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Supporting information

C-Terminal lactamization of peptides

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Materials and methods

All chemicals were acquired from commercial providers and used as received. All solvents were HPLC quality. Peptides were synthesized via SPPS in filtration columns equipped with polyethylene frits. A fluoren-9-ylmethyloxycarbonyl (Fmoc) strategy was used for SPPS. The resin of choice was the poly(ethylene glycol)-poly-(*N*,*N*-dimethylacrylamide) copolymer (PEGA). The PL-PEGA$_{800}$ resin (0.4 mmol/g, 150-300 µm) was provided by Agilent Technologies, Inc. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker instrument at 500 MHz and 126 MHz, respectively. Chemical shift values are quoted in ppm and coupling constants (*J*) in Hz. The residual solvent peak from DMSO-d_6 (^{1}H: 2.50 ppm, ^{13}C: 39.5 ppm), acetonitrile-d_3 (^{1}H: 1.94 ppm, ^{13}C: 118.26 ppm), or methanol-d_4 (^{1}H: 4.87 ppm, ^{13}C: 49.0 ppm) was used as reference. HPLC-UV spectra were recorded on an Agilent 1100 HPLC instrument with a 100 mm XBridge C_{18} column. LC-MS spectra were recorded on a Bruker instrument with an Acclaim™ RSLC 120 C_{18} column (2.2 µm, 120 Å, 2.1×100 mm). HRMS spectra were recorded on a Bruker ESP-MALDI-FT-ICR instrument. Peptide purification was performed using a semipreparative Gilson RP-HPLC with a UV detector and a stationary phase consisting of C_{18}-modified silica (XTetra RP 18 column, 10 µm, 19×150 mm). For the mobile phase, two buffers were used: buffer A (0.1 % trifluoroacetic acid in water) and buffer B (0.1 % trifluoroacetic acid in 9:1 MeCN/water).

General procedure A: Solid-phase synthesis of peptides

PL-PEGA$_{800}$ (dry resin) in a polyethylene filtration column was swelled in DMF (50 mL/mmol resin). The resin was washed with 3 × DMF and then treated with 20 % piperidine in DMF for 0.5 h. The resin was washed with 3 × DMF, 3 × CH_2Cl_2, and 3 × DMF. 4-hydroxymethyl benzoic acid (HMBA) (3 equiv.) and *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methyl methanaminiumtetrafluoroborate (TBTU) (2.88 equiv.) were dissolved in DMF (25 mL/mmol resin), and *N*-ethylmorpholine (NEM) (4 equiv.) was added. After 5 min, the mixture was added to the resin and the reaction was gently shaken for 2 h. The resin was washed with 6 × DMF and 6 × anhydrous CH_2Cl_2, and dried under N_2 flow. The first amino acid (3 equiv.) and 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT) (3 equiv.) were mixed in anhydrous CH_2Cl_2 (50 mL/mmol resin), and *N*-methylimidazole (MeIm) (2.25 equiv.) was added. After complete dissolution, the mixture was shaken 5 min and then added to the resin. The reaction was gently shaken for 1 h, washed with 1 × anhydrous CH_2Cl_2, 1 × DMF, and 1 × anhydrous CH_2Cl_2, and the MSNT coupling was repeated once. The resin was washed with 3 × CH_2Cl_2, 3 × DMF, and 3 × CH_2Cl_2, and dried under N_2 flow. The resin loading (amount of NH_2 groups/mass of dry resin) was measured after MSNT-coupling of the first amino acid. An aliquot of the dry resin (typically 5-8 mg for PEGA$_{800}$) was transferred to a 4 mL glass vial, and 3.5 mL 20 % piperidine in DMF was added and shaken for 30 min at r.t. Absorbance of fluorene at 300 nm was measured using a NanoDrop 2000 spectrophotometer from Thermo Scientific. Absorbances were converted to fluorene concentration using a calibration curve with the linear function A = 0.7529 × c - 0.009 (A = absorbance, c = concentration of fluorene in mmol/L). Based on the volume of piperidine solution and the mass of dry resin, the resin loading can be calculated from the fluorene concentration. The dry resin was swollen in DMF and washed with 2 × DMF. A mixture of 20 % piperidine in DMF was added to remove the Fmoc protecting group. After 2 min, the piperidine solution was

removed, and fresh 20 % piperidine in DMF was added. This was removed after 18 min, and the resin was washed with 5 × DMF. The remaining Fmoc-protected amino acids or acetic acid was coupled in the same way as the HMBA linker, using TBTU and NEM. To minimize the extent of diketopiperazine formation during removal of the Fmoc protecting group of the second amino acid, the resin was only treated with 20 % piperidine in DMF for 5 min once and then washed with 3 × DMF for a total of 5 min before adding the activated Fmoc-protected amino acid or acetic acid.

General procedure B: Lactam formation using DIPEA.

The dry resin was swelled in DMF 30 min, then washed with 3 × DMF and 3 × CH_2Cl_2. A mixture of TFA/H_2O/PhOH/TIPS (88:5:5:2), or TFA/CH_2Cl_2/H_2O/$PhSCH_3$/DTT/TIPS (66.5:20:5:5:2.5:1) for the cysteine-containing peptides, was added. After 3 h the resin was washed with 6 × CH_2Cl_2 and 6 × MeCN. DIPEA (17 %) in MeCN (1 mL) was added and after 1 h, the MeCN/DIPEA product solution was isolated by filtration. The remaining peptide product was extracted from the resin using water with 0.1 % TFA (1 mL), MeCN/H_2O (1:1) with 0.1 % TFA (2 × 1 mL), MeCN/H_2O (9:1) with 0.1 % TFA (1 mL), and water with 0.1 % TFA (1 mL). The extracts were combined with the isolated MeCN phase, and the combined liquid phase was freeze-dried. The resulting crude product was purified using a semipreparative RP-HPLC.

General procedure C: Lactam formation using DBU.

The dry resin was swelled in DMF 30 min, then washed with 3 × DMF and 3 × CH_2Cl_2. A mixture of TFA/H_2O/PhOH/TIPS (88:5:5:2) was added. After 3 h, the resin was washed with 6 × CH_2Cl_2 and 6 × MeCN. DBU (2 %) in MeCN (5 mL) was added and the mixture with resin was shaking for 20 h. The MeCN phase was isolated, and lactamized peptide was extracted from the resin using water with 0.1 % TFA (5 mL), MeCN/H_2O (1:1) with 0.1 % TFA (5 mL), and MeCN/H_2O (9:1) (5 mL). The lactamization step was repeated once for 4 h using a fresh 5 mL portion of 2 % DBU in MeCN, after which the MeCN phase was combined with the original extracts. Lactamized peptide was again extracted from the resin. All combined extracts were freeze-dried. The resulting crude product was purified using a semipreparative RP-HPLC.

Synthesis of Ac-Trp(Boc)-L-Dab(Boc)-HMBA-$PEGA_{800}$ (**1**), Ac-Trp(Boc)-L-Orn(Boc)-HMBA-$PEGA_{800}$ (**2**), and Ac-Trp(Boc)-L-Lys(Boc)-HMBA-$PEGA_{800}$ (**3**).

The peptides were synthesized by general procedure A using PL-$PEGA_{800}$ (250 mg dry resin, 0.1 mmol)., HMBA (46.5 mg, 0.30 mmol), TBTU (92.5 mg, 0.288 mmol), and NEM (50.3 µL, 0.40 mmol). Followed by Fmoc-L-Dab(Boc)-OH (132 mg, 0.30 mmol), Fmoc-L-Orn(Boc)-OH (136 mg, 0.30 mmol), or Fmoc-L-Lys(Boc)-OH (141 mg, 0.30 mmol), MSNT (88.9 mg, 0.30 mmol) and MeIm (18 µL). The loading was found to be 0.249 mmol/g (for Fmoc-L-Dab-HMBA-PEGA), 0.209 mmol/g (for Fmoc-L-Orn-HMBA-PEGA), and 0.218 mmol/g (for Fmoc-L-Lys-HMBA-PEGA). Fmoc-Trp(Boc)-OH (0.30 mmol) and acetic acid (0.30 mmol) were coupled using TBTU (92.5 mg, 0.288 mmol, 2.88 equiv.) and NEM (50.3 µL, 0.40 mmol, 4 equiv.).

Test-lactamization of Ac-Trp-L-Dab

Dried resin (8.0 mg) was swelled in DMF and washed with 3 × DMF, 3 × CH_2Cl_2. The side-chains were deprotected using TFA/H_2O/PhOH/TIPS (88:5:5:2, reagent B) for 3 h, and washed with 6 × CH_2Cl_2 and 6 × MeCN. *C*-terminal lactamization was performed using one of the following conditions, respectively: MeCN (100 µL) at r.t. for 1 h; 2 % DIPEA in MeCN (100 µL) at r.t. for 1 h; 17 % DIPEA in MeCN (100 µL) at r.t. for 1 h; 17 % DIPEA in MeCN (100 µL) at 60 °C for 2 h; 10 % Et_3N in MeCN (100 µL) at r.t. for 1 h; and 2 % DBU in MeCN (100 µL) at r.t. for 1 h. After reaction, the MeCN phase was isolated and diluted to 1 mL using 25 % MeCN/ H_2O with 0.1 % TFA. The diluted MeCN phases were then used for HPLC-UV-analysis. The resin was washed with 3 × MeCN and 3 × H_2O, and 0.1 M NaOH in water (100 µL) was added. After 2 h, 0.1 M HCl in water (100 µL) was added to neutralize the solution, and the aqueous phase was isolated. Any remaining, non-lactamized peptide was extracted from the resin using 5:5 MeCN/ H_2O with 0.1 % TFA (2 × 125 µL), 9:1 MeCN/ H_2O with 0.1 % TFA (250 µL), and water with 0.1 % TFA (300 µL); all extracts were combined with the original neutralized aqueous phase to a total volume of 1 mL, and the sample was used for HPLC-UV-analysis.

Test-lactamization of Ac-Trp-L-Orn

Test-lactamization of Ac-Trp-L-Orn was conducted in the same way as for Ac-Trp-L-Dab using 8.0 mg resin aliquots

Test-lactamization of Ac-Trp-L-Lys

Test-lactamization of Ac-Trp-L-Lys was conducted in the same way as for Ac-Trp-L-Dab using 8.0 mg resin aliquots. Apart from the conditions studied for lactamization of Ac-Trp-L-Dab, a few others were tried with Ac-Trp-L-Lys: 2 % DBU in MeCN (100 µL) at r.t. for 20 h, and 100 µL, and potassium hexamethyldisalazane in THF (22.5 % w/w) at r.t. for 1 h.

Ac-Trp-L-Dab(-NHCO-) (**4**)

Ac-Trp-L-Dab(-NHCO-) (**4**) was synthesized from Ac-Trp(Boc)- L-Dab (Boc)-HMBA-$PEGA_{800}$ (98.1 mg) dry resin via General procedure B. The yield of **4** was$(\frac{4.7 \times 10^{-3}\text{g}}{328.37200\ \text{g/mol}})/(0.249 \times 10^{-3}\text{mol/g} \times 0.0981\ \text{g}) = 59\ \%$.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (d, *J* = 2.4 Hz, 1H), 8.23 (d, *J* = 8.2 Hz, 1H), 7.94 (d, *J* = 8.4 Hz, 1H), 7.82 (s, 1H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.17 (d, *J* = 2.4 Hz, 1H), 7.04 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H), 6.96 (ddd, *J* = 7.9, 6.9, 1.0 Hz, 1H), 4.53 (td, *J* = 8.8, 4.4 Hz, 1H), 4.32 (dt, *J* = 10.5, 8.5 Hz, 1H), 3.18 (dd, *J* = 9.1, 6.0 Hz, 2H), 3.12 (dd, *J* = 14.8, 4.5 Hz, 1H), 2.91 (dd, *J* = 14.7, 9.2 Hz, 1H), 2.28 (dddd, *J* = 11.7, 8.6, 6.0, 2.6 Hz, 1H), 1.81 (m, 1H), 1.76 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.6, 172.0, 169.3, 136.1, 127.50, 123.8, 120.9, 118.6, 118.3, 111.4, 110.3, 53.4, 49.7, 38.2, 28.3, 28.2, 22.7. TOF MS (ESI+) *m/z*calcd.for $C_{17}H_{21}N_4O_3^+$ $[M+H]^+$ 329.1608, found 329.1610. *m/z*calcd. for $C_{17}H_{20}N_4NaO_3^+$ $[M+Na]^+$ 351.1433, found 351.1430. *m/z*calcd. for $C_{34}H_{40}N_8NaO_6^+$ $[2M+Na]^+$ 679.2963, found 679.2963.

Ac-Trp-L-Orn(-NHCO-) (**5**)

Ac-Trp-L-Orn(-NHCO-) (**5**) was synthesized from Ac-Trp(Boc)-L-Orn(Boc)-HMBA-PEGA$_{800}$ (449.4 mg) dry resin via General procedure B. The product **5** was isolated in a yield of $(\frac{32.8\times10^{-3}\text{g}}{342.39900\text{ g/mol}})/(0.226\times10^{-3}\text{mol/g}\times 0.4494\text{ g}) = 94\ \%$.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.77 (d, *J* = 2.4 Hz, 1H), 8.13 (d, *J* = 8.0 Hz, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.60 (d, *J* = 8.1 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.17 (d, *J* = 2.3 Hz, 1H), 7.04 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H), 7.00 – 6.93 (m, 1H), 4.52 (td, *J* = 8.9, 4.3 Hz, 1H), 4.16 (ddd, *J* = 10.9, 8.0, 6.0 Hz, 1H), 3.14 (ddt, *J* = 8.7, 4.8, 2.9 Hz, 3H), 2.90 (dd, *J* = 14.8, 9.3 Hz, 1H), 2.03 – 1.90 (m, 1H), 1.88 – 1.68 (m, 5H), 1.67 – 1.54 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.4, 169.7, 168.9, 136.0, 127.4, 123.6, 120.7, 118.4, 118.1, 111.2, 110.3, 53.3, 48.9, 41.0, 28.0, 27.6, 22.6, 21.0. HRMS (TOF, ESI+) *m/z* calcd. for $C_{18}H_{22}N_4NaO_3^+$ $[M+Na]^+$ 365.1584, found 365.1583.

Ac-Trp-L-Lys(-NHCO-) (**6**)

Ac-Trp-L-Lys(-NHCO-) (**6**) was synthesized from Ac-Trp(Boc)-L-Orn(Boc)-HMBA-PEGA$_{800}$ dry resin (309.5 mg) via General procedure C at 60 °C. The yield of **6** was $(\frac{10.9\times10^{-3}\text{g}}{356.42600\text{ g/mol}})/(0.218\times10^{-3}\text{mol/g}\times 0.3095\text{ g}) = 45\ \%$.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (d, *J* = 2.6 Hz, 1H), 8.14 (dd, *J* = 13.6, 8.1 Hz, 1H), 7.92 – 7.68 (m, 2H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.36 – 7.25 (m, 1H), 7.13 (t, *J* = 2.0 Hz, 1H), 7.05 (ddd, *J* = 8.2, 6.9, 1.4 Hz, 1H), 6.96 (ddd, *J* = 7.9, 6.9, 1.0 Hz, 1H), 4.61 – 4.43 (m, 1H), 4.40 – 4.26 (m, 1H), 3.23 – 2.97 (m, 3H), 2.88 (ddd, *J* = 14.5, 9.2, 5.4 Hz, 1H), 1.94 – 1.84 (m, 1H), 1.83 – 1.68 (m, 5H), 1.69 – 1.59 (m, 1H), 1.43 – 1.08 (m, 2H).

^{13}C (126 MHz, DMSO-d_6) δ 174.0, 170.6, 169.2, 136.0, 127.3, 123.5, 120.8, 118.3, 118.2, 111.3, 110.3, 53.7, 51.4, 40.6, 31.0, 28.8, 27.6, 27.5, 22.5. HRMS (TOF, ESI+) *m/z* calcd. for $C_{19}H_{25}N_4O_3^+$ $[M+H]^+$ 357.1921, found 357.1922. HRMS (TOF, ESI+) *m/z* calcd. for $C_{19}H_{24}N_4O_3Na^+$ $[M+Na]^+$ 379.1741, found 379.1741.

Ac-Trp-D-Orn(-NHCO-) (**7**)

Ac-Trp(Boc)-D-Orn(Boc)-HMBA-PEGA$_{800}$ was synthesized by general procedure A using PL-PEGA$_{800}$ (250 mg dry resin, 0.1 mmol). Ac-Trp-D-Orn(-NHCO-) (**7**) was synthesized from Ac-Trp(Boc)-D-Orn(Boc)-HMBA-PEGA$_{800}$ (104.6 mg) dry resin via General procedure B. The product (**7**) was isolated in a yield of $(\frac{6.9\times10^{-3}\text{g}}{342.39900\text{ g/mol}})/(0.209\times10^{-3}\text{mol/g}\times 0.1059\text{ g}) = 91\ \%$.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.76 (d, *J* = 2.4 Hz, 1H), 8.18 (d, *J* = 7.8 Hz, 1H), 8.04 (d, *J* = 8.5 Hz, 1H), 7.69 – 7.49 (m, 2H), 7.39 – 7.22 (m, 1H), 7.13 (d, *J* = 2.4 Hz, 1H), 7.04 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H), 6.95 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H), 4.57 (td, *J* = 8.6, 5.6 Hz, 1H), 4.09 (ddd, *J* = 10.4, 7.7, 5.9 Hz, 1H), 3.18 – 3.00 (m, 3H), 2.86 (dd, *J* = 14.5, 8.7 Hz, 1H), 1.88 – 1.73 (m, 4H), 1.73 – 1.58 (m, 2H), 1.42 (dtd, *J* = 12.6, 10.4, 4.5 Hz, 1H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 171.2, 169.5, 168.9, 136.0, 127.4, 123.5, 120.7, 118.5, 118.1, 111.2, 110.2, 53.2, 48.9, 41.0, 28.3, 27.4, 22.6, 20.6. HRMS (TOF, ESI+) *m/z* calcd for $C_{18}H_{23}N_4O_3^+$ $[M+H]^+$ 343.1765, found 343.1767. *m/z*calcd. for $C_{18}H_{22}N_4NaO_3^+$ $[M+Na]^+$ 365.1584, found 365.1586.

Solid-phase synthesis of ciliatamide A (**10**)

The precursor peptide for ciliatamide A (**8**) was synthesized by general procedure A using dry resin (654.6 mg). Resin loading was found to be 0.218 mmol/g. Ciliatamide A (**10**) was synthesizes from the precursor peptide **8** via General procedure C. Ciliatamide A (**10**) was isolated as a colorless gel in a yield of $(\frac{1.6\times10^{-3}\text{g}}{441.616\ \text{g/mol}})/(0.218 \times 10^{-3}\text{mol/g} \times 0.6546\ \text{g}) = 3\ \%$.

^{1}H NMR (500 MHz, Methanol-d_4) δ 7.86 (m, NH), 7.61 (m, NH), 7.37 – 7.13 (m, 5H), 5.83 (ddt, *J* = 14.3, 10.4, 7.6 Hz, 1H), 5.40 (dq, *J* = 10.6, 5.2 Hz, 1H), 5.00 (dd, *J* = 17.0, 2.0 Hz, 1H), 4.68 – 4.51 (m, 1H), 3.14 – 3.01 (m, 1H), 3.01 – 2.89 (m, 3H), 2.36 – 2.16 (m, 2H), 2.11 – 1.93 (m, 4H), 1.92 – 1.77 (m, 2H), 1.76 – 1.02 (m, 12H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 176.9, 176.7, 171.6, 140.1, 138.8, 130.3, 130.0, 129.7, 129.5, 127.7, 114.7, 59.7, 53.5, 42.5, 35.1, 34.9, 34.4, 33.1, 32.3, 30.4, 30.3, 30.2, 30.1, 29.9, 29.1, 26.1. TOF MS (ESI+) *m/z* calcd.for $C_{26}H_{40}N_3O_3^+$ $[M+H]^+$ 442.3064, found 442.3064. TOF MS (ESI+) *m/z* calcd.for $C_{26}H_{39}N_3O_3Na^+$ $[M+Na]^+$ 464.2884, found 464.2883.

Solid-phase synthesis of ciliatamide C (**11**)

The precursor peptide for ciliatamide C (**9**) was synthesized by general procedure A using dry resin (276.5 mg). Resin loading was found to be 0.231 mmol/g. Ciliatamide C (**11**) was synthesizes from the precursor peptide **9** via General procedure B. Ciliatamide C (**11**) was isolated as a colorless gel in a yield of$(\frac{24.7\times10^{-3}\text{g}}{427.589\text{g/mol}})/(0.231 \times 10^{-3}\text{mol/g} \times 0.2765\text{g}) = 90\ \%$.

^{1}H NMR (500 MHz, acetonitrile-d_3) δ 7.30 – 7.14 (m, 5H), 6.84 (d, *J* = 7.5 Hz, 1H), 6.50 (s, 1H), 5.84 (ddt, *J* = 17.0, 10.2, 6.7 Hz, 1H), 5.37 (dd, *J* = 10.8, 5.3 Hz, 1H), 5.00 (dt, *J* = 17.2, 1.9 Hz, 1H), 4.93 (ddt, *J* = 10.2, 2.4, 1.3 Hz, 1H), 4.23 – 4.16 (m, 1H), 3.24 (m, 3H), 2.90 (dd, *J* = 14.6, 10.8 Hz, 1H), 2.82 (s, 2H), 2.79 (s, 1H), 2.28 – 2.07 (m, 2H), 2.07 – 1.98 (m, 2H), 1.84 (ddq, *J* = 15.5, 7.6, 4.3 Hz, 2H), 1.78 – 1.57 (m, 1H), 1.34 (qt, *J* = 7.6, 3.9 Hz, 4H), 1.29 – 1.17 (m, 3H), 1.17 – 1.08 (m, 2H), 1.08 – 0.96 (m, 1H). ^{13}C NMR (126 MHz, acetonitrile-d_3) δ 175.1, 171.9, 171.1, 140.2, 139.2, 130.2, 129.9, 129.4, 129.2, 127.2, 114.7, 58.5, 50.6, 42.4, 34.4, 34.4, 33.9, 32.2, 29.9, 29.8, 29.7, 29.6, 28.3, 25.6, 22.0. TOF MS (ESI+) *m/z*calcd.for$C_{25}H_{38}N_3O_3^+$ $[M+H]^+$ 428.2908, found 428.2922. *m/z* calcd. for$C_{25}H_{37}N_3NaO_3^+$ $[M+Na]^+$ 450.2727, found 450.2745.

Synthesis and test-lactamization of Ac-Trp-L-Dab(N_3) and Ac-Trp-L-Orn(N_3)

The azido peptides were synthesized by general procedure A. 8.0 mg resin aliquots were used for test-lactamization. The resin aliquots were washed with 3 × DMF and 3 × CH_2Cl_2 prior to treatment with reagent B. After treatment with reagent B and washing, test-lactamization was conducted using the respective conditions: in 200 µL MeCN at 60 °C for 1 h; in 200 µL 0.75 M DTT in MeCN at 60 °C for 1 h; in 200 µL 0.75 M DIPEA in MeCN at 60 °C for 1 h; in 200 µL 0.5 M DTT/0.5 M DIPEA in MeCN at 60 °C for 1 h; and in 200 µL 0.75 M DTT/0.75 M DIPEA in MeCN at 60 °C for 1 h. After reaction, the MeCN phase was isolated and diluted to 1 mL using 25 % MeCN/H_2O with 0.1 % TFA. The diluted MeCN phases were then used for HPLC-UV-analysis. The resin was washed with 3 × MeCN and 3 × H_2O, and 100 µL 0.1 M NaOH in water was added subsequently. After

2 h, 100 µL 0.1 M HCl in water was added to neutralize the solution, and the aqueous phase was isolated. Any remaining, non-lactamized peptide was extracted from the resin using 2 × 125 µL 5:5 MeCN/H_2O with 0.1 % TFA, 250 µL 9:1 MeCN/H_2O, and 300 µL water with 0.1 % TFA; all extracts were combined with the original neutralized aqueous phase to a total volume of 1 mL, and the sample was used for HPLC-UV-analysis.

Ac-HfRW-L-Dab with *C*-terminal *γ*-lactam (**12**)

The peptide was synthesized by general procedure A using dry resin (223.9 mg). Resin loading was found to be 0.217 mmol/g. The lactamized product **12** was synthesized by General procedure B. The yield of **12** was $(\frac{19.9 \times 10^{-3}\text{g}}{768.880\ \text{g/mol}})/(0.217 \times 10^{-3}\text{mol/g} \times 0.2239\ \text{g}) = 53\ \%$. TOF MS (ESI+) *m/z* calcd.for $C_{38}H_{49}N_{12}O_6^+$ $[M+H]^+$ 769.3893, found 769.3905. *m/z* calcd. for $C_{38}H_{48}N_{12}NaO_6^+$ $[M+Na]^+$ 791.3712, found 791.3728. *m/z* calcd. for $C_{38}H_{50}N_{12}O_6^{2+}$ $[M+2H]^{2+}$ 385.1983, found 385.1989.

Ac-HfRW-D-Orn with *C*-terminal *δ*-lactam (**13**)

The peptide was synthesized by general procedure A using dry resin (208.1 mg). Resin loading was found to be 0.242 mmol/g. The lactamized product **13** was synthesized by General procedure B. The yield of **13** was $(\frac{25.7 \times 10^{-3}\text{g}}{782.9070\ \text{g/mol}})/(0.242 \times 10^{-3}\text{mol/g} \times 0.2081\ \text{g}) = 65\ \%$**.** TOF MS (ESI+) *m/z* calcd.for $C_{39}H_{51}N_{12}O_6^+$ $[M+H]^+$ 783.4049, found 783.4058. *m/z* calcd. for $C_{39}H_{50}N_{12}NaO_6^+$ $[M+Na]^+$ 805.3869, found 805.3884. *m/z* calcd. for $C_{39}H_{52}N_{12}O_6^{2+}$ $[M+2H]^{2+}$ 392.2061, found 392.2068.

