Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 27 August 2008, Revised 14 January 2009, Accepted 28 March 2009 Published online 3 July 2009 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1604

The first synthesis of [^{11}C]oseltamivir: a tool for elucidating the relationship between Tamiflu and its adverse effects on the central nervous system

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Oseltamivir phosphate (Tamiflu®) is an anti-influenza drug approved in many countries. Recently, in Japan, adverse effects on the central nervous system have been reported in younger patients administrated with Tamiflu. As a tool for elucidating the relationship between Tamiflu and its adverse effects, ^{11}C-labeled oseltamivir was synthesized through a two-step reaction involving [^{11}C]acetylation with [1-^{11}C]acetyl chloride. Starting from approximately 37.0 GBq of [^{11}C]CO_2, 1.2–1.8 GBq ($n = 5$) of [^{11}C]oseltamivir was obtained at the end of synthesis (EOS) 36–39 min after the end of bombardment. Radiochemical purity and specific activity were greater than 98% and 2.7–6.3 GBq/μmol at EOS, respectively.

Keywords: [^{11}C]oseltamivir; [1-^{11}C]acetyl chloride; PET

Introduction

Oseltamivir phosphate (Tamiflu®, **1**·H_3PO_4, Figure 1), an ethyl ester prodrug of the potent and selective neuraminidase inhibitor Ro 64-0802, is an anti-influenza drug approved in many countries for the treatment and prevention of influenza types A and B. The number of Tamiflu prescriptions has reached approximately 10 million in Japan, which accounted for 70–80% of the world total in 2006. Recently, in Japan, abnormal behavior such as suicide attempts, delirium and self-injury has been reported, especially in younger patients administrated with Tamiflu. According to the report by the Ministry of Health, Labor and Welfare, abnormal behavior associated with Tamiflu administration was observed in 211 patients (0.002% of all patients), which becomes a serious problem; however, the relationship between Tamiflu and abnormal behavior has not been clearly elucidated.

Adverse effects on the central nervous system (CNS) are generally induced by the accumulation of a drug and/or its metabolite(s) in the brain through the blood–brain barrier (BBB). However, it is believed that Tamiflu and its active metabolite Ro 64-0802 do not easily pass through the BBB. Recent studies demonstrated that the penetration of Tamiflu into the brain was limited by the drug efflux transporter P-glycoprotein (P-gp) at the BBB.[1] On the other hand, P-gp expression level was significantly lower in fetal or immature experimental animals than in adults[2,3] and its functional activity can be affected by several factors, such as genetic polymorphisms of P-gp,[4] drug–drug or drug–food interaction[5–7] and CNS inflammation.[8,9] These reports suggested that these factors can increase the brain permeability of Tamiflu and its metabolites, leading to adverse effects on the CNS.

Positron Emission Tomography (PET) is an advanced technology to study drug biodistribution and interaction with target proteins directly in experimental animals and humans using radiotracers labeled with short-lived positron emitters such as ^{11}C, ^{13}N, ^{15}O and ^{18}F. PET imaging has emerged as a powerful scientific and clinical tool to investigate the behavioral, therapeutic and toxic properties of drugs. PET provides a new perspective on drug research to directly assess both the pharmacokinetic and pharmacodynamic properties in animals and humans. Recent studies on the adverse effects of Tamiflu on the human CNS led us to synthesize **1** labeled with a positron emitter as a tool for studying the distribution of the drug. In this paper, we report the first synthesis of (3*R*,4*R*,5*S*)-ethyl 4-[*carbonyl*-^{11}C]acetamido-5-amino-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate ([^{11}C]**1**) using an automated production system. [1-^{11}C]acetyl chloride ([^{11}C]AcCl) was synthesized by reacting [^{11}C]CO_2 with methyl magnesium bromide (MeMgBr) in a polyethylene loop, followed by chlorination with oxalyl chloride ($(COCl)_2$) and distilled efficiently into the second reaction vessel containing a solution of precursor **2**. After the [^{11}C]acetylation of **2** (Figure 2), the Boc group in [^{11}C]**4** was easily removed with 4 M HCl at 120°C in 3 min (Scheme 1). We believe

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that our study will help to elucidate the apparent relationship between Tamiflu and its adverse effects on the CNS.

