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Research Article

Synthesis of 3′-deoxy-3′-[^{18}F]fluoro-1-β-D-xylofuranosyluracil ([^{18}F]-FMXU) for PET

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Summary

The synthesis of a pyrimidine analog, 3′-deoxy-3′-[^{18}F]-fluoro-1-β-D-xylofuranosyluracil ([^{18}F]-FMXU) is reported. 5-Methyluridine **1** was converted to its dimethoxytrityl derivatives **2** and **3** as a mixture. After separation the 2′, 5′-di-methoxytrityluridine **2** was converted to its 3′-triflate **4** followed by derivatization to the respective N^3-*t*-Boc product **5**. The triflate **5** was reacted with tetrabutylammonium[^{18}F]fluoride to produce **6**, which by acid hydrolysis yielded compound **7**. The crude preparation was purified by HPLC to obtain the desired product [^{18}F]-FMXU. The radiochemical yields were 25–40% decay corrected (d. c.) with an average of 33% in four runs. Radiochemical purity was >99% and specific activity was >74 GBq/μmol at the end of synthesis (EOS). The synthesis time was 67–75 min from the end of bombardment (EOB). Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: fluorine-18; nucleoside; PET

Introduction

We and others have been developing and testing several radiofluorinated pyrimidine nucleoside analogs as potential agents for imaging tumor proliferative activity or HSV-tk reporter gene expression.[1–8] Initially, many fluorinated analogs of pyrimidine nucleosides have been synthesized and studied as potential antitumor and antiviral agents.[9–13] Among these, 2′-deoxy-2′-fluoro-5-methyl-1-β-D-arabinofuranosyluracil (FMAU) and other 5-substituted derivatives are known to be phosphorylated by human and other mammalian nucleoside kinases including thymidine kinase TK1 and TK2, as well as viral kinase such as herpes simplex virus (HSV) type 1 and 2, and

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hepatitis B virus.[14,15] In particular, ^{11}C and ^{18}F radiolabeled FMAU is currently undergoing clinical studies in multiple centers for imaging tumor proliferation in a variety of cancer types.[16–18] Also, 2′-deoxy-2′-fluoro-5-methyl-1-β-D-ribofuranosyluracil (FMRU) and 5-substituted analogs have been synthesized and tested as potential imaging agents for tumor proliferation.[19,20] The only nucleoside analog radiolabeled in the 3′-position reported to date is 3′-[^{18}F]-fluoro-3′-deoxy-thymidine ([^{18}F]-FLT), which is currently under clinical investigation as a potential PET imaging agent for tumor proliferation.[21,22] However, no information is available regarding the 3′-deoxy-3′-fluoro-5-methyl-1-β-D-xylofuranosyluracil (FMXU), no radiolabeling procedure has been described in the literature, and the biological properties of this compound are unknown. Recently, 3′-deoxy-3′-fluoro-*xylo*-cytidine derivatives have been reported to exhibit antiviral activity against hepatitis C virus.[23] This prompted us to attempt the synthesis and radiofluorination of pyrimidine nucleosides in the 3′-fluoro *xylo*-configuration. In contrast to 2′-deoxy-2′-fluoro-*arabino*-pyrimidine nucleosides, in which the direct radiolabeling of 2′-position in the *arabino*-configuration is not feasible using a one step radiolabeling procedure, the direct fluorination in the 3′-*xylo* configuration of the sugar moiety of a pyrimidine nucleoside is feasible as described in the current report. Using the protected nucleoside precursor 2′,5′-di-methoxytrityl-3′-trifluoromethanesulfonyl-N^3-*t*-Boc-5-methyluridine and conventional nucleophilic radiofluorination and purification, we have synthesized 3′-deoxy-3′-[^{18}F]fluoro-5-methyl-1-β-D-xylofuranosyluracil in high yield, high specific activity and purity.

