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# EFFECT OF LEAVING GROUP SUBSTITUENTS ON THE MICROFLUIDIC SYNTHESIS OF [$^{18}$F]3-FLUORO-5-[(PYRIDIN-3-YL)ETHYNYL]BENZONITRILE ([$^{18}$F]FPEB)

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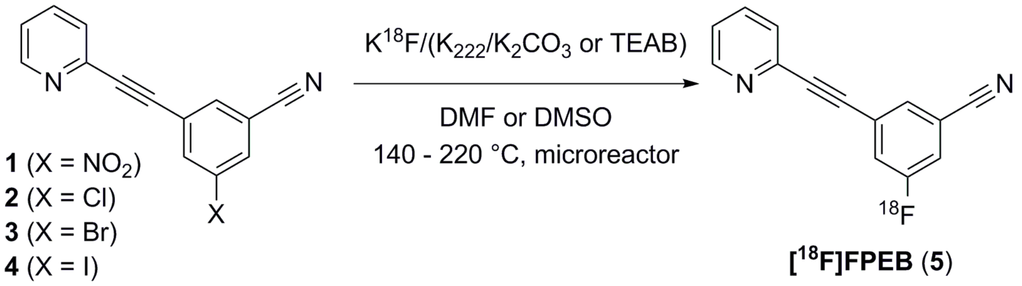
**Abstract** – A commercial microfluidic reactor system has been used to synthesize the mGLUR$_5$ receptor imaging agent [$^{18}$F]FPEB. To study the effect of leaving group substituents on the synthesis of the desired compound, the chloro-, bromo-, iodo-, and nitro-substituted precursors for FPEB were evaluated. Precursor concentrations of 4 – 10 mg/mL were evaluated in various solvents, with temperature ranges between 120 and 220 °C, and total processing times of less than five minutes. Optimized incorporation yields ranged from 5% to 69.4% depending on the precursor used.

## INTRODUCTION

Experiments conducted under microfluidic conditions often provide a number of benefits related to product yields and reaction times when compared to macroscale vial chemistry (experiments performed at millimolar or greater scale in glass flasks or vials). Several types of microfluidic reactors have been developed to take advantage of increased yields and lower processing times and are routinely used in synthesis of radiotracers. Positron emission tomography (PET) has become a useful diagnostic tool in the medical field. Of prime importance to successful PET imaging is the efficient synthesis and purification of radiolabeled products while retaining sufficient activity to obtain a useful image. For this reason, the commonly used PET isotope, [$^{18}$F]fluoride (half life = 109.7 minutes), requires well optimized preparation procedures. Previous experiments have demonstrated the utility of microfluidic devices in the rapid (processing time under five minutes) and high yielding (incorporation yields up to 95%) syntheses of a number of PET radiotracers from nitro precursors using no-carrier-added [$^{18}$F]KF ([$^{18}$F]KF with no added non-radioactive KF).[1] Scheme 1 describes the nucleophilic aromatic substitution reaction used in this study to produce [$^{18}$F]3-fluoro-5-[(pyridin-3-yl)ethynyl]benzonitrile ([$^{18}$F]FPEB). Although substitution of electron withdrawing groups in the *para* position led to the most efficient radiolabeling under microfluidic conditions, several compounds provided evidence for improved labeling of *meta*-substituted compounds.[1] Preliminary studies also demonstrate that nitro displacements are more efficient than iodo displacements. 4-Nitrobenzonitrile and 4-iodobenzonitrile provided [$^{18}$F]4-fluorobenzonitrile in yields of 89% and 31%, respectively, when labeled using microfluidic technology, suggesting nitro displacements are more efficient under microfluidic conditions than halide displacements.[2] Since [$^{18}$F]FPEB is commonly produced from fluorodehalogenations, a comparison between three halide precursors and the nitro precursor will be made under microfluidic conditions.



