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A novel method for stereospecific fluorination at the 2′-arabino-position of pyrimidine nucleoside: synthesis of [^{18}F]-FMAU

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Direct fluorination of a pyrimidine nucleoside at the 2′-arabino-position has been deemed to be extremely difficult, if not impossible. The conventional synthesis of 2′-deoxy-2′-fluoro-5-methy-1-β-D-arabinofuranosyluracil (FMAU) and its 5-substituted analogs involves stereospecific fluorination of the 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose-2-sulfonate ester followed by bromination at the C_1-postion, and then coupling with pyrimidine-bis-trimethylsilyl ether. Several radiolabeled nucleoside analogs, including [^{18}F]FMAU, and other 5-substituted analogs, were developed according to this methodology. However, routine production of these compounds using this multi-step process is inconvenient and limits their clinical application. We developed a novel precursor and method for direct fluorination of preformed nucleoside analogs at the 2′-arabino position, exemplified via radiosynthesis of [^{18}F]FMAU. The 2′-methylsulfonyl-3′,5′-*O*-tetrahydropyranyl-N^3-Boc-5-methyl-1-β-D-ribofuranosiluracil was synthesized in multiple steps. Radiofluorination of this precursor with K^{18}F/kryptofix produced 2′-deoxy-2′-[^{18}F]fluoro-3′,5′-*O*-tetrahydropyranyl-N^3-Boc-5-methyl-1-β-D-arabinofuranosiluracil. Acid hydrolysis followed by high-performance liquid chromatography purification produced the desired [^{18}F]FMAU. The average radiochemical yield was 2.0% (decay corrected, $n = 6$), from the end of bombardment. Radiochemical purity was >99%, and specific activity was >1800 mCi/μmol. Synthesis time was 95–100 min from the end of bombardment. This direct fluorination is a novel method for synthesis of [^{18}F]FMAU, and the method should be suitable for production of other 5-substituted pyrimidine analogs, including [^{18}F]FEAU, [^{18}F]FIAU, [^{18}F]FFAU, [^{18}F]FCAU, and [^{18}F]FBAU.

Keywords: stereospecific fluorination; Fluorine-18; pyrimidine nucleoside; PET

Introduction

Several 2′-deoxy-2′-fluoro-5-substituted-1-β-D-arabinofuranosyluracils are known to be antiviral agents against herpes simplex virus type 1 (HSV-1),[1–3] and anticancer agents against leukemia.[4] Some of these pyrimidine nucleoside analogs have been radiolabeled with [^{18}F] for PET imaging of tumor proliferation and herpes simplex virus thymidine kinase (HSV1-tk) reporter gene expression.[5–7] On the basis of many reports from the laboratory of J. J. Fox and K. A. Watanabe, at Memorial Sloan Kettering Cancer Center, New York, it was reported that the direct introduction of a fluoro group in the 2′-up (arabino) position from a preformed nucleoside would be 'difficult, if not impossible',[8] (p. 233) because of neighboring-group participation of the carbonyl group at C-2 of the pyrimidine moiety.[9,10] Direct SN2-type reactions with inversion of configuration at the 2′-position of a pyrimidine were not possible owing to the formation of an intermediate 2,2′-anhydronucleoside (Figure 1).[11] Thus, reaction of 5′-trityl-2′-tosyl-5-methyluridine with sodium iodide produced the corresponding 2′-iodo-ribofuranose.[11,12] However, when the 2,2′-anhydro intermediate was used under the same conditions (sodium iodide in acetonylacetone at 100°C) no reaction occurred. It was explained by Fox and Miller,[12] and Codington *et al.*[13] that the successful conversion of the 2′-tosyloxy derivative to its 2′-iodo analog through the anhydro intermediate was probably due to the presence of a small amount of *p*-toluenesulfonic acid liberated during the formation of the 2,2′-anhydro intermediate. Since that report, all 2′-arabino-fluoro-pyrimidine nucleoside analogs have been synthesized using an alternative multi-step methodology such as stereospecific fluorination of 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose-2-sulfonate ester followed by bromination at the C_1-postion; and then coupling the protected 1-bromosugar with pyrimidine-bis-trimethylsilyl ether, and then hydrolysis of the protecting groups.[1,3,8,14–16] Previously, Alauddin *et al.* developed [^{18}F]-labeled 2′-fluoro-arabinofuranosyluracil derivatives, such as 2′-deoxy-2′-[^{18}F]fluoro-5-methyl-1-β-D-arabinofuranosyluracil ([^{18}F]-FMAU) and some other 2′-deoxy-2′-[^{18}F]fluoro-5-substituted-1-β-D-arabinofuranosyluracil derivatives,[5,6] following this multi-step process. The radiosynthesis of these compounds has also been reported by others using the same methodology.[7]

