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Efficient sequential synthesis of PET Probes of the COX-2 inhibitor [^{11}C]celecoxib and its major metabolite [^{11}C]SC-62807 and in vivo PET evaluation

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ABSTRACT

Synthesis of [^{11}C]celecoxib, a selective COX-2 inhibitor, and [^{11}C]SC-62807, a major metabolite of celecoxib, were achieved and the potential of these PET probes for assessing the function of drug transporter in biliary excretion was evaluated. The synthesis of [^{11}C]celecoxib was achieved in one-pot by reacting [^{11}C]methyl iodide with an excess of the corresponding pinacol borate precursor using $Pd_2(dba)_3$, P(*o*-tolyl)$_3$, and K_2CO_3 (1:4:9) in DMF. The radiochemical yield of [^{11}C]celecoxib was 63 ± 23% (decay-corrected, based on [^{11}C]CH_3I) (n = 7) with a specific radioactivity of 83 ± 23 GBq/μmol (n = 7). The average time of synthesis from end of bombardment including formulation was 30 min with >99% radiochemical purity. [^{11}C]SC-62807 was synthesized from [^{11}C]celecoxib by further rapid oxidation in the presence of excess $KMnO_4$ with microwave irradiation. The radiochemical yield of [^{11}C]SC-62807 was 55 ± 9% (n = 3) (decay-corrected, based on [^{11}C]celecoxib) with a specific radioactivity of 39 ± 4 GBq/μmol (n = 3). The average time of synthesis from [^{11}C]celecoxib including formulation was 20 min and the radiochemical purity was >99%. PET studies in rats and the metabolite analyzes of [^{11}C]celecoxib and [^{11}C]SC-62807 showed largely different excretion processes, and consequently, [^{11}C]SC-62807 was rapidly excreted via hepatobiliary excretion without further metabolism. [^{11}C]SC-62807 was shown to have a high potential as a PET probe for evaluating drug transporter function in biliary excretion.

1. Introduction

It is now accepted that drug transporters play important roles in tissue distribution and excretion of drugs and their metabolites. Clinical studies have shown that variation in drug transporter activity caused by genetic polymorphisms or drug–drug interactions can affect the variability in therapeutic efficacy and the incidence of adverse effects.[1,2] The information on the functional characteristics of drug transporters in vivo allows improvements in drug delivery or drug design by targeting specific transporter proteins.[3] Accordingly, the methods that allow quantitative estimation of the tissue concentration of the drugs in vivo have been required for the investigation of such variations in the tissue distribution or disposition processes of drugs.

Positron emission tomography (PET) is a powerful and widely accepted noninvasive method for molecular imaging in living systems.[4–6] The high sensitivity and exceptional spatial-temporal resolution of PET make it particularly useful for in vivo estimation of the function of drug transporters in various tissues.[7]

Celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazole-1-yl]benzenesulfonamide) is a selective cyclooxygenase (COX)-2 inhibitor that has analgesic and anti-inflammatory effects in patients with rheumatoid arthritis, but has no effect on COX-1 activity at therapeutic plasma concentrations.[8] In humans, celecoxib is extensively metabolized in the liver via sequential two-step oxidative pathways, initially to a hydroxymethyl metabolite (SC-60613), and upon subsequent further oxidation to a carboxylic acid metabolite (SC-62807) (Fig. 1).[9,10] The majority of celecoxib is excreted into the bile as SC-62807. In this context, Wu et al. reported that SC-62807 is a substrate of drug transporters, such as Organic Anion Transporting Polypeptide 1B1 (OATP1B1) and Breast Cancer Resistance Protein (BCRP), which presumably mediate its hepatobiliary transport.[11] Therefore, celecoxib or SC-62807 radiolabeled with a short-lived positron-emitting radionuclide could be a potential PET probe for evaluating the function of these drug transporters in hepatobiliary excretion.

Celecoxib and SC-62807 have two readily accessible possible positions for ^{11}C and ^{18}F radiolabeling, and Prabhakaran et al. reported the synthesis of both [^{11}C]celecoxib and [^{18}F]celecoxib.[12,13] However, chemical and metabolic instabilities derived from [^{18}F]defluorination on the benzylic position of [^{18}F]celecoxib resulted in undesirable bone imaging due to [^{18}F]fluorine

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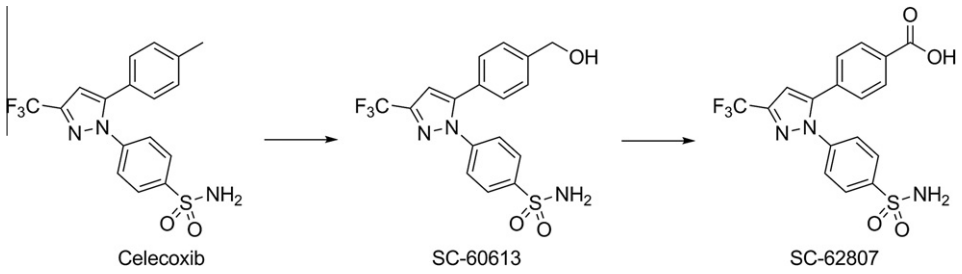


Figure 1. Two-step metabolic pathway from celecoxib to SC-62807.

generation. Therefore, ^{11}C-labeling of celecoxib would be preferable to ^{18}F-labeling.

The palladium(0)-mediated rapid *C*–[^{11}C]methylation reaction developed by our group and based on the use of [^{11}C]CH_3I and tributyl stannyl substrate is used in the synthesis of [^{11}C]celecoxib (Scheme 1).[14] Protection of the sulfonamide group is necessary to promote such a reaction. We recently reported a new type of palladium(0)-mediated rapid *C*–[^{11}C]methylation that uses an organoboron compound.[15] This reaction proceeds in the presence of $Pd_2(dba)_3$, P(*o*-tolyl)$_3$, and K_2CO_3 in DMF for 5 min under mild temperature and without the use of a microwave.[16,17] In general, organoboron compounds exhibit higher reactivity in the coupling reaction than organostannyl compounds,[18,19] and are also less toxic. Accordingly, the synthesis of [^{11}C]celecoxib reported draws extensively from our brief report[15] and advances made since its publication.

SC-62807 has a benzoic acid structure that can be labeled with ^{11}C. Efficient ^{11}C labeling of a benzoic acid structure has been reported that involves: (1) [^{11}C]CO insertion to the corresponding aryl halide,[20] (2) reaction of the Grignard reagent with [^{11}C]CO_2,[21] and (3) coupling of the [^{11}C]cyanide ion and the corresponding aryl halide, and hydrolysis.[22] Here, we explored the novel possibility of ^{11}C labeling using a combination of rapid *C*–[^{11}C]methylation and rapid oxidation, starting with a common organoboron precursor to carry out the sequential transformation of [^{11}C]celecoxib to [^{11}C]SC-62807.

