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Synthesis of [^{11}C]dehydropravastatin, a PET probe potentially useful for studying OATP1B1 and MRP2 transporters in the liver

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ABSTRACT

Drug transporters mediate the uptake and elimination of drugs in various organs; therefore, having knowledge of how a transporter functions in the body would play a key role in ensuring drug efficacy in in vivo systems. In this context, we designed and synthesized [^{11}C]dehydropravastatin, a novel PET probe that would be potentially useful for evaluation of the functions of the OATP1B1 and MRP2 transporters, based on the use of palladium(0)-mediated rapid C-[^{11}C]methylation (viz., the rapid cross-coupling between [^{11}C]methyl iodide and a boron intermediate).

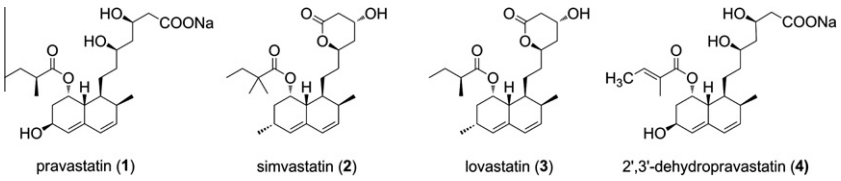
1. Introduction

Positron emission tomography (PET)[1–3] is an important molecular imaging diagnostic technique with excellent disease sensitivity, and it is routinely used to detect and assess oncologic, neurologic, and cardiologic abnormalities. This noninvasive molecular imaging technique is used to study physiology by visualizing the distribution of radiopharmaceuticals in in vivo vital systems. It is also expected that PET probes could be applicable to revolutionizing drug development by introducing a human microdose study at an early stage (phase 0) to efficiently suppress the large drop-out (>90%) of drug candidates during clinical trials (phases I–III).

One of the major functions of the liver is to remove various endogenous and exogenous compounds from the blood circulation. This clearance process involves the uptake of compounds across the sinusoidal membrane of the hepatocyte and the efflux across the bile canalicular membrane; therefore, research of how a transporter functions in the liver is important for drug development[4] as well as for diagnoses of hepatic diseases associated with a particular transporter dysfunction.

Among various drug transporters, we focused on organic anion-transporting polypeptides (OATPs)[5,6] and multidrug resistance-associated protein 2 (MRP2),[7] which play roles in the uptake of drugs into the liver and the canalicular efflux, respectively.[8]

Pravastatin (**1**),[9] a member of the statin class of drugs, has been used widely to lower the cholesterol concentration in blood by inhibiting the action of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which plays a central role in the production of cholesterol in the liver. In humans, the uptake of pravastatin into the liver is mediated by the OATP1B1 transporter,[10–12] and the drug is then excreted into the bile by MRP2 without metabolism.[13] Therefore, pravastatin would be a good molecular probe for PET to track and evaluate the functions of these two transporters in vivo.



A comparison of the structures of simvastatin (**2**)[14] and lovastatin (**3**),[15] both belonging to the statin class of drugs, suggests that the chiral center at C(2′) and the number of methyl groups in the

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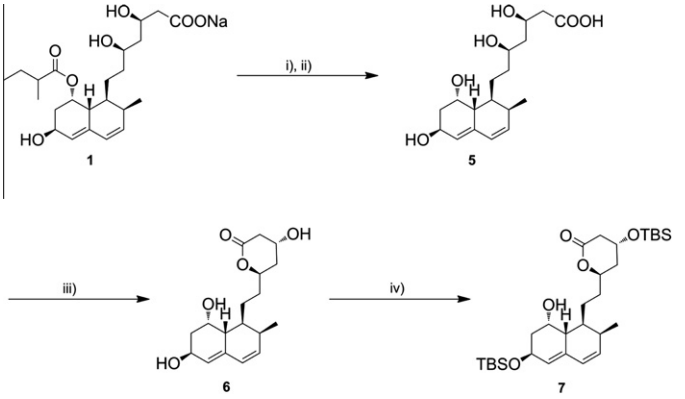
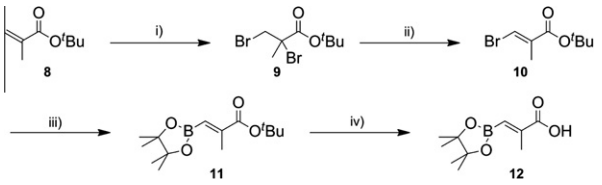


Scheme 1. Synthesis of the alcoholic intermediate **7**. (i) NaOMe, MeOH, reflux, 60 h; (ii) H_2O, HCl, 0 °C; (iii) $(EtO)_2P(O)CN$, Et_3N, THF, 1.5 h, 0 °C to room temperature; (iv) TBDMSCl, imidazole, DMF, 2 h, 0 °C to room temperature, overall yield 32%.

ester moiety of the statin structure do not have much influence on the efficacies of these drugs. Therefore, we instead chose dehydropravastatin (**4**) because it lacks a chiral center at C(2′), thus making it a suitable candidate for the radiolabeling in terms of both its easier synthetic accessibility compared with that of pravastatin and it being an adequate target for our new synthetic methodology to synthesize a ^{11}C-labeled PET molecular probe.

Short-lived radionuclides such as ^{11}C and ^{18}F, with physical half-lives of 20.4 and 109.8 min, respectively, are usually used for radiolabeling probes for PET. The carbon atom is an essential element in organic molecules such as drugs, and therefore, ^{11}C labeling is the most important method for the synthesis of a PET probe. In this context, we have developed various types of palladium(0)-mediated rapid *C*-[^{11}C]methylations onto organic frameworks, based on the cross-coupling reaction of methyl iodide and the corresponding organostannanes or organoboron compounds.

