Modification of N-Terminal α-Amine of Proteins *via* Biomimetic *ortho*-quinone-mediated Oxidation

Supporting Information

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1. General information

All organic reactions were carried out in Nitrogen atmosphere. Anhydrous reaction solvent was purchased from suppliers and kept in Sure-seal bottles. All chemical reagents were purchased from Sigma Aldrich. Flash chromatography was performed on silica gel (0.040 – 0.060 mm) manufactured by Qingdao Hailang Inc. Fmoc amino acid building blocks and resins were purchased from GL biochem. The NMR spectra of synthesized alkoxyamine were recorded on a Bruker Advance 500 spectrometer at 300 K. Chemical shifts are reported in parts per million (ppm) and are referenced to solvent residue signals ^{1}H assignment ($CDCl_3$, 7.26 ppm), relative integral, multiplicity (s = singlet, br.s = broad singlet, d = doublet, q = quartet, t = triplet, m = multiplet, etc.) and coupling constants (*J* Hz).[1]

High-performance liquid chromatography-mass spectrometry (HPLC-MS) was performed on a Shimadzu® HPLC-MS 2020 system using LC-2020 separation module. Eluent solvent is H_2O as solvent A and MeCN as solvent B with 0.1% formic acid. High High-resolution ESI mass spectra were obtained on a Waters Vion® IMS QTOF spectrometer equipped with an Acquity® UHPLC separation module.

HPLC separations were performed on a Shimadzu® HPLC system equipped with SPD-20A UV detector and LC-20AR semi-prep system. Analytical HPLC traces were recorded with a Waters® HPLC 2790 separation module equipped with a 2487 UV detector. Eluent solvent used in both two systems is H_2O as solvent A and MeCN as solvent B with 0.1% trifluoroacetic acid.

2. General procedure for Fmoc-SPPS

2.1. Loading of 2-chlorotrityl (2-CTC) resin

2-CTC resin (0.25 g, loading capacity 1.0 mmol/g) was swelled in $SOCl_2$ in DCM solution (2.0% v/v) at 25 °C for 30 min. After quick washing with DCM (5 mL), a solution of Fmoc-AA-OH (1.0 mmol, 4 equiv.), DIPEA (0.35 mL, 2.0 mmol, 8 equiv.) in DMF (4 mL) was added to the resin. The mixture was agitated in reaction vessel for 2 h at 25 °C. After the loading was completed, the resin was washed with DMF (3 × 5 mL), DCM (3 × 5 mL) and DMF (3 × 5 mL).

2.2. Fmoc-loading assay

Once the loading was completed, the Fmoc group was removed by treatment with piperidine in DMF solution (1/4, v/v, 5 mL, 2 × 5 min, 25 °C). The reaction solution was collected in a 25 mL volumetric flask and topped up with piperidine (25 vol% in DMF) solution to 25 mL. The solution was then diluted to a factor of 100 before measuring its absorbance at 301 nm (fulvene-piperidine adduct: ε = 7800 M^{-1} cm^{-1}).[2] The reading was subjected to equation (n / mol = 2500 × Abs / 7800) to estimate the loading of amino acid on target resin.

2.3. Coupling of Fmoc-AA-OH

Fmoc-AA-OH (4.0 equiv.), DIC (4.1 equiv.) and Oxyma (4.2 equiv.) were dissolved in DMF with a final concentration of 0.3 M.[3] The solution was added to the resin and the reaction was agitated at 50 °C for 30 min. After the reaction was completed, the resin was washed with DMF (3 × 5 mL), DCM (3 × 5 mL) and DMF (3 × 5 mL).

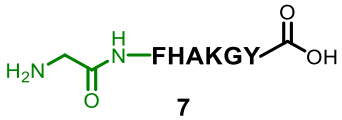
2.4. Fmoc-deprotection

The Fmoc group removal was conducted in piperidine (20 vol%) in DMF solution (5 mL, 2 × 5 min) at 50 °C. After the reaction was completed, the resin was washed with DMF (3 × 5 mL), DCM (3 × 5 mL) and DMF (3 × 5 mL).

2.5. Acidolytic peptide cleavage and deprotection (global deprotection)

After washing the resin with DCM thoroughly (10 × 5 mL), resin-bound peptide was mixed with TFA/*i*-Pr_3SiH/H_2O (18:1:1, v/v/v, 10 mL). The mixture was agitated at 25 °C for 2 h. Once the reaction was completed, the acidic filtrate containing target peptide was collected and concentrated under reduced pressure. The concentrated solution was then added into ice-cold ether (50 mL) and centrifuged. After decanting the organic liquid, the resulting solid was washed further with ether (2 × 50 mL). The crude peptide was dissolved in $MeCN/H_2O$ and purified with RP-HPLC.

2.6. Synthesis and analytical data for model peptide **7** and **10**-**28**



7

Gly peptide **7** was synthesized on 2-CTC resin (0.2 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 0.2 g peptide was obtained as a lyophilized solid (200 μmol, 10%).

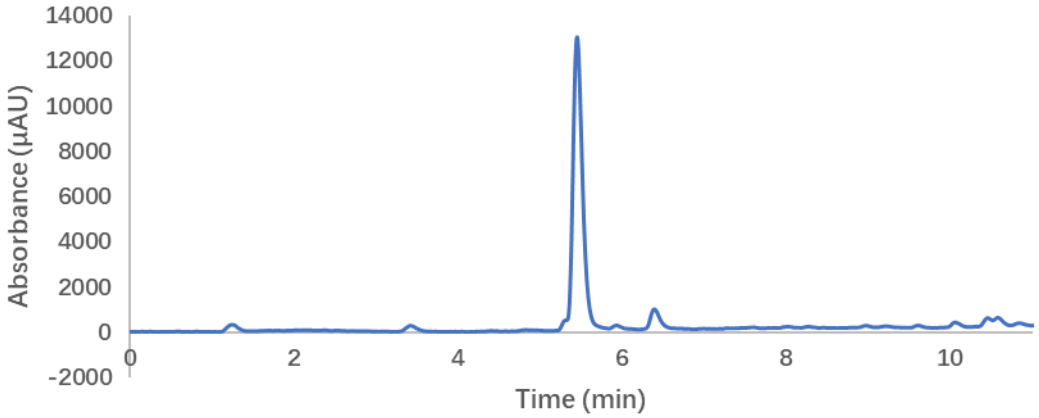


Figure 1: Analytical HPLC-MS trace of purified peptide **7** (t_R = 5.5 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical

column).

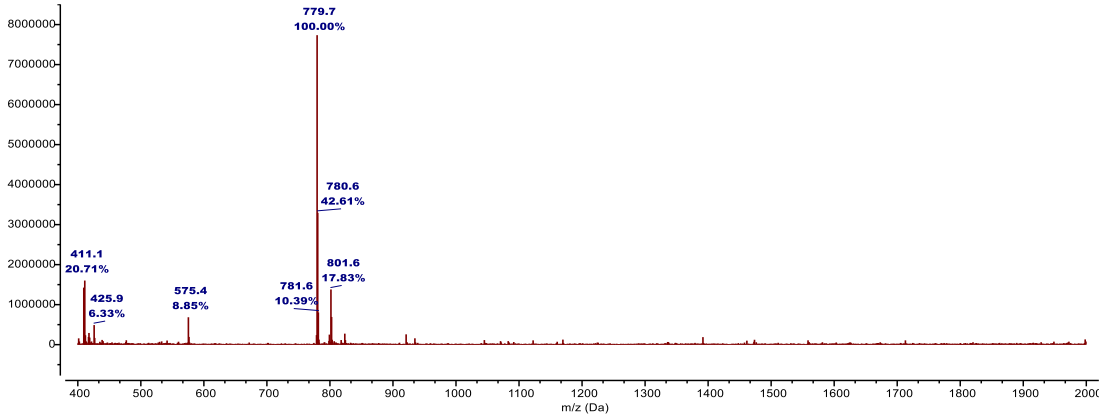
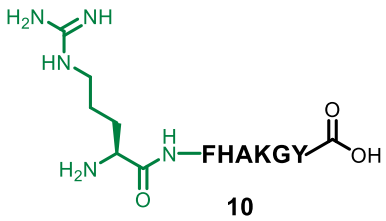


Figure 2: Low-resolution MS spectrum of peptide **7**, *m/z* (ESI$^+$) calcd M_{mono} = 778.4, found 779.7 [*M* + H]$^+$, 801.6 [*M* + Na]$^+$.



10

Arg peptide **10** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 76 mg peptide was obtained as a lyophilized solid (74 μmol, 7%).

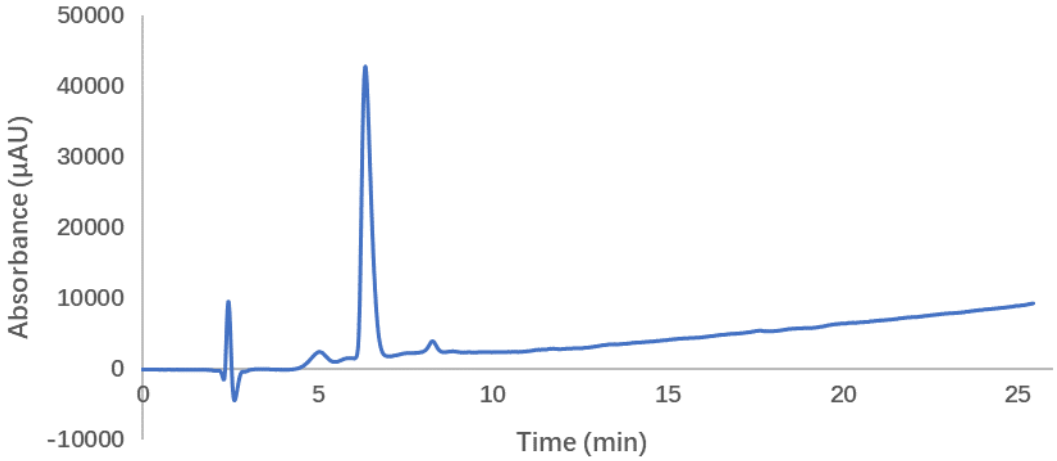


Figure 3: Analytical HPLC-MS trace of purified peptide **10** (t_R = 6.4 min, 0% B for 1 min and then 0 to 15% B over 25 min with a flow rate of 0.2 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

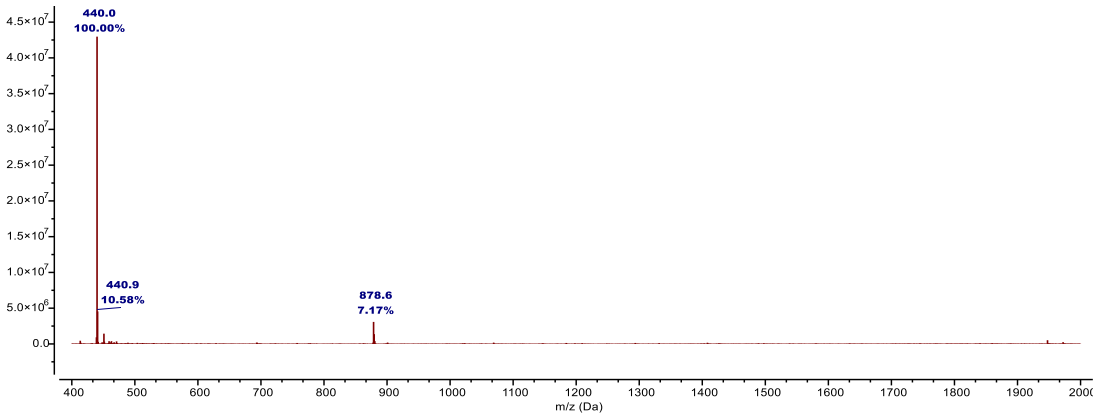
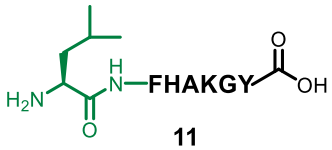


Figure 4: Low-resolution MS spectrum of peptide **10**, *m/z* (ESI$^+$) calcd M_{mono} = 877.5, found 878.6 [M + H]$^+$, 440.0 [M + 2H]$^{2+}$.



11

Leu peptide **11** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 72 mg peptide was obtained as a lyophilized solid (71 µmol, 7%).

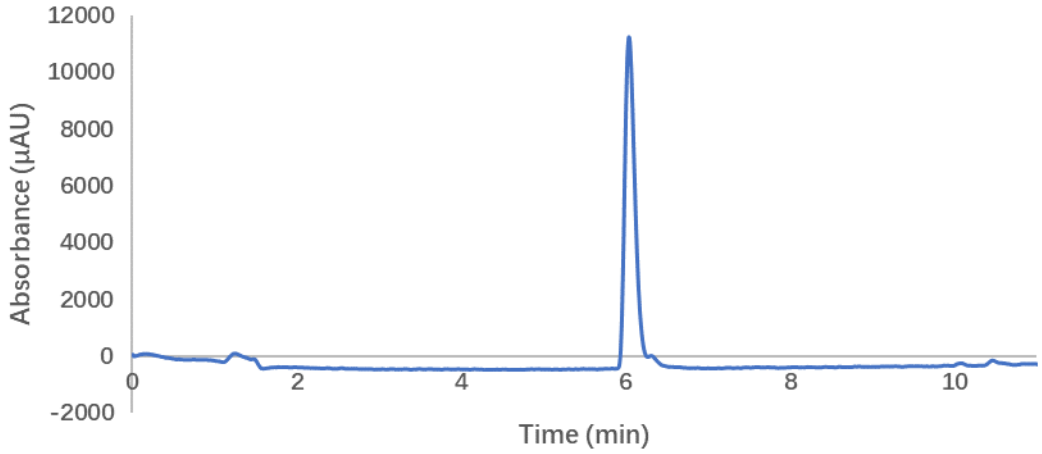


Figure 5: Analytical HPLC-MS trace of purified peptide **11** (t_R = 6.0 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

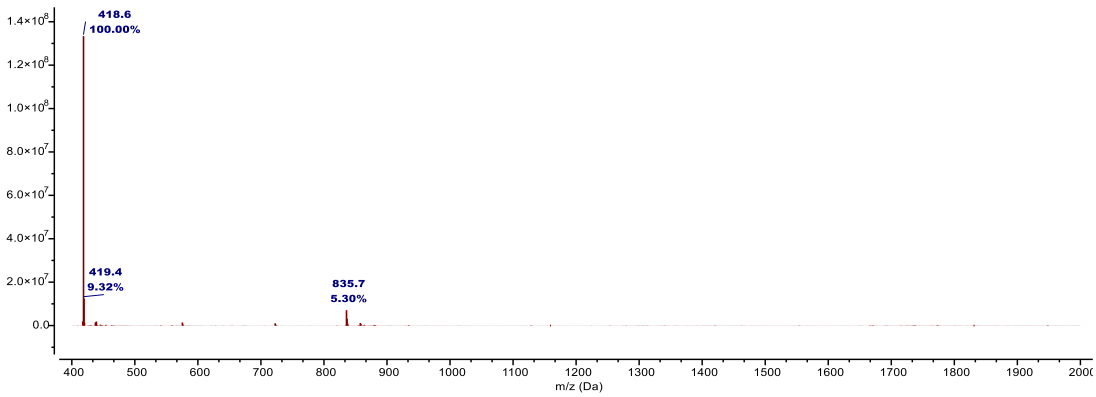
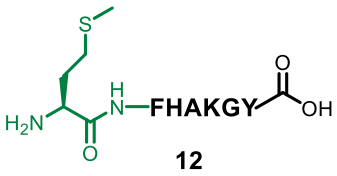


Figure 6: Low-resolution MS spectrum of peptide **11**, *m/z* (ESI+) calcd M_{mono} = 834.4, found 835.7 $[M + H]^+$, 418.6 $[M + 2H]^{2+}$.



12

Met peptide **12** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 80 mg peptide was obtained as a lyophilized solid (79 µmol, 8%).

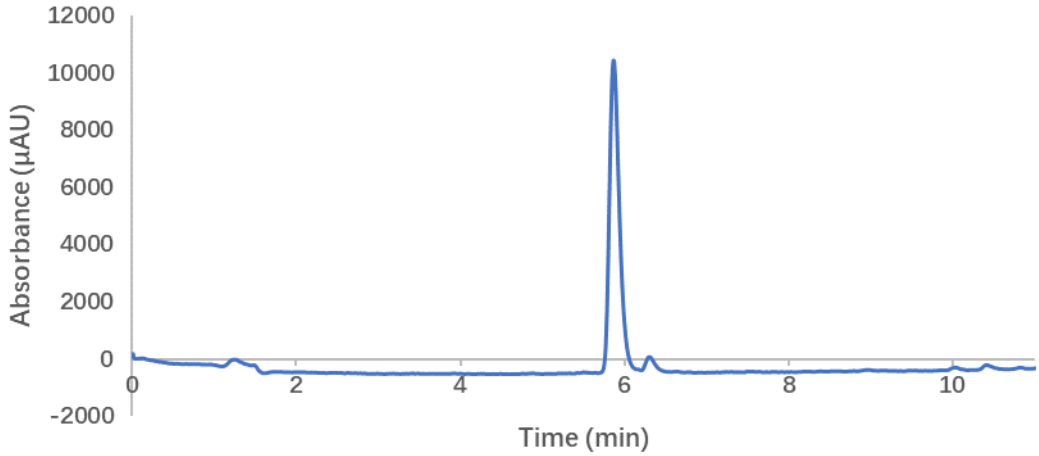


Figure 7: Analytical HPLC-MS trace of purified peptide **12** (t_R = 5.9 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

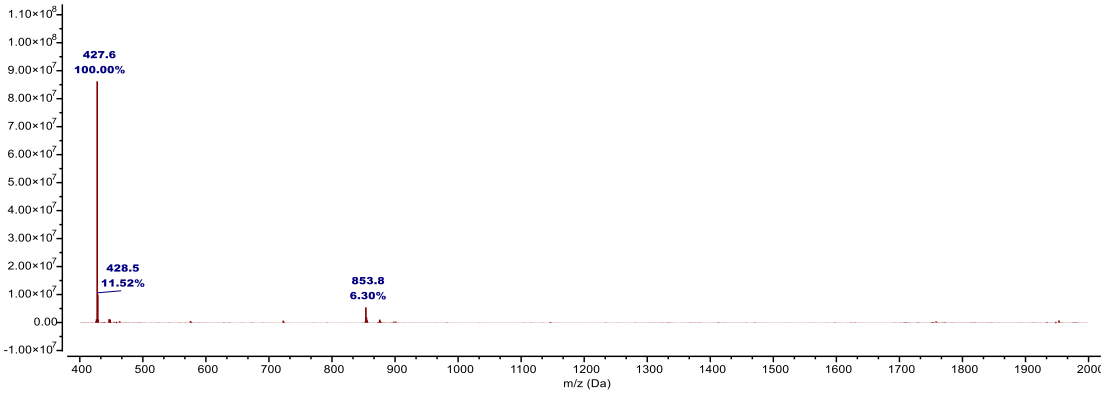
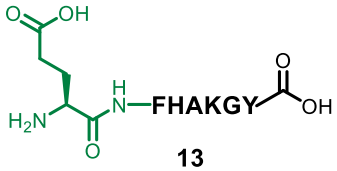


Figure 8: Low-resolution MS spectrum of peptide **12**, *m/z* (ESI+) calcd M_{mono} = 852.4, found 853.8 [M + H]$^{+}$, 427.6 [M + 2H]$^{2+}$.



13

Glu peptide **13** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 70 mg peptide was obtained as a lyophilized solid (70 μmol, 7%).

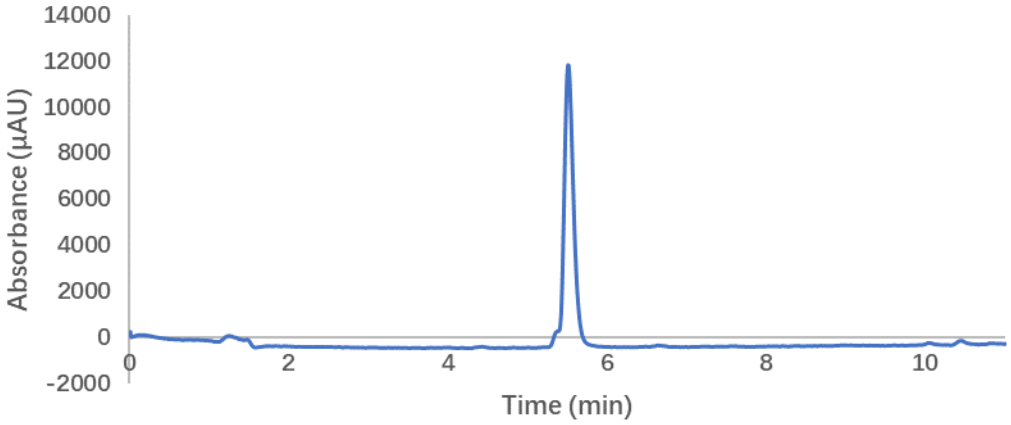


Figure 9: Analytical HPLC-MS trace of purified peptide **13** (t_R = 5.5 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

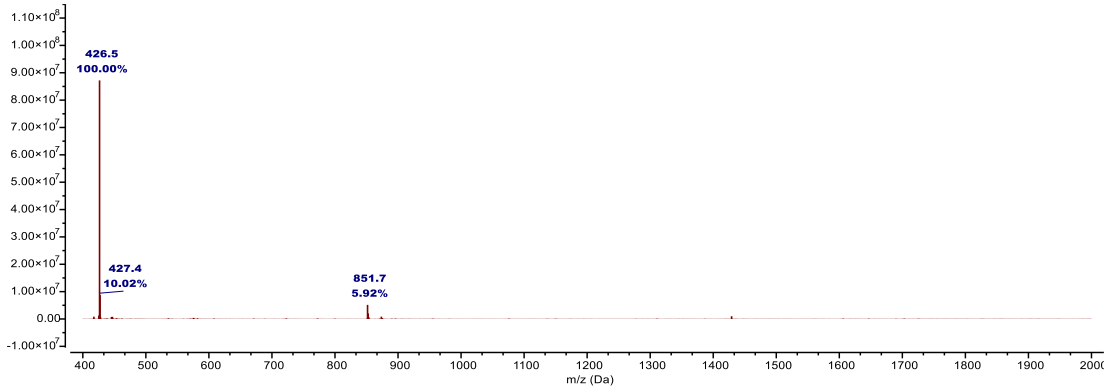
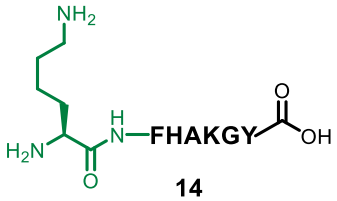


Figure 10: Low-resolution MS spectrum of peptide **13**, *m/z* (ESI⁺) calcd M_{mono} = 850.4, found 851.7 [M + H]⁺, 426.5 [M + 2H]²⁺.



14

Lys peptide **14** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 65 mg peptide was obtained as a lyophilized solid (64 µmol, 6%).

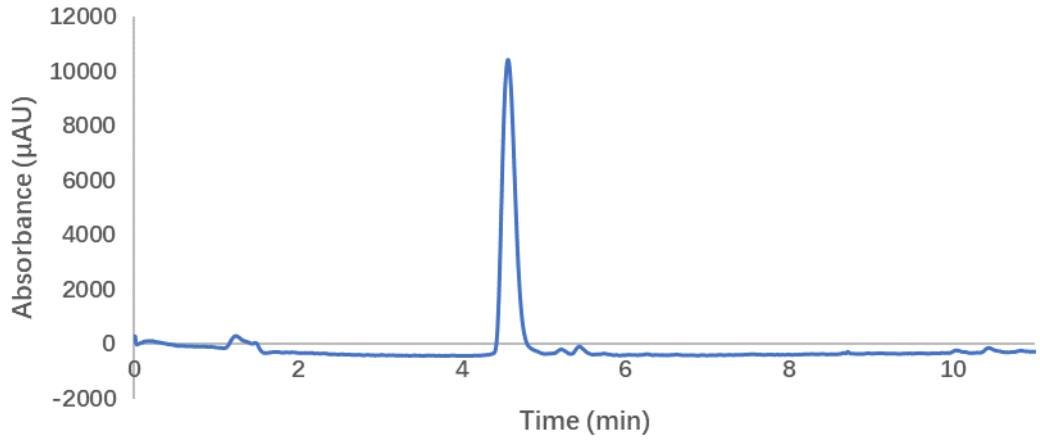


Figure 11: Analytical HPLC-MS trace of purified peptide **14** (t_R = 4.6 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

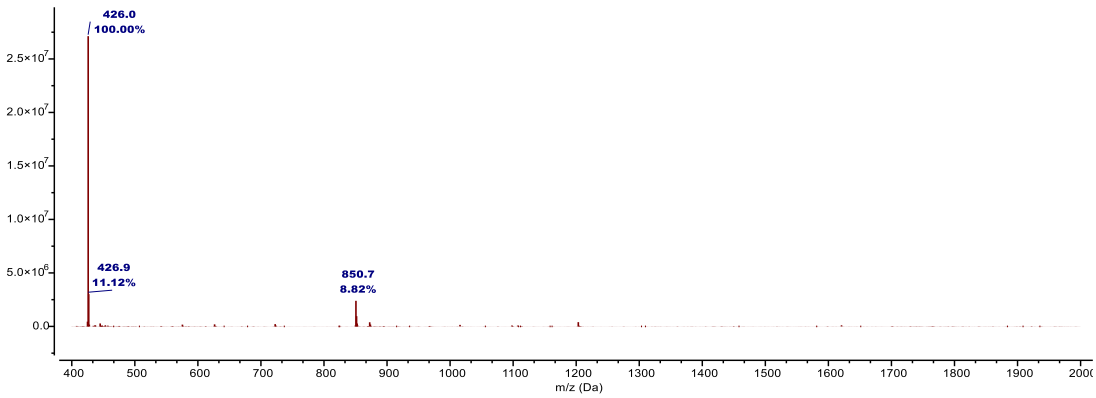
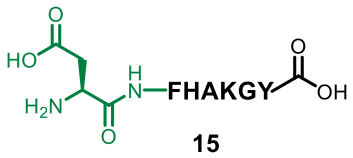


Figure 12: Low-resolution MS spectrum of peptide **14**, *m/z* (ESI⁺) calcd M_{mono} = 849.5, found 850.7 [M + H]⁺, 426.0 [M + 2H]²⁺.



15

Asp peptide **15** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 65 mg peptide was obtained as a lyophilized solid (68 µmol, 7%).

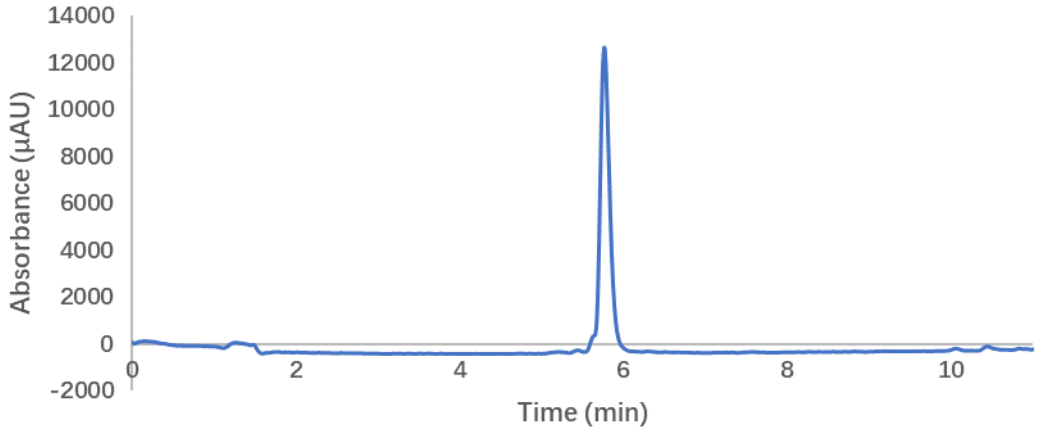


Figure 13: Analytical HPLC-MS trace of purified peptide **15** (t_R = 5.8 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

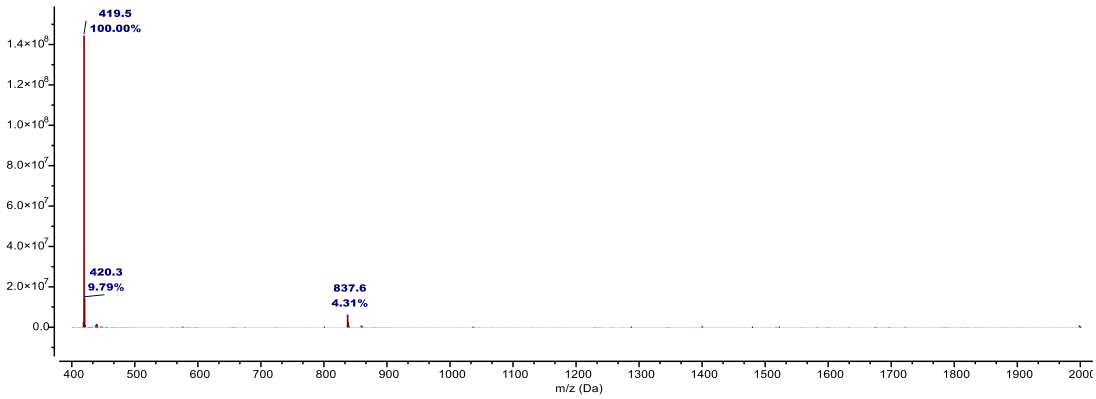
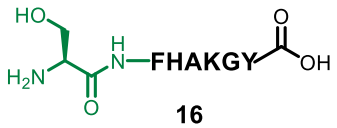


Figure 14: Low-resolution MS spectrum of peptide **15**, *m/z* (ESI⁺) calcd M_{mono} = 836.4, found 837.6 [M + H]⁺, 419.5 [M + 2H]²⁺.



16

Ser peptide **16** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 72 mg peptide was obtained as a lyophilized solid (72 μmol, 7%).

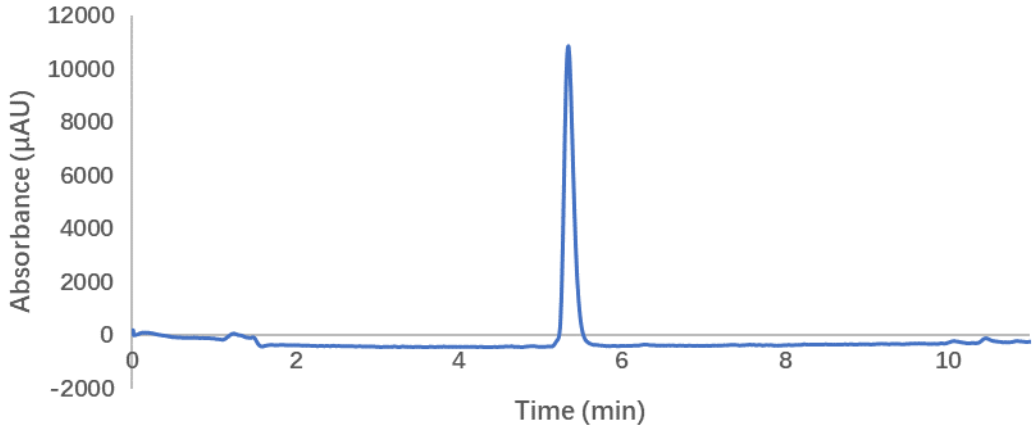


Figure 15: Analytical HPLC-MS trace of purified peptide **16** (t_R = 5.4 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

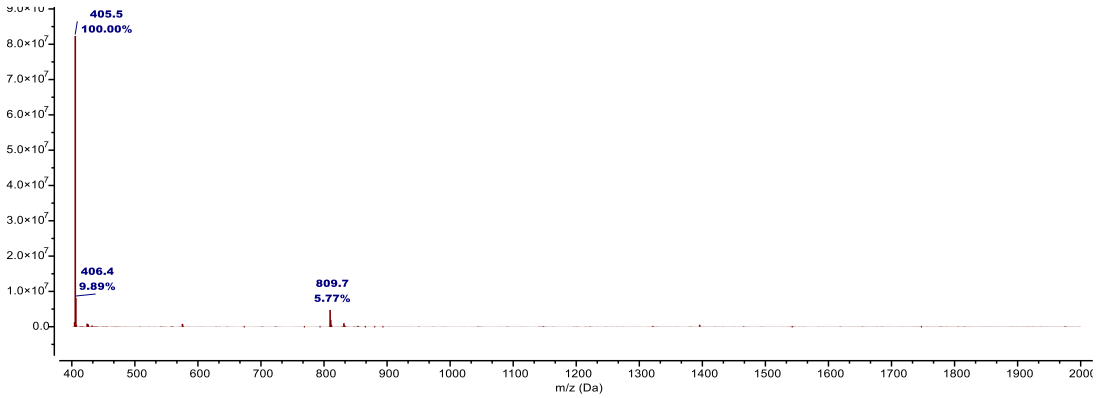
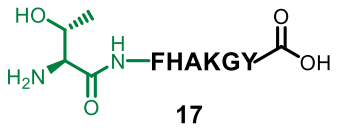


Figure 16: Low-resolution MS spectrum of peptide **16**, *m/z* (ESI⁺) calcd M_{mono} = 808.4, found 809.7 [M + H]⁺, 405.5 [M + 2H]²⁺.



17

Thr peptide **17** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 85 mg peptide was obtained as a lyophilized solid (85 µmol, 8%).

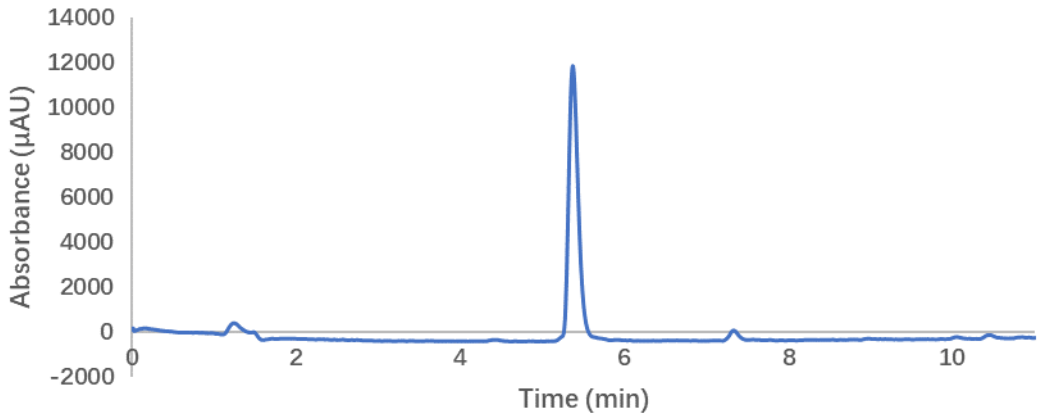


Figure 17: Analytical HPLC-MS trace of purified peptide **17** (t_R = 5.4 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

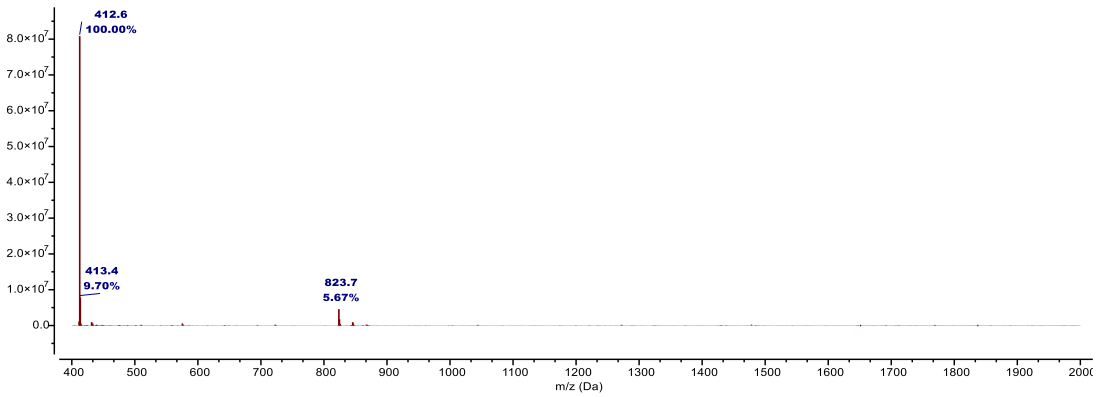
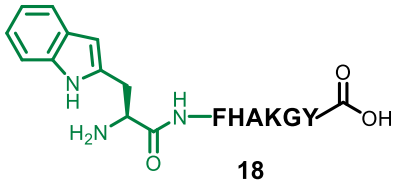


Figure 18: Low-resolution MS spectrum of peptide **17**, *m/z* (ESI⁺) calcd M_{mono} = 822.4, found 823.7 [M + H]⁺, 412.6 [M + 2H]²⁺.



18

Trp peptide **18** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 73 mg peptide was obtained as a lyophilized solid (70 µmol, 7%).

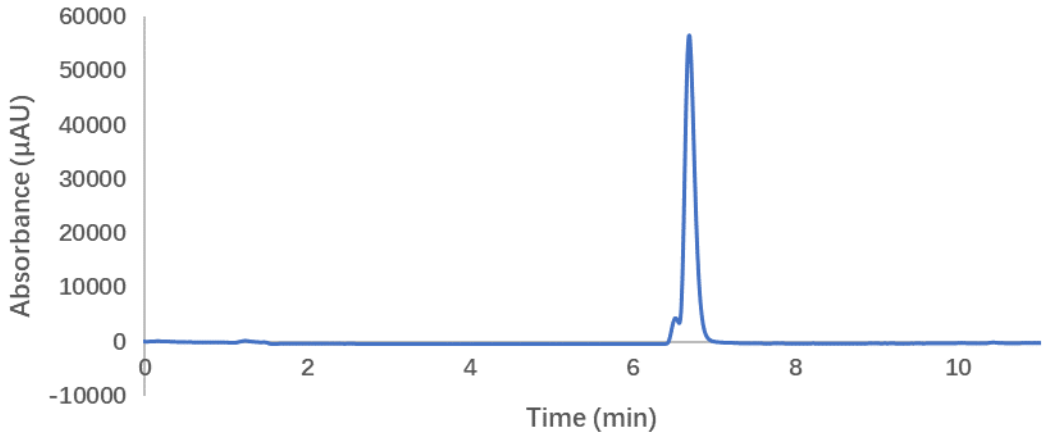


Figure 19: Analytical HPLC-MS trace of purified peptide **18** (t_R = 6.7 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

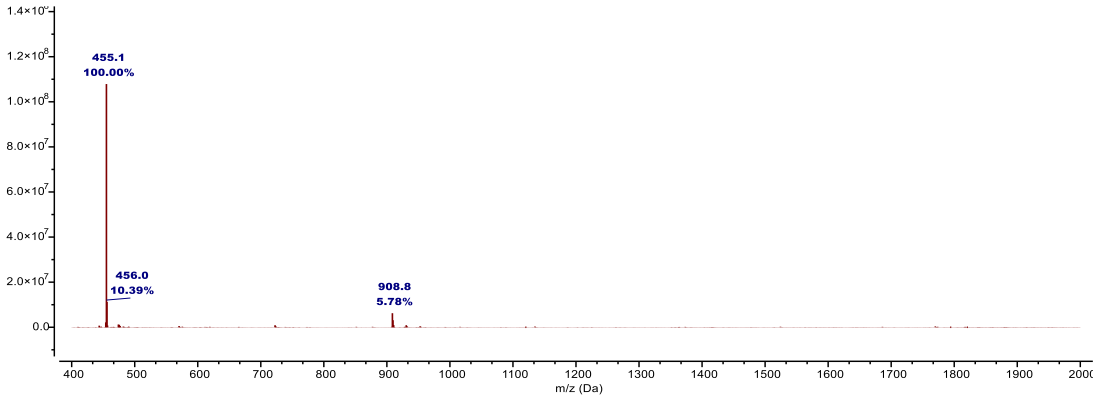


Figure 20: Low-resolution MS spectrum of peptide **18**, *m/z* (ESI⁺) calcd M_{mono} = 907.4, found 908.8 [M + H]⁺, 455.1 [M + 2H]²⁺.



19

Ala peptide **19** was synthesized on 2-CTC resin (0.1 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 80 mg peptide was obtained as a lyophilized solid (80 µmol, 8%).

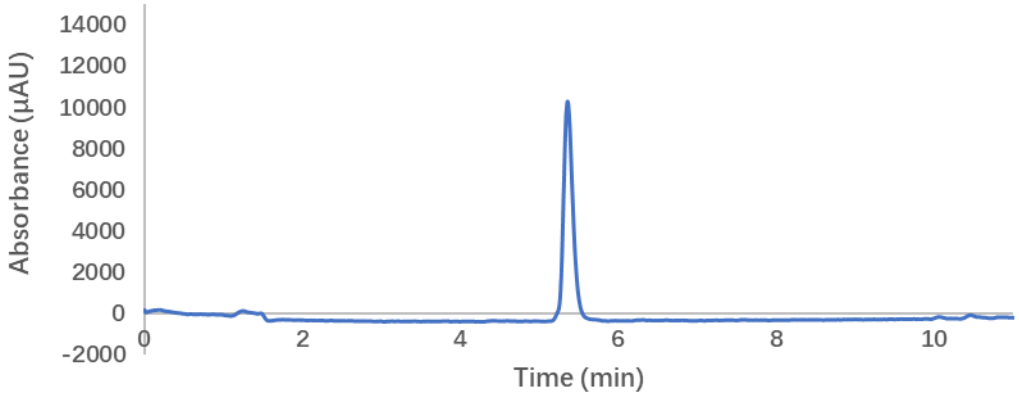


Figure 21: Analytical HPLC-MS trace of purified peptide **19** (t_R = 5.3 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

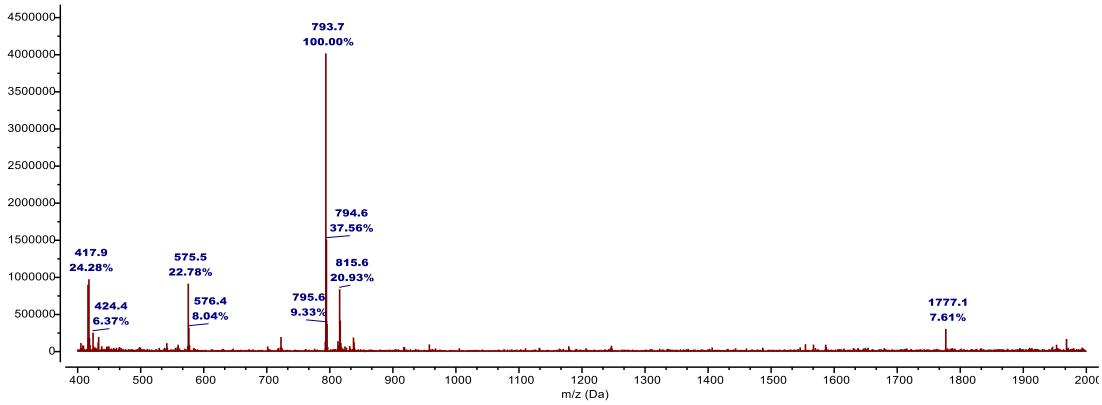
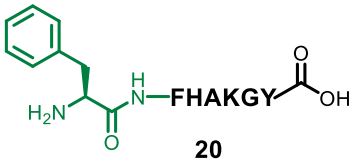


Figure 22: Low-resolution MS spectrum of peptide **19**, *m/z* (ESI⁺) calcd M_{mono} = 792.4, found 793.7 [M + H]⁺, 815.6 [M + Na]⁺.



20

Phe peptide **20** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 75 mg peptide was obtained as a lyophilized solid (75 µmol, 7.5%).

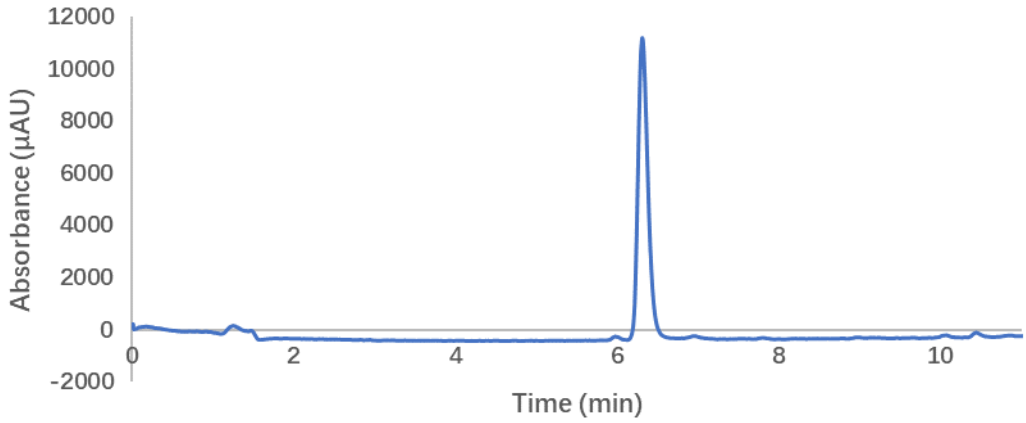


Figure 23: Analytical HPLC-MS trace of purified peptide **20** (t_R = 6.5 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

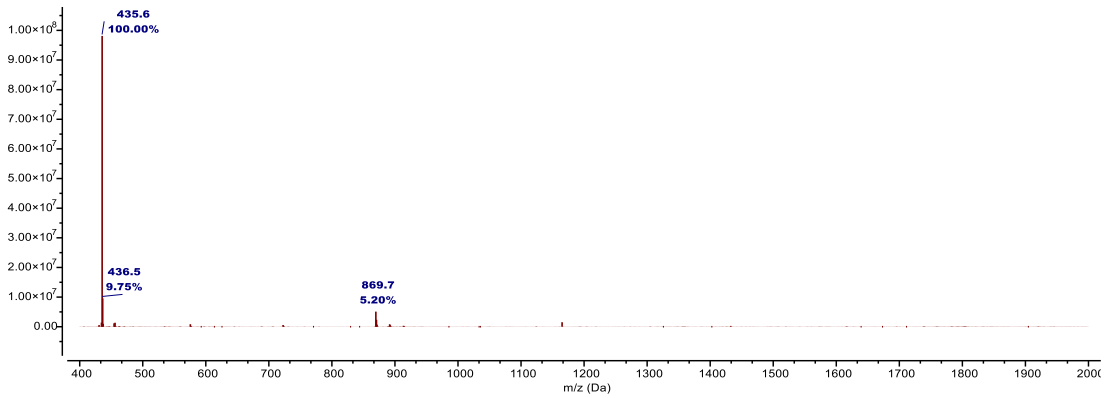
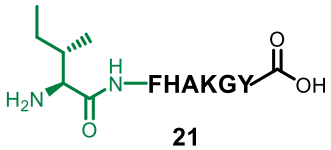


Figure 24: Low-resolution MS spectrum of peptide **20**, *m/z* (ESI⁺) calcd M_{mono} = 868.4, found 869.7 [M + H]⁺, 435.6 [M + 2H]²⁺.



21

Ile peptide **21** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 62 mg peptide was obtained as a lyophilized solid (62 μmol, 6%).

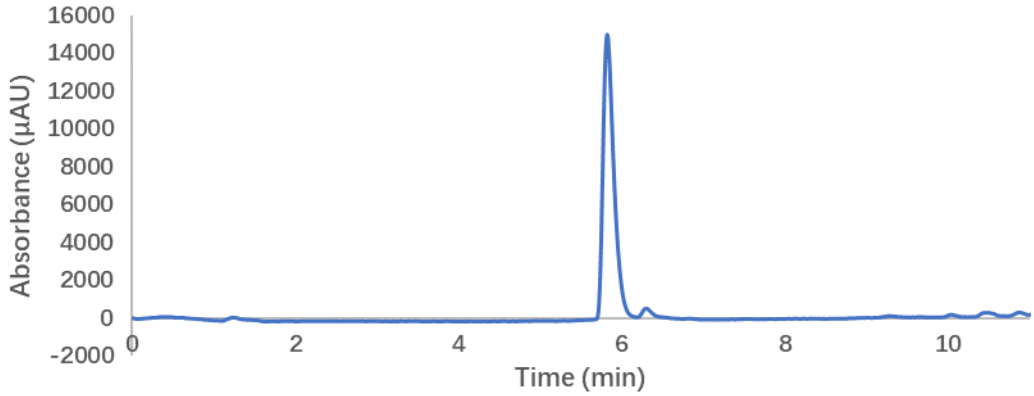


Figure 25: Analytical HPLC-MS trace of purified peptide **21** (t_R = 5.8 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

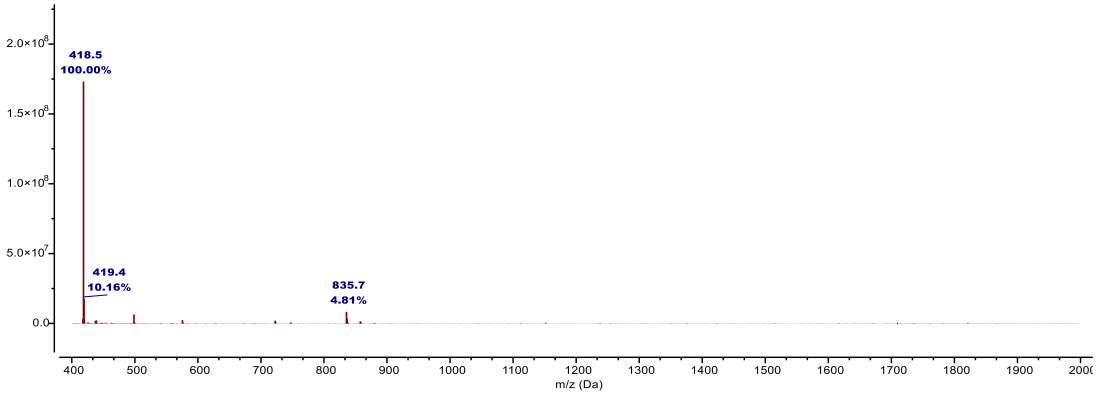
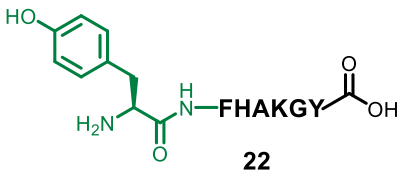


Figure 26: Low-resolution MS spectrum of peptide **21**, *m/z* (ESI⁺) calcd M_{mono} = 834.4, found 835.7 [M + H]⁺, 418.5 [M + 2H]²⁺.



22

Tyr peptide **22** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 66 mg peptide was obtained as a lyophilized solid (65 µmol, 7%).

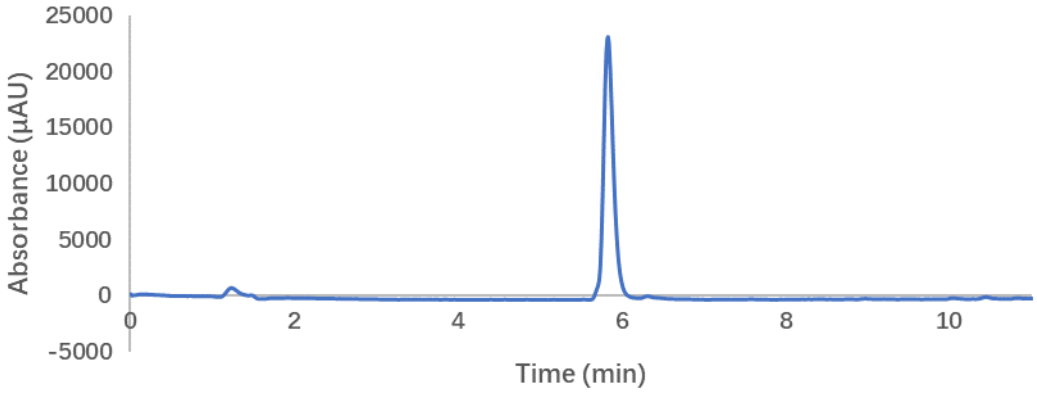


Figure 27: Analytical HPLC-MS trace of purified peptide **22** (t_R = 5.8 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

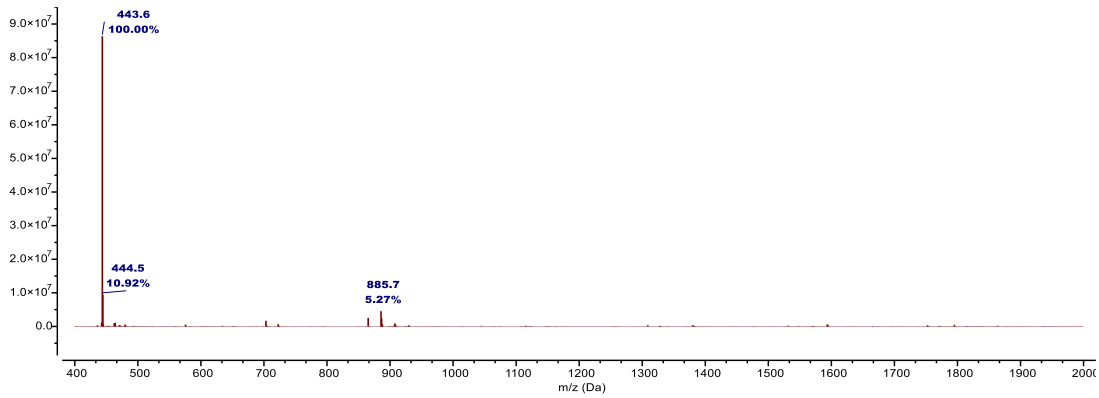
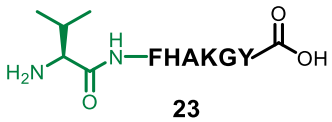


Figure 28: Low-resolution MS spectrum of peptide **22**, *m/z* (ESI⁺) calcd M_{mono} = 884.4, found 885.7 [M + H]⁺, 443.6 [M + 2H]²⁺.



23

Val peptide **23** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 80 mg peptide was obtained as a lyophilized solid (80 µmol, 8%).

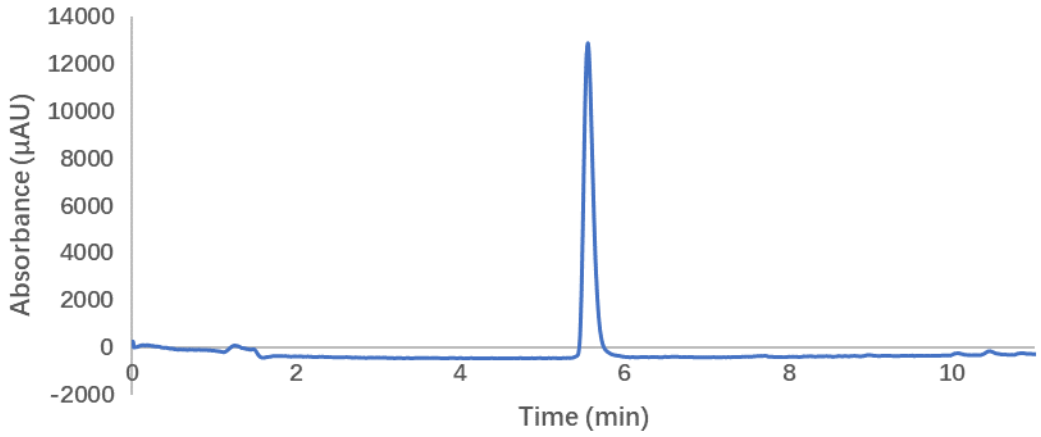


Figure 29: Analytical HPLC-MS trace of purified peptide **23** (t_R = 5.5 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

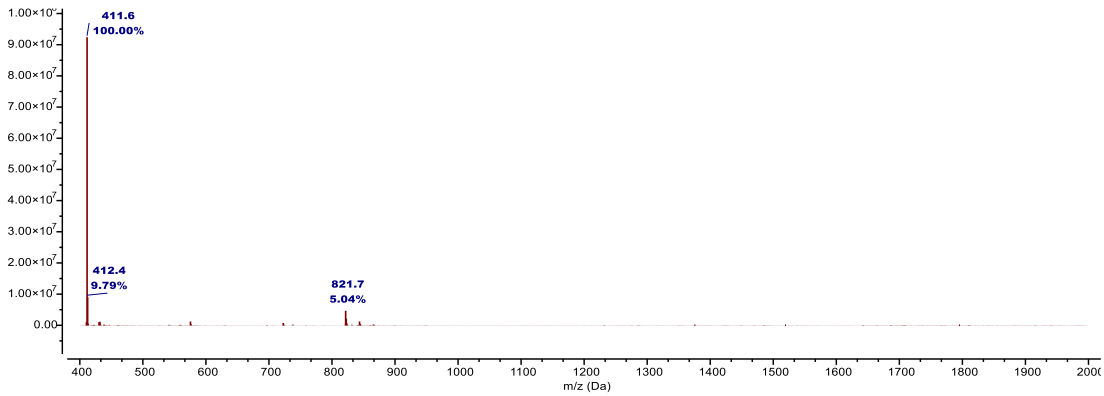
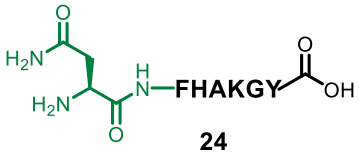


Figure 30: Low-resolution MS spectrum of peptide **23**, *m/z* (ESI⁺) calcd M_{mono} = 820.4, found 821.7 [M + H]⁺, 411.6 [M + 2H]²⁺.



24

Asn peptide **24** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 75 mg peptide was obtained as a lyophilized solid (74 µmol, 7%).

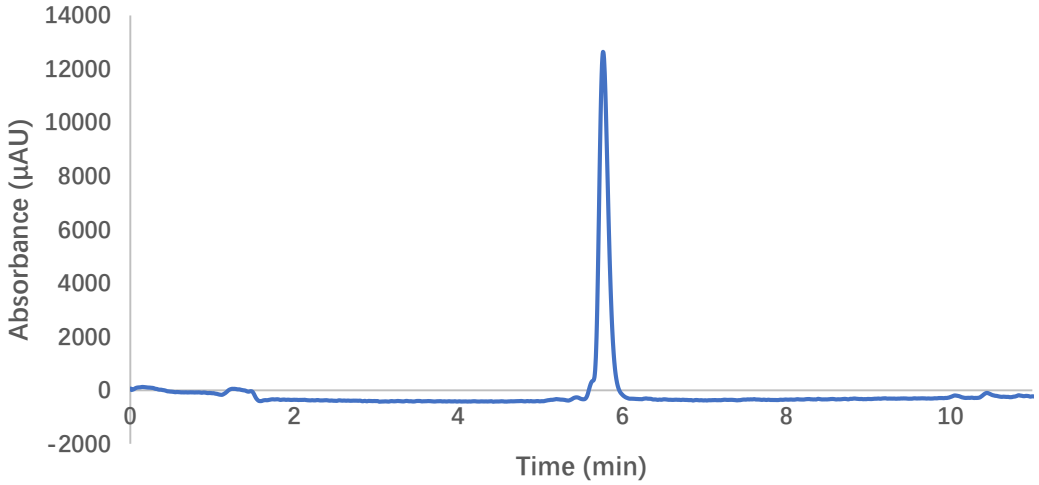


Figure 31: Analytical HPLC-MS trace of purified peptide **24** (t_R = 5.8 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

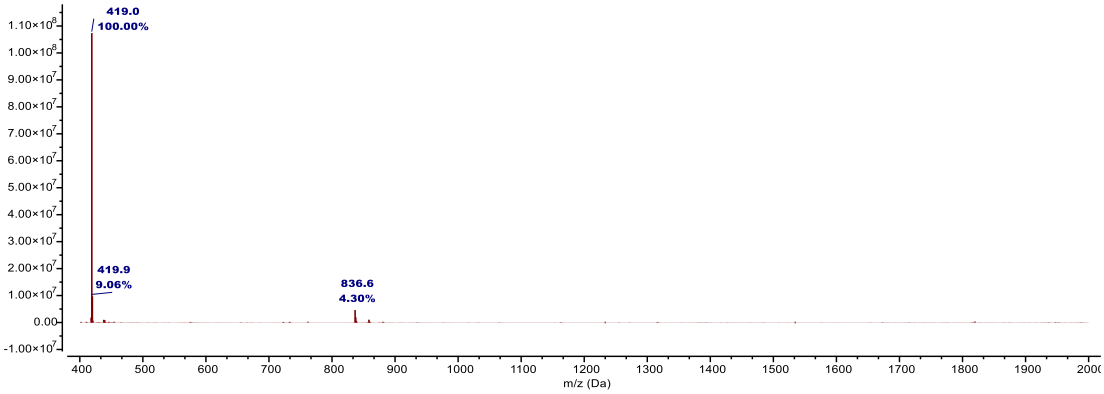
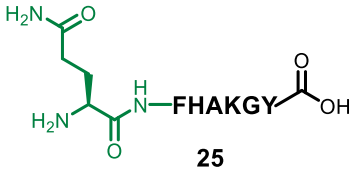


Figure 32: Low-resolution MS spectrum of peptide **24**, *m/z* (ESI⁺) calcd M_{mono} = 835.4, found 836.6 [M + H]⁺, 419.0 [M + 2H]²⁺.



25

Gln peptide **25** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 66 mg peptide was obtained as a lyophilized solid (66 µmol, 7%).

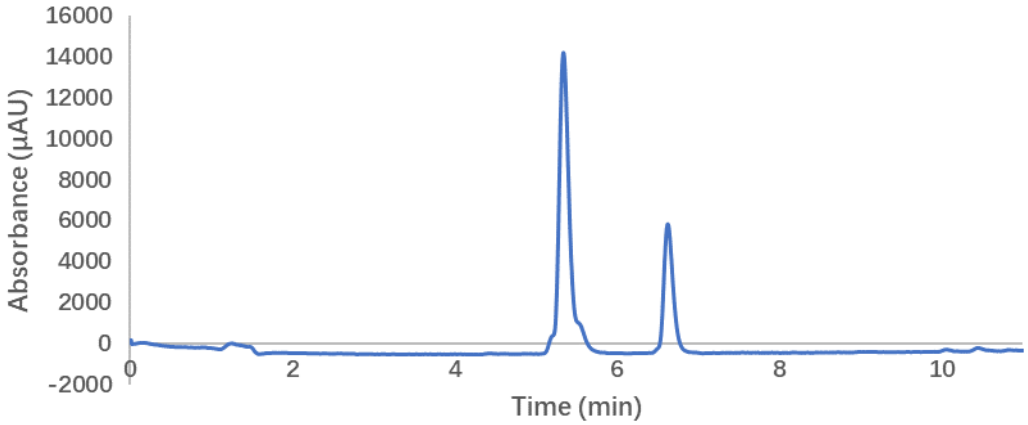


Figure 33: Analytical HPLC-MS trace of purified peptide **25** (t_R = 5.3 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column). By-product: pyroglytamic acid peptide **25(py)** (t_R = 6.6 min).

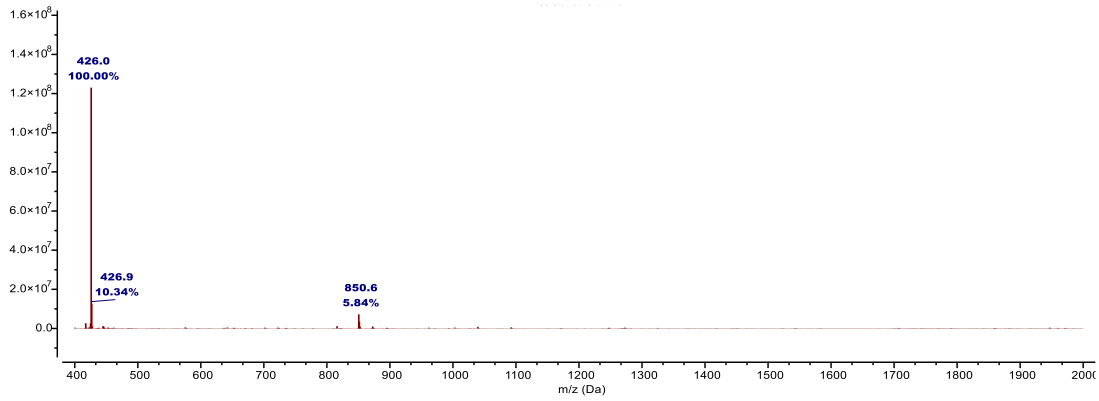
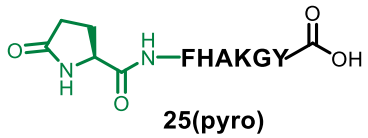


Figure 34: Low-resolution MS spectrum of peptide **25**, *m/z* (ESI⁺) calcd M_{mono} = 849.4, found 850.6 [M + H]⁺, 426.0 [M + 2H]²⁺.



25(pyro)

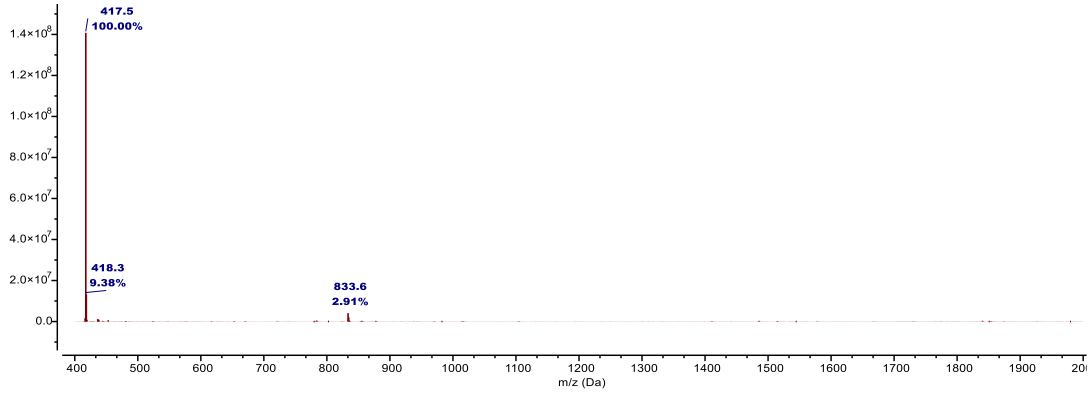
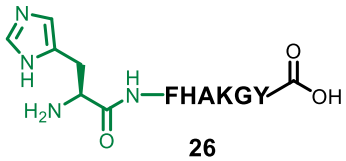


Figure 35: Low-resolution MS spectrum of peptide **25(pyro)**, *m/z* (ESI⁺) calcd M_{mono} = 832.4, found 833.6 [M + H]⁺, 417.5 [M + 2H]²⁺.