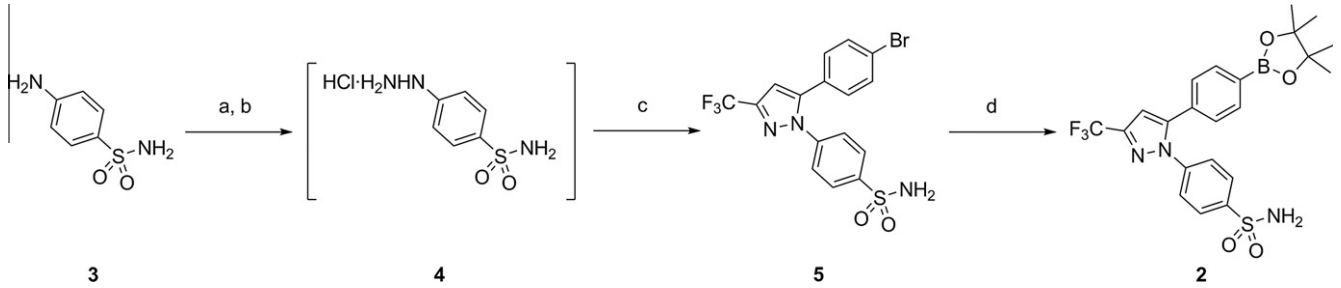
This report describes the highly efficient synthesis of [^{11}C]celecoxib and as an expanding of rapid *C*–[^{11}C]methylation, the synthesis of [^{11}C]SC-62807 by applying it to rapid *C*–[^{11}C]carboxylation. In addition, we describe evaluation of the in vivo behavior of each ^{11}C-labeled compound in rats using PET to determine whether these radiotracers enable the visualization of hepatobiliary excretion for the quantitative assessment of drug transporter function.

2. Results and discussion

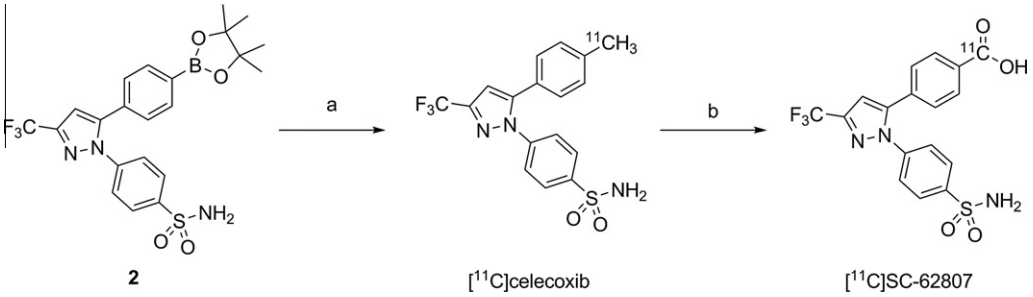
2.1. Synthesis of [^{11}C]celecoxib and [^{11}C]SC-62807 PET probes

The synthesis of the pinacol borate precursor, (4-[5-[4-(4,4,5,5-tetramethyl-[1.3.2]dioxaborolan-2-yl)phenyl]-3-trifluoromethyl-1*H*-pyrazol-1-yl]-1-phenylsulfonamide, (**2**) was achieved as described in Scheme 1. 4-[5-(4-Bromophenyl)-3-(trifluoromethy)-1*H*-pyrazol-1-yl]-1-phenylsulfonamide (**5**) was prepared from 1-(4-bromophenyl)-4,4,4-trifluorobutane-1,3-dione and hydrazine (**4**), which was generated from aniline (**3**) in one-pot based on previously described method with minor modifications.[12,23] Compound **5** was converted into **2** using bis(pinacolato)diboron and Pd catalyst in DMSO.[24]

Synthesis of [^{11}C]celecoxib was previously reported by Prabhakaran et al.,[12] using an organotin compound with a sulfonamide group protected by a dimethoxytrityl (DMT) group as a precursor for radiolabeling. The authors succeeded in synthesizing [^{11}C]celecoxib by the rapid methylation reaction using a DMT-protected stannyl precursor and [^{11}C]CH_3I in the presence of $Pd_2(dba)_3$ and P(*o*-tolyl)$_3$ in DMF at 135 °C for 5 min followed by deprotection of the DMT group in the presence of trifluoroacetic acid (TFA) at 60 °C for 5 min. Prabhakaran et al. also reported the reaction between the organostannyl-precursor without the protecting group and CH_3I in the presence of $Pd_2(dba)_3$, P(*o*-tolyl)$_3$, CuCl, and K_2CO_3 in DMF, but the reaction yielded starting material together with undesired destannylated product.[25] We concluded that in the synthesis of a short-lived PET probe, a direct one-step reaction without deprotection is necessary to reduce the total synthesis time. In a context related approach, we employed the rapid *C*–[^{11}C]methylation reaction recently developed by our group that uses an organoboron precursor. Thus, [^{11}C]celecoxib was synthesized by treating pinacol borate precursor (**2**) with [^{11}C]CH_3I in DMF in the presence of $Pd_2(dba)_3$/P(*o*-tolyl)$_3$/K_2CO_3 (1:4:9) at 65 °C for 4 min (Scheme 2). This reaction produced a 98% analytical yield by HPLC (Fig. 2A). The total synthesis time was 30 min from the end of bombardment (EOB). The average decay-corrected radiochemical yield (DCY) based on [^{11}C]CH_3I was 63 ± 23% (n = 7), and the specific radioactivity at the end of synthesis was 84 ± 23 GBq/μmol (n = 7) with >98% radiochemical purity (Fig. 2B). The chemical identity of [^{11}C]celecoxib was confirmed by co-injection with the reference standard celecoxib in analytical HPLC and it showed the same retention time peaks on the UV and the radioactive chromatograms. Thus, we efficiently synthesized [^{11}C]celecoxib from the corresponding pinacol borate precursor without the protection of a sulfonamide group.



Scheme 1. Synthesis of the boron precursor for [^{11}C]celecoxib. Reagents and conditions: (a) $NaNO_2$, concd HCl, −5 °C, 15 min; (b) $SnCl_2 \cdot H_2O$, −20 °C, 1 h; (c) compound **3**, C_2H_5OH, reflux, 16 h, 75% (overall in three steps); (d) bis(pinacolato)diboron, $PdCl_2(dppf) \cdot CH_2Cl_2$, AcOK, DMSO, 80 °C, 16 h, 78%.



Scheme 2. Synthesis of [^{11}C]celecoxib and [^{11}C]SC-62807. Reagents and conditions: (a) [^{11}C]CH_3I, $Pd_2(dba)_3$, P(*o*-tolyl)$_3$, DMF, 65 °C, 4 min. (b) $KMnO_4$, 0.2 M NaOH, heating, 5 min.

