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An investigation on stereospecific fluorination at the 2′-arabino-position of a pyrimidine nucleoside: radiosynthesis of 2′-deoxy-2′-[^{18}F]fluoro-5-methyl-1-β-D-arabinofuranosyluracil

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ABSTRACT

Direct fluorination at the 2′-arabino-position of a pyrimidine nucleoside has been a long-standing challenge, yet we recently reported such a stereospecific fluorination for the first time in the synthesis of [^{18}F]FMAU, albeit in low yields. Herein we report the results of an investigation on stereospecific fluorination on a variety of precursors for synthesis of [^{18}F]FMAU. Several precursors were synthesized in multiple steps and fluorination was performed at the 2′-arabino-position using K[^{18}F]/kryptofix 2.2.2. All precursors produced [^{18}F]FMAU in low yields.

1. Introduction

2′-Deoxy-2′-fluoro-5-substituted-1-β-D-arabinofuranosyluracils are biologically important analogues of thymidine, because of their anticancer[1] and antiviral properties.[2–4] Therefore, many attempts have been made to develop facile methods for single-step fluorination of various protected pyrimidine nucleoside precursors.[5–7] In an attempt to prepare 2′-iodo-arabinothymidine from 5′-trityl-2′-tosyl-5-methyluridine by reaction with sodium iodide, an intermediate 2,2′-anhydronucleoside product was formed.[5,6] Upon further heating of the crude reaction mixture from this preparation 2′-iodo-ribofuranose was produced instead of an arabino-derivative. Fox and Miller[6] and Codington et al.[7] explained that the conversion of the 2′-tosyloxy derivative to its 2′-iodo-ribo analogue via the 2,2′-anhydro intermediate was catalyzed by the presence of a small amount of *p*-toluenesulfonic acid (TSOH) liberated during the formation of the 2,2′-anhydro intermediate. Thus, direct nucleophilic substitution (S_N2-type) reactions with inversion of configuration at the 2′-position of a pyrimidine were not possible due to the formation of an intermediate 2,2′-anhydronucleoside.[5–9]

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' because of neighboring-group participation of the carbonyl oxygen at C_2-position of the pyrimidine moiety.[10] Therefore, the synthesis of these 2′-arabino-fluoro-pyrimidine nucleoside analogues was developed using a multistep methodology, such as stereospecific fluorination of 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose-2-sulfonate ester to produce 1,3,5-tri-*O*-benzoyl-β-D-2-fluoro-arabinofuranose, bromination of the 1,3,5-tri-*O*-benzoyl-β-D-2-fluoro-arabinosugar at the C_1-postion, and then coupling of the 1,3,5-tri-*O*-benzoyl-β-D-2-fluoro-1-bromo-arabinofuranose with pyrimidine-bis-trimethylsilyl ether to produce the protected pyrimidine nucleoside analogue. Finally hydrolysis of the protecting groups with a strong base and purification produced the desired 2′-fluoro-1-β-D-arabino-pyrimidine nucleoside.[2,4,11–13]

Because of the anticancer and antiviral properties, some of these fluorinated pyrimidine nucleoside analogues have been radiolabeled with ^{18}F for positron emission tomography (PET) of tumor proliferation[14–16] and herpes simplex virus type 1-thymidine kinase (HSV1-tk) reporter gene expression.[17–22] 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) for the first time[23] and some other 2′-deoxy-2′-[^{18}F]fluoro-5-substituted-1-β-D-arabinofuranosyluracil derivatives,[24] by modification of the multistep process, such as radiofluorination of 1,3,5-tri-*O*-

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benzoyl-α-D-ribofuranose-2-trifluoromethylsulfonate ester, followed by bromination of the radiolabeled sugar and coupling of the radiolabeled 1-bromosugar with pyrimidine-bis-trimethylsilyl ether. The coupled product was hydrolyzed and purified by high-performance liquid chromatography (HPLC). Other researchers have also reported the radiosynthesis of these compounds using the same methodology.[25] This multistep synthetic method is currently used for synthesizing [^{18}F]FMAU and other 5-substituted analogues for preclinical and clinical applications;[14–17,20–22,26,27] however, this method requires multiple steps after radiofluorination of the sugar and cannot be applied to a commercial automated synthesis module for routine production. Therefore, there is a need for one-step stereospecific fluorination at the 2′-arabino-position of the intact nucleoside, especially for radiosynthesis of ^{18}F-labeled compounds using automated synthesis module.

Direct S_N2-type fluorination reactions with inversion of configuration at the 2′-position of the sugar moiety in a purine nucleoside have been successful;[28] however, for pyrimidine nucleoside, these S_N2-type reactions have seldom been performed or attempted because of neighboring-group participation of the carbonyl oxygen at C_2-position of the pyrimidine moiety. The assumption that substitution of the N^3-position with a suitable protecting group would prevent the formation of a 2,2′-anhydro compound and therefore increase the likelihood of an S_N2 reaction at the 2′-position is reasonable. Surprisingly, this strategy has rarely been used, and only a few attempts have been made to stereospecifically substitute at the 2′-arabino-position on a pyrimidine nucleoside.[29–31] In some instances, the N^3-position was protected by substituting the hydrogen with an electron withdrawing group, such as benzoate;[30] however, subsequent substitution of the 2′-hydroxyl group with a triflate was not successful.[31] In the other instance the N^3-proton was substituted with a nitro-group and successfully prepared a 2′-triflate using a one-step reaction.[29] Thus, an *N*^{3}-nitro-3′,5′-*O*-1,1,3,3-tetraisopropyl-1,3-disiloxane-2′-triflate was prepared and the precursor was reacted with tetrabutylammonium halides (Bu_4NX); the halogens were iodide, chloride, and bromide. The corresponding 2′-halo-arabinopyrimidine nucleosides were obtained in reasonably good yields. This was the first demonstration of an S_N2 substitution at the 2′-position of an intact pyrimidine nucleoside with the arabino-configuration; however, in this methodology no 2′-arabino-fluorinated compound has been reported.

