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RESEARCH ARTICLE

Title:

Copper-mediated radioiodination reaction through aryl boronic acid or ester precursor and its application to direct radiolabeling of a cyclic peptide

Running head:

Direct labeling of a peptide by copper-mediated radioiodination

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ABSTRACT

A copper-mediated radioiodination using aryl boronic precursors is attracting attention as a solution to oxidative iododestannylation and nickel-mediated radioiodination drawbacks. The copper-mediated radiolabeling method allows radioiodination at room temperature with stable aryl boronic precursors without preparing complex starting materials or reagents, and can be performed in a reaction vessel exposed to air. This method has good potential in radiochemistry; however, studies on the scope of copper-mediated radioiodination through boronic precursors are insufficient. In particular, few reports have demonstrated the effect of protecting groups on radiolabeling efficiency. Therefore, the effect of the protecting group of aryl boronic acids on the copper-mediated radioiodination was investigated. In addition, this method, which does not require heating, is expected to be useful for direct radiolabeling of peptides. Thus, we attempted direct radioiodination of c(RGDyk) as an example. The resulting radioiodination method was well tolerated in various substrates and was unaffected by the pinacol ester-type protecting group. Also, c(RGDyk) was labeled with ^{125}I via copper-mediated radioiodination using an aryl boronic acid precursor. The reaction time and yield were improved, compared with the indirect method. Furthermore, the large difference in polarity between the boronic acid precursor and the radiolabeled compound facilitated purification.

KEYWORDS

copper-mediated, radioiodination, aryl boronic precursor, ^{125}I, cyclic peptide, c(RGDyk), αVβ3 integrin inhibitor.

1. INTRODUCTION

An oxidative iododestannylation of aryl stannanes is widely used as a radioiodination method because of its high radiochemical purity.[1,2] However, this method allows the presence of toxic organotin residues in clinical preparations of radioiodinated imaging agents, and the instability of some organotin precursors prevents long-term storage.[3] In addition, hydrogen peroxide (H_2O_2) and *N*-chlorosuccinimide, used as oxidizing agents, require careful handling.[4,5]

Alternative approaches have been developed to improve these issues. For example, in the nickel-mediated direct radioiodination of aryl and heteroaryl bromides,[6] this halogen-exchange reaction carried out at high temperatures (e.g., 180°C) employs $Ni(cod)_2$, an air-sensitive metal complex requiring storage at 0°C and inert atmosphere techniques for handling. Therefore, Ni-based precursors are not trivial to prepare by non-chemist professionals performing (pre)clinical studies.

An excellent radiolabeling method, copper-mediated radioiodination using aryl boronic precursors through a Chen–Evans–Lam cross-coupling reaction, was reported in 2016.[3,7] Makvandi and Mach group found that the copper complex $[Cu(pyridine)_4(OTf)_2]$ (OTf =

trifluoromethanesulfonate) was suitable for the radioiodination reaction.[8] This reaction process does not require preparation of complex starting materials or reagents and can be performed in a reaction vessel exposed to air. Recently, a reaction without a copper catalyst was also reported. This report has enabled the development of a simple base-catalyzed halodeboronation suitable for the preparation of ^{125}I-labeled products for imaging applications.[20]

Copper-mediated transformations between aryl boronic precursors and various coupling partners have already been investigated[9,10] and their translations into radiochemistry have gained considerable attention. Copper-mediated ^{18}F-fluorination,[11,12] $^{77/76}$Br-bromination,[13] $^{123/125/131}$I-radioiodination,[3,7] ^{211}At-astatination,[8] and ^{11}C-cyanation[14] methods via aryl boronic precursors have been reported. Tracers labeled with various radioactive elements could be synthesized through a common aryl boronic precursor.[3] Notably, its applicability to ^{211}At-astatination is extremely valuable from the treatment viewpoint.

FDA-approved medicines containing boron have already been used in clinical practice, for example, Kerydin® (tavaborole), VELCADE® (bortezomib), and Eucrisa® (crisaborole).[20] For treatment, a high dose of the medicine is administered, compared with the medicine dose used for single-photon emission computed tomography (SPECT). Therefore, the tolerability of humans to medicines containing boronic acid has been proven; further, boronic acid precursors overcome the issue of toxicity in clinical use.

Several protecting groups have been available for boronic acid, which are essential for the synthesis of boronic precursors. Occasionally, the protecting groups may be unexpectedly removed during the precursor synthesis. Conversely, the reaction to remove protecting groups may not proceed efficiently. Therefore, in designing precursors, how the protecting groups of boronic acid affect the radiolabeling efficiency should be determined; however, few studies have been conducted on this data and they have not been investigated in an integrated manner.[3,7,8]

In recent years, studies on the scope of the copper-mediated ^{18}F-labeling method and preparation of ^{18}F-labeled PET radiotracers from a diverse range of boronic precursors have been in progress.[11,12,15] There are also studies on labeling method of small molecules,[3,8] peptides, and antibodies[22] via copper-mediated radioiodination using a boronic precursor. In particular, its application to small molecule labeling using the copper-mediated radioiodination reaction is increasing, but studies on the scope of copper-mediated radioiodination using boronic precursors are insufficient so far. Notably, few studies have been conducted on the direct radiolabeling of peptides via the copper-mediated radioiodination reaction using a boronic precursor. First, the direct labeling method of peptides with high specificity was limited. Direct labeling of c(RGDfK) with ^{125}I or ^{211}At using tin precursors has been reported,[23] but the issue of toxicity derived from residual tin is unavoidable. Vaidyanathan et al. reported that ^{131}I-iodinated MIBG-octreotate was yielded by heating a mixture of brominated precursor and

radioiodine in acetic acid at 100°C [Radio chemical yield (RCY): 3%–36%, n = 10].[17] In general, since the brominated peptide precursor and radioiodinated peptide have similar polarities, it is difficult to separate them by high performance liquid chromatography (HPLC). When this method is applied to longer-chain peptides, the isolation and purification are expected to become more difficult. Also, a heating operation should be avoided for application to a wide variety of peptides. On the contrary, copper-mediated radioiodination via boronic precursors does not require oxidizing reagents such as chloramine-T and can be carried out at room temperature.[8] In addition, the precursor does not contain heavy metals, and the polarity between the boronic acid precursor and the iodine compound is significantly different. Therefore, this method would be a highly versatile direct labeling reaction for peptides (Figure 1).