The labeling of a highly functionalized complex molecule is a fascinating and challenging subject of our rapid *C*-[^{11}C]methylation reaction, and its realization proves the high potentiality of the new methodology. We describe herein the synthesis of [^{11}C]2′,3′-dehydropravastatin, which was well executed by our rapid *C*-[^{11}C]



Scheme 2. Synthesis of the boronic acid unit 12. (i) Bromine, CCl_4, 0 °C to room temperature, 16 h, quant.; (ii) DBU, THF, 0 °C to room temperature, 16 h, 85%; (iii) $(BPin)_2$, $PdCl_2(dppf)\cdot CH_2Cl_2$, KOAc, DMSO, 80 °C, 18 h, 69%; iv) TFA, CH_2Cl_2, room temperature, 4 h, 59%, recovery 14%.

methylation reaction between $sp^2_{(vinyl)}$-sp^3 carbons using [^{11}C]methyl iodide and an organoboron precursor in the ^{11}C-labeling step.

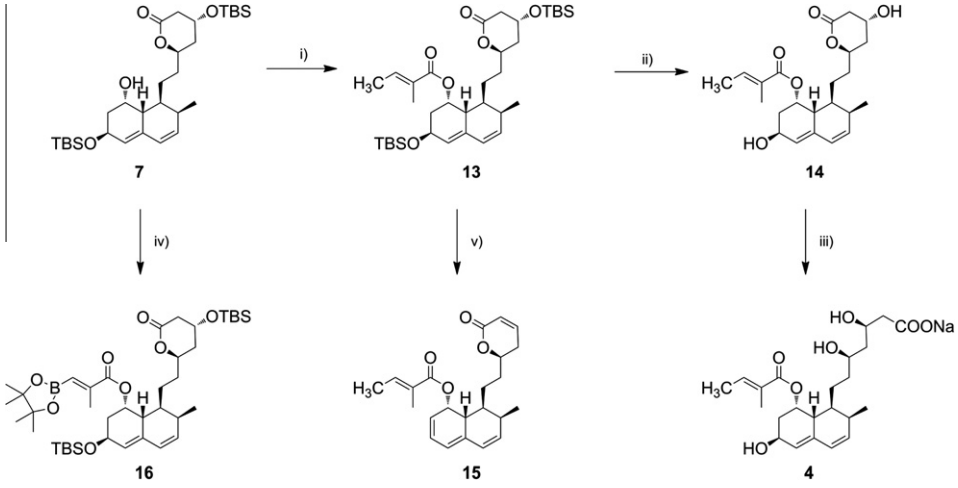
2. Results and discussion

2.1. PET probe synthesis

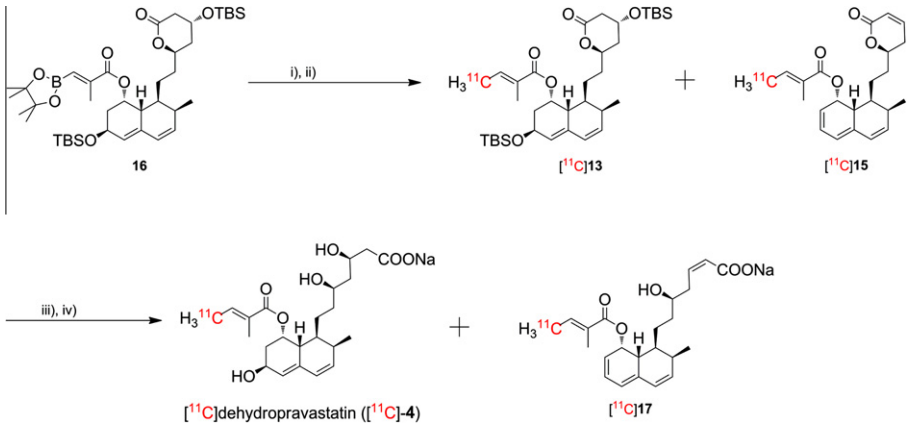
The syntheses of dehydropravastatin (**4**) and radioactive [^{11}C]dehydropravastatin ([^{11}C]**4**) were performed via the deconstruction–reconstruction of an ester moiety, as shown in Schemes 1–4. In Scheme 1, pravastatin sodium salt undergoes methanolysis with NaOMe to remove an ester moiety, and this is followed by lactonization using diethyl cyanophosphonate treatment in the presence of triethylamine and the protection of hydroxy groups by a *tert*-butyldimethylsilyl (TBS) group to render the alcoholic intermediate **7**.[16]

As shown in Scheme 2, the acid unit was synthesized as follows. Commercially available *tert*-butyl methacrylate (**8**) was dibrominated with bromine in CCl_4, at 0 °C to room temperature, to give intermediate **9** quantitatively, which was then debrominated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at 0 °C in tetrahydrofuran (THF), giving vinylic bromide (**10**) in 85% yield.[17,18] The vinyl bromide (**10**) was subsequently converted to 3-pinacol boronated methacrylate (**11**) in 69% yield, which underwent de-esterification with trifluoroacetic acid to give the desired boronated acid (**12**) in 59% yield.[19]

The reconstruction of the whole dehydropravastatin structure was conducted as follows. The alcohol **7** was esterified with commercially available tiglic acid using diethyl chlorophosphate and



Scheme 3. Synthesis of 2′,3′-dehydropravastatin (**4**) and boronic ester precursor (**16**) for [^{11}C]dehydropravastatin formation. (i) Tiglic acid, $(EtO)_2P(O)Cl$, 4-pyrrolidinopyridine, Et_3N, benzene, 0 °C for 30 min, then canulation to substrate, reflux, 2 h, 86%; (ii) TBAF, AcOH, THF, 0 °C to room temperature (rt), 90%; (iii) 0.1 M NaOH, H_2O, 1,4-dioxane, room temperature, 15 min, quant.; (iv) 12, $(EtO)_2P(O)Cl$, 4-pyrrolidinopyridine, Et_3N, benzene, 0 °C to reflux, 2 h, 86%; (v) TBAF, THF, room temperature.



