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Radiosynthesis and quality control of [^{11}C]TASP457 as a clinically useful PET ligand for imaging of histamine H_3 receptors in human brain

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ABSTRACT

Introduction: Recently, 6-[(1-cyclobutylpiperidin-4-yl)oxy]-1-(6-[^{11}C]methoxypyridin-3-yl)-3,4-dihydroquinolin-2(1*H*)-one ([^{11}C]TASP457, [^{11}C]**2**) has been developed as a novel PET ligand for histamine H_3 receptors in brain. [^{11}C]**2** is potentially suitable for imaging H_3 receptors in rat and monkey brains, which has motivated us to perform first-in-human study of [^{11}C]**2** for qualifying H_3 receptors in human brain. In this paper, we report an efficient radiosynthesis of [^{11}C]**2** to obtain sufficient radioactivity and high quality for clinical application.
Methods: In manual synthesis, we optimized the reaction conditions of desmethyl precursor **1**, which contains a 2-hydroxypyridine moiety, with [^{11}C]MeI or [^{11}C]MeOTf. After optimization, we performed automated synthesis and quality control of [^{11}C]**2**.
Results: Bubbling [^{11}C]MeOTf into a heated mixture of precursor **1** and cesium carbonate in DMF at 100 °C for 90 s produced [^{11}C]**2** with decay-corrected radiochemical yields of (based on [^{11}C]CO_2) 7.9 ± 1.8% (n = 78). The specific activity of [^{11}C]**2** was 156 ± 52 GBq/μmol (n = 78) at the end of synthesis. The total synthesis time was approximately 35 min from the end of bombardment. All the quality control results of [^{11}C]**2** were in compliance with our in-house quality control/assurance specifications.
Conclusion: We radiosynthesized [^{11}C]TASP457 ([^{11}C]**2**) with sufficient amounts of radioactivity and high quality for clinical usefulness. This radioligand is being used for PET assessment of H_3 receptors in human brain in our facility.

1. Introduction

Histaminergic neurotransmission is involved in numerous pathologies of the central nervous system, such as sleep, addiction, depression, schizophrenia, pain, and neurodegeneration, for example in Alzheimer's and Parkinson's diseases [1]. Histamine H_3 receptors have been reported to play a role in the synthesis and regulation of release of histamine, and are widely expressed in the mammalian brain, with the highest density expressed in the areas involved with cognitive processes and arousal [2].

Molecular imaging is an advancing technology that allows for visualization of interactions between molecular probes and biological targets. Positron emission tomography (PET), in particular, is a useful modality that enables *in vivo* biological information to be obtained in a noninvasive manner using a variety of radioligands [3,4]. Since 1994, several PET ligands for imaging of H_3 receptors have been synthesized and evaluated [5–12]. The carbodithioate analog *S*-[^{11}C]methylthioperamide was the first reported H_3-targeting PET ligand [6], but the brain uptake of this radioligand demonstrated a high level of nonspecific binding [6]. As the first reported ^{18}F-labeled H_3 ligand, the imidazolylbenzophenone analog [^{18}F]FUB272 showed low and non-specific binding in brain [7]. On the other hand, because non-imidazole-based H_3 receptor antagonists seemed to be more promising clinical candidates as drugs with higher target selectivity than the previous imidazole analogs, pharmacophores in the development of PET ligands have been converted to non-imidazole analogs. The first reported non-imidazole-based radioligand was derived from the benzylmorpholine analog JNJ-10181457 [8]. Followed by the JNJ compound, some promising PET ligands for H_3 receptors have been reported [9–12]. Among them, [^{11}C]GSK189254 [9] and [^{11}C]MK-8278 [10], both belonging to non-imidazole analogs, have recently been developed and used for clinical studies of H_3 receptors in human brain (Fig. 2). [^{11}C]GSK189254 has an extremely high affinity for H_3 receptors, but has slow brain kinetics that limits the accuracy and precision of PET quantification of H_3 density in human brain [9]. [^{11}C]MK-8278 has shown large test–retest variability especially in the high density H_3 regions of human

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brain [10]. Therefore, a novel PET ligand with more suitable pharmacokinetics and better binding characteristics is required for imaging and assessment of H_3 receptors in clinical research.