26

His peptide **26** was synthesized on 2-CTC resin (0.10 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 72 mg peptide was obtained as a lyophilized solid (71 µmol, 7%).

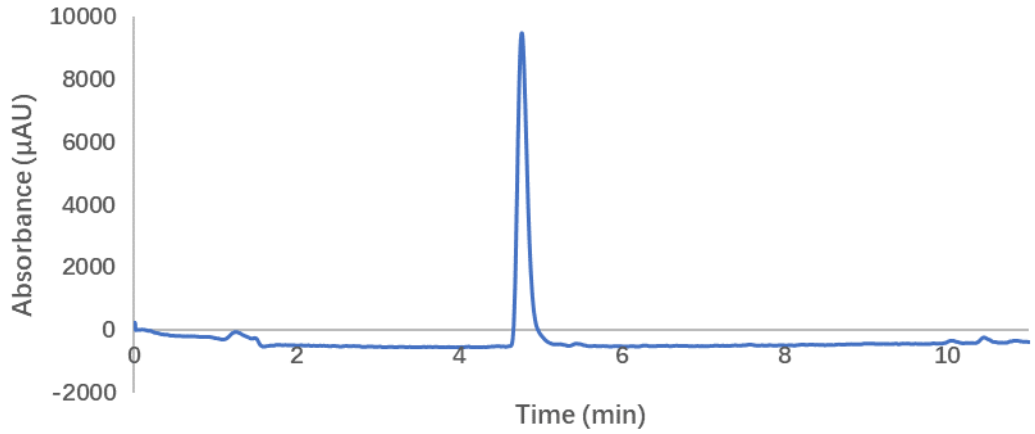


Figure 36: Analytical HPLC-MS trace of purified peptide **26** (t_R = 4.8 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

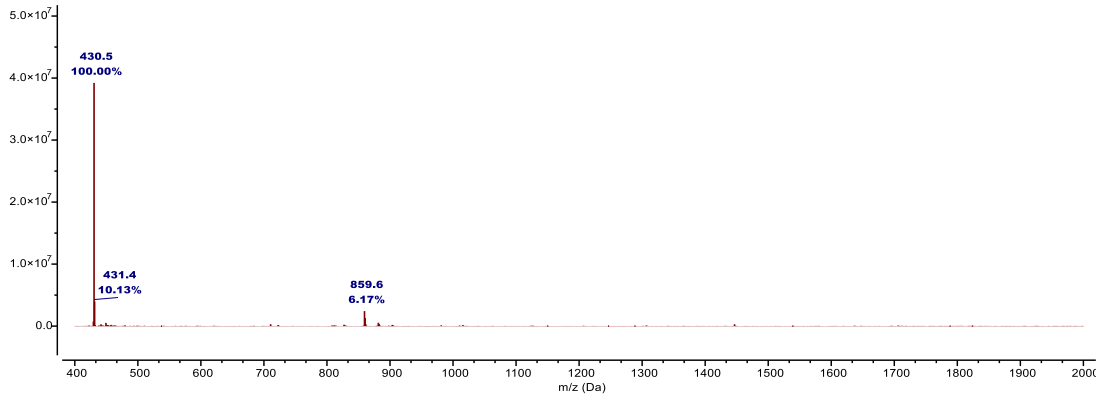


Figure 37: Low-resolution MS spectrum of peptide **26**, *m/z* (ESI⁺) calcd M_{mono} = 858.4, found 859.6 [M + H]⁺, 430.5 [M + 2H]²⁺.



27

Pro peptide **27** was synthesized on 2-CTC resin (0.05 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 34 mg peptide was obtained as a lyophilized solid (34 µmol, 7%).

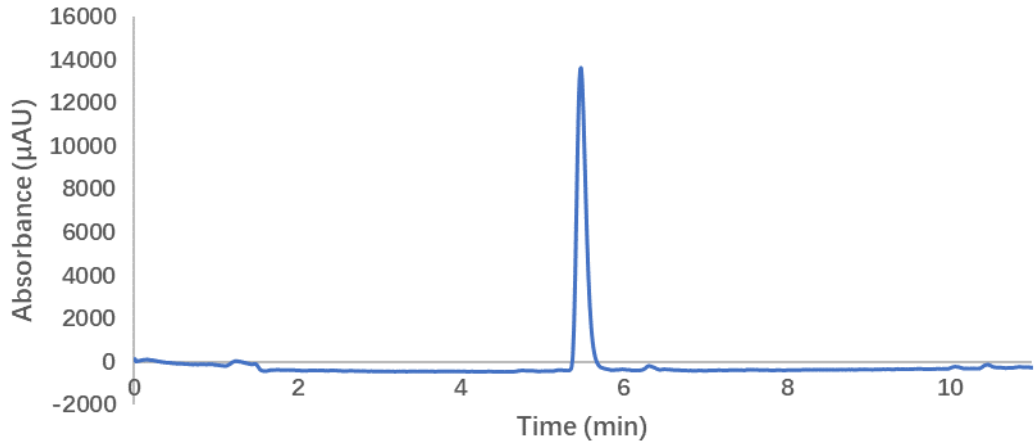


Figure 38: Analytical HPLC-MS trace of purified peptide **27** (t_R = 5.5 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

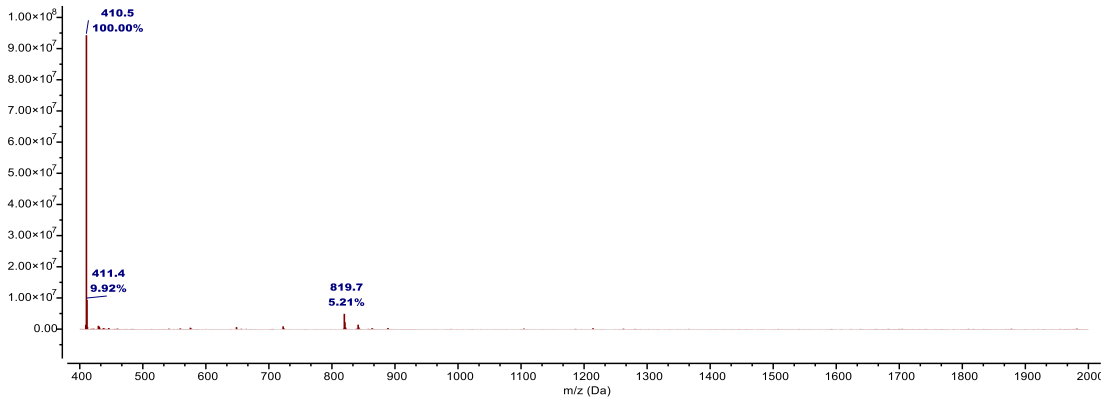
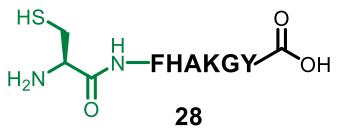


Figure 39: Low-resolution MS spectrum of peptide **27**, *m/z* (ESI⁺) calcd M_{mono} = 818.4, found 819.7 [M + H]⁺, 410.5 [M + 2H]²⁺.



28

Cys peptide **28** was synthesized on 2-CTC resin (0.05 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 35 mg peptide was obtained as a lyophilized solid (35 µmol, 7%).

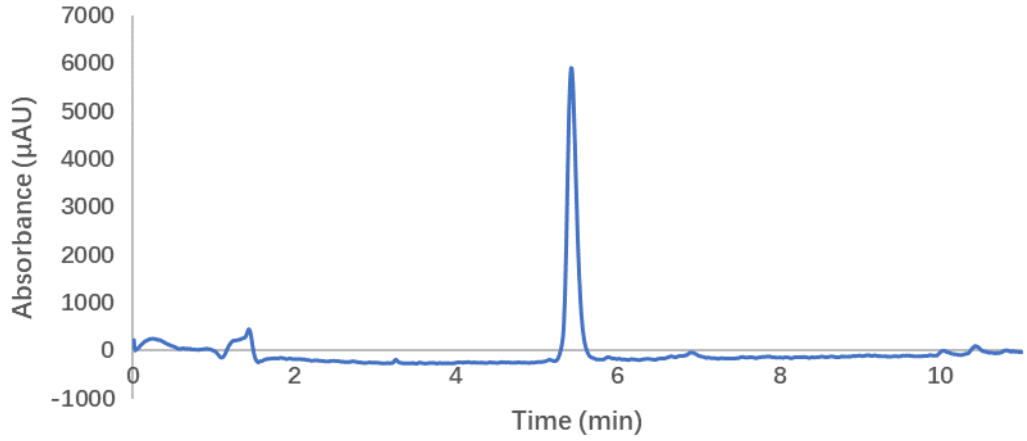


Figure 40: Analytical HPLC-MS trace of purified peptide **28** (t_R = 5.4 min, 0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

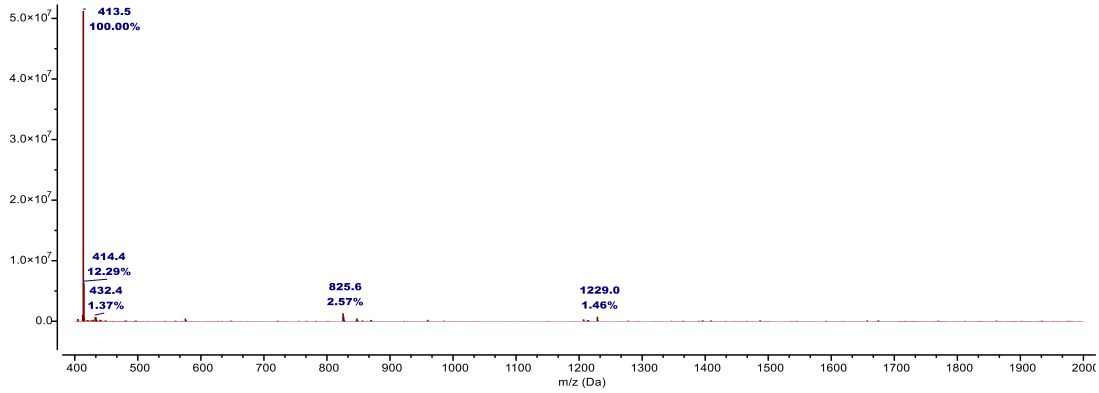


Figure 41: Low-resolution MS spectrum of peptide **28**, *m/z* (ESI⁺) calcd M_{mono} = 824.4, found 825.6 [M + H]⁺, 413.5 [M + 2H]²⁺.

2.7. Synthesis and analytical data for COVID-19 RBD peptide fragment **S1**



S1

COVID-19 RBD peptide **S1** was synthesized on 2-CTC resin (0.20 g, loading capacity 1.0 mmol/g) according to standard Fmoc-SPPS protocol. After HPLC purification, 57 mg peptide was obtained as a lyophilized solid (70 µmol, 7%).

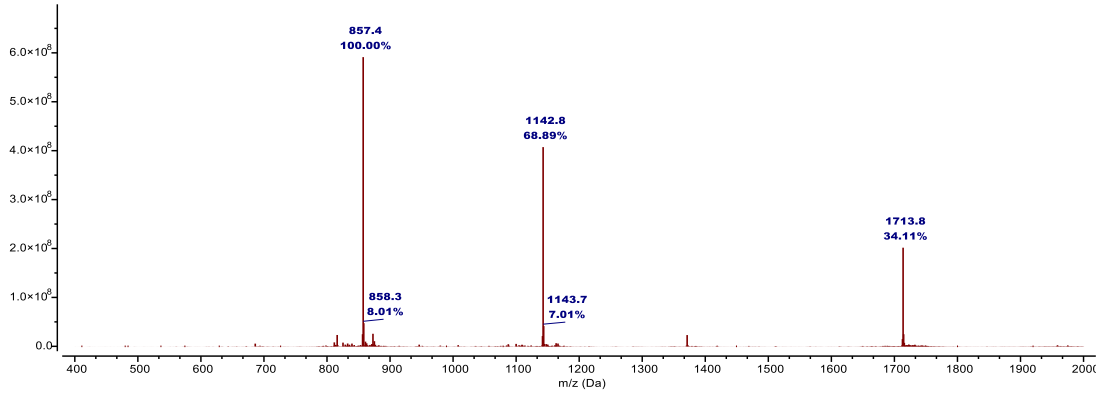
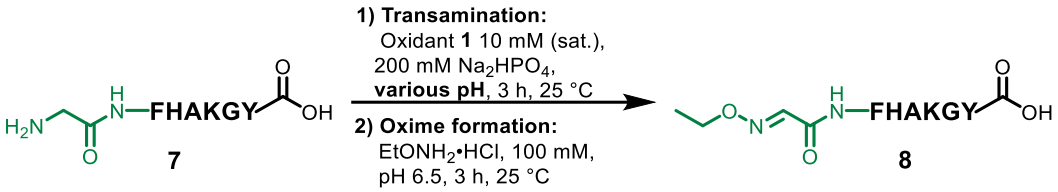


Figure 42: Low-resolution MS spectrum of peptide **S1**, *m/z* (ESI$^+$) calcd M_{av} = 3424.9, found 1713.8 [M + 2H]$^{2+}$, 1142.8 [M + 3H]$^{3+}$, 857.4 [M + 4H]$^{4+}$.

3. N-terminal transamination and oxime ligation

3.1. Optimization for transamination of model peptide

3.1.1. pH optimization for transamination of model peptide with quinone **1**



Model peptide **7** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at the described pH, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS to deduce the conversion rate. The entire experiment was repeated for three times.

Table 1: A summary of pH optimization experiment with quinone **1**.

pH	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
4.0	60.3	55.7	57.3	57.8	2.0
5.0	81.2	73.2	77.5	77.3	3.7
5.5	85.3	84.3	82.1	83.9	1.3
6.0	90.7	89.2	88.9	89.6	0.8
6.5	92	91.6	89.2	90.9	1.2
7.0	86.3	87.7	87.5	87.2	0.6
7.5	80.4	82	83.8	82.1	1.4
8.0	57.5	61.9	60.7	60.0	1.9

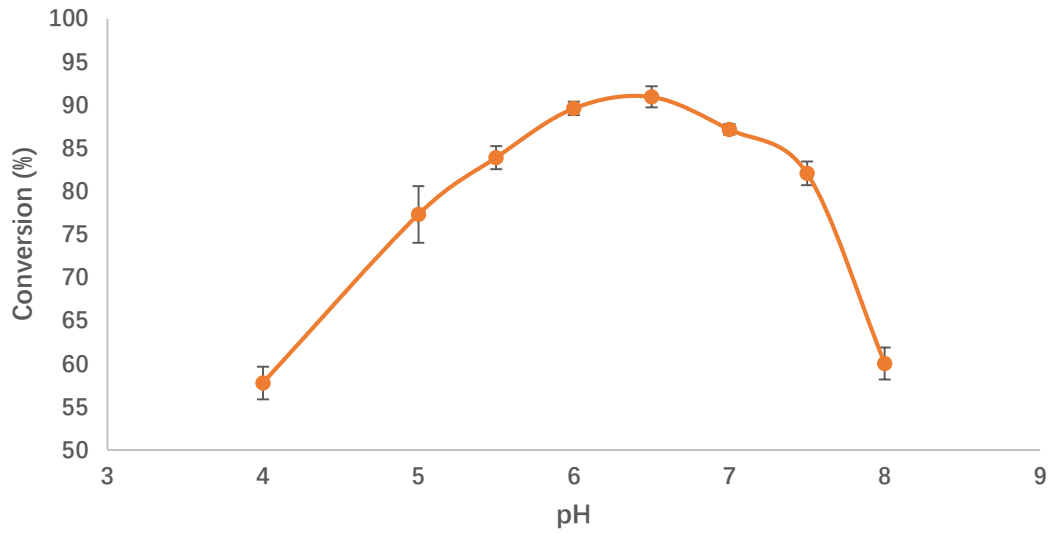


Figure 43: A summary chart of pH optimization of oxidant **1** transamination.

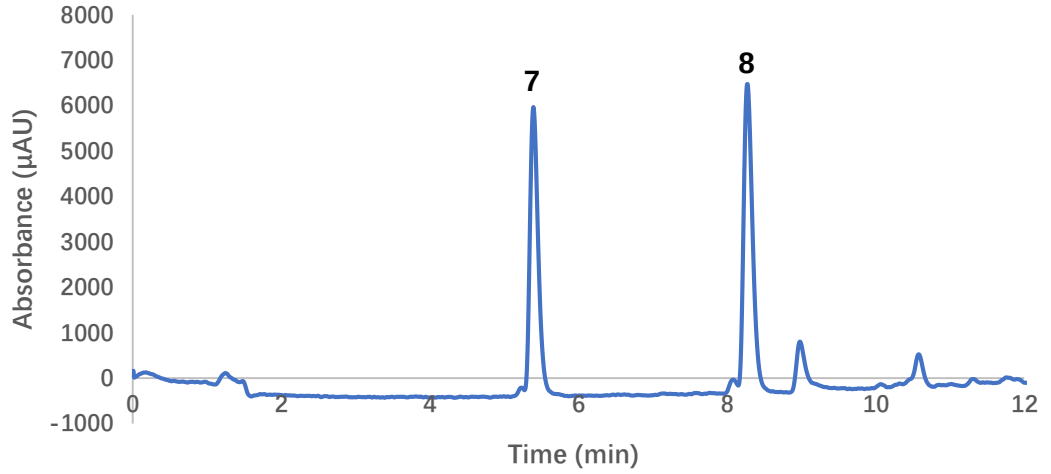


Figure 44: Analytical HPLC-MS trace of reaction at pH 4.0. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

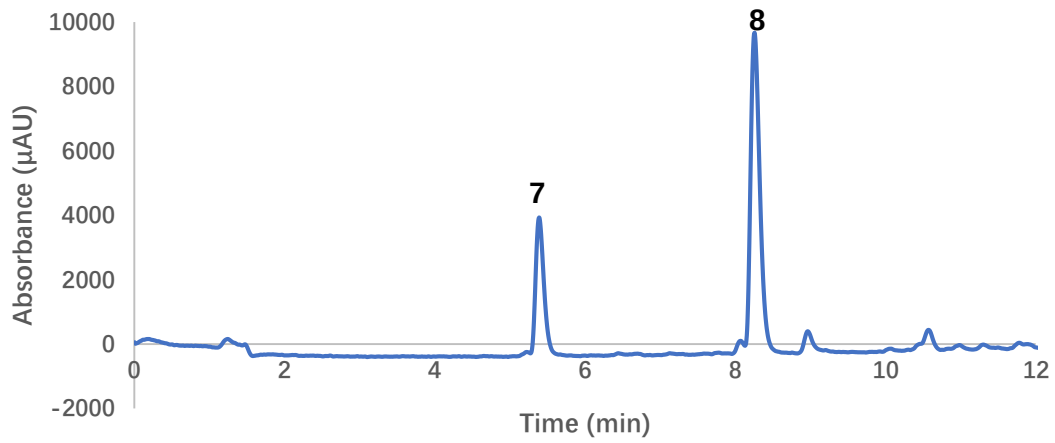


Figure 45: Analytical HPLC-MS trace of reaction at pH 5.0. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

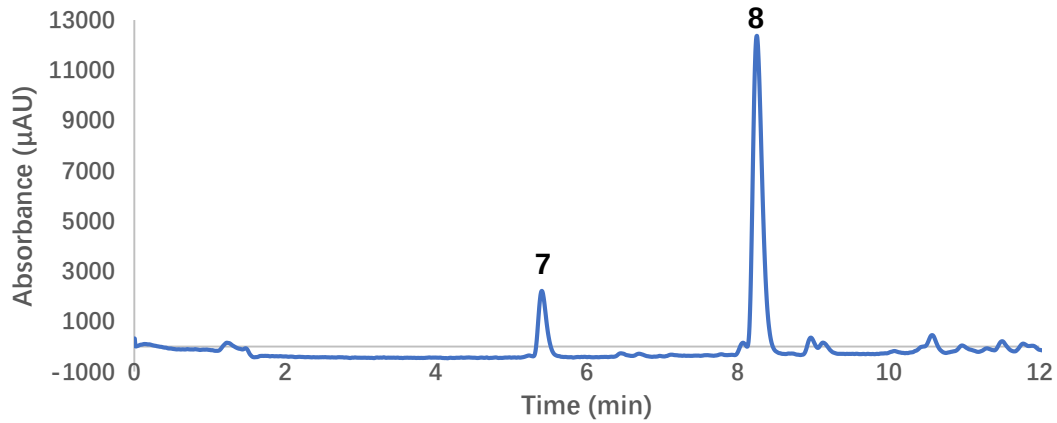


Figure 46: Analytical HPLC-MS trace of reaction at pH 5.5. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

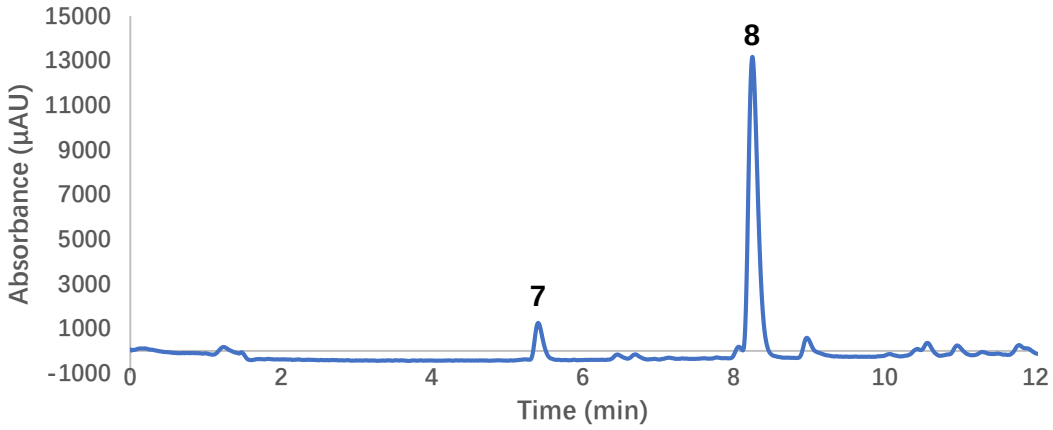


Figure 47: Analytical HPLC-MS trace of reaction at pH 6.0. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

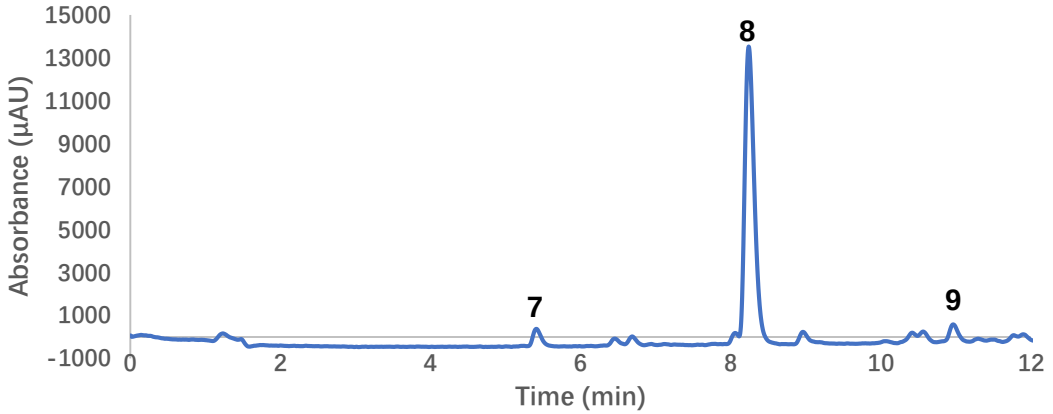
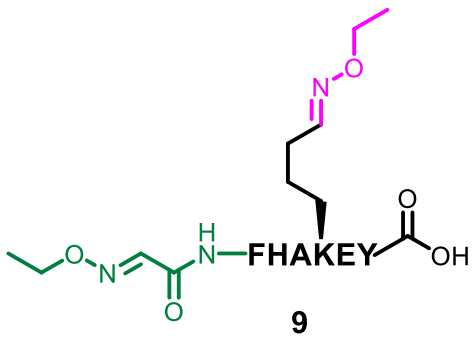


Figure 48: Analytical HPLC-MS trace of reaction at pH 6.5. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min; side product **9**: t_R = 11.0 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).



9

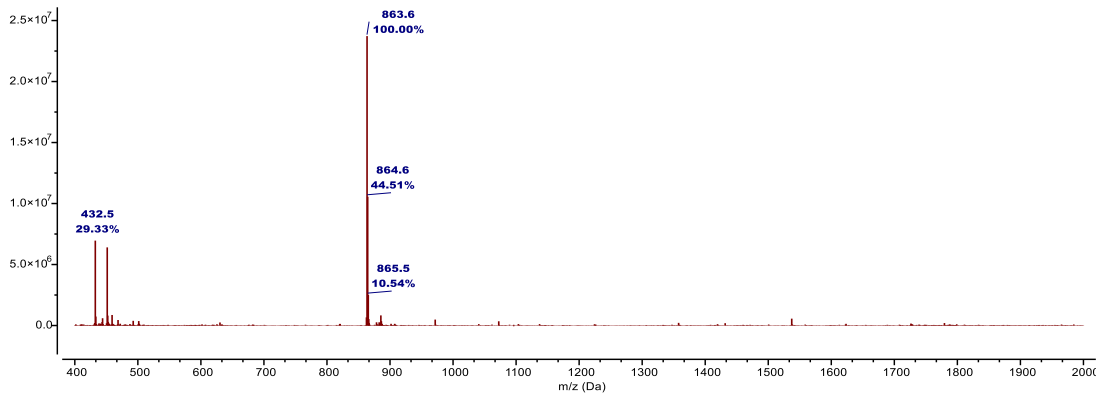


Figure 49: Low-resolution MS spectrum of byproduct **9**, *m/z* (ESI⁺) calcd M_{mono} = 862.4, found 863.6 [M + H]⁺, 432.5 [M + 2H]²⁺.

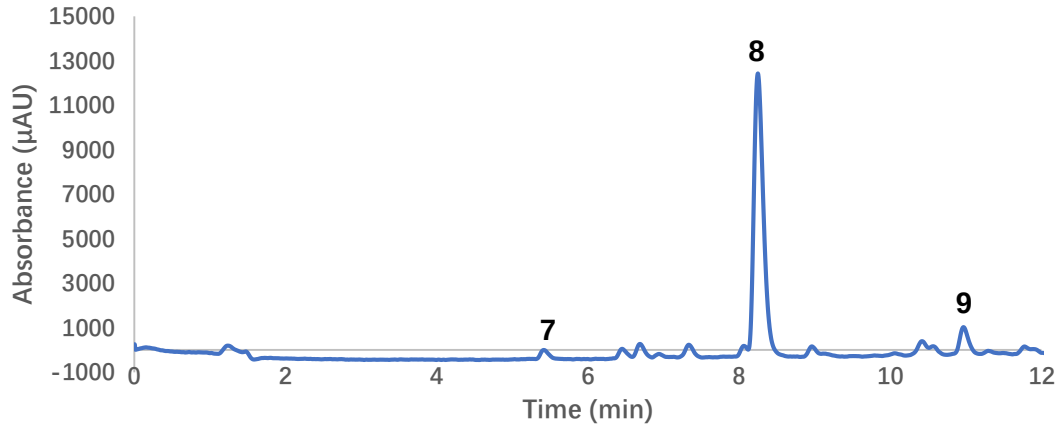


Figure 50: Analytical HPLC-MS trace of reaction at pH 7.0. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min; side product **9**: t_R = 11.0 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

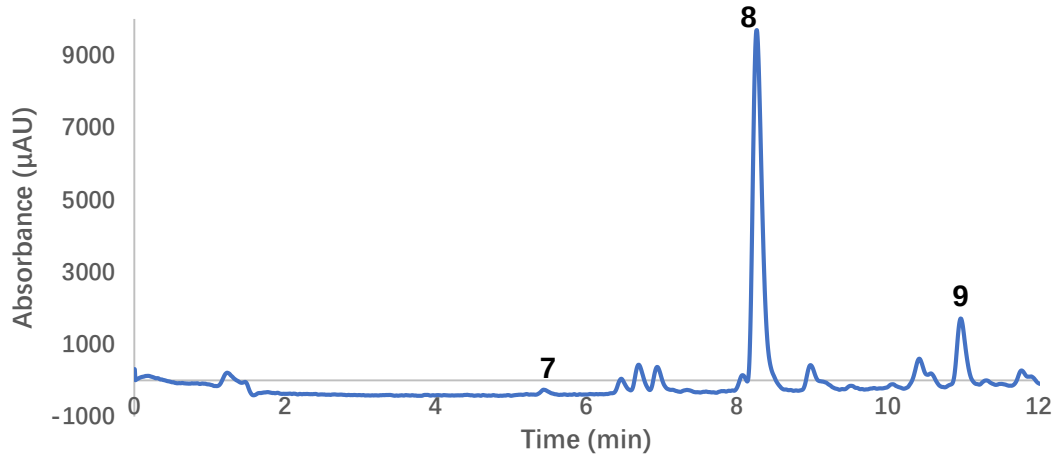


Figure 51: Analytical HPLC-MS trace of reaction at pH 7.5. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min; side product **9**: t_R = 11.0 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

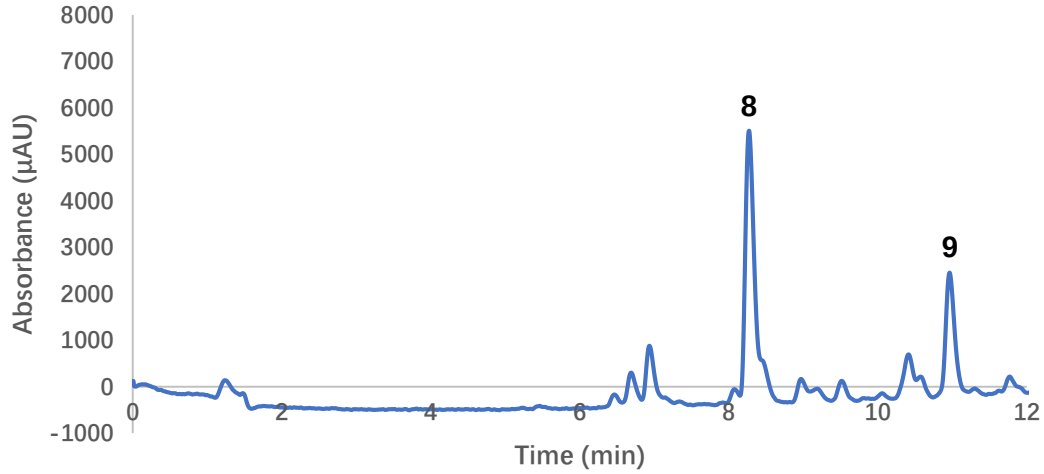


Figure 52: Analytical HPLC-MS trace of reaction at pH 8.0. Starting peptide **7** (hardly observed); desired product **8**: t_R = 8.3 min; side product **9**: t_R = 11.0 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

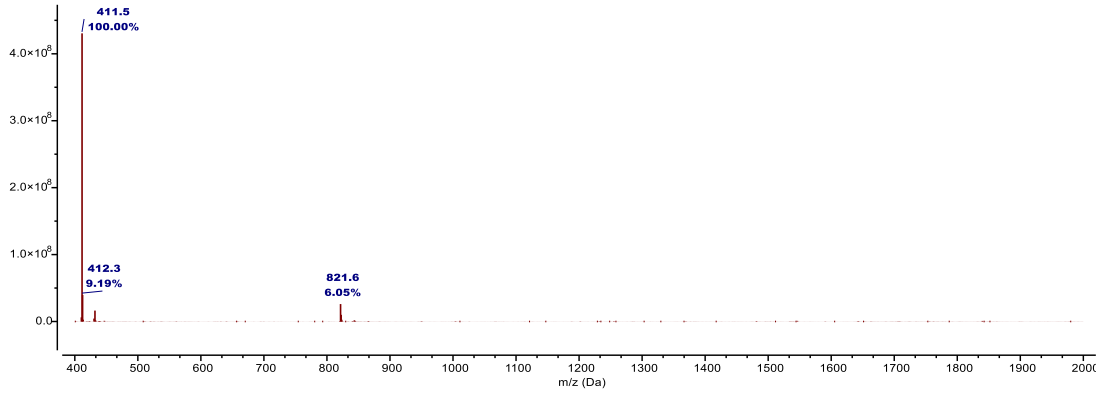
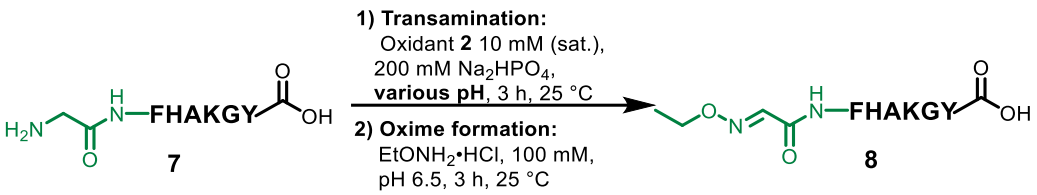


Figure 53: Low-resolution MS spectrum of desired peptide product **8**, *m/z* (ESI⁺) calcd M_{mono} = 820.4, found 821.6 $[M + H]^+$, 411.5 $[M + 2H]^{2+}$.

3.1.2. pH optimization for transamination of model peptide with quinone **2**



Model peptide **7** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at the described pH, followed by the addition of oxidant **2** (1.7 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analysed with LCMS to deduce the conversion rate. The entire experiment was repeated for three times.

Table 2: A summary of pH optimization experiment with quinone **2**.

pH	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
4.0	87.3	89.5	89.6	88.8	1.1
5.0	88.4	83.6	82.8	84.9	2.5
5.5	83.8	85.3	79.9	83.0	2.3
6.0	49.6	69.4	61.1	60.0	8.1
6.5	38	55.1	53.3	48.8	7.7
7.0	60.9	44.6	36	47.2	10.3
7.5	42	33.2	36.4	37.2	3.6
8.0	2.3	17.5	6.7	8.8	6.4

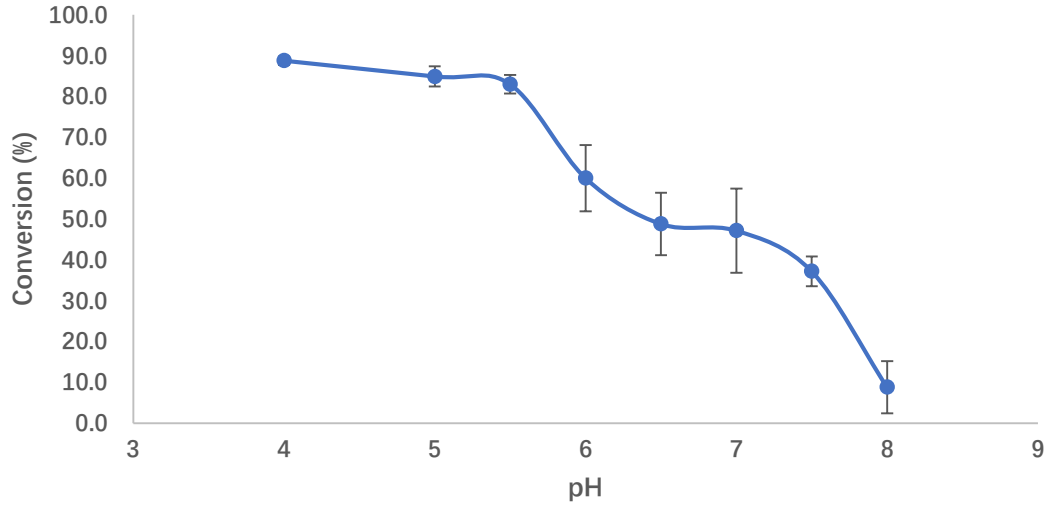


Figure 54: A summary chart of pH optimization of oxidant **2** transamination.

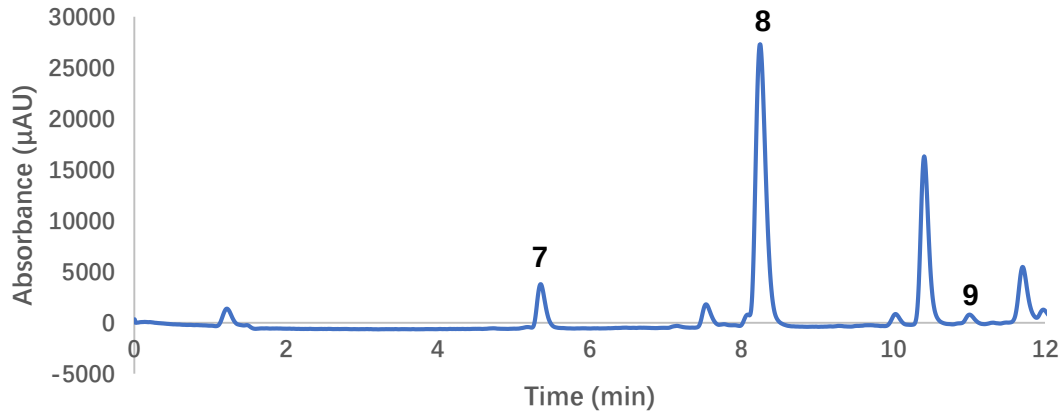


Figure 55: Analytical HPLC-MS trace of reaction at pH 4.0. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min; side product **9**: t_R = 11.0 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

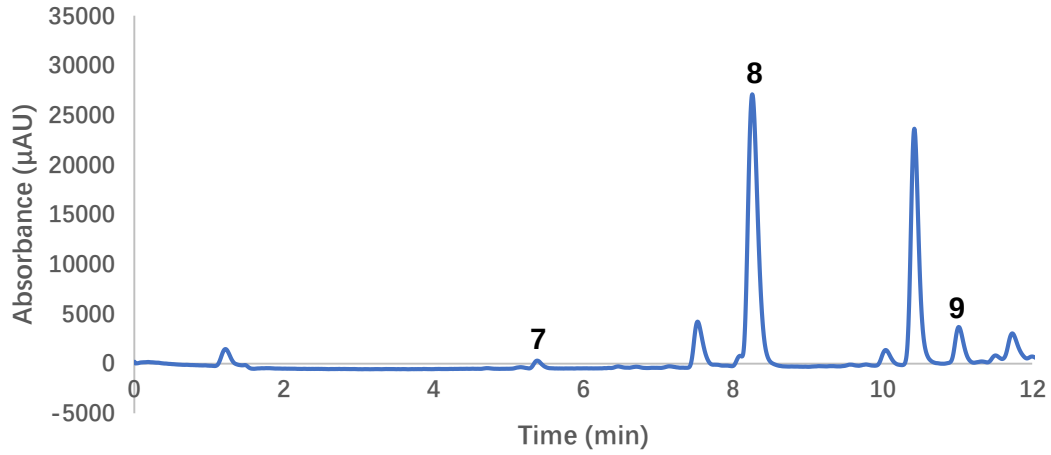


Figure 56: Analytical HPLC-MS trace of reaction at pH 5.0. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min; side product **9**: t_R = 11.0 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

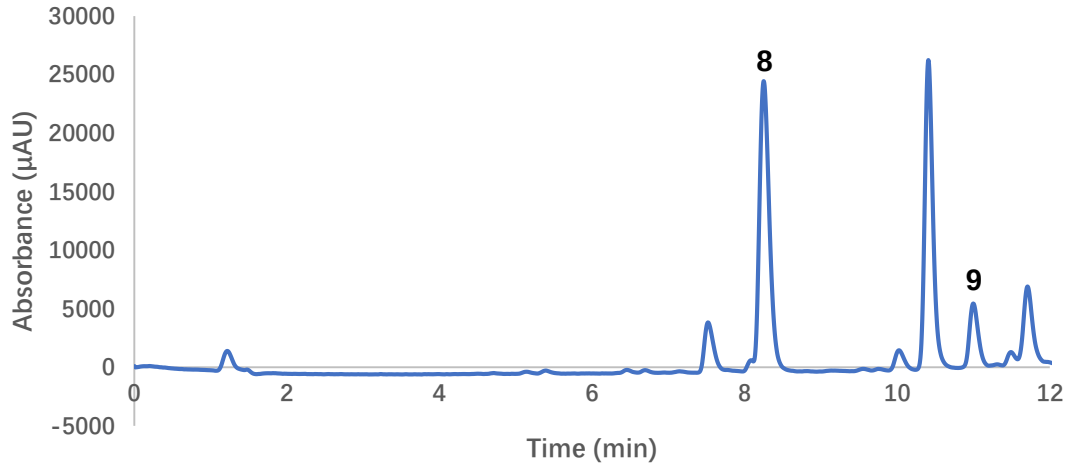


Figure 57: Analytical HPLC-MS trace of reaction at pH 5.5. Starting peptide **7** (hardly observed); desired product **8**: t_R = 8.3 min; side product **9**: t_R = 11.0 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

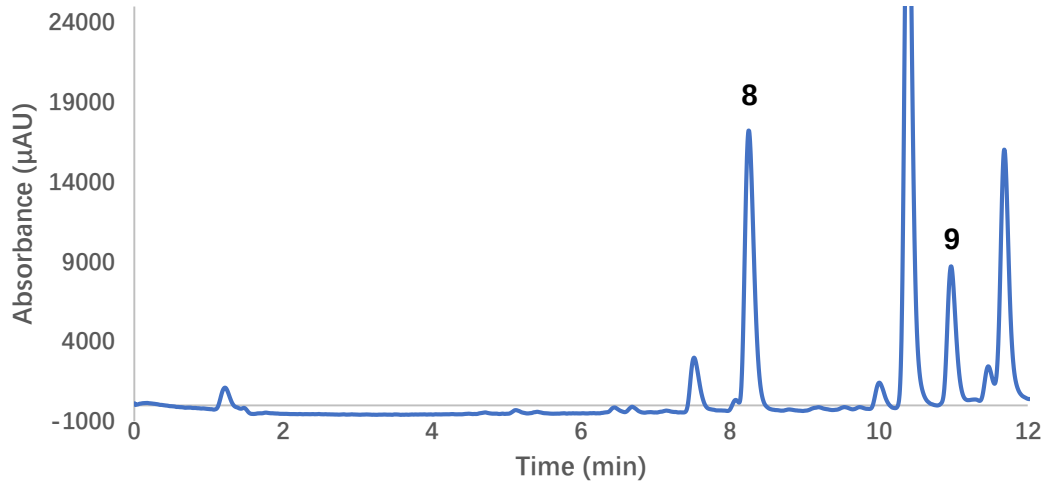


Figure 58: Analytical HPLC-MS trace of reaction at pH 6.0. Starting peptide **7** (hardly observed); desired product **8**: t_R = 8.3 min; side product **9**: t_R = 11.0 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

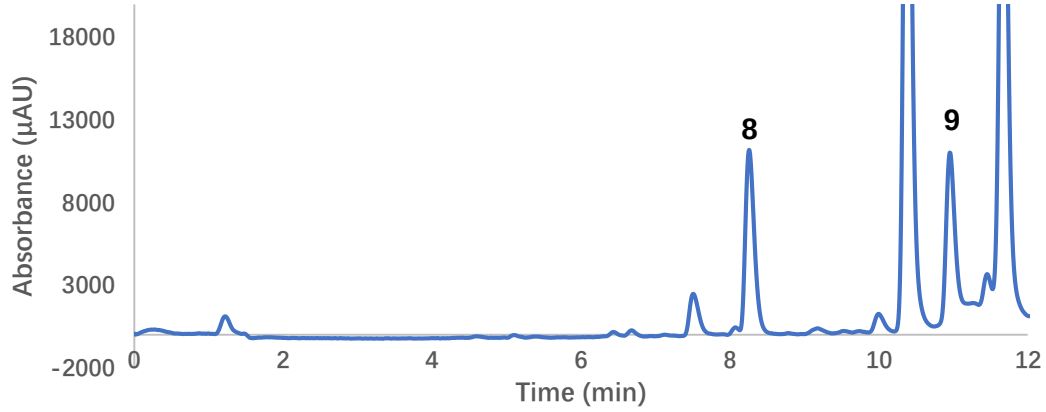


Figure 59: Analytical HPLC-MS trace of reaction at pH 6.5. Starting peptide **7** (hardly observed); desired product **8**: t_R = 8.3 min; side product **9**: t_R = 11.0 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

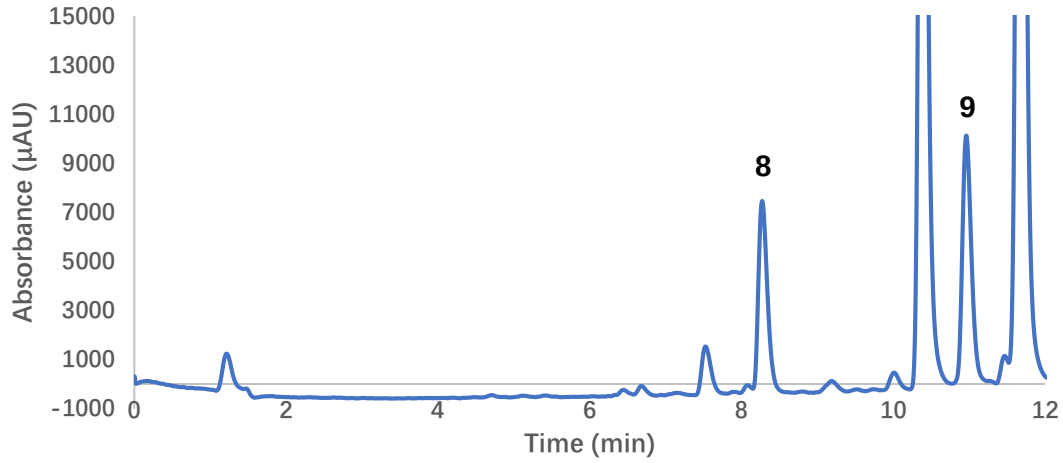


Figure 60: Analytical HPLC-MS trace of reaction at pH 7.0. Starting peptide **7** (hardly observed); desired product **8**: t_R = 8.3 min; side product **9**: t_R = 11.0 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

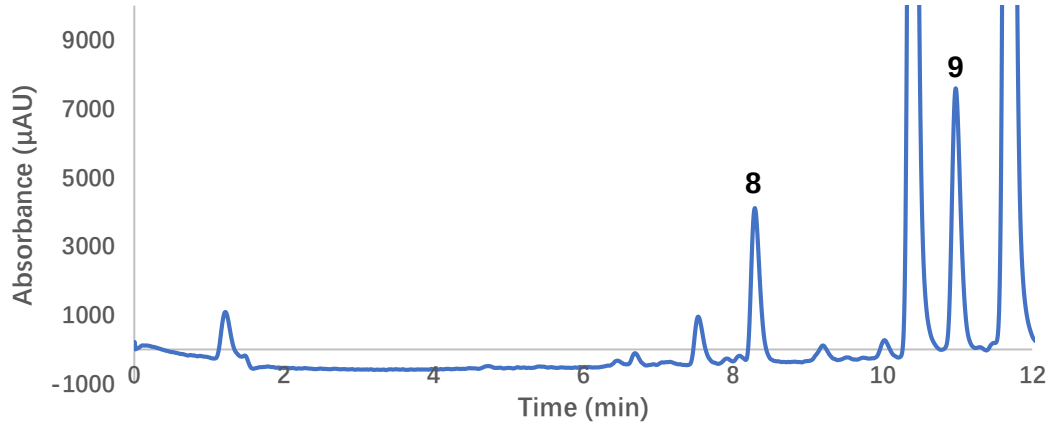


Figure 61: Analytical HPLC-MS trace of reaction at pH 8.0. Starting peptide **7** (hardly observed); desired product **8**: t_R = 8.3 min; side product **9**: t_R = 11.0 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

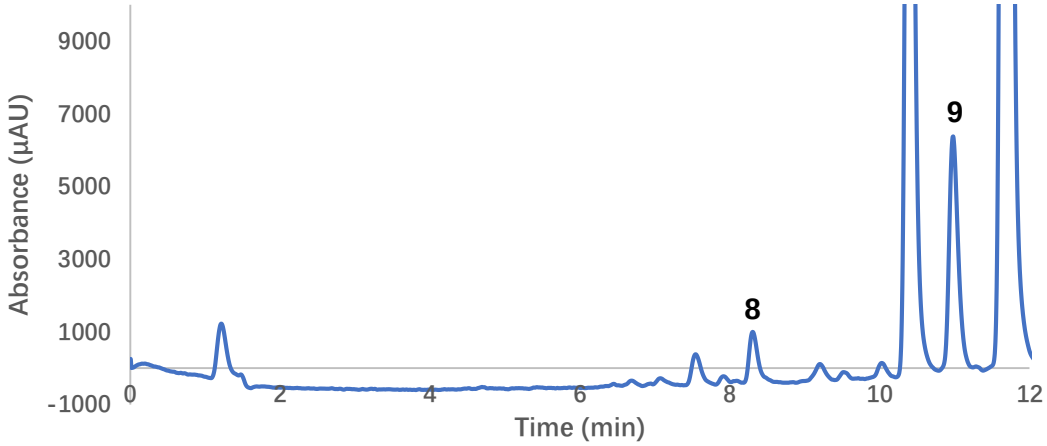
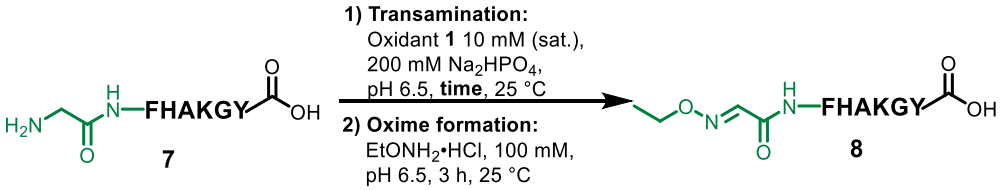


Figure 62: Analytical HPLC-MS trace of reaction at pH 8.0. Starting peptide **7** (hardly observed); desired product **8**: t_R = 8.3 min; side product **9**: t_R = 11.0 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

3.1.3. Time course experiment for transamination of model peptide with quinone **1**



Model peptide **7** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at the described pH, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C. Time points were taken by quenching 20 µL of reaction mixture with 20 µL of $EtONH_2$·HCl (0.2 M, pH 6.5) and incubated at 25 °C for another 3 h. The reaction mixture was analysed with LCMS to deduce the conversion rate. The entire experiment was repeated for three times.

Table 3: Results for time course experiment (3 repeats).

Time (min)	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
5	14.3	8.6	11.8	11.6	2.33
10	23.3	20.7	27.6	23.9	2.85
15	33.0	33.0	37.0	34.3	1.89
20	39.8	37.5	45.3	40.9	3.27
25	45.2	44.8	50.4	46.8	2.55
30	49.3	49.7	56.5	51.8	3.30
35	54.2	54.8	60.2	56.4	2.70
40	57.8	58.4	63.2	59.8	2.42
45	60.9	61.8	66.9	63.2	2.64
50	64.0	64.8	70.8	66.5	3.03
60	68.3	69.8	75.2	71.1	2.96
70	71.9	75.2	77.7	74.9	2.38

80	75.2	78.3	81.4	78.3	2.53
90	77.7	80.5	82.7	80.3	2.05
100	80.0	83.1	85.2	82.8	2.14
120	82.9	86.7	88.0	85.9	2.16
140	86.5	88.8	89.9	88.4	1.42
160	86.9	90.8	90.3	89.3	1.73
180	89.3	92.3	91.9	91.2	1.33

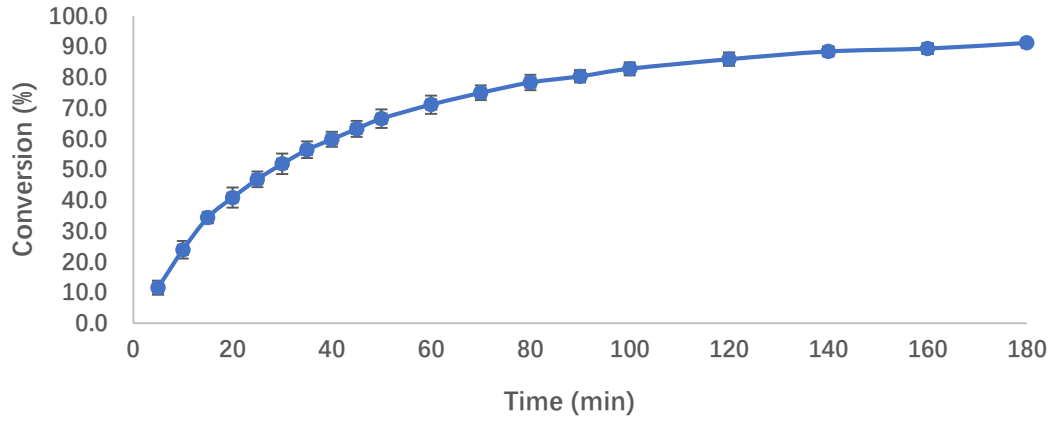


Figure 63: Results of time course experiment of quinone **1**.

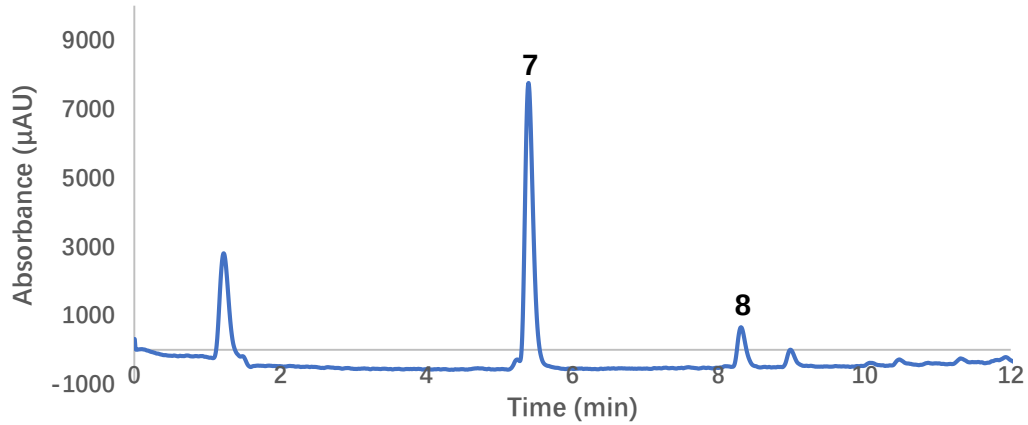


Figure 64: Analytical HPLC-MS trace of the reaction at 5 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

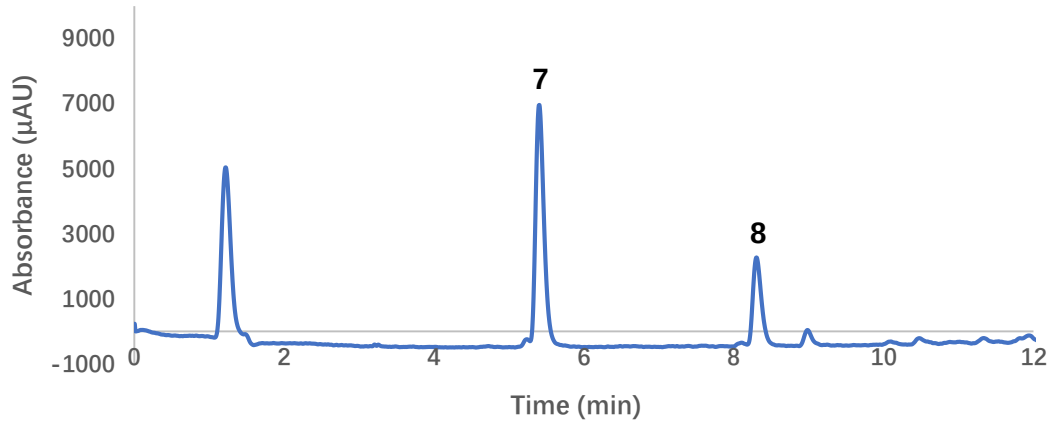


Figure 65: Analytical HPLC-MS trace of the reaction at 10 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

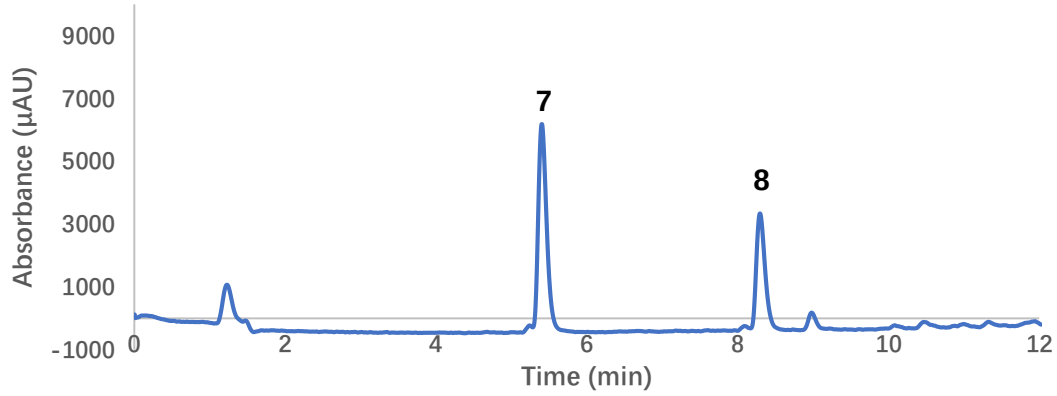


Figure 66: Analytical HPLC-MS trace of the reaction at 15 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

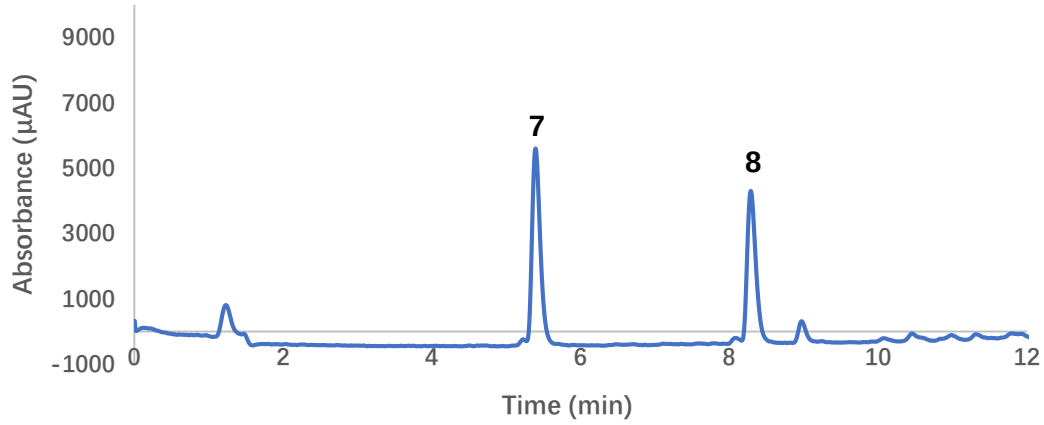


Figure 67: Analytical HPLC-MS trace of the reaction at 20 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

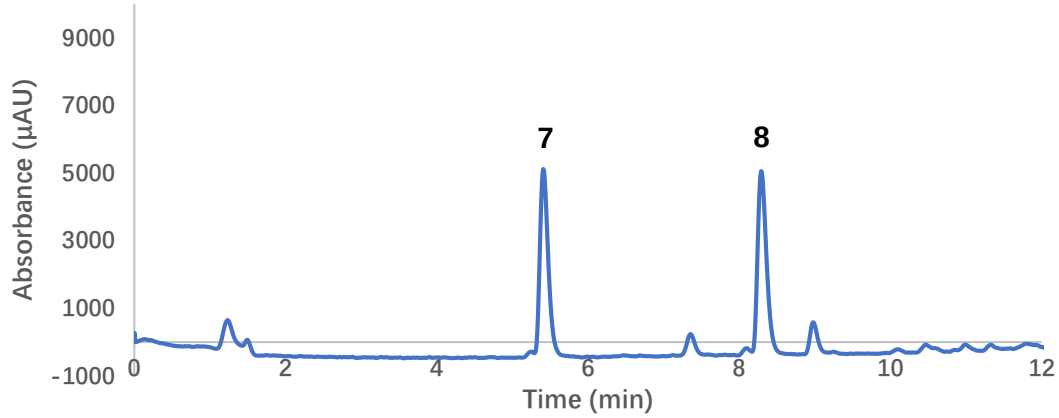


Figure 68: Analytical HPLC-MS trace of the reaction at 25 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

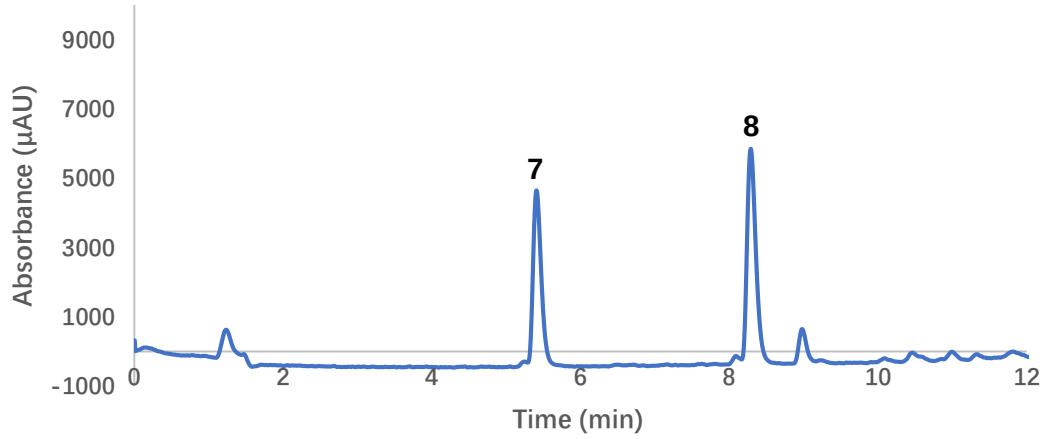


Figure 69: Analytical HPLC-MS trace of the reaction at 30 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

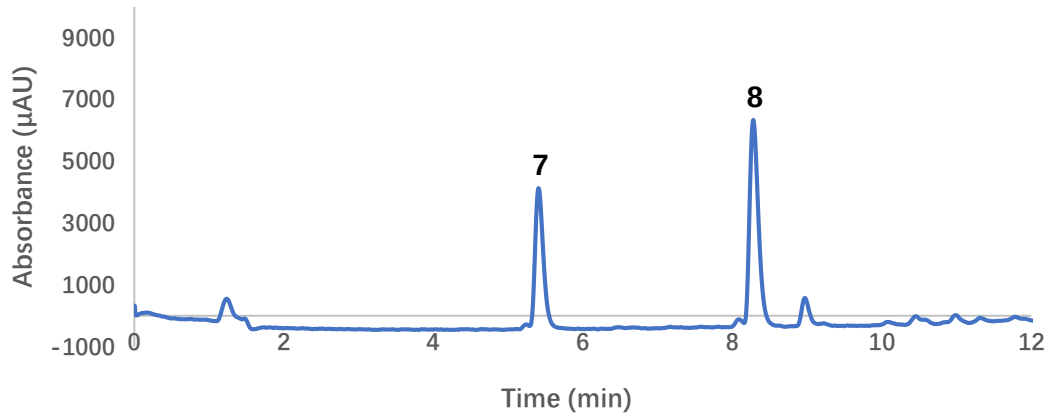


Figure 70: Analytical HPLC-MS trace of the reaction at 35 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

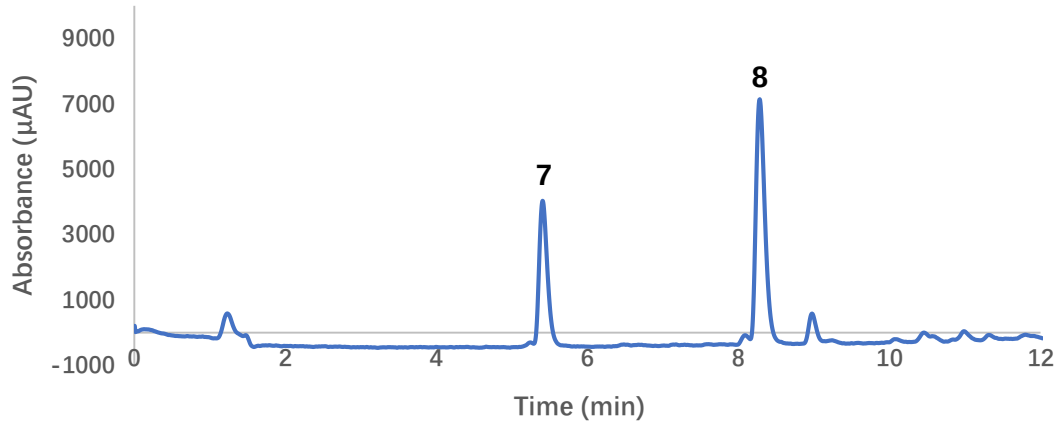


Figure 71: Analytical HPLC-MS trace of the reaction at 40 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