Results and discussion

Figure 1 represents the scheme for the synthesis of the pyrimidine nucleoside analog, FMXU. Compounds **2** and **3** were prepared from 5-methyluridine following a method similar to the synthesis of fluoro-adenosine analogs.[24] Reaction of 5-methyluridine with methoxytrityl chloride (MTrCl) in pyridine in the presence of *N*,*N*-dimethylaminopyridine (DMAP) produced a mixture of 2 compounds, which were separated by column chromatography. The early eluting compound was the desired product 2′,5′-di-methoxytrityl derivative **2**. Compounds **2** and **3** were isolated in 50% and 28% yields, respectively, with a combined yield of 78%. The chromatographic elution of these compounds was similar to that of adenosine derivatives.[24] Both compounds were characterized by ^1H NMR spectroscopy, and compound **2** was further analyzed by high-resolution mass spectrometry.

Compound **4** was prepared by reaction of **2** with trifluoromethanesulfonic anhydride ($(Tf)_2O$) in pyridine at room temperature. However, addition of $(Tf)_2O$ should be performed at 0°C, since it is highly reactive and exothermic. There after the reaction may proceed at room temperature. Yield in this step

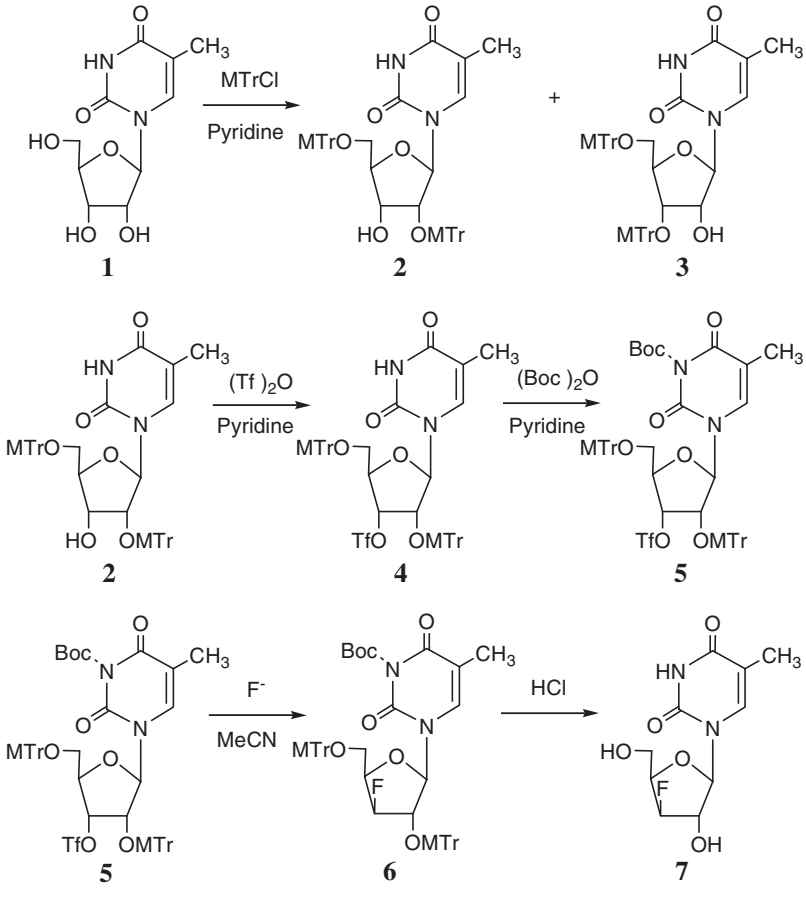

Figure 1. Synthetic scheme of 3′-deoxy-3′-fluoro-5-methyl-1-β-D-xylofuranosyluracil (FMXU)

was 82% after chromatographic purification. This compound was characterized by ^{1}H and ^{19}F NMR spectroscopy, and high-resolution mass spectrometry. Chemical shift of the 3′ H was significantly changed from 2.79 to 4.72 ppm due to the electronegativity of the sulfonic ester. ^{19}F NMR spectrum showed a singlet at −75.678 ppm, consistent with those of other sugar triflates.[24]

Compound **5** was prepared by reaction of **4** with di-*tert*-butyl dicarbonate (Boc) in pyridine at room temperature. Yield in this step was 60% after chromatographic purification. This compound was characterized by ^{1}H and ^{19}F NMR spectroscopy, and high-resolution mass spectrometry. ^{19}F NMR spectra showed a singlet at −75.678 ppm.

