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Radiofluorination and reductive amination using a microfluidic device

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A commercial microfluidic device (NanoTek, Advion) was used as a synthesis platform for the preparation of fluorine-18 labelled tertiary amines in two consecutive steps. Firstly, the nucleophilic radiofluorination of an aromatic aldehyde and secondly, the reductive amination to produce the corresponding amine. Fluorine-18 labelled [^{18}F]fluorobenzaldehyde ([^{18}F]2) was obtained in an analytical radiochemical yield (rcy) of 93% and a preparative yield of 60% (decay corrected). The produced [^{18}F]2 was applied in two model reactions yielding [^{18}F]5 and [^{18}F]6 in analytical rcy 70 and 75%, respectively. To further test the utility of this methodology, a delta opioid agonist, [^{18}F]8, was also radiolabelled using the same setup in an analytical rcy of 29%.

In a preparative run, 1050 MBq (28.4 mCi) isolated product ([^{18}F]6) was obtained in a 37.5% decay corrected overall rcy calculated from [^{18}F]fluoride. The radiochemical purity of [^{18}F]6 was greater than 99% and the specific radioactivity 298 GBq/μmol (8052 Ci/mmol) at end of synthesis.

Keywords: microfluidics; reductive amination; radiofluorination; fluorine-18

Introduction

Positron emission tomography (PET) is a powerful tool using radiolabelled compounds as molecule probes to study biological processes in vivo. The use of PET in medical applications and drug discovery has stimulated the development of new radiolabelling strategies and tracer molecules labelled with ^{11}C ($t_{1/2}$ = 20.3 min) and ^{18}F ($t_{1/2}$ = 109.7 min) as positron emitting radionuclides.[1–3] It has been increasingly recognized that micro-reactors (microfluidic) may present considerable advantages in the radiochemistry of PET tracer labelled with positron emitting radionuclides.[4,5] The advantages of using microfluidic reactors for organic chemistry are well-documented and include benefits associated with miniaturization, which offer advantages over the conventional reaction settings, such as reduced chemical consumption, high surface-area-to-volume ratios, automation, and improved control over mass and heat transfer.[6–9] Microfluidic reactors for PET radiosynthesis have generated considerable interest primarily because miniaturization reaction systems have the potential to address the challenges of increasing the speed of labelling reaction and improving the overall efficiency of the radiolabelling reaction process.[10–13]

Throughout the past decennium, several studies on the application of microfluidics for PET have been reported, mainly focusing on reactor design and proof-of-principle reactions, as in the multiple reports on the carbonylation reaction of carbon 11-labelled amides using [^{11}C]carbon monoxide[14–16] and the full radiosynthesis of the well-established PET radioligand [^{18}F]**2**-fluoro-2-deoxy-D-glucose.[17–19]

Amines are an important class of compounds found in many natural products, pharmaceuticals, and other valuable organic molecules. Although there are many synthetic methods that can be used to produce amines, there is still a need for alternative ways that are mild and reproducible. Of these, reductive amination is particularly important, direct reductive amination of carbonyl compounds, mostly aldehydes or ketons, is one of the most powerful methods for the synthesis of substituted amines.[20–24]

We report here the use of a commercially available microfluidic radiochemistry system as synthetic platform applied in a two-step synthetic preparation of fluorine 18-labelled fluorobenzyl amines, via the aromatic nucleophilic radiofluorination and followed by the direct reductive amination starting from a mono substituted benzaldehyde compound. (Scheme 1)

Results and discussion

Radiosynthesis of [^{18}F]fluorobenzaldehyde ([^{18}F]2) (Scheme 1)

As a model substrate for the reductive amination, we used the aromatic aldehyde, [^{18}F]**2** as a labelling precursor, a strategy first published by Wilson and co-workers in 1990.[25]

The first step involves the nucleophilic aromatic substitution of the para-substituted 4-trimethylammoniumbenzaldehyde triflate precursor.

