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Synthesis of ^{11}C-Labeled Thiamine and Fursultiamine for in Vivo Molecular Imaging of Vitamin B$_1$ and Its Prodrug Using Positron Emission Tomography

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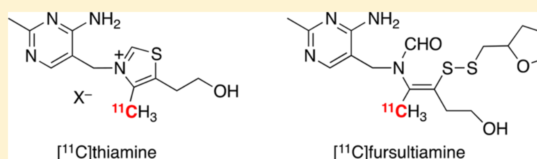
Supporting Information

ABSTRACT: To enable in vivo analysis of the kinetics of vitamin B$_1$ (thiamine) and its derivatives by positron emission tomography (PET), ^{11}C-labeled thiamine ([^{11}C]-**1**) has been synthesized. This was carried out via a rapid, multistep synthesis consisting of Pd^0-mediated *C*-[^{11}C]methylation of a thiazole ring for 3 min and benzylation with 5-(bromomethyl)pyrimidine for 7 min. The [^{11}C]-**1** was also converted to ^{11}C-labeled fursultiamine ([^{11}C]-**2**), a prodrug of vitamin B$_1$, by disulfide formation with *S*-tetrahydrofurfurylthiosulfuric acid sodium salt. Characterization of [^{11}C]-**1** and [^{11}C]-**2** showed them to be suitable for use as PET probes for in vivo pharmacokinetic and medical studies. The total durations of the preparations of [^{11}C]-**1** and [^{11}C]-**2** were shorter than 60 and 70 min, respectively. The [^{11}C]CH$_3$I-based decay-corrected radiochemical yields of [^{11}C]-**1** and [^{11}C]-**2** were 9−16% and 4−10%, respectively. The radioactivities of the final injectable solutions of [^{11}C]-**1** and [^{11}C]-**2** were 400−700 and 100−250 MBq, respectively. The radiochemical purity of both [^{11}C]-**1** and [^{11}C]-**2** was 99%, and the chemical purities of [^{11}C]-**1** and [^{11}C]-**2** were 99% and 97−99%, respectively. In vivo PET imaging of normal rats was illustrated by the distribution of [^{11}C]-**1** and [^{11}C]-**2** following intravenous injection.



INTRODUCTION

Thiamine (vitamin B$_1$) was first reported in 1911 by Dr. Umetaro Suzuki, having been isolated from rice bran, which had been found to be an effective therapeutic agent against the disease beriberi.[1,2] Notably, this is also considered to be the first discovery of a vitamin. In the early 1950s, allithiamine was developed as the first artificial vitamin B$_1$ prodrug with moderate lipophilicity, allowing improved gut absorption.[3,4] Fursultiamine (thiamine tetrahydrofurfuryl disulfide) has been available in Japan since the early 1960s and was developed as an improved vitamin B$_1$ prodrug.[5−7] Fursultiamine is considered a commercially successful vitamin product in Japan and Asia and has also been sold in Europe and the United States.

Positron emission tomography (PET) is a powerful bioscientific and clinical technique that is used to image the molecular events involved in biochemical, physiological, and pathological processes in both animals and humans.[8,9] Among the short-lived positron-emitting radionuclides, ^{11}C—with a half-life of 20.4 min—has been extensively used to radiolabel both bioactive organic compounds and drugs of medical and pharmaceutical significance.

Vitamins must be obtained through the diet because the human body is unable to synthesize these compounds. From the biological and medical perspective, there is little information on why humans do not have the biosynthetic capabilities in the body and need to ingest such tiny amounts of these biogenic compounds. In this regard, we supposed that the use of PET imaging could provide valuable scientific information and insight by evaluating the in vivo pharmacokinetics of thiamine and fursultiamine. Therefore, we focused on synthesizing PET probes of thiamine and fursultiamine by labeling with the ^{11}C radionuclide ([^{11}C]-**1** and [^{11}C]-**2**), which is a reliable radiolabeling scheme for low molecular weight bioactive organic compounds (Scheme 1).

Here, we report the successful synthesis of ^{11}C-labeled thiamine and fursultiamine. These compounds are suitable for medical PET studies in animals and humans and represent an important advance in the context of time-sensitive and synthetic process-restricted ^{11}C-labeling chemistry.

RESULTS AND DISCUSSION

Regarding the synthesis of ^{11}C-labeled thiamine and fursultiamine, the initial synthetic strategy involved ^{11}C labeling at a late stage by use of stannyl precursors **3** and **4**, possessing the basic structure of thiamine, but we were obliged to abandon the preparation of **3** and **4** because of difficulties synthesizing such

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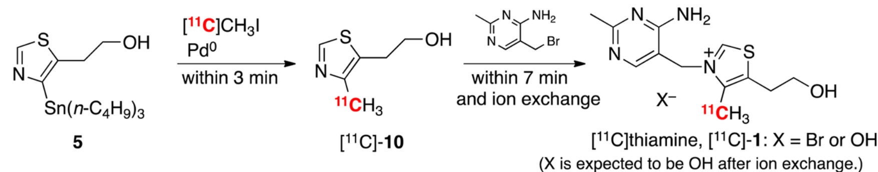
Scheme 1. Strategy for Synthesis of ^{11}C-Labeled Thiamine [^{11}C]-1 (vitamin B_1) and Fursultiamine [^{11}C]-2 (a prodrug of vitamin B_1)

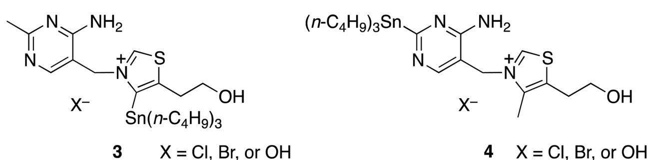
First Process: synthesis of ^{11}C-labeled thiamine



Second Process: synthesis of ^{11}C-labeled fursultiamine

[^{11}C]-1 base within 3 min

[^{11}C]fursultiamine, [^{11}C]-2

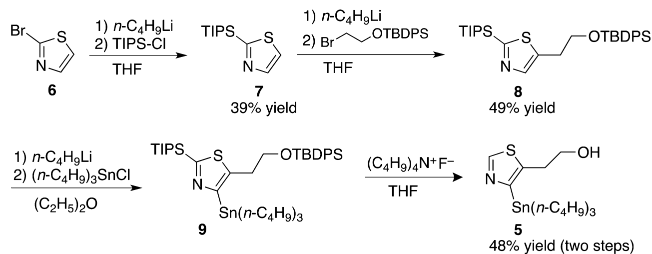


ionic compounds possessing the hydrophobic tri-*n*-butylstannyl group. Therefore, the synthetic route shown in Scheme 1 was considered the first-line strategy to achieve ^{11}C labeling of thiamine and fursultiamine. This synthetic strategy involved the following key steps: (1) incorporation of a [^{11}C]methyl group into a heteroaromatic thiazole ring via rapid Pd^0-mediated *C*-[^{11}C]methylation,[10−12] (2) rapid benzylation using 4-amino-5-(bromomethyl)-2-methylpyrimidine hydrobromide, and (3) rapid formation of a disulfide with *S*-tetrahydrofurfurylthiosulfuric acid, sodium salt (TFT−Na). It was intended that each of these three reactions be completed after a reaction time of only a few minutes.

