Supporting Information

Cysteine Pseudoprolines for Thiol Protection and Peptide Macrocyclization Enhancement in Fmoc-Based Solid-Phase Peptide Synthesis

Tobias M. Postma[†,‡] and Fernando Albericio*[,†, ‡, §, ‖]

[†] Institute for Research in Biomedicine, Baldiri Reixac 10, Barcelona, 08028, Spain; [‡] CIBER-BBN, 08028, barcelona, Spain; [§]Department of Organic Chemistry, Martí i Franquès 1, 08028, Barcelona, Spain; [‖] School of Chemistry University of KwaZulu Natal, 4001, Durban, South Africa

* fernando.albericio@irbbarcelona.org

1. General Procedures

Fmoc-amino acids, Cys pseudoprolines and Fmoc-Rink Amide AM resin were obtained from IRIS Biotech (Marktredwitz, Germany). Rink-Amide-Chemmatrix Low LOA was obtained from PCAS BioMatrix Inc. (Quebec, Canada). DIPEA, diisopropylcarbodiimide (DIC) and TFA were obtained from Aldrich (Milwaukee, USA). Oxyma Pure was obtained from Luxembourg Industries Ltd. (Tel Aviv, Isreal). DMF, CH_2Cl_2, Et_2O, acetonitrile, DMSO and piperidine (HLPC grade) were obtained from SDS (Peypin, France). All reagents and solvents were used as received.

Room temperature (rt) refers to ambient temperature. Solid-phase syntheses were carried out manually in polypropylene syringed containing a polyethylene frit. Solvents and soluble reagents were removed by suction. Deprotection of the Fmoc group was achieved by treatment of the resin with 20% piperidine/DMF (1 × 1 min and 2 × 5 min). Washings between deprotection and coupling were performed with DMF (5 × 1 min), CH_2Cl_2 (5 × 1 min) and DMF (5 × 1 min). Following the final coupling or deprotection the resin was washed with DMF (5 × 1 min), CH_2Cl_2 (5 × 1 min) and dried under a stream of air. Yields for peptides refer to the area of the chromatographic product peak recorded at 220 nm.

High resolution mass spectrometry (HRMS) measurements were recorded on Thermo Scientific LTQ-FT Ultra spectrometer. Mass values are quoted within the error limits of ±5 ppm mass units. ESI refers to the electrospray ionization technique.

Analytical high pressure liquid chromatography (HPLC) was carried out on a Waters instrument comprising a separation module (Waters 2695), automatic injector, photodiode array detector (Waters 2998) and system controller (Empower login), with an Xbridge BEH130 C18 reversed-phase analytical column (4.6 mm × 100 mm, 3.5 µm). All UV measurements were recorded at a wavelength of 220 nm, and linear gradients of acetonitrile (0.036% TFA) into water (0.045% TFA) over 8 min were used at a flow rate of 1.0 $mL \cdot min^{-1}$ and a run time of 11 min.

LCMS was carried out on a Waters Micromass ZQ spectrometer using a SunFire C18 analytical reversed-phase HPLC column (2.1 mm × 100 mm, 5 µm). Linear gradients of acetonitrile (0.07% formic acid) into water (0.1% formic acid) over 8 min were used at a flow rate of 1.0 $mL \cdot min^{-1}$ and a run time of 11 min.

2. General Methods Peptide Synthesis

General Method 1: Peptide Synthesis on Rink Amide AM Resin

Fmoc-Rink-Amide AM resin (0.45 mmol/gram, 1 equiv) was washed with DMF (5 × 1 min), CH_2Cl_2 (5 × 1 min) and DMF (5 × 1 min). Deprotection of the Fmoc group was achieved by treatment of the resin with 20% piperidine/DMF (1 × 1 min and 2 × 5 min) followed by washing with DMF (5 × 1 min), CH_2Cl_2 (5 × 1 min) and DMF (5 × 1 min). The protected Fmoc-amino acids (3 equiv) were incorporated using DIC (3 equiv.) and Oxyma (3 equiv) in DMF, as a coupling system, with 5 min preactivation for 1 h at rt. Washes between couplings and deprotections were performed with DMF (5 × 1 min), CH_2Cl_2 (5 × 1 min) and DMF (5 × 1 min). The disappearance of amine was monitored by the Kaiser test to indicate completion of the coupling.[1]

General Method 2: Peptide Synthesis on Rink Chemmatrix Resin

Rink-Amide-Chemmatrix Low LOA resin (0.53 mmol/g, 1 equiv) was washed with with DMF (3 × 1 min), CH_2Cl_2 (3 × 1 min), TFA/CH_2Cl_2 (1:99) (5 × 1 min), CH_2Cl_2 (3 × 1 min), DIPEA/CH_2Cl_2 (5:95) (5 × 1 min), CH_2Cl_2 (5 × 1 min). Deprotection of the Fmoc group was achieved by treatment of the resin with 20% piperidine/DMF (1 × 1 min and 2 × 5 min) followed by washing with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min). The protected Fmoc-amino acids (3 equiv.) were incorporated using DIC (3 equiv.) and Oxyma (3 equiv.) in DMF, as a coupling system, with 5 min preactivation for 1 h at rt. Washes between couplings and deprotections were performed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min). The disappearance of amine was monitored by the Kaiser test to indicate completion of the coupling.[1]

General Method 3: Allyl Protection Removal

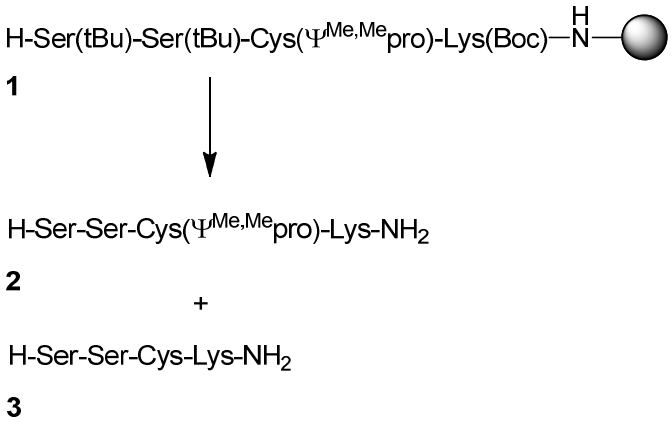
The resin was washed with DMF (5 × 1 min), CH_2Cl_2 (5 × 1 min). The resin was suspended in dry CH_2Cl_2, phenylsilane (24 equiv) was added and the mixture was bubbled with N_2 for 10min. $Pd(PPh_3)_4$ (0.1 equiv) was added and the bubbled with N_2 for 10 min under the exclusion of light. The resin was washed with CH_2Cl_2 (5 × 1 min). This process was repeated twice.

General Method 4: Microcleavage

Dry resin (5 mg) was treated with TFA/TIS/H_2O (95:2.5:2.5) for 1 h at rt. The cleavage mixture was evaporated with a stream of argon, precipitated with Et_2O, centrifuged and the pellet was redissolved in H_2O/CH_3CN (1:1) for analysis by HPLC and LCMS.

3. Peptide Synthesis

H-Ser-Ser-Cys($\psi^{Me,Me}$pro)-Lys-NH_2 (2)



Peptide **2** was synthesized according to General Method 1 using Fmoc-Rink-Amide AM resin (222.2 mg, 0.1 mmol, 0.45 mmol/gram). Following peptide elongation, the resin was treated with 20% piperidine/DMF (1 × 1 min and 2 × 5 min) to remove Fmoc, and washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min). Microcleavage of resin **1** (5 mg resin): the peptide was cleaved from the resin according to General Method 4 and subsequent HPLC analysis found that peptide **2** was obtained in 65**%** purity and peptide **3** in 35% (linear gradient from 0% to 30% acetonitrile over 8 min, t_R peptide **2** : 5.0 min; peptide **3**: 1.9 min). **LCMS** peptide **2** observed $[M+H]^+$ 463.2, required $[M+H]^+$ 463.2; peptide **3** observed $[M+H]^+$ 423.2, required $[M+H]^+$ 423.2.

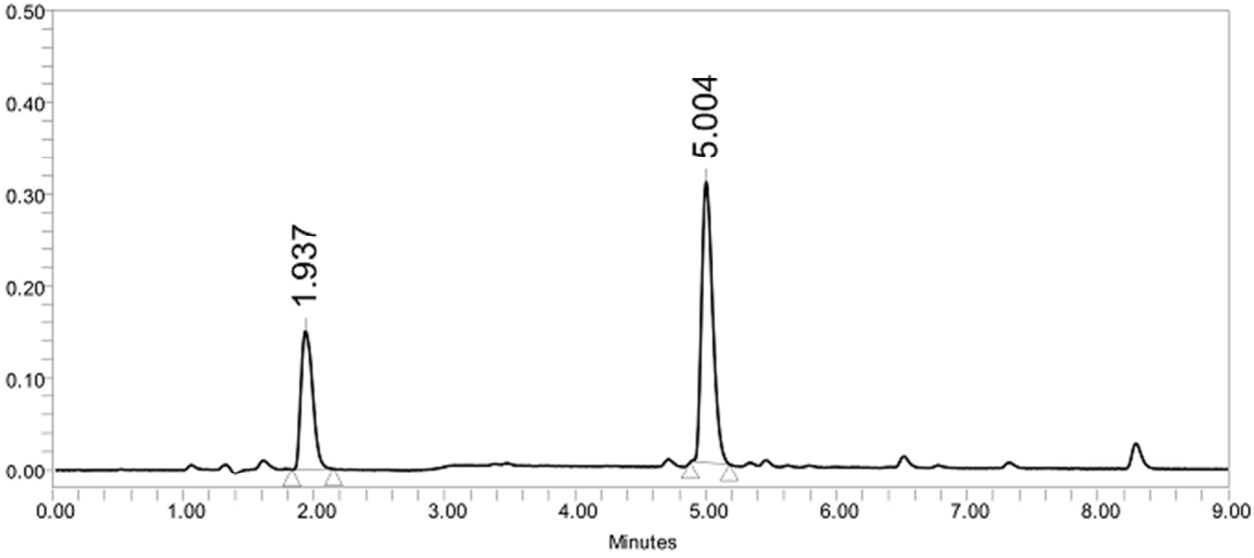
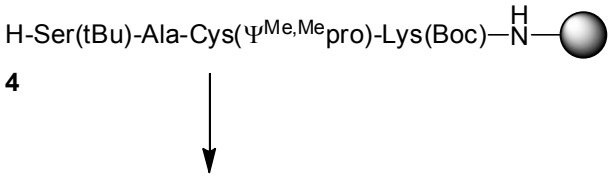


