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CONTINUOUS FLOW MICROFLUIDIC CHEMISTRY: SYNTHESIS OF [^{18}F]-3-FLUORO-2-(4-((2-NITRO-1*H*-IMIDAZOL-1-YL)METHYL)-1*H*-1,2,3-TRIAZOL-1-YL)PROPAN-1-OL, [^{18}F]F-HX4, A POTENT HYPOXIA AGENT

Murthy Akula[1], David Blevins[1], and George Kabalka[1,2]*

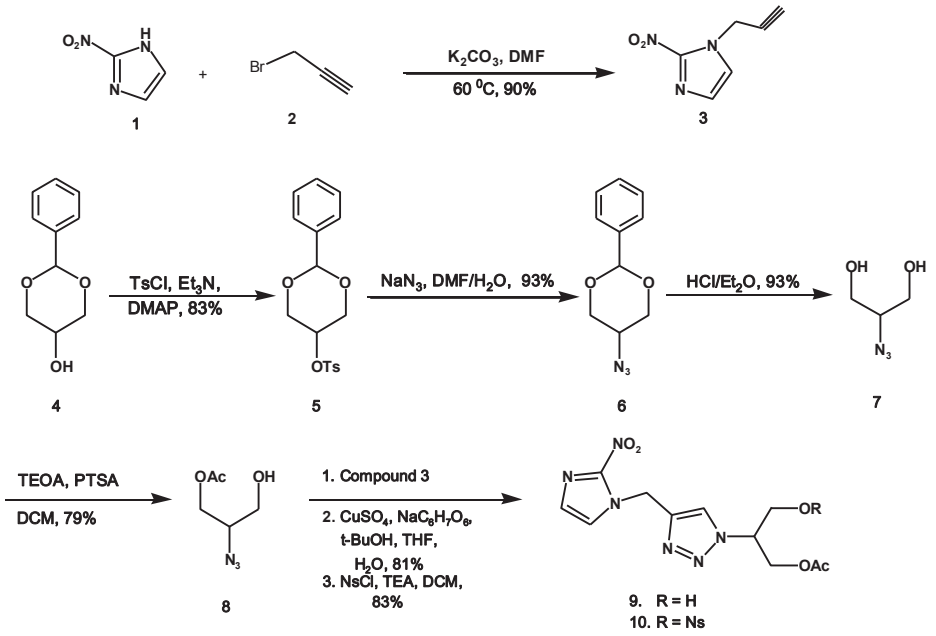
1. Department of Radiology, The University of Tennessee Medical Center, Knoxville, TN, USA 37919; 2. Department of Chemistry, The University of Tennessee, Knoxville, USA. kabalka@utk.edu

Abstract – The microfluidic synthesis of the promising hypoxia imaging agent [^{18}F]-3-fluoro-2-(4-((2-nitro-1*H*-imidazol-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl)propa-n-1-ol, [^{18}F]F-HX4, was successfully accomplished using a commercial microfluidic system. A decay corrected radiochemical yield of 48±4% (n=4) and radiochemical purity of ≥ 98% was obtained. The total reaction time including the HPLC purification was 40 min.

Due to their rapid and irregular growth, solid neoplastic tumors often develop disorganized vascularization which may lead to hypoxic environments within the tumor. Hypoxic tumors are difficult to treat since they resist both radiation and cytotoxic therapies and thus require an alternative treatment plan[1]. Several types of cancers such as breast, cervical, and non-small cell lung cancer are known to develop hypoxic tumors. Hypoxia is related to aggressive cancer metastasis. Determining the hypoxia level within a tumor is crucial since it determines the proper treatment regimen as well as the prognosis for the patient. There are several bioreducible imaging agents that can detect hypoxic cells *in-vivo* including [^{18}F]FMISO, which is one of the most often used hypoxia markers.[2] [^{18}F]FMISO allows the visualization of hypoxic tumors, but it has a slow diffusion rate into hypoxic tumors and a high background uptake, which diminishes the tumor to background ratio. 3-[^{18}F]-Fluoro-2-(4-((2-nitro-1*H*-imidazol-1-yl) methyl)-1*H*-1,2,3-triazol-1-yl)propan-1-ol, [^{18}F]F-HX4, **15** is a PET radiotracer that has a low background uptake due to rapid renal clearance and yields a high tumor to background ratio. A recent study of the efficacy of [^{18}F]F-HX4 for imaging hypoxia in head and neck tumors found that, in a direct comparison with [^{18}F]FMISO, [^{18}F]F-HX4 exhibited higher specificity and sensitivity, faster clearance, and shorter injection-acquisition time than [^{18}F]FMISO.[3] The only

