

# Supporting information for

## **Selenopeptide transamidation and metathesis**

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## 1. General methods

### Reagents and solvents

2-(1*H*-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium fluorophosphate (HBTU) and *N*-Fmoc protected amino acids were obtained from Iris Biotech GmbH. Side-chain protecting groups used for the amino acids were Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(*O**t*Bu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(*O**t*Bu)-OH, Fmoc-His(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Thr(*t*Bu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Tyr(*t*Bu)-OH, Fmoc-Cys(*S**t*Bu)-OH or Fmoc-Cys(Trt)-OH. Synthesis of *bis*(2-sulfanylethyl)aminotriyl polystyrene (SEA PS) resin was carried out as described elsewhere.<sup>1</sup> Rink-PEG-PS resin (NovaSyn TGR) resin was obtained from Novabiochem. 4-Mercaptophenylacetic acid (MPAA), 3-mercaptopropionic acid (MPA), *tris*(2-carboxyethyl)phosphine hydrochloride (TCEP), triisopropylsilane (TIS), dimethyl sulfide (DMS), guanidine hydrochloride were purchased from Sigma-Aldrich. All other reagents were purchased from Acros Organics or Merck and were of the purest grade available.

Peptide synthesis grade *N,N*-dimethylformamide (DMF), dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>), diethylether (Et<sub>2</sub>O), acetonitrile (CH<sub>3</sub>CN), heptane, LC-MS-grade acetonitrile (CH<sub>3</sub>CN, 0.1% TFA), LC-MS-grade water (H<sub>2</sub>O, 0.1% TFA), *N,N*-diisopropylethylamine (DIEA), acetic anhydride (Ac<sub>2</sub>O) were purchased from Biosolve and Fisher-Chemical. Trifluoroacetic acid (TFA) was obtained from Biosolve. Water was purified with a Milli-Q Ultra Pure Water Purification System.

### Analyses and purifications

Products were characterized by analytical LC-MS (Waters 2695 LC/ZQ 2000 quadrupole) on a reverse phase column XBridge BEH300 C18 (3.5 μm, 300 Å, 4.6 × 150 mm) at 30 °C using a linear gradient of 0-100% of buffer B in buffer A over 30 min at a flow rate of 1 mL/min (buffer A = 0.1% TFA in H<sub>2</sub>O; buffer B = 0.1% TFA in CH<sub>3</sub>CN/H<sub>2</sub>O: 4/1 by vol). The column eluate was monitored by UV at 215 nm and by evaporative light scattering (ELS, waters 2424). The peptide masses were measured by on-line LC-MS: Ionization mode: ES+, *m/z* range 350–2040, capillary voltage 3 kV, cone voltage 30 V, extractor voltage 3 V, RF lens 0.2 V, source temperature 120 °C, desolvation temperature 350 °C. Calculated masses were based on average isotope composition. Samples were prepared using 10 μL aliquots of the reaction mixtures. The aliquots were quenched by adding 90 μL of 10% aqueous TFA, extracted with Et<sub>2</sub>O to remove MPAA or MPA before analysis.

MALDI-TOF mass spectra were recorded with a Bruker Autoflex Speed using alpha cyano 4-hydroxycinnamic acid or sinapinic acid as matrix. The observed *m/z* corresponded to the monoisotopic ions, unless otherwise stated.

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<sup>1</sup>N. Ollivier, J. Dheur, R. Mhidia, A. Blanpain, O. Melnyk, *Org. Lett.* **2010**, *12*, 5238-5241.

Preparative reverse phase HPLC of crude peptides were performed with an Autopurification prep HPLC–MS Waters system using a reverse phase column XBridge ODB prep C18 (5  $\mu\text{m}$ , 300  $\text{\AA}$ , 19  $\times$  100 mm) and appropriate linear gradient of increasing concentration of buffer B in buffer A (flow rate of 25 mL/min). The fractions containing the purified target peptide were identified on-line using MS (ZQ 2000) quadripole. Selected fractions were then combined and lyophilized.

## 2. Synthesis of SEA<sup>off</sup> peptides 1a-h

The peptides **1a-h** H-ILKEPVHGX-SEA<sup>off</sup> (X= Phe (a), Ser (b), Thr (c), Arg (d), Ala (e), Glu (f), Cys (g), Gly (h)) were synthesized using SEA PS resin as described elsewhere.<sup>1</sup>

### *Typical experimental procedures*

#### *Coupling of the first amino acid on SEA PS resin*

Briefly, the first amino acid (10 eq) was coupled to SEA PS resin (0.1 mmol, 0.15 mmol/g) using HATU (379 mg, 1 mmol, 10 eq)/DIEA (387  $\mu\text{L}$ , 2 mmol, 20 eq) activation in the minimal volume of DMF. The amino acid was preactivated (2 min) and then added to the resin swelled in the minimal volume of DMF. The beads were agitated during 1 h 30 at room temperature (rt). Then, the resin was washed with DMF (3  $\times$  2 min) and drained. The absence of unreacted secondary amino groups was verified using chloranil colorimetric assay. The resin was acetylated using Ac<sub>2</sub>O/DIEA/DMF: 10/5/85 by vol (5 min, 10 min) and then washed with DMF (3  $\times$  2 min).

#### *Automated peptide elongation*

Peptide elongation was performed using standard Fmoc/*tert*-butyl chemistry on an automated peptide synthesizer without microwaves (0.1 mmol scale for **1a-d** and **1f-h**, 0.25 mmol scale for **1e**). Couplings were performed using 4-fold molar excess of each Fmoc L-amino acid, 3.6-fold molar excess of HBTU, and 8-fold molar excess of DIEA. A capping step was performed after each coupling with Ac<sub>2</sub>O/DIEA. At the end of the synthesis, the resin was washed with CH<sub>2</sub>Cl<sub>2</sub>, diethyl ether (2  $\times$  2 min) and dried *in vacuo*.

#### *Final peptide deprotection and cleavage*

Peptides **1a-h** were cleaved using TFA/TIS/DMS/water: 92.5/2.5/2.5/2.5 by vol for 1 h 30 (10 mL for 0.1 mmol scale, 20 mL for a 0.25 mmol scale).

The crude peptides were precipitated in cold diethyl ether/heptane: 1/1 by vol (150 mL for 0.1 mmol scale, 300 mL for 0.25 mmol scale), solubilized in deionised water and lyophilized.

#### *Oxidation (SEA<sup>on</sup> $\rightarrow$ SEA<sup>off</sup>) and purification*

Crude peptide (21.95  $\mu\text{mol}$ ) was dissolved in AcOH/water 1/4 (45 mL). I<sub>2</sub> (43.9  $\mu\text{mol}$ , 530  $\mu\text{L}$  of a 82.7 mM solution in DMSO, 2 eq) was added to the previous solution. After 30 seconds of stirring, dithiothreitol (DTT, 43.9  $\mu\text{mol}$ , 677.5  $\mu\text{L}$  of a 64.8 mM solution in AcOH/water: 1/4 by vol, 2 eq) was added to consume excess I<sub>2</sub>. Then the peptide was purified immediately by RP-HPLC as described in the

general procedure (buffer A = water containing 0.05% TFA, buffer B=acetonitrile in water 4/1 containing 0.05% TFA, rt, detection at 215 nm).

Peptide	C-terminal amino acid	Scale (mmol)	Isolated yield (%)	Calcd. monoisotopic [M+H] <sup>+</sup>	MALDI-TOF [M+H] <sup>+</sup>
<b>1a</b>	Phe	0.1	25	1156.6	1156.6
<b>1b</b>	Ser	0.1	9	1096.6	1096.6
<b>1c</b>	Thr	0.1	10	1110.6	1110.6
<b>1d</b>	Arg	0.1	27	1165.6	1165.6
<b>1e</b>	Ala	0.25	34	1080.5	1080.5
<b>1f</b>	Glu	0.1	15	1138.6	1138.6
<b>1g</b>	Cys (StBu)	0.1	18	1200.6	1200.6
<b>1h</b>	Gly	0.1	22	1066.6	1066.6

### 3. Synthesis of TCEP=Se

#### *Synthetic procedure*

Tris(2-carboxyethyl)phosphine hydrochloride (TCEP.HCl, 60.6 mg, 0.21 mmol, 200 mM final concentration) and selenium (16.53 mg, 0.21 mmol, 1 eq) were mixed together at rt in phosphate buffer (0.1 M, 1.05 mL). After 2 h, the reaction medium was diluted with water containing 0.05 % TFA by vol (4 mL), filtered and purified by RP-HPLC (rt, detection at 215 nm, buffer A = water containing 0.05% TFA, buffer B=acetonitrile in water 4/1 containing 0.05% TFA, gradient=0 to 5% B in 1 min, then 5 to 35% B in 25 min) to afford 54.5 mg of TCEP=Se (78%).

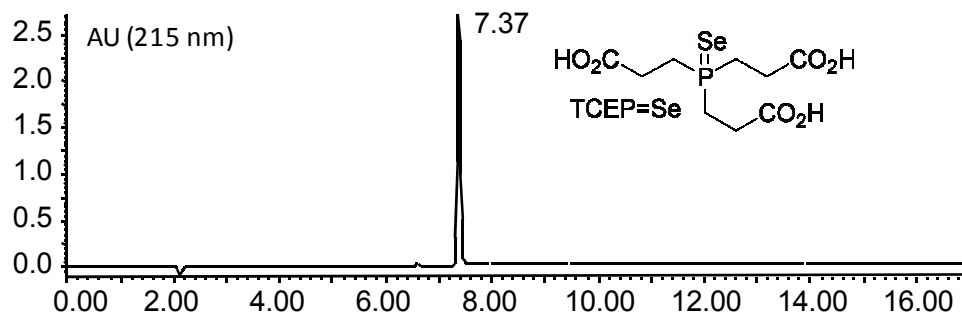


Figure S1. Analytical LC-MS analysis of TCEP=Se (HPLC trace). ESI-MS detection (positive ion mode) [M-H<sub>2</sub>O + H]<sup>+</sup> calcd 313.15, found 313.0.

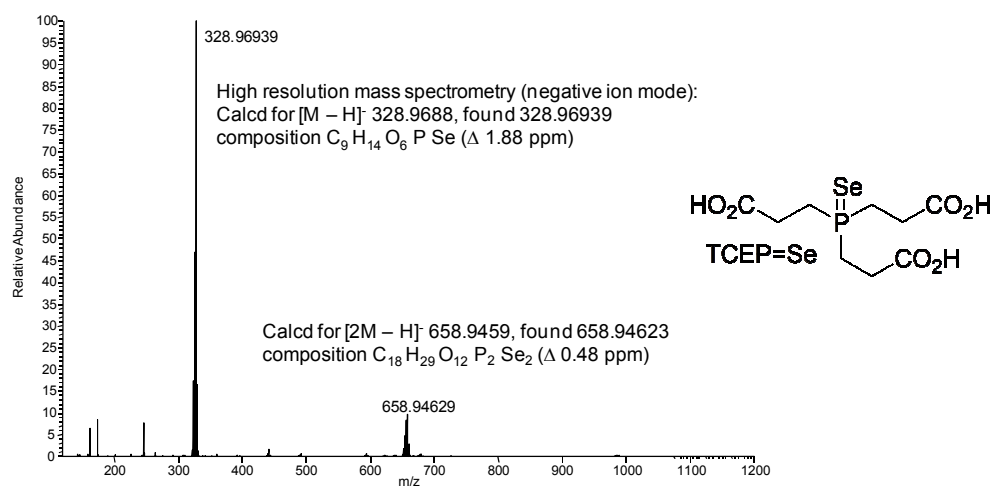


Figure S2. High resolution mass spectrometry of TCEP=Se (negative ion mode).

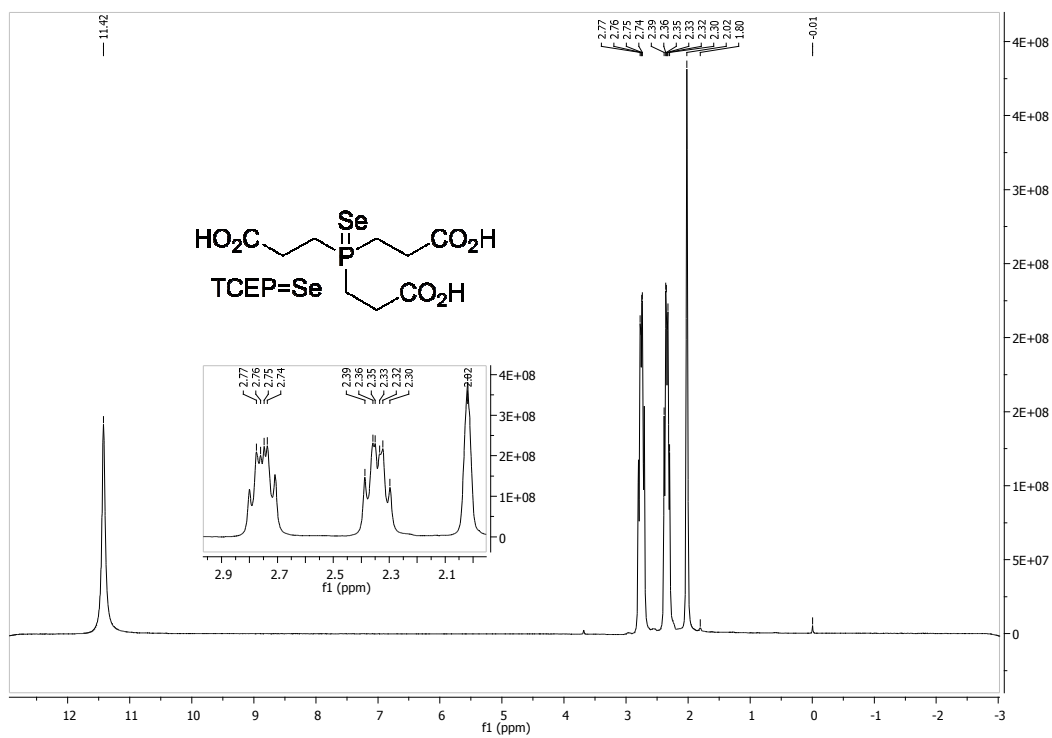


Figure S3.  $^1H$  NMR spectrum (300.0 MHz) of TCEP=Se

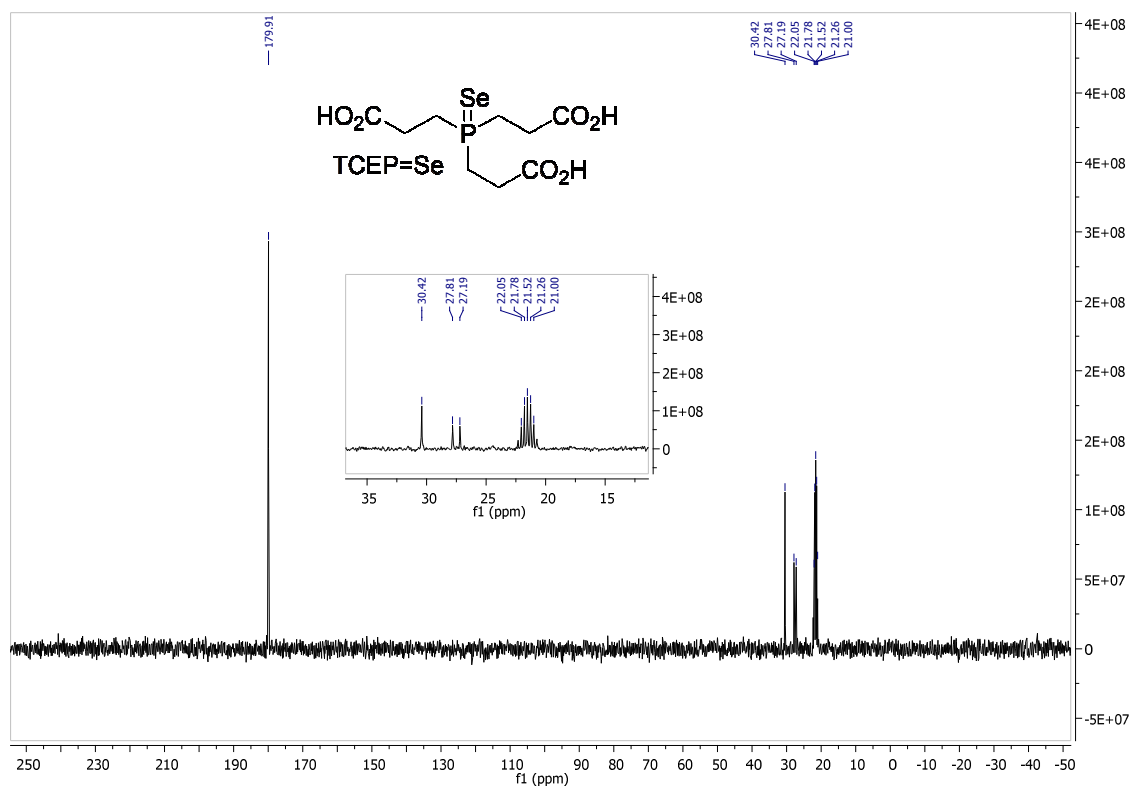


Figure S4. <sup>13</sup>C NMR spectrum (75 MHz) of TCEP=Se.

