Lighting up peptides: direct dehydroalanine formation from diselenides via Rose Bengal photocatalysis

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1. Reagents and Materials

All commercially available reagents were used without further purification. Water was deionized using a reverse osmosis system (Hydrolab). The following solvents and reagents for synthesis—N,N-dimethylformamide (DMF), dichloromethane (DCM), methanol (MeOH), tetrahydrofuran (THF), diethyl ether (Et_2O), N,N-diisopropylethylamine (DIPEA), piperidine (PIP), formic acid (HCOOH), trifluoroacetic acid (TFA), triisopropylsilane (TIS), and 1,2-ethanedithiol (EDT)—were of analytical grade and purchased from Sigma-Aldrich. Acetic anhydride was obtained from Lachner. Fmoc-protected amino acid derivatives used for solid-phase peptide synthesis (SPPS) were purchased from PeptideWeb (Peptydy.pl). The coupling reagent PyBOP (benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate) and the TentaGel® M RAM resin (0.2 mmol/g) were obtained from Sigma-Aldrich. Rose Bengal, (Ir[dF(CF_3)ppy]2(dtbpy))PF_6 and disodium phosphate were also obtained from Sigma-Aldrich. Sodium hydroxide (NaOH) was purchased from Stanlab, and hydrochloric acid (HCl) of analytical grade from Sigma-Aldrich. Solvents used for LC-MS analysis—acetonitrile (MeCN), water (H_2O), and formic acid (HCOOH) (HPLC grade)—were purchased from Sigma-Aldrich. Methanol (MeOH) for LC-MS was obtained from J.T. Baker.

2. HPLC analysis

HPLC analyses were performed using a Shimadzu Nexera X2 UPLC system equipped with a photodiode array (PDA) detector. Chromatograms were recorded in the wavelength range of 190–380 nm. The liquid chromatography system operated with a mobile phase consisting of solvent A: 0.1% formic acid in water, and solvent B: 0.1% formic acid in acetonitrile (MeCN). Three different gradient conditions were used depending on the peptide type:

- **Gradient 1**: 0–20% B over 15 minutes
- **Gradient 2** 0–25% B over 15 minutes
- **Gradient 3** 0–30% B over 15 minutes
- **Gradient 4**: 0–40% B over 15 minutes

The flow rate was set at 0.3 mL/min, and the injection volume was 1 µL. Separations were carried out on a Phenomenex Aeris Peptide XB-C18 column (100 mm × 2.1 mm, 3.6 µm particle size). Peptide samples were dissolved in 400 µL of a water/acetonitrile mixture (95:5, v/v) prior to injection.

3. LC-MS analysis

LC-MS analyses of the obtained peptides were conducted using a Shimadzu 9030 mass spectrometer, a hybrid system combining a quadrupole and a time-of-flight (Q-TOF) mass analyzer. The instrument was equipped with an electrospray ionization (ESI) source. The potential between the spray needle and the orifice was set at 4.5 kV. Mass spectra were acquired in the m/z range of 200–1000. Fragmentation was performed by collision-induced dissociation (CID) using argon as the collision gas. MS/MS spectra were recorded under the following conditions: accurate *m/z* measurement, a mass range of 200–1000 m/z, an isolation window of 5 Da, ion accumulation time of 20 ms, and collision energy individually optimized for each precursor ion within the range of 20–30 eV.

Liquid chromatography was carried out using a Nexera X2 UPLC system. The mobile phase consisted of solvent A: 0.1% formic acid in water, and solvent B: 0.1% formic acid in acetonitrile (MeCN). Three gradient methods were applied depending on the sample type:

- **Gradient 1**: 0–20% B over 15 minutes
- **Gradient 2** 0–25% B over 15 minutes
- **Gradient 3** 0–30% B over 15 minutes
- **Gradient 4**: 0–40% B over 15 minutes

For synthetic peptides, the flow rate was 0.3 mL/min with an injection volume of 1 µL Chromatographic separation was performed on a Phenomenex Aeris Peptide XB-C18 column (100 mm × 2.1 mm) with a particle size of 3.6 µm for model peptides. Peptide samples were dissolved in 400 µL of a water/acetonitrile mixture (95:5, v/v) prior to injection

4. Mass spectrometry analysis

High-resolution mass spectra were acquired at the Mass Spectrometry Laboratory, Department of Chemistry, University of Wrocław, using a Bruker qTOF Compact mass spectrometer. The instrument is equipped with an electrospray ionization (ESI) source and a hybrid mass analyzer comprising a quadrupole coupled with a time-of-flight (TOF) analyzer, providing a resolution of approximately 30,000 FWHM.

Prior to each measurement, mass calibration was performed using the quadratic method. Fragmentation experiments were carried out in a CID-type collision cell using argon as the collision gas. The collision energy was individually optimized for each precursor ion and ranged between 20 and 30 eV.

Samples were prepared by dissolving approximately 100 µg of the analyte in 1 mL of a 50:50 (v/v) acetonitrile/water mixture containing 0.1% formic acid. All solvents used were of LC-MS grade purity. Spectra were recorded in the positive ionization mode over an *m/z* range of 200–3000.

5. Purification of peptides.

All products obtained via solid-phase synthesis were purified after cleavage using preparative reversed-phase high-performance liquid chromatography (RP-HPLC). The purification was performed on a TOSOH BIOSCIENCE TSKgel ODS-120T C18 column (21.5 mm × 30 cm, 10 µm particle size, 120 Å pore size). The mobile phase consisted of:

- **Solvent A (S1):** 0.1% trifluoroacetic acid (TFA) in water
- **Solvent B (S2):** 80% acetonitrile in water with 0.1% TFA

A linear gradient from 5% to 70% B over 50 minutes was applied at a flow rate of 7.0 mL/min. Elution was monitored by UV absorbance at 210 nm. Collected fractions were subsequently lyophilized.

The identity of the purified products was confirmed by high-resolution mass spectrometry using the previously described Bruker qTOF Compact instrument equipped with an electrospray ionization (ESI) source.

Peptide purity was assessed by analytical HPLC using a Shimadzu Nexera system with photodiode array (PDA) detection at 210 nm. Analyses were performed on a Phenomenex Aeris Peptide XB-C18 column (100 mm × 2.1 mm, 3.6 µm particle size).

6. General procedure for preparation of model peptides

All the peptides **1-6** were synthesized on solid support using standard Fmoc (9-fluorenylmethyloxycarbonyl) chemistry and a TentaGel® M RAM resin (loading 0.2 mmol/g). After swelling the resin in *N,N*-dimethylformamide (DMF) for 30 minutes, Fmoc-protected amino acids (3 equivalents) were coupled using PyBOP (3 equivalents) and DIPEA (6

equivalents) in a syringe reactor. The coupling reaction was performed for 20 minutes under ultrasound-assisted conditions, followed by filtration and five washes with DMF. Fmoc deprotection was carried out by adding 25% piperidine in DMF (v/v), and placing the syringe in an ultrasonic bath for 3 minutes. The resin was then filtered and washed seven times with DMF. The completeness of each coupling step was monitored using the Kaiser test. These steps were repeated sequentially for each amino acid residue until the desired hexapeptide sequence was assembled. Upon completion of the synthesis, the resin was shrunk using a series of solvents: DMF/DCM, DCM, DCM/MeOH, and finally MeOH alone. The resin was then dried overnight in a desiccator. Cleavage of the peptide from the resin was performed using a standard cleavage cocktail composed of trifluoroacetic acid (TFA)/H_2O/triisopropylsilane (TIS)/1,2-ethanedithiol (EDT) in a 92.5:2.5:2.5:2.5 (v/v) ratio for 2 hours at room temperature. The crude peptide was precipitated with cold diethyl ether and collected by centrifugation. In the next step, the methoxybenzyl (Mob) group was removed. For this purpose, the peptide was dissolved in 1 ml of a 5% DMSO/TFA solution and left overnight. After this time, the peptide was precipitated with cold ether, collected by centrifugation and subsequently purified by preparative-scale reversed-phase HPLC. The collected fractions were analyzed by mass spectrometry and proper fractions pooled and lyophilized.

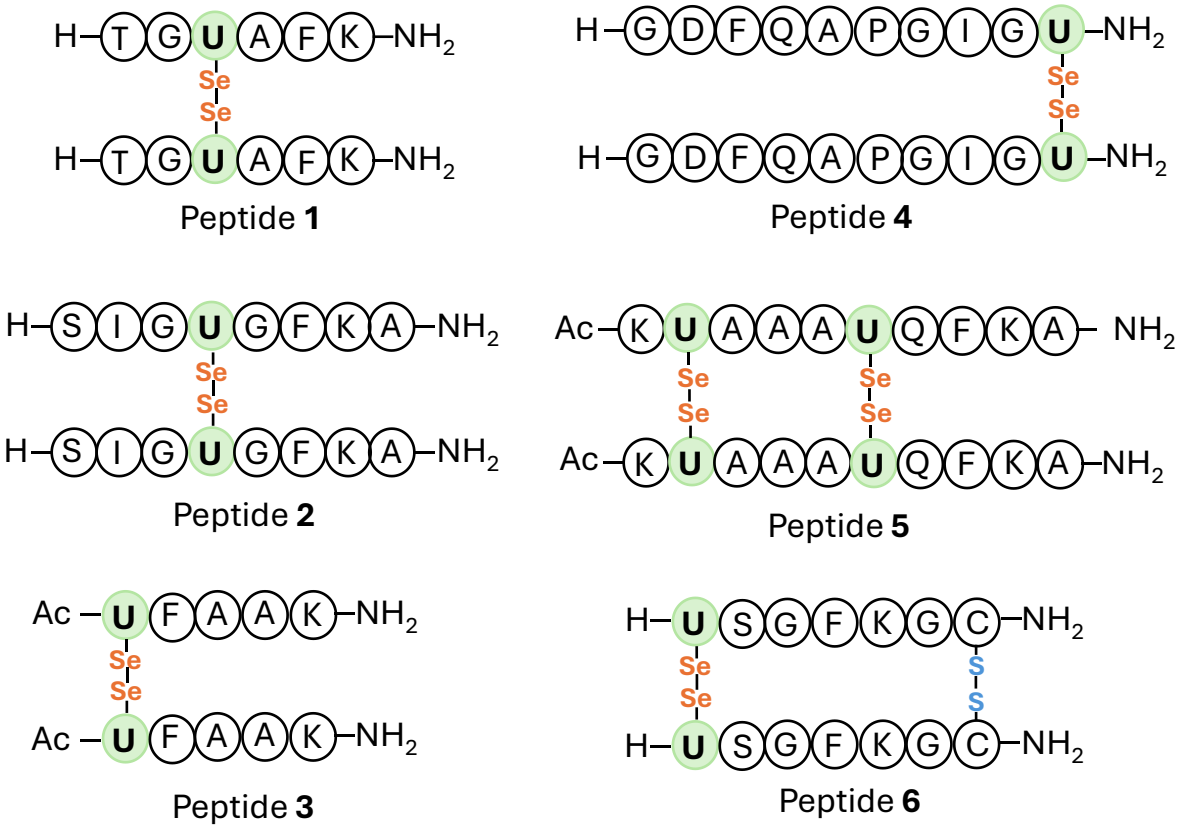


Fig S 1. Schematic representation of synthesized peptide sequences

7. General (optimized) procedure for photochemical conversion of peptide diselenide to dehydroalanine

A peptide sample (1 mg), containing an oxidized selenocysteine residue in the form of a diselenide homodimer, was placed in a 2 mL HPLC vial made of transparent borosilicate glass The vial must not be sealed with a cap in order to allow the access of atmospheric oxygen. The sample was dissolved in 900 µL of aqueous buffer consisting of 8 M guanidine hydrochloride and 0.2 M Na_2HPO_4, prepared with deionized water obtained from a reverse osmosis system (Hydrolab, Poland). The pH of the solution was adjusted to 6 using 6 M HCl. Subsequently, 0.3 equivalents of Rose Bengal were added from a freshly prepared aqueous stock solution (2 mg/mL). The stock solution can be stored for up to one week; however, its long-term stability has not been tested. The vial was placed on a magnetic stirrer and gently heated until the solution reached 50 °C. The sample was then irradiated for over 1 hour using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K).

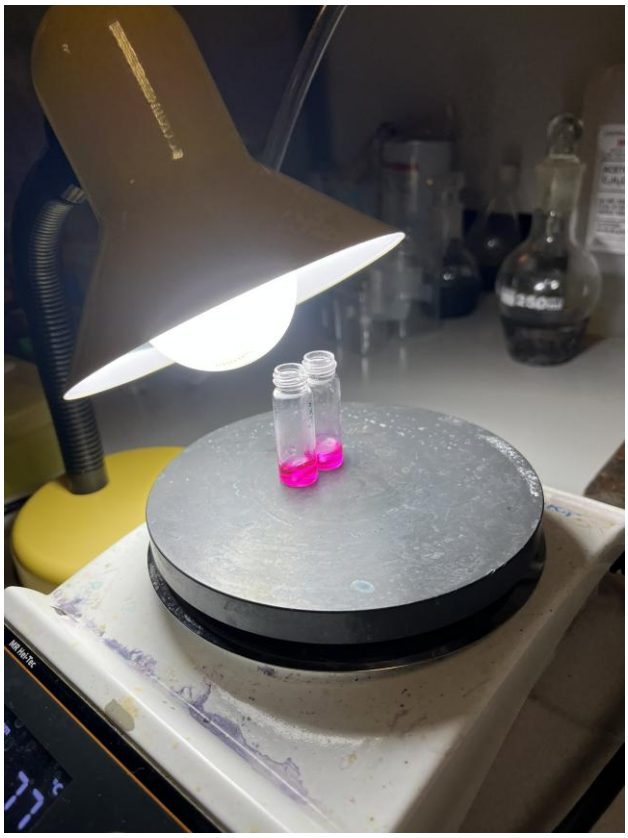


Fig S 2. A photograph showing the execution of the experiment

8. The procedure for immobilization of Rose Bengal on solid support

TentaGel® M RAM resin (loading 0.2 mmol/g) was swelled in *N,N*-dimethylformamide (DMF) for 30 minutes. Fmoc deprotection was carried out by adding 25% piperidine in DMF (v/v), and placing the syringe in an ultrasonic bath for 3 minutes. The resin was then filtered and washed seven times with DMF. Rose Bengal (6 equivalents) was coupled using DIC (6 equivalents) in a syringe reactor. The coupling reaction was performed for 3 hours using rotary, followed by filtration and five washes with DMF. Upon completion of the synthesis, the resin was shrunk utilizing a series of solvents: DMF/DCM, DCM, DCM/MeOH, and finally MeOH alone. The resin was then dried overnight in a desiccator.

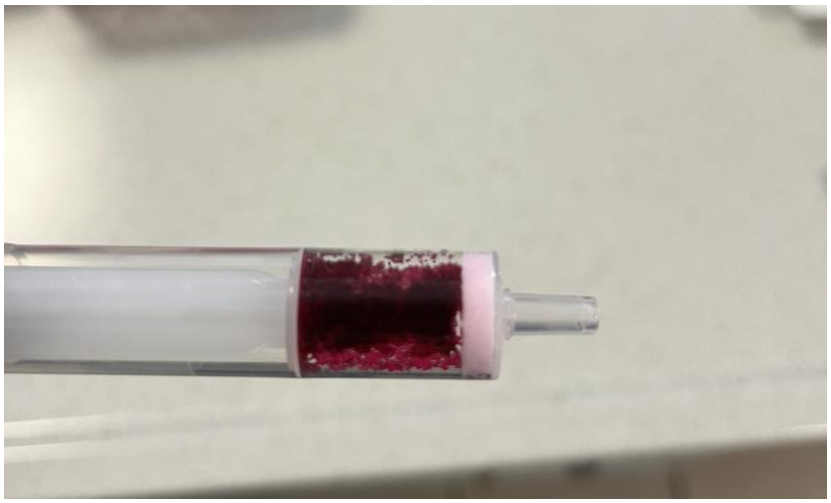


Fig S 3. The syringe with the immobilized RB on solid support

9. Analytical data of the synthesized model peptides

9.1 The synthesis of model peptide 1

The model peptide **1** was assembled according to the procedure described in section 6.

HR-ESI-MS calcd *m/z* 672.2494, found 672.2500 $[M+2H]^{2+}$, **HPLC**: R_t= 6.9 min (gradient 2), **yield:** 70%

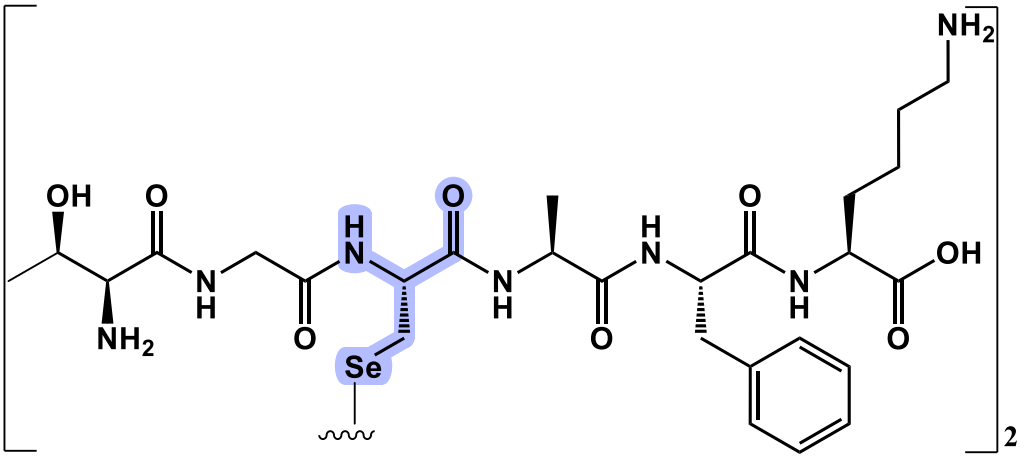


Fig S 4. Structure of model peptide 1

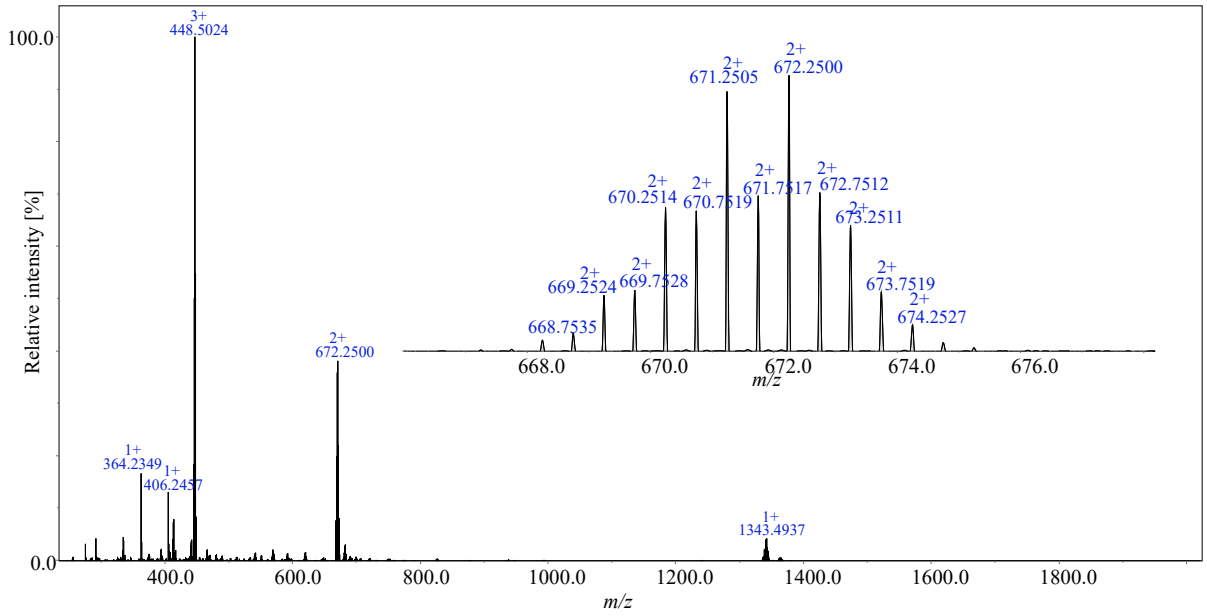


Fig S 5 ESI-MS spectrum obtained for model peptide 1

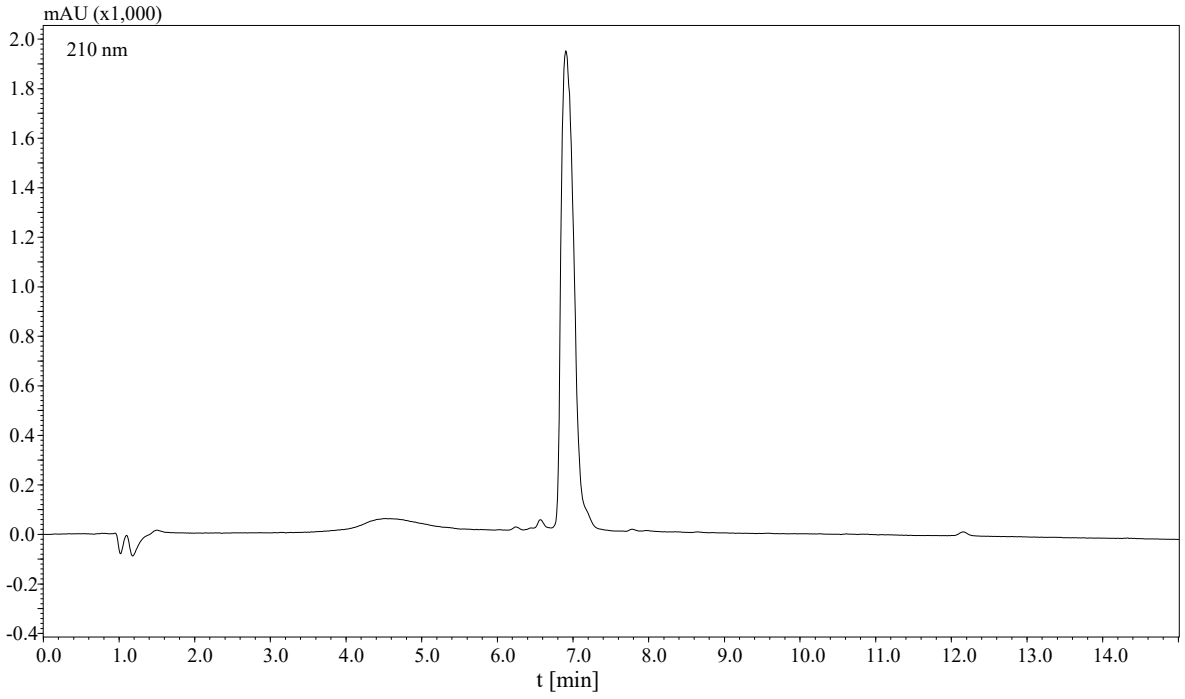


Fig S 6. HPLC chromatogram obtained for model peptide 1 (210 nm)

9.2 The synthesis of model peptide 2

The model peptide **2** was assembled according to the procedure described in section 6.