Scheme 4. Synthesis of [^{11}C]dehydropravastatin ([^{11}C]-**4**). (i) $^{11}CO_2$, LAH, HI; (ii) $Pd_2(dba)_3$, P(*o*-toly)$_3$, K_2CO_3, THF, 65 °C, 5 min; (iii) TBAF, 60 °C, 2 min; iv) 0.1 M NaOH, room temperature, 1 min.

triethylamine to give the ester **13** in 86% yield. The reaction gave a 7:3 mixture of (*E*), (*Z*) isomers, which were treated by normal-phase high-performance liquid chromatography (HPLC) to give the pure (*E*) isomer **13**.[20] The resulting isomer **13** was deprotected by treatment with tetrabutylammonium fluoride (TBAF) in the presence of a large amount of acetic acid in order to neutralize the basicity of the tetrabutylammonium ion to give compound **14** in 90% yield.[21] Without acetic acid, the desired compound **14** was not obtained; instead, an undesired aromatic compound **15** was produced via dehydrogenation. The deprotected lactone structure in compound **14** was opened by sodium hydroxide to give dehydropravastatin (**4**) in quantitative yield.

It is expected that dehydropravastatin sodium (**4**) would be a good substrate for the OATP transporter, similar to natural pravastatin sodium (the detailed in vitro and in vivo data will be shown in a separate biological paper). With the consideration of such potential biological information in mind, we continued to label the dehydropravastatin (**4**) with a ^{11}C radionuclide.

The alcoholic intermediate **7** was reacted with the boronated acrylic acid **12** under the same conditions as the synthesis of compound **13**, giving boronic ester **16**, a precursor for ^{11}C labeling. Since compound **16** was very unstable under TBAF conditions, we selected it as a substrate for the ^{11}C-labeling reaction without TBS group deprotection.

Thus, boronated ester **16** was reacted with [^{11}C]methyl iodide in the presence of $Pd_2(dba)_3$, P(*o*-toly)$_3$, and K_2CO_3 (in 1:3:4 molar ratio) at 65 °C for 5 min in THF to give a mixture of the desired ^{11}C-incorporated **13** ([^{11}C]**13**) and the further dehydroxylated (undesired) [^{11}C]**15**, which were detected by HPLC using a UV and radio detector.[22,23] Successive treatments of the resulting mixture with a solution of TBAF in THF, and then with an aqueous NaOH solution, gave a mixture that included the desired [^{11}C]**4**. It should be noted that it was difficult to deprotect silyl groups using TBAF in dimethylformamide (DMF). Such deprotection in THF gave the desired [^{11}C]**4**, in addition to another radioactive

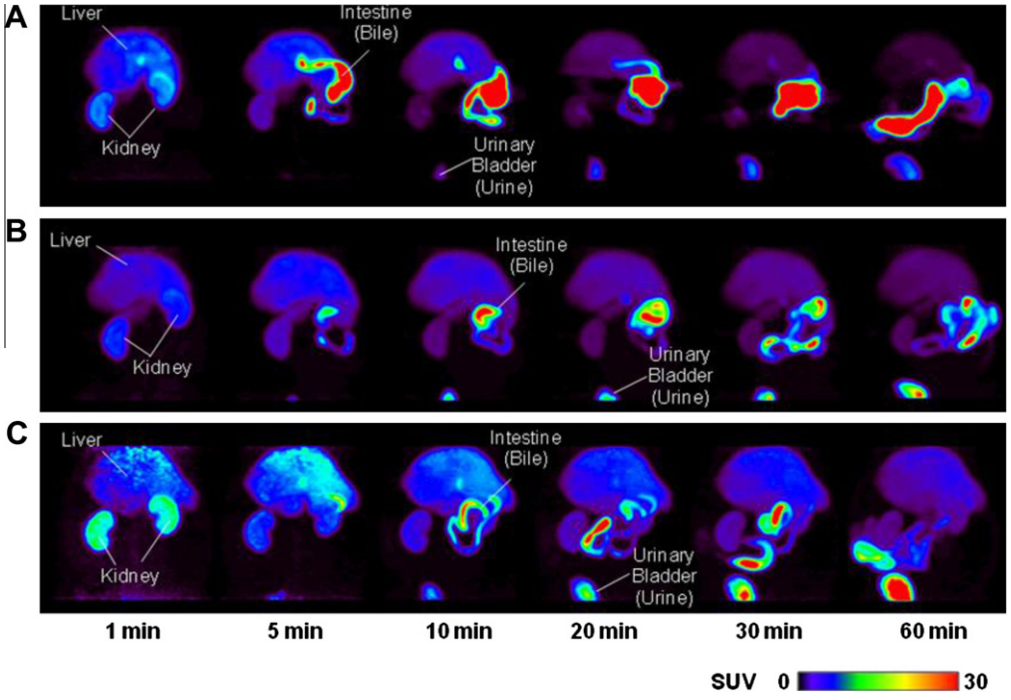


Figure 1. Color-coded PET images of the abdominal regions of rats after administration of [^{11}C]**4**. Coronal maximum intensity projection PET images of radioactivity in the abdominal region were captured at 1, 5, 10, 20, 30, and 60 min in control rats (A), in rats treated with rifampicin at an infusion rate of 1.5 μmol min^{-1} kg^{-1} (B), and in Mrp2 hereditary-deficient rats (C) after intravenous administration of [^{11}C]**4**.

byproduct that was identified by liquid chromatography—mass spectrometry to be the dehydrated structure [^{11}C]**17**.

