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An Efficient Radiosynthesis of [^{18}F]Fluoromisonidazole

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An efficient preparation of the hypoxic cell tracer [^{18}F]3-fluoro-1-(2′-nitro-1′-imidazolyl)-2-propanol ([^{18}F]fluoromisonidazole) is reported. This radiopharmaceutical is of interest to probe hypoxic tissue in infarcts and tumors. One-step radiolabeling and rapid protection group removal provided 55–80% yield in 50 min. The process is similar to common fluorine labeling procedures, simplifying the procedure for most laboratories, and offers an improvement over more difficult previous methods. The labeling precursor was prepared in five steps from readily available materials in a straightforward reaction scheme.

Introduction

Much interest has been focused on developing radiosensitizer agents based upon nitroaromatics for detection of hypoxia. Among them misonidazole and its analogs have received the most attention in recent years. It has been shown that fluoromisonidazole labeled with carbon-14, fluorine-18 and tritium is metabolically trapped by viable cells according to their degree of hypoxia (Hoffman *et al.*, 1987; Grunbaum *et al.*, 1987; Rasey *et al.*, 1987, 1989, 1990; Martin *et al.*, 1989, 1990). The accepted mechanism (Franko, 1986) involves the diffusion of the nitro derivative into the cell where enzymatic reduction forms a reactive and non-diffusible radical species. In the presence of oxygen, a futile cycle exists because the radical species is oxidized to re-form the diffusible parent nitro compound. In hypoxic tissue, the reactive anion becomes irreversibly trapped by covalent binding to macromolecules or by further reduction, and therefore accumulates in the cell. Positron-emitting [^{18}F]fluoromisonidazole was proposed as a potential radiopharmaceutical for the non-invasive detection of hypoxia in malignant tumors, myocardial infarcts or cerebral ischemia using positron emission tomography (PET). The synthesis of [^{18}F]fluoromisonidazole (Jerabek *et al.*, 1986; Hwang *et al.*, 1989; Grierson *et al.*, 1989) and promising results from its use in humans (Valk *et al.*, 1991; Koh *et al.*, 1991) have been reported. We were motivated by reports of early success to prepare this compound and apply it to clinical research studies. However, the reported procedures for synthesis of [^{18}F]fluoromisonidazole were relatively tedious, required considerable technical skill to perform routinely, and were reported to give low yields. We concluded that daily preparation of [^{18}F]fluoromisonidazole for full-scale clinical studies would not be practical within a schedule which included several other research and clinical studies.

In this work, we describe a new synthetic precursor for the preparation of [^{18}F]fluoromisonidazole through a high yield single step process. We have eliminated the post-labeling condensation step and introduced labeled fluoride directly into the final product. Thus, fluoromisonidazole was produced in an efficient fashion suitable for routine PET imaging.

Experimental

Reagents and solvents were obtained from Aldrich Chemical Co. and Fisher Scientific and used without further purification unless otherwise noted. Melting points were recorded on an Electrothermal melting point apparatus and are uncorrected. NMR spectroscopy was carried out on either a Varian XL-200 (200 MHz) or a Gemini-300 (300 MHz) instrument using tetramethylsilane (TMS) as internal standard and the chemical shifts were reported in parts per million (ppm) from TMS. Infrared spectra were obtained on a Beckman FT-1100 spectrophotometer. High-resolution mass spectra were obtained on a MS 25RFA Kratos spectrometer operating at 70 eV. Tetrahydrofuran (THF) was freshly distilled from sodium benzophenone ketyl under argon prior to use. Acetonitrile, pyridine and dimethylformamide (DMF) were freshly distilled from calcium hydride prior to use. 2-Nitroimidazole is commercially available (Aldrich); it was also prepared from 2-aminoimidazole using the procedure of Beaman *et al.* (1966). In either case, 2-nitroimidazole was sublimed (0.1 mmHg, 110–120°C) prior to use. Column chromatography was performed on either 230 mesh silica

gel or neutral alumina of Brockman I activity. Thin layer chromatography (TLC) was carried out on E. Merck silica gel 60 F_{254} analytical plates. TLC elution conditions varied and are specified individually, with R_f values, for each compound below. High performance liquid chromatography (HPLC) was performed on a Hewlett Packard 1050 system with a multiple wavelength u.v./visible detector using a reverse phase analytical column (Altech Econosil-C18 10 μm, 4.6 × 250 mm) eluted at 2 mL/min with 20% acetonitrile/water.

