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Research Article

N^3-Substituted thymidine analogues III: radiosynthesis of N^3-[(4-[^{18}F]fluoromethyl-phenyl)butyl]thymidine ([^{18}F]-FMPBT) and N^3-[(4-[^{18}F]fluoromethyl-phenyl)pentyl] thymidine ([^{18}F]-FMPPT) for PET

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Abstract: Radiosyntheses of two N^3-substituted thymidine analogues, N^3-[(4[^{18}F]fluoromethyl-phenyl)butyl]thymidine ([^{18}F]-FMPBT) and N^3-[(4[^{18}F]fluoromethyl-phenyl)pentyl]thymidine ([^{18}F]-FMPPT), are reported. The precursor compounds **9** and **10** were synthesized in six steps and the standard compounds **13** and **14** were synthesized from these precursors. For radiosynthesis, compounds **9** and **10** were fluorinated with *n*-Bu_4N[^{18}F] to produce [^{18}F]-**11** and [^{18}F]-**12**, which by acid hydrolysis yielded [^{18}F]-**13** and [^{18}F]-**14**, respectively. The crude products were purified by high-performance liquid chromatography to obtain [^{18}F]-FMPBT and [^{18}F]-FMPPT. The average decay-corrected radiochemical yield for [^{18}F]-**13** was 15% in five runs, and that for [^{18}F]-**14** was 10% in four runs. The radiochemical purity was >99% and the specific activity was >74 GBq/μmol at the end of synthesis. The synthesis time was 80–90 min from the end of bombardment. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: fluorine-18; N^3-substituted thymidine; TK1; PET

Introduction

Since the late 1970s, many fluorinated analogues of pyrimidine nucleosides have been synthesized and studied for their antitumor and antiviral activities.[1–5] Among these compounds, 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 the thymidine kinases TK1 and TK2, and are effective against viruses such as herpes simplex virus (HSV) type 1 and 2, and the hepatitis B-virus.[6–8] We and others have been developing and testing several radiofluorinated pyrimidine nucleoside analogues for use in imaging tumor proliferation, DNA synthesis, and HSV-tk reporter gene expression.[6,9–15] [^{11}C]- and [^{18}F]-labeled FMAU are currently in clinical trials at several centers for imaging tumor proliferation or DNA synthesis in a variety of types of cancer.[16–18] 2′-Deoxy-2′-fluoro-5-methyl-1-β-D-ribofuranosyluracil (FMRU) and 5-substituted analogues have also been synthesized and tested for their ability to visualize tumor proliferation.[19,20] Nucleoside analogues radiolabeled in the 3′-position reported to date are 3′-[^{18}F]-fluoro-3′-deoxythymidine ([^{18}F]-FLT)[11] and 3′-deoxy-3′-[^{18}F]fluoro-5-methyl-1-β-D-xylofuranosyluracil ([^{18}F]-FMXU).[21] [^{18}F]-FLT is currently under clinical investigation for tumor proliferation by positron emission tomography (PET).[22,23] Although both [^{18}F]-FMAU and [^{18}F]-FLT are in clinical investigation, their rates of phosphorylation by TK1 are relatively low compared with that of thymidine. Therefore, there is a desire for radiotracers, which are substrates for TK1 with high rates of phosphorylation and being stable *in vivo*.

During the past decade, a series of N^3-substituted thymidine analogues carrying a carboranyl moiety at the N^3-position, with spacers of various lengths, have been developed as boron delivery agents for boron neutron capture therapy.[24–28] Some of these N^3-substituted thymidine analogues are phosphorylated by TK1 at an efficacy of 50–89% of that of thymidine but are poor substrates for thymidine phosphorylase.[26–28]

