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Radiosyntheses of two positron emission tomography probes: [^{11}C]Oseltamivir and its active metabolite [^{11}C]Ro 64-0802

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Abstract—Oseltamivir phosphate (Tamiflu®, **1**·H_3PO_4 is an orally active anti-influenza drug, which is hydrolyzed by esterase to its carboxylate metabolite Ro 64-0802 (**2**) with potent activity inhibiting neuraminidase. In this study, for the first time, we synthesized carbon-11-labeled oseltamivir ([^{11}C]**1**) and Ro 64-0802 ([^{11}C]**2**) as two novel positron emission tomography probes and demonstrated that [^{11}C]**1** had twofold higher radioactivity concentration in the mouse brains than [^{11}C]**2**.

Oseltamivir phosphate (Tamiflu®, **1**·H_3PO_4, Scheme 1) is an orally active anti-influenza drug for the treatment of influenza types A and B.[1] It is a prodrug which is hydrolyzed by esterase to its carboxylate metabolite Ro 64-0802 (**2**, Scheme 1), a potent inhibitor for neuraminidase.[2] This drug is regularly prescribed as a treatment for seasonal influenza in Japan and Japanese consumption accounts for up to 75% of all Tamiflu use worldwide. However, the safety of **1** is questioned because suicidal or abnormal behaviors especially in younger patients have been reported after Tamiflu ingestion.[3]

The mechanisms for the abnormal behaviors remain uncertain. A high dose of **1** damaged the brain in experimental animals, and was likely caused by uptake of **1** and/or **2** into the central nervous system (CNS). It has been reported that **2** affects the CNS because neuraminidase, a key enzyme inhibited by **2**, plays a role in the CNS development and impulse conduction.[3] In vitro study using brain slices also revealed that **2** had clear effects on neuronal excitability.[4] Therefore, although it is believed that **1** and **2** do not easily pass the blood–brain barrier, they may enter the brain if the BBB is immature or damaged. However, the relationship between the presence of **1** and **2** in the CNS and the side effects of Tamiflu has not been elucidated clearly.

Positron emission tomography (PET) is a useful way of medical imaging using radioactive agents labeled with positron emission nuclides such as carbon-11 (^{11}C), fluorine-18, nitrogen-13 and oxygen-15.[5] PET is a non-invasive imaging modality that provides functional information on physiological, biochemical and pharmacological processes in vivo. PET can be used to assess the drug action in humans from the drug development and evaluation process to clinical therapy. It has the ability to give pharmacokinetic and pharmacodynamic information about a drug to determine the drug efficacy, and proof of potential biochemical mechanisms of drug action, including the side and toxic effects. Thus, positron emitter-labeled **1** and **2** may be useful to determine their presence in the brain and to elucidate the cause of their CNS side effects in humans.

The aim of this study was to synthesize ethyl (*3R,4R,5S*)-4-[carbonyl-^{11}C]acetamido-5-amino-3-(1-ethylpropoxy)-cyclohex-1-ene-1-carboxylate ([^{11}C]**1**, Scheme 1) and (*3R,4R,5S*)-4-[carbonyl-^{11}C]acetamido-5-amino-3-(1-ethylpropoxy)cyclohex-1-ene-1-carboxylic acid ([^{11}C]**2**) using [1-^{11}C]acetyl chloride[6] ([^{11}C]AcCl) and to determine their

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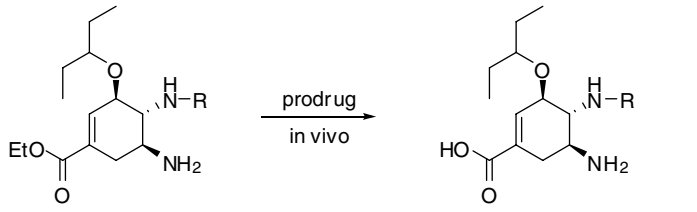


Oseltamivir (**1**): R = -$COCH_3$ Ro 64-0802 (**2**): R = -$COCH_3$

[^{11}C]**1**: R = -$^{11}COCH_3$ [^{11}C]**2**: R = -$^{11}COCH_3$

Scheme 1. Chemical structures of oseltamivir (**1**), Ro 64-0802 (**2**) and their labeled compounds [^{11}C]**1**, [^{11}C]**2**.

