Published in final edited form as:

Nucl Med Biol. 2012 November ; 39(8): 1182–1188. doi:10.1016/j.nucmedbio.2012.06.008.

An improved strategy for the synthesis of [^{18}F]-labeled arabinofuranosyl nucleosides

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Abstract

The expression of the herpes simplex virus type-1 thymidine kinase (HSV1-tk) gene can be imaged efficaciously using a variety of 2'-[^{18}F]fluoro-2'-deoxy-1-b-D-arabinofuranosyl-uracil derivatives [[^{18}F]-FXAU, X= I(iodo), E(ethyl), and M(methyl)]. However, the application of these derivatives in clinical and translational studies has been impeded by their complicated and long syntheses (3–5 h). To remedy these issues, in the study at hand we have investigated whether microwave or combined catalysts could facilitate the coupling reaction between sugar and nucleobase and, further, have probed the feasibility of establishing a novel approach for [^{18}F]-FXAU synthesis.

We have demonstrated that the rate of the trimethylsilyl trifluoromethanesulfonate (TMSOTf)-catalyzed coupling reaction between the 2-deoxy-sugar and uracil derivatives at 90°C can be significantly accelerated by microwave-driven heating or by the addition of Lewis acid catalyst (SnCl_4). Further, we have observed that the stability of the α - and β -anomers of [^{18}F]-FXAU derivatives differs during the hydrolysis step. Using the microwave-driven heating approach, overall decay-corrected radiochemical yields of 19–27% were achieved for [^{18}F]-FXAU in 120 min at a specific activity of >22 MBq/nmol (595 Ci/mmol). Ultimately, we believe that these high yielding syntheses of [^{18}F]-FIAU, [^{18}F]-FMAU and [^{18}F]-FEAU will facilitate routine production for clinical applications.

INTRODUCTION

Non-invasive reporter-gene imaging using radiolabeled PET probes is a powerful tool to quantitatively determine the location, duration, and extent of expression of reporter/therapeutic genes in humans and animals [1–3]. One such gene, herpes simplex virus-1 thymidine kinase (HSV1-), has been used successfully as a reporter gene for imaging several biological processes, including transcriptional regulation, lymphocyte migration, and stem-cell tracking [4–7]. The HSV1-*tk* gene is also a safer reporter gene to use in the clinic,

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because cells transduced with HSV1-tk can be selectively killed via treatment with anti-herpes drugs such as ganciclovir and acyclovir [8–9]. These favorable attributes have prompted many research groups to develop radiolabeled substrates for the HSV1-*tk* enzyme that can be used for imaging the expression of the HSV-*tk* gene [10–13].

A number of ^{18}F -labeled pyrimidine-based nucleosides have shown promise as reporter probes for non-invasive imaging of HSV1-tk gene expression, including 2'-fluoro-2'-deoxy-1- β -D-arabinofuranosyl-5-iodo uracil (FIAU) [14]; 2'-[^{18}F]-fluoro-2'-deoxy-1- β -D-arabinofuranosyl-5-methyluracil ([^{18}F]-FMAU) [15–16]; and 2'-[^{18}F]-fluoro-2'-deoxy-1- β -D-arabinofuranosyl-5-ethyluracil ([^{18}F]-FEAU) [17]. Although direct fluorination of the nucleoside at the 2'-position of the sugar is the simplest way to synthesize these ^{18}F -labeled pyrimidine derivatives, this approach cannot be applied to routine clinical production, because the reported overall yields are less than 1%, quite low for routine clinical use [18].

The original multi-step approach for the synthesis of 2'- ^{18}F labeled nucleosides involved bromination at the C1-position of the fluorinated arabinosugar using HBr/HOAc in order to couple the sugar to the silylated pyrimidine derivative with high β -anomeric selectivity [19–20]. Unfortunately, the highly corrosive nature of HBr/HOAc can result in sugar hydrolysis and a shortened shelf-life of automated equipment employed in the synthesis. Further, the overall yield for this approach was low, considering 3–5 hours were needed for the 4-step radiosynthesis. Recently, we have developed a trimethylsilyl trifluoromethanesulfonate (TMSOTf)-catalyzed methodology to carry out an *in situ* 2'- ^{18}F -fluorosugar base condensation reaction in one-step without the aforementioned bromination step. Our radiochemical yield was $10 \pm 6\%$ (decay-corrected) with a synthesis time of 110 min [21]. This approach was confirmed by Li, *et al.* in their radiosynthesis of [^{18}F]-FMAU and subsequently extended to other derivatives [22]. Their reaction time for the coupling step was 60 min, which led to overall synthesis time of 150 min. Their overall yields for [^{18}F]-FAU, [^{18}F]-FEAU, [^{18}F]-FFAU, [^{18}F]-FCAU, [^{18}F]-FBAU and [^{18}F]-FIAU varied from $4.1 \pm 0.8\%$ to $10.1 \pm 1.9\%$ (decay-corrected) [22]. More recently they have adapted the methodology to single reactor system for the synthesis of [^{18}F]-FMAU with a yield of $12 \pm 3\%$ (decay-corrected) [23].