The 4′-thioanalogue of the pyrimidine nucleoside underwent fluorinated at the 2′-position with an arabino-configuration using diethylaminosulfur trifluoride, whereby the 4′-sulfur behaved differently than the 4′-oxygen. In this reaction, sulfur took an active part during fluorination and assisted to produce the desired 2′-arabino-fluoro-compound.[32] The synthesis of a 4′-seleno-2′-fluoro-arabinofuranosyluracil has also been reported,[33] in which selenium played a role similar to that of the sulfur in 4′-thio-pyrimidine. Most recently, we reported a synthesis of 2′-arabino-fluoro-derivative ([^{18}F]FMAU) for the 4′-oxo-nucleoside by stereospecific fluorination.[34] In this method the N^3-proton was substituted with *tert*-butyloxycarbonyl (Boc) and a precursor compound, 2′-methylsulfonyl-3′,5′-*O*-tetrahydropyranyl-*N*^{3}-Boc-5-methyl-1-β-D-ribofuranosyluracil, was synthesized in multiple steps. Radiofluorination of this precursor was performed with K[^{18}F]/kryptofix 2.2.2. to produce 2′-deoxy-2′-[^{18}F]fluoro-3′,5′-*O*-tetrahydropyranyl-*N*^{3}-Boc-5-methyl-1-β-D-arabinofuranosyluracil. Acid hydrolysis of the intermediate compound produced the desired product [^{18}F]FMAU, which was purified by HPLC. The average radiochemical yield was 2.0% (decay corrected, d.c.) from the end of bombardment. To improve the yield of this fluorination reaction, we have synthesized several new precursor compounds with two different leaving groups and various protecting groups at the 3′- and 5′-postions, and tested them for direct fluorination at the 2′-position to synthesize [^{18}F]FMAU. In this article, we report syntheses of those new precursors and results of direct fluorination of the intact pyrimidine nucleoside precursors at the 2′-carbon with an arabino-configuration under various reaction conditions.

2. Results

Fig. 1 shows the synthetic scheme for preparation of the precursor compounds and radiosynthesis of [^{18}F]FMAU **9**. Chemical yields for compounds **2**, **3**, **4**, **5**, **6a**, **7a**, and **8a** were comparable as reported previously.[34] Compound **6b** was obtained in 70% yield, which was lower than that of **6a** (90%). Hydrolysis of compounds **6a** and **6b** produced **7a** and **7b** in 97% and 70% yields, respectively. The yield of compound **7b** was also lower than that of **7a**. The 3′- and 5′-hydroxyl groups of compounds **7a** and **7b** were protected using the appropriate protecting groups, such as acetate, benzoate, methoxy methyl (Mom), and tetrahydropyranyl (THP) groups, and the products **8b**–**d**, **8e**, and **8f**–**g** were obtained in 60%, 57%, and 50% yields, respectively. Radiofluorination of compounds **8a**–**g** using K^{18}F/kryptofix 2.2.2. produced the desired product [^{18}F]FMAU in very low yields (Table 1).

Table 1 represents the results of radiofluorination reactions with radiochemical yields of [^{18}F]FMAU (**9**) from all precursors **8a**–**g** at various temperatures. All precursors produced the desired product [^{18}F]FMAU in variable yields with an average ranging from 0.08 to 2.00%. At 90 °C, **8a** produced the highest yield of 2.0% (d.c.) with radiochemical purity >99% and specific activity >1500 mCi/μmol. The synthesis time was 95–100 min from the end of bombardment.

Fig. 2 represents a quality-control analysis of [^{18}F]FMAU using HPLC, which shows that the product (radioactive trace) was co-eluted with standard FMAU (UV trace).

3. Discussion

Stereospecific fluorination at the 2′-arabino-position of a pyrimidine nucleoside has been a long-standing challenge. We recently reported such a stereospecific fluorination for the first time in the synthesis of [^{18}F]FMAU in low yields. To improve the yield of the fluorination reaction we have investigated several precursor compounds through their synthesis and application in radiofluorination reactions. Syntheses of the precursor compounds **8a**–**g** (Fig. 1) involved a sequence of protection, deprotection, and derivatization. Protection of 4′- and 5′-hydroxyl groups of 5-methyluridine **1** produced compound **2**; then protection of 2′-hydroxyl group with trimethylsilane (TMS) produced compound **3**, and finally protection of the NH proton with Boc produced compound **4**. Deprotection of the 2′-hydroxyl group of **4** produced compound **5**, which by derivatization with mesylate and nosylate produced compounds **6a** and **6b**, respectively. Deprotection of 4′- and 5′-hydroxyl groups of **6a** and **6b** produced compounds **7a** and **7b**, which were then derivatized with a variety of protecting groups to obtain compounds **8a**–**g**. The purpose of protecting the NH proton was to prevent its participation to form the 2,2′-anhydro-compound during fluorination as previously reported.[5]