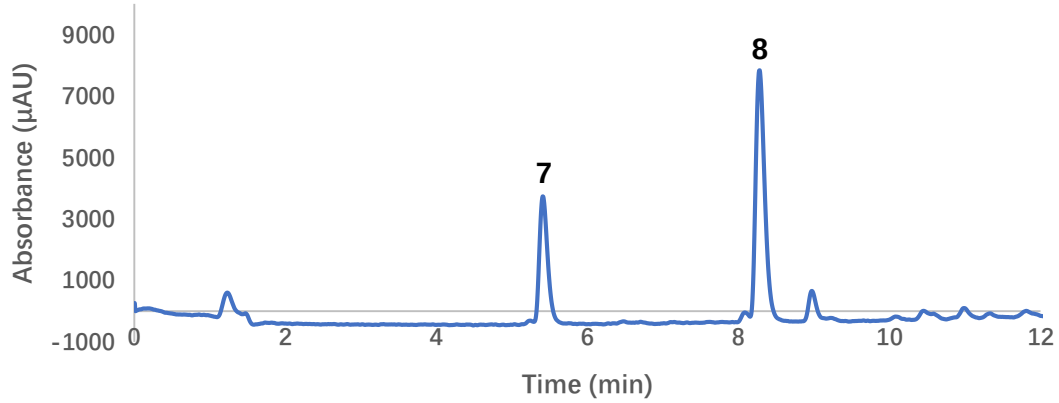


Figure 72: Analytical HPLC-MS trace of the reaction at 45 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

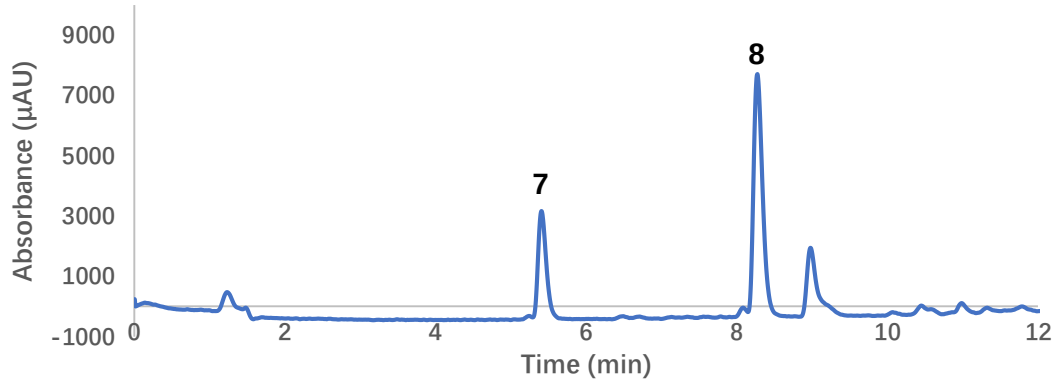


Figure 73: Analytical HPLC-MS trace of the reaction at 50 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

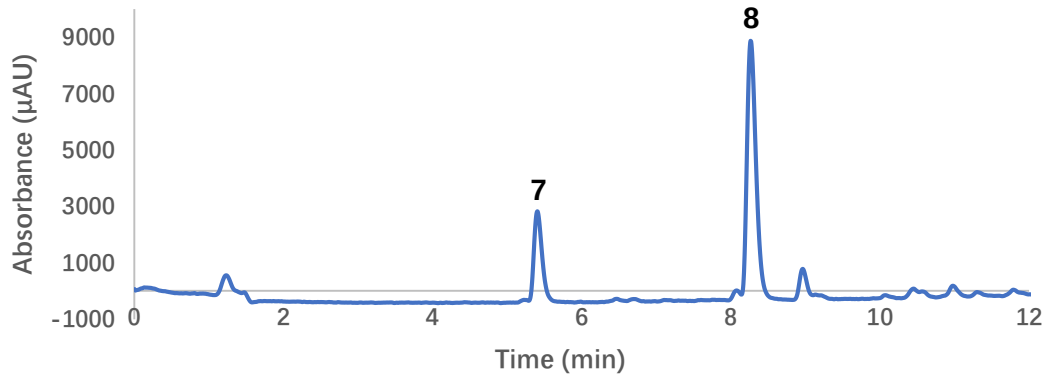


Figure 74: Analytical HPLC-MS trace of the reaction at 60 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

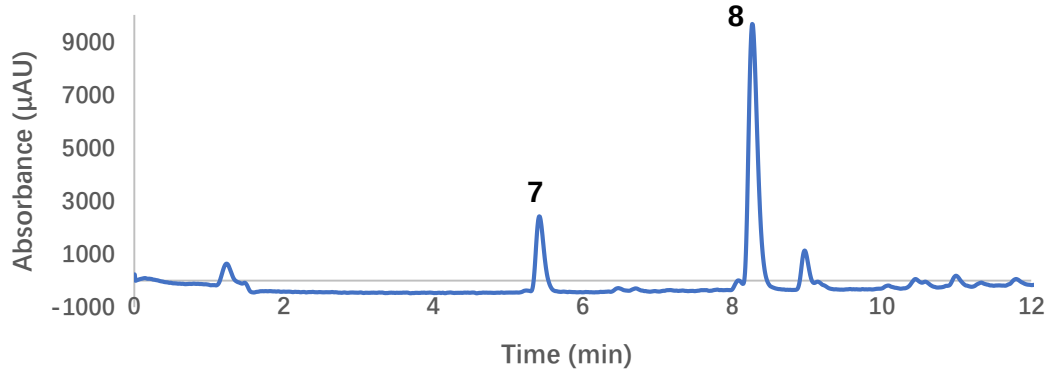


Figure 75: Analytical HPLC-MS trace of the reaction at 70 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

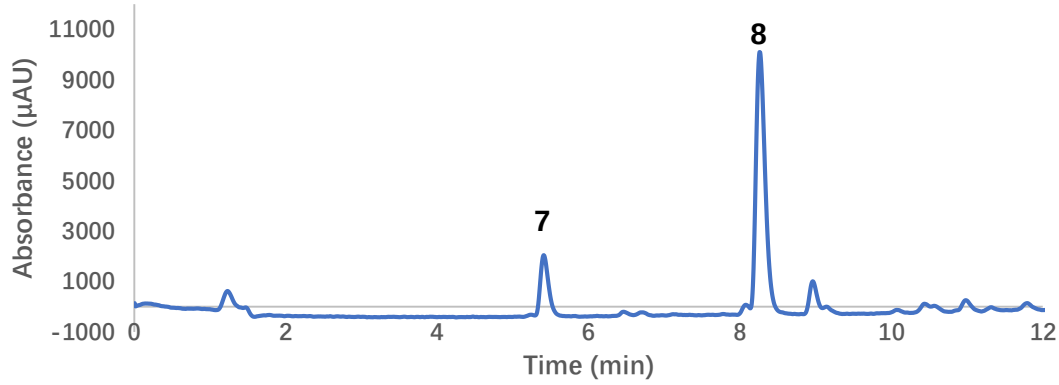


Figure 76: Analytical HPLC-MS trace of the reaction at 80 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

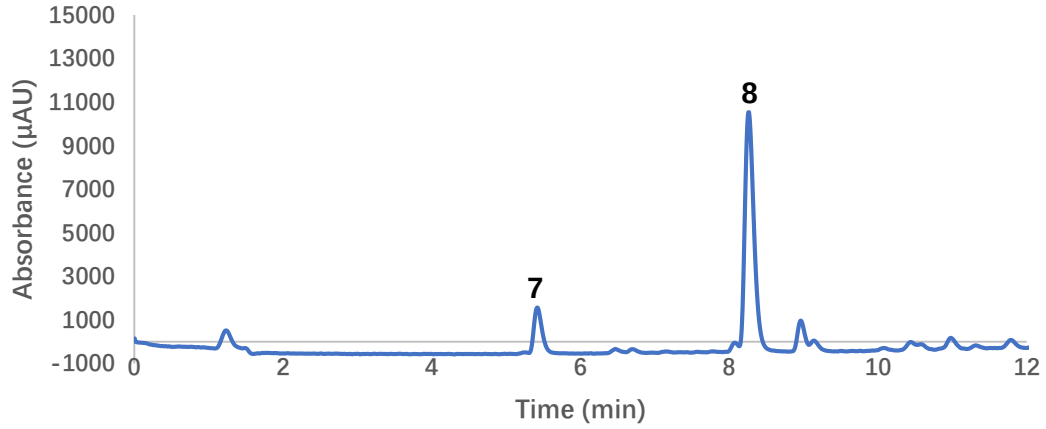


Figure 77: Analytical HPLC-MS trace of the reaction at 90 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

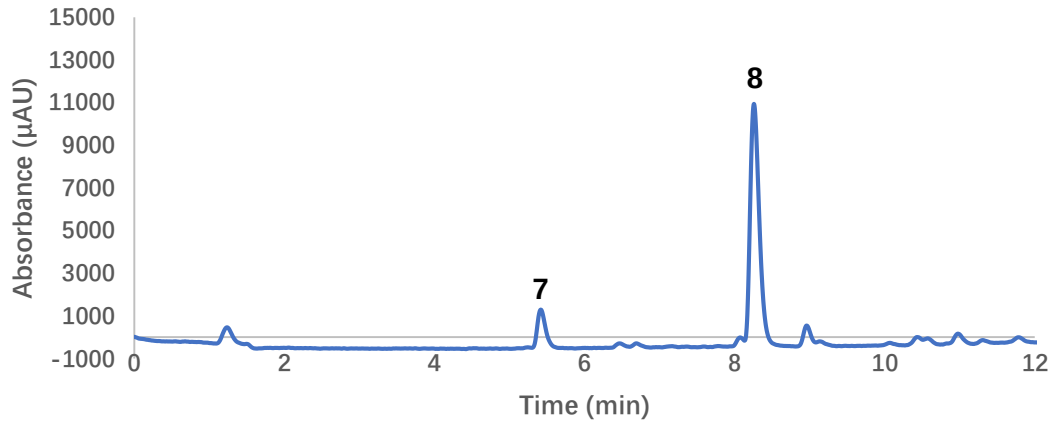


Figure 78: Analytical HPLC-MS trace of the reaction at 100 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

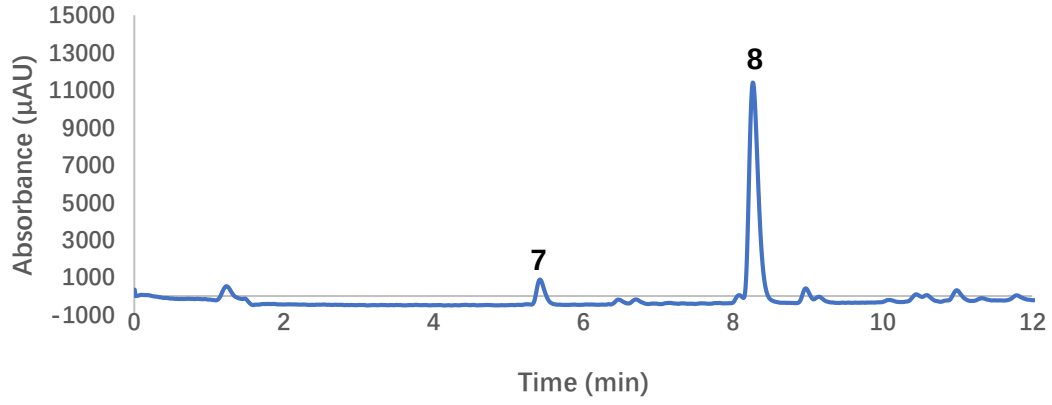


Figure 79: Analytical HPLC-MS trace of the reaction at 120 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

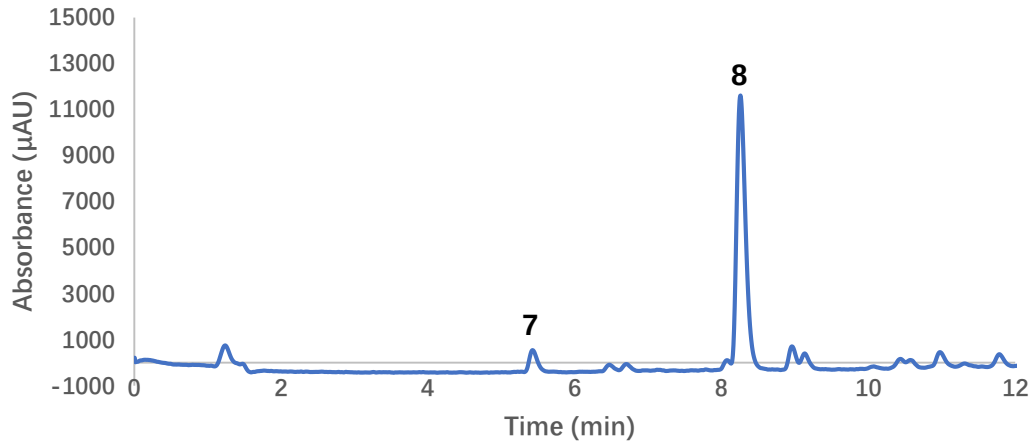


Figure 80: Analytical HPLC-MS trace of the reaction at 140 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

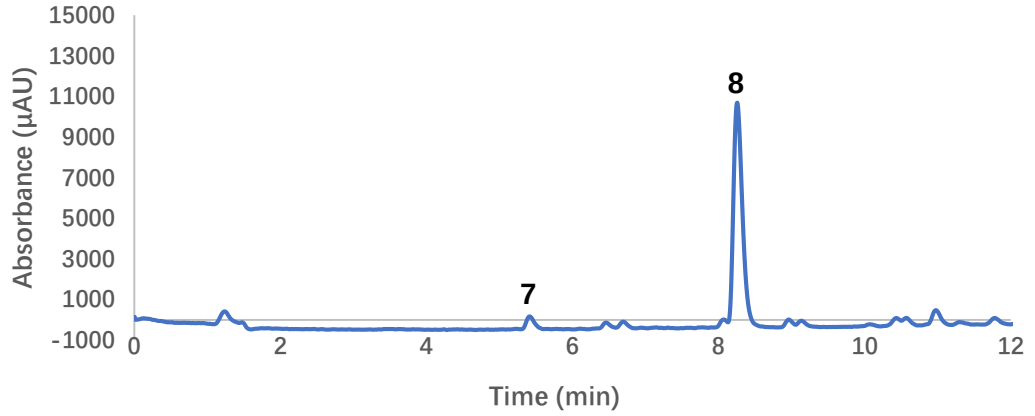


Figure 81: Analytical HPLC-MS trace of the reaction at 160 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

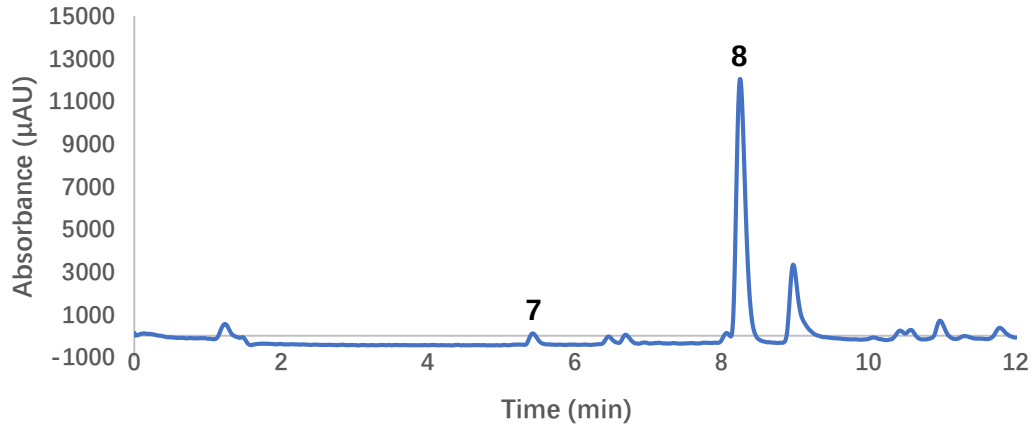
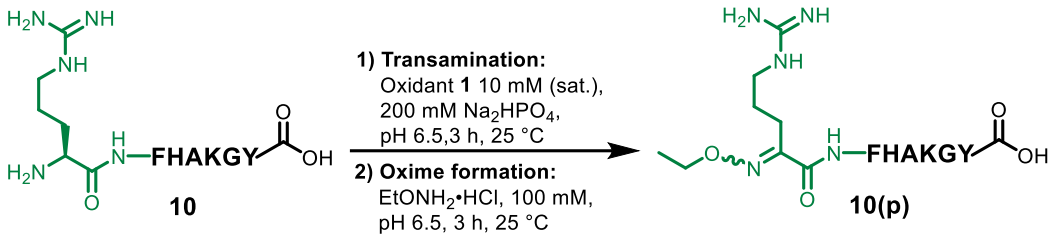


Figure 82: analytical HPLC-MS trace of the reaction at 180 min. Starting peptide **7**: t_R = 5.4 min; desired product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

3.2. Transamination of other model peptides **10-28**



Arg peptide **10** (1.0 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

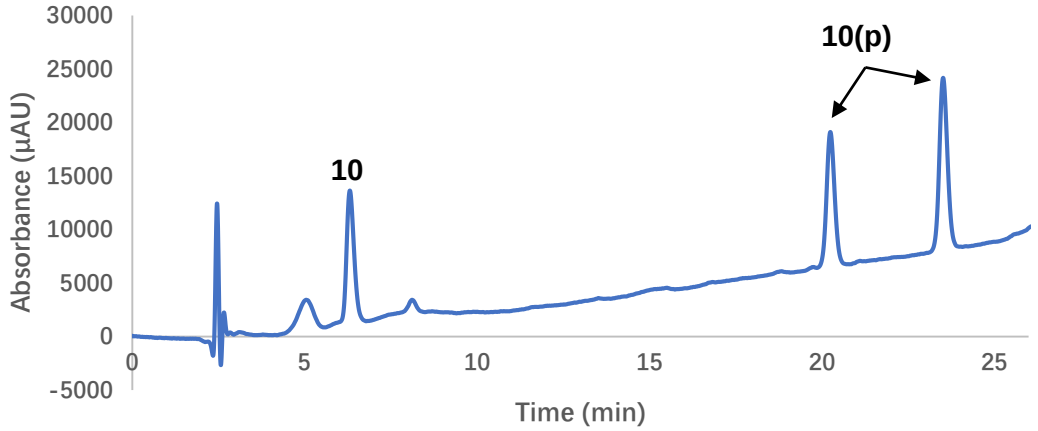


Figure 83: Analytical HPLC-MS trace of the transamination of Arg peptide **10**. Starting peptide **10**: t_R = 6.3 min; desired product **10(p)** peak 1: t_R = 20.2 min; desired product **10(p)** peak 2: t_R = 23.5 min. (0% B for 1 min and then 0 to 15% B over 25 min with a flow rate of 0.2 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

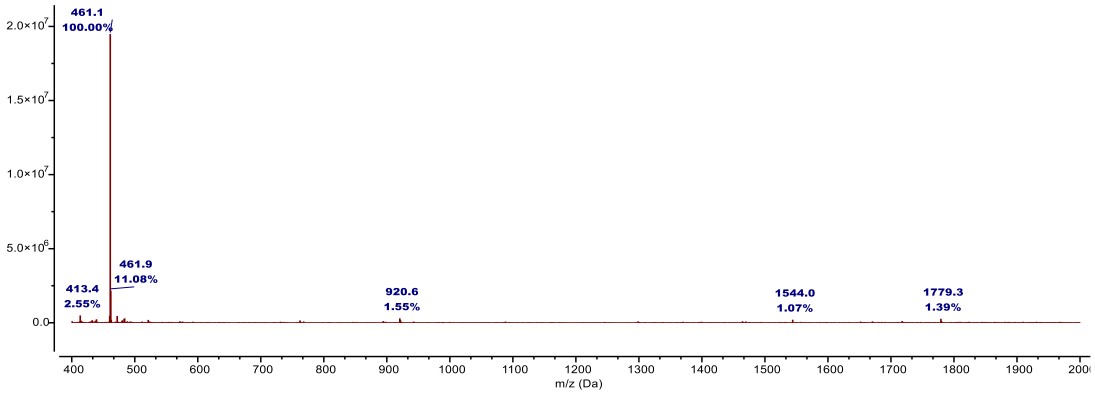


Figure 84: Low-resolution MS spectrum of desired peptide product **10(p)** peak 1 (extracted at 20.2 min), *m/z* (ESI⁺) calcd M_{mono} = 919.5, found 920.6 $[M + H]^+$, 461.1 $[M + 2H]^{2+}$.

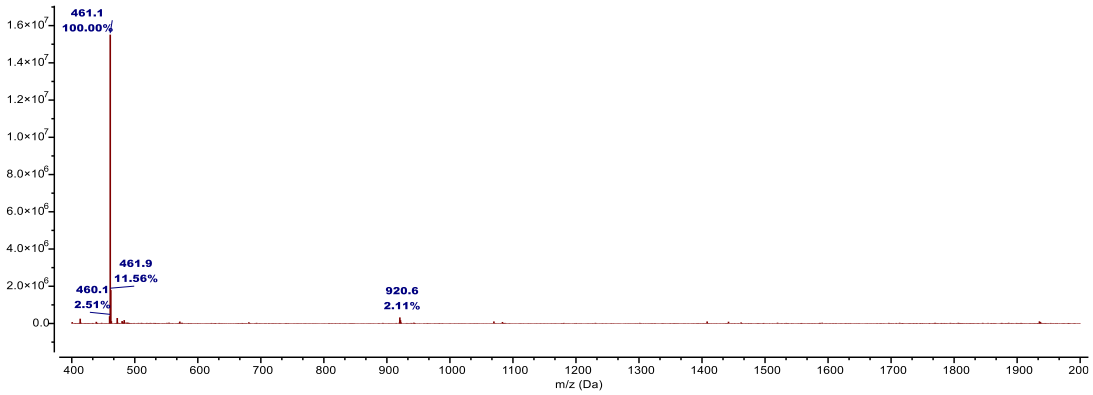
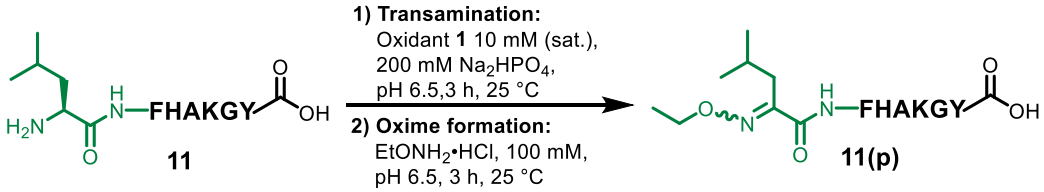


Figure 85: Low-resolution MS spectrum of desired peptide product **10(p)** peak 2 (extracted at 23.5 min), *m/z* (ESI⁺) calcd M_{mono} = 919.5, found 920.6 $[M + H]^+$, 461.1 $[M + 2H]^{2+}$.

Table 4: A summary of the transamination of Arg peptide **10** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **10(p)**	70.3	70.6	72.9	71.3	1.2



Leu peptide **11** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

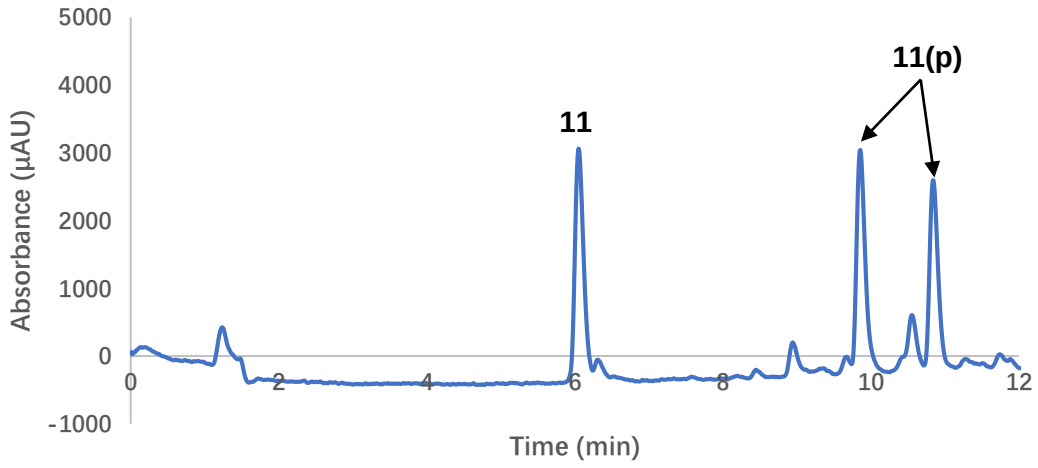


Figure 86: Analytical HPLC-MS trace of the transamination of Leu peptide **11**. Starting peptide **11**: t_R = 6.0 min; desired product **11(p)** peak1: t_R = 9.8 min; desired product **11(p)** peak 2: t_R = 10.8 min. (0% B

for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

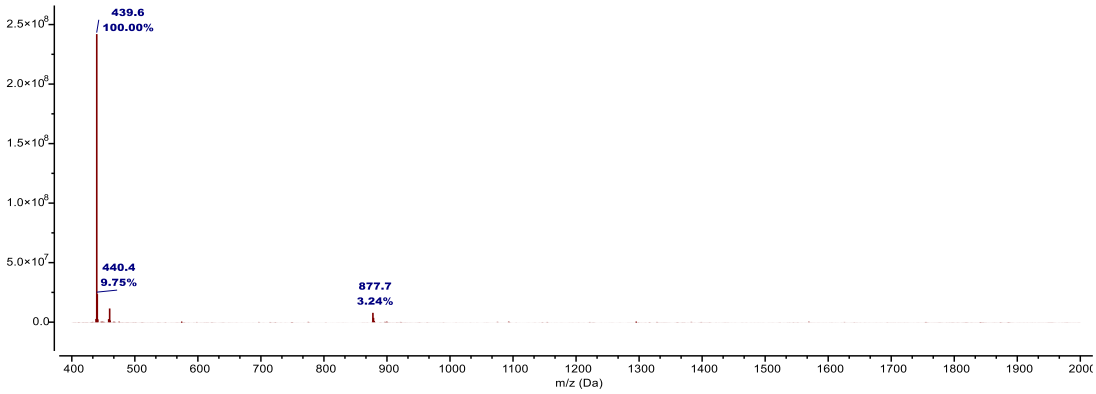


Figure 87: Low-resolution MS spectrum of desired peptide product **11(p)** peak 1 (extracted at 9.8 min), *m/z* (ESI⁺) calcd M_{mono} = 876.4, found 877.7 $[M + H]^+$; 439.6 $[M + 2H]^{2+}$.

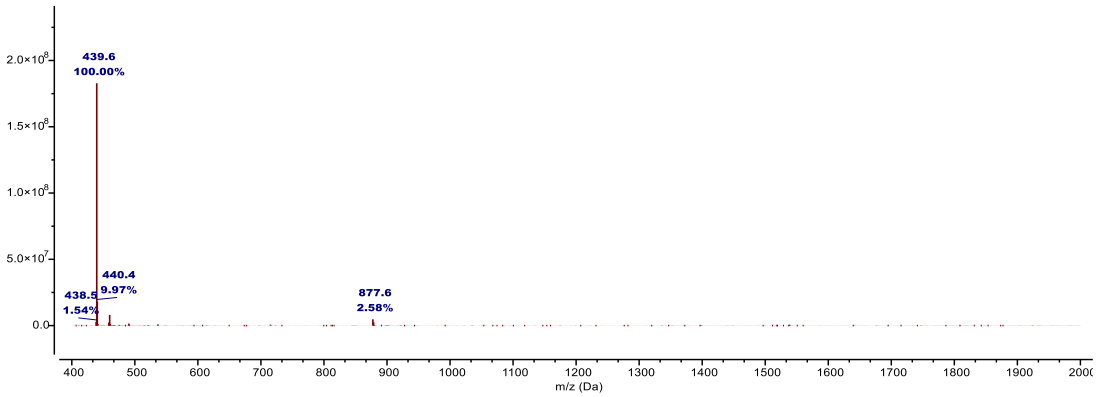
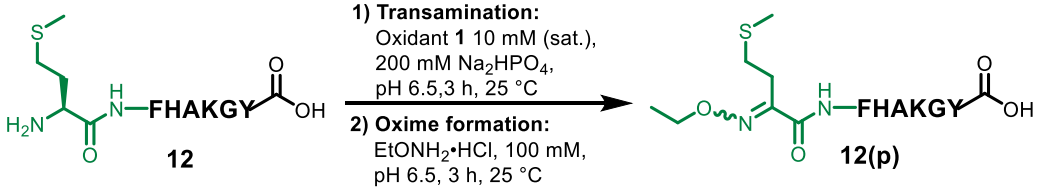


Figure 88: Low-resolution MS spectrum of desired peptide product **11(p)** peak 2 (extracted at 10.8 min), *m/z* (ESI⁺) calcd M_{mono} = 876.4, found 877.6 $[M + H]^+$; 439.6 $[M + 2H]^{2+}$.

Table 5: A summary of the transamination of Leu peptide **11** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **11(p)**	68.5	71.4	68.4	69.4	1.4



Met peptide **12** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

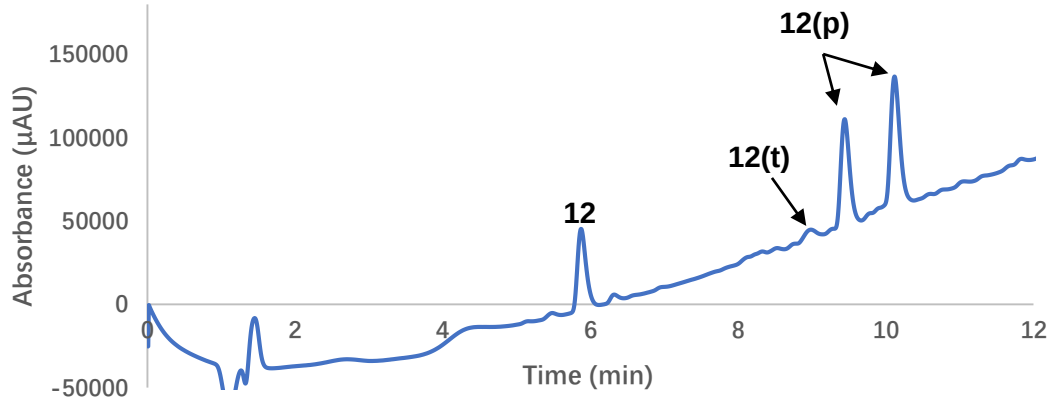


Figure 89: Analytical HPLC-MS trace of the transamination of Met peptide **12** at **230** nm. Starting peptide **12**: t_R = 5.9 min; desired product **12(p)** peak1: t_R = 9.4 min; desired product **12(p)** peak 2: t_R = 10.1 min; transaminated product **12(t)**: t_R = 9.0 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

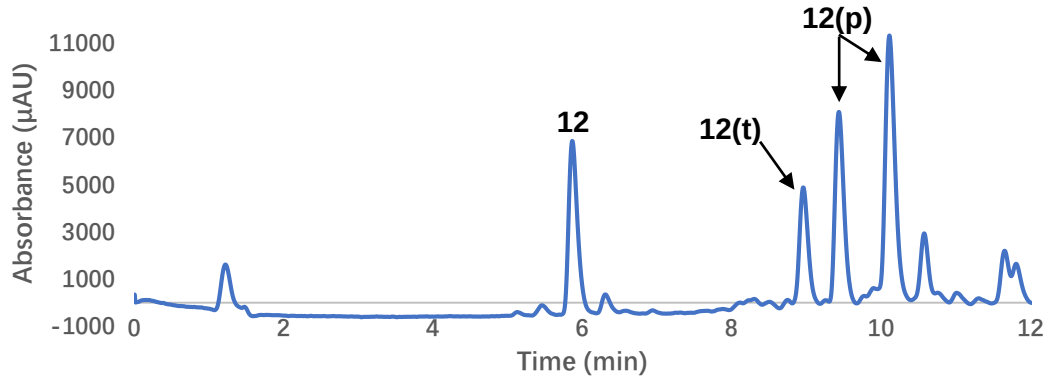


Figure 90: Analytical HPLC-MS trace of the transamination of Met peptide **12** at **280** nm. Starting peptide **12**: t_R = 5.9 min; desired product **12(p)** peak1: t_R = 9.4 min; desired product **12(p)** peak 2: t_R = 10.1 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

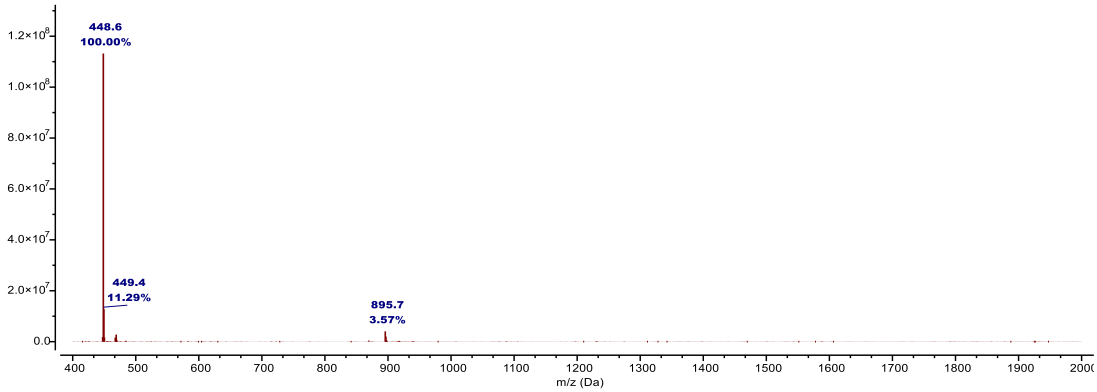


Figure 91: Low-resolution MS spectrum of desired peptide product **12(p)** peak 1 (extracted at 9.4 min), *m/z* (ESI$^+$) calcd M_{mono} = 894.4, found 895.7 $[M + H]^+$; 448.6 $[M + 2H]^{2+}$.

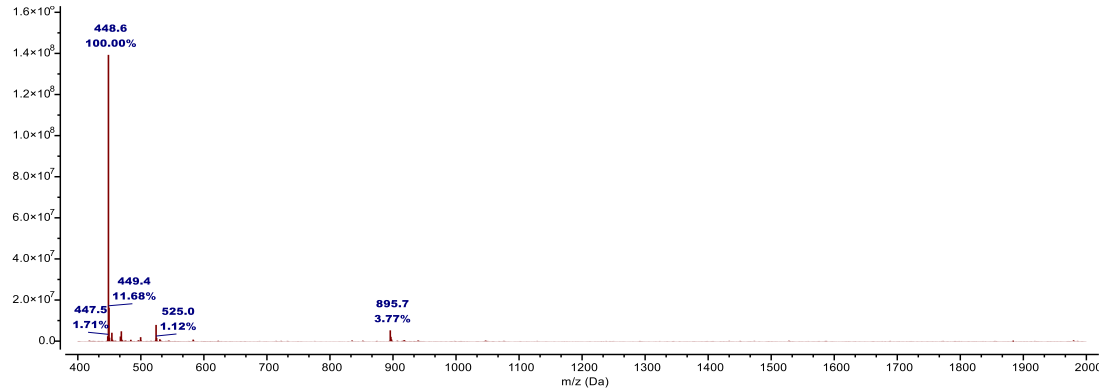
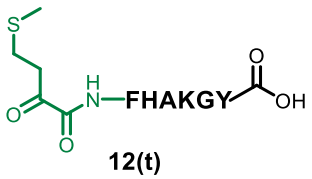


Figure 92: Low-resolution MS spectrum of desired peptide product **12(p)** peak 2 (extracted at 10.1 min), *m/z* (ESI⁺) calcd M_{mono} = 894.4, found 895.7 $[M + H]^+$; 448.6 $[M + 2H]^{2+}$.



12(t)

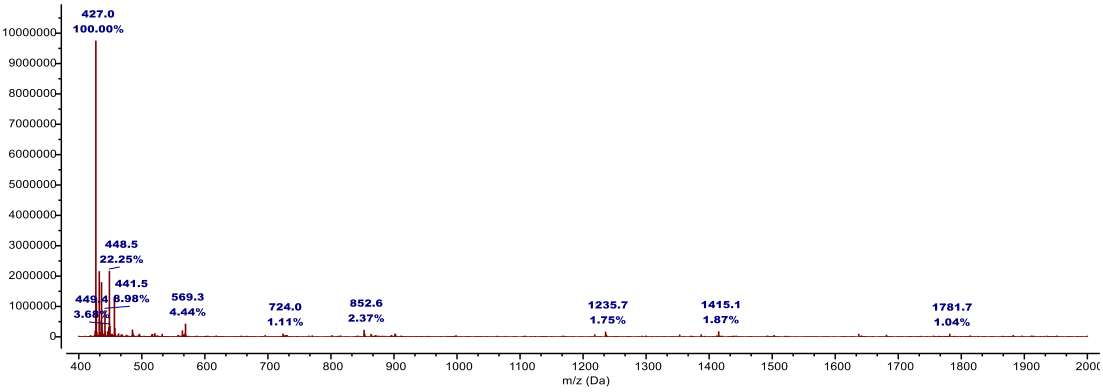
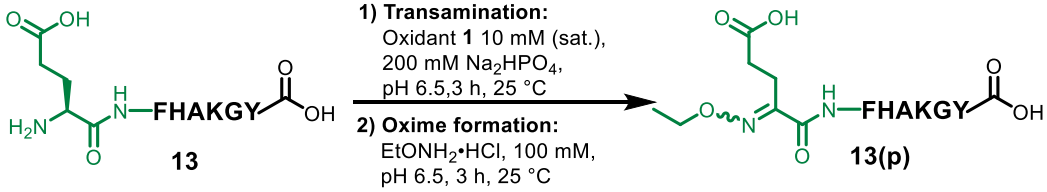


Figure 93: Low-resolution MS spectrum of peptide product **12(t)** (extracted at 9.0 min), *m/z* (ESI⁺) calcd M_{mono} = 851.4, found 852.6 $[M + H]^+$; 427.0 $[M + 2H]^{2+}$.

Table 6: A summary of the transamination of Met peptide **12** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **12(p)**	69	67.2	69.6	68.6	1.0
Product **12(t)**	4	3.6	4	3.9	0.2



13 **13(p)**

Glu peptide **13** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture

was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

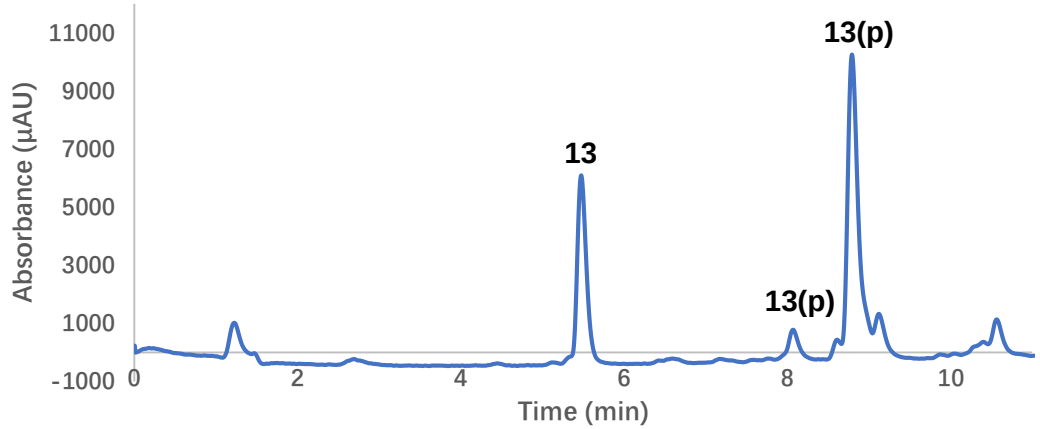


Figure 94: Analytical HPLC-MS trace of the transamination of Glu peptide **13**. Starting peptide **13**: t_R = 5.5 min; desired product **13(p)**: product peak 1:t_R = 8.1 min: product peak 2: t_R = 8.8 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

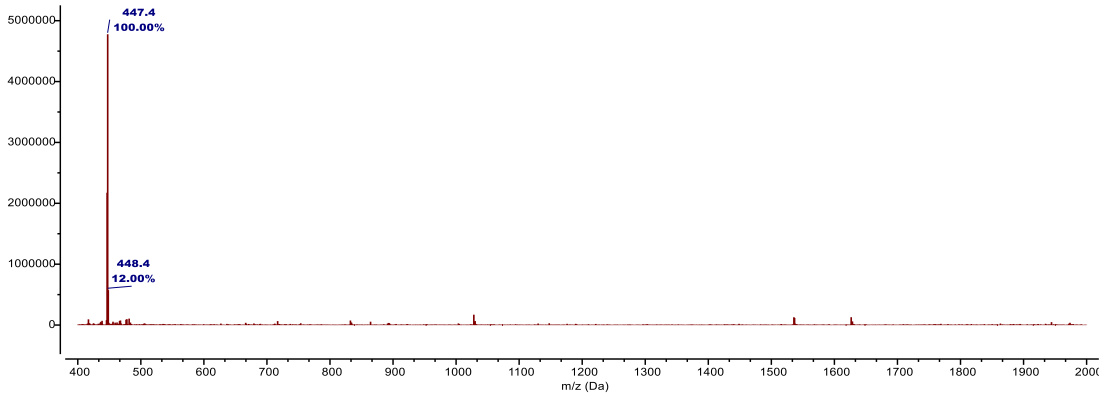


Figure 95: Low-resolution MS spectrum of desired peptide product **13(p)** peak 1 (extracted at 8.0 min), *m/z* (ESI⁺) calcd M_{mono} = 892.4, found 447.4 $[M + 2H]^{2+}$.

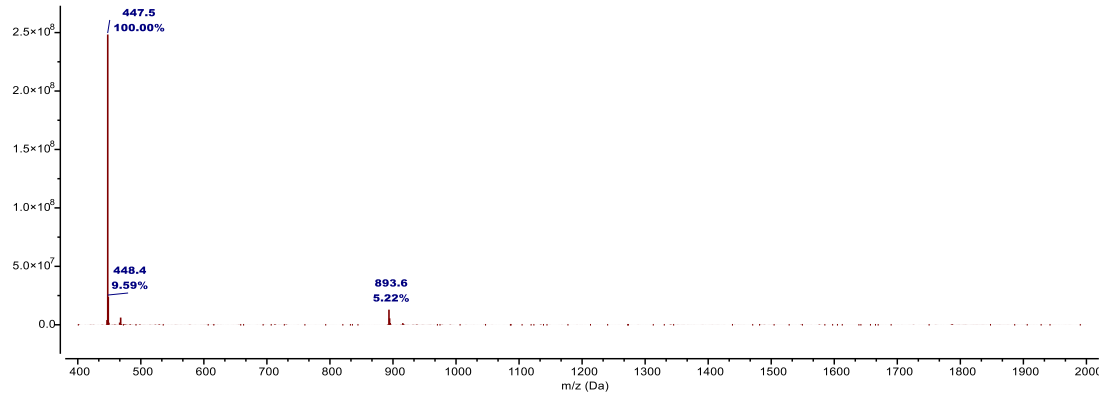
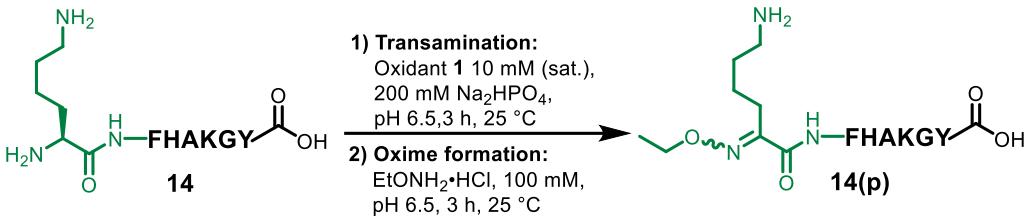


Figure 96: Low-resolution MS spectrum of desired peptide product **13(p)** peak 2 (extracted at 8.8 min),

m/z (ESI$^+$) calcd M_{mono} = 892.4, found 893.6 [*M* + H]$^+$, 447.5 [*M* + 2H]$^{2+}$.

Table 7: A summary of the transamination of Glu peptide **13** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **13(p)**	67.3	65.9	66.7	66.6	0.6



Lys peptide **14** (1.0 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

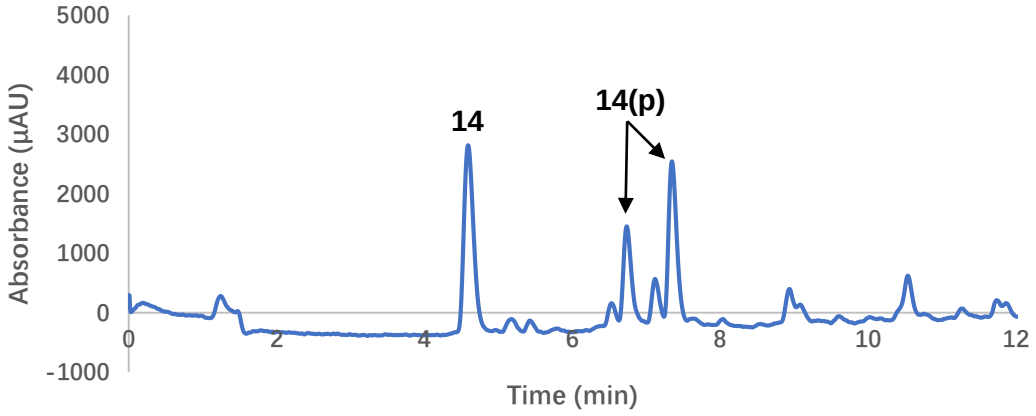


Figure 97: Analytical HPLC-MS trace of the transamination of Lys peptide **14**. Starting peptide **14**: t_R = 4.6 min; desired product **14(p)** peak1: t_R = 6.8 min; desired product **14(p)** peak 2: t_R = 7.4 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

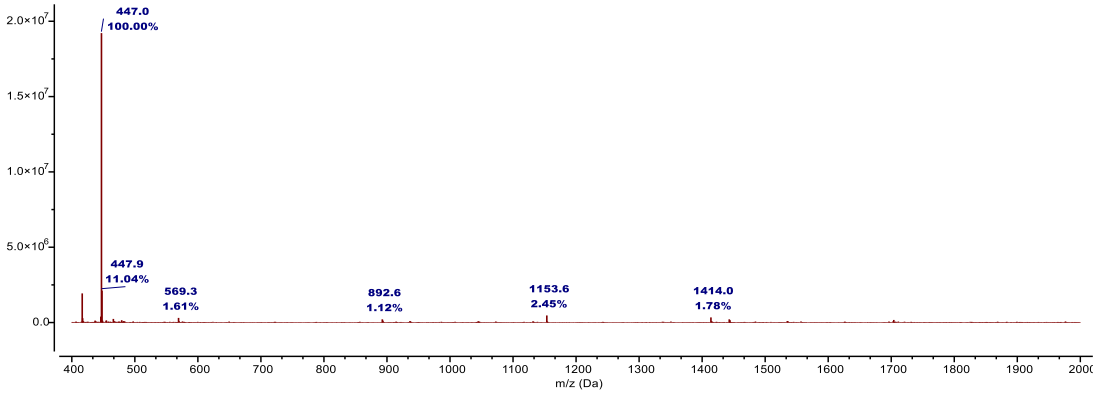


Figure 98: Low-resolution MS spectrum of desired peptide product **14(p)** peak 1 (extracted at 6.8 min), *m/z* (ESI⁺) calcd M_{mono} = 891.5, found 892.6 $[M + H]^+$; 447.0 $[M + 2H]^{2+}$.

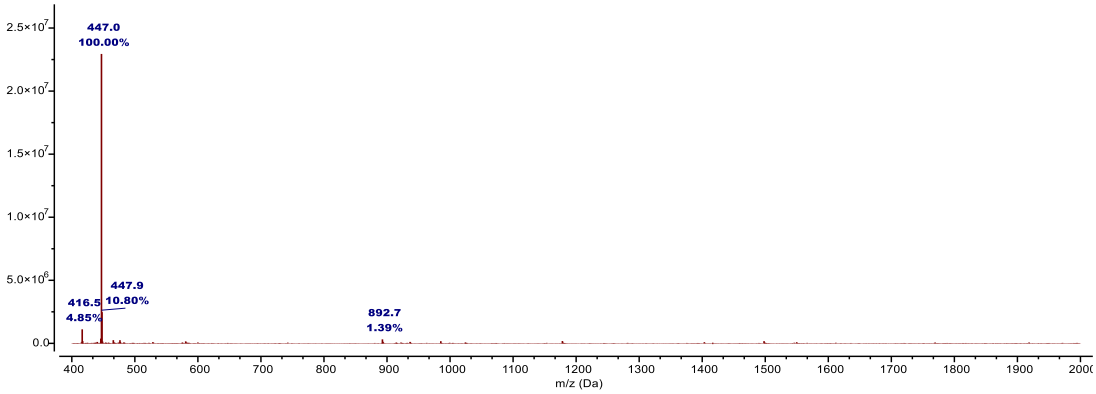
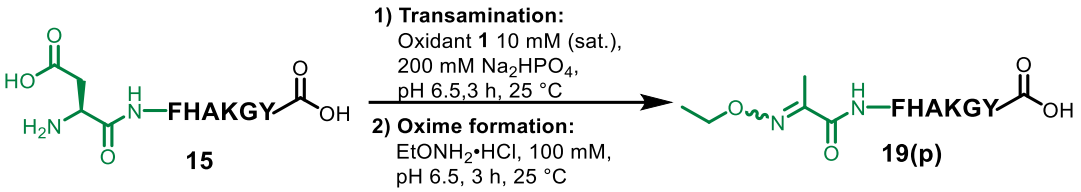


Figure 99: Low-resolution MS spectrum of desired peptide product **14(p)** peak 2 (extracted at 7.4 min), *m/z* (ESI⁺) calcd M_{mono} = 891.5, found 892.7 $[M + H]^+$; 447.0 $[M + 2H]^{2+}$.

Table 8: A summary of the transamination of Lys peptide **14** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **14(p)**	58.7	58.9	59.4	59.0	0.3



Asp peptide **15** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

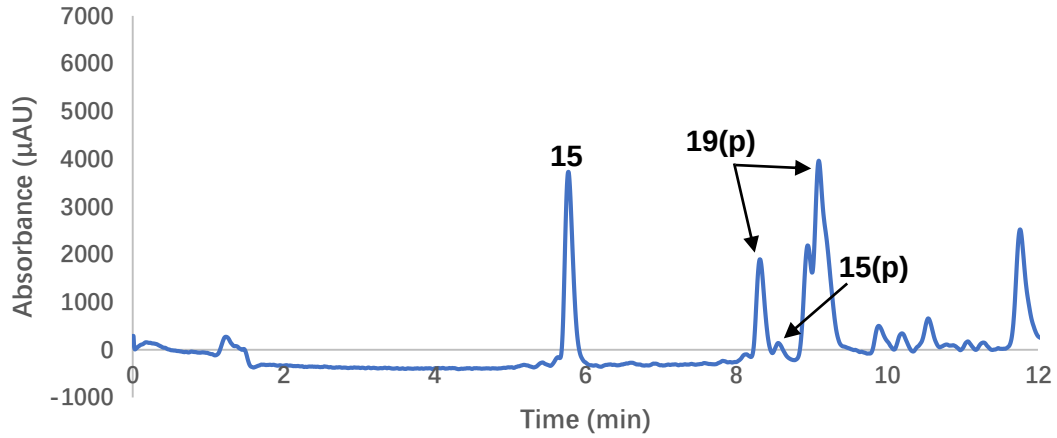


Figure 100: Analytical HPLC-MS trace of the transamination of Asp peptide **15**. Starting peptide **15**: t_R = 5.8 min; product **19(p)** (decarboxylated, which resulted in a compound with the same structure as the product of Ala transamination): product peak 1: t_R = 8.3 min; product peak 2: t_R = 9.1 min, desired product peak **15(p)**: t_R = 8.6 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

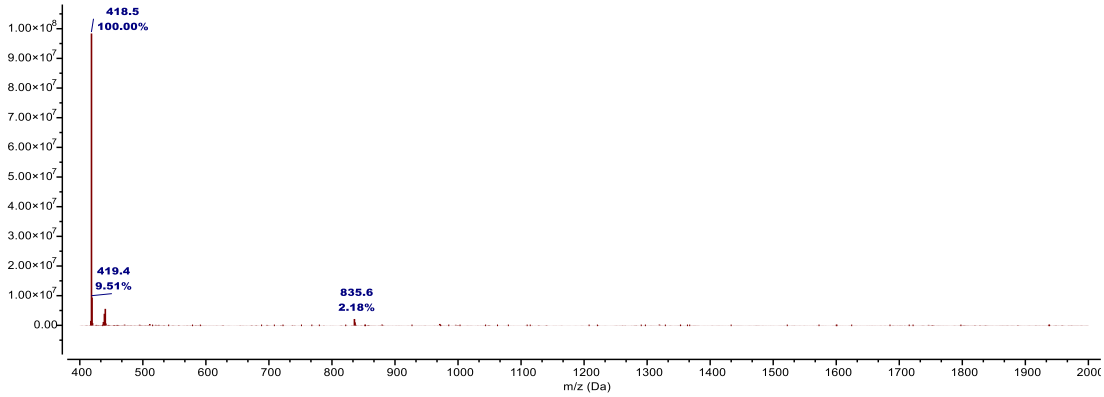


Figure 101: Low-resolution MS spectrum of desired peptide product **19(p)** peak 1 (extracted at 8.3 min), *m/z* (ESI⁺) calcd M_{mono} = 834.4, found 835.6 $[M + H]^+$, 418.5 $[M + 2H]^{2+}$.

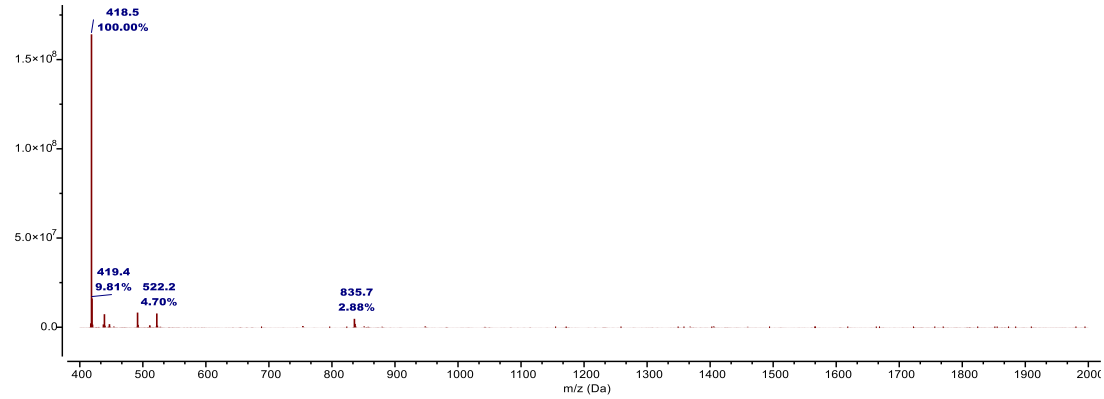
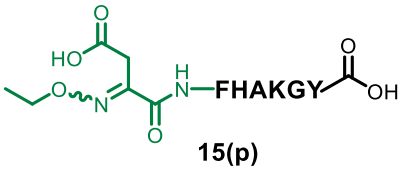


Figure 102: Low-resolution MS spectrum of desired peptide product **19(p)** peak 2 (extracted at 9.1 min), *m/z* (ESI⁺) calcd M_{mono} = 834.4, found 835.7 $[M + H]^+$, 418.5 $[M + 2H]^{2+}$.



15(p)

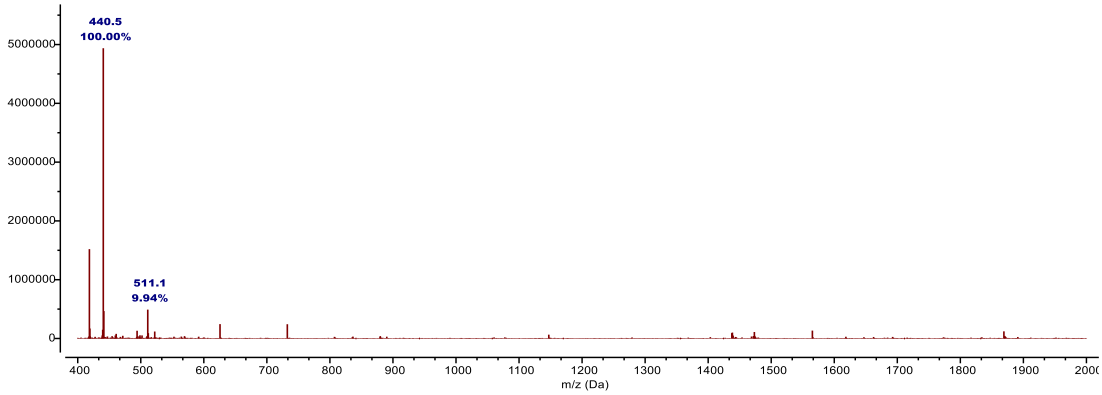
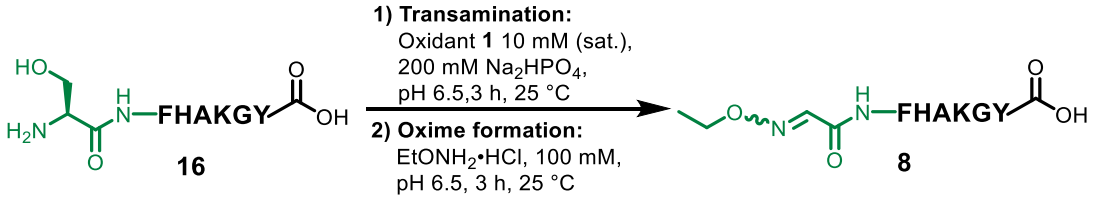


Figure 103: Low-resolution MS spectrum of desired peptide product **15(p)** (extracted at 8.6 min), *m/z* (ESI+) calcd M_{mono} = 878.4, found 440.5 $[M + 2H]^{2+}$.

Table 9: A summary of the transamination of Asp peptide **15** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **19(p)** (decarboxylated)	61.1	56.2	61	59.4	2.3
Product **15(p)**	1.7	2	2	1.9	0.1

The decarboxylated product **19(p)** generated from transamination of Asp peptide **15** aligned with the observation reported by Francis *et al.*[4]



Ser peptide **16** (1.0 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

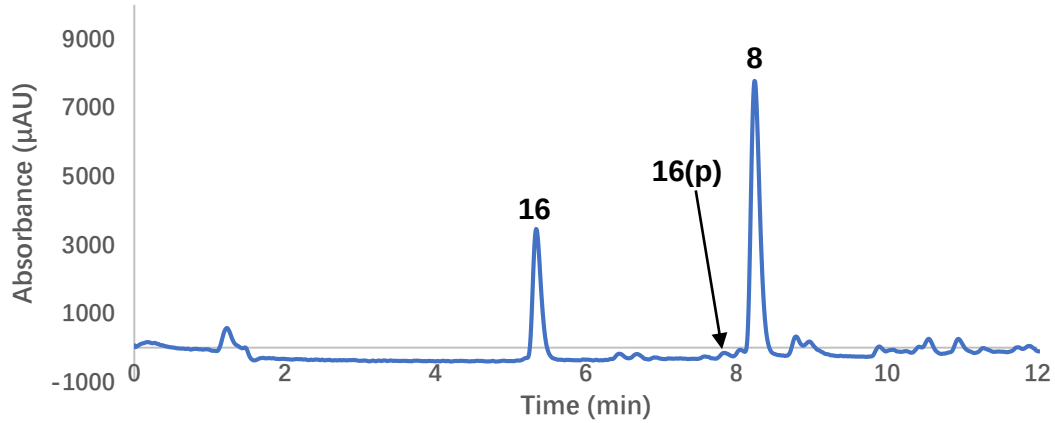


Figure 104: Analytical HPLC-MS trace of the transamination of Ser peptide **16**. Starting peptide **16**: t_R = 5.4 min; side-chain cleaved product **8**: t_R = 8.3 min; product **16(p)**: t_R = 7.8 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

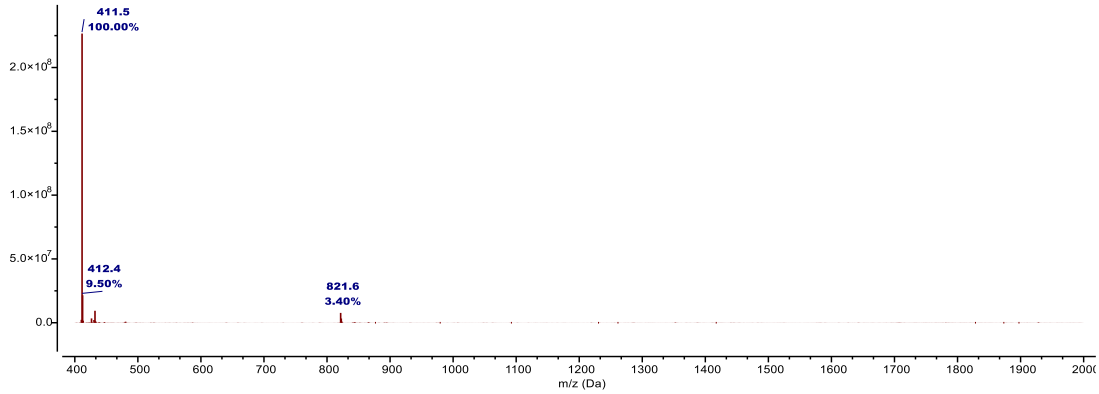
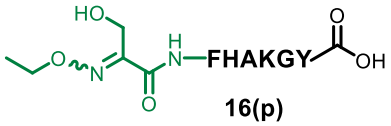


Figure 105: Low-resolution MS spectrum of side-chain cleaved peptide product **8** (extracted at 8.3 min), *m/z* (ESI⁺) calcd M_{mono} = 820.4, found 821.6 $[M + H]^+$, 411.5 $[M + 2H]^{2+}$.



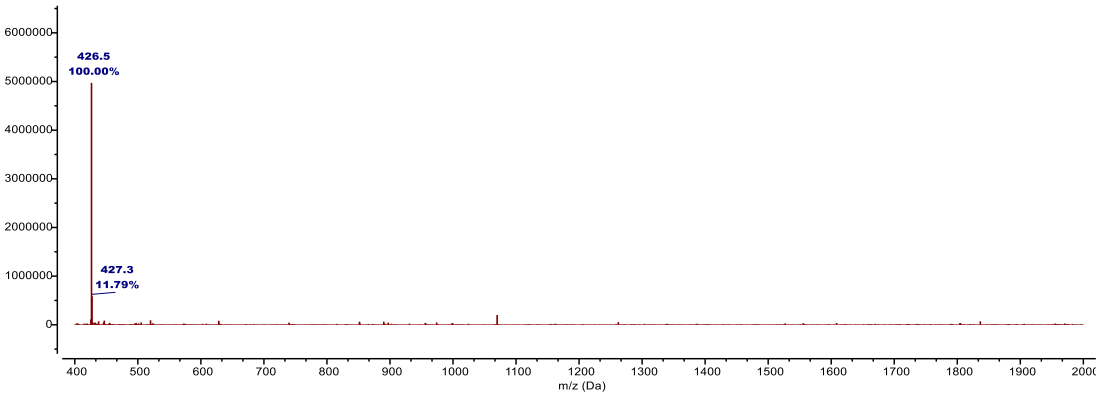
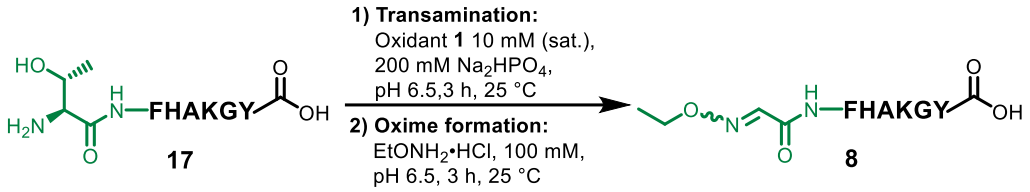


Figure 106: Low-resolution MS spectrum of peptide product **16(p)** (extracted at 7.8 min), *m/z* (ESI⁺)

calcd M_{mono} = 850.4, found 426.5 $[M + 2H]^{2+}$.

