ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Effect of Water on Direct Radioiodination of Small Molecules/Peptides Using Copper-Mediated Iododeboronation in Water−Alcohol Solvent

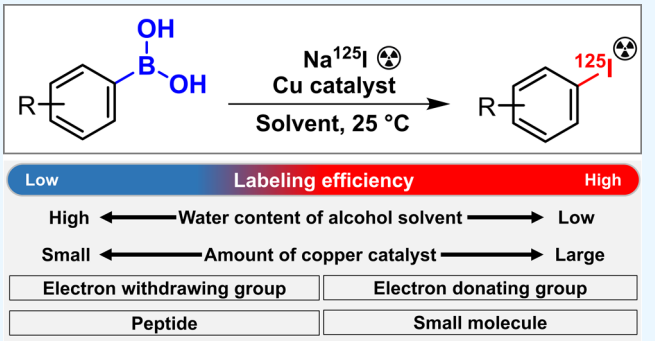
Yuto Kondo, Hiroyuki Kimura,* Minon Sasaki, Sumina Koike, Yusuke Yagi, Yasunao Hattori, Hidekazu Kawashima, and Hiroyuki Yasui

Cite This: *ACS Omega* 2023, 8, 24418−24425

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Direct radioiodination of peptides using copper-mediated iododeboronation is a promising radiosynthetic method for solving issues of classical direct radiolabeling, such as toxicity of the organotin precursor (iododestannylation) or formation of radio byproducts (by electrophilic iodination of a tyrosine residue). However, the parameters for optimizing the reaction conditions for various peptides are not completely understood. In particular, considering peptide solubility, the effects of water-containing solvents on labeling efficiency should be thoroughly investigated. Herein, we describe the effect of water on copper-mediated radioiododeboronation and the key factors for ensuring the successful radiolabeling of small molecules and peptides in water−organic solvents. ^{125}I-labeled substrates containing peptides ([^{125}I]*m*/*p*-IBTA) were obtained with high radiochemical conversions (RCCs: >95%) using an alcohol solvent, and a decrease in these RCCs was observed with increasing water content in the methanol solvent. Additionally, when using water−methanol solvents, a difference in RCC due to the substituent effect was also observed. However, the RCCs can be improved without the use of other additives by adjusting the copper catalyst and time of the labeling reaction or by utilizing substituent effects. This study contributes to the improvement of the design of boronic peptide precursors and radiolabeling protocols using copper-mediated iododeboronation.



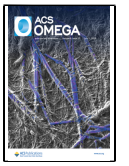
INTRODUCTION

Copper-mediated nucleophilic reactions using a boronic precursor are promising radiosynthetic methods for labeling an aromatic ring with radioiodine.[1−5] Copper-mediated radioiododeboronation is performed in a reaction vessel exposed to air at room temperature, leading to the formation of the desired radiolabeled compound without the preparation of complex starting materials or reagents. In addition, copper-mediated radioiododeboronation can also be adapted for direct radiolabeling of peptide mimetics.[4,5] Thus, it solves the issues of classical direct radiolabeling of peptides using radioiododestannylation or electrophilic radioiodination of tyrosine residues, such as toxicity of the organotin precursor or formation of byproducts.[6] However, information for optimizing reaction conditions for various peptides is ambiguous, as the method has scarcely been used for this type of compounds.

The solubility of peptides necessitates investigating the effect of water on labeling efficiency to enable reproducible direct radiolabeling for various types of peptides. Additionally, as iodine-123, -125, and -131 are often used as aqueous solutions of sodium iodide that contain sodium hydroxide, and radiolabeling reactions are performed on a smaller scale than general chemical reactions, the radioiodination reaction is affected by the aqueous solution from the radioisotope supply. Although water derived from radioisotope supply can be removed before radiolabeling, concentrating processes are undesirable due to exposure and limited working time caused by the half-life of radioisotopes.

Thus, investigating the effect of water on the radiolabeling reaction leads to the optimization of the procedure. In this study, we investigated the effect of water on copper-mediated iododeboronation at the tracer level. Before the radiosynthesis of ^{125}I-labeled octreotate derivatives ([^{125}I]*m*/*p*-IBTA), the fundamental investigation of the reaction parameters using small molecules (1−10) was performed as a model reaction.

Received: March 24, 2023
Accepted: June 14, 2023
Published: June 27, 2023



RESULTS AND DISCUSSION

The target compounds ([^{125}I]**2** and [^{125}I]**4**) were synthesized as shown in Figure 1. The radiolabeling protocol was prepared

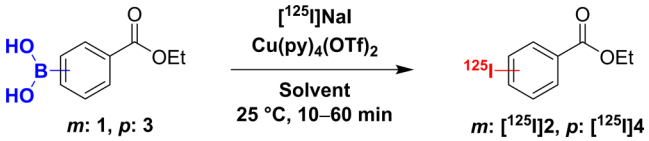


Figure 1. Radiosynthesis of [^{125}I]**2** or [^{125}I]**4** using copper-mediated iododeboronation. Reaction conditions: boronic precursor (**1** or **3**; 100 nmol), $Cu(py)_4(OTf)_2$ (0–40 nmol), solvent (0–50% v/v H_2O/methanol, ethanol, acetonitrile, *N*,*N*-dimethylformamide, or dimethyl sulfoxide; 100 μL). Legend: Et = ethyl, py = pyridine, OTf = trifluoromethanesulfonate.

according to previously reported methods with some modifications.[3−5] In this study, $Cu(py)_4(OTf)_2$ (py = pyridine, OTf = trifluoromethanesulfonate) was used as it was reported as the optimal copper catalyst for this labeling reaction.[3] A boronic precursor (**1** or **3**) and $Cu(py)_4(OTf)_2$ dissolved in a solvent, water (H_2O), or an organic solvent to control the H_2O content of the reaction solvent were added to a microtube in this precise order. A solution of [^{125}I]NaI (ca. 629 GBq/mg as iodine, 3.33−4.07 GBq/mL) was then added to the mixture, and the radiolabeling reaction occurred at 25 °C. The reaction solutions were analyzed using radio-thin-layer chromatography (radio-TLC) and radio-high-performance liquid chromatography (radio-HPLC) to calculate the radiochemical conversion (RCC). This study was performed using a $Cu(py)_4(OTf)_2$ within the parenteral permitted daily exposure (300 μg/day copper) defined in the draft guidance ICH Q3D[7] for the clinical use of copper-mediated radioiodination.

