Supporting Information for

Synthesis and preliminary biological evaluations of [^{18}F]-1-deoxy-1-fluoro-scyllo-inositol

Neil Vasdev,[a,b,*] Jason Chio[c] Erik M. van Oosten[a,c] Mark Nitz,[c] JoAnne McLaurin,[d] Douglass C. Vines,[e] Sylvain Houle[a,b] Raymond M. Reilly,[f] and Alan A. Wilson[a,b]

neil.vasdev@camhpet.ca

General Information

A Scanditronix MC 17 cyclotron was used for radionuclide production. Analyses of products and intermediates were performed via HPLC with an in-line UV detector (Waters 486) in series with a coincidence radioactivity detector (Bioscan Type B6I6/1.SB in conjunction with a Flowcount) and/or a radio-TLC system (Bioscan System 200 Imaging Scanner with Autochanger 1000). Isolated radiochemical yields were determined with a dose calibrator (Capintec CRC-712M). Proton nuclear magnetic resonance spectra (^{1}H NMR) and fluorine nuclear magnetic resonance spectra (^{19}F NMR) were recorded on a Varian Mercury 400 spectrometer. Chemical shifts for protons are reported in parts per million (δ scale) downfield from tetramethylsilane (TMS: δ 0.00) and are referenced to residual proton in the NMR solvent (CD_2HOD: δ 3.31). The ^{19}F NMR spectra were referenced relative to $CFCl_3$. Electrospray ionization mass spectrometry was conducted by the Advanced Instrumentation for Molecular Structure Laboratory at the University of Toronto. Elemental analysis was performed by the Analytical Laboratory for Environmental Science Research and Training, University of Toronto. All chemicals were obtained from Aldrich or Caledon. All animal experiments were carried out under humane conditions, with approval from the Animal Care Committee at the Centre for Addiction and Mental Health or the University Health Network and in accordance with the guidelines set forth by the Canadian Council on Animal Care.

1-*O*-Benzoyl-4-deoxy-4-fluoro-*scyllo*-inositol (3)

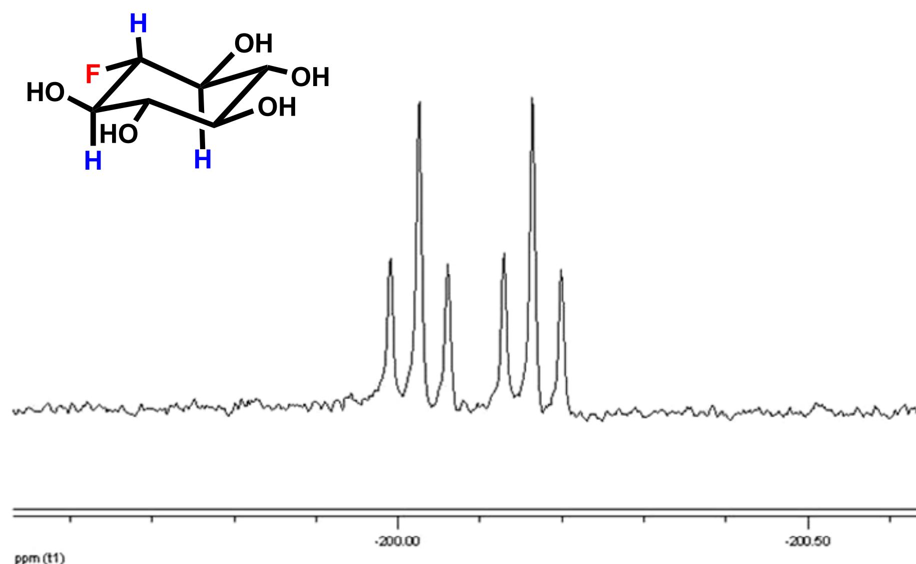
Compound **2** (25 mg, 0.05 mmol) was added to a stirred solution of 95% trifluoroacetic acid (TFA; 5 mL) at 0 ºC. The yellow mixture was warmed to room temperature and stirred for 3.5 h. Subsequently, the mixture was taken to dryness with N_2 (g). Co-evaporation with toluene (3 x 10 mL) under reduced pressure removed traces of TFA and 2,3-butanedione and afforded a beige solid (7 mg, 49%); mp 208-209ºC; R_f 0.73 (5:2:1 EtOAc:MeOH:H_2O); ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm): 8.09-8.07 (m, 2H), 7.60 (tt, 1H, J = 7.5 Hz, 1.3 Hz), 7.50-7.46 (m, 2H), 5.09 (t, 1H, J = 9.5 Hz), 4.18 (dt, 1H, $^1J_{HF}$ = 51.6 Hz, J_{HH} = 9.0 Hz), 3.67 – 3.48 (m, 4H) ppm; ^{19}F NMR (CD_3OD, 375 MHz) δ: -199.7 ppm (dt, $^1J_{HF}$ = 51.7 Hz, $^3J_{HF}$ = 12.7 Hz); HRMS *m/z* (ESI) calculated for $C_{13}H_{15}FO_6$ $(M+H)^+$ 287.0925; found: 287.0918; Anal. Calcd for $C_{13}H_{15}FO_6$: C, 54.55; H, 5.28. Found: C, 54.34; H, 5.26.

