067 mmol) as a white solid (46 mg, 99%) following general procedure D: ^1H NMR (CD_3CN) δ 8.13–7.95 (m, 6 H), 7.79–7.47 (m, 10 H), 6.31 (dd, 1 H, $J_1 = 3.2$ Hz, $J_2 = 20.6$ Hz), 5.69 (ddd, 1 H, $J_1 = 1.0$ Hz, $J_2 = 3.8$ Hz, $J_3 = 18.8$ Hz), 5.45 (ddd, 1 H, $J_1 = 1.0$ Hz, $J_2 = 3.3$ Hz and $J_3 = 44.0$ Hz), 4.79 (dd, 1 H, $J_1 = 12.1$ Hz and $J_2 = 22.9$ Hz), 4.77 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 24.1$ Hz), 4.65–4.59 (m, 1 H), 4.13 (t, 2 H, $J = 6.2$ Hz), 2.97 (s, 3 H), 2.36–2.27 (m, 2 H), 1.89–1.75 (m, 2 H); ^{13}C NMR (CD_3CN) δ 170.3, 167.1, 166.3, 163.4, 150.1, 138.65, 138.57, 136.6, 134.9, 134.6, 132.6, 131.4, 130.9, 130.8, 130.7, 130.6, 130.5, 130.1, 129.9, 129.8, 113.7, 94.4 (d, $J = 189.8$ Hz), 85.7 (d, $J = 16.4$ Hz), 81.5 (d, $J = 1.3$ Hz), 77.8 (d, $J = 30.2$ Hz), 70.9, 64.3, 37.5, 28.7, 24.1; HRMS calcd for $\text{C}_{34}\text{H}_{31}\text{FN}_2\text{O}_{11}\text{SNa}$ ($[\text{M} + \text{Na}]^+$) 717.1530, found 717.1527; HRMS calcd for $\text{C}_{34}\text{H}_{32}\text{FN}_2\text{O}_{11}\text{S}$ ($[\text{M} + \text{H}]^+$) 695.1711, found 695.1709.

3-N-Benzoyl-3',5'-di-O-benzoyl-2'-fluoro-2'-deoxy-5-(4-methanesulfonyloxybutyl)-1- β -D-arabinofuranosyl uracil (8e):

Compound **8e** was prepared from alcohol **7e** (134 mg, 0.21 mmol) as a white solid (127 mg, 84%) following general procedure D: ^1H NMR (CD_3CN) δ 8.12–7.93 (m, 6 H), 7.79–7.48 (m, 10 H), 6.31 (dd, 1 H, $J_1 = 3.2$ Hz, $J_2 = 20.7$ Hz), 5.69 (dd, 1 H, $J_1 = 3.1$ Hz, $J_2 = 19.0$ Hz), 5.44 (ddd, 1 H, $J_1 = 1.0$ Hz, $J_2 = 3.2$ Hz, $J_3 = 50.3$ Hz), 4.79 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 29.7$ Hz), 4.77 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 31.0$ Hz), 4.64–4.58 (m, 1 H), 4.12 (t, 2 H, $J = 5.4$ Hz), 2.97 (s, 3 H), 2.32–2.15 (m, 2 H), 1.65–1.38 (m, 4 H); ^{13}C NMR (CD_3CN) δ 170.4, 167.1, 166.4, 163.5, 150.1, 136.1, 135.3, 133.9, 132.6, 131.7, 130.7, 130.0, 129.8, 129.7, 129.4, 129.2, 129.1, 128.8, 114.6, 94.5 (d, $J = 189.9$ Hz), 85.6 (d, $J = 16.4$ Hz), 81.5, 77.8 (d, $J = 30.3$ Hz), 71.5, 64.3, 37.5, 29.2, 27.1, 25.4; HRMS calcd for $\text{C}_{35}\text{H}_{33}\text{FN}_2\text{O}_{11}\text{SNa}$ ($[\text{M} + \text{Na}]^+$) 731.1687, found 731.1681; HRMS calcd for $\text{C}_{35}\text{H}_{34}\text{FN}_2\text{O}_{11}\text{S}$ ($[\text{M} + \text{H}]^+$) 709.1817, found 709.1897.