The multi-step synthetic method for the 2′-fluoro-arabionucleoside is currently used for radiosynthesis of [^{18}F]-FMAU and the other 5-substituted analogs for clinical and pre-clinical applications;[17–22] however, this method is inconvenient owing

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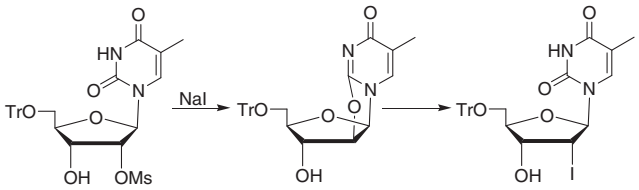

Figure 1. Formation of the 2,2′-anhydro-5-methyluridine in the reaction of 2′-tosyl-5′-trityl-5-methyluridine with NaI.

to the multi-step synthesis after radiofluorination. Therefore, there is a need for one-step stereospecific direct fluorination at the 2′-arabino position of the intact nucleoside, especially for radiosynthesis of [^{18}F]-labeled compounds. Direct SN2-type reactions with inversion of configuration at the 2′-position of a purine nucleoside have been successful;[23] however, for pyrimidine nucleoside, these SN2 type reactions have seldom been performed or attempted because of neighboring-group participation of the carbonyl group at C-2 of the pyrimidine moiety. It is reasonable to assume that substitution of the N^3-position by a suitable protecting group would prevent the formation of a 2,2′-anhydro compound (Figure 1) and therefore increase the chance of an SN2 reaction at the 2′-position. Surprisingly, this strategy has rarely been utilized, and only a few attempts have been made for stereospecific substitution at the 2′-arabino position on a pyrimidine nucleoside.[24–26] In those studies, one group of investigators protected the N^3-position by substituting the hydrogen with an electron-withdrawing group such as benzoate;[25] however, subsequent substitution of the 2′-hydroxy group with a triflate was not successful.[26] The other investigators substituted the N^3-proton with a nitro group and successfully prepared a 2′-triflate using one-step reaction.[24] Thus, a N^3-nitro-3′,5′-*O*-1,1,3,3-tetraisopropyl-1,3-disiloxane-2′-triflate was prepared and reacted with tetrabutylammonium halides (Bu_4NX); the halogens used were iodide, chloride, and bromide; and the corresponding 2′-halo-arabinopyrimidine nucleosides were obtained. This was the first example of an SN2 substitution at the 2′-position of an intact pyrimidine nucleoside with the arabino configuration; however, no 2′-arabino-fluorinated compound could be prepared by this method.