^{19}F NMR spectrum of 1-deoxy-1-fluoro-*scyllo*-inositol (4)

Compound **4** was synthesized and characterized as previously described.[1]

^{19}F NMR (D_2O, 375 MHz) δ: -200.1 ppm (dt, $^1J_{HF}$ = 52.0 Hz, $^3J_{HF}$ = 13.1 Hz) relative to $CFCl_3$ (Fig. 1).

Figure 1. ^{19}F NMR spectrum of **4**.



Preparation of dry K[^{18}F]/[K_{222}]

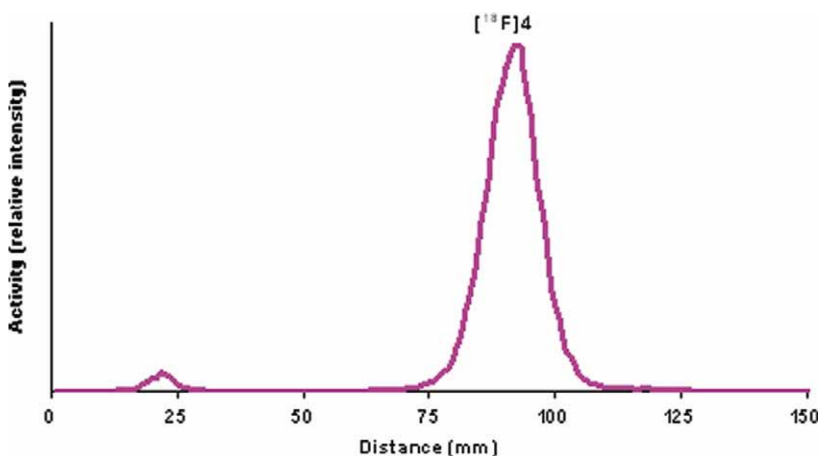
Aqueous [^{18}F]-fluoride was produced by the $^{18}O(p,n)^{18}F$ nuclear reaction on [^{18}O]H_2O using a Scanditronix MC17 cyclotron as previously described.[2] The [^{18}F]-fluoride was trapped on a Chromafix ion-exchange 30-PS-HCO_3 resin 10 (activated with 1 mL EtOH followed by 1 mL sterile H_2O) and back-flushed using a 1 mL solution (14 mg 2,2,2-crypt (Kryptofix®, K_{222}), 1.35 mg K_2CO_3, 0.1 mL deionized H_2O, 0.9 mL MeOH) into a glass BD Vacutainer™ serum tube (7 mL, 13 x 100 mm, no additive). The reaction mixture was azeotropically dried with 3 x 0.5 mL anhydrous acetonitrile and N_2 flow over ca. 8 min.

