Palladium Oxidative Addition Complexes for Peptide and Protein Crosslinking

Koji Kubota[†], Peng Dai[†], Bradley L. Pentelute*, Stephen L. Buchwald*

Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts, 02139, United States

e-mail: blp@mit.edu, sbuchwal@mit.edu

Table of Contents

1. General information

General reagent information

1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-oxide hexafluorophosphate (HATU), Fmoc-L-Gly-OH, Fmoc-L-Leu-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Ala-OH, Fmoc-L-Cys(Trt)-OH, Fmoc-L-Gln(Trt)-OH, Fmoc-L-L-Asn(Trt)-OH, Fmoc-L-Glu(OtBu)-OH, Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Phe-OH, Fmoc-L-Ser(*t*Bu)-OH, Fmoc-L-Thr(*t*Bu)-OH, Fmoc-L-Tyr(*t*Bu)-OH, and Fmoc-L-His(Trt)-OH were purchased from Chem-Impex International (Wood Dale IL). H-Rink Amide ChemMatrix resin was obtained from PCAS BioMatrix Inc. (Quebec, Canada). Peptide synthesis-grade *N*,*N*'-dimethylformamide (DMF), dichloromethane (CH_2Cl_2), diethyl ether, HPLC-grade acetonitrile, and guanidine hydrochloride were obtained from VWR International (Philadelphia, PA). All deuterated solvents were purchased from Cambridge Isotopes and used

without further purification. All other reagents were purchased from Sigma-Aldrich and used as received. MDM2 (**9**) protein [in 1.02% Imidazole, 0.002% phenylmethylsulfonyl fluoride (PMSF), 0.82% Sodium phosphate, 0.004% DTT, 1.75% Sodium chloride, 25% Glycerol, pH 7.0] was purchased from Abcam (ab167941), and used directly as received in the reactions. Sortase A P94S/D160N/K196T [SrtA* (**7**)] was expressed as previously reported[1] and used as a stock solution (150 μM in 20 mM Tris and 150 mM NaCl, pH 8.0) for the crosslinking reaction. CCA (**13**) (C-terminal domain of HIV-1 capsid assembly polyprotein) was expressed as previously reported[2] and used as a stock solution (200 μM in 50 mM phosphate, pH 8.0) for the crosslinking reaction.

All reactions with peptides and proteins were set up on the bench top and carried out under ambient conditions. Pentane and cyclohexane were purchased from Sigma-Aldrich in Sure/Seal™ bottles and were purged with argon before use.

General analytical information

All small-molecule organic and organometallic compounds were characterized by ^{1}H, ^{13}C NMR, and high-resolution mass spectrometry. ^{31}P NMR spectroscopy was used for characterization of palladium complexes. Copies of the ^{1}H, ^{13}C, and ^{31}P NMR spectra can be found at the end of the Supporting Information. Nuclear Magnetic Resonance spectra were recorded on a Bruker 400 MHz instrument. Unless otherwise noted, ^{1}H NMR experiments are reported in δ units, parts per million (ppm), and were measured relative to the signals of the residual proton resonances $CHCl_3$ (7.23 ppm), unless otherwise stated. All ^{31}P NMR spectra are measured decoupled from ^{1}H nuclei and are reported in ppm relative to H_3PO_4 (0.00 ppm).

LC-MS analysis

LC-MS chromatograms and associated mass spectra were acquired using Agilent 6520 ESI-Q-TOF mass spectrometer. Mobile phases used for LC-MS analysis are solvent **A** (0.1% formic acid in water) and solvent **B** (0.1% formic acid in acetonitrile). The following LC-MS method was used.

LC-MS analysis. LC conditions: Zorbax 300SB C_3 column: 2.1 x 150 mm, 5 μm was used with column temperature set at 40 °C. gradient: 0-2 min 5% **B**, 2-10 min 5-95% **B**, 10-11 min 95% **B**, flow rate: 0.8 mL/min. MS conditions: positive electrospray ionization (ESI) extended dynamic mode in mass range 300 – 3000 *m/z*, temperature of drying gas = 350 ^{o}C, flow rate of drying gas =

11 L/min, pressure of nebulizer gas = 60 psi, the capillary, fragmentor, and octupole rf voltages were set at 4000, 175, and 750, respectively.

Data were processed using Agilent MassHunter software package. Deconvoluted masses of proteins were obtained using a maximum entropy algorithm.

Y-axis in all chromatograms shown in supplementary figures represents total ion current (TIC); mass spectrum insets correspond to the maxima point of the TIC peak.

Determination of reaction conversion by LC-MS

The conversion related to peptide substrates was determined by integrating TIC spectra. First, using Agilent MassHunter software package, the peak area for all relevant peptidic species on the chromatogram were integrated. The conversion was calculated as following: peptide conversion = 1 - S_t/S_0 where S_t is the peak area of the corresponding peptide at time t, and S_0 is the peak area of the peptide at time 0.

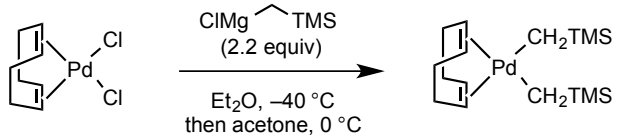
For protein substrates, the conversion was calculated from the relative peak intensity of starting material and products in the deconvoluted mass spectrum as follows: protein conversion = 1 - $I_{\text{starting material}}/I_{\text{all relevant species}}$ where $I_{\text{starting material}}$ is the peak intensity of protein starting material, and $I_{\text{all relevant species}}$ is the sum of the peak intensities of all relevant species (starting material and products) in the deconvoluted mass spectra.

2. Procedure for preparing the oxidative addition complex 1

Synthesis of oxidative addition complex 1

[(1,5-COD)$PdCl_2$] was prepared according to the literature procedure.[3] [(1,5-COD)Pd($CH_2TMS)_2$] was prepared according to a modified literature procedure.[4] COD = 1,5-cycloocatadiene

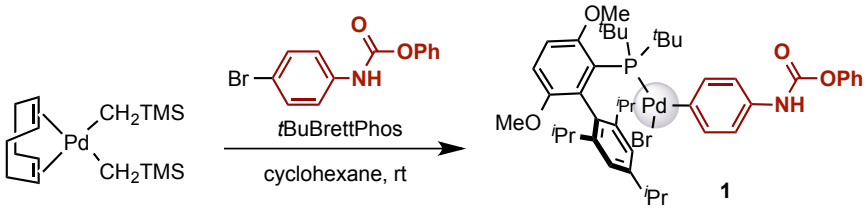
Synthesis of [(1,5-CODPd($CH_2TMS)_2$).



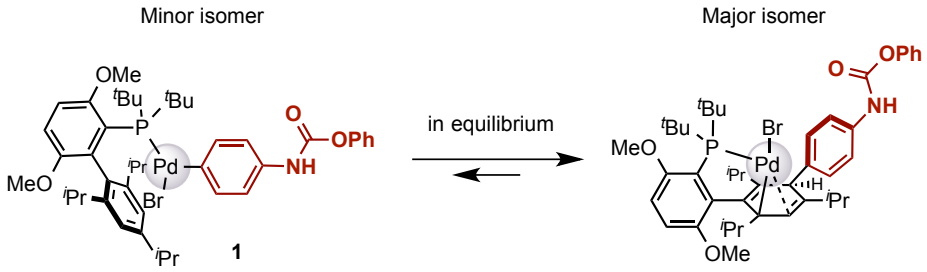
A flame-dried Schlenk flask, equipped with a magnetic stir bar, was filled with argon and charged with (1,5-COD)$PdCl_2$ (3.15 g, 11.05 mmol). The flask was put under vacuum and backfilled with argon (this procedure was repeated a total of three times). Diethyl ether (49.4 mL) was introduced via syringe, the reaction was cooled to –40 °C (acetonitrile/dry ice bath) and

$TMSCH_2MgCl$ (23.4 mL, 1.0 M) was added dropwise over 10–20 min. The reaction mixture was stirred at –40 °C for 1 h and then at 0 °C for an additional 20 min. Acetone (1.3 mL) was added via syringe at 0 °C, the reaction mixture was stirred for 5 min, after which time the solvent was removed under high vacuum connected through a needle with an external trap (the flask was kept at 0 °C by water/ice bath). The flask was then opened to air, pentane (100 mL) was added and the crude material was filtered through a pad of Celite into a new round-bottom flask (500 mL) cooled to 0 °C. The filter cake was washed with pentane (50 mL × 2). Pentane from the combined washes was removed with the aid of a rotary evaporator at 0 °C (ice/water bath). The resulting white solid was dried under vacuum for 2 h at 0 °C and brought into a nitrogen-filled glovebox where the solid was transferred into a 20 mL scintillation vial and stored at -20 °C (3.00 g, 64%). The ^1H and ^{13}C NMR spectra of the obtained material are identical to those reported in the literature.[4]

Synthesis of oxidative addition complex 1.



In a nitrogen-filled glovebox, an oven-dried scintillation vial (10 mL), equipped with a magnetic stir bar, was charged with *t*BuBrettPhos (67.9 mg, 0.14 mmol), phenyl (4-bromophenyl)carbamate (40.9 mg, 0.14 mmol) and cyclohexane (1.5 mL). Solid $(COD)Pd(CH_2TMS)_2$ (54.5 mg, 0.13 mmol) was added rapidly in one portion and the resulting solution was stirred for 16 h at room temperature. After this time, pentane (3 mL) was added and the resulting mixture was placed into –20 °C freezer for 3 h. The vial was then removed from the glovebox, and the resulting precipitate was filtered, washed with pentane (3 mL × 3), and dried under reduced pressure to afford the oxidative addition complex **1** (94.1 mg, 92%) as an orange solid.



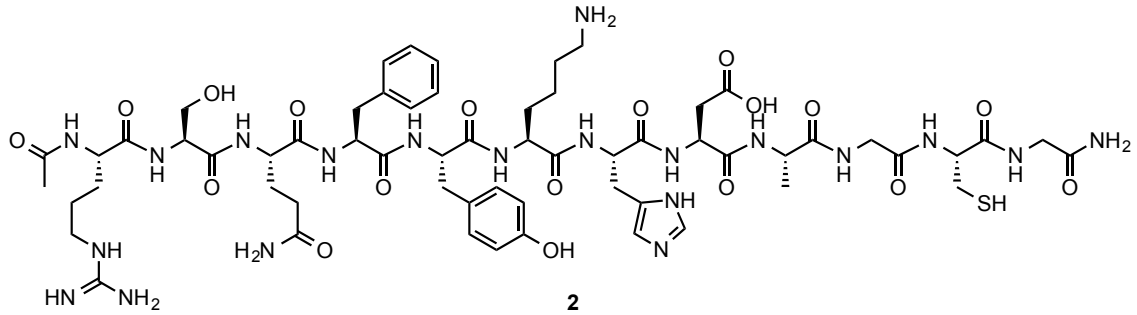
^1H NMR (400 MHz, $CDCl_3$): major isomer δ 8.82 (dd, J = 8.5, 2.2 Hz, 1H), 7.71 (d, J = 7.4 Hz, 1H), 6.83–7.55 (m, 10H), 5.85 (s, 1H), 3.85 (s, 3H), 3.76 (s, 3H), 2.55–2.70 (m, 1H), 2.37–2.50 (m, 1H),

1.91–2.04 (m, 1H), 1.42–1.48 (m, 18H), 1.18–1.22 (m, 6H), 1.28 (d, J = 6.6 Hz, 3H), 1.13 (d, J = 6.9 Hz, 3H) 0.78 (d, J = 6.5 Hz, 3H), 0.07 (d, J = 7.3, 3H) ppm; minor isomer δ 6.83–7.55 (m, 13H), 3.84 (s, 3H), 3.38 (s, 3H), 3.21–3.23 (m, 1H), 3.12–3.14 (m, 1H), 2.37–2.50 (m, 1H), 1.61–1.66 (m, 6H), 1.57 (s, 9H), 1.54 (s, 9H), 1.37–1.40 (m, 6H), 0.85–0.89 (m, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 174.0, 154.9, 154.0, 151.4, 151.3, 150.8, 150.6, 139.7, 136.6, 136.4, 136.2, 135.6, 135.5, 132.2, 132.1, 132.0, 129.37, 129.35, 129.3, 129.2, 129.0, 126.0, 125.8, 125.6, 125.4, 124.1, 121.81, 121.77, 121.7, 121.3, 118.9, 113.7, 110.2, 110.1, 100.0, 98.6, 68.0, 67.9, 54.7, 51.6, 41.1, 41.0, 39.8, 39.7, 39.2, 39.1, 34.4, 33.72, 33.69, 32.7, 32.6, 32.33, 32.27, 32.24, 32.18, 31.3, 30.2, 30.1, 26.9, 25.5, 25.4, 24.4, 24.3, 22.3, 22.2, 22.0, 21.1, 21.07, 21.00, 20.01, 19.95, 19.9 ppm (observed complexity is due to C–P coupling and equilibrium with the rearranged complex); ^{31}P NMR (162 MHz, $CDCl_3$) δ 81.2, 70.1 ppm. HRMS–ESI (m/z): $[M–Br]^+$ calcd for $C_{44}H_{59}NO_4PPd$, 802.3217; found, 802.3263.

3. Peptide synthesis and LC-MS characterization

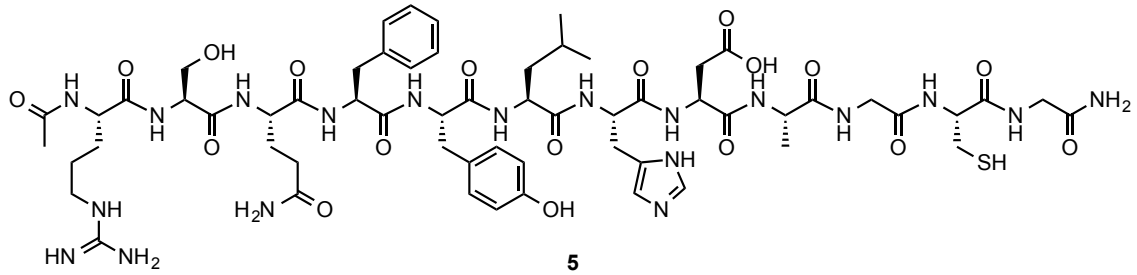
Peptide synthesis

All peptides were synthesized on a 0.1 mmol scale on H-Rink Amide ChemMatrix resin. Solid-phase peptide synthesis (SPPS) was carried out on a synthesizer for automated flow peptide synthesis (AFPS).[5] *N*-terminal acetylation was carried out under batch condition as described below. Acetic acid (1 mmol) was dissolved in a HATU solution in DMF (2.5 mL, 0.38 M), and diisopropylethylamine (DIEA) (500 µL) was added. The mixture was added to resin and allowed to react at room temperature for 20 min, followed by washing with DMF (3x). After completion of the synthesis, the resin was washed thoroughly with CH_2Cl_2 and dried under vacuum. The resins were transferred to a 50-mL plastic tube and the peptide was simultaneously cleaved from the resin and the side-chain was deprotected by treatment with 2.5% (v/v) water, 2.5% (v/v) 1,2-ethanedithiol (EDT), and 1% (v/v) triisoproprylsilane in neat trifluoroacetic acid (TFA) for 2 h at rt. The resulting solution containing peptide was triturated and washed with cold diethyl ether (pre-chilled in -80 °C freezer) (2x). The obtained gummy-like solid was dissolved in 50% H_2O: 50% acetonitrile containing 0.1% TFA and lyophilized. The following peptides were synthesized following this procedure.