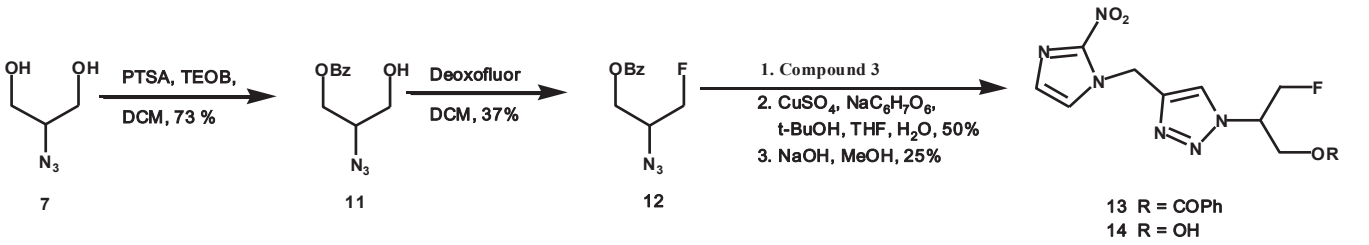
synthesis of [^{18}F]F-HX4 known to date is based on vial chemistry that was performed on a commercially available chemistry module. During the past decade radiochemists have shown a growing interest in using microfluidic chemistry in the development of radiopharmaceuticals.[4] The two types of microfluidics are micro-vessel systems (MVS), miniature versions of batch reactor modules, and micro-channel systems (MCS) in which reactions are performed using flow chemistry in a microenvironment.[5] Recently [^{18}F]fallypride and [^{18}F]FMISO were prepared by MVS and MCS devices for human use.[6,7] Several known radiotracers were prepared using different microfluidic systems that include a variety of radionuclides such as fluorine-18, carbon-11, nitrogen-13, copper-64, and gallium-68.[8] Thus, microfluidic technology presents a viable alternative to conventional vessel based methods and is a versatile platform for the optimization of reaction parameters using very small amounts of expensive precursors and radioactivity. We wish to report the microfluidic synthesis of the title compound **16** using a MCS system which results in in higher yields than the previously reported vial synthesis.

Chemistry. Nosylate precursor **10** and an authentic standard HX4 **14** were synthesized following known procedures with minor modifications.[9] The preparation of nosylate **10** is outlined in **Scheme 1**. Alkyne **3** was prepared from 2-nitroimidazole **1** by N-alkylation using propargyl bromide **2** and potassium carbonate in DMF at 60 °C in 90% yield. *cis/trans*-1,3-O-Benzylidineglycerol **4** was converted to tosylate **5** by treatment with *p*-toluenesulfonyl chloride and trimethylamine in presence of a catalytic amount of DMAP. Displacement of tosylate with azide was carried out using sodium azide in a mixture of DMF and water to afford azide **6** (93%). The benzylidine group was removed in the presence of ethereal HCl to obtain the diol **7** (93%).



Scheme 1

Monoacetylation of diol **7** was performed using triethyl orthoacetate and *p*-toluenesulfonic acid in DCM to obtain acetoxy azide **8**. The copper sulphate mediated click reaction of alkyne **3** and azide **8** in the presence of sodium ascorbate in a mixture of *t*-butanol-THF-water afforded the triazole **9**. Nosylation of **9** with 2-nitrosulfonyl chloride in triethylamine resulted in nosylate **10**. Thus the requisite nosylate precursor **10** was prepared in seven steps with an overall yield of 34%.

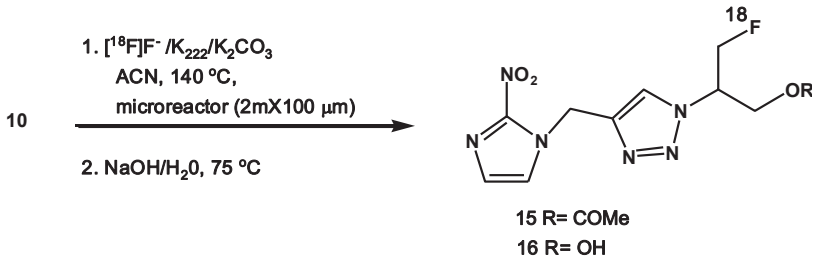


Scheme 2

Synthesis of the reference standard **14** was accomplished by converting diol **7** into benzoate **11** using triethyl orthobenzoate and *p*-toluenesulfonic acid in DCM. Benzoate **11** was fluorinated using deoxofluor [bis(2-methoxyethyl)aminosulfur trifluoride] in DCM to obtain fluoroazide **12** in 37% yield. The click reaction of alkyne **3** with fluoroazide **12** yielded the benzoyl protected HX4 **13** which was then hydrolyzed using dilute sodium hydroxide in methanol to obtain HX4 **14** [**Scheme 2**].

