Synthesis and Evaluation of F-18 Labeled 2'-Deoxy-2'-fluoro-5-methyl-1-β-L-arabinofuranosyluracil (L-[^{18}F]FMAU)

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L-[^{18}F]FMAU ([^{18}F]**1b**) was prepared from the precursor 2-*O*-[(trifluoromethyl)-sulfonyl]-1,3,5-tri-*O*-benzoyl-α-L-ribofuranose, by coupling the radioactive fluoro-sugar with the corresponding silylated thymine in 4 steps. The final products, including the α and β anomers, were purified using reverse phase HPLC with an appropriate solvent (5% CH_3CN/H_2O) at a flow rate of 3.0 mL/min. The total elapsed time of synthesis was about 180-200 min from EOB. The α/β anomeric ratio of the compounds was about 1:9, and the radiochemical purity of the product (β-form) was >98% with decay-corrected yields of 25-35%. All radioactive samples were confirmed using co-injection with pure non-radioactive analogues in every step. In the cellular uptake in vitro test of herpes simplex virus-thymidine kinase (HSV1-TK) gene expressed cells, the percent uptake of injected dose (%ID) of L- and D-FMAU was 37.28 and 65.86, respectively after 240 min incubation. However, the relative uptake (MCA-TK/MCA cellular uptake ratio) of L-FMAU was higher than that of D-FMAU (%ID of L-FMAU, 0.36 and D-FMAU, 0.93 after 240 min incubation in MCA cells). This means that L-FMAU will show better specific HSV1-TK gene expressed cell uptake for selective HSV1-TK gene imaging.

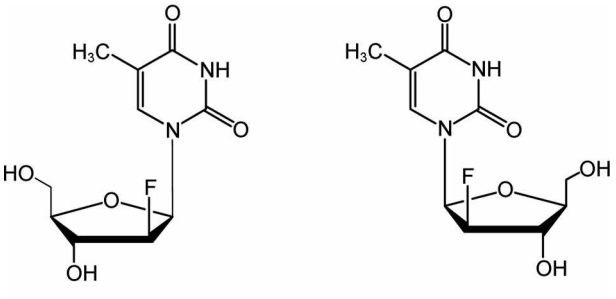
Key Words : L-[^{18}F]FMAU, D-[^{18}F]FMAU, Nucleoside, Fluorine-18, PET

Introduction

Recently, nucleosides with the unnatural L-configuration have been studied as potent chemotherapeutic agents against human immunodeficiency virus (HIV), hepatitis B virus (HBV), and certain forms of cancer.[1,2] A number of radionuclide labeled pyrimidine nucleoside analogues have been evaluated as potential antitumor and antiviral agents.[3,4] C-11 labeled 2'-deoxy-2'-fluoro-5-[^{11}C-methyl]-1-β-D-arabinofuranosyluracil ([^{11}C]-FMAU) was developed as a radiotracer for cell proliferation by positron emission tomography (PET).[5] However, C-11 labeled radiopharmaceuticals have a limited clinical application because of its short half life ($t_{1/2}$ = 20 min).[6] Therefore, other radioactive analogues with longer half-life, such as fluorine-18 labeled analogues ($t_{1/2}$ = 110 min) were developed for effective clinical application. Some fluorinated analogues of pyrimidine nucleosides have been studied as potential agents for imaging tumor cell proliferation or HSV-tk reporter gene expression.[6,7] Accordingly, Alauddin *et al.* developed the F-18 labled 2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyluracil (D-[^{18}F]FMAU).[3] There are four kinds of stereoisomers in FMAU (β-D, α-D, β-L and α-L), among them, L-FMAU has demonstrated high antiviral activity against HBV and Epstein Barr virus (EBV).[8,9] Therefore, we prepared the authentic L-FMAU compound and its precursor for the direct introduction of fluorine-18.