The synthesis of ^{11}C-labeled PET probes is very different from conventional organic synthesis; the specific characteristics of PET chemistry—short radioactive half-lives, extremely small amounts of available radionuclide, and relatively high-energy radiation—impose severe restrictions on the synthesis of PET probes. In general, the synthesis of a high-purity and injectable ^{11}C-labeled probe needs to be accomplished within a maximum of three half-lives (i.e., 60 min) because of the rapid radioactive decay of ^{11}C. The process of preparing a ^{11}C-labeled probe involves the following steps: (1) derivatization of a cyclotron-produced ^{11}C-labeled primary precursor to an appropriate labeling precursor, such as $^{11}CH_3I$,[13−16] (2) ^{11}C labeling of the target probe, (3) workup and chromatographic purification of the desired ^{11}C-labeled probe, and (4) preparation of an injectable solution for use in an animal/human PET study. The synthesis of a ^{11}C-labeled PET probe is also made difficult by the small amount of ^{11}C-labeling precursor used, which is generally in the range of 10−500 nmol.[17] Therefore, the labeling reaction is usually carried out with an excess of substrate (several milligrams, micromole scale) to accelerate the reaction rate. In addition, a remote-controlled radiolabeling system is needed for probe synthesis in order to protect personnel from harmful radiation. Finally, purification of the probe needs to be efficient, so that the final preparation of the probe is safe for ultimate intravenous administration into an animal or human. To be useful in a clinical study, the final preparation of the PET probe needs to be of high purity and have a minimum radioactivity of several hundred MBq.

For the incorporation of ^{11}C into organic carbon frameworks, we previously developed various types of Pd^0-mediated rapid *C*-[^{11}C]methylations of [^{11}C]CH_3I with organostannanes or organoboranes.[10] These reactions allow regioselective ^{11}C labeling through the formation of a C−C bond. A tri-*n*-butyltin-substituted thiazole precursor **5**, a prerequisite for Pd^0-mediated rapid *C*-[^{11}C]methylation, was prepared, as shown in Scheme 2. Initially, 2-bromothiazole **6** was converted to TIPS-protected thiazole **7** in 39% yield via a 2-lithiothiazole intermediate. Subsequently, **7** underwent further lithiation at the 5 position of the thiazole ring, and the subsequent reaction with 2-bromoethoxy(*tert*-butyldiphenyl)silane gave the ethoxy-group-containing thiazole **8** in 49% yield. The introduction of a $(n\text{-}C_4H_9)_3Sn$ group was carried out by reacting the product of the lithiation of **8** with tri-*n*-butyltin chloride to produce the stannylated compound **9**. Finally, the deprotection of two silyl groups of **9** gave the desired tri-*n*-butylstannyl precursor **5** in 48% yield (two steps).

Scheme 2. Synthesis of Stannyl Precursor 5 Used for Pd^0-Mediated Rapid *C*-[^{11}C]Methylation



Using relatively low levels of radioactivity, approximately 8.0 GBq of [^{11}C]CH_3I, the radiolabeling efficiency of the Pd^0-mediated rapid *C*-[^{11}C]methylation reaction using the stannylthiazole precursor **5** was investigated. As shown in Table 1, the use of standard conditions for the rapid *C*-[^{11}C]methylation of heteroaromatics,[11] $Pd_2(dba)_3$ (dba, dibenzylideneacetone) and P(*o*-tolyl)$_3$ (1:16) in the presence of CuBr and CsF in NMP for 5 min at 100 °C, was effective at producing the target [^{11}C]methylated thiazole [^{11}C]-**10** with a sufficiently high radioactivity of 2.4 GBq following purification by semipreparative HPLC (entry 1). Interestingly, the use of

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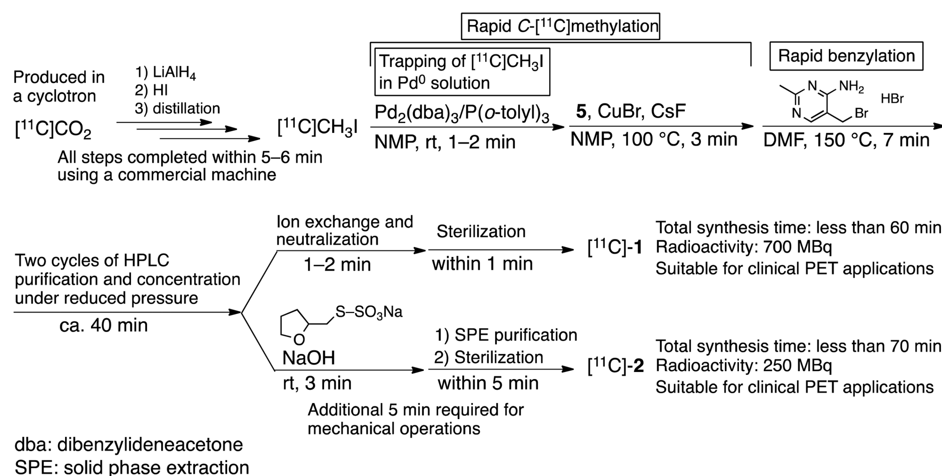
Table 1. Confirmation of Pd^0-Mediated Rapid C-[^{11}C]Methylation of Thiazole: Effects of Additives and Reaction Temperature

5 → [^{11}C]-10 ([^{11}C]CH_3I (ca. 8 GBq), $Pd_2(dba)_3$/P(*o*-tolyl)$_3$ (1:16), CuBr and F$^-$ as additives; NMP)

entry[a]	Cu^I and F$^-$ additives (equivalent ratio to $Pd_2(dba)_3$ in brackets)	reaction temperature	reaction time	isolated radioactivity[b]	DCY[c] based on [^{11}C]CH_3I
1	CuBr/CsF (5:12.5)	100 °C	5 min	2.4 ± 0.2 GBq	86 ± 7%
2	CuBr/TBAF (5:12.5)	100 °C	5 min	2.3 ± 0.3 GBq	82 ± 11%
3	CuBr/KF/Kryptofix 2.2.2 (5:12.5:25)	100 °C	5 min	2.3 ± 0.1 GBq	82 ± 4%
4	CuBr/CsF (5:12.5)	60 °C	3 min	1.3 ± 0.2 GBq	46 ± 7%
5	CuBr/CsF (5:12.5)	80 °C	3 min	1.9 ± 0.4 GBq	68 ± 14%
6	CuBr/CsF (5:12.5)	100 °C	3 min	2.4 ± 0.2 GBq	86 ± 7%
7	CuBr/TBAF (5:12.5)	60 °C	3 min	1.7 ± 0.3 GBq	61 ± 11%
8	CuBr/TBAF (5:12.5)	80 °C	3 min	2.6 ± 0.4 GBq	93 ± 14%
9	CuBr/TBAF (5:12.5)	100 °C	3 min	2.0 ± 0.1 GBq	71 ± 4%

[a]Reaction of [^{11}C]CH_3I (approximately 8.0 GBq) and **5** (11.9 μmol) was carried out in the presence of $Pd_2(dba)_3$ (1.2 μmol; dba, dibenzylideneacetone), P(*o*-tolyl)$_3$ (19.1 μmol), CuBr (5.9 μmol), and F$^-$ additive (14.9 μmol) in NMP solvent (0.2 mL for entries 1−3 and 0.5 mL for entries 4−9). [b]The target [^{11}C]methylated thiazole [^{11}C]-**10** was isolated by semipreparative HPLC, and the radioactivity was measured by a γ-ray dose calibrator at the end of the 30 min synthesis. The radioactivity of purified [^{11}C]-**10** was calculated as the average of two or three experiments (rounded to one decimal place). [c]DCY (decay-corrected radiochemical yield) was calculated from the isolated radioactivity of [^{11}C]-**10** based on [^{11}C]CH_3I (8.0 GBq), taking into consideration the natural decay of the ^{11}C radionuclide (half-life = 20.4 min).

