Direct ^{11}CN-Labeling of Native Peptides via Palladium-Mediated Sequential Cross-Coupling Reactions

Wenjun Zhao,[†,‡,¶] Hong Geun Lee,[§,¶] Stephen L. Buchwald,[,§] Jacob M. Hooker*[*,†,‡]*

† Division of Nuclear Medicine and Molecular Imaging, Department of Radiology, Massachusetts General Hospital, Boston, Massachusetts 02114, United States

‡ Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts 02129, United States

§ Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, United States

E-mail: sbuchwal@mit.edu, hooker@nmr.mgh.harvard.edu

SUPPORTING INFORMATION

Table of Contents

I. General Experimental.

Anhydrous dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich in Sure-Seal^{TM} bottles. HPLC grade acetonitrile and water were obtained from Fisher Chemical. All deuterated solvents were purchased from Cambridge Isotopes and used without further purification. [(1,5-COD)Pd($CH_2TMS)_2$] was prepared according to the literature procedure and stored in a nitrogen-filled glovebox at –20 °C when not in use.[1] All the peptides used in this study were synthesized by Massachusetts General Hospital Peptide/Protein Core Facility. RuPhos and *t*-BuBrettPhos used in this work were received as gifts from Sigma-Aldrich, for which we are grateful. All other reagents were purchased from commercial sources and used as received, or prepared as described below.

Pd complexes were characterized by ^{1}H, ^{13}C, and ^{31}P NMR. ^{1}H NMR and ^{13}C NMR were taken on a Varian Inova 500 MHz spectrometer or a Bruker Avance-400 MHz spectrometer in $CDCl_3$ and calibrated using residual solvent as an internal reference (^{1}H NMR: δ = 7.24 ppm and ^{13}C NMR: δ = 77.0 ppm). $^{31}P\{^{1}H\}$ spectra were recorded on a Varian Inova 500 MHz spectrometer in $CDCl_3$ and calibrated to an external standard of neat H_3PO_4 (δ 0.0 ppm). The following abbreviations were used to explain multiplicities: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, p = pentet, sext = sextet, sept = septet, m = multiplet. ICP-MS analysis of the purified product was conducted using an Agilent 8800-QQQ ICP-MS system.

Peptide identities were analyzed by LC-MS. LC-MS chromatograms and associated mass spectra were acquired using Agilent 1200 series LC and Agilent 6310 Ion Trap mass spectrometer. A general mobile phase combination of 0.1% formic acid

(FA) in water (solvent **I**) and 0.1% FA in acetonitrile (solvent **II**) were used. LC conditions: Zorbax Eclipse XDB-C8 column: 2.1 × 150 mm, 5 μm, room temperature, gradient: 0-10 min 5-95% **II**, 10-15 min 95% **II**, flow rate: 0.3 mL/min. MS conditions: positive electrospray ionization (ESI) extended dynamic mode in mass range 70 – 2200 m/z, temperature of drying gas = 300 °C, flow rate of drying gas = 7 L/min, pressure of nebulizer gas = 30 psi, the skimmer, cap exit, Oct 1 DC and Oct 2 DC voltages were set at 40, 136.7, 12 and 1.77, respectively. The octupole rf, lens 1 and lens 2 voltages were set at 200, -5 and -60, respectively. The Trap Drive was set at 57.1.

Determination of Reaction Yields for non-radioactive reactions (Table 1): All reported yields in Table 1 were determined by calculation based on a calibration curve and integration of LC spectra obtained from LC-MS system. The peak areas on the chromatogram were integrated using Agilent ChemStation software.

Radioactivity was produced by an Eclipse HP 11 MeV cyclotron (Siemens Healthcare, Munich, Germany) using a ^{11}C gas target (N_2 gas containing 2.5% O_2) irradiated at 52 μA to generate [^{11}C]CO_2. The [^{11}C]CO_2 was delivered to a Siemens Healthcare Explora CN synthesis module and first converted to [^{11}C]CH_4 with H_2 on Ni at 400 °C, and then to [^{11}C]HCN with NH_3 on Pt at 900 °C. Helium was used as a carrier gas. The [^{11}C]HCN was trapped as a solution in H_2O. The amount of captured [^{11}C]CN was measured by attaching the vent line of the reaction vial to a charcoal trap. Passing the [^{11}C]CN gas mixture through H_2O leads to ~98% trapping of [^{11}C]CN, while residual, unreacted [^{11}C]CH_4 from the production of [^{11}C]CN is not trapped. Beginning from approximately 250 mCi of $^{11}CO_2$ (52 μA · 5 min), approximately 110 mCi of [^{11}C]CN can be routinely captured in solution at 15 min post bombardment. For high activity

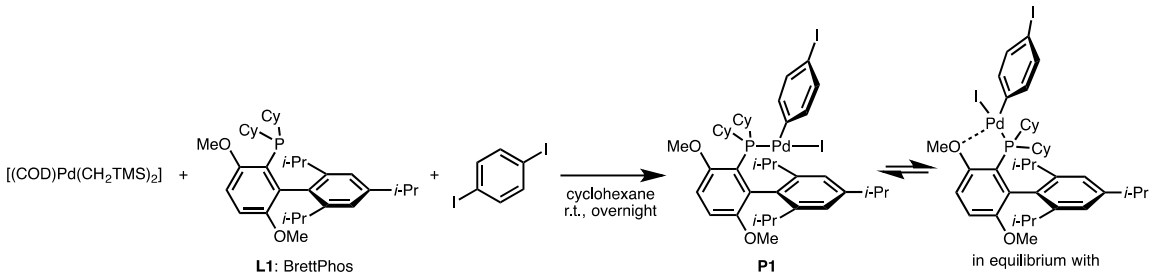
syntheses, the point at which the addition of [^{11}C]HCN concluded was designated as the start of synthesis, and the moment when the radioactivity of the final purified product was measured was designated as the end of synthesis. Semi-preparative HPLC purification was performed on an Agilent 1200 HPLC equipped with a VICI sample injector, quaternary pump, vacuum degasser, variable wavelength detector, and semi-preparative HPLC column (Agilent ZORBAX Eclipse XDB-C18, 9.4 × 250 mm, 5 μm). Radioactive analytes were detected with a Carroll & Ramsey radiation detector (Model 105S). Analytical HPLC analyses were performed with an Agilent 1200 series HPLC equipped with a quaternary pump, vacuum degasser, diode-array detector, Carroll & Ramsey radiation detector (Model 105S), and analytical HPLC column (Agilent ZORBAX Eclipse XDB-C18, 4.6 × 150 mm, 5 μm). The identities of the final products were confirmed by either analysis of the sample via LC-MS, or retention time comparison to previously analyzed standards using identical conditions. Typically, ca. 0.25 min separation between the UV peak and the corresponding radioactive peak (flow rate dependent) was observed, due to void volume between detectors. Specific radioactivity (SA) was calculated by analytical HPLC analysis of purified radioactive products. The peak area (UV 280 nm) of the desired analyte was used to determine the concentration of mass versus radioactivity. A calibration curve of four known mass quantities versus HPLC peak area (280 nm) was generated to calculate the mass concentration in the final radioactive formulation.

II. Preparation of Pd complexes (P1-P4).

General procedure for Pd complex preparation:

In a nitrogen-filled glovebox, to an oven-dried scintillation vial (10 mL) equipped with a magnetic stir bar were added the biarylphosphine ligand (0.220 mmol, 1.10 equiv), 1,4-diiodobenzne (659 mg, 2.00 mmol, 10.0 equiv), and cyclohexane (4 mL). Then [(COD)Pd($CH_2TMS)_2$] (77.8 mg, 0.200 mmol, 1.00 equiv) was added rapidly into the reaction vial in one portion. The vial was then sealed and removed from the glovebox. After the resulting solution was stirred for 16 h at rt, pentane (3 mL) was added and the resulting mixture was placed into a –20 °C freezer for 3 h. The resulting precipitate was filtered, washed with pentane (5 × 3 mL), and dried under reduced pressure to afford the oxidative addition complex.

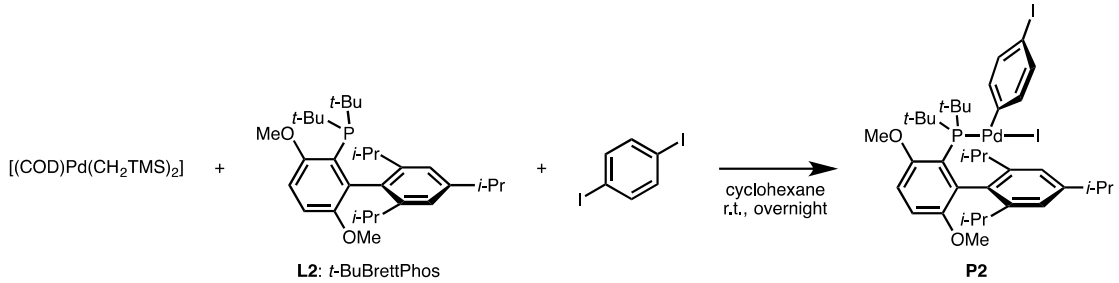
*Preparation of **P1**:*



The general procedure for Pd-complex preparation was followed with BrettPhos (118 mg, 0.220 mmol, 1.10 equiv). General work up afforded **P1** as a light yellow solid (150 mg, 0.154 mmol, 77%). Spectral data for **P1**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.13 (d, *J*=8.8 Hz, 2H), 7.09 (s, 2H), 6.86 (dd, *J*=8.8, 2.4 Hz, 1H), 6.80 (br d, *J*=8.8 Hz, 2H), 6.78 (d, *J*=9.2 Hz, 1H), 3.80 (s, 3H), 3.30 (s, 3H), 3.20-3.09 (m, 1H), 2.79-2.67 (m, 2H), 2.52-2.40 (m, 2H), 1.95-1.85 (m, 2H), 1.56 (br d, *J*=6.4 Hz, 6H), 1.36 (d, *J*=6.8 Hz, 6H), 0.76 (d, *J*=6.8 Hz, 6H) ppm (major isomer; the other peaks in the region of 1.80-0.50 ppm are not assigned due to complexity caused by the overlap of the two isomers); δ 7.15 (d, *J*=8.3 Hz, 2H), 7.06 (br d, *J*=7.8 Hz, 2H), 7.00 (s, 2H), 6.99 (dd, *J*=9.3, 3.9 Hz, 1H), 6.95 (d, *J*=9.3 Hz, 1H), 4.42 (s, 3H), 3.56 (s, 3H), 2.96-2.85 (m, 1H), 2.38-2.25 (m, 2H), 1.24 (d, *J*=6.8 Hz, 6H), 1.22 (d, *J*=8.3 Hz, 6H), 0.89 (d, *J*=6.4 Hz, 6H), 0.68-0.51 (m, 2H) ppm (minor isomer; the other peaks in the region of 1.80-0.76 ppm are not assigned due to complexity caused by the overlap of the two isomers); ^{13}C NMR (126 MHz, $CDCl_3$): δ 157.18, 156.57, 156.49, 154.68, 154.66, 153.83, 153.78, 152.12, 151.93, 151.81, 149.06, 146.66, 140.97, 140.94, 140.76, 140.73, 138.00, 137.83, 134.62, 134.54, 133.37, 131.38, 131.35, 130.22, 125.19, 121.36, 116.80, 113.11, 113.09, 112.93, 112.92, 111.41, 111.36, 110.96, 110.93, 88.06, 87.83, 64.68, 54.91, 54.61, 54.36, 36.56, 36.39, 35.20, 35.01, 34.28, 34.10, 31.56, 31.48, 30.83, 30.61, 30.58, 29.31, 29.29, 29.01, 28.99, 27.89, 27.84,

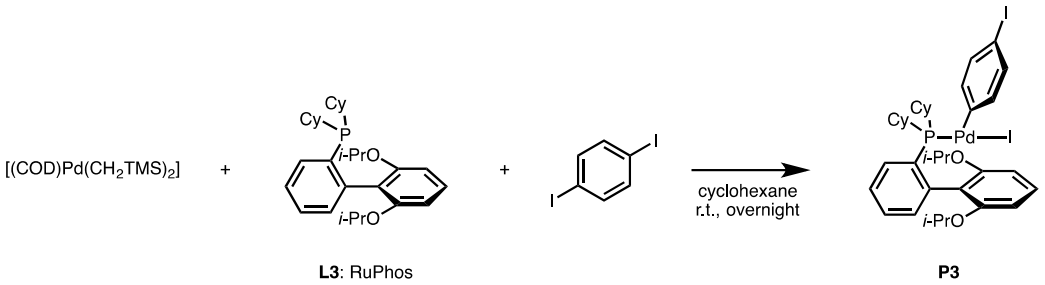
27.79, 27.75, 26.78, 26.67, 26.45, 26.35, 26.05, 25.46, 25.38, 25.27, 24.71, 24.44, 23.98, 23.35, 22.31, 14.03 ppm. (Observed complexity is due to C-P splitting and isomer formation); ^{31}P NMR (121 MHz, $CDCl_3$): δ 41.7, 31.8 ppm.

*Preparation of **P2**:*



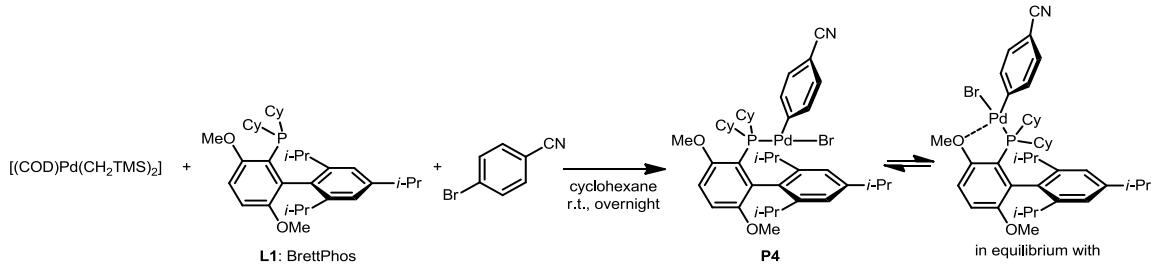
The general procedure for Pd-complex preparation was followed with *t*-BuBrettPhos (107 mg, 0.22 mmol, 1.1 equiv). General work up afforded **P2** as a light yellow solid (99 mg, 0.11 mmol, 55%). Due to the instability of the complex in solution state, which is documented in the literature, clean ^{13}C NMR spectra could not be obtained.[2] Spectral data for **P2**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.96-7.10 (m, 4H), 6.76-6.94 (m, 4H), 3.28 (s, 3H), 3.76 (s, 3H), 2.96-3.15 (m, 1H), 2.52 (hept, 2H, *J* = 7.0 Hz), 1.56 (d, 6H, *J* = 7.0 Hz), 1.20-1.43 (m, 24H), 0.76 (d, 6H, *J* = 7.0 Hz)ppm; ^{31}P NMR (121 MHz, $CDCl_3$) δ 65.5 ppm.

*Preparation of **P3**:*



The general procedure for Pd-complex preparation was followed with RuPhos (103 mg, 0.220 mmol, 1.10 equiv). General work up afforded **P3** as a light yellow solid (138 mg, 0.153 mmol, 76%). Spectral data for **P3**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.66 - 7.54 (m, 2H), 7.43 - 7.38 (m, 1H), 7.38 - 7.32 (m, 1H), 7.23 - 7.13 (m, 2H), 6.83 (br d, J=6.7 Hz, 2H), 6.82 - 6.79 (m, 1H), 6.64 (d, J=8.2 Hz, 2H), 4.63 - 4.48 (m, 2H), 2.17 - 2.06 (m, 2H), 1.81 - 1.49 (m, 12H), 1.39 - 1.31 (m, 6H), 1.25 - 1.06 (m, 8H), 1.03 - 0.95 (m, 6H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.25, 144.56, 144.41, 140.56, 140.53, 139.32, 134.93, 134.92, 134.87, 133.13, 132.87, 132.61, 132.52, 130.85, 130.59, 130.57, 126.36, 126.32, 111.11, 111.08, 107.80, 88.37, 71.03, 33.90, 33.70, 28.09, 27.54, 27.52, 27.25, 27.15, 26.88, 26.79, 25.98, 25.98, 22.16, 21.64 ppm. (Observed complexity is due to C–P coupling); ^{31}P NMR (121 MHz, $CDCl_3$) δ 27.51 ppm.

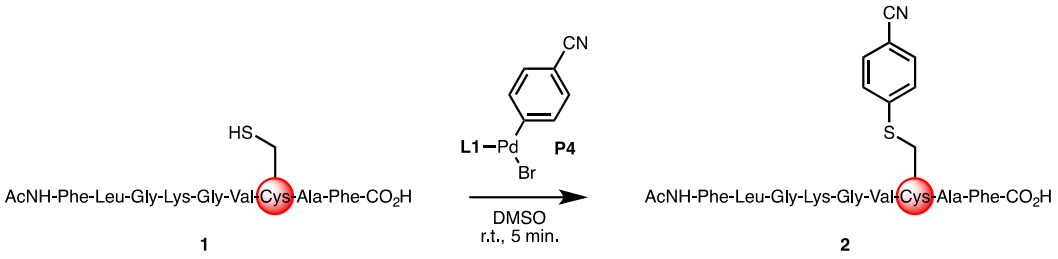
*Preparation of **P4**:*



The general procedure for Pd-complex preparation was followed with BrettPhos (118 mg, 0.220 mmol, 1.10 equiv), and 4-bromobenzonitrile (43.7 mg, 0.240 mmol, 1.20 equiv) in place of 1,4-diiodobenzene. General work up afforded P4 as a light yellow solid (106 mg, 0.128 mmol, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (dd, *J*=8.3, 1.8 Hz, 2H), 7.25 (br dd, *J*=7.8, 1.2 Hz, 2H), 7.13 (br d, *J*=14.5 Hz, 2H), 7.10 (br d, *J*=14.7 Hz, 2H), 7.07 (s, 2H), 7.03 (dd, *J*=9.2, 4.3 Hz, 1H), 7.01 (s, 2H), 6.98 (d, *J*=9.2 Hz, 1H), 6.87 (dd, *J*=9.0, 2.9 Hz, 1H), 6.81 (d, *J*=8.8 Hz, 1H), 4.37 (s, 3H), 3.81 (s, 3H), 3.58 (s, 3H), 3.33 (s, 3H), 3.12-3.00 (m, 1H), 2.97-2.85 (m, 1H), 2.69 (br dd, *J*=23.5, 12.0 Hz, 2H), 2.51-2.39 (m, 2H), 2.35-2.22 (m, 2H), 1.89-1.51 (m, 18H), 1.56 (d, *J*=6.7 Hz, 6H), 1.48-1.40 (m, 4H), 1.35 (d, *J*=7.0 Hz, 6H), 1.24 (d, *J*=6.8 Hz, 6H), 1.22 (d, *J*=6.8 Hz, 6H), 1.20-1.05 (m, 10H), 0.98-0.74 (m, 8H), 0.90 (d, *J*=6.6 Hz, 6H), 0.79 (d, *J*=6.7 Hz, 6H), 0.66-0.54 (m, 2H) (a mixture of two isomers with a 1:1 ratio); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.83, 156.33, 156.24, 154.54, 154.51, 154.05, 153.98, 152.44, 152.01, 151.86, 149.56, 149.53, 149.22, 146.61, 146.06, 138.88, 138.85, 138.56, 138.53, 138.18, 137.97, 133.48, 133.46, 129.94, 128.70, 128.54, 124.65, 124.60, 124.32, 121.42, 120.10, 119.90, 117.98, 117.70, 115.84, 115.80, 113.46, 113.43, 113.32, 111.22, 111.16, 111.02, 110.98, 106.26, 106.04, 77.32, 77.20, 77.00, 76.68, 65.82, 62.39, 54.95, 54.66, 54.43, 36.40, 36.17, 35.44, 35.18, 34.36, 34.26, 31.56, 31.43, 30.86, 30.79, 30.76, 29.31, 29.29, 27.87, 27.77, 27.75,

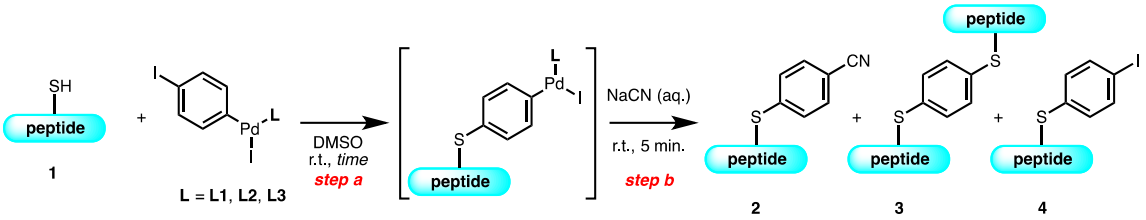
27.65, 27.61, 26.66, 26.52, 26.31, 26.19, 26.02, 25.41, 25.39, 25.24, 24.79, 24.44, 23.96, 23.33, 15.25, 14.09. ^{31}P NMR (121 MHz, $CDCl_3$) δ 46.67, 36.65.