Previously, we reported protection of the 2′-hydroxyl group by TMS followed by protection of the NH proton with Boc,[34] because our attempt to protect the NH proton after protection of the 2′-hydroxyl group with mesylate was not successful. However, now we have successfully protected the NH proton after protecting the 2′-OH with an electronegative group such as mesylate. Fig. 3 shows the scheme for preparation of compound **6a** from compound **2** via **10**. This is a significant improvement over the previously reported synthesis of **6a**, because this method involves only two steps, which is three steps shorter than the previous one, and both steps produced very high yields, 93% and 90% for compounds **10** and **6a**, respectively. However, an attempt to adapt this synthetic

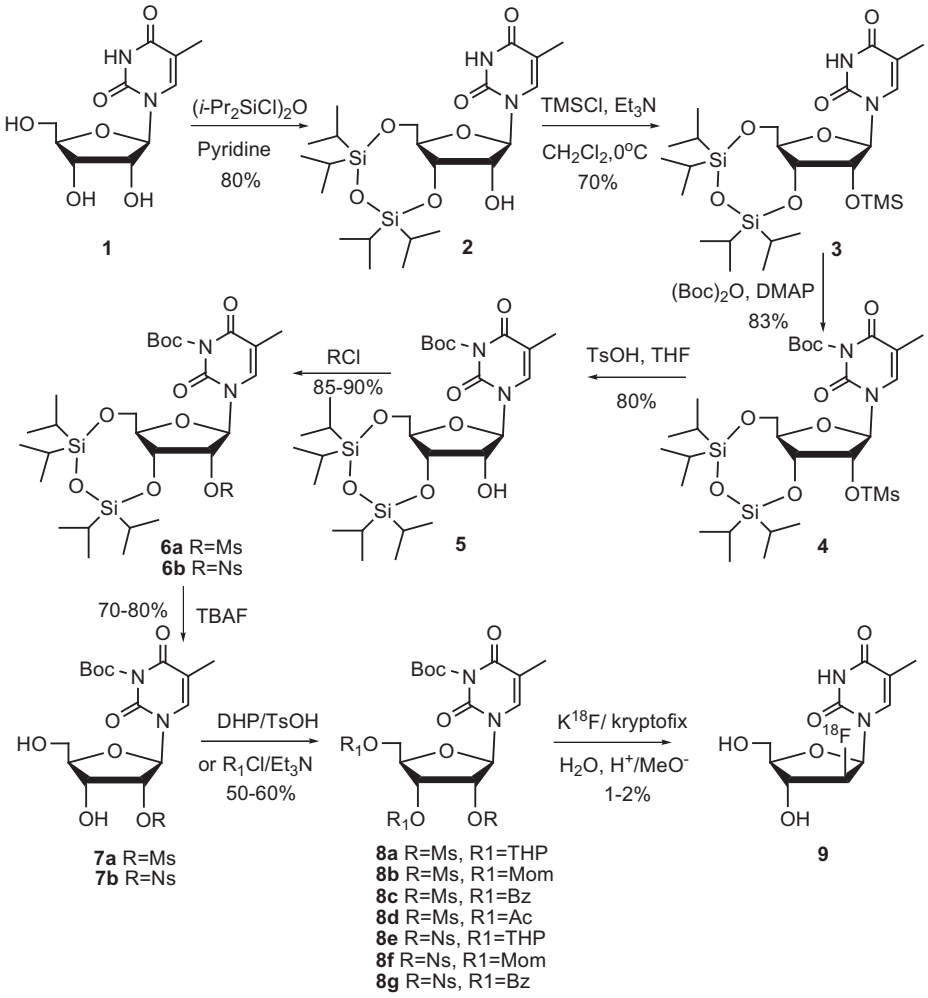

Fig. 1. Scheme for syntheses of the precursors and radiosynthesis of [^{18}F]FMAU.

Table 1
Results of radiofluorination reactions on various precursor compounds **8a**–**g** under various conditions

Experiment #	Compound #	Temperature (20 min), °C	Yield, %	Average yield, %
1	**8a**	90	2.0	
2	**8a**	85	1.2	1.56
3	**8a**	100	1.5	
4	**8b**	100	0.9	
5	**8b**	90	0.5	0.60
6	**8b**	90	0.4	
7	**8c**	90	0.1	
8	**8c**	90	0.11[a]	0.08
9	**8c**	90	0.08[a]	
10	**8d**	90	0.06[a]	
11	**8d**	90	0.2[a]	0.22
12	**8d**	90	0.4[a]	
13	**8e**	90	0.4[a]	
14	**8e**	90	0.9[a]	0.63
15	**8e**	95	0.6[a]	
16	**8f**	90	1.00	
17	**8f**	90	0.92	1.00
18	**8f**	100	1.08	
19	**8g**	90	0.9[a]	
20	**8g**	90	1.8	1.10
21	**8g**	90	0.8[a]	

[a] The yield of [^{18}F]FMAU was calculated from the analytical HPLC chromatograms of the crude reaction mixtures.

