A Radiosynthesis of Fluorine-18 Fluoromisonidazole

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A new preparation of [^{18}F]fluoromisonidazole [1H-1-(3-[^{18}F]fluoro-2-hydroxypropyl)-2-nitroimidazole] is presented as a two-step, two-pot reaction sequence. The method is useful for the production of 20–30 mCi quantities of this compound from [^{18}F]fluoride, available in 40% radiochemical yield at end of bombardment (EOB) with a specific activity (nca) of > 650 Ci/mmol (EOB) and a synthesis time of ~140 min. The key feature of the reaction scheme is the preparation of a new fluoroalkylating agent, [^{18}F]epifluorohydrin.

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Misonidazole and its congeners are metabolically trapped in cells that are alive but at low oxygen tension (*1*). As an example of this biologic property, studies with tritiated fluoromisonidazole (F-MISO) have shown accumulation of this drug in hypoxic tissues of tumors, stroke, and ischemic myocardium (*2–5*). This has led to the suggestion that the fluorine-18- (^{18}F) labeled compound might be useful for in vivo positron emission tomography (PET) imaging of these tissues. Although the radiosynthesis of [^{18}F]F-MISO and its biodistribution in rats has been reported (*6*), the yield by this procedure is too low (<1% EOS) for widespread application. In pursuit of a method which affords a higher radiochemical yield of this compound we have investigated the [^{18}F]fluoride displacement of (2 *R*)-(-)glycidyl tosylate (GOTS) to prepare [^{18}F]epifluorohydrin (EPI-F) and subsequently the nucleophilic ring opening of the latter compound with 2-nitroimidazole (2-NIM) to afford [^{18}F]F-MISO (*7*) (Fig. 1). This paper describes a two-pot synthetic preparation of ^{18}F-labeled F-MISO as well as a description of some exploratory reactions performed in the development of this radiosynthesis.

MATERIALS AND METHODS

Potassium fluoride dihydrate, tetrabutylammonium fluoride (TBAF) trihydrate, anhydrous potassium carbonate, 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane [Kryptofix(2.2.2)], (2 *R*)-(-)-glycidyl tosylate, 2-NIM, EPI-F, 1,8-bis-(dimethylamino)naphthalene (Proton Sponge), acetonitrile (CH_3CN), dimethylsulfoxide (DMSO), and dimethylformamide (DMF) were obtained commercially (Aldrich Chemical Co., Milwaukee, WI). F-MISO was obtained from the National Cancer Institute (NSC# 292930) and was purified by recrystallization from ethanol. Proton sponge was purified by repeated recrystallization from ethanol to afford a white crystalline material, mp 48–49°C, and was stored in the dark. EPI-F was purified by distillation and stored at −10°C. GOTS was purified by partitioning between saturated aqueous $NaHCO_3$ and diethyl ether, followed by isolation from the ether solution and recrystallization from that solvent to afford a material which exhibited mp 46–48°C. This material was stored at −10°C. All other reagents and solvents were used as received.

Gas liquid chromatography (GLC) was performed using a 6 ft. × 1/8 in. o.d. stainless steel column packed with 80–100 mesh HayeSep-Q (Alltech Associates, Deerfield, IL) operated at 200°C and a helium carrier gas flow of 30 ml/min. Thermal conductivity and NaI(Tl)-scintillation detectors were used to monitor the column effluent. Using this column EPI-F could be cleanly separated (R_t EPI-F 2 min, k′ 9.0) from solutions with both acetonitrile and dimethylformamide.

Solutions of [^{18}F]EPI-F of low specific activity (20 μmol carrier) were routinely analyzed by GLC, high performance liquid chromatography (HPLC), and thin layer chromatography (TLC). Only GLC was unambiguous as a method for determining the chemical identity of EPI-F. Once correlated to GLC, HPLC served to evaluate the conversion of EPI-F into F-MISO in trial reactions. TLC was useful only to the extent that the absence of fluoride in preparations of EPI-F could be established. Using this method it was observed that labeled EPI-F could not be visualized due to a co-evaporation

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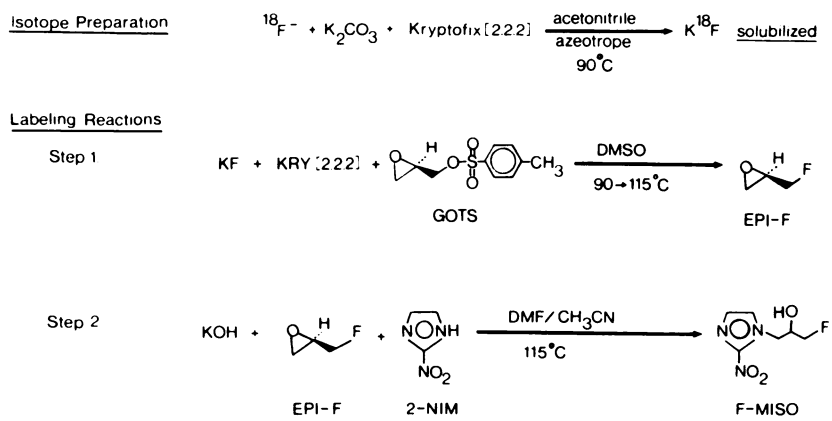

