JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2006; **49**: 1079–1088.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.1127

Research Article

Synthesis of [^{18}F]-labeled N-3(substituted) thymidine analogues: N-3([^{18}F]fluorobutyl) thymidine ([^{18}F]-FBT) and N-3([^{18}F]fluoropentyl) thymidine ([^{18}F]-FPT) for PET

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Summary

Syntheses of N-3(substituted) analogues of thymidine, N-3([^{18}F]fluorobutyl)thymidine ([^{18}F]-FBT) and N-3([^{18}F]fluoropentyl)thymidine ([^{18}F]-FPT) are reported. 1,4-Butane diol and 1,5 pentane diol were converted to their tosyl derivatives **2** and **3** followed by conversion to benzoate esters **4** and **5,** respectively. Protected thymidine **1** was coupled separately with **4** and **5** to produce **6** and **7**, which were hydrolyzed to **8** and **9**, then converted to their mesylates **10** and **11**, respectively. Compounds **10** and **11** were fluorinated with *n*-Bu_4N[^{18}F] to produce **12** and **13**, which by acid hydrolysis yielded **14** and **15**, respectively. The crude products were purified by HPLC to obtain [^{18}F]-FBT and [^{18}F]-FPT. The radiochemical yields were 58–65% decay corrected (d.c.) for **14** and 46–57% (d.c.) for **15** with an average of 56% in three runs per compound. Radiochemical purity was $>99\%$ and specific activity was >74 GBq/μmol at the end of synthesis (EOS). The synthesis time was 65–75 min from the end of bombardment (EOB). Copyright © 2006 John Wiley & Sons, Ltd.

Received 2 June 2006; Revised 3 August 2006; Accepted 4 August 2006

Key Words: fluorine-18; thymidine; TK1; PET

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Contract/grant sponsor: The University of Texas MD Anderson Cancer Center
Contract/grant sponsor: CCSG; contract/grant number: CA016672

Introduction

We and others have been developing and testing several radiofluorinated pyrimidine nucleoside analogues as potential agents for imaging tumor proliferative activity or HSV-tk reporter gene expression.[1–8] Initially, many fluorinated analogues 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-arabinofuranosyl-uracil (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 hepatitis B-virus.[14,15] In particular, ^{11}C and ^{18}F radiolabeled FMAU are currently undergoing clinical studies in multiple centers for imaging tumor proliferation in a variety of cancer types[16,17] and DNA synthesis.[18] Also, 2′-deoxy-2′-fluoro-5-methyl-1-β-D-ribofuranosyl-uracil (FMRU) and 5-substituted analogues have been synthesized and tested as potential imaging agents for tumor proliferation.[19,20] The nucleoside analogues radiolabeled in the 3′-position reported to date are 3′-[^{18}F]-fluoro-3′-deoxy-thymidine ([^{18}F]-FLT)[4] and 3′-deoxy-3′-[^{18}F]fluoro-5-methyl-1-β-D-xylo-furanosyluracil ([^{18}F]-FMXU).[21] [^{18}F]-FLT is currently under clinical investigation as a PET imaging agent for tumor proliferation.[22,23] Although both [^{18}F]-FMAU and [^{18}F]-FLT are in clinical investigation, their phosphorylation rates by TK1 are relatively low compared to that of thymidine.

Recently a series of N-3(substituted)thymidine analogues carrying a carborane moiety at the N-3-position with various spacer length have drawn attention in boron neutron capture therapy.[24–28] It has been reported that some of the N-3(substituted)thymidine analogues are phosphorylated by TK1 with 50–89% efficiency as compared to thymidine, while being poor substrates for thymidine phosphorylase.[25–27] Furthermore, some simple N-3(substituted)thymidine derivatives such as ethyl, *n*-butyl, acetylenic, etc. have also been shown to be substrates of TK1 with high phosphorylation rates.[25,26,29] This prompted us to synthesize ^{18}F-labeled N-3(substituted) thymidine analogues for PET imaging of TK1 activity and DNA synthesis during tumor proliferation. Here we report synthesis and radiosynthesis of two N-3(substituted)thymidine analogues, N-3([^{18}F]-fluorobutyl)thymidine ([^{18}F]-FBT) and N-3([^{18}F]-fluoropentyl)thymidine ([^{18}F]-FPT) in high yield, high specific activity and purity.

