Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 4287

www.rsc.org/obc

PAPER

Highly efficient syntheses of [methyl-^{11}C]thymidine and its analogue 4′-[methyl-^{11}C]thiothymidine as nucleoside PET probes for cancer cell proliferation by Pd^0-mediated rapid *C*-[^{11}C]methylation†‡

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Received 24th December 2010, Accepted 14th March 2011
DOI: 10.1039/c0ob01249a

Pd^0-mediated rapid couplings of CH_3I (and then [^{11}C]CH_3I) with excess 5-tributylstannyl-2′-deoxyuridine and -4′-thio-2′-deoxyuridine were investigated for the syntheses of [methyl-^{11}C]thymidine and its stable analogue, 4′-[methyl-^{11}C]thiothymidine as PET probes for cancer diagnosis. The previously reported conditions were attempted using $Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$ (1 : 4 in molar ratio) at 130 °C for 5 min in DMF, giving desired products only in 32 and 30% yields. Therefore, we adapted the current reaction conditions developed in our laboratory for heteroaromatic compounds. The reaction using CH_3I/stannane/$Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$/CuCl/K_2CO_3 (1 : 25 : 1 : 32 : 2 : 5) at 80 °C gave thymidine in 85% yield. Whereas, CH_3I/stannane/$Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$/CuBr/CsF (1 : 25 : 1 : 32 : 2 : 5) including another CuBr/CsF system promoted the reaction at a milder temperature (60 °C), giving thymidine in 100% yield. Chemo-response of thiothymidine-precursor was different from thymidine system. Thus, the above optimized conditions including CuBr/CsF system gave 4′-thiothymidine only in 40% yield. The reaction using 5-fold amount of CuBr/CsF at 80 °C gave much higher yield (83%), but unexpectedly, the reaction was accompanied by a considerable amount of undesired destannylated product. Such destannylation was greatly suppressed by changing to a CuCl/K_2CO_3 system using CH_3I/stannane/$Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$/CuCl/K_2CO_3 (1 : 25 : 1 : 32 : 2 : 5) at 80 °C, giving the 4′-thiothymidine in 98% yield. The each optimized conditions were successfully applied to the syntheses of the corresponding PET probes in 87 and 93% HPLC analytical yields. [^{11}C]Compounds were isolated by preparative HPLC after the reaction conducted under slightly improved conditions, exhibiting sufficient radioactivity of 3.7–3.8 GBq and specific radioactivity of 89–200 GBq μmol^{-1} with radiochemical purity of ≥99.5% for animal and human PET studies.

Introduction

Positron emission tomography (PET) is a non-invasive, *in vivo* molecular imaging method enabling the analysis of the dynamic behavior of a radiotracer in living systems such as the brain, the heart, and other active tissues and organs.[2] Thymidine (**1**) is one of the fundamental building blocks for deoxyribonucleic acid, and hence, the labeling of thymidine and its derivatives attracts much interest for cancer diagnosis.[3] Actually, metabolically stable ^{11}C or ^{18}F-labeled thymidine derivatives have been synthesized as PET probes[4,5] for imaging tumor cell proliferation in living systems.[3] Among the ^{11}C-incorporation methods, particularly focusing on the methyl group in the structure of thymidine, [^{11}C]methylation could be ideal in view of the directness, efficiency, and practicability using the rapid cross-coupling of [^{11}C]CH_3I and a soft metalloid thymidine precursor.[5c] In this context, Långström and coworkers synthesized 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-[methyl-^{11}C]thymine ([^{11}C]FMAU, [^{11}C]**2**) in 28 ± 5% decay-corrected radiochemical yield calculated from [^{11}C]methyl iodide using 5-*trimethylstannyl* precursors **3** by a Stille-type cross-coupling reaction with [^{11}C]methyl iodide.[5c] However, this method possesses several problems to be solved from practical points of view; (1) excess use of the potentially toxic trimethylstannyl derivative; actually, highly toxic trimethylstannyl iodide[6] could be produced as a by-product after cross-coupling reaction with [^{11}C]methyl iodide; (2) suffering from the production of undesired volatile [^{11}C]ethane by scrambling between [^{11}C]CH_3I and a $(CH_3)_3Sn$ unit[1a] to cause safety and environmental problems; (3) a decrease in the yield of the desired

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† Rapid methylation on carbon frameworks for PET tracer synthesis, Part 10; for parts 1–9, see refs. 1a–i.

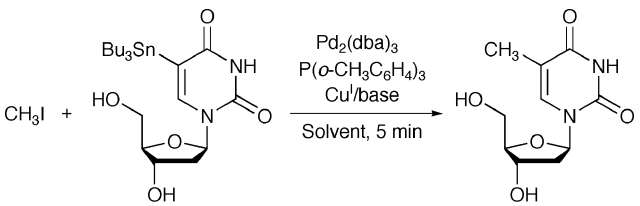
‡ Electronic supplementary information (ESI) available: HPLC charts after the rapid *C*-[^{11}C]methylation and purification. See DOI: 10.1039/c0ob01249a

product to a considerable extent in nature by such a scramble reaction.[7] The labeled compound of 4′-thiothymidine ([^{11}C]**4**), which closely resembles the biological properties of thymidine and is resistant to phosphorylase cleavage,[8] has been first reported by J. Toyohara and co-workers as an attractive PET probe for tumor imaging.[9] They synthesized [^{11}C]**4** by a reaction using the *tributylstannyl* precursor **5** in order to avoid the strong toxicity of trimethylstannyl compound under the conditions of heating at 130 °C for 5 min using $Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$ in DMF.[9b] In this context, we have so far found that the tributylstannyl precursor is much less reactive than the trimethyl one and can be efficiently activated under mild conditions (60–80 °C) using Pd^0–bulky phosphine complexes mixed with the synergic combinations of Cu^I/K_2CO_3 or Cu^I/CsF to realize the desired rapid cross coupling (rapid *C*-methylations) in high yield without scrambling.[1a,f,7b] The rapid *C*-methylations have covered the use of aryl-,[1a] alkenyl-,[1g] alkynyl-,[1e] and hetero-aromatic tributylstannyl precursors[1i] in addition to aryl- and alkenylboranes.[1h] Taking our accumulated information on the Stille-type rapid cross-coupling reactions into consideration, the reported reaction conditions in both groups still seem insufficient. Thus, we have made an effort to optimize the reaction conditions based on the introduction of two kinds of synergic effects on the synthesis of thymidine and 4′-thiothymidine. Described herein are truly efficient syntheses of [methyl-^{11}C]thymidine and 4′-[methyl-^{11}C]thiothymidine by the Pd^0-mediated rapid *C*-[^{11}C]methylation.[1j]

