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Automated synthesis of 2′-deoxy-2′-[^{18}F]fluoro-5-methyl-1-β-D-arabinofuranosyluracil ([^{18}F]-FMAU) using a one reactor radiosynthesis module

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Abstract

2′-Deoxy-2′-[^{18}F]fluoro-5-methyl-1-β-D-arabinofuranosyluracil ([^{18}F]-FMAU) is an established PET probe used to monitor cellular proliferation. For clinical applications, a fully automated cGMP-compliant radiosynthesis would be preferred. However, the current synthesis of [^{18}F]-FMAU requires a multistep procedure, making the development of an automated protocol difficult and complicated. Recently, we have developed a significantly simplified one-pot reaction condition for the synthesis of [^{18}F]-FMAU in the presence of Friedel-Crafts catalysts. Here, we report a fully automated synthesis of [^{18}F]-FMAU based on a one reactor radiosynthesis module using our newly developed synthetic method. The product was purified on a semi-preparative high-performance liquid chromatography integrated with the synthesis module using 6% EtOH in 10 mM phosphate buffer or 8% MeCN/water. [^{18}F]-FMAU was obtained in 12±3% radiochemical yield (decay corrected overall yield based on [^{18}F]-F^-, n=4) with 383±33 mCi/μmol specific activity at the time of injection. The α/β anomer ratio was 4:6. The overall reaction time was about 150 min from the end of bombardment and the radiochemical purity was >99%. This automated synthesis should also be suitable for the production of other 5-substituted thymidine analogues.

Keywords: [^{18}F]-FMAU; PET probe; Friedel-Crafts catalysts; F-18 labeling; Automated synthesis

1. Introduction

A number of radiolabeled 2′-deoxy-2′-fluoro-5-substituted-1-β-D-arabinofuranosyl-uracil derivatives have been evaluated as probes for imaging tumor proliferative activity and HSV1-tk reporter gene expression with positron emission tomography (PET) [1–12]. Among these, 2′-deoxy-2′-[^{18}F]fluoro-5-methyl-1-β-D-arabinofuranosyl-uracil ([^{18}F]-FMAU), 2′-deoxy-2′-fluoro-5-[^{11}C]methyl-1-β-D-arabinofuranosyl-uracil and 2′-deoxy-2′-[^{18}F]fluoro-5-bromo-1-β-D-arabinofuranosyl-uracil are markers for DNA synthesis through phosphorylation by human and other mammalian nucleoside kinases, including thymidine kinases TK1 and TK2 [3,4,13]. Although ^{18}F-3′-deoxy-3′-fluorothymidine (^{18}F-FLT) has been widely used for cell proliferation imaging by taking advantage of the pyrimidine salvage pathway [14,15], ^{18}F-FLT-triphosphate is not significantly incorporated into DNA [16–20] and the majority of ^{18}F-FLT persists as mono- and triphosphates in the cytosol [15,16]. Preclinical studies have shown that FMAU retention in tumors and nontumor tissues with rapid cell turnover (e.g., marrow and small intestine) reflects its incorporation into DNA [1,2,4,13]. FMAU may be useful for imaging tumor cell proliferation with PET and that further clinical investigation of C-11 and F-18 FMAU, in comparison with ^{18}F-FLT, is warranted. FMAU is undergoing preclinical and clinical studies for imaging tumor proliferation in a variety of cancer types [3,4,13,21]. The other uracil derivatives, such as 2′-deoxy-2′-[^{18}F]-fluoro-5-iodo-1-β-D-arabinofuranosyluracil, 2′-deoxy-2′-[^{18}F]fluoro-5-fluoro-1-β-D-arabinofuranosyl-uracil and 2′-deoxy-2′-[^{18}F]-fluoro-5-chloro-1-β-D-arabinofuranosyl-uracil are excellent substrates for the viral kinases such as herpes simplex virus Types 1 and 2, and 2′-deoxy-2′-[^{18}F]-fluoro-5-iodo-1-β-D-arabinofuranosyluracil (FIAU), is also a substrate for hepatitis B virus and Epstein Barr virus thymidine kinase