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These favorable characteristics of N^3-substituted thymidine analogues make them attractive candidates for development as radiolabeled agents for imaging proliferative activity. Some simple N^3-substituted thymidine derivatives with simple substitutions at the N^3-position, e.g. ethyl, *n*-butyl and acetylenic have also been shown to be effective substrates for TK1, with high phosphorylation rates.[26,27,29] These favorable properties of N^3-substituted thymidine analogues prompted us to explore the development of [^{18}F]-labeled N^3-substituted thymidine analogues for use in PET imaging of TK1 activity. Recently, we and others have reported a few [^{18}F]-labeled N^3-substituted thymidine analogues with open chains of different lengths at the N^3-position.[30,32] We hypothesize that a bulky lipophilic group, such as a phenyl group, at the end of the tether may mimic the carboranyl group of the previously reported compounds, which have been reported to be substrates for TK1 with 50–89% efficiency relative to thymidine but do not react with thymidine phosphorylase. Such compounds, radiolabeled with [^{18}F], would thus be good candidates for PET imaging of cellular proliferation. Here, we report the synthesis and radiosynthesis of two new N^3-substituted thymidine analogues with chains of different lengths containing an aromatic substituent at the end of the spacer: N^3-[(4-[^{18}F]-fluoromethyl-phenyl)butyl]thymidine ([^{18}F]-FMPBT) and N^3-[4-([^{18}F]-fluoromethyl-phenyl)pentyl]thymidine ([^{18}F]-FMPPT). Our synthesis produced these tracers in good yields with high purity and high specific activity.

Results and Discussion

The scheme for synthesizing the N^3-substituted thymidine analogues [^{18}F]-FMPBT and [^{18}F]-FMPPT is shown in Figure 1. Compound **1** was prepared from thymidine according to a published method, with 95% yield.[32]

Compound **2** was prepared by reaction of 4-bromobenzyl alcohol with *tert*-butyldimethylsilyl chloride (TBDMSCl) in dichloromethane in the presence of triethylamine. Compound **2** was purified by column chromatography and isolated in 79% yield. The ^{1}H NMR spectrum of **2** was consistent with that published in the literature.[33] Compounds **3** and **4** were prepared by treating **2** with *n*-butyllithium in dry tetrahydrofuran (THF) at −78°C followed by addition of 1-chloro-4-iodobutane and 1-chloro-5-iodopentane, respectively. Compounds **3** and **4** were both purified by flash chromatography on silica gel columns. Yields in this step were 73% and 61% for **3** and **4**, respectively.