Oxytocin with *C*-terminal *γ*-lactam (**14**)

The linear peptide (CYIQNCP-L-Dab) was synthesized by general procedure A using dry resin (245.7 mg). The side chain deprotection and the lactamization were obtained by general procedure B. Half of the resulting raw peptide was transferred to a round-bottomed flask and oxidized using 20 % DMSO in H_2O (100 mL) with air gently bubbled through the solution under stirring for 18 h at r.t. AcOH (200 µL) was added to acidify the solution, which was subsequently freeze-dried, and the solid residue was purified using a semipreparative RP-HPLC, yielding 7.1 mg of pure, cyclized peptide **7**, corresponding to a yield of $(\frac{7.1 \times 10^{-3}\text{g}}{1033.231\ \text{g/mol}})/(0.232 \times 10^{-3}\text{mol/g} \times 0.2457\ \text{g}) \times \frac{1}{2} = 23\ \%$. TOF MS (ESI+) *m/z* calcd.for $C_{45}H_{69}N_{12}O_{12}S_2^+$ $[M+H]^+$ 1033.4594, found 1033.4575. *m/z*calcd. for $C_{45}H_{70}N_{12}O_{12}S_2^{2+}$ $[M+2H]^{2+}$ 517.2333, found 517.2329.

Desmopressin with *C*-terminal *γ*-lactam (**15**)

The peptide was synthesized as by the same method as for the oxytocin derivative **15** using dry resin (329.7 mg). The yield was $(\frac{19.6 \times 10^{-3}\text{g}}{1095.262\ \text{g/mol}})/(0.232 \times 10^{-3}\text{mol/g} \times 0.3297\ \text{g}) \times \frac{1}{2} = 44\ \%$.

TOF MS (ESI+) *m/z* calcd.for $C_{48}H_{67}N_{14}O_{12}S_2^+$ $[M+H]^+$ 1095.4499, found 1095.4510. *m/z* calcd. for $C_{48}H_{68}N_{14}O_{12}S_2^+$ $[M+2H]^{2+}$ 548.2286, found 548.2285. *m/z* calcd. for $C_{48}H_{67}N_{14}NaO_{12}S_2^{2+}$ $[M+H+Na]^{2+}$ 559.2196, found 559.2196.

GFP reporter gene assay[1]

Cells housing a GFP reporter gene sensitive to melanocortin receptor activity were seeded in a 96 well fluorescence-compatible plate (50,000 cells per well). Cells were grown to 70 % confluency and then given fresh growth media plus or minus agonist/compound or vehicle (DMSO). Cells were then incubated overnight for 18 hours, washed with a buffered Saline solution, and the fluorescence of the reporter gene was measured using a i3x platereader/minimax 300 cytometer.

The images (brightfield and fluorescence) were collected and submitted to the image analysis program we use to normalize our fluorescence values to cellular content/coverage.

To characterize the peptides, a 7-point dose-response curve was drawn for all. The range is from 10 nM at the highest to 50 pM at the lowest using serial dilution of the most concentrated ligand solution. A standard reference molecule, alpha-MSH, was also characterized in parallel on each plate.

Plasma stability studies[2]

Human plasma serum (Sigma) was stored at -80 °C and thawed on ice prior to the experiment. Neat plasma (200 uL) was spiked with test compound (1.00 uL, 1.00 mM in DMSO) and incubated at 37 °C in a circulating water bath. Aliquots (20.0 uL) were taken at 0, 5, 15, 60 and 90 min and added to 9:1 MeCN/water (80.0 uL), the mixture was vortexed immediately and placed on crushed ice. The cold mixtures were sonicated for 1 min and centrifuged at 7,000 RPM for 3 min. The supernatant was collected and analyzed by LC-MS/MS for the presence of test compound.

Oxytocin binding assay

Oxytocin *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit (ab133050, Abcam) was applied for assaying the binding of compound **14** towards polyclonal rabbit antibody specific to oxytocin. The assay was conducted in triplicates for compound **14** and duplicates for the oxytocin standard. Initially, the compound **14** and the oxytocin standard, respectively, were dissolved in the assay buffer at 0.10 mg/ml. The stock solutions was diluted to 1000, 750, 500, 250, 125, 62.5 and 31.25 pg/ml. The assaying was conducted according to the kit protocol. The assay data was obtained by measuring absorbance at 405 nm using a Spectramax i3x (Molecular Devices) plate reader. The binding was determined by plotting the data in GraphPad Prism 6 and applying a semi logarithmic best fit curve. The R^2-value of the curve fit was 0.996 for the oxytocin standard and the 50 % response dose was determined to be 336 pg/ml (reported value 228 pg/ml). The R^2-value of the curve fit was 0.980 for compound **14** and the 50 % response dose was determined to be 640 pg/ml.

Test-lactamization of Ac-Trp-L-Dab

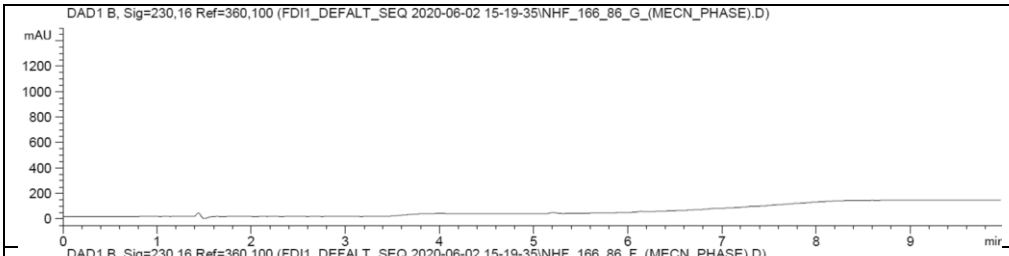
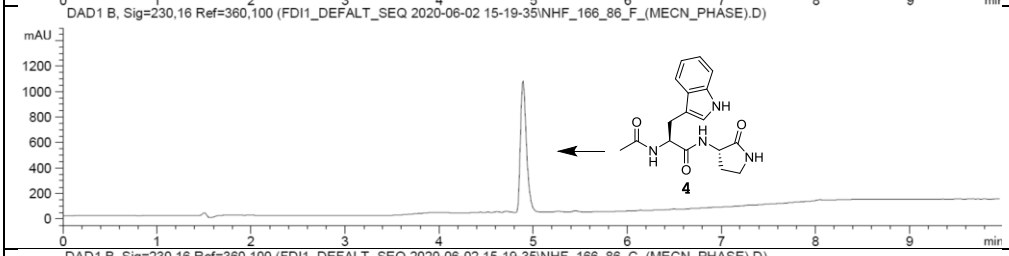
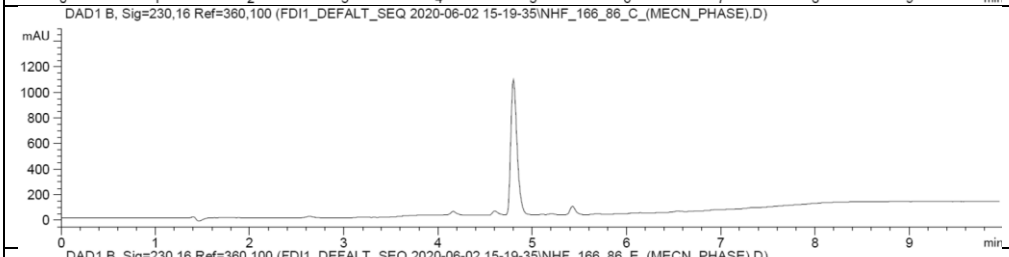
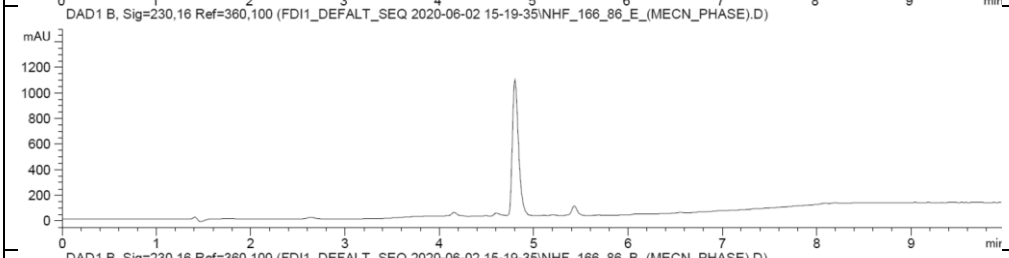
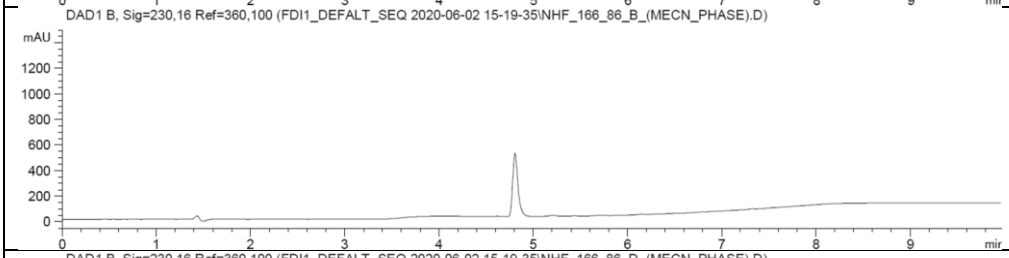
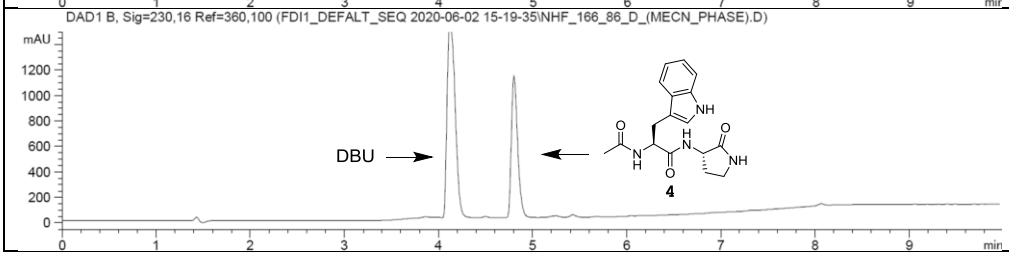
	**MeCN, 1 h, r.t.**
	**10 % Et_3N in MeCN, 1 h, r.t.**
	**17 % DIPEA in MeCN, 1 h, r.t.**
	**17 % DIPEA in MeCN, 2 h, 60 °C**
	**2 % DIPEA in MeCN, 1 h, r.t.**
	**2 % DBU in MeCN, 1 h, r.t.**

Table S 1. UV traces of diluted MeCN phases containing Ac-Trp-L-Dab cleaved by lactamization, recorded at 230 nm. The MeCN phases (100 µL) were diluted to 1 mL using 25 % MeCN/H_2O with 0.1 % TFA.

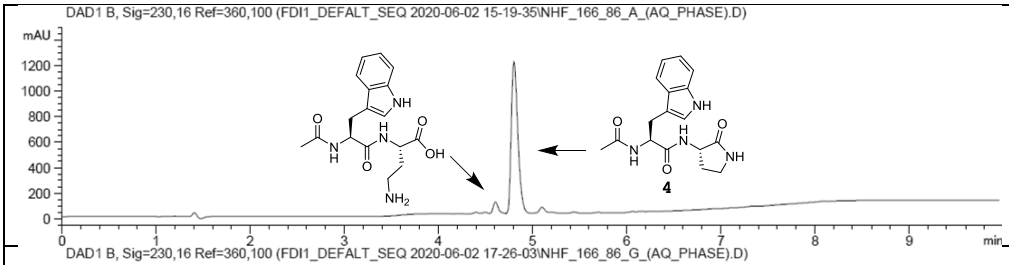
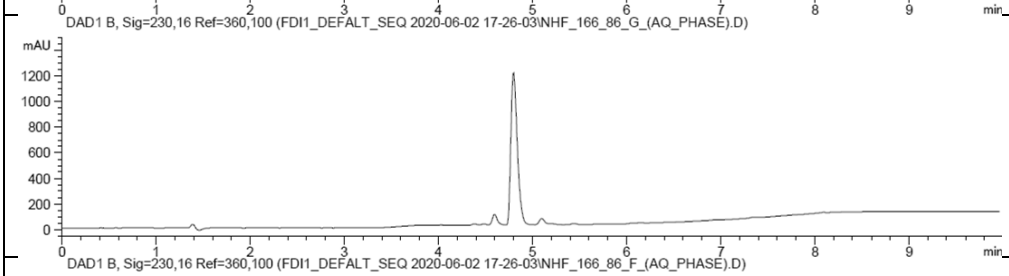
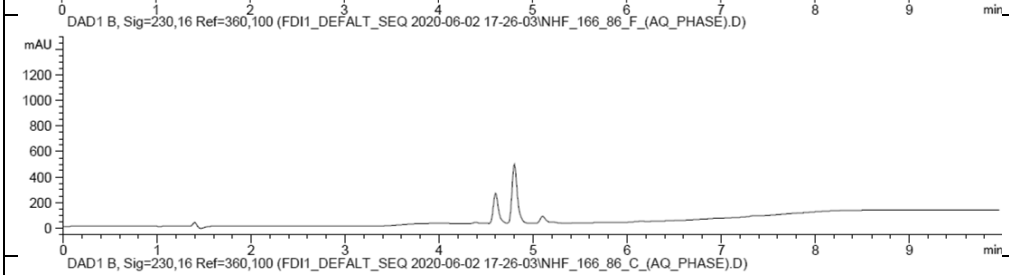
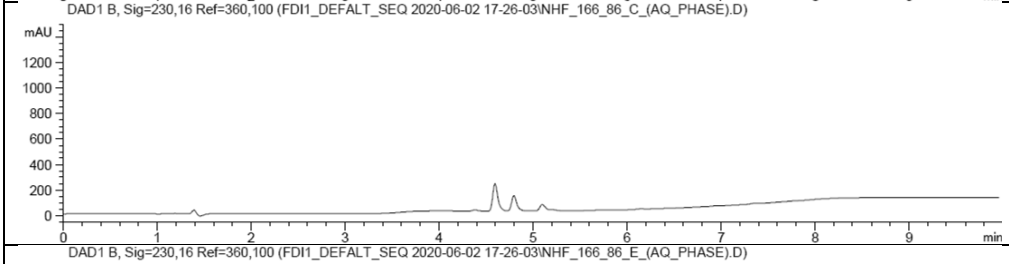
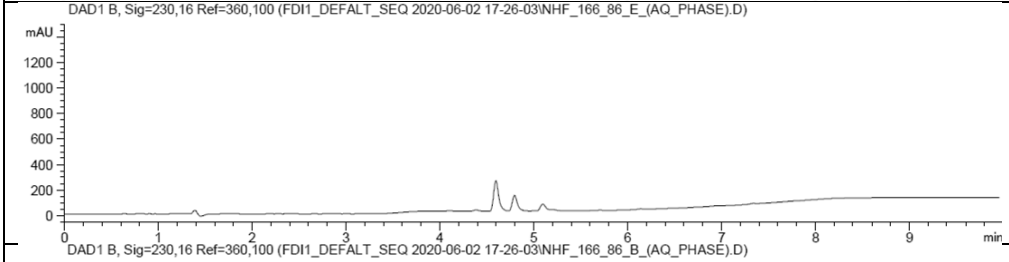
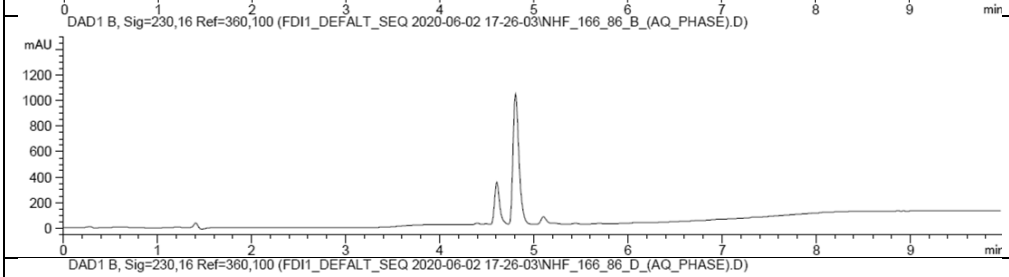
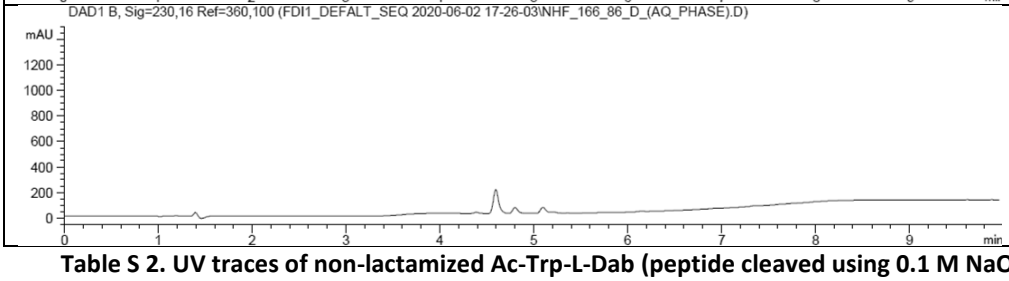
	**N/A**
	**MeCN, 1 h, r.t.**
	**10 % Et_3N in MeCN, 1 h, r.t.**
	**17 % DIPEA in MeCN, 1 h, r.t.**
	**17 % DIPEA in MeCN, 2 h, 60 °C**
	**2 % DIPEA in MeCN, 1 h, r.t.**
	**2 % DBU in MeCN, 1 h, r.t.**

Table S 2. UV traces of non-lactamized Ac-Trp-L-Dab (peptide cleaved using 0.1 M NaOH (aq) after the treatment specified in the right column) recorded at 230 nm.

Test-lactamization of Ac-Trp-L-Orn

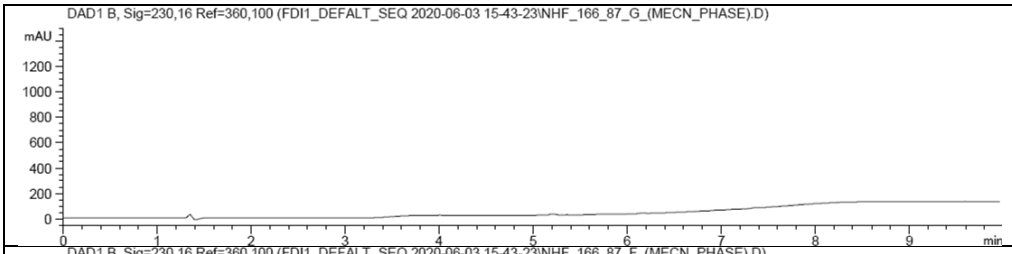
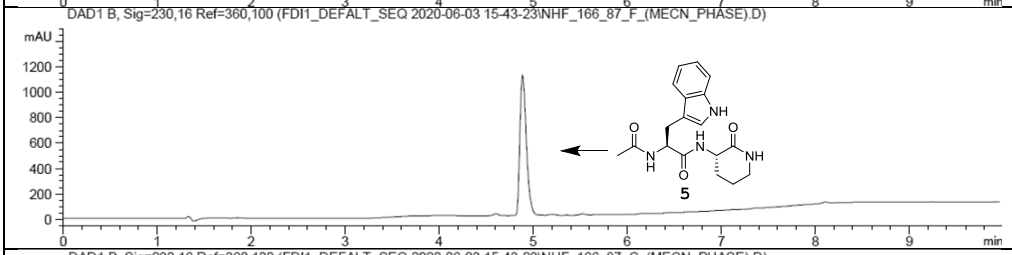
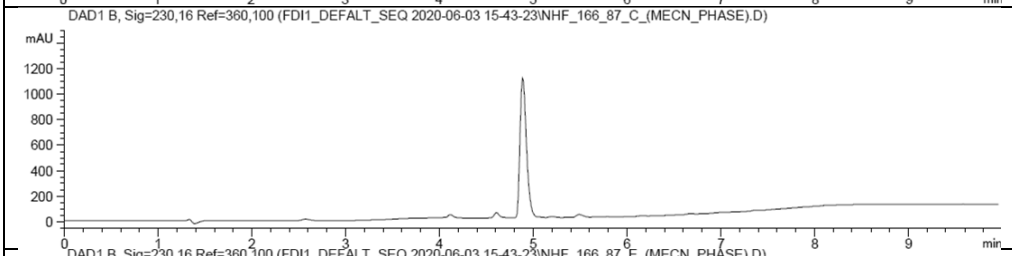
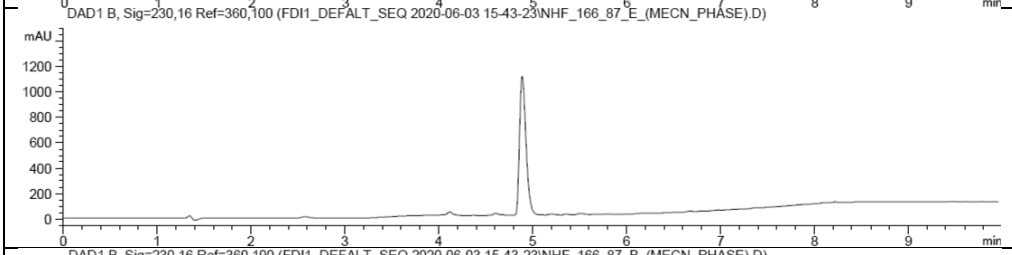
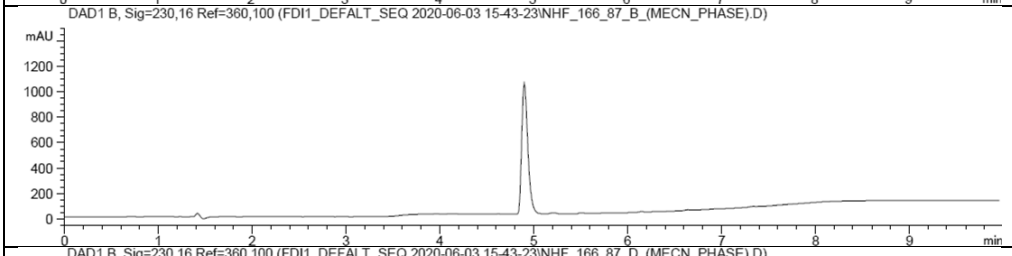
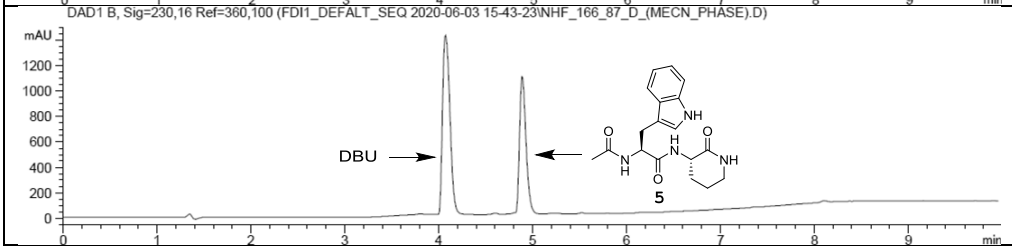
	**MeCN, 1 h, r.t.**
	**10 % Et_3N in MeCN, 1 h, r.t.**
	**17 % DIPEA in MeCN, 1 h, r.t.**
	**17 % DIPEA in MeCN, 2 h, 60 °C**
	**2 % DIPEA in MeCN, 1 h, r.t.**
	**2 % DBU in MeCN, 1 h, r.t.**

Table S 3. UV traces of diluted MeCN phases containing Ac-Trp-L-Orn cleaved by lactamization, recorded at 230 nm. The MeCN phases (100 µL) were diluted to 1 mL using 25 % MeCN/H_2O with 0.1 % TFA.

