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An efficient new method for the synthesis of 3-[^{18}F]fluoro-4-aminopyridine via Yamada-Curtius rearrangement

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Abstract

4-Aminopyridine is a clinically approved drug to improve motor symptoms in multiple sclerosis. A fluorine-18-labeled derivative of this drug, 3-[^{18}F]fluoro-4-aminopyridine, is currently under investigation for positron emission tomography (PET) imaging of demyelination. Herein, the Yamada-Curtius reaction has been successfully applied for the preparation of this PET radioligand with a better radiochemical yield and improved specific activity. The overall radiochemical yield was 5 to 15% (n = 12, uncorrected) with a specific activity of 37 to 148 GBq/μmol (end of synthesis) in a 90 minute synthesis time. It is expected that this 1 pot Yamada-Curtius reaction can be used to prepare similar fluorine-18-labeled amino substituted heterocycles.

Keywords

aminopyridine; F-18; multiple sclerosis; PET; Yamada-Curtius rearrangement

1 | INTRODUCTION

Multiple sclerosis is a common immune-mediated disease of the central nervous system in which myelin, the insulating membrane that wraps around axons, is damaged. This damage —which is often repaired through the process of remyelination—disrupts the ability of parts of the nervous system to communicate, resulting in neurological deficits that may include physical, mental, and sometimes psychiatric problems. 4-Aminopyridine is a clinically

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SUPPORTING INFORMATION

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approved drug to improve walking ability in multiple sclerosis patients.[1] Its fluorinated derivative 3-[^{18}F]fluoro-4-aminopyridine is currently being studied as a potential tracer for demyelination.[2–4]

Pyridine derivatives are common in pharmaceuticals and radiopharmaceuticals for a variety of biological activities.[5–8] Compared with other heterocycles, these derivatives are the most widely studied for fluorine-18 labeling due to the availability of the precursor and the ease of incorporation of fluorine-18 at the *ortho* and/or *para* position(s). Unlike in benzene, nucleophilic aromatic substitution (S_NAr) at the *ortho* or *para* position in a pyridine ring does not require additional electron-withdrawing groups.[9,10] The nitrogen atom of the pyridine lowers the aromatic stability and stabilizes the intermediate Meisenheimer complex by both inductive and mesomeric effects.[11,12] However, substitution at *meta* position remains challenging, often requiring an electron-withdrawing group at *ortho* and/or *para* to the leaving group for successful fluorination.[13,14]

Chun and Pike reported a radiosynthesis of *meta*-[^{18}F] fluoropyridine from its iodonium salt precursor.[15] Abrahim et al reported *meta* fluorination of pyridine via displacement of a Br or Cl substituent with an activating group (cyano, nitro, or carboxamide) at the *ortho* position to the pyridine nitrogen.[16] In this substitution, the 4-bromo-2-nitro derivative does not yield the desired fluorinated nitro pyridine, as the nitro group is preferentially displaced by fluorine. Brugarolas et al reported a novel approach to direct the fluorination at the position *ortho* to the nitro group by using pyridine *N*-oxide derivative. They prepared the fluorine-18 analog of 3-fluoro-4-aminopyridine (Scheme 1) by halogen (Br or F) exchange followed by hydrogenation. Nevertheless, this method suffered from low overall radiochemical yield or low specific activity.[17] Therefore, new methods to prepare fluorine-18 labeled 3-fluoro-4-aminopyridine in better radiochemical yield and higher specific activity are warranted.

