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[^{18}F]-EF5, a marker for PET detection of hypoxia: synthesis of precursor and a new fluorination procedure

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Abstract

There is a great deal of clinical and experimental interest in determining tissue hypoxia using non-invasive imaging methods. We have developed EF5, 2-(2-nitro-1[H]-imidazol-1-yl)-*N*-(2,2,3,3,3-pentafluoropropyl)-acetamide, with both invasive and non-invasive hypoxia detection in mind. EF5 and other 2-nitroimidazoles are used to detect hypoxia, because the rate of their bioreductive metabolism is inversely dependent on oxygen partial pressure. Such metabolism leads to the formation of covalent adducts within the metabolizing cells. Previously, we have described the invasive detection of these adducts by highly specific monoclonal antibodies after tissue biopsy. In this report, we demonstrate the synthesis of ^{18}F-labeled EF5, [^{18}F]-2-(2-nitro-1[H]-imidazol-1-yl)-*N*-(2,2,3,3,3-pentafluoropropyl)-acetamide, in greater than 10% yield by direct fluorination of the newly synthesized precursor 2-(2-nitro-1[H]-imidazol-1-yl)-*N*-(2,3,3-trifluoroallyl)-acetamide by [^{18}F]-F_2 in trifluoroacetic acid. Our objective was to optimize the electrophilic fluorination of the fluorinated alkene bond with fluorine gas, a new method of ^{18}F-labeling of polyfluorinated molecules. Previous biodistribution studies in mice have demonstrated uniform access of EF5 to all tissues with bioelimination dominated by renal excretion. When [^{18}F]-EF5 was injected into a rat followed by urine collection and analysis, we found no detectable metabolism to other radioactive compounds. Thus, [^{18}F]-EF5 should be well suited for use as a non-invasive hypoxia marker with detection using positron emission tomography (PET).

1. Introduction

Tissue hypoxia has important biological and clinical implications in various pathological states. In tumors, the level of tumor oxygenation correlates with response to radiation therapy (Brizel et al., 1997; Hockel et al., 1993; Nordsmark et al., 1996). Therefore, the in vivo measurement of the level of tumor oxygenation is clinically important. We have previously developed a 2-nitroimidazole marker of hypoxia named EF5 (2-(2-nitroimidazol-1[H]-yl)-*N*-(2,2,3,3,3-pentafluoropropyl)-acetamide) (Lord et al., 1993; Evans et al., 1995; Koch et al., 1995). The reductive intracellular metabolism of EF5 leads to its covalent binding with cellular molecules. This process is inhibited by increasing oxygen concentration (Chapman et al., 1983; Koch et al., 1984; Varghese et al., 1976), providing specific drug

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binding in hypoxic tissue. Fluorescent labeled monoclonal antibodies allow the detection of the bound adducts and determination of the level and location of hypoxia (Evans et al., 1995; Koch et al., 1995).

Incorporation of ^{18}F into 2-nitroimidazole molecules provides an opportunity to use these agents for the detection of hypoxia by positron emission tomography (PET) (Jerabek et al., 1986; Mathias et al., 1987). Several groups have developed ^{18}F-labeled nitroimidazole-based PET assays, for example, [^{18}F]-fluoromisonidazole (Rasey et al., 1987, 1996; Grierson et al., 1989; Koh et al., 1992), [^{18}F]-fluoroerythronitroimidazole (Yang et al., 1995) and [^{18}F]-fluoroetanidazole (Tewson, 1997). The first described and most investigated compound of this type is [^{18}F]-fluoromisonidazole (Rasey et al., 1987). This agent has been studied in several anatomic sites in humans including gliomas (Valk et al., 1992), lung cancer (Koh et al., 1994) and nasopharyngeal carcinoma (Yeh et al., 1996).

Despite the extensive investigations, none of the currently developed compounds is accepted clinically as a PET marker of hypoxia. A possible general problem is that the compounds described above are new products with relatively unknown pharmacological properties (Stöcklin, 1998), e.g. the structure of [^{18}F]-fluoromisonidazole is substantially different from misonidazole, [^{18}F]-fluoroetanidazole has the hydroxyl group of etanidazole substituted by a fluorine atom. It has only recently been shown that [^{18}F]-fluoromisonidazole is not stable in vivo, and produces multiple radioactive products distinct from parent drug following renal clearance (Rasey et al., 1999). These differences and the use of very low drug concentrations for the PET studies may therefore lead to results not predicted from studies with the parent compounds or at much higher drug concentrations. In contrast, labeling of the EF5, molecule with ^{18}F provides a unique opportunity to develop a true PET analog of a hypoxia marker with well documented pharmacological properties which has been shown to predict radiotherapy resistance in individual rodent tumors (Laughlin et al., 1996; Evans et al., 1996).