Initially, we performed a comparative study using different solvents. [^{125}I]**2** and [^{125}I]**4** were synthesized at 25 °C for 10 min using $Cu(py)_4(OTf)_2$ (5 nmol) and boronic precursors **1** and **3** (100 nmol), respectively. The RCC of [^{125}I]**2** and [^{125}I]**4** exceeded 97% when methanol (MeOH) was used as the solvent (Table 1, entries 1 and 6). Their RCCs exceeded 95% when ethanol (EtOH) was used (Table 1, entries 2 and 7). Thus, using MeOH or EtOH as solvents resulted in similar RCCs of the positional isomers. However, a significant decrease in the RCCs was observed when acetonitrile (MeCN), *N*,*N*-dimethylformamide (DMF), and dimethyl sulfoxide (DMSO) were used as solvents (Table 1). We further investigated the effects of adding protic polar solvents (MeOH and H_2O) to aprotic polar solvents on labeling efficiency (Table 2). The addition of MeOH to aprotic polar solvents improved the RCCs (Table 2, entries 1−3), while that of H_2O did not (Table 2, entries 4−6). The results in Tables 1 and 2 show that alcohol solvents such as MeOH are the optimal solvents for copper-mediated radioiodination, which prompted us to use H_2O/MeOH mixtures for our further investigation.

Table 1. Solvent Effect on Copper-Mediated Radioiododeboronation[b]

entry	precursor	solvent	RCC (%)[a]
1	**1**	MeOH	97.4 ± 0.8
2	**1**	EtOH	95.2 ± 0.6
3	**1**	MeCN	7.4 ± 0.6
4	**1**	DMF	-
5	**1**	DMSO	-
6	**3**	MeOH	97.7 ± 0.3
7	**3**	EtOH	95.9 ± 0.1
8	**3**	MeCN	3.3 ± 0.6
9	**3**	DMF	-
10	**3**	DMSO	-

[a]Data are presented as mean ± standard deviation (N = 3). [b]Reaction conditions: precursor (100 nmol), $Cu(py)_4(OTf)_2$ (5 nmol), 25 °C/10 min.

Table 2. Effect of MeOH and H_2O on the Labeling Efficiency[b]

entry	precursor	solvent	RCC (%)[a]
1	**1**	MeCN/MeOH = 1:1	75.7 ± 5.0
2	**1**	DMF/MeOH = 1:1	39.6 ± 1.5
3	**1**	DMSO/MeOH = 1:1	24.9 ± 1.1
4	**1**	MeCN/H_2O = 1:1	-
5	**1**	DMF/H_2O = 1:1	4.0 ± 0.2
6	**1**	DMSO/H_2O = 1:1	2.9 ± 0.5

[a]Data are presented as mean ± standard deviation (N = 3). [b]Reaction conditions: precursor (100 nmol), $Cu(py)_4(OTf)_2$ (5 nmol), 25 °C/10 min.

Then, [^{125}I]**2** or [^{125}I]**4** was synthesized using a mixture solvent comprising 0−50% v/v H_2O/MeOH for 10 min in the presence of $Cu(py)_4(OTf)_2$ (10 nmol). Consequently, the RCCs decreased with the increase in the H_2O content of the solvent (Table 3). Since a decrease in RCCs might appear due to the poor solubility of boronic precursors (**1** and **3**) or target compounds ([^{125}I]**2** and [^{125}I]**4**), a study using solvents with a water content of 50% or more was not performed. When the solvent mixture H_2O/MeOH was used, the RCC of [^{125}I]**4** was lower than that of [^{125}I]**2**. Furthermore, the RCC of [^{125}I]**2** and [^{125}I]**4** were investigated as functions of the copper catalyst concentration (0−40 nmol) and time of labeling reaction (0−60 min) (Figure 2). Consequently, the RCCs increased with the amount of $Cu(py)_4(OTf)_2$ and exceeded 85% for 40 nmol of $Cu(py)_4(OTf)_2$. We evaluated the influence of the reaction time (10, 30, or 60 min) on the RCC using a mixture solvent comprising 20% v/v H_2O/MeOH. A labeling time-dependent increase in the RCCs of [^{125}I]**2** and [^{125}I]**4** was observed (Figure 3). Their RCCs exceeded 90% at 30 min when using 20 nmol of $Cu(py)_4(OTf)_2$ and at 60 min when using 10 nmol of $Cu(py)_4(OTf)_2$. These results show that H_2O decreases the reaction rate of copper-mediated radioiododeboronation and the RCCs can be improved by

Table 3. Effect of H_2O Content of MeOH Solvent on the Labeling Efficiency[b]

entry	precursor	H_2O/MeOH (%)	RCC (%)[a]
1	**1**	0	97.2 ± 0.1
2	**1**	20	57.1 ± 3.8
3	**1**	50	20.1 ± 1.5
4	**3**	0	97.6 ± 0.5
5	**3**	20	45.1 ± 2.4
6	**3**	50	14.5 ± 0.6

[a]Data are presented as mean ± standard deviation (N = 3). [b]Reaction conditions: precursor (**1** or **3**; 100 nmol), $Cu(py)_4(OTf)_2$ (10 nmol), 25 °C/10 min.

https://doi.org/10.1021/acsomega.3c01974
ACS Omega 2023, 8, 24418−24425

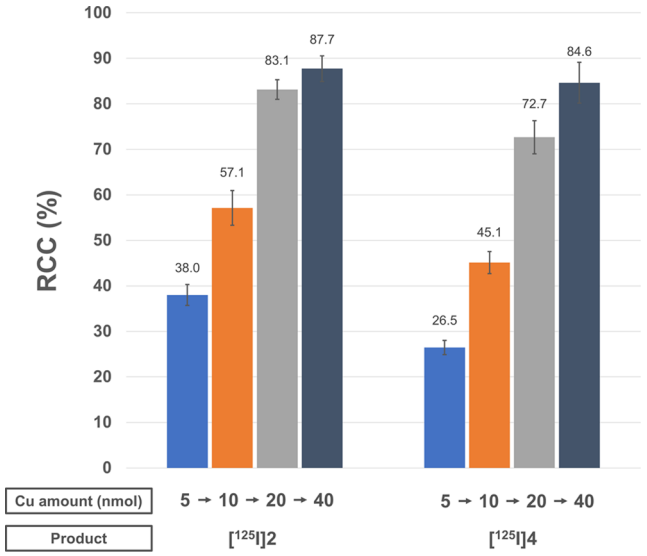


Figure 2. Effect of the copper catalyst amount (5−40 nmol) on RCCs of [^{125}I]**2** and [^{125}I]**4**. Reaction conditions: precursor (**1** or **3**; 100 nmol), 20% v/v H_2O/MeOH (100 μL), 25 °C/10 min.

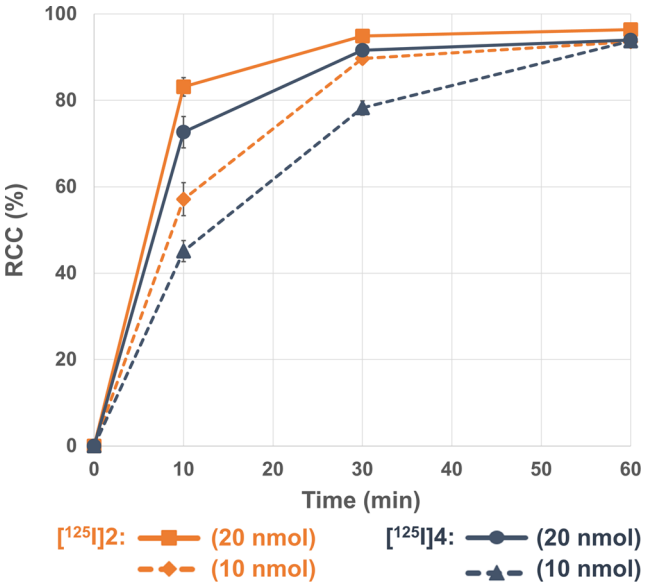


Figure 3. Effect of the labeling reaction time (0−60 min) on RCCs (%) of [^{125}I]**2** and [^{125}I]**4**. Reaction conditions: precursor (**1** or **3**; 100 nmol), $Cu(py)_4(OTf)_2$ (10 nmol: dashed lines, 20 nmol: full lines), 20% v/v H_2O/MeOH (100 μL), 25 °C.

adjusting the amount of copper catalyst and the time of the labeling reaction. Thus, to perform rapid radiolabeling, the water content in solvents should be decreased as much as possible. Additionally, for radioiodine recovered in water-containing solvents, highly concentrated radioiodine should be used.