Here, we explore the possibility of labeling methyl-3-nitroisonicotinate followed by hydrolysis of the ester and conversion of the acid to the amine via the Yamada-Curtius rearrangement (Scheme 2). Nonradioactive fluorination of the methyl-3-nitroisonicotinate has been reported using CsF with 38% yield.[18] To our knowledge, the Yamada-Curtius rearrangement has never been used in the synthesis of PET radiopharmaceuticals. This method may be a superior alternative to other literature methods for preparing fluorine-18-labeled aminoheterocycles.[17,19]

2 | MATERIALS AND METHODS

Precursors and nonradioactive cold standards were obtained from Combi-Blocks (San Diego, CA, USA). All other chemicals and solvents were received from Sigma- Aldrich (St Louis, MO, USA) and used without further purification. Fluorine-18 was obtained from National Institutes of Health cyclotron facility (Bethesda, MD, USA). Chromafix 30-PS-HCO_3 anion-exchange cartridges were purchased from Macherey-Nagel (Düren, Germany). A semipreparative column for the purification of the final labeled product was obtained from Phenomenex (Torrance, CA, USA). Columns, iTLC-SG plates, and all other Sep-Pak cartridges used in this synthesis were obtained from Agilent Technologies (Santa Clara, CA, USA) and Waters (Milford, MA, USA), respectively. Alumina N cartridge was conditioned

with 5 mL of water. High-performance liquid chromatography (HPLC) purification and analytical HPLC analyses for radiochemical work were performed on an Agilent 1200 Series instrument equipped with multiwavelength detectors. iTLC-SG papers were developed by using acetonitrile. The papers were read in an Eckert & Ziegler TLC scanner (B-AR2000-1).

2.1 | Experimental procedure for manual synthesis

2.1.1 | Synthesis of 3-[^{18}F]fluoroisonicotinic acid (2)—Methyl 3-nitroisonicotinate (10 mg) in acetonitrile (0.3 mL) was added to the azeotropically dried mixture of [^{18}F]KF/K_{222} (2 mg K_2CO_3, 10 mg K_{222}) and heated at 80°C for 15 minutes. The reaction mixture was cooled to 40°C and treated with 100 μL sodium hydroxide (0.25 N) followed by heating at 100°C for 10 minutes. The solution was cooled to 40°C and acidified with hydrochloric acid (150 μL, 0.25 N). About 200 μL of triethylamine in 0.5 mL acetonitrile was added to the solution and evaporated to dryness. The residue was further dried by coevaporation with 1 mL acetonitrile.

2.1.2 | Synthesis of 3-[^{18}F]fluoro-4-aminopyridine (3)—A solution of 20 μL diphenylphosphoryl azide (DPPA) in dimethyl sulfoxide (300 μL) was added to the dried residue of intermediate (**2**). The mixture was heated at 130°C for 10 minutes, then diluted with 2 mL HPLC solvent and passed through an activated (5 mL water) alumina Sep-Pak plus and washed with 2 mL HPLC solvent. The combined crude product was purified by HPLC using a semipreparative column. HPLC conditions: Phenomenex Luna column, 5 μm, 9.4 × 250 mm; 5% ethanol in water (0.1% triethylamine); flow rate, 4 mL/min, t_R = ~16 minutes.

2.2 | Experimental procedure for automated synthesis

2.2.1 | Azeotropic drying of [^{18}F]fluoride—Typically, 11.1 GBq [^{18}F]fluoride in 2.5 mL of target water was passed through the PS-HCO_3 cartridge, and the cartridge was rinsed with 1 mL of acetonitrile. [^{18}F]Fluoride was eluted from the cartridge into Reactor 1 with the eluent (2 mg K_2CO_3, 10 mg K_{222} in 1 mL MeOH, and 200 μL water) in Vial 1 and dried under N_2/vacuum at 75°C for 4 minutes. Reactor 1 was cooled to 50°C, acetonitrile in Vial 2 was added, and the activity was azeotropically dried at 55°C for 3 minutes, then at 95°C for 3 minutes under N_2/vacuum. The activity was further dried by using a vacuum for 3 minutes. The [^{18}F]fluoride drying cycle took about 20 minutes.