Results and discussion

There are several restrictions on the production of radiotracers labeled with a short-lived positron-emitting radionuclide. The production of radiotracers must be achieved within a short time and requires a remote-controlled system for radiation protection. In addition, Tamiflu is rapidly hydrolyzed to its active form Ro 64-0802 by carboxylesterase *in vivo*. Therefore, ^{11}C-labeling of the acetamide group in **1** with [^{11}C]AcCl was considered to be the most appropriate method.

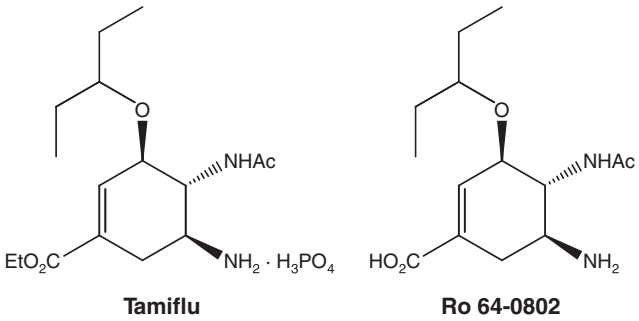


Figure 1. Chemical structures of Tamiflu and Ro 64-0802.

We initially considered to synthesize [^{11}C]**1** through a one-step reaction between the diamine **3** and [^{11}C]AcCl. In a preliminary study using nonradioactive reagents, however, the acetylation of **3** with an equimolar amount of AcCl occurred preferentially in the sterically less hindered 5-amino position, resulting in very poor yield of **1**.[10] Therefore, precursor **2** protecting the 5-amino group with the Boc group was synthesized from **1** by a new route (Scheme 2). The two-step synthesis of **2** was accomplished with an overall yield of 28%. The structural and stereochemical assignment of **2** was confirmed by a comparison of ^{1}H NMR, ^{13}C NMR and optical rotation data for **1** synthesized from **2** with those of an authentic sample[11]. Physical and spectroscopic data for **4** were also identical to those reported previously.[12] Additionally, precursor **2** showed no contamination by the target compound **1** in the HPLC analysis.

The synthesis of [^{11}C]**1** was achieved through a two-step reaction consisting of the [^{11}C]acetylation of **2** and the removal of the Boc group in the resultant [^{11}C]**4** (Scheme 1). Figure 2 shows a

