

# Syntheses of $^{11}\text{C}$ - and $^{18}\text{F}$ -labeled carboxylic esters within a hydrodynamically-driven micro-reactor

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Carboxylic esters were successfully labeled with one of two short-lived positron-emitters, carbon-11 or fluorine-18, within a hydrodynamically-driven micro-reactor. The non-radioactive methyl ester **4a** was obtained at room temperature; its yield increased with higher substrate concentration and with reduced infusion rate. Radioactive methyl ester **4b** was obtained from the reaction of **1** (10 mM) with **2b** in 56% decay-corrected radiochemical yield (RCY) at an infusion rate of  $10 \mu\text{L min}^{-1}$ , and when the infusion rate was reduced to  $1 \mu\text{L min}^{-1}$ , the RCY increased to 88%. The synthesis of the non-radioactive fluoroethyl ester **5a** from **1** and **3a** required heating of the micro-reactor on a heating block at  $80^\circ\text{C}$  (14–17% RCY), whilst the corresponding radioactive **5b** from **1** and **3b** was obtained in 10% RCY. The radioactive ‘peripheral’ benzodiazepine receptor ligand **7b** was obtained from the reaction of acid **6** with labeling agent **2b** in 45% RCY at an infusion rate of  $10 \mu\text{L min}^{-1}$ . When the infusion rate was reduced to  $1 \mu\text{L min}^{-1}$ , the RCY increased to 65%. The results exemplify a new methodology for producing radiotracers for imaging with positron emission tomography that has many potential advantages, including a requirement for small quantities of substrates, enhanced reaction, rapid reaction optimisation and easy product purification.

## Introduction

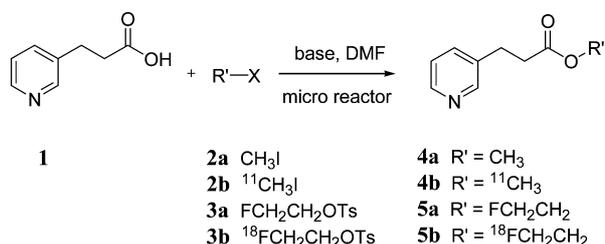
Positron-emission tomography (PET) is a radiotracer imaging modality that is used to provide quantitative information on physiological and biochemical phenomena in animals and human subjects *in vivo*.<sup>1–3</sup> Hence, this technique is valuable for clinical research<sup>4</sup> and also for drug development.<sup>5–7</sup> The biochemical scope and specificity of PET is determined by the available array of positron-emitting radiotracers, which are generally labeled with either carbon-11 ( $t_{1/2} = 20.4$  min) or fluorine-18 ( $t_{1/2} = 109.7$  min) at high (no-carrier-added; NCA) specific radioactivity.<sup>†</sup> These radiotracers, because of their short half-lives, must be produced as needed from cyclotron sources of carbon-11 and fluorine-18, which are usually [ $^{11}\text{C}$ ]carbon dioxide and [ $^{18}\text{F}$ ]fluoride, respectively. The reliable and regular production of PET radiotracers is a major challenge to chemists.<sup>6</sup> Such production must be performed in a lead-shielded hot-cell with remotely controlled and preferably automated equipment<sup>8–12</sup> that is capable of (i) synthesis of the radiotracer from high initial levels of radioactivity (up to 5 Ci; 1 Ci = 37 GBq), (ii) purification of the radiotracer and (iii) formulation of the radiotracer for intravenous injection, all within only two to three half-lives of the radioisotope.

Primary cyclotron irradiation products, such as [ $^{11}\text{C}$ ]carbon dioxide ([ $^{11}\text{C}$ ]CO<sub>2</sub>) and [ $^{18}\text{F}$ ]fluoride ([ $^{18}\text{F}$ ]F<sup>−</sup>), may be converted rapidly into alkylating agents such as

[ $^{11}\text{C}$ ]iodomethane<sup>13–15</sup> and [ $^{18}\text{F}$ ]2-fluoroethyl tosylate.<sup>16,17</sup> These are versatile labeling agents for introducing the radioisotope into target radiotracers.<sup>18</sup> Typically, a very large ( $10^2$ – $10^4$ -fold) excess of the non-radioactive reactant (precursor) is used in an alkylation reaction to promote rapid and efficient incorporation of the radioisotope into the target radiotracer. The reaction volume is typically 0.3 to 1.0 mL with the vessel sealed and heated. These conditions necessitate rapid separation of a low quantity of radioactive product (typically a few  $\mu\text{g}$ ) from a large excess of unreacted precursor (typically a few mg); this represents a considerable challenge that is usually met with single pass HPLC on either a semi-preparative or full preparative column. Moreover, in practice, efficient transfers of radioactive product to HPLC are not always possible, since they may require intervening concentration of the reaction mixture by evaporation or the use of solid phase extraction.