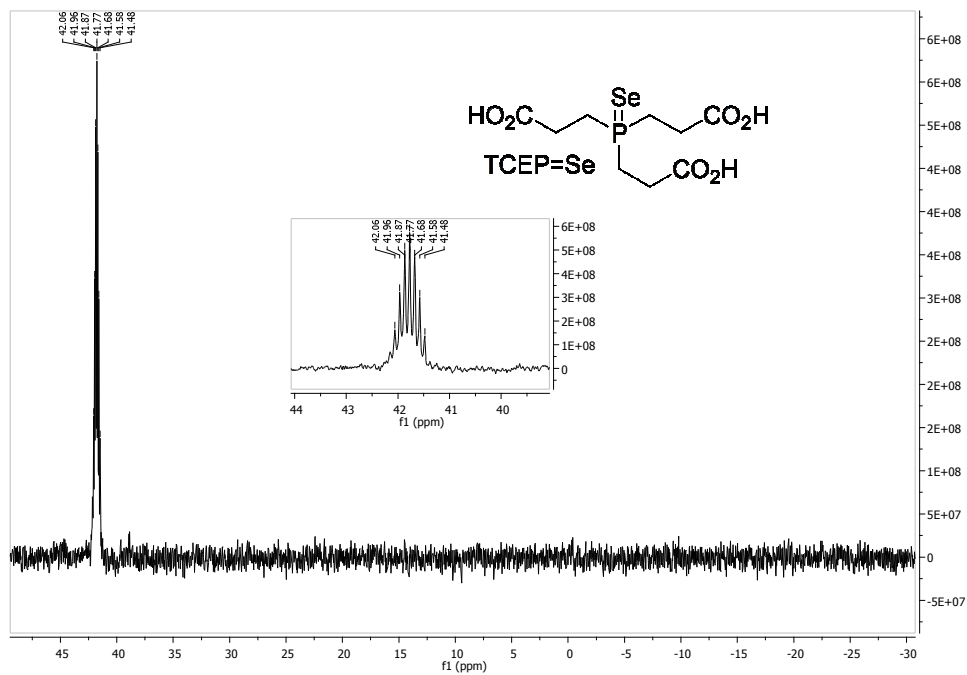


Figure S5. <sup>31</sup>P NMR spectrum (121.4 MHz) of TCEP=Se (<sup>1</sup>H coupled).

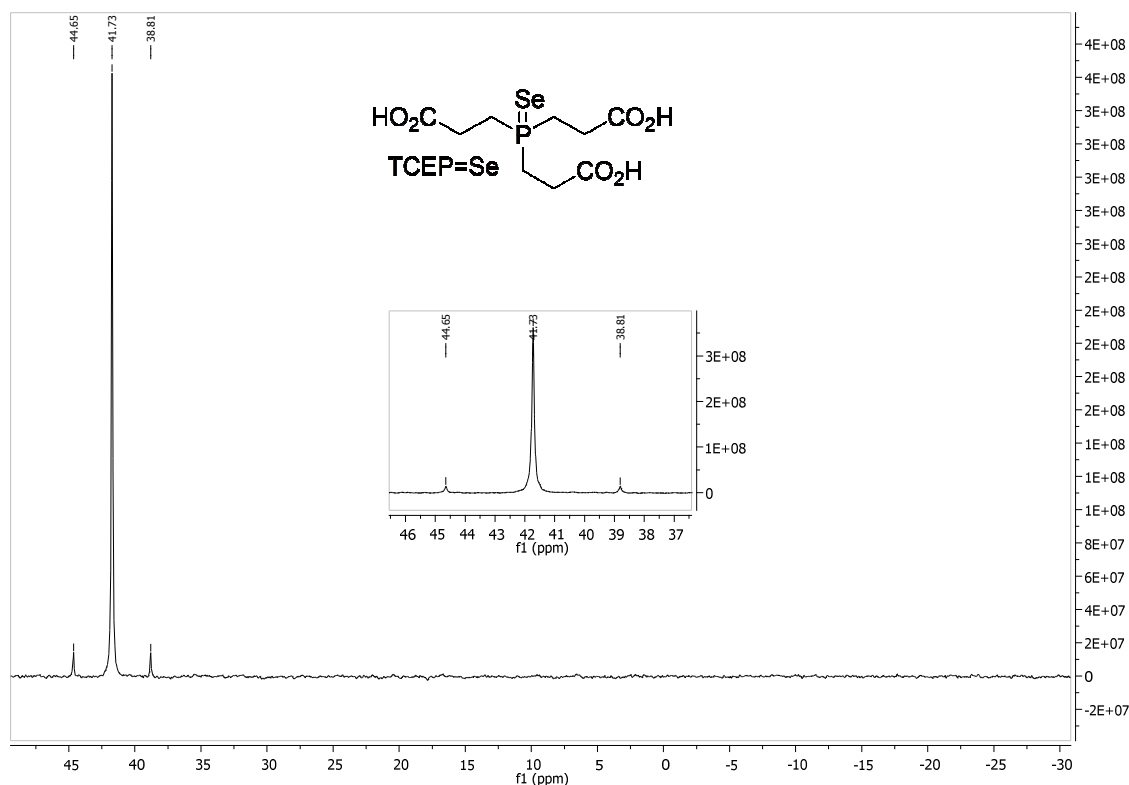


Figure S6.  $^{31}\text{P}$  NMR spectrum (121.4 MHz) of TCEP=Se ( $^1\text{H}$  uncoupled).

### 3. Synthesis of selenopeptides 3a-h

The general procedure is illustrated with the synthesis of peptide 3d (*H-ILKEPVHGRU-OH*)<sub>2</sub>

Peptide **1d** (12.24 mg, 7.55  $\mu\text{mol}$ , 7 mM final concentration) and selenocystine (5.05 mg, 15.1  $\mu\text{mol}$ , 2eq) were dissolved together in a solution of 200 mM MPAA, 200 mM DTT and 6 M Gdn.HCl in 0.1 M phosphate buffer (pH 7). The pH was adjusted to 5.5 by addition of 6 N NaOH (final volume 1.08 mL). The reaction mixture was stirred overnight under nitrogen atmosphere.

The reaction mixture was then diluted with aqueous AcOH (500  $\mu\text{L}$  in 6 mL water) and extracted three times with diethylether, filtered and purified on preparative RP-HPLC (rt, 215 nm, column C18 XBridge autoprep, buffer A = water containing 0.05% TFA, buffer B=acetonitrile in water 4/1 containing 0.05% TFA, gradient 0 to 5% B in 1 min, then 5 to 25% B in 25 min, flow 25 mL/min) to give 8.5 mg of diselenide **3d** (yield = 69 %).

#### *Synthesis of peptide 3a (H-ILKEPVHGFU-OH)*<sub>2</sub>

Starting from peptide **1a** (X = Phe, 7.8 mg, 5.2  $\mu\text{mol}$ ), the same protocol furnished 7.61 mg (97 %) of diselenide **3a** (gradient 0 to 15% B in 1 min, then 15 à 35% B in 25 min).

D-Phe content (0.34 %) was determined by chiral GC-MS analysis after acid hydrolysis (CAT, Germany) as described elsewhere.<sup>2</sup>

<sup>2</sup> 1. W. Amelung and S. Brodowski, *Anal. Chem.*, 2002, **74**, 3239-3246.



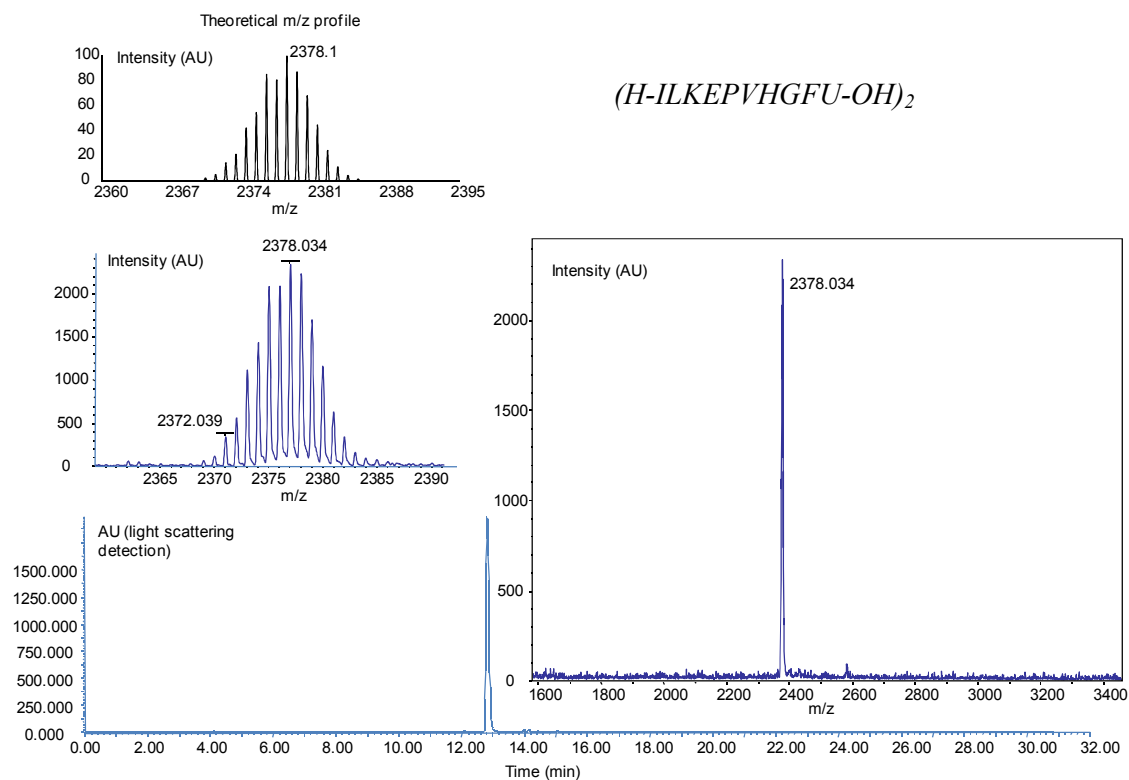


Figure S7. HPLC and MALDI-TOF analysis of compound **3a** (diselenide). Calcd. for  $[M+H]^+$  (monoisotopic resolution, most abundant isotope composition) 2378.1, found 2378.03.

**Synthesis of peptide 3b ( $H-ILKEPVHGSU-OH$ )<sub>2</sub>**

Starting from peptide **1b** (X = Ser, 16.1 mg, 11.2  $\mu$ mol), the same protocol furnished 9.5 mg (59 %) of diselenide **3b** (gradient 10 to 30% B in 25 min).

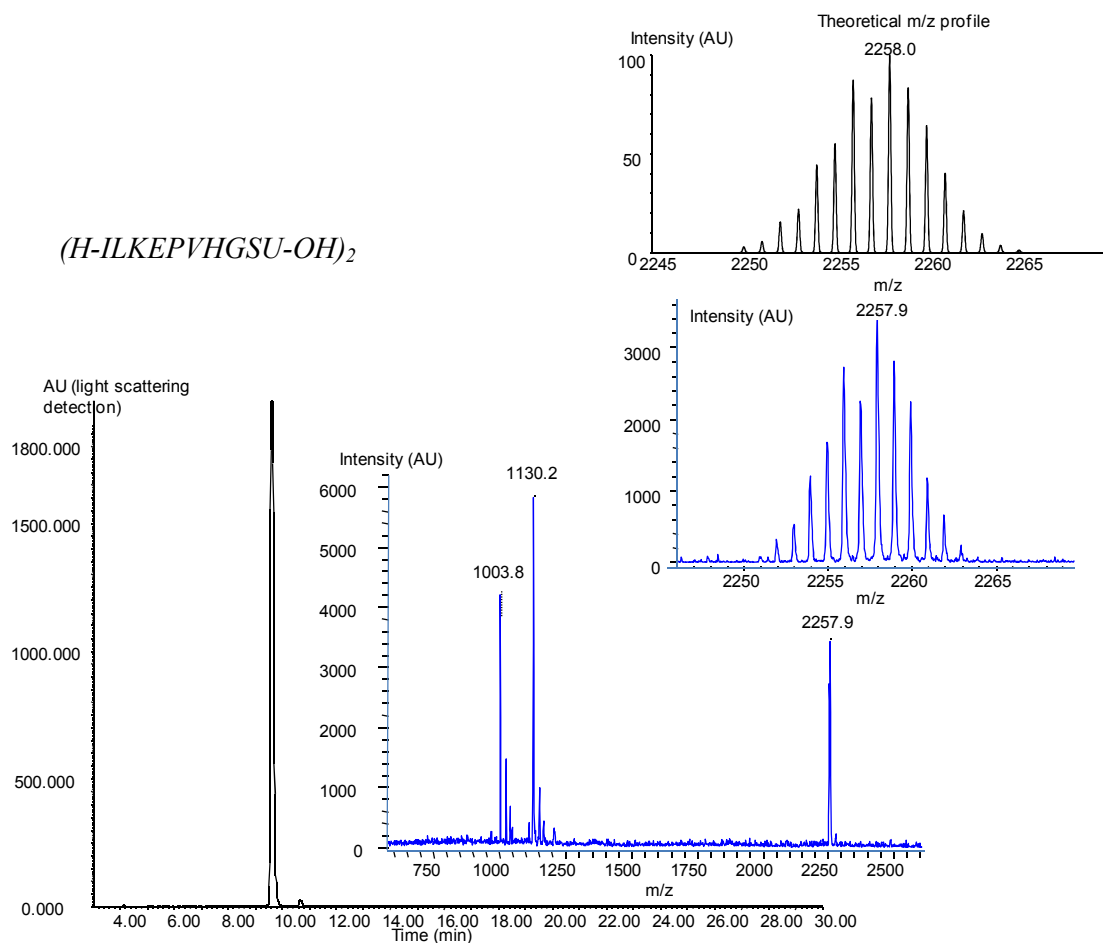


Figure S8. HPLC and MALDI-TOF analysis of compound **3b** (diselenide). Calcd. for  $[M+H]^+$  (monoisotopic resolution, most abundant isotope composition) 2258.0, found 2257.9

*Synthesis of peptide 3c (H-ILKEPVHGTU-OH)<sub>2</sub>*

Starting from peptide **1c** (X = Thr, 13.4 mg, 9.2  $\mu$ mol), the same protocol furnished 6.9 mg (51 %) of diselenide **3c** (gradient 10 to 30% B in 25 min).