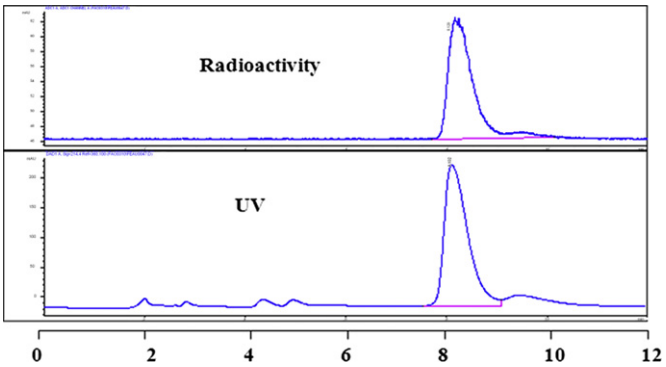

Fig. 2. HPLC chromatogram of [^{18}F]FMAU co-injected with standard FMAU; analytical C_{18} column, 9% MeCN/water, flow 1 mL/min.

methodology to prepare the 2′-nosylate precursor **6b** from compound **2** was not successful.

The yield of compound **6b** from **5** (Fig. 1) was lower (70%) than that of **6a** (90%). ^{1}H NMR spectrum of **6b** showed characteristic peaks in the aromatic region due to the nosylate group in addition to the other peaks, which were consistent with the identity and structure of the compound **6b**, and the compound was further

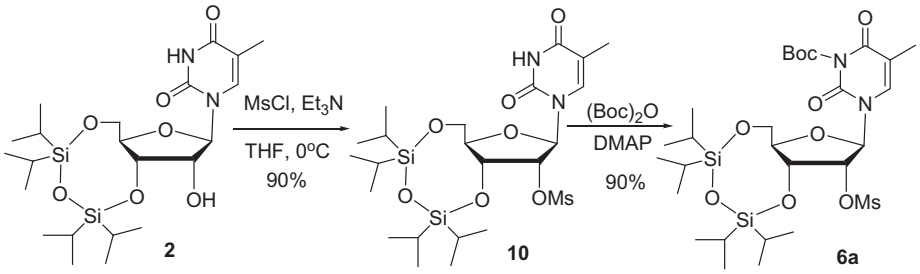

Fig. 3. Scheme for alternative improved synthesis of **6a.**

characterized by high-resolution mass spectrometry (HRMS). Hydrolysis of the siloxane group from **6b** also produced lower yield (70%) of **7b** compared with that of **7a** (97%). This lower yield of **7b** may be due to higher reactivity of nosylate compared with that of mesylate. During the preparation and work-up of **7b**, an unidentified by-product was obtained in variable yields (15–20%) from run to run. The unknown compound was less polar than **7b** as observed by thin-layer chromatography (TLC). The ^{1}H NMR spectrum of the unknown compound clearly showed the absence of the peak at 8.35 ppm for the 3,5-protons next to the nitro-group of the 4-nitro-phenylsulphonyl moiety; and reappearance of a new peak in the higher field at 8.10 ppm, which clearly indicates the absence of the nitro-group. Unfortunately, the mass spectrum of the unknown compound could not yield the molecular ion or any reasonable ion to interpret; therefore, the compound could not be completely identified. Due to the formation of this unknown by-product, the yield of the desired product **7b** was much lower than that of the mesylate **7a**.

The 4′- and 5′-hydroxyl groups of **7a** and **7b** were protected with various protecting groups to produce compounds **8a**–**g**. The THP group was inserted into **7a** and **7b** to produce **8a** and **8e** according to a previously reported method[34] using a catalytic amount of TsOH. Compound **8e** was obtained in slightly higher yield (57%) compared with that of **8a** (48%). However, these yields were low in general for both compounds compared with the other steps. One of the reasons for the lower yields of **8a** and **8e** was incomplete formation of the disubstituted THP-ethers; a small amount of the monosubstituted THP-ether was also isolated during this preparation, as a result **8a** and **8e** were produced in lower yields. Furthermore, low yield may be due to partial hydrolysis of the *N*-Boc by TsOH used during the preparation of THP-ethers. We have synthesized precursors **8b** and **8f** with another protecting group, Mom, at the 4′- and 5′-positions. In these preparations, yield of **8f** was lower (50%) than that of **8b** (60%), probably due to the more reactive nosylate group at the 2′-position of **8f** compared with the mesylate in **8b**. The benzoate protected precursors **8c** and **8g** were obtained in reasonably good yields, 60% and 50%, respectively. Similarly, compound **8d** with the mesylate group was obtained in higher yield (60%) than that of the nosylate. In general, compounds containing the nosylate group had lower yields in the subsequent steps compared with the mesylate group, probably due to the difference in reactivity between these two leaving groups.

In this study, we investigated stereospecific fluorination of intact pyrimidine nucleoside toward the radiosynthesis of [^{18}F]FMAU using two different leaving groups, mesylate and nosylate, and various other protecting groups at the 4′- and 5′-positions. We anticipated that nosylate would be more reactive than mesylate and would therefore provide better yields of [^{18}F]FMAU. However, no significant improvement in fluorination was achieved using these nosylate-containing precursors. In some instances (expt. #16–21), nosylate-containing precursors produced relatively better yields, but lower than **8a**. Moreover, the precursors containing nosylate produced several radioactive by-products after fluorination as observed by HPLC, and these were not related to the nucleoside; rather, one of this product was *p*-nitrophenylsulfonyl-^{18}F-fluoride, which was verified by co-injection with the standard compound in HPLC. The precursors containing mesylate produced a clean desired product and appeared to be better than those with nosylate in stereospecific fluorination of pyrimidine nucleoside. The most reactive leaving group known, triflate, could not be prepared for this study.