FIGURE 1
Isotope preparation and labeling scheme for the [^{18}F]F-MISO synthesis.

of the compound from the plate with the development solvents. This fact had originally led to the overestimation of TLC based radiochemical yields of labeled F-MISO.

Thin layer chromatography was performed with silica on aluminum backed plates (Merck, Art. 5534, Darmstadt, West Germany). To facilitate the localization of labeled F-MISO on developed TLC plates, samples of the unlabeled compound were co-spotted on the plates prior to their development. In each case, the compound was visualized by exposure of the developed plate to ultraviolet light. The distribution of radioactivity on the plate was visualized by exposure of high speed instant film (Polaroid, Type 57, 9 × 12 cm format, Cambridge, MA) to the plate placed directly on the film package, followed by processing. Alternately, the zone on the plate visualized as unlabeled F-MISO was excised and the silica was extracted with ethanol. The extract was concentrated and analyzed by HPLC (vide infra). The radioactivity eluted from the HPLC column corresponded to the elution of the unlabeled standard, demonstrating that the activity on the TLC plate with an R_f of unlabeled standard was labeled F-MISO.

The isolation of labeled F-MISO from crude reaction mixtures was performed using a semipreparative HPLC column (Whatman, P/10, ODS-3, M9/25, (25 cm × 9 mm id), Clifton, NJ) whereas the radiochemical purity and specific activity of the purified product was determined using an analytic HPLC column (Beckman-Altex Scientific, Ultrasphere ODS, 5μm, (25 cm × 4.6 mm id), Berkeley, CA). HPLC columns were eluted with 5% ethanol in water and postcolumn detection was performed with ultraviolet absorbance (254 mm) and NaI(Tl)-scintillation detectors in series.

Specific activity values were calculated by comparing the mass response per unit volume of solution (uv absorbance) with the quantity of activity per unit volume, decay corrected to the end of bombardment (EOB). For a 20-μl injection a mass detection threshold (S/N = 4.5) of 1 nmol/ml of F-MISO was determined. For each determination of a volumetric solution containing both 2-NIM (2.6 mg/l) and F-MISO (2.5 mg/l) was used as a (uv) calibration standard. Further, the identity of the labeled compound was established by co-injection of the product solution with the standard solution.

Melting points were determined by the capillary method and are uncorrected. ^{1}H, ^{19}F, and ^{13}C (proton decoupled) NMR spectra were recorded at 300, 282 and 75 MHz, respectively, using a Varian VXR-300 multinuclear spectrometer. Resonances are reported in ppm relative to tetramethylsilane for ^{1}H and ^{13}C spectra and $CFCl_3$ for ^{19}F spectra. Fluorobenzene (-113.1ppm) was used as an internal standard for ^{19}F NMR and resonances expressed as negative values were observed upfield relative to $CFCl_3$.

Preparation of Analytic Standards

Samples of both EPI-F and F-MISO were obtained from reactions conducted with reagents and conditions resembling those for their radiosynthesis.

Preparation of Epifluorohydrin

A solution of GOTS (250 mg, 1.1 mmol) and TBAF trihydrate (852 mg, 2.7 mmol) in DMSO (2.5 ml) was heated at 80°C and purged with 100 ml/min of argon. For 30 min, the effluent volatile materials were passed, via teflon tubing, into 2 ml of cold (-78°C) deuteriochloroform. The chloroform mixture was isolated, warmed to room temperature, and passed through anhydrous Na_2SO_4. Spectroscopic examination (^{1}H, ^{13}C and ^{19}F NMR) of this solution revealed that the product was identical to commercially available EPI-F. Aside from traces of DMSO and water, EPI-F was isolated uncontaminated. With the addition of 1,4-dioxane (14 mg), as an integration standard, it was estimated that the yield of EPI-F was 56%. NMR: ^{1}H ($CDCl_3$) δ: 2.64 (m,1H), 2.83 (m, 1H), 3.23 (m, 1H), 4.26 (dddd, J = 45.7 Hz, J = 10.8 Hz, J = 5.9 Hz, J = 1.1 Hz, 1H $-CH_2F$), 4.63 (dddd, J = 45.7 Hz, J = 10.7 Hz, J = 2.4 Hz, J = 1.1 Hz, 1H, $-CH_2F$); ^{13}C ($CDCl_3$) δ: 50.37 (d, J = 23.7 Hz, $-CH_2F$), 82.95 and 85.20 (epoxide carbons); ^{19}F ($CDCl_3$) δ: —224.0 (td, J = 47 Hz, J = 11 Hz).