Table 10: A summary of the transamination of Ser peptide **16** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **8**	68.4	70.2	70.6	69.7	68.4
Product **16(p)**	1	0.9	0.9	0.9	0.0



Thr peptide **17** (1.0 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

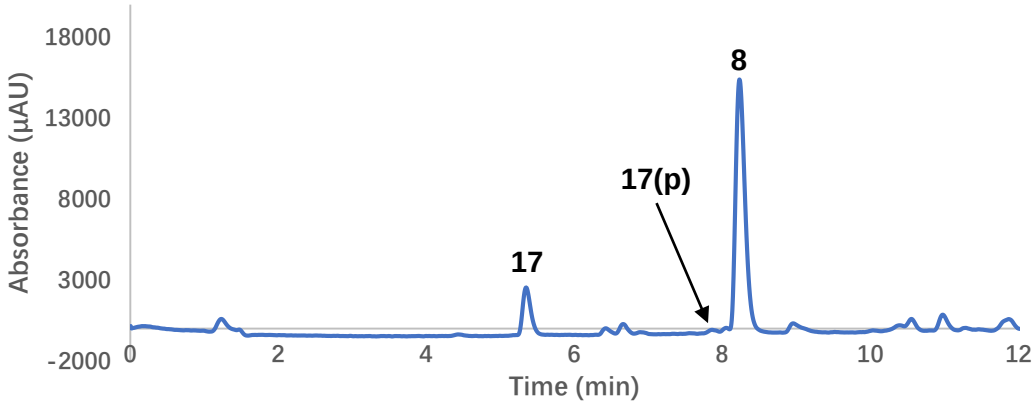


Figure 107: Analytical HPLC-MS trace of the transamination of Thr peptide **17**. Starting peptide **17**: t_R = 5.3 min; side-chain cleaved product **8**: t_R = 8.2 min; product **17(p)**: t_R = 7.9 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

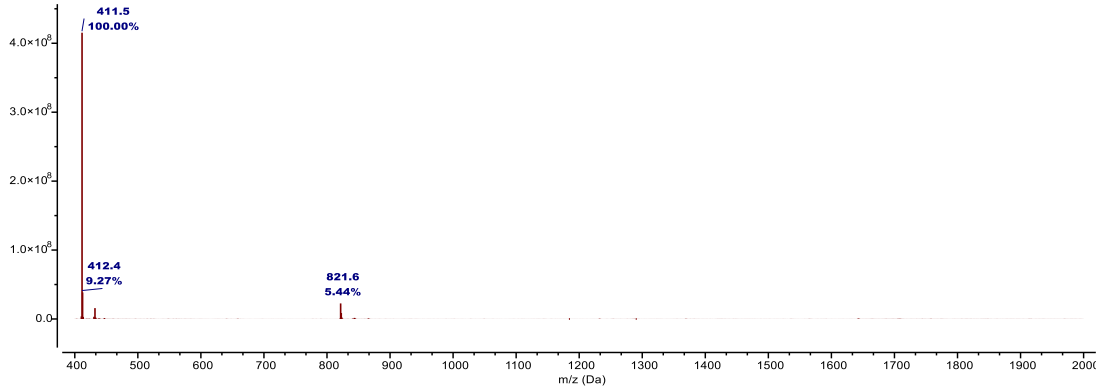
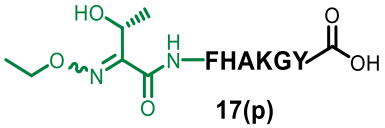


Figure 108: Low-resolution MS spectrum of side-chain cleaved peptide product **8** (extracted at 8.3 min), *m/z* (ESI+) calcd M_{mono} = 820.4, found 821.6 $[M + H]^+$, 411.5 $[M + 2H]^{2+}$.



17(p)

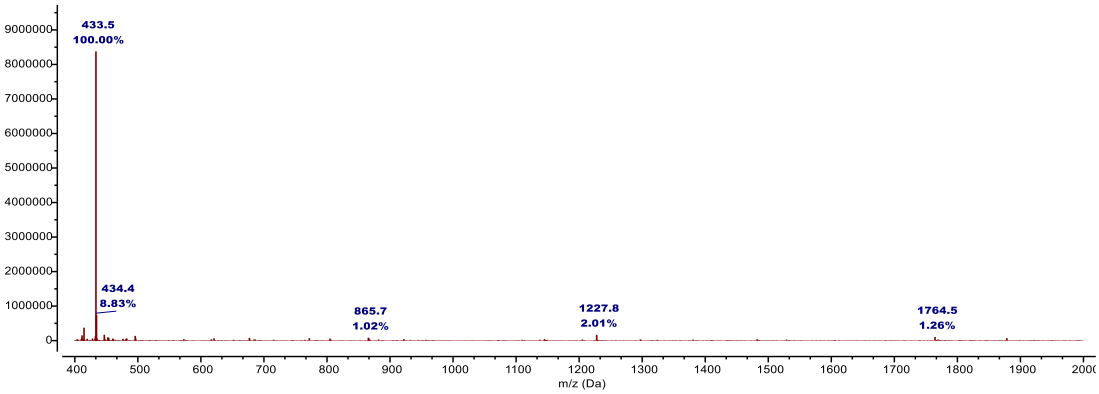
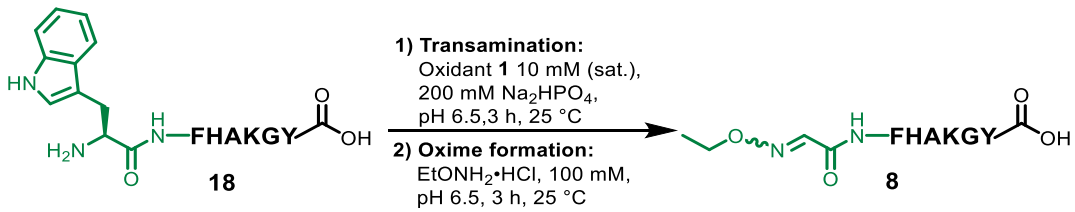


Figure 109: Low-resolution MS spectrum of peptide product **17(p)** (extracted at 7.9 min), *m/z* (ESI+) calcd M_{mono} = 864.4, found 865.7 $[M + 2H]^+$, 433.5 $[M + 2H]^{2+}$.

Table 11: A summary of the transamination of Thr peptide **17** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **8**	82.9	84.2	85.6	84.2	1.1
Product **17(p)**	0.6	0.6	0.6	0.6	0.0



Trp peptide **18** (1.0 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture

was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

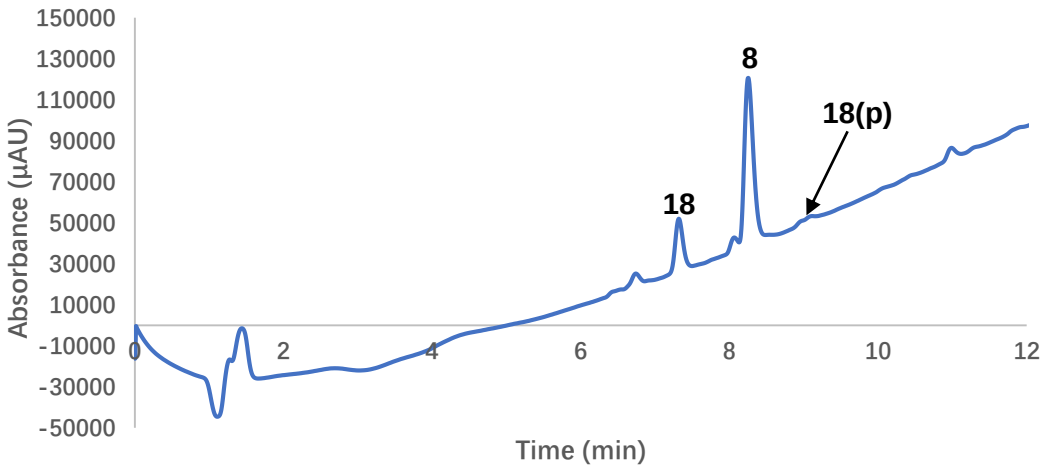


Figure 110: Analytical HPLC-MS trace of the transamination of Trp peptide **18** at **230** nm. Starting peptide **18**: t_R = 7.5 min; side-chain cleaved product **8**: t_R = 8.3 min; product **18(p)**: t_R = 9.1 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

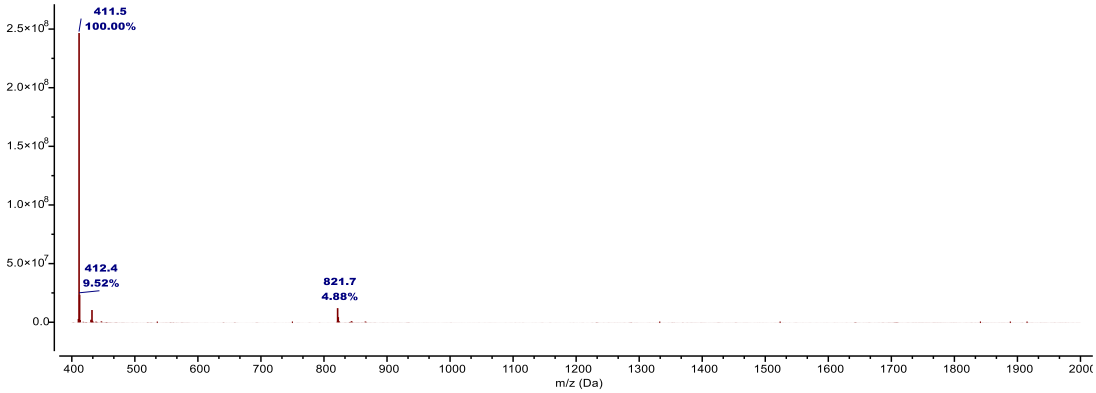
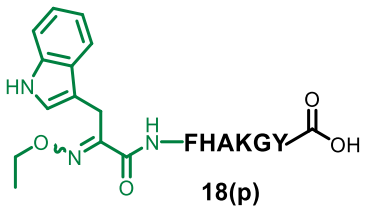


Figure 111: Low-resolution MS spectrum of side-chain cleaved peptide product **8** (extracted at 8.3 min), *m/z* (ESI⁺) calcd M_{mono} = 820.4, found 821.7 $[M + H]^+$, 411.5 $[M + 2H]^{2+}$.



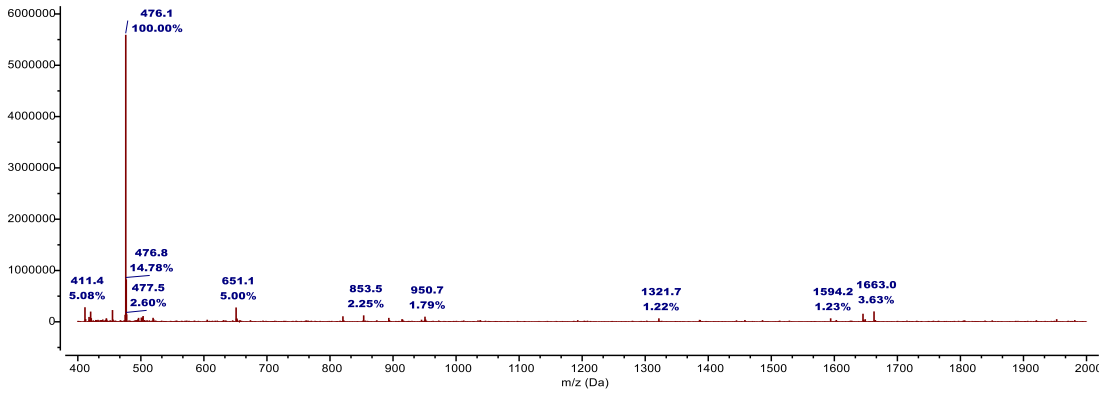
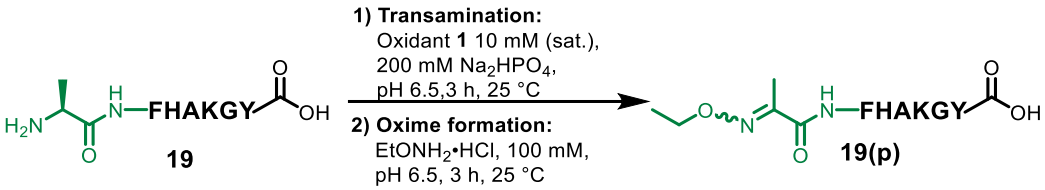


Figure 112: Low-resolution MS spectrum of peptide product **18(p)** (extracted at 9.1 min), *m/z* (ESI$^+$) calcd M_{mono} = 949.4, found 950.7 $[M + H]^+$, 476.1 $[M + 2H]^{2+}$.

Table 12: A summary of the transamination of Trp peptide **18** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **8**	93.7	95.1	94.8	94.5	0.6
Product **18(p)**	0.8	0.5	0.9	0.7	0.2



Ala peptide **19** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

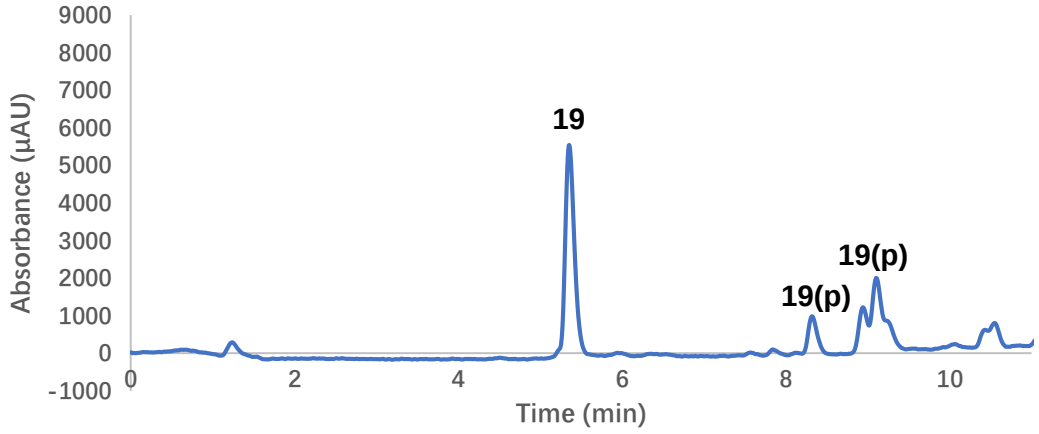


Figure 113: Analytical HPLC-MS trace of the transamination of Ala peptide **19**. Starting peptide **19**: t_R = 5.4 min; desired product **19(p)**: product peak 1:t_R = 8.3 min: product peak 2: t_R = 9.1 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid,

Dubhe C18 analytical column).

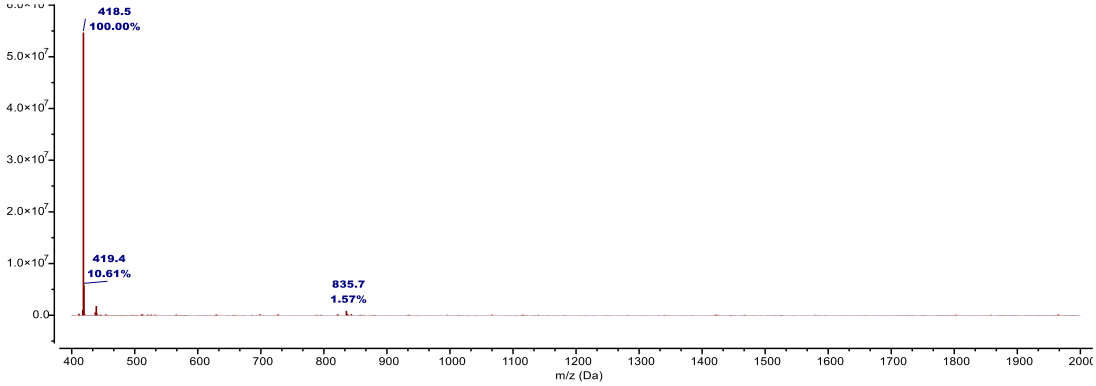


Figure 114: Low-resolution MS spectrum of desired peptide product **19(p)** peak 1 (extracted at 8.3 min), *m/z* (ESI+) calcd M_{mono} = 834.4, found 835.7 $[M + H]^+$, 418.5 $[M + 2H]^{2+}$.

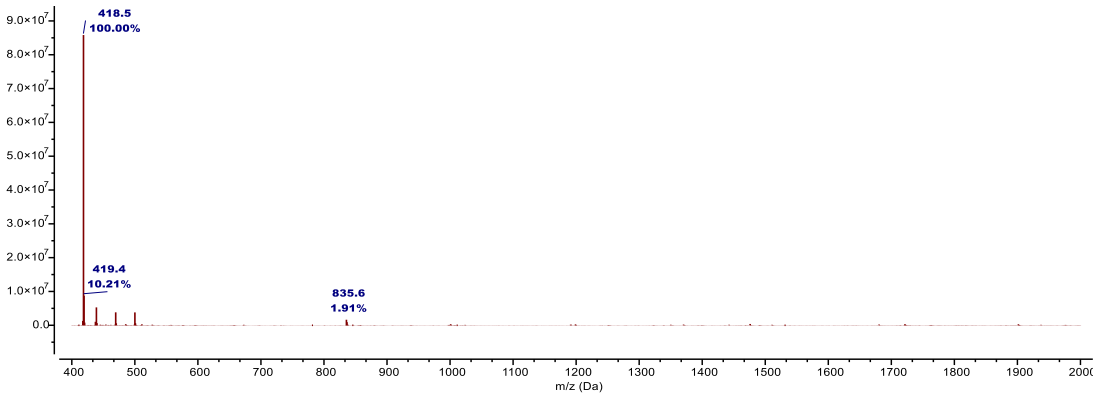
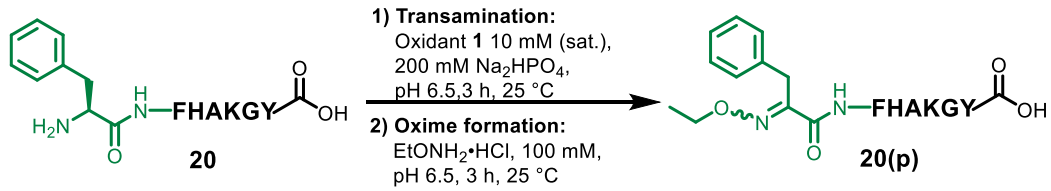


Figure 115: Low-resolution MS spectrum of desired peptide product **19(p)** peak 2 (extracted at 9.1 min), *m/z* (ESI+) calcd M_{mono} = 834.4, found 835.6 $[M + H]^+$, 418.5 $[M + 2H]^{2+}$.

Table 13: A summary of the transamination of Ala peptide **19** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **19(p)**	33	30.8	33.7	32.5	1.2



Phe peptide **20** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

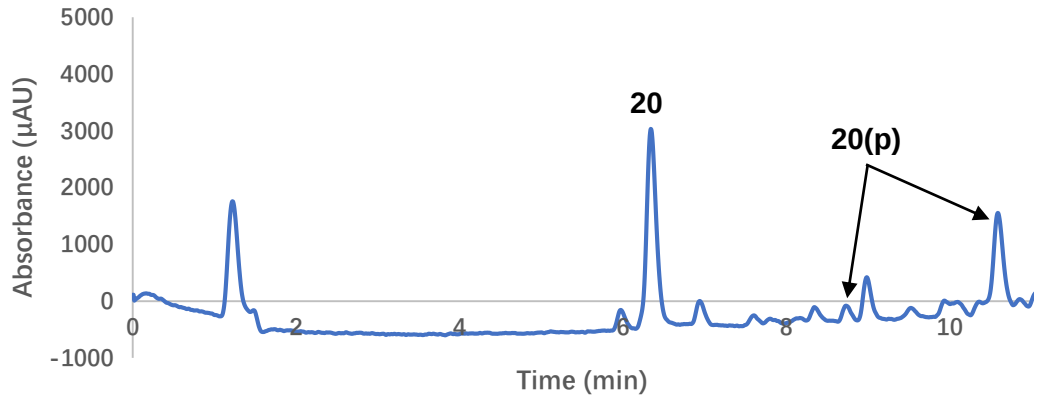


Figure 116: Analytical HPLC-MS trace of the transamination of Phe peptide **20**. Starting peptide **20**: t_R = 5.5 min; desired product **20(p)**: product peak 1:t_R = 8.7 min: product peak 2: t_R = 10.5 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

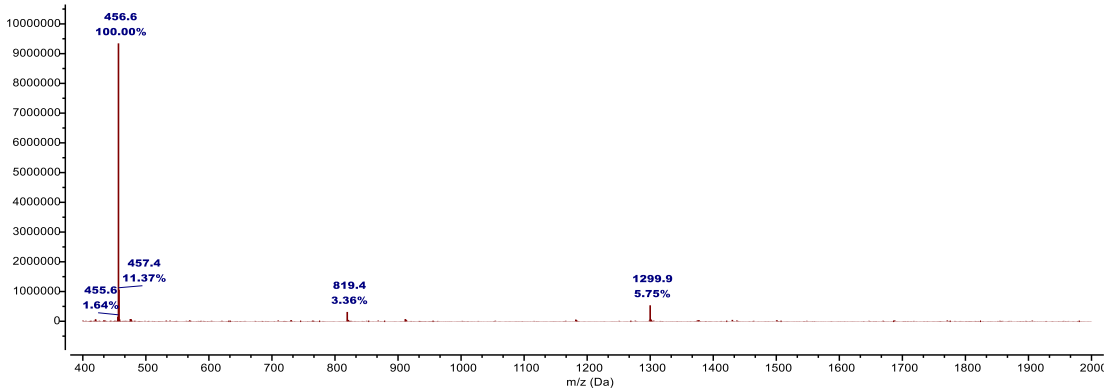


Figure 117: Low-resolution MS spectrum of desired peptide product **20(p)** peak 1 (extracted at 8.7 min), *m/z* (ESI⁺) calcd M_{mono} = 910.4, found 456.6 $[M + 2H]^{2+}$.

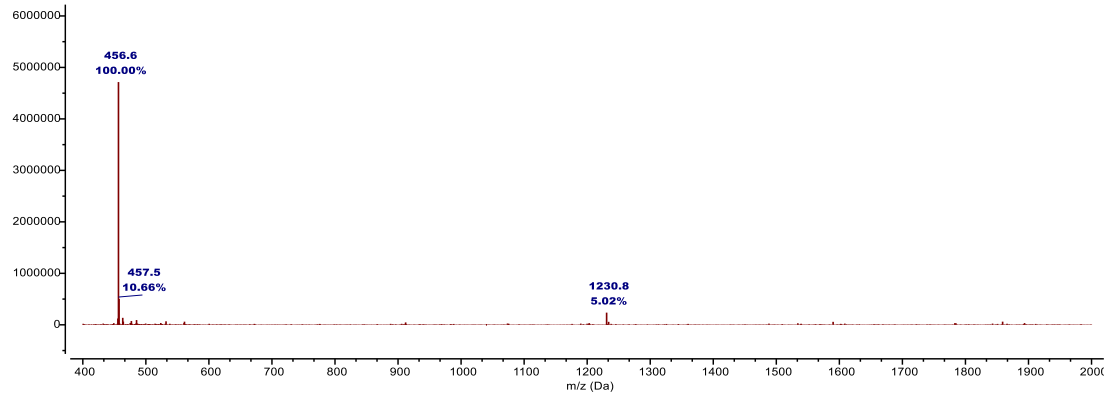
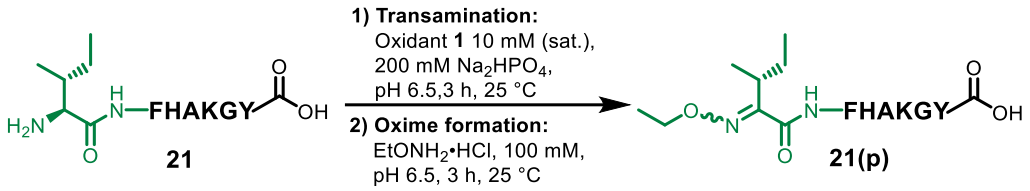


Figure 118: Low-resolution MS spectrum of desired peptide product **20(p)** peak 2 (extracted at 10.5 min), *m/z* (ESI⁺) calcd M_{mono} = 910.4, found 456.6 $[M + 2H]^{2+}$.

Table 14: A summary of the transamination of Phe peptide **20** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD

Product **20(p)**	29.9	28.9	31.1	30.0	0.9



Ile peptide **21** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

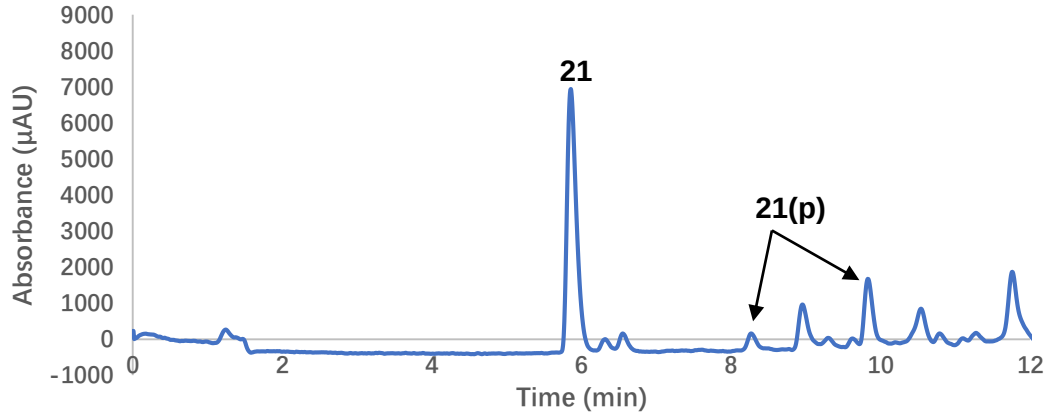


Figure 119: Analytical HPLC-MS trace of the transamination of Ile peptide **21**. Starting peptide **21**: t_R = 5.9 min; desired product **21(p)** peak1: t_R = 8.3 min; desired product **21(p)** peak 2: t_R = 9.8 min;. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

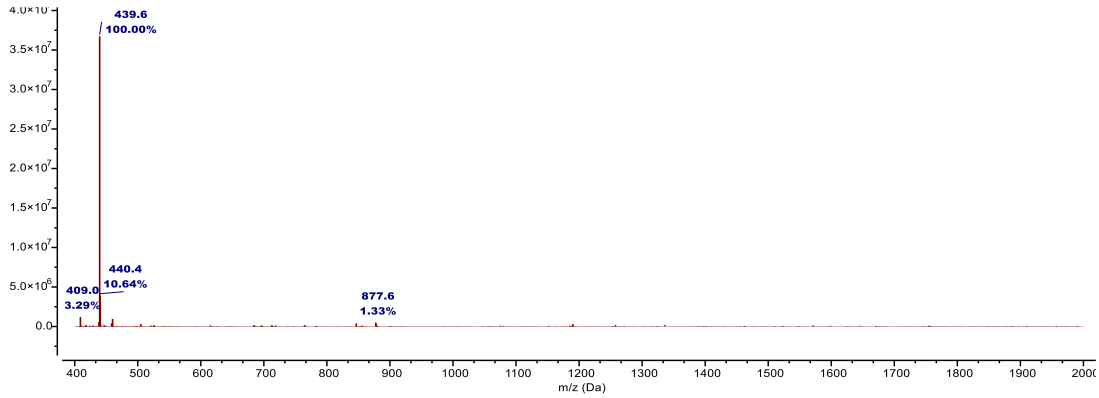


Figure 120: Low-resolution MS spectrum of desired peptide product **21(p)** peak 1 (extracted at 8.3 min), *m/z* (ESI⁺) calcd M_{mono} = 876.4, found 877.6 $[M + H]^+$; 439.6 $[M + 2H]^{2+}$.

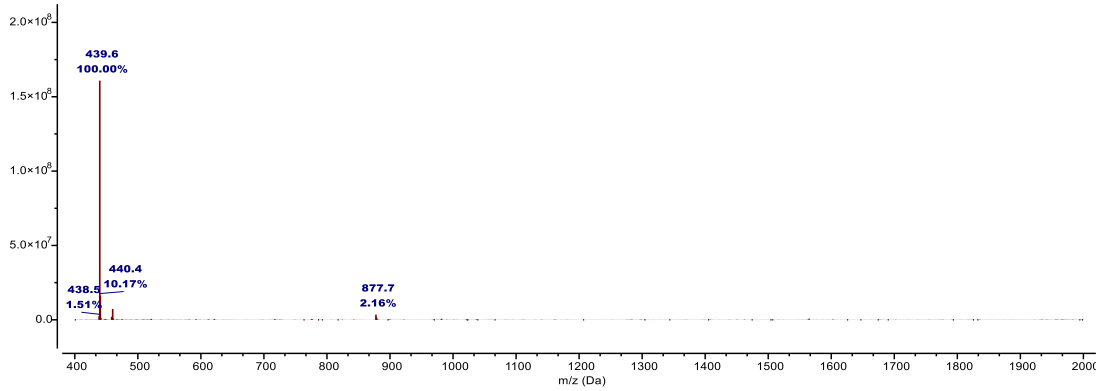
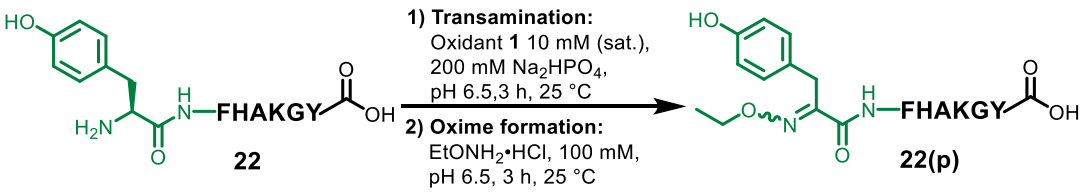


Figure 121: Low-resolution MS spectrum of desired peptide product **21(p)** peak 2 (extracted at 9.8 min), *m/z* (ESI⁺) calcd M_{mono} = 876.4, found 877.7 $[M + H]^+$; 439.6 $[M + 2H]^{2+}$.

Table 15: A summary of the transamination of Ile peptide **21** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **21(p)**	25	25.5	29.7	26.7	2.1



Tyr peptide **22** (1.0 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

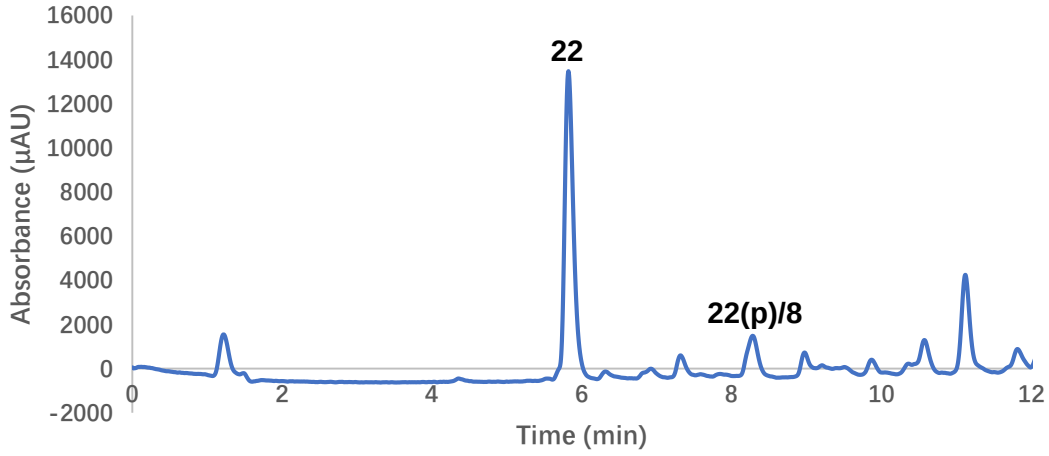


Figure 122: Analytical HPLC-MS trace of the transamination of Tyr peptide **22**. Starting peptide **22**: t_R = 5.8 min; peptide product **22(p)** was mixed with side chain cleaved product **8**: t_R = 8.3 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

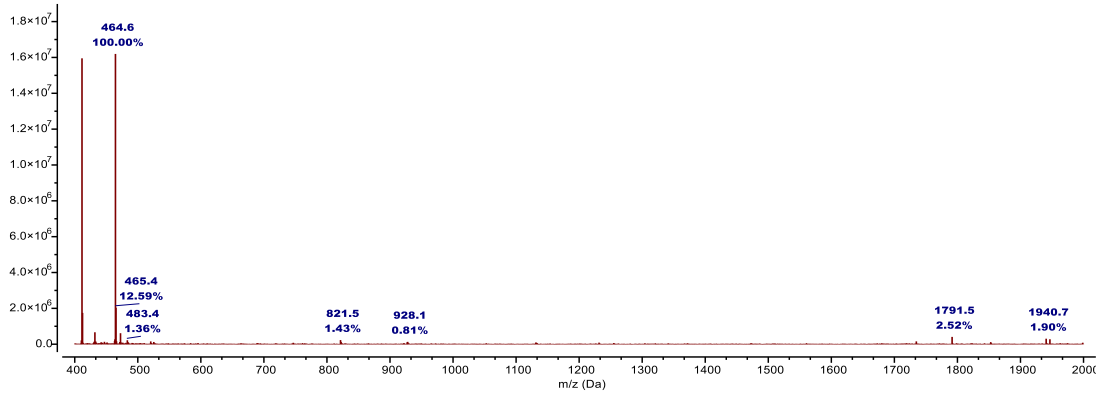
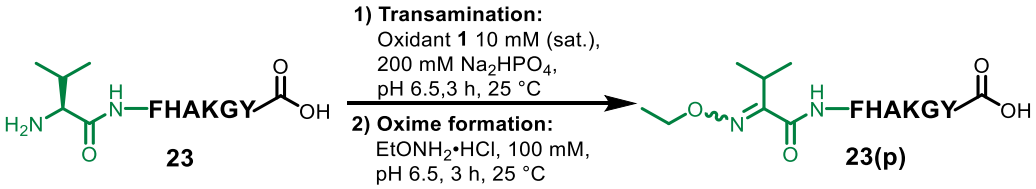


Figure 123: Low-resolution MS spectrum extracted at 8.3 min. Desired peptide product **22(p)**, *m/z* (ESI+) calcd M_{mono} = 820.4, found 926.4 $[M + H]^+$, 464.6 $[M + 2H]^{2+}$; side-chain cleaved product **8**, *m/z* (ESI+) calcd M_{mono} = 820.4, found 821.5 $[M + H]^+$, 411.5 $[M + 2H]^{2+}$. The ratio of two products was approximately 1:1 according to peak intensity.

Table 16: A summary of the transamination of Tyr peptide **22** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **8**	10.6	10.5	11.3	10.8	0.4
Product **22(p)**	9.3	9.6	11	10.0	0.7



Val peptide **23** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

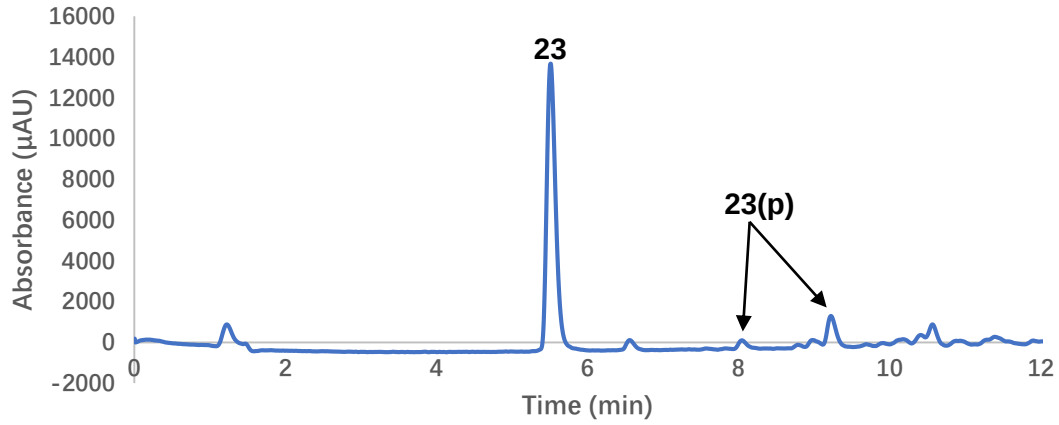


Figure 124: Analytical HPLC-MS trace of the transamination of Val peptide **23**. Starting peptide **23**: t_R = 5.5 min; desired product **23(p)** peak 1: t_R = 8.0 min; desired product **23(p)** peak 2: t_R = 9.2 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

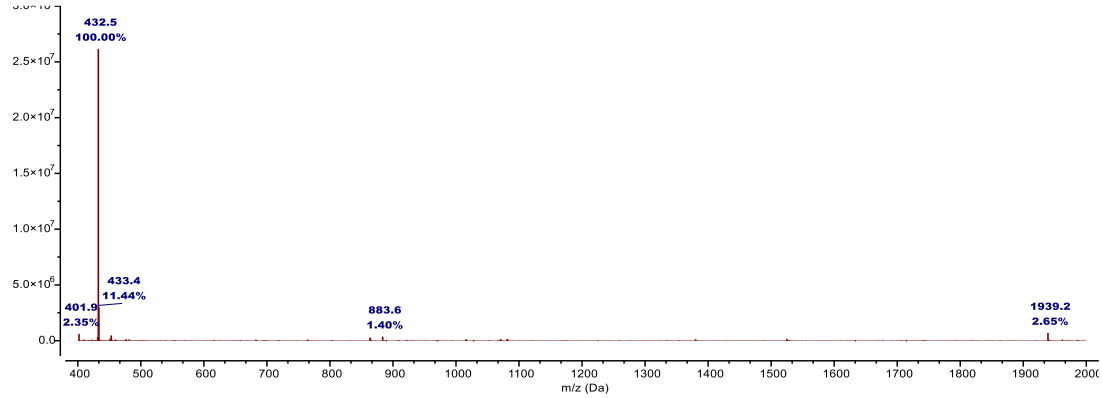


Figure 125: Low-resolution MS spectrum of desired peptide product **23(p)** peak 1 (extracted at 8.0 min), *m/z* (ESI+) calcd M_{mono} = 862.4, found 883.6 $[M + Na]^+$, 432.5 $[M + 2H]^{2+}$.

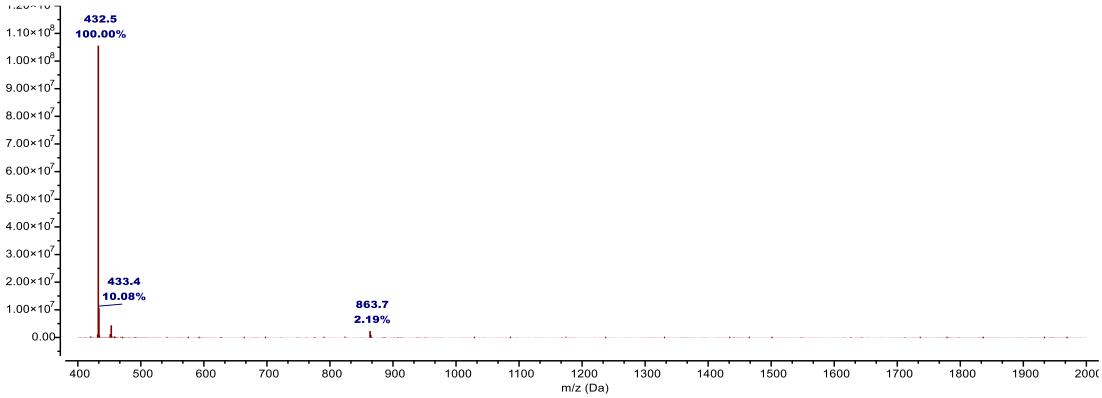
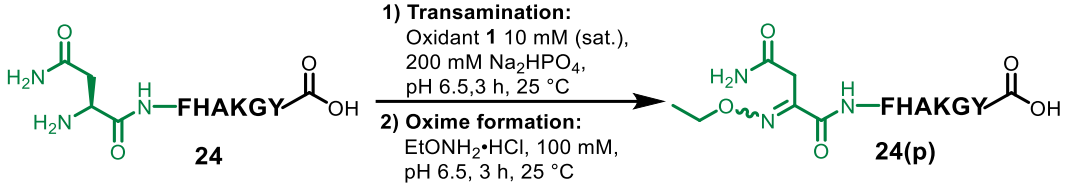


Figure 126: Low-resolution MS spectrum of desired peptide product **23(p)** peak 2 (extracted at 9.2 min), *m/z* (ESI+) calcd M_{mono} = 862.4, found 863.7 $[M + H]^+$, 432.5 $[M + 2H]^{2+}$.

Table 17: A summary of the transamination of Val peptide **23** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **23(p)**	11.5	11.6	10.6	11.2	0.4



Asn peptide **24** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

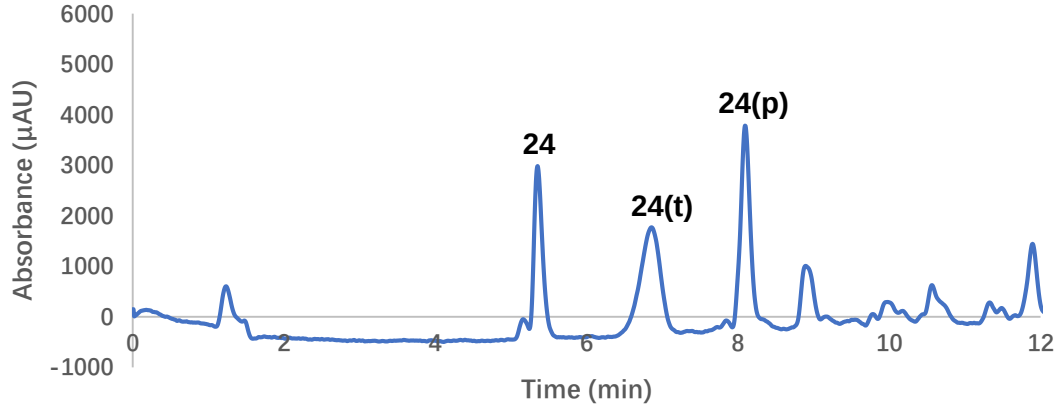


Figure 127: Analytical HPLC-MS trace of the transamination of Asn peptide **24**. Starting peptide **24**: t_R = 5.4 min; desired product **24(p)**: t_R = 8.1 min; transaminated product **24(t)**: t_R = 6.9 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

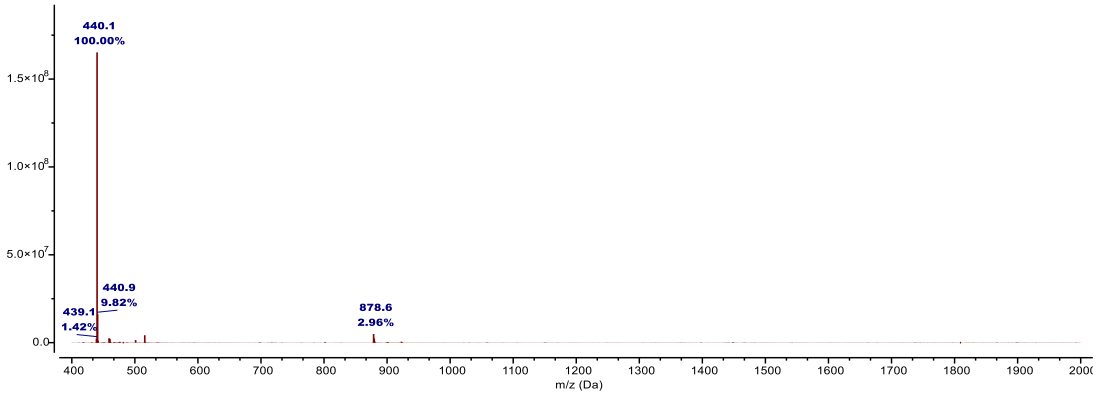


Figure 128: Low-resolution MS spectrum of desired peptide product **24(p)** (extracted at 6.9 min), *m/z* (ESI⁺) calcd M_{mono} = 877.4, found 878.6 $[M + H]^+$; 440.1 $[M + 2H]^{2+}$.

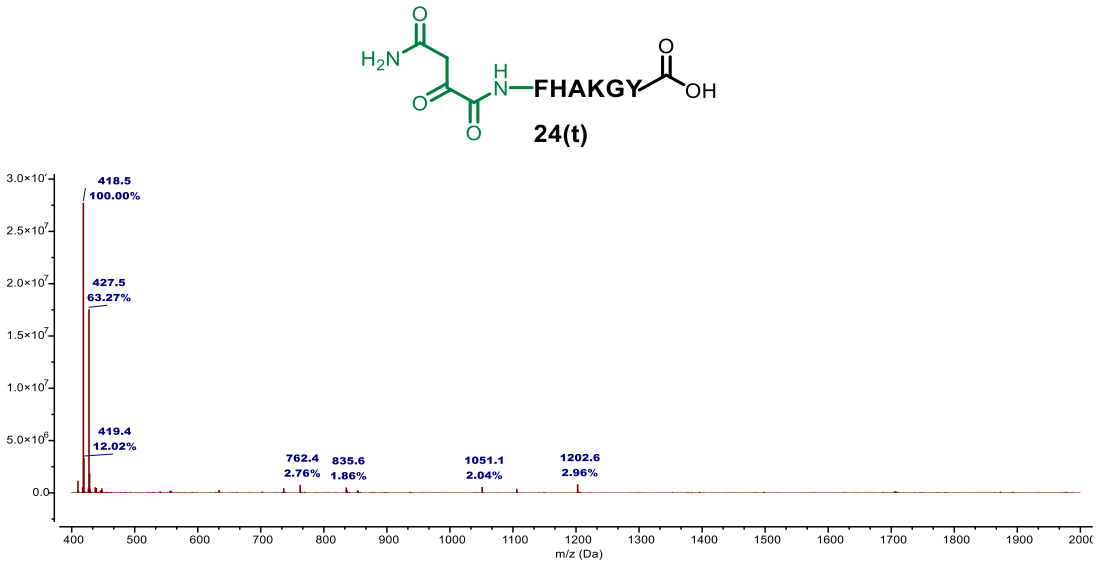
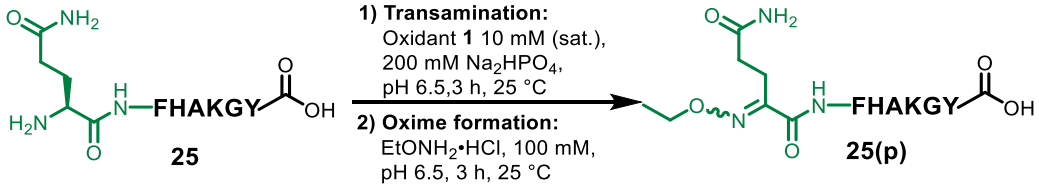


Figure 129: Low-resolution MS spectrum of peptide product **24(t)** (extracted at 6.9 min), *m/z* (ESI$^+$) calcd M_{mono} = 834.4, found 835.6 $[M + H]^+$; 418.5 $[M + 2H]^{2+}$.

Table 18: A summary of the transamination of Asn peptide **24** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **24(p)**	37.9	40.8	44.6	41.1	2.7
Product **24(t)**	37.9	33.8	25.1	32.3	5.3



Gln peptide **25** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

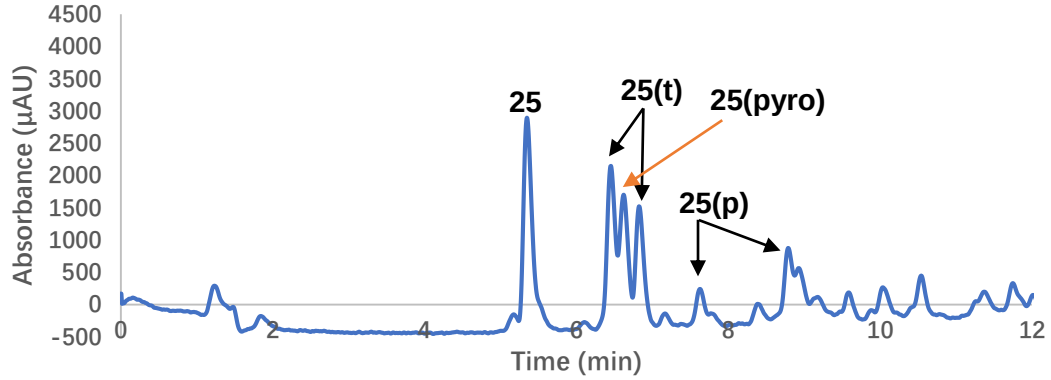


Figure 130: Analytical HPLC-MS trace of the transamination of Gln peptide **25**. Starting peptide **25**: t_R = 5.4 min; desired product **25(p)** peak 1: t_R = 7.6 min; desired product **25(p)** peak 2: t_R = 8.8 min; transaminated product **25(t)** peak 1: t_R = 6.5 min; transaminated product **25(t)** peak 2: t_R = 6.8 min; formation of pyroglytamate **25(pyro)**: t_R = 6.6 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

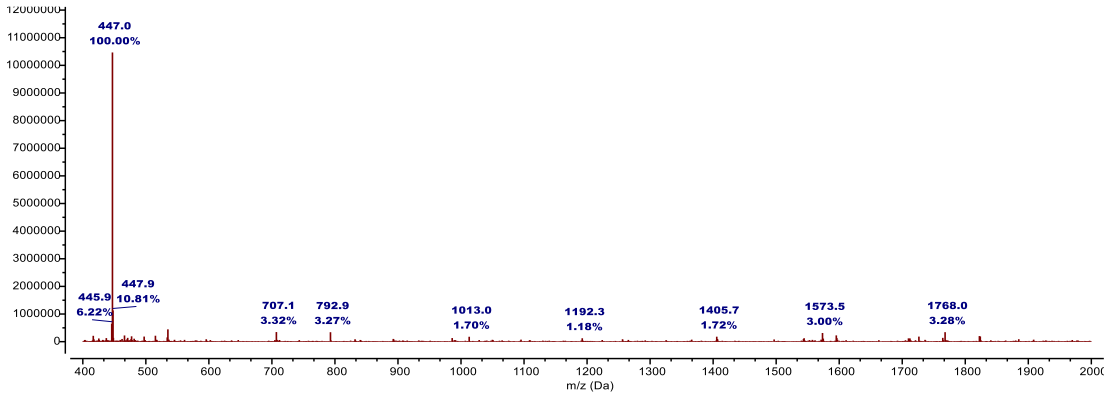


Figure 131: Low-resolution MS spectrum of desired peptide product **25(p)** peak 1 (extracted at 7.6 min), *m/z* (ESI+) calcd M_{mono} = 891.4, found 447.0 $[M + 2H]^{2+}$.

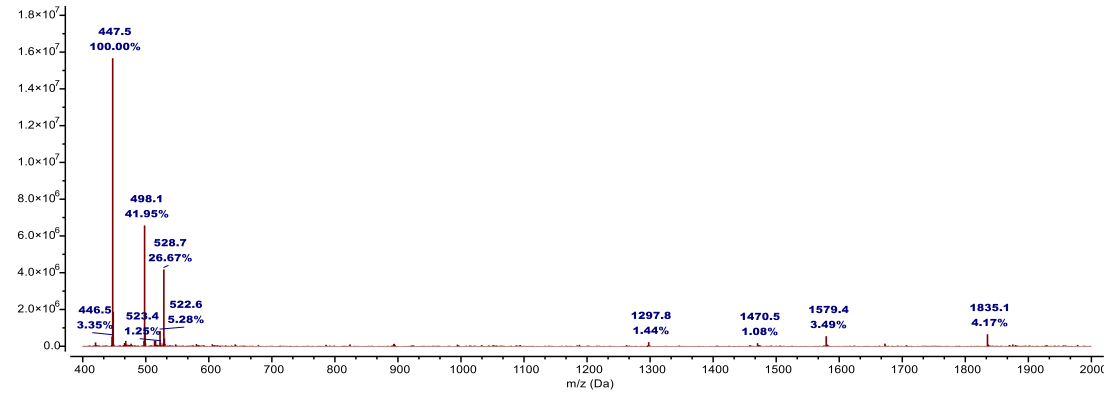
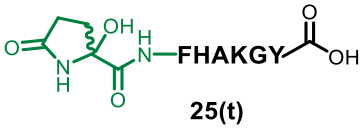


Figure 132: Low-resolution MS spectrum of desired peptide product **25(p)** peak 1 (extracted at 8.8 min), *m/z* (ESI+) calcd M_{mono} = 891.4, found 447.5 $[M + 2H]^{2+}$.



25(t)

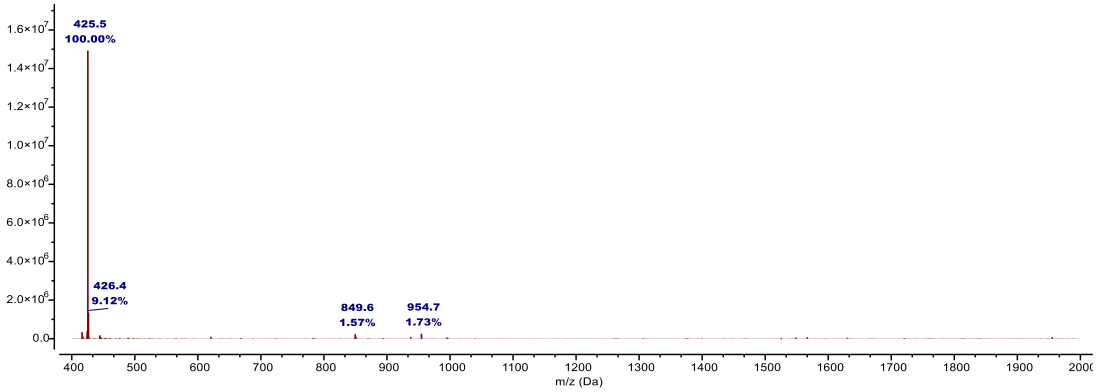


Figure 133: Low-resolution MS spectrum of transaminated peptide product **25(t)** peak 1 (extracted at 6.5 min), *m/z* (ESI⁺) calcd M_{mono} = 848.4, found 849.6 $[M + H]^+$, 425.5 $[M + 2H]^{2+}$.

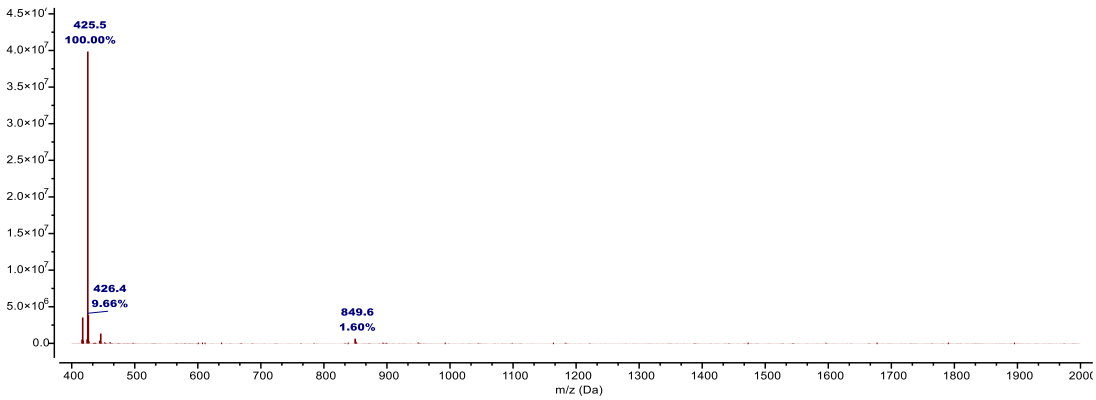
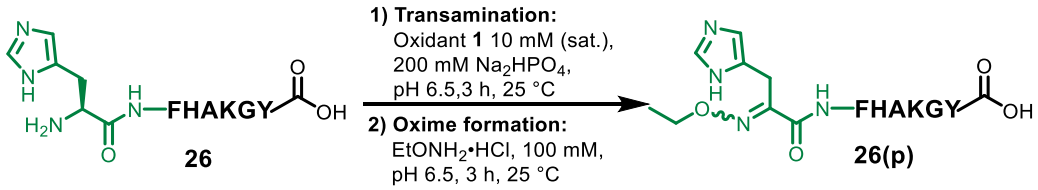


Figure 134: Low-resolution MS spectrum of transaminated peptide product **25(t)** peak 2 (extracted at 6.8 min), *m/z* (ESI⁺) calcd M_{mono} = 891.4, found 849.6 $[M + H]^+$, 425.5 $[M + 2H]^{2+}$.

Table 19: A summary of the transamination of Gln peptide **25** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **25(p)**	12.4	13.8	11.1	12.4	1.1
Product **25(t)**	30.2	31.3	29.4	30.3	0.8



26 **26(p)**

His peptide **26** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with

LCMS. The entire experiment was repeated for three times.

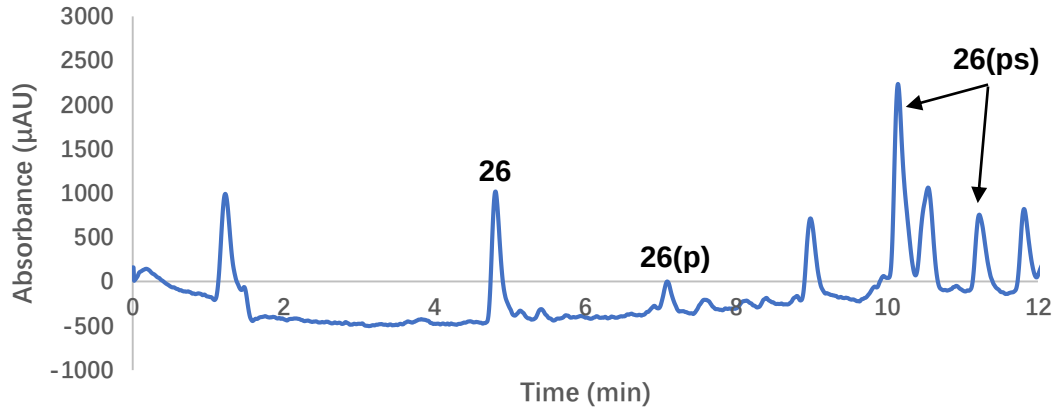


Figure 135: Analytical HPLC-MS trace of the transamination of His peptide **26**. Starting peptide **26**: t_R = 4.8 min; desired product **26(p)**: t_R = 7.1 min; Pictet-Spengler product **26 (ps)** peak 1: t_R = 10.1 min; Pictet-Spengler product **26(ps)** peak 2: t_R = 11.2 min. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

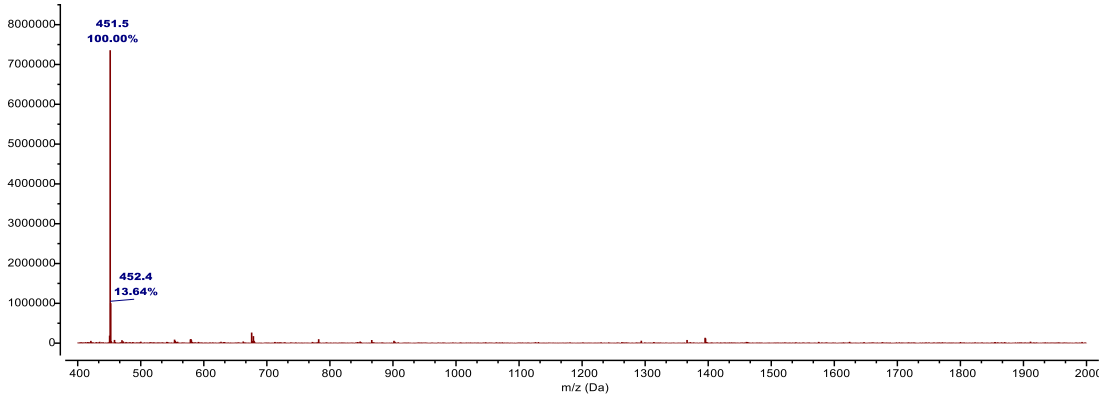
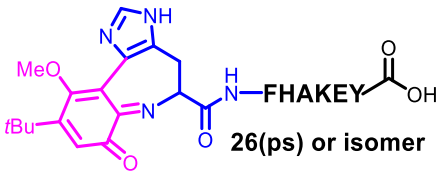


Figure 136: Low-resolution MS spectrum of desired peptide product **26(p)** (extracted at 7.1 min), *m/z* (ESI$^+$) calcd M_{mono} = 900.4, found 451.5 $[M + 2H]^{2+}$.



Pictet-Spengler product **26(ps)** or its isomer was observed as the major byproduct during the transamination of His peptide **26**. This transformation aligned with the observation reported by Francis *et al.*[5]

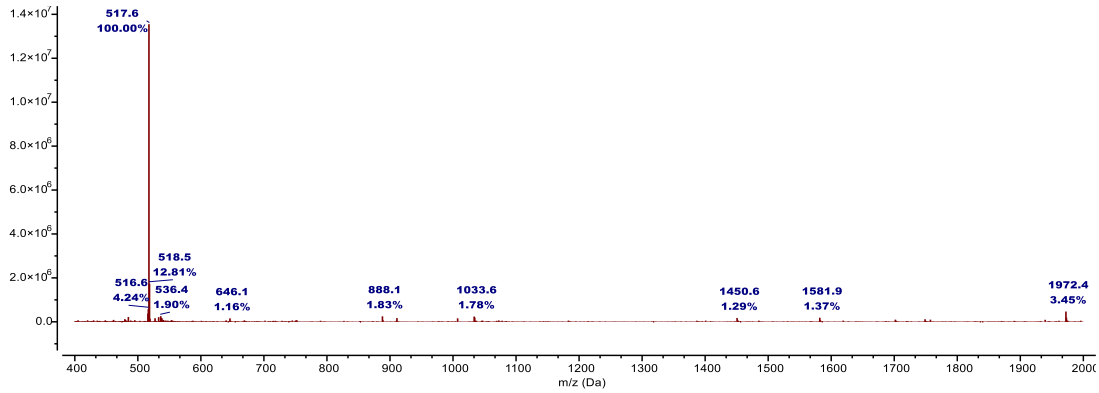


Figure 137: Low-resolution MS spectrum of desired peptide product **26(ps)** (extracted at 10.1 min), *m/z* (ESI⁺) calcd M_{mono} = 1032.4, found 1033.6 $[M + H]^+$; 517.6 $[M + 2H]^{2+}$.

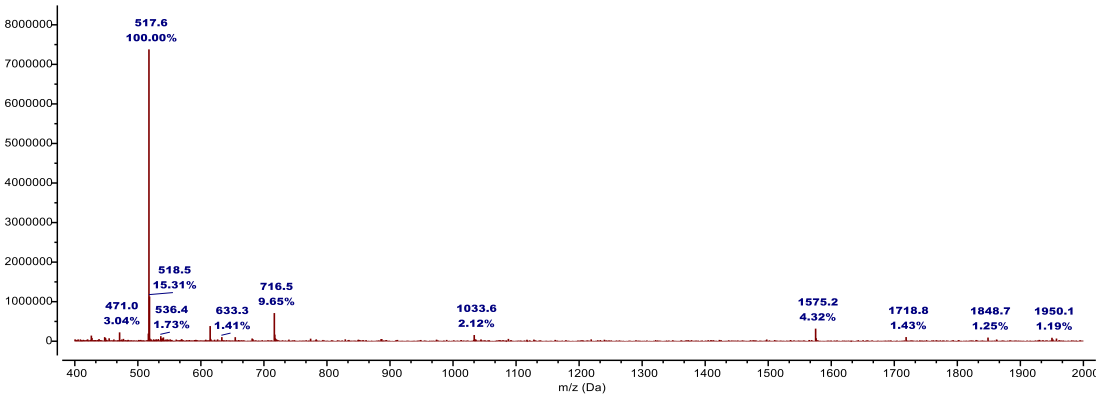
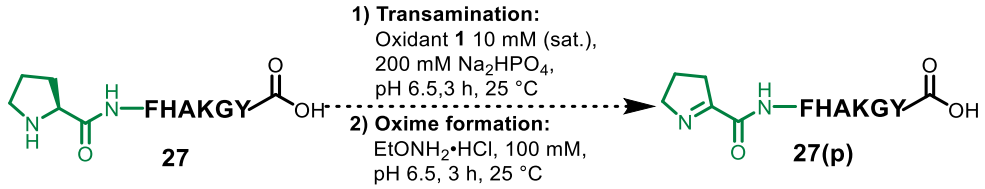


Figure 138: Low-resolution MS spectrum of desired peptide product **26(ps)** (extracted at 11.2 min), *m/z* (ESI⁺) calcd M_{mono} = 1032.4, found 1033.6 $[M + H]^+$; 517.6 $[M + 2H]^{2+}$.

Table 20: A summary of the transamination of His peptide **26** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **26(p)**	5.6	5.9	4.8	5.4	0.5
Product **26(ps)** Pictet-Spengler	66.3	66.7	66	66.5	0.3



Pro peptide **27** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times.

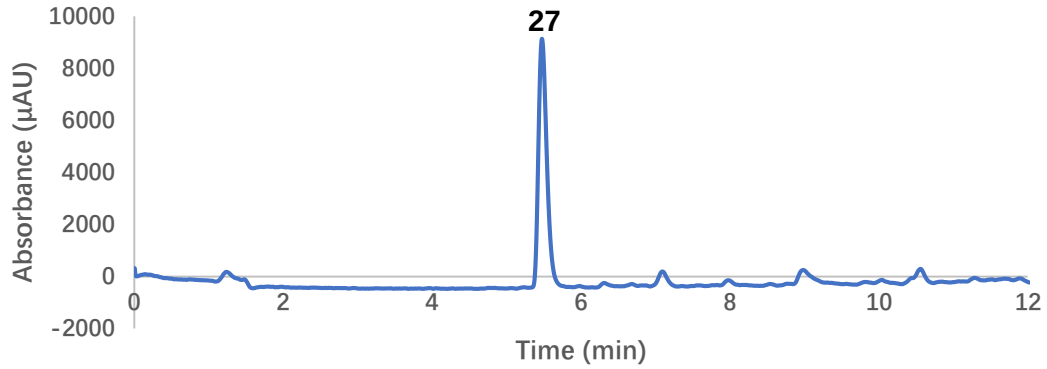


Figure 139: Analytical HPLC-MS trace of the transamination of Pro peptide **27**. Starting peptide **27**: t_R = 5.5 min; desired product **27(p)** was not observed in this reaction. (0% B for 1 min and then 0 to 40% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

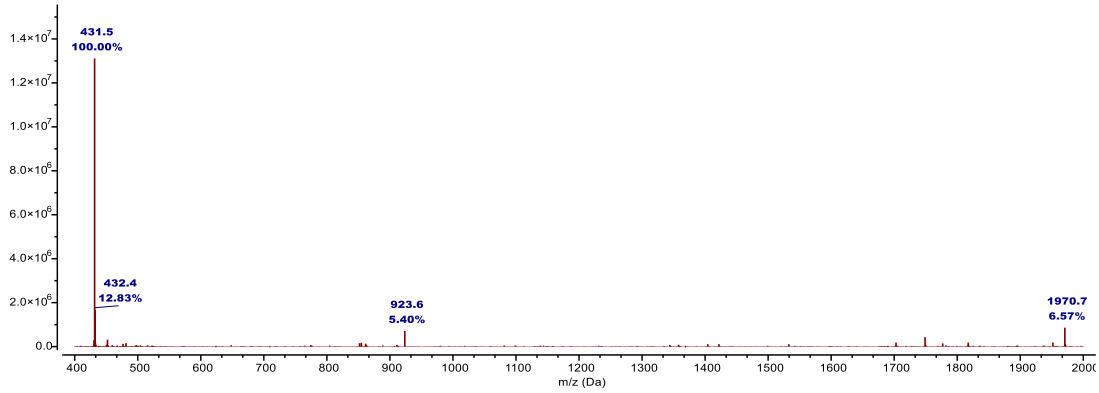
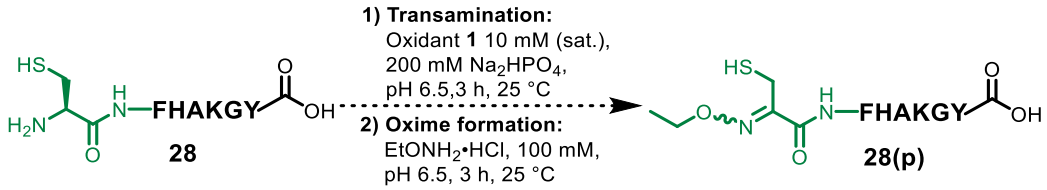


Figure 140: Low-resolution MS spectrum of peptide oxime product **27(p)** (extracted at 8.0 min), *m/z* (ESI$^+$) calcd M_{mono} = 922.4, found 923.6 $[M + H]^+$; 431.5 $[M + 2H]^{2+}$.