III. Synthesis of peptide 2 standard.



A modified protocol of a reported strategy was followed.[3] To a solution of peptide **1** (2.0 mg, 1.9 µmol) in 300 µL DMSO was added a solution of the palladium complex **P4** (1.6 mg, 1.9 µmol) in 250 µL DMSO. The resulting reaction mixture was mixed by vortexing for 5 s and left at room temperature for 5 min. The reaction was quenched by the addition of a solution of 0.1% TFA in H_2O (1.3 mL), which was subjected to semi-preparative HPLC purification. Semi-preparatory HPLC conditions: mobile phase **I** = water (0.1 % TFA), mobile phase **II** = acetonitrile (0.1 % TFA); gradient method, 5-95 % **II** 0-10 min, 95 % **II** 10-15 min; flow rate = 5 mL/min (Agilent ZORBAX Eclipse XDB-C18, 9.4 × 250 mm, 5 µm). The collected fractions containing the product (t = ~ 7.4 min) was combined and concentrated to a volume of 1 mL and then dried via speed vac evaporator, which afforded the product **2** as a white powder (1.7 mg, 1.5 µmol) in 79% yield. The identity of the product was confirmed by LCMS (calculated mass for $[M+H]^+$: 1141.6; found: 1141.6).

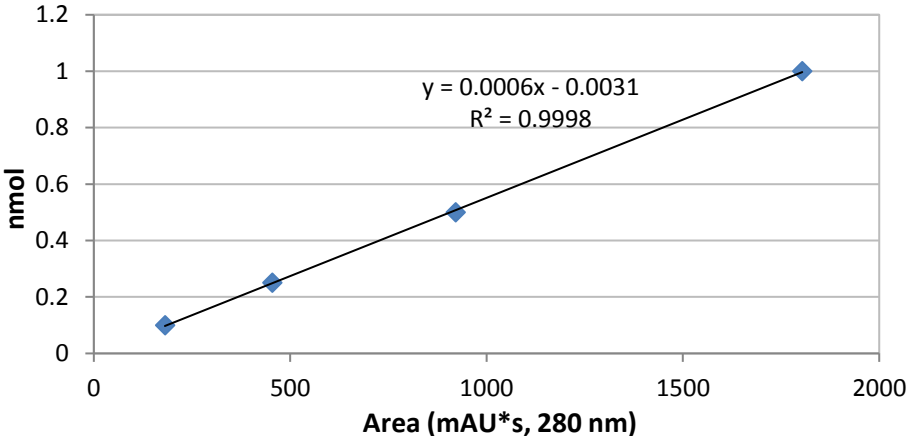
IV. General Procedure and Results for Table 1.



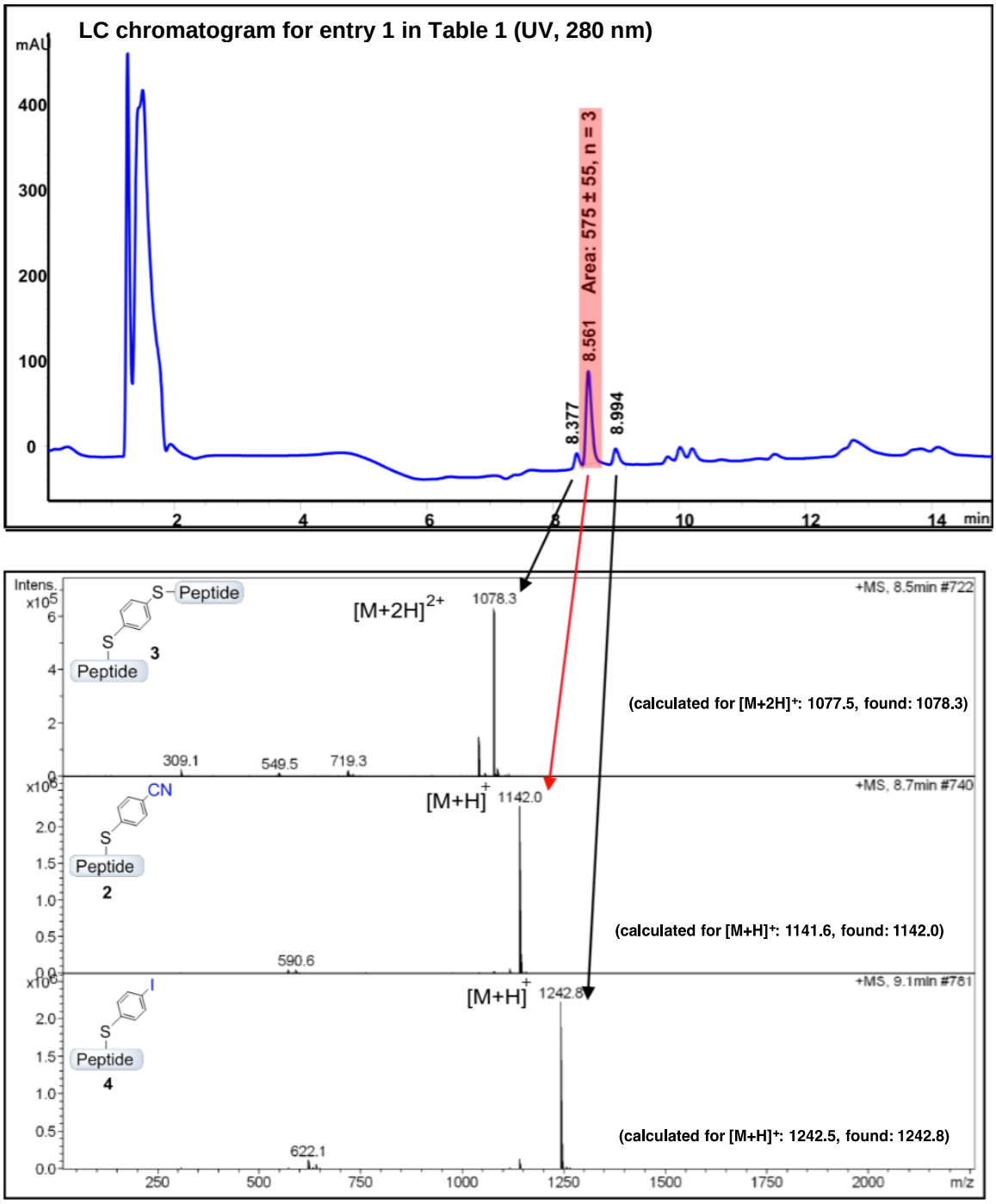
Step a: To a 1.0 mL Eppendorf tube were added DMSO (180 µL) and peptide **1** (20 nmol, 10 µL, 2.0 mM in DMSO). The resulting solution was mixed by vortexing for 10 s, then a solution of palladium complex (20 nmol, 10 µL, 2.0 mM in DMSO, 1.0 equiv) was added. The reaction mixture was vortexed for 5 s and left at rt for 10 min. Step b: To this solution was added aqueous NaCN (20 nmol, 10 µL, 2.0 mM in H_2O, 1.0 equiv) and the mixture was mixed well by vortexing for 10 s. After it was left at rt for 5 min, a solution of 0.1% TFA in H_2O (790 µL) was added to the mixture. A fraction of the resulting mixture was subjected to LC-MS analysis with an injection volume of 50 µL (n=3). LC conditions: see general experimental. Peptides **3** and **4** were identified by mass spectra shown below. The identity of Peptide **2** (product) was confirmed by comparison with the independently prepared sample in addition to the mass spectrum. Retention time of peptides **2**, **3** and **4** are ~ 8.6 min, 8.4 min and 9.0 min, respectively, on LC chromatogram. There is *ca.* 0.11 min separation between the UV peak and the corresponding mass peak with a flow rate of 0.3 mL/min, which is due to void volume between detectors. The yields are calculated based on the area of peptide **2** (x) and the calibration curve (y = 0.0006x - 0.0031) shown below: yield = y*1000 µL /50 µL/(amount of limiting reagent)*100%.

Calibration curve for LC-MS:

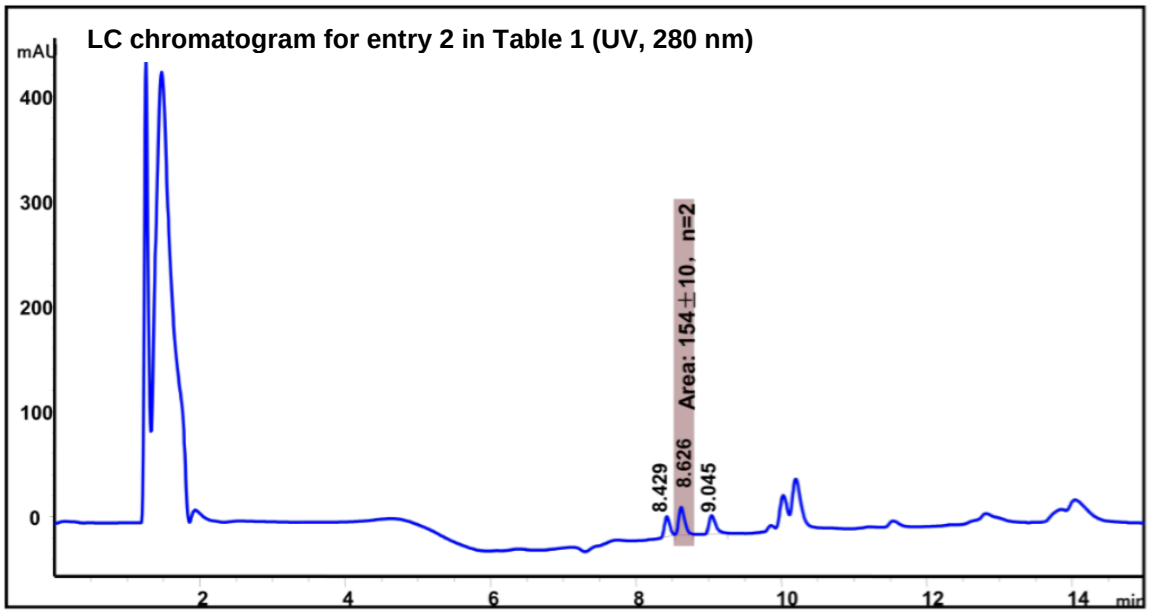
Solutions of different concentrations were injected at constant volume on the LC-MS. By multiplying the concentration by the volume (50 µL injection) the amount of peptide **2** in nmol was obtained. Molar amount vs peak area calibration curve was generated as described below. Four concentrations (2.0, 5.0, 10.0, 20.0 µM) of peptide **2** standard were prepared. The samples were analyzed by LC-MS and the peak area (UV 280 nm) of peptide **2** was recorded and used to construct a calibration curve. The LC conditions described in general experimental part (Part I) are followed.



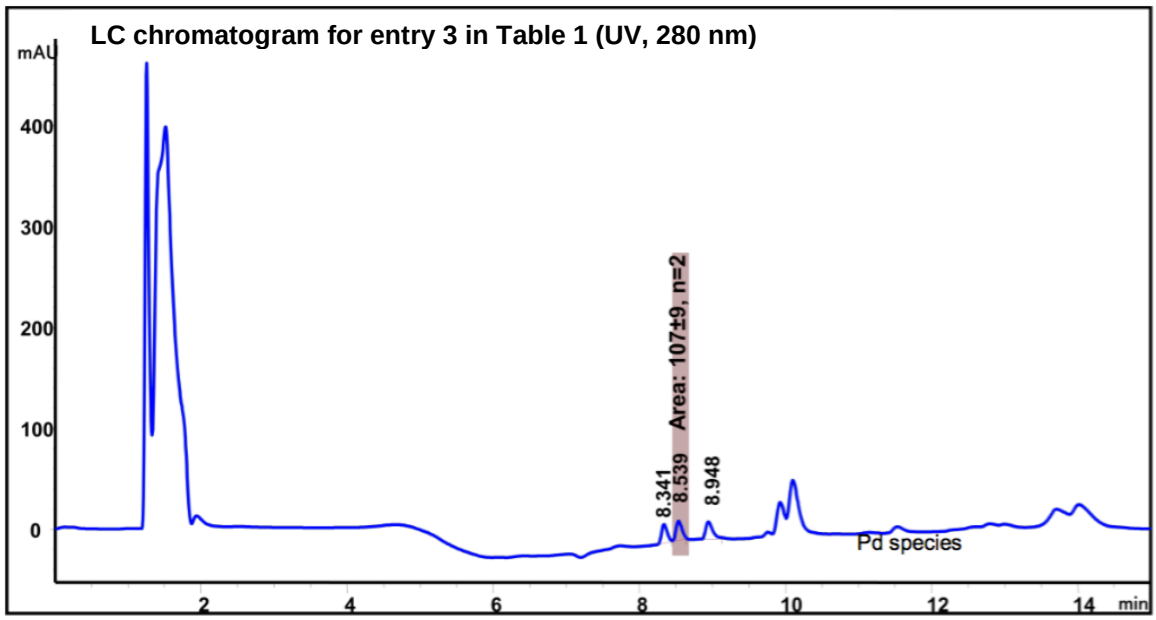
Entry 1: The general procedure for peptide labeling in Table 1 described above was conducted with **P1** (20 nmol, 10 µL, 2.0 mM in DMSO, 1.0 equiv) and NaCN (20 nmol, 10 µL, 2.0 mM in H_2O, 1.0 equiv). The average yield of entry 1 is 34% (n=3, 37%, 31%, 34%).



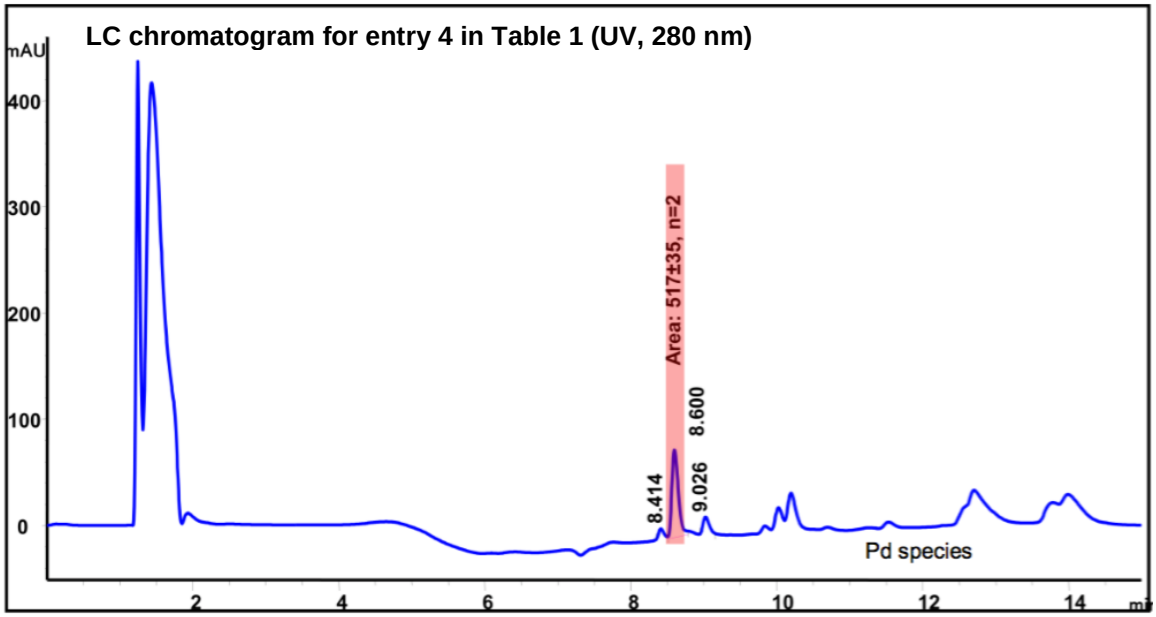
Entry 2: The general procedure for peptide labeling in Table 1 described above was conducted with **P1** (20 nmol, 10 µL, 2.0 mM in DMSO, 1.0 equiv) and NaCN (4.0 nmol, 10 µL, 0.40 mM in H_2O, 0.2 equiv). The average yield of entry 2 is 46% (n=2, 42%, 49%).



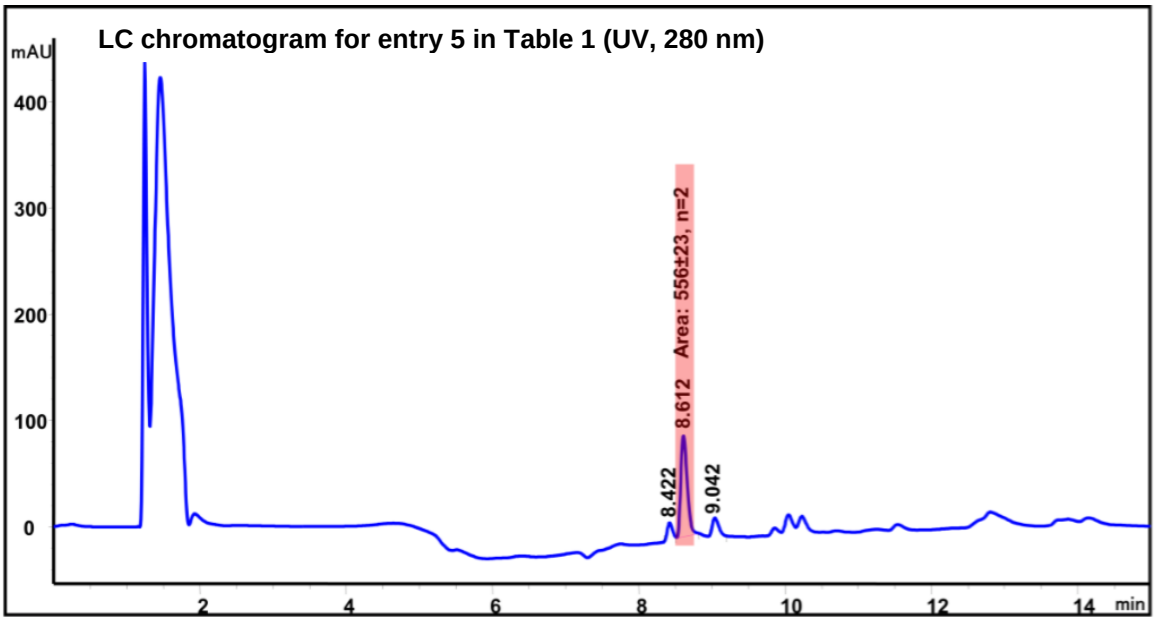
Entry 3: The general procedure for peptide labeling in Table 1 described above was conducted with **P1** (20 nmol, 10 µL, 2.0 mM in DMSO, 1.0 equiv) and NaCN (2.0 nmol, 10 µL, 0.20 mM in H_2O, 0.1 equiv). The average yield of entry 3 is 61% (n=2, 56%, 66%).



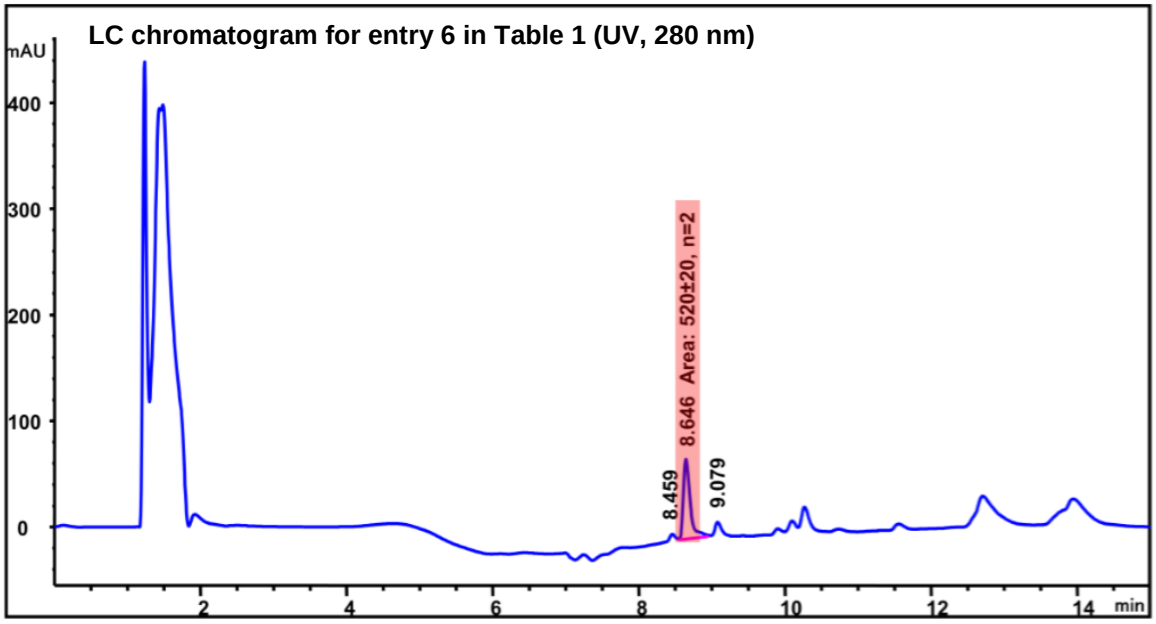
Entry 4: The general procedure for peptide labeling in Table 1 described above was conducted with **P1** (30 nmol, 15 µL, 2.0 mM in DMSO, 1.5 equiv), DMSO (175 µL) and NaCN (20 nmol, 10 µL, 2.0 mM in H_2O, 1.0 equiv). The average yield of entry 4 is 31% (n=2, 28%, 33%).



