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Synthesis of 1-[2-[^{18}F]Fluoro-1-(hydroxymethyl)-ethoxy]methyl-2-nitroimidazole ([^{18}F]FENI), a Potential Agent for Imaging Hypoxic Tissues by PET

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SUMMARY

Fluorine-18 labeled 1-[2-fluoro-1-(hydroxymethyl)ethoxy]-mehyl-2-nitroimidazole ([^{18}F]FENI), a novel, potential radiotracer for imaging hypoxic tissue by PET, was synthesized. The [^{18}F]FENI was prepared by nucleophilic ^{18}F-fluorination of the precursor, 1-[2-(toluene-4-sulfoxy)-1-(acetoxymethyl)ethoxy]methyl-2-nitroimidazole, followed by deprotection and HPLC purification. Radiochemically pure [^{18}F]FENI was obtained in overall yields of 3-11% (EOB) with specific activity of >26 GBq/μmol (EOS) within 90 min.

Key words: 1-[2-fluoro-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole, FENI, fluorine-18, hypoxic cell marker, tumor imaging, PET

INTRODUCTION

Since nitroimidazoles are metabolically trapped by hypoxic cells, they have been used as radiosensitizers. Based on this selective binding and retention in hypoxic regions, it is expected that labeled nitroimidazoles can be used as hypoxia markers. The misonidazole analog, fluoromisonidazole (FMISO), was first labeled with ^{18}F (1-5) and evaluated for clinical use. It is said that [^{18}F]FMISO has two problems:

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relatively low concentration within the lesion and the need to wait several hours to permit clearance of the agent from the normoxic background tissue (6). In attempts to seek an alternative to [^{18}F]FMISO radiosyntheses of [^{18}F]fluoroetanidazole (7) and [^{18}F]EF1 (8) have been reported.

The 2-nitroimidazole nucleoside analog, RP-170, was developed as a potential radiosensitizer for hypoxic tumor cells. It is reported that the reduction potential and radiosensitizing activity of RP-170 are similar to those of misonidazole and etanidazole (9-11). Since its favorable distribution and sensitizing activity for hypoxic tissue and low neurotoxicity have been confirmed by *in vivo* and *in vitro* studies, it was expected that the radiolabeled derivative of RP-170 would serve as a useful probe for tissues that are oxygen deficient but still viable.

In this study, a fluorinated analog of RP-170, 1-[2-fluoro-1-(hydroxymethyl)-ethoxy]methyl-2-nitroimidazole (FENI), was labeled with ^{18}F using no-carrier-added [^{18}F]fluoride in order to be evaluated as a potential agent for clinical PET studies of hypoxia.

RESULTS AND DISCUSSION

A general synthetic method for the preparation of [^{18}F]FENI ([^{18}F]**2**) is shown in Figure 1. The strategy for the synthesis was straightforward, based on the commonly

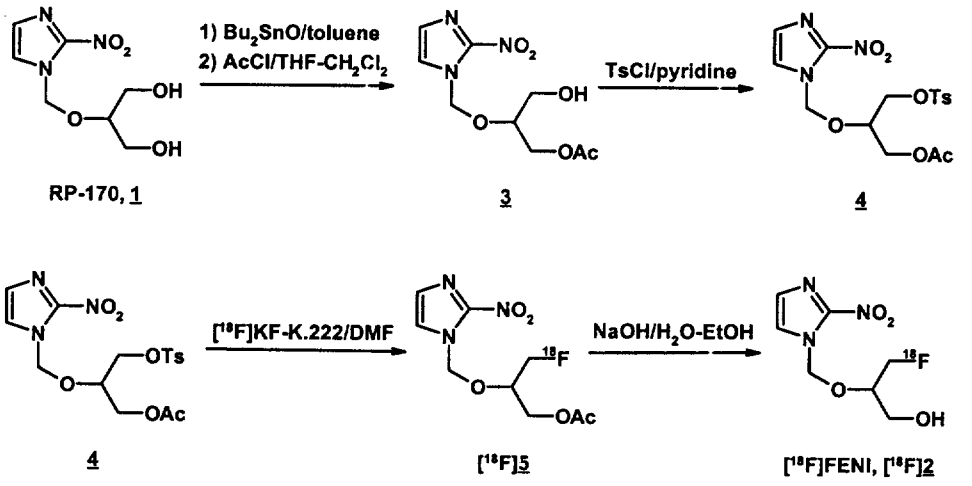

Figure 1. Synthetic method for [^{18}F]FENI.