A copper-mediated radiolabeling reaction using a boronic precursor is well tolerated at the tracer level in both electron-rich and electron-poor substrates, including sterically crowded substrates.[3,4] However, in this study, using a mixture of solvents containing H_2O/MeOH resulted in differences between positional isomers. These observations prompted us to investigate the effect of the substituents on labeling efficiency at the tracer level in a mixture solvent containing H_2O/MeOH. Thus, the syntheses of [^{125}I]**6**, [^{125}I]**8**, and [^{125}I]**10** were additionally carried out (Figure 4). When using MeOH as a solvent, the target compounds were obtained in high yields (RCCs = 94.9−97.6%) in arylboronic acids with electron-donating or electron-withdrawing groups, which is consistent with previous reports.[3,4] In contrast, when using a mixture solvent comprising 20 or 50% v/v H_2O/MeOH, the RCCs of substrates with electron-donating groups ([^{125}I]**8** and [^{125}I]**10**) were higher than those of substrates with electron-withdrawing groups ([^{125}I]**4** and [^{125}I]**6**). Previous mechanistic studies on Chan−Evans−Lam (CEL) etherification[8,9] and amination[10,11] have shown that transmetalation is the turnover-limiting step of the catalytic reaction, and electron-rich arylboronic acids undergo fast catalyst turnover. The ability of electron-donating groups to promote transmetalation from arylboronic acids has been documented.[9,12] The results illustrated in Figure 4 agree with those of previous reports on the CEL coupling reaction and explain the difference in RCCs between [^{125}I]**2** and [^{125}I]**4**.

The reaction mechanism of copper-mediated radioiodination was not clearly described to the best of our knowledge although the reaction mechanisms of CEL etherification and amination were investigated in detail.[8−11] We summarized the radioiodination mechanism in Figure 5 based on the reaction mechanism study of CEL etherification[8,9] and amination[10,11] and the results of this study. The formation of complexes (**11**, **12**, and **13**) has been proposed as the first step in transmetalation. Sulfonate[13] or hydroxy[10,11] groups enable bridging coordination with boron and facilitate transmetalation. Complex **13** is present when using MeOH as solvent[8,9] and the electron-donating effect of the methyl group on the oxygen atom may promote the formation of the complex required for transmetalation. Therefore, we believe that the reaction-promoting effect of the alcohol was confirmed by its facilitation of transmetalation. Conversely, when using a water−methanol mixed solvent, the formation of complex **13** decreases due to competition with that of complex **12**. Consequently, increasing water content in methanol affects the RCCs of target compounds, as shown in Table 3.

Based on the above results, direct radiolabeling of the peptides was performed using copper-mediated radioiododeboronation (Figure 6). The boronic precursors (*m*/*p*-PBTA) and standard compounds (*m*/*p*-IBTA) were synthesized using solid-phase peptide synthesis (Figure 7). The linear peptides were manually assembled using the standard 9-fluorenylmethyloxycarbonyl (Fmoc) protocol based on Fmoc-amino acid derivatives and carboxyphenylboronic acid (Cpb-OH) or iodobenzoic acid (Ibz-OH). After the linear peptides were deprotected and removed from the resin, the desired peptides were precipitated with cold diethyl ether and purified using HPLC. The linear peptides were further cyclized by air oxidation in a DMF solution of saturated ammonia hydrogen carbonate (NH_4HCO_3), and the mixture was purified using HPLC to yield *m*/*p*-PBTA and *m*/*p*-IBTA. Studies on boronic peptides and synthetic protocols using solid-phase peptide synthesis are abundant in the literature.[14] The synthesis of peptide boronic precursors is simpler and easier to automate than that of tributyltin or iodonium salt precursors. These are significant features of the copper-mediated radioiodination method using a peptide boronic precursor.

[^{125}I]*m*/*p*-IBTA was directly synthesized using the same protocol as for [^{125}I]**2** and [^{125}I]**4**. In a preliminary study, based on the results obtained for [^{125}I]**2** and [^{125}I]**4**, the

https://doi.org/10.1021/acsomega.3c01974
ACS Omega 2023, 8, 24418−24425

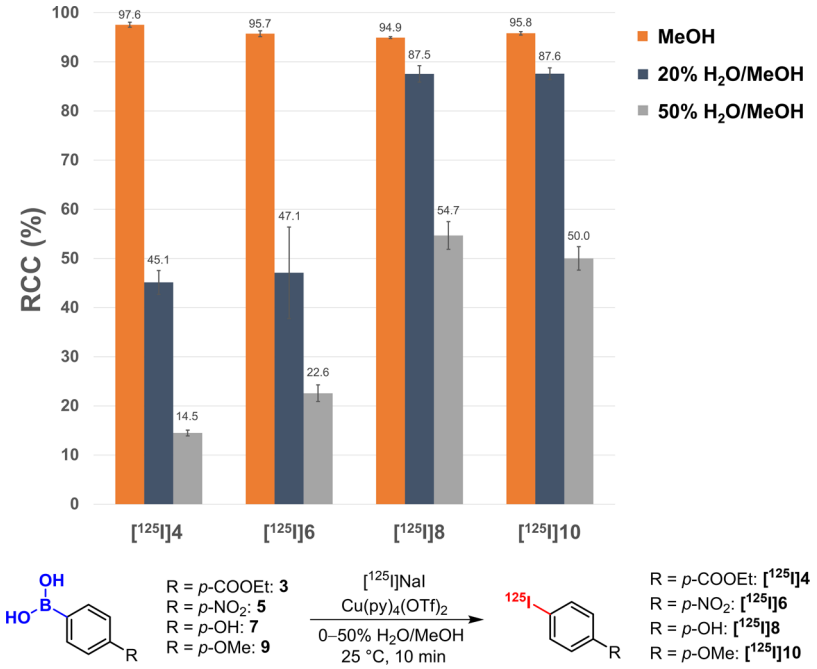


Figure 4. Substituent effect on copper-mediated radioiododeboronation. Reaction condition: precursor (**3**, **5**, **7**, and **9**; 100 nmol), $Cu(py)_4(OTf)_2$ (10 nmol), mixture solvent comprising 0–50% v/v H_2O/MeOH (100 μL), 25 °C/10 min.

