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Research Article

Efficient *O*- and *N*-(*β*-fluoroethylation)s with NCA [^{18}F]*β*-fluoroethyl tosylate under microwave-enhanced conditions

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Summary

Reactions of no-carrier-added (NCA) [^{18}F]*β*-fluoroethyl tosylate with amine, phenol or carboxylic acid to form the corresponding [^{18}F]*N*-(*β*-fluoroethyl)amine, [^{18}F]*β*-fluoroethyl ether or [^{18}F]*β*-fluoroethyl ester, were found to be rapid (2–10 min) and efficient (51–89% conversion) under microwave-enhanced conditions. These conditions allow reactants to be heated rapidly to 150°C in a low boiling point solvent, such as acetonitrile, and avoid the need to use high boiling point solvents, such as DMSO and DMF, to promote reaction. The microwave-enhanced reactions gave about 20% greater radiochemical yields than thermal reactions performed at similar temperatures and over similar reaction times. With a bi-functional molecule, such as DL-pipecolinic acid, [^{18}F]*β*-fluoroethyl tosylate reacts exclusively with the amino group. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: fluorine-18; [^{18}F]*β*-fluoroethylation; amine; phenol; ester; pipecolinic acid; microwave

Introduction

In the field of labeling compounds with fluorine-18 ($t_{1/2} = 109.8$ min; $\beta^+ = 96.9\%$) for positron emission tomography (PET), no-carrier-added (NCA) [^{18}F]*β*-fluoroethyl tosylate is widely used as a reagent for *O*-, *N*- and *S*-fluoroalkylations.[1–13] This reagent is attractive for several reasons, including its (a) ease of preparation, (b) good stability, (c) wide applicability and (d) suitability for one-pot radiosyntheses.[13] However, applications of this labeling agent are restricted by some disadvantages, including lower reactivity

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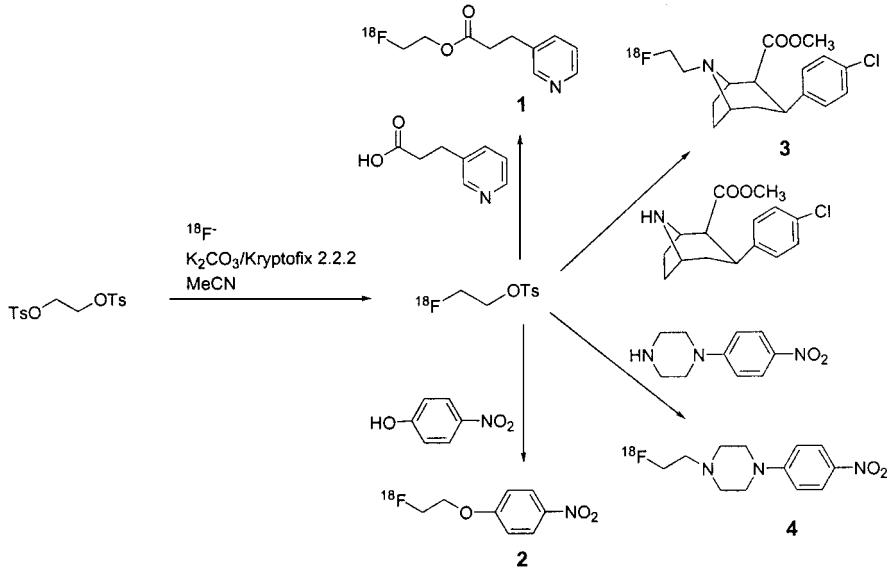
compared to alternative labeling agents (*e.g.* [^{18}F]β-fluoroethyl triflate[14,15]) and its sensitivity to some solvents and bases that are used in the labeling reactions. For example, it was observed that reactions with some secondary aliphatic and aromatic amines were particularly sluggish in acetonitrile and that polar, high boiling point solvents, such as DMF or DMSO, were required to complete reactions.[14] Consequently, alternative labeling agents,[14,15] catalysts[16,17] or new radiochemistry[18,19] have been explored to overcome the lack of reactivity. Another noted drawback of this labeling reagent is its lack of selectivity in reactions with multi-functional molecules. For example, a base-supported [^{18}F]β-fluoroethylation of an H-acidic group, like a phenolic hydroxyl or carboxyl group, may be accompanied by the alkylation of a coexisting nucleophilic amino group, making the use of appropriate protection necessary.[19]