Previously, we described the preparation of a new PET hypoxia marker [^{18}F]-EF1, [^{18}F]-2-(2-nitroimidazol-1[H]-yl)-*N*-(3-fluoropropyl)-acetamide, with a structure similar to EF5 (Kachur et al., 1999). This compound was synthesized using nucleophilic substitution of the bromine atom of a precursor 2-(2-nitroimidazol-1[H]-yl)-*N*-(3-bromopropyl)-acetamide, by [^{18}F]-F^-. [^{18}F]-EF1 has shown good potential for labeling of hypoxic tumors and a relatively uniform biodistribution limited by slow equilibration with brain tissue (Evans et al., 2000). In the work to be described herein, we initially attempted to utilize the same technique. Unfortunately, nucleophilic substitution of a bromine substituted precursor to EF5 was not successful. Thus, we developed a new approach, namely an electrophilic fluorination of an allyl precursor. Here, we report the procedure for the preparation of [^{18}F]-EF5 [^{18}F]-2-(2-nitroimidazol-1[H]-yl)-*N*-(2,2,3,3,3-pentafluoropropyl)-acetamide by addition of [^{18}F]-fluorine gas to 2-(2-nitro-1[H]-imidazol-1-yl)-*N*-(2,3,3-trifluoroallyl)-acetamide. Subsequent extraction and HPLC purification allow a facile synthesis which should be suitable for human use. Preliminary rodent biostability data demonstrated renal excretion of [^{18}F]-EF5 with no evidence for breakdown products excreted in urine.

2. Experimental

Reagents and solvents were purchased from Aldrich and used without additional purification unless otherwise noted. ^1H-NMR spectra were recorded on a Bruker-AMX-300 using $CDCl_3$ or acetone-d_6 as solvent and tetramethylsilane as an internal standard; ^{19}F-NMR spectra were measured on a Varian XL at 282 MHz, referenced to external CF_3COOH in D_2O. HPLC was performed on a Waters system (with Waters UV detector and radioactivity detector from IN/US Service, Fairfield, NJ) using an Altima C-18 column (5 μm particle size, 4 mm × 250 mm) and ammonia–acetate buffer containing 40% CH_3OH (pH = 4.7, final concentration 0.1 M) as a mobile phase (flow rate 1 ml/min) with serial detection of 325 nm absorbency (specific for 2-nitroimidazole moiety) and radioactivity. The same HPLC conditions were used for the purification of [^{18}F]-EF5.

2.1. 2,3,3-Trifluoroallylamine hydrochloride

2,3,3-Trifluoroallylamine hydrochloride was prepared as described in literature (Castelhano, 1988) using the following intermediate compounds: *N*-(benzyloxycarbonyl)-chloroglycinate was synthesized according to Williams et al. (1990) and converted into 3,4,4-trifluoro-2-benzyloxycarbonylamino-but-3-enoic acid methyl ester as described by Castelhano et al. (1986). NMR data of the product correspond to literature information (Castelhano, 1988): ^1H (300 MHz, $CDCl_3$) δ 3.84 (*dm*, J = 21.3 Hz, 1H); ^{19}F (282 MHz, D_2O) δ −96.94 (*dd*, J = 32, 68 Hz, 1F), −115.15 (*dd*, J = 68, 115 Hz, 1F), −178.9 (*ddt*, J = 21, 32.1, 115 Hz, 1F).

2.2. 2-(2-Nitro-1[H]-imidazol-1-yl)-N-(2,3,3-trifluoroallyl)-acetamide

N-Methylmorpholine (1.01 g, 10 mmol) was added to 2-(2-nitro-1[H]-imidazol-1-yl)-acetic acid (1.71 g, 10 mmol) in 150 ml of dry THF under nitrogen at 0°C and stirred for 10 min. Later, isobutyl chloroformate