Figure S-1: HPLC chromatogram of peptide **2** and **3**

H-Ser-Ala-Cys($\psi^{Me,Me}$pro)-Lys-NH_2 (5)



4

H-Ser-Ala-Cys($\Psi^{Me,Me}$pro)-Lys-NH_2

5

H-Ser-Ala-Cys-Lys-NH_2

6

Peptide **5** was synthesized according to General Method 1 using Fmoc-Rink-Amide AM resin (222.2 mg, 0.1 mmol, 0.45 mmol/gram). Following peptide elongation, the resin was treated with 20% piperidine/DMF (1 × 1 min and 2 × 5 min) to remove Fmoc, and washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min). Microcleavage of resin **4** (5 mg resin): the peptide was cleaved from the resin according to General Method 4 and subsequent HPLC analysis found that peptide **5** was obtained in 85**%** purity and peptide **6** in 15% (linear gradient from 0% to 30% acetonitrile over 8 min, t_R peptide **5**: 5.2 min and peptide **6** : 1.5 min). **LCMS** peptide **5** observed $[M+H]^+$ 447.3, required $[M+H]^+$ 447.2; peptide **6** observed $[M+H]^+$ 407.2, required $[M+H]^+$ 407.2.

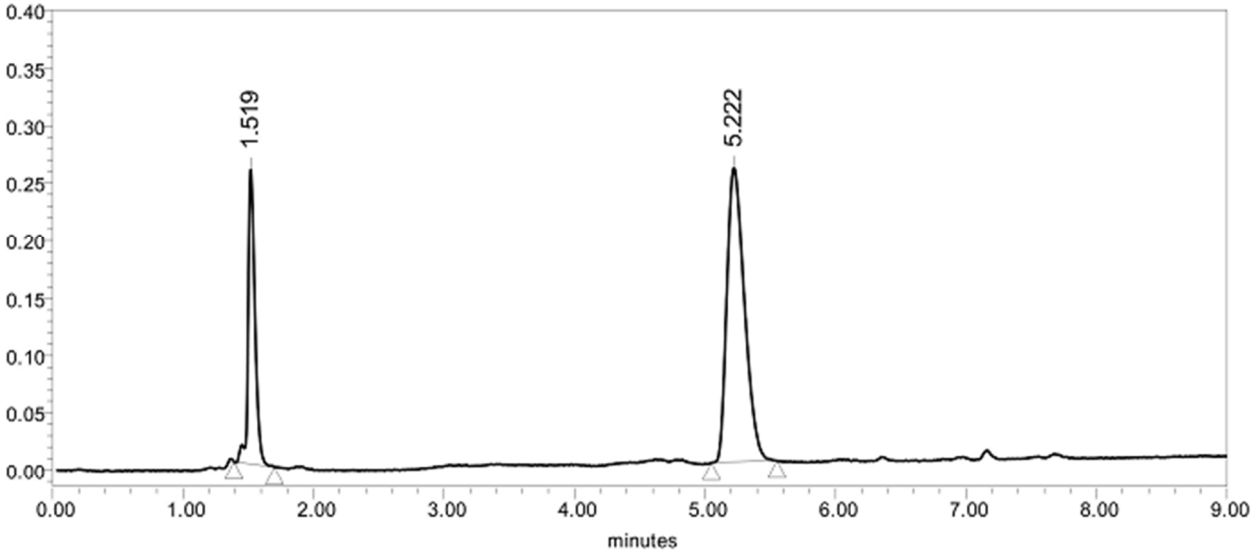


Figure S-2: HPLC chromatogram of peptide **5** and **6**

Peptide (8)

H-Cys(Trt)-Asp(tBu)-Thr(tBu)-Gln(Trt)-Ser(tBu)-Cys($\psi^{Me,Me}$pro)-Thr(tBu)-Thr(tBu)-Dap(Boc)-Phe–N(H)-●

7

H-Cys-Asp-Thr-Gln-Ser-Cys($\psi^{Me,Me}$pro)-Thr-Thr-Dap-Phe-NH_2

8

H-Cys-Asp-Thr-Gln-Ser-Cys-Thr-Thr-Dap-Phe-NH_2

9

Peptide **8** was synthesized according to General Method 1 using Fmoc-Rink-Amide AM resin (222.2 mg, 0.1 mmol, 0.45 mmol/gram). The coupling of Fmoc-Ser-Cys($\psi^{Me,Me}$pro)-OH needed a recoupling to achieve complete conversion. Following peptide elongation, the resin was treated with 20% piperidine/DMF (1 × 1 min and 2 × 5 min) to remove Fmoc, and washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min). Microcleavage of resin **7** (5 mg resin): The peptide was cleaved from the resin according to General Method 4 and subsequent HPLC analysis found that peptide **9** was obtained in 88**%** purity

(linear gradient from 5% to 100% acetonitrile over 8 min, t_R : 3.4 min). **LCMS** peptide **8** not observed; peptide **9** observed $[M+H]^+$ 1090.5, required $[M+H]^+$ 1090.4.

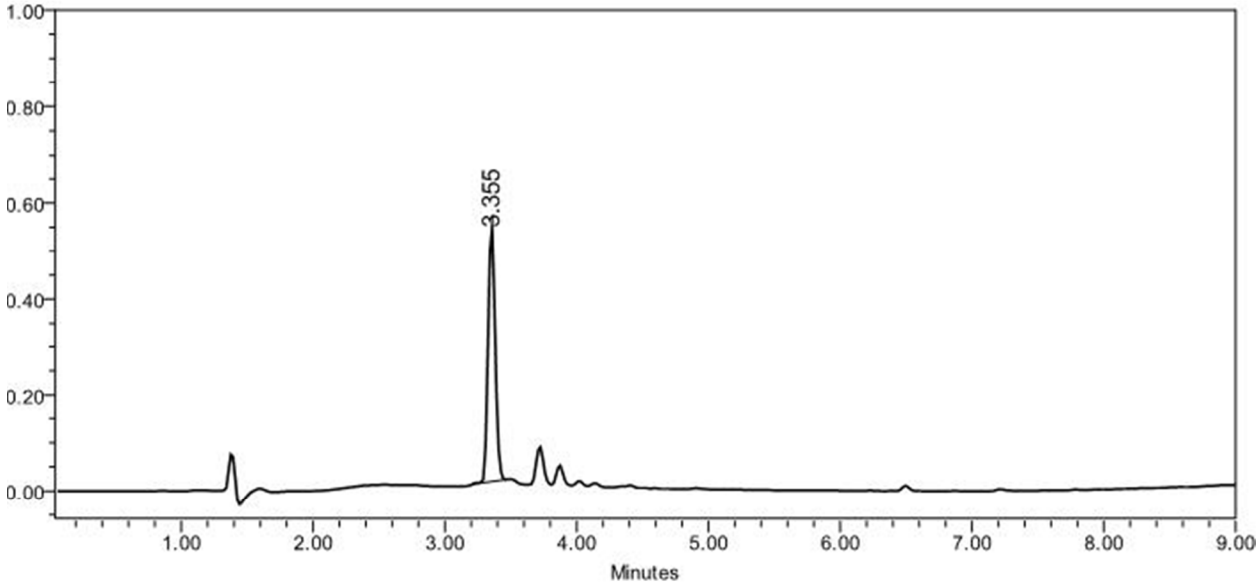


Figure S-3: HPLC chromatogram of peptide **9**

Seritocin (11)

H-Cys(Trt)-Tyr(tBu)-Ile-Gln(Trt)-Ser(tBu)-Cys($\Psi^{Me,Me}$pro)-Pro-Ile-Gly–N(H)– 

10

H-Cys-Tyr-Ile-Gln-Ser-Cys($\Psi^{Me,Me}$pro)-Pro-Ile-Gly-NH_2

11

H-Cys-Tyr-Ile-Gln-Ser-Cys-Pro-Ile-Gly-NH_2

12

Peptide **11** was synthesized according to General Method 1 using Fmoc-Rink-Amide AM resin (222.2 mg, 0.1 mmol, 0.45 mmol/gram). Following peptide elongation, the resin was treated with 20% piperidine/DMF (1 × 1 min and 2 × 5 min) to remove Fmoc, and washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min). Microcleavage of resin **10** (5 mg resin): The peptide was cleaved from the resin according to

General Method 4 and subsequent HPLC analysis found that peptide **12** was obtained in 98% purity (linear gradient from 5% to 100% acetonitrile over 8 min, t_R : 4.0 min). **LCMS** peptide **11** not observed; peptide **12** observed $[M+H]^+$ 982.5, required $[M+H]^+$ 982.5.