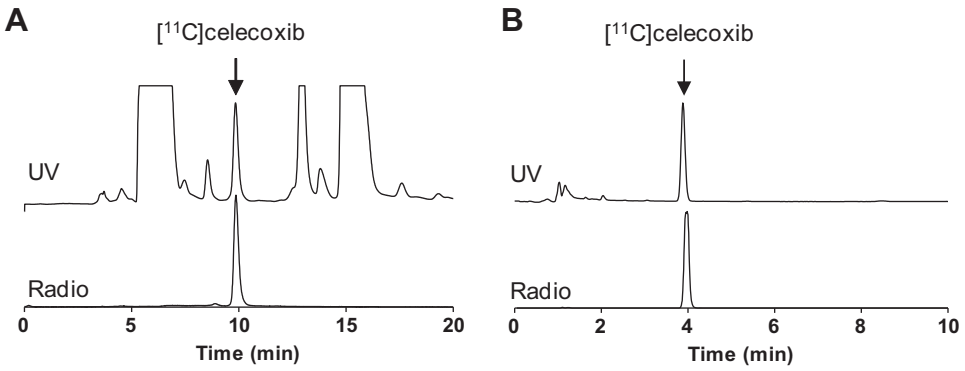


Figure 2. (A) HPLC chromatogram before [^{11}C]celecoxib purification; (B) HPLC chromatogram after [^{11}C]celecoxib purification. UV absorbance: 254 nm.

Table 1
Rapid oxidation reaction conditions. Reaction time = 5 min

Entry	Temp.[a] (°C)	HPLC analytical yield[b] (%)	Radiochemical yield[c] (%)
1	120 (n = 5)	55 ± 30	32 ± 18
2	120, microwave (n = 4)	79 ± 13	47 ± 11
3	140, microwave (n = 3)	87 ± 5	55 ± 9

[a] Values in parentheses show number of runs.
[b] Not decay-corrected, calculated by the peak area ratio of the desired [^{11}C]methylated product obtained by radio-HPLC analysis of the reaction mixture.
[c] Decay-corrected, calculated based on [^{11}C]celecoxib.

Scheme 2 also illustrates the synthesis of ^{11}C-labeled SC-62807 via [^{11}C]celecoxib based on sequential rapid *C*–[^{11}C]methylation and oxidation. [^{11}C]celecoxib was dissolved in 0.2 M NaOH and transferred to a reaction vessel containing $KMnO_4$ (the amount of [^{11}C]celecoxib transferred by this method was ~20–60% of the synthesized radioactivity). The reaction was carried out by setting the time to 5 min in order to optimize the heating conditions (Table 1). Consequently, [^{11}C]celecoxib was converted to [^{11}C]SC-62807 in 32 ± 18% (n = 5) DCY[26] based on [^{11}C]celecoxib (HPLC analytical yield[27]: 55 ± 30% (n = 5)) by conventional heating at 120 °C. The process had poor reproducibility, presumably due to the presence of different concentrations of [^{11}C]celecoxib in each reaction. Therefore, we used microwave irradiation as an alternative heating method based on prior experiments in which microwave irradiation tended to enhance reactivity at low concentrations. As expected, microwave irradiation was extremely effective in increasing both the yield and the reproducibility of the reaction. Finally, [^{11}C]SC-62807 was efficiently synthesized by rapid oxidation of [^{11}C]celecoxib at 140 °C for 5 min under microwave irradiation with 55 ± 9% (n = 3) DCY based on [^{11}C]celecoxib (HPLC analytical yield: 87 ± 5% (n = 3)) (Fig. 3A). The total synthesis time was 20 min from the [^{11}C]celecoxib transfer, with a specific radioactivity at the end of synthesis of 39 ± 4 GBq/μmol (n = 3) >98% radiochemical purity (Fig. 3B). The chemical identity of [^{11}C]SC-62807 was confirmed by co-injection with the reference

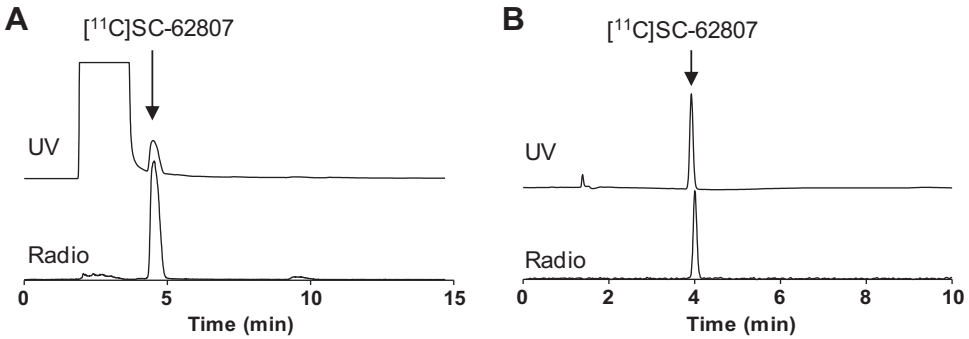


Figure 3. (A) HPLC chromatogram before [^{11}C]SC-62807 purification; (B) HPLC chromatogram after [^{11}C]SC-62807 purification. UV absorbance: 254 nm.