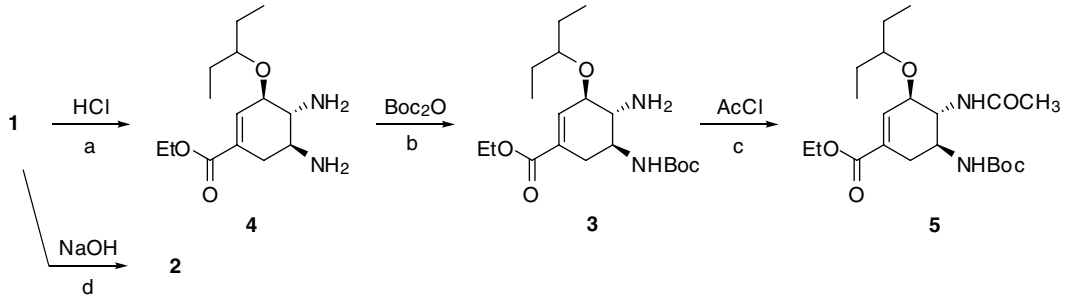
radioactivity concentrations in mouse brains. The labeling strategy did not change their chemical structures and maintained their pharmacological profiles. So far, chemical syntheses of non-radioactive **1** have been successfully performed[7] and a method for the synthesis of [^{11}C]**1** has also been reported;[8] however, radiosyntheses of [^{11}C]**1** and [^{11}C]**2** have not been achieved. Radiosynthesis for PET probes involving short-lived positron emitters such as ^{11}C (20.4 min), which is different from those used in conventional organic synthesis, requires practical methods and techniques under radiation protection. The radiosynthesis must be accomplished within a short time compatible to the half-life, using only a limited step sequences. Another important characteristic of radiosynthesis is the efficient production and application of the radioactive labeling agents such as [^{11}C]AcCl,[9] [^{11}C]CH_3I[10] and [^{11}C]CH_3NO_2,[11] which is not commercially available like the non-radioactive AcCl, CH_3I and CH_3NO_2.

Here, we report: 1) chemical synthesis of a precursor (**3**) for radiosynthesis using a new route, 2) radiosyntheses of [^{11}C]**1** and [^{11}C]**2**, 3) radioactivity concentrations of [^{11}C]**1** and [^{11}C]**2** in the brain and blood of mice.

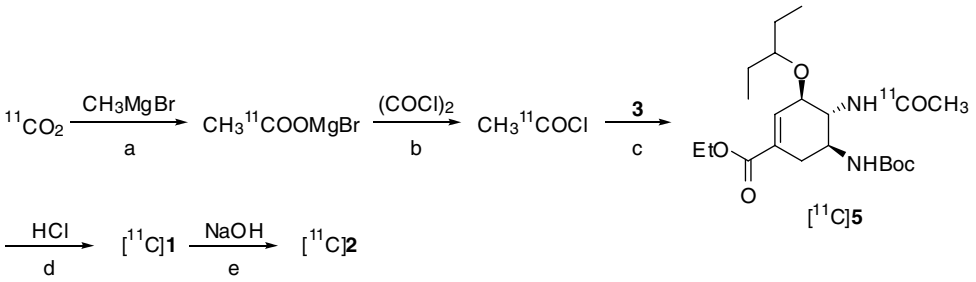
Precursor **3** for radiosynthesis was prepared using a new route from **1**, as shown in Scheme 2. The diamine **4** was prepared by heating **1** with concentrated HCl in EtOH. Excess treatment with HCl led to hydrolysis of the ester group. Direct acetylation of **4** using an equimolar amount of AcCl was simulated for radiosynthesis but the desired **1** was not afforded. This result was supported by that the preferable acylation took place in the sterically less hindered 5-amine position.[7d] Therefore, the 5-amine group was firstly protected with Boc group to give precursor **3**. The structure of **3** was assigned by NMR study and was further derived to **5**, identical to the analytic data previously reported.[7c] On the other hand, the non-radioactive **2** was prepared by hydrolyzing **1** with 2 N NaOH in almost quantitative yield.

