Note

Application of Microreactor to the Preparation of C-11-Labeled Compounds *via O*-[^{11}C]Methylation with [^{11}C]CH_3I: Rapid Synthesis of [^{11}C]Raclopride

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A new radiolabeling method using a microreactor was developed for the rapid synthesis of [^{11}C]raclopride. A chip bearing a Y-shaped mixing junction with a 200 μm (width)×20 μm (depth)×250 mm (length) flow channel was designed, and the efficiency of *O*-[^{11}C]methylation was evaluated. Dimethyl sulfoxide solutions containing the *O*-desmethyl precursor or [^{11}C]CH_3I were introduced into separate injection ports by infusion syringes, and the radiochemical yields were measured under various conditions. The decay-corrected radiochemical yield of microreactor-derived [^{11}C]raclopride reached 12% in 20 s at 25°C, which was observed to increase with increasing temperature. In contrast, batch synthesis at 25°C produced a yield of 5%: this indicates that this device could effectively achieve *O*-[^{11}C]methylation in a shorter period of time. The microreactor technique may facilitate simple and efficient routine production of ^{11}C-labeled compounds *via O*-[^{11}C]methylation with [^{11}C]CH_3I.

Key words microreactor; [^{11}C]raclopride; *O*-[^{11}C]methylation; [^{11}C]CH_3I; positron emission tomography

Positron emission tomography (PET) imaging is utilized in nuclear medicine applications, allowing for noninvasive, early-stage diagnosis of diseases. Radiopharmaceuticals labeled with short half-life, positron-emitting radionuclides such as carbon-11 ($t_{1/2}$=20.4 min) or fluorine-18 ($t_{1/2}$=109.8 min) are used as PET imaging tracers. These radiopharmaceuticals must be prepared *via* radiolabeling reaction and purification steps as rapidly as possible.

Microfluidic technologies, known as "lab-on-a-chip," have recently been shown to be useful in the synthesis of PET radiotracers.[1–4] A microreactor consists of a network of micron-sized channels, typically 10–300 μm in dimension, which are etched into a solid substrate such as quartz glass (microfluidic chip).[5,6] Microreactors can be more effective than conventional reactors owing to their ability to control the transfer of small volumes of liquid, and due to the large surface area of the device that may promote efficient mixing of reagents as well as efficient heat conduction; consequently, reaction rates are typically observed to be faster in microreactors. In addition, microreactors carry a number of advantages specifically for radiochemical reactions, particularly with regards to safety: notably, they are small enough to fit in the internal space of a hot cell (a lead-shielded cabinet used in radiopharmaceutical synthesis) that protects the operator from radiation exposure.

In this study, we report the radiosynthesis of [^{11}C]raclopride using a simple microchip bearing a Y-shaped mixing junction (Fig. 1). [^{11}C]Raclopride is extensively used as a radiopharmaceutical for PET imaging of the dopamine D_2 receptor in the human brain,[7,8] and its synthesis was carried out by *O*-[^{11}C]methylation with [^{11}C]methyl iodide ([^{11}C]CH_3I) or [^{11}C]-methyl triflate based on the knowledge that *O*-[^{11}C]methylation preferentially proceeds compared to *N*-[^{11}C]methylation (formation of quaternary ammonium cation).[9–13] Synthesis of [^{11}C]raclopride by conventional methods typically involves *O*-[^{11}C]methylation of desmethylraclopride with [^{11}C]methyl triflate, a more reactive species than [^{11}C]CH_3I. However, the use of [^{11}C]methyl triflate is generally not so easy because silver triflate, which converts [^{11}C]CH_3I to [^{11}C]methyl triflate, is inactivated by oxidizing agents rapidly. We expected that preparation of [^{11}C]raclopride would be more facilitated by using a microreactor which can negate the less reactivity of [^{11}C]CH_3I as compared to [^{11}C]methyl triflate. Although synthesis of [^{11}C]raclopride on a microchip with [^{11}C]CH_3I has been recently demonstrated by Haroun *et al.*,[14] this report does not address two important issues required for the development of practical methods: firstly, in order to enhance the reactivity, the method required premixing of reaction solutions before introduction into the microchannel; secondly, the reaction efficiency was estimated by the relative radioactivity or conversion. Therefore, this study aimed to optimize a more practical method for the microchip-based synthesis of [^{11}C]raclopride by calculating the decay-corrected radiochemical yield with a microreactor system in which two precursors were introduced into each inlet separately.