(1.43 ml, 11 mmol) was added. After 30 min, 2,3,3-trifluoroallylamine hydrochloride (1.62 g, 11 mmol) and *N*-methylmorpholine (1.21 g, 12 mmol) were added to the solution and the mixture was stirred at room temperature overnight. The solution was then filtered and the organic solvent was evaporated to give a pale yellow solid. Purification by chromatography (silica gel, $CH_3OH/CHCl_3 = 10:1$) gave a white solid (1.2 g, 48%) with a melting point of 141–143°C. ^1H (300 MHz, CD_3COCD_3) δ 4.24 (*dm*, J = 21.3 Hz, 1H), 5.34 (*s*, 2H), 7.19 (*s*, 1H), 7.56 (*s*, 1H), 8.10 (*br*, 1H); ^{19}F (282 MHz, CD_3COCD_3) −102.2 (*dd*, J = 32, 81 Hz, 1F), −118.6 (*dd*, J = 81, 113 Hz, 1F), −176.0 (*ddt*, J = 21.4, 32, 113 Hz, 1F); anal. calcd. for $C_8H_7F_3N_4O_3$: C, 36.36; H, 2.65; N, 21.21. Found: C, 36.84; H, 2.60; N, 20.71.

2.3. 3-Bromo-2,2,3,3-tetrafluoropropylamine and 2-(2-nitro-1[H]-imidazol-1-yl)-N-(3-bromo-2,2,3,3-tetrafluoropropyl)-acetamide

3-Bromo-2,2,3,3-tetrafluoropropylamine was prepared through the intermediate 4-bromo-4,4,3,3-tetrafluorobutanoic acid using the literature method (Wei Yuan et al., 1990). $BrCF_2CF_2COOH$ (1.2 g, 5 mmol) was dissolved in 3 ml of H_2SO_4. Sodium azide (0.8 g, 12 mmol) was added in portion to the mixture at 80°C. After addition was completed, the reaction was continued for 20 h. The mixture was then cooled to 0°C. The solution was diluted with dichloromethane followed by addition of sodium carbonate solution (4 g in 20 ml of water). The organic layer was separated and the water layer was extracted with CH_2Cl_2 (20 ml × 2). The combined dichloromethane was dried over magnesium sulfate overnight and gaseous HCl bubbled into the solution. 0.79 g of white solid was collected by filtration and vacuum dried. ^1H-NMR δ 3.82 (*t*, J = 16 Hz, 2H); ^{19}F-NMR δ −66.8 (*t*, J = 16 Hz, 2H), −113.74 (*m*, 2F); anal. calcd. for $C_3H_5BrClF_4N$: C, 14.6; H, 2.03; N, 5.68. Found: C 14.57; H 1.96; N 5.56.

3-Bromo-2,2,3,3-tetrafluoropropylamine was conjugated with 2-(2-nitro-1H-imidazol-1-yl)-acetic acid yielding 2(2-nitro-1H-imidazol-1-yl)-*N*-(3-bromo-2,2,3,3-tetrafluoropropyl)-acetamide using isobutylchloroformate mixed anhydride method as is described above for 2-(2-nitro-1[H]-imidazol-1-yl)-*N*-(2,3,3-trifluoroallyl)-acetamide. The purity of the final product was confirmed by HPLC.

2.4. Synthesis of EF5 from allyl precursor by addition of F_2

2-(2-Nitro-1[H]-imidazol-1-yl)-*N*-(2,3,3-trifluoroallyl)-acetamide (50 mg, 0.20 mmol) was dissolved in 4 ml of trifluoroacetic acid at room temperature. 10% F_2/Ne was bubbled into the solution for 30 min (flow rate = 10 ml/min; 1.2 mmol F_2). The solvent was evaporated and the residue was triturated in the presence of ethyl acetate. A white solid was filtered and the organic solvent evaporated to get the residue, which was purified by chromatography (silica gel, $CH_3OH/CHCl_3$: 8:1) to give 2-(2-nitro-1[H]-imidazol-1-yl)-*N*-(2,2,3,3,3-pentafluoropropyl)-acetamide (18 mg, 32%). ^1H (300 MHz, CD_3COCD_3) δ 4.06 (*dt*, 2H), 5.37 (*s*, 2H), 7.15 (*s*, 1H), 7.54 (*s*, 1H), 8.22 (*br*, 1H); ^{19}F-NMR (282 MHz, CD_3COCD_3) δ −81.70 (*s*, 3F), −118.76 (*t*, J = 16 Hz, 2F).