Preparation of Fluoromisonidazole

A mixture of 2-NIM (250 mg, 2.2 mmol), proton sponge (237 mg, 1.1 mmol), EPI-F (0.315 ml, 4.4 mmol) and DMSO (1 ml) was stirred and heated at 80°C for 1 hr in a sealed 2-ml vial. As the reaction proceeded the mixture gradually deepened in color. The mixture was isolated and the volatile materials were removed by air-bath distillation (80–90°C, 0.1mm). The colorless distillate was discarded and the stillpot residue (~0.5 ml) was rapidly chromatographed over silica (230–400 mesh, 3 × 15 cm column) eluting with 20% CH_3CN/$CHCl_3$ for the first 200 ml followed by 40% CH_3CN/$CHCl_3$ for the remainder of the elution. The eluted fractions were examined by TLC and selected fractions were combined and concentrated to a solid residue. This material was dried at room temperature under vacuum for 0.5 hr to afford 0.271 g (71%) of material, mp 133–140°C. Two recrystallizations of the product from ethanol raised the melting point to 139–141°C (lit (*8*) mp 139.5–140°C). The ^{1}H, ^{19}F, and ^{13}C NMR

spectra of this material were identical to those of an authentic sample obtained from the National Cancer Institutes. Anal. calcd. for $C_6H_8FN_3O_3$: C(38.09), H(4.26), N(22.01), F(10.41). Found: C(37.93), H(4.21), N(22.08), F(10.11). NMR: ^1H (DMSO) δ: 4.00–4.10 (m, 1H, $-C\underline{H}$(OH)-), 4.32–4.46 (m, 2H), 4.48–4.58 (m, 1H), 4.65 (dd, J = 13.7 Hz, J = 3.6 Hz, 1H), 5.64 (d, J = 7.8 Hz, 1H, $-C\underline{H}$(OH)-), 7.22 (s, imid-H), 7.64 (s, imid-H); ^{13}C (DMSO) δ: 51.41 (d, J = 7.6 Hz, imid-$\underline{C}H_2CH$(OH)-), 67.70 (d, J = 19.3 Hz, $-\underline{C}H$(OH)-), 84.58 (d, J = 169.1 Hz, -$\underline{C}H_2$-F), 127.35 (s, imid-C(4 or 5)), 128.52 (s, imid-C(5 or 4)) 144.91 (s, imid-C(2)); ^{19}F (DMSO) δ: -230.7 (td, J = 46 Hz, J = 19 Hz).

Fluorine-18 Fluoride Ion Production

Target irradiations were done using the University of Washington cyclotron (Scanditronix AB, MC 50 cyclotron, Uppsala, Sweden) and [^{18}F]fluoride was obtained from enriched [^{18}O]water via the $^{18}O(p,n)^{18}F$ reaction. The target (*9*) consists of a front 1.0 cm aluminum window (He cooled), a titanium insert between 1.1 mil Ti foils which forms the target chamber, and a rear water-cooled section. The thick window is used to degrade the 50.5-MeV proton beam to 12.2 ± 2.2 MeV. A typical 30-min irradiation using 2.8 ml of 20% enriched water with 22 μA on target products 110 mCi of ^{18}F at EOB, with 264 mCi of ^{13}N and 2 μCi of ^{48}V as contaminants. A practical limit to beam current on the thick Al window is 22 μA. The use of higher currents result in lower ^{18}F yields, presumably a result of heating and target thinning. Activity was transferred to the radiochemistry laboratory under vacuum through 130 ft. of 1/16 in. o.d. (0.5 mm id) teflon tubing.

Preparation of Labeled Fluoromisonidazole

In a typical experiment a 10 ml Vacutainer (Becton Dickson, Plain (z), BD#6530, Rutherford, NJ) was charged with ^{18}O-enriched water containing the [^{18}F]fluoride activity (1.5 ml), anhydrous K_2CO_3 (4.1 mg, 30 μmol), Kryptofix(2.2.2) (22.5 mg, 60 μmol), KF dihydrate (1.8 mg, 20 μmol) and acetonitrile (1.5 ml). This mixture was concentrated at 90°C while directing a rapid stream of argon across the surface of the solution. The residue (100 μl) was taken up into a 1.5 ml portion of acetonitrile and concentrated as before. This process was repeated (×5) and the final residue was taken up into 200 μl of DMSO at 90°C for 2–3 min. The mixture was transferred under forced siphon, through teflon tubing, to the reaction vessel containing the tosylate compound (40 mg, 175 μmol). The Vacutainer was rinsed with an additional 100 μl of DMSO followed by transfer of the rinse to the reaction vessel.

