Supporting Information for

Accelerating chemoselective peptide bond formation using *bis*(2-selenylethyl)amido peptide selenoester surrogates

Laurent Raibaut,‡[a] Marine Cargoët,‡[a] Nathalie Ollivier,[a] Yun Min Chang,[a] Hervé Drobecq,[a] Emmanuelle Boll,[a] Rémi Desmet,[a] Jean-Christophe M. Monbaliu,*[b] Oleg Melnyk*[a]

[a] UMR CNRS 8161 CNRS, Université de Lille, Institut Pasteur de Lille, 1 rue du Pr Calmette, 59021 Lille Cedex, France.

[b] Center for Integrated Technology and Organic Synthesis, Department of Chemistry, Building B6a, Room 3/16a, University of Liège, Sart-Tilman, B-4000 Liège, Belgium.

‡ Laurent Raibaut and Marine Cargoët contributed equally to this work

Corresponding authors:

Dr Oleg Melnyk, E-mail : oleg.melnyk@ibl.cnrs.fr
Web site: http://olegmelnyk.cnrs.fr

Phone: +33 (0)3 20 87 12 14; ORCID
Cent Nat de la Recherche Scientifique (CNRS)
Institut de Biologie de Lille
1 rue du Pr Calmette, CS 50447, 59021 Lille cedex, France

Dr Jean-Christophe M. Monbaliu, PhD.
Center for Integrated Technology and Organic Synthesis - CiTOS
University of Liège, Department of Chemistry, Building B6a, Room 3/16a
Quartier Agora, Allée du six Aout, 13B-4000 Liège (Sart Tilman), Belgium

t +32 (0) 4 366 35 10 - jc.monbaliu@ulg.ac.be www.citos.ulg.ac.be

Table of Contents

1. General Methods

Reagents and solvents

2-(1*H*-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium fluorophosphate (HBTU) and *N*α-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)aminotrityl polystyrene (SEA PS) resin was carried out as described elsewhere.[1] Rink-PEG-PS resin (NovaSyn TGR) and Wang resin (100-200 Mesh) were obtained from Novabiochem. 4-mercaptophenylacetic acid (MPAA), 3-mercaptopropionic acid (MPA), *tris*(2-carboxyethyl)phosphine hydrochloride (TCEP), metallic selenium and sodium borohydride ($NaBH_4$) 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_2Cl_2), diethylether (Et_2O), acetonitrile (CH_3CN), heptane, LC–MS-grade acetonitrile (CH_3CN, 0.1% TFA), LC–MS-grade water (H_2O, 0.1% TFA), *N,N*-diisopropylethylamine (DIEA), acetic anhydride (Ac_2O) 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

The reactions were monitored by analytical LC–MS (Waters 2695 LC/ZQ 2000 quadripole) on an reverse phase column XBridge BEH300 C18 (3.5 μm, 300 Å, 4.6 × 150 mm) unless otherwise stated at 30 °C using a linear gradient of 0-100% of eluent B in eluent A over 30 min at a flow rate of 1 mL/min (eluent A = 0.1% TFA in H_2O; eluent B = 0.1% TFA in CH_3CN/H_2O: 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, dessolvation temperature 350 °C. Samples were prepared using 10 μL aliquots of the reaction mixtures. The aliquots were quenched by adding 90 μL of 1% aqueous TFA, extracted with Et_2O to remove MPAA or MPA before analysis.

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

^1H and ^{13}C NMR spectra were recorded on a Bruker Advance-300 spectrometer operating at 300 MHz and 75 MHz respectively. The spectra are reported as parts per million (ppm) down field shift using tetramethylsilane or dimethylselenide as an internal reference. The data are reported as chemical shift (δ), multiplicity, relative integral, coupling constant (J Hz) and assignment where possible.

The determination of optical purity of the C-terminal amino acid was done by chiral GC-MS following total acid hydrolysis in deuterated aqueous acid (C.A.T. GmbH & Co. Chromatographie und Analysentechnik

KG, Heerweg 10, D-72070 Tübingen, Germany).[2]

HPLC purification

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 C-18 (5 μm, 300 Å, 19 × 100 mm) and appropriate gradient of increasing concentration of eluent B in eluent 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. Peptide synthesis

General procedure for automated peptide synthesis

Peptide elongation was performed using standard Fmoc/*tert*-butyl chemistry on an automated peptide synthesizer (0.2 mmol scale). Couplings were performed using 5-fold molar excess of each Fmoc-L- amino acid, 4.5-fold molar excess of HBTU, and 10-fold molar excess of DIEA. A capping step was performed after each coupling with Ac_2O/DIEA in DMF. At the end of the synthesis, the resin was washed with CH_2Cl_2, diethylether (2 × 2 min) and dried in vacuo.

2.1 Synthesis of SEA^{off} peptide segments (9a-c, 18, 19)

Peptide elongation was performed on SEA PS resin (0.2 mmol, 0.16 mmol/g) using standard Fmoc/*tert*-butyl chemistry on an automated peptide synthesizer. Typical procedures for the synthesis of SEA^{off} peptide segments were described in previous papers.[1, 3] For a detailed protocol see the protocol article [4]. The analytical HPLC and MS analyses of the purified synthetic SEA^{off} peptides segments **(9a-c, 18, 19)** are shown in Figure S1.

Yields for the HPLC purified SEA^{off} peptides **(9a-c, 18, 19)**:

-**Peptide 9a**: 50 mg (35% yield, 0.1 mmol scale), MALDI-TOF calcd. for $[M+H]^+$: 1080.6, observed mass: 1080.5 (monoisotopic).

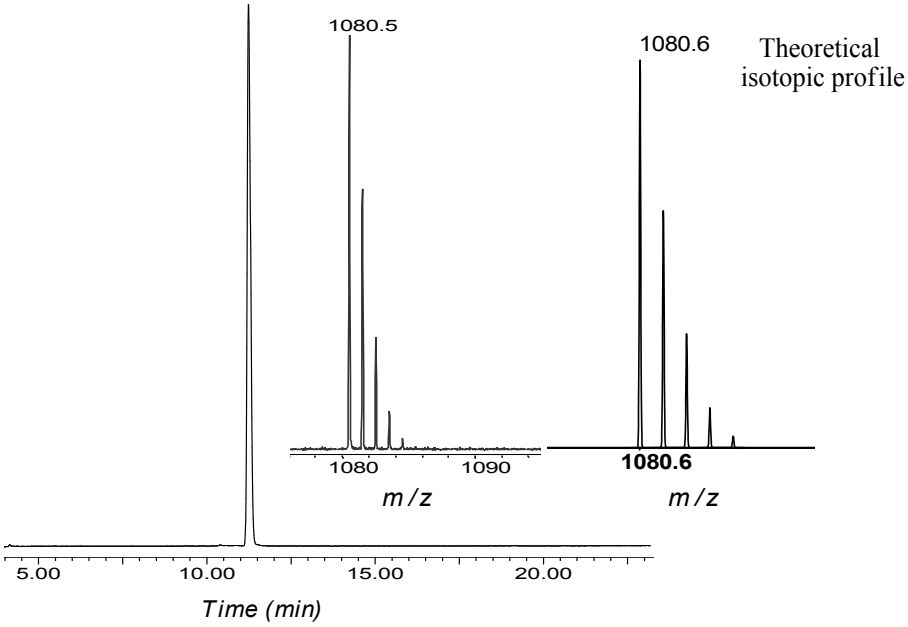
-**Peptide 9b**: 56 mg (39% yield, 0.1 mmol scale), MALDI-TOF calcd. for $[M+H]^+$: 1108.6, observed mass: 1108.5 (monoisotopic).

-**Peptide 19**: 35 mg (25% yield, 0.1 mmol scale), MALDI-TOF calcd. for [M+H]+: 1054.4, observed mass: 1054.3 (monoisotopic).

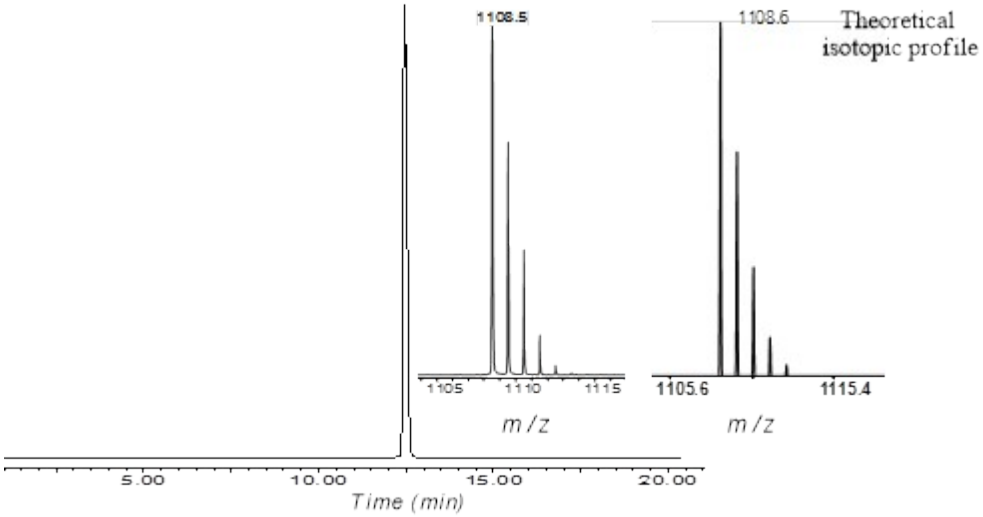
-**Peptide 9c**: 53 mg (30% yield, 50 μmol scale), MALDI-TOF calcd. for $[M+H]^+$: 2784.5, observed mass: 2784.1 (monoisotopic).

-**Peptide 18**: 47 mg (23% yield, 50 μmol scale), MALDI-TOF calcd. for $[M+H]^+$: 3541.6, observed mass: 3541.9 (monoisotopic).

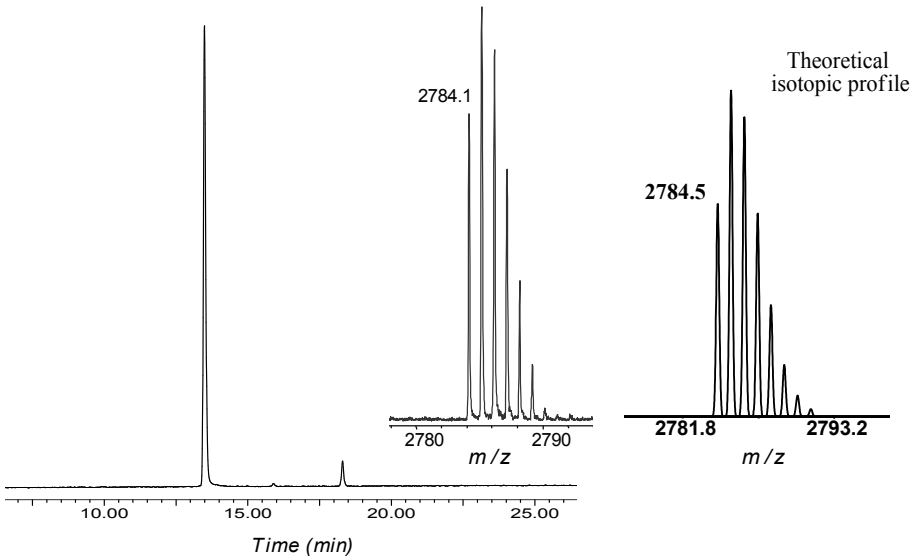
ILKEPVHGA-SEA^{off} (**9a**)



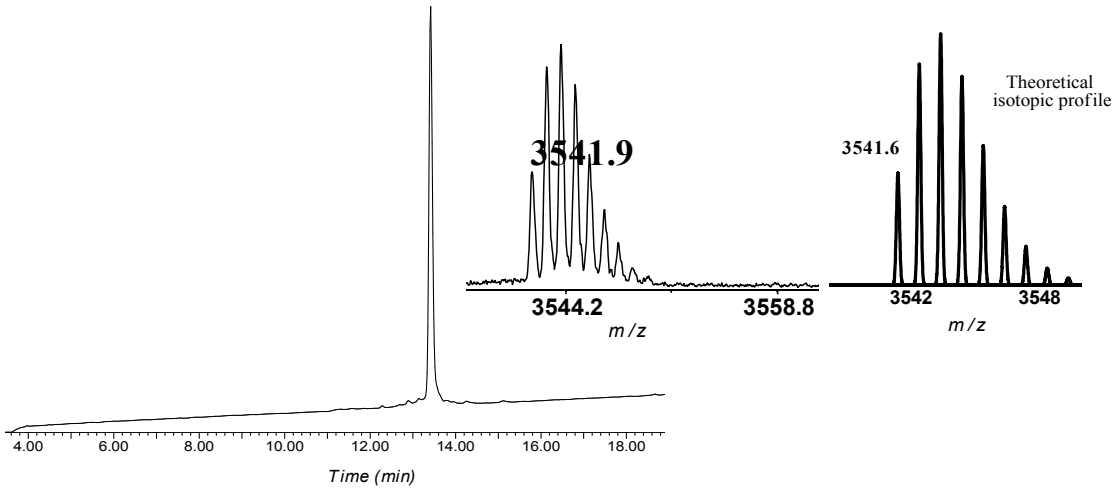
ILKEPVHGV-SEA^{off} (**9b**)



IRNC(S*t*Bu)IIGKGRSYKGTVSITKSGIK-SEA^{off} (**9c**)



C(S*t*Bu)QPWSSMIPHEHSFLPSSYRGKDLQENY-SEA^{off} (**18**)



C(S*t*Bu)HHLEPGG-SEA^{off} (**19**)

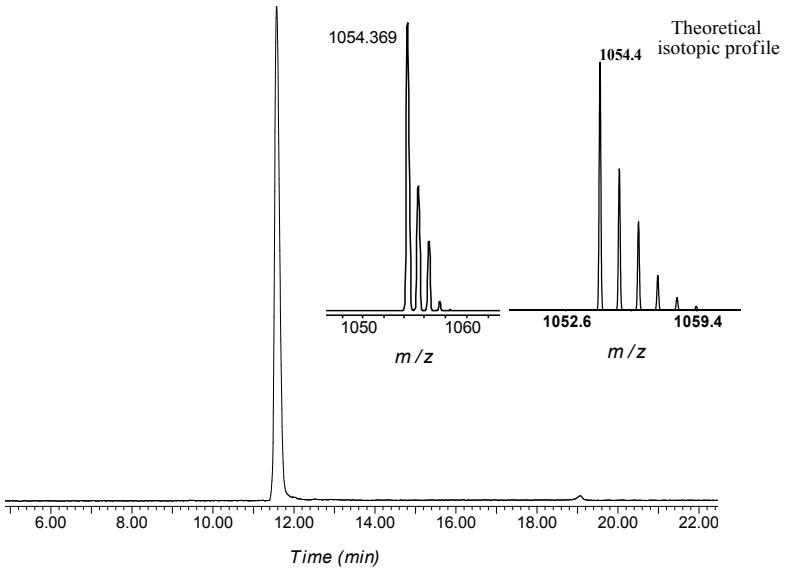


Figure S1. Analytical HPLC profiles (λ=215 nm) for purified synthetic peptide segments **(9a-c, 18, 19)** and MALDI-TOF data corresponding to each product.

2.2 Synthesis of MPA thioesters 12a,b

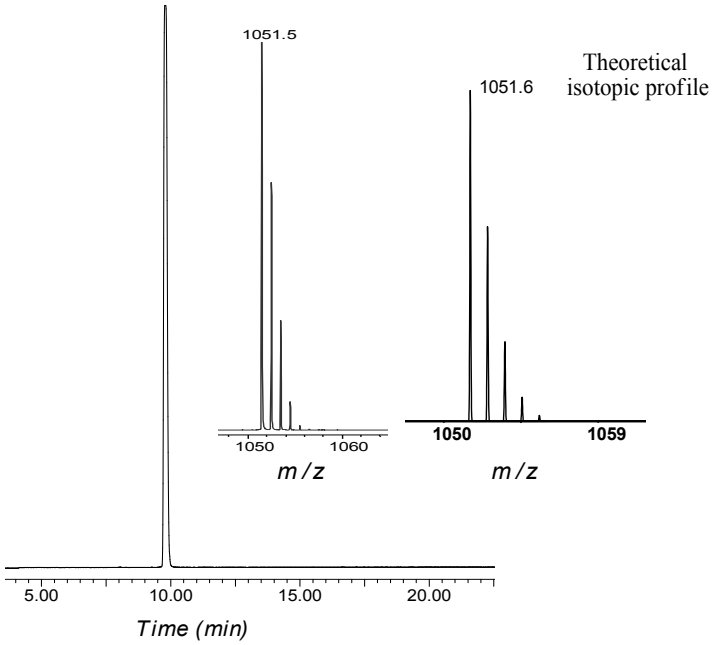
Typical procedures for the synthesis of MPA thioester peptides using SEA^{off} peptides **9a,b** were described in detail elsewhere.[4, 5] The analytical HPLC and MS analyses of the purified MPA thioester peptides **12a,b** are shown in Figure S2.

Yields for the HPLC purified MPA peptide thioesters

-**Peptide 12a**: 9.5 mg (68% yield, 10 μmol scale), MALDI-TOF calcd. for $[M+H]^+$: 1051.6, observed mass: 1051.5 (monoisotopic).

-**Peptide 12b**: 7 mg (49% yield, 10 μmol scale), MALDI-TOF calcd. for $[M+H]^+$: 1079.6, observed mass: 1079.5 (monoisotopic).

ILKEPVHGA-S$(CH_2)_2$COOH (**12a**)



ILKEPVHGV-S$(CH_2)_2$COOH (**12b**)

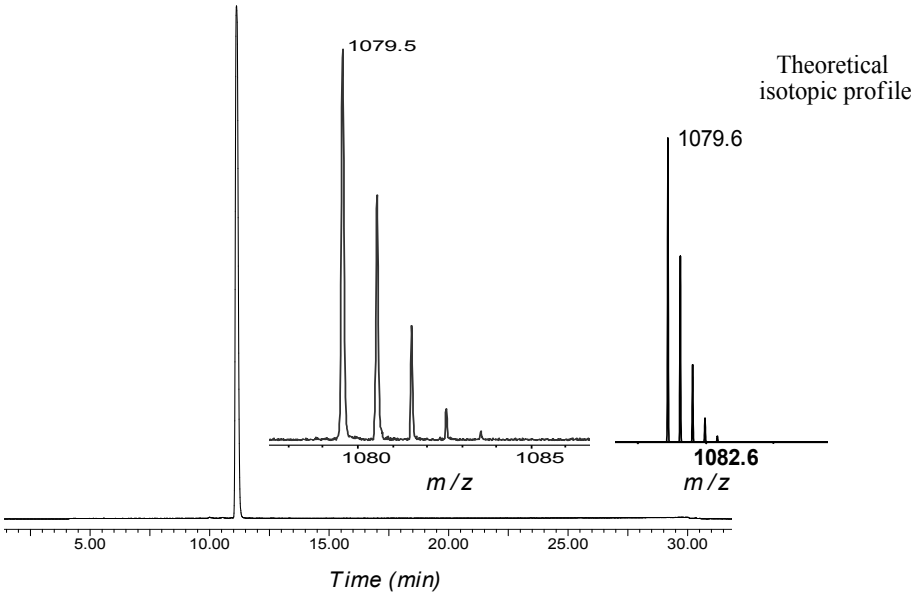


Figure S2. Analytical HPLC profiles (λ=215 nm) for purified synthetic peptide segments **12a,b** and MALDI-TOF data corresponding to each product.

2.3 Synthesis of peptide 13d and Cys peptides 14, 21, 22

Peptide elongation were performed on Rink-PEG-PS resin (NovaSyn TGR, 0.25 mmol, 0.25 mmol/g) or on pre-loaded Fmoc-Gly-Wang resin (0.1 mmole, 0.61 mmol/g) using standard Fmoc/*tert*-butyl chemistry on an automated peptide synthesizer. The analytical HPLC and MS analyses of the purified peptides **13d**, **14, 21** and **22** are shown in Figure S3.

Isolated yield for the HPLC purified peptides **13d**, **14**, **21**, **22**:

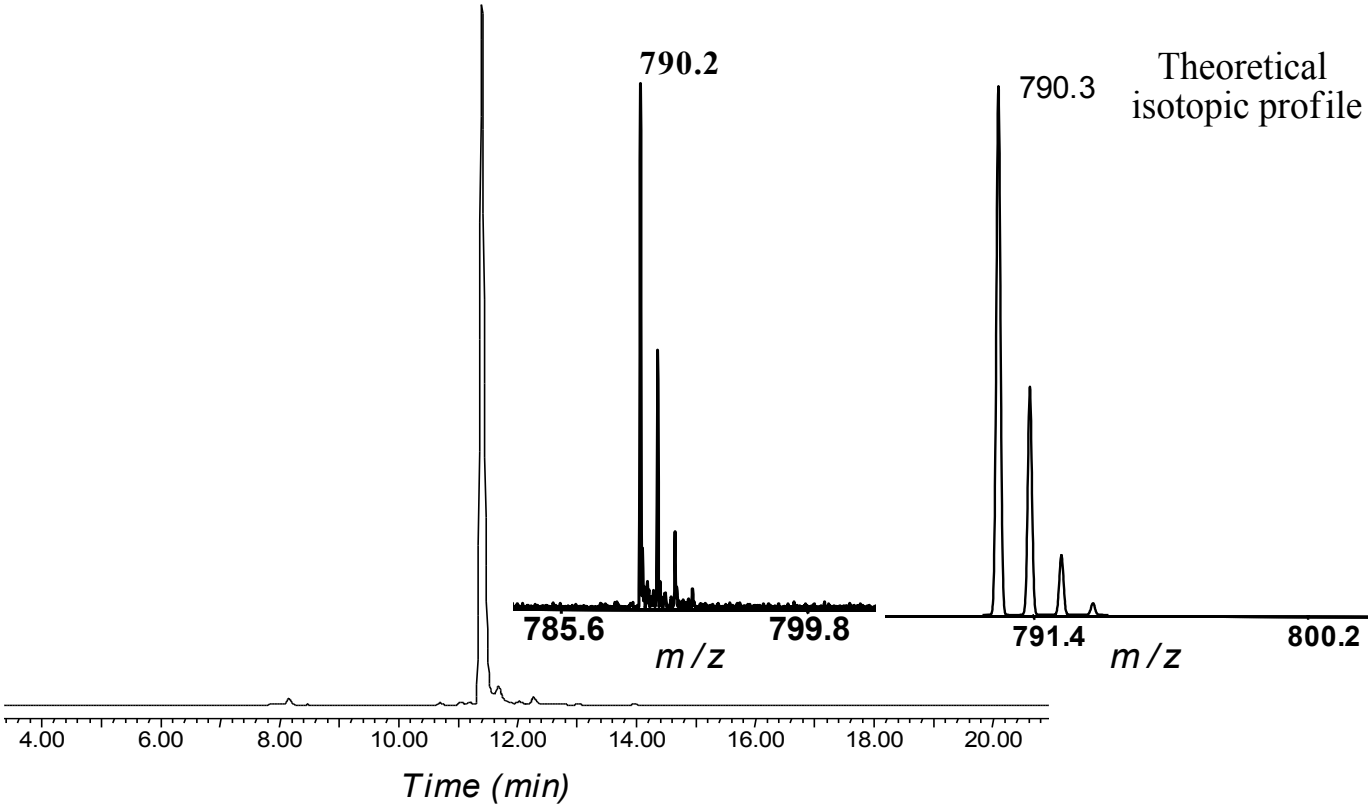
Peptide **13d**: 33 mg (43% yield, 0.1 mmol scale), Maldi-TOF calc. for $[M+Na]^+$: 790.3, observed mass: 790.2 (monoisotopic).

Peptide **14**: 89 mg (62% yield, 0.1 mmol scale), MALDI-TOF calcd. for $[M+H]^+$: 1093.6, observed mass: 1093.6 (monoisotopic).