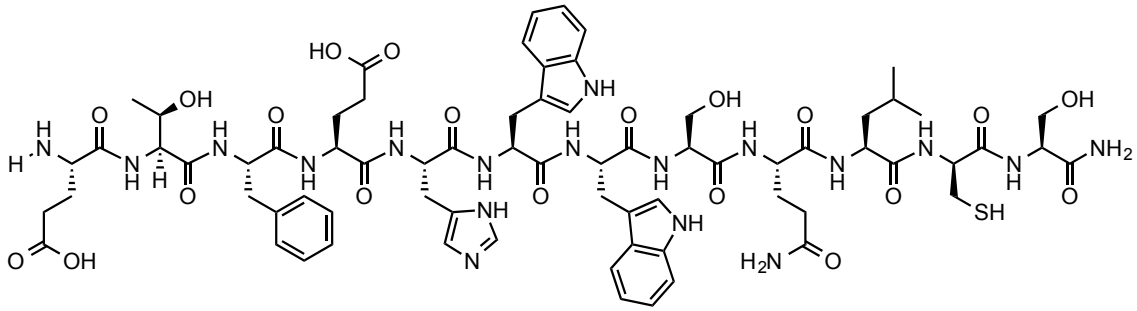
2

Ac-RSQFYKHDAGCG



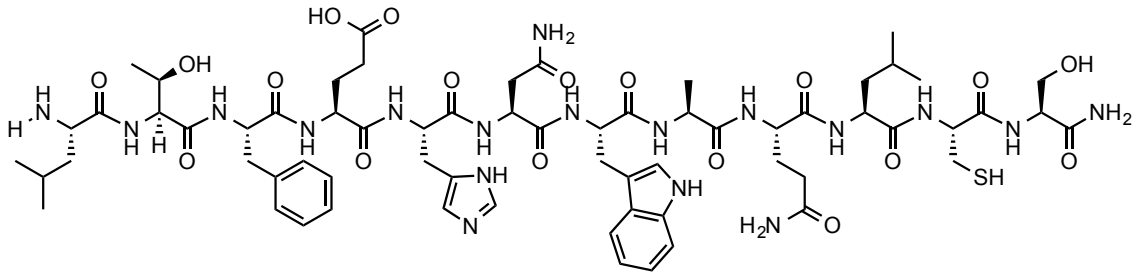
5

Ac-RSQFYLHDAGCG



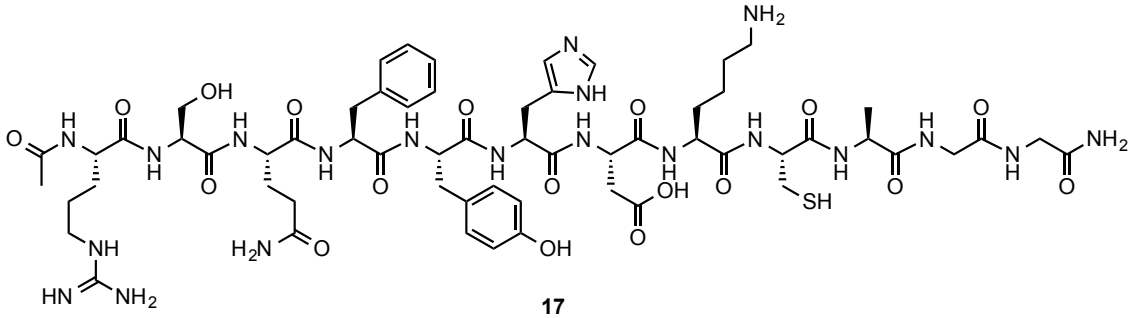
10

ETFEHWWSQLCS



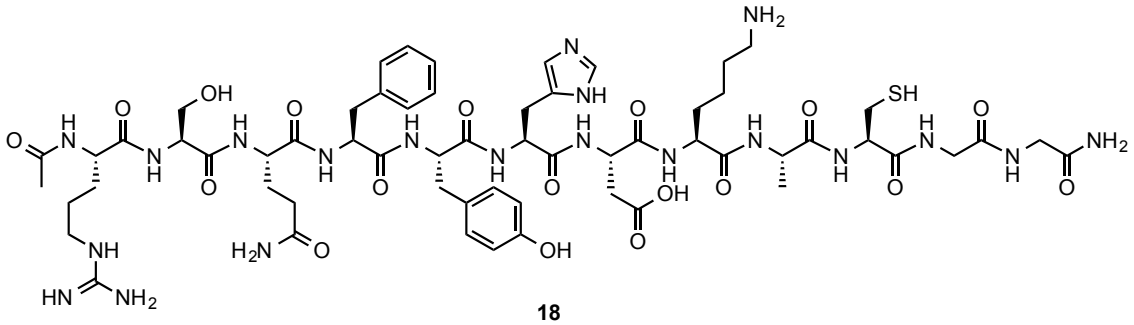
15

LTFEHNNWAQLCS



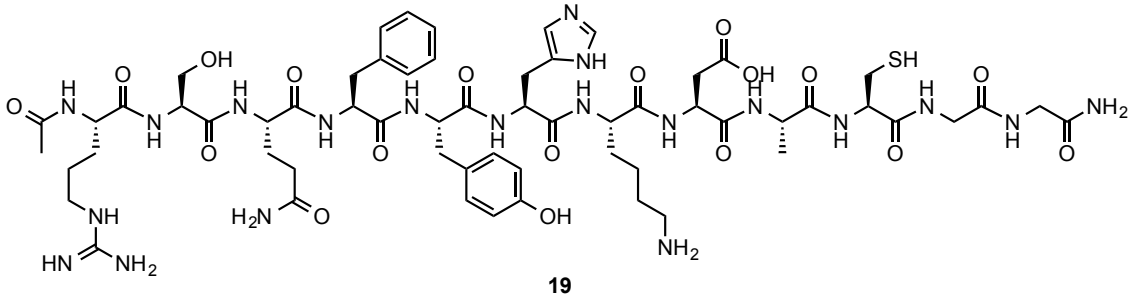
17

Ac-RSQFYHDKCAGG



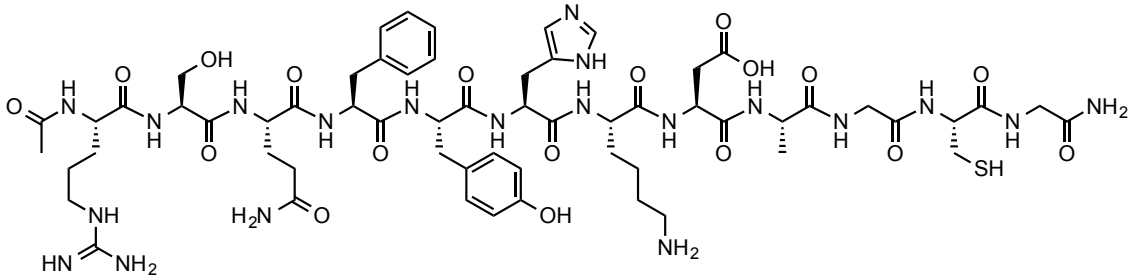
18

Ac-RSQFYHDKACGG



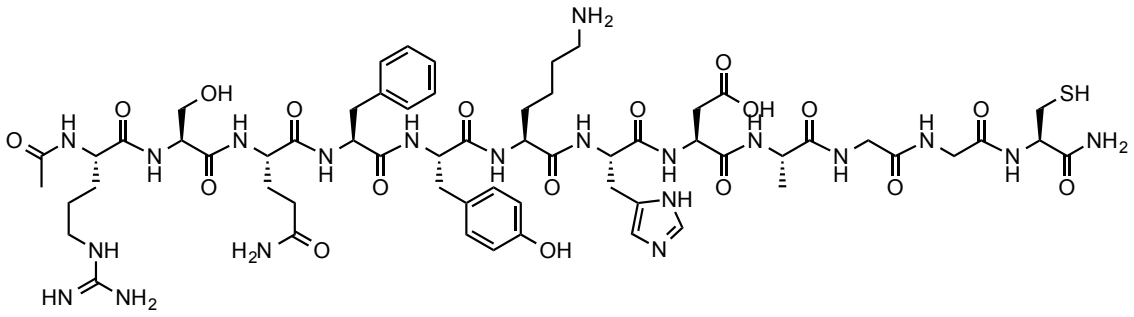
19

Ac-RSQFYHKDACGG



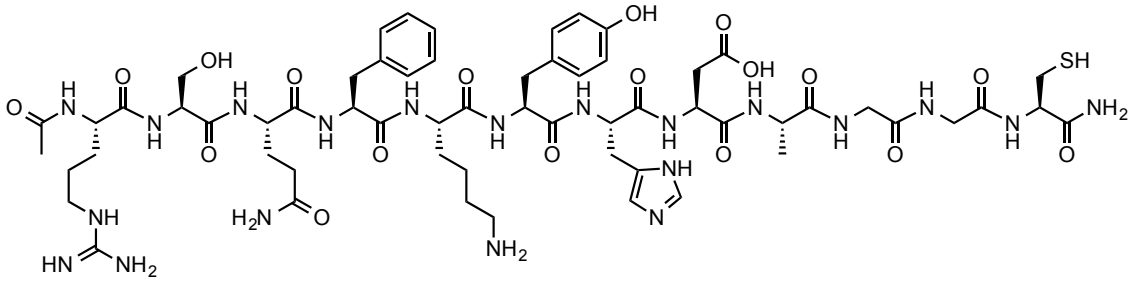
20

Ac-RSQFYHKDAGCG



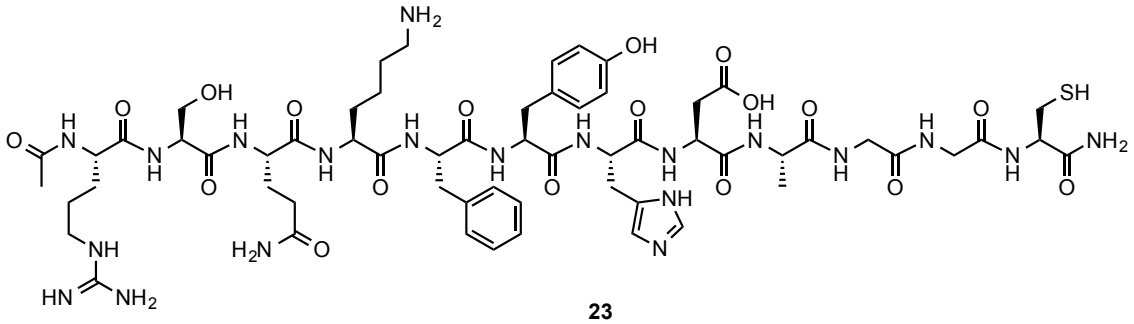
21

Ac-RSQFYKHDAGGC



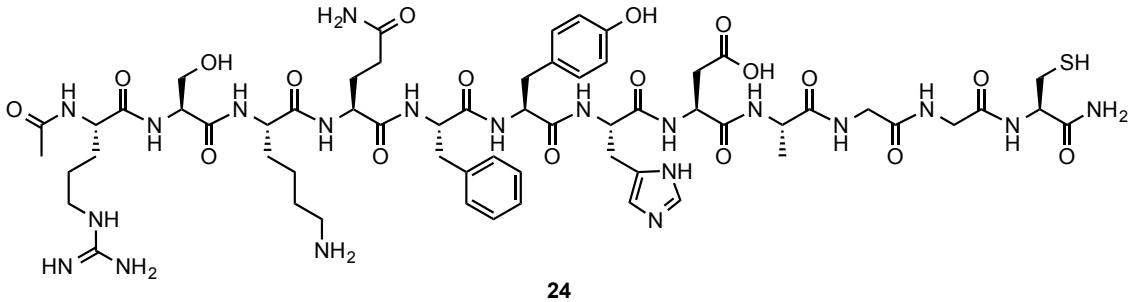
22

Ac-RSQFKYHDAGGC



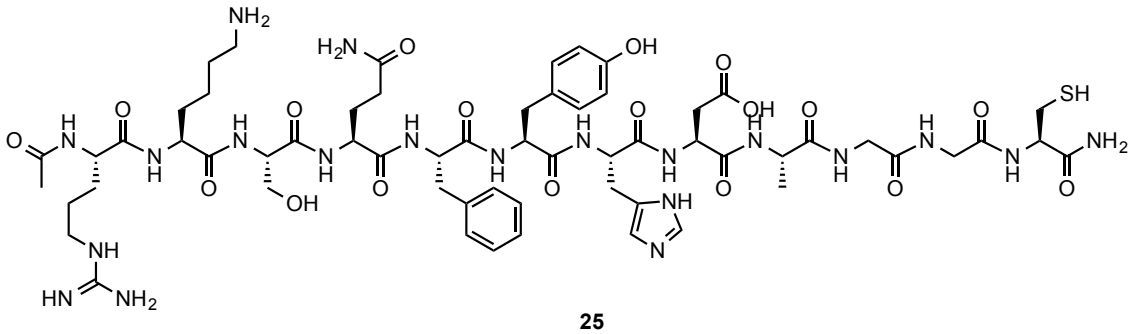
23

Ac-RSQKFYHDAGGC



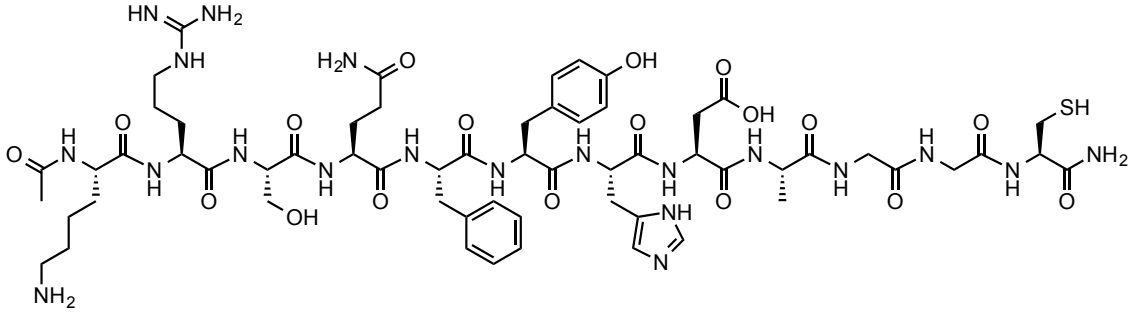
24

Ac-RSKQFYHDAGGC



25

Ac-RKSQFYHDAGGC



26

Ac-SRKQFYHDAGGC

Peptide purification

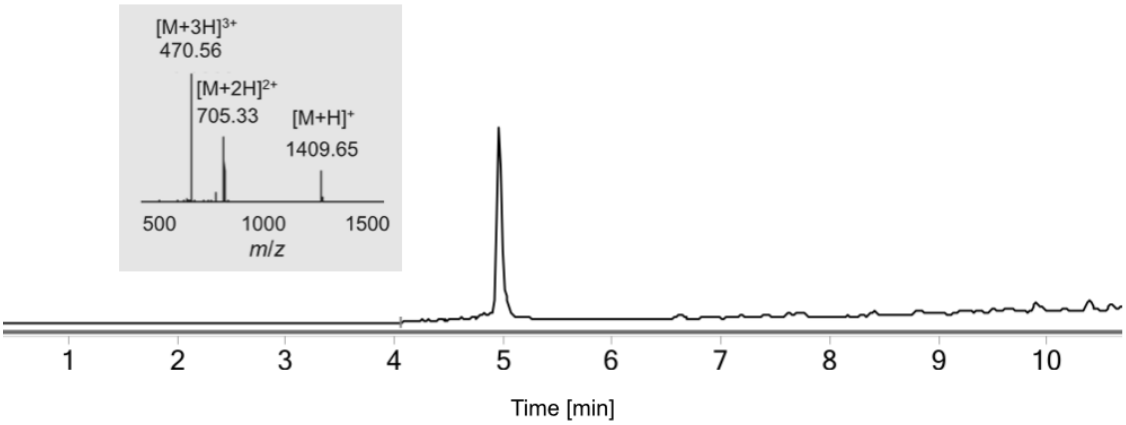
Peptides **2** and **5** were purified by the following procedure. Solvent compositions for reverse phase HPLC (RP-HPLC) purification were water with 0.1% TFA (solvent **C**) and acetonitrile with 0.1% TFA (solvent **D**). The crude peptide was dissolved in 95% **C**: 5% **D** with 6 M guanidinium hydrochloride and purified on Agilent 1260 Infinity Automated LC/MS Purification System, with a semi-preparative Reverse Phase-HPLC column (Agilent Zorbax 300SB C_3 column: 21.2 x 250 mm, 7 μm, linear gradient: 1-41% **D** over 80 min, flow rate: 4 mL/min). The purity of fractions was confirmed by LC-MS analysis. The fractions containing pure cyclized peptide were combined and lyophilized. Peptides **17–26** were purified by the following procedure. Solvent compositions for reverse phase HPLC (RP-HPLC) purification are water with 0.1% TFA (solvent **C**) and acetonitrile with 0.1% TFA (solvent **D**). The crude peptide was dissolved in 95% **C**: 5% **D** with 6 M guanidinium hydrochloride and purified on Agilent 1260 Infinity Automated LC/MS Purification System, with a semi-preparative Reverse Phase-HPLC column (Agilent Zorbax 300SB C_3 column: 21.2 x 250 mm, 7 μm, linear gradient: 1-31% **D** over 42 min, flow rate: 4 mL/min). The purity of fractions was confirmed by LC-MS analysis. The fractions containing pure cyclized peptide

were combined and lyophilized. The crude peptides **10** and **15** were used for the next palladium-mediated arylation step without purification. Peptides synthesized using manual SPPS are listed in Table S1.