2.2. PET study of [^{11}C] 2′,3′-dehydropravastatin ([^{11}C]4)

PET images of the radioactivity in the abdominal region over time, following administration of [^{11}C]**4** (22 ± 1 MBq/body) to rats, are shown in Figure 1. Radioactivity was identifiable in the liver and kidneys within 2 min after the radiotracer administration, at which point the radioactivity began to decrease rapidly. On the other hand, the radioactivity was localized mainly in the intestine (via the bile excreted into the intestine), and some radioactivity was also observed in the urinary bladder (via the urine excreted into the urinary bladder) by 60 min (Fig. 1a). The radioactivity excreted into the bile or the urine increased until the end of the scan and reached 67.9 ± 8.9% and 1.51 ± 0.33% of the original dose, respectively. When rifampicin, a typical OATP inhibitor, was co-administered with [^{11}C]**4**, the distribution of the radioactivity in the liver and that excreted into the bile was decreased (Fig. 1b). In addition, the total radioactivity in the bile of Mrp2 hereditary-deficient rats was reduced as compared with that in control animals (Fig. 1c). The radiometabolite of [^{11}C]**4** was detected in the blood, liver, and bile sampled within 20 min after radiotracer administration, and its composition was much lower than that of [^{11}C]**4** in blood sampled at 2 min. [^{11}C]**4** formed the major portion of the radioactivity in the blood, liver, and bile sampled at 20 min, but the existence of its radiometabolite was not negligible in rats. In bile, at least four metabolites were detected, three of which were polar than unchanged compound **4**. LC/MS/MS analysis revealed that ions with a *m*/*z* of 437.3 (+16 Da) and a *m*/*z* of 455.3 (+34 Da) were found in the extracts of bile sampled after IV injection of compound **4**. It is speculated that a *m*/*z* 437.3 may be an oxidative form of compound **4**, and a *m*/*z* 455.3 may be a dihydrodiol form in the alkene position of compound **4**, However, the identification of these metabolites was not pursued further as it accounted for a very small proportion of the dose. In addition, in vitro metabolism using the hepatocyte suspension system has shown that the metabolic stability in human hepatocytes is much higher than that in rat hepatocytes (data not shown). Thus, [^{11}C]**4** has great potential to be a suitable PET probe to evaluate the functions of OATPs (ideally OATP1B1) and MRP2 in hepatobiliary excretion. Further investigation using [^{11}C]**4** for evaluating the functions of these drug transporters in rats and in vitro transport study is ongoing.

3. Conclusion

In conclusion, 2′,3′-dehydropravastatin (**4**) and radiolabeled [^{11}C]**4** were designed and synthesized using palladium(0)-mediated *C*-methylation and rapid *C*-[^{11}C]methylation, respectively. In radiosynthesis, the cross-coupling reaction and deprotection of the TBS group as well as hydrolysis were carried out in a single container so that the loss of intermediates in the transfer process could be minimized. The physicochemical properties of [^{11}C]**4** were as follows: (1) total radioactivity, 1.2 GBq; (2) radio purity, >99%; chemical purity, 98%; (3) radio chemical yield, 64%; decay correct yield, 19.7%; specific radioactivity, 79 GBq/μmol. The total synthesis time was 34 ± 2 min. The application of [^{11}C]**4** to PET studies on animals and humans will be reported in due course.

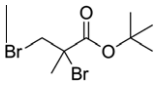
4. Experimental section

4.1. General

All manipulations were carried out under an argon atmosphere unless otherwise noted. Argon gas was dried by passage through P_2O_5 (Merck, SICAPENT). NMR spectra were recorded on a JEOL AL-400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C). Chemical shifts are reported in δ parts per million referenced to an internal tetramethylsilane standard for ^{1}H NMR. Chloroform-d_1 (δ 77.0 for ^{13}C) was used as an internal reference for ^{13}C NMR. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD. Mass spectra were recorded on a Thermofisher Scientific LCQ Fleet mass spectrometer. High resolution mass spectra were recorded on a JEOL JMS-T100LC mass spectrometer. Optical rotation was recorded on a JASCO P-2100 polarimeter. Melting point was measured by an AS-ONE ATM-01 melting point apparatus.

The [^{11}C]methylation was conducted in a lead-shielded hot cell with remote control of all operations. [^{11}C]Carbon dioxide was produced by a ^{14}N(p,α)^{11}C reaction using a CYPRIS HM-12S cyclotron (Sumitomo Heavy Industries) and then converted into [^{11}C]methyl iodide by treatment with lithium aluminum hydride followed by hydriodic acid using an original automated synthesis system for ^{11}C-labeling in RIKEN Center for Molecular Imaging Science. The obtained [^{11}C]methyl iodide was used for the palladium(0)-mediated rapid [^{11}C]methylation. The analytical HPLC system used for the [^{11}C]methylated product consisted of an Aloka radioanalyzer (RLC-700) and a Shimadzu HPLC system with a system controller (CBM-20A), an online degasser (DGU-20A$_3$), a solvent delivery unit (LC-20AB), a column oven (CTO-20AC), a photodiode array detector (SPD-M20A), and software (LC-Solution). The columns used for analytical and semipreparative HPLC were COSMOSIL C_{18} MS-II 4.6 × 100 mm and 10 × 250 mm (Nacalai Tesque). The radioactivity was quantified with an ATOMLAB™ 300 dose calibrator (Aloka).