HR-ESI-MS calcd *m/z* 828.3394, found 828.3366 $[M+2H]^{2+}$, **HPLC**: R_t= 10.4 min (gradient 2), **yield:** 67%

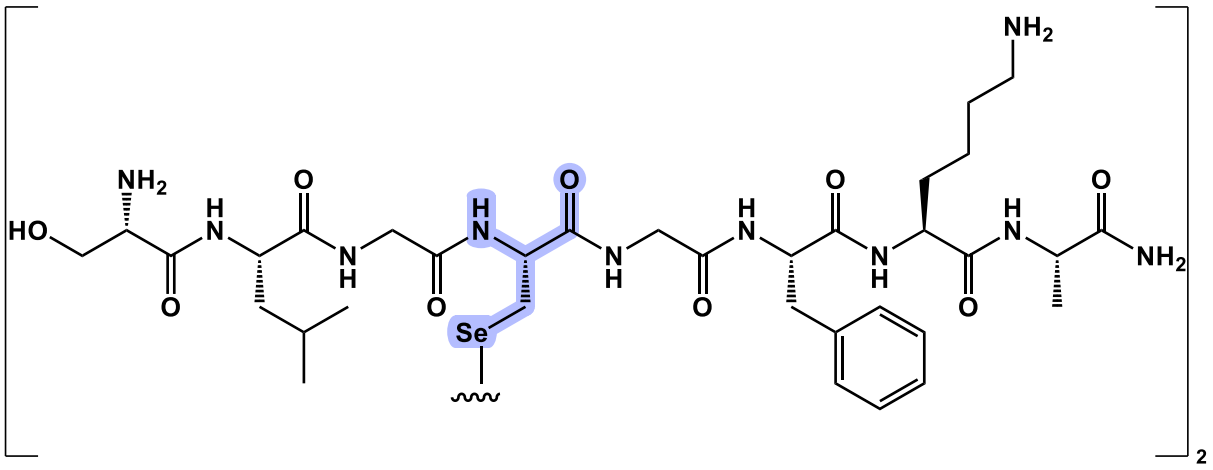


Fig S 7. Structure of model peptide 2

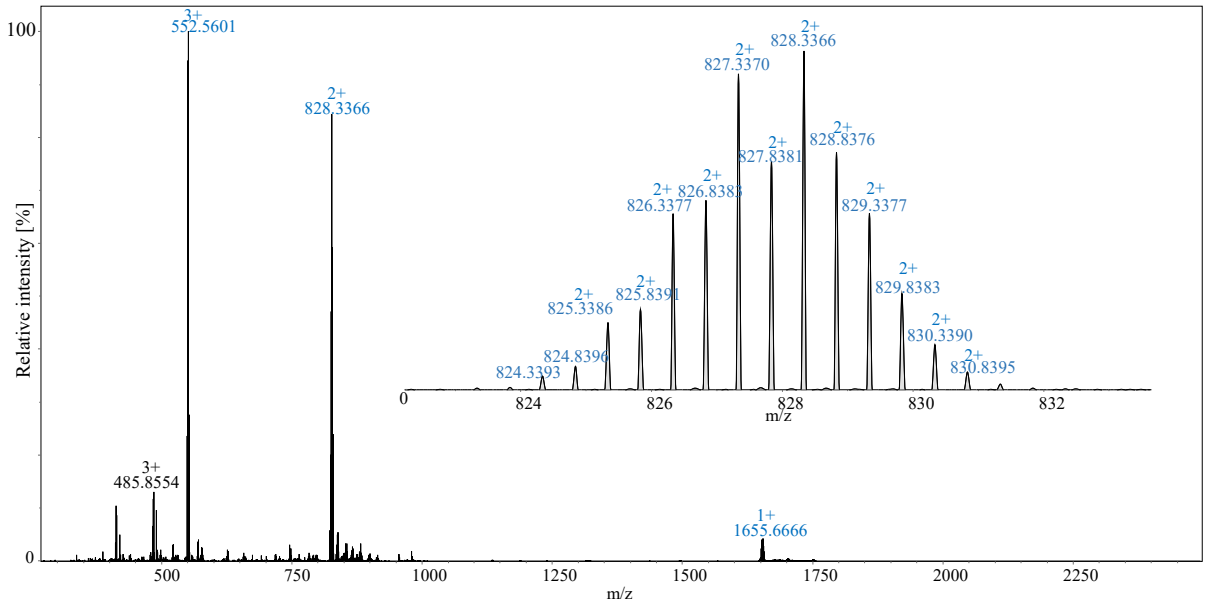


Fig S 8. ESI-MS spectrum obtained for model peptide 2

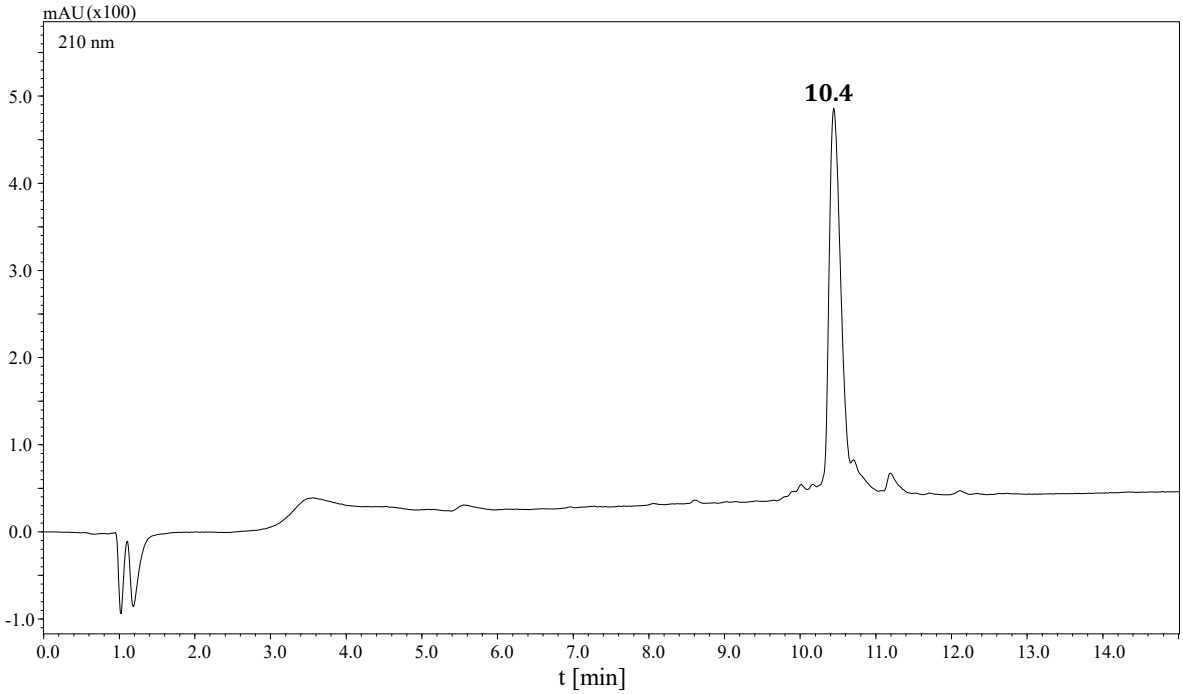


Fig S 9. HPLC chromatogram obtained for model peptide 2 (210 nm)

9.2 The synthesis of model peptide 3

The model peptide **3** was assembled according to the procedure described in section 6.

HR-ESI-MS calcd *m/z* 627.2272, found 627.2280 $[M+2H]^{2+}$, **HPLC**: R_t= 9.9 min (gradient 3), **yield:** 72%

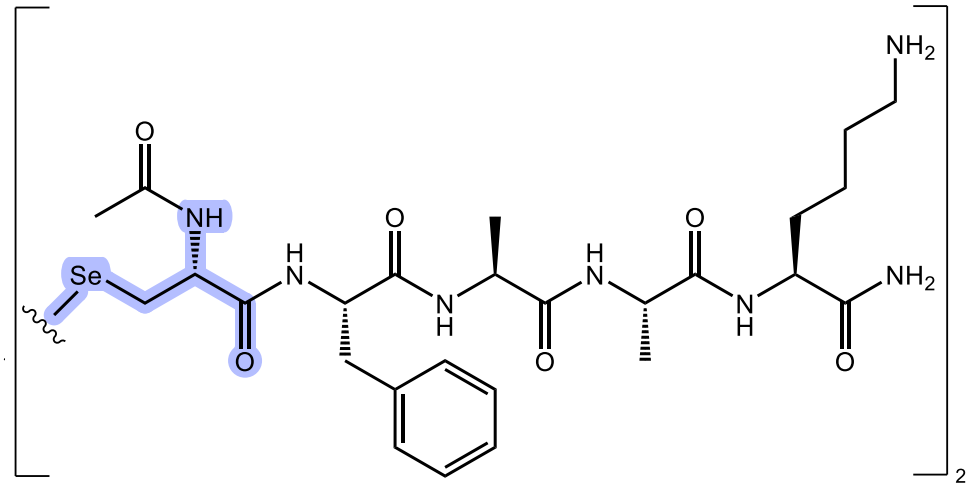


Fig S 10. Structure of model peptide 3

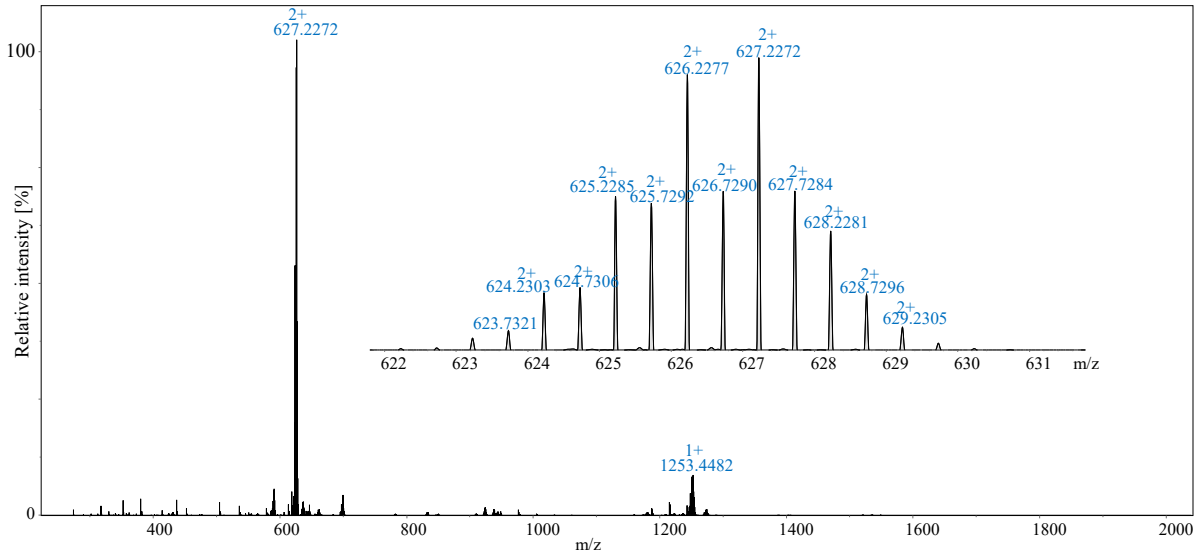


Fig S 11. ESI-MS spectrum obtained for model peptide 3

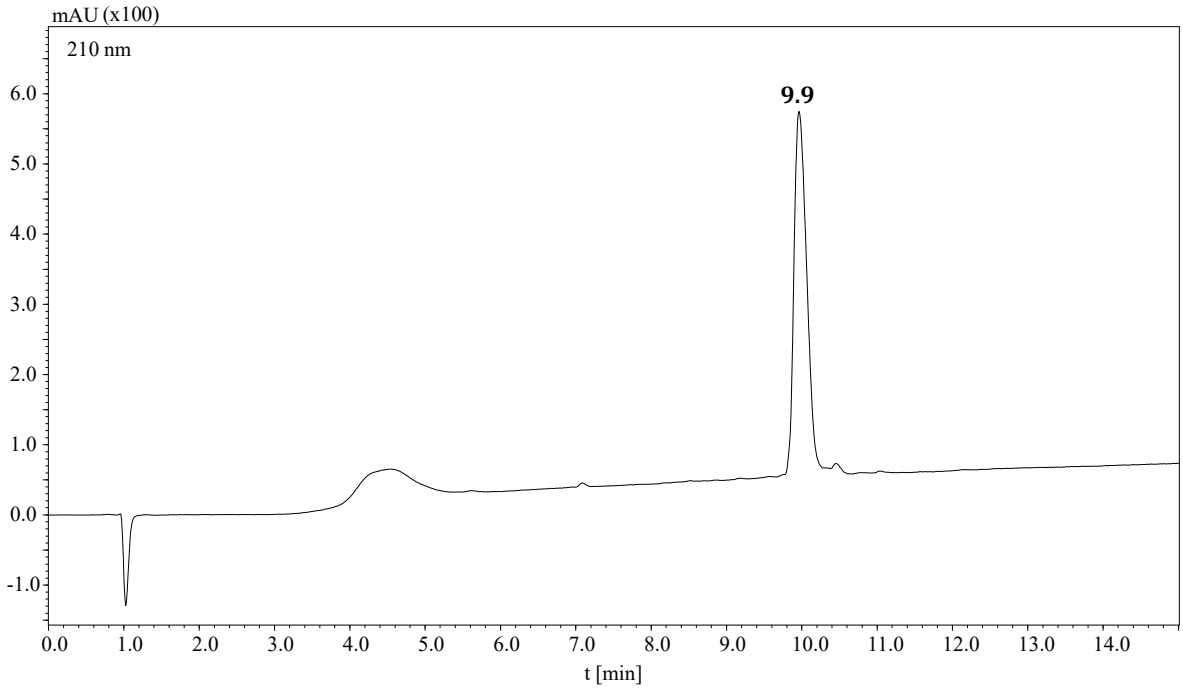


Fig S 12. HPLC chromatogram obtained for model peptide 3 (210 nm)

9.4 The synthesis of model peptide 4

The model peptide **4** was assembled according to the procedure described in section 6.

HR-ESI-MS calcd *m/z* 683.261, found 683.255 $[M+3H]^{3+}$, **HPLC**: R_t= 10.1 min (gradient 4), **yield:** 65%

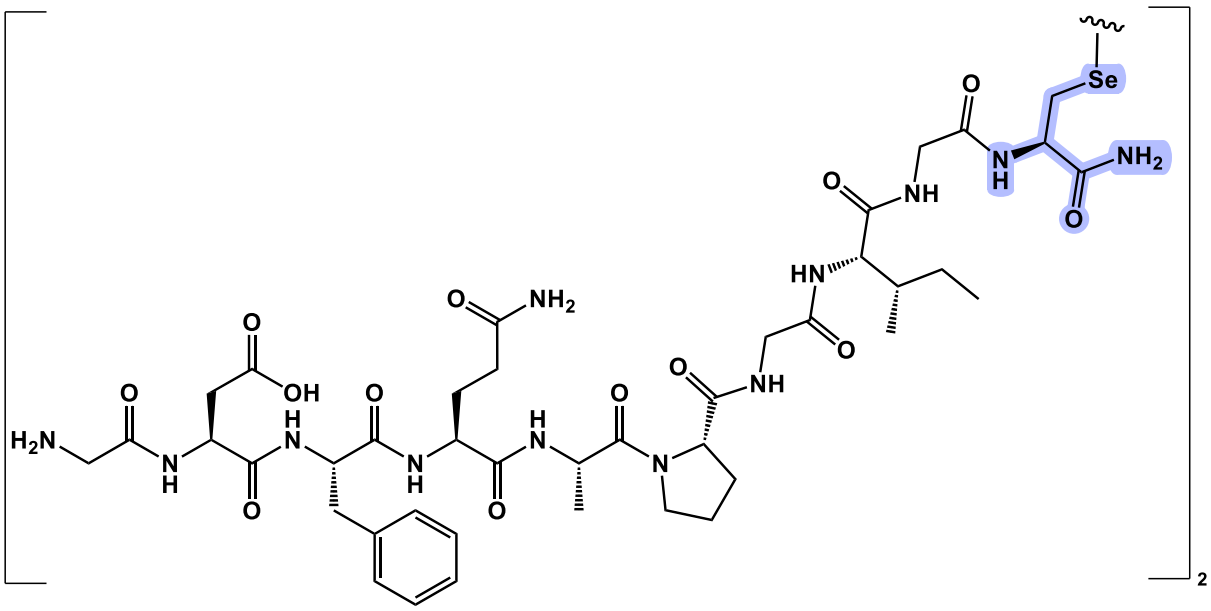


Fig S 13. Structure of model peptide 4

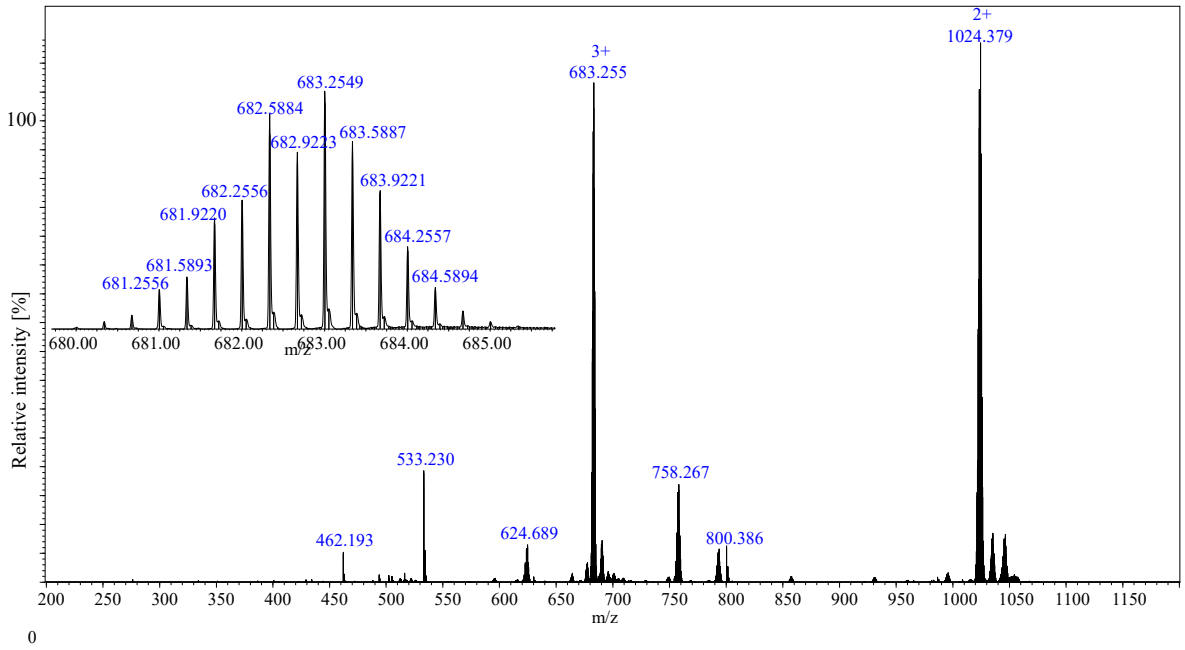


Fig S 14. ESI-MS spectrum obtained for model peptide 4

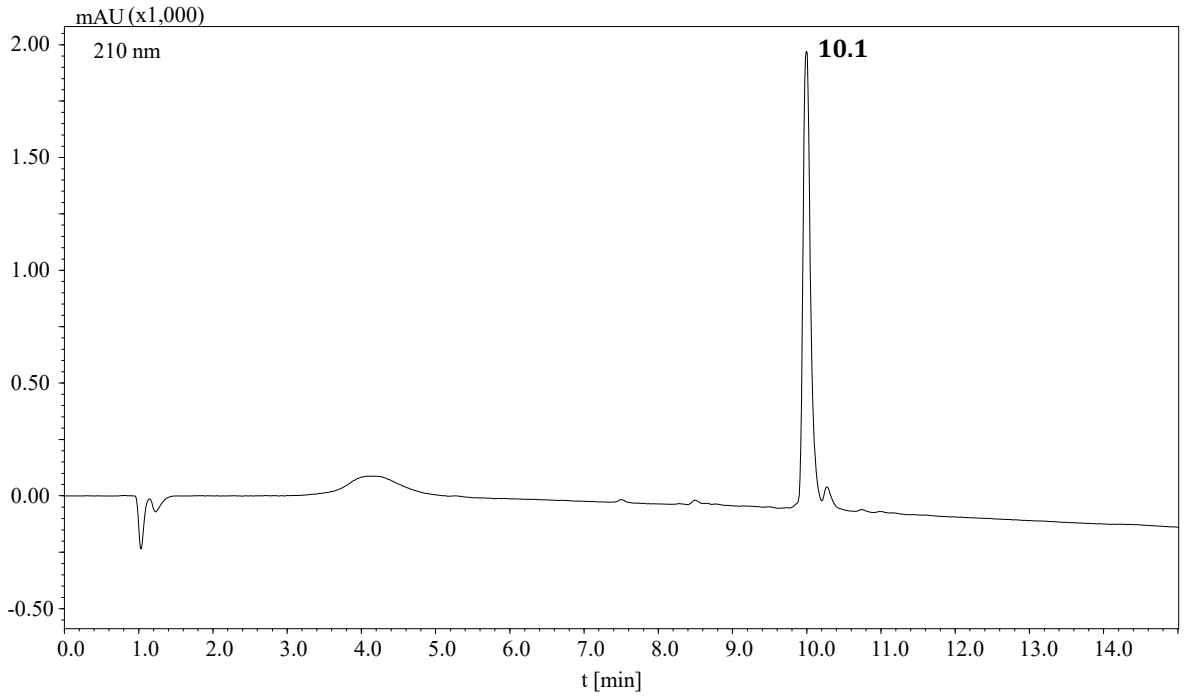


Fig S 15. HPLC chromatogram obtained for model peptide 4 (210 nm)

9.5 The synthesis of model peptide 5

The model peptide **5** was assembled according to the procedure described in section 6.

HR-ESI-MS calcd *m/z* 588.2057, found 588.2047 $[M+4H]^{4+}$, **HPLC**: R_t= 8.9 min (gradient 4) , **yield:** 65%

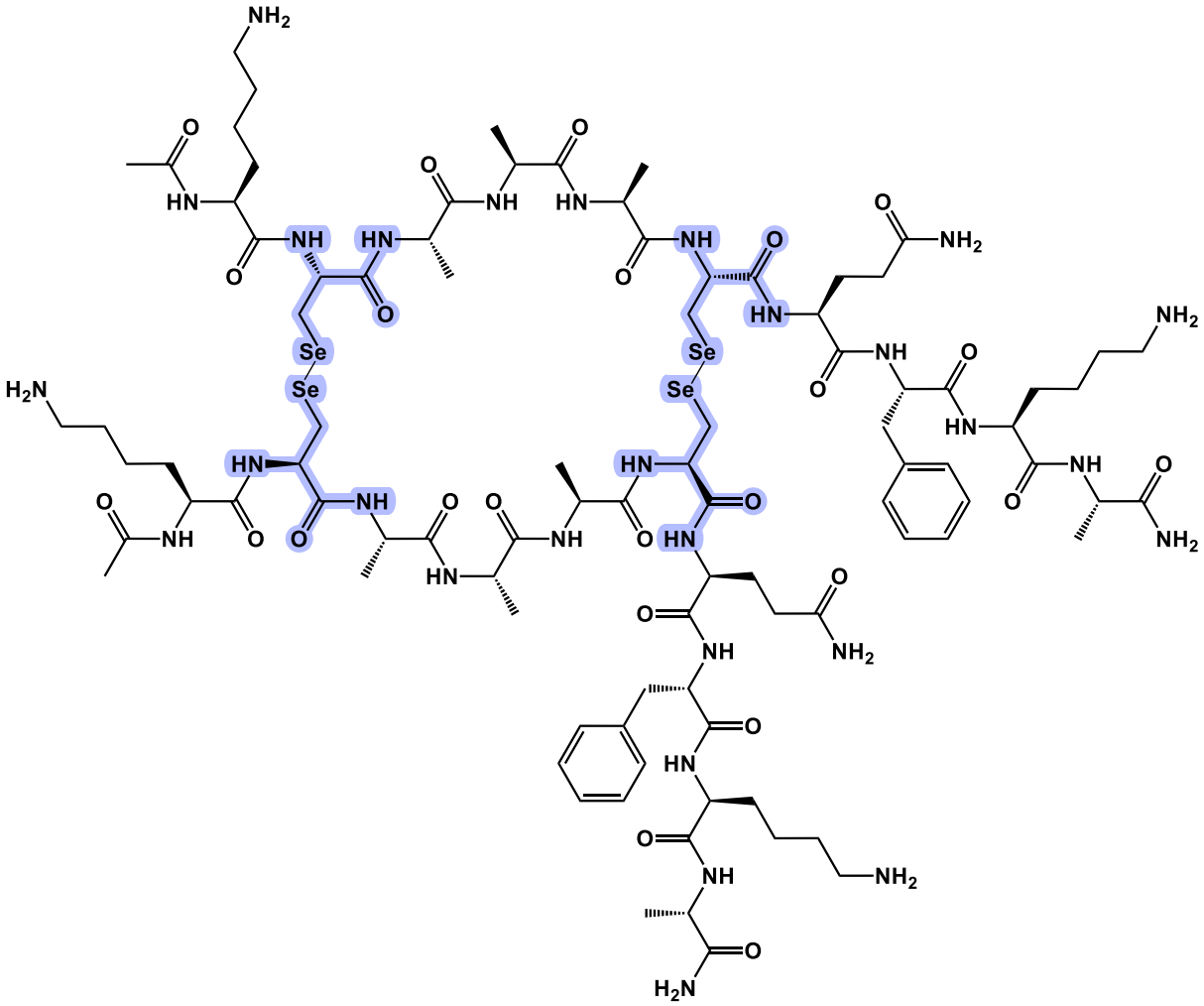


Fig S 16. Structure of model peptide 5.