In the field of nucleoside synthesis, SnCl_4 is a popular Lewis acid catalyst that has been widely used in the coupling reaction step to synthesize nucleoside derivatives [24–27]. The mechanism for this coupling reaction is that SnCl_4 promotes the leaving of the C1 benzoate from the sugar ring [28]. In recent years, microwave energy has been widely utilized to accelerate organic reactions and radiolabeling [29–30]. We hypothesized that a Lewis acid catalyst or microwave energy heating may accelerate the leaving of C1 benzoate from the sugar ring, resulting in a faster rate of the TMSOTf-catalyzed coupling reaction between 2-[^{18}F]-fluoro-2-deoxy-1,3,5-tri-O-benzoyl-1- α -D-ribofuranose and silylated uracil derivatives. Very recently, we reported that both SnCl_4 and microwave energy heating significantly shortened the synthetic time for [^{18}F]-FIAU preparation [31], and Chen, *et al.* [32] also revealed that microwave energy heating enhanced the coupling efficiency of [^{18}F]-sugar and silylated methyl-uracil in [^{18}F]-FMAU synthesis.

In the study at hand, we report our investigations into the coupling reaction between the 2- ^{18}F -sugar and 5-substitute-uracil (iodo, ethyl or methyl), the base hydrolysis, and the purification steps in order to shorten the overall reaction time. Further we establish non-HBr/HOAc approach for the synthesis of ^{18}F -labeled pyrimidine-based nucleosides in satisfactory yields for clinical application.

Materials and Methods

General

All reagents and solvents were obtained from commercial available sources and used without further purification. Triflate sugar (2-*O*-[(trifluoromethyl)sulfonyl]-1,3,5-tri-*O*-benzoyl- α -D-ribofuranose) **1** was purchased from ABX GmbH (Radeberg, Germany) or made in house [33]. Uracil derivatives were purchased from Sigma-Aldrich (St. Louis, MO). ^{18}F -fluoride ion was obtained from MSKCC radiochemistry core facility or from IBA (NJ). Radioactivity was measured using Capintec (CRC-30BC, Ramsey, NJ) dose calibrator. Microwave systems were from Biotage Inc (Initiator 2.5, Charlotte, NC) or CEM Inc (SP-1537, Matthews, NC). ^1H NMR spectra were obtained at 600 MHz (Bruker AVIII 600 spectrometers). Chemical shifts are reported as δ values (parts per million) relative to remaining protons in deuterated solvent. Coupling constants are reported in hertz. The multiplicity is defined by s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), br (broad) or m (multiplet). LC-MS was performed on a Waters Autopurification system with PDA, MicroMass ZQ and ELSD detector, together with a waters xBridge C18 column (150 mm $\times$ 4.6 mm). HPLC system equipped with Shimadzu LC-20 AB binary pumps, SPD-20A Prominence UV/vis detector, and a BioScan Flow Count radiodetector was used for preparative and analytical HPLC.

Synthesis of 2-Deoxy-2- ^{18}F Fluoro- α -D-Arabinofuranose (**2**)

Compound **2** was prepared manually according to a published procedure [21, 34]. Briefly, no-carrier added ^{18}F -fluoride (2.59–12.95 GBq) was released into a 5-mL V-vial from QMA cartridge with anhydrous acetonitrile (0.9 mL), H_2O (0.2 mL) and 4% tetrabutylammonium bicarbonate (TBAHCO_3 , 100 μL , 13 μmol). The collected ^{18}F was dried on a heating block at 86–90°C under a mild stream of nitrogen, and the azeotropic drying was repeated three times with further addition of 1 mL acetonitrile. Triflate sugar **1** (10 mg, 17 μmol in 500 μL anhydrous acetonitrile) was added to the dried ^{18}F -fluoride and heated at 86–90°C for 10–20 min with microwave system or heating block. The resulting yellow-brown mixture of **2** was then used for the following synthesis without any purification. An aliquot of crude **2** was analyzed with analytical HPLC system (**Method 1** column: Alltima HP C18 (250mm $\times$ 4.6mm); UV detector: 280 nm; flow rate: 1 mL/min; mobile phase: 0.05% TFA in water and **B** (0.05% TFA in $\text{AcCN}/\text{H}_2\text{O}$ (v/v=95/5), or 0.09% NaCl in water and EtOH ; gradient: 0–1 min, 50%–50% **B** (or EtOH), 1–19 min, 100% **B** (or EtOH), 19–24 min, 100% **B** (or EtOH), 24–25 min, 50% **B** (or EtOH)).