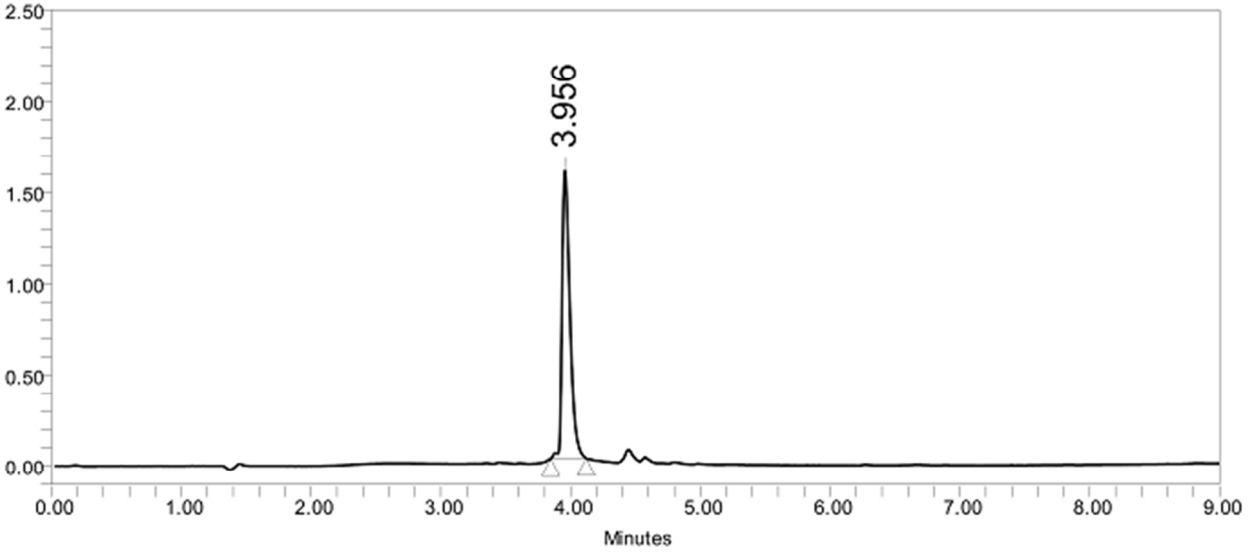


Figure S-4: HPLC chromatogram of peptide **12**

Peptide (14)

H-Cys(Trt)-Thr(tBu)-Ile-Gln(Trt)-Ala-Cys($\Psi^{Me,Me}$pro)-Pro-Ile-Gly–N(H)—●

13

↓

H-Cys-Thr-Ile-Gln-Ala-Cys($\Psi^{Me,Me}$pro)-Pro-Ile-Gly-NH_2

14

H-Cys-Thr-Ile-Gln-Ala-Cys-Pro-Ile-Gly-NH_2

15

Peptide **14** was synthesized according to General Method 1 using Fmoc-Rink-Amide AM resin (222.2 mg, 0.1 mmol, 0.45 mmol/gram). Following peptide elongation, the resin was treated with 20% piperidine/DMF (1 × 1 min and 2 × 5 min) to remove Fmoc, and washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min). Microcleavage of resin **13** (5 mg resin): The peptide was cleaved from the resin according to General Method 4 for 2h and subsequent HPLC analysis found that peptide **15** was obtained in 94%

purity (linear gradient from 5% to 100% acetonitrile over 8 min, t_R : 4.0 min). **LCMS** peptide **14** not observed; peptide **15** observed $[M+H]^+$ 904.3, required $[M+H]^+$ 904.4.

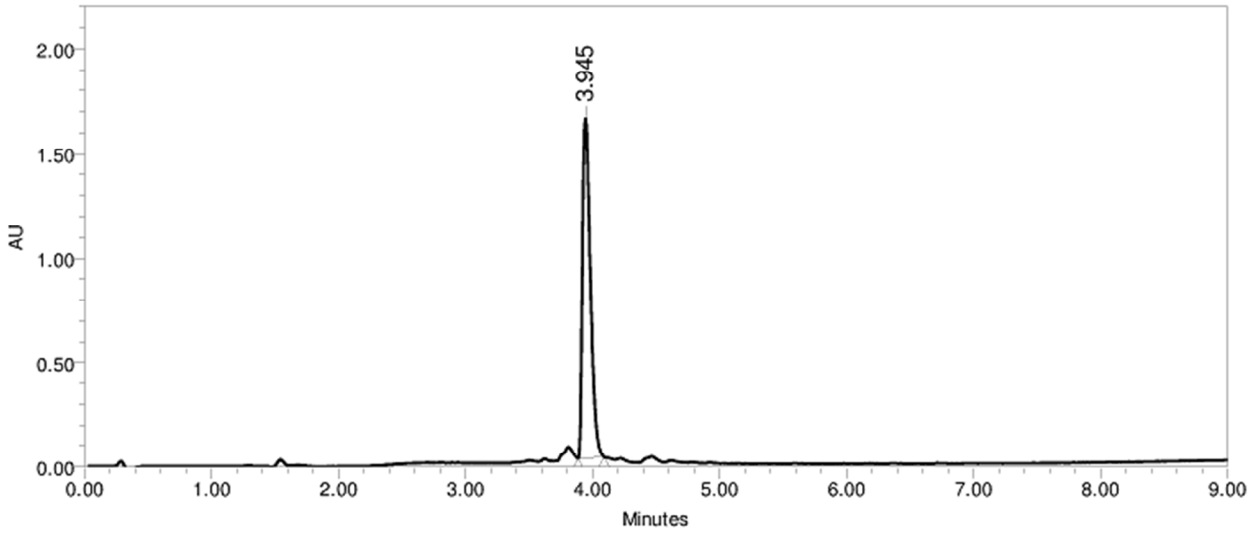


Figure S-5: HPLC chromatogram of peptide **15**

Peptide (17)

H-Cys(Trt)-Thr(tBu)-Ile-Gln(Trt)-Asp(tBu)-Cys($\Psi^{Me,Me}$pro)-Pro-Ile-Gly–N(H)—●

16

↓

H-Cys-Thr-Ile-Gln-Asp-Cys($\Psi^{Me,Me}$pro)-Pro-Ile-Gly-NH_2

17

H-Cys-Thr-Ile-Gln-Asp-Cys-Pro-Ile-Gly-NH_2

18

Peptide **17** was synthesized according to General Method 1 using Fmoc-Rink-Amide AM resin (222.2 mg, 0.1 mmol, 0.45 mmol/gram). Following peptide elongation, the resin was treated with 20% piperidine/DMF (1 × 1 min and 2 × 5 min) to remove Fmoc, and washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min). Microcleavage of resin **16** (5 mg resin): The peptide was cleaved from the resin according to General Method 4 for 2h and subsequent HPLC analysis found that peptide **18** was obtained in 95% purity (linear gradient from 5% to 100% acetonitrile over 8 min, t_R : 3.9 min). **LCMS** peptide **17** not observed; peptide **18** observed $[M+H]^+$ 948.4, required $[M+H]^+$ 948.4.

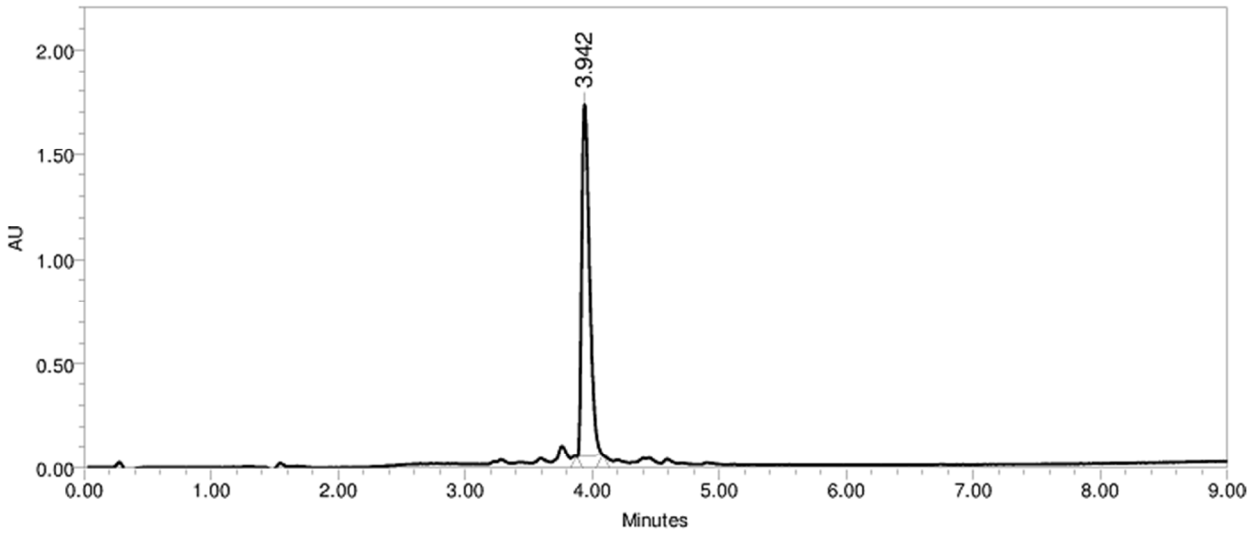


Figure S-6: HPLC chromatogram of peptide **18**

Peptide (20)

H-Cys(Trt)-Thr(tBu)-Ile-Arg(Pbf)-Asp(tBu)-Cys($\Psi^{Me,Me}$pro)-Pro-Glu(tBu)–N(H)— 

19

H-Cys-Thr-Ile-Arg-Asp-Cys($\Psi^{Me,Me}$pro)-Pro-Glu-NH_2

20

H-Cys-Thr-Ile-Arg-Asp-Cys-Pro-Glu-NH_2

21

Peptide **20** was synthesized according to General Method 1 using Fmoc-Rink-Amide AM resin (222.2 mg, 0.1 mmol, 0.45 mmol/gram). Following peptide elongation, the resin was treated with 20% piperidine/DMF (1 × 1 min and 2 × 5 min) to remove Fmoc, and washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min). Microcleavage of resin **19** (5 mg resin): The peptide was cleaved from the resin according to General Method 4 for 2h and subsequent HPLC analysis found that peptide **21** was obtained in 91% purity (linear gradient from 5% to 100% acetonitrile over 8 min, t_R : 3.4 min). **LCMS** peptide **20** not observed; peptide **21** observed $[M+H]^+$ 935.4, required $[M+H]^+$ 935.4.