With respect to the other protecting groups at the 4′- and 5′-positions, we anticipated that the THP group might have steric hindrance because of its ring size when folded over the sugar ring and prevents the incoming fluoride ion from the same side of the sugar; therefore, selected a smaller and linear protecting group Mom. However, Mom did not show any significant improvement of the yield and had a similar effect in terms of product formation. Changing the leaving group from mesylate to nosylate in these Mom-containing precursors led to a similar yield. We also used another type of protecting groups, esters such as acetate and benzoate, at the 4′- and 5′-position. In both protecting groups, neither acetate nor benzoate with either mesylate or nosylate improved the yield.

For hydrolysis of the protecting groups from the crude products, two methods were used depending on the protecting groups. For precursors **8a**, **8b**, **8e**, and **8f**, hydrolysis was performed using HCl in MeOH and all protecting groups were hydrolyzed in a single step. For precursors **8c**, **8d**, and **8g**, hydrolysis required two steps: acid hydrolysis of the *N*-Boc using trifluoroacetic acid followed by basic hydrolysis of the esters using NaOMe. Thus, precursors **8a**, **8b**, and **8e** have the benefit of one-step hydrolysis that saves time in radiosynthesis. Compound **8a** appeared to be the best precursor in the synthesis of [^{18}F]FMAU as reported earlier.[34] This precursor produced higher yields and requires one-step hydrolysis of the protecting groups; therefore, this precursor may be used for routine production of the compound in an automated synthesis module. However, further studies, especially those using a triflate, as leaving group, are needed to improve the yields.

All precursors produced the desired product in variable low yields. The reactions that produced yields>1% were purified by HPLC using a semipreparative column and reported as isolated yields, and the reactions with yield <1% were calculated from the analytical HPLC chromatograms of the crude reaction mixtures. Reactions in MeCN at 90 °C for 20 min appeared to be optimal. This synthetic method with precursor **8a** using an automated synthesis module may be suitable in production of [^{18}F]FMAU for clinical application.

4. Summary

Several precursor compounds of intact pyrimidine nucleoside have been investigated for stereospecific fluorination at the 2′-arabino-position. Out of seven precursors, all produced the desired product in variable low yields. Compound **8a** appeared to be the best precursor in terms of product yield and simplicity of its

synthesis. Precursors like **8a** and this methodology should be applicable for radiosynthesis of the other 2′-deoxy-2′-fluoro-5-substituted-1-β-D-arabinofuranosyluracil analogues, including [^{18}F]FEAU, [^{18}F]FIAU, [^{18}F]FFAU, [^{18}F]FCAU, and [^{18}F]FBAU for PET imaging.

5. Experimental

5.1. 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). FMAU was prepared in-house for the HPLC standard.

TLC was performed on pre-coated Kieselgel 60 F_{254} (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, and ^{13}C NMR spectra were recorded on a Bruker 600 MHz spectrometer at The University of Texas M.D. Anderson Cancer Center. HRMS were obtained on a Bruker BioTOF II mass spectrometer at the University of Minnesota using the 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 a single-channel analyzer (Bioscan, Washington, DC), using a semipreparative C_{18} reverse-phase column (Alltech, Econosil, 10x250 mm) and an analytical C_{18} column (Alltech, Econosil, 4.6×250 mm). An acetonitrile/water (MeCN/H_2O) solvent system (9% 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.

5.2. Methods

*5.2.1. Compounds **1**–**5**, **6a**, **7a**, and **8a**.* Compounds **1**–**5**, **6a**, **7a**, and **8a** were synthesized as described previously.[34]

*5.2.2. Preparation of N^3-Boc-3′,5′-O-1,1,3,3-tetraisopropyl-1,3-disiloxane-2′-O-p-nitrophenylsulfonyl-5-methyluridine **6b**.* Compound **5** (0.50 g, 0.83 mmol) was dissolved in dichloromethane (5.0 mL) and pyridine (5.0 mL) was added, followed by the addition of 4-nitrobenzensulfonyl chloride (0.37 mL, 2 mmol). The mixture was stirred at rt for 12 h. The reaction mixture was filtered, and the solvent was removed under reduced pressure to render oil that was purified by flash chromatography on a silica gel column using 20% ethyl acetate (EtOAc) in hexane to give **6b** as white foam in 90% yield. ^{1}H NMR ($CDCl_3$) δ: 8.35 (d, J=9.0 Hz, 2H, aromatic, C_3H and C_5H), 8.22 (d, J=9.0 Hz, 2H, aromatic, C_2H and C_6H), 7.53 (s, 1H, C_6H), 5.58 (s, 1H, 1′H), 5.15 (d, 1H, J=4.5 Hz, 2′H), 4.40 (m, 1H, 3′H), 4.20 (m, 1H, 4′H), 4.11 (m, 1H, 5′H), 4.03 (m, 1H, 5′H), 1.92 (s, 3H, CH_3), 1.63 (s, 9H, *t*-Bu), 1.06–1.09 (m, 28H, *i*-Pr). ^{13}C NMR ($CDCl_3$) δ: 161.09, 150.73, 147.78, 147.56, 142.90, 133.73, 129.40, 124.31, 110.62, 88.68, 87.21, 84.00, 81.93, 66.82, 58.87, 27.41, 17.38, 17.32, 17.21, 16.92, 16.82, 16.81, 13.55, 12.86, 12.79, 12.73, 12.61. HRMS (m/z): [M+Na] for $C_{33}H_{51}N_3O_{13}SSi_2$, calculated, 808.2579; found, 808.2567.