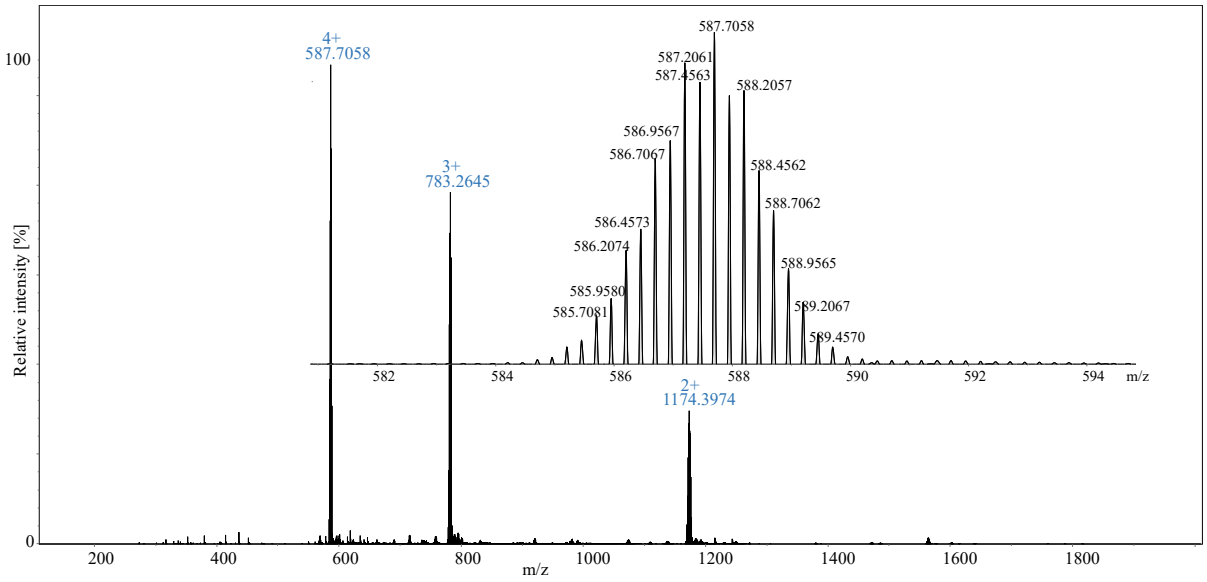


Fig S 17. ESI-MS spectrum obtained for model peptide 5

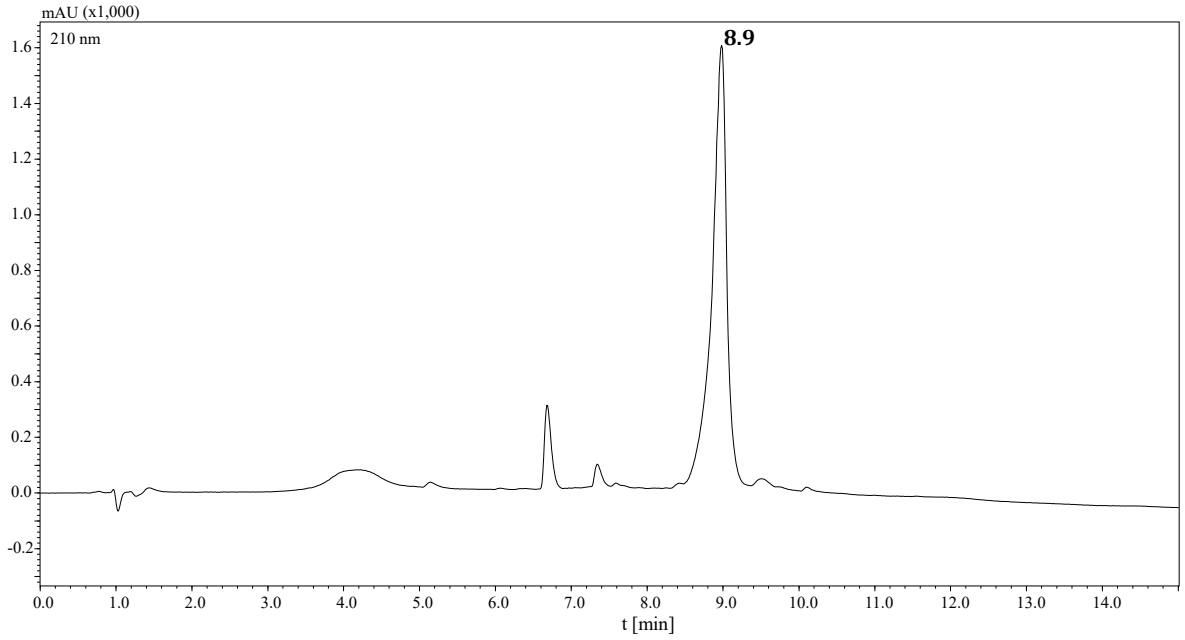


Fig S 18. HPLC chromatogram (210) obtained for model peptide 5*

*additional peak at 6.7 min results from intramolecular diselenide formation → illumination with visible light leads to a shift toward intramolecular diselenide bond

9.6 The synthesis of model peptide 6

The model peptide **6** was assembled according to the procedure described in section 6.

HR-ESI-MS calcd *m/z* 497.8156, found 497.8151 $[M+4H]^{4+}$, **HPLC**: R_t= 5.4 min (gradient 1) , **yield:** 61%

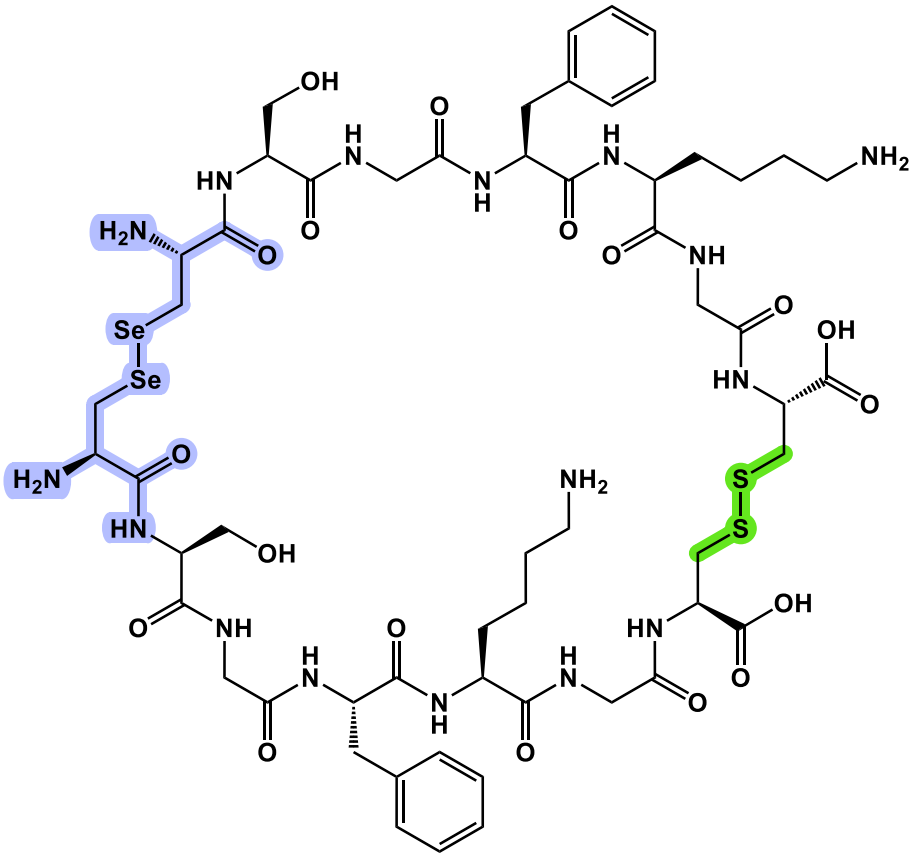


Fig S 19. Structure of model peptide 6

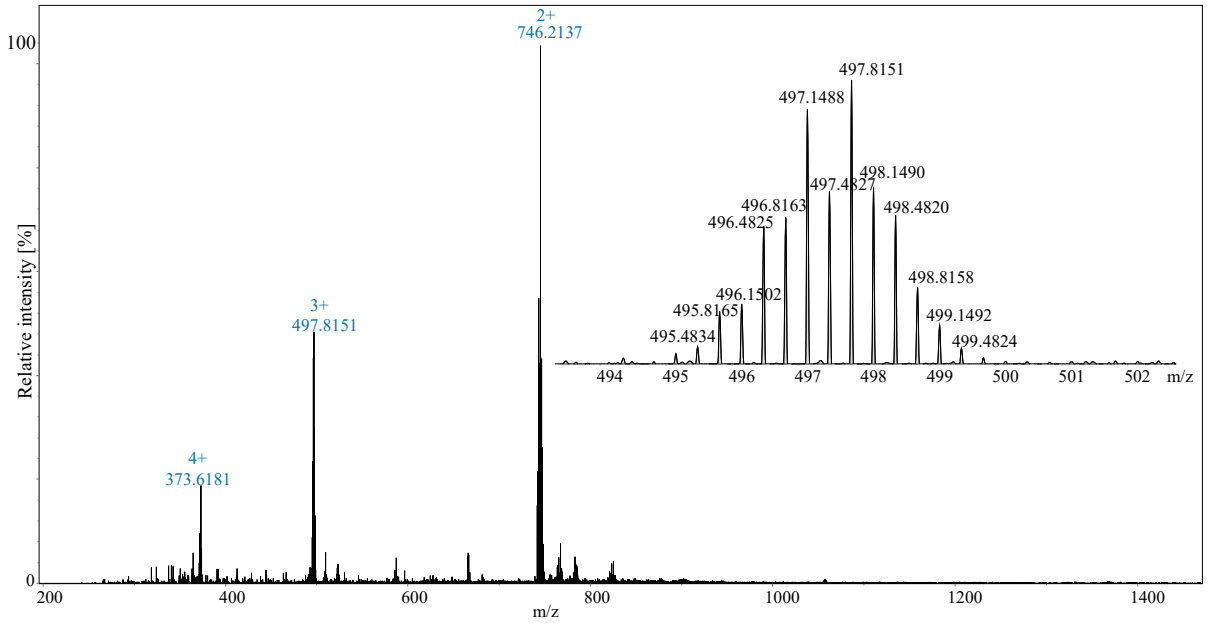


Fig S 20. ESI-MS spectrum obtained for model peptide 6

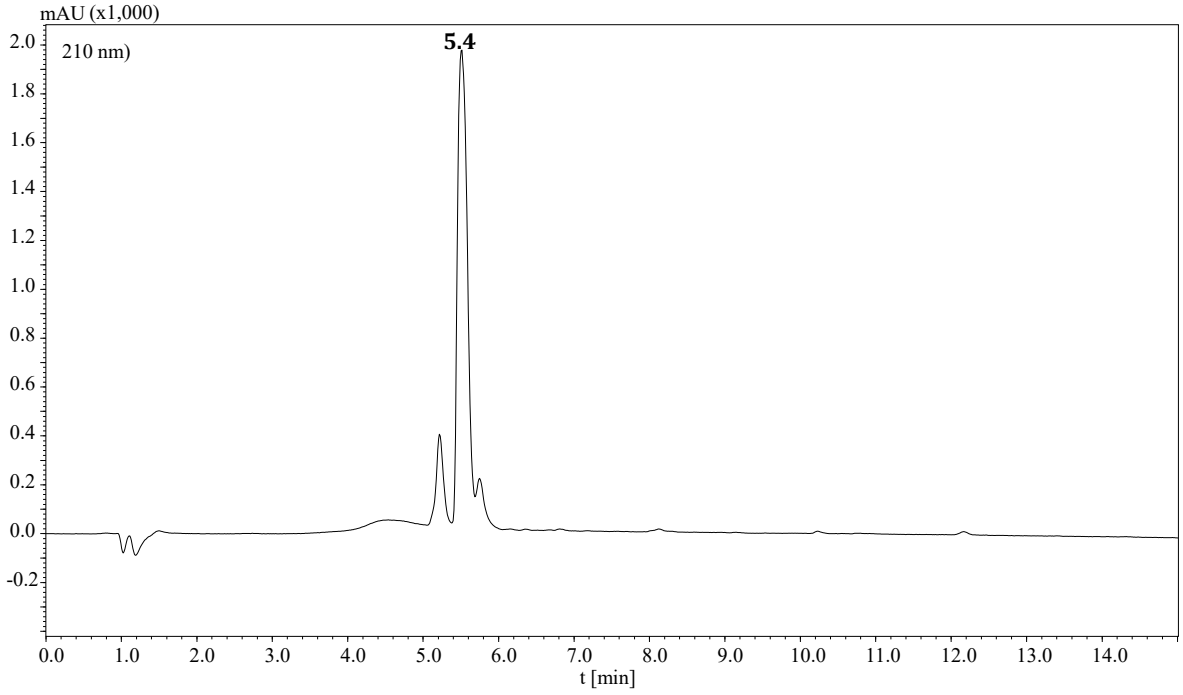


Fig S 21. HPLC chromatogram obtained for model peptide 6 (210 nm)

10. Optimization of RB-assisted photochemical conversion

10.1. Optimization of RB-assisted photochemical conversion – pH

Peptide **1** sample (0.5 mg), containing an oxidized selenocysteine residue in the form of a diselenide homodimer, was placed in a 2 mL HPLC vial made of transparent borosilicate glass The vial must not be sealed with a cap in order to allow the access of atmospheric oxygen. The sample was dissolved in 450 µL of aqueous buffer consisting of 8 M guanidine hydrochloride and 0.2 M Na_2HPO_4, prepared with deionized water obtained from a reverse osmosis system (Hydrolab, Poland). The pH of the solution was adjusted to 5, 6 , 7 and 8 (each experiment at a single pH) using 6 M HCl or 6M NaOH. Subsequently, 0.3 equivalents of Rose Bengal were added from a freshly prepared aqueous stock solution (2 mg/mL). The stock solution can be stored for up to one week; however, its long-term stability has not been tested. The sample was then irradiated for over 3 hours using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K).

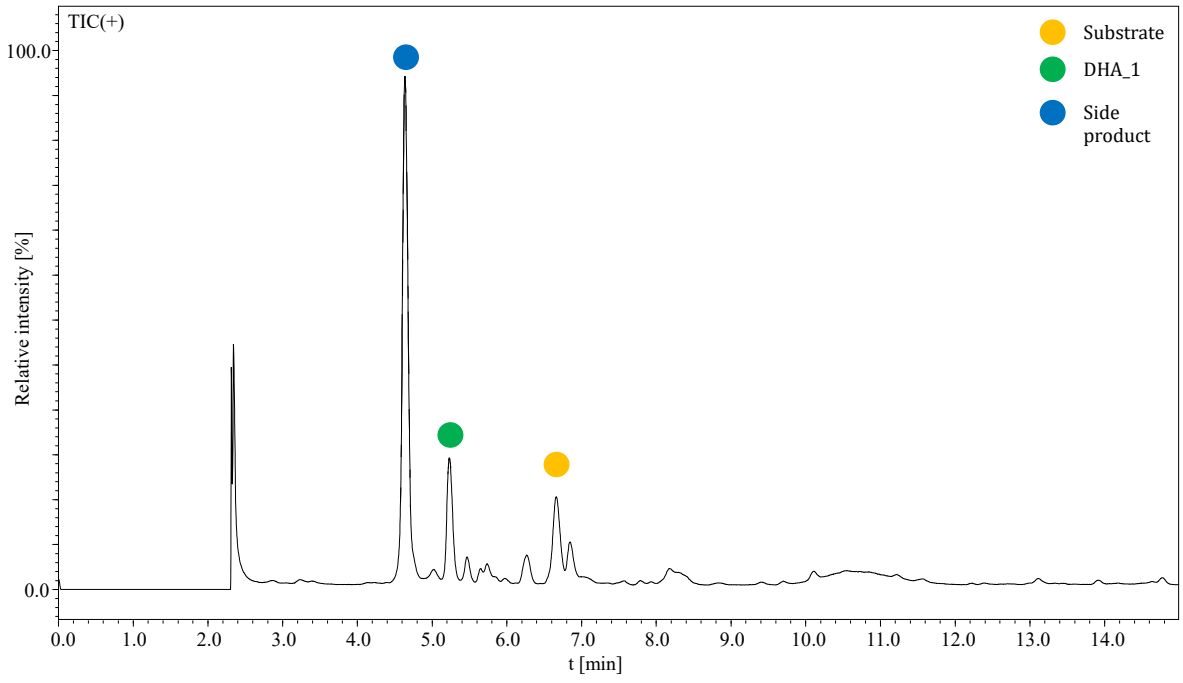


Fig S 22. RB-mediated conversion of peptide 1 to its Dha-analog – pH 5 (LC-MS chromatogram – gradient 2)

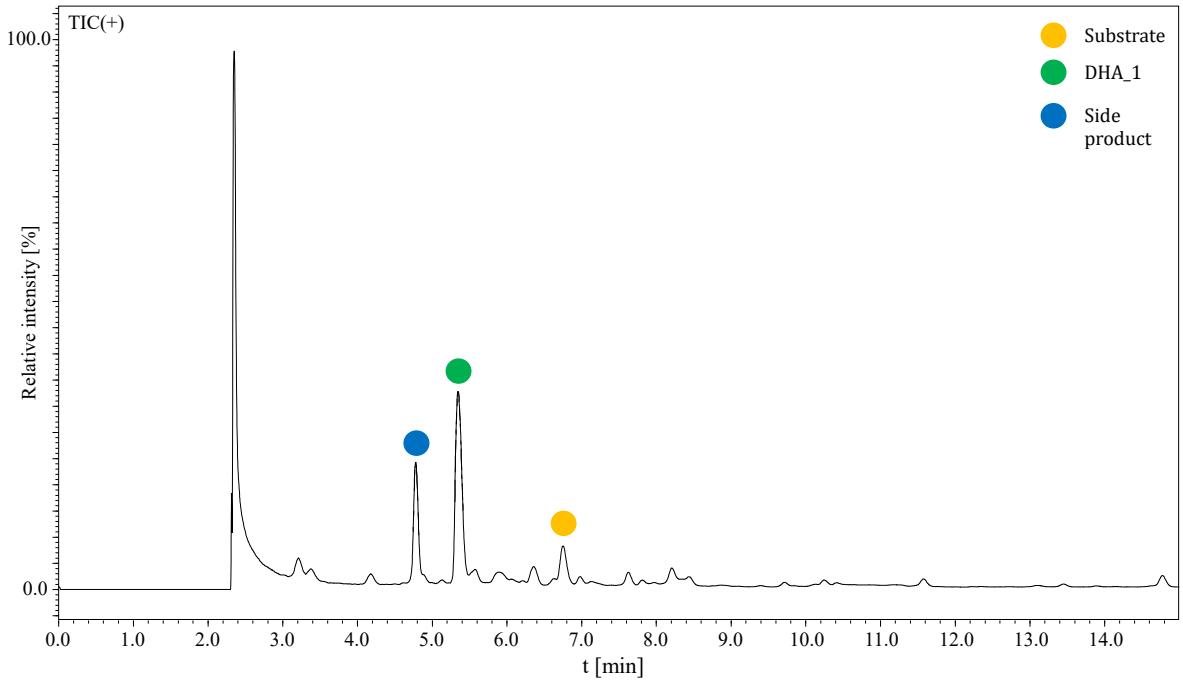


Fig S 23. RB-mediated conversion of peptide 1 to its Dha-analog – pH 6 (LC-MS chromatogram – gradient 2)

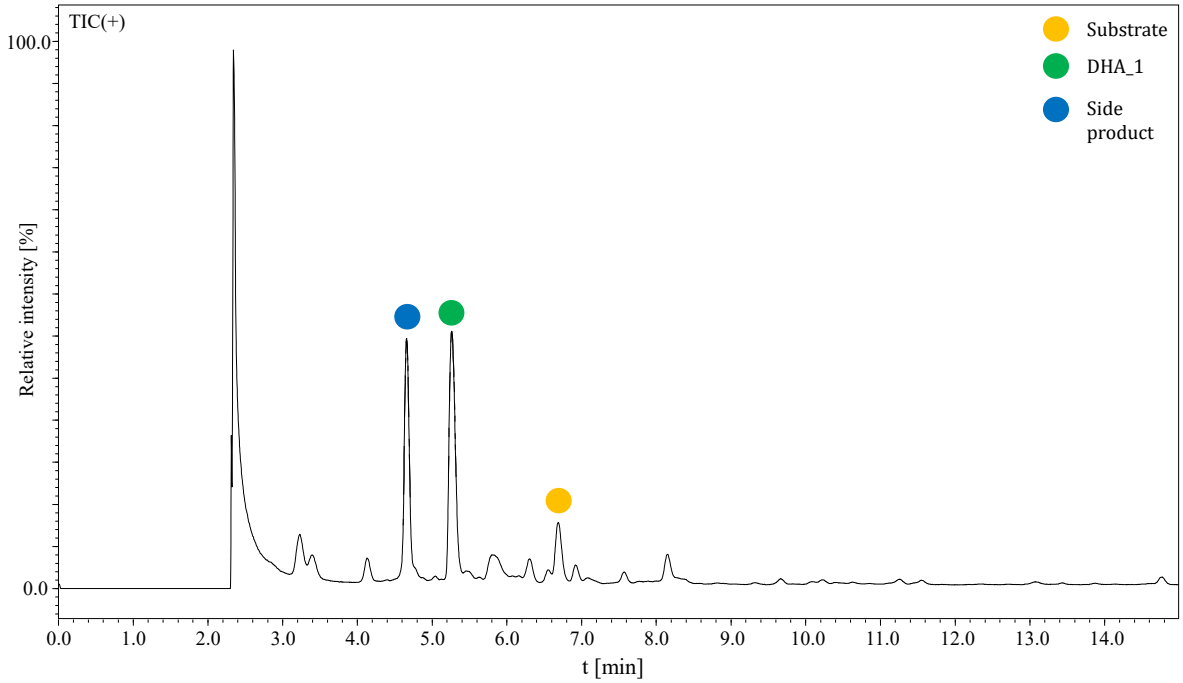


Fig S 24. RB-mediated conversion of peptide 1 to its Dha-analog – pH 7 (LC-MS chromatogram – gradient 2)

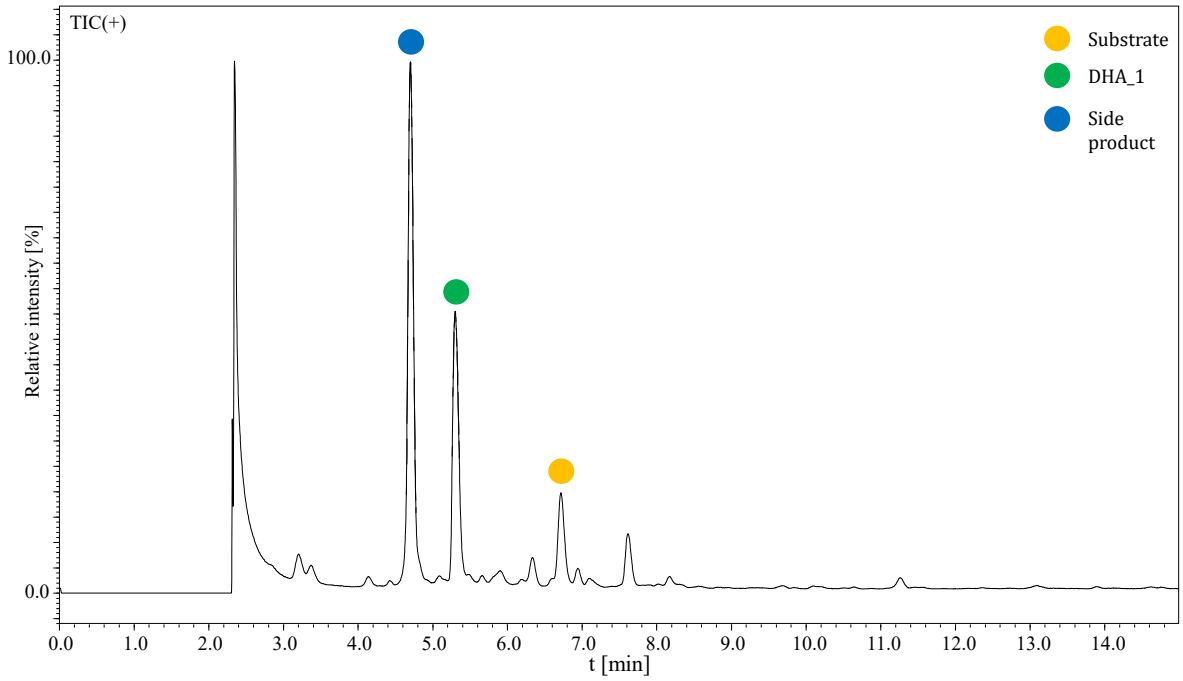


Fig S 25. RB-mediated conversion of peptide 1 to its Dha-analog – pH 7 (LC-MS chromatogram – gradient 2)