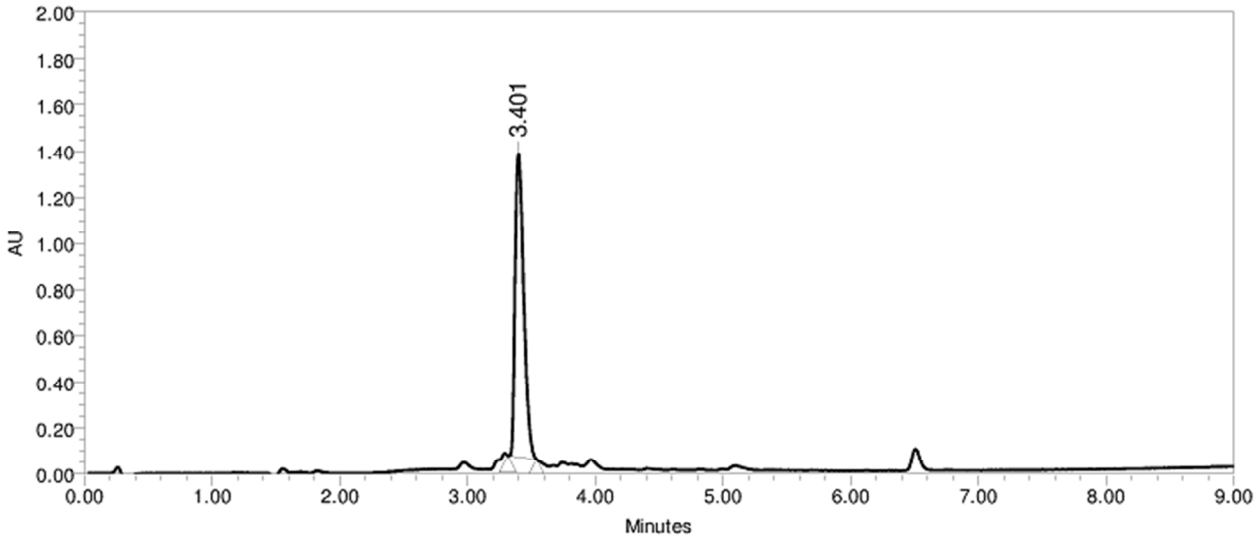


Figure S-7: HPLC chromatogram of peptide **7-dp**

Peptide (23)

H-Cys(Trt)-Thr(tBu)-Ile-Gln(Trt)-Lys(Boc)-Cys($\Psi^{Me,Me}$pro)-Pro-Ile-Gly–N(H)—●

22

↓

H-Cys-Thr-Ile-Gln-Lys-Cys($\Psi^{Me,Me}$pro)-Pro-Ile-Gly-NH_2

23

H-Cys-Thr-Ile-Gln-Lys-Cys-Pro-Ile-Gly-NH_2

24

Peptide **23** was synthesized according to General Method 1 using Fmoc-Rink-Amide AM resin (222.2 mg, 0.1 mmol, 0.45 mmol/gram). Following peptide elongation, the resin was treated with 20% piperidine/DMF (1 × 1 min and 2 × 5 min) to remove Fmoc, and washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min). Microcleavage of resin **22** (5 mg resin): The peptide was cleaved from the resin according to General Method 4 for 2h at 45°C and subsequent HPLC analysis found that peptide **24** was obtained in 93% purity (linear gradient from 5% to 100% acetonitrile over 8 min, t_R : 3.7 min). **LCMS** peptide **23** not observed; peptide **24** observed $[M+H]^+$ 961.5, required $[M+H]^+$ 961.5.

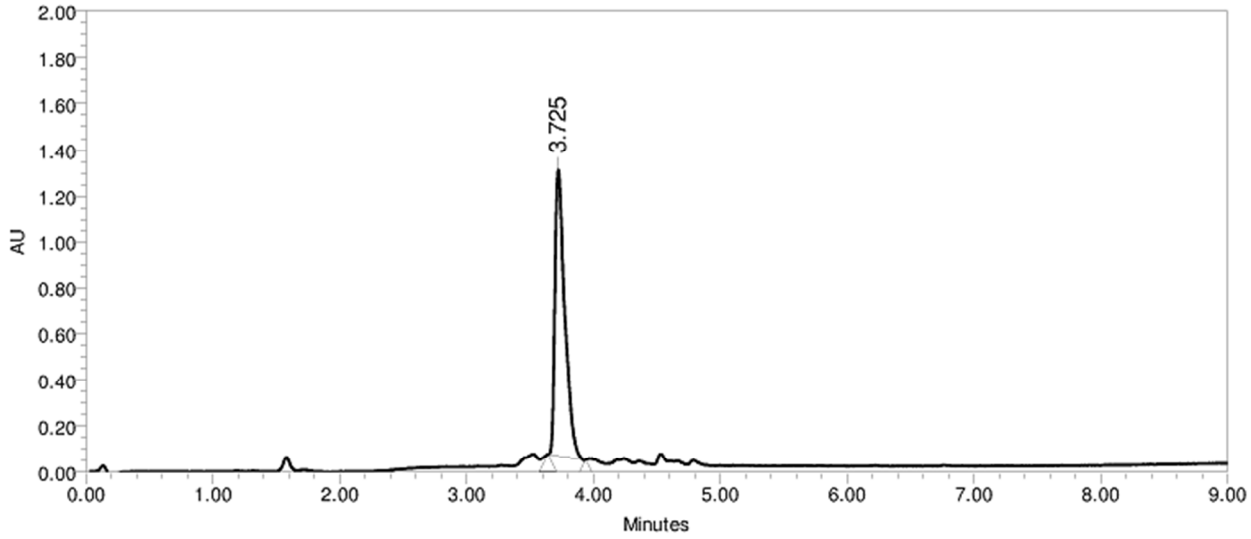


Figure S-8: HPLC chromatogram of peptide **24**

Peptide (26)

H-Lys(Boc)-Thr(tBu)-Lys(Boc)-Cys($\Psi^{Me,Me}$pro)-Lys(Boc)-Phe-Leu-Lys(Boc)-Lys(Boc)-Cys(Trt)–N(H)– 

25

H-Lys-Thr-Lys-Cys($\Psi^{Me,Me}$pro)-Lys-Phe-Leu-Lys-Lys-Cys-NH_2

26

H-Lys-Thr-Lys-Cys-Lys-Phe-Leu-Lys-Lys-Cys-NH_2

27

Peptide **26** was synthesized according to General Method 1 using Fmoc-Rink-Amide AM resin (222.2 mg, 0.1 mmol, 0.45 mmol/gram). Following peptide elongation, the resin was treated with 20% piperidine/DMF (1 × 1 min and 2 × 5 min) to remove Fmoc, and washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min). Microcleavage of resin **25** (5 mg resin): The peptide was cleaved from the resin according to General Method 4 for 3h at 45°C and subsequent HPLC analysis found that peptide **27** was obtained in 91% purity (linear gradient from 5% to 100% acetonitrile over 8 min, t_R : 3.5 min). **LCMS** peptide **26** not observed; peptide **27** observed $[M+H]^+$ 1225.7, required $[M+H]^+$ 1225.7.

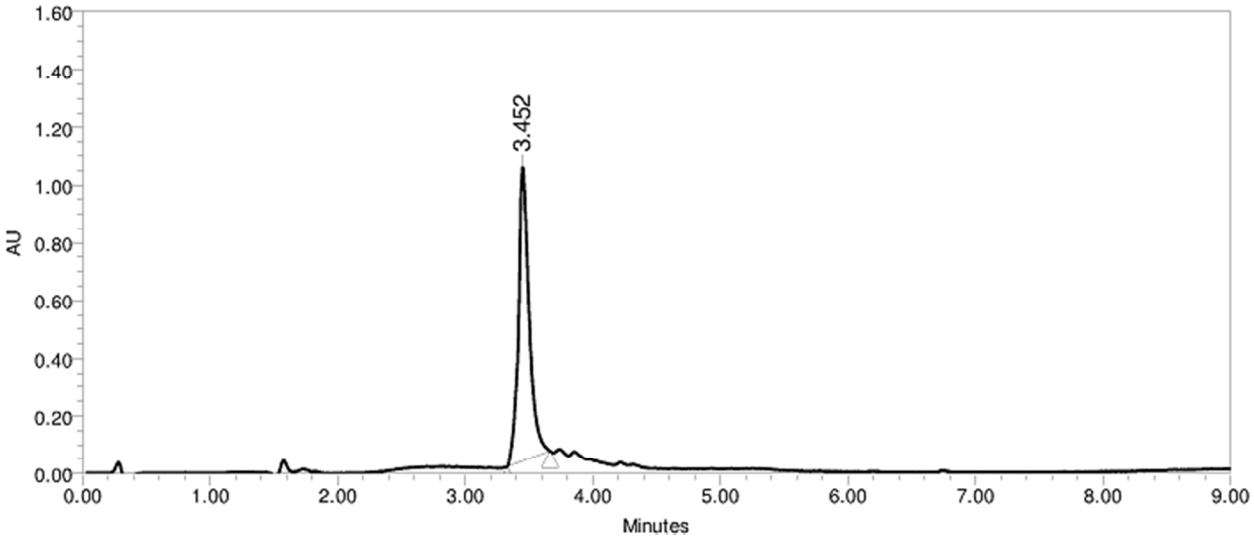
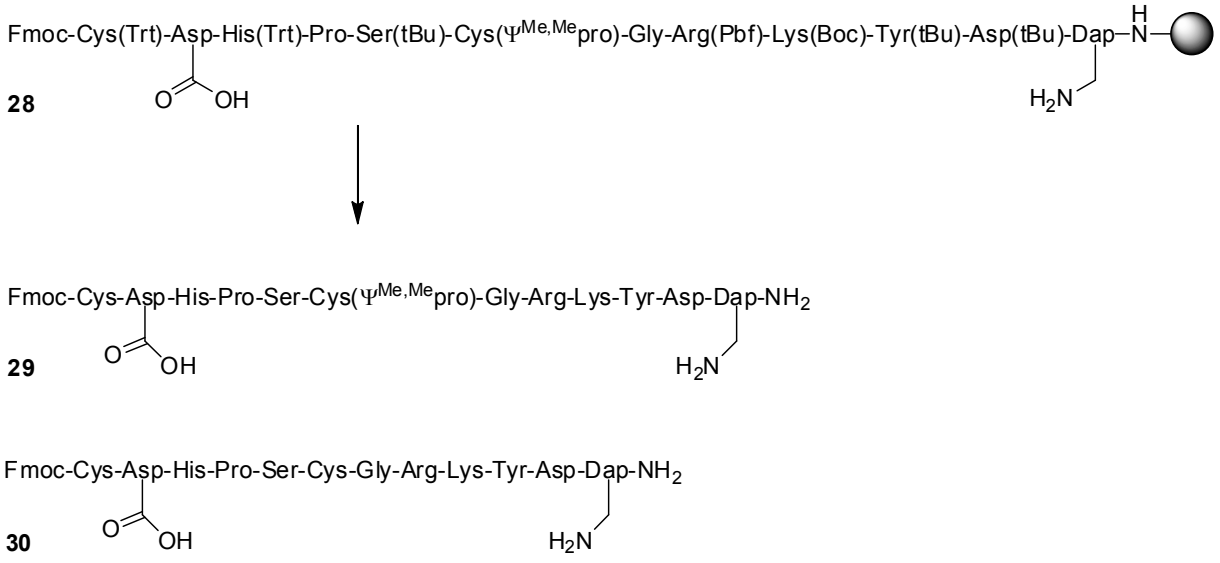