3-*N*-Benzoyl-3',5'-di-*O*-benzoyl-2'-fluoro-2'-deoxy-5-(5-methanesulfonyloxy-pentyl)-1- β -D-arabinofuranosyl uracil (8f): Compound **8f** was prepared from alcohol **7f** (110 mg, 0.18 mmol) as a white solid (111 mg, 89%) following general procedure D: ^1H NMR (CD_3CN) δ 8.18–7.93 (m, 6 H), 7.79–7.48 (m, 10 H), 6.30 (dd, 1 H, $J_1 = 3.3$ Hz, $J_2 = 20.7$ Hz), 5.70 (ddd, 1 H, $J_1 = 0.8$ Hz, $J_2 = 3.5$ Hz, $J_3 = 19.1$ Hz), 5.44 (ddd, 1 H, $J_1 = 0.9$ Hz, $J_2 = 3.3$ Hz, $J_3 = 50.2$ Hz), 4.79 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 32.4$ Hz), 4.77 (dd, 1 H, $J_1 = 12.1$ Hz, $J_2 = 33.8$ Hz), 4.64–4.59 (m, 1 H), 4.13 (t, 2 H, $J = 6.4$ Hz), 2.98 (s, 3 H), 2.22–2.12 (m, 2 H), 1.69–1.55 (m, 2 H), 1.47–1.23 (m, 4 H); ^{13}C NMR (CD_3CN) δ 170.4, 167.1, 166.4, 163.5, 150.1, 138.1, 138.0, 136.5, 135.0, 134.7, 132.6, 131.3, 130.8, 130.5, 130.1, 129.9, 129.8, 114.9, 94.5 (d, $J = 189.8$ Hz), 85.6 (d, $J = 16.3$ Hz), 81.4, 77.8 (d, $J = 30.3$ Hz), 71.7, 64.2, 37.4, 29.5, 28.7, 27.3, 25.6; HRMS calcd for $\text{C}_{36}\text{H}_{35}\text{FN}_2\text{O}_{11}\text{SNa}$ ($[\text{M} + \text{Na}]^+$) 745.1843, found 745.1833; HRMS calcd for $\text{C}_{36}\text{H}_{36}\text{FN}_2\text{O}_{11}\text{S}$ ($[\text{M} + \text{H}]^+$) 723.2024 found 723.2018.

III. Radiochemistry. General Procedure for the Preparation of 2'-fluoro-2'-deoxy-5- $[\text{I}^{125}]$ iido-1- β -D-arabinofuranosyluracil ($[\text{I}^{125}]$ FIAU)

$[\text{I}^{125}]$ FIAU was prepared by the iododestannylation method as reported previously.⁴⁸ Briefly, to a solution of 5-tri-*n*-butylstannyl derivative of FIAU (100 μg , 0.19 μmol) in MeOH (50 μL) was added $[\text{I}^{125}]\text{NaI}$ (18.5–37 MBq (0.5–1 mCi) in 0.1 M NaOH solution, pH 12–14) followed by the addition of 30% H_2O_2 /acetic acid (1:3, v/v) (25 μL). The reaction was vortexed and left at room temperature for 30 min. Saturated sodium bisulfite solution (0.1 mL) was added to quench the reaction, followed by saturated sodium bicarbonate (0.2 mL). The reaction mixture was loaded onto a Sep-Pak C_{18} cartridge (pre-conditioned with 5 mL EtOH followed by 10 mL H_2O). The C_{18} cartridge was eluted with water (3×10 mL), followed by MeOH (5 mL) to isolate $[\text{I}^{125}]$ FIAU. The methanol was evaporated under a stream of N_2 and the $[\text{I}^{125}]$ FIAU was reformulated in MeOH/ H_2O (2:8) (100 μL), and purified by HPLC [Phenomenex Gemini C18 analytical column (4.6 mm $\times$ 250 mm, 5 μm), MeOH/ H_2O (2:8), flow rate 1 mL/min, $t_R = 8.2$ min]. The radiolabeled product was isolated in 74–83% radiochemical yield (RCY) (radiochemical purities (RCP) > 99%). $[\text{I}^{125}]$ FIAU was prepared under a no-carrier added condition, thus any ^{129}I products formed are below the detection limit of the UV spectrophotometer. As such, we assume that the specific activity (SA) of $[\text{I}^{125}]$ FIAU are close to the theoretical maximum value of 81.4×10^4 GBq/mmol (2,200 Ci/mmol) for ^{125}I .