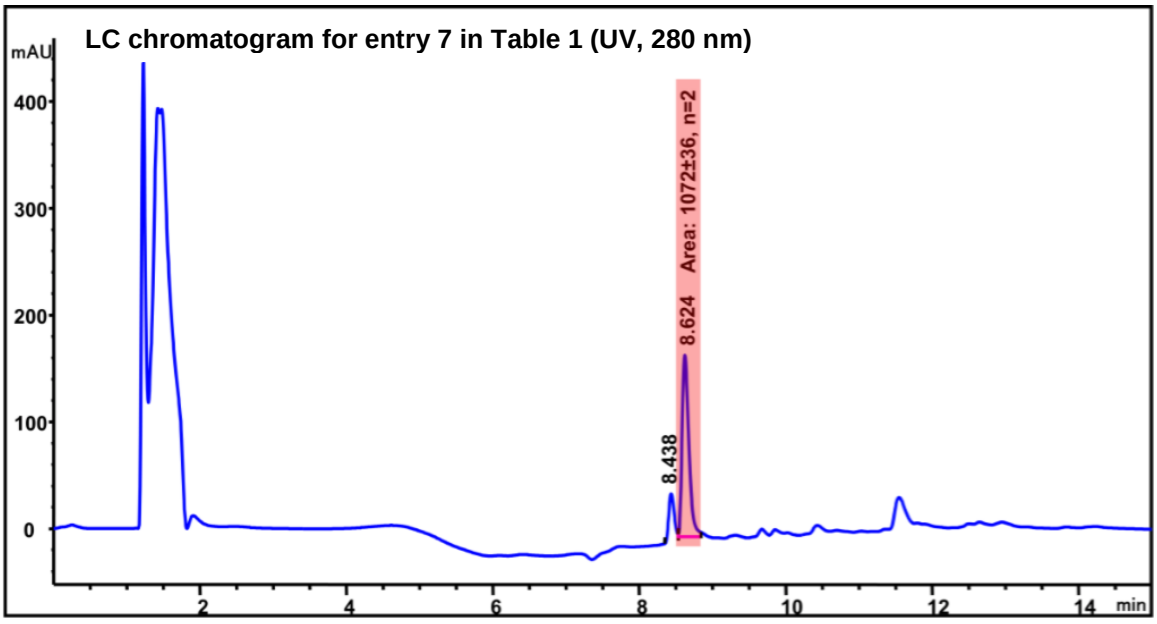
Entry 5: The procedure for peptide labeling in entry 1 of Table 1 described above was followed except that the reaction time for step a was 30 min. The average yield of entry 5 is 33% (n=2, 34%, 32%).



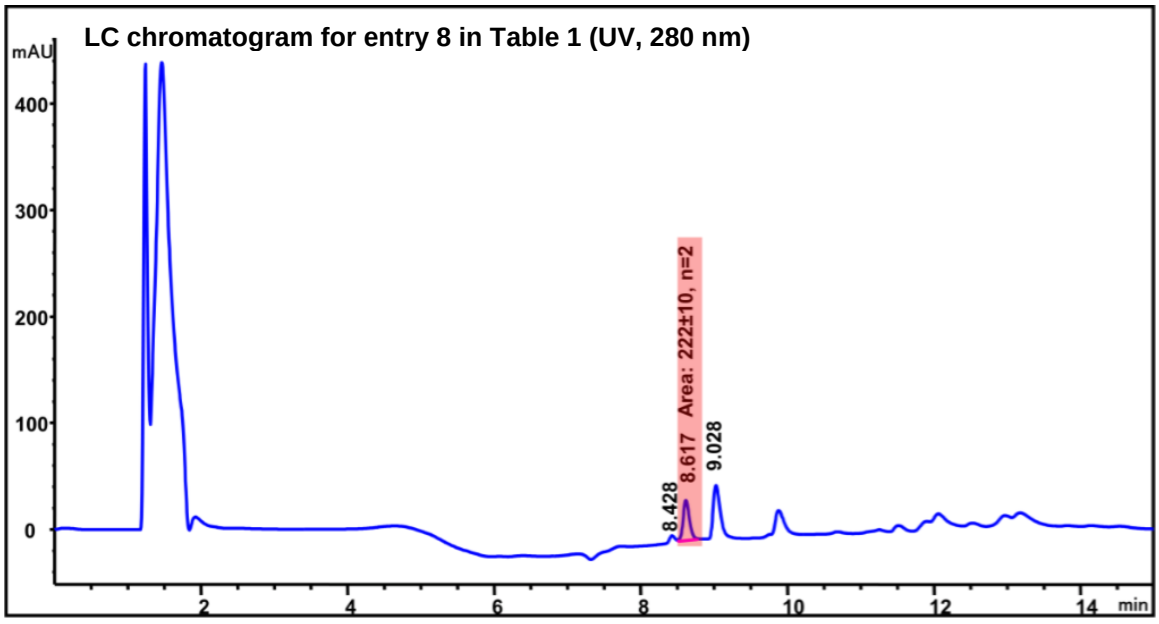
Entry 6: The procedure for peptide labeling in Table 1 described above was followed with 160 µL of DMSO and 20 µL of Tris buffer (1.0 M in H_2O, pH 7.5) in place of 180 µL of DMSO. The average yield of entry 6 is 31% (n=2, 32%, 29%).



Entry 7: The procedure for peptide labeling in entry 1 of Table 1 described above was followed with **P2** (20 nmol, 10 µL, 2.0 mM in DMSO, 1.0 equiv) instead of **P1**. The average yield of entry 7 is 64% (n=2, 66%, 62%).



Entry 8: The procedure for peptide labeling in entry 1 of Table 1 described above was followed with **P3** (20 nmol, 10 µL, 2.0 mM in DMSO, 1.0 equiv) instead of **P1**. The average yield of entry 8 is 13% (n=2, 14%, 12%).



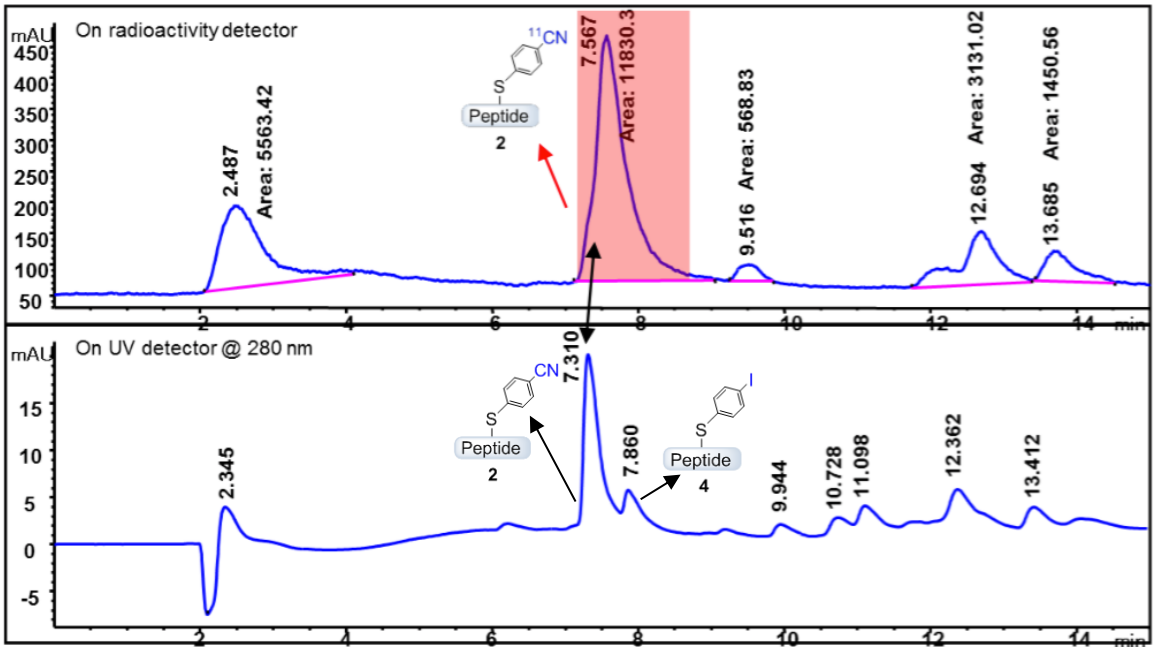
V. General Procedure and Results for Table 2 and Table 3.

To a 1.0 mL Eppendorf tube were added DMSO, H_2O, Tris buffer (1.0 M in H_2O, pH 7.5) in indicated amount if mentioned, and a solution of peptide **1** in DMSO. The resulting solution was mixed by vortexing for 10 s, followed by the addition of a solution of the palladium complex in DMSO (1 equiv with respect to peptide **1**). Then the reaction mixture was vortexed for 5 s and left at rt for 10 min. To this solution was added ^{11}CN in H_2O (20 μL, 1-10 mCi) and the mixture was mixed well by pipetting the solution in and out several times. After it was left at rt for 5 min, a solution of 0.1% TFA in H_2O (200-400 μL) was added to the mixture. A fraction of the resulting mixture was subjected to HPLC analysis. HPLC conditions: mobile phase **I** = water (0.1 % TFA), mobile phase **II** = acetonitrile (0.1 % TFA); gradient method, 5-95 % **II** 0-10 min, 95 % **II** 10-15 min; 50 μL injection volume (1.0 mL/min, Agilent ZORBAX Eclipse XDB-C18, 4.6 × 150 mm, 5 μm). Typically, *ca.* 0.25 min separation between the UV peak and the corresponding radioactive peak with a flow rate of 1.0 mL/min was observed, due to void volume between detectors. The identity of peptide **2** was confirmed by LC-MS analysis of the reaction mixture.

Entries and Radio-traces for Table 2:

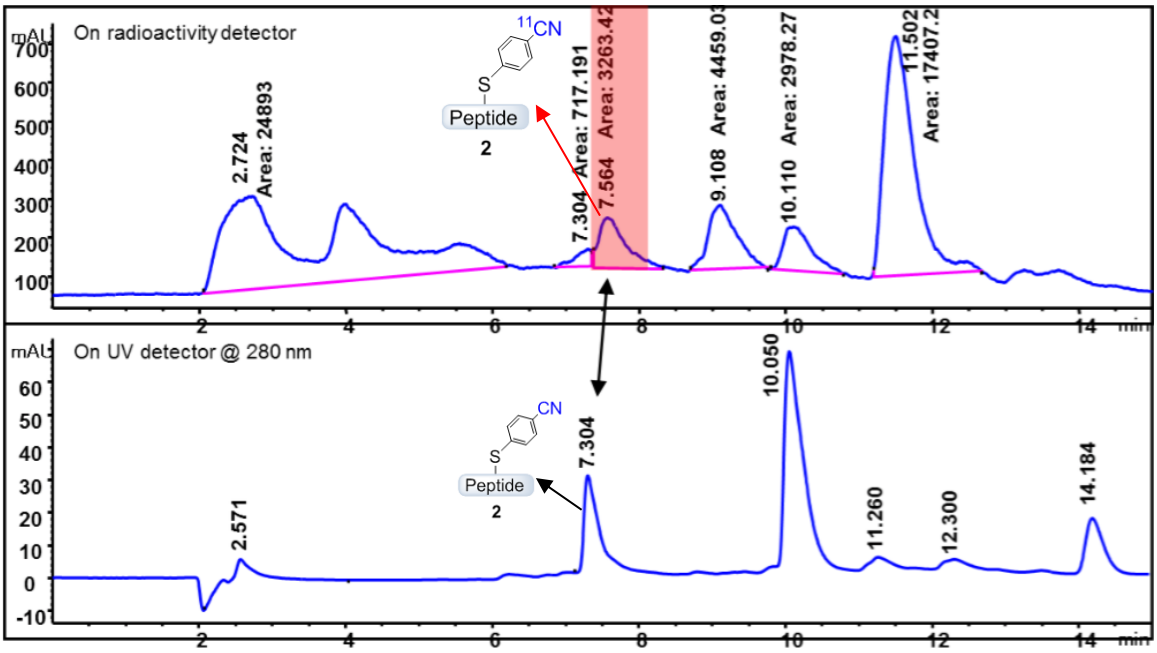
Entry 1: The reaction was carried out following the general procedure for peptide labeling in Table 2 with peptide **1** (2.0 mM, 10 µL, 20 nmol), Pd complex **P1** (2.0 mM, 10 µL, 20 nmol), and DMSO (180 µL). The labeled peptide **2** was obtained in 50% yield based on HPLC radio trace (non-decay corrected).

LC chromatograms for entry 1 in Table 2



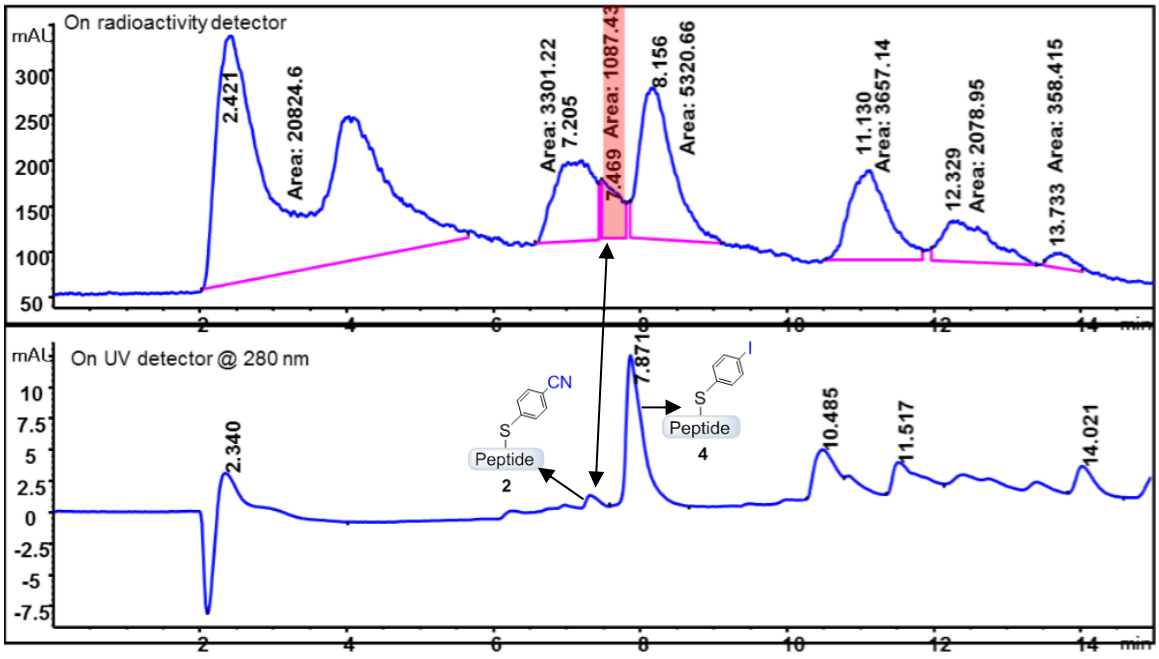
Entry 2: The reaction was carried out following the general procedure for peptide labeling in Table 2 with peptide **1** (2.0 mM, 10 µL, 20 nmol), Pd complex **P2** (2.0 mM, 10 µL, 20 nmol), and DMSO (180 µL). The labeled peptide **2** was obtained in 7% yield based on HPLC radio trace (non-decay corrected).

LC chromatograms for entry 2 in Table 2



Entry 3: The reaction was carried out following the general procedure for peptide labeling for Table 2 with DMSO (180 µL), peptide **1** (2 mM, 10 µL, 20 nmol), and Pd complex **P3** (2 mM, 10 µL, 20 nmol). The product was not effectively separated from other radiolabeled impurities present in the same area. The area corresponding to the product was calculated to be 3% yield based on HPLC radio trace (non-decay corrected).

LC chromatograms for entry 3 in Table 2



Control experiment: ***Investigation of the radioactive compounds generated from peptide labeling condition***

Three reactions were carried out:

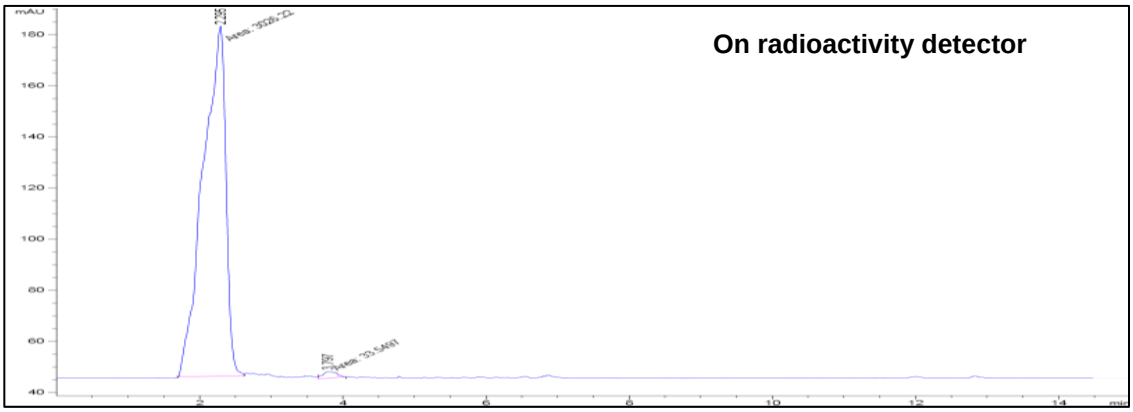
a) The reaction was carried out following the general procedure for peptide labeling for Table 2 with DMSO (178 μL) and ^{11}CN in H_2O (20 μL, 1-10 mCi);

b) The reaction was carried out following the general procedure for peptide labeling for Table 2 with DMSO (178 μL), Pd complex **P1** (2 mM, 1 μL, 2 nmol), and ^{11}CN in H_2O (20 μL, 1-10 mCi);

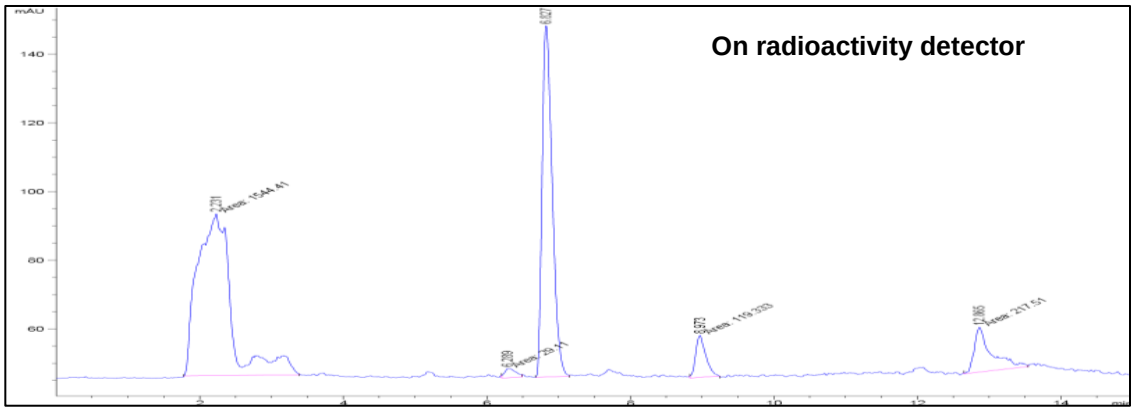
c) The reaction was carried out following the general procedure for peptide labeling for Table 2 with DMSO (178 μL), peptide **1** (2 mM, 1 μL, 2 nmol), Pd complex **P1** (2 mM, 1 μL, 2 nmol), and ^{11}CN in H_2O (20 μL, 1-10 mCi).