Experimental

Materials All chemicals used in synthesis and analysis were reagent-grade and were purchased from Merck (Darmstadt, Germany), Sigma-Aldrich (St. Louis, MO, U.S.A.), or Nacalai Tesque (Kyoto, Japan); *O*-desmethylraclopride and lithium aluminum hydride (LAH) were purchased from

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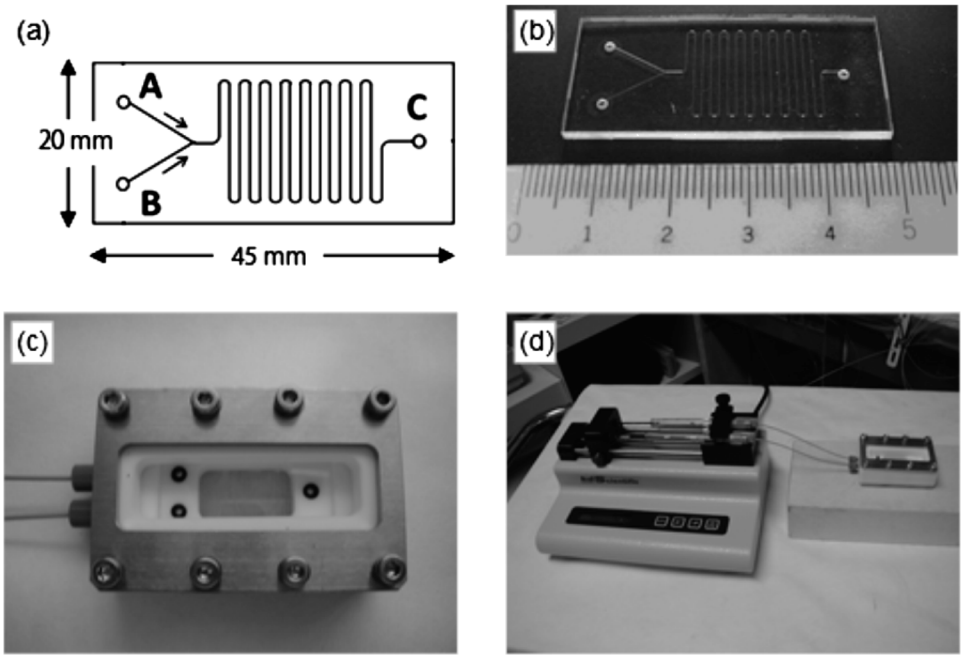

Fig. 1. (a) Layout of the Chip Bearing a Y-Shaped Mixing Junction with a Channel of Dimensions 200 μm (Width)×20 μm (Depth)×250 mm (Length) Channel (Total Volume: 1 μL)

The chip size was 20 mm×45 mm, with two inlets, **A** and **B**, for the precursors and one outlet **C**. (b) Optical photograph of the chip. (c) The chip was covered with a glass plate and fixed using a teflon/stainless plate jig and (d) subsequently connected to gas-tight microsyringes powered by a precision pump.

ABX (Radeberg, Germany). All chemicals were used without further purification. Shimadzu (Kyoto, Japan) prepared the microreactor chip bearing a Y-shaped mixing junction with 200 μm (width)×20 μm (depth)×250 mm (length) channel dimensions (total volume: 1 μL) on quartz glass using the sandblasting method. The angle between two inlet channels was 70°. The chip was covered with a glass plate, interposed in a Teflon/stainless plate jig, and firmly secured.

Radiolabeling by General Batch Method C-11 was produced *via* a $^{14}N(p,\alpha)^{11}C$ reaction by bombarding a nitrogen gas target with 16 MeV protons in a cyclotron (CYPRIS model 325R; Sumitomo Heavy Industry). The $[^{11}C]CO_2$ was transported to an automated C-11-labeled compound synthesis system (CUPID C-100; Sumitomo Heavy Industry) followed by LAH reduction and iodination. $[^{11}C]CH_3I$ was synthesized by the conventional method and trapped in 200 μL of dimethyl sulfoxide (DMSO). A solution of *O*-desmethyl precursor **1** (1.0 mg) and sodium hydroxide (NaOH, 0.6 mg) in DMSO (400 μL) was placed in a sealed vial and sonicated for 10 min. The solution was then allowed to mix and react with an equal volume of $[^{11}C]CH_3I$ in DMSO (1.5 GBq/200 μL) (final concentration of **1**: 3.75 mM). The reaction time was adjusted over the range 20–300 s and an oil bath was used to heat the vial at 25°C or 60°C. The reaction mixture was then injected to HPLC column (YMC-Pack Pro C18 AS-302, 4.6×150 mm; YMC) and eluted with acetonitrile/0.14 M ammonium formate=40/60 at 1.0 mL/min. The eluates were collected every 30 s, and the radioactivity of each fraction was measured using an automatic gamma counter (COBRAII, Packard), from which the decay-corrected radiochemical yields could then be calculated.