With the vessel held in a 90°C oil bath a stream of argon was bubbled (20 ml/min) through the DMSO solution of the tosylate. After 2 min, 100 μl of acetonitrile was added to the mixture and the temperature was raised to 115°C. Thereafter, two additional 100-μl portions of acetonitrile were added at 5-min intervals. All materials exiting the reaction vessel were directed, through teflon tubing, into 200 μl of cold (0°C) DMF. After a total of 15 min, a warm solution of 2-NIM (40 mg, 350 μmol) and aqueous KOH (10 μl, 14.8 *M*, 150 μmol) in DMF (200 μl) was added to the DMF trapping solution. The vial containing the resultant solution and a teflon coated stirring bar was sealed and the mixture was rapidly stirred and heated at 115°C for 15 min.

The cooled reaction mixture was taken up into 1.5 ml of water and 70 μl of glacial acetic acid was added to precipitate excess 2-NIM. The mixture was diluted with 20 ml of water and then passed through a 0.8-μm membrane filter (Millipore, Millex-PF (SLAAV255F), Bedford, MA) followed by two C-18 SEP-PAKs (Millipore-Waters, Milford, MA). The unit was rinsed with 5 ml of water. The SEP-PAKs strongly retained a brown material while a faintly yellow solution was obtained for the combined filtrate. The solution was concentrated at 70°C under reduced pressure to an orange residue which was taken up into 1.5 ml of warm water and passed through a 0.8-μm membrane filter. The filter was rinsed with an additional 0.5 ml of water and the combined filtrates were applied to a semipreparative HPLC column at a rate of 2 ml/min for the first minute, followed by elution at 6 ml/min with 5% aqueous ethanol. A fraction of the eluate containing the labeled F-MISO was collected after 6-7 min in 8-14 ml. The isolated compound was found to be radiochemically pure and the only observable chemical contaminant was a small amount of 2-NIM (<1.5 μmol). The concentration of this contaminant depends upon the efficiency of the separation.

An identical procedure to that described above was used for the no-intentionally-carrier-added preparations, the only exceptions being the elimination of KF as a source of carrier and increased amounts of 2-NIM and concentrated aqueous KOH. For one experiment, a 50% excess of base (KOH) was used over that for the carrier-added procedure, while for another a 100% excess of base and a 50% excess of 2-NIM were used (Table 1, Exp B and C)

A mock-carrier-added radiolabeling experiment was performed using isotopically stable fluoride (20 μmol) and d^3-acetonitrile, for the co-distillation of EPI-F. Spectroscopic examination of the isolated distillate by ^{19}F NMR revealed that EPI-F was the exclusive fluorine containing species (δ: -70 to -300 ppm). Further, the ^{1}H spectrum revealed that EPI-F, DMSO and water were all present in trace amounts, with a notable absence of KRY(2.2.2) (δ: 3.60 ppm, $-OC\underline{H}_2C\underline{H}_2O-$) and species containing the tosyl functionality. In fact, it was determined that at a detection threshold of 60 nmol no KRY(2.2.2) was present.

RESULTS AND DISCUSSION

Based on a report (*8*) for the synthesis of F-MISO from EPI-F, we have labeled the latter compound with ^{18}F by the reaction of glycidyl tosylate (GOTS) and [^{18}F] fluoride, with the aim of finding suitable reaction conditions for the transformation of [^{18}F]EPI-F into [^{18}F] F-MISO. From a synthetic point of view, the preparation of EPI-F from GOTS is a reasonable choice realizing that the reaction of a nucleophilic source of fluoride ion at either C-1 or C-3 of this ambident electrophile would be expected to afford the same product, EPI-F, although with a difference in absolute stereochemistry. Further, reaction of nucleophiles at the internal epoxide carbon atom (C-2) in structurally related compounds such as epichlorohydrin is not generally observed (*10–12*).