As indicated by the radio-HPLC spectra, in the peptide product region (0-10 min), all other radioactive peaks resulted from reactions of ^{11}CN with Pd complex.

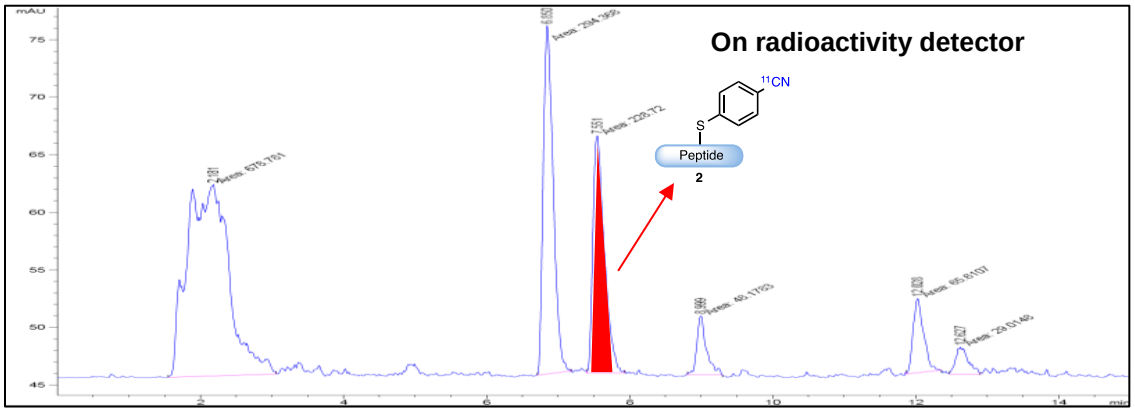
a) Reaction without Pd complex and peptide **1**



b) Reaction without peptide **1**



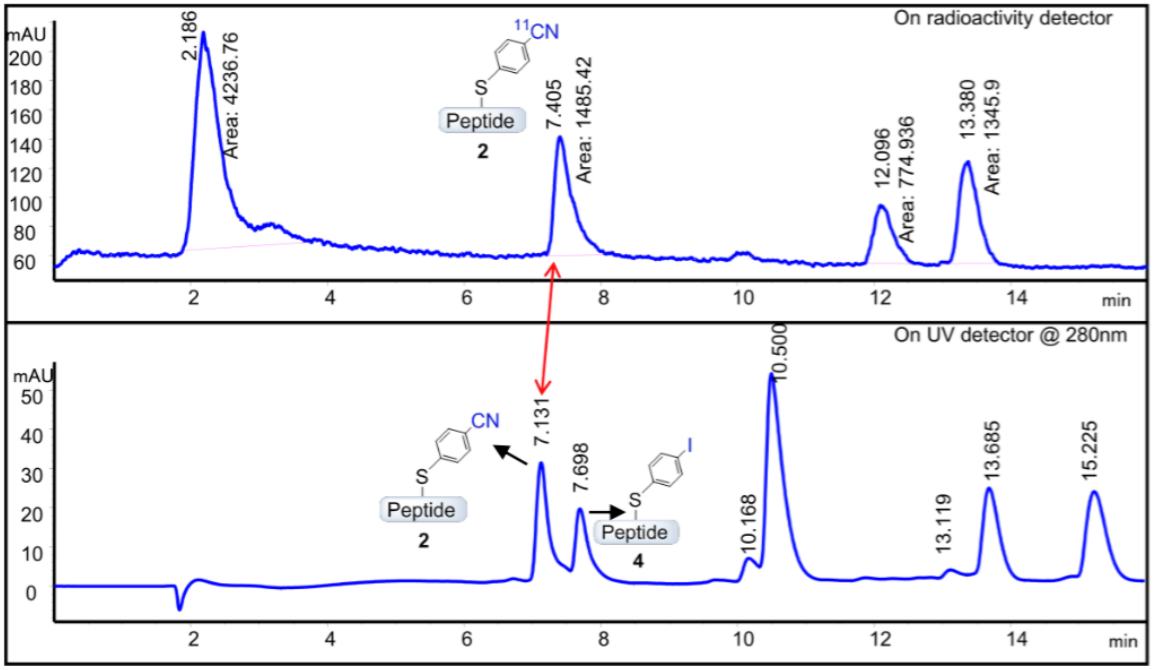
c) Standard reaction



Entries and Radio-traces for Table 3:

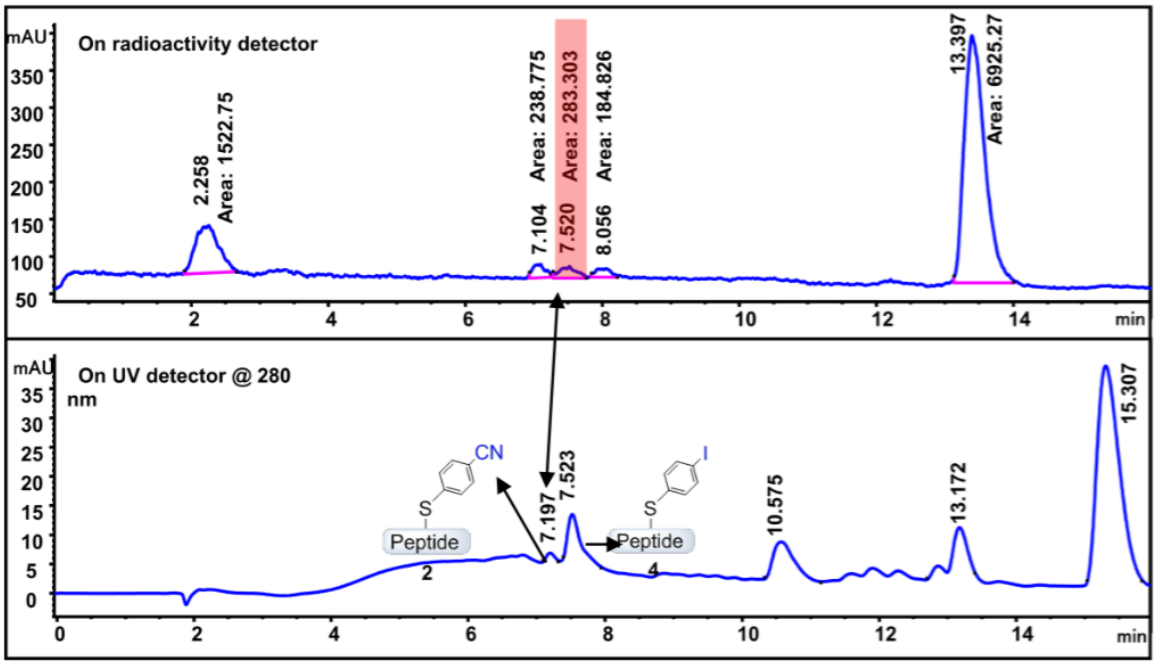
Entry 1: The reaction was carried out following the general procedure for peptide labeling for Table 2 with DMSO (170 µL), Tris buffer (20 µL, 1.0 M, pH 7.5), peptide **1** (20 mM, 5 µL, 100 nmol), and Pd complex **P1** (20 mM, 5 µL, 100 nmol). The labeled peptide **2** was obtained in 19% yield based on HPLC radio trace (non-decay corrected). Typically, *ca.* 0.25 min separation between the UV peak and the corresponding radioactive peak with a flow rate of 1.0 mL/min was observed, due to void volume between detectors.

LC chromatograms for entry 1 in Table 3



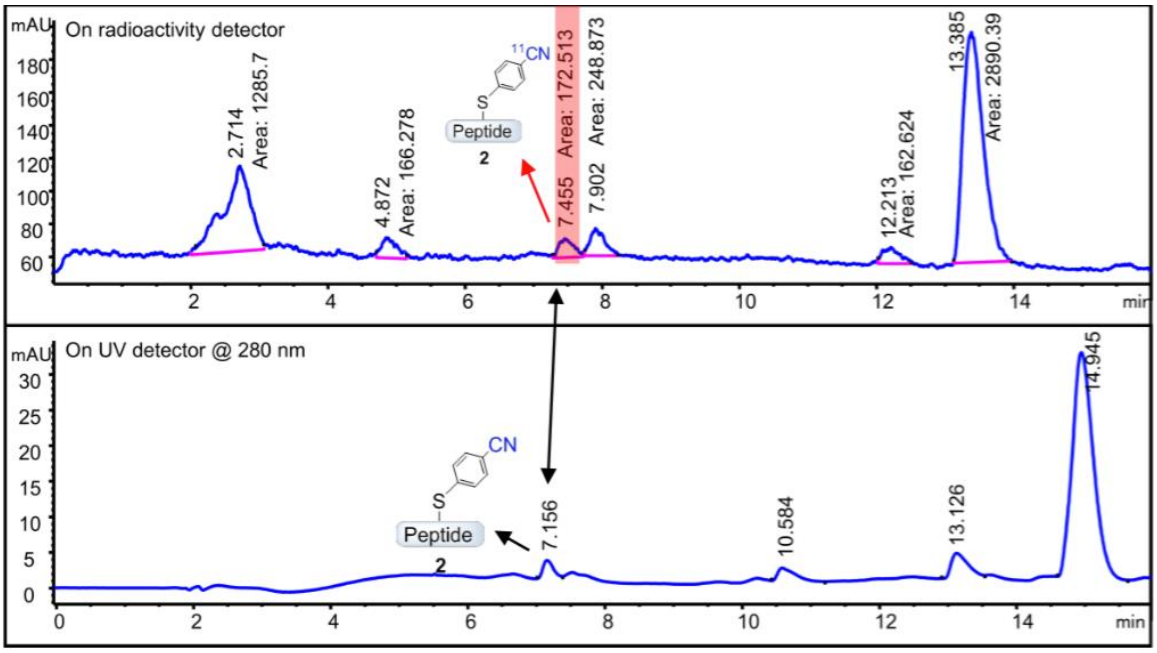
Entry 2: The reaction was carried out following the general procedure for peptide labeling for Table 2 with DMSO (57 µL), H_2O (113 µL), Tris buffer (20 µL, 1.0 M, pH 7.5), peptide **1** (20 mM, 5 µL, 100 nmol), and Pd complex **P1** (20 mM, 5 µL, 100 nmol). The labeled peptide **2** was obtained in 3% yield based on HPLC radio trace (non-decay corrected).

LC chromatograms for entry 2 in Table 3



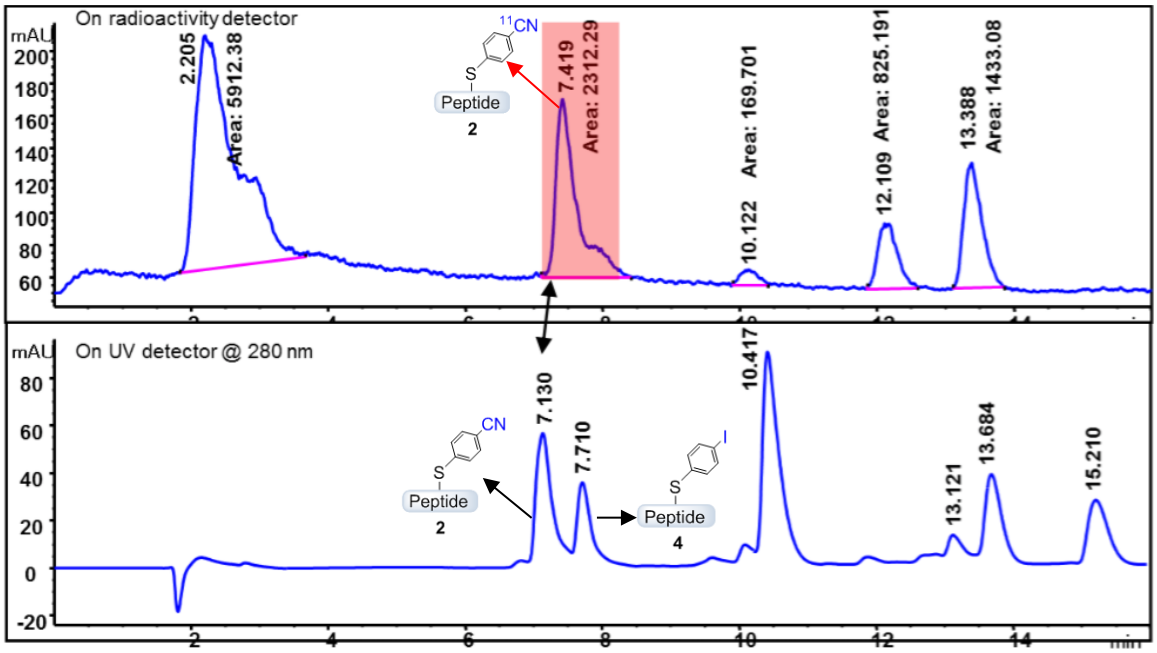
Entry 3: The reaction was carried out following the general procedure for peptide labeling in Table 2 with H_2O (170 µL), Tris buffer (20 µL, 1.0 M, pH 7.5), peptide **1** (20 mM, 5 µL, 100 nmol), and Pd complex **P1** (20 mM, 5 µL, 100 nmol). The labeled peptide **2** was obtained in 4% yield based on HPLC radio trace (non-decay corrected).

LC chromatograms for entry 3 in Table 3



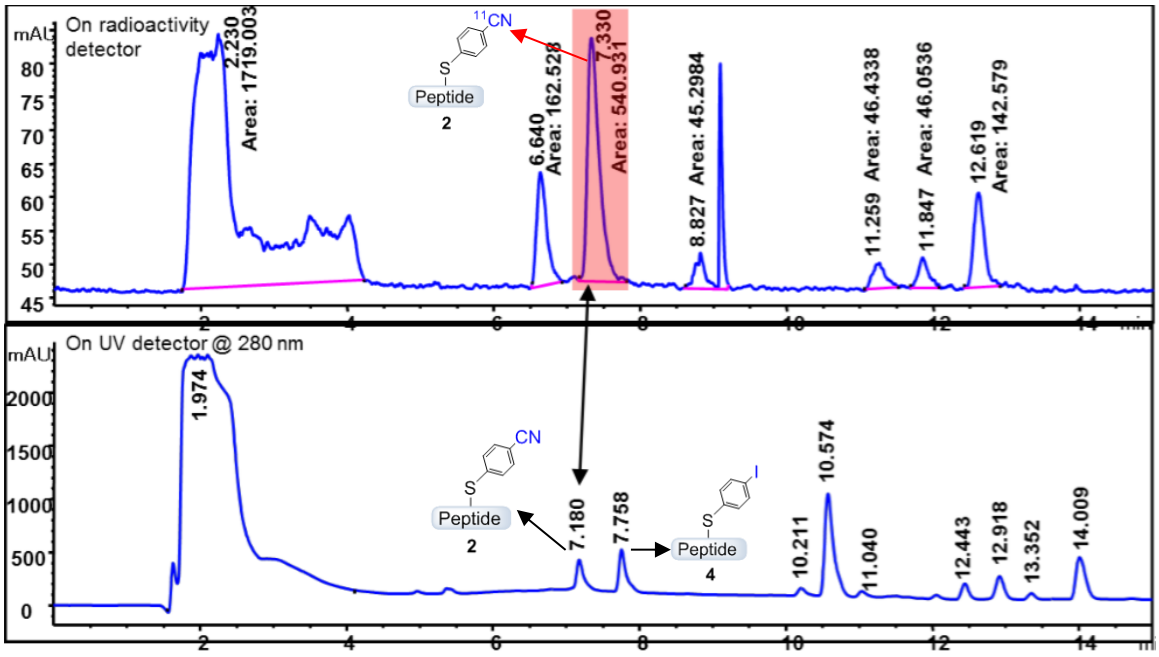
Entry 4: The reaction was carried out following the general procedure for peptide labeling for Table **2** with DMSO (175 µL), Tris buffer (20 µL, 1.0 M, pH 7.5), peptide **1** (20 mM, 2.5 µL, 50 nmol), and Pd complex P1 (20 mM, 2.5 µL, 50 nmol). The labeled peptide **2** was obtained in 22% yield based on HPLC radio trace (non-decay corrected).

LC chromatograms for entry 4 in Table 3



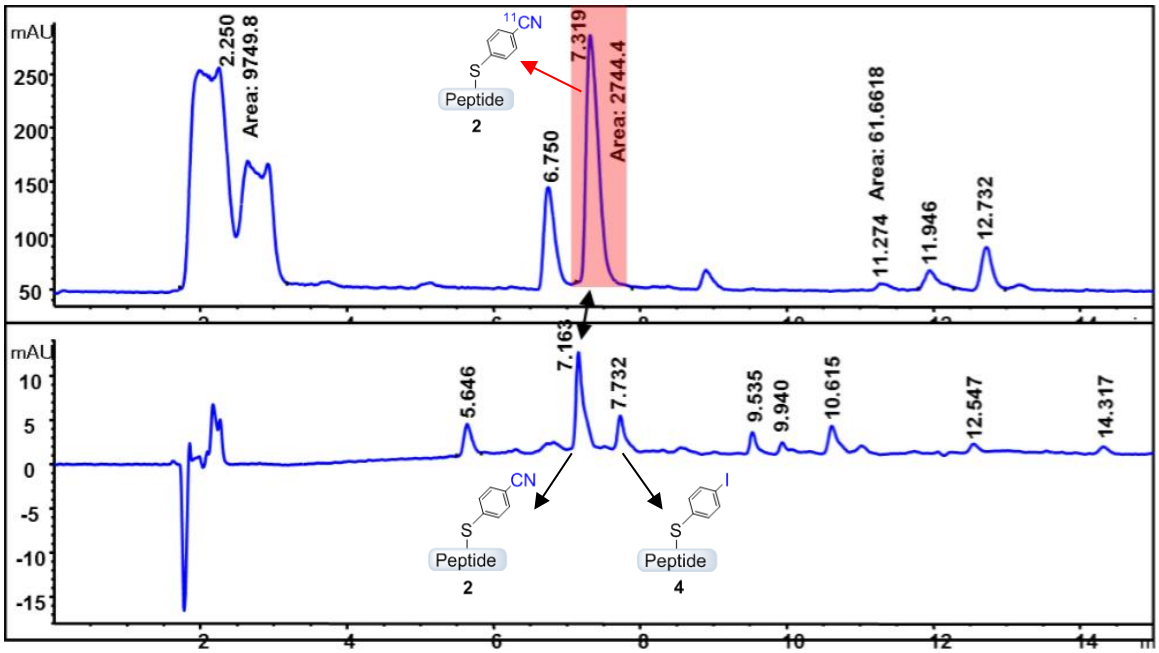
Entry 5: The reaction was carried out following the general procedure for peptide labeling for Table 2 with DMSO (170 µL), Tris buffer (20 µL, 1.0 M, pH 7.5), peptide **1** (2 mM, 5 µL, 10 nmol), and Pd complex **P1** (2 mM, 5 µL, 10 nmol). The labeled peptide **2** was obtained in 20% yield based on HPLC radio trace (non-decay corrected).

LC chromatograms for entry 5 in Table 3



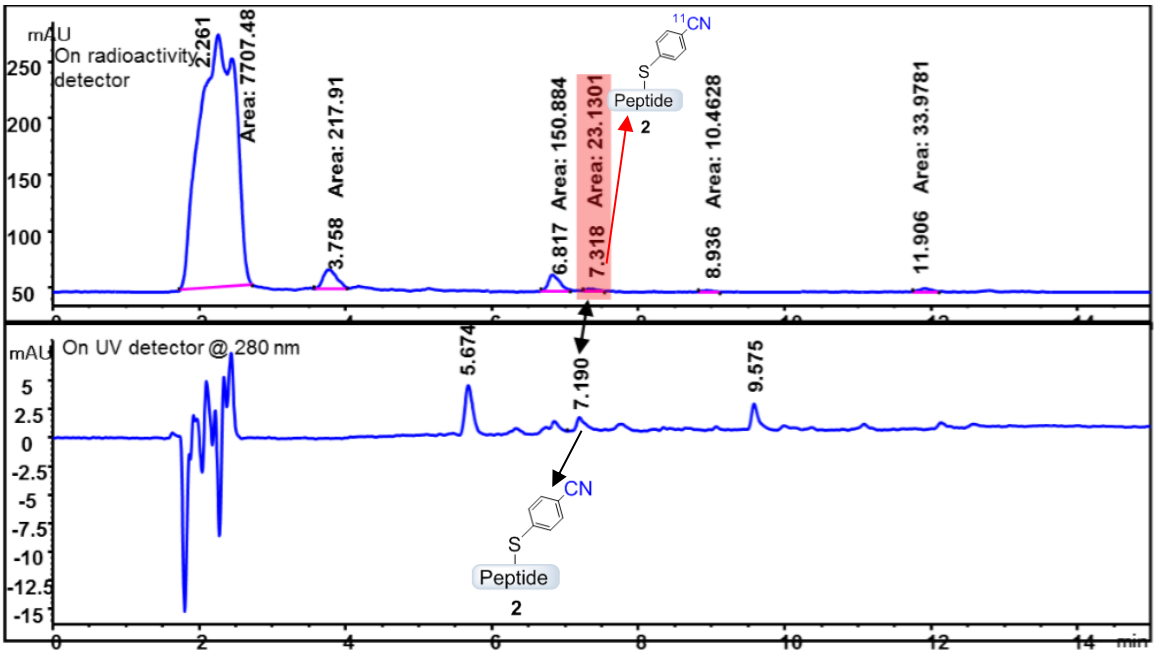
Entry 6: The reaction was carried out following the general procedure for peptide labeling for Table 2 with DMSO (178 µL), Tris buffer (20 µL, 1.0 M, pH 7.5), peptide **1** (2 mM, 1 µL, 2 nmol), and Pd complex **P1** (2 mM, 1 µL, 2 nmol). The labeled peptide **2** was obtained in 19% yield based on HPLC radio trace (non-decay corrected).