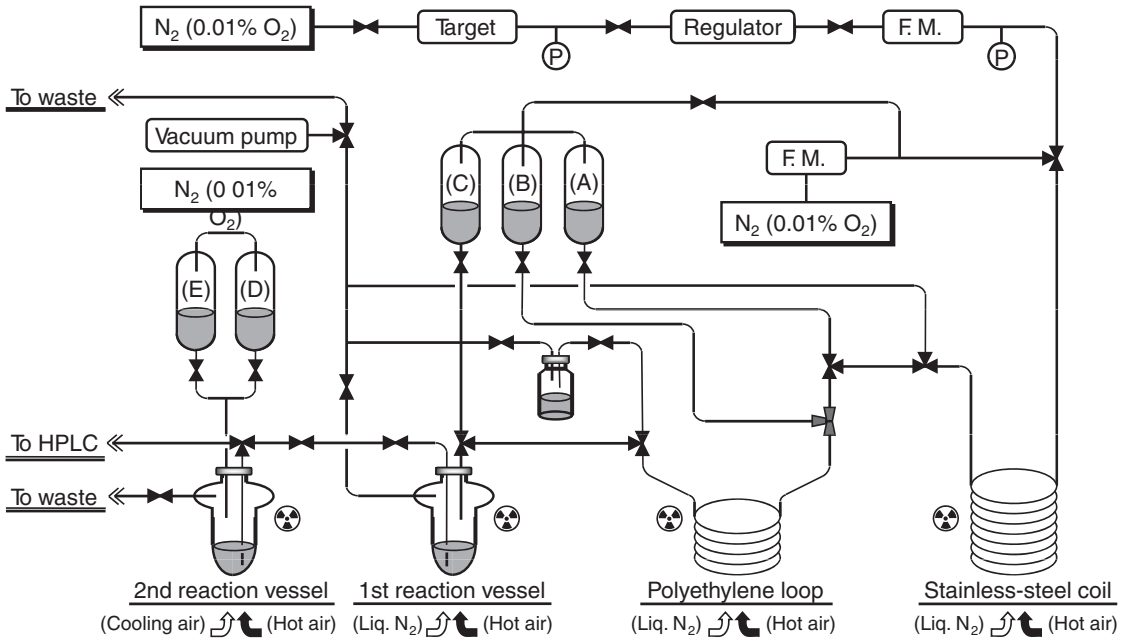
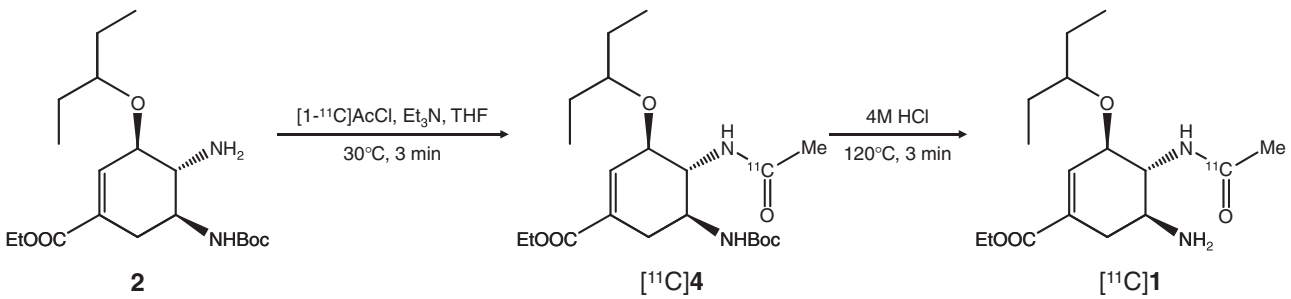
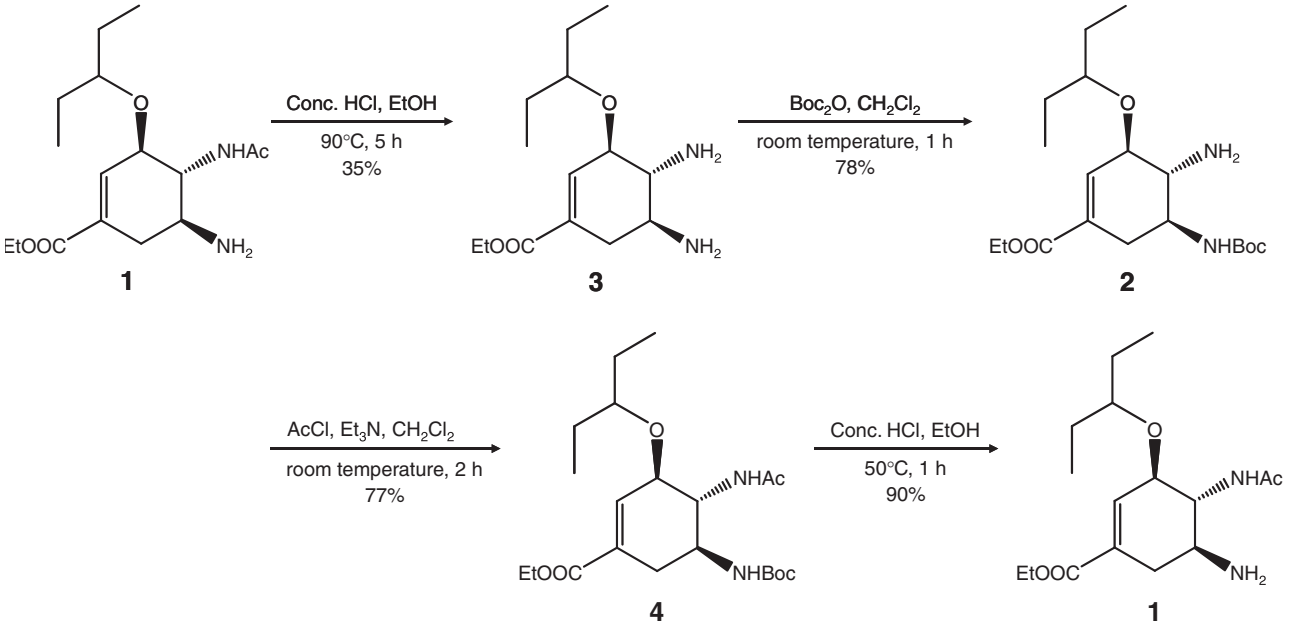