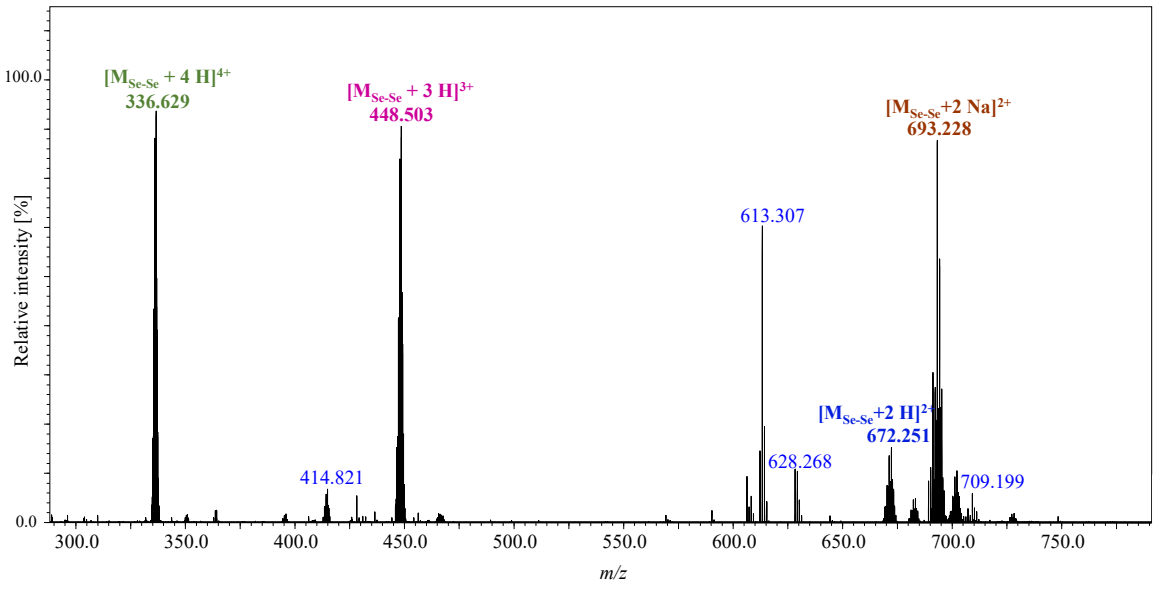


Fig S 26. LC-ESI-MS spectrum obtained in 6.7 min - substrate (peptide 1)

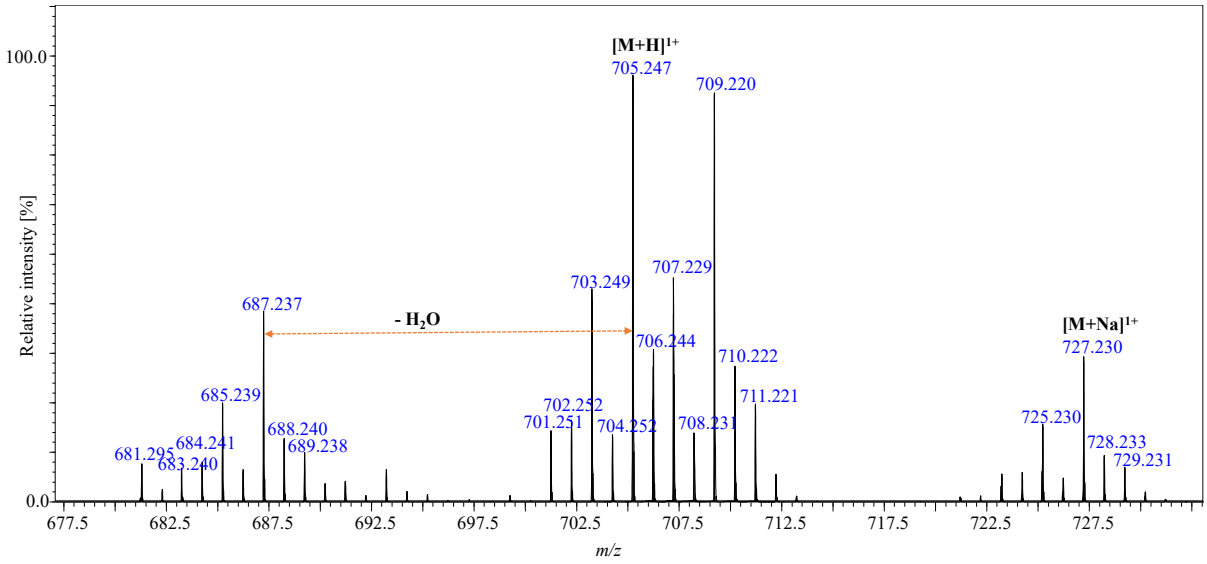


Fig S 27. LC-ESI-MS spectrum obtained in 4.6 min – side product

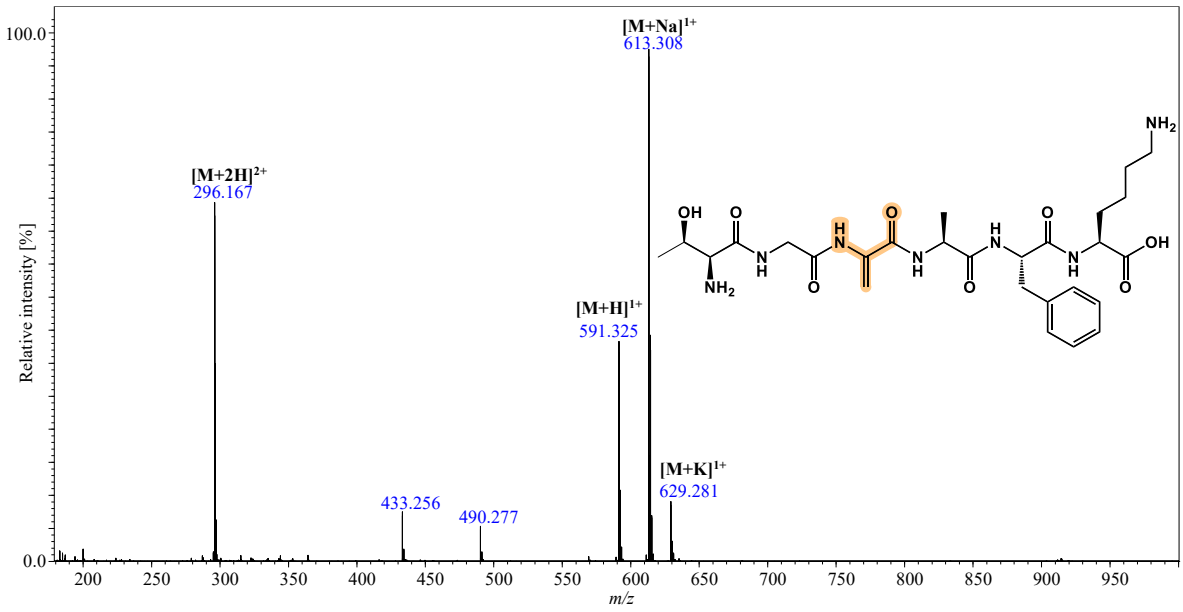


Fig S 28. LC-ESI-MS spectrum obtained in 5.4 min – desired product

10.2 Optimization of RB-assisted photochemical conversion – deoxygenation of sample

A peptide sample (0.5 mg), containing an oxidized selenocysteine residue in the form of a diselenide homodimer, was placed in a 2 mL HPLC vial made of transparent borosilicate glass The sample was dissolved in 450 µL of aqueous buffer consisting of 8 M guanidine hydrochloride and 0.2 M Na_2HPO_4, prepared with deionized water obtained from a reverse osmosis system (Hydrolab, Poland). The pH of the solution was adjusted to 6 using 6 M HCl.

Subsequently, 0.3 equivalents of Rose Bengal were added from a freshly prepared aqueous stock solution (2 mg/mL). The stock solution can be stored for up to one week; however, its long-term stability has not been tested. The vial must was sealed with a cap and a needle was inserted into the sample and then nitrogen was passed through for 3 min to deoxygenate the sample. The sample was then irradiated for over 3 hours using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K).

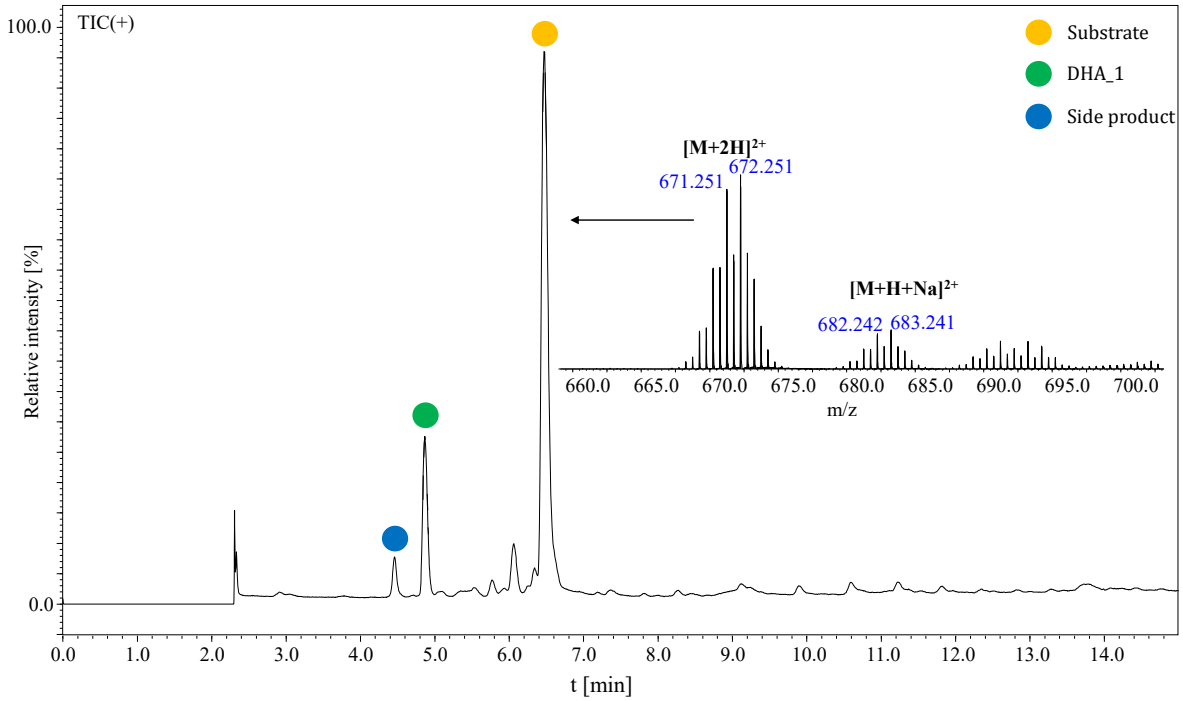


Fig S 29. RB-mediated conversion of peptide 1 to its Dha analog in pH 6 (nitrogen was passed through the sample before the reaction - 3 min) – gradient 2

10.3 Optimization of RB-assisted photochemical conversion – amount of Rose Bengal

Peptide **1** sample (0.5 mg), containing an oxidized selenocysteine residue in the form of a diselenide homodimer, was placed in a 2 mL HPLC vial made of transparent borosilicate glass. The vial must not be sealed with a cap in order to allow the access of atmospheric oxygen. The sample was dissolved in 450 µL of aqueous buffer consisting of 8 M guanidine hydrochloride and 0.2 M Na_2HPO_4, prepared with deionized water obtained from a reverse

osmosis system (Hydrolab, Poland). The pH of the solution was adjusted to 6 using 6 M HCl. Subsequently, 0.1, 0.2, 0.3, 0.4 and 0.5 equivalents of Rose Bengal (one RB equivalent value was tested for each sample) were added from a freshly prepared aqueous stock solution (2 mg/mL). The stock solution can be stored for up to one week; however, its long-term stability has not been tested. The sample was then irradiated for over 3 hours using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K).

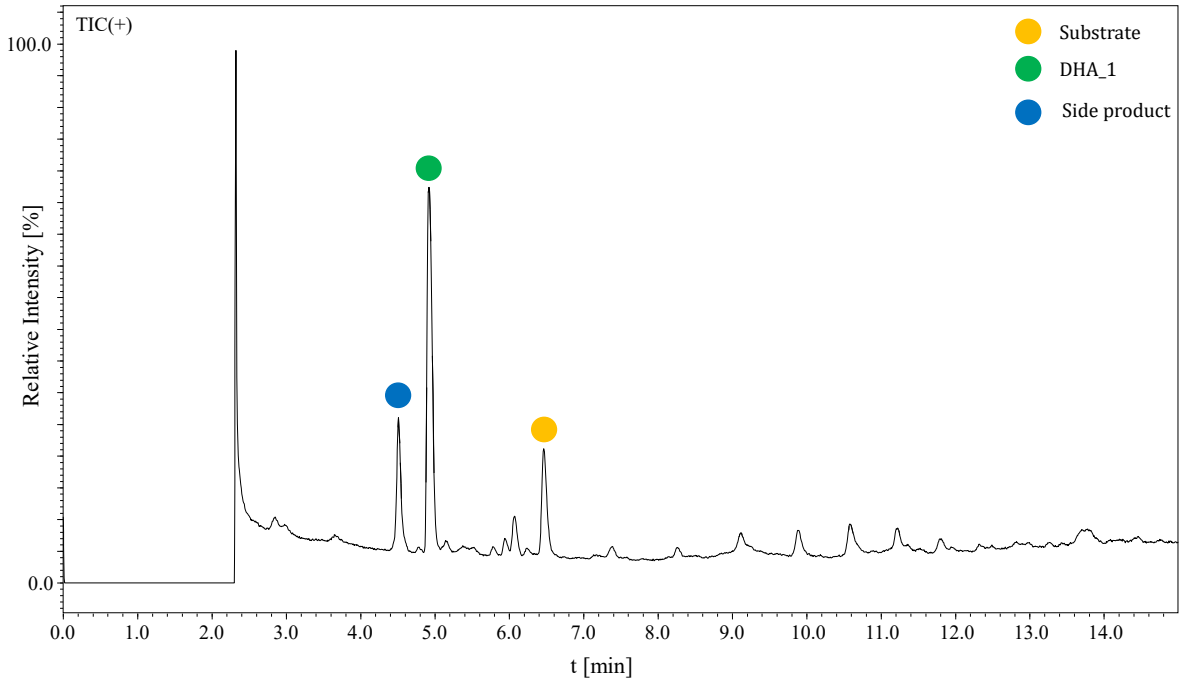


Fig S 30. RB-mediated conversion (pH 6) of peptide 1 to its Dha analog – 0.1 equiv. RB (LC-MS chromatogram – gradient 2)

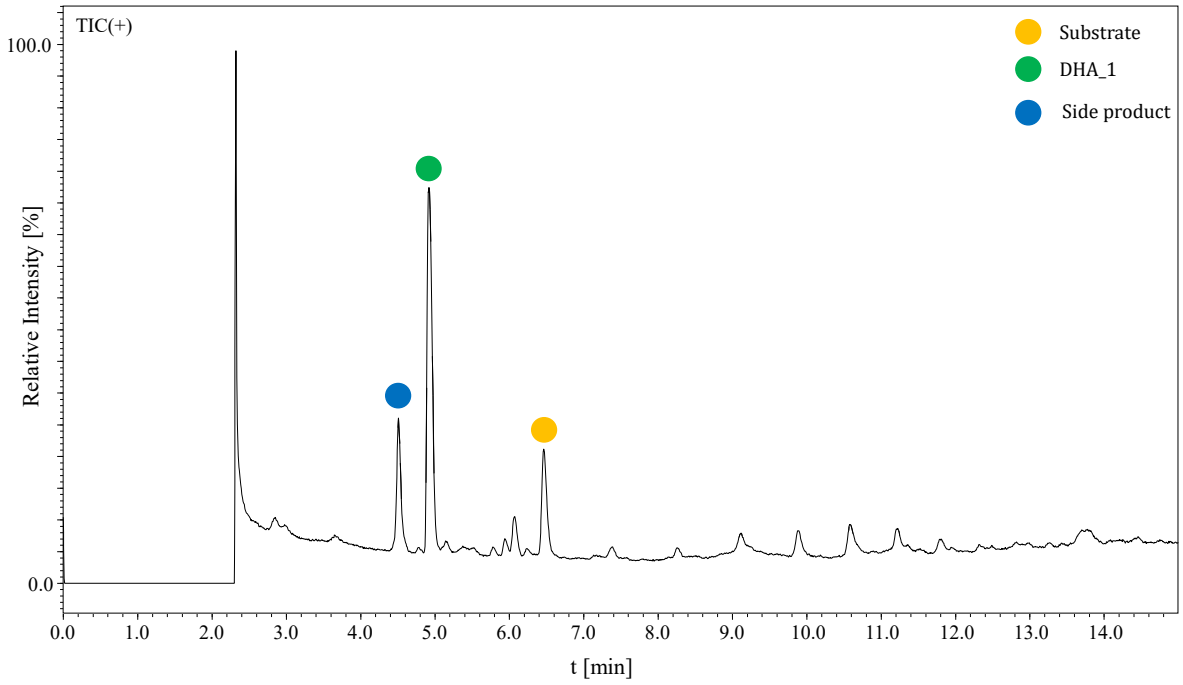


Fig S 31. RB-mediated conversion (pH 6) of peptide 1 to its Dha analog – 0.2 equiv. RB (LC-MS chromatogram – gradient 1)

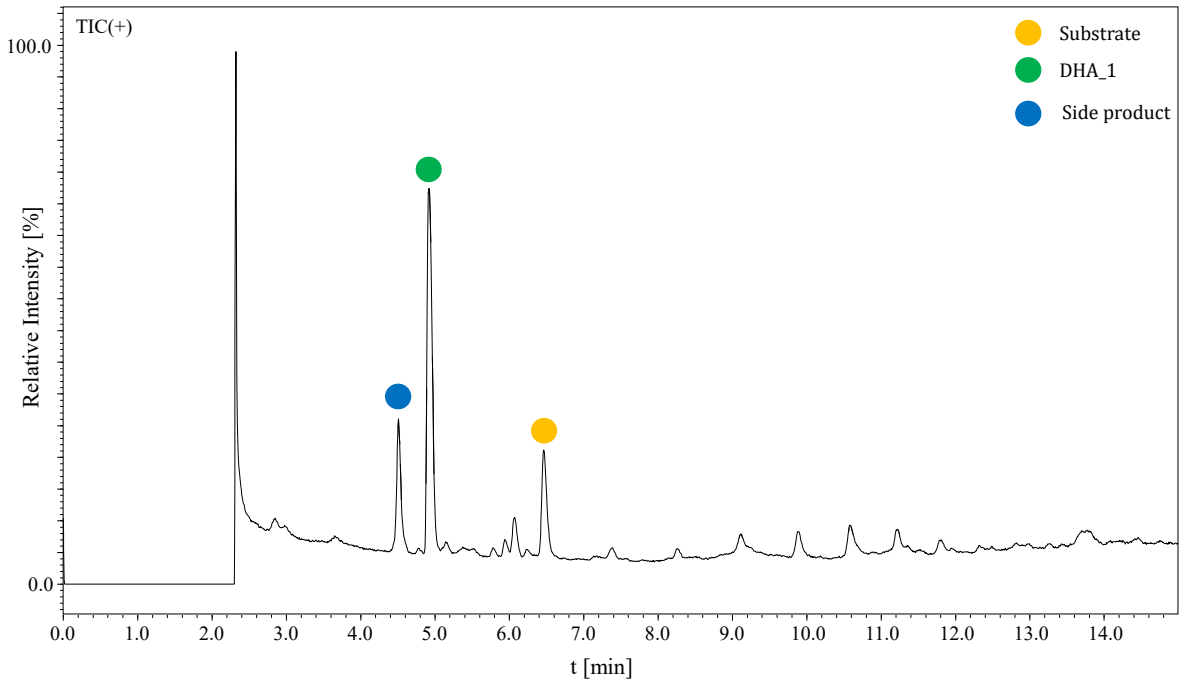


Fig S 32. RB-mediated conversion (pH 6) of peptide 1 to its Dha analog – 0.3 equiv. RB (LC-MS chromatogram – gradient 2)

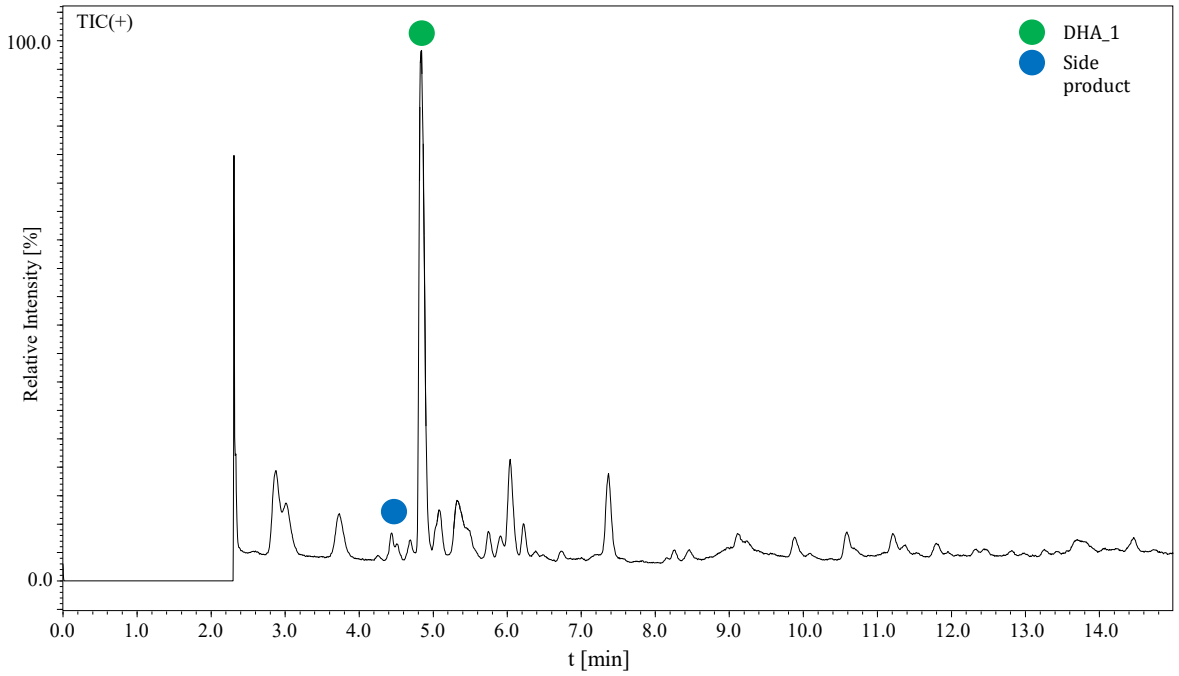


Fig S 33. RB-mediated conversion (pH 6) of peptide 1 to its Dha analog – 0.4 equiv. RB (LC-MS chromatogram – gradient 2)

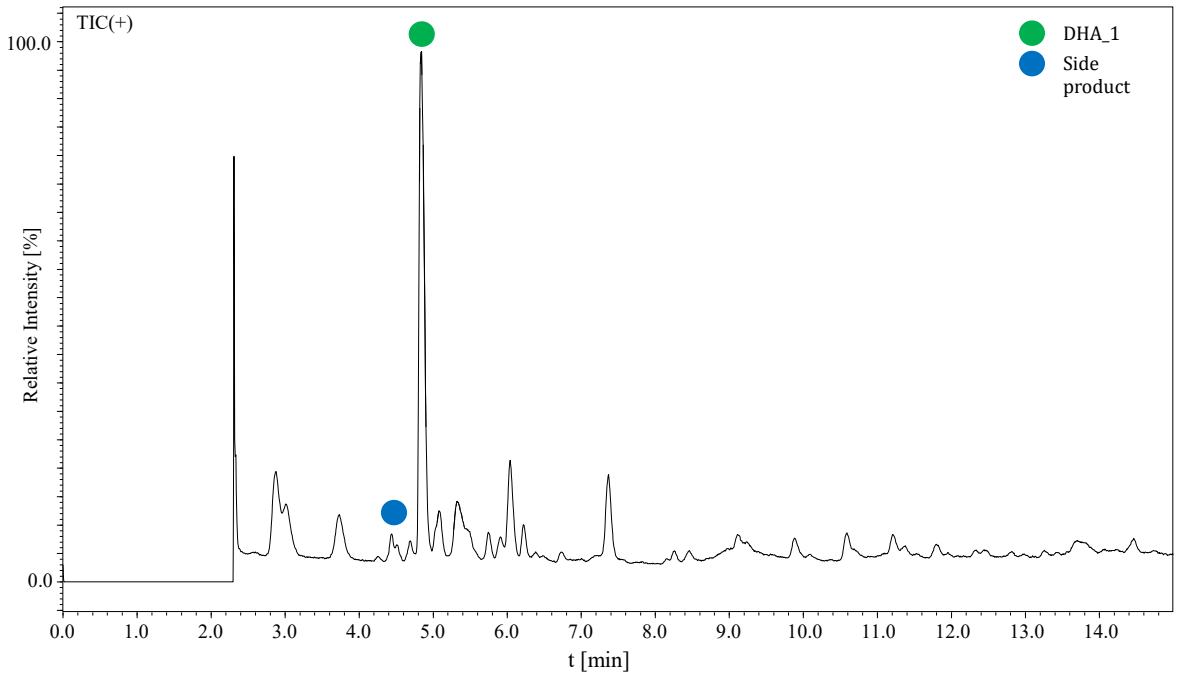


Fig S 34. RB-mediated conversion (pH 6) of peptide 1 to its Dha analog – 0.5 equiv. RB (LC-MS chromatogram – gradient 2)

10.4 Optimization of RB-assisted photochemical conversion –the influence of temperature

Peptide **2** (0.5 mg), containing an oxidized selenocysteine residue in the form of a diselenide homodimer, was placed in a 2 mL HPLC vial made of transparent borosilicate glass The vial must not be sealed with a cap in order to allow the access of atmospheric oxygen. The sample was dissolved in 450 µL of aqueous buffer consisting of 8 M guanidine hydrochloride and 0.2 M Na_2HPO_4, prepared with deionized water obtained from a reverse osmosis system (Hydrolab, Poland). The pH of the solution was adjusted to 6 using 6 M HCl. Subsequently, 0.3 equivalents of Rose Bengal were added from a freshly prepared aqueous stock solution (2 mg/mL). The stock solution can be stored for up to one week; however, its long-term stability has not been tested.