4.1.2. Synthesis of tert-butyl 2,3-dibromo-2-methylpropanoate (9)



A solution of *tert*-butyl methacrylate (8.15 mL, 50 mmol) in CCl_4 was cooled to 0 °C and added 2.65 mL of bromine (51 mmol) and stirred for 16 h at room temperature. The reaction mixture was then quenched with saturated sodium bisulfate and diluted with ether. The organic phase was extracted with ether three times and dried over Na_2SO_4. Combined organic layer was concentrated under reduced pressure to afford 15 g (quant. as light yellow oil) of desired compound **9**.

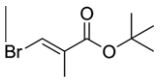
^{1}H NMR ($CDCl_3$) δ 4.18(dd, J = 0.49, 9.75 Hz, 1H), 3.70 (d, J = 9.75 Hz, 1H), 1.98(d, J = 0.49 Hz, 3H), 1.51(s, 9H).

^{13}C NMR ($CDCl_3$) δ 167.0, 82.8, 56.6, 38.2, 27.2, 25.9.

Mass spectra (EI) 289 (M+4-CH_3), 287 (M+2-CH_3), 285 (M-CH_3), 231 (M+4-O^{t}Bu), 229 (M+2-O^{t}Bu), 227 (M-O^{t}Bu), 203 (M+4-O^{t}Bu-CO), 201 (M+2-O^{t}Bu-CO), 119 (M-O^{t}Bu-CO).

HRMS (EI) *m*/*z* Calcd for $C_7H_{11}O_2{}^{81}Br_2$: 288.9085. Found 288.9088 [M-CH_3], *m*/*z* Calcd for $C_7H_{11}O_2{}^{79}Br^{81}Br$: 286.9105. Found 286.9102 [M-CH_3], *m*/*z* Calcd for $C_7H_{11}O_2{}^{79}Br_2$: 284.9125. Found 284.9135 [M-CH_3].

4.1.3. Synthesis of (E)-tert-butyl 3-bromo-2-methylacrylate (10)



A solution of *tert*-butyl 2,3-dibromo-2-methylpropanoate (3.03 g, 10 mmol) in THF was cooled at to 0 °C and added DBU (1.65 mL, 11 mmol) and stirred for 16 h at room temperature. The reaction mixture was diluted with ether and then washed with water. The

organic phase was washed with 5% of citric acid three times, saturated sodium hydrogen carbonate solution twice, and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford 1.86 g (8.46 mmol, 85% as colorless oil) of desired compound **10**.

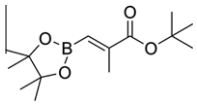
^{1}H NMR ($CDCl_3$) δ 7.41(q, J = 1.6 Hz, 1H), 1.96(d, J = 1.6 Hz, 3H), 1.49(s, 9H).

^{13}C NMR ($CDCl_3$) δ 164.0, 135.4, 121.6, 81.4, 27.9, 15.5.

Mass spectra (EI) 222 (M+2), 220 (M) 149, (M+2-O^{t}Bu), 147 (M-O^{t}Bu), 121 (M+2-O^{t}Bu-CO), 119 (M-O^{t}Bu).

HRMS (EI) m/z Calcd for $C_4H_4O_1{}^{81}Br$: 148.9425. Found 148.9423 [M-O^{t}Bu], m/z Calcd for $C_4H_4O_1{}^{79}Br$: 146.9445. Found 148.9444 [M-O^{t}Bu].

4.1.4. Synthesis of *(E)-tert*-butyl 2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)acrylate (11)



A solution of *(E)-tert*-butyl 3-bromo-2-methylacrylate (221 mg, 1 mmol) and KOAc (196 mg, 2 mmol) and bis (pinacolato) diboron (508 mg, 2 mmol) in 3.5 mL of 1,4-dioxane was added 10 mg of $PdCl_2$(dppf)$.CH_2Cl_2$, and then reaction mixture was heated to 80 °C for 16 h. The reaction mixture was diluted with water and extracted with ether 4 times. Combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated. Resulting crude was purified by SiO_2 column chromatography to afford 184 mg (68.6% as white solid) of desired compound **11**.

^{1}H NMR ($CDCl_3$) δ 6.43(q, J = 1.2 Hz, 1H), 2.11(d, J = 1.2 Hz, 3H), 1.48(s, 9H), 1.29(s, 12H).

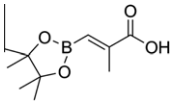
^{13}C NMR ($CDCl_3$) δ 167.9, 150.1, 84.3, 84.3, 81.3, 28.8, 28.8, 28.8, 25.7, 25.7, 25.7, 25.7, 17.8.

MS (ESI) 291 (M+Na).

HRMS (TOF) m/z Calcd for $C_{14}H_{25}B_1O_4Na_1$: 291.1746. Found 291.1743 [M+Na].

Melting point 62 °C.

4.1.5. Synthesis of *(E)*-2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)acrylic acid (12)



A solution of *(E)-tert*-butyl 2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)acrylate (268 mg, 1 mmol) in 5 mL of CH_2Cl_2 was added 0.5 mL of trifluoroacetic acid at 0 °C and then stirred at room temperature for 2 h. The reaction mixture was purified by PLC (hexane/EtOAc 1/1) to afford 126 mg (59% as light yellow powder) of desired compound **12** and 37 mg (14%) of the starting boron substrate.

^{1}H NMR (CD_3OD) δ 11.1(br, 1H), 6.40(q, J = 1.2 Hz, 1H), 2.09(d, J = 1.2 Hz, 3H), 1.29(s, 12H).

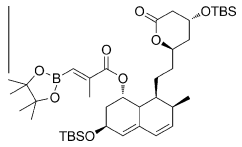
^{13}C NMR (CD_3OD) δ 171.8, 145.6, 83.3, 83.3, 24.4, 24.4, 24.4, 24.4, 16.3.

MS (ESI) 213 (M+H) [positive mode], 211(M−1) [negative mode].