LC chromatograms for entry 6 in Table 3

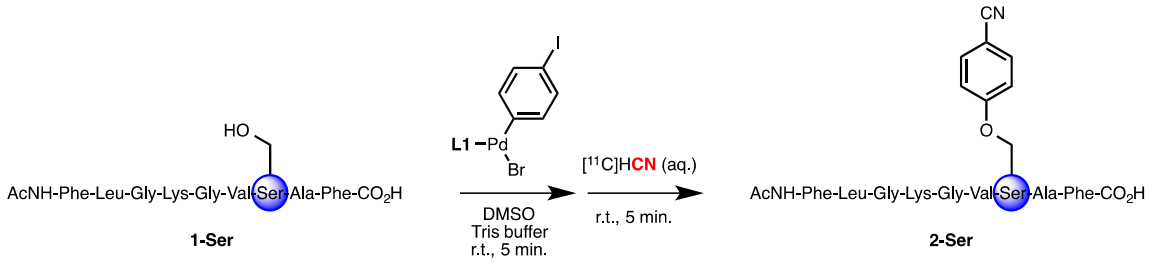


Entry 7: The reaction was carried out following the general procedure for peptide labeling for Table 2 with DMSO (172 µL), Tris buffer (20 µL, 1.0 M, pH 7.5), peptide **1** (0.1 mM, 4 µL, 0.4 nmol), and Pd complex **P1** (0.1 mM, 4 µL, 0.4 nmol). The labeled peptide **2** was obtained in 0.28% yield based on HPLC radio trace (non-decay corrected).

LC chromatograms for entry 7 in Table 3

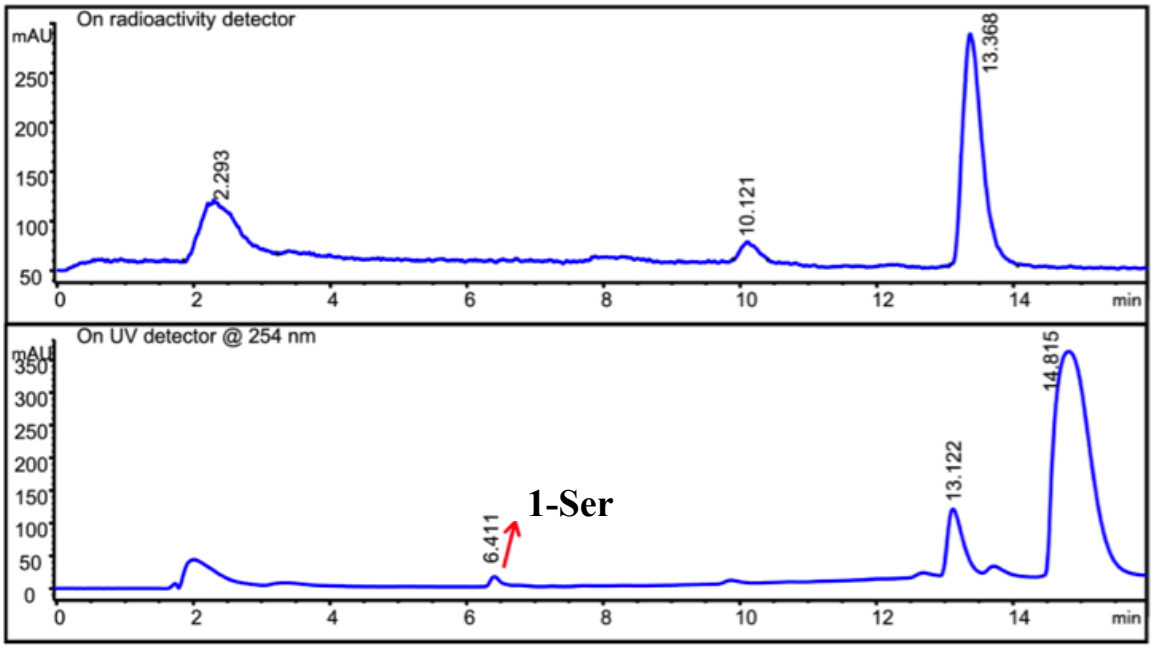


Entry 8:



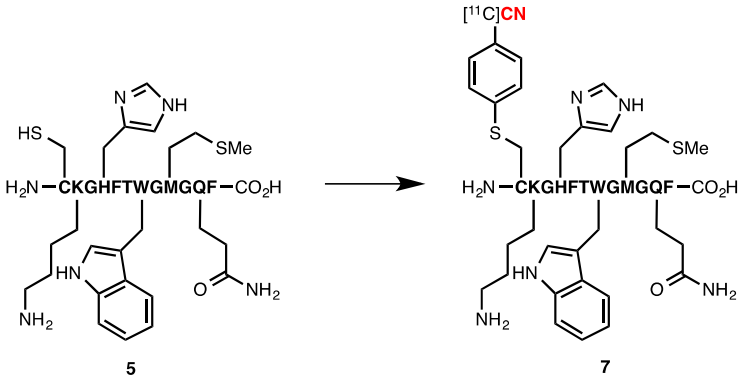
To a 1.0 mL Eppendorf tube were added DMSO (170 µL), Tris buffer (20 µL, 1.0 M) and a solution of peptide **1-Ser** in DMSO (20 mM, 5.00 µL, 100 nmol). The resulting solution was mixed by vortexing for 10 s, followed by the addition of a solution of the palladium complex in DMSO (20.0 mM, 5 µL, 100 nmol, 1.0 equiv). Then the reaction mixture was vortexed for 5 s and left at rt for 10 min. To this solution was added ^{11}CN in H_2O (20 µL, 1-10 mCi) and the mixture was mixed well by pipetting the solution in and out several times. After it was left at rt for 5 min, a solution of 0.1% TFA in H_2O (200-400 µL) was added to the mixture. A fraction of the resulting mixture was subjected to HPLC analysis. In the peptide region, only unreacted peptide **1-Ser** was identified on the UV trace (254 nm).

LC chromatograms for entry 8 in Table 3



VI. Radiosynthesis of Peptides 7 & 8 (Figure 3).

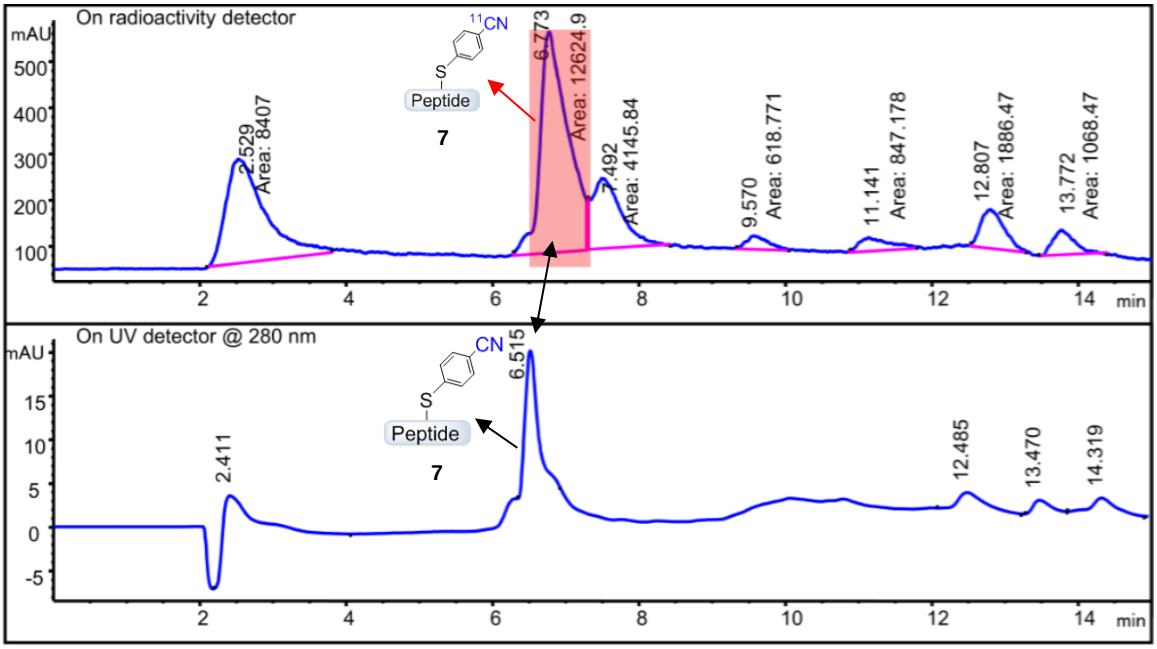
*Radiosynthesis of peptide **7**:*

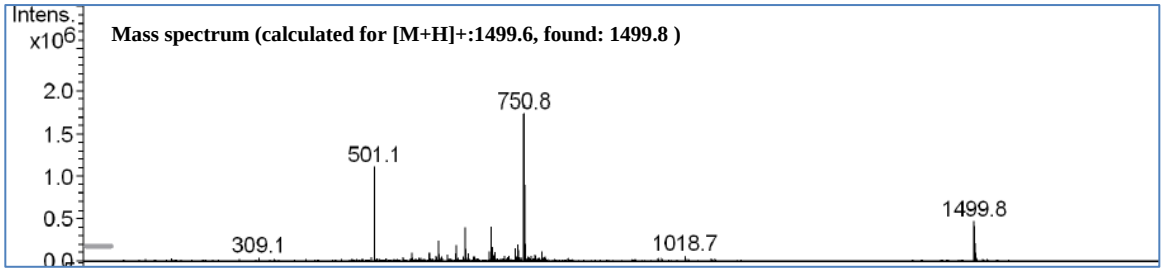


5 **7**

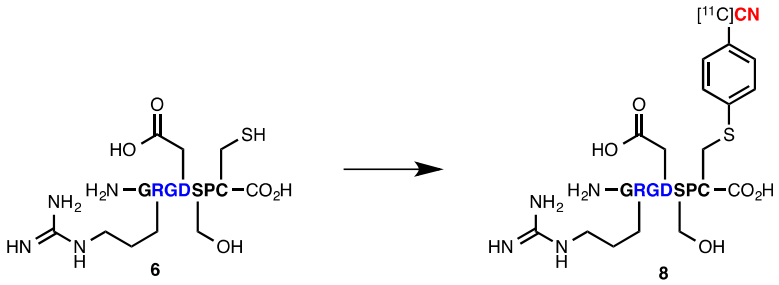
To a 1.0 mL Eppendorf tube were added DMSO (180 µL) and a solution of the precursor peptide **5** in DMSO (2.0 mM, 10 µL, 20 nmol). The resulting solution was mixed by vortexing for 10 s, followed by the addition of a solution of the palladium complex **P1** in DMSO (2.0 mM, 10 µL, 20 nmol). Then the reaction mixture was vortexed for 5 s and left at rt for 10 min. To this solution was added ^{11}CN in H_2O (20 µL, 1-5 mCi) and the mixture was mixed well by shaking the tube for 5 s. After it was left at rt for 5 min, a solution of 0.1% TFA in H_2O (200-400 µL) was added to the mixture. A fraction of the resulting mixture was subjected to HPLC analysis. Typically, *ca.* 0.25 min separation between the UV peak and the corresponding radioactive peak with a flow rate of 1.0 mL/min was observed, due to void volume between detectors. The RCY for peptide **7** (t = 6.773 min on radio trace; t = 6.515 min on UV trace @ 280 nm) was determined to be 43%. The identity of peptide **7** was confirmed by LC-MS analysis of the reaction mixture.

LC chromatograms for radiosynthesis of peptide 7 in Figure 3



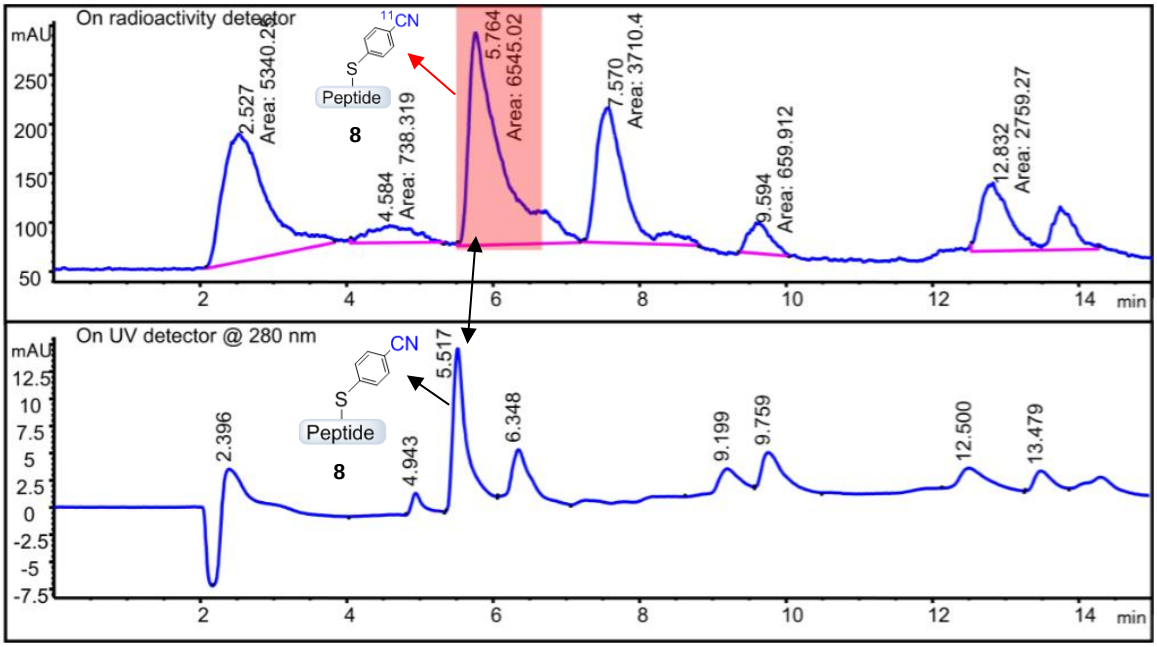


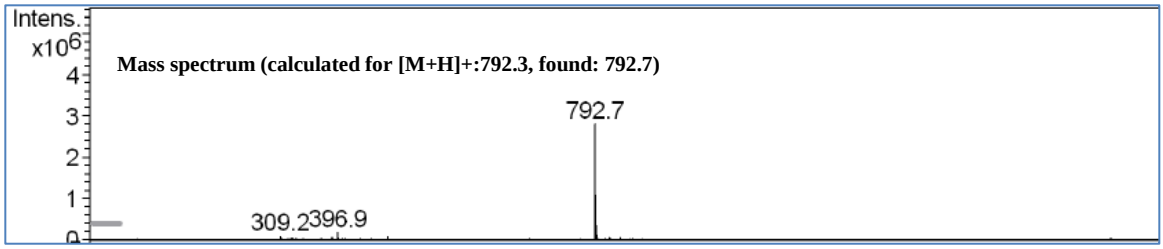
*Radiosynthesis of peptide **8**:*



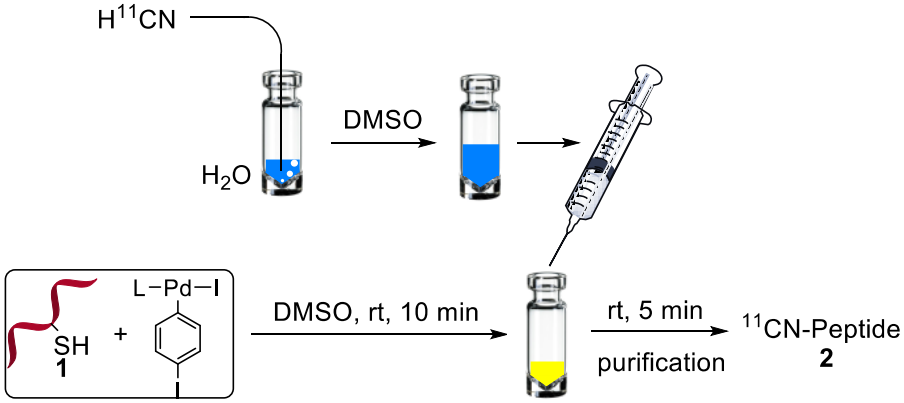
The procedure for radiosynthesis of peptide **7** was followed with the corresponding precursor peptide **6** in DMSO (2.0 mM, 10 µL, 20 nmol). The RCY for peptide **8** (t = 5.764 min on radio trace; t = 5.517 min on UV trace @ 280 nm) was determined to be 33%. The identity of peptide **8** was confirmed by LC-MS analysis of the reaction mixture.

LC chromatograms for radiosynthesis of peptide 8 in Figure 3




Intens.
x10^6
4
3
2
1
0
Mass spectrum (calculated for [M+H]+:792.3, found: 792.7)
792.7
309.2
396.9

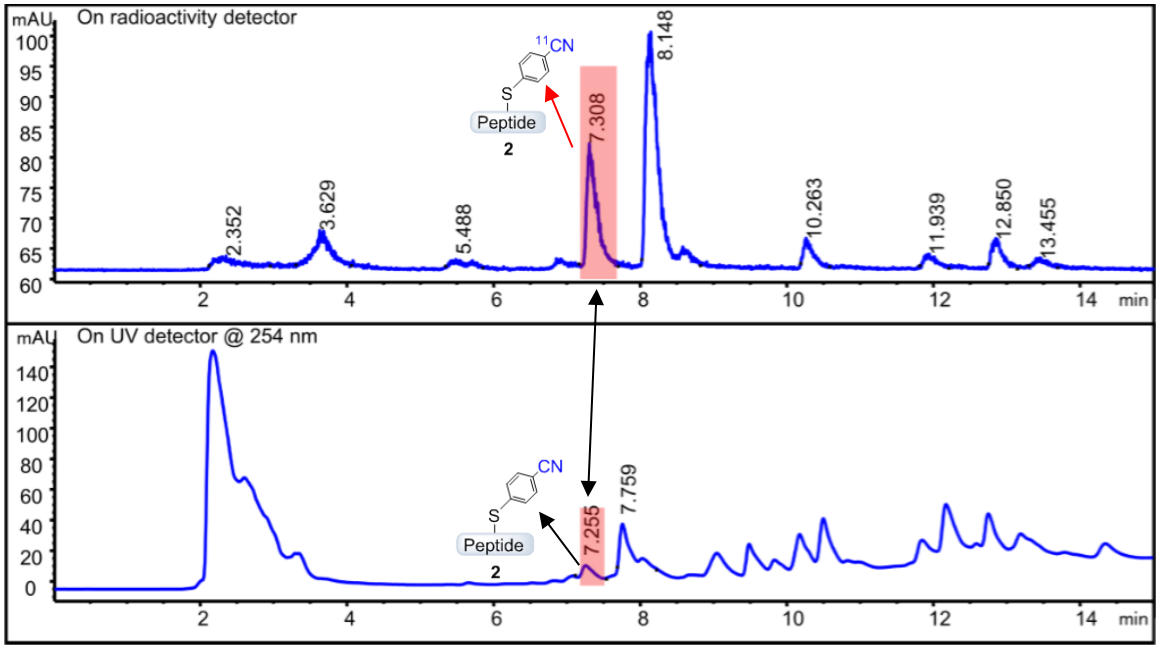
VII. High-Radioactivity synthesis of [^{11}CN]Peptide 2 (Figure 4).