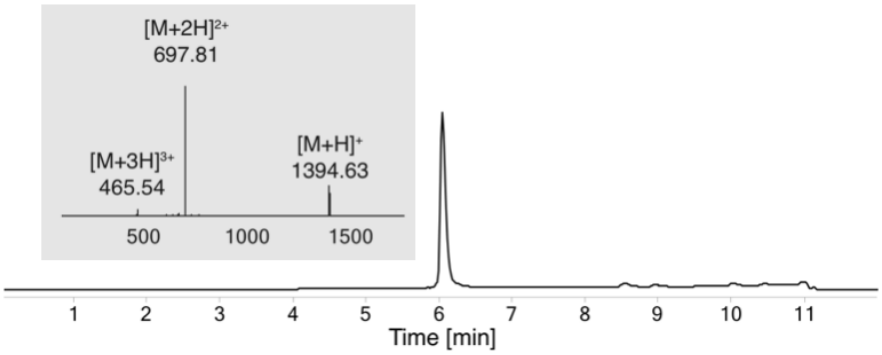
Table S1. Sequences and masses of peptides synthesized by manual fast flow SPPS.

Peptide	Sequence	Calculated Mass	Observed Mass $[M+H]^+$
2	AcNH-**RSQFYKHDAGCG**-C(O)NH_2	1408.63	1409.65
5	AcNH-**RSQFYLHDAGCG**-C(O)NH_2	1393.62	1394.63
10	NH_2-**ETFEHWWSQLCS**-C(O)NH_2	1550.66	1551.66
15	NH_2-**ITCEDLLDYYGP**-C(O)NH_2	1446.67	1447.68
17	AcNH-**RSQFYHDKCAGG**-C(O)NH_2	1408.63	1409.65
18	AcNH-**RSQFYHDKACGG**-C(O)NH_2	1408.63	1409.65
19	AcNH-**RSQFYHKDACGG**-C(O)NH_2	1408.63	1409.65
20	AcNH-**RSQFYHKDAGCG**-C(O)NH_2	1408.63	1409.65
21	AcNH-**RSQFYKHDAGGC**-C(O)NH_2	1408.63	1409.65
22	AcNH-**RSQFKYHDAGGC**-C(O)NH_2	1408.63	1409.65
23	AcNH-**RSQKFYHDAGGC**-C(O)NH_2	1408.63	1409.65
24	AcNH-**RSKQFYHDAGGC**-C(O)NH_2	1408.63	1409.65
25	AcNH-**RKSQFYHDAGGC**-C(O)NH_2	1408.63	1409.65
26	AcNH-**KRSQFYHDAGGC**-C(O)NH_2	1408.63	1409.65

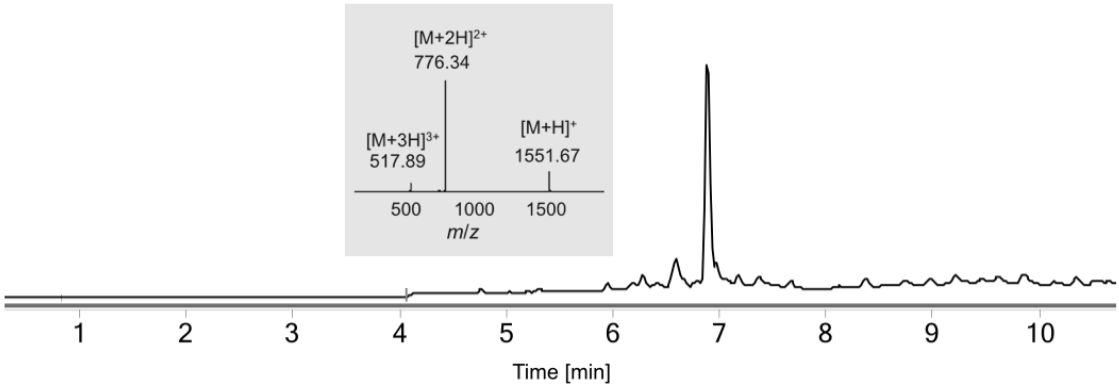
LC-MS trace of peptide 2 (Calculated Mass: 1408.63)



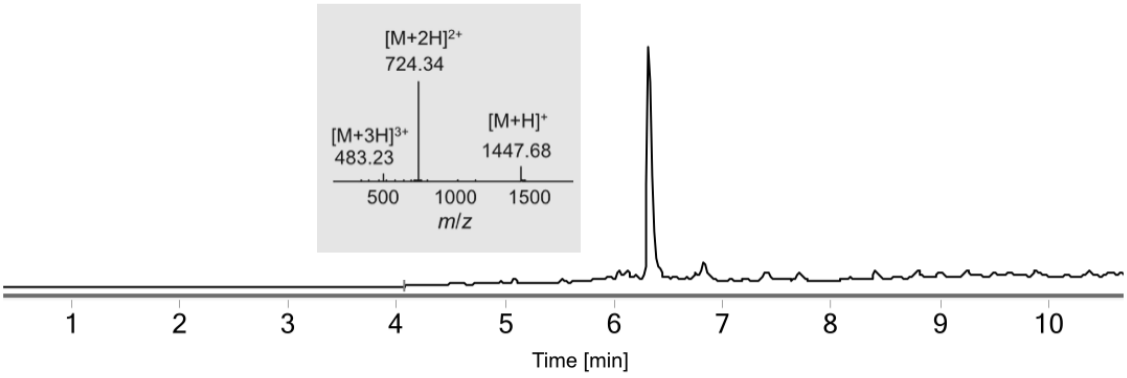
LC-MS trace of peptide 5 (Calculated Mass: 1393.62)



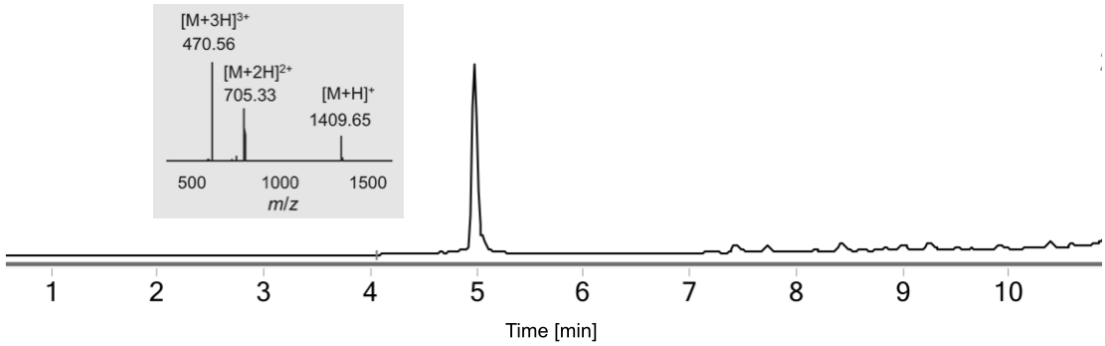
LC-MS trace of crude peptide 10 (Calculated Mass: 1550.66)



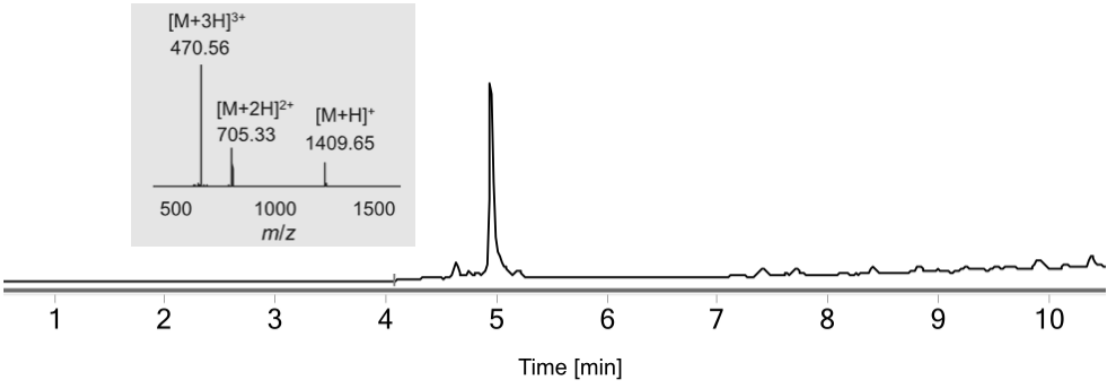
LC-MS trace of crude peptide 15 (Calculated Mass: 1446.67)



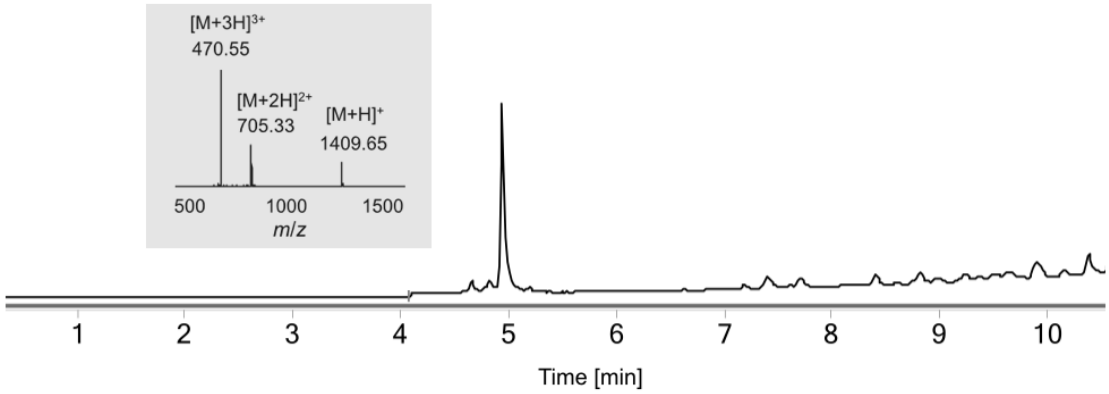
LC-MS trace of peptide 17 (Calculated Mass: 1408.63)



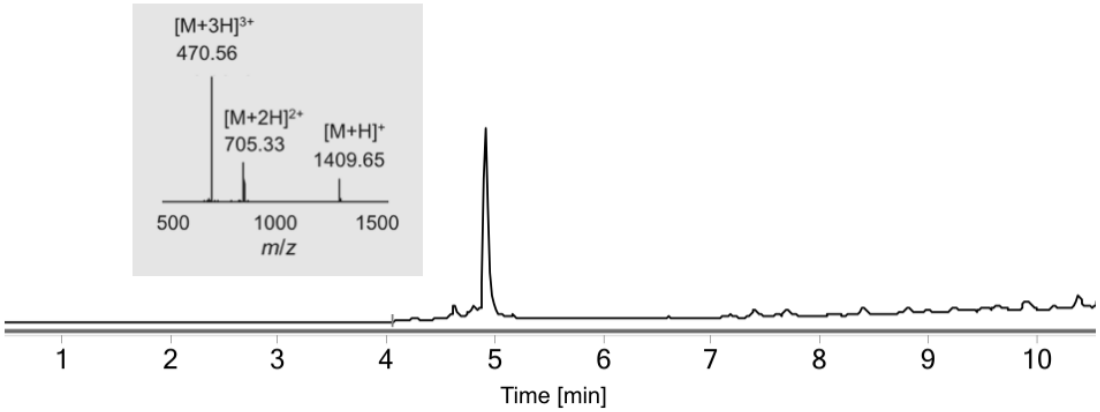
LC-MS trace of peptide 18 (Calculated Mass: 1408.63)



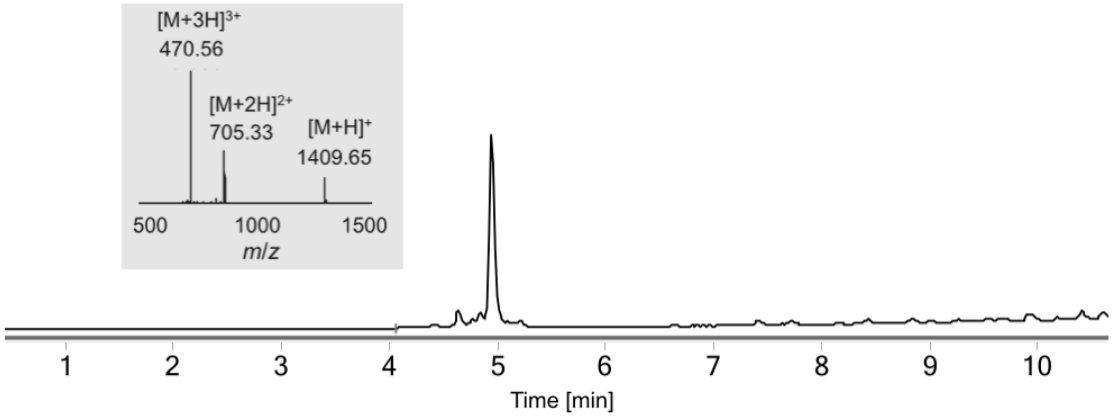
LC-MS trace of peptide 19 (Calculated Mass: 1408.63)



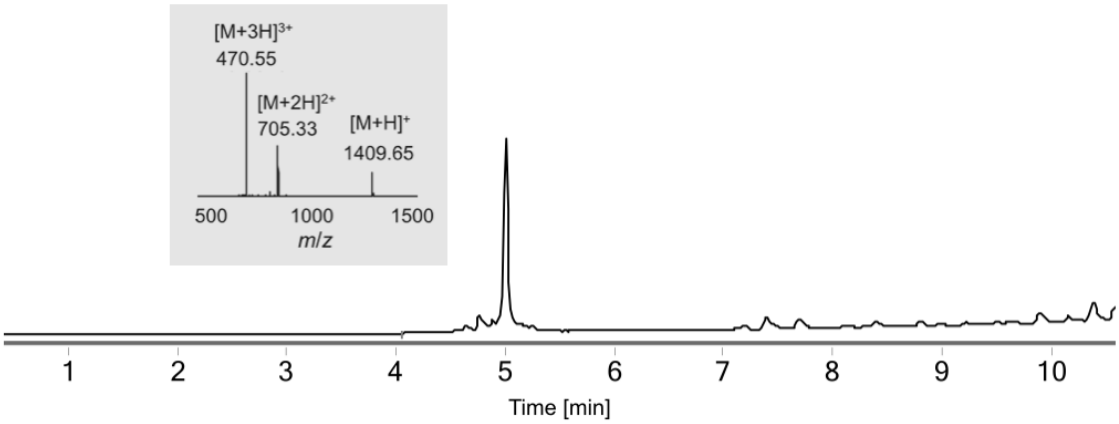
LC-MS trace of peptide 20 (Calculated Mass: 1408.63)



