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Supporting Information

Spontaneous Head-to-Tail Cyclization of Unprotected Linear Peptides with the KAHA Ligation

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1. General Methods

1.1. Reagents and solvents

Fmoc-amino acids with suitable side-chain protecting groups, HCTU (*O*-(1*H*-6-Chlorobenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluroniumhexafluorophosphate) and HATU (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) were purchased from Peptides International (Louisville, KY, USA) and ChemImpex (Wood Dale, IL, USA). COMU (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)-dimethylamino-morpholino-carbenium hexafluorophosphate) was obtained from Luxembourg Bio Technologies (Rehovot, Israel). Solvents for flash chromatography (EtOAc, MeOH) were of technical grade and distilled prior to use. HPLC grade CH_3CN from Sigma-Aldrich was used for analytical and preparative HPLC purification. DMF (> 99.8%) from Sigma-Aldrich was directly used without further purification for solid phase peptide synthesis. Other commercially available reagents and solvents were purchased from Sigma- Alrich (Buchs, Switzerland), Acros Organics (Geel, Belgium) and TCI Europe (Zwijndrecht, Belgium). Fmoc-(*S*)-5-oxaproline[1] and Boc-(*S*)-5-oxaproline[2] were prepared as previously reported by our group.

1.2. Characterization

^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX400, Bruker AVIII400 and Bruker AVIII600 spectrometers. Chemical shifts for ^{1}H NMR (400 and 600 MHz) and ^{13}C NMR (101 and 150 MHz) are expressed in parts per million and are referred to residual undeuterated solvent signals. Coupling constants are reported in Hertz (Hz) and the corresponding splitting patterns are indicated as follows: s, singlet; bs, broad singlet; d, doublet; dd, doublet of doublet; ddd, doublet of doublet of doublet; td, triplet of doublet; t, triplet; m, multiplet; appt d, apparent triplet of a doublet; d apt, doublet of an apparent triplet; d appt d, doublet of an apparent triplet of a doublet. High- resolution mass spectra were recorded by the Mass Service of the Laboratory of Organic Chemistry at ETH Zurich either with a Bruker maXis instrument (ESI-MS measurements) equipped with an ESI source and a Qq-TOF detector or with a Bruker solariX instrument (MALDI-FTICR-MS) using 4-hydroxy-α-cyanocinnamic acid as matrix.

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(1) Ogunkoya, A. O.; Pattabiraman, V. R.; Bode, J. W. *Angew. Chem. Int. Ed.* **2012**, *51*, 9693– 9697.
(2) Pattabiraman, V. R.; Ogunkoya, A. O.; Bode, J. W. *Angew. Chem. Int. Ed.* **2012**, *51*, 5114–5118.

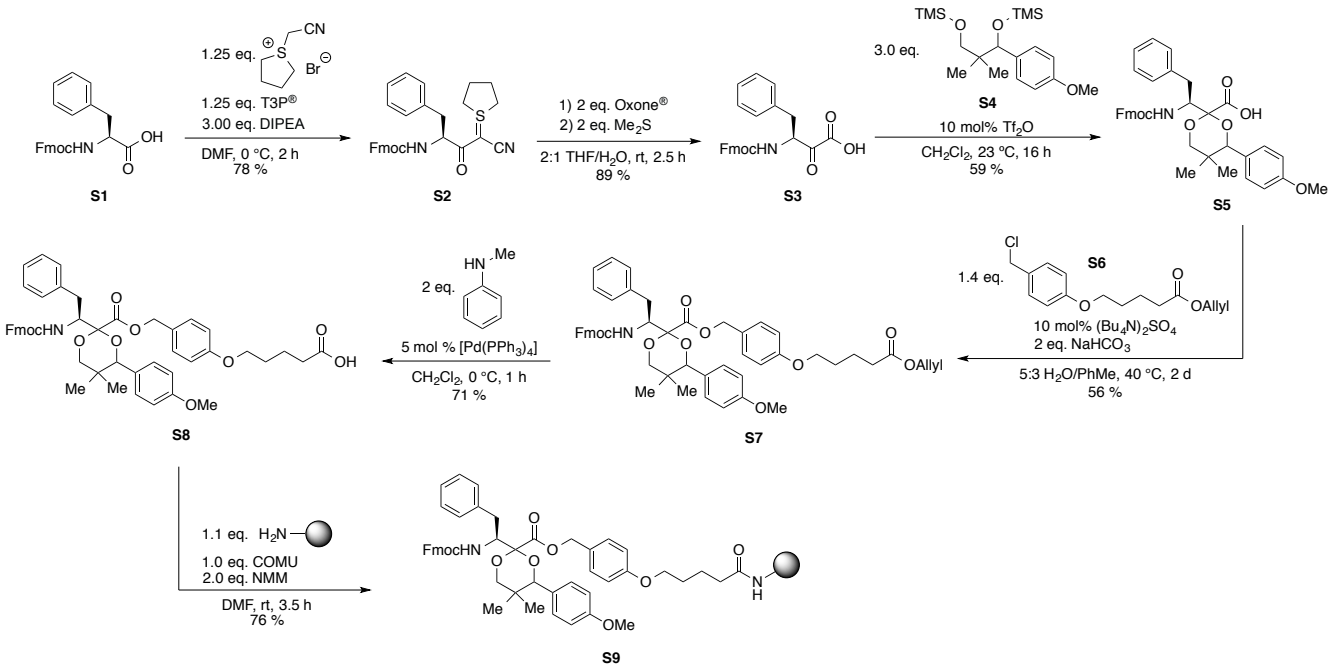
1.3. Reactions and purification

All reactions were performed using standard techniques under an atmosphere of N_2. Reactions and fractions from flash chromatography were monitored by thin layer chromatography using precoated glas plates (Merck, silica 60 F254) and visualized by staining with basic $KMnO_4$ solution. Flash chromatography was performed on Silicycle SiO_2 Type F60 (230-400 mesh) using a forced flow of air at 0.5-1.0 bar. Unless otherwise stated, peptides and protein fragments were analyzed and purified by reversed phase high performance liquid chromatography (RP-HPLC) on Jasco analytical and preparative instruments equipped with dual pumps, mixer and in-line degasser, a variable wavelength UV detector (simultaneous monitoring of the eluent at 220 nm, 254 nm and 301 nm) and a Rheodyne injector fitted with a 20 or 1000 µl injection loop. If required, the columns were heated using an Alltech column heater or a water bath (preparative HPLC). The mobile phase for RP-HPLC were Milipore-H_2O containing 0.1 % (v/v) TFA and HPLC grade CH_3CN containing 0.1 % (v/v) TFA. Analytical HPLC was performed on Shiseido Capcell Pak C18 MGII (5 µm, 4.6 mm I.D. x 250 mm) or Shiseido Capcell Pak C18 (UG 80, 5 µm, 4.6 mm I.D. x 250 mm) columns at a flow rate of 1 ml/min. Preparative HPLC was performed on Shiseido Capcell Pak MGII (5 µm, 20 mm I.D. x 250 mm) or Vydac 248MS C18 (10 µm, 22 mm I.D. x 250 mm) columns at a flow rate of 10 ml/min.

1.4. Solid phase peptide synthesis

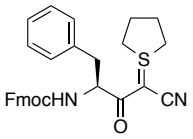
Peptides were synthesized on a Multisyntech Syro I parallel synthesizer using Fmoc SPPS chemistry. The following Fmoc amino acids with side-chain protection groups were used: Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc- His(1-Trt)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc- Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Val-OH. Fmoc-deprotections were performed with 20 % piperidine in DMF (2 x 6 min). Couplings were performed with Fmoc amino acid (4.0 equiv to resin substitution), HCTU (3.9 equiv) and *N*-methylmorpholine (8.0 equiv) in DMF for 30 min and repeated once. After coupling, unreacted free amine was capped by treatment with 20 % acetic anhydride and *N*-methylmorpholine (1.5 equiv to acetic anhydride).

2. Preparation of protected Fmoc-Leu α-ketoacid resin S9



Scheme S1: Preparation of protected Fmoc-Leu α-ketoacid resin **S9**.

2.1. Fmoc-(*S*)-Phe sulfur ylide S2



S2

Fmoc-(*S*)-phenylalanine (30.0 g, 77.4 mmol, 1.00 equiv) and 1-cyanomethyltetrahydrothiophenium bromide (20.1 g, 96.8 mmol, 1.25 equiv) were dissolved in DMF (500 mL). The mixture was cooled to 0 °C and *N*,*N*-diisopropylamine (40.5 mL, 232 mmol, 3.00 equiv) was added. T3P® (50% in EtOAc, 57.6 mL, 96.8 mmol, 1.25 equiv) was added within 1 h and the mixture was stirred for 1 h at 0 °C. H_2O (300 mL) was added and the mixture was extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (5 x 100 mL), H_2O (5 x 100 mL) and brine (2 x 100 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford **S2** (30.0 g, 60.4 mmol, 78 %) as an off-white solid, which was used for the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.4 Hz, 2H), 7.57 (t, *J* = 7.0 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.35 – 7.16 (m, 7H), 5.61 (d, J = 8.1 Hz, 1H), 4.93 (q, J = 7.1 Hz, 1H), 4.43 – 4.33 (m, 1H), 4.26 (dd, J = 10.4, 7.1 Hz, 1H), 4.24 – 4.12 (m, 1H), 3.40 – 2.97 (m, 6H), 2.59 – 2.44 (m, 2H), 2.10 – 1.93 (m, 2H).

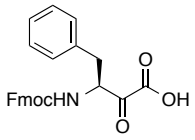
13C NMR (101 MHz, $CDCl_3$) δ 188.4, 155.5, 144.1, 141.4, 136.7, 129.8, 128.4, 127.7, 127.1, 126.8, 125.4, 125.3, 120.0, 119.2, 66.9, 56.9, 47.3, 45.2, 44.9, 39.6, 28.6, 28.5.

$[\alpha]_D^{28}$ = +26.2°(c = 0.4, $CHCl_3$).

IR (thin film): 3411, 3289, 2948, 2246, 2170, 1714, 1593, 1496, 1449, 1248, 1081, 1046, 844, 760 cm^{-1}.

HR-MS (ESI): calculated for $C_{30}H_{29}N_2O_3S$ $[M+H]^+$: 497.1893, found: 497.1886.

2.2. Fmoc-(*S*)-Phe α-ketoacid S3



S3

Fmoc-(*S*)-Phe sulfur ylide **S2** (10.0 g, 20.1 mmol, 1.0 equiv) was dissolved in a mixture of THF (320 mL) and H_2O (160 mL). Oxone® (24.8 g, 40.3 mmol, 2.0 equiv) was added and the mixture was stirred for 2.5 h at rt. Dimethyl sulfide (2.96 mL, 40.1 mmol, 2.0 equiv) was added and the mixture was stirred for 5 min. The reaction mixture was diluted with H_2O (100 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with saturated aqueous NH_4Cl (1 x 100 mL), H_2O (3 x 100 mL) and brine (1 x 50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was suspended in a mixture of CH_2Cl_2 (50 mL) and hexanes (50 mL). The white solid was collected by filtration to afford **S3** (7.5 g, 18 mmol, 89 %), which was used for the next step without further purification.

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.98 (d, *J* = 7.9 Hz, 1H), 7.88 (d, *J* = 7.5 Hz, 2H), 7.64 (dd, *J* = 7.5, 4.3 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.36 – 7.18 (m, 7H), 4.80 (ddd, *J* = 10.1, 8.0, 4.2 Hz, 1H), 4.33 – 4.08 (m, 3H), 3.13 (dd, *J* = 13.9, 4.2 Hz, 1H), 2.78 (dd, *J* = 13.9, 10.1 Hz, 1H).

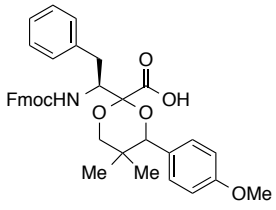
^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ 195.1, 163.0, 155.9, 143.7, 140.7, 137.5, 129.1, 128.3, 127.7, 127.1, 126.5, 125.2, 120.1, 65.8, 58.3, 46.6, 34.5.

$[\alpha]_D^{27}$ = +29.3°(c = 0.4, $CHCl_3$).

IR (thin film): 3315, 3063, 1706, 1520, 1450, 1335, 1254, 1033, 758 cm^{-1}.

HR-MS (ESI): calculated for $C_{25}H_{21}NNaO_5$ $[M+Na]^+$: 438.1312, found: 438.1306.

2.3. Protected Fmoc-Phe α-ketoacid S5



S5

Fmoc-(*S*)-Phe α-ketoacid **S3** (500 mg, 1.2 mmol, 1.0 equiv) and 2,2,2-trifluoroacetic anhydride (20 µL, 0.12 mmol, 0.1 equiv) were suspended in anhydrous CH_2Cl_2. A solution of 4-(4-methoxyphenyl)-2,2,5,5,8,8-hexamethyl-3,7-dioxa-2,8-disilanonane **S4**[3] (1.28 g, 3.6 mmol, 3.0 equiv) in anhydrous CH_2Cl_2 (15 mL) was added dropwise within 12 h and the mixture was stirred for further 4 h. The reaction mixture was washed with H_2O (10 mL) and brine (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (SiO_2, hexanes/EtOAc/MeOH/AcOH 50:40:3:1) to afford **S5** (inseparable mixture of diastereomers, 433 mg, 0.71 mmol, 59 %) as a white solid. Note: In the NMR data, not all signals of the diastereomers are resolved.

^{1}H NMR (400 MHz, ($CDCl_3$) δ 7.76 – 7.64 (m, 2H), 7.49 – 7.10 (m, 13H), 6.92 – 6.80 (m, 2H), 5.95 (s, broad, 1H), 5.32 – 5.18 (m, 1H), 4.73 – 4.38 (m, 2H), 4.36 – 3.94 (m, 3H), 3.95 – 3.82 (m, 1H), 3.83 – 3.76 (m, 3H), 3.76 – 3.61 (m, 1H), 3.58 – 3.31 (m, 1H), 2.97 – 2.75 (m, 1H), 1.02 – 0.90 (m, 3H), 0.71 – 0.61 (m, 3H).

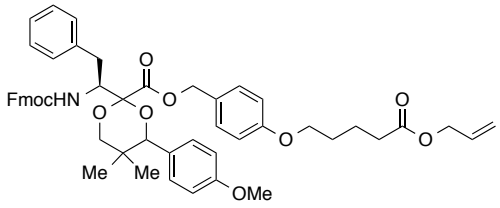
^{13}C NMR (101 MHz, ($CDCl_3$) δ 159.4, 156.9, 143.8, 141.3, 137.5, 129.4, 129.1, 128.8, 128.6, 127.7, 127.1, 126.7, 125.3, 120.0, 113.3, 100.6, 83.4, 82.8, 75.0, 74.7, 67.6, 57.8, 57.5, 55.4, 47.0, 39.5, 35.1, 34.04, 34.00, 21.90, 21.89, 18.7, 18.6.

IR (thin film): 3029, 2961, 2934, 1725, 1613, 1514, 1465, 1249, 1174, 1063, 1034, 909, 759 cm^{-1}.

HR-MS (ESI): calculated for $C_{37}H_{37}NNaO_7$ $[M+Na]^+$: 630.2462, found: 630.2460.

(3) Prepared as recently reported by our group: Wucherpfennig, T.G.; Pattabiraman, V. R.; Limberg, F. R. P.; Ruiz-Rodríguez, J.; Bode, J. W. *Angew. Chem. Int. Ed.* **2014**, DOI: 10.1002/anie.201407014.

2.4. Protected Fmoc-Phe α-ketoacid with linker allyl ester S7



S7

Protected Fmoc-Phe α-ketoacid **S5** (3.54 g, 5.82 mmol, 1.0 equiv), allyl 5-(4-(chloromethyl) phenoxy) pentanoate **S6**[3] (2.31 g, 8.17 mmol, 1.4 equiv) and tetrabutylammonium sulfate (as 50 % aqueous solution, 0.69 g, 1.19 mmol, 0.1 equiv) were stirred at 40 °C in a biphasic mixture of toluene (6 mL) and saturated aqueous $NaHCO_3$ solution (10 mL, 978 mg $NaHCO_3$, 11.6 mmol, 2.0 equiv) for 2 d. The mixture was extracted with EtOAc (30 mL) and the organic phase was washed with brine (10 mL), dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure and the crude material purified by flash chromatography (SiO_2, hexanes/EtOAc 3:1 to 5:2) to afford **S7** (inseparable mixture of diastereomers, 2.75 g, 3.22 mmol, 56 %) as off-white foam. Note: In the NMR data, not all signals of the diastereomers are resolved.

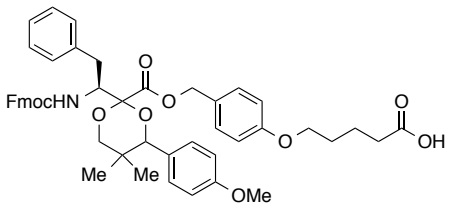
^{1}H NMR (400 MHz, ($CDCl_3$) δ 7.79 – 7.67 (m, 2H), 7.51 – 7.07 (m, 15H), 6.92 – 6.70 (m, 4H), 6.00 – 5.85 (m, 1H), 5.36 – 4.97 (m, 5H), 4.67 – 4.35 (m, 4H), 4.21 – 4.10 (m, 1H), 4.10 – 3.92 (m, 2H), 3.91 – 3.62 (m, 7H), 3.45 – 3.21 (m, 1H), 2.87 – 2.68 (m, 1H), 2.44 – 2.35 (m, 2H), 1.85 – 1.71 (m, 4H), 1.05 – 0.90 (m, 3H), 0.71 – 0.58 (m, 3H).

^{13}C NMR (101 MHz, ($CDCl_3$) δ 173.1, 168.92, 168.88, 159.35, 159.33, 159.26, 159.20, 155.76, 155.74, 144.22, 144.19, 144.06, 144.03, 141.28, 141.26, 137.74, 137.72, 132.4, 130.8, 130.6, 129.6, 129.5, 129.2, 128.8, 128.45, 128.43, 127.74, 127.65, 127.56, 127.12, 127.11, 127.07, 126.5, 125.45, 125.41, 125.35, 125.30, 119.9, 118.4, 114.62, 114.60, 114.54, 114.51, 113.24, 113.20, 101.2, 101.0, 83.2, 82.4, 74.9, 74.6, 67.49, 67.45, 67.44, 67.39, 67.08, 67.04, 65.2, 57.6, 57.2, 55.41, 55.39, 47.17, 47.14, 35.5, 35.4, 34.02, 33.98, 33.95, 28.72 22.0, 21.7, 18.7, 18.6.

IR (thin film): 2953, 2873, 1737, 1613, 1514, 1451, 1248, 1173, 1063, 834, 760 cm^{-1}.

HR-MS (ESI): calculated for $C_{52}H_{56}NO_{10}$ $[M+H]^+$: 854.3899, found: 854.3893.

2.5. Protected Fmoc-Phe α-ketoacid with linker S8



S8

Allyl ester **S7** (439 mg, 514 µmol, 1.0 equiv) and freshly distilled *N*-methylaniline (110 mg, 1.03 mmol, 2.0 equiv) were dissolved in degassed CH_2Cl_2. The solution was cooled to 0 °C and $[Pd(PPh_3)_4]$ (29.7 mg, 25.7 µmol, 0.05 equiv) was added. The mixture was stirred for 1 h at 0 °C. Saturated aqueous NH_4Cl (10 mL) and the mixture was extracted with EtOAc (20 mL). The organic layer was separated, washed with brine (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude material was purified by flash chromatography (SiO_2, CH_2Cl_2 to CH_2Cl_2/MeOH 9:1) to afford **S8** (295 mg, 363 µmol, 71 %) as off-white foam. Note: In the NMR data, not all signals of the diastereomers are resolved.

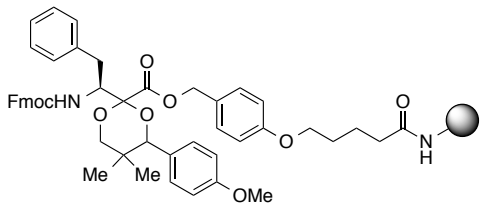
^{1}H NMR (400 MHz, ($CDCl_3$) δ 7.82 – 7.66 (m, 2H), 7.54 – 7.00 (m, 15H), 6.96 – 6.67 (m, 4H), 5.48 – 5.17 (m, 1H), 5.16 – 5.00 (m, 2H), 4.70 – 4.29 (m, 2H), 4.25 – 3.91 (m, 3H), 3.91 – 3.60 (m, 7H), 3.48 – 3.21 (m, 1H), 2.87 – 2.68 (m, 1H), 2.49 – 2.35 (m, 2H), 1.88 – 1.70 (m, 4H), 1.01 – 0.88 (m, 3H), 0.68 – 0.58 (m, 3H).

^{13}C NMR (101 MHz, ($CDCl_3$) δ 178.6, 169.0, 168.9, 159.34, 159.33, 159.2, 159.2, 155.80, 155.76, 144.23, 144.20, 144.05, 144.02, 141.29, 141.26, 137.74, 137.72, 130.8, 130.73, 130.65, 130.63, 129.6, 129.5, 129.2, 128.8, 128.5, 128.4, 127.8, 127.7, 127.6, 127.12, 127.08, 126.54, 125.46, 125.43, 125.36, 125.32, 120.0, 114.63, 114.61, 114.55, 114.52, 113.3, 113.2, 101.2, 101.1, 83.2, 82.4, 74.9, 74.6, 67.5, 67.42, 67.41, 67.10, 67.07, 57.6, 57.2, 55.42, 55.40, 47.2, 47.1, 35.5, 35.4, 34.03, 33.98, 33.6, 28.6, 22.0, 21.6, 18.7, 18.6.

IR (thin film): 2956, 1737, 1712, 1712, 1613, 1514, 1451, 1249, 1174, 1062, 910, 760 cm^{-1}.

HR-MS (ESI): calculated for $C_{49}H_{52}NO_{10}$ $[M+H]^+$: 814.3586, found: 814.3573.

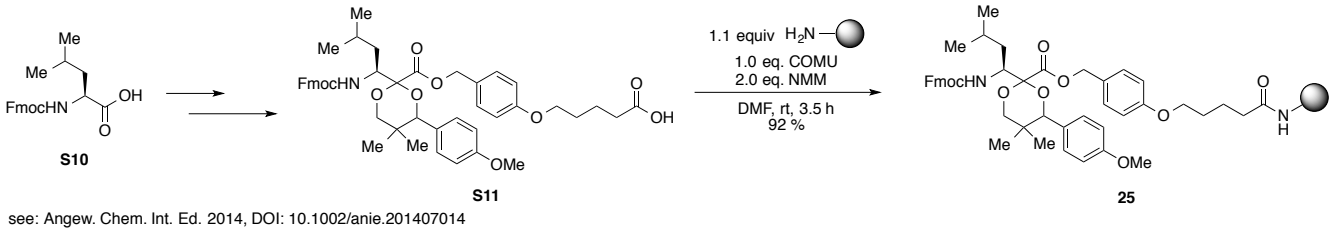
2.6. Protected Fmoc-Phe α-ketoacid resin S9



S9

Aminomethyl polystyrene resin (300 mg, loading according to manufacturer: 1.17 mmol/g, 424 µmol, 1.1 equiv) was placed in a plastic syringe equipped with a fritted filter, preswelled for 15 min with DMF and the solvent discharged. In a separate vial, protected Fmoc-Phe α-ketoacid with linker **S8** (300 mg, 368 µmol, 1.0 equiv) and COMU (158 mg 386 µmol, 1.00 equiv) were dissolved in 4 ml DMF and *N*-methylmorpholine (74.6 mg, 737 µmol, 2.0 equiv) was added. The yellow solution was immediately added to the resin and shaken for 3.5 h at rt. The solvent was discharged, the resin washed with DMF (3 x) and excess amine groups on the solid support capped by treatment of the resin with a mixture of Ac_2O/*N*-methylmorpholine/DMF 1:1:4 (2 x 10 min). After more washings (3 x DMF, 3 x CH_2Cl_2) the resin was dried under a forced flow of N_2 to afford resin **S9** (586 mg). The loading was determined to be 0.501 mmol/g (76% based on **S9**) by UV(λ = 304 nm) quantification of dibenzofulvene group released after treating with 2% DBU in DMF.

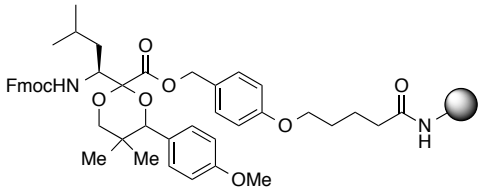
3. Preparation of protected Fmoc-Leu α-ketoacid resin 25



see: Angew. Chem. Int. Ed. 2014, DOI: 10.1002/anie.201407014

Scheme S2: Preparation of protected Fmoc-Leu α-ketoacid resin **25**.

3.1. Protected Fmoc-Leu α-ketoacid resin 25



25

Aminomethyl polystyrene resin resin (1.20 g, loading according to manufacturer: 1.17 mmol/g, 1.41 mmol, 1.1 equiv) was placed in a plastic syringe equipped with a fritted filter, preswelled for 15 min with DMF and the solvent discharged. In a separate vial, protected Fmoc-Leu α-ketoacid with linker **S11**[3] (1.00 g, 1.28 mmol, 1.0 equiv) and COMU (549 mg 1.28 mmol, 1.00 equiv) were dissolved in 10 ml DMF and *N*-methylmorpholine (259 mg, 2.56 mmol, 2.0 equiv) was added. The yellow solution was immediately added to the resin and shaken for 3.5 h at rt. The solvent was discharged, the resin washed with DMF (3 x) and excess amine groups on the solid support capped by treatment of the resin with a mixture of Ac_2O/*N*-methylmorpholine/DMF 1:1:4 (2 x 10 min). After more washings (3 x DMF, 3 x CH_2Cl_2) the resin was dried under a forced flow of N_2 to afford resin **25** (2.05 g). The loading was determined to be 0.576 mmol/g (92% based on **S11**) by UV(λ = 304 nm) quantification of dibenzofulvene group released after treating with 2% DBU in DMF.

4. Library synthesis

4.1. General methods

4.1.1. General method for solid phase peptide synthesis

The peptides were synthesized in parallel by a Syro II Peptide Parallel Synthesis System (Biotage) on a 50 µmol scale following standard solid phase Fmoc-peptide chemistry: All reagents were of peptide grade. Fmoc-Hse(Trt)-OH was purchased from Chem-Impex, the resins H-Leu-2-Cl-Trt- and H-Phe-2-Cl-Trt were purchased from Merck Biosciences. A typical procedure[4] for peptide elongation involved for each cycle the removal of Fmoc-protecting group with 20% piperidine/DMF and a double coupling (2x 40 min) with standard coupling reagents (3.6 eq) in DMF.

4.1.2. General method for HPLC Analyses and purification

Analytical HPLC retention times (RT, in minutes) were determined on an Ultimate 3000 RS (Thermo Scientific) using Xbridge BEH C8 XP(Waters Part No 186006041), 50x2.1 mm, 2.5 µm particles size, 130Å with the following solvents A (H_2O+0.1% TFA) and B(CH_3CN + 0.085% TFA) and the gradient: 0-0.5 min: 97% A, 3% B; 2.8 min-3.18 min: 3% A, 97%B; 3.2-3.3 min: 97% A, 3%B. Flow rate 1.4 mL/min. Column oven temperature 55°C. All peptides were purified by semi-automatic reverse phase HPLC-MS (C18 Waters XSelect) with semi-automatic adapted gradients from 30% to 70% CH_3CN/H_2O with 0.1% TFA over 15 min.

4.1.3. General method for side chain protected cyclization (Method A)

After completion of the peptide elongation (50 µmol scale), the 2-Cl-Trt resins were washed with CH_2Cl_2 and treated with 1% TFA in CH_2Cl_2 (3 x 1 ml, 2 min). The filtrates were neutralized with DIPEA in CH_2Cl_2 and evaporated to give the linear protected peptides. The products were cyclized overnight at rt in DMF (8 mL, 6.25 mM) with DIPEA (6 eq) and HATU (2 eq). After evaporation of the solvent and co-evaporation with toluene, the cyclic protected peptides were treated with a mixture of 95:2.5:2.5 TFA/TIS/H_2O (7 ml) at rt for 2.5 h. The volatiles were partially evaporated and the crude cyclic peptides were precipitated by addition of Et_2O/*n*-pentane 1:1 precooled to 0 °C. After centrifugation and removal of the solvents, the crude peptides were washed twice with Et_2O/*n*-pentane 1:1 (7 ml). The solid residues were air-dried and dissolved in 4:1 DMSO/H_2O before purification.

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(4) Atherton, E.; Sheppard, R. C. Solid-phase Peptide Synthesis-A Practical Approach; IRL Press: Oxford, 1989.

4.1.4. General method for KAHA cyclization (Method B)

For SPPS, protected Fmoc-Phe α-ketoacid resin **S9** (loading: 0.50 mmol/g) or protected Fmoc-Leu α-ketoacid resin **5** (loading 0.58 mmol/g) were used. After completion of the peptide elongation (50 µmol scale), the resins were treated with a mixture of 95:2.5:2.5 TFA/DODT/H_2O (7 mL) at rt for 3 h. The volatiles were partially evaporated and the crude linear peptides were precipitated by addition of Et_2O/*n*-pentane 1:1 precooled to 0 °C. After centrifugation and removal of the solvents, the crude peptides were washed 3 times with Et_2O/*n*-pentane 1:1 (7 ml). The solid residues were air-dried and dissolved in a mixture of CH_3CN (5.33 mL) and 0.05 M aqueous oxalic acid (2.66 mL). The mixtures were stirred at 50 °C for 18 h. After cooling to rt, aqueous ammonia (25 % in H_2O, 978 µL, 13.1 mmol) was added and the mixtures were stirred at rt for 3 h. After addition of TFA (978 µL, 12.7 mmol) the volatiles were removed and the crude peptides were dissolved in 4:1 DMSO/H_2O before purification.

4.2. Summary table S1

Compound	Sequence	Mw (g/mol)	Method A				Method B			
			crude purity (%)	yield			crude purity (%)	yield		
				mg	µmol	%		mg	µmol	%
amide-**S13**	cyclo(T§TILTPGL)	797.0	63	2.0	2.5	5	46	4.3	5.3	11
amide-**S14**	cyclo(T§FGPLAPF)	831.0	74	21.0	25.3	51	61	4.9	5.9	12
amide-**S15**	cyclo(T§ASYSSKPF)	969.1	62	15.9	16.4	33	40	2.4	2.5	5
amide-**S16**	cyclo(T§pPTRLFPL)	1023.2	26	11.4	11.2	22	66	18.7	18.3	37
amide-**S17**	cyclo(T§AATRLFPL)	971.2	24	8.5	8.7	17	55	11.1	11.4	23
amide-**4**	cyclo(T§WEFpPFEWL)	1333.5	48	23.4	17.5	35	68	12.9	9.70	19
amide-**S18**	cyclo(T§WEFAAFEWL)	1281.4	42	15.1	11.8	24	43	8.3	6.5	13
amide-**S19**	cyclo(T§DWYASTWTSGDF)	1518.6	46	7.3	4.8	10	69	6.0	4.0	8
amide-**28**	cyclo(T§NIHWpPVSNKAL)	1458.7	40	31.1	21.3	43	54	15.4	10.6	21
amide-**S20**	cyclo(T§NIHWpKVSNKAL)	1489.7	45	37.3	25.1	50	41	20.4	13.7	27
amide-**S21**	cyclo(T§NIHWAKVSNKAL)	1463.7	4	4.1	2.8	6	11	5.0	3.4	7
amide-**S22**	cyclo(T§YQKLQWFNpYAKF)	1816.1	56	16.0	8.81	18	55	8.7	4.8	10
amide-**S23**	cyclo(T§YQKLQWFNAAAKF)	1698.0	6	2.7	1.6	3	51	9.9	5.8	12
amide-**S24**	cyclo(T§WNPFKAQSpPGYLKL)	1829.1	33	12.6	6.87	14	59	12.9	7.07	14
amide-**S25**	cyclo(T§WNPFKAQSpAGYLKL)	1803.1	29	17.6	9.74	19	53	9.7	5.4	11
amide-**S26**	cyclo(T§WNPFKAQSKAGYLKL)	1834.2	17	2.7	1.5	3	50	7.0	3.8	8
amide-**S27**	cyclo(T§RTNpPKKEKVGpKRL)	1831.2	31	21.6	11.8	24	75	38.7	21.1	42
amide-**S28**	cyclo(T§RTNAAKKEKVGpKRL)	1779.1	35	18.9	10.6	21	57	11.4	6.42	13
amide-**S29**	cyclo(T§VFQpPFT§RKRFKGRPFL)	2204.7	16	11.6	5.27	11	68	22.9	10.4	21
amide-**S30**	cyclo(T§VFQpYFT§RKRFKGRPFL)	2270.7	18	26.0	11.5	23	83	29.4	12.9	26
amide-**S31**	cyclo(T§VFQAAFT§RKRFKGRPFL)	2152.6	20	11.7	5.42	11	60	9.1	4.2	8
amide-**S32**	cyclo(T§WPRLQFT§HRLpPAEWFKAL)	2476.9	24	14.3	5.79	12	54	13.0	5.23	10
amide-**S33**	cyclo(T§WPRLQFT§HRLpKAEWFKAL)	2508.9	21	9.9	3.9	8	32	20.2	8.03	16
amide-**S34**	cyclo(T§WPRLQFT§HRLAAAEWFKAL)	2424.8	21	0	0	0	47	5.2	2.1	4

4.3. Synthesis and analytical data

In the following sections HPLC chromatograms for each step of Method A and Method B are shown to compare both methods at relevant stages of the process.

