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Iodonium Ylide Mediated Radiofluorination of ^{18}F-FPEB and Validation for Human Use

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Shortened Title: ^{18}F-FPEB *via* an iodonium ylide

ABSTRACT

Translation of new methodologies for labeling non-activated aromatic molecules with fluorine-18 remains a challenge. Here, we report a one-step, regioselective, metal-free ^{18}F-labeling method that employs a hypervalent iodonium(III) ylide precursor, to prepare the radiopharmaceutical ^{18}F-FPEB . **Methods**: Automated radiosynthesis of ^{18}F-FPEB was achieved by reaction of the ylide precursor (4 mg) with ^{18}F-NEt_4F in DMF at 80 °C for 5 minutes, and formulated for injection within 1 hour. **Results**: ^{18}F-FPEB was synthesized in 15 – 25% (n = 3) uncorrected radiochemical yields relative to ^{18}F-fluoride, with specific activities of 666 ± 51.8 GBq/μmol (18 ± 1.4 Ci/μmol) at the end-of-synthesis (EOS) and was validated for human use. . **Conclusions:** Radiofluorination of iodonium (III) ylides proved to be an efficient radiosynthetic strategy for synthesis of ^{18}F-labeled radiopharmaceuticals.

KEYWORDS: ^{18}F-FPEB; $mGlu_5$; iodonium ylide; PET; fluorination

Introduction

Historically, the formation of aromatic C-F bonds has been challenging in the field of synthetic organic chemistry, and even more so in radiochemistry, with the short-lived radionuclide fluorine-18 (^{18}F; $t_{½}$ = 109.7 min) for molecular imaging by positron emission tomography (PET). Electrophilic fluorination reactions with carrier-added ^{18}F-F_2 gas and its derivatives (e.g. ^{18}F-CH_3CO_2F) have enabled the development of ^{18}F-labeled aromatics by direct electrophilic substitution or demetalation reactions with organometallic reagents such as aryl stannanes (*1, 2*). Electrophilic radiosynthesis with ^{18}F-F_2 and its derivatives involve the use of carrier-added fluorine gas and consequently result in products with relatively low specific activities. Such reactions also require specialized equipment as well as technical expertise for the safe handling of F_2(g). Commercial availability of high specific activity, no-carrier added ^{18}F-fluoride has led to this reagent becoming the most widely used radiofluorinating species. Synthesis of aromatic molecules with ^{18}F-fluoride is typically achieved by nucleophilic aromatic substitution (S_NAr) reactions with electron-deficient (activated) aromatics, and these reactions have been used extensively to prepare high specific activity radiopharmaceuticals (*3*). However, labeling of electron-rich (non-activated or deactivated) aromatics with ^{18}F-fluoride remains a long-standing and unmet challenge in routine PET radiopharmaceutical production.

Early efforts to expand the scope of reactions of electron-rich aromatics with ^{18}F-fluoride include inefficient and low yielding thermal decomposition processes such as the Balz–Schiemann and Wallach reactions (*3*). Isotopic exchange *via* ^{18}F-for-^{19}F displacement reactions can be used to label electron-rich aromatics, and have been

applied to prepare low specific activity radiotracers (*3*, *4*). Strategies that convert electron-withdrawing aryl substituents to electron-donating groups following radiofluorination, (e.g., reduction of fluoronitrobenzene or fluorobenzaldehydes , decarbonylation reactions, etc.), are useful to access electron-rich ^{18}F-fluoroaromatics in high specific activity but are known to have limited chemical scope, involve multi-step labeling approaches and are challenging to adapt to radiopharmaceutical production. Recent strategies for reactions of ^{18}F-fluoride with electron-rich aromatics include the development of transition metal-mediated reactions with isolable aryl palladium or nickel complexes, copper-mediated fluorinations with aryl borate esters, oxidative fluorination with phenolic substrates , fluorination of diaryliodonium or sulfonium salts as well as diarylsulfoxides, and the majority of these methods have been recently reviewed (*5*). These newer methods appear promising for preparing radiolabeled aromatics. However, drawbacks include the use of air-sensitive and/or toxic transition metals, limited substrate scope, or poor regioselectivity. Furthermore, radiotracer syntheses by the above-mentioned methods generally result in low isolated radiochemical yields. Such limitations have hampered the use of these alternative labeling reactions for clinical translation. To our knowledge none of these methodologies have been applied to radiolabel a non-activated aromatic ring with ^{18}F-fluoride for synthesis of a radiopharmaceutical and validated it for human use.