Microwave dielectric heating transforms electromagnetic energy directly to heat in reaction media. In recent years, microwaves have had a huge impact on how experimental organic or medicinal chemistries are performed.[20,21] Microwave-enhanced radiochemistry can provide a faster, cleaner, more selective and highly atom-efficient methodology. The shorter reaction time coupled with other benefits, such as higher product purity, due to reduced reaction mixture decomposition and the promotion of otherwise sluggish reactions, make it an ideal tool to be explored in radiochemistry with short-lived positron-emitters.[22–24] Here we report how improved radiochemical yields and practical convenience derive from the use of microwaves when using [^{18}F]β-fluoroethyl tosylate to prepare [^{18}F]*N*-β-fluoroethyl-amines, ethers and esters. We also report that, under microwave conditions, [^{18}F]β-fluoroethyl tosylate is chemoselective in reacting exclusively with the amino group of bi-functional DL-pipecolinic acid.

Results and discussion

Benefits of using microwaves extend to a wider choice of solvents, including acetonitrile. Acetonitrile is an attractive aprotic polar solvent for use in PET radiochemistry, because it is easy to maintain in a dry state and is easily removable. Thus, in this work, acetonitrile was first used for the azeotropic removal of the proton-irradiated [^{18}O]water and recovery of the reactive 'naked' [^{18}F]fluoride ion for the preparation of [^{18}F]β-fluoroethyl tosylate. Acetonitrile was also a component of the mobile phase for the preparation and purification of the [^{18}F]β-fluoroethyl tosylate with solid phase extraction. Under microwave conditions, it was possible to heat reaction mixtures containing [^{18}F]β-fluoroethyl tosylate rapidly to 150°C in low boiling point (b.p. 82°C) acetonitrile. This avoids the need for high boiling point solvents, such as DMF or DMSO, to drive reactions to completion under thermal conditions. Such solvents are more difficult to remove and indeed quite



Scheme 1. Examples of the preparation of NCA [^{18}F]*N*-*β*-fluoroethylamine, [^{18}F]*β*-fluoroethyl ether and [^{18}F]*β*-fluoroethyl ester using [^{18}F]*β*-fluoroethyl tosylate as labeling agent in a microwave-enhanced procedure

unwieldy extraction techniques have been required for this purpose.[13] Although acetonitrile is not considered a good microwave solvent on its own, mainly due to its small dielectric loss (ε'') to dielectric constant (ε') ratio ($\tan\delta = 0.062$)[21], dissolved metal salts (Cs^+ or Na^+) dramatically improve the absorption and transfer of the microwave energy to heat by the ionic conduction mechanism.[25,26]

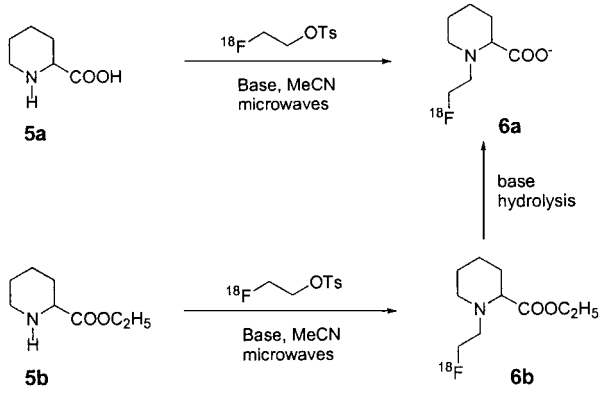
3-(3-Pyridyl)propionic acid (**1**), 4-nitrophenol (**2**), 2*β*-carbomethoxy-3*β*-(4-chlorophenyl)nortropane (**3**) and 1-(4-nitrophenyl)piperazine (**4**) were selected as examples of compounds containing carboxyl, hydroxyl and amino groups, as commonly encountered in substrates to be labeled with fluorine-18. Reaction of [^{18}F]*β*-fluoroethyl tosylate with the acid, phenol and amines proceeded smoothly in acetonitrile under microwave-enhanced conditions (Scheme 1). The standard Pyrex reaction vessel (10 ml), which can be crimp-sealed,[†] was used and no loss of solvent or radioactivity was observed during the microwave irradiation. The procedure was safe and reliable; no vessel failure incident occurred. Improvement in decay-corrected radiochemical yields (RCY) of the [^{18}F]*β*-fluoroethylated compounds under microwave-enhanced conditions compared to thermal heating in acetonitrile are

[†] Radiation exposure to the worker is reduced by replacing the screw-capped vials, which require more hand manipulation during radiosynthesis, with the simpler, crimp-sealed microwave reaction tube.