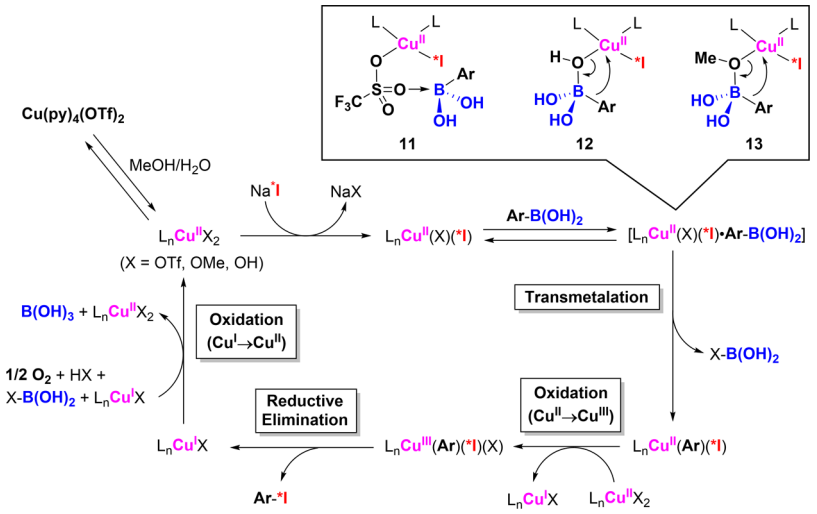


Figure 5. Proposed mechanism of copper-mediated radioiododeboronation (*I = ^{125}I).

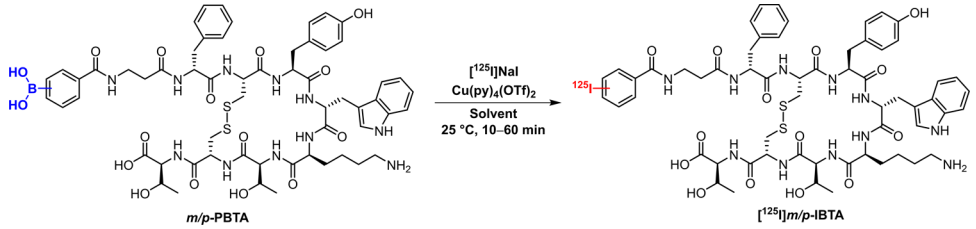


Figure 6. Radiosynthesis of [^{125}I]*m*/*p*-IBTA using copper-mediated radioiododeboronation. Reaction conditions: precursor (*m*/*p*-PBTA; 100 nmol) and solvent (MeOH or mixture comprising 20% v/v H_2O/MeOH; 100 μL).

synthesis of [^{125}I]*m*/*p*-IBTA was performed by using $Cu(py)_4(OTf)_2$ (5 nmol) in MeOH; however, the RCCs were low (p = 7.8%, m = 4.0%). Thus, the amount of $Cu(py)_4(OTf)_2$ was increased to 40 nmol, to improve the RCCs of [^{125}I]*m*/*p*-IBTA, as shown in Figure 8. Consequently, the RCC of [^{125}I]*m*-IBTA and [^{125}I]*p*-IBTA reached 98.6 ± 1.3% and 98.3 ± 1.7% when using 80 nmol and 160 nmol of $Cu(py)_4(OTf)_2$, respectively. The synthesis of [^{125}I]*m*/*p*-IBTA required a larger amount of copper catalyst for the RCCs to exceed 97% than that of [^{125}I]**2** and [^{125}I]**4**. Furthermore, the

https://doi.org/10.1021/acsomega.3c01974
ACS Omega 2023, 8, 24418−24425

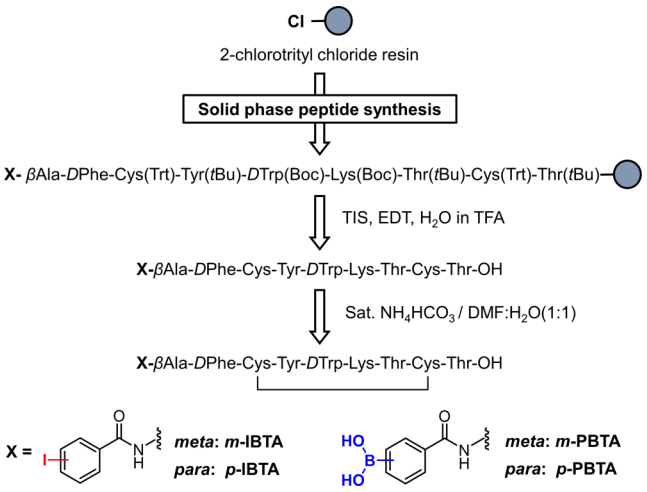


Figure 7. Synthesis of octreotate derivatives using solid-phase peptide synthesis method with 9-fluorenylmethyloxycarbonyl-based protocol.

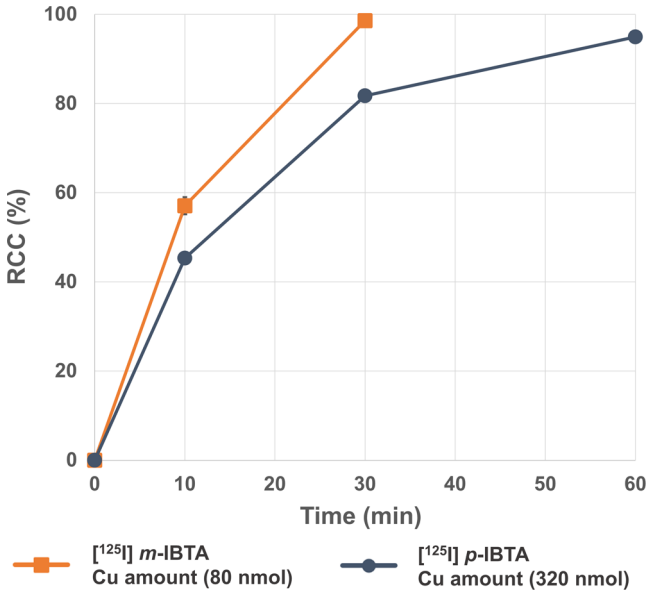


Figure 9. Effect of radiolabeling time (0−60 min) on RCCs of [^{125}I] *m*/*p*-IBTA. Reaction condition: precursor (*m*/*p*-PBTA; 100 nmol), solvent: 20% v/v H_2O/MeOH (100 μL), 25 °C.

synthesis of [^{125}I]*m*/*p*-IBTA in H_2O/MeOH solvent was conducted. Subsequently, the RCCs of [^{125}I]*m*/*p*-IBTA decreased when using a mixture solvent comprising 20% v/v H_2O/MeOH; however, they were improved by increasing the amount of the copper catalyst (Figure 8). In addition, a time-dependent increase in the RCCs was observed (Figure 9), similar to that observed for the synthesis of [^{125}I]**2** and [^{125}I]**4**. The RCC of [^{125}I]*m*-IBTA reached 98.6 ± 1.3% at 30 min when using 80 nmol of $Cu(py)_4(OTf)_2$ and that of [^{125}I]*p*-IBTA reached 94.8 ± 0.5% at 60 min when using 320 nmol of $Cu(py)_4(OTf)_2$. The synthesis of [^{125}I]*p*-IBTA required a larger amount of copper catalyst and a longer time of the labeling reaction than that of [^{125}I]*m*-IBTA to exceed 95% RCC. These results are similar to those observed for the synthesis of [^{125}I]**2** and [^{125}I]**4**. However, compared to [^{125}I]**2** and [^{125}I]**4**, [^{125}I]*m*/*p*-IBTA presented a significant difference in the RCCs between position isomers; thus, the effect of the substituents appears more clearly in the direct radiolabeling of the peptide.