Synthesis of 2'- ^{18}F -fluoro-2'-deoxy-3',5'-di-*O*-benzoyl-1- α/β -D-arabinofuranosyl-5-*X*-uracil (**3**, *X*= Iodo, Ethyl and Methyl)

Briefly, 20 mg of **5-*X*-uracil** (*X*= Iodo, ethyl, and methyl) was added to a 5-mL V-vial containing anhydrous acetonitrile (400 μL), 1,1,1,3,3,3-hexamethyldisilazane (HMDS, 100 μL), and trimethylsilyl trifluoromethanesulfonate (TMSOTf , 100 μL) or $(\text{NH}_4)_2\text{SO}_4$ (9 mg) or without catalyst, which was sealed and heated at 120°C for 20 min using microwave system. Fresh **2** was added to the reaction vial with 1 mL syringe. After the mixture was sealed and heated at the elevated temperature for a certain time with microwave, NaHCO_3 saturated H_2O (1 mL) was added to quench reaction, and a DCM or C18 cartridge was used to extract the anomeric mixture of **3a** and **3b** and the unreacted **2**. For heating with traditional heating block, Lewis acid catalyst, SnCl_4 or AlCl_3 in methanol (1 M, 200 μL), was added for coupling. An aliquot of the crude **3** was used for characterization with analytical HPLC system if needed (**Method 1**).

Synthesis of 2'-Deoxy-2'-[¹⁸F]Fluoro-1- α/β -D-arabinofuranosyl-5-X-uracil (**4**, X= Iodo, Ethyl and Methyl)

The crude product **3**, was concentrated and MeONa (500 μ L, 0.5 M) was added and heated at 50°C for deprotection of the benzoyl groups on the arabinose. The reaction mixture was cooled and quenched with HCl (500 μ L, 1.25 M in methanol) and solvents were removed by heating at 80°C under a slow stream of argon. The crude mixture containing the product (**4**) was dissolved in AcCN/H₂O solution (v/v=2/98) and loaded for purification with semi-preparative HPLC system (**Method 2** column: Phenomenex® Luna C18(2) (250 mm $\times$ 10.0 mm, 5 mm, 100 Å); UV detector: 280 nm; flow rate: 7 mL/min; mobile phase: 0.1% TFA in water and AcCN); gradient: 0–1 min, 2%–10% AcCN, 1–16 min, 10%–20% AcCN, 16–18.5 min, 20%–100% AcCN, 24.5min, 100% AcCN, 25min, 2% AcCN). An aliquot of the crude **4** or the purified [¹⁸F]-FXAU was characterized with analytical HPLC system (**Method 3**, column: Alltima HP C18 (250 mm $\times$ 4.6 mm) flow rate: 1 mL/min; mobile phase: 0.1% TFA in water and AcCN; gradient: 0–2 min, 2–10% AcCN, 14 min, 10% AcCN, 14–16.5 min, 10–100% AcCN, 24 min, 100% AcCN, 25 min, 2% AcCN).

Synthesis of 2'-Deoxy-2'-Fluoro-1- α/β -D-arabinofuranosyl-5-X-uracil (FXAU, X= Iodo, Ethyl and Methyl)

FXAU was synthesized with a similar procedure that was utilized for radiosynthesis. The obtained crude intermediates, Bz-FXAU were analyzed with analytical HPLC system (**Method 1**) to determine conversion percentage, characterized with LC-MS (flow rate: 1.2 mL/min; mobile phase: 0.1% TFA in water and AcCN; gradient: 0–10.5 min, 50%–85% AcCN, 11 min, 95% AcCN, 11–12 min, 50% AcCN). The experimentally determined masses of benzoyl protected derivatives (Bz-FIAU, Bz-FEAU and Bz-FMAU) were found to be in agreement with calculated masses for these compounds.