Scheme 3. Continuous Reaction Procedure Carried Out Using a Remote-controlled Radiolabeling System to Synthesize ^{11}C-Labeled Thiamine [^{11}C]-1 and Fursultiamine [^{11}C]-2



TBAF or KF/Kryptofix 2.2.2 in place of CsF gave [^{11}C]-**10** with a similar radioactivity of 2.3 GBq (entries 2 and 3). The total synthesis time (including HPLC purification) for each of entries 1−3 was approximately 30 min. The [^{11}C]CH_3I-based decay-corrected radiochemical yield (DCY)[18] for each of entries 1−3 was high (82−86%); however, when KF/Kryptofix 2.2.2 was used as an additive, the subsequent in situ benzylation with 4-amino-5-(bromomethyl)-2-methylpyrimidine hydrobromide did not proceed, supposedly due to inhibition caused by the residual KF/Kryptofix 2.2.2. Therefore, the conditions shown in entries 1 and 2 were considered the most suitable for the rapid *C*-[^{11}C]methylation when followed by the benzylation reaction.

The effect of reaction temperature was investigated using a shorter, fixed reaction time of 3 min (Table 1, entries 4−9). Fortunately, every condition used for the Pd^0-mediated rapid *C*-[^{11}C]methylation resulted in a satisfactory yield of [^{11}C]-**10** with a radioactivity of more than 1.3 GBq (entries 4−9). Ultimately, the use of CuBr and CsF at 100 °C (entry 6) was adopted as the standard conditions for rapid *C*-[^{11}C]-methylation of the thiazole ring, because of reproducibility of the reaction under PET radiolabeling conditions.

As shown in Scheme 3, the synthesis of ^{11}C-labeled thiamine [^{11}C]-**1** was conducted under high radioactivity conditions using approximately 35 GBq of [^{11}C]CH_3I. The [^{11}C]CH_3I was transferred in a stream of He gas and trapped in a solution of $Pd_2(dba)_3$ and P(*o*-tolyl)$_3$ in NMP, and then a solution of the stannyl precursor **5**, CuBr, and CsF in NMP was added to it.[12,19] After heating the mixture at 100 °C for 3 min, a solution of 4-amino-5-(bromomethyl)-2-methylpyrimidine hydrobromide in DMF was added. The resulting mixture was further heated at 150 °C for 7 min by bubble mixing with a N_2 gas stream to increase the reaction efficiency. The reaction was stopped after 7 min because of the obligatory time limit imposed by the use of ^{11}C, even though the benzylation reaction was incomplete.

Subsequently, two cycles of purification using semipreparative HPLC were used in order to obtain the desired ^{11}C-labeled thiamine [^{11}C]-**1** with purity high enough for biological or medical use. The reaction mixture was directly added to the

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first semipreparative HPLC column, and the fraction containing the desired [^{11}C]-**1** was collected and concentrated by evaporation under reduced pressure. The resultant solution was subjected to a second semipreparative HPLC cycle using another column. The desired fraction was again collected and concentrated by evaporation. The resultant solution was passed through a solid-phase extraction (SPE) cartridge for ion exchange and neutralization and then sterilized via filtration. Finally, an injectable solution of [^{11}C]-**1** was collected in a sterilized vial. The [^{11}C]-**1** was identified by HPLC analysis using phosphate buffer eluent by coinjecting with a commercially available thiamine hydrogen chloride salt **11**. This synthesis has so far been conducted more than 23 times for the use of [^{11}C]-**1** in animal PET studies. The radioactivity of [^{11}C]-**1** after formulation of the injectable solution was at a satisfactory level of 400−700 MBq. The total synthesis time was less than 60 min. The radiochemical purity was more than 99%. The chemical purity was estimated by converting thiamine [^{11}C]-**1** to thiochrome by oxidation with potassium ferricyanide[20,21] (Figure 1), as the tiny amount of thiamine [^{11}C]-**1** could not be detected using the normal UV-HPLC method due to its weak UV absorption. On the UV-HPLC chromatogram shown in Figure 1b, peaks of impurities, which could be attributed to the stannyl substrate, palladium complexes, additives, and byproducts of organic compounds, were not observed. Thus, the chemical purity of [^{11}C]-**1** was estimated to be more than 99%. The specific activity of [^{11}C]-**1** contained in the injectable solution was calculated to be 50−150 GBq/μmol, based on the quantitative analysis of fluorescent thiochrome by HPLC (Figure 1c). Therefore, the quality of [^{11}C]-**1** was judged to be suitable for animal and human PET studies. The [^{11}C]CH_3I-based DCY of [^{11}C]-**1** was calculated to be 9−16%.[22]

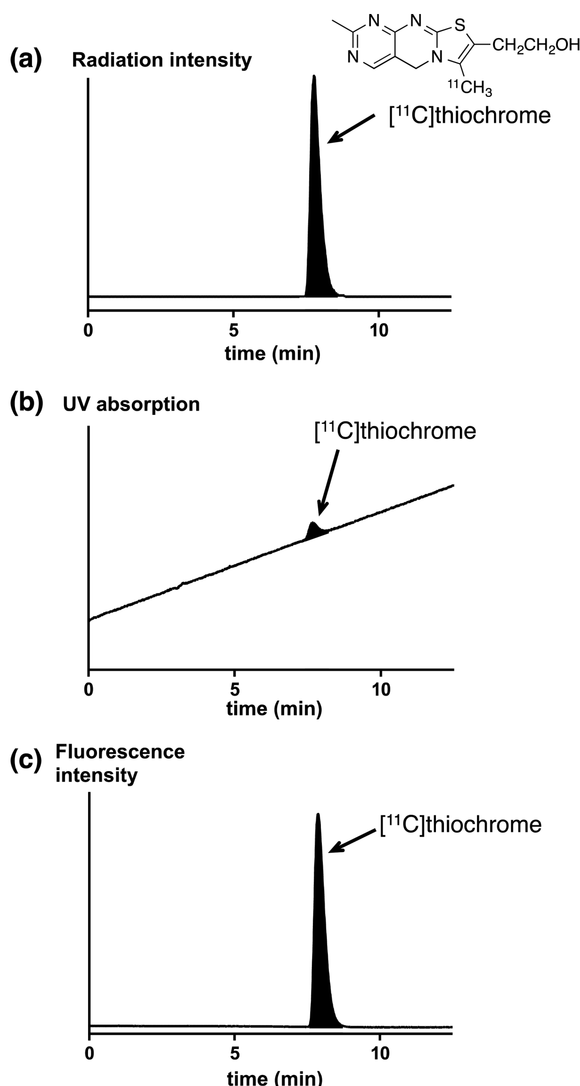

Figure 1. Purity analysis of ^{11}C-labeled thiamine ([^{11}C]-**1**) by quantitative conversion of [^{11}C]-**1** to fluorescent [^{11}C]thiochrome. (a) Radio-HPLC analysis. (b) UV-HPLC analysis. (c) Fluorescence-HPLC analysis.