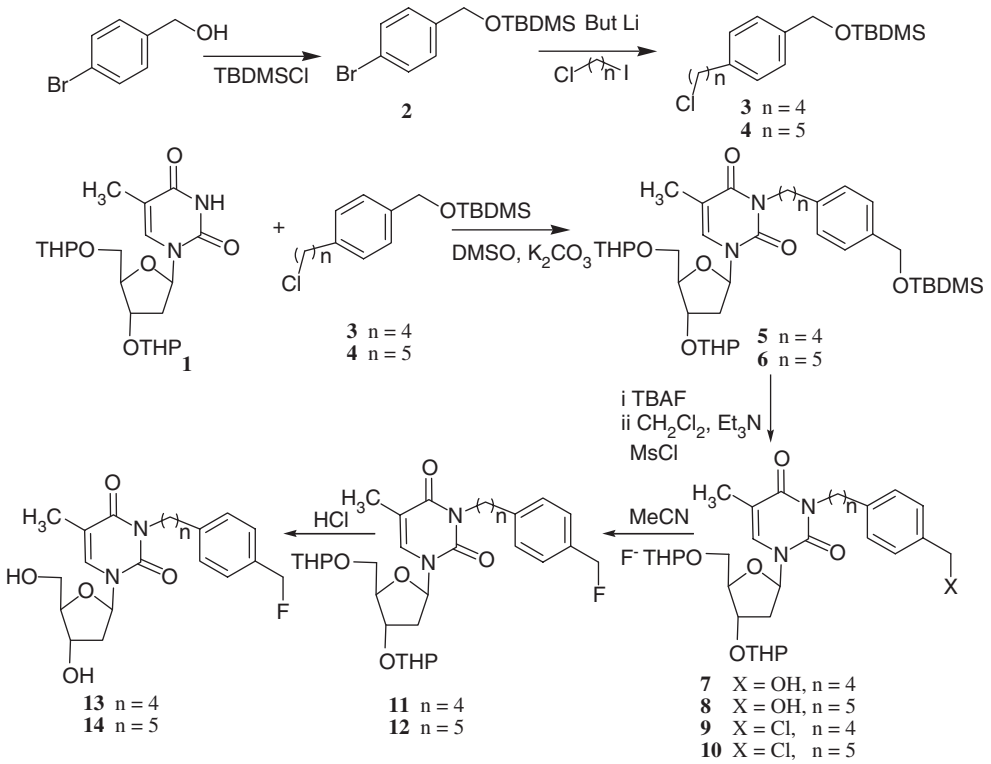

Figure 1 Synthetic scheme for N^3-[(4-[^{18}F]fluoromethyl-phenyl)butyl]thymidine ([^{18}F]-FMPBT); compound [^{18}F]-**13** and N^3-[(4-[^{18}F]fluoromethyl-phenyl)pentyl]thymidine ([^{18}F]-FMPPT); compound [^{18}F]-**14**.

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Compounds **5** and **6** were prepared by reacting **3** and **4** with thymidine tetrahydropyranyl ether (THP) **1** in acetone and dimethylsulfoxide (DMSO) (1:1) and potassium carbonate at 50°C following a published method.[24] Purified yields in this step were 69% for **5** and 59% for **6**. Compounds **5** and **6** were hydrolyzed by Bu_4NF in THF and purified by flash chromatography to obtain compound **7** in 66% yield, and **8** in 68% yield.

Compounds **9** and **10** were prepared by reaction of **7** and **8** with methanesulfonyl chloride in dichloromethane in the presence of triethylamine and *N*,*N*-dimethyl aminopyridine (DMAP). Although our aim was to prepare mesylate derivatives, the reaction at room temperature eventually produced the respective chloroderivatives **9** and **10**. The mesylates were quite unstable to isolate; they were readily converted to the respective chlorides. Small amount of the mesylate was isolated in some reactions. Yields in this step were 42% and 47% for compounds **9** and **10**, respectively.

Compounds **11** and **12** were prepared by reacting **9** and **10** with *n*-Bu_4NF in dry acetonitrile at 90°C for 25 min. Yields in this step were 31% and 26% for **11** and **12**, respectively. These compounds were characterized by ^{19}F NMR spectroscopy in addition to ^{1}H NMR and mass spectrometry. The ^{19}F NMR spectra (coupled) of compounds **11** and **12** showed multiplets centered at −204.08 ppm and −203.92 ppm, respectively.

Compounds **13** and **14** were prepared by acid hydrolysis of compounds **11** and **12**, respectively, followed by purification with high-performance liquid chromatography (HPLC). Yields in this step were 70% for **13** and 66% for **14**. These compounds were also characterized by ^{1}H and ^{19}F NMR spectroscopy and by high-resolution mass spectrometry. The ^{1}H NMR spectrum of compound **13** showed a peak (d) at 5.35 ppm with $J = 48.3$ Hz, a typical geminal coupling constant between fluorine and hydrogen. Similarly, compound **14** showed a peak at 5.36 ppm (d) with the similar characteristic F–H geminal coupling constant of 48.0 Hz.

Radiolabeled compounds **13** and **14** were prepared by radiofluorination of precursors **9** and **10** separately with *n*-Bu_4N[^{18}F] followed by acid hydrolysis and HPLC purification. After radiofluorination of the precursors, the crude reaction mixture was passed through a silica gel cartridge (900 mg, Alltech), and the crude product was eluted with ethyl acetate. The recovered [^{18}F]-labeled intermediate compounds **11** and **12** were readily hydrolyzed with acid to remove the protecting groups, and the desired labeled N^3-substituted thymidine derivatives [^{18}F]-**13** ([^{18}F]-FMPBT) and [^{18}F]-**14** ([^{18}F]-FMPPT) were isolated by HPLC purification. Two different HPLC solvent systems were required, 35% MeCN in water for **13** and 40% MeCN in water for **14**, because of the difference in lipophilicity between the compounds. The retention times for both products, 11.5–12.5 min, were quite similar under these solvent systems. Pure products [^{18}F]-**13** and [^{18}F]-**14** showed a single radioactive peak that co-eluted with their cold standard in analytical HPLC. The radiochemical yields were 14–17% decay corrected (d.c.) for [^{18}F]-**13**, with an average of 15% in five runs, and 8–12% (d.c.) for [^{18}F]-**14**, with an average of 10% in four runs. The radiochemical purity was >99%, and the specific activity was >74 GBq/μmol. The synthesis time was 80–90 min from the end of bombardment (EOB).