*(H-ILKEPVHGTU-OH)<sub>2</sub>*

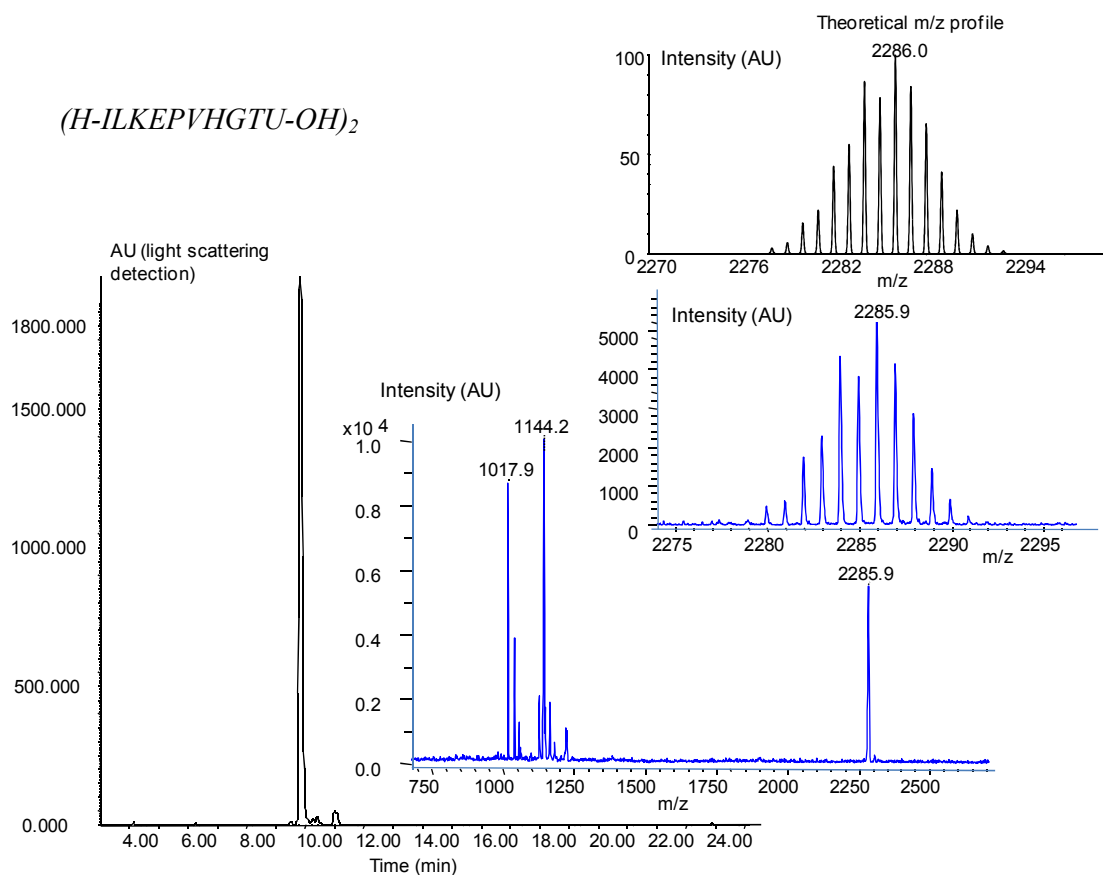


Figure S9. HPLC and MALDI-TOF analysis of compound **3c** (diselenide). Calcd. for  $[M+H]^+$  (monoisotopic resolution, most abundant isotope composition) 2286.0, found 2285.9.

### *Synthesis of peptide 3d (H-ILKEPVHGRU-OH)<sub>2</sub>*

D-Arg content (0.19 %) was determined by chiral GC-MS analysis after acid hydrolysis (CAT, Germany) as described elsewhere.<sup>3</sup>

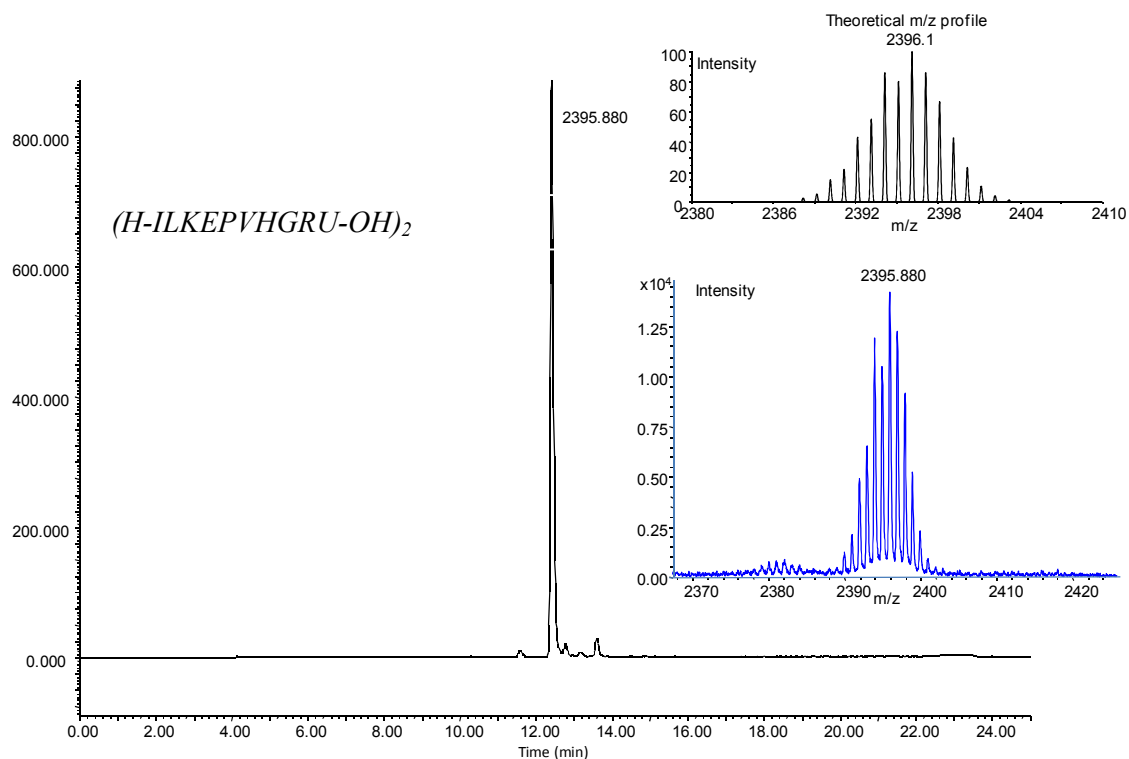


Figure S10. HPLC and MALDI-TOF analysis of compound **3d** (diselenide). Calcd. for  $[M+H]^+$  (monoisotopic resolution, most abundant isotope composition) 2396.1, found 2395.88.

#### *Synthesis of peptide 3e (H-ILKEPVHGAU-OH)<sub>2</sub>*

Starting from peptide **1e** (X = Ala, 19.3 mg, 13.6  $\mu$ mol), the same protocol furnished 10.2 mg (52 %) of diselenide **3e** (gradient 0 to 5% B in 1 min, then 5 to 25% B in 25 min).

D-Ala content (0.36 %) was determined by chiral GC-MS analysis after acid hydrolysis (CAT, Germany) as described elsewhere.<sup>3</sup>

*(H-ILKEPVHGAU-OH)<sub>2</sub>*

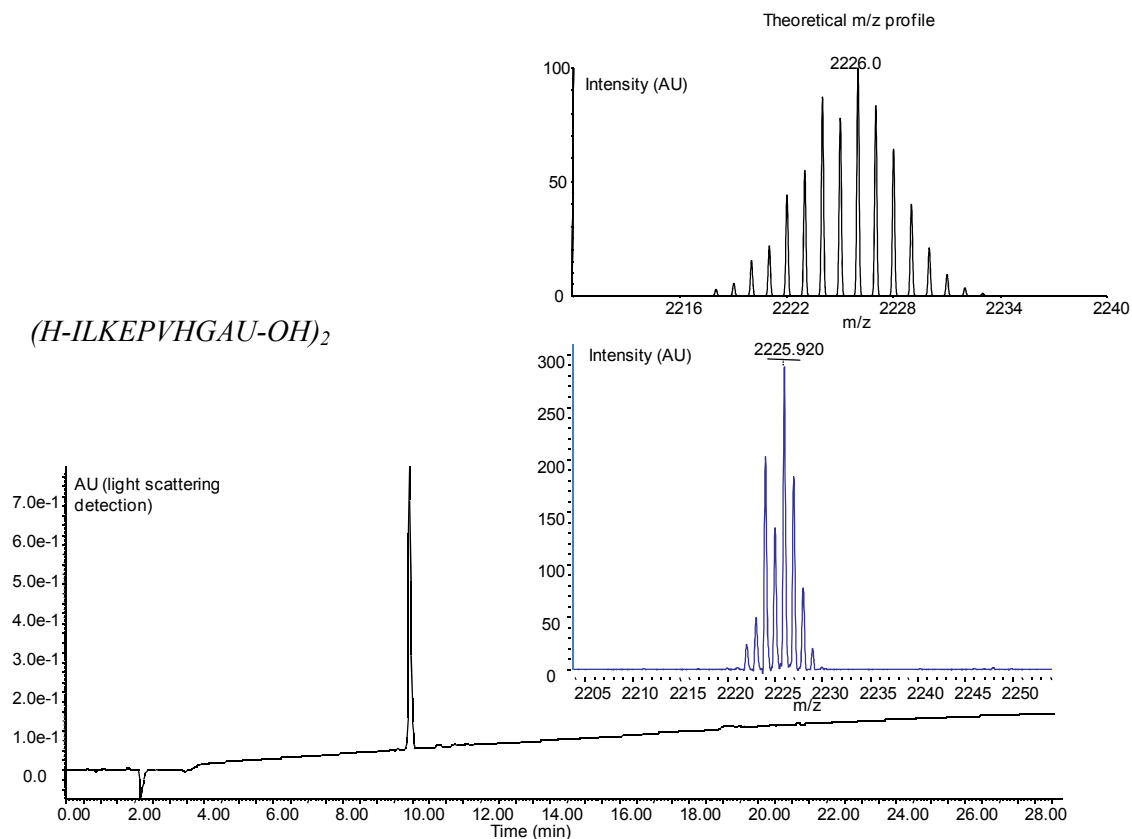


Figure S11. HPLC and MALDI-TOF analysis of compound **3e** (diselenide). Calcd. for  $[M+H]^+$  (monoisotopic resolution, most abundant isotope composition) 2226.0, found 2225.92

#### *Synthesis of peptide 3f (H-ILKEPVHGEU-OH)<sub>2</sub>*

Starting from peptide **1f** (X = Glu, 4.3 mg, 2.9  $\mu$ mol), the same protocol furnished 3.7 mg (86 %) of diselenide **3f** (gradient 0 to 5% B in 1 min, then 5 to 25% B in 25 min).

D-Glu content (0.95 %) was determined by chiral GC-MS analysis after acid hydrolysis (CAT, Germany) as described elsewhere.<sup>3</sup>

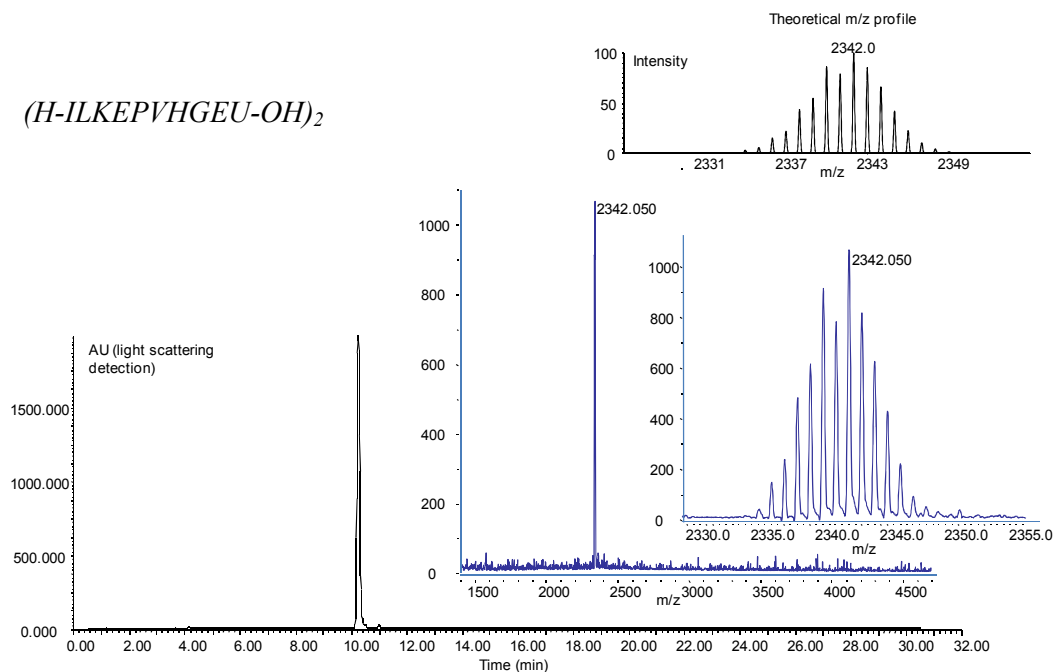


Figure S12. HPLC and MALDI-TOF analysis of compound **3f** (diselenide). Calcd. for  $[M+H]^+$  (monoisotopic resolution, most abundant isotope composition) 2342.0, found 2342.05.