Radiosynthesis of [^{18}F]4

All radiosynthetic steps were carried out at 90°C. A solution of **1** (5 mg, 0.0078 mmol) in anhydrous CH_3CN (1 mL) was added to the dry K[^{18}F]/[K_{222}] and stirred for 15 min. A solution of TFA in anhydrous CH_3CN (2:3, 0.5 mL) was immediately added to the reaction mixture and stirred for 15 min. The mixture (yellow) was evaporated to dryness under N_2 flow, followed by addition of 2.0 N NaOH (1.5 mL) which was continually stirred and heated for 15 min. The resulting brown reaction mixture was diluted with 3 mL of deionized water (dH_2O) and removed from the bath, and then passed through a solid phase purification system consisting of 2.6 g Dowex® 50WX8-400 anion exchange resin (pre-activated with 10 mL 95% EtOH and 10 mL dH_2O), a C-18 Sep-Pak® (solid phase extraction cartridge), an Alumina N Sep-Pak® Plus, and a Millex®-Gs 0.22µm Filter Unit. The purified product was collected in a 10 mL sterile dose vial, pre-charged with 0.5 – 1 mL of 8.4% $NaHCO_3$ solution (10% of volume) and 0.3 – 0.6 mL of 14.6% NaCl, adjusted accordingly to provide isotonic solution, and was washed with dH_2O (2 x 2 mL). Analytical HPLC was performed on intermediates [^{18}F]**2** and [^{18}F]**3** using a Phenomenex Luna C18(2), 150 mm x 4.60 mm, 5 µm, eluted with CH_3CN:H_2O (65:35 v/v) + 0.1 N ammonium formate (AF) using a flow rate of 2.0 mL/min using a wavelength of 220 nm. Specific activity was determined after initial fluorination of **1** from a quenched solution containing [^{18}F]**2** using a calibrated coincidence γ-detector as this intermediate was readily separated by HPLC and contains a chromophore (HPLC conditions as above except no buffer used in mobile phase and flow rate was 2.2 mL/min). Conversion from [^{18}F]**3** to the product [^{18}F]**4** was analyzed on Phenomenex Luna NH_2, 250 mm x 4.60 mm, 5 µm; eluted with mobile phase consisting of

CH_3CN:H_2O (80:20 v/v) at a flow rate of 2.0 mL/min. Radio-TLC (stationary phase: silica gel; eluent: EtOAc:MeOH:H_2O, 5:2:1) of authentic **4** (phosphomolybdic acid stain) and [^{18}F]**4** had coincidental R_f values (0.6; Figure 2). The Log *D* of [^{18}F]**4** was carried out as previously described[3] by octanol extraction from phosphate buffer at pH 7.4 .

Figure 2. Radio-TLC chromatogram of [^{18}F]**4** after purification. Stationary phase: silica gel; mobile phase: 5:2:1 EtOAc:MeOH:H_2O; R_f 0.6.



Carrier-added synthesis of [^{18}F]4

From a 1 mg/mL solution of potassium fluoride in water, 0.1 mL (5:1 mole equivalent of compound **1**:KF) was added to the reaction tube containing [^{18}F]-fluoride and a 1 mL solution containing K_{222} and the mixture was azeotropically dried. The radiosynthesis of [^{18}F]**4** was carried out as previously described (vide supra).

Cold synthesis of 4 with KF as the fluoride source

The previous reaction (vide supra) was repeated in the absence of [^{18}F]-fluoride, using 0.1 mL of a 1 mg/mL KF solution in water and was repeated two more times. The purified products were concentrated, combined, and characterized by ^{19}F NMR spectroscopy. The ^{19}F NMR spectrum conformed to that of authentic **4** (vide supra). ^{19}F NMR (D_2O, 375 MHz) δ: -200.1 ppm (dt, $^1J_{HF}$ = 52.0 Hz, $^3J_{HF}$ = 13.1 Hz).