3-Fluoro-1-(2'-nitro-1'-imidazolyl)-2-propanol (1)

Unlabeled fluoromisonidazole was prepared according to Grierson *et al.* (1989) and used as a reference sample (m.p. 130–135°C). After one recrystallization from ethanol the melting point rose to 135–137°C [Grierson *et al.* (1989), 139–140°C]. HPLC, RT = 2.6 min. TLC: EtOAc, $R_f = 0.65$. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.20 (s, 1H, imidazolyl), 7.15 (s, 1H, imidazolyl), 5.30 (br s, 1H, OH), 4.77 (dd, 1H, $J = 3$, 13), 4.67–4.54 (m, 1H), 4.52–4.32 (2 overlapping m, 2H), 4.30–4.26 (m, 1H, CH). i.r. (KBr) 3255, 3104, 1534, 1482, 1364, 1279, 1161, 1116, 1024, 919, 834, 795 cm^{-1}.

1,3-O-Benzylidenepropanetriol (2)

A mixture of glycerol (22 g, 0.24 mol) and benzaldehyde (20 g, 0.19 mol) in benzene (25 mL) in the presence of a catalytic amount of *p*-toluenesulfonic acid was refluxed using a Dean-Stark trap. When 2.5 mL of water (*ca* 70% theoretical) had been removed azeotropically the reaction mixture was cooled to room temperature. Benzene was removed *in vacuo* at room temperature and the reaction mixture was stored at −5°C overnight. The solid thus formed was filtered from the glycerol solution and recrystallized from a 1:1 mixture of benzene and petroleum ether to afford a white solid (8.5 g, 25%): m.p. 60–65°C [Baggett *et al.* (1960), m.p. 63–65°C]. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.50 (m, 3H), 7.40 (m, 3H), 5.80 and 5.46 (2s, 1H, benzyl), 4.34 (m, 1H), 4.18 (dd, 2H, $J = 11$, 7), 4.04 (br s, 1H, OH), 3.63 (t, 2H, $J = 10$). i.r. (KBr) 3400 (br, OH), 2860, 1453, 1395, 1220, 1150, 1082, 1030, 980, 760, 695 cm^{-1}.

1,3,-O-Benzylidene-2-O-tetrahydropyranylpropanetriol (3)

A mixture of **2** (7.2 g, 40 mmol), pyridinium *p*-toluenesulfonate (2 g, 8 mmol), prepared according to the method of Miyashita *et al.* (1977), and 3,4-dihydro-2*H*-pyran (DHP, 7.4 g, 8.8 mmol) in anhydrous THF (100 mL) was stirred at room temperature overnight. A white precipitate was formed and the reaction was followed by TLC: EtOAc/petroleum ether (3/7), $R_f = 0.6$. The reaction mixture was diluted with half saturated brine to remove the catalyst and extracted with EtOAc. Removal of the solvent *in vacuo* provided a clear liquid (11 g) which was chromatographed on 80 g of silica gel eluted with 40% EtOAc/petroleum ether to give a white solid (8 g, 75%): m.p. 46–48°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.53 (m, 2H), 7.35 (m, 3H), 5.57 (s, 1H, benzyl), 4.85 (t, 1H, $J = 4$) 4.35 (t, 2H, $J = 11$), 4.15 (d, 1H, $J = 13$), 4.03 (d, 1H, $J = 13$), 3.93 (dt, 1H, $J = 8$, 4), 3.67 (m, 1H), 3.52 (m, 1H), 1.80–1.53 (3 overlapping m, 6H). i.r. (KBr) 2940, 2856, 1390, 1340, 1162, 1138, 1074, 1030, 800, 750, 702 cm^{-1}.

2-O-Tetrahydropyranylpropanetriol (4)

To a stirred suspension of **3** (1 g, 3.8 mmol) in 25 mL of refluxing liquid ammonia under argon was added a total of 0.45 g of sodium (20 mmol) in portions over 20 min by which time the characteristic deep blue color persisted. The reaction was allowed to stir for an additional 10 min. The blue color was then carefully discharged by adding solid ammonium chloride and the ammonia was evaporated under a stream of argon. The solid residue was triturated with 40 mL of petroleum ether to remove dibenzyl and the diol **4** was taken up in 40 mL of methylene chloride (Reist *et al.*, 1964). Evaporation of the solvent *in vacuo* afforded a yellowish oil (0.5 g, 75%). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 4.62 (m, 1H), 4.00 (m, 1H), 3.80–3.53 (3 overlapping m, 6H), 2.89 (br s, 2H, OH), 1.84 (m, 2H), 1.55 (m, 4H). i.r. (neat) 3400 (br, OH), 2940, 2865, 1450, 1139, 1120, 1070, 1032, 988 cm^{-1}.

