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A PHOTOCHEMICAL TRANSFORMATION OF CYCLIC PEPTIDES LEADING TO FORMATION OF SELENOLANTHIONINE BRIDGES

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EXPERIMENTAL

Reagents

Solvents for peptide synthesis (analytical grade) were obtained from Sigma-Aldrich (dimethylformamide-DMF; trifluoroacetic acid-TFA) and J. T. Baker (diethyl ether). The H-Rink amide Chemmatrix® resin was purchased from Sigma-Aldrich. The (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) coupling reagent were obtained from Navoabiochem®. All standard amino acids derivatives were purchased from Peptydy.pl. Fmoc-Sec(pMeOBz) was purchased from Merck. Solvents for LC-MS and MS measurements: acetonitrile (MeCN), methanol (MeOH), formic acid (HCOOH) were purchased from VWR Chemicals.

Peptide synthesis

All the presented peptides were prepared on a solid support, according to the standard Fmoc protocol. The coupling of respective amino acids residues were carried out using a PyBOP in DMF. Both the amino acid derivatives and the coupling agent were used in 3-fold excess. The selenocysteine residue was introduced as Fmoc-Sec(Mob)-OH derivative. Additionally, the coupling reaction and Fmoc deprotection (25%piperydine/DMF) were supported by sonication over 15 min and 3 min, respectively [1]. The crude product was cleaved from the resin using mixture of TFA/H_2O/TIS (95:2.5:2.5, v/v/v) for 2h at room temperature. The standard cleavage procedure allow to obtain the peptide without protecting group, except of selenolanthionine Mob protection. Therefore, the additional treatment with mixture consisting of 5%DMSO/TFA over 5h was required. This procedure led to formation of oxidized product. The obtained peptides were precipitated in cold diethyl ether, and subsequently lyophilized.

Mass spectrometric analysis

The mass spectrometry measurements were carried out on Apex Ultra FT-ICR (Bruker, Germany) equipped in electrospray ion source (ESI) ion funnel. The mass spectrometer was calibrated before the run with the Tunemix mixture (Bruker Daltonics) by a quadratic method. All the measurements were performed in the positive ion mode. For the CID (collision induced dissociation) during the MS/MS experiments was optimized the collision energy (10–20 eV) for the best fragmentation (the voltage over the hexapole collision cell varied from 15 to 30 V). Argon was used as a collision gas. An acetonitrile/water/formic acid (50:50:0.1) mixture were used as the solvents for recording the mass spectra. The potential between the spray needle and the orifice was set to 4.5 kV.

HPLC analysis

After release from the resin, the crude peptide products were analyzed using a Thermo Separation HPLC system with a UV detection (210 nm) on a Vydac Protein RP C18 column (4.6 × 250 mm, 5 µm), with a gradient elution of 0%–80% S2 in S1 (S1 = 0.1% aqueous TFA; S2 = 80% acetonitrile + 0.1%) for 40 min (flow rate 1 mL/min) – Gradient 1

LC-MS

The LC-MS analysis of obtained peptides were carried out on Shimadzu IT-TOF, which is hybrid system consisting of ion trap and time of flight mass analyzer. This instrument is also equipped in electrospray (ESI) ion source. The potential between the spray needle and the orifice was set to 4.5 kV. The LC system was operated with mobile phase, consisting of solvent A: 0.1% formic acid in H_2O and solvent B: 0.1% formic acid in MeCN. The following separation conditions were used: The gradient conditions (B %) were from 5 to 60% B within 15 min-Gradient 2. The flow rate was 0.2 mL/min and the injection volume 1 µL. The separation was performed on a Aeris Peptide XB-C18 column (50 mm × 2.1 mm) 3.6 µm bead diameter. The samples of peptide were dissolved in 400 µl of water : acetonitrile mixture (95 : 5). For experiments involving nisin-dieselenide analogue, the HPLC with PDA detector (the same instrument) was performed,

Purification and characterization of peptides.

The nisin diselenide analogue and its irradiation product were purified by preparative reversed-phase HPLC on a Vydac C18 column (22 mm x 9 250 mm), using the following solvent systems: S1 0.1% aqueous TFA, S2 80% acetonitrile + 0.1% TFA, linear gradient from 5 to 70% of S2 for 50 min, flow rate 7.0 ml/min, UV detection at 210 and 280 nm. The resulting fractions were collected and subjected to a lyophilization process. The identities of the products were confirmed by MS analysis using a Apex Ultra FT-ICR (Bruker, Germany) mass spectrometer equipped with an electrospray (ESI) ionization source. The purity of peptides were analyzed using a Thermo Separation HPLC system with a UV detection (210 nm) on a Vydac Protein RP C18 column (4.6 × 250 mm, 5 μm), with a gradient elution of 0%–80% S2 in S1 (S1 = 0.1% aqueous TFA; S2 = 80% acetonitrile + 0.1%) for 40 min (flow rate 1 mL/min). The nisin-selenolanthionine analogue (C-ring fragment) was analyzed by HPLC on a Aeris Peptide XB-C18 column (50 mm × 2.1 mm) 3.6 μm bead diameter using Shimadzu IT-TOF instrument equipped in PDA detector. The separation conditions were described in section LC-MS. The purity of the purified substrate and selenolathionine analogue were 95% and 97%, respectively. These values were calculated basing on peak integration (HPLC, UV absorption, 210 nm).

Irradiation experiment

The sample of peptide (15 mg) was dissolved in 2 ml of methanol and placed in quartz cuvette. Then, the sample was irradiated using UV lamp (254 nm) over 1h. Finally, the solvent was evaporated in the stream of nitrogen.