*5.2.3. Preparation of N^3-Boc-2′-O-p-nitrophenylsulfonyl-5-methyluridine **7b**.* A solution of **6b** (0.27 g, 0.4 mmol) in THF (10 mL) was cooled to 0 °C and *n*-Bu_4NF (1 M in THF, 0.5 mL, 0.5 mmol) was added. The reaction mixture was stirred at rt for 15 min, when TLC showed no starting material remained. The reaction mixture was concentrated under vacuum and the residue purified by flash chromatography on a silica gel column eluted with 10% methanol in dichloromethane to give **7b** as white foam in 70% yield. Based on ^{1}H NMR spectrum, the compound was >98% pure. ^{1}H NMR ($CDCl_3$) δ: 8.35 (d, J=9.0 Hz, 2H, aromatic, C_3H and C_5H), 8.22 (d, J=9.0 Hz, 2H, aromatic, C_2H and C_6H), 7.49 (s, 1H, C_6H) 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), 2.20–2.80 (br s, 2H, OH), 1.95 (s, 3H, CH_3), 1.62 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$) δ: 161.09, 150.73, 147.78, 147.56, 142.90, 133.73, 129.40, 124.31, 110.62, 88.62, 87.21, 84.00, 81.93, 66.82, 58.87, 27.14, 12.61. HRMS: (m/z): [M+Na] for $C_{21}H_{25}N_3O_{12}S$, calculated, 566.1051; found, 566.1046.

*5.2.4. Preparation of N^3-Boc-3′,5′-O-bis-methoxymethyl-2′-O-methylsulfonyloxy-5-methyluridine **8b**.* To a solution of compound **7a** (100.0 mg, 0.23 mmol) in dry CH_2Cl_2 (5 mL), chloromethyl methyl ether (0.5 mL, 36 mmol) and *N*,*N*-diisopropylethylamine (0.5 mL) were added. The reaction mixture was stirred at rt for 16 h, and the solvent was evaporated. The residue was purified by flash chromatography on a silica gel column using 30% EtOAc in hexane to obtain **8b** as white foam in 60% yield. Based on HPLC chromatogram, the compound was>98% pure. ^{1}H NMR ($CDCl_3$) δ: 7.49 (s, 1H, C_6H) 5.89 (d, 1H, J=4.5 Hz, 1′H), 5.27 (t, 1H, J=4.5 Hz, 2′H), 4.75 (s, 4H, methylene), 4.60 (t, 1H, J=5.2 Hz, 3′H), 4.20 (m, 1H, 4′H), 4.08–3.83 (m, 2H, 5′H), 3.44 (s, 6H, OMe), 3.23, (s, 3H, Ms), 1.95 (s, 3H, CH_3), 1.62 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$) δ: 161.20, 148.10, 147.63, 133.77, 110.56, 89.03, 87.23, 84.86, 84.00, 82.86, 81.92, 66.74, 58.90, 57.56, 57.50, 39.21, 27.47, 12.64. HRMS (m/z): [M+H] for $C_{21}H_{35}N_2O_{12}S$, calculated, 437.1224; found, 437.1222.

*5.2.5. Preparation of N^3-Boc-3′,5′-O-bis-benzoyl-2′-O-methylsulfonyloxy-5-methyluridine **8c**.* Compound **7a** (100.0 mg, 0.23 mmol) was dissolved in dry THF (5 mL), triethylamine (0.5 mL) and benzoyl chloride (0.5 mL, 46 mmol) were added. The reaction mixture was stirred at RT for 10 h. The reaction mixture was filtered and the solvent was evaporated. The residue was purified by flash chromatography on a silica gel column using 30% EtOAc in hexane to give **8c** as white foam in 60% yield. Based on HPLC chromatogram, the compound was >98% pure. ^{1}H NMR ($CDCl_3$) δ: 7.93 (m, 4H, aromatic), 7.63 (m, 2H, aromatic), 7.49 (s, 1H, C_6H), 7.45 (m, 4H, aromatic), 5.89 (d, 1H, J=2.7 Hz, 1′H), 5.27 (dd, 1H, J=5.7 and 7.2 Hz, 3′H), 4.60 (dd, 1H, J=2.7 and 5.7 Hz, 2′H), 4.84 (dd, 1H, J=2.7, 12.7 Hz), 4.72–4.67 (m, 1H, 4′H), 4.57 (dd, 1H, J=2.7, 12.7 Hz, 5′H), 3.10, (s, 3H, Ms), 1.66 (s, 3H, CH_3), 1.62 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$) δ: 165.91, 165.42, 161.00, 148.20, 147.37, 133.77, 133.63, 133.41, 129.93, 129.86, 129.67, 129.63, 128.63, 128.60, 128.49, 110.56, 89.03, 87.23, 82.86, 81.92, 66.74, 58.90, 39.21, 27.43, 12.64. HRMS (m/z): [M+Na] for $C_{30}H_{32}N_2O_{12}S$, calculated, 667.1575; found, 667.1579.