Figure S-9: HPLC chromatogram of peptide **27**

Conotoxin Derivative (28)



Peptide **28** was synthesized according to General Method 2 using Rink-Amide-Chemmatrix Low LOA resin (188.7 mg, 0.1 mmol, 0.53 mmol/g). Fmoc-Ser-Cys($\psi^{Me,Me}$pro)-OH was coupled for 2 h to achieve complete conversion. Following peptide elongation, Allyl protection was removed according to General Method 4. Microcleavage of resin **28** (5 mg resin): The peptide was cleaved from the resin according to

General Method 4 and subsequent HPLC analysis found that peptide **29** was obtained in 35**%** purity and peptide **30** in 65% (linear gradient from 25% to 50% acetonitrile over 8 min, t_R peptide **29** : 4.9 min and peptide **30** : 4.4 min). **LCMS** peptide **29** observed $[M+H]^+$ 1590.8, required $[M+H]^+$ 1590.7 and peptide **30** observed $[M+H]^+$ 1550.8, required $[M+H]^+$ 1550.6.

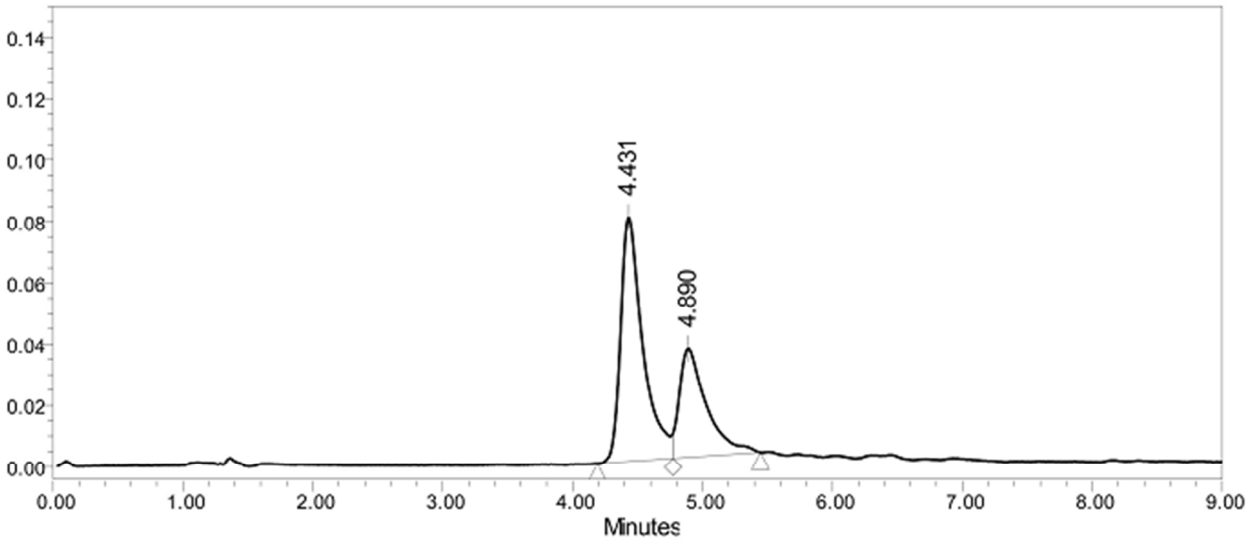
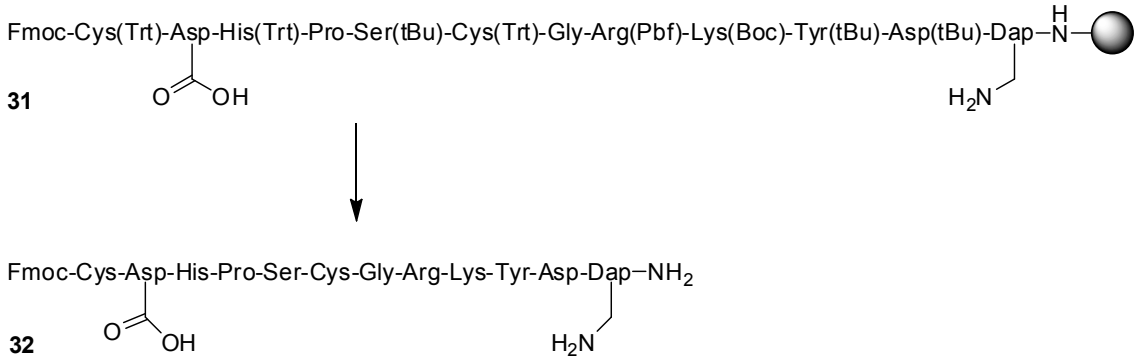


Figure S-10: HPLC chromatogram of peptide **29**

Conotoxin Derivative (31)



Peptide **31** was synthesized according to General Method 2 using Rink-Amide-Chemmatrix Low LOA resin (188.7 mg, 0.1 mmol, 0.53 mmol/g). Following peptide elongation, Allyl protection was removed according to General Method 4. Microcleavage of resin **31** (5 mg resin): The peptide was cleaved from the resin according to General Method 4 and subsequent HPLC analysis found that peptide **32** was

obtained in 97**%** purity (linear gradient from 25% to 50% acetonitrile over 8 min, t_R : 4.3 min). **LCMS** peptide **32** observed $[M+H]^+$ 1550.8, required $[M+H]^+$ 1550.6.

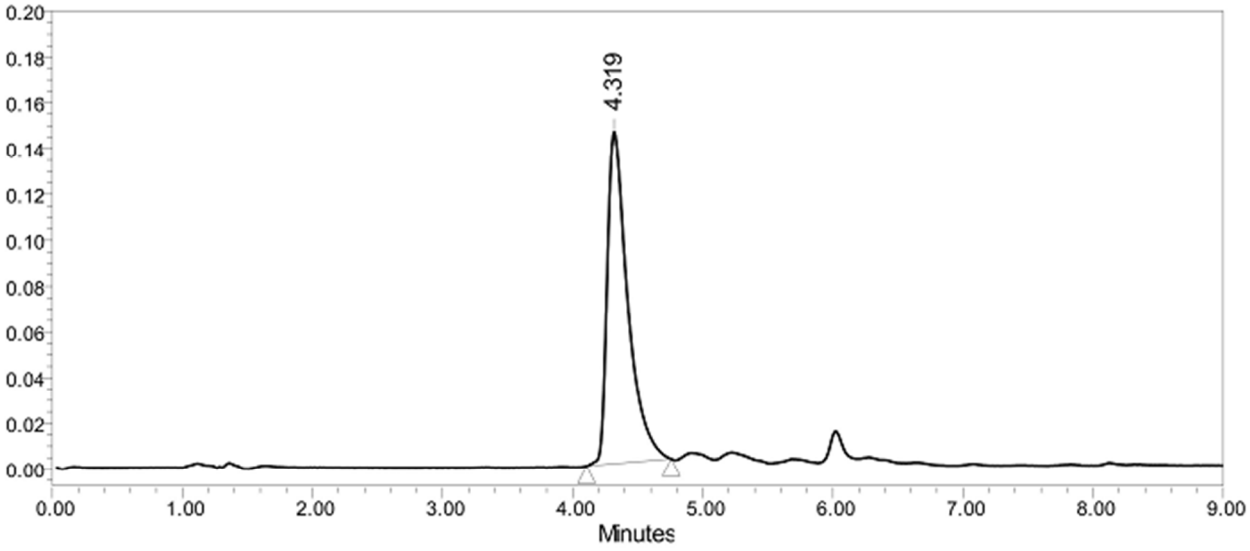
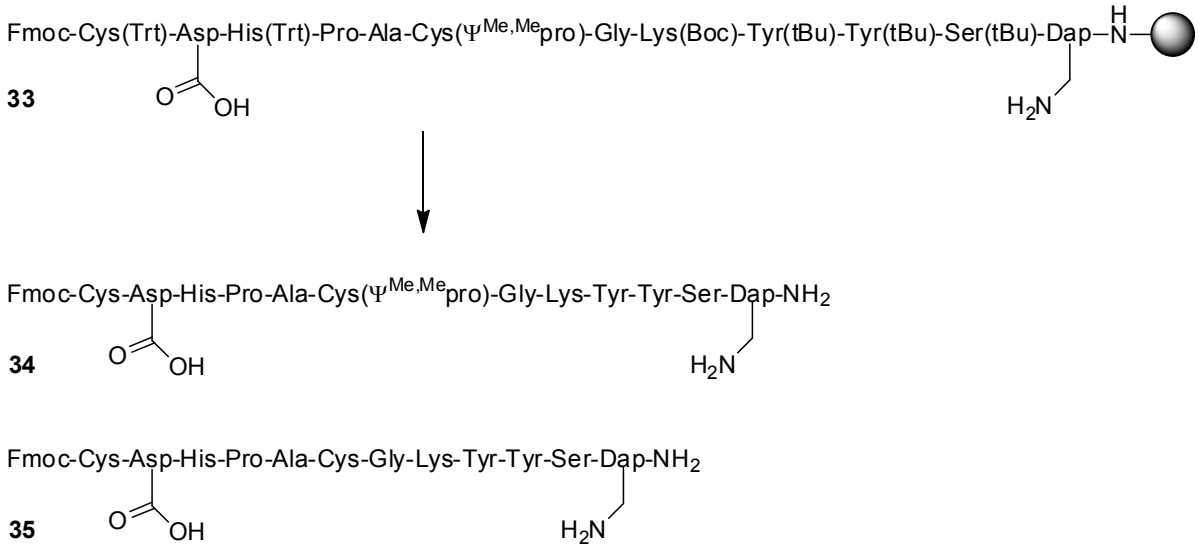