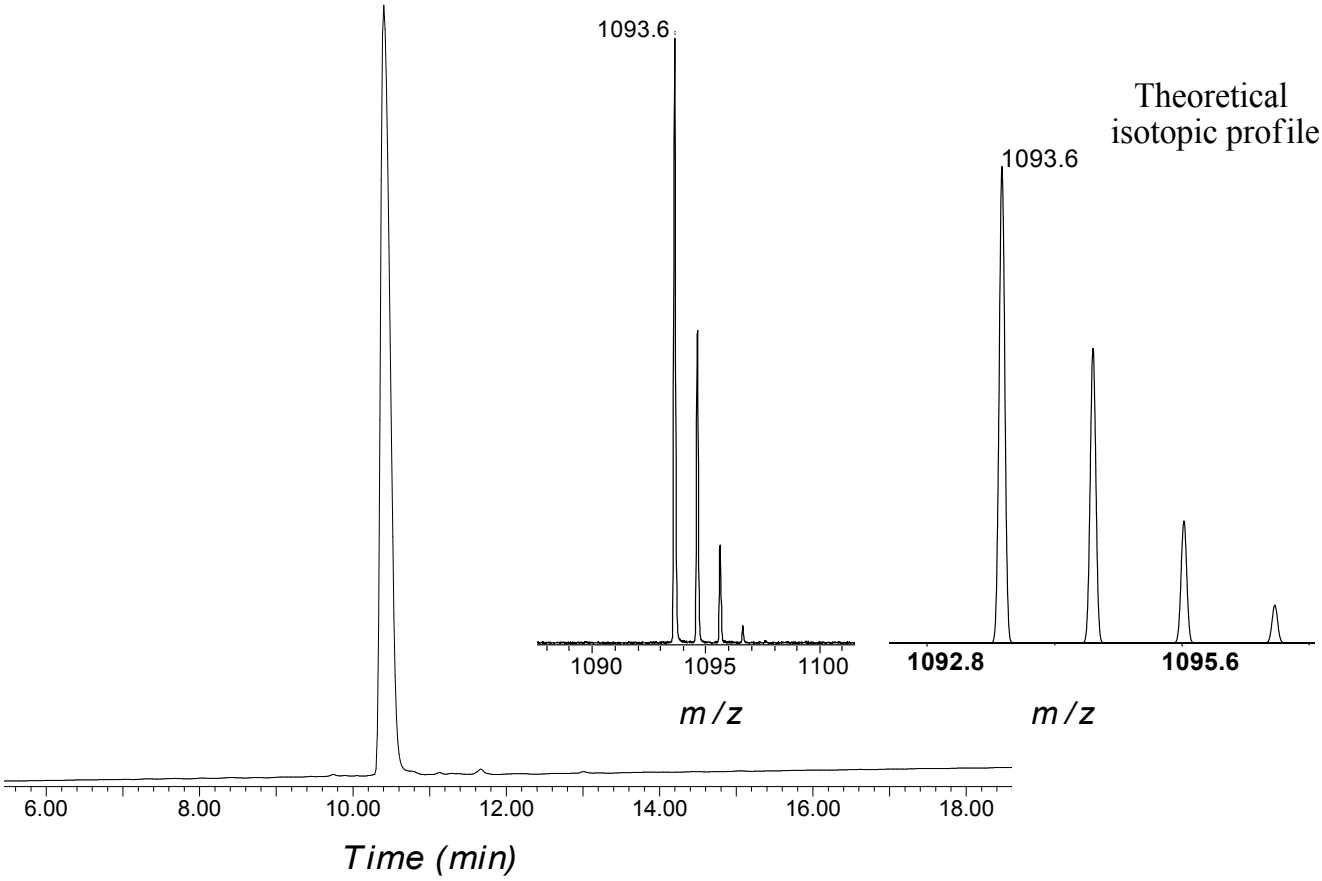
Peptide **21**: 134 mg (13% yield, 0.25 mmol scale), MALDI-TOF calcd. for $[M+H]^+$: 3755.6, observed mass: 3755.8 (monoisotopic).

Peptide **22**: 77 mg (55% yield, 0.1 mmol scale), MALDI-TOF calcd. for $[M+H]^+$: 1065.5, observed mass: 1065.6 (monoisotopic).

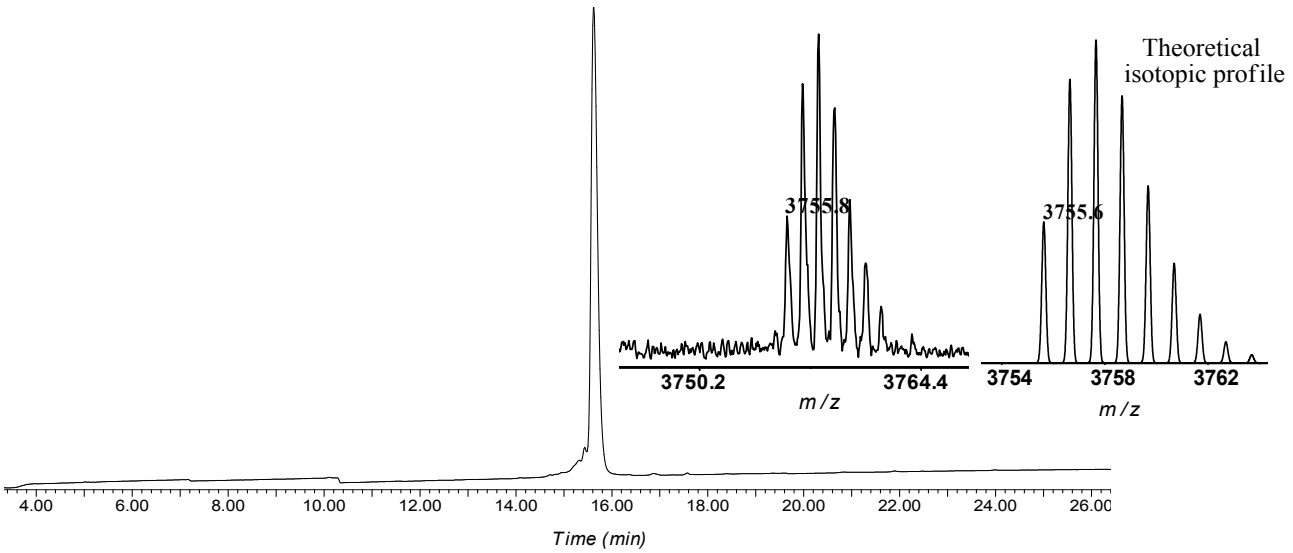
Ac-GFGQGFGG-COOH (**13d**)



CILKEPVHGV-NH_2 (**14**)



CRNPRGEEGGPWCFTSNPEVRYEVCDIPQCSEV-NH_2 (**21**)



CILKEPVHGA-NH_2 (**22**)

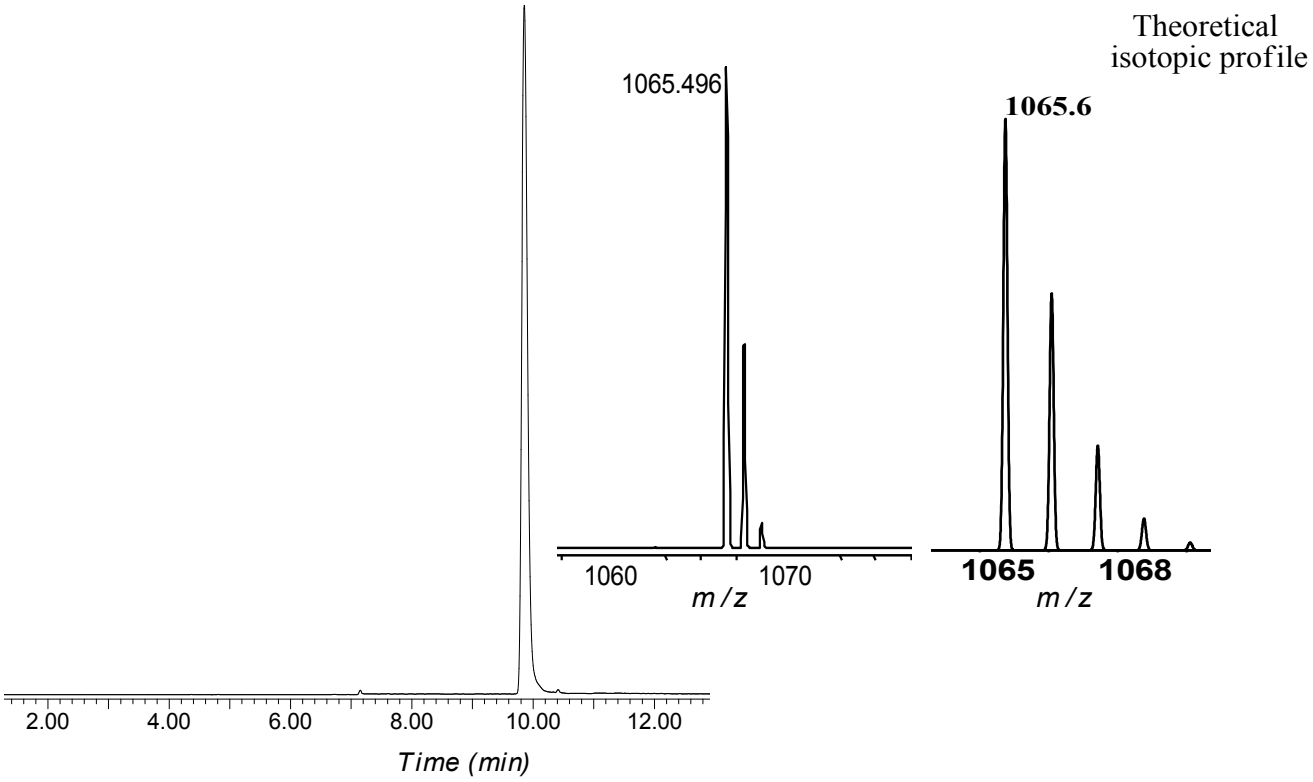
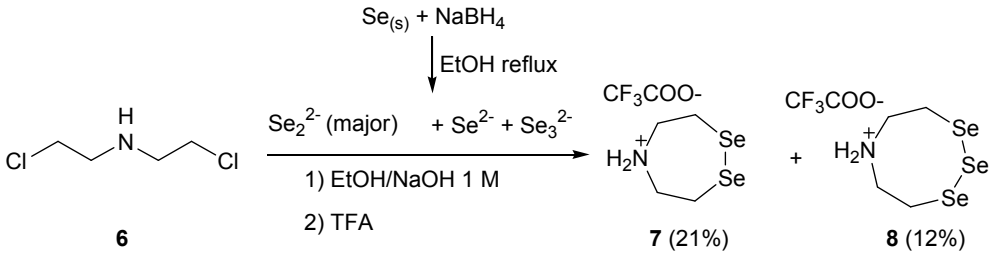


Figure S3. Analytical HPLC profiles (λ=215 nm) for the purified synthetic peptide segments **13d**, **14**, **21** and **22** and MALDI-TOF data corresponding to each product.

3. Synthesis of diselenide 7 & triselenide 8



Scheme S1. Synthesis of diselenide **7** & triselenide **8**.

CAUTION: H_2Se is highly toxic. The reaction must be performed in an efficient fume hood with appropriate protection (glasses, lab coat and gloves).

1-Preparation of sodium diselenide: Absolute ethanol (27 ml) was added dropwise with magnetic stirring to metallic selenium powder (1.5 g, 19.8 mmol, 1.5 eq) and sodium borohydride (0.5 g, 13.2 mmol) cooled in an ice bath. After the vigorous exothermic reaction had occurred, the mixture was further stirred and heated at reflux for 1.5 hr with N_2 passing into the liquid in order to expel H_2Se. The nitrogen flow containing H_2Se and going out of the reaction vessel is passed through NaOH and NaOCl traps respectively. The resulting brown/red colored solution was then cooled down at room temperature and used immediately in the next step.

2- Preparation of compounds 7 and 8: The *bis*(2-chloroethyl)amine hydrochloride **6** (1.2 g, 6.6 mmol, 0.6 eq) was dissolved in 1 M NaOH/EtOH: 1/1 by volume (5 mL) and then was added dropwise over a period of 20 min to the above solution of Na_2Se_2 or Na_2Se_3. The solution was then stirred for 1 hour under argon at room temperature. The reaction mixture was diluted with 1 M NaOH (15 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic phase was dried over solid Na_2SO_4 and evaporated to dryness in vacuo. The crude product was purified by reversed-phase HPLC using a linear water-acetonitrile gradient to give 495 mg (21%) of 1,2,5-diselenazepane as the trifluoroacetate salt **7** and 80 mg (12%) of 1,2,3,6-triselenazocane as the trifluoroacetate salt **8** (yellow powders).

The characterization of diselenide **7** can be found elsewhere.[6]

HR-MS, NMR $^{1}H/^{13}C$ and HPLC analyses for the purified triselenide **8** are shown below in Figures S4-S12.

The presence of ^{77}Se atoms in compound **8** and the formation of aggregates make the NMR spectra of compound **8** complex. The same complexity was observed for compound **7** as discussed elsewhere.[6] Compound **8** was found to degrade partially into **7** upon storage (see below), but this did not alter the usefulness of triselenide **8** for accessing SeEA peptides.

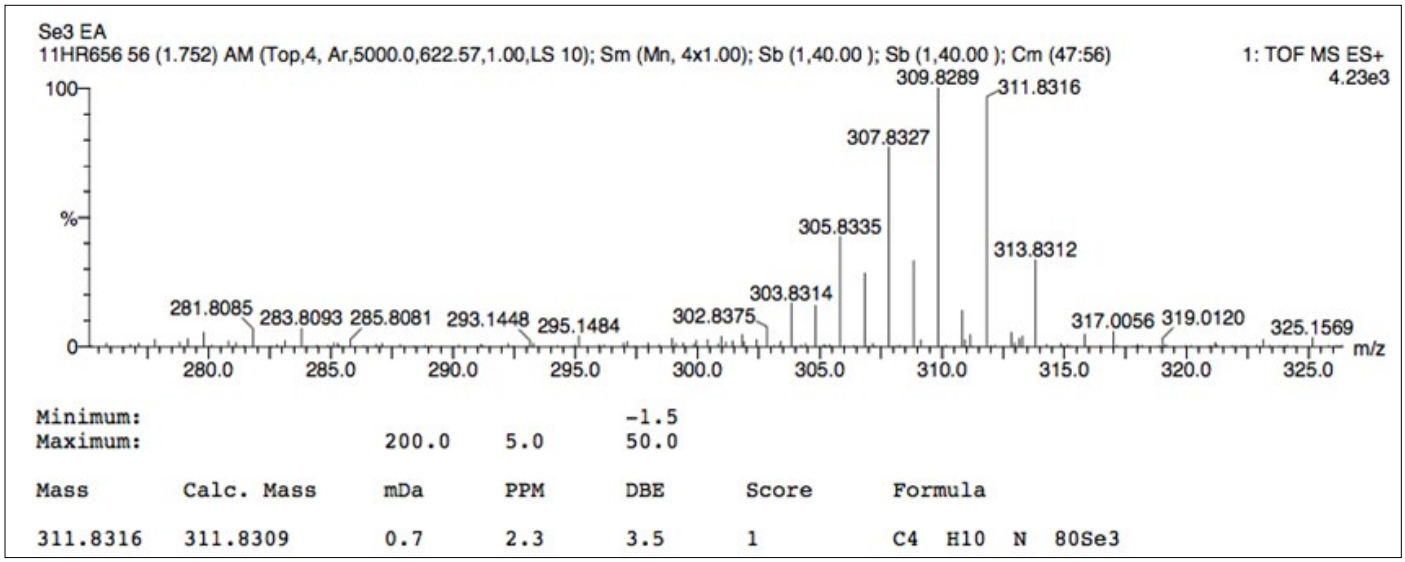


Figure S4. HR-MS analysis for purified triselenide compounds **8**. Calcd. for $[M+H]^+$: 311.831, observed mass: 311.830 (monoisotopic).

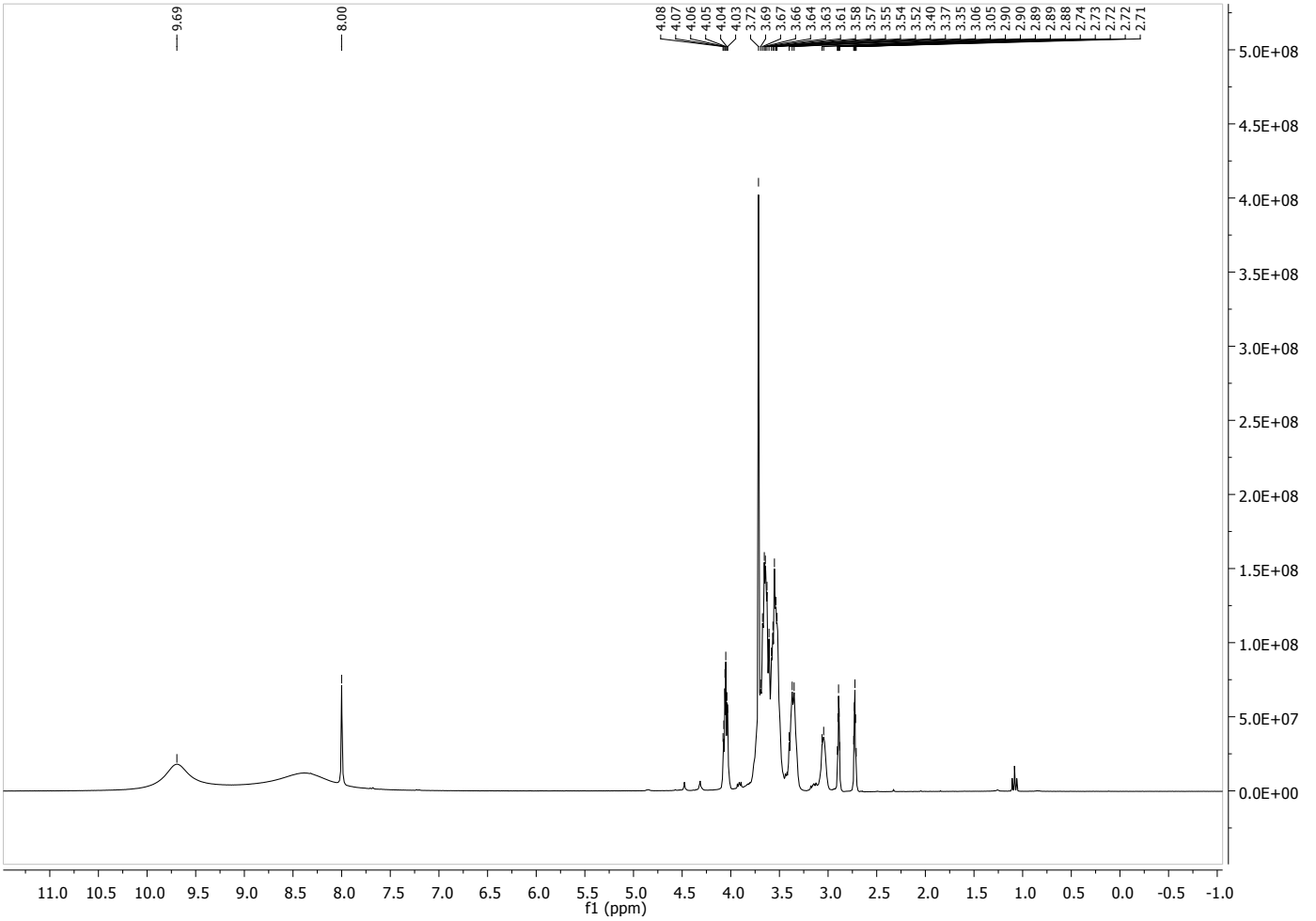


Figure S5. ^{1}H NMR (300 MHz) spectrum for compound **8** (DMF-d7).

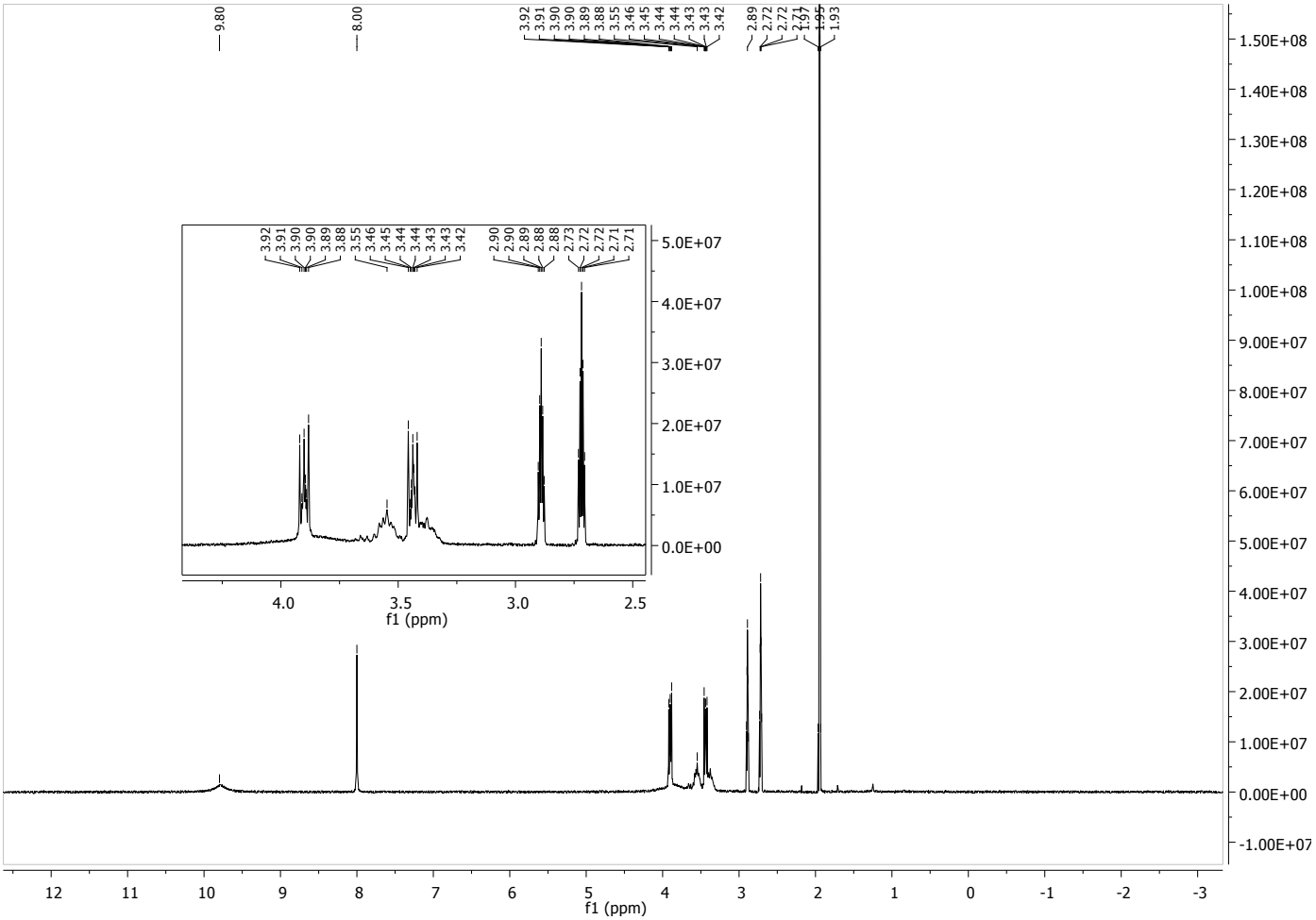


Figure S6. [1]H NMR (300 MHz) spectrum for compound **7** (DMF-d7). See ref [6]

