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Efficient syntheses of [^{11}C]zidovudine and its analogs by convenient one-pot palladium(0)–copper(I) co-mediated rapid *C*-[^{11}C]methylation

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The nucleosides zidovudine (AZT), stavudine (d4T), and telbivudine (LdT) are approved for use in the treatment of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) infections. To promote positron emission tomography (PET) imaging studies on their pharmacokinetics, pharmacodynamics, and applications in cancer diagnosis, a convenient one-pot method for Pd(0)–Cu(I) co-mediated rapid *C–C* coupling of [^{11}C]methyl iodide with stannyl precursor was successfully established and applied to synthesize the PET tracers [^{11}C]zidovudine, [^{11}C]stavudine, and [^{11}C]telbivudine. After HPLC purification and radiopharmaceutical formulation, the desired PET tracers were obtained with high radioactivity (6.4–7.0 GBq) and specific radioactivity (74–147 GBq/μmol) and with high chemical (>99%) and radiochemical (>99.5%) purities. This one-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation also worked well for syntheses of [methyl-^{11}C]thymidine and [methyl-^{11}C]4′-thiothymidine, resulting twice the radioactivity of those prepared by a previous two-pot method. The mechanism of one-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation was also discussed.

Keywords: [^{11}C]Zidovudine ([^{11}C]AZT); [^{11}C]Telbivudine ([^{11}C]LdT); [^{11}C]Stavudine ([^{11}C]d4T); [Methyl-^{11}C]thymidine; [Methyl-^{11}C]4′-thiothymidine ([^{11}C]4DST); Rapid *C*-[^{11}C]methylation

Introduction

Positron emission tomography (PET) has been used for molecular imaging studies in disease diagnosis and as a powerful, non-invasive tool for pharmacokinetics and pharmacodynamics investigations *in vivo*.[1–3] To this end, the PET tracers 2-deoxy-2-[^{18}F]fluoro-*D*-glucose ([^{18}F]FDG) and [^{18}F]fluorothymidine ([^{18}F]FLT) have been developed and used in the clinic for early cancer diagnosis.[1] Some PET tracers derived from therapeutic agents such as [^{11}C]verapamil,[4] [^{11}C]topotecan,[5] [^{18}F]fluoroestradiol,[6] and [^{11}C]ketoprofen methyl ester[7] have also been synthesized and used for PET imaging studies on pharmacokinetics, drug resistance,[4,5] and cancer characteristics,[5,6] as well as on neuroinflammation.[8] These studies could help physicians select the therapy and therapeutic agent best suited for the patient as soon as possible, resulting in great improvements in disease treatment.[1,6,9] It is probable that PET imaging studies on HIV and HBV infections and on anti-HIV/anti-HBV agents could promote the development of personalized therapies to enhance the quality of life for patients with HIV and HBV infections. However, such PET imaging studies on HIV and HBV infections as well as on anti-HIV and anti-HBV agents are still very limited because of the lack of a suitable PET tracer.[10,11]

The synthetic thymidine nucleoside analogs zidovudine (AZT), stavudine (d4T),[12] and telbivudine (LdT)[13] have been approved for use in the treatment of HIV and HBV infections. These antiviral agents can be phosphorylated by cellular kinases to triphosphate derivatives, resulting in the interruption of HIV or HBV replication by the inhibition of both HIV or HBV reverse transcriptase and viral DNA synthesis.[12–14] Similarly, it was found that AZT could also be incorporated into the DNA of cancer cells to terminate the DNA chain, suppressing cancer growth.[15] In order to promote PET imaging studies on these anti-HIV and anti-HBV agents, we present synthesis methods for [^{11}C]zidovudine ([^{11}C]AZT), [^{11}C]stavudine ([^{11}C]d4T), and [^{11}C]telbivudine ([^{11}C]LdT), (Figure 1).

Recently, for syntheses of ^{11}C-labeled PET tracers, four types of rapid *C*-[^{11}C]methylation methods (sp–sp^3, sp^2(alkenyl)–sp^3, sp^2(arenyl)–sp^3, and sp^3–sp^3 couplings) were developed, using the Pd(0)-mediated rapid reaction of [^{11}C]CH_3I with organoboron or organostannyl precursors.[16–22] When organostannyl compounds

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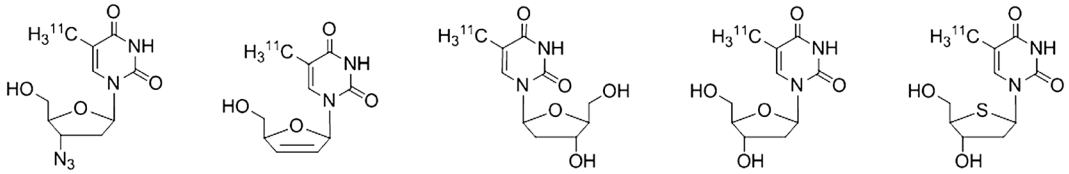

Figure 1. Structures of PET tracers [^{11}C]zidovudine and its analogs.

were used as precursors, copper(I) salt was used in combination to activate the precursors. A one-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation method has been attempted previously, but the method demonstrated a low yield and low reproducibility for PET tracer synthesis.[18,20] To overcome this problem, a two-pot process was required until now.[20,21] In the two-pot process, [^{11}C]CH_3I was trapped in the first reactor containing a solution of $Pd_2(dba)_3$ and P(*o*-tolyl)$_3$ at room temperature, and then the resulting {[^{11}C]CH_3PdI} complex was transferred to the second reactor containing an organostannyl precursor and copper(I) salt for a *C*-[^{11}C] methylation reaction to generate the desired PET tracer [^{11}C]CH_3-R. Recently, we also improved syntheses for the PET tracers [methyl-^{11}C]thymidine ([^{11}C]Thy) and [methyl-^{11}C]4′-thiothymidine ([^{11}C]4DST) by two-pot Pd(0)–Cu(I) co-mediated rapid coupling of [^{11}C]CH_3I with 5-tributylstannyl-2′-deoxyuridine and 5-tributylstannyl- 4′-thio-2′-deoxyuridine, respectively.[23] However, for two-pot Pd(0)–Cu(I) co-mediated *C*-[^{11}C]methylation, modifications of the PET tracer synthesizers are required; therefore, it was not easy to translate it into a general method for clinical use. Thus, it is very important to establish a much more practical one-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation method. Herein, we reported a convenient and temperature-controlled one-pot Pd(0)–Cu(I) co-mediated *C*-[^{11}C]methylation process for the syntheses of [^{11}C]AZT and its analogs.

Materials and methods

General remarks

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, Tokyo, Japan) and then converted into [^{11}C]CH_3I by $LiAlH_4$ reduction followed HI treatment, using an original automated synthesis system for ^{11}C-labeling in RIKEN CLST.[22,23] The obtained [^{11}C]CH_3I was used for the Pd(0)–Cu(I) co-mediated rapid [^{11}C]methylation. The analytical HPLC system used for the [^{11}C]methylated products, consisted of a Shimadzu HPLC system with a system controller (CBM-20A), an online degasser (DGU-20A3), a solvent delivery unit (LC-20AD), a column oven (CTO-20AC), a photodiode array detector (SPD-M20A), an Aloka radioanalyzer (RLC-700), and software (LC-Solution). The radioactivity was quantified with an ATOMLAB™ 300 dose calibrator (Aloka, Tokyo, Japan).

Materials

Tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$) and tri (*o*-tolyl)phosphine (P(*o*-tolyl)$_3$) were purchased from Sigma-Aldrich (Tokyo, Japan). Tetrakis(triphenylphoshine)palladium(0) (Pd(PPh_3)$_4$) was obtained from Strem Chemicals (Newburyport, MA, USA). 2′-Deoxy-*L*-uridine was brought from Ark Pharm (Libertyville, IL, USA). Other chemicals and solvents were purchased from Wako Pure Chemical Industries (Osaka, Japan), Tokyo Kasei Kogyo (Tokyo, Japan), and Nacala Tesque (Kyoto, Japan).