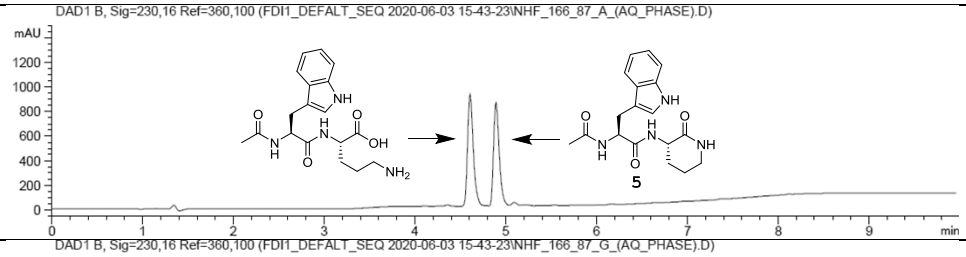
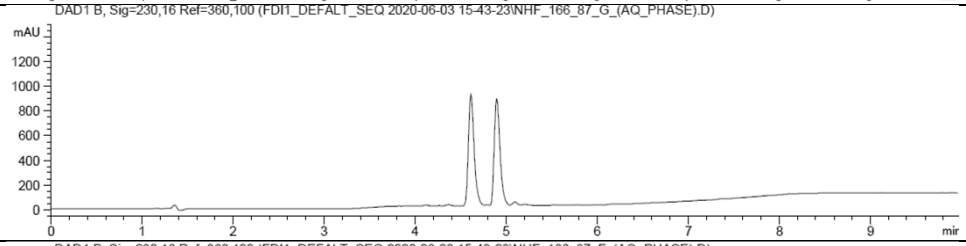
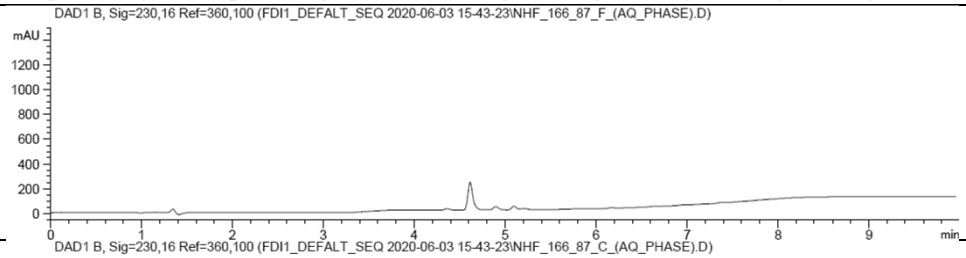
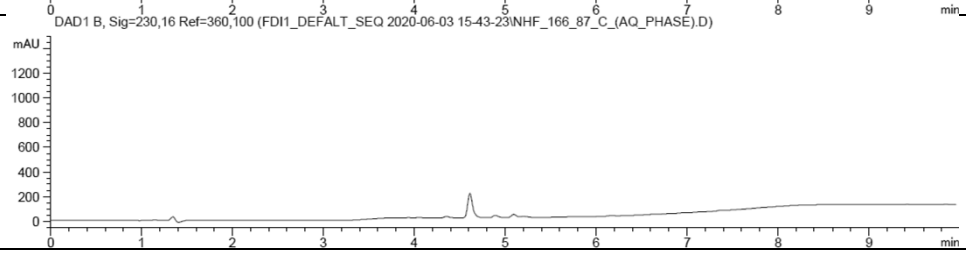
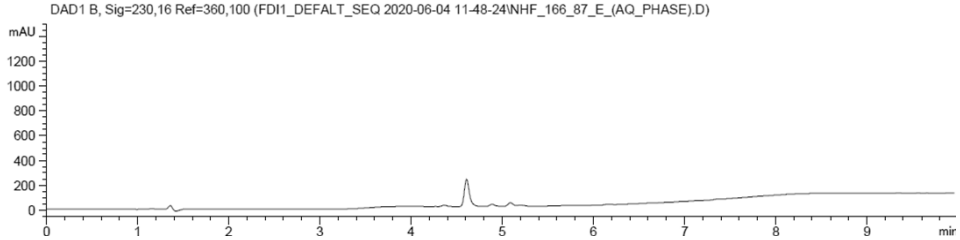
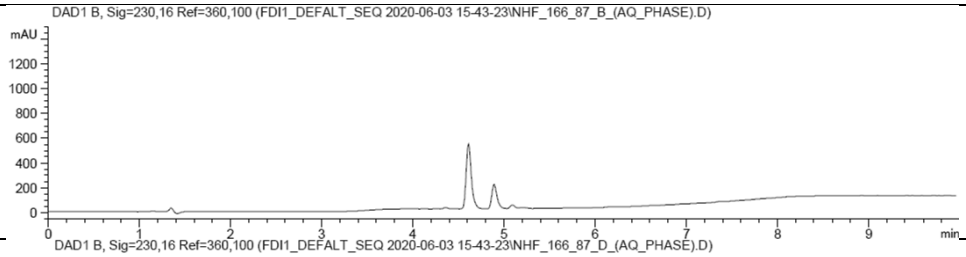
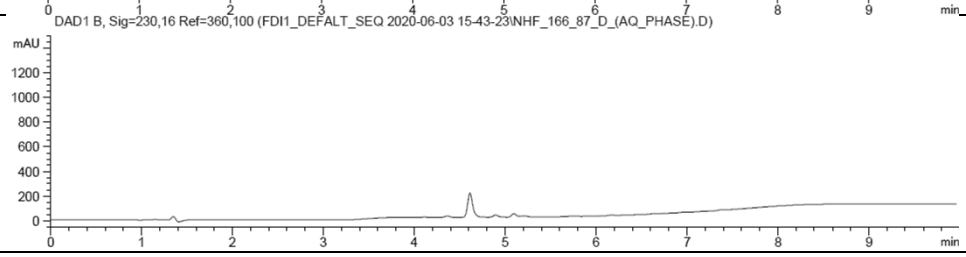
	**N/A**
	**MeCN, 1 h, r.t.**
	**10 % Et_3N in MeCN, 1 h, r.t.**
	**17 % DIPEA in MeCN, 1 h, r.t.**
	**17 % DIPEA in MeCN, 2 h, 60 °C**
	**2 % DIPEA in MeCN, 1 h, r.t.**
	**2 % DBU in MeCN, 1 h, r.t.**

Table S 4. UV traces of non-lactamized Ac-Trp-L-Orn (peptide cleaved using 0.1 M NaOH (aq) after the treatment specified in the right column) recorded at 230 nm.

Test-lactamization of Ac-Trp-L-Lys

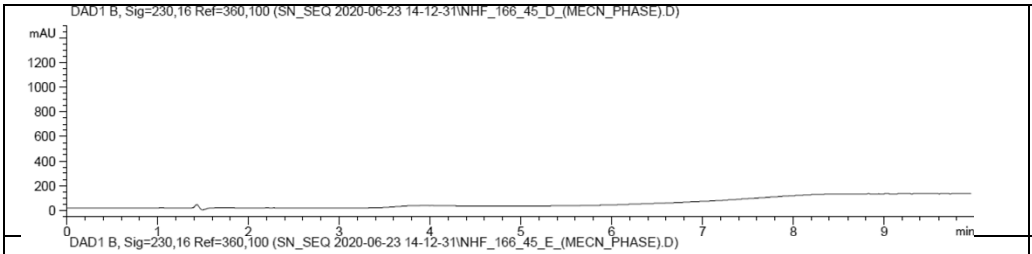
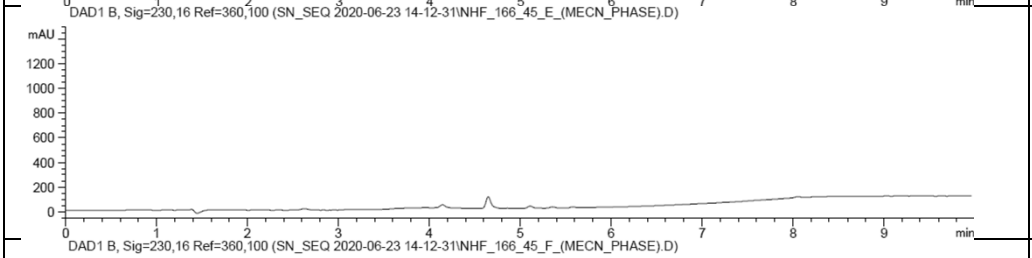
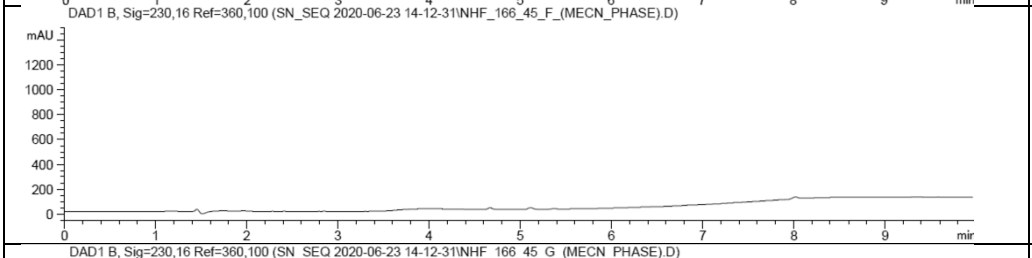
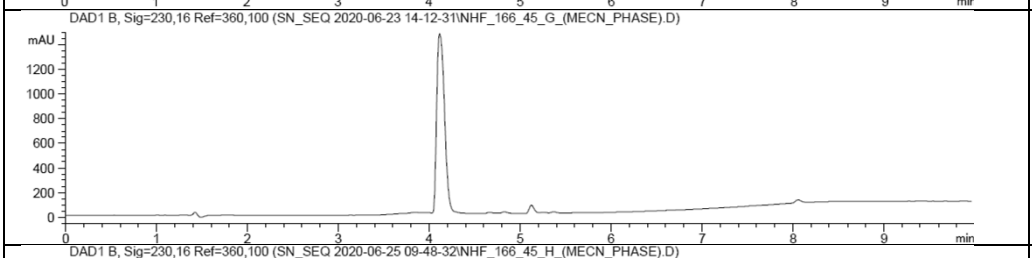
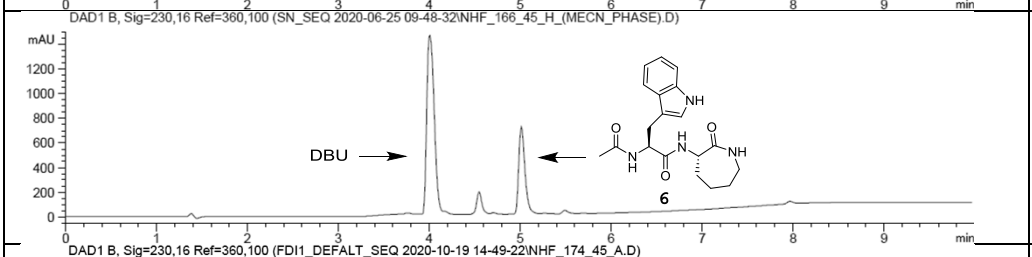
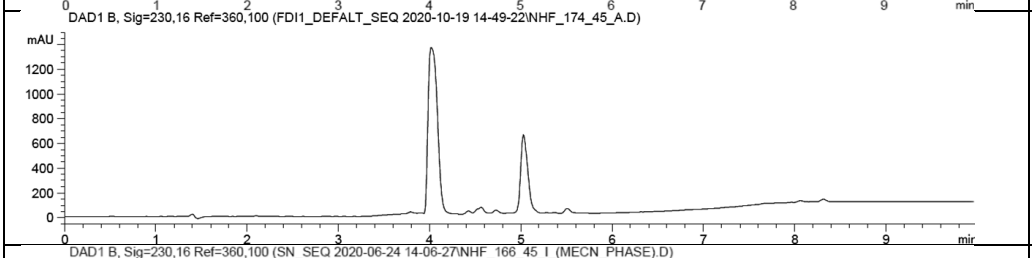
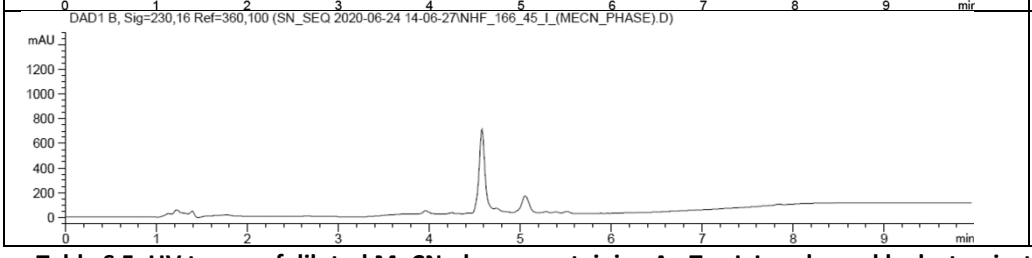
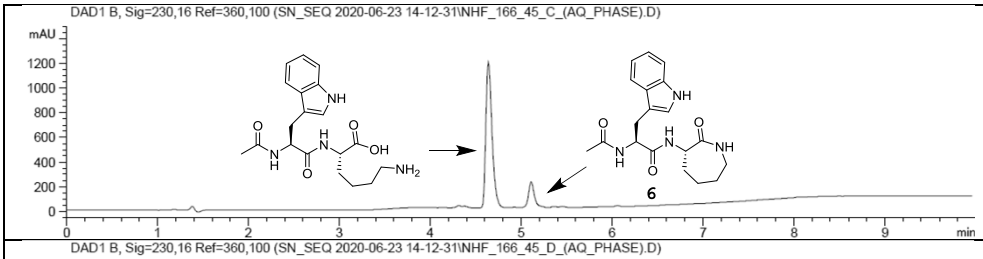
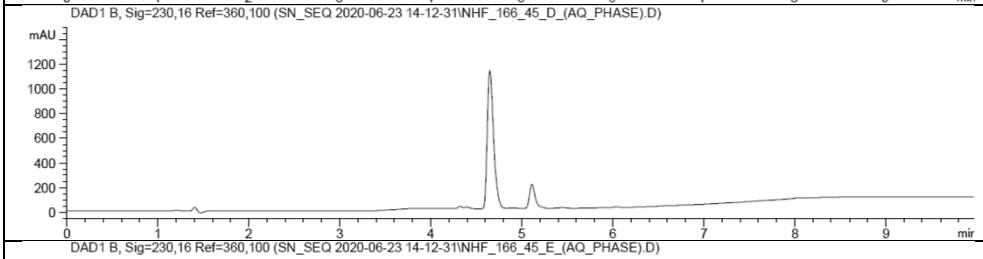
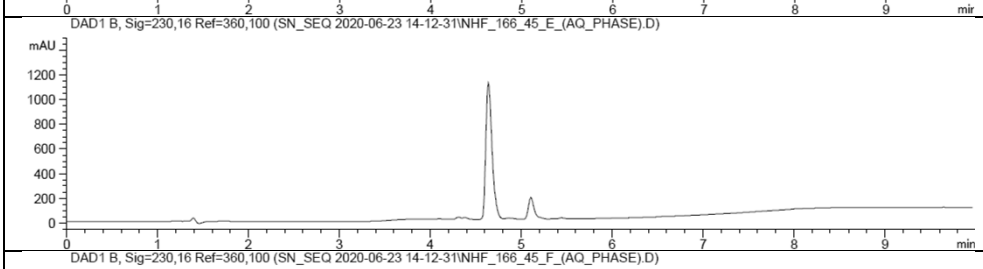
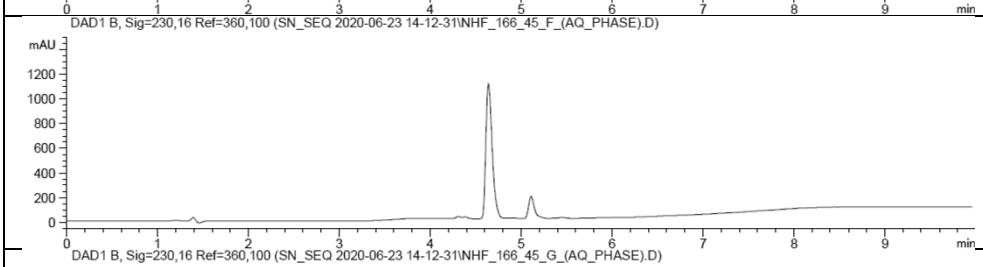
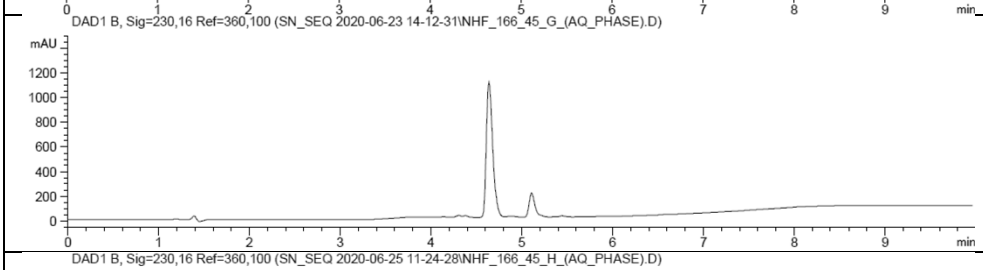
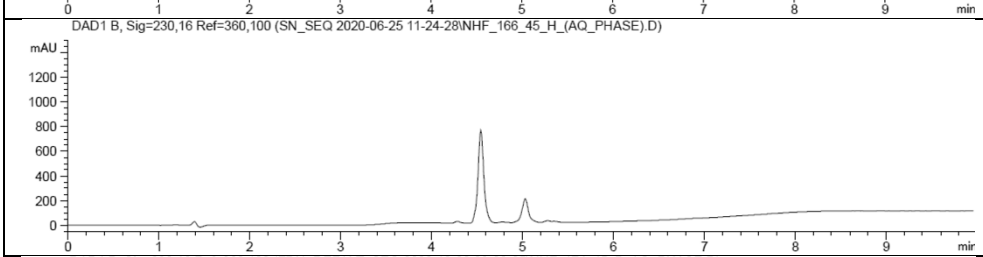
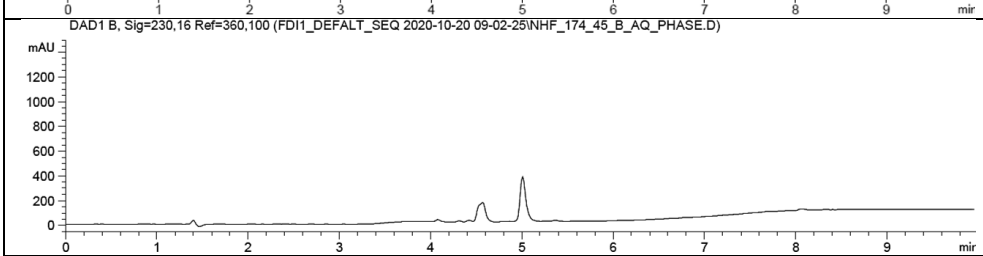
	**MeCN, 1 h, r.t.**
	**1 M DIPEA in MeCN, 1 h, r.t.**
	**10 % Et_3N in MeCN, 1 h, r.t.**
	**2 % DBU in MeCN, 1 h, r.t.**
	**2 % DBU in MeCN, 20 h, r.t.**
	**2 % DBU in MeCN, 20 h, 60 °C**
	**1 M KHMDS in THF, 1 h, r.t.**

Table S 5. UV traces of diluted MeCN phases containing Ac-Trp-L-Lys cleaved by lactamization, recorded at 230 nm. The MeCN phases (100 µL) were diluted to 1 mL using 25 % MeCN/H_2O with 0.1 % TFA.

	**N/A**
	**MeCN, 1 h, r.t.**
	**1 M DIPEA in MeCN, 1 h, r.t.**
	**10 % Et_3N in MeCN, 1 h, r.t.**
	**2 % DBU in MeCN, 1 h, r.t.**
	**2 % DBU in MeCN, 20 h, r.t.**
	**2 % DBU in MeCN, 20 h, 60 °C**

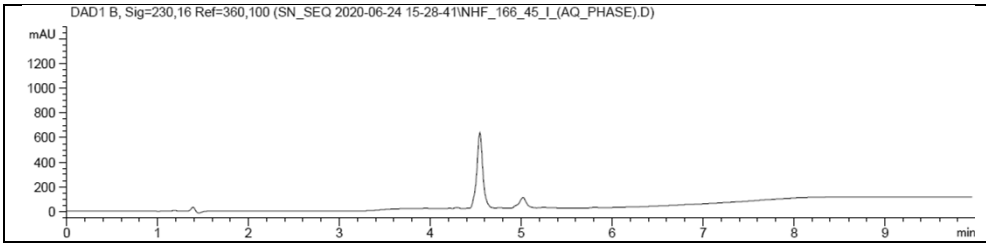
	**1 M KHMDS in THF, 1 h, r.t.**

Table S 6. UV traces of non-lactamized Ac-Trp-L-Lys (peptide cleaved using 0.1 M NaOH (aq) after the treatment specified in the right column) recorded at 230 nm.

Synthesis and test-lactamization of Ac-Trp-L-Dab(N_3)

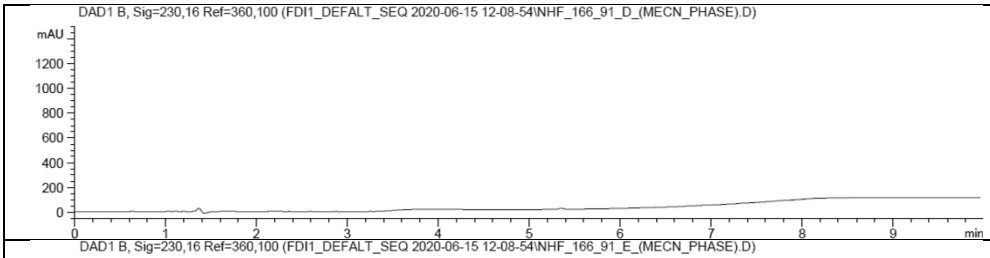
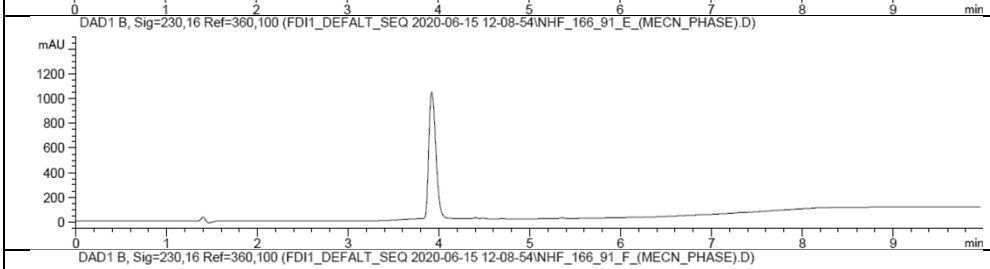
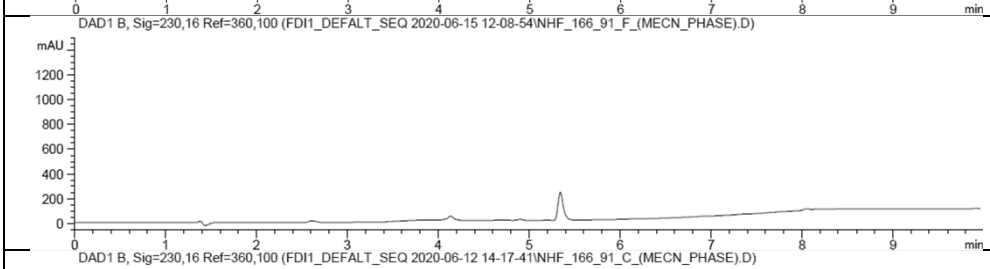
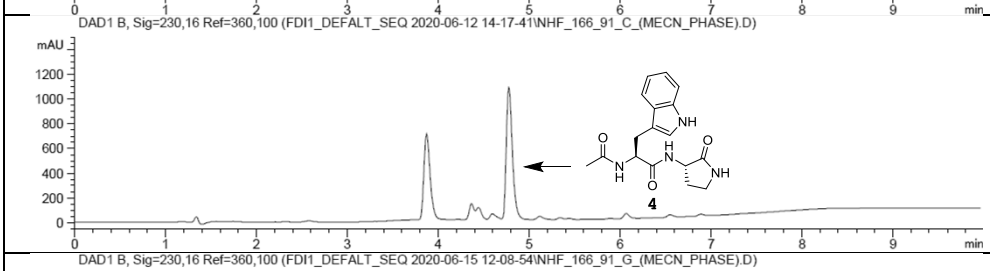
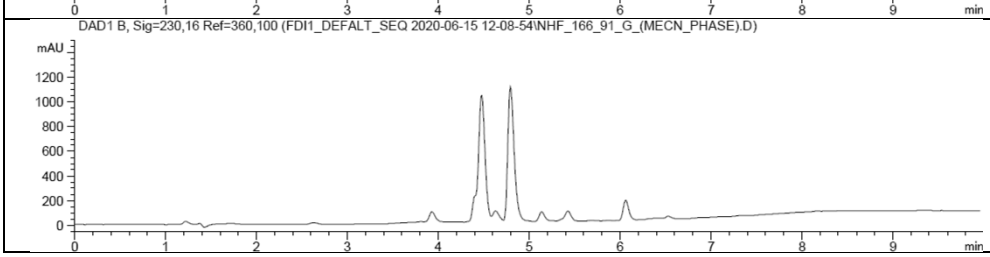
	**MeCN, 60 °C, 1 h**
	**15 % w/w DTT in MeCN, 60 °C, 1 h**
	**13 % DIPEA in MeCN, 60 °C, 1 h**
	**10 % w/w DTT, 13 % DIPEA in MeCN, 60 °C, 1 h**
	**15 % w/w DTT, 13 % DIPEA in MeCN, 60 °C, 1 h**

Table S 7. UV traces of diluted MeCN phases containing Ac-Trp-L-Dab(N_3) cleaved by reduction andlactamization, recorded at 230 nm. The MeCN phases (100 µL) were diluted to 1 mL using 25 % MeCN/H_2O with 0.1 % TFA.

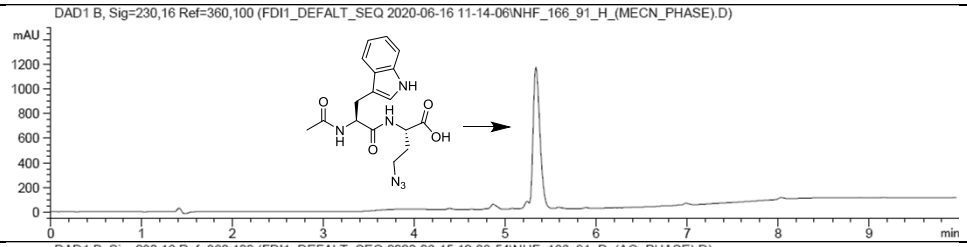
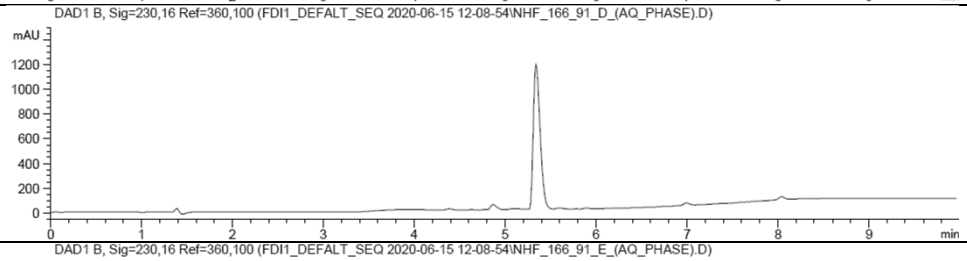
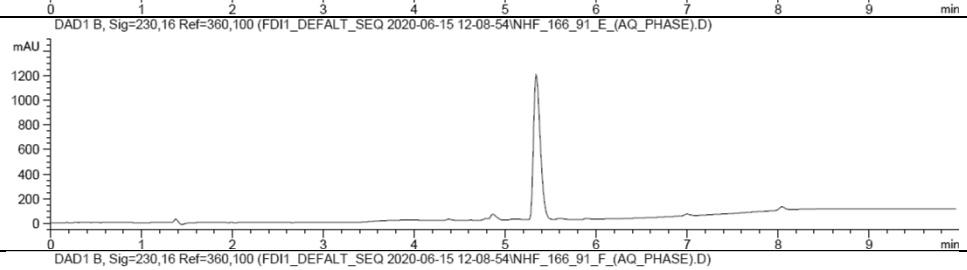
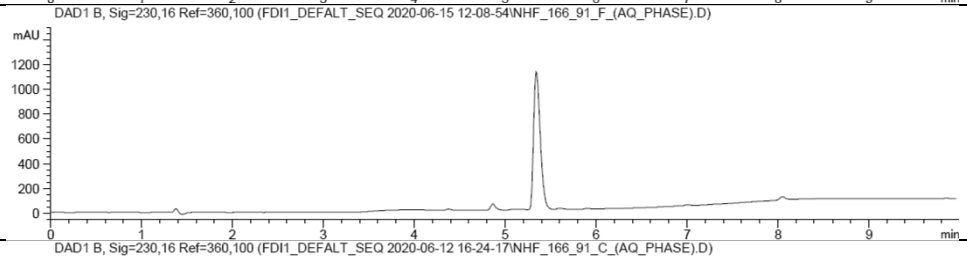
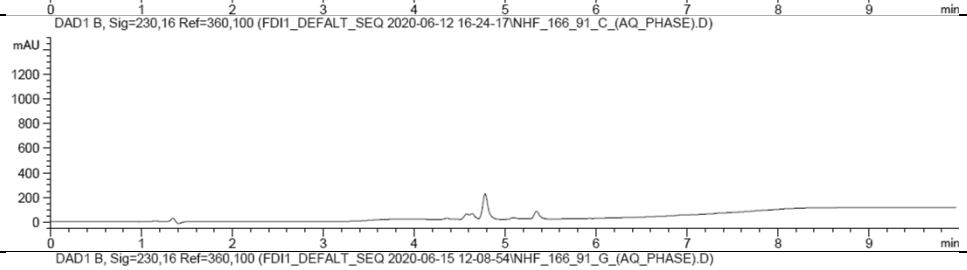
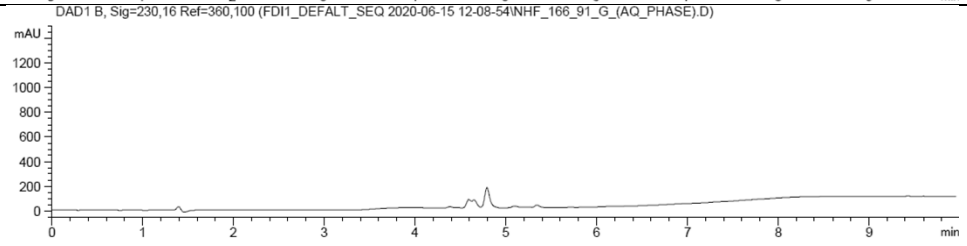
	**N/A**
	**MeCN, 60 °C, 1 h**
	**15 % w/w DTT in MeCN, 60 °C, 1 h**
	**13 % DIPEA in MeCN, 60 °C, 1 h**
	**10 % w/w DTT, 13 % DIPEA in MeCN, 60 °C, 1 h**
	**15 % w/w DTT, 13 % DIPEA in MeCN, 60 °C, 1 h**

Table S 8. UV traces of non-lactamized Ac-Trp-L-Dab(N_3) (peptide cleaved using 0.1 M NaOH (aq) after the treatment specified in the right column) recorded at 230 nm.

