Rapid Synthesis of 3-[^{18}F]Fluoro-1-(2′-Nitro-1′-Imidazolyl)-2-Propanol ([^{18}F]Fluoromisonidazole)

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INTRODUCTION

Because tumor cells are more sensitive to radiation in the presence of oxygen than in its absence, even a small percentage of hypoxic cells within a tumor could limit response to radiation (1,2). Hypoxic radioresistance has been demonstrated in many experimental and animal tumors but in man hypoxia has been directly demonstrated in only a few tumor types (3,4). The occurrence of hypoxia in human tumors has in most cases been inferred from histologic findings and from animal tumor studies. *In vivo* demonstration of hypoxia has required tissue measurements with oxygen electrodes and the invasiveness of these techniques has limited its application. Most attempts to increase the radiosensitivity of tumors by administration of chemical radiosensitizers have been unsuccessful (5–7). However, there has been no clinically applicable means of demonstrating tumor hypoxia, and it has not been possible to identify the patients who could potentially benefit from radiosensitizing therapy. The potential advantage of neutrons over more conventional radiation is less dependent on oxygenation of the tumor and less variable of cell sensitivity to neutrons around the cell cycle.

3-[^{18}F]Fluoro-1-(2′-nitro-1′-imidazolyl)-2-propanol ([^{18}F]fluoromisonidazole; FMISO) has been used with positron emission tomography (PET) to differentiate a hypoxic but metabolically active tumor from a well-oxygenated metabolically active tumor. [^{18}F]FMISO is metabolized by intracellular nitroreductases and acts as a competing electron acceptor at low oxygen levels. It is reduced and subsequently incorporated into hypoxic, but metabolically active, cells by covalent bonding to various macromolecules. Recent studies have shown that PET, with its ability to monitor cell oxygen content through ^{18}F-FMISO, has a high potential to predict tumor response to radiation (8–11).

Although there is a great demand for PET [^{18}F]FMISO, a simple and efficient synthesis method to produce sufficient radioactivity of [^{18}F]FMISO has been demonstrated less often. Most studies used [^{18}F]epifluorohydrin to react with 2-nitroimidazole (12–14). The reaction takes a longer time (90 min) and provides a lower radiochemical yield (7–12%) because of using a two-step, two-pot reaction sequence. Another approach was to use a 2-*O*-tetrahydropyranyl tosyl misonidazole analogue to prepare [^{18}F]FMISO (15). In that report, glycerol 1,3-positions were protected with benzaldehyde. Position 2-OH of glycerol was protected with dihydropyran. The 1,3-positions were then deprotected, followed by reacting with tosyl chloride. The 1,3-ditosyl-*O*-tetrahydropyran was reacted with 2-nitroimidazole. Several steps are needed to prepare the precursor. Here we report an alternative approach for the rapid synthesis of [^{18}F]FMISO (Scheme I).

EXPERIMENTAL

Nuclear magnetic resonance (NMR) spectra (^{1}H and ^{13}C) were recorded at ambient temperature on an IBM-Bruker Model NR/200 AF spectrometer in the Fourier transform mode in $CDCl_3$ with tetramethylsilane as an internal reference. Chemical shifts (δ) are reported as parts per million (ppm) and coupling constants (*J*) as hertz. Mass spectral analyses were conducted at the University of Texas, Health Science Center at Houston. The mass data were obtained by fast-atom bombardment on a Kratos MS50 instrument. The elemental analyses were conducted at Galbraith Laboratories, Inc. (Knoxville, TN). High-resolution mass spectroscopy (HRMS) was performed at the Midwest Center for Mass Spectrometry (Lincoln, NE). All chemical reactions were conducted in dry glassware and were protected from atmospheric moisture. Solvents were dried over freshly activated (300°C, 1 hr) molecular sieves (type 4A). The homogeneity of the products was determined by ascending thin-layer chromatography (TLC) on silica-coated glass plates (silica gel 60 F 254, Merck) with mixtures of $CHCl_3$–MeOH as the eluting solvent. Preparative separations were performed by column chromatography on silica gel (Merck; 230–400 mesh) with mixtures of $CHCl_3$–MeOH as eluant.