2.2.2 | Synthesis of [^{18}F]fluoroisonicotinic acid (2)—Methyl 3-nitroisonicotinate (10 mg) in acetonitrile (0.5 mL) in Vial 3 was added to the azeotropically dried mixture of [^{18}F]KF/K_{222} in Reactor 1. The mixture was heated at 80°C for 15 minutes. The reaction mixture was cooled to 40°C, and 150 μL sodium hydroxide (0.25 N) in Vial 4 was added, followed by heating at 100°C for 10 minutes. The solution was cooled to 40°C and acidified with hydrochloric acid (200 μL, 0.25 N) in Vial 5. The content in Reactor 1 was transferred to Reactor 2. Reactor 1 was rinsed by adding triethylamine (200 μL) in 0.5 mL acetonitrile in Vial 6; the mixture was also transferred to Reactor 2. The combined mixture in Reactor 2 was evaporated to dryness under N_2 and vacuum. The residue was further dried with 1 mL acetonitrile in Vial 7.

2.2.3 | Synthesis of 3-[^{18}F]fluoro-4-aminopyridine (3)—A solution of DPPA (20 μL) in dimethyl sulfoxide (300 μL) in Vial 8 was added to the dried residue of intermediate (**2**). The mixture was heated at 130°C for 10 minutes, diluted with 2 mL ammonium hydroxide (5%) in water in Vial 9, and passed through an activated (5 mL water) Alumina Sep-Pak plus. HPLC solvent in Vial 10 (2 mL) was added to wash Reactor 2, which was also passed through the Alumina Sep-Pak plus. The combined crude product was purified by HPLC by using a semipreparative column. HPLC conditions: Phenomenex Luna C18^2 column, 5 μm, 9.4 × 250 mm; 5% ethanol in water (0.1% ammonium hydroxide); flow rate, 4 mL/min, t_R = 15 to 17 minutes. The fraction containing the product was collected in a product vial through a 0.22 μm sterile filter. The pH value of the final dose was adjusted to ~7 by using 0.1 MH_3PO_4 solution in water. The identity of the product was checked by analytical HPLC. HPLC conditions: Agilent XDB-C18 column, 5 μm, 4.6 × 150 mm; 5% acetonitrile in water (0.1% triethylamine), 1 mL/min. The total synthesis time is approximately 90 to 95 minutes.

3 | RESULTS AND DISCUSSION

Fluorine 18 labeling was achieved in 3 steps (Scheme 2). Labeling efficiency was first standardized manually by using low amounts of starting activity (0.11-0.37 GBq). Briefly, nitro-precursor **1** (10 mg) was heated with [^{18}F] KF/K_{222} at 80°C for 15 minutes. Fluorine-18 incorporation was monitored by analytical HPLC analysis (Figure 1A). The identity of the fluorinated ester was confirmed by comparing its HPLC retention time with authentic nonradioactive methyl 3-fluoroisonicotinate coinjected in analytical HPLC (Figure 1B). In the HPLC system, radiodetector is connected after UV detector. The slight difference (0.2 min) of retention time between UV and radiation trace is due to the dead volume between these 2 detectors.

Deprotection of the methyl ester group was achieved with sodium hydroxide at 100°C for 5 minutes in the same pot with no need for purification of the methyl ester. Progress of the reaction was monitored by analytical HPLC (Figure 1C).

Reaction of the intermediate 3-[^{18}F]fluoroisonicotinic acid **2** with DPPA at 130°C in the presence of triethylamine produced the final product 3-[^{18}F]fluoro-4-aminopyridine **3** via Yamada-Curtius rearrangement. This reaction was also carried out on the same flask without the need for purification of the intermediate. The final product (**3**) was purified by HPLC using a semipreparative column. The overall radiochemical yield was 5 to 15% (uncorrected, n = 12) in 90 minutes, with >98% radiochemical purity by analytical HPLC (Figure 2A). The identity of the final product (**3**) was confirmed by comparing its HPLC retention time with authentic nonradioactive 3-fluoro-4-aminopyridine coinjected in analytical HPLC (Figure 2B). The final product was also checked by radio TLC and found to be free of unreacted fluoride- 18 (Figure S1).