Industrially, fursultiamine is synthesized from thiamine by disulfide formation with TFT−Na with a reaction time of several hours.[23] With the aim of accelerating the conversion of thiamine to fursultiamine in order to make it more appropriate to the synthesis of a PET probe, the most efficient conditions were determined by adjusting both the pH and the amount of TFT−Na. As shown in Table 2, reactions were carried out using thiamine hydrochloride salt **11** and a varying amount of a 28.5% aq solution of TFT−Na at rt with a reaction time of 10 min. Unexpectedly, the yield of fursultiamine decreased as the amount of TFT−Na increased (entries 1−5 in Table 2). When its pH was measured, the reaction mixture described in entry 1 had a pH value of 14. However, the pH values of entries 2−5 showed a gradual acidification as the amount of TFT−Na was increased. This acidification might be due to sodium hydrogen sulfate produced by the partial decomposition of TFT−Na. When the pH of the reaction mixture reached a value higher than 11 by adding NaOH, the product yield of fursultiamine was increased as the amount of TFT−Na was increased (entries 6−10). These results indicated that it was possible to carry out the disulfide formation in a few minutes under PET radiolabeling conditions.

Table 2. Optimization of the Rapid Conversion of Thiamine to Fursultiamine by Disulfide Formation

thiamine hydrochloride **11** → (28.5% aq S–SO_3Na (TFT–Na), NaOH, rt, 10 min) → fursultiamine **12**

entry[a]	amount of TFT−Na (28.5% aq)	product yield (%) of **12**	pH value of the reaction mixture
1[b]	200 μL	27	14
2[b]	400 μL	3	12
3[b]	600 μL	2	6
4[b]	800 μL	trace	5
5[b]	1000 μL	0	5
6[c]	200 μL	32	>11
7[c]	400 μL	39	>11
8[c]	600 μL	64	>11
9[c]	800 μL	56	>11
10[c]	1000 μL	66	>11

[a]Reaction was carried out at rt for 10 min using thiamine hydrochloride **11** (4.0 mg, 11.9 μmol) and a corresponding amount of 28.5% aq *S*-tetrahydrofurfurylthiosulfuric acid, sodium salt (TFT−Na.). [b]1.0 M aq NaOH solution (36 μL, 36 μmol) was added, and the pH value of the reaction mixture was monitored at the end of the reaction for 10 min. [c]The pH value of the reaction mixture was kept higher than 11 by modulating the amount of NaOH.

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The ^{11}C-labeled thiamine that was obtained after two cycles of HPLC purification was reacted with TFT−Na (28.5% aq solution) at rt for 3 min by bubble mixing with a N_2 gas stream at a pH above 11 using NaOH (30% aq solution). The reaction mixture was purified using a SPE cartridge. Highly polar impurities were washed away from the cartridge using a solution (5.0 mL) of sterile saline and propylene glycol (90:10), and then the desired ^{11}C-labeled fursultiamine [^{11}C]-**2** was eluted using a solution (2.0 mL) of sterile saline and propylene glycol (70:30). However, it was found that, during the HPLC analysis, the obtained [^{11}C]-**2** gradually decomposed back to [^{11}C]-**1** after a few minutes (Figure 2). The cause of the decomposition was unclear because this phenomenon was never observed for nonradiolabeled fursultiamine. The cause was thought to be the dissociation of the S−S bond of ^{11}C-labeled fursultiamine triggered by radicals that would be generated by radiolysis, as often observed in PET radiochemistry. It is probable that subsequent recyclization of vinylsulfur radical to a 5-membered ring would occur to produce thermodynamically stable thiazolium compound [^{11}C]-**1** (Scheme 4).

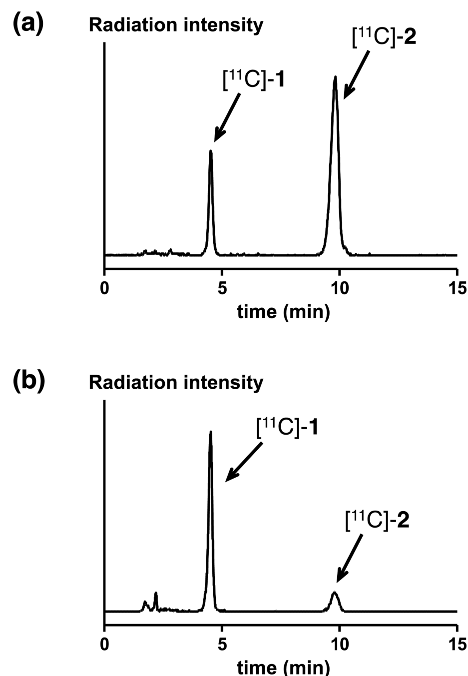


Figure 2. Radio-HPLC chromatogram of a [^{11}C]-**2**-containing solution after synthesis. (a) Analysis within 2 min of synthesis of [^{11}C]-**2**; trace shows decomposition of [^{11}C]-**2** to give [^{11}C]-**1**. (b) Analysis 30 min after the synthesis of [^{11}C]-**2**; decomposition of most [^{11}C]-**2** to give [^{11}C]-**1**.

In order to overcome such unpredictable radiolysis, attention was focused on the use of biocompatible antioxidant additives, such as sodium ascorbate, ascorbic acid, and citric acid, which could act as radical scavengers and suppress the decomposition of [^{11}C]-**2**. In Table 3, the percentage of [^{11}C]-**2** remaining after synthesis in the absence or presence of these additives is shown. The rate of decomposition was calculated from the peak area ratio of [^{11}C]-**2** and [^{11}C]-**1** observed using radio-HPLC analysis both 30 and 60 min after the synthesis of [^{11}C]-**2**. The initial value of [^{11}C]-**2** at 0 min was defined as 100%. Obviously, [^{11}C]-**2** decomposed in a time-dependent manner (Table 4, entry 1). Sodium ascorbate, a frequently used additive for the prevention of radiolysis, was not effective (entry 2). Interestingly, ascorbic acid and citric acid effected a remarkable suppression of the decomposition, resulting in 97−98% [^{11}C]-**2** remaining after 30 min and 91−92% remaining after 60 min (entries 3 and 4). In light of future medical applications, the use of ascorbic acid is considered preferable to citric acid.