Synthesis of 5′-*O*-(*tert*-butyldimethylsilyl)-2,3′-anhydro-2′-deoxy-5-iodouridine (3)

5-Iodo-2′-deoxyuridine (**1**) was selectively protected by *tert*-butyldimethylsilyl group (TBS) to give 5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-5-iodouridine (**2**).[24] To a solution of compound **2** (1.81 g, 3.88 mmol) and triphenylphosphine (2.03 g, 7.76 mmol) in DMF (15 mL) diisopropyl azodicarboxylate (DIAD), (1.56 mL, 7.76 mmol) was added dropwise. The reaction mixture was kept stirring at room temperature overnight, then quenched with aq. NH_4Cl solution and extracted with EtOAc. After drying over Na_2SO_4, the solvent was removed under vacuum. The crude was purified by silica gel column chromatography (EtOAc) to give product **3** as a white solid (1.6 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.07 (s, 3H), 0.87 (s, 9H), 2.44–2.49 (m, 1H), 2.67–2.70 (m, 1H), 3.71–3.86 (m, 2H), 4.11–4.31 (m, 1H), 5.23 (s, 1H), 5.53 (d, J = 4.0 Hz, 1H), 7.56 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.46, 18.25, 25.78, 33.48, 61.01, 81.42, 85.96, 88.04, 143.92, 154.13, 167.46; HRMS (ESI) calcd for $C_{15}H_{23}IN_2O_4SiNa$ $(M+Na)^+$ 473.0369, found 473.0363.

Synthesis of 5′-*O*-(*tert*-butyldimethylsilyl)-3′-azido-3′-deoxy-5-iodouridine (4)

A mixture of **3** (900 mg, 2 mmol) and sodium azide (900 mg, 13.8 mmol) in DMF (8 mL) was heated to 110 °C and kept stirring for 6 h. After cooling at room temperature, the reaction mixture was quenched with aq. NH_4Cl solution and extracted with EtOAc. After drying over Na_2SO_4, the solvent was removed under vacuum. The crude was purified by flash chromatography (Hex/EtOAc = 3:1) to give product **4** as a white solid (480 mg, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.18 (s, 3H), 0.96 (s, 9H), 2.22–2.27 (m, 1H), 2.47–2.53 (m, 1H), 3.79–4.05 (m, 2H), 4.22–4.26 (m, 1H), 6.16 (t, J = 6.4 Hz, 1H), 8.05 (s, 1H), 8.28 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.22, 18.49, 26.16, 33.61, 60.77, 63.02, 68.55, 85.04, 85.48, 143.91, 149.64, 159.70; HRMS (ESI) calcd for $C_{15}H_{24}IN_5O_4SiNa$ $(M+Na)^+$ 516.0540, found 516.0530.

Synthesis of 5′-*O*-(*tert*-butyldimethylsilyl)-3′-azido-2′,3′-dideoxy-5-(tributylstannyl)-uridine (5)

A solution of **4** (250 mg, 0.5 mmol) in dry THF (3 mL) was treated with sodium hydride (20 mg, 60% in paraffin) for 10 min at RT. The reaction mixture was then cooled to −78 °C and treated with 2 equiv. of *n*-BuLi (1.63 M, 0.61 mL, 1.0 mmol) for 30 min. The resulted reaction mixture was added dropwise tributyltin chloride (0.82 mL, 3.0 mmol) and hexamethylphosphoric triamine (0.54 mL). After stirring at −78 °C for 1 h, the reaction mixture was warmed-up at room temperature and kept stirring for another 6 h. Then

the reaction solution was quenched with aq. NH_4Cl, extracted with EtOAc. The organic phase was evaporated, and the residue was purified by flash chromatography (Hex/EtOAc = 1:4) to give product **5** as a solid (223 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.10 (s, 3H), 0.88–1.64 (m, 36H), 2.25–2.28 (m, 1H), 2.45–2.50 (m, 1H), 3.69–3.99 (m, 3H), 4.21–4.22 (m, 1H), 6.06 (t, J = 6.8 Hz, 1H), 7.12 (s, 1H), 8.14 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.44, −5.32, 9.89, 13.57, 13.66, 17.50, 18.30, 25.88, 26.82, 26.93, 27.24, 27.82, 28.82, 28.92, 29.02, 37.27, 61.50, 63.22, 84.44, 85.78, 112.85, 143.10, 150.58, 165.95; HRMS (ESI) calcd for $C_{27}H_{51}N_5O_4SiSnNa$ $(M + Na)^+$ 680.2634, found 680.2640.

Synthesis of 3′-azido-2′,3′-dideoxy-5-(tributylstannyl)-uridine (AZT–Sn)

A solution of **5** (60 mg, 0.091 mmol) in THF (1 mL) was cooled to 0 °C and treated with tetra-*n*-butylammonium fluoride (TBAF) solution in THF (1 M, 1 mL). After stirring for 1 h, the reaction mixture was quenched with aq. NH_4Cl and extracted with EtOAc. The organic phase was evaporated, and the residue was purified by flash chromatography ($MeOH/CHCl_3$ = 1:20) to give product AZT–Sn as a white solid (45 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.88–1.55 (m, 27H), 2.30–2.40 (m, 1H), 2.61–2.67 (m, 1H), 3.80–3.99 (m, 3H), 4.41–4.43 (m, 1H), 5.98 (t, J = 6.8 Hz, 1H), 7.20 (s, 1H), 8.40 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 9.89, 13.68, 16.40, 27.05, 27.28, 27.84, 28.94, 37.02, 60.48, 62.36, 84.67, 87.96, 113.30, 145.09, 150.69, 165.76; HRMS (ESI) calcd for $C_{21}H_{37}N_5O_4SnNa$ $(M + Na)^+$ 566.1769, found 566.1769.

Synthesis of 5′-*O*-(*tert*-butyldimethylsilyl)-5-bromouridine (7)

A solution of 5-bromouridine (**6**), (1.95 g, 5 mmol) in DMF (12 mL) was cooled to 0 °C and treated with imidazole (1.0 g, 14.7 mmol) and *tert*-butyldimethylchlorosilane (TBSCl) (0.945 g, 6.3 mmol). Then, the reaction mixture was warmed at room temperature and stirred overnight. The reaction was quenched with aq. NH_4Cl solution and extracted with EtOAc. The organic phase was evaporated, and the residue was purified by silica chromatography ($MeOH/CHCl_3$ = 1:9) to give product **7** as a white solid (1.73 g, 66%). ^{1}H NMR (400 MHz, CD_3OD) δ: 0.19 (s, 6H), 0.98 (s, 9H), 3.84–4.15 (m, 5H), 5.93 (q, J = 1.6 Hz, 1H), 7.90 (s, 1H), 8.13 (s, 1H). ^{13}C NMR (100 MHz; CD_3OD) δ: −4.85, 19.71, 26.92, 64.43, 71.88, 76.56, 86.95, 90.45, 97.79, 141.26, 152.07, 161.73. MS (HR-ESI) calcd for $C_{15}H_{25}BrN_2O_6SiNa$ $(M + Na)^+$ 461.0544, found 461.0536.