Results and discussion

Figure 1 represents the scheme for synthesis of N-3(substituted)thymidine analogues, [^{18}F]-FBT and [^{18}F]-FPT. Compound **1** was prepared from thymidine following a literature method in 95% yield.[30] Compounds **2** and

DOI: 10.1002/jlcr

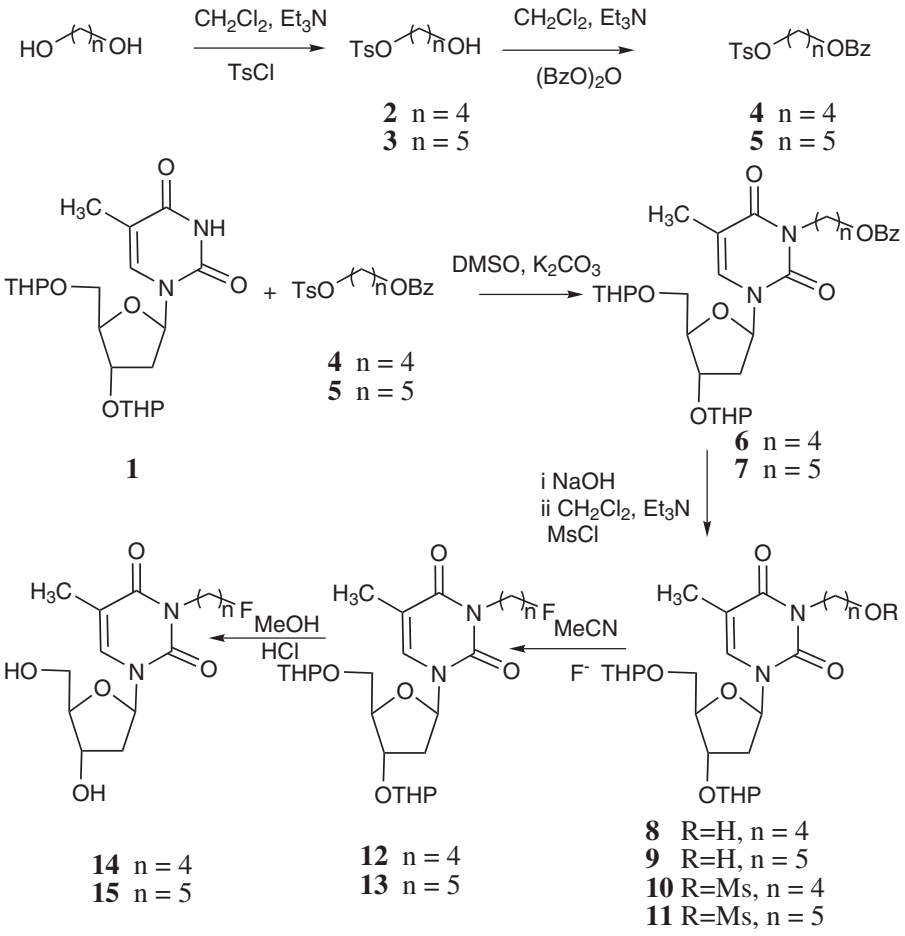

Figure 1. Synthetic scheme of N-3([18F]fluorobutyl)thymidine ([18F]-FBT) and N-3([18F]fluoropentyl)thymidine ([18F]-FPT)

3 were prepared following literature methods,[31,32] in 33 and 40% yields, respectively.

Compounds **4** and **5** were prepared from **2** and **3** by reaction with benzoic anhydride in dichloromethane in the presence of triethylamine at 50°C. Yields in this step were 63 and 76% for **4** and **5**, respectively.

Compounds **6** and **7** were prepared by reaction of **4** and **5** with thymidine THP ether **1** following a literature method.[28] Yields in this step were 80% for **6** and 85% for **7**, respectively. Basic hydrolysis of the benzoate esters **6** and **7** produced **8** and **9** in 92% and 95% yields, respectively.

Compounds **10** and **11** were prepared by reaction of **8** and **9** with methanesulfonyl chloride in dichloromethane in the presence of triethylamine and N,N-dimethyl aminopyridine (DMAP) at 0°C. Cooling the reaction

DOI: 10.1002/jlcr

mixture to 0°C was necessary to obtain high yields. Yields in this step were 86 and 91% for compounds **10** and **11,** respectively.

Compounds **12** and **13** were prepared by the reaction of **10** and **11** with *n*-Bu_4NF in dry acetonitrile at 90°C for 25 min. Yields in this step were 70 and 76% for compounds **12** and **13**, respectively. These compounds were characterized by ^{19}F NMR spectroscopy in addition to ^{1}H NMR, and high resolution mass spectrometry. ^{19}F NMR spectra (coupled) showed multiplets centered at −218.26 and −218.30 ppm for **12** and **13**, respectively.