Table 21: A summary of the transamination of Pro peptide **27** (3 repeats)

Conversion	Run 1 (%)	Run 2 (%)	Run 3 (%)	Average (%)	SD
Product **27(p)**	1.6	1.8	1.7	1.7	0.1

Considering possible transamination of Lys side chain, oxime product **27(p)** is not recognized as N-terminal transaminated product.



Cys peptide **28** (0.9 mg, 1 nmol) was dissolved in an aqueous solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture

was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS. The entire experiment was repeated for three times. No peak corresponding to the product was observed.

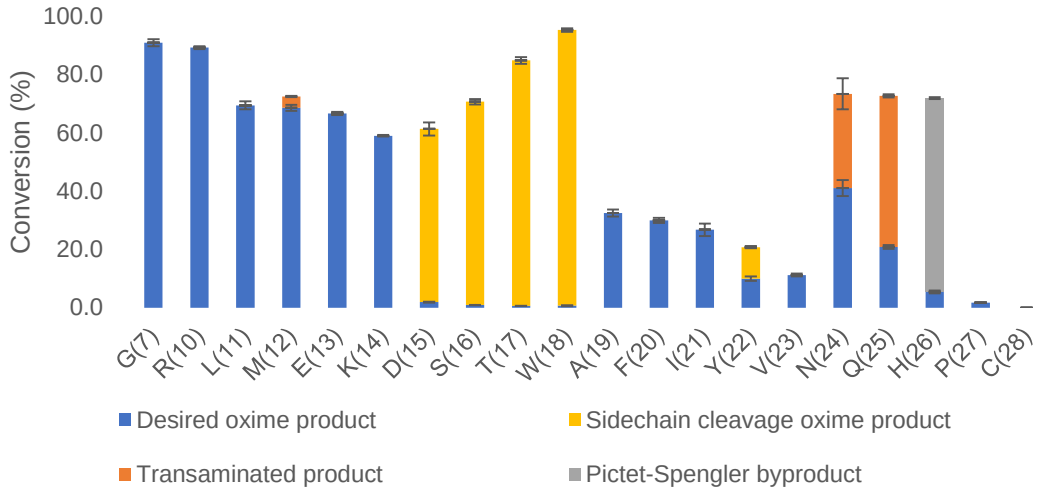
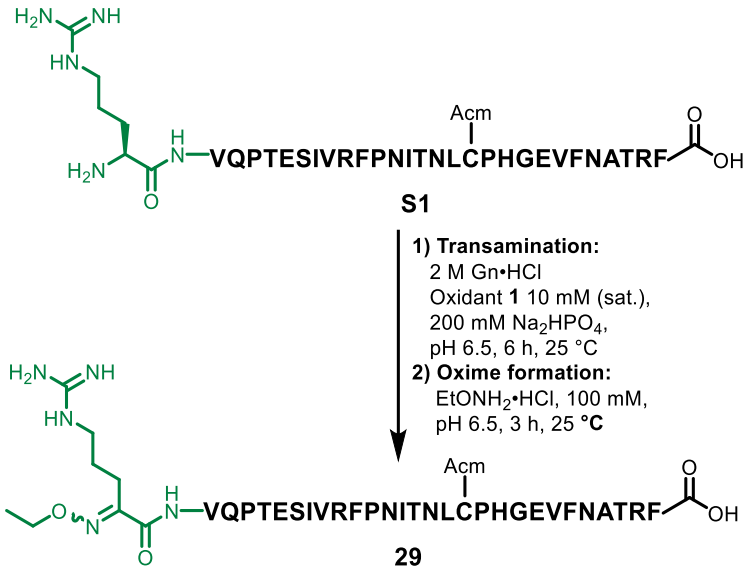


Figure 141: A summary chart of the conversion rates of peptide with different N-terminal residues.

3.3. Transamination of COVID-19 spike protein 319-347 fragment **S1**



COVID-19 spike protein 319-347 fragment **S1** (1.0 mg, 1 nmol) was dissolved in 2 M Gn·HCl solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 6 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS.

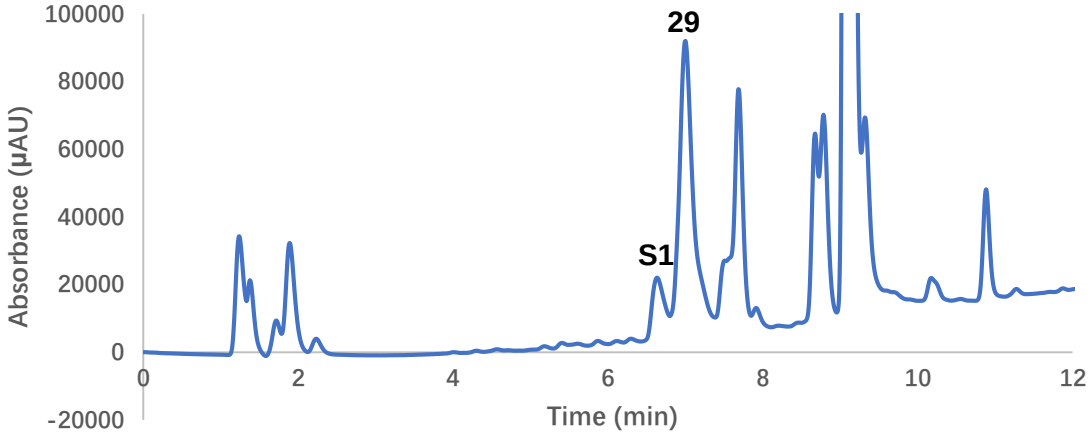


Figure 142: Analytical HPLC-MS trace of the transamination of S-protein fragment **S1**. Starting peptide **S1**: t_R = 6.7 min; oxime product **29**: t_R = 7.0 min. All other peaks were corresponding to non-peptidic materials (0% B for 1 min and then 0 to 95% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

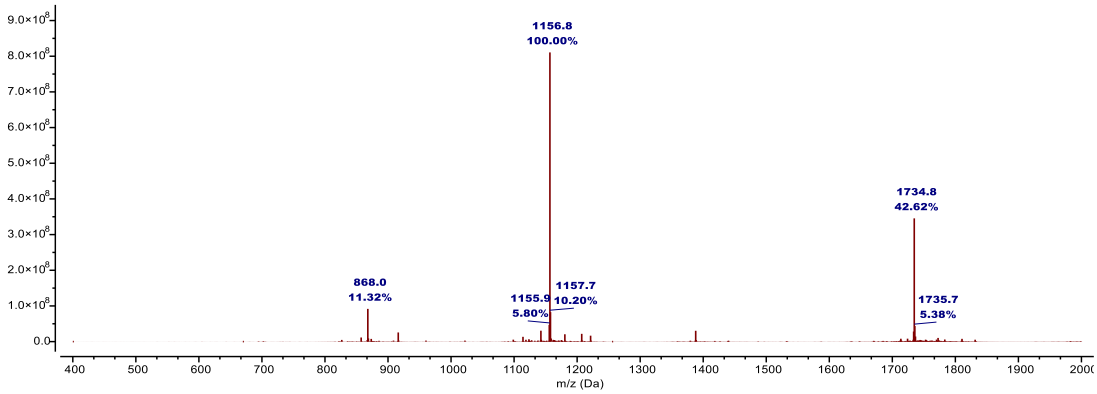
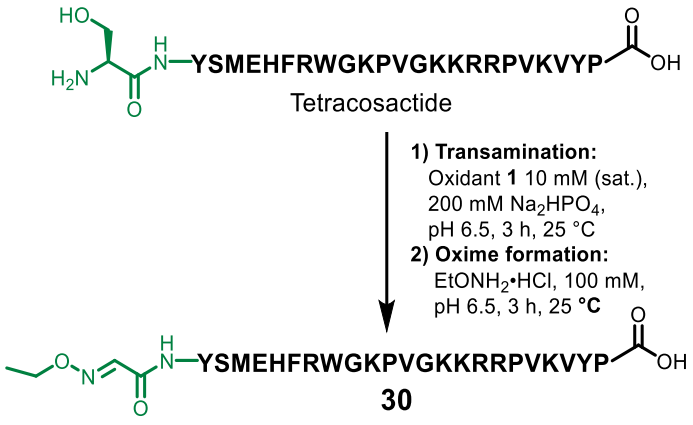


Figure 143: Low-resolution MS spectrum of peptide **29**, *m/z* (ESI⁺) calcd M_{av} = 3465.0, found 1734.8 $[M + 2H]^{2+}$, 1156.8 $[M + 3H]^{3+}$, 868.0 $[M + 4H]^{4+}$.

3.4. Transamination of Tetracosactide



Tetracosactide (1.0 mg, 1 nmol) was dissolved in 2 M Gn·HCl solution buffered with Na_2HPO_4 at pH 6.5, followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 6 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS.

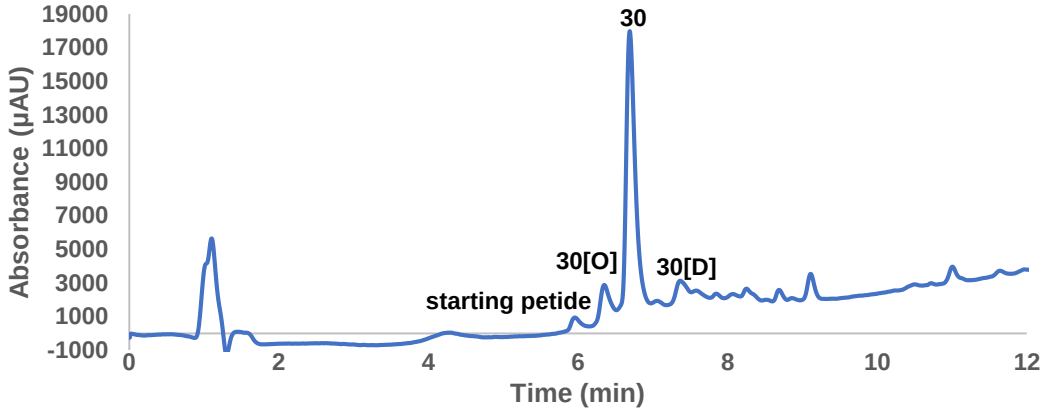


Figure 144: Analytical HPLC-MS trace of the transamination of tetracosactide. Starting peptide: t_R = 5.9 min; oxime product **30**: t_R = 6.7 min; Met[O] oxime product **30[O]**: t_R = 6.4 min; doubly transaminated oxime product **30[D]**: t_R = 7.4 min. (0% B for 1 min and then 0 to 95% B over 10 min with a flow rate of 0.3 mL/min buffered with 0.1% formic acid, Dubhe C18 analytical column).

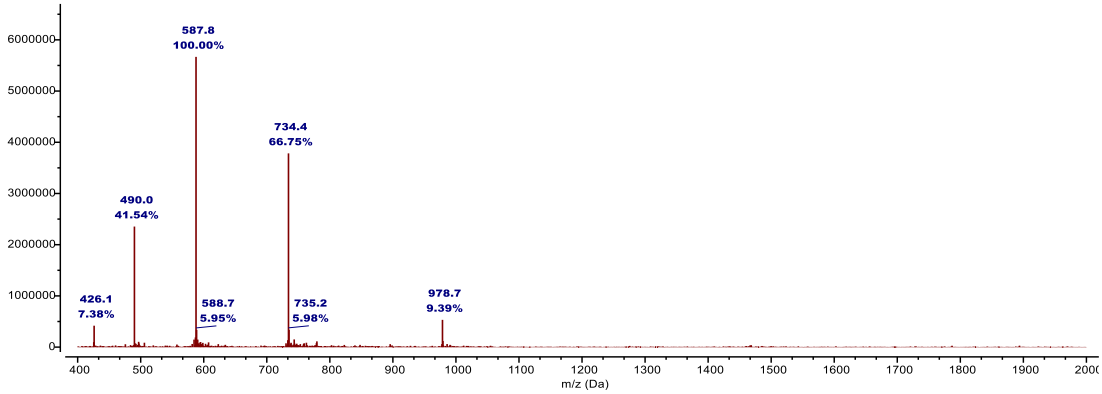


Figure 145: Low-resolution MS spectrum of extracted at 5.9 min. Tetracosactide, *m/z* (ESI⁺) calcd M_{av} = 2933.5, found 978.7 $[M + 3H]^{3+}$, 734.4 $[M + 4H]^{4+}$, 587.8 $[M + 5H]^{5+}$, 490.0 $[M + 6H]^{6+}$.

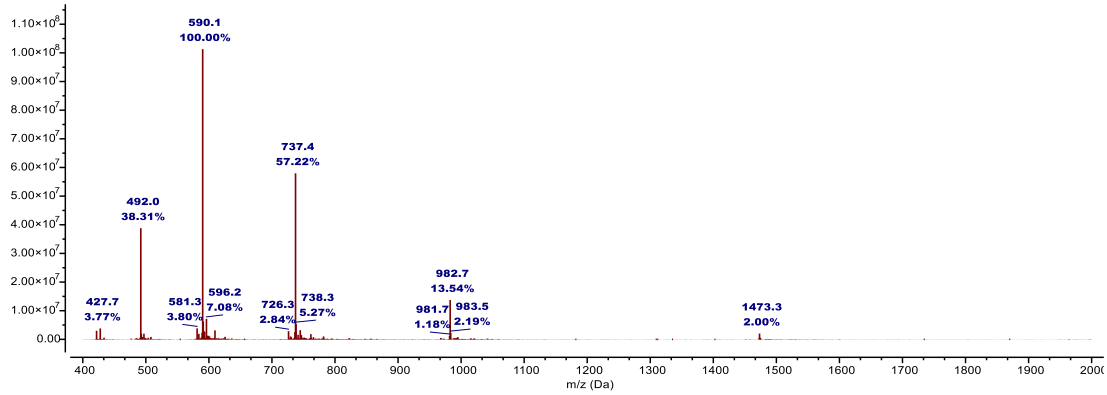


Figure 146: Low-resolution MS spectrum of extracted at 6.7 min. Desired oxime product **30**, *m/z* (ESI$^+$) calcd M_{av} = 2945.5, found 1473.3 $[M + 2H]^{2+}$, 982.7 $[M + 3H]^{3+}$, 737.4 $[M + 4H]^{4+}$, 590.1 $[M + 5H]^{5+}$, 492.0 $[M + 6H]^{6+}$.

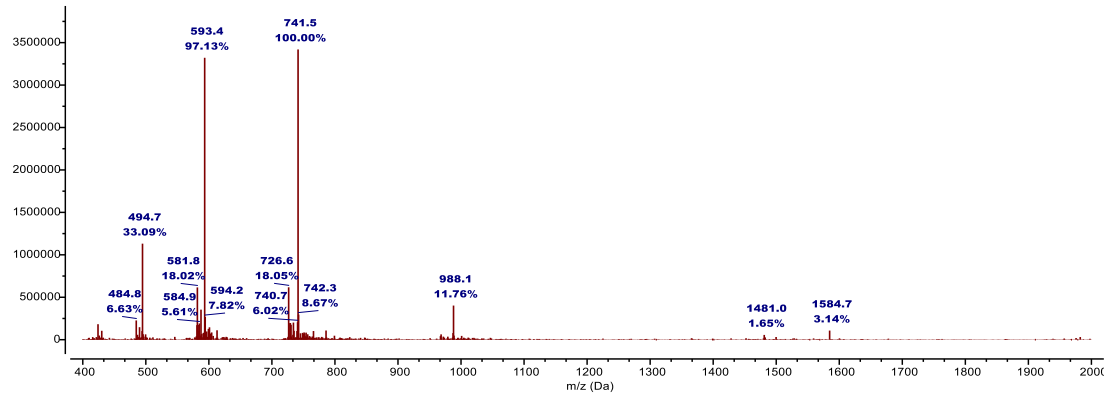


Figure 147: Low-resolution MS spectrum of extracted at 6.4 min. Oxidised oxime product **30[O]**, *m/z* (ESI$^+$) calcd M_{av} = 2987.5, found 996.7 $[M + 3H]^{3+}$, 747.9 $[M + 4H]^{4+}$, 598.6 $[M + 5H]^{5+}$, 499.0 $[M + 6H]^{6+}$.

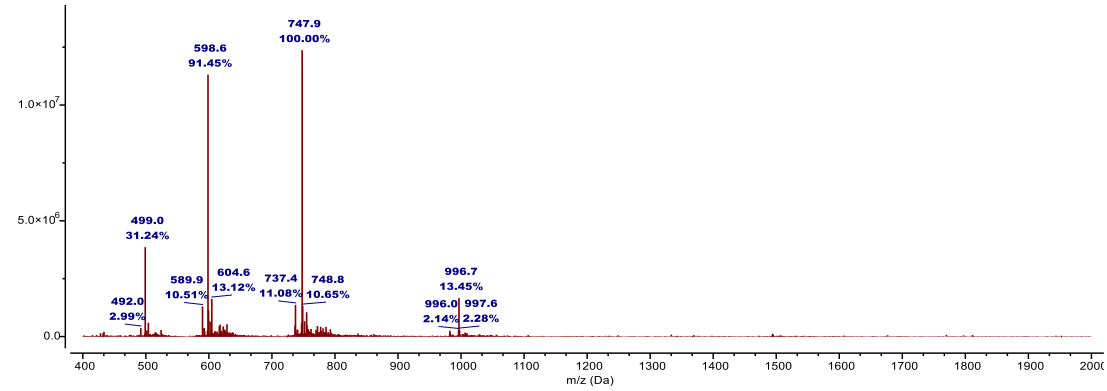
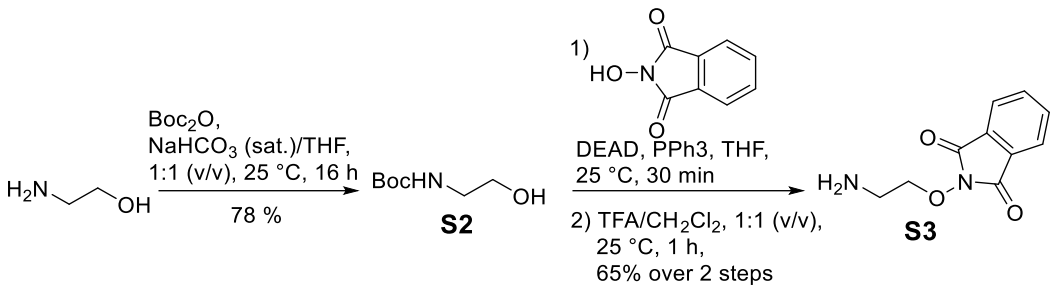


Figure 148: Low-resolution MS spectrum of extracted at 7.4 min. Doubly oxime product **30[D]**, *m/z* (ESI$^+$) calcd M_{av} = 2961.5, found 1481.0 $[M + 2H]^{2+}$, 988.1 $[M + 3H]^{3+}$, 741.5 $[M + 4H]^{4+}$, 593.4 $[M + 5H]^{5+}$, 494.7 $[M + 6H]^{6+}$.

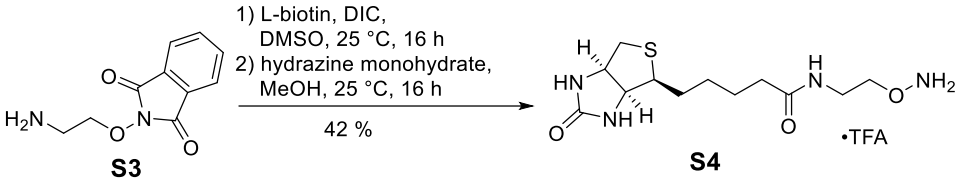
3.5. Transamination of Myoglobin with $EtONH_2$ or Biotinylated hydroxylamine

3.5.1. Synthesis of biotinylated hydroxylamine



2-aminoethanol (10.0 mL, 163 mmol) was added to the mixture of saturated $NaHCO_3$ aqueous solution (50 mL) and THF (50 mL), followed by addition of Boc anhydride (34.2 mL, 326 mmol). The mixture was stirred at 25 °C for 16 h. After the reaction was completed, the mixture was concentrated under reduced pressure to remove the organic phase. The residue was extracted with EtOAc (3 x 100 mL). The combined organic phase was washed with 0.1 M HCl (3 x 50 mL), brine (100 mL) and dried over anhydrous Na_2SO_4. The organic solution was concentrated under reduced pressure to afford Boc protected 2-aminoethanol **S2** (20.4 g, 127 mmol).

S2 (10 g, 62 mmol), PPh_3 (21.2 g, 80 mmol) and *N*-hydroxyphthalimide (14.6 g, 90 mmol) were mixed in anhydrous THF (100 mL). DEAD (11 mL, 70 mmol) was added dropwise at 0 °C over a period of 5 min. The solution was allowed to warm up to 25 °C and stirred for a further 30 min. The mixture was then concentrated *in vacuo* to 1/3 of its original volume. The residue was re-dissolved in a solution of CH_2Cl_2 (50 mL) and TFA (50 mL) and stirred at 25 °C for 30 min. After the reaction was completed, the acidic solution was concentrated under reduced pressure to 1/3 of its original volume. The residue was dripped into diethyl ether (200 mL). Target product **S3** was precipitated as TFA salt. After centrifuging, the ether layer was decanted. The crystal was washed with ether (2 x 100 mL) and re-dissolved in distilled water (100 mL). The aqueous solution was neutralized by addition of solid $NaHCO_3$, followed by extraction with EtOAc (3 x 100 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to afford the desired amine **S3** as a colorless solid (8.7 g, 42.2 mmol).



L-biotin (2.4 g, 10 mmol) and DIC (1.6 mL, 10 mmol) were dissolved in DMSO (5 mL). After stirring for 10 min, **S3** (2 g, 9.7 mmol) was added to the solution, and the resulting solution was stirred at 25 °C for 16 h. After the reaction was completed, the DMSO solution was added into distilled water (100 mL). The precipitated solid was collected by centrifuging and re-dissolved in MeOH (50 mL). Hydrazine monohydrate (2 mL) was added to the solution and stirred at 25 °C for 16 h. After the reaction was completed, the solution was acidified with TFA and purified with RP-HPLC to afford desired product **S4** as a lyophilized solid (1.7 g, 4.0 mmol, 42%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.44 (dd, *J* = 7.5, 5.0 Hz, 1H), 4.25 (dd, *J* = 7.5, 5.0 Hz, 1H), 4.04 (dt, *J* = 26.8, 4.8 Hz, 2H), 3.21 – 3.05 (m, 3H), 2.87 (dd, *J* = 12.8, 4.8 Hz, 1H), 2.64 (d, *J* = 12.8 Hz, 1H), 2.18 (dt, *J* = 56.8, 7.2 Hz, 2H), 1.72 – 1.47 (m, 4H), 1.39 (q, *J* = 7.2

Hz, 2H). ^{13}C NMR (100 MHz, Methanol-d_4) δ 176.2 (rotamer), 166.1, 72.7 (rotamer), 63.3, 61.6, 56.9 (rotamer), 41.0, 39.0 (rotamer), 34.0 (rotamer), 29.6 (rotamer), 29.4 (rotamer), 26.1 (rotamer).

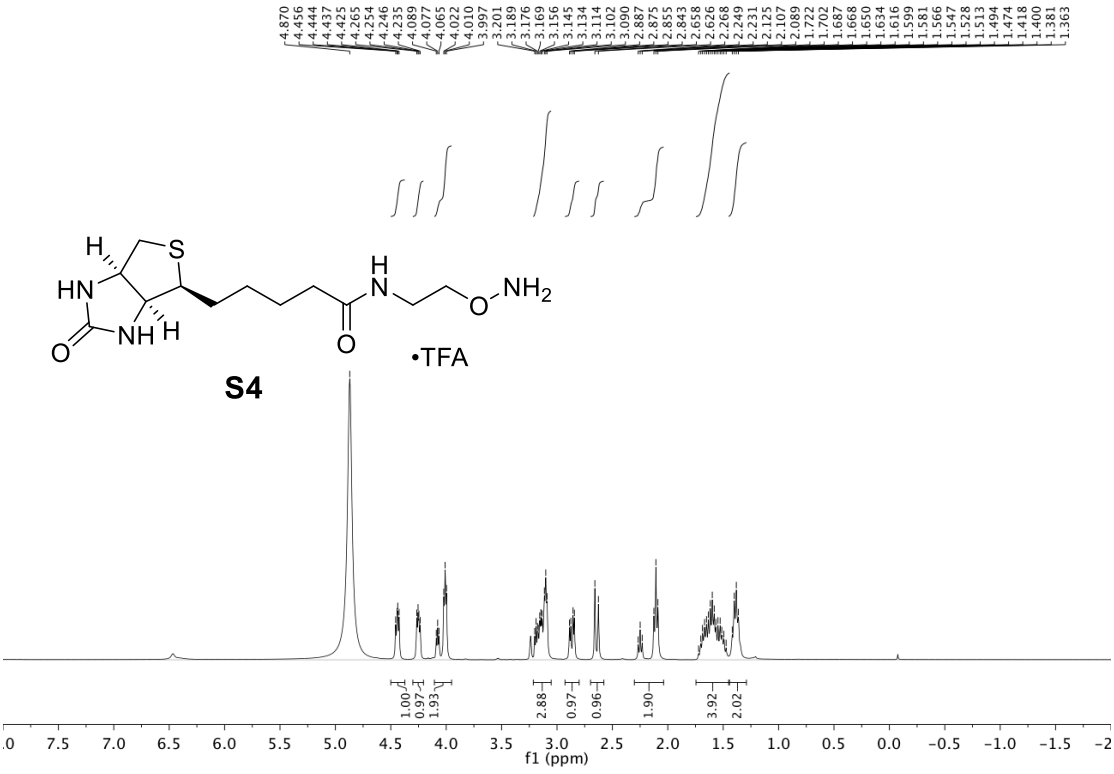


Figure 149: ^{1}H NMR spectrum for **S4**

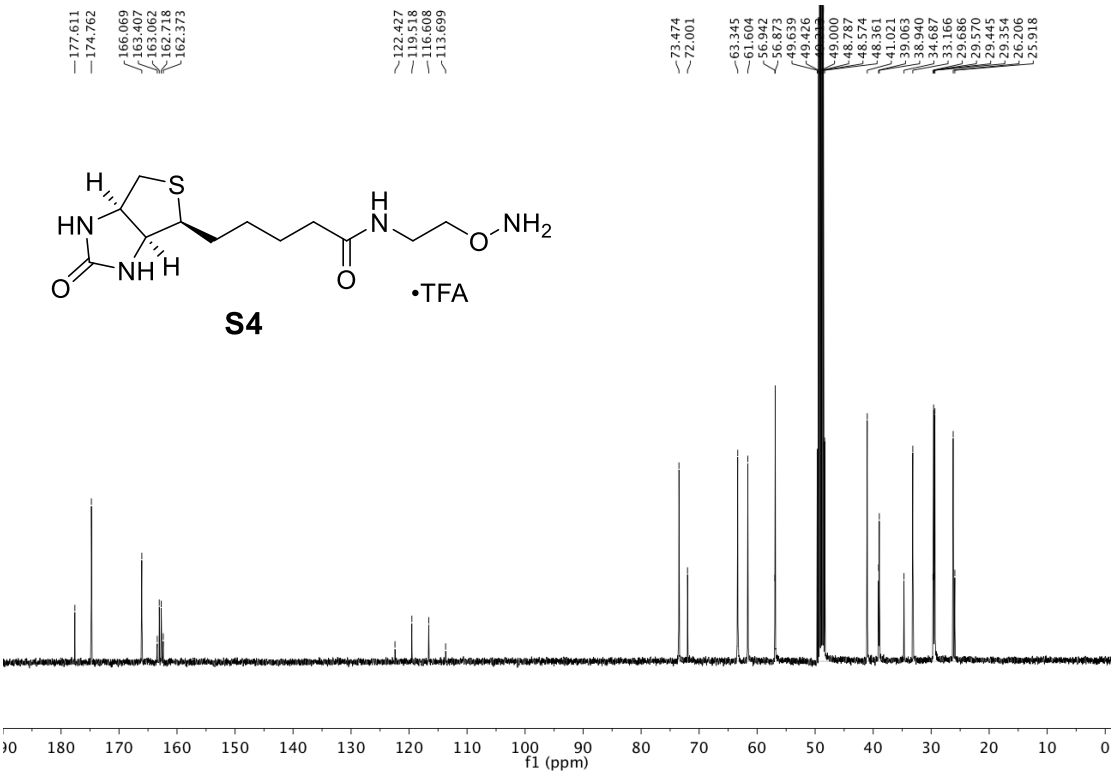
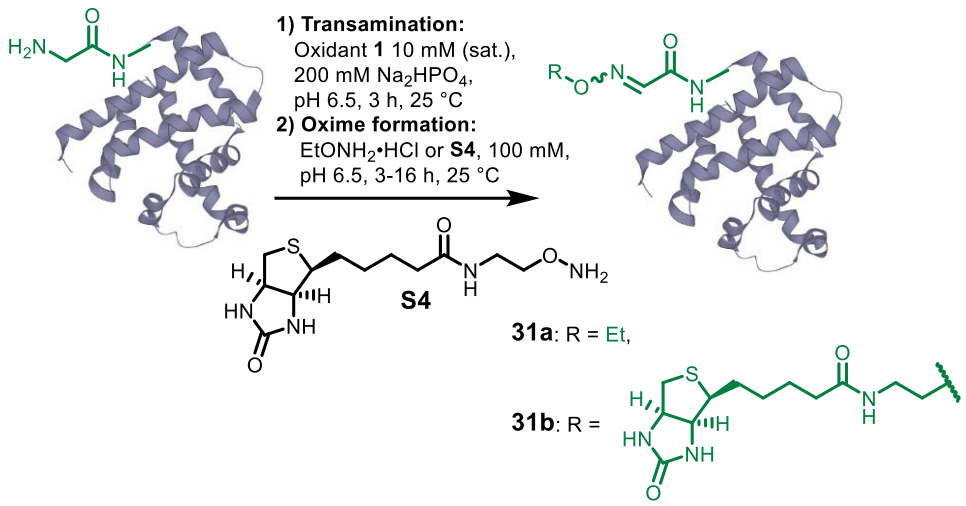


Figure 150: ^{13}C NMR spectrum for **S4**

3.5.2. Transamination of Myoglobin



Myoglobin (1.0 mg, 1 nmol) was dissolved in aqueous solution buffered with Na_2HPO_4 at pH 6.0 (1 mL), followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 6 h.

For generating ethyl oxime variants **31a**: the reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 16 h. The reaction mixture was analyzed with LCMS.

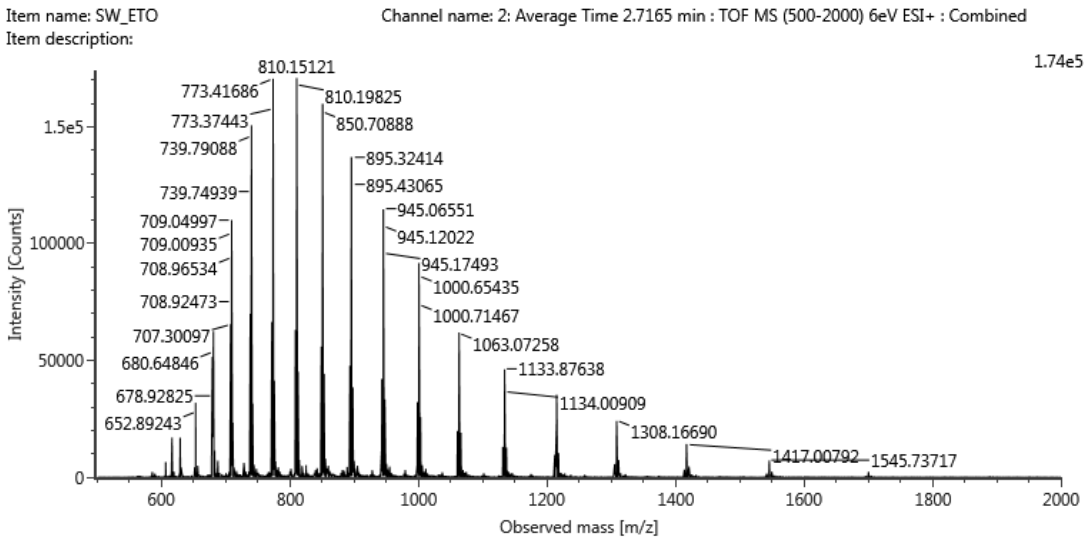


Figure 151: High-resolution MS spectrum of **31a**. *m/z* (ESI+) calcd M_{av} = 16993.6, found 1545.7 $[M + 11H]^{11+}$, 1417.0 $[M + 12H]^{12+}$, 1308.2 $[M + 13H]^{13+}$, 1214.8 $[M + 14H]^{14+}$, 1133.9 $[M + 15H]^{15+}$, 1063.1 $[M + 16H]^{16+}$ 1000.6 $[M + 17H]^{17+}$, 945.1 $[M + 18H]^{18+}$, 895.4 $[M + 19H]^{19+}$, 850.7 $[M + 20H]^{20+}$, 810.2 $[M + 21H]^{21+}$, 773.4 $[M + 22H]^{22+}$, 739.7 $[M + 23H]^{23+}$, 709.0 $[M + 24H]^{24+}$.

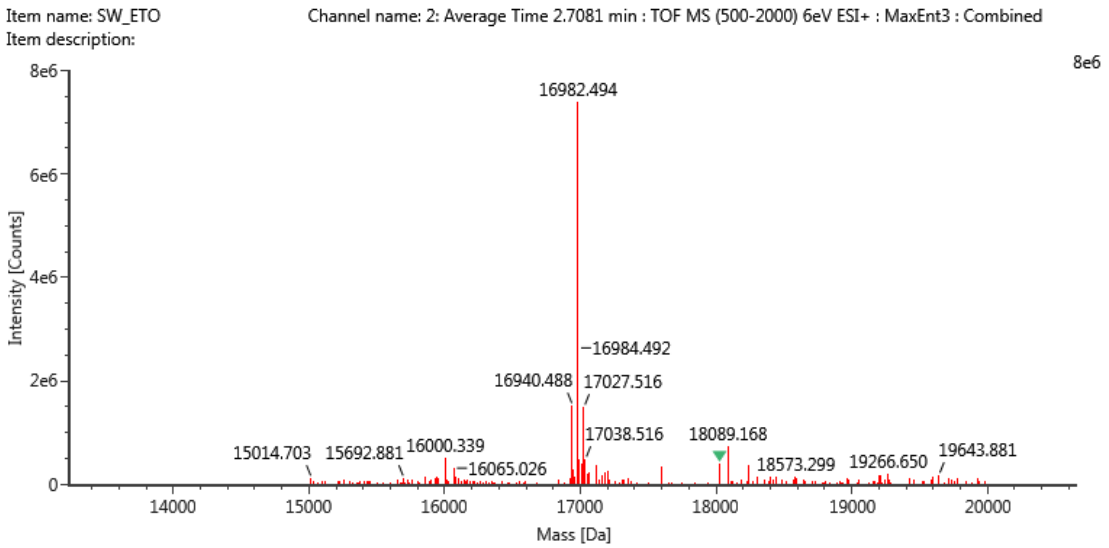


Figure 152: High-resolution deconvoluted MS spectrum of **31a**, *m/z* (ESI$^+$) calcd $[M + H]^+_{mono}$ = 16982.9, found 16982.5 $[M + H]^+$.

For generating biotinylated oxime variants **31b**: the reaction was quenched by extraction with ethyl acetate (2 x 1.0 mL) followed by addition of 0.5 mL of **S4** (0.1 M, pH 6.5) to the aqueous solution. The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 16 h. The reaction mixture was analyzed with LCMS.

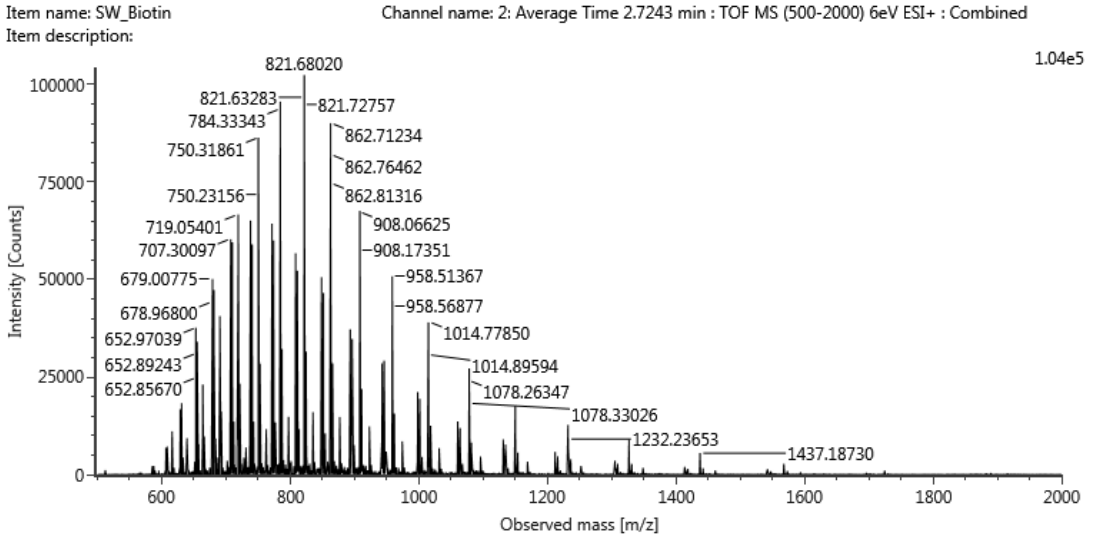


Figure 153: High-resolution MS spectrum of **31b**. *m/z* (ESI$^+$) calcd M_{av} = 17234.9, found 1437.2 $[M + 12H]^{12+}$, 1326.7 $[M + 13H]^{13+}$, 1232.2 $[M + 14H]^{14+}$, 1150.2 $[M + 15H]^{15+}$, 1078.3 $[M + 16H]^{16+}$ 1014.8 $[M + 17H]^{17+}$, 958.5 $[M + 18H]^{18+}$, 908.2 $[M + 19H]^{19+}$, 862.8 $[M + 20H]^{20+}$, 821.7 $[M + 21H]^{21+}$, 784.3 $[M + 22H]^{22+}$, 750.2 $[M + 23H]^{23+}$, 719.1 $[M + 24H]^{24+}$.

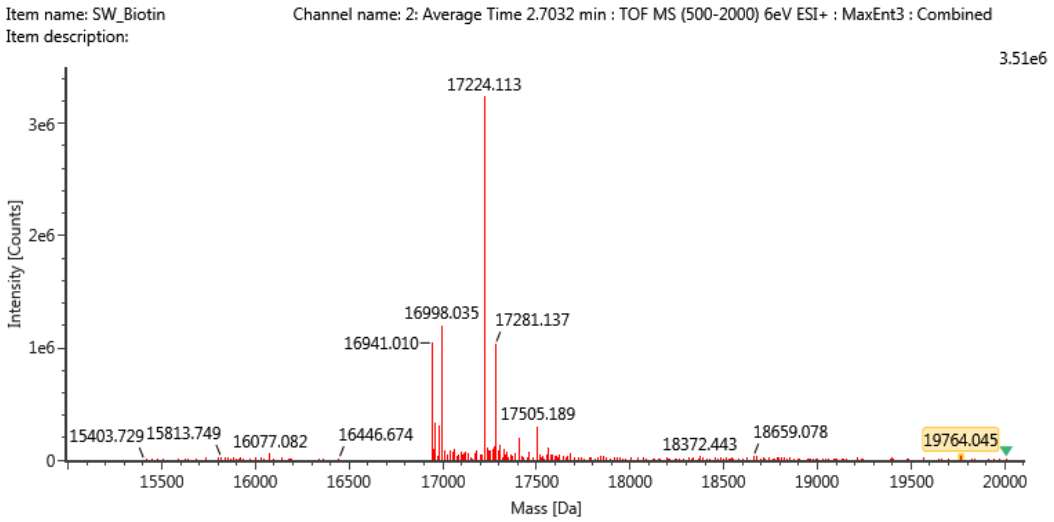
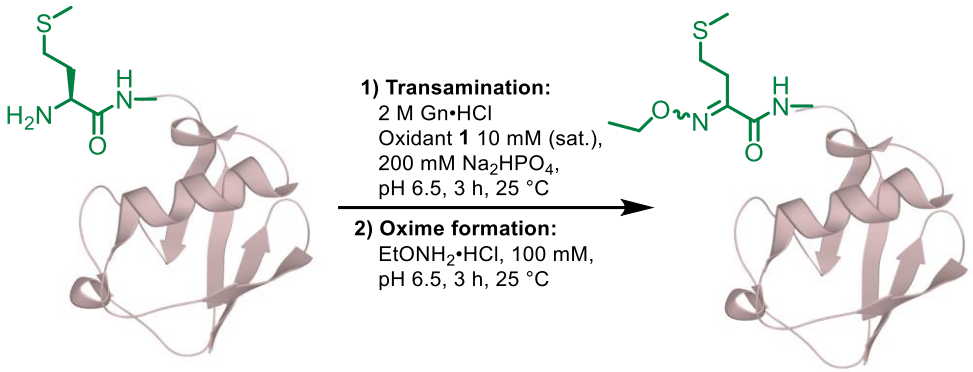


Figure 154: High-resolution deconvoluted MS spectrum of **31b**, *m/z* (ESI⁺) calcd $[M + H]^+_{mono}$ = 17224.1, found 17224.1$[M + H]^+$.

3.6. Transamination of Ubiquitin



32

Myoglobin (1.0 mg, 1 nmol) was dissolved in 2 M Gn·HCl solution buffered with Na_2HPO_4 at pH 6.5 (1 mL), followed by the addition of oxidant **1** (2.0 mg, 10 nmol, saturated). The reaction was incubated at 25 °C for 3 h. The reaction was quenched by addition of 0.5 mL of $EtONH_2$·HCl (0.2 M, pH 6.5). The mixture was adjusted at pH 6.5 and incubated at 25 °C for another 3 h. The reaction mixture was analyzed with LCMS.

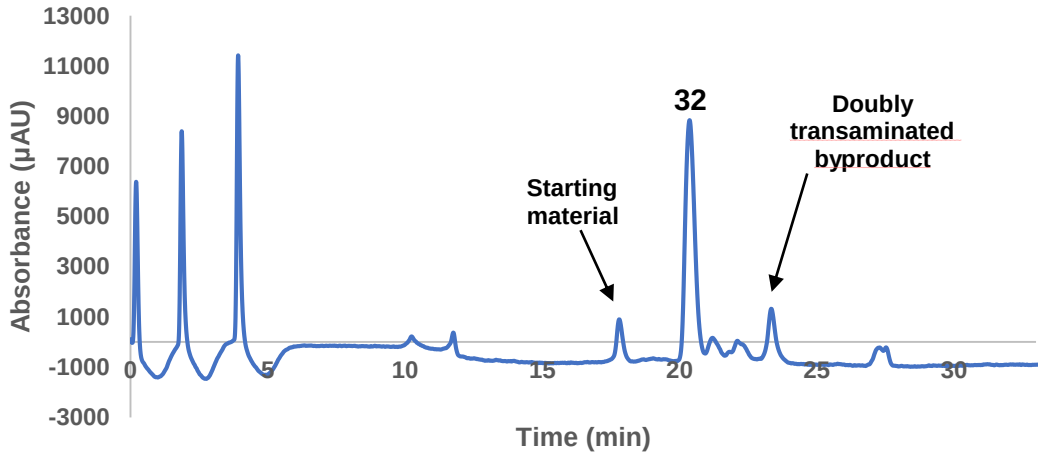


Figure 155: Analytical HPLC-MS trace of the transamination of Ubiquitin. Starting material: t_R = 17.9 min; oxime product **32**: t_R = 20.6 min, doubly transaminated byproduct (t_R = 23.4 min). (0% B for 3 min and then 20 to 50% B over 30 min with a flow rate of 0.2 mL/min buffered with 0.1% TFA, Waters BEH300 analytical column).

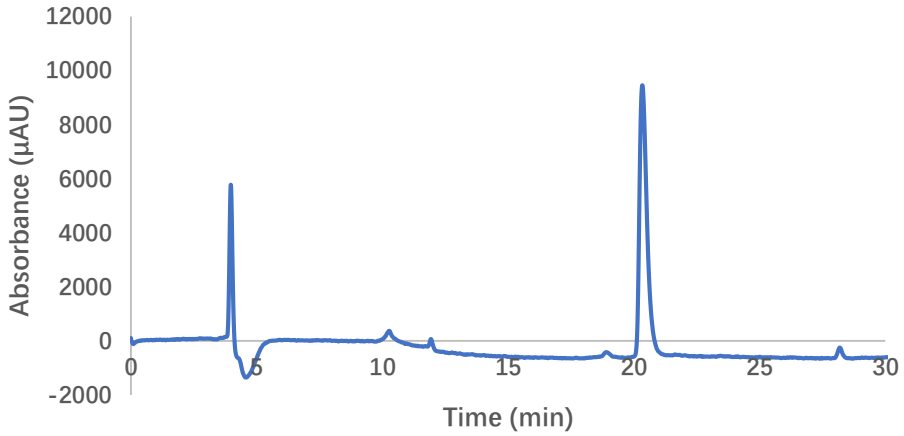


Figure 156: Analytical HPLC trace of purified **32** (t_R = 20.6 min, 0% B for 3 min and then 20 to 50% B over 30 min with a flow rate of 0.2 mL/min buffered with 0.1% TFA, Waters BEH300 analytical column).

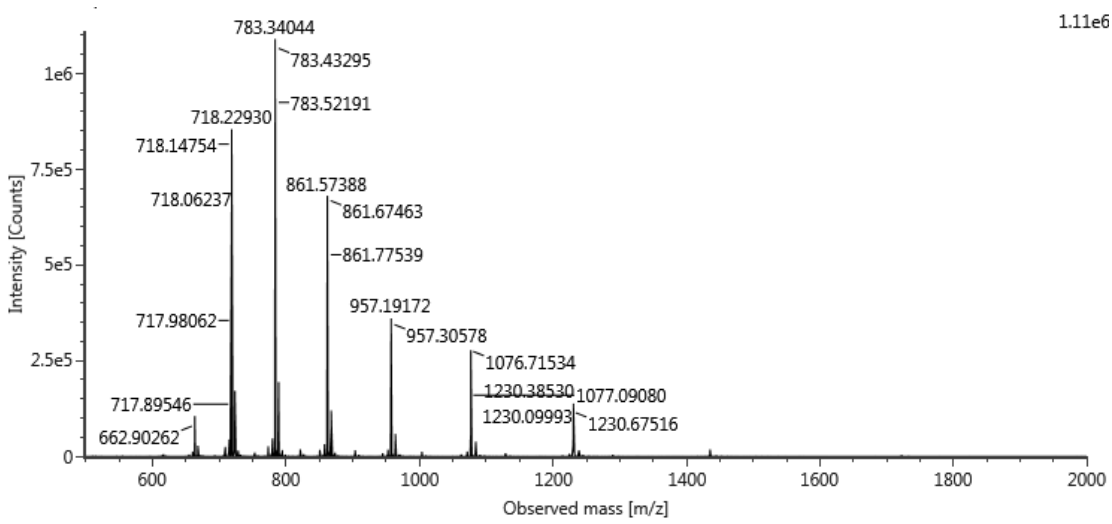


Figure 157: High-resolution MS spectrum of oxime product **32**, *m/z* (ESI$^+$) calcd M_{AV} = 8601.6, found 1230.7 $[M + 7H]^{7+}$, 1076.7 $[M + 8H]^{8+}$, 957.2 $[M + 9H]^{9+}$, 861.7 $[M + 10H]^{10+}$, 718.1 $[M + 11H]^{11+}$.

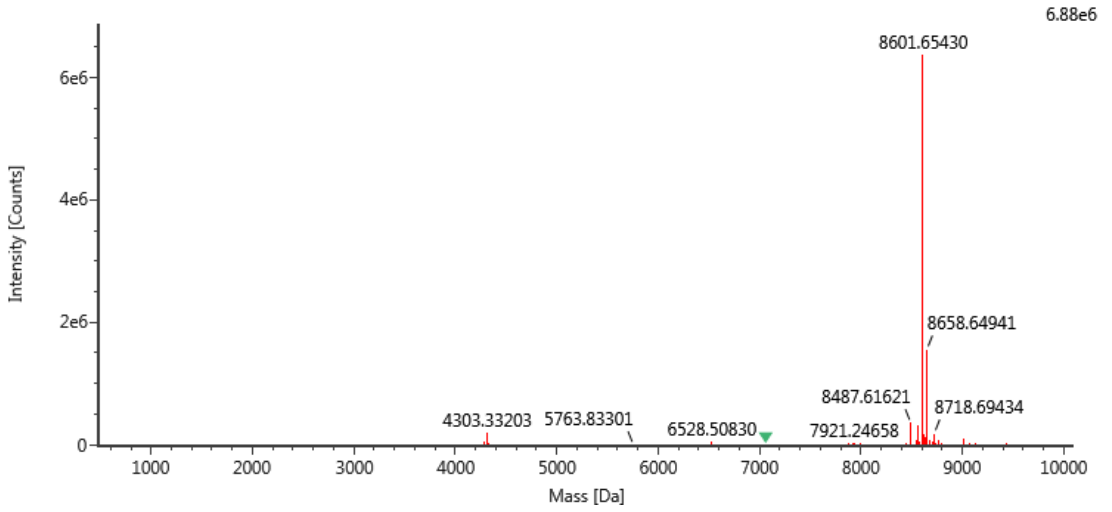


Figure 158: High-resolution deconvoluted MS spectrum of oxime product **32**, *m/z* (ESI$^+$) calcd $[M + H]^+_{mono}$ = 8601.6, found 8601.7 $[M + H]^+$.

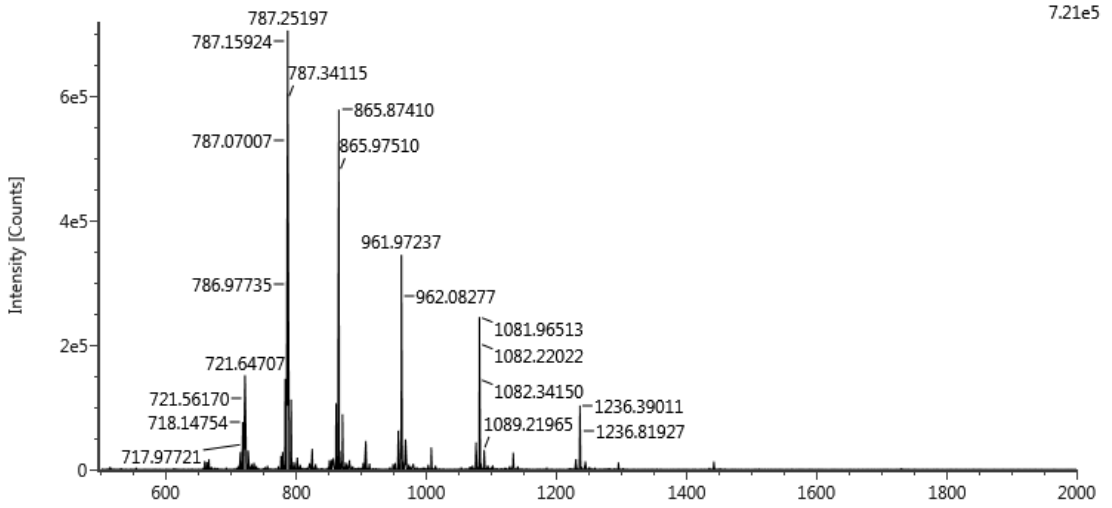


Figure 159: High-resolution MS spectrum of doubly transaminated byproduct (t_R = 23.4 min), *m/z* (ESI$^+$) calcd M_{AV} = 8648.9, found 1236.4 $[M + 7H]^{7+}$, 1082.0 $[M + 8H]^{8+}$, 962.0 $[M + 9H]^{9+}$, 865.9 $[M + 10H]^{10+}$, 787.3 $[M + 11H]^{11+}$.

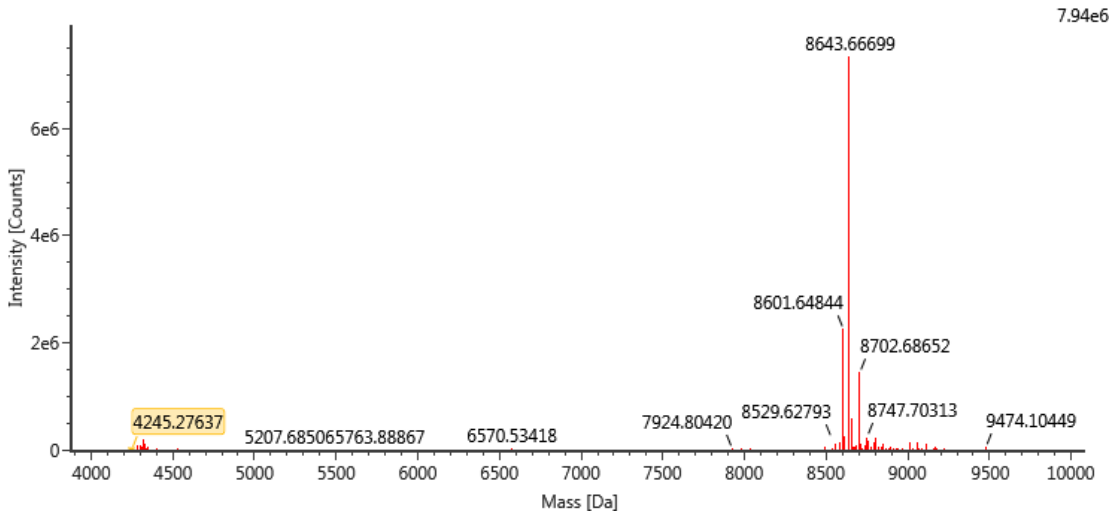


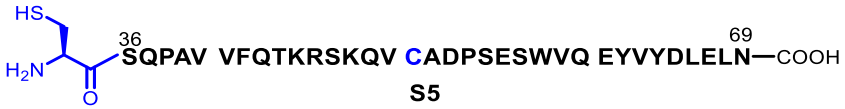
Figure 160: High-resolution deconvoluted MS spectrum of doubly transaminated byproduct (t_R = 23.4 min), *m/z* (ESI$^+$) calcd $[M + H]^+_{mono}$ = 8643.6, found 8643.7 $[M + H]^+$.

4. Syntheses of MIP-1β analogs

4.1. Selective peptide cleavage and C-terminal thioester generation

After washing the resin with DCM thoroughly (10 × 5 mL), resin-bound peptide was treated with HFIP/DCM (3:7, v/v, 5 mL, 4 x 30 min) at 25 °C. The combined solution was evaporated under reduced pressure. The crude fully-protected peptide was mixed with ethyl 3-mercaptopropinoate (100 equiv.), DIPEA (5 equiv.) in DCM with a final concentration of 0.3 mM (of peptide) in solution. After cooling the reaction mixture to -50 °C, PyBOP (5 equiv.) was added. The resulting solution was stirred at -30 °C for 3 h. After the reaction was completed, the mixture was concentrated under reduced pressure and subjected to acidolytic deprotection as previously mentioned.

4.2. Synthesis and analytical data for peptide **S5**



S5

Rink-amide resin (0.5 g, loading capacity 0.5 mmol/g) was swelled in DCM (5 mL) for 30 min. The Fmoc group was deprotected by treatment with piperidine in DMF (1/4, v/v, 5 mL) solution (2 × 5 min, 25 °C). The resin was subsequently washed with DMF (3 × 5 mL), DCM (3 × 5 mL) and DMF (3 × 5 mL). Fmoc-Asp-O*t*Bu (0.42 g, 1.0 mmol, 4 equiv.), DIC (0.16 mL, 1.02 mmol, 4.1 equiv.) and oxyma (0.15 g, 1.04 mmol, 4.2 equiv.) were dissolved in DMF (4 mL) and added to the reaction vessel containing the resin. The reaction mixture was agitated at 50 °C for 25 min. Once the reaction was completed, the resin was

washed with DMF (3 × 5 mL), DCM (3 × 5 mL) and DMF (3 × 5 mL). Coupling of the rest of the amino acids was performed in accordance with general Fmoc-SPPS protocols. Gln43-Thr44, Glu56-Ser57 were both coupled as pseudoproline dipeptides, respectively.[6] Once the elongation was completed, acidolytic cleavage was performed. The resulting solid was purified with RP-HPLC (Dubhe C18 column, 0 to 50% B over 25 min with a flow rate of 20 mL/min) and yielded to desired MIP-1β (35-69) peptide **S5** as a lyophilized solid (250 mg, 56 nmol, 23%).

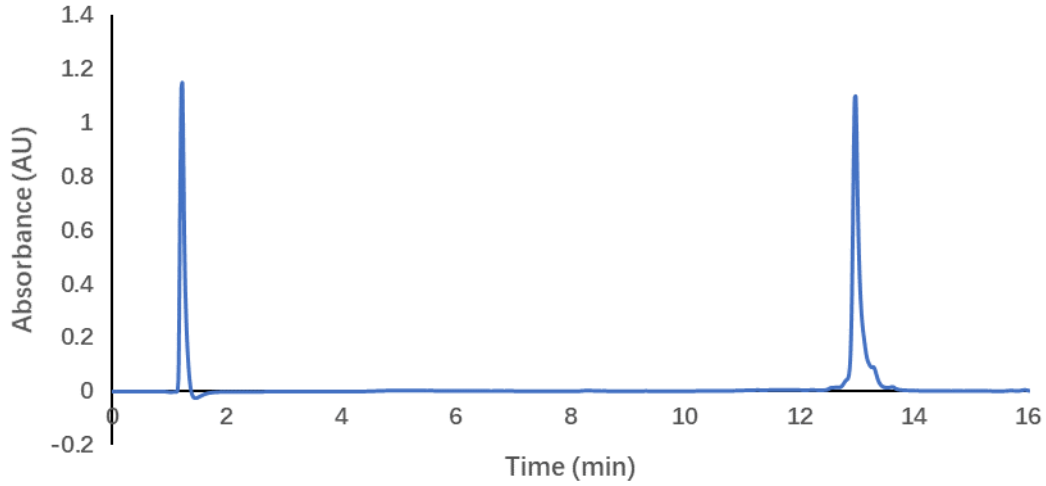


Figure 161: Analytical HPLC trace of purified MIP-1β (35-69) peptide **S5** (t_R = 13.0 min, 0% B for 1 min and then 0 to 50% B over 15 min with a flow rate of 0.4 mL/min, Dubhe C18 analytical column).

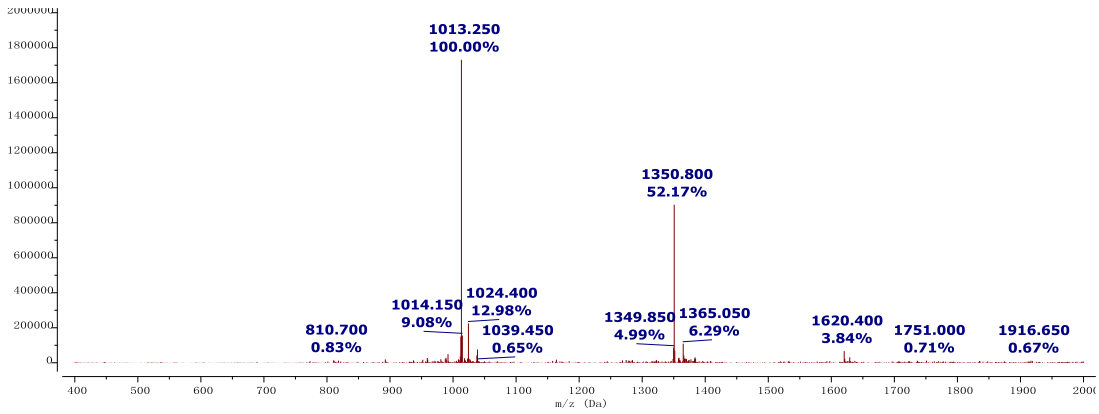


Figure 162: Low-resolution MS spectrum of MIP-1β (35-69) peptide **S5**, *m/z* (ESI⁺) calcd M_{AV} = 4048.5, found 1350.8 $[M + 3H]^{3+}$, 1013.3 $[M + 4H]^{4+}$, 810.7 $[M + 5H]^{5+}$.

4.3. Synthesis and analytical data for peptide **S6**



Synthesis of peptide thioester **S6** was performed on 2-CTC resin (0.18 nmol scale) according to Fmoc-SPPS protocol. Tyr15-Thr16, Gly30-Thr31 were both coupled as pseudoproline dipeptides, respectively.[6] Cys12 was coupled as Boc-Thz-OH. Once the peptide was fully elongated, the peptide was

cleaved with HFIP (30 vol% in DCM), followed by thioesterification and acidolytic deprotection. After ether precipitation, the resulting solid was purified with RP-HPLC (Dubhe C18 column, 0 to 50% B over 25 min with a flow rate of 20 mL/min) and yielded to desired MIP-1β (12-34) peptide thioester **S6** as a lyophilized solid (107 mg, 33 nmol, 19%).

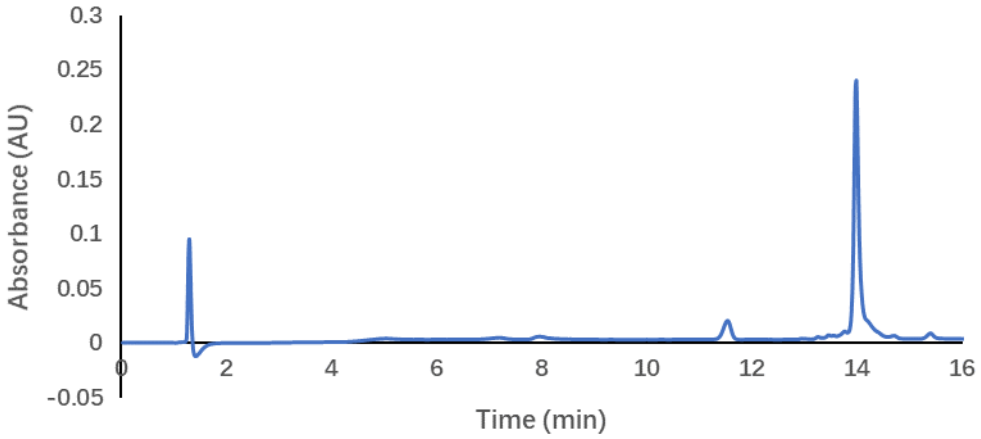


Figure 163: Analytical HPLC trace of purified MIP-1β (12-34) peptide thioester **S6** (t_R = 14.0 min, 0% B for 1 min and then 0 to 50% B over 15 min with a flow rate of 0.4 mL/min, Dubhe C18 analytical column).

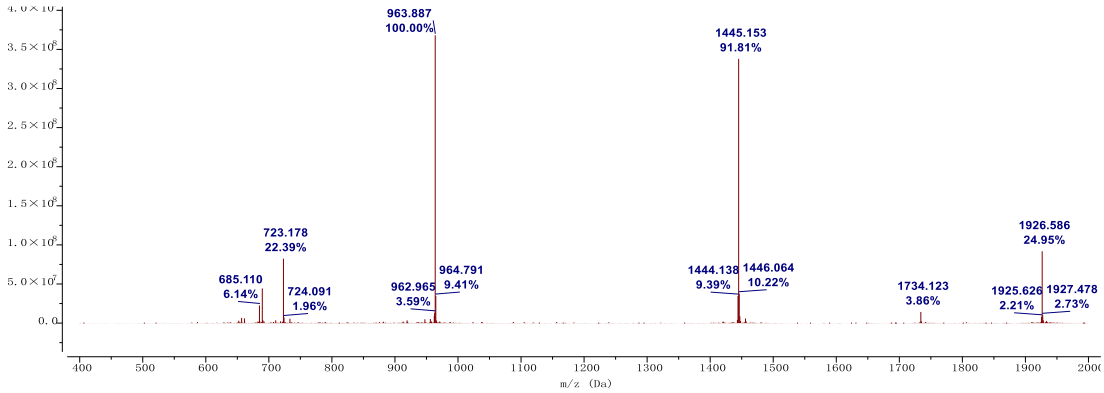
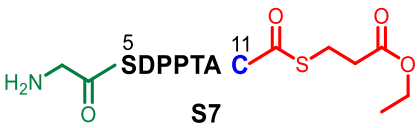


Figure 164: Low-resolution MS spectrum of MIP-1β (12-34) peptide thioester **S6**, *m/z* (ESI⁺) calcd M_{AV} = 2888.3, found 1926.6 $[2M + 3H]^{3+}$,1445.2 $[M + 2H]^{2+}$, 963.9 $[M + 3H]^{3+}$, 723.2 $[M + 4H]^{4+}$.