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). *n*-Bu_4NHCO_3 solution was prepared by bubbling CO_2 gas into a solution of *n*-Bu_4NOH (1% by wt) to pH 7.0.

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 with tetramethylsilane used as an internal reference and hexafluorobenzene as an external reference at The University of Texas M. D. Anderson Cancer Center. Low-resolution mass spectral analysis was performed in house with an HPLC-mass spectrometer (Applied Biosystems Q-Trap LC/MS/MS). High-resolution mass spectra were obtained on a Bruker BioTOF II mass spectrometer at the University of Minnesota by using electrospray ionization (ESI) technique.

HPLC was performed with an 1100 series pump (Agilent, Germany), with a built-in UV detector operated at 254 nm, and a radioactivity detector with single-channel analyzer (Bioscan, Washington, DC) with 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). An acetonitrile/water (MeCN/H_2O) solvent system (35% MeCN/H_2O for FMPBT and 40% MeCN for FMPPT) was used for purification of the radiolabeled products at a flow of 4 mL/min. Quality control analyses were performed on an analytical HPLC with the same solvents at a flow of 1 mL/min.

Preparation of 3′,5′-*O*-Bis-tetrahydropyranyl-thymidine: 1

3′,5′-*O*-bis-tetrahydropyranyl-thymidine **1** was prepared according to a published method.[32]

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Preparation of 4-bromobenzyl *tert*-butyldimethylsilyl ether: 2

4-Bromobenzylalcohol (0.5 g, 2.67 mmol) was dissolved in dichloromethane (15 mL) in a dry flask under argon; triethylamine (1.9 mL, 13.35 mmol) was added, followed by addition of DMAP (98 mg, 0.80 mmol). TBDMSCl (0.605 g, 4.0 mmol) was added and the reaction mixture was stirred for 3 h at room temperature, when TLC showed that no starting material remained. The solvent was evaporated under vacuum, the residue was dissolved in CH_2Cl_2 (60 mL), and the solution was washed with H_2O (3×60 mL). The organic phase was dried ($MgSO_4$), evaporated to dryness and the crude product was purified on a silica gel column by using 10% ethyl acetate in hexane as eluent. This afforded product **2** as a colorless oil (630 mg, 79% yield). ^{1}H NMR ($CDCl_3$) δ: 7.48 (d, 2H, $J = 8.4$ Hz, aromatic), 7.22 (d, 2H, $J = 8.7$ Hz, aromatic), 4.71 (s, 2H, benzylic H), 0.97 (s, 9H, *tert*-but), 0.12 (s, 6H, CH_3). MS: M + 1, calculated 301.10, found 301.24.

Preparation of 1-chloro-4-(4-*tert*-butyl-dimethylsilyl-benzyl)butane and 1-chloro-5-(4-*tert*-butyl-dimethylsilyl-benzyl)pentane: 3, 4

Compounds **3** and **4** were prepared with the same method; a representative procedure is described below. A solution of compound **2** (0.5 g, 1.67 mmol) in dry THF (7 mL) was cooled to −78°C for 15 min under argon. *n*-Butyllithium (1.6 M in hexanes, 1.7 mL, 2.67 mmol) was slowly added using a syringe over a period of 5 min. After the mixture was stirred at the same temperature for 3 min, a solution of 1-chloro-4-iodobutane (0.27 mL, 2.17 mmol) in THF (0.5 mL) was added dropwise using a syringe over 5 min. The reaction mixture was stirred at −78°C for 20 min, warmed to room temperature, and continued stirring for another 20 min, and then quenched with H_2O (50 μL). The solvent was evaporated under vacuum; the residue was purified on a silica gel column using 5% acetone in hexane as eluent. The pure compound **3** (380 mg) was obtained in 73% yield. Compound **4** was obtained in 61% yield. ^{1}H NMR **3** ($CDCl_3$) δ: 7.23 (d, 2H, $J = 8.4$ Hz, aromatic), 7.16 (d, 2H, $J = 8.1$ Hz, aromatic), 4.73 (s, 2H, benzylic H), 3.56 (t, 2H, $J = 4.8$ Hz, C_1H), 2.65 (t, 2H, $J = 6.3$ Hz, C_4H), 1.80 (t, 4H, $J = 3.0$ Hz, $C_{2,3}$H), 0.95 (s, 9H, *tert*-but), 0.11 (s, 6H, CH_3). MS: $M + NH_4$, calculated 330.23, found 330.30.