Radiosyntheses of [^{11}C]**1** and [^{11}C]**2** were carried out as shown in Scheme 3. The radioactive agent [^{11}C]AcCl was synthesized by reacting methylmagnesium bromide (CH_3MgBr) with [^{11}C]CO_2 on the inner surface of a narrow polyethylene tube, followed by chlorination with oxalyl chloride.[9] The radiochemically pure [^{11}C]AcCl was purified by distillation and trapped in a solution of THF containing **3** and Et_3N at −15 °C. After this reagent trapping ended, the acetylation required a further 5 min at 80 °C to give [^{11}C]**5**. Without purifying this mixture, 6 N HCl (300 μL) was added to the reaction to eliminate the Boc group of [^{11}C]**5**. After the treatment, the radioactive mixture was purified by HPLC (Waters XBridge Prep C18 5 μm column, 10 mm ID ×50 mm, $CH_3CN/H_2O/Et_3N$ (30/70/1), 5 mL/min, 254 nm) to afford [^{11}C]**1**. These sequences were accomplished using an automated synthesis system. Moreover, [^{11}C]**1** was hydrolyzed with 1% NaOH for 5 min at 100 °C to give [^{11}C]**2**.

The identity, radiochemical purity and specific activity of [^{11}C]**1** and [^{11}C]**2** were determined using HPLC and radio-TLC. In the HPLC analysis, the retention time was 6.6 min for [^{11}C]**1** (CAPCELL PAK C_{18} column: 4.6 mm ID ×250 mm, $CH_3CN/H_2O/Et_3N$ (30/70/0.1), 1.5 mL/min, 254 nm) and 5.2 min for [^{11}C]**2** (CAPCELL PAK C_{18} column: 4.6 mm ID ×250 mm, CH_3OH/pH 6.8 phosphate buffer (4/6), 1 mL/min, 210 nm), respectively. The identity was confirmed by co-injecting with the corresponding non-radioactive sample. The radiochemical purity of [^{11}C]**1** or [^{11}C]**2** was higher than 98% and the specific activity was about 4 GBq/μmol as determined from the mass mea-



Scheme 2. Chemical synthesis. Reagents and conditions: (a) EtOH, 90 °C, 5 h, 37%; (b) CH_2Cl_2, 25 °C, 1 h, 78%; (c) cat. DMAP, pyridine, 90 °C, 1 h, 95%; (d) EtOH, 90 °C, 2 h, 99%.



Scheme 3. Radiosynthesis. Reagents and conditions: (a) THF, −5 to 0 °C; (b) THF, 75 °C, min; (c) THF, 80 °C, 5 min; (d) 80 °C, 5 min, 10% from $^{11}CO_2$; (e) 100 °C, 5 min, 100%.

sured from the HPLC UV analysis. In the final product solution, no significant peak relative to **3** and its decomposition product was detected by HPLC. The two products were further determined by radio-TLC with a mixture as developing solvents: $CHCl_3$/CH_3OH/AcOH (5/2/0.1), R_f 0.8 ([^{11}C]**1**), 0.4 ([^{11}C]**2**). Co-elution with authentic standards on TLC supported their identity. Moreover, the radiochemical purity of the products remained >95% after maintenance of the preparations at 25 °C for 3 h, and they were stable for performing evaluation. From the end of bombardment, the synthesis times were 30 min for [^{11}C]**1** and 35 min for [^{11}C]**2**, respectively. Starting from the cyclotron-produced [^{11}C]CO_2 with total radioactivity of 30–37 GBq, [^{11}C]**1** or [^{11}C]**2** was obtained with about 740 MBq (n > 3) as an injectable solution. This amount of radioactivity is enough for animal experiments.