4.4. Synthesis and analytical data for peptide **S7**



Synthesis of peptide thioester **S7** was performed on 2-CTC resin (0.10 nmol scale) according to Fmoc-SPPS protocol. Once the peptide was fully elongated, the peptide was cleaved with HFIP (30 vol% in DCM), followed by thioesterification and acidolytic deprotection. After ether precipitation, the resulting solid was purified with RP-HPLC (Dubhe C18 column, 0 to 50% B over 25 min with a flow rate of 20

mL/min) and yielded to desired MIP-1β (4-11) peptide thioester **S7** as a lyophilized solid (17 mg, 19 nmol, 20%).

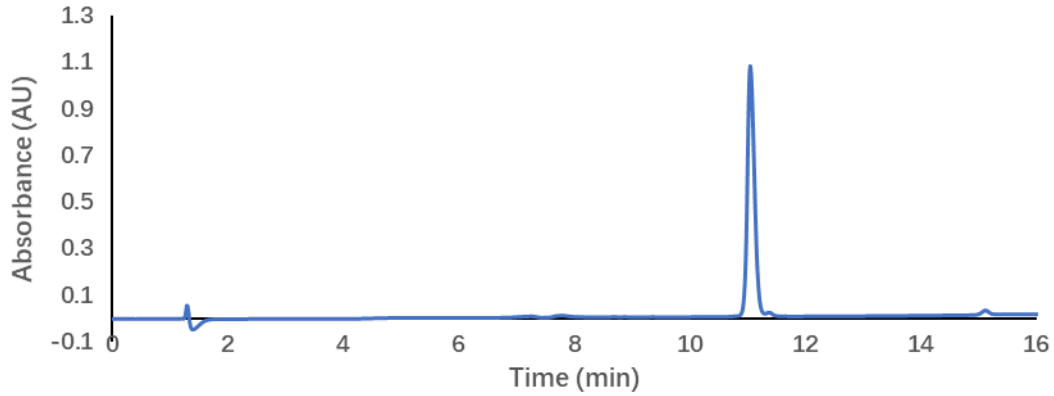


Figure 165: Analytical HPLC trace of purified MIP-1β (4-11) peptide thioester **S7** (t_R = 11.1 min, 0% B for 1 min and then 0 to 50% B over 15 min with a flow rate of 0.4 mL/min, Dubhe C18 analytical column).

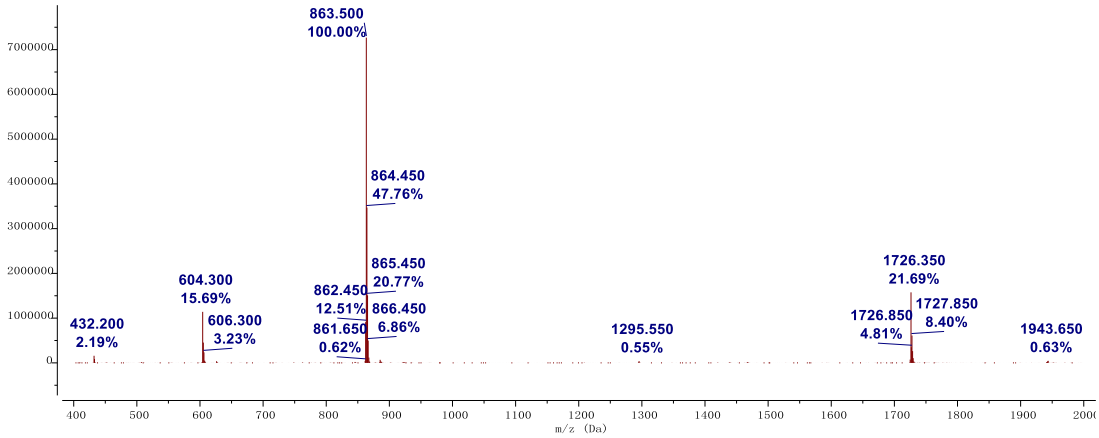
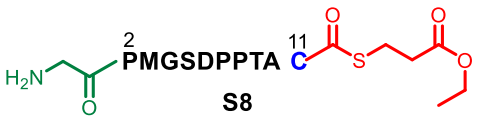


Figure 166: Low-resolution MS spectrum of MIP-1β (4-11) peptide thioester **S7**, *m/z* (ESI⁺) calcd M_{AV} = 862.3, found 863.5 $[M + H]^+$.

4.5. Synthesis and analytical data for peptide **S8**



Synthesis of peptide thioester **S8** was performed on 2-CTC resin (0.10 nmol scale) according to Fmoc-SPPS protocol. Once the peptide was fully elongated, the peptide was cleaved with HFIP (30 vol% in DCM), followed by thioesterification and acidolytic deprotection. After ether precipitation, the resulting solid was purified with RP-HPLC (Dubhe C18 column, 0 to 50% B over 25 min with a flow rate of 20 mL/min) and yielded to desired MIP-1β (Gly1-11) peptide thioester **S8** as a lyophilized solid (18 mg, 16 nmol, 16%).

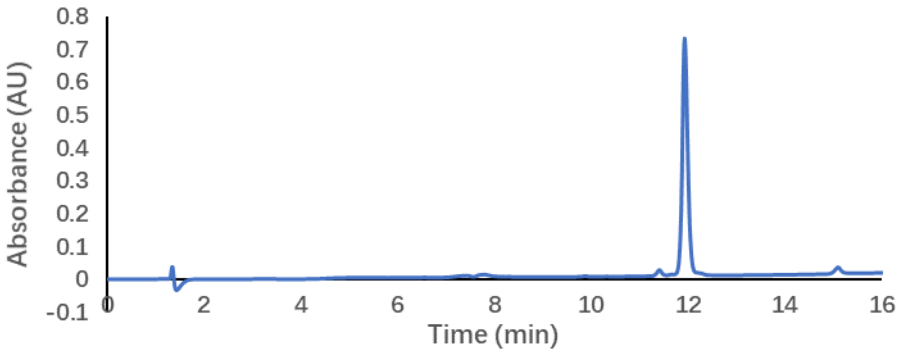


Figure 167: Analytical HPLC trace of purified MIP-1β (Gly1-11) peptide thioester **S8** (t_R = 12.0 min, 0% B for 1 min and then 0 to 50% B over 15 min with a flow rate of 0.4 mL/min, Dubhe C18 analytical column).

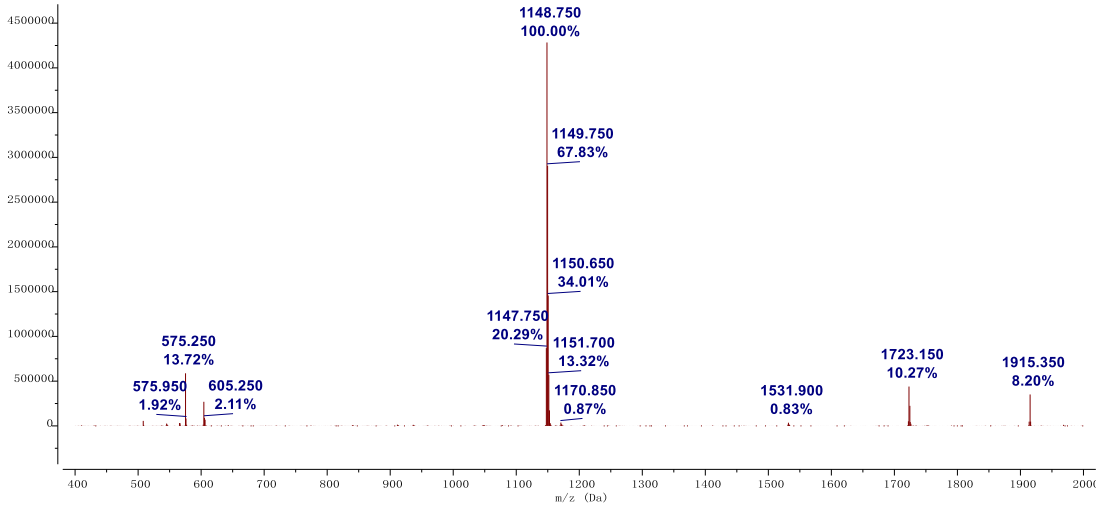
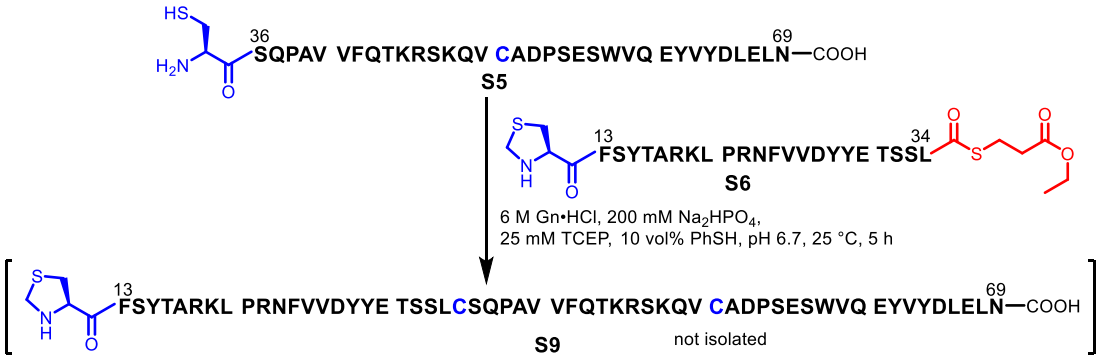


Figure 168: Low-resolution MS spectrum of MIP-1β (Gly1-11) peptide thioester **S8**, *m/z* (ESI⁺) calcd M_{AV} = 1147.4, found 1148.8 $[M + H]^+$.

4.6. Protein assembly by NCL



Fragment **S5** (48 mg, 11 µmol, 1.1 equiv.) and thioester **S6** (34 mg, 10 µmol, 1.0 equiv.) were added to an aqueous solution (6 mL) containing 6 M Gn·HCl, 200 mM Na_2HPO_4, 25 mM TCEP. The final pH of this

solution was 6.7, followed by the addition of PhSH (0.6 mL). The mixture was agitated at 25 °C for 5 h. Aliquot was taken for LCMS analysis to confirm that the product **S9** was formed. The mixture was subjected to the next reaction without further purification.

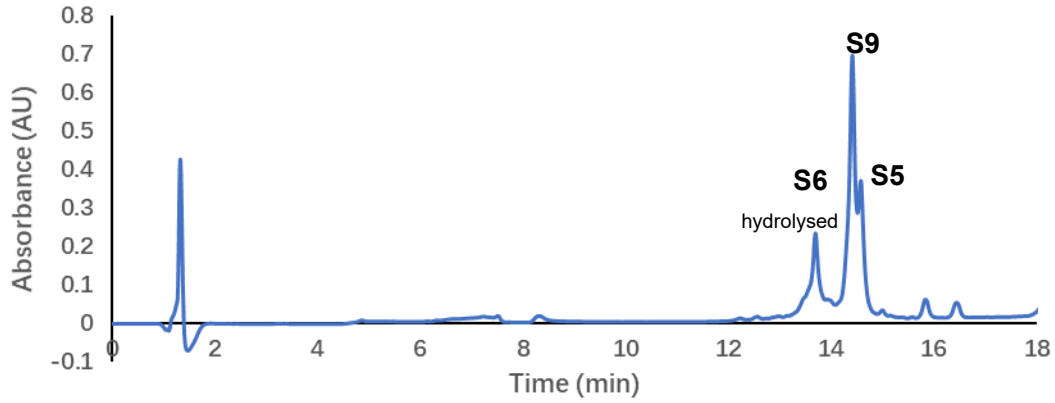


Figure 169: Crude analytical HPLC trace of the ligation between peptide **S5** and peptide thioester **S6**, desired ligation product **S9** was observed (t_R = 14.4 min, 0% B for 1 min and then 0 to 50% B over 15 min with a flow rate of 0.4 mL/min, Dubhe C18 analytical column).

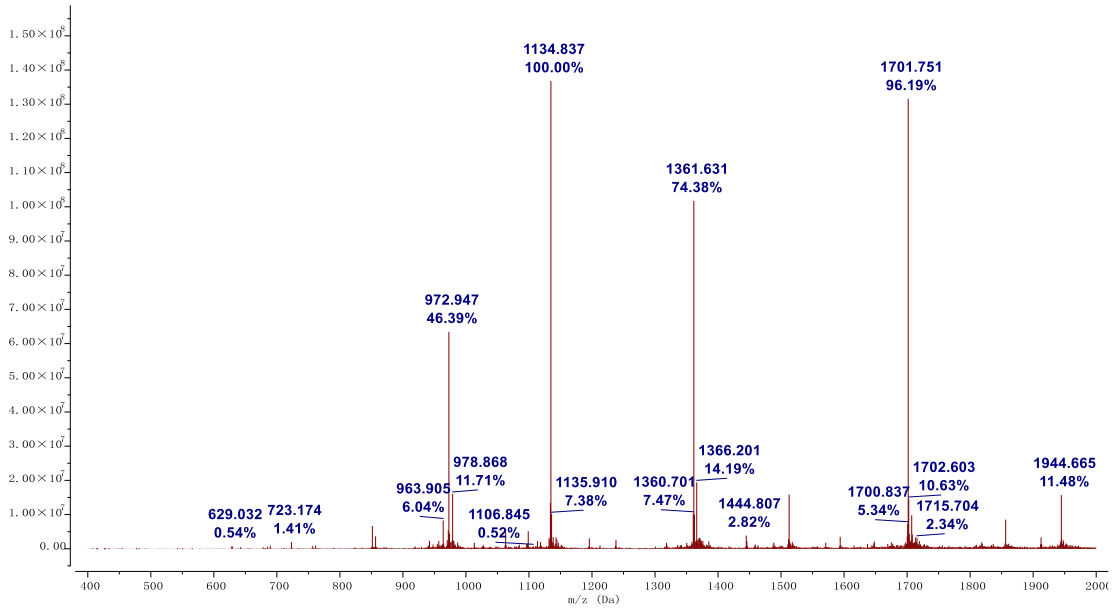
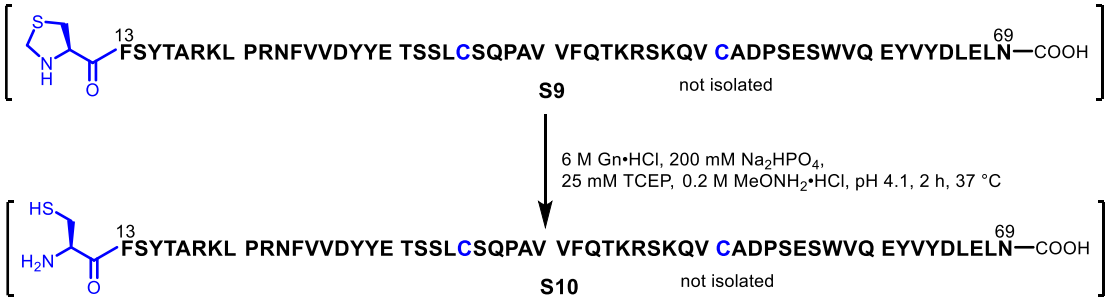


Figure 170: Low-resolution MS spectrum of ligation product peptide **S9**, *m/z* (ESI⁺) calcd M_{AV} = 6802.6, found 1701.8 $[M + 4H]^{4+}$, 1361.6 $[M + 5H]^{5+}$, 1134.8 $[M + 6H]^{6+}$, 972.9 $[M + 7H]^{7+}$.



$MeONH_2$·HCl (103 mg, 1.2 mmol) was added to the previous reaction mixture containing fragment **S9**, the final pH of the solution was adjusted to pH 4.1 by addition of conc. HCl. The mixture was incubated at 37 °C for 2 h. The desired product **S10** was observed in LCMS analysis as major product. The reaction mixture was subjected to the next step without further purification.

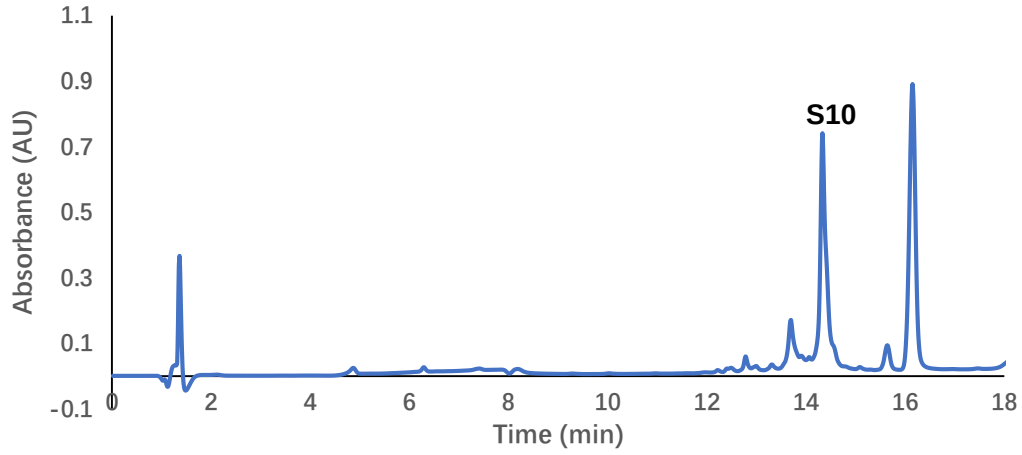


Figure 171: Crude analytical HPLC trace of the Thz deprotection, desired ligation product **S10** was observed (t_R = 14.3 min, 0% B for 1 min and then 0 to 50% B over 15 min with a flow rate of 0.4 mL/min, Dubhe C18 analytical column).

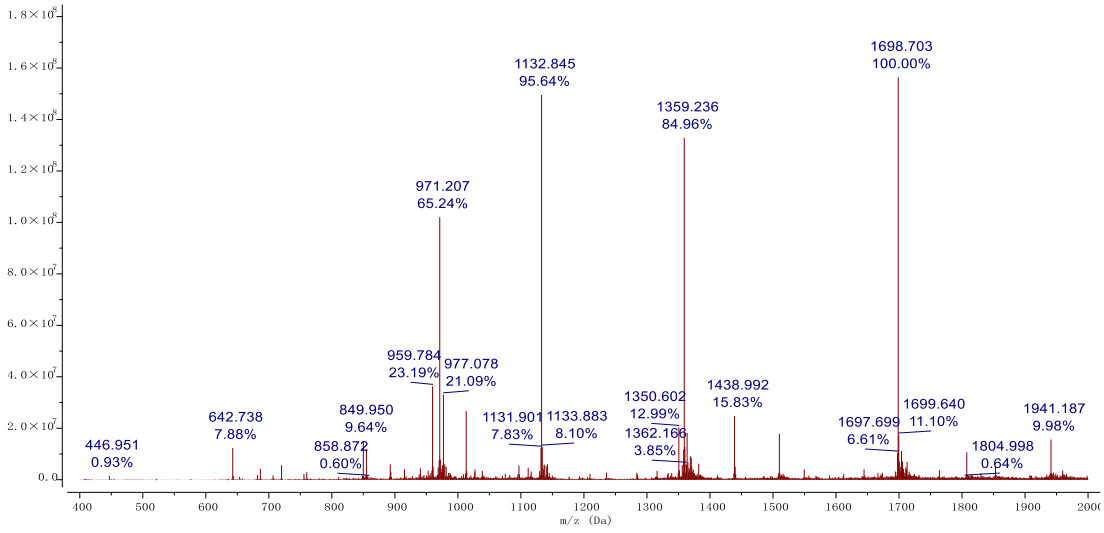
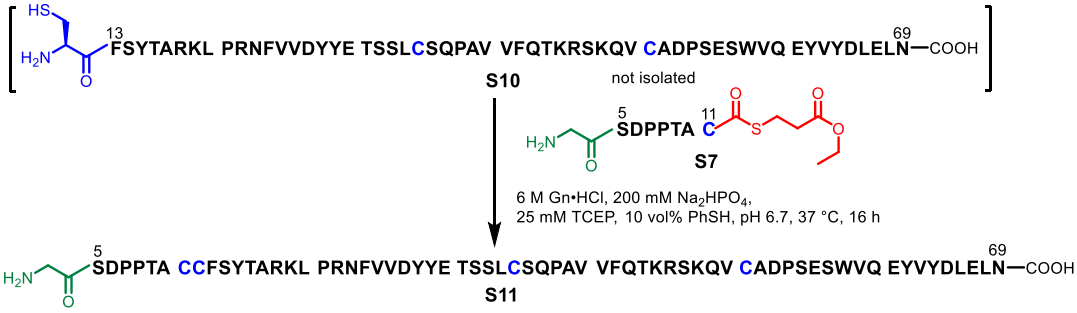


Figure 172: Low-resolution MS spectrum of ligation product peptide **S10**, *m/z* (ESI⁺) calcd M_{AV} = 6790.6, found 1698.7 $[M + 4H]^{4+}$, 1359.2 $[M + 5H]^{5+}$, 1132.8 $[M + 6H]^{6+}$, 971.2 $[M + 7H]^{7+}$.



The previous reaction mixture containing peptide **S10** was adjusted to pH 6.7. Peptide thioester **S7** (11 mg, 12 µmol, 12 equiv.) was added to the mixture. The mixture was incubated at 37 °C for 16 h. Desired product reduced protein **S11** was observed as major product. The reaction mixture was purified with RP-HPLC (Dubhe C18 column, 0 to 50% B over 25 min with a flow rate of 20 mL/min) and yielded to desired MIP-1β (4-69) reduced protein **S11** as a lyophilized solid (34 mg, 4.1 µmol, 41%).

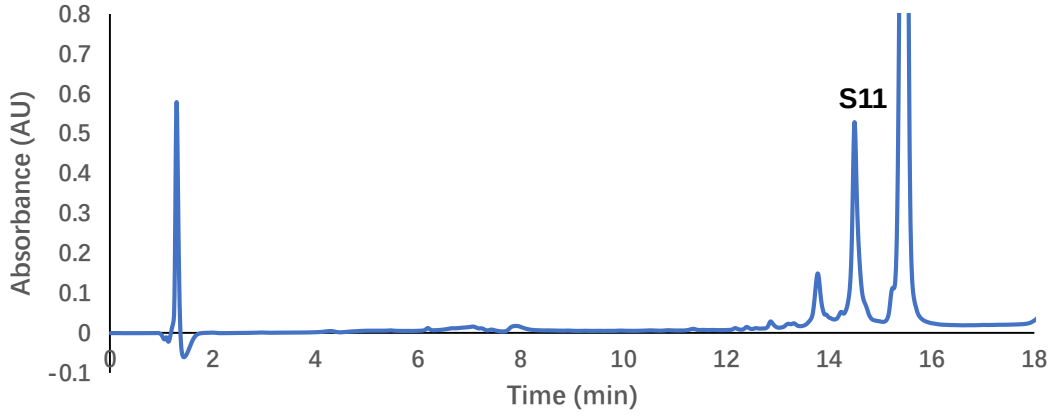


Figure 173: Crude analytical HPLC trace of 2nd ligation between peptide **S10** and peptide thioester **S7**, desired ligation product **S11** was observed (t_R = 14.5 min, 0% B for 1 min and then 0 to 50% B over 15 min with a flow rate of 0.4 mL/min, Dubhe C18 analytical column).

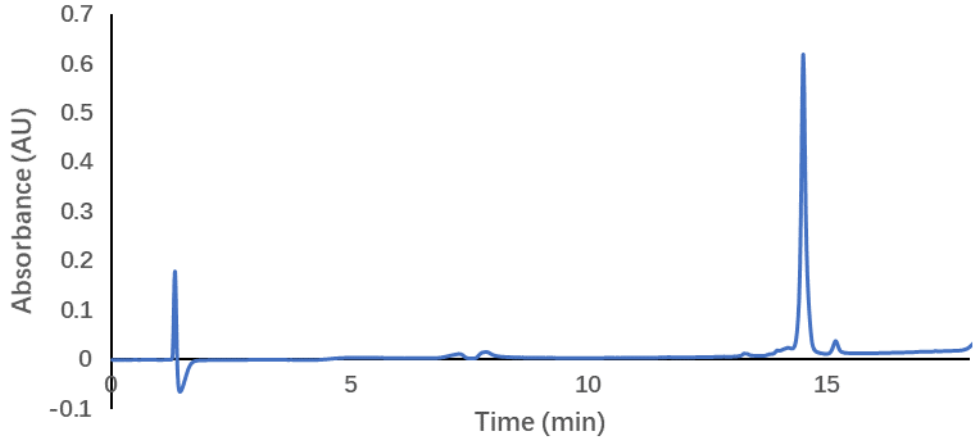


Figure 174: Analytical HPLC trace of purified MIP-1β (4-69) reduced protein **S11** (t_R = 14.5 min, 0% B for 1 min and then 0 to 50% B over 15 min with a flow rate of 0.4 mL/min, Dubhe C18 analytical column).

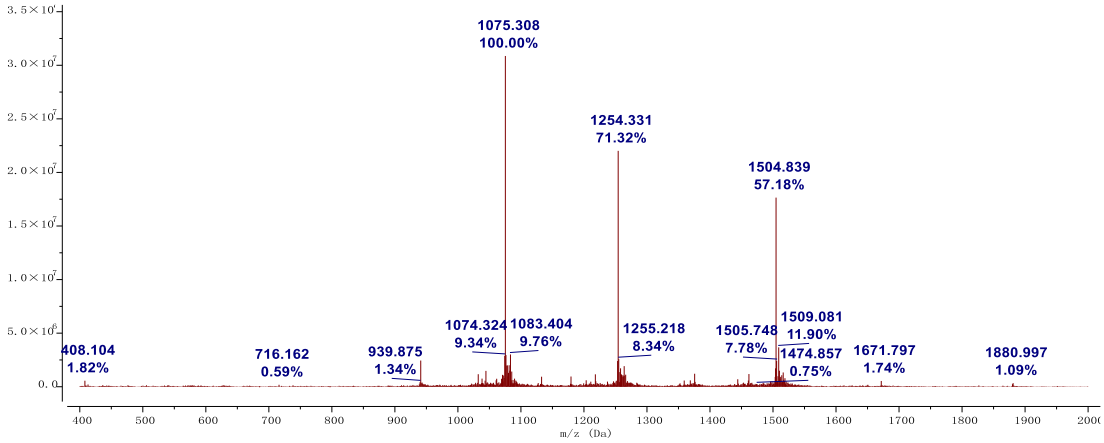
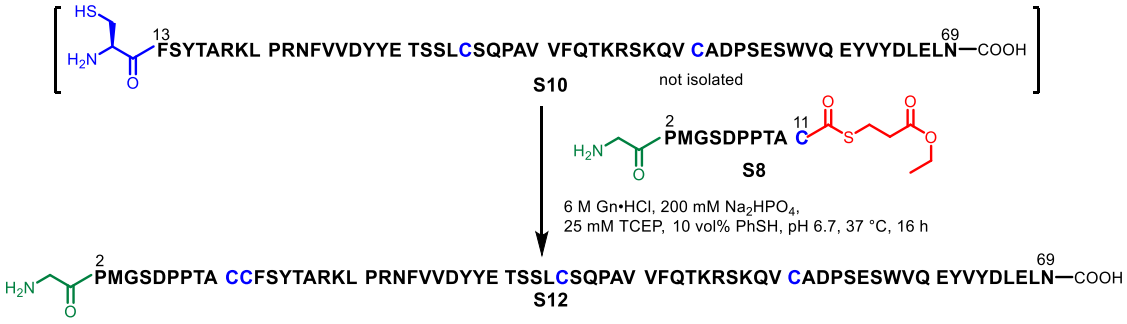


Figure 175: Low-resolution MS spectrum of ligation product peptide **S11**, *m/z* (ESI⁺) calcd M_{AV} = 7519.4, found 1881.0 $[M + 4H]^{4+}$, 1504.8 $[M + 5H]^{5+}$, 1254.3 $[M + 6H]^{6+}$, 1075.3 $[M + 7H]^{7+}$.



The previous reaction mixture containing peptide **S10** was adjusted to pH 6.7. Peptide thioester **S8** (15 mg, 12 µmol, 12 equiv.) was added to the mixture. The mixture was incubated at 37 °C for 16 h. Desired product reduced protein **S12** was observed as major product. The reaction mixture was purified with RP-HPLC (Dubhe C18 column, 0 to 50% B over 25 min with a flow rate of 20 mL/min) and yielded to desired MIP-1β (Gly1-69) reduced protein **S12** as a lyophilized solid (38 mg, 4.5 µmol, 45%).

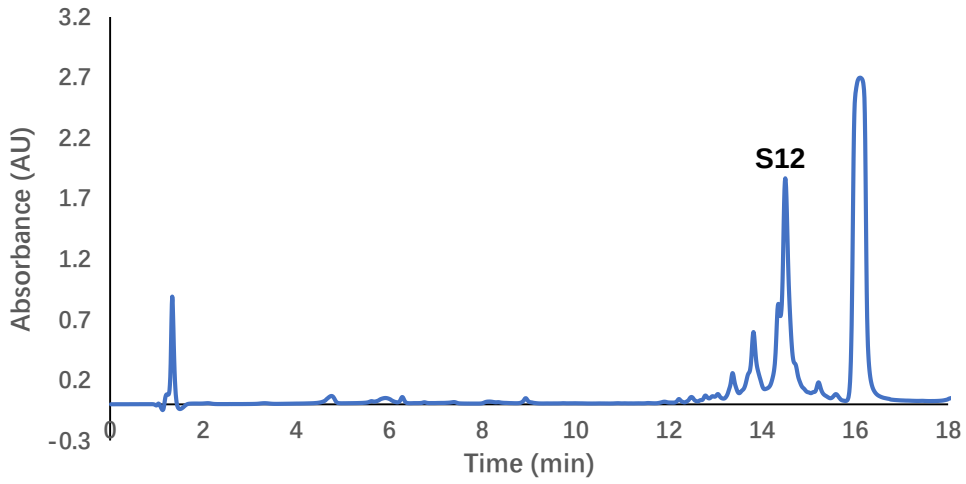


Figure 176: Crude analytical HPLC trace of 2nd ligation between peptide **S10** and peptide thioester **S8**, desired ligation product **S12** was observed (t_R = 14.6 min, 0% B for 1 min and then 0 to 50% B over 15 min with a flow rate of 0.4 mL/min, Dubhe C18 analytical column).

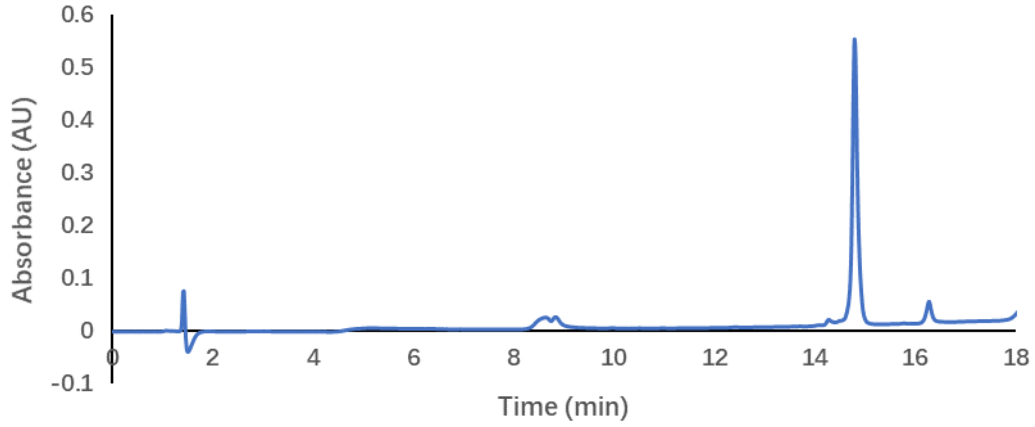


Figure 177: Analytical HPLC trace of purified MIP-1β (Gly1-69) reduced protein **S12** (t_R = 14.7min, 0% B for 1 min and then 0 to 60% B over 15 min, Dubhe C18 analytical column).

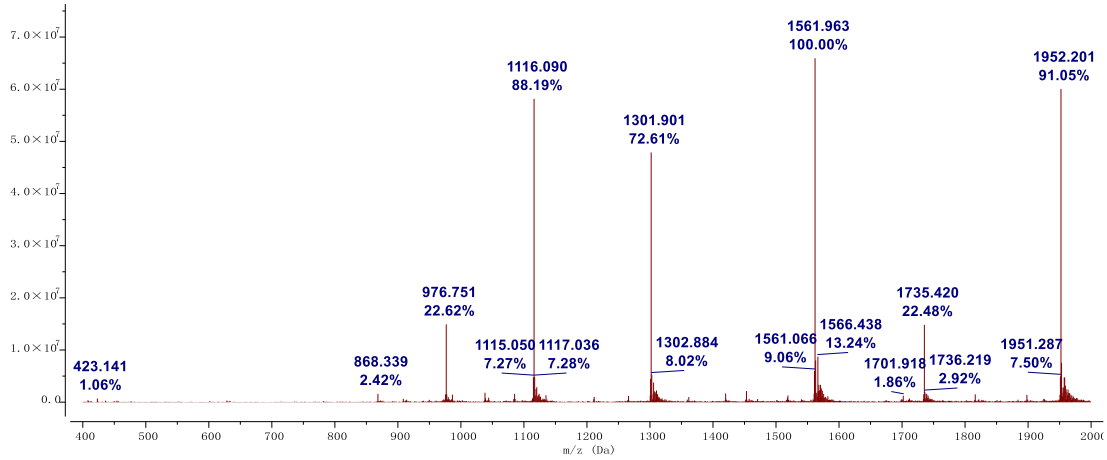
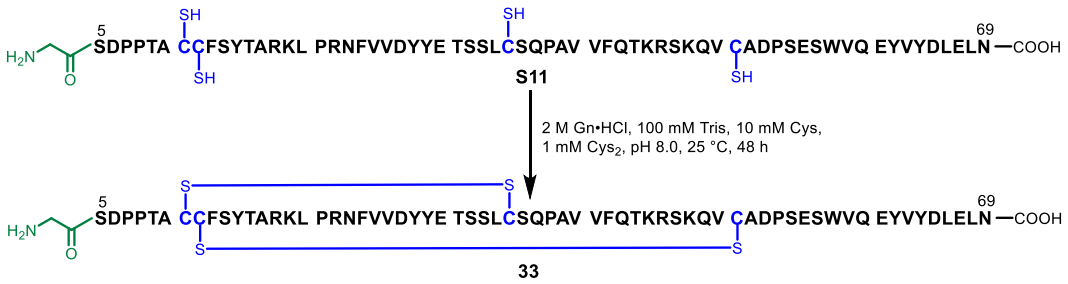


Figure 178: Low-resolution MS spectrum of ligation product peptide **S12**, *m/z* (ESI⁺) calcd M_{AV} = 7804.7, found 1952.2 $[M + 4H]^{4+}$, 1562.0 $[M + 5H]^{5+}$, 1301.9 $[M + 6H]^{6+}$, 1116.1 $[M + 7H]^{7+}$.

4.7. Refolding



Reduced MIP-1β 4-69 protein **S11** (30 mg, 3.6 μmol) was added to an aqueous solution (60 mL, with a final protein conc. = 0.5 mg/mL) containing 2 M Gn·HCl, 100 mM Tris, 10 mM cysteine and 1 mM cystine buffered at pH 8.0. The solution was stirred at 25 °C for 48 h. The reaction mixture was analyzed with LC-MS. Once the reaction was completed, the mixture was purified with RP-HPLC (Dubhe C18 column, 0 to 50% B over 25 min with a flow rate of 20 mL/min) and yielded to desired MIP-1β 4-69 protein **33** as a lyophilized solid. (9.6 mg, 1.14 μmol, 38%)

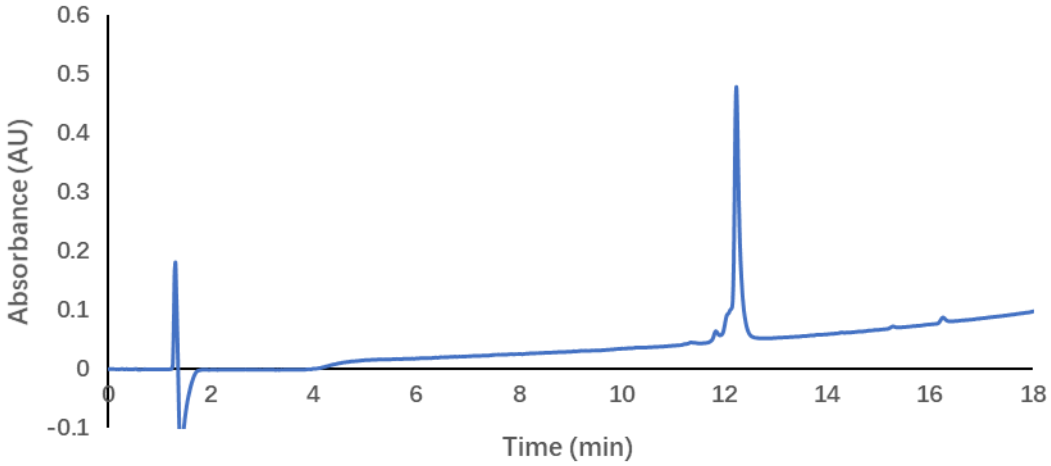


Figure 179: Analytical HPLC trace of purified MIP-1β (4-69) protein **33** (t_R = 12.2 min, 0% B for 1 min and then 0 to 60% B over 15 min with a flow rate of 0.4 mL/min, Dubhe C18 analytical column).

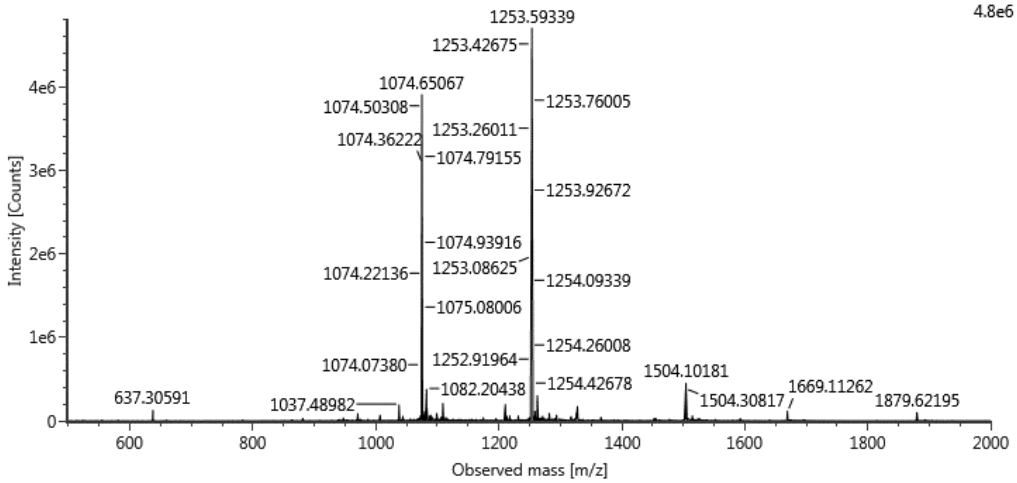


Figure 180: High-resolution MS spectrum of MIP-1β (4-69) protein **33**, *m/z* (ESI⁺) calcd M_{AV} = 7515.4, found 1879.6 $[M + 4H]^{4+}$, 1504.1 $[M + 5H]^{5+}$, 1253.6 $[M + 6H]^{6+}$, 1074.6 $[M + 7H]^{7+}$.

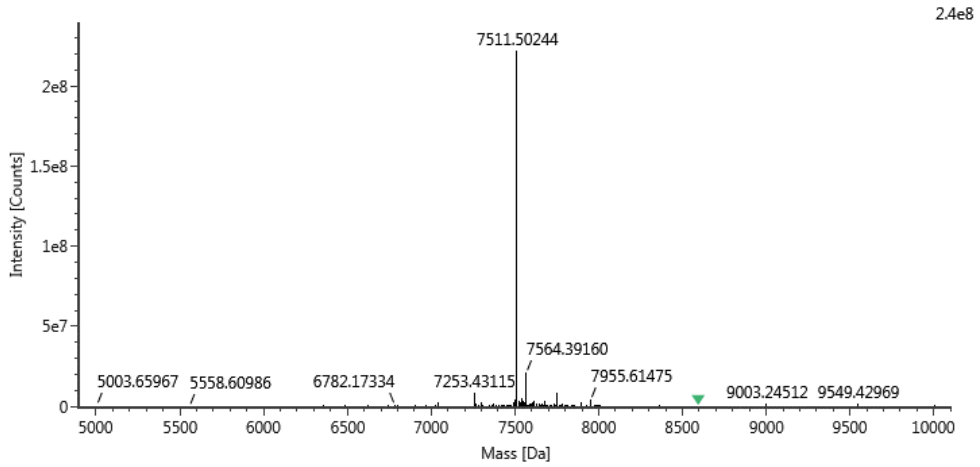
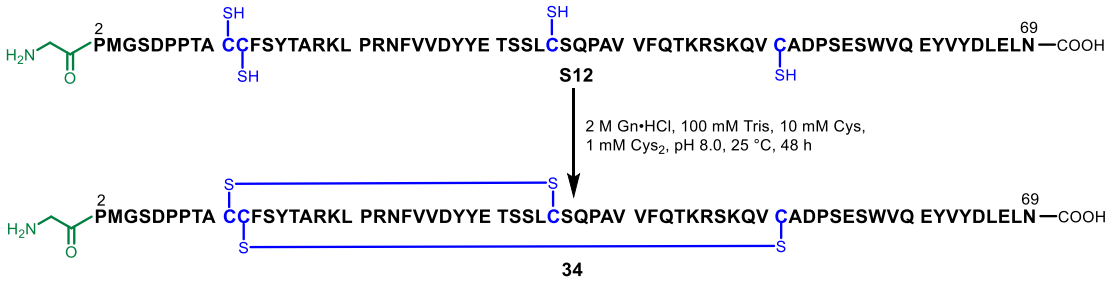


Figure 181: High-resolution deconvoluted MS spectrum of MIP-1β (4-69) protein **33**, *m/z* (ESI⁺) calcd $[M + H]^{+}_{mono}$ = 7511.5, found 7511.5 $[M + H]^{+}$.



Reduced MIP-1β (Gly1-69) protein **S12** (30 mg, 3.3 μmol) was added to an aqueous solution (60 mL, with a final protein conc. = 0.5 mg/mL) contained 2 M Gn·HCl, 100 mM Tris, 10 mM cysteine and 1 mM cystine buffered at pH 8.0. The solution was stirred at 25 °C for 48 h. The reaction mixture was analyzed with LC-MS. Once the reaction was completed, the mixture was purified with RP-HPLC (Dubhe C18 column, 0 to 50% B over 25 min with a flow rate of 20 mL/min) and yielded to desired MIP-1β (Gly1-69) protein **34** as a lyophilized solid. (12.3 mg, 1.44 μmol, 41%)

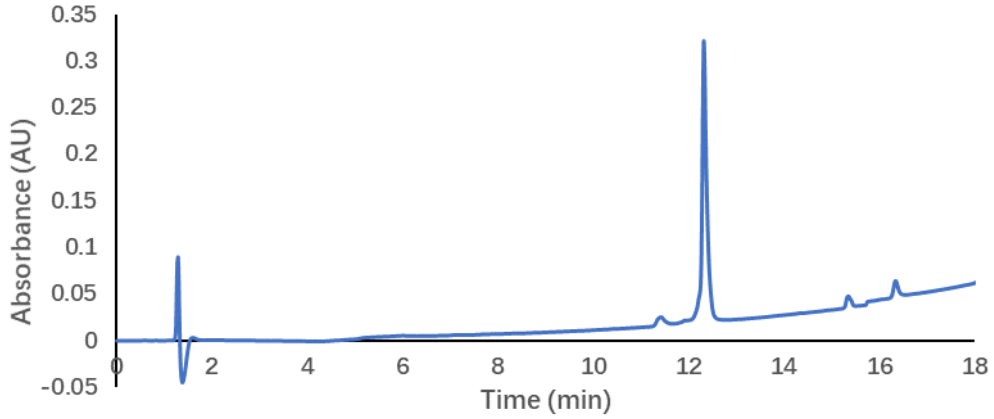


Figure 182: Analytical HPLC trace of purified MIP-1β (Gly1-69) protein **34** (t_R = 12.3 min, 0% B for 1 min and then 0 to 60% B over 15 min with a flow rate of 0.4 mL/min, Dubhe C18 analytical column).

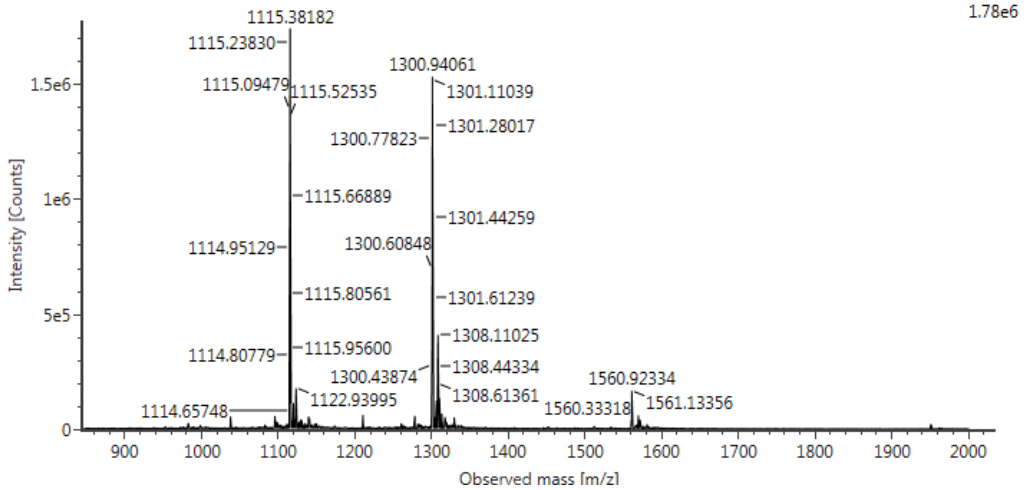


Figure 183: High-resolution MS spectrum of MIP-1β (Gly1-69) protein **34**, *m/z* (ESI⁺) calcd M_{AV} = 7800.7, found 1561.0 $[M + 5H]^{5+}$, 1300.9 $[M + 6H]^{6+}$, 1115.4 $[M + 7H]^{7+}$.

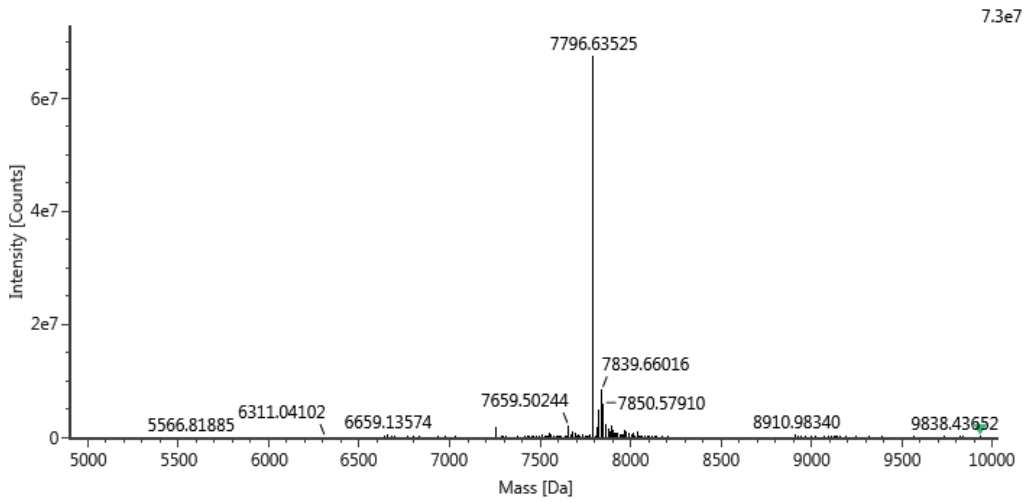
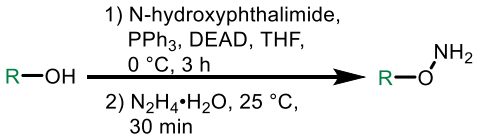


Figure 184: High-resolution deconvoluted MS spectrum of MIP-1β (Gly1-69) protein **34**, *m/z* (ESI⁺) calcd $[M + H]^{+}_{mono}$ = 7796.6, found 7796.6 $[M + H]^{+}$.

5. Synthesis of alkoxyamine

5.1. General procedure for generating alkoxyamine



To a solution of alcohol (1.0 equiv.) in dry THF (0.25 M) was added PPh_3 (1.1 equiv.), *N*-hydroxyphthalimide (1.1 equiv.), followed by the addition of DEAD (1.1 equiv.) dropwise at 0 °C. The resulting mixture was stirred at 0 °C for 3 h. $N_2H_4 \cdot H_2O$ (80%, 2.0 equiv.) was added dropwise and the reaction was stirred for another 30 min, then H_2O was added, and extracted with ethyl acetate. The organic layer was then washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography to give the corresponding product.

5.1.1. ***O*-hexylhydroxylamine**



This compound was prepared according to the **General procedure** by treating **hexan-1-ol** (1.02 g, 10 mM). The residue was purified by flash chromatography (petroleum ether/ethyl acetate 100:1 → 20:1) to give the product as colorless oil (760 mg, 65% yield). R_f (petroleum ether/ethyl acetate 20:1): 0.50; **^{1}H NMR** (500 MHz, DMSO-d_6): δ 5.85 (2H, br.s), 3.39 (2H, t, J = 6.5 Hz), 1.49-1.43 (2H, m), 1.29-1.22 (6H, m), 0.86 (3H, t, J = 7.0 Hz); **^{13}C NMR** (125 MHz, DMSO-d_6): δ 74.9, 31.2, 28.0, 25.3, 22.1, 13.9.

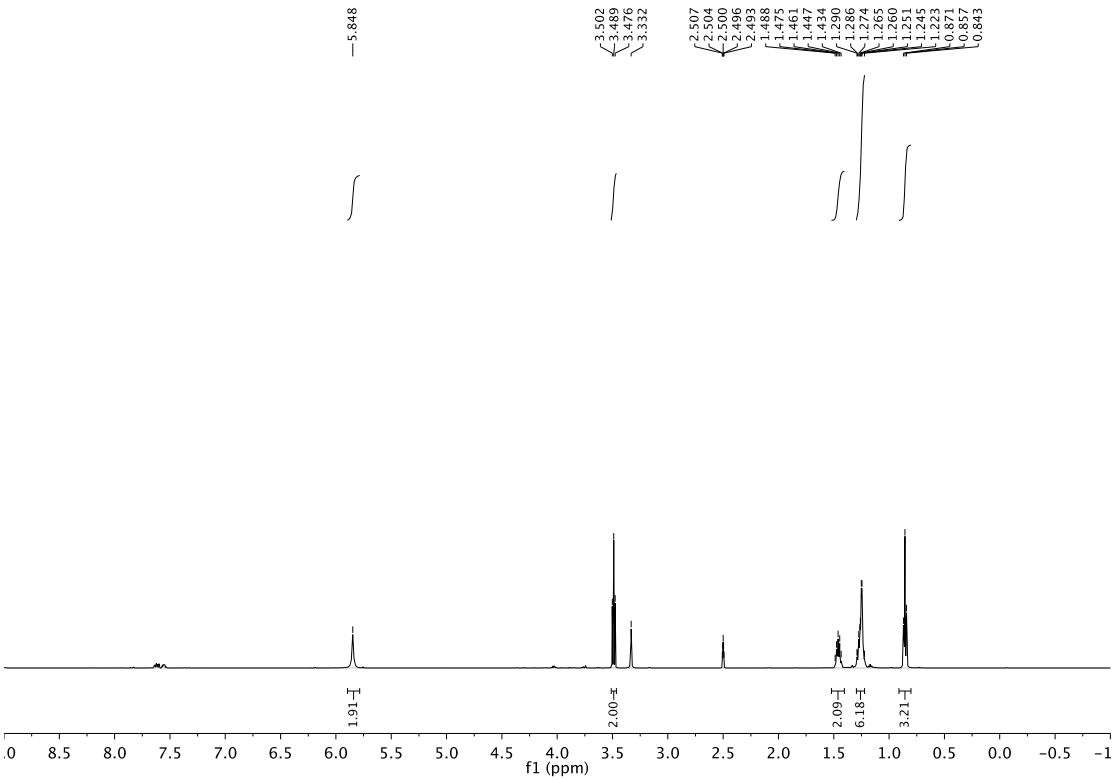


Figure 185: ^{1}H NMR spectrum for *O*-hexylhydroxylamine

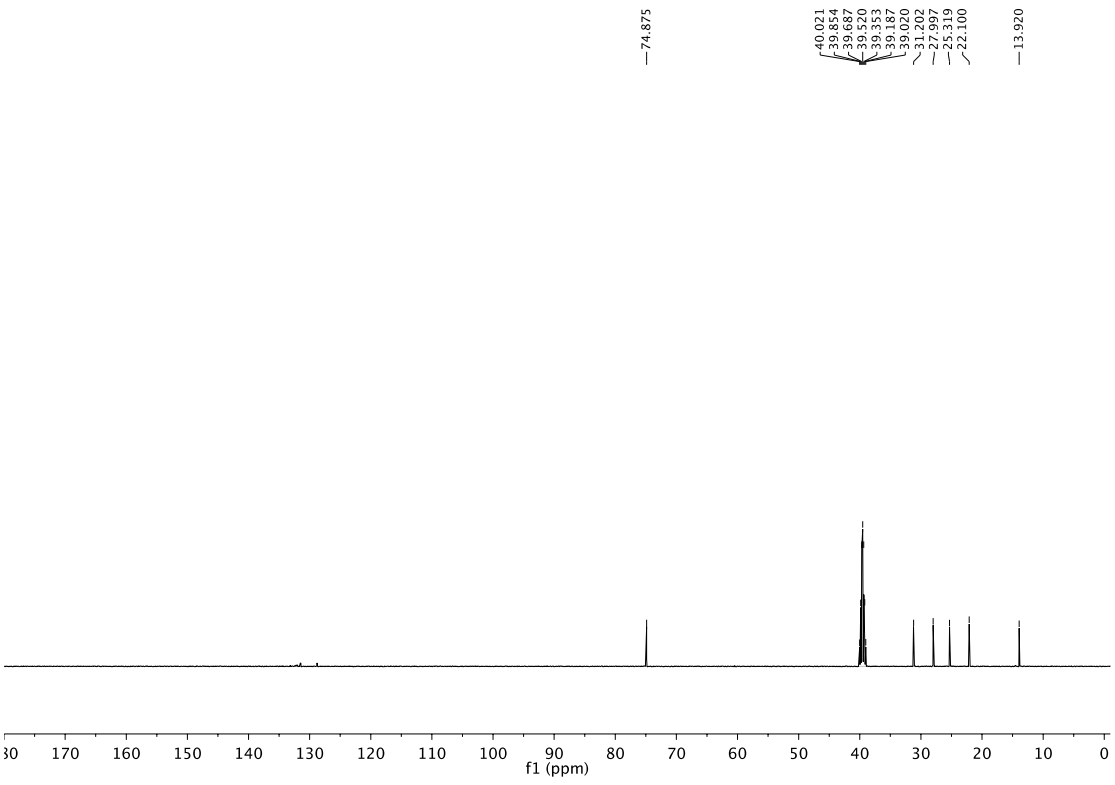


Figure 186: ^{13}C NMR spectrum for *O*-hexylhydroxylamine

5.1.2. **(*E*)-*O*-(hex-4-en-1-yl)hydroxylamine**



This compound was prepared according to the **General procedure** by treating **(*E*)-hex-4-en-1-ol** (1.0 g, 10 mM). The residue was purified by flash chromatography (petroleum ether/ethyl acetate 100:1 → 20:1) to give the product as colorless oil (805 mg, 70% yield). R_f (petroleum ether/ethyl acetate 20:1): 0.50; **^{1}H NMR** (500 MHz, $CDCl_3$): δ 5.45-5.38 (2H, m), 4.85 (2H, s), 3.65 (2H, t, J = 6.5 Hz), 2.03-1.99 (2H, m), 1.64-1.59 (5H, m); **^{13}C NMR** (125 MHz, $CDCl_3$): δ 130.7, 125.4, 75.7, 29.0, 28.3, 18.0.

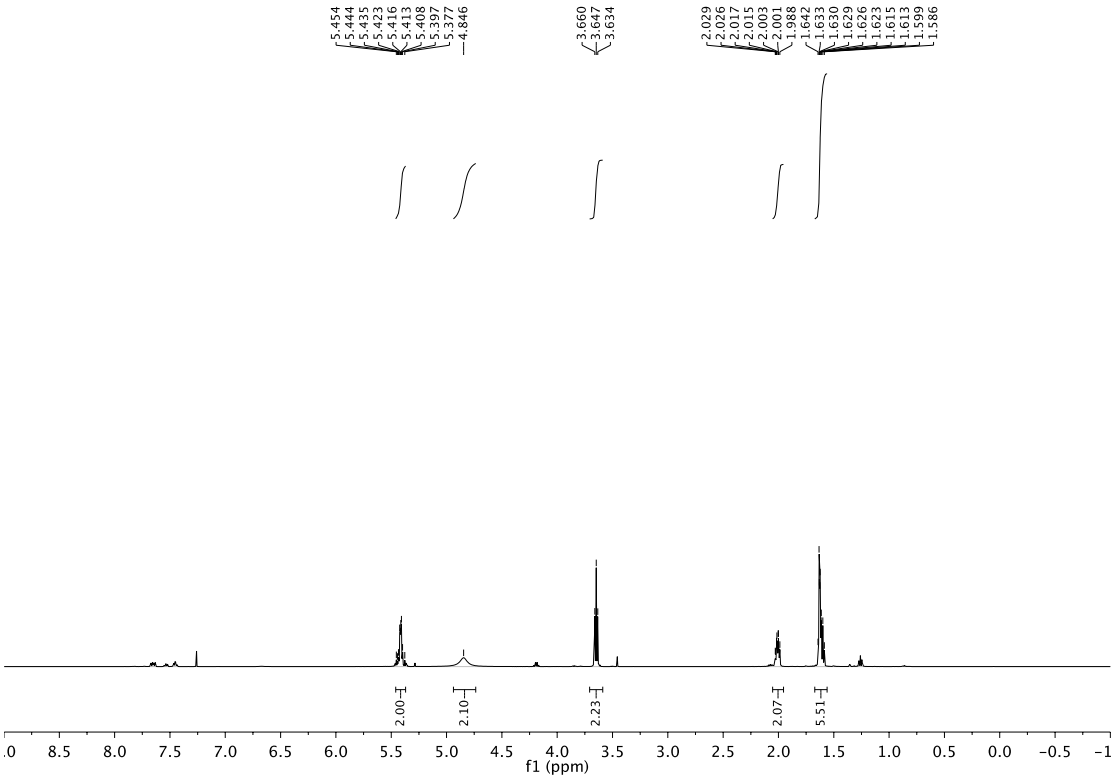


Figure 187: ^{1}H NMR spectrum for (*E*)-*O*-(hex-4-en-1-yl)hydroxylamine

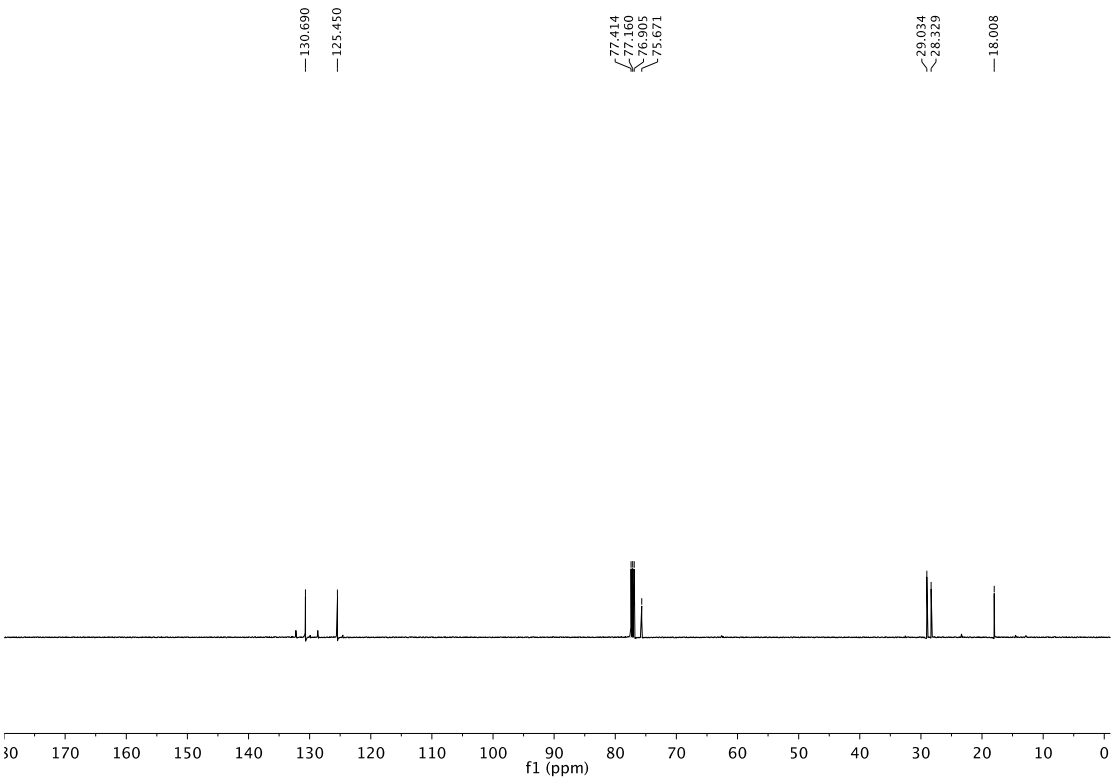
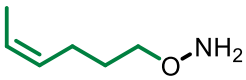


Figure 188: ^{13}C NMR spectrum for (*E*)-*O*-(hex-4-en-1-yl)hydroxylamine

5.1.3. **(*Z*)-*O*-(hex-4-en-1-yl)hydroxylamine**



This compound was prepared according to the **General procedure** by treating **(*Z*)-hex-4-en-1-ol** (1.0 g, 10 mM). The residue was purified by flash chromatography (petroleum ether/ethyl acetate 100:1 → 20:1) to give the product as colorless oil (782 mg, 68% yield). R_f (petroleum ether/ethyl acetate 20:1): 0.50; **^{1}H NMR** (500 MHz, $CDCl_3$): δ 5.45-5.40 (1H, m), 5.37-5.31 (1H, m), 5.09 (2H, s), 3.63 (2H, t, J = 6.5 Hz), 2.05 (2H, q, J = 7.5 Hz), 1.63-1.56 (5H, m); **^{13}C NMR** (125 MHz, $CDCl_3$): δ 129.8, 124.5, 75.5, 28.2, 23.3, 12.7.