More recently, we have labeled a new class of non-imidazole H_3 receptor ligands and evaluated their potentials for PET imaging of brain H_3 receptors *in vivo*. Among these radioligands, 6-[(1-cyclobutylpiperidin-4-yl)oxy]-1-(6-[^{11}C]methoxypyridin-3-yl)-3,4-dihydroquinolin-2(1*H*)-one ([^{11}C]TASP457 or [^{11}C]TASP0410457, [^{11}C]**2**) (Fig. 1) showed high binding affinity for H_3 receptors (IC_{50} = 2.34 nM for rat and 1.80 nM for monkey) and high selectivity for compared to other receptors (IC_{50} = 379 nM for σ_1 and 1000 nM for adrenergic α_{2c} receptors) [13]. Radioligand [^{11}C]**2** has shown to be suitable for PET imaging of H_3 receptors in rat and monkey brains, as it offered robust quantitative measurement of H_3 receptor binding throughout the H_3-enriched brain regions [13]. These results have motivated us to translate [^{11}C]**2** for the first-in-human study in our facility [14].

In this paper, we report an efficient synthesis of [^{11}C]**2** that provides with sufficient radioactivity for clinical research use that routinely meets our quality control and release criteria.

2. Materials and methods

Desmethyl precursor 6-[(1-cyclobutylpiperidin-4-yl)oxy]-1-(6-hydroxypyridin-3-yl)-3,4-dihydroquinolin-2(1*H*)-one (**1**, Fig. 2), 6-[(1-cyclobutylpiperidin-4-yl)oxy]-1-(6-methoxypyridin-3-yl)-3,4-dihydroquinolin-2(1*H*)-one (TASP457, TASP0410457, **2**, Fig. 1), and *N*-methylated byproduct 6-[(1-cyclobutylpiperidin-4-yl)oxy]-1-(1-methyl-6-oxo-1,6-dihydropyridin-3-yl)-3,4-dihydroquinolin-2(1*H*)-one (**3**, Fig. 2) were synthesized and kindly supplied from Taisho Pharmaceutical (Tokyo, Japan). Commercially available reagents and organic solvents were purchased from Sigma-Aldrich (St. Louis, MI, USA) and Wako Pure Chemical Industries (Osaka, Japan) without further purification. Injection water and sodium phosphate corrective injection (0.5 mol/L) were purchased from Otsuka Pharmaceutical Factory (Naruto, Tokushima, Japan). Semi-preparative HPLC was performed using a Jasco HPLC system (Jasco, Tokyo, Japan). Analytical HPLC was performed using the Jasco HPLC system or a Waters HPLC system (Milford, MA, USA). The ^{11}C radioactivity was produced using a cyclotron (CYPRIS HM-18; Sumitomo Heavy Industries, Tokyo, Japan).

2.1. Radiolabeling of **1** by manual synthesis using [^{11}C]methyl iodide

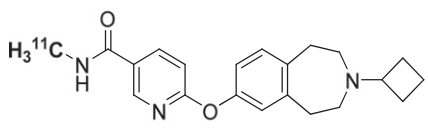
[^{11}C]Carbon dioxide ([^{11}C]CO_2) was produced using the ^{14}N(p,α)^{11}C nuclear reaction in a 0.01% oxygen-containing nitrogen gas with 18 MeV proton beams (15.8 MeV on target). Following the bombardment process, [^{11}C]CO_2 was transferred to a reaction vessel containing a 50 mmol/L solution of lithium aluminium hydride in tetrahydrofuran (500 μL) at a temperature in the range −10 to −15 °C. The resulting solution was concentrated to dryness and a 57% solution of hydriodic acid (400 μL) was added to the vessel. The mixture was then heated for 150 °C to produce [^{11}C]methyl iodide ([^{11}C]MeI). The [^{11}C]MeI was purified by passing it through a small column filled with Ascarite and phosphorus pentoxide, and collected in a reaction vial containing a solution of **1** (1.0 mg) and base in *N*,*N*-dimethylformamide (DMF, 300 μL) at a flow rate of 30 mL/min at room temperature. The reaction mixture was then heated at 100 °C for 3 min, followed by addition of water (100 μL) to terminate the reaction. The detailed reaction conditions are showed in Table 1. Aliquots of the reaction mixtures were analyzed by reverse-phase HPLC using an X-terra C-18 column (4.6 mm i.d. × 150 mm, 5 μm, Waters). A flow rate of 1 mL/min was used with an isocratic mobile phase (acetonitrile/50 mmol/L phosphoric acid (25/75, *v*/v)). The absorbance was monitored at a wavelength of 254 nm. The identity of reaction products was confirmed by co-injection of unlabeled sample **2** or **3** with the corresponding reaction mixture.