HRMS (TOF) m/z Calcd for $C_{10}H_{16}B_1O_4$: 211.1143. Found 211.1150 [M−H].

Melting point 152 °C.

4.1.6. Synthesis of (*E*)-(1*S*,3*S*,7*S*,8*S*)-3-((*tert*-butyldimethylsilyl)oxy)-8-(2-((2*R*,4*R*)-4-((*tert*-butyldimethylsilyl)oxy)-6-oxotetrahydro-2H-pyran-2-yl)ethyl)-7-methyl-1,2,3,7,8,8*a*-hexahydronaphthalen-1-yl 2-methylbut-2-enoate (13)



To a solution of 89 mg (0.6 mmol) of 4-pyrrolidinopyridine in 0.6 mL of benzene was added 112 μL (0.8 mmol) of triethylamine and 40 mg (0.4 mmol) of tiglic acid at 0 °C. Then, 86 μL (0.5 mmol) of diethyl chlorophosphate was added to the resulting solution. The mixture was stirred at room temperature for 30 min. A solution of (4*R*, 6*R*)-6-{2-[(1*S*, 2*S*, 6*S*, 8*S*, 8*aR*)-1,2,6,7,8,8*a*-hexahydro-6-*tert*-butyldimethylsilyloxy-8-hydroxy-2-methyl-1-naphthyl]ethyl}tetrahydro-4-*tert*-butyldimethylsilyloxy-2H-pyran-2-one (110 mg; 0.2 mmol) dissolved in 0.2 mL of benzene was added to the reaction mixture, and the mixture was refluxed for a further 2.5 h. At the end of this time, the reaction mixture was cooled to room temperature, and diluted with ethyl acetate. The diluted mixture was washed with 5% citric acid three times, saturated sodium hydrogen carbonate two times, and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting oil was purified by silica gel column chromatography, to give 108 mg (172 μmol, 86% as colorless oil) of desired compound **13**.

^{1}H NMR ($CDCl_3$) δ 6.77(m, 1H), 5.99(d, 1H, J = 9.77 Hz), 5.85(dd, 1H, J = 5.85, 9.77 Hz), 5.49(br s, 1H), 5.41(br s, 1H), 4.57(m, 1H), 4.42(m, 1H), 4.26(m, 1H), 2.34–2.40(m, 5H), 1.26–1.85(m, 15H), 0.88(s, 18H), 0.88(s, 3H), 0.07(s, 12H).

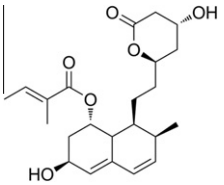
^{13}C NMR ($CDCl_3$) δ 170.7, 167.9, 137.6, 135.6, 135.0, 129.1, 128.1, 127.6, 76.5, 70.1, 66.1, 64.0, 39.7, 38.0, 37.5, 37.2, 33.3, 31.5, 18.7, 18.4, 14.9, 14.2, 12.5, −4.47.

$[\alpha]_d^{25}$ +69.6 (c = 18.9, $CHCl_3$).

MS (ESI) 655 (M+Na), 633(M+H).

HRMS (TOF) m/z Calcd for $C_{35}H_{60}O_6Si_2Na_1$: 655.3826. Found 655.3826 [M+Na].

4.1.7. Synthesis of (*E*)-(1*S*,3*S*,7*S*,8*S*)-3-hydroxy-8-(2-((2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl)ethyl)-7-methyl-1,2,3,7,8,8*a*-hexahydronaphthalen-1-yl 2-methylbut-2-enoate (14)



To a solution of 40 mg (63 μmol) (*E*)-(1*S*,3*S*,7*S*,8*S*)-3-((*tert*-butyldimethylsilyl)oxy)-8-(2-((2*R*,4*R*)-4-((*tert*-butyldimethylsilyl)oxy)-6-oxotetrahydro-2H-pyran-2-yl)ethyl)-7-methyl-1,2,3,7,8,8*a*-hexahydronaphthalen-1-yl 2-methylbut-2-enoate in 0.5 mL of THF was added 86 μL (1.5 mmol) of acetic acid and 1 mL (1 mmol) of 1.0 M tetrabuthylammonium fluoride THF solution at 0 °C. The mixture was then stirred for 14 h at room temperature. At the end of this time, the mixture was diluted with ethyl acetate, and washed with saturated sodium hydrogen carbonate two times and brine. The

organic phase was dried with Na_2SO_4 and concentrated under reduced pressure. The resulting oil was purified by silica gel column chromatography to give 23 mg (56.9 μmol, 90% as white powder) of desired compound **14**.

^{1}H NMR ($CDCl_3$) δ 6.78(m, 1H), 6.01(d, 1H, J = 9.51 Hz), 5.89(dd, 1H, J = 5.83, 9.51 Hz), 5.59(br s, 1H), 5.44(br s, 1H), 4.60(m, 1H), 4.42(m, 1H), 4.35(m, 1H), 2.57–2.75(m, 3H), 2.34–2.42(m, 2H), 1.90(m, 1H), 1.29–1.79(m, 15H), 0.91(d, 3H, J = 7.07 Hz).

^{13}C NMR ($CDCl_3$) δ 170.2, 167.5, 137.7, 135.6, 135.2, 128.4, 127.3, 126.0, 75.8, 69.3, 64.9, 62.5, 30.2, 38.4, 37.6, 36.7, 36.5, 35.8, 32.5, 30.9, 23.4, 14.3, 13.5, 11.9.

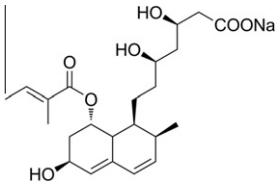
$[\alpha]_d^{25}$ +266.4 (c = 0.50, $CHCl_3$).