4.3.1. Cyclo(HseTILTPGL) S13

S13 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 2.0 mg (2.5 µmol; 5 %; Method A); 4.3 mg (5.3 µmol; 11 %; Method B).

Method A

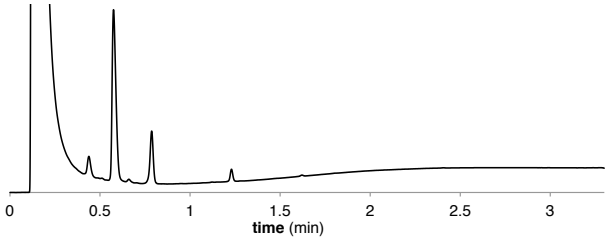


Figure S1-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

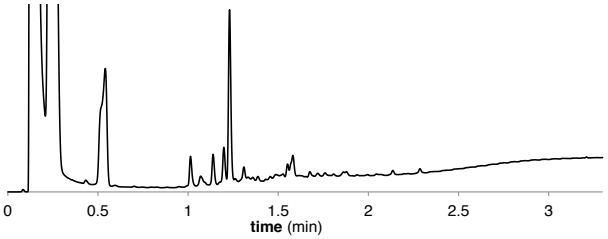


Figure S1-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

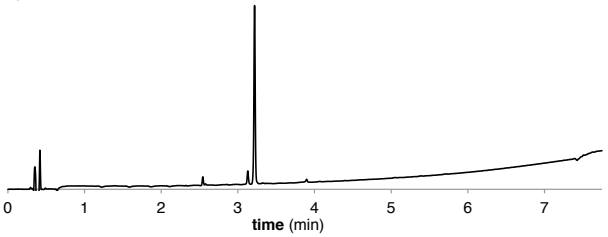


Figure S1-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S13**.

Method B

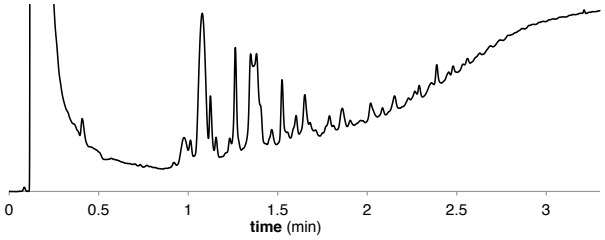


Figure S1-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

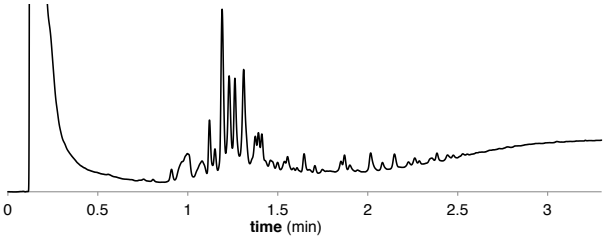


Figure S1-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

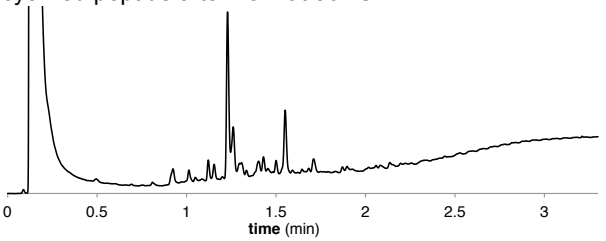


Figure S1-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

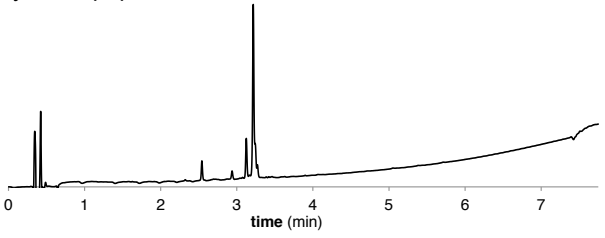


Figure S1-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S13**.

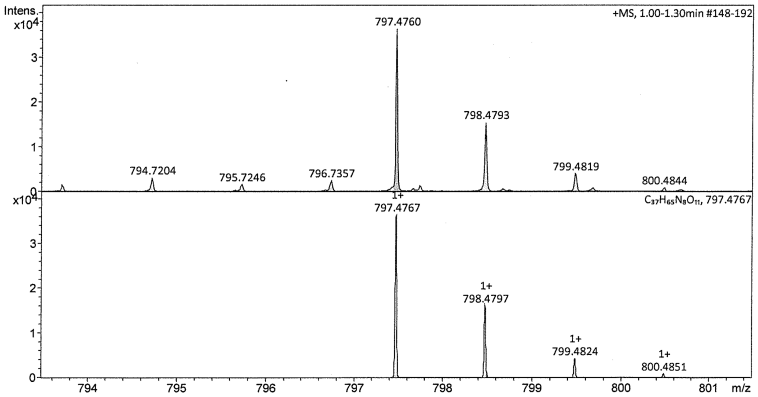


Figure S1-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S13**.

HR-MS (ESI): calculated molecular weight ($C_{37}H_{65}N_8O_{11}$) $[M+H]^+$: 797.4767 m/z; found: 797.4760 m/z.

4.3.2. Cyclo(HseFGPLAPF) S14

S14 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 21 mg (25.3 µmol; 51 %; Method A); 4.9 mg (5.9 µmol; 12 %; Method B).

Method A

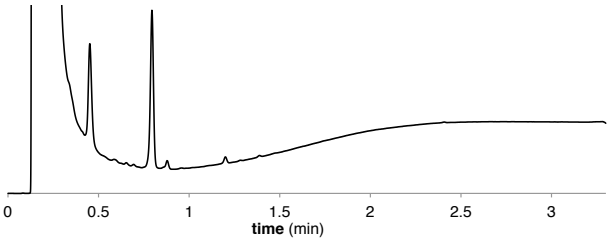


Figure S2-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

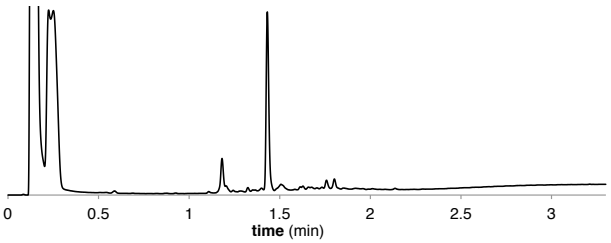


Figure S2-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

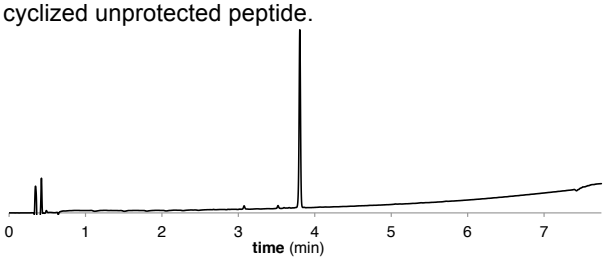


Figure S2-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S14**.

Method B

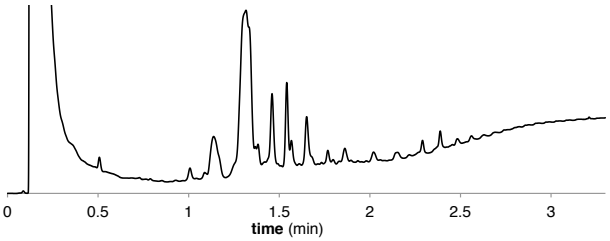


Figure S2-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

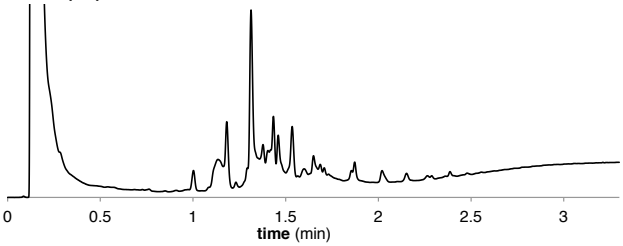


Figure S2-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

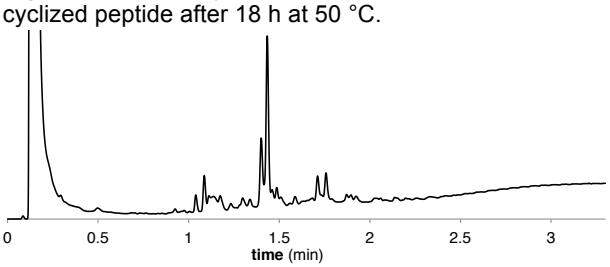


Figure S2-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

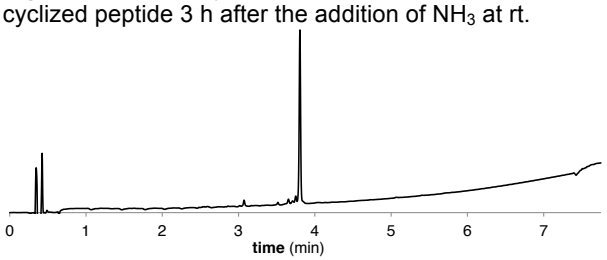


Figure S2-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S14**.

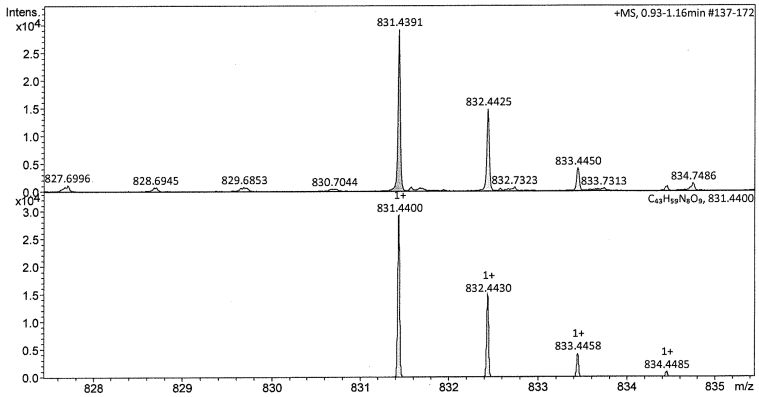


Figure S2-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S14**.

HR-MS (ESI): calculated molecular weight ($C_{43}H_{59}N_8O_9$) $[M+H]^+$: 831.4400 m/z; found: 831.4391 m/z.

4.3.3. Cyclo(HseASYSSKPF) S15

S15 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B).Yield: 15.9 mg (16.4 µmol; 33 %; Method A); 2.4 mg (2.5 µmol; 5 %; Method B).

Method A

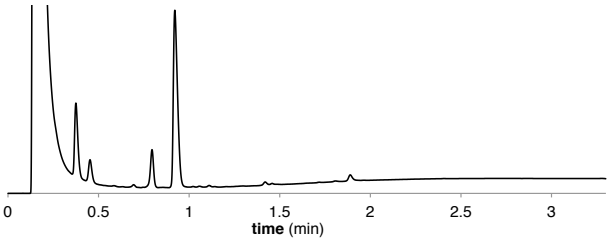


Figure S3-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

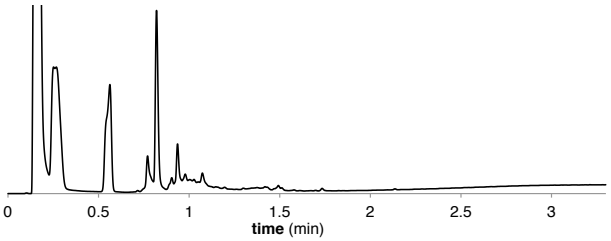


Figure S3-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

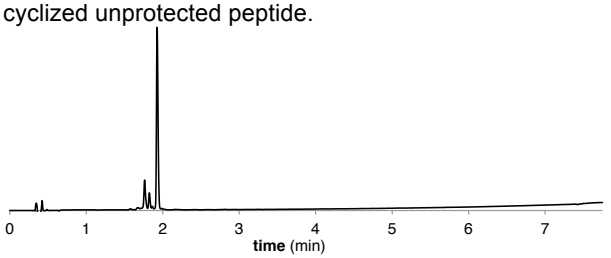


Figure S3-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S15**.

Method B

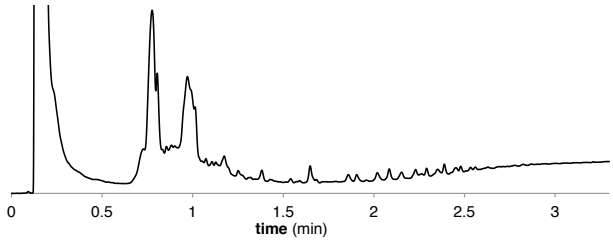


Figure S3-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

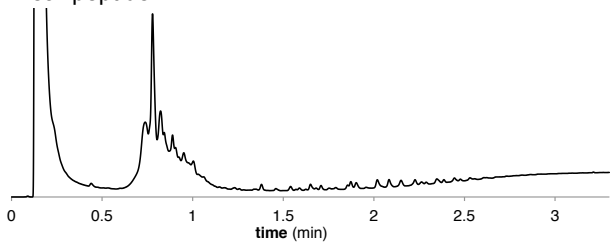


Figure S3-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

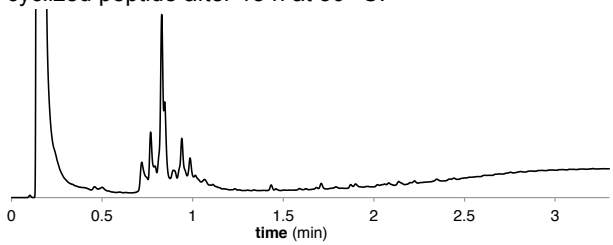


Figure S3-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

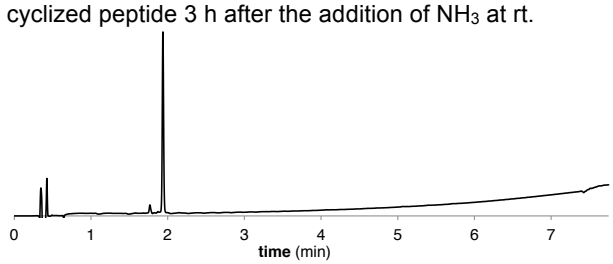


Figure S3-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S15**.

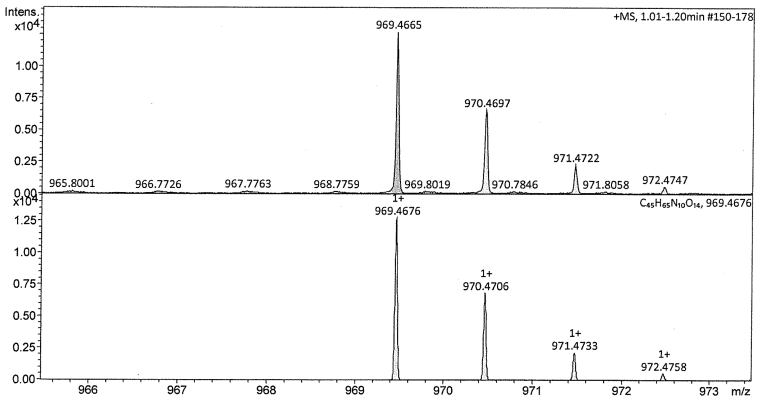


Figure S3-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S15**.

HR-MS (ESI): calculated molecular weight ($C_{45}H_{65}N_{10}O_{14}$) $[M+H]^+$: 969.4676 m/z; found: 969.4665 m/z.

4.3.4. Cyclo(Hse-pPTRLFPL) S16

S16 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 11.4 mg (11.2 µmol; 22 %; Method A); 18.7 mg (18.3 µmol; 37 %; Method B).

Method A

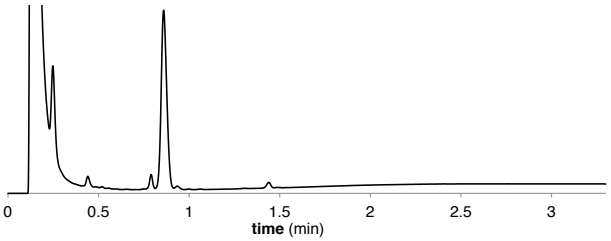


Figure S4-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

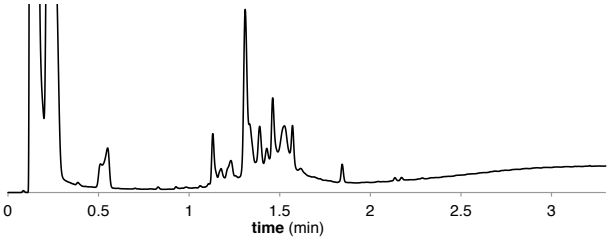


Figure S4-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

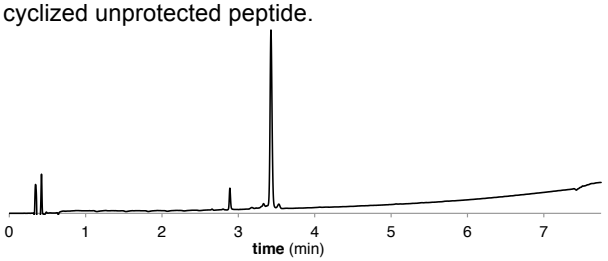


Figure S4-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S16**.

Method B

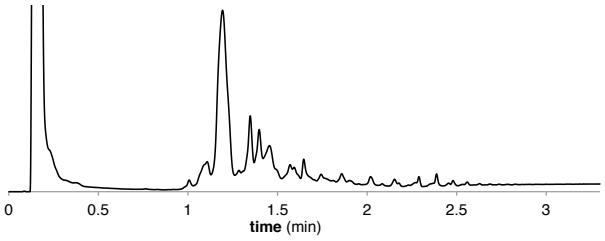


Figure S4-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

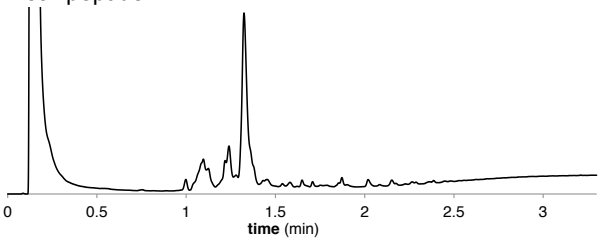


Figure S4-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

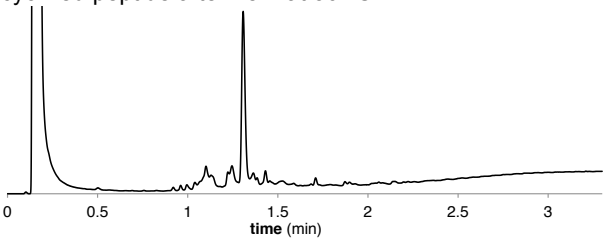


Figure S4-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

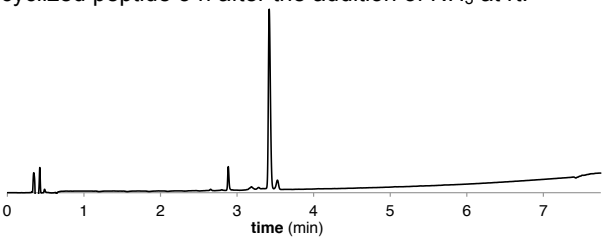


Figure S4-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S16**.

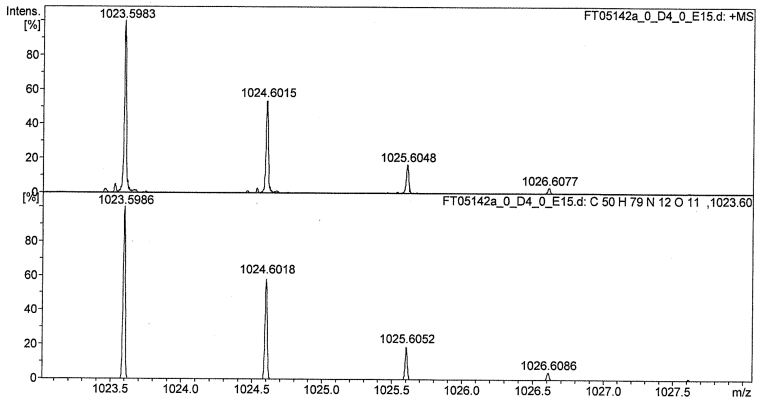


Figure S4-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S16**.

HR-MS (MALDI): calculated molecular weight ($C_{50}H_{79}N_{12}O_{11}$) $[M+H]^+$: 1023.5986 m/z; found: 1023.5983 m/z.

4.3.5. Cyclo(Hse HseAATRLFPL) S17

S17 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 8.5 mg (8.7 µmol; 17 %; Method A); 11.1 mg (11.4 µmol; 23 %; Method B).

Method A

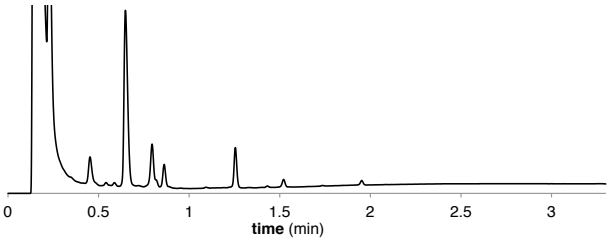


Figure S5-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

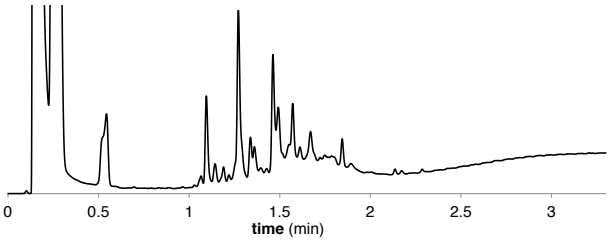


Figure S5-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

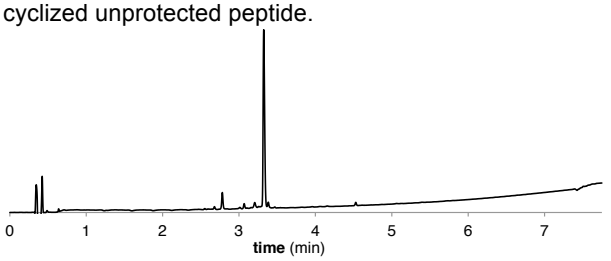


Figure S5-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S17**.

Method B

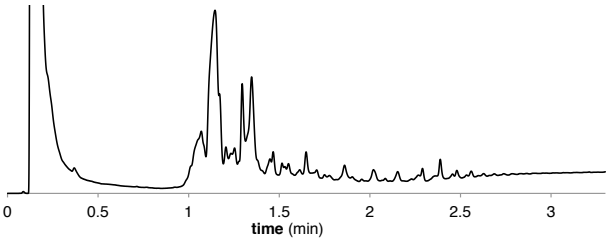


Figure S5-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

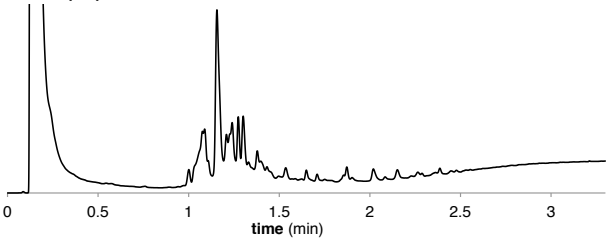


Figure S5-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

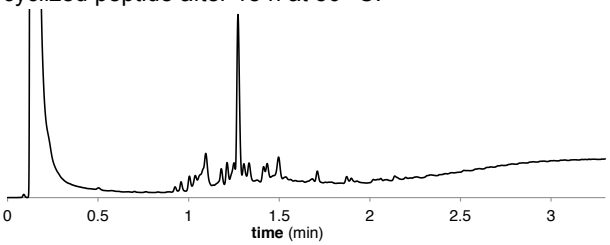


Figure S5-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

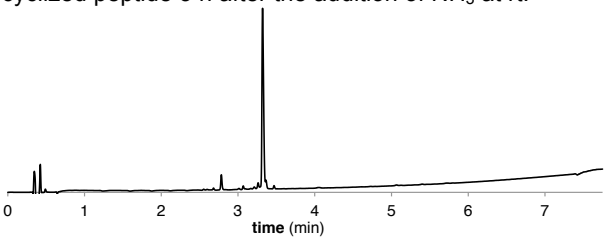


Figure S5-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S17**.

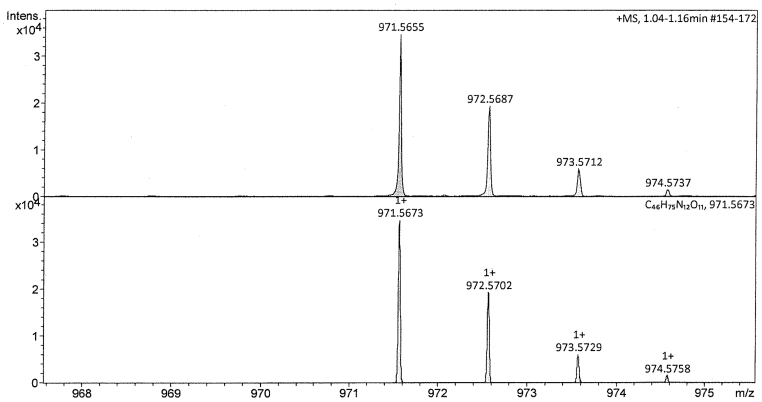


Figure S5-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S17**.

HR-MS (ESI): calculated molecular weight ($C_{46}H_{75}N_{12}O_{11}$) $[M+H]^+$: 971.5673 m/z; found: 971.5655 m/z.

4.3.6. Cyclo(HseWEFpPFEWL) 4

4 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 23.4 mg (17.5 µmol; 35 %; Method A); 12.9 mg (9.7 µmol; 19 %; Method B).

Method A

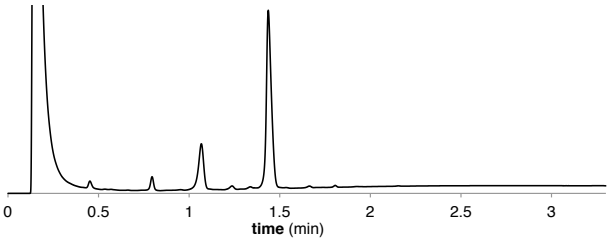


Figure S6-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

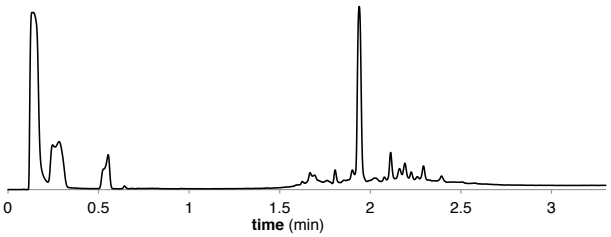


Figure S6-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

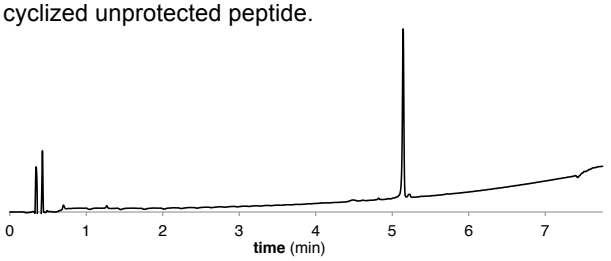


Figure S6-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**4**.

Method B

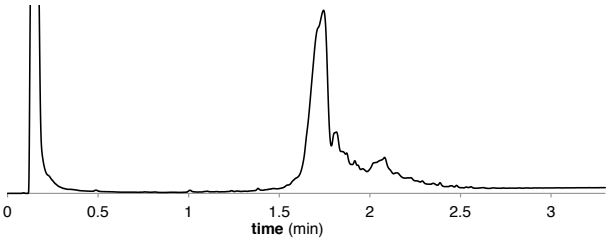


Figure S6-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

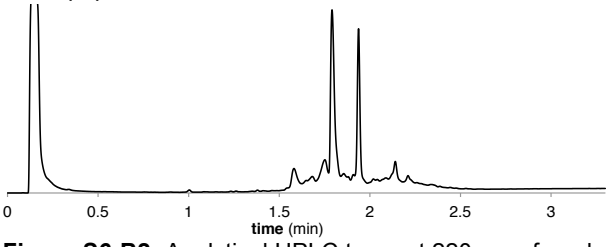


Figure S6-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

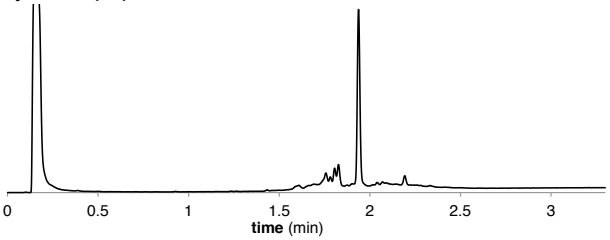


Figure S6-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

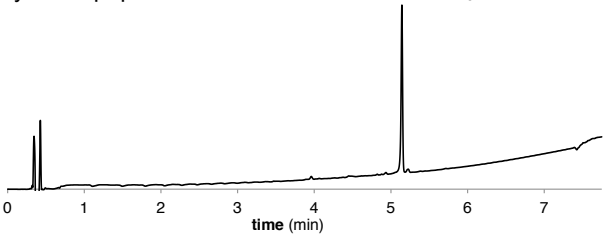


Figure S6-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**4**.

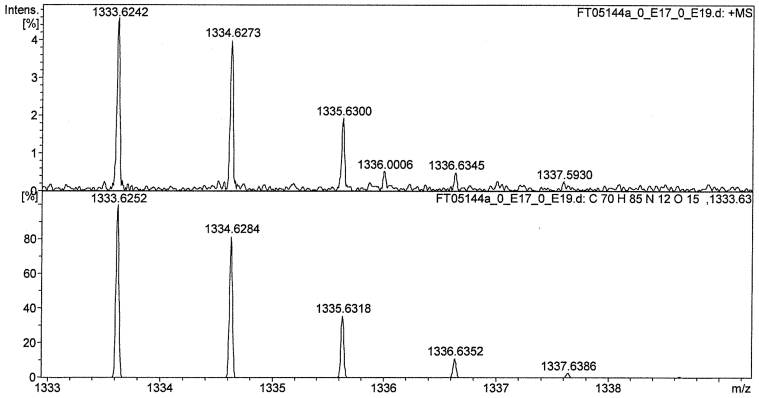


Figure S6-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**4**.

HR-MS (MALDI): calculated molecular weight ($C_{70}H_{85}N_{12}O_{15}$) $[M+H]^+$: 1333.6252 m/z; found: 1333.6242 m/z.