The preliminary study on the radioactivity concentrations of [^{11}C]**1** and [^{11}C]**2** in the mouse brain and blood was performed. A solution of [^{11}C]**1** and [^{11}C]**2** (average of 20 MBq/400 μL) was injected into Wister mice (30 g, 7 weeks, male) through the tail vein. Three mice for each time point were sacrificed by cervical dislocation at 1, 5, 15, 30 or 60 min after injection. The whole brain and blood samples were quickly removed. The radioactivities present in these tissues were measured and expressed as a percentage of the injected dose per gram of wet tissue. All radioactivity measurements were corrected for decay.

Table 1 summarizes the uptakes of [^{11}C]**1** and [^{11}C]**2** in the brain and blood. As can be seen, [^{11}C]**1** and [^{11}C]**2** had relatively low uptakes into the mouse brain and their radioactivity concentration in the brain was much lower than some useful PET probes[12] developed by us for clinical brain imaging. Despite their low uptakes into the brain, [^{11}C]**1** had higher levels in the brain than [^{11}C]**2** from 15 to 60 min after injection. Within this period, the two probes displayed slow washout of radioactivity from the brains, respectively, and the concentration of [^{11}C]**1** was about twofold higher than that of [^{11}C]**2**. The ratios of brain/blood radioactivity, which is an index determining the actual accumulation of a radioligand in the brain, increased from 0.07 to 0.19 for [^{11}C]**1** and from 0.06 to 0.14 for [^{11}C]**2** at the end of this experiment, respectively. Compared to [^{11}C]**2**, this result revealed that [^{11}C]**1** could pass through the BBB and be retained in the brain, although **1** has been reported to a substrate of P-glycoprotein substrate.[13]

Table 1. Radioactivity (% of injection dose/g tissue: mean ± SD, $n = 3$) in the brain and blood of mice at 1, 5,15, 30 and 60 min after injection

Tissue	Time (min)	[^{11}C]**1**	[^{11}C]**2**
Brain	1	0.20 ± 0.01	0.35 ± 0.03
	5	0.15 ± 0.01	0.21 ± 0.04
	15	0.14± 0.01	0.07 ± 0
	30	0.10 ± 0.01	0.07 ± 0.01
	60	0.07 ± 0.02	0.03 ± 0
Blood	1	2.89 ± 0.35	6.23 ± 0.34
	5	1.58 ± 0.08	3.31 ± 0.58
	15	1.36 ± 0.05	1.31 ± 0.51
	30	0.79 ± 0.06	0.59 ± 0.03
	60	0.37 ± 0.04	0.22 ± 0.04

In conclusion, [^{11}C]**1** and its active metabolite [^{11}C]**2** were synthesized for the first time as two novel PET probes. Preliminary evaluation showed that these probes had low uptakes into the mouse brain, although it was unclear whether the low concentrations in the brain are enough to induce the side CNS effects of Tamiflu. The next step of our study is to determine the detail distribution pattern and to acquire PET images of animal and human brains of different ages. We believe that our study is helpful to elucidate the cause of the side effects of Tamiflu using the two PET probes.

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References and notes

1. (a) Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, W. G.; Stevens, R. C.