Another class of nucleoside, the 4′-thioanalog of the pyrimidine nucleoside, has been fluorinated at the 2′-position with an arabino configuration using diethylaminosulfure trifluoride (DAST), in which the 4′-sulfur behaves differently than the 4′-oxygen. In this reaction, sulfur takes an active part during fluorination.[27] Most recently, synthesis of 4′-seleno-arabinofuranosyluracil has been reported,[28] in which selenium played a role similar to that of the sulfur in 4′-thio-pyrimidine. Although 2′-arabino-fluorinated compounds have been successfully synthesized in the case of 4′-thio- and 4′-seleno-nucleoside analogs, no 2′-arabino-fluoro-derivative has yet been successful for the 4′-oxo- nucleoside. In this paper, we report for the first time, direct fluorination of the intact pyrimidine nucleoside analog at the 2′-carbon with an arabino configuration, which has been remained a challenge for more than 40 years.

Experimental

Reagents and instrumentation

All reagents and solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI), and used without further purification. Solid-phase extraction cartridges (Sep-Pak, silica gel, 900 mg) were purchased from Alltech Associates (Deerfield, IL). Nonradioactive 2′-deoxy-2-fluoro-5-methyl-1-β-D-ribofuranosyluracil (FMRU) was purchased from ABX (Radeberg, Germany). FMAU was prepared in-house for the high-performance liquid chromatography (HPLC) standard.

Thin layer chromatography (TLC) was performed on pre-coated Kieselgel 60 F254 (Merck, Darmstadt, Germany) glass plates. Proton and ^{19}F NMR spectra were recorded on a Bruker 300 MHz spectrometer with tetramethylsilane used as an internal reference and hexafluorobenzene as an external reference at The University of Texas M. D. Anderson Cancer Center. High-resolution mass spectra (MS) were obtained on a Bruker BioTOF II mass spectrometer at the University of Minnesota using electrospray ionization technique.

HPLC was performed with an 1100 series pump, Agilent Technologies, (Stuttgart, Germany), with a built-in UV detector operated at 254 nm, and a radioactivity detector with single-channel analyzer (Bioscan, Washington, DC) with a semipreparative C_{18} reverse-phase column (Alltech, Econosil, 10 × 250 mm) and an analytical C_{18} column (Alltech, Econosil, 4.6 × 250 mm). An acetonitrile/water (MeCN/H_2O) solvent system (10% MeCN/H_2O) was used for purification of the radiolabeled product at a flow of 4 mL/min. Quality control analyses were performed on an analytical HPLC column with the same solvents at a flow of 1 mL/min.

Methods

Preparation of 3′, 5′-O-1,1,3,3-tetraisopropyl-1,3-disiloxane-5-methyluridine 2

A solution of 5-methyluridine **1** (1.6 g, 6.2 mmol) in pyridine (10 mL) was cooled to 0°C. Dichloro-1,1,3,3-tetraisopropyldisyloxane (1.89 g, 6.0 mmol) was added dropwise to the cold solution, and the mixture was warmed slowly to room temperature, then stirred for 6 h. The reaction mixture was filtered and the solvent evaporated, and the residue was purified by flash chromatography on a silica gel column with 0–50% ethyl acetate in hexane as gradient elution to give **2** as a white foam in 80% yield. Based on ^{1}H NMR spectrum, the compound was >98% pure. ^{1}H NMR ($CDCl_3$): 7.99 (s, 1H, NH). 7.76 (s, 1H, C_6–H), 5.66 (s, 1H, 1′-H), 4.25 (d, 1H, J = 13.8 Hz, 5′-H), 4.11–4.18 (m, 3H, 2′-, 3′- and 4′-H), 4.00 (dd, 1H, J = 2.4, 13.8 Hz, 5′-H), 2.79 (s, 1H, –OH), 1.92 (s, 3H, CH_3-H), 1.03–1.07 (m, 28H, *i*-Pr-H).