Figure S-11: HPLC chromatogram of peptide **32**

Conotoxin Derivative (33)



Peptide **33** was synthesized according to General Method 2 using Rink-Amide-Chemmatrix Low LOA resin (188.7 mg, 0.1 mmol, 0.53 mmol/g). Fmoc-Ala-Cys($\psi^{Me,Me}$pro)-OH was coupled for 2 h to achieve

complete conversion. Following peptide elongation, Allyl protection was removed according to General Method 4. Microcleavage of resin **33** (5 mg resin): The peptide was cleaved from the resin according to General Method 4 and subsequent HPLC analysis found that peptide **34** was obtained in 16**%** purity and peptide **35** in 84% (linear gradient from 25% to 50% acetonitrile over 8 min, t_R peptide **34** : 4.1 min and peptide **35** : 3.7 min). **LCMS** peptide **34** observed $[M+H]^+$ 1627.8, required $[M+H]^+$ 1627.7 and peptide **35** observed $[M+2H]^+$ 794.3, required $[M+2H]^+$ 794.3.

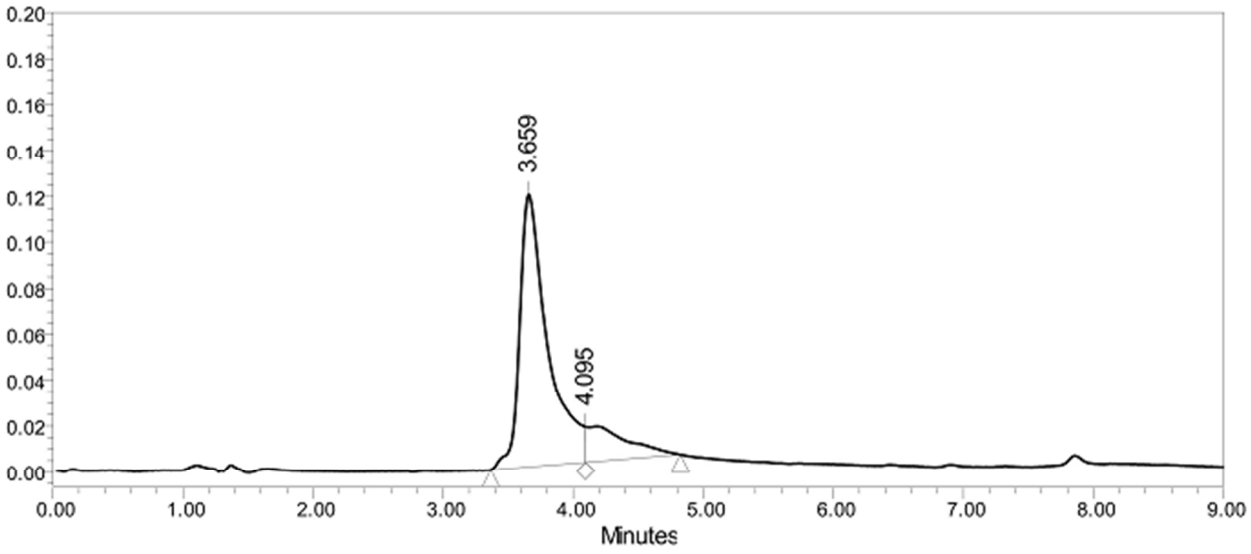
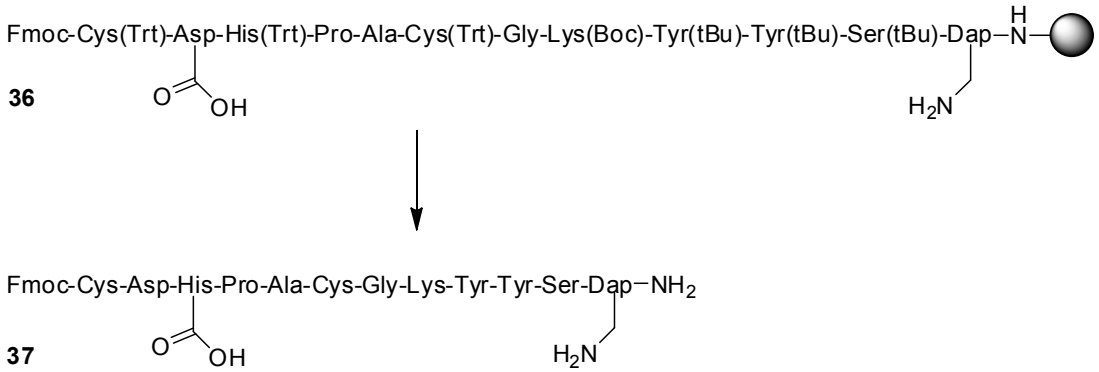


Figure S-12: HPLC chromatogram of peptide **34**

Conotoxin Derivative (36)



Peptide **36** was synthesized according to General Method 2 using Rink-Amide-Chemmatrix Low LOA resin (188.7 mg, 0.1 mmol, 0.53 mmol/g). Fmoc-Ser-Cys[Psi(Me,Me)Pro]-OH was coupled for 2 h to

achieve complete conversion. Following peptide elongation, Allyl protection was removed according to General Method 4. Microcleavage of resin **36** (5 mg resin): The peptide was cleaved from the resin according to General Method 4 and subsequent HPLC analysis found that peptide **37** was obtained in 98**%** purity (linear gradient from 25% to 50% acetonitrile over 8 min, t_R : 3.5 min). **LCMS** peptide **37** observed $[M+2H]^+$ 794.4, required $[M+2H]^+$ 794.3.

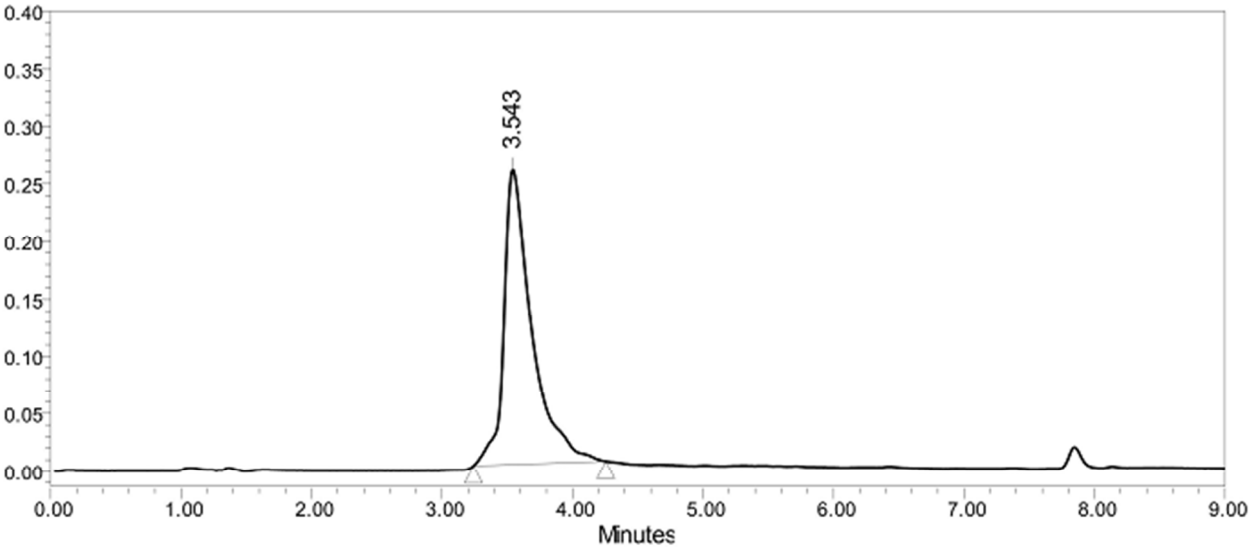
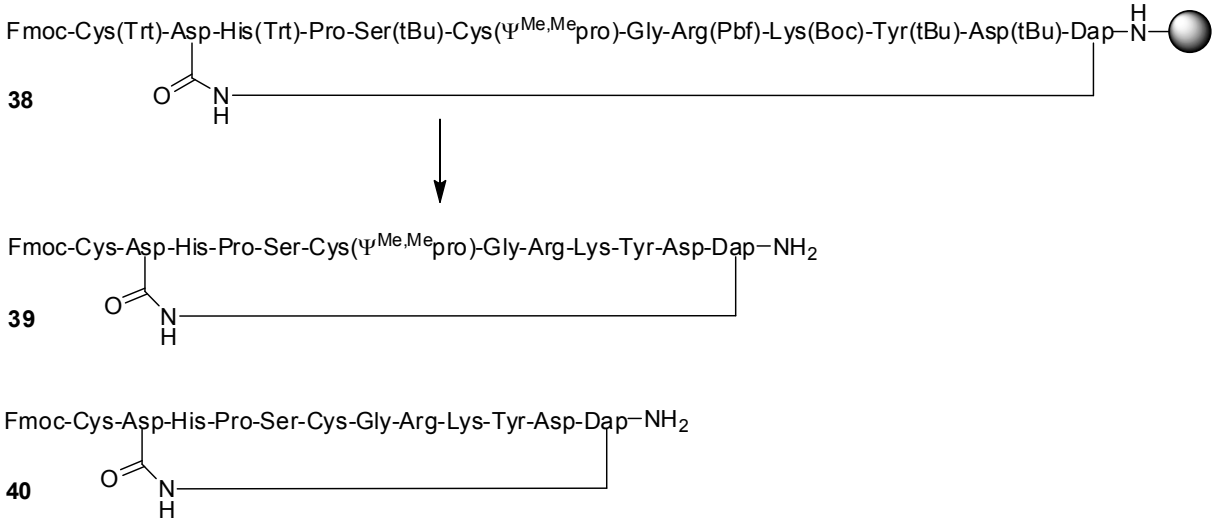