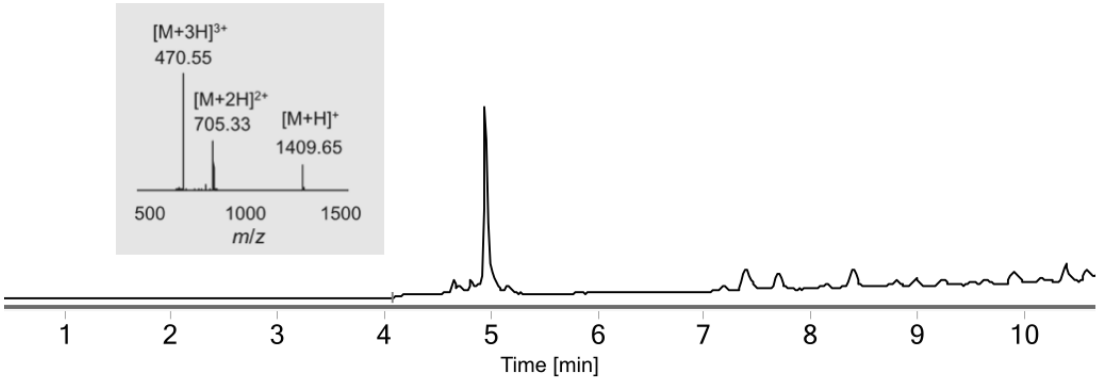
LC-MS trace of peptide 21 (Calculated Mass: 1408.63)



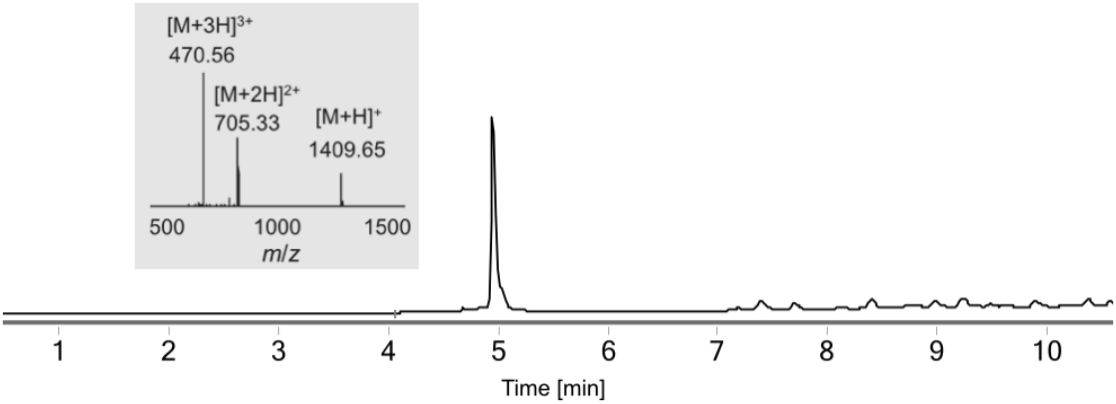
LC-MS trace of peptide 22 (Calculated Mass: 1408.63)



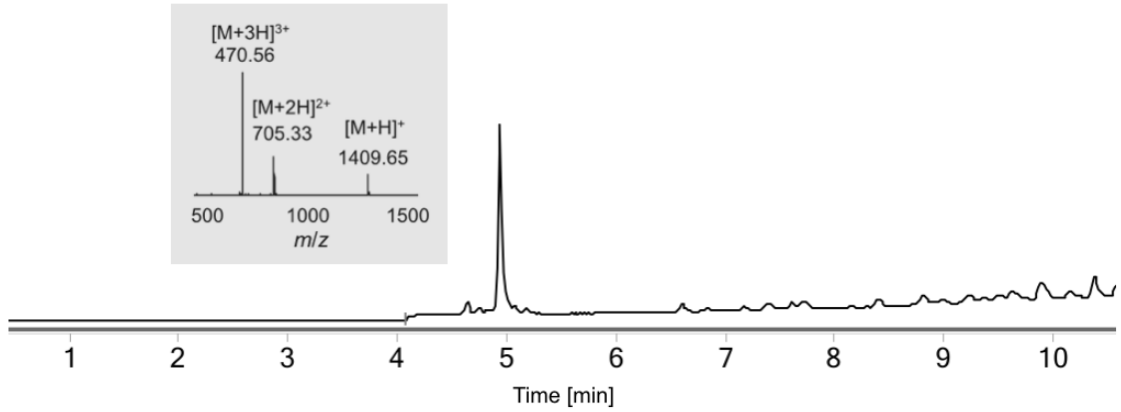
LC-MS trace of peptide 23 (Calculated Mass: 1408.63)



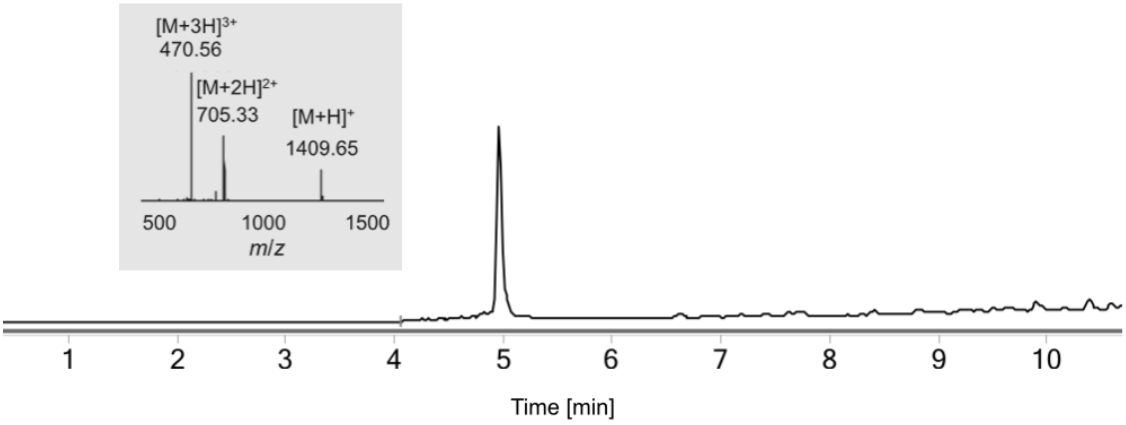
LC-MS trace of peptide 24 (Calculated Mass: 1408.63)



LC-MS trace of peptide 25 (Calculated Mass: 1408.63)

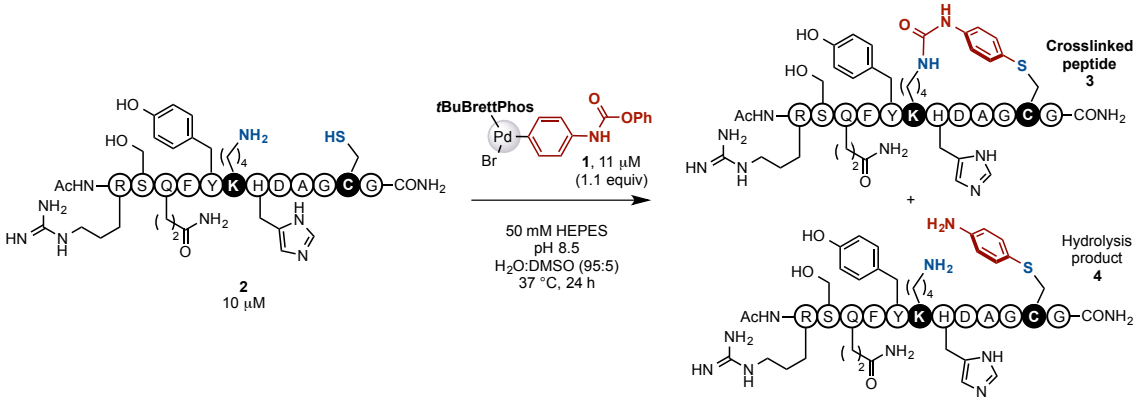


LC-MS trace of peptide 26 (Calculated Mass: 1408.63)



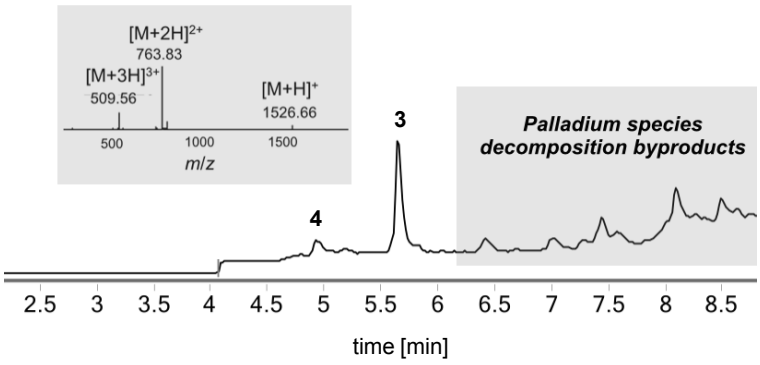
4. Crosslinking with model peptide

Experimental procedure for crosslinking of 2



Peptide **2** (4 μL, 150 μM in water), H_2O (47 μL), DMSO (1.9 μL), and HEPES buffer (6 μL, 500 mM, pH 8.5) were combined in a 0.6 mL plastic Eppendorf tube and the resulting solution was mixed in a vortex for 10 s. A solution of the palladium complex **1** (1.1 μL, 600 μM in DMSO) was added in one portion, the reaction tube was vortexed to ensure proper reagent mixing and let stand at 37 °C for 24 h. The reaction was quenched by the addition of 3/50/50 (v/v/v) TFA/MeCN/H_2O (60 μL), then the mixture was analyzed by LC-MS.

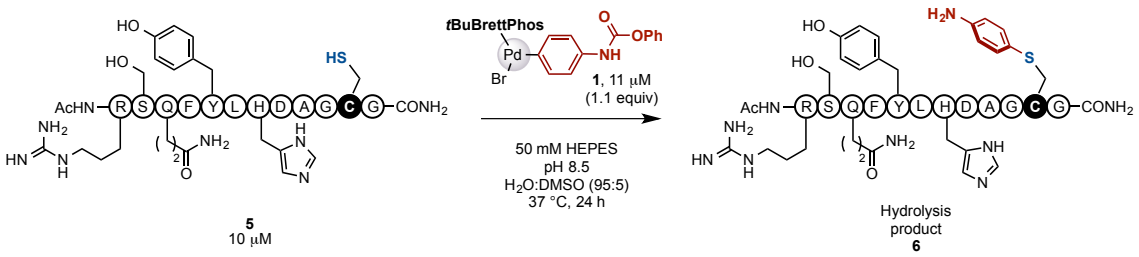
LC-MS trace of the crude reaction mixture



Final concentrations of the reaction before quenching:

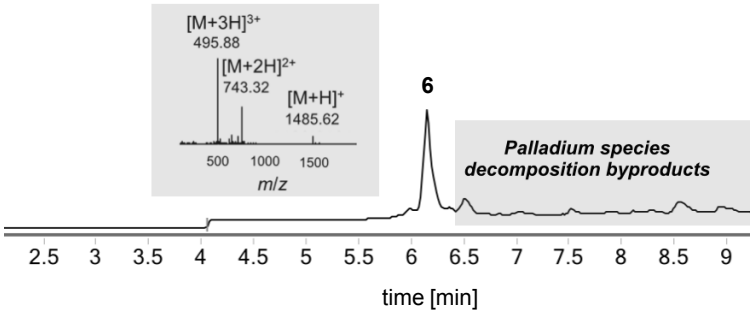
Peptide **2** – 10 μM, Pd-complex **1** – 11 μM, HEPES buffer – 50 mM, DMSO:H_2O = 5:95.

Crosslinking of Lys to Leu mutation (peptide 5) under the optimized conditions

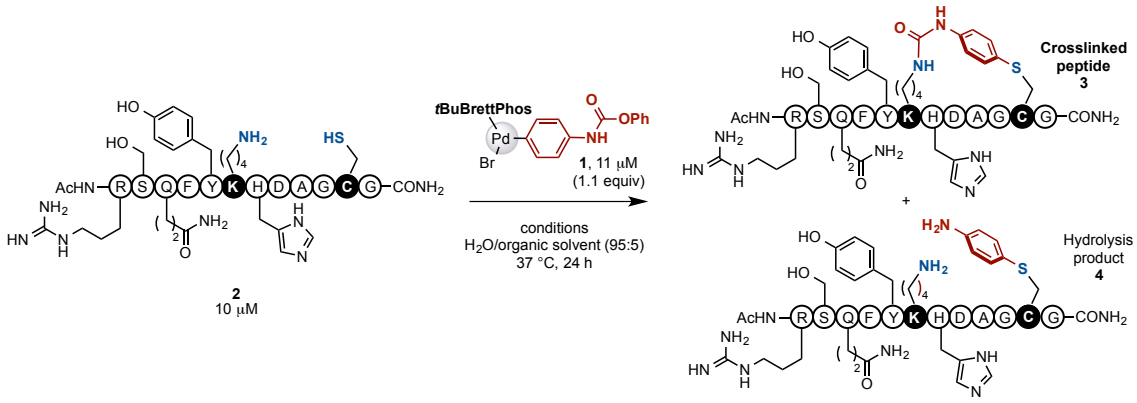


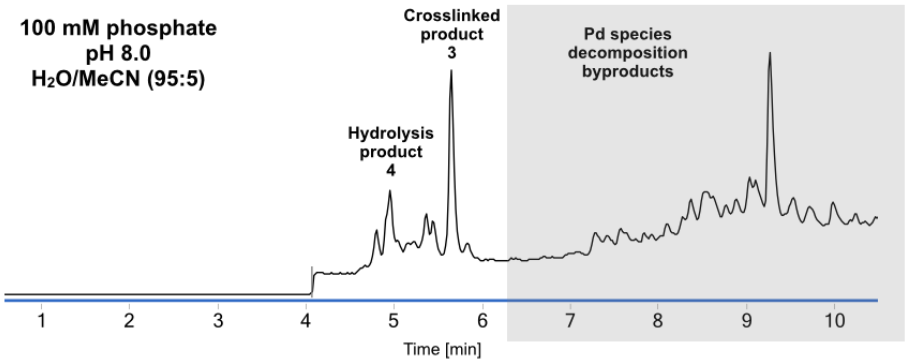
The crosslinking reaction of **5**, the analog of peptide **2** in which the lysine residue of **2** was replaced by leucine, with the palladium complex was investigated to check the chemoselectivity of this reaction. As a result, this reaction afforded the corresponding hydrolysis product **6** in full conversion, which suggested that the crosslinking proceeded between cysteine-lysine residues with high selectivity.