Radiochemsitry. The radiosynthesis leading to the title compound **16** was performed in a commercially available micro reactor apparatus (NanoTek; Advion Biosicences). This system has the unique ability to dispense the reagents through metered delivery, to efficiently control the temperature gradients, and to use relatively small amounts of expensive nonradioactive precursors for radiolabelling as compared to the vial synthesis. Also, a series of reactions can be performed expeditiously with very low fixed quantities of reagents under precisely controlled conditions of time, temperature, and reaction stoichiometry. As the reagents pass through the micro channel (100 mμ channel), the reactor is heated to a controlled temperature in a very short time span, and the reaction can be carried out at above the boiling point of the solvent due to the elevated pressure of the system. Typically these micro reactors have low internal volumes (15.7 μL and 31.4 μL for 2m and 4m length respectively). As a result, the reactant solutions rapidly mix under laminar flow. The residence time of the reactants in the micro reactor equals the reaction time and is determined by the flow rates of the reactants. Mechanically driven syringes determine the flow rates very precisely. The system is comprised of three different module types called concentrator module, reactor module and reagent module. The isotope is dried in the concentrator module and the reagent modules are used to store the isotope and precursor solutions. The reactant solutions are then sent through the micro reactor contained in the reactor module.

Nosylate **10** was radiofluorinated in the microfluidic system using [^{18}F]F$^-$/K_{222}/K_2CO_3 to obtain acetate **15** which was then hydrolyzed with dilute NaOH at 75 °C to afford [^{18}F]F-HX4 **16 [Scheme 3]**.



Scheme 3

The optimum reaction temperature for fluoride incorporation was determined in the Discovery Mode (NanoTek LF 1.4 software) by using a constant flow rate of (20 µL/min) through the reactor at different temperatures (100-180 °C) and a precursor **10** concentration of 5 mg/mL in acetonitrile. The crude incorporation yields were obtained using radio-TLC [**Figure 1**].

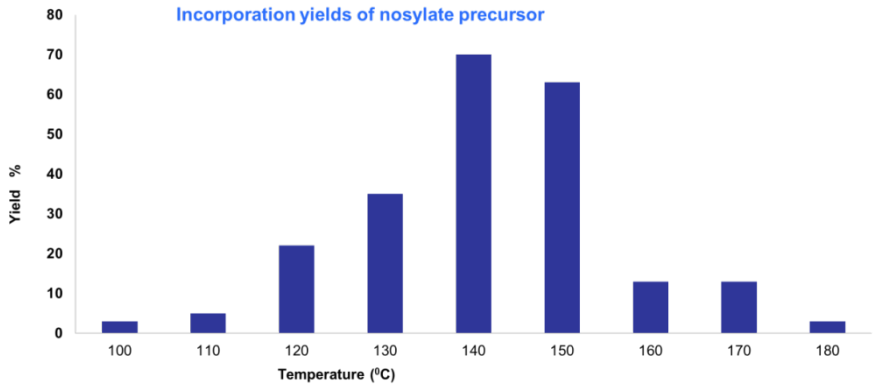


Figure 1. [^{18}F]fluoride incorporation yields

Fluoride incorporation peaked at 71% at 140 °C and diminished rapidly above 150 °C. Thus the temperature was kept at 140 °C and the optimum combined flow rate was found to be 200 µL/min (100 µl/min each of isotope and precursor solution). These conditions were then applied to the synthesis of **15**, and a typical radio-TLC of the reaction mixture is shown [**Figure 2**].