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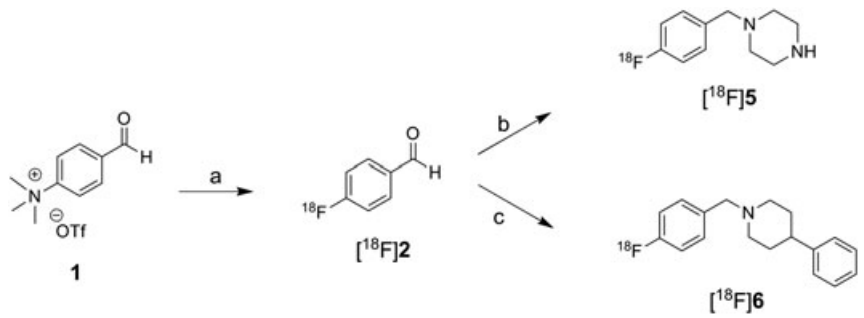


Scheme 1. The two-step synthesis route for two fluorine 18-labelled fluorobenzyl amines, [^{18}F]**5** and [^{18}F]**6**. (a) ^{18}F/K+/K_{222} in MeCN; (b) piperazine, sodium cyanoborohydride, acidic acid in 1:1 mixture between MeCN/MeOH; (**c**) 4-phenyl-piperidine, sodium cyanoborohydride, acidic acid in 1:1 mixture between MeCN/MeOH.

Reaction parameter screening (Figure 1)

A series of reactions was conducted in a 2-meter micro-reactor (i.d. 100 μm) using acetonitrile as solvent, to establish conditions where [^{18}F]**2** is produced rapidly in high and reproducible yield. The flow rate and volume of the two reagents, precursor solution and [^{18}F]fluoride ion solution, were set equal at 50 μL/min and 20 μL, respectively. The reaction mixtures leaving the micro-reactor were collected in a vial containing ethanol before the product mixture was analyzed by radio-thin layer chromatography (TLC) to calculate the radiochemical yield (rcy). The influence of temperature on the rcy was studied at different precursor concentrations, the results are displayed in Figure 1. An 80% rcy was achieved when using a precursor concentration of 3.2 mM, which is about 4–6 times lower than the reported by other groups using conventional methods.[25–27] The reaction/residence time for a particular liquid plug inside the micro-reactor was 9.4 s, thus allowing for a rapid screening of the reaction parameters as well as decreasing the number of required cyclotron productions. The highest rcy based on [^{18}F]fluoride, 93%, was obtained at 200°C with a precursor concentration of 31.9 mM. However, because a lower concentration of precursor would be beneficial for the subsequent reductive amination, we chose to use 4.8 mM at a reaction temperature of 160°C for the continued study. The lower temperature was selected to increase the stability of the reaction.

Batch production of [^{18}F]2

As opposed to in the reaction parameter screening, we experienced several plugged reactors when setting up the full batch production of [^{18}F]**2**, that is, when using a larger reagent volume for the reaction, 400 μL versus 20 μL. After decreasing the amounts of potassium carbonate and kryptofix (personal communication, Shuiyu Lu, NIMH), we did not experience any more plugged reactors. [^{18}F]**2** was produced in a decay corrected rcy of 60 ± 3% approximately 8 min after start of reaction. The radiochemical purity was greater than 99%.

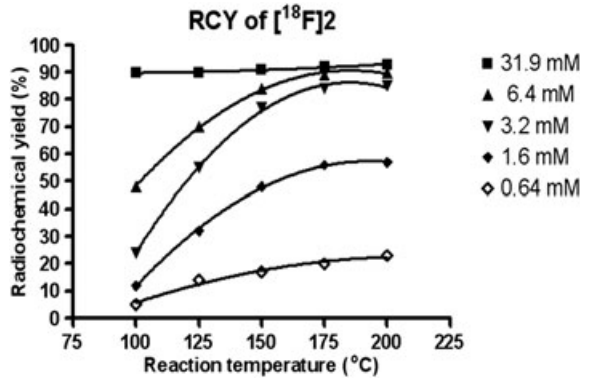


Figure 1. Radiochemical yield based on [^{18}F]fluoride is presented as a function of temperature and precursor concentration for the radiofluorination step, establish by analytical radio-TLC.