Preliminary small animal imaging in tumour bearing mice

The MDA-MB-231 cell line was chosen for preliminary imaging in human breast cancer xenografts in athymic mice as it rapidly proliferates and we have shown that it avidly sequesters [^{18}F]FDG.[4] MDA-MB-231 cells were purchased from the American Type Culture Collection (Manassas, VA). MDA-MB-231 cells were cultured in Dulbecco's minimal essential medium (Ontario Cancer Institute, Toronto, ON) supplemented with 10% FBS (Invitrogen) containing 100 U/mL penicillin and 100 µg/mL streptomycin under a 5% CO_2 atmosphere at 37ºC.

MDA-MB-231 tumour xenografts were established in female athymic CD1 *nu/nu* mice (Charles River, Wilmington, MA). The mice were inoculated subcutaneously (s.c.) in the right shoulder with 5 × 10^6 MDA-MB-231 cells in 200 µL of a 1:1 mixture of Matrigel (BD Biosceiences, Bedford, MA) and serum-free culture medium. Mice (n = 2) once tumours had reached an appropriate size underwent small microPET and microCT imaging. Images were acquired on a Focus 220 microPET (Siemens Preclinical Solutions, Knoxville, TN). Mice were fasted, with access to water, for 15-20 h prior to [^{18}F]**4** administration and imaging. Mice were placed on a heating pad 1 h prior to

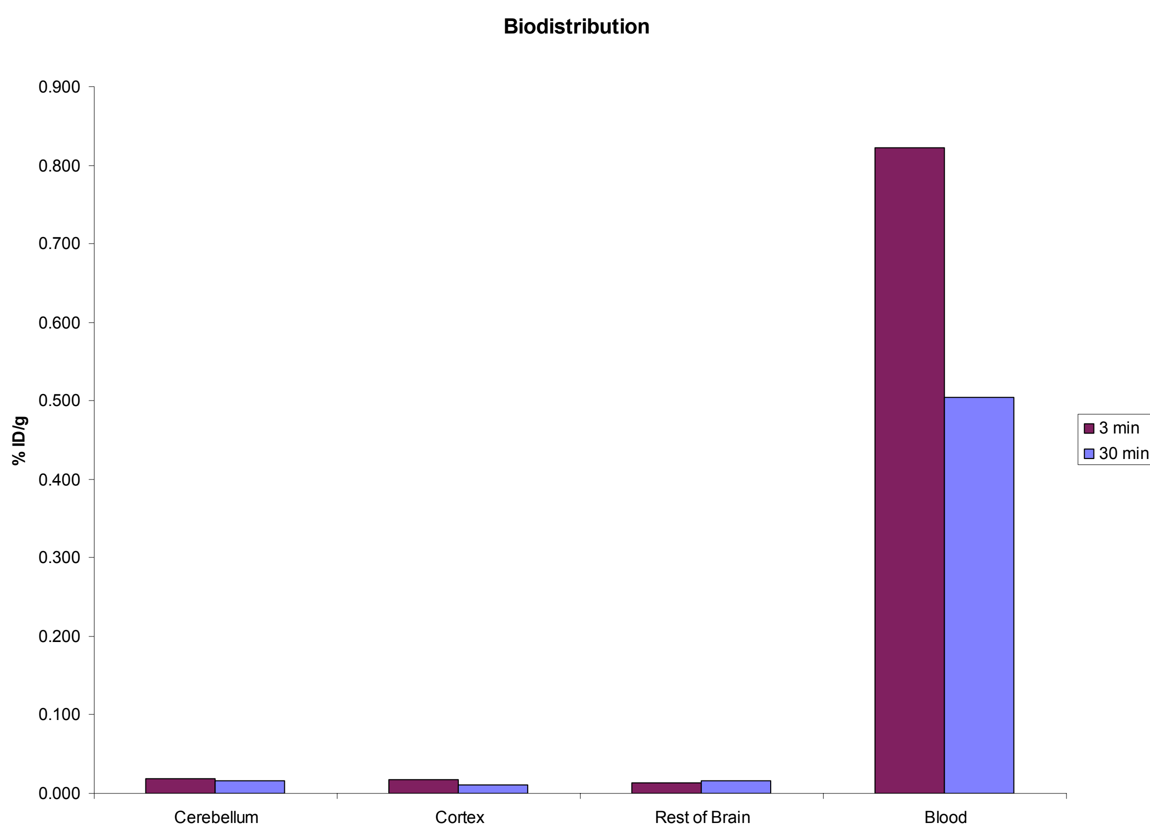
injection and body weight was measured. Blood glucose levels were measured (Ascensia Contour, Bayer, Tarrytown, NY) by a needle prick to the tail vein (ca. 4). Conscious mice were injected i.v. with 240 ± 80 μCi of [^{18}F]**4**. Following injection, the mice were anesthetized by inhalation of 2% isoflurane in oxygen and warmed using a heat lamp while in the scanner over a 2 hour continuous imaging scan. Images were reconstructed using an ordered subset expectation maximization followed by maximum *a posteriori* probability reconstruction algorithm with no attenuation correction and no correction for partial volume effects as the tumours had diameters > 4 mm, which was greater than 2.5 times the full-width-at-half-maximum resolution of the tomograph (Siemens Preclinical Solutions). Quantification was performed by volume-of-interest (VOI) analysis using Inveon Research Workplace software (Siemens) and tumour uptake was expressed as the mean ± SD percent injected dose per gram (% i.d./g). Immediately following microPET, microCT was performed on a GE eXplore Locus Ultra Preclinical CT Scanner (GE Healthcare, Chalfont St. Giles, UK) with routine acquisition parameters (80 kVp, 70 mA, voxel size of 150 μm × 150 μm × 150 μm). Co-registration of microPET and microCT images was performed using Inveon Research Workplace software (Siemens).