2.2. Radiolabeling of **1** by manual synthesis using [^{11}C]methyl triflate

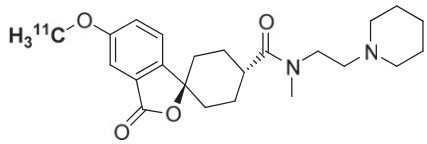
[^{11}C]Methyl triflate ([^{11}C]MeOTf) for radiosynthesis was prepared from [^{11}C]MeI as previously described [15]. Briefly, [^{11}C]MeOTf was generated by the reaction of fresh [^{11}C]MeI with silver triflate in an on-line flow-through process at 150 °C under a N_2 gas flow of 30 mL/min. Reaction condition surveys of [^{11}C]**2** were carried out according to the same procedures with [^{11}C]MeI. The detailed reaction conditions are showed in Table 2. Aliquots of the reaction mixtures were analyzed by reverse-phase HPLC.

2.3. Automated production of [^{11}C]**2** by remote-controlled synthesis

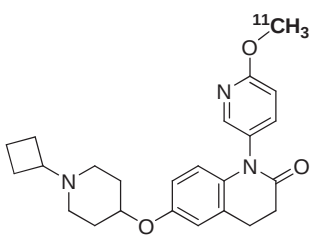
[^{11}C]MeOTf was synthesized using an automated synthesis system developed in house [16]. The produced [^{11}C]MeOTf was transferred



[^{11}C]GSK189254



[^{11}C]MK8278



[^{11}C]TASP457 ([^{11}C]2)

Fig. 1. Chemical structures of PET ligands for imaging histamine H_3 receptors in human brain.

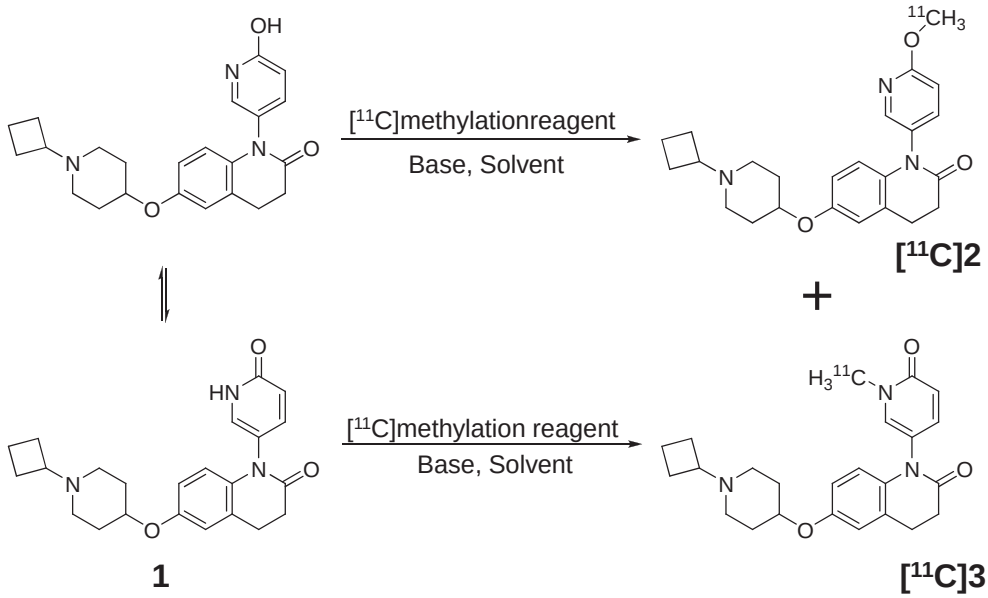


Fig. 2. Formation of *O*-[^{11}C]**2** and undesired byproduct *N*-[^{11}C]**3** by the reaction of desmethyl precursor **1** with [^{11}C]methylation reagent.