Synthesis of Glycerol-1,3-ditosylate (Compound 2)

Compound 2 was synthesized according to the previously reported method with modifications (16). *p*-Toluenesulfonyl chloride (10.29 g, 54 mmol) dissolved in dry pyridine was added to a stirred solution of anhydrous glycerol (2.48 g, 27 mmol) in dry pyridine (30 mL at 0°C). The solution was added slowly and left to react for 44 hr in the refrigerator (0–3°C). The pink mixture was poured over crushed ice and acidified with concentrated HCl. The organic layer was separated and the aqueous layer was washed with methylene chloride (2 × 50 mL). The combined organic extracts were washed successively with 2 *N* HCl (2 × 10 mL) and distilled water (2 × 10 mL) and dried over anhydrous Na_2SO_4. The filtrate was evaporated and the residual crude product was purified by column chromatography on silica gel to afford Compound 2 as an oil: 9.72 g (24.3 mmol), 90% yield. ^{1}H NMR [chemical shift (δ), multiplicity, coupling constant (Hz), number of protons, atom]: 7.75 (d; J = 8.2 Hz; 4H, Har.); 7.35 (d; J = 8.2 Hz; 4H; Har.); 4.1 (bs; 4H; $H_{1,3}$); 3.35

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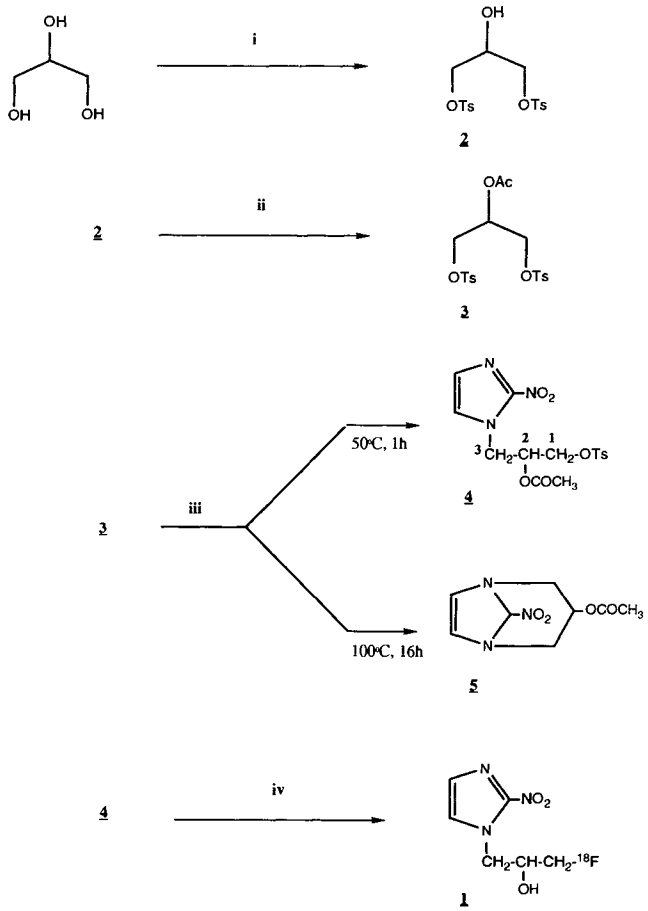

Scheme I. Radiosynthesis of [^{18}F]fluoromisonidazole. Reagents and conditions: (i) TsCl (2 Eq), pyridine, 0°C, 48 hr (90%); (ii) $(CH_3CO)_2O$ (1.5 Eq), $BF_3 \cdot Et_2O$ (0.1 Eq) ether, 0°C, 30 min (100%); (iii) 2-nitroimidazole (0.9 Eq), Cs_2CO_3 (0.9 Eq), DMF (48%); (iv) [^{18}F]/kryptofix, CH_3CN, 95°C, 10 min, H^+, 90°C, 5 min (12–18%).

(bs; 1H; H_2); 2.4 (s; 6H; $2CH_3$). ^{13}C NMR (ppm): 145 (C ar.); 131.7 (c ar.); 129.7 (CH ar.); 127.6 (CH ar.); 69.3 ($C_{1,3}$); 66.7 (C_2); 21.2 (CH_3).

Glycerol-1,3-ditosylate-2-*O*-acetylate (Compound 3)

Glycerol 1,3-ditosylate (Compound 2; 5 g, 12.5 mmol) was added a drop at a time, stirring 5 min at 0°C, to a solution of acetic anhydride (20 ml, 200 mmol) and $BF_3 \cdot$ etherate (1 mL) in anhydrous ether (50 mL). The reaction mixture was stirred for 10 min, washed successively with 25% sodium acetate solution (10 mL) and water (2 × 15 mL), and dried over anhydrous sodium sulfate. The solvent was evaporated to yield a white solid (5.48 g, 12.4 mmol). The structure of the product was determined by ^{1}H and ^{13}C NMR, mass spectral data, and elemental analysis. ^{1}H NMR: 7.8 (d; J = 8.0 Hz; 4H; Har.); 7.3 (d; J = 8.0 Hz; 4H; Har.); 5.05 (t; J = 4.8 Hz; 1H; H_2); 4.1 (d; J = 4.8 Hz; 4H; $H_{1,3}$); 2.4 (s; 6H; 2CH3ar.); 1.8 (s; 3H; CH_3 ac.). ^{13}C NMR (ppm): 169.6 ($OCOCH_3$); 145.3 (C ar.); 132.2 (C ar.); 130 (CH ar.); 127.9 (CHar.); 68.1 (C2); 66.5 ($C_{1,3}$); 21.6 (CH_3 ar.); 20.5 ($OCOCH_3$). Mass [FAB; $(C_{19}H_{22}S_2O_8)^+$] *m/z:* 441 (M^+, 5%); 383 (M^+-$OCOCH_3$, 4%) 271 (M^+-tosylate, 100%), 229 (M^+ + 1-tosylate-$COCH_3$; 3%). *Anal.* Calc. ($C_{19}H_{22}S_2O_8$): C, 51.58; H, 4.97 Found: C, 51.50; H, 4.93.