Figure S-8: HPLC chromatogram of peptide **37**

Macrocyclization of peptide resin 28 (39)



Peptide resin **28** (10 mg, 5.3 µmol) was washed with DMF (5 × 1 min), CH_2Cl_2 (5 × 1 min) and DMF (5 × 1 min). The macrocyclization was performed using DIC (4 equiv.) and Oxyma Pure (4 equiv) in DMF (300 µl), as a coupling system, for 2 h at rt. The peptide was cleaved from the resin **38** according to General Method 4 and subsequent HPLC analysis found that peptide **39** was obtained in 18**%** purity and peptide **40** was obtained in 77% (linear gradient from 25% to 50% acetonitrile over 8 min, t_R peptide **39** : 5.5 min and peptide **40** : 4.7 min). **LCMS** peptide **39** observed $[M+H]^+$ 1609.8, required $[M+H]^+$ 1609.7; peptide **40** observed $[M+H]^+$ 1569.4, required $[M+H]^+$ 1569.6.

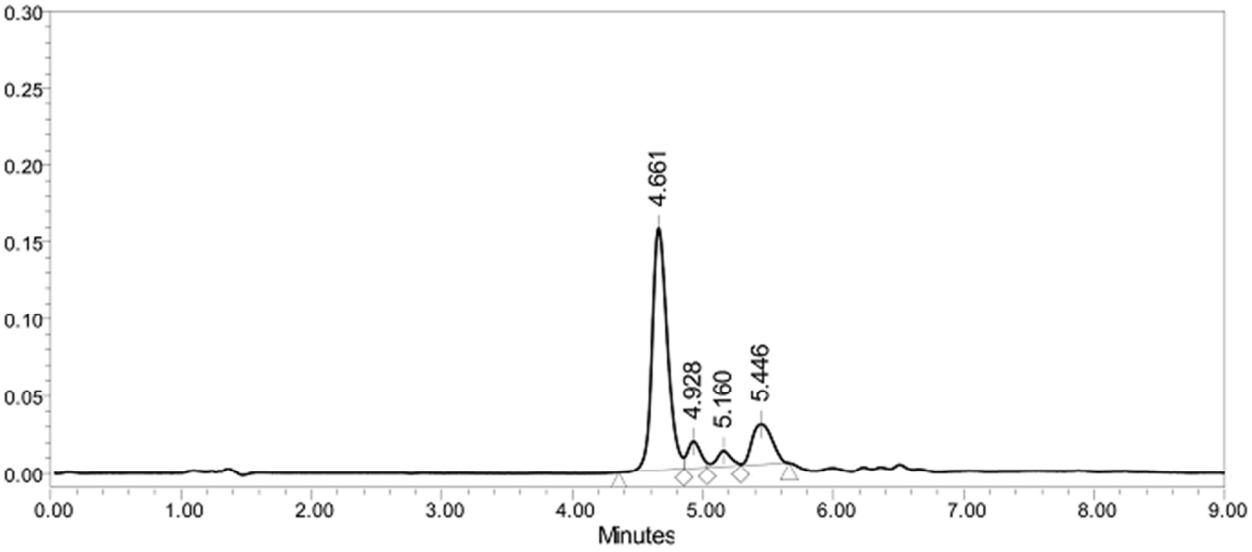
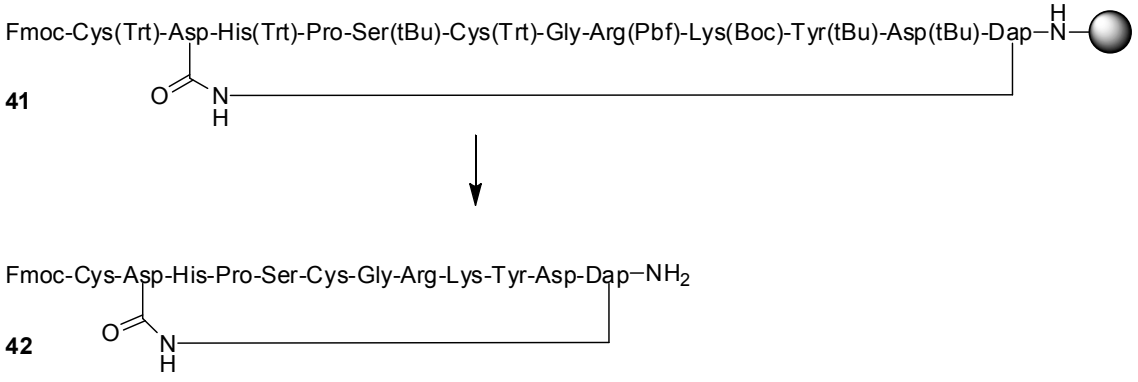


Figure S-14: HPLC chromatogram of peptide **39**

Macrocyclization of peptide resin 31 (42)



Peptide resin **31** (10 mg, 5.3 µmol) was washed with DMF (5 × 1 min), CH_2Cl_2 (5 × 1 min) and DMF (5 × 1 min). The macrocyclization was performed using DIC (4 equiv.) and Oxyma Pure (4 equiv) in DMF (300

µl), as a coupling system, for 2 h at rt. The peptide **41** was cleaved from the resin according to General Method 4 and subsequent HPLC analysis found that peptide **42** was obtained in 45**%** purity (linear gradient from 25% to 50% acetonitrile over 8 min, t_R : 4.7 min). **LCMS** peptide **42** observed $[M+H]^+$ 1569.9, required $[M+H]^+$ 1569.6.

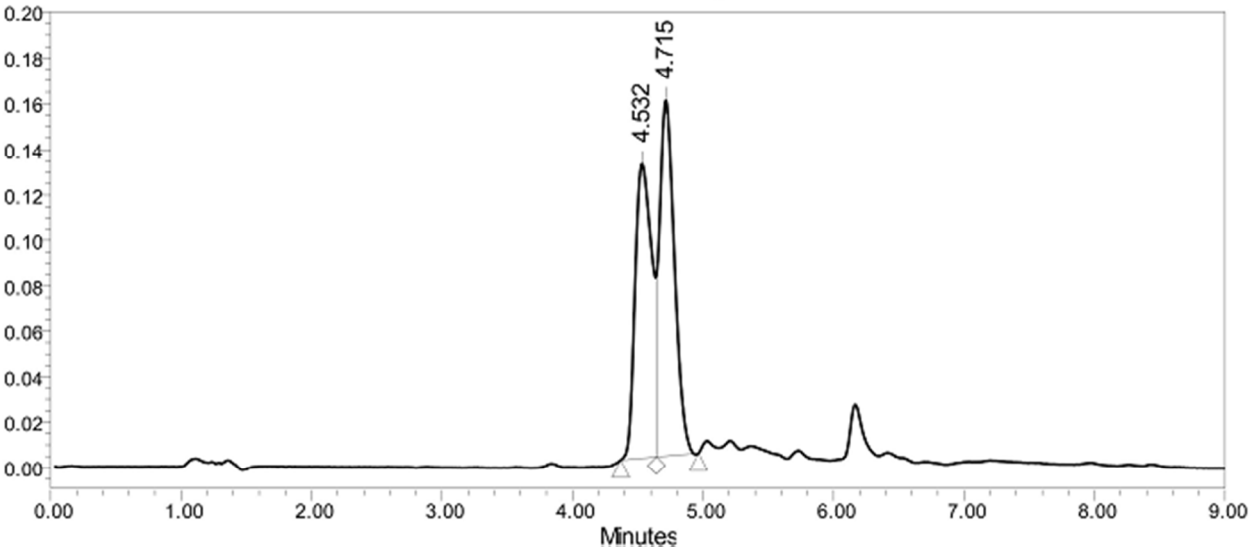
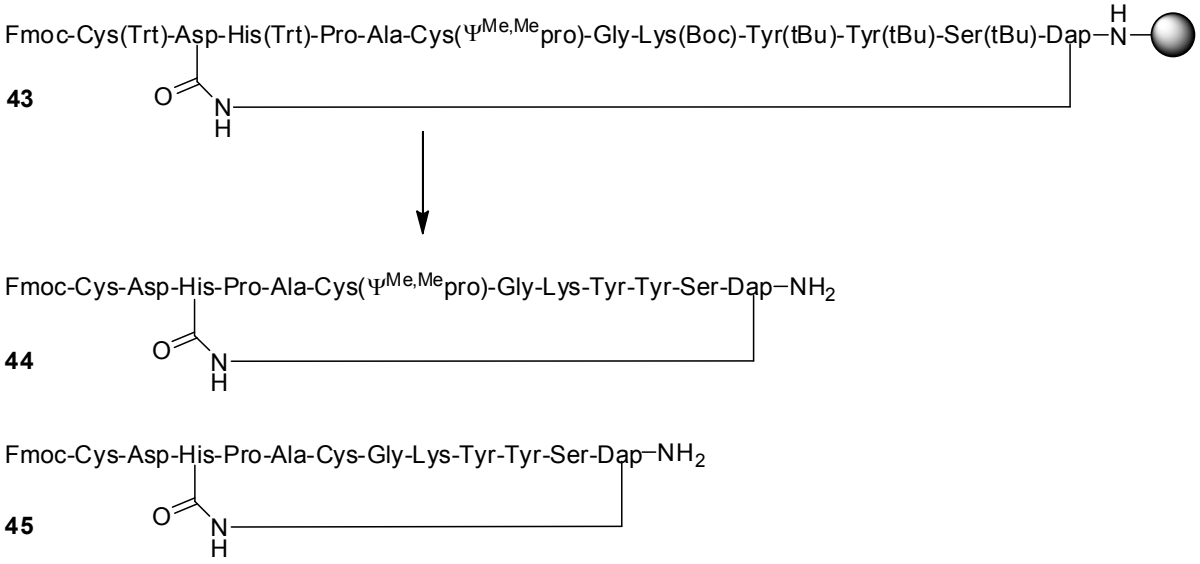