*5.2.6. Preparation of N^3-Boc-3′,5′-O-bis-acetyl-2′-O-methylsulfonyloxy-5-methyluridine **8d**.* To a solution of compound **7a** (100.0 mg, 0.23 mmol) in dry pyridine (5 mL) was added acetic anhydride (0.1 mL, 1.15 mmol). The reaction mixture was stirred at rt for 8 h and the solvent was evaporated. The residue was purified by flash chromatography on a silica gel column using 30% EtOAc in hexane to give **8d** as white foam in 60% yield. ^{1}H NMR ($CDCl_3$) δ: 7.49 (s, 1H, C_6H) 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), 2.21 (s, 3H, acetyl), 1.95 (s, 3H, CH_3), 1.62 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$) δ: 170.16, 169.89, 161.00, 148.20, 147.39, 135.11, 110.93, 91.22, 87.22, 79.14, 78.83, 68.40, 61.63, 38.35, 27.43, 20.72, 20.49, 12.65. HRMS (m/z): [M+H] for $C_{21}H_{35}N_2O_{12}S$, calculated, 437.1224; found, 437.1222.

*5.2.7. Preparation of N^3-Boc-3′,5′-O-bis-tetrahydropyranyl-2′-O-p-nitrophenylsulfonyl-5-methyluridine **8e**.* Compound **7b** (50.0 mg, 0.09 mmol) was dissolved in dry THF (5 mL), a catalytic amount of

TsOH (10.0 mg) and 3,4-dihydro-2*H*-pyrane (DHP, 0.20 mL, 2.2 mmol) were added. The reaction mixture was stirred at rt for 16 h, and then neutralized by the addition of triethylamine (30.0 μL), and the solvent was evaporated. The residue was purified by flash chromatography on a silica gel column using 30% EtOAc in hexane to give **8e** (a mixture of diastereomers) as a colorless oil in 57% yield. ^{1}H NMR ($CDCl_3$) δ: 8.35 (d, J=9.0 Hz, 2H, aromatic, C_3H and C_5H), 8.22 (d, J=9.0 Hz, 2H, aromatic, C_2H and C_6H), 7.79, 7.78, 7.68, 7.67 (4s, 1H, C_6H), 6.06, 5.98 (2d, J=3.3, 2.4 Hz, 1H, 1′H), 4.55–4.47 (m, 1H, 2′-H), 4.72–3.54 (m, 9H, 3′-5′H and THP), 1.96, 1.92 (2s, 3H, CH_3), 1.83–1.70 (m, 4H, THP), 1.62 (m, 9H, *t*-Bu), 1.63–1.58 (m, 8H, THP). ^{13}C NMR ($CDCl_3$) δ: 161.09, 150.73, 147.78, 147.56, 142.90, 133.73, 129.40, 124.31, 110.62, 99.93, 98.80, 97.98, 97.37, 88.68, 87.21, 85.31, 85.65, 84.65, 84.09, 83.69, 81.93, 76.93, 66.82, 62.42, 58.87, 39.24, 38.00, 30.70, 27.41, 25.26, 25.23, 19.31, 19.09, 12.39. HRMS (*m*/*z*): [M+Na] for $C_{31}H_{41}N_3O_{14}S$, calculated, 734.2207; found, 734.2233.

*5.2.8. Preparation of N^3-Boc-3′,5′-O-bis-methoxymethyl-2′-O-p-nitrophenylsulfonyl-5-methyluridine **8f**.* To a solution of compound **7b** (100.0 mg, 0.18 mmol) in dry THF (5 mL) was added chloromethyl methyl ether (0.5 mL, 36 mmol). The reaction mixture was stirred at rt for 16 h, and the solvent was evaporated. The residue was purified by flash chromatography on a silica gel column using 30% EtOAc in hexane to give **8f** as colorless oil in 50% yield. ^{1}H NMR ($CDCl_3$) δ: 8.35 (d, J=9.0 Hz, 2H, aromatic, C_3H and C_5H), 8.22 (d, J=9.0 Hz, 2H, aromatic, C_2H and C_6H), 7.49 (s, 1H, C_6H) 5.89 (d, 1H, J=4.5 Hz,1′H), 5.27 (t, 1H, J=4.5 Hz, 2′H), 4.75 (s, 4H, methylene), 4.60 (t, 1H, J=5.2 Hz, 3′H), 4.20 (m, 1H, 4′H), 4.08–3.83 (m, 2H, 5′H), 3.44 (s, 6H, OMe), 1.95 (s, 3H, CH_3), 1.62 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$) δ: 161.09, 150.73, 147.78, 147.56, 142.90, 133.73, 129.40, 124.31, 110.62, 88.68, 87.21, 85.10, 84.61, 84.00, 81.93, 66.82, 58.87, 57.56, 57.00, 27.41, 12.61. HRMS (*m*/*z*): [M+Na] for $C_{25}H_{33}N_3O_{14}S$, calculated, 654.1581; found, 654.1714.