The introduction of fluorine-18 into the L-ribofuranose configuration and the consequent coupling with the pyrimidine base was found to be successful for the syntheses of the target nucleoside.[10-12] In this research, we tried to search for various suitable incorporation of radiofluorine using K^{18}F (K_2CO_3, Kryptofix 2.2.2., $^{18}F^-/H_2{}^{18}O$), CsF (Cs_2CO_3, Kryptofix 2.2.2., $^{18}F^-/H_2{}^{18}O$), TBA^{18}F (TBAOH, $^{18}F^-/H_2{}^{18}O$), TBA^{18}F (TBAHCO$_3$, $^{18}F^-/H_2{}^{18}O$) in the 2-position of the benzoyl protected sugar with mesyl-, tosyl- and imidazole sulfonyl- and nosyl-group as a leaving group but couldn't obtain the satisfactory results except for the triflate precursor. So, we describe here a detailed synthetic scheme for L-[^{18}F]-FMAU using triflate precursor as a model for general synthesis of the 2'-deoxy-2'-[^{18}F]fluoro-1-β-D-arabinofuranosyluracil nucleoside.[13]

Results and Discussion



D-FMAU (**1a**) L-FMAU (**1b**)

Figure 1. Stucture of D-[^{18}F]FMAU (**1a**) and L-[^{18}F]FMAU (**1b**).

Synthesis

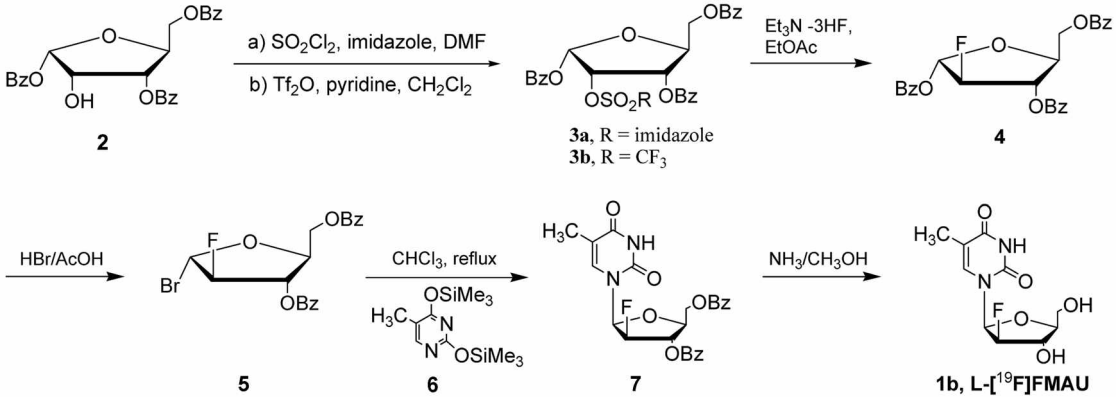
2'-Deoxy-2'-[^{19}F]fluoro-5-methyl-1-β-L-arabinofuranosyluracil (L-[^{19}F]FMAU, [^{19}F]1b). 2'-Deoxy-2'-fluoro-5-methyl-β-L-arabinofuranosyluracil ([^{19}F]**1b**, L-[^{19}F]FMAU) was prepared according to literature procedure (Scheme 1).[14] 1,3,5-Tri-*O*-benzoyl-α-L-ribofuranose (**2**) was reacted with sulfuryl chloride and imidazole in DMF-CH_2Cl_2 in order to introduce a good leaving group in compound **3a** in 85% yield. The precursor triflated **3b** was prepared with trifluoromethanesulfonic anhydride and pyridine in conditions adjusted for radiolabeling.[12] To synthesize the standard compound, the imidazole derivative (**3a**) was converted to the 1,3,5-tri-*O*-benzoyl-2-deoxy-2-fluoro-α-L-ribofuranose (**4**) in the presence of 6-7 equiv. of Et_3N·3HF in ethyl acetate at 70 °C.[14,15] 1-α-Bromo sugar moiety (**5**) was synthesized from fluorinated sugar (**4**) by reaction with 33% HBr/AcOH at room temperature for 24 h. 2'-Deoxy-2'-fluoro-3',5'-di-*O*-benzoyl-5-methyl-1-β-L-arabinofuranosyluracil (**7**) was obtained by the coupling of bis(*O*-trimethylsilyl)thymine (**6**) with 1-α-bromo sugar moiety (**5**) in $CHCl_3$ at 80 °C for 24 h in 65% yield. The benzoyl groups were hydrolyzed with NH_3 in MeOH, thereby producing the reference product, L-[^{19}F]FMAU ([^{19}F]**1b**), in 85% yield.