Synthesis and test-lactamization of Ac-Trp-L-Orn(N_3)

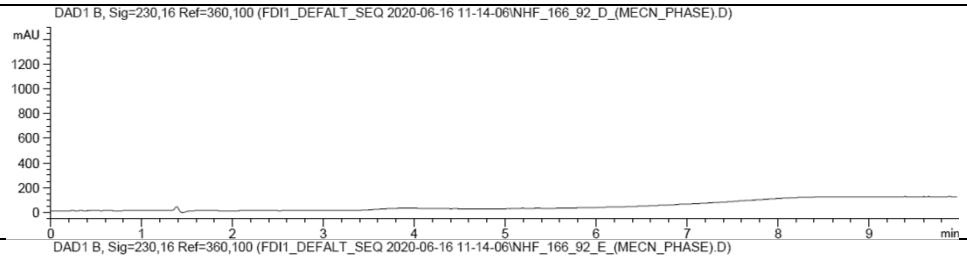
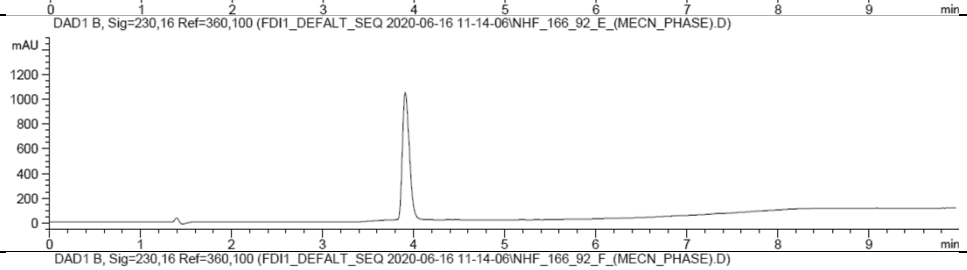
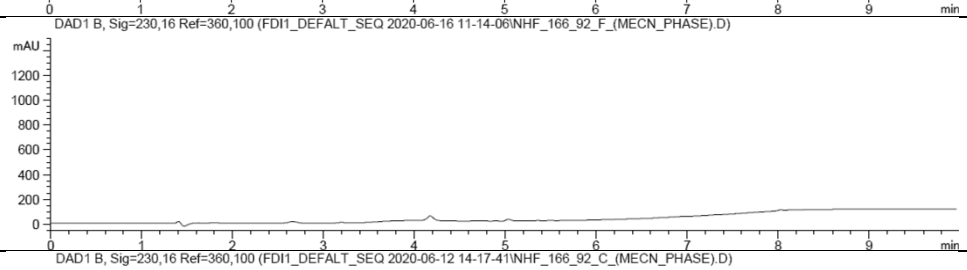
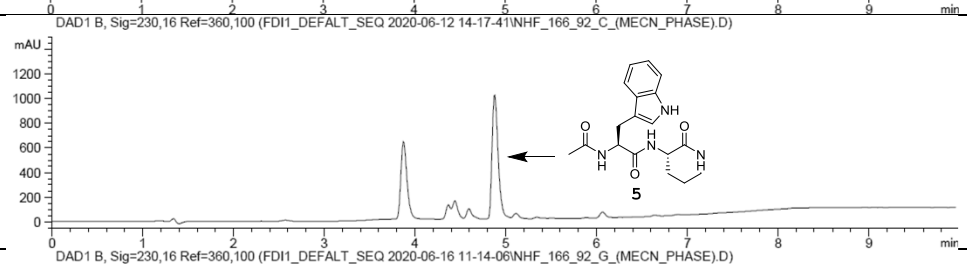
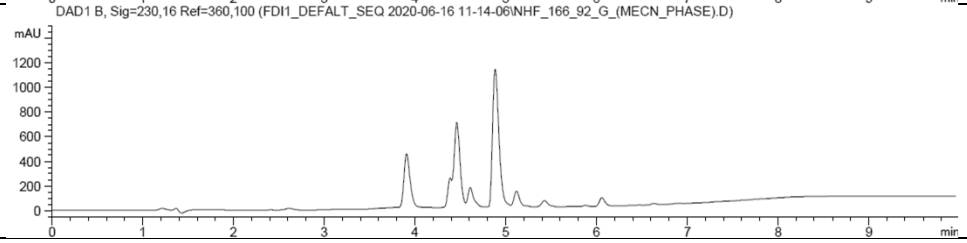
	**MeCN, 60 °C, 1 h**
	**15 % w/w DTT in MeCN, 60 °C, 1 h**
	**13 % DIPEA in MeCN, 60 °C, 1 h**
	**10 % w/w DTT, 13 % DIPEA in MeCN, 60 °C, 1 h**
	**15 % w/w DTT, 13 % DIPEA in MeCN, 60 °C, 1 h**

Table S 9. UV traces of diluted MeCN phases containing Ac-Trp-L-Orn(N_3) cleaved by reduction andlactamization, recorded at 230 nm. The MeCN phases (100 µL) were diluted to 1 mL using 25 % MeCN/H_2O with 0.1 % TFA.

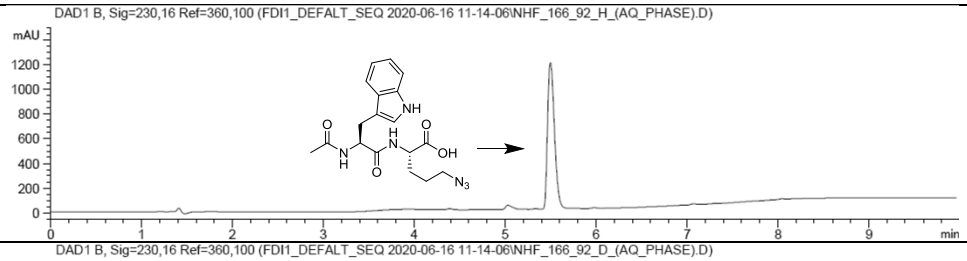
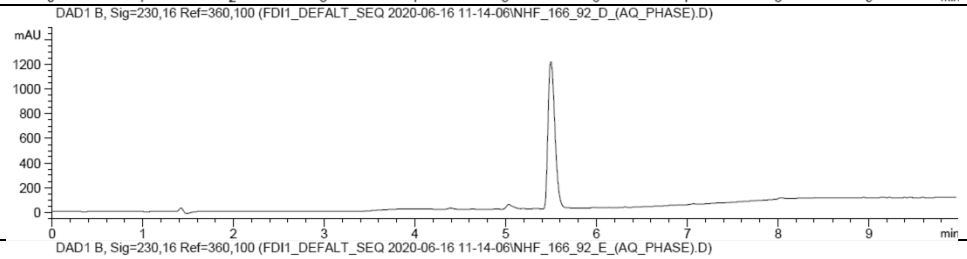
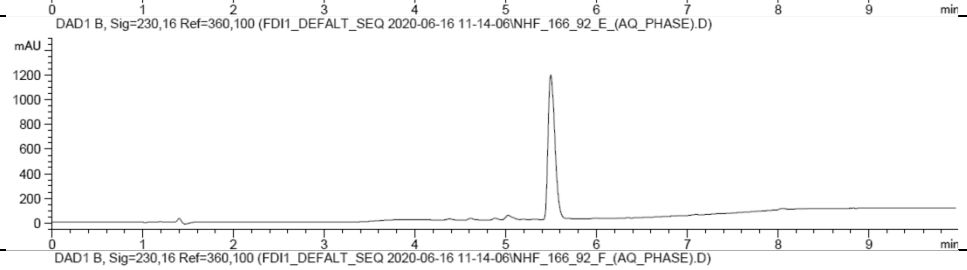
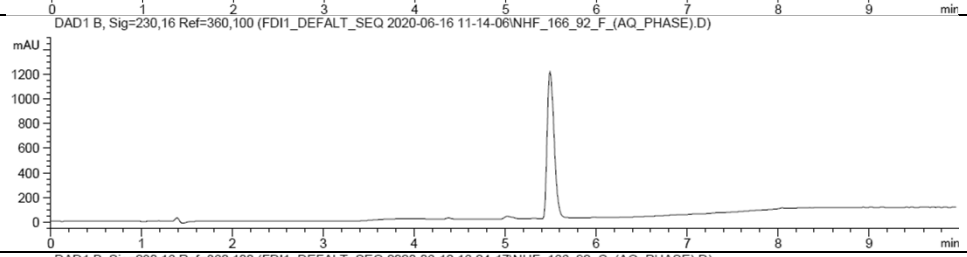
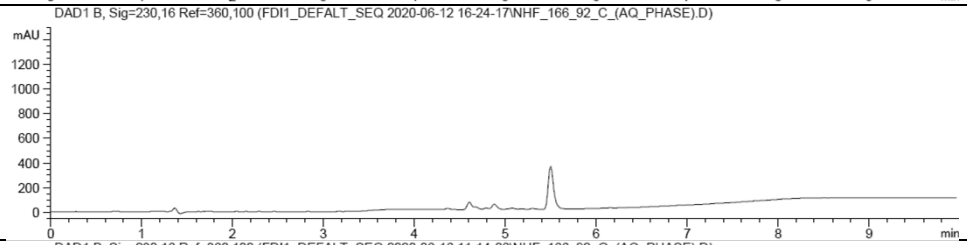
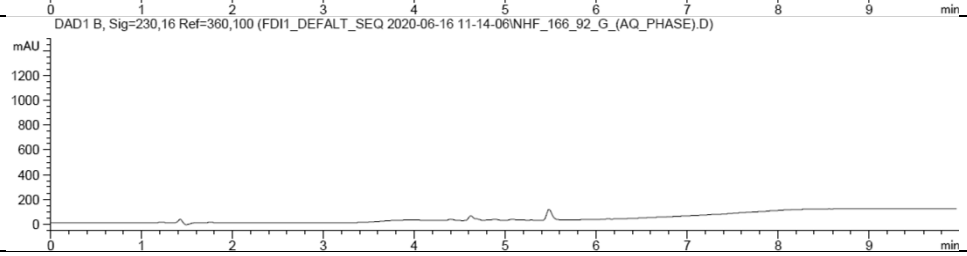
	**N/A**
	**MeCN, 60 °C, 1 h**
	**15 % w/w DTT in MeCN, 60 °C, 1 h**
	**13 % DIPEA in MeCN, 60 °C, 1 h**
	**10 % w/w DTT, 13 % DIPEA in MeCN, 60 °C, 1 h**
	**15 % w/w DTT, 13 % DIPEA in MeCN, 60 °C, 1 h**

Table S 10. UV traces of non-lactamized Ac-Trp-L-Orn(N_3) (peptide cleaved using 0.1 M NaOH (aq) after the treatment specified in the right column) recorded at 230 nm.

C-terminal lactamization of Ac-Trp-L-Dab (**4**)

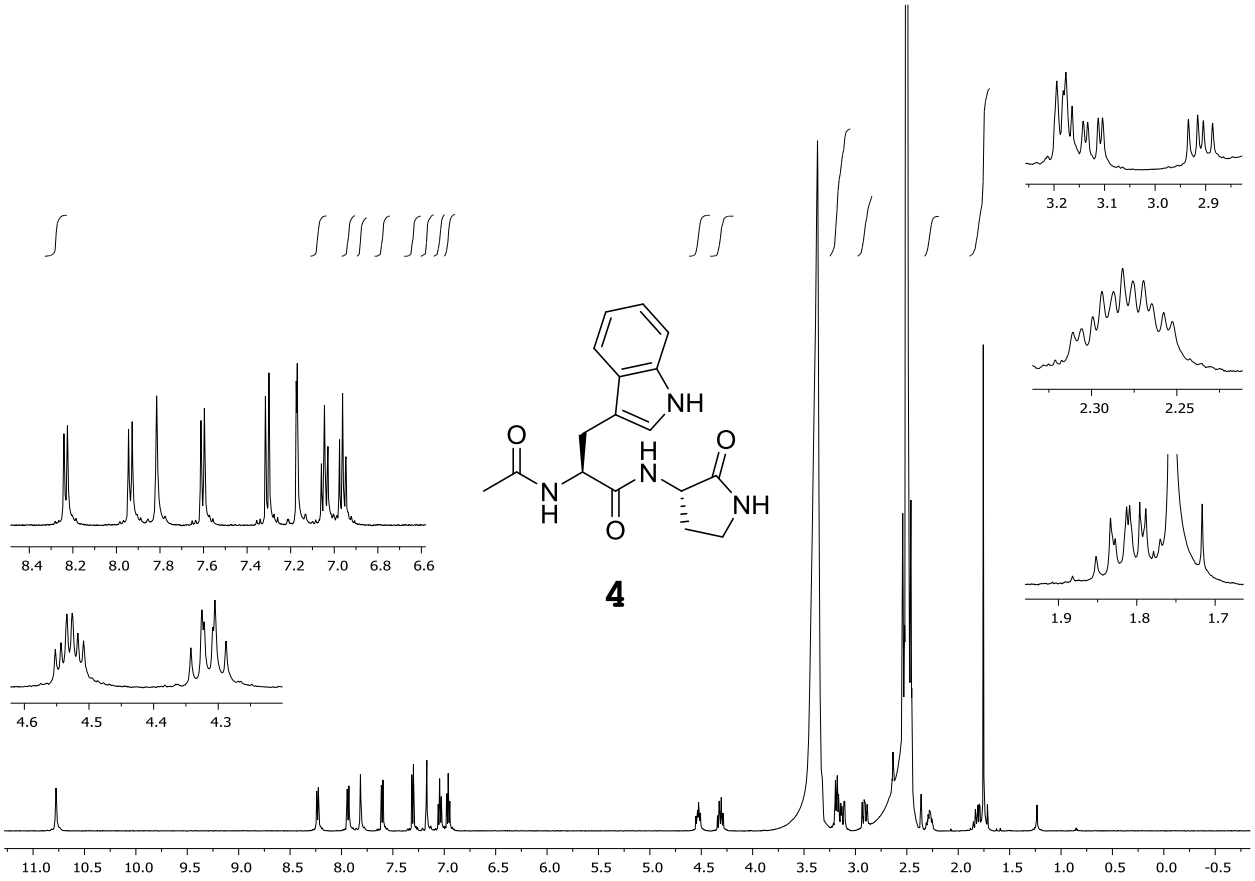


Figure S 1. ^{1}H NMR spectrum of peptide 4 recorded in DMSO-d_6 at 500 MHz.

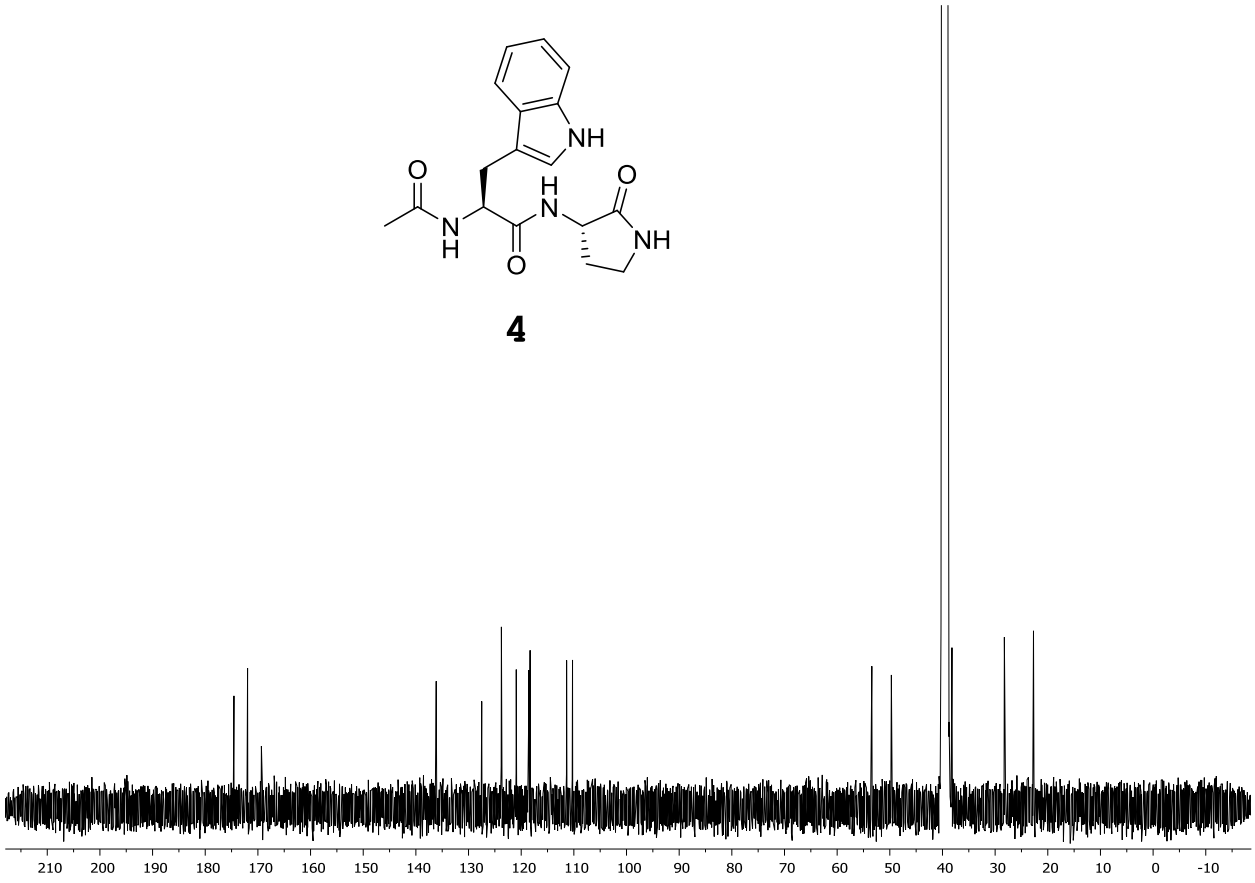


Figure S 2. ^{13}C NMR spectrum of peptide 4 recorded in DMSO-d_6 at 126 MHz.

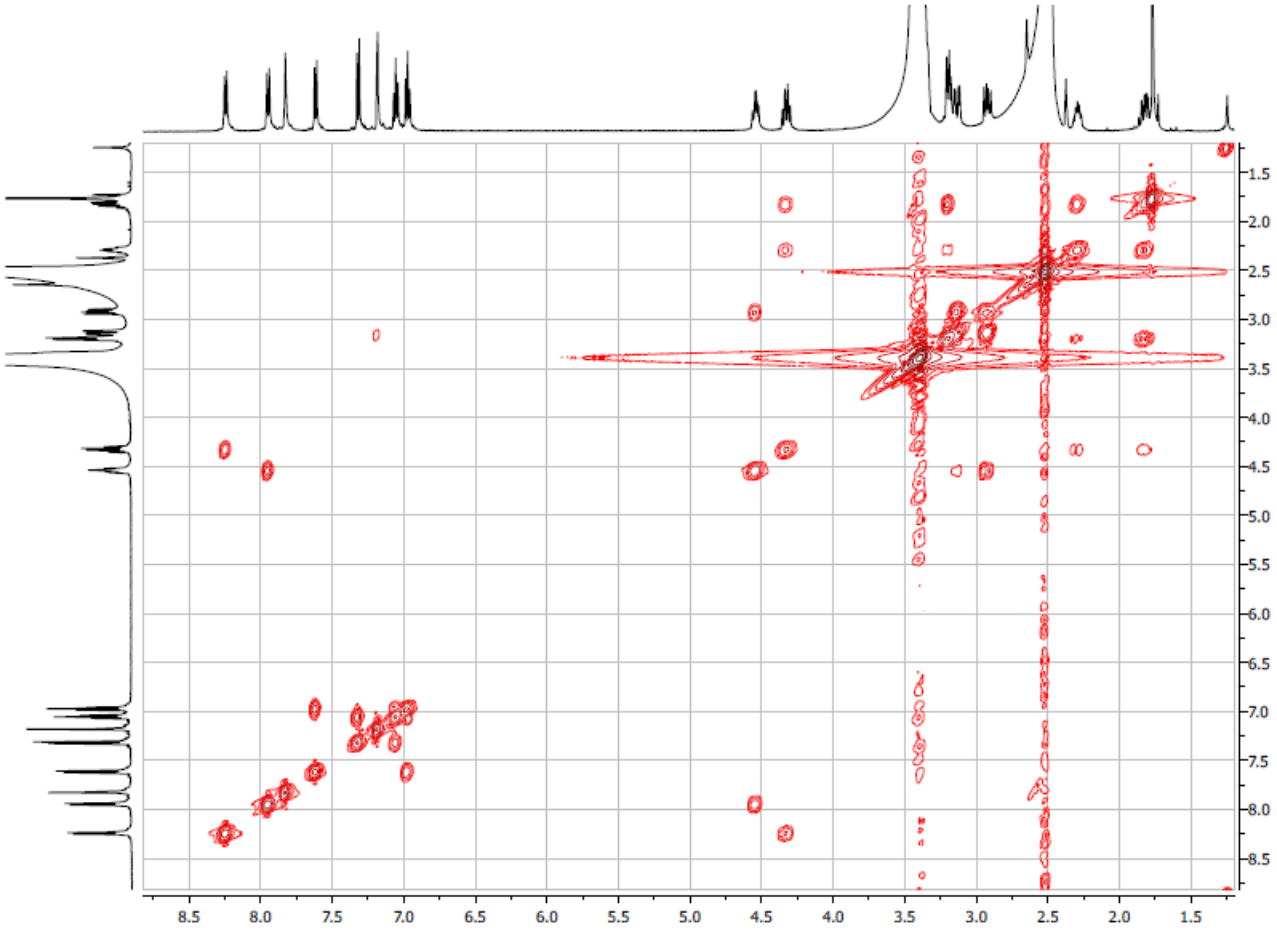


Figure S 3. COSY spectrum of peptide 4 recorded in DMSO-d_6 at 500 MHz.

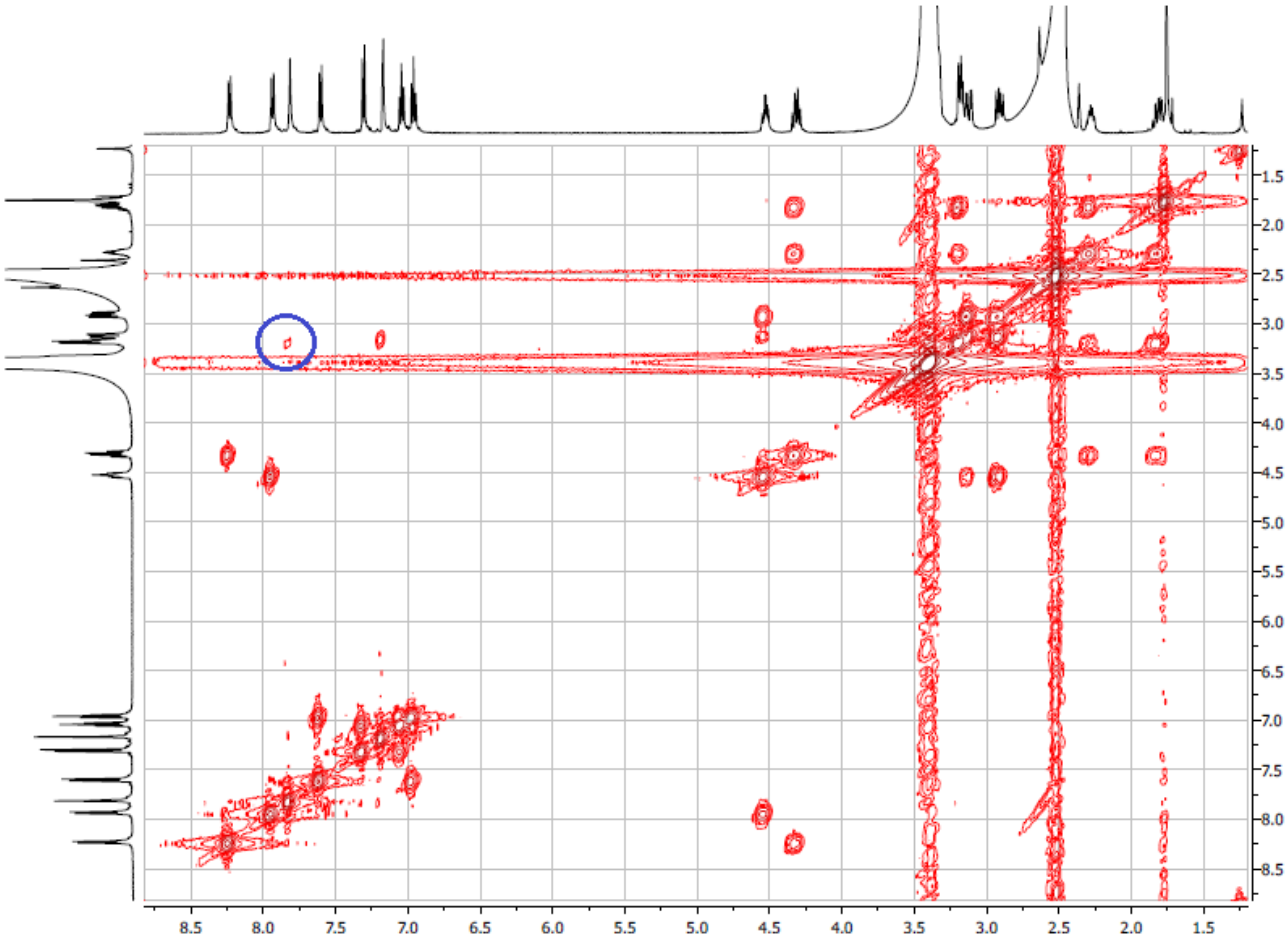


Figure S 4. COSY spectrum of peptide 4 recorded in DMSO-d_6 at 500 MHz. The cross-peak showing coupling between *γ*-lactam N-H and neighboring methylene group is marked with a blue circle.