Decreasing the fluorine concentration of the gas mixture slightly decreases the overall EF5 yield, but simultaneously causes more efficient consumption of fluorine. Reaction of 25 mg of precursor (0.1 mmol) in 5 ml of trifluoroacetic acid with an equivalent amount of 0.1% F_2 (flow rate 100 ml/min for 25 min, 0.1 mmol F_2) causes a complete consumption of allyl precursor, yielding 11% EF5 as determined by HPLC. The rationale for using an initially lower precursor concentration in this case was more intense TFA evaporation caused by the increase in total gas flow. This decreased the final solution volume to 1.5 ml, simultaneously increasing the concentration.

2.5. [^{18}F]-2-(2-Nitro-1[H]-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl)-acetamide

[^{18}F]-F_2 was prepared by the $^{20}Ne(d, \alpha)^{18}F$ reaction using a 50 ml target filled with 0.3% F_2/Ne with total pressure 10 atm. The [^{18}F]-F_2 (20 mCi, specific activity 300 mCi/mmol) was bubbled through 5 ml of trifluoroacetic acid containing 2-(2-nitro-1[H]-imidazol-1-yl)-*N*-(2,3,3-trifluoroallyl)-acetamide (25 mg, 0.10 mmol). HPLC analysis shows that radioactive EF5 is the major ^{18}F-containing product of the reaction (Fig. 1). The solution was neutralized by 10 N NaOH and extracted by a tetrahydrofuran–chloroform mixture (1:1, 3 × 10 ml). At this stage, about 30% of the activity was transferred to the organic phase, and the radiochemical yield of [^{18}F]-EF5 was 17%. The organic phase was collected, evaporated, dissolved in 200 μl of methanol and purified by HPLC with collection of the 11–13 min fraction, which includes EF5. The fraction collected was evaporated and reanalyzed by HPLC, showing a high degree of chemical and radiochemical purity (Fig. 2). Although we do not require compound with high specific activity at present, since animals are administered EF5 at whole body doses of 100 μmol/kg (Evans et al., 1996, 2000), the specific activity can be varied at will by changing the proportion of radioactive to non-radioactive fluorine gas.

2.6. Biostability of [^{18}F]-EF5

A rat was administered a mixture of purified ^{18}F-labeled EF5 with non-radioactive compound in physiological saline (total concentration 10 mM) by tail vein injection (total dose 100 μmol/g, 30 μCi). Urine was collected for 2 h after injection and the animal was sacrificed. Urine was mixed with an equal volume of 10% TCA, the solution centrifuged, and clear supernatant analyzed by HPLC as described above. Unmodified EF5 was the only major radioactive product (Fig. 2).

3. Discussion

Incorporation of ^{18}F into 2-nitroimidazole molecules provides an opportunity to use these agents for hypoxia detection by non-invasive PET technique. Our early attempt to develop a PET agent [^{18}F]-EF1 with a structure similar to EF5 (Kachur et al., 1999) was quite successful. This marker was found to give a tumor to muscle ratio of about 3 in hypoxic tumors, but only 1.2 in non-hypoxic tumors (Evans et al., 2000). However, we have almost no data on EF1 metabolism, while EF5 is well investigated. Additionally, the low lipophilicity of EF1 slows penetration of the brain-blood barrier which may limit its use in diseases of the brain (Evans et al., 2000). At the same time, EF5 has been shown to have uniform access to all tissues and its pharmacokinetic properties are well documented (Laughlin et al., 1996). Finally, EF5 has been approved for use in human cancer patients. Thus, our goal was to develop ^{18}F-labeled EF5.

Initially, we tried to apply the method of nucleophilic substitution of bromine atom by fluorine anion,