After successful manual standardization of the procedure, the fully automated synthesis was performed in a GE Tracerlab FX-N Pro module (Figure 3). Azeotropic drying, fluorination, and hydrolysis were performed in Reactor 1. After hydrolysis, compound **2** was transferred to Reactor 2 and converted to the final product, 3-[^{18}F] fluoro-4-aminopyridine (**3**). The overall radiochemical yield was comparable with the manual synthesis with a specific

activity of 37 to 148 GBq/μmol (n = 12). As reported in the recent literature,[17] this tracer was synthesized with 2.5% (noncorrected) of radiochemical yield and with a specific activity of 0.37 to 3.7 GBq/μmol.

4 | CONCLUSIONS

3-[^{18}F]Fluoro-4-aminopyridine (**3**) is a PET radioligand under investigation for imaging demyelination and remyelination. Despite the simple nature of this compound, its labeling is challenging due to the *meta* position of the fluorine substituent. Previous syntheses solved this problem by starting from pyridine *N*-oxide, but this required hydrogen gas and suffered from low radiochemical yield or low specific activity. We improved the synthesis by employing the Yamada-Curtius rearrangement and here describes a fully automated method to prepare **3** with moderate radiochemical yield and high specific activity in 90 minutes of synthesis time. Successful radiolabeling performed at multiple institutions indicates that this radiolabeling method is robust. Finally, the automated synthesis allows facile translation to clinical production of tracer, repeatedly, in a safe and sterile manner for patient applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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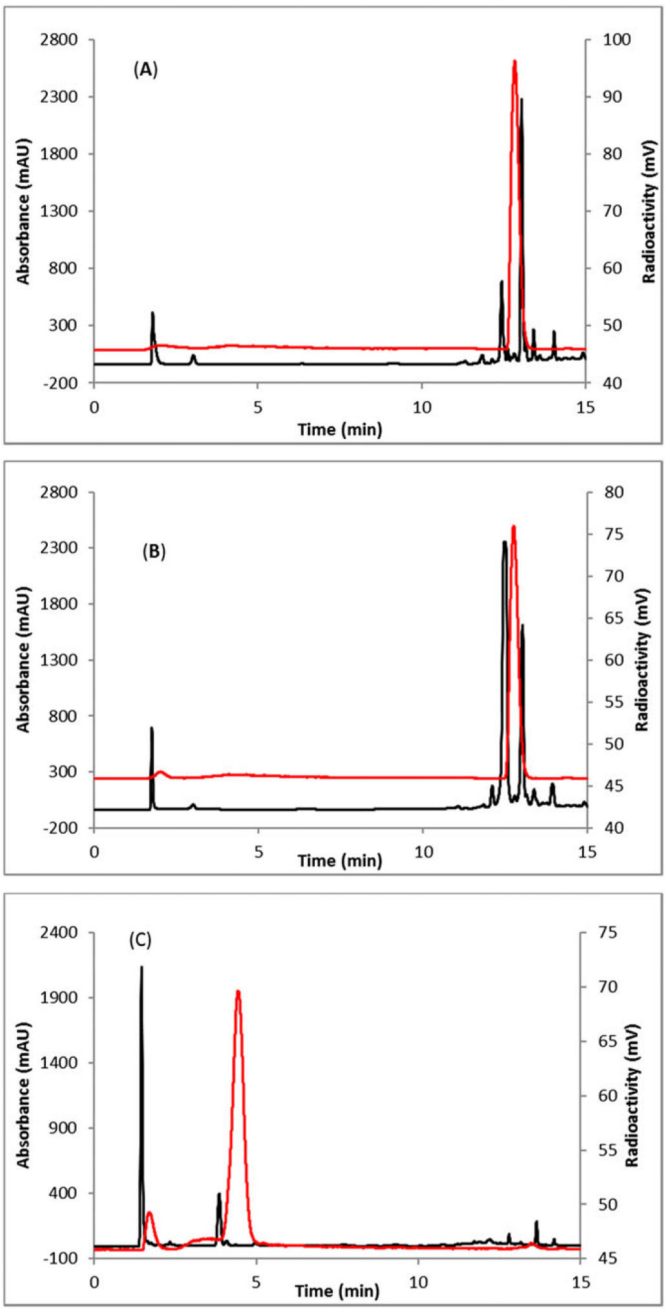


FIGURE 1.