Compounds **14** and **15** were prepared by acid hydrolysis of the compounds **12** and **13**, respectively, followed by HPLC purification, and characterized by ^{1}H and ^{19}F NMR spectroscopy, and high-resolution mass spectrometry. ^{1}H NMR spectrum of **14** showed a peak (dt) at 4.48 ppm with $J = 47.4$ Hz, a typical geminal coupling constant between fluorine and hydrogen. Similarly, compound **15** showed a peak at 4.46 ppm (dt) with the similar characteristic F-H geminal coupling constant 47.1 Hz. Interestingly, the C_6 proton and the methyl (CH_3) protons showed as doublets with small coupling constants in the range of 0.9 to 1.2 Hz for both compounds. ^{19}F NMR spectrum (coupled) of **14** and **15** showed multiplets centered at −218.30 and −218.36 ppm, respectively, due to long range coupling between fluorine and hydrogen in addition to their geminal coupling, which was also observed in ^{1}H NMR spectra.

Radiolabeled compounds **14** and **15** were prepared by fluorination of the precursors **10** and **11** separately with *n*-$Bu_4N^{18}F$ followed by acid hydrolysis and HPLC purification. *n*-$Bu_4N^{18}F$ was prepared *in situ* from *n*-Bu_4NHCO_3 and aqueous $H^{18}F$ using 0.40 ml (1% solution, ~7 μmol) of *n*-Bu_4NHCO_3 to elute the activity from the ion exchange cartridge.[1,2] Following 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 **12** and **13** were readily hydrolyzed with acid to remove the protecting groups, and the desired labeled N-3(substituted)thymidine derivatives **14** and **15** isolated by HPLC purification. Two different HPLC solvent systems, 25 and 30% MeCN in water for **14** and **15**, respectively, were required due to the difference in lipophilicity between the compounds. The radiochemical yields were 58–65% (d.c.) for **14** and 46–57% (d.c.) for **15** with an average of 56%, in three runs per compound. The radiochemical purity was >99% with specific activity >74 GBq/μmol. The synthesis time was 65–75 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

DOI: 10.1002/jlcr

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 using tetramethylsilane as an internal reference and hexafluorobenzene as an external reference, respectively, at The University of Texas MD Anderson Cancer Center. High resolution mass spectra 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 (25% MeCN for FBT and 30% MeCN for FPT) was used for purification of the radiolabeled products. For quality control analysis on analytical HPLC, 20% MeCN and 25% MeCN were used for FBT and FPT, respectively.

Preparation of 1-p-toluenesulfonyl-4-benzoyl-n-butane and 1-p-toluenesulfonyl-5-benzoyl-n-pentane-: **4**, **5**

Both compounds **4** and **5** were prepared using the same methodology, a representative procedure is described below. Compound **2** (0.375 g, 1.5 mmol) was dissolved in dichloromethane (7 ml) under argon. Triethylamine (1.0 ml, 7.7 mmol) was added followed by addition of benzoic anhydride (0.45 mg, 2.0 mmol). The reaction mixture was refluxed at 50°C for 1.2 h when TLC showed no starting material remained. Solvent was evaporated under vacuum, the residue was re-dissolved in CH_2Cl_2 (50 ml) and washed with H_2O (3×50 ml). The organic phase was dried ($MgSO_4$), evaporated to dryness and purified by flash column chromatography using 20% acetone in hexane as eluent. The pure compound **4**, 332 mg was obtained in 63% yield. Compound **5** was obtained in 76% yield. ^{1}H NMR **4** ($CDCl_3$) δ: 8.02 (d, 2 H, $J = 7.2$ Hz, aromatic), 7.81 (d, 2 H, $J = 8.1$ Hz, aromatic), 7.64–7.55 (m, 1 H, aromatic), 7.48 (d, 2 H, $J = 7.2$ Hz, aromatic), 7.35 (d, 2 H, $J = 8.1$ Hz, aromatic), 4.30 (t, 2 H, $J = 6.0$ Hz, C_4H), 4.12 (t, 2 H, $J = 6$ Hz, C_1H), 2.45 (s, 3 H, CH_3), 1.84 (quint, 4 H, $C_{2-3}H$). MS: M + 1, 349.50. ^{1}H NMR **5** ($CDCl_3$) δ: 8.04 (d, 2 H, $J = 8.4$ Hz, aromatic), 7.80 (d, 2 H, $J = 8.4$ Hz, aromatic), 7.61–7.55 (m, 1 H, aromatic), 7.48–7.43 (m, 2 H, aromatic), 7.34 (d, 2 H, $J = 8.4$ Hz, aromatic), 4.29 (t, 2 H, $J = 6.3$ Hz, C_5H), 4.07 (t, 2 H, $J = 12.6$ Hz, C_1H), 2.44 (s, 3 H, CH_3), 1.76 (quint, 4 H, $C_{2,4}H$), 1.52 (quint, 2 H, C_3H). MS: M + 1, 363.30.