Results and discussion

The reaction using the 1 : 25 ratio of methyl iodide and stannyl substrate **6** was set up by keeping the actual PET tracer synthesis in mind.[10] To begin with, we evaluated the conditions reported by B. Långström and coworkers for the synthesis of [^{11}C]**2**.[5c] Thus, the reaction was conducted using $Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$ (1 : 4 in molar ratio) in DMF at 130 °C for 5 min to give thymidine **1** only in 32% yield (HPLC analytical yield based on the consumption of CH_3I, HPLC: t_R = 6.1 min,[11] Table 1 entry 1). Continuously, we conducted the reaction under our previously developed conditions for the sp^3–sp^2(phenyl) rapid coupling[1a] using a combination of CH_3I/**6**/$Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$/CuCl/K_2CO_3 (1 : 25 : 0.5 : 2 : 2 : 2 in molar ratio) at 60 °C for 5 min, giving **1** in 71% yield (Table 1 entry 2). The reaction conducted at 100 °C did not improve the yield even at such a high temperature (74%, Table 1 entry 2). Instead, the increasing of the quantity of the added phosphine[1g] from 2 to 16 equiv was not effective at 60 °C (60%, Table 1 entry 3), but the use of 2-fold amount of Pd/phosphine (1 : 32 ratio) resulted in increasing the yield at 80 °C to a considerable extent (85%, Table 1 entry 4). On the other hand, the CuBr/CsF combination has also been found to be an efficient synergic combination[12] for the sp^3–sp^2(vinyl) type rapid coupling reaction. Actually, though the reaction using CH_3I/**6**/$Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$/CuBr/CsF (1 : 25 : 1 : 4 : 2 : 5 in molar ratio) at 60 °C in DMF for 5 min gave **1** in 53% yield (in Table 1 entry 5), the enhancement of the reaction temperature to 80 °C improved the yield to a great extent (97%, Table 1 entry 5). In addition, the increase in the quantity of CuBr/CsF enhanced the reaction even at a lower temperature (60 °C, 87%, Table 1 entry 6). To our surprise, it was found that the use of 8-fold quantity of phosphine (32 equiv) tremendously promoted the reaction to give the desired methylated product **6** quantitatively, even at lower temperature (60 °C) without an increase in the quantity of Cu^I/CsF. Thus, the rapid

Table 1 Synthesis of a thymidine (**1**) by the rapid trapping of methyl iodide with 5-tributylstannyl-2′-deoxyuridine (**6**)[a]

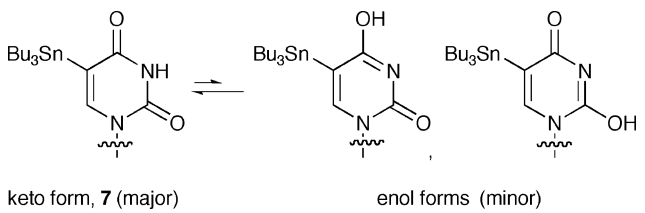


6 **1**

Entry	$Pd_2(dba)_3$(equiv)	P(*o*-$CH_3C_6H_4)_3$ (equiv)	Cu^I/base (molar ratio)	Solvent	Yield of **1** (%)[b] Temp. (°C) 60	80	100	130
1[c,d]	1	4	none	DMF	0	—	—	32
2[e]	0.5	2	CuCl/K_2CO_3 (2 : 2)	DMF	71	—	**74**	—
3	0.5	16	CuCl/K_2CO_3 (2 : 5)	DMF	60	—	—	—
4	1	32	CuCl/K_2CO_3 (2 : 5)	DMF	67	85	—	—
5	1	4	CuBr/CsF (2 : 5)	DMF	53	**97**	—	—
6	1	4	CuBr/CsF (10 : 25)	DMF	87	—	93	—
7	1	32	CuBr/CsF (2 : 5)	DMF	**100**	—	**97**	—
8	0.75	24	CuBr/CsF (2 : 5)	DMF	88	89	—	—
9	0.5	16	CuBr/CsF (2 : 5)	DMF	69	—	—	—
10	1	4	CuBr/CsF (2 : 5)	NMP	—[f]	**100**	—	—

[a] Reaction was carried out with 25 equiv of **6** relative to methyl iodide (2.0 μmol). [b] The yields were determined by HPLC analysis based on the CH_3I consumption using acridine as an internal standard. [c] Reaction was carried out with 5 equiv of **6**. [d] See reference 5c. [e] See reference 1a. [f] The mixture was insoluble at this reaction temperature giving a product with low reproducibility.