(2′-Nitro-1′-imidazolyl)-2-*O*-acetyl-3-*O*-tosylpropanol (Compound 4)

A mixture of ditosylate (Compound 3) (0.44 g, 1 mmol), 2-nitroimidazole (0.1 g, 0.9 mmol), and cesium carbonate (0.29 g, 0.9 mmol) in 10 mL of dry DMF was heated at 50°C for 1 hr. The reaction was then cooled and DMF was carefully removed under reduced pressure. The residue was taken up in ethyl acetate and filtered. Removal of ethyl acetate *in vacuo* produced a yellow oil which was chromatographed on silica gel eluted with 60–70% ethyl acetate/petroleum ether to afford a white solid (0.17 g, 0.43 mmol, 48% yield). The structure of the product was determined by ^{1}H and ^{13}C NMR and mass spectral data. HPLC showed the retention time of the title compound to be 9.83 min (UV 310 nm, C-18 reverse-phase column, eluted with water/methanol, 0–80%, at a flow rate of 1.5 mL/min). However, if the reaction was heated at 100°C for 16 hr, a major by-product (Compound 5) was isolated.

Compound 4. IR (Nujol) (cm^{-1}): 1760, 1610, 1560, 1500, 1480, 1300, 1240, 1200. ^{1}H NMR: 7.8 (d; 2H; J = 8.1 Hz; 2Har), 7.4 (d; 2H; J = 8.1 Hz; 2Har), 7.1 (d; 2H; J = 2.9; H-imidazolyl), 5.3 (m; 1H; H_2), 4.85 (dd; 1H; J = 14.4, 3.5 Hz; H_{3a}), 4.5 (dd; 1H; J = 14.4, 8.4 Hz; H_{3b}), 4.2 (dd; 2H; J = 5, 4.1 Hz; H_1), 2.45 (s; 3H; CH3tosyl), 1.95 (s; 3H; CH3ac). ^{13}C NMR (50 MHz), d(ppm): 169.3 (C=0), 145.6 (Car), 132 (Car), 130.1 (CHar), 129.9 (C-imidazolyl), 128.3 (CH-imidazolyl), 127.9 (CHar), 126.5 (CH-imidazolyl), 68.8 (C_2), 67.2 (C_1), 49.3 (C_3), 21.6 (CH_3tosyl), 20.3 (CH_3ac). HRMS calc. for $C_{15}H_{18}N_3SO_7$, 384.08655; found, 384.0869.

Compound 5. ^{1}HNMR: 7.15 (2H, H-imidazolyl); 4.9 (S, 1H, H-imidozolyl); 4.8 (m, 1H, H_2); 4.2 (d, 4H, J = 3.5 Hz), 2.1 (S, 3H, CH_3). ^{13}C NMR: 171.1 (C=0), 127.7 (CH-imidazolyl); 68.2 (CH–NO_2, $H_{1,3}$ 3), 65.4 (C_2); 52.8 ($C_{1,3}$), 21 (CH_3 ac). HRMS calcd for $C_8H_{12}N_3O_4$, 214.08278; found, 214.0827.

Radiosynthesis of [^{18}F]FMISO

[^{18}F]Fluoride was produced in the cyclotron facility at the University of Texas M. D. Anderson Cancer Center by proton irradiation of enriched [^{18}O]water in a small-volume silver target. Aliquots containing 250–500 mCi of ^{18}F activity was combined with kryptofix-2,2,2 (26 mg) and anhydrous potassium carbonate (4.6 mg), heated under reduced pressure to remove [^{18}O]water, and dried by azeotropic distillation with acetonitrile (3 × 1.5 mL). The tosyl analogue of misonidazole (Compound 4; 5 mg), prepared from 1,3-ditosyl-2-*O*-acetylated glycerol (Compound 3) was dissolved in acetonitrile (1.5 mL), added to the kryptofix–fluoride complex, and then warmed at 95°C for 10 min. After cooling, the reaction mixture was passed through a silica gel Sep-pak column (Whatman Lab, Clifton, NJ) and eluted with ether (2 × 2.5 mL). The solvent was evaporated and the resulting mixture was hydrolyzed with 1 *N* HCl (2 mL) at 90°C for 5 min. The mixture was cooled under N_2 and neutralized with 2 *N* NaOH (0.8 mL) and 1 *N* $NaHCO_3$ (1 mL). The mixture was passed through a short alumina column, a C-18 Sep-Pak column, and a 0.22-μm Millipore filter, followed by eluting 5