Radiofluorination of non-activated aromatics can be achieved with aryliodonium ylide-based precursors bearing various β-dicarbonyl auxiliaries (*6)*. This technology was introduced by Satyamurthy and Barrio using barbituric or Meldrum's acid auxiliaries (7). A recent study by Coenen and coworkers demonstrated the satisfactory application of this

method using Meldrum's acid auxiliaries, but led to non-regiospecific labeling and formation of several byproducts (*8*). Concurrently, we explored the application of iodonium ylide precursors with barbituric acid and Meldrum's acid auxiliaries for ^{18}F-labeling but also found yields to be sub-optimal (*9*). We discovered that spirocyclic iodonium(III) ylide precursors, in conjunction with systematic optimization of the reaction conditions, led to efficient, regiospecific, one-step radiosyntheses of non-activated aromatics with ^{18}F-fluoride (*9*). These bench-stable precursors involved facile synthesis and demonstrated a broad substrate scope for non-activated ^{18}F-aromatics including hindered alkyl substituents, benzyl azides, anisoles, amides, heterocycles, and halogenated aromatics. The radiofluorination is operationally simple and was shown to be suitable for routine and automated production of ^{18}F-labeled aromatics. The conceptual advantages of excellent regioselectivity and viability of incorporating ^{18}F into a wide array of non-activated (hetero)arenes makes this methodology attractive for routine radiopharmaceutical production.

^{18}F-3-Fluoro-5-[(pyridin-3-yl)ethynyl] benzonitrile (^{18}F-FPEB) is a metabotropic glutamate receptor subtype 5 ($mGlu_5$) antagonist used in preclinical (*10, 11*) and clinical PET neuroimaging research (*12, 13*). Radiosynthesis of ^{18}F-FPEB and several structurally related radiotracers for $mGlu_5$ has been challenging because nucleophilic aromatic substitution by ^{18}F-fluoride is not a favored reaction due to the absence of electron-withdrawing groups located *ortho* or *para* to the labeling site. High temperatures are generally required and several chemical impurities are generated during the labeling reactions. Isolated ^{18}F-FPEB is typically obtained in low radiochemical yields (1−5 % uncorrected yield relative to ^{18}F-fluoride) (*10, 14-16*) as shown in Scheme 1.

The goals of the present work were to exploit our new spirocyclic iodonium(III) ylide precursor technology to develop a high yield radiosynthesis of ^{18}F-FPEB, and to demonstrate that this methodology is suitable for routine radiopharmaceutical production.

MATERIALS AND METHODS

Full details of precursor synthesis and characterization, radioisotope production, analytical methods, spectra and radiosynthesis with a GE medical systems commercial TRACERlab™ FX_{FN} radiosynthesis module, as well as human validation data are available in the supplemental materials.

Manual and Automated Radiosynthesis of ^{18}F-FPEB

Precursor (**1**, 4 mg) was dissolved in *N*,*N*-dimethylformamide (DMF; 400 μL) and added to a glass V-vial containing azeotropically dried ^{18}F-Et_4NF (typically 1 – 3 mCi). The reaction was heated at 80 °C for 5 min. The reaction mixture was cooled for 3 min and then quenched with high performance liquid chromatography (HPLC) mobile phase (60:40 CH_3CN:H_2O + 0.1 N ammonium formate, 2 mL). The reaction was further diluted with water (16 mL) and passed through a pre-activated (ethanol [1 mL] and water [5 mL]) Waters solid phase extraction (SPE; C18 Sep-Pak®) cartridge. The SPE cartridge was flushed with water (2 mL) and the product was eluted with ethanol (1 mL). Product identity and purity were confirmed by radio-HPLC and radio-TLC (100% EtOAc). The product was >99% radiochemically pure. Radiochemical yield was determined as the percentage of radioactivity that was isolated as the final product from the amount of activity present in the V-vial before addition of iodonium precursor to dried ^{18}F-Et_4NF, and is not decay-corrected.