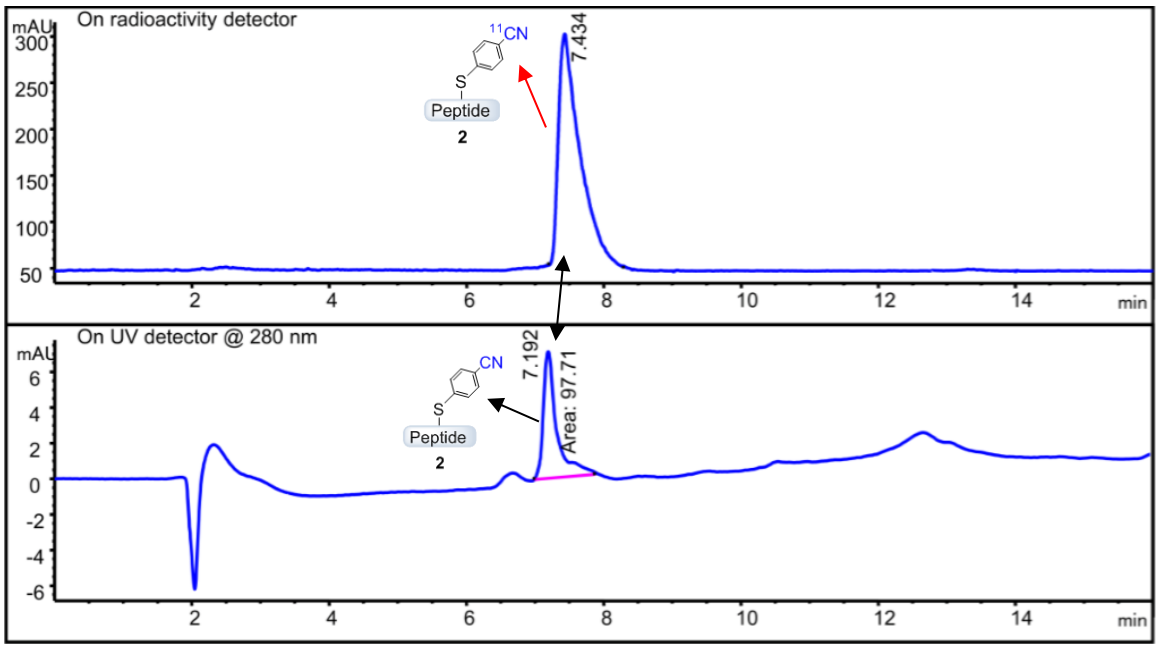
To a 4.0 mL vial were added DMSO (400 µL) and a solution of peptide **1** in DMSO (2 mM, 10 µL, 20 nmol). The resulting solution was mixed by vortexing for 10 s, followed by the addition of a solution of the palladium complex **P1** in DMSO (2 mM, 10 µL, 20 nmol). Then the reaction mixture was vortexed for 5 s and left at rt for 10 min. In the interim, [^{11}C]HCN was generated (10 min beam) and bubbled into H_2O (100 µL). Then the solution was diluted with DMSO (300 µL).[4] A fraction of this solution (200 µL, 20.1 mCi, start of synthesis) was quickly added to the reaction mixture from peptide **1** and Pd complex **P1**. The vial was well shaken to give a homogeneous solution, which was kept at rt for 5 min. After dilution with a solution of 0.1% TFA in H_2O (1 mL), the mixture was subjected to semi-preparatory HPLC purification. Semi-preparatory HPLC condition: mobile phase **I** = water (0.1 % TFA), mobile phase **II** = acetonitrile (0.1 % TFA); gradient method, 5-95 % **II** 0-10 min, 95 % B 10-15 min; flow rate = 5 mL/min; Agilent ZORBAX Eclipse XDB-C18, 9.4 × 250 mm, 5 µm). Typically, *ca.* 0.05 min separation between the UV peak and the corresponding radioactive peak with a flow rate of 5.0 mL/min was observed, due to void volume between detectors. After 7.6 min, the radioactivity of the collected fraction (3.3 mL) corresponding to the desired product was

measured (2.0 mCi, End of synthesis). The radiochemical yield of peptide **2** was 10% (non-decay corrected). Specific activity measurement: 100 µL out of the purified product solution (3.3 mL) was injected onto analytical HPLC and the peak area (UV 280 nm) of peptide **2** was recorded and applied to the calibration curve shown below. Analytical HPLC conditions: mobile phase **I** = water (0.1 % TFA), mobile phase **II** = acetonitrile (0.1 % TFA); gradient method, 5-95 % **II** 0-10 min, 95 % **II** 10-15 min; 50 µL injection volume (1.0 mL/min, Agilent ZORBAX Eclipse XDB-C18, 4.6 × 150 mm, 5 µm). Typically, *ca.* 0.25 min separation between the UV peak and the corresponding radioactive peak with a flow rate of 1.0 mL/min was observed, due to void volume between detectors. Calculation of SA: (2 mCi)/[(Area*0.0006+0.0017)*(3300 µL)/(100 µL)] = 1.0 Ci/µmol

Semi-preparatory HPLC traces:

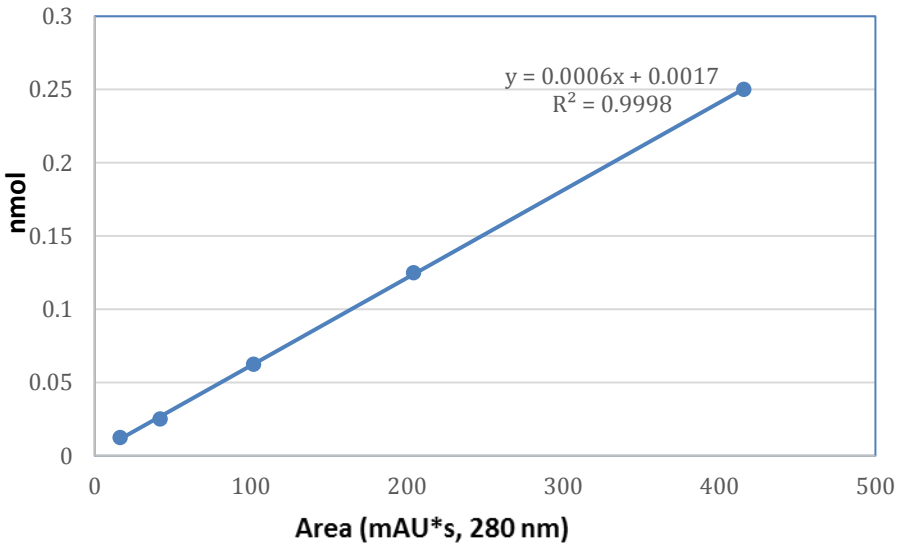


Analytical HPLC traces of purified product:

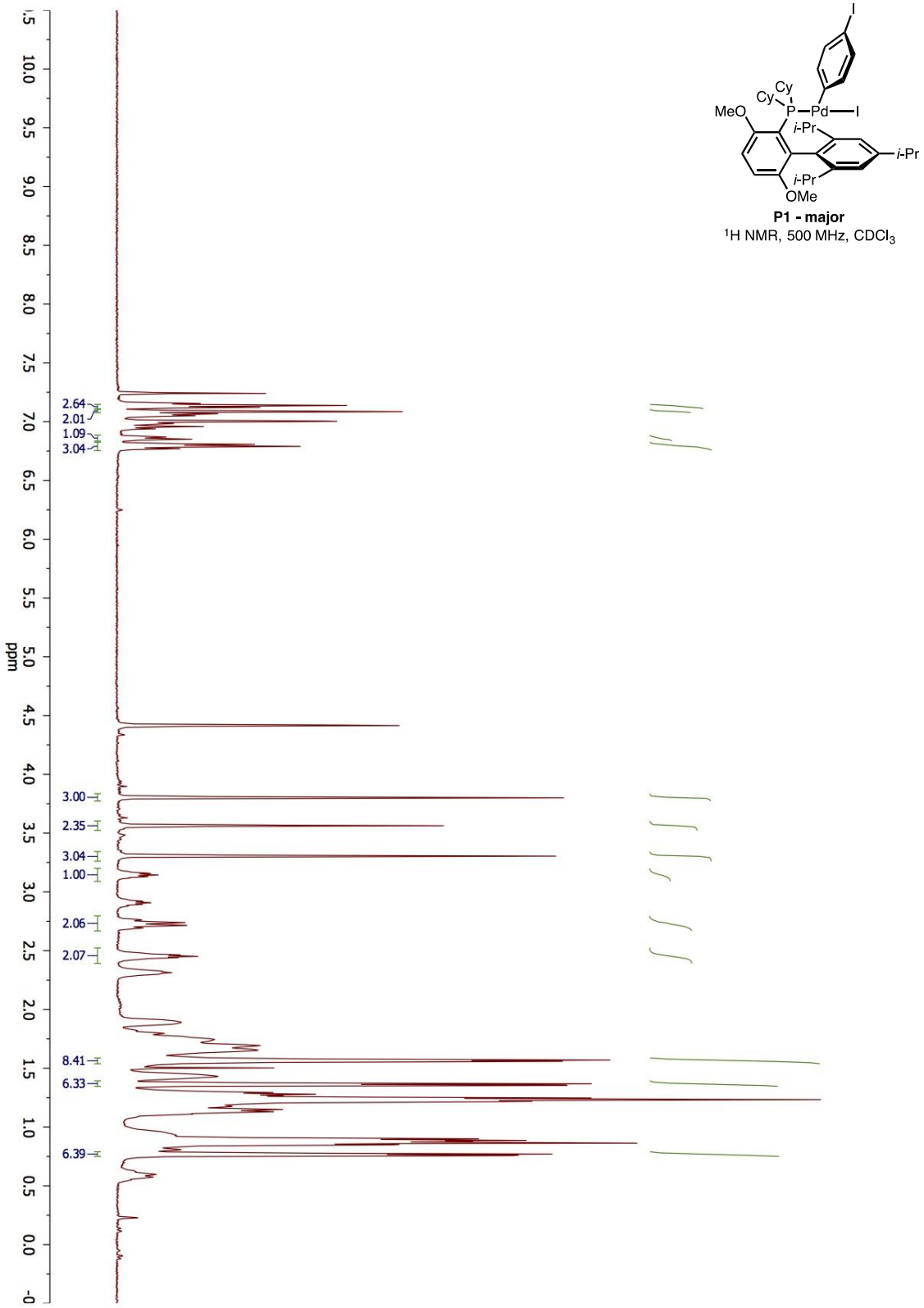


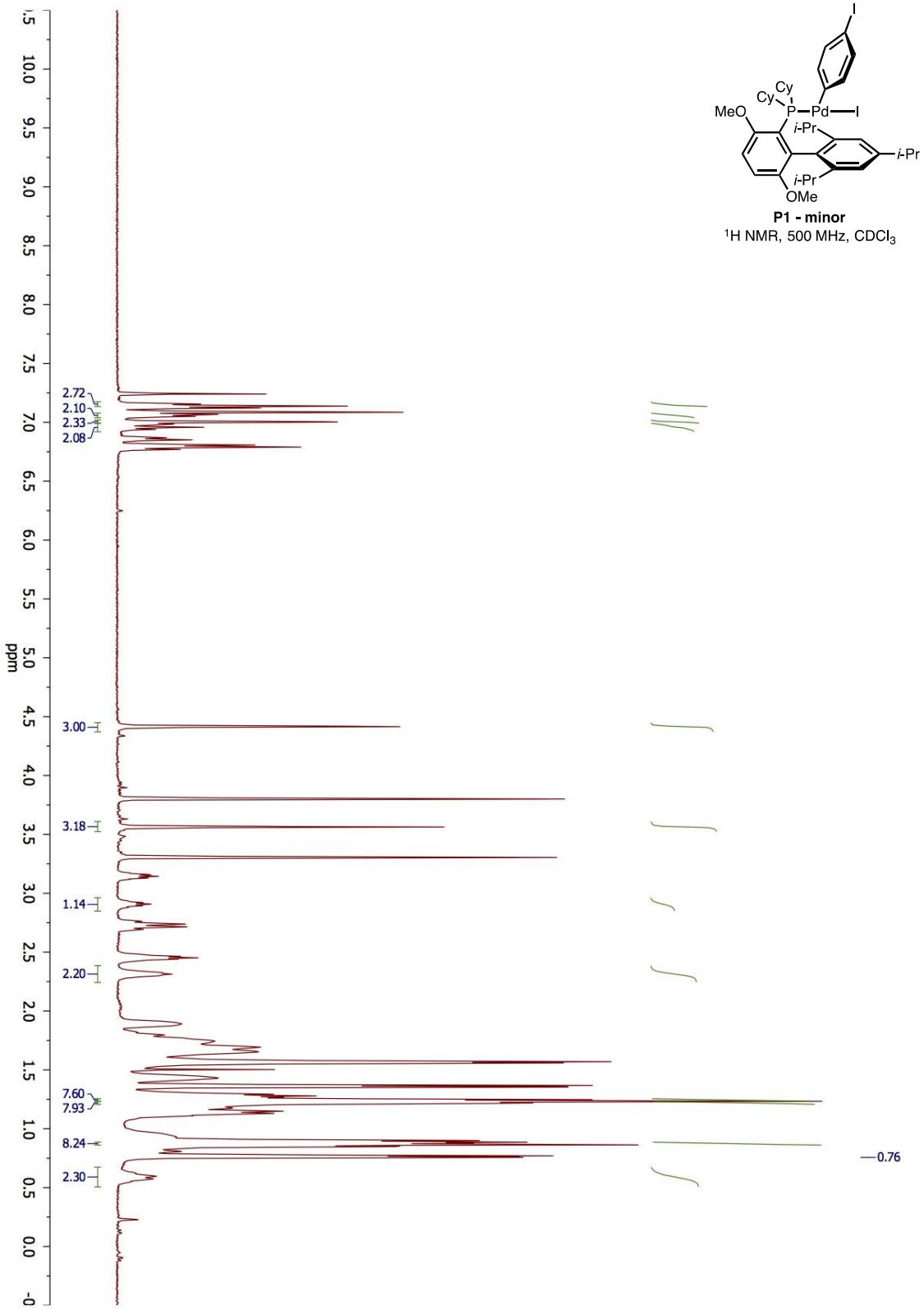
Calibration curve for specific activity (SA):

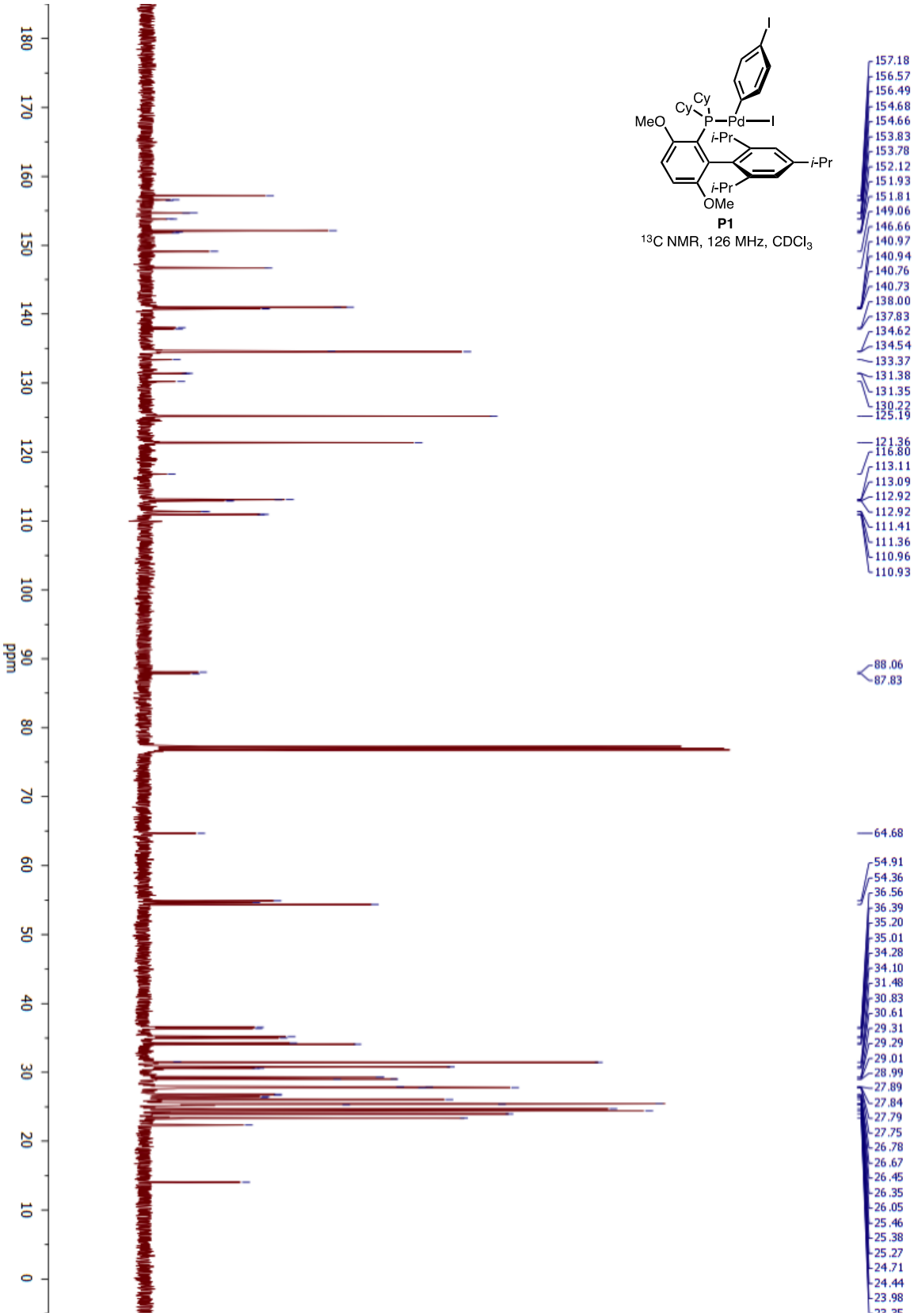
Solutions of different concentrations were injected at constant volume on analytical HPLC. By multiplying the concentration by the volume (50 µL injection) the amount of peptide **2** in nmol was obtained. Five concentrations (0.25, 0.5, 1.25, 2.5, 5.0 µM) of peptide **2** standard were prepared. The samples were analyzed by HPLC and the peak area (UV 280 nm) of peptide **2** was recorded to construct a calibration curve (molar amount vs peak area calibration curve). HPLC conditions: mobile phase **I** = water (0.1 % TFA), mobile phase **II** = acetonitrile (0.1 % TFA); gradient method, 5-95 % **II** 0-10 min, run for 8 min; 50 µL injection volume (1.0 mL/min, Agilent ZORBAX Eclipse XDB-C18, 4.6 × 150 mm, 5 µm).