Several trial experiments with unlabeled materials were carried out to establish a rapid overall synthesis of

TABLE 1
Exploratory Reaction Conditions for the F-MISO Synthesis in DMSO

	Reagents* (equivalents)†						Conditions	Products (% total)†
1.	GOTS	+	TBAF					EPI-F
	(2)	:	(1)				(a) r.t., 20 min	(5)
							(b) 80°C, 20 min	(95)
2.	GOTS	+	KF	+	KRY(2.2.2)			EPI-F
	(2)	:	(1)	:	(1)		(a) 80°C, 20 min	(95)
3.	GOTS	+	KF					EPI-F
	(2)	:	(1)				(a) 80°C, 20 min	(0)
4.	EPI-F	+	2-NIM					F-MISO
	(1)	:	(2)				(a) 80°C, 60 min	(4)
5.	EPI-F	+	2-NIM	+	PS			F-MISO
(a)	(1)	:	(2)	:	(0.2)		(a) 80°C, 20 min	(9)
(b)	(1)	:	(2)	:	(0.2)		(b) 80°C, 60 min	(21)
(c)	(1)	:	(2)	:	(1)		(c) 80°C, 20 min	(19)
(d)	(1)	:	(2)	:	(2)		(d) 80°C, 20 min	(22)
6.	GOTS	+	TBAF					
	(2)	:	(1)				(a) 80°C, 20 min	
		then					then	F-MISO
	2-NIM	+	PS				(b) 80°C, 20 min	(27)
	(4)	:	(2)					

* F = Fluoride ion, GOTS = glycidyl tosylate, EPI-F = epifluorohydrin, 2-NIM = 2-nitroimidazole, KRY(2.2.2) = Kryptofix(2.2.2), TBAF = tetrabutylammonium fluoride, F-MISO = fluoromisonidazole, KF = potassium fluoride dihydrate, PS = proton sponge.

† One equivalent = 175 μmol, all reaction solutions used 300 μl of DMSO as the solvent regardless of reagent volume and all NMR samples were of entire reaction mixtures to which an additional 300 μl of DMSO was added prior to spectroscopic examination.

F-MISO which was compatible with the half-life of ^{18}F. Reaction mixtures were either stirred at room temperature or heated in sealed vials and then analyzed by ^{19}F NMR. The results of those experiments are summarized in Table 1. Entries 1-3 relate to the synthesis of EPI-F from fluoride; entries 4-6 relate to the further conversion of EPI-F into F-MISO. Comparison of the first set of entries reveals that KF alone, or in combination with a base, is a poor source of nucleophilic fluoride, while either TBAF or the combination of KF/KRY(2.2.2) may be used to rapidly and efficiently prepare EPI-F. Comparison of entries 4–6 reveals that the reaction between EPI-F and 2-NIM alone is sluggish compared to the half-life of ^{18}F and that this reaction may be considerably accelerated by base catalysis (cf Entries 4 and 5c,d). Coupling of the more favorable reaction conditions for the overall synthetic scheme may be used to obtain a useful yield of F-MISO in a relatively short overall time (Entry 6).

When solutions of various forms of fluoride ion were examined by ^{19}F NMR, nearly identical chemical shifts were observed for fluoride ion as TBAF (-102.5 ppm) or KF in the presence of 1–3 equivalents of KRY(2.2.2) (-102.8 ppm) in DMSO. These shifts were observed considerably downfield of KF in 1:2 water/DMSO (-125.3 ppm) or DMSO saturated with KF dihydrate (-116.2 ppm). Given that TBAF and KF/KRY(2.2.2) are effective sources of nucleophilic fluoride and that TBAF is essentially a source of naked fluoride ion then perhaps the degree of fluoride ion hydration, and hence its nucleophilicity, may be gauged spectroscopically. The complex KF/KRY(2.2.2) has previously been described as a source of naked fluoride and used effectively for the preparations of labeled fluorodeoxyglucose and fluorinated long chain fatty acids (*13*).

Examination of reaction mixtures including carrier-added [^{18}F]-EPI-F in trial experiments revealed a smooth conversion of [^{18}F]-EPI-F to [^{18}F]F-MISO. This was the near exclusive reaction, unless the temperature was excessively high (>120°C). The course of these reactions were monitored by HPLC. Although yields uncorrected for recovered activity of injected material were obtained, the results of parallel reactions clearly indicated preferred reaction conditions. Proton Sponge (PS) was initially used as a source of a non-nucleophilic, organic soluble, strong base (pKa 12.5). This was intended to provide a large concentration of the 2-nitroimidazole anion to increase the rate of reaction between 2-NIM and EPI-F. Ultimately, the substitution of other bases for PS in this reaction revealed that K_2CO_3 and KOH were more effective as catalysts. Reaction mixtures in DMSO held at 80°C for 20 min afforded 4% and 13% of F-MISO using PS and KOH, respectively. Using a temperature of 110°C for the same period raised these same values to 13% and 73%. Further, K_2CO_3 was found to be equally effective as KOH