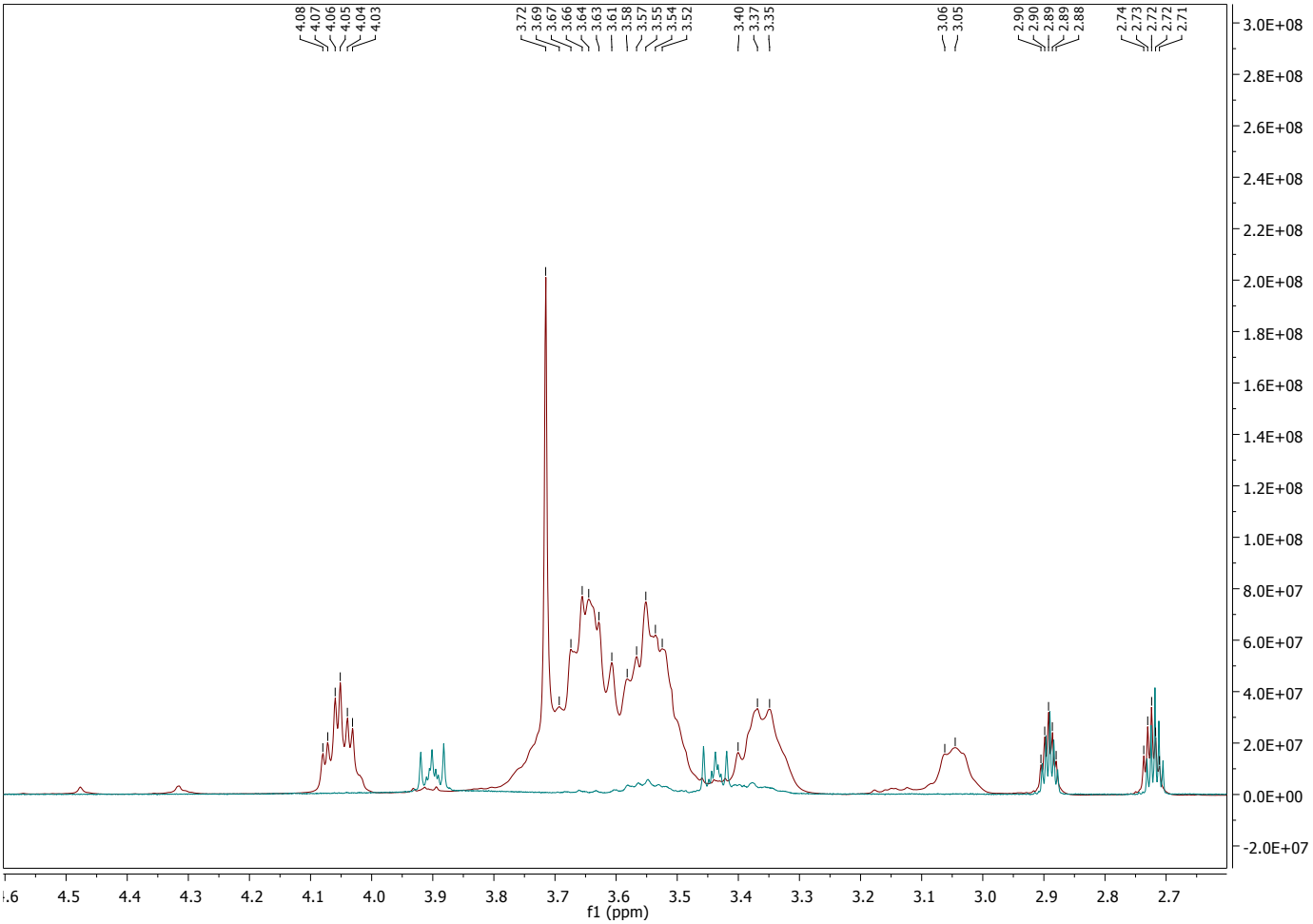
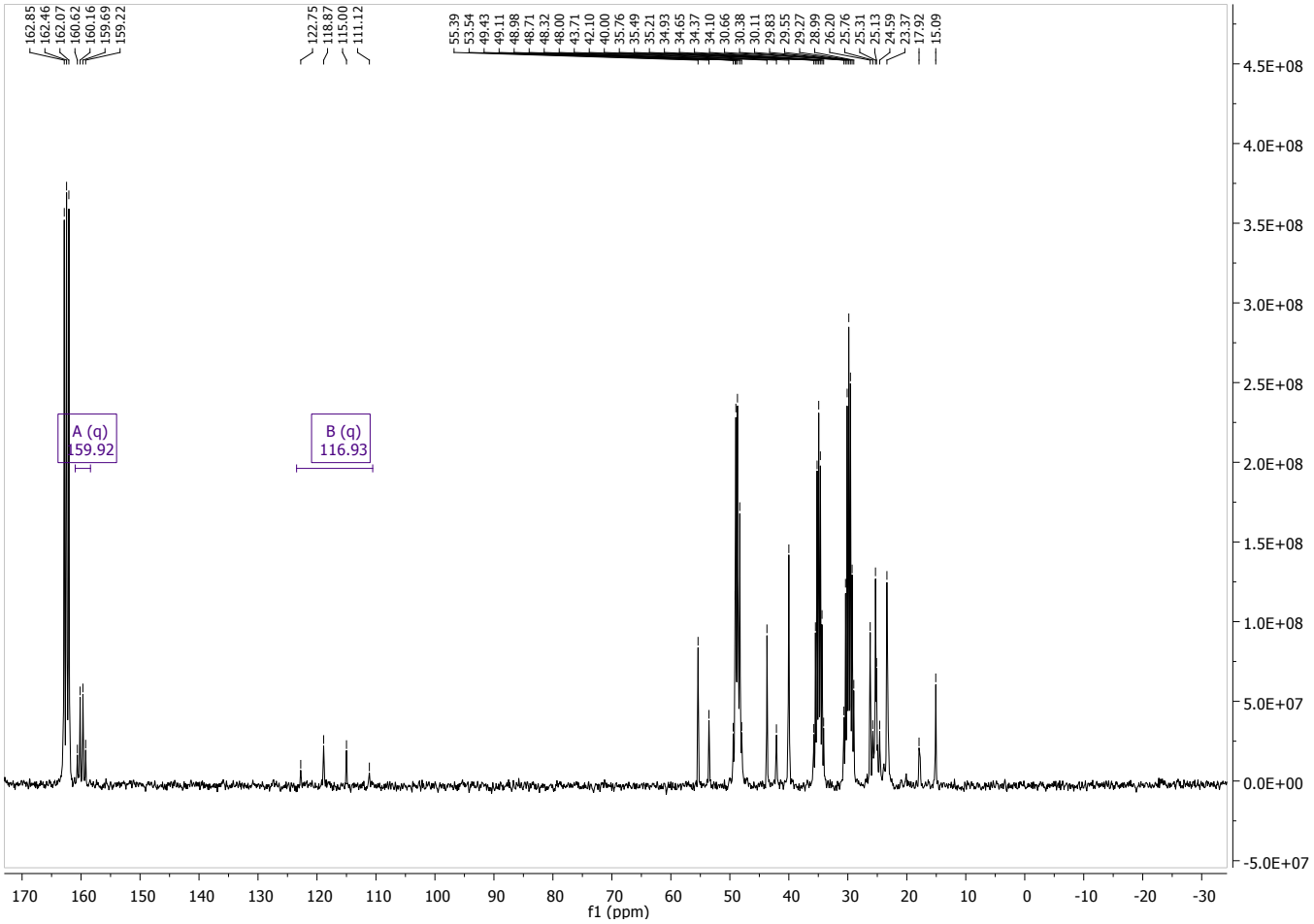


Figure S7. ^{1}H NMR (300 MHz, DMF-d7) spectrum for compounds **7** and **8** (superposition of NMR spectra acquired separately, see Figures S5 & S6).

This figure shows that the triselenide compound **8** isolated by HPLC contains minor amounts of the diselenide **7**.



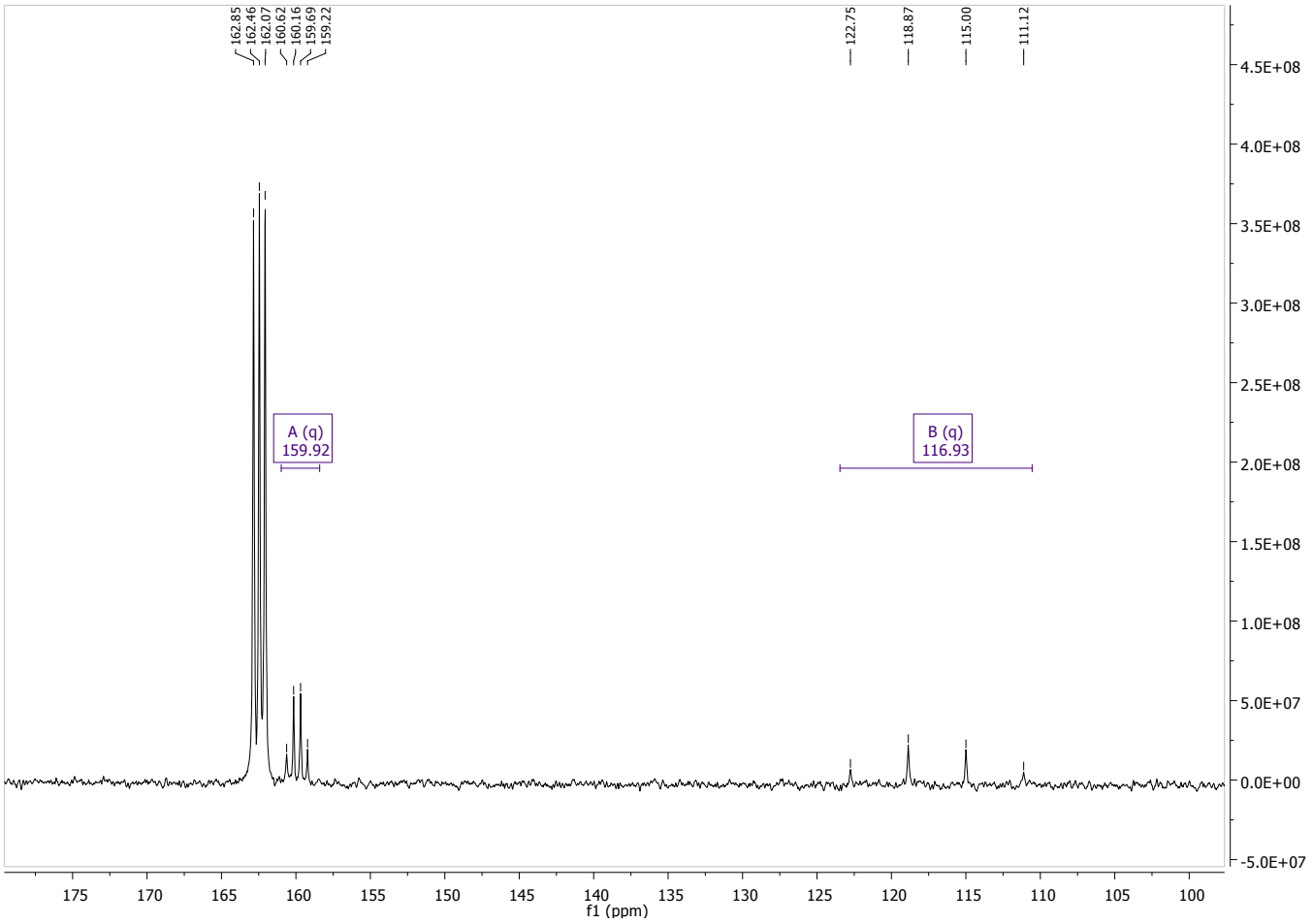


Figure S8. ^{13}C NMR (75 MHz) spectrum for HPLC purified compound **8** (DMF-d7). The quartets at 159.92 and 116.93 ppm are for the trifluoroacetate ion.

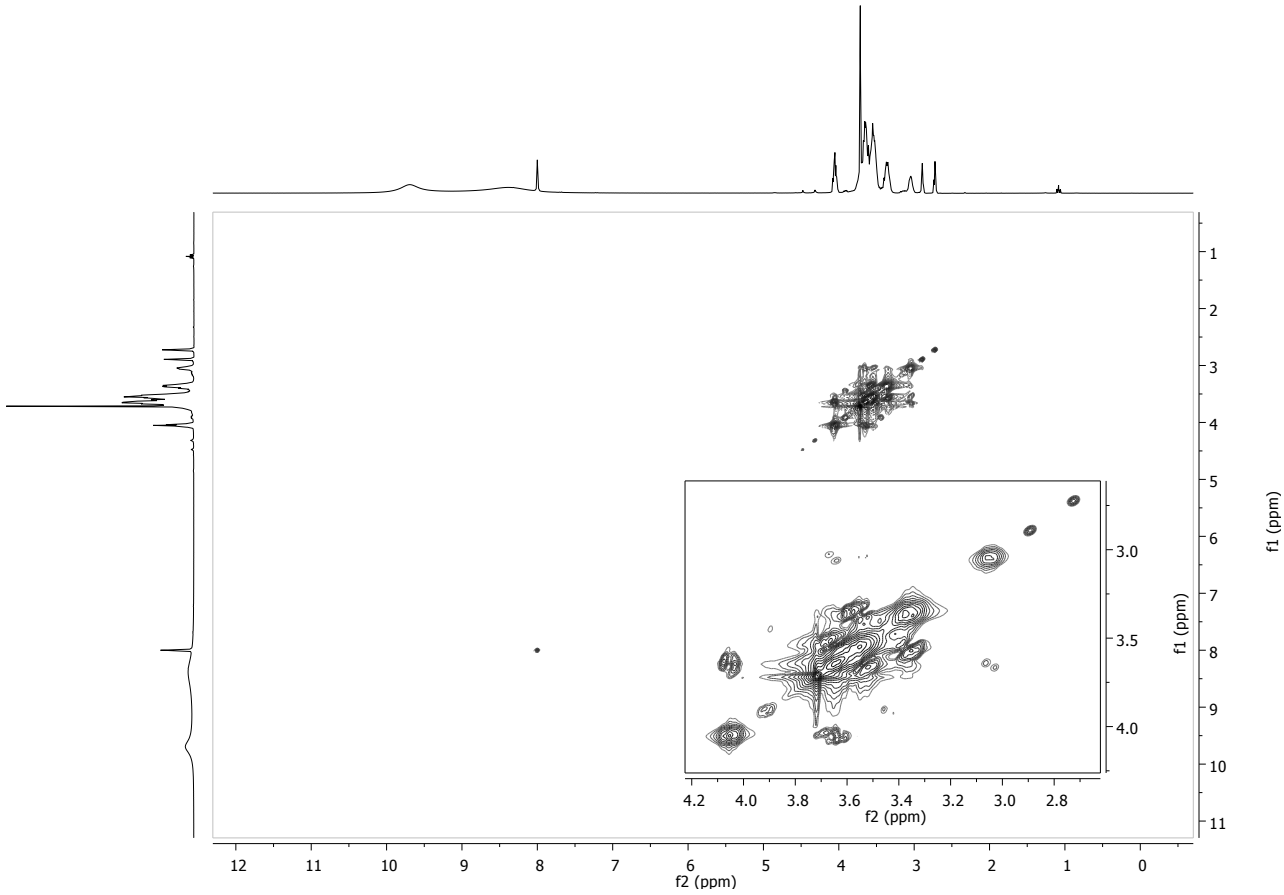


Figure S9. ^{1}H-^{1}H COSY spectrum for compound **8** (DMF-d7).

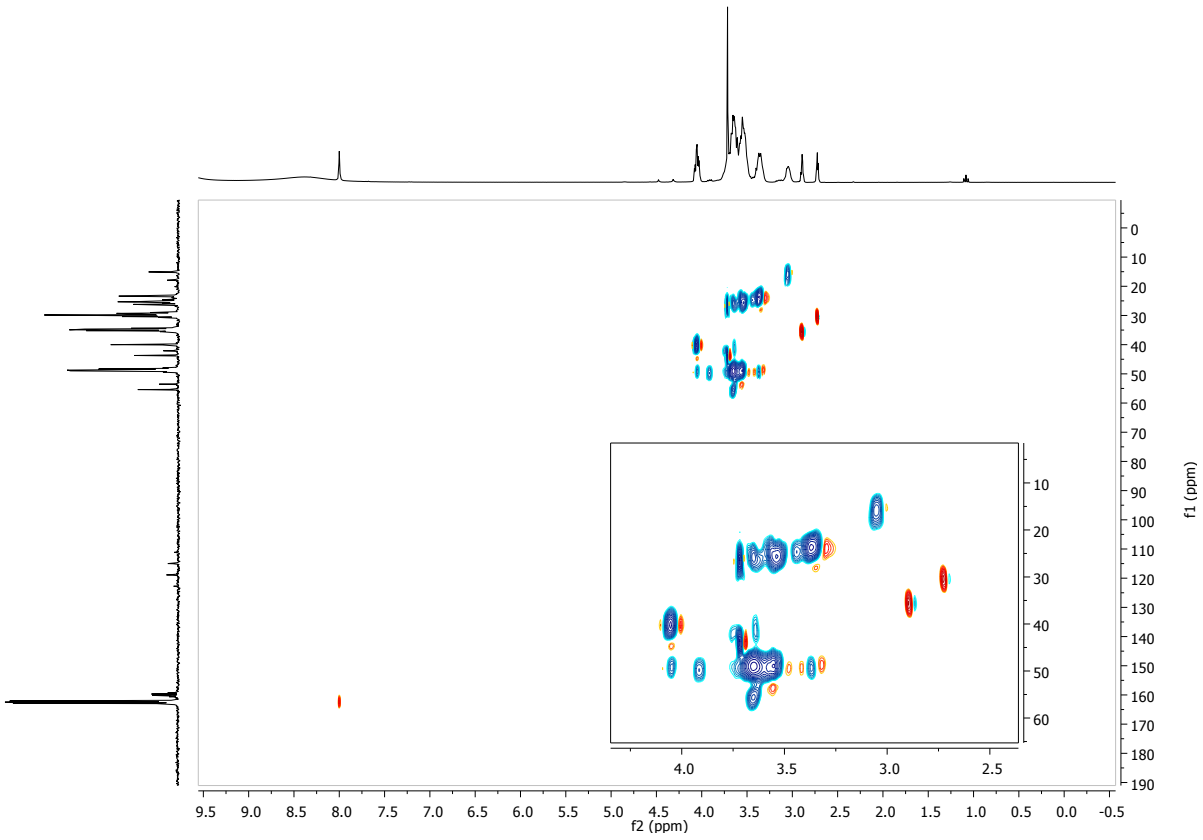


Figure S10. ^{1}H-^{13}C HSQC spectrum for compound **8** (DMF-d7).

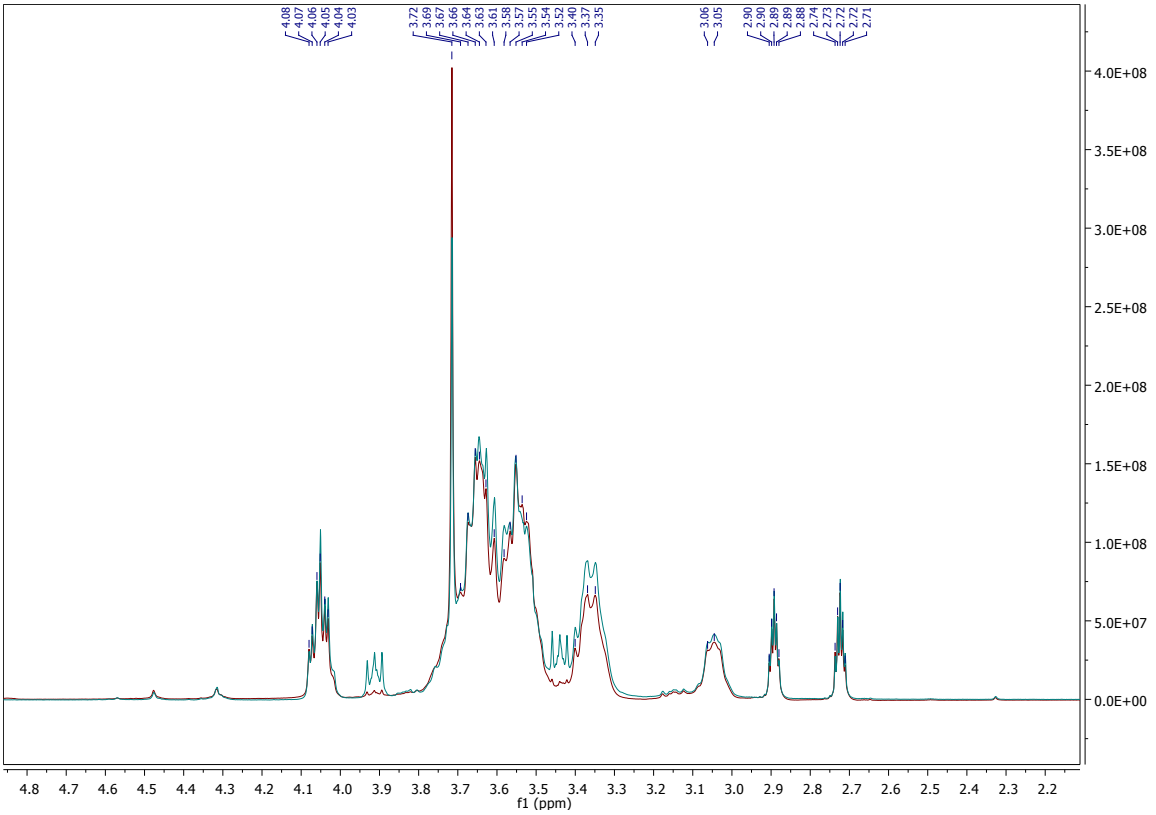


Figure S11. ^{1}H NMR (300 MHz, DMF-d7) spectrum for compound **8** after 20 h in DMF-d7 at 27°C (blue trace) and superposition with the ^{1}H NMR spectrum of triselenide **8** taken immediately after HPLC purification (red trace, see Fig. S5 & S7).

This figure shows that the triselenide compound **8** isolated by HPLC is partially converted into diselenide **7** in DMF-d7 solution.

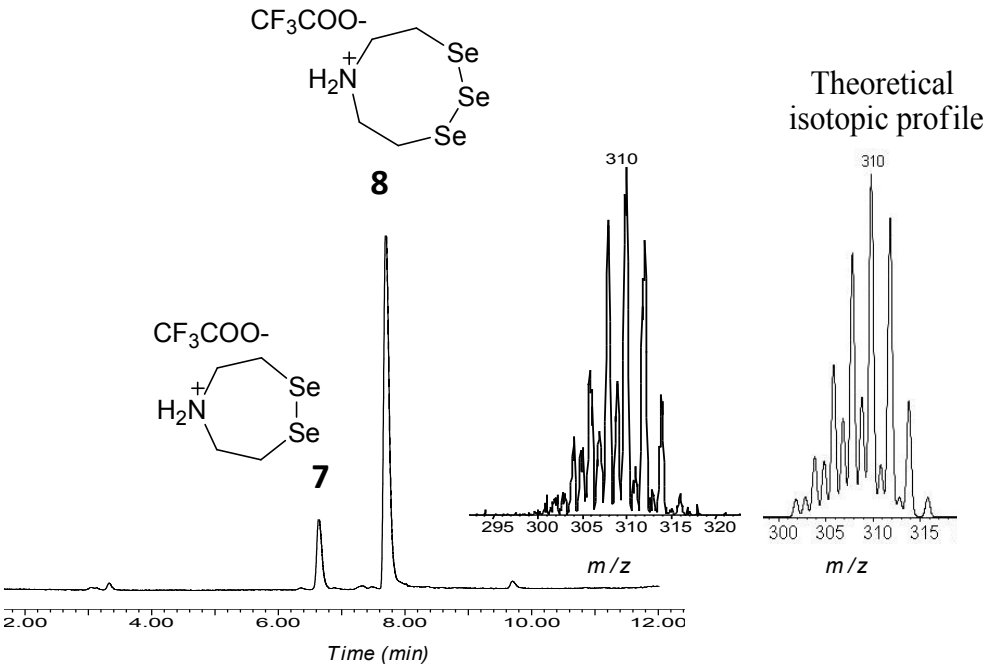


Figure S12. Analytical HPLC profile (λ=215 nm) of purified triselenide compounds **8** after several days in lyophilized form. Note that triselenide compound **8** degraded partially into diselenide compound **7** during storage. The loss of selenium from polyselenides of the type RSe_nR (n > 3) is well documented, see ref [7].

4. Synthesis of SeEA^{off} peptide segments

4.1 Protocol for the synthesis of SeEA^{off} peptides 10a-c using triselenide 8

4.1.1 Optimization of the exchange reaction (Fig. 1). Synthesis of SeEA^{off} peptide 10a starting from SEA^{off} peptide 9a using selenide compounds 7 or 8.

TCEP-HCl (29 mg, 0.1 mmol) was dissolved in 0.2 M pH 4.2 sodium acetate buffer (1 mL). NaOH (5 M) was then used to adjust the pH to 5.5. Peptide **9a** (10 mg, 0.7 μmol) and selenide compound **8** (24 mg, 7 μmol, 10 eq) or a mixture of **7** (30 mg, 7 μmol, 10 equiv) and metallic selenium (1.6 mg, 3 equiv) were dissolved in the above solution. The final peptide concentration was 10 mM (pH 5.5). The reactions were performed at 37°C under nitrogen atmosphere and monitored by LC-MS.