Preparation of 3′,5′-O-1,1,3,3-tetraisopropyl-1,3-disiloxane-2′-O-(trimethylsilyl)-5-methyluridine 3

Compound **2** (2.0 g, 4.0 mmol) was dissolved in dry CH_2Cl_2 (50 mL), and the solution was cooled to 0°C. Triethylamine (2.80 mL, 20.2 mmol) and trimethylsilyl chloride (TMSCl) (1.52 mL, 12.0 mmol) were added slowly to this solution via a syringe. The solution was warmed slowly to room temperature over the course of 1.5 h. The reaction mixture was poured into a cold aqueous solution of $NaHCO_3$ (1.0 M, 10 mL) and extracted twice with CH_2Cl_2 (25 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure to afford **3** as a white foam in quantitative crude yield. Compound **3** was used for the next step without further purification: ^{1}H NMR ($CDCl_3$)δ: 7.99 (s, 1H, NH). 7.76 (s, 1H, C_6–H), 5.66 (s, 1H, 1′-H), 4.25 (d, 1H, J = 13.8 Hz, 5′-H), 4.11–4.18 (m, 3H, 2′-, 3′- and 4′-H,), 4.00 (dd, 1H, J = 2.4, 13.8 Hz, 5′-H), 1.92 (s, 3H, CH_3–H), 1.03–1.07 (m, 28H, *i*-Pr-H), 0.21 (s, 9H, TMS).

Preparation of N^3-Boc-3′,5′-O-1,1,3,3-tetraisopropyl-1,3-disiloxane-5-methyluridine 4

Compound **3** (2.29 g, 4.0 mmol) was dissolved in tetrahydrofuran (THF, 50 mL), then di-*tert*-butyl dicarbonate (1.0 g, 4.8 mmol) and *N*,*N*-dimethylaminopyridine (DMAP, 0.49 g, 4.0 mmol) were added. The reaction mixture was stirred at room temperature for 12 h, when TLC showed a complete reaction ($R_f = 0.7$: 20% ethylacetate in hexane). To the above solution was added *p*-toluenesulfonic acid (TsOH) (1.52 g, 8.0 mmol), and the mixture was stirred at room temperature for 30 min. The reaction mixture was then cooled to 0°C, and triethylamine (2.2 mL, 16.0 mmol) was added dropwise; and the reaction mixture was stirred for another 10 min. The solution was filtered and diluted with 1 M aqueous $NaHCO_3$ (15 mL) and extracted with ethyl acetate (3×50 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated to give a colorless oil that was purified by flash chromatography on a silica gel column using 0–50% ethyl acetate in hexane with gradient elution. The appropriate fractions were combined and evaporated to give a colorless thick oil, which was re-dissolved in CH_2Cl_2 and evaporated under high vacuum to give **4** as a white foam in 83% yield. Based on ^{1}H NMR spectrum, the compound was >98% pure. ^{1}H NMR ($CDCl_3$)δ: 7.40 (d, 1H, $J = 1.2$ Hz, C_6–H), 5.78 (s, 1H, 1′-H), 4.40 (m, 1H), 4.20 (m, 2H), 4.11 (m, 1H), 4.03 (m, 1H), 2.79 (d, 1H, $J = 0.7$ Hz, –OH), 1.94 (d, 3H, $J = 1.0$ Hz, CH_3–H), 1.62 (s, 9H, N-Boc), 1.06–1.09 (m, 28H, *i*-Pr-H). High-resolution MS (*m/z*): [M+Na] for $C_{27}H_{48}N_2O_9Si_2$, calculated, 623.2796; found, 623.2830.

Preparation of N^3-Boc-3′,5′-O-1,1,3,3-tetraisopropyl-1,3-disiloxane-2′-O-methylsulfonyloxy-5-methyluridine 5