Automated synthesis of ^{18}F-FPEB was carried out on a GE medical systems commercial TRACERlab™ FX_{FN} radiosynthesis module. The final product was formulated and found suitable for injection in compliance with quality control protocols and guidelines of The International Conference of Harmonization of Technical Requirement of Pharmaceuticals for Human Use (see supplementary materials).

RESULTS

The iodonium ylide precursor, **1**, was synthesized in six steps starting with 4-amino 3,5, diiodobenzoic acid. A Sandmeyer reaction furnished 3,5-diiodobenzoic acid which was converted to the nitrile via acid chloride formation, amidation and dehydration. Sonogashira coupling with 3-ethynylpyridine provided the aryl iodide (IPEB). We anticipated that oxidation of the iodine could be problematic in the presence of a pyridine functional group, which is known to undergo efficient transformation to the pyridine *N*-oxide under conditions analogous to those used in aryl iodide oxidation. Oxidants such as *m*-chloroperoxybenzoic acid (mCPBA) and H_2O_2/urea in acetic acid resulted in complex mixtures of products. To our delight, Oxone® in TFA was successfully used to oxidize IPEB partially attributed to a protonation event on nitrogen of pyridine, which prevented the formation of *N*-oxide. Removal of TFA, resolubilization in ethanol, treatment with the auxiliary in aqueous sodium carbonate, and subsequent purification with silica gel chromatography, provided **1** in 40% yield.

Initial efforts toward the radiosynthesis of ^{18}F-FPEB using precursor **1** (2 mg) were carried out manually with 1 – 3 mCi of starting ^{18}F-fluoride. Using our previously optimized radiolabeling conditions for these reactions (*9*), with Et_4NHCO_3 (7 mg) in

DMF (400 μL) at 120 °C, only 6% radiochemical conversion (RCC; via radio-TLC) of ^{18}F-fluoride to ^{18}F-FPEB was attained, along with formation of a radioactive by-product (**2**; *vide infra*) in 21% RCC (Scheme 1, entry I). Monitoring the reaction over time indicated that ^{18}F-FPEB formed with high conversions during the initial 3 minutes of the reaction, and subsequently decomposed. Control reactions with non-radioactive ("cold") FPEB demonstrated that the compound was stable in DMF at 120 ºC. However, in the presence of excess (2 equiv.) of Et_4NHCO_3 at the same temperature, FPEB underwent a rapid, quantitative base-mediated hydrolysis of the nitrile group to form 3-fluoro-5-(pyridin-2-ylethynyl)benzamide, **2**. In order to suppress amide formation, the concentration of Et_4NHCO_3 was reduced from 90 mM to 40 mM. Reduced base concentration resulted in increased ^{18}F-fluoride incorporation into products and favored distribution between ^{18}F-FPEB and by-product **2**. Increased RCC from 6% to 27% for ^{18}F-FPEB was observed and formation of byproduct **2** decreased to 12% (Scheme 1, entry II). Furthermore, the temperature was lowered to 80 ºC and the reaction time was reduced to 5 minutes, affording ^{18}F-FPEB in excellent RCC of 49 ± 6% (n = 3; Scheme 1, entry III).

In light of these promising results, automated radiosynthesis of ^{18}F-FPEB, validation was subsequently performed to demonstrate the utility of the iodonium(III) ylide precursor for clinical translation. Three consecutive productions of ^{18}F-FPEB were isolated with >200 mCi, at the end of synthesis and formulated for injection, within 1 hour. Analysis of the formulated product (10% ethanol in 0.9% sodium chloride) by HPLC showed high specific activity 666 ± 51.8 GBq/μmol (18 ± 1.4 Ci/μmol) as well high radiochemical purity (≥99%) and chemical purity (≥98%). Validation via an

established quality control protocol (*16*) demonstrated that ^{18}F-FPEB synthesized from iodonium ylide precursor **1** is suitable for human injection (see supplementary materials for full validation data).