and bubbled into a heated mixture of **1** (1.0 mg) and cesium carbonate (Cs_2CO_3, 10 mg) in DMF (300 μL) at 100 °C with N_2 gas (50 mL/min). After radioactivity in the reaction vial remained unchanged, the HPLC solvent (1.0 mL) was immediately added to the reaction vial to terminate this reaction. The reaction mixture was transferred to a semi-preparative HPLC system. [^{11}C]**2** was separated from the reaction mixture by HPLC on a X-Bridge RP18 column (10 mm i.d. × 250 mm, 5 μm, Waters) eluted with mobile phase (acetonitrile/50 mmol/L phosphoric acid (23/77), (*v*/v)) at a flow rate of 4.0 mL/min. The eluent was monitored for its UV absorbance (254 nm) and radioactivity. The retention time for [^{11}C]**2** was approximately 8.5 min. The radioactive fraction corresponding to [^{11}C]**2** was collected into a rotary evaporator flask containing polysorbate 80/ethanol (1/4, (*v*/v), 100 μL). The solvent was removed *in vacuo* at 150 °C. The resulting residue was dissolved with phosphate solution (injection water/sodium phosphate corrective injection 13/2, (*v*/v), 15 mL) and sterilized with a Millex-GS filter (Millipore, Billerica, MA, USA) to obtain [^{11}C]**2** as an injectable solution.

2.4. Quality control of [^{11}C]**2**

All quality control procedures for [^{11}C]**2** were performed according to our in-house PET radiopharmaceuticals standards authorized by the Japanese Society of Nuclear Medicine (JSNM) and the Japanese Pharmacopeia, sixteenth edition (JP16) [17]. The quality control specifications include visual inspection, radioactivity measurement, residual solvents, pyrogens (endotoxin) burden and sterility testing. A dose calibrator (IGC-3R Curiemeter; Aloka, Tokyo, Japan) was used for all the radioactivity measurements (unless otherwise stated). HPLC effluent radioactivity was measured using a NaI (Tl) scintillation detector system (Ohyo Koken Kogyo, Tokyo, Japan). The pH value was measured by pH meter (F-71; HORIBA, Kyoto, Japan) equipped with a probe (9618-10D; HORIBA). The endotoxin content in the injectable sample was measured using a toxinometer (ET-6000, Wako Pure Chemical Industries). Soybean-casein digest broth and fluid thioglycollate medium were purchased from Merck Millipore (Darmstadt, German). Residual organic solvent analyses were conducted on a gas chromatography 7890 system (Agilent Technologies, Santa Clara, CA, USA). Radiochemical purity was assayed by analytical HPLC [18]. Aliquots of the final radioactive products were analyzed by reverse-phase HPLC onto an X-Bridge shield RP18 column (3.0 mm i.d. × 50 mm, 2.5 μm). An isocratic mobile phase (90% acetonitrile/100 mmol/L ammonium phosphate buffer (pH 2.0) containing 5 mmol/L sodium 1-octanesulfonate (30:70, *v*/v)) was used at a flow rate of 1 mL/min. The absorbance was monitored at a wavelength of 254 nm.

3. Results and discussion

3.1. Optimization of reaction conditions

In the present radiosynthesis of [^{11}C]**2**, compound **1**, which contains a 2-hydroxypyridine moiety (Fig. 2), was used as the desmethyl precursor for radiolabeling.

Table 1
Ratios of [^{11}C]**2** under various reaction conditions by using [^{11}C]MeI[a].