According to our results, as the synthesis and preparation of radiolabeled probes require a rapid radiolabeling method considering their half-life, the radioiodination of peptides needs a larger amount of copper catalyst than that of small molecules. Thus, careful attention must be paid to the copper contamination in the sample solution. Therefore, for translational studies, inductively coupled plasma mass spectrometry (ICP-MS) analysis was performed on *m*-IBTA purified by a conventional HPLC technique to investigate the presence of residual copper (note: nonradioactive sodium iodide was used due to the regulation of this facility). However, we must highlight that the amount of $Cu(py)_4(OTf)_2$ used in this study

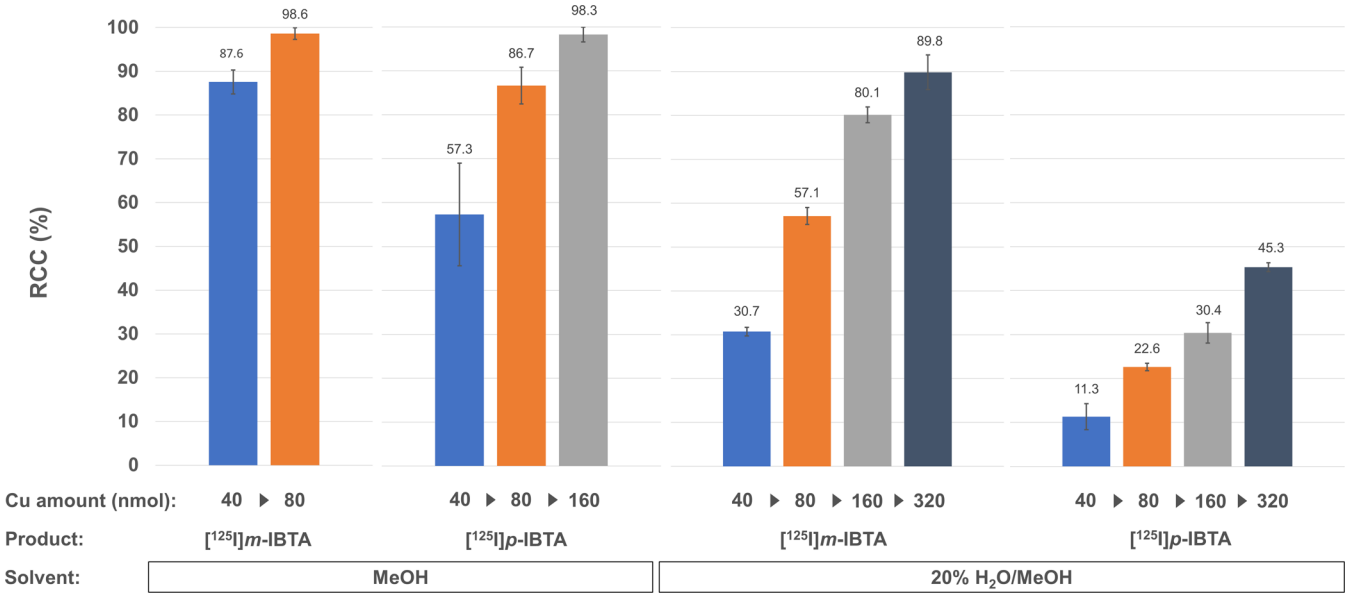


Figure 8. Effect of copper catalyst (40−320 nmol) on RCCs of [^{125}I]*m*/*p*-IBTA. Reaction condition: precursor (*m*/*p*-PBTA; 100 nmol), 25 °C/10 min.

https://doi.org/10.1021/acsomega.3c01974
ACS Omega 2023, 8, 24418−24425

was lower than the levels of concern defined in the draft guidance ICH Q3D.[7] The result of the ICP-MS analysis indicated that $Cu(py)_4(OTf)_2$ can be removed effectively as the labeled material contained 0.3 ng Cu^{63} residue (n = 4), an amount substantially lower than any level of concern.

CONCLUSIONS

We investigated the effect of water on copper-mediated radioiododeboronation and described key factors for the successful radiolabeling of small molecules and peptides in H_2O/MeOH solvents. The target ^{125}I-labeled compounds were obtained with high RCCs using an alcohol solvent, and a decrease in RCCs was observed with increasing the H_2O content in the MeOH solvent. Additionally, a difference in RCC owing to the substituent effect was confirmed when using H_2O/MeOH solvents. However, their RCCs can be improved without the use of other additives by adjusting the amount of the copper catalyst and the time of the labeling reaction or by utilizing the substituent effects. In this study on the direct labeling of peptides, the RCCs of [^{125}I]*m*/*p*-IBTA exceeded 94%, although they were affected by the content of H_2O in the mixture solvent. This study improves the design of boronic precursors and boronic precursor-based radiolabeling protocols using copper-mediated radioiodination, and its results might be useful not only for the preparation of radioiodinated probes but also for that of other radiohalogen-labeled probes.[15−18]

METHODS

General. All reagents were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), Nacalai Tesque, Inc. (Kyoto, Japan), Fujifilm Wako Chemical Corp. (Osaka, Japan), and Sigma-Aldrich (St. Louis, MO). They were used without further purification unless otherwise indicated. Carrier-free [^{125}I]NaI solution was purchased from PerkinElmer, Inc. (Waltham, MA).

High-resolution mass spectra were obtained using liquid chromatography coupled to ion trap time-of-flight mass spectrometry (ESI; Shimadzu, Kyoto, Japan). Cu concentrations were quantified using ICP-MS (Agilent 7700X/Mass Hunter; Agilent Technologies, Inc., Santa Clara, CA).

An autoradiograph of the reagents on the TLC sheet (5.0 cm × 2.0 cm; TLC silica gel 60 F_{254} aluminum plate; Merck, Kenilworth, NJ) was acquired using an image analyzer (Typhoon 9410; GE Healthcare, Waukesha, WI). The radioactive signal intensities of each TLC were evaluated from the respective images using ImageQuant TL software (GE Healthcare).