*Synthesis of peptide 3g (H-ILKEPVHGCU-OH)<sub>2</sub>*

Starting from peptide **1g** (X = Cys(*S*tBu), 8.9 mg, 5.8  $\mu$ mol), the same protocol furnished 4.7 mg (52 %) of diselenide **3g** (gradient 10 to 30% B in 25 min).

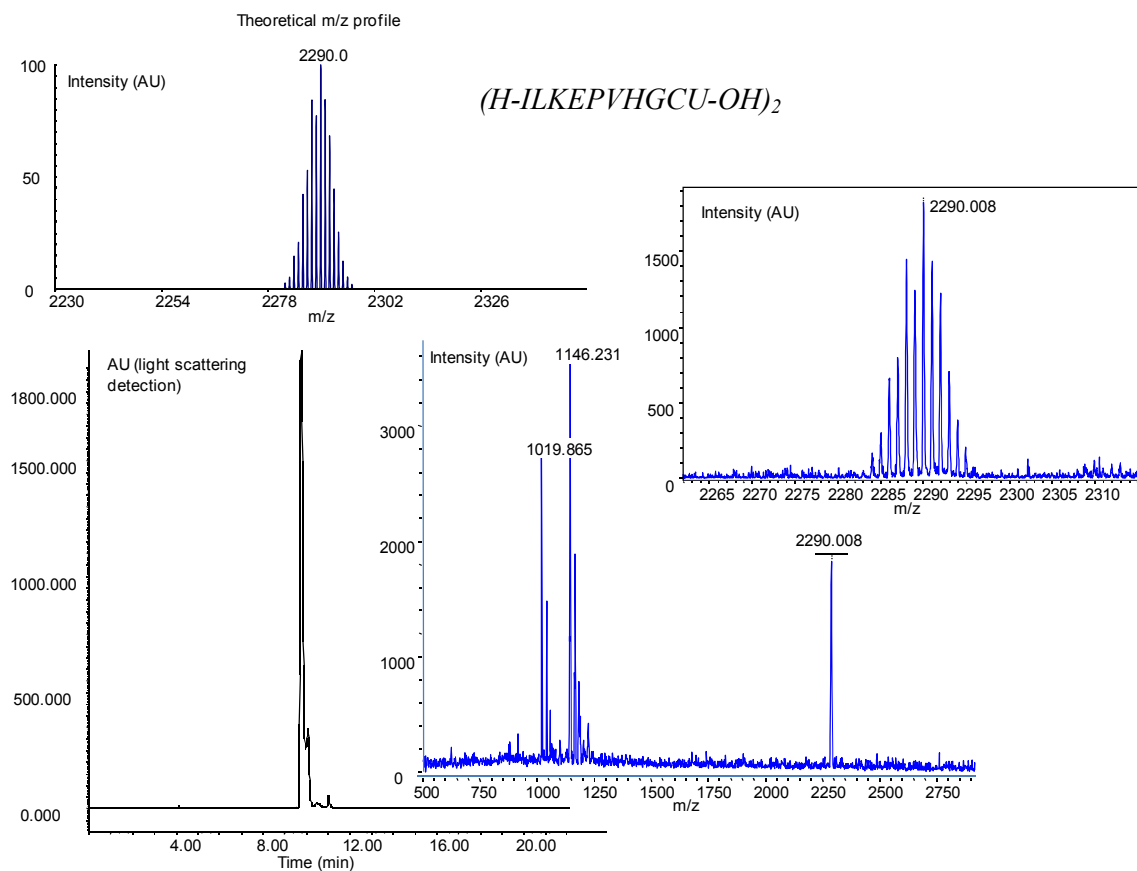


Figure S13. HPLC and MALDI-TOF analysis of compound **3g** (diselenide). Calcd. for  $[M+H]^+$  (monoisotopic resolution, most abundant isotope composition) 2290.0, found 2290.0.

### Synthesis of peptide **3h** (*H-ILKEPVHGGU-OH*)<sub>2</sub>

Starting from peptide **1h** (X = Gly, 17.3 mg, 12.3  $\mu$ mol), the same protocol furnished 9.8 mg (56 %) of diselenide **3h** (gradient 10 to 30% B in 25 min).

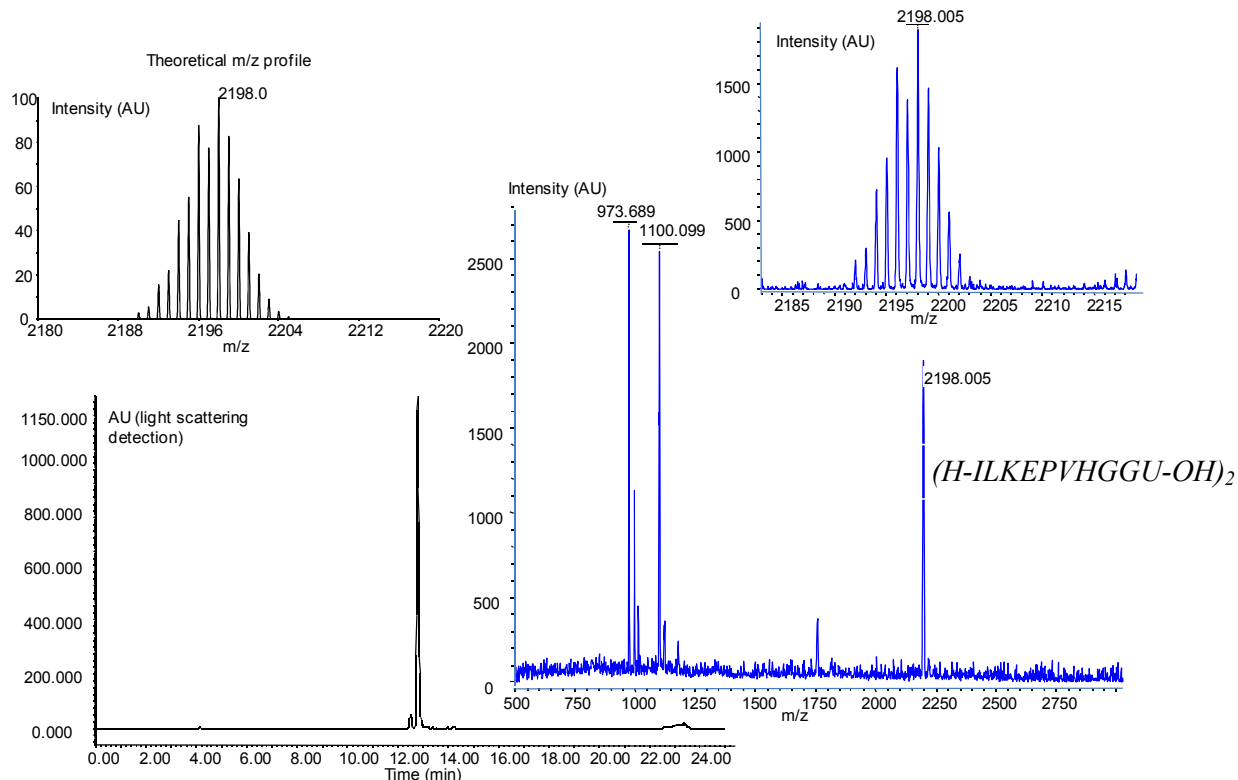


Figure S14. HPLC and MALDI-TOF analysis of compound **3h** (diselenide). Calcd. for  $[M+H]^+$  (monoisotopic resolution, most abundant isotope composition) 2198.0, found 2198.0.

### Synthesis of peptides **5d,e**

#### Synthesis of peptide (*H-UILKEPVRGA-NH*)<sub>2</sub>

Peptide elongation was performed on NovaSyn TGR resin (667 mg, 0.1 mmol, 0.2 mmol/g) using standard Fmoc/*tert*-butyl chemistry on a peptide synthesizer. Couplings were performed using 4-fold molar excess of each Fmoc L-amino acid, 3.6-fold molar excess of HBTU, and 8-fold molar excess of DIEA. A capping step was performed after each coupling with Ac<sub>2</sub>O/DIEA. At the end of the synthesis, the resin was washed with DMF.

Coupling of Fmoc-Sec(*p*MeOBzl)-OH (127.61 mg, 0.25 mmol, 2.5 eq) was performed using DIC (39.1  $\mu$ L, 0.25 mmol, 2.5 eq) and HOBT (33.78 mg, 0.25 mmol, 2.5 eq) during 2 h in DMF. The resin was washed with DMF and Fmoc protecting group was removed using 20% piperidine in DMF. The resin was washed with DMF, CH<sub>2</sub>Cl<sub>2</sub> and Et<sub>2</sub>O.

Final deprotection and cleavage from the solid support were performed using TFA/thioanisole/EDT/water: 92.5/2.5/2.5/2.5 by vol for 1 h 30 (10 mL). The crude peptide was precipitated in cold diethyl ether/ *n*-heptane: 1/1 by vol., solubilized in deionised water and lyophilized to give 171 mg of crude diselenide peptide (62.9 %).

Purification was carried out by preparative RP-HPLC (rt, 215 nm, column C18 XBridge autoprep, buffer A = water containing 0.05% TFA, buffer B=acetonitrile/water: 4/1 by vol containing 0.05% TFA,

gradient 10 to 30% B in 25 min, flow 25 mL/min) afforded 66.7 mg of diselenide peptide (H-UILKEPVRGA-NH<sub>2</sub>)<sub>2</sub> (24.5 %). LC-MS M calcd. (mean mass) 2260.49; found 2261.40±0.16.

#### Ligation with peptide **1e**

Peptide **1e** (X = Ala) (29.03 mg, 20.4 μmol, 7 mM final concentration) and diselenide peptide (H-UILKEPVRGA-NH<sub>2</sub>)<sub>2</sub> (27.72 mg, 10.2 μmol, 0.5 eq.) were dissolved in a solution of 200 mM MPAA, 100 mM DTT and 6 M Gdn.HCl in 0.1 M phosphate buffer (pH 7, 2.91 mL). The pH was adjusted to 5.55 after using 6 N NaOH. The reaction was stirred 4 days at 37°C under nitrogen atmosphere.

The reaction mixture was then diluted with water (6 mL), acidified with AcOH (500 μL), extracted with diethylether, and purified by preparative RP-HPLC (rt, 215 nm, column C18 XBridge autoprep, buffer A = water containing 0.05% TFA, buffer B=acetonitrile/water: 4/1 by vol containing 0.05% TFA, gradient 10 to 30% B in 25 min, flow 25 mL/min) to afford 21.6 mg of diselenide **5e** (40%).

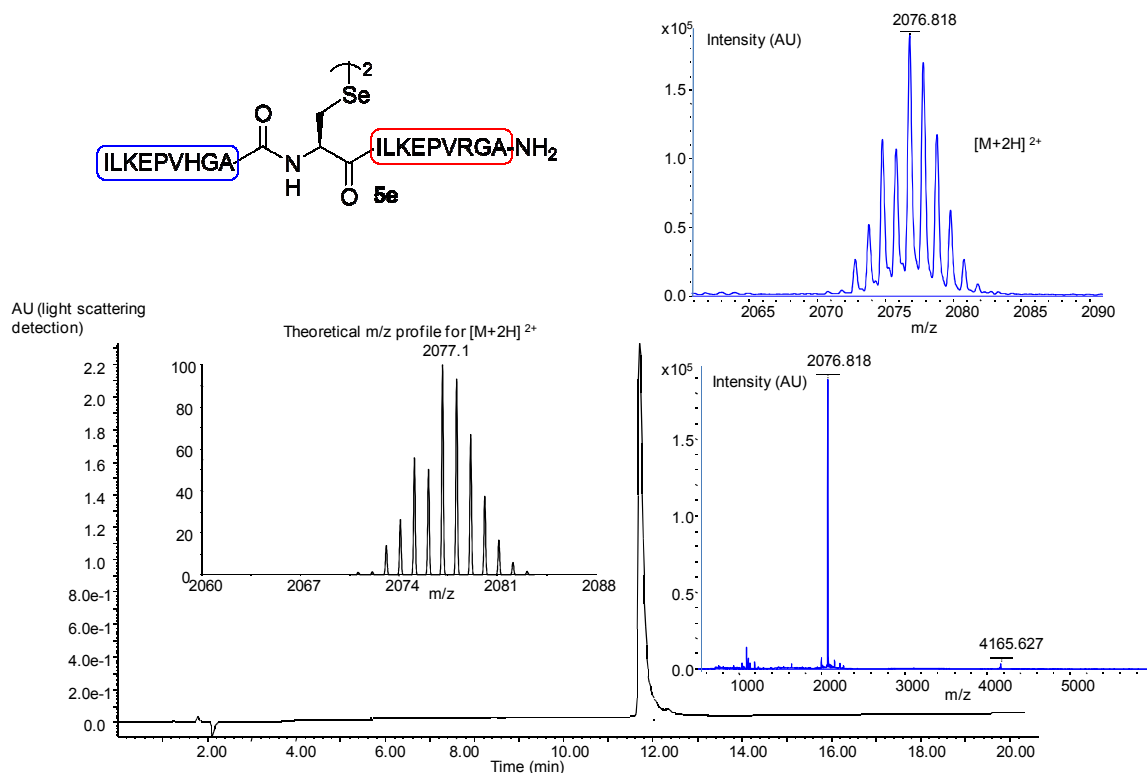


Figure S15. HPLC and MALDI-TOF analysis of compound **5e** (diselenide). Calcd. for [M+H]<sup>+</sup> (selenol, monoisotopic resolution, most abundant isotopic composition) 2077.1, found 2076.8.

Starting from peptide **1d** (X = Arg, 7.2 mg, 4.44 μmol) and diselenide peptide (H-UILKEPVRGA-NH<sub>2</sub>)<sub>2</sub> (6.53 mg, 2.22 μmol, 0.5 eq.), the same protocol furnished 7.9 mg (62.5%) of diselenide **5d** after purification by preparative RP-HPLC (rt, 215 nm, column C18 XBridge, buffer A = water containing 0.1% TFA, buffer B=acetonitrile/water: 4/1 by vol containing 0.1% TFA, gradient 10 to 30% B in 60 min, flow 6 mL/min).