2-O-Tetrahydropyranyl-1,3-O-ditoluenesulfonylpropanetriol (5a)

To a mixture of diol **4** (0.4 g, 2.3 mmol) in 10 mL of anhydrous pyridine cooled with an ice bath was added dropwise a solution of *p*-toluenesulfonyl chloride (1 g, 5.75 mmol) in 10 mL of dry pyridine. After the addition the reaction mixture was allowed to slowly warm to room temperature and stir overnight. The mixture was then poured into an ice–water mixture. The precipitate was collected by filtration (1 g, 90%): m.p. 108–110°C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.78 (d, 4H, H_2 H_6), 7.74 (d, 4H, H_3 H_5), 4.60 (m, 1H), 4.10–3.98 (3 overlapping m, 6H), 3.69 (m, 1H), 3.41 (m, 1H), 2.46 (s, 6H, CH_3), 1.60 (m, 2H), 1.46 (m, 4H). i.r. (KBr) 2940, 1600, 1360, 1180, 1098, 1037, 1020, 1000, 980, 830, 814, 669 cm^{-1}.

2-O-Tetrahydropyranyl-1,3-O-dimethanesulfonylpropanetriol (5b)

The procedure described above for **5a** was followed using **4** (0.4 g, 2.3 mmol) and methanesulfonyl chloride (0.78 g, 6.9 mmol) to give **5b** as an oil (0.11 g, 15%) after chromatography on silica eluted with 60% EtOAc/petroleum ether. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 4.76 (m, 1H), 4.39 (m, 2H), 4.33 (m, 2H), 4.26–4.16 (m, 1H), 3.95–3.85 (m, 1H), 3.60–3.50 (m, 1H), 3.09 (s, 3H, CH_3), 3.07 (s, 3H, CH_3), 1.78 (m, 2H), 1.64–1.55 (m, 4H). i.r. (neat) 2940, 1442, 1360, 1180, 1120, 1035, 1000, 970, 835, 820 cm^{-1}.

*1-(2'-Nitro-1'-imidazolyl)-2-O-tetrahydropyranyl-3-O-toluenesulfonylpropanediol (**6a**)*

A mixture of disulfonate **5a** (0.47 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, through which argon was bubbled for 10 min prior to use, was heated at 110°C for 1 h. The reaction was then cooled and DMF was carefully removed under reduced pressure. The residue was taken up in EtOAc and filtered. Removal of EtOAc *in vacuo* gave a yellow oil which was chromatographed on neutral alumina eluted with 60–70% EtOAc/petroleum ether to afford a light yellow oil (0.16 g, 41%) which was dried *in vacuo* and stored at 0° under argon. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.80 (t, 2H, $J = 8$, H_2 and H_6), 7.37 (d, 2H, $J = 8$, H_3 and H_5), 7.10 (s, 2H, imidazolyl), 4.79 (dd, 1H, $J = 3$, 14), 4.36 (dd, 1H, $J = 7$, 14), 4.27–4.17 (m, 2H), 4.09–4.00 (m, 2H), 3.62 (m, 1H), 3.32 (m, 1H), 2.46 (s, 3H, CH_3), 1.67–1.53 (m, 2H), 1.46–1.32 (m, 4H). i.r. (neat) 2941, 2862, 1672, 1534, 1482, 1364, 1174, 1116, 1089, 1037, 958, 828, 664 cm^{-1}. High resolution MS calcd for $C_{18}H_{23}N_2O_5S$ (M^+-NO_2): 379.1328. Found: 379.1315.

*1-(2'-Nitro-1'-imidazolyl)-2-O-tetrahydropyranyl-3-O-methanesulfonylpropanediol (**6b**)*

The procedure described above for **6a** was followed using **5b** (0.1 g, 0.3 mmol), 2-nitroimidazole (0.035 g, 0.3 mmol) and cesium carbonate (0.1 g, 0.3 mmol) to give **6b** as an oil (0.015 g, 15%) after chromatography on alumina eluted with 80% EtOAc/petroleum ether. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.17 (s, 2H, imidazolyl), 4.85 (dd, 1H, $J = 4$, 14), 4.46 (dd, 1H, $J = 7$, 14), 4.29 (m, 2H), 3.98 (m, 2H), 3.61 (m, 1H), 3.30 (m, 1H), 3.10 (s, 3H, CH_3), 1.80–1.71 (m, 2H), 1.58–1.51 (m, 4H). i.r. (neat) 2941, 2870, 1544, 1488, 1357, 1174, 1122, 1031, 971, 828, 808, 664 cm^{-1}.