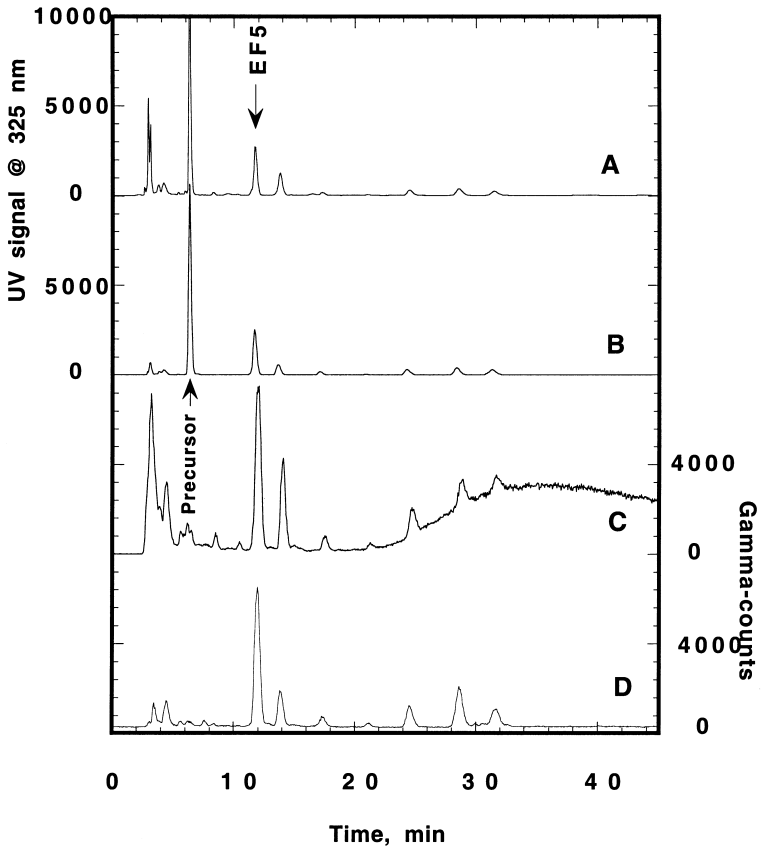


Fig. 1. HPLC analysis of the reaction mixture after fluorination of 2-(2-nitro-1[H]-imidazol-1-yl)-*N*-(2,3,3-trifluoroallyl)-acetamide with [^{18}F]-F_2. Serial detection of UV absorption at 325 nm (A) and radioactivity (C) indicates that [^{18}F]-EF5 is the major radioactive product. Free ^{18}F is eluted after 23 min, forming a broad signal of radioactivity due to absorption on the surface of the detector. HPLC analysis of organic phase after chloroform–tetrahydrofuran extraction (curves B and D represent UV absorption and radioactivity, respectively) shows decreased level of hydrophilic radioactive impurities (eluted before 5 min) and complete absence of ^{18}F.

analogous to the reaction used for the preparation of [^{18}F]-labeled EF1 (Kachur et al., 1999). We attempted to incorporate fluorine into the appropriate precursor 2-(2-nitro-1[H]-imidazol-1-yl)-*N*-(3-bromo-2,2,3,3-tetrafluoropropyl)-acetamide using F–Br exchange with potassium-kryptofix fluoride in DMSO at high temperature. This method was not successful, a result which may have been anticipated due to the high stability of C–Br bond in CF_2CF_2Br moiety. We were similarly unsuccessful in obtaining a direct exchange of [^{18}F]-F^- for any of the fluorines on authentic EF5. Therefore, we decided to attempt electrophilic addition of [^{18}F]-F_2 gas to precursor molecules with terminal double bond. This required the preparation of 2,3,3-trifluoroallylamine and/or 2-(2-nitro-1[H]-imidazol-1-yl)-*N*-(2,3,3-trifluoroallyl)-acetamide.

The synthesis of 2,3,3-trifluoroallylamine, which had been reported earlier (Castelhano, 1988), was accomplished via a six-step synthesis. Initially, we tried to prepare the intermediate, methyl *N*-(benzyloxycarbonyl)-chloroglycinate, by the method described by Bernstein and Ben-Ishai (1977), but found problems with neutralization of intermediate non-aqueous solution and purification of the desired product from other compounds produced in side reactions. Thus, we adopted another synthetic pathway (Williams et al., 1990) which proceeded smoothly and gave very pure chlorinated compound. Importantly, this chemistry was repeatable and suitable for scale-up.

A critical aspect of the [^{18}F]-labeling by fluorine gas was deciding the step in which it can be accomplished. Certainly, addition in the final step would be best, considering the short half-life of ^{18}F, but whether the imidazole ring or side chain can survive treatment with fluorine gas seemed questionable. In fact, it has been reported that the reaction of amide with fluorine in $CFCl_3$ at -78°C gave the corresponding acid (Purrington and Woodard, 1990), suggesting possible cleavage of the side chain of EF5 analogs under these conditions. Considering this information, we initially investigated the reaction of fluorine gas with 2,3,3-trifluoroallylic amine hydrochloride with the idea of distilling radioactive pentafluoropropylamine (bp 49°C) and its further conjugation with activated 2-nitroimidazole acetate in a manner analogous to a recent literature report (Tewson, 1997).