MS spectra and chromatograms

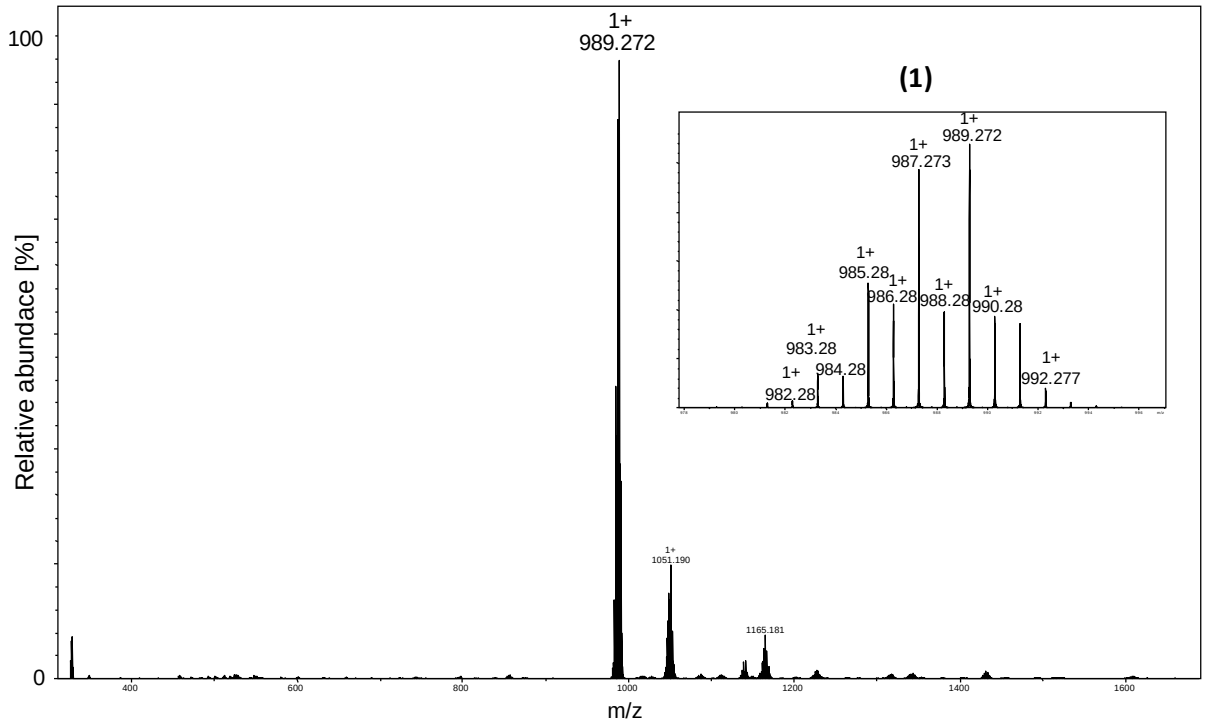

Fig. S 1 ESI-MS spectrum of purified nisin diselenide (C-ring) analogue (1). The main signal was expanded to show the isotopic pattern.

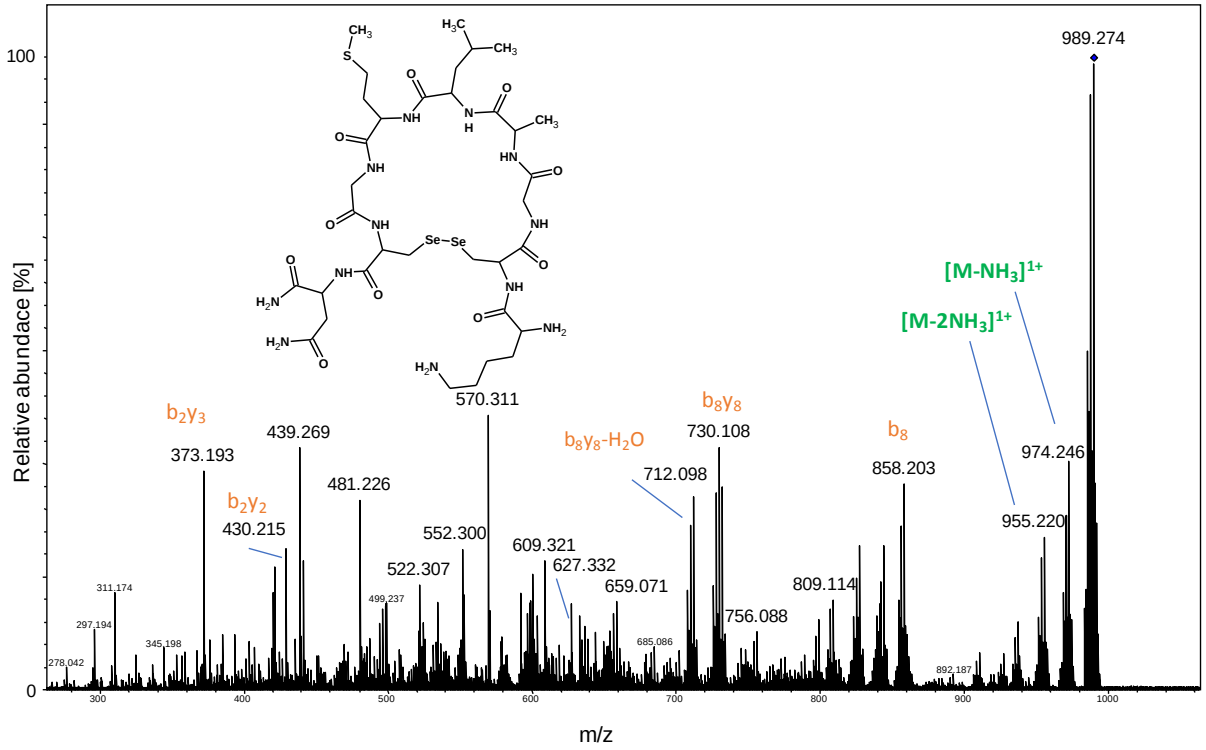

Fig. S 2 ESI-MS/MS spectrum of purified nisin diselenide (C-ring) (1) analogue.

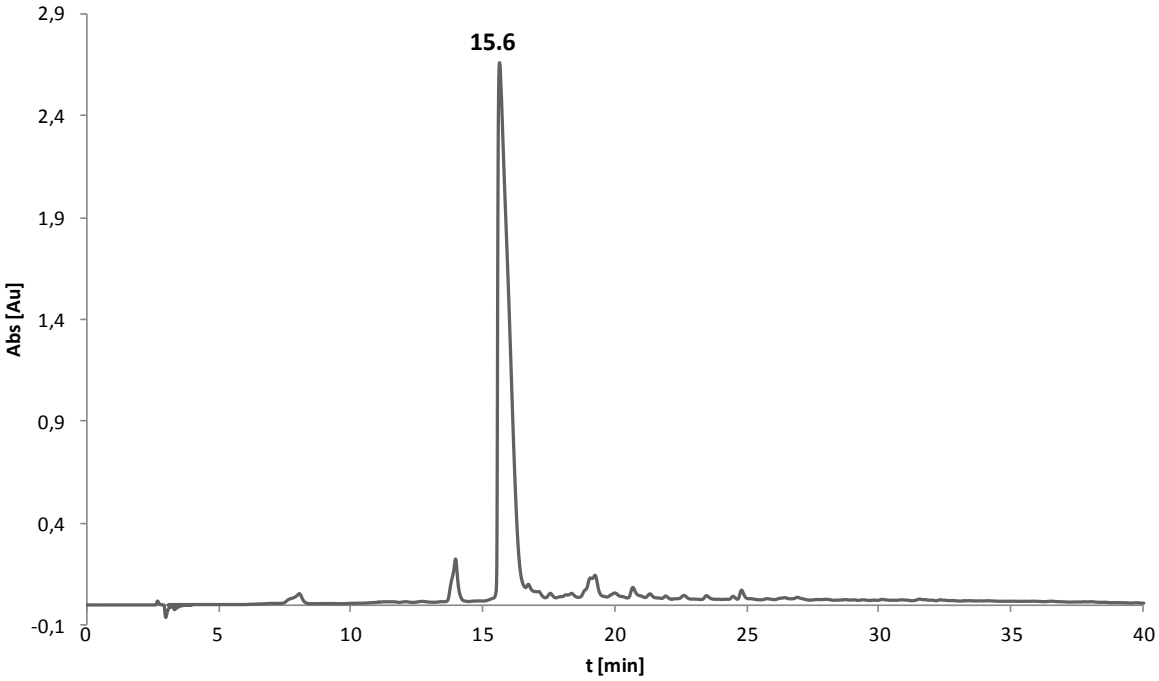

Fig. S 3 HPLC chromatogram of purified nisin diselenide (C-ring) analogue (1) – Gradient 1.