Fluorination of the triflate precursor **5** with *n*-Bu_4NF produced the 3′-deoxy-3′-fluoro-5-methyl-1-β-D-xylofuranosyluracil derivative **6** in 40–50%

yields. Compound 6 was purified by column chromatography, and characterized by ^{1}H and ^{19}F NMR spectroscopy, and high-resolution mass spectrometry. ^{1}H NMR spectrum of **6** was identical with that reported for 3′-deoxy-3′-fluoro-1-β-D-xylofuranosyluracil (FXU),[25] especially the coupling between fluorine and 4′ H vs fluorine and 2′ H. The coupling constant between F and 4′ H (34 Hz) was significantly larger than that between F and 2′ H (16 Hz), which suggests the trans orientation of F and 4′ H. The coupling constant between F and 3′ H was 50.7 Hz, a typical geminal coupling between F and H. Comparing the ^{1}H NMR spectrum with that of FXU, the orientation of F in compound **6** appeared to be in the *xylo*-configuration.[25] ^{19}F NMR spectrum (decoupled) of **6** showed a single peak at −198.38 ppm, and the coupled spectrum showed a multiplate (doublet of doublet of doublet) with coupling constants as observed in ^{1}H NMR spectrum.

Compound **7** (FMXU) was prepared by acid hydrolysis of the protecting groups from **6** following the method reported earlier.[24] Both *t*-Boc and methoxytrityl groups were hydrolyzed under the same reaction condition. The crude product was purified by HPLC and characterized by ^{1}H and ^{19}F NMR spectroscopy, and high-resolution mass spectrometry. ^{1}H NMR spectrum of FMXU showed a typical coupling of 3′ H and F (geminal coupling) with coupling constant 50.7 Hz. The vicinal coupling constant between F and 4′ H was larger than that of F and 2′ H. ^{19}F NMR spectrum showed 8 peaks (a doublet of doublet of doublets).

Radiolabeled compound **7** was prepared by fluorination of the triflate precursor **5** with *n*-$Bu_4N^{18}F$, prepared *in situ* from *n*-Bu_4NHCO_3 and aqueous $H^{18}F$. Either $Bu_4N^{18}F$ or $K^{18}F$ can be used as fluorinating agent; however, $K^{18}F$ requires kryptofix to generate naked fluoride. $Bu_4N^{18}F$ has advantages, such as, it is more soluble in organic solvents, and fluorination takes place at lower temperature. In this reaction 0.40 ml (1% solution, ∼7 μmol) of *n*-Bu_4NHCO_3 was used based on our earlier experience of an optimum quantity.[1,2,24] Evaporation of water from the aqueous $Bu_4N^{18}F$ and anhydrous conditions are critical for the fluorination reaction. Overheating (>80°C) of $Bu_4N^{18}F$ resulted in low yields of the desired product. Higher yields were obtained when drying the $Bu_4N^{18}F$ was performed by adding multiple sequential aliquots of acetonitrile during azeotropic evaporation of water from aqueous $Bu_4N^{18}F$. Unreacted [^{18}F]-fluoride was removed by passing the crude reaction mixture through a silica-gel cartridge (900 mg, Alltech), and the product was eluted with 10% methanol in dichloromethane. After solvent evaporation the recovered ^{18}F-labeled compound **6** was readily hydrolyzed with acid, and the desired labeled nucleoside **7** isolated by HPLC purification using 10% MeCN. Figure 2 represents a typical purification of [^{18}F]-FMXU. The radioactive peak at 10 min is the desired product [^{18}F]-FMXU. The UV peak corresponding to the radioactive peak was quite low,

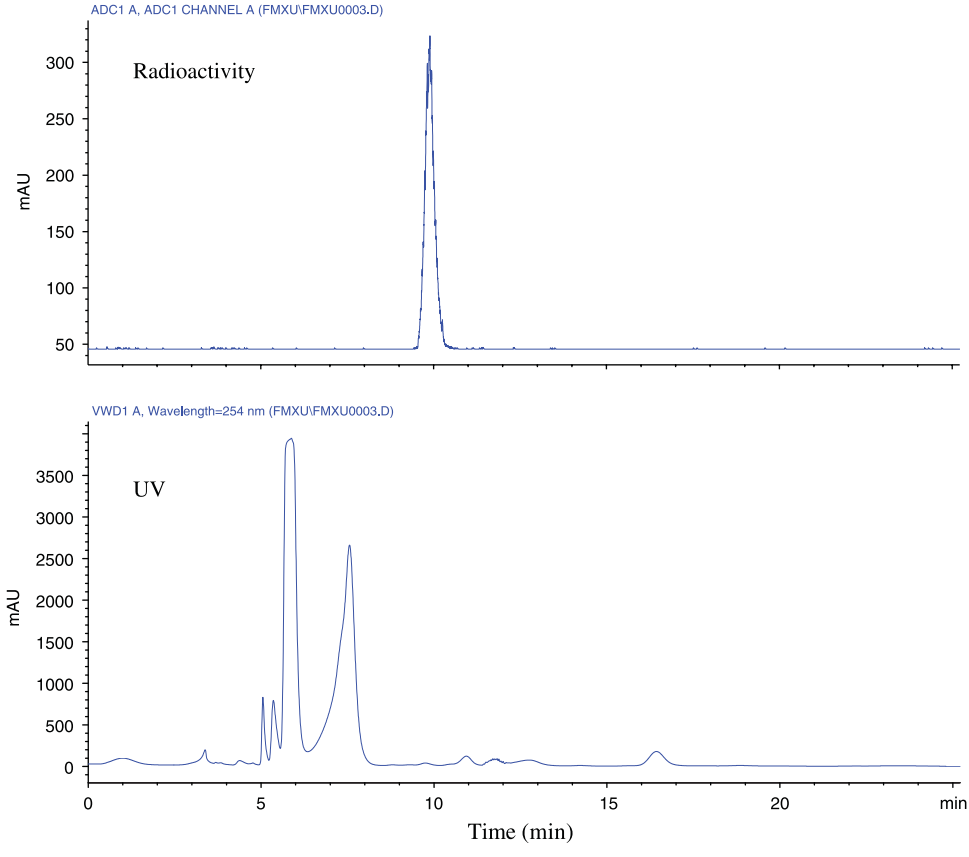

Figure 2. HPLC purification of [^{18}F]-FMXU: Semi-prep C_{18} Column; 10% MeCN/H_2O; flow 4.0 ml/min. Capacity factor (k') = 3.3

almost undetectable since there was no carrier added in the synthesis. The product could also be purified by HPLC using 10% ethanol in water. In this solvent system [^{18}F]-FMXU eluted at 12 min with the same flow rate as 10% MeCN.

The radiochemical yield of this synthesis was 25–40% (d. c.) with an average of 33 $\pm$ 7% in four runs. The radiochemical purity was $>$99% with specific activity $>$74 GBq/μmol. The specific activity in this no-carrier-added synthesis was comparable with that of [^{18}F]-FMAU and 2-fold higher than [^{18}F]-FLT (37 GBq/μmol), which are produced for PET imaging of animals and cancer patients.[1,4] The synthesis time was 67–75 min from end of bombardment (EOB). In a representative synthesis, 5 mCi (185 MBq) of labeled product **7** was obtained starting from 20 mCi (740 MBq) of [^{18}F]-fluoride. Analytical sample of **7** (HPLC) showed a single radioactive peak that co-eluted at 7.5 min with an authentic sample (Figure 3).

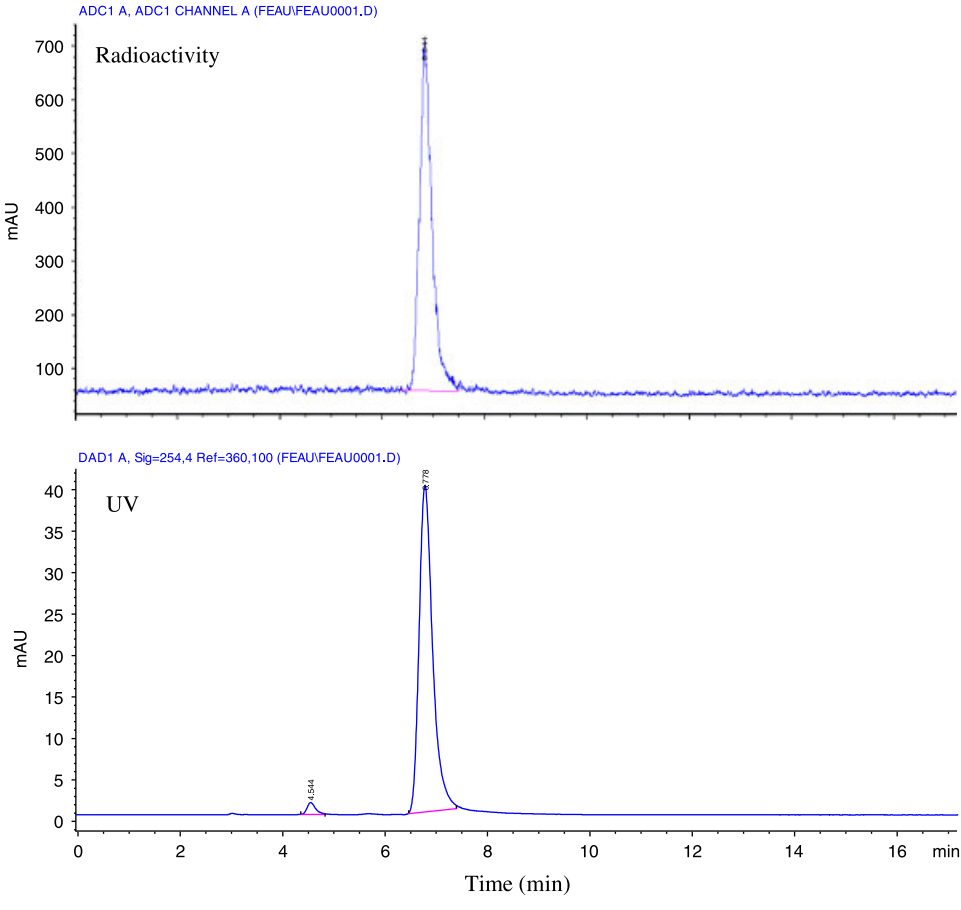

Figure 3. HPLC chromatogram of [^{18}F]-FMXU, co-injected with standard FMXU: Analytical C_{18} Column; 10% MeCN/H_2O; flow 1.0 ml/min. Capacity factor (k') = 2.2

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 (silica gel, 900 mg) were purchased from Alltech Associates (Deerfield, IL).

Thin layer chromatography (TLC) was performed on pre-coated Kieselgel 60 F254 (Merck) glass plates. Proton and ^{19}F NMR spectra were recorded on a Brucker 300 MHz spectrometer using tetramethylsilane as an internal reference and hexafluorobenzene as an external reference, respectively. High-resolution mass spectra (MS) were obtained on a Bruker BioTOF II mass

spectrometer at the University of Minnesota using electrospray ionization (ESI) technique.

High-performance liquid chromatography (HPLC) was performed on a 1100 series pump (Agilent, Germany), with built in UV detector operated at 254 nm, and a radioactivity detector with single-channel analyzer (Bioscan, Washington DC) using a semi-preparative C_{18} reverse phase column (Alltech, Econosil, 10×250 mm, Deerfield, IL) and an analytical C_{18} column (Rainin, Microsorb-MV, 4.6×250 mm, Emeryville, CA). A acetonitrile/water (MeCN/H_2O) solvent system (10% MeCN) was used for purification of the radiolabeled nucleoside, and for quality control analysis on analytical HPLC.

Preparation of 2′,5′-di-methoxytrityl-3′-hydroxy-5-methyluridine: **2**

Methyluridine (520 mg, 2.02 mmol), methoxytrityl chloride (1.4 g, 4.5 mmol) and *N*,*N*-dimethylaminopyridine (250 mg, 2.04 mmol) were taken into a dry flask under argon. Pyridine (10 ml) was added and the mixture was heated with stirring at 90°C for 16 h when TLC showed no starting material remained. The reaction mixture was cooled to room temperature and solvent was evaporated under vacuum. The residue was dissolved in 50% EtOAc/hexane (250 ml) and the solution washed with water (3×250 ml). The organic phase was dried in $MgSO_4$ and solvent was evaporated to dryness. The crude product was purified on a silica-gel column and eluted with 30% ethyl acetate in hexane. The solvent was evaporated to get the desired product in 50% yield. ^{1}H NMR ($CDCl_3$) δ: 8.59 (s, 1 H, NH), 6.69–7.51 (m, 29 H, aromatic & C_6H), 6.58 (d, 1 H, $J = 6$ Hz, 1′ H), 4.49–4.53 (m, 1 H, 2′ H), 3.98 (s, 1 H, 4′ H), 3.78 (s, 6 H, OMe), 3.25 (d, 1 H, $J_{gem} = 9.0$ Hz, 5′ H), 2.99, (d, 1 H, $J_{gem} = 9.0$ Hz, 5′ H), 2.79 (d, 1 H, $J = 3$ Hz, 3′ H), 1.22 (s, 3 H, Me). High-resolution MS: M + Na, calculated 825.3146; found 825.3150.

Preparation of 2′,5′-di-methoxytrityl-3′-trifluoromethanesulfonyl-5-methyluridine: **4**

Compound **2** (0.450 g, 0.56 mmol) was dissolved in pyridine (7 ml) under argon and cooled in an ice bath. Trifluoromethanesulfonic anhydride (0.2 ml, 2 equiv) was added and the reaction mixture was stirred for 10 min in the cold and subsequently for 50 min at room temperature when TLC showed no starting material remained. The reaction mixture was quenched with cold water (50 ml) and extracted with 50% EtOAc/hexane (3×50 ml). The organic extract was dried ($MgSO_4$) and evaporated to dryness, and the crude product purified by chromatography on a silica-gel column using 40% ethyl acetate in hexane. Pure compound (540 mg) was obtained in 82% yield. ^{1}H NMR ($CDCl_3$) δ: 8.45 (s, 1 H, NH), 6.70–7.45 (m, 29 H, aromatic & C_6H), 6.49 (d, 1 H, $J = 9.0$ Hz, 1′ H), 4.69–4.73 (m, 1 H, 2′ H), 4.29 (d, 1 H, $J = 3$ Hz, 3′ H),

4.24 (s, 1 H, 4′ H), 3.82 (s, 3 H, OMe), 3.77 (s, 3 H, OMe), 3.36 (d, 1 H, J_{gem} = 9.0 Hz, 5′ H), 3.09 (d, J_{gem} = 9.0 Hz, 5′ H), 1.37 (s, 3 H, Me). ^{19}F NMR (δ): −75.678 (s). High-resolution MS: M + Na, calculated 957.2639; found 957.2618.

Preparation of 2′,5′-di-methoxytrityl-3′-trifluoromethanesulfonyl-N^3-t-Boc-5-methyluridine: **5**

Compound **4** (140 mg, 0.15 mmol) and di-*tert*-butyl dicarbonate (120 mg, 3.6 equiv) were taken into a flask under argon. Dry pyridine (5 ml) was added and the reaction mixture was stirred at room temperature for 16 h when TLC showed no starting material remained. Solvent was evaporated under high vacuum, and the residue was dissolved in 50% EtOAc/hexane (40 ml), then washed with water (3 × 40 ml). The organic phase was dried ($MgSO_4$) and evaporated to isolate the crude product, which was purified by flash chromatography on silica-gel column using 25% EtOAc/hexane. Pure compound (0.103 g) was obtained in 62% yield. ^{1}H NMR ($CDCl_3$) δ: 6.72–7.43 (m, 29 H, aromatic & C_6H), 6.49 (d, 1 H, J = 8.1 Hz, 1′ H), 4.69–4.73 (m, 1 H, 2′ H), 4.24–4.29 (m, 2 H, 3′ H & 4′ H), 3.80 (s, 3 H, OMe), 3.74 (s, 3 H, OMe), 3.38 (d, 1 H, J_{gem}= 9.0 Hz 5′ H), 3.0 (d 1 H, J_{gem}= 9.0 Hz, 5′ H), 1.62 (s, 3 H, Me), 1.52 (s, 9 H, *t*-but). ^{19}F NMR (δ): −75.678 (s). High-resolution MS: M + Na, calculated 1057.3164; found 1057.3201.

*Preparation of 2′,5′-di-methoxytrityl-3′-fluoro-N^3-Boc-5-methyl-1-β-*D*-xylofuranosyluracil:* **6**

Compound **5** (34 mg, 0.033 mmol) was dissolved in dry MeCN (2.0 ml) in a sealed V-vial. To the above solution, *n*-Bu_4NF (1 M, 70 µl) was added and the mixture was heated for 20 min at 72–74°C in a heating block. The reaction mixture was cooled to room temperature. The solvent was evaporated in a stream of air and the residue purified on a short silica-gel column using 30% ethyl acetate in hexane. Pure compound **6** (14 mg) was obtained in 46% yield. ^{1}H NMR ($CDCl_3$) δ: 6.79–7.50 (m, 29 H, aromatic & C_6H), 6.48 (d, 1 H, J = 2.1 Hz 1′ H), 4.07–4.13 (m, 2 H, 2′ & 4′ H), 3.78 (s, 3 H, OMe), 3.77 (s, 3 H, OMe), 3.55 (dd, 1 H, J = 50.7 and 1.8 Hz, 3′ H), 3.36–3.42 (m, 1 H, 5′ H), 3.18–3.23 (m, 1 H, 5′ H), 1.79 (s, 1 H, CH_3), 1.65 (s, 9 H, *t*-But). ^{19}F NMR (δ): −198.38. High-resolution MS: M + Na, calculated 927.3627; found 927.3659.

*Preparation of 3′-deoxy-3′-fluoro-5-methyl-1-β-*D*-xylofuranosyluracil:* **7**

Compound **6** (30 mg) was placed in a small flask and dissolved in methanol (5 ml). Hydrochloric acid (1 M, 0.3 ml) was added to the above solution and refluxed for 10 min. The reaction mixture was cooled and solvent evaporated.

The crude product was washed with CH_2Cl_2 (3 × 10 ml), which was discarded, and the residue was purified by HPLC to obtain the unlabeled FMXU (6 mg) in 70% yield. ^{1}H NMR (D_2O) δ: 7.45 (s, 1 H, C_6H), 5.82 (d, 1 H, $J = 2.1$ Hz, 1′ H), 5.01 (dd, 1 H, $J = 50.7$ and 3 Hz, 3′ H), 4.47 (d, 1 H, $J = 15$ Hz, 2′ H), 4.37 (dt, $J = 30$ Hz, 4′ H), 3.92–3.96 (m, 2 H, 5′ H), 1.79 (s, 1 H, CH_3). High-resolution MS: M + Na, calculated 283.0701 found 283.0718.

*Preparation of 3′-deoxy-3′[^{18}F]fluoro-5-methyl-1-β-*D*-xylofuranosyluracil*

The aqueous [^{18}F]fluoride was trapped in anion exchange cartridge (ABX, Germany) and eluted with a solution of *n*-Bu_4NHCO_3 (400 µl, 1% by wt) into a V-vial and the solution evaporated azeotropically with acetonitrile (1.0 ml) to dryness at 79–80°C under a stream of argon. To the dried *n*-$Bu_4N^{18}F$, a solution of **5** (5–6 mg) in anhydrous acetonitrile (0.5 ml) was added, and the mixture was heated at 79–80°C for 10 min. The reaction mixture was cooled, passed through a silica-gel cartridge (Alltech), and eluted with 10% methanol in dichloromethane (2.5 ml). After evaporation of the solvent with a stream of argon at ~80°C, the residue was dissolved in methanol (0.4 ml). Hydrochloric acid solution in methanol (1 N, 0.1 ml) was added and the mixture was refluxed for 10 min. The crude mixture was neutralized with 1 N sodium bicarbonate solution (0.1 ml), diluted with HPLC solvent (1.0 ml) and purified by HPLC. The desired product was isolated and radioactivity was measured in a dose calibrator (Capintec, Ramsey, NJ). The product was co-injected with an authentic unlabeled sample onto an analytical column to confirm its identity.

Conclusion

We have developed a synthesis of a new radiotracer 3′-deoxy-3′-[^{18}F]fluoro-5-methyl-1-β-D-xylofuranosyluracil ([^{18}F]-FMXU) at high yield, high specific activity and purity. The described method may be suitable for the preparation of other 3′-deoxy-3′-[^{18}F]fluoro-*xylo*-nucleoside analogs.

Acknowledgements

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