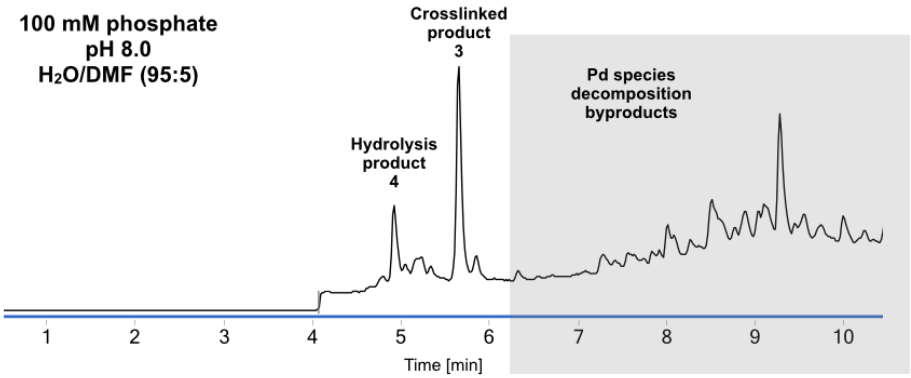
LC-MS trace of the reaction crude mixture

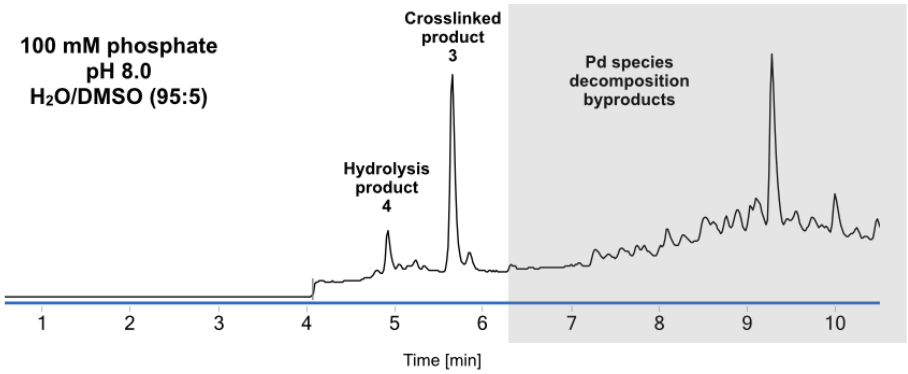


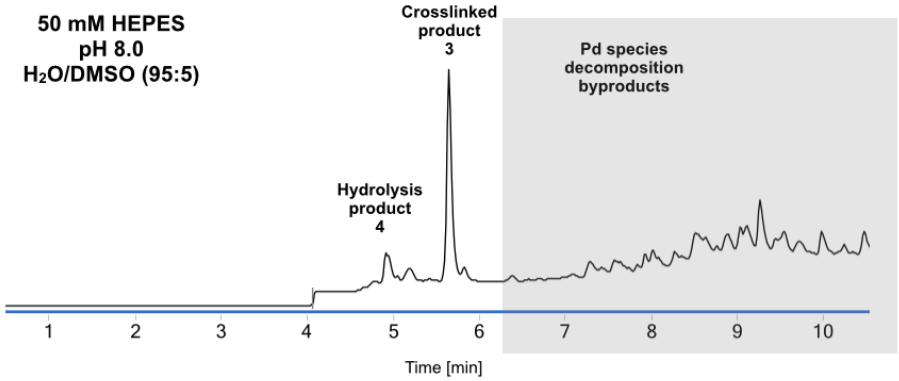
Optimization study on the reaction conditions

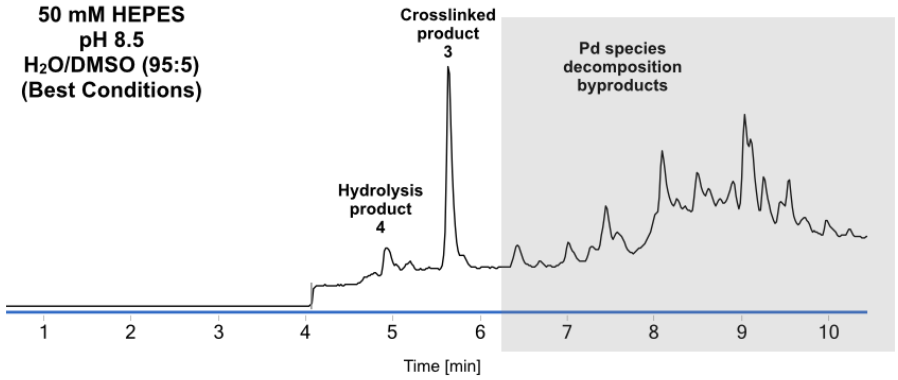







100 mM phosphate
pH 8.0
H2O/DMSO (95:5)
Crosslinked product 3
Hydrolysis product 4
Pd species decomposition byproducts
1
2
3
4
5
6
7
8
9
10
Time [min]


50 mM HEPES
pH 8.0
H2O/DMSO (95:5)
Crosslinked product 3
Hydrolysis product 4
Pd species decomposition byproducts
1
2
3
4
5
6
7
8
9
10
Time [min]

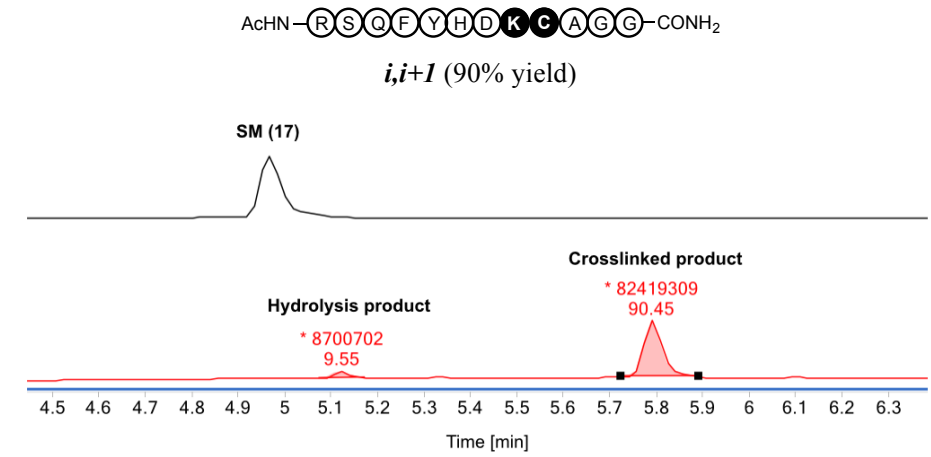

50 mM HEPES
pH 8.5
H2O/DMSO (95:5)
(Best Conditions)
Crosslinked product 3
Hydrolysis product 4
Pd species decomposition byproducts
1
2
3
4
5
6
7
8
9
10
Time [min]

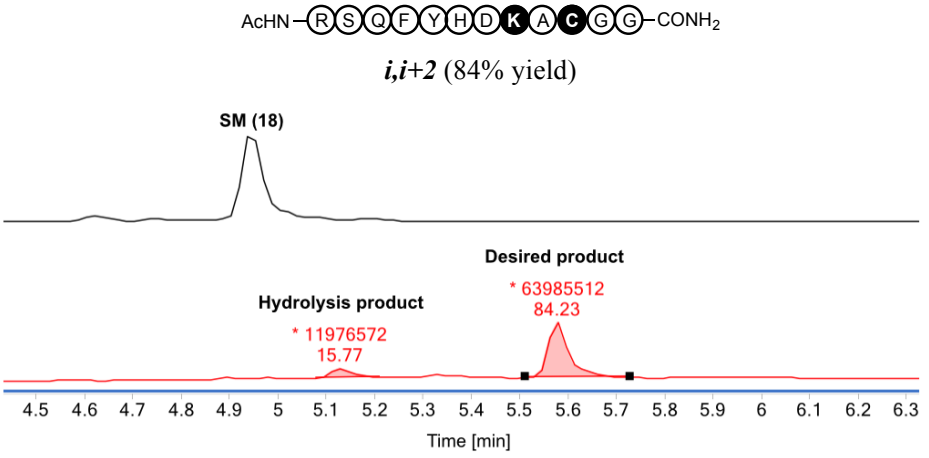
5. Tuning the size of the crosslinked peptide

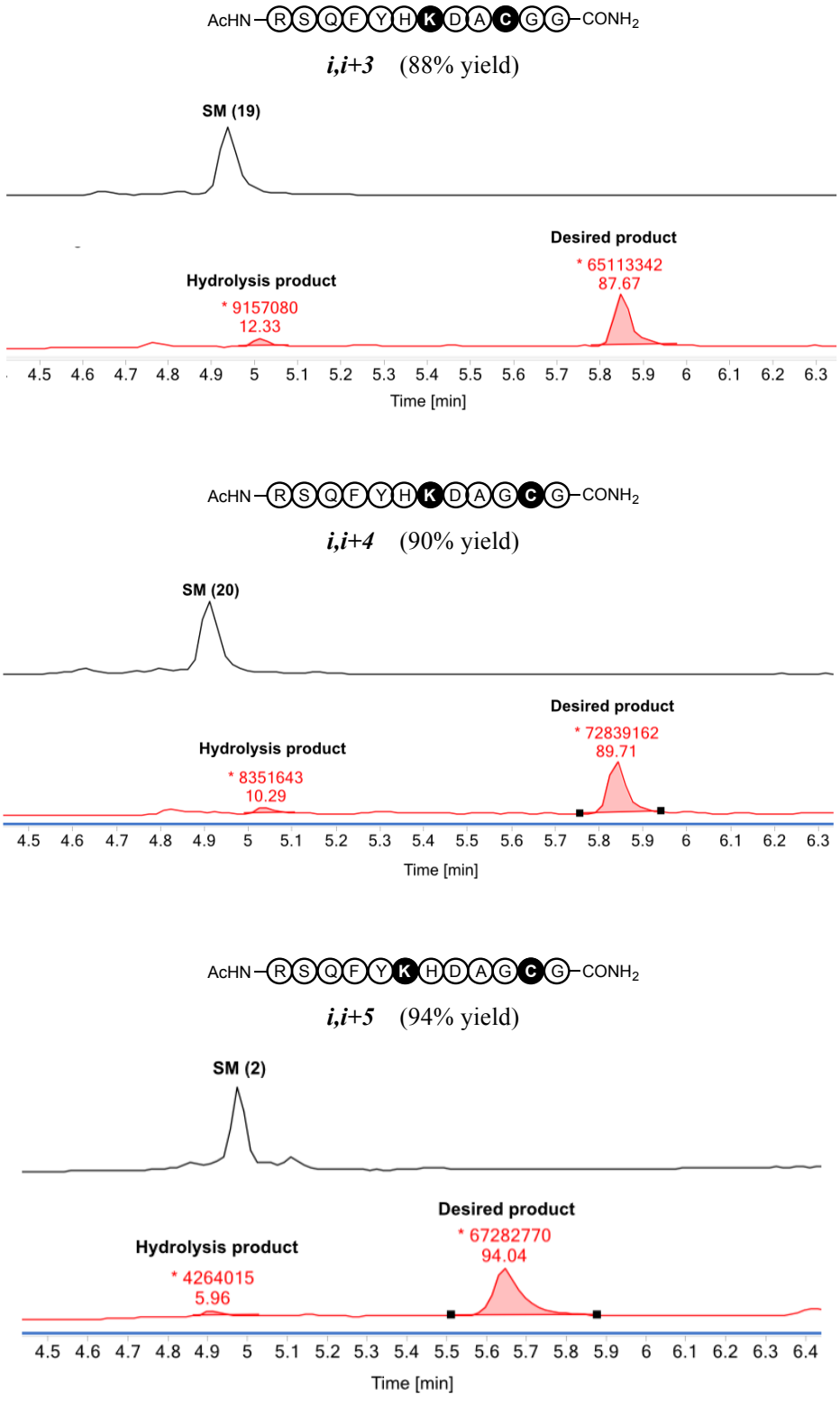
Determination of reaction yield by LC-MS

The reaction yield related to the intramolecular crosslinked peptide was determined by integrating the TIC spectra. First, using Agilent MassHunter software package, the peak area for all relevant peptidic species on the chromatogram were integrated. The yield was calculated as following: yield (%) = $P_t/(P_t + H_t + S_t)$ where P_t is the peak area of the corresponding crosslinked product at time t, H_t is the peak area of the corresponding hydrolysis byproduct at time t and S_t is the peak area of the remaining peptide substrate at time t.

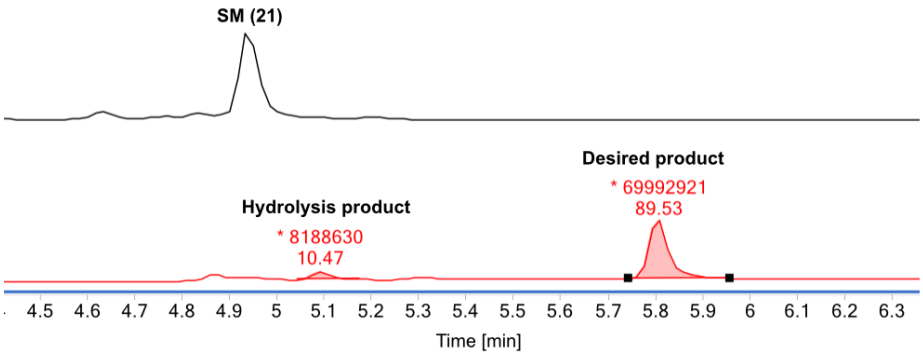
LC-MS trace of the crosslinking reaction of peptide analogs of 2 by varying the relative position of cysteine and lysine residues (from i,i+1 to i,i+11)



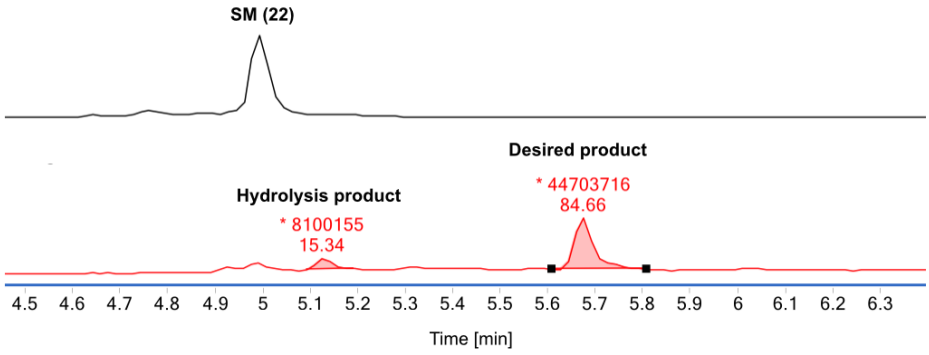



AcHN–RSQFYHKDACGG–CONH2
i,i+3 (88% yield)
SM (19)
Desired product
* 65113342
87.67
Hydrolysis product
* 9157080
12.33
4.5 4.6 4.7 4.8 4.9 5 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6 6.1 6.2 6.3
Time [min]
AcHN–RSQFYHKDAGCG–CONH2
i,i+4 (90% yield)
SM (20)
Desired product
* 72839162
89.71
Hydrolysis product
* 8351643
10.29
4.5 4.6 4.7 4.8 4.9 5 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6 6.1 6.2 6.3
Time [min]
AcHN–RSQFYKHDAGCG–CONH2
i,i+5 (94% yield)
SM (2)
Desired product
* 67282770
94.04
Hydrolysis product
* 4264015
5.96
4.5 4.6 4.7 4.8 4.9 5 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6 6.1 6.2 6.3 6.4
Time [min]

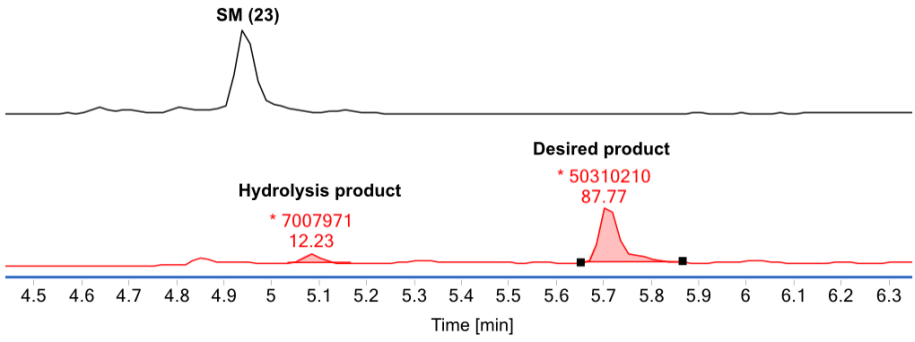
i,i+6 (90% yield)