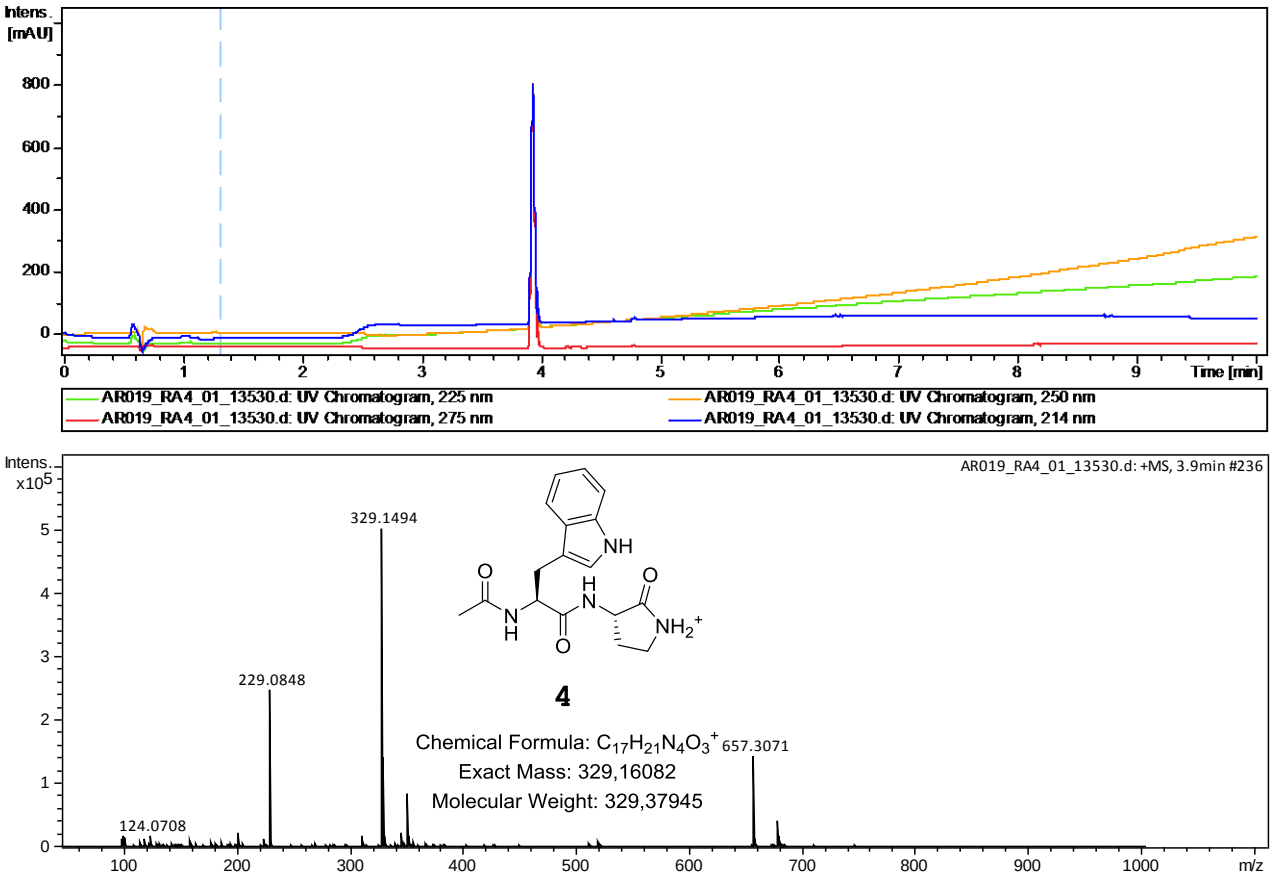


Figure S 5. LC-UV-MS spectrum of peptide 4.Blue: UV trace at 214 nm. Green: UV trace at 225 nm. Orange: UV trace at 250 nm. Red: UV trace at 275 nm.

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C-terminal lactamization of Ac-Trp-L-Orn (**5**)

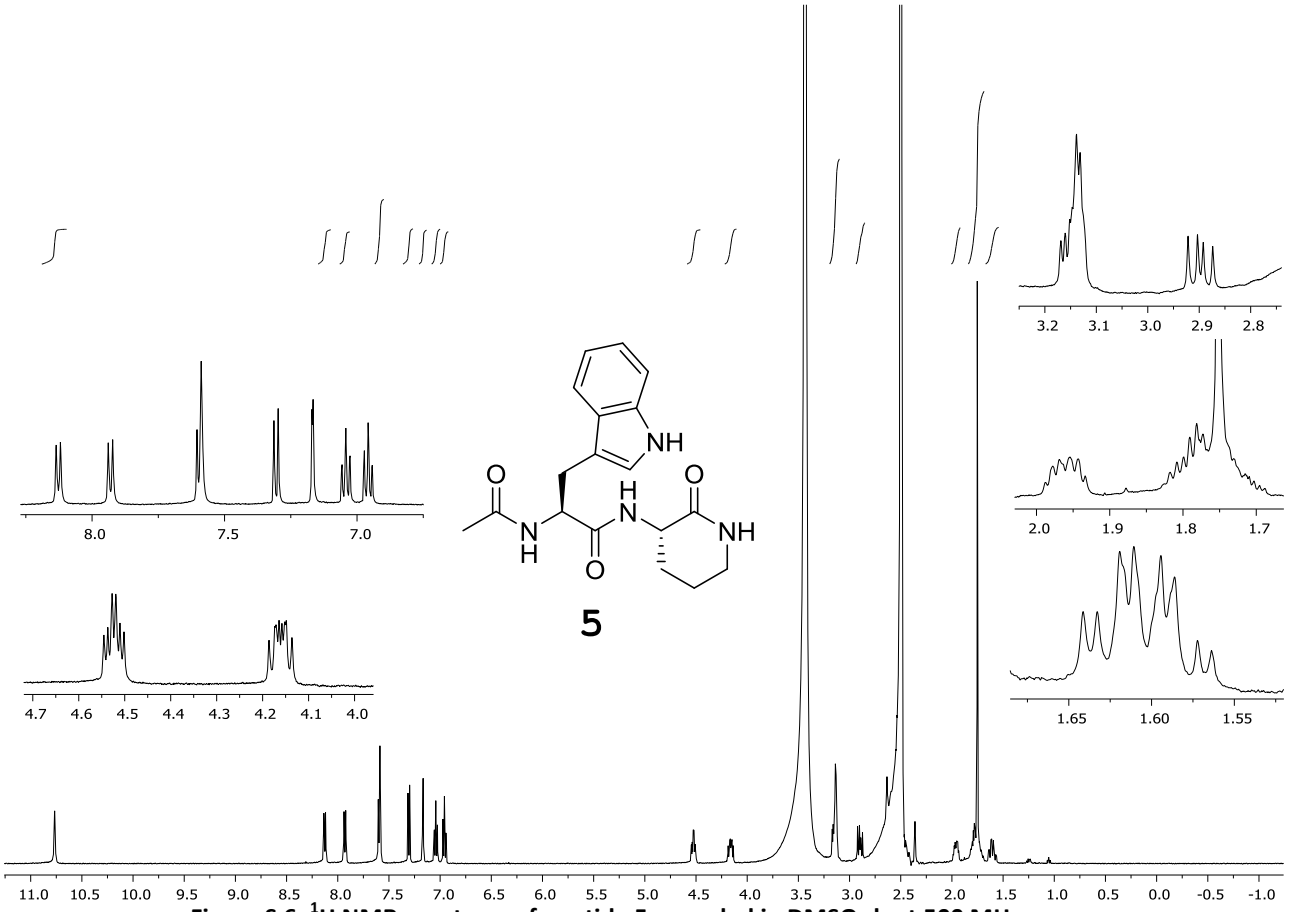


Figure S 6. ^{1}H NMR spectrum of peptide 5 recorded in DMSO-d_6 at 500 MHz.

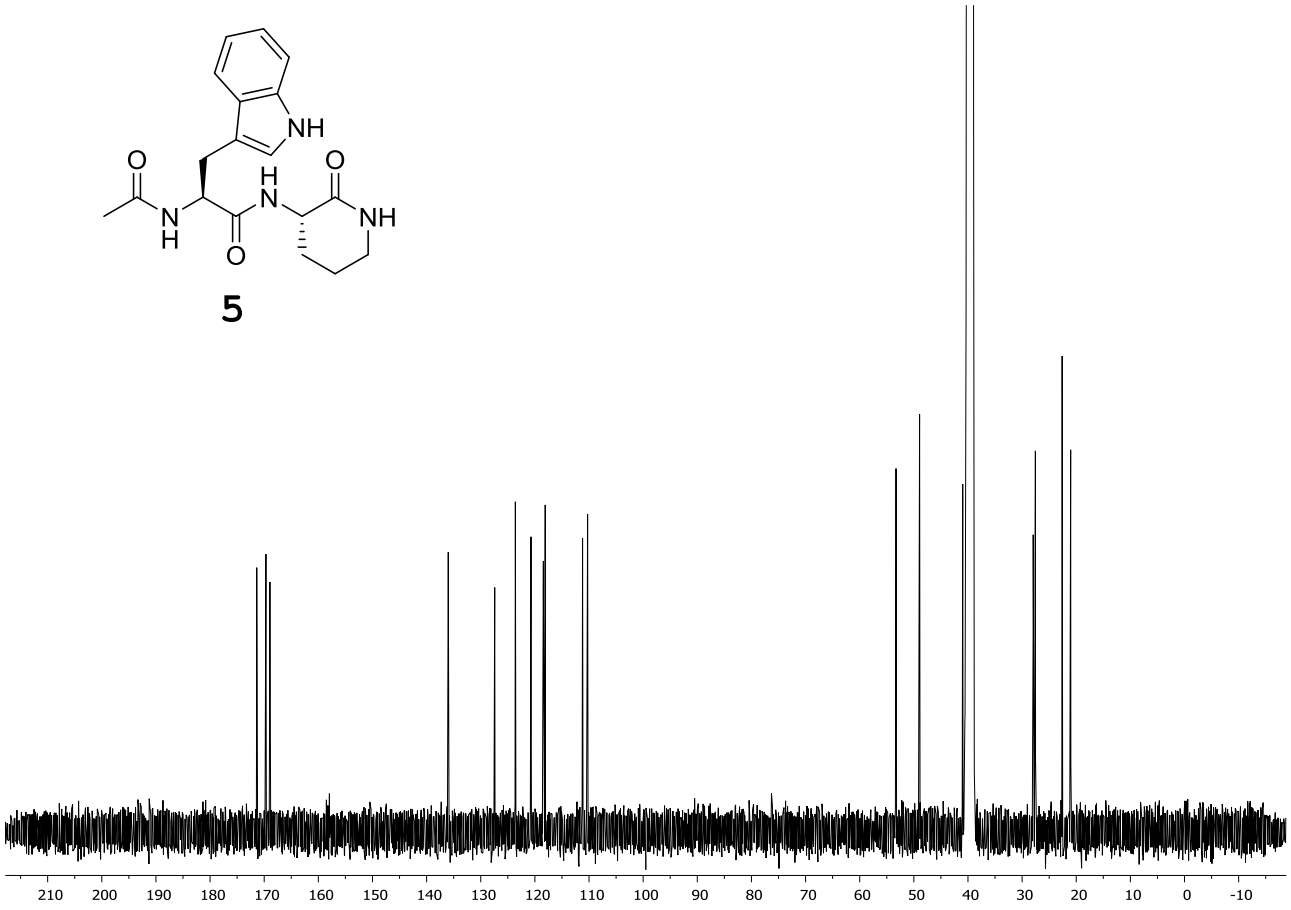


Figure S 7. ^{13}C NMR spectrum of peptide 5 recorded in DMSO-d_6 at 126 MHz.

Figure S 8. COSY spectrum of peptide 5 recorded in DMSO-d_6 at 500 MHz.

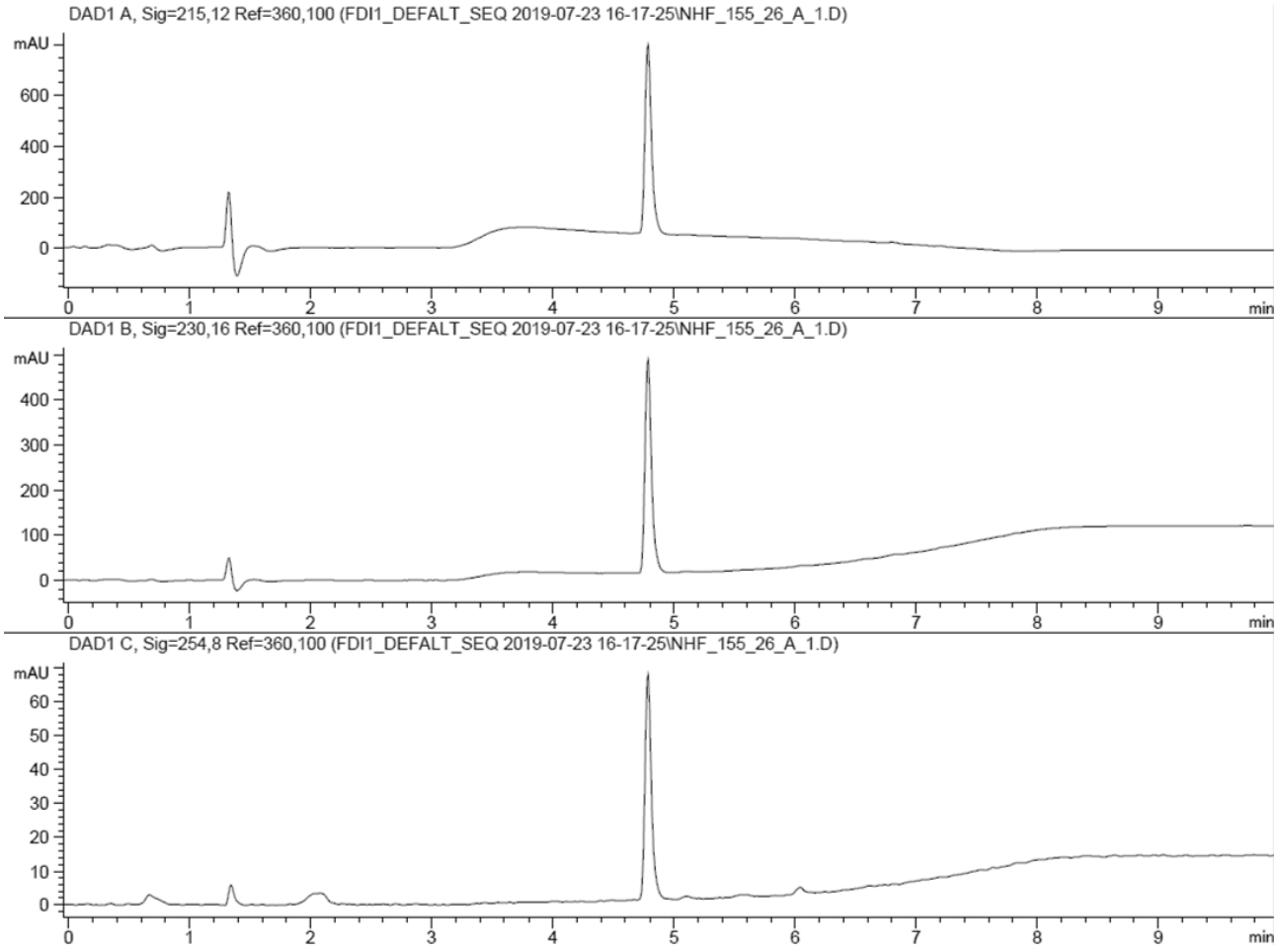


Figure S 9. HPLC-UV spectra of peptide 5 recorded at (from top to bottom) 215, 230, and 254 nm.

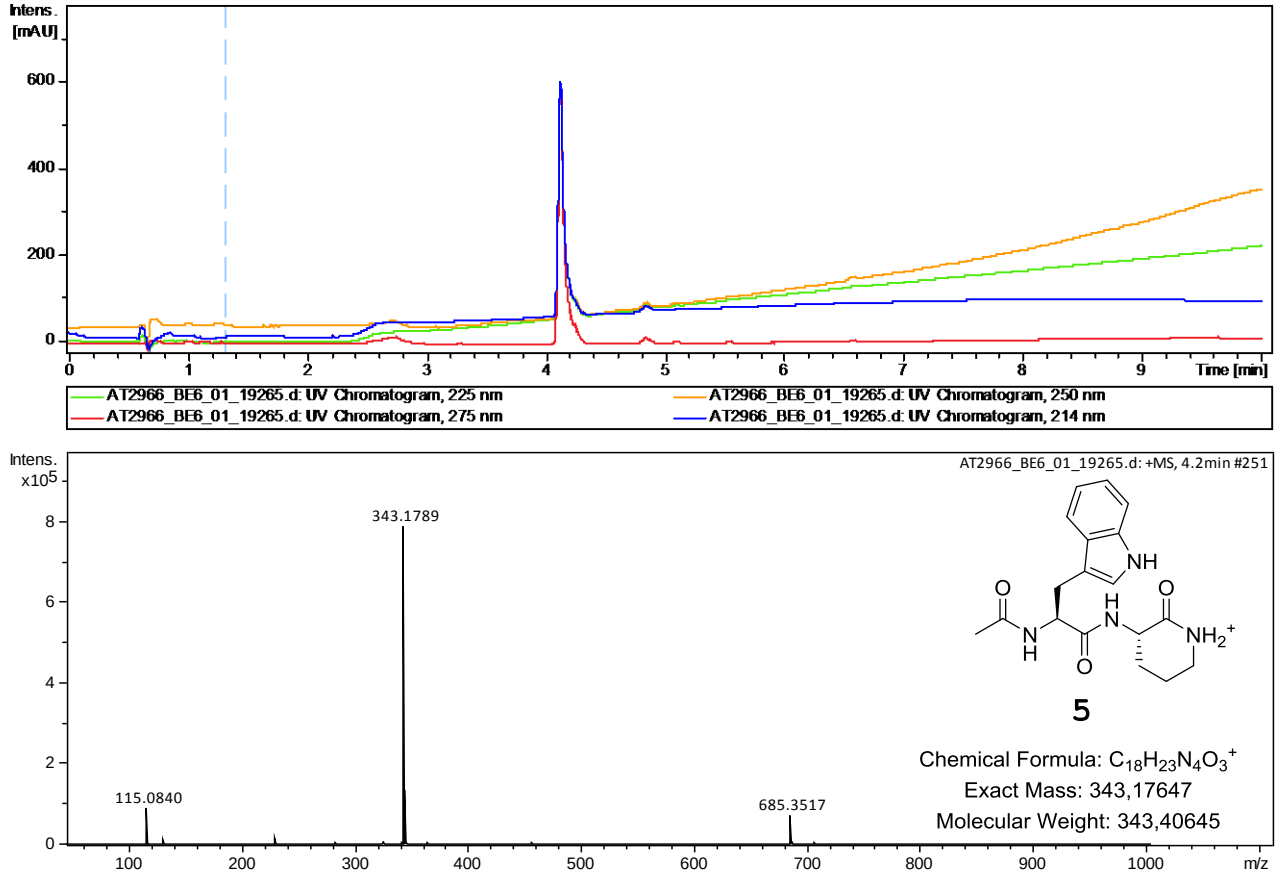


Figure S 10. LC-UV-MS spectrum of peptide 5. Blue: UV trace at 214 nm. Green: UV trace at 225 nm. Orange: UV trace at 250 nm. Red: UV trace at 275 nm.

C-terminal lactamization of Ac-Trp-L-Lys (**6**)

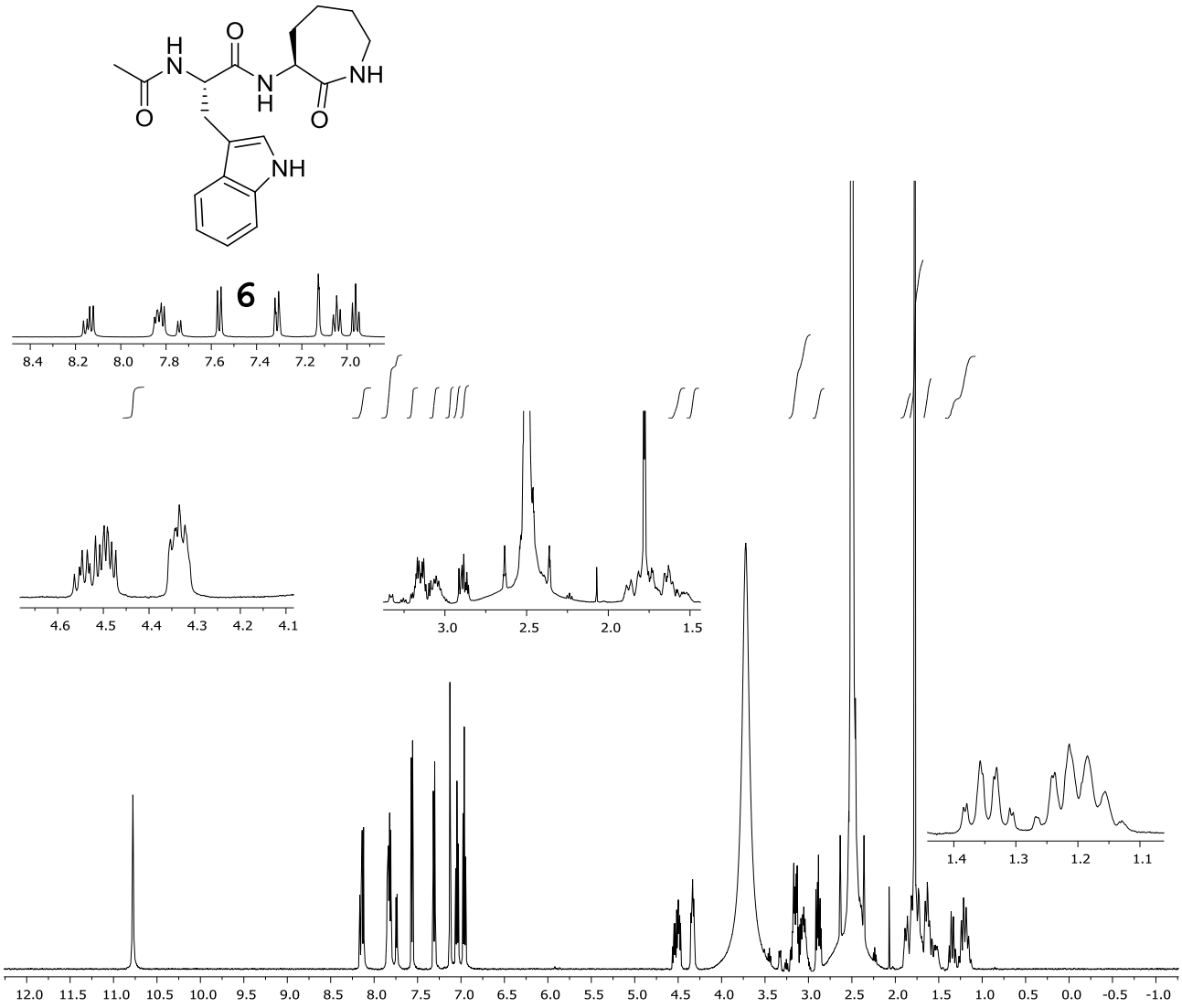
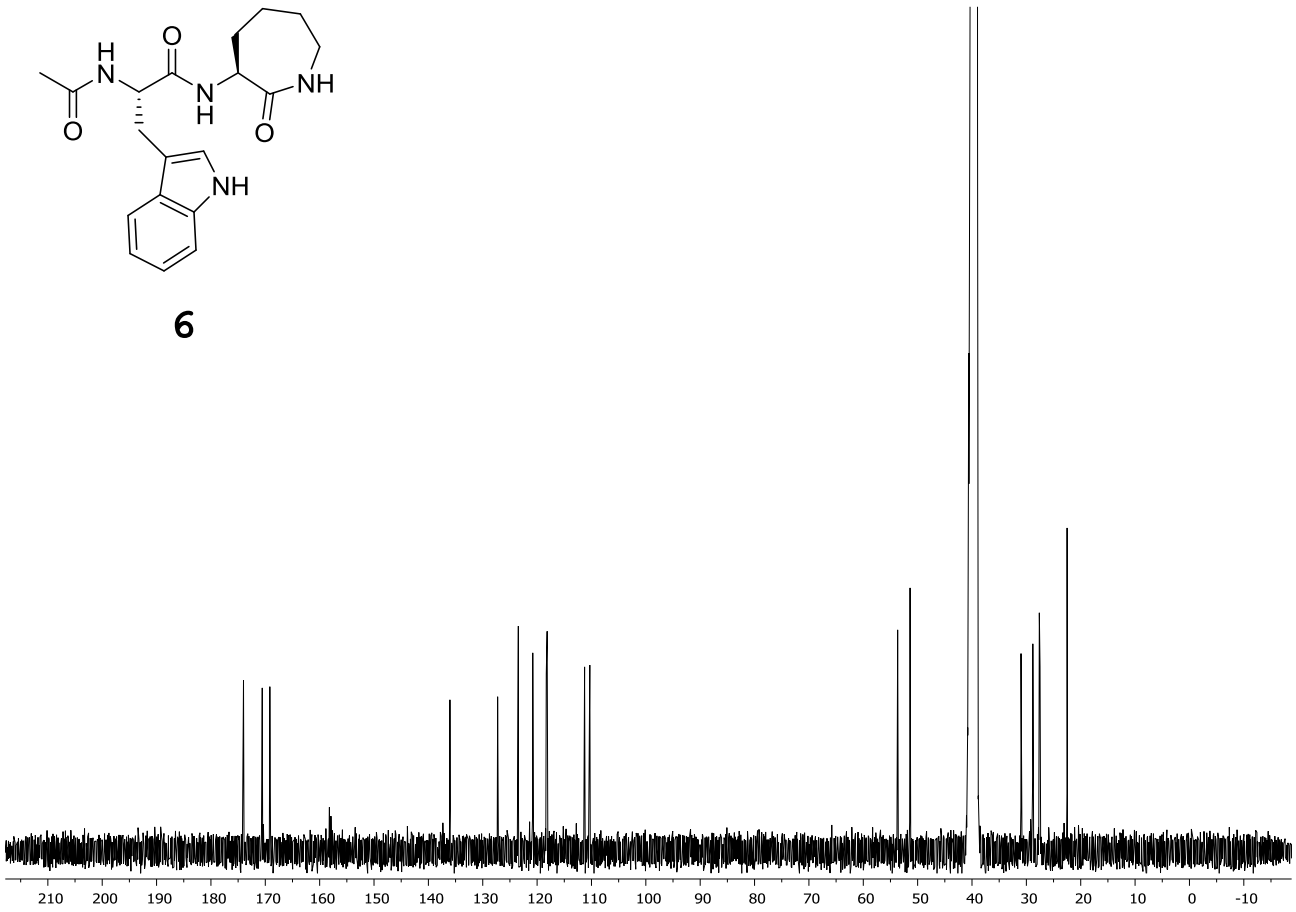


Figure S 11. ^{1}H NMR spectrum of peptide 6 recorded in DMSO-d_6 at 500 MHz.