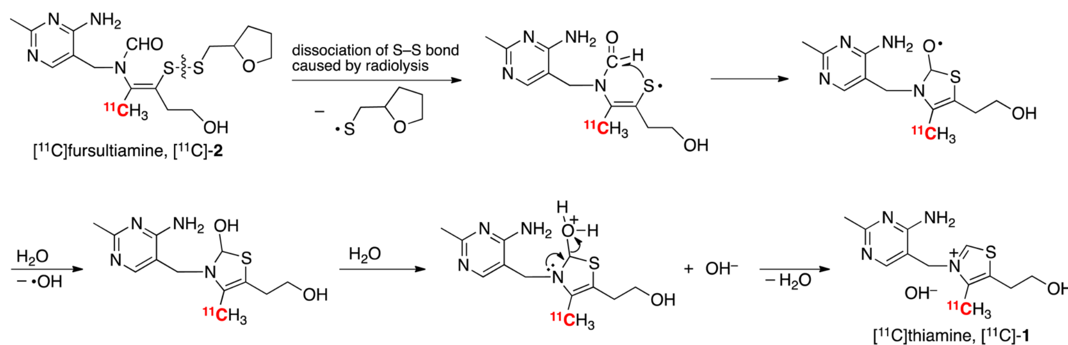
Table 3. Effect of Antioxidant Additives on the Percentage of ^{11}C-Labeled Fursultiamine [^{11}C]-2 Remaining after Synthesis

entry[a]	additive	30 min after the synthesis	60 min after the synthesis
1	none	66%	29%
2	sodium ascorbate	61%	0%
3	ascorbic acid	97%	91%
4	citric acid	98%	92%

[a]Percentage of ^{11}C-labeled fursultiamine [^{11}C]-**2** remaining was calculated from the peak area ratio of [^{11}C]-**2** by HPLC analysis at 30 and 60 min after the synthesis of [^{11}C]-**2**. The amount remaining 0 min after the synthesis of [^{11}C]-**2** was defined as 100%. As an additive, a 25% aq solution (0.2 mL) of sodium ascorbate, ascorbic acid, or citric acid was used for each reaction of entries 2−4.

Ultimately, ^{11}C-labeled fursultiamine [^{11}C]-**2** with satisfactory stability was successfully synthesized with the addition of ascorbic acid. An injectable solution of [^{11}C]-**2** was prepared by eluting the reaction mixture from a SPE cartridge with a mixed solution (2.2 mL) composed of (A) a solution (2.0 mL) of sterile saline and propylene glycol (70:30) and (B) a 25% aq ascorbic acid solution (0.2 mL). The eluate was sterilized via membrane filtration. [^{11}C]-**2** was identified by HPLC analysis

Scheme 4. Possible Reaction Mechanism of Production of [^{11}C]Thiamine ([^{11}C]-1) by Radicalic Decomposition of [^{11}C]Fursultiamine ([^{11}C]-2)



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with coinjection of commercially available fursultiamine **12**. This synthesis has so far been conducted more than 23 times for use of [^{11}C]-**2** in animal PET studies. The radioactivity of [^{11}C]-**2** after radiopharmaceutical formulation of the injectable solution was 100−250 MBq. The total synthesis time was less than 70 min. The radiochemical purity was up to 99%, and the chemical purity was 97−99% (Figure 3). The specific activity

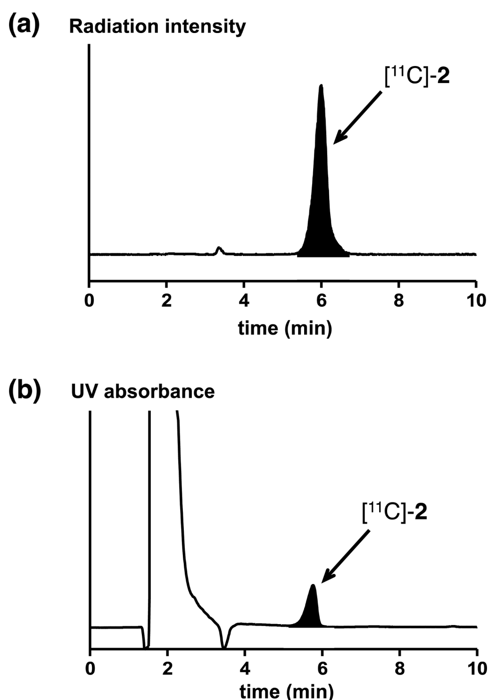

Figure 3. Purity analysis of ^{11}C-labeled fursultiamine ([^{11}C]-**2**). (a) Radio-HPLC analysis. (b) UV-HPLC analysis; the peak at 1.5−3.5 min is largely attributed to the use of ascorbic acid as an additive.

after preparation of the injectable solution was 15−35 GBq/μmol. Therefore, the quality of [^{11}C]-**2** was judged to be suitable for use as a probe in animal and human PET studies. The [^{11}C]CH_3I-based DCY of [^{11}C]-**2** was calculated to be 4−10%.[22]

Figure 4 shows the synthesis of [^{11}C]-**1** and [^{11}C]-**2** by remote-controlled operation using radiolabeling machines.

To assess the PET probes in vivo, each of [^{11}C]thiamine [^{11}C]-**1** (approximately 30 MBq, 49−54 GBq/μmol, 0.2 mL) and [^{11}C]fursultiamine [^{11}C]-**2** (approximately 15 MBq, 18−22 GBq/μmol, 0.2 mL) was intravenously injected into the tail vein of rats, and whole-body PET images were obtained (Figure 5a and 5b). High accumulation of [^{11}C]-**1** was observed in the liver, kidney, and urinary bladder (Figure 5a). On the other hand, it is notable that [^{11}C]-**2** initially accumulated extensively in the heart before accumulating in the liver and kidney and finally in the urinary bladder (Figure 5b). Indeed, in a dissection experiment to analyze the tissue distribution of [^{11}C]-**2**, the accumulation of radioactivity in the removed and washed rat heart remained high compared to other major tissues. Additional animal PET studies using [^{11}C]-**1** and [^{11}C]-**2** are currently in progress, and a human clinical PET study is planned. The detailed results of biological and medical studies conducted using [^{11}C]-**1** and [^{11}C]-**2** will be reported separately.

■ CONCLUSION

In summary, we succeeded in synthesizing ^{11}C-labeled thiamine [^{11}C]-**1** and fursultiamine [^{11}C]-**2** with high purity and decent yields for use in animal and human PET studies. Synthetic procedures consisting of rapid Pd^0-mediated C-[^{11}C]-methylation, rapid benzylation, rapid disulfide formation, effective HPLC purification, and successful suppression of radiolysis were established. The entire synthesis of [^{11}C]-**1** or [^{11}C]-**2** was accomplished within 60 or 70 min, respectively, by remote controlling our conventional radiolabeling system.

The preparation of ^{11}C-labeled thiamine and fursultiamine via a multistep synthesis can be seen as both a demonstration of advanced ^{11}C-labeling chemistry and an example of the potential of organic chemistry. We are now in the process of developing a specialized synthesizer for more efficient synthesis of [^{11}C]-**1** or [^{11}C]-**2** with the aims of shortening the current synthesis by at least 10 min, decreasing the loss of radioactivity that occurs during practical manipulations, and increasing the purification efficiency by HPLC. Studies on intestinal absorption of [^{11}C]-**1** or [^{11}C]-**2** via oral administration in humans are already in the planning stage.

■ EXPERIMENTAL SECTION

General Methods. The ^{1}H NMR spectra were measured with a 400 MHz instrument. The chemical shifts in deuterated chloroform and DMSO ($CDCl_3$ and DMSO-d_6) are given as δ values using TMS as an internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, sept = septet, m = multiplet), integration, coupling constants, and assignment. The ^{13}C NMR spectra were recorded on a 100 MHz instrument with complete proton decoupling. HRMS spectra were recorded on a TOF mass spectrometer. All reagents and solvents were used as received from their commercial source without further purification. Silica gel column chromatography was carried out using spherical and neutral silica gels (particle size, 30−60 μm). TLC was performed with 0.2 mm silica gel plates. [^{11}C]Carbon dioxide was produced by a ^{14}N(p,α)^{11}C nuclear reaction using a 12 MeV cyclotron. [^{11}C]CH_3I was prepared by reduction of [^{11}C]carbon dioxide with LAH followed by iodination with hydroiodic acid using an automated radiolabeling system, which involved heating the reaction mixture, dilution, HPLC injection, fractional collection, evaporation, and sterile filtration. Radioactivity was quantified with a dose calibrator. Semipreparative purification and purity analysis by HPLC was performed on a system equipped with pumps, a UV detector, and a fluorescence detector, and radioactivity in the effluent was determined using a radioanalyzer.