Table 1. RCYs of [^{18}F]β-fluoroethyl derivatives under microwave-enhanced conditions or thermal heating in acetonitrile

Substrate	Base	Microwave		Thermal heating	
		Conditions	RCY (%)[a]	Conditions	RCY (%)[a]
1	Cs_2CO_3	300 W, 2 min	89	120°C, 10 min	66
2	Cs_2CO_3	300 W, 6 min	77	120°C, 10 min	59
3	$NaHCO_3$	300 W, 150°C, 10 min	78	120°C, 45 min	60
4	$NaHCO_3$	300 W, 150°C, 10 min	51	70°C, 25 min	5[b]

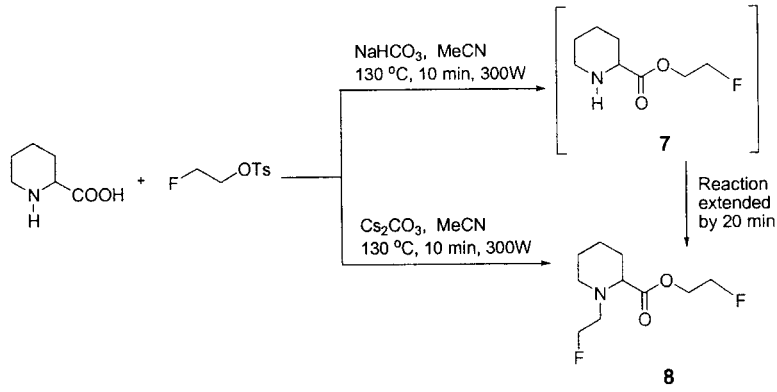
[a] RCY based on [^{18}F]β-fluoroethyl tosylate.
[b] This data is from Ref. 31 for similar reaction.



Scheme 2. Reactions of [^{18}F]β-fluoroethyl tosylate with DL-pipecolinic acid and its ethyl ester under microwave-enhanced conditions

summarized in Table 1. Generally, the microwave-enhanced reactions gave about 20% greater RCYs than thermal reactions under similar conditions of temperature (the highest achievable with a heating block) and reaction time.

DL-Pipecolinic acid (**5a**) was selected as a model substrate for examination of the reactivity and selectivity of [^{18}F]β-fluoroethyl tosylate towards a bi-functional compound. In the presence of sodium bicarbonate or cesium carbonate a single radioactive product, the [^{18}F]*N*-(β-fluoroethyl)amine **6a**, was obtained in very high RCY (90% or 96%, respectively, by radio-TLC analysis) within 5–10 min of microwave heating at 130°C. In the absence of base the RCY was around 10% (Scheme 2). In reactions of DL-pipecolinic acid (**5a**) with one equivalent of *non-radioactive* β-fluoroethyl tosylate in the presence of sodium bicarbonate, mono-substituted ester **7** was the major product after 10 min, and the di-substituted compound **8** appeared if the reaction was extended for another 20 min. When cesium carbonate was used as base, di-substituted compound **8** was the major product at 10 min (Scheme 3).



Scheme 3. Influence of base on the reaction of one molar equivalent of *non-radioactive* β-fluoroethyl tosylate with DL-pipecolinic acid under microwave-enhanced conditions

Table 2. [^{18}F]β-Fluoroethylation of pipecolinic acid, salts and ethyl ester, under microwave-enhanced conditions

Entry	Substrate	Base	Irradiation time (min)	RCY (%)[a]
1	**5a**	—	5	10
2	**5a**	—	10	11
3	**5a**	Cs_2CO_3	10	96 ± 1 ($n=3$)
4	**5a**	$NaHCO_3$	10	90 ± 1 ($n=3$)
5	**5b**	—	5	28
6	**5b**	Cs_2CO_3	5	54
7	**5b**	Cs_2CO_3	10	95

[a] By radio-TLC, $n=1$ unless otherwise stated.

Under the described microwave conditions, whether the carboxylic acid group is blocked, as with an ethyl group in **5b**[27–29] (Table 2, entries 6 and 7), or not (Table 2, entries 3 and 4), [^{18}F]β-fluoroethyl tosylate reacts exclusively with the amino group.