6

Figure S 12. ^{13}C spectrum of peptide 6 recorded in DMSO-d_6 at 126 MHz.

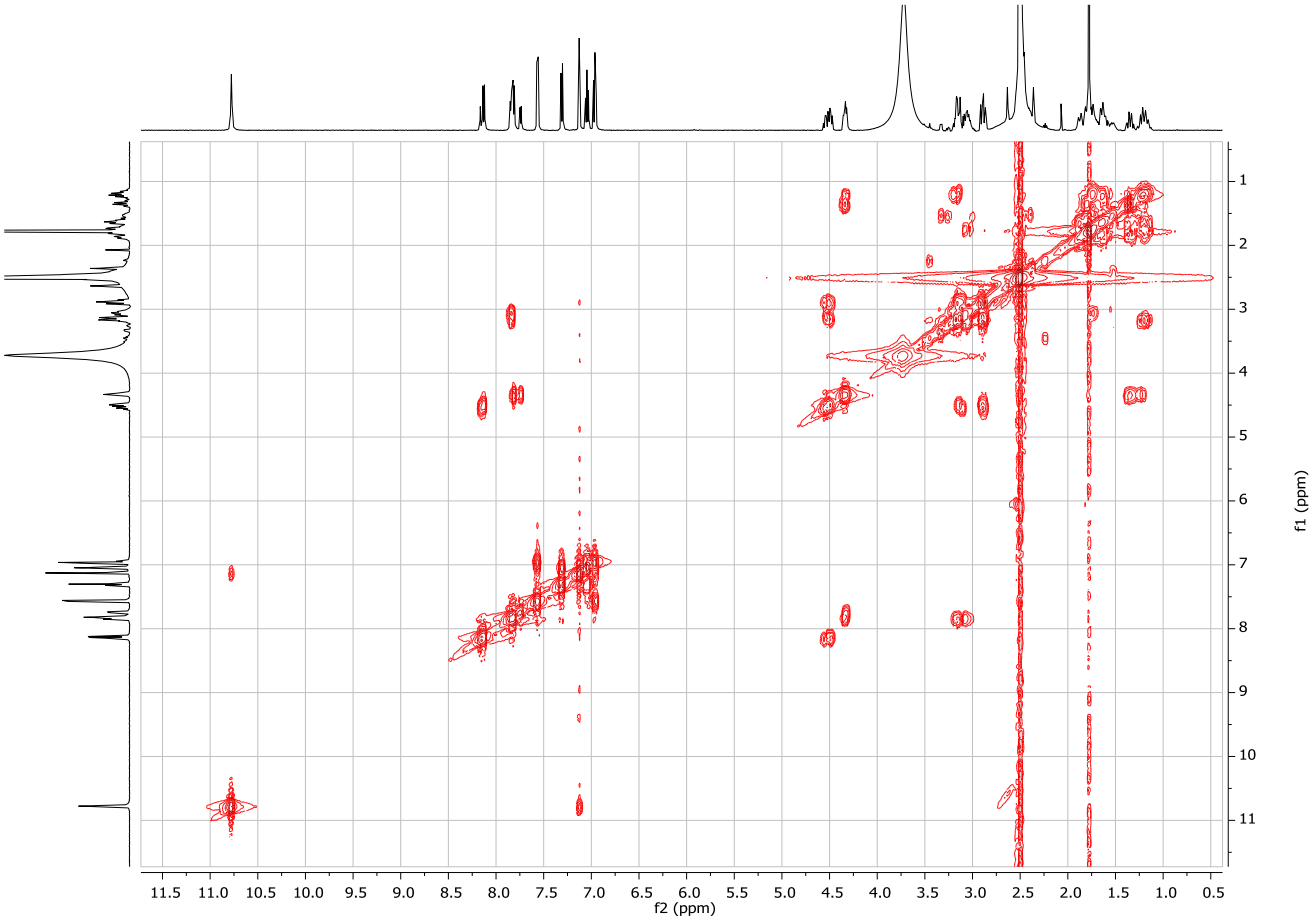


Figure S 13. COSY spectrum of peptide 6 recorded in DMSO-d_6 at 500 MHz.

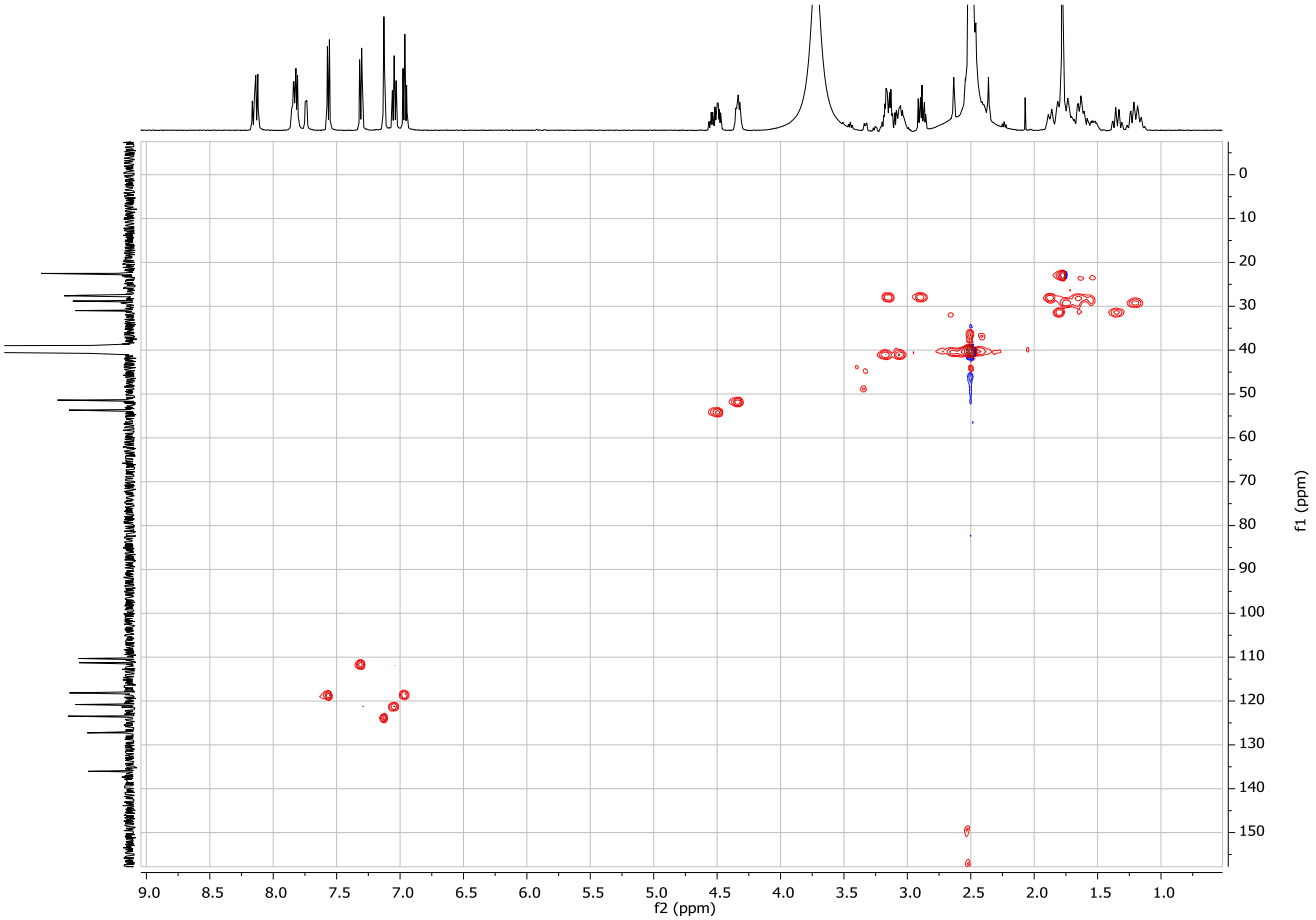


Figure S 14. HSQC spectrum of peptide 6 recorded in DMSO-d_6.

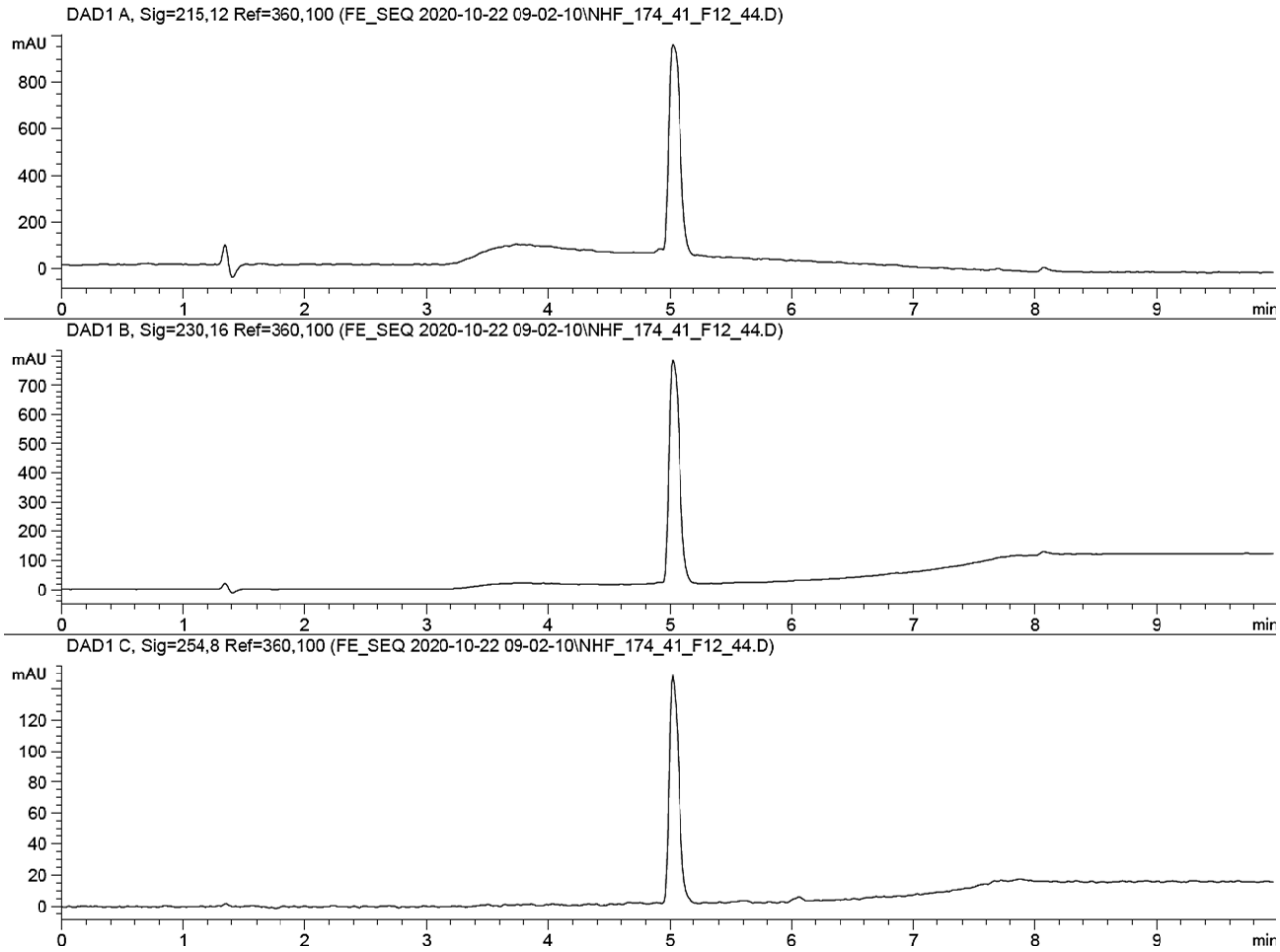


Figure S 15. HPLC-UV spectrum of peptide 6 lactamized at 60 °C recorded at (from top to bottom) 215, 230, and 254 nm.

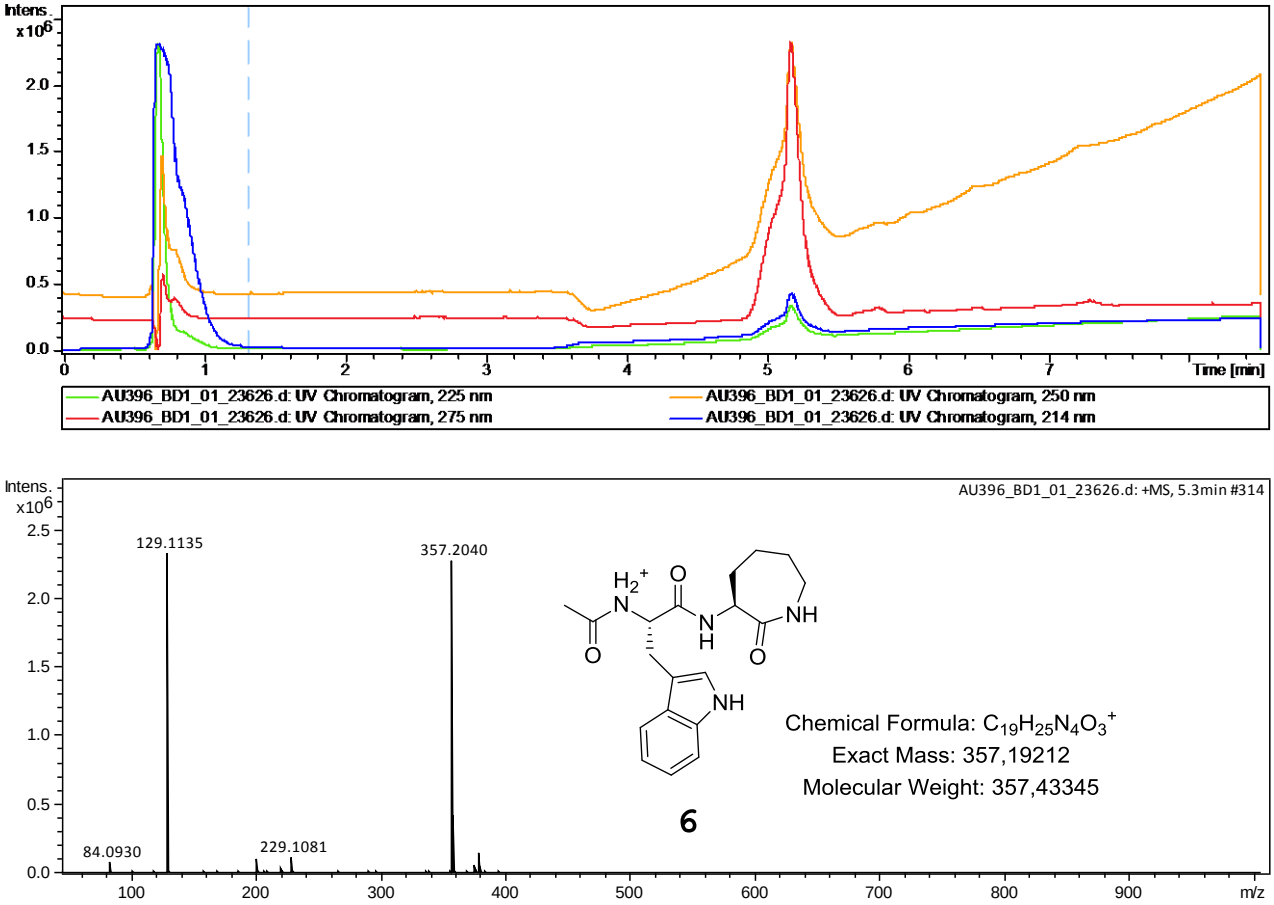


Figure S 16. LC-UV-MS spectrum of peptide 6. Blue: UV trace at 214 nm. Green: UV trace at 225 nm. Orange: UV trace at 250 nm. Red: UV trace at 275 nm.

C-terminal lactamization of Ac-Trp-D-Orn (**7**)

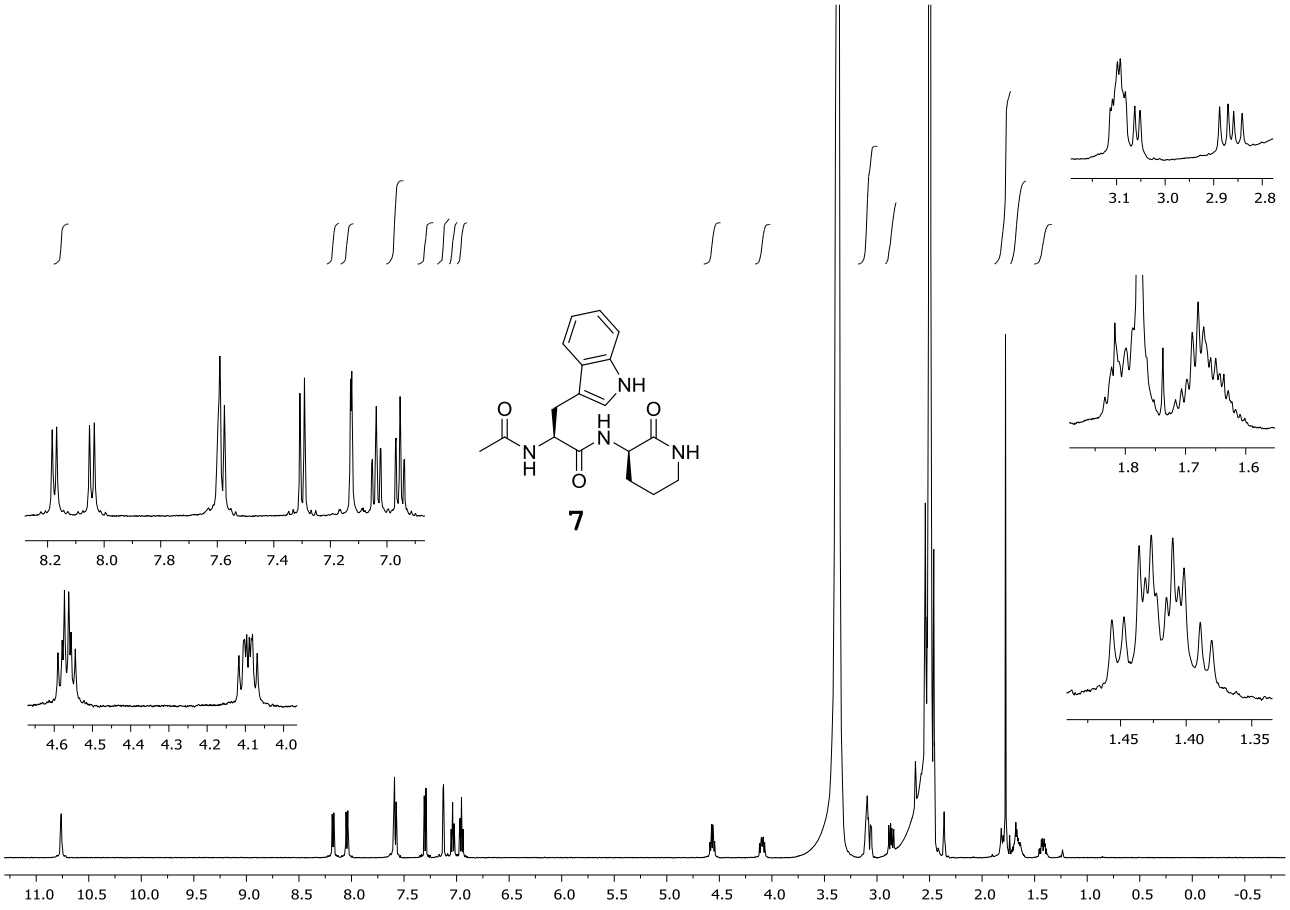


Figure S 17. ^{1}H NMR spectrum of peptide 7 recorded in DMSO-d_6 at 500 MHz.

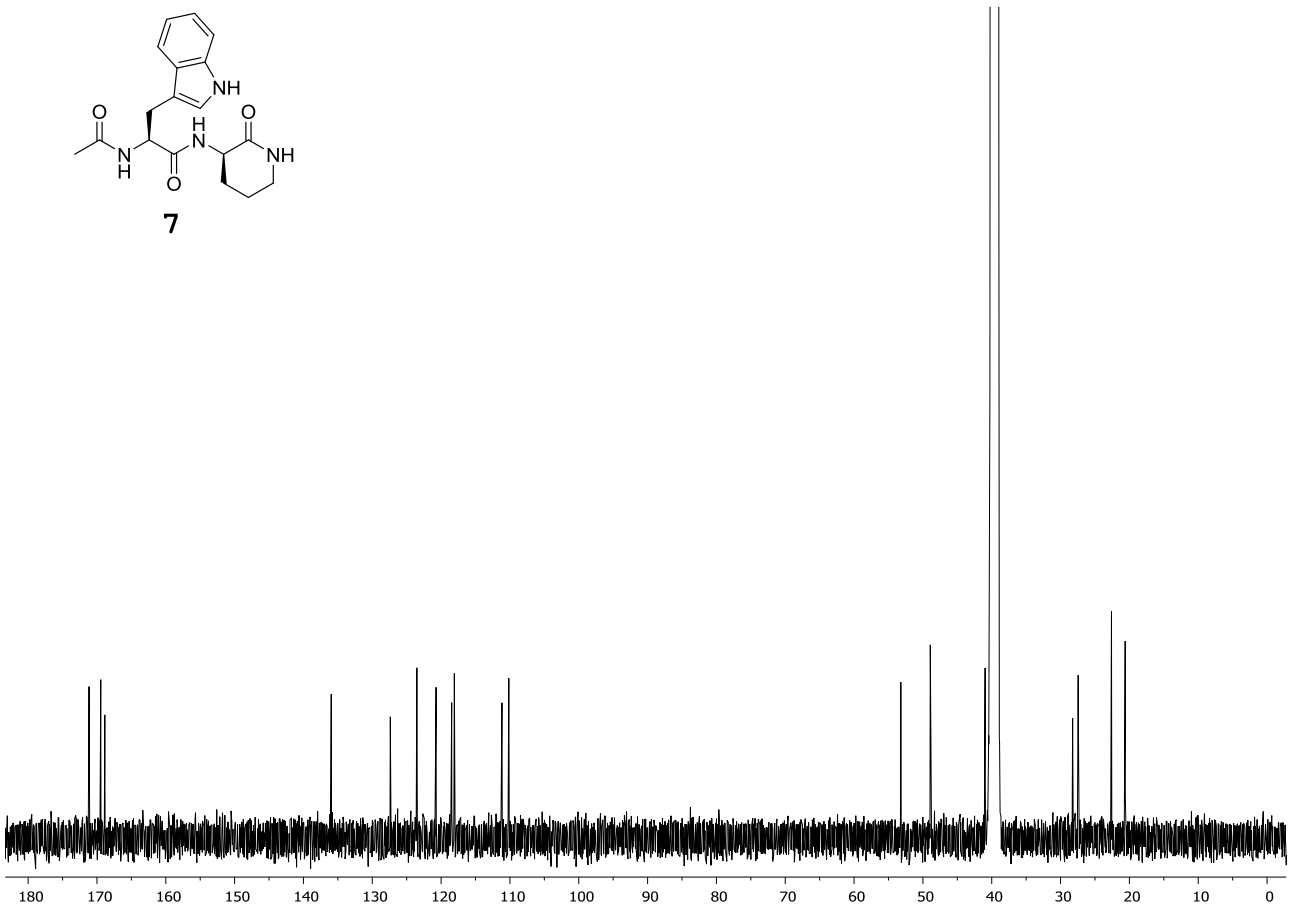


Figure S 18. ^{13}C NMR spectrum of peptide 7 recorded in DMSO-d_6 at 126 MHz.