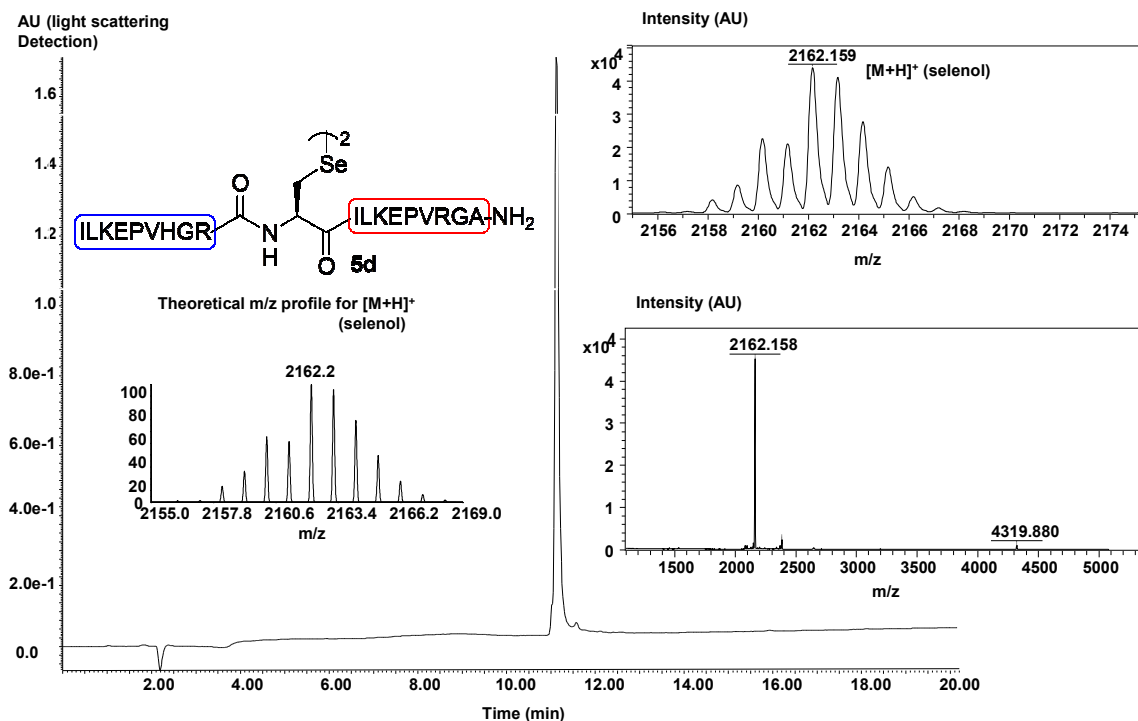


Figure S16. HPLC and MALDI-TOF analysis of compound **5d** (diselenide). Calcd. for  $[M+H]^+$  (detected as the selenol, monoisotopic resolution, most abundant isotopic composition) 2162.2, found 2161.99.

#### 4. Study of the transamidation reaction

##### *Typical experimental procedure*

This procedure was used for the determination of the kinetic rates or for the isolation of peptides **7a**, **7d**, **7e**, **7f**. For this the reaction mixtures were analyzed by LC-MS using light scattering detection.

The typical experimental procedure is illustrated with the synthesis of peptide **7e**

The diselenide **3e** (7.96 mg, 2.74  $\mu\text{mol}$ , 3.5 mM final concentration) and Cys peptide **6** (7.86 mg, 5.48  $\mu\text{mol}$ , 2 eq) were dissolved in a solution of 200 mM MPAA, 70 mM TCEP and 210 mM TCEP=Se in 0.1 M phosphate buffer (pH 7, 783  $\mu\text{L}$ ). The pH was adjusted to 5.53 by addition of 6 N NaOH. The mixture was stirred at 37°C under nitrogen atmosphere.

The reaction mixture was diluted with aqueous acetic acid (500  $\mu\text{L}$  AcOH in 8 mL water) to precipitate MPAA. The mixture was extracted with diethylether three times to remove MPAA, filtered and then purified by preparative RP-HPLC (rt, 215 nm, column C18 XBridge autoprep, buffer A = water containing 0.05% TFA, buffer B=acetonitrile/water: 4/1 containing 0.05% TFA, gradient 15 to 30% B in 20 min, flow 25 mL/min) to afford 6.2 mg (43.4 %) of peptide H-ILKEPVHGACILKEPVHGV-NH<sub>2</sub> **7e**

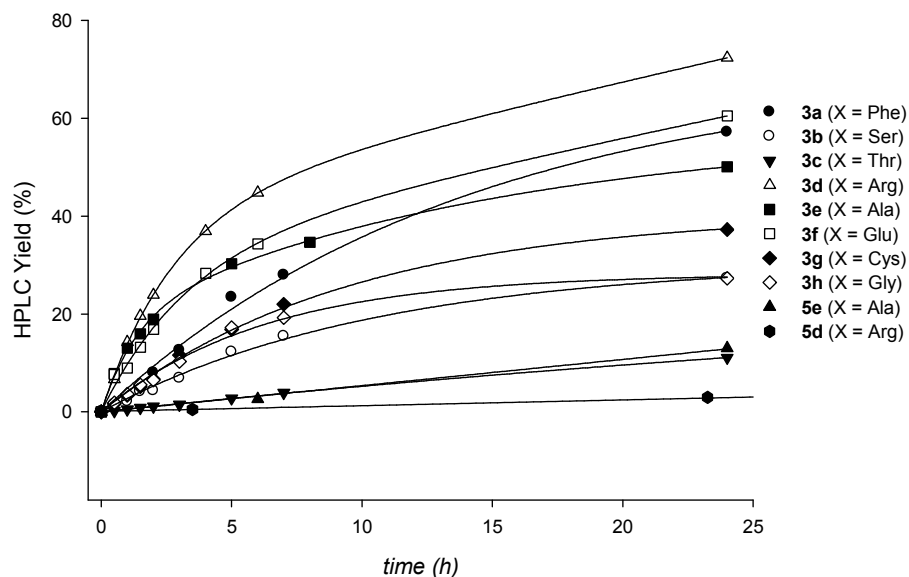


Figure S17. Kinetic profiles for the transamidation of selenopeptides **4a-h** and **5d,e** by Cys peptide **6**.

#### *Formation of peptide 7a by transamidation (3a + 6 → 7a)*

Peptide **7a** was found to be identical by HPLC and MALDI-TOF to a reference peptide synthesized by standard Fmoc SPPS (data not shown, available on request).

D-Phe content (4.5 %) was determined by chiral GC-MS analysis after acid hydrolysis (CAT, Germany) as described elsewhere.<sup>3</sup>

<sup>3</sup> I. W. Amelung and S. Brodowski, *Anal. Chem.*, 2002, **74**, 3239-3246.

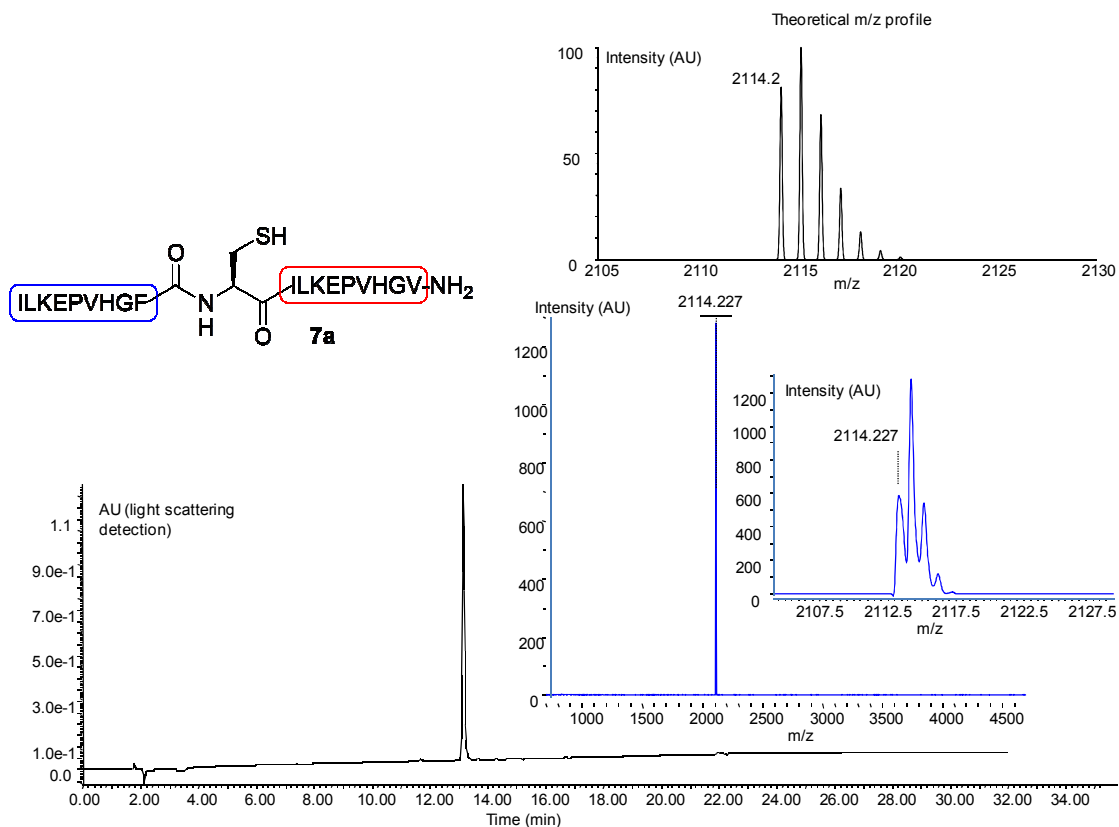


Figure S18. HPLC and MALDI-TOF analysis of peptide **7a**. Calcd. for [M+H]<sup>+</sup> (monoisotopic) 2114.2, found 2114.2.

### Formation of peptide **7d** by transamidation (**3d** + **6** → **7d**)

Peptide **7d** was found to be identical by HPLC and MALDI-TOF to a reference peptide synthesized by standard Fmoc SPPS (data not shown, available on request).

D-Arg content (1.1 %) was determined by chiral GC-MS analysis after acid hydrolysis (CAT, Germany) as described elsewhere.<sup>3</sup>

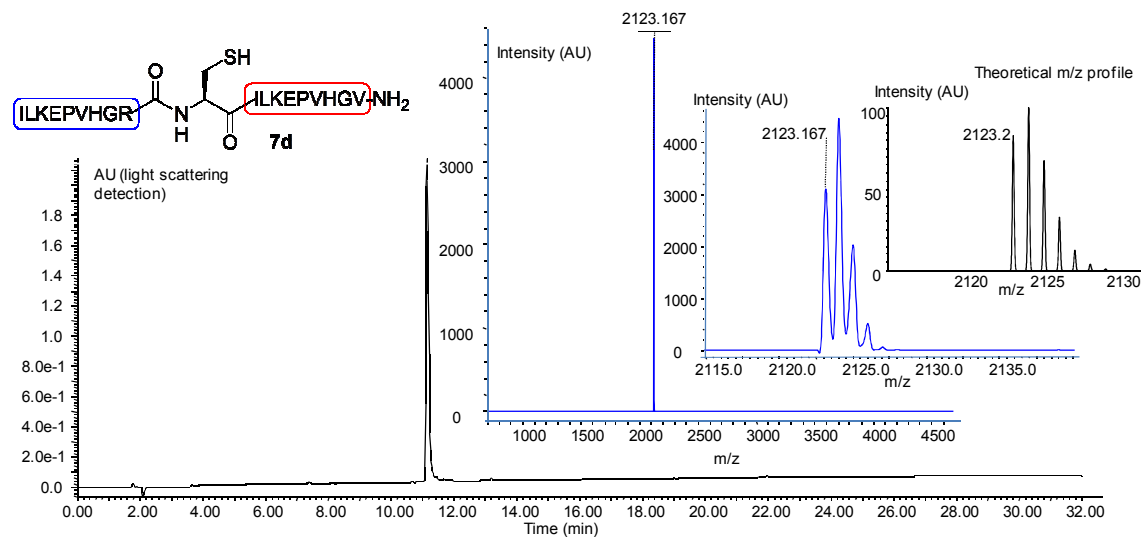


Figure S19. HPLC and MALDI-TOF analysis of peptide **7d**. Calcd. for [M+H]<sup>+</sup> (monoisotopic) 2123.2, found 2123.17.

### Formation of peptide 7e by transamidation (3e + 6 → 7e)

Peptide **7e** was found to be identical by HPLC and MALDI-TOF to a reference peptide synthesized by standard Fmoc SPPS (data not shown, available on request).

D-Ala content (2.2 %) was determined by chiral GC-MS analysis after acid hydrolysis (CAT, Germany) as described elsewhere.<sup>3</sup>

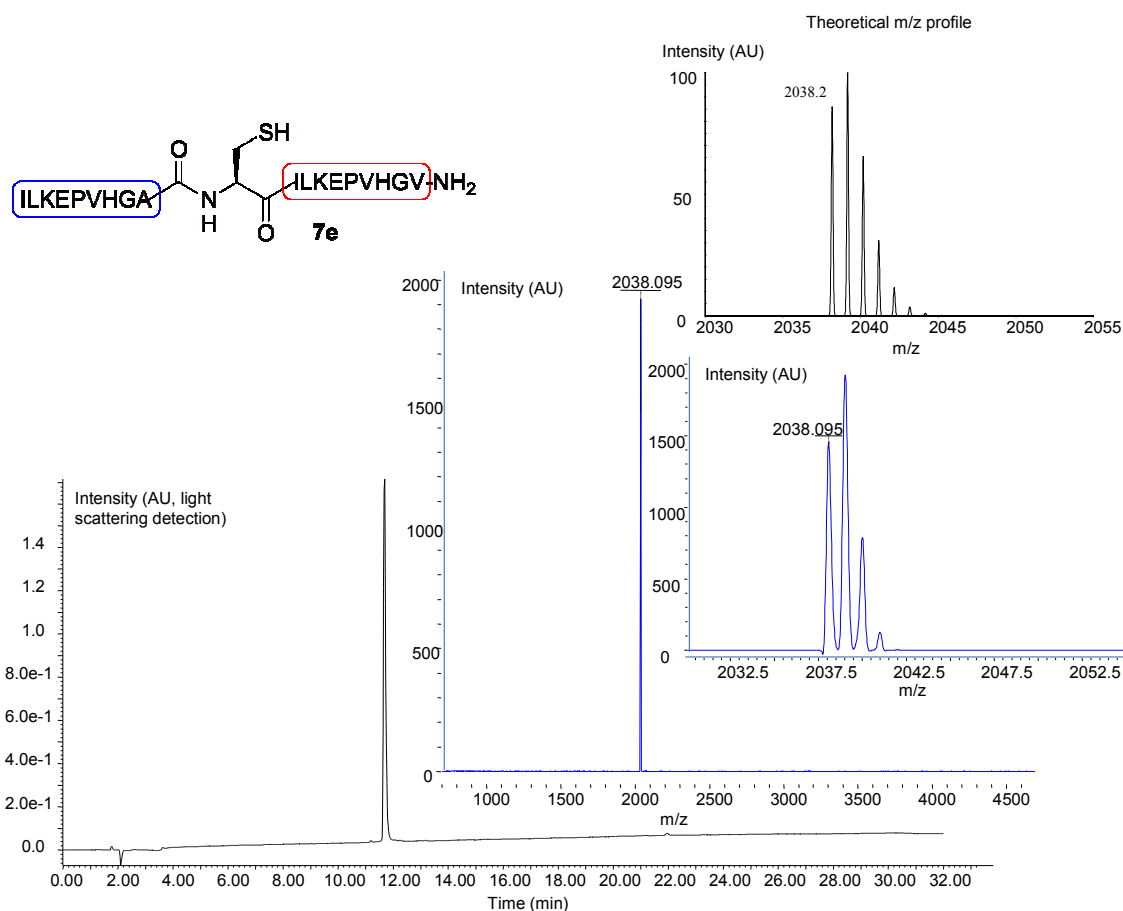


Figure S20. HPLC and MALDI-TOF analysis of peptide **7e**. Calcd. for  $[M+H]^+$  (monoisotopic) 2038.2, found 2038.1.