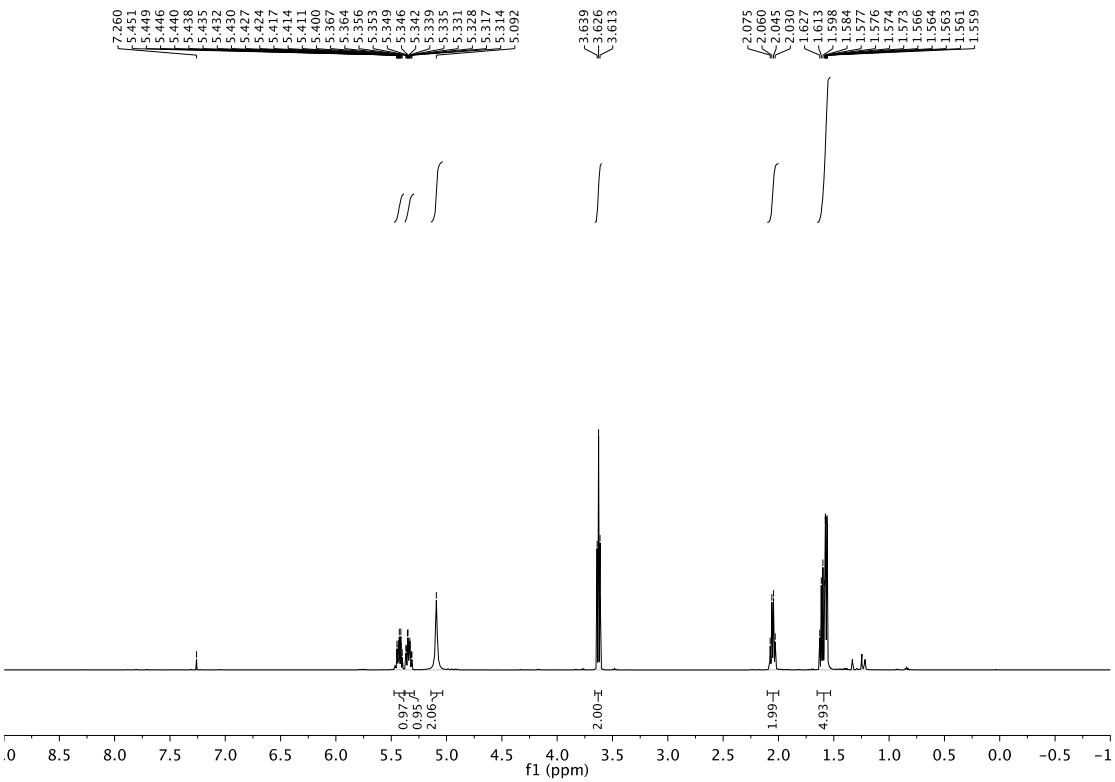


Figure 189: ^{1}H NMR spectrum for (*Z*)-*O*-(hex-4-en-1-yl)hydroxylamine

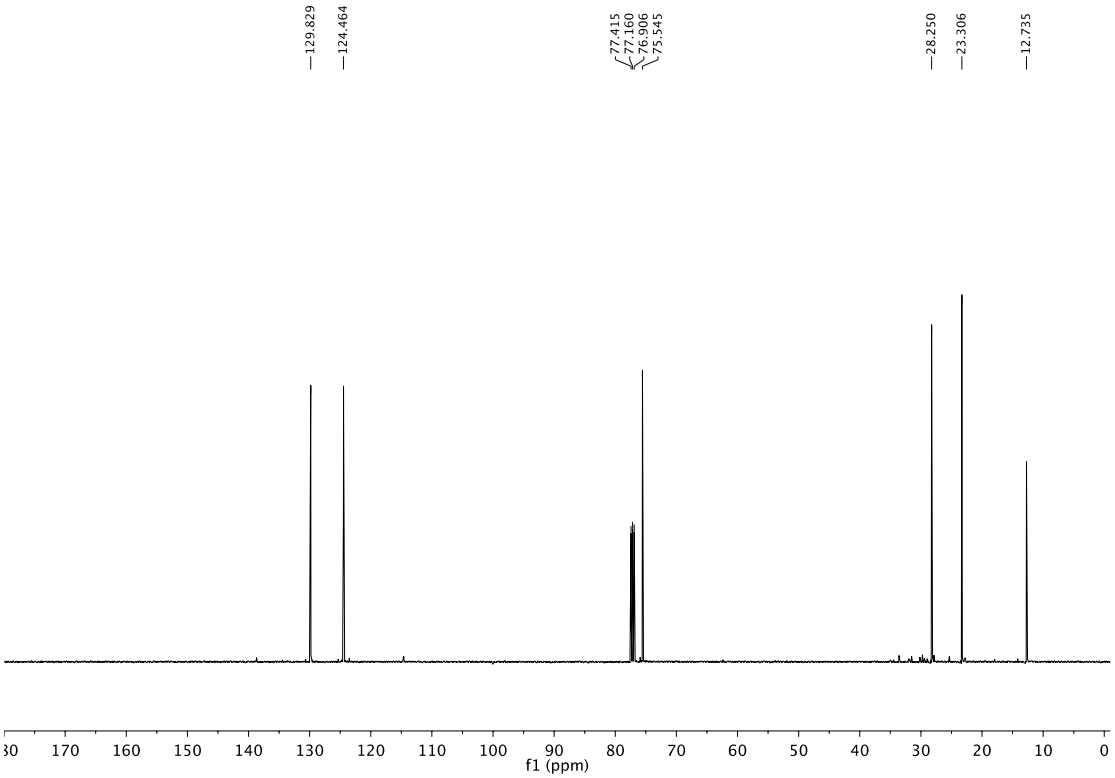
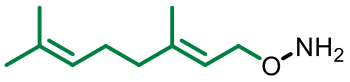


Figure 190: ^{13}C NMR spectrum for (*Z*)-*O*-(hex-4-en-1-yl)hydroxylamine

5.1.4. **(*E*)-*O*-(3,7-dimethylocta-2,6-dien-1-yl)hydroxylamine**



This compound was prepared according to the **General procedure** by treating **(*E*)-3,7-dimethylocta-2,6-dien-1-ol** (1.54 g, 10 mM). The residue was purified by flash chromatography (petroleum ether/ethyl acetate 100:1 → 20:1) to give the product as colorless oil (1.35 g, 80% yield). R_f (petroleum ether/ethyl acetate 20:1): 0.55; **^{1}H NMR** (500 MHz, DMSO-d_6): δ 5.82 (2H, br.s), 5.27 (1H, dt, J = 1.5, 7.0 Hz), 5.07 (1H, tt, J = 1.5, 7.0 Hz), 4.04 (2H, d, J = 7.0 Hz), 2.08-2.04 (2H, m), 1.99-1.96 (2H, m), 1.64 (3H, s), 1.61 (3H, s), 1.56 (3H, s); **^{13}C NMR** (125 MHz, DMSO-d_6): δ 139.2, 130.9, 123.9, 120.5, 71.5, 39.1, 25.9, 25.5, 17.5, 16.2.

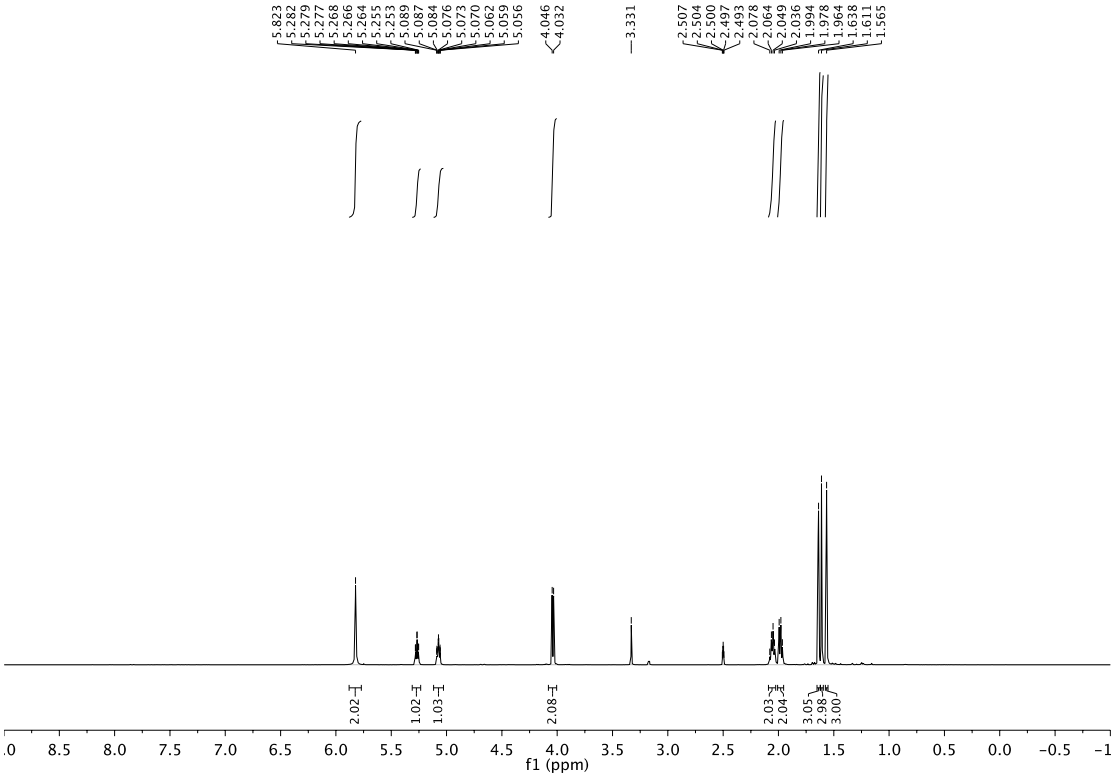


Figure 191: ^{1}H NMR spectrum for (*E*)-*O*-(3,7-dimethylocta-2,6-dien-1-yl)hydroxylamine

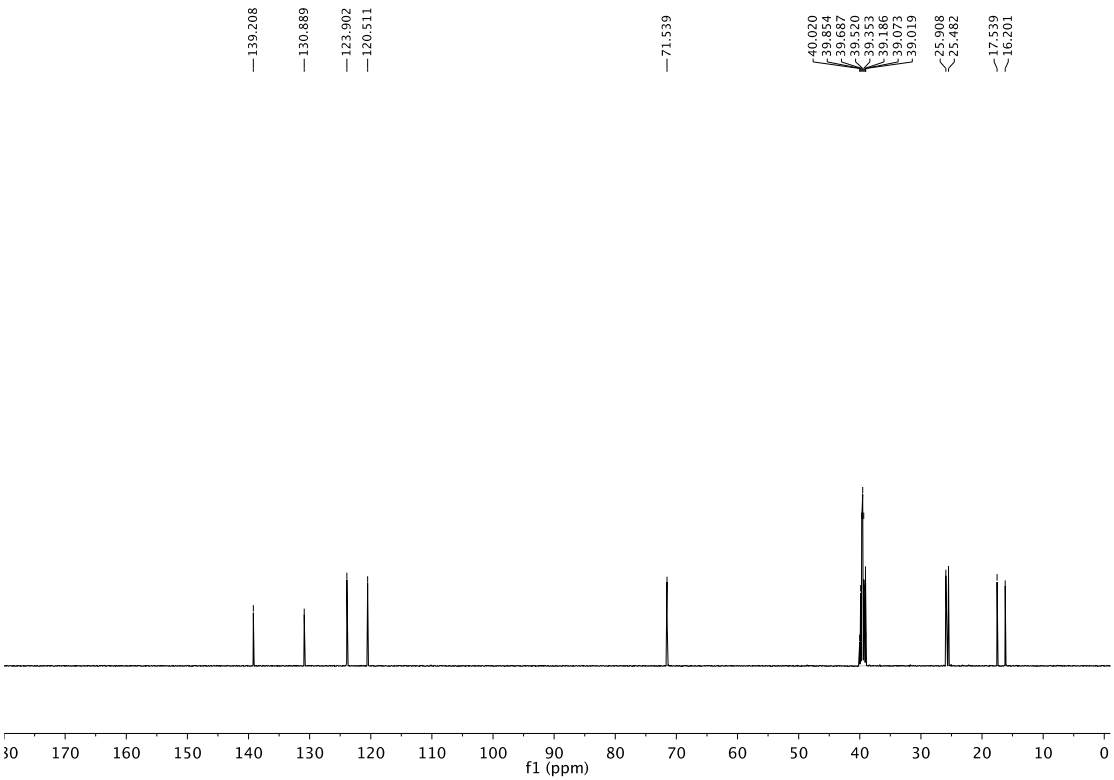


Figure 192: ^{13}C NMR spectrum for (*E*)-*O*-(3,7-dimethylocta-2,6-dien-1-yl)hydroxylamine

5.1.5. ***O*-decylhydroxylamine**



This compound was prepared according to the **General procedure** by treating **decan-1-ol** (1.58 g, 10 mM). The residue was purified by flash chromatography (petroleum ether/ethyl acetate 100:1 → 20:1) to give the product as colorless oil (1.33 g, 77% yield). R_f (petroleum ether/ethyl acetate 20:1): 0.55; **^{1}H NMR** (500 MHz, $CDCl_3$): δ 4.95 (2H, br.s), 3.64 (2H, t, J = 7.0 Hz), 1.58-1.52 (2H, m), 1.31-1.25 (14H, m), 0.86 (3H, t, J = 7.0 Hz); **^{13}C NMR** (125 MHz, $CDCl_3$): δ 76.4, 32.0, 29.7 (two overlapping peaks), 29.6, 29.4, 28.5, 26.1, 22.8, 14.2.

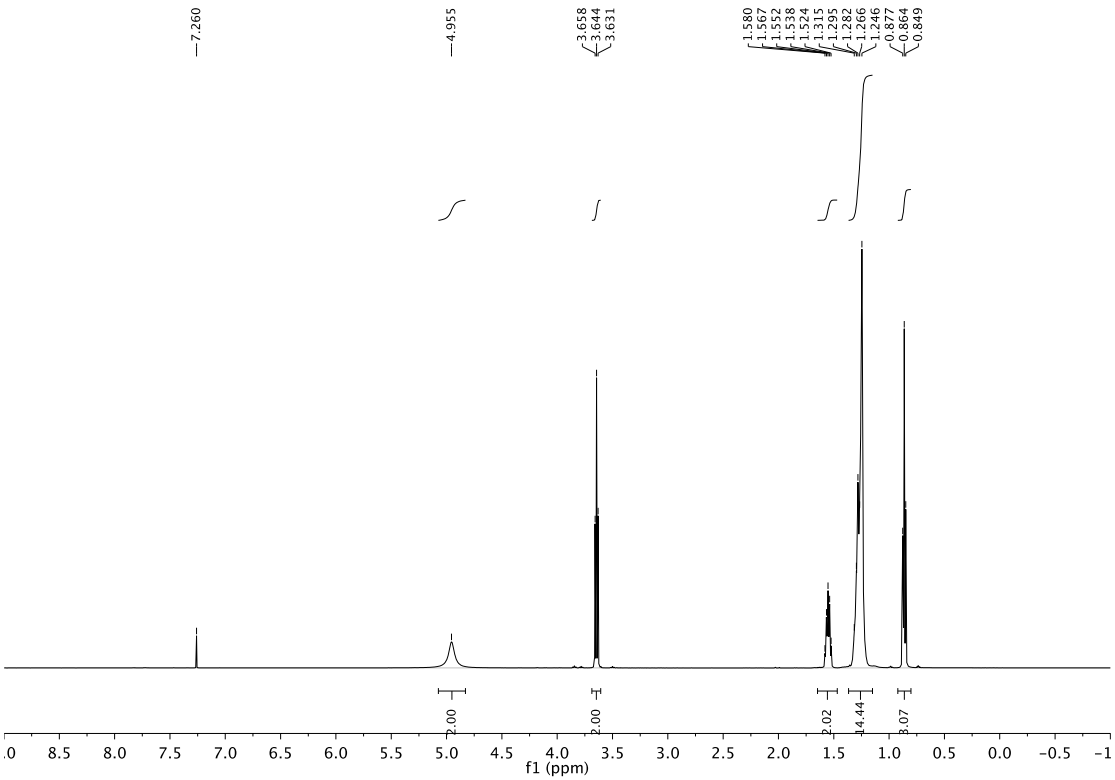


Figure 193: ^{1}H NMR spectrum for *O*-decylhydroxylamine

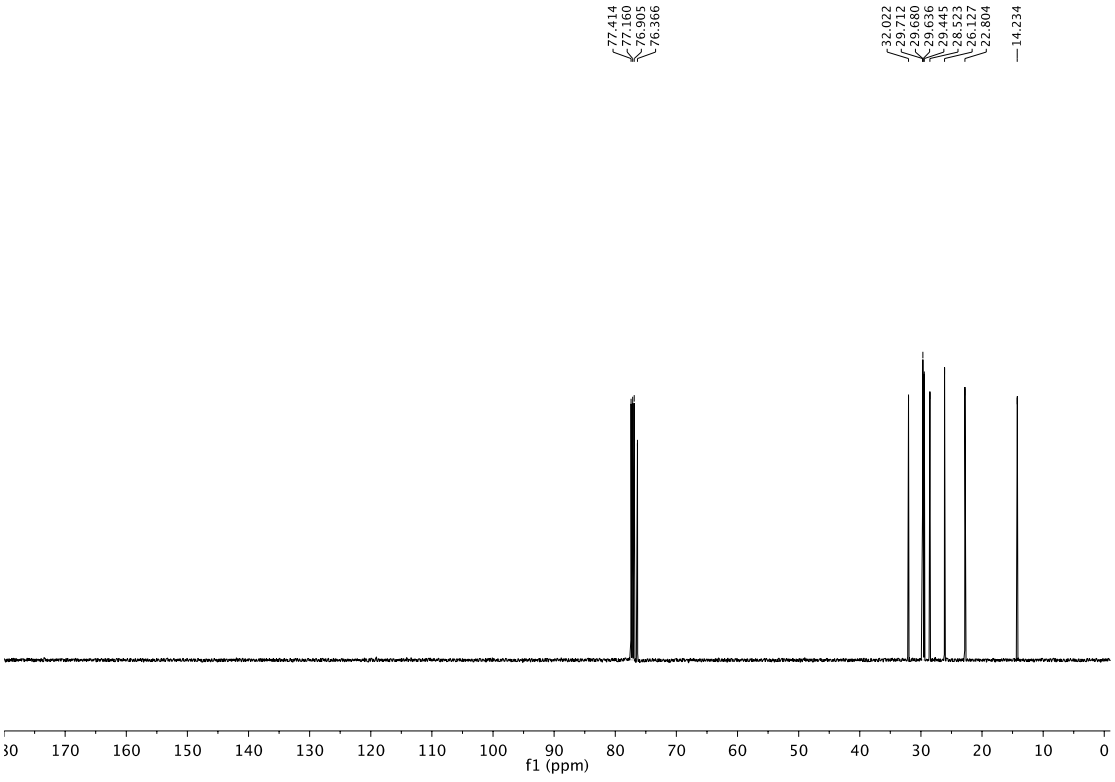


Figure 194: ^{13}C NMR spectrum for *O*-decylhydroxylamine

5.1.6. ***O*-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)hydroxylamine**



This compound was prepared according to the **General procedure** by treating **2-(2-(2-methoxyethoxy)ethoxy)ethan-1-ol** (1.64 g, 10 mM). The residue was purified by flash chromatography (CH_2Cl_2/MeOH 100:1 → 25:1) to give the product as pale-yellow oil (1.07 g, 60% yield). R_f (CH_2Cl_2/MeOH 20:1): 0.40; **^{1}H NMR** (500 MHz, $CDCl_3$): δ 4.88 (2H, br.s), 3.81-3.79 (2H, m), 3.64-3.59 (8H, m), 3.52-3.50 (2H, m), 3.33 (3H, s); **^{13}C NMR** (125 MHz, $CDCl_3$): δ 74.8, 71.9, 70.6, 70.5 (two overlapping peaks), 69.6, 59.0.

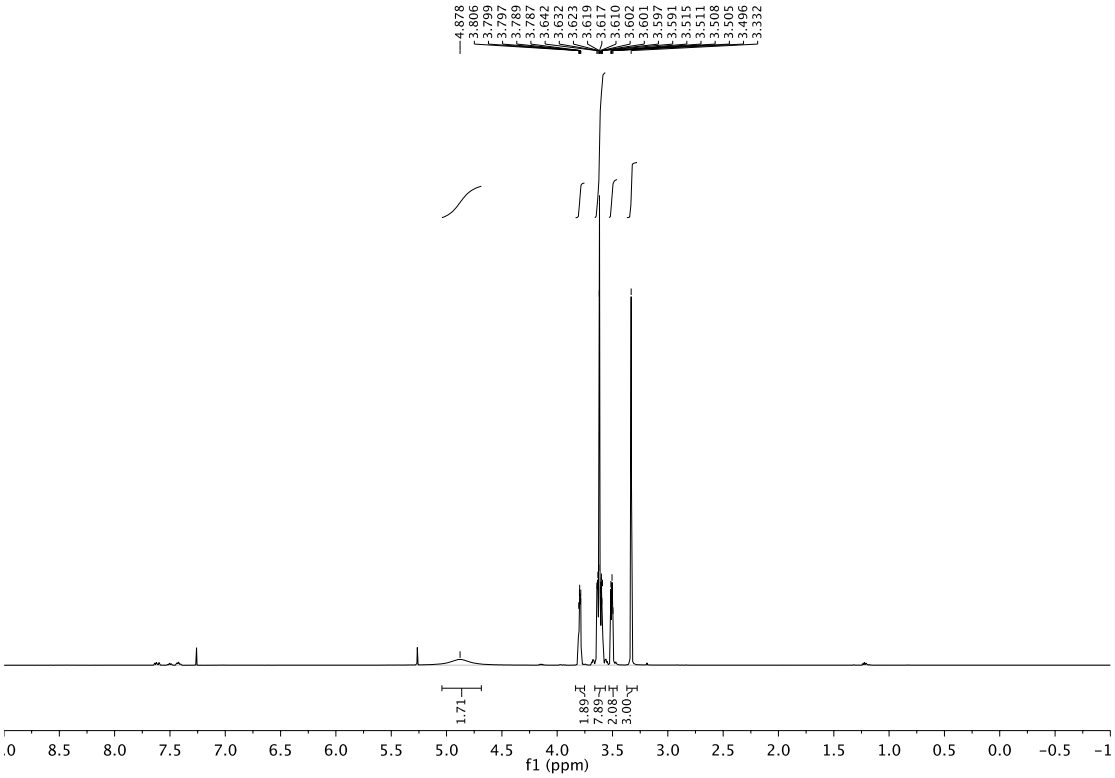


Figure 195: ^{1}H NMR spectrum for *O*-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)hydroxylamine

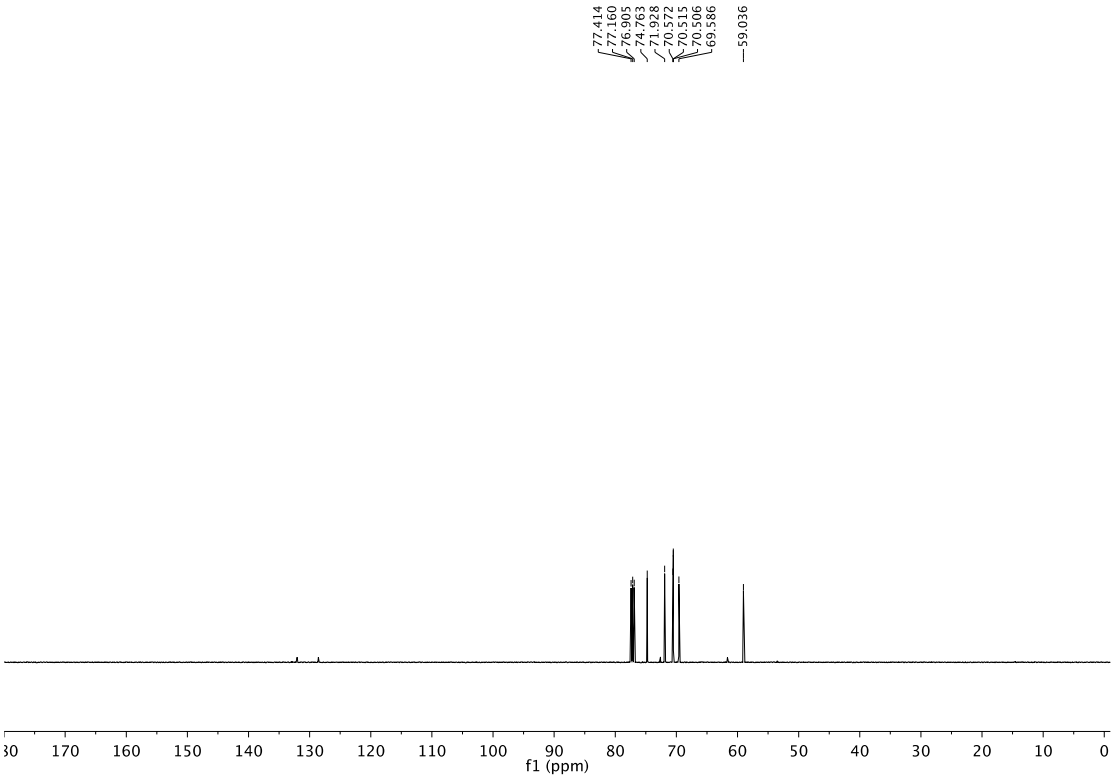
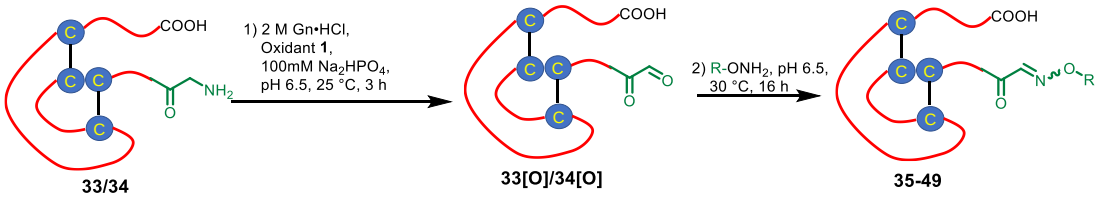


Figure 196: ^{13}C NMR spectrum for *O*-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)hydroxylamine

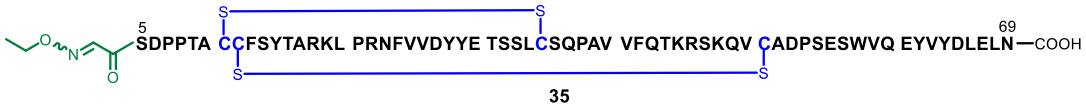
6. Transamination of MIP-1β analogs



To an aqueous buffer of 2 M Gn·HCl and 50 mM Na_2HPO_4 (500 µL), Protein (1 mg, approx. 0.11 nmol) was dissolved and the pH of the solution was adjusted to 6.5. Then oxidant **1** (1.0 mg 5.0 nmol) was added to the solution. The resulting solution was incubated at 37 °C for 16 h.

For addition of $MeONH_2$·HCl and $EtONH_2$·HCl, stock solution of 0.4 M alkoxyamine in 2 M Gn·HCl solution buffered with 50 mM Na_2HPO_4 at pH 6.5 was added (400 µL) to the protein mixture. The mixture was incubated at 37 °C for another 16 h. The resulting solution was analyzed with LCMS and purified with RP-HPLC.

For addition of the rest of fatty alkoxyamine, 50 µL of the neat liquid was added directly into the reaction mixture. The suspension was agitated vigorously at 37 °C in an oven shaker for 16 h. The resulting solution was analyzed with LCMS and purified with RP-HPLC.



Reaction was carried out with MIP-1β (4-69) protein **33** (2.0 mg, 0.24 nmol). After RP-HPLC purification, protein **35** was obtained as a lyophilized solid (0.86 mg, 0.10 nmol, 43%).

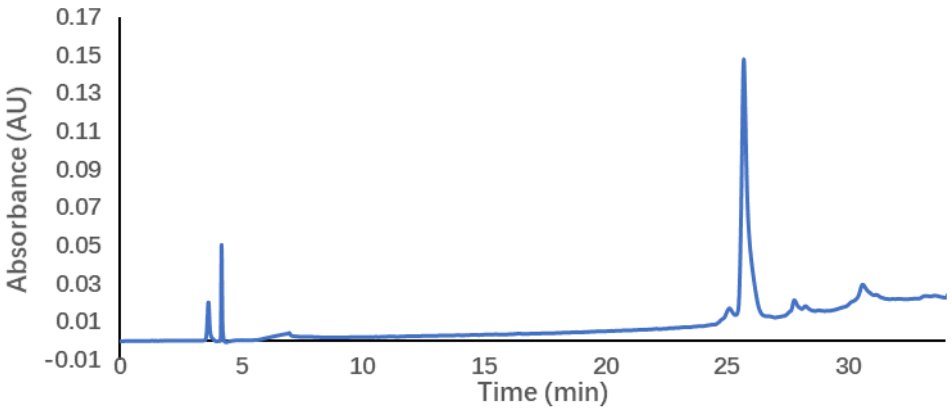


Figure 197: Analytical HPLC trace of purified MIP-1β (4-69) modified protein **35** (t_R = 25.7 min, 0% B for 1 min and then 0 to 50% B over 30 min with a flow rate of 1 mL/min, GL Sciences Inertsil® C4 analytical column).

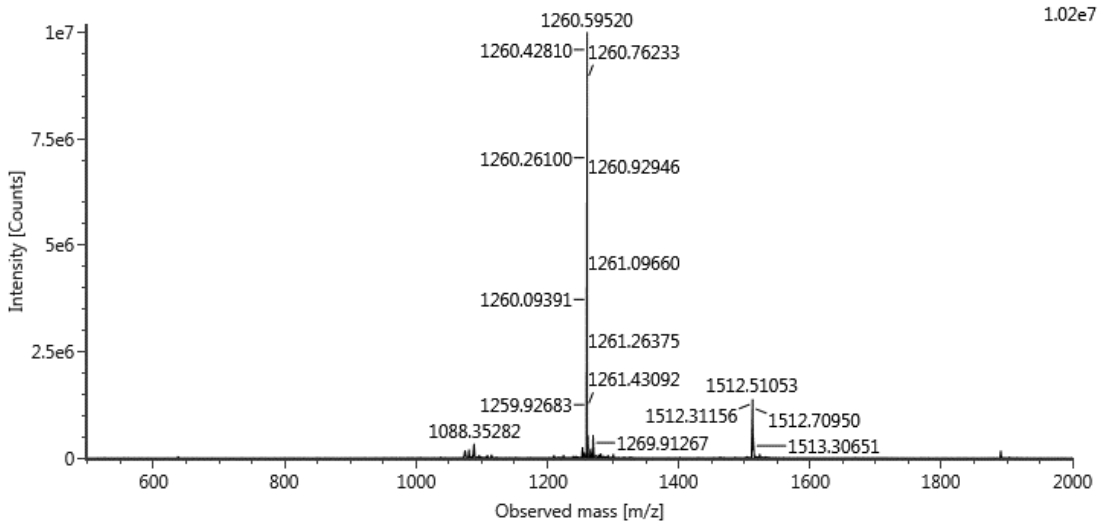


Figure 198: High-resolution MS spectrum of MIP-1β (4-69) modified protein **35**, *m/z* (ESI⁺) calcd M_{AV} = 7557.4, found 1512.5 $[M + 5H]^{5+}$, 1260.6 $[M + 6H]^{6+}$.

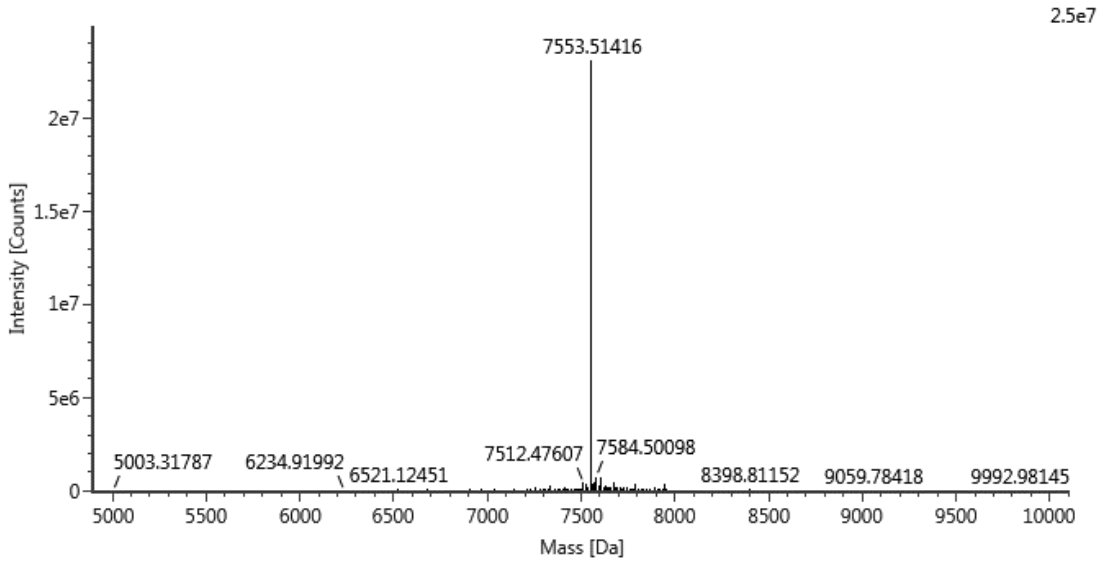
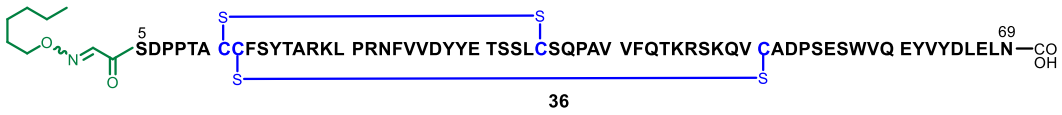


Figure 199: High-resolution deconvoluted MS spectrum of MIP-1β (4-69) modified protein **35**, *m/z* (ESI⁺) calcd $[M + H]^{+}_{mono}$ = 7553.5, found 7553.5 $[M + H]^{+}$.



Reaction was carried out with MIP-1β (4-69) protein **33** (4.3 mg, 0.52 nmol). After RP-HPLC purification, modified protein **36** was obtained as a lyophilized solid (1.3 mg, 0.15 nmol, 29%).

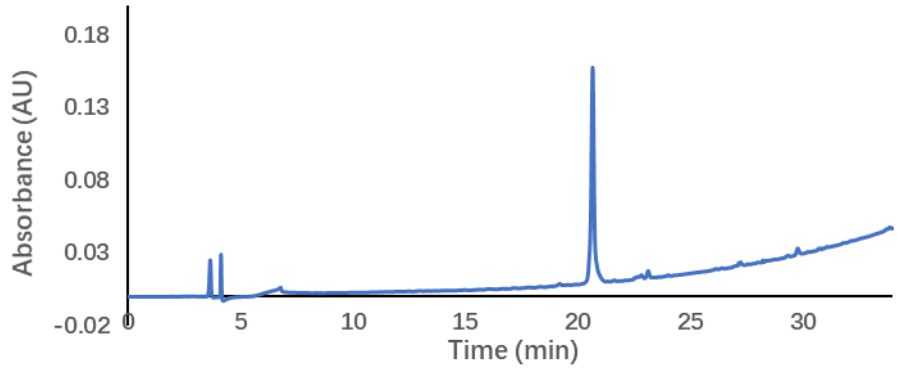


Figure 200: Analytical HPLC trace of purified MIP-1β (4-69) modified protein **36** (t_R = 20.7 min, 0% B for 1 min and then 0 to 70% B over 30 min with a flow rate of 1 mL/min, GL Sciences Inertsil® C4 analytical column).

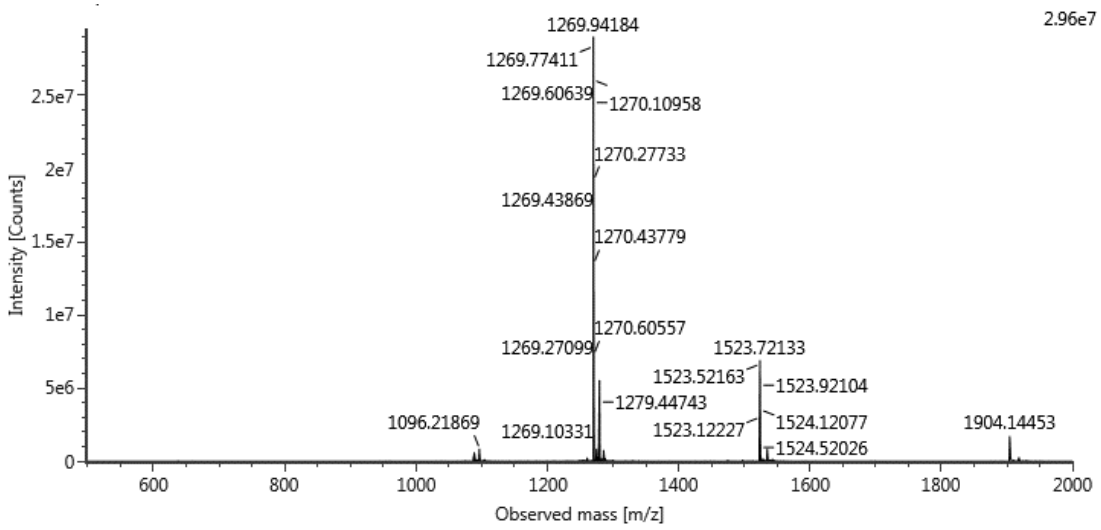


Figure 201: High-resolution MS spectrum of MIP-1β (4-69) modified protein **36**, *m/z* (ESI⁺) calcd M_{AV} = 7613.5, found 1904.1 $[M + 4H]^{4+}$, 1523.7 $[M + 5H]^{5+}$, 1269.9 $[M + 6H]^{6+}$.

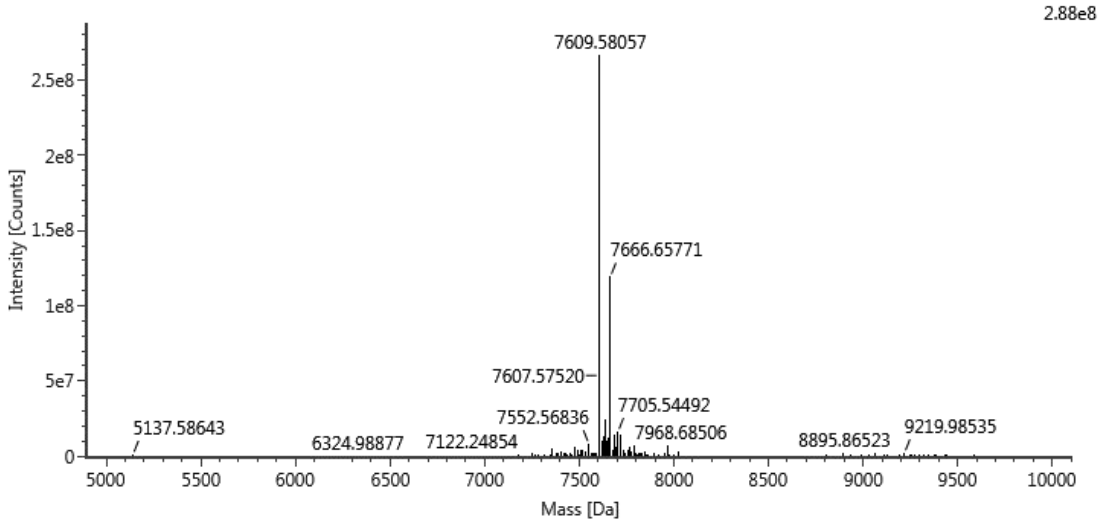
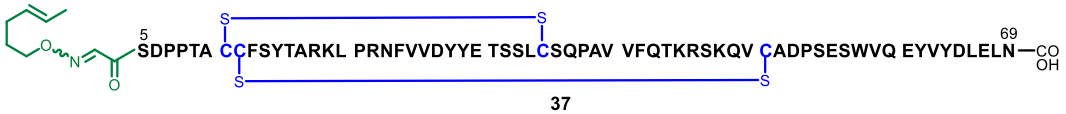


Figure 202: High-resolution deconvoluted MS spectrum of MIP-1β (4-69) modified protein **36**, *m/z* (ESI⁺) calcd $[M + H]^{+}_{mono}$ = 7609.6, found 7609.6 $[M + H]^{+}$.



Reaction was carried out with MIP-1β (4-69) protein **33** (4.8 mg, 0.58 nmol). After RP-HPLC purification, modified protein **37** was obtained as a lyophilized solid (1.7 mg, 0.20 nmol, 35%).

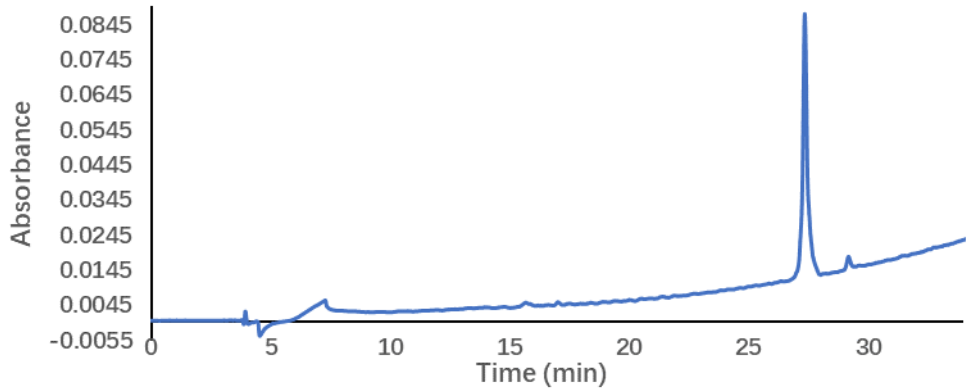


Figure 203: Analytical HPLC trace of purified MIP-1β (4-69) modified protein **37** (t_R = 20.7 min, 0% B for 1 min and then 0 to 70% B over 30 min with a flow rate of 1 mL/min, GL Sciences Inertsil® C4 analytical column).

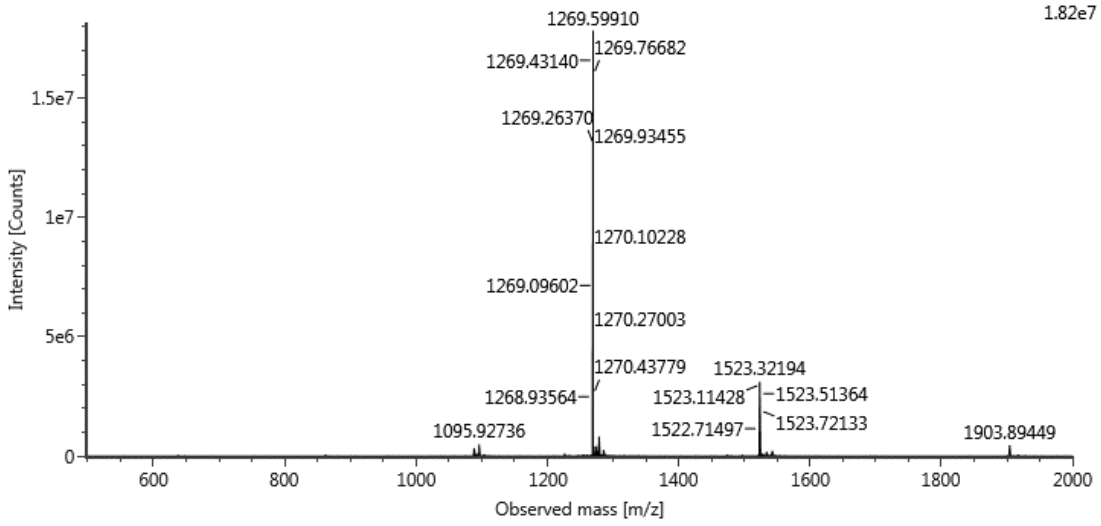


Figure 204: High-resolution MS spectrum of MIP-1β (4-69) modified protein **37**, *m/z* (ESI⁺) calcd M_{AV} = 7611.5, found 1903.9 $[M + 4H]^{4+}$, 1523.3 $[M + 5H]^{5+}$, 1269.6 $[M + 6H]^{6+}$.

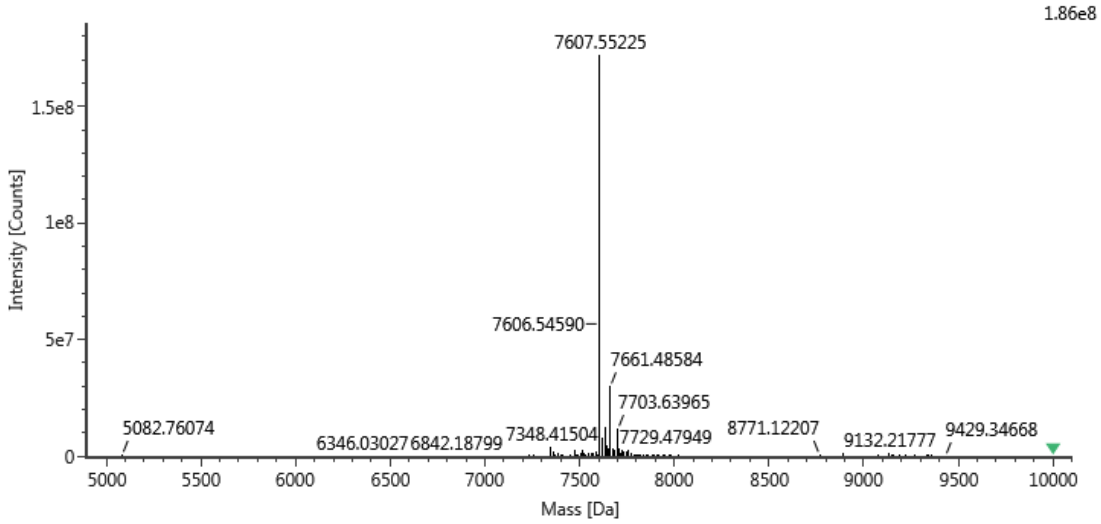
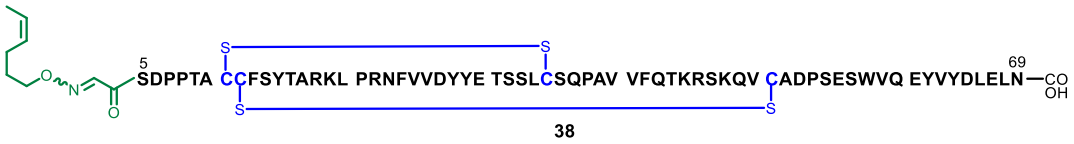


Figure 205: High-resolution deconvoluted MS spectrum of MIP-1β (4-69) modified protein **37**, *m/z*

(ESI$^+$) calcd $[M + H]^+_{mono}$ = 7607.6, found 7607.6 $[M + H]^+$.



Reaction was carried out with MIP-1β (4-69) protein **33** (4.8 mg, 0.59 nmol). After RP-HPLC purification, modified protein **38** was obtained as a lyophilized solid (1.5 mg, 0.18 nmol, 31%).

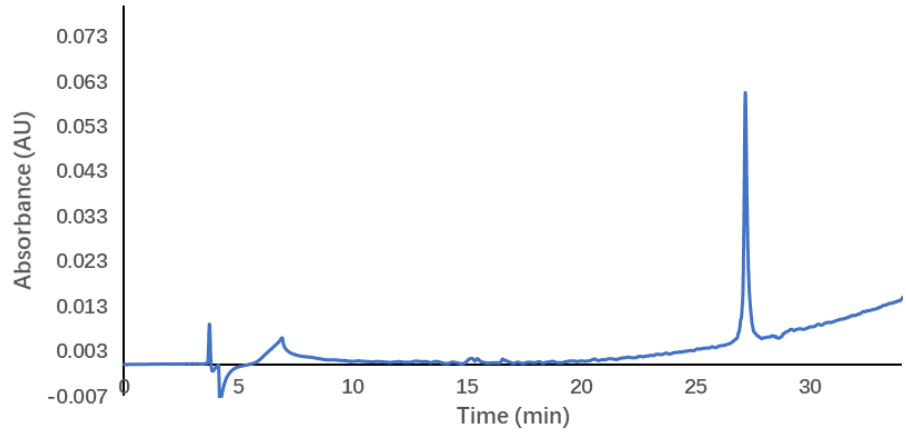


Figure 206: Analytical HPLC trace of purified MIP-1β (4-69) modified protein **38** (t_R = 27.2 min, 0% B for 1 min and then 0 to 70% B over 30 min with a flow rate of 1 mL/min, GL Sciences Inertsil® C4 analytical column).

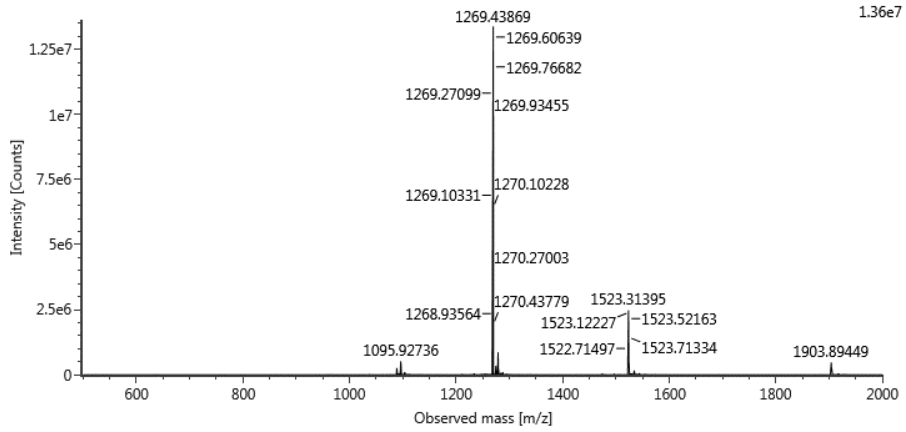


Figure 207: High-resolution MS spectrum of MIP-1β (4-69) modified protein **38**, *m/z* (ESI$^+$) calcd M_{AV} = 7611.5, found 1903.9 $[M + 4H]^{4+}$, 1523.3 $[M + 5H]^{5+}$, 1269.4 $[M + 6H]^{6+}$.

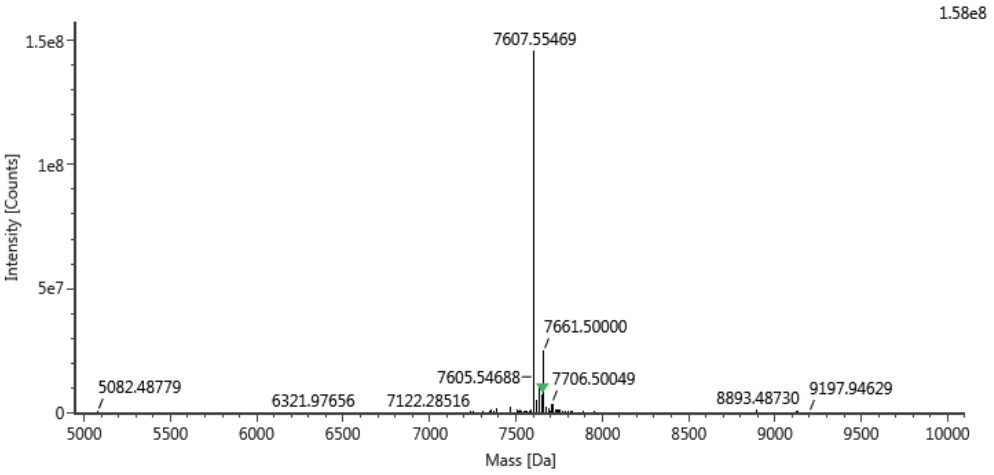
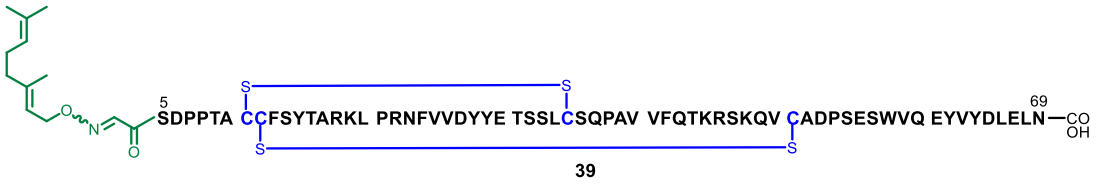


Figure 208: High-resolution deconvoluted MS spectrum of MIP-1β (4-69) modified protein **38**, *m/z* (ESI⁺) calcd $[M + H]^{+}_{mono}$ = 7607.6, found 7607.6 $[M + H]^{+}$.



39

Reaction was carried out with MIP-1β (4-69) protein **33** (5.2 mg, 0.62 nmol). After RP-HPLC purification, modified protein **39** was obtained as a lyophilized solid (2.1 mg, 0.24 nmol, 38%).

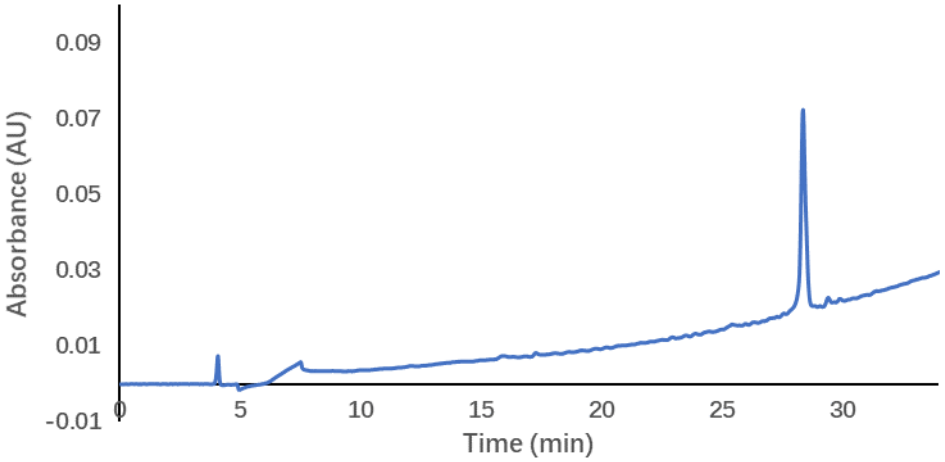


Figure 209: Analytical HPLC trace of purified MIP-1β (4-69) modified protein **39** (t_R = 28.4 min, 0% B for 1 min and then 0 to 50% B over 30 min with a flow rate of 1 mL/min, GL Sciences Inertsil® C4 analytical column)

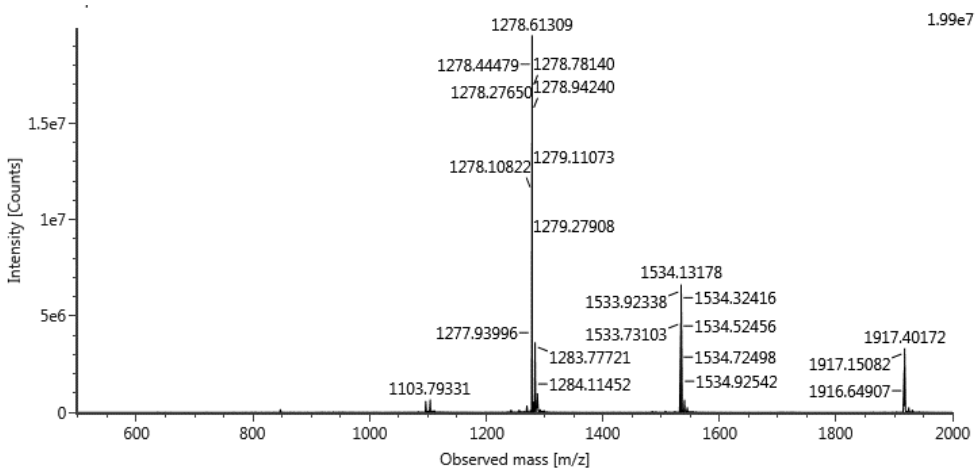


Figure 210: High-resolution MS spectrum of MIP-1β (4-69) modified protein **39**, *m/z* (ESI⁺) calcd M_{AV} = 7665.6, found 1917.4 $[M + 4H]^{4+}$, 1534.1 $[M + 5H]^{5+}$, 1278.6 $[M + 6H]^{6+}$.

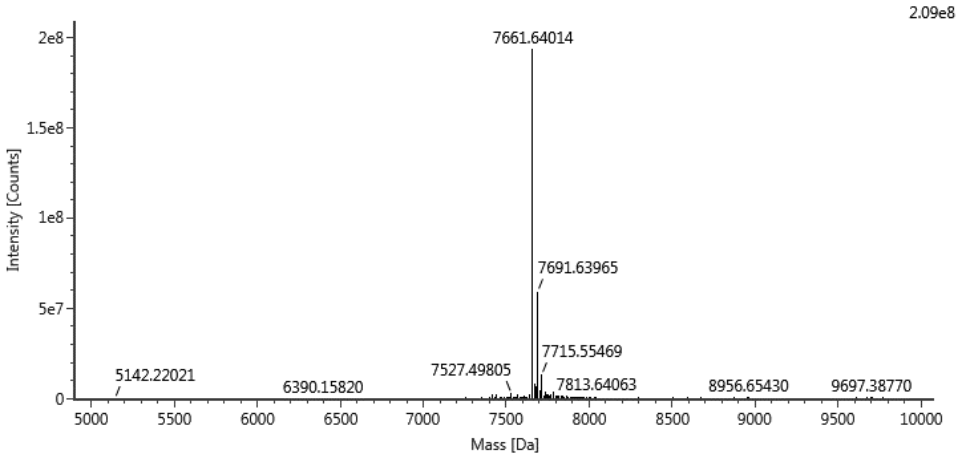
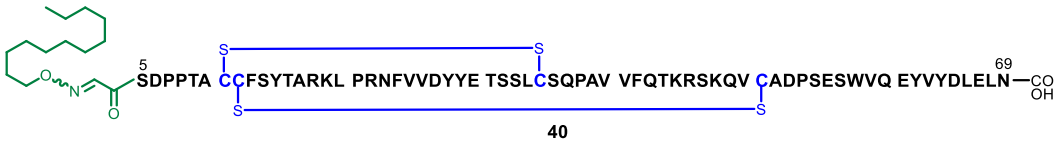


Figure 211: High-resolution deconvoluted MS spectrum of MIP-1β (4-69) modified protein **39**, *m/z* (ESI⁺) calcd $[M + H]^{+}_{mono}$ = 7661.6, found 7661.6 $[M + H]^{+}$.



Reaction was carried out with MIP-1β (4-69) protein **33** (2.9 mg, 0.35 nmol). After RP-HPLC purification, modified protein **40** was obtained as a lyophilized solid (1.1 mg, 0.13 nmol, 36%).

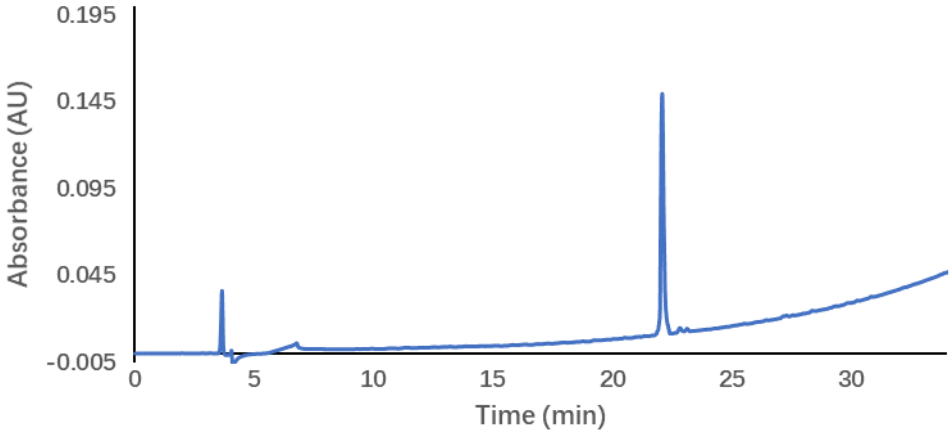


Figure 212: Analytical HPLC trace of purified MIP-1β (4-69) modified protein **40** (t_R = 22.1 min, 0% B for 1 min and then 0 to 70% B over 30 min with a flow rate of 1 mL/min, GL Sciences Inertsil® C4 analytical column)

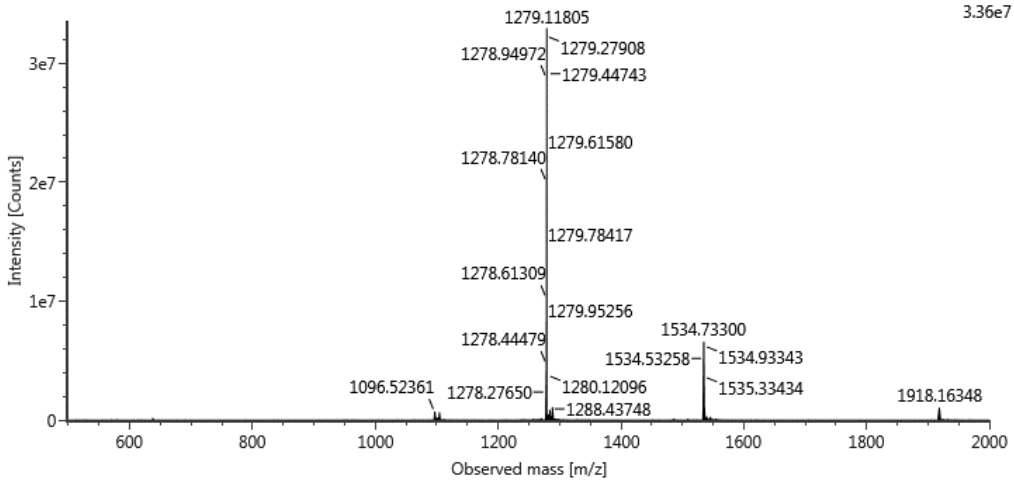


Figure 213: High-resolution MS spectrum of MIP-1β (4-69) modified protein **40**, *m/z* (ESI⁺) calcd M_{AV} = 7669.2, found 1918.2 $[M + 4H]^{4+}$, 1534.7 $[M + 5H]^{5+}$, 1279.1 $[M + 6H]^{6+}$.

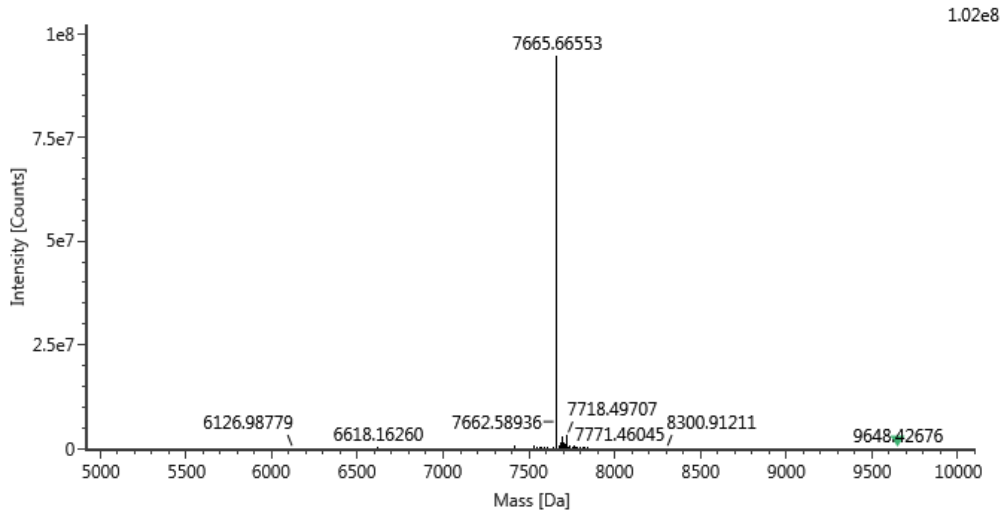
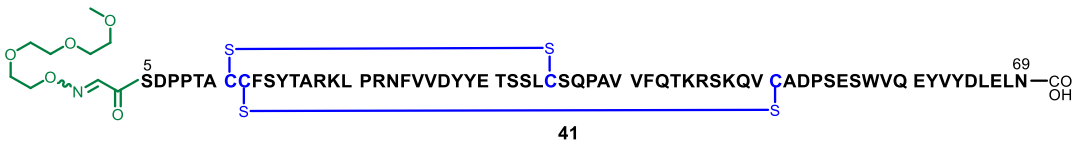


Figure 214: High-resolution deconvoluted MS spectrum of MIP-1β (4-69) modified protein **40**, *m/z* (ESI⁺) calcd $[M + H]^{+}_{mono}$ = 7665.6, found 7665.7 $[M + H]^{+}$.



Reaction was carried out with MIP-1β (4-69) protein **33** (4.4 mg, 0.53 nmol). After RP-HPLC purification, modified protein **41** was obtained as a lyophilized solid (0.98 mg, 0.12 nmol, 23%).

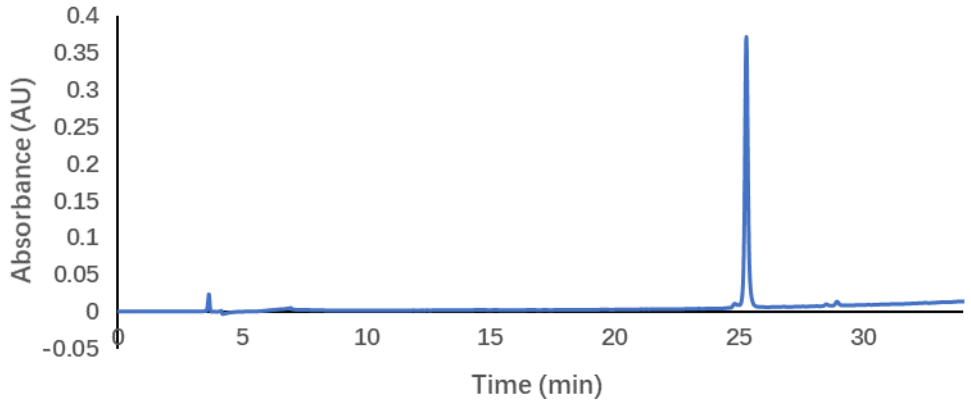


Figure 215: Analytical HPLC trace of purified MIP-1β (4-69) modified protein **41** (t_R = 25.8 min, 0% B for 1 min and then 0 to 50% B over 30 min with a flow rate of 1 mL/min, GL Sciences Inertsil® C4 analytical column)

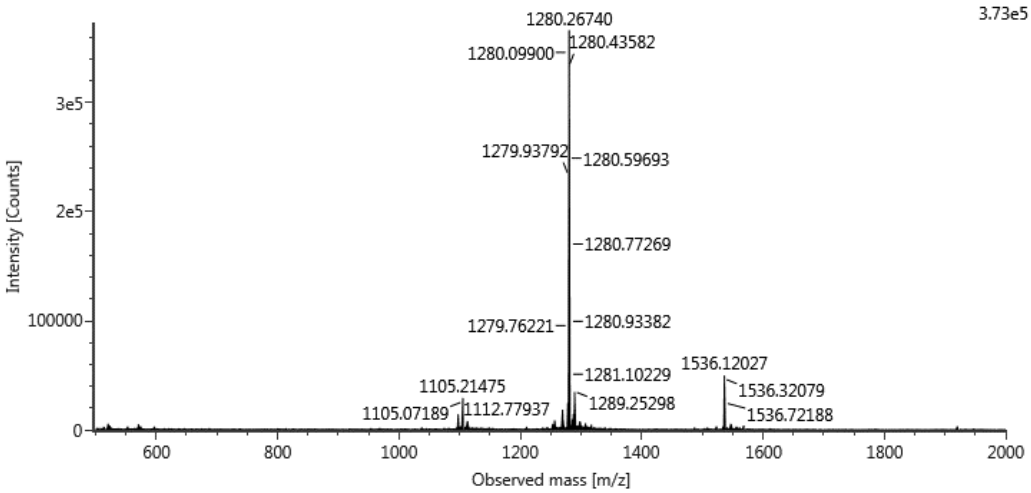


Figure 216: High-resolution MS spectrum of MIP-1β (4-69) modified protein **41**, *m/z* (ESI⁺) calcd M_{AV} = 7675.5, found 1536.1 $[M + 5H]^{5+}$, 1280.3 $[M + 6H]^{6+}$.