Synthesis of 5′-*O*-(*tert*-butyldimethylsilyl)-5-bromouridine 2′,3′-*O*-thiocarbonate (8)

A solution of 1,1′-thiocarbonylimidazole (**7**), (0.63 g, 3.55 mmol) in dry CH_2Cl_2 (5 mL) was dropped into a solution of **7** (1.4 g, 3.2 mmol) in dry DMF (5 mL). The reaction mixture was stirred for 3 h at room temperature, then diluted with EtOAc (30 mL) and extracted with H_2O. The organic layer was dried over Na_2SO_4 and reduced under vacuum. The crude product was purified by flash column chromatography (EtOAc/Hex, 25 → 47%) to give product **8** as a white solid (1.33 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.09 (s, 6H), 0.89 (s, 9H), 3.84–3.94 (m, 2H), 4.60 (q, J = 4 Hz, 1H), 5.55 (q, J = 4 Hz, 1H), 5.83 (d, J = 2 Hz, 1H), 7.71 (s, 1H), 8.66 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −4.76, 18.96, 26.55, 63.32, 86.01, 88.14, 89.08, 94.48, 98.30, 140.91, 150.05, 159.49, 189.79. MS (HR-ESI) calcd $C_{16}H_{23}BrN_2O_6SSiNa$ $(M + Na)^+$ 501.0127, found 501.0128.

Synthesis of 5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-didehyro-2′,3′-dideoxy-5-bromouridine (9)

Compound **8** (0.82 g, 1.7 mmol) was treated with trimethyl phosphite (7 mL) at 110 °C for 6 h. The reaction mixture was concentrated under reduced pressure, then purified by flash column chromatography (EtOAc/Hex = 1:2) to give product **9** as a white solid (0.4 g, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.11 (s, 6H), 0.90 (s, 9H), 3.84–3.95 (m, 2H), 4.92 (d, J = 3.2 Hz, 1H), 5.89 (d, J = 3.2 Hz, 1H), 6.28 (d, J = 3.2 Hz, 1H), 6.92 (d, J = 3.2Hz, 1H), 7.97 (s, 1H), 8.48 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.17, −5.06, 18.72, 26.07, 64.31, 87.67, 90.46, 96.92, 126.14, 134.82, 139.95, 149.70, 158.74. MS (HR-ESI) calcd for $C_{15}H_{23}BrN_2O_4SiNa$ $(M + Na)^+$ 427.0490, found 427.0502.

Synthesis of 5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-didehydro-2′,3′-dideoxy-5-(tributylstannyl)-uridine (10)

A solution of **9** (200 mg, 0.5 mmol), $Pd(PPh_3)_4$ (58 mg, 0.05 mmol), and *bis*(tributyltin), (0.5 mL, 1 mmol) in dioxane (10 mL) was refluxed for 48 h. The reaction mixture was concentrated under reduced pressure, the residue was purified by a flash column chromatography (EtOAc/Hex = 1:3) to give product **10** as a clear oil (82 mg, 23%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.07 (s, 3H), 0.08 (s, 3H), 0.88–1.58 (m, 36 H), 3.56–3.79 (m, 1H), 3.79–3.83 (m, 1H), 4.86 (d, J = 3.6 Hz, 1H), 5.86 (d, J = 6 Hz, 1H), 6.44 (d, J = 6 Hz, 1H), 6.99 (q, J = 3.6 Hz, 1H), 7.94 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.37, −5.27, 9.87, 13.67, 18.32, 25.85, 26.95, 27.24, 27.55, 28.95, 29.05, 65.79, 86.83, 90.09, 113.00, 125.66, 135.30, 143.25, 151.33, 161.12. MS (HR-ESI) calcd for $C_{27}H_{50}N_2O_4SiSnNa$ $(M + Na)^+$ 637.2464, found 637.2474.

Synthesis of 2′,3′-didehydro-2′,3′-dideoxy-5-(tributylstannyl)-uridine (d4T–Sn)

A solution of **10** (50 mg, 0.07 mmol) in THF (0.5 mL) was cooled to 0 °C and treated with TBAF solution in THF (1 M, 0.5 mL). After stirring for 1 h, the reaction mixture was quenched with aq. NH_4Cl solution and extracted with EtOAc. The organic phase was dried over Na_2SO_4 and evaporated under vacuum. The residue was purified by a flash chromatography ($MeOH/CHCl_3$ = 1:20) to give product d4T–Sn as a white solid (40 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86–1.57 (m, 27 H), 3.77–3.90 (m, 2H), 4.94 (bs, 1H), 5.88 (d, J = 6, 1H), 6.32 (q, J = 2.8 Hz, 1H), 6.99 (q, J = 3.2 Hz, 1H), 7.31 (s, 1 H), 7.97 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 9.76, 13.67, 27.23, 28.93, 63.81, 86.97, 90.13, 112.84, 126.63, 134.09, 144.33, 165.89. MS (HR-ESI) calcd for $C_{21}H_{36}N_2O_4SnNa$ $(M + Na)^+$ 523.1599, found 523.1602.

Synthesis of 3′,5′-*bis*-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-*L*-uridine (12)

To a solution of 2′-deoxy-*L*-uridine (**11**), (0.5 g, 2.2 mmol) in DMF (5 mL) was added imidazole (0.84 g, 12 mmol), followed by (0.82 g, 5.5 mmol) TBSCl. The mixture was stirred at room temperature overnight then quenched with aq. NH_4Cl solution and extracted with EtOAc. The organic phase was dried over Na_2SO_4 and evaporated under vacuum. The residue was purified by flash chromatography (EtOAc/Hex, 5 → 30%) to give **12** (1.0 g, 100%) as a white foam powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.08–0.11 (m, 12H), 0.88–0.92 (m, 18H), 2.04–2.09 (m, 1H), 2.29–2.35 (m, 1H), 3.75–3.92 (m, 3H), 4.40–4.43 (m, 1H), 5.68 (q, J = 5.6Hz, 1H), 6.28 (t, J = 6 Hz, 1H), 7.89 (d, J = 8 Hz, 1H), 8.44

(bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.59, −4.88, −4.63, 17.96, 18.33, 25.70, 25.85, 41.83, 62.41, 71.18, 85.18, 87.76, 102.11, 140.18, 150.03, 162.96. MS (HR-ESI) calcd for $C_{21}H_{40}N_2O_5Si_2Na$ $(M+Na)^+$ 479.2373, found 479.2371.

Synthesis of 3′,5′-*bis*-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-*L*-5-bromouridine (13)

A solution of **12** (1.0 g, 2.2 mmol) in dimethoxyethane (20 mL) was added NaN_3 (0.57 g, 8.8 mmol) and *N*-bromosuccinimide (0.428 g, 2.42 mmol). The reaction mixture was stirred at room temperature overnight then diluted with EtOAc and washed with water. The organic phase was dried over Na_2SO_4 and concentrated under reduce pressure. The crude mixture was purified by a flash chromatograph (EtOAc/Hex, 15 → 30%) to give product **13** (0.83 g, 70%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.08–0.16 (m, 12H), 0.89–0.95 (m, 18H), 1.99–2.06 (m, 1H), 2.29–2.35 (m, 1H), 3.76–4.00 (m, 3H), 4.40–4.42 (m, 1H), 6.28 (q, J = 5.6 Hz, 1H), 8.08 (s, 1H), 8.29 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.37,−5.29, −4.85, 17.99, 18.47, 25.72, 26.06, 42.03, 63.04, 72.47, 85.88, 88.47, 96.76, 139.47, 149.23, 158.68. MS (HR-ESI) calcd for $C_{21}H_{39}BrN_2O_5Si_2Na$ $(M+Na)^+$ 557.1479, found 557.1473.