After deprotection, the crude FXAU was purified with semi-preparative HPLC system (**Method 2** column: Phenomenex® Luna C18(2) (250 mm $\times$ 10.0 mm, 5 μ m, 100 Å); flow rate: 5 mL/min; mobile phase: 0.05% TFA in water and AcCN). The purified FXAU were characterized with NMR analysis and LC-MS (flow rate: 1.2 mL/min; mobile phase: 0.1% TFA in water and AcCN; gradient: 0–2 min, 5% AcCN, 2–8 min, 5%–30% AcCN, 10.5 min, 95% AcCN, 11–12 min, 5% AcCN). **FIAU(β)**: ¹H NMR (600 MHz, D₂O), δ 8.13 (s, 1 H), 6.10 (d, 1 H, J = 18 Hz), 5.08 (d, 1 H, J = 54 Hz), 4.28 (d, 1 H, J = 18 Hz), 3.90 (s, 1 H), 3.79–3.66 (d, m, 2 H, J = 66 Hz). ¹³C NMR (600 MHz, D₂O) δ 163.04, 150.98, 146.54, 146.52, 95.21, 93.94, 83.72, 83.60, 83.22, 83.19, 73.02, 72.85, 67.41, 60.07. **FIAU(α)**: ¹H NMR (600 MHz, D₂O), δ 7.99 (s, 1 H), 5.88 (d, 1 H, J = 12 Hz), 5.20 (d, 1 H, J = 48 Hz), 4.35 (d, 1 H, J = 6 Hz), 4.30 (s, 1 H), 3.67–3.50 (d, m, 2 H, J = 60 Hz). ¹³C NMR (600 MHz, D₂O) δ 163.28, 151.15, 146.65, 99.42, 98.19, 90.89, 90.64, 87.29, 87.27, 73.26, 73.09, 67.29, 60.56. **FEAU(β)**: ¹H NMR (600 MHz, D₂O), δ 7.51 (s, 1 H), 6.18 (d, 1 H, J = 18 Hz), 5.13 (d, 1 H, J = 54 Hz), 4.33 (d, 1 H, J = 8 Hz), 3.92 (s, 1 H), 3.82–3.70 (d, m, 2 H, J = 60 Hz), 2.21 (q, 2 H), 0.98 (t, 3 H). ¹³C NMR (600 MHz, D₂O) δ 166.02, 151.26, 137.38, 137.37, 116.35, 95.51, 94.23, 83.31, 83.20, 82.77, 82.74, 73.07, 72.91, 59.98, 30.15, 19.49, 11.89. **FEAU(α)**: ¹H NMR (600 MHz, D₂O), δ 7.37 (s, 1 H), 5.89 (d, 1 H, J = 16 Hz), 5.19 (d, 1 H, J = 51 Hz), 4.34 (d, 1 H, J = 5 Hz), 4.31 (s, 1 H), 3.67–3.58 (d, m, 2 H, J = 51 Hz), 2.16 (q, 2 H), 0.93 (t, 3 H). ¹³C NMR (600 MHz, D₂O) δ 166.24, 151.37, 137.76, 116.56, 99.52, 98.29, 90.55, 90.30, 86.84, 86.82, 73.42, 73.25, 60.52, 19.42, 11.97. **FMAU(β)**: ¹H NMR (600 MHz, D₂O), δ 7.50 (s, 1 H), 6.10 (d, 1 H, J = 17 Hz), 5.10 (d, 1 H, J = 52 Hz), 4.26 (d, 1 H, J = 25 Hz), 3.88 (s, 1 H), 3.78–3.68 (d, m, 2 H, J = 57 Hz), 1.73 (s, 3 H). ¹³C NMR (600 MHz, D₂O) δ 166.43, 151.33, 138.04, 110.62, 95.42, 94.15, 83.32, 82.98, 73.32, 73.16, 60.28, 11.44. **FMAU(α)**: ¹H NMR (600 MHz, D₂O), δ 7.41 (s, 1 H), 5.87 (d, 1 H, J = 16 Hz), 5.17 (d, 1 H, J = 51 Hz), 4.34 (d, 1 H, J = 5 Hz), 4.28 (s, 1 H), 3.68–3.57 (d, m, 2 H, J = 55 Hz), 1.73 (s, 3 H). ¹³C NMR (600 MHz, D₂O) δ 166.63, 151.46, 138.10,

110.89, 99.54, 98.31, 90.32, 89.72, 86.64, 73.33, 73.17, 60.53, 11.43. Calculated mass of FXAU (X= Iodo, ethyl, and methyl is 371.96, 274.10 and 260.08, respectively); Found mass was 372.8([M+H]⁺), 394.9([M+Na]⁺) for FIAU, 275.2([M+H]⁺), 297.1([M+Na]⁺) for FEAU, and 261.1([M+H]⁺), 283.0([M+Na]⁺) for FMAU.