- The sample was then irradiated for over 3 hours using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K).

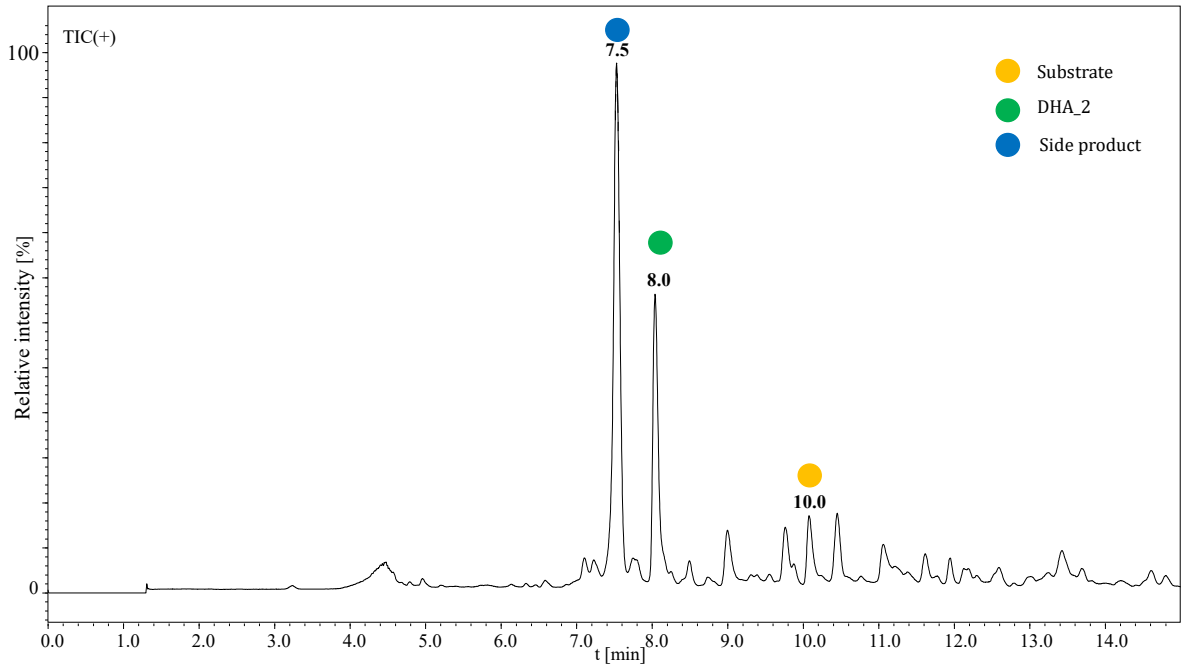


Fig S 35. RB-mediated conversion (pH 6) of peptide 2 to its Dha analog – 0.3 equiv. RB, 3h (without heating) – gradient 2

- The sample was then irradiated for over 3 hours using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K). Then, The vial was placed on a magnetic stirrer and gently heated until the solution reached 50 °C (1 hour)

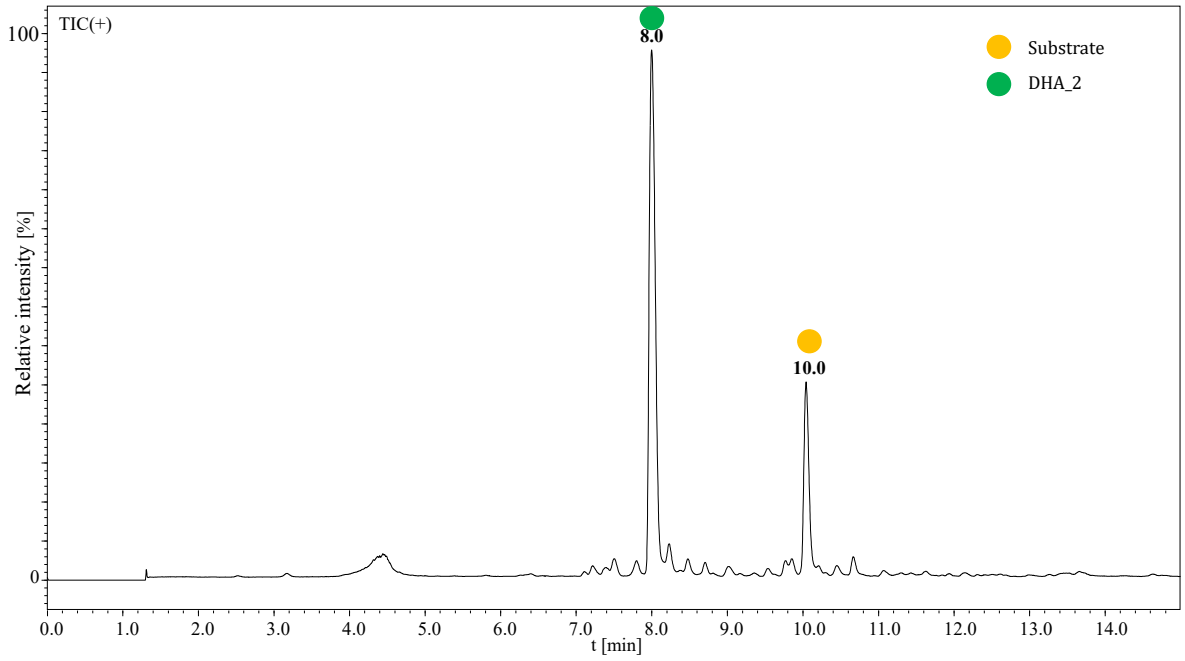


Fig S 36. RB-mediated conversion (pH 6) of peptide 2 to its Dha analog – 0.3 equiv. RB, 3h - followed by 1 hour heating at 50 °C (gradient 2)

- The vial was placed on a magnetic stirrer and gently heated until the solution reached 50 °C. Simultaneously, the sample was then irradiated for over 30 min using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K).

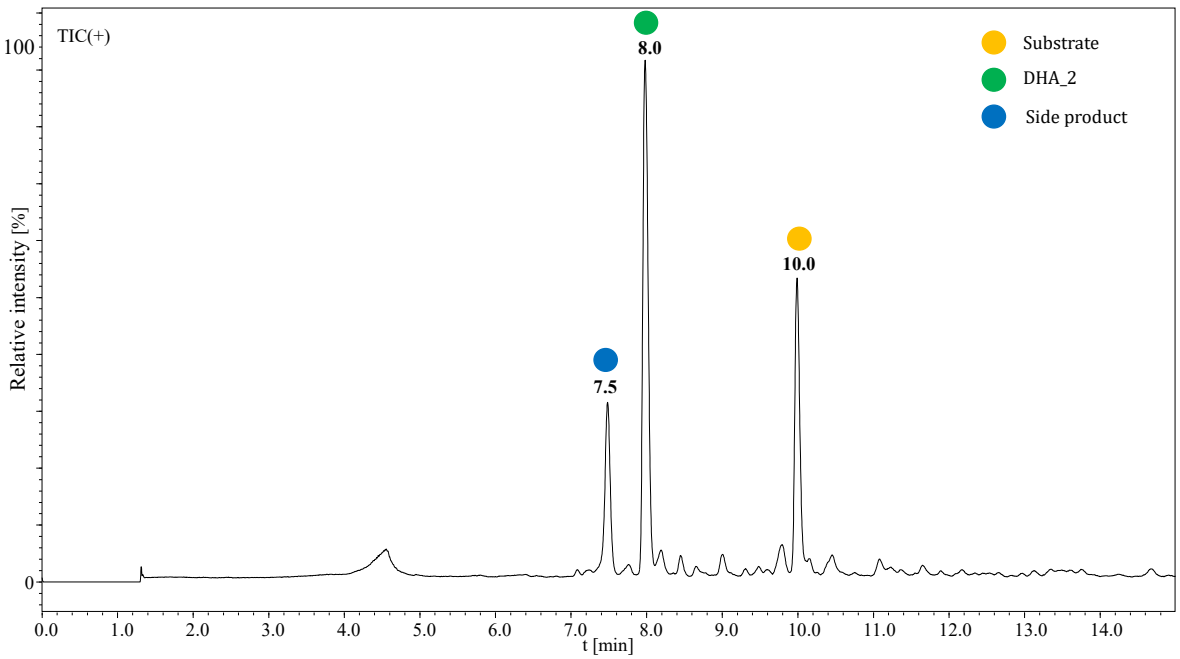


Fig S 37. RB-mediated conversion (pH 6) of peptide 2 to its Dha analog – 0.3 equiv. RB, 30 min with simultaneous heating at 50 °C (gradient 2)

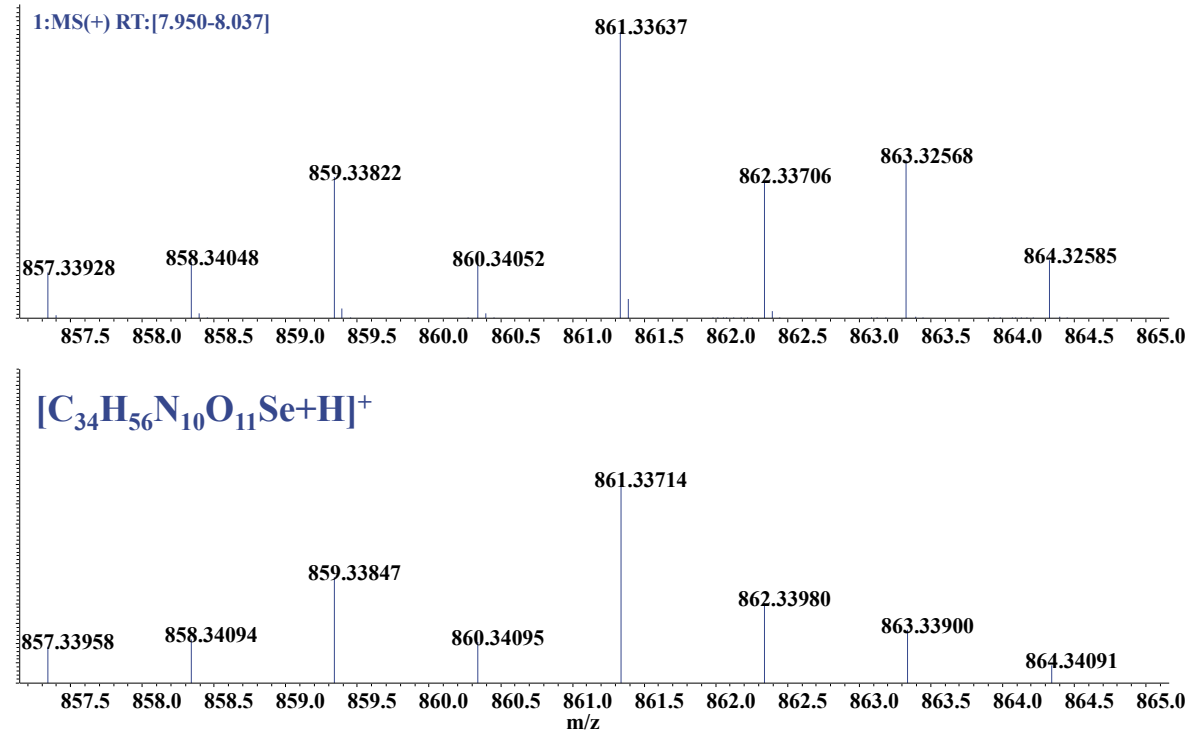


Fig S 38. ESI-MS spectrum obtained for selenium-based intermediate (peptide 2 with oxidized selenium) compared with the theoretical one (7.5 min)

- The vial was placed on a magnetic stirrer and gently heated until the solution reached 50 °C. Simultaneously, the sample was then irradiated for over 1 hour using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K).

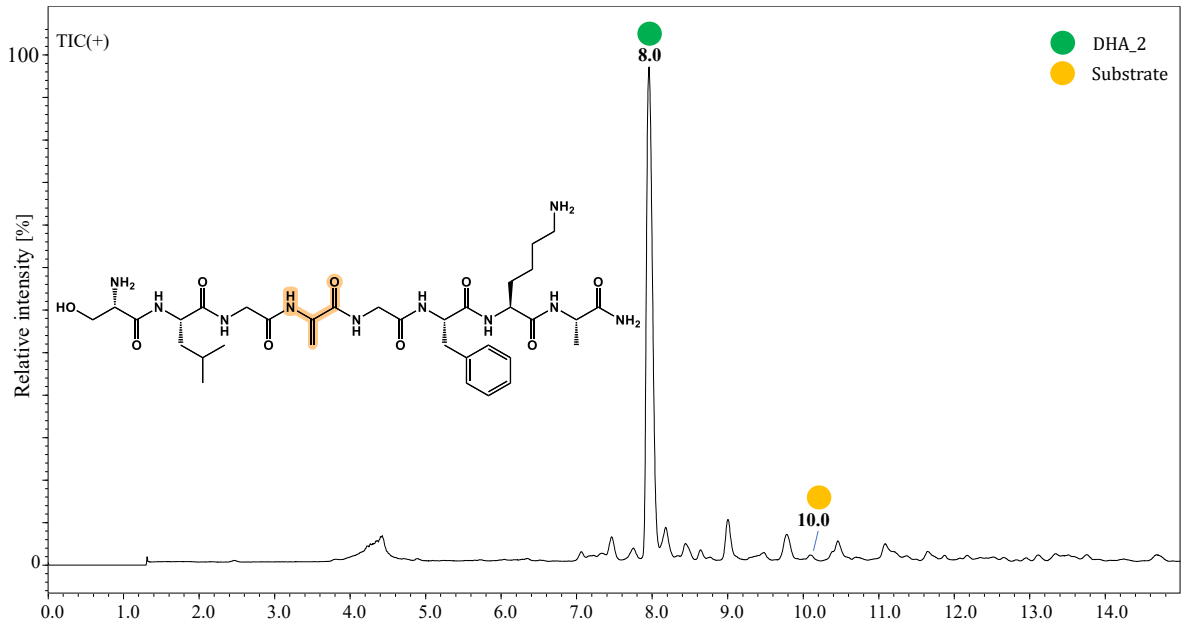


Fig S 39. RB-mediated conversion (pH 6) of peptide 2 to its Dha analog – 0.3 equiv. RB, 60 min with simultaneous heating at 50 °C

10.5 Optimization of RB-assisted photochemical conversion – addition of organic solvent

Peptide **2** sample (0.5 mg), containing an oxidized selenocysteine residue in the form of a diselenide homodimer, was placed in a 2 mL HPLC vial made of transparent borosilicate glass. The vial must not be sealed with a cap in order to allow the access of atmospheric oxygen. The sample was dissolved in 330 µL of aqueous buffer consisting of 8 M guanidine hydrochloride and 0.2 M Na_2HPO_4, prepared with deionized water obtained from a reverse osmosis system (Hydrolab, Poland). The pH of the solution was adjusted to 6 using 6 M HCl. Then, 120 µL of acetonitrile was added to the buffer. Subsequently, 0.3equiv. of Rose Bengal were added from a freshly prepared aqueous stock solution (2 mg/mL). The vial was placed on a magnetic stirrer and gently heated until the solution reached 50 °C. Simultaneously, the sample was then

irradiated for over 1 hour using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K).

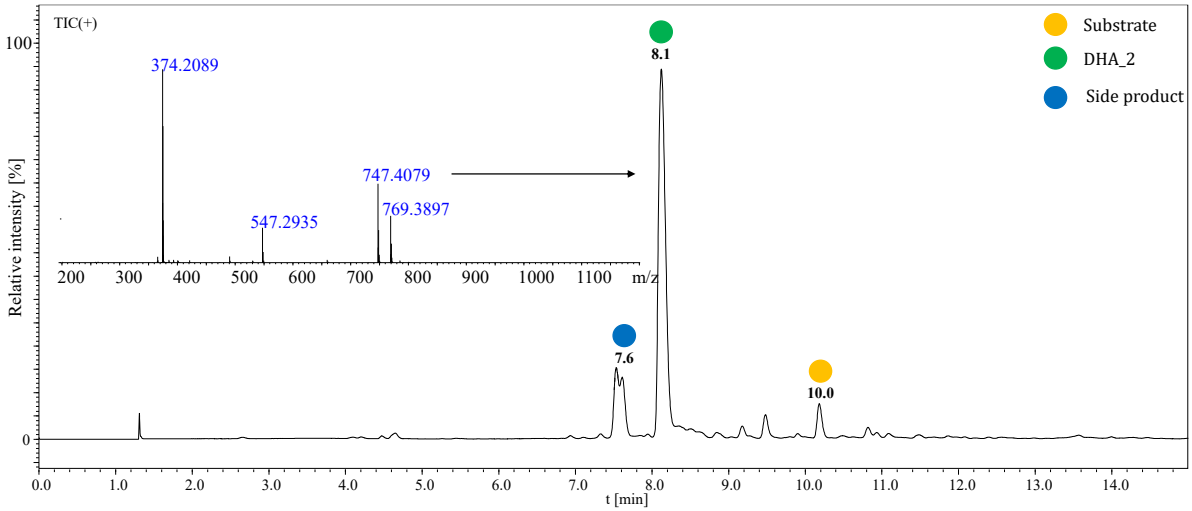


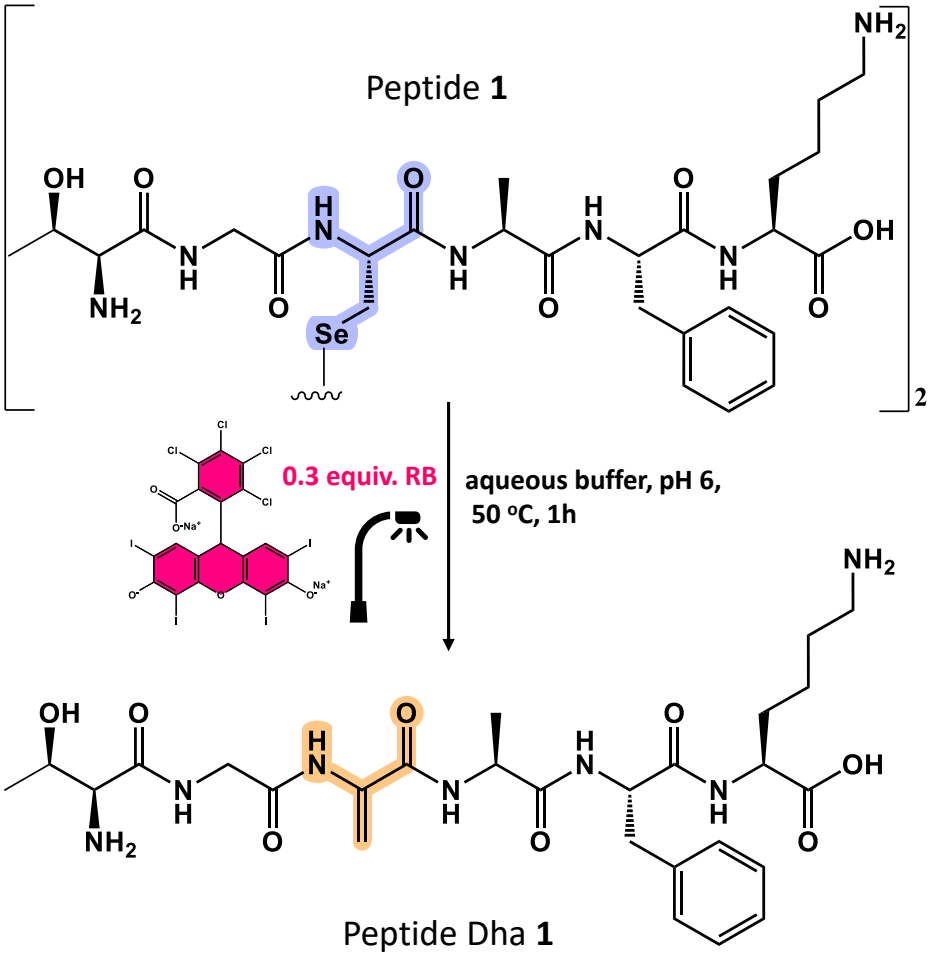
Fig S 40. RB-mediated conversion (pH 6) of peptide 2 to its Dha analog in the presence 25% acetonitrile co-solvent– 0.3 equiv. RB, 60 min with simultaneous heating at 50 °C (gradient 2)

11 RB-mediated photochemical conversion – optimized procedure

11.1 RB-mediated photochemical conversion of model peptide 1

1 mg of model peptide **1** containing an oxidized selenocysteine residue in the form of a diselenide homodimer, was placed in a 2 mL HPLC vial made of transparent borosilicate glass The vial must not be sealed with a cap in order to allow the access of atmospheric oxygen. The sample was dissolved in 900 µL of aqueous buffer consisting of 8 M guanidine hydrochloride and 0.2 M Na_2HPO_4, prepared with deionized water obtained from a reverse osmosis system (Hydrolab, Poland). The pH of the solution was adjusted to 6 using 6 M HCl. Subsequently, 0.3 equivalents of Rose Bengal were added from a freshly prepared aqueous stock solution (2 mg/mL). The stock solution can be stored for up to one week; however, its long-term stability has not been tested. The vial was placed on a magnetic stirrer and gently heated until the solution reached 50 °C. Simultaneously, the sample was then irradiated for over 1 hour using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K).