Synthesis of 3′,5′-*bis*-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-*L*-5-(tributylstannyl)-uridine (14)

A solution of **13** (273 mg, 0.5 mmol) in dioxane (15 mL) was added $Pd(PPh_3)_4$ (58 mg, 0.05 mmol) and *bis*(tributyltin), (0.5 mL, 1 mmol). The reaction mixture was refluxed for 48 h. The reaction mixture was concentrated under reduced pressure then purified by a flash column chromatography (EtOAc/Hex, 10 → 30%) to give product **14** as a clear oil (146 mg, 39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.07–0.09 (m, 12H), 0.87–1.55 (m, 45H), 1.90–2.02(m, 1H), 2.29–2.32(m, 1H), 3.60–3.78 (m, 2H), 3.94 (d, J = 1.2 Hz, 1H), 4.38 (t, J = 2.8 Hz,1H), 6.23 (t, J = 6 Hz, 1H), 7.16 (s, 1H), 8.16 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: −5.70, −5.69, −5.15, 9.54, 13.35, 16.95, 17.66, 18.04, 25.60, 26.43,27.93, 28.60, 40.02, 63.01, 72.20, 85.19, 87.28, 112.14, 142.91, 150.33, 165.65. MS (HR-ESI) calcd for $C_{33}H_{66}N_2O_5Si_2SnNa$ $(M+Na)^+$ 769.3436, found 769.3414.

Synthesis of 2′-deoxy-*L*-5-(tributylstannyl)-uridine (LdT-Sn)

Compound **14** (120 mg, 0.16 mmol) in THF (2 mL) was cooled to 0 °C and treated with TBAF in THF (1 M, 1 mL). After stirring for 2 h, the reaction mixture was quenched with aq. NH_4Cl solution and extracted with EtOAc. The organic phase was dried over Na_2SO_4 and evaporated under vacuum. The residue was purified by flash chromatography (MeOH/$CHCl_3$, 5 → 9%) to give the product LdT–Sn as a white solid (80 mg, 97%).^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.87–1.54 (m, 27H), 2.23–2.53(m, 2H), 3.790–4.04 (m, 3H), 4.60 (t, J = 3.6 Hz, 1H), 6.12 (t, J = 6.8 Hz,1H), 7.24 (s, 1H), 8.70 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 9.85, 13.66, 27.25, 28.92, 39.83, 62.62, 71.92, 87.13, 87.96, 113.05, 145.31, 151.00, 166.30. MS (HR-ESI) calcd for $C_{21}H_{38}N_2O_5SnNa$ $(M+Na)^+$ 541.1704, found 541.1696.

Synthesis of [methyl-^{11}C]toluene by a one-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation method

Method A

$Pd_2(dba)_3$ (1.0 mg, 1.1 μmol) and P(*o*-tolyl)$_3$ (3.4 mg, 11 μmol) were dissolved in DMF (250 μL) with sonication. The resultant reaction solution was mixed with CuCl (0.8 mg, 8 μmol) and K_2CO_3 (1.4 mg, 10 μmol) and then transferred to the reactor by using a syringe. Then, tributylphenylstannane (3–8 μmol) in DMF (50 μL) was added to the reactor and kept at room temperature while waiting for the [^{11}C]CH_3I preparation. Subsequently, [^{11}C]CH_3I with N_2 gas was bubbled into the reaction mixture by distillation. The resultant reaction mixture was quickly heated to 65 °C for 5 min. The reaction mixture was diluted with 50% CH_3CN (1 mL). After being passed through a syringe filter (filter media, PVDF; pore size, 0.2 mm; Whatman Inc., NJ, USA), the filtrate (20 μL) was sampled for HPLC analysis (analytical column, COSMOSIL 5C18 MS-II 4.6 ø × 150 mm [Nacalai Tesque]; mobile phase, 65% CH_3CN; flow rate, 1.0 mL/min; detection, UV 254 nm). The retention time of [methyl-^{11}C]toluene was 5.1 min. The yield of [methyl-^{11}C]toluene (i.e., conversion of [^{11}C]methyl iodide into the desired product [methyl-^{11}C]toluene) was determined from the radio-HPLC peak area percentage of the sum of all radioactive peak areas. Additionally, the side product 1, 1′-biphenyl generated by homocoupling of tributylphenylstannane was also detected by the aforementioned HPLC with a retention time of 8.0 min.

Method B

The reaction mixture of $Pd_2(dba)_3$, P(*o*-tolyl)$_3$, CuCl, and K_2CO_3 was prepared in the same way as in Method A and then transferred to the reactor by using a syringe. After this, the reaction mixture was cooled to −10 °C, tributylphenylstannane (3–8 μmol) in DMF (50 μL) was added and kept below −10 °C by cooling while waiting for the [^{11}C]CH_3I preparation. This reaction mixture was further cooled to −20 °C to trap [^{11}C]CH_3I gas, followed by the mixture being quickly heated to 65 °C for 5 min. The resulting reaction mixture was diluted with 50% CH_3CN and sampled for radio-HPLC analysis using the procedure described in Method A.

Synthesis of [methyl-^{11}C]2-picoline

[Methyl-^{11}C]2-picoline was synthesized following a similar process described in Method B for [methyl-^{11}C]toluene. [^{11}C]CH_3I was trapped in the reaction mixtures of $Pd_2(dba)_3$ (1.0 mg, 1.1 μmol), P(*o*-tolyl)$_3$ (5.0 mg, 16 μmol), CuBr (1.0 mg, 7 μmol), CsF (2.0 mg, 13 μmol), and the precursor 2-tributylstannylpyridine (3.0 mg, 8.1 μmol) in *N*-methylpyrrolidone (250 μL) at −20 °C. The resulting mixture was then quickly heated to 80 °C for 5 min, followed by dilution with 50% CH_3CN (1 mL). After being passed through a syringe filter (filter media, PVDF; pore size, 0.2 mm; Whatman Inc.), the filtrate (20 μL) was sampled for HPLC analysis (analytical column, COSMOSIL 5C18 MS-II 4.6 ø × 150 mm [Nacalai Tesque]; mobile phase, 20% CH_3CN; flow rate, 1.0 mL/min; detection, UV 254 nm). The retention time of [methyl-^{11}C]2-picoline was 5.0 min. From the radio-HPLC peak area percentage of the sum of all radioactive peak areas, the yield of [methyl-^{11}C]2-picoline was determined as 89 ± 2% (n = 2). In addition, the side product pyridine generated via demetallation of 2-tributylstannylpyridine was also detected by the aforementioned HPLC method with a retention time of 3.3 min.

Synthesis of [methyl-^{11}C]3-picoline

[Methyl-^{11}C]3-picoline was synthesized and analyzed following the same process for [methyl-^{11}C]2-picoline. Precursor 3-

tributylstannylpyridine (3.0 mg, 8.1 μmol) was used. The desired tracer [methyl-^{11}C]3-picoline was detected by HPLC with a retention time of 6.0 min. From the radio-HPLC peak area percentage of the sum of all radioactive peak areas, the yield of [methyl-^{11}C]3-picoline was determined as 92 ± 2% (n = 2). Additionally, the side product 3,3′-bipyridine generated via homocoupling of 3-tributylstannylpyridine was detected by HPLC with a retention time of 4.9 min.