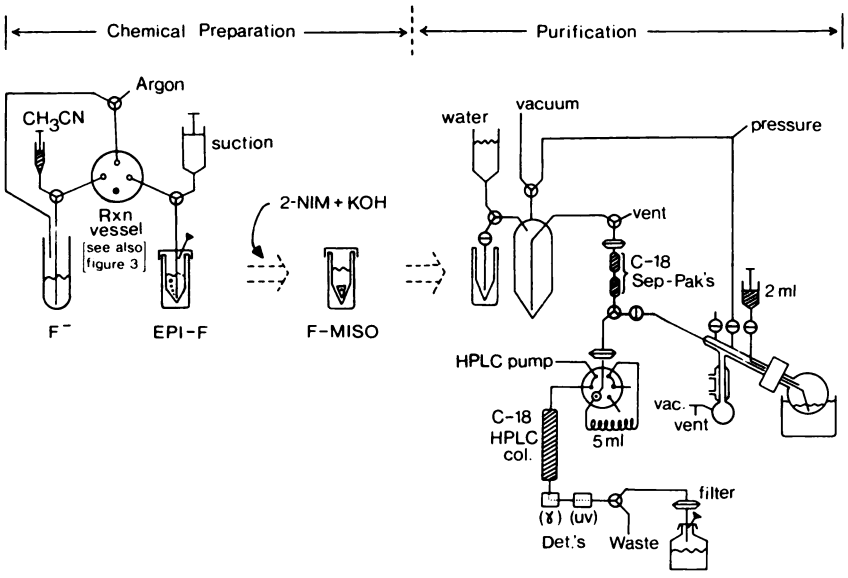

FIGURE 2
Apparatus for the synthesis of [^{18}F] EPI-F and [^{18}F]F-MISO.

at 110°C for 20 min. The use of DMF in place of DMSO as a solvent afforded a 78% yield of F-MISO. In overview, it was decided that DMSO was a suitable solvent for the preparation of EPI-F while DMF was most appropriate for the further transformation of EPI-F into F-MISO. The use of DMF had an additional benefit in that it could be readily removed from the crude product during workup.

Figure 1 illustrates the scheme used for the preparation of labeled F-MISO. Labeled fluoride is prepared for chemical reaction by azeotropic drying of the irradiated enriched water containing the activity, followed by reconstitution of the residue into the aprotic solvent DMSO. This is done in the presence of K_2CO_3 and KRY(2.2.2), with or without carrier (*13*). Labeling of F-MISO is conducted as a two-pot reaction sequence. In the first step, utility is made of the low boiling point of EPI-F (85°C) by removing the [^{18}F]EPI-F as it is generated from the [^{18}F]fluoride solution. This is accomplished by co-distillation with a comparatively large mass of acetonitrile (300 μl). As a result, the isolated EPI-F is obtained free of the bulk of the chemical and radiochemical materials present in the solution used for its preparation. In fact, preparation of epifluorohydrin on a macroscopic scale (authentic standard synthesis with stable fluoride) and [^{18}F]EPI-F (from carrier added [^{18}F]fluoride) both revealed that the reaction product between GOTS and fluoride was exclusively EPI-F (GLC, TLC, NMR-collectively). There was no indication of the presence of 1,3-difluoro-2-propanol or 3-fluoro-1,2-dihydroxypropane in the distillate, as a result of further reaction of EPI-F with either additional fluoride or water (epoxide hydrolysis), respectively. It was therefore assumed that the reaction of no carrier added [^{18}F]fluoride to give [^{18}F]EPI-F proceeded in a similar way as the carrier added reaction of [^{18}F]fluoride (20 μmol carrier). This assumption is reasonable based on the fact that yields of [^{18}F]EPI-F and F-MISO are not diminished at the no-carrier-added level versus the carrier added level (vide infra). This implies that the side reactions that would limit the yield of these compounds at the no-carrier-added level are not as important a factor as the use of lower specific activities. For the second step the EPI-F (an epoxide) is reacted with 2-NIM in the presence of KOH as a base catalyst in DMF/CH_3CN at 115°C (15 min) within a sealed vial. The use of DMF as a co-solvent for this reaction permitted the dissolution of a relatively large quantity of 2-NIM (40 to 60 mg) and, as well, was volatile enough to be readily removed before HPLC purification of the crude product.

The apparatus used in the overall procedure is illustrated in Figure 2. In the figure, the reaction vessel and the collection vial act as a micro-distillation apparatus which is used to prepare a small volume solution of [^{18}F]EPI-F. Once sealed, the vial served as the reaction vessel for the further conversion of EPI-F to F-MISO. It was a benefit to keep the head space of this vial small. Since the use of a larger vessel results in a reduced yield, presumably a result of less of the volatile EPI-F being in solution. The remainder of the system relates to the isolation of the [^{18}F]F-MISO from the crude reaction mixture. In short, the crude product is neutralized with acid then diluted with water to precipitate the bulk of the 2-NIM. The mixture was filtered, extracted with C-18 SEP-PAKs, and concentrated to a small volume for HPLC purification.