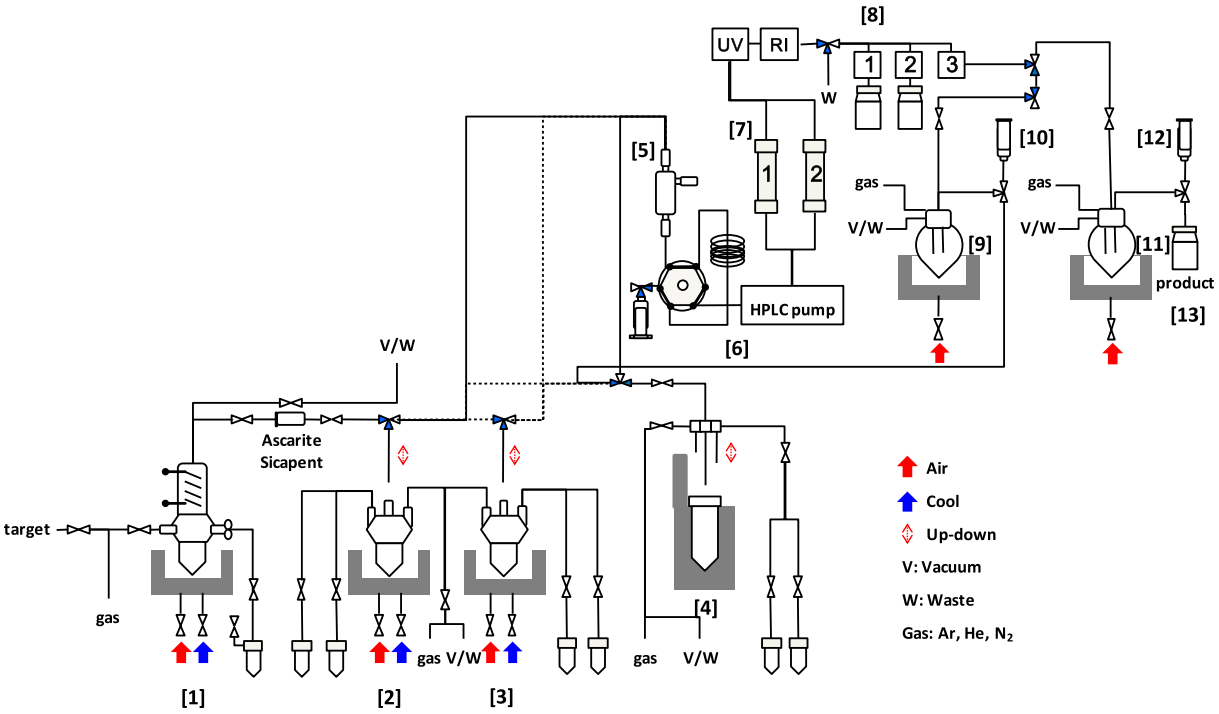


Figure 4. Automated radiolabeling system designed for sequential two-step-radiosyntehsis of [^{11}C]SC-62807. [1] = reactor for [^{11}C]CH_3I production, [2] and [3] = reactor, [4] = microwave apparatus, [5] = reservoir, [6] = HPLC pump, [7] = column switching, [8] = fraction collector, [9] and [11] = evaporator, [10] and [12] = syringe, [13] = vial.

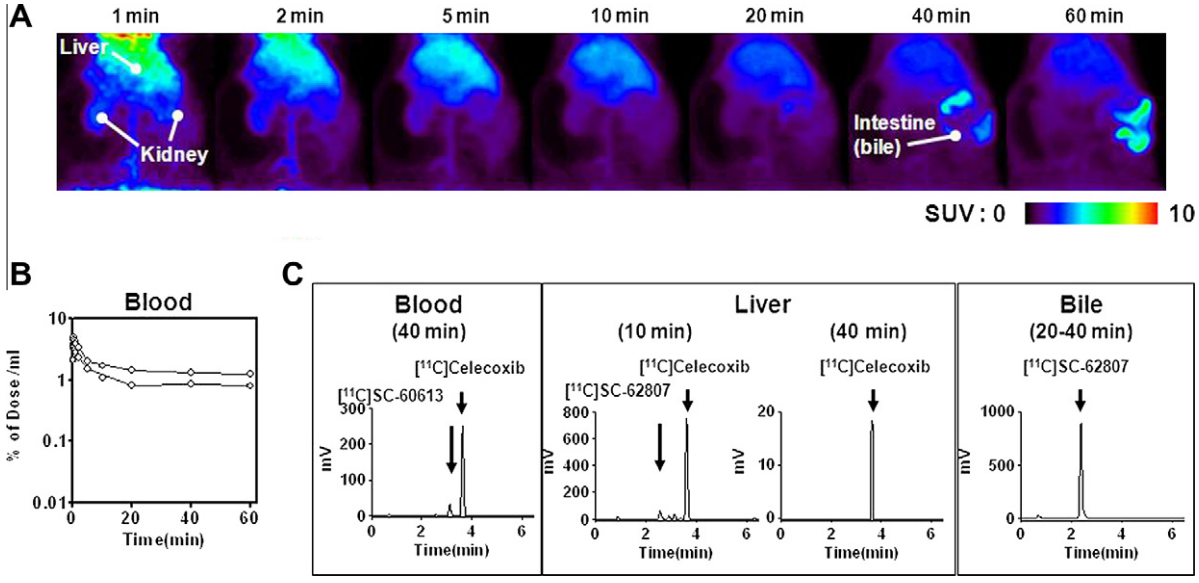


Figure 5. PET images, blood time–activity curve, and radiometabolite analysis following intravenous administration of [^{11}C]celecoxib to male Sprague–Dawley rats. (A) PET images of radioactivity in the abdominal region at 1, 2, 5, 10, 20, 40, and 60 min after administration. SUV = standardized uptake value; (B) the time profiles of radioactivity in the blood were determined by blood sampling over a 60-min period after administration of [^{11}C]celecoxib. The data represent the individual data of two rats; (C) representative HPLC chromatograms of blood, liver, and bile extracts.

standard SC-62807 in analytical HPLC and it showed the same retention time peaks on the UV and the radioactive chromatograms. Thus, we successfully synthesized [^{11}C]celecoxib and [^{11}C]SC-62807 by rapid *C*–[^{11}C]methylation of the corresponding pinacol borate precursor without the protection of a sulfonamide group, and sequential combination rapid oxidation of the [^{11}C]methyl group, respectively. The total time required for this sequential synthesis was 50 min from EOB.

2.2. Construction of an automated two-step radiolabeling system for the synthesis of [^{11}C]-SC-62807

Figure 4 shows the radiolabeling system we developed to carry out two-step synthesis of [^{11}C]SC-62807 using microwave irradiation. The system consists of a reactor for $^{11}CH_3I$ production using an established method,[28] two reactors for cooling and heating, one reactor for rapid oxidation under microwave irradiation (Initi-