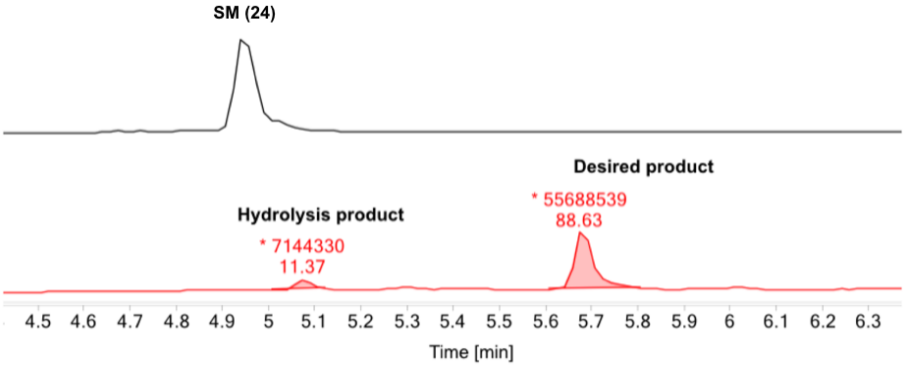
i,i+7 (85% yield)



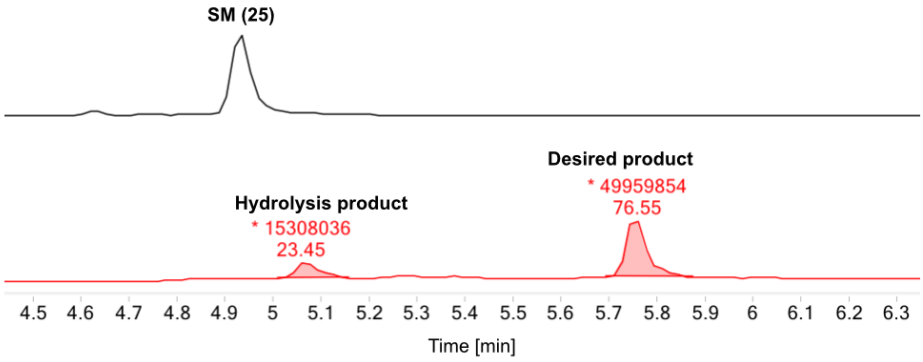
i,i+8 (88% yield)



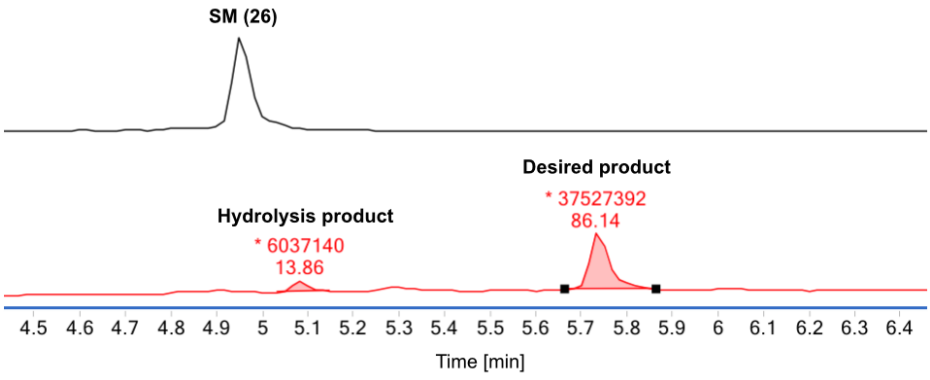
i,i+9 (89% yield)



i,i+10 (77% yield)



i,i+11 (86% yield)



6. Study of the stability of the crosslinked peptide

Stability evaluation in the presence of base, acid or an external thiol nucleophile

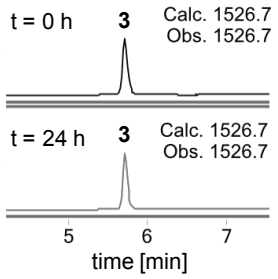
Intramolecular crosslinked peptide **3** was pre-dissolved in water in a plastic Eppendorf tube to make a 1 mM stock solution used in the stability evaluation experiments. In separate Eppendorf tubes charged with peptide **3** and the reagent or reaction conditions were allowed to stand for 24 h. Then the individual reactions were quenched with a solution of 50% **A** : 50% **B** : 0.1% TFA and the resulting samples were analyzed by LC-MS. The conditions for each experiment are shown in below.

Basic conditions:

Stability test reagent: K_2CO_3 (1 µL, 50 mM in H_2O);

Final conditions before quenching: 100 µM peptide, 500 µM K_2CO_3; 24 h at rt.

The LC-MS shows no degradation after 24 h.

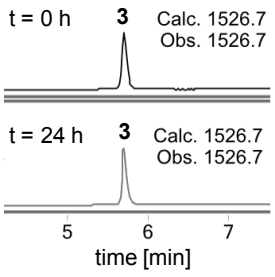


Acidic conditions:

Stability test reagent: HCl (1 µL, 1 M in H_2O);

Final conditions before quenching: 100 µM peptide, 10 mM HCl, 24 h at rt.

The LC-MS shows no degradation after 24 h.

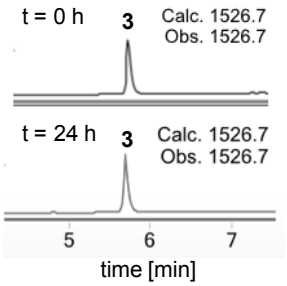


Oxidative conditions:

Stability test reagent: Oxygen (air)

Final conditions before quenching: 100 µM peptide, 0.1M Tris, pH 7.5, 24 h at 37 °C.

The LC-MS shows no degradation after 24 h.

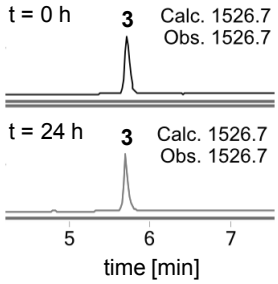


Presence of external thiol nucleophile: GSH:

Stability test reagent: Glutathione (1 µL, 50 mM in 1M Tris; pH 7.5);

Final conditions before quenching: 100 µM peptide, 500 µM GSH, 0.1M Tris, pH 7.5; 24 h at rt.

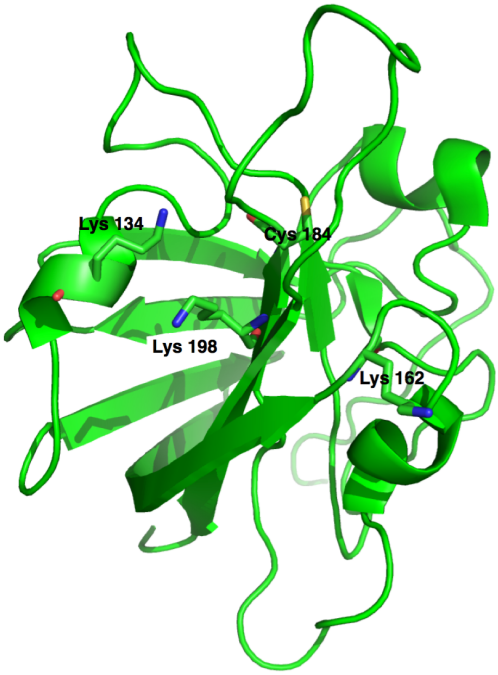
The LC-MS shows no degradation after 24 h.



7. Intramolecular crosslinking of SrtA*

The X-ray crystal structure (PDB ID 2KID) of wild type SrtA shows that cysteine184 is relatively close to lysine 134, lysine 162 and lysine 198 (Figure S1).

Figure S1. X-Ray crystal structure analysis of wild type SrtA.

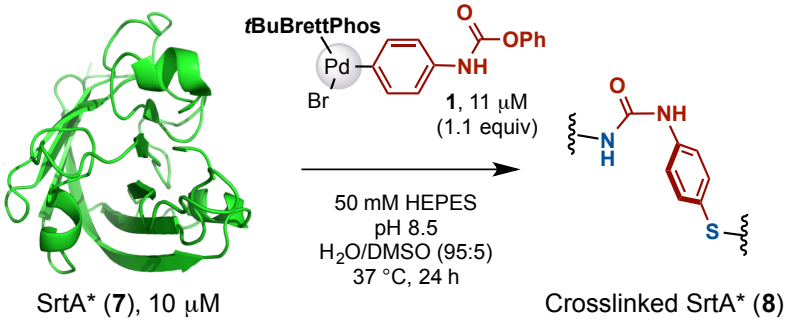


Sortase A P94S/D160N/K196T [SrtA* (**7**)], which was used in this study, was expressed as previously reported.[1] The full sequence of the SrtA*(**7**) is shown below:

ASMTGGQQMGRDPNSQAKPQIPKDKSKVAGYIEIPDADIKEPVYPGPATSEQLNRGVSFAEE
NESLDDQNISIAGHTFIDRPNYQFTNLKAAKKGSMVYFKVGNETRKYKMTSIRNVKPTDVE
VLDEQKGKDKQLTLITCDDYNEKTGVWETRKIFVATEVKLEHHHHHH

The cysteine 184 in the SrtA* is highlighted.

Experimental procedure for crosslinking of SrtA*



SrtA* (**7**) (4 μL, 150 μM in aqueous buffer), H_2O (47 μL), DMSO (1.9 μL), and the HEPES buffer (6 μL, 500 mM, pH 8.5) were combined in a 0.6 mL plastic Eppendorf tube and the resulting solution was vortexed for 10 s. A solution of palladium complex **1** (1.1 μL, 600 μM in DMSO) was added in one portion, the reaction tube was vortexed to ensure proper reagent mixing and left at 37 °C for 24 h. The reaction was quenched by the addition of 3/50/50 (v/v/v) TFA/MeCN/H_2O (60 μL), then the mixture was analyzed by LC-MS.

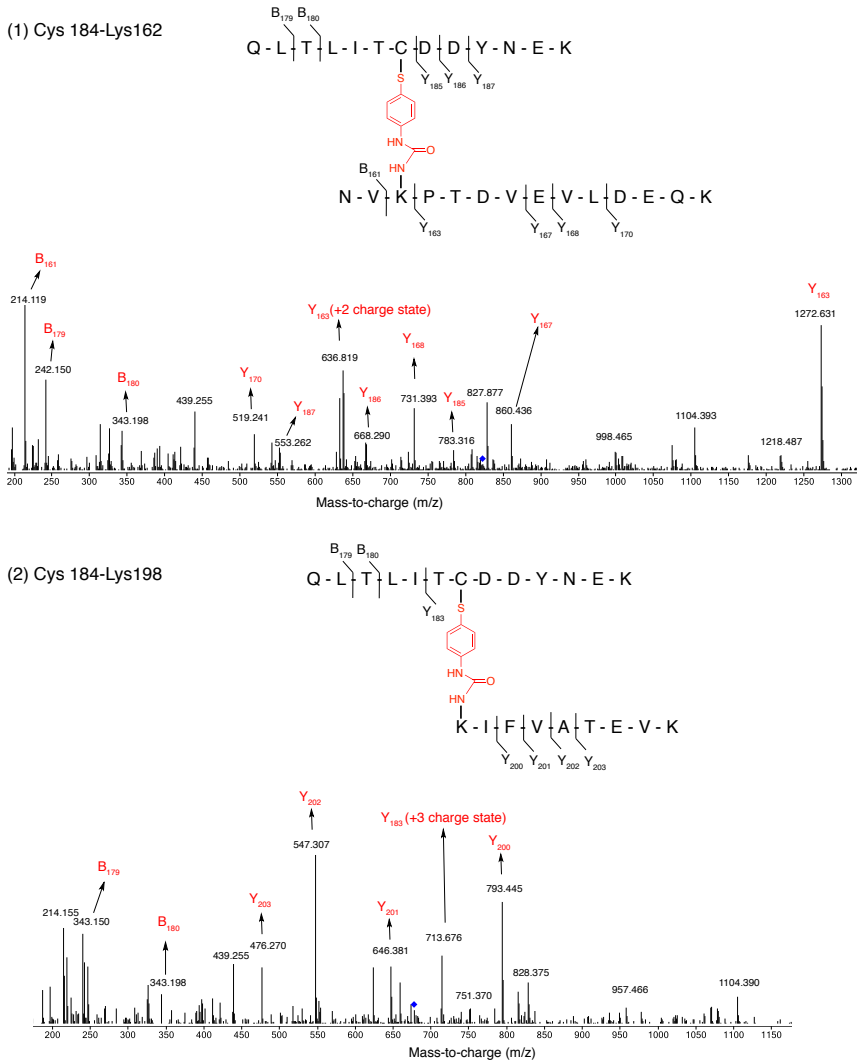
Final concentrations of the reaction before quenching:
SrtA* (**7**) – 10 μM, Pd-complex **1** – 11 μM, HEPES buffer – 50 mM, DMSO:H_2O = 5:95.

Determination of the crosslinking sites in SrtA* crosslinking reaction

To determine the site of crosslinking, the reaction mixture was digested by trypsin. SrtA* reaction mixture (20 μL) was mixed with NH_4HCO_3 (1.3 μL, 1.5 M), Trypsin stock solution (2 μL 2 mg/mL) and H_2O (17 μL). The reaction was heated at 37 °C for 3 h. Then 4 μL buffer containing 2 M phosphate, 200 mM TCEP at pH 8.0 was added to the reaction mixture for 15 min to reduce the disulfides. The reaction mixture was finally quenched by the addition of water containing 3% TFA (20 μL) and analyzed by LC-MS/MS (Agilent 6550 ESI-Q-TOF mass spectrometer).

Assuming all of the 18 lysines could react with Cys184, we calculated the mass for the digested fragments containing Lys-Cys crosslinking for the cases in which each lysine residue was labeled. Only the masses corresponding to Lys162-Cys184 and Lys198-Cys184 crosslinking was observed. As a negative control, these two fragments are not observed in the SrtA* only digestion experiment.

Figure S2. MS/MS fragmentation of the trypsin digested crosslinked peptides derived from the SrtA* (7) crosslinking reaction.

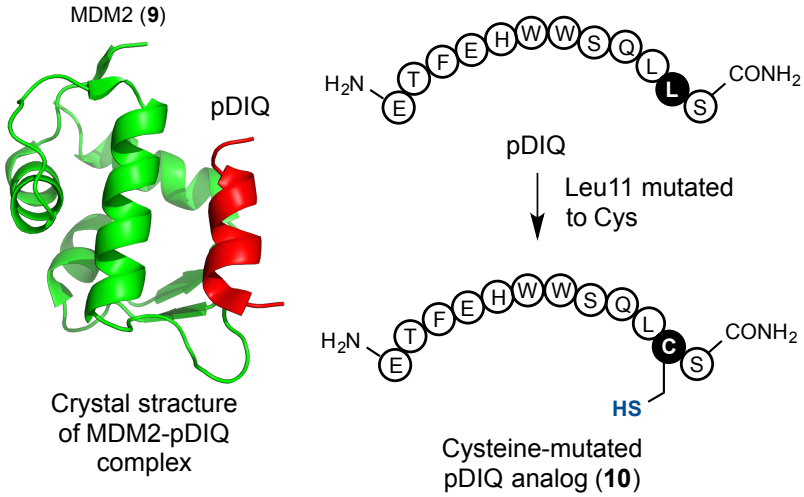


The peak at 822.15 and 677.35 are parent ions correspond to the crosslinked peptide (+ 4H) after digestion. B_n indicates the b ion from *N*-terminal of the crosslinked peptide to residue n. Y_n indicates the y ion from the residue n to the C-terminal of the crosslinked peptide.