Entry	Base	Solvent (200 μL)	[^{11}C]**2**/[^{11}C]**3** ratio ($n = 4$)[b]
1	1 mol/L NaOH (10 μL)	DMF	1.1: 98.9
2	NaOH (10 mg)	DMF	0.9: 99.1
3	1 mol/L TBAOH (10 μL)	DMF	1.4: 98.6
4	CsF (10 mg)	DMF	no reaction
5	K_2CO_3 (10 mg)	DMF	0: 100
6	Cs_2CO_3 (10 mg)	DMF	5.3: 94.7

[a] Reaction condition: [^{11}C]MeI/DMF (37 MBq–370 MBq, 100 μL), **1** (1 mg, 2.5 μmol), 100 °C, 3 min.
[b] Determined from the radioactivity areas in the analytical radio-HPLC chromatograms.

Table 2
Ratios of [^{11}C]**2** under various reaction conditions by using [^{11}C]MeOTf[a].

Entry	Base	Temperature (°C)	Solvent (200 μL)	[^{11}C]**2**/[^{11}C]**3** ratio ($n = 4$)[b]
1	None	100	DMF	No reaction
2	1 mol/L NaOH (10 μL)	100	DMF	13.4: 86.6
3	Cs_2CO_3 (10 mg)	100	DMF	28.8: 71.2
4	Cs_2CO_3 (10 mg)	100	DMSO	27.4: 72.6
5	Rb_2CO_3 (10 mg)	100	DMF	22.9: 77.1
6	$CsHCO_3$ (10 mg)	100	DMF	20.6: 79.4
7	Cs_2CO_3 (10 mg)	Room temperature	DMF	No reaction
8[c]	Cs_2CO_3 (10 mg)	100	DMF	47.9: 52.1

[a] Reaction condition: [^{11}C]MeOTf/DMF or DMSO (37 MBq–370 MBq, 100 μL), **1** (1 mg, 2.5 μmol), 3 min.
[b] Determined from the radioactivity areas in the analytical radio-HPLC chromatograms.
[c] [^{11}C]MeOTf was collected into a heated mixtures of **1** and Cs_2CO_3 in DMF (300 μL) at 100 °C with N_2 gas (50 mL/min), $n = 1$.

Because the desmethyl precursor **1** contains two putative methylating sites on the 2-hydroxypyridyl group, it is difficult to obtain only the *O*-[^{11}C]methylated product [^{11}C]**2** by reacting **1** with conventional [^{11}C]methylating agents (Fig. 2). The *N*-[^{11}C]methylation is often a competing process due to the 2-pyridone isomeric structure. So far, in cold organic chemistry, ratio control of *N*- or *O*-alkylation in 2-hydroxypyridine has been extensively investigated [19–23]. Selective *O*-methylation of 2-hydroxypyridine has been achieved using Mitsunobu reaction with MeOH [24]. However, in hot radiochemistry, [^{11}C]MeOH is not available for synthesis of PET ligands because reliable production of [^{11}C]MeOH and following [^{11}C]methylation invariably occur over long time periods relative to the short half-life of ^{11}C [25].

In this study, we first used [^{11}C]MeI as a radiolabeling agent to perform the *O*-[^{11}C]methylation of **1**. [^{11}C]MeI was trapped into a mixture of **1** with different bases in DMF and all the reaction mixtures were heated at 100 °C for 3 min. After the reaction, the mixture was cautiously treated to prevent loss of radioactivity from the reaction vial. By radio-HPLC analysis, the ratios of *O*-[^{11}C]methylation over *N*-[^{11}C]methylation were quantitatively assayed. Table 1 summarizes the ratios of *O*-[^{11}C]methylated **2** to *N*-[^{11}C]methylated **3** under different reaction conditions. It was noted that all the reactions (except entry 4) listed in this table were proceeded with >95% *O*/*N*-[^{11}C]methylated efficiency for **1**.