General Procedure for the $[\text{F}^{18}]$ -Radiolabeling of 5-*O*-Mesylate Precursors **8a–f—** $[\text{F}^{18}]$ fluoride was passed through a Sep-Pak Light QMA cartridge (pre-conditioned with 10 mL NaHCO_3 followed by 10 mL H_2O) and the cartridge dried by airflow. The ^{18}F activity was eluted with 1.2 mL of a Kryptofix 222 ($\text{K}[2,2,2]$)/ K_2CO_3 solution (22 mg $\text{K}[2,2,2]$ and 4.6 mg K_2CO_3 in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (1.77/0.23)). The solvent was removed at 120 $^\circ\text{C}$ under an N_2 stream. The residue was azeotropically dried with 1 mL of anhydrous CH_3CN twice at 120 $^\circ\text{C}$ under an N_2 stream. The ^{18}F activity was redissolved in anhydrous DMF (0.2 mL) and transferred to a borosilicate glass vial and the solution pre-heated for 30 sec using an oil bath set at 135 $^\circ\text{C}$. A solution of the 5-*O*-mesylate precursor **8a–f** (1–2 mg) in anhydrous DMF (0.15 mL) was added to the reaction vessel containing the ^{18}F activity. The reaction was heated at 135 $^\circ\text{C}$ for 5 min. To the cooled reaction mixture was added

anhydrous MeOH (0.1 mL) followed by anhydrous 0.5 N NaOMe/MeOH (0.05 mL), and the mixture heated at 135 °C for 5 min. The reaction was neutralized with 10% HCl (0.1 mL). Water (10 mL) was added, and the solution was passed through an Oasis HLB (6 mL) cartridge (pre-conditioned with 10 mL EtOH, followed by 10 mL H₂O). The cartridge was washed with water (2 × 5 mL), and the radiolabeled compound eluted with MeOH (5 mL). The solvent was removed at 120 °C under an N₂ stream and the residue reconstituted in MeOH/H₂O (3:7). The compound was purified by HPLC [Phenomenex Gemini C18 semi-preparative column (10 mm × 250 mm, 5 μm), 268 nm, MeOH/H₂O (3:7) (for compounds [¹⁸F]1a–b, d–e) or MeOH/H₂O (4:6) (for compounds [¹⁸F]1c and 1f), flow rate 2–4 mL/min, *t_R* = 10.5–23.3 min]. Isolated RCY were 17.3–35.2%. To determine RCP and SA of the HPLC-purified materials, analytical HPLC was employed [Phenomenex Gemini C18 analytical column (4.6 mm × 250 mm, 5 μm), 268 nm, MeOH/H₂O (3:7) (for compounds [¹⁸F]1a–b, d–e) or MeOH/H₂O (4:6) (for compounds [¹⁸F] 1c and 1f), flow rate 1 mL/min, *t_R* = 6.0–21.6 min]. SA was estimated by comparing the UV peak intensity of the purified [¹⁸F]labeled compound with calibration curve for the nonradioactive standard of known concentration.

IV. 1-Octanol/Water Partition Coefficient

Partition coefficients were determined for [¹²⁵I]FIAU or 5-[¹⁸F]fluoroalkyl nucleosides [¹⁸F]1a–f by measuring the distribution of radiolabeled compound in 1-octanol and buffer (0.1 M NaH₂PO₄, pH 7.4). A 20 μL sample of radiolabeled nucleoside (in water), was added to a glass vial containing 3 g each of 1-octanol and buffer. The vial was vortexed for 3 min at room temperature, followed by centrifugation for 5 min to allow for complete separation of the two layers. Two weighed samples (0.5 g each) from the 1-octanol and buffer layers were then counted on a gamma counter. Samples of the 1-octanol layer were repartitioned until consistent partition coefficient values were obtained. The measurements were done in triplicate and repeated three times. The partition coefficient was determined by calculating the ratio of counts (cpm) per unit weight of the 1-octanol layer to that of the buffer layer. Log *P* values were calculated using the formula:

$$\log P = \log \left(\frac{\text{cpm/g of 1-octanol}}{\text{cpm/g of buffer}} \right)$$

V. Biology. Cell Culture

Rat glioma cells (RG2) expressing the HSV1-*tk* gene (RG2TK+) were used for these studies as previously reported.^{37,38,48} Non-transduced (wild-type) RG2 cell were used as controls. Cells were cultivated in Dulbecco's modified Eagle medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 1% penicillin/streptomycin. RG2TK+ cells were additionally given 250 mg/L G418 for HSV1-*tk*+ cell selection. Cells were incubated at 37 °C with 5% CO₂, and subcultured by trypsinization using 0.25% Trypsin-EDTA.