Preparation of fluorine-18 labelled [^{18}F]fluorobenzyl amines (Scheme 1)

Having defined the radiofluorination conditions, we proceeded to study [^{18}F]**2** reactivity in the subsequent reductive amination reaction.

Direct reductive amination involves the initial formation of an intermediate hemiaminal that dehydrates to form an imine. Under acidic condition, the imine is protonated to form an iminium ion and is subsequently reduced to produce the corresponding alkylated amine. The reduction of the imine is usually achieved by borohydride reducing agents or catalytic hydrogenation. In this study, we utilized sodium cyanoborohydride as reductant.

Reaction parameter screening (Figure 2)

We conducted a series of reactions using [^{18}F]**2** and a secondary amine mixture including the reducing agent sodium cyanoborohydride dissolved in methanol. The secondary amine solution was pH adjusted by adding droplets of acetic acid, with a final pH between 3 and 5, in accordance with earlier publications.[22] The crude reaction mixture from the first step was mixed online with reagent mixture 2 at the inlet of the second micro-reactor (4 m long). The overall labelling occurred as a single process without any intermediate purification step.

The influence of temperature and amine concentration on the rcy was performed using test compounds **3** and **4** by varying the amine concentration between 10 and140 mM and at a temperature interval of 100–175°C, whereas the reaction conditions from the first step were kept constant. For the second step, the flow rate from respective syringe pump was kept at 30 μL/min, with a final reaction time of 31.4 s. The reaction mixture leaving the micro-reactor was collected in a vial containing ethanol before the product mixture was analyzed by radio-HPLC to assess the rcy; results are displayed in Figure 2.

The highest rcy of [^{18}F]**5** was 70% and the corresponding value for [^{18}F]**6** was 75%. The reproducibility in the preparation

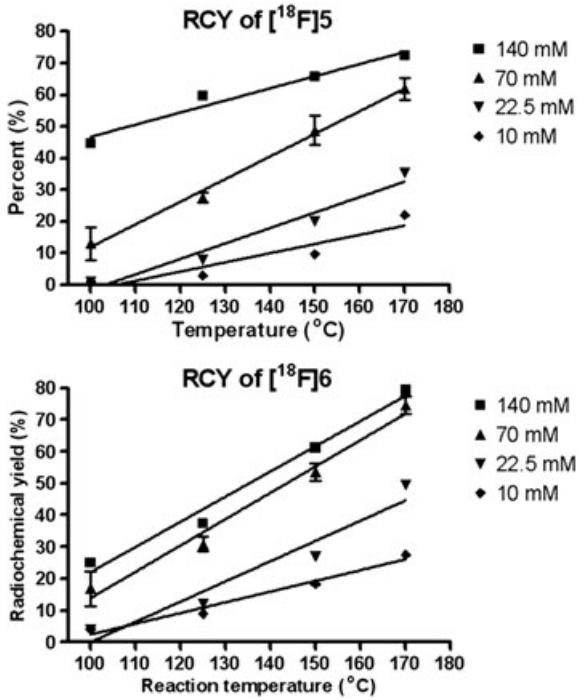


Figure 2. Radiochemical yields (Rcy) of [^{18}F]**5** and [^{18}F]**6** based on [^{18}F]**2** are presented as a function of temperature and precursor concentration for the reductive amination step, establish by analytical radio-HPLC. Rcy values at 70 mM is a mean from three consecutive runs, $n = 3$.

of [^{18}F]**5** and [^{18}F]**6** was also studied by three consecutive productions cycles at an amine concentration of 70 mM.