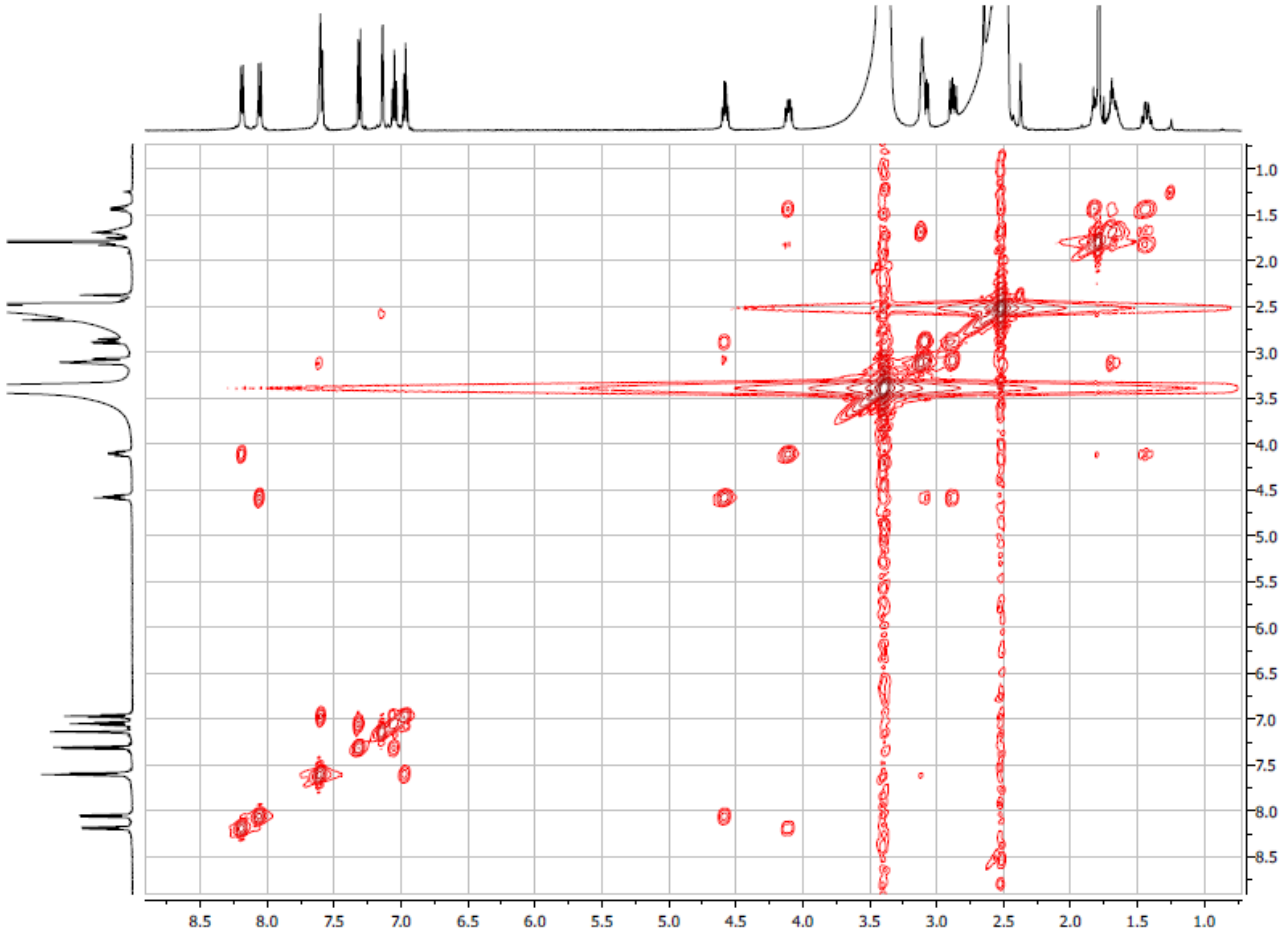


Figure S 19. COSY spectrum of peptide 7 recorded in DMSO-d_6 at 500 MHz.

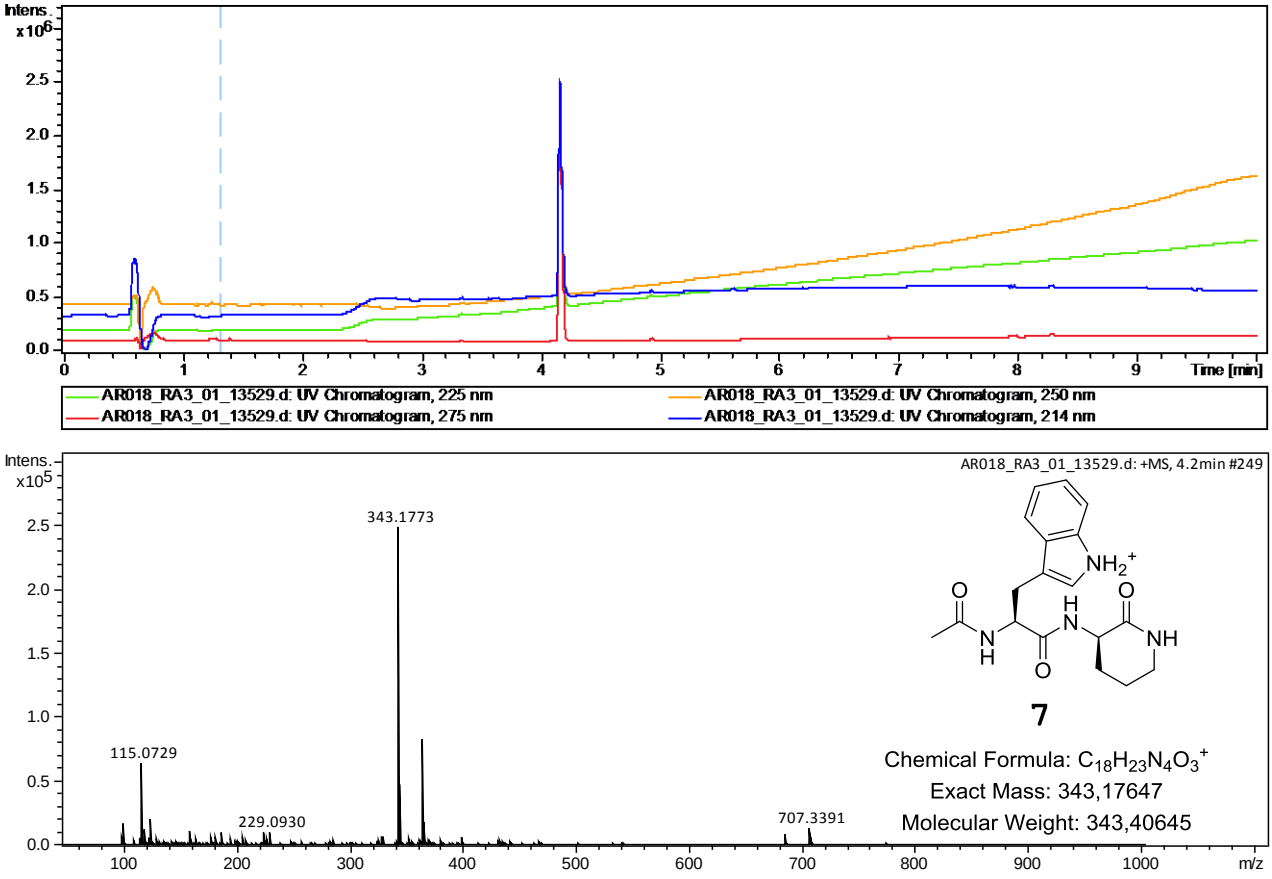


Figure S 20. LC-UV-MS spectrum of peptide 7.Blue: UV trace at 214 nm.Green: UV trace at 225 nm.Orange: UV trace at 250 nm.Red: UV trace at 275 nm.

Solid-phase synthesis of ciliatamide A (**10**)

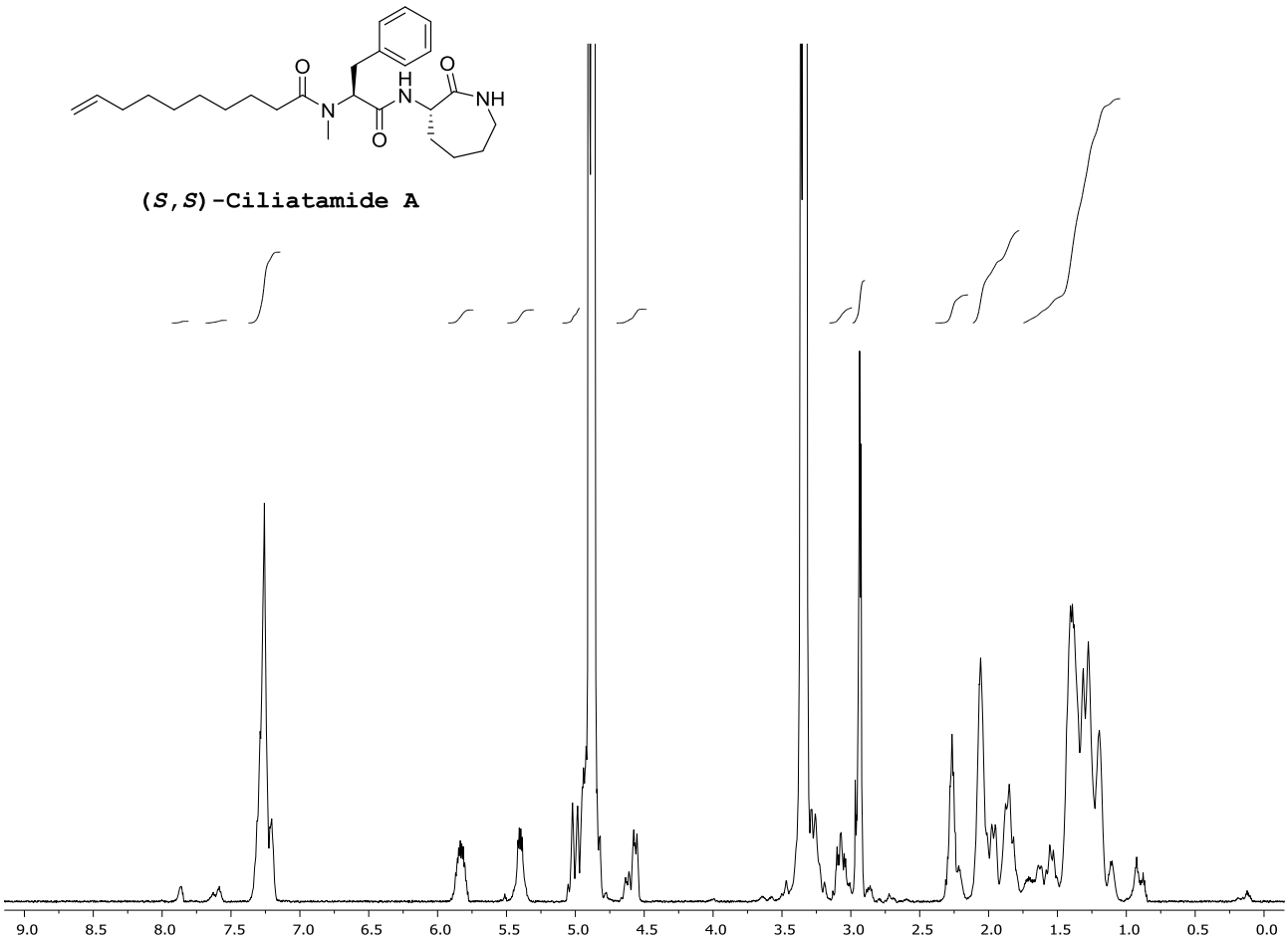


Figure S 21. ^{1}H NMR spectrum of ciliatamide A recorded in methanol-d_4 at 500 MHz.

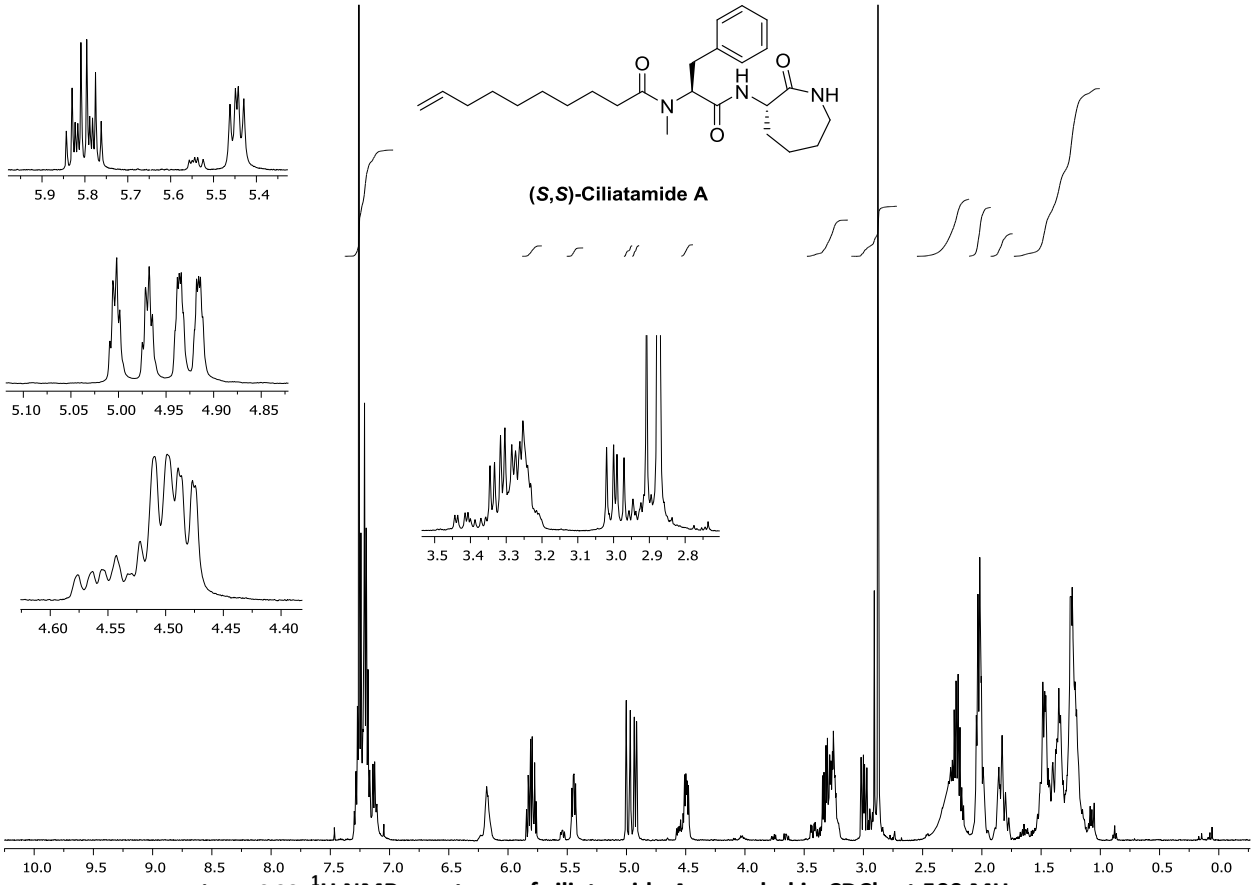


Figure S 22. ^{1}H NMR spectrum of ciliatamide A recorded in $CDCl_3$ at 500 MHz.

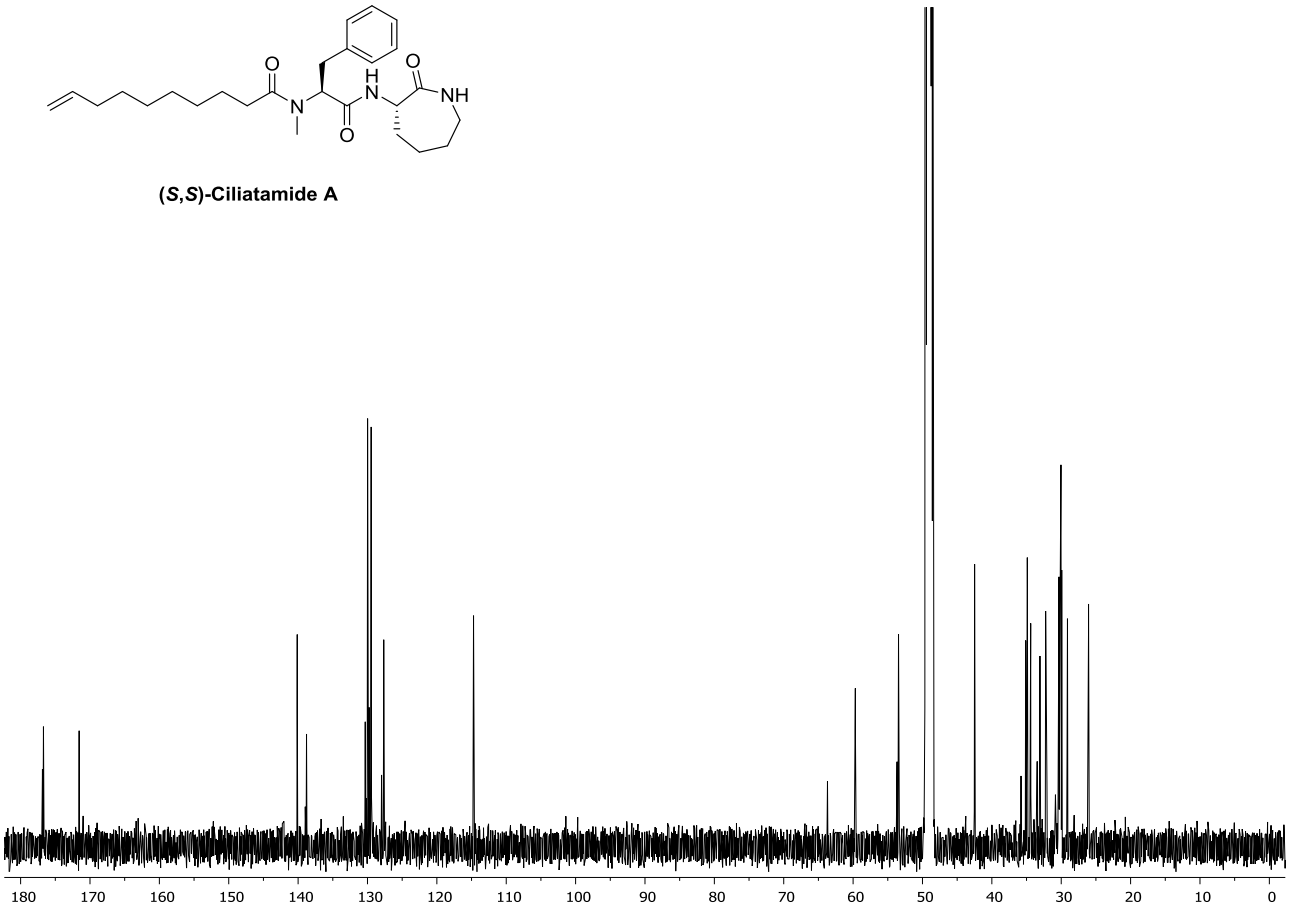


Figure S 23. ^{13}C NMR spectrum of ciliatamide A recorded in methanol-d_4 at 126 MHz.

Solid-phase synthesis of ciliatamide C (**11**)

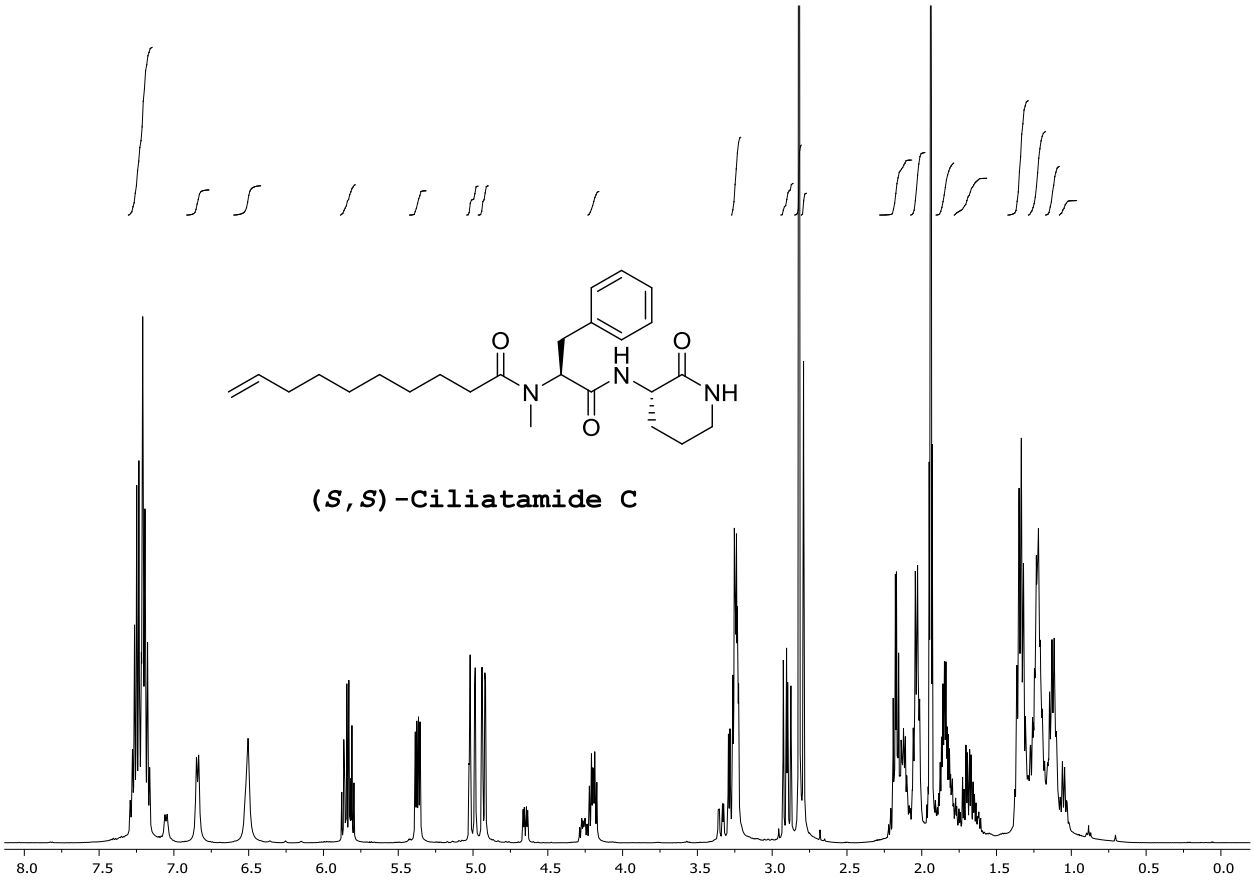


Figure S 24. ^{1}H NMR spectrum of ciliatamide C recorded in acetonitrile-d_3 at 500 MHz.

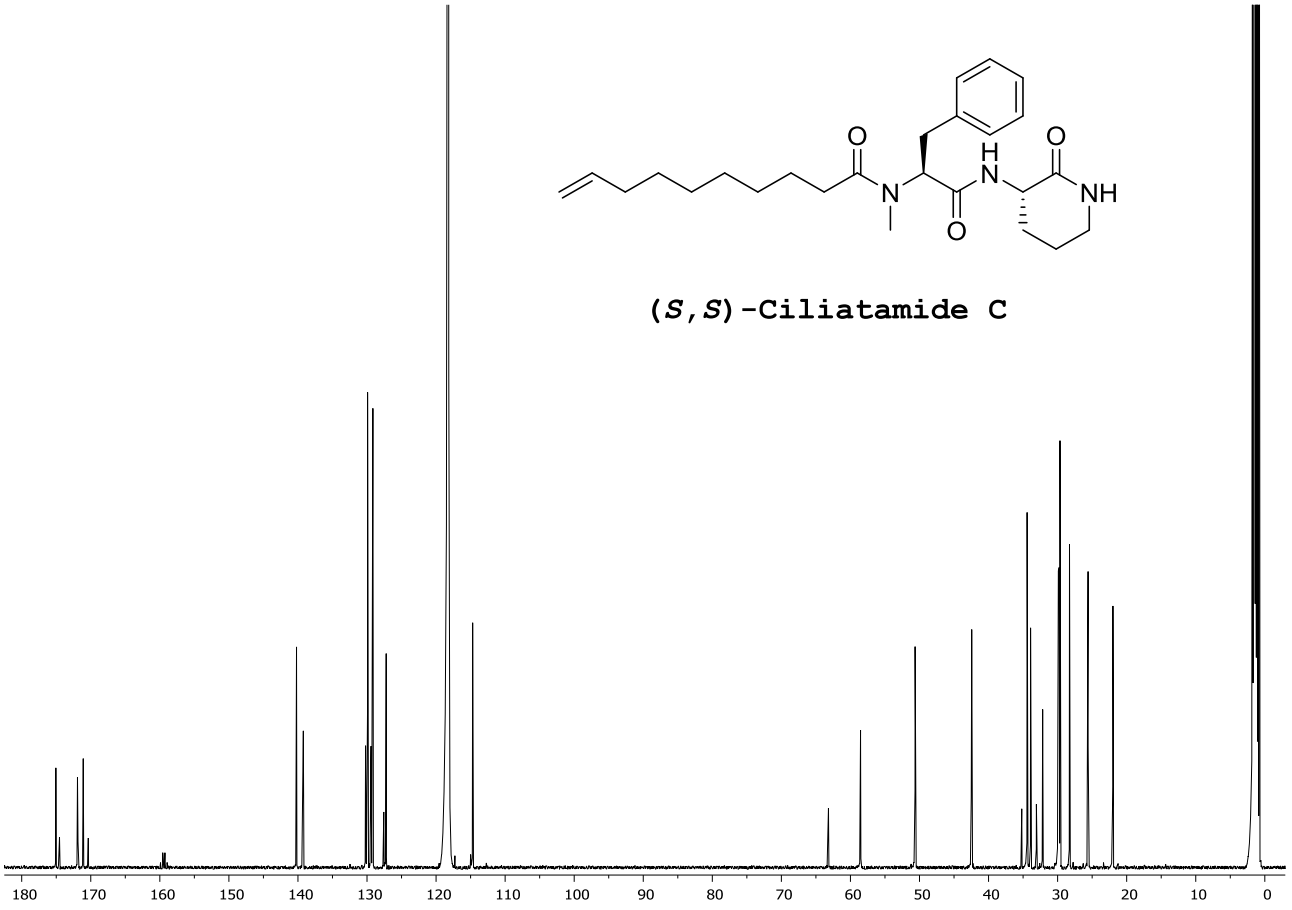


Figure S 25. ^{13}C NMR spectrum of ciliatamide C recorded in acetonitrile-d_3 at 126 MHz.