An LD-20AD (Shimadzu, Kyoto, Japan) was used for HPLC, along with a CBM-20A (Shimadzu) communication bus module, DGU-20A3R (Shimadzu) degassing unit, CTO-20AC (Shimadzu) column oven, SPD-20A (Shimadzu) ultraviolet detector (k = 254 nm), and γ-survey meter TCS-172 (ALOKA, Mitaka, Japan) or GABI Star (Elysia-Raytest GmbH, Straubenhardt, Germany) detector of radioisotope. COSMOSIL $5C_{18}$-AR-II column (4.6 ID × 150 mm or 10.0 ID × 150 mm; Nacalai Tesque, Inc.) was used for reversed-phase (RP-) HPLC.

^{1}H NMR and ^{13}C NMR spectra were measured with DMSO-d_6 as a solvent on an Ascend 500 (Bruker, Billerica, MA).

Synthesis of *m*-PBTA. The linear peptide was manually assembled from 2-chlorotrityl chloride resin using a standard Fmoc-protocol with Fmoc-amino acid derivatives and *m*Cpb-OH.

Coupling of First Fmoc-Amino Acids. In total, 2-chlorotrityl chloride resin (188.7 mg, loading capacity 1.06 mmol/g) was swelled in dichloromethane for 16 h. The resin was thoroughly washed with fresh DMF (1 × 3 mL). Fmoc-Thr(tBu)-OH (238.5 mg, 0.6 mmol, 3.0 equiv), *N*,*N*-diisopropylethylamine (DIEA; 102.0 μL, 0.6 mmol, 3.0 equiv), and DMF (1.5 mL) were mixed and added to the reaction vessel. The reaction mixture was agitated at 20 °C for 16 h. After the solution was removed, the resin was thoroughly washed with DMF (5 × 3 mL).

Capping 2-Chlorotrityl Chloride Resin. DMF (1.5 mL), MeOH, and DIEA (102.0 μL, 0.6 mmol, 3.0 equiv) were added to the resin. The reaction mixture was then agitated for 1 h at 25 °C. After the reaction solution was removed, the resin was thoroughly washed with DMF (5 × 3 mL). Piperidine (20%) in DMF (1.5 mL) was added to the reaction vessel and agitated for 30 min to deprotect the Fmoc groups of the amino acids. Once the reaction was completed, the resin was thoroughly washed with DMF (5 × 3 mL). Kaiser test showed that the resin was deprotected.

Coupling of the Second and Subsequent Amino Acids. Fmoc-Cys(Trt)-OH (351.4 mg, 0.6 mmol, 3.0 equiv) 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]-pyridinium 3-oxide hexafluorophosphate (HATU; 228.1 mg, 0.6 mmol, 3.0 equiv), DIEA (102.0 μL, 0.6 mmol, 3.0 equiv), and DMF (1.5 mL) were mixed and added to the reaction vessel. The reaction mixture was agitated for 3 h at 25 °C. After the reaction solution was removed, the resin was thoroughly washed with DMF (5 × 3 mL). A Kaiser test was performed to confirm the completion of coupling by showing the absence of free amines on the resin. Piperidine (20%) in DMF (1.5 mL) was added to the reaction vessel and agitated for 30 min to deprotect the Fmoc groups of the amino acids. Once completed, the resin was thoroughly washed with DMF (5 × 3 mL). Kaiser test showed that the resin was deprotected. Cycles of coupling, washing, deprotection, and washing were repeated until the desired peptide sequence was obtained. Finally, the resin was washed with MeOH (5 × 3 mL) and dried under reduced pressure.

Deprotection and Removal of the Peptide from the Resin. The linear peptide was cleaved from the solid support by the addition of trifluoroacetic acid (TFA)/triisopropylsilane (TIS)/1,2-ethanedithiol (EDT)/H_2O (94.0:1.0:2.5:2.5) for 3 h at 25 °C. The resin was removed by filtration and washed with TFA. The obtained solution was concentrated using a rotary evaporator. The residual oily compound was treated with cold diethyl ether to obtain a precipitate. The precipitate was washed thrice with diethyl ether. Finally, the mixture was purified using RP-HPLC [column: COSMOSIL $5C_{18}$-AR-II (10.0 ID × 150 mm); phase: MeCN:H_2O (containing 0.1% v/v TFA) = 27:73; flow rate: 4.5 mL/min; temperature: 25 °C] to yield the crude linear *m*-PBTA (*m*Cpb-*b*Ala-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-OH) (47.0 mg).

Cyclization by Air Oxidation. Crude linear peptide (47.0 mg) was dissolved in 4.7 mL (10 mg/mL) of saturated NH_4HCO_3/DMF:H_2O (1:1). The reaction solution was air-oxidized for 16 h at room temperature. Finally, the mixture was purified using RP-HPLC [column: COSMOSIL $5C_{18}$-AR-II (10.0 ID × 150 mm); phase: MeCN/H_2O (containing 0.1% v/v TFA) = 27:73; flow rate: 4.5 mL/min; temperature: 25 °C] to yield *m*-PBTA (16.2 mg, 12.8 μmol). The calculated mass of

https://doi.org/10.1021/acsomega.3c01974
ACS Omega 2023, 8, 24418−24425

$(M - 2H_2O + 2H)^{2+}$ was 616.7397 *m/z*. The detected mass of $(M - 2H_2O + 2H)^{2+}$ was 616.7395 *m/z*. ^1H and ^{13}C NMR (DMSO-d_6; see the Supporting Information).

Synthesis of *p*-PBTA. The linear peptide was manually assembled from 2-chlorotrityl chloride resin (188.7 mg, loading capacity 1.06 mmol/g) according to the standard Fmoc-protocol using Fmoc-amino acid derivatives and *p*Cpb-OH. After deprotection and removal of the peptide from the resin using TFA/TIS/EDT/H_2O (94.0:1.0:2.5:2.5), the obtained mixture was purified using RP-HPLC [column: COSMOSIL 5C_{18}-AR-II (10.0 ID × 150 mm); phase: MeCN/H_2O (containing 0.1%v/v TFA) = 28:72; flow rate: 4.5 mL/min; temperature: 25 °C] to evaluate the yield of the crude linear *p*-PBTA (*p*Cpb-*b*Ala-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-OH) (35.0 mg).

The crude linear peptide (35.0 mg) was dissolved in 3.5 mL (10 mg/mL) of solvent comprising saturated NH_4HCO_3/DMF:H_2O (1:1). The reaction solution was air-oxidized for 16 h at 25 °C. Finally, the mixture was purified using RP-HPLC [column: COSMOSIL 5C_{18}-AR-II (10.0 ID × 150 mm); phase: MeCN:H_2O (containing 0.1% v/v TFA) = 28:72; flow rate: 4.5 mL/min; temperature: 25 °C] to yield *p*-PBTA (15.4 mg, 12.1 μmol). The calculated mass of $(M - H_2O + 2H)^{2+}$ was 625.7450 *m/z*. The detected mass of $(M - H_2O + 2H)^{2+}$ was 625.7441 *m/z*. ^1H and ^{13}C NMR (DMSO-d_6; see the Supporting Information).