Figure 2. Schematic diagram of an automated production system for [^{11}C]**1**. (a) $(COCl)_2$/THF; (b) 1 M MeMgBr/THF; (C) DMF; (D) 4 M HCl; (E) 10% NH_3 solution; F.M.: flow meter; P: pressure gauge; ⋈: two-way solenoid valve; ⋈: three-way solenoid valve; ⋈: manual three-way valve; ⌒: cooler; ⌒: heater; ☢: radioactivity sensor.



Scheme 1. Synthesis of [^{11}C]**1**.



Scheme 2. Synthesis of precursor **2**.

schematic diagram of an automated production system for [^{11}C]**1**. Synthesis involves the following steps: (1) trapping [^{11}C]CO_2 in a stainless-steel coil; (2) transferring [^{11}C]CO_2 into the polyethylene loop coated with MeMgBr; (3) passing $(COCl)_2$/THF through the loop and transferring the radioactive mixture into the first reaction vessel; (4) distilling [^{11}C]AcCl into the second reaction vessel containing a solution of precursor **2**; (5) [^{11}C]acetylation of **2**; (6) adding 4 M HCl into the second reaction vessel to remove the Boc group in the resultant [^{11}C]**4**; (7) purification of [^{11}C]**1** by HPLC.

Starting from approximately 37.0 GBq of [^{11}C]CO_2, 1.2–1.8 GBq ($n=5$) of [^{11}C]**1** was obtained at the end of synthesis (EOS). The total synthesis time was 36–39 min from the end of bombardment. Radiochemical purity and specific activity were greater than 98% and 2.7–6.3 GBq/μmol at EOS, respectively. No peaks corresponding to precursor **2** and other chemical impurities were detected by HPLC. Its identity was confirmed by HPLC co-injection with an authentic sample and LC-MS analysis. The retention time of [^{11}C]**1** matched that of an authentic sample. Furthermore, the LC-MS spectra showed an [M+H$^+$] ion at *m/z* 313.2 corresponding to oseltamivir. Although the small radioactive peak that had a retention time identical to that of possible by-product 5-acetylamino derivative **5** was observed in HPLC analysis of the final reaction mixture, [^{11}C]**1** and this impurity were completely separated by semipreparative HPLC (Figure 3). Previously, we found that the nonradioactive CO_2 present in the MeMgBr solution acted as a source of contamination of the [^{11}C]CO_2 and reduced the specific activity of the product.[13] Although only a small amount of MeMgBr was used for the synthesis of [^{11}C]AcCl, the specific activity of [^{11}C]**1** was unexpectedly low. The cause of this is currently under investigation.