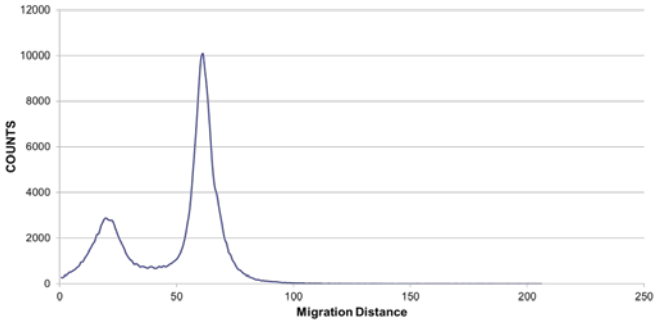


Figure 2. Radio-TLC of acetate **15**

Nosylate **9** (2.5 mg) in anhydrous acetonitrile (0.5 mL) and [^{18}F]F$^-$/K_{222}/K_2CO_3 (100 mCi) in acetonitrile (0.5 mL) were then allowed to react in the micro reactor (2 m x 100 μm) at 140 °C at a combined flow rate of 200 μL/min. The radiofluorinated product was collected in a vial in the concentrator module containing aqueous NaOH (0.1 *N*, 1.0 mL). The resulting mixture was heated at 70 °C for 5 min. The crude product was purified by semi-preparative HPLC using a Phenomenex Luna reverse phase column (250 X 10 mm, 10 μ), employing 6% aqueous ethanol at a flow rate of 4.0 mL/min. The peak at 18-20 min was collected, and the purity of the product was confirmed using analytical HPLC [**Figure 3**] by co-elution with the standard.

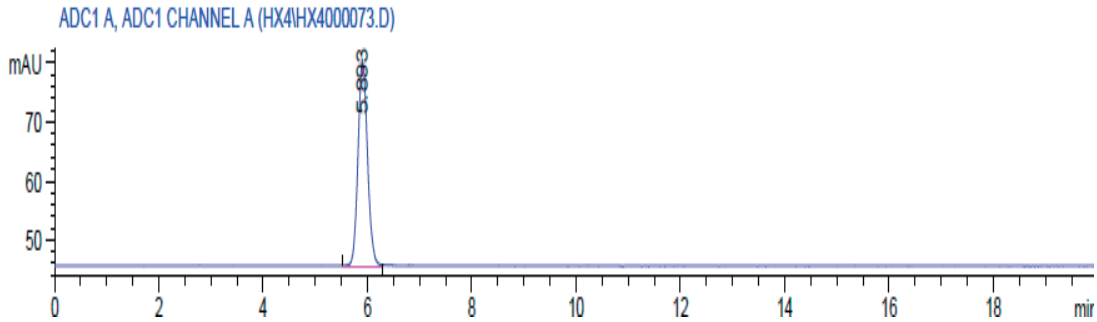


Figure 3. Radio-HPLC chromatogram of [^{18}F]F-HX4. Retention time is 5.89 min.

CONCLUSIONS

The synthesis of [^{18}F]-3-fluoro-2-(4-((2-nitro-1*H*-imidazol-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl) propan-1-ol **16** was successfully accomplished by continuous flow chemistry in a microfluidic environment with a decay corrected radiochemical yield of 48±4% (n = 4) and a radiochemical purity of ≥ 98%. The yield of the labelled product is higher than the previously reported vial synthesis. The microfluidic process allows the use of very low amounts of the expensive precursor. The total reaction time, including the HPLC purification, was 40 min. The specific activity of the product was determined to be 2 Ci/μmol.

EXPERIMENTAL

All reagents and solvents were purchased from Acros or Aldrich and were used as received. Column chromatography was performed using silica gel (60 Å, 230–400 mesh, Sorbent Technologies, USA). Analytical thin-layer chromatography was performed using 250 μm silica plates (Analtech, Inc., Newark, DE). ^{1}H-NMR and ^{13}C-NMR spectra were recorded at (300 or 500) and 125 MHz, respectively. Chemical shifts for ^{1}H-NMR and ^{13}C-NMR spectra were referenced to the residual protons of the deuterated solvents or to TMS. High Resolution Mass Spectrometry was performed using a JEOL AccuTOF™ DART Mass Spectrometer.

No-carrier-added [^{18}F]F$^-$, produced from recycled [^{18}O] water, was obtained from PET Net (Knoxville, TN). Thin-layer chromatography visualization was performed with radiation detectors using a BioScan AR-2500 radio-TLC reader and Win Scan 1.3 software. All radio-TLC plates were developed using methanol as the eluent. Analytical radio-HPLC analyses were performed on an Agilent 1200 series instrument employing a 254 nm UV detector and a Phenomenex Luna C_{18} column, 5μ, 4.6 x 250 mm, using 6% ethanol/94% water as the eluent at a flow rate of 1mL/min. F-18 labelling was performed in 100 μm x 2m reactor using Advion NanoTek Microfluidic Synthesis System controlled by NanoTek LF 1.4 Software.