DOI: 10.1002/jlcr

Preparation of 3′,5-′-O-bis-tetrahydropyranyl-N-3(4-benzoyl-n-butyl)thymidine and 3′,5′-O-bis-tetrahydropyranyl-N-3(5-benzoyl-n-pentyl)thymidine: **6**, **7**

Both compounds **6** and **7** were prepared using the same methodology, a representative procedure is described below. Compound **4** (0.10 g, 0.29 mmol) was dissolved in acetone (3 ml) and DMSO (3 ml) (1:1) under argon. Potassium carbonate (0.144 g, 1.45 mmol) and thymidine THP ether **1** (0.119 g, 0.29 mmol) were added and the reaction mixture heated with stirring at 50°C for 30 h when TLC showed no significant starting material remained. The reaction mixture was filtered and evaporated under vacuum, and the residue was dissolved in CH_2Cl_2 (30 ml). The solution was washed with H_2O (3×30 ml). The organic phase was dried ($MgSO_4$), evaporated to dryness and purified on a silica gel column using 20% acetone in hexane. The pure compound **6** (136 mg) was obtained in 80% yield. Compound **7** was obtained in 85% yield. ^{1}H NMR **6** ($CDCl_3$) δ: 8.06–8.03 (m, 2 H, aromatic), 7.66–7.50 (m, 2 H, C_6H and aromatic), 7.46–7.38 (m, 2 H, aromatic), 6.41–6.37 (m, 1 H, 1′H), 4.75–3.52 (m, 14 H, N-3$C_{1,4}$H, THP and 3′-5′H), 2.59–2.0 (m, 2 H, 2′H), 1.96, 1.95, 1.93, 1.92 (4 s, 3 H, CH_3), 1.90–1.48 (m, 16 H, N-3$C_{2,3}$H and THP). High resolution MS: M + Na, calculated 609.2783; found 609.2799. ^{1}H NMR **7** ($CDCl_3$) δ: 8.05 (d, 2 H, $J = 8.1$ Hz, aromatic), 7.65–7.51 (m, 2 H, C_6H and aromatic), 7.47–7.44 (m, 2 H, aromatic), 6.41–6.37 (m, 1 H, 1′H), 4.78–3.48 (m, 14 H, N-3$C_{1,5}$H, THP and 3′-5′H), 2.59–2.00 (m, 2 H, 2′H) 1.96, 1.95, 1.93, 1.92 (4 s, 3 H, CH_3), 1.98–1.48 (m, 18 H, N-3C_{2-4}H and THP). MS: M + 1, 601.60.

Preparation of 3′,5′-O-bis-tetrahydropyranyl-N-3(4-hydroxy-n-butyl)thymidine and 3′,5′-O-bis-tetrahydropyranyl-N-3(5-hydroxy-n-pentyl)thymidine: **8**, **9**

Both compounds **8** and **9** were prepared in the same methodology. Compound **6** (80 mg, 0.14 mmol) was placed in a small flask and dissolved in MeOH (2 ml). Aqueous sodium hydroxide solution (1 M, 0.2 ml) was added to the above solutions and refluxed for 30 min at 70°C when TLC showed no starting material remained. The reaction mixture was cooled and solvent evaporated. The residue was purified on a silica gel column and eluted with 30% acetone in hexane to produce **8** (62 mg) in 92% yield. Compound **9** was obtained in 95% yield. ^{1}H NMR **8** ($CDCl_3$) δ: 7.66, 7.60, 7.56, 7.54 (4d, $J = 1.2$ Hz, 1 H, C_6H), 6.43–6.34 (m, 1 H, 1′H), 4.72–3.48 (m, 14 H, N-3$C_{1,4}$H, THP and 3′-5′H), 2.58–2.10 (m, 2 H, 2′H), 1.96, 1.95, 1.93, 1.92 (4 s, 3 H, CH_3), 1.89–1.48 (m, 16 H, N-3$C_{2,3}$H and THP). MS: M + 1, 483.40. ^{1}H NMR **9** ($CDCl_3$) δ: 7.65, 7.59, 7.55, 7.53 (4d, 1 H, $J = 1.2$ Hz, C_6H), 6.41–6.37 (m, 1 H, 1′H), 4.76–3.48 (m, 14 H, N-3$C_{1,5}$H, THP and 3′-5′H), 2.58–2.00 (m, 2 H, 2′H), 1.96, 1.95, 1.93, 1.92 (4 s, 3 H, CH_3), 1.89–1.39 (m, 18 H, N-3C_{2-4} and THP). High resolution MS: M + Na, calculated 519.2677; found 519.2686.