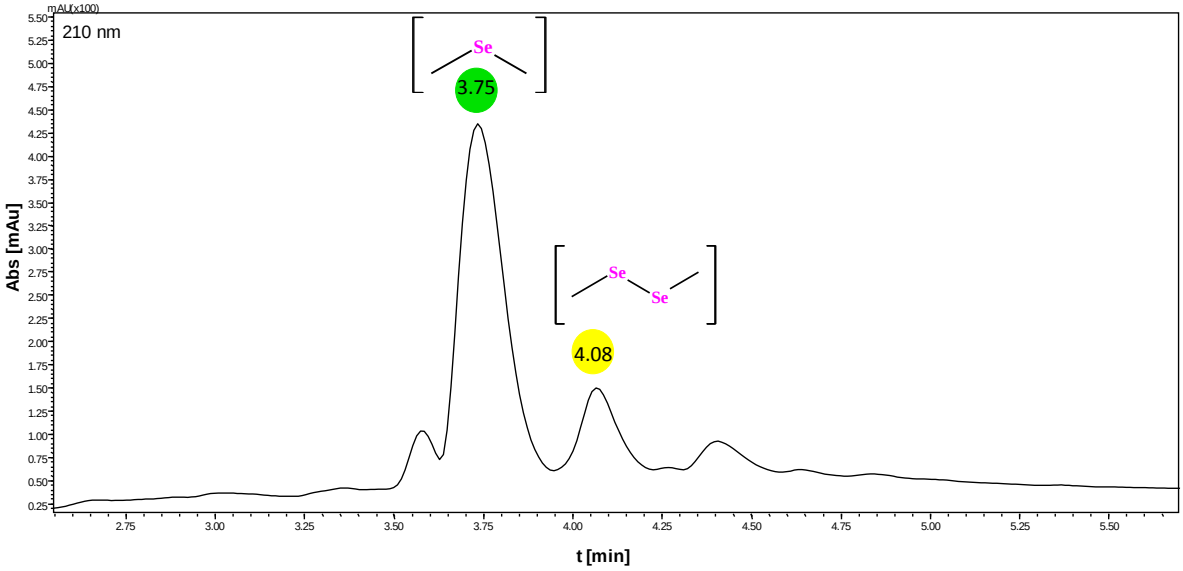

Fig. S 4 HPLC chromatogram of the mixture obtained after UV irradiation of nisin diselenide (C-ring) analogue (1) – Gradient 1

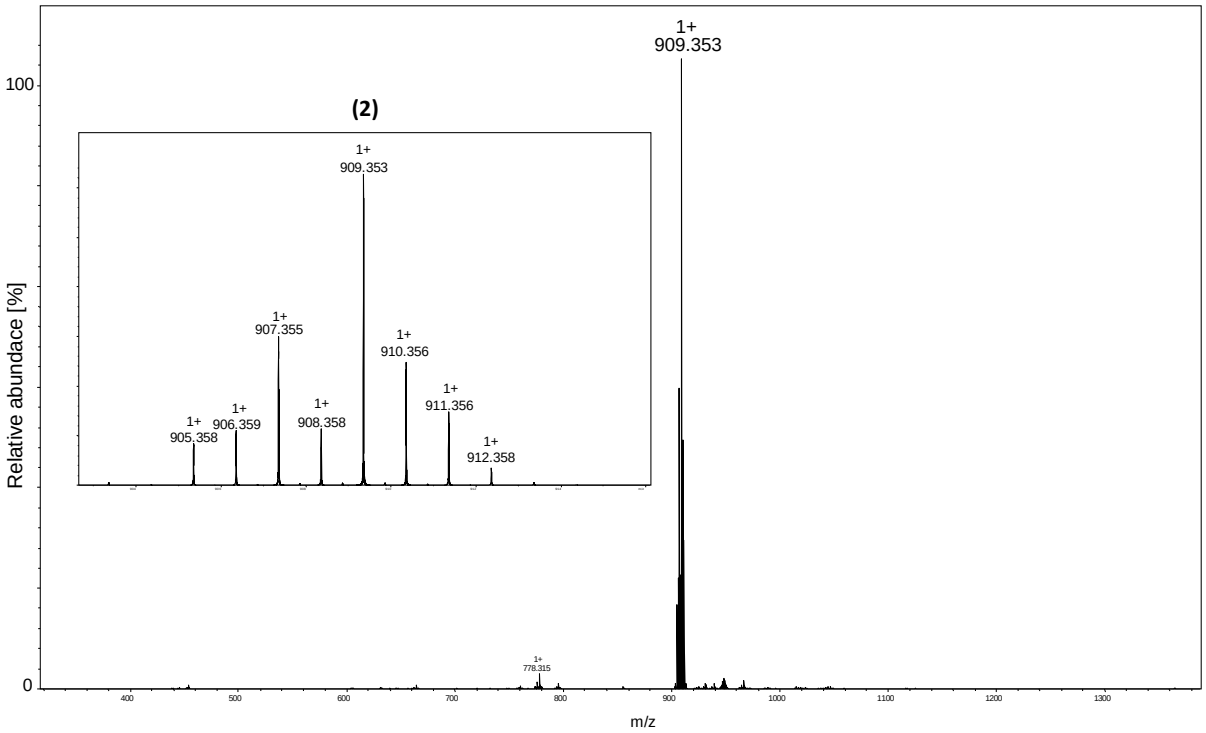

Fig. S 5 ESI-MS spectrum of purified nisin-selenolanthionine (C-ring) analogue (2).