Batch production of [^{18}F]6

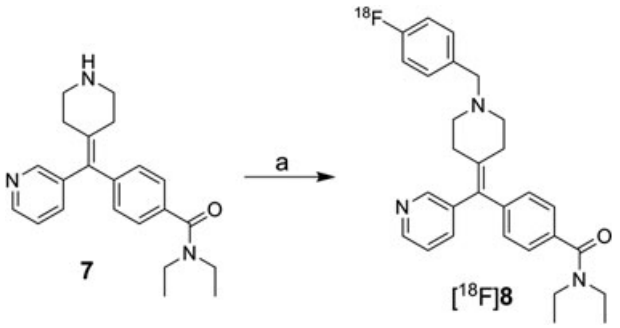
In a preparative run, 1050 MBq (28.4 mCi) isolated product ([^{18}F]**6**) was obtained in a 37.5% decay corrected overall rcy from [^{18}F] fluoride. The radiochemical purity was greater than 99%, and the specific radioactivity was 298 GBq/μmol (8052 Ci/mmol). The relatively low radioactivity recovery could partly be explained by [^{18}F]fluoride ion absorption onto inner surfaces, for example, micro-reactor, tubing, and drying vial. Absorption is a common phenomenon in reactions of dry NCA [^{18}F]fluoride ion.[28]

Radiosynthesis of [^{18}F]AZ12439516 ([^{18}F]8) (Scheme 2)

To further test the utility of this methodology, we also radiolabelled a delta opioid agonist using the optimal conditions established for compounds [^{18}F]**5** and [^{18}F]**6**. The analytical rcy of the crude product was 29%. The rather low rcy could be a consequence of a poor drying step for that particular experiment.

Conclusion

A method for the two-step preparation of ^{18}F-fluorobenzyl amines using reductive amination in a commercially available microfluidic radiochemistry system has been developed. The microfluidic apparatus allowed for a rapid parameter optimization and were scalable to produce adequate radioactivities for PET applications. The method is simple and provides good yields and was successfully applied in the radiolabelling of a potentially new radioligand for the delta opioid receptor.



Scheme 2. The two-step synthesis route of AZ12439516 ([^{18}F]**8**) via the reductive amination of [^{18}F]Fluorobenzaldehyde ([^{18}F]**2**). (a) step (1) ^{18}F/K+/K_{222}, 4-trimethylammoniumbenzaldehyde triflate in MeCN; step (2) amine precursor (**7**), sodium cyanoborohydride, acidic acid in 1:1 mixture between MeCN/MeOH.

Experimental

General procedures

MP-1 anionic rasin cartridges were supplied by ORTG (Tennessee, USA). All chemicals and solvents were purchased from commercial sources and used without further purification, except for 4-trimethylammoniumbenzaldehyde triflate precursor, which was prepared according to the literature.[25] High pressure liquid chromatographic analysis (HPLC) was performed using a Hitachi L-6200 gradient pump and a Hitachi L-4000 variable wavelength UV-detector in a series with a Bioscan β^+-flow detector. Analytical HPLC analysis was performed using a reverse phase column (Waters μBondapak, C18, 10 μm, 3.9 × 300 mm) eluted with a gradient between acetonitrile (A) and 10 mM H_3PO_4 (B). The gradient was linear between 20 and 100% over 10 min at a flow rate of 2 ml/min. Thin-Layer Chromatograhpy (TLC) was performed on a Raytest MiniGita TLC scanner (Raytest, Munich, Germany).