RESULTS AND DISCUSSION

The central hypothesis of this investigation is that microwave-driven heating or combined Lewis acid catalysis can accelerate the rate of the coupling reaction between sugar and base and consequently can increase the overall yields of the final product. Our studies have revealed that ¹⁸F-labeled pyrimidine-based nucleosides (the beta-anomer) can be obtained in a reasonable radiochemical yields using our approach to the coupling reaction (Scheme 1, Approach iii), followed by hydrolysis and HPLC purification of the product.

2'-[¹⁸F]-fluoro-2'-deoxy-3',5'-di-O-benzoyl-1-α/β-D-arabinofuranosyl-5-iodo-uracil (3)

Our kinetic studies on the reaction in which only TMSOTf was used as a catalyst indicated that the yield of the coupling reaction between **2** and the pyrimidine bis-trimethylsilyl ether (Table 1) was time-dependent. Coupling of the sugar to the pyrimidine was complete after 1 h incubation at 85–90°C, as reported earlier [21, 23]. It has been reported in the literature that bromination at the C1 position of **2** does not accelerate the coupling reaction significantly [15]. Mangner, *et al.* [16] also reported that 30 min of incubation was used for the coupling reaction at 150°C after the bromination step. Our experiments revealed that while the use of a stronger Lewis acid catalyst (AlCl₃) resulted in more byproducts and reduced the conversion yield (Table 1), a “mild” Lewis acid catalyst (SnCl₄) was able to significantly enhance the coupling reaction. When the combined intermediate Lewis acids such as (NH₄)₂SO₄/SnCl₄ or TMSOTf/SnCl₄ were used, the coupling reaction time was shortened from 1 h to 15 min or less (Table 1) at 85–90°C with little or no byproducts (Figure 1). If the reaction mixture was heated using the microwave system, the coupling reaction also proved time- and temperature- dependent, and the conversion percentage at 90°C was much higher than when subjected to conventional heating (Table 1).

Both of our new methods offered a more efficient and faster conversion rate than using previously reported approaches [16, 21, 23]. The results have revealed that both the presence of a Lewis acid catalyst and the energy delivered by microwave are important factors in facilitating rapid quantitative conversions under mild conditions. This data also implied that a key issue in the coupling reaction was whether the intermediate (Scheme 2) for **3α** and **3β** could form more efficiently. By employing either an additional Lewis acid (SnCl₄) or microwave-driven heating, the intermediate could form much faster in the TMSOTf-catalyzed coupling reaction. In addition, mild reaction conditions can preclude the formation of any potential decomposition products; in contrast, when high temperature was utilized, decomposition of **2** and **3** was observed. Moreover, the use of a brominated sugar as a reactive intermediate at a higher temperature (>90 °C) resulted in additional byproducts and reduced the conversion yield.

We analyzed the crude product **3** and Bz-FXAU with HPLC (Method 1 and 0.05% TFA in water/acetonitrile as the mobile phase) to investigate both the anomeric ratio obtained from the coupling reaction and the impact on anomeric selectivity of variant groups on the 5-position of uracil. As expected, LC-MS analysis of Bz-FXAU (α and β anomers) derivatives showed identical molecular weights but eluted with different retention times. The anomeric ratio obtained for [¹⁸F]-Bz-FXAU derivatives closely matched the ratio obtained for synthesis of the non-radioactive derivatives (Figure 1) and corroborated our previous results [21]. The combined Lewis acid catalysts or microwave-driven heating did not change the anomeric ratio in favor of either anomer (Table 2), unlike the brominated-sugar approach,