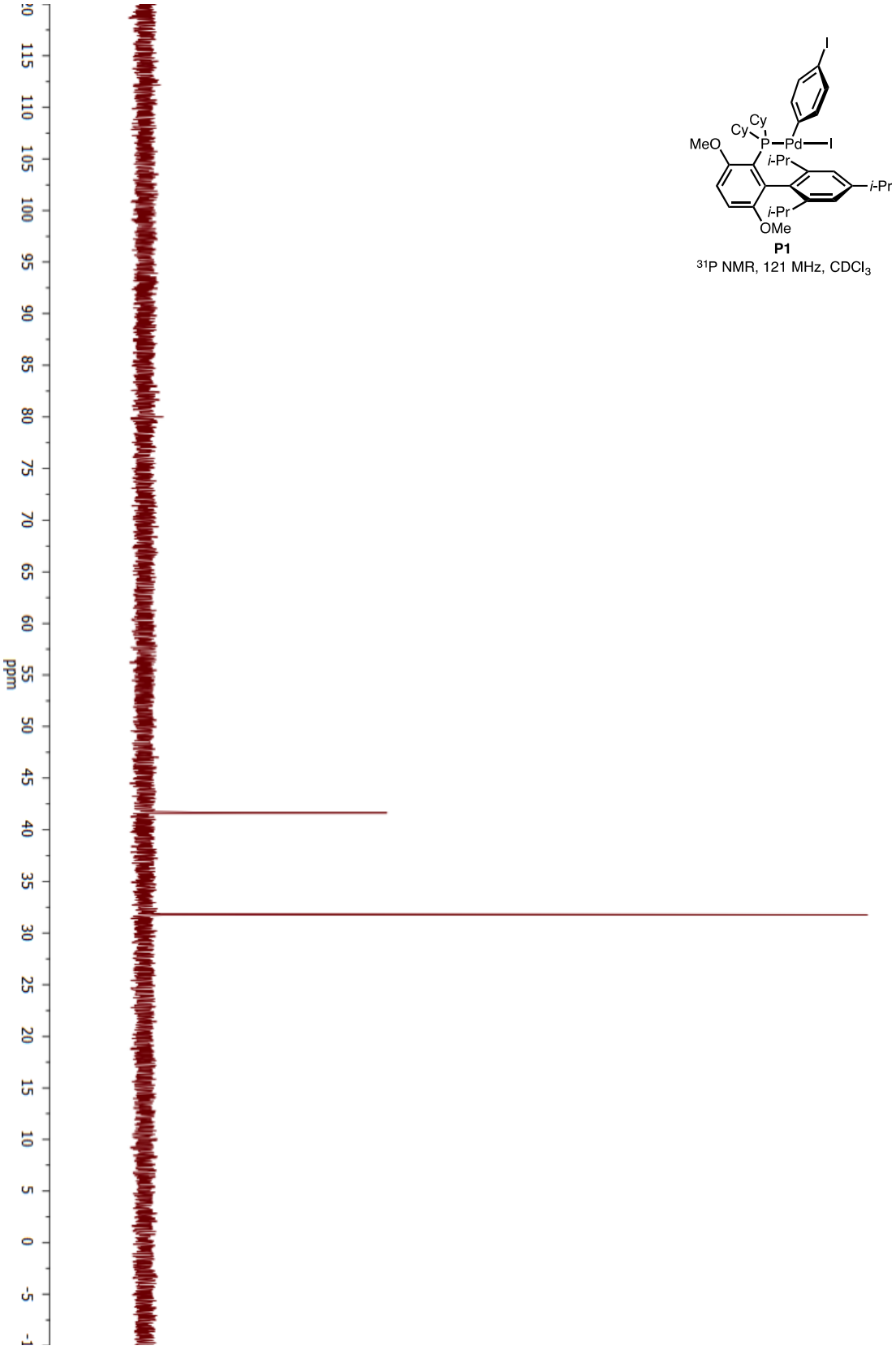


VIII. NMR spectra of Pd complexes.


I
Cy
Cy
P
Pd
I
MeO
i-Pr
i-Pr
i-Pr
OMe
P1 - major
1H NMR, 500 MHz, CDCl3
ppm
2.64
2.01
1.09
3.04
3.00
2.35
3.04
1.00
2.06
2.07
8.41
6.33
6.39

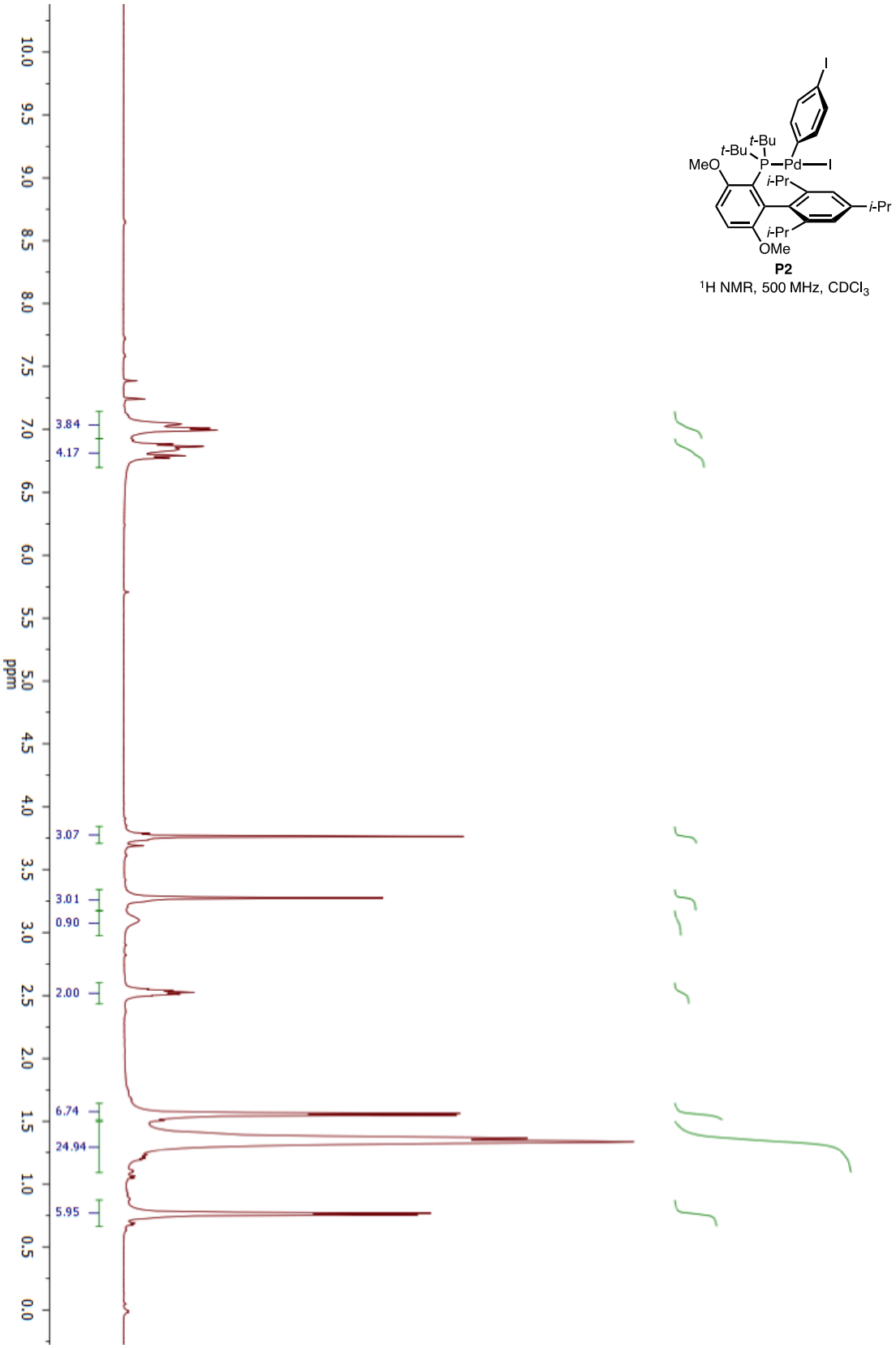

I
Cy
Cy
P
Pd
I
MeO
i-Pr
i-Pr
i-Pr
OMe
P1 - minor
1H NMR, 500 MHz, CDCl3
ppm
2.72
2.10
2.33
2.08
3.00
3.18
1.14
2.20
7.60
7.93
8.24
2.30
0.76

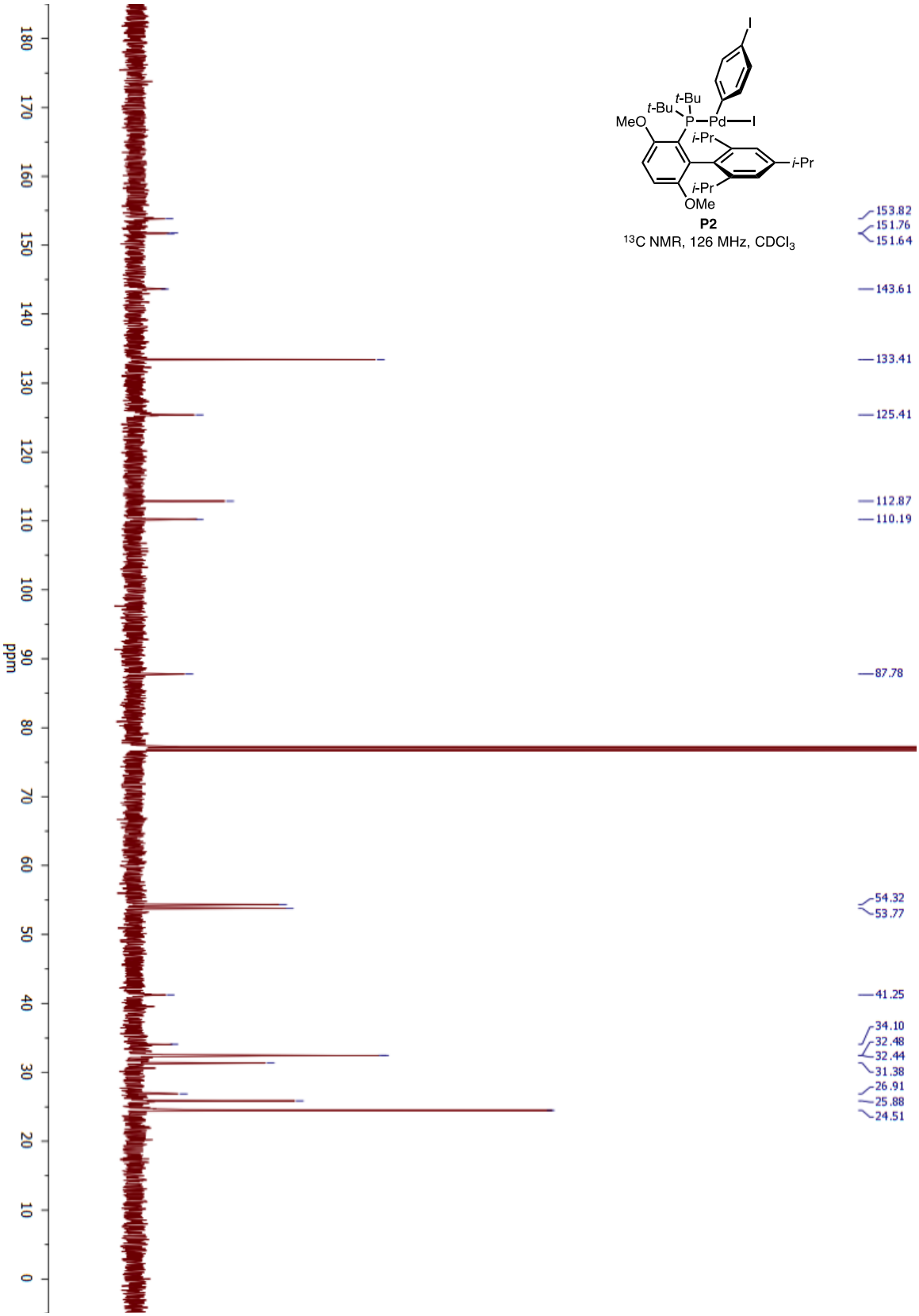

I
Cy
Cy
MeO
P
Pd
I
i-Pr
i-Pr
i-Pr
OMe
P1
13C NMR, 126 MHz, CDCl3
ppm
157.18
156.57
156.49
154.68
154.66
153.83
153.78
152.12
151.93
151.81
149.06
146.66
140.97
140.94
140.76
140.73
138.00
137.83
134.62
134.54
133.37
131.38
131.35
130.22
125.19
121.36
116.80
113.11
113.09
112.92
112.92
111.41
111.36
110.96
110.93
88.06
87.83
64.68
54.91
54.36
36.56
36.39
35.20
35.01
34.28
34.10
31.48
30.83
30.61
29.31
29.29
29.01
28.99
27.89
27.84
27.79
27.75
26.78
26.67
26.45
26.35
26.05
25.46
25.38
25.27
24.71
24.44
23.98

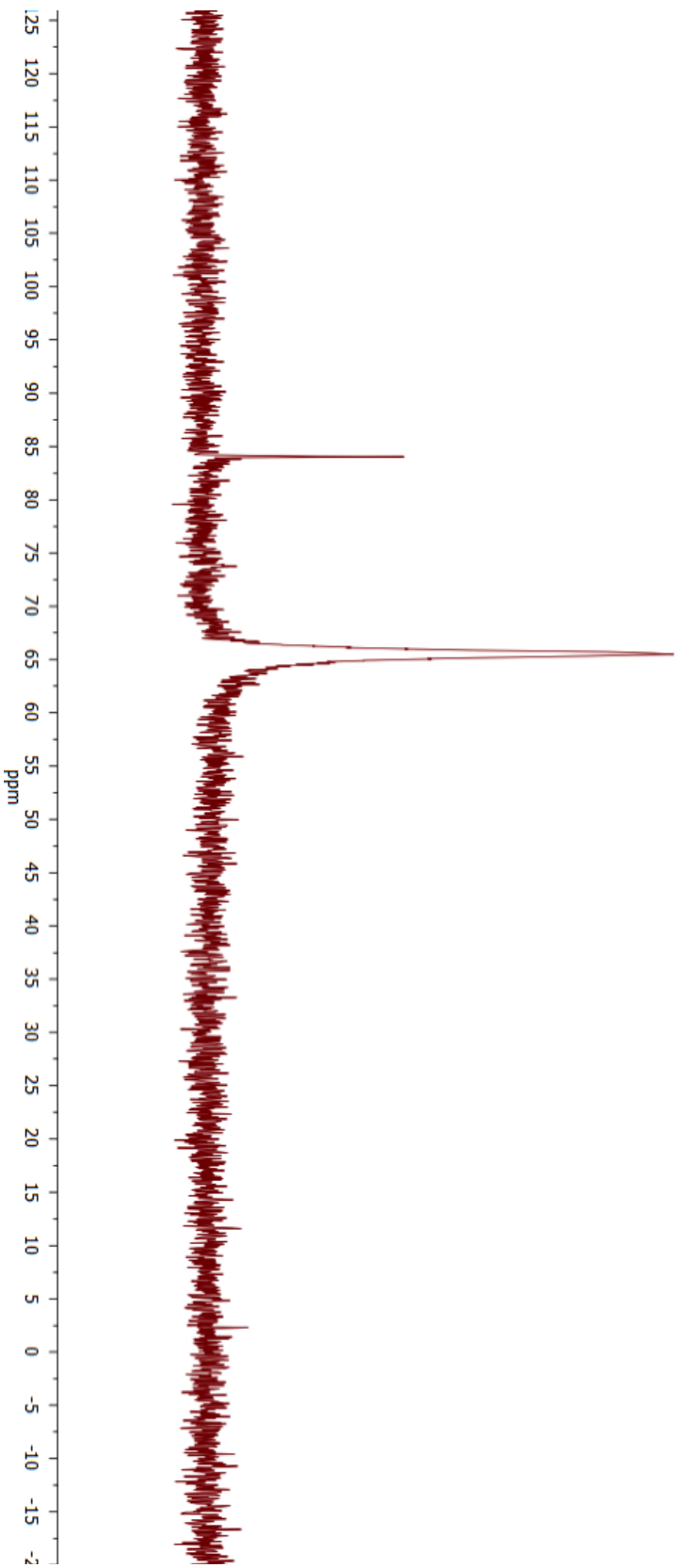


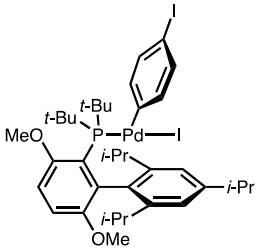
P1

^{31}P NMR, 121 MHz, $CDCl_3$


I
t-Bu
t-Bu
MeO
P
Pd
I
i-Pr
i-Pr
i-Pr
OMe
P2
1H NMR, 500 MHz, CDCl3
ppm
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
3.84
4.17
3.07
3.01
0.90
2.00
6.74
24.94
5.95

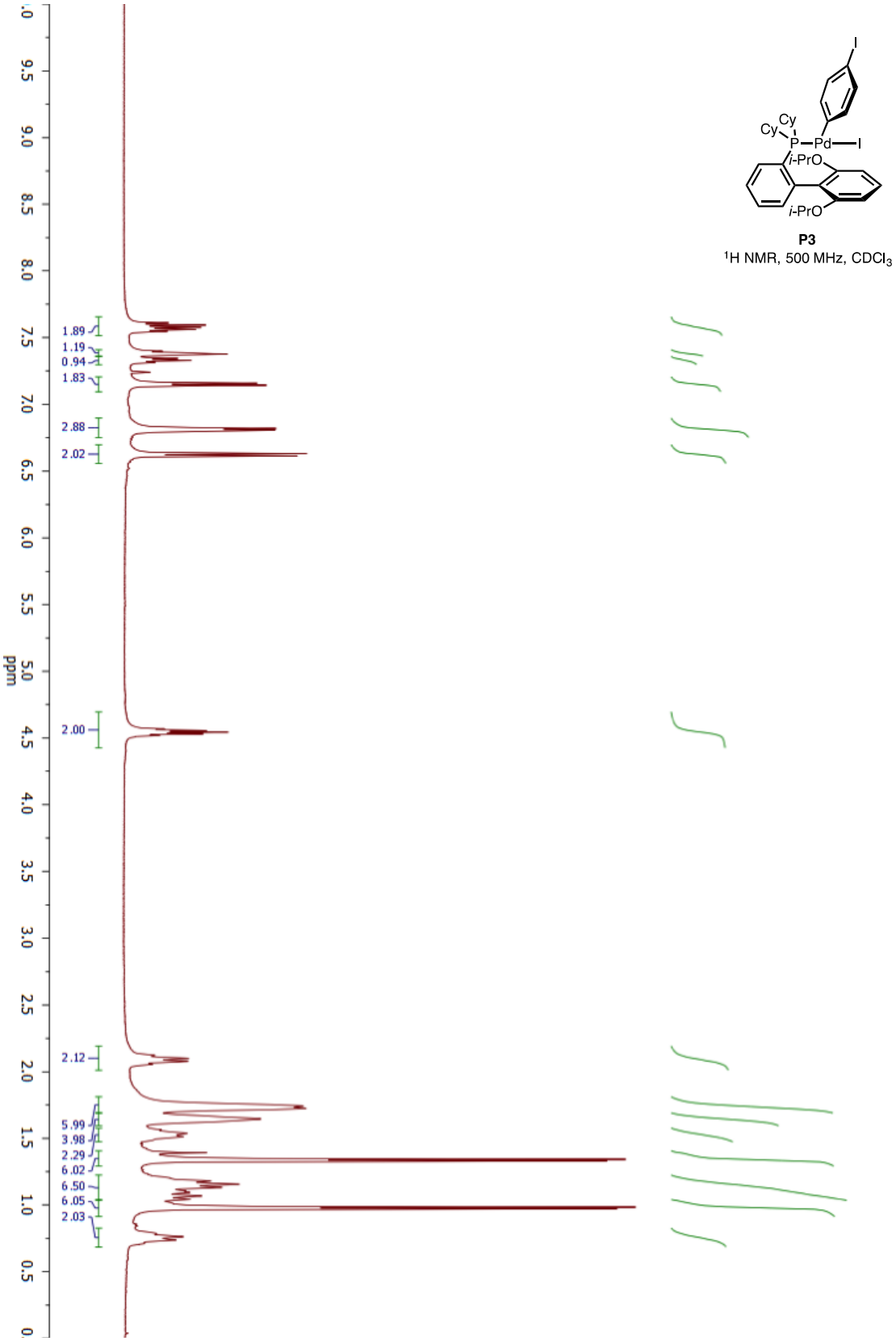

I
t-Bu
t-Bu
MeO
P
Pd
I
i-Pr
i-Pr
i-Pr
OMe
P2
13C NMR, 126 MHz, CDCl3
153.82
151.76
151.64
143.61
133.41
125.41
112.87
110.19
87.78
54.32
53.77
41.25
34.10
32.48
32.44
31.38
26.91
25.88
24.51
ppm
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