HR-ESI-MS calcd *m/z* 591.325, found 591.319 $[M+H]^{1+}$; **ESI-MS/MS** (CE 20eV): b_4 (calcd *m/z* 299.135, found 299.132), b_5 (calcd *m/z* 446.203, found 446.199), b_5-H_2O (calcd *m/z* 428.192, found 428.189), y_2-NH_3 (calcd *m/z* 276.170, found 276.168), y_2 (calcd *m/z* 293.197, found 293.194), y_4 (calcd *m/z* 433.255, found 433.252), **HPLC**: R_t= 5.7 min (gradient 2), **yield:** 90%



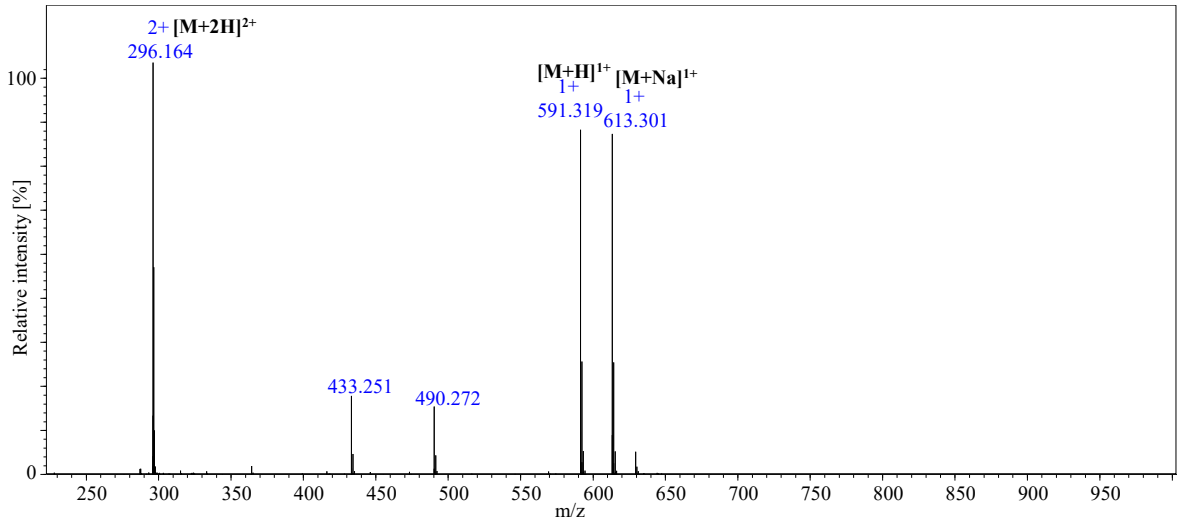


Fig S 41. ESI-MS spectrum obtained for peptide Dha 1

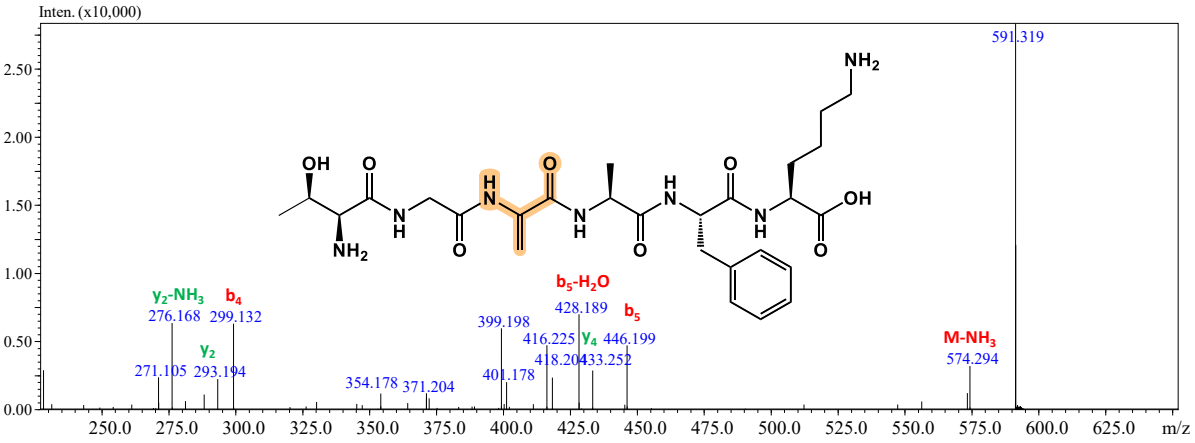


Fig S 42. ESI-MS/MS (20 eV) spectrum obtained for peptide Dha 1

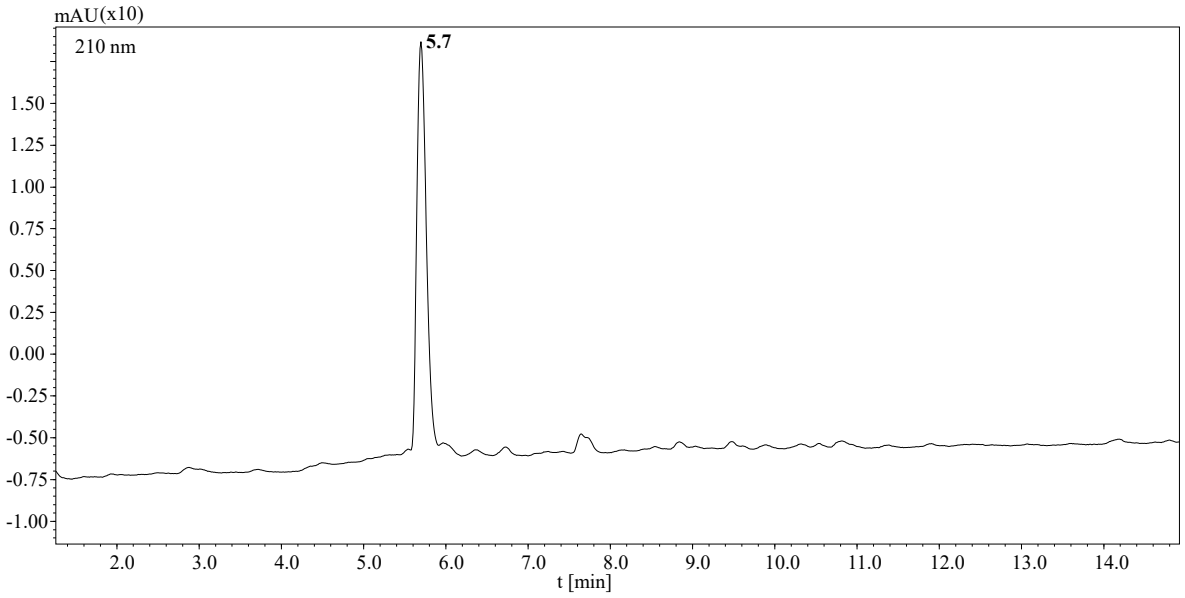
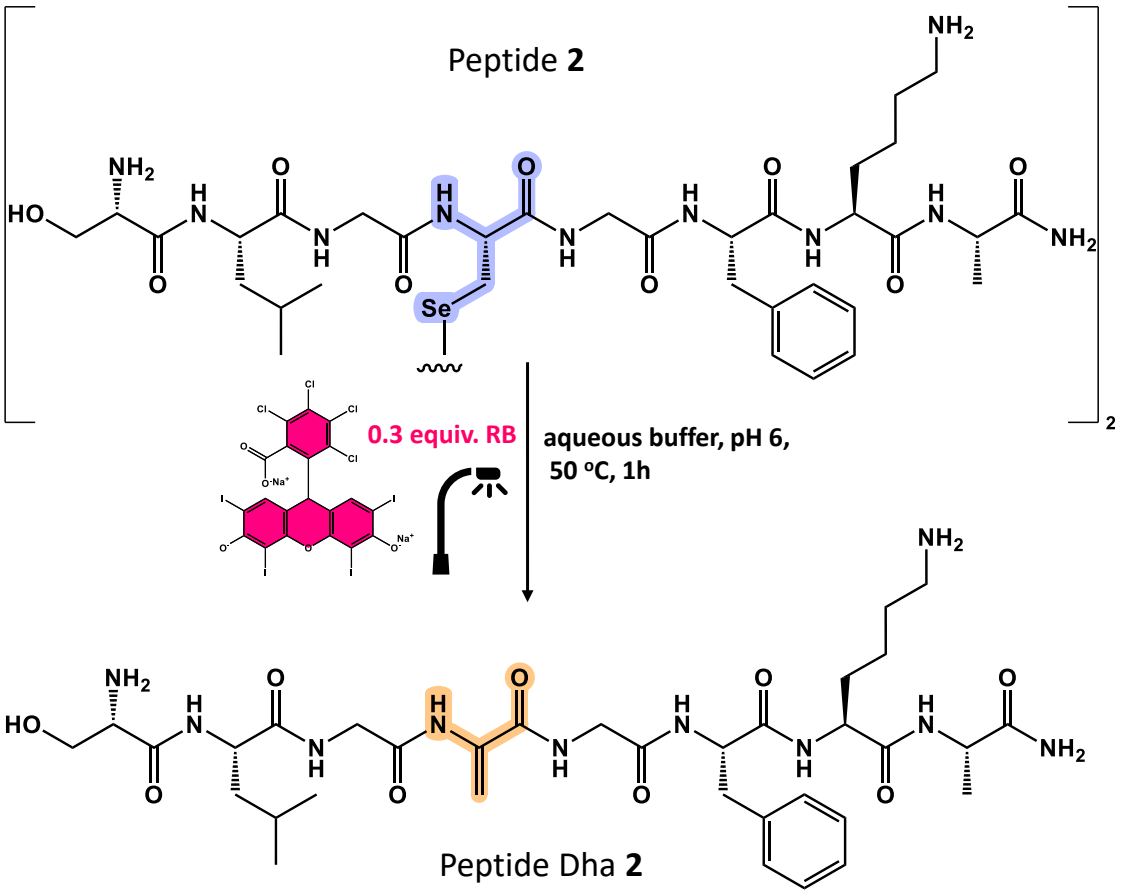


Fig S 43. HPLC chromatogram obtained for peptide Dha 1 (gradient 2)

11.2 RB-mediated photochemical conversion of model peptide 2

5 mg of model peptide **1** containing an oxidized selenocysteine residue in the form of a diselenide homodimer, was placed in a 5 mL HPLC vial made of transparent borosilicate glass The vial must not be sealed with a cap in order to allow the access of atmospheric oxygen. The sample was dissolved in 4 mL of aqueous buffer consisting of 8 M guanidine hydrochloride and 0.2 M Na_2HPO_4, prepared with deionized water obtained from a reverse osmosis system (Hydrolab, Poland). The pH of the solution was adjusted to 6 using 6 M HCl. Subsequently, 0.3 equivalents of Rose Bengal were added from a freshly prepared aqueous stock solution (2 mg/mL). The stock solution can be stored for up to one week; however, its long-term stability has not been tested. The vial was placed on a magnetic stirrer and gently heated until the solution reached 50 °C. Simultaneously, the sample was then irradiated for over 1 hour using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K). The product was purified with preparative-scale RP-HPLC.

HR-ESI-MS calcd *m/z* 747.414, found 747.407 $[M+H]^{1+}$; **ESI-MS/MS** (CE 25eV): b_6-H_2O (calcd *m/z* 513.245, found 513.241), b_6 (calcd *m/z* 531.256, found 531.251), b_7 (calcd *m/z* 659.351, found 659.345), y_2 (calcd *m/z* 217.165, found 217.163), y_5 (calcd *m/z* 490.277, found 491.273), M-NH_3 (calcd *m/z* 730.388, found 730.381), **HPLC**: R_t= 8.5 min (gradient 2), **yield:** 93%



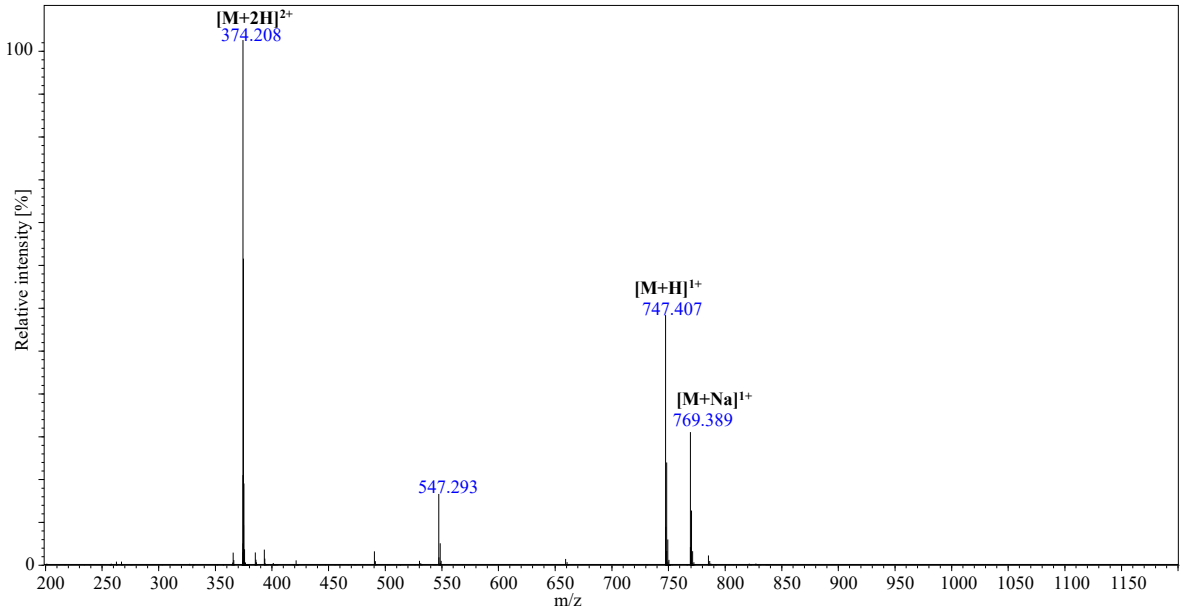


Fig S 44. ESI-MS spectrum obtained for peptide Dha 2

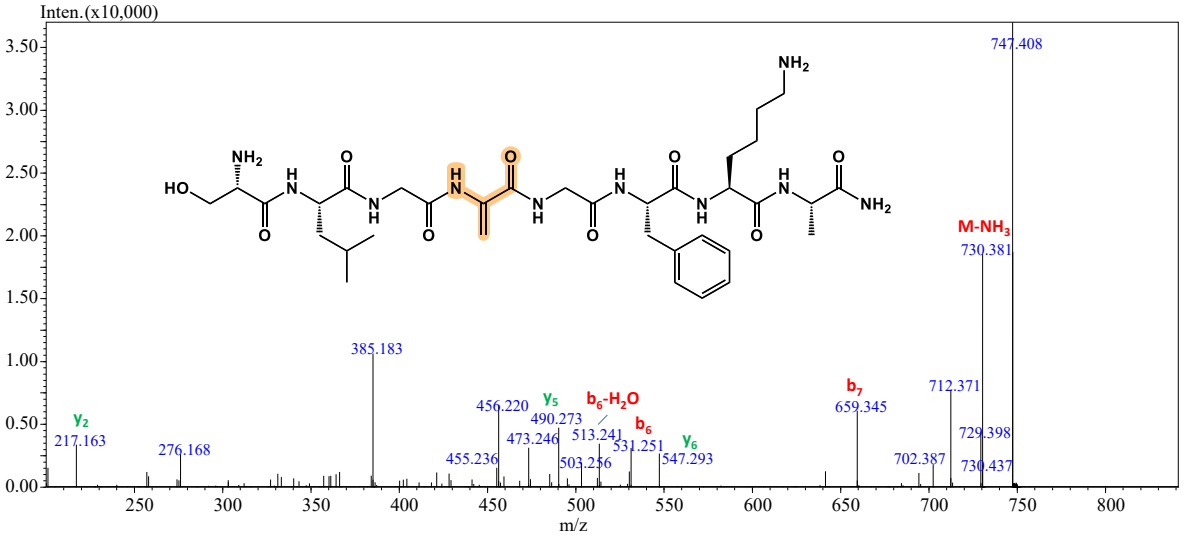


Fig S 45. ESI-MS/MS (25 eV) spectrum obtained for peptide Dha 2.

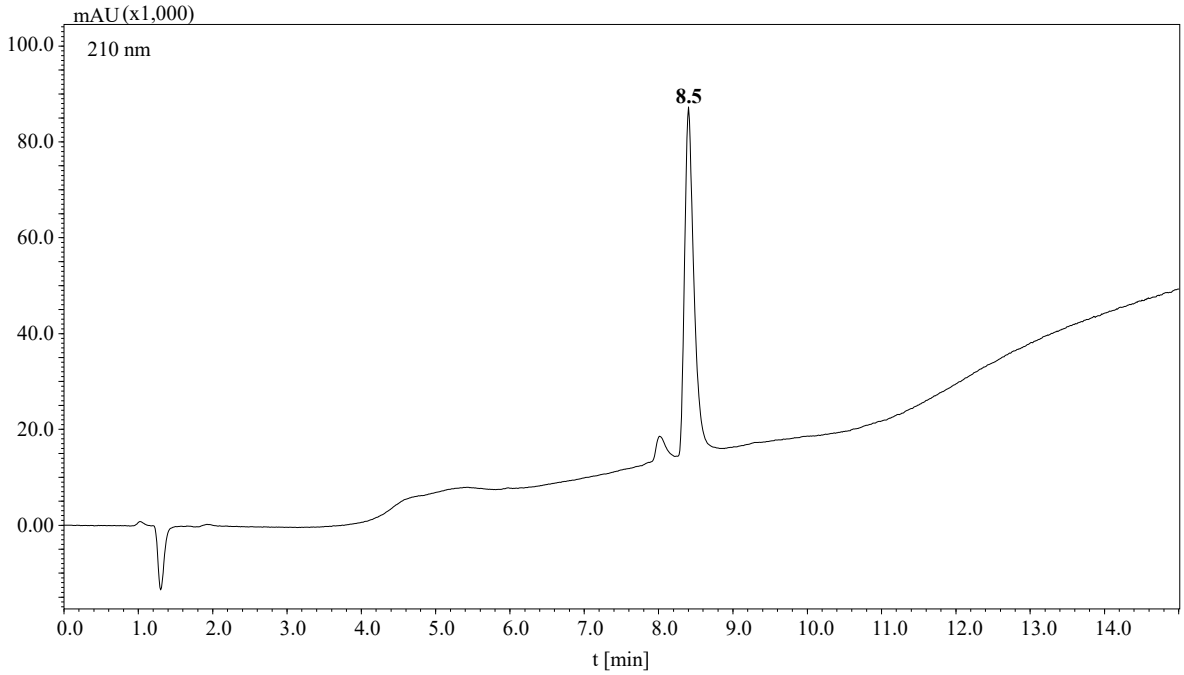
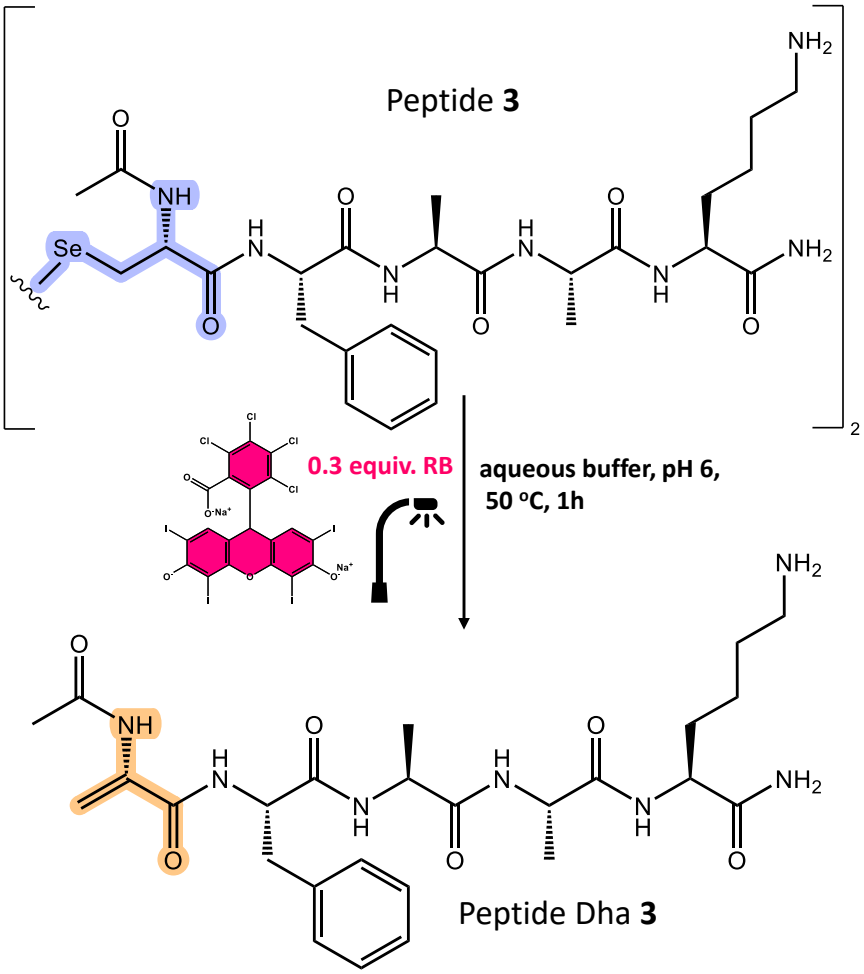


Fig S 46. HPLC chromatogram obtained for peptide Dha 2

11.3 RB-mediated photochemical conversion of model peptide 3

1 mg of model peptide **3** containing an oxidized selenocysteine residue in the form of a diselenide homodimer, was placed in a 2 mL HPLC vial made of transparent borosilicate glass The vial must not be sealed with a cap in order to allow the access of atmospheric oxygen. The sample was dissolved in 900 µL of aqueous buffer consisting of 8 M guanidine hydrochloride and 0.2 M Na_2HPO_4, prepared with deionized water obtained from a reverse osmosis system (Hydrolab, Poland). The pH of the solution was adjusted to 6 using 6 M HCl. Subsequently, 0.3 equivalents of Rose Bengal were added from a freshly prepared aqueous stock solution (2 mg/mL). The stock solution can be stored for up to one week; however, its long-term stability has not been tested. The vial was placed on a magnetic stirrer and gently heated until the solution reached 50 °C. Simultaneously, the sample was then irradiated for over 1 hour using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K).