C-methylation was accomplished using CH_3I/**6**/$Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$/CuBr/CsF (1 : 25 : 1 : 32 : 2 : 5 in molar ratio) at 60 °C for 5 min in 100% yield (Table 1 entry 7). Such a combination was tolerant of a wide range of the reaction temperature (60–100 °C) to give **1** in high yield (Table 1 entry 7). The decrease of the Pd^0/CH_3I ratio from 2 to 1.5 and 1 showed the lesser effect in the order (Table 1 entries 8 and 9), indicating that the reaction favors the use of an excess amount of Pd^0 (Pd^0/CH_3I >2). This tendency matches well with actual PET probe synthesis using an extremely small amount of $[^{11}C]CH_3I$.[1g,10] Here, the solvent was changed from DMF to *N*-methyl-2-pyrrolidinone (NMP) known as a better solvent for general rapid *C*-methylation of heteroaromatic frameworks of heteroaryl(tributyl)stannane.[1i] Because the reaction mixture was not dissolved in NMP at 60 °C, the reaction was conducted at 80 °C to give the methylated product quantitatively (Table 1 entry 10) similar to the result obtained in DMF shown in entry 5 of Table 1.[13] The effect of solvent compared between entries 5 and 10 indicates that NMP is comparable to DMF in the thymidine case,[1i] implying that the properties of 2,4-pyrimidinedione structure **7** (keto form) in thymidine are different from those of pyridine and its derivative as basic heteroaromatic compounds and are rather regarded as phenol-type proton-donor structures (enol forms) as illustrated by the equilibrium shown in Scheme 1.[14] A vinylstannyl moiety in the 2,4-pyrimidinedione structure would also favor the reaction conditions including the Cu^I/CsF synergic system over those including Cu^I/K_2CO_3 (Table 1 entries 7 vs. 4) as previously reported for the rapid *C*-methylation of alkenylstannane precursors.[1g,12] Although an excess amount of phosphine is needed at low temperature to obtain high yield (60 °C, Table 1 entry 7), such an excess phosphine was not necessary at elevated temperature (80 °C, see Table 1 entry 5). Overall, we concluded that the optimized conditions for rapid *C*-methylation for **6** are CH_3I/**6**/$Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$/CuBr/CsF (1 : 25 : 1 : 32 : 2 : 5 in molar ratio) at 60 °C for 5 min.



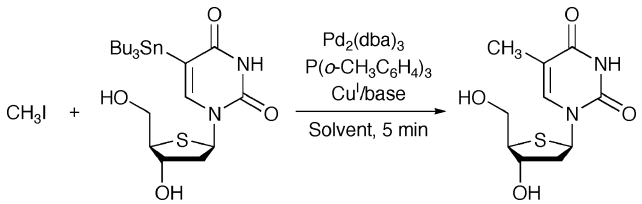
Scheme 1 2,4-Pyrimidinedione structure **7** (keto form) is equilibrated with two kinds of enol forms.

The chemo-response of 5-tributylstannyl-4′-thio-2′-deoxyuridine (**5**) in the Pd^0-mediated reaction was considerably different from that of **6**. The reaction was conducted similarly to that with **6** by using methyl iodide and excess stannyl substrate **5** (1 : 25), which was synthesized from 2-deoxy-D-ribose *via* 12 steps according to the previous paper.[15] As seen in Table 2, we first evaluated the reaction conditions reported by J. Toyohara and co-workers using CH_3I/**5**/$Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$ (1 : 25 : 1 : 4 in molar ratio) at 130 °C for 5 min,[9b] giving the desired 4′-thiothymidine (**4**) in only 30% yield (Table 2 entry 1, HPLC: t_R = 12.0 min).[11] Next, the optimized conditions elaborated in the study on **6** (Table 1 entry 7) were applied to this stannyl compound **5** using a combination of CH_3I/**5**/$Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$/CuBr/CsF (1 : 25 : 1 : 32 : 2 : 5 in molar ratio) at 60 °C for 5 min, unexpectedly giving the desired product **4** in only 40% yield (Table 2 entry 2). Increasing the quantity of CuBr/CsF at this temperature improved the yield considerably but was still insufficient (64%, Table 2 entry 3). The use of NMP as the solvent revealed a similar result (Table 2 entry 4).[16] Here, we raised the temperature (80 °C) in DMF to substantially accelerate the reaction, giving **4** in 83% yield (Table 2 entry 3). However, HPLC analysis of the reaction mixtures obtained in entries 2 and 3 showed that the reaction generated the destannylated compound **8** (HPLC: t_R = 6.8 min)[11] as a byproduct to a considerable extent (20–49% for an initial amount of **5**).[17] This observation prompted us to change the use of the CuBr/CsF synergic system to $CuCl/K_2CO_3$ with the aim of suppressing such destannylation. Thus, application of our previously reported conditions[1a] for the synthesis of toluene using the combination of CH_3I/**5**/$Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$/$CuCl/K_2CO_3$ (1 : 25 : 0.5 : 2 : 2 in molar ratio) in DMF at 60 and 80 °C for 5 min was attempted to give the desired product **4** in 57 and 69% yields, respectively (Table 2 entry 5). Further, the reaction with increased Pd^0/CH_3I ratio (2 : 1) using CH_3I/**5**/$Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$/$CuCl/K_2CO_3$ (1 : 25 : 1 : 4 : 2 : 5 in molar ratio) at 80 °C for 5 min in DMF gave **4** in 78% yield (Table 2 entry 6). Although the yield was lower than that of the reaction shown as entry 3 in Table 2, it was noted that the formation of the destannylated compound **8** was much decreased (5–8%) compared with the CuBr/CsF system.[17] Finally, the use of an increased amount of the phosphine (32 equiv) promoted the reaction markedly to give the desired product **4** in 83% yield at 60 °C and then in 98% yield at a slightly raised temperature (80 °C, Table 2 entry 8). Decreasing the amount of phosphine (16 equiv) gave slightly lower yield (Table 2 entry 7).

The promotion of destannylation in the CuBr/CsF system and its suppression in the $CuCl/K_2CO_3$ system could be rationalized by the following explanation as shown in Scheme 2. In the presence of a Cu^I salt, the stannyl substrate **5** containing a sulfide ring could establish an equilibrium with $\mathbf{5}_{Cu1}$ and $\mathbf{5}_{Cu2}$ formed by the first coordination of a Cu^I salt with a sulfur atom in the sugar moiety ($\mathbf{5}_{Cu1}$), and then by further coordination with the carbonyl of the 2,4-pyrimidinedione part ($\mathbf{5}_{Cu2}$, Scheme 2).[18] By such copper(I) behavior, the transmetallation of Sn/Cu could be slowed and the N–H (or O–H in enol form, see Scheme 1) bond in pyrimidinedione would become more acidic than that of non Cu^I-coordinated substrate **5**. As a result, the compound $\mathbf{5}_{Cu2}$ could be a strong proton donor capable of protonating a C–Cu bond in organo copper compound **8**, generated with one more Cu^I salt, giving destannylated product **9** as a byproduct. On the contrary, in the system of the $CuCl/K_2CO_3$, the stannyl substrate **5** was changed to a proton acceptor **10** by deprotonation with a base, resulting in suppressing the destannylation observed in the CuBr/CsF system.[19] We consider that excess phosphine serves to dissociate the coordination of various heteroatoms in the structure of substrate with Pd^0 and/or Cu^I complexes to regenerate the reactivity of native tin(IV) and copper(I) species to promote the reaction (see also Table 1 entry 7).[1i]

Thus, we found that appropriate selection of the CuBr/CsF and $CuCl/K_2CO_3$ synergic systems is quite important to realize the highly efficient rapid *C*-methylations of **6** and **5**, respectively.