4.3.7. Cyclo(HseWEFAAFEWL) S18

S18 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 15.1 mg (11.8 µmol; 24 %; Method A); 8.3 mg (6.5 µmol; 13 %; Method B).

Method A

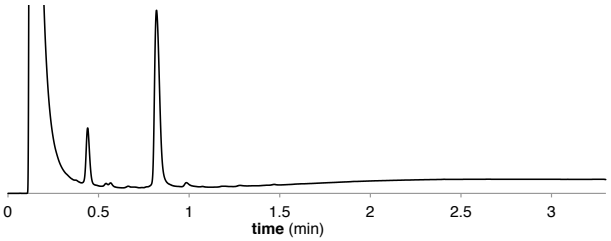


Figure S7-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

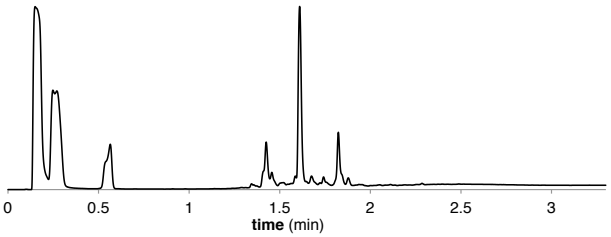


Figure S7-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

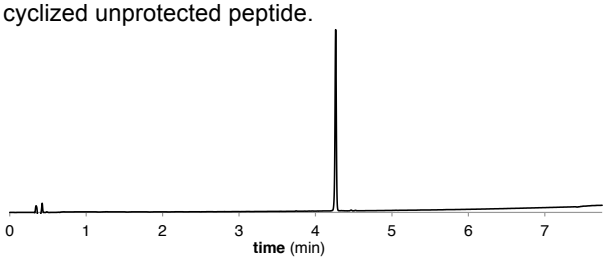


Figure S7-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S18**.

Method B

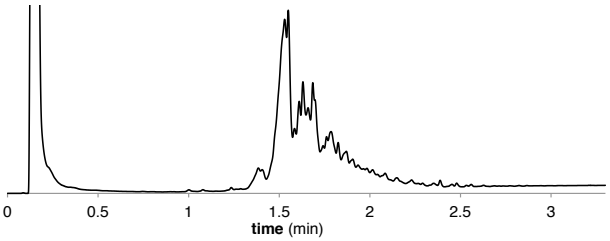


Figure S7-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

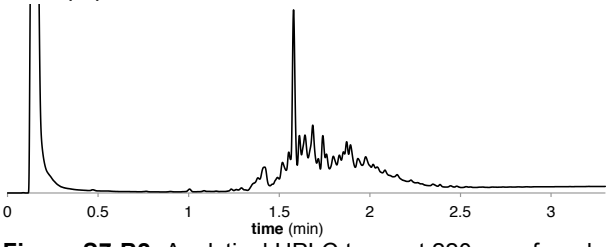


Figure S7-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

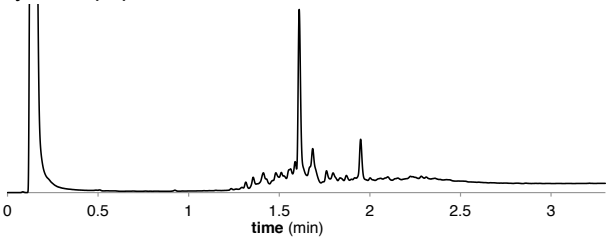


Figure S7-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

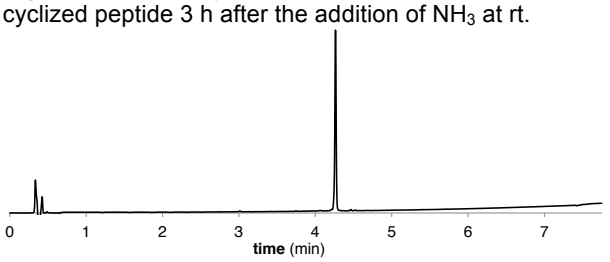


Figure S7-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S18**.

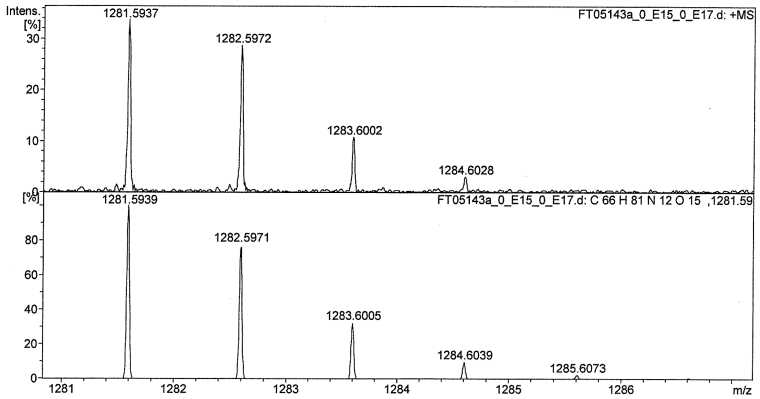


Figure S7-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S18**.

HR-MS (MALDI): calculated molecular weight ($C_{66}H_{81}N_{12}O_{15}$) $[M+H]^+$: 1281.5939 m/z; found: 1281.5937 m/z.

4.3.8. Cyclo(HseDWYASTWTSGDF) S19

S19 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 7.3 mg (4.8 µmol; 10 %; Method A); 6.0 mg (4.0 µmol; 8 %; Method B).

Method A

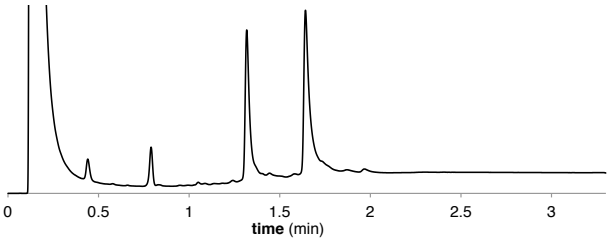


Figure S8-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

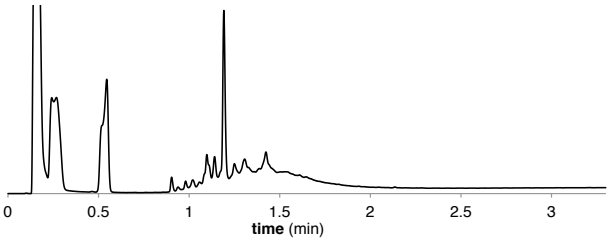


Figure S8-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

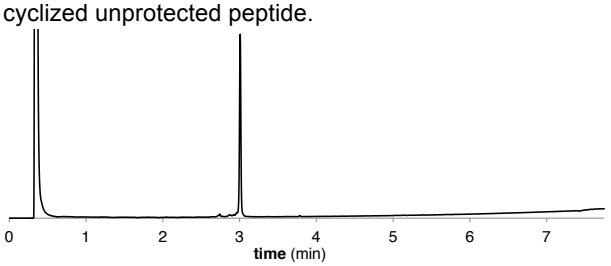


Figure S8-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S19**.

Method B

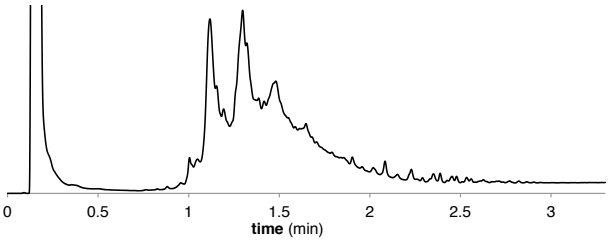


Figure S8-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

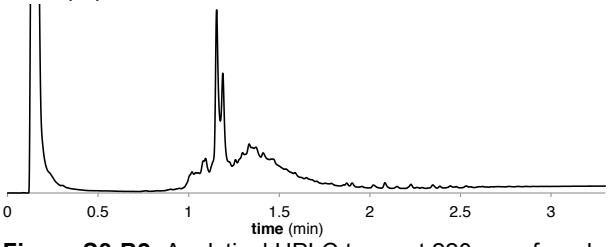


Figure S8-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

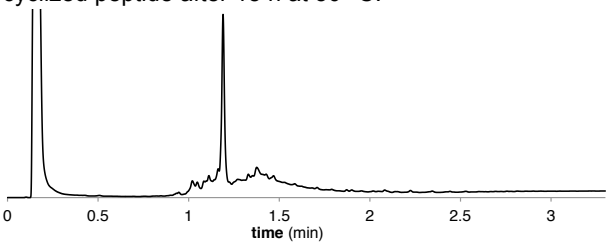


Figure S8-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

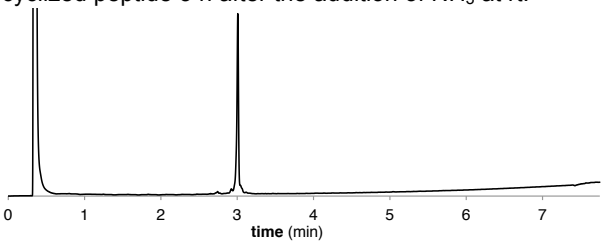


Figure S8-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S19**.

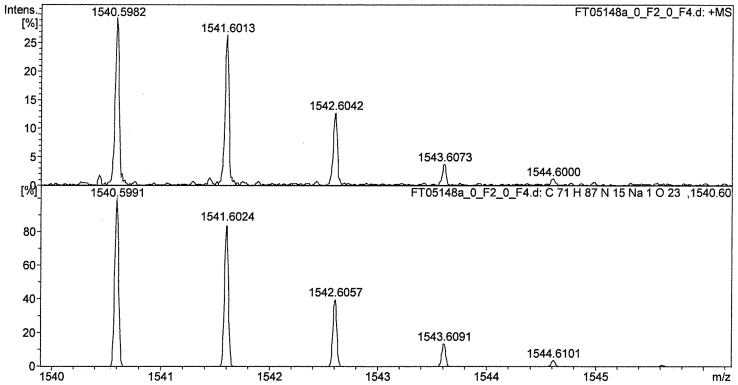


Figure S8-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S19**.

HR-MS (MALDI): calculated molecular weight ($C_{71}H_{87}N_{15}NaO_{23}$) $[M+Na]^+$: 1540.5991 m/z; found: 1540.5982 m/z.

4.3.9. Cyclo(HseNlHWpPVSNKAF) 28

8 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 31.1 mg (21.3 µmol; 43% %; Method A); 15.4 mg (10.6 µmol; 21%; Method B).

Method A

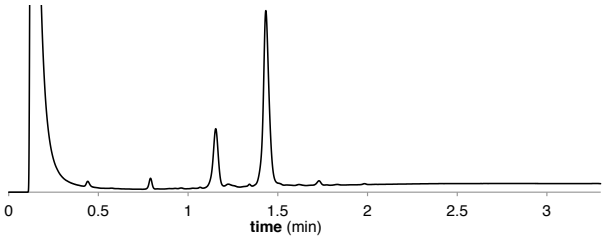


Figure S9-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

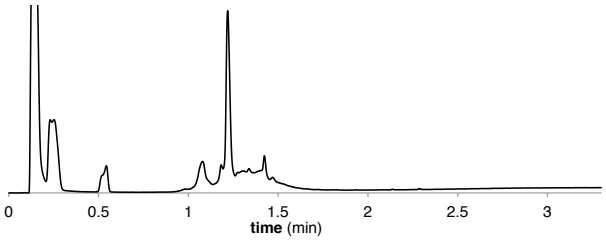


Figure S9-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

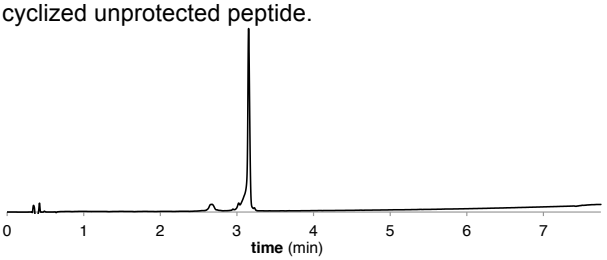


Figure S9-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**28**.

Method B

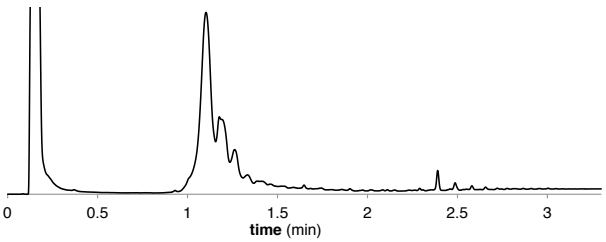


Figure S9-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

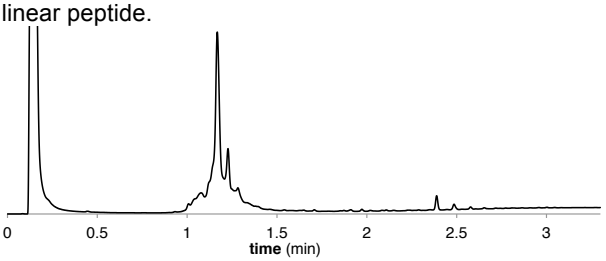


Figure S9-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

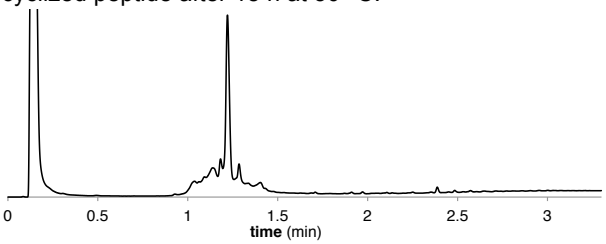


Figure S9-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

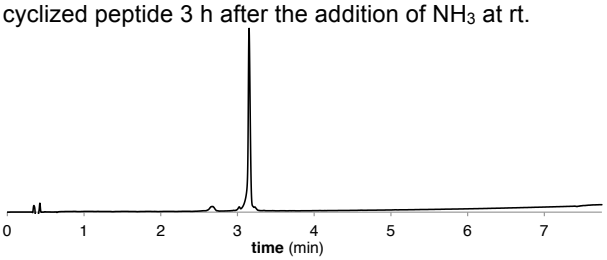


Figure S9-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**8**.

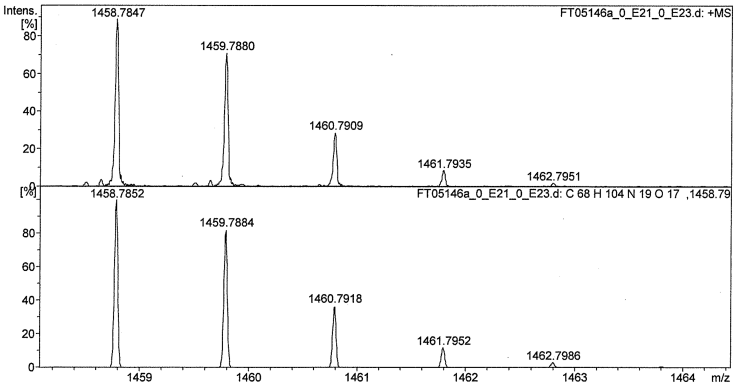


Figure S9-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**28**.

HR-MS (MALDI): calculated molecular weight ($C_{68}H_{104}N_{19}O_{17}$) $[M+H]^+$: 1458.7852 m/z; found: 1458.7847 m/z.

4.3.10. Cyclo(HseNlHWpKVSNKAF) S20

S20 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 37.3 mg (25.1 µmol; 50 %; Method A); 20.4 mg (13.7 µmol; 27 %; Method B).

Method A

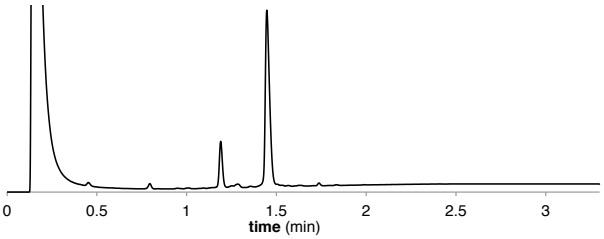


Figure S10-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

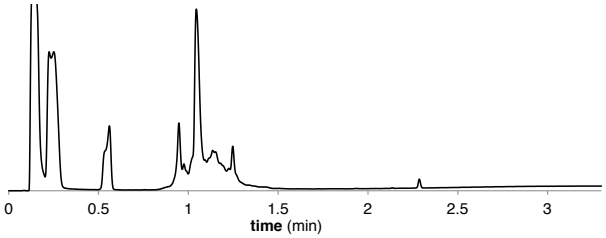


Figure S10-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

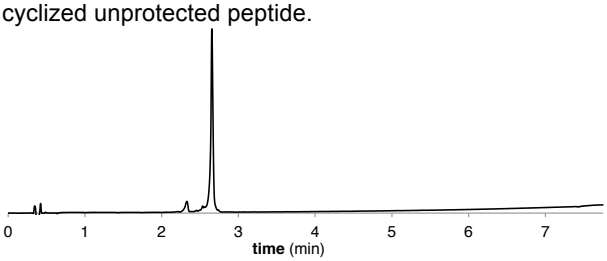


Figure S10-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S20**.

Method B

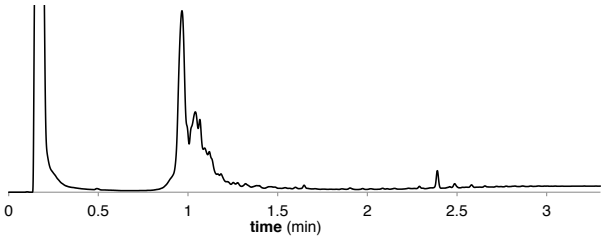


Figure S10-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

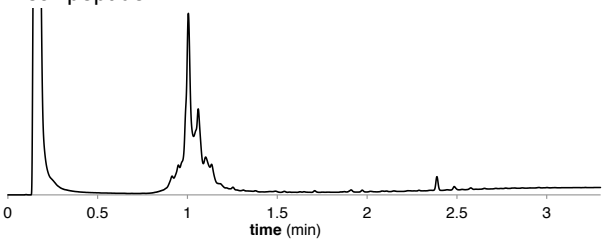


Figure S10-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

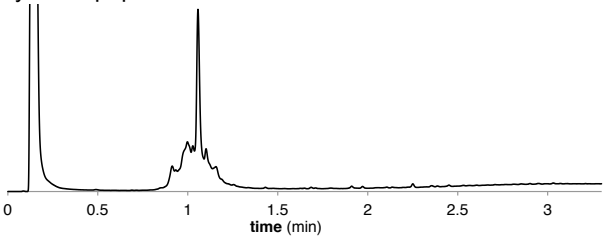


Figure S10-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

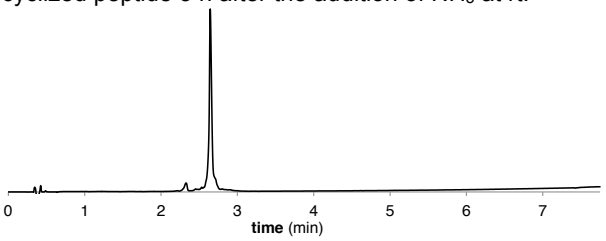


Figure S10-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S20**.

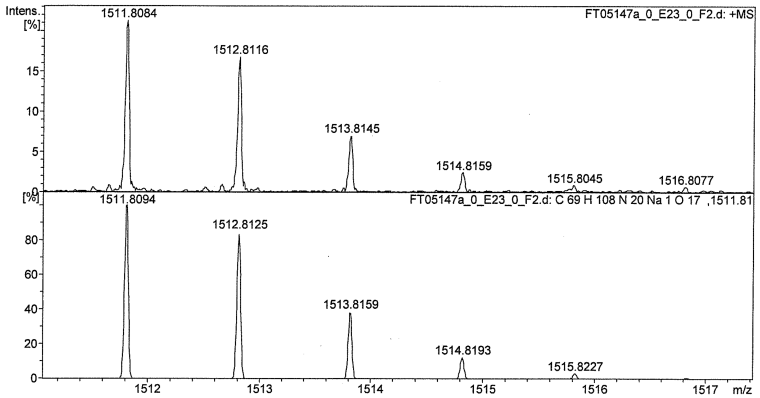


Figure S10-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S20**.

HR-MS (MALDI): calculated molecular weight ($C_{69}H_{108}N_{20}NaO_{17}$) $[M+Na]^+$: 1511.8094 m/z; found: 1511.8084 m/z.

4.3.11. Cyclo(HseNlHWAKVSNKAF) S21

S21 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 4.1 mg (2.8 µmol; 6 %; Method A); 5.0 mg (3.4 µmol; 7 %; Method B).

Method A

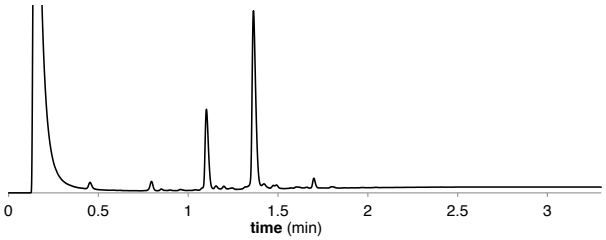


Figure S11-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

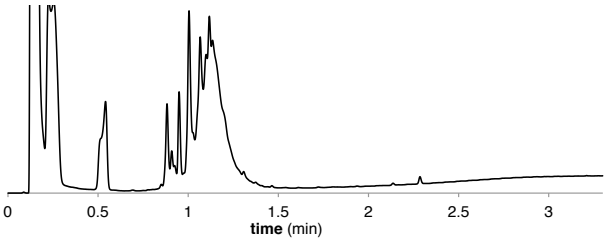


Figure S11-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

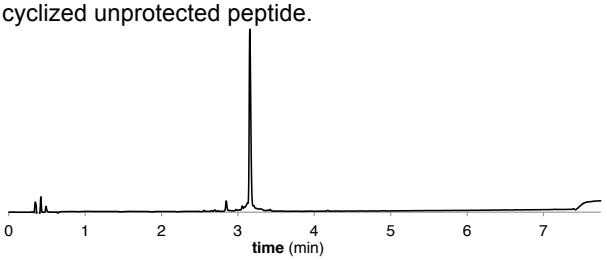


Figure S11-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S21**.

Method B

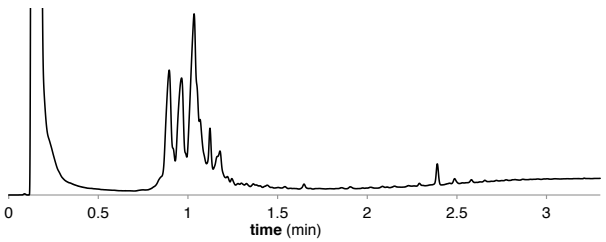


Figure S11-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

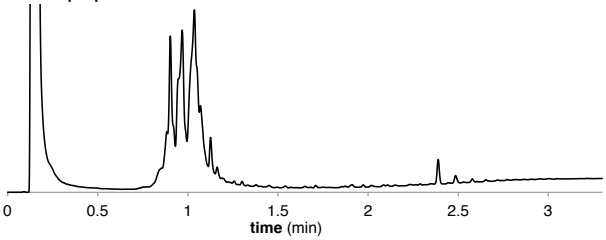


Figure S11-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

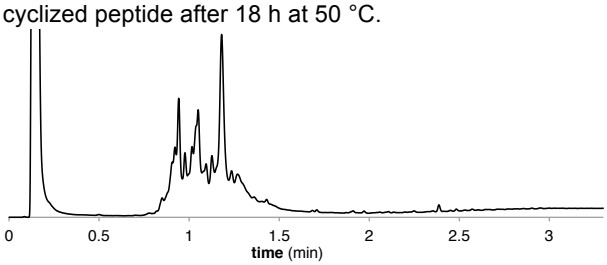


Figure S11-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

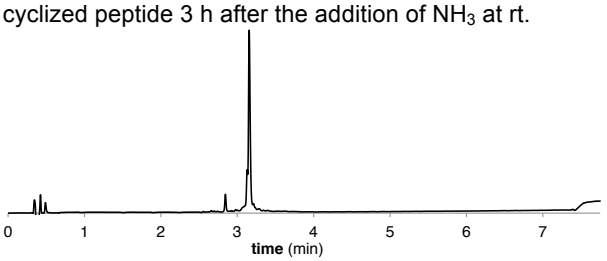


Figure S11-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S21**.

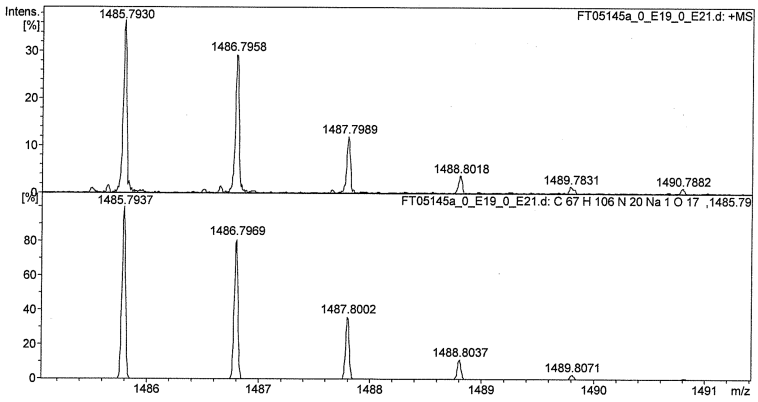


Figure S11-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S21**.

HR-MS (MALDI): calculated molecular weight ($C_{67}H_{106}N_{20}NaO_{17}$) $[M+Na]^+$: 1485.7937 m/z; found: 1485.7930 m/z.

4.3.12. Cyclo(HseYQKLQWFNpYAKF) S22

S22 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 16.0 mg (8.81 µmol; 18 %; Method A); 8.7 mg (4.8 µmol; 10 %; Method B).

Method A

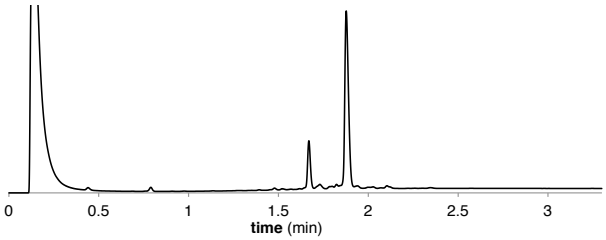


Figure S12-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

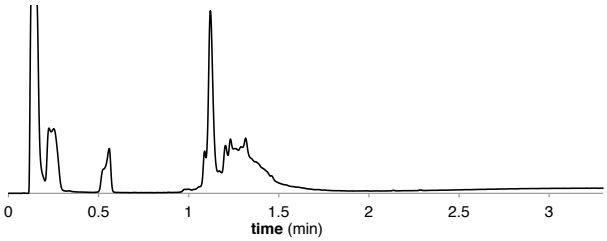


Figure S12-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

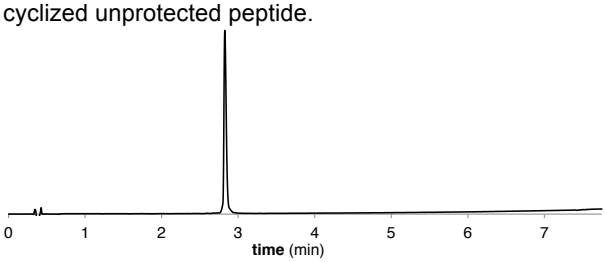


Figure S12-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S22**.

Method B

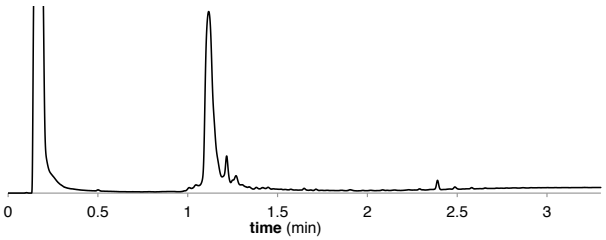


Figure S12-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

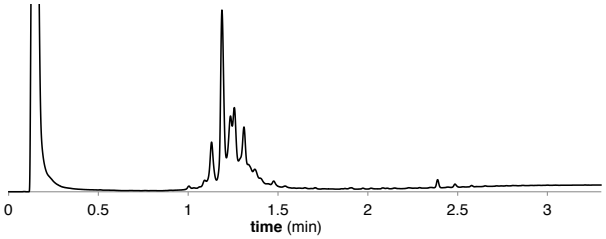


Figure S12-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

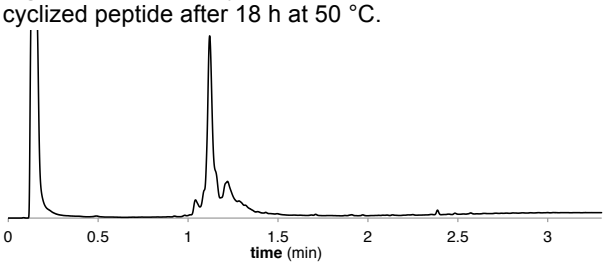


Figure S12-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

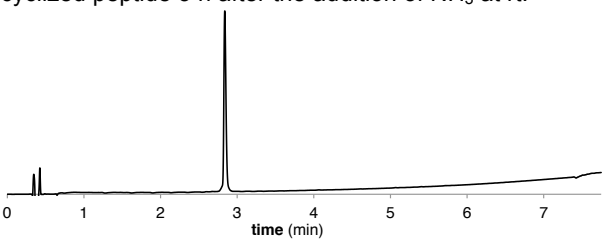


Figure S12-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S22**.

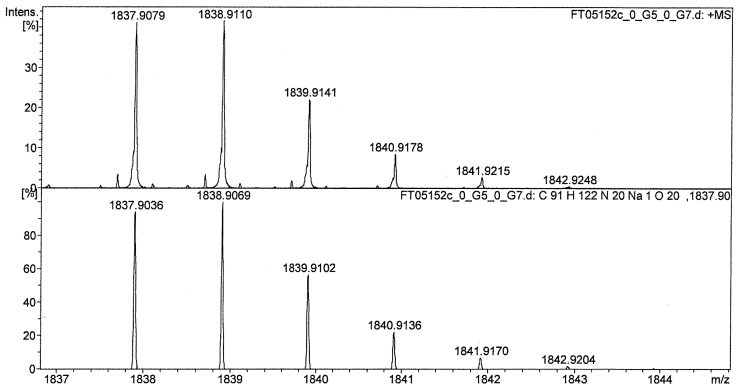


Figure S12-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S22**.

HR-MS (MALDI): calculated molecular weight ($C_{91}H_{122}N_{20}NaO_{20}$) $[M+Na]^+$: 1837.9036 m/z; found: 1837.9079 m/z.

4.3.13. Cyclo(HseYQKLQWFNAAAKF) S23

S23 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 2.7 mg (1.6 µmol; 3 %; Method A); 9.9 mg (5.8 µmol; 12 %; Method B).

Method A

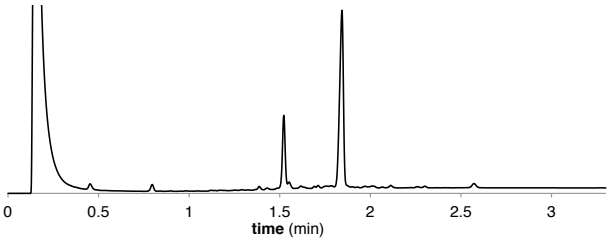


Figure S13-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

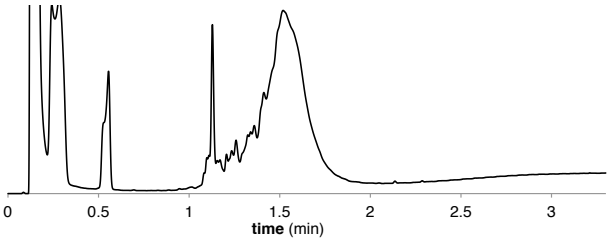


Figure S13-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

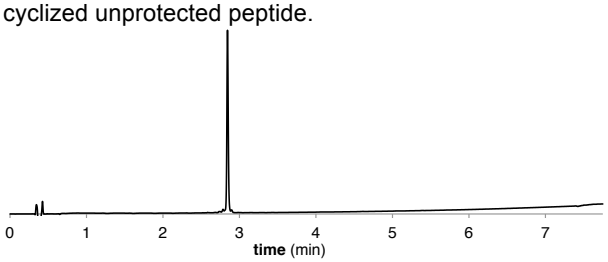


Figure S13-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S23**.