Although the reaction of fluorine with double-bond containing hydrocarbon systems has been well studied, there have been virtually no reports of successful synthetic use of this reaction since the original paper by Rozen. His method suggested the use of polar media and low temperatures (i.e., $CFCl_3/CHCl_3$/EtOH 5/4/1 at -78°C) to minimize the free radical behavior of molecular F_2 (Rozen and Brand, 1986). Certainly, there have been no reports of direct addition of F_2 to a partially fluorinated double bond. This is not a trivial reproduction of alkene fluorination due to decreased electron density on the double bond. Because the trifluoroallylamine hydrochloride was insoluble in Rozen's mixed solvent system, several

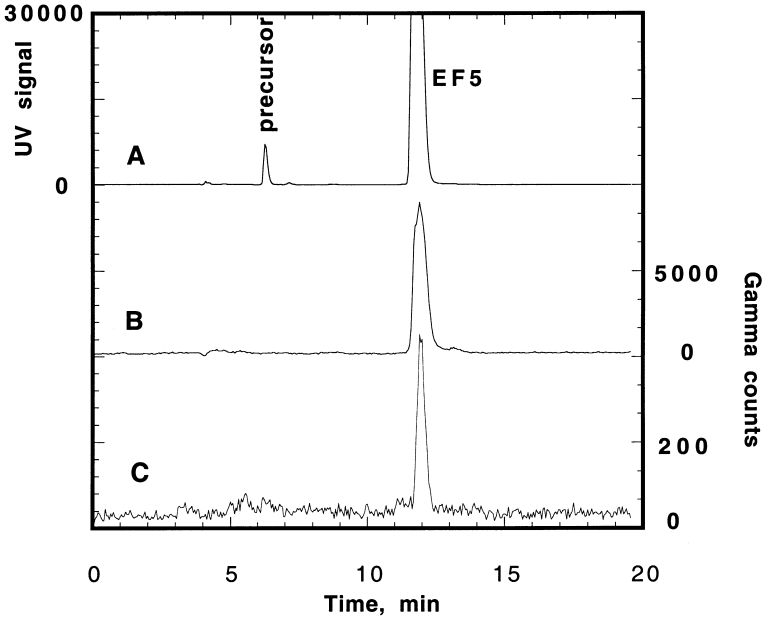


Fig. 2. HPLC analysis of purified [^{18}F]-EF5 sample with serial detection of UV (A) and radioactivity (B), and radioactivity of urine (C).

others were examined; e.g. methanol, trifluoroethanol, acetonitrile, mixture of $CFCl_3/CH_3OH$, $CFCl_3/CH_3CN$, HCOOH, H_2SO_4, CF_3COOH. It was found that trifluoroacetic acid was the best choice, since it dissolved the salt well and gave rise to a cleaner reaction. When the addition of fluorine to the amine was carried out in CF_3COOH at room temperature, two products were obtained: pentafluoropropylamine and a chlorine containing side-product most likely derived from the oxidation of chloride ion by fluorine gas. Attempting to use the salt with the counter ion BF_4^- instead of Cl^-, supposedly inert in fluorination reaction, produced an unexpectedly large number of products. Although the reaction should have been clean with $CF_2{=}CFCH_2NH_3F$ as the substrate, this would require the use of anhydrous HF as a solvent, creating very difficult handling problems. Thus, although we found it possible to label the allylic trifluoropropylamine, it appeared that the subsequent purification was going to be quite difficult and time consuming.

Therefore, we decided to explore the possibility of adding fluorine in the last step of EF5 preparation according to Scheme 1.