4.1.2 Synthesis of SeEA^{off} peptide 10a starting from peptide 9a

TCEP-HCl (29 mg, 0.1 mmol) was dissolved in 0.2 M pH 4.2 sodium acetate buffer (1 mL). NaOH (5 M) was then added to adjust the pH to 4.1.

Peptide **9a** (4 mg, 2.8 μmol) and selenide compound **8** (12 mg, 28 μmol, 10 equiv) were dissolved in the above solution (312 μL, final peptide concentration 9 mM). The reaction mixture was shaken at 37°C under nitrogen atmosphere and monitored by LC-MS (Figure S13). After 24 h, the mixture was diluted with water-TFA 1% (2 mL) and purified by reversed-phase HPLC using a linear water-acetonitrile gradient containing 0.05% TFA to give the purified product **10a** (2.2 mg, 51% yield).

The determination of the optical purity for the C-terminal amino acid residue within peptide **10a** was performed by chiral GC-MS analysis after acid hydrolysis in deuterated acid. The analysis was done by C.A.T. GmbH & Co. company (Chromatographie und Analysen technik KG, Heerweg 10, D-72070 Tübingen, Germany). The analysis indicated a D-Ala content of 0.23%.

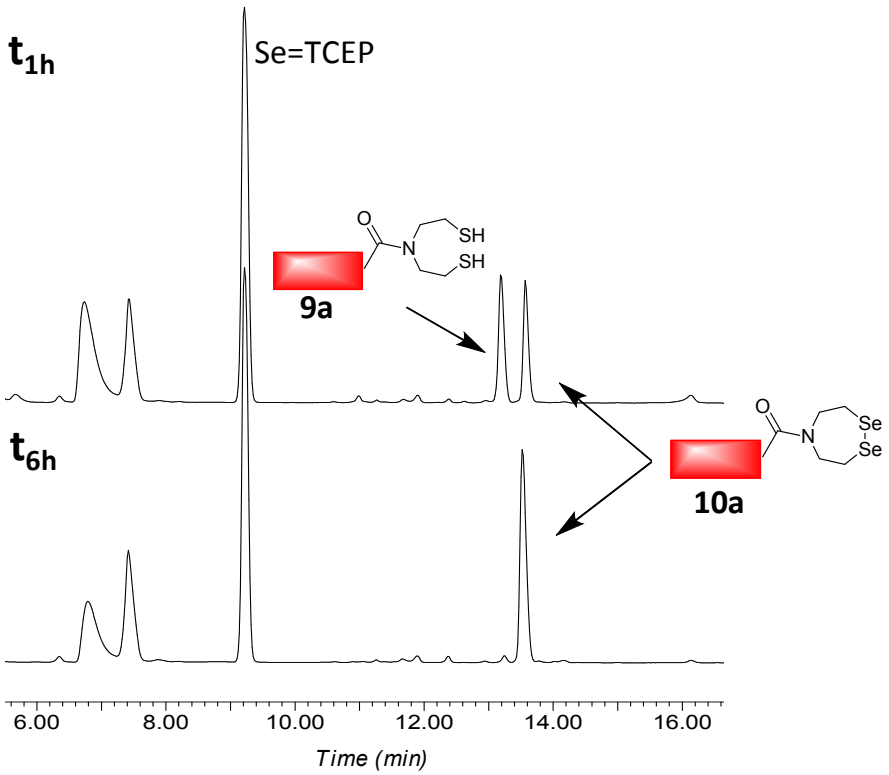


Figure S13. LC-MS analysis of the crude exchange reaction after 24 h (**9a**→**10a**).

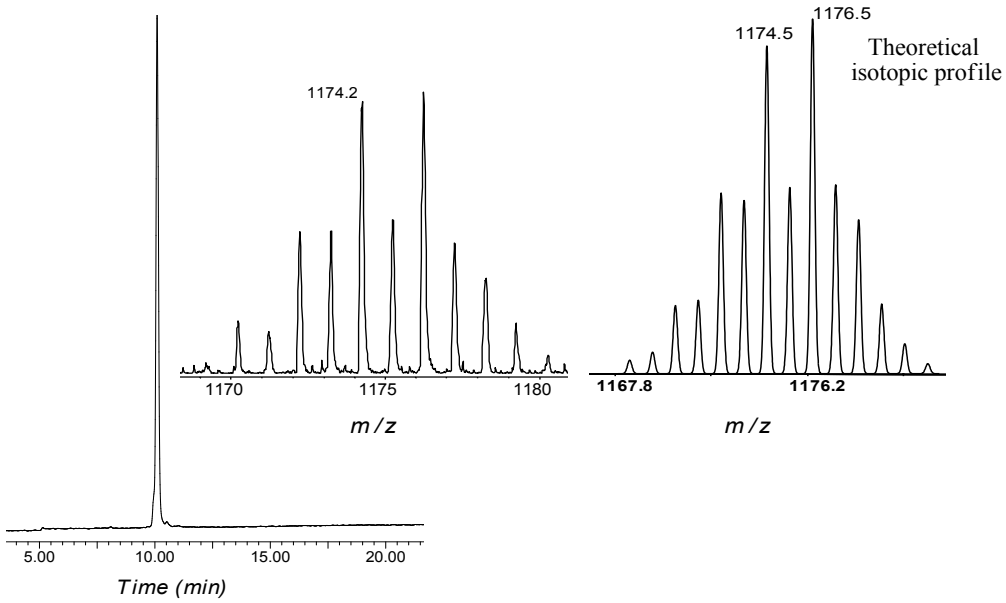


Figure S14. HPLC (λ=215 nm) and MALDI-TOF analysis of the purified peptide **10a**.

4.1.3 Synthesis of SeEA^{off} peptide 10a by using peptide thioester 12a as starting material

TCEP-HCl (29 mg, 0.1 mmol) was dissolved in 0.2 M pH 4.2 sodium acetate buffer (1 mL). NaOH (5 M) was then added to adjust the pH to 5.5.

Peptide **12a** (5 mg, 2.8 μmol) and selenide compound **8** (15 mg, 28 μmol, 10 equiv) were dissolved in the above solution (398 μL, final peptide concentration 9 mM). The reaction mixture was shaken at 37°C under nitrogen atmosphere and monitored by LC-MS (Figure S15). After 30 h, the mixture was diluted with water-TFA 1% (2 mL) and purified by reversed-phase HPLC using a linear water-acetonitrile gradient containing 0.05% TFA to give the purified product **10a** (2.5 mg, 46% yield).

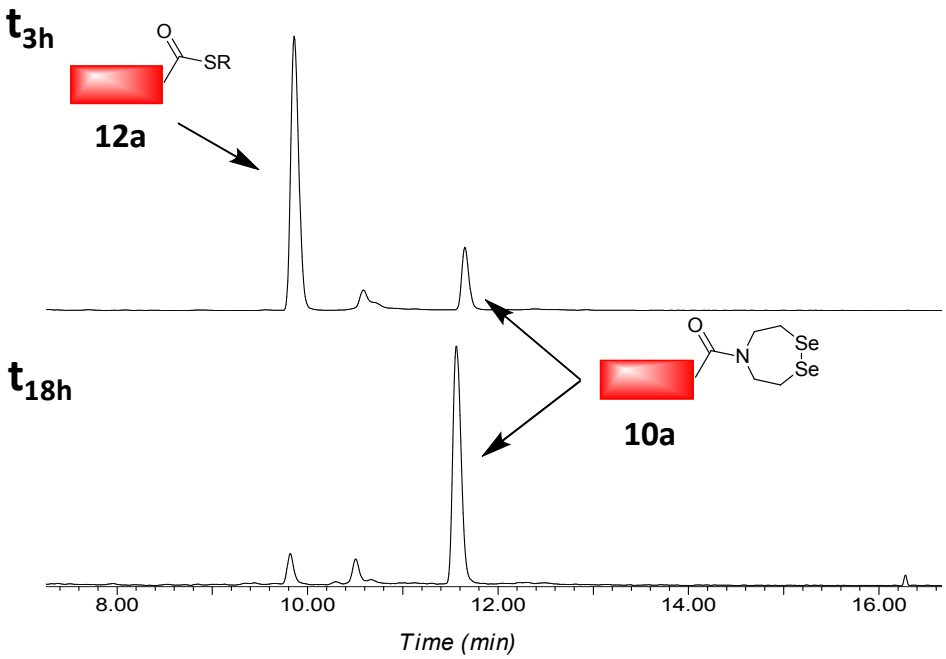


Figure S15. LC-MS analysis of the crude exchange reaction after 18 h (**12a**→**10a**).

4.1.4 Synthesis of SeEA^{off} peptide 10b

Peptide **10b** was synthesized on a 6.9 µmol scale starting from peptide **9b** by using the same procedure as described above for the conversion of **9a** into **10a**. The reaction mixture was shaken at 37°C under nitrogen atmosphere and monitored by LC-MS (Figure S16). After 72 h, the mixture was diluted with water-TFA 1% (2 mL) and purified by reversed-phase HPLC using a linear water-acetonitrile gradient containing 0.05% TFA to give the purified product **10b** (4.3 mg, 40% yield).

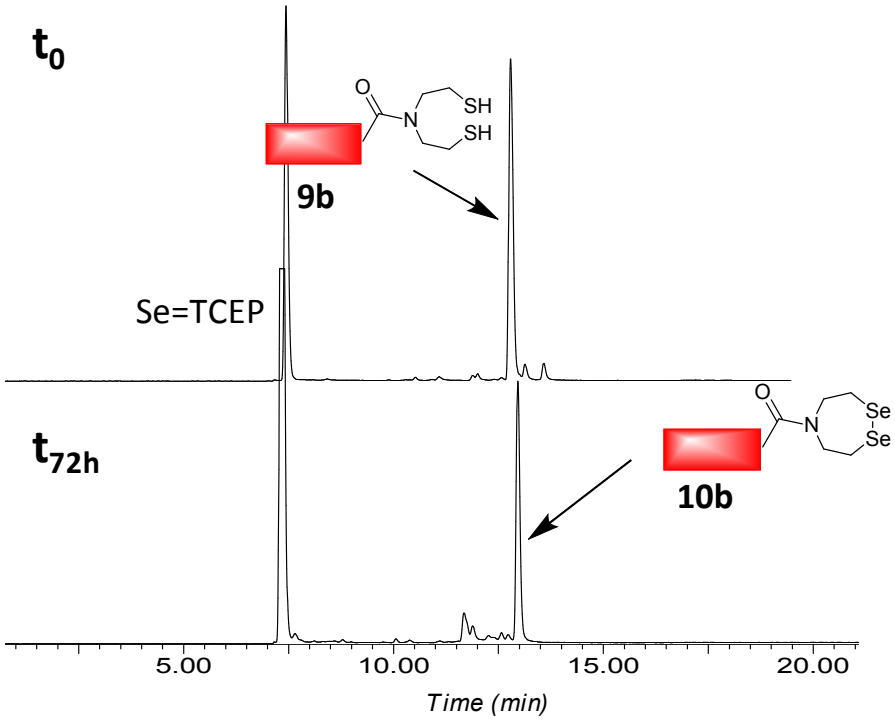


Figure S16. LC-MS analysis of the crude exchange reaction after 72 h (**9b**→**10b**).

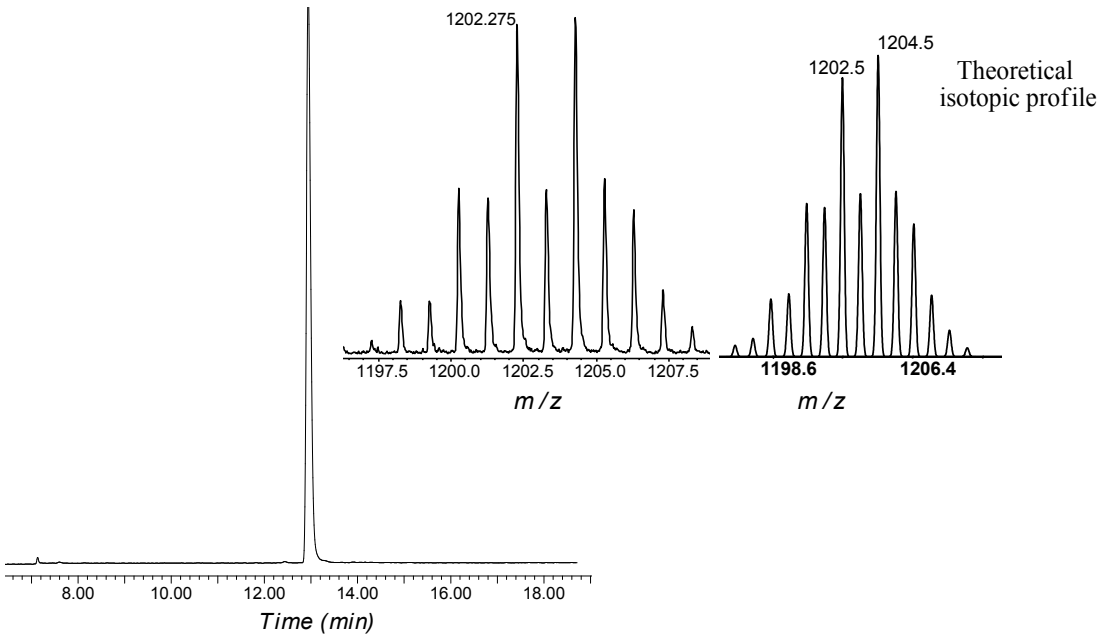


Figure S17. HPLC (λ=215 nm) and MALDI-TOF analysis of the purified peptide **10b**.

4.1.5 Synthesis of SeEA^{off} peptide 10c

TCEP-HCl (6 mg, 0.02 mmol) was dissolved in 0.2 M pH 4.2 sodium acetate buffer (1 mL). NaOH (5 M) was then used to adjust the pH to 4.1.

Peptide **9c** (5 mg, 1.4 μmol) and selenide compound **8** (5.9 mg, 14 μmol, 10 eq), were dissolved in the above solution (698 μL, final peptide concentration 2 mM). The reaction mixture was shaken at 37°C under nitrogen atmosphere and monitored by LC-MS (Figure S18). After 32 h, the mixture was diluted with water-TFA 1% (2 mL) and purified by reversed-phase HPLC using a linear water-acetonitrile gradient containing 0.05% TFA to give the purified product **10c** (1.9 mg, 38% yield).

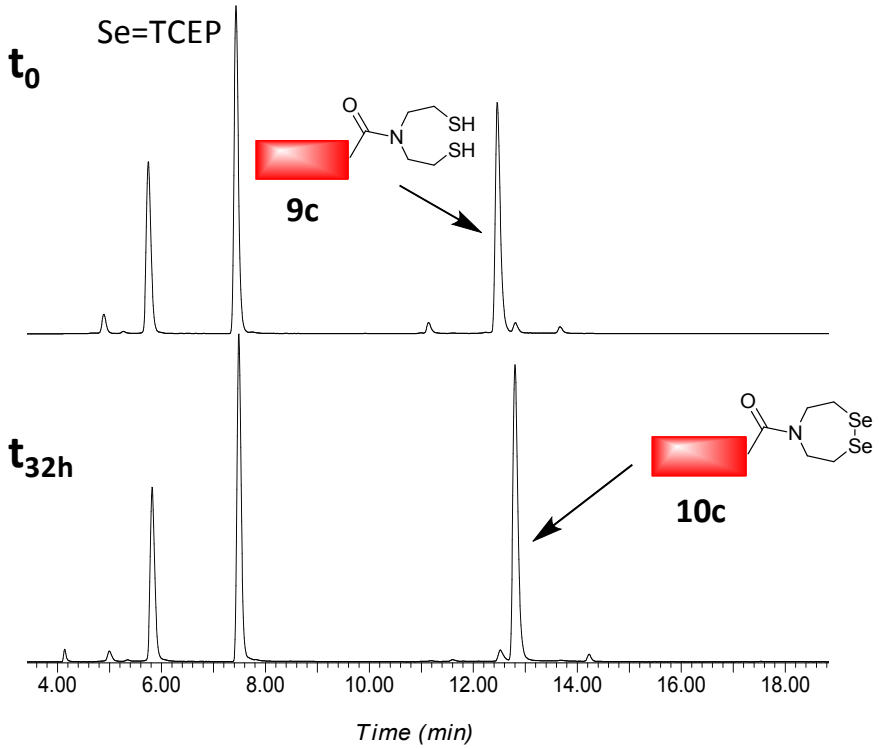


Figure S18. LC-MS analysis of the crude exchange reaction after 32 h (**9c**→**10c**).

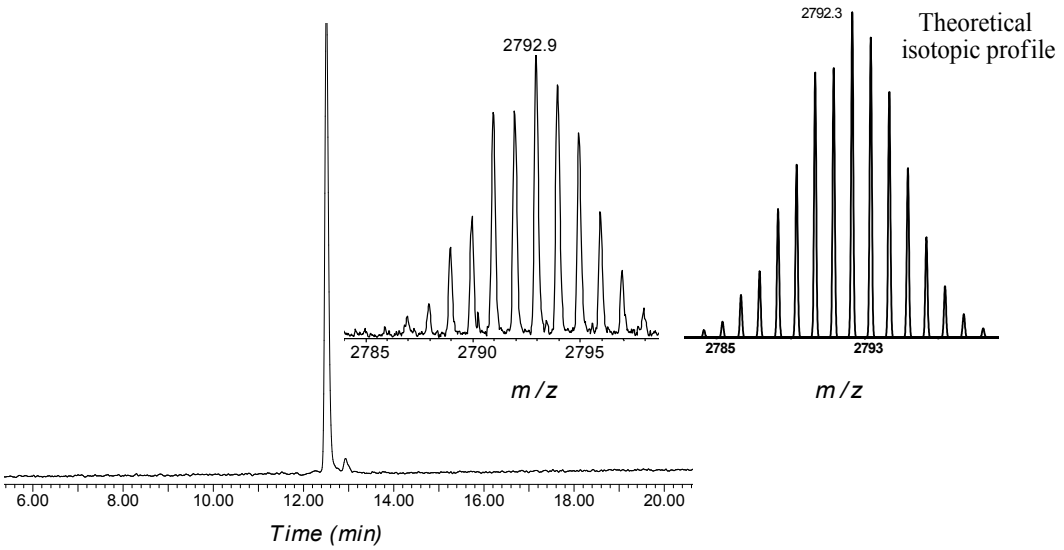


Figure S19. HPLC (λ=215 nm) and MALDI-TOF analysis of the purified peptide **10c**.

4.2 Protocol for the synthesis of SeEA^{off} peptide 10d by using peptide acid 13d as starting material

Diselenide **7** (4.5 mg, 14 μmol, 2 eq) was added to a solution of peptide **13d** (5 mg, 7 μmol) dissolved in anhydrous DMF (0.6 mL, final peptide concentration 10 mM). (Benzo-triazol-1-yloxy)tri-pyrrolidinophosphoniumhexafluorophosphate (PyBOP, 6.8 mg, 14 μmol, 2 equiv) and DIEA (6.8 μL, 0.39 mmol, 6 equiv) were then added to the solution. The mixture was stirred overnight at room temperature under argon atmosphere and the progress of the reaction was monitored by LC-MS. The solvent was then evaporated in vacuo, and the crude product was directly purified by reversed-phase HPLC using a linear water-acetonitrile gradienttogive 2.2 mg (34%) of peptide **10d**.

Ac-GFGQGFGG-SeEA^{off} (**10d**)

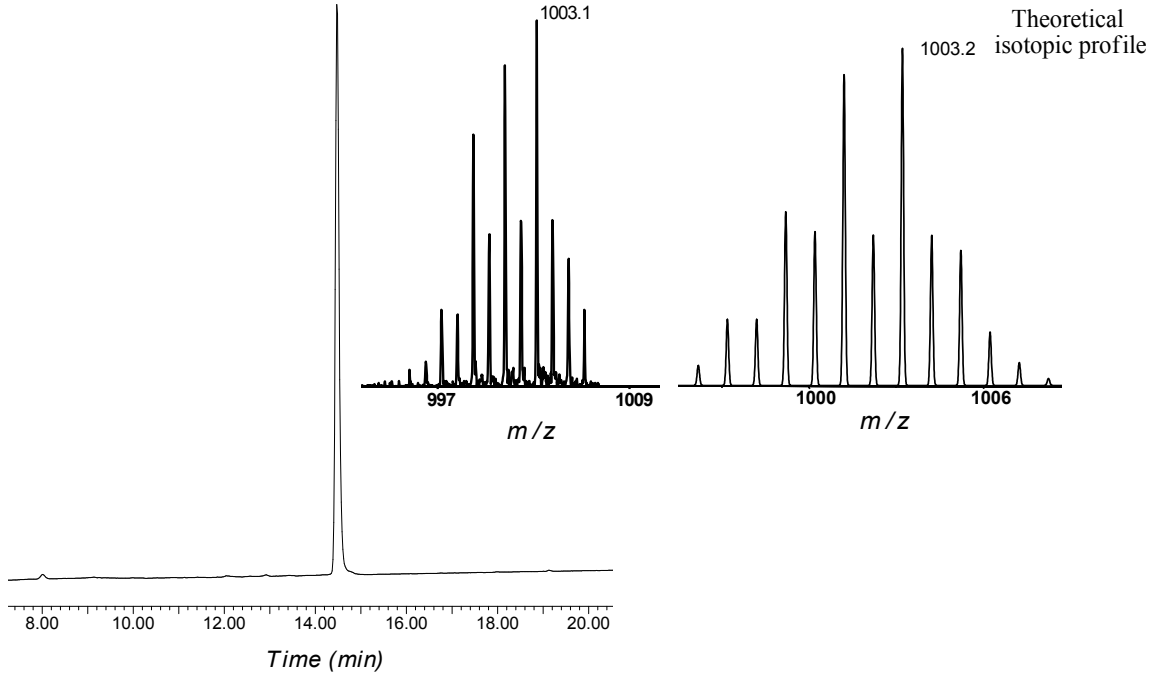


Figure S20. Analytical HPLC profile (λ=215 nm) and MALDI-TOF data for the purified synthetic peptide segments **10d**. MALDI-TOF calc. for $[M+Na]^+$: 1003.2, observed mass: 1003.1 (monoisotopic).

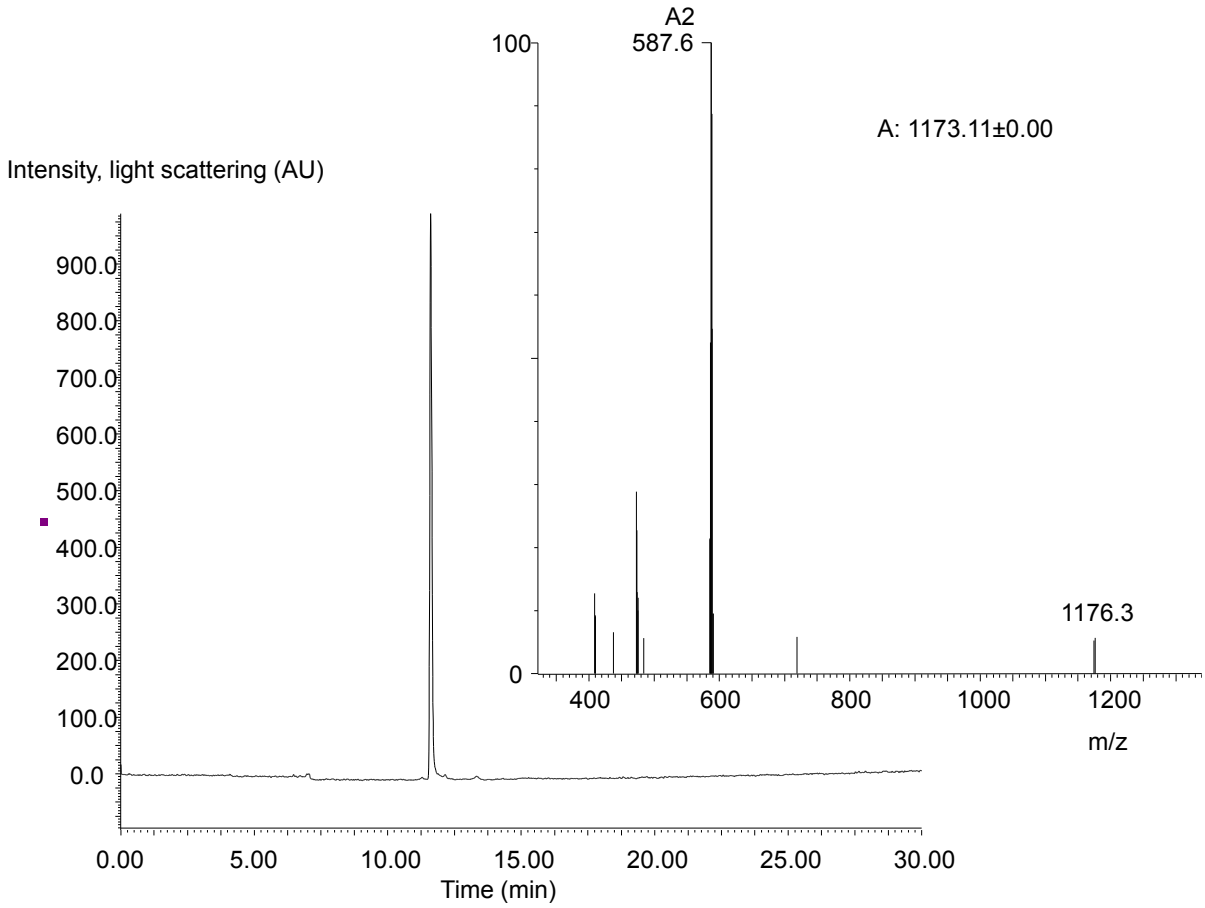
4.3 Protocol for the synthesis of SeEA^{off} peptide 10a by exchange using diselenide 7

4.3.1 Protocol for the synthesis of SeEA^{off} peptides using diselenide 7 + $Se_{(s)}$

TCEP-HCl (28.7 mg, 0.1 mmol), was dissolved in 0.2 M pH 4.2 sodium acetate buffer (1 mL).
Diselenide compound **7** (24.1 mg, 70 μmol, 10 equiv) and metallic selenium (1.6 mg, 3 equiv) were suspended in the above solution (703 μL). NaOH (5 M) was then added to adjust the pH to 5.5.
Peptide **9a** (10 mg, 7 μmol) was dissolved in the above solution (703 μL, final peptide concentration 10 mM). The reaction mixture was shaken at 37 °C under nitrogen atmosphere and monitored by LC-MS. After

20 h, the mixture was diluted with water-TFA 1 % (2 mL) and purified by reversed-phase HPLC (eluent A = water containing 0.1% TFA, eluent B=acetonitrile in water 4/1 containing 0.1 % TFA, 50 °C, detection at 215 nm, 6 mL/min, 0 to 10 % eluent B in 10 min, then 10 to 25 % eluent B in 45 min, C18 XBridge column) to give 5.92 mg of pure product (56 %).