Liquid chromatography - Mass Spectrometry (LC-MS) was performed on a Waters QTof Premier coupled with a Waters Acquity UPLC system (Waters, Sollentuna, Sweden). Semi-preparative HPLC purification was performed on a reversed-phase column (ACE – C18, 5 μm, 10 × 250 mm) eluted with an isocratic mobile phase consisting of MeCN and 50 mM H_3PO_4 (30:70, v/v) at a flow rate of 6 ml/min. The eluate from the HPLC column was monitored for UV absorbance (254 nm) and radioactivity using a Knauer smartline UV-detector 2600 and an uncalibrated GM-tube, and the signal was recorded using a flatbed recorder (Kipp & Zonen, Holland). No-carrier-added (NCA) [^{18}F]fluoride ion was obtained from the ^{18}O(n, p)^{18}F nuclear reaction by irradiating [^{18}O]water for 2 min with a proton beam (16.4 MeV, 35 μA) generated with a GEMS PETtrace cyclotron (GE, Uppsala, Sweden). [^{18}F]Fluoride ion was isolated onto an anion resin (MP-1) cartridge and subsequently eluted with a solution of K_2CO_3 (0.45 mg, 3.3 μmol) and Kryptofix 2.2.2 (4.5 mg, 11.9 μmol) in MeCN-H_2O (450 μl, 1:1 v/v) into a vial (2 ml, Alltech). The solvents were azeotropically dried at 105°C in 15 min. The formed [^{18}F]$F^-/K^+/K_{222}$ complex was diluted with anhydrous MeCN and loaded onto storage loop (P3, 400 μl) in the apparatus. The automatic microfluidic system was either setup for one-step or two-step radiosynthesis (Figure 3). For more detail description of the apparatus, we refer to earlier studies.[10,11]

Microfluidic synthesis of [^{18}F]2

Dry [^{18}F]$F^-/K^+/K_{222}$ solution and 4-trimethylammoniumbenzaldehyde triflate precursor (0.64–31.9 mM), both in anhydrous acetonitrile (400 μl), were loaded into their respective storage loop. The precursor solution was filtered (filter, 0.45 μm, Waters) before use. About 20 μl of solution from each loop was simultaneously dispensed through a coiled silica glass micro-reactor (100 μm i.d., 2 m long; internal volume 15.7 μl) using variable temperature (100–200°C) at a set flow rate (50 μl/min). The product mixture was analyzed by radio-TLC on silica gel (Merck), developed with CH_2Cl_2, and quantified using a radio-TLC scanner. In the full batch production, 400 μl of each solution was dispensed through the micro-reactor at the optimal conditions established in the test stage. [^{18}F]**2** was isolated on a conditioned (10 ml EtOH, 15 ml H_2O) reversed phase SPE cartridge

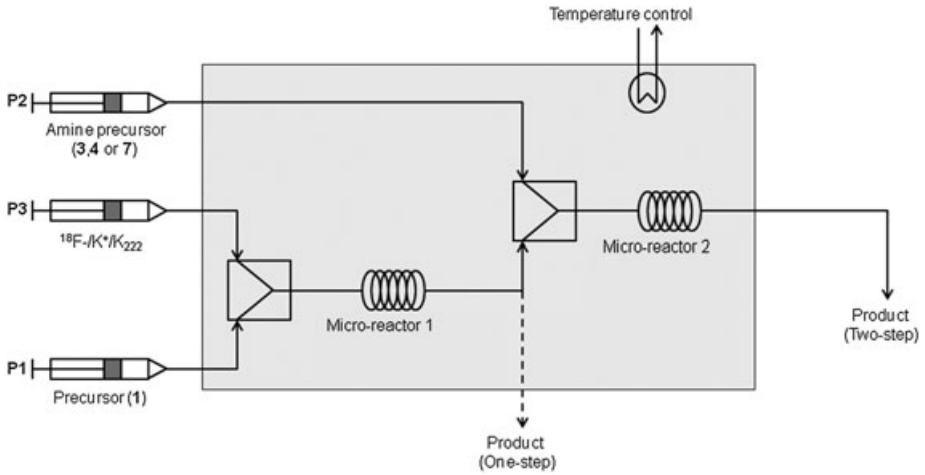


Figure 3. Microfluidic apparatus setup, (**P3**) ^{18}F-/K+/K$_{222}$ complex solution; (**P1**) precursor solution; (**P2**) amine precursor solution.