Experimental

Materials

2β-Carbomethoxy-3β-(4-chlorophenyl)nortropane and its *N*-fluoroethyl derivative (**3**) were obtained from Dr. M. Goodman (Emory University). Ethylene glycol di-*p*-tosylate (99%), 1-(4-nitrophenyl)piperazine (97%), 4-nitrophenol (99 + %), 3-(3-pyridinyl)propionic acid (98%), DL-pipecolinic acid (98%) (**5a**), ethyl pipecolinate (98%) (**5b**), sodium hydrogen carbonate (99.7%), cesium carbonate (99.95%), sodium hydroxide (97%) and Kryptofix 2.2.2® (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo-[8,8,8]hexacosane), all from Aldrich,

plus ammonium formate (96%; J.T. Baker), ethyl acetate (HPLC grade; Fisher Scientific), hexane (OmniSolv; EMD), methanol (HPLC grade; Fisher Scientific) and acetonitrile (high purity solvent; Burdick & Jackson) were all used as received. β-Fluoroethyl tosylate was prepared using a literature method.[30]

General analytical methods

^{1}H-NMR spectra were recorded on an Avance-400 spectrometer (Bruker). LC-MS analysis was performed on a Surveyor LC system (Thermo Finnigan) equipped with a Quest LC Q_{DECA} ESI probe (Finnigan).

Radioactivity was measured with a calibrated dose calibrator (Atomlab™ 300; Biodex Medical Systems). Radio-TLC analysis was performed on silica gel coated glass plates (Uniplates; AnalTech), which were developed in ethyl acetate–hexane (2:1 v/v) and scanned with a sensitive quantitative γ-detecting instrument (Bioscan AR-2000). HPLC analysis was performed on a system comprising a Gold HPLC module [System Gold 126 solvent module coupled with a 166 UV absorbance detector (single wavelength); Beckman Coulter] plus a Flow Count radioactivity detector (diode or PMT; Bioscan). The HPLC system was equipped with a reverse phase column (Luna C18; 10 μ; 100 Å; 250 × 4.6 mm i.d.; Phenomenex) eluted with acetonitrile–ammonium formate solution (0.1 M) at 1 ml/min.

Non-UV absorbing radioactive compounds were identified with radio-TLC and UV absorbing compounds with radio-HPLC. In each method, co-migration of radioactive product and reference compound was verified. RCYs were estimated by integration of radioactive peaks and represent conversions, not isolated yields. The area of the product peak (P, in units of V min) was compared to the integral of radioactivity from all eluted peaks (T, in units of V min) and the RCY calculated as $[P/T \times R_2/R_1 \times 100\%]$, where R_2 is the total activity transferred out of the reaction vessel and R_1 was the starting activity of $^{18}FCH_2CH_2OTs$, both decay corrected. In HPLC, no corrections were made for radioactive decay in the short time between the first and last eluted peak ($\sim$12 min). Regular checks were performed, confirming that all radioactive compounds injected onto HPLC column were eluted during the course of analysis.

Reference compounds

Non-radioactive counterparts of compounds **1**, **2**, **4**[11], **6b**, **7** and **8** were prepared by treating the des-β-fluoroethyl compounds with β-fluoroethyl tosylate (1:1 molar ratio). Each product was isolated by column chromatography, and characterized by TLC, LC-MS and ^{1}H-NMR (Table 3).