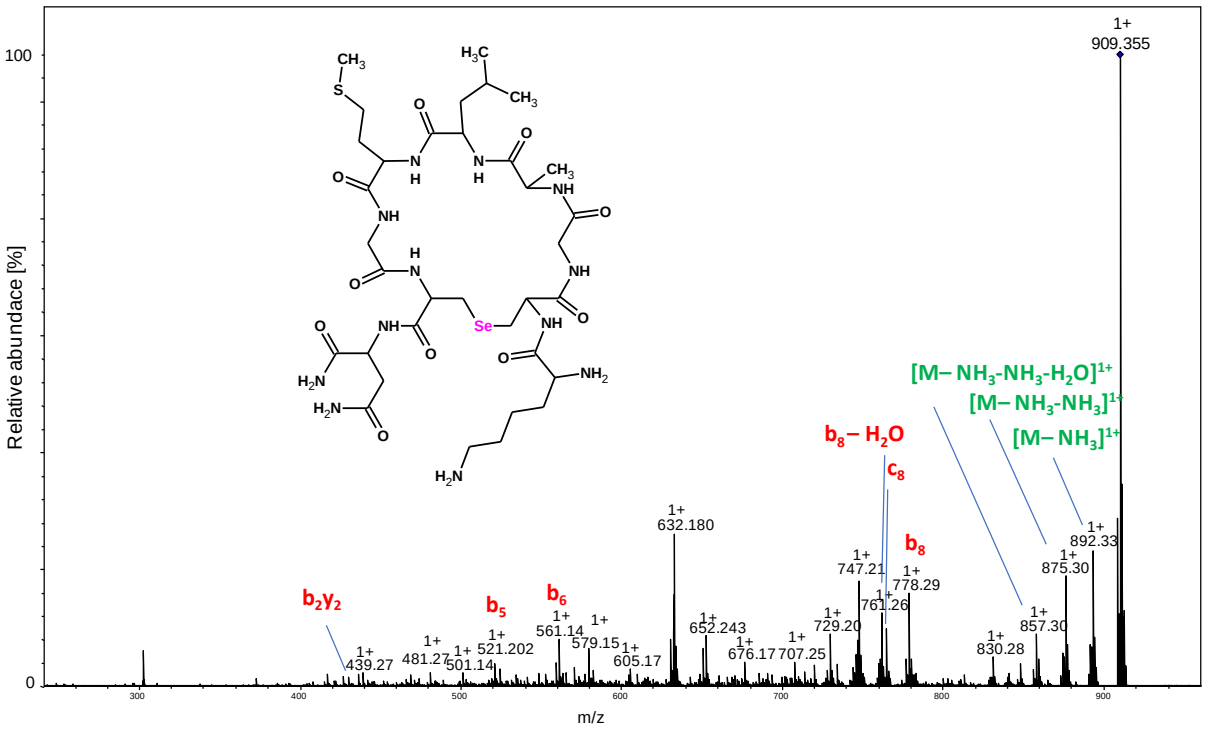

Fig. S 6 ESI-MS/MS spectrum of purified nisin-selenolanthionine analogue (C-ring fragment) (2).

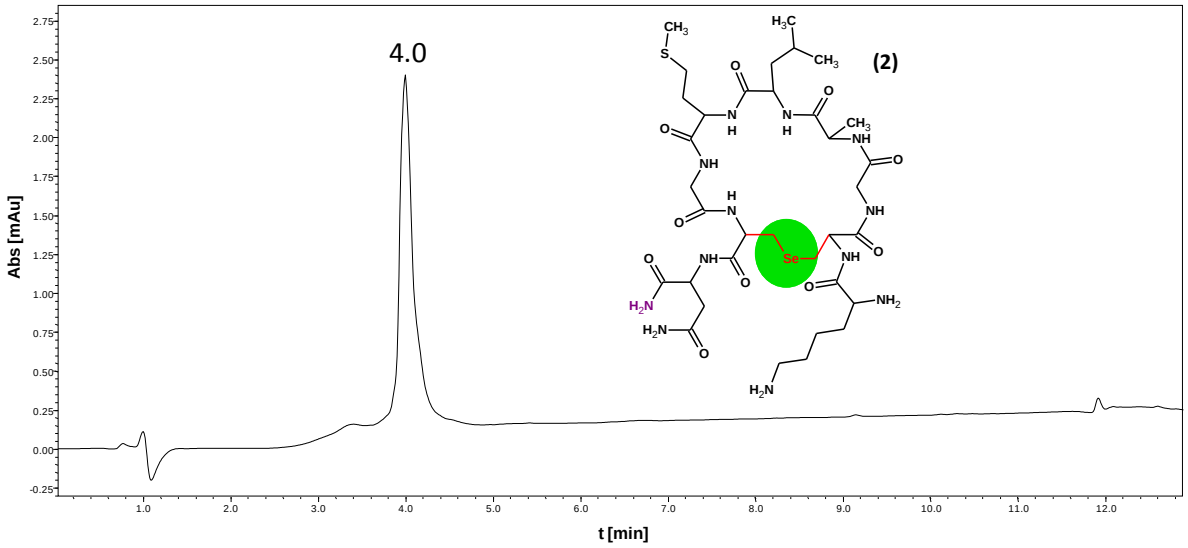

Fig. S 7 HPLC chromatogram of purified nisin-selenolanthionine analogue (C-ring fragment) (2) – Gradient 2