### Formation of peptide 7f by transamidation (3f + 6 → 7f)

Peptide **7f** was found to be identical by HPLC and MALDI-TOF to a reference peptide synthesized by standard Fmoc SPPS (data not shown, available on request).

D-Glu content (1.4 %) was determined by chiral GC-MS analysis after acid hydrolysis (CAT, Germany) as described elsewhere.<sup>3</sup>

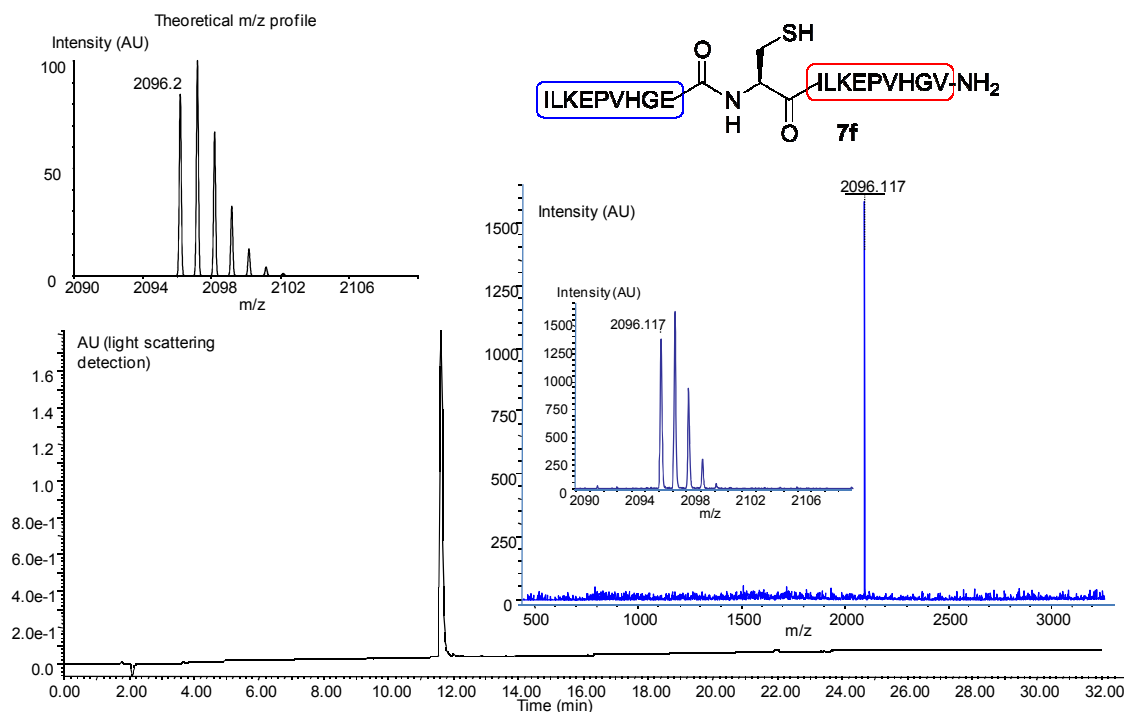


Figure S21. HPLC and MALDI-TOF analysis of peptide **7f**. Calcd. for  $[M+H]^+$  (monoisotopic) 2096.2, found 2096.12.

#### *Formation of peptide 7e by transamidation (5e + 6 → 7e)*

The same protocol with peptide **5e** (10 mg, 1.89  $\mu$ mol) furnished peptide 4.3 mg of peptide **7e** (42%).

Peptide **7e** was found to be identical by HPLC and MALDI-TOF analysis to a reference peptide synthesized by standard Fmoc SPPS (data not shown, available on request).

D-Ala content (0.86 %) was determined by chiral GC-MS analysis after acid hydrolysis (CAT, Germany) as described elsewhere.<sup>3</sup>

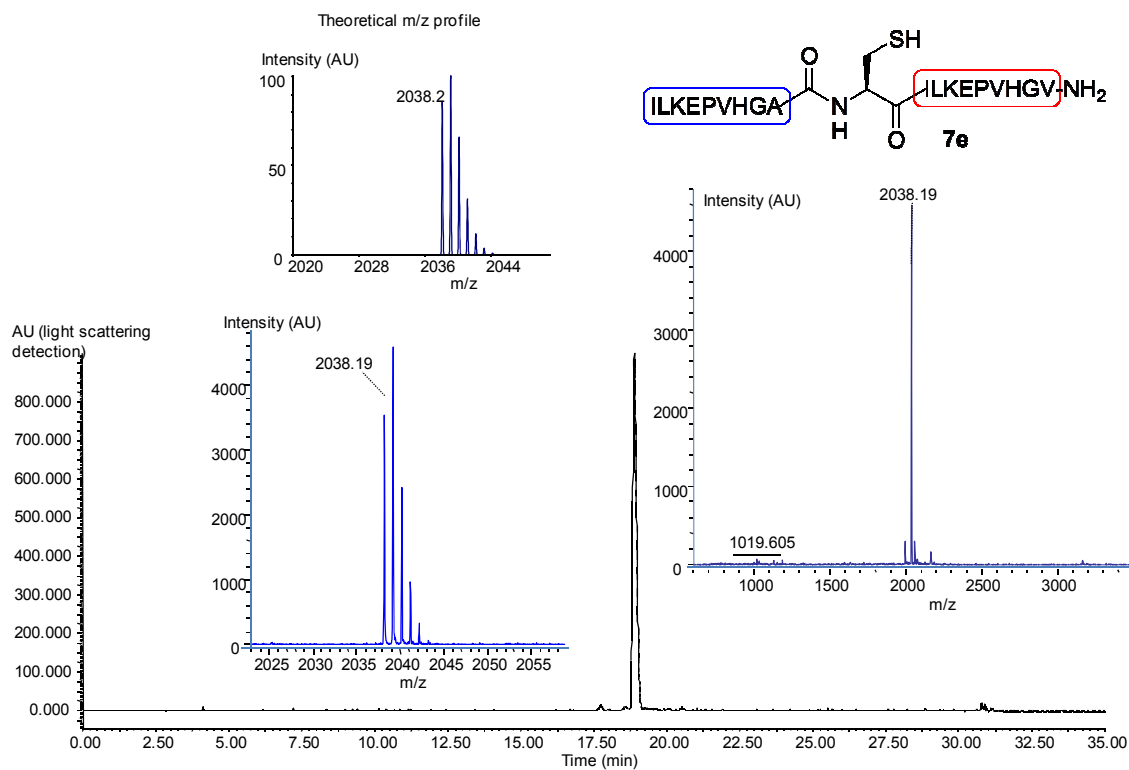


Figure S22. HPLC and MALDI-TOF analysis of peptide **7e**. Calcd. for  $[M+H]^+$  (monoisotopic) 2038.2, found 2038.19.

Peptide **7e** obtained by transamidation was also sequenced by MALDI-TOF as an additional proof of identity.

In this experiment, the presence of expected b and y ion series demonstrate the presence of the Ala-Cys bond formed in the transamidation process.

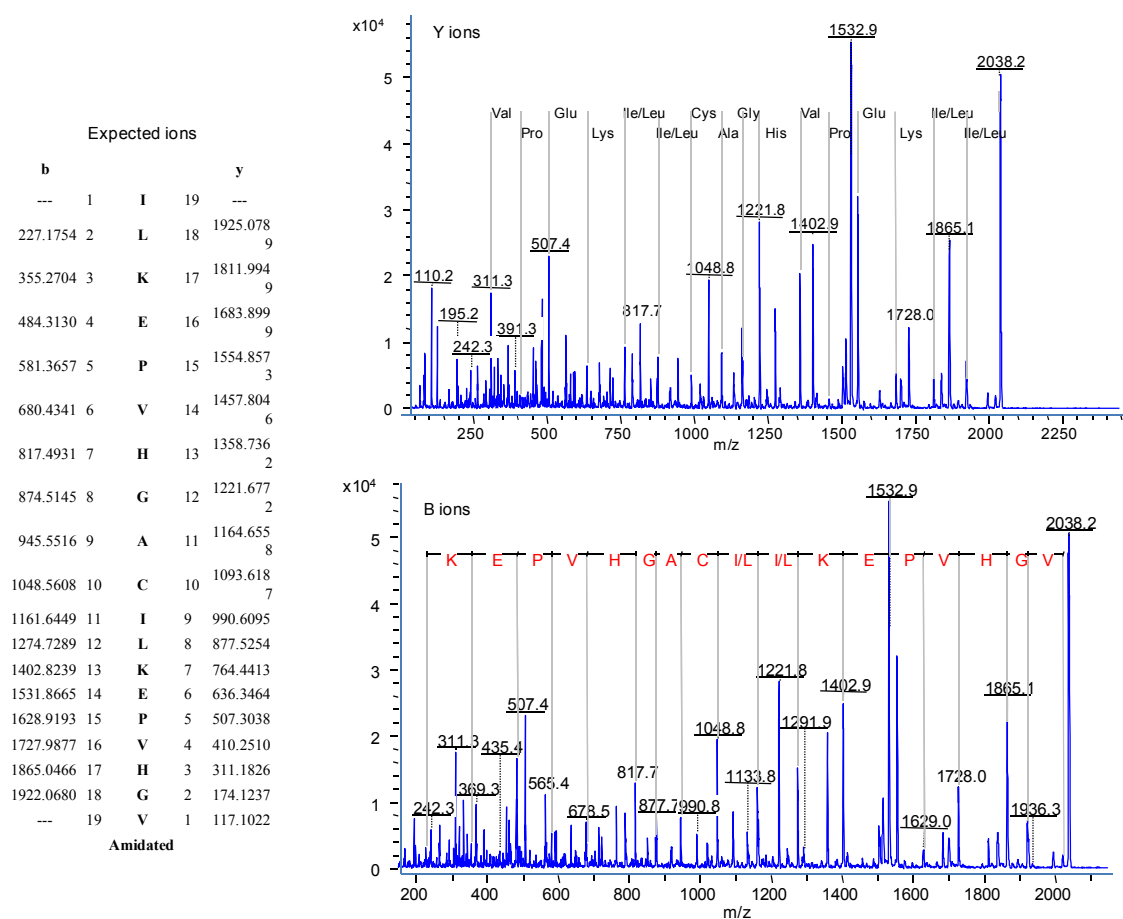
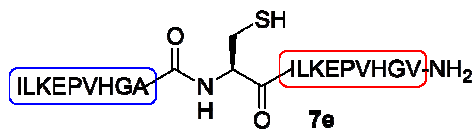


Figure S23. MALDI-TOF sequencing of peptide **7e** (b and y ion series).

## 5. Study of the metathesis reaction



### Typical experimental procedure

The reaction mixtures were analyzed by LC-MS using UV detection (215 nm).

The diselenide **5e** (2.07 mg, 0.39  $\mu\text{mol}$ , 3.9 mM final concentration) and diselenide **3d** (1.29 mg, 0.39  $\mu\text{mol}$ , 1 eq) were dissolved in a solution of 200 mM MPAA, 70 mM TCEP, 210 mM TCEP=Se and 50 mM sodium ascorbate in 0.1 M phosphate buffer (pH 7, 100  $\mu\text{L}$  total). The pH was adjusted to 5.6 by addition of 6N NaOH. The mixture was stirred at 37°C under nitrogen atmosphere.

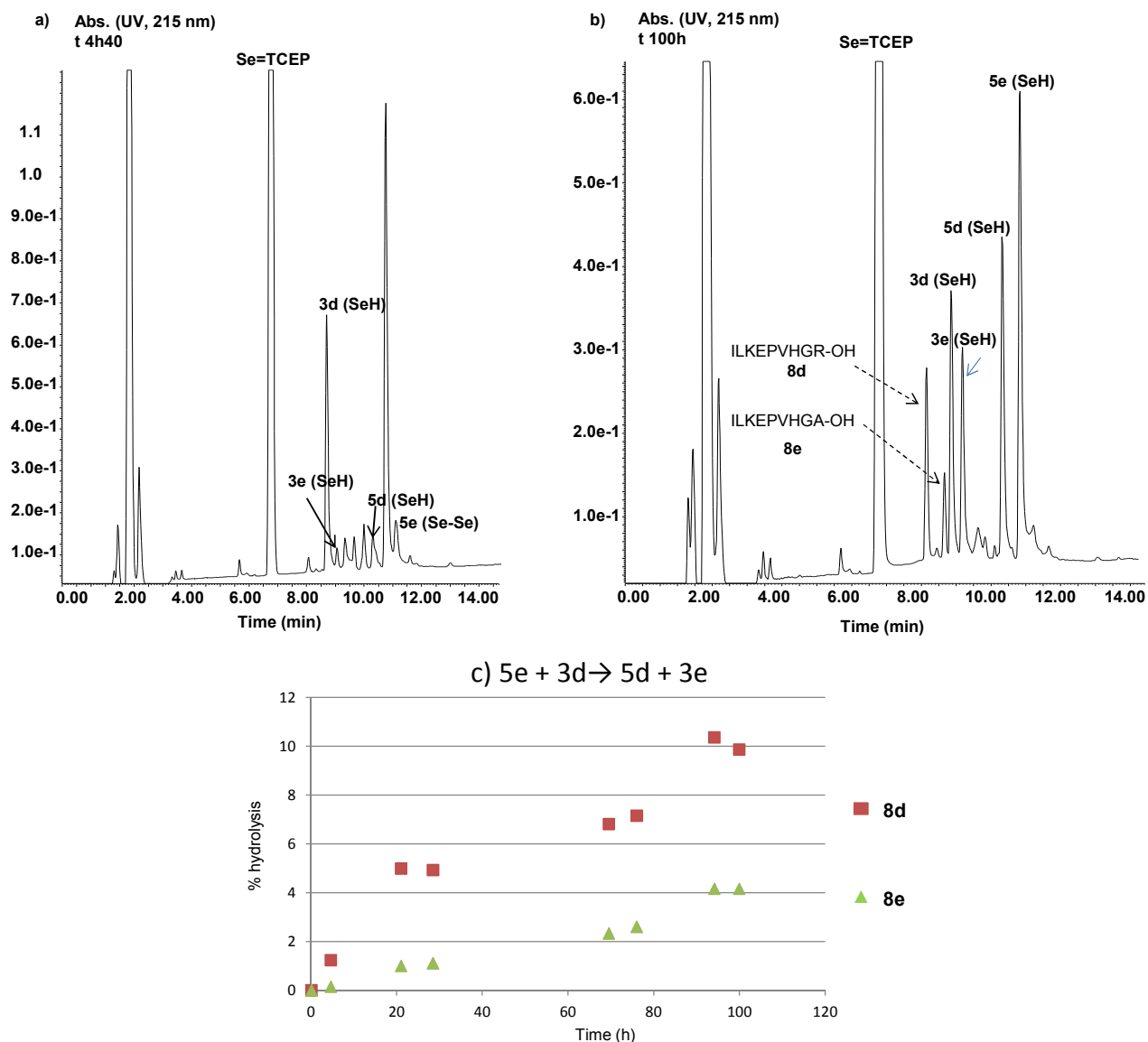


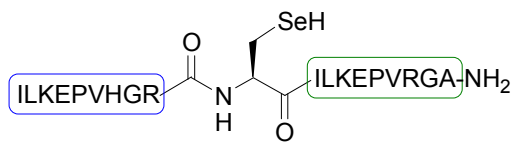
Figure S24. HPLC traces of the crude methathesis reaction between **5e** and **3d** after a) 4 h 40, b) 100 h. c) Time course of the formation of hydrolysis products **8d** and **8e**. Peptides **5d** and **3e** (selenol) were found to be identical by HPLC with peptide **5d** or **3e** obtained by peptide synthesis (diselenide) after reduction by TCEP (co-injection of the crude methathesis reaction with reduced authentic samples).