**Scheme 1.** Typical conditions for nucleophilic aromatic substitution reaction using various FPEB precursors

[$^{18}$F]FPEB (**5**) has been shown to be a high affinity binding agent to the metabotropic glutamate receptor 5 (mGluR$_5$). Autoradiography studies have shown a regional distribution with the highest binding sites density in the cortex, striatum, and hippocampus, moderate in the thalamus and the lowest in the cerebellum of three mammalian species.[3] Recently presented studies have shown that [$^{18}$F]FPEB could be a useful PET imaging agent for *in vivo* evaluation of neurological conditions involving defects in metabotropic glutamate receptors, including pain and addiction diseases as well as neurodegenerative diseases.[4] Human evaluation of [$^{18}$F]FPEB as a PET tracer has been conducted and gave positive results both in safety and repeatability.[5] Vial synthesis under microwave conditions and subsequent purification of [$^{18}$F]FPEB generally requires 1 – 2 hours with low radiochemical yield starting from the chloro precursor.[6] Synthesis of [$^{18}$F]FPEB has also been reported by copper mediated displacement of boronic acids, however these reactions required 20 minutes and radiochemical conversions of 4 – 8% depending on the conditions.[7] Related studies using the nitro precursor **1** have produced [$^{18}$F]FPEB with high specific activity and in sufficient quantity for *in vitro* and *in vivo* studies using microfluidic reactors but a direct comparison with halide precursors using identical conditions has not been made.[8] A recent study presented a high yielding synthesis of [$^{18}$F]FPEB from iodonium ylide precursors, but only halide and nitro precursors are examined here.[9] In addition, a variety of fluoride eluents and solvents have been tested to determine their effects on the fluorination reaction to produce [$^{18}$F]FPEB.

## RESULTS AND DISCUSSION

The FPEB precursor ($NO_2$, Cl, Br, or I) (**1 – 4**) was dissolved in anhydrous dimethyl sulfoxide (DMSO) or *N*,*N*-dimethylformamide (DMF), at a concentration of 4 – 10 mg/mL. [$^{18}$F]Fluoride solution was prepared using a concentrator module of the NanoTek® system. Potassium carbonate - Kryptofix® ($K_{222}$) or tetraethylammonium bicarbonate (TEAB) was used to elute the [$^{18}$F]fluoride from the Bio-Rad AG® MP-1M Anion Exchange Resin trap (MP1), a strongly basic quaternary ammonium anion exchanger with a carbonate counter ion, or QMA fluoride trap, a silica-based hydrophilic anion exchanger with 300 Å pore size and a carbonate counter ion, followed by the azeotropic drying step (3 x 0.25 mL acetonitrile at 105 °C) and dilution with the solvent used to dissolve the FPEB precursor. Both precursor and the fluoride solutions were then loaded onto storage loops and passed through the microfluidic reactor (100 µm x 2 m) using the Discovery mode of the NanoTek® system.[10] Initial experiments were started at a temperature of 140 °C and then increased in 20 degree increments using an initial fluoride solution (Fluoride pump; "P3") flow rate of 30 µL/min and ratio of precursor (Precursor; "P1") to fluoride (P3) = 1:1. This resulted in a combined flow rate through the microfluidic reactor of 60 µL/min. The latter two variables (P3 flow rate and P1: P3 ratio) were then adjusted using the temperature found to generate the highest yield among the initial experiments until optimized reaction conditions were attained. Incorporation yields (crude radiochemical yields) were determined by radio-TLC. Product identity was confirmed by co-injection with an authentic sample using an analytical HPLC equipped with dual gamma and UV detector.

Experiments employing FPEB precursor at 4 mg/mL (**Tables 1** and **2**) showed that the nitro group was a far more effective leaving group than any of the halides. At 4 mg/mL precursor concentration, **1** gave much better isotope incorporation in DMF than DMSO (52.3% and 24.6%, respectively; **Table 1**, entry 1 and **Table 2**, entry 1). However, upon increasing the precursor concentration to 10 mg/mL, DMSO incorporations increased dramatically to 69.4% (**Table 4**, entry 5). Precursor **2** gave better optimized isotope incorporation in DMF than DMSO (16.1% and 7.3%, respectively; **Table 1**, entry 2 and **Table 2**, entry 2). Neither DMSO nor DMF provided any notable solvent effect on the optimized radiochemical yields for precursors **3** and **4** (**Table 1**, entries 3 and 4, **Table 2**, entries 3 and 4). Fluoride eluent composition (potassium carbonate-$K_{222}$, potassium oxalate-$K_{222}$, or tetraethylammonium bicarbonate) affected the incorporation yields of the fluorodenitration of **1**, with potassium carbonate-$K_{222}$ (**Table 4**, entries 2 and 5) giving better results than potassium oxalate-$K_{222}$ (**Table 4**, entries 3 and 6) consistently.