DISCUSSION

Radiosynthesis of ^{18}F-FPEB is low yielding by most traditional S_NAr reactions (<5% radiochemical yields), because nucleophilic displacement of common leaving groups (e.g., Cl , Br or NO_2) by ^{18}F-fluoride is not favored when the electron-withdrawing group, i.e., nitrile, is at the *meta* position. Harsh conditions, including high temperatures and prolonged reaction times are generally required and several chemical and radiochemical impurities are usually generated during these reactions, thereby complicating purification. The original radiosynthesis of ^{18}F-FPEB used a chlorinated precursor (Scheme 2, entry I) (*14*). We and other laboratories (*10, 15, 16*) have validated a reproducible radiosynthesis of ^{18}F-FPEB *via* 3-nitro-5-(pyridin-2-ylethynyl)benzonitrile (Scheme 2, entry II) which resulted in 1 – 5 % radiochemical yield for clinical research studies. Notably, our efforts to further optimize the radiochemical yield of ^{18}F-FPEB by use of the nitro-precursor in the presence of reduced base concentrations still required high temperatures to proceed (ca. 150 ^{o}C) and continued to yield a problematic ^{18}F-labeled hydrolysis product as well as chemical byproducts that are difficult to separate (for comparison of semi-preparative HPLC chromatograms see supporting information).. Use of the bromo-precursor , or employing microfluidic technologies demonstrated that the radiotracer could be prepared suitably for human use by conventional radiofluorination or flow chemistry, albeit without an increase in isolated radiochemical yield or simplified purification (*16*). In the present work, attempts to prepare a trimethyl ammonium triflate precursor (*17*), proved to

be a chemical challenge and consistently led to the formation of an undesired methyl pyridinium salt, as predicted to be the thermodynamically favored product (see supplementary materials). The spirocyclic iodonium ylide (**1**) was explored as a novel precursor for ^{18}F-FPEB based on our recent demonstration of the viability of this strategy for radiolabeling a wide range of compounds (Scheme 1, entry III) (*8*).

Radiofluorination of unsymmetrical diaryliodonium compounds are believed to involve a distinct mechanistic pathway compared to traditional S_NAr type reactions, i.e. ^{18}F-fluoride capture followed by reductive elimination to produce ^{18}F-labeled aromatics with C–^{18}F bond formation occurring at the more electron-deficient substituent (*18*). Diaryliodonium precursors for structurally related $mGlu_5$ radiotracers have been radiolabeled (*19*) but were not pursued herein because of the lack of regioselectivity. Nonetheless, diaryliodonium salts have been widely used for preclinical studies, and were recently shown to be suitable for human use with an activated (electron-deficient) aromatic precursor of ^{18}F-flumazenil (*20*). Unlike diaryliodonium salts, which rely on an aryl auxiliary, diaryl iodonium ylides employ an electron-rich β-dicarbonyl auxiliary, resulting in a selective C–^{18}F bond formation and increased chemical stability (*8*). Iodonium ylides were easily purified by silica flash chromatography, which is a challenge with diaryliodonium salts, and are bench stable compounds at room temperature.

A high yield radiosynthesis of ^{18}F-FPEB, via the iodonium ylide based precursor **1**, resulted in 20 ± 5% (n = 3) radiochemical yield ready for injection (Scheme 2), entry III) and represents a 10-fold increase over our previous methodology based on the NO_2 precursor (*16*). The present method is achieved in 60 minutes ready for injection, compared with 90 min by our previous method, and greater than 2-fold increase in

specific activity (18 Ci/μmol). The production was easily automated and validated for routine radiopharmaceutical production, passing our quality control protocol (*16*). Reduced base concentration and temperature suppressed formation of an amide impurity (**2**), identified as a major byproduct formed by base-promoted hydrolysis of ^{18}F-FPEB (Scheme 1). Compound **1** has been stored at room temperature for 2 months and has not shown signs of decomposition by structural characterization and chromatography**,** and has not lost labeling efficiency when reacted with ^{18}F-fluoride. The radiochemical methodology demonstrated herein should prove to be widely applicable to several diagnostic PET imaging agents for $mGlu_5$ that share a similar structural scaffold to ^{18}F-FPEB (*13*).

CONCLUSION

The use of a spirocyclic hypervalent iodine(III)-mediated radiofluorination was shown to provide a high-yielding synthesis of the non-activated aromatic ring of ^{18}F-FPEB and is validated for human imaging studies. A 10-fold increase in radiochemical yield (20%, non-decay corrected) and more than 2-fold increase in specific activity (18 Ci/μmol) compared with our established clinical production procedure was achieved. The methodology described herein should not only facilitate widespread preclinical and clinical use of ^{18}F-FPEB but represents the utility of iodonium ylides as a viable strategy for the practical radiofluorination of non-activated aromatics with ^{18}F-fluoride, and is suitable for human use.