used two-step procedure, ^{18}F-substitution and deprotection. In our preliminary radiosynthesis, the ditosylated derivative of RP-170 (**1**) was examined as a precursor, but the ^{18}F-fluorinated intermediate resisted deprotection under basic conditions. Therefore, in the preparation of the precursor, one of the two equivalent hydroxy groups of **1** was converted to an acetyl protecting group and the other to tosylate as a leaving group for ^{18}F-substitution. Conversion of **1** into cyclostannioacetal with dibutyl tin oxide followed by treatment with acetyl chloride and hydrolysis afforded the mono-acetylated derivative **3** (12). Compound **4** was prepared using the literature procedure with some modifications (13, 14). The reaction of **3** and tosyl chloride gave the precursor **4**. The overall yield of **4** based on **1** was 44 %.

The cold fluorination of **4** was carried out in *N,N*-dimethylformamide (DMF) at 110°C. Potassium fluoride (KF) and 18-Crown-6 were used as a fluorinating reagent to produce **5**. After 6 hours heating, the reaction was quenched to give **5**. The deprotection of **5** was carried out in a 0.05 M water-ethanol (50:50 v/v) solution of NaOH in 1 minute at 40°C. This was followed by treatment with cation-exchange resin and purification by column chromatography on silica gel to give 1-[2-fluoro-1-(hydroxymethyl)ethoxy]methyl- 2-nitroimidazole (FENI, **2**) in the overall yield of 20% based on **4**.

Radiosynthesis was performed in a similar way to the cold synthesis described above except that Kryptofix 2.2.2 was used instead of 18-Crown-6. The substitution reaction of [^{18}F]fluoride with **4** to afford the intermediate [^{18}F]**5** was followed by radio-thin layer chromatography (TLC). As shown in Figure 2a, it was observed that

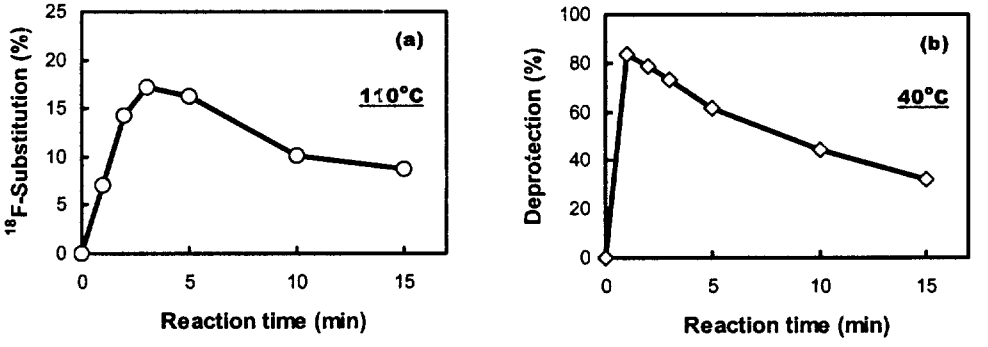

Figure 2. Optimization of ^{18}F-substitution (a) and deprotection (b).

the radiochemical yield of [^{18}F]**5** increased during the first 4 min and then gradually decreased while more hydrophilic degradation products were found to increase in the reaction solution. It is suggested that the ether bond of **3** is likely to be cleaved by prolonged heating under the basic conditions.

Figure 2b shows that the removal of the acetyl group from [^{18}F]**5** proceeds rapidly and quantitatively. However, a rapid decrease in the yield of [^{18}F]**2** was again observed. Optimal hydrolysis time was thus determined to be 1 min.

The desired product was isolated by semi-preparative C_{18} high performance liquid chromatography (HPLC) (see Figure 3). The radioactive peak eluting at 6.5-7.0 min, corresponding to [^{18}F]FENI ([^{18}F]**2**), was collected and evaporated to dryness under reduced pressure. The residue was dissolved in saline. The overall synthesis time including HPLC purification and formulation was less than 90 min.

As can be seen in Figure 3, the radioactive peak of [^{18}F]**2** had no corresponding UV peak and high radiochemical purity of the collected product was also confirmed by analytical HPLC. The specific activity was estimated to be higher than 26 GBq/μmol (0.7 Ci/μmol) at the end of the synthesis by comparison of the UV response of cold FENI. The average decay-corrected overall radiochemical yield was 6.2% (3-11%, 12 runs).