Ex vivo Biodistribution and In vivo SIC Probe Studies in Rats

Ex vivo biodistribution studies following administration of [^{18}F]**4** in male Sprague-Dawley rats (400 – 500 g) in conjunction with in vivo SIC probe studies were conducted as previously described by our group.[5, 6] The *in vivo* SIC probe experiment was carried out in the frontal cortex and cerebellum from 0-40 min post-injection of [^{18}F]**4**. For *ex vivo* biodistribution studies, conscious rats received ca. 260 μCi of [^{18}F]**4** in 0.3 mL of

buffered saline via the tail vein and were sacrificed by decapitation at 3 and 30 min post-injection (n=1 per time-point). The brains were removed and regions of interest (cortex, cerebellum, rest of brain) were excised, blotted and weighed, while whole blood was collected from the trunk in a heparinized tube (Figure 3).

Figure 3. *Ex vivo* biodistribution evaluation of [^{18}F]**4** in rats.



This journal is (c) The Royal Society of Chemistry 2009

References

1. Y. Sun, G. Zhang, C. A. Hawkes, J. E. Shaw, J. McLaurin and M. Nitz, *Bioorg. Med. Chem.*, 2008, **16**, 7177-7184.
2. N. Vasdev, P. Seeman, A. Garcia, W. T. Stableford, J. N. Nobrega, S. Houle and A. A. Wilson, *Nucl. Med. Biol.*, 2007, **34**, 195-203.
3. A. A. Wilson, L. Jin, A. Garcia, J. N. DaSilva and S. Houle, *Appl. Radiat. Isot.*, 2001, **54**, 203-208.
4. K. McLarty, A. Fasih, D. A. Scollard, S. J. Done, D. C. Vines, D. E. Green, D. L. Contantini and R. M. Reilly, *J. Nucl. Med.*, submitted.
5. N. Vasdev, S. Natesan, L. Galineau, A. Garcia, W. T. Stableford, P. McCormick, P. Seeman, S. Houle and A. A. Wilson, *Synapse*, 2006, **60**, 314-318.
6. A. A. Wilson, J. N. DaSilva and S. Houle, *Nucl. Med. Biol.*, 1996, **23**, 141-146.