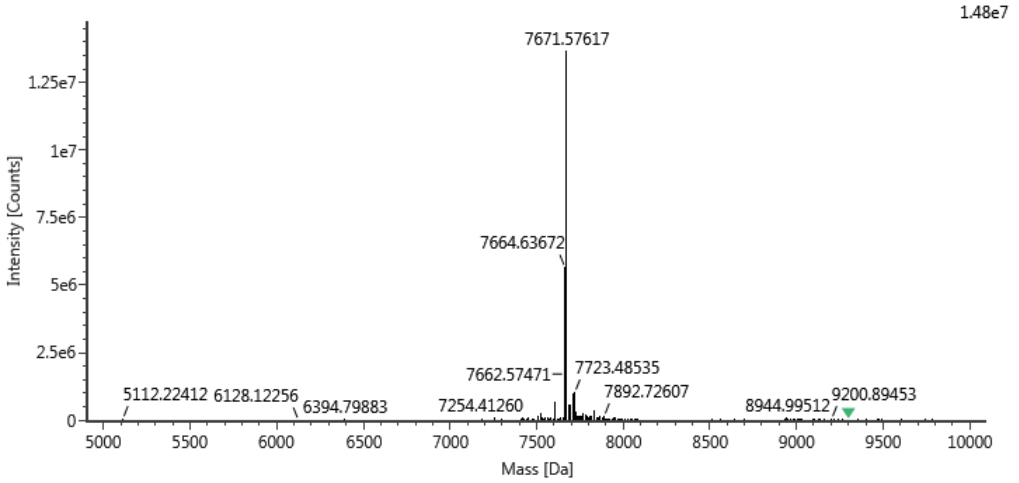
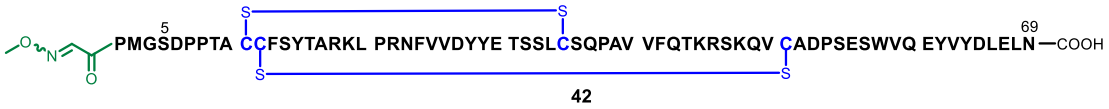


Figure 217: High-resolution deconvoluted MS spectrum of MIP-1β (4-69) modified protein **41**, *m/z* (ESI⁺) calcd $[M + H]^{+}_{mono}$ = 7671.6, found 7671.6 $[M + H]^{+}$.



Reaction was carried out with MIP-1β (Gly1-69) protein **34** (4.6 mg, 0.54 nmol). After RP-HPLC purification, modified protein **42** was obtained as a lyophilized solid (2.0 mg, 0.24 nmol, 44%).

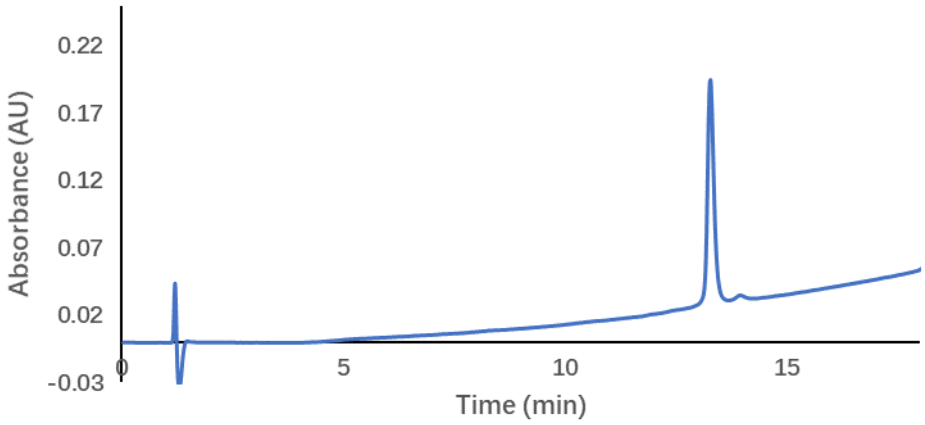


Figure 218: Analytical HPLC trace of purified MIP-1β (Gly1-69) modified protein **42** (t_R = 20.7 min, 0% B for 1 min and then 0 to 50% B over 15 min with a flow rate of 0.4 mL/min, Dubhe C4 analytical column).

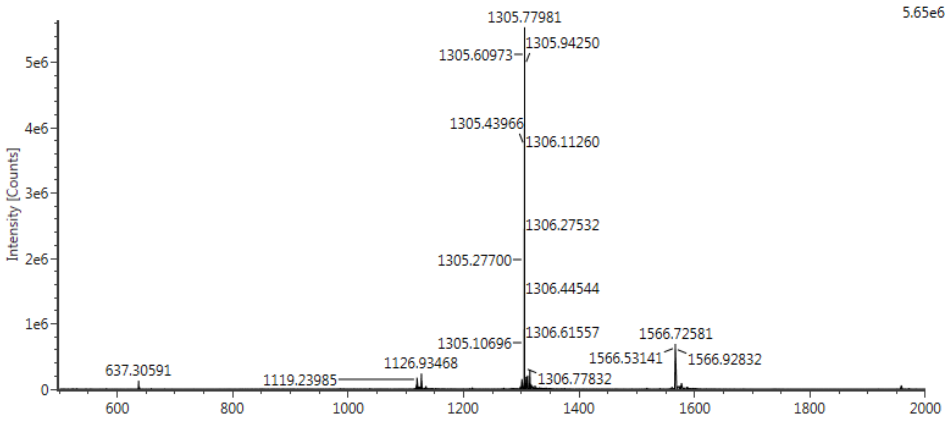


Figure 219: High-resolution MS spectrum of MIP-1β (Gly1-69) modified protein **42**, *m/z* (ESI⁺) calcd M_{AV} = 7828.7, found 1566.7 $[M + 5H]^{5+}$, 1305.8 $[M + 6H]^{6+}$.

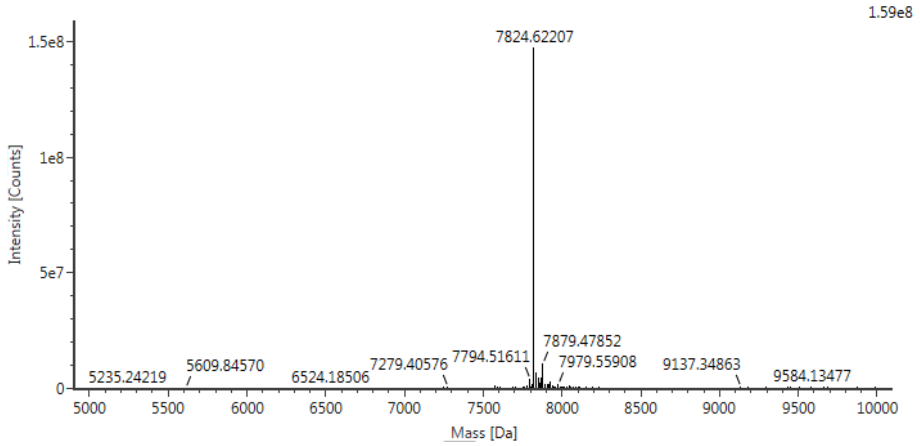
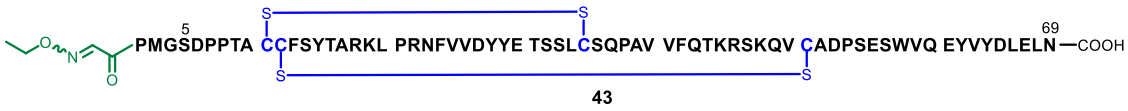


Figure 220: High-resolution deconvoluted MS spectrum of MIP-1β 1Gly -69 modified protein **42**, *m/z* (ESI⁺) calcd $[M + H]^{+}_{mono}$ = 7824.6, found 7824.6 $[M + H]^{+}$.



Reaction was carried out with MIP-1β (Gly1-69) protein **34** (3.4 mg, 0.41 nmol). After RP-HPLC purification, modified protein **43** was obtained as a lyophilized solid (1.7 mg, 0.19 nmol, 47%).

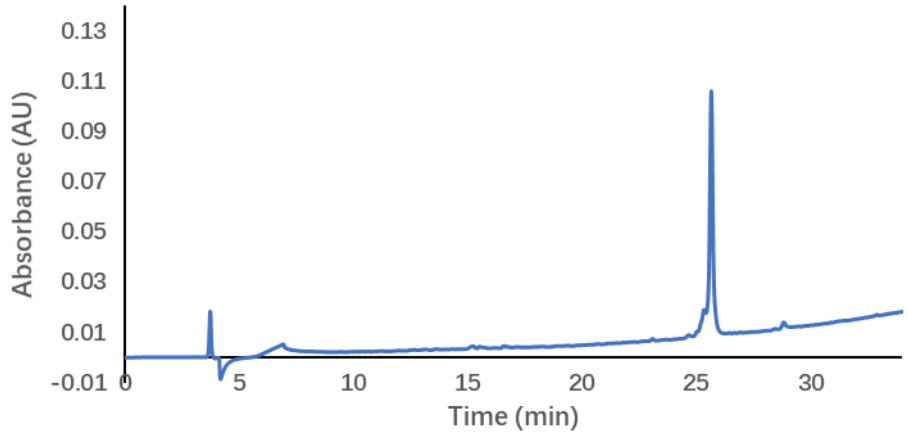


Figure 221: Analytical HPLC trace of purified MIP-1β (Gly1-69) modified protein **43** (t_R = 25.7 min, 0% B for 1 min and then 0 to 50% B over 30 min with a flow rate of 1 mL/min, GL Sciences Inertsil® C4 analytical column).

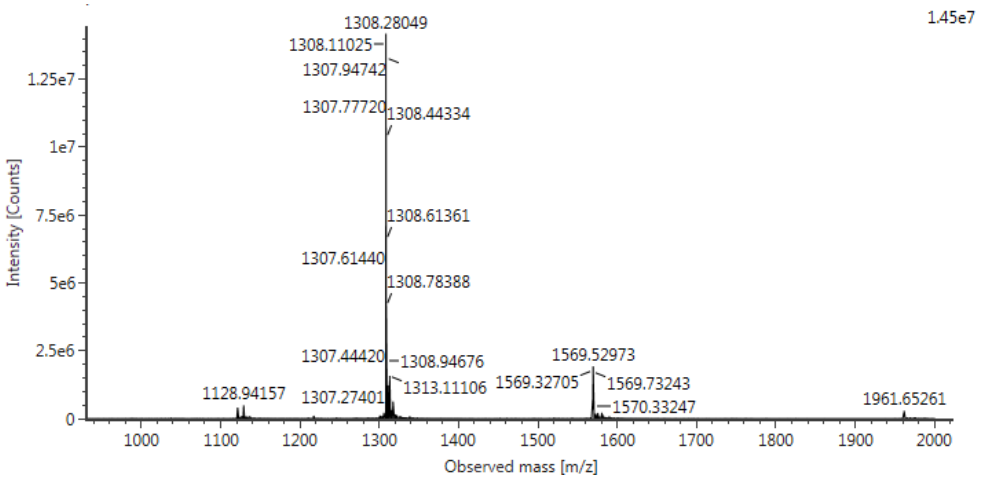


Figure 222: High-resolution MS spectrum of MIP-1β (Gly1-69) modified protein **43**, *m/z* (ESI⁺) calcd M_{AV} = 7842.8, found 1569.5 $[M + 5H]^{5+}$, 1308.3 $[M + 6H]^{6+}$.

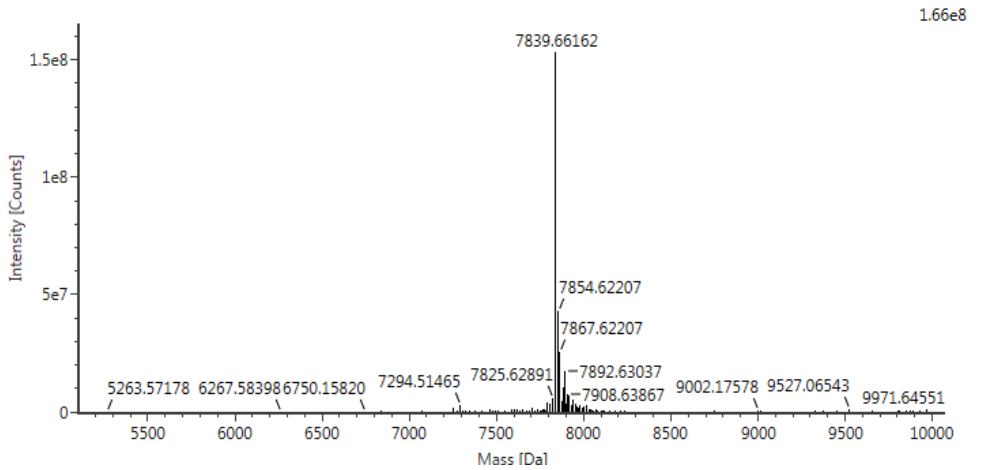
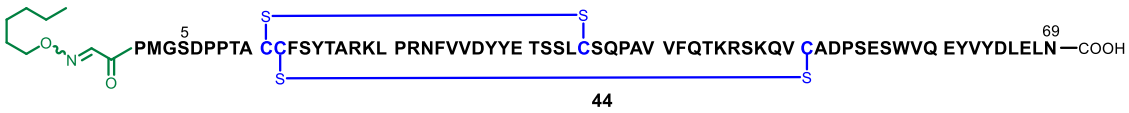


Figure 223: High-resolution deconvoluted MS spectrum of MIP-1β (Gly1-69) modified protein **43**, *m/z* (ESI⁺) calcd $[M + H]^{+}_{mono}$ = 7838.7, found 7839.7 $[M + H]^{+}$.



Reaction was carried out with MIP-1β (Gly1-69) protein **34** (5.7 mg, 0.67 nmol). After RP-HPLC purification, modified protein **44** was obtained as a lyophilized solid (1.8 mg, 0.21 nmol, 32%).

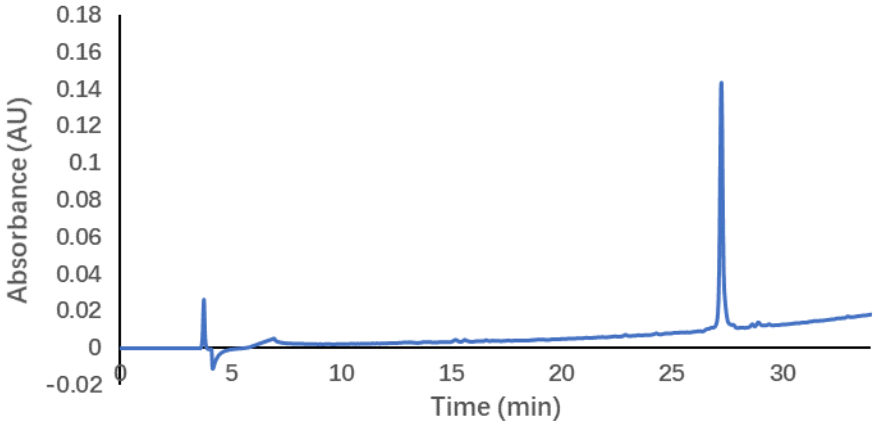


Figure 224: Analytical HPLC trace of purified MIP-1β (Gly1-69) modified protein **44** (t_R = 27.2 min, 0% B for 1 min and then 0 to 50% B over 30 min with a flow rate of 1 mL/min, GL Sciences Inertsil® C4 analytical column).

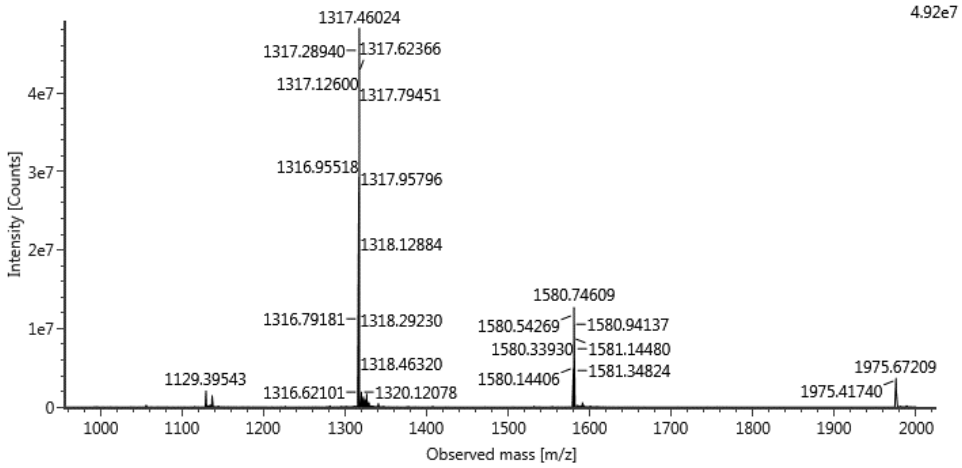


Figure 225: High-resolution MS spectrum of MIP-1β (Gly1-69) modified protein **44**, *m/z* (ESI⁺) calcd M_{AV} = 7898.9, found 1975.7 $[M + 4H]^{4+}$, 1580.7 $[M + 5H]^{5+}$, 1317.5 $[M + 6H]^{6+}$.

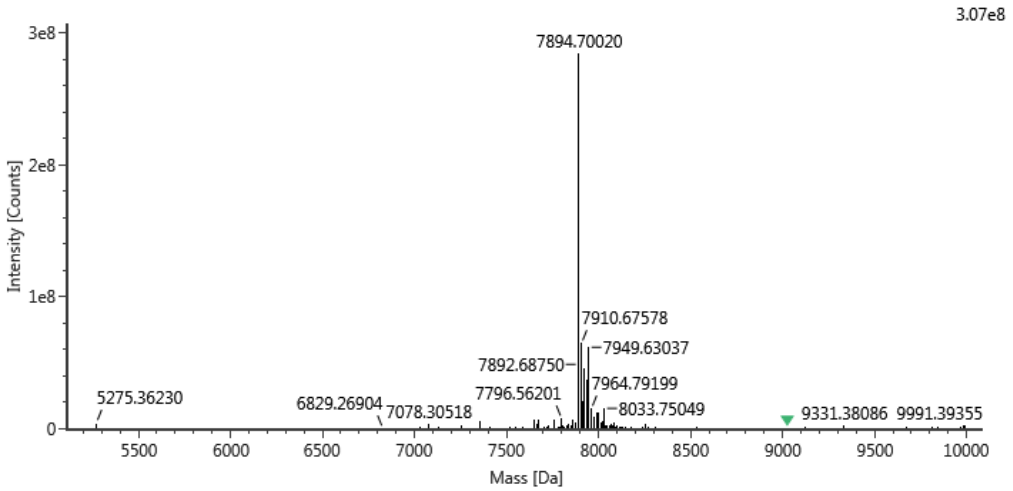
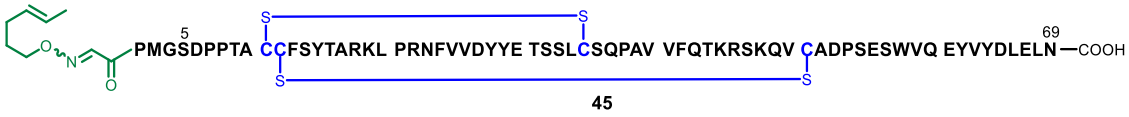


Figure 226: High-resolution deconvoluted MS spectrum of MIP-1β (Gly1-69) modified protein **44**, *m/z* (ESI⁺) calcd $[M + H]^{+}_{mono}$ = 7894.7, found 7894.7 $[M + H]^{+}$.



Reaction was carried out with MIP-1β (Gly1-69) protein **34** (5.0 mg, 0.58 nmol). After RP-HPLC purification, modified protein **45** was obtained as a lyophilized solid (1.7 mg, 0.19 nmol, 33%).

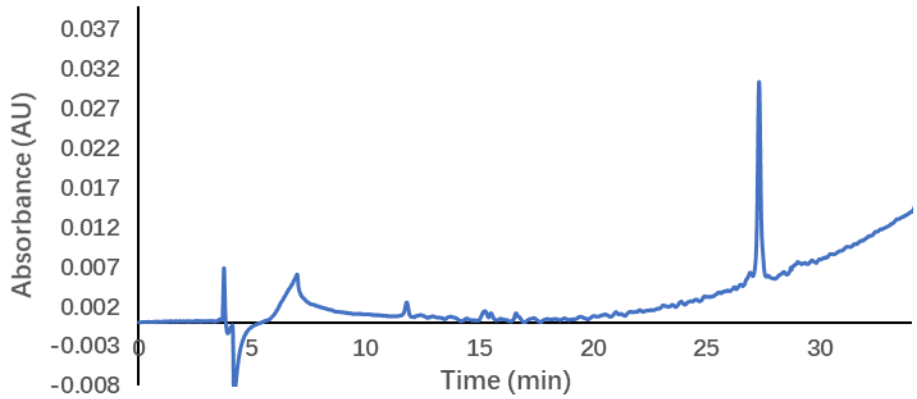


Figure 227: Analytical HPLC trace of purified MIP-1β (Gly1-69) modified protein **45** (t_R = 27.4 min, 0% B for 1 min and then 0 to 50% B over 30 min with a flow rate of 1 mL/min, GL Sciences Inertsil® C4 analytical column).

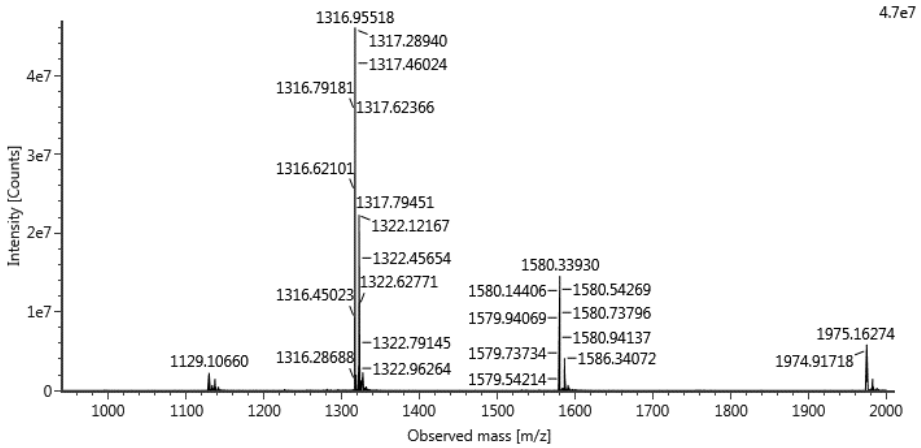


Figure 228: High-resolution MS spectrum of MIP-1β (Gly1-69) modified protein **45**, *m/z* (ESI⁺) calcd M_{AV} = 7896.9, found 1975.2 $[M + 4H]^{4+}$, 1580.3 $[M + 5H]^{5+}$, 1317.0 $[M + 6H]^{6+}$.

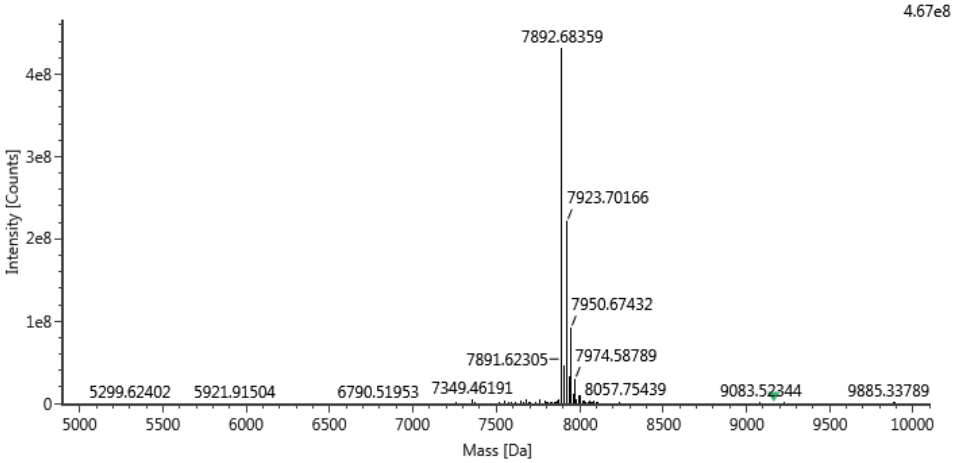
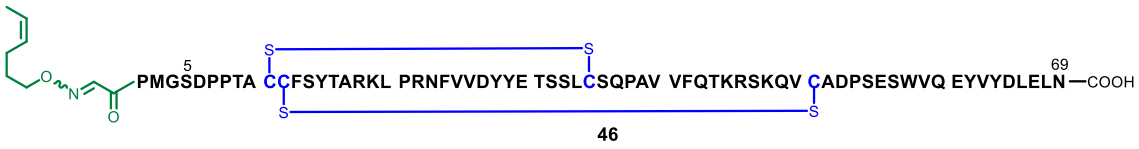


Figure 229: High-resolution deconvoluted MS spectrum of MIP-1β (Gly1-69) modified protein **45**, *m/z* (ESI⁺) calcd $[M + H]^{+}_{mono}$ = 7892.7, found 7892.7 $[M + H]^{+}$.



Reaction was carried out with MIP-1β (Gly1-69) protein **34** (4.8 mg, 0.57 nmol). After RP-HPLC purification, modified protein **46** was obtained as a lyophilized solid (1.8 mg, 0.21 nmol, 36%).

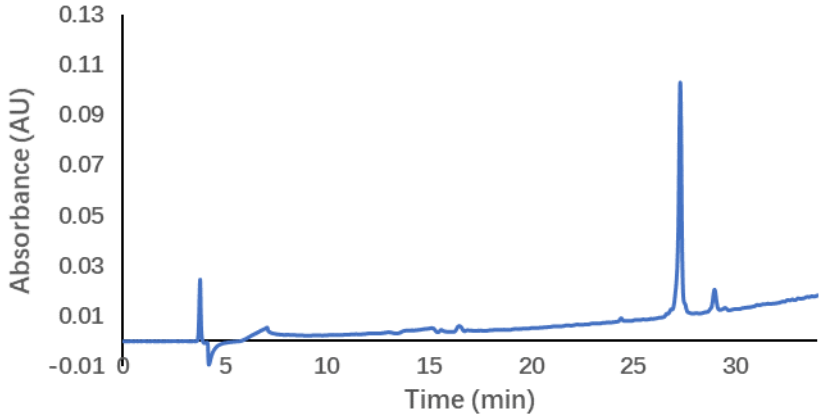


Figure 230: Analytical HPLC trace of purified MIP-1β (Gly1-69) modified protein **46** (t_R = 27.5 min, 0% B for 1 min and then 0 to 50% B over 30 min with a flow rate of 1 mL/min, GL Sciences Inertsil® C4 analytical column).

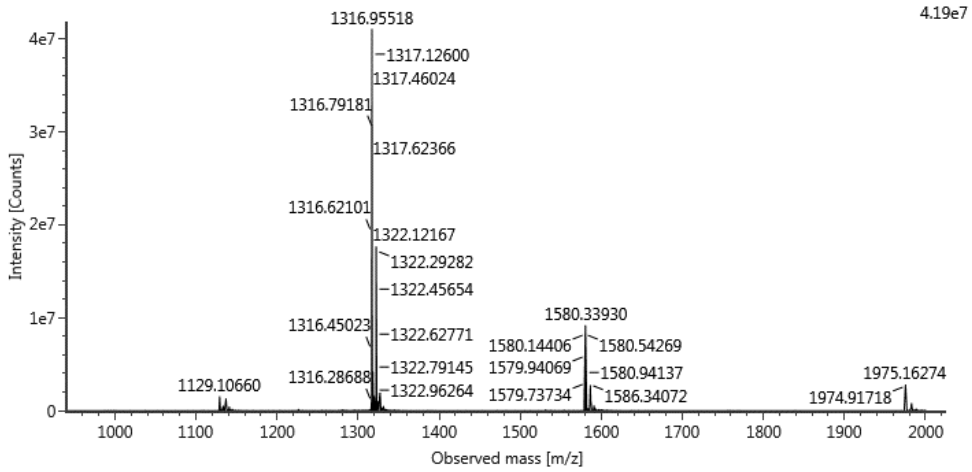


Figure 231: High-resolution MS spectrum of MIP-1β (Gly1-69) modified protein **46**, *m/z* (ESI⁺) calcd M_{AV} = 7896.9, found 1975.2 $[M + 4H]^{4+}$, 1580.3 $[M + 5H]^{5+}$, 1317.0 $[M + 6H]^{6+}$.

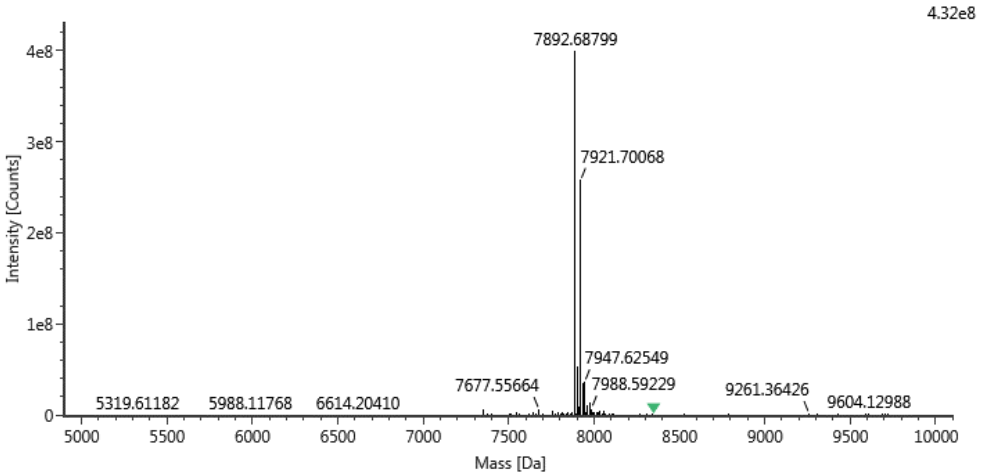
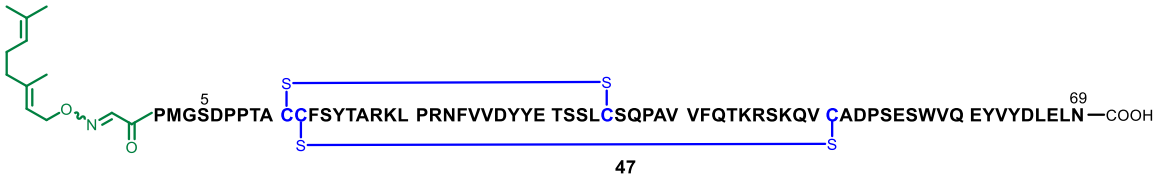


Figure 232: High-resolution deconvoluted MS spectrum of MIP-1β (Gly1-69) modified protein **46**, *m/z* (ESI⁺) calcd $[M + H]^+_{mono}$ = 7892.7, found 7892.7 $[M + H]^+$.



Reaction was carried out with MIP-1β (Gly1-69) protein **34** (4.4 mg, 0.52 nmol). After RP-HPLC purification, modified protein **47** was obtained as a lyophilized solid (1.5 mg, 0.18 nmol, 35%).

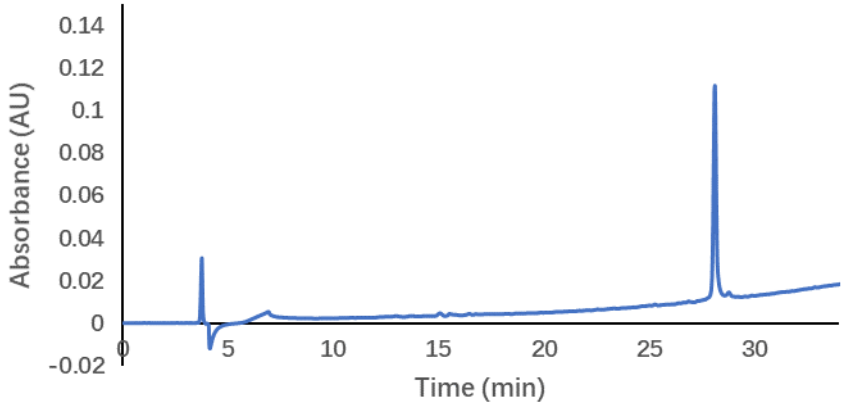


Figure 233: Analytical HPLC trace of purified MIP-1β (Gly1-69) modified protein **47** (t_R = 28.1 min, 0% B for 1 min and then 0 to 50% B over 30 min with a flow rate of 1 mL/min, GL Sciences Inertsil® C4 analytical column).

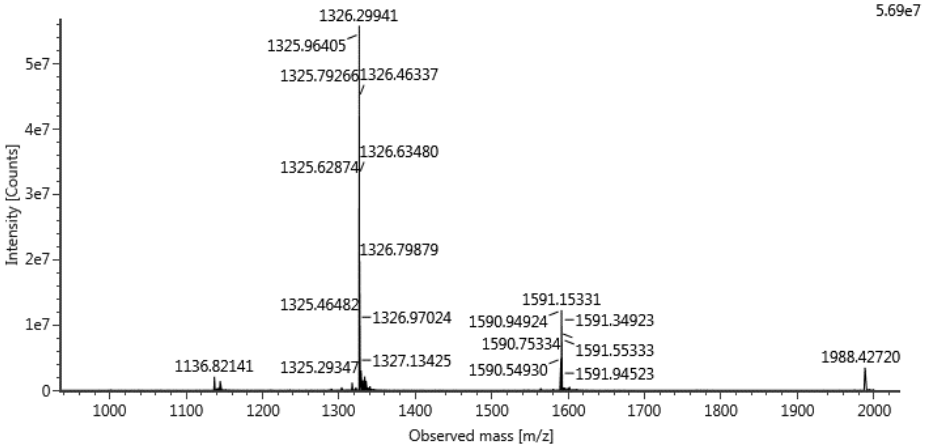


Figure 234: High-resolution MS spectrum of MIP-1β (Gly1-69) modified protein **47**, *m/z* (ESI⁺) calcd M_{AV} = 7951.0, found 1988.4 $[M + 4H]^{4+}$, 1591.1 $[M + 5H]^{5+}$, 1326.3 $[M + 6H]^{6+}$.

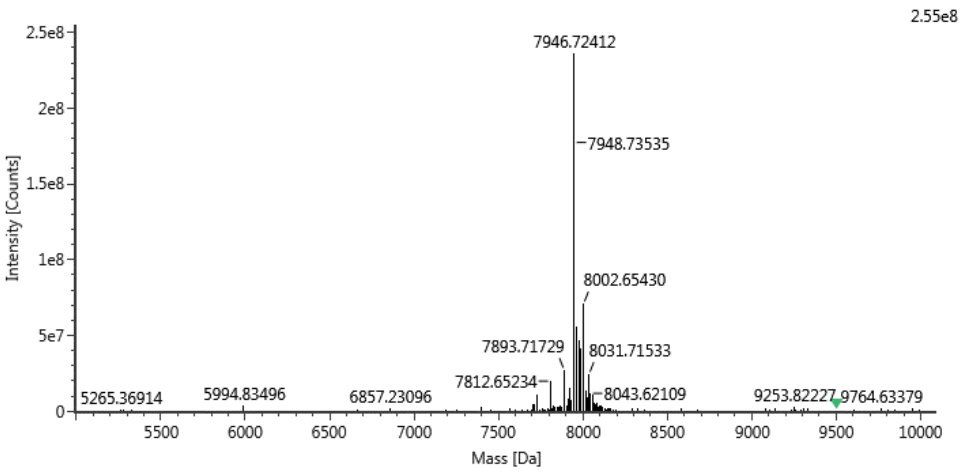
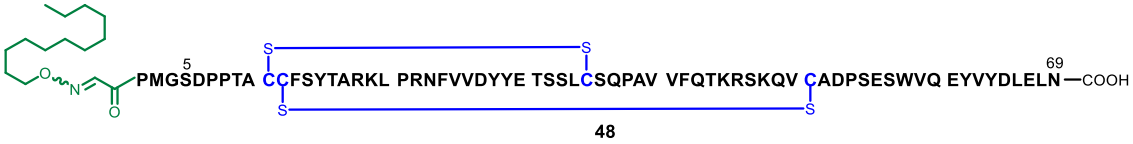


Figure 235: High-resolution deconvoluted MS spectrum of MIP-1β (Gly1-69) modified protein **47**, *m/z* (ESI⁺) calcd $[M + H]^{+}_{mono}$ = 7946.7, found 7946.7 $[M + H]^{+}$.



Reaction was carried out with MIP-1β (Gly1-69) protein **34** (3.2 mg, 0.38 nmol). After RP-HPLC purification, modified protein **48** was obtained as a lyophilized solid (0.91 mg, 0.11 nmol, 28%).

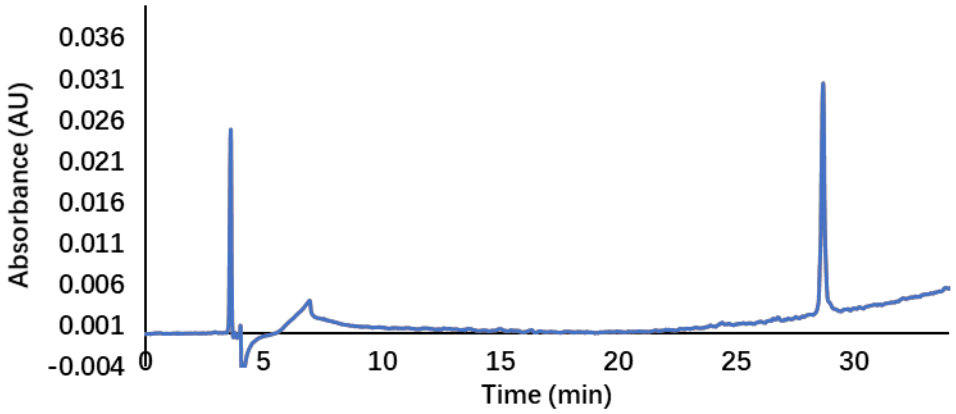


Figure 236: Analytical HPLC trace of purified MIP-1β (Gly1-69) modified protein **48** (t_R = 28.1 min, 0% B for 1 min and then 0 to 50% B over 30 min with a flow rate of 1 mL/min, GL Sciences Inertsil® C4 analytical column).

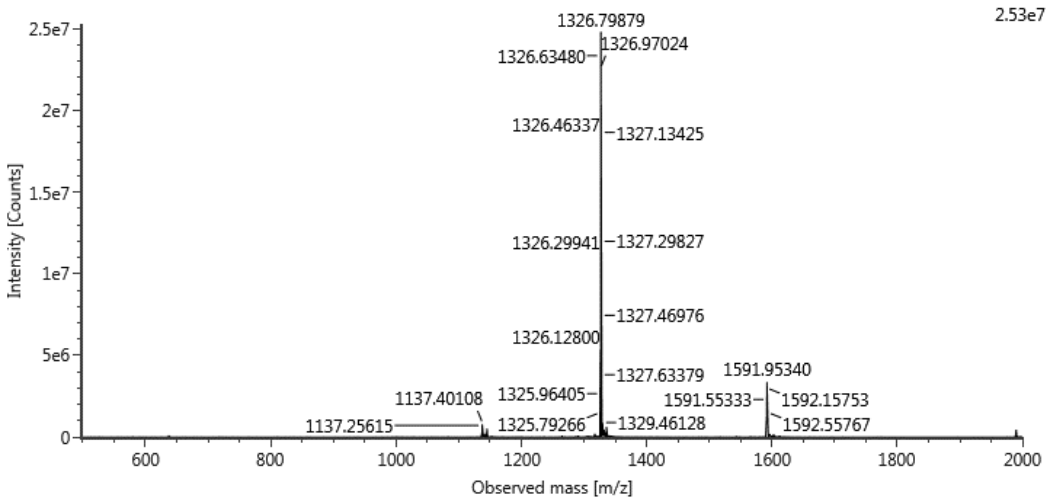


Figure 237: High-resolution MS spectrum of MIP-1β (Gly1-69) modified protein **48**, *m/z* (ESI⁺) calcd M_{AV} = 7955.0, found 1591.9 $[M + 5H]^{5+}$, 1326.8 $[M + 6H]^{6+}$.

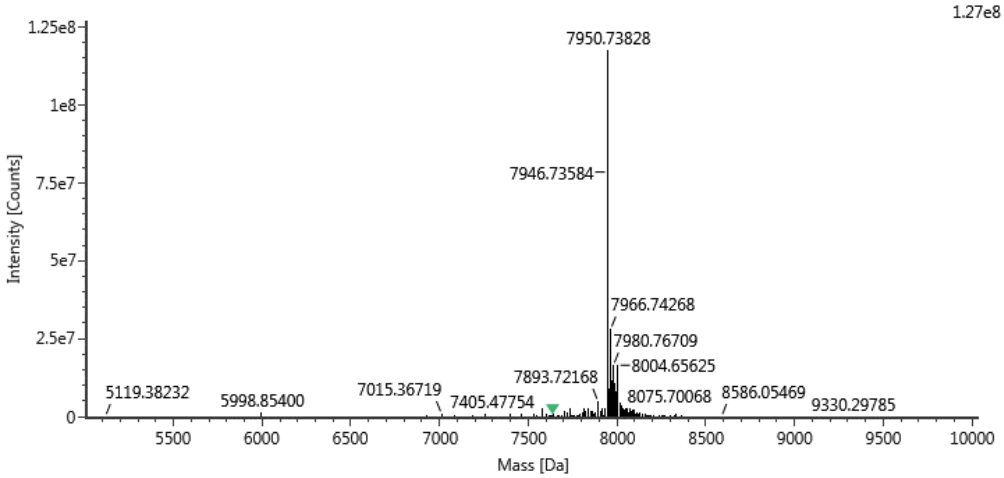
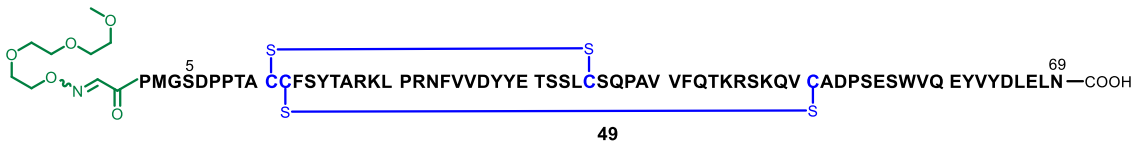


Figure 238: High-resolution deconvoluted MS spectrum of MIP-1β (Gly1-69) modified protein **48**, *m/z* (ESI⁺) calcd $[M + H]^{+}_{mono}$ = 7950.8, found 7950.7 $[M + H]^{+}$.



Reaction was carried out with MIP-1β (Gly1-69) protein **34** (5.4 mg, 0.63 nmol). After RP-HPLC purification, modified protein **49** was obtained as a lyophilized solid (1.3 mg, 0.15 nmol, 25%).

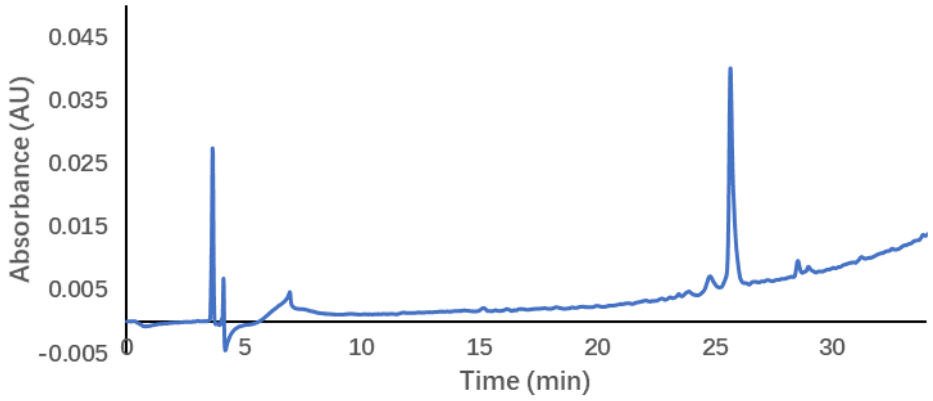


Figure 239: Analytical HPLC trace of purified MIP-1β (Gly1-69) modified protein **49** (t_R = 25.6 min, 0% B for 1 min and then 0 to 50% B over 30 min with a flow rate of 1 mL/min, GL Sciences Inertsil® C4 analytical column).

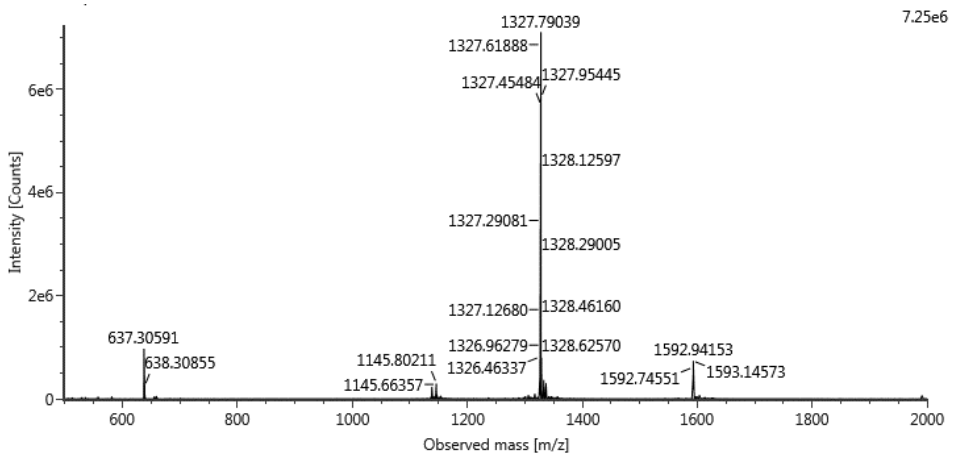


Figure 240: High-resolution MS spectrum of MIP-1β (Gly1-69) modified protein **49** *m/z* (ESI⁺) calcd M_{AV} = 7960.9, found 1592.9 $[M + 5H]^{5+}$, 1327.8 $[M + 6H]^{6+}$.

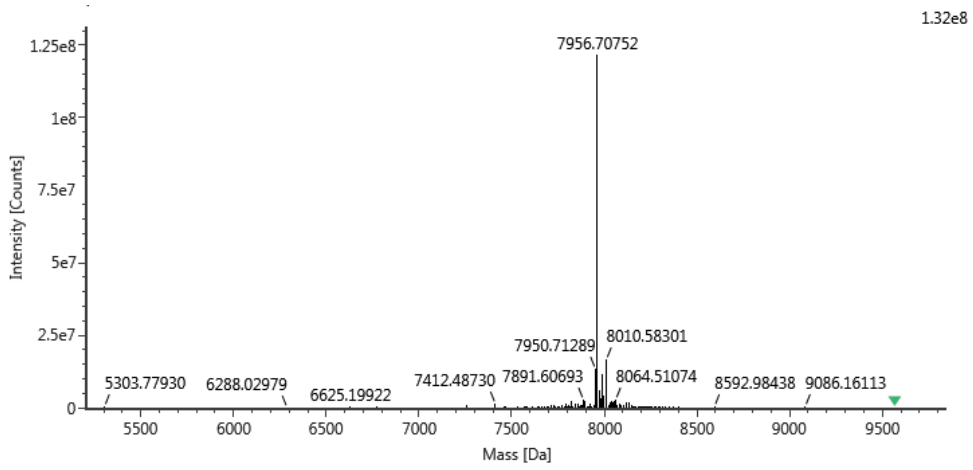


Figure 241: High-resolution deconvoluted MS spectrum of MIP-1β (Gly1-69) modified protein **49**, *m/z* (ESI⁺) calcd $[M + H]^+_{mono}$ = 7956.7, found 7956.7 $[M + H]^+$.

7. Experimental information for biological evaluation

7.1. Materials

Positive control compound Maraviroc (MVC), MTT (3,(4,5-dimethylthiazol 2 yl)-2,5-diphenyl tetrazolium bromide) and penicillin were purchased from Sigma Aldrich. Streptomycin sulfate was purchased from Amresco. DMEM medium and FBS were both purchased from Invitrogen. Recombinant MIP-1β was purchased from Amyjet Scientific. Tzm-bl cells were recovered and passaged according to general procedures: Cells were recovered from freezing and were washed with PBS to remove the freezing medium. Complete DMEM medium with 10% FBS was then added, mixed and incubated at 37°C with 5% CO_2. The cells were subcultured every two days and were subcultured one day before the assay. Strain HIV-1Ba-L was obtained from NIH and cultured as usual.

All protein samples were dissolved in non-serum median with a concentration of 500 μM. The solution was filtered with 0.22 μM membrane filter, aliquoted and stored at -20 °C. MVC was dissolved in DMSO at 2-8 °C with a concentration of 50 mM.

7.2. Medium

Complete DMEM medium, 100,000 IU Penicillin, 100μg/ml Streptomycin, with or without 10% FBS.

7.3. Cytotoxicity with MTT assay

100 μL of Tzm-bl suspension culture with cell density at 3×10^5 viable cells/mL was exposed to different proteins with 6 dilutions in triplicate. 6 individual cell cultures without any protein served as negative control, and 6 wells with only medium as blank. The culture was incubated at 37°C with 5% CO_2 for 3 days before the MTT colorimetric assay. Optical density (OD) was measured at 490 nm with reference wavelength at 620 nm using an ELx800 plate reader. CC_{50} (50 % Cytotoxic Concentration) for each protein was calculated and reported.

7.4. Inhibition of HIV-1Ba-L replication in Tzm-bl cells

Each protein was prepared with 5 dilutions, 100 μL of each dilution was transferred to a 96-well plate. 50 μL of Tzm-bl culture with cell density at 6×10^5 viable cells/mL was added, followed by the addition of HIV-1Ba-L dilution with multiplicity of infection (MOI) at 0.03. Cells with only virus served as positive control, and cells without virus served as negative control, and MVC was used as a positive drug control. All experiments were performed in triplicate. After incubation at 37 °C with 5% CO_2 for 48 hours, the supernatant was removed, and cells were washed with PBS twice. Then, the cells were lysed by adding 100 μL of luciferase lysis buffer and incubating at 4 °C for 30 min. After the addition of the luminescent substrate with 1/1 ratio, fluorescent signal was recorded using a FLEX Station3 plate reader. EC_{50} was calculated and reported.

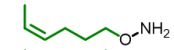
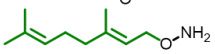
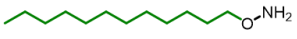
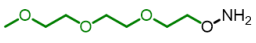
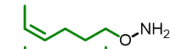
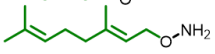
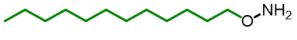
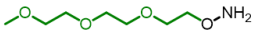
7.5. Calculations

EC_{50} and CC_{50} were calculated according to Reed and Muench's method. Therapeutic index (TI) was defined as TI = CC_{50}/EC_{50}

Relative cell viability (%) = OD of treated wells/ OD of negative control wells × 100%

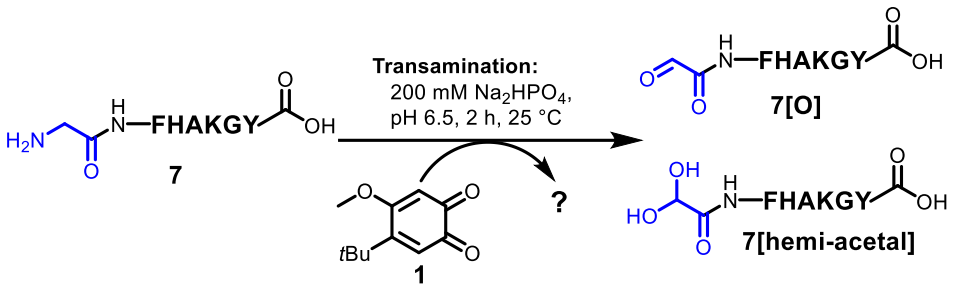
Inhibition of HIV-1 induced luciferase expression (%) = [1- (treated RLU - negative control RLU)/(positive control RLU - negative control RLU)]×100%

Table 22: Anti-HIV activity and cytotoxicity of MIP-1β analogs.

Starting material	Alkoxyamine R-ONH_2	Product Code	EC_{50} (nM)	TI	CC_{50} (μM)
Rb-CCL4	NA	**NA**	3306.12±1404.07	>15.12	>50
33 (4-69)	NA	**33**	>5000	ND	>50
33 (4-69)	EtONH_2·HCl	**35**	200.52±17.68	>249.36	>50
33 (4-69)		**36**	>5000	ND	>50
33 (4-69)		**37**	>5000	ND	>50
33 (4-69)		**38**	>5000	ND	>50
33 (4-69)		**39**	>5000	ND	>50
33 (4-69)		**40**	>5000	ND	>50
33 (4-69)		**41**	>5000	ND	>50
34 (Gly1-69)	NA	**34**	157.48±48.80	>317.51	>50
34 (Gly1-69)	MeONH_2·HCl	**42**	2762.23±554.63	>18.10	>50
34 (Gly1-69)	EtONH_2·HCl	**43**	2312.93±645.07	>21.62	>50
34 (Gly1-69)		**44**	>5000	ND	>50
34 (Gly1-69)		**45**	>5000	ND	>50
34 (Gly1-69)		**46**	>5000	ND	>50
34 (Gly1-69)		**47**	>5000	ND	>50
34 (Gly1-69)		**48**	>5000	ND	>50
34 (Gly1-69)		**49**	>5000	ND	>50

8. Mechanism study

To study the mechanism, we performed the following experiments:



Reaction were carried out with peptide **7** (1 mg, 1.2 µmol) and oxidant **1** (2.0 mg, 10 µmol) in 1 mL of aqueous buffer. Reaction was kept in identical condition detailed in section **3**. After 2 hours, the reaction mixture was analyzed with LCMS directly.

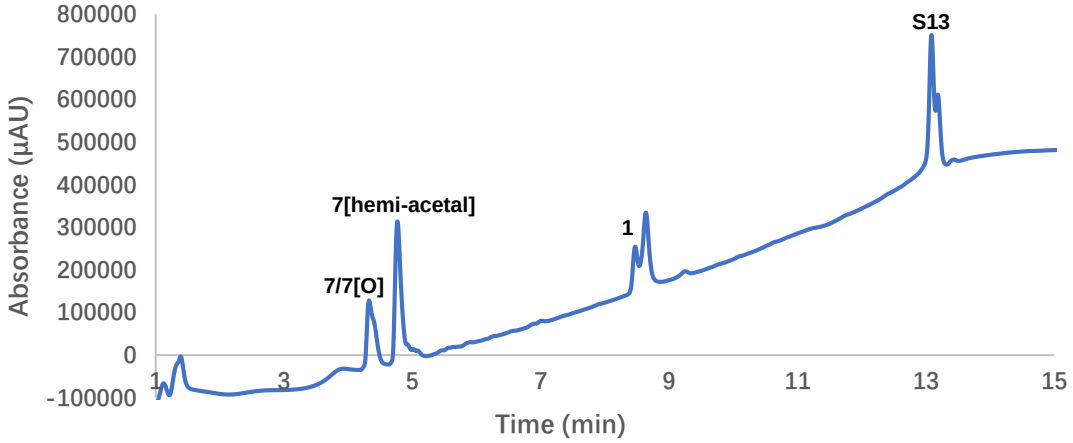


Figure 242: Analytical LC trace of the reaction mixture. (0% B for 1 min and then 0 to 100% B over 10 min with a flow rate of 0.3 mL/min, buffered with 0.1% formic acid, Dubhe C18 analytical column).

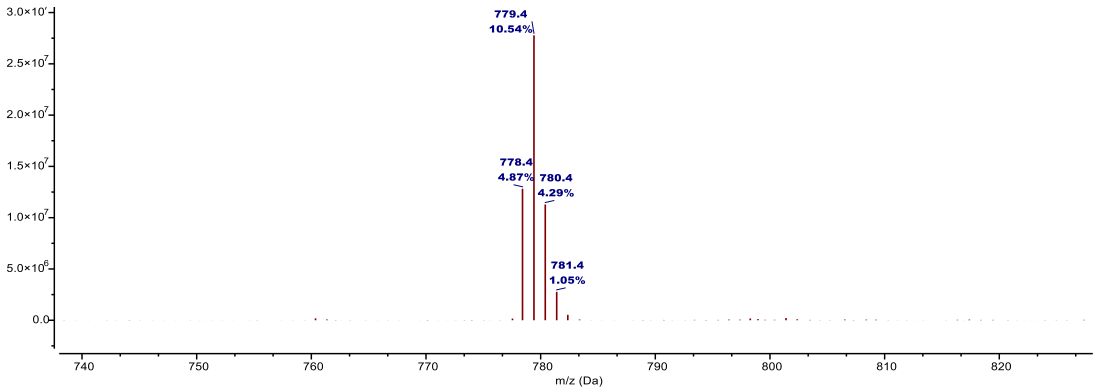


Figure 243: Expanded MS spectrum extracted at t_R = 4.3 min. The result showed a mixture of starting peptide **7** ([M+H]$^+$ 779.4) with a small fraction of **7[O]** ([M+H]$^+$ 778.4)

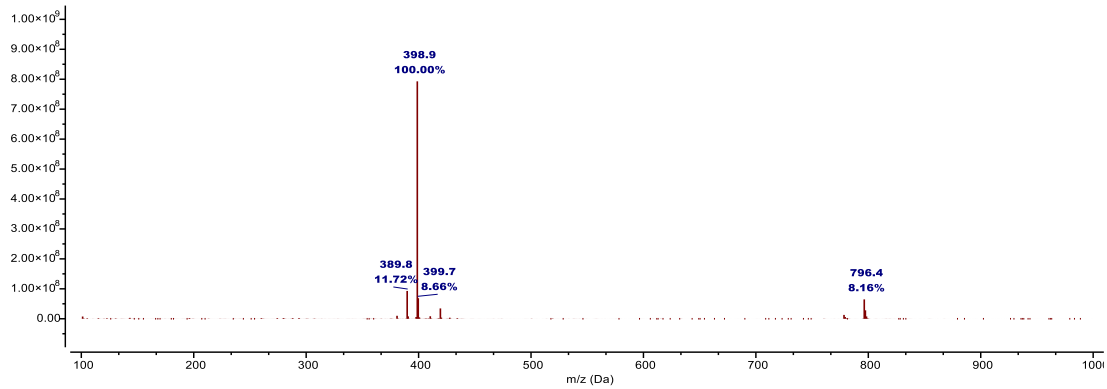


Figure 244: MS spectrum extracted at t_R = 4.8 min, which attributed it to the hemiacetal of **7[O]** ([M+H]$^+$ 796.4)

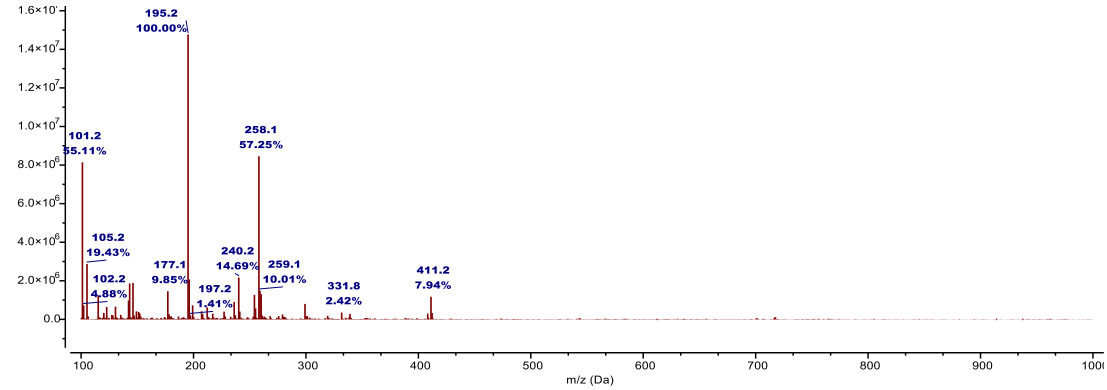


Figure 245: MS spectrum extracted at t_R = 8.5 min. molecular ion of oxidant **1** was observed ([M+H]$^+$ 195.2).

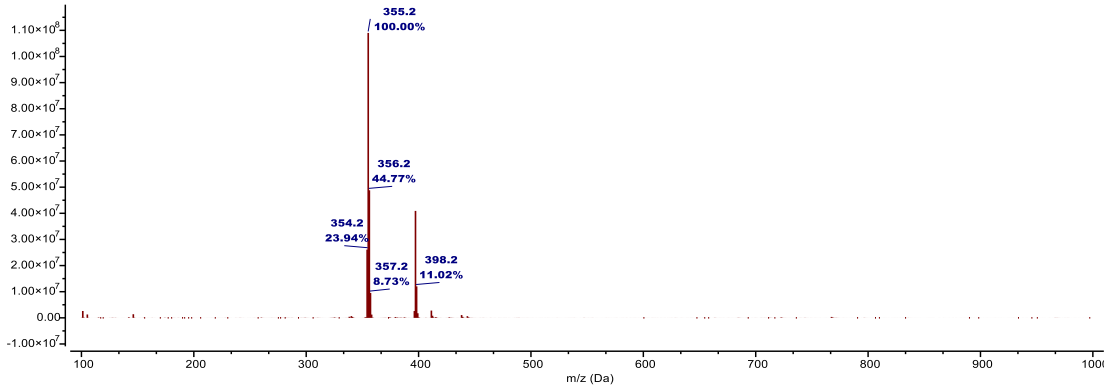
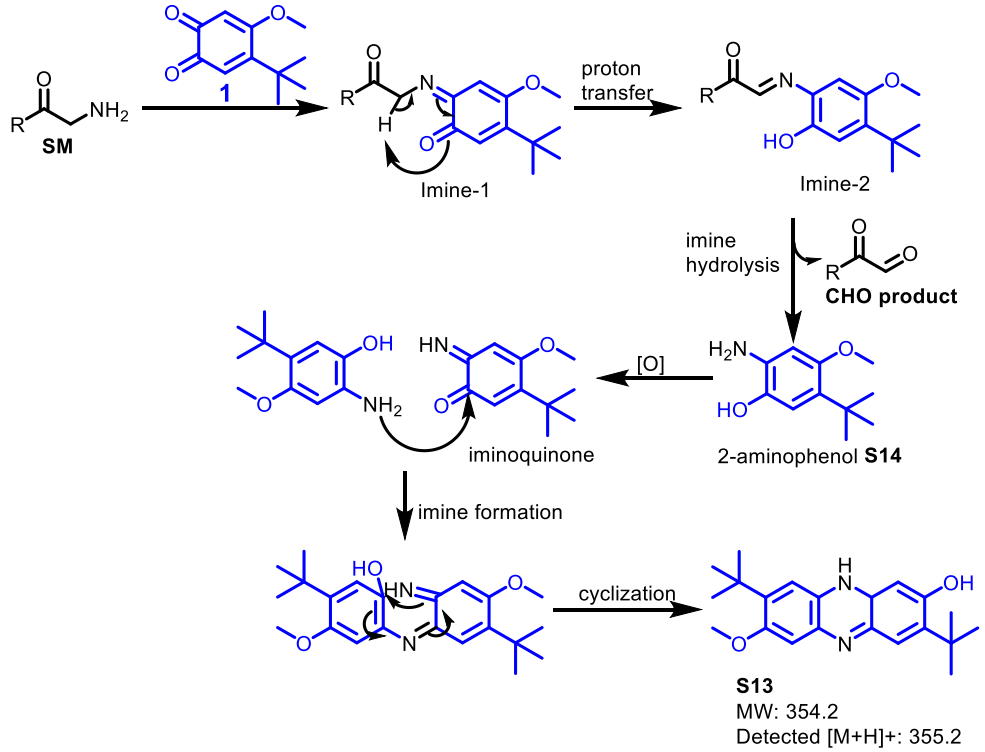


Figure 246: MS spectrum extracted at t_R = 13.1 min. MS spectrum extracted at t_R = 13.1 min. This peak was marked as byproduct **S13** with molecular ion [M+H]$^+$ 355.2.

A similar reaction was conducted with glycine (0.1 g, 1.3 mmol) and oxidant (0.2 g,1.0 mmol) reacted in 100 mL of aqueous solution buffered at pH 6.5. After 1 hour, the mixture was also analyzed with LCMS and the eluted peaks have identical molecular ions to that of peptide experiments.

Considering the mechanism reported by Luo and co-workers, we purposed that this transamination reaction still proceeds *via* imine intermediates **1** and **2** to release the desired aldehyde, which also converted ortho-quinone **1** to 2-aminophenol **S14**. However, our LCMS experiments didn't observe the molecular ion corresponding to **S14**. Instead, two products with molecular ion 355.2 were observed. Therefore, we believe that the **S13** was continuously reacted in this oxidative reaction to form **S13**.



Scheme 1: purposed mechanism of quinone **1** oxidized N-terminal glycine peptide.

Reference

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