Radiolabeling

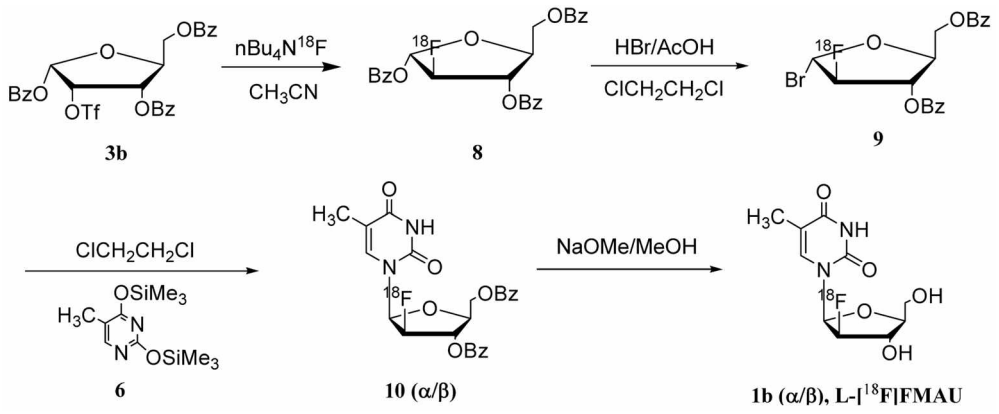
2'-Deoxy-2'-[^{18}F]fluoro-5-methyl-1-β-L-arabinofuranosyluracil (L-[^{18}F]FMAU, [^{18}F]1b). L-[^{18}F]FMAU was prepared by coupling the radiolabeled fluoro-sugar with the corresponding silylated thymine following the preparation procedure for D-[^{18}F]FMAU reported by Alauddin *et al.* with a little modification as shown in Scheme 2.[3,13] For the preparation of L-[^{18}F]FMAU, the tribenzoyl triflate sugar (**3b**) was used as a precursor because sometimes the sulfonyl imidazole (**3a**) precursor could not be detected by radio-TLC or gave low labeling yields. Use of similar radiofluorination conditions with the tribenzoyl triflate (**3b**), however, showed evidence of product formation about >85% (by radio-TLC).

F-18 fluoride was eluted from QMA cartridge using 50 μL of 4% $TBAHCO_3$. The solvent was completely removed by azeotrope with acetonitrile. To the reaction v-vial, the solution of triflate precursor **3b** in acetonitrile was added. The reaction mixture was heated at 80 °C for 25 min and cooled to room temperature. Unreacted F-18 was removed with two silica Sep-pak (light) and eluted with ethyl acetate. This mixture was checked by reverse phase HPLC system with authentic compound (**4**) as shown in Figure 2.

The ethyl acetate solution was dried with stream of argon gas. After complete dissolution of the mixture was dissolved with 1,2-dichloroethane, 33% HBr in acetic acid was added and the mix was heated at 80 °C for 10 min (**9**, checked by radioTLC). After the solvent was removed by azeotropic distillation with toluene, silylated thymine in chloroform was added under an argon atmosphere. The solution was heated to 110 °C for 45 min and then cooled to room



Scheme 1. Synthesis of reference compound, L-[^{19}F]FMAU ([^{19}F]**1b**).