Radiolabeling Using Microreactor The microreactor was equipped with two inlets (Fig. 1(a) **A** and **B**), each connected to a gas-tight microsyringe (Hamilton) powered by a precision syringe pump (KDS101; KD Scientific); the outlet (**C**) led to a collection reservoir. Syringe **A** and **B** were loaded with reagents prepared similarly to the batch method: **A**, *O*-desmethyl precursor **1** with NaOH in DMSO (1.0 mg/400 μL); **B**, $[^{11}C]CH_3I$ in DMSO (1.5 GBq/200 μL). Importantly, the setting of syringe **B** was completed within 2 min after the radioactivity measurement. Each solution was then injected into the corresponding inlet simultaneously at an equal given rate (in the range 0.1–3.0 μL/min). The jig used to secure the microfluidic chip was placed onto a heating plate for the reactions conducted at 25°C or 60°C and the temperature was controlled using feedback from a laminated sensor on the chip. The reaction mixture collected from outlet **C** was continuously quenched by dilution on ice. A portion of the reaction mixture was analyzed by HPLC to calculate the decay-corrected radiochemical yield with the following formula:

Radiochemical yield (%)
= {Decay-corrected radioactivity of $[^{11}C]$raclopride obtained by HPLC purification} / {Radioactivity of $[^{11}C]CH_3I$ loaded into the chip}×100

Statistical Analyses Data are presented as mean values with standard deviation (S.D.). Comparisons were performed using the unpaired Student's *t*-test. $p<0.05$ was considered as a statistically significant difference.

Results and Discussion

A previous report detailing the microreactor-based synthesis of C-11-labeled carboxylic esters by mixing carboxylic acids and $[^{11}C]CH_3I$ used a microreactor with a T-shaped mixing junction and channel dimensions of 220 μm (width)×60 μm (depth)×14 mm (length), yielding a total volume of *ca.* 0.2 μL.[1] Moreover, for both T- and Y-shaped microchips, mixing be-

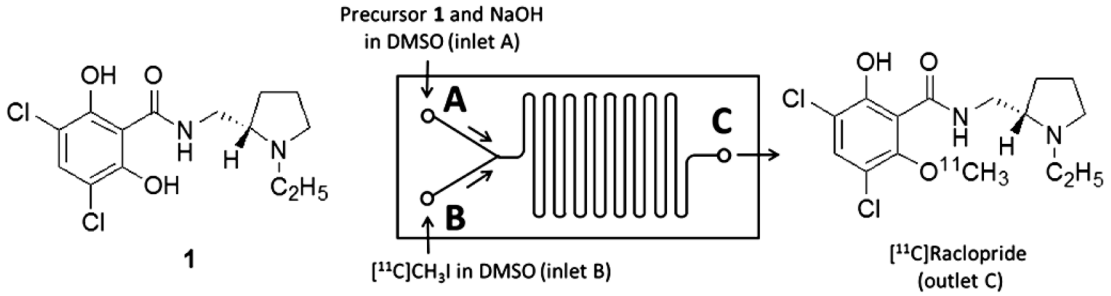

Fig. 2. Synthetic Scheme for the Preparation of [^{11}C]Raclopride Using a Microreactor Equipped with Two Inlets for the Precursors (**A** and **B**) and One Outlet (**C**)

Table 1. Radiochemical Yields of [^{11}C]Raclopride at 25°C: Effect of Labeling Method and the Reaction Time

Reaction time (s)		10	20	60	300
Yield (%)[a)]	Microreactor	5.5±1.1	11.7±4.3[#]	14.5±2.3	15.5±4.7
	Batch	N.P.	4.8±0.75	10.6±6.0	14.1±8.1

a) All values represent the mean±S.D. for three to seven independent experiments. [#]$p<0.05$ compared to the value obtained from the batch system. N.P.: Not performed.

tween reactants solely depends on the diffusion of the molecules at the interface between two solvents; as such, the time required for mixing is proportional to the square of diffusion length.[15] Therefore, in order to achieve the efficient mixing of solutes, a microchip with shallow-depth and a longer flow channel (yielding a total volume of 1 μL) was designed for this study; the efficacy of the microreactor was then evaluated by testing [^{11}C]raclopride synthesis *via* *O*-[^{11}C]methylation under varying reaction conditions. A solution of *O*-desmethyl precursor **1** in DMSO in the presence of NaOH was loaded into the inlet **A**, and a solution of [^{11}C]CH_3I in DMSO was simultaneously loaded into the inlet **B**. The reaction mixture was then collected from the outlet **C** (Fig. 2).