Method B

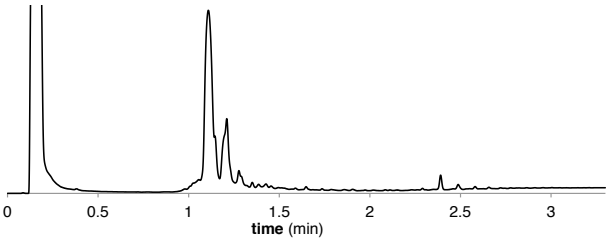


Figure S13-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

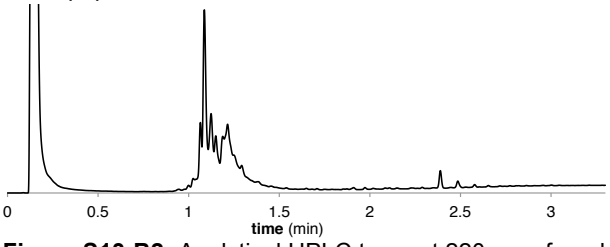


Figure S13-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

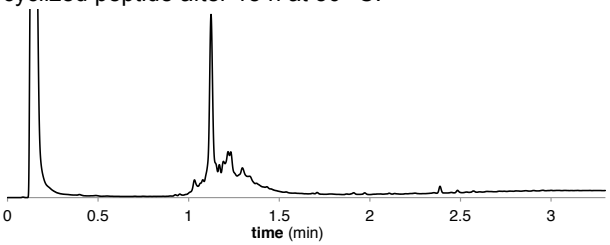


Figure S13-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

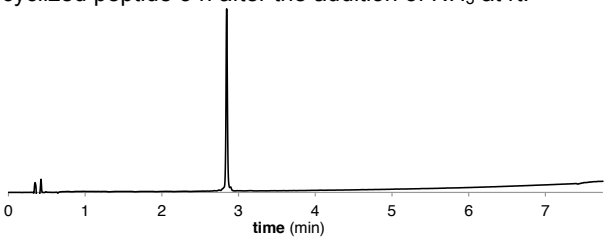


Figure S13-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S23**.

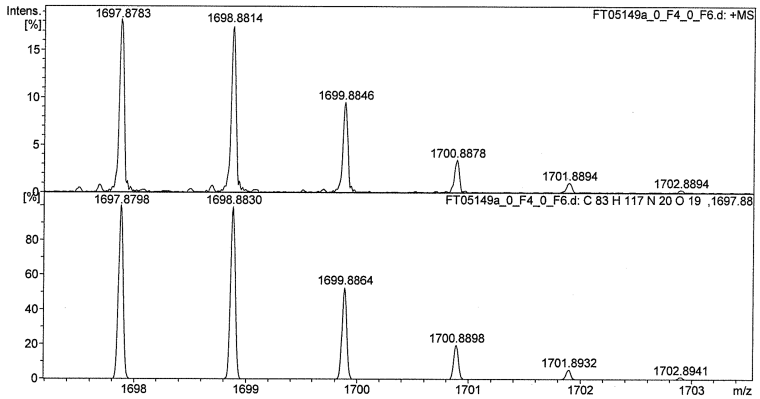


Figure S13-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S23**.

HR-MS (MALDI): calculated molecular weight ($C_{83}H_{117}N_{20}O_{19}$) $[M+H]^+$: 1697.8798 m/z; found: 1697.8783 m/z.

4.3.14. Cyclo(HseWNPFKAQSpPGYLKL) S24

S24 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 12.6 mg (6.87 µmol; 14 %; Method A); 12.9 mg (7.07 µmol; 14 %; Method B).

Method A

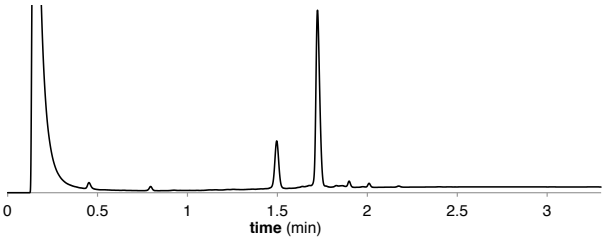


Figure S14-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

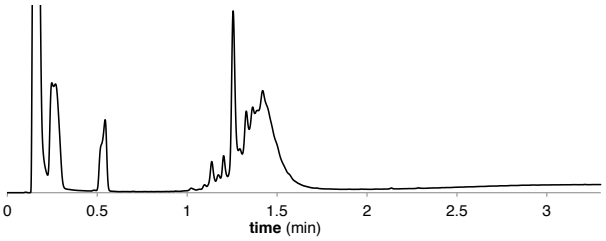


Figure S14-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

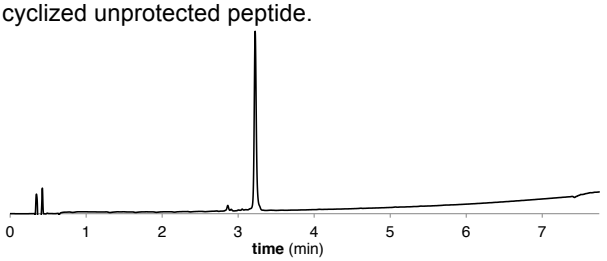


Figure S14-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S24**.

Method B

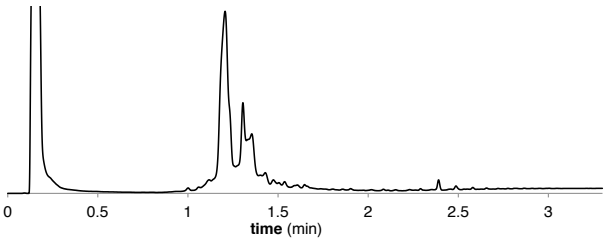


Figure S14-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

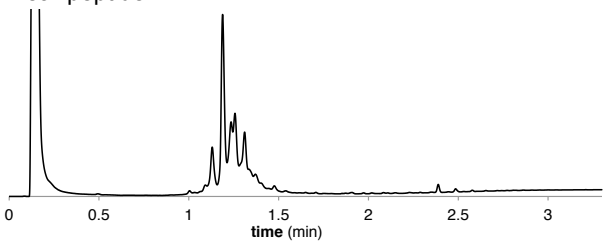


Figure S14-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

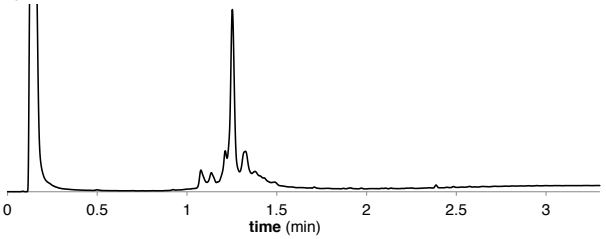


Figure S14-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

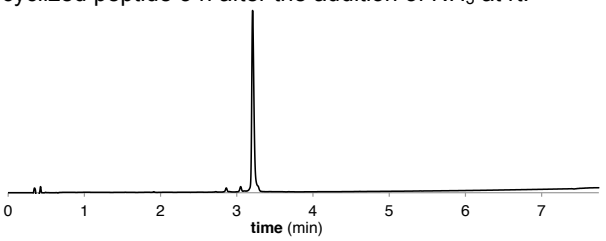


Figure S14-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S24**.

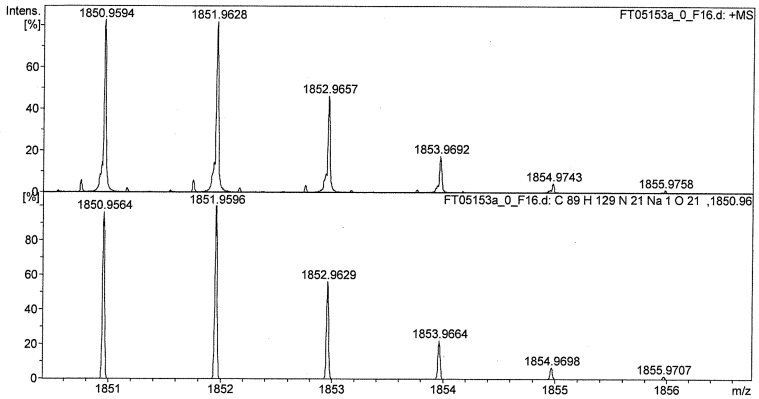


Figure S14-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S24**.

HR-MS (MALDI): calculated molecular weight ($C_{89}H_{129}N_{21}NaO_{21}$) $[M+Na]^+$: 1850.9564 m/z; found: 1850.9594 m/z.

4.3.15. Cyclo(HseWNPFKAQSpAGYLKL) S25

S25 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 17.6 mg (9.74 µmol; 19 %; Method A); 9.7 mg (5.4 µmol; 11 %; Method B).

Method A

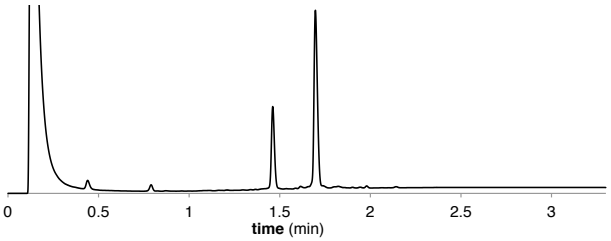


Figure S15-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

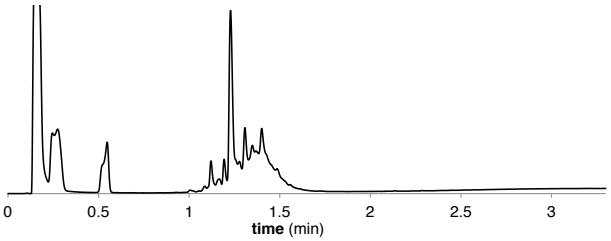


Figure S15-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

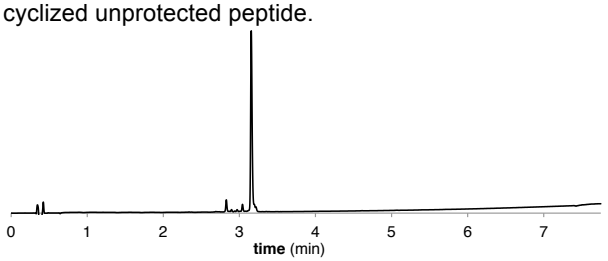


Figure S15-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S25**.

Method B

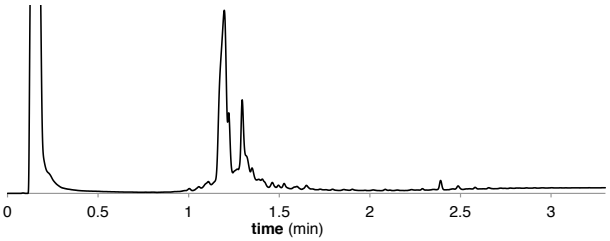


Figure S15-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

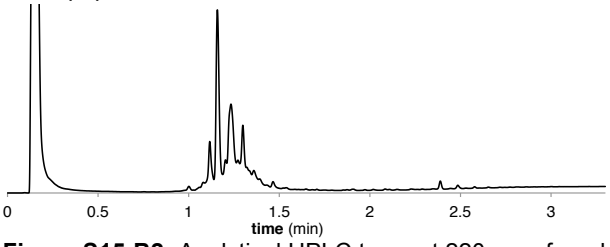


Figure S15-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

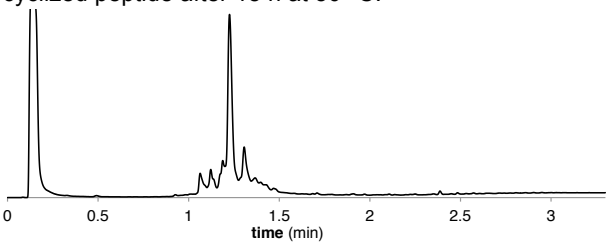


Figure S15-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

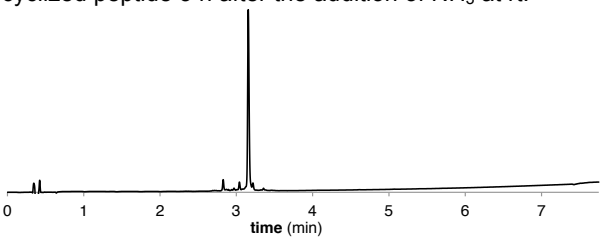


Figure S15-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S25**.

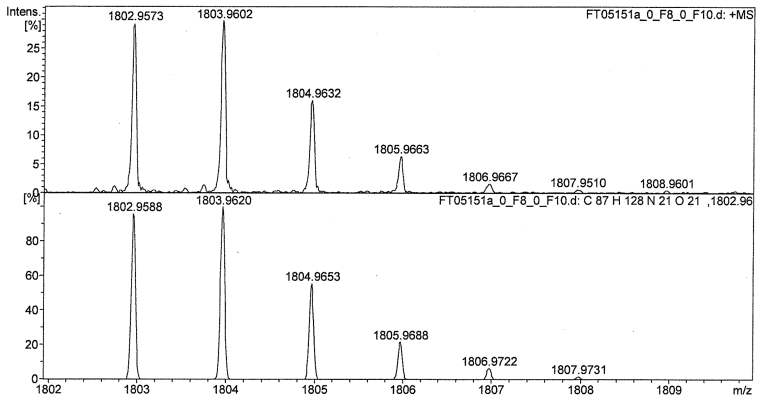


Figure S15-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S25**.

HR-MS (MALDI): calculated molecular weight ($C_{87}H_{128}N_{21}O_{21}$) $[M+H]^+$: 1802.9588 m/z; found: 1802.9573 m/z.

4.3.16. Cyclo(HseWNPFKAQSKAGYLKL) S26

S26 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 2.7 mg (1.5 µmol; 3 %; Method A); 7.0 mg (3.8 µmol; 8 %; Method B).

Method A

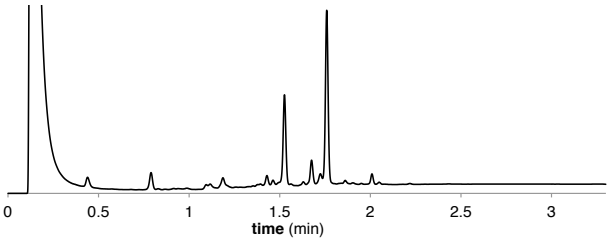


Figure S16-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

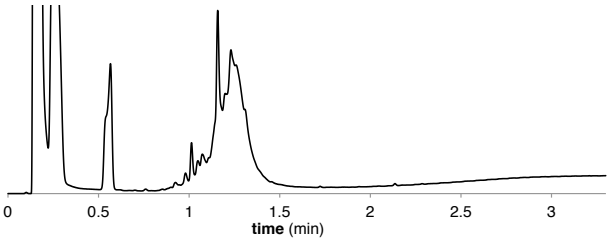


Figure S16-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

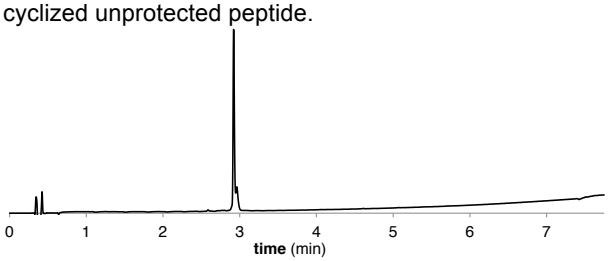


Figure S16-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S26**.

Method B

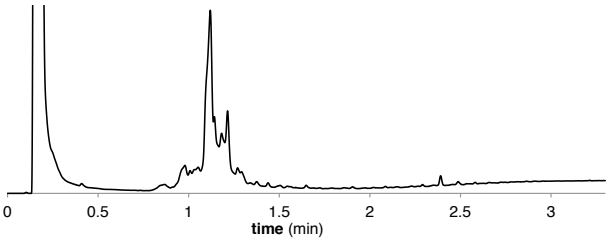


Figure S16-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

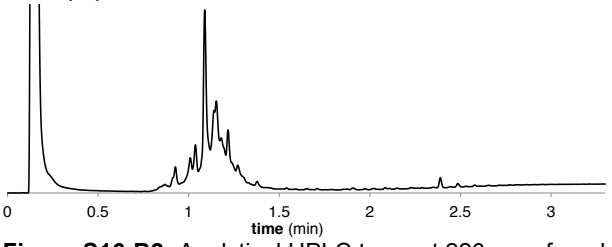


Figure S16-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

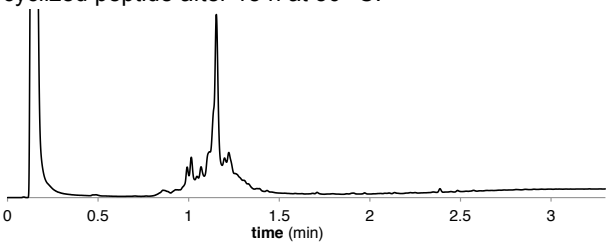


Figure S16-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

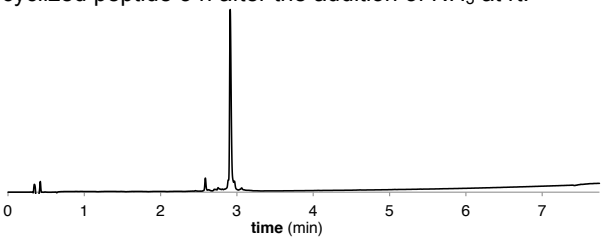


Figure S16-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S26**.

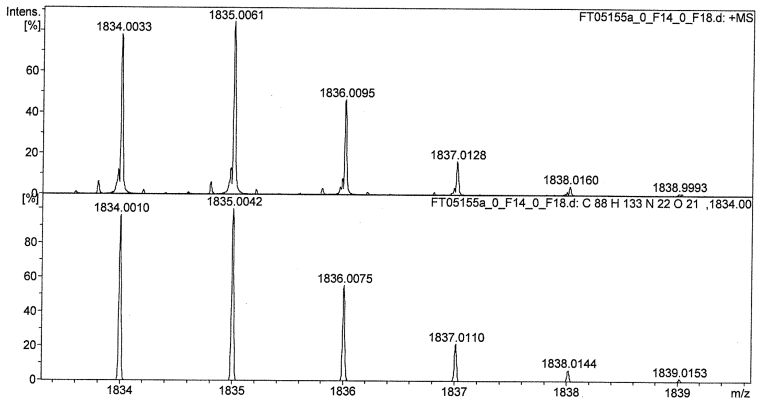


Figure S16-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S26**.

HR-MS (MALDI): calculated molecular weight ($C_{88}H_{133}N_{22}O_{21}$) $[M+H]^+$: 1834.0010 m/z; found: 1834.0033 m/z.

4.3.17. Cyclo(HseRTNpPKKEKVGpKRL) S27

S27 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 21.6 mg (11.8 µmol; 24 %; Method A); 38.7 mg (21.1 µmol; 42 %; Method B).

Method A

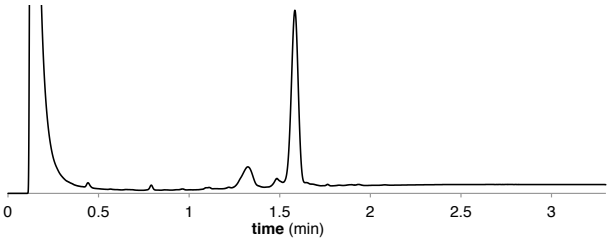


Figure S17-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

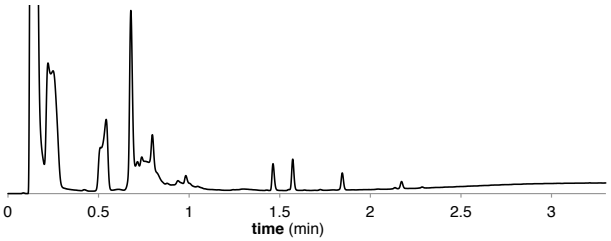


Figure S17-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

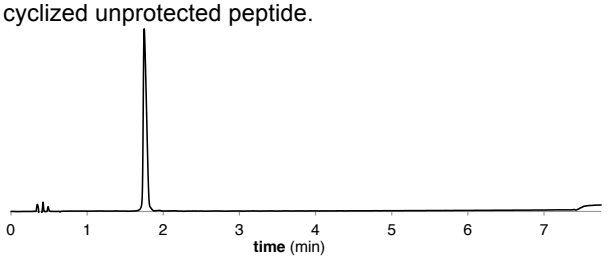


Figure S17-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S27**.

Method B

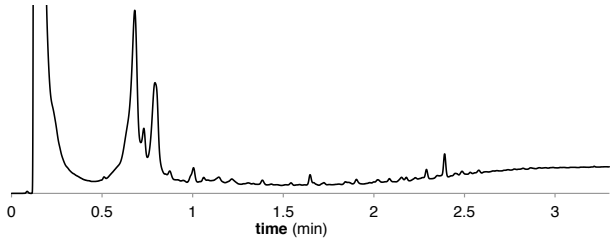


Figure S17-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

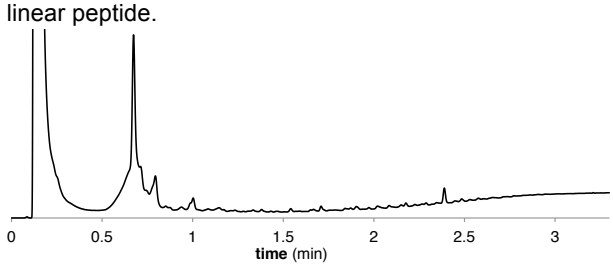


Figure S17-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

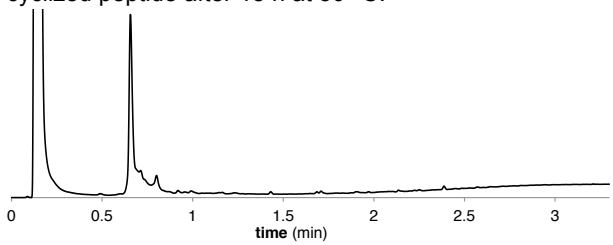


Figure S17-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

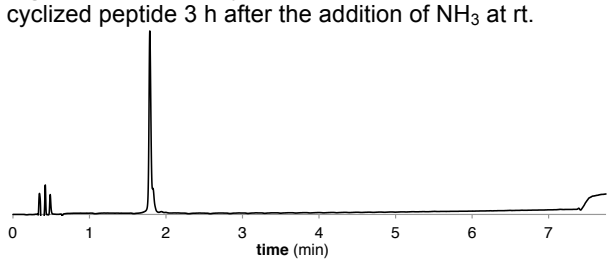


Figure S17-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S27**.

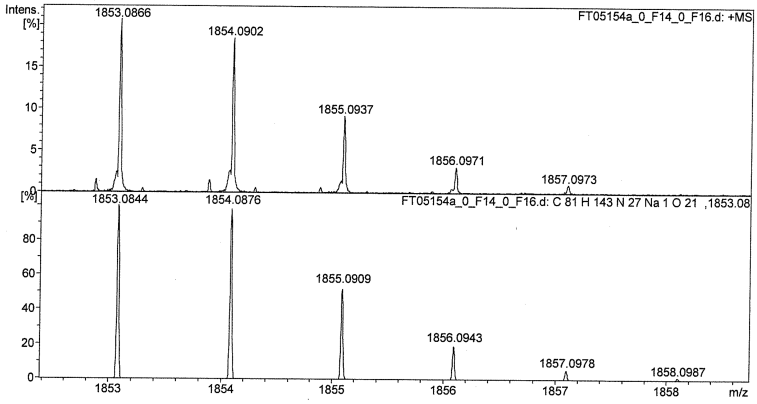


Figure S17-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S27**.

HR-MS (MALDI): calculated molecular weight ($C_{81}H_{143}N_{27}NaO_{21}$) $[M+Na]^+$: 1853.0844 m/z; found: 1853.0866 m/z.

4.3.18. Cyclo(HseRTNAAKKEKVGpKRL) S28

S28 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 18.9 mg (10.6 µmol; 21 %; Method A); 11.4 mg (6.42 µmol; 13 %; Method B).

Method A

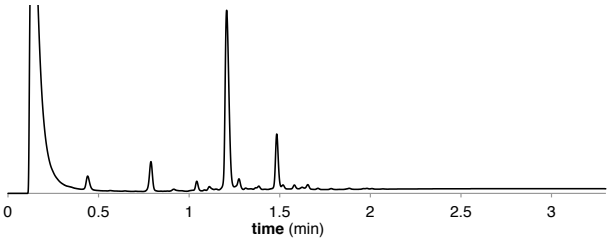


Figure S18-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

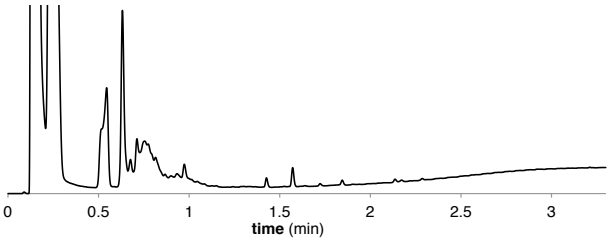


Figure S18-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

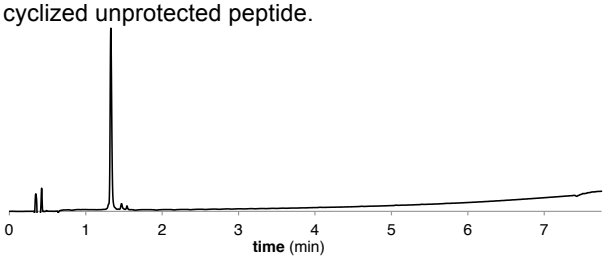


Figure S18-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S28**.

Method B

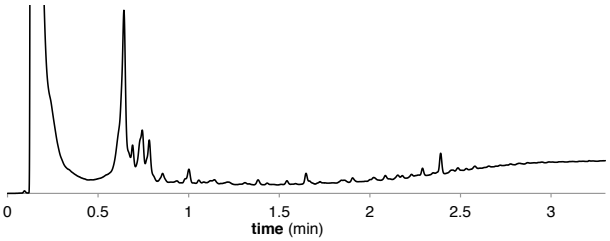


Figure S18-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

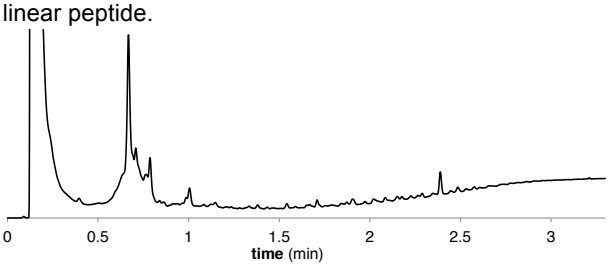


Figure S18-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

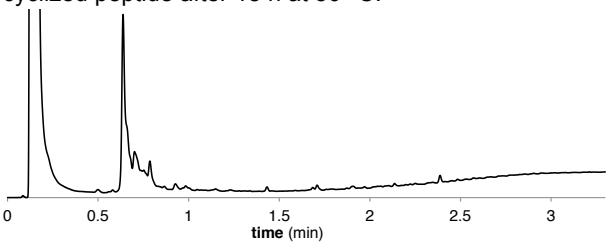


Figure S18-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

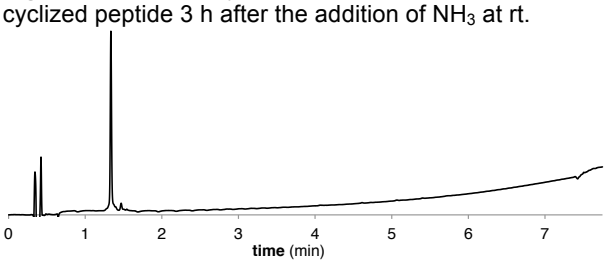


Figure S18-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S28**.

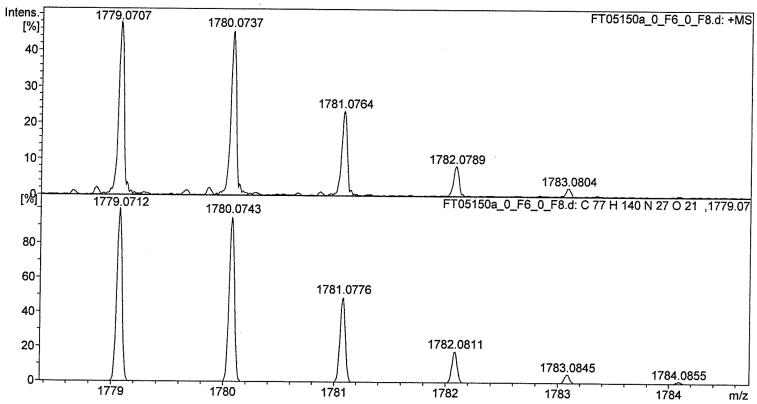


Figure S18-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S28**.

HR-MS (MALDI): calculated molecular weight ($C_{77}H_{140}N_{27}O_{21}$) $[M+H]^+$: 1779.0712 m/z; found: 1779.0707 m/z.

4.3.19. Cyclo(HseVFQpPFHseRKRFKGRPFL) S29

S29 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 11.6 mg (5.27 µmol; 11 %; Method A); 22.9 mg (10.4 µmol; 21 %; Method B).

Method A

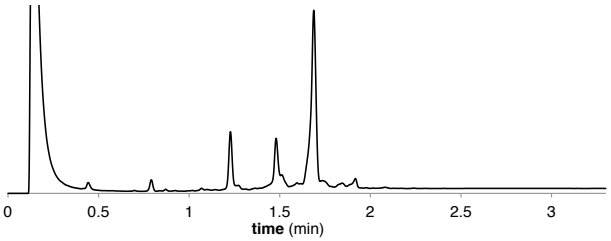


Figure S19-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

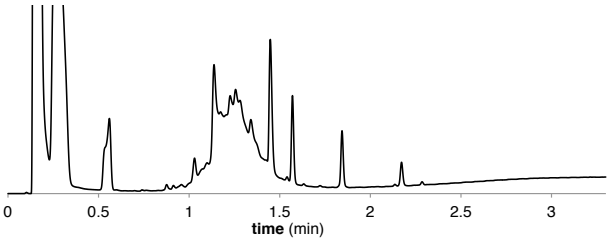


Figure S19-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

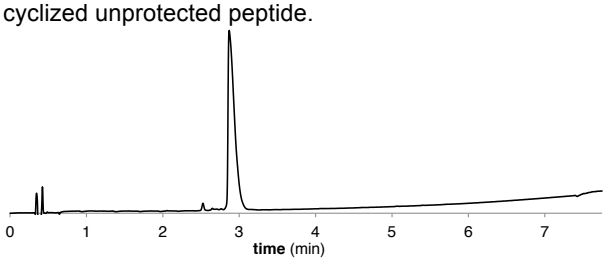


Figure S19-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S29**.

Method B

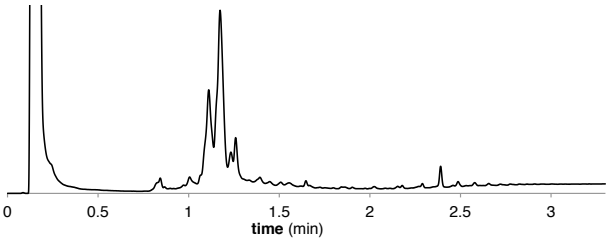


Figure S19-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

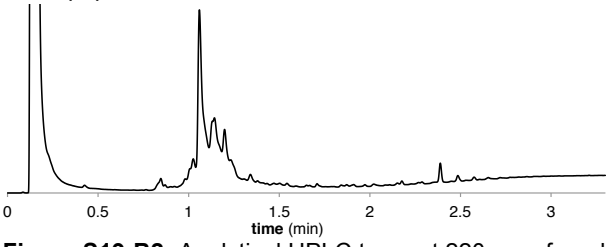


Figure S19-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

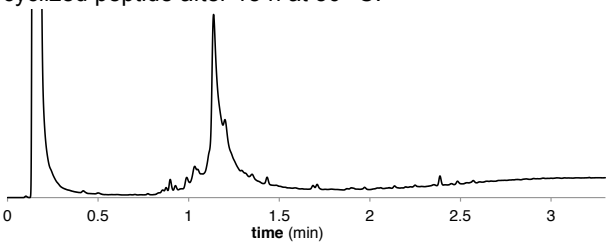


Figure S19-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

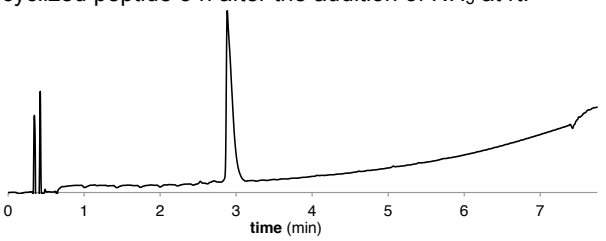


Figure S19-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S29**.