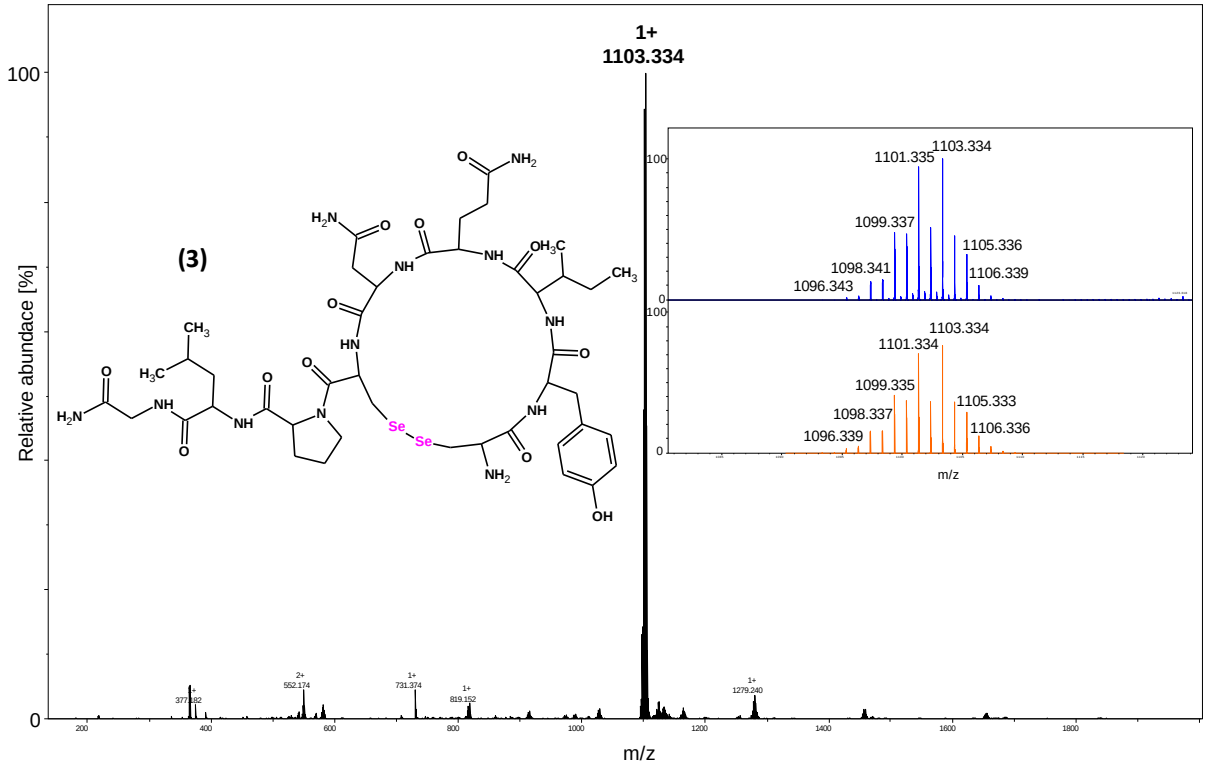

Fig. S 8 ESI-MS spectrum of purified oxitocin selenolanthionine analogue (3).

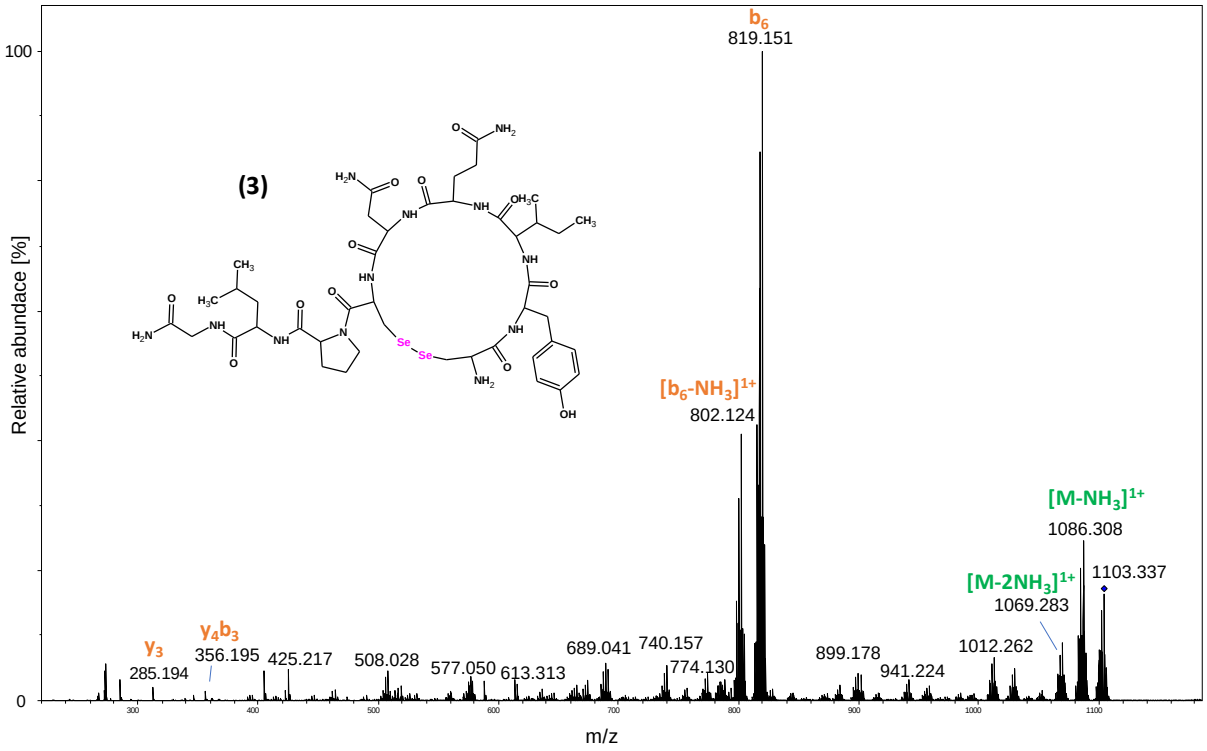

Fig. S 9 ESI-MS/MS spectrum of purified oxitocin selenolanthionine analogue (3).

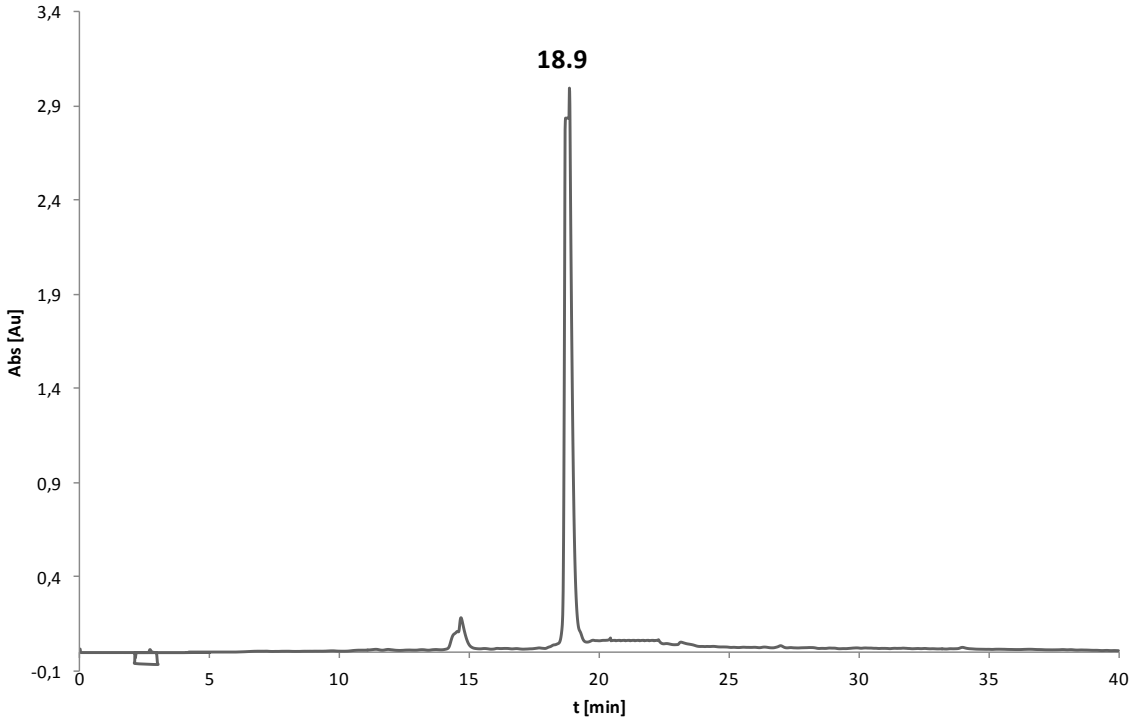

Fig. S 10 HPLC chromatogram of purified oxitocin diselenide analogue (3)– Gradient 1.