Miniaturization of radiosyntheses with carbon-11 or fluorine-18 might lead to several benefits such as the use of less materials (especially precursor, which may be precious or difficult to obtain) and easier and faster purification with greater conservation of radioactive product and its specific radioactivity. Micro-reactor devices,<sup>19,20</sup> consisting of a network of micron-sized channels (typical dimensions in the range 10–300  $\mu\text{m}$ ) etched into a solid substrate such as glass, are now emerging as an extremely useful technology for the intensification and miniaturization of chemical processes. The ability to manipulate reagent concentrations and reaction interfaces in both space and time within the channel network of a micro-reactor provides the fine level of reaction control that is desirable in PET radiochemistry practice. However, the application of micro-reactors in radiochemistry is so far very little explored, being limited to a single patent application.<sup>21</sup>

In this communication we report the use of a simple hydrodynamically-driven micro-reactor for radiosynthesis with carbon-11 and fluorine-18, exemplified by radiosyntheses of NCA  $^{11}\text{C}$ - and  $^{18}\text{F}$ -labeled esters (Scheme 1). Esters are commonly encountered functional groups in PET radiotracers, and they may be labeled by reactions of carboxylic acid salts with, for example, [ $^{11}\text{C}$ ]iodomethane (**2b**)<sup>22</sup> or [ $^{18}\text{F}$ ]2-fluoroethyl tosylate (**3b**).<sup>23</sup>



Scheme 1 Labeling of carboxylic esters with  $^{11}\text{C}$  or  $^{18}\text{F}$ .

## Experimental

All chemicals were used as received from commercial sources except where stated otherwise. Compound **6** was synthesized according to a published method.<sup>24</sup>

The micro-reactor is a T-shaped channel [220  $\mu\text{m}$  (W)  $\times$  60  $\mu\text{m}$  (D)  $\times$  14 mm (L); total volume  $\sim$  0.2  $\mu\text{L}$ ] located at the interface of two bonded borosilicate glass layers. It has two entry ports (A and B), each connected to a gas-tight micro-syringe (Hamilton) powered by a precision syringe pump (Harvard), and one exit port (C) leading to a collection reservoir (Scheme 2).

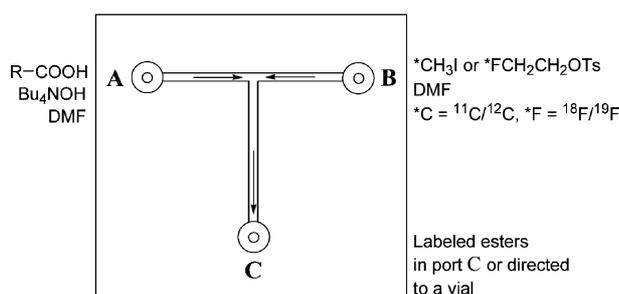
Reactions in the micro-reactor were first investigated with non-radioactive reagents. In a typical experiment, syringe A was loaded with a solution of **1** (concentration set in the range 2–10 mM) plus tetra-*n*-butylammonium hydroxide of equal concentration in DMF (200  $\mu\text{L}$ ) and syringe B was loaded with a solution of the alkylating agent (**2a** or **3a**) of the same concentration in DMF (200  $\mu\text{L}$ ). A portion of the solution (10  $\mu\text{L}$ ) in each syringe was then infused simultaneously and at an equal set rate (in the range 0.5 to 10  $\mu\text{L min}^{-1}$ ) through the micro-reactor. The reaction mixture output at port C was quenched continuously by dilution in acetonitrile (0.5 mL).<sup>‡</sup> A portion (25  $\mu\text{L}$ ) of the quenched reaction mixture was injected onto a HPLC column (C 18; Luna; 10  $\mu\text{m}$ ; 4.6  $\times$  250 mm; Phenomenex) eluted with acetonitrile (B) – 0.01 M aqueous ammonium formate (A) with a gradient of 30%B to 80%B over 10 min at 1 mL  $\text{min}^{-1}$ . Conversions of acid into ester were estimated from the UV absorbance areas in the chromatogram (Beckman Gold). Decay-corrected radiochemical yields (RCYs) were calculated from the peak areas of the product and unreacted starting material (Bioscan). The products were identified by both co-elution of reference product and LC-MS analysis of the selected fraction.