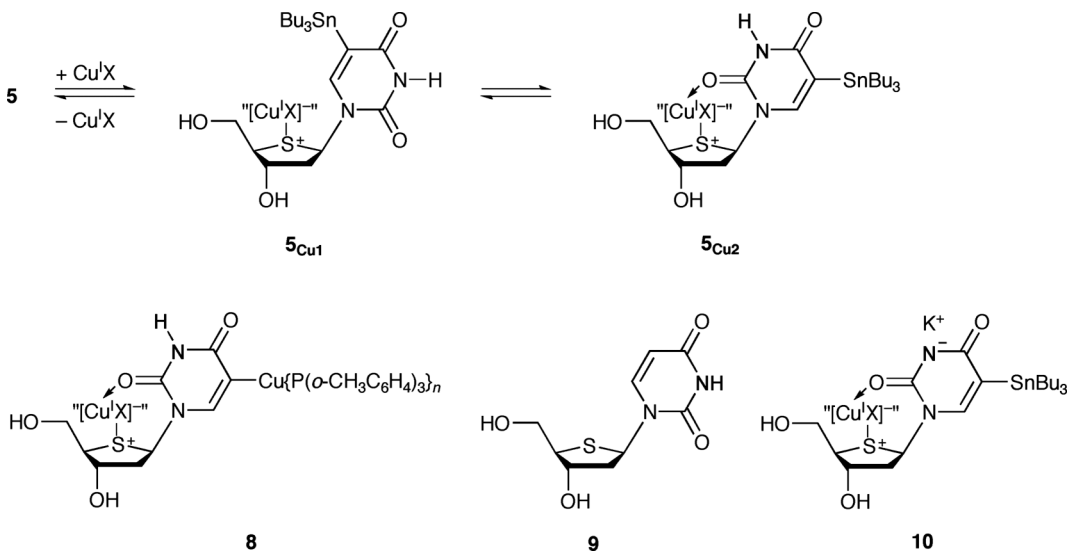
Table 2 Synthesis of a 4′-thiothymidine (**4**) by the rapid trapping of methyl iodide with 5-tributylstannyl-4′-thio-2′-deoxyuridine (**5**)[a]



5 **4**

Entry	$Pd_2(dba)_3$ (equiv)	$P(o\text{-}CH_3C_6H_4)_3$ (equiv)	Cu^I/base (molar ratio)	Solvent	Yield of 4[b] (%) Temp. (°C) 60	80	130
1	1	4	none	DMF	—	—	30
2	1	32	CuBr/CsF (2 : 5)	DMF	40	—	—
3	1	32	CuBr/CsF (10 : 25)	DMF	64	83	—
4	1	32	CuBr/CsF (10 : 25)	NMP	60	—	—
5	0.5	2	$CuCl/K_2CO_3$ (2 : 2)	DMF	57	69	—
6	1	4	$CuCl/K_2CO_3$ (2 : 5)	DMF	—	78	—
7	1	16	$CuCl/K_2CO_3$ (2 : 5)	DMF	—	**94**	—
8	1	32	$CuCl/K_2CO_3$ (2 : 5)	DMF	83	**98**	—

[a] Reaction was carried out with 25 equiv of **5** relative to methyl iodide (1.0 μmol). [b] The yields were determined by HPLC analysis based on the CH_3I consumption using acridine as an internal standard.



Scheme 2 Assumed equilibration between a stannyl thiothymidine **5** and a Cu^I salt.

The utility of the established reaction conditions was well demonstrated by the syntheses of [methyl-^{11}C]thymidine ([^{11}C]**1**) and 4′-[^{11}C-methyl]thiothymidine ([^{11}C]**4**). In our experience with actual PET studies, we had found that the continuous two-pot operations to minimize the negative secondary effect of CuI generated in situ[1f] gave a better result in terms of the reproducibility. Therefore, the reaction was conducted according to such two-pot operation using $Pd_2(dba)_3/P(o\text{-}CH_3C_6H_4)_3/CuBr/CsF$ (1 : 32 : 2 : 5 in molar ratio, see Table 1 entry 7)[10] system. Thus, the successive mixing of the Pd^0/phosphine complex with [^{11}C]methyl iodide in DMF and then with the stannane **6** in the presence of CuBr and CsF in DMF followed by heating at 65 °C for 5 min gave [^{11}C]**1** in 87% analytical yield[20] as judged by HPLC (Figs. 1a and b), indicating the high reaction efficiency.[21,22] The ^{11}C-labeled 4′-thiothymidine ([^{11}C]**4**) was also synthesized using the corresponding tin substrate (**5**) under the similar procedure as the synthesis of [^{11}C]**1** using the $Pd_2(dba)_3/P(o\text{-}CH_3C_6H_4)_3/CuCl/K_2CO_3$ (1 : 32 : 2 : 5 in molar ratio, see Table 2 entry 8)[10] system at 80 °C for 5 min to give [^{11}C]**4** in 93% HPLC analytical yield[20] (Figs. 1c and d). The reactions for the isolation of pure [^{11}C]**1** and [^{11}C]**4** were conducted by slightly improved conditions using a half amount of phosphine (16 equiv) from a practical point of view[23] under the conditions $Pd_2(dba)_3/P(o\text{-}CH_3C_6H_4)_3/CuBr/CsF$ (1 : 16 : 2 : 10 in molar ratio)[10] at 80 °C[24]