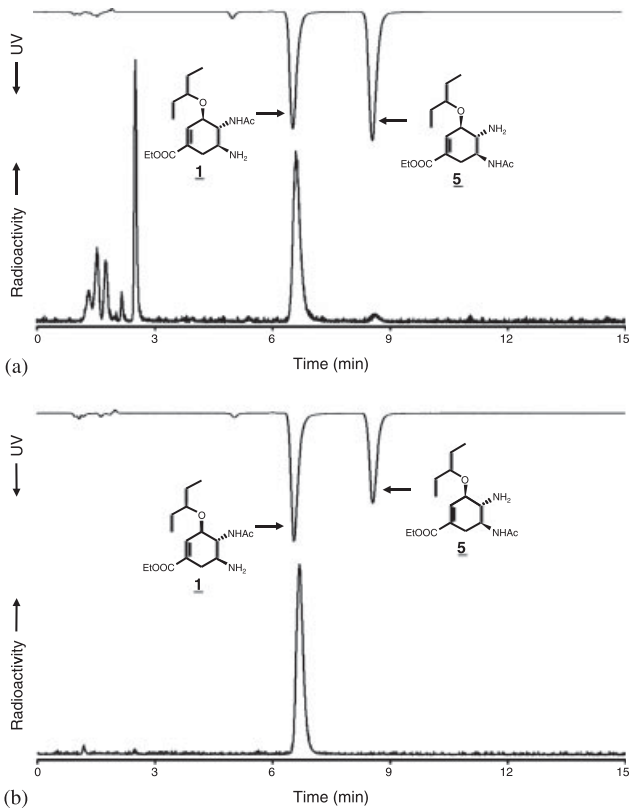


Figure 3. HPLC profiles of (a) the final reaction mixture and (b) [^{11}C]**1** isolated from the reaction mixture by HPLC, co-injected with authentic samples of **1** and 5-acetylamino derivative. HPLC conditions: column, CAPCELL PAK C18 (Shiseido, 4.6 mm i.d. × 250 mm); eluent, MeCN/H_2O/Et_3N (30/70/0.1); flow rate, 1.5 mL/min; UV 254 nm.

Experimental

Materials and methods

Carbon-11 was produced by the ^{14}N(p, α)^{11}C nuclear reaction using the CYPRIS HM-18 cyclotron (Sumitomo Heavy Industry, Tokyo, Japan). [^{11}]Carbon dioxide was produced by the bombardment of dry N_2 gas (1.5 MPa; Nippon Sanso, Tokyo, Japan) containing 0.01% O_2 (Nippon Sanso) with a beam

(15–20 μA) of 18 MeV protons (14.2 MeV on target). Radioactivity was measured by a dose calibrator (IGC-3R Curiemeter; Aloka, Tokyo, Japan). HPLC was performed using a JASCO HPLC system (JASCO, Tokyo, Japan): the eluate was monitored for radioactivity (NaI (Tl) scintillation detector system) and UV absorption at 254 nm. For analytical HPLC, CAPCELL PAK C18 column (4.6 mm i.d. × 250 mm, Shiseido, Tokyo, Japan) was used and eluted with MeCN/H_2O/Et_3N (30/70/0.1) at a flow rate of 1.5 mL/min (**1**; retention time = 6.6 min). For semipreparative HPLC, XBridge C18 column (10 mm i.d. × 250 mm, Waters Co., Milford, MA, USA) was used and eluted with MeCN/H_2O/Et_3N (30/70/1) at a flow rate of 5.0 mL/min (**1**; retention time = 7.2 min). LC-MS was performed in positive ion mode on an Agilent 1100 Series HPLC system (Agilent Technologies, Walldbron, Germany) coupled with an Applied Biosystem 4000 QTrap mass spectrometer (Applied Biosystems, Darmstadt, Germany). The LC system was equipped with an XBridge C18 column (3 mm i.d. × 100 mm, Waters Co.), eluted with MeCN/5 mM HCOOH (25/75) at a flow rate of 0.3 mL/min (retention time = 5.2 min).

Melting points (m.p.) were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JNM-GX-270 spectrometer. High-resolution FAB mass spectra (HRMS (FAB)) were obtained on a JEOL NMS-SX 102-SX spectrometer. Optical rotations were recorded on a JASCO P-2200 digital polarimeter. Column chromatography was performed on Merck Kieselgel gel 60 F_{254} (70–230 mesh). TLC was carried out on Merck Kieselgel 60 F_{254} plates.

Chemicals and solvents were obtained commercially and used without further purification. Oseltamivir phosphate was purchased from Sequoia Research Products Ltd. (Oxford, UK), MeMgBr in THF (about 1.0 M) from Kanto Chemical Co., Inc. (Tokyo, Japan) and $(COCl)_2$ and 2,6-di-*tert*-butylpyridine from Sigma-Aldrich Chemical Co., Ltd. (Milwaukee, WI, USA) at the highest grade available.