Synthesis of *m*-IBTA. The linear peptide was manually assembled from 2-chlorotrityl chloride resin (188.7 mg, loading capacity 1.06 mmol/g) according to the standard Fmoc-protocol using Fmoc-amino acid derivatives and *m*Ibz-OH. After deprotection and removal of the peptide from the resin using TFA/TIS/EDT/H_2O (94.0:1.0:2.5:2.5), the obtained mixture was purified using RP-HPLC [column: COSMOSIL 5C_{18}-AR-II (10.0 ID × 150 mm); phase: MeCN:H_2O (containing 0.1% v/v TFA) = 37:63; flow rate: 4.5 mL/min; temperature: 25 °C] to yield the crude linear *m*-IBTA (*m*Ibz-*b*Ala-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-OH) (14.6 mg).

The crude linear peptide (14.6 mg) was dissolved in 1.5 mL (10 mg/mL) of a solvent comprising saturated NH_4HCO_3/DMF:H_2O (1:1). The reaction solution was air-oxidized for 16 h at 25 °C. Finally, the mixture was purified using RP-HPLC [column: COSMOSIL 5C_{18}-AR-II (10.0 ID × 150 mm); phase: MeCN/H_2O (containing 0.1% v/v TFA) = 37:63; flow rate: 4.5 mL/min; temperature: 25 °C] to yield *m*-IBTA (8.4 mg, 6.2 μmol). The calculated mass of $(M + 2H)^{2+}$ was 675.6946 *m/z*. The mass detected for $(M + 2H)^{2+}$ is 675.6956 *m/z*. ^1H and ^{13}C NMR (DMSO-d_6; see the Supporting Information).

Synthesis of *p*-IBTA. The linear peptide was manually assembled from 2-chlorotrityl chloride resin (188.7 mg, loading capacity 1.06 mmol/g) according to the standard Fmoc-protocol using Fmoc-amino acid derivatives and *p*Ibz-OH. After deprotection and removal of peptide from the resin using TFA/TIS/EDT/H_2O (94.0:1.0:2.5:2.5), the obtained mixture was purified using RP-HPLC [column: COSMOSIL 5C_{18}-AR-II (10.0 ID × 150 mm); phase: MeCN:H_2O (containing 0.1%v/v TFA) = 37:63; flow rate: 4.5 mL/min; temperature: 25 °C] to yield the crude linear *p*-IBTA (*p*Ibz-*b*Ala-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-OH) (13.2 mg).

The crude linear peptide (13.2 mg) was dissolved in 1.3 mL (10 mg/mL) of solvent comprising saturated NH_4HCO_3/DMF:H_2O (1:1). The reaction solution was air-oxidized for 16 h at 25 °C. Finally, the mixture was purified using RP-HPLC [column: COSMOSIL 5C_{18}-AR-II (10.0 ID × 150 mm); phase: MeCN:H_2O (containing 0.1% v/v TFA) = 37:63; flow rate: 4.5 mL/min; temperature: 25 °C] to yield *p*-IBTA (7.1 mg, 5.3 μmol). The calculated mass of $(M + 2Na)^{2+}$ is 697.6765 *m/z*. The detected mass of $(M + 2Na)^{2+}$ is 697.6789 *m/z*. ^1H and ^{13}C NMR (DMSO-d_6; see the Supporting Information).

General Procedure to Radiosynthesize [^{125}I]2, 4, 6, 8, and 10. Boronic precursor (100 nmol) in the solvent and $Cu(py)_4(OTf)_2$ (0−40 nmol) in the solvent were added to a microtube; H_2O or MeOH was further added to adjust the H_2O content (the final solution volume = 100 μL). A 10 μM NaOH aqueous solution of ^{125}I (reductant free, ca. 629 GBq/mg as iodine, 3.33−4.07 GBq/mL, 0.5 μL, 1−1.3 MBq) was added to the mixture. The tube was then gently vortexed for 10 s. The reaction proceeded at 25 °C for 10−60 min. The mixture was analyzed using radio-TLC and radio-HPLC. RCCs were determined using radio-TLC analysis. Note: The reaction contained water (ca. 0.5%) derived from an aqueous solution of ^{125}I.

Radiosynthesis of [^{125}I]*m*-IBTA and [^{125}I]*p*-IBTA. *m*/*p*-PBTA (100 nmol) in MeOH (40 μL) and $Cu(py)_4(OTf)_2$ (0−320 nmol) in MeOH (40 μL) were added to a microtube; H_2O (20 μL) or MeOH (20 μL) and a 10 μM NaOH aqueous solution of ^{125}I (reductant free, ca. 629 GBq/mg as iodine, 3.33−4.07 GBq/mL, 0.5 μL, 1−1.3 MBq) were added to the mixture. The tube was gently vortexed for 10 s. The reaction proceeded at 25 °C for 10−60 min. The mixture was analyzed using radio-HPLC. RCCs were determined using radio-HPLC analysis. Note: The reaction contained water (ca. 0.5%) derived from an aqueous solution of ^{125}I.

ICP-MS Analysis. *m*-PBTA (100 nmol) in MeOH (40 μL) and $Cu(py)_4(OTf)_2$ (80 nmol) in MeOH (40 μL) were added to a microtube; MeOH (20 μL) and 10 μM NaOH aqueous solutions of NaI (5 nmol, 0.5 μL) were further added. The reaction was performed at 25 °C for 10 min. After the reaction, the solution was concentrated in vacuo and dissolved in 35% MeCN+0.1% TFA (50 μL). The target compound, *m*-IBTA, was isolated by RP-HPLC [column:5C_{18}-AR-II (4.6 ID × 150 mm); phase: MeCN/H_2O (containing 0.1% v/v TFA) = 35:65 (0−15 min), 100:0 (16 min), 100:0 (16−25 min); flow rate: 1.0 mL/min; temperature: 40 °C]. The isolated sample solution was heated at 160 °C, and 60% nitric acid (HNO_3), 60% perchloric acid ($HClO_4$), and 30% hydrogen peroxide (H_2O_2) were added. This procedure was repeated until all organic materials were removed. After the samples were cooled to 25 °C, the residues were resuspended in 5 mL of 5% HNO_3 (5 mL). The solutions were then used to quantify the copper concentrations using ICP-MS. Standard curves were plotted using a 5% HNO_3 solution with final metal concentrations of 0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, and 200 ng/mL (ppb), prepared using 1000 μg/mL (ppm) standard solutions of Cu (FUJIFILM Wako Pure Chemical Corporation). For quality control, 1 ng/mL (ppb) of a reference internal standard (In) was measured in parallel with the samples. All of the tall beakers and sample cups used in this experiment were pretreated with 1% HNO_3 to avoid metal contamination.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its online supplementary material.

https://doi.org/10.1021/acsomega.3c01974
ACS Omega 2023, 8, 24418−24425

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c01974.