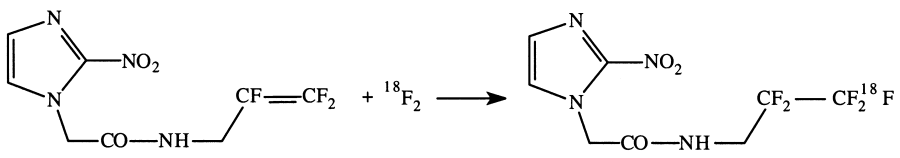
This reaction seems very unlikely to proceed in the required manner, since one could envision numerous side reactions: addition of fluorine to the ring, cleavage of any of C–H, C–C and C–N bonds, radical polymerization involving allyl and ring double bonds, etc. The reaction of fluorine with 2-(2-nitro-1[H]-imidazol-1-yl)-*N*-(2,3,3-trifluoroallyl)-acetamide was performed in different solvents, such as CH_3CN, CF_3CH_2OH, HCOOH, CF_3COOH, and we found that the addition in trifluoroacetic acid was much cleaner than in other solvents. The usefulness of highly acidic media may be related to the basic nature of the 2-nitroimidazole ring. Trifluoroacetic acid causes protonation of the nitrogen atom in position 3, thereby decreasing the electron density in the nitroimidazole ring. This would protect the nitroimidazole ring and its nitro group from electrophilic attack by fluorine gas, making the allyl double bond a more exclusive target. It should be noted that initially the amount of the fluorine gas was titrated carefully to prevent fluorination of the product. However, we found that authentic EF5 was only moderately decomposed in trifluoroacetic acid bubbled with a mole equivalent of fluorine gas, supporting our conclusion about the protective effect of acidic media on the 2-nitroimidazole moiety.

The EF5 yield vs. allyl precursor using 10% F_2 may be as high as 32%, but the degree of fluorine consumption was only 5% at these conditions since a six-fold excess of fluorine gas was used. This may be related to gas solubilization but poses a difficult monitoring problem. Decreasing the fluorine concentration to 0.1% makes the fluorine consumption more effective, requiring equivalent amounts of reagents. This decreased the total yield of EF5, but increased the product yield vs. F_2 up to 11%, making the incorporation of target ^{18}F into the product more efficient. In our radiochemical reaction, we used 0.1% [^{18}F]-F_2 and a two-fold excess of allyl precursor, which resulted in even higher radiochemical yield of product (17%).

The described method of electrophilic addition of fluorine gas to an allyl precursor could be of general use for the preparation of new PET agents with structure similar to EF5. Using precursors with differing number of fluorine atoms at carbon atoms adjacent to the allylic double bond, six different compounds with two to five fluorine atoms in the side chain can be produced. Furthermore, the method discussed herein may be generally useful for the preparation of other 2-nitroimidazole PET hypoxia markers and other types of polyfluorinated PET agents — protection strategies similar to our use of TFA would need to be employed however.

Product purification was performed in two steps. Initially, the solution contained a large proportion of $^{18}F^-$, which is a highly undesired impurity due to high binding in bone. Extraction by organic solvents completely eliminated the fluoride ion (Fig. 1). HPLC purification of [^{18}F]-EF5 with regular analytical column allowed substantial preparation of chemically and radiochemically pure product (Fig. 2). Further improvements are obviously required for clinical use, but there seems to be no impediment to obtaining high purity labeled EF5.

Previous results indicated renal clearance as the main path of EF5 excretion in mice (Laughlin et al., 1996) and we have found no change for other species tested, including human (data not shown). In the current study, we performed urine analysis in order to demonstrate the metabolic stability of [^{18}F]-EF5. Puri-



Scheme 1.

fied [^{18}F]-EF5 was injected intravenously into a rat in combination with authentic non-radioactive EF5. Total drug (30 mg/kg) was equivalent to 100 μM under these conditions, as used previously for our studies employing immunohistochemical detection techniques (Evans et al., 1996). At these concentrations, EF5 is eliminated by simple exponential decay with a half-life of 150 min in rats (Evans et al., 1996). HPLC analysis of urine showed that the only radioactive peak was unmodified parental compound (Fig. 2). This result indicates an absence of metabolic breakdown of [^{18}F]-EF5 with production of other [^{18}F]-labeled compounds. The biological stability of [^{18}F]-EF5 represents a significant advantage in comparison with [^{18}F]-fluoromisonidazole, which is excreted in urine as several radioactive products (Rasey et al., 1999). These results, together with biodistribution data for EF5 labeled at the ring position 2 by ^{14}C (Laughlin et al., 1996), suggest that [^{18}F]-EF5 can be useful as a non-invasive hypoxia marker with detection by PET.

4. Conclusion

We developed the procedure of fluorine addition to alkene double bond in trifluoroacetic acid at room temperature. This may be a useful synthetic method for the fluorination of multifunctional aromatic compound with basic function. We also demonstrated the possibility of the direct addition of fluorine gas to partially fluorinated double bond.

The proposed method may be used for the preparation of other PET agents. The results indicate our ability to prepare clinically useful amounts of [^{18}F]-EF5, a PET analog of a hypoxia marker currently used for in vivo assessment of tumor hypoxia.

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