Based on previous reports illustrating the Chan–Evans–Lam reaction to access radioiodination via boronic reagents, we focused on the most frequently used pinacol ester-type protecting group and aimed at investigating the effect of the protecting group of an aryl boronic acid on copper-mediated radioiodination. In addition, to evaluate the effectiveness of copper-mediated radioiodination via boronic precursor in direct labeling peptides, we report the direct radioiodination method of c(RGDyk), a selective αVβ3 integrin inhibitor.

2. RESULTS AND DISCUSSION

Labeling the aromatic ring with radioiodine is a common in radiotracer synthesis from the viewpoint of stability of the iodinated compound *in vivo*.[2,16] Hence, the effect of substituents on the aromatic ring and their positional isomers on the copper-mediated radioiodination through the boronic acid or ester should be investigated. The precursors **1a–1j** were selected to complement those of previous reports.[3,7,8] Precursors without protecting groups **1a–1j(i)** or with pinacol ester-type protecting groups **1a–1j(ii)** were labeled with ^{125}I by the method shown in Figure 2. Cu(pyridine)$_4$(OTf)$_2$, which can be labeled efficiently without the presence of 1,10-phenanthroline as a ligand, was used.[8] As copper-catalyzed transformations of functional groups from aryl boronic acids proceed under solvents containing water,[18] an aqueous solution of Na^{125}I was used for radiolabeling. Radiochemical conversions (RCCs) shown in Figure 2 were determined by radio-thin layer chromatography (radio-TLC).

As a result, all compounds tested (**2a–2j)** showed high RCCs (99.7%–84.6%), and no significant difference was observed between the case of radiolabeling via boronic precursors without protecting groups **2a–2j(i)** and with protecting groups **2a–2j(ii)**. This labeling method is well tolerated in both electron-rich (**2a–c, 2h–2j**) and electron-poor substrates (**2d–2f, 2g**), including sterically crowded substrates (**2c** and **2f**). There are studies on radioiodination of boronic precursors containing nitrogen atoms using the copper-mediated radiolabeling method,[3,8] however, reports on radioiodination of precursors containing sulfur atoms, which

are important for drug development, are limited. In our study, this labeling method was applied to precursors with sulfur atoms (**2i** and **2j**) and showed high RCCs (97.2%–99.0%). In addition, approximately no difference in RCCs was observed between positional isomers, allowing flexibly designing precursors. Boronic acids are used in well-known reactions, such as Suzuki–Miyaura cross-coupling,[19] and various types of boronic acid compounds can be purchased. Therefore, the boronic acid precursor is easy to synthesize using commercial compounds. In addition, it is highly stable and can be stored for a long time. If application data is accumulated, the copper-mediated radioiodination method becomes an indispensable radiolabeling method. Then, as an application example of this labeling method, c(RGDyk) was directly labeled with radioiodine through the boronic precursor. The cyclic peptide c(RGDyk) is a selective αVβ3 integrin inhibitor. Zhang et al. indirectly labeled c(RGDyk) using ^{125}I-labeled *N*-hydroxysuccinimide ester of iodobenzoic acid ([^{125}I]IB-NHS) synthesized by the copper-mediated radioiodination from a boronic acid precursor, and the SPECT imaging results confirmed the good targeting ability and *in vivo* stability of radiopharmaceuticals.[7] In general, the indirect radiolabeling method takes a long time to label and increases the exposure risk of researchers; however, labeling methods that can directly label peptides with high specificity are limited. The development of effective and versatile direct radiolabeling methods for peptides is required. Therefore, we evaluated whether copper-mediated radioiodination via boron precursors, which can react under mild conditions, can be applied to direct radiolabeling

of peptides such as c(RGDyk).

First, [^{125}I]IB-c(RGDyk) was synthesized by the indirect radiolabeling method. Simultaneously, we investigated the reaction condition of ^{125}I-labeled *N*-hydroxysuccinimide ester of iodobenzoic acid ([^{125}I]IB-NHS, **4**), commonly employed in indirect radiolabeling of peptides, proteins, and antibodies.[16] *N*-Hydroxysuccinimide esters of boronobenzoic acid (PB-NHS, **3**) can be easily handled because they have high crystallinity. In addition, boronic acid precursors are highly polar and have significantly different retention times than the target tracer labeled with radioiodine; therefore, they can be easily separated and removed by HPLC. Thus, we decided to synthesize [^{125}I]IB-NHS (**4**) using PB-NHS (**3**) as a precursor. The reaction was carried out using a methanol solvent (Figure 3). The RCCs of **4** showed a high value after 10 min. However, RCCs decreased with time. Then, the reaction solvent was changed from methanol (MeOH) to ethanol (EtOH), acetonitrile, or acetone and we briefly investigated RCCs of the target compound and by-product with time using radio-TLC (Table S1). At 10 min after the reaction, the RCCs of MeOH and EtOH showed high values (97.7%–98.8%). The increased rate of by-products with time when EtOH was used as the reaction solvent was smaller than that when MeOH was used. Therefore, EtOH was used as a reaction solvent for the synthesis of [^{125}I]IB-NHS (**4**) through PB-NHS (**3**). Finally, c(RGDyk) was indirectly labeled using [^{125}I]IB-NHS as a radiotracer to give [^{125}I]IB-c(RGDyk) [radioisolated yield: 36.5%–39.3% (n = 3), working time: 140.5–157.3 min (n = 3), Scheme 1B]. Since the synthesis and purification

process of [^{125}I]IB-NHS are required, this indirect radiolabeling method requires a long time to yield [^{125}I]IB-c(RGDyk). Thus, applying an indirect radiolabeling method to the labeling of ^{123}I ($t_{1/2}$ = 13.27 h), which has a half-life shorter than ^{125}I ($t_{1/2}$ = 59.40 day), is inappropriate.