N-Propargyl-2-nitroimidazole (**3**)

2-Nitroimidazole (0.50 g, 4.4 mmol), anhydrous K_2CO_3 (0.73g, 5.3 mmol), and propargyl bromide in toluene (80% w/v, 0.80 mL, 5.3 mmol) were added to a round bottomed flask. The flask was flushed with argon, and 5 mL of dry DMF was added. The mixture was refluxed at 60 ºC for 2 h. The reaction mixture was then added to 30 mL of water and extracted with ethyl acetate (3 x 20 mL). The combined organic layers were washed with water (2 x 20 mL) and then brine (15 mL) and dried over sodium sulfate. The solvent was evaporated, and then the product was purified by silica gel column chromatography using 1:1 hexanes/EtOAc as the eluent. A light yellow solid, 0.61g (91%), was collected. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.20 (s, 1H), 7.47 (s, 1H), 5.27 (d, *J*=2.4 Hz, 2H), 2.66 (t, *J*=2.4, 1 H).

5-*p*-Toluenesulfonyl-2-phenyl-1,3-dioxolane (**5**). In a round bottom flask, *cis/trans*-2-phenyl-[1,3]dioxan-5-ol, **4**, (5.15g, 27.7 mmol) was dissolved in 100 mL of DCM. Triethylamine (5.8 mL, 41.6 mmol) and DMAP (10 mol%, 339 mg) were added and the solution was cooled to 0 °C. *p*-Toluenesulfonyl chloride (5.82g, 30.5 mmol) was then added. The reaction was allowed to warm to room temperature and stirred for 16 h. The organic layer was separated, and the aqueous layer was extracted with DCM (30 mL x 2). The combined organic layer was washed with brine and dried over sodium sulfate to yield 6.96g (75%) of toluene-4-sulfonic acid 2-phenyl-[1,3]dioxan-5-yl ester as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.44 (s, 3H), 4.09 (dd, *J*=13.6, 2.0 Hz, 2H), 4.27 (dd, *J*=13.6, 1.6 Hz, 2H), 4.51 (t, *J*=1.6 Hz, 2H), 5.49-5.51 (s, 1H), 7.33-7.36 (m, 5H), 7.44-7.45 (m, 2H), 7.85 (d, *J*=8.4 Hz, 2H).

5-Azido-2-phenyl-1,3-dioxolane (**6**). In a round bottomed flask, tosylate **5** (3.87g, 11.6 mmol) was dissolved in 45 mL of DMF. A sodium azide solution (3.02g in 18 mL of water) was added, and the resulting mixture was refluxed at 105 ºC overnight. The reaction solution was added to water (120 mL) and extracted with EtOAc (30 mL x 3). The organic layer was washed with water (20 mL x 3) followed by brine (20 mL), and then dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* to yield crude 5-azido-2-phenyl-1, 3-dioxolane which was then recrystallized from hexanes/ethyl acetate to obtain

the product (2.21g, 93%) as a faint yellow solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.67 (t, *J*=11.6 Hz, 2H), 3.78-3.88 (m, 1H), 4.36-4.04 (m, 2H), 5.40-5.418 (s, 1H), 7.36-7.39 (m, 3H), 7.45-7.47 (m, 2H).

2-Azido-1,3-dihydroxypropane (**7**). Benzylidine azide **6** (2.21g, 10.7 mmol) was dissolved in 20 mL of diethyl ether and 9 mL of concentrated hydrochloric acid was then added. The resulting solution was stirred overnight at room temperature. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography using a70% EtOAc/30% hexanes as the eluent. Diol **6** (1.17g) was collected as a yellow oil. ^{1}H-NMR (500 MHz, MeOH-d_4): δ 1.91 (t, *J*=9.0 Hz, 2H), 3.64-3.7 (q, *J*=9.0 Hz, 1H), 3.73-3.89 (m, *J*=9.0 Hz, 4H).

2-Azido-3-acetoxypropan-1-ol (**8**). Diol **6** (1.05 g, 8.9 mmol) was added to a round bottomed flask and dissolved in 45 mL of DCM. Triethyl orthoacetate (2.50 mL, 13.4 mmol) and PTSA (34.4 mg, 2.00 mol %) were added at room temperature. The reaction mixture was stirred for one hour and then water (0.5 mL) was added; the stirring was continued for an additional 40 min. The DCM and water were removed *in vacuo*, and the residue was purified by silica gel column chromatography to yield 1.12g (79.0%) of **7** as a colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.12 (s, 3H), 3.63-3.80 (m, *J*=10.5; 5.5 Hz, 3H), 4.19-4.31 (m, 2H).