which preferentially yields the β -anomer ($\alpha:\beta \sim 1:3$). However, slightly different $\alpha:\beta$ anomeric ratios were observed when the group at the 5-position of uracil was varied, most likely due to steric or electronic factors (Table 2). The α and β anomers were equally formed when TMSOTf (or with SnCl_4 as additional catalyst) was utilized to couple 2- ^{18}F -fluoro-2-deoxy-1,3,5-di-O-benzoyl-D-arabinofuranose to uracil analogs. Fox et al [35] found that when $\text{SnCl}_4/\text{TMSCl}$ (trimethylchlorosilane) was utilized as a catalyst to couple 1-acetyl-2-chloro-2-deoxy-3,5-di-O-benzoyl-D-arabinofuranose to cytosine, the α anomer was exclusively obtained, probably due to formation of the 1,2-chloronium salt intermediate above the plane of sugar. We can therefore deduce that no 1,2-fluoronium salt formed above the plane of sugar in our reaction, and instead that the different active intermediate (Scheme 2) was formed without any anomeric selectivity. Pyrimidine bis-trimethylsilyl ether (activated 5-X-uracil) attacked the active C1 of the sugar unit ($\alpha/\beta = 1/1$) with a $\text{S}_{\text{N}}2$ mechanism, resulting in **3** with a 50% yield for the β anomer.

2'- ^{18}F -fluoro-2'-deoxy-1- α/β -D-arabinofuranosyl-5-iodo-uracil (**4**, X = iodo)

Deprotection of **3** was achieved using MeONa or NaOH as a base, affording crude **4** quantitatively after 5–10 min incubation at elevated temperatures (Table 3). It has been shown that NH_3 can efficiently remove the benzoyl group of protected uridine when the incubation was performed at 4°C for 24–48 h [24–25]; however, in our case, incubation with NH_3 at 50°C for 5 min resulted in incomplete deprotection and unidentified byproducts. We also observed that presence of SnCl_4 reduced the overall yield of the desired final product (β -anomer) than expected from the coupling step. One possible explanation is that the β -anomer is prone to decomposition during the hydrolysis step in the presence of SnCl_4 . Therefore, extraction of SnCl_4 and other ionic impurities using water was performed before hydrolysis, which improved our overall yields (Table 3). Interestingly, after extraction with DCM/ H_2O or a C_{18} Sep-PakTM cartridge, deprotection of the benzoyl group caused a more rapid decomposition of the α anomer, especially at higher temperatures (Table 3). This, in turn, results in a preferable α/β ratio for β anomer product, but the overall yield remains the same. Mild hydrolysis conditions, such as 5 min at 50°C , caused minimum decomposition of both anomers and the best yield of β -anomer (Table 3).

Overall radiosynthesis of 2'- ^{18}F -fluoro-2'-deoxy-1- β -D-arabinofuranosyl-5-X-uracil (β anomer, ^{18}F -FXAU, X = I(iodo), E(ethyl), and M(methyl))

Generating a patient-level dose of ^{18}F -FXAU using approach **ii** and **iii** (Scheme 1) is fraught with synthetic complexity, as the radiosynthesis lasts 3–5 hours (depending on the method used), and the overall yield of the final product is low and variable. However, with our newly established approach, ^{18}F -FXAU could be synthesized successfully with shorter reaction times and higher overall yields. In these current studies, we performed the complete synthesis of three ^{18}F -FXAU analogues with the microwave system (Table 4, Figure 2 and 3). Although our current approach can only produce an equal amount of α and β anomer, the absolute radiochemical yield of our final desired β anomer is higher than earlier approaches. Compared to the published results (Table 4), radiosynthesis of ^{18}F -FXAU with microwave-driven heating required almost the same amount of time as the direct fluorination approach **i** and required much less time than approach **ii** and **iii** (Scheme 1). Our microwave approach consistently afforded higher radiochemical yields of the β anomer of ^{18}F -FXAU (Table 4). Using this approach, 0.74–1.48 GBq (20–40 mCi) of final product was obtained starting from 7.4–12.95 GBq (200–350 mCi) of ^{18}F -fluoride. Further, we are performing the manual synthesis and therefore if automated, the overall reaction time can be reduced considerably.

The combined catalysts approach (e.g., TMSOTf/ SnCl_4) using conventional heating can be readily translated to commercial automatic synthesizers, and the microwave assisted heating

approach could be used for manual synthesis. Currently, we use TMSOTf as the catalyst and the microwave as the heating system in our manual clinical production. The advantages of using TMSOTf are that it is less corrosive than HBr/HOAc, it eliminates the need for an additional step involving synthesis of brominated sugar derivative and removal of HBr/HOAc, it is able to increase solubility of the uracil analogues in acetonitrile, and it affords the mostly “clean” product. However, TMSOTf is highly moisture sensitive, and should be handled with care. Further, the fluorine exchange from TMSOTf or one of the byproducts may result in lower specific activity. Our [^{18}F]-FMAU and [^{18}F]-FEAU (Table 4) synthesis afforded 2–3 fold lower specific activities than the highest specific activity reported in the literature (Table 4) [15, 18, 33, 36]. We obtained the highest specific activity with this method for [^{18}F]-FIAU. It is important to note that since PET probe-target interaction is enzymatic in nature, [^{18}F]-FXAU is recognized, phosphorylated, and trapped into the HSV1-*tk* gene transduced cells continuously by thymidine kinase, a product of the HSV1-*tk* gene. This process should be affected only by the HSV1-*tk* gene expression levels and radiotracer concentration in circulation but not the specific activity of [^{18}F]-FXAU.