DISCLOSURE

The costs of publication of this article were defrayed in part by the payment of page charges. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18-USC section 1734. No other potential conflict of interest relevant to this article was reported.

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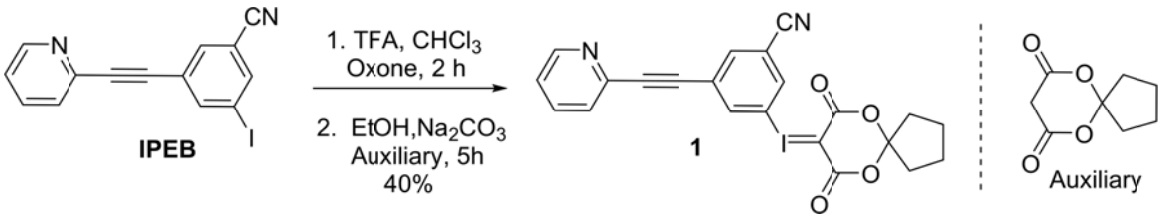
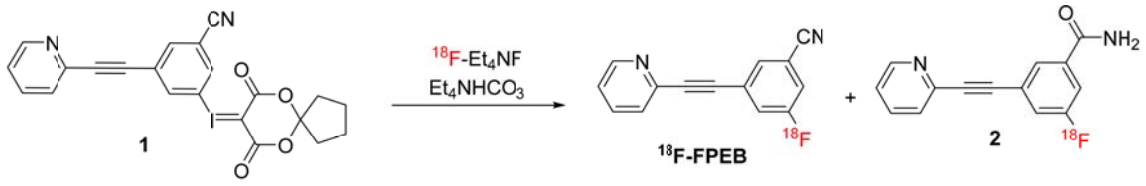
Figure 1. Synthesis of iodonium ylide precursor (1) for radiofluorination.



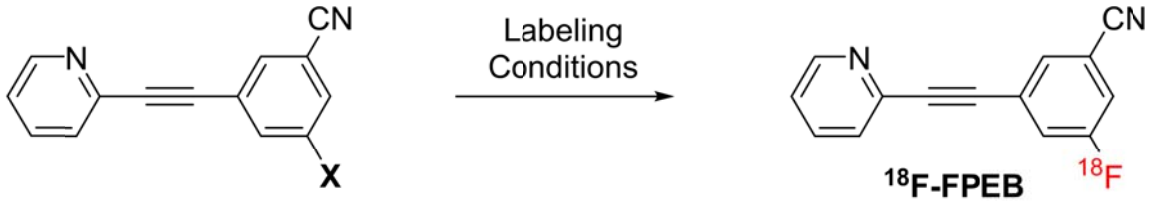
Scheme 1. Manual radiosynthesis of ^{18}F-FPEB[*]

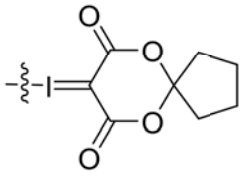


Entry	Et_4NHCO_3 / mg	Temp/ °C	Time / min	^{18}F-FPEB [†]	2[b]
I	7	120	10	6 ± 1 %	18 ± 4 %
II	3	120	10	27 ± 1%	12 ± 4 %
III	3	80	5	49 ± 6 %	≤ 1%

[*]Manual optimization of ^{18}F-FPEB radiosynthesis based on crude reaction mixtures. Conditions: precursor **1** (2 mg), Et_4NHCO_3, DMF (400 µL). [†]Incorporation: % Radiochemical conversion as determined by radio-TLC (*n* = 3). Product identity was confirmed by co-injections of authentic **1** and **2** via radio-HPLC.

Scheme 2. Comparison of ^{18}F-FPEB production yields for clinical research



Entry	X	Labeling Conditions	Reported RCY of ^{18}F-FPEB*	Reference
I	Cl	^{18}F-KF/ K_{222} MW(45 W)	**4%**	**14**
II	NO_2	^{18}F-KF/ K_{222}, 150 °C or ^{18}F-TBAF, 150 °C	**1 -5 %**	**10, 15, 16**
III		^{18}F-Et4NF, 80 °C	**20 %**	This work

*Non-decay corrected radiochemical yield at EOS relative to starting ^{18}F-fluoride.