Supplementary Information

Enhanced Aqueous Suzuki-Miyaura Coupling Allows Site-specific Polypeptide $^{18}$F-labeling

Zhanghua Gao, Veronique Gouverneur and Benjamin G. Davis*

Department of Chemistry, University of Oxford, Chemistry Research Laboratory,
Mansfield Road, Oxford OX1 3TA, U.K.
E-mail: Ben.Davis@chem.ox.ac.uk
Fax: +44 (0)1865 275674; Tel: +44 (0)1865 275652

Table of Contents

1. General Experimental Information. ........................................................................................................2
   1.1 General considerations ...............................................................................................................................2
   1.2 Protein mass spectrometry .......................................................................................................................2

2. Synthetic protocols (small molecules) ......................................................................................................3
   2.1 Palladium catalyst preparation ................................................................................................................3
   2.2 Preparation of small molecular SMC product reference .........................................................................4
   2.3 Synthesis of decamer peptide ................................................................................................................4
   2.4 SMC of decamer peptide: reference compound preparation ..................................................................7
   2.5 SMC of decamer peptide: cold optimization ...........................................................................................8
   2.6 Synthesis and SMC of octamer peptide ....................................................................................................9
   2.7 Optimization of small molecular SMC with reversed stoichiometry (non-radioactive) ......................10
   2.8 Preparation of $^{18}$F-fluorination precursor: Di(4-iodophenyl)iodonium triflate (5) ......................11
   2.9 Cold optimization of borylation .............................................................................................................12

3. Protein purification and SMC (non-radioactive) ....................................................................................13
   3.1 Preparation of SBL-ArI in high concentration .....................................................................................13
   3.2 Typical cold SMC of SBL-ArI .................................................................................................................13
   3.3 Ligand comparison (Scheme 1 in manuscript) .....................................................................................13
   3.4 Optimization of protein SMC (Protein:boronic acid = 1:2, cold) .......................................................14
   3.5 Other boronic acids: Protein SMC modification with low boronic acid loadings (20 equivalents, 1 mM) ...........................................................................................................................15
   3.6 Palladium content assay .......................................................................................................................17
1. General Experimental Information.

1.1 General considerations

All NMR spectra were recorded on Bruker DPX200, DPX300, DPX400, DQX400 or AVN400 spectrometers. Proton and carbon-13 NMR spectra are reported as chemical shifts (δ) in parts per million (ppm) relative to the solvent peak as the internal standard (1H NMR: D$_2$O = 4.79; DMSO-d$_6$ = 2.50 and 13C NMR: DMSO-d$_6$ = 39.5). Fluorine-19 NMR spectra are referenced relative to CFCl$_3$ in CDC$_3$. Coupling constants (J) are reported in units of hertz (Hz). The following abbreviations are used to describe multiplicities – s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet) brs (broad singlet). High resolution mass spectra (HRMS, m/z) were recorded on a Bruker MicroTOF spectrometer using positive electrospray ionization (ESI+/ESI-). Infrared spectra were recorded either as the neat compound or in a solution using a Bruker Tensor 27 FT-IR spectrometer. Absorptions are reported in wavenumbers (cm$^{-1}$) and only peaks of interest are reported. Melting points of solids were measured on a Griffin apparatus and are uncorrected. IUPAC names were obtained using the ACD/I Lab service. Solvents were purchased from Fisher, Rathburn or Sigma-Aldrich. When dry solvents were required they were purified by expression through an activated alumina column built according to the procedures described by Pangborn and Grubbs.1 Chemicals were purchased from Acros, Alfa Aesar, Fisher, Fluorochem, Sigma-Aldrich and used as received. Reactions were monitored by thin-layer chromatography (TLC) carried out on Merck Kiesegel 60 F254 plates, silica gel column chromatography was performed over Merck silica gel C60 (40-60 μm). Deionized water was used for chemical reactions and Milli-Q purified water for protein manipulations. Protein concentrations were determined by BCA (bicinechonic acid) assay (Pierce) and/or OD$_{280}$. SDS-PAGE gels were run using pre-cast gels purchased from Invitrogen (NuPAGE 10 % Bis-Tris gel), and stained using InstantBlueTM (Expedeon).

1.2 Protein mass spectrometry

Liquid chromatography-mass spectrometry (LC-MS) was performed on a Micromass LCT (ESITOF-MS) coupled to an Agilent 1100 Series HPLC using a Phenomenex Jupiter 5 μm C4 column (100 × 4.6 mm). Water:acetonitrile, 95:5 (solvent A) and acetonitrile (solvent B), each containing 1% formic acid by volume, were used as the mobile phases at a flow rate of 1.2 mL/min. The gradient was programmed

REFERENCES.

Review of Prior Metal-Mediated Methods.

Radiochemical Procedures

1. General Information

2. HPLC method

3. General procedure – fluoride drying using K$^{18}$F/Kryptofix® 222

4. [$^{18}$F]Fluoride incorporation

5. Radiosynthesis of [$^{18}$F]1

6. SMC of [$^{18}$F]1 with small molecules

7. SMC of [$^{18}$F]1 with peptides

8. Protein $^{18}$F-labelling and purification (with control)

9. Preparation of [$^{18}$F]1 without purification of intermediate

5. Review of Prior Metal-Mediated Methods
as follows: 5% B for 1 min to desalt and then a linear gradient to 95% B over 10 min followed by 95% B for an additional 3 min. A linear gradient over 3 minutes back to 5% B followed by 3 minutes 5% B to re-equilibrate the column. The electrospray source was operated with a capillary voltage of 3.2 kV and a cone voltage of 25 V. Nitrogen was used as the nebulizer and desolvation gas at a total flow of ca 600 L/hr. For reaction analysis, the mass spectra for all protein material in the spectra were combined using MassLynx software (v. 4.1 from Waters). Mass spectra were then calibrated using a calibration curve constructed from a minimum of 15 matched peaks from the multiply charged ion series of equine myoglobin obtained at a cone voltage of 25V. The calibrated, combined ion series was deconvoluted using a maximum entropy (MaxEnt 1) algorithm that is preinstalled on the MassLynx software. For SBL-ArI, the output Mass Ranges were set to 20,000-30,000, damage mode was set to Uniform Gaussian width at half height as 0.6 Da. These MaxEnt 1 settings gave clear final deconvoluted mass spectrometry and helped to resolve the product (SM – 32) from side peaks when the conversion is low.