Conceivably, the entire synthetic scheme could be reduced to a one-pot procedure. However, the use of a two-pot procedure greatly simplifies the chromatographic isolation of [^{18}F]F-MISO from crude reaction mixtures. Since most of the organic and radiolabeled components that can complicate the chromatography, notably DMSO and [^{18}F]fluoride, are removed during

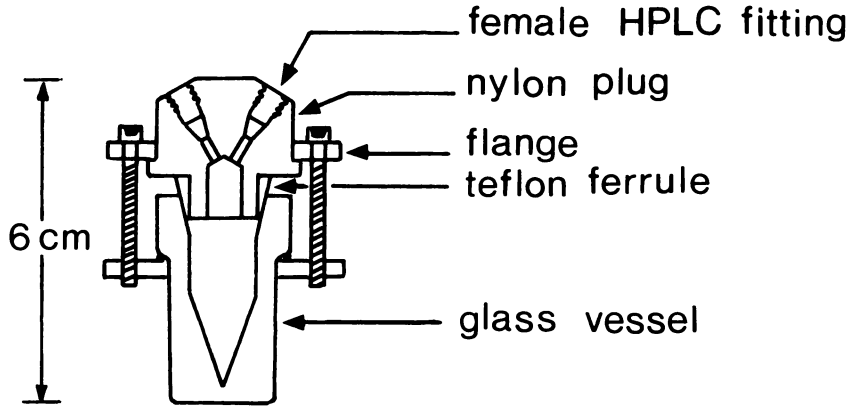

FIGURE 3
Reaction vessel used for the preparation of [^{18}F]F-MISO.

the distillation of [^{18}F]EPI-F. One added advantage to this approach is the flexibility of isolate radiochemically pure [^{18}F]EPI-F for use in alternate syntheses.

The reaction vessel and all other assemblies within the system are interconnected using 1/16-in. o.d. teflon tubing and fluid or gas paths are directed using inexpensive three-way slider valves (Rheodyne, Model 5302, Conti, CA). Transfers are accomplished either with the aid of pressure or suction. The reaction vessel is shown in detail in Figure 3. The assembly consists of a machined nylon cap-plug, a ½-in teflon front ferrule, a custom made 2-ml glass vial and a set of flanges secured by eight bolts. The cap-plug has four radially arranged, threaded openings for receiving standard fingertight HPLC (Kel-F) tube fittings (Alltech Associates) (or plugs) for the introduction or removal of materials via 1/16-in. o.d. teflon tubing. The inner glass surface on the vial mates of the ½-in. teflon ferrule and was formed

TABLE 2
Activity Distribution During the F-MISO Synthesis and Reaction Summary

Item	% Total activity (EOB)		
	A	B	C
1. Fluoride/KRY(2.2.2)/K_2CO_3	100	100	100
2. Evaporated activity	99	92	93
3. Nonsolubilized activity	10	2	6
4. Reaction activity	89	90	87
5. Trapped EPI-F	69	83	77
6. Depleted DMSO activity	14	4	4
	83	87	81
7. Crude F-MISO mixture	68	83	77
8. C-18 SEP PAKs and filter	3	10	14
9. Crude filtered product	25	61	48
10. HPLC isolated F-MISO	23	43	40
11. Sterile injectable solution	22	-	-
Summary*	A	B	C
Fluoride activity (EOB)	85.1 mCi	29.5 mCi	142.4 mCi
Carrier level	20 μmol	nca	nca
F-MISO activity (EOS)	8.0 mCi	4.3 mCi	24.9 mCi
Radiochemical yield (EOB)	23%	43%	40%
Preparation time	142 min	175 min	133 min
Specific activity (EOB)	3.9 Ci/mmol	90 Ci/mmol	670 Ci/mmol

*A = Typical carrier-added reaction (n=3): 40 mg 2-NIM/ 10 μl(150 μmol) KOHaq.
B = No-carrier-added reaction: 40 mg 2-NIM/ 15 μl KOHaq.
C = No-carrier-added reaction: 60 mg 2-NIM/ 20 μl KOHaq.