***In Vitro* Cell Uptake Studies of Radiolabeled Nucleosides**—RG2TK+ and RG2 cells were seeded at 5 × 10⁵ cells/well in 12-well tissue culture plates. After 24 h at 37 °C, monolayers had grown. Cells were washed with warmed phosphate buffered saline (PBS)

solution (2×1 mL) and incubated with 0.5 mL serum-free media containing approximately 0.02 MBq (0.5 μ Ci) of [125 I]FIAU (SA 81.4×10^4 GBq/mmol, 2,200 Ci/mmol) or approximately 0.2 MBq (5 μ Ci) of [18 F]FPrDU, [18 F]FFPrAU, [18 F]FBuDU, [18 F]FFBuAU, [18 F]FPeDU, or [18 F]FFPeAU (SA from 18.5–925 GBq/ μ mol, 500–25000 mCi/ μ mol). Nucleoside accumulation experiments were run for 5, 15, 30, 60, 90 and 120 min at 37 °C. After incubation, the cell culture medium was quickly removed and the monolayers washed three times with cold PBS. The cells were released from the culture plates with 0.25% Trypsin-EDTA (250 μ L/well) and the trypsin neutralized with cell culture medium (350 μ L/well). A 50 μ L sample was taken and mixed with 50 μ L trypan blue to count the number of viable cells. The cell-associated radioactivity was measured in gamma counter and normalized to the number of viable cells and to the total activity administered. Results are expressed as the percentage of radioactivity accumulated per 10^6 cells. Each value represents the mean $\pm$ S.D. of three or more independent experiments and each independent experiment was performed in duplicate.

Cytotoxicity Assays—RG2TK+ and RG2 cells were seeded in a 96-well plate at a density of 1.5×10^3 cells/well and left to incubate overnight at 37 °C. Cells were treated with increasing concentrations of drug in culture media (0.001–1000 μ mol/L, 100 μ L/well) in quadruplicate. Control groups consisted of cells in media, without chemical treatment, and blank wells contained media but no cells. All wells were processed identically and incubated simultaneously as the treated groups. After incubation for 72 h at 37 °C, MTT solution (20 μ L/well of 1 mg/mL) was added to each well and cells incubated for 3 h. After this time, the medium was rapidly removed and the formazan crystals produced by viable cells were solubilized using DMSO (100 μ L/well). The plates were optically scanned at 490 nm (650 nm reference wavelength). Absorbance readings were subtracted from the value of the blank wells. The reduction of cell growth was expressed as a percentage of the control group. Data are expressed as the mean of three independent experiments $\pm$ S.E.M.

VI. Statistical Analysis

Descriptive statistics of the differences of cellular accumulation of the tracers were performed using univariate analysis with GraphPad Prism 4.0 and Microsoft Excel 2004. Group data were compared using ANOVA analysis, and a two-sided unpaired Student's *t*-test; statistical significance was set at $P < 0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

HSV1	herpes simplex virus type-1
<i>tk</i>	thymidine kinase gene
PET	positron emission tomography
TK	thymidine kinase protein
TdR	thymidine
GCV	ganciclovir
IDU	5-iodo-2'-deoxyuridine; 2'-fluoro-2'-deoxy-5-[¹²⁴ I]iodo-1-β-D-arabinofuranosyluracil
FIAU	5-bromovinyl-2'-deoxyuridine
FHBG	9-(4-[¹⁸ F]fluoro-3-hydroxymethyl)butyl)guanine
FEAU	2'-fluoro-2'-deoxy-5-ethyl-1-β-D-arabinofuranosyluracil
FFEAU	2'-fluoro-2'-deoxy-5-(2-fluoroethyl)-1-β-D-arabinofuranosyluracil
FPrDU	5-(3-fluoropropyl)-2'-deoxyuridine
FBuDU	5-(4-fluorobutyl)-2'-deoxyuridine
FPeDU	5-(5-fluoropentyl)-2'-deoxyuridine
FFPrAU	2'-fluoro-2'-deoxy-5-(3-fluoropropyl)-1-β-D-arabinofuranosyluracil
FFBuAU	2'-fluoro-2'-deoxy-5-(4-fluorobutyl)-1-β-D-arabinofuranosyluracil
FFPeAU	2'-fluoro-2'-deoxy-5-(5-fluoropentyl)-1-β-D-arabinofuranosyluracil