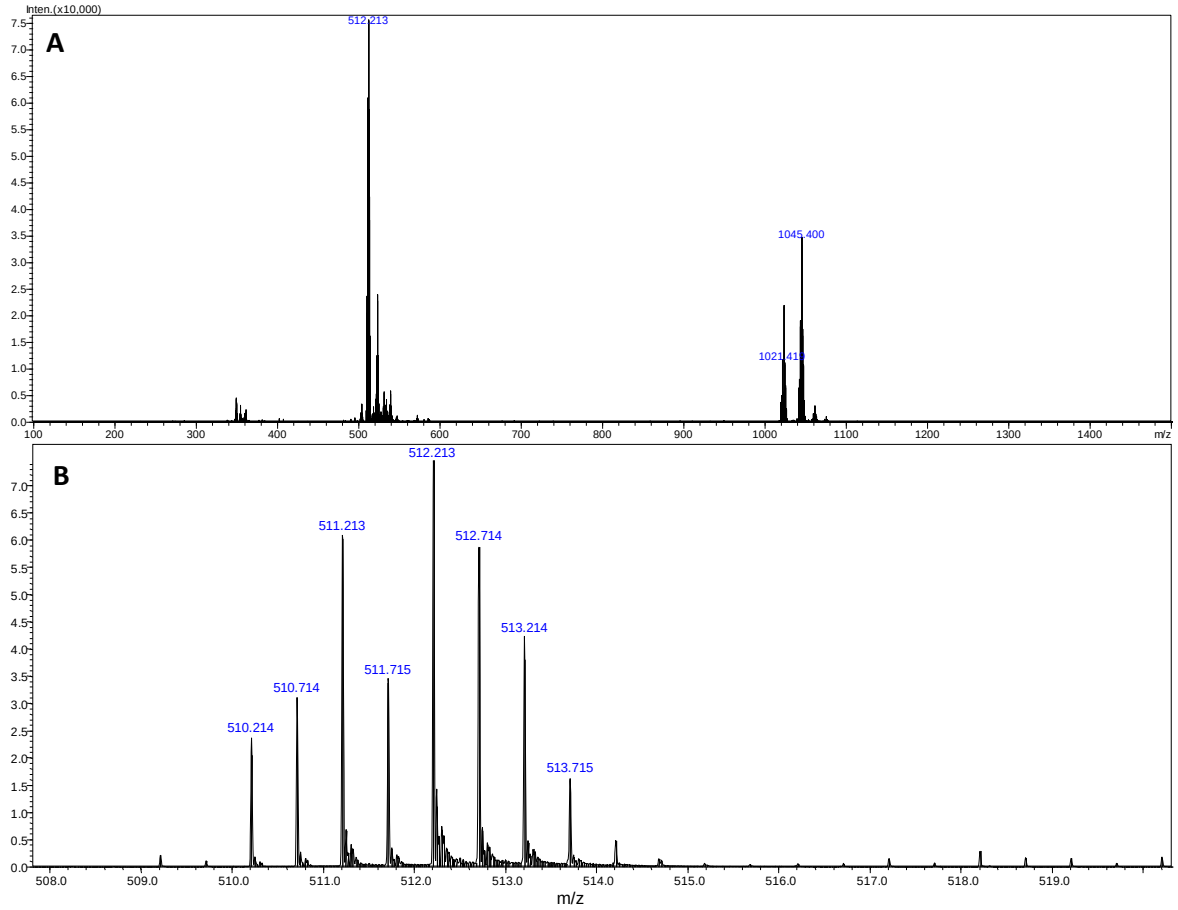

Fig. S 11 A. LC-ESI-MS spectrum obtained for the irradiation product (4)(oxitocin selenolanthionine analogue). B. Expanded peak at *m/z* 512.213