3-Hydroxy-2-(4-((2-nitro-1*H*-imidazol-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl)propyl acetate (**9**). Alkyne **3** (577 mg, 3.8 mmol) and azide **7** (604 mg, 3.8 mmol) were placed in a round bottomed flask along with 13 mL of 1:1:1 *t*-BuOH/THF/water. Copper(II) sulfate pentahydrate (47.4 mg, 5.0 mol%) and sodium ascorbate (75.2 mg, 10.0 mol %) were added. The reaction mixture was stirred overnight and the solvent was removed *in vacuo.* The residue was purified by silica gel column chromatography using 1:1 hexanes/EtOAc and then 10% MeOH/90% DCM as the eluents. 3-Hydroxy-2-(4-((2-nitro-1H-imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propyl acetate, **8,** (953 mg, 81%) was isolated as a light yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$/DMSO-d_6): δ 1.91 (s, 3H), 3.78-3.85 (t, *J*=5.0 Hz, 2H), 4.39-4.44 (d, *J*=5.5 Hz, 2H), 4.79-4.88 (m, *J*=5.5 Hz, 1H), 5.14-5.19 (t, *J*=5.0 Hz, 1H), 5.70 (s, 2H), 7.12 (s, 1H), 7.62 (s, 1H), 8.14 (s, 1H).

3-(2-Nitrobenzenesulfonyloxy)-2-[4-((2-nitro-1*H*-imidazol-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl]propyl Acetate (**10**). Alcohol **9** (0.500 g, 1.6 mmol) along with 110 mg of 4Å molecular sieves, were added to a round bottomed flask followed by 10 mL of dry DCM. The solution was cooled to 0 ºC, and then triethylamine (0.45 mL, 3.2 mmol) was added. The reaction mixture was stirred for 1 hour at which time 2-nitrophenylsulfonyl chloride (441 mg, 1.93 mmol) was added. The stirring was continued for an additional 2 hours while warming to room temperature. The solvent was removed *in vacuo*, and the residue was purified by silica gel column chromatography using 2% MeOH/DCM as the eluent. Nosylate **10** (577 mg, 83.0%) was isolated as a light yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$/DMSO-d_6): δ 1.93 (s,

3H), 4.42-4.51 (d, *J*=6.0 Hz), 4.69-4.83 (dt, *J*=11.0 Hz, 7.0 Hz, 2H), 5.25-5.35 (m, 1H), 5.70 (s, 2H), 7.15 (s, 1H), 7.63 (s, 1H), 7.83-8.07 (m, 4H), 8.24 (s, 1H).

2-Azido-3-hydroxypropyl benzoate (**11**)

In a round bottomed flask, diol **7** (1.03 g 8.8 mmol) was dissolved in 90 mL of DCM. Triethyl orthobenzoate (3.0 mL,13.5 mmol) and *p*-toluenesulfonic acid (32.0 mg 2 mol%) were added. The reaction mixture was stirred at room temperature for one hour. Water (0.4 mL) was added and the mixture stirred vigorously for an additional 40 minutes. The solvent was removed *in vacuo*, and the residue was purified by silica gel column chromatography using 25% ethyl acetate/hexanes as the eluent. Benzoate **10** (1.02 g, 52%) was obtained as a yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 2.15-2.20 (t, *J*=6.0 Hz, 1H), 3.71-3.78 (dd, *J*=6.0, 12.0 Hz, 1H), 3.78-3.84 (dd, *J*= 5.0, 12.0 Hz, 1H), 3.86-3.93 (quin, J=5.0 Hz, 1H), 4.44-4.51 (dd, J=7.0, 12.0 Hz, 1H), 4.53-4.59 (dd, J=4.5, 12.0 Hz, 1H), 7.44-7.50 (dd, *J*=5.0 Hz, 7.0 Hz, 2H), 7.57-7.62 (t, *J*=7.5 Hz, 1H), 8.03-8.09 (d, *J*=7.5 Hz, 2H).