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Figure 1.
Chemical structures of 5-[¹⁸F]fluoroalkyl pyrimidine nucleosides [¹⁸F]1a-f

Figure 2.

In vitro cellular uptake of [^{18}F]FPrDU, [^{18}F]FBuDU, [^{18}F]FPeDU, [^{18}F]FFPrAU, [^{18}F]FFBuAU, and [^{18}F]FFPeAU (**[^{18}F]1a–f**, respectively) in RG2TK+ and RG2 cells. Data are expressed as the mean $\pm$ S.D. of three or more independent experiments performed in duplicate.

Figure 3. [¹²⁵I]FIAU accumulation in RG2TK+ cells with increasing concentrations of inhibitors (0–300 μM): FPrDU (**1a**), FBuDU (**1b**), FPeDU (**1c**), FFPrAU (**1d**), FFBuAU (**1e**), and FFPeAU (**1f**), in comparison to FIAU. Data are expressed as the mean ± S.D. of three or more independent experiments performed in duplicate.

Figure 4.

Cellular uptake selectivity index of [¹⁸F]FPrDU, [¹⁸F]FBuDU, [¹⁸F]FPeDU, [¹⁸F]FFPrAU, [¹⁸F]FFBuAU, and [¹⁸F]FFPeAU (average tracer accumulation ratio between RG2TK+ and RG2 cells). ([¹⁸F]FPrDU versus [¹⁸F]FBuDU, **, $P < 0.007$; [¹⁸F]FPrDU versus [¹⁸F]FPeDU, n.s., $P > 0.05$; [¹⁸F]FBuDU versus [¹⁸F]FPeDU, *, $P < 0.05$; [¹⁸F]FBuDU versus [¹⁸F]FFBuAU, ***, $P < 0.001$; [¹⁸F]FFPrAU versus [¹⁸F]FFBuAU and [¹⁸F]FFPrAU versus [¹⁸F]FFPeAU, and [¹⁸F]FFBuAU versus [¹⁸F]FFPeAU, n.s., $P > 0.05$; [¹²⁵I]FIAU versus [¹⁸F]FPrDU and FBuDU and FPeDU, ***, $P < 0.001$; [¹²⁵I]FIAU versus [¹⁸F]FFPrAU, **, $P < 0.005$; [¹²⁵I]FIAU versus [¹⁸F]FFPeAU, *, $P < 0.02$; [¹²⁵I]FIAU versus [¹⁸F]FFPeAU, n.s., $P > 0.05$).

Scheme 1.

^a Synthesis of 5-fluoroalkyl pyrimidine nucleosides **1a–f**

^aReagents and conditions: (a) $\text{IZn}(\text{CH}_2)_3\text{F}$ or $\text{BrZn}(\text{CH}_2)_4\text{F}$ or $\text{BrZn}(\text{CH}_2)_5\text{F}$, $\text{Pd}(\text{P}(t\text{-Bu})_3)_2$, DMA, rt; (b) 0.5 N NaOMe, MeOH, 80 °C.

Scheme 2.

^a Synthesis of 5-*O*-mesylate precursors **8a–f**

^aReagents and conditions: (a) Zn, I₂, DMA, 80 °C; (b) Pd(P(*t*-Bu)₃)₂, DMA, rt; (c) 1% HCl, EtOH, rt; (d) MsCl, Et₃N, CH₂Cl₂, 0 °C → rt.

Scheme 3.

^a Radiofluorination of 5-*O*-mesylate precursors **8a–f** to afford [¹⁸F]**1a–f**

^aReagents and conditions: (a) [¹⁸F]KF, K[2,2,2], K₂CO₃, DMF, 135 °C, 5 min; (b) 0.5 N NaOMe, MeOH, 135 °C, 5 min.