*1,3-O-Benzylidene-2-O-benzylpropanetriol (**7**)*

A mixture of **2** (4 g, 22 mmol) and freshly cut sodium (~1 g, 43 mg-atoms) in toluene (30 mL) was heated to reflux for 4 h using a Dean-Stark trap. To the cooled and decanted solution, benzyl chloride (5 g, 40 mmol) was added and the mixture was further refluxed overnight. The reaction mixture was washed twice with water, dried over magnesium sulfate and evaporated *in vacuo* to yield a solid which was recrystallized from 20% EtOAc/petroleum ether (3.7 g, 62%): m.p. 73–76°C [Baggett *et al.* (1960), m.p. 76–77°C]. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.51 (m, 2H), 7.41–7.33 (m, 8H), 5.56 (s, 1H, O_2CH), 4.71 (s, 2H, benzyl), 4.36 (d, 2H, $J = 11$), 4.15 (q, 1H, $J = 11$), 4.04 (d, 2H, $J = 11$). i.r. (KBr) 3380 (br, OH), 2990, 2880, 1452, 1390, 1218, 1150, 1080, 1028, 980, 756, 700 cm^{-1}.

*2-O-Benzylpropanetriol (**8**)*

A solution of methanol (30 mL) and 4 N hydrochloric acid (10 mL) containing **7** (3.5 g, 13 mmol) was boiled under reflux for 1 h. The cooled mixture was poured into ice–water (150 mL) and benzaldehyde was removed by extraction with petroleum ether. The water phase was then extracted with methylene chloride and the extracts were dried over magnesium sulfate. Removal of the solvent *in vacuo* afforded **8** as an oil (1 g, 42%). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.32 (s, 5H), 4.60 (s, 2H, benzyl), 4.80–4.60 (2 overlapping m, 4H), 3.50 (m, 1H, CH), 2.76 (br s, 2H, OH). i.r. (neat) 3380, 2140, 2080, 1450, 1120, 1052, 744, 100 cm^{-1}.

*2-O-Benzyl-1,3-O-ditoluenesulfonylpropanetriol (**9a**)*

The procedure described above for **5a** was followed using **8** (0.5 g, 2.7 mmol) and *p*-toluenesulfonyl chloride (2 g, 10 mmol) to give **9a** as a white solid (1 g, 74%) after recrystallization from 1:1 EtOAc/petroleum ether: m.p. 106–107°C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.73 (d, 4H, $J = 8$), 7.33–7.26 (2 overlapping m, 7H), 7.19–7.15 (m, 2H), 4.47 (s, 2H, benzyl), 4.10–4.02 (m, 4H), 3.80 (m, 1H), 2.44 (s, 6H, CH_3). i.r. (KBr) 2920, 1600, 1450, 1374, 1357, 1188, 1178, 1092, 1057, 975, 948, 840, 812, 744, 676, 662 cm^{-1}.

*2-O-Benzyl-1,3-O-dimethanesulfonylpropanetriol (**9b**)*

The procedure described above for **5a** was followed using **8** (0.33 g, 1.8 mmol) and methanesulfonyl chloride (0.83 g, 0.56 mmol) to give **9b** as a white solid (0.4 g, 60%) after chromatography on alumina eluted with 60% EtOHc/petroleum ether: m.p. 77–80°C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.35 (s, 5H), 4.68 (s, 2H, benzyl), 4.39–4.25 (m, 4H), 3.95 (m, 1H, CH), 3.01 (s, 6H, CH_3). i.r. (KBr) 3020, 2920, 1354, 1176, 1000, 963, 820, 740, 690 cm^{-1}.