Compound **4** (0.50 g, 0.83 mmol) was dissolved in 5 mL of THF and cooled to 0°C, then triethylamine (0.56 mL, 4.1 mmol) was added, followed by the addition of methanesulfonyl chloride (0.15 mL, 1.9 mmol). The mixture was stirred at 0°C for 10 min, warmed to room temperature, and stirred for an additional 1.5 h. The reaction mixture was filtered, and THF was removed under reduced pressure to give a colorless oil that was purified by flash chromatography on a silica gel column with 20% ethyl acetate in hexane to give **5** as a white foam in 90% yield. Based on ^{1}H NMR spectrum, the compound was >98% pure. ^{1}H NMR ($CDCl_3$) δ: 7.53 (s, 1H, C_6–H), 5.78 (s, 1H), 5.04 (d, 1H, $J = 4.5$ Hz), 4.40 (m, 1H), 4.20 (m, 1H), 4.11 (m, 1H), 4.03 (m, 1H), 3.25 (s, 3H, -Ms), 1.94 (s, 3H, CH_3), 1.63 (s, 9H), 1.06–1.09 (m, 28H). High-resolution MS (*m/z*): [M+Na] for $C_{28}H_{50}N_2O_{11}SSi_2$, calculated, 701.2572; found, 701.2605.

Preparation of N^3-Boc-2′-O-methylsulfonyloxy-5-methyluridine 6

To a solution of **5** (0.27 g, 0.4 mmol) in THF (10 mL) was added *n*-Bu_4NF (1M in THF, 1.0 mL, 1.0 mmol). The reaction mixture was stirred at room temperature for 30 min, when TLC showed no starting material remained. The reaction mixture was concentrated and the residue purified by flash chromatography on a silica gel column with 10% methanol in dichloromethane to give **6** as a white foam in 97% yield and 98% purity.^{1}H NMR ($CDCl_3$)δ: 7.49 (s, 1H, C_6–H) 5.89 (d, 1H, $J = 4.5$ Hz,1′-H), 5.27 (t, 1H, $J = 4.5$ Hz, 2′-H), 4.60 (t, 1H, $J = 5.2$ Hz, 3′-H), 4.20 (m, 1H, 4′-H), 4.08−3.83 (m, 2H, 5′-H), 3.23, (s, 3H, -Ms), 2.20–2.80 (broad s, 2H, –OH), 1.95 (s, 3H, CH_3), 1.62 (s, 9H, N-Boc). High-resolution MS (*m/z*): [M+H] for $C_{16}H_{24}N_2O_{10}S$, calculated, 437.1224; found, 437.1222.

Preparation of N^3-Boc-3′,5′-O-bis-tetrahydropyranyl-2′-O-methylsulfonyloxy-5-methyluridine 7

To a solution of compound **6** (62.0 mg, 0.14 mmol) in dry THF (5 mL) and a catalytic amount of TsOH (10.0 mg) was added 3,4-dihydro-2*H*-pyrane (DHP, 0.36 mL, 0.4 mmol). The reaction mixture was stirred at room temperature for 16 h, and then neutralized by the addition of triethyl amine (20.0 μL), and the solvent was evaporated. The residue was purified by flash chromatography on a silica gel column with 20% ethyl acetate in hexane to give **7** (a mixture of diasteriomers) as a colorless oil in 48% yield. Based on HPLC chromatogram, the compound was >98% pure. ^{1}H NMR ($CDCl_3$)δ: 7.79, 7.78, 7.68, 7.67 (4s, 1H, C_6–H), 6.06, 5.98 (2d, $J = 3.3$ Hz, 2.4 Hz, 1H, 1′-H), 4.55−4.47 (m, 1H, 2′-H), 4.72−3.54 (m, 10H, 3′-5′-H and THP), 3.23, 3.22 (2s, 3H, Ms), 2.43−2.37 (m, 1H, 2′-H), 1.96, 1.92 (2s, 3H, CH_3), 1.83−1.70 (m, 4H, THP), 1.62 (m, 9H), 1.63−1.58 (m, 8H, THP). High-resolution MS (*m/z*): [M+Na] for $C_{26}H_{40}N_2O_{12}S$, calculated, 627.2200; found, 627.2235.