mL of 10% ethanol/saline. A yield of 20–40 mCi of pure product was isolated (12–18% yield, decay corrected) with the end of bombardment (EOB) at 60–70 min. HPLC was performed on a C-18 Radial-Pak column, 8 × 100 mm, with pure water, using a flow rate of 1 mL/min. The no-carrier-added product corresponded to the retention time (4.8 min) of the unlabeled FMISO under similar conditions. The radiochemical purity was greater than 99%. Under the UV detector (310 nm), there were no other impurities. A radio-TLC scanner (Bioscan, Washington, DC) showed a retardation factor of 0.5 for the final product using a silica gel plate G/UV 254, 5 × 20 cm (Whatman, Anaspec, MI), eluted with chloroform:methanol (7:3), which corresponds to the unlabeled FMISO. In addition, kryptofix-2,2,2 was not visualized (developed in iodine chamber) on the silica gel-coated plate using 0.1% (v/v) triethylamine in methanol as an eluant. The specific activity of [^{18}F]FMISO ranged from 1 to 2 Ci/μmol based upon UV and radioactivity detection of a sample of known mass and radioactivity.

Half-Life Test of [^{18}F]Fluoromisonidazole

After the [^{18}F]fluoromisonidazole was prepared, an aliquot (10 μCi) of this radioactive product was counted on a gamma counter at various time intervals (30, 60, 120 min). To assure that the correct isotope (^{18}F) was incorporated into the nitroimidazole analogue, the radioactivity of the final product at different time intervals was counted and decay was corrected.

Sterility and Pyrogenicity

To demonstrate the sterility of the product, each batch of product was tested using Bactec culture vials with aerobic and anaerobic materials (NR6 and NR7; Towson, MD). An aliquot (0.3 mL) of the final solution was incubated in vials for 7 days at 37°C. Sterility was examined every day and assayed by visualizing the cloudiness of the solution. Our 10 samples were consistently shown to be sterile.

To assure pyrogenicity, a LAL kit (Whittaker Bioproduct, Walkersville, MD) was used. To prove that the drug solution did not enhance or inhibit the LAL test, we used three 1:1 dilutions (0.25 mL of drug solution to 0.25 mL of sterile water) to test the pyrogenicity of each batch of product. Compared to standard solutions (12.5 Eμ/mL, 1.25 Eμ/mL, and negative), the product solution proved to be pyrogen-free (0 Eμ/mL) and did not interfere with the sensitivity of the assay. Our 10 samples were shown to be pyrogen-free.

RESULTS AND DISCUSSION

The synthetic approach to obtain [^{18}F]FMISO is shown in Scheme I. [^{18}F]FMISO precursor was prepared in a simplified three-step procedure. Glycerol-1,3-ditosylate (Compound 2) was obtained by treating glycerol with tosyl chloride. Compound 3 was prepared by reacting this glycerol-1,3-ditosylate with anhydrous acetic anhydride and BF_3 etherate; the yield was 100%. From a mixture of Compound 3 (2-nitroimidazole and Cs_2CO_3 in dry DMF at 50°C for 1 hr), Compound 4 was prepared in a 48% yield. The only other product isolated was the starting material. HPLC and TLC of labeled Compound 1 correspond to those of unlabeled Compound 1 under the same conditions. The final radiolabeled product proved to be sterile and pyrogen-free, suitable for intravenous injection to patients. The ^{18}F half-life test indicated that no other radioisotope was incorporated in the molecule.

It has been reported that kryptofix must be eluted from a C-18 column under acidic conditions (17). In our final product, the pH value was in the range of 7.4–8.0. In TLC analysis, the final product did not show the presence of kryptofix.

The key feature of this design was to use the right temperature for the last step. Heating the reaction mixture (Compound 3; 2-nitroimidazole and cesium carbonate) at 100°C for several hours produced a bisalkylation cyclized by-product (Compound 5). The spectroscopic data, ^{1}H and ^{13}C NMR, and HRMS, confirmed the structure of Compound 5.

In summary, we have developed an alternative synthesis of [^{18}F]FMISO. Our method produces [^{18}F]FMISO with a high specific activity, a short synthesis time, and a reasonable yield, that is sterile and pyrogen-free, which should be useful for the evaluation of hypoxic tumors with PET.

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