The *O*-[^{11}C]methylation of **1** was performed in a microreactor at 25°C with a total flow rate of 6.0 μL/min (reaction time: 10 s), producing [^{11}C]raclopride with a yield of 5.5±1.1%. Furthermore, flow rates of 3.0 μL/min, 1.0 μL/min, and 0.2 μL/min produced reaction times of 20 s, 60 s, and 300 s, where the desired product was obtained in 11.7±4.3%, 14.5±2.3%, and 15.5±4.7% radiochemical yields, respectively (Table 1). As unreacted **1** and [^{11}C]CH_3I were observed in the reaction mixture, it is considered that a longer reaction time facilitated the diffusion of these solutes, which contributed to the increase in the yield. On the other hand, radioactive decay depending on the physical half-life of carbon-11 (20.4 min) also needs to be taken into account; moreover, longer residence time induces the formation of radioactive byproducts through undesired [^{11}C]methylation and/or decomposition of [^{11}C]raclopride.[16] Thus, the flow rate should be optimized to increase the radiochemical yield: it should be rapid enough to give a reasonably short reaction time and thereby minimize radioactive decay, yet to some extent slow enough to permit diffusion of the reactants. For a given reaction time, the yields obtained using microreactor were higher than those obtained by the batch method; in particular for a reaction time of 20 s, the yield achieved statistical significance.

In addition, the synthesis of [^{11}C]raclopride was performed in a microreactor heated at 60°C (total flow rate: 3.0 μL/min, reaction time: 20 s). Under these conditions, [^{11}C]raclopride was obtained in a radiochemical yield of 20.3±2.0%, which

Table 2. Radiochemical Yields of [^{11}C]Raclopride in a 20 s Reaction Time: Effect of Labeling Method and the Temperature

Temperature (°C)		25	60
Yield (%)[a)]	Microreactor	11.7±4.3	20.3±2.0[#]
	Batch	4.8±0.75	17.1±3.0[##]

a) All values represent the mean±S.D. for three to six independent experiments. [#]$p<0.05$, [##]$p<0.01$ compared to the value at 25°C.

was a significant increase compared with the reaction conducted at room temperature (Table 2). However, it was not statistically higher than the yield obtained from the batch system operated at 60°C. Moreover, when the reaction was carried out at 90°C under the same total flow rate (3.0 μL/min), radiochemical yield of [^{11}C]raclopride was observed to decrease (11.9±3.4%, n=3). Because *O*-desmethylraclopride is known to be unstable in DMSO, the high reaction temperature combined with the micro-space of the reactor may cause the decomposition of the precursor and thereby decrease the yield. These results indicate that the microreactor used in this study was better suited to lower reaction temperatures.

In this study, low yield of [^{11}C]raclopride was a bottleneck in the microreactor synthesis. When the reaction was performed at 60°C with a total flow rate of 3.0 μL/min, the radioactivity of [^{11}C]raclopride obtained after HPLC purification was 5–10 MBq. In previous batch reaction studies, the radiochemical yields of [^{11}C]raclopride with [^{11}C]CH_3I in the presence of NaOH fell in the ranges 25–50% and 8.3–50% based on [^{11}C]CO_2 and [^{11}C]CH_3I, respectively.[9,10,15,16] Net radioactivity values obtained in these experiments were much higher than the results reported here; however, low reproducibility of the yield due to inter/intra-institutional differences has been problematic in these studies. It is considered that microfluidic devices with simple structures can overcome this problem and achieve stable and repeatable incorporation of [^{11}C]methyl group into the precursors. However, to improve the reaction efficiency, further optimization of the chip design, flow rate, temperature control, as well as increase the number of reactor (multi-scale approach) are necessary steps. The diffusion of solute is proportional to temperature, while inversely

proportional to the solvent viscosity in some mathematical models,[17] and thus, heating-control of the system under conditions in which reagents are stable could increase the reaction rate substantially. In addition, channel size and form (such as surface roughness of the wall) influence the solute mixing.[18,19] Molding of a roughened channel surface will evoke artificial swirling of the fluid flow, potentially enhancing the overall reaction yield.

Additionally, synthesis of [^{11}C]raclopride in this investigation was achieved using an extremely simple microchip architecture: the results of this study suggest that the solutes were able to diffuse widely enough to achieve *O*-[^{11}C]methylation effectively in this reaction vessel; therefore, this basic Y-shaped microchip might be applied to other radiolabeling reactions such as *N*-[^{11}C]methylation and *S*-[^{11}C]methylation by adjusting the reaction time and/or temperature.

Conclusion

The microreactor produced a higher yield of [^{11}C]raclopride than the corresponding batch method. [^{11}C]Raclopride was conveniently prepared in a yield exceeding 20% (total flow rate of 3.0 μL/min, 60°C), and therefore microreactor-based synthesis may potentially be applied to simplify and improve the efficiency of the routine production of C-11-labeled compounds. Although the radiochemical yields obtained by this method should be improved, the microfluidic device described here is useful for the radiosynthesis of PET imaging radiopharmaceuticals.

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Conflict of Interest The authors declare no conflict of interest.

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