**Table 1.** Experiments conducted in DMF with potassium carbonate-$K_{222}$ fluoride eluent

| Precursor | Precursor concentration (mg/mL) | Solvent | Fluoride eluent | Optimum temperature (°C) | Optimum flow rate (µL/min) | Optimum Precursor: Fluoride ratio | Optimized yield (%) |
|---|---|---|---|---|---|---|---|
| **1** | 4 | DMF | $K_{222}/K_2CO_3$ | 200 | 5 | 2 | 52.3 |
| **2** | 4 | DMF | $K_{222}/K_2CO_3$ | 210 | 20 | 1 | 16.1 |
| **3** | 4 | DMF | $K_{222}/K_2CO_3$ | 210 | 30 | 1 | 10.6 |
| **4** | 4 | DMF | $K_{222}/K_2CO_3$ | 200 | 20 | 2 | 12.7 |

**Table 2.** Experiments conducted in DMSO with potassium carbonate-$K_{222}$ fluoride eluent

| Precursor | Precursor concentration (mg/mL) | Solvent | Fluoride eluent | Optimum temperature (°C) | Optimum flow rate (µL/min) | Optimum Precursor: Fluoride ratio | Optimized yield (%) |
|---|---|---|---|---|---|---|---|
| **1** | 4 | DMSO | $K_{222}/K_2CO_3$ | 210 | 15 | 2 | 24.6 |
| **2** | 4 | DMSO | $K_{222}/K_2CO_3$ | 200 | 20 | 3 | 7.3 |
| **3** | 4 | DMSO | $K_{222}/K_2CO_3$ | 210 | 5 | 2 | 13.4 |
| **4** | 4 | DMSO | $K_{222}/K_2CO_3$ | 210 | 5 | 3 | 12.5 |

**Table 3.** Experiments conducted at 10 mg/mL precursor concentration in DMSO with tetraethylammonium bicarbonate fluoride eluent

| Precursor | Precursor concentration (mg/mL) | Solvent | Fluoride eluent | Optimum temperature (°C) | Optimum flow rate (µL/min) | Optimum Precursor: Fluoride ratio | Optimized yield (%) |
|---|---|---|---|---|---|---|---|
| **1** | 10 | DMSO | TEAB | 220 | 5 | 1 | 65.2 |
| **2** | 10 | DMSO | TEAB | 210 | 5 | 1 | 26.2 |
| **3** | 10 | DMSO | TEAB | 220 | 5 | 2 | 29.5 |
| **4** | 10 | DMSO | TEAB | 220 | 5 | 1 | 12.5 |

**Table 4.** Experiments conducted with nitro precursor with various solvents and fluoride eluents

| Precursor | Precursor concentration (mg/mL) | Solvent | Fluoride eluent | Optimum temperature (°C) | Optimum flow rate (µL/min) | Optimum Precursor: Fluoride ratio | Optimized yield (%) |
|---|---|---|---|---|---|---|---|
| **1** | 4 | DMF | $K_{222}/K_2CO_3$ | 200 | 5 | 2 | 52.3 |
| **1** | 4 | DMSO | $K_{222}/K_2CO_3$ | 210 | 15 | 2 | 24.6 |
| **1** | 4 | DMSO | $K_{222}/K_2C_2O_4$ | 210 | 30 | 1 | 8.5 |
| **1** | 5 | DMSO | $K_{222}/K_2C_2O_4$ | 210 | 10 | 1 | 20.6 |
| **1** | 10 | DMSO | $K_{222}/K_2CO_3$ | 210 | 5 | 1 | 69.4 |
| **1** | 10 | DMSO | $K_{222}/K_2C_2O_4$ | 200 | 5 | 1 | 32.1 |
| **1** | 10 | DMSO | TEAB | 220 | 5 | 1 | 65.2 |

At 10 mg/mL precursor concentration, the incorporation yields of the fluorodenitration of **1** were virtually identical when employing potassium carbonate-$K_{222}$ (**Table 4**, entry 5) and tetraethylammonium bicarbonate (**Table 4**, entry 7) in DMSO, with both above 65%, whereas potassium oxalate-$K_{222}$ provided only 32.1% incorporation (**Table 4**, entry 6). Optimized conditions for the 4 mg/mL precursor series of reactions ranged between 200 – 210 °C with P3 flow rates between 5 – 30 µL/min and ratios of precursor solution to fluoride solution between 1:1 and 3:1.