*1-(2'-Nitro-1'-imidazolyl)-2-O-benzyl-3-O-toluenesulfonylpropanediol (**10a**)*

The procedure described above for **6a** was followed using **9a** (0.13 g, 0.3 mmol), 2-nitroimidazole (0.03 g, 0.2 mmol) and cesium carbonate (0.06 g, 0.2 mmol) to provide **10a** as an oil (0.025 g, 30%) after chromatography on alumina eluted with 70% EtOAc/petroleum ether. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.73 (d, 4H, $J = 8$), 7.31 (m, 5H), 7.17 (m, 2H), 4.48 (s, 2H, benzyl), 4.03 (m, 4H), 3.79 (quintet, 1H, $J = 5$), 2.45 (s, 3H, CH_3). i.r. (neat) 2921, 1655, 1488, 1449, 1358, 1168, 1122, 1089, 1004, 815, 664 cm^{-1}.

*1-(2'-Nitro-1'-imidazolyl)-2-O-benzyl-3-O-methanesulfonylpropanediol (**10b**)*

The procedure described above **6a** was followed using **9b** (0.19 g, 0.6 mmol), 2-nitroimidazole (0.06 g, 0.5 mmol) and cesium carbonate (0.06 g, 0.2 mmol) to provide **10b** as an oil (0.07 g, 40%) after chromatography on alumina eluted with 70% EtOAc/petroleum ether. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.29 (m, 5H), 7.14 (m, 1H), 7.09 (m, 1H), 4.71 (2 overlapping d, 2H, benzyl), 4.44–4.26 (2 overlapping m, 4H), 4.03–3.96 (2 overlapping m, 1H, CH), 3.05 (s, 3H, CH_3). i.r. (neat) 3030, 2934, 1650, 1540, 1482, 1358, 1168, 1044, 965, 834, 743, 697 cm^{-1}.

[^{18}F]3-Fluoro-1-(2′-nitro-1′-imidazolyl)-2-propanol ([^{18}F]fluoromisonidazole)

The [^{18}F]fluoride obtained by proton bombardment of [^{18}O]water (Tewson *et al.*, 1988) was added to 1.8 mg of potassium carbonate and 13 mg of Kryptofix 222. Excess water was removed *in vacuo* at 110°C. The reaction mixture was dried further by azeotropic distillation with 4 mL of anhydrous acetonitrile under a helium stream. A solution of dry acetonitrile (2 mL) containing 5 mg of tosylate precursor **6a** was introduced into the reaction vessel and refluxed at 100°C for 10 min. While the temperature was dropped to 50°C the reaction mixture was concentrated to half the original volume using a He gas stream. Ether (2 mL) was added and the mixture was loaded onto a silica SepPak (Waters, Millipore) and eluted with an additional 4 mL of ether to remove Kryptofix (Chaly *et al.*, 1990). The solvent was then evaporated and the residue was hydrolyzed in 2 mL of 1 N hydrochloric acid with heating at 100°C for 3 min. The reaction mixture was cooled to 35°C, neutralized with 1 mL of 2 N sodium hydroxide and buffered by 1 mL of 1 N sodium bicarbonate. The mixture was passed through a short alumina column (0.3 × 2 cm), a C18 SepPak (Waters, Millipore) and a 0.22 μm sterile and sterilizing filter followed by 4 mL of 10% ethanol/sterile water. [^{18}F]Fluoromisonidazole was thus obtained as a sterile and isotonic solution in 5% of ethanol [radiochem. yield, uncorr. 40–65% at EOS, 45–50 min EOB, chem yield (from $^{18}F^-$) 55–80%, specific activity lower limit 600 Ci/mmol]. Quality control was performed on reverse phase HPLC with radiation with u.v. (320 and 254 nm) detection [RT (min): **1**, 2.6; hydrolyzed **6a**, 1-(2′-nitro-1′-imidazolyl)-2,3-propanediol, 5.5; **6a**, 5.8] and by TLC (EtOAc, $R_f = 0.65$–0.7). The radiolabeled product co-eluted with authentic fluoromisonidazole on both systems. No radiochemical impurities were observed by HPLC or TLC, and no u.v.-absorbing products, including fluoromisonidazole, were detected.