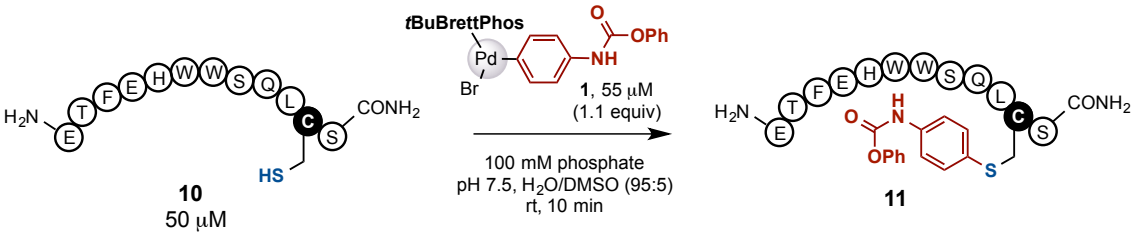
8. Intramolecular crosslinking of MDM2

MDM2 protein (**9**) binds to pDIQ peptide with high affinity. Therefore, we synthesized the pDIQ analog **10** in which the leucine residue is replaced by a cysteine residue. To incorporate a phenyl carbamate group into **10**, we carried out the arylation reaction of the peptide substrate **10**.

Design of pDIQ analog for cysteine arylation



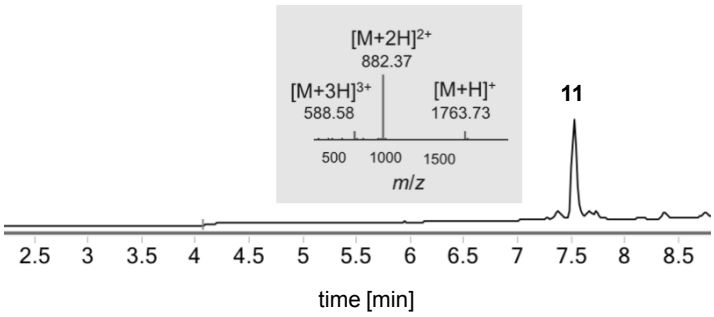
Experimental procedure for arylation of pDIQ analog 10



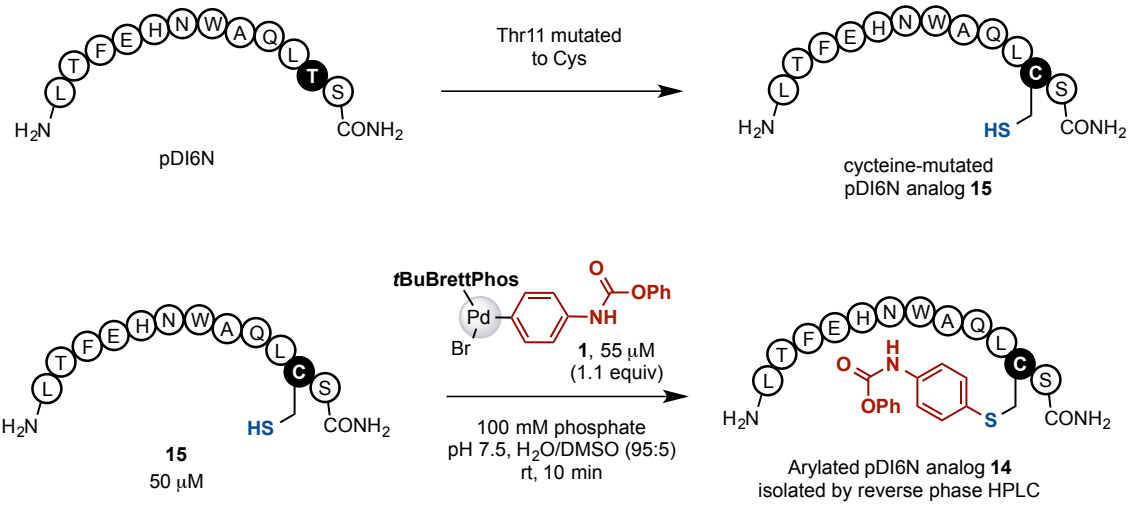
A 100 mL round bottom flask, equipped with a stir bar was charged with peptide **10** (690 μL, 2 mM in water), H_2O (23.5 mL), phosphate buffer (540 μL, 1 M, pH 7.5). A solution of the palladium complex **1** (2.25 mL, 600 μM in DMSO) was then added and stirred at room temperature for 10 min. The reaction was filtered and purified by RP-HPLC. The solvent compositions for reverse phase HPLC (RP-HPLC) purification were water with 0.1% TFA (solvent **C**) and acetonitrile with 0.1% TFA (solvent **D**). The crude mixture was directly injected into an Agilent 1260 Infinity Automated LC/MS Purification System, with a semi-preparative Reverse Phase-HPLC column

(Agilent Zorbax 300SB C_3 column: 21.2 x 250 mm, 7 µm, linear gradient: 5-65% **D** over 82 min, flow rate: 4 mL/min). The purity of fractions was confirmed by LC-MS analysis. The fractions containing pure cyclized peptide were combined and lyophilized.

LC-MS trace of arylated pDIQ binder analog 11

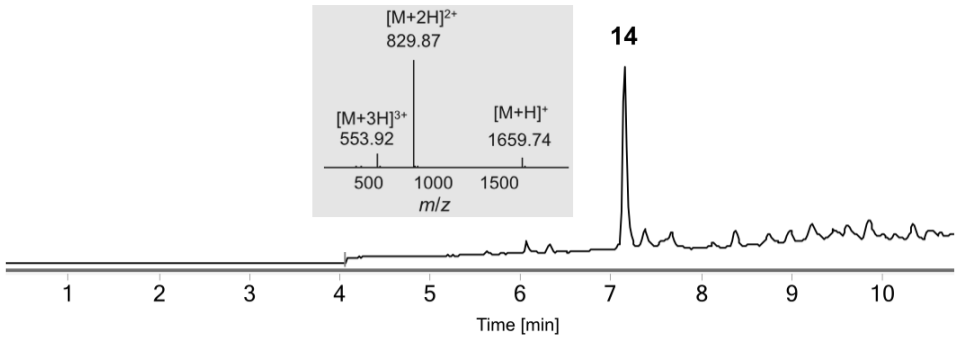


Design of pDI6N analog 15 and experimental procedure for arylation of pDI6N analog 15

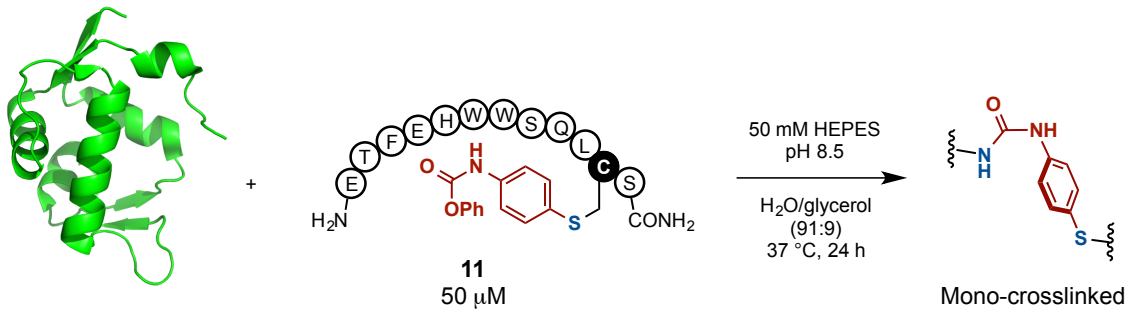


The arylation of pDI6N analog **15** was conducted according to the general procedure as described above. The crude peptide **15** solution was directly injected into the semi-preparative RP-HPLC (Agilent Zorbax 300SB C18 column: 21.2 x 250 nm, 7 µm, liner gradient: 5-65% **B** over 82 min, flow rate: 5 mL/min). Each HPLC fraction was analyzed by mass-directed preparative LC-MS, combined, and lyophilized.

LC-MS trace of arylated pDIQ binder analog 14

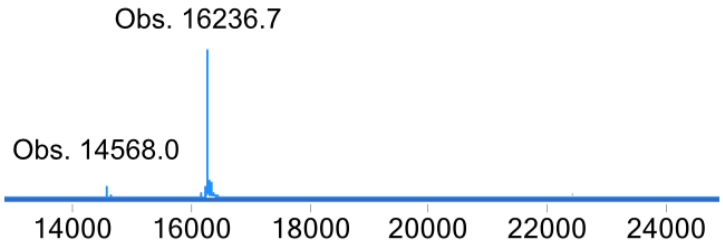


Experimental procedure for crosslinking of MDM2 (9) with 11



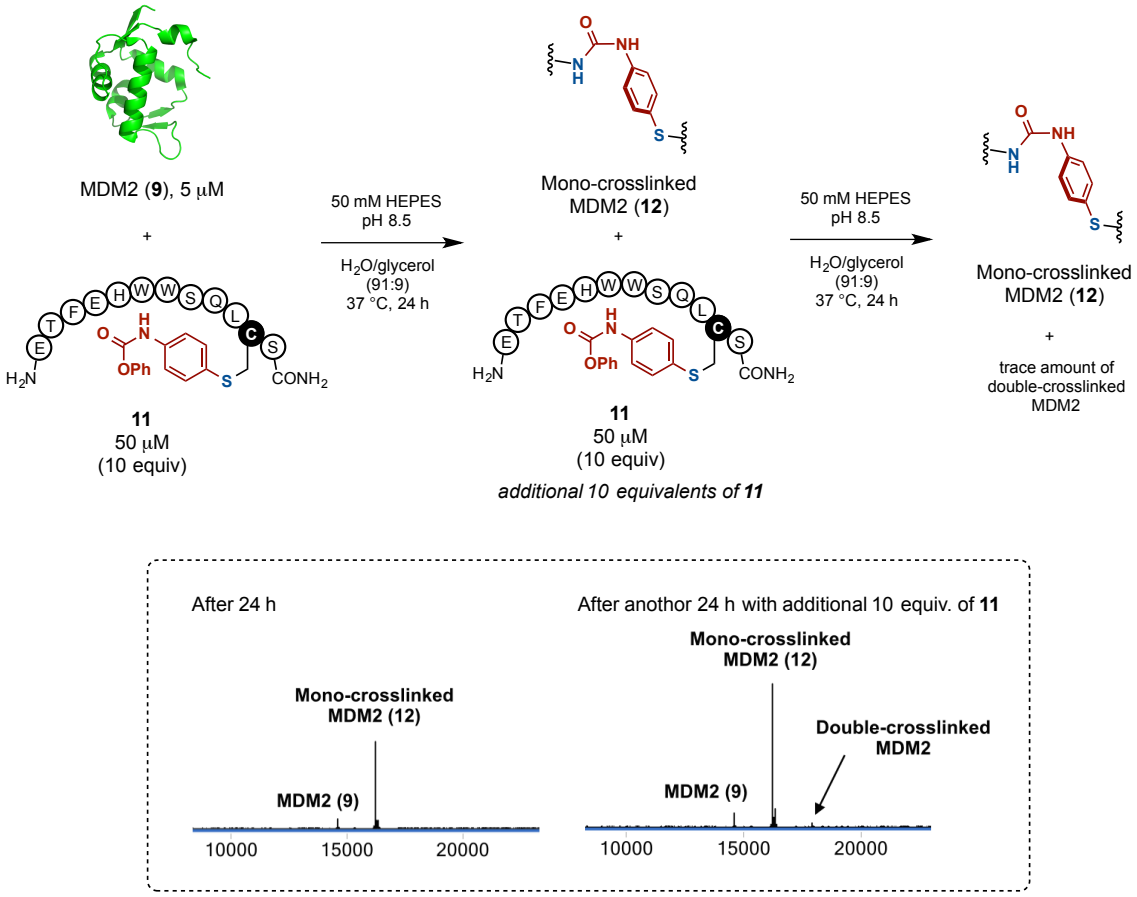
MDM2 (**9**) [3.5 μL, 14 μM in water/glycerol (3:1) buffer], H_2O (4.5 μL), and the HEPES buffer (1.0 μL, 500 mM, pH 8.5) were combined in a 0.1 mL plastic Eppendorf tube. A stock solution of the arylated pDIQ analog **11** (1.1 μL, 600 μM in water) was added in one portion, the reaction tube was vortexed for short time to ensure proper reagent mixing and left at 37 °C for 24 h. After 24 h, the reaction mixture was quenched with 0.1/50/50 (v/v/v) TFA/MeCN/H_2O, then the mixture was analyzed by LC-MS.

Deconvoluted mass spectra of the crude reaction mixture of MDM2 (9)

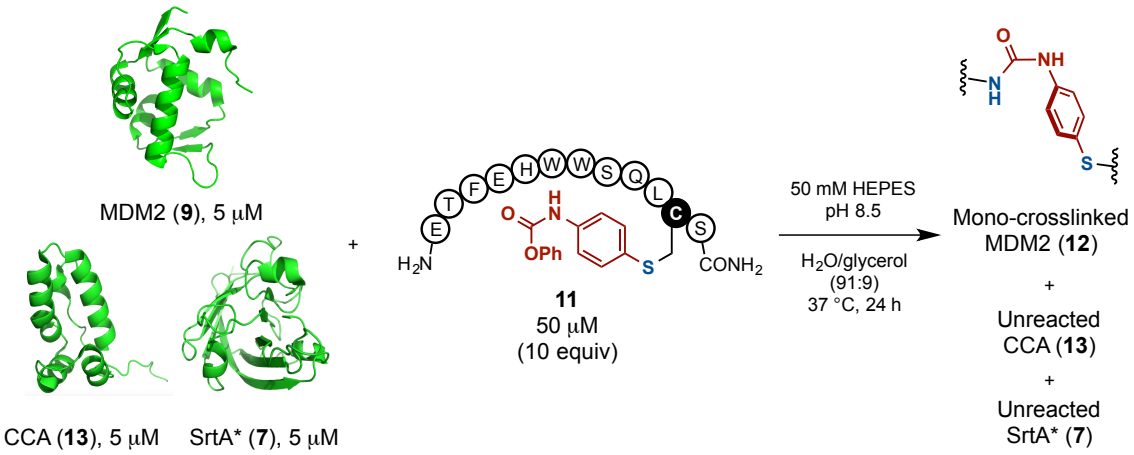


In addition, the mono-crosslinked MDM2 (**12**) was subjected to the crosslinking reaction with an additional 10 equivalents of **11**, and let stand for an another 24 h. Even in this case, only trace amount of the double-crosslinked product was observed (Figure S3), highlighting the high selectivity of this reaction.

Figure S3. The crosslinking reaction of the mono-crosslinked MDM2 (12) with an additional 10 equivalents of 11.