Chemistry

*Synthesis of (3R,4R,5S)-ethyl 4,5-diamino-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (**3**)*

To a solution of **1** (114 mg, 0.37 mmol) in EtOH (2 mL) was added concentrated HCl (1 mL). The reaction mixture was stirred at 90°C for 5 h. After evaporation of the solvent, the residue was dissolved with H_2O (3 mL) and washed with AcOEt (2 mL). The layers were separated and the aqueous phase was basified with saturated $NaHCO_3$ and extracted with $CHCl_3$ (5 mL × 3). The combined organic extracts were dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography on silica gel with 2% MeOH/$CHCl_3$ to give **3** (34.9 mg, 35%) as a pale yellow oil: IR (ATR) ν_{max} 1714, 3292, 3373 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.923 (t, J = 7.3 Hz, 3H), 0.93 (t, J = 7.3 Hz, 3H), 1.28 (t, J = 7.2 Hz, 3H), 1.44–1.69 (m, 4H), 1.97–2.08 (m, 1H), 2.60–2.82 (m, 3H), 3.37 (quintet, J = 5.7 Hz, 1H), 3.77–3.81 (m, 1H), 4.18 (q, J = 7.2 Hz, 2H), 6.78 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 9.4, 9.7, 14.1, 25.7, 26.4, 34.0, 51.1, 58.5, 60.7, 79.0, 80.9, 129.4, 137.6, 166.5; HRMS (FAB): *m/z* calculated for $C_{14}H_{27}N_2O_3$ [M+H$^+$]: 271.2022, found 271.2056; $[\alpha]_D^{22}$ −40.3 (*c* 0.55, $CHCl_3$).

*Synthesis of (3R,4R,5S)-ethyl 4-amino-5-(tert-butoxycarbonylamino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (**2**)*

To a solution of **3** (46.5 mg, 0.17 mmol) in CH_2Cl_2 (3 mL) was added di-*tert*-butyldicarbonate (41.4 mg, 0.19 mmol) in CH_2Cl_2 (1.5 mL). The reaction mixture was stirred at room temperature for 1 h. After evaporation of the solvent, the residue was purified by column chromatography on silica gel with $CHCl_3$ to give **2** (49.5 mg, 78%) as colorless prisms: m.p. 51–52°C; IR (ATR) ν_{max} 1699, 1715 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.93 (t, J = 7.3 Hz, 6H), 1.29 (t, J = 7.2 Hz, 3H), 1.45 (s, 9H), 1.48–1.63 (m, 6H), 2.16–2.27 (m, 1H), 2.76–2.89 (m, 2H), (m, 1H), 3.40 (quintet, J = 5.7 Hz, 1H), 3.72 (br, 1H), 3.82–3.85 (m, 1H), 4.20 (q, J = 7.2 Hz, 2H), 5.01 (br, 1H), 6.81 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 9.5, 9.6, 14.2, 25.7, 26.3, 28.3, 55.1, 60.8, 78.5, 81.2, 129.3, 136.6, 155.7, 166.3; HRMS (FAB): *m/z* calculated for $C_{19}H_{35}N_2O_5$ [M+H$^+$]: 371.2546, found 371.2581; $[\alpha]_D^{27}$ −27.3 (*c* 0.36, $CHCl_3$).

*Synthesis of (3R,4R,5S)-ethyl 4-acetamido-5-(tert-butoxycarbonylamino)-3-(pentan-3-yloxy)cyclohex-1-ene1-carboxylate (**4**)*

To a solution of **2** (49.5 mg, 0.13 mmol) and Et_3N (23.6 μL, 0.17 mmol) in CH_2Cl_2 (3 mL), acetyl chloride (10.2 μL, 0.14 mmol) in CH_2Cl_2 (2 mL) was added dropwise at 0°C. The reaction mixture was stirred at room temperature for 2 h. After evaporation of the solvent, the residue was purified by column chromatography on silica gel with 40% AcOEt/hexane to gave **4** (42.4 mg, 77%) as colorless needles: m.p. 140–141°C; IR (ATR) ν_{max} 1670, 1690, 1705 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.88 (t, J = 7.3 Hz, 3H), 0.90 (t, J = 7.3 Hz, 3H), 1.29 (t, J = 7.2 Hz, 3H), 1.43 (s, 9H), 1.48–1.54 (m, 4H), 1.99 (s, 3H), 2.25–2.34 (m, 1H), 2.74 (dd, J = 17.8, 5.3 Hz, 1H), 3.36 (quintet, J = 5.6 Hz, 1H), 3.74–3.85 (m, 1H), 3.96–3.98 (m, 1H), 4.03–4.16 (m, 1H), 4.20–4.26 (m, 2H), 5.14 (d, J = 9.0 Hz, 1H), 5.88 (d, J = 9.2 Hz, 1H), 6.79 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 9.2, 9.5, 14.1, 23.3, 25.7, 26.1, 28.3, 30.9, 49.1, 54.4, 60.9, 75.8, 79.6, 82.2, 129.3, 137.6, 156.3, 165.9, 170.8; MS (FAB): *m/z* 413 [M+H$^+$]; $[\alpha]_D^{27}$ −94.4 (*c* 0.20, $CHCl_3$).