In conclusion, we have demonstrated that the energy delivered by a microwave or additional Lewis catalyst with traditional heating accelerates the coupling reaction significantly affording quantitative yields with >75% shorter reaction time for the coupling step. Our investigations also revealed that the α and β anomers of [^{18}F]-FXAU [X= I(iodo), E(ethyl), and M(methyl)] derivatives displayed different stabilities during the hydrolysis step, especially in the presence of SnCl_4 . Two new general radiosynthetic approaches have been developed for the synthesis of 2'-deoxy-2'-[^{18}F]-fluoro-1-b-D-arabinofuranosyl-5-substituted-uracil analogues and have been optimized. The combined Lewis acid catalyst approach (*i.e.*, TMSOTf/ SnCl_4) can be translated to automatic synthesizers easily, whereas the microwave-driven heating approach is currently only suitable for manual syntheses. Using the microwave-driven heating approach, [^{18}F]-FIAU, [^{18}F]-FMAU and [^{18}F]-FEAU have been successfully synthesized within 120 min, affording a radiochemical yield of 19–27% (decay corrected) at a specific activity of >22 MBq/nmol (595 Ci/mmol). [^{18}F]-FIAU, [^{18}F]-FMAU, and [^{18}F]-FEAU synthesized with this newly established methodology could both fulfill the requirements of PET imaging of the HSV1-*tk* gene expression in patients and be suitable for routine clinical production.

Acknowledgments

The authors gratefully acknowledge Dr. Brian Zeglis for editorial comments/corrections and scientific discussions regarding the manuscript. This work was financially supported in part by the Office of Science (BER) - U.S. Department of Energy (Award DE-SC0002456; JSL) and the NIH/NCI ICMIC grant to MSKCC (P50 CA086438) awards. We also thank personnel of MSKCC Cyclotron-Radiochemistry Core (NIH Center Grant P30-CA08748) for their technical services.

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Figure 1. HPLC monitoring of coupling reaction between ^{18}F -sugar and 5-Iodo-uracil, heated using microwave for 15 min. **Method 1** (details in method section), 0.05% TFA in water/AcCN as mobile phase. Red line: sample was from radiosynthesis; Black line: sample from the synthesis of cold **3**.

Figure 2.

A typical HPLC chromatogram showing purification of [^{18}F]-FIAU using a semi-preparative HPLC system. Red line: radioactive signal from radio-detector (Rad); Black line: UV signal from UV detector (UV) @ 254 nm.

Figure 3.

Quality control analysis of [^{18}F]-FIAU with analytical HPLC system. A) [^{18}F]-FIAU coinjected with cold standard, FIAU; B) [^{18}F]-FIAU. Red line: radioactive signal from radio-detector (Rad); Black line: UV signal from UV detector (UV) @ 280 nm.

Scheme 1.

Scheme 2.

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Table 1

E Results of conversion of **2** to Benzoyl protected [¹⁸F]-FIAU under different conditions

entry	temperature (°C)	time (min)	catalyst(s)	heating model [‡]	conversion [‡] (%)
1	86–90	15	AlCl ₃	HB	4±1 (n = 2)
2	86–90	15	SnCl ₄	HB	68±0 (n = 2)
3	86–90	15	TMSOTf	HB	65±3 (n=6)
4	86–90	22	TMSOTf	HB	74±1 (n=2)
5	86–90	30	TMSOTf	HB	81±0 (n=2)
6	86–90	45	TMSOTf	HB	91±1 (n=2)
7	86–90	60	TMSOTf	HB	98±1 (n=2)
8	86–90	15	(NH ₄) ₂ SO ₄ + SnCl ₄	HB	92±2 (n=2)
9	86–90	15	TMSOTf + AlCl ₃	HB	8±2 (n=2)
10	86–90	15	TMSOTf + SnCl ₄	HB	98±1(n=5)
11	74–76	15	TMSOTf	MW	83±0 (n = 2)
12	89–91	10	TMSOTf	MW	95±1 (n=2)
13	89–91	15	TMSOTf	MW	98±2 (n=5)
14	104–106	15	TMSOTf	MW	99±1 (n=3)

Note:

[‡] heating model, HB: block fitting on heating plate, and MW: microwave system;

[‡] conversion percentage was from the analysis with radiodetector integrated analytical HPLC system (method 1).