## Results and discussion

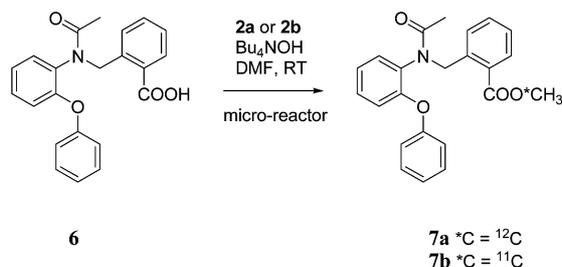
We selected reactions of 3-(3-pyridinyl)propionic acid (**1**) with **2b** or **3b** to test the feasibility of radiosyntheses with short-lived radioisotopes within a simple micro-reactor. Reactions were also run similarly with the precursor acid **6** to produce the peripheral benzodiazepine receptor (PBR) ligand, **7a** (Scheme 3).<sup>24,25</sup>

Radiosyntheses with **1** were conducted similarly, except that syringe B was loaded with a solution of **2b** in DMF or **3b** in DMF (1 mCi;  $\sim$  375 or  $\sim$  200 mCi  $\mu\text{mol}^{-1}$ , corresponding to 0.03 mM or 0.05 mM, respectively; 100  $\mu\text{L}$ ) and the volume in syringe A was similarly reduced to 100  $\mu\text{L}$ .<sup>§</sup>

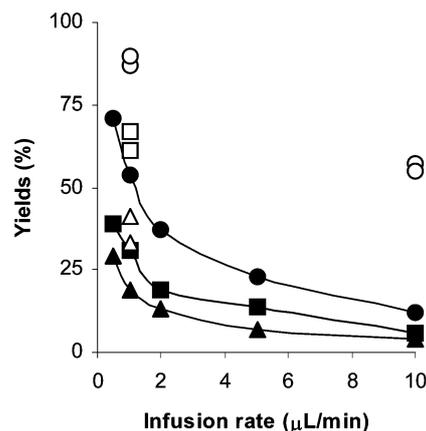
For the non-radioactive reactions of **1** with **2a**, the methyl ester **4a** was obtained at room temperature, and its yield increased with higher reagent concentration and with reduced infusion rate (Fig. 1). The same trend is also observed in reactions with radioactive materials. For example, as illustrated in Fig. 1, at an infusion rate of 10  $\mu\text{L min}^{-1}$  the



**Scheme 2** Schematic representation of the reagent placement and flow direction within the micro reactor.



**Scheme 3** Synthesis of non-radioactive or <sup>11</sup>C-labeled peripheral benzodiazepine receptor ligand.



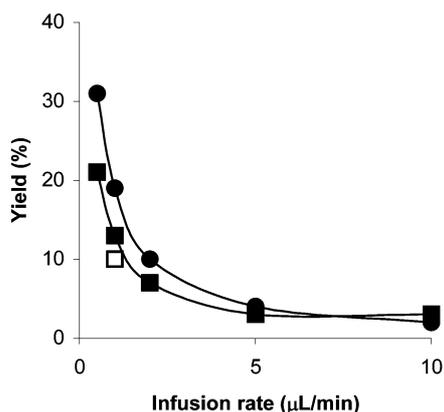
**Fig. 1** Effect of infusion rate and reagent concentration on the yield of ester **4a** from the reactions of acid **1** (10 mM) with **2a** (10 mM) (●); **1** (5 mM) with **2a** (5 mM) (■); **1** (2 mM) with **2a** (2 mM) (▲) and RCYs of <sup>11</sup>C-labeled ester **4b** from the reaction of acid **1** (10 mM) with **2b** (○), **1** (5 mM) with **2b** (□) and **1** (2 mM) with **2b** (△). All reactions were performed at room temperature.

radioactive methyl ester **4b** was obtained from the reaction of **1** (10 mM) with **2b** in 56% RCY ( $n = 2$ ), and when the infusion rate was reduced to 1  $\mu\text{L min}^{-1}$ , the RCY increased to 88% ( $n = 2$ ). The lower infusion rate allows more time in the micro-reactor (residence time) for reactant mixing by diffusion, and for reaction. At 1  $\mu\text{L min}^{-1}$  the residence time is about 12 s and total processing time 10 min, the latter is comparable to reaction times currently used in PET radiotracer synthesis. With new micro-reactor designs<sup>26</sup> it should be possible to achieve thorough mixing of reagents at higher infusion rates, so further accelerating the process.