As shown in Table 1, the undesired [^{11}C]**3**, which was formed by *N*-[^{11}C]methylation of 2-hydroxypyridine, predominated in the all reactions. When NaOH solution was used as a base, the ratio of [^{11}C]**2** in the reaction mixture was very low (entry 1). There was no discernible improvement on the ratio of [^{11}C]**2** when other representative bases were used, respectively (entries 2–5). Only utilization of Cs_2CO_3 gave a 5% ratio of [^{11}C]**2** in the reaction mixture (Table 1, entry 6), as determined in the analytic radio-HPLC chromatogram (Fig. 3A). This result gave us a hint that use of Cs_2CO_3 may be helpful for improving the selectivity of *O*-[^{11}C]methylation over *N*-[^{11}C]methylation. In fact, during our determination for the reaction conditions, two papers were published regarding the synthesis of 2-[^{11}C]methoxypyridine radioligands by the reaction of 2-hydroxypyridine precursor with [^{11}C]MeI in the presence of Cs_2CO_3[22,23]. Because of very low ratios of *O*-[^{11}C]methylation in the present reactions, we ceased to optimize the reaction conditions of **1** with [^{11}C]MeI.

We next performed the [^{11}C]methylation of **1** with [^{11}C]MeOTf. This radiolabeling agent has been used in synthesis of many PET ligands for clinical usefulness [25]. The produced [^{11}C]MeOTf was trapped into a mixture of **1** in the presence of bases and the ratios of *O*/*N*-[^{11}C]methylation were analyzed for each reaction mixture. Table 2 summarizes the ratios of [^{11}C]**2** to [^{11}C]**3** under different reaction conditions using [^{11}C]MeOTf. After the reaction, >95% of total radioactivity was left in the reaction mixture without significant loss of radioactivity.

As shown in Table 2, the reaction of **1** with [^{11}C]MeOTf in DMF did not proceed without use of base (entry 1). After the reaction was conducted in the presence of 1 mol/L NaOH (entry 2), the ratio of [^{11}C]**2** in the reaction mixture reached 13%. Interestingly, when the reaction was performed using Cs_2CO_3 as the base, drastic increase in the ratio of [^{11}C]**2** was achieved (entry 3). The improvement by Cs_2CO_3 was similar with the result which was obtained by the reaction of **1** with [^{11}C]MeI (Table 1, entry 6).

When the [^{11}C]methylation reaction was performed in DMSO, a similar ratio of [^{11}C]**2** was achieved (entry 4). In place of Cs_2CO_3, when Rb_2CO_3 or $CsHCO_3$ was evaluated as alternative bases, similar synthetic results were obtained (entries 5 and 6). The effect of reaction temperature on the [^{11}C]methylation efficiency was also evaluated (entry 7). Comparison of entry 7 with entries 4–6 indicated that heating the reaction mixture was indispensable for achieving this radiosynthesis.

To shorten reaction time and minimize loss of radioactivity as possible during the transferring and trapping process, we directly bubbled [^{11}C]MeOTf into a heated mixture of **1** and Cs_2CO_3 in DMF at 100 °C with N_2 gas (50 mL/min) (entry 8). After the radioactivity remained at a steady state, this reaction was immediately terminated by addition of water. It was surprising that the ratio of [^{11}C]**2** was significantly improved, compared to the other reaction entries. The analytical radio-HPLC chromatogram showed that in addition to the *O*/*N*-[^{11}C]methylated products, [^{11}C]methanol (decomposed from the unreacted [11C]MeOTf) was observed (Fig. 3B). Although formation of [^{11}C]MeOH decreased the *O*/*N*-[^{11}C]methylated efficiency of **1** to some extent, the ratio of [^{11}C]**2** was increased to 48%, which was the highest among all reaction entries conducted in the present study. Obviously, directly bubbling [^{11}C]MeOTf into the heated solution of desmethyl precursor **1** was efficient for improving the selectivity of *O*-[^{11}C]methylation over *N*-[^{11}C]methylation, compared to bubbling [^{11}C]MeOTf at room temperature and then heating the reaction mixture (entry 3), which is a conventional synthetic procedure with [^{11}C]MeOTf. The main reason for this improvement was considered as that the equilibrium from the ketone in compound **1** to the hydroxide form of the *O*-hydroxy pyridine was shifted using a strong base and under heating. To our knowledge, this is the first report utilizing a heated trapping process for [11C]MeOTf in the radiosynthesis of PET ligands. The present protocol paved the way for routine production of [^{11}C]**2** with a sufficient amount of radioactivity for clinical use. Moreover, this technique can be used to label the ligands containing a 2 or 4-methoxypyridine moiety with [^{11}C]MeOTf efficiently.