J. Am. Chem. Soc. **1997**, *119*, 681; (b) Kim, C. U.; Lew, W.; Williams, M. A.; Wu, H.; Zhang, L.; Chen, X.; Escarpe, P. A.; Mendel, D. B.; Laver, W. G.; Stevens, R. C. *J. Med. Chem.* **1998**, *41*, 2451.
2. He, J.; Massarella, P.; Ward, P. *Clin. Pharmacokinet.* **1999**, *37*, 471.
3. (a) http//www.nature.com/news/2007/070319/full/446358a.html; (b) http//www.fda.gov/cder/foi/label/2006/021087s0331b1.pdf.
4. Izumi, Y.; Tokuda, K.; O'dell, K. A.; Zorumski, C. F.; Narahashi, T. *Neurosci. Lett.* **2007**, *426*, 54.
5. Fowler, J. S.; Volkow, N. D.; Wang, G.-J.; Ding, Y.-S.; Dewey, S. L. *J. Nucl. Med.* **1999**, *40*, 1154.
6. (a) McCarron, J. A.; Turton, D. R.; Pike, V. W.; Poole, K. G. *J. Label. Compd. Rad.* **1996**, *38*, 941; (b) Matarrese, M.; Sudati, F.; Soloviev, D.; Todde, S.; Turolla, E. A.; Kienle, M. G.; Fazio, F. *Appl. Radiat. Isot.* **2002**, *57*, 675.
7. (a) Abrecht, S.; Harrington, P.; Iding, H.; Karpf, M.; Trussardi, R.; Wirz, B.; Zutter, U. *Chimia* **2004**, *58*, 621; (b) Farina, V.; Brown, J. D. *Angew. Chem., Int. Ed.* **2006**, *45*, 7330; (c) Yeung, Y.-Y.; Hong, S.; Corey, E. J. *J. Am. Chem. Soc.* **2006**, *128*, 6310; (d) Fukuta, Y.; Mita, T.; Fukuda, N.; Kanai, M.; Shibasaki, M. *J. Am. Chem. Soc.* **2006**, *128*, 6312; (e) Mita, T.; Fukuda, N.; Roca, F. X.; Kanai, M.; Shibasaki, M. *Org. Lett.* **2007**, *9*, 259; (f) Yamatsugu, K.; Kamijo, S.; Suto, Y.; Kanai, M.; Shubasaki, M. *Tetrahedron Lett.* **2007**, *48*, 1403; (g) Satoh, N.; Akiba, T.; Yokoshima, S.; Fukuyama, T. *Angew. Chem., Int. Ed.* **2007**, *46*, 5734; (h) Bromfield, K. M.; Graden, H.; Hagberg, D. P.; Olsson, T.; Kann, N. *Chem. Commun.* **2007**, 3183; (i) Shie, J.-J.; Fang, J.-M.; Wang, S.-Y.; Tsai, K.-C.; Cheng, Y.-S. E.; Yang, A.-S.; Hsiao, S.-C.; Su, C.-Y.; Wong, C.-H. *J. Am. Chem. Soc.* **2007**, *129*, 11892.
8. Morita, M.; Sone, T.; Yamatsugu, K.; Sohtome, Y.; Matsunaga, S.; Kanai, M.; Watanabe, Y.; Shibasaki, M. *Bioorg. Med. Chem. Lett*. **2007**, doi:10.1016/j.bmcl.2007.11.079.
9. (a) Zhang, M.-R.; Ogawa, M.; Yoshida, Y.; Suzuki, K. *Appl. Radiat. Isot.* **2006**, *64*, 216; (b) Arai, T.; Zhang, M.-R.; Ogawa, M.; Fukumura, T.; Kato, K.; Suzuki, K. *submitted*.
10. Suzuki, R.; Inoue, O.; Hashimoto, K.; Yamasaki, T.; Kuchiki, M.; Tamate, T. *Appl. Radiat. Isot.* **1985**, *36*, 971.
11. Kato, K.; Zhang, M.-R.; Suzuki, K. *Mol. Biosyst.* **2008**, *1*, 53.
12. (a) Zhang, M.-R.; Kida, T.; Noguchi, J.; Furutsuka, K.; Maeda, J.; Suhara, T.; Suzuki, K. *Nucl. Med. Biol.* **2003**, *31*, 649; (b) Zhang, M.-R.; Ogawa, M.; Maeda, J.; Ito, T.; Noguchi, J.; Kumata, K.; Okauchi, T.; Suhara, T.; Suzuki, K. *J. Med. Chem.* **2006**, *49*, 2735; (c) Zhang, M.-R.; Kumata, K.; Maeda, J.; Yanamoto, K.; Hatori, A.; Okada, M.; Higuchi, M.; Obayashi, S.; Suhara, T.; Suzuki, K. *J. Nucl. Med.* **2007**, *48*, 1853.
13. (a) Morimoto, K.; Nakakariya, M.; Shirasaka, Y.; Kakinuma, C.; Fujita, T.; Tamai, I.; Ogihara, T. *Drug Metab. Dispos.* **2008**, *36*, 6; (b) Ose, A.; Kusuhara, H.; Yamatsugu, K.; Kanai, M.; Shibasaki, M.; Fujita, T.; Yamamoto, A.; Sugiyama, Y. *Drug Metab. Dispos*. **2007**, dmd.107.018556v2.