MS (ESI) 427 (M+Na), 831(2 M+Na).

HRMS (TOF) m/z Calcd for $C_{23}H_{32}O_6Na_1$: 427.2097. Found 427.2105 [M+Na].

Melting point 156 °C.

4.1.8. Synthesis of sodium (3*R*,5*R*)-3,5-dihydroxy-7-((1*S*,2*S*,6*S*,8*S*)-6-hydroxy-2-methyl-8-(((*E*)-2-methylbut-2-enoyl)oxy)-1,2,6,7,8,8*a*-hexahydronaphthalen-1-yl)heptanoate (4)



To a solution of 23 mg (56.9 μmol) (*E*)-(1*S*,3*S*,7*S*,8*S*)-3-hydroxy-8-(2-((2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl)ethyl)-7-methyl-1,2,3,7,8,8*a*-hexahydronaphthalen-1-yl 2-methylbut-2-enoate in 1 mL of 1,4-dioxane and 480 μL of water was added 569 μL (56.9 μmol) of 0.1 N NaOH solution. The mixture was stirred for 30 min at room temperature. At the end of this time, reaction mixture was freeze dried to give 27 mg of white powder. The powder was purified by HPLC giving 23 mg of pure compound **4**.

^{1}H NMR (CD_3OD) δ 6.77(m, 1H), 5.99(d, 1H, J = 9.51 Hz), 5.88(m, 1H), 5.51(br s, 1H), 5.38(br s, 1H), 4.26(m, 1H), 4.05(m, 1H), 3.65(br s, 1H), 2.20–2.53(m, 6H), 1.78(s, 3H), 1.77(s, 3H), 1.22–1.70(m, 7H), 0.91(d, 3H, J = 7.1 Hz).

^{13}C NMR (CD_3OD) δ 180.4, 169.1, 138.7, 136.8, 136.7, 130.0, 128.6, 72.3, 71.3, 69.4, 68.1, 65.5, 45.5, 44.9, 39.0, 38.5, 37.1, 35.4, 32.4, 25.0, 14.4, 14.0, 12.1.

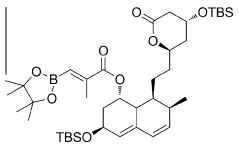
$[\alpha]_d^{25}$ +131.3 (c = 0.44, EtOH).

MS (ESI) 421 (M-Na) [negative mode].

HRMS (TOF) m/z Calcd for $C_{23}H_{33}O_7$: 421.2226. Found 421.2221 [M-Na].

Melting point 134 °C.

4.1.9. Synthesis of (*E*)-(1*S*,3*S*,7*S*,8*S*)-3-((*tert*-butyldimethylsilyl)oxy)-8-(2-((2*R*,4*R*)-4-((*tert*-butyldimethylsilyl)oxy)-6-oxotetrahydro-2H-pyran-2-yl)ethyl)-7-methyl-1,2,3,7,8,8*a*-hexahydronaphthalen-1-yl 2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)acrylate (16)



A solution of (*E*)-2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)acrylic acid (121 mg; 0.57 mmol) and 4-pyrrolidinopyridine (134 mg; 0.9 mmol) and Et_3N (168 μL; 1.2 mmol) in 0.8 mL of benzene was added diethyl chlorophosphate (130 μL; 0.75 mmol) at 0 °C for 30 min. The reaction mixture was added to the solution of (4*R*, 6*R*)-6-{2-[(1*S*, 2*S*, 6*S*, 8*S*, 8*aR*)-1,2,6,7,8,8*a*-hexahydro-6-*tert*-butyldimethylsilyloxy-8-hydroxy-2-methyl-1-naphthyl]ethyl}tetrahydro-4-*tert*-butyldimethylsilyloxy-2H-pyran-2-one (165 mg; 0.3 mmol) was added to the reaction mixture, and then the mixture was refluxed for a further 2.5 h. At the end of this time, the reaction mixture was cooled to room temperature, and then diluted with ethyl acetate. The diluted mixture was washed with 5% citric acid three times, saturated sodium hydrogen carbonate two times, and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting oil was purified by silica gel column chromatography to give 192 mg (86.0% as light yellow solid) of desired compound **16**.

^{1}H NMR ($CDCl_3$) δ 6.41(s, 1H), 5.97(d, 1H, J = 9.76 Hz), 5.84(dd, 1H, J = 5.85, 9.76 Hz), 5.47(br s, 1H), 5.41(br s, 1H), 4.56(m, 1H), 4.41(m, 1H), 4.26(m, 1H), 2.35–2.57(m, 5H), 2.17(s, 2H), 2.12(s, 3H), 1.85–1.64(m, 4H), 1.30–1.26(m, 15H), 0.88(s, 18H), 0.88(s, 3H), 0.06(s, 12H).

^{13}C NMR ($CDCl_3$) δ 170.3, 167.2, 147.3, 135.1, 134.4, 127.7, 127.7, 127.2, 83.7, 83.7, 76.3, 70.5, 65.5, 63.5, 39.3, 37.6, 36.9, 36.7, 36.6, 32.9, 31.0, 25.9, 25.9, 25.9, 25.9, 25.7, 25.7, 25.7, 24.8, 24.8, 23.6, 18.2, 18.0, 17.1, 13.7, −4.57, −4.66, −4.87, −4.91.

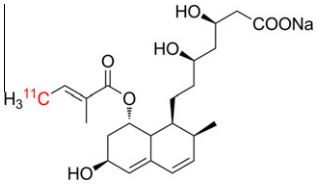
$[\alpha]_d^{25}$ +93.0 (c = 0.60, $CHCl_3$).

MS (ESI) 744(M+H), 767 (M+Na)

HRMS (TOF) m/z Calcd for $C_{40}H_{69}BO_8Si_2Na$: 767.4529. Found 767.4526 [M+Na].