Preparation of [^{18}F]FMAU 8

The aqueous [^{18}F]fluoride produced from the cyclotron by the reaction of ^{18}O(p, n)[^{18}F] was trapped on an ion-exchange cartridge (Chromafix 30-PS-HCO_3, ABX) and eluted with an aqueous solution of K_2CO_3 (2.75 mg/mL) into a V-vial contaning kryptofix 2.2.2 solution (12.0 mg/mL) in acetonitrile. Water was removed by an azeotropic evaporation at 80°C with acetonitrile (1.0 mL) under a stream of argon. A solution of **7** (2–3 mg) in acetonitrile (0.3 mL) was added to the dried $K^{18}F$/kryptofix 2.2.2. The reaction mixture was heated at 80°C for 20 min. The crude reaction mixture was passed through a silica Sep-Pak cartridge followed by elution with two portions of ethyl acetate (2.5 mL, total) which was evaporated at 80°C under a stream of argon. The residue was dissolved in methanol (0.3 mL), 1M methanol/HCl solution (0.1 mL) was added, and the mixture was heated at 80°C for 10 min. The solvent was evaporated, and the residue was dissolved in HPLC solvent (9% acetonitrile/water, 1.0 mL) and purified by HPLC using a semipreperative column. The product was eluted with 9% acetonitrile/water at a flow of 4 mL/min. The appropriate fraction (radioactive) was collected between 11.5 and 12.5 min, and the solvent was evaporated under reduced pressure. The product was dissolved in saline, and an aliquot of the product [^{18}F]FMAU **8** was analyzed on an analytical HPLC column to verify its identity and purity by coinjection with the nonradioactive authentic samples FMAU and FMRU.

A carrier-added synthesis was performed following the above method, and the product [$^{18/19}$F]FMAU **8** was saved for decay. Two days later, the product [^{19}F]FMAU **8** was submitted for analysis by mass spectrometry. MS (*m/z*): [M+H] for $C_{10}H_{14}N_2O_5F$, 261.12.

Results and discussion

Figure 2 presents the synthetic scheme for the preparation of [^{18}F]FMAU **8**. The synthesis began with a selective sequence of protection and deprotection on 5-methyluridine functionalities. We first prepared the 3′,5′-*O*-1,1,3,3-tetraisopropyl-1,3-disiloxane-5-methyluridine **2** from the 5-methyluridine according to published methods.[24,29] Compound **2** was obtained in 80% yield. The ^{1}H NMR spectrum was in agreement with previously published results.[29] This one-step protection of the 3′- and 5′-hydroxyl groups turned out to be advantageous; it allowed for