*5.2.9. Preparation of N^3-Boc-3′,5′-O-bis-benzoyl-2′-O-p-nitrophenylsulfonyl-5-methyluridine **8g**.* Compound **7b** (100.0 mg, 0.18 mmol) was dissolved in dry THF (5 mL), triethylamine (0.1 mL), 4-dimethylaminopyridine (45 mg, 0.37 mmol), and benzoyl chloride (0.2 mL) were added. The reaction mixture was stirred at rt for 1 h, and the solvent was evaporated. The residue was purified by flash chromatography on a silica gel column using 30% EtOAc in hexane to give **8g** as colorless oil in 50% yield. ^{1}H NMR ($CDCl_3$) δ: 8.18 (d, J=9.0 Hz, 2H, aromatic, C_3H and C_5H), 8.10 (d, J=9.0 Hz, 2H, aromatic, C_2H and C_6H), 7.93 (m, 4H, benzoyl), 7.63 (m, 2H, benzoyl), 7.45 (m, 4H, benzoyl), 7.07 (s, 1H, C_6H), 5.93 (d, 1H, J=3.6 Hz, 1′H), 5.71 (t, 1H, J=5.7 Hz, 3′H), 5.50 (dd, 1H, J=3.6, 5.4 Hz, 2′H), 4.80 (dd, 1H, J=12.3, 2.7 Hz, 5′H), 4.65 (m, 1H, 4′H), 4.59 (dd, 1H, J=12.3, 3.9 Hz, 1H, 5′H), 1.7 (s, 3H, CH_3), 1.62 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$) δ: 165.91, 165.42, 161.20, 148.10, 147.63, 142.90, 133.77, 133.63, 133.41, 129.93, 129.86, 129.67, 129.63, 129.22, 128.67, 128.60, 124.31, 110.62, 88.63, 87.21, 84.00, 81.92, 66.82, 58.87, 27.47, 12.64. HRMS (*m*/*z*): [M+Na] for $C_{35}H_{33}N_3O_{14}S$, calculated, 774.1575; found, 774.1587.

*5.2.10. Preparation of 3′,5′-O-1,1,3,3-tetraisopropyl-1,3-disiloxane-2′-O-methylsulfonyloxy-5-methyluridine **10**.* Compound **2** (0.50 g, 1.0 mmol) was dissolved in THF (5 mL) 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 rt and stirred for an additional 1.5 h. The reaction mixture was filtered and THF was removed under reduced pressure to give colorless oil, which was purified by flash chromatography on a silica gel column eluted with 20% ethyl acetate in hexane to give **10** as white foam in 90% yield. ^{1}H NMR ($CDCl_3$) δ: 8.83 (s, 1H, NH), 7.54 (d, 1H, C_6H), 5.80 (s, 1H, 1′H), 5.04 (d, 1H, J=4.5 Hz, 2′H), 4.40 (m, 1H, 3′H), 4.20 (m, 1H, 4′H), 4.11 (m, 1H, 5′H), 4.03 (m, 1H, 5′H), 3.25 (s, 3H, Ms), 1.94 (s, 3H, CH_3), 1.06–1.09 (m, 28H, *i*-Pr). ^{13}C NMR ($CDCl_3$) δ: 161.20, 148.10, 147.63, 133.77, 110.56, 89.03, 87.23, 82.86, 81.92, 58.87, 39.21, 17.39, 17.33, 17.22, 17.20, 16.95, 16.82, 16.78, 13.54, 12.86, 12.75, 12.64. HRMS (*m*/*z*): [M+Na] for $C_{23}H_{42}N_2O_9SSi_2Na$, calculated, 601.2047; found, 601.2060.

*5.2.11. Fluorination of the precursors: preparation of [^{18}F]FMAU **9**.* All precursors were reacted with $K^{18}F$/kryptofix 2.2.2. under similar conditions with a slight variation in temperature. The aqueous solution of [^{18}F]fluoride/kryptofix 2.2.2. was purchased from Cyclotope Inc. (Houston, TX) (*CAUTION*: A hot cell or highly shielded area should be used for using radioactive material.). Water was removed by an azeotropic evaporation at 90 °C with acetonitrile (1.0 mL) under a stream of argon. A solution of the precursors **8a**–**g** (3–5 mg) in dry acetonitrile (0.3 mL) was added to the dried $K^{18}F$/kryptofix 2.2.2. The reaction mixture was heated at 90 °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 90 °C under a stream of argon. The residue from reactions of **8a**, **8b**, **8e**, and **8f** was dissolved in methanol (0.3 mL), 1 M 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 semipreparative column. The residue from reactions of **8c**, **8d**, and **8g** was dissolved in trifluoracetic acid (0.3 mL), heated for 5 min at 80 °C then trifluoracetic acid was evaporated completely. The residue was dissolved in MeOH (0.3 mL) and NaOMe solution (0.5 M, 0.2 mL) was added. The reaction mixture was heated for 7 min at 80 °C. Solvent was evaporated and the crude product was neutralized with 1 M HCl (0.2 mL) and purified by HPLC as described above. 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 partially evaporated under reduced pressure. An aliquot of the product [^{18}F] FMAU **9** was analyzed on an analytical HPLC column to verify its purity and identity by co-injection with the nonradioactive authentic sample of FMAU. For those reactions with very low yield, the crude products were analyzed on an analytical column and the % yield was calculated from the radiochromatograms.

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