Then, c(RGDyk) was directly labeled with radioiodine via copper-mediated radioiodination using the boronic precursor (Scheme 1A). PB-NHS (**3a**) was reacted with c(RGDyk) in triethylamine/dimethylformamide (DMF) to give PB-c(RGDyk) (Yield: 43.6%). We then explored appropriate conditions for direct radioiodination of PB-c(RGDyk) as a precursor (Table S2). As a result, [^{125}I]IB-c(RGDyk) was obtained in a methanol solution of Cu(pyridine)$_4$(OTf)$_2$ at 25°C (RCC: >99%). When dimethyl sulfoxide (DMSO) or DMF was used as the solvent, the radiolabeling efficiency was poor (RCC: 0%–15.3%). After optimizing radiolabeling conditions, [^{125}I]IB-c(RGDyk) was synthesized three times to determine the radioisolated yield [64.4%–69.0% (n = 3)]. The working time was 27.7–32.1 min (n = 3), which is faster than the indirect radiolabeling method. This direct radiolabeling method is particularly effective when ^{123}I is used as a tracer. Retention times of the boronic precursor PB-c(RGDyk) and the tracer labeled with radioiodine [^{125}I]IB-c(RGDyk) were significantly different, which facilitates easy separation by HPLC (Figure 4). These results indicated that the copper-mediated radiolabeling method via aryl boronic precursors was an effective method to achieve direct radioiodination of c(RGDyk), a cyclic peptide.

There are few reports on direct peptide labeling via copper-mediated radioiodination using boronic precursors. Therefore, an accumulation of further application examples is very important. Our results facilitate the application of this synthetic method to direct labeling of peptides.

3. CONCLUSION

This study explored the effect of pinacol ester-type protecting group of aryl boronic acids on copper-mediated radioiodination and found that the radiolabeling method is well tolerated in both electron-rich and electron-poor substrates, including sterically crowded substrates, unaffected by protecting groups. In addition, we adapted the copper-mediated radiolabeling methodology to achieve direct radioiodination of c(RGDyk). The reaction time and yield were improved, compared with the indirect method. Also, the large difference in polarity between the boronic acid precursor and the radiolabeled target tracer facilitated purification. These results indicate that the copper-mediated radiolabeling reaction via aryl boronic precursors is an effective radiolabeling method for direct radioiodination of peptides. We hope that our report promotes development of peptide tracers.

4. EXPERIMENTAL

4.1 General

All reagents were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), Nakalai Tesque Inc. (Kyoto, Japan), Fujifilm Wako Chemical Corp. (Osaka, Japan), and Fluorochem Ltd. (Hadfield, Derbyshire, UK), and were used without further purification unless otherwise indicated. The cyclic peptide, c(RGDyk), was provided by Nard Institute, Ltd. (Hyogo, Japan). Sodium [^{125}I]iodide ([^{125}I]NaI) (carrier-free) solution was purchased from PerkinElmer, Inc. (Waltham, MA, USA).

^{1}H NMR and ^{13}C NMR spectra were measured on Ascend^{TM} 500 (Bruker, Billerica, Massachusetts, USA) with $CDCl_3$ or DMSO-d_6 as a solvent. Data were reported as follows: chemical shifts are reported as δ in units of parts per million (ppm) relative to an internal standard (tetramethylsilane); multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quintet), br. s (broad singlet), dd (doublet of doublets), or m (multiplet); coupling constants are reported as a *J* value in Hertz (Hz); the number of protons (*n*) for a given resonance is indicated as *n*H, based on spectral integration values.

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

images.

An LD-20AD (Shimadzu, Kyoto, Japan) was used for HPLC, along with an SPD-20A (Shimadzu) ultraviolet (UV) detector (k = 254 nm) and γ-survey meter TCS-172 (ALOKA, Mitaka, Japan) or Gavi Nova (M&S Instruments Inc., Osaka, Japan) RI detector. A Cosmosil $5C_{18}$-AR-II column (4.6ID × 150 mm or 10.0ID × 150 mm; Nacalai Tesque Inc.) was used for reverse phase HPLC.

Low-resolution mass spectra were obtained by Agilent LC/MS 6130B (Agilent Technologies, California, USA). High-resolution mass spectra were obtained using LCMS-IT-TOF (ESI; Shimadzu, Kyoto, Japan), GC mate II (EI, JEOL, Tokyo, Japan), or SX-102A (FAB; JEOL, Tokyo, Japan).

4.2 Synthesis of precursor 3a

4-Carboxyphenylboronic acid (*p*-PB, 745.0 mg, 4.5 mmol) and *N*-hydroxy succinimide (NHS, 559.2 mg, 4.9 mmol) were dissolved in tetrahydrofuran (THF, 22.0 mL), and then dicyclohexylcarbodiimide (DCC, 1.0 g, 4.9 mmol) was added into the solution. The reaction was stirred at room temperature under nitrogen for 3 h. The reaction solution was concentrated by the rotary evaporator and dissolved in ethyl acetate (200 mL). The organic layer was washed with sat. NH_4Cl aq. (100 mL × 2), sat. $NaHCO_3$ aq. (100 mL × 2), and brine (50 mL × 1). The obtained organic solution was dried over $MgSO_4$ and concentrated using a rotary evaporator. Residual water was removed by the freeze-drying to yield *p*-PB-NHS (682.0 mg, 2.6 mmol).

Yield: 57.8%; mp 262.5°C–266.1°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.90 (br. s, 4H), 8.01 (d, J = 8.2 Hz, 2H), 8.05 (d, J = 8.4 Hz, 2H), 8.46 (br. s., 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 24.93, 124.91, 128.12, 134.19, 161.33, 169.73; LRESIMS: *m/z* 286.0510 $[M + Na]^+$; HRESIMS: calcd for $C_{11}H_{10}NO_6B$ $[M + Na]^+$ 286.0495, found 286.0497.

4.3 Synthesis of precursor 3b

3-Carboxyphenylboronic acid (*m*-PB, 401.2 mg, 2.4 mmol) was dissolved in dichloromethane (DCM, 5.0 mL) and added with *N*-hydroxy succinimide (NHS, 231.9 mg, 2.0 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, 386.3 mg, 2.0 mmol), and triethylamine (279.3 μL, 2.0 mmol). The reaction was stirred at room temperature for 4 h. The solvent was removed under reduced pressure and dissolved in ethyl acetate (100 mL). The organic layer was washed with water (50 mL × 2), sat. NH_4Cl aq. (50 mL × 2), sat. $NaHCO_3$ aq. (50 mL × 2), and brine (50 mL × 1). The obtained organic solution was dried over $MgSO_4$ and removed under reduced pressure. The residual water was removed by the freeze-drying to yield *m*-PB-NHS (453.8 mg, 1.7 mmol) as a white powder. Yield: 71.4%; mp 252.6°C–254.5°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.90 (br. s., 4H), 7.63 (t, J = 7.6 Hz, 1H), 8.11 (d, J = 7.8 Hz, 1H), 8.20 (d, J = 7.3 Hz, 1H), 8.44 (br. s., 2H), 8.52 (s, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 25.56, 123.79, 128.66, 131.48, 135.59, 140.99, 162.09, 170.41; LRESIMS: *m/z* 286.0503 $[M + Na]^+$; HRESIMS: calcd for $C_{11}H_{10}NO_6B$ $[M + Na]^+$ 286.0495, found 286.0492.

4.4 Synthesis of standard compound 4a

4-Iodobenzoic acid (*p*-IB, 103.5 mg, 0.42 mmol) was dissolved in tetrahydrofuran (THF, 4.1 mL), and *N*-hydroxy succinimide (NHS, 61.5 mg, 0.53 mmol) and *N*,*N*'-dicyclohexylcarbodiimide (DCC, 111.9 mg, 0.54 mmol) were then added into the solution. The reaction was stirred at room temperature under nitrogen for 4 h. The reaction solution was concentrated using a rotary evaporator and dissolved in ethyl acetate (50 mL). The organic layer was washed with sat. NH_4Cl aq. (30 mL × 2), sat. $NaHCO_3$ aq. (30 mL × 2), and brine (20 mL × 1). The obtained organic solution was dried over $MgSO_4$ and concentrated using a rotary evaporator. The residual water was removed by freeze-drying to yield 2,5-dioxopyrrolidin-1-yl 4-iodobenzoate (*p*-IB-NHS, 142.5 mg, 0.41 mmol). Yield: 98.4%; mp 225.6°C–227.7°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.92 (br. s., 4H), 7.84 (d, J = 8.5 Hz, 2H), 7.90 (d, J = 8.5 Hz, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 25.65, 103.39, 124.55, 131.67, 138.30, 161.58, 169.05; HREIMS: calcd for $C_{11}H_8INO_4$ $[M]^+$ 344.9498, found 344.9498.

4.5 Synthesis of standard compound 4b

3-Iodobenzoic acid (*m*-IB, 1.9 g, 7.8 mmol) was dissolved in tetrahydrofuran (THF, 20 mL), and then with *N*-hydroxysuccinimide (NHS, 1.0 g, 8.6 mmol) and *N*,*N*'-dicyclohexylcarbodiimide (DCC, 1.8 g, 8.6 mmol). The reaction was stirred at room temperature for 22 h. The solution was filtered, and then the mother liquid was concentrated using a rotary evaporator and purified using medium pressure liquid chromatography (SiO_2,

hexane:ethyl acetate = 1:1) to yield 2,5-dioxopyrrolidin-1-yl 3-iodobenzoate (*m*-IB-NHS, 2.6 g, 7.6 mmol) as white solid. Yield: 96.8%; mp 141.2°C–143.1°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.90 (br. s., 4H), 7.46 (t, *J* = 7.9 Hz, 1H), 8.11 (d, *J* = 8.1 Hz, 1H), 8.22 (d, *J* = 8.1 Hz, 1H), 8.35 (s, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 25.56, 95.53, 126.40, 129.29, 131.61, 137.85, 144.10, 160.58, 170.22; LRFABMS: *m/z* 345.7 $[M + H]^+$; HRFABMS: calcd for $C_{11}H_8INO_4$ $[M + H]^+$ 345.9578, found 345.9577.

4.6 Synthesis of IB-c(RGDyk), the standard sample

c(RGDyk) (20.0 mg, 32.3 μmol) and **4a** (10.6 mg, 32.3 μmol) were dissolved in DMF (2.0 mL), and then triethylamine (100 μL) was added into the solution and reacted at room temperature for 16 h. The reaction solvent was removed using a rotary evaporator and purified by HPLC [method F; Cosmosil 5C_{18}-AR-II (10.0 ID × 150 mm); Flow: 4.0 mL/min; Temp.: 40°C; t_R: 16.8 min] to yield IB-c(RGDyk) (16.7 mg) as white powder; Yield: 60.8%; Purity: >99%; ^{1}H NMR (DMSO-d_6, 500 MHz) *δ*: 1.10 (m, 2H), 1.27-1.51 (m, 6H), 1.58 (m, 1H), 1.65-1.74 (m, 1H), 2.34-2.41 (m, 1H), 2.62-2.74 (m, 2H), 2.74-2.81 (m, 1H), 3.02-3.13 (m, 2H), 3.15- 3.27 (m, 3H), 3.88-3.95 (m, 1H), 3.99-4.06 (m, 1H), 4.11-4.18 (m, 1H), 4.35 (q, *J* = 7.2 Hz, 1H), 4.59-4.66 (m, 1H), 6.63 (d, *J* = 8.4 Hz, 2H), 6.91 (d, *J* = 8.4 Hz, 2H), 7.49 (t, *J* = 5.7 Hz, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.95 (d, *J* = 7.2 Hz, 1H), 8.06 (d, *J* = 7.0 Hz, 1H), 8.11 (d, *J* = 8.5 Hz, 1H), 8.43 (dd, J_1 = 6.8, J_2 = 4.8 Hz, 1H), 8.55 (t, *J* = 5.5 Hz, 1H), 9.19 (br. s., 1H); ^{13}C NMR (DMSO-d_6, 126 MHz) *δ*: 1.63,

23.39, 25.69, 29.00, 31.29, 35.45, 37.15, 43.69, 49.33, 52.31, 55.07, 55.15, 99.21, 115.38, 127.68, 129.61, 130.47, 134.42, 137.60, 156.26, 157.00, 158.57, 165.91, 170.03, 170.38, 171.36, 171.67, 172.16, 172.64; LRESIMS *m/z* 850.0 $[M + H]^+$; HRESIMS: calcd for $C_{34}H_{44}N_9O_9I$ $[M + H]^+$ 850.2379 found 850.2359.

4.7 Synthesis of PB-c(RGDyk)

c(RGDyk) (20.0 mg, 32.3 μmol) and **3a** (8.0 mg, 32.3 μmol) were dissolved in DMF (2.0 mL), and then triethylamine (100 μL) was added into the solution and reacted at room temperature for 16 h. The reaction solvent was removed using a rotary evaporator and purified by HPLC [method F; Cosmosil 5C_{18}-AR-II (10.0 ID × 150 mm); Flow: 4.0 mL/min; Temp.: 40°C; t_R: 11.4 min] to yield PB-c(RGDyk) (10.8 mg, 14.1 μmol) as white powder. Yield: 43.6%; Purity: 98.5%; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.07-1.17 (m, 1H), 1.29-1.53 (m, 6H) 1.54-1.62 (m, 1H) 1.65-1.75 (m, 1H), 2.34-2.42 (m, 1H), 2.62-2.74 (m, 2H), 2.79 (m, J = 8.9 Hz, 1H), 3.03-3.13 (m, 2H), 3.16-3.28 (m, 3H), 3.92 (t, J = 9.4 Hz, 1H), 4.03 (m, J_1 = 14.9, J_2 = 7.7 Hz, 1H), 4.16 (q, J = 7.5 Hz, 1H), 4.36 (q, J = 7.3 Hz, 1 H), 4.58-4.66 (m, 1H), 6.63 (d, J = 8.2 Hz, 2H), 6.91 (d, J = 8.2 Hz, 2H), 7.49 (br. s., 1H), 7.60 (d, J = 6.7 Hz, 1H), 7.79 (d, J = 7.9 Hz, 2H), 7.85 (d, J = 7.8 Hz, 2H), 7.94 (d, J = 7.0 Hz, 1H), 8.07 (d, J = 7.2 Hz, 1H), 8.12 (d, J = 8.4 Hz, 1H), 8.23 (s, 2H), 8.42 (dd, J_1 = 6.4, J_2 = 4.6 Hz, 1H) 8.48 (t, J = 5.6 Hz, 1H), 9.19 (s, 1H), 12.29 (br. s., 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 23.42, 25.69, 28.99, 29.13, 31.33, 37.16, 43.69, 49.34, 52.30, 55.13, 115.39, 126.50, 127.69, 130.48, 134.39,

136.32, 156.27, 157.03, 166.77, 170.01, 170.41, 171.37, 171.69, 172.19, 172.67; LRESIMS: *m/z* 790.1 $[M + Na]^+$; HRESIMS: calcd for $C_{34}H_{46}N_9O_{11}B$ $[M + Na]^+$ 790.3308 found 790.3309.

4.8 General procedure of ^{125}I-radiolabeling using boronic acid or ester precursors (1a–1j, 3a–b)

Boronic acid or ester precursor (0.5 mg) and $Cu(pyridine)_4(OTf)_2$ in MeOH or EtOH (2.2 mg/mL, 100 μL) were added into a 1.5-mL microtube. About 10-μM NaOH aq. solution of ^{125}I (0.5 μL, 1–4 MBq) was added to the mixture in the microtube. Then, the tube was gently vortexed for 5 s. The reaction was left at room temperature for 10 min. RCCs were determined by radio-TLC (Supplementary information). The reaction mixture was analyzed by radio-HPLC (Supplementary information).

4.9 Indirect ^{125}I-labeling of c(RGDyk) using [^{125}I]IB-NHS

p-PB-NHS (**3a**, 0.2 mg) and $Cu(pyridine)_4(OTf)_2$ in EtOH (2.2 mg/mL, 50 μL) were added to a 1.5-mL microtube. About 10-μM NaOH aq. solution of ^{125}I (1.0 μL, 2.4–2.9 MBq) was added to the mixture in the microtube. Then, the tube was gently vortexed for 5 s. The reaction was left to set at room temperature for 10 min. The obtained solution was dried under a flow of nitrogen for 2 min and dissolved in acetonitrile (40 μL). **4a** was isolated using HPLC [method B, t_R: 7.42 min]. The solution of **4a** was diluted with water (30 mL), passed through a C_{18} Sep-Pak column, and washed with water (4 mL). Finally, **4a** was eluted with acetonitrile (0.5 mL) and dried under flow of nitrogen for 10–20 min. c(RGDyk) (10 mg/mL, 20 μL), triethylamine

(2.5 μL), and DMF (20 μL) were added into the microtube containing **4a**. The reaction was left at room temperature for 1 h. [^{125}I]IB-c(RGDyk) (0.9–1.1 MBq) was isolated by HPLC (method F; radioisolated yield: 36.5%–39.3%; working time: 140.5–157.3 min, n = 3). The mixture of purified and cold samples was analyzed by UV- and radio-HPLC, showing a single peak (t_R: 16.92 min) corresponding to cold sample (t_R: 16.85 min).

4.10 Direct ^{125}I-radiolabeling through PB-c(RGDyk)

PB-c(RGDyk) (0.1 mg) and Cu(pyridine)$_4$(OTf)$_2$ in MeOH (2.2 mg/mL, 50.0 μL) were added to a microtube. About 10-μM NaOH aq. solution of ^{125}I (1.0 μL, 2.6-2.8 MBq) was added to the mixture. Then, the tube was gently vortexed for 5 s. The reaction was left at room temperature for 10 min. [^{125}I]IB-c(RGDyk) (1.8-1.9 MBq) was isolated by HPLC [method F]. RCCs were determined by radio-HPLC analysis; RCC: 82.4%–99.9% (n = 3); radioisolated yield: 64.4%–69.0% (n = 3); and working time: 27.7–32.1 min (n = 3). The mixture of purified and cold samples was analyzed using UV- and radio-HPLC, which showed a single peak (t_R: 16.91 min) corresponding to cold sample (t_R: 16.83 min).

CONFLICT OF INTEREST

The authors declare no competing financial interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

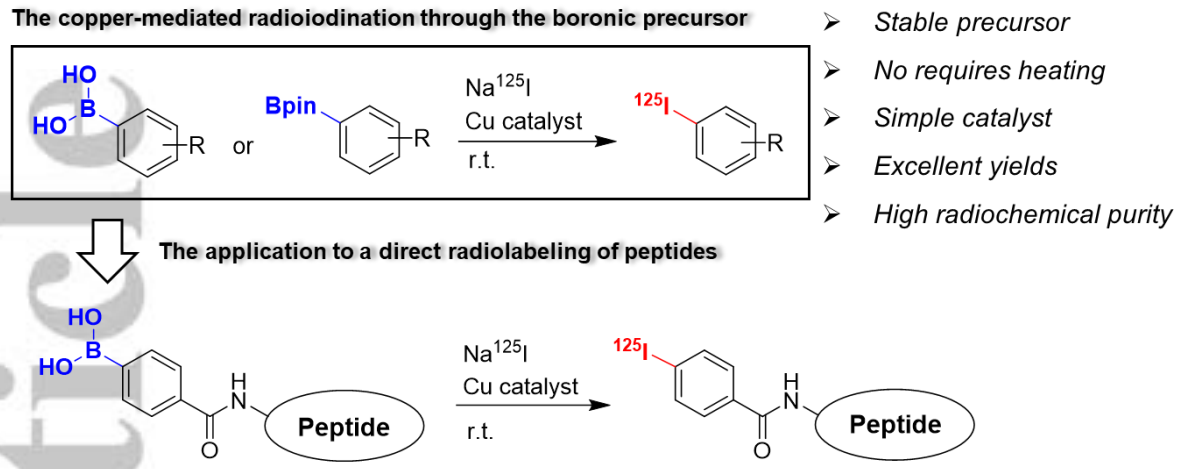


FIGURE 1 Application of the copper-mediated radioiodination through the boronic precursor for direct radiolabeling of peptides.

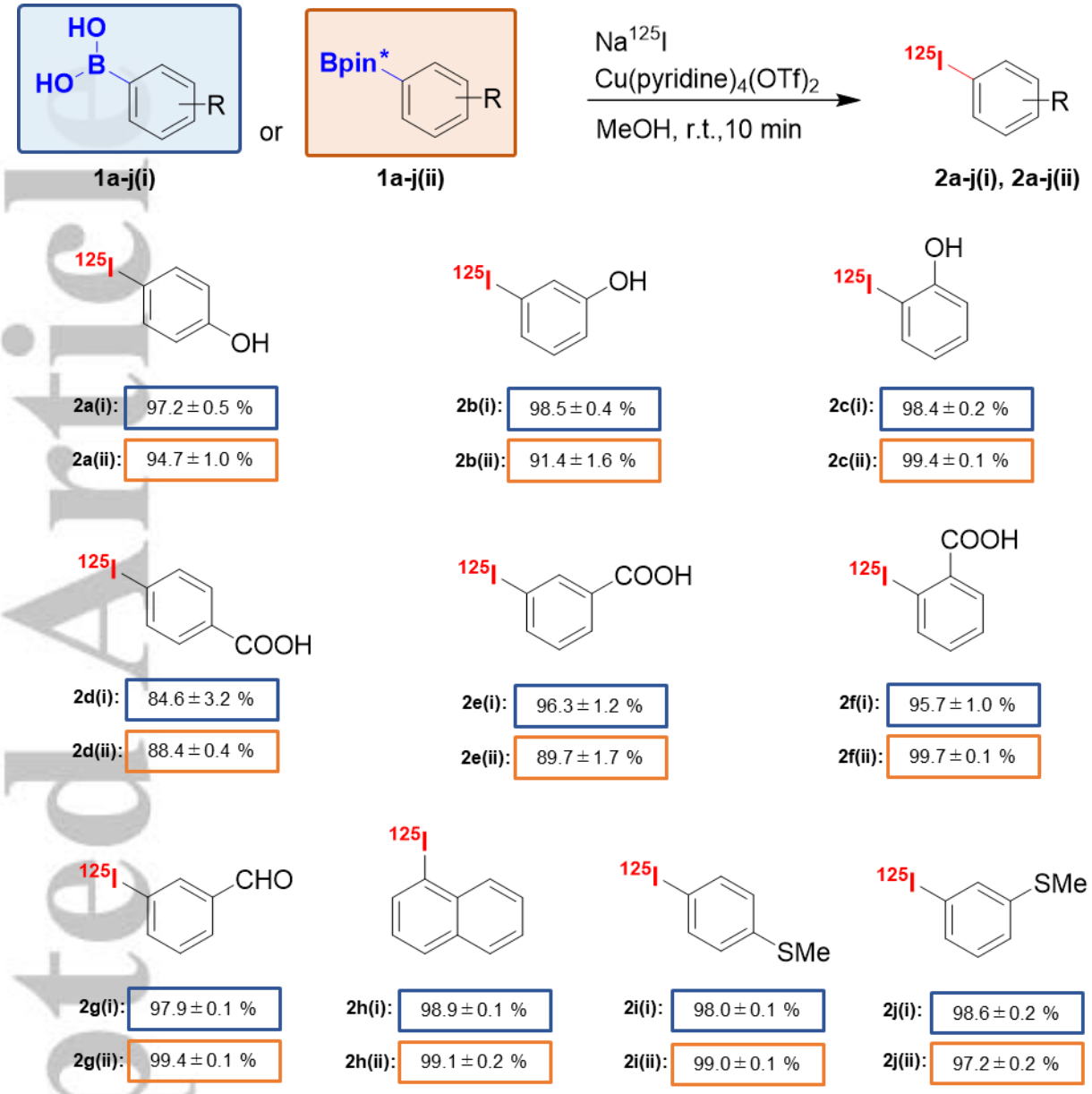


FIGURE 2 The scope of copper-mediated ^{125}I-iodination via aryl boronic acid or pinacol ester. RCCs of radiolabeled compounds were determined by radio-TLC (n = 3). The reaction solution was analyzed using the radio-HPLC to confirm identity of ^{125}I-radiolabeled compounds. *pinacol boronic ester group = Bpin.

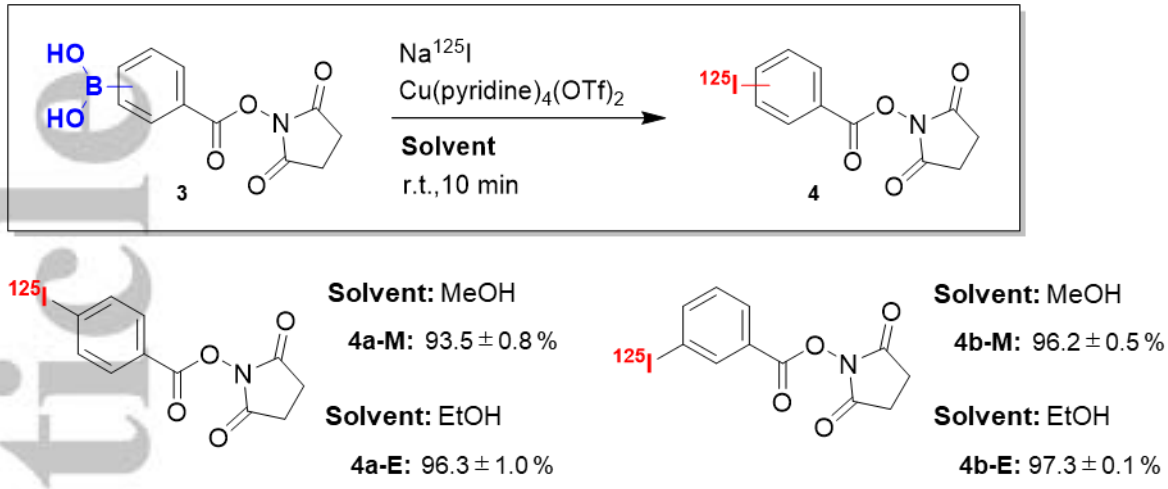


FIGURE 3 Copper-mediated ^{125}I-radiolabeling of the active ester of carboxyphenylboronic acid (n = 3).

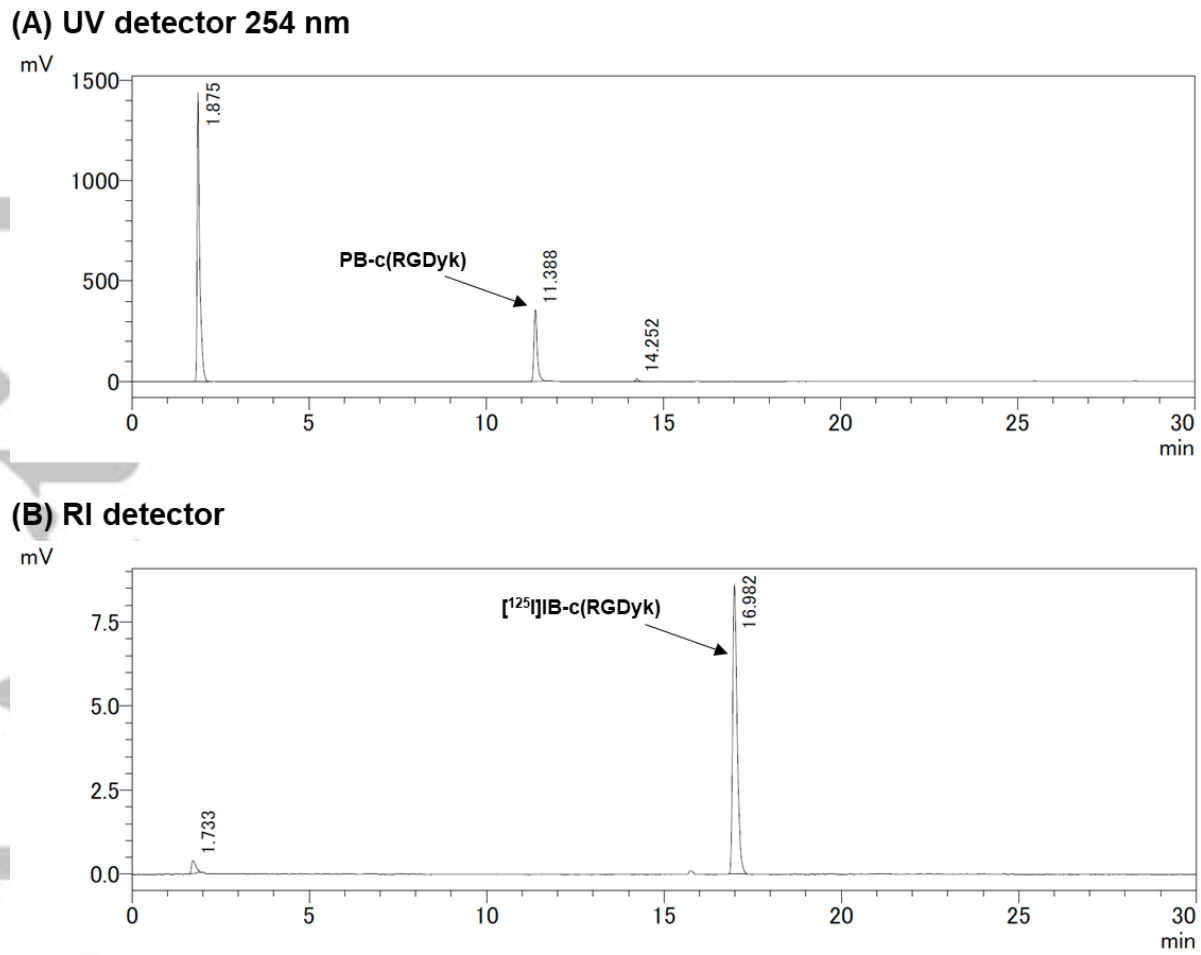
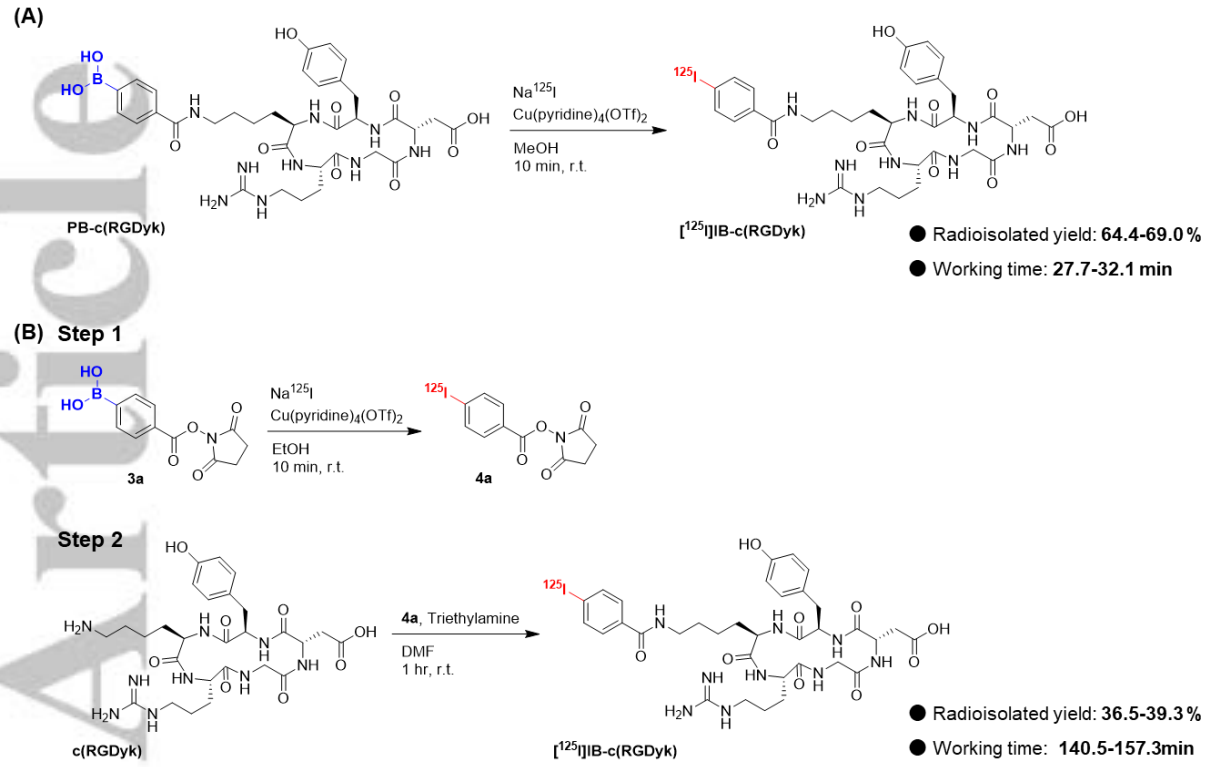


FIGURE 4 (A) UV- and (B) radio-HPLC chromatogram of the reaction solution after the copper-mediated direct radiolabeling of c(RGDyk) via PB-c(RGDyk). PB-c(RGDyk) t_R = 11.39 min; [^{125}I]IB-c(RGDyk) t_R = 16.98 min. The first peak detected at 1.50–2.00 min is a peak derived from the copper catalyst or [^{125}I]NaI.



SCHEME 1 (A) Direct radioiodination of c(RGDyk) through the boronic precursor, PB-c(RGDyk), (n = 3). (B) Indirect radioiodination of c(RGDyk) using ^{125}I-labeled *N*-hydroxysuccinimide ester of iodobenzoic acid (n = 3).

Graphical Abstract