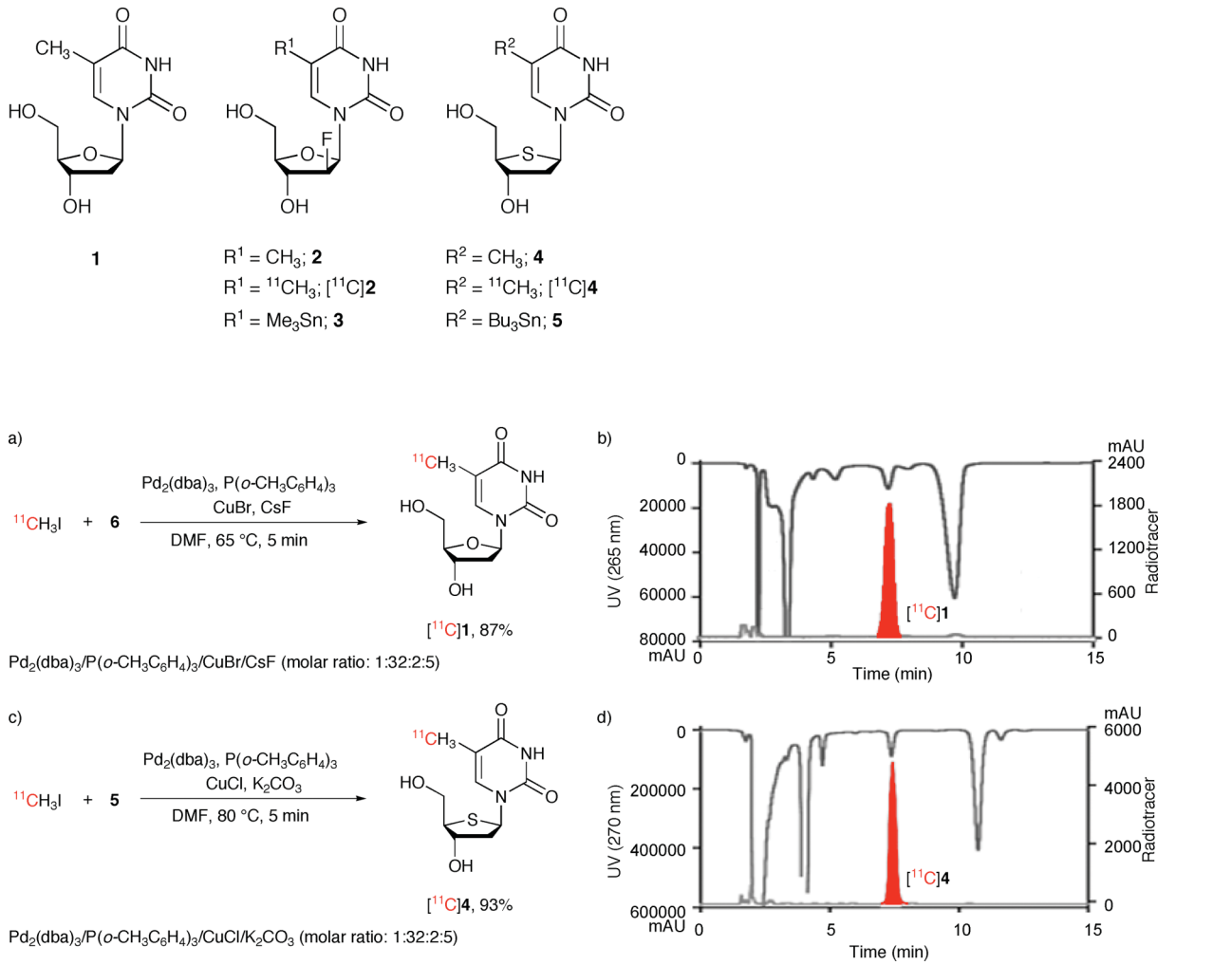


Fig. 1 a) Synthetic scheme of [methyl-^{11}C]thymidine ([^{11}C]**1**), and b) the chart of high performance liquid chromatography in the analysis of [^{11}C]**1** (radioactivity and UV vs. time). c) Synthetic scheme of 4′-[methyl-^{11}C]thiothymidine ([^{11}C]**4**), and d) the chart of high performance liquid chromatography in the analysis of [^{11}C]**4** (radioactivity and UV vs. time). The peak at a retention time of 7.6 min shown in b) is [^{11}C]**1** and the peak at a retention time of 7.4 min shown in d) is [^{11}C]**4**.

and $Pd_2(dba)_3/P(o\text{-}CH_3C_6H_4)_3/CuCl/K_2CO_3$ (1 : 16 : 3 : 8 in molar ratio)[10] at 80 °C. Purifications by preparative HPLC gave pure single products with high radiochemical purity (≥99.5% each, see ESI‡). Total synthesis time was 42 min in each case until the radiopharmaceutical formulation, exhibiting radioactivity of 3.7 and 3.8 GBq, respectively.

Decay-corrected radiochemical yields of [^{11}C]**1** and [^{11}C]**4** based on the radioactivity of [^{11}C]CH_3I trapped in the Pd solution[25] were 45 and 42–59%, respectively.[26] Specific radioactivity of [^{11}C]**1** and [^{11}C]**4** after the formulation was in the range of 89–200 GBq μmol^{-1}. Thus, the quantity of radioactivity and the radiochemical quality of [^{11}C]**1** and [^{11}C]**4** obtained above are sufficiently satisfactory for animal and human PET studies. We are currently in the process of applying these synthetic procedures to the synthesis under the guideline of Good Manufacturing Practice.