3.2. Automated production of [^{11}C]**1**

Based on the reaction conditions optimized in the above studies we performed the remote-controlled radiosynthesis of [^{11}C]**2** by the reaction of **1** with [^{11}C]MeOTf using an automated production system. The produced [^{11}C]MeOTf was bubbled into a heated mixture of **1** and Cs_2CO_3 in DMF at 100 °C. Followed by the trapping of [^{11}C]MeOTf for

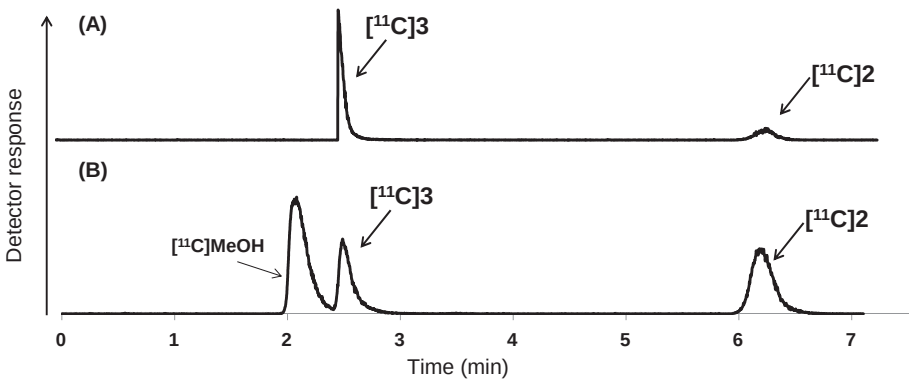


Fig. 3. Comparison of analytical radio-HPLC chromatograms of [^{11}C]**2** and [^{11}C]**3** under two reaction conditions. (A) Bubbling [^{11}C]MeI into a mixture of **1** and Cs_2CO_3 at room temperature and then heating the reaction mixture at 100 °C for 3 min, (B) bubbling [^{11}C]MeOTf into a heated mixture of **1** and Cs_2CO_3 at 100 °C for 90 s.

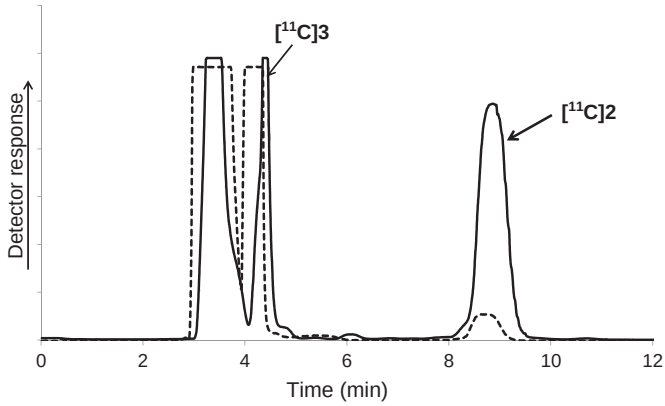


Fig. 4. Preparative HPLC chromatogram of [^{11}C]**2**. The black line represents the radioactivity peak, and the black dotted line represents the UV peak at 254 nm.

90 s, the reaction was immediately stopped and the mixture was transferred to the semi-preparative HPLC system. After the reaction, HPLC separation (Fig. 4) and formulation, [^{11}C]**2** was obtained with a sufficient amount of radioactivity. At the end of synthesis, a sterile injectable solution of [^{11}C]**2** in normal saline after 30 min of proton bombardment at a beam current of 18 μA, starting from the cyclotron-produced [^{11}C]CO_2 of 25.1–39.9 GBq. The decay-corrected radiochemical yield of [^{11}C]**2** based on [^{11}C]CO_2 was 7.9 ± 1.8% at the end of bombardment, and the specific activity was 156 ± 52 GBq/μmol ($n = 78$) at the end of synthesis. The averaged radiochemical purity of the [^{11}C]**2** product (retention time: 2.6 min) for each batch was greater than 99% and remained greater than 95% at 60 min after synthesis (Fig. 5). No significant No significant UV peak corresponding to unreacted **1** was observed in the final product solution. The total synthesis time was approximately 30 min from the end of bombardment.