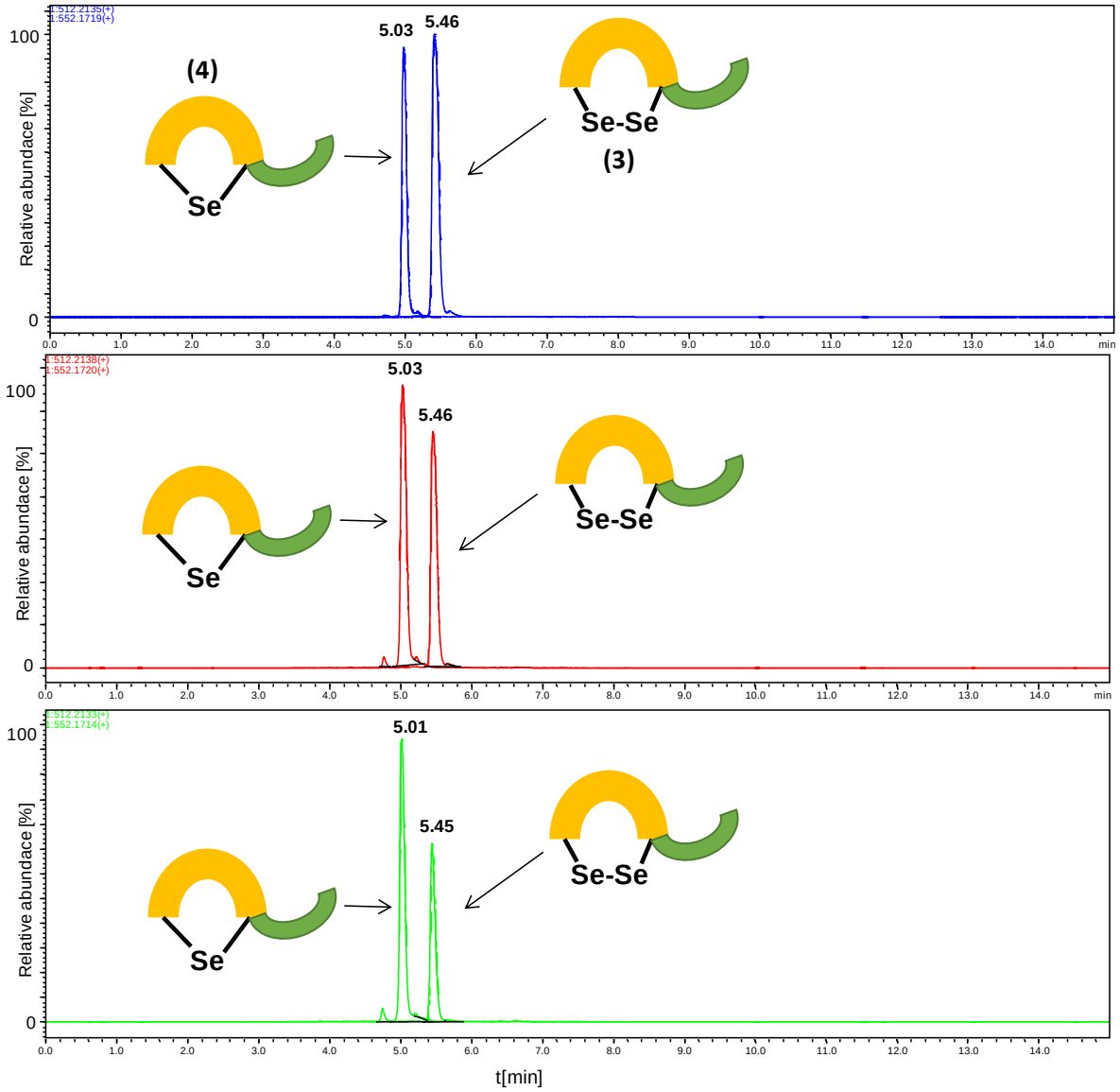

Fig. S 12 LC-ESI-MS chromatograms (XIC) obtained after UV irradiation of purified oxitocin diselenide analogue (3) over: 10, 30, 60 min (blue, red, green chromatograms respectively).

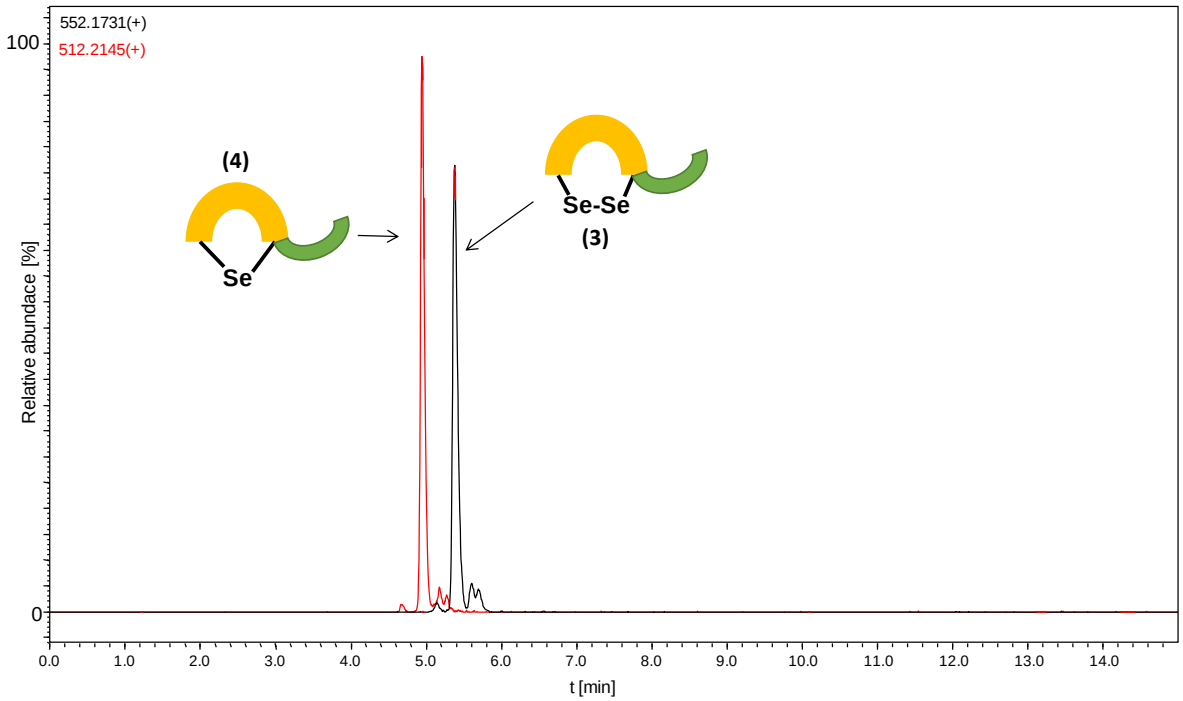

Fig. S13 LC-ESI-MS chromatograms (XIC) obtained after UV irradiation (60 min) of purified oxitocin diselenide analogue (3). The oxygen was removed from the solution.

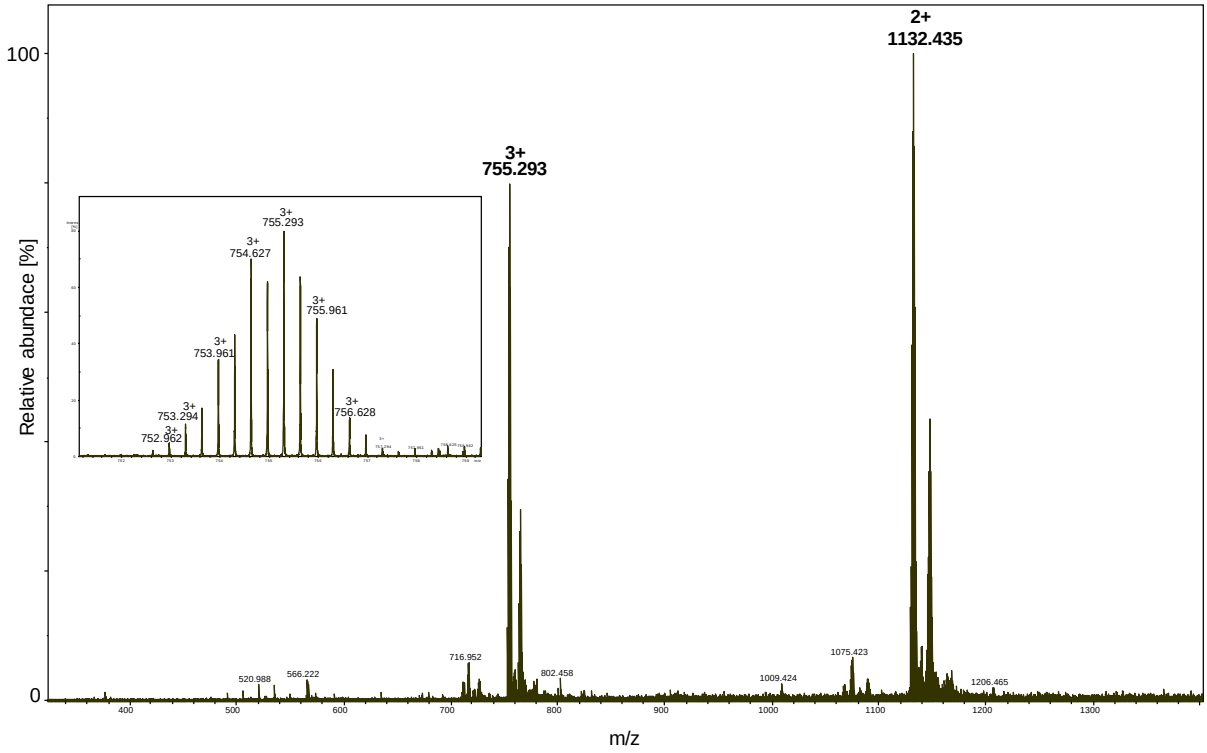

Fig. S 14 ESI-MS spectrum obtained for peptide (5) containing disulfide and diselenide bridge.