In conclusion, we have developed a convenient method for the preparation of

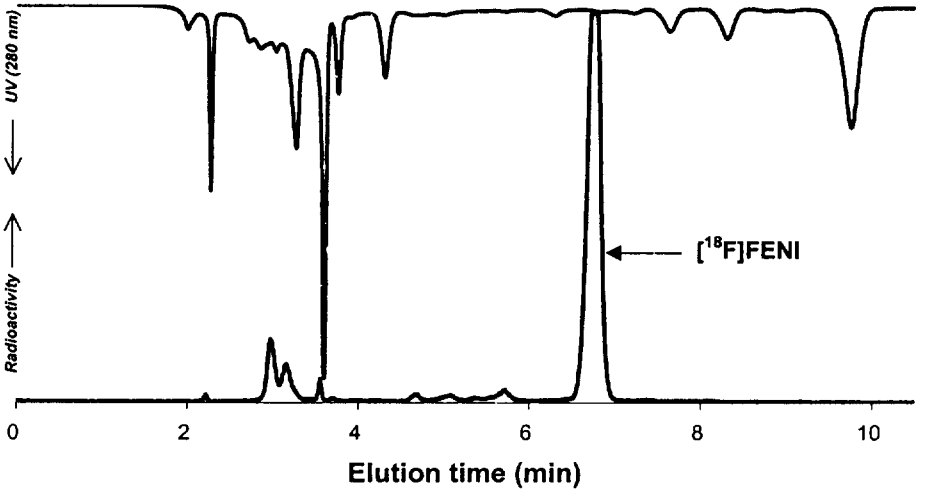

Figure 3. Semi-preparative HPLC separation.

[^{18}F]FENI, ^{18}F-labeld fluoro analog of RP-170 from [^{18}F]fluoride. By this method, [^{18}F]FENI is obtained in a moderate radiochemical yield with sufficiently high radiochemical purity for clinical PET investigations of hypoxia. Preliminary results in the evaluation of [^{18}F]FENI using small animals are promising and details will be reported elsewhere.

EXPERIMENTAL

General Method

The starting material, RP-170 (**1**), was supplied by POLA Chemical Industries, Inc. Acetyl chloride was purchased from Wako Pure Chemicals and purified by distillation from P_2O_5. Other chemicals and solvents were commercially available and used without further purification.

TLC was performed on precoated Kieselgel 60 F_{254} aluminum sheets (Merck) and visualized with UV radiation. Autoradiographic images of TLC plates were obtained using a BAS 3000 system (Fuji Film, Ltd.). A radio-HPLC system consisted of a Waters 6000A pump, a Rheodyne injector, a Gilson Model 111 UV detector and a radioactivity detector. The UV detector was operated at 280 nm. ^{1}H NMR and ^{19}F NMR were recorded on Varian XL-200 and Bruker ARX-300 spectrometers, respectively, with tetramethylsilane and hexafluorobenzene as references. Mass spectra were recorded on Hitachi M-2500S, JEOL JMSDX303, and JEOL JMSAX 500 spectrometers.

1-[2-Hydoroxy-1-(acetoxymethyl)ethoxy]methyl-2-nitroimidazole (3)

Compound **3** was prepared by a literature procedure (4).

To a flask containing **1** (594 mg, 2.73 mmol), dibutyl tin oxide (714 mg, 2.87 mmol) and molecular sieves 4 Å (4 g) was added dry toluene (20 mL) and the mixture was heated to reflux for 2 h. After the heating, the molecular sieves were filtered off. The volatiles of the solution were removed under vacuum, and dry CH_2Cl_2 (15 mL), dry tetrahydrofuran (4 mL), and acetyl chloride (0.20 mL, 0.22 g, 2.8 mmol) were added to the residue. The solution was stirred for 0.5 h at room temperature. After the stirring, sodium phosphate buffer (pH 7.1; 10 mL) was added

and the entire mixture was filtered through a celite pad and extracted with $CHCl_3$ (3 x 30 mL). The organic extract was dried with Na_2SO_4 and the volatiles were removed under vacuum. The crude mixture was purified by silica gel column chromatography (mobile phase: ethyl acetate) and **3** (410 mg, 1.58 mmol) was obtained as a colorless oil in 58 % yield. ^{1}H NMR (400 MHz, CD_3CN) δ/ppm 1.93 (s, 3H, C(O)C*H*$_3$), 3.04 (br, 1H, OH), 3.47-3.57 (m, 2H, C*H*$_2$OH), 3.75-3.81 (m, 1H, NCH$_2$OC*H*), 3.91-4.11 (m, 2H, C*H*$_2$OAc), 5.79, 5.85 (AB pattern, 2H, $^2J_{H\text{-}H}$ = 10.8 Hz, NC*H*$_2$OCH), 7.12 (d, 1H, $^3J_{H\text{-}H}$ = 1.1 Hz, imidazolyl H), 7.50 (d, 1H, $^3J_{H\text{-}H}$ = 1.1 Hz, imidazolyl H). MS (FAB) *m/z* 260 (23, M^++H), 154 (100, M^+-CH_2OAc-CH_2OH-H). Anal. Calcd for $C_9H_{13}N_3O_6$: C 41.70, H 5.06, N 16.21. Found: C 41.36, H 5.16, N 15.74 %.