Synthesis of 5-[methyl-^{11}C]-3′-azido-2′,3′-dideoxy-thymidine ([^{11}C]AZT)

[^{11}C]AZT was synthesized following the same process described in Method B for [methyl-^{11}C]toluene. [^{11}C]CH_3I was trapped in the reaction mixtures of $Pd_2(dba)_3$ (1.0 mg, 1.1 μmol), P(*o*-tolyl)$_3$ (3.4 mg, 11 μmol), precursor AZT–Sn (2.0 mg, 3.7 μmol), CuCl (0.8 mg, 8 μmol), and K_2CO_3 (1.4 mg, 10 μmol) in DMF (300 μL) at −20 °C. The resulting mixture was then quickly heated to 80 °C for 5 min, followed by dilution with 10% CH_3CN (1 mL). The mixture was passed through cotton and then syringe filter (filter media, PVDF; pore size, 0.2 mm; Whatman Inc.). The filtrate was injected into a preparative HPLC (semipreparative column, COSMOSIL 5C18 MS-II 20 ø × 250 mm [Nacalai Tesque Inc.]; mobile phase, 13.3% CH_3CN; flow rate, 9.9 mL/min; detection, UV 265 nm). The retention time of [^{11}C]AZT was 12.5 min. The desired fraction was collected into a flask and concentrated to remove the organic solvent under reduced pressure. The residue was then diluted in a mixture of 25% ascorbic acid (200 μL) and saline (2 mL). The total time of the synthesis was 35 min from end of bombardment (EOB). The decay-corrected radiochemical yield (DCY) was 58 ± 6% (n = 3) from [^{11}C]carbon dioxide. Product [^{11}C]AZT was identified by an analytical HPLC (column, COSMOSIL 5C18 AR-II 4.6 ø × 150 mm [Nacalai Tesque Inc.]; mobile phase, 14% CH_3CN; flow rate, 1.0 mL/min; detection, UV 265 nm) with a retention time of 6.0 min.

Synthesis of 5-[methyl-^{11}C]-2′,3′-didehydro-2′-deoxy-thymidine ([^{11}C]d4T)

[^{11}C]d4T was synthesized following the same process for [^{11}C]AZT. Precursor d4T–Sn (2.0 mg, 4.0 μmol) was used. The product was purified by a preparative HPLC (semipreparative column, Gemini 5C18 21.2 ø × 250 mm [Phenomenex Inc., CA, USA]; mobile phase, 9% CH_3CN; flow rate, 9.9 mL/min; detection, UV 265 nm). The retention time of [^{11}C]d4T was 12.2 min. The total time of the synthesis was 35 min from EOB. The DCY was 54 ± 3% (n = 3) from [^{11}C]carbon dioxide. Product [^{11}C]d4T was identified by an analytical HPLC (column, Luna 5C18 4.6 ø × 150 mm [Phenomenex Inc.]; mobile phase, 7% CH_3CN; flow rate, 1.0 ml/min; detection, UV 265 nm) with a retention time of 6.8 min.

Synthesis of 5-[methyl-^{11}C]-2′-deoxy-*L*-thymidine ([^{11}C]LdT)

[^{11}C]LdT was synthesized following the same process for [^{11}C]AZT. Precursor LdT–Sn (4.0 mg, 7.7 μmol) was used. The product was purified by a preparative HPLC (semipreparative column, Gemini 5C18 21.2 ø × 250 mm [Phenomenex Inc.]; mobile phase, 4% CH_3CN containing 100 mM NH_4HCO_3; flow rate, 9.9 mL/min; detection, UV 265 nm). The retention time of [^{11}C]LdT was 13.7 min. The total time of the synthesis was 35 min from EOB. The DCY was 53 ± 3% (n = 3) from [^{11}C]carbon dioxide. Product [^{11}C]LdT was identified by an analytical HPLC (column, Luna 5C18 4.6 ø × 150 mm [Phenomenex Inc.]; mobile phase, 5% CH_3CN containing 100 mM NH_4HCO_3; flow rate, 1.0 mL/min; detection, UV 265 nm) with a retention time of 5.4 min.

Synthesis of [^{11}C]Thy

[^{11}C]Thy was synthesized following the same process for [^{11}C]AZT. Precursor 5-tributylstannyl-2′-deoxyuridine (5.5 mg, 10.6 μmol) was used. The product was purified by a preparative HPLC (semipreparative column, Gemini 5C18 21.2 ø × 250 mm [Phenomenex Inc.]; mobile phase, 4% CH_3CN containing 100 mM NH_4HCO_3; flow rate, 9.9 mL/min; detection, UV 265 nm). The retention time of [^{11}C]Thy was 12.5 min. The total time of the synthesis was 39 min from EOB. The DCY was 75 ± 2% (n = 3) from [^{11}C]carbon dioxide. Product [^{11}C]Thy was identified by an analytical HPLC (column, Luna 5C18 4.6 ø × 150 mm [Phenomenex Inc.]; mobile phase, 4% CH_3CN containing 100 mM NH_4HCO_3; flow rate, 1.0 mL/min; detection, UV 265 nm) with a retention time of 6.9 min.

Synthesis of [^{11}C]4DST

[^{11}C]4DST was synthesized following the same process for [^{11}C]AZT. Precursor 5-tributylstannyl-4′-thio-2′-deoxyuridine (4.0 mg, 7.5 μmol) was used. The product was purified by a preparative HPLC (semipreparative column, Gemini 5C18 21.2 ø × 250 mm [Phenomenex Inc.]; mobile phase, 7% CH_3CN containing 100 mM NH_4HCO_3; flow rate, 9.9 mL/min; detection, UV 265 nm). The retention time of [^{11}C]4DST was 13.5 min. The total time of the synthesis was 36 min from EOB. The DCY was 65 ± 4% (n = 3) from [^{11}C]carbon dioxide. Product [^{11}C]4DST was identified by an analytical HPLC (column, Luna 5C18 4.6 ø × 150 mm [Phenomenex Inc.]; mobile phase, 7% CH_3CN containing 100 mM NH_4HCO_3; flow rate, 1.0 mL/min; detection, UV 265 nm) with a retention time of 7.4 min.

Results and discussion

Optimization of a one-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation method of a stannyl precursor

In order to optimize the one-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation method, the stannyl precursor tributyl-phenylstannane was used as a model compound for synthesizing the PET tracer [methyl-^{11}C]toluene. In the previous one-pot method (Method A), the reaction mixture of $Pd_2(dba)_3$, P(*o*-tolyl)$_3$, CuCl, K_2CO_3, and the stannyl precursor (3–4 μmol) was prepared and kept at room temperature while waiting for the [^{11}C]CH_3I preparation. Subsequently, [^{11}C]CH_3I with nitrogen gas was bubbled into the reaction mixture by distillation. The resultant reaction mixture was then heated 5 min at 65 °C for [methyl-^{11}C]toluene generation. We found that the yield of [methyl-^{11}C]toluene (conversion of [^{11}C]CH_3I into [methyl-^{11}C]toluene) was only around 0.8–12.4% (Figure 2, Method A). Although the amount of tributylphenylstannane was increased to 5–8 μmol, the yield of [methyl-^{11}C]toluene was still less than 50% and much lower than the yield (~90%) obtained in an ordinary organic chemical reaction in our previous study.[17] In the ordinary organic chemical reaction, after the reaction mixtures of $Pd_2(dba)_3$, P(*o*-tolyl)$_3$), tributylphenylstannane, and copper(I) chloride in DMF were prepared within 3 min at room temperature, they were immediately added to the solution of CH_3I in DMF and then

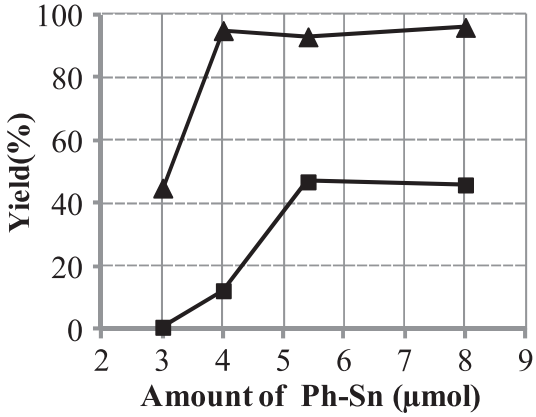

Figure 2. The yield of [methyl-^{11}C]toluene generated by Method A (squares) and Method B (triangles) using various amounts of the precursor tributylphenylstannane (Ph–Sn). The yield of [methyl-^{11}C]toluene was determined from the radio-HPLC peak area percentage of the sum of all radioactive peak areas. In Method A, the reaction mixture of $Pd_2(dba)_3$, P(*o*-tolyl)$_3$, CuCl, K_2CO_3, and Ph–Sn was prepared and kept at room temperature while waiting for [^{11}C]CH_3I preparation; it was not cooled while [^{11}C]CH_3I gas was bubbling. In Method B, the reaction mixture of $Pd_2(dba)_3$, P(*o*-tolyl)$_3$, CuCl, K_2CO_3, and Ph–Sn was controlled below −10 °C before [^{11}C]CH_3I bubbling and further cooled to −20 °C during [^{11}C]CH_3I gas bubbling. For details, please see the text.

quickly heated for a coupling reaction. In contrast, during hot PET tracer synthesis, [^{11}C]CH_3I was synthesized from cyclotron-generated [^{11}C]CO_2 and then bubbled into the mixture of Pd(0)–Cu(I) and the stannyl precursor by distillation. Additionally, all reactions had to be carried out in a shielded hot cell. Thus, the mixture of Pd(0)–Cu(I) and the stannyl precursor had to be prepared before [^{11}C]CH_3I was generated.

