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# $N^3$-Substituted thymidine analogues V: Synthesis and preliminary PET imaging of $N^3$-[$^{18}$F]fluoroethyl thymidine and $N^3$-[$^{18}$F]fluoropropyl thymidine

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**Abstract**

**Introduction:** [$^{18}$F]-Labeled analogues of thymidine have demonstrated efficacy for PET imaging of cellular proliferation. We have synthesized two [$^{18}$F]-labeled $N^3$-substituted thymidine analogues, $N^3$-[$^{18}$F]fluoroethyl thymidine ($N^3$-[$^{18}$F]-FET) and $N^3$-[$^{18}$F]fluoropropyl thymidine ($N^3$-[$^{18}$F]-FPrT), and performed preliminary PET imaging studies in tumor-bearing mice.

**Methods:** Thymidine was converted to its 3′,5′-*O*-bis-tetrahydropyranyl ether, which was then converted to the $N^3$-ethyl and propyl-substituted mesylate precursors. Reactions of these mesylate precursors with n-$Bu_4N$[$^{18}$F] or K[$^{18}$F]/kryptofix followed by acid hydrolysis and HPLC purification yielded $N^3$-[$^{18}$F]-FET and $N^3$-[$^{18}$F]-FPrT, respectively. Subcutaneous (sc) xenografts of H441 human non–small cell lung cancer were established in two groups of mice (each $n$=6). Micro-PET images of the tumor-bearing animals were acquired after intravenous injection of $N^3$-[$^{18}$F]-FET or $N^3$-[$^{18}$F]-FPrT (3700 KBq/animal).

**Results:** The radiochemical yields were 2–12% (d.c.) for $N^3$-[$^{18}$F]-FET and 30–38% (d.c.) for $N^3$-[$^{18}$F]-FPrT. Radiochemical purity was >99% and calculated specific activity was >74 GBq/μmol at the end of synthesis. The accumulation of $N^3$-[$^{18}$F]-FET and $N^3$-[$^{18}$F]-FPrT in the tumor tissue at 2 h postinjection was 1.81±0.78 and 2.95±1.14 percent injected dose per gram (%ID/g), respectively; tumor/muscle ratios were 5.57±0.82 and 7.69±2.18, respectively; the unidirectional influx rates ($K_i$) were 0.013 and 0.018 ml/g per minute, respectively.

**Conclusion:** Two novel [$^{18}$F]- $N^3$-substituted thymidine analogues have been synthesized in good yields, high purity and high specific activity. Preliminary in vivo studies demonstrated the efficacy of these [$^{18}$F]- $N^3$-substituted thymidine analogues for PET imaging of tumors.



*Keywords:* Fluorine-18; Thymidine; Tumor; Proliferation; PET

## 1. Introduction

Over the past two decades, a number of $^{18}$F-labeled analogues of thymidine have been developed for positron emission tomography (PET) of cellular proliferative activity in vivo [1–8]. Some of the $^{18}$F-labeled nucleoside analogues have been developed for imaging tumor HSV-tk reporter gene expression [9–13]. Initially, most of the fluorinated pyrimidine nucleoside analogues have been synthesized and evaluated as antitumor and antiviral agents [14–18]. Among these, 2′-deoxy-2′-fluoro-5-methyl-1-β-D-arabinofuranosyluracil (FMAU) and other small side-chain 5-substituted derivatives (i.e., 5-halogenated) are phosphorylated with different efficacy by human and other mammalian nucleoside kinases including thymidine kinases TK1 and/or TK2; viral kinases such as herpes simplex virus type 1 and 2 (HSV1-TK and HSV2-TK); and inhibit viral DNA polymerases, such as in case of hepatitis B virus [16–20].

Currently, [$^{11}$C]- and [$^{18}$F]-radiolabeled FMAU are undergoing clinical studies for imaging tumor proliferative activity in a variety of cancer types [1–3,21,22]. 2′-Deoxy-2′-fluoro-5-methyl-1-β-D-ribofuranosyluracil and 5-substituted analogues have also been synthesized and tested for imaging tumor proliferation with PET [23,24].

To date, two nucleoside analogues have been radiolabeled in the 3′-position: 3′-[$^{18}$F]-fluoro-3′-deoxy-thymidine ([$^{18}$F]-FLT) [5] and 3′-deoxy-3′-[$^{18}$F]fluoro-5-methyl-1-β-

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D-xylofuranosyluracil ([$^{18}$F]-FMXU) [25]. [$^{18}$F]-FLT is currently widely used in clinical investigations as an agent for PET imaging of tumor proliferation [4,26]. Although both [$^{18}$F]-FMAU and [$^{18}$F]-FLT are used in clinical research, their phosphorylation rates by TK1 are relatively low (10–13%), as compared with that of thymidine [27]. Therefore, there is a need for a more sensitive agent for PET imaging of cellular proliferation with higher phosphorylation rates by TK1, and resistance to degradation by thymidine phosphorylase (TPase).