Reactions with precursor concentration of 10 mg/mL (**Table 3**) were then performed to ascertain whether increasing the amount of precursor would increase incorporation yield. These higher concentration reactions employed tetraethylammonium bicarbonate as fluoride eluent to decrease the likelihood of clogging the microfluidic reactors at high temperatures and low flow rates due to salt precipitation as a result of thermal decomposition of Kryptofix® complexes. At 4 mg/mL of precursor in DMF, the order of reactivity was found to be $NO_2$>Cl>I>Br (**Table 1**). At 4 mg/mL of precursor in DMSO, the order of reactivity was found to be $NO_2$>Br>I>Cl (**Table 2**). At 10 mg/mL of precursor in DMSO, the order of reactivity was found to be $NO_2$>Br>Cl>I (**Table 3**). Optimized reaction conditions at the 10 mg/mL precursor concentration ranged from 200 – 220 °C, with P3 flow rates of 5 – 10 µL/min, and precursor to fluoride ratios of 1:1 or 2:1. Attempts at experiments using DMF solvent with 10 mg/mL precursor led to microfluidic reactor clogging. A possible reason for the low reactivity of the iodo compound, **4**, is that the steric bulk of the iodine atom prevents the formation of the intermediate complex despite the fact that iodide is normally considered a good leaving group.

Since the nitro precursor proved to be the best yielding, experiments to test various fluoride eluents were performed using nitro precursor **1**, presented in **Table 4**. Fluoride eluent solutions of potassium carbonate-$K_{222}$, potassium oxalate-$K_{222}$, and tetraethylammonium bicarbonate were tested. At 4 mg/mL precursor concentration in DMSO, potassium carbonate-$K_{222}$ proved superior to potassium oxalate-$K_{222}$, with optimized incorporation yields of 24.6% (**Table 4**, entry 2) and 8.5% (**Table 4**, entry 3) respectively. By increasing the concentration of precursor, optimized incorporation yields using potassium oxalate-$K_{222}$ improved to 20.6% at 5 mg/mL precursor (**Table 4**, entry 4), and to 32.1% with 10 mg/mL precursor (**Table 4**, entry 6). However, when the precursor concentration was increased from 4 mg/mL to 10 mg/mL using potassium carbonate-$K_{222}$, there was a much greater improvement in incorporation yield, from 24.6% (**Table 4**, entry 2) to 69.4% (**Table 4**, entry 5). Tetraethylammonium bicarbonate was employed in higher temperature reactions since it is less likely to thermally degrade than a Kryptofix® complex, which can cause blockage of the microfluidic channel. The incorporation yield using tetraethylammonium bicarbonate was comparable to that using potassium carbonate-$K_{222}$, 65.2% and 69.4% respectively (**Table 4**, entries 5 and 7).

## CONCLUSION

Experimental procedures for microfluidic fluorodenitration developed on model radiotracers have been applied to the synthesis of [$^{18}$F]FPEB and the fluorodenitration reactions provided superior incorporation yields of the product. [$^{18}$F]FPEB was synthesized from four different precursors in crude labeling yields of up to 69.4%. The optimum conditions for this reaction employed potassium carbonate - Kryptofix® $K_{222}$ as fluoride eluent, DMSO as solvent, precursor **1** at a concentration of 10 mg/mL. The optimum temperature and flow rate were 210 °C and 5 μL/min and optimum precursor:fluoride solution ratio of 1:1 to produce an incorporation yield of 69.4%. Total reaction times were less than five minutes providing an advantage over current literature procedures. The ability to synthesize [$^{18}$F]FPEB more efficiently provides the opportunity to increase its role in *in vitro*, autoradiographical, and *in vivo* testing.

## EXPERIMENTAL

The FPEB precursors were provided by Molecular NeuroImaging (New Haven, CT). [$^{18}$F]Fluoride was made from recycled [$^{18}$O]water ([$^{18}$O]water that has been re-purified after its initial use to remove organic and inorganic impurities so it may be reused as a cyclotron target) and purchased from PETNET (Knoxville, TN). Anhydrous solvents were purchased from Acros and used as received. Inorganic salts were purchased from Fisher and used as received. Radio thin layer chromatography was performed with radiation detection using a BioScan AR-2500 radio-TLC reader and WinScan 1.3 software. TLC plates were developed in a mixture of 9:1 $CHCl_3$:MeOH. Analytical radio-HPLC analyses were performed on an Agilent 1200 series instrument employing a 254 nm UV detector and a Phenomenex phenylhexyl column, 5 μm, 4.6 x 250 mm, using a 60:40 mixture of MeCN:0.1 *M* ammonium formate buffer. Fluorine-18 labeling was performed in 100 μm x 2 m reactors using the Discovery mode of the Advion NanoTek$^{®}$ Microfluidic Synthesis System (Ithaca, NY) controlled by NanoTek$^{®}$ LF 1.4 Software. Samples were collected in plastic, capped sample vials upon exit from the reactor for analysis. The potassium carbonate-K$_{222}$ solution used was made by dissolving 300 mg of Kryptofix$^{®}$ K$_{222}$ and 45 mg of potassium carbonate in 10 mL of 95:5 MeCN:H$_2$O. The potassium oxalate-K$_{222}$ solution used was made by dissolving 100 mg of Kryptofix$^{®}$ K$_{222}$ and 13.3 mg potassium oxalate in 10 mL of 87.5:12.5 MeCN:H$_2$O. Tetraethylammonium bicarbonate solution was 5 mg/mL in 90:10 MeCN:H$_2$O.