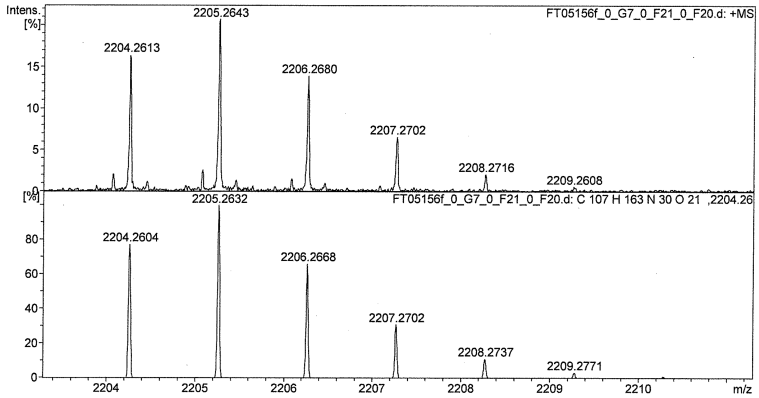


Figure S19-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S29**.

HR-MS (MALDI): calculated molecular weight ($C_{107}H_{163}N_{30}O_{21}$) $[M+H]^+$: 2204.2604 m/z; found: 2204.2613 m/z.

4.3.20. Cyclo(HseVFQpYFHseRKRFKGRPFL) S30

S30 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 26.0 mg (11.5 µmol; 23 %; Method A); 29.4 mg (12.9 µmol; 26 %; Method B).

Method A

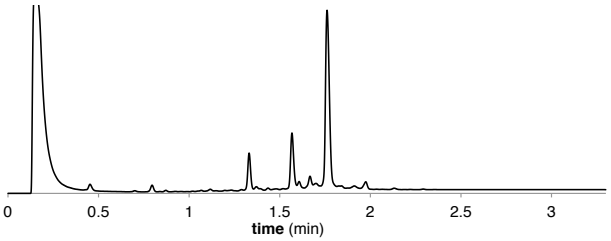


Figure S20-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

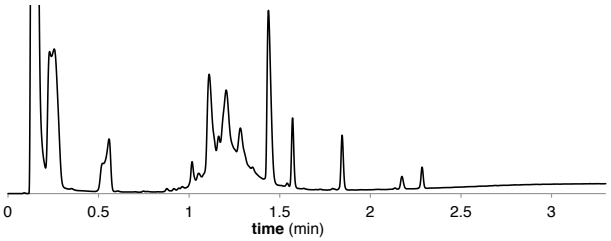


Figure S20-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

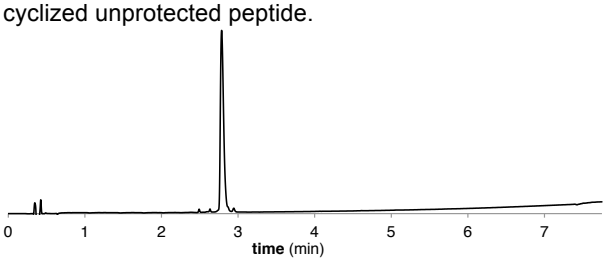


Figure S20-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S30**.

Method B

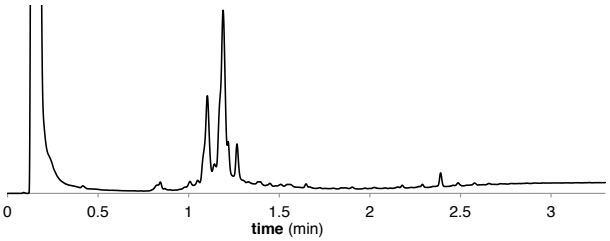


Figure S20-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

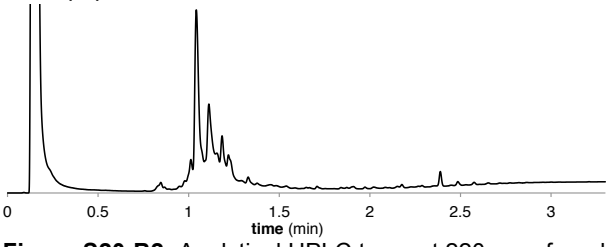


Figure S20-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

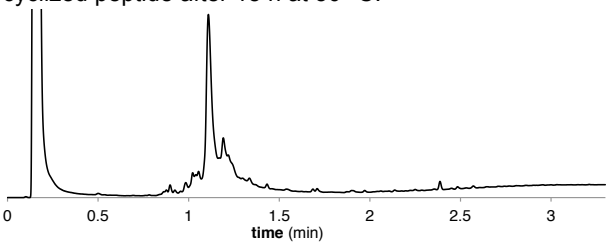


Figure S20-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

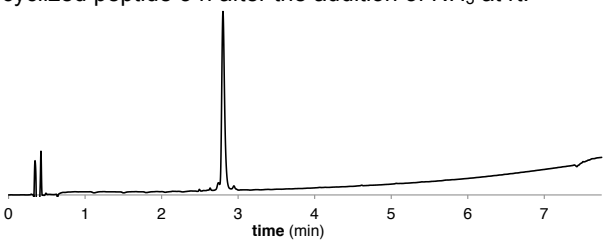


Figure S20-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S30**.

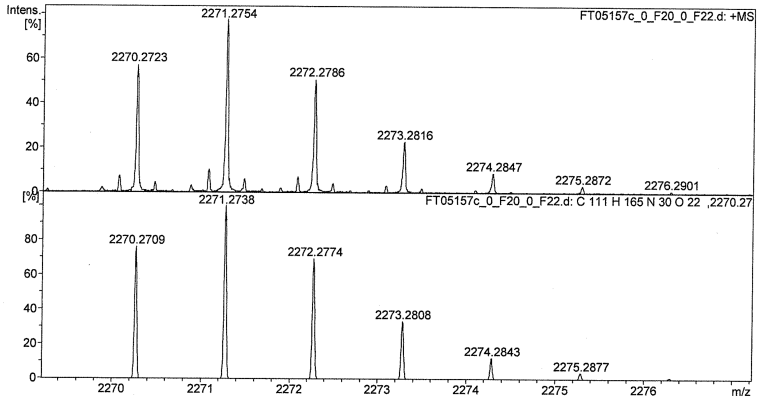


Figure S20-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S30**.

HR-MS (MALDI): calculated molecular weight ($C_{111}H_{165}N_{30}O_{22}$) $[M+H]^+$: 2270.2709 m/z; found: 2270.2723 m/z.

4.3.21. Cyclo(HseVFQAAFHseRKRFKGRPFL) S31

S31 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 11.7 mg (5.42 µmol; 11 %; Method A); 9.1 mg (4.2 µmol; 8 %; Method B).

Method A

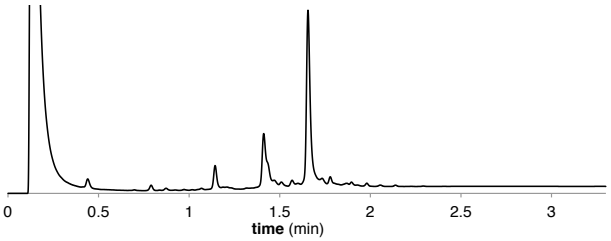


Figure S21-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

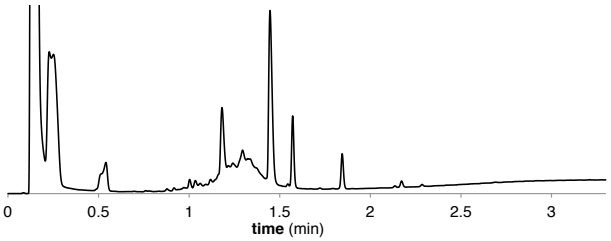


Figure S21-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

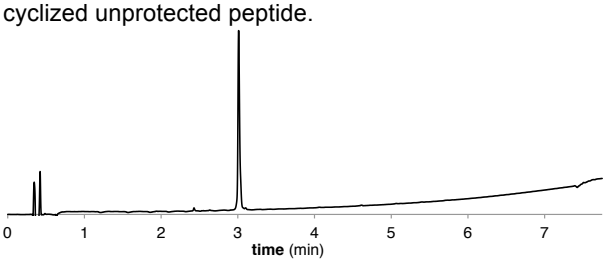


Figure S21-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S31**.

Method B

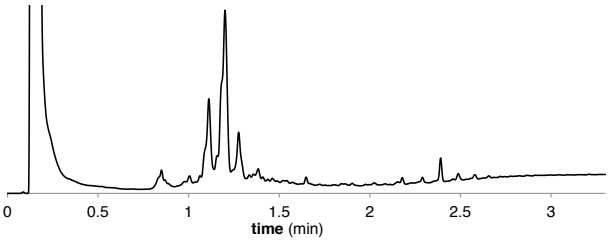


Figure S21-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

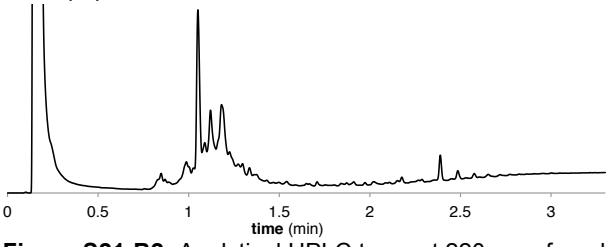


Figure S21-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

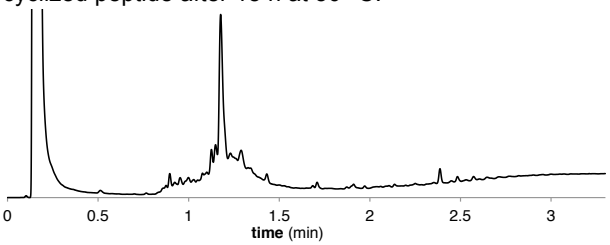


Figure S21-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

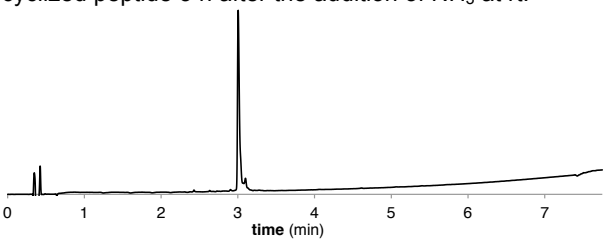


Figure S21-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S31**.

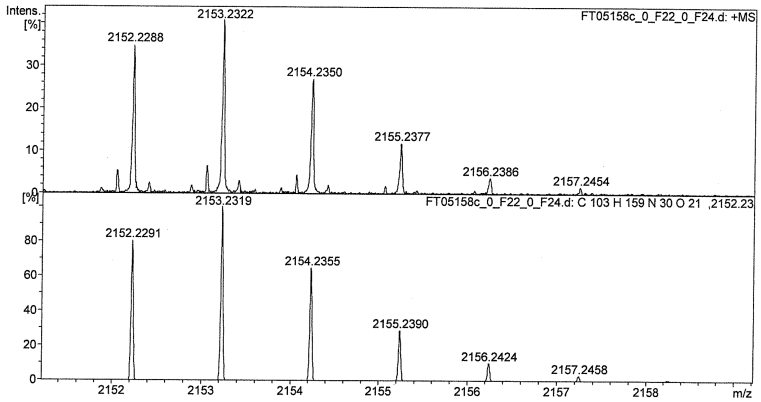


Figure S21-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S31**.

HR-MS (MALDI): calculated molecular weight ($C_{103}H_{159}N_{30}O_{21}$) $[M+H]^+$: 2152.2291 m/z; found: 2152.2288 m/z.

4.3.22. Cyclo(HseWPRLQFHseHRLpPAEWFKAL) S32

S32 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 14.3 mg (5.79 µmol; 12 %; Method A); 13.0 mg (5.23 µmol; 10 %; Method B).

Method A

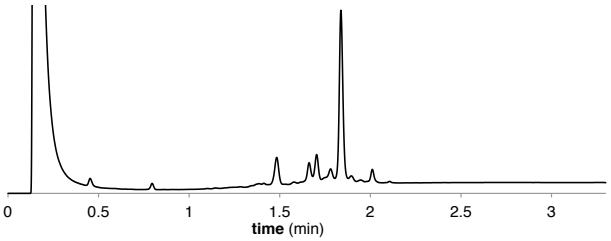


Figure S22-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

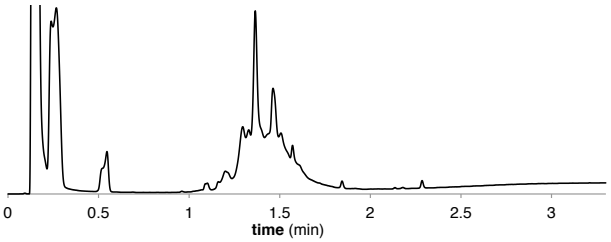


Figure S22-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

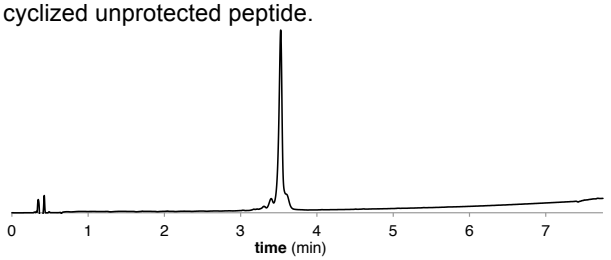


Figure S22-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S32**.

Method B

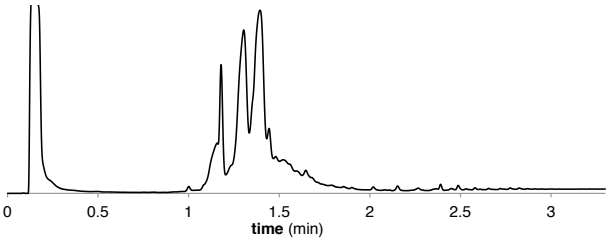


Figure S22-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

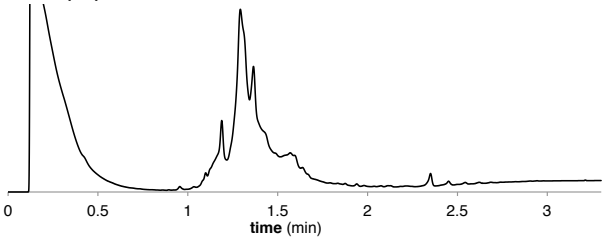


Figure S22-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

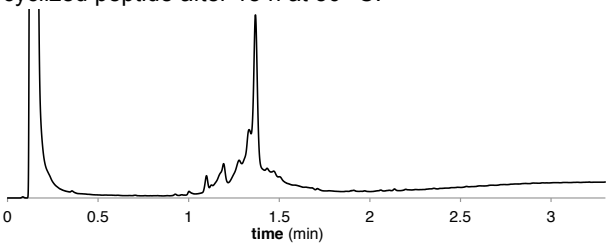


Figure S22-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

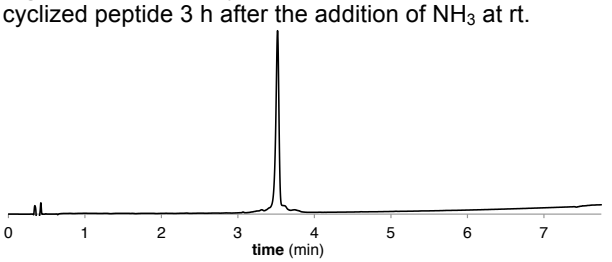


Figure S22-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S32**.

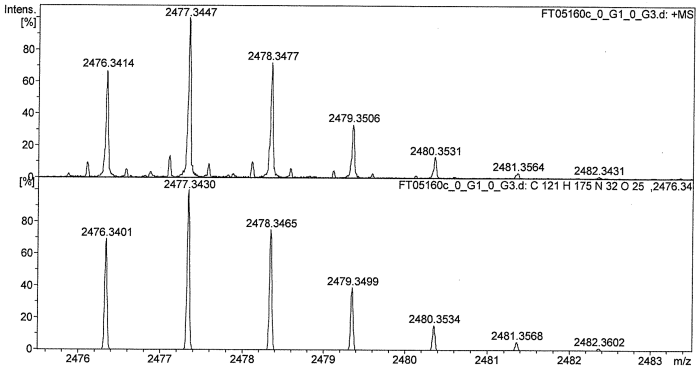


Figure S22-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S32**.

HR-MS (MALDI): calculated molecular weight ($C_{121}H_{175}N_{32}O_{25}$) $[M+H]^+$: 2476.3401 m/z; found: 2476.3414 m/z.

4.3.23. Cyclo(HseWPRLQFHseHRLpKAEWFKAL) S33

S33 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). Yield: 9.9 mg (3.9 µmol; 8 %; Method A); 20.2 mg (8.03 µmol; 16 %; Method B).

Method A

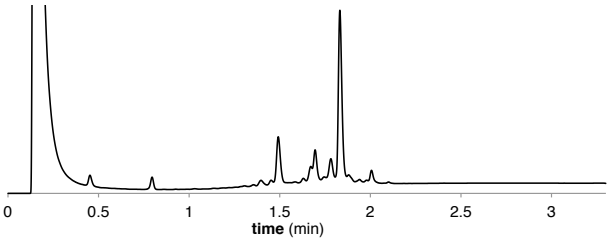


Figure S23-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

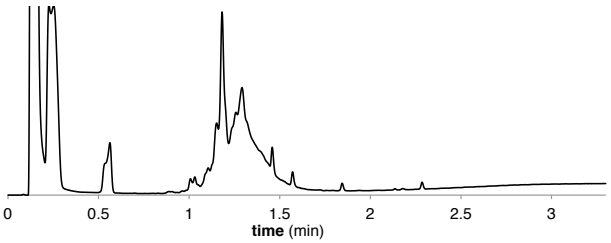


Figure S23-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

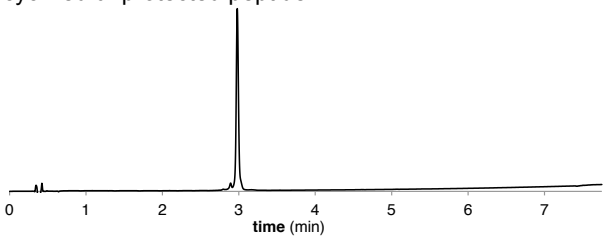


Figure S23-A3: Analytical HPLC trace at 220 nm of purified product *amide*-**S33**.

Method B

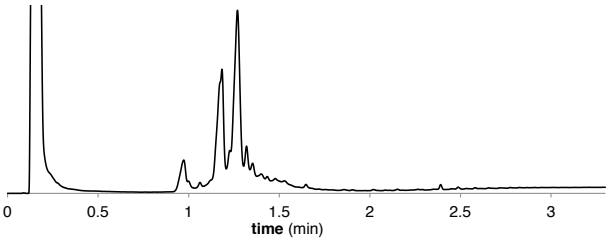


Figure S23-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

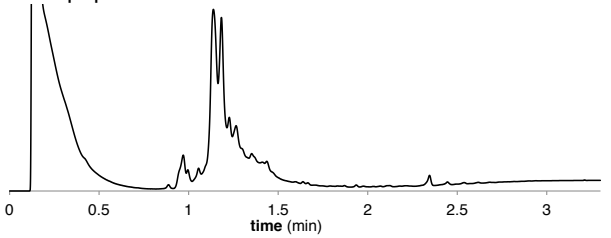


Figure S23-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

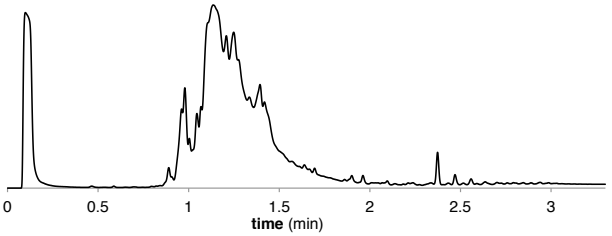


Figure S23-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

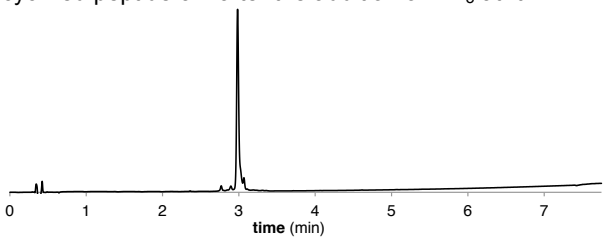


Figure S23-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S33**.

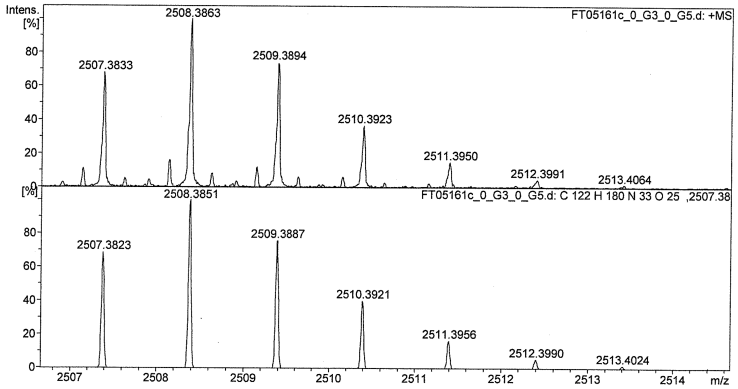


Figure S23-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S33**.

HR-MS (MALDI): calculated molecular weight ($C_{122}H_{180}N_{33}O_{25}$) $[M+H]^+$: 2507.3823 m/z; found: 2507.3833 m/z.

4.3.24. Cyclo(HseWPRLQFHseHRLAAAEWFKAL) S34

S34 was prepared following the general procedure for sidechain-protected cyclization (Method A) and KAHA cyclization (Method B). With Method A no product was obtained. Yield: 5.2 mg (2.1 µmol; 4 %; Method B).

Method A

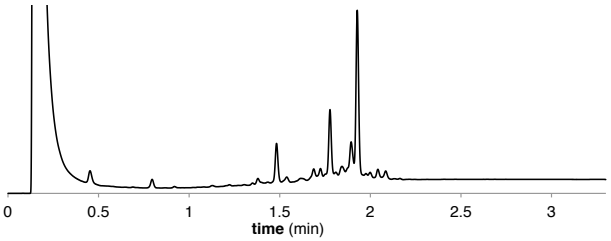


Figure S24-A1: Analytical HPLC trace at 220 nm of crude protected linear peptide.

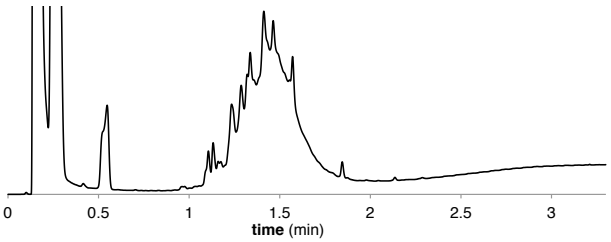


Figure S24-A2: Analytical HPLC trace at 220 nm of crude cyclized unprotected peptide.

Product could not be isolated.

Method A

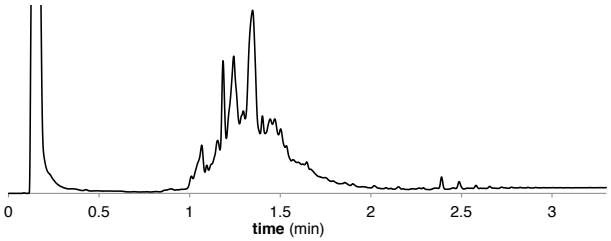


Figure S24-B1: Analytical HPLC trace at 220 nm of crude linear peptide.

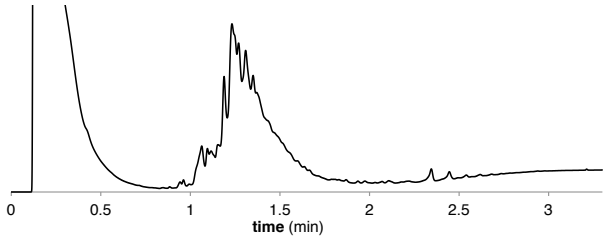


Figure S24-B2: Analytical HPLC trace at 220 nm of crude cyclized peptide after 18 h at 50 °C.

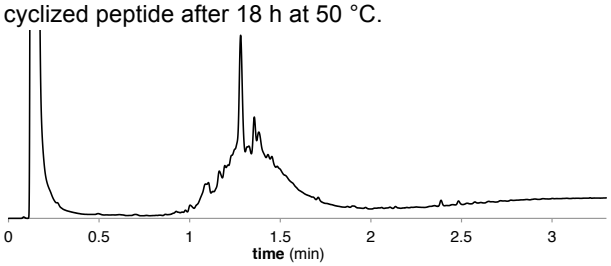


Figure S24-B3: Analytical HPLC trace at 220 nm of crude cyclized peptide 3 h after the addition of NH_3 at rt.

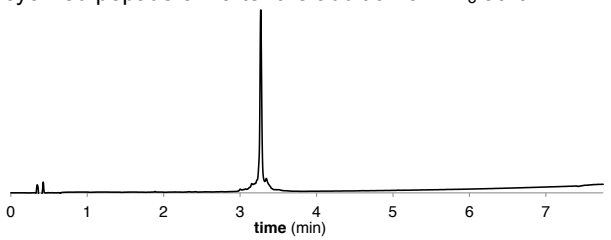


Figure S24-B4: Analytical HPLC trace at 220 nm of purified product *amide*-**S34**.

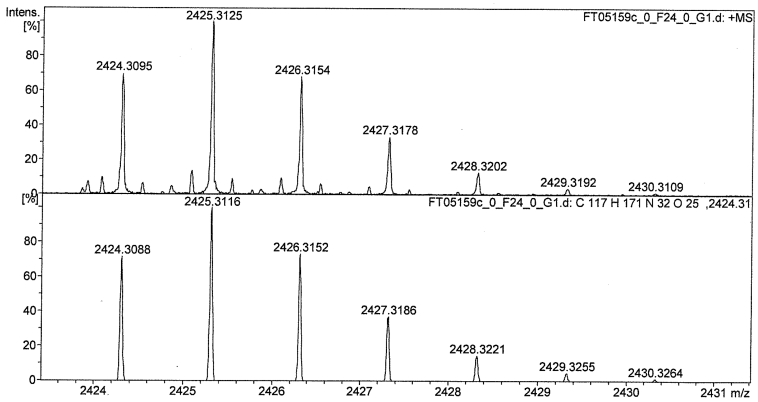


Figure S24-AB1: Measured (top) and calculated (bottom) high-resolution mass spectrum of *amide*-**S34**.

HR-MS (MALDI): calculated molecular weight ($C_{117}H_{171}N_{32}O_{25}$) $[M+H]^+$: 2424.3088 m/z; found: 2424.3095 m/z.

5. Initial Optimization and Characterization

5.1. Synthesis of OprWEFpPFEWL-α-ketoacid 3

Linear peptide α-ketoacid **3** was synthesized by Fmoc SPPS starting from protected Fmoc-Leu α-ketoacid resin **5** (loading 0.181 mmol/g) on 0.35 mmol scale using the procedure described in the general methods section. The last aminoacid was coupled for 3 h using Boc-(*S*)-oxaproline (152 mg, 700 µmol, 2.00 equiv.), COMU (297 mg, 693 µmol, 1.98 equiv) and *N*-methylmorpholine (142 mg, 1.40 mmol, 4.00 equiv). The peptide was cleaved using 95:2.5:2.5 TFA/H_2O/EDT (30 mL) for 3 h. The solvent was evaporated under reduced pressure and the peptide was precipitated with Et_2O. The precipitate was washed with Et_2O (3 x 30 mL) and dried in the air. To obtain a pure sample of **3**, 10 mg of the crude material was purified by preparative HPLC using a Shiseido Capcell Pak MGII C18 column (20 x 250 mm) with a gradient from 40 % to 70 % CH_3CN with 0.1 % TFA in 30 min to afford pure **3** (1.5 mg, 1.1 µmol).

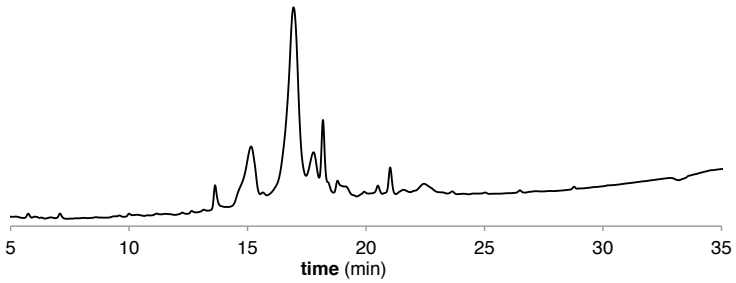


Figure S25: HPLC trace at 220 nm showing the crude peptide after cleavage from resin.

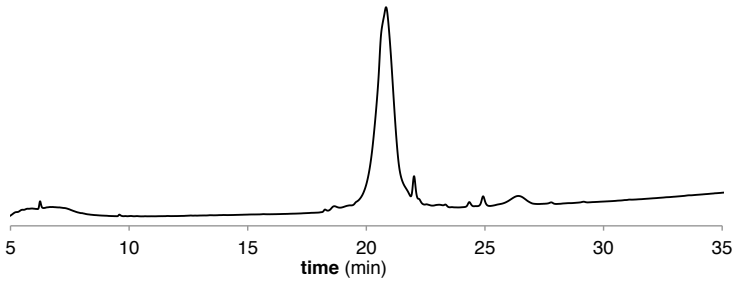


Figure S26: HPLC trace at 220 nm of purified **3**.

5.2. Cyclization of OprWEFpPFEWL-α-ketoacid 3 to *depsi*-cyclo(T[§]WEFpPFEWL) *depsi*-4

Purified **3** (0.6 mg, 0.4 µmol) and oxalic acid (3.9 mg, 44 µmol) were dissolved in a mixture of DMSO (305 µL) and H_2O (131 µL). The mixture was shaken at 60 °C for 2 h. The mixture was purified by preparative HPLC using a Shiseido Capcell Pak MGII C18 column (20 x 250 mm) with a gradient from 45 % to 75 % CH_3CN with 0.1 % TFA in 30 min to afford pure *depsi*-**4** (ca. 0.4 mg).

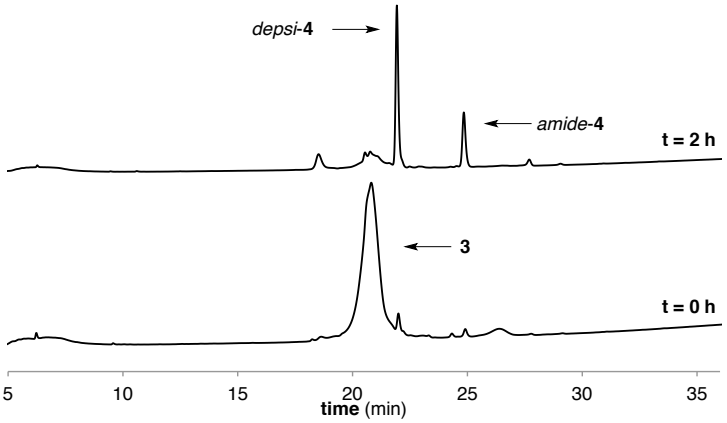


Figure S27: HPLC trace at 220 nm showing the cyclization at t = 0 h and t = 2 h.