HR-ESI-MS calcd *m/z* 546.303, found 546.298 $[M+H]^{1+}$; **ESI-MS/MS** (CE 20eV): b_2 (calcd *m/z* 259.107, found 259.105), b_3 (calcd *m/z* 331.144, found 331.145), b_4 (calcd *m/z* 402.182, found 402.181), y_1-NH_3 (calcd *m/z* 129.102, found 129.101), y_2-NH_3 (calcd *m/z* 200.139, found 200.137), **HPLC**: R_t= 8.3 min (gradient 3), **yield:** 92%



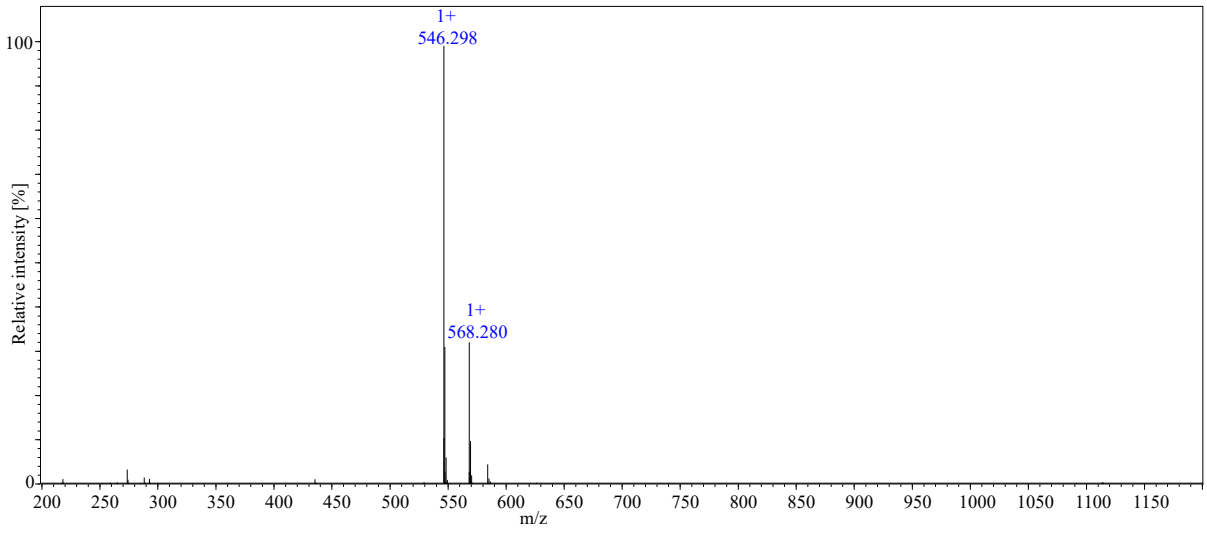


Fig S 47. ESI-MS spectrum obtained for peptide Dha 3

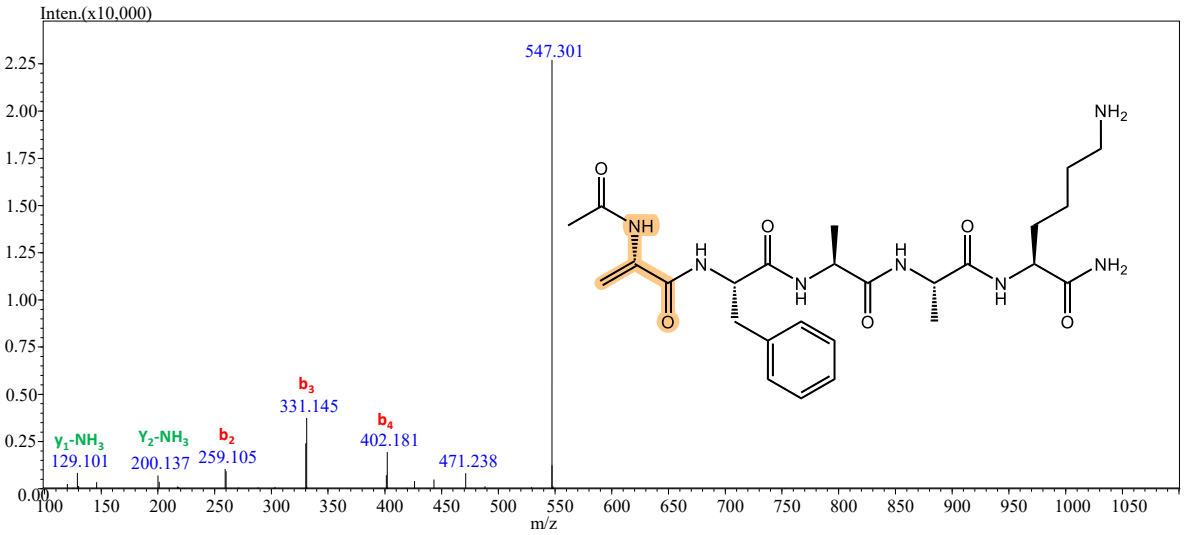


Fig S 48. ESI-MS/MS (20 eV) spectrum obtained for peptide Dha 3

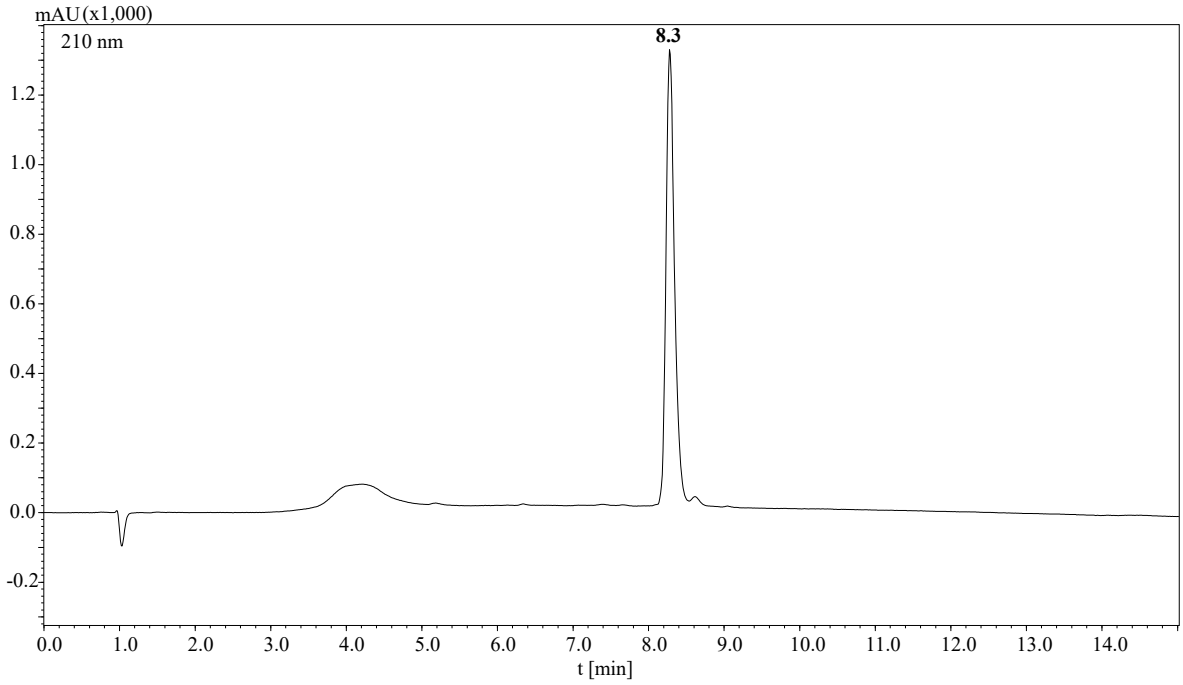
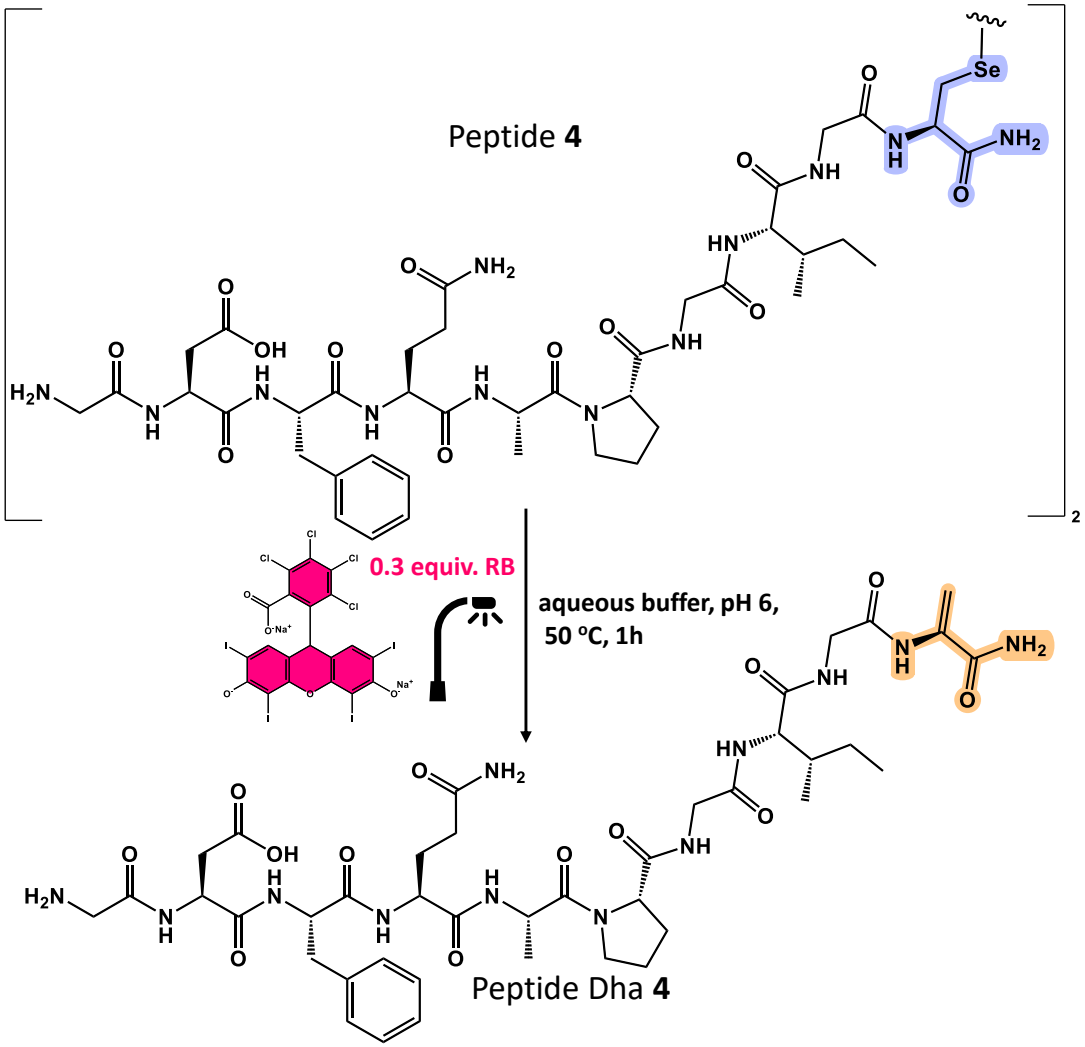


Fig S 49. HPLC chromatogram obtained for peptide Dha 3

11.4 RB-mediated photochemical conversion of model peptide 4

1 mg of model peptide **4** containing an oxidized selenocysteine residue in the form of a diselenide homodimer, was placed in a 2 mL HPLC vial made of transparent borosilicate glass The vial must not be sealed with a cap in order to allow the access of atmospheric oxygen. The sample was dissolved in 900 µL of aqueous buffer consisting of 8 M guanidine hydrochloride and 0.2 M Na_2HPO_4, prepared with deionized water obtained from a reverse osmosis system (Hydrolab, Poland). The pH of the solution was adjusted to 6 using 6 M HCl. Subsequently, 0.3 equivalents of Rose Bengal were added from a freshly prepared aqueous stock solution (2 mg/mL). The stock solution can be stored for up to one week; however, its long-term stability has not been tested. The vial was placed on a magnetic stirrer and gently heated until the solution reached 50 °C. Simultaneously, the sample was then irradiated for over 1 hour using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K).

HR-ESI-MS calcd *m/z* 472.235, found 472.231 $[M+2H]^{2+}$; **ESI-MS/MS** (CE 25eV): b_5-NH_3 (calcd *m/z* 516.208, found 516.204), b_5 (calcd *m/z* 533.235, found 533.230), b_7 (calcd *m/z* 687.309, found 687.302), b_8(calcd *m/z* 800.393, found 800.386), y_6 (calcd *m/z* 482.272, found 482.267), y_8 (calcd *m/z* 757.399, found 757.392), M-NH_3 (calcd *m/z* 926.436, found 926.427), **HPLC**: R_t= 8.7 min (gradient 4), **yield:** 90%



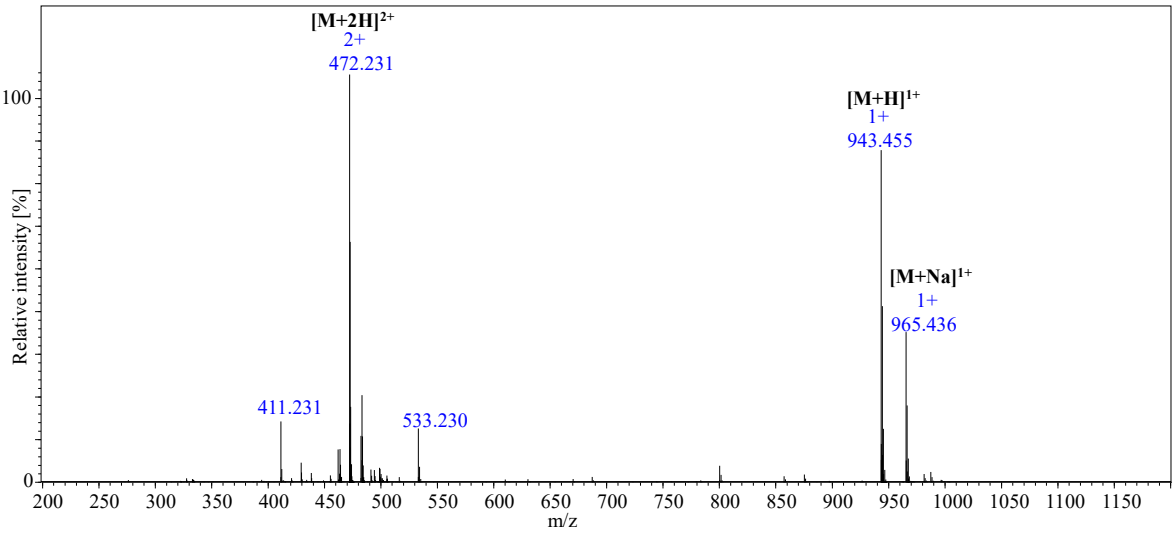


Fig S 50. ESI-MS spectrum obtained for peptide Dha 4

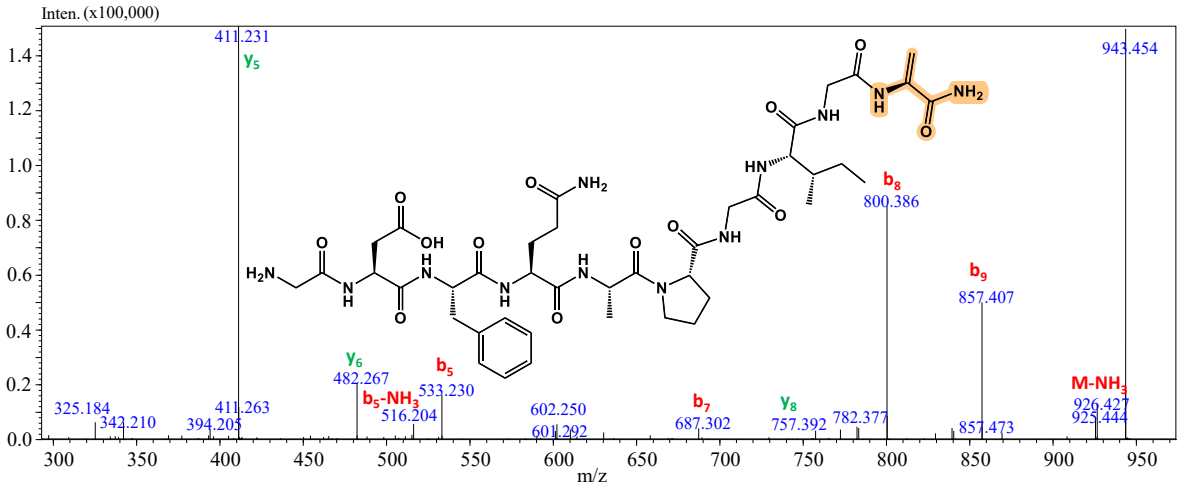


Fig S 51. ESI-MS/MS (25 eV) spectrum obtained for peptide Dha 4

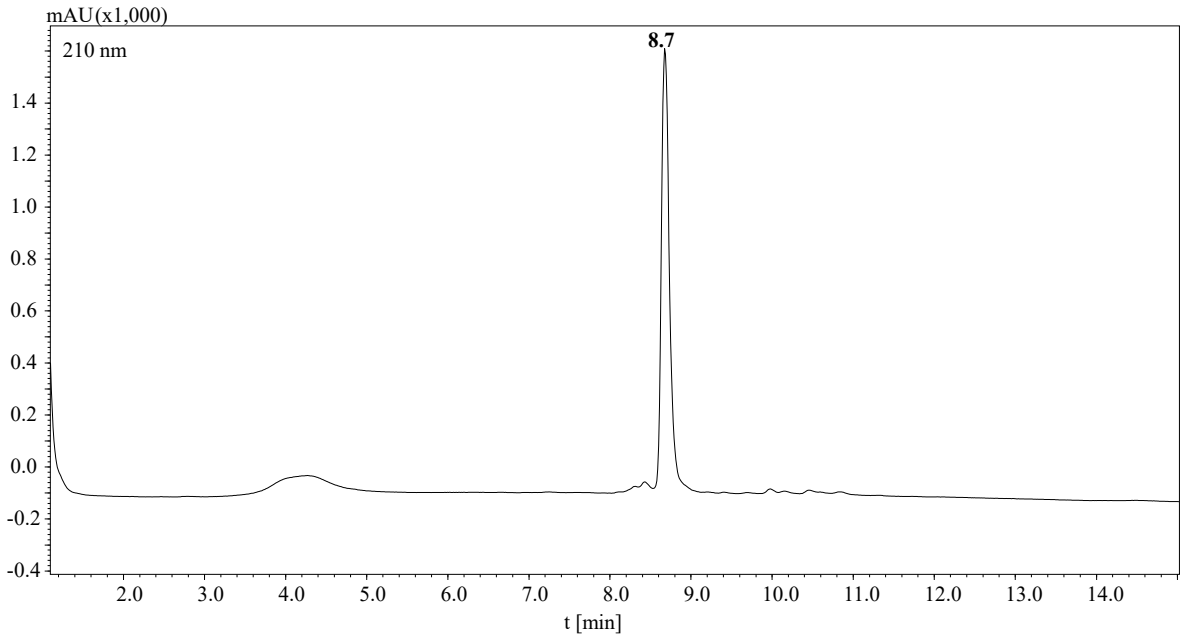
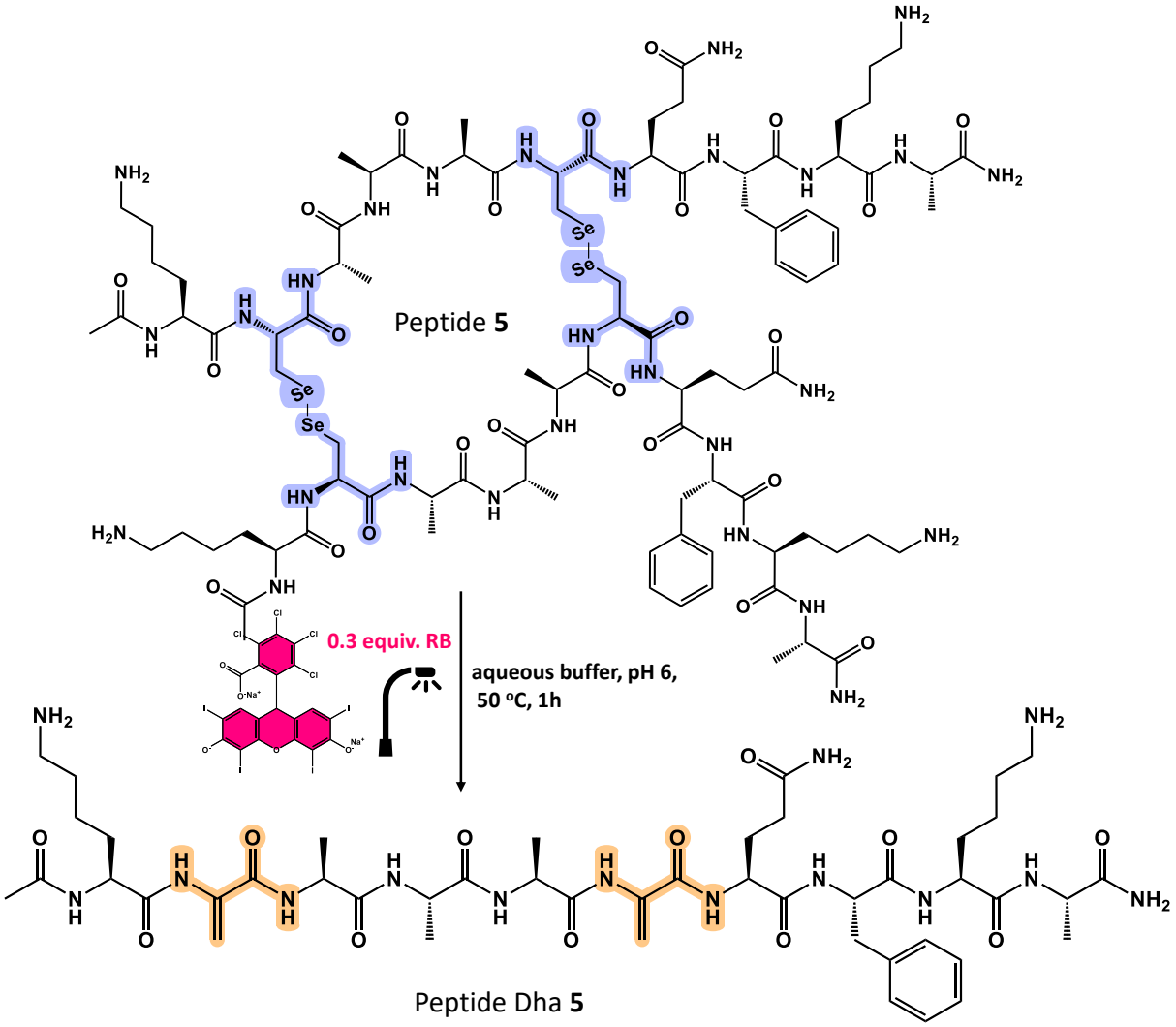


Fig S 52. HPLC chromatogram obtained for peptide Dha 4

11.1 RB-mediated photochemical conversion of model peptide 5

1 mg of model peptide **5** containing an oxidized selenocysteine residue in the form of a diselenide homodimer, was placed in a 2 mL HPLC vial made of transparent borosilicate glass The vial must not be sealed with a cap in order to allow the access of atmospheric oxygen. The sample was dissolved in 900 µL of aqueous buffer consisting of 8 M guanidine hydrochloride and 0.2 M Na_2HPO_4, prepared with deionized water obtained from a reverse osmosis system (Hydrolab, Poland). The pH of the solution was adjusted to 6 using 6 M HCl. Subsequently, 0.3 equivalents of Rose Bengal were added from a freshly prepared aqueous stock solution (2 mg/mL). The stock solution can be stored for up to one week; however, its long-term stability has not been tested. The vial was placed on a magnetic stirrer and gently heated until the solution reached 50 °C. Simultaneously, the sample was then irradiated for over 1 hour using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K).