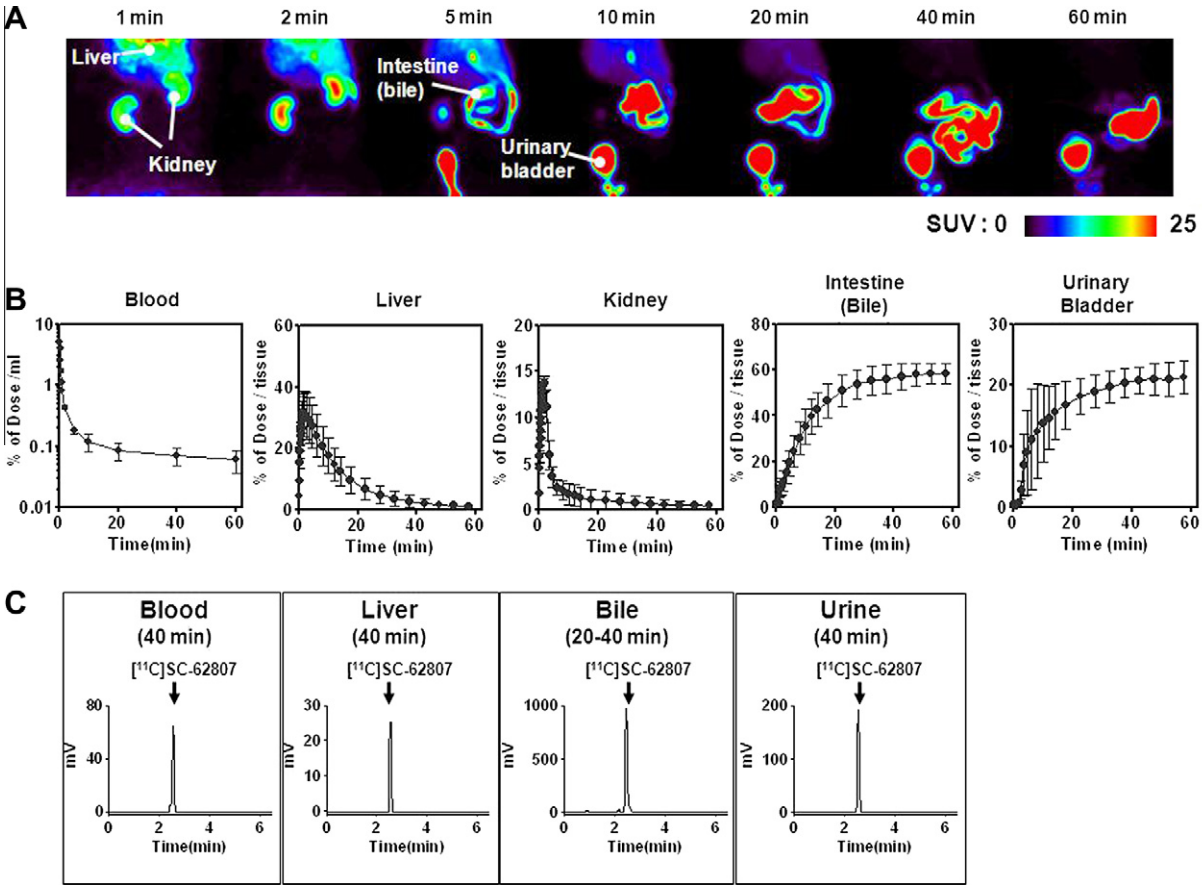


Figure 6. PET images, time–activity curves, and radiometabolite analysis following intravenous administration of [^{11}C]SC-62807 to male Sprague–Dawley rats. (A) PET images of radioactivity in the abdominal region at 1, 2, 5, 10, 20, 40, and 60 min after administration. SUV = standardized uptake value; (B) the time profiles of radioactivity in the blood, liver, kidney, intestine (bile), and urinary bladder were determined by PET imaging and blood sampling over a 60-min period after administration of [^{11}C]SC-62807. The data represent the mean ± S.D. (n = 3); (C) representative HPLC chromatograms of blood, liver, bile, and urine extracts.

ator™, Biotage), multiple reservoirs for the addition of reagents, column switching (six columns available), and four fraction collectors, two of which play the role of evaporators attached to the formulation system. Any combination of reactors and fraction collectors can be chosen by changing the connections. This remote-controlled synthesis apparatus was utilized for the preparation of [^{11}C]SC-62807 using the rapid sequential *C*–[^{11}C]methylation–oxidation reaction. The automated multistep labeling system could be utilized for the synthesis of a wide range of ^{11}C-incorporated PET probes.

2.3. PET study and radiometabolite analyzes of [^{11}C]celecoxib

Time course maximum intensity projection PET images of the abdominal region of a rat following administration of [^{11}C]celecoxib are shown in Figure 5A. Radioactivity initially localized in the liver, and subsequently part of the radioactivity moved to the intestine, although the amount of radioactivity in the entire abdominal region was relatively high. Radioactivity in the blood gradually decreased, however, the radioactivity was still higher level (more than 1% of dose/ml blood) even at the end of the scan (Fig. 5B). Figure 5C shows representative radiochromatograms of blood, liver, and bile extracts prepared after administration of [^{11}C]celecoxib to rats. At 40 min after administration, [^{11}C]celecoxib was detected predominantly in the blood and the liver, and a very small amount of radiometabolites, [^{11}C]SC-60613 (hydroxymethyl form of celecoxib) and [^{11}C]SC-62807 (carboxylic acid form), were detected in the blood and the liver, respectively. In contrast, the [^{11}C]SC-62807 radiometabolite was the major component found in the bile after administration of [^{11}C]celecoxib. These pharmacokinetic profiles of [^{11}C]celecoxib will make the analysis of biliary excretion difficult for the following reasons: (1) since PET cannot discriminate between [^{11}C]celecoxib and its radiometabolite, [^{11}C]celecoxib PET images of hepatobiliary excretion will be the result of two different pharmacokinetic functions, metabolism and biliary excretion of [^{11}C]celecoxib, and (2) the very high concentration of [^{11}C]celecoxib in the blood may affect the tissue concentration determined by PET image analysis. Therefore, [^{11}C]celecoxib should not be used as a PET probe for the evaluating drug transporter function in biliary excretion.

2.4. PET study and radiometabolite analyzes of [^{11}C]SC-62807

Figure 6A shows time course maximum intensity projection PET images of radioactivity in the rat abdominal region following administration of [^{11}C]SC-62807. Radioactivity localized primarily in the liver and kidneys within 2 min after [^{11}C]SC-62807 administration. By 60 min, radioactivity was localized in the intestine (derived from bile excreted into the intestine) and the urinary bladder.

Time–activity curves for blood and tissues in the abdominal region of rats are shown in Figure 6B. The radioactivity in blood rapidly decreased. A maximum of 35 ± 6% and 14 ± 1% of the dose was distributed in the liver and kidney, respectively, by 2 min post-administration, at which point the amount of radioactivity began to decline rapidly. The radioactivity in the intestine (derived from radioactivity in bile excreted into the intestine) and the urinary bladder (derived from radioactivity excreted into the urine) increased until 60 min, reaching 58 ± 6% and 22 ± 3% of the dose,

respectively. Metabolites of [^{11}C]SC-62807 were not detected in the blood, liver, bile, or urine within 40 min after administration of [^{11}C]SC-62807 (Fig. 6C). These results show that the radioactivity of [^{11}C]SC-62807 is rapidly excreted via hepatobiliary and renal excretion without further metabolism.

There are some key transporters in hepatobiliary transporter including MRP2 (multidrug resistance-associated protein 2), BCRP, and OATPs. Some probes such as ^{99m}Tc-mebrofenin, *N*-[^{11}C]acetyl-leukotriene E4, and (15*R*)-16-*m*-tolyl-17,18,19,20-tetranorisocarbacyclin (15*R*-[^{11}C]TIC-Me) for molecular imaging technology have been reported for evaluating the function of MRP2 or OATPs in hepatobiliary excretion.[29–31] Compared to these probes, the study using [^{11}C]SC-62807 may have the potentials for evaluating the other transporter BCRP at least in hepatobiliary transport and renal excretion. In addition, [^{11}C]SC-62807 is superior to the other probes in the use of diagnosis because of the rapid excretion without further metabolism, which may enable the functional analysis more simple. Further investigations using [^{11}C]SC-62807 are ongoing to evaluate the function of these drug transporters using knockout animal models or specific drug transporter inhibitors.