A)



B)

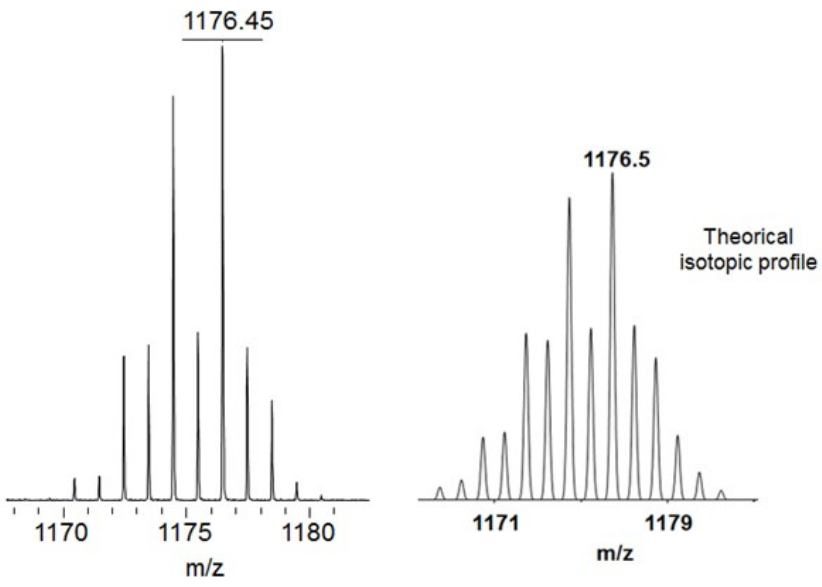


Figure S21. A) LC-MS analysis of peptide **10a**. LC trace, eluent A 0.10 % TFA in water, eluent B 0.10 % TFA in CH_3CN/water: 4/1 by vol. C18 Xbridge BEH 300 Å 5 µm (4.6 x 250 mm) column, gradient 0-100 % B in 30 min (1 mL/min, detection 215 nm). MS trace: $[M+2H]^{2+}$ m/z calcd. 589.3, obs. 587.6. B) MALDI-TOF analysis and comparison with the theoretical profile.

5. Kinetic measurements (Fig. 2)

5.2 General procedure for kinetic measurements

Table S4: Peptides used for the kinetic study

SEA^{off}, SeEA^{off} or thioester peptide	
9a	H-ILKEPVHG**A-**SEA
12a	H-ILKEPVHG**A-**S$(CH_2)_2$COOH
10a	H-ILKEPVHG**A-**SeEA
9b	H-ILKEPVHG**V-**SEA
12b	H-ILKEPVHG**V-**S$(CH_2)_2$COOH
10b	H-ILKEPVHG**V-**SeEA

For SEA peptides **9a,b** and thioester peptides **12a,b**: TCEP-HCl (29 mg, 0.1 mmol) and MPAA (17 mg, 0.1 mmol) were dissolved in 0.1 M pH 7.2 sodium phosphate buffer (1 mL). NaOH (5 M) was then added to adjust the pH to 7.1.

For SeEA peptides **10a,b**: TCEP-HCl (29 mg, 0.1 mmol), MPAA (17 mg, 0.1 mmol) and Se=TCEP (12 mg, 35 μmol) were dissolved in 0.1 M pH 7.2 sodium phosphate buffer (1 mL). NaOH (5 M) was then added to adjust the pH to 7.1. Se=TCEP is used to inhibit the deselenization of SeEA peptides by TCEP.[8]

Peptides **9a-b or 10a-b or 12a-b** (1 μmol) and peptide **14** (1.5 μmol, 1.5 equiv) were dissolved in the above solution (300 μL, final peptide concentration 3.5 mM, pH 7.1). The ligations were performed at 37°C under nitrogen atmosphere and monitored by RP-HPLC. For each time point, 10 µL aliquots were withdrawn, quenched by adding 90 µL of 1% aqueous TFA and extracted with Et_2O to remove MPAA. The samples were stored at –20°C until analysis.

5.3 Synthesis of peptide 15a by reaction of SEA^{off} peptide 10a with Cys peptide 14

For the ligation of SeEA^{off} peptide peptide **10a** with Cys peptide **14** on a preparative scale, the reaction was scaled up to 3.3 μmol. In this reaction, TCEP-HCl (29 mg, 0.1 mmol), MPAA (17 mg, 0.1 mmol) and Se=TCEP (26 mg, 0.08 mmol) were dissolved in 0.1 M pH 7.2 sodium phosphate buffer (1 mL). NaOH (5 M) was then added to adjust the pH to 7.1. Peptide **10a** (5 mg, 3.3 μmol) and peptides **14** (7 mg, 4.9 μmol, 1.5 eq) were dissolved in the above solution (942 μL, 3.5 mM final concentration). After completion of the ligation, the mixture was diluted with water-TFA 5% (2 mL), extracted with Et_2O (3 × 500 μL) to remove MPAA and purified by reversed-phase HPLC using a linear water-acetonitrile gradient containing 0.05% TFA to give the purified ligation product **15a** (4.8 mg, 56% yield)**.**

Determination of optical purity of amino acid derivative of peptide ligation product **15a** by chiral GC-MS after acid hydrolysis showed 0.43% of D-Ala C-terminus.

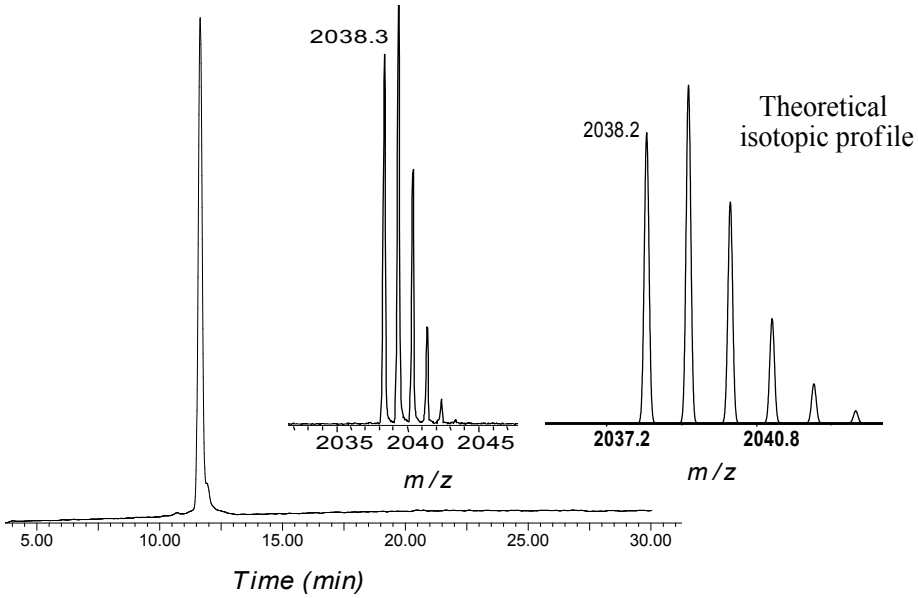


Figure S22. Analytical HPLC profile (λ=215 nm) and MALDI-TOF data for the purified peptide **15a**. Calcd. for $[M+H]^+$: 2038.3, observed mass: 2038.2 (monoisotopic).

.

6. SeEA/SEA kinetically Controlled Ligation

6.1 Synthesis of peptide 23d

TCEP-HCl (58 mg, 0.2 mmol) and MPAA (33 mg, 0.2 mmol) were dissolved in 6 M guanidine-HCl, 0.1 M pH 7.2 sodium phosphate buffer (1 mL). NaOH (5 M) was then added to adjust the pH to 7.1.

Peptide **10d** (5.3 mg, 5.4 μmol) and peptide **19** (7.6 mg, 5.4 μmol) were dissolved in the above solution (780 μL, final peptide concentration 7 mM) resulting in the reduction of $SeEA^{off}$ and SEA^{off} groups and in the removal of cysteine S*t*Bu protecting group. The mixture was stirred at 37 °C under nitrogen atmosphere and the progress of the ligation was monitored by LC-MS. Figure S23A was obtained after 4 h of reaction. After 7 h, peptide segment **22** (11 mg, 8.1 μmol, 1.5 eq) was added to the reaction mixture which was further stirred at 37°C under nitrogen. Figure S23B corresponds to the LC-MS analysis of the crude after 48 h. After 48 h, the reaction mixture was diluted with water (2 mL), acidified with 5% aqueous TFA (2 mL) and extracted with diethylether to remove the excess of MPAA. The crude product was directly purified by reversed-phase HPLC using a linear water-acetonitrile gradient containing 0.05% TFA to give the purified ligation product **23d** (4.5 mg, 27% yield) (Figure S24).

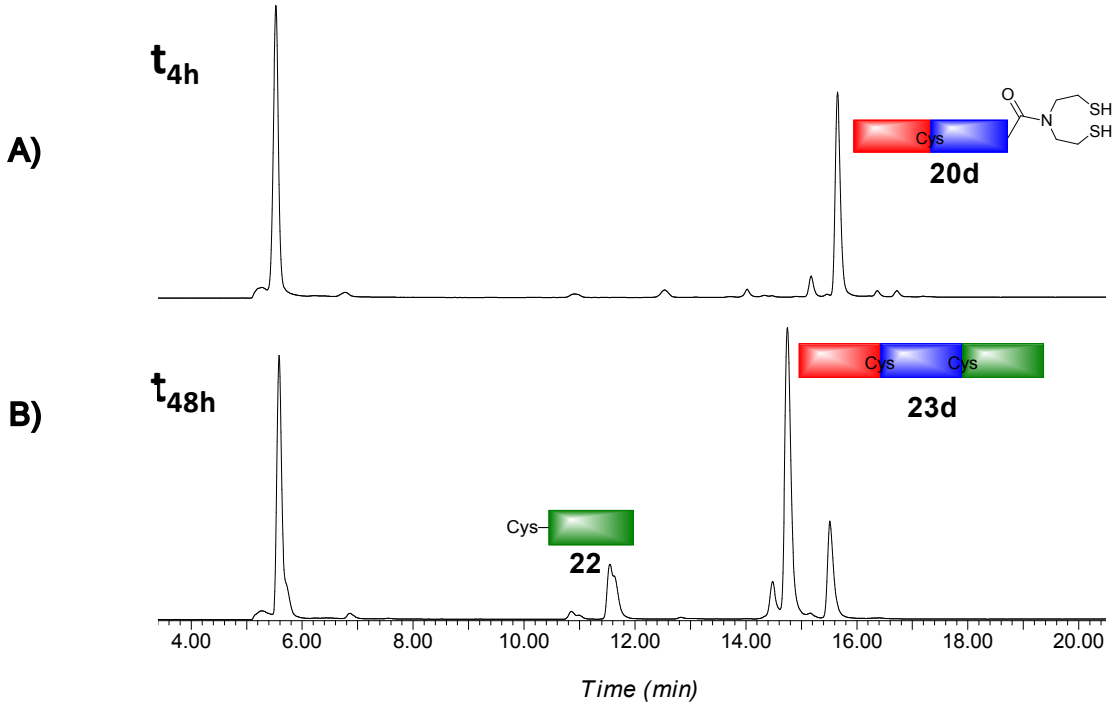


Figure S23. HPLC analysis (λ=215 nm) of the crude kinetically controlled assembly process leading to the formation of peptide **23d** after 4 h (SeEA ligation step, up) and after 48 h (SEA ligation step, down).

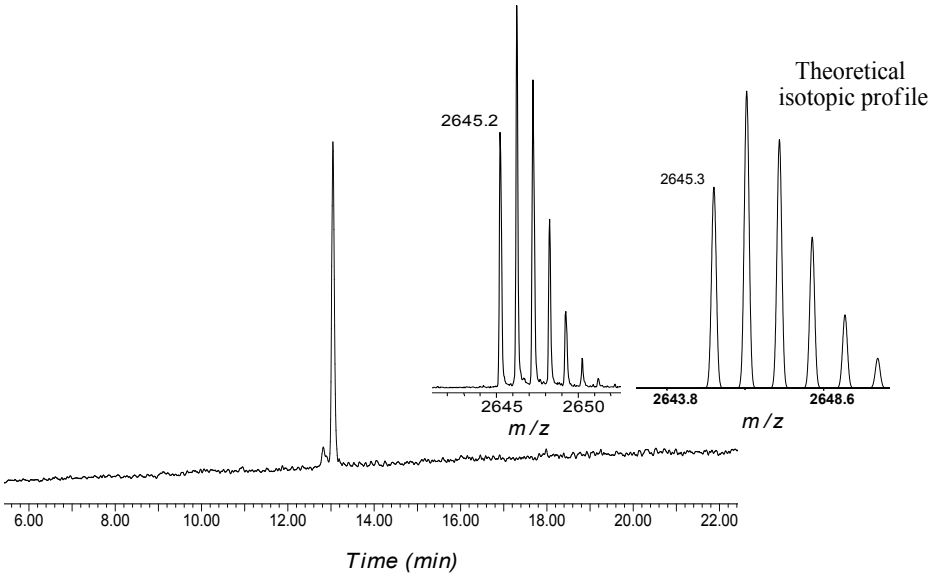


Figure S24. Analytical HPLC profile (λ=215 nm) and MALDI-TOF data for the purified peptide **23d**. Calcd. for $[M+H]^+$: 2645.3, observed mass: 2645.2 (monoisotopic).

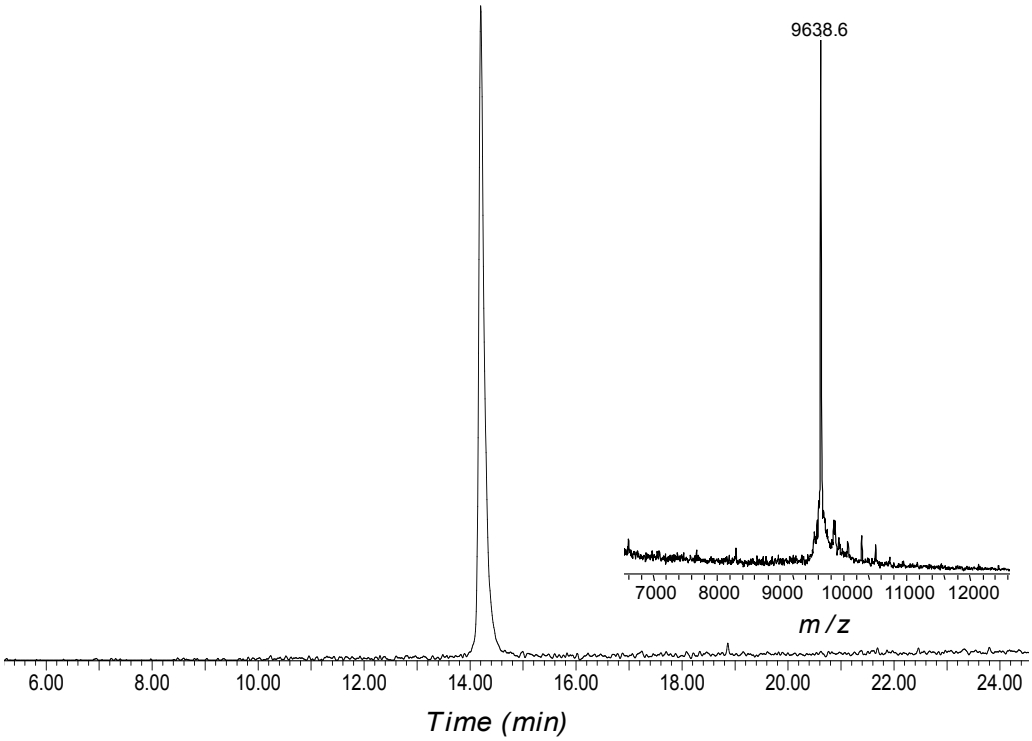
6.2 Total synthesis of K1 domain of human hepatocyte growth factor (23c, Fig. 5)

TCEP-HCl (29 mg, 0.1 mmol), MPAA (17 mg, 0.1 mmol) and Se=TCEP (26 mg, 0,08 mmol) were dissolved in 6 M guanidine-HCl, 0.1 M pH 7.2 sodium phosphate buffer (1 mL). NaOH (5 M) was then added to adjust the pH to 7.1.

Peptide **10c** (5 mg, 1.4 μmol) and peptide **18** (7 mg, 1.7 μmol, 1.2 eq) were dissolved in the above solution (348 μL, final peptide concentration 4 mM). The mixture was stirred at 37 °C under nitrogen atmosphere

and the progress of the ligation was monitored by LC-MS (see Figure 6). After 30 h, peptide segment **21** (7 mg, 1.7 µmol, 1.2 equiv) was added to the reaction mixture which was further stirred at 37°C under nitrogen for 72 h. The reaction mixture was then diluted with water (2 mL), acidified with 5% aqueous TFA (2 mL) and extracted with diethylether to remove the excess of MPAA. The crude product was directly purified by reversed-phase HPLC using a linear water-acetonitrile gradient containing 0.05% TFA to give K1 polypeptide **23c** (3.3 mg, 21% yield) (Figure S25).

a)



b)

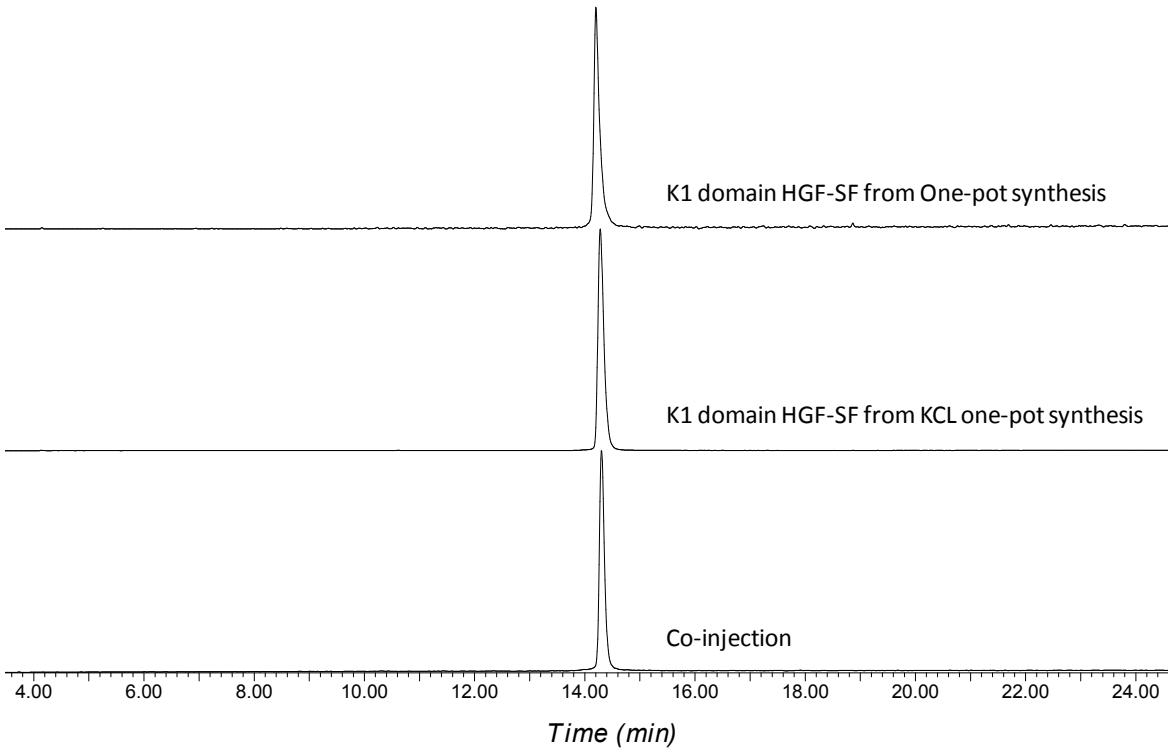


Figure S25. a) Analytical HPLC profile (λ=215 nm) and MALDI-TOF data for the purified K1 polypeptide **23c**. Calc. for $[M+H]^+$: 9639.8, observed mass: 9638.6 (average isotopic composition). b) Comparison between synthetic K1 produced by "one-pot" three-segment ligation (see ref [3]) and by the SeEA/SEA KCL method discussed in this paper.

7. Synthesis of SeEA peptide 31

7.1 One-pot synthesis of MPA peptide thioester 26 (one-pot process I)

The synthesis of peptides **24** and **25** was performed as described elsewhere.[9]

6 M Gdn.HCl 0.1 M pH 7.2 sodium phosphate buffer was degassed during 30 min. MPAA was dissolved in this solution to a final concentration of 200 mM. NaOH (5 M) was then added to adjust the pH to 7.1.

Peptide thioester **24** (13.09 mg, 2.12 µmol) and SEA^{off} peptide **25** (8.84 mg, 2.12 µmol) were dissolved in the above solution (650 µL, final peptide concentration 3.3 mM). The mixture was stirred at 37 °C under nitrogen atmosphere. After 20 h, the exchange of the SEA group by MPA was started by adding a 20 % MPA solution in water containing 0.2 M TCEP (pH=3.9, 650 µL). After 20 h, the reaction mixture was diluted with 10 % aqueous acetic acid (22 mL), extracted with Et_2O (3 x 15 mL) to remove MPAA and purified by RP-HPLC (eluent A = water containing 0.1 % TFA, eluent B=acetonitrile in water 4/1 containing 0.1 % TFA, 50 °C, detection at 215 nm, 6 mL/min, 0 to 10 % eluent B in 5 min, then 10 to 40 % eluent B in 60 min, C18 XBridge column) to give 9.84 mg of thioester peptide **26** (46.5 %).