5.3. Acyl shift of *depsi*-cyclo(T[§]WEFpPFEWL) *depsi*-4 to *amide*-4

Purified *depsi*-**4** (ca. 0.4 mg) was dissolved in CH_3CN (250 µL) and aqueous phosphate buffer (pH 11, 200 mM, 125 µL). The mixture was shaken at rt for 18 h. Within this time, the starting material was converted quantitatively to *amide*-**4**.

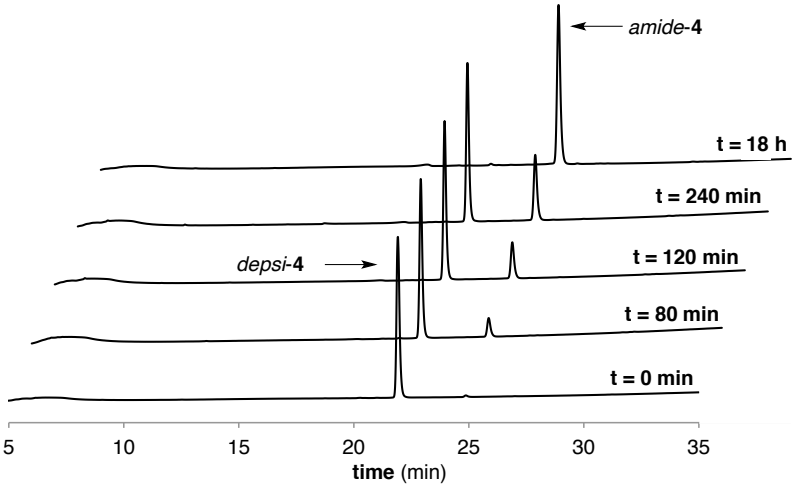


Figure S28: HPLC trace at 220 nm showing the N,O-acyl shift between t = 0 h and t = 18 h.

5.4. Large-scale cyclization of crude OprWEFpPFEWL-α-ketoacid 3 to *depsi*-cyclo(T[§]WEFpPFEWL) *depsi*-4

Crude **3** (100 mg, ca. 72 µmol) and oxalic acid (65.2 mg, 724 µmol) were dissolved in CH_3CN (4.83 mL) and H_2O (2.41 mL). The mixture was shaken at 60 °C for 4 h. The mixture was purified by preparative HPLC using a Shiseido Capcell Pak C18 column (50 x 250 mm) with a gradient from 45 % to 75 % CH_3CN with 0.1 % TFA in 40 min to afford pure *depsi*-**4** (17.8 mg, 13.4 µmol, 19 % based on crude **3**) and *amide*-**4** (11.2 mg, 8.40 µmol, 12 % based on crude **3**).

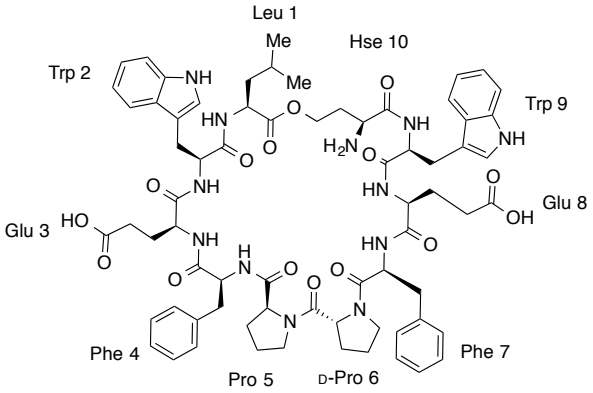
5.5. Full NMR characterization of *depsi*-4

Spectra were recorded on a Bruker AVIII600 Spectrometer at 600 MHz (1H) and 150 MHz (^{13}C) at rt. The sample was measured in CD_3OH with presaturation to suppress the solvent O-H signal. ^{1}H and ^{13}C Signals are referenced to residual undeuterated solvent signals and assigned with the use of TOCSY, COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{15}N HSQC, HMBC and ROESY spectra.

Table S2: 1H, 13C and 15N assignments for *depsi*-**4**

Residue	N-H (15N)	Cα-H (13C)	Cβ-H (13C)	H_{other} (13C)	Carbonyl 13C
Leu 1	8.90-8.93 (122.82)	3.59-3.63 (53.09)	1.47-1.53, 1.64-1.70 (39.32)	γ: 0.67-0.71 (24.84), δ1: 0.58-0.63 (20.75), δ2: 0.65-0.68 (23.85)	174.28
Trp 2	9.09-9.12 (123.52)	4.99-5.05 (54.83)	2.99-3.01, 3.25-3.29 (30.57)	1: 10.33-10.35 (N 138.09), 2: 6.98-6.99 (124.6) 3: (110.35) 3.1: (128.37) 4: 7.60-7.63 (119.46) 5: 6.89-6.92 (119.77) 6: 7.00-7.04 (122.28) 7: 7.25-7.27 (112.26) 7.1: (138.09)	172.97
Glu 3	8.72-8.74 (120.47)	5.21-5.26 (53.3)	1.94-2.03 (30.26)	γ: 2.31-2.40 (31.26), δ: (175.93)	173.02
Phe 4	7.96-8.01 (119.03)	4.87-4.90 (55.75)	3.22-3.24, 3.30-3.32 (39.25)	1: (139.06), 2,6: 7.47-7.50 (130.43), 3,57.32-7.34 (129.39), 4: 7.23-7.26 (127.75)	173.07
Pro 5	-	4.39-4.43 (61.85)	1.71-1.76, 1.86-1.90 (30.32)	γ: 0.88-0.93, 1.60-1.64 (23.85), δ:3.46-3.52, 3.79-3.85 (48.18)	172.95
D-Pro 6	-	4.54-4.59 (59.41)	1.84-1.90, 2.10-2.16 (28.99)	γ: 1.78-1.82, 2.08-2.11 (26.26), δ: 3.43-3.47, 3.55-3.59 (48.68)	173.47
Phe 7	8.96-9.00 (123.69)	5.07-5.12 (53.42)	3.01-3.04, 3.16-3.20 (39.54)	1: (138.34), 2,6: 7.28-7.31 (130.34), 3,5 7.21-7.25 (129.39), 4: 7.09-7.12 (127.60)	170.86
Glu 8	8.75-8.77 (121.7)	5.11-5.16 (53.73)	2.01-2.08 (29.63)	γ: 2.37-2.44 (31.54) δ: (176.28)	173.01
Trp 9	8.73-8.75 (119.46)	4.91-4.96 (55.61)	3.31-3.31 (30.65)	1: 10.36-10.38 (N 127.97) 2: 7.17-7.19 (124.93) 3: (111.03) 31: (128.92) 4: 7.80 (119.67) 5: 7.00-7.03 (119.83) 6: 7.06-7.09 (122.25) 7: 7.29-7.32 (112.36) 71: (138.07)	173.48
Hse 10	8.30 (at 0°C)	4.13-4.17 (52.31)	1.88-1.91, 2.18-2.24 (35.09)	γ: 4.09-4.13, 4.68-4.74 (60.26)	170.14

Table S3: Characteristic NMR spectra of ester-linked homoserine in *depsi*-**4**



depsi-**4**

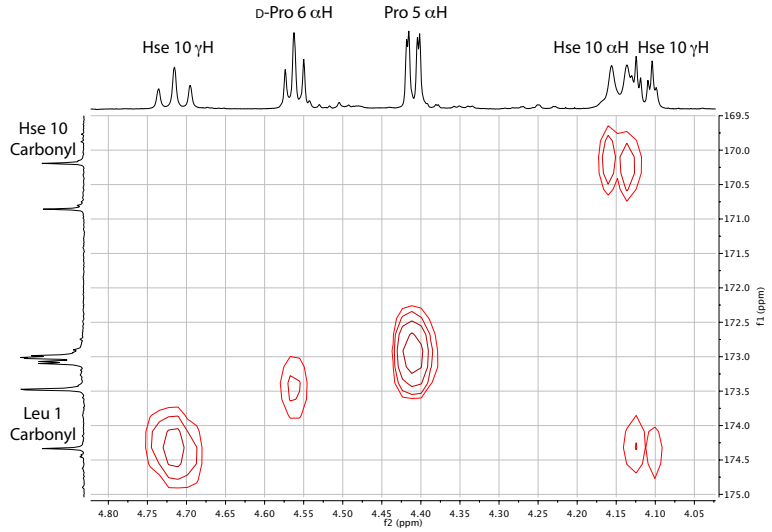
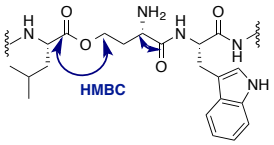
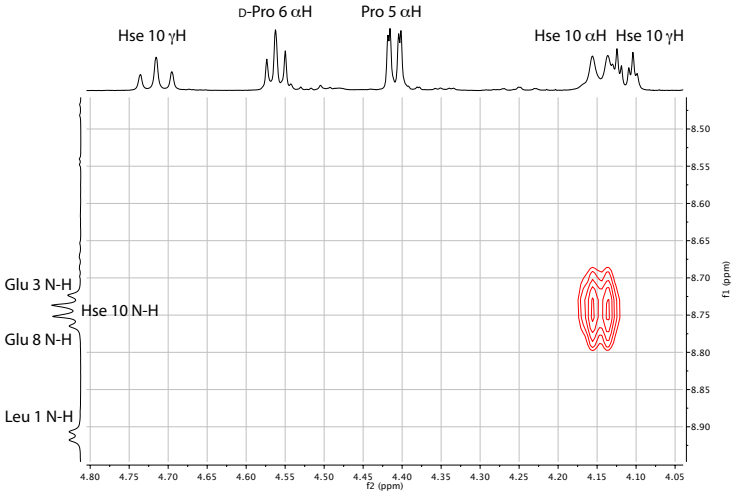
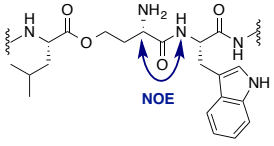
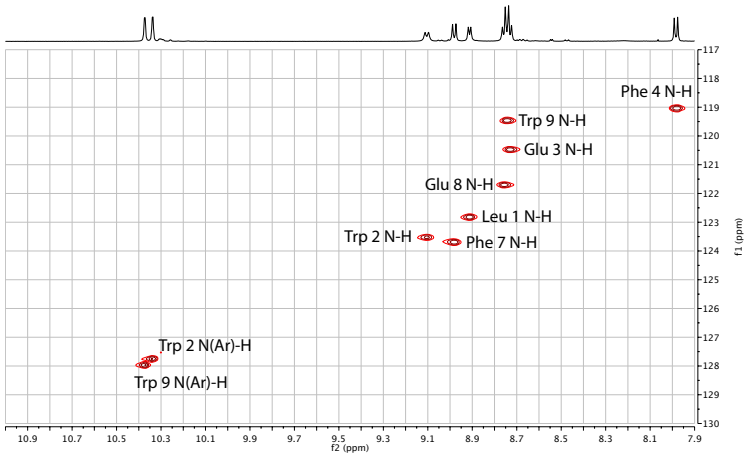
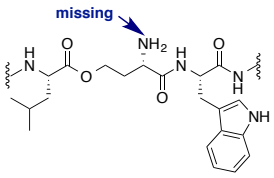
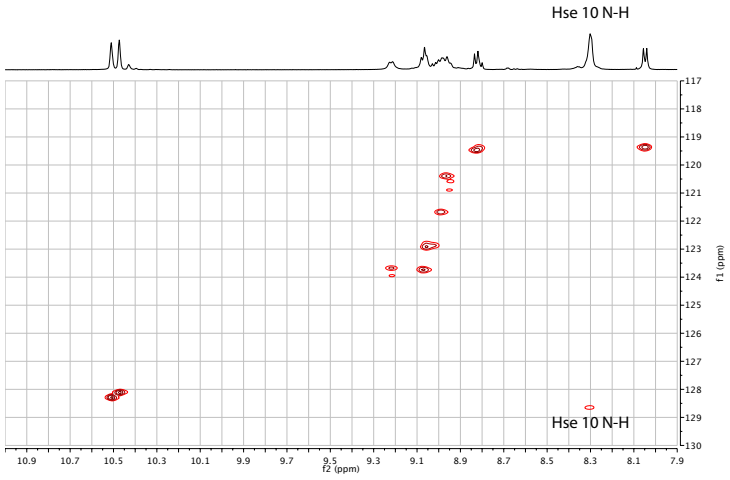
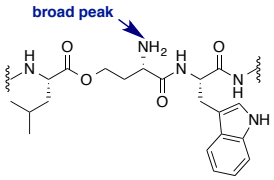
Experiment	**Spectrum**	**Illustration**
HMBC at rt Crosspeaks between: Hse-αH and Hse-Carbonyl and Hse-γH and Leu-Carbonyl		
ROESY at rt NOE between Hse-αH and Trp-NH		

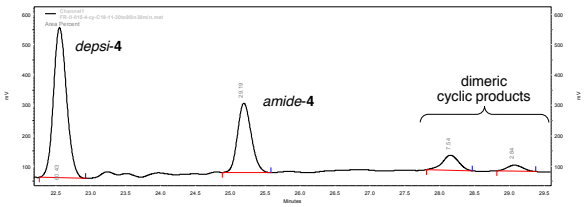
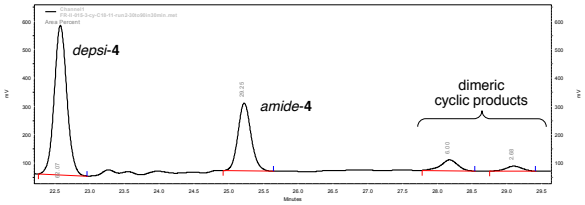
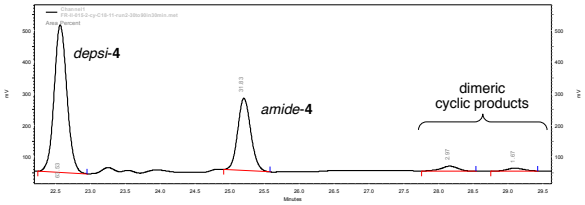
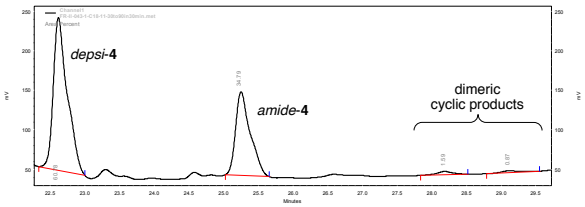
Table S3 (continued): Characteristic NMR spectra of ester-linked homoserine in *depsi*-**4**

Experiment	Spectrum	Illustration
^{1}H-^{15}N HSQC at rt All N-H (cross)peaks are visible exept for Hse-NH_2.		
^{1}H-^{15}N HSQC at 0°C acidified with TFA Hse-NH_2 is now visible as broad peak due to slower exchange at 0 °C and low pH.		

5.6. Influence of concentration on the formation of dimers

Crude **3** was dissolved in 2:1 CH_3CN/H_2O (0.1 M oxalid acid), shaken at 60 °C for 4 h and analyzed by HPLC.

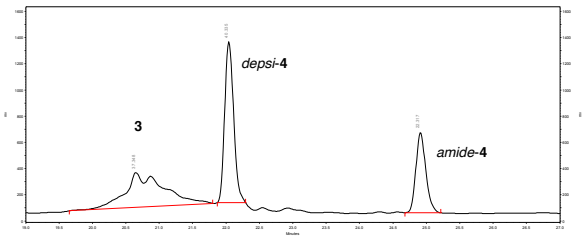
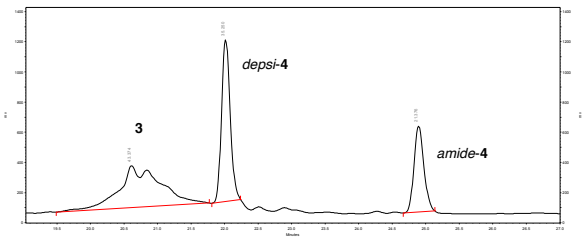
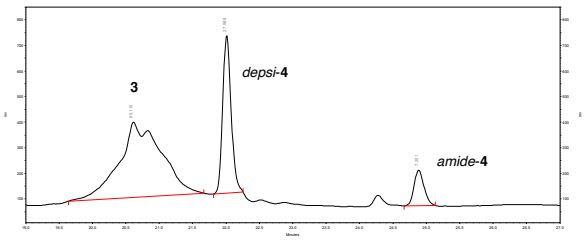
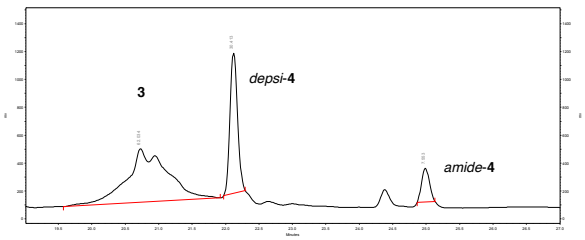
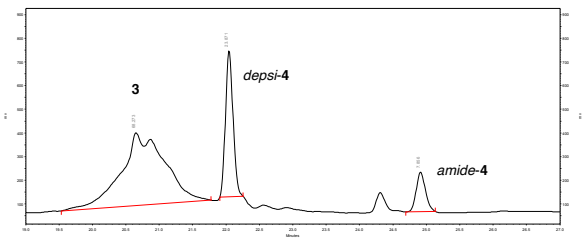
Table S4: Influence of concentration on the formation of dimers

Details	Integrated (relative area %) HPLC trace at 220 nm	Product distibution
3: 2.0 mg, 1.45 µmol Solvent: 72.5 µL Concentration: 20 mM		*depsi*-**4**: 60.4 % *amide*-**4**: 29.2 % cyclodimers: 10.4 %
3: 1.0 mg, 725 nmol Solvent: 72.5 µL Concentration: 10 mM		*depsi*-**4**: 62.1 % *amide*-**4**: 29.2 % cyclodimers: 8.7 %
3: 1.0 mg, 725 nmol Solvent: 145 µL Concentration: 5 mM		*depsi*-**4**: 63.5 % *amide*-**4**: 31.8 % cyclodimers: 4.7 %
3: 1.0 mg, 725 nmol Solvent: 725 µL Concentration: 1 mM		*depsi*-**4**: 62.0% *amide*-**4**: 35.5% cyclodimers: 2.5 %

5.1. Influence of solvents on the conversion

3 (0.3 mg, 218 nmol) was dissolved in a solvent (218 µL), shaken at 60 °C for 30 min and analyzed by HPLC. The conversion was determined by comparing the relative area % of starting material and products.

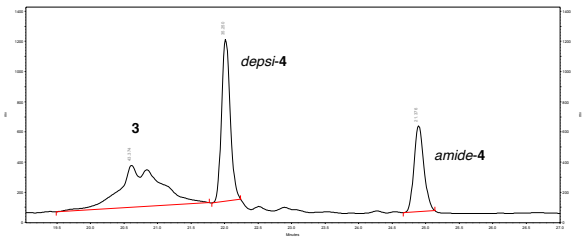
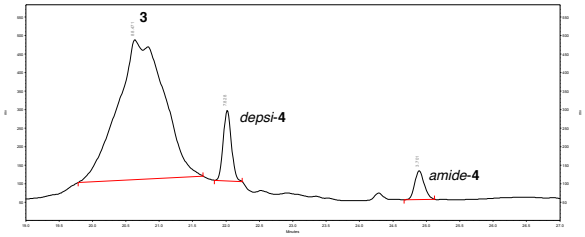
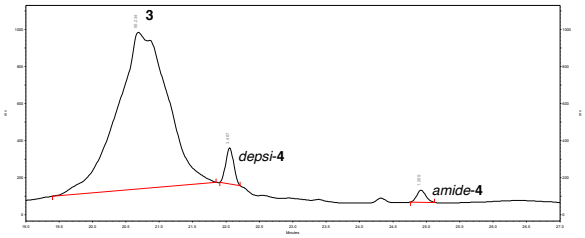
Table S5: Influence of solvents on the conversion

Solvent	Integrated (relative area %) HPLC trace at 220 nm	Product distibution
4:1 CH_3CN/H_2O (H_2O: 0.1 M oxalic acid)		Starting material **3**: 37.3 % *amide*-**4**: 40.3 % *depsi*-**4**: 22.3 % Sum products: 62.7 %
2:1 CH_3CN/H_2O (H_2O: 0.1 M oxalic acid)		Starting material **3**: 43.3 % *amide*-**4**: 35.3 % *depsi*-**4**: 21.4 % Sum products: 56.7 %
2:1 $DMSO/H_2O$ (H_2O: 0.1 M oxalic acid)		Starting material **3**: 65.1 % *amide*-**4**: 27.6 % *depsi*-**4**: 7.3 % Sum products: 34.9 %
2:1 DMF/H_2O (H_2O: 0.1 M oxalic acid)		Starting material **3**: 62.0 % *amide*-**4**: 30.4 % *depsi*-**4**: 7.6 % Sum products: 38.0 %
2:1 NMP/H_2O (H_2O: 0.1 M oxalic acid)		Starting material **3**: 68.2 % *amide*-**4**: 23.9 % *depsi*-**4**: 7.9 % Sum products: 31.8 %

5.2. Influence of temperature on the conversion

3 (0.3 mg, 218 nmol) was dissolved in 218 µL of 2:1 CH_3CN/H_2O (0.1 M oxalic acid), shaken at different temperatures for 30 min and analyzed by HPLC. The conversion was determined by comparing the relative area % of starting material and products.

Table S6: Influence of temperature on the conversion

Temperature	Integrated (relative area %) HPLC trace at 220 nm	Product distibution
60 °C		Starting material **3**: 43.3 % *amide*-**4**: 35.3 % *depsi*-**4**: 21.4 % Sum products: 56.7 %
40 °C		Starting material **3**: 88.5 % *amide*-**4**: 7.8 % *depsi*-**4**: 3.7 % Sum products: 11.5 %
22 °C		Starting material **3**: 95.0 % *amide*-**4**: 3.5 % *depsi*-**4**: 1.3 % Sum products: 4.8 %

6. Epimerization Analysis

6.1. Chiral GC/MS

6.1.1. Method

Samples were analyzed by "C.A.T. GmbH & Co Chromatographie und Analysetechnik KG" (Tübingen, Germany) using their established method. In brief, peptides were hydrolyzed with DCl in D_2O, converted into volatile derivatives and analyzed by chiral GC/MS. The amino acid of interest was identified via retention time and mass spectrum.

6.1.2. Results

Table S7: Epimerization analysis by chiral GC-MS.

Entry	Compound	Description	Content of D-Leu or D-Phe
1	*depsi*-**4**	isolated directly from reaction mixture[a]	5.6 %
2	*amide*-**4**		13 %
3	*amide*-**4**	after rearrangement of isolated *depsi*-**4**[b]	4.7 %
4	*amide*-**4**	prepared by method B (see page 14)	8.1 %
5	*amide*-**4**	from reaction in 1:1 $(CH_3)_3COH/H_2O$[c]	18 %
6	*amide*-**4**	prepared by method A (see page 13)	1.3 %
7	*amide*-**S19**	prepared by method A (see page 13)	4.9 %
8	*amide*-**S19**	prepared by method B (see page 14)	9.7 %

[a]2:1 CH_3CN/0.05 M oxalic acid in H_2O, 50 °C, 15 h; [b]2:1 CH_3CN/0.05 M oxalic acid in H_2O, 1.6 M NH_3, 23 °C, 3 h; [c]1:1 $(CH_3)_3COH/H_2O$, 0.1 M oxalic acid, 60°C, 15 h.

6.2. Deuterium Incorporation

6.2.1. Method

After cyclization of **3** using the conditions specified below, an aliquot of the reaction mixture was analyzed by analytical HPLC. The product-containing fractions were collected and incubated at 60 °C for 3 h. lyophilized and analyzed by MALDI-MS. Each set of MS signals was normalized to 100 %. For each compound of interest (*depsi*-**4** and *amide*-**4**) a reference MS spectrum without deuterium incorporation was recorded to exclude

machine-related systematic errors. The variable m is defined as the monoisotopic mass of the compound of interest. X can be Na^+ or H^+. Other variables are defined as follows:

Reference mass spectrum

X_R: Normalized Intensity of $[m+X]^+$ peak

Y_R: Normalized Intensity of $[m+1+X]^+$ peak

Z_R: Normalized Intensity of $[m+2+X]^+$ peak

Sample mass spectrum

X_R: Normalized Intensity of $[m+X]^+$ peak

Y_R: Normalized Intensity of $[m+1+X]^+$ peak

Z_R: Normalized Intensity of $[m+2+X]^+$ peak

The deuterium incorporation was calculated using following equations:

$$r_{m+1} = \frac{Y_T - (X_T Y_R)}{X_T + Y_T - (X_T Y_R)}$$

for the $[m+1+H]^+$ peak and

$$r_{m+2} = \frac{Z_T - (X_T Z_R)}{(X_T Y_R) + Z_T - (X_T Z_R)}$$

for the $[m+2+H]^+$ peak. The resulting average incorporation was calculated as:

$$r_{all} = \frac{r_{m+1}^{H^+} + r_{m+2}^{H^+} + r_{m+1}^{Na^+} + r_{m+2}^{Na^+}}{4}$$

with a standard derivation of:

$$\sigma = \sqrt{(r_{m+1}^{H^+})^2 + (r_{m+2}^{H^+})^2 + (r_{m+1}^{Na^+})^2 + (r_{m+2}^{Na^+})^2}$$

Peaks with a higher m/z than $[m+2+H]^+$ were omitted due to the decreasing signal to noise ratio.

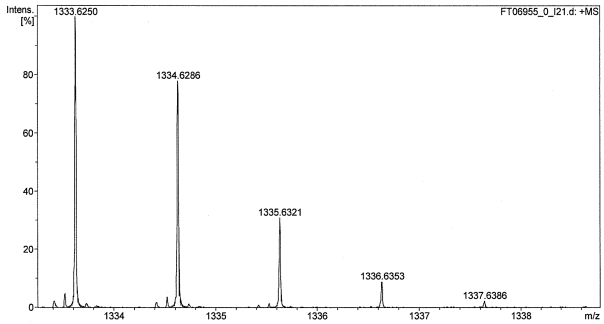
6.2.2. Results

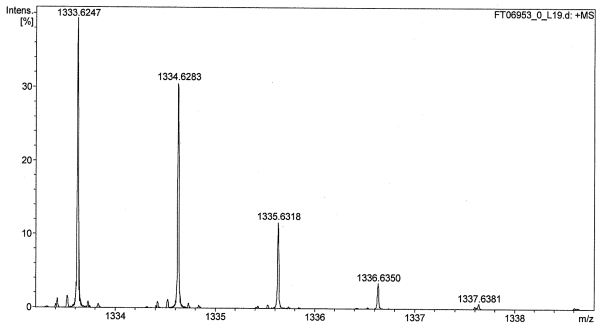
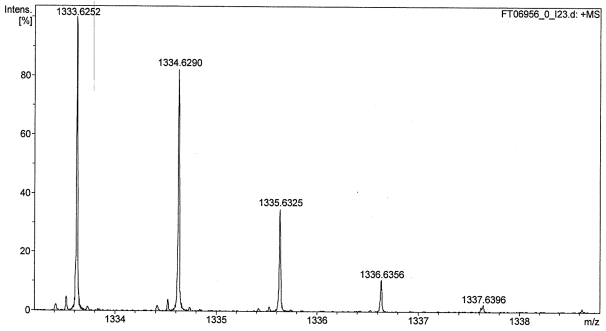
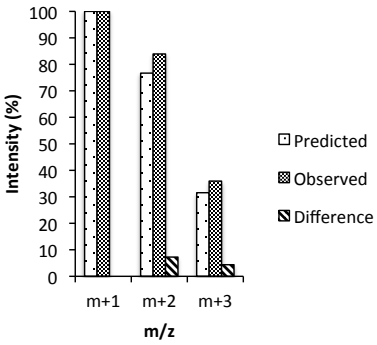
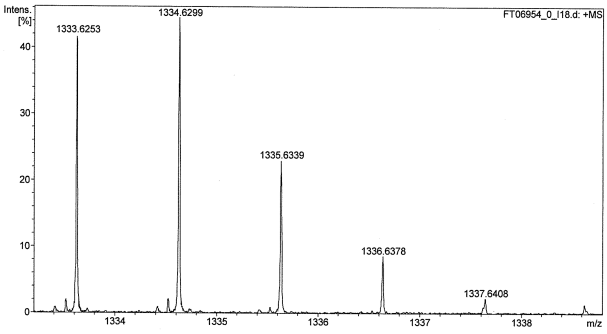
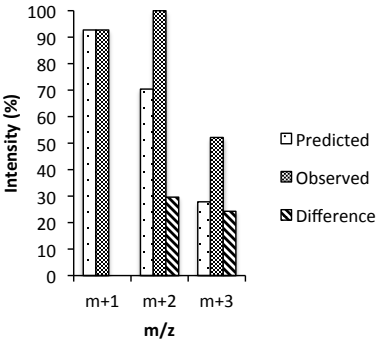
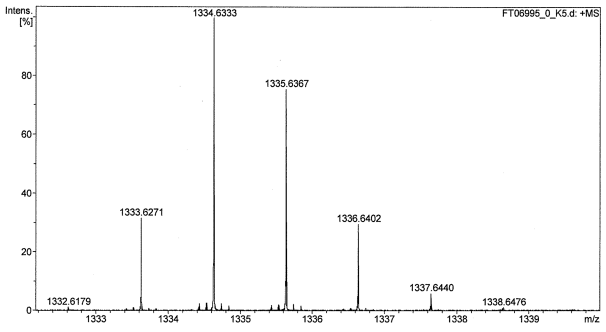
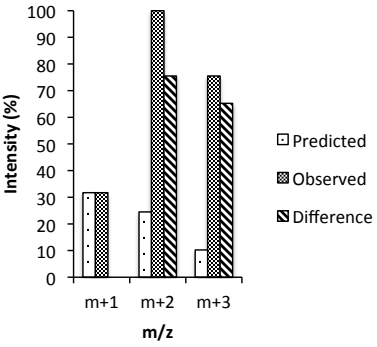
Table S8: Deuterium incorporation into *depsi*-**4** and *amide*-**4** measured by MALDI HR-MS analysis.

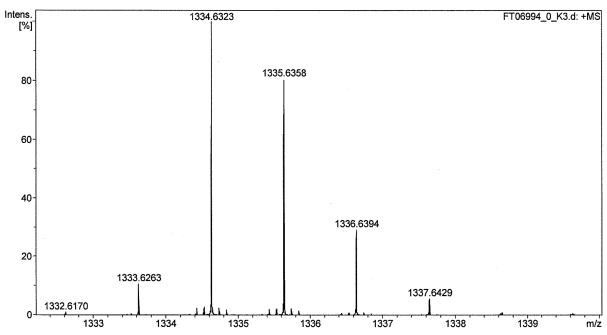
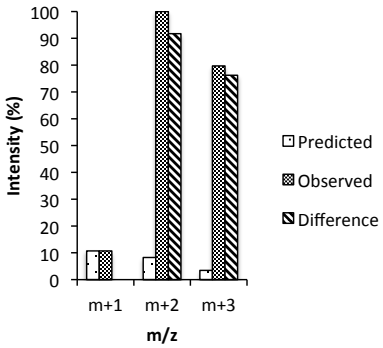
Entry			Intensity[a] [%] m	m+1	m+2	Deuterium incorporation
Reference	depsi	H^+	100	77.2	32.3	-
		Na^+	100	76.1	30.8	
	amide	H^+	100	75.2	30.0	-
		Na^+	100	76.6	30.1	
CH_3CN/D_2O[b]	depsi	H^+	100	82.8	36.1	**6±2 %**
		Na^+	100	85.1	35.7	
	amide	H^+	91.5	100	50.5	**25±1 %**
		Na^+	94.0	100	53.7	
$CH_3CN/MeOD$[c,d]	depsi[e]	H^+	31.7	100	75.5	**72±2 %**
	depsi[f]	H^+	10.7	100	79.9	**90±1 %**

[a]The signal intensity of each data row was normalized to 100 %. [b]2:1 CH_3CN/0.05 M oxalic acid in D_2O, 60 °C, 5 h; [c]2:1 CH_3CN/CH_3OD, 0.1 M oxalic acid, 60 °C; [d]the sample was analyzed using a narrow-scan MALDI-MS method (1320-1350 m/z) and thus only the H^+ adducts were measured. [e]aliquot analyzed after 45 min. [f]aliquot analyzed after 4 h.