Conclusions

We elaborated the rapid *C*-methylations with high-yield and practical reaction conditions for the syntheses of thymidine and its metabolically stable analog, 4′-thiothymidine, with the aim of incorporating a short-lived ^{11}C in these biologically significant compounds. The optimized reaction conditions are CH_3I/**6**/$Pd_2(dba)_3/P(o\text{-}CH_3C_6H_4)_3$/CuBr/CsF (1 : 25 : 1 : 32 : 2 : 5 in molar ratio) at 60 °C for 5 min and CH_3I/**5**/$Pd_2(dba)_3/P(o\text{-}CH_3C_6H_4)_3/CuCl/K_2CO_3$ (1 : 25 : 1 : 32 : 2 : 5 in molar ratio), giving **1** and **4** in 100 and 98% yields, respectively. The utility of these rapid *C*-methylations was demonstrated by efficient syntheses of the PET probes, [methyl-^{11}C]thymidine and 4′-[methyl-^{11}C]thiothymidine, giving [^{11}C]**1** and [^{11}C]**4** in 87 and 93% (HPLC analytical yields), respectively. Pure [^{11}C]**1** and [^{11}C]**4** were obtained under slightly improved reaction conditions followed by preparative HPLC separation, and the radiopharmaceutical formulation for administration, exhibiting sufficient radioactivity of 3.7–3.8 GBq and high radiochemical purities (≥99.5% each). These radioactivities and radiochemical qualities are adequate for both animal and human PET studies.[27,28] These methods could readily be applicable to ^{11}C-labeling of the thymidine derivatives such as [^{11}C]FMAU[3], [^{11}C]FLT[3] and their thiothymidine analogues[29] as well as thymidine-included artificial oligonucleotides with high

metabolic stability and binding affinity such as Bridged Nucleic Acids as a bridged nucleic acid analogue possessing a fixed N-type sugar conformation.[30]

Experimental section

General remarks

All reactions were performed under argon (Ar) with Schlenk techniques. Solvents and solutions were transformed by syringe–septum and cannula techniques. Methyl iodide (Nacalai) was distilled over P_4O_{10} prior to use. Dehydrated *N*,*N*-dimethylformamide (DMF; Kanto), *N*-methyl-2-pyrrolidinone (NMP; Kanto), tris(dibenzylideneacetone)dipalladium(0) (Aldrich), tri-*o*-tolylphosphine (Aldrich), copper(I) chloride (Wako), copper(I) bromide (Wako), potassium carbonate (Wako), and cesium fluoride (Aldrich) were commercial grade. Stannane **6** was prepared according to a literature procedure.[31] Methyl iodide was distilled over P_4O_{10} prior to use. The [^{11}C]methylation reactions in Fig. 1 were conducted in a lead-shielded hot cell with remote control of all operations. [^{11}C]Carbon dioxide was produced by a $^{14}N(p,\alpha)^{11}C$ reaction using a Sumitomo CYPRIS HM-12S cyclotron (Sumitomo Heavy Industries) and then converted into [^{11}C]methyl iodide by hydriodic acid using an original automated synthesis system for ^{11}C-labeling in RIKEN CMIS. The [^{11}C]methyl iodide obtained was used for the palladium(0)-mediated rapid *C*-[^{11}C]methylation reaction shown in Fig. 1.

Product analysis

Yields of the reaction were determined by HPLC analysis using following conditions.

Conditions A. Instrument: Shimadzu HPLC system with a system controller (SCL-10AVP), a degasser (DGU-12A), a liquid chromatograph (LC-10AT and LC-10ATVP), a column oven (CTO-10AVP), an UV-vis detector (SPD-10A), and software (CLASS-VP); column, SHIM-PACK CLC-ODS (6.0 mm I.D. × 150 mm, SHIMADZU); mobil phase, CH_3CN:100 mM NaH_2PO_4 containing 200 mM $NaClO_4$ (pH 2) (6 : 94, v/v); flow rate, 1.0 mL min^{-1}; detection, UV 260 nm; column temperature, 40 °C; retention time, thymidine (**1**), 6.1 min, 2′-deoxyuridine, 4.7 min.

Conditions B. Instrument: JASCO HPLC system with a chromatography interface (LC-Net II/ADC), a quaternary HPLC pump (PU-2089Plus), a diode array detector (MD-2010Plus), a column oven (CO-2065Plus), a recycling valve unit (RV-2080-02), and software (chrom NAV chromatography data system); column, SHIM-PACK CLC-ODS (6.0 mm I.D. × 150 mm, SHIMADZU); mobil phase, CH_3CN/100 mM NaH_2PO_4 containing 200 mM $NaClO_4$ (pH 2) (6 : 94, v/v); flow rate, 1.0 mL min^{-1}; detection, UV 260 nm; column temperature, 40 °C; retention time, 4′-thiothymidine (**4**), 12.0 min; 2′-deoxy-4′-thiouridine (**8**), 6.8 min.

Conditions C. Instrument: Shimadzu HPLC system with a system controller (CBM-20A), an online degasser (DHU-20A$_3$), a solvent delivery unit (LC-20AD), a column oven (CTO-20AC), a photodiode array detector (SPD-20A), and software (LC solution) and an Aloka radioanalyzer (RLC-700); column, Luna 5 μ C18 (2) (4.6 mm I.D. × 150 mm, Phenomenex); mobil phase, CH_3CN/100 mM NH_4HCO_3 (pH 7.8) (3 : 97, v/v); flow rate, 1.0 mL min^{-1}; detection, UV 265 nm; column temperature, 40 °C; retention time, [^{11}C]**1**, 7.6 min; 2′-deoxyuridine, 5.8 min; mobil phase, CH_3CN/100 mM NH_4HCO_3 (pH 7.8) (8 : 92, v/v); flow rate, 1.0 mL min^{-1}; detection, UV 270 nm; column temperature, 30 °C; retention time, [^{11}C]**4**, 7.4 min.