Scheme 2. Synthesis of L-[^{18}F]FMAU.

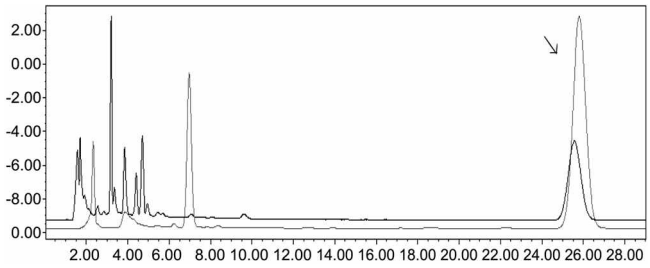


Figure 2. HPLC analysis of authentic **4** with reaction mixture (**8**) (Black:UV, Red:Radioactivity, mBondapak RP-18, 10 μ, 3.9 mm × 300 mm, CH_3CN/H_2O = 60/40 [v/v], flow rate: 1.0 mL/min, R_t: 25.7 min).

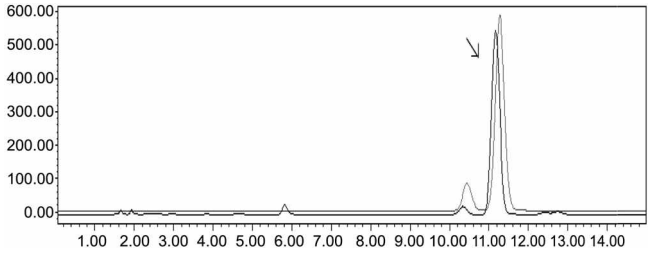


Figure 3. HPLC analysis of authentic **7** with reaction mixture (**10**) (Black:UV, Red:Radioactivity, mBondapak RP-18, 10 m, 3.9 mm × 300 mm, CH_3CN/H_2O = 5:5 (0 min) to 7:3 (20 min) [v/v], flow rate: 1.0 mL/min, R_t: 11.2 min).

temperature. The reaction mixture was passed through a silica Sep-pak (plus) and eluted with 10% MeOH/CH_2Cl_2. This mixture was checked by reverse phase HPLC system with authentic compound (**7**) as shown in Figure 3.

Finally, the 10% MeOH/CH_2Cl_2 solvent was removed with a stream of argon and 0.5 M sodium methoxide in MeOH was added. The reaction mixture was heated at 80 °C for 10 min and then cooled to room temperature. The solution was neutralized with 2 N HCl in MeOH and the solvent was removed under reduced pressure. The mixture was checked by reverse phase HPLC system with authentic compound ([^{19}F]**1b**) as shown in Figure 4.

After that, the mixture containing the α and β anomers, was purified by reverse phase HPLC using a semi-preparative Xterra C18 column (7.9 × 250 mm) with 5% CH_3CN/H_2O at a flow rate of 3.0 mL/min. The fraction eluted at 12-14 min was collected (Figure 5). The α/β anomeric ratio of the synthetic compounds was found to be about 1:9 ratio. Finally, the collected sample was confirmed using analytical HPLC system by co-injection with authentic compound ([^{19}F]**1b**).

Cellular uptake test. Both of L-FMAU and D-FMAU showed little uptake in the wild type MCA cells. However, cellular uptake of [^{18}F]L- and D-FMAU was significantly increased in the HSV1-TK expressing cells (MCA-TK) up to 240 min, depending on the time elapsed (Fig. 6). In the HSV1-TK expressing cells, the %ID of L-FMAU and D-FMAU was 37.28 and 65.86, respectively after 240 min (in MCA cells, %ID of L-FMAU and D-FMAU was 0.36 and 0.93 after 240 min). The cellular uptake of L-FMAU was lower than D-FMAU in MCA-TK cells, but relative uptake