HR-ESI-MS calcd *m/z* 507.772, found 507.778 $[M+2H]^{2+}$; **ESI-MS/MS** (CE 25eV): b_2 (calcd *m/z* 240.134, found 240.132), b_3 (calcd *m/z* 311.171, found 311.168), y_5-NH_3 (calcd *m/z* 544.271, 544.283), y_5 (calcd *m/z* 561.298, found 561.309), y_7 (calcd *m/z* 703.372, found 703.382), y_8 (calcd *m/z* 774.409, found 774.418), y_9-NH_3 (calcd *m/z* 826.404, found 826.413) y_9 (calcd *m/z* 843.431, found 843.440), **HPLC**: R_t= 7.4 min (gradient 4), **yield:** 90%



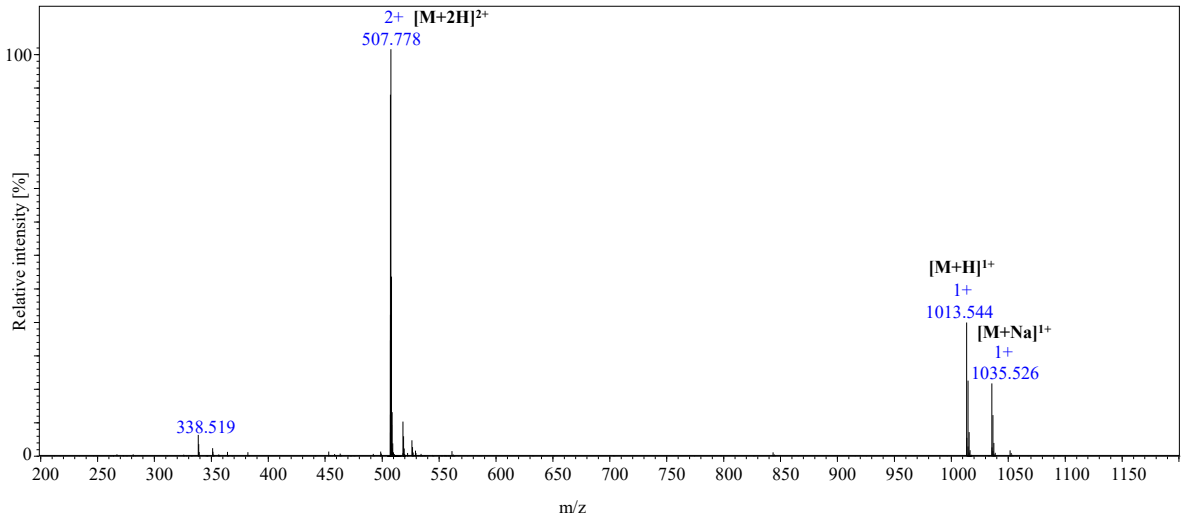


Fig S 53. ESI-MS spectrum obtained for peptide Dha 5

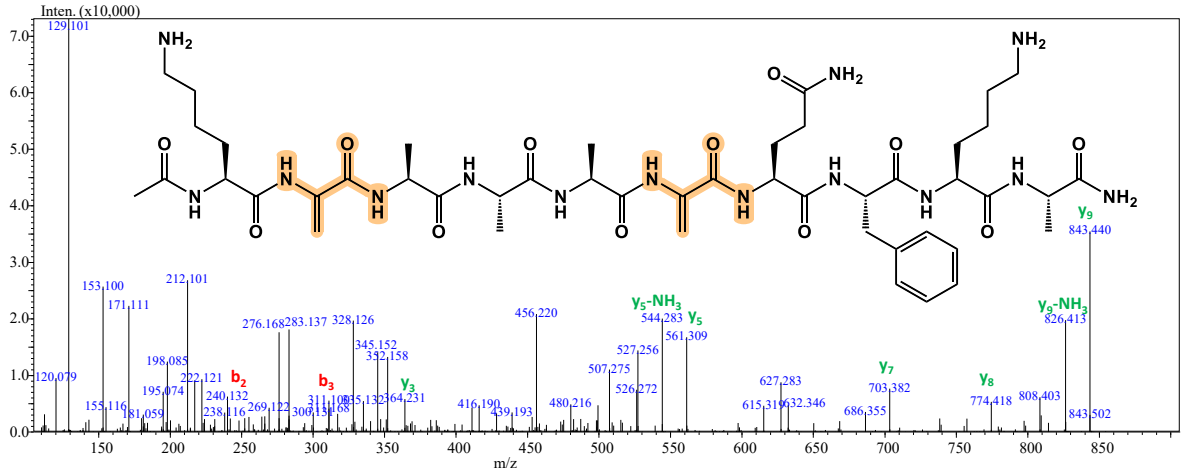


Fig S 54. ESI-MS/MS (25 eV) spectrum obtained for peptide Dha 5

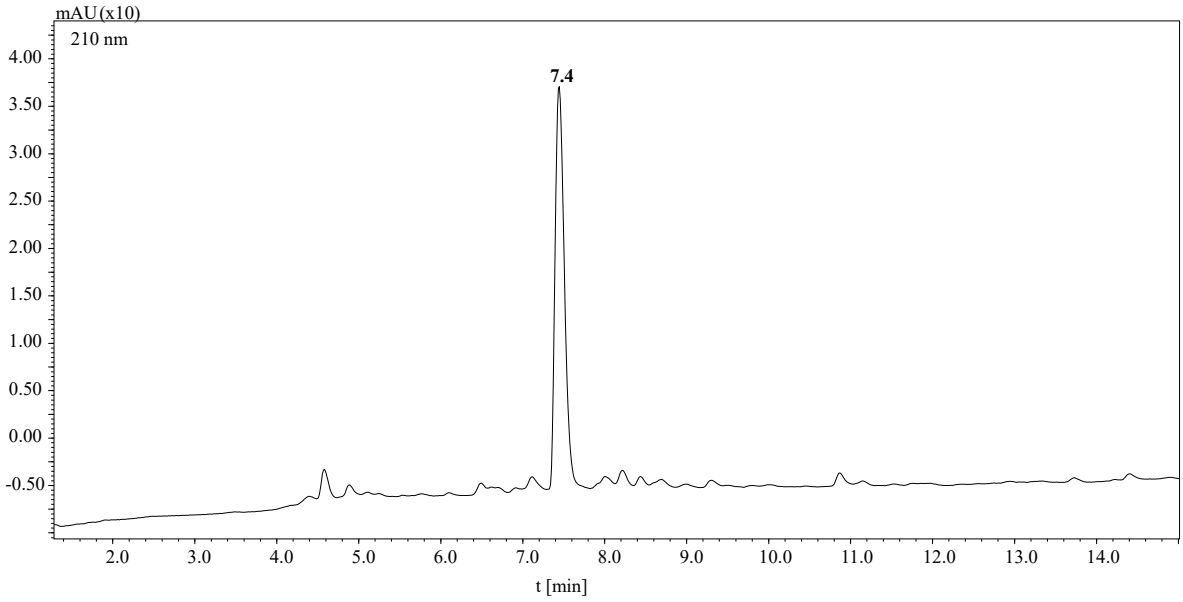


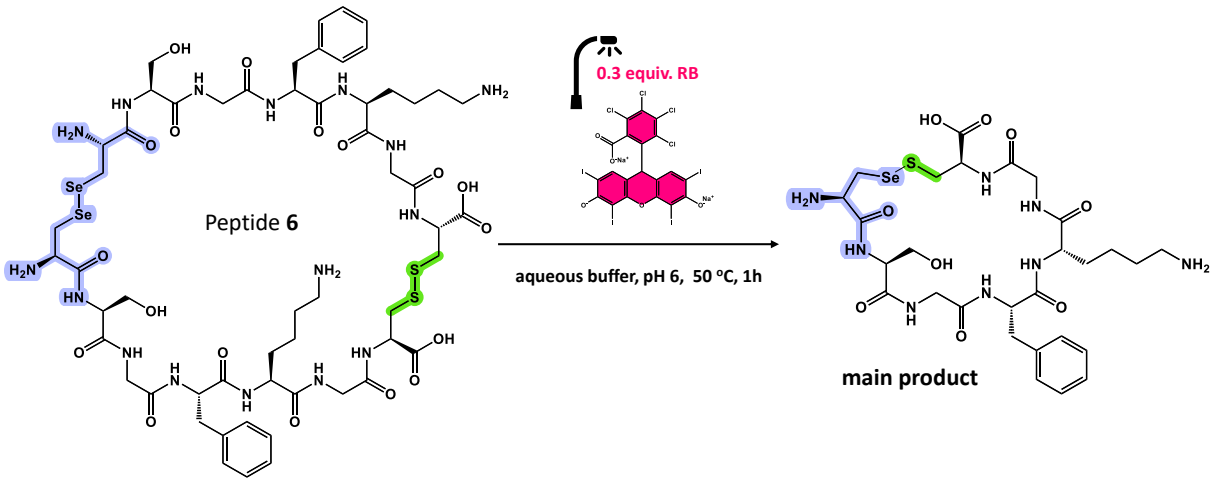
Fig S 55. HPLC chromatogram obtained for peptide Dha 5

11.6 RB-mediated photochemical conversion of model peptide 6

1 mg of model peptide **6** containing an oxidized selenocysteine residue in the form of a diselenide homodimer, was placed in a 2 mL HPLC vial made of transparent borosilicate glass The vial must not be sealed with a cap in order to allow the access of atmospheric oxygen. The

sample was dissolved in 900 µL of aqueous buffer consisting of 8 M guanidine hydrochloride and 0.2 M Na_2HPO_4, prepared with deionized water obtained from a reverse osmosis system (Hydrolab, Poland). The pH of the solution was adjusted to 6 using 6 M HCl. Subsequently, 0.3 equivalents of Rose Bengal were added from a freshly prepared aqueous stock solution (2 mg/mL). The stock solution can be stored for up to one week; however, its long-term stability has not been tested. The vial was placed on a magnetic stirrer and gently heated until the solution reached 50 °C. Simultaneously, the sample was then irradiated for over 1 hour using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K).

HR-ESI-MS calcd *m/z* 746.219, found 746.212 $[M+H]^{1+}$, **HPLC**: R_t= 6.2 min (gradient 1)



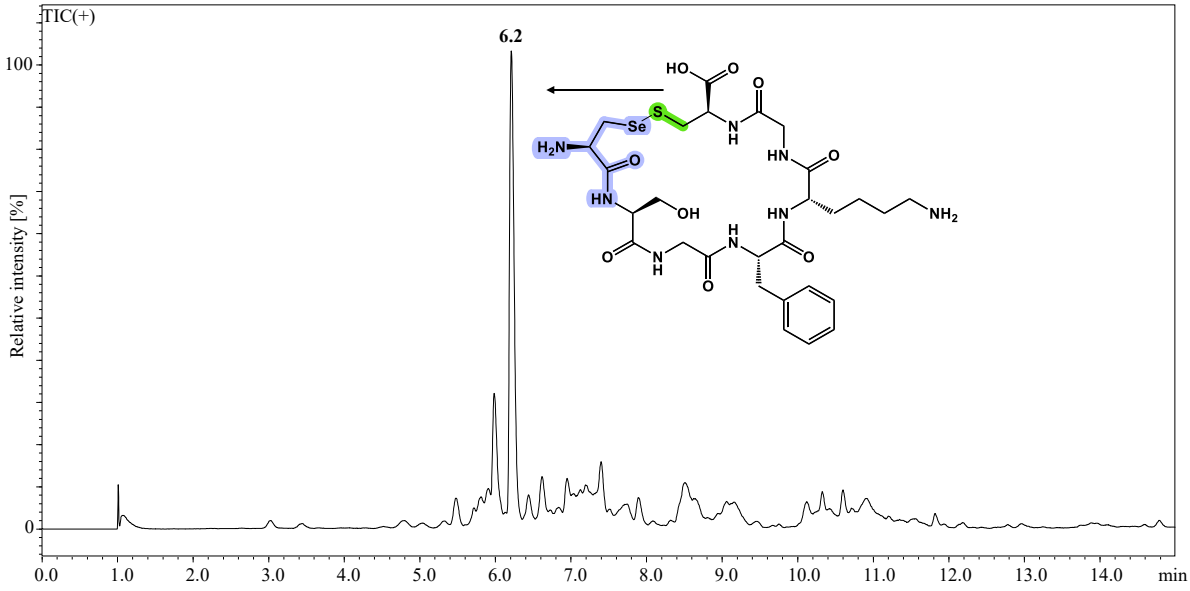


Fig S 56. LC-MS chromatogram obtained for the mixture after irradiation of model peptide 6 with RB

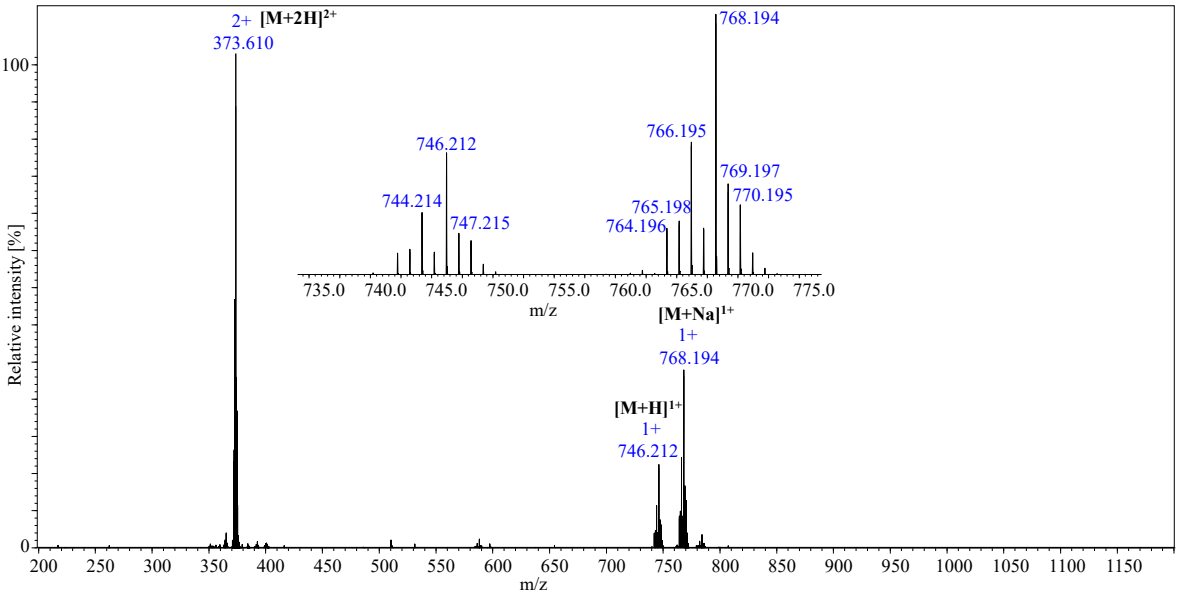


Fig S 57. MS spectrum obtained for the main product (6.2 min)

12. Photochemical conversion of model peptide 2 with Ir (III) catalyst.

0.5 mg of peptide **2** containing an oxidized selenocysteine residue in the form of a diselenide homodimer, was placed in a 2 mL HPLC vial made of transparent borosilicate glass The sample was dissolved in 450 µL of aqueous buffer consisting of 8 M guanidine hydrochloride and 0.2 M Na_2HPO_4, prepared with deionized water obtained from a reverse osmosis system (Hydrolab, Poland). The pH of the solution was adjusted to 6 using 6 M HCl. Subsequently, 5% mol (Ir[dF(CF_3)ppy]2(dtbpy))PF_6 was added from a freshly prepared stock solution (1 mg/mL) in acetonitryle. The vial was placed on a magnetic stirrer and gently heated until the solution reached 50 °C. The sample was then irradiated for over 1 hour using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K).

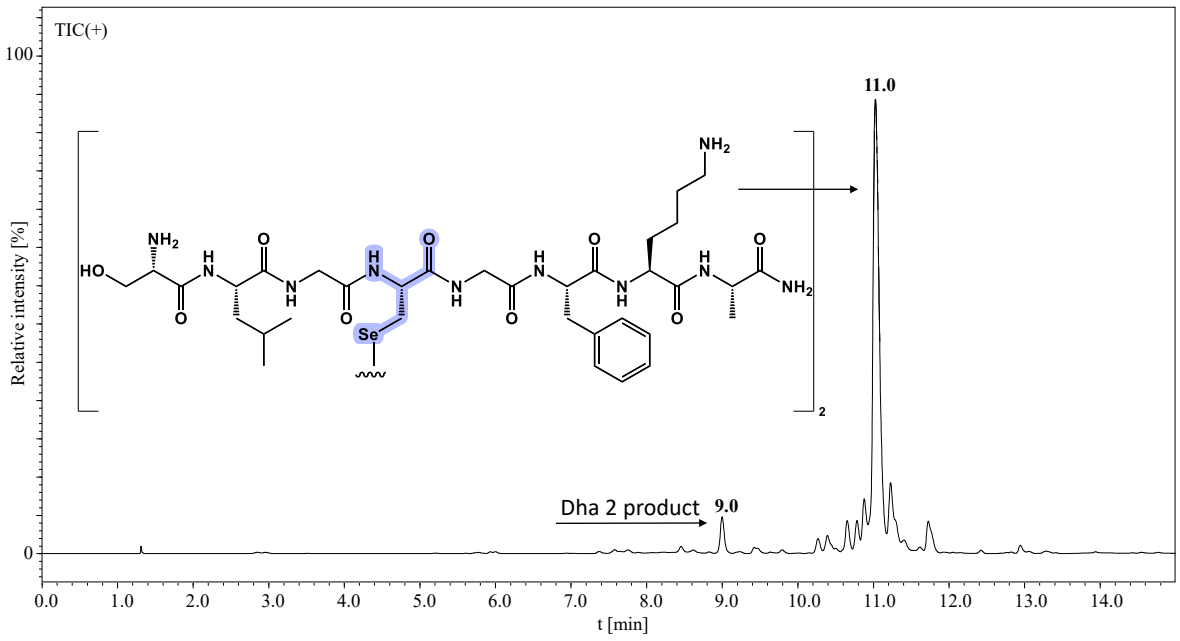


Fig S 58. LC-MS chromatogram (gradient 2) obtained for the mixture after irradiation of peptide 2 in the presence of Ir (III) catalyst

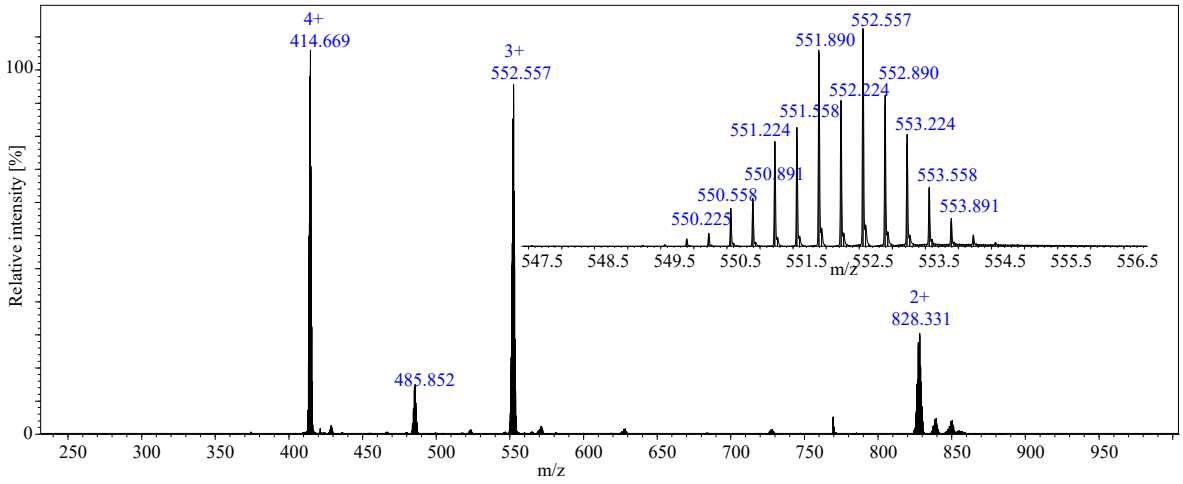


Fig S 59. MS spectrum obtained in 11.0 min (substrate)

13. Photochemical conversion of model peptide 2 using immobilized Rose Bengal

0.5 mg of peptide **2** containing an oxidized selenocysteine residue in the form of a diselenide homodimer, was placed in a 2 mL HPLC vial made of transparent borosilicate glass The sample was dissolved in 450 µL of aqueous buffer consisting of 8 M guanidine hydrochloride and 0.2 M Na_2HPO_4, prepared with deionized water obtained from a reverse osmosis system (Hydrolab, Poland). The pH of the solution was adjusted to 6 using 6 M HCl. Subsequently, immobilized RB was added (20% v/v). The vial was placed on a magnetic stirrer and gently heated until the solution reached 50 °C. Simultaneously, the sample was then irradiated for over 2 hours using an office desk lamp (Kobi Light Sp. z o.o., model KX3087, 230 V, 50 Hz) equipped with a Philips LED bulb (6500 K).

HR-ESI-MS calcd *m/z* 747.414, found 747.407, **HPLC**: R_t= 8.4 min (gradient 2), **yield:** 90%

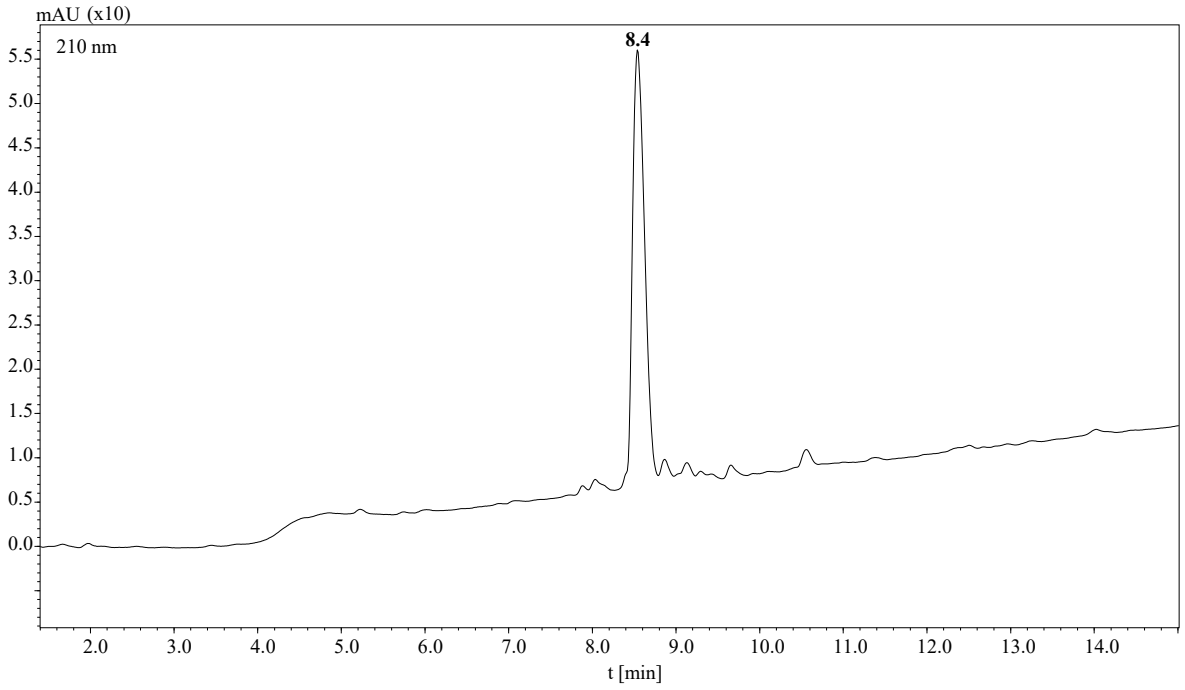


Fig S 60. HPLC chromatogram obtained after conversion of peptide 2 to Dha analog using immobilized RB (reaction time -2 hours)

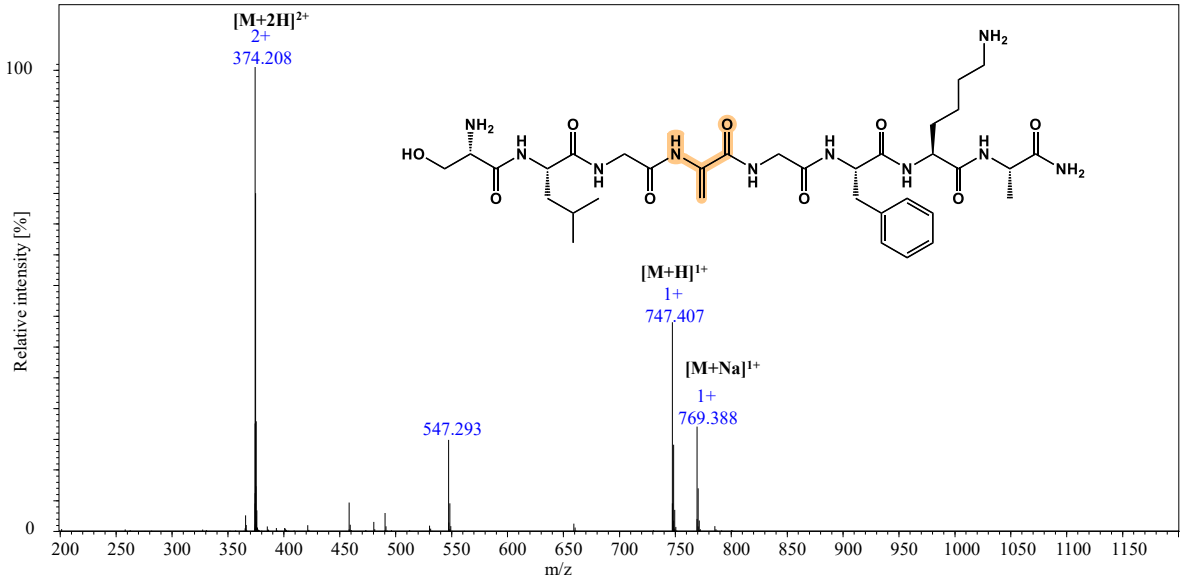


Fig S 61. MS spectrum obtained for desired product (peptide Dha 2)