Figure S-15: HPLC chromatogram of peptide **42**

Macrocyclization of peptide resin 33 (44)



Peptide resin **33** (10 mg, 5.3 µmol) was washed with DMF (5 × 1 min), CH_2Cl_2 (5 × 1 min) and DMF (5 × 1 min). The macrocyclization was performed using DIC (4 equiv.) and Oxyma Pure (4 equiv) in DMF (300 µl), as a coupling system, for 2 h at rt. The peptide **43** was cleaved from the resin according to General Method 4 and subsequent HPLC analysis found that peptide **44** was obtained in 14% purity and peptide **45** in 84% (linear gradient from 25% to 50% acetonitrile over 8 min, t_R peptide **44**: 3.8 min and peptide **45** 3.5 min). LCMS peptide **44** observed $[M+H]^+$ 1572.9, required $[M+H]^+$ 1572.6; peptide **45** observed $[M+H]^+$ 1532.8, required $[M+H]^+$ 1532.6.

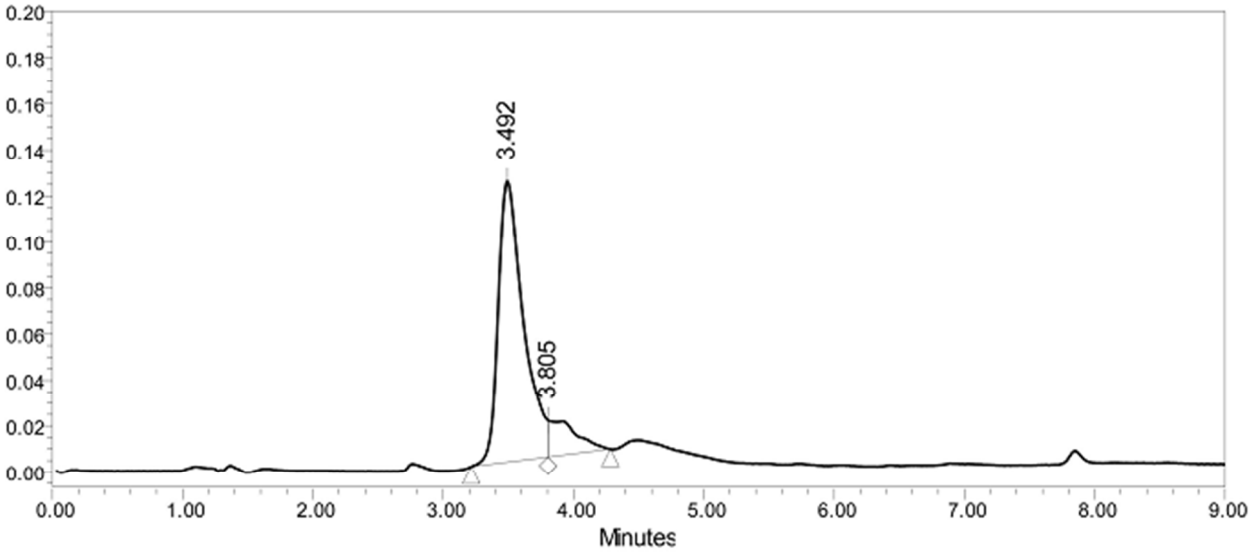
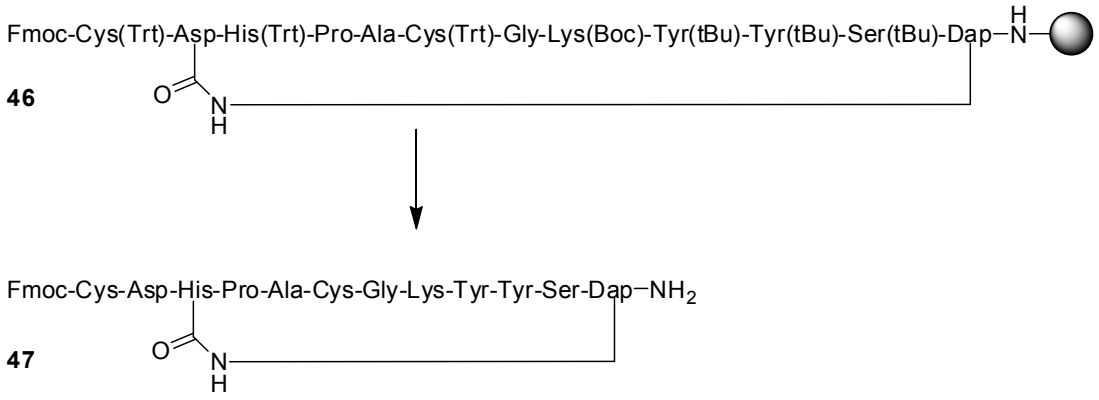


Figure S-16: HPLC chromatogram of peptide **44**

Macrocyclization of peptide resin 36 (47)



Peptide resin **36** (10 mg, 5.3 µmol) was washed with DMF (5 × 1 min), CH_2Cl_2 (5 × 1 min) and DMF (5 × 1 min). The macrocyclization was performed using DIC (4 equiv.) and Oxyma Pure (4 equiv) in DMF (300 µl), as a coupling system, for 2 h at rt. The peptide **46** was cleaved from the resin according to General Method 4 and subsequent HPLC analysis found that peptide **47** was obtained in 72**%** purity (linear gradient from 25% to 50% acetonitrile over 8 min, t_R : 3.5 min). LCMS peptide **47** observed $[M+H]^+$ 1532.8, required $[M+H]^+$ 1532.6.

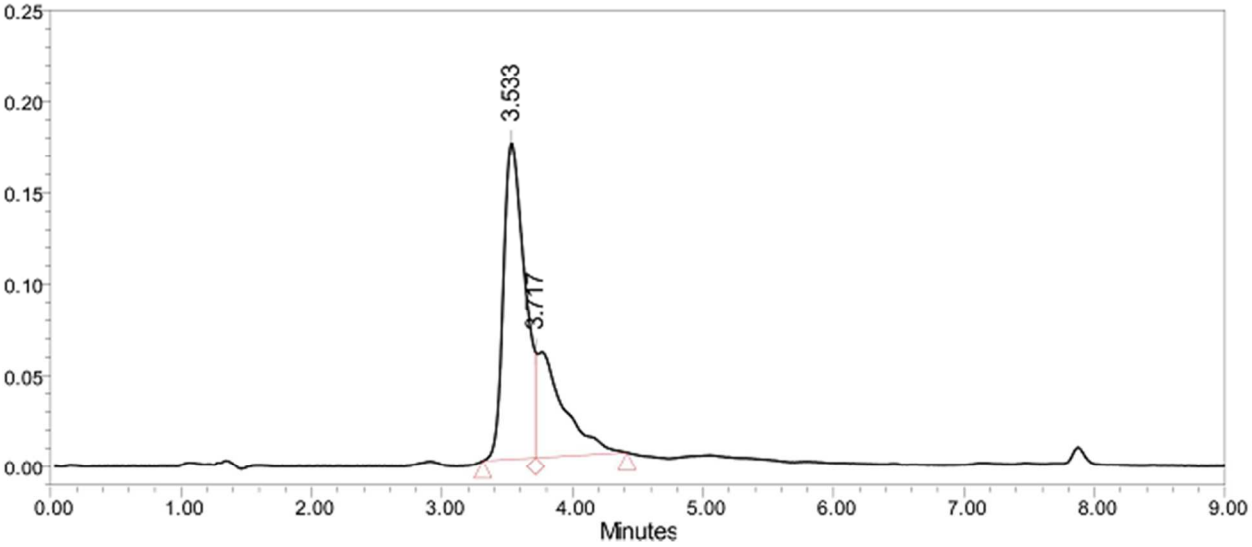


Figure S-17: HPLC chromatogram of peptide **47**

Kaiser test of Macrocyclization Experiments

After 2 h coupling a Kaiser test was performed to show whether amines were present or not. The peptides containing Cys pseudoproline groups gave negative Kaiser tests and indicated completion of macrocyclization whereas the peptides with standard protection gave positive Kaiser tests and indicated incomplete reaction (Fig. S-13). Number 1 is peptide **38**, 2 is peptide **41**, 3 is peptide **43** and 4 is peptide **46**.

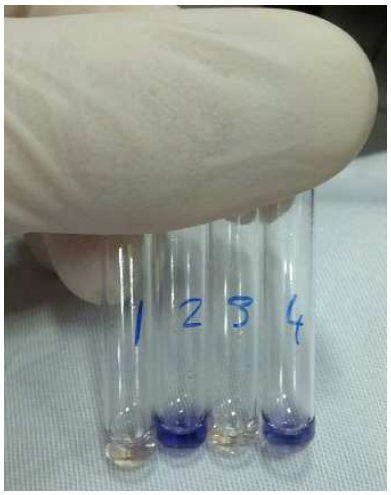


Figure S-13: Kaiser test of macrocyclization experiment

4. References

1. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970,** *34*, 595-598.