An analytical sample of peptides **5d** and **3e** (selenol form) obtained by metathesis and separated by HPLC were sequenced by MALDI-TOF as an additional proof of identity.

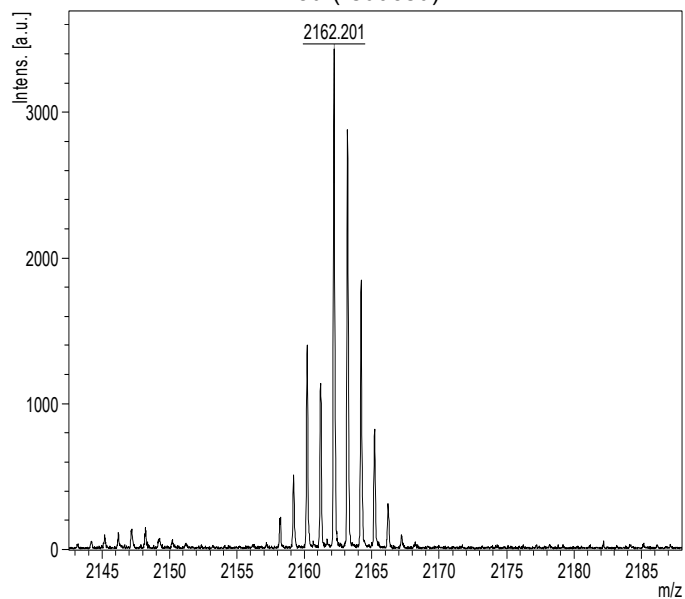
In this experiment, fragmentation of peptide **5d** showed the presence of expected b and y ion series demonstrating the presence of the Arg-SeC-Ileu bond formed in the metathesis process.

Likewise, fragmentation of peptide **3e** formed by metathesis agreed with the proposed structure.





**5d (reduced)**



Abs. Int. \* 1000

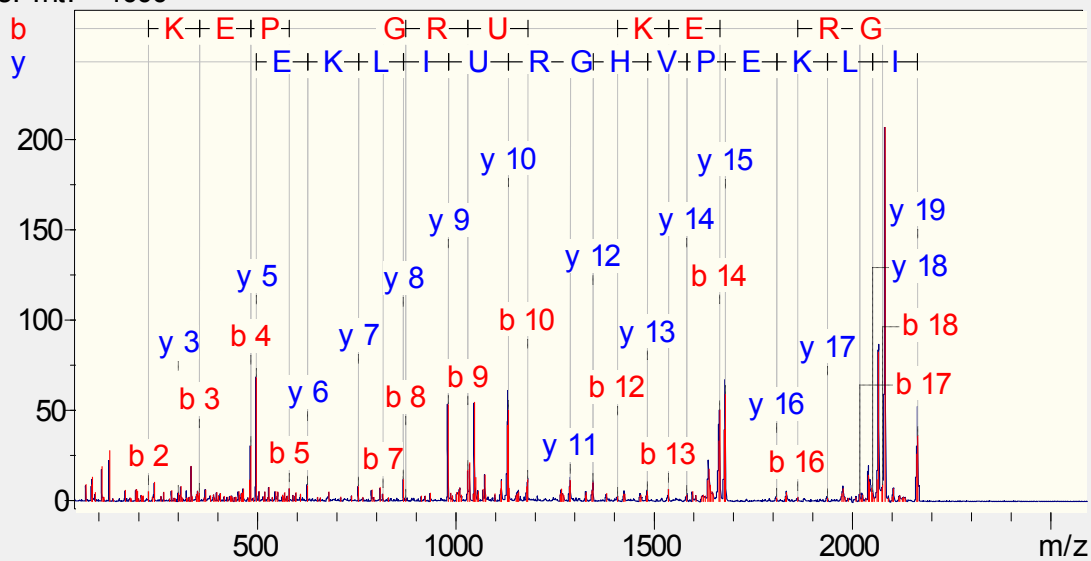


Figure S25. MALDI-TOF sequencing of peptide **5d** selenol (b and y ion series) formed during the metathesis reaction. Matrix: 2,5-dihydroxybenzoic acid.

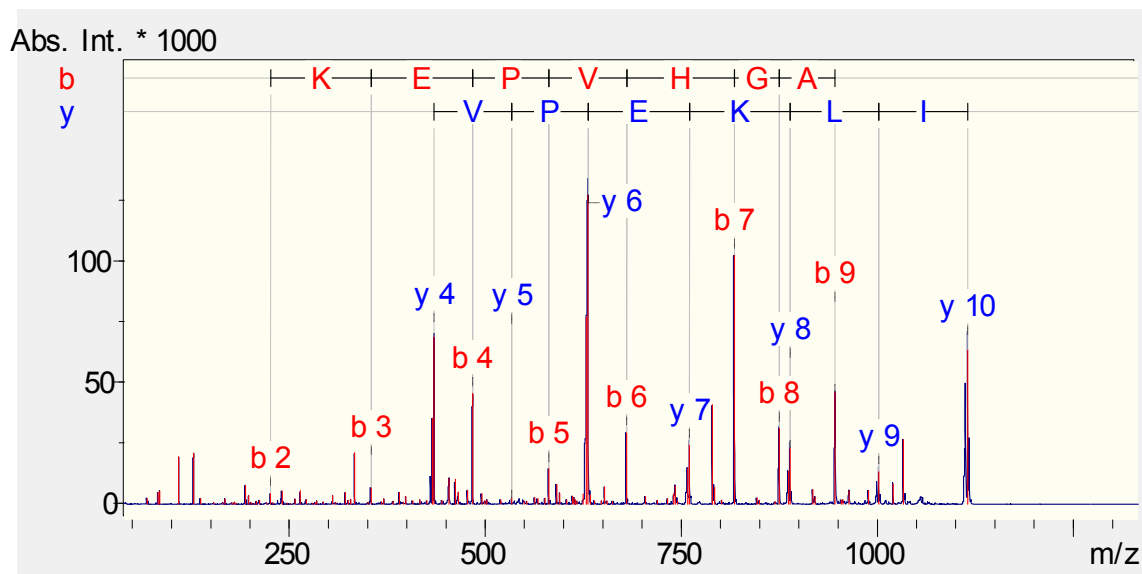
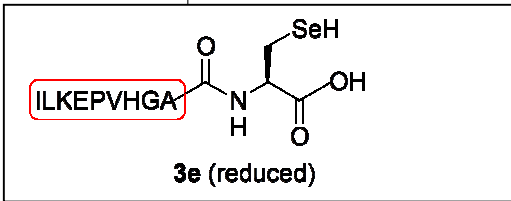
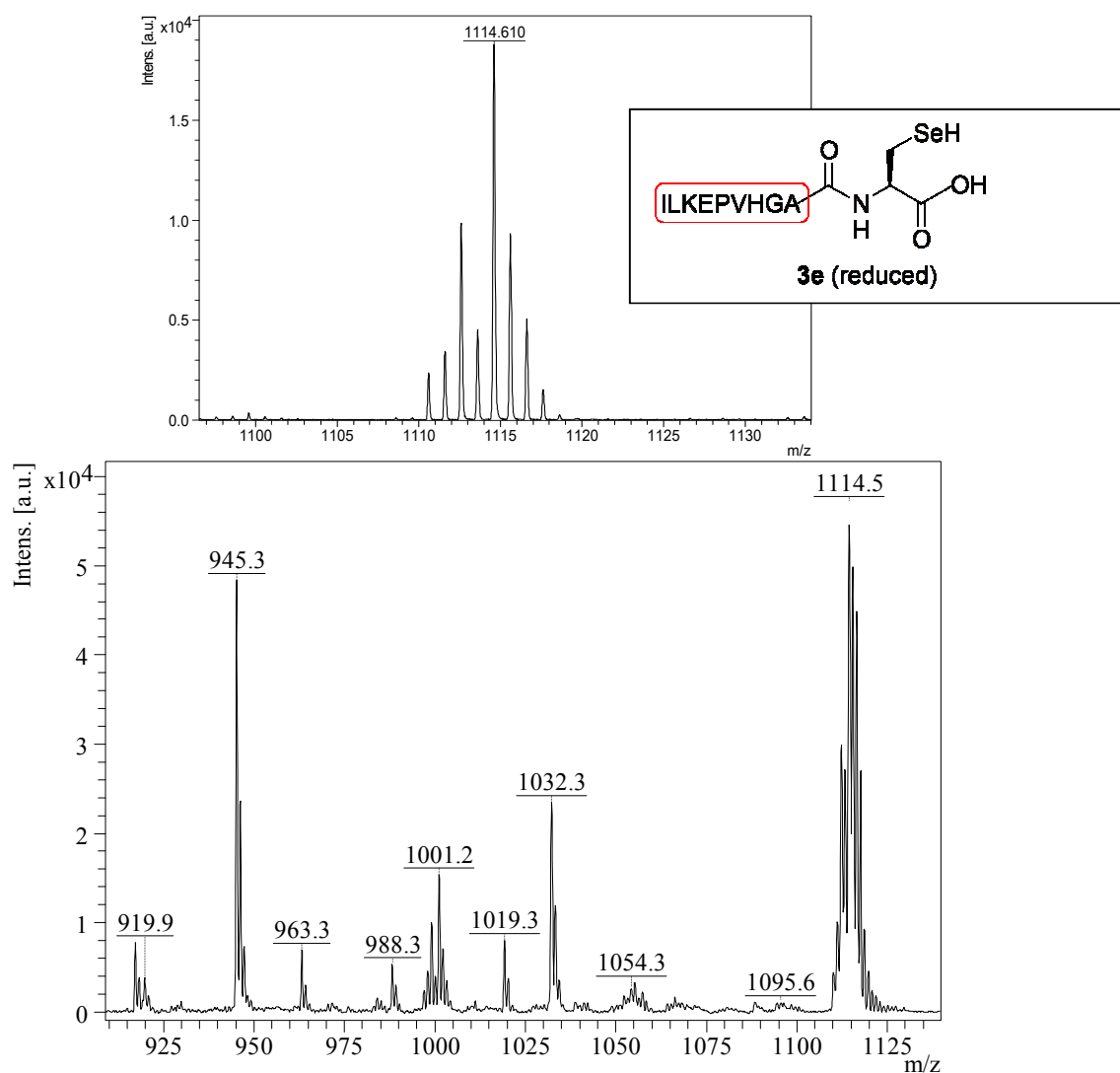


Figure S26. MALDI-TOF sequencing of peptide **3e** selenol (b and y ion series) formed during the metathesis reaction. Matrix: 2,5-dihydroxybenzoic acid.

### 5d + 3e → 5e + 3d

The same protocol with diselenide peptide **5d** (2.21 mg, 0.39 μmol, 3.9 mM final concentration) and diselenide **3e** (1.13 mg, 0.39 μmol) furnished peptides **5e** and **3d** (selenol form) by metathesis.

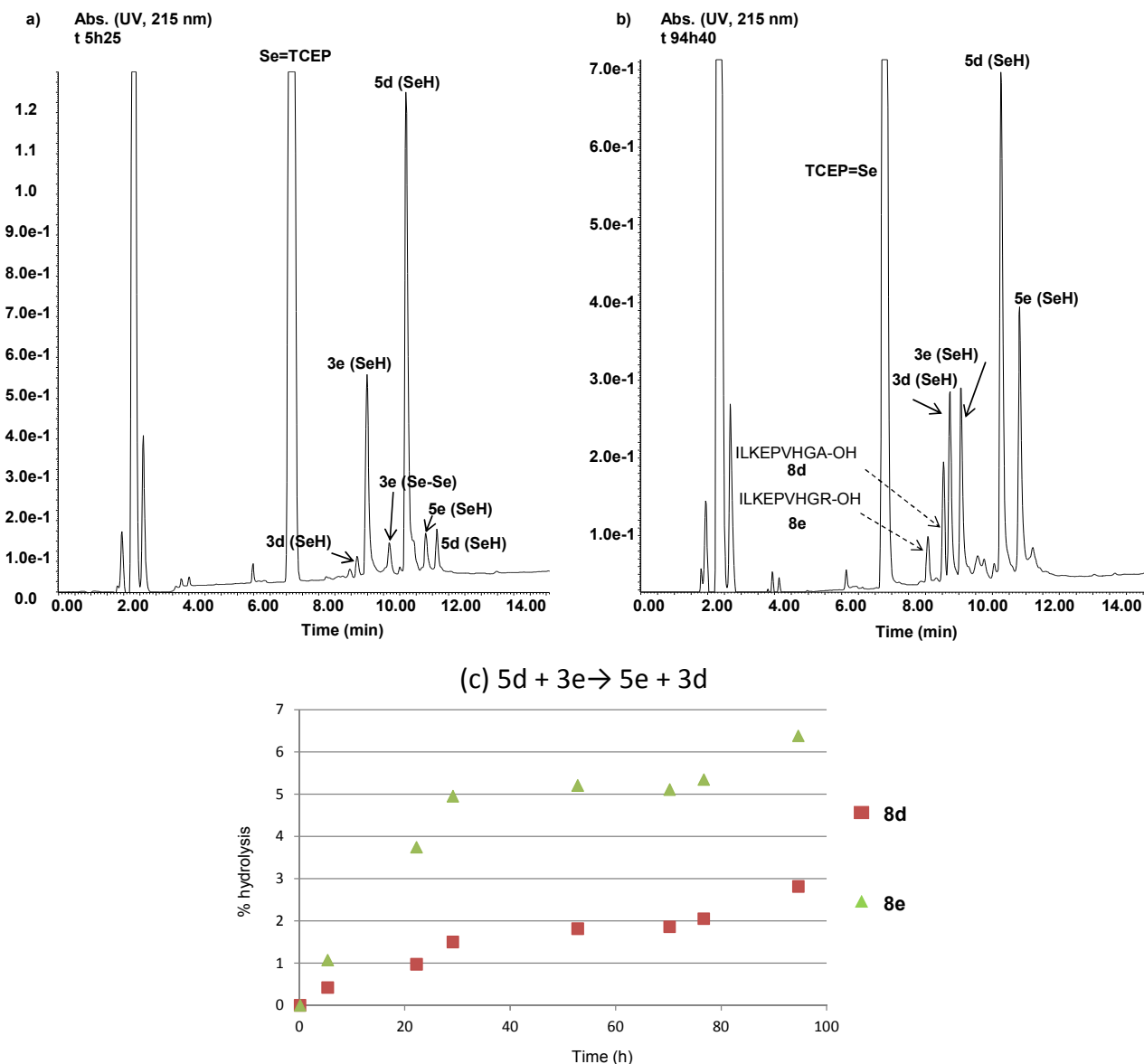


Figure S27. HPLC traces of the crude methathesis reaction between **5d** and **3e** after a) 5 h 25, b) 94 h 40. c) Time course of the formation of hydrolysis products **8d** and **8e**. Peptides **5e** and **3d** (selenol) were found to be identical by HPLC with peptide **5e** or **3d** obtained by peptide synthesis (diselenide) after reduction by TCEP (co-injection of the crude metathesis reaction with reduced authentic samples).