Results and Discussion

Choice of precursor

An ideal method for preparing [^{18}F]fluoromisonidazole would use a precursor which, in a single rapid step, incorporated ^{18}F directly into the product structure. An early report by Jerabek *et al.* (1986) used such a one-step labeling strategy. 1-(2′,3′-Epoxypropyl)-2-nitroimidazole was used as labeling precursor and consistently provided <1% radiochemical yield of the desired product. This poor result has been cited as an argument against the general one-step labeling approach which we have used. Two recent papers (Agrawal *et al.*, 1979; Suto *et al.*, 1991) in which similar glycidyl-2-nitroimidazole derivatives were used in base-catalyzed substitutions, reported that imidazol[2,1-b]oxazole derivatives were isolated as side products, and that in aprotic solvents the oxazole derivatives became the major products. These compounds resulted from attack of an alkoxide, formed from the epoxide ring opening, on the imidazole ring to displace the nitro group. Also in the report of Jerabek *et al.* (1986) was the preparation of [^{18}F]1-(2′-fluoroethyl)-2-nitroimidazole in 23% radiochemical yield from the corresponding triflate. We concluded that one-step labeling of fluoromisonidazole in the presence of 2-nitroimidazole could be successful with a good leaving group, but that the 2-hydroxyl group must be protected to prevent undesired intramolecular reactions. It was then necessary to prepare a precursor which meets the above criteria. The functionality of the desired precursor is equivalent to that of glycerol. Initially, we attempted to prepare the cyclic sulfate of the readily available 1-(2′-nitro-3′-imidazolyl)-2,3-propanediol as the labeling precursor. All attempts to prepare the sulfate directly or via the corresponding cyclic sulfite (Berridge *et al.*, 1990; Gao and Sharpless, 1988) failed due to the high reactivity of the sulfate. It was therefore necessary to prepare a precursor using glycerol as the starting material.

Precursor synthesis

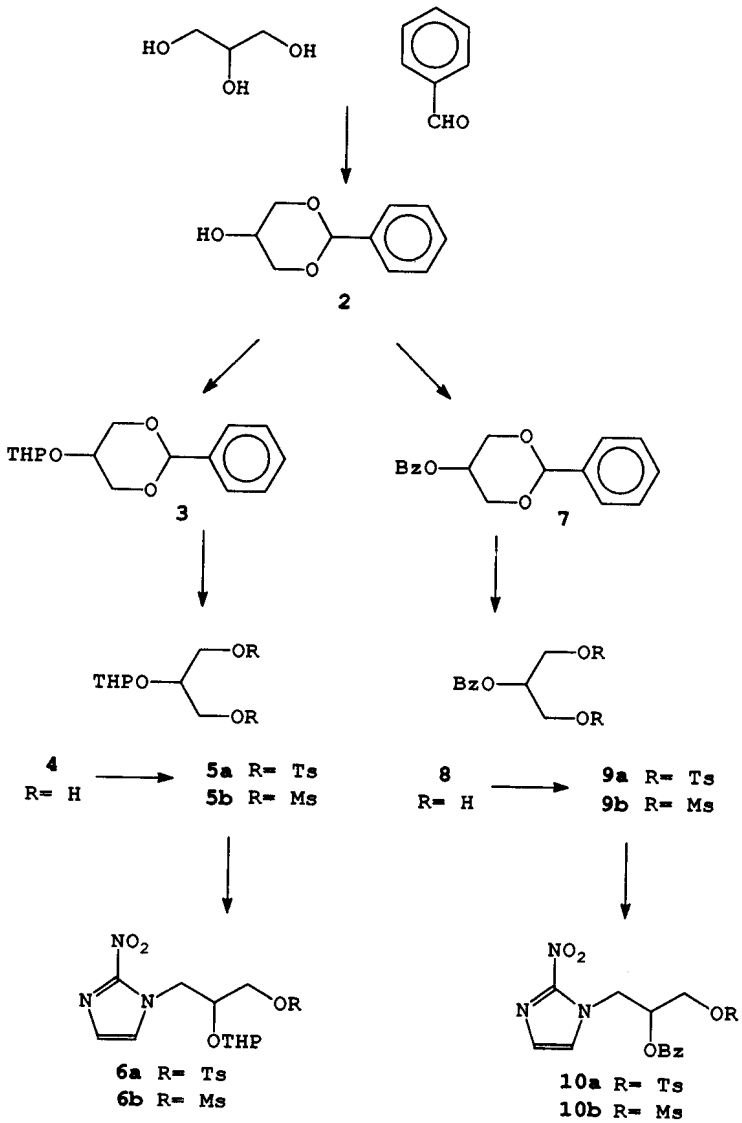
The synthetic problem in preparing the precursor from glycerol is that one of the two primary hydroxyl groups of glycerol must be replaced by the nucleophilic imidazole, the other must be converted to an effective leaving group for fluorine-18 labeling, while the secondary hydroxyl needs to be protected throughout the process and then readily deprotected after [^{18}F] incorporation. Two different hydroxyl protecting groups were used for the two types of hydroxyls in the glycerol. Benzylidene **2** was obtained in 25% yield by condensation of glycerol and benzaldehyde. The reaction gave a low yield, and was not driven to completion to prevent the formation of side products. Nevertheless, the starting materials were sufficiently available and inexpensive that **2** was easily prepared in 100-g quantities. Treatment of **2** with dihydropyran in the presence of pyridinium *p*-toluenesulfonate gave **3** in 80% yield. Acid catalysts which are more typically used for alcohol protection were not successful due to cleavage of the benzylidene on workup, while base catalysis is not effective for the reaction. The use of the weaker acid pyridinium *p*-toluenesulfonate avoided this problem. Alternatively, the secondary hydroxyl group was protected with a benzyl ether by reaction of **2** with benzyl chloride to give **7** in 62% yield. The added stability of this group allowed exploration of the subsequent chemistry with relative freedom. However, the added lability of the tetrahydropyranyl group is required for a production method since hydrogenolysis of the benzyl group is not practical for hot cell use.