^{1}H NMR **4** ($CDCl_3$) δ: 7.26 (d, 2H, $J = 8.4$ Hz, aromatic), 7.16 (d, 2H, $J = 8.1$ Hz, aromatic), 4.73 (s, 2H, benzylic H), 3.54 (t, 2H, $J = 6.9$ Hz, C_1H), 2.63 (t, 2H, $J = 7.5$ Hz, C_5H), 1.82 (quint, 2H, C_2H), 1.66 (quint, 2H, C_4H), 1.46 (m, 2H, C_3H), 0.96 (s, 9H, *tert*-but), 0.12 (s, 6H, CH_3). MS: $M + NH_4$, calculated 344.18, found 344.36.

Preparation of 3′,5′-*O*-bis-tetrahydropyranyl-N^3-[4-(4-*tert*-butyl-dimethylsilyl-benzyl)butyl]thymidine and 3′,5′-*O*-bis-tetrahydropyranyl-N^3-[5-(4-*tert*-butyl-dimethylsilyl-benzyl)pentyl]thymidine: 5, 6

Compounds **5** and **6** were prepared by the same methodology; a representative procedure is described below. Compound **3** (0.400 g, 1.28 mmol) was dissolved in a mixture of acetone and DMSO (20 mL, 1:1) under argon. Potassium carbonate (0.634 g, 6.4 mmol) and thymidine THP ether **1** (0.525 g, 1.28 mmol) were added, and the reaction mixture was heated with stirring at 50°C for 27 h, when TLC showed that no significant starting material remained. The reaction mixture was filtered and evaporated under vacuum, and the residue was dissolved in CH_2Cl_2 (60 mL). The solution was washed with H_2O (3×60 mL). The organic phase was dried ($MgSO_4$), evaporated to dryness, and purified on a silica gel column using 25% acetone in hexane. The pure compound **5** (605 mg) was obtained in 69% yield. Compound **6** was obtained in 59% yield. ^{1}H NMR **5** ($CDCl_3$) δ: 7.68–7.52 (4s, 1H, C_6H), 7.23 (d, 2H, $J = 7.8$ Hz, aromatic), 7.14 (d, 2H, $J = 8.1$ Hz, aromatic), 6.44–6.33 (m, 1H, 1′H), 4.78–3.48 (m, 14H, benzylic H, N^3–C_1H, THP and 3′–5′ H), 2.70–2.60 (m, 2H, N^3–C_4H), 2.58–2.01 (m, 2H, 2′H), 1.94 and 1.92 (2s, 3H, CH_3), 1.89–1.77 (m, 4H, N^3–$C_{2,3}$H), 1.72–1.49 (m, 12H, THP), 0.95 (s, 9H, *tert*-but), 0.11(s, 6H, CH_3). MS: M + 1, calculated 687.95, found 687.83.

^{1}H NMR **6** ($CDCl_3$) δ: 7.65–7.52 (4s, 1H, C_6H), 7.23 (d, 2H, $J = 7.5$ Hz, aromatic), 7.14 (d, 2H, $J = 8.1$ Hz, aromatic), 6.44–6.33 (m, 1H, 1′H), 4.78–3.49 (m, 14H, benzylic H, N^3–C_1H, THP and 3′–5′H), 2.65–2.55 (m, 2H, N^3–C_5H), 2.53–2.00 (m, 2H, 2′H), 1.96 and 1.93 (2s, 3H, CH_3), 1.89–1.77 (m, 4H, N^3–$C_{2,4}$H), 1.71–1.51 (m, 12H, THP), 1.48–1.38 (m, 2H, N^3–C_3H), 0.95 (s, 9H, *tert*-but), 0.10 (s, 6H, CH_3). MS: $M + NH_4$, calculated 718.41, found 718.93.