Table 1

¹⁸F-Labeling yields, preparation times, and log *P*^a for [¹⁸F]1a-f

Cmpd	RCY ^a (%)	RCP ^b (%)	Preparation time (min)	Log <i>P</i> ^c	<i>n</i> ^d
[¹⁸ F]FPrDU ([¹⁸ F]1a)	21.6 ± 2.4	>99	60	-0.57	3
[¹⁸ F]FBuDU ([¹⁸ F]1b)	20.8 ± 4.7	>99	60	-0.24	4
[¹⁸ F]FPeDU ([¹⁸ F]1c)	35.2 ± 2.6	>99	70	0.23	4
[¹⁸ F]FPrAU ([¹⁸ F]1d)	19.3 ± 4.9	>99	60	-0.12	3
[¹⁸ F]FBuAU ([¹⁸ F]1e)	20.4 ± 3.4	>99	60	0.27	3
[¹⁸ F]FPeAU ([¹⁸ F]1f)	17.3 ± 2.3	>99	70	0.74	8

^aRCY: Radiochemical yield, decay-corrected; Results are reported as the mean ± S.D.;

^bRCP: Radiochemical purity;

^cLog *P* = log([1-octanol]/[0.1 M NaH₂PO₄ pH 7.47]);

^d*n*: number of experiments performed.

Table 2

Inhibition of [125 I]FIAU uptake after 2 h incubation with 5-fluoroalkyl pyrimidine nucleosides **1a–f** in murine glioma cells, RG2TK+

Cmpd	IC ₅₀ ^a in RG2TK+ cells
FPrDU, 1a	7.6 ± 0.5
FBuDU, 1b	34.5 ± 3.7
FPeDU, 1c	36.8 ± 8.3
FFPrAU, 1d	51.2 ± 5.6
FFBuAU, 1e	23.0 ± 2.0
FFPeAU, 1f	22.9 ± 1.9
FIAU	0.7 ± 0.1

^aIC₅₀ (μM), inhibitory concentration required to inhibit [125 I]FIAU uptake by 50%; Results are reported as the mean ± S.D. of at least three independent experiments performed in duplicate.

Table 3

Comparison of cellular uptake of tracers after 2 h incubation in RG2TK+ and RG2 cells.

Cmpd	% Uptake per 10 ⁶ Cells ^a		Uptake Selectivity Index (RG2TK+/RG2)
	RG2TK+	RG2	
[¹⁸ F]FPrDU	8.2 ± 1.5	0.41 ± 0.07	20.5 ± 4.4
[¹⁸ F]FBuDU	8.4 ± 0.7	0.59 ± 0.16	14.9 ± 4.0
[¹⁸ F]FPeDU	4.4 ± 1.1	0.23 ± 0.07	19.9 ± 6.9
[¹⁸ F]FFPrAU	1.9 ± 0.3	0.38 ± 0.03	5.0 ± 0.7
[¹⁸ F]FFBuAU	3.7 ± 0.7	0.66 ± 0.13	7.8 ± 4.3
[¹⁸ F]FFPeAU	1.2 ± 0.3	0.24 ± 0.01	5.5 ± 2.3
[¹²⁵ I]FIAU	29.4 ± 7.4	3.4 ± 2.0	9.9 ± 4.6

^aResults are reported as the mean ± S.D. of at least three independent experiments performed in duplicate.

Table 4

Cytotoxic activity^a of 5-fluoroalkyl pyrimidine nucleosides **1a–f** against murine glioma cells, RG2TK+ and RG2 as determined by MTT assay

Cmpd	Cytotoxic Activity (CC ₅₀)	
	RG2TK+	RG2
FPrDU, 1a	>1000	>450
FBuDU, 1b	>800	>800
FPeDU, 1c	>650	>750
FFPrAU, 1d	209	121
FFBuAU, 1e	346	355
FFPeAU, 1f	375	>1000
GCV	5.86	377
FIAU	0.79	80

^aCC₅₀ (μM), cytotoxic concentration required to inhibit cell proliferation by 50%. Results are reported as the mean ± S.E.M. of three independent experiments performed in duplicate.