3. Conclusion

A novel procedure for the synthesis of a [^{11}C]benzoic acid structure via sequential rapid *C*–[^{11}C]methylation and oxidation reactions was established. The first step involves rapid and highly efficient ^{11}C-labeling of celecoxib from a pinacol boron precursor. The subsequent rapid oxidation under microwave irradiation produces [^{11}C]SC-62807. Microwave irradiation significantly enhances both radiochemical yield and reproducibility. The protocol for this two-step rapid radiosynthesis can be executed in a fully remote-controlled manner by using the radiolabeling system we developed.

PET image analysis in parallel with radiometabolite analyzes of rats indicated that [^{11}C]celecoxib is not suitable for evaluating biliary excretion because it shows high blood concentration and its biliary excretion includes the mixture of two different pharmacokinetic functions, metabolism and biliary excretion of [^{11}C]celecoxib. On the other hand, [^{11}C]SC-62807 enables the visualization of hepatobiliary excretion as well as renal excretion without further metabolism, and therefore is a potentially useful PET probe for the quantitative determination of the drug transporter. Further evaluation of [^{11}C]SC-62807 in terms of its utility in functional analyzes of drug transporters in hepatobiliary and renal excretion is in progress.

4. Materials and methods

4.1. Chemistry

All chemicals and solvents were purchased from Sigma–Aldrich Japan (Tokyo, Japan), Wako Pure Chemical Industries (Osaka, Japan), Tokyo Kasei Kogyo (Tokyo, Japan), Nacalai Tesque (Kyoto, Japan), and ABX (Radeberg, Germany), and were used without further purification. Celecoxib, SC-60613, and SC-62807 as cold standards were purchased from Tronto Research Chemicals (North York, Canada). Flash chromatography was performed on Teledyne Isco CombiFlash Companion (Lincoln, USA). Nuclear magnetic resonance (NMR) spectra were recorded on JEOL JNM-ECX400P spectrometer (Tokyo, Japan) at ambient temperature. The chemical shifts are expressed in parts per million (ppm) downfield from tetramethylsilane or in ppm relative to $CHCl_3$ (δ 7.26 in ^{1}H NMR and 77.0 in ^{13}C NMR). Signal patterns are indicated as follows: s, singlet; d, doublet; t, tripled; q, quartet; m, multiplet; br, broad signal. Coupling constants (*J* values) are given in hertz (Hz). Mass spectra (MS) was measured on a ThermoFinnigan LCQ ion trap mass spectrometer (San Jose, CA, USA) equipped with a turbo ESI ion source. Carbon-11 was produced by an ^{14}N(p,α)^{11}C nuclear reaction using a CYPRIS HM-12S Cyclotron (Sumitomo Heavy Industry, Tokyo, Japan). An automated radiolabeling system was used for heating the reaction mixture, dilution, HPLC injection, fraction collection, evaporation, and sterile filtration. Purification with semipreparative HPLC was performed on a GL Science system (Tokyo, Japan). Microwave irradiation was carried out in a Biotage Initiator™ (Tokyo, Japan) using a sealed vessel. Radioactivity was quantified with an ATOMLAB™ 300 dose calibrator (Aloka, Tokyo, Japan). Analytical HPLC was performed on a Shimadzu system (Kyoto, Japan) equipped with pumps and a UV detector, and effluent radioactivity was measured with an RLC700 radio analyzer (Aloka). The columns used for analytical and semipreparative HPLC were COSMOSIL C_{18} MS-II and AR-II (Nacalai Tesque), respectively.

4.1.1. 4-[5-(4-bromophenyl)-3-(trifluoromethy)-1*H*-pyrazol-1-yl]1-phenylsulfonamide (5)

A solution of sodium nitrate (1.38 g, 20 mmol) was added into a mixture of sulfanilamide (6.8 g, 20 mmol) in concd HCl (10 mL) and water (2.5 mL) over 15 min at −5 °C. The mixture was rapidly added to a cooled (−20 °C) solution of tin(II) chloride dyhydrate (10 g, 44 mmol) in conc. HCl (15 mL). The resulting mixture was stirred for 1 h at room temperature. A solution of 1-(4-bromophenyl)-4,4,4-trifluorobutane-1,3-dione (4.19 g, 14.2 mmol) in ethanol (20 mL) was added. The mixture was stirred under reflux for 16 h. It was evaporated under the reduced pressure and the residue was extracted with ethyl acetate (30 mL, three times). Organic layer was washed with brine (30 mL), dried over sodium sulfate, filtered, and evaporated. The crude product was purified by flash chromatography eluting with hexane/ethyl acetate = 2:1 to give the title compound (4.5 g, 71%) as a white solid. ^{1}H NMR spectrum was identified with the data of Ref. 12.

4.1.2. 4-[5-[4-(4,4,5,5-Tetramethyl-[1.3.2]dioxaborolan-2-yl)-phenyl]-3-trifluoromethyl-1*H*-pyrazol-1-yl]-1-phenylsulfonamide (2)

A mixture of **5** (2.23 g, 5.0 mmol), bis(pinacolato)diboron (1.40 g, 5.5 mmol), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium (122 mg, 0.15 mmol), and potassium acetate (1.47 g, 15 mmol) in anhydrous DMSO (20 mL) was stirred at 80 °C for 3 h. It was partitioned quenched with water (50 mL) and then extracted with diethylehter (50 mL, two times). Organic layer was dried over sodium sulfate, filtered, and evaporated. The crude product was purified by flash chromatography eluting with hexane/ethyl acetate = 4:1 to give the title compound (1.5 g, 40%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.90 (d, *J* = 8.8 Hz, 2H), 7.79 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 8.8 Hz, 2H), 7.22(d, *J* = 8.0 Hz, 2H), 6.80 (s, 1H), 5.04 (br s, 2H), 1.35 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 145.0, 144.4, 144.0, 142.4, 141.4, 135.3, 131.0, 128.1, 127.6, 125.5, 121.0 (q, *J* = 268.7 Hz), 106.8, 84.2, 24.6. MS (ESI): *m*/*z* 494.35 $[M+H]^+$, 492.40 $[M-H]^-$.

4.1.3. Synthesis of [^{11}C]celecoxib

[^{11}C]CO_2 was converted to [^{11}C]CH_3I by treatment with lithium aluminum hydride followed by hyrdoiodic acid using an automated synthesis system.[28] [^{11}C]CH_3I was trapped in a solution of pinacol borate precursor **2** (3.8 mg, 7.7 μmol), $Pd_2(dba)_3$ (2.9 mg, 3.2 μmol), P(*o*-tolyl)$_3$ (3.8 mg, 12.7 μmol), and K_2CO_3 (4.0 mg, 28.9 μmol) in DMF (400 μL) at 30 °C. Next, the mixture was heated to 65 °C for 4 min and then diluted with CH_3CN (700 μL) and water (300 μL). After filtration through a 0.2 μm PVDF filter (Millipore), the resulting mixture was injected onto a preparative HPLC column (AR-II C_{18}, 20 mm i.d. × 250 mm, 5 μm (COSMOSIL, Nacalai Tesque) using a mobile phase of CH_3CN/water = 70:30 at a flow rate

of 10 mL/min with UV detection at 254 nm. The [^{11}C]celecoxib retention time was 9.6 min. The desired fraction was collected in a flask and evaporated to dryness.