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[7,8,21-24]. Many of these 2′-fluoro-5-substitued arabinosyluracil derivatives were synthesized and evaluated earlier as antiviral agents [25-27]. The first radiochemical synthesis of FMAU with PET isotope [^{11}C] was reported by us [28]. However, due to the short half-life of [^{11}C] ($t_{1/2}$=20 min), there was a need to develop an [^{18}F]-labeled derivative. Subsequently, we reported the radiosynthesis of [^{18}F]-labeled FMAU and other 5-substituted thymidine analogues [29,30]. In this procedure, the radiosynthesis of [^{18}F]-FMAU involves radiofluorination of 2-trifluoromethane-sulfonyl-1,3,5-tri-*O*-benzoyl ribofuranose to the 2-[^{18}F]-fluoro-1,3,5-tri-*O*-benzoyl arabinofuranose derivative, followed by conversion to the 1-bromo-2-[^{18}F]-fluoro-1,3,5-tri-*O*-benzoyl derivative, and then coupling of the 1-bromo-2-[^{18}F]fluoro-2,3,-di-*O*-benzoylarabinofuranose with 2,4-*bis*-trimethylsilyluracil derivatives. Finally, hydrolysis of the protecting groups from the sugar moiety was performed and high-performance liquid chromatography (HPLC) purification yielded the desired products. Following our synthesis, another group of investigators also reported the [^{18}F]-labeled synthesis of these pyrimidine nucleoside analogues [31]. Although we and other researchers in the field have demonstrated these reactions are very reliable and reproducible [32–34], the complexity of this method often requires significant modification of existing commercial automated modules, accompanied by frequent production failures. In order to find an efficient fully automated cGMP-compliant radiosynthesis methodology for the production of these probes, our group has been optimizing the reaction conditions in order to reduce synthetic time and simplify reaction conditions [35]. Recently, we reported the use of Friedel-Crafts catalysts for an improved synthesis of [^{18}F]-FMAU, which also included a significantly simplified one-pot reaction condition (Scheme 1) [36,37]. In this paper, we report for the first time an automated synthesis of [^{18}F]-FMAU using a one-reactor radiosynthesis module. The method is also compatible with most commercially available modules typically used for production of cGMP-compliant radiotracers for clinical applications.

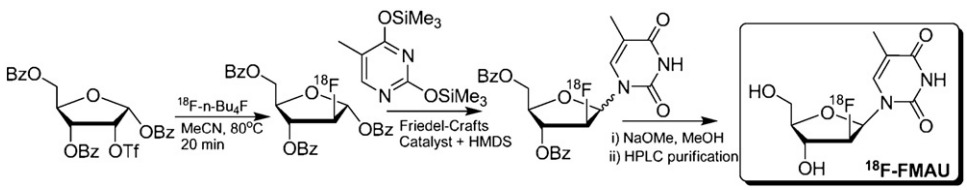
2. Experimental

2.1. Reagents and instrumentation

All reagents and solvents were purchased from Aldrich Chemical (Milwaukee, WI, USA), and used without further purification. Solid-phase extraction cartridges were purchased from Waters. Ion exchange cartridges were purchased from ABX (Germany). 2-Trifluoromethanesulfonyl-1,3,5-tri-*O*-benzoyl-α-D-ribofuranose (precursor) and bis-2,4-trimethylsilyl-5-methyluracil were purchased from ABX (Germany). Non-radioactive FMAU anomers were prepared in house and used as HPLC standards. Analysis was performed on an analytical reversed-phase HPLC system equipped with a dual UV absorbance detector (Waters 2487) using a phenomenex C18 RP (250×4.6 mm 5 micron). [^{18}F]-FMAU purification was performed on an isocratic HPLC with UV detector operated at 254 nm and radioactivity detector. A semipreparative C18 reverse phase column (phenomenex C18, 250×10 mm, 10 μm) was used in the separation. A solution of 6% ethanol in phosphate buffer (10 mM, pH 6.5) or 8% MeCN/water was used for the purification of [^{18}F]-FMAU. A solution of 8% MeCN in water was used for the quality control of [^{18}F]-FMAU on an analytical HPLC.

2.2. Automated [^{18}F]-FMAU synthesis

The solutions of potassium carbonate and Kryptofix K2.2.2 [or tetrabutylammonium bicarbonate (TBAB) and MeCN] were loaded into Reservoirs, respectively. Other Reservoirs were filled with precursor 1 (5.0-10 mg sugar triflate in 600 μl anhydrous MeCN), precursor 2 [a solution of 20 mg TMS-uracil, 100 μl hexamethyldisilizane (HMDS), and 150 μl trimethylsilyl trifluoromethanesulfonate (TMSOTf), in 300 μl dichloroethane], KOMe solution (0.4 ml, 2.0 N in MeOH), and HCl (0.2 ml, 4.0 N HCl + 1.0 ml HPLC solvent), respectively. The target water containing ^{18}F was passed through a preconditioned QMA cartridge where the ^{18}F-F$^-$ was trapped. The ^{18}F was released from the QMA cartridge by passing K_2CO_3 or TBAB solution through the cartridge and allowed to enter into the reactor. Kryptofix solution or MeCN was added into the reactor and the whole mixture was dried at 95°C in combination of nitrogen flow and vacuum. The precursor solution was added to the dried ^{18}F ion and heated at 80°C for 20 min. The MeCN was then evaporated and precursor 2 solution was added to the reactor. The reaction mixture was heated for 1 h at 85°C. The solvent was removed and KOMe solution was then added. The mixture was heated for 7 min at 80°C and MeOH was removed under vacuum. The HCl and mobile phase solution was then added to the reactor and passed through an alumina cartridge to a V-vial.



Scheme 1. One-pot synthesis of [^{18}F]-FMAU.