2. Synthetic protocols (small molecules)

2.1 Palladium catalyst preparation

\[
\begin{align*}
\text{ADHP (L1)} & \quad \text{DMADHP (L2)} \\
\text{Na}_2\text{O} & \quad \text{Na}_2\text{O} \\
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{N} \\
\text{O} & \quad \text{O} \\
\text{ADHP (L1)} & \quad \text{DMADHP (L2)}
\end{align*}
\]

\[(\text{L1})_2\text{Pd(OAc)}_2 \quad \text{and} \quad (\text{L2})_2\text{Pd(OAc)}_2\]:

These 10 mM palladium catalysts were prepared following previous reports.\textsuperscript{1-3}

\[
\begin{align*}
\text{DMG (L3)} \\
\text{H}_2\text{N} & \quad \text{NH} \\
\text{DMG (L3)}
\end{align*}
\]

\[(\text{L3})_2\text{Pd(OAc)}_2\]:

1,1-Dimethylguanidine sulfate (L3(H\text{2SO}_4)_{0.5}, 13.6 mg, 0.10 mmol) was mixed with NaOH (0.1 M, 2 mL) and water (1 mL). Palladium acetate (11.2 mg, 0.050 mmol) was then added and the suspension was stirred at 65°C for 45 minutes, vortexing intermittently to give an orange-brown solution. After this period the solution was cooled and diluted with water to a total volume of 5 mL to give a 10 mM catalyst stock solution.

\[
\begin{align*}
\text{TMG (L4)} \\
\text{HN} & \quad \text{NMe}_2 \\
\text{TMG (L4)}
\end{align*}
\]

\[(\text{L4})_2\text{Pd(OAc)}_2\]:

1,1,3,3-Tetramethylguanidine (L4, 11.5 mg, 0.10 mmol) was mixed with NaOH (0.1 M, 1 mL) and water (2 mL). Palladium acetate (11.2 mg, 0.050 mmol) was then added and the suspension was stirred at 65°C for 45 minutes and vortexed intermittently to give a brown solution, which was diluted with water to a total volume of 5 mL to give a 10 mM catalyst stock solution.
2.2 Preparation of small molecular SMC product reference

This reference compound was prepared as described in our previous report.1

4-Iodobenzoic acid (9, 0.50 mmol), 4-fluorophenylboronic acid (1, 84 mg, 0.60 mmol, 1.2 eq) and disodium hydrogen phosphate (355 mg, 2.5 mmol) were mixed in water (5 mL) and methanol (5 mL) at 37 °C for 5 min. (L4)2Pd(OAc)2 (10 mM, 0.05 mL, 0.001 eq) was added and the reaction mixture stirred at 37°C for 18 h before being diluted with water (40 mL) and acidified with 1 N HCl (2.5 mL). After filtration, the dry residue was washed with water (2 × 5 mL) and petroleum ether (2 × 2 mL) to give a white solid. The crude material was dissolved in DCM-Et2O (1:1, 50 mL), filtered through a short silica plug and concentrated to give product as a white solid, m.p. 234-236 °C.1

1H NMR (DMSO-d6, 200MHz): δ = 8.01 (d, J=8.4 Hz, 2 H), 7.69 - 7.87 (m, 4 H), 7.33 ppm (t, J=8.9 Hz, 2 H).

13C NMR (DMSO-d6, 50MHz): δ = 167.13, 162.38 (d, J=245.8 Hz), 143.26, 135.51 (d, J=3.1 Hz), 130.00, 129.59, 129.11 (d, J=8.3 Hz), 126.80, 115.96 (d, J=21.5 Hz) ppm.

19F NMR (DMSO-d6, 376MHz): δ = -114.20 ppm.

2.3 Synthesis of decamer peptide

Peptide synthesis was carried out with TCP-resin (1.3 mmol/g) following a standard Fmoc-strategy on a CEM peptide synthesizer.

H2N-Ala-pI-Phe-Ala-Val-Asn-Thr(Bu)-Ala-Asn-Ser(Bu)-Thr(Bu)-OH

Scheme S1 Synthesis of AXAVNTANST peptide (X = p-I-Phe)
**Loading of TCP-resin**

Fmoc-Thr(tBu)-OH (1.5 eq, 2.1 g) was attached to the TCP-resin (1.3 mmol, 1.0 g) by shaking with DIEA (2.5 eq, 1.8 mL) in anhydrous DCM (15 mL) at room temperature for 1.5 h. After filtration the remaining trityl chloride groups were capped by a solution of DCM/MeOH/DIEA (20 mL) (17/2/1; V/V/V) for 15 min. The resin was filtered and washed thoroughly with DCM (× 2), DMF (× 3) and MeOH (× 5) to give the Thr loaded resin (1.13 g) for the following SPPS. The loading (0.56 mmol/g) was measured by Fmoc deprotection and UV analysis against the deprotection of a standard Fmoc-Thr(tBu)-OH.

**Peptide synthesizer settings**

**Fmoc Deprotection.** The resin-bound Fmoc peptide (initial weight 446 mg, 0.25 mmol) was treated with 20% piperidine in NMP (V/V) for 15 min (twice). The resin was washed with DMF and DCM.

**HBTU/HOBt Coupling.** A solution of Fmoc-Xaa-OH (3 eq), HBTU (3 eq), HOBt (3 eq), DIEA (6 eq) in DMF was added to the resin-bond free amine peptide for 15 min in a microwave reactor.