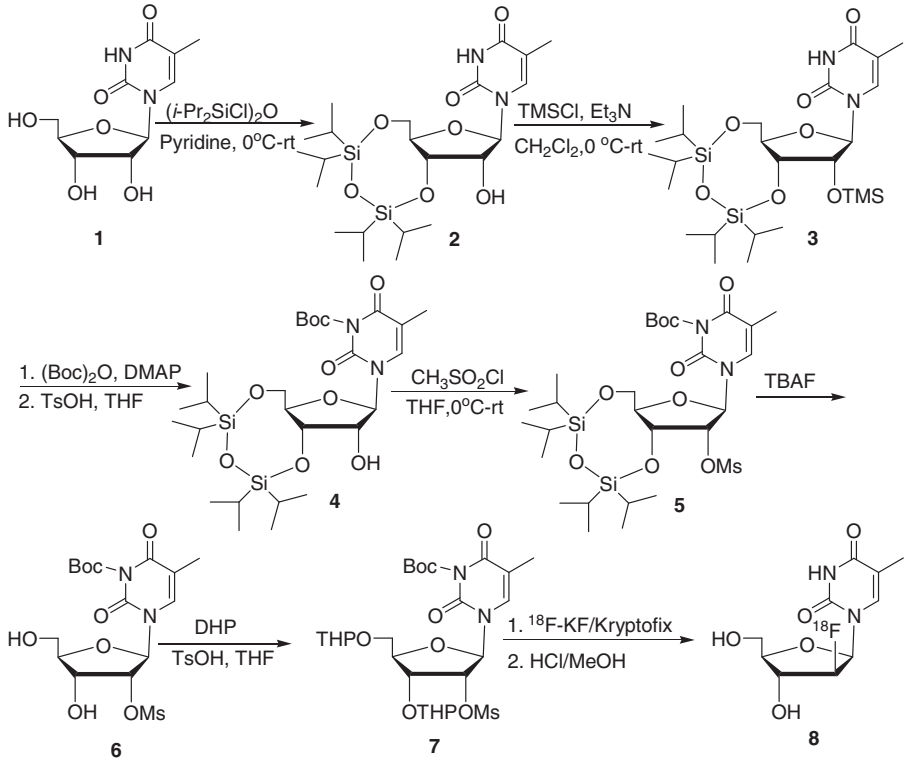

Figure 2. Synthetic scheme for the preparation of 2′-deoxy-2′-[^{18}F]fluoro-5-methyl-1-β-D-arabinofuranosyluracil ([^{18}F]FMAU).

the selective protection of the 2′-hydroxyl group with another protecting group. The protecting group, 1,1,3,3-tetraisopropyl-1,3-disiloxane, was stable under mildly acidic conditions. In the following step, reaction of **2** with TMSCl afforded the 2′-*O*-trimethylsilyl derivatve **3** in quantitative yield as a crude product. It is worth noting that the TMS group gets hydrolyzed readily on the silica gel column during purification; therefore, compound **3** was used in the following step without further purification. The ^{1}H NMR spectrum of **3** showed all the characteristic peaks, including the TMS (0.21 ppm, s, 9H).

Compound **4** was prepared from **3** by reaction with di-*tert*-butyl dicarbonate (Boc anhydride) in the presence of a stoichiometric amount of DMAP, and obtained in 83% yield. The reaction was monitored by TLC, which confirmed the completion of the reaction within 12 h. After the completion of the N-Boc protection, the 2′-*O*-trimethylsilyl intermediate was not isolated because of the sensitivity of the TMS group to chromatographic purification on a silica gel column. Instead, the TMS group was hydrolyzed using TsOH at room temperature and the free 2′-hydroxy compound **4** was purified.

Compound **5** was prepared in 90% yield by reaction of **4** with methanesulfonyl chloride in THF in the presence of triethyl amine. The ^{1}H NMR spectrum of compound **5** showed the peak characteristic of the Ms group at 3.25 ppm. In addition, compound **5** was further analyzed by high-resolution MS. The 3′,5′-*O*-1,1,3,3-tetraisopropyl-1,3-disiloxane of **5** was selectively removed by using 2.0 equivalents of *n*-Bu_4NF in THF, and compound **6** was obtained in 97% yield. Compound **7** was prepared by reaction of **6** with DHP in the presence of a catalytic amount of TsOH in THF according to a previously published method[30] in 48% yield. Although the reported yield for similar protection was quite high,[30,31] we obtained moderate yield in this experiment, because of the steric hindrance of the 2′-*O*-Ms and 5′-*O*-THP limiting the accessibility of the DHP to the 3′-OH. Another compound was isolated from this reaction as a by-product, which was identified to be N^3-Boc-5′-*O*-bis-tetrahydropyranyl-2′-*O*-methylsulfonyloxy-5-methyluridine (a mono-THP ether), characterized by ^{1}H NMR and high-resolution MS. Isolation of this compound supports the steric hindrance of the 2′-*O*-Ms and 5′-*O*-THP during the formation of **7**. The addition of excess DHP and TsOH and a relatively longer reaction time did not significantly increase the yield.