Preparation of 3′,5′-*O*-bis-tetrahydropyranyl-N^3-[(4-hydroxybenzyl)butyl]thymidine and 3′,5′-*O*-bis-tetrahydropyranyl-N^3-[(4-hydroxybenzyl)pentyl]thymidine: 7, 8

Compounds **7** and **8** were prepared by the same method. Compound **5** (800 mg, 1.16 mmol) was taken in a round-bottom flask and dissolved in THF (15 mL). Bu_4NF solution (1 M, 2.33 mL) was added to the above solution and stirred for 2 h at room temperature, when TLC showed that no starting material remained.

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Solvent was evaporated and the residue was purified on a silica gel column using 30% acetone in hexane to isolate **7** (440 mg) in 66% yield. Compound **8** was obtained in 68% yield. ^{1}H NMR **7** ($CDCl_3$) δ: 7.66–7.52 (4s, 1H, C_6H), 7.29 (d, 2H, $J = 7.8$ Hz, aromatic), 7.17 (d, 2H, $J = 8.1$ Hz, aromatic), 6.43–6.32 (m, 1H, 1′H), 4.78–3.51 (m, 14H, benzylic H, N^3–C_1H, THP and 3′–5′H), 2.66 (t, 2H, N^3–C_4H), 2.58–2.01 (m, 2H, 2′H), 1.95 and 1.92 (2s, 3H, CH_3), 1.90–1.76 (m, 4H, N^3–$C_{2,3}$H), 1.72–1.51 (m, 12H, THP). MS: M + 1, calculated 573.69, found 573.74.

^{1}H NMR **8** ($CDCl_3$) δ: 7.67–7.51 (4s, 1H, C_6H), 7.27 (d, 2H, $J = 7.8$ Hz, aromatic), 7.16 (d, 2H, $J = 7.8$ Hz, aromatic), 6.43–6.32 (m, 1H, 1′H), 4.77–3.48 (m, 14H, benzylic H, N^3–C_1H, THP and 3′–5′H), 2.68–2.58 (m, 2H, N^3–C_5H), 2.49–2.23 (m, 2H, 2′H), 1.95 and 1.92 (2s, 3H, CH_3), 1.90–1.76 (m, 4H, N^3–$C_{2,4}$H), 1.69–1.51 (m, 12H, THP) 1.49–1.35 (m, 2H, N^3–C_3H). MS: M + 1, calculated 587.30, found 587.61.

Preparation of 3′,5′-*O*-bis-tetrahydropyranyl-N^3-[(4-chloro-benzyl)butyl]thymidine and 3′,5′-*O*-bis-tetrahydropyranyl-N^3-[(4-chloro-benzyl)pentyl]thymidine: 9, 10

Compounds **9** and **10** were prepared by the same method. Compound **7** (0.650 g, 1.13 mmol) was dissolved in dichloromethane (15 mL) under argon, and triethylamine (0.8 mL, 5.65 mmol) was added, followed by addition of DMAP (42 mg, 0.33 mmol). The reaction mixture was cooled to 0°C, and then methane sulfonyl chloride (265 μL, 3.40 mmol) was added. The reaction mixture was warmed to room temperature and stirred for 2 h, when TLC showed that no starting material remained. The solvent was evaporated under vacuum, the residue was dissolved in CH_2Cl_2 (60 mL), and the solution was washed with H_2O (3 × 60 mL). The organic phase was dried ($MgSO_4$), evaporated to dryness, and purified on a silica gel column. Appropriate fractions were combined and evaporated to produce 280 mg of the compound **9** in 47% yield. Compound **10** was obtained in 34% yield. ^{1}H NMR **9** ($CDCl_3$) δ: 7.65–7.53 (4s, 1H, C_6H), 7.30 (d, 2H, $J = 8.1$ Hz, aromatic), 7.18 (d, 2H, $J = 7.8$ Hz, aromatic), 6.44–6.33 (m, 1H, 1′H), 4.78–3.50 (m, 14H, benzylic H, N^3–C_1H, THP and 3′–5′H), 2.66 (t, $J = 6.9$ Hz, 2H, N^3–C_4H), 2.48–2.01 (m, 2H, 2′H), 1.96 and 1.92 (2s, CH_3), 1.89–1.76 (m, 4H, N^3–$C_{2,3}$H), 1.73–1.59 (m, 12H, THP). High-resolution MS: M + Na, calculated 613.2651, found 613.2658.