According to the HPLC analysis of the reaction mixture, we found that during the waiting period for the [^{11}C]CH_3I preparation, part of the tributylphenylstannane had been transformed into the compound 1,1′-biphenyl via homocoupling. Such a side reaction consumes the stannyl precursor and greatly impedes the subsequent *C*-[^{11}C]methylation to generate the desired tracer [methyl-^{11}C]toluene. In order to inhibit this side reaction, we optimized the temperature for the hot labeling process, in which the reaction mixture of the Pd(0)–Cu(I) catalyst and tributylphenylstannane was controlled below −10 °C by cooling before the [^{11}C]CH_3I was generated, further cooled to −20 °C during [^{11}C]CH_3I bubbling, and then followed by quickly heating the mixture to 65 °C for 5 min. Using this optimized one-pot process (Method B), the yield of [methyl-^{11}C]toluene was greatly improved to 93–96% when 4–8 μmol tributylphenylstannane was used (Figure 2). This optimized one-pot method also worked well for the *C*–*C* coupling of [^{11}C]CH_3I with the heteroaryl compounds 2-tributylstannylpyridine and 3-tributylstannylpyridine. From the radio-HPLC peak area percentage of the sum of all radioactive peak areas, the yields of [methyl-^{11}C]2-picoline and [methyl-^{11}C]3-picoline were determined as 89 ± 2% (n = 2) and 92 ± 2% (n = 2), respectively.

Synthesis of the PET tracer [^{11}C]AZT

The aforementioned optimized one-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation was successfully applied to label the anti-HIV agents AZT and d4T and the anti-HBV agent LdT to generate the PET tracers [^{11}C]AZT, [^{11}C]d4T, and [^{11}C]LdT, respectively. For synthesis of the PET tracer [^{11}C]AZT, AZT–Sn was synthesized as a precursor (Figure 3A). 5-Iodo-2′-deoxyuridine (**1**) was selectively protected by TBS to give 5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-5-iodouridine (**2**).[24] Compound **2** was treated with triphenylphosphine and DIAD to yield 5′-*O*-(*tert*-butyldimethylsilyl)-2,3′-anhydro-2′-deoxy-5-iodouridine (**3**), which reacted with sodium azide to produce 5′-*O*-(*tert*-butyldimethylsilyl)-3′-azido-3′-deoxy-5-iodouridine (**4**). Subsequently, **4** was treated with sodium hydride, *n*-BuLi, and tributyltin chloride to yield 5′-*O*-(*tert*-butyldimethylsilyl)-3′-azido-2′,3′-dideoxy-5-(tributylstannyl)-uridine (**5**). Following deprotection of TBS with TBAF, the stannyl precursor AZT–Sn was obtained. By the optimized one-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation method, the precursor AZT–Sn was efficiently coupled with [^{11}C]CH_3I to generate the PET tracer [^{11}C]AZT. After HPLC purification and radiopharmaceutical formulation, the desired product [^{11}C]AZT was obtained with a radioactivity of 7.0 ± 0.8 GBq (n = 3) and a specific radioactivity in the range of 74–128 GBq/μmol at the end of synthesis (see Table 1). The total time of the synthesis was 35 min from EOB. The DCY was 58 ± 6% (n = 3) from [^{11}C]carbon dioxide. The chemical and radiochemical purities of the product were >99% and >99.5%, respectively. Thus, by using the optimized one-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation, the PET probe [^{11}C]AZT was synthesized successfully with sufficient radioactivity and specific radioactivity and high chemical and radiochemical purities, which could meet the requirements for preclinical PET studies.

Synthesis of the PET tracer [^{11}C]d4T

[^{11}C]d4T was synthesized previously by Alan J. Fischman and his colleagues[25] by using a tedious process. In brief, 5′-*O*-(tetrahydro-2*H*-pyran-2-yl)-2′,3′-dideoxy-5-bromouridine was first treated with *n*-BuLi at −78 °C, then reacted with [^{11}C]CH_3I, and finally deprotected by HCl to generate [^{11}C]d4T with a radioactivity of about 0.38 GBq.[25] We improved the preparation process of [^{11}C]d4T by an optimized one-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation method. d4T–Sn was designed and prepared as a precursor (see Figure 3B). The 5-bromouridine (**6**) was protected by TBS to give 5′-*O*-*tert*-butyldimethylsilyl-5-bromouridine (**7**), which was then transformed into 5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-didehyro-2′-3′-dideoxy-5-bromouridine (**9**) by a Corey–Winter reaction. Compound **9** then reacted with *bis*(tributyltin) and deprotected by TBAF to generate the precursor d4T–Sn. d4T–Sn was then utilized in the one-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation method to successfully produce the PET tracer [^{11}C]d4T. Purification by preparative HPLC followed by radiopharmaceutical formulation gave the desired product [^{11}C]d4T with a radioactivity of 6.6 ± 0.3 GBq (n = 3) and a specific radioactivity in the range of 126–135 GBq/μmol at the end of synthesis. The total time of the synthesis was 35 min from EOB. The DCY was 54 ± 3% (n = 3) from [^{11}C]carbon dioxide. The chemical and radiochemical purities of the product were >99% and >99.5%, respectively.

Synthesis of the PET tracer [^{11}C]LdT

Similarly, by the same one-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation method, the anti-HBV agent LdT was also successfully labeled with the PET nuclide ^{11}C to generate [^{11}C]LdT. As a precursor, LdT–Sn was synthesized following the route shown in Figure 3C. 2′-Deoxy-*L*-uridine (**11**) was protected with TBS to give 3′,5′-*bis*-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-*L*-uridine