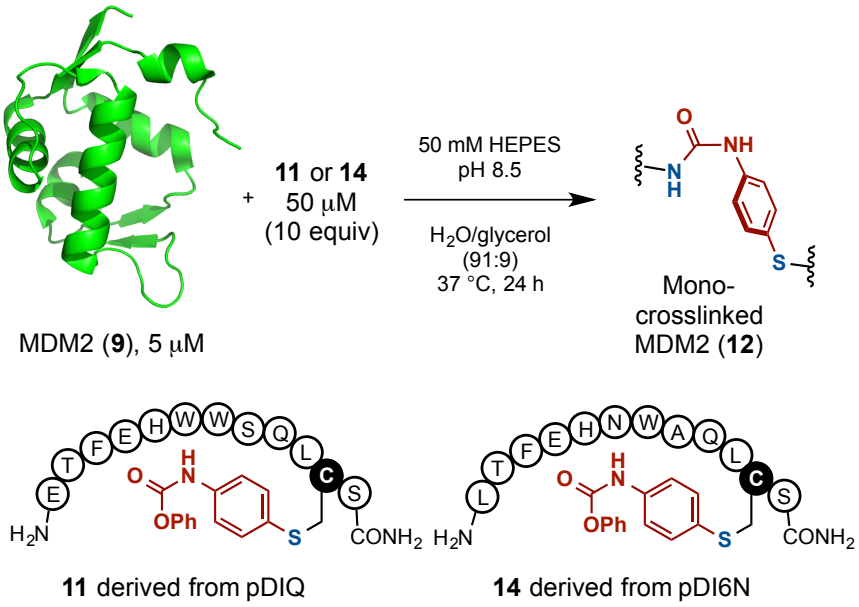


Experimental procedure for crosslinking of MDM2 (9) in protein mixture



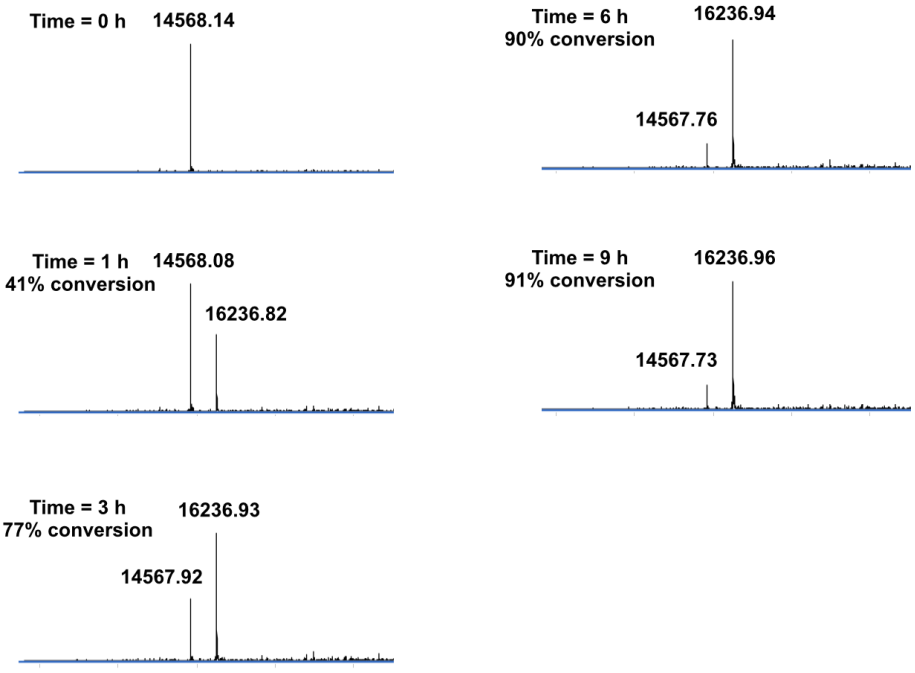
MDM2 (**9**) [7.1 μL, 14 μM in water/glycerol (3:1) buffer], CCA (**13**) (0.5 μL, 200 μM in aqueous buffer), SrtA* (**7**) (0.67 μL, 150 μM in aqueous buffer), arylated pDIQ analog **11** (2 μL, 500 μM in water), H_2O (7.7 μL), and the HEPES buffer (2.0 μL, 500 mM, pH 8.5) were combined in a 0.1 mL plastic Eppendorf tube and the resulting solution was mixed by pipetting the solution up and down (10x). The mixture was allowed to stand at 37 °C for 24 h. After 24 h, the reaction mixture was quenched with 0.1/50/50 (v/v/v) TFA/MeCN/H_2O, then the mixture was analyzed by LC-MS. CCA (C-terminal domain of HIV-1 capsid assembly polyprotein) was expressed as previously reported.[5]

Experimental procedure for the intermolecular crosslinking of MDM2 (9) with the arylated pDIQ analog (11) and the arylated pDI6N analog (14)

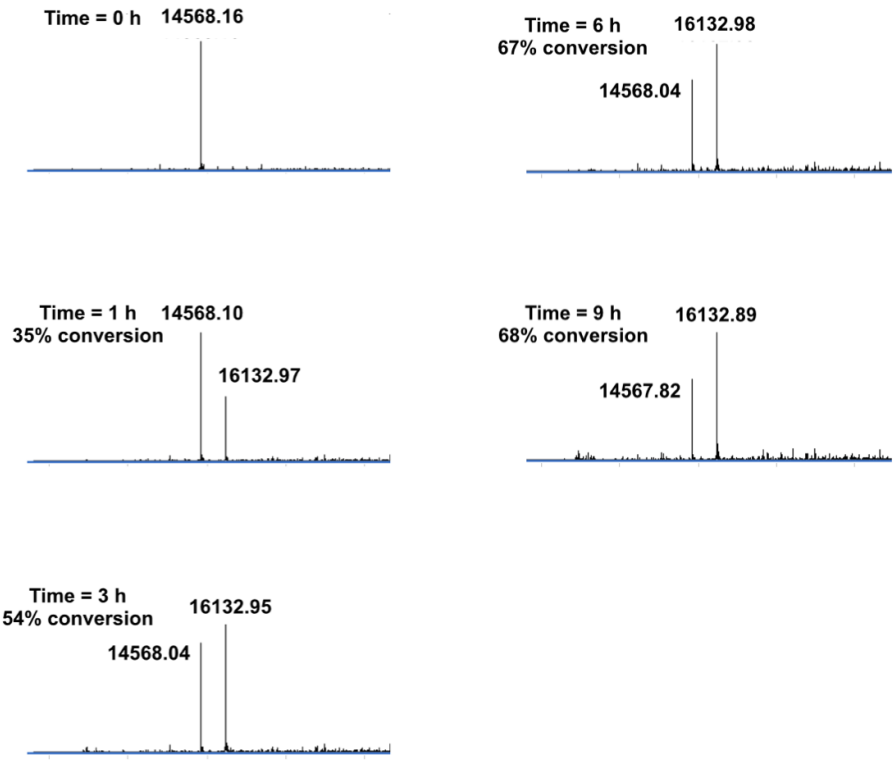


MDM2 (**9**) [3.5 μL, 14 μM in water/glycerol (3:1) buffer], H_2O (4.5 μL), and the HEPES buffer (1.0 μL, 500 mM, pH 8.5) were combined in a 0.1 mL plastic Eppendorf tube. A stock solution of the arylated pDIQ analog **11** (1.0 μL, 600 μM in H_2O) or the arylated pDI6N analog **14** (1.0 μL, 600 μM in H_2O) was added in one portion, the reaction tube was vortexed for a short time to ensure proper reagent mixing and left at 37 °C for 24 h. For each time point (0, 1, 3, 6, 9 h), 1.0 μL of the reaction mixture was diluted with 0.1/50/50 (v/v/v) TFA/MeCN/H_2O (29 μL), then the mixture was analyzed by LC-MS.

Deconvoluted mass spectra of the reaction mixture of MDM2 (9) crosslinking with the arylated pDIQ analog (11)



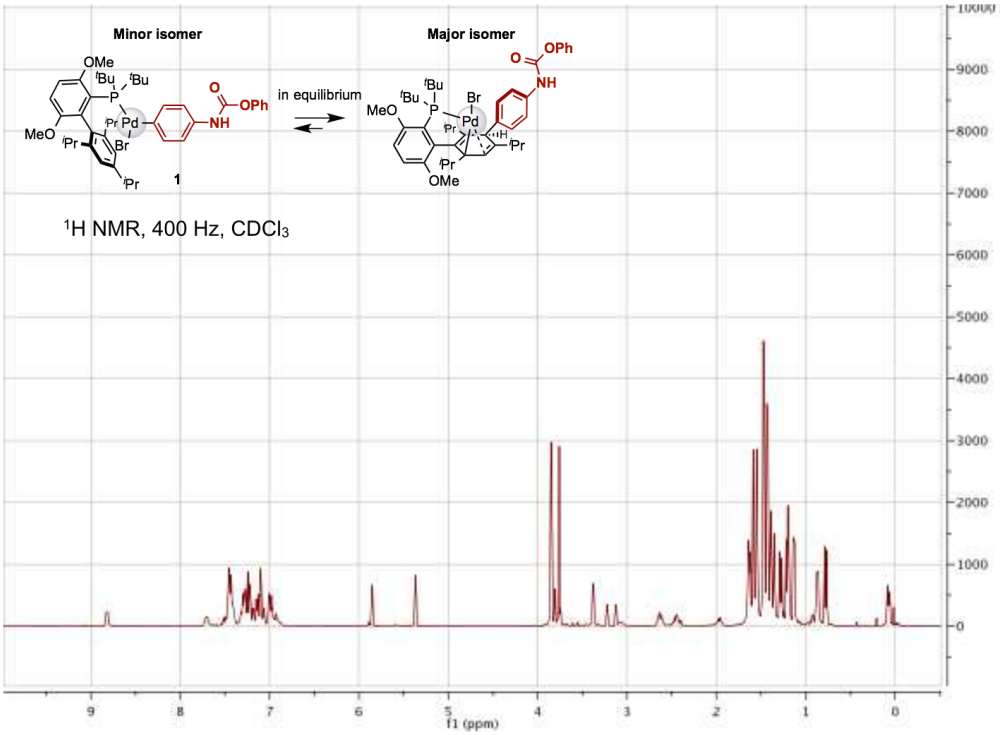
Deconvoluted mass spectra of reaction mixture of MDM2 (9) crosslinking with the arylated pDI6N analog (14)

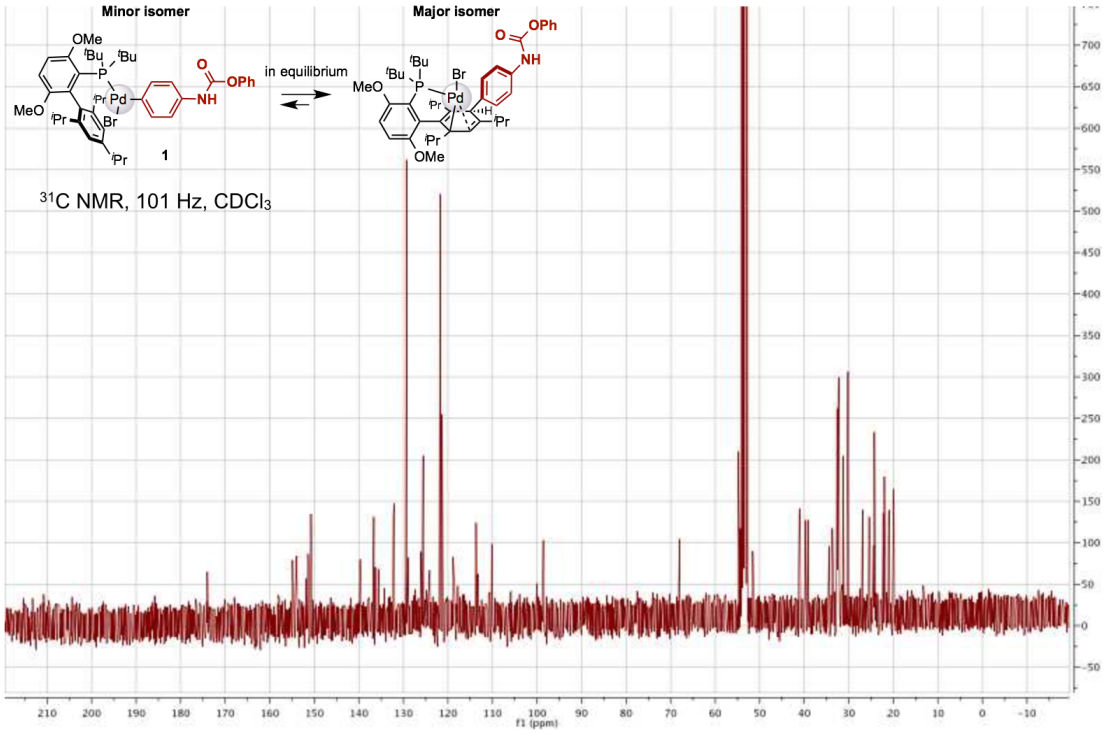


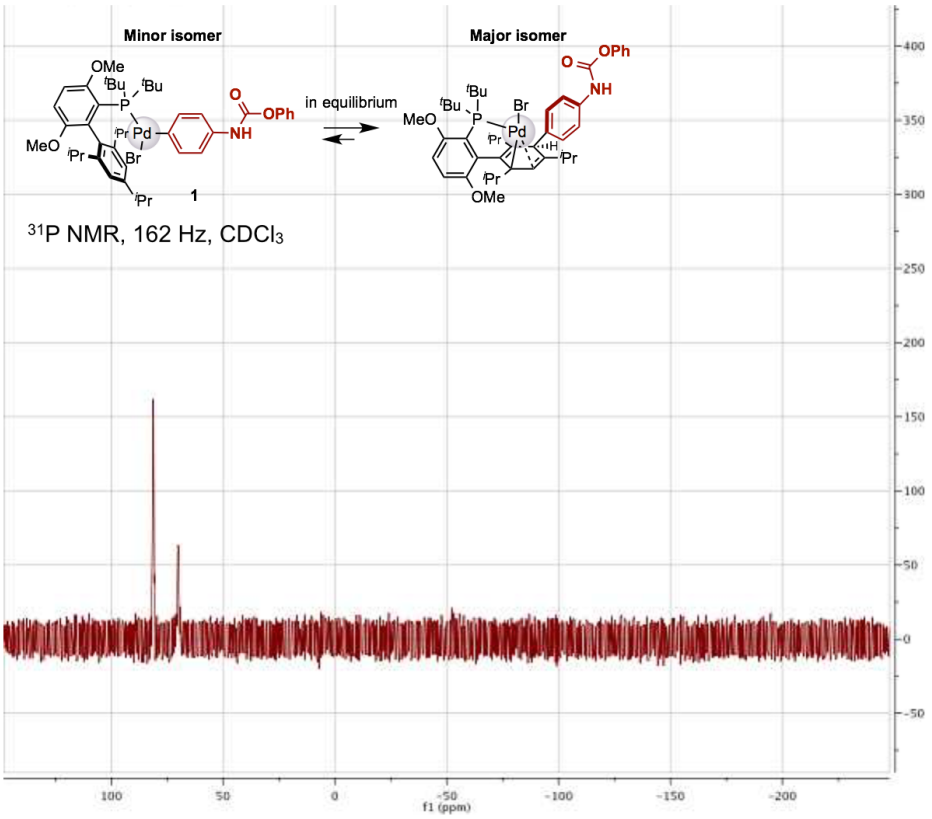
9. References

1. J. J. Ling, R. L. Policarpo, A. E. Rabideau, X. Liao, B. L. Pentelute, *J. Am. Chem. Soc.* **2012**, *134*, 10749–10752.
2. A. A. Vinogradov, Z. N. Choo, K. A. Totaro, B. L. Pentelute, *Org. Lett.* **2016**, *18*, 1226–1229.
3. R. Angelici, *Inorg. Synth.* **1990**, *28*, 346–349.
4. J. R. McAtee, S. E. S. Martin, D. T. Ahneman, K. A. Johnson, D. A. Watson, *Angew. Chem. Int. Ed.* **2012**, *51*, 3663–3667.
5. A. J. Mijalis, D. A. Thomas, M. D. Simon, A. Adamo, R. Beaumont, K. F. Jensen, B. L. Pentelute, *Nat. Chem. Biol.* **2017**, *13*, 464–466.

10. NMR spectra






Minor isomer
Major isomer
in equilibrium
1
31P NMR, 162 Hz, CDCl3
f1 (ppm)