Table 2

Results of anomeric ratio of Benzoyl protected [¹⁸natF]-FXAU under different conditions

X =	temperature (°C)	time (min)	catalyst(s)	heating model [‡]	3α/3β [‡]
Iodo	86–90	60	TMSOTf	HB	1.0/1.0
Iodo	86–90	15	TMSOTf + SnCl ₄	HB	1.0/1.0
Iodo	89–91	15	TMSOTf	MW	1.0/1.0
Ethyl	89–91	15	TMSOTf	MW	1/0.74
Methyl	89–91	15	TMSOTf	MW	1/0.77

Note:

[‡] heating model HB: blocker fitting on heating plate, and MW: microwave system;

[‡] the average value of α/β anomer ratio was calculated with the results of HPLC analysis (n >= 4, UV = 280nm or radiossignals, **method 2**).

Table 3

Results of hydrolysis of Benzoyl protected [¹⁸F]-FIAU under different conditions

entry	catalyst(s) for coupling step	extraction [‡]	hydrolysis base	heating model [†]	temperature (°C)	time (min)	α/β ratio	% of β anomer [#]
1	TMSOTf + SnCl ₄	no	MeONa	HB	86–90	10	2/1	25±5 (n=2)
2	TMSOTf + SnCl ₄	yes	MeONa	HB	86–90	10	1.1/1	42±4 (n=3)
3	TMSOTf	no	MeONa	HB	86–90	10	1/1	37±3 (n=2)
4	TMSOTf	yes	MeONa	HB	86–90	10	1/2	45±1 (n=2)
5	TMSOTf	yes	MeONa	MW	90	10	1/1.5	42±3 (n=2)
6	TMSOTf	yes	MeONa	MW	90	5	1/1.1	46±2 (n=3)
7	TMSOTf	yes	MeONa	MW	50	5	1/1.1	46±2 (n=6)
8	TMSOTf	yes	MeONa		RT	5	1/1	41±1 (n=2)
9	TMSOTf	yes	NaOH	MW	50	5	1/1	39±1 (n=2)
10	TMSOTf	yes	NH ₃	MW	50	5	ND	ND

Note:

[‡] extraction was performed before hydrolysis or not;

[†] heating model, HB: block fitting on heating plate, and MW: microwave system;

[#] after hydrolysis, [¹⁸F]-FIAU crude solution was analyzed with radiodetector integrated analytical HPLC system (**method 3**) to determine % of β anomer on over all radioactivity.

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Table 4

Radiosynthesis of [¹⁸F]-FXAU (X = I(iodo), E(ethyl) and M(methyl))

X=	Approach [‡]	synthetic time (min)	radiochemical yield (%) [†]	specific activity (MBq/nmol) [#]	reference
Iodo	ii	180	35±7 (n=5)	0.11	[16]
	iii	110	10±6 (n=5)	4.6	[21]
	iii-MW	120	22±3 (n=3)	>418	current
Methyl	i	95–100	2 (n=6)	66	[18]
		180	42±12 (n=9)	0.11	[16]
	ii	210–240	25 (n=4)	85	[15]
	iii	150	12±3 (n=4)	14	[23]
	iii-MW	120	23±2 (n=3)	26	current
Ethyl	ii	330	5±1 (n=3)	48	[33]
		150	4±1 (n=7)	46	[36]
		210	15–20	-	[20]
	iii	193	9±3 (n=7)	27	current
	iii-MW	120	25±2 (n=3)	22	current

Note:

[‡] synthetic approaches were listed in scheme 1: **i**, direct fluorination (3 steps); **ii**, indirect fluorination via bromination of ¹⁸F-sugar (5 steps); **iii**, indirect fluorination without bromination step (4 steps); **iii-MW**, indirect fluorination without bromination step, and microwave system was used (4 steps).

[†] yields were calculated from the amount of [¹⁸F]-FXAU after evaporation of the fraction from HPLC purification with decay correction.

[#] specific activity was measured with HPLC analysis (UV = 280nm), which was precalibrated with standard compound.