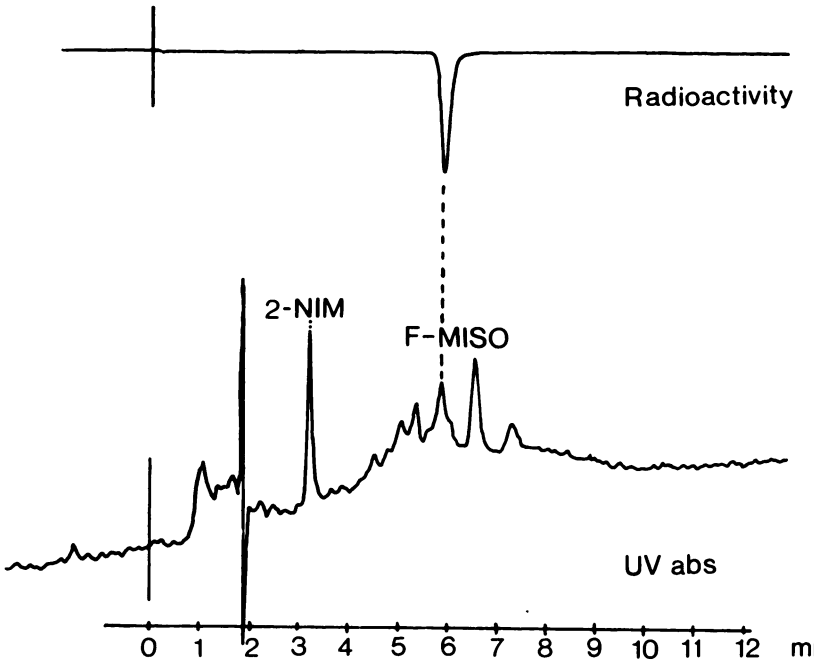

FIGURE 4
An analytical HPLC radiochromatogram of purified nca. [^{18}F]F-MISO obtained for the product isolated from Reaction C (Table 2, 10 μmol injection, AUFS = 0.005 @ 254 nm, F-MISO = 6 nmol/ml, specific activity = 670 Ci/mmol, 2-NIM = 20 nmol/ml).

using a ½-in. o.d. stainless steel ferrule as a mandrel during the glass blowing process. The glass body is desirable for conducting reactions sensitive to metal surfaces and the entire unit can be pressurized to >80 psi. For the present application the repeated use of this vessel with carrier added fluoride (20 μmol) has not resulted in a decrease in the specific activity of the isolated F-MISO. In fact, subsequent use of the vessel for a no-carrier-added preparation afforded a product with a specific activity of 670 Ci/mmol.

The distribution of activity during typical preparations of labeled F-MISO are presented in Table 2. Entry 1 is a normalized starting point for the procedure. Entries 2–4 represent the activity remaining as a residue after azeotropic distillation of the recovered target water, the activity remaining on the wall of the Vacutainer after the residue had been taken up into DMSO, and the activity available for EPI-F synthesis, respectively. Overall, within 80 min over 85% of the decay corrected activity could be isolated in a useful form for synthetic use. Entries 5 and 6 represent the activity of trapped EPI-F and the activity that could not be distilled from the reaction vessel, respectively. From several experiments independent analyses of the trapping solution revealed it to be free of fluoride (TLC) and the EPI-F was the only observable labeled compound (GLC). Entries 7–9 represent the activity in the crude reaction mixture containing the labeled F-MISO, the combined activity remaining on the spent filter and C-18 SEP-PAKs, and the activity in the processed product prior to HPLC purification, respectively. Entries 10 and 11 represent the activity of the final purified product isolated as a solution in 5% aqueous ethanol or alternately as a sterile and isotonic solution, respectively. For the carrier added reaction (column A) most of the losses of activity occur during the concentration of the SEP-PAK extracted aqueous solution of the crude product (compare Entries 7 and 9). It is during this stage that unreacted EPI-F is removed from solution. This was established by comparative examination (HPLC) of the solution before and after concentration. For the no-carrier-added reactions the losses were dominated by the presence of by-products removed by the SEP-PAKs and HPLC purification (compare entries 7–10).

From Table 2 some general trends are clear for both the carrier-added and no-carrier-added reactions. However, the lower radiochemical yield for the carrier-added reaction (A) relative to the no-carrier-added reactions (B and C) was surprising. This may arise from a mass effect or more directly from the use of increased concentrations of the 2-nitroimidazole anion (2-NIM/KOH) for the no-carrier-added reactions. The optimum conditions for the second step in the synthetic sequence have not been fully established. Nevertheless, useful quantities of labeled F-MISO with quite good specific activity may be obtained by this overall procedure. Figure 4 illustrates a HPLC radiochromatogram of purified, no-carrier-added, [^{18}F]F-MISO (reaction C, Table 2). With a specific activity of 670 Ci/mmol, the total injectable dose (14 ml) contained 84 nmol of F-MISO and 280 nmol of 2-NIM.

In conclusion, the present study describes a procedure useful for the preparation of the labeled fluoroalkylation reagent, EPI-F. Presently, we are using this reagent for the production of labeled F-MISO in quantities sufficient for PET imaging. The reagent may find

application in other areas for the alkylation of nucleophilic heteroatoms on biologically interesting compounds.

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