2-Azido-3-fluoropropyl benzoate (**12**)

Benzoate **11** (1.02 g, 4.6 mmol) was dissolved in 25 mL of dry DCM in a round bottomed flask. The flask was flushed with argon and cooled to 0 °C. Deoxofluor (1.28 mL, 6.90 mmol) was added dropwise in three equal portions over 3 h. Then the reaction was allowed to warm to room temperature and stirred overnight. The reaction was quenched with saturated aqueous sodium hydrogen carbonate while stirring. The organic layer was separated from the aqueous layer and was washed with water (10 mL x 3) followed by brine (10 mL). The solvent was dried over anhydrous sodium sulfate, filtered, and then removed *in vacuo*. The residue was purified by silica gel column chromatography using 5% ethyl acetate/95% hexanes as the eluent. Fluoroazidobenzoate **12** was isolated as a colorless oil (143 mg, 14.0%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.95-4.11 (pseudo dt, *J*(^{1}H, ^{19}F)=30.5 Hz, *J*(^{1}H, ^{1}H)=8.0, 9.5 Hz, 1H), 4.39-4.48 (ddd, *J*(^{1}H, ^{19}F)=20.0 Hz, *J*(^{1}H, ^{1}H)=10.0 Hz, *J*(^{1}H, ^{1}H)=2.0 Hz, 1H), 4.50-4.59 (m, 2H), 4.63-4.74 (m, 1H), 7.43-7.51 (t, *J*=13.5 Hz, 2H), 7.57-7.64 (t, *J*=11.5 Hz, 1H), 8.02-8.09 (d, J=13 Hz), 2H.

3-Fluoro-2-(4-((2-nitro-1*H*-imidazol-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-1-*O*-benzoylpropane (**13**)

In a round bottomed flask, azidobenzoate **12** (143.0 mg, 0.64 mmol) and alkyne **3** (97.5 mg 0.64 mmol) were dissolved in 3 mL of a 1:1:1 mixture of *t*-BuOH, THF, and water. Copper(II) sulfate (8.0 mg, 5.00 mol%) and sodium ascorbate (12.7 mg,10 mol%) were added, and the reaction mixture was stirred overnight at room temperature. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography using 50% EtOAc/50% hexanes as the eluent. Benzoyl protected **13** (118mg, 49.0%) was collected as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.80-4.83 (d, *J*=6.5 Hz, 2H), 4.84-5.06 (ddd, J(^{1}H, ^{19}F)=81.5 Hz, J(^{1}H, ^{1}H)=10.5 Hz, J(^{1}H, ^{1}H)=5 Hz, 1H), 4.92-4.98 (pseudo sext, *J*=5 Hz, 5.5 Hz, 1H), 5.20-5.29 (pseudo dquin, *J*(^{1}H, ^{19}F)=21 Hz, *J*(^{1}H, ^{1}H)=5, 5.5 Hz, 1H), 5.72 (s, 2H), 7.14 (s, 1H), 7.35 (s, 1H), 7.42-7.48 (t, *J*=7.5 Hz, 2H), 7.57-7.62 (t, *J*=7.5 Hz), 7.88-7.92 (d, *J*=7.5 Hz, 2H), 7.94

(s, 1H). ^{13}C-NMR (125.7 MHz, $CDCl_3$): δ 44.7, 60.0 (d, J(^{13}C, ^{19}F)=20.5 Hz, 1C), 62.0 (d, J(^{13}C, ^{19}F)=6.8 Hz, 1C), 80.6, 82.0, 123.5, 126.3, 128.7, 128.8, 129.6, 133.8, 141.6, 165.6. HRMS for $C_{16}H_{15}FN_6O_4$ (MH+): 374.1139. Found: 374.1132.

3-Fluoro-2-(4-((2-nitro-1*H*-imidazol-1-yl)methyl)-1*H*-1,2,3-triazol-1-yl)propan-1-ol (**14**).

In a round bottomed flask, compound **13** (117 mg, 0.310 mmol) was dissolved in 5 mL of MeOH, and 3 mL of 0.25*M* sodium hydroxide was added. The reaction was stirred at room temperature for 2 h. The pH was brought to 7 by adding 1*M* HCl, the solvent removed *in vacuo*, and the residue was purified by silica gel column chromatography using 10% MeOH/90% DCM as the eluent. Non radioactive **14** was obtained (43.3 mg, 25%) a as a white solid. ^{1}H-NMR (500 MHz, acetone-d_6): δ 4.03 (t, J=5.6 Hz, 2H), 4.17-4.45 (m, 1H), 4.82 (dd, J =4.0, 10.0 Hz, 1H), 4.87-5.05 (m, 2H), 5.81 (s, 2H), 7.12 (s, 1H), 7.58 (s. 1H), 8.16 (s, 1H).