HPLC analysis of crude A, methyl 3-[^{18}F] fluoroisonicotinate, B, methyl 3-[^{18}F]fluoroisonicotinate coinjected with nonradioactive cold standard, and C, [^{18}F]fluoroisonicotinic acid (**2**). HPLC conditions: Agilent Eclipse XDB C18 column (5 μm, 4.6 × 150 mm), mobile phase: 1 to 5% B in 8 minutes, 5 to 90% B in 15 minutes. A = water (0.1% TFA), B = acetonitrile, with a flow rate of 1 mL/min. Red line, in-line radiodetection; black line, UV detection at 254 nm

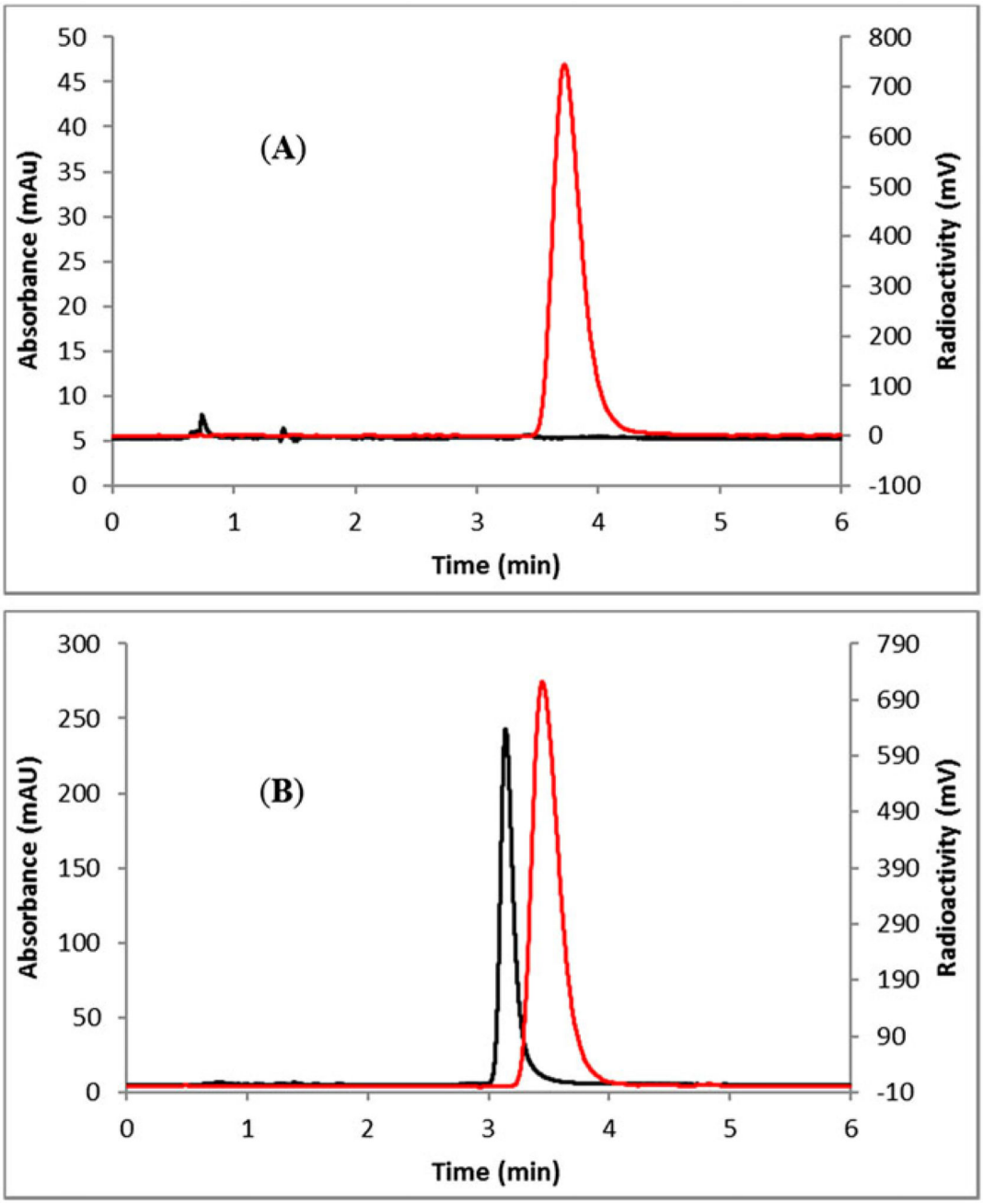


FIGURE 2.

HPLC analysis of A, 3-[^{18}F]fluoro-4-aminopyridine (**3**) and B, 3-[^{18}F]fluoro-4-aminopyridine (**3**) coinjected with the nonradioactive standard. HPLC conditions: Phenomenex Luna C18 column (5 μm, 4.6 × 100 mm), mobile phase: 5% acetonitrile in water (0.1% triethylamine), with a flow rate of 1 mL/min. Red line, in-line radiodetection; black line, UV detection at 254 nm