Selective cleavage of the benzylidene group in the presence of the tetrahydropyranyl ether was easily accomplished with sodium in liquid ammonia to

provide the diol **4** in 75% yield. This diol was converted to the ditoluenesulfonate **5a** in 90% yield, which in turn gave the desired labeling precursor **6a** by displacement with 2-nitroimidazole in 45% yield after purification. The precursor decomposed slowly at room temperature and in solution so it was dried and stored under inert atmosphere at 0°C, under which conditions it was stable for several months. An alternate approach, which was also successful, was to prepare the monotoluenesulfonate of **4** by maintaining **4** in excess during the tosylation. This tosylate was then condensed with 2-nitroimidazole and the methane- or toluenesulfonate of the resulting alcohol produced in the usual fashion. This method potentially allowed greater purity and better product identification and would avoid potential side reactions of the ditosylate **5a**, but did not give enough practical advantage to justify the extra effort. Overall, THP-tosylate precursor **6a** was prepared in five steps with 5% yield from readily available and inexpensive materials. The synthetic route (Scheme 1) is straightforward, however, each intermediate requires purification either by crystallization or column chromatography. It is worth noting that the majority of yield loss is in the first step which involves inexpensive reagents, and that the only limiting reagent with regard to availability and expense, 2-nitroimidazole, is not introduced until the final step.

Radiolabeling

Radiolabeling by S_N2 displacement with [^{18}F]fluoride ion of the four sulfonate precursors (**6a** and **b, 10a** and **b**) used essentially the same procedure as a commonly used method for no-carrier-added labeling of [^{18}F]2-deoxy-2-fluoroglucose FDG (Hamacher *et al.*, 1986). Table 1 summarizes our



Scheme 1. Preparation of precursors for synthesis of [^{18}F]fluoromisonidazole. Ts, toluenesulfonyl; Ms, methanesulfonyl.

Table 1. Reaction yields as a function of reaction parameters

Precursor Cmpd (mg)	Reflux time (min)	Temperature (°C)	Radiochemical yield
5a (2)	5	100	10 (1)
5a (2)	10	100	14 (1)
5a (2)	15	100	26 (1)
5a (5)	5	100	36 (1)
5a (5)	10	100	43 (1)
5a (5)	15	100	57 (1)
5a (5)	10	80	65 ± 4 (4)
5a (5)	10	100	71 ± 3 (4)
***5a** (5)	10	100	44 ± 1 (4)
***5a** (10)	10	100	50 ± 1 (2)
5b (5)	10	100	23 ± 11 (3)
6a (10)	10	100	82 ± 2 (4)
6b (10)	10	100	84 ± 3 (3)

The precursor used is designated as compound number (mg used). Radiochemical yields are expressed as percent ± standard deviation (number of observations) at EOS based on labeled fluoride, uncorrected for decay. Yields of fluoromisonidazole are given except for **6a** and **b**, where the hydrolysis of the benzyl ether was not performed.