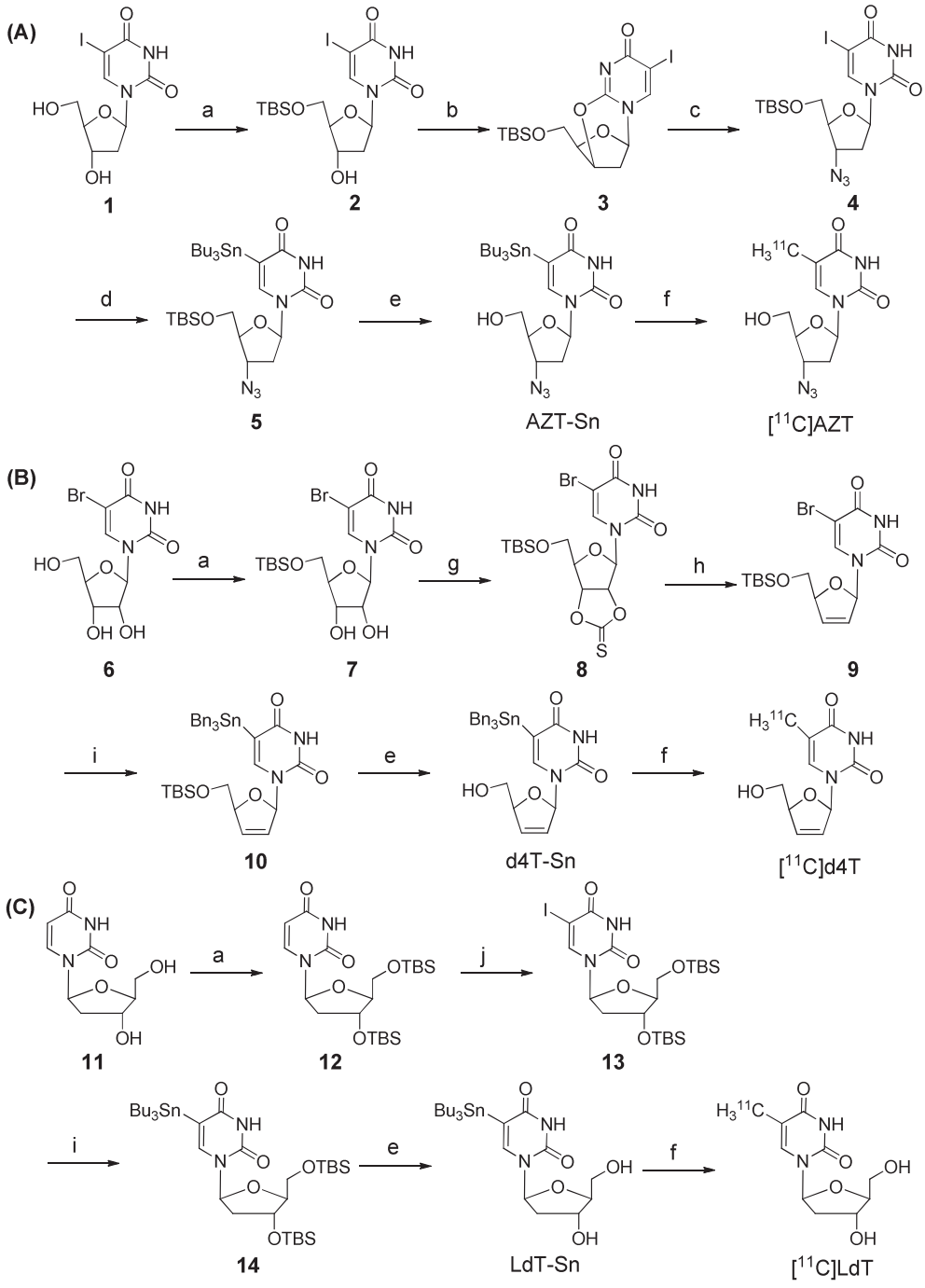

Figure 3. Syntheses of [^{11}C]AZT (A), [^{11}C]d4T (B), and [^{11}C]LdT (C). Reagents and conditions: a) TBSCl, imidazole; b) DIAD, PPh_3; c) NaN_3, 110 °C; d) NaH, *n*-BuLi, –78 °C, then Bu_3SnCl and HAMP; e) TBAF, 0 °C; f) $Pd_2(dba)_3$, P(*o*-tolyl)$_3$, CuCl, K_2CO_3, [^{11}C]CH_3I trapping at –20 °C, then 80 °C, 5 min; g) 1,1′-thiocarbonyl imidazole; h) trimethyl phosphite, 118 °C; i) $Pd(PPh_3)_4$, $(Bu_3Sn)_2$, reflux in dioxane; and j) NaN_3, NBS.

(**12**), which was then brominated with *N*-bromosuccinimide to yield 3′,5′-*bis*-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-*L*-5-bromouridine (**13**). Compound **13** was then reacted with *bis*(tributyltin) in the presence of the catalyst $Pd(PPh_3)_4$ to produce 3′,5′-*bis*-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-*L*-5-(tributylstannyl)-uridine (**14**), which was deprotected to give the stannyl precursor LdT–Sn. The precursor LdT–Sn was coupled with [^{11}C]CH_3I to generate the PET tracer [^{11}C]LdT. Purification by preparative HPLC followed by radiopharmaceutical formulation gave the product [^{11}C]LdT with a radioactivity of 6.4 ± 0.3 GBq ($n = 3$) and a specific radioactivity in the range of 75–94 GBq/μmol at the end of synthesis. The total time of the synthesis was 35 min from EOB. The DCY was 53 ± 3% ($n = 3$) from [^{11}C]carbon dioxide. The chemical and radiochemical purities of the product were >97% and >99.5%, respectively.

Syntheses of [^{11}C]Thy and [^{11}C]4DST

[^{11}C]Thy and [^{11}C]4DST are effective PET probes for monitoring cell proliferation and cancer growth.[26,27] Recently, we improved

Table 1. [^{11}C]zidovudine and its analogs synthesized by one-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation

PET tracers	Radioactivity (GBq)	DCY (%)[a]	Specific radioactivity (GBq/μmol)
[^{11}C]Zidovudine	7.0 ± 0.8	58 ± 6	74–128
[^{11}C]Stavudine	6.6 ± 0.3	54 ± 3	126–135
[^{11}C]Telbivudine	6.4 ± 0.3	53 ± 3	75–94
[^{11}C]Thy	7.9 ± 0.2	75 ± 2	86–147
[^{11}C]4DST	7.6 ± 0.4	65 ± 4	85–216

Data are expressed as the mean ± SD (n = 3). All the values were measured at the end of synthesis.
[a]The decay-corrected radiochemical yield (DCY) was based on [^{11}C]carbon dioxide.

their preparation by the two-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation method with the precursors 5-tributylstannyl-2′-deoxyuridine and 5-tributylstannyl-4′-thio-2′-deoxyuridine.[23] By this two-pot method, after HPLC purification and radiopharmaceutical formulation, [^{11}C]Thy and [^{11}C]4DST were prepared with radioactivities of around 3.7–3.8 GBq and DCY of around 39–40% (based on the radioactivity of [^{11}C]carbon dioxide). Here, we found that the optimized one-pot method was much more convenient and efficient than the previous two-pot method. Using the optimized one-pot method, [^{11}C]Thy was obtained with a radioactivity of 7.9 ± 0.2 GBq (n = 3) and a specific radioactivity in the range of 86–147 GBq/μmol at the end of synthesis. Similarly, [^{11}C]4DST was also effectively prepared with a radioactivity of 7.6 ± 0.4 GBq (n = 3) and a specific radioactivity in the range of 85–216 GBq/μmol at the end of synthesis. The DCYs of [^{11}C]Thy and [^{11}C]4DST were 75 ± 2% and 65 ± 4% (n = 3), respectively. Thus, through the one-pot method, the yields of [^{11}C]Thy and [^{11}C]4DST were greatly improved, approximately twice as high as those obtained by the two-pot process. In this optimized one-pot method, [^{11}C]CH_3I was trapped in the reaction mixture and cooled to −20 °C, which showed higher [^{11}C] CH_3I capture efficiency than at room temperature. The one-pot method also shortened the total synthesis time and greatly reduced the loss of radioactivity adsorbed by the reaction vessels, resulting in a much higher product radiochemical yield than the two-pot method.