^{1}H NMR **10** ($CDCl_3$) δ: 7.63–7.52 (4s, 1H, C_6H), 7.28 (d, 2H, $J = 8.1$ Hz, aromatic), 7.16 (d, 2H, $J = 7.8$ Hz, aromatic), 6.43–6.32 (m, 1H, 1′H), 4.77–3.48 (m, 14H, benzylic H, N^3–C_1H, THP and 3′–5′H), 2.67–2.55 (m, 2H, N^3–C_5H), 2.29–2.00 (m, 2H, 2′H), 1.96 and 1.92 (2s, 3H, CH_3), 1.89–1.78 (m, 4H, N^3–$C_{2,4}$H), 1.73–1.50 (m, 12H, THP), 1.48–1.30 (m, 2H, N^3–C_3H). High-resolution MS: M + Na, calculated 627.2808, found 627.2819.

Preparation of 3′,5′-*O*-bis-tetrahydropyranyl-N^3-[(4-fluoromethyl-phenyl)butyl]thymidine and 3′,5′-*O*-bis-tetrahydropyranyl-N^3-[(4-fluoromethyl-phenyl)-pentyl]thymidine: 11, 12

As the fluorination reactions of both precursor compounds **9** and **10** were similar, a representative procedure is described here. Compound **9** (40 mg, 0.07 mmol) was dissolved in dry MeCN (1.0 mL) in a sealed v-vial under argon. To the above solution, *n*-Bu_4NF (1 M, 50 μL) was added and the mixture was heated at 90°C for 25 min in a heating block. The reaction mixture was cooled to room temperature and the solvent was evaporated under a stream of air. The residue was purified on a short silica gel column using 25% acetone in hexane as the eluent. Pure compound **11** (12 mg) was obtained in 31% yield. Compound **12** was obtained in 26% yield. ^{1}H NMR **11** ($CDCl_3$) δ: 7.64–7.53 (4s, 1H, C_6H), 7.30 (d, 2H, $J = 8.1$ Hz, aromatic), 7.22 (d, 2H, $J = 8.4$ Hz, aromatic), 6.44–6.33 (m, 1H, 1′H), 5.35 (d, 2H, $J = 48.0$ Hz, benzylic H), 4.77–3.50 (m, 12H, N^3–C_1H, THP and 3′–5′H), 2.75–2.60 (m, 2H, N^3–C_4H), 2.58–2.00 (m, 2H, 2′H), 1.96 and 1.92 (2s, 3H, CH_3), 1.87–1.75 (m, 4H, N^3–$C_{2,3}$H), 1.71–1.53 (m, 12H, THP). ^{19}F NMR ($CDCl_3$) (δ): −204.08 (t, $J = 51$ Hz, coupled). MS: M + 1, calculated 575.72, found 575.65.

^{1}H NMR **12** ($CDCl_3$) δ: 7.65–7.52 (4s, 1H, C_6H), 7.30 (d, 2H, $J = 8.1$ Hz, aromatic), 7.17 (d, 2H, $J = 8.4$ Hz, aromatic), 6.44–6.33 (m, 1H, 1′H), 5.35 (d, 2H, $J = 48.3$ Hz, benzylic H), 4.78–3.49 (m, 12H, N^3–C_1H, THP and 3′–5′H), 2.68–2.59 (m, 2H, N^3–C_5H), 2.57–2.00 (m, 2H, 2′H), 1.96 and 1.92 (2s, 3H, CH_3), 1.90–1.78 (m, 4H, N^3–$C_{2,4}$H), 1.74–1.50 (m, 12H, THP), 1.48–1.36 (m, 2H, N^3–C_3H). ^{19}F NMR (δ): −203.92 (t, $J = 51$ Hz, coupled). MS: M + 1, calculated 589.34, found 589.60.