## GENERAL PROCEDURE FOR PRODUCTION OF [$^{18}$F]FPEB (5)

The FPEB precursor (**1**, **2**, **3**, or **4**) was placed in a 1 mL glass tapered bottom vial and sealed with a septum. Solvent (DMSO or DMF) was added to create a solution of the desired concentration. [$^{18}$F]Fluoride (~50 mCi, specific activity >1 Ci/μmol) was loaded onto the system and eluted from the ion exchange trap (MP1, or QMA trap if tetraethylammonium bicarbonate fluoride eluent was used) with one of the following eluents: acetonitrile/water potassium carbonate-K$_{222}$ solution, acetonitrile/water potassium oxalate-K$_{222}$ solution, or tetraethylammonium bicarbonate solution. Optimum temperatures ranged between 200 – 210 °C. Optimum flow rates ranged from 5 – 30 μL/min and optimum ratios of precursor solution to fluoride solution ranged between 1:1 and 3:1.

## THE MICROFLUIDIC REACTOR

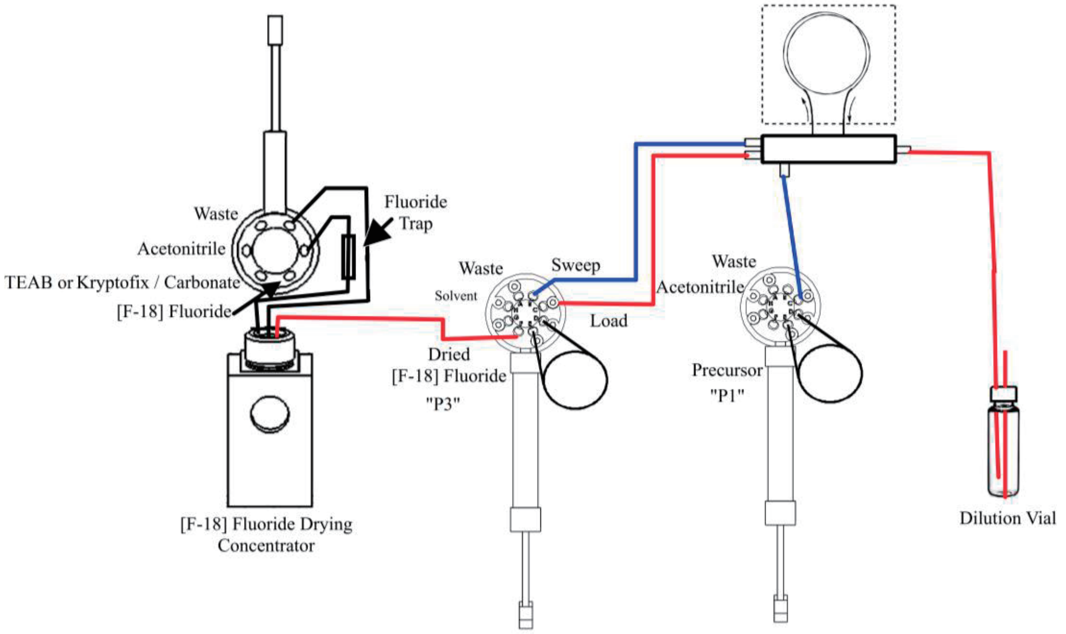


**Figure 1.** The configuration of the NanoTek$^{®}$ microfluidic reactor system

The microfluidic reactor system (Figure 1) utilized in these studies consists of two modules: a concentrator and reactor module and a base module. The system was placed in a fume hood and the concentrator and reactor module enclosed within a lead lined box. The modules are controlled via computer software which monitors the cleaning, fluoride drying, reagent loading, reaction, and sweep stages after several manual inputs. Before and after daily operations, the system was cleaned with dry MeCN. After setup, vials of reagent solutions and solvents were connected to tubing for loading into the appropriate modules. The fluoride was dried according to standard NanoTek® drying protocols (sequential addition/evaporation of anhydrous MeCN). The reagents were then loaded into the system and optimum reaction conditions were then determined by performing up to 30 reactions using the same reagents. The NanoTek® LF system configuration and its operation have been previously described.[10-11]

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