Melting point 63 °C.

4.1.10. Radiosynthesis of sodium (3*R*,5*R*)-3,5-dihydroxy-7-((1*S*,2*S*,6*S*,8*S*)-6-hydroxy-2-methyl-8-((1-[^{11}C]-(*E*)-2-methylbut-2-enoyl)oxy)-1,2,6,7,8,8*a*-hexahydronaphthalen-1-yl)heptanoate [^{11}C]-4



[^{11}C]Methyl iodide formed from [^{11}C]CO_2 according to the established method[24] was trapped in a solution of (*E*)-(1*S*,3*S*,7*S*,8*S*)-3-((*tert*-butyldimethylsilyl)oxy)-8-(2-((2*R*,4*R*)-4-((*tert*-butyldimethylsilyl)oxy)-6-oxotetrahydro-2H-pyran-2-yl)ethyl)-7-methyl-1,2,3,7,8,8*a*-hexahydronaphthalen-1-yl 2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)acrylate (2.0 mg; 2.7 μmol), $Pd_2(dba)_3$ (2.0 mg; 2.2 μmol), P(o-toly)$_3$ (2.0 mg; 6.6 μmol), and K_2CO_3 (1.2 mg; 8.7 μmol) in THF (300 μL) at room temperature. The resulting mixture was heated to 65 °C for 5 min. The reaction mixture was cooled to 0 °C. Then 200 μL of 1 M TBAF solution was added to the reaction mixture and heated to 60 °C for 2 min. The reaction vessel was cooled by air to room temperature and 400 μL of 0.1 M NaOH solution was added, and then placed 1 min. After dilution with 400 μL of 10 mM NH_4OAc, the mixture was injected onto a semipreparative HPLC column with a mobile phase consisting of 10 mM NH_4OAc and CH_3CN (70:30) under the flow rate of 5 mL/min with equipped UV detection at 230 nm. The [^{11}C]**4** retention time was 12.5 min. The desired fraction was collected in a flask and the organic solvent was removed under reduced pressure.

For the radio pharmaceutical formulation of [^{11}C]**4** for use in a PET study, [^{11}C]**4** was dissolved with a 4 mL of saline. The total synthesis time from end of bombardment (EOB) was 34 min. The decay-corrected radiochemical yield was 19.7% (n = 3) with specific radioactivity (SA) of 79 GBq/μmol (n = 3). We assigned [^{11}C]**4** by

LC-MS using the remaining [^{12}C]**4**. Observed peak was 421 (M-Na) in negative mode.

4.2. Imaging

4.2.1. Experimental animals

Male Sprague-Dawley (SD) rats weighing 180–242 g and male Mrp2 hereditary-deficient Eisai hyperbilirubinemic rats (EHBRs) weighing 287 g were purchased from Japan SLC Inc. 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).

4.2.2. 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]**4** (approximately 22 ± 1 MBq per animal) via a venous catheter inserted into the tail vein, a 60 min emission scan was performed. The chemical amount of [^{11}C]**4** for the bolus injection was calculated as 1.0 ± 0.8 nmol/body (0.45 ± 0.37 μg/body). Emission data were acquired in list mode, and the data were reconstructed with standard 2D filtered back projection (Ramp filter, cutoff frequency at 0.5 cycles per pixel). Region of interests (ROIs) were placed on liver, intestine, kidney, or urinary bladder using image processing software (Pmod ver.3.3, PMOD Technologies Ltd, Zurich, Switzerland). Regional uptake of radioactivity in the tissue and blood radioactivity was decay-corrected to the injection time and expressed as % dose/tissue or % dose/mL blood, normalized for injected radioactivity. For the functional analysis of Oatp in hepatic uptake, rifampicin, a typical Oatp inhibitor, was intravenously infused at a rate of 1.5 μmol/min/kg for at least 90 min before the administration of [^{11}C]**4**, and then a constant infusion was kept until the end of the PET scan.

Radiometabolite analysis of rat blood, liver, and bile was performed separately from the PET study to evaluate the composition of the radioactivity in the tissue. Equal volumes of acetonitrile were added to an aliquot of the samples (blood, liver homogenate, and bile) and then the resulting mixture was centrifuged at 12,000 rpm for 2 min at 4 °C. The supernatant was diluted with HPLC mobile phase, and then analyzed for radioactive components by using an HPLC system (Shimadzu Corporation) with a coupled NaI(Tl) positron detector UG-SCA30 (Universal Giken, Kanagawa, Japan) to measure intact radiotracer and its metabolites. Chromatographic separation was carried out using a Waters Atlantis T3 column (4.6(i.d.) × 50 mm; Waters, Milford, MA). The flow was 2.0 mL/min at initial conditions of 80% solvent A (5% acetonitrile in 10 mM ammonium acetate) and 20% solvent B (90% acetonitrile in 10 mM ammonium acetate). Analytes were eluted using the following gradient conditions: 0–0.5 min: 0% solvent B in solvent A; 0.5–2.5 min: 0–100% solvent B in solvent A; 2.5–4.5 min: 100% solvent B. Following the elution, the column was returned to 0% solvent B in solvent A over 2 min. The elution was monitored by UV absorbance at 254 nm and coupled NaI positron detection. The amount of radioactivity associated with intact radiotracer and its metabolite was calculated as a percentage of the total amount radioactivity.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.04.051.

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20. The mixture of (*E*), (*Z*) isomers was injected onto a recycle HPLC with a mobile phase consisting of hexane and ethyl acetate (80:20) under the flow rate of 5 mL/min with equipped UV detection at 230 nm. The column used for semipreparative HPLC was COSMOSIL SL-II 10 × 250 mm (Nacalai Tesque).
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