For the subsequent oxidation reaction, [^{11}C]celecoxib was dissolved in 0.2 M NaOH, while for the isolation and radiochemical formulation of [^{11}C]celecoxib for use in the PET study, it was reconstituted with a mixture of polysorbate 80, propylene glycol, and saline (0.1:1:10, v/v/v, 4.0 mL). The total synthesis time from EOB until formulation was 30 min. The decay-corrected radiochemical yield was 63 ± 23% (n = 7) based on [^{11}C]CH_3I with a specific radioactivity of 83 ± 23 GBq/μmol (n = 7). The chemical identity of [^{11}C]celecoxib was confirmed by co-injection with reference standard celecoxib in analytical HPLC (column: AR-II C_{18}, 4.6 mm i.d. × 100 mm, 5 μm (COSMOSIL, Nacalai Tesque); mobile phase: CH_3CN/water (pH 7.4) = 65:35; flow rate: 1 mL/min; UV detection: 254 nm; retention time: 3.8 min) and it showed the same retention time peaks on the UV and the radioactive chromatograms. Both the chemical purity analyzed at 254 nm and the radiochemical purity were always greater than 98%.

4.1.4. Synthesis of [^{11}C]SC-62807

[^{11}C]celecoxib in 0.2 M NaOH was mixed with potassium permanganate (20 mg). The resulting mixture was heated under microwave irradiation to 140 °C for 5 min. The reaction was quenched with 30% sodium hydrogen sulfite aqueous solution (400 μL) and then the mixture was acidified with 2 M HCl (200 μL). After dilution with CH_3OH (600 μL), the mixture was injected onto a preparative HPLC column (AR-II (COSMOSIL), C_{18}, 10 mm i.d. × 250 mm, 5 μm with a mobile phase consisting of CH_3CN and 0.2% HCOOH (60:40) and a flow rate of 6 mL/min with UV detection at 254 nm. The [^{11}C]SC-62807 retention time was 4.4 min. The desired fraction was collected in a flask and the organic solvent was removed under reduced pressure.

For the radiochemical formulation of [^{11}C]SC-62807 for use in PET analyzes, it was reconstituted with a mixture of polysorbate 80, propylene glycol, and saline (0.1:1:10, v/v/v, 2.0 mL). The total synthesis time until formulation from [^{11}C]celecoxib was 20 min, thus, the total synthesis time from EOB was about 50 min. The decay-corrected radiochemical yield was 55 ± 9% (n = 3) with specific radioactivity of 39 ± 4 GBq/μmol (n = 3). The chemical identity of [^{11}C]SC-62807 was confirmed by co-injection with the reference standard celecoxib in analytical HPLC (column: AR-II, 4.6 mm i.d. × 100 mm, 5 μm; mobile phase: CH_3CN and 0.2% HCOOH = 50:50; flow rate: 1 mL/min; UV detection: 254 nm) and it showed the same retention time peaks on the UV and the radioactive chromatograms. The [^{11}C]SC-62807 retention time was 4.2 min. Both the chemical purity analyzed at 254 nm and the radiochemical purity were greater than 99%.

4.2. Experimental animals

Male Sprague–Dawley (SD) rats weighing 210–290 g (7–8 weeks old, n = 2 or 3) were purchased from Japan SLC Inc. (Shizuoka, Japan). The animals were kept in a temperature- and light-controlled environment and had ad libitum access to standard food and tap water. All experimental protocols were approved by the Ethics Committee on Animal Care and Use of the Center for Molecular Imaging Science in RIKEN, and were performed in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985).

4.3. PET studies

Rats were anesthetized with a mixture of 1.5% isoflurane and nitrous oxide/oxygen (7:3) and then placed on the PET scanner gantry (MicroPET Focus 220, Siemens Co., Ltd, Knoxville, TN, USA). The PET scanner has a spatial resolution of 1.4 mm FWHM at the center of the field of view, which is 220 mm in diameter with an axial extent 78 mm in length. After intravenous bolus injection of [^{11}C]celecoxib (approximately 26–33 MBq per animal) or [^{11}C]SC-62807 (approximately 11–20 MBq per animal) via a venous catheter inserted into the tail vein, a 60-min emission scan was performed. The chemical amounts of [^{11}C]celecoxib and [^{11}C]SC-62807 contained in the bolus injections were calculated to be 0.29–1.3 nmol/body (0.11–0.51 μg/body) and 0.20–1.4 nmol/body (0.10–0.72 μg/body), respectively. Arterial blood was sampled via the cannulated femoral artery at the following time points: 10, 20, 30, 40, and 50 s, and 1, 2, 5, 10, 20, 40, and 60 min after administration of the radiotracers. Blood radioactivity was measured using a 1470 WIZARD® Automatic Gamma Counter (PerkinElmer, Waltham, MA, USA). Emission data were acquired in list mode, and the data were reconstructed with standard 2D filtered back projection (Ramp filter, cutoff frequency of 0.5 cycles per pixel). Region of interests (ROIs) were placed on liver, intestine, kidney, or urinary bladder using image processing software (Pmod ver.3.0, PMOD Technologies Ltd, Zurich, Switzerland). Regional uptake of radioactivity in the tissue and blood radioactivity were decay-corrected to the injection time and expressed as % dose/tissue or % dose/ml blood, normalized for injected radioactivity.

4.4. Radiometabolite analyzes

Rats were anesthetized with 1.5% isoflurane before administration of the radiotracers. After intravenous injection of [^{11}C]celecoxib (approximately 26–48 MBq per animal) or [^{11}C]SC-62807 (approximately 10–32 MBq per animal), blood, urine, and liver samples were taken at 10, 20, and 40 min post-administration. The liver was removed quickly and then homogenized. To sample bile, the bile duct was cannulated before administration of the radiotracer, and bile was collected over the periods 0–10, 10–20, and 20–40 min post-administration. A two-fold volume of CH_3CN was added to an aliquot of each sample and the resulting mixture was centrifuged at 12,000 rpm for 2 min at 4 °C. The supernatant was diluted with HPLC mobile phase and analyzed for intact radiotracers and metabolites using a Shimadzu HPLC system coupled to a NaI(Tl) positron detector UG-SCA30 (Universal Giken, Kanagawa, Japan). Chromatographic separation was carried out using a 4.6 mm i.d. × 50 mm Waters Atlantis T3 column (Waters, Milford, MA). The flow was 2.0 mL/min at an initial condition of 80% solvent A (5% CH_3CN in 10 mM CH_3COONH_4) and 20% solvent B (90% CH_3CN in 10 mM CH_3COONH_4). Analytes were eluted using the following gradient conditions: 0–0.5 min: 20% solvent B in solvent A; 0.5–2.5 min: 20–100% solvent B in solvent A; 2.5–4 min: 100% solvent B. Following analyte elution, the column was returned to 20% solvent B in solvent A over 2 min. The elution was monitored by UV absorbance at 254 nm and coupled with NaI positron detection. The amount of radioactivity associated with each intact radiotracer and its metabolite was calculated as a percentage of the total amount radioactivity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.03.020.