An analytical sample of peptides **5e** and **3d** (selenol) obtained by metathesis was also sequenced by MALDI-TOF as an additional proof of identity.

In this experiment, the presence of expected b and y ion series demonstrate the presence of the Ala-SeC-Ileu bond formed in the metathesis process.

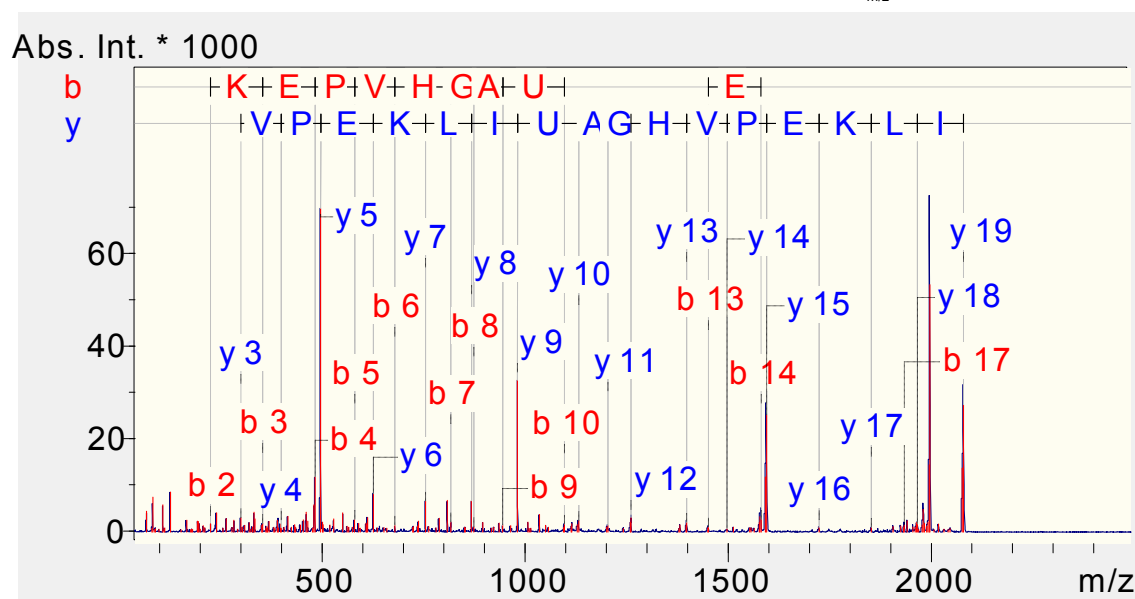
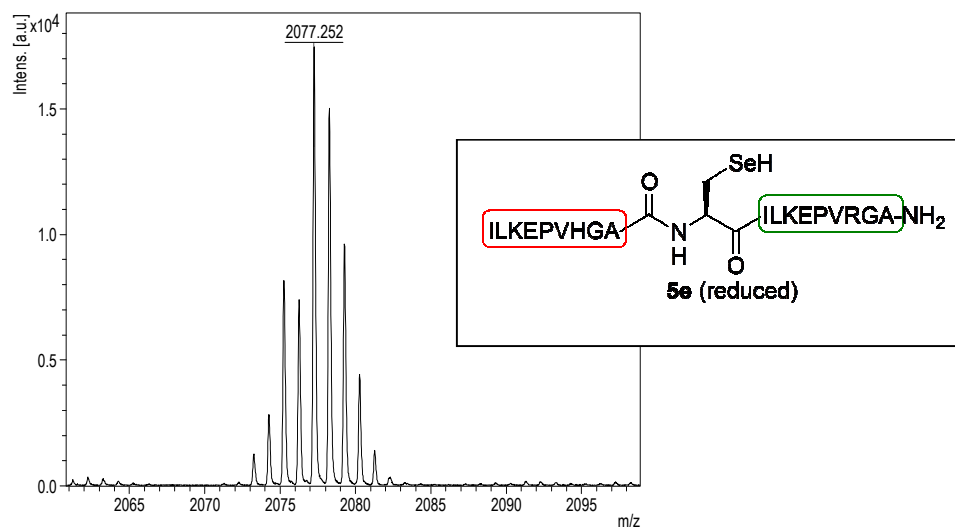


Figure S28. MALDI-TOF sequencing of peptide **5e** selenol (b and y ion series) formed during the metathesis reaction (isolated by HPLC). Matrix: 2,5-dihydroxybenzoic acid.

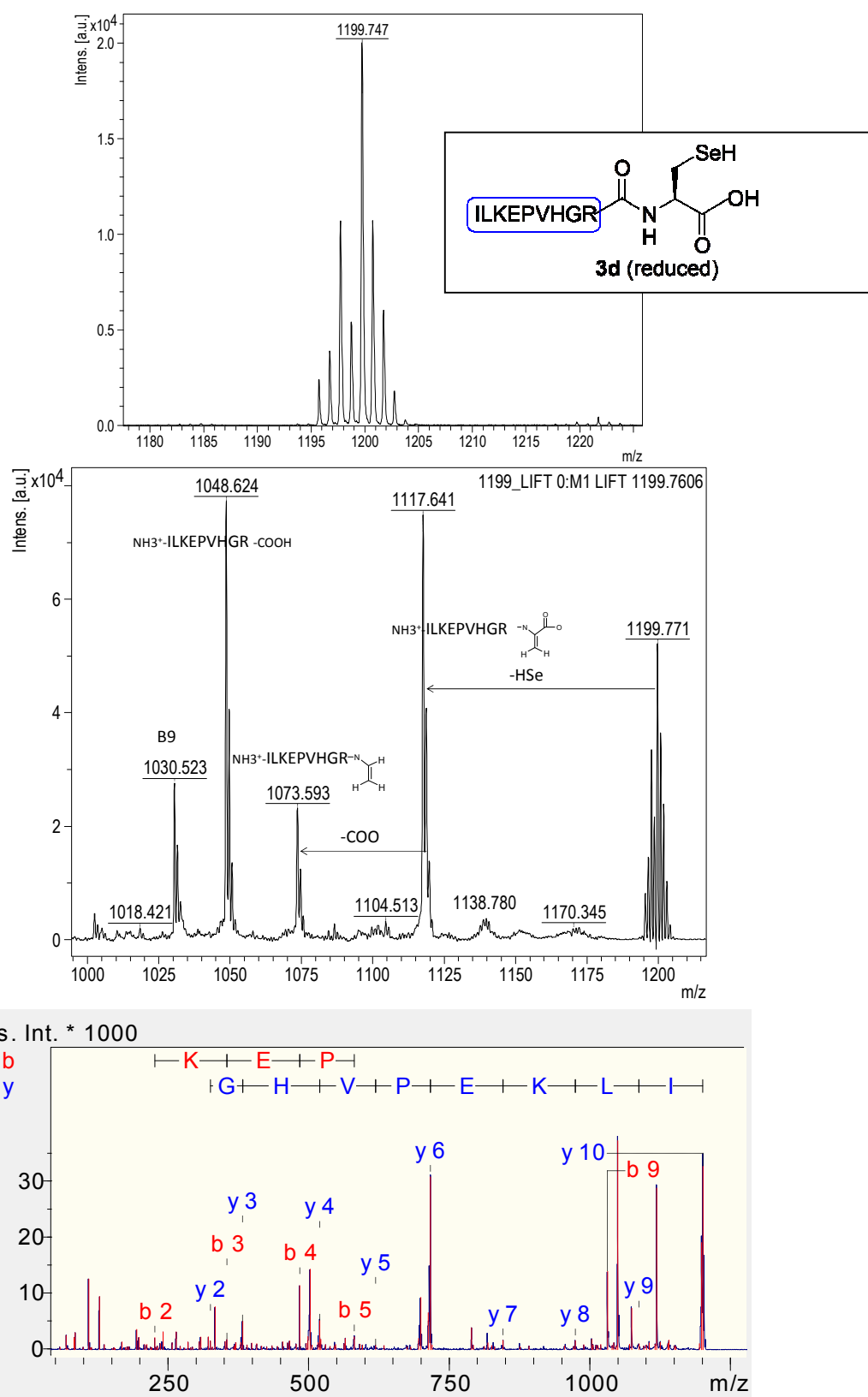


Figure S29. MALDI-TOF analysis and fragmentation of peptide **3d** selenol (b and y ion series) formed during the metathesis reaction (isolated by HPLC). Matrix: 2,5-dihydroxybenzoic acid.

## 6. Effect of TCEP=Se concentration on the TCEP-induced deselenization reaction

Fig. S30 below describes the model reaction which allowed us to study the effect of TCEP=Se concentration on the deselenization reaction.

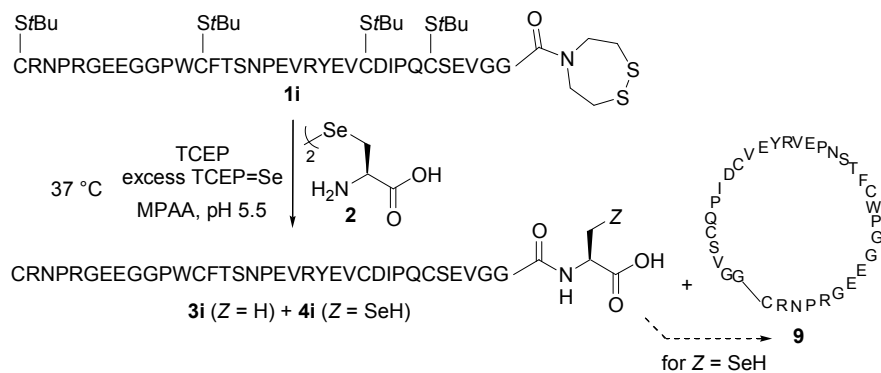


Figure S30. Synthesis of protected selenopeptide **4i** by SEA ligation of peptide **1i** with 2 equiv of selenocystine. Two side-products in this reaction are the formation of the deselenized product **3i** and the formation of the cyclic peptide **9**. Peptide **9** is formed by intramolecular transamidation reaction of the C-terminal Sec residue.

Fig. S31 below shows that the inhibitory effect of TCEP=Se on the formation of the deselenized product **3i** is not significant unless the concentration of TCEP=Se is greater than ~ 150 mM.

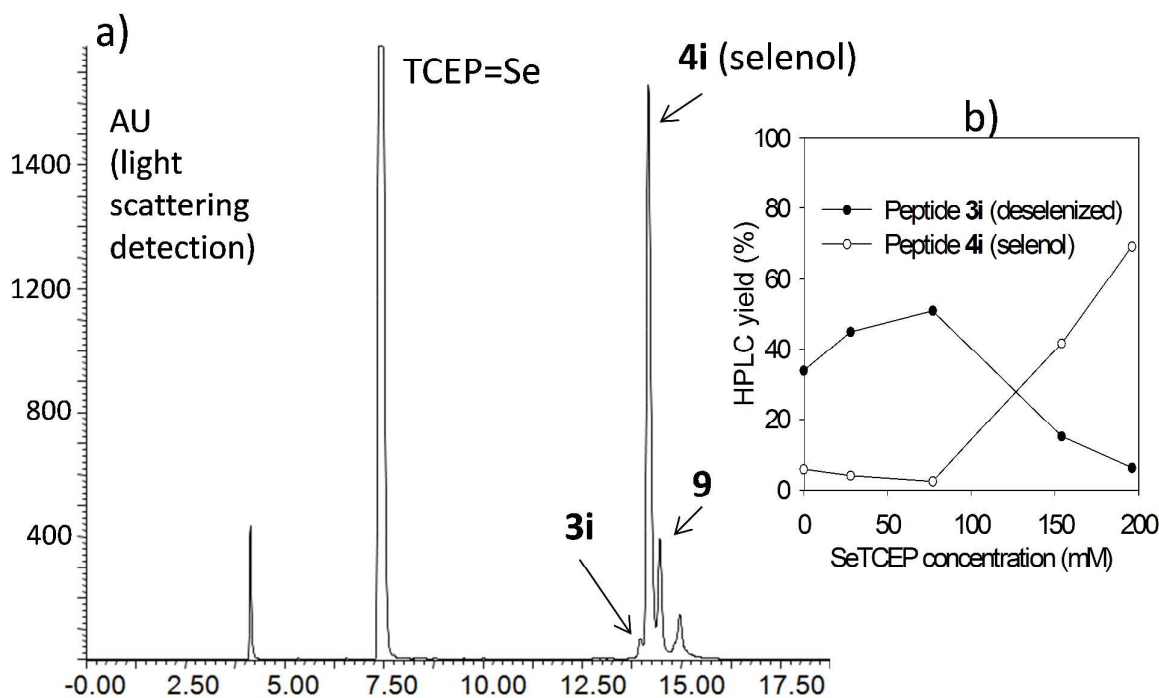


Figure S31. Effect of TCEP=Se concentration on the proportion of deselenized product **3i** formed during the ligation of peptide **1i** with selenocystine **2** (two equiv). The HPLC trace was obtained using 200 mM of TCEP=Se.

The experiment was carried out under nitrogen atmosphere. Guanidine hydrochloride (Gdn.HCl, 574 mg, 6.00 mmol) was dissolved in phosphate buffer (0.1 M, pH=7) to obtain 1 mL of 6 M Gdn.HCl solution. MPA (33.7 mg, 200  $\mu$ mol), TCEP (57.5 mg, 200  $\mu$ mol) were dissolved together in the above solution.

NaOH (6 M in water) was added to adjust the pH to 5.7. TCEP=Se (78.7 mg, 239  $\mu\text{mol}$ ) was dissolved in previous solution (1.138 mL). NaOH (6 M in water) was added to adjust the pH to 5.51. SEA<sup>off</sup> peptide **1i** (36.6 mg, 7.6  $\mu\text{mol}$ , 7 mM final concentration) and selenocystine (25.9 mg, 76.2  $\mu\text{mol}$ , 10 eq) were dissolved together in above solution (1.2 mL). The final concentration of TCEP=Se was varied from 0 to 200 mM. The reaction mixture was heated overnight at 37°C. The reaction was monitored by LC-MS as indicated in the general section.