Table 3. ^{1}H-NMR (400 MHz) and LC-MS data of the reference compounds

^{19}F-Compound	^{1}H-NMR	LC-MS $[M+1]^{+}$
1	CD_3CN, 8.52 (s, 1 H), 8.47 (d, 1H), 7.67 (d, 1H), 7.31 (dd, 1H), 4.62 (m, 2 H, $J_{HF}=47$ Hz), 4.32 (m, 2 H, $J_{HF}=30$ Hz), 2.99 (t, 2H), 2.75 (t, 2H)	198
2	CD_3CN, 8.21 (d, 2 H), 7.08 (d, 2 H), 4.77 (m, 2 H, $J_{HF}=46$ Hz), 4.35 (m, 2 H, $J_{HF}=29$ Hz)	185
4	CD_3CN, 8.08 (d, 2 H), 6.93 (d, 2 H), 4.57 (m, 2 H, $J_{HF}=47$ Hz), 3.43 (t, 4 H), 2.69 (m, 2 H, $J_{HF}=29$ Hz), 2.62 (t, 4 H)	254
6b	$CDCl_3$, 4.56 (m, 2 H, $J_{HF}=47$ Hz), 4.19 (q, 2 H), 3.23 (m, 1 H), 3.11 (m, 1 H), 2.86 (m, 2 H, $J_{HF}=29$ Hz), 2.74 (M, 1 H), 2.38 (m, 1 H), 1.85 (m, 2 H), 1.66 (m, 2 H), 1.41 (m, 1 H), 1.26 (t, 3 H)	204
7	Mixture with fluoroethyl tosylate not isolated in a pure state for NMR	176
8	CD_3CN, 4.59 (m, 2 H, $J_{HF}=47$ Hz), 4.47 (m, 2 H, $J_{HF}=47$ Hz), 4.31 (m, 2 H, $J_{HF}=29$ Hz), 3.33 (t, 1 H), 3.27 (d, 1 H), 3.01 (m, 1 H), 2.80 (m, 2H, $J_{HF}=47$ Hz), 2.70 (m, 1 H), 2.41 (m, 1 H), 1.77 (m, 2 H), 1.56 (m, 2 H)	222

Preparation of NCA [^{18}F]fluoride ion

NCA [^{18}F]fluoride ion was prepared through the $^{18}O(p,n)^{18}F$ nuclear reaction by irradiating [^{18}O]water (95 atom %) for 120 min with a proton beam (17 MeV; 20 μA) from a commercial cyclotron (PETtrace; GE).

Preparation of [^{18}F]β-fluoroethyl tosylate

Non-automated method. The one-pot procedure was based on a literature method with some modification.[12] Cyclotron-produced NCA [^{18}F]fluoride ion was mixed with Kryptofix 2.2.2 (5 mg; 13.3 μmol) and potassium carbonate (0.5 mg; 3.6 μmol) in acetonitrile-water (95:5 v/v; 0.1 ml) and dried azeotropically by alternating evaporations and additions of acetonitrile (0.5 ml × 3). Ethylene glycol di-*p*-tosylate (2 mg; 5.4 μmol) in acetonitrile (250 μl) was added to the reaction mixture then heated at 110°C for 10 min to afford [^{18}F]β-fluoroethyl tosylate. The solution was used directly for the next reaction without further purification.

Automated method. The automated procedure was carried out on a Tracerlab FX module (GE). The preparation and azeotropic drying of the [^{18}F]fluoride ion-Kryptofix 2.2.2-K_2CO_3 complex were nearly identical to the manual method but completed in the module. The reaction mixture containing [^{18}F]β-fluoroethyl tosylate was diluted in water (100 ml) and passed through a reverse phase silica gel cartridge (C-18 Sep-Pak; Waters). The cartridge was washed with water again (3 ml) and the radioactivity eluted off with acetonitrile (1–1.5 ml).

General procedure for [^{18}F]β-fluoroethylation

To a standard Pyrex glass microwave reaction vessel containing substrate (~0.01 mmol), base (1 equiv to substrate) was added [^{18}F]β-fluoroethyl tosylate solution in acetonitrile (radioactivity $R_1 = 0.2$–2 mCi; 200–300 µl). The contents were crimp sealed with a PTFE-coated septum and irradiated in a single mode microwave cavity (Discover^{TM}; CEM) for a pre-set period of time, temperature and power. At the end of the microwave heating, the reaction mixture was cooled to room temperature, transferred out of the reaction vessel and further diluted by acetonitrile or methanol (radioactivity R_2). A fraction of the solution was injected into the radio-HPLC or spotted onto the radio-TLC plate for analysis.

Conclusion

Examples of NCA [^{18}F]*N*-β-fluoroethylamine, [^{18}F]β-fluoroethyl ether and [^{18}F]β-fluoroethyl ester were obtained using [^{18}F]β-fluoroethyl tosylate as labeling agent in a microwave-enhanced, and reliable procedure. An otherwise sluggish reaction in acetonitrile, a low boiling point and medium polar solvent, became possible by changing the energy input mode, *i.e.* to microwave heating. The use of high boiling point solvents, such as DMSO and DMF, which are difficult to remove, is avoided. RCYs for these reactions are at least 20% greater under microwave-enhanced condition than under thermal heating at similar temperatures and reaction times. For reaction with a bi-functional compound, such as DL-pipecolinic acid, [^{18}F]β-fluoroethyl tosylate reacts exclusively with the amino group.

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