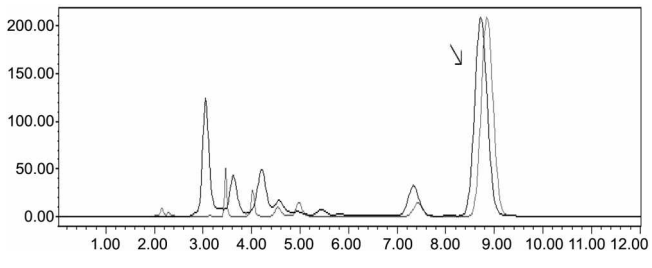


Figure 4. HPLC analysis of authentic [^{19}F]**1b** with reaction mixture ([^{18}F]**1b**) (Black:UV, Red:Radioactivity, μBondapak RP-18, 10 μ, 3.9 mm × 300 mm, CH_3CN/H_2O = 8/92 [v/v], flow rate: 1.0 mL/min, R_t: 8.8 min).

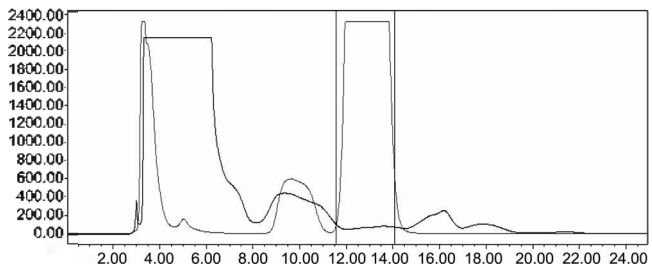


Figure 5. Semi-preparative HPLC analysis of L-[^{18}F]FMAU reaction mixture (Waters, Xterra RP-18, 10 μ, 7.9 mm × 250 mm, CH_3CN/H_2O = 5/95 [v/v], flow rate: 3.0 mL/min, R_t: 12-14 min).

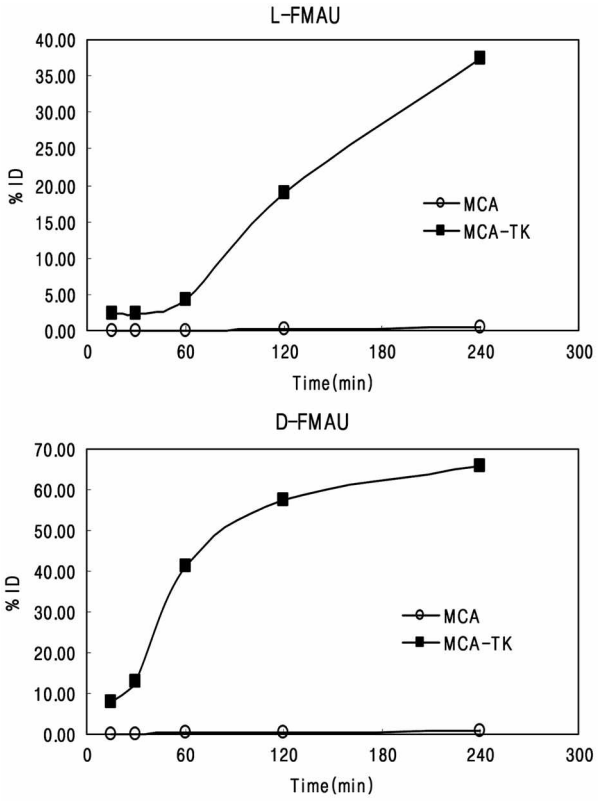


Figure 6. The cellular uptake of L-FMAU and D-FMAU in MCA and MCA-TK cell lines.

ratio (MCA-TK/MCA) of L-FMAU was more higher than D-FMAU. Relative uptake ratio of L-FMAU and D-FMAU was 100 and 70, respectively.