Prior to use in the synthesis reaction, an aq solution of TFT−Na (28.5%, 1.0 mL, 1.65 mmol) was twice passed through a C_{18} SPE cartridge, which had been preconditioned with methanol (5 mL) and water (10 mL). This procedure was necessary to improve the conversion of [^{11}C]-**1** to [^{11}C]-**2**. TFT−Na (>95% purity) was supplied by Takeda Pharmaceutical Co.

All animal experiments were approved by and performed in accordance with the guidelines of the Animal Care and Use Committee of RIKEN Kobe Institute (MAH21-13). A small-animal PET scanner was used for the imaging of rats; during the scan, their body temperature was maintained at 37 °C using a heat pad.

Synthesis of 2-Triisopropylsilylthiazole (7). In a dry flask, a solution of 1.65 M *n*-butyllithium in hexane (20.3 mL, 33.5 mmol) in THF (90 mL) was placed under argon at −78 °C. After addition of a solution of 2-bromothiazole **6** (5.00 g, 30.4 mmol) in THF (10 mL), the mixture was stirred under argon at −78 °C for 45 min, followed by addition of triisopropylsilyl chloride (8.39 mL, 39.5 mmol). The resulting mixture was further stirred under argon at −78 °C for 15 min. The reaction mixture was gradually warmed to rt and quenched with a saturated solution of $NaHCO_3$. The organic layer was extracted with ethyl acetate, washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography (hexane/

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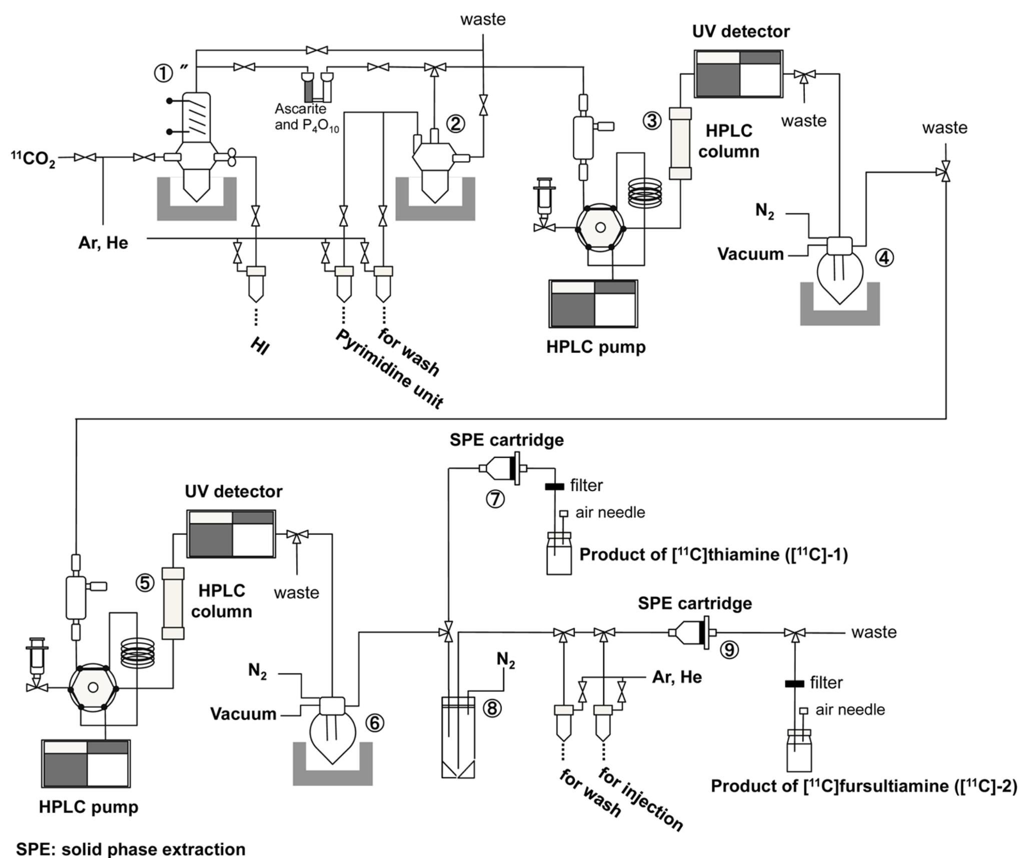

Figure 4. Flowchart showing the synthesis of ^{11}C-labeled thiamine ([^{11}C]-**1**) and fursultiamine ([^{11}C]-**2**). (1) Preparation of [^{11}C]CH_3I. (2) Rapid *C*-[^{11}C]methylation of thiazole. (3) First purification of [^{11}C]thiamine ([^{11}C]-**1**). (4) Concentration. (5) Second purification of [^{11}C]thiamine ([^{11}C]-**1**). (6) Concentration. (7) Pharmaceutical formulation for [^{11}C]thiamine ([^{11}C]-**1**). (8) Reaction with *S*-tetrahydrofurfurylthiosulfuric acid, sodium salt. (9) Purification and pharmaceutical formulation for [^{11}C]fursultiamine ([^{11}C]-**2**).

ethyl acetate = 100:0 and 70:30) produced the target compound **7** as a yellow oil. Yield: 2.84 g (39%, 11.7 mmol). TLC R_f 0.5 (hexane/ethyl acetate = 10:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17(d, 1H, $J_{4,5}$ = 2.8 Hz, thiazole H-4), 7.55(d, 1H, $J_{5,4}$ = 2.8 Hz, thiazole H-5), 1.47(sept, 3H, J = 7.2 Hz), 1.14(d, 18H, J = 7.6 Hz, $\underline{CH_3}$ × 6).

Synthesis of 5-{2-[(*tert*-Butyldiphenylsilyl)oxy]ethyl}-2-triisopropylsilylthiazole (8). In a dry flask, a solution of 1.65 M *n*-butyllithium in hexane (1.4 mL, 2.32 mmol) in THF (2.5 mL) was placed under argon at −40 °C. After addition of a solution of **7** (0.5 g, 2.07 mmol) in THF (2.5 mL), the mixture was stirred under argon at −40 °C for 50 min, followed by addition of a solution of 2-bromoethoxy(*tert*-butyldiphenyl)silane (1.13 g, 3.10 mmol) in THF (2.5 mL). The resulting mixture was further stirred under argon at rt overnight. The reaction mixture was quenched with an aq ammonium chloride solution. The organic layer was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography (hexane/chloroform = 100:0 and 10:90) produced the target compound **8** as a yellow oil. Yield: 489 mg (49%, 0.93 mmol). TLC R_f 0.28 (hexane/ethyl acetate = 10:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85(s, 1H, thiazole H-4), 7.63−7.60(m, 4H, Ph-$\underline{H}$), 7.42−7.34(m, 6H, Ph-$\underline{H}$), 3.85(t, 2H, J = 6.0 Hz, O-$\underline{CH_2}$), 3.12(t, 2H, J = 6.0 Hz, CH_2), 1.44(sept, 3H, J = 7.2 Hz), 1.14(d, 18H, J = 7.2 Hz, $\underline{CH_3}$ × 6), 1.03(s, 9H, Si-$\underline{CH_3}$ × 3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 143.9, 138.1, 135.7, 133.6, 129.8, 127.8, 64.3, 30.3, 26.9, 19.3, 18.6, 17.9, 12.4, 11.8. HRMS (positive mode) found: m/z 524.2842 $[M + H]^+$, calcd for $C_{30}H_{45}NOSSi_2$: 524.2839.