Rapid coupling of methyl iodide with 5-tributylstannyl-2′-deoxyuridine (6) to afford thymidine (1; Table 1 entry 7)

In a dry Schlenk tube (10 mL), $Pd_2(dba)_3$ (1.8 mg, 2.0 μmol), P(*o*-$CH_3C_6H_4)_3$ (19.5 mg, 64.0 μmol), CuBr (0.6 mg, 4 μmol) and CsF (1.5 mg, 10 μmol) were placed under Ar. After the addition of DMF (250 μL), the mixture was stirred for 5 min at RT, followed by successive additions of the solutions of stannane **6** (25.9 mg, 50.0 μmol) in DMF (150 μL) and methyl iodide (0.20 M DMF solution, 10 μL, 2.0 μmol). After stirring at 60 °C for 5 min, the mixture was rapidly cooled in an ice bath. The resulting mixture was loaded onto a short column of silica-gel (0.5 g) and eluted with DMF (*ca.* 1 mL), followed by the addition of acridine (0.10 M DMF solution, 10 μL, 1.0 μmol) as an internal standard. The resulting solution was analyzed HPLC under *Conditions A*. Yield of **1**: 100%. The product was identified by HPLC with an added authentic reference (see ESI‡). The methylation reactions under other conditions in Table 1 were conducted by the same procedure as those in entry 7.

Rapid coupling of methyl iodide with 5-tributylstannyl-4′-thio-2′-deoxyuridine (5) to afford 4′-thiothymidine (4; Table 2 entry 8)

In a dry Schlenk tube (10 mL), $Pd_2(dba)_3$ (0.9 mg, 1 μmol), P(*o*-$CH_3C_6H_4)_3$ (9.8 mg, 32 μmol), CuCl (0.2 mg, 2 μmol) and K_2CO_3 (0.7 mg, 5 μmol) were placed under Ar. After the addition of DMF (150 μL), the mixture was stirred for 3 min at RT, followed by successive additions of the solutions of stannane **5** (13 mg, 25 μmol) in DMF (100 μL) and methyl iodide (40 mM DMF solution, 25 μL, 1.0 μmol). After stirring at 80 °C for 5 min, the mixture was rapidly cooled in an ice bath. The resulting mixture was loaded onto a short column of silica-gel (0.3 g) and eluted with DMF (*ca.* 0.5 mL), followed by the addition of acridine (0.10 M DMF solution, 20 μL, 2.0 μmol) as an internal standard. The resulting solution was analyzed by HPLC under *Conditions B*. Yield of **4**: 98%. The product was identified by HPLC with an added authentic reference (see ESI‡). The methylation reactions under other conditions in Table 2 were conducted by the same procedure as those in entry 8.

Rapid coupling of methyl iodide with 5-tributylstannyl-2′-deoxyuridine (6) to afford [methyl-^{11}C]thymidine ([^{11}C]1) in order to determine the HPLC analytical yield

[^{11}C]Methyl iodide was prepared from [^{11}C]CO_2 *via* reduction with $LiAlH_4$, followed by HI treatment according to the established method.[2] [^{11}C]Methyl iodide was trapped in a solution of $Pd_2(dba)_3$ (0.9 mg, 1 μmol) and P(*o*-$CH_3C_6H_4)_3$ (9.7 mg, 32 μmol) in DMF (300 μL) and the mixture was heated at 40 °C for 1 min. The resulting solution was added to a solution of stannane **6** (2.0 mg, 3.9 μmol), CuBr (0.3 mg, 2 μmol), and CsF (0.8 mg, 5 μmol) in DMF (130 μL). The combined mixture was heated at 65 °C for 5 min and then diluted with water (1 mL). The mixture was passed through cotton and then Syringe Filter (filter

media PVDF, pore size 0.2 μm, Whatman Inc.) and analyzed by HPLC under *Conditions C*. HPLC analytical yield of [^{11}C]**1**: 87%, calculated by peak area ratio of the [^{11}C]product distributions. The identifications of [^{11}C]**1** was conducted by co-injecting with the corresponding non-radioactive thymidine (see ESI‡).

Synthesis of [methyl-^{11}C]thymidine ([^{11}C]1) for a PET study

[^{11}C]Methyl iodide was trapped in a solution containing $Pd_2(dba)_3$ (1.2 mg, 1.3 μmol) and P(*o*-$CH_3C_6H_4)_3$ (2.5 mg, 8.2 μmol)[23,32] in DMF (300 μL) at RT. The solution was added to the mixture of stannane **6** (5.5 mg, 11 μmol), P(*o*-$CH_3C_6H_4)_3$ (3.7 mg, 12 μmol),[32] CuBr (0.4 mg, 3 μmol), and CsF (2.0 mg, 13 μmol) in DMF (80 μL) and then apparatus was rinsed with DMF (250 μL). The radioactivity of [^{11}C]CH_3I trapped in the Pd solution was measured as approximately 24 GBq. The combined mixture was heated at 80 °C for 5 min, and then salts and palladium residue in the reaction mixture were removed by passing through cotton and then Syringe Filter (pore size 0.2 μm) and washed with 8% aqueous ascorbic acid solution (1.5 mL). The combined elutes were injected into preparative HPLC {semi-preparative column, Gemini 5 C18 (21.2 mm I.D. × 250 mm, Phenomenex); mobile phase, CH_3CN/100 mM NH_4HCO_3 (pH 7.8) (4 : 96, v/v); flow rate, 9.9 mL min^{-1}; detection, UV 265 nm} to give a radioactive fraction corresponding to [^{11}C]**1** (retention time, 12.5 min, see ESI‡) with radioactivity of 5.4 GBq. The decay-corrected radiochemical yield based on the radioactivity of [^{11}C]CH_3I trapped in the Pd solution was calculated to be 45%. The desired fraction was corrected into a flask containing 25% ascorbic acid solution (400 μL) and evaporated under reduced pressure. The residue was diluted with saline solution (4 mL). The total synthesis time including HPLC purification and radiopharmaceutical formulation was 42 min. The radioactivity of a formulated injection solution of saline (4–5 mL) was 3.7 GBq with specific radioactivity of up to 89 GBq μmol^{-1}. The chemical identity of [^{11}C]**1** was confirmed by HPLC under *Conditions C*. The chemical purity was ≥98% and the radiochemical purity was ≥99.5%.