Conclusion

These synthetic results demonstrate that the labeling procedure for D-[^{18}F]FMAU can easily be applied to L-[^{18}F]FMAU without a significant loss in yield or anomeric ratio. The reference compound, L-[^{19}F]FMAU, was prepared in 14 steps from a commercially available L-arabinose. F-18 labeled L-[^{18}F]FMAU was synthesized by coupling the radiolabeled fluoro-sugar with the corresponding silylated thymine. The total elapsed time was about 180-200 min and the radiochemical purity was shown to be >98% with decay-corrected yields of 25-35%. The α/β anomeric ratio of L-[^{18}F]FMAU was about 1:9 and the average specific activity was greater than 55.0 GBq/mmol. In spite of double uptake of D-[^{18}F]FMAU, L-[^{18}F]FMAU showed higher MCA-TK/MCA cellular uptake ratio than D-[^{18}F]FMAU. This means that L-[^{18}F]FMAU will show lower toxicity and higher specific imaging ability for HSV1-TK reporter gene imaging.

Experimental

General. All reagents and solvents were purchased from Aldrich Chemical Co. and used without further purification. The solid phase extraction cartridge (Sep-pak, silica) was obtained purchased from Waters Associates. The QMA cartridge (SPE cartridge Chromafix 30-PS-HCO_3) was from Macherey-Nagel Ins. (U.S.A.). 2-*O*-[(Trifluoromethyl)-sulfonyl]-1,3,5-tri-*O*-benzoyl-α-L-ribofuranose (**3b**) and thymine-2,5-bis-trimethylsilyl ether (**6**) were prepared following reported methods with a little modification. Thin layer chromatography (TLC) was performed on Merck 60 F_{254} silica plates and the corresponding reference compounds were previously characterized by NMR. Radio-TLC was monitored on a Bioscan AC-3000 scanner (Washington D.C., U.S.) and high performance liquid chromatograph (HPLC) was performed on a Waters system using a 515 pump, 2487 UV detector (254 nm), and Raytest GABI γ-detector using a semi-preparative C18 reverse phase column (Waters, Xterra C18, 7.9 × 250 mm) and an analytical C18 column (Waters, mbondapak-C18, 3.9 × 300 mm). F-18 was produced with MC-50 cyclotron by irradiation of $H_2{}^{18}O$ at Korea Institute of Radiological and Medical Sciences (KIRAMS).

The preparation of 2-*O*-[(trifluoromethyl)sulfonyl]-1,3,5-tri-*O*-benzoyl-α-L-ribofuranose (3b). Trifluoromethanesulfonyl anhydride (40 μL, 0.237 mmol) was added into anhydrous pyridine (5 mL) containing 1,3,5-tri-*O*-benzoyl-α-L-ribofuranose (**2**) (100 mg, 0.216 mmol) through a syringe at 0 °C. After the reaction mixture was stirred for 10 h at room temperature, the resulting solution was poured into ice-water and the aqueous layer was extracted with methylene chloride. The extract was washed with H_2O and 3 N H_2SO_4 and cold saturated $NaHCO_3$. The combined organic layer was dried and purified by flash column chromatography (EtOAc:Hexane = 1:3) to give **3b** as a yellow oil in 85% yield. ^{1}H-NMR ($CDCl_3$): δ 4.70 (m, 2H, 5'-H), 4.87 (q, 1H, 4'-H), 5.55 (dd, 1H, 3'-H), 5.79 (q, 1H, 3'-H), 6.88 (d, 1H, 1'-H), 7.40-7.52 (m, 6H, Ar-H), 7.60-7.70 (m, 1H, Ar-H), 8.02-8.17 (m, 6H, Ar-H).

The preparation of 2'-deoxy-2'-[^{18}F]fluoro-5-methyl-1-β-L-arabinofuranosyluracil (L-[^{18}F]FMAU, [^{18}F]1b).