Synthesis of 5-Hydroxyethyl-4-(tri-*n*-butylstannyl)thiazole (5). In a dry flask, a solution of **8** (3.27 g, 6.24 mmol) in THF (25 mL) was placed under argon at −78 °C. After addition of 1.65 M *n*-butyllithium in hexane (6.05 mL, 9.98 mmol), the mixture was stirred under argon at 0 °C for 30 min, followed by successive addition of tri-*n*-butyltin chloride (5.35 mL, 18.7 mmol). The resulting mixture was further stirred under argon at rt for 2 h. The reaction mixture was quenched with an aq ammonium chloride solution. The organic layer was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography (hexane/ethyl acetate = 100:0 and 50:1) produced **9** as a yellow oil. The oil was dissolved in THF (5 mL) and cooled to 0 °C. After addition of 1.0 M TBAF in THF (24.6 mL, 24.6 mmol), the mixture was stirred at rt for 1 h. The organic layer was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography (hexane/ethyl acetate = 5:1) produced the target compound **5** as a yellow oil. Yield: 1.25 g (48%, 2.99 mmol; two steps). TLC R_f 0.35 (hexane/ethyl acetate = 3:1). ^{1}H NMR (400 MHz, DMSO) δ 9.02(s, 1H, thiazole H-2), 4.93(t, 1H, J = 5.2 Hz, CH_2−$\underline{OH}$), 3.55(q, 2H, J = 5.2 Hz, CH_2), 2.96(t, 2H, J = 6.4 Hz, CH_2), 1.55−1.47(m, 6H, Sn−($\underline{CH_2}$)$_3$), 1.32−1.23(m, 6H, Sn−($\underline{CH_2}$)$_3$), 1.11−1.07(m, 6H, Sn−($\underline{CH_2}$)$_3$), 0.84(t, 9H, J = 6.8 Hz, Sn−(CH_2)$_3$$\underline{CH_3}$ × 3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 152.6, 141.1, 64.0, 32.0, 29.2, 27.4, 13.8, 10.7. HRMS (positive mode) found: m/z 420.1380 $[M + H]^+$, calcd for $C_{17}H_{33}NOSSn$: 420.1383.

Synthesis of ^{11}C-Labeled Thiamine ([^{11}C]-1). [^{11}C]CH_3I (ca. 35 GBq) was trapped in a solution of $Pd_2(dba)_3$ (1.1 mg, 1.2 μmol) and $P(o\text{-tolyl})_3$ (5.8 mg, 19.1 μmol) in NMP (200 μL) at rt. After addition of a solution of stannyl precursor **5** (5.0 mg, 11.9 μmol), CuBr (0.8 mg, 5.9 μmol), and CsF (2.3 mg, 14.9 μmol) in NMP (100 μL), the mixture was heated for 3 min at 100 °C using hot air. To this mixture, a solution of 4-amino-5-(bromomethyl)-2-methylpyrimidine hydrobromide (50.0 mg, 176.7 μmol) in DMF (100 μL) was added, and the resulting mixture was further heated at 150 °C for 7 min by bubble mixing with a N_2 gas stream (40 mL/min). After the addition of acetonitrile (400 μL) and water (200 μL), the mixture was injected into a semipreparative HPLC system (mobile phase: acetonitrile/50 mM ammonium acetate = 40:60; column: COSMOSIL, HILIC, 20

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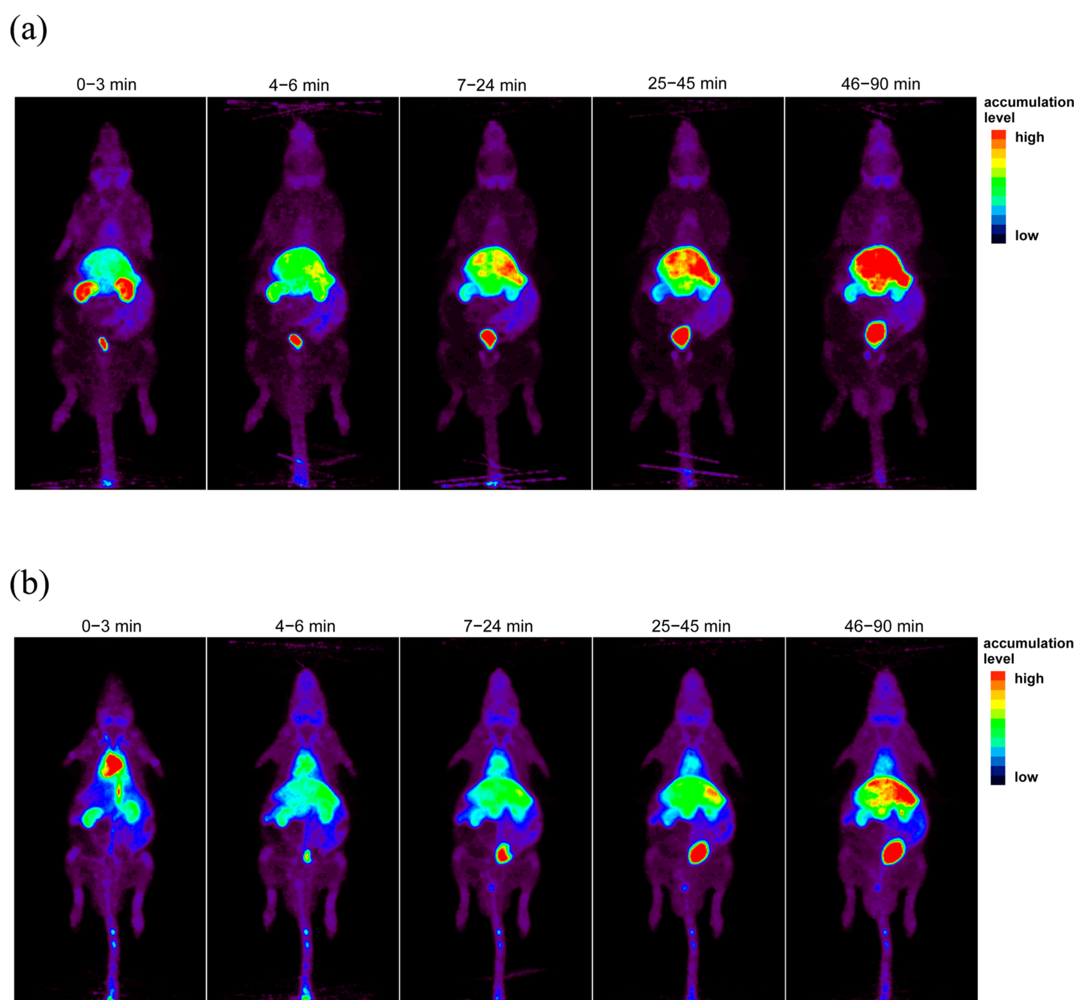