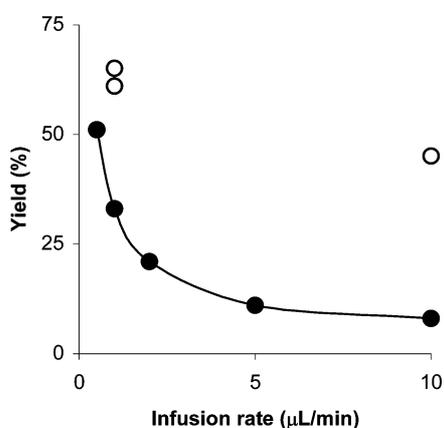
The synthesis of the fluoroethyl ester **5a** from **1** and **3a** did not proceed at room temperature and required heating of the micro-reactor on a heating block at 80 °C to achieve moderate yields (14–17%). The reaction can be performed with as low as 0.75  $\mu\text{g}$  (5 nmol) of **1** in 10  $\mu\text{L}$  of solution (Fig. 2). At an infusion rate of 1  $\mu\text{L min}^{-1}$ , reactions of **1** with the labeling agent **3b** at 80 °C gave the <sup>18</sup>F-labeled ester **5b** in 10% RCY.

This approach was further exemplified by the synthesis of a ligand **7a** and its labeling with carbon-11 as a prospective radioligand **7b** for PET imaging of brain 'peripheral' benzodiazepine receptors. At an infusion rate of 10  $\mu\text{L min}^{-1}$ , **7b** was obtained from the reaction of acid **6** with labeling agent **2b** in 45% RCY, and when the infusion rate was reduced to 1  $\mu\text{L min}^{-1}$ , the RCY increased to 65% (Fig. 3).

It should be noted that the radioactive reaction mixtures obtained from the micro-reactor are easily and rapidly separable on an analytical HPLC column, because only low amounts of material are present in a low volume. The radiotracer may be obtained from the HPLC in a smaller volume which in turn facilitates its easier formulation for safe intravenous administration.



**Fig. 2** Effect of infusion rate and reagent concentration on the yield of ester **5a** from the reaction of acid **1** (10 mM) with **3a** (10 mM) (●) and **1** (5 mM) with **3a** (5 mM) (■) at 80 °C in DMF, and the RCY of ester **5b** from the reaction of acid **1** (5 mM) with **3b** at 80 °C (□).



**Fig. 3** The effect of infusion rate on the yield of ester **7a** from the reaction of acid **6** (5 mM) with **2a** (5 mM) (●) and the RCYs of ester **7b** from the reaction of acid **6** (5 mM) with **2b** (○). All reactions were performed at room temperature.

## Conclusions

We have demonstrated, as proof of principle, that the synthesis of NCA  $^{11}\text{C}$  and  $^{18}\text{F}$ -labeled carboxylic esters is rapid and efficient in a simple hydrodynamically-driven glass micro-reactor. These results exemplify some of the potential advantages of this methodology for radiotracer synthesis, which should be amenable to greater sophistication to encompass entire radiosyntheses in a versatile high throughput manner.

## References

† Specific radioactivity is the ratio of the amount of radioactive compound (Ci) to non-radioactive counterpart known as carrier (mol). The specific radioactivities of no-carrier-added  $^{11}\text{C}$ - or  $^{18}\text{F}$ -labeled compounds typically far exceed  $1 \text{ Ci } \mu\text{mol}^{-1}$  at the end of synthesis. The specific radioactivity is a time-variable parameter which decreases with radioactive decay.

‡ Dilution in acetonitrile was shown to be a convenient and simple means for quenching the progress of the alkylation reactions. For

example, a reaction of **1** with **2a** giving a yield of 38%, progressed by only a further 3% over 70 min after quenching by dilution in acetonitrile. The effect of acetonitrile addition on the reaction of **1** with **3a** was similar.

§ These radiosyntheses were conducted with low radioactivity for ease of safe experimentation. Several hundred-fold higher radioactivity concentration would be feasible, the upper limit only being determined by the technique of producing the labeling agent.

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