Preparation of N^3-[(4-fluoromethyl-phenyl)butyl]thymidine and N^3-[(4-fluoromethyl-phenyl) pentyl] thymidine: 13, 14

Compounds **13** and **14** were prepared by the same method; a representative procedure is described here for **13**. Compound **11** (15 mg, 0.03 mmol) was placed in a small flask and dissolved in MeOH (1 mL). Hydrochloric acid (1 M in MeOH, 0.1 mL) was added to the above solution and the reaction mixture was refluxed for 5 min at 80°C. The reaction mixture was cooled and the solvent evaporated. The residue was purified on a short silica gel column using 40% acetone in hexane as

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the eluent. The pure compound **13** (7.4 mg) was obtained in 70% yield. Compound **14** was obtained in 66% yield.

^{1}H NMR **13** ($CDCl_3$) δ: 7.32 (s, 1H, C_6H), 7.30 (d, 2H, $J = 8.1$ Hz, aromatic), 7.22 (d, 2H, $J = 8.1$ Hz, aromatic), 6.18 (t, 1H, $J = 6.9$ Hz, 1′H), 5.35 (d, 2H, $J_{HF} = 48.3$ Hz, benzylic H), 4.63–4.58 (m, 1H, 3′H), 4.03–3.82 (m, 5H, N^3–C_1H, 4′ and 5′H), 2.67 (bs, 2H, N^3–C_4H), 2.51–2.26 (m 2H, 2′H), 1.94 (s, 3H, CH_3), 1.72–1.64 (m, 4H, N^3–$C_{2,3}$H). ^{19}F NMR (δ): −203.92 (t, $J = 51$ Hz, coupled). High-resolution MS: M + Na, calculated 429.1796, found 429.1802.

^{1}H NMR **14** ($CDCl_3$) δ: 7.29 (s, 1H, C_6H), 7.31 (d, 2H, $J = 8.4$ Hz, aromatic), 7.21 (d, 2H, $J = 8.1$ Hz, aromatic), 6.16 (t, 1H, $J = 7.2$ Hz, 1′H), 5.36 (d, 2H, $J_{HF} = 48.0$ Hz, benzylic H), 4.64–4.62 (m, 1H, 3′H), 4.04–3.83 (m, 5H, N^3–C_1H, 4′ and 5′H), 2.64 (t, 2H, $J = 6.9$ Hz, N^3–C_5H), 2.54–2.44 (m, 2H, 2′H), 1.94 (s, 3H, CH_3), 1.74–1.60 (m, 4H, N^3–$C_{2,4}$H), 1.45–1.34 (m, 2H, N^3–C_5H). ^{19}F NMR (δ): −203.73 (t, $J = 51$ Hz, coupled). High-resolution MS: M + Na, calculated 443.1953, found 443.1960.

Preparation of N^3-[(4-[^{18}F]fluoromethyl-phenyl)butyl]thymidine and N^3-[(4-[^{18}F]fluoromethyl-phenyl)-pentyl]thymidine: [^{18}F]-13, [^{18}F]-14

The aqueous [^{18}F]fluoride was trapped in an 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 was evaporated azeotropically with anhydrous MeCN (1.0 mL) to dryness at 80°C under a stream of argon. To the dried *n*-Bu_4N[^{18}F], a solution of **9** or **10** (5–6 mg) in anhydrous MeCN (0.5 mL) was added, and the mixture was heated at 90°C for 25 min. The reaction mixture was cooled, passed through a silica gel cartridge (Alltech), and eluted with ethyl acetate (2.5 mL). After evaporation of the solvent under 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 5 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 the radioactivity was measured in a dose calibrator (Capintec, Ramsey, NJ). The solvent was evaporated and the product was redissolved in saline and filtered through a 0.22 μm Millipore filter. The final product was analyzed by HPLC onto an analytical column and co-injected with an authentic standard compound to confirm its identity and purity.

Conclusion

We have synthesized two new radiolabeled N^3-substituted analogues of thymidine, N^3-[(4-[^{18}F]fluoromethyl-phenyl)butyl] thymidine ([^{18}F]-FMPBT) and N^3-[(4-[^{18}F]fluoromethyl-phenyl)pentyl]thymidine ([^{18}F]-FMPPT) in good yields, with high purity and high specific activity. These compounds may be useful agents for PET imaging of tumor proliferation and DNA synthesis.

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