Proposed mechanism of the one-pot Pd(0)–Cu(I) co-mediated C-[^{11}C]methylation with a stannyl precursor

We previously found that although hot [^{11}C]CH_3I gas was trapped in the reaction mixture of the catalyst Pd(0)–Cu(I) and the stannyl precursor at room temperature, the one-pot process for Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C] methylation lacked reproducibility.[18,20,21] In this study, we succeeded in solving this problem by cooling the reaction mixture before [^{11}C]CH_3I was introduced. The effect of cooling could be explained by the mechanism shown in Figure 4. The transmetallation of the stannyl precursor (R–$SnBu_3$) with CuCl gave a highly reactive intermediate organocopper R–Cu and the Lewis acid Bu_3SnCl. Bu_3SnCl was neutralized by K_2CO_3, and the organocopper R–Cu could react with the {[^{11}C]CH_3PdI} complex to generate the desired PET tracer [^{11}C]CH_3–R. During such reaction processes, CuCl and K_2CO_3 work synergistically. The neutralization of Bu_3SnCl with K_2CO_3 could assist the Sn/Cu transmetallation to accelerate the *C*-[^{11}C]methylation.[17,20,21] However, it should be noted that

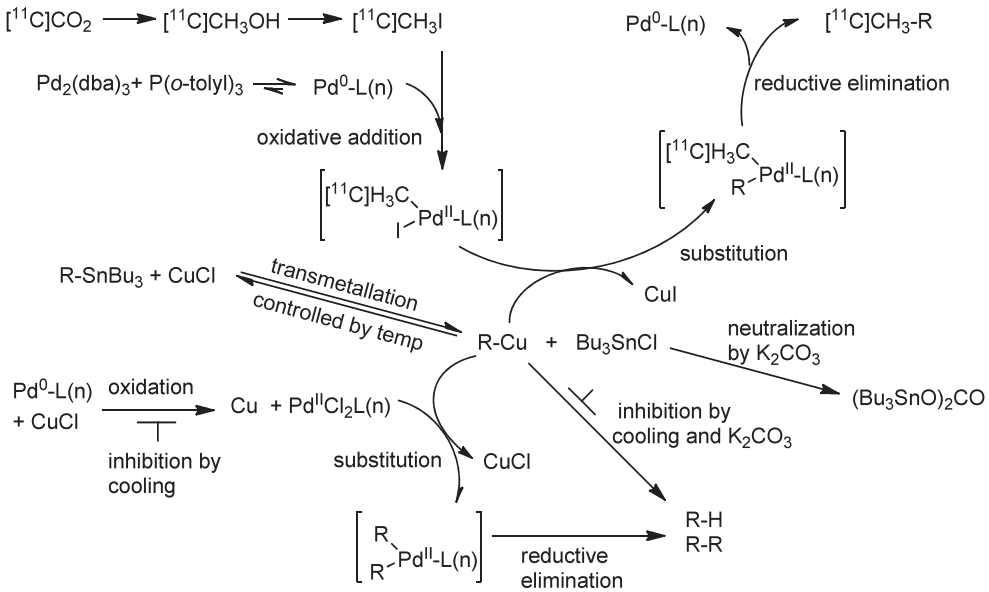

Figure 4. The assumed mechanism of the one-pot Pd(0)–Cu(I) co-mediated reaction of [^{11}C]CH_3I with a stannyl precursor. L = P(*o*-tolyl)$_3$.

the highly reactive organocopper R–Cu could also take part in side reactions such as demetallation to yield RH and homocoupling to yield R–R. In this context, we previously demonstrated that the demetallation process could be partly suppressed by K_2CO_3 and an excess amount of P(*o*-tolyl)$_3$.[23]

Here, we observed that tributylphenylstannane and 3-tributylstannylpyridine could react with CuCl at room temperature to yield the homocoupling products 1,1′-biphenyl and 3,3′-bipyridine, respectively. When 2-tributylstannylpyridine reacted with CuCl, the demetallation product pyridine could be detected. We also found that 5-tributylstannyl-2′-deoxyuridine could react with CuCl to generate 2′-deoxyuridine and 5,5′-bi(2′-deoxyuridine) at room temperature, and this reaction could be accelerated by heating. Such Cu-mediated homocoupling and destannylation of stannyl compounds were also reported in several recent papers.[28–30] Furthermore, we found that Sn/Cu transmetallation and R–Cu homocoupling occurred much more quickly to yield 1,1′-biphenyl when $Pd_2(dba)_3$ and P(*o*-tolyl)$_3$ were added into the reaction mixtures of tributylphenylstannane and CuCl. This should be attributed to the oxidation of Pd[P(*o*-tolyl)$_3$]$_n$ ($Pd^0L_{(n)}$) by CuCl to yield the active $PdCl_2$[P(*o*-tolyl)$_3$]$_n$ ($Pd^{II}Cl_2L_{(n)}$), which greatly promoted the homocoupling of the organocopper R–Cu via substitution and reductive elimination. Therefore, in the previous one-pot method without cooling, part of the stannyl precursor (R–$SnBu_3$) would be transformed into the side products R–R or RH via homocoupling or demetallation while waiting for the addition of [^{11}C]CH_3I. Moreover, when the hot nitrogen gas containing [^{11}C]CH_3I was bubbled into the reaction mixture without cooling, these side reactions could be aggravated, even to the extent of exhausting the precursor R–$SnBu_3$ before *C*-[^{11}C]methylation. Thus, the previous one-pot method without cooling gave a low yield and suffered from low reproducibility.[18,20,21] In the two-pot process, the catalyst $Pd^0L_{(n)}$ was present in the first reactor to capture the [^{11}C]CH_3I gas, and the precursor R–$SnBu_3$ in the second reactor was cooled to 0 °C. In this way, the side reactions of R–Cu/R–$SnBu_3$ could be well controlled before they met with the {[^{11}C]CH_3PdI} complex. Additionally, this ensured that in the subsequent heating, R–Cu or R–$SnBu_3$ could efficiently react with the {[^{11}C]CH_3PdI} complex to yield the desired product [^{11}C]CH_3–R, although side reactions through homocoupling or demetallation would also occur. In contrast, in our optimized one-pot method, the reaction mixture of the Pd(0)–Cu(I) catalyst and the stannyl precursor was cooled below −10 °C before [^{11}C]CH_3I bubbling and further cooled to −20 °C during [^{11}C]CH_3I bubbling. Through cooling, the side reactions of R–$SnBu_3$/R–Cu were also greatly suppressed, and the oxidative addition of [^{11}C]CH_3I with Pd(0) could selectively be carried out to form the {[^{11}C]CH_3PdI} complex during [^{11}C]CH_3I bubbling. Thus, in the subsequent quick heating, the major *C*-[^{11}C]methylation of the {[^{11}C]CH_3PdI} complex with R–Cu or R–$SnBu_3$ could proceed smoothly to generate the desired product [^{11}C]CH_3–R with a high yield and good reproducibility.

Conclusion

A convenient and efficient one-pot method for the Pd(0)–Cu(I) co-mediated rapid *C*–*C* coupling of [^{11}C]CH_3I with a stannyl precursor (R–$SnBu_3$) was successfully established through a temperature controlling process. By cooling the reaction mixture before and during the addition of [^{11}C]CH_3I, the side reactions (homocoupling and demetallation) of R–$SnBu_3$/R–Cu were greatly inhibited; and this ensured that in the subsequent heating, R–$SnBu_3$/R–Cu could effectively react with the [^{11}C]CH_3I to generate the desired PET tracer [^{11}C]CH_3-R. By this optimized one-pot Pd(0)–Cu(I) co-mediated rapid *C*-[^{11}C]methylation, the anti-HIV agents AZT and d4T and the anti-HBV agent LdT were efficiently labeled with ^{11}C to generate the PET tracers [^{11}C]AZT, [^{11}C]d4T, and [^{11}C]LdT. Furthermore, the preparations of the PET tracers [^{11}C]Thy and [^{11}C]4DST were also greatly improved. All of these obtained PET probes with sufficient radioactivity and specific radioactivity and high purity could meet the requirements for preclinical PET studies.

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Conflict of Interest

The authors did not report any conflict of interest.

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