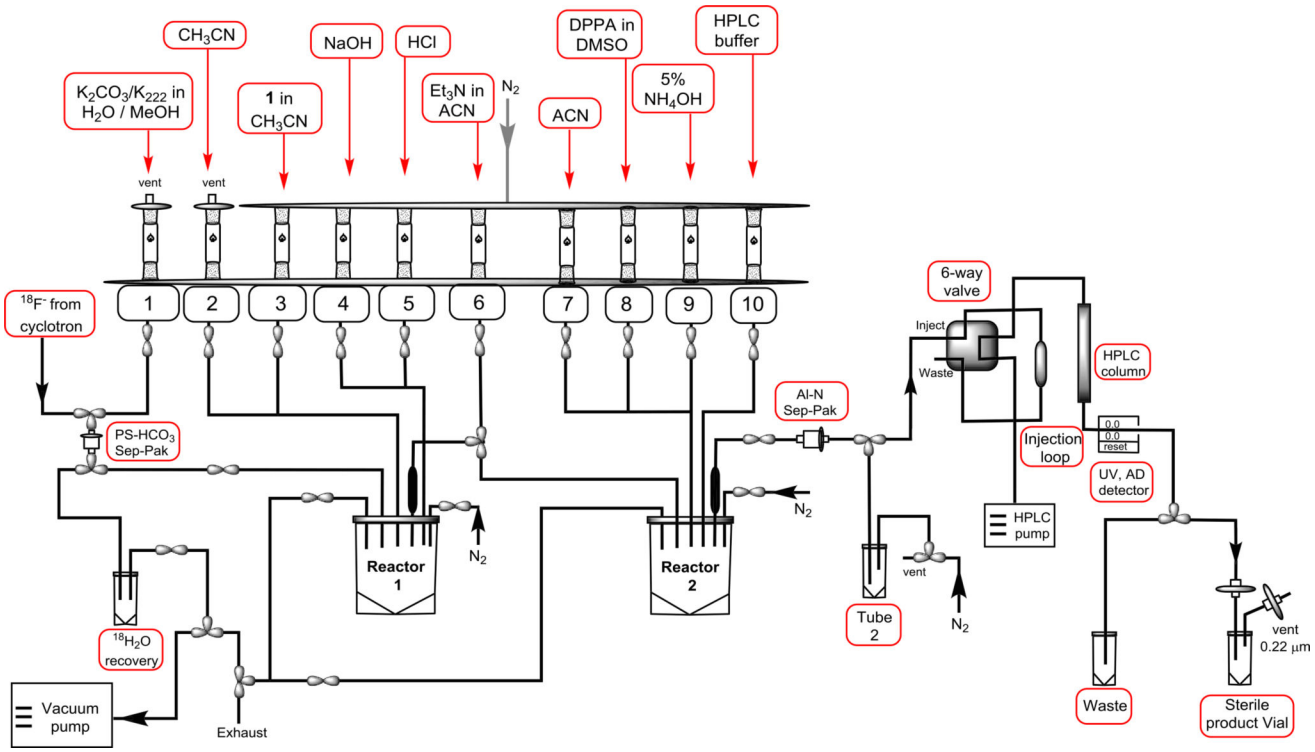
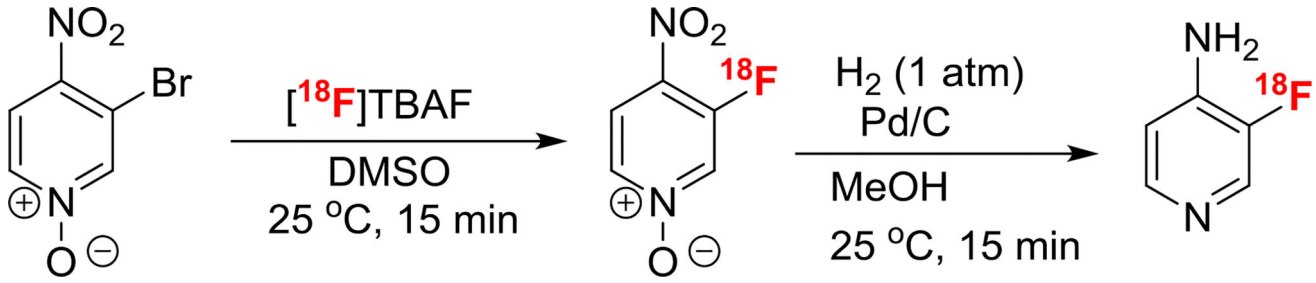
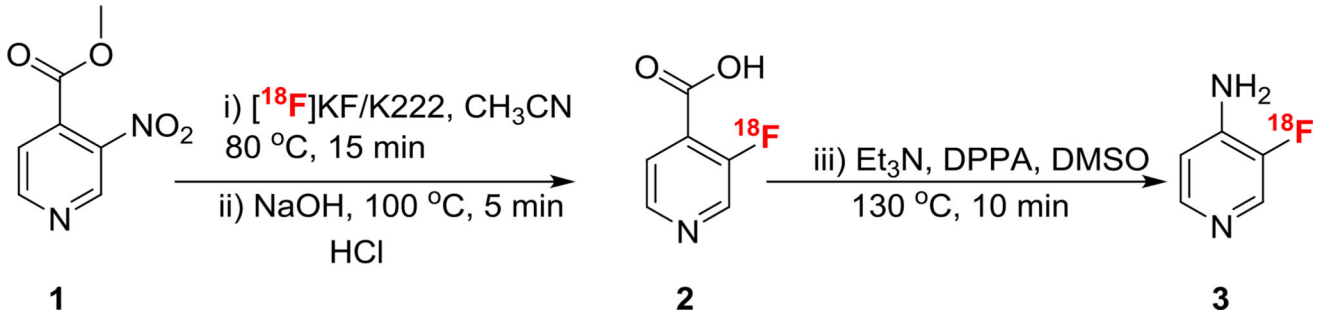


FIGURE 3.
Schematic diagram of the automated synthesis on GE FX-N Pro Module



SCHEME 1.
Literature method[17] for the preparation of 3-[^{18}F] fluoro-4-aminopyridine



SCHEME 2.

Preparation of 3-[^{18}F] fluoro-4-aminopyridine via Yamada- Curtius rearrangement