(Waters, Sep-pak tC18 PLUS) using the following procedure: The crude reaction mixture was diluted with 25 ml H_2O before it was pushed through the SPE. The isolated product ([^{18}F]**2**) was eluted with 2 ml of ethanol. The radiochemical purity was established with radio-HPLC.

*Microfluidic optimization of [^{18}F]**5** and [^{18}F]**6***

Solutions of 4-trimethylammoniumbenzaldehyde triflate precursor (4.8 mM) in MeCN (400 µl), dry [^{18}F]F$^-$/K$^+$/K$_{222}$ solution in MeCN (400 µl) and a solution of amine (10–140 mM, **3** or **4**), sodium cyanoborohydride (8 mg, 127.3 µmol), acetic acid (10 µl, 158.7 µmol) in MeOH (400 µl), was loaded onto their respective storage loop. The radiolabelling step conditions were kept constant (reagent volume, 20 µl; temperature, 160°C; syringe flow rate, 30 µl/min). Amine solution (40 µl) and the crude reaction mixture solution from step one (40 µl) was simultaneously dispensed through a micro-reactor (100 µm i.d., 4 m long; internal volume 31.4 µl), the micro-reactor was kept at a fixed temperature (100–175°C) using a set flow rate of 30 µl/min. Radiochemical purities were analyzed by analytical radio-HPLC.

*Batch production of [^{18}F]**6***

[^{18}F]**6** was synthesized in a full scale two-step production. Solutions of 4-trimethylammoniumbenzaldehyde triflate precursor (4.8 mM) in MeCN (200 µl), dry [^{18}F]F$^-$/K$^+$/K$_{222}$ solution in MeCN (200 µl), and a solution of amine (70 mM, **4**), sodium cyanoborohydride (8 mg, 127.3 µmol), acetic acid (10 µl, 158.7 µmol) in MeOH (400 µl) was loaded onto their respective storage loop. Solutions used for the radiofluorination step was simultaneously infused at 15 µl/min into a micro-reactor (100 µm i.d., 2 m long; internal volume 15.7 µl), the micro-reactor was kept at a fixed temperature of 160°C. Furthermore, amine solution and the crude reaction mixture solution from step one was dispensed simultaneously through a micro-reactor (100 µm i.d., 4 m long; internal volume 31.4 µl), the micro-reactor was kept at a fixed temperature 175°C using a set flow rate of 30 µl/min. The overall labelling occurred as a single process without any intermediate purification (Figure 3). The crude reaction mixture was purified and isolated using preparative liquid chromatography. The specific radioactivity and radiochemical purity of the final product was established with HPLC-UV respective radio-HPLC, and the identification of synthesized compound was confired by LC-MS of associated carrier [m/z = 270.16 (M + 1)+] in the formulated preparation.

*Synthesis of [^{18}F]AZ12439516 ([^{18}F]**8**)*

Solutions of 4-trimethylammoniumbenzaldehyde triflate precursor (4.8 mM) in MeCN (400 µl), dry [^{18}F]F$^-$/K$^+$/K$_{222}$ solution in MeCN (400 µl), and a solution of amine (70 mM, **7**), sodium cyanoborohydride (8 mg, 127.3 µmol), acetic acid (10 µl, 158.7 µmol) in MeOH (400 µl) was loaded onto their respective storage loop. The radiolabeling step conditions were kept constant (reagent volume, 20 µl; temperature, 160°C; syringe flow rate; 30 µl/min). Amine solution (40 µl) and the crude reaction mixture solution from step one (40 µl) was simultaneously dispensed through a micro-reactor (100 µm i.d., 4 m long; internal volume 31.4 µl), the micro-reactor was kept at 175°C and a set flow rate of 30 µl/min. Radiochemical purities were analyzed by analytical radio-HPLC.

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Conflict of Interest

The authors did not report any conflict of interest.

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