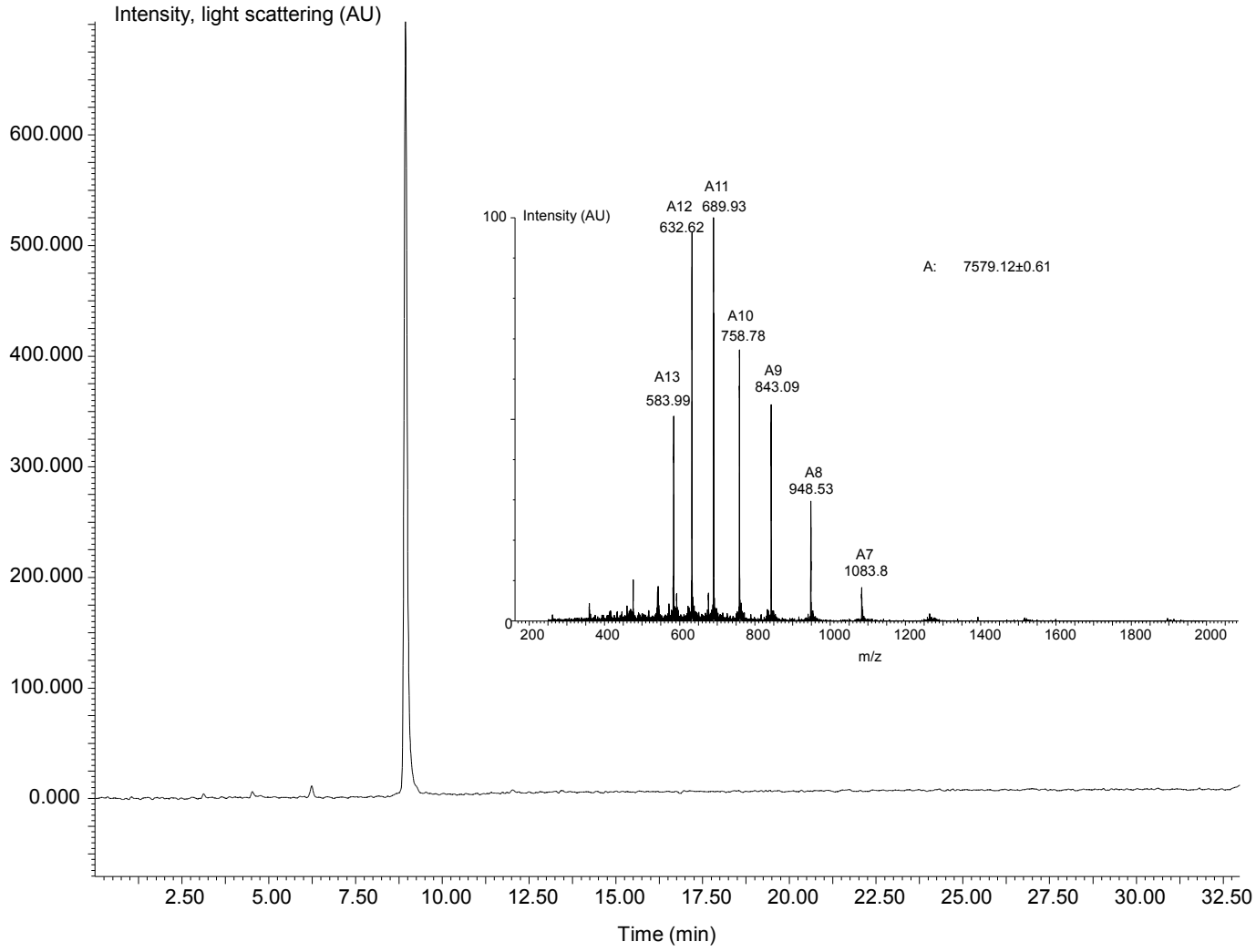


Figure S26. LC-MS analysis of thioester peptide **26.** LC trace, eluent A 0.10 % TFA in water, eluent B 0.10 % TFA in CH_3CN/water: 4/1 by vol. C18 Xbridge BEH 300 Å 5 µm (4.6 x 250 mm) column, gradient 0-100 % B in 30 min (1 mL/min, detection 215 nm). MS trace: M calculated (mean) 7580.01, found 7579.12±0.61.

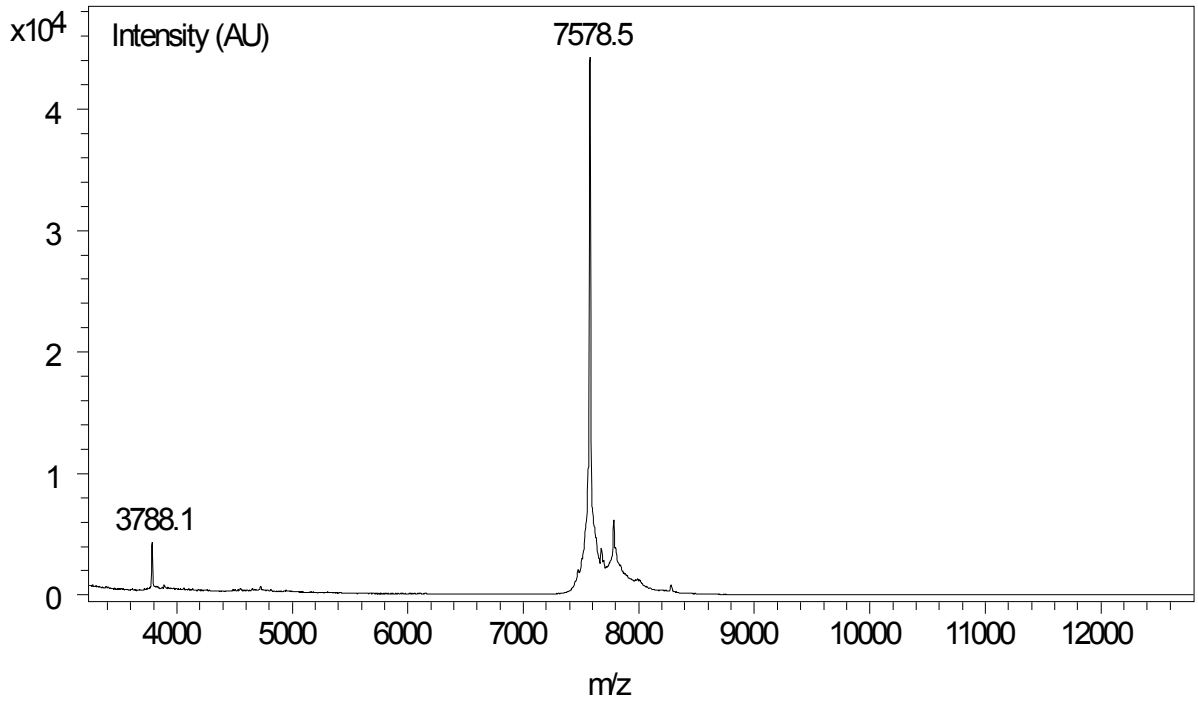
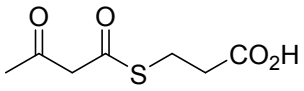


Figure S27. MALDI-TOF analysis of thioester peptide **26**. Matrix : 2,5-dihydroxybenzoic acid (DHB), positive mode. $[M+H]^+$ calcd. (mean) 7580.01, found 7578.5.

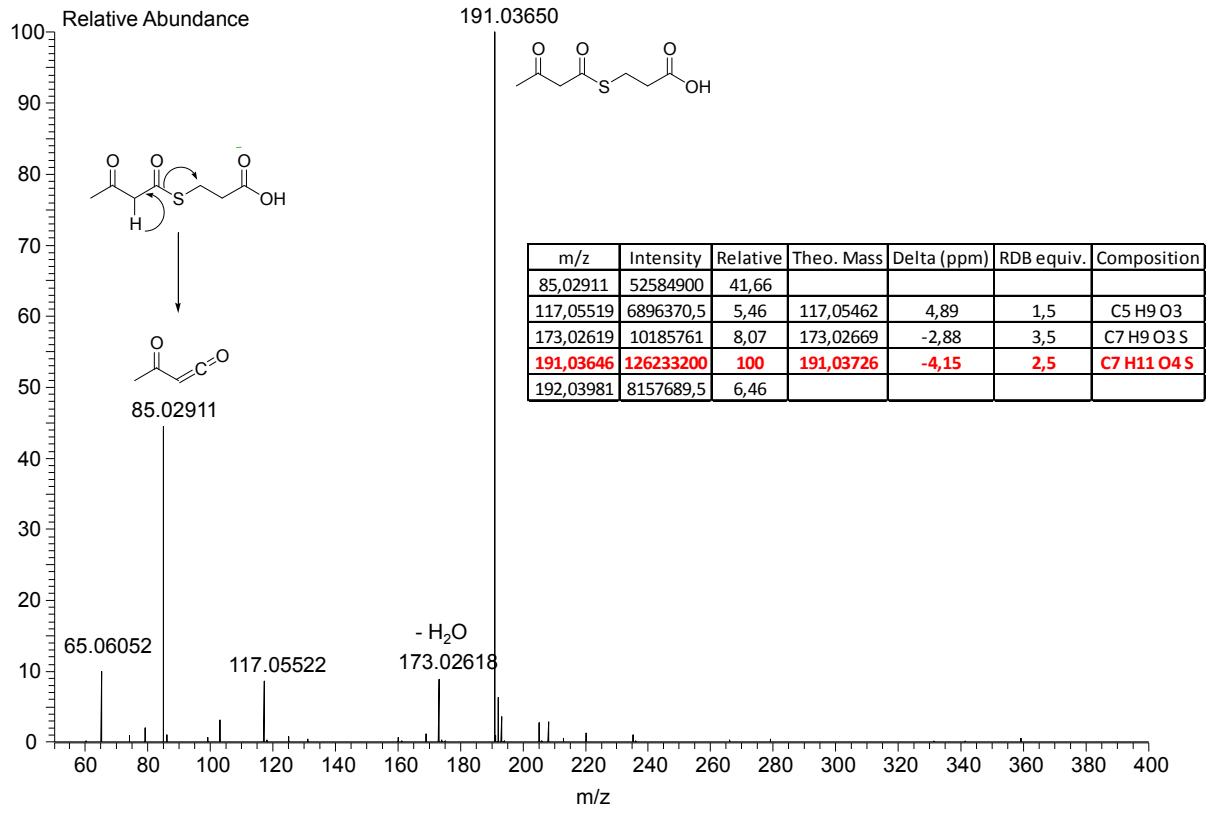
7.2 One-pot synthesis of SeEA peptide 30

7.2.1 Synthesis of AcA-MPA



AcA-MPA

N-hydroxysuccinimidyl acetoacetate (265.4 mg, 1.33 mmol) was dissolved in CH_2Cl_2 (4 mL) at rt under argon. 3-Mercaptopropionic acid (116 µL, 1.33 mmol) and *N*-methylmorpholine (292.8 µL, 2.66 mmol) were added in one portion and the reaction medium was stirred overnight. Then, the solvent was evaporated and the resulting yellow oil was purified by silica gel chromatography (ethyl acetate/cyclohexane : 4/6 v/v containing 1 % acetic acid) to give 49.0 mg of **AcA-MPA** (19.4 %).



m/z	Intensity	Relative	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
85,02911	52584900	41,66				
117,05519	6896370,5	5,46	117,05462	4,89	1,5	C5 H9 O3
173,02619	10185761	8,07	173,02669	-2,88	3,5	C7 H9 O3 S
191,03646	**126233200**	**100**	**191,03726**	**-4,15**	**2,5**	**C7 H11 O4 S**
192,03981	8157689,5	6,46				

Figure S28. High resolution mass spectrometry of AcA-MPA (positive ion mode).

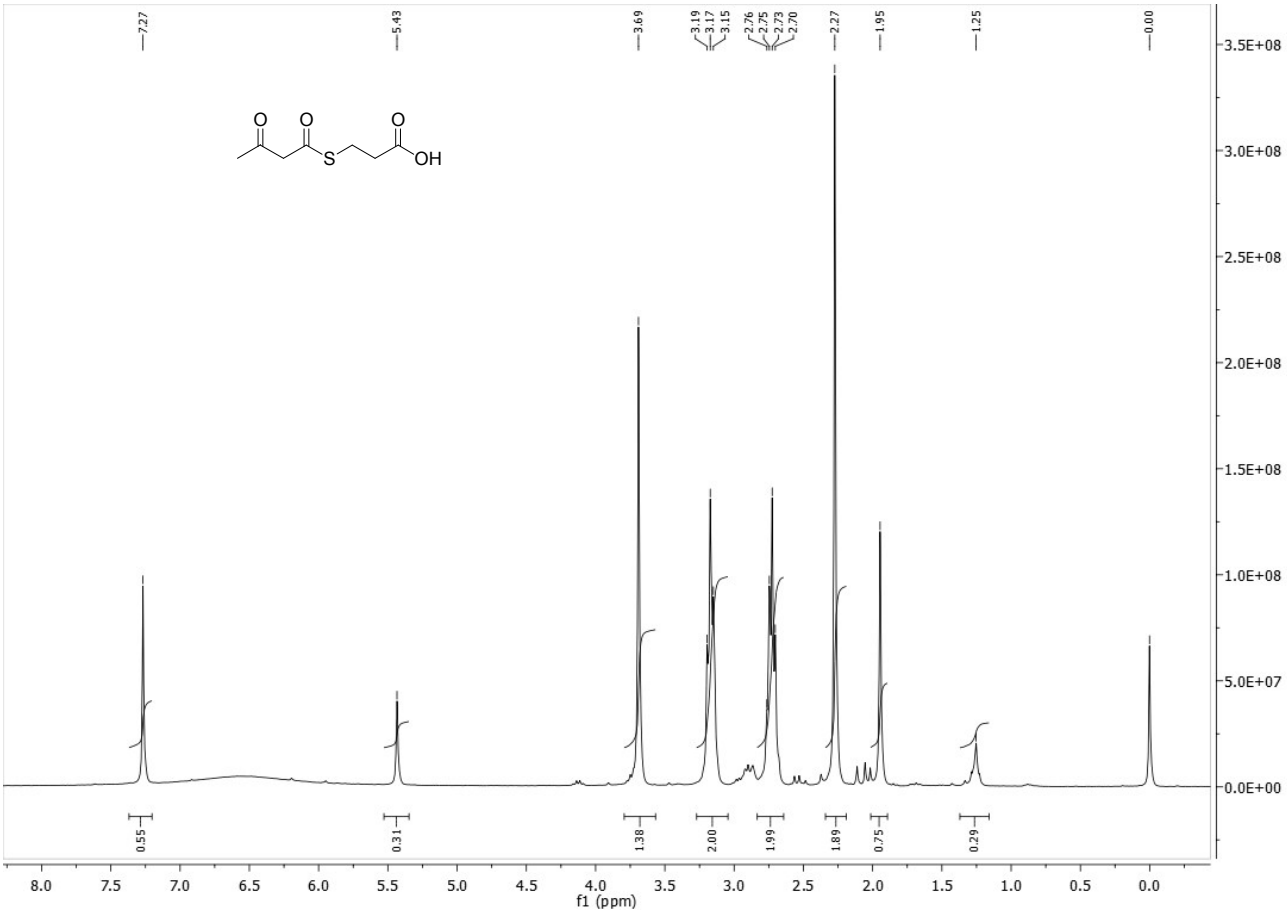


Figure S29. ^{1}H NMR spectrum (300.0 MHz, $CDCl_3$) of AcA-MPA

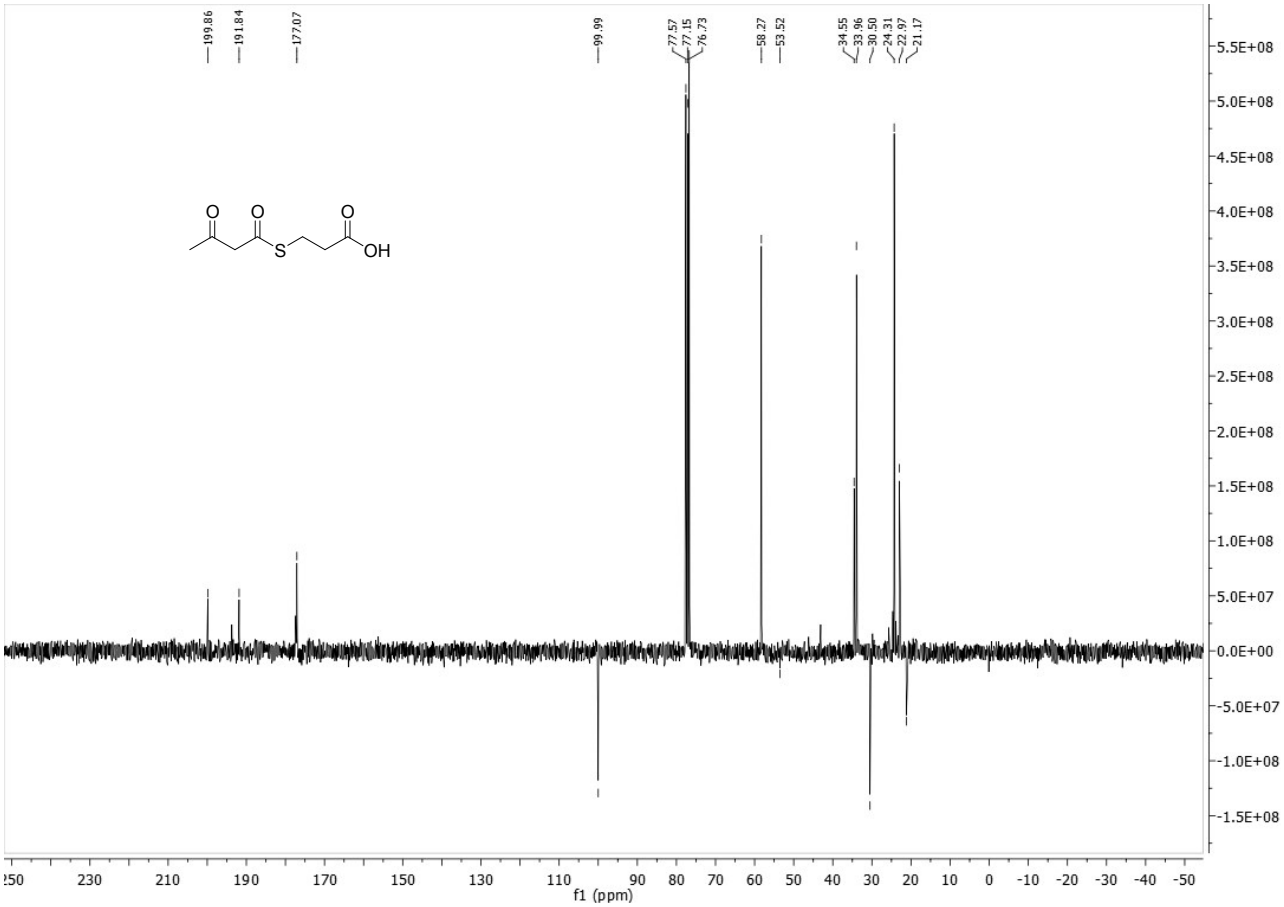


Figure S30. ^{13}C NMR spectrum (75 MHz, $CDCl_3$) of AcA-MPA.

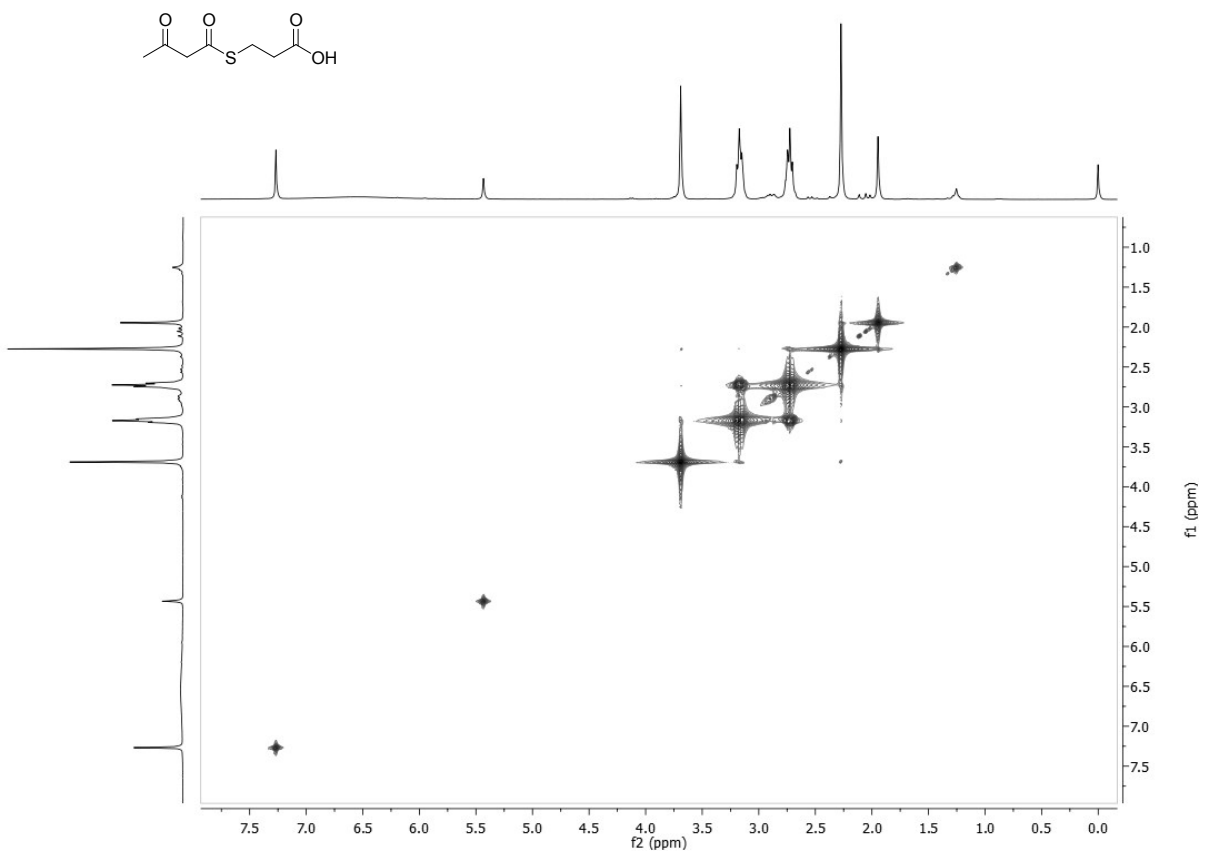


Figure S31. ^{1}H-^{1}H COSY NMR spectrum of AcA-MPA.

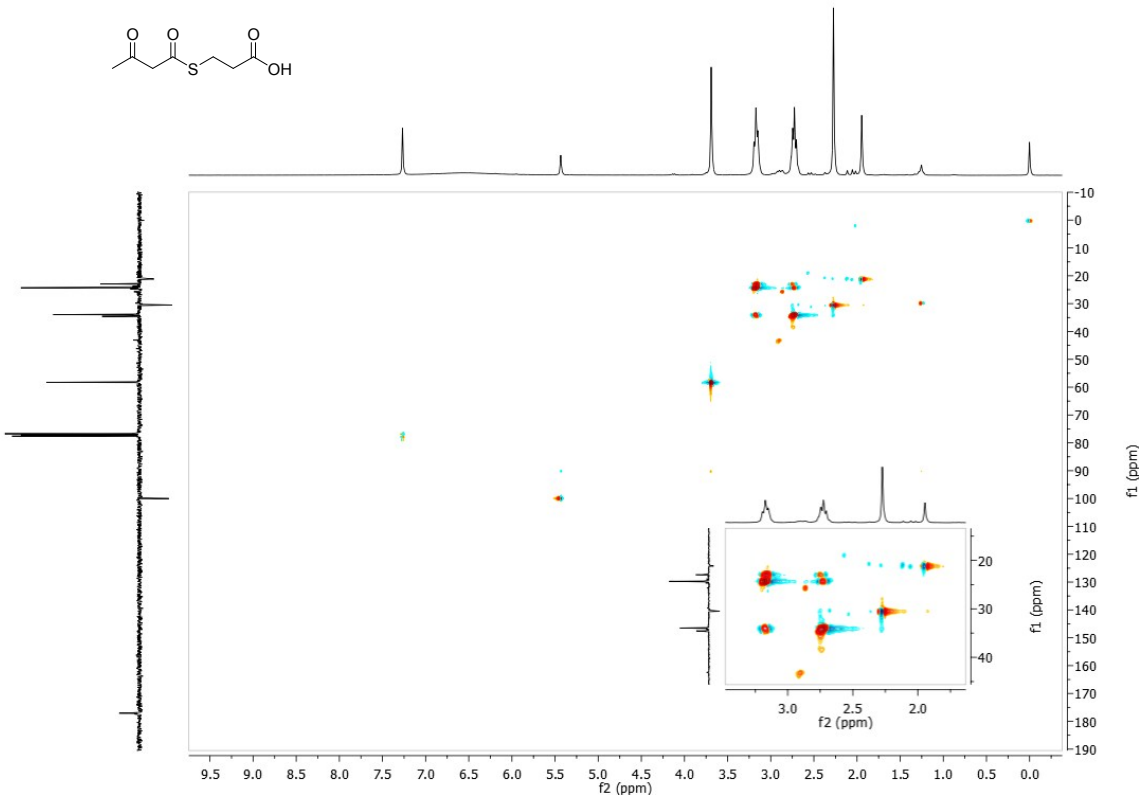


Figure S32. ^{1}H-^{13}C HSQC NMR spectrum of AcA-MPA.