3.3. Quality control of [^{11}C]**2**

Quality control of [^{11}C]**2** was carried out in accordance with the standard protocols, which were designated for a PET radiopharmaceutical produced in house [26]. Table 3 summarizes the results of quality control assessment for three different batches of the [^{11}C]**2** production. The product was clear, colorless and free of particulate matters. The pH value was 6.3. In sterility testing, no viable bacteria or microorganisms were observed in soybean-casein digest broth or fluid thioglycollate medium. The endotoxin content was undectable and all the samples were free of pyrogens. The radiochemical purity of [^{11}C]**2** was 99.6 ± 0.1% ($n = 3$) at the end of synthesis and was maintained

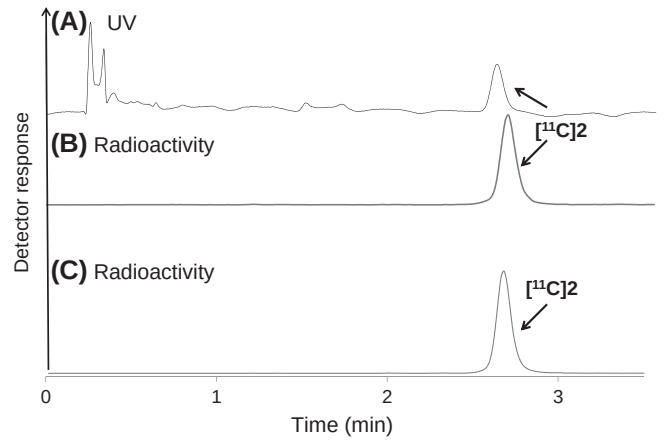


Fig. 5. Analytical HPLC chromatograms of the [^{11}C]**2** production. Immediately after synthesis: (A) UV at 254 nm; (B) radioactivity peak. At 60 min after synthesis: (C) radioactivity peak.

Table 3
Tests, specifications and results for three consecutive preparation batches of [^{11}C]**2**.

Test	Specifications	Batch 1	Batch 2	Batch 3
Radioactivity (GBq)	>0.185	2.18	2.48	2.47
Specific activity (GBq/μmol)	>3.7	85.8	117.7	103.7
Bacterial endotoxin (international units/maximum dose)	<175	0.50	0.57	0.52
Sterility	No bacterial growth	Pass	Pass	Pass
pH	4.5–8.0	6.3	6.3	6.3
Radiochemical purity after 60 min	>95%	99.2%	99.3%	99.0%
Residual solvent (ppm)				
Ethanol	<5000	4.8	326	149
Acetonitrile	<410	0.2	12	6.9
N,*N*-Dimethylformamide	<880	1.3	287	245

within the range of 99.1 ± 0.1% at 60 min after synthesis. The residual amounts of ethanol, acetonitrile and DMF in the [^{11}C]**2** injection sample were within our acceptance criteria. All the quality control results of [^{11}C]**2** were in compliance with our in-house quality control/assurance specifications. These results have guaranteed reliable supply of [^{11}C]**2** with sufficient amounts of radioactivity and high quality for clinical usefulness.

4. Conclusion

[^{11}C]TASP457 ([^{11}C]**2**), a clinically useful PET ligand for the imaging of histamine H_3 receptors in human brain, was successfully synthesized by the reaction of desmethyl precursor **1**, having the labeling site on the hydroxyl group in 2-hydroxypyridine moiety, with [^{11}C]MeOTf in the presence of Cs_2CO_3. We have so far achieved more than 100 productions of [^{11}C]**2** with sufficient amounts of radioactivity and high quality in our facility. This radioligand is being used for a variety of research purposes, including PET imaging of non-human primates and clinical research PET imaging of volunteers and clinical research subjects. In addition, the labeling technique determined in this study could be used to develop novel PET ligands by labeling candidates with a 2- or 4-[^{11}C]methoxypyridine moiety with [^{11}C]MeOTf efficiently.

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