References and notes

1. Shitara, Y.; Sato, H.; Sugiyama, Y. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 689.
2. Maeda, K.; Sugiyama, Y. *Drug Metab. Pharmacokinet.* **2008**, *23*, 223.
3. Willmann, J. K.; van Bruggen, N.; Dinkelborg, L. M.; Gambhir, S. S. *Nat. Rev. Drug. Disc.* **2008**, *7*, 591.
4. Ametamey, S. M.; Honer, M.; Schubiger, P. A. *Chem. Rev.* **2008**, *108*, 1501.
5. Fowler, J. S.; Wolf, A. P. *Acc. Chem. Res.* **1997**, *30*, 181.
6. Miller, P. W.; Long, N. J.; Vilar, R.; Gee, A. D. *Angew. Chem., Int. Ed.* **2008**, *47*, 8998.
7. Riemann, B.; Schäfers, K. P.; Schober, O.; Schäfers, M. *Q. J. Nucl. Med. Mol. Imaging* **2008**, *52*, 215.
8. Penning, T. D.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1347.
9. Paulson, S. K.; Zhang, J. Y.; Breau, A. P.; Hri bar, J. D.; Liu, N. W. K.; Jessen, S. M.; Lawal, Y. M.; Cogburn, J. N.; Gresk, C. J.; Markos, C. S.; Maziasa, T. J.; Schoenhard, G. L.; Burton, E. G. *Drug. Metab. Dispos.* **2000**, *28*, 514.
10. Paulson, S. K.; Zhang, J. Y.; Jessen, S. M.; Lawal, Y.; Liu, N. W. K.; Dudkowski, C. M.; Wang, Y.-F.; Chang, M.; Yang, D.; Findlay, J. W. A.; Berge, M. A.; Markos, C. S.; Breau, A. P.; Hribar, J. D.; Yuan, J. *Xenobitica* **2000**, *30*, 731.
11. Wu, C.; Kusuhara, H.; Sugiyama, Y.; Takashima, T.; Takashima-Hirano, M.; Doi, H.; Suzuki, M.; Watanabe, Y. *Jpn. Phamacol. Ther.* **2009**, *37*, S37.
12. Prabhakaran, J.; Majo, V. J.; Simpson, N. R.; Van Heertum, R. K.; Mann, J. J.; Kumar, J. S. D. *J. Labelled Compd. Radiopharm.* **2005**, *48*, 887.
13. Prabhakaran, J.; Underwood, M. D.; Parsey, R. V.; Arango, V.; Majo, V. J.; Simpson, N. R.; Heertum, R. V.; Mann, J. J.; Kumar, J. S. D. *Bioorg. Med. Chem.* **2007**, *15*, 1802.
14. Suzuki, M.; Doi, H.; Björkman, M.; Andersson, Y.; Långström, B.; Watanabe, Y.; Noyori, R. *Chem. Eur. J.* **1997**, *3*, 2039.
15. Doi, H.; Ban, I.; Nonomiya, A.; Sumi, K.; Kuang, C.; Hosoya, T.; Tsukada, H.; Suzuki, M. *Chem. Eur. J.* **2009**, *15*, 4165.
16. Hostetler, E. D.; Terr, G. E.; Burns, H. D. *J. Labelled Compd. Radiopharm.* **2005**, *48*, 629.
17. Hamill, T. J.; Krause, S.; Ryan, C.; Bonnefous, C.; Govek, S.; Seiders, T. J.; Cosford, N. D. P.; Roppe, J.; Kamenecka, T.; Patel, S.; Gibson, R. E.; Sanabria, S.; Riffel, K.; Eng, W.; King, C.; Yang, Z.; Green, M. D.; O'Malley, S. S.; Hargreaves, R.; Burns, H. D. *Synapse* **2005**, *56*, 205.
18. Aldous, D. J.; Bower, S.; Moorcroft, N.; Todd, M. *Synlett* **2001**, 150.
19. Morris, G. A.; Nguyen, S. T. *Tetrahedron Lett.* **2001**, *42*, 2093.
20. Långström, B.; Itsenko, O.; Rahman, O. *J. Labelled Comp. Radiopharm.* **2007**, *50*, 794.
21. Långström, B.; Antoni, G.; Gullberg, P.; Halldin, C.; Malmborg, P.; Någren, K.; Rimland, A.; Svärd, H. *J. Nucl. Med.* **1987**, *28*, 1037.
22. Ponchant, M.; Hinnen, F.; Demphel, S.; Crouzel, C. *Appl. Radiat. Isot.* **1997**, *48*, 755.
23. Ando, K.; Kato, T.; Kawai, A.; Nonomura, T. WO9964415, 1999.
24. Ishiyama, T.; Murata, M.; Miyaura, N. *J. Org. Chem.* **1995**, *60*, 7508.
25. Further details concerning with the yield, purity, etc. have not been reported in Ref. 6.
26. Decay-corrected yield (DCY) in the oxidation reaction was calculated based on the radioactivity of [^{11}C]celecoxib. In oxidation reaction, a part of [^{11}C]celecoxib which was isolated in the rapid *C*–[^{11}C]methylation reaction was used, therefore, DCY based on [^{11}C]CH_3I in oxidation reaction was not able to calculate.
27. HPLC analytical yield indicates the reaction efficiency. The reaction efficient is calculated by the peak area ratio of the desired [^{11}C]methylated product obtained by a radio-HPLC analysis of the reaction mixture. See Ref. 32.
28. Långström, B.; Antoni, G.; Gullberg, P.; Halldin, C.; Malmborg, P.; Nagren, K.; Rimland, A.; Svard, H. *J. Nucl. Med.* **1987**, *28*, 1037.
29. Ghibellini, G.; Leslie, E. M.; Pollack, G. M.; Brouwer, L. R. *Pharm. Res.* **2008**, *25*, 1851.
30. Guhlmann, A.; Krauss, K.; Oberdorfer, F.; Siegel, T.; Scheuber, P. H.; Muller, J.; Csuk-Glanzer, B.; Ziegler, S.; Ostertag, H.; Keppler, D. *Hepatology* **1995**, *21*, 1568.
31. Takashima, T.; Nagata, H.; Nakae, T.; Cui, Y.; Wada, Y.; Kitamura, S.; Doi, H.; Suzuki, M.; Maeda, K.; Kusuhara, H.; Sugiyama, Y.; Watanabe, Y. *J. Pharmacol. Exp. Ther.* **2010**, *37*, 314.
32. Suzuki, M.; Doi, H. *J. Synth. Org. Chem. Jpn.* **2010**, *68*, 1195.