7.2.2 Synthesis of peptide 29

7.2.2.1 Synthesis of AcA-HGF 128-149-SEA^{off}

HGF 128-149: CIIGKGRSYK GTVSITKSGI K

0.1 M pH 7.2 sodium phosphate buffer was degassed during 30 min. Gdn.HCl (2.86 g, 0.03 mol) and MPAA (168.3 mg, 1 mmol) were dissolved in this buffer (5 mL qsp). NaOH (6 M) was then added to adjust the pH to 7-7.5.

Peptide CIIGKGRSYK GTVSITKSGI K-SEA^{off} (19.84 mg, 6.43 µmol) and AcA-MPA (1.47 mg, 7.72 µmol) were dissolved in the above solution (1.5 mL). The mixture was stirred at 37 °C under nitrogen atmosphere overnight.

The reaction mixture was then acidified with glacial acetic acid (400 µL), diluted with water (6.1 mL) and extracted with Et_2O (3 x 2 mL) to remove MPAA. The crude peptide was purified by RP-HPLC (eluent A = water containing 0.1 % TFA, eluent B = acetonitrile in water 4/1 containing 0.1 % TFA, 30 °C, detection at 215 nm, 6 mL/min, 0 to 20 % eluent B in 5 min, then 20 to 40 % eluent B in 60 min, C18 XBridge column) to give 11.9 mg of peptide AcA-CIIGKGRSYK GTVSITKSGI K-SEA^{off} (62.4 %).

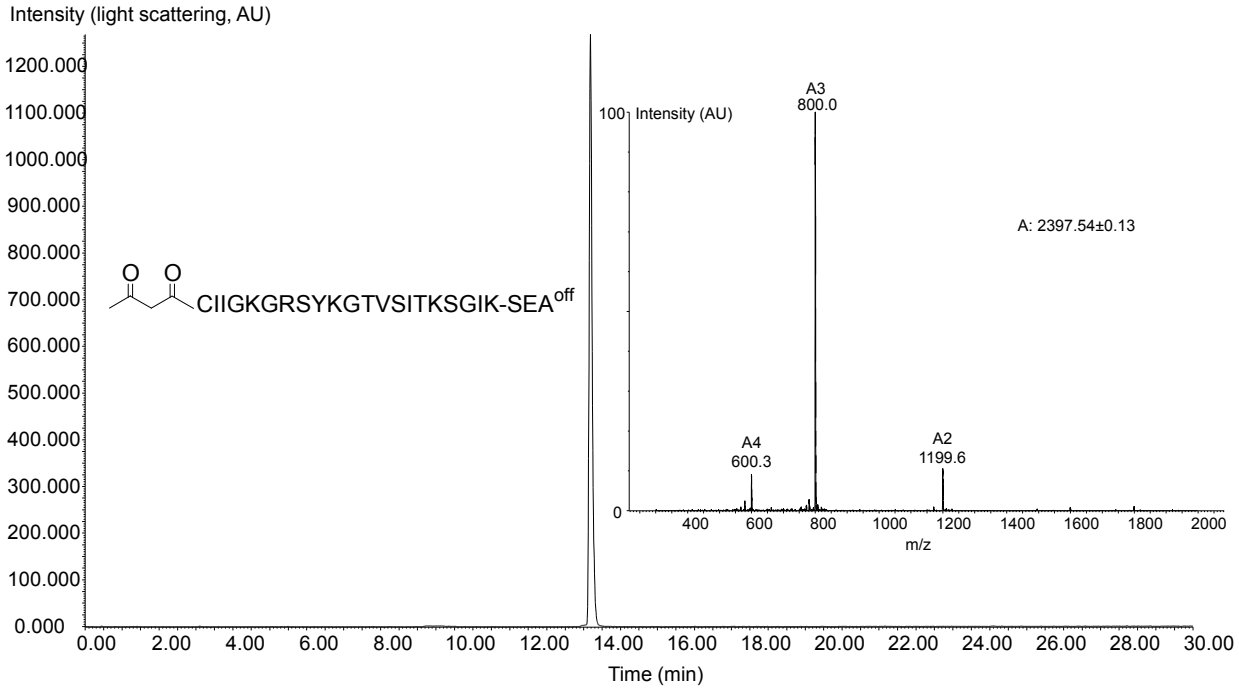


Figure S33. LC-MS analysis of peptide AcA-HGF 128-149-SEA^{off}**.** LC trace, eluent A 0.10 % TFA in water, eluent B 0.10 % TFA in CH_3CN/water: 4/1 by vol. C18 Xbridge BEH 300 Å 5 µm (4.6 x 250 mm) column, gradient 0-100 % B in 30 min (1 mL/min, detection 215 nm). MS trace: $[M+2H]^{2+}$ m/z calcd. 1199.98, obs. 1199.6, $[M+3H]^{3+}$ m/z calcd. 800.32, obs. 800.0, $[M+4H]^{4+}$ m/z calcd. 600.49, obs. 600.3.

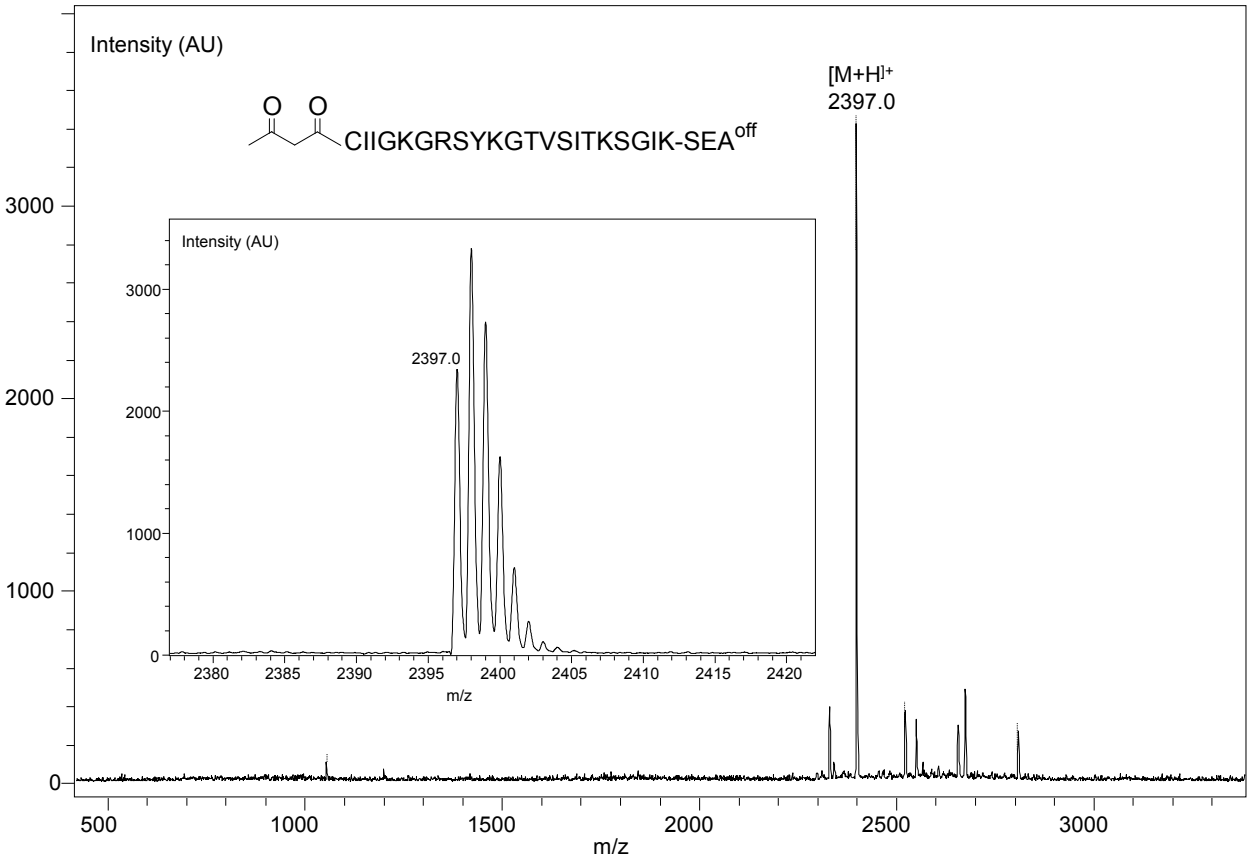


Figure S34. MALDI-TOF analysis of peptide AcA-HGF 128-149-SEA^{off}. Matrix, 2,5-dihydroxybenzoic acid (DHB) positive mode, $[M+H]^+$ calcd. (monoisotopic) 2397.28, found 2397.0

7.2.2.2 Exchange of the SEA group by MPA

A typical procedure for the exchange of the SEA group by MPA was described in detail elsewhere.[4, 5] The analytical HPLC and MS analyses of the purified thioester peptide **29** are shown below.

Yields for the HPLC purified MPA peptide thioester **29**

Peptide 29: 6.41 mg (40% yield, 5.6 μmol scale), MALDI-TOF calcd. for $[M+H]^+$: 2368.3, observed mass: 2368.3 (monoisotopic).

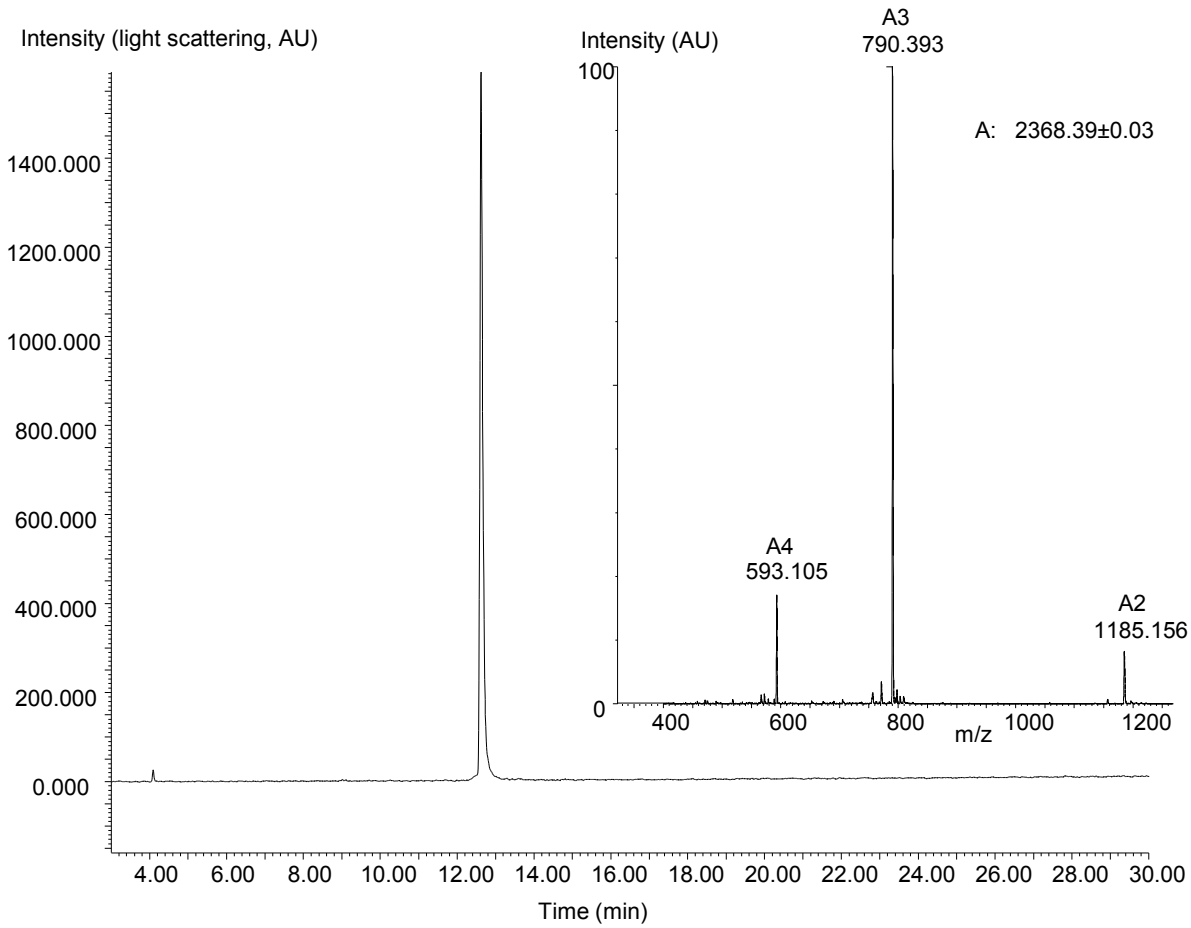


Figure S35. LC-MS analysis of peptide **29**. LC trace, eluent A 0.10 % TFA in water, eluent B 0.10 % TFA in CH_3CN/water: 4/1 by vol. C18 Xbridge BEH 300 Å 5 µm (4.6 x 250 mm) column, gradient 0-100 % B in 30 min (1 mL/min, detection 215 nm). MS trace: M calculated (mean) 2368.84, found 2368.39±0.03.

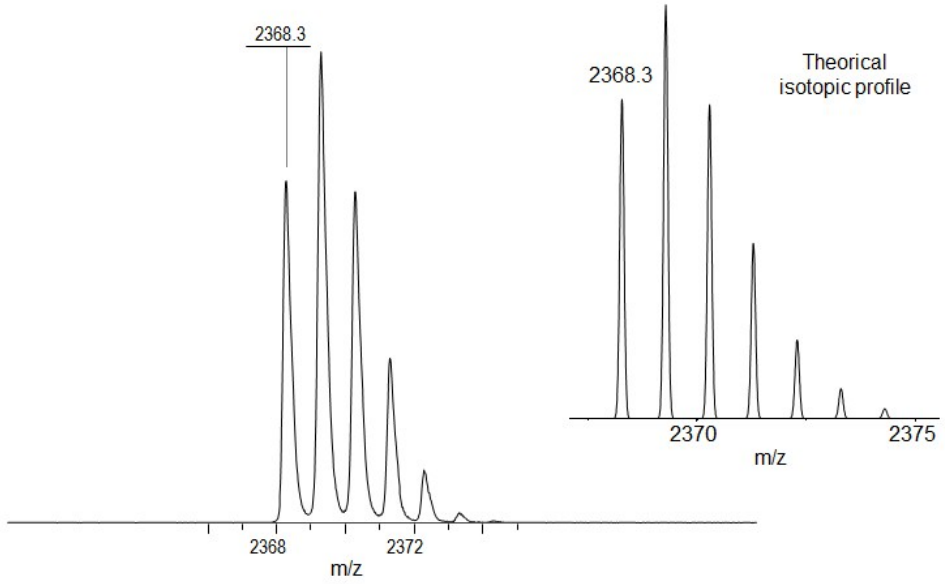


Figure S36. MALDI-TOF analysis of thioester peptide **29**. Matrix, 2,5-dihydroxybenzoic acid (DHB) positive mode. $[M+H]^+$ calcd. (monoisotopic) 2368.27, found 2368.3.

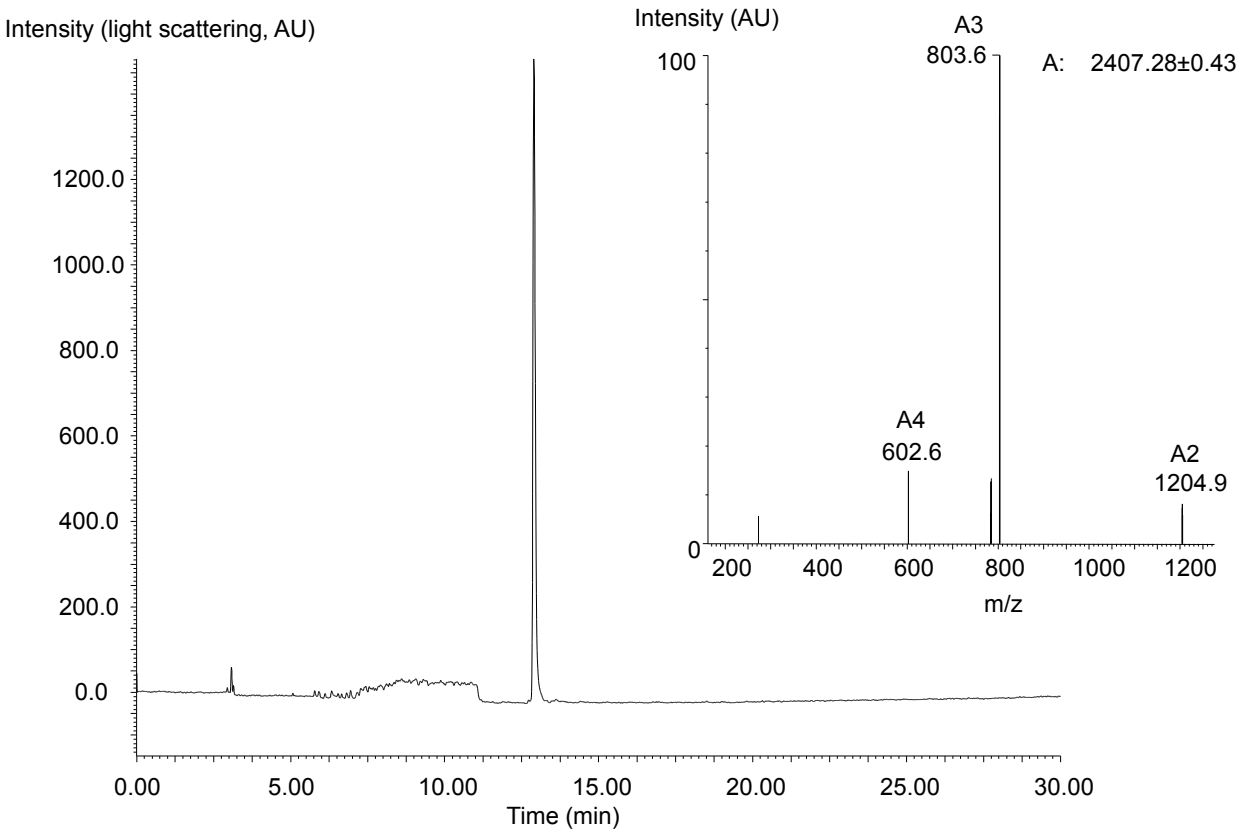
7.2.3 Preparation of SeEA^{off} peptide 30 (one-pot process II)

TCEP-HCl (2.89 mg, 0.1 mmol), selenide compound **7** (3.42 mg, 0.01 mmol, 5 eq) and metallic selenium (0.237 mg, 1.5 equiv) were dissolved in 0.2 M pH 4.2 sodium acetate buffer (1 mL). NaOH (5 M) was then added to adjust the pH to 5-5.5.

Peptide **29** (5.65 mg, 1.94 µmol) was dissolved in the above solution (972 µL, final peptide concentration 2 mM). The reaction mixture was shaken at 37°C under nitrogen atmosphere and monitored by LC-MS.

After 20 h of exchange, DMSO (105.8 µL) was added (10% of DMSO by vol) to inactivate the SeEA group by formation of a diselenide bond. The reaction mixture was stirred 30 min at 37°C. Then, acetic acid (275.7 µL) was added to have a final 20% acetic acid solution. 0.43 M hydroxylamine solution in water (45 µL) was then added to remove AcA protecting group. The reaction medium was heated at 37°C for 1 h 30 min and then diluted with water (11 mL final volume). The aqueous solution was extracted with diethyl ether (3 x 3 mL) and then purified by RP-HPLC (eluent A = water containing 0.1% TFA, eluent B=acetonitrile in water 4/1 containing 0.1% TFA, 30°C, detection at 215 nm, 6 mL/min, 0 to 20% eluent B in 5 min, then 20 to 40% eluent B in 60 min, C18XBridge column) to give 2.4 mg of SeEA^{off} peptide **30** (40%).

A)



B)

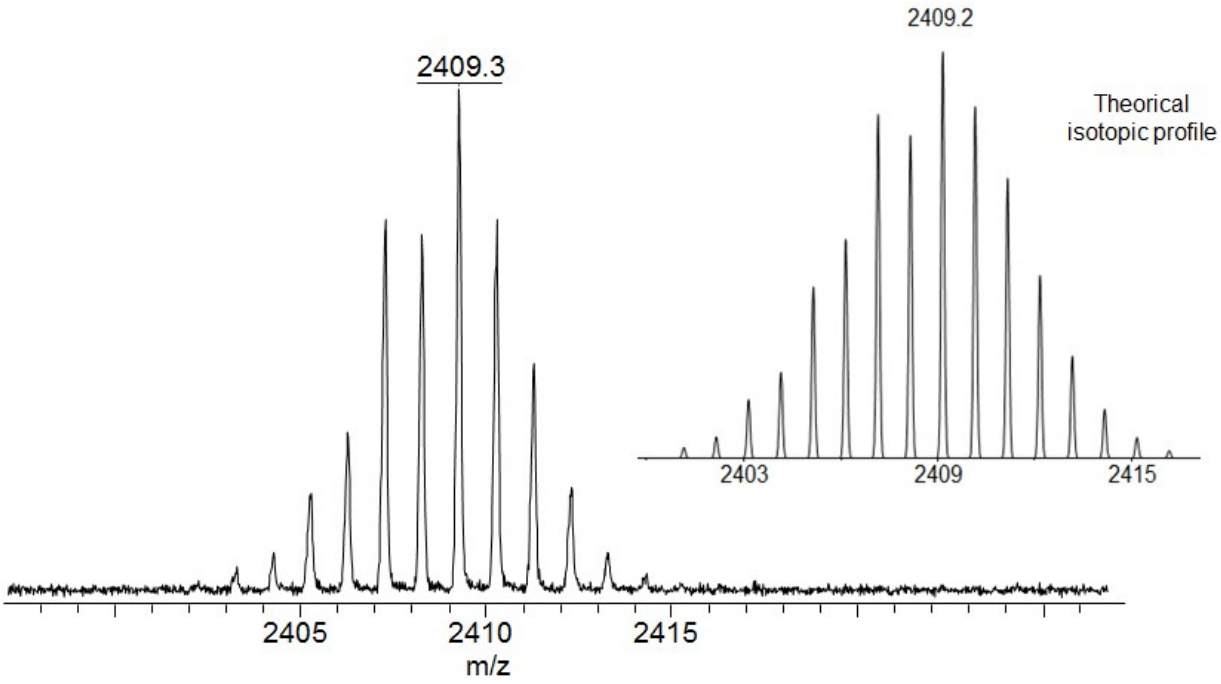


Figure S37. A) LC-MS analysis of peptide **30**. LC trace, eluent A 0.10 % TFA in water, eluent B 0.10 % TFA in CH_3CN/water: 4/1 by vol. C18 Xbridge BEH 300 Å 5 µm (4.6 x 250 mm) column, gradient 0-100 % B in 30 min (1 mL/min, detection 215 nm).B) MALDI-TOF analysis of thioester peptide **30**. Matrix, 2,5-dihydroxybenzoic acid (DHB) positive mode.

7.3 One-pot assembly of peptide 31 (one-pot process III)

Step 1

MPAA (3.34 mg, 0.2 mmol) and Se=TCEP (6.58 mg, 0.02 mmol) were dissolved in 6 M Gdn.HCl, 0.1 M pH 7.2 sodium phosphate buffer (100 µL). NaOH (5 M) was then added to adjust the pH to 7-7.5.