**Cleavage from Resin and Deprotection of tBu Group.** Starting from 565 mg of crude material, the peptide was cleaved off the resin and deprotected in TFA/TIPS-H2O/water (95/2.5/2.5, V/V, 14 mL) at RT for 3 hours. After filtration and washing with TFA (× 2), the filtrate was concentrated to c.a. 4 g, then treated with cold diethyl ether (24 mL). After filtration and washing with diethyl ether (3 × 3 mL), the filtrate was concentrated again to 1 g. Diethyl ether (3 mL) was then added to the residue. The precipitate was filtered and washed with diethyl ether (3 × 3 mL). The combined solids (150 mg) were dissolved in 3% MeOH-water containing 0.1% TFA (100 mL) and loaded on to a Biotage RP 100 g column for purification (2-30% MeCN-water with 0.1% TFA). The fractions were analyzed by HPLC and the product collected by rotavapor concentration. The residue was re-suspended in deionized water (3 mL) and lyophilized to give the final product (121 mg in total, 0.11 mmol, yield: 43% from Thr-resin). The sequence was confirmed by MS-MS analysis.

```
HRMS m/z (El+): Found 1121.3768 (M+H+); C42H66IN12O16 requires 1121.3759. 1H NMR (500 MHz, DMSO) δ 12.57 (s, 1H), 8.54 (d, J = 8.3 Hz, 1H), 8.33 (d, J = 7.2 Hz, 1H), 8.23 (d, J = 7.4 Hz, 1H), 8.08 – 7.92 (m, 5H), 7.84 (d, J = 8.8 Hz, 1H), 7.79 – 7.67 (m, 3H), 7.61 (d, J = 8.2 Hz, 2H), 7.46 (s, 1H), 7.39 (s, 1H), 7.11 (d, J = 8.3 Hz, 2H), 6.98 (d, J = 9.2 Hz, 2H), 4.88 (s, 3H), 4.62 (dd, J = 14.1, 6.9 Hz, 1H), 4.60 – 4.52 (m, 2H), 4.35 (dt, J = 7.7, 6.4 Hz, 2H), 4.29 – 4.20 (m, 2H), 4.18 (dd, J = 8.6, 3.2 Hz, 1H), 4.16 – 4.08 (m, 2H), 4.08 – 4.01 (m, 1H), 3.76 (s, 1H), 3.60 (ddd, J = 21.2, 10.9, 5.6 Hz, 2H), 2.99 (dd, J = 13.9, 3.4 Hz, 1H), 2.73 (dd, J = 13.8, 9.9 Hz, 1H), 2.60 (dt, J = 15.1, 6.5 Hz, 2H), 2.43 (ddd, J = 22.4, 15.5, 7.0 Hz, 2H), 1.96 (dd, J = 13.2, 6.6 Hz, 1H), 1.31 (d, J = 7.0 Hz, 3H), 1.23 (dd, J = 7.0, 3.2 Hz, 6H), 1.05 (d, J = 6.4 Hz, 3H), 1.02 (d, J = 6.3 Hz, 3H), 0.84 (d, J = 6.8 Hz, 3H), 0.81 (d, J = 6.8 Hz, 3H). 13C NMR (126 MHz, DMSO) δ 172.16 (s), 172.02 (s), 171.92 (s), 171.75 (s), 171.74 (s), 171.23 (s), 170.72 (s), 170.71 (s), 170.16 (s), 169.78 (s), 169.50 (s), 137.37 (s), 136.85 (s), 131.81 (s), 92.49 (s), 66.42 (s), 66.16 (s), 61.52 (s), 58.23 (s), 57.81 (s), 57.16 (s), 54.84 (s), 53.75 (s), 49.72 (s), 49.63 (s), 48.50 (s), 48.18 (s), 47.95 (s), 37.12 (s), 36.96 (s), 36.90 (s), 30.95 (s), 20.31 (s), 19.67 (s),
```
19.24 (s), 18.04 (s), 17.87 (s), 17.83 (s), 17.25 (s).

**MS-MS analysis**

All the peptide fragmentation b and y ions were found by MS-MS analysis.

YW_ZG307_30min_MSMS_1

1: TOF MSMS ES+

TIC

4.97e4

X = 4-iodophenylalanine
2.4 SMC of decamer peptide: reference compound preparation

The iodo-decamer peptide (4, 11.2 mg, 0.010 mmol) and 4-fluorophenylboronic acid (1, 14.0 mg, 0.10 mmol) were sonicated in pH 8.0 phosphate buffer (50 mM, 48 mL) at 50 °C to give a clear solution. After cooling down to 37 °C, (L3)_2Pd(OAc)_2 (10 mM, 1.0 mL, 0.2 mM in reaction solution) was added and the reaction mixture was incubated at 37 °C for 30 min. Additional (L3)_2Pd(OAc)_2 (10 mM, 1.5 mL) was added. The resulting reaction mixture was incubated for another 30 min before the addition of 3-mercaptopropionic acid (5 µL in 1 mL of water, 57 mM, 1 mL). The reaction mixture was loaded on to a 12-g C18 column and eluted with 2-30% acetonitrile in water (+0.1% formic acid). The product containing fractions (monitored by LCMS) were combined and subjected to another 30-g C18 column and eluted with 5-80% MeOH in water (+0.1% formic acid). The product was concentrated to dryness and re-suspended in deionized water (1 mL), lyophilized to give 12 as a white powder (2.53 mg, yield 23%). HRMS m/z (EI+): Found 1089.4978 (M+H+) C_{48}H_{70}FN_{12}O_{16} requires 1089.5011.

The sequence was confirmed by MSMS analysis with all the peptide fragmentation b and y ions being found.
2.5 SMC of decamer peptide: cold optimization

General method: The iododecamer peptide stock solution (0.10-0.60 mM) was prepared in pH 8 buffer at 50°C and stored at 37 °C prior to use. The peptide (0.20 mM in pH 8.0 phosphate buffer, 25 µL, 5 nmol) and fluoroarylboronic acid (20 mM, 0.5-2.5 μL, 10-50 nmol) were mixed and diluted with pH 8.0 phosphate buffer (50 mM) to a total volume of 40 μL. (L3)2Pd(OAc)2 (10 mM, 0.8-4 μL) was then added. The reaction mixture was vortexed and shaken at 37 °C for 30 minutes before being treated with
3-mercaptopropionic acid (57 mM, 5.7 eq to Pd) for 5 minutes. The crude reaction mixture was then analyzed by LCMS and the conversion was measured by the mass intensity ratio between the product and the starting material.

The yield based on the peptide conversion is given in the table below. (37 °C, 30 min)

Table S1 SMC of peptide 4 (0.1 mM) with 10 or 2 equivalents of boronic acid 1(1.0/0.2 mM).

| [Pd] | 1.0 mM | 0.2 mM |
|------|--------|--------|
| 2.0  | 97%    | 56%    |
| 1    | 98%    | 70%    |
| 0.5  | 95%    | 62%    |
| 0.3  | 98%    | 33%    |
| 0.2  | 84%    | 24%    |
| 0.1  | 41%    | 7%     |
| 0    | 0%     | 0%     |

Typical LCMS spectrums are shown below:

Figure S1 LCMS with (bottom) or without (top) scavenger (3-mercaptopropionic acid or dithiothreitol).

Figure S2 Mass spectrum of an uncompleted reaction ([4] = 0.1 mM, [1] = 0.2 mM, [(L3)2Pd(OAc)2] = 1.0 mM, conversion = 70%).

2.6 Synthesis and SMC of octamer peptide

The octamer peptide was synthesized following the same SPPS methods as described for the decamer peptide 4. HRMS m/z (EI+): Found 979.3019 (M+H+); C_{36}H_{50}IN_{10}O_{14} requires 979.3017.
SMC reference compound preparation

The iodo-octamer peptide (5, 9.9 mg, 0.010 mmol) and 4-fluorophenylboronic acid (1, 7.0 mg, 0.05 mmol) were sonicated in pH 8.0 phosphate buffer (50 mM, 48 mL) at 60 °C to give a clear solution. After cooling down to 37 °C, (L3)2Pd(OAc)2 (10 mM, 1.0 mL, 0.2 mM in reaction solution) was added and the reaction mixture was incubated at 37 °C for 30 min. A freshly made dithiothreitol (DTT) solution (1 M, 60 µL) was then added. The resulting reaction mixture was incubated for 10 min before the reaction mixture was loaded on to a 12-g C18 column and eluted with 2-20% acetonitrile in water (+0.1% formic acid). The product containing fractions (monitored by LCMS) were combined and subjected to semi-prep HPLC purification (Synergi 4u Fusion-RP 80A, 0-30% acetonitrile in water (+0.1% formic acid). The product containing fractions were concentrated to dryness and re-suspended in deionized water (1 mL), then lyophilized to give 13 as a white powder (3.2 mg, yield 34%). HRMS m/z (EI+): Found 485.2078 (M+2H+); C42H60FN10O14 requires 485.2081.