Table S9: Visualization of observed MALDI HR mass spectra.

Entry	MALDI HR mass spectrum of 4	Graphic deconvolution
Reference *depsi* H^+ adduct		-

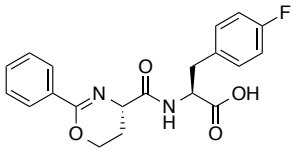
Reference *amide* H^+ adduct		-
CH_3CN/D_2O[a] *depsi* H^+ adduct		
CH_3CN/D_2O[a] *amide* H^+ adduct		
$CH_3CN/MeOD$[b,c] *depsi* after 45 min H^+ adduct		

$CH_3CN/MeOD$[b,d]		
depsi		
after 4 h		
H^+ adduct		

[a]2:1 CH_3CN/0.05 M oxalic acid in D_2O, 60 °C, 5 h; [b]2:1 CH_3CN/CH_3OD, 0.1 M oxalic acid, 60 °C; [c]aliquot analyzed after 45 min. [d]aliquot analyzed after 4 h.

7. Mechanistic Studies

7.1. Synthesis of iminoether 24 by KAHA ligation



24

(*S*)-3-(4-fluorophenyl)-2-((*S*)-isoxazolidine-3-carboxamido)propanoic acid **23** (10.0 mg, 35.4 μmol, 1.00 equiv) and benzoylformic acid **22** (10.6 mg, 70.8 μmol, 2.00 equiv) were dissolved in dry CH_3OH. The mixture was heated to 60 °C for 35 min and purified by preparative HPLC (Shiseido Capcell Pak MGII (5 µm, 20 mm I.D. x 250 mm) using a gradient of 5 % to 90 % of CH_3CN in 27 min with a flow rate of 10 mL/min. Both eluents contained 0.01 % (v/v) HCO_2H. The use of 0.1 % TFA as acidic additive results in partial hydrolysis of the product. The product containing fractions were lyophilized to afford **24** (4.1 mg, 11 μmol, 31 %) as a white solid.

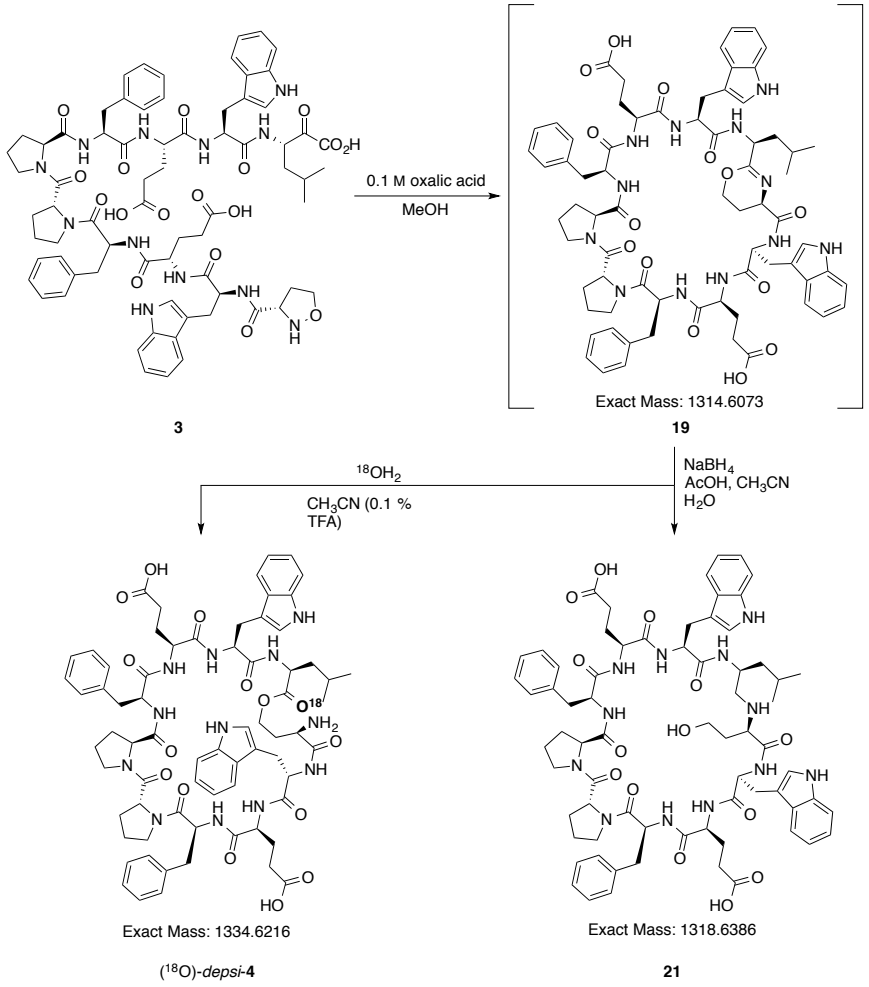
^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 12.99 (bs, 1H), 7.90 – 7.82 (m, 3H), 7.55 – 7.49 (m, 1H), 7.47 – 7.41 (m, 2H), 7.18 (m, 2H), 7.01 – 6.90 (m, 2H), 4.55 (appt d, *J* = 8.1, 5.2 Hz, 1H), 4.33 (appt d, *J* = 10.4, 3.4 Hz, 1H), 4.26 (d appt, *J* = 10.6, 4.4 Hz, 1H), 4.10 (dd, *J* = 9.2, 4.9 Hz, 1H), 3.11 (dd, *J* = 13.8, 5.2 Hz, 1H), 3.04 (dd, *J* = 13.8, 8.1 Hz, 1H), 2.17 (d appt d, *J* = 13.5, 4.7, 3.4 Hz, 1H), 1.66 (d appt d, *J* = 13.5, 9.5, 4.5 Hz, 1H).

^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ 172.5, 171.1, 161.0 (d, J_{CF} = 243 Hz), 154.6, 133.3 (d, J_{CF} = 3.1 Hz), 133.0, 131.0 (d, J_{CF} = 8.1 Hz), 130.9, 128.1, 126.9, 114.8 (J_{CF} = 21.0 Hz), 64.3, 53.8, 52.6, 35.6, 23.8.

IR (thin film): 3347, 2932, 1719, 1655, 1644, 1625, 1524, 1508, 1220, 1196, 1150, 699 cm^{-1}.

HR-MS (ESI): calculated for $C_{20}H_{20}FN_2O_4$ $[M+H]^+$: 371.1402, found: 371.1400.

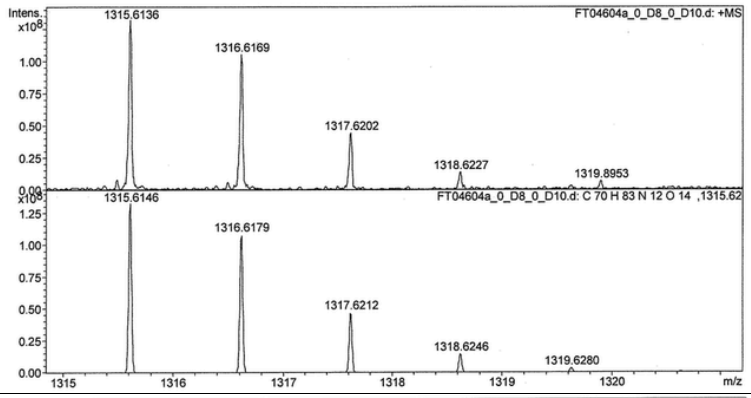
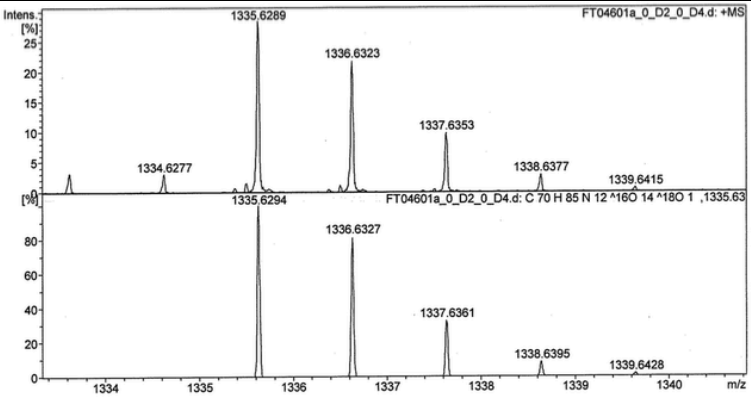
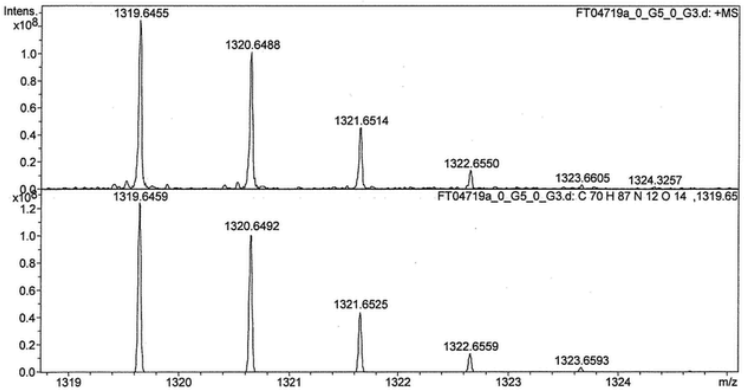
7.2. Observation of intermediates by mass spectroscopy



Scheme S3: Synthesis and reactions of iminoether **19**.

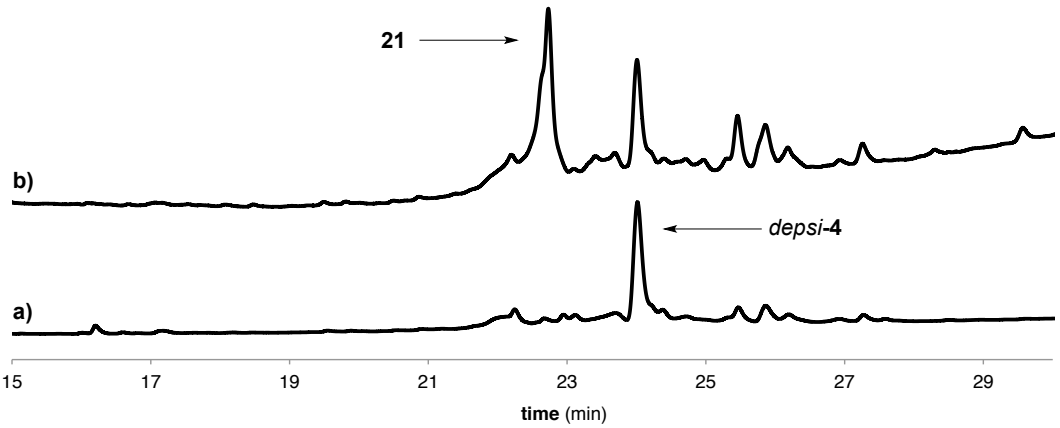
3 (1.0 mg, 726 nmol) and oxalic acid (0.9 mg, 97 μmol) were dissolved in dry MeOH (290 µL) and shaken at 60 °C for 1.5 h. An aliquot of the reaction mixture was analyzed by MALDI HR-MS (m/z calculated for **19** ($C_{70}H_{83}N_{12}O_{14}$) $[M+H]^+$: 1315.6146, found: 1315.6136). Another aliquot (20 µL) of the reaction mixture was mixed with CH_3CN (20 µL, containing 0.1 % TFA) and $^{18}OH_2$ (20 µL, 97 atom % ^{18}O, 1.1 mmol), shaken at 40 °C for 20 min and analyzed by MALDI HR-MS (m/z calculated for (^{18}O)-*depsi*-**4** ($C_{70}H_{85}N_{12}{}^{16}O_{14}{}^{18}O_1$) $[M+H]^+$: 1335.6294, found: 1335.6289). A third aliquot (20 μL) of the reaction mixture was mixed with $NaBH_4$ (2.0 mg, 53 µmol) and CH_3CO_2H (2.5 μL). After 20 min, CH_3CN (20 μL) and H_2O (20 μL) were added and the mixture was analyzed by analytical HPLC. The newly formed peak was collected and analyzed by MALDI HR-MS (m/z calculated for **7** ($C_{70}H_{87}N_{12}O_{14}$) $[M+H]^+$: 1319.6459, found: 1319.6455).

Table S10: MALDI HR-MS Results.

Entry	MALDI HR-MS spectra[a]
Iminoether **19**	
depsi-**4** with ^{18}O incorporation	
Amine **21** after isolation by analytical HPLC	

[a]In each row the top spectrum corresponds to the observed MALDI HR-MS spectrum and the lower spectrum to the calculated spectrum.

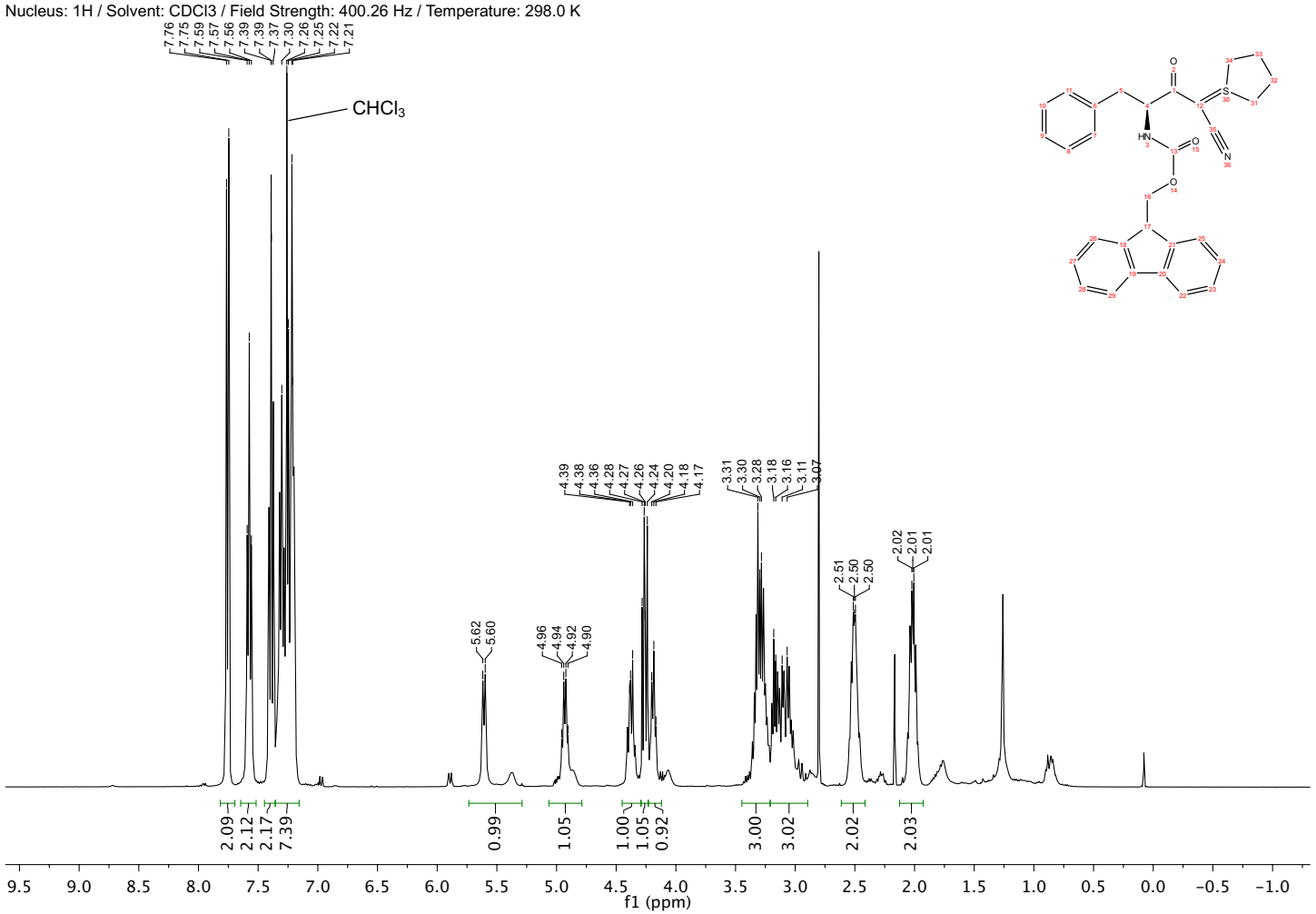
Figure S29: analytical HPLC traces at 220 nm of a) crude mixture reaction mixture after heating in CH_3OH[a] and b) after reduction with $NaBH_4$.

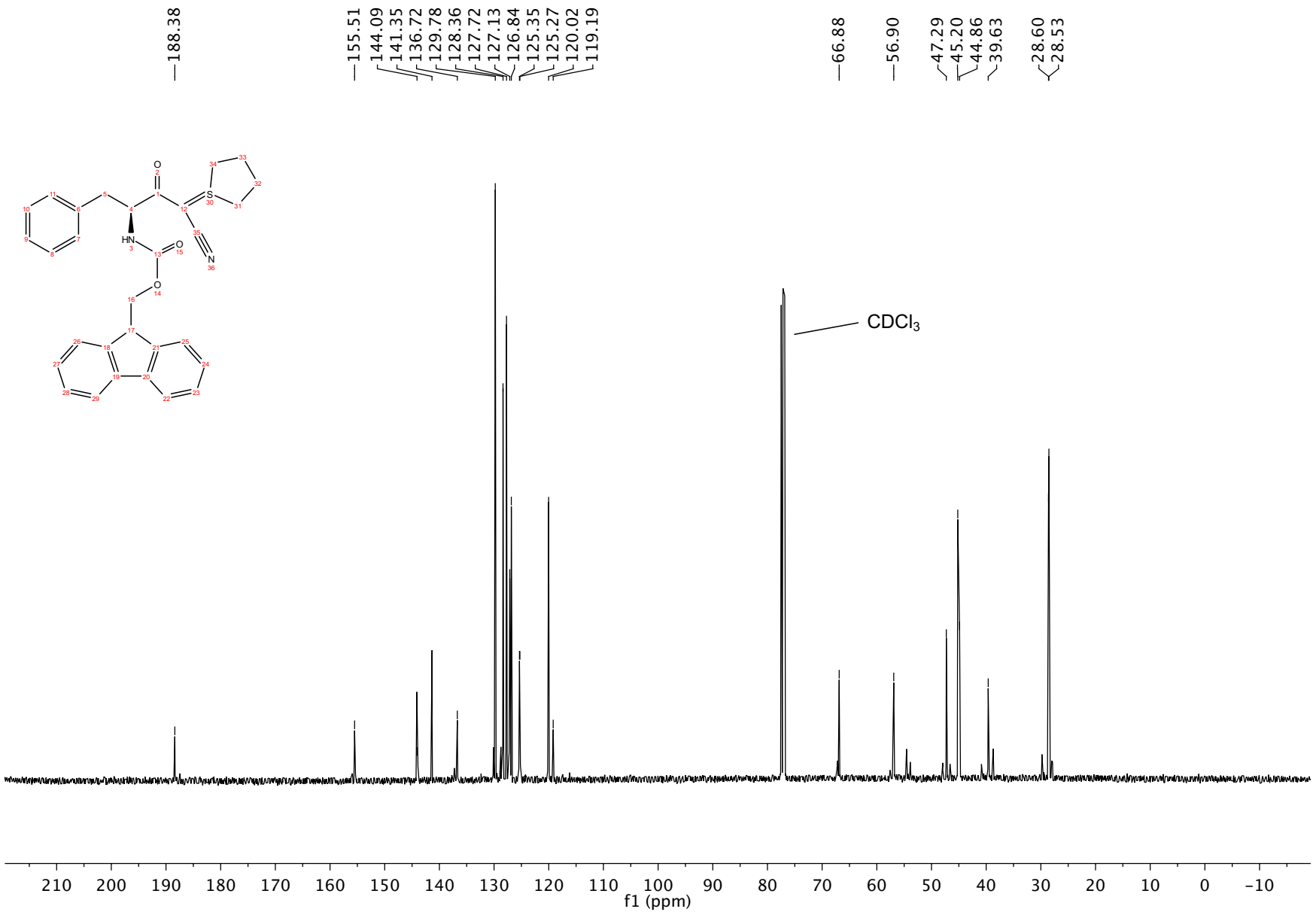


[a]Injection to analytical HPLC results in hydrolysis of iminoether **19** and affords almost exclusively depsi-**4**.

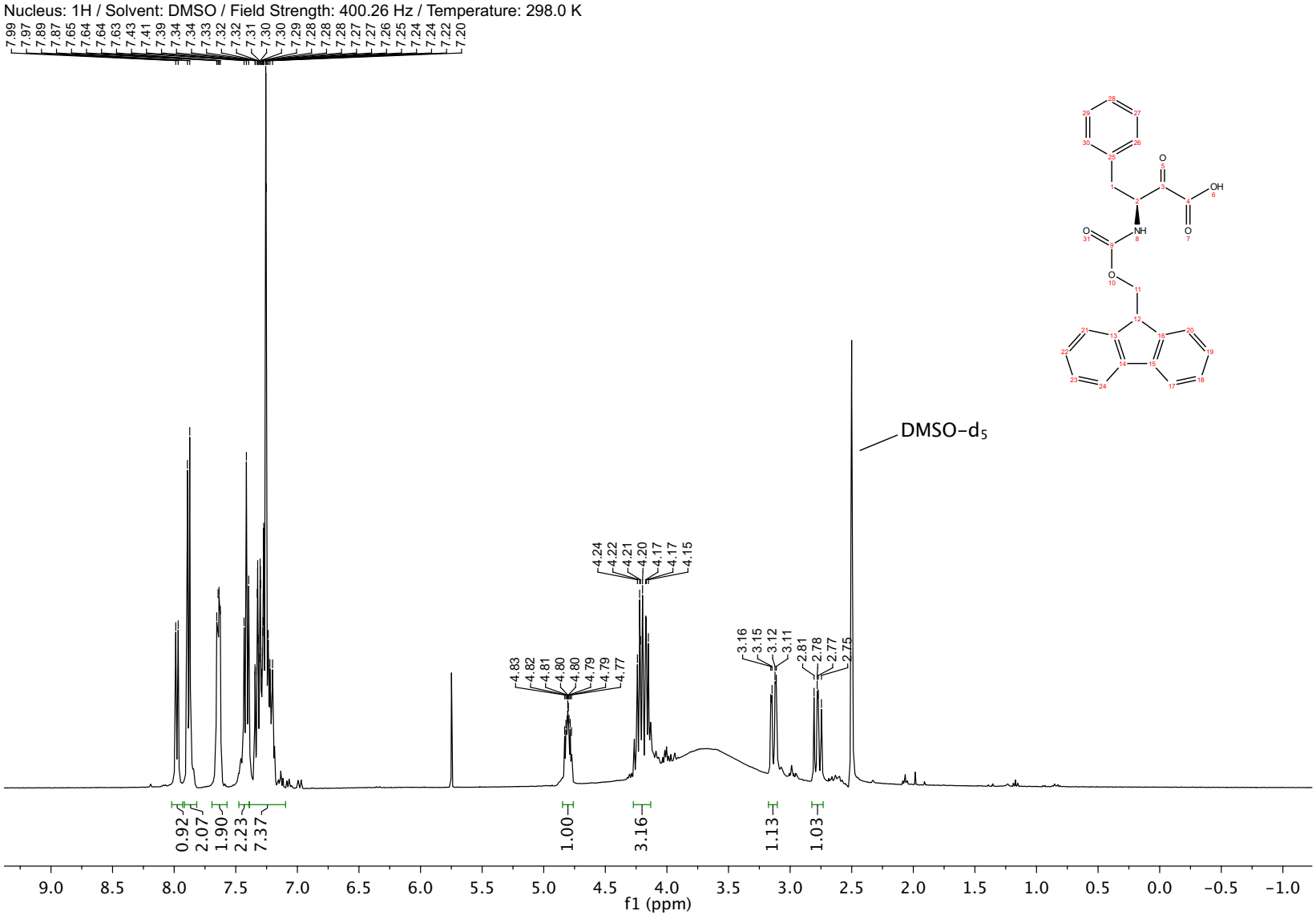
8. NMR Spectra

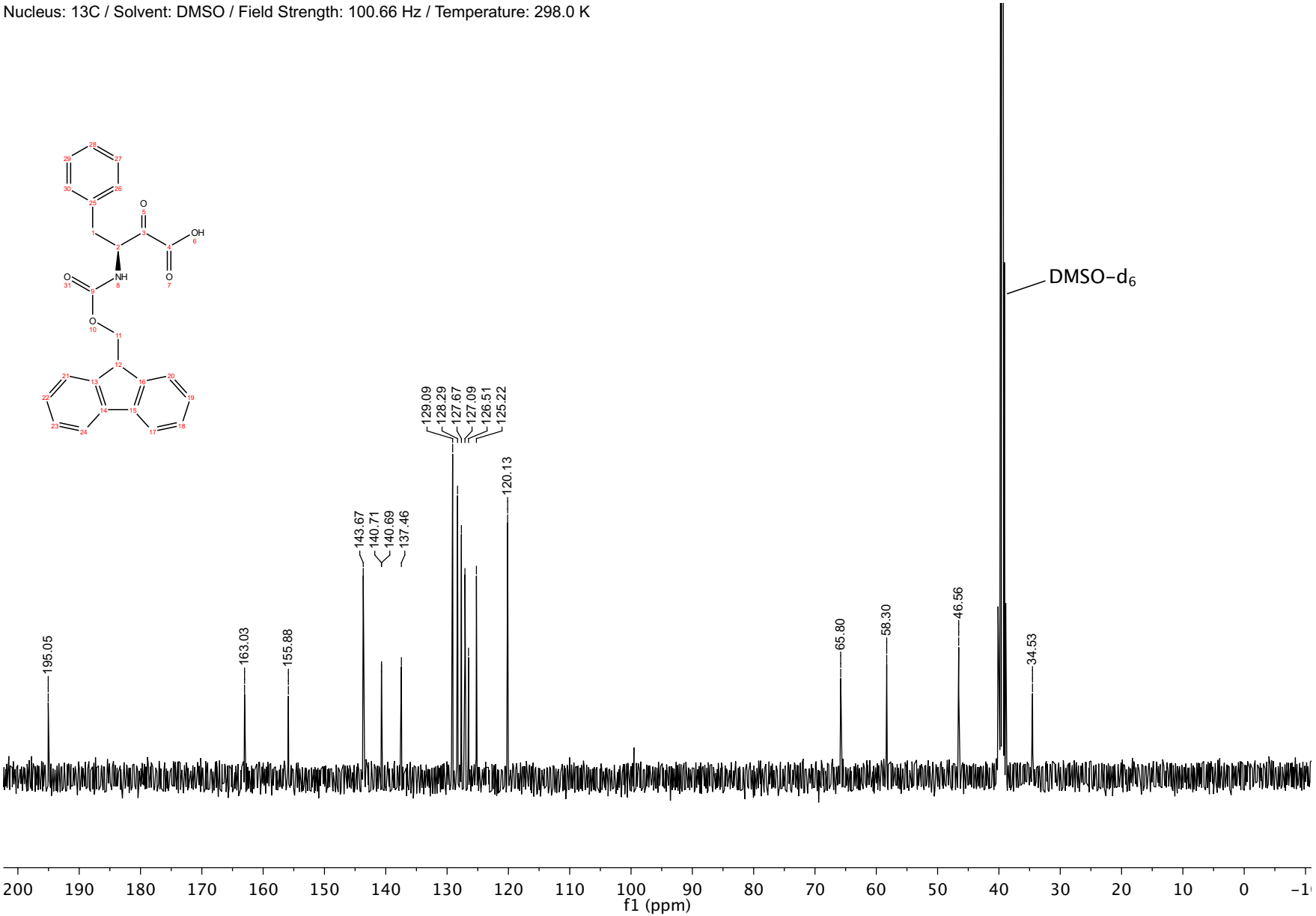
8.1. Fmoc-(*S*)-Phe sulfur ylide S2




188.38
155.51
144.09
141.35
136.72
129.78
128.36
127.72
127.13
126.84
125.35
125.27
120.02
119.19
66.88
56.90
47.29
45.20
44.86
39.63
28.60
28.53
CDCl3
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