*Synthesis of (3R,4R,5S)-ethyl 4-acetamido-5-amino-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (**1**)*

To a solution of **4** (40 mg, 0.10 mmol) in EtOH (2 mL) was added concentrated HCl (1 mL). The reaction mixture was stirred at 50°C for 1 h. After evaporation of the solvent, the residue was dissolved with H_2O (2 mL) and washed with AcOEt (1 mL). The layers were separated and the aqueous phase was basified with saturated $NaHCO_3$ and extracted with $CHCl_3$ (3 mL × 3). The combined organic extracts were dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography on silica gel with 5% MeOH/$CHCl_3$ to give **1** (27.2 mg, 90%) as colorless prisms: m.p. 107–108°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.90 (t, J = 7.3 Hz, 3H), 0.91 (t, J = 7.3 Hz, 3H), 1.29 (t, J = 7.0 Hz, 3H), 1.46–1.57 (m, 4H), 2.05 (s, 3H), 2.10–2.21 (m, 1H), 2.76 (dd, J = 17.6, 5.1 Hz, 1H), 3.23 (quintet, J = 5.5 Hz, 1H), 3.31–3.38 (m, 1H), 3.48–3.57 (m, 1H), 4.21 (dd, J = 14.3, 7.0 Hz, 2H), 4.21–4.26 (m, 1H), 5.68 (d, J = 8.1 Hz, 1H), 6.79 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 9.3, 9.5, 14.2, 23.7, 25.7, 26.2, 33.6, 49.2, 59.0, 60.8, 74.8, 81.6, 129.6, 137.5, 166.3, 170.9; MS (FAB): *m/z* 313 [M+H$^+$]; $[\alpha]_D^{26}$ −60.9 (*c* 0.36, $CHCl_3$). Comparison of **1** with an authentic sample revealed that ^{1}H NMR, ^{13}C NMR and optical rotation data were identical. For an authentic sample, m.p. 107–108°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.90

(t, J = 7.3 Hz, 3H), 0.91 (t, J = 7.3 Hz, 3H), 1.29 (t, J = 7.0 Hz, 3H), 1.46–1.57 (m, 4H), 2.05 (s, 3H), 2.09–2.21 (m, 1H), 2.76 (dd, J = 17.2, 5.1 Hz, 1H), 3.23 (quintet, J = 5.5 Hz, 1H), 3.31–3.38 (m, 1H), 3.48–3.56 (m, 1H), 4.21 (dd, J = 14.3, 7.0 Hz, 2H), 4.21–4.24 (m, 1H), 5.62 (d, J = 8.1 Hz, 1H), 6.79 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 9.3, 9.5, 14.2, 23.7, 25.7, 26.2, 33.7, 49.2, 59.1, 60.8, 74.8, 81.6, 129.6, 137.5, 166.3, 170.9; $[\alpha]_D^{27}$ −60.8 (*c* 0.36, $CHCl_3$).

*(3R,4R,5S)-ethyl 5-acetamido-4-amino-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (**5**)*

Colorless needles; m.p. 160–161°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.92 (t, J = 7.3 Hz, 3H), 0.93 (t, J = 7.3 Hz, 3H), 1.29 (t, J = 7.0 Hz, 3H), 1.47–1.62 (m, 4H), 1.98 (s, 3H), 2.26–2.34 (m, 1H), 2.76–2.83 (m, 1H), 2.90–2.95 (m, 1H), 3.42 (quintet, J = 5.9 Hz, 1H), 3.81–3.82 (m, 1H), 4.02–4.11 (m, 1H), 4.20 (dd, J = 14.3, 7.0 Hz, 2H), 6.44 (d, J = 7.7 Hz, 1H), 6.85 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 9.5, 14.1, 23.4, 25.8, 26.2, 29.5, 48.4, 54.0, 60.8, 77.9, 81.4, 129.4, 135.9, 166.3, 170.0; MS (FAB): *m/z* 313 $[M+H^+]$; $[\alpha]_D^{27}$ −24.7 (*c* 0.35, $CHCl_3$).