Ac-HfRW-L-Dab with *C*-terminal *γ*-lactam (**12**)

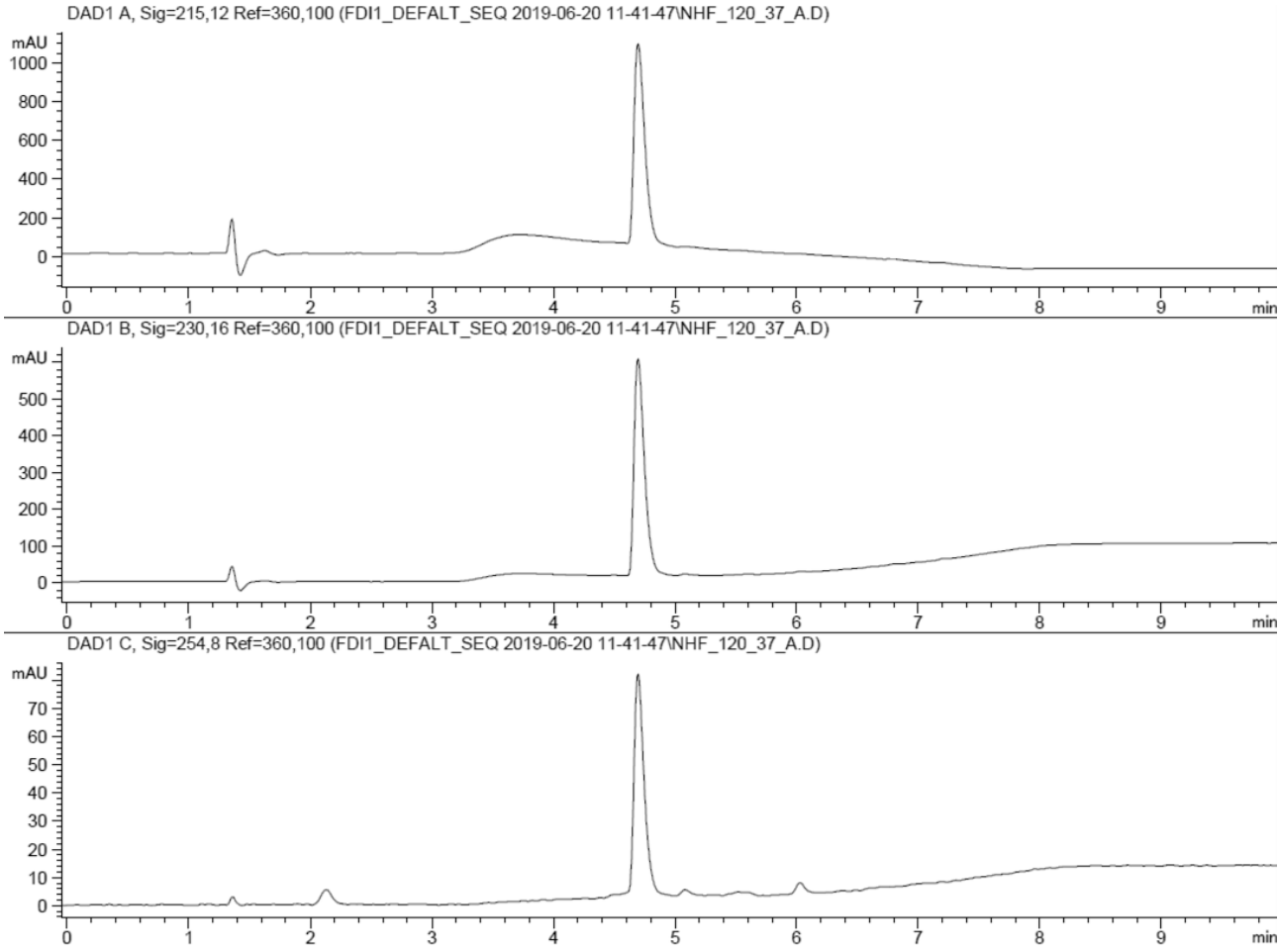


Figure S 26. LC-UV spectra of peptide 12 recorded at (from top to bottom) 215, 230, and 254 nm.

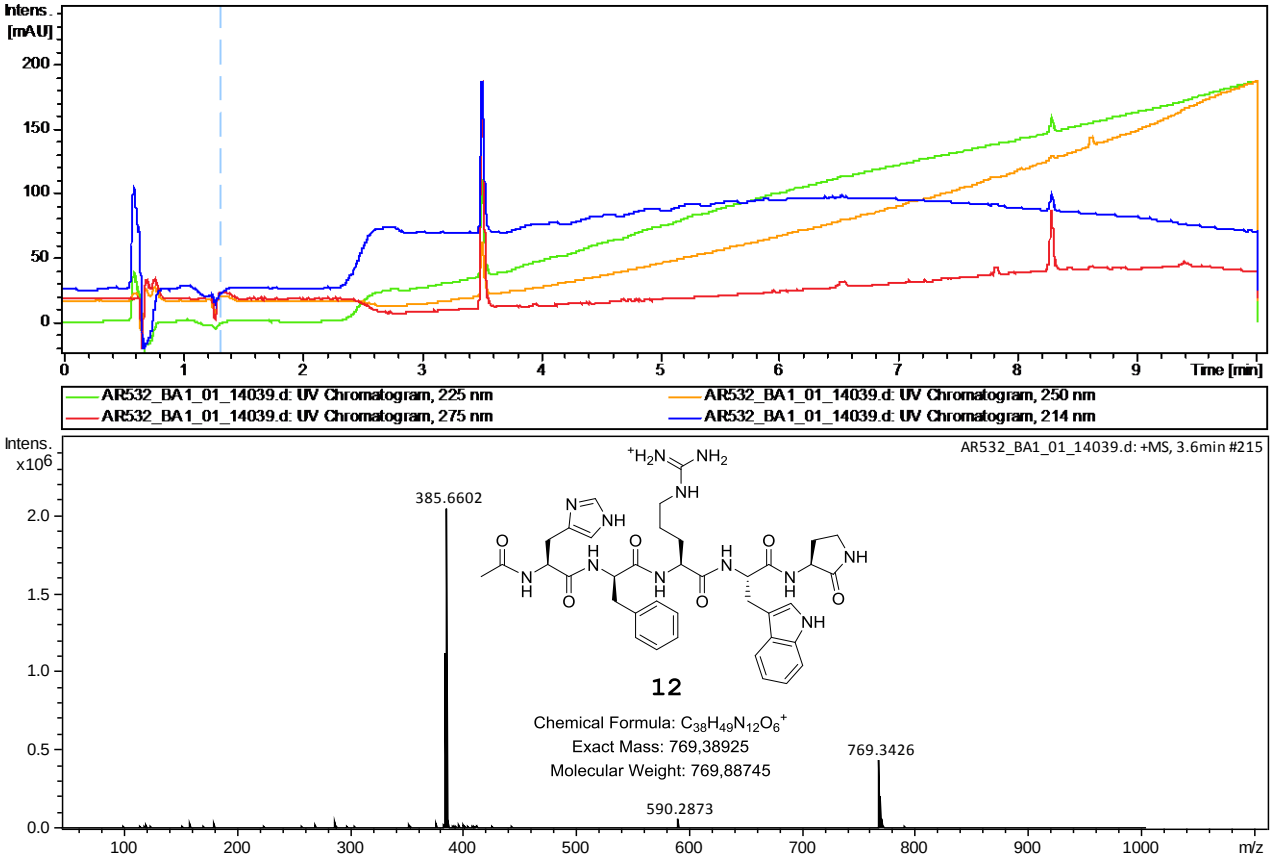


Figure S 27. LC-UV-MS spectrum of peptide 12.Blue: UV trace at 214 nm. Green: UV trace at 225 nm. Orange: UV trace at 250 nm. Red: UV trace at 275 nm.

Ac-HfRW-D-Orn with *C*-terminal *δ*-lactam (**13**)

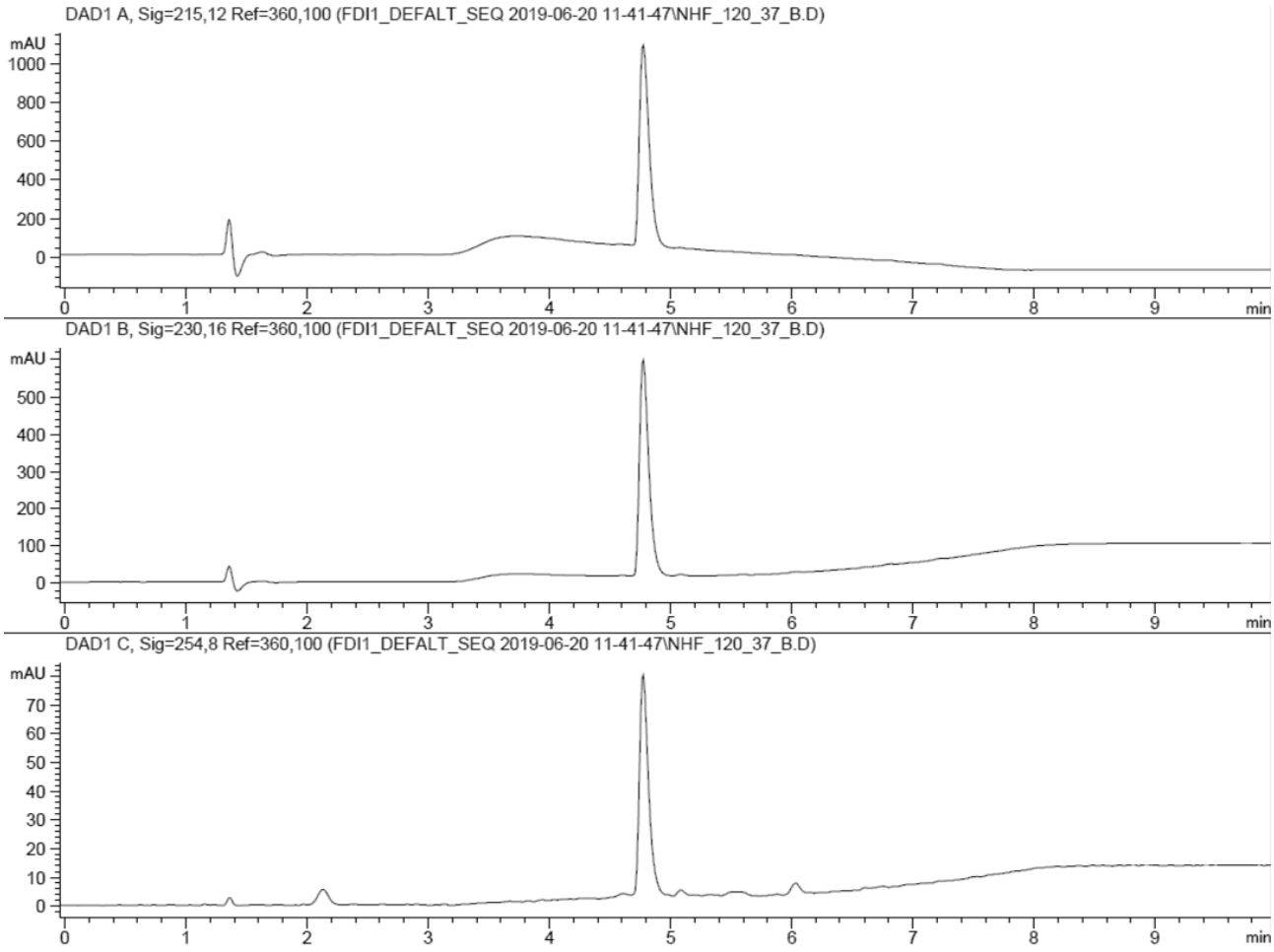


Figure S 28. LC-UV spectra of peptide 13 recorded at (from top to bottom) 215, 230, and 254 nm.

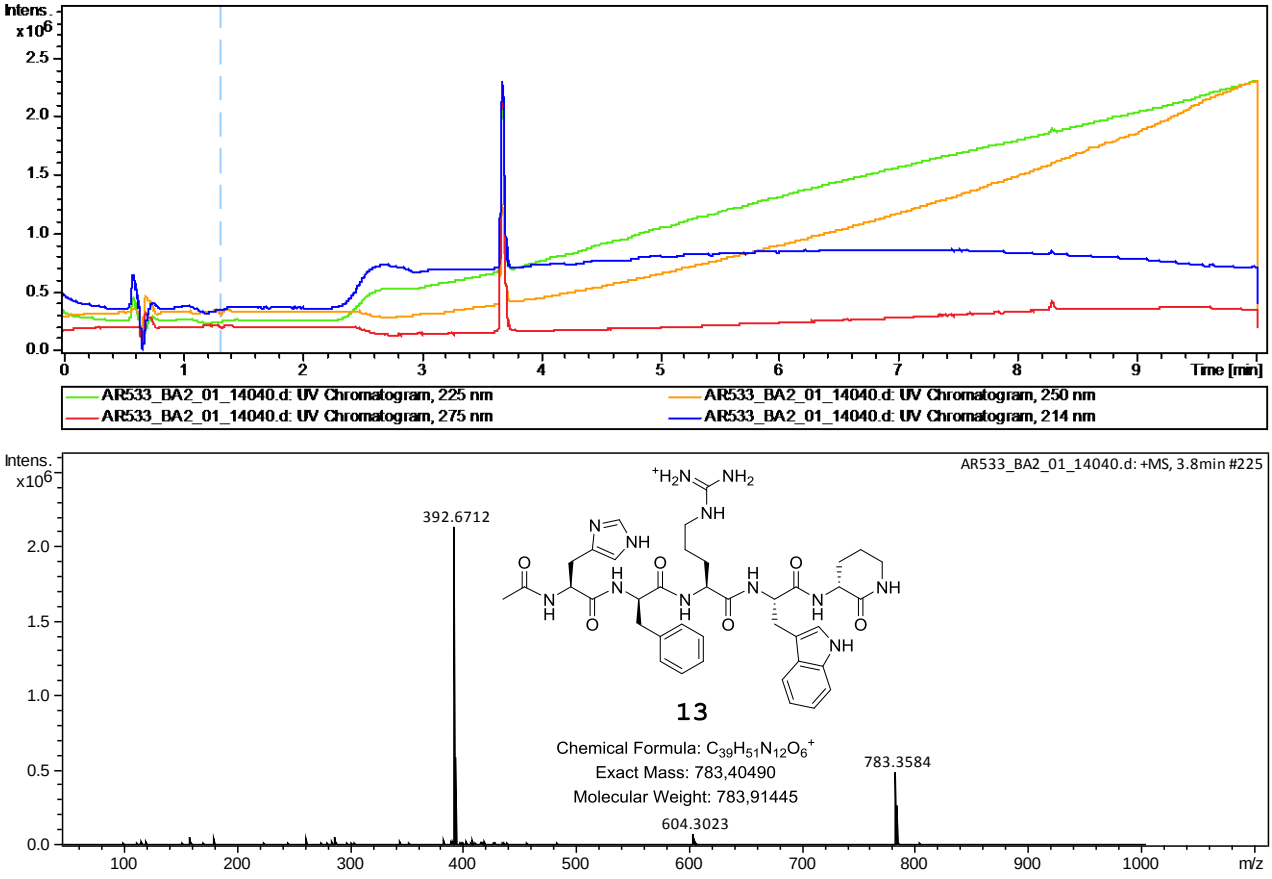


Figure S 29. LC-UV-MS spectrum of peptide 13. Blue: UV trace at 214 nm. Green: UV trace at 225 nm. Orange: UV trace at 250 nm. Red: UV trace at 275 nm.

Oxytocin with *C*-terminal *γ*-lactam (**14**)

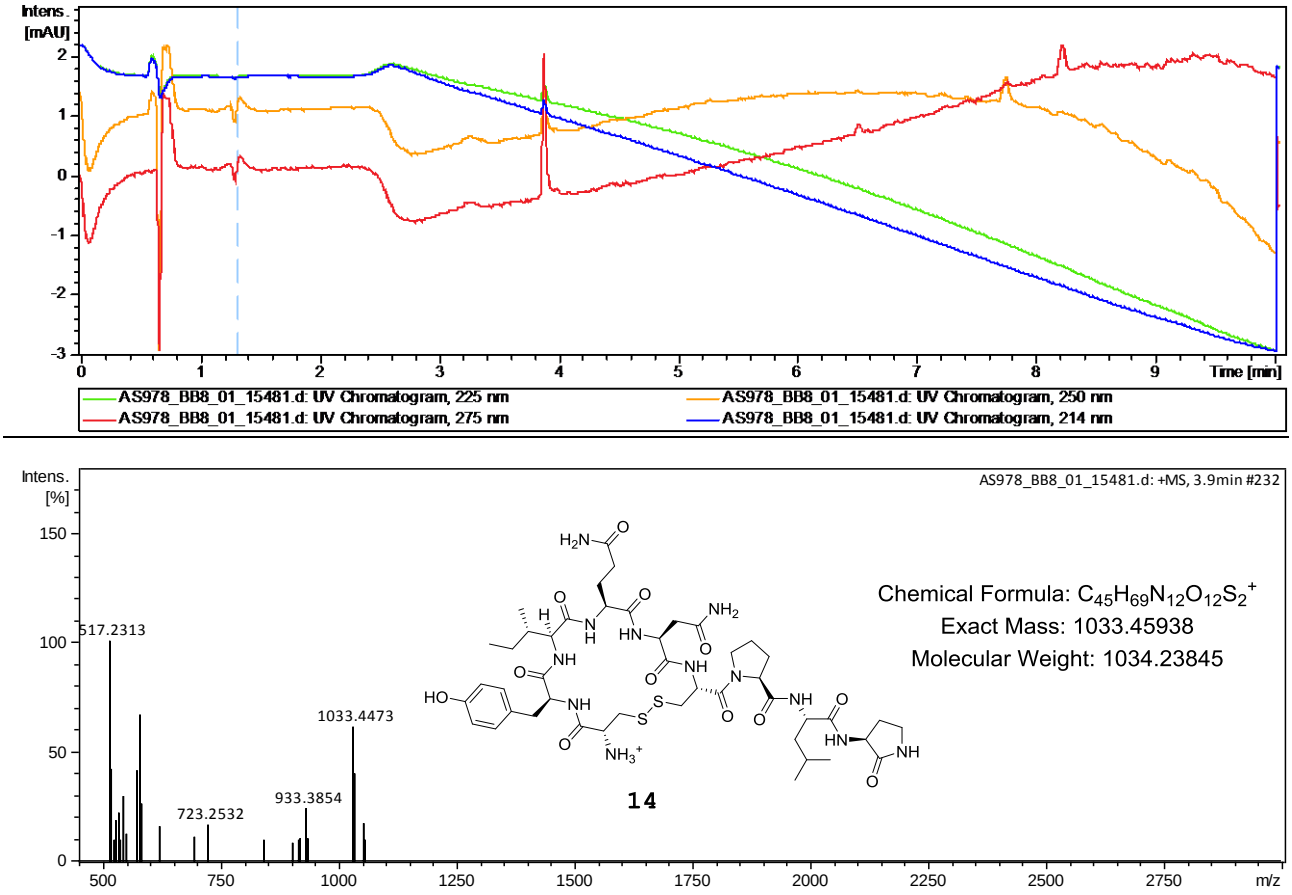


Figure S 30. LC-UV-MS spectrum of peptide 14. Blue: UV trace at 214 nm. Green: UV trace at 225 nm. Orange: UV trace at 250 nm. Red: UV trace at 275 nm.

Desmopressin with *C*-terminal *γ*-lactam (**15**)

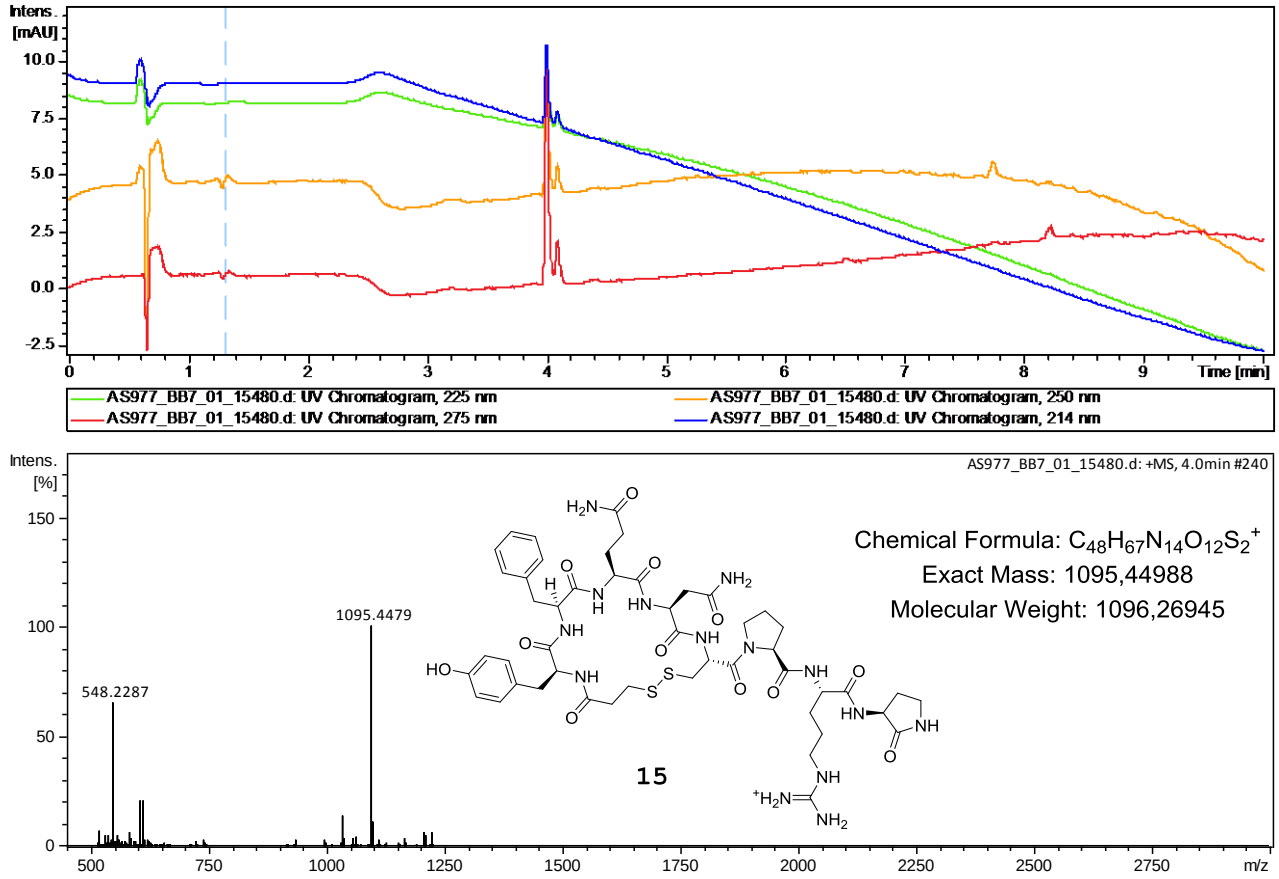


Figure S 31. LC-UV-MS spectrum of peptide 15. Blue: UV trace at 214 nm. Green: UV trace at 225 nm. Orange: UV trace at 250 nm. Red: UV trace at 275 nm.

Binding assay of oxytocin with *C*-terminal *γ*-lactam (**14**)

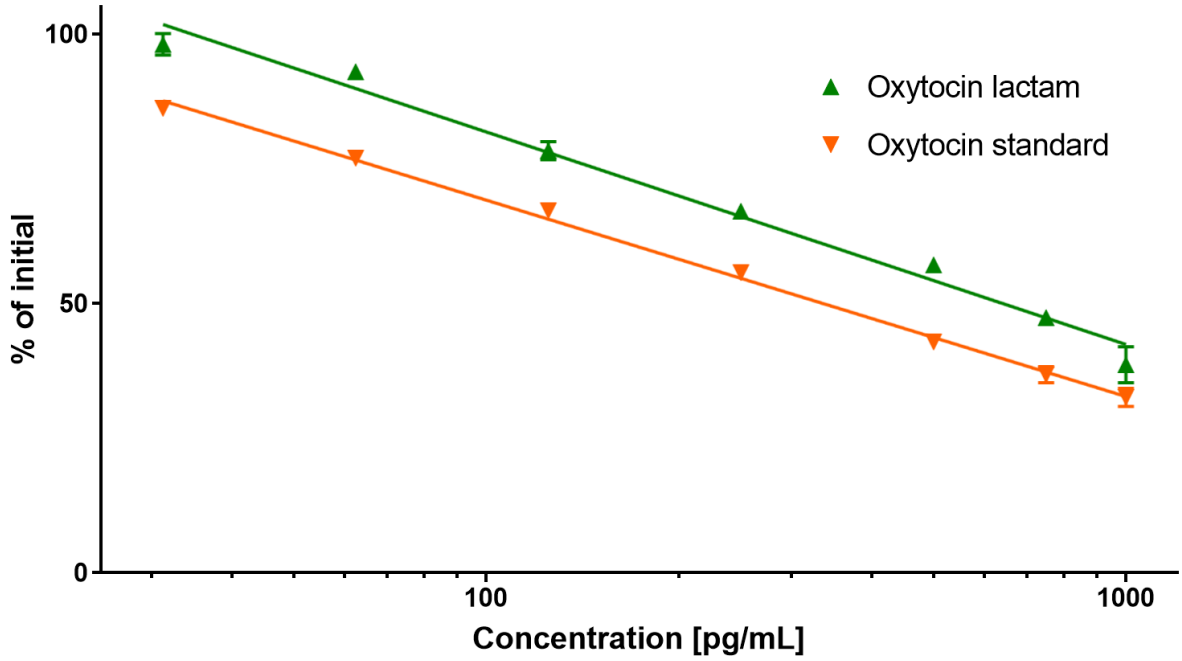


Figure S 32. Graph of normalized binding data for binding of compound 14 and oxytocin standard towards polyclonal rabbit antibody specific to oxytocin.

References:

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[2] *(a)* D. S. Nielsen, R. Lohman, H. N. Hoang, T. A. Hill, A. Jones, A. Lucke, and D. P. Fairlie, *ChemBioChem*, 2015, **16**, 2289-2293; *(b)* D. S. Nielsen, H. N. Hoang, R. Lohman, T. A. Hill, *et. al.*, *Angew. Chem. Int. Ed.*, 2014, **53**, 12059-12063.