1-[2-(Toluene-4-sulfoxy)-1-(acetoxymethyl)ethoxy]methyl-2- nitroimidazole (4)

Compound **3** was tosylated according to literature procedures with some modifications (13, 14).

Dry pyridine (20 mL) was added to a flask containing **3** (401 mg, 1.55 mmol) and *p*-toluene sulfonyl chloride (893 mg, 4.68 mmol), and the mixture was stirred at room temperature for 5 h. Ethyl acetate (50 mL) was added to the solution and the mixture was washed with water (2 x 50 mL). The aqueous phase was extracted with ethyl acetate (50 mL). The combined organic phase was dried with anhydrous Na_2SO_4 and the volatiles were removed under vacuum. The crude product was purified by silica gel column chromatography (mobile phase: ethyl acetate) to give **4** (488 mg, 1.18 mmol) as a yellow oil in 76% yield. ^{1}H NMR (400 MHz, CD_3CN) δ/ppm 1.88 (s, 3H, C(O)C*H*$_3$), 2.44 (s, 3H, $SO_2C_6H_4CH_3$), 3.89-3.95 (m, 1H, NCH$_2$OC*H*), 3.96-4.11 (m, 4H, C*H*$_2$OAc and C*H*$_2$OTs), 5.68, 5.78 (AB pattern, 2H, $^2J_{H\text{-}H}$ = 10.7 Hz, NC*H*$_2$OCH), 7.11 (d, 1H, $^3J_{H\text{-}H}$ = 1.0 Hz, imidazolyl H), 7.39 (d, 1H, $^3J_{H\text{-}H}$ = 1.0 Hz, imidazolyl H), 7.42 (d, 1H, $^3J_{H\text{-}H}$ = 8.5 Hz, phenyl H), 7.73 (d, 1H, $^3J_{H\text{-}H}$ = 8.5 Hz, phenyl H). MS (EI, 70 eV) *m/z* 413 (1.2, M^+), 271 (100, [CH(CH_2OAc)-CH_2OTs]$^+$). Anal. Calcd for $C_{16}H_{19}N_3O_8S$: C 46.49, H 4.63, N 10.16. Found: C 46.52, H 4.69, N 9.70%.

1-[2-Fluoro-1-(acetoxymethyl)ethoxy]methyl-2-nitroimidazole (5)

Acetonitrile (20 mL) and water (3 mL) were added to a flask containing KF (87.0 mg, 1.50 mmol) and 18-crown-6 (150 mg, 568 μmol), and the solution was evaporated to dryness under vacuum. A dry DMF (20 mL) solution of **4** (196 mg, 474 μmol) was added to the residue, and the solution was heated at 110°C for 6 h. Ethyl acetate (30 mL) was added to the solution, and the mixture was washed with water (2 x 30 mL). The organic phase was extracted with ethyl acetate (30 mL), and the combined organic phase was evaporated to dryness under vacuum. The crude product was purified by silica gel column chromatography (mobile phase: ethyl acetate) to give **5** (60.1 mg, 230 μmol) as a yellow oil in 49% yield. ^{1}H NMR (400 MHz, CD_3CN) δ/ppm 1.94 (s, 3H, C(O)C*H*$_3$), 3.98-4.14 (m, 3H, C*H*OC*H*$_2$OAc), 4.38-4.58 (dm, 2H, $^2J_{H\text{-}F}$ = 47.0 Hz, C*H*$_2$F), 5.79, 5.86 (AB pattern, 2H, $^2J_{H\text{-}H}$ = 10.8 Hz, NC*H*$_2$OCH), 7.13 (d, 1H, $^3J_{H\text{-}H}$ = 1.2 Hz, imidazolyl H), 7.47 (d, 1H, $^3J_{H\text{-}H}$ = 1.2 Hz, imidazolyl H). ^{19}F NMR (282 MHz, CD_3CN) δ/ppm -230.6 (t, $^2J_{F\text{-}H}$ = 47.0 Hz). MS (EI, 70 eV) *m/z* 261 (0.69, M^+), 220 (2.0, M^+-H), 43 (100, Ac^+). Exact MS *m/z* Found: 261.0743. Calcd for $C_9H_{12}FN_3O_5$ (M^+): 261.0760.