A series of $N^3$-substituted thymidine analogues carrying a carborane moiety at the $N^3$ position with various spacer lengths have been developed for boron neutron capture therapy [28–32]. Some of these $N^3$-substituted analogues have been reported to be phosphorylated by TK1 with 50–89% efficiency, as compared with thymidine, while being resistant to TPase [29–32]. Furthermore, several simple $N^3$-substituted derivatives, such as ethyl, *n*-butyl and acetylene, have also been reported as effective substrates of TK1, with high phosphorylation rates [30,31,33]. These favorable properties of N3-substituted thymidine analogues prompted us and others to synthesize [$^{18}$F]-labeled $N^3$-substituted thymidine analogues and assess their efficacy for PET imaging of TK1 activity and DNA synthesis during tumor proliferation. Recently, we reported a few [$^{18}$F] long-chain $N^3$-substitued analogues of thymidine [34,35]. Previously, $N^3$-[$^{18}$F]-FET and nonradiolabeled FPrT with shorter $N^3$-side chains have also been reported by others [36,37]. Using an in vitro enzyme assay, Toyohara et al. [36] demonstrated that FET and FPrT have phosphorylation rates of 47% and 26%, as compared to thymidine, respectively. However, a follow-up in vivo PET imaging study conducted by the same group of investigators demonstrated the lack of accumulation of $N^3$-[$^{18}$F]-FET in sc tumor xenografts in mice, which contradicted their previously reported in vitro enzyme assay results [37]. Another study demonstrated that modification of $N^3$ position in 1-β-D-arabinosylthymdine and FLT results in a marked decrease in both antiviral and cytostatic activity of these compounds [38]. Therefore, the utility of radiolabeled $N^3$-substituted thymidine analogues for PET imaging of proliferative activity remains unclear and requires further investigation.

In this paper, we report the radiosynthesis of two $N^3$-substituted thymidine analogues, $N^3$-[$^{18}$F]fluoroethyl thymidine ($N^3$-[$^{18}$F]-FET) and $N^3$-[$^{18}$F]fluoropropyl thymidine ($N^3$-[$^{18}$F]-FPrT), as well as the results of preliminary PET imaging studies in human tumor xenograft-bearing immunocompromised mice.

## 2. Materials and methods

### 2.1. Reagents and instrumentation

All reagents and solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and used without further purification. Solid-phase extraction cartridges (silica gel, 900 mg) were purchased from Alltech Associates (Deerfield, IL, USA).

Thin layer chromatography (TLC) was performed on precoated Kieselgel 60 F254 (Merck, Germany) 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, at The University of Texas MD Anderson Cancer Center, and chemical shifts are expressed in parts per million. High-resolution mass spectra were obtained on a Brucker BioTOF II mass spectrometer at the University of Minnesota using electrospray ionization (ESI) technique.

High-performance liquid chromatography (HPLC) was performed on an 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, USA) using a semi-preparative $C_{18}$ reverse-phase column (Econosil, 10×250 mm, Alltech, Deerfield, IL, USA) and an analytical $C_{18}$ column (Microsorb-MV, 4.6×250 mm, Rainin, Emeryville, CA, USA). An acetonitrile/water (MeCN/$H_2O$) solvent system (10% MeCN for FET and 15% MeCN for FPrT) was used for purification on a semi-prep column, and quality control analysis on analytical HPLC.

### 2.2. Preparation of 3′,5′-O-bis-tetrahydropyranyl-thymidine (1)

This compound was prepared following a literature method [39]. Briefly, thymidine (1.0 g, 4.12 mmol) was dissolved in dry THF (25 ml) under argon. P-Toluenesulfonic acid (60 mg, catalytic amount) was added to the reaction flask followed by the addition of 3,4-dihydro-*2H*-pyran (3.7 ml, 41.2 mmol). The reaction mixture was stirred at room temperature for 2 h, when TLC showed that no starting material remained. The reaction was quenched by addition of triethylamine (0.2 ml). Solvent was evaporated, and the crude product was purified by flash chromatography using a silica gel column and 50% acetone in hexane as eluent. White solid, 1.66 g of pure compound was obtained as a mixture of four diastereomers in 95% yield. $^1$H NMR ($CDCl_3$) $\delta$: 8.28 (s, 1H, NH), 7.70, 7.69, 7.64, 7.63 (4s, 1H, $C_6$H), 6.39–6.35 (m, 1H, 1′H), 4.72–3.54 (m, 10H, 3′-5′H and THP), 2.55–2.47 (m, 1H, 2′H), 2.43–2.37 (m, 1H, 2′H), 1.96, 1.95, 1.93, 1.92 (4s, 3H, $CH_3$), 1.83–1.70 (m, 4H, THP), 1.63–1.58 (m, 8H, THP). High-resolution MS: M +Na, calculated 433.1945; found 433.1954.

### 2.3. Preparation of 1-bromoethyl-2-benzoate (2) and 1-bromopropyl-3-benzoate (3)

Both Compounds **2** and **3** were prepared using the same methodology, a representative procedure is described below. 1-Bromo-2-ethanol (0.5 g, 4.00 mmol) was dissolved in dichloromethane (10 ml) under argon. Triethylamine (2.8 ml, 20 mmol) was added followed by the addition of benzoic anhydride (1.36 mg, 6.01 mmol). The reaction mixture was