*These experiments were performed using the entire amount of fluoride produced by the cyclotron target. All other experiments were performed using 1–40 mCi of fluoride withdrawn from target batches of 200–700 mCi.

fluoromisonidazole labeling results as a function of the reaction parameters. The reaction proceeded in 55–80% chemical yield except in the case of the THP-protected mesylate **6b** (25%). Higher yields (70–80%) were obtained in syntheses which used portions of the fluoride from a cyclotron run, as opposed to the entire batch of fluoride. The low incorporation observed with **6b** may be due to its instability, or higher reactivity with trace impurities. This may also explain the low yields obtained in the preparation of **6b** (15%), and the THP-protected dimesylate **5b** (15%), as compared to the corresponding tosylate derivatives (45 and 90%, respectively). Because of the low labeling yield obtained using methanesulfonate as a leaving group, we did not attempt to optimize any of these syntheses.

The radiolabeling yield was influenced by three factors (Table 1). Increasing the amount of precursor from 5 to 10 mg improved the fluorination yield by 6% when entire fluoride batch runs were used. When the reaction was performed using portions of a cyclotron run (1–40 mCi from 200–700 mCi, 2 vs 5 mg of precursor) the yield improvement ranged from 26 to 31%. There was apparently a stoichiometric competition with impurities in the reaction solution for fluoride ion, such as has been observed previously (Tewson *et al.*, 1988) for the labeling of FDG. Temperature and reaction time also affected the yields, giving small improvements with increases from 80 to 100°C and 10 to 15 min. We considered 5 mg of precursor and a 10 min reaction at 100°C to be the optimum compromise between consumption of precursor and production of useful amounts of product. The typical product yields obtained in production for routine use were near 50% (uncorrected). Injectable [^{18}F]fluoromisonidazole in the range 100–300 mCi was obtained from 210–620 mCi of $^{18}F^-$ (300 mCi EOS, 50 min, from bombardment of 15 μA · 45 min, 620 mCi $^{18}F^-$ EOB).

Hydrolysis

Hydrolysis of the THP protecting group after labeling of **6a** proceeded rapidly with quantitative yield. It was important to avoid high concentrations of aqueous acid or extended reflux times. A 10 min reaction in 1 N hydrochloric acid produced 20% of an un-characterized labeled polar byproduct. This product may arise from the reaction of [^{18}F]fluoromisonidazole with excess of tosylate **6a** or its hydrolysed products. An acid concentration of 0.1 N is sufficient to cleave the protecting group, though we used a 1 N solution with a short reaction time (3–5 min) in order to generate a slightly hypertonic solution for injection at the end of the process.

By contrast, the benzyl protecting group is relatively inert in aqueous acid. As expected, even with 5 N hydrochloric acid only partial hydrolysis (20%) occurred. At higher acid concentration substantial decomposition took place. Precursors **10a** and **b** are therefore not serious candidates for the preparation of [^{18}F]fluoromisonidazole. However, their chemistry was originally better characterized than the THP-protected intermediates, and their stability afforded us an opportunity to test the labeling approach with a minimum of complications. The purification of the product was accomplished without chromatography by using small silica, alumina and C-18 cartridges. A silica SepPak was used to remove Kryptofix (Chaly *et al.*, 1990) and any other polar materials before the hydrolysis. Following hydrolysis the C-18 cartridge was used to remove possible unhydrolyzed material and an alumina cartridge was used to remove fluoride ion and also removed 1-(2′-nitro-1′-imidazolyl)-2,3-propanediol, the hydrolysis product of the unreacted precursor. The final product contained no detectable radiochemical impurities. The u.v. chromatograms of the product mixture contained only four very small (< 1 nmol total) peaks which were found to be normal components of the USP sterile isotonic saline used to formulate the product. The interference of the saline in the chromatogram limited the detection sensitivity for fluoromisonidazole, resulting in the sp. act. lower limit of 600 Ci/mmol.

Conclusion

We have labeled fluoromisonidazole via one step fluorination of an appropriately protected precursor **6a**, followed by rapid removal of the protecting group. After synthesis and purification, identity and purity were assayed by HPLC and TLC and no radiochemical or chemical impurities were detected. In routine PET use, up to 300 mCi of [^{18}F]fluoromisonidazole has been prepared within 45–50 min.

The operational similarity of our approach for the production of labeled fluoromisonidazole to the commonly used preparation of [^{18}F]FDG provides several advantages in addition to the obvious improvements in chemistry. This preparation can be carried out by any existing apparatus that produces [^{18}F]FDG using the triflate precursor of Hamacher *et al.* (1986). No

additional expertise, equipment, or HPLC is needed. Moreover, the radiochemical reaction is reproducible and in our hands has been more reliable than that of FDG. No labeling failure has yet been observed.

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