The radiolabeled compound **8** ([^{18}F]-FMAU) was synthesized by no-carrier-added nucleophilic fluorination of **7** using K^{18}F/kryptofix 2.2.2. The radiochemical yield was low and variable (1.5–2.5%), with an average yield of 2.0 % ($n = 6$, decay corrected). The crude reaction mixture after radiofluorination was passed through a silica Sep-Pak cartridge to remove the unreacted fluoride then eluted with ethyl acetate. The crude product was hydrolyzed with 1 M HCl/methanol solution by heating at 80°C for 10 min. HPLC purification of the crude product produced the pure compound [^{18}F]-FMAU **8**. After removal of the HPLC solvent under reduced pressure, the product was dissolved in saline and then analyzed by analytical HPLC. The purity of **8** was checked on an analytical column, and its identity was confirmed by a coinjection of the radioactive product with an authentic nonradioactive standard compound, FMAU. Furthermore, we performed a carrier-added synthesis and analyzed the isolated product by MS that showed the product to be FMAU.

One can envision that compound **7** might form the 2,2′-anhydro intermediate through the donation of a lone pair of electrons on the N^3-nitrogen, as presented by Serra *et al.*[24] This intermediate may react with the [^{18}F]-fluoride to produce the ribo-analog (FMRU). In order to prove the correct stereochemistry, we further analyzed the final product [^{18}F]-FMAU **8** by HPLC, coinjected with a mixture of nonradioactive standards

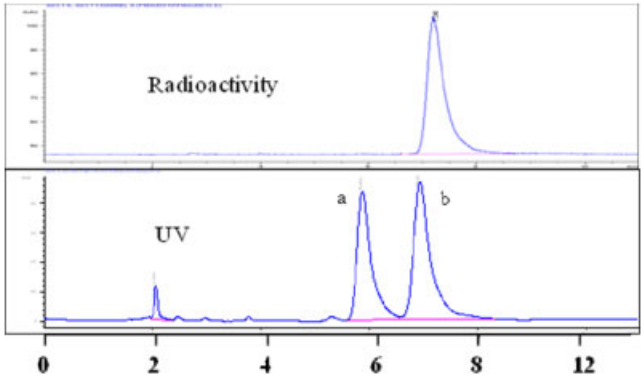

Figure 3. HPLC chromatogram of [^{18}F]-FMAU, coinjected with standard solution of (a) FMRU and (b) FMAU: Analytical C_{18} column, 10% MeCN/H_2O; flow 1.0 mL/min. FMRU and FMAU were eluted at 5.87 and 6.94 min, respectively.

FMRU (ABX) and FMAU. The HPLC chromatogram (Figure 3) shows that the product was co-eluted with FMAU at 6.94 min, whereas the ribo-analog FMRU eluted much earlier at 5.87 min. Thus, the radioactive product was confirmed to be a 2′-up (arabino) nucleoside. The HPLC chromatogram of the crude product did not show any evidence of formation of the ribo-analog FMRU. Therefore, we conclude that the fluorine was inserted only into the 2′-arabino position of the nucleoside. This is the first example of stereospecific fluorination at the 2′-arabino position of a preformed pyrimidine nucleoside.

The average radiochemical yield in this synthesis was 2.0% (decay corrected) with radiochemical purity of >99% and specific activity of >1800 mCi/μmol. The synthesis time was 95–100 min from the end of bombardment. Although the overall yield in this synthesis is low, this approach is not still insufficient for routine synthesis. Further optimization of the reaction conditions including the preparations of other precursors, is in progress.

Conclusion

A novel method for stereospecific fluorination of the intact pyrimidine nucleoside at the 2′-arabino position has been achieved for the first time. This method is applicable for radiosynthesis of the 2′-deoxy-2′-fluoro-5-methyl-1-β-D-arabino-furanosyluracil ([^{18}F]FMAU) and its 5-substituted analogs, including [^{18}F]FEAU, [^{18}F]FIAU, [^{18}F]FFAU, [^{18}F]FCAU, and [^{18}F]FBAU for PET. Further optimization for improvement on the radiochemical yield is in progress.

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