2-Deoxy-2-[^{18}F]fluoro-1,3,5-tri-*O*-benzoyl-α-L-arabinofuranose (8). F-18 fluoride was eluted (about 11.0 GBq, 0.5 mL) from QMA cartridge (SPE cartridge Chromafix 30-PS-HCO_3) using 50 μL of 4% TBAHCO_3 in methanol (1.0 mL). The solvent was completely removed by azeotrope with acetonitrile (x 3). To the reaction v-vial, the solution of triflate precursor (**3b**) (15 mg) in 700 μL of acetonitrile was added. The reaction mixture was heated to 80 °C for 25 min and then cooled to room temperature. After acetonitrile was removed, the mixture was dissolved with ethyl acetate and the unreacted F-18 was removed with two silica Sep-pak (light). Ethyl acetate was removed with an argon flow and the residue was used for the next step without further purification.

2-Deoxy-2-[^{18}F]fluoro-3,5-di-*O*-benzoyl-α-L-arabinofuranosyl bromide (9). The radiolabeled fluoro-sugar (**8**) was dissolved in 0.4 mL of 1,2-dichloroethane and then HBr (33 wt% in acetic acid, 0.1 mL) was added. The mixture was heated at 80 °C for 10 min and cooled to room temperature. This solvent was evaporated with toluene (1 mL) at 80 °C under a stream of argon to aid in the azeotropic removal of HBr/AcOH traces. The crude product was used for the next step without further purification.

2'-Deoxy-2'-[^{18}F]fluoro-3',5'-di-*O*-benzoyl-5-methyl-1-β-L-arabinofuranosyluracil (10 α/β). To 2-Deoxy-2-[^{18}F]-fluoro-3,5-di-*O*-benzoyl-α-L-arabinofuranosyl bromide (**9**) was added a solution of the silylated thymine (**6**) (75-85 μmol, 8-9 equiv.) in chloroform (0.7 mL). The reaction mixture was heated for 45 min at 110 °C and cooled to room temperature. This solution was passed through a Sep-Pak (silica plus) and eluted with 10% MeOH/CH_2Cl_2 (2.5 mL). The solvent was evaporated under argon gas and the crude product was used for the next step without further purification.

2'-Deoxy-2'-[^{18}F]fluoro-5-methyl-1-β-L-arabinofuranosyluracil ([^{18}F]1b, β form). The crude mixture of 2'-deoxy-2'-[^{18}F]fluoro-3,5-di-*O*-benzoyl-5-methyl-1-β-L-arabinofuranosyluracil (**10**) was added to methanolic solution of sodium methoxide (0.5 M NaOMe in methanol, 0.5 mL). The mixture was heated to 80 °C for 10 min and cooled to room temperature. The solution was neutralized with 2 N HCl in methanol, the solvent was removed with an argon flow. The crude material was diluted with 5% CH_3CN/H_2O and filtered with 0.45 μm HPLC filter. The mixture, including the α and β anomers, was purified by RP-HPLC system using a semi-preparative Xterra-C18 column (7.9 × 250 mm) with 5% CH_3CN/H_2O at a flow rate of 3.0 mL/min. The product was collected at 12-14 min. An aliquot of the final product ([^{18}F]**1b**, β-form) was analyzed by analytical HPLC and confirmed by co-injection with [^{19}F]**1b**.

Cell line. The MCA cell line is a MCA RH7777 hepatoma cell line, and MCA-TK cells are a cell line derived from HSV1-TK expression cells using a retroviral vector. Both

cell lines were supported by Dr. Kwon of Molecular Oncology Laboratory, KIRAMS.

Cellular uptake test. MCA and MCA-TK cells were grown to 5 × 10^5 cells/well in 6-well culture plates and incubated at 37 °C for 24 h. [^{18}F]L-/D-FMAU was added to each well (20 μC^3/2 mL) and the mixture was incubated for 15, 30, 60, 120 and 240 min at 37 °C with 5% CO_2. After that, the medias were removed, the cells rinsed with PBS, and adherent cells were harvested. Finally, the radioactivity was determinded by gamma counter.

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