Radiosynthesis of [^{18}F]F-HX4 (**16**).

The detailed configuration and operation of the NanoTek Microfluidic System have been described previously.[10] Cyclotron-produced no-carrier-added [^{18}F]fluoride ion (100 mCi) in [^{18}O]water (225–350 μL) was first adsorbed onto an anion exchange resin ORTG cartridge within the concentrator module of a NanoTek apparatus (Advion Biosciences), and then released with a solution of K_2CO_3 (1.8 mg) plus $K_{2.2.2}$ (12.0 mg) in MeCN/H_2O (9.5:0.5 v/v; 400 μL) into a 5 mL V-vial. The solution was dried by three cycles of azeotropic evaporation with MeCN (0.45 mL) at 100 °C. The dry $^{18}F^-$-$K_{2.2.2}$-K^+ complex (70 mCi) was dissolved in MeCN.(0.5 mL). The isotope solution was loaded into the loop of the reactor module (431 μL), and the nosylate precursor **10** (2.5 mg in 0.5 mL) solution was loaded into the loop on the reagent module (431 μL). These solutions were concurrently infused into a 2 m long micro reactor coil (100 μm) at a combined flow rate of 200 μL/min. The radiofluorinated product exiting the micro reactor was collected in a vial, in the concentrator module, containing aqueous sodium hydroxide (0.1 *N*, 1 mL), and the resulting reaction mixture was heated at 75 °C for 5 min. The product was neutralized with sodium acetate (3 *N*, 1 mL) and purified by semi-preparative HPLC column (Phenomenex Luna reverse phase column, 250 x 10 mm, 10 μ), and 6% aqueous EtOH at flow rate of 4.0 mL/min. The peak at 18-20 min was collected (33±2 mCi). The identity of the product was confirmed using analytical HPLC.

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REFERENCES

1. R. Torres, E. Arstad, and P. Blower, *Targ. Oncol*., 2009, **4**, 183.

2. I. Serganova, J. Humm, C. Ling, and R. Blasberg, *Clin. Cancer Res.*, 2006, **12**, 5260.
3. L. Chena, Z. Zhang, H. C. Kolb, J. C. Walsh J. Zhang, and Y. Guana, *Nucl. Med. Commun.*, 2012, **33**, 1096.
4. (a) S. Lu, P. Watts, F. Chin, T. Hong, J. Masachio, E. Briard, and V. Pike, *Lab Chip*, 2004, **4**, 523; (b) C. Lee, G. Sui, A. Alizarov, C. Shu, Y. Dooley, J. Huang, A. Daridon, P. Wyatt, D. Stout, H. Kolb, O. Witte, N. Satyamurthy, J. Heath, M. Phelps, S. Quake, and H. Tseng, *Science*, 2005, **310**, 1793; (c) L. Collier, M. Akula, and G. Kabalka, *J. Nucl. Med*., 2011, **52**, 86P; (d) M. Akula, L. Collier, and G. Kabalka, *J. Nucl. Med*., 2012, **53**, 175P; (e) T. Moore, M. Akula, L. Collier, and G. Kabalka, *Appl. Radiat. Isot*., 2013, **71**, 47.
5. G. Pascali, P. Watts, and P. Salvadori, *Nucl. Med. Biol.*, 2013, **40**, 776.
6. A. Lebedev, R. Miraghaie, K. Kotta, C. E. Ball, J. Zhang, M. Buchsbaum, H. C. Kolb, and A. Elzarov, *Lab Chip*, 2013, 13.
7. M. Zheng, L. Collier, F. Bois, O. Keleda, K. Hammond, J. Ropcham, M. Akula, D. Carlson, G. Kabalka, and Y. Huang, *Nucl. Med. Biol*., 2015, **42**, 578
8. B. C. Rensch, A. Jackson, S. Linder, R. Salvamoser, V. Samper, S. Riese, S. Bartenstein, B. Wangler, and C. Wangler, *Molecules*, 2013, **18**, 7930.
9. T. C. Collier, B. A. Duclos, U. B. Gangadharmath, Z. Gao, F. Karimi, D. Kasi, H. C. Kolb, Q. Liang, H. C. Padgett, J. C. Walsh, and T. Zhao, US Patent, 2011, # US 7,977361 B.
10. S. Lu, A. Giamis, and V. Pike, *Curr. Radiopharm*., 2009, **2**, 49. J. Chun, S. Lu, Y. Lee, and V. Pike, *J. Org. Chem*., 2010, **75**, 49.