Figure 5. Whole-body PET images of rats using (a) ^{11}C-labeled thiamine [^{11}C]-**1** and (b) fursultiamine [^{11}C]-**2** as probes. Each picture shows integrated images at the corresponding time point after injection. Rats were anesthetized with 1.5% isoflurane prior to insertion of an indwelling catheter in the tail vein for radiotracer injection.

mm internal diameter [i.d.] × 250 mm, 5 μm; flow rate: 8.0 mL/min; UV detection: 267 nm; retention time: 7.7 min). The desired fraction was collected in a flask and concentrated in vacuo. The collected solution was once again injected into a semipreparative HPLC system (mobile phase: acetonitrile/5 mM sodium 1-hexanesulfonate [1.0% H_3PO_4] = 5:95; column: COSMOSIL, 5C$_{18}$-AR-II, 10 mm i.d. × 250 mm, 5 μm; flow rate: 5.0 mL/min [0−6 min] to 6.5 mL/min [7−30 min]; UV detection: 267 nm; retention time: 16.4 min). The desired fraction was collected in a flask and concentrated in vacuo. The collected solution of radiotracer was passed through a short anion-exchange resin cartridge and collected in a vial containing an aq $NaHCO_3$ solution (1.0 M, 400 μL, 0.4 mmol) via filtration sterilization. The pH values of the final samples were 4−6, and the volumes were 2.0−3.0 mL. The total synthesis time, including HPLC purification and radiopharmaceutical formulation for intravenous administration, was less than 60 min. The radioactivity of the final samples was 400−700 MBq, and the specific activity was 50−150 GBq/μmol. The [^{11}C]CH$_3$I-based DCY was 9−16%. The chemical identity of ^{11}C-labeled thiamine [^{11}C]-**1** was confirmed by coinjection with an authentic sample of thiamine into an analytical HPLC system, after postcolumn fluorescence derivatization[20,21] (mobile phase: methanol/10 mM NaH_2PO_4 containing 0.15 M $NaClO_4$ [pH 2.2 adjusted with $HClO_4$] = 2:98; column: COSMOSIL, 5C$_{18}$-AR-II, 4.6 mm i.d. × 250 mm, 5 μm; flow rate: 1.0 mL/min; reagent: 15% aq NaOH solution containing 0.01% $K_3[Fe(CN)_6]$; flow rate of the reagent: 0.4 mL/min; UV detection: 350 nm; fluorescence detection: ex at 375 nm, em at 440 nm; retention time: 7.5 min). The chemical purity was analyzed by UV-HPLC at 350 nm and the radiochemical purity was analyzed by HPLC with a radiodetector, and both were found to be greater than 99%.

Synthesis of ^{11}C-Labeled Fursultiamine ([^{11}C]-2). The concentrated solution of ^{11}C-labeled thiamine [^{11}C]-**1** after the HPLC isolation was transferred to another reaction vial. After addition of an aq solution of TFT−Na (28.5%, 1.0 mL, 1.65 mmol) and an aq solution of NaOH (30%, 400 μL, 3.75 mmol), the mixture was stirred at rt for 3 min by bubble mixing with a N_2 gas stream (400 mL/min). The resulting mixture was trapped using a C$_{18}$ SPE cartridge, which was preconditioned with methanol (5 mL) and water (5 mL). The SPE cartridge was washed with a mixture of saline and propylene glycol (90:10 v/v, 5 mL) and then eluted with a mixture of saline (1.4 mL), propylene glycol (0.6 mL), and a 25% aq ascorbic acid solution (0.2 mL). The eluate was collected into a vial via filtration sterilization. The pH values of the final samples were 4.0−4.5, and the volumes were 2.2−2.4 mL. The total synthesis time, including HPLC purification and radiopharmaceutical formulation, was less than 70 min. The radioactivity of the final samples was 100−250 MBq, and the specific activity was 15−35 GBq/μmol. The [^{11}C]CH$_3$I-based DCY was 4−10%. The chemical identity of ^{11}C-labeled fursultiamine [^{11}C]-**2** was confirmed by coinjection with an authentic sample of fursultiamine using analytical HPLC (mobile phase: acetonitrile/water [each containing 0.3% heptafluorobutyric acid] = 25:75; column: COSMOSIL 5C$_{18}$-AR-II, 4.6 mm i.d. × 150 mm, 5 μm; flow rate: 1.0 mL/min; UV detection: 241 nm; retention time: 5.8 min). The chemical purity, according to UV-HPLC at 241 nm, was

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97–99%, and the radiochemical purity, according to HPLC with a radiodetector, was up to 99%.

■ ASSOCIATED CONTENT

Supporting Information

Possible reaction mechanism of production of [^{11}C]-fursultiamine ([^{11}C]-**2**) using [^{11}C]thiamine ([^{11}C]-**1**) and *S*-tetrahydrofurfurylthiosulfuric acid, sodium salt (TFT–Na); Characterization data for compound **5** (^{1}H, ^{13}C{^{1}H}, and 2D NMR spectra, and HRMS analysis); Characterization data for compound **8** (^{1}H, ^{13}C{^{1}H}, and 2D NMR spectra, and HRMS analysis); Characterization data for compound **7** (^{1}H and 2D NMR spectra). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b00685.

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Notes

The authors declare no competing financial interest.

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(13) Among the common precursors for ^{11}C-labeling, [^{11}C]CH_3I was chosen due to its high chemical reactivity. For preparation of [^{11}C]CH_3I by basic methods, see refs 14–16.
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(17) In a typical experiment, the amount of ^{11}C-labeling precursor is equivalent to 60 nmol when it has a radioactivity of 30 GBq and a specific activity of 500 GBq/μmol at the start of the probe synthesis.
(18) DCY shows the conversion yield based on the decay-corrected radioactivity of the target ^{11}C-labeled product from [^{11}C]CH_3I. DCY is useful for discussion of the production efficiency of the ^{11}C-labeled compound using remote-controlled mechanical synthesis under radiolabeling conditions.
(19) We usually adopt a stepwise procedure where [^{11}C]CH_3I is first trapped in a Pd^0 solution and the addition of a stannyl substrate to the solution is carried out second. For the stepwise procedure, see: Suzuki, M.; Doi, H.; Kato, K.; Björkman, M.; Långström, B.; Watanabe, Y.; Noyori, R. *Tetrahedron* **2000**, *56*, 8263–8273.
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(22) DCY tends to be lower for increasingly complicated synthetic operations because losses of radioactivity result from the use of the PET probe synthesizer. These losses are due to such things as incomplete transfer of the reaction mixture from vessel to vessel, insufficient injection of the reaction mixture into semipreparative HPLC, and incomplete isolation of the ^{11}C-labeled product by semipreparative HPLC.
(23) Information from Takada Pharmaceutical Company. The details of the fursultiamine synthesis have not been announced. For a possible reaction mechanism of production of [^{11}C]fursultiamine ([^{11}C]-**2**) using [^{11}C]thiamine ([^{11}C]-**1**) and TFT–Na, see Supporting Information.

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