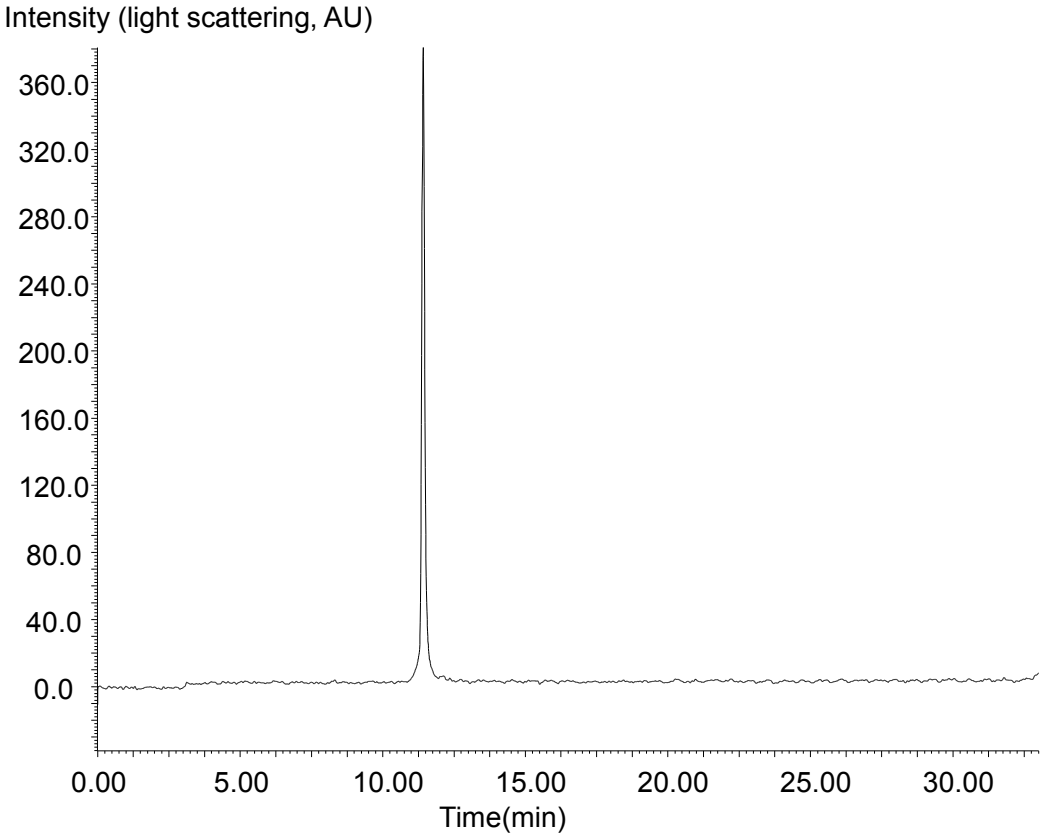
Peptide **26** (5.27 mg, 0.53 µmol) and peptide **27** (2.51 mg, 0.53 µmol) were dissolved in the above solution (75 µL, final peptide concentration 7 mM). The reaction mixture was shaken at 37°C under nitrogen atmosphere and monitored by LC-MS.

Step 2

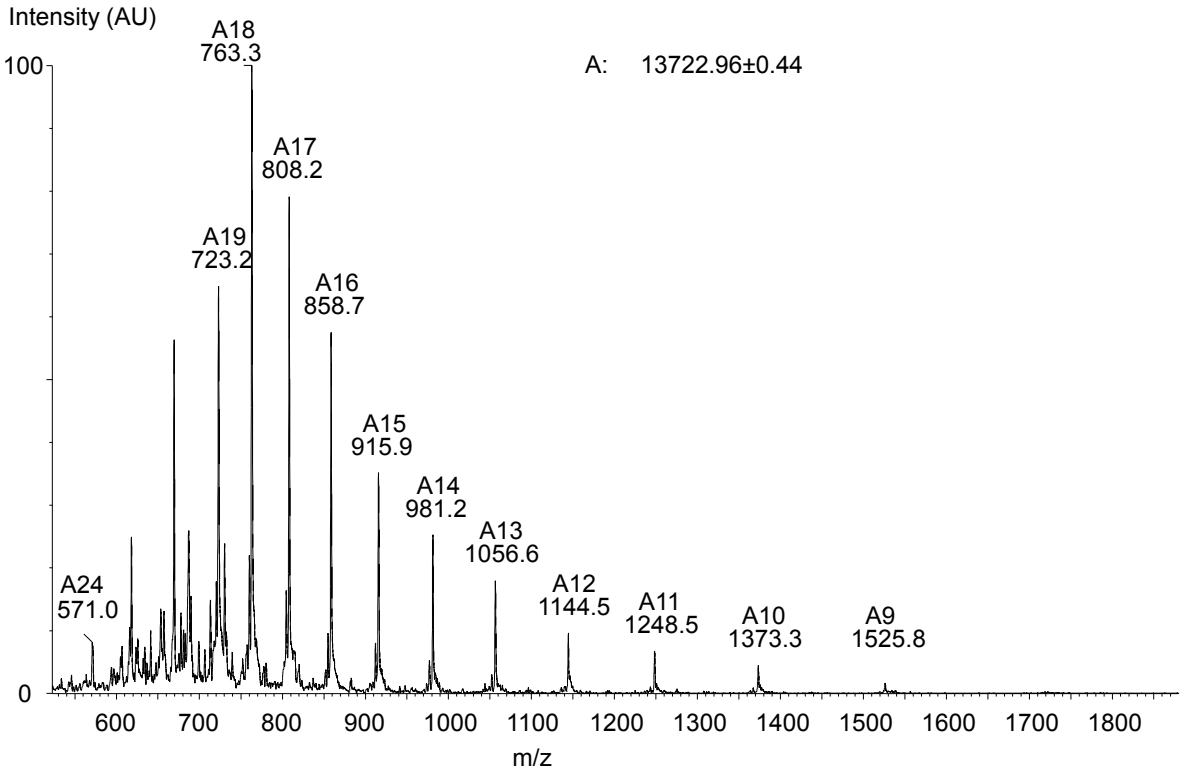
After 20 h, the reaction mixture was divided in two equal portions. To one of these, DTT (0.59 mg, 100 mM) and $SeEA^{off}$ peptide **30** (1.04 mg, 0.3 µmol) were added in this order. The reaction mixture was shaken at 37°C under nitrogen atmosphere and monitored by LC-MS.

After 42 h, the reaction mixture was diluted with 5 % aqueous acetic acid (4 mL), extracted with Et_2O (5 x 2 mL) to remove MPAA and purified by RP-HPLC (eluent A = water containing 0.1 % TFA, eluent B=acetonitrile in water 4/1 containing 0.1 % TFA, 50 °C, detection at 215 nm, 6 mL/min, 0 to 20 % eluent B in 5 min, then 20 to 50 % eluent B in 90 min, C18 XBridge column) to give 0.94 mg of $SeEA^{off}$ peptide **31** (21 % overall starting from peptide **26**).

A)



B)



C)

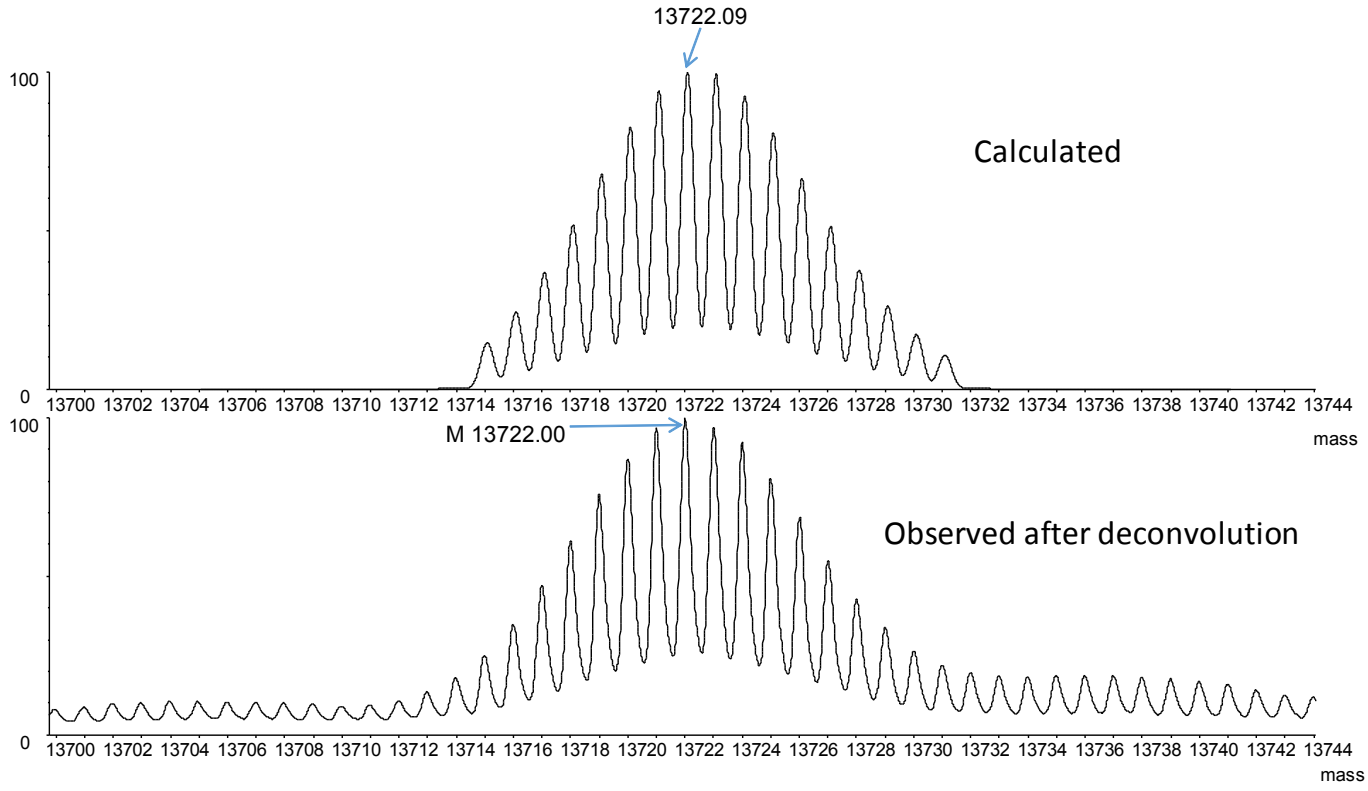


Figure S38. LC-MS analysis of thioester peptide **31**. A) LC trace, eluent C 0.10 % FA in water, eluent D 0.10 % FA in CH_3CN/water: 4/1 by vol. C3 Zorbax 300SB 3.5 µm (4.6 x 150 mm) column, gradient 0-100 % D in 30 min (1 mL/min, detection 215 nm). B) ESI MS trace: M calculated (mean) 13722.89, found 13722.96. C) High resolution ESI MS with experimental and calculated profile after deconvolution.

7.4 One-pot synthesis of NK1-B (one-pot process IV)

TCEP.HCl (2.87 mg, 0.01 mmol), MPAA (1.29 mg, 0.01 mmol) and Se=TCEP (2.63 mg, 0.008 mmol) were dissolved in 6 M guanidine-HCl, 0.1 M pH 7.2 sodium phosphate buffer (100 µL). NaOH (5 M) was then added to adjust the pH to 7-7.5.

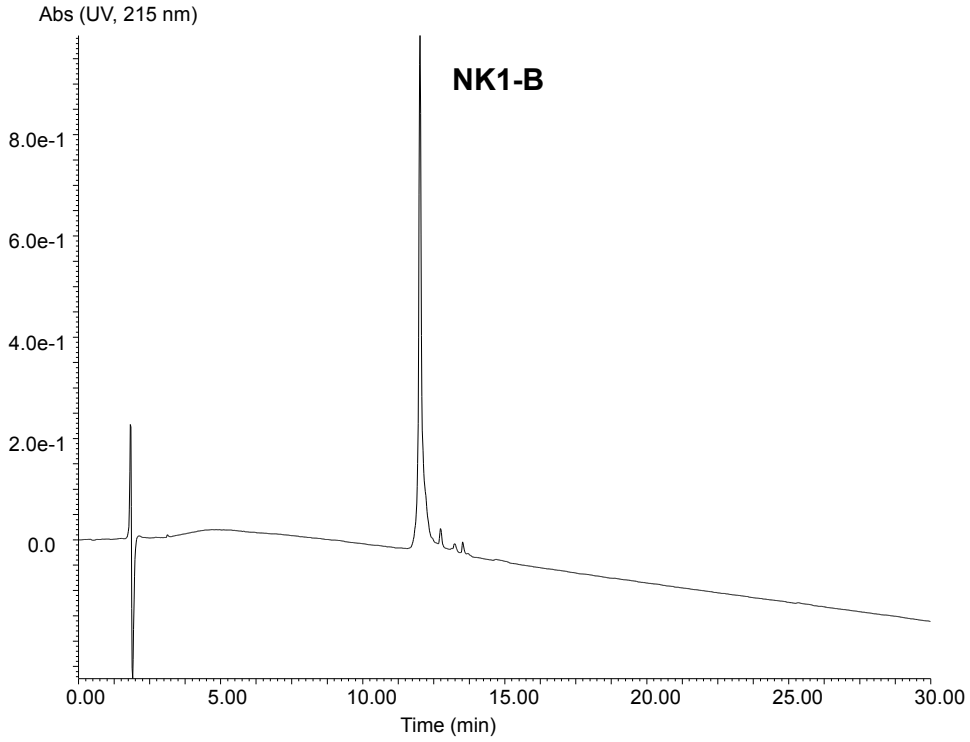
Peptide **31** (0.82 mg, 0.047 µmol) and peptide **18** (0.23 mg, 0.057 µmol, 1.2 equiv) were dissolved in the above solution (12 µL, final peptide concentration 4 mM). The reaction mixture was shaken at 37°C under nitrogen atmosphere and monitored by LC-MS.

After 18 h, TCEP.HCl (2.87 mg, 0.01 mmol) and MPAA (1.29 mg, 0.01 mmol) were dissolved in 6 M Gdn.HCl, 0.1 M pH 7.2 sodium phosphate buffer (100 µL). NaOH (5 M) was then added to adjust the pH to 5.

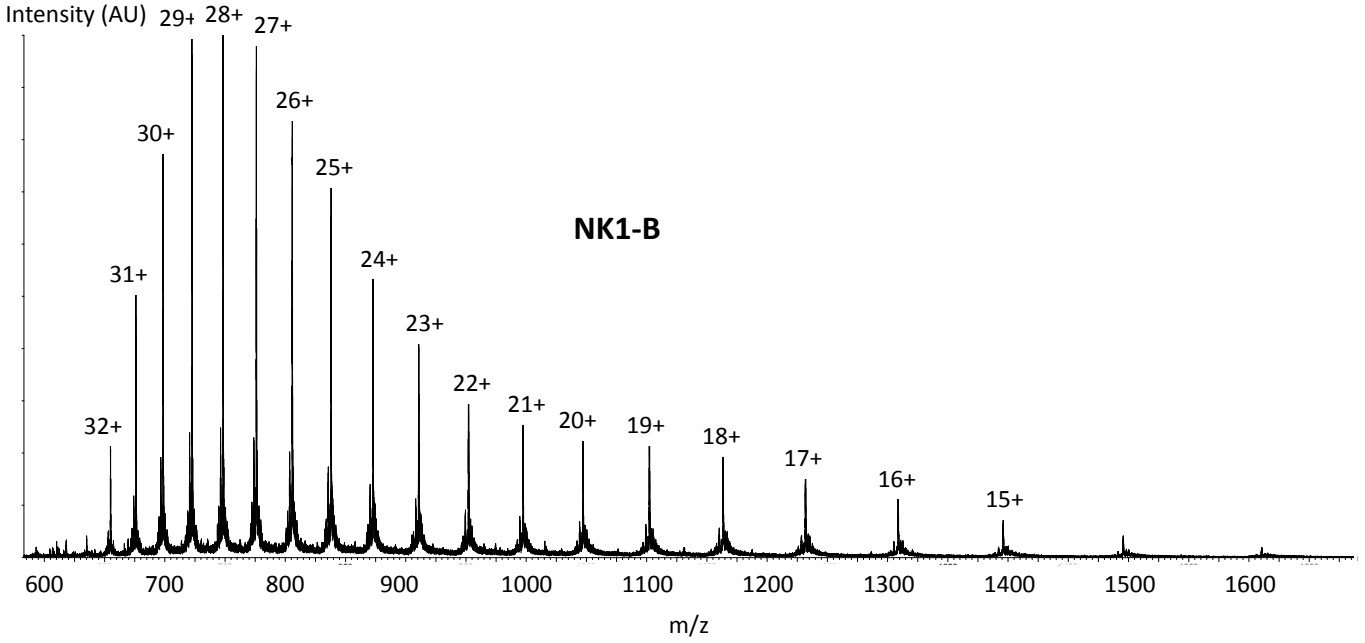
Peptide **33** (0.43 mg, 0.095 µmol, 2 equiv) was dissolved in the above solution (12 µL) and then added to the reaction mixture which was shaken at 37°C under nitrogen atmosphere.

After 72 h, the reaction mixture was diluted with 5 % aqueous acetic acid (4 mL), extracted with Et_2O (3 x 2 mL) to remove MPAA and purified by RP-HPLC (eluent C = water containing 0.1 % formic acid (FA), eluent D=acetonitrile in water 4/1 containing 0.1 % FA, 50 °C, detection at 215 nm, 6 mL/min, 0 to 20 % eluent B in 5 min, then 20 to 50 % eluent B in 90 min, C3 Zorbax column) to give 335 µg of **NK1-B** (32% overall starting from peptide **31**).

A)



B)



C)

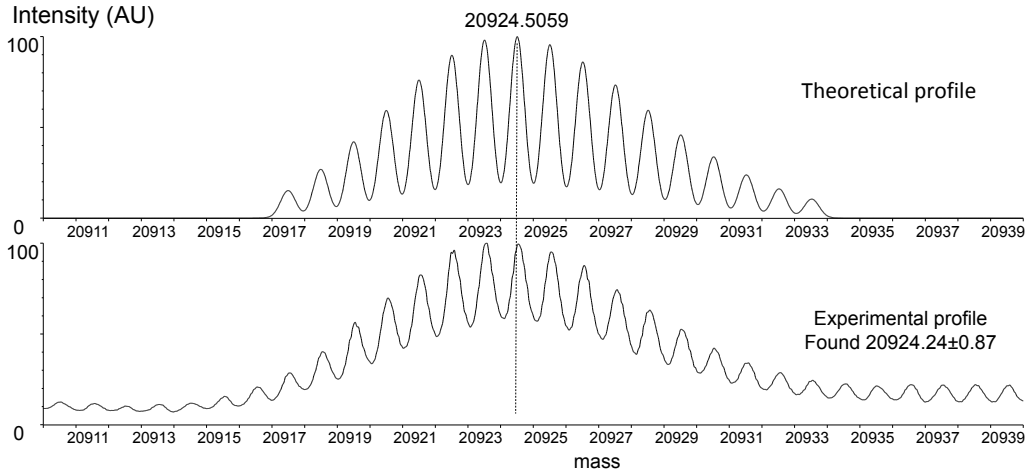


Figure S39. A) LC analysis of NK1-B. A) LC trace, eluent A 0.10 % formic acid (FA) in water, eluent B 0.10 % FA in CH_3CN/water: 4/1 by vol. C3 Zorbax 300SB 3.5 µm (4.6 x 150 mm) column, gradient 0-100 % B in 30 min (1 mL/min, detection 215 nm). B) ESI MS high resolution spectrum. C) Deconvoluted spectrum and comparison with the theoretical profile.

8. Computational analysis

Quantum chemical calculations were performed using the Gaussian 09 package of programs.[10] DFT computations were carried out using the B3LYP hybrid functional employing the 6-31+G* basis set with 5 pure d functions. Gradient techniques using internal coordinates with very tight optimization convergence criteria (each component of the first energy derivative below 2.0 10^{-6}Hartree/Bohr or radian) were used for both geometry optimization and computation of vibrational properties. The transitions states were localized using the Newton-Raphson algorithm, and the nature of the stationary points was determined by analysis of the Hessian. The activation and reaction energies were calculated from the thermochemical output (298.150 Kelvin, 1 atm) computed for the reagents, transition states and products, using standard thermochemistry as implemented in Gaussian 09. Intrinsic reaction coordinate (IRC) calculations were performed in the gas phase to localize the nearest local minima on the reactant and product sides of the reaction coordinate.[11] Solvent effects (water) were taken into account using Tomasi's polarizable continuum model (PCM).[12]

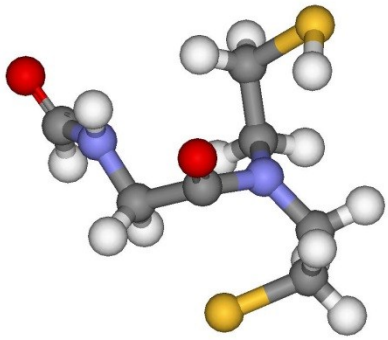
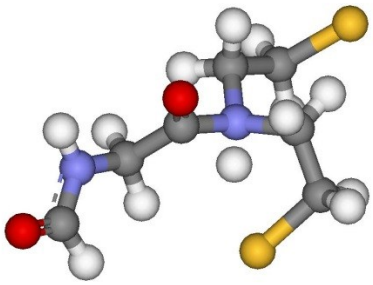
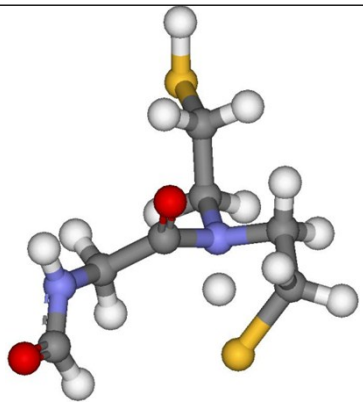
TS SEA	**TS SEA_2**	**TS SEA_3**
		
H = -1330.731085 Hartree G = -1330.789404 Hartree	H = -1330.703070 Hartree G = -1330.763103 Hartree	H = -1331.221528 Hartree G = -1331.281239 Hartree

Figure S40 Structures and absolute energies for anionic (**TS SEA**, **TS SEA_2**) and neutral (**TS SEA_3**) transitions states (gas phase). **TS SEA** is discussed in the main manuscript (one neutral 2-mercaptoethyl limb protonates the amide nitrogen, while the other is anionic and attacks the amide carbonyl). In the other anionic transition state TS SEA_2, one neutral 2-mercaptoethyl limb protonates the amide nitrogen, while the other is anionic and spectator. The third transition state **TS SEA_3** is neutral (one neutral 2-mercaptoethyl limb protonates the amide nitrogen, while the other is neutral too and spectator). The activation barrier for TS SEA is about 20 kcal mol^{-1} less than for **TS SEA_2** and **TS SEA_3**.

References

1. N. Ollivier, J. Dheur, R. Mhidia, A. Blanpain and O. Melnyk, *Org. Lett.*, 2010, **12**, 5238-5241.
2. W. Amelung and S. Brodowski, *Anal. Chem.*, 2002, **74**, 3239-3246.

3. N. Ollivier, J. Vicogne, A. Vallin, H. Drobecq, R. Desmet, O. El-Mahdi, B. Leclercq, G. Goormachtigh, V. Fafeur and O. Melnyk, *Angew. Chem., Int. Ed.*, 2012, **51**, 209-213.
4. N. Ollivier, L. Raibaut, A. Blanpain, R. Desmet, J. Dheur, R. Mhidia, E. Boll, H. Drobecq, S. L. Pira and O. Melnyk, *J. Pept. Sci.*, 2014, **20**, 92–97.
5. J. Dheur, N. Ollivier, A. Vallin and O. Melnyk, *J. Org. Chem.*, 2011, **76**, 3194-3202.
6. L. Raibaut, H. Drobecq and O. Melnyk, *Org. Lett.*, 2015, **17**, 3636-3639.
7. L. Henriksen, in *The Chemistry of Organic Selenium and Tellurium Compounds*, ed. S. Patai, John Wiley & Sons, Chichester, 1987, vol. 2, pp. 393-420.
8. N. Ollivier, A. Blanpain, L. Raibaut, H. Drobecq and O. Melnyk, *Org. Lett.*, 2014, **16**, 3032-4035.
9. L. Raibaut, J. Vicogne, B. Leclercq, H. Drobecq, R. Desmet and O. Melnyk, *Bioorg. Med. Chem.*, 2013, **21**, 3486-3494.
10. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, Izmaylov A. F., J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, J. Peralta, J. E., F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, *Gaussian 09. Revision A.02*, Gaussian, Inc., Wallingford (CT, USA), 2009.
11. K. Fukui, *Acc. Chem. Res.*, 1981, **14**, 363-368.
12. J. Tomasi and M. Persico, *Chem. Rev.*, 1994, **94**, 2027-2094.