1-[2-Fluoro-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole (FENI, 2)

A water-ethanol (50:50 v/v) solution of NaOH (0.05 N, 15 mL) was added to a flask containing **5** (56.6 mg, 217 μmol) and the solution was stirred at 40°C for 1 min. The reaction mixture was passed through a Maxi-Clean IC-H Plus cartridge (Alltech, #30256) to remove sodium cation. The cartridge was washed with ethanol (2 mL), and the combined mixture was evaporated to dryness under vacuum. The crude product was purified by silica gel column chromatography (mobile phase: ethyl acetate) to give **2** (19.6 mg, 47.0 μmol) as a yellow oil in 41 % yield. ^{1}H NMR (400 MHz, CD_3CN) δ/ppm 3.01 (br, 1H, CH_2OH), 3.49-3.53 (m, 2H, C*H*$_2$OH), 3.81-3.88 (dm, 1H, $^3J_{H\text{-}F}$ = 19.8 Hz, NCH_2OCH), 4.32-4.54 (dm, 2H, $^2J_{H\text{-}F}$ = 50.4 Hz, CH_2F), 5.83, 5.85 (AB pattern, 2H, $^2J_{H\text{-}H}$ = 10.8 Hz, NC*H*$_2$OCH), 7.11 (d, 1H, $^3J_{H\text{-}H}$ = 1.1 Hz, imidazolyl H), 7.51 (d, 1H, $^3J_{H\text{-}H}$ = 1.1 Hz, imidazolyl H). ^{19}F NMR (282 MHz, CD_3CN) δ/ppm -230.6 (t, $^2J_{H\text{-}F}$ = 50.4 Hz). MS (FAB) *m/z* 220 (29, M^++H), 154 (100,

M^+-CH_2OH-CH_2F-H). Exact MS *m/z* Found: 220.0744. Calcd for $C_7H_{11}FN_3O_4$ (M^++H): 220.0733.

1-[2-[^{18}F]Fluoro-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole ([^{18}F]FENI, [^{18}F]2)

No-carrier-added [^{18}F]fluoride was produced with a Cypris HM12 cyclotron (Sumitomo Heavy Ind.) by proton-irradiation of ^{18}O-enriched water. To a Wheaton glass vial (10 mL) containing Kryptofix 2.2.2. (30 mg, 80 μmol) in acetonitrile (1 mL) was added [^{18}F]fluoride (2.6-7.0 GBq) dissolved in an aqueous solution of K_2CO_3 (33 mM). The solution was heated at 110°C and evaporated to dryness with the aid of a He flow. This process was repeated three times by adding acetonitrile (1 mL). To the residue was added a solution of **4** (5-6 mg, 12-15 μmol) in dry DMF (1 mL), and the reaction mixture was heated at 110°C for 5 min. The reaction was quenched by adding water (10 mL), and the resulting mixture was passed through a Sep-Pak Plus C18 cartridge (Waters). The Sep-Pak cartridge was washed with 0.05 N HCl (10 mL) and water (10 mL). and [^{18}F]**5** retained by the C18 was eluted with MeCN (4 mL). The MeCN eluate was evaporated to dryness under reduced pressure. To the residue was added a 0.05 N NaOH solution of 50% ethanol-in-water and the mixture was heated for 1 min at 40°C. The reaction mixture was passed through an IC-H Plus cartridge for neutralization, and the eluate was evaporated to dryness under reduced pressure. The residue was dissolved in an HPLC solvent (acetonitrile-water: 15/85) and injected onto a semi-preparative HPLC C18 column (A-324, 10 mm x 300 mm, YMC). The effluent at a flow rate of 6.0 mL/min was monitored with both radioactivity and UV detectors, and the fraction containing the desired product was collected. Chemical and radiochemical purity was assayed by analytical HPLC on a reverse phase C18 column (Inertsil ODS 80A, 4.6 mm x 250 mm, GL Sciences) with a solvent system of acetonitrile-water (30/70). No other radioactive peak than [^{18}F]FENI or no UV peak corresponding to FENI was found.

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