DOI: 10.1002/jlcr

Preparation of 3′,5′-O-bis-tetrahydropyranyl-N-3(4-methane-sulfonyl-n-butyl) thymidine and 3′,5′-O-bis-tetrahydropyranyl-N-3(5-methanesulfonyl-n-pentyl) thymidine: **10**, **11**

Both compounds **10** and **11** were prepared using the same methodology, a representative procedure is described below. Compound **8** (0.150 g, 0.31 mmol) was dissolved in dichloromethane (5 ml) under argon and triethylamine (0.30 ml, 2.2 mmol) was added, followed by addition of dimethyl aminopyridine (38 mg, 0.31 mmol). The reaction mixture was cooled to 0°C then methane sulfonyl chloride (34 µl, 0.43 mmol) was added. The reaction mixture was stirred for 40 min at 0°C when TLC showed no starting material remained. Solvent was evaporated under vacuum, the residue was dissolved in CH_2Cl_2 (25 ml) and the solution washed with H_2O (3 × 25 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 149 mg of the compound **10** in 86% yields. Compound **11** was obtained in 91% yield. ^{1}H NMR **10** ($CDCl_3$) δ: 7.66, 7.60, 7.56, 7.54 (4d, 1 H, J = 1.2 Hz, C_6H), 6.39–6.35 (m, 1 H, 1′H), 4.74–3.48 (m, 14 H, N-3$C_{1,4}$H, THP and 3′-5′H), 3.01 (s, 3 H, S-CH_3), 2.58–2.00 (m, 2 H, 2′H), 1.95, 1.94, 1.91, 1.90 (4 s, 3 H, CH_3), 1.89–1.49 (m, 16 H, N-3$C_{2,3}$H andTHP). High resolution MS: M + Na, calculated 583.2296; found 583.2300. ^{1}H NMR **11** ($CDCl_3$) δ: 7.65, 7.59, 7.55, 7.53 (4d, J = 1.2 Hz, 1 H, C_6H), 6.42–6.31 (m, 1 H, 1′H), 4.78–3.50 (m, 14 H, N-3$C_{1,5}$H, THP and 3′-5′H), 3.01 (s, 3 H, S-CH_3), 2.51–2.00 (m, 2 H, 2′H), 1.96, 1.95, 1.93,1.92 (4 s, 3 H, CH_3), 1.59-1.40 (m, 18 H, N-3C_{2-4}H and THP). High resolution MS: M + Na, calculated 597.2452; found 597.2465.

Preparation of 3′,5′-O-bis-tetrahydropyranyl-N-3(4-fluoro-n-butyl)thymidine and 3′,5′-O-bis-tetrahydropyranyl-N-3(5-fluoro-n-pentyl)thymidine: **12**, **13**

The fluorination reactions of both precursor compounds **10** and **11** are similar, and a representative procedure is described. Compound **10** (30 mg, 0.05 mmol) was dissolved in dry MeCN (3 ml) in a sealed v-vial under argon. To the above solution, *n*-Bu_4NF (1 M, 30 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 20% acetone in hexane as eluent. The pure compound **12** (20 mg) was obtained in 70% yield. Compound **13** was obtained in 76% yield. ^{1}H NMR **12** ($CDCl_3$) δ: 7.66, 7.60, 7.56, 7.54 (4d, J = 0.9 Hz, 1 H, C_6H), 6.41–6.37 (m, 1 H, 1′H), 4.76–3.48 (m, 14 H, N-3$C_{1,4}$H, THP and 3′-5′H), 2.60–2.00 (m, 2 H, 2′H), 1.96, 1.95, 1.93, 1.92 (4 s, 3 H, CH_3), 1.85–1.41 (m, 16 H, N-3C_{2-3}H and THP). ^{19}F NMR **12** (δ): −218.26 (s, decoupled), −218.09 to −218.43 (m, coupled). High resolution MS: M + K, calculated 523.2216; found 523.2213. ^{1}H NMR **13** ($CDCl_3$) δ:

DOI: 10.1002/jlcr

7.65, 7.59, 7.55, 7.53 (4d, $J = 0.9$ Hz, 1 H, C_6H), 6.41–6.37 (4 s, 1 H, 1H), 4.79–3.52 (m, 14 H, N-3$C_{1,5}$H, THP and 3′-5′H), 2.61–2.00 (m, 2 H, 2′H), 1.96, 1.95, 1.93, 1.92 (4 s, 3 H, CH_3), 1.90–1.40 (m, 18 H, N-3C_{2-4}H and THP). ^{19}F NMR **13** (δ): −218.33 (s, decoupled), −218.16 to −218.58 (m, coupled). High resolution MS: M + Na, calculated 521.2634; found 521.2654.

Preparation of N-3(4-fluoro-n-butyl)thymidine and N-3(5-fluoro-n-pentyl) thymidine: **14**, **15**

Compounds **14** and **15** were prepared in the same methodology and a representative preparation is described below. Compound **12** (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 solvent evaporated. The crude product was diluted with 25% MeCN in water (1.2 ml) and purified by HPLC. After solvent evaporation 6.7 mg of the product was obtain in 68% yield. Compound **15** was purified with 30% MeCN/H_2O to obtain 73% yields. ^{1}H NMR **14** ($CDCl_3$) δ: 7.34 (d, 1 H, $J = 1.2$ Hz, C_6H), 6.19 (t, 1 H, $J = 6.9$ Hz, 1′H), 4.62–4.59 (m, 1 H, 3′H), 4.48 (dt, 2 H, $J_{HF} = 47.4$ Hz and $J_{H-H} = 5.7$ Hz, N-3C_4H), 4.04–3.83 (m, 5 H, N-3C_1H, 4′ and 5′H), 2.51–2.42 (m, 2 H, 2′H), 1.95 (d, 3 H, $J = 1.2$ Hz, CH_3), 1.81–1.69 (m, 4 H, N-3C_{2-3}H). ^{19}F NMR **14** (δ): −218.30 (s, decoupled), −218.04 to −218.55 (m, coupled). High resolution MS: M + Na, calculated 339.1327; found 339.1347. ^{1}H NMR **15** ($CDCl_3$) δ: 7.32 (d, 1 H, $J = 1.2$ Hz, C_6H), 6.18 (t, 1 H, $J = 6.9$ Hz, 1′H), 4.64–4.61 (m, 1 H, 3H), 4.46 (dt, 2 H, $J_{HF} = 47.1$ Hz and $J_{H-H} = 6.0$ Hz, N-3C_5H), 4.04–3.85 (m, 5 H, N-3C_1H, 4′ and 5′H), 2.52–2.43 (m, 2 H, 2′H), 1.94 (d, 3 H, $J = 0.9$ Hz, CH_3), 1.85–1.65 (m, 6 H, N-3C_{2-4}H). ^{19}F NMR **15** (δ): −218.36 (s, decoupled), −218.11 to −218.62 (m, coupled). High resolution MS: M + Na, calculated 353.1483; found 353.1486.

Preparation of N-3(4-[^{18}F]fluoro-n-butyl)thymidine and N-3(5-[^{18}F]fluoro-n-pentyl)thymidine

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 **10** or **11** (5–6 mg) in anhydrous acetonitrile (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

DOI: 10.1002/jlcr

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). Solvent was evaporated and the product was redissolved in saline. The final product was analyzed onto an analytical column and co-injected with an authentic standard compound to confirm its identity.

Conclusion

We have synthesized two new radiotracers, N-3(substituted) analogues of thymidine, N-3([^{18}F]fluorobutyl)thymidine ([^{18}F]-FBT) and N-3([^{18}F]fluoropentyl)thymidine ([^{18}F]-FPT) with high yield, high specific activity and purity. The described method may be suitable for the preparation of other N-3(substituted) analogues of thymidine.

Acknowledgements

This work was supported by start up funds from The University of Texas MD Anderson Cancer Center. NMR spectra were taken at the NMR facility supported by CCSG core grant CA016672.

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