UV- and radio-HPLC chromatograms; imaging data of radio-TLC; and ^{1}H, ^{13}C NMR spectra of products (PDF)

AUTHOR INFORMATION

Corresponding Author

Hiroyuki Kimura − *Department of Analytical and Bioinorganic Chemistry, Division of Analytical and Physical Sciences, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan;* orcid.org/0000-0002-4291-3524; Email: hkimura@mb.kyoto-phu.ac.jp

Authors

Yuto Kondo − *Department of Analytical and Bioinorganic Chemistry, Division of Analytical and Physical Sciences, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan*

Minon Sasaki − *Department of Analytical and Bioinorganic Chemistry, Division of Analytical and Physical Sciences, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan*

Sumina Koike − *Department of Analytical and Bioinorganic Chemistry, Division of Analytical and Physical Sciences, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan*

Yusuke Yagi − *Department of Analytical and Bioinorganic Chemistry, Division of Analytical and Physical Sciences, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan; Department of Radiological Technology, Faculty of Medicinal Science, Kyoto College of Medical Science, Nantan, Kyoto 622-0022, Japan;* orcid.org/0000-0003-0937-3746

Yasunao Hattori − *Center for Instrumental Analysis, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8412, Japan*

Hidekazu Kawashima − *Radioisotope Research Center, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8412, Japan*

Hiroyuki Yasui − *Department of Analytical and Bioinorganic Chemistry, Division of Analytical and Physical Sciences, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c01974

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This study was supported by JSPS KAKENHI (Grant Number: JP20K08061).

REFERENCES

(1) Wilson, T. C.; McSweeney, G.; Preshlock, S.; Verhoog, S.; Tredwell, M.; Cailly, T.; Gouverneur, V. Radiosynthesis of SPECT Tracers via a Copper Mediated ^{123}I Iodination of (Hetero) Aryl Boron Reagents. *Chem. Commun.* **2016**, *52*, 13277−13280.

(2) Zhang, P.; Zhuang, R.; Guo, Z.; Su, X.; Chen, X.; Zhang, X. A Highly Efficient Copper-Mediated Radioiodination Approach Using Aryl Boronic Acids. *Chem. − Eur. J.* **2016**, *22*, 16783−16786.

(3) Reilly, S. W.; Makvandi, M.; Xu, K.; Mach, R. H. Rapid Cu−Catalyzed [^{211}At] Astatination and [^{125}I] Iodination of Boronic Esters at Room Temperature. *Org. Lett.* **2018**, *20*, 1752−1755.

(4) Kondo, Y.; Kimura, H.; Fukumoto, C.; Yagi, Y.; Hattori, Y.; Kawashima, H.; Yasui, H. Copper-Mediated Radioiodination Reaction Through Aryl Boronic Acid or Ester Precursor and Its Application to Direct Radiolabeling of a Cyclic Peptide. *J. Labelled Compd. Radiopharm.* **2021**, *64*, 336−345.

(5) Kondo, Y.; Kimura, H.; Sasaki, I.; Watanabe, S.; Ohshima, Y.; Yagi, Y.; Hattori, Y.; Koda, M.; Kawashima, H.; Yasui, H.; Ishioka, N. S. Copper-Mediated Radioiodination and Radiobromination via Aryl Boronic Precursor and Its Application to ^{125}I/^{77}Br-Labeled Prostate-Specific Membrane Antigen Imaging Probes. *Bioorg. Med. Chem.* **2022**, *69*, No. 116915.

(6) Oliveira, M. C.; Correia, J. D. G. Biomedical Applications of Radioiodinated Peptides. *Eur. J. Med. Chem.* **2019**, *179*, 56−77.

(7) European Medicines Agency. ICH Guideline Q3D (R1) on Elemental Impurities, 2019. https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-32.pdf.

(8) King, A. E.; Brunold, T. C.; Stahl, S. S. Mechanistic Study of Copper-Catalyzed Aerobic Oxidative Coupling of Arylboronic Esters and Methanol: Insights Into an Organometallic Oxidase Reaction. *J. Am. Chem. Soc.* **2009**, *131*, 5044−5045.

(9) King, A. E.; Ryland, B. L.; Brunold, T. C.; Stahl, S. S. Kinetic and Spectroscopic Studies of Aerobic Copper(II)-Catalyzed Methoxylation of Arylboronic Esters and Insights Into Aryl Transmetalation to Copper(II). *Organometallics* **2012**, *31*, 7948−7957.

(10) Vantourout, J. C.; Miras, H. N.; Isidro-Llobet, A.; Sproules, S.; Watson, A. J. B. Spectroscopic Studies of the Chan-Lam Amination: a Mechanism-Inspired Solution to Boronic Ester Reactivity. *J. Am. Chem. Soc.* **2017**, *139*, 4769−4779.

(11) Bose, S.; Dutta, S.; Koley, D. Entering Chemical Space With Theoretical Underpinning of the Mechanistic Pathways in the Chan-Lam Amination. *ACS Catal.* **2022**, *12*, 1461−1474.

(12) Nishikata, T.; Yamamoto, Y.; Miyaura, N. 1,4-Addition of Arylboronic Acids and Arylsiloxanes to α,β-Unsaturated Carbonyl Compounds via Transmetalation to Dicationic Palladium(II) Complexes. *Organometallics* **2004**, *23*, 4317−4324.

(13) Hardouin Duparc, V.; Bano, G. L.; Schaper, F. Chan-Evans-Lam Couplings With Copper Iminoarylsulfonate Complexes: Scope and Mechanism. *ACS Catal.* **2018**, *8*, 7308−7325.

(14) Tan, Y.; Wu, J.; Song, L.; Zhang, M.; Hipolito, C. J.; Wu, C.; Wang, S.; Zhang, Y.; Yin, Y. Merging the Versatile Functionalities of Boronic Acid With Peptides. *Int. J. Mol. Sci.* **2021**, *22*, 12958.

(15) Tredwell, M.; Preshlock, S. M.; Taylor, N. J.; Gruber, S.; Huiban, M.; Passchier, J.; Mercier, J.; Génicot, C.; Gouverneur, V. A General Copper-Mediated Nucleophilic ^{18}F Fluorination of Arenes. *Angew. Chem., Int. Ed.* **2014**, *53*, 7751−7755.

(16) Zhou, D.; Chu, W.; Voller, T.; Katzenellenbogen, J. A. Copper-Mediated Nucleophilic Radiobromination of Aryl Boron Precursors: Convenient Preparation of a Radiobrominated PARP- 1 Inhibitor. *Tetrahedron Lett.* **2018**, *59*, 1963−1967.

(17) Ellison, P. A.; Olson, A. P.; Barnhart, T. E.; Hoffman, S. L. V.; Reilly, S. W.; Ellison, P. A.; Olson, A. P.; Barnhart, T. E.; Hoffman, S. L. V.; Reilly, S. W.; Makvandi, M.; Bartels, J. L.; Murali, D.; DeJesus, O. T.; Lapi, S. E.; Bednarz, B.; Nickles, R. J.; Mach, R. H.; Engle, J. W. Improved Production of ^{76}Br, ^{77}Br and ^{80}mBr via CoSe Cyclotron Targets and Vertical Dry Distillation. *Nucl. Med. Biol.* **2020**, *80*−*81*, 32−36.

(18) Ma, L.; Placzek, M. S.; Hooker, J. M.; Vasdev, N.; Liang, S. H. [^{11}C]Cyanation of Arylboronic Acids in Aqueous Solutions. *Chem. Commun.* **2017**, *53*, 6597−6600.