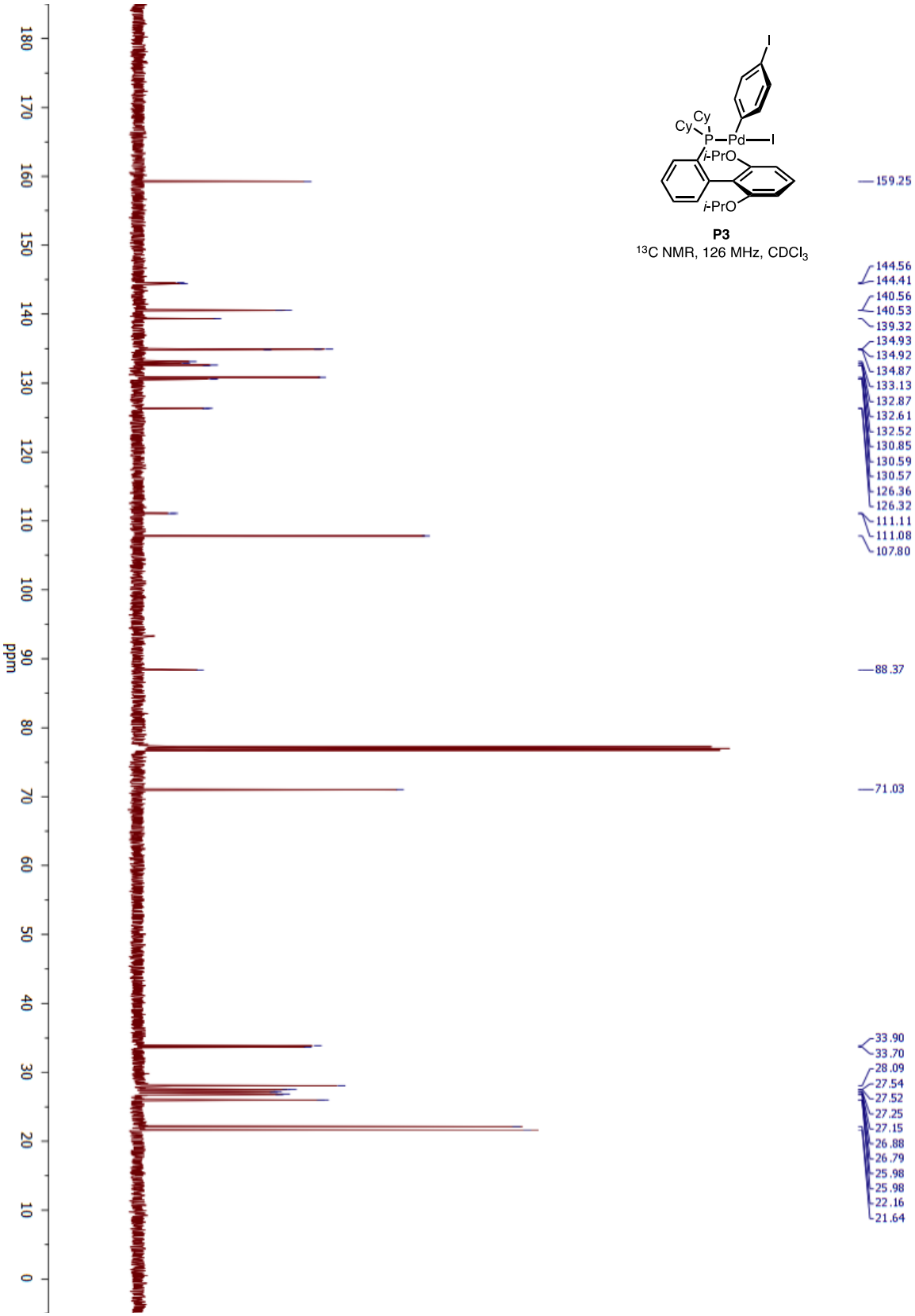


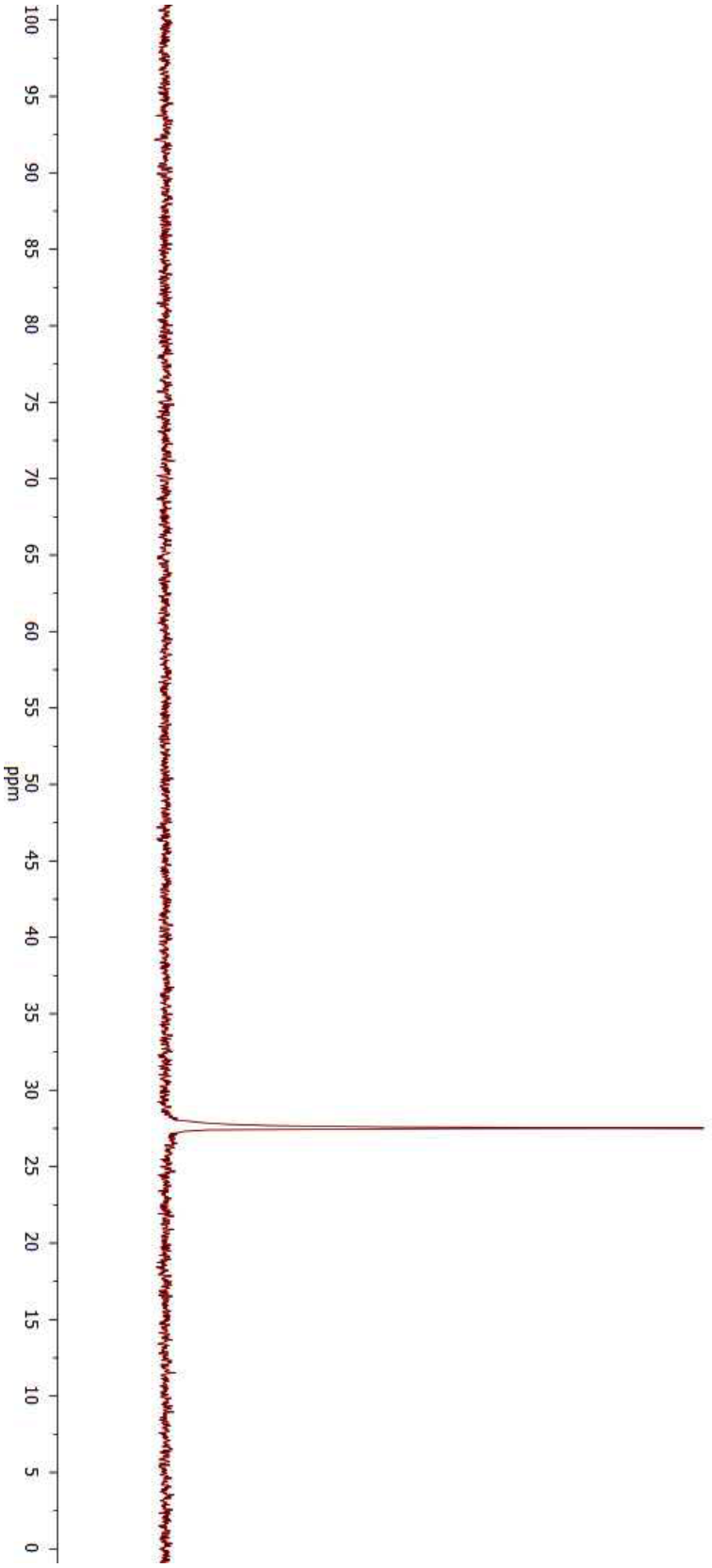


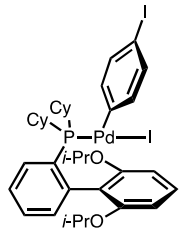
P2

^{31}P NMR, 121 MHz, $CDCl_3$


I
Cy
Cy
P
Pd
I
i-PrO
i-PrO
P3
1H NMR, 500 MHz, CDCl3
ppm
1.89
1.19
0.94
1.83
2.88
2.02
2.00
2.12
5.99
3.98
2.29
6.02
6.50
6.05
2.03

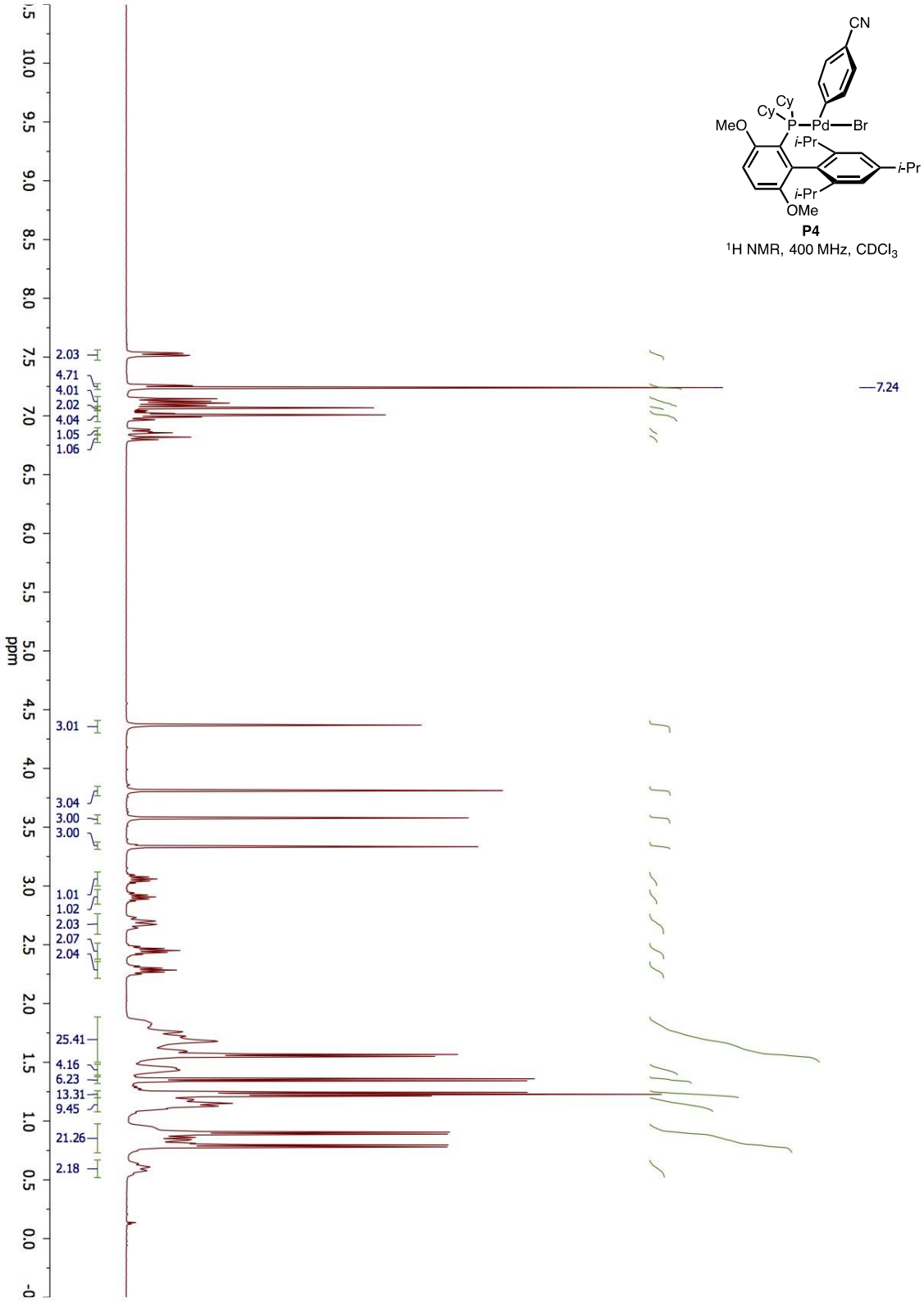

I
Cy
Cy
P
Pd
I
i-PrO
i-PrO
P3
13C NMR, 126 MHz, CDCl3
ppm
159.25
144.56
144.41
140.56
140.53
139.32
134.93
134.92
134.87
133.13
132.87
132.61
132.52
130.85
130.59
130.57
126.36
126.32
111.11
111.08
107.80
88.37
71.03
33.90
33.70
28.09
27.54
27.52
27.25
27.15
26.88
26.79
25.98
25.98
22.16
21.64

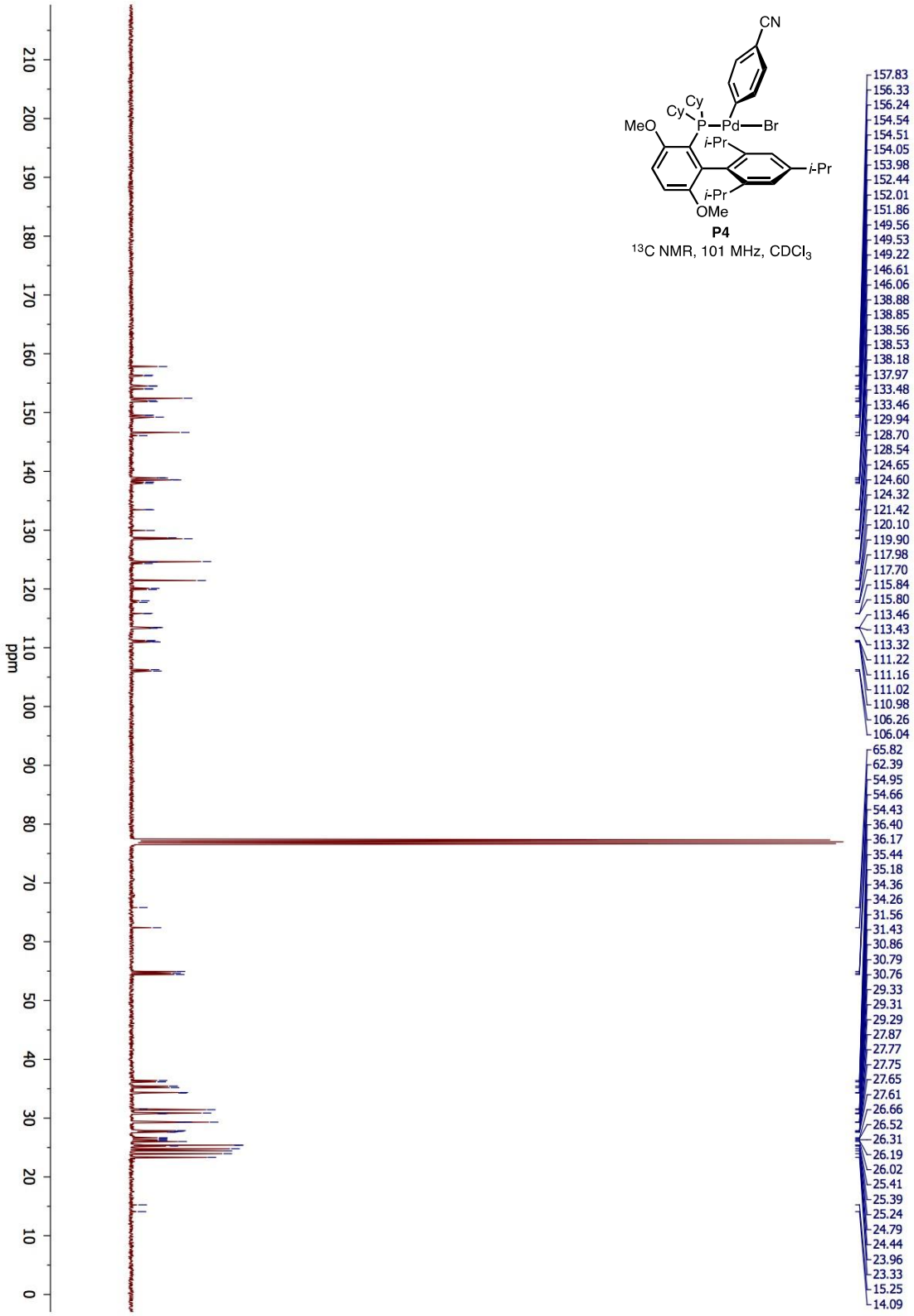


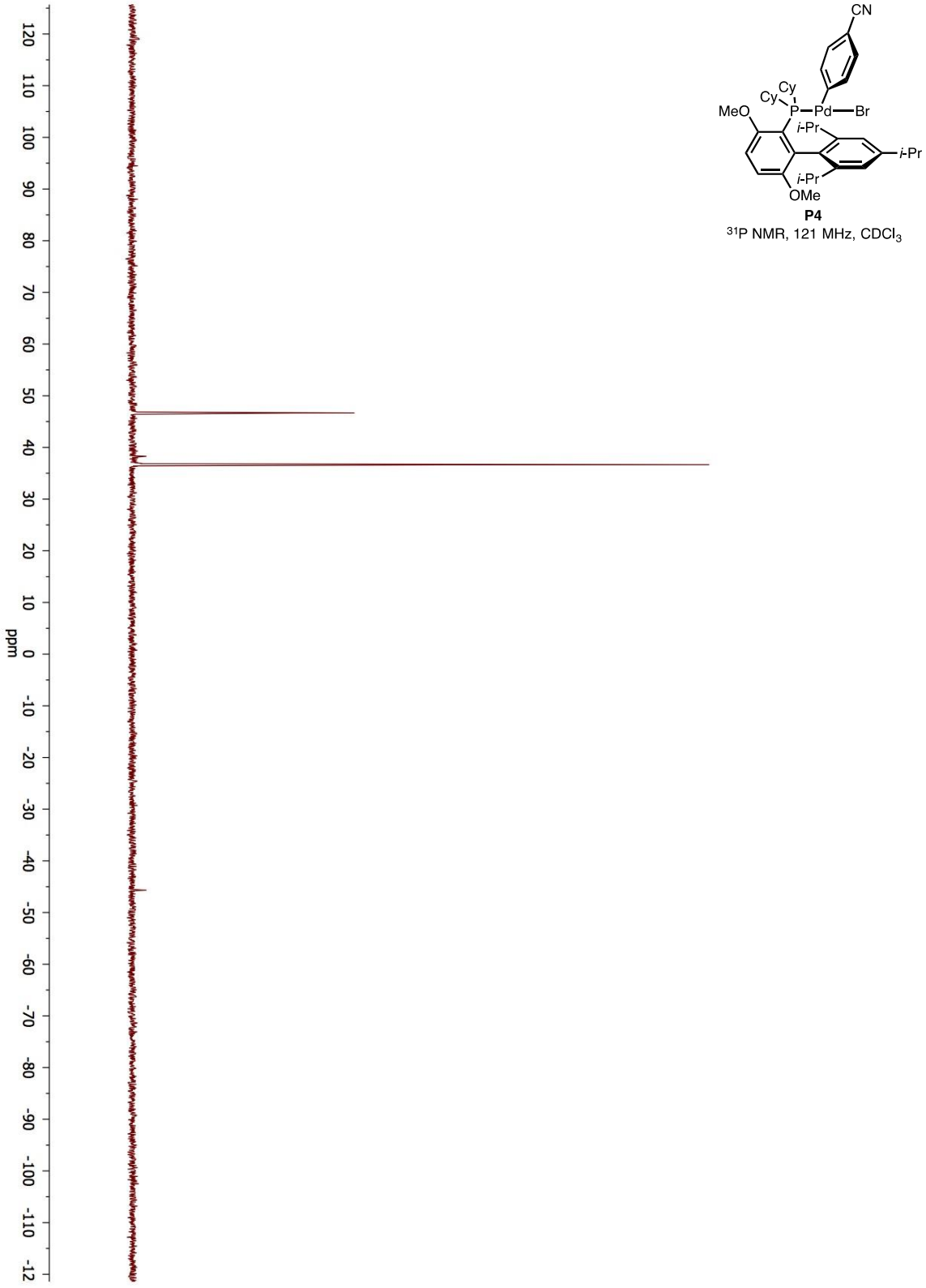


P3

^{31}P NMR, 121 MHz, $CDCl_3$


CN
Cy
Cy
MeO
P
Pd
Br
i-Pr
i-Pr
i-Pr
i-Pr
OMe
P4
1H NMR, 400 MHz, CDCl3
7.24
ppm
2.03
4.71
4.01
2.02
4.04
1.05
1.06
3.01
3.04
3.00
3.00
1.01
1.02
2.03
2.07
2.04
25.41
4.16
6.23
13.31
9.45
21.26
2.18


CN
Cy
Cy
P
Pd
Br
MeO
i-Pr
i-Pr
i-Pr
OMe
P4
13C NMR, 101 MHz, CDCl3
ppm
157.83
156.33
156.24
154.54
154.51
154.05
153.98
152.44
152.01
151.86
149.56
149.53
149.22
146.61
146.06
138.88
138.85
138.56
138.53
138.18
137.97
133.48
133.46
129.94
128.70
128.54
124.65
124.60
124.32
121.42
120.10
119.90
117.98
117.70
115.84
115.80
113.46
113.43
113.32
111.22
111.16
111.02
110.98
106.26
106.04
65.82
62.39
54.95
54.66
54.43
36.40
36.17
35.44
35.18
34.36
34.26
31.56
31.43
30.86
30.79
30.76
29.33
29.31
29.29
27.87
27.77
27.75
27.65
27.61
26.66
26.52
26.31
26.19
26.02
25.41
25.39
25.24
24.79
24.44
23.96
23.33
15.25
14.09


CN
Cy
Cy
P
Pd
Br
MeO
i-Pr
i-Pr
i-Pr
OMe
P4
31P NMR, 121 MHz, CDCl3
ppm
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-12

IX. References.

1. McAtee, J. R.; Martin, S. E. S.; Ahneman, D. T.; Johnson, K. A.; Watson, D. A. *Angew. Chem. Int. Ed.* **2012**, *51*, 3663.
2. Milner, P. J.; Maimone, T. J.; Su, M.; Chen, J.; Müller, P.; Buchwald, S. L. *J. Am. Chem. Soc.* **2012**, *134*, 19922.
3. Vinogradova, E. V.; Zhang, C.; Spokoyny, A. M.; Pentelute, B. L.; Buchwald, S. L. *Nature*, **2015**, *526*, 687.
4. [^{11}C]HCN without dilution by DMSO resulted in lower yield of labeled peptide **2**.