Radiochemistry

*Automated production system for [^{11}C]**1***

The automated production system used for the synthesis of [^{11}C]**1** was developed at the National Institute of Radiological Sciences (NIRS). The unit is versatile and equipped for the synthesis of multiple PET radiopharmaceuticals (Figure 2).[14]

The coil is a stainless-steel trap (SUS316; length: 1.5 m; o.d.: $\frac{1}{8}$ in; i.d.: 1 mm) and the loop is a polyethylene tube (length: 40 cm; o.d.: $\frac{1}{16}$ in; i.d.: 0.75 mm). Major parts, such as the coil, loop, reaction vessels and solenoid valves, are connected by $\frac{1}{16}$ in Teflon tubes. Quick cooling and heating (monitored by a temperature sensor) during these processes is realized by using a cooling device (Vortex, OH, USA) with liquid N_2 and a heating device (Nakazawa Seisakusho, Chiba, Japan) with hot air, respectively.

Synthesis of [^{11}C]AcCl

During the production of [^{11}C]CO_2, MeMgBr (1 M in THF, 500 μL) was passed through the polyethylene loop (cooled between −5 and 0°C) previously flushed with N_2. N_2 (3 mL/min) was then passed through the loop for 30 s to remove the excess MeMgBr solution and to leave a thin film of MeMgBr. After irradiation, [^{11}C]CO_2 was carried from the target with a stream of N_2 (1000 mL/min) and trapped in the stainless-steel coil cooled between −170 and −165°C. After trapping of [^{11}C]CO_2, this coil was heated at 50°C and concentrated [^{11}C]CO_2 was transferred in an N_2 stream (3.0 mL/min) into the loop (maintained between −5 and 0°C) coated with MeMgBr. By passing $(COCl)_2$/THF (10/400, 400 μL) through the loop, the radioactive mixture was transferred into the first reaction vessel containing 2,6-di-*t*-butylpyridine (30 μL).

*Synthesis of [^{11}C]**1***

After the addition of DMF (300 μL) into the first reaction vessel, synthesized [^{11}C]AcCl was distilled into the second reaction vessel (maintained between −10 and −5°C) containing a solution of **2** (2 mg) and Et_3N (30 μL) in THF (300 μL) by heating the first reaction vessel at 120°C in an N_2 stream (150 mL/min). The entire distillation step lasted for approximately 5 min. After distillation, the second reaction vessel was heated at 30°C for 3 min to facilitate the [^{11}C]acetylation of **2**. Into the second reaction vessel, 4 M HCl (300 μL) was added to remove the Boc group in the resultant [^{11}C]**4**. After reaction at 120°C for 3 min, the reaction mixture was treated with 10% NH_3 solution (500 μL) and applied onto a semipreparative HPLC column. A radioactive fraction with a retention time of approximately 7 min was collected, evaporated and dissolved in sterile water for injection.

*Analysis of [^{11}C]**1***

The identity of [^{11}C]**1** was confirmed by HPLC co-injection with an authentic sample and LC-MS analysis. Radiochemical purity analysis of [^{11}C]**1** was performed on an analytical HPLC column. Specific activity was calculated by determining the area of the UV absorbance peak of the carrier ligand in an aliquot of known radioactivity compared with the area of a standard sample.

Conclusion

Through a two-step reaction consisting of the [^{11}C]acetylation of **2** with [^{11}C]AcCl, followed by the removal of the Boc group in the resultant [^{11}C]**4**, [^{11}C]**1** was synthesized repeatedly and reliably in adequate radiochemical yield and purity suitable for routine use in animal and human studies.

Acknowledgement

The authors thank the cyclotron crew of NIRS and Mr M. Takei, Tokyo Nuclear Service, for their technical support in the operation of the cyclotron and production of radioisotopes. We also thank Mr K. Furutsuka and T. Ito, SHI Accelerator Service, for their excellent assistance in the MS analysis. This study was partially supported by a consignment grant for the Molecular Imaging Program on Research Base for PET Diagnosis from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT).

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