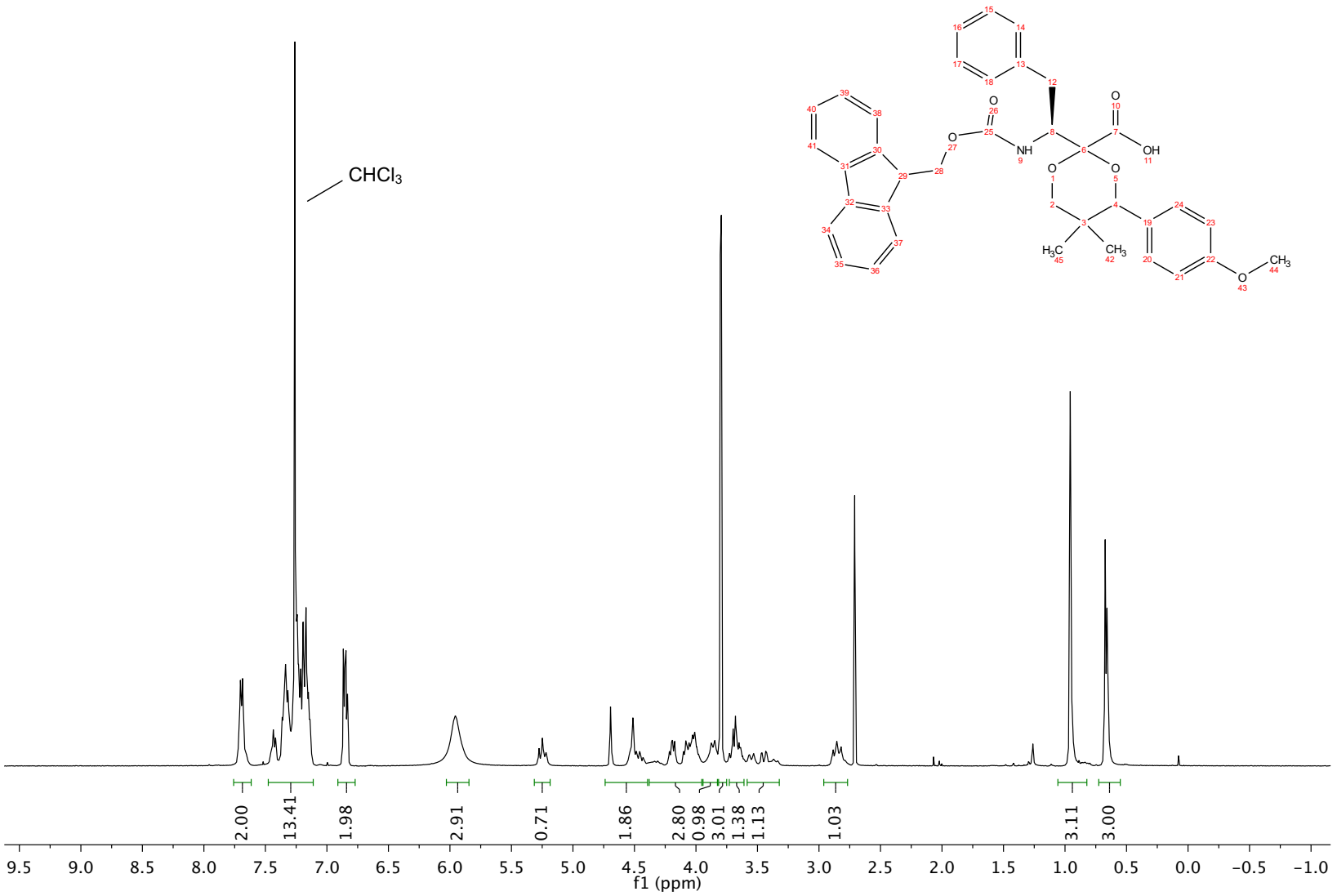
8.2. Fmoc-(*S*)-Phe α-ketoacid S3



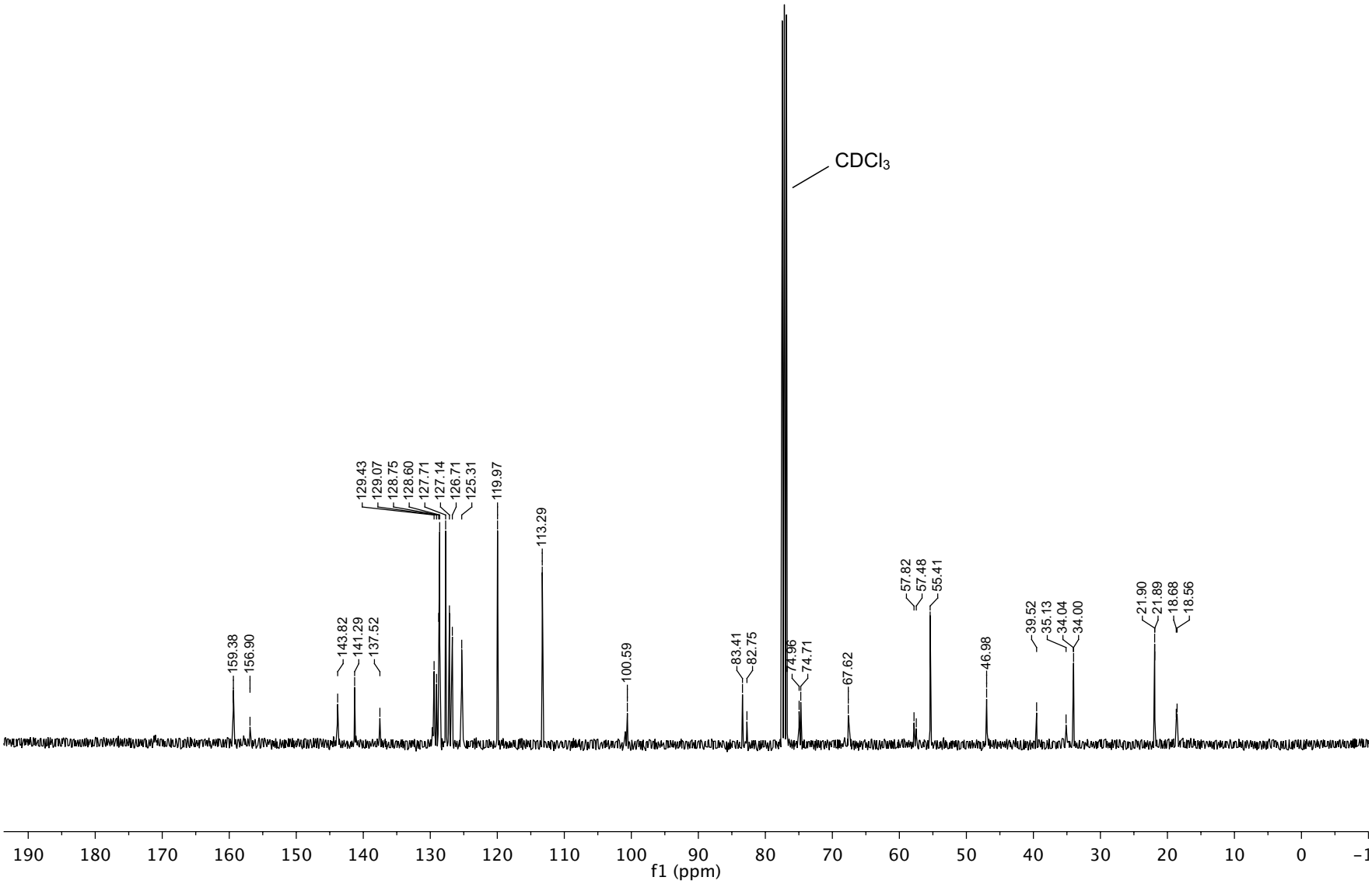

Nucleus: 13C / Solvent: DMSO / Field Strength: 100.66 Hz / Temperature: 298.0 K
DMSO-d6
195.05
163.03
155.88
143.67
140.71
140.69
137.46
129.09
128.29
127.67
127.09
126.51
125.22
120.13
65.80
58.30
46.56
34.53
f1 (ppm)

8.3. Protected Fmoc-Phe α-ketoacid S5

Nucleus: 1H / Solvent: CDCl3 / Field Strength: 400.26 Hz / Temperature: 298.0 K

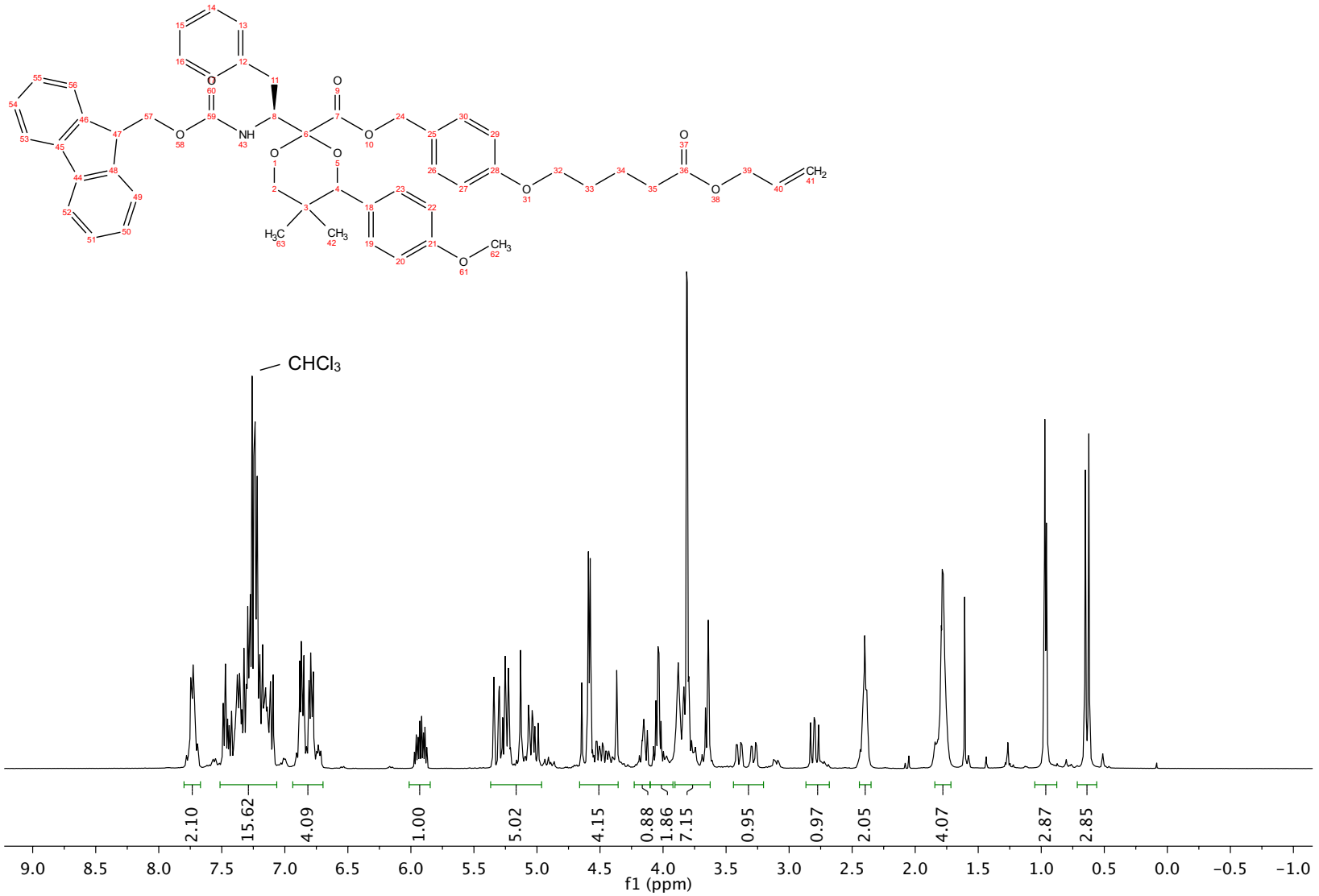


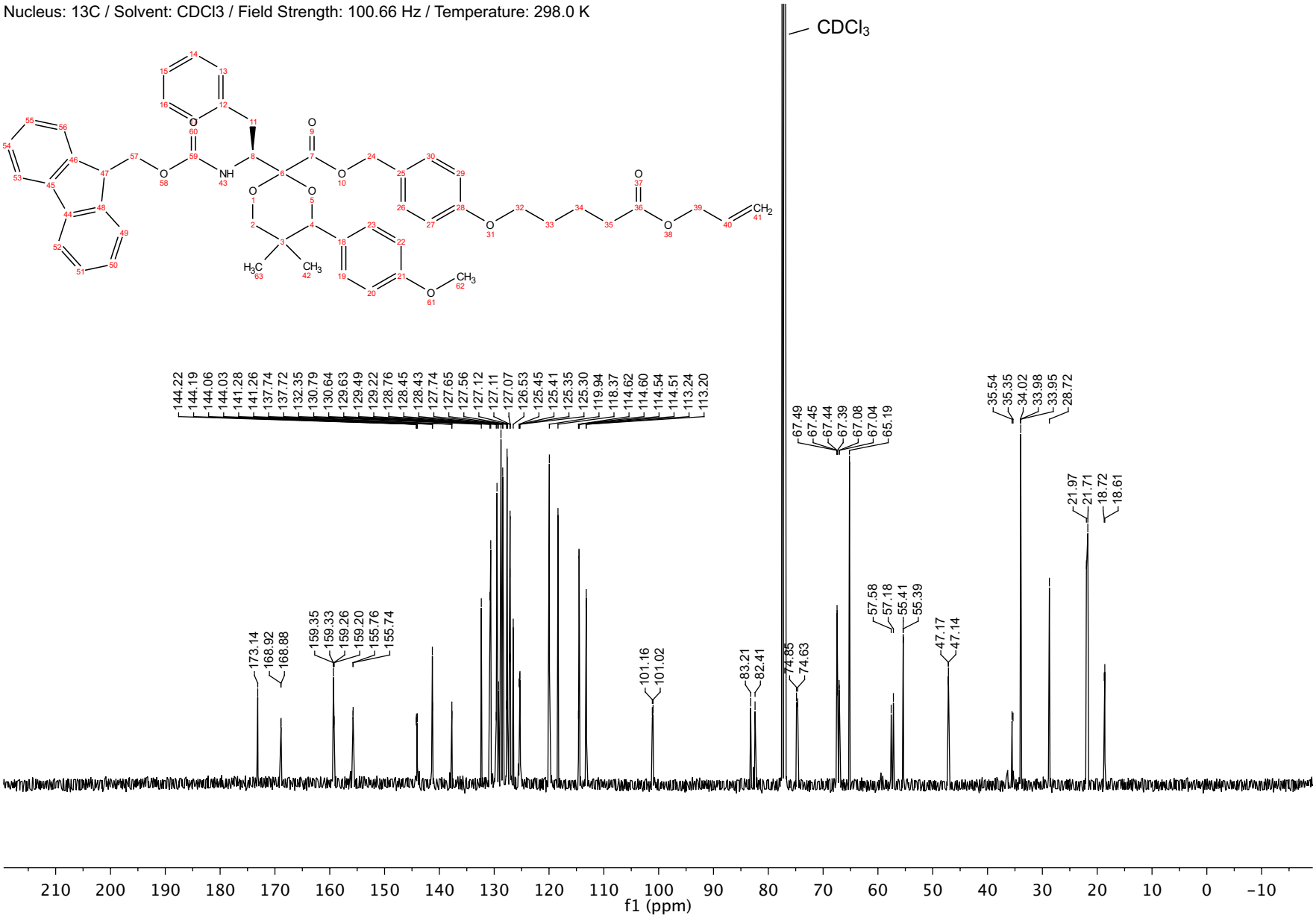
Nucleus: 13C / Solvent: CDCl3 / Field Strength: 100.66 Hz / Temperature: 298.0 K



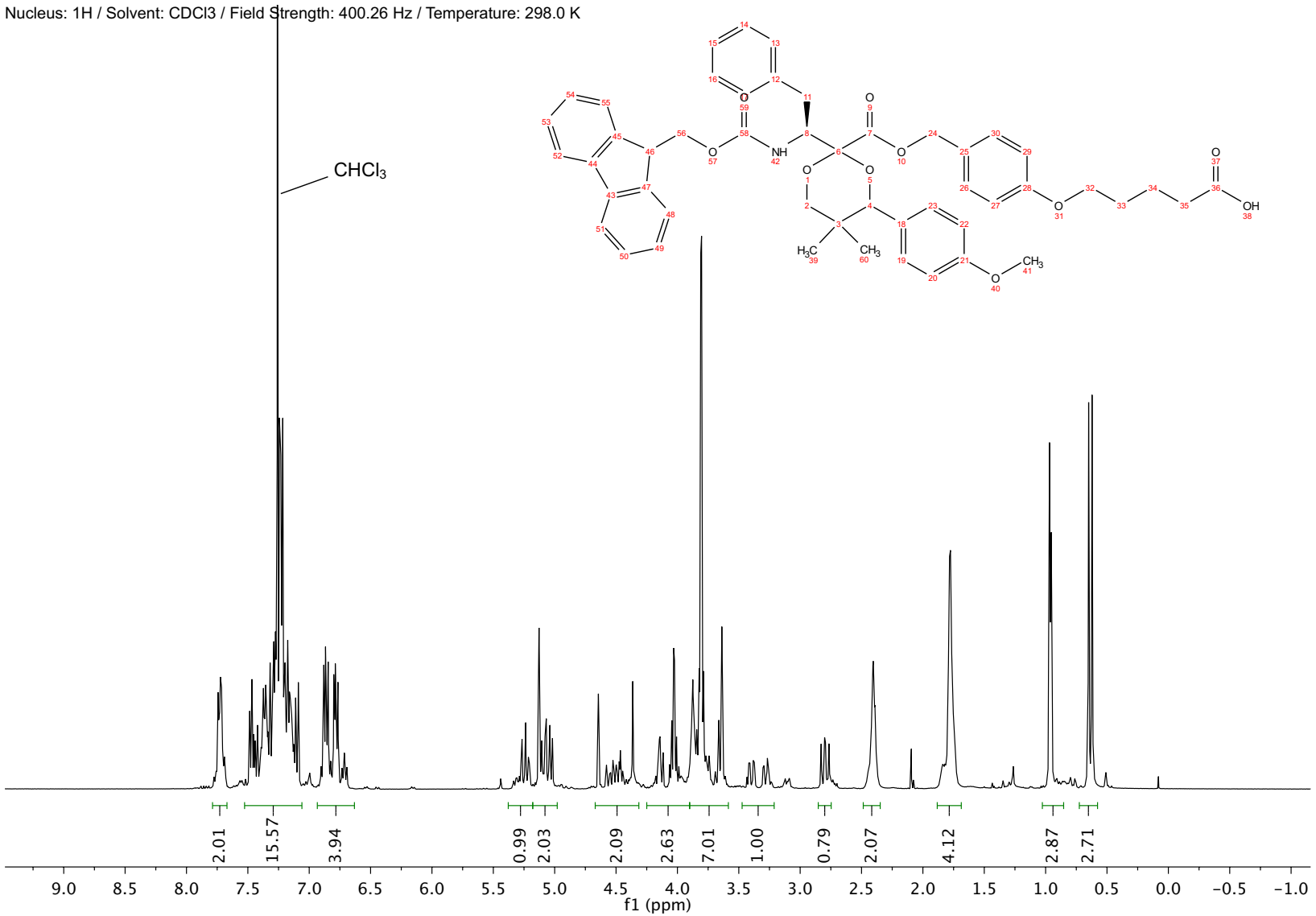
8.4. Protected Fmoc-Phe α-ketoacid with linker allyl ester S7

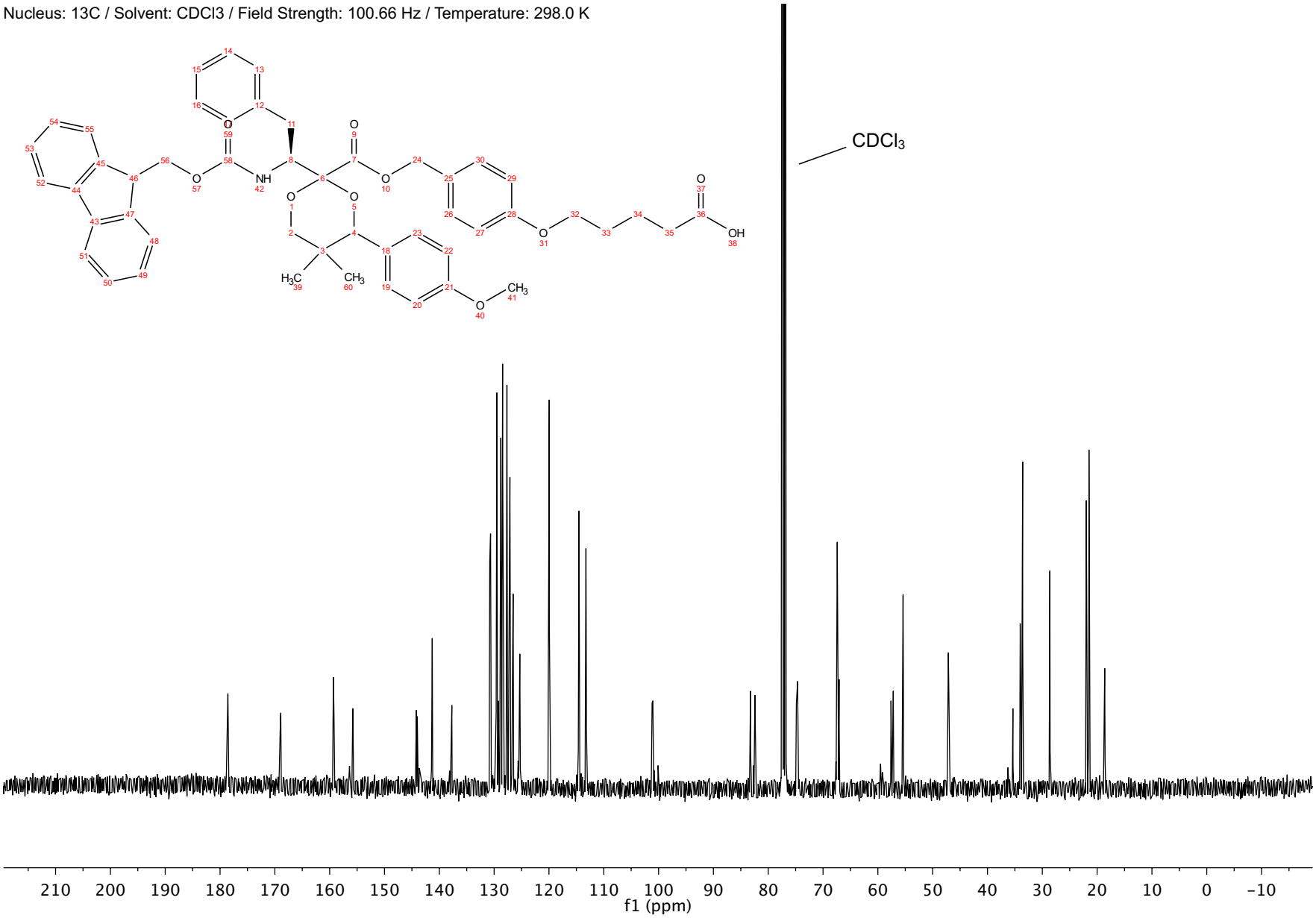
Nucleus: 1H / Solvent: CDCl3 / Field Strength: 400.26 Hz / Temperature: 298.0 K



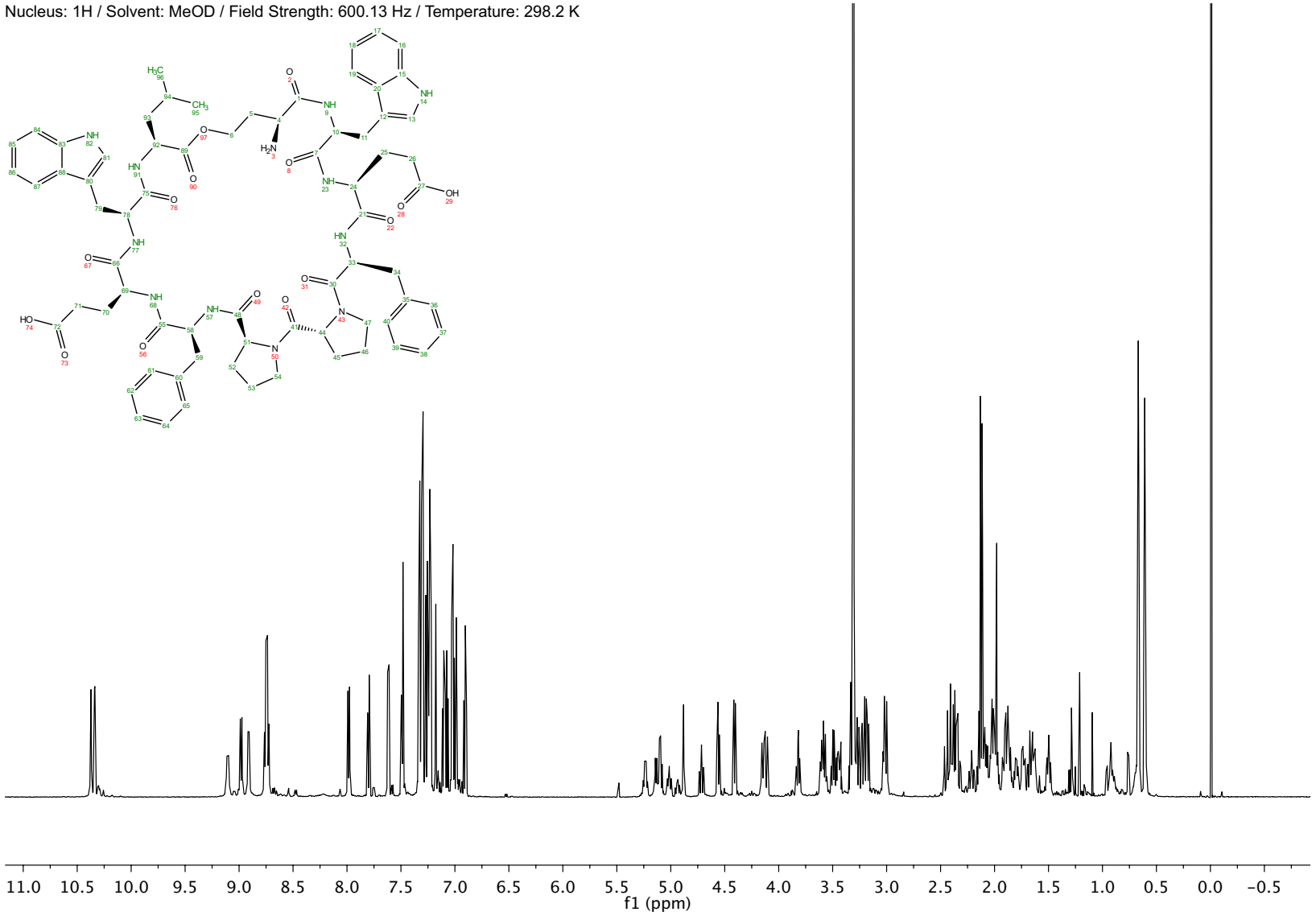

Nucleus: 13C / Solvent: CDCl3 / Field Strength: 100.66 Hz / Temperature: 298.0 K
CDCl3
144.22
144.19
144.06
144.03
141.28
141.26
137.74
137.72
132.35
130.79
130.64
129.63
129.49
129.22
128.76
128.45
128.43
127.74
127.65
127.56
127.12
127.11
127.07
126.53
125.45
125.41
125.35
125.30
119.94
118.37
114.62
114.60
114.54
114.51
113.24
113.20
67.49
67.45
67.44
67.39
67.08
67.04
65.19
35.54
35.35
34.02
33.98
33.95
28.72
21.97
21.71
18.72
18.61
173.14
168.92
168.88
159.35
159.33
159.26
159.20
155.76
155.74
101.16
101.02
83.21
82.41
74.85
74.63
57.58
57.18
55.41
55.39
47.17
47.14
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

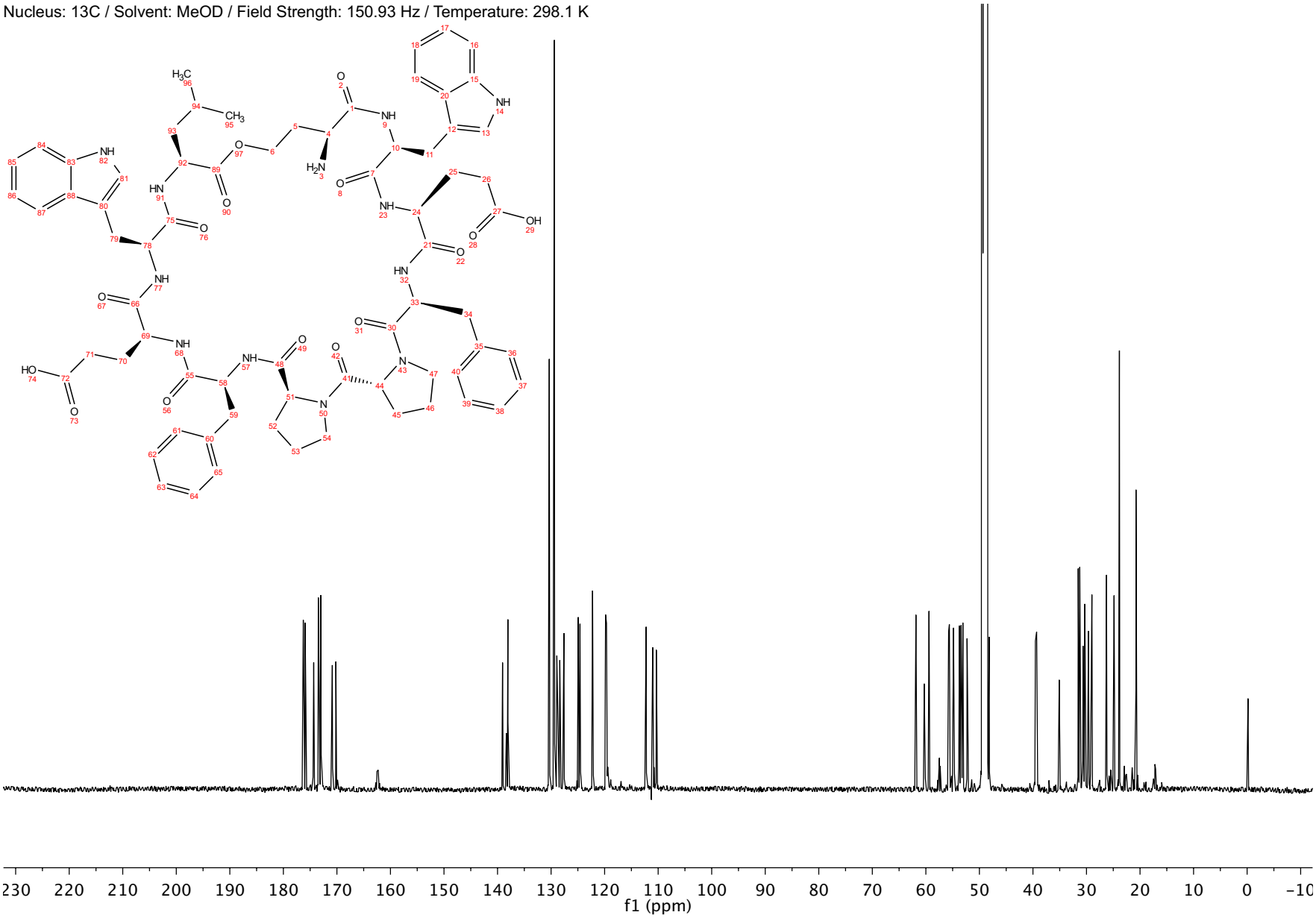
8.5. Protected Fmoc-Phe α-ketoacid with linker S8

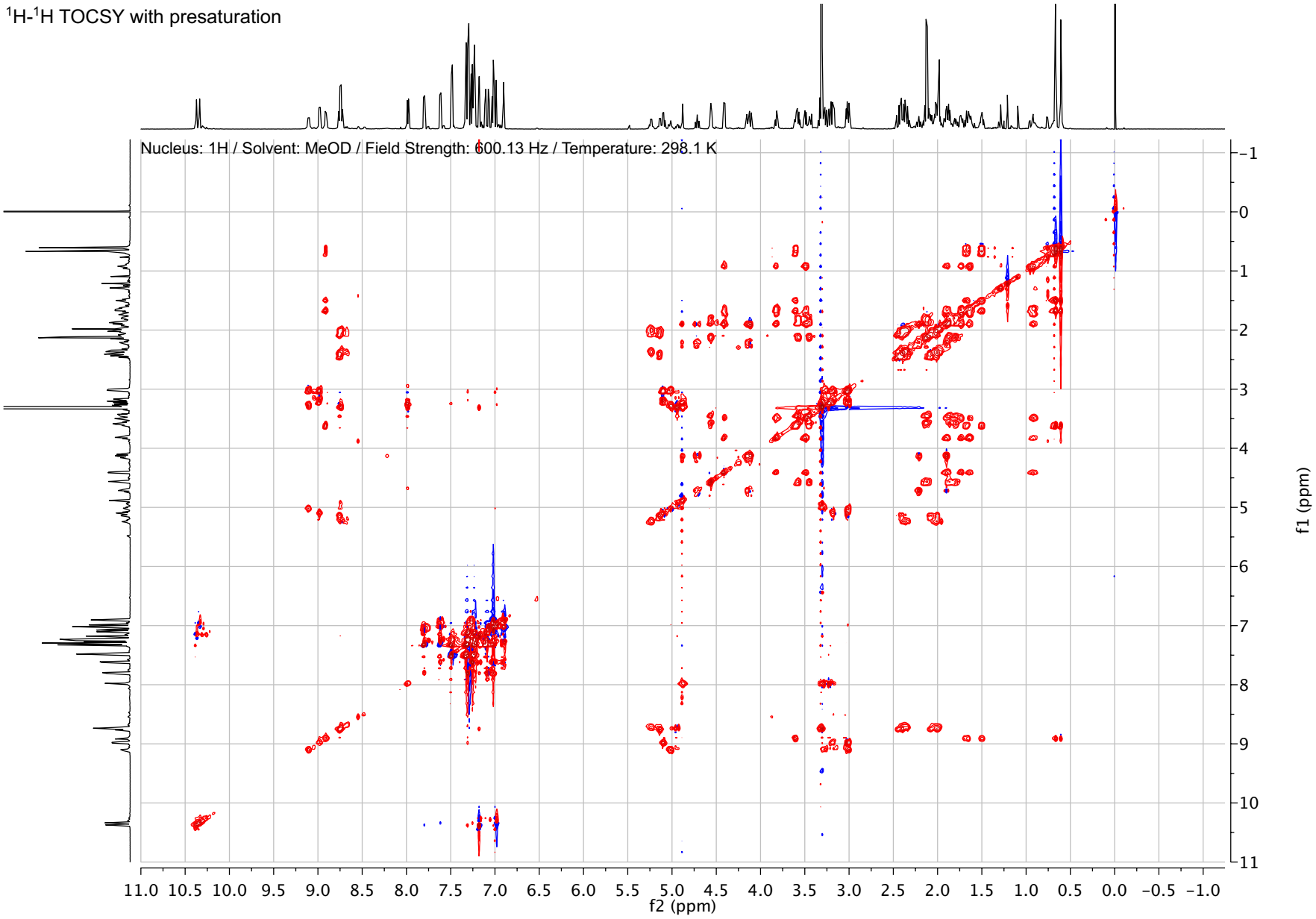