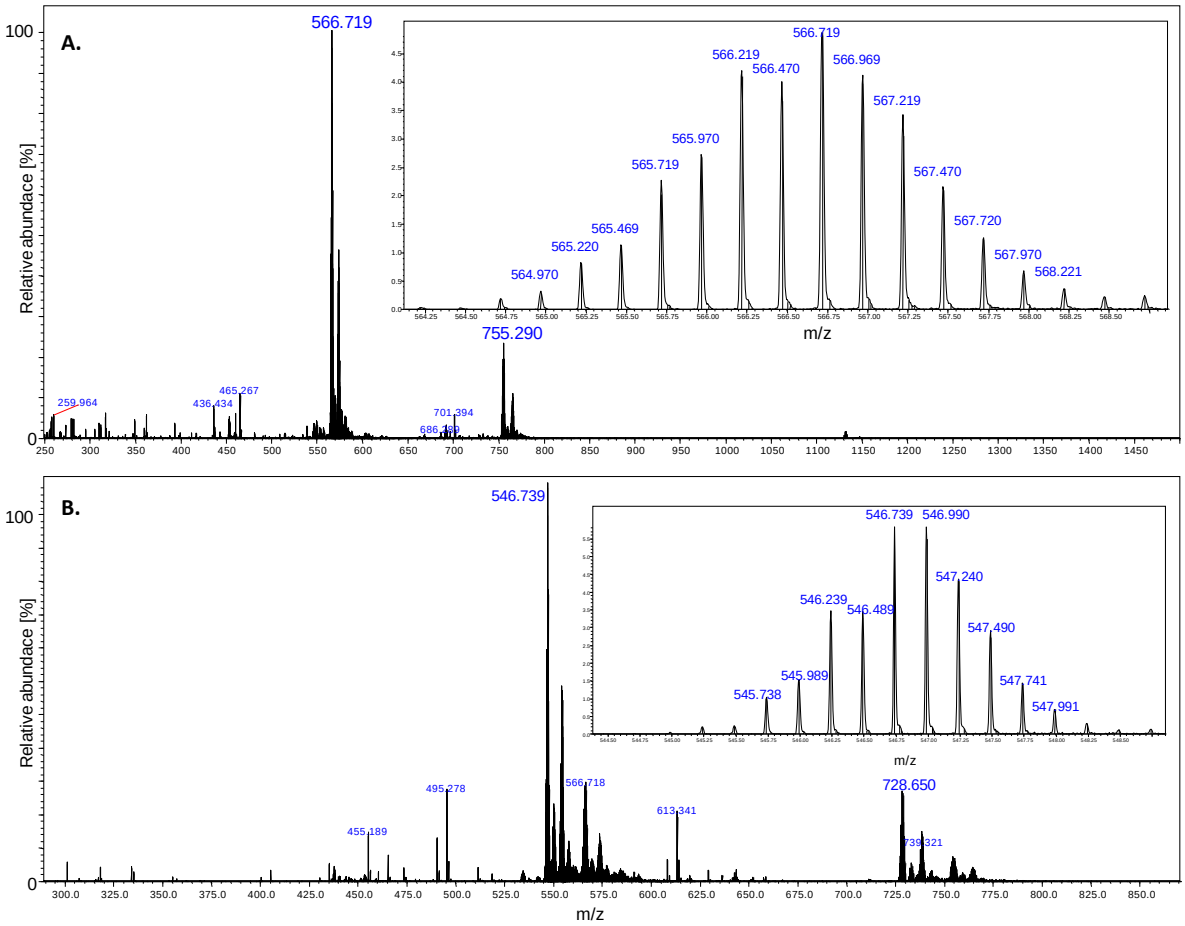

Fig. S 15 LC-MS spectrum (XIC) acquired for the peptide (5)with diselenide bond (A) and its selenolathionine (6) analogue (B).

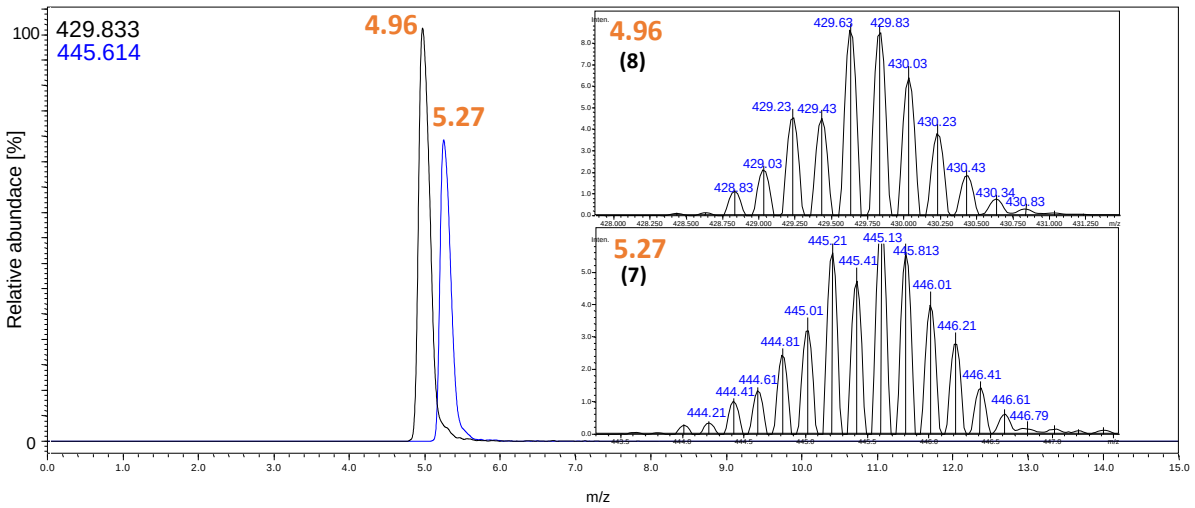

Fig. S 16 LC-MS spectrum (XIC) acquired for the mixture obtained after UV irradiation of peptide (2) (H-Ile-Leu-Lys-Glu-Pro-Val-His-Gly-Ala-Sec-NH_2)$_2$ containing diselenide bond. The signal at 4.96 represents the selenolanthionine analogue (8), while the blue marked derived from peptide with diselenide bond (7).

[1]G. Wołczański, H. Płóciennik, M. Lisowski and P. Stefanowicz, *Tetrahedron Lett.,* 2019, **60**, 1814