Rapid coupling of methyl iodide with 5-tributylstannyl-4′-thio-2′-deoxyuridine (5) to afford 4′-[methyl-^{11}C]thiothymidine ([^{11}C]4) in order to determine the HPLC analytical yield

[^{11}C]Methyl iodide was trapped in a solution of $Pd_2(dba)_3$ (1.8 mg, 2.0 μmol) and P(*o*-$CH_3C_6H_4)_3$ (19 mg, 64 μmol) in DMF (300 μL) at RT. The solution was added to a solution of stannane **5** (4.3 mg, 8.0 μmol), CuCl (0.4 mg, 4 μmol), and K_2CO_3 (1.4 mg, 10 μmol) in DMF (100 μL). The resulting mixture was heated at 80 °C for 5 min. The reaction mixture were passed through cotton and then Syring Filter and were washed with water (1 mL). The combined elutes were analyzed by HPLC under *Conditions C*. HPLC analytical yield of [^{11}C]**4**: 93%, calculated by peak area ratio of the [^{11}C]product distributions. The identifications of [^{11}C]**4** was conducted by co-injecting with the corresponding non-radioactive 4′-thiothymidine (see ESI‡).

Synthesis of 4′-[methyl-^{11}C]thiothymidine ([^{11}C]4) for a PET study

[^{11}C]Methyl iodide was trapped in the solution of $Pd_2(dba)_3$ (1.1 mg, 1.2 μmol) and P(*o*-$CH_3C_6H_4)_3$ (2.1 mg, 6.9 μmol)[23,32] in DMF (300 μL) at RT. The solution was transferred into the mixture of stannane **5** (4.3 mg, 8.0 μmol), P(*o*-$CH_3C_6H_4)_3$ (3.7 mg, 12 μmol),[32] CuCl (0.4 mg, 4 μmol), and K_2CO_3 (1.4 mg, 10 μmol) in DMF (80 μL) and then apparatus was rinsed with DMF (250 μL). The radioactivity of [^{11}C]CH_3I trapped in the Pd solution was measured as approximately 24 GBq. The combined mixture was heated at 80 °C for 5 min, and then the salts and palladium residue in the reaction mixture were removed by passing through cotton and then Syringe Filter (pore size 0.2 μm) and washed with 10% aqueous acetonitrile (1.5 mL). The combined elutes were injected into preparative HPLC {semi-preparative column, Gemini 5 C18 (21.2 mm I.D. × 250 mm, Phenomenex); mobil phase, CH_3CN/100 mM NH_4HCO_3 (pH 7.8) (7 : 93, v/v); flow rate, 9.9 mL min^{-1}; detection, UV 270 nm} to give a radioactive fraction corresponding to [^{11}C]**4** (retention time, 12.5 min, see ESI‡) with radioactivity of 6.9 GBq. The decay-corrected radiochemical yield based on the radioactivity of [^{11}C]CH_3I trapped in the Pd solution was calculated to be 59%. The desired fraction was collected into a flask, and the organic solvent was removed under reduced pressure. The desired [^{11}C]product was dissolved in a mixture of 25% ascorbic acid (200 μL) and saline (4 mL). The total synthesis time including HPLC purification and radiopharmaceutical formulation was 42 min. The radioactivity of a formulated injection solution of saline (4–5 mL) was 3.8 GBq with the specific radioactivity in the range of 89–200 GBq μmol^{-1}. The chemical purity of the product was ≥98% and the radiochemical purity of the product was ≥99.5%.

Acknowledgements

This work was supported in part by the Research & Development of Life Science Fields responding to the needs of society, Molecular Imaging Research Program, of the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan. We thank Mr. Jeongwan Son for his technical assistance in part of the study for preparation of the tin substrate **6**.

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10 The synthesis of PET probes is rather different from the usual organic syntheses. The reaction involves the trapping of an extremely small amount of $^{11}CH_3I$ (approximately 100 nmol level containing $^{12}CH_3I$) with a large amount (mg level, >1000 equiv) of reacting substrate. Therefore, we here set up the reaction using an excess amount of a tributylstannyl substrate for CH_3I ([^{11}C]CH_3I) to meet the demand.

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20 Yield was obtained according to the equation of 100% × (radioactivity of desired product)/(total radioactivity of the products) based on the radioactivity observed in HPLC.

21 The same reaction was conducted using the conditions previously reported[5c] for $Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$ (1 : 4 in molar ratio) in DMF at 130 °C for 5 min, giving [methyl-^{11}C]thymidine [^{11}C]**1** in 17% (HPLC analytical yield).

22 [^{11}C]**1** was obtained in 91% (HPLC analytical yield) under the $Pd_2(dba)_3$/P(*o*-$CH_3C_6H_4)_3$/CuBr/CsF (1 : 32 : 10 : 25 in molar ratio) system in DMF, 65 °C, 5 min. Thus the increase of the amount of the CuBr/CsF tends to give the desired product in slightly higher yield.

23 We improved the reaction conditions to some extent under keeping the specified smaller-sized reaction vial combined with a preparative HPLC column for an actual PET probe synthesis in mind. Particularly, the limited solubility of the phosphine was compelled to the tedious preparative HPLC operations to purify small amounts of PET probes from an insoluble heterogeneous mixture. See experimental section and reference 1i.

24 See also Table 1 entry 5.

25 This study is focused on the rapid *C*-[^{11}C]methylation. In this context, $^{11}CH_3PdI$-phosphine complex is involved directly in the cross-coupling reaction with a stannyl substrate, and therefore, the radioactivity of non-volatile $^{11}CH_3PdI\{P(o\text{-}CH_3C_6H_4)_3\}_n$ formed by trapping $^{11}CH_3I$ with the Pd^0 complex was selected as a first checking point of total radioactivity.

26 Various factors such as radiolysis inducing radical reactions under high concentration during the evaporation of the solvent and the separation by preparative HPLC, the absorption on the HPLC solid support, time elongation, *etc.* are considered to be reflected on lowering the isolated yield. As a good example about the deviation of the isolated yield and efforts to suppress such a yield decreasing by adding a radical trapping agent to be sacrificed, see the synthesis of various ^{11}C-labeled 2-arylpropionic acids and their esters, see: M. Takashima-Hirano, M. Shukuri, T. Takashima, M. Goto, Y. Wada, Y. Watanabe, H. Onoe, H. Doi and M. Suzuki, *Chem.–Eur. J.*, 2010, **16**, 4250–4258.

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