2.7 Optimization of small molecular SMC with reversed stoichiometry (non-radioactive)

General method: The palladium catalyst (10 mM, up to 10 µL) was added to a solution (200 µL) containing 4-fluorophenylboronic acid (1, 0.05 mM) and 3 (0.10 mM). The mixture was stirred at the assigned temperature for the assigned time before HPLC analysis. The yield was obtained from the UV integration of the product 11 using a standard calibration curve.

| Entry | [Pd] mM | Pd/ArI equiv. | Ligand | Time min | 1 | Product % |
|-------|---------|---------------|--------|----------|---|-----------|
| 1.    | 2.0     | 20            | L3     | 30       | 0 | 16        |
| 2.    | 1.0     | 10            | L3     | 30       | 0 | 28        |
| 3.    | 0.5     | 5             | L3     | 30       | 0 | 39        |
| 4.    | 0.25    | 2.5           | L3     | 30       | 0 | 51        |
| 5.    | 0.10    | 1             | L3     | 30       | 0 | 75        |
| 6.    | 0.05    | 0.5           | L3     | 30       | 17| 64        |
| 7.    | 0.05    | 0.5           | L3     | 45       | 10| 74        |
| 8.    | 0.05    | 0.5           | L3     | 60       | 0 | 72        |
2.8 Preparation of $^{18}$F-fluorination precursor: Di(4-iodophenyl)iodonium triflate (5)

\[
\begin{array}{c}
\text{I} \\
\text{I} \\
\end{array} \quad \text{1. mCPBA, TFOH, DCM,} \\
\begin{array}{c}
\text{I} \\
\text{I} \\
\end{array} \quad \text{2. PhI, TFOH} \\
\begin{array}{c}
\text{I} \\
\text{I} \\
\text{+} \\
\text{TFO}^{-} \\
\end{array} \\
\begin{array}{c}
\text{6} \\
\text{6b} \\
\end{array}
\]

Commercial mCPBA (4.7 g, 77% max, from Sigma-Aldrich) and anhydrous sodium sulphate (10 g) were suspended in DCM (20 mL). The liquid was decanted and the solid was washed with DCM (2×15 mL). The combined DCM solutions were filtered through an anhydrous sodium sulphate pad (1 g). The filtrate obtained was titrated (excess NaI, then sodium thiosulphate). This mCPBA solution (0.278 M) was stored in a -18°C freezer and no obvious decomposition was observed after 4 weeks. Crystallisation occurs when cool. It is necessary to warm up the solution to RT and dissolve all the crystals before use.

A 100-mL RB flask was charged with diiodobenzene (3.63 g, 11 mmol), DCM (10 mL) and a solution of mCPBA in DCM (0.278 M, 37 mL, 10 mmol) under N$_2$. The solution was cooled in an ice-water bath and triflic acid (0.80 mL, 9 mmol) was added dropwise over 2 min. Once the addition was complete, the ice bath was removed and the reaction mixture was stirred at RT for 45 min. The resulting yellow-green suspension was cooled to 0°C. Triflic acid (1.85 mL, 2.1 mmol) was added, followed by the addition of iodobenzene (1.23 mL, 11 mmol). The mixture was vigorously stirred at 0°C for 45 min, then RT for 10 min before dilution with DCM (30 mL) and stirred until most of the solid was dissolved. The reaction mixture was filtered through celite (3 g) under N$_2$ and washed with DCM (2×5 mL). The filtrate was concentrated to give 12.8 g of brown solid, which was suspended in diethyl ether (50 mL) in an ultrasound bath for 3 min, then subjected to centrifugation (20°C, 3000 G, 5 min). The supernatant was then discarded. The sonication-centrifugation ether wash was repeated twice (30 mL, then 10 mL). The solid was dried in vacuo to give a grey solid 2.9 g, crude yield 43%. Further recrystallization (1.33 g) from isopropanol (29 mL) gave the product (0.97 g). Overall yield 31%. $^1$H NMR (400 MHz, DMSO-d$_6$) δ 7.90 (appr d, $J=8.6$ Hz, 4 H) 7.98 (appr d, $J=8.6$ Hz, 4 H) ppm; $^{13}$C NMR (101MHz, DMSO-d$_6$) δ 140.44, 136.76, 116.05, 100.43 ppm; $^{19}$F NMR (376 MHz, DMSO-d$_6$) δ -77.72 ppm.
2.9 Cold optimization of borylation

Borylation was optimized with a reaction time of up to 20 min by varying solvent and base. All reactions were carried out with [6] (0.40 mmol), [7] (0.60 mmol) and base (0.80 mmol) in 4 mL of solvent. The crude reaction mixture was heated or irradiated with microwave at the 60-90°C for 5-90 minutes before diluted in methanol (to 50 mL) for HPLC analysis.

| Entry | Solvent | Base   | Temp  | Time | Yield (1) |
|-------|---------|--------|-------|------|-----------|
| 1     | CH₃CN  | KOAc   | 60°C  | 90   | 92%       |
| 2     | MeOH   | KOAc   | 60°C  | 90   | 4%        |
| 3     | EtOH   | KOAc   | 60°C  | 20   | 76%       |
| 4     | tPrOH  | KOAc   | 60°C  | 20   | 90%       |
| 5     | DMF    | KOAc   | 60°C  | 20   | 88%       |
| 6     | DMSO   | KOAc   | 60°C  | 20   | 0%        |
| 7     | DMSO   | Cs₂CO₃ | 60°C  | 20   | 29%       |
3. Protein purification and SMC (non-radioactive)

3.1 Preparation of SBL-ArI in high concentration

Protein Sequence: \((X = S-(para\text{-}iodobenzyl)cysteine)\)

\[
\begin{align*}
AQSVP & \text{ WGISR VQAPA AHNRG LTGSG VKVAV LDTGI STHPD LNIRG GASFV PGEPS TQDG} \\
& \text{ NGTH VAGTI AALNN SIGVL GVAPS AELYA VKVLG ASGSG SVSSI AQQLE WAGNN GMHVA} \\
& \text{ NLSLG SPSPS ATLEQ AVNSA TSRGV LVVAA SGNXG AGSIS YPARY ANAMA VGATD QNNNR} \\
& \text{ ASFSQ YGAGL DIVAP GVNVQ STYPG STYAS LNTS MATPH VAGAA ALVKQ KNPSW SNVQI} \\
& \text{ RNHLK NTATS LGSTN LYGSG LVNAE AATR}
\end{align*}
\]

A 2.0 mg/mL SBL-ArI solution was prepared as described in our previous report. Such a protein sample was then concentrated with a Vivaspin 10K tube to give a high concentration sample (7.3 mg/mL, determined by BCA assay).

3.2 Typical cold SMC of SBL-ArI

Typical method: SBL-156ArI (2.0 mg/mL, 20 \(\mu\)L), FPBA (1, 10 mM, 3 \(\mu\)L) and \((L3)_2\text{Pd(OAc)}_2\) (10 mM, 1.5 \(\mu\)L) were mixed and shaken at 37 °C for 30 minutes. 3-MPrAc (6 eq to Pd, 37°C, 30 min) was then added and incubated for additional 30 min. The reaction mixture was then analyzed by LCMS. The conversion was measured by the mass intensity ratio between the product and the starting material.

A typical mass spectrum is given below.
3.3 Ligand comparison (Scheme 1 in manuscript)

Ligands were compared under fixed protein concentration ([1] = 0.05 mM) and palladium catalyst loading ([L2Pd(OAc)2] = 1 mM).

Table S2 SMC of 1 with model proteins SBL-ArI (2) under constrained conditions ([1] = 0.10–10.0 mM)

| [1]   | 0.10 mM | 0.50 mM | 1.0 mM | 2.0 mM | 10.0 mM |
|-------|---------|---------|--------|--------|---------|
| L1    | 0       | 0       | 0      | 5      | 72      |
| L2    | 1       | 38      | 69     | 83     | 91      |
| L3    | 1       | 58      | 79     | 85     | 95      |
| L4    | 3       | 12      | 73     | 83     | 88      |

3.4 Optimization of protein SMC (Protein:boronic acid = 1:2, cold)

1. Optimal pH: pH 8.0.

SMC of SBL-ArI and 1 were first carried out at different pHs at concentrations of [SBL-ArI] = 0.20 mM, [1] = 0.40 mM, ([L3]Pd(OAc)2] = 1.0 mM. While the same protein conversions (1%) were obtained at pH 8.0 and pH 8.5, no SMC product was detected at pH lower than 8 or higher than 8.5.
2. Optimal Pd-catalyst concentration: \([{(\text{L3})^2}\text{Pd(OAc)}_2] = 2 \text{ mM}\)

At higher [Pd] higher product/sm ratios were obtained. However, high Pd loadings (4 mM) caused substantial protein degradation /precipitation. The SDS Page gel analysis suggested the degradation of protein under such conditions. The relative concentration of the remained protein was estimated against an SBL-ArI sample by gel-densitometry and used to correct all the yields/conversions of the SMC. A maximum 10% protein conversion was obtained with 2 mM palladium catalyst.

![Figure S4 SMC of SBL-ArI and 1 with different [Pd]
(pH 8.0, [SBL-ArI] = 0.20 mM, [1] = 0.40 mM, \([(\text{L3})^2]\text{Pd(OAc)}_2] = 0.5-4.0 \text{ mM})](image)

3. Protein concentration

In this experiment, boronic acid 1/protein 2 ratio was set to 2 while [2] varies. The maximum achievable protein concentration is between 0.26 - 0.3 mM, so 0.2 mM was set as the optimal protein concentration.\(^6\)

![Figure S5 SMC of SBL-ArI and 1 at different protein concentration (pH 8.0, [SBL-ArI] = 0.20 mM, [1] = 0.40 mM, \([(\text{L3})^2]\text{Pd(OAc)}_2] = 4.0 \text{ mM})](image)

With 2 eq of 1, the optimized conditions for SMC of SBL-ArI ([Protein] = 0.20 mM, [Pd] = 2 mM, pH 8.0, 37°C, 30 min) gave a 10% yield based on protein. Therefore, the corresponding yield based on 1 is 5%.

3.5 Other boronic acids: Protein SMC modification with low boronic acid loadings (20 equivalents, 1 mM)

![Image of Pic156 reaction with arylboronic acid and (L3)2Pd(OAc)2 catalyst](image)

2 (0.05 mM)
Protein modifications were conducted at a fixed protein concentration ([2] = 0.05 mM) and palladium catalyst loading ([L3]2Pd(OAc)2] = 1 mM).

Table S3 SMC of model protein SBL-ArI (2) with 1 mM boronic acids

| Entry | Boronic acid ArB(OH)2 | Conversion | Remaining 2 | Deiodination | Calculated mass | Mass found |
|-------|-----------------------|------------|-------------|--------------|----------------|------------|
| 1     | ![Boronic acid 1](image1) | 84%        | 0           | 16%          | 27053          | 27055      |
| 2     | ![Boronic acid 2](image2) | 85%        | 0           | 15%          | 27025          | 27027      |
| 3     | ![Boronic acid 3](image3) | 74%        | 0           | 26%          | 27060          | 27062      |
| 4     | ![Boronic acid 4](image4) | 65%        | 13%         | 22%          | 27035          | 27037      |
| 5     | ![Boronic acid 5](image5) | 90%        | 0           | 10%          | 27065          | 27067      |
| 6     | ![Boronic acid 6](image6) | 89%        | 0           | 11%          | 27095          | 27097      |
| 7     | ![Boronic acid 7](image7) | 84%        | 0           | 16%          | 27049          | 27051      |

ESI-MS

**CRL315874 209 (3.560)**

Entry 1, ArB(OH)2 =
Calc. mass 27053
Found 27055

**TOF MS ES+**
3.92e4

S16
3.6 Palladium content assay
A mixture of SBL-ArI (7.3 mg/mL, 72.4 µL, 20 nmol), (L3)2Pd(OAc)2 (10 mM, 20 µL, 200 nmol) and [19F]I (0.075 mM in pH 8.0 50 mM phosphate buffer, 20 µL) was shaken at 37°C for 30 min. After this time 3-MPrAc (0.5% in water, 20 µL, 1.2 µmol) was added to dissolve the precipitation. After a short incubation time (1 minute), the reaction mixture (132.4 µL) was applied slowly in the middle of the
packed bed of a PD SpinTrap G25 equilibrated with pH 8.0 50 mM phosphate buffer (a stacker volume of 7.6 µL was applied for the sample volume to reach 140 µL). The sample was eluted by centrifugation at 800 × G for 2 minutes. The PD SpinTrap purification was repeated 4-6 times (including the initial purification). A sample of the collected product (100 µL) was mixed with distilled nitric acid (200 µL) and diluted with deionised water to 10.0 mL for palladium content analysis. Palladium was analysed at 340.458 nm using a Perkin Elmer Optima 5300 DV ICP-OES (analysed in axial view, Argon flow rates: Plasma 15 L/min; Aux 0.45 L/min; Neb 0.75 L/min; RF power 1400W).

Table S4 Results of palladium removal using PD SpinTrap G-25. (n = number of SpinTrap centrifugations)

| n  | 0   | 1   | 4   | 5   | 6   |
|----|-----|-----|-----|-----|-----|
| Palladium content (ppm) | 149 | 74.9 | 6.4 | 2.6 | 1.0 |

4. Radiochemical Procedures

4.1 General Information

$[^{18}\text{F}]$Fluoride was produced by PETNet Solutions at Mount Veron Hospital (UK) via the $^{18}\text{O}(p,n)^{18}\text{F}$ reaction and delivered as $[^{18}\text{F}]$fluoride in $[^{18}\text{O}]$water. Radiosynthesis and azeotropic drying was performed on a NanoTek® automated microfluidic device (Advion). $[^{18}\text{F}]$Fluoride was separated from $^{18}\text{O}$-enriched-water using anion exchange cartridges (MP1, ORTG, Tennessee, USA or $^{18}\text{F}$ separation cartridges, 45 mg, Synthra, Hamburg, Germany).

HPLC analysis was performed with a Dionex Ultimate 3000 dual channel HPLC system equipped with shared autosampler, parallel UV-detectors and LabLogic NaI/PMT-radiodetectors with Flowram analog output. Radio-TLC was performed on Merck Kiesegel 60 F254 plates. Analysis was performed using a plastic scintillator/PMT detector. All isolated radiochemical yields quoted are decay corrected. Radiochemical yields are calculated from activity isolated from SPE purification relative to the activity used in the reaction. For small molecule and peptide SMC labeling, the radiochemical yields are calculated from the HPLC radiotrace. The product fraction was collected and re-injected to determine the radiochemical purity (RCP).

4.2 HPLC method

**HPLC gradient 1** (A: Water with 0.1% formic acid; B: Methanol with 0.1% formic acid, 1.6 mL/min, Merck Chromolith C18 Column, 4.6 × 100 mm)

- 0-1 min 5% B isocratic
- 1-11 min (5% B to 95% B) linear increase
- 11-12 min (95% B) isocratic
- 12-13 min (95% B to 5% B) linear decrease
- 13-15 min (5% B) isocratic

**HPLC gradient 2** (A: Water:acetonitrile, 95:5 with 0.1% formic acid; B: acetonitrile with 0.1% formic acid, 1.0 mL/min, Waters Nova-Pak C18 Column, 4 µm, 3.9 × 150 mm).

- 0-0.5 min 0% B isocratic
- 0.5-10 min (0% B to 100% B) linear increase
- 10-11 min (100% B) isocratic
- 11-12 min (100% B to 0% B) linear decrease
- 12-15 min (0% B) isocratic
HPLC gradient 3 (A: Water:acetonitrile, 95:5 with 0.1% trifluoroacetic acid; B: acetonitrile with 0.1% trifluoroacetic acid, 1.6 mL/min, Merck Chromlith C18 Column, 4.6 × 100 mm)

0-0.5 min 5% B isocratic
0.5-10 min (5% B to 95% B) linear increase
10-11 min (95% B) isocratic
11-12 min (95% B to 5% B) linear decrease
12-15 min (5% B) isocratic

HPLC gradient 4 (A: Water with 0.1% formic acid; B: acetonitrile with 0.1% formic acid, 1.0 mL/min, Waters Nova-Pak C18 Column, 4 µm, 3.9 × 150 mm).

0-1 min 5% B isocratic
1-11 min (5% B to 95% B) linear increase
11-12 min (95% B) isocratic
12-13 min (95% B to 0% B) linear decrease
13-14 min (5% B) isocratic

4.3 General procedure – fluoride drying using K\textsuperscript{18}F/Kryptofix® 222

The fluoride drying was carried out with a NanoTek (Advion BioSystems) device. Cyclotron-produced non-carrier-added aqueous \([\text{\textsuperscript{18}F}]\)fluoride (3~4 GBq) was first trapped in an MP1 anion-exchange cartridge and subsequently released with kryptofix 222 (12 mg/mL) and K\textsubscript{2}CO\textsubscript{3} (2.2 mg/mL) in acetonitrile and water (4/1=v/v, 5 × 100 µL). The solvent was then evaporated under a stream of nitrogen (0.4 L/min) at 105 °C. The fluoride was further dried azeotropically with 6 × 50 µL of acetonitrile. The total drying process took 15 – 20 minutes.

4.4 \textsuperscript{18}F Fluoride incorporation

A solution of the bis(4-iodophenyl)iodonium triflate (6, 10 mg) in anhydrous DMF (400 µL) was added to the dry \([\text{\textsuperscript{18}F}]\)fluoride. The reaction mixture was heated at 145°C for 20 min, then cooled down for 1 min. The reaction mixture was diluted with water (4.6 mL) and transferred through a LiChrolut RP18 cartridge (500 mg, activated with 5 mL of methanol then 10 mL of water) over 2-5 min. The vial was rinsed with 5 mL of water and transferred through the cartridge.

With 2.5 mL of 70% MeCN/water, the \([\text{\textsuperscript{18}F}]\)FPhI product was eluted into another 20 mL dilution vial containing 12 mL of deionized water. The well-shaken mixture was passed through a Sep-Pak C18 light cartridge. The cartridge was rinsed with water (5 ml) and dried by passing 5-10 mL of air through.

DMSO (0.10 mL) was injected to the Sep-Pak C18 light cartridge and the filtrate was discarded. The cartridge was further eluted with DMSO (0.30 mL) to give the solution of \([\text{\textsuperscript{18}F}]\)7 (typically 300 MBq – 1 GBq). Radiochemical yield 15–44%. Radiochemical purity (>95%) and identity was confirmed by HPLC analysis.
Figure S6 Identification of $[^{18}\text{F}]$FPhI (7) (top: radioactivity; bottom: UV with co-injected reference compound. HPLC gradient 3)

4.5 Radiosynthesis of $[^{18}\text{F}]$1

The solution of $[^{18}\text{F}]$7 in DMSO was transferred into a 5-mL vial containing KOAc (7 mg), B$_2$(OH)$_4$ (7, 4.5 mg) and Pd(dppf)Cl$_2$ (0.7 mg) and heated at 90°C for 20 min then cooled down for 1 min. The reaction mixture was diluted with water (4.6 mL) and transferred through two successive Sep-Pak plus C18 cartridges (360 mg each, activated with 5 mL of methanol and 10 mL of water) over 2 min. The cartridges were rinsed with water (5 mL) and 20% methanol/water (12 mL), dried by passing 5 mL of air through. Methanol (1.4 mL) was slowly injected to the air-dried cartridges and the filtrate was discarded. Another 0.9 mL of methanol was passed through the cartridges and the product $[^{18}\text{F}]$1 was collected. The product was concentrated at 90°C with a stream of nitrogen and resolubilized in 50 mM pH 8.0 phosphate buffer (200 µL) for further SMC study. Radiochemical yield 28–39% (typically 70 – 140 MBq).

To determine the specific activity, the above $[^{18}\text{F}]$1 (~ 1 MBq, 20 µL) was injected to the HPLC and the chemical concentration was determined using the UV integration at 217 nm. The above protocol gave a specific activity of 9.7 GBq/µmol (ranging 3.1–14.4 GBq/µmol).
4.6 SMC of [18F]1 with small molecules

**Typical procedure**: A total reaction mixture volume of 30 - 40 µL was maintained by dilution with pH 8.0 phosphate buffer (50 mM, 0-20 µL) if needed. [18F]1 (5-60 MBq/mL, 4-32 µL), stock ArI solution (0.1-1 mM for 3 and 9, 4-32 µL) and palladium catalyst (10 mM, 4 µL) were mixed and incubated at 37 °C for 30 min before HPLC analysis. The radiochemical yield was determined by integration of radio-HPLC.

**Table S5. SMC of [18F]1**

| Entry | ArI | [ArI] | [Pd] | L   | RCY | [18F]1 |
|-------|-----|-------|------|-----|-----|-------|
| 1     | 9   | 0.1   | 1.0  | L3  | 83% | 0%    |
| 2     | 3   | 0.1   | 1.0  | L3  | 87% | 1%    |
| 3     | 9   | 0.1   | 2.0  | L3  | 78% | 0%    |
Figure S8 RCP analysis of N-Boc-4-iodophenylalanine labeling (top: radioactivity; bottom: UV of a reference compound. HPLC gradient 4)

Figure S9 SMC [\(^{18}\)F]-labelling of 9 (top: radioactivity; bottom: UV of reference compound. HPLC gradient 2)
Figure S10 RCP analysis of 4-iodobenzoic acid labeling (top: radioactivity; bottom: UV of a reference compound. HPLC gradient 4)

4.7 SMC of [18F]I with peptides
The decamer 4 or octamer 5 peptide stock solution (0.10-0.60 mM) was prepared in pH 8.0 buffer and stored at 37 °C prior to use.

Typical procedure: A total reaction mixture volume of 50 µL was controlled by dilution with pH 8.0 phosphate buffer (50 mM) if needed. [18F]I (5-60 MBq/mL, 5 µL), stock peptide solution (0.5 mM, 40 µL) and Palladium solution (10 mM, 10 µL) were mixed and incubated at 37 °C for 30 min before a freshly prepared DTT solution (60 mM, 10 µL) was added. The reaction mixture was incubated 5 minutes before HPLC analysis. The radiochemical yield was determined by integration of radio-HPLC. (4: RCY 33% as an average of 9 reactions; 5: RCY 48% as an average of 3 reactions).

Table S6. Peptide SMC labeling with [18F]I

| Entry | Peptide | [Peptide] / mM | [Pd] / mM | RCY | Entry | Peptide | [Peptide] / mM | [Pd] / mM | RCY |
|-------|---------|----------------|-----------|-----|-------|---------|----------------|-----------|-----|
| 1     | 4       | 0.1            | 1.0       | 7%  | 1     | 5       | 0.1            | 1.0       | 30% |
| 2     | 4       | 0.2            | 1.0       | 13% | 2     | 5       | 0.4            | 1.0       | 22% |
| 3     | 4       | 0.4            | 1.0       | 19% | 3     | 5       | 0.1            | 2.0       | 48% |
| 4     | 4       | 0.8            | 1.0       | 4%  |       |         |               |           |     |
| 5     | 4       | 0.4            | 0.5       | 17% |       |         |               |           |     |
| 6     | 4       | 0.4            | 2         | 33% |       |         |               |           |     |
Figure S11 SMC $[^{18}\text{F}]$-labelling of decamer peptide 4 (top: radioactivity; bottom: UV of reference compound. HPLC gradient 1) ([4] = 0.10 mM, [(L3)$_2$Pd(OAc)$_2$] = 2.0 mM, 37°C, 30 min)

Figure S12 RCP analysis of labeled decamer peptide 12 (top: radioactivity; bottom: UV of a reference compound. HPLC gradient 4)
4.8 Protein $^{18}$F-labelling and purification (with control)

A mixture of SBL-Arl (7.3 mg/mL, 72.4 µL, 20 nmol, ~0.2 mM in reaction mixture of a total volume 97.2-112.2 µL), ($\text{L3}_2\text{Pd(OAc)}_2$) (10 mM, 20 µL, 200 nmol) and $[^{18}\text{F}]$1 (5~20 µL, ~15 MBq) was mixed and shaken at 37°C for 30 min. Then 3-MPrAc (0.5% in water, 20 µL, 1.2 µmol) was added to dissolve the precipitation. After a short incubation time (1 minute), the reaction mixture sample was applied slowly in the middle of the packed bed of a PD SpinTrap equilibrated with pH 8.0 buffer (a stacker volume was applied for sample volume to reach 140 µL). The sample was eluted by centrifugation 800 $\times$ G for 2 minutes. The collected product was subjected to HPLC analysis after activity measurement (0.2-0.5 MBq). Radioactivity in the trap was also measured to work out the radiochemical yield. The decay corrected RCY obtained were 2-5%.
Figure S15 $[^{18}\text{F}]$-labeled protein 14 (top: radioactivity of purified protein; bottom: 277 nm UV of the same sample. HPLC gradient 3).

The control experiment was carried out same as the protein labelling method except the Palladium catalyst was replaced with pH 8.0 buffer. The fluorophenylboronic acid was the only radioactive component detectable, which showed negligible non-specific FPBA binding.

4.9 Preparation of $[^{18}\text{F}]$1 without purification of intermediate 7

The purification of the radiolabelling intermediate $[^{18}\text{F}]$7 requires two C18 cartridges for purification and formulation. This takes 20-30 minutes in a typical experiment. We also tested the preparation of $[^{18}\text{F}]$1 without such purification. Thus, the crude fluorination mixture was transferred directly in to the borylation mixture ($\text{B}_2(\text{OH})_4$, Pd(dppf)Cl$_2$ and KOAc). After heating at 90°C for 20 min and SPE purification, $[^{18}\text{F}]$1 was obtained in a 5-20% radiochemical yield with 59-80% radiochemical purity.

Method 1

Fluorination was carried out as described in the standard protocol (4.4-4.5), from 10 mg of precursor 6 and 200 μL of DMF. A 100 μL aliquot of this reaction mixture (486 MBq, RCP 67% excluding the $[^{18}\text{F}]$fluoride, by HPLC) and dry DMSO (300 μL) were added to the borylation vial containing 4.5 mg of $\text{B}_2(\text{OH})_4$, 0.7 mg of Pd(dppf)Cl$_2$ and 7.0 mg of KOAc. After heating at 90°C for 20 min SPE purification was undertaken following the standard protocol (4.5), $[^{18}\text{F}]$1 was obtained in a 42% decay corrected radiochemical yield (123 MBq) with 59% radiochemical purity.

Method 2

A solution of the bis(4-iodophenyl)iodonium triflate (6, 5 mg) in anhydrous DMF (200 μL) was added to the dry $[^{18}\text{F}]$fluoride. The reaction mixture was heated at 145°C for 20 min, then cooled down for 1 min. The reaction mixture was transferred to a 5-mL vial containing KOAc (7 mg), $\text{B}_2(\text{OH})_4$ (8, 4.5 mg) and Pd(dppf)Cl$_2$ (0.7 mg) and heated at 90°C for 20 min then cooled down for 1 min. The reaction mixture was then diluted with water (4.6 mL) and purified through three successive Sep-Pak plus C18 cartridges (360 mg each, activated with 5 mL of methanol and 10 mL of water) over 2 min. The cartridges were rinsed with water (5 mL) and 20% methanol/water (20 mL) and dried by passing 5 mL of air through. 50% methanol/water (2 mL) was slowly injected in to the air-dried cartridges and the filtrate was discarded. Another 2 mL of 50% methanol/water was passed through the cartridges and the product
[^18]F]I was collected. The product was analyzed by HPLC, giving a 80% RCP and decay corrected RCY of 5% over 2 steps.

The product was concentrated at 90°C with a stream of nitrogen and resolubilized in 50 mM pH 8.0 phosphate buffer (200 µL) for use in SMC reactions.

**SMC test**

To test its performance in SMC,[^18]F]I prepared as method 2 (RCP 80%) was reacted with 4-iodobenzoic acid (9, 0.1 mM in reaction), offering a RCY of 60% (compared to RCY 83% from a radiochemically pure 7).

![HPLC chromatogram](image)

Figure S16 Simplified preparation of[^18]F]I (top: radiotracer of[^18]F]I (method 1); middle: radiotracer of[^18]F]I (method 2); bottom: SMC of[^18]F]I (method 2) with 4-iodobenzoic acid (0.1 mM) in presence of 1 mM of (L3)2Pd(OAc)2. HPLC gradient 4)

**5. Review of Prior Metal-Mediated Methods.**

Many currently employed Cu(I)-catalyzed triazole-forming protocols used for introducing 18F to small peptides still use great excesses and often elevated temperatures (60 °C and higher) that are not compatible with many proteins. As a result perhaps of these difficulties and those outlined in the main text, effective site-selective applications of this method to proteins are rare. For example, to the best our knowledge, there is only one example of direct, site-selective 18F prosthetic incorporation into proteins using the Cu(I)-catalyzed triazole reaction\(^\text{11}\) and a second example that is two-step process that relies initially on Cys-modification chemistry\(^\text{12}\) and then a second triazole-forming step. The single direct site-selective Cu(I) triazole method\(^\text{11}\) is an overall less efficient process than the Pd-mediated method that we present here. Other methods have been used to label e.g. lysines indiscriminately and without site control (thus generating a mixed population of proteins) but the goal of this work is to create pure proteins with control of site and label.
6. References.

1 Chalker, J. M.; Wood, C. S. C.; Davis, B. G. *J. Am. Chem. Soc.* **2009**, *131*, 16346-16347.

2 Dumas, A.; Spicer, C. D.; Gao, Z.; Takehana, T.; Lin, Y. A.; Yasukohchi, T.; Davis, B. G. *Angew. Chem. Int. Ed.* **2013**, *52*, 3916.

3 Li, N.; Lim, R. K. V.; Edwardraja, S.; Lin, Q. *J. Am. Chem. Soc.* **2011**, *133*, 15316.

4 (a) Li, X.; Abell, C.; Ladlow, M. *J. Org. Chem.* **2003**, *68*, 4189. (b) Bright, T. V.; Dalton, F.; Elder, V. L.; Murphy, C. D.; O'Connor, N. K.; Sandford, G. *Org. Biomol. Chem.* **2013**, *11*, 1135.

5 The product contains 7% or asymmetric product (5b, characteristic $^1$H NMR(400 MHz, DMSO-$d_6$) δ ppm 8.24 (appr d, $J$=8.2 Hz, 2 H) 7.67 (appr t, $J$=8.5 Hz, 1 H) 7.53 (appr t, $J$=8.5 Hz, 2 H)). Fluorination of this compound gave a 85:15 fluorination selectivity (FPhI vs FPh) according to Coennen’s report (Shah, A.; Pike, V. W.; Widdowson, D. A., *J. Chem. Soc., Perkin Trans. 1* **1998**, *2043-2046*.). Although a high purity synthesis of symmetric precursor 5 has been reported, (Cardinale, J.; Ermert, J.; Coenen, H. H. *Tetrahedron* **2012**, *68*, 4112.), decomposition of this precursor would generate iodobenzene (Wuest, F. R.; Kniess, T., *J. Labelled Compd. Radiopharm.* **2003**, *46*, 699-713). No effort was taken to improve the purity of 5 further.

6 The protein stock solution in use contains 7.3 mg/mL of SBL-ArI (~0.26 mM), which was diluted by other reagents and chemicals in the reaction mixture. It was found impractical to increase the protein concentration further due to protein precipitation.

7 This experiment was carried out with 4 mM of [Pd] prior to gel densitometry assay. When protein degradation/precipitation was not accounted, same level of conversions were obtained with 2 mM an 4 mM of [(L3)$_2$Pd(OAc)$_2$].

8 When the reaction was conducted at 60 °C (optimal temperature for cold borylation), remaining starting [$^{18}$F]6 was occasionally found. Constantly full consumption of [$^{18}$F]6 was given at elevated temperature (90 °C).

9 DTT (DL-1,4-Dithiothreitol) was used as a scavenger to replace 3-mercaptopropionic acid. Palladium complex with the latter scavenger showed strong UV background overlapping with the product peptide.

10 3-Mercaptopropionic acid effectively resolubilized solid in the reaction mixture.

11 Boutureira, O.; D’Hooge, F.; Fernandez-Gonzalez, M.; Bernardes, G. J. L.; Sanchez-Navarro, M.; Koeppke, J. R.; Davis, B. G. *Chem. Commun.* **2010**, *46*, 8142-8144.

12 Ramenda, T.; Kniess, T.; Bergmann, R.; Steinbach, J.; Wuest, F., *Chem. Commun.*, **2009**, *45*, 7521-7523.