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Research Article

Synthesis of [^{18}F]-labeled 2′-deoxy-2′-fluoro-5-methyl-1-β-D-arabinofuranosyluracil ([^{18}F]-FMAU)

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Summary

Synthesis of 2′-deoxy-2′-[^{18}F]fluoro-5-methyl-1-β-D-arabinofuranosyluracil ([^{18}F]-FMAU) is reported. 2-Deoxy-2-[^{18}F]fluoro-1,3,5-tri-O-benzoyl-α-D-arabinofuranose **2** was prepared by the reaction of the respective triflate **1** with tetrabutylammonium[^{18}F]fluoride. The fluorosugar **2** was converted to its 1-bromo-derivative **3** and coupled with protected thymine **4**. The crude product mixture (**5a** and **5b**) was hydrolyzed in base and purified by HPLC to obtain the radiolabeled FMAU **6a**. The radiochemical yield of **6a** was 20–30% decay corrected (d.c.) in four steps with an average of 25% in four runs. Radiochemical purity was >99% and average specific activity was 2300 mCi/μmol at the end of synthesis (EOS). The synthesis time was 3.5–4.0 h from the end of bombardment (EOB). Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: fluorine-18; nucleoside; FMAU

Introduction

2′-Deoxy-2′-fluoro-5-[^{11}C-methyl]-1-β-D-arabinofuranosyluracil ([^{11}C]-FMAU)[1] is being studied as a marker for cell proliferation by positron

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emission tomography (PET).[2] However, the short half-life of ^{11}C ($t_{1/2}$= 20 min) limits the clinical application of the compound. The corresponding analogue labeled with fluorine-18 ($t_{1/2}$= 110 min) would be more advantageous.

Attempted syntheses of 2′-deoxy-2′-fluoro-1-β-D-arabinofuranosyluracils by the direct introduction of fluorine have hitherto failed.[3,4] However, the incorporation of fluorine in the *arabino* configuration at C-2 of the sugar followed by coupling with the pyrimidine base has been successful towards the syntheses of these nucleosides.[3–6] The conventional methods for the stereospecific (*arabino*) incorporation of fluorine involve use of KHF_2 or $Et_3N \cdot 3HF$ in excess (6 equiv) as the fluorinating agent and require long reaction times (6 h).[4,5] Such conditions are not satisfactory for incorporation of radiofluorine. We reported, however, a method suitable for stereospecific (*arabino*) incorporation of radioactive fluorine in the 2-position of the protected sugar.[7]

Coupling of the sugar with a pyrimidine base often involves conversion of the protected sugar to its 1-bromo-derivative, which is then treated with a protected pyrimidine over a lengthy period of time ranging from 16 h to 7 days.[3–6] As in the case of the fluorination reaction discussed above these conditions are not appropriate for a synthesis using a reactant labeled with short half-life isotopes such as ^{18}F. Another method of coupling the glycosyl moiety to the pyrimidine base used a Friedel–Crafts catalyst and a 2-substituted ribofuranose to produce exclusively the β-anomer of the nucleoside.[8] Very limited work has been done on pyrimidine glycosylation with protected 2-deoxyarabinose.[8,9] However, we developed a method that should be suitable for coupling a radiolabeled 2-deoxy-2-fluoro-arabinofuranose with protected pyrimidines.[10] Early work in our laboratory on the incorporation of radiofluorine in the *arabino* configuration at C-2 of the sugar followed by coupling with the pyrimidine base resulted in ^{18}F-labeled FMAU, albeit in low yield.[11] In that synthesis the [^{18}F]-arabinofluorosugar was prepared from the respective 2-fluorosulfonyl ester followed by coupling with the protected pyrimidine in the presence of a Friedel–Crafts catalyst. Most recently a high yield synthesis of [^{18}F]-FMAU has been reported in an abstract, which involves high temperature in all stages, and may require special equipment.[12] We report here a synthesis of [^{18}F]-FMAU, which can be performed under milder conditions. This method is suitable for general syntheses of [^{18}F]-labeled 2′-deoxy-2′-fluoro-1-β-D-arabinofuranosyluracil nucleosides.

Results and discussion

Figure 1 represents the scheme for the [^{18}F]-labeled synthesis of the nucleoside, [^{18}F]-FMAU. Compound **2** was prepared by fluorination of **1** with *n*-$Bu_4N^{18}F$, prepared *in situ* from *n*-Bu_4HCO_3 and aqueous $H^{18}F$. In this reaction the amount of *n*-Bu_4NHCO_3 is critical. For 5–6 mg ($\sim$10 μmol) of the precursor **1**, 45–50 μl of the *n*-Bu_4NHCO_3 (4% soln., $\sim$7 μmol) produced a radiochemical yield of 58–68%. Any variation from this stoicheometry lowers the yields of the desired product. Tetrabutylammonium fluoride (*n*-$Bu_4N^{18}F$) is extremely hygroscopic and the degree of dryness achieved for the reagent was difficult to ascertain. That may explain the small variability of the yields observed. Unreacted fluoride was removed by passing the crude reaction mixture through a Sep-Pak cartridge (silica), and the pure product could be isolated by high performance liquid chromatography (HPLC) purification. However, further purification was not necessary since the product was found to be >90% radiochemically pure by analytical HPLC.

Compound **3** was prepared by the literature method as modified in our laboratory.[11] The reaction was complete in 10 min at 80–82°C.

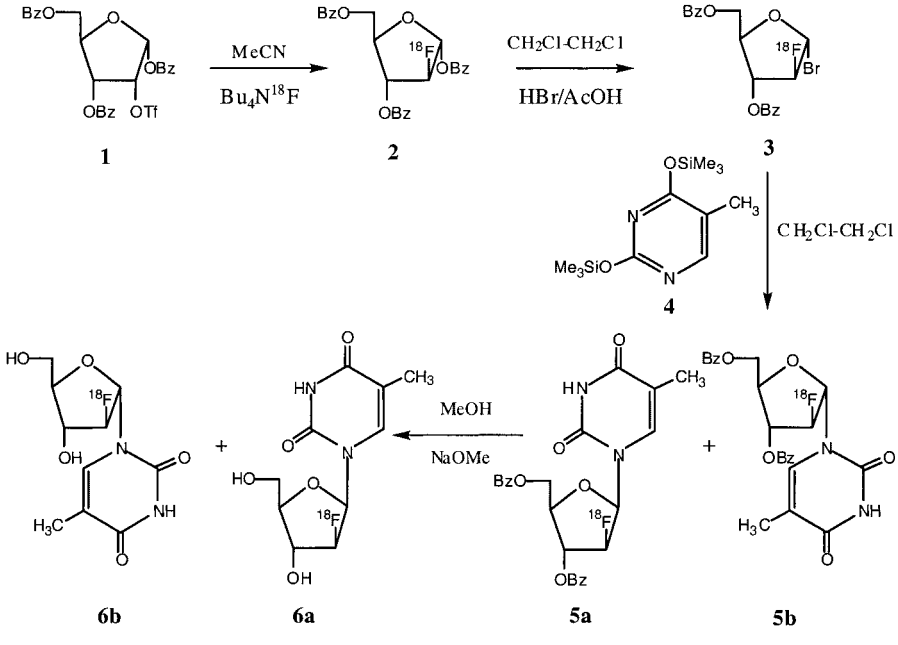

Figure 1. Synthetic Scheme of [^{18}F]-FMAU

In aprotic solvents compound **3** is stable at temperatures higher than 100°C. A trace of acetic acid and water converts the compound **3** to equal amounts of 1-hydroxy and 1-acetoxy derivatives at room temperature. Direct evaporation of the reaction mixture to remove solvent and excess reagents converts the 1-bromosugar **3** to 1-acetate exclusively. This decomposition was avoided by first adding toluene (1 ml) to the reaction mixture followed by evaporation. Acids are removed azeotropically at the initial stage of concentration leaving the product unaffected. Crude **3** was used directly to reduce the synthesis time, since it was found to be >90% radiochemically pure (HPLC).

Coupling of the 1-bromo-2-fluorosugar **3** with thymine silyl ether **4** produced a mixture of protected nucleoside anomers **5a** and **5b** as observed earlier.[4,10] In a less polar solvent such as 1,2-dichloroethane the ratio of the anomers $\beta:\ \alpha$ was 9 : 1, however, the required reaction time was 1 h to obtain maximum yield. The thymine silyl ether is susceptible to hydrolysis. During addition of the thymine silyl ether solution to the reaction vial partial hydrolysis occurs as indicated by the appearance of solvent turbidity. Use of an excess of thymine silyl ether compensates for this loss and the coupling reaction proceeds without significant reduction in yield based on the amount of the fluorosugar.

Compounds **6a** and **6b** were produced by basic hydrolysis of **5a** and **5b** and could be isolated by HPLC. However, trace amounts of thymine remains as a contaminant in the final product in this process. To avoid the presence of thymine, the crude reaction mixture containing **5a** and **5b** was passed through a Sep-Pak cartridge (silica) and eluted with 10% methanol in dichloromethane. The recovered **5a** and **5b** after hydrolysis and HPLC purification produced the desired nucleoside **6a** (Figure 2). This treatment eliminates the presence of thymine in the [^{18}F]-FMAU.

Analysis of the pure product, **6a** by HPLC showed a single radioactive peak that co-eluting with an authentic sample of FMAU (Figure 3). The apparent difference in the retention times (Figure 3) is an artefact to physical separation of the radioactivity and UV detectors.

The radiochemical yield of this synthesis was 20–30% decay corrected (d.c.) from the EOB in four steps. The radiochemical purity was >99% with an average specific activity 2300 mCi/μmol. The synthesis time was 3.5–4.0 h from the EOB. In a typical synthesis, 18 mCi of labeled product was obtained starting from 246 mCi of [^{18}F]-fluoride.

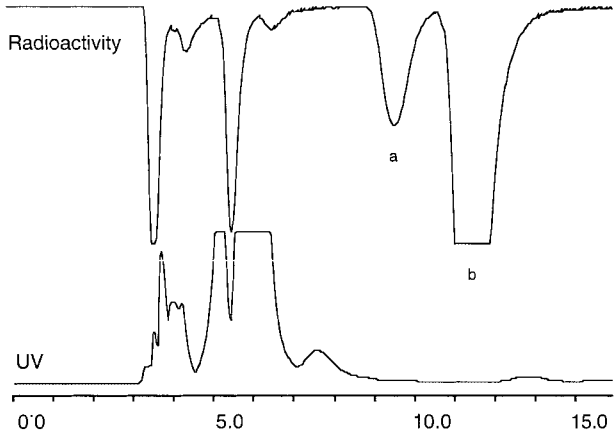

Figure 2. Purification of [^{18}F]-FMAU: Semi-prep Column; 7.5% MeCN/H_2O; flow 4.05 ml/min. a = α anomer of [^{18}F]-FMAU; b = [^{18}F]-FMAU

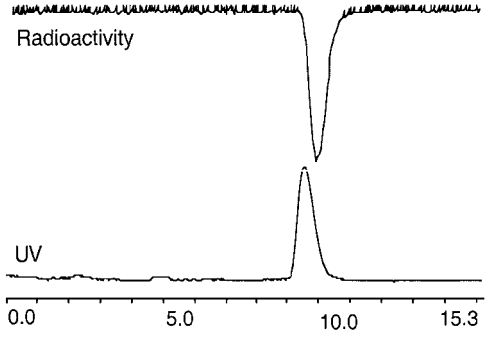

Figure 3. HPLC chromatogram of [^{18}F]-FMAU, co-injected with standard FMAU: Anal Column; 7.5% MeCN/H_2O; flow 1.13 ml/min

Experimental

Reagents and instrumentation

All reagents and solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI), and used without further purification. Solid phase extraction cartridge (Sep-Pak) was purchased from Waters Associates (Milford, MA). 2-O-(trifluoromethylsulfonyl)-1,3,5-tri-O-benzoyl-α-D-ribofuranose **1** and thymine-2,5-bis-trimethylsilyl ether **4** were prepared following literature methods.[5,6] Compounds **2**, **3** and **5** were not isolated but used directly in the subsequent steps. The corresponding unlabeled

compounds were previously characterized by NMR and mass spectrometry.[7,10]

High performance liquid chromatography (HPLC) was performed on a system using a 510 pump (Waters Associates, Milford, MA), UV detector (Isco, Lincoln, NE) operated at 254 nm, and a radioactivity detector with single-channel analyzer (Technical Associate, Woodland Hills, CA) using a semi-preparative C_{18} reverse phase column (Alltech, Econosil, 10×250 mm, Deerfield, IL) and an analytical C_{18} column (Alltech, Econosil 4×250 mm). A acetonitrile/water (MeCN/H_2O) solvent system (75% MeCN) was used for purification of [^{18}F]-arabinofuranose, and (7.5% MeCN) for purification of the radiolabeled nucleoside.

2-Deoxy-2-[^{18}F]fluoro-1,3,5-tri-O-benzoyl-α-D-arabinofuranose **2**

This compound was prepared following the method developed in our laboratory with modification.[7] Briefly, aqueous [^{18}F] fluoride (0.4 ml) was added to a solution of *n*-Bu_4NHCO_3 (50 µl, 4% by wt.) in a v-vial and evaporated azeotropically with acetonitrile (1.0 ml), to dryness at 80°C under a stream of argon. To the dry residue, *n*-$Bu_4N^{18}F$, a solution of **1** (5–6 mg, 8–10 µmol) in dry acetonitrile (0.5 ml) was added, and the reaction mixture was heated at 80–82°C for 30 min. The reaction mixture was cooled to room temperature, passed through a Sep-Pak cartridge (silica gel), and eluted with ethyl acetate (2.5 ml). After evaporation of solvent with a stream of argon at 80°C, the residue was used for the next step without further purification.

1-Bromo-2-deoxy-2-[^{18}F]fluoro-3,5-di-O-benzoyl-α-D-arabinofuranose **3**

The radiolabeled fluorosugar **2** was dissolved in 1,2-dichloroethane (0.4 ml) under argon. Hydrogen bromide (HBr) in acetic acid (30%, 0.1 ml) was added, and the reaction mixture was heated for 10 min at 80–82°C. The reaction mixture was diluted with toluene (1 ml) and the HBr/AcOH was evaporated under a stream of argon. The dry crude product was used for the coupling experiment without purification.

2′-Deoxy-2′-[^{18}F]fluoro-3′,5′-di-O-benzoyl-5-methyl-1-β/β-D-arabinofuranosyluracil **5a**/**5b**

To 1-bromo-2-deoxy-2-[^{18}F]fluoro-3,5-di-O-benzoylarabinofuranose **3** as obtained in the previous step was added a solution of freshly

prepared 2,4-bis-O-(trimethylsilyl)thymine **4** (75–85 μmol, 8–9 equiv.) in 1,2-dichloroethane (0.5 ml). The vial was heated in a heating block at 97–100°C for 60 min. The reaction mixture was cooled to room temperature, passed through a Sep-Pak cartridge (silica) and eluted with 10% methanol in dichloromethane (2.5 ml). The solvent was evaporated at ~100°C under a stream of argon and the crude product was recovered.

2′-Deoxy-2′[^{18}F]fluoro-5-methyl-1-β-D-arabinofuranosyluracil **6a**

The crude mixture of 2′-deoxy-2′-[^{18}F]fluoro-3,5-di-O-benzoyl-5-methyl-1-β-D-arabinofuranosyluracil **5a** and the α-anomer **5b** was dissolved in methanol (0.3 ml). Sodium methoxide (1 M solution in methanol, 0.03 ml) was added, and the mixture was heated for 5 min at reflux. The reaction mixture was cooled and neutralized with HCl (2N in methanol, 0.15 ml). After evaporation of methanol, the crude material was diluted with HPLC solvent and purified by HPLC as described earlier at a flow of 4.05 ml/min. The appropriate fraction specified by the prior determined retention time of an authentic sample eluted at around 11 min (Figure 2) was collected and evaporated to dryness. The pure product was re-dissolved in saline (Abbott Lab., Chicago, IL) and filtered through a 0.22 μm filter (Millipore, Badford, MA). An aliquot of the final product was analyzed by analytical HPLC, and found to be co-eluting with the authentic compound (Figure 3).

Conclusion

A convenient synthesis of 2′-deoxy-2′-[^{18}F]fluoro-5-methyl-1-β-D-arabinofuranosyluracil ([^{18}F]-FMAU) has been developed. The radiochemical yield of this synthesis was 20–30% d.c. from the EOB in four steps. The radiochemical purity was >99% with an average specific activity 2300 μmCi/μmol. The synthesis time was 3.5–4.0 h from the EOB. This method appears suitable for general syntheses of other 2′-deoxy-2′-[^{18}F]fluoro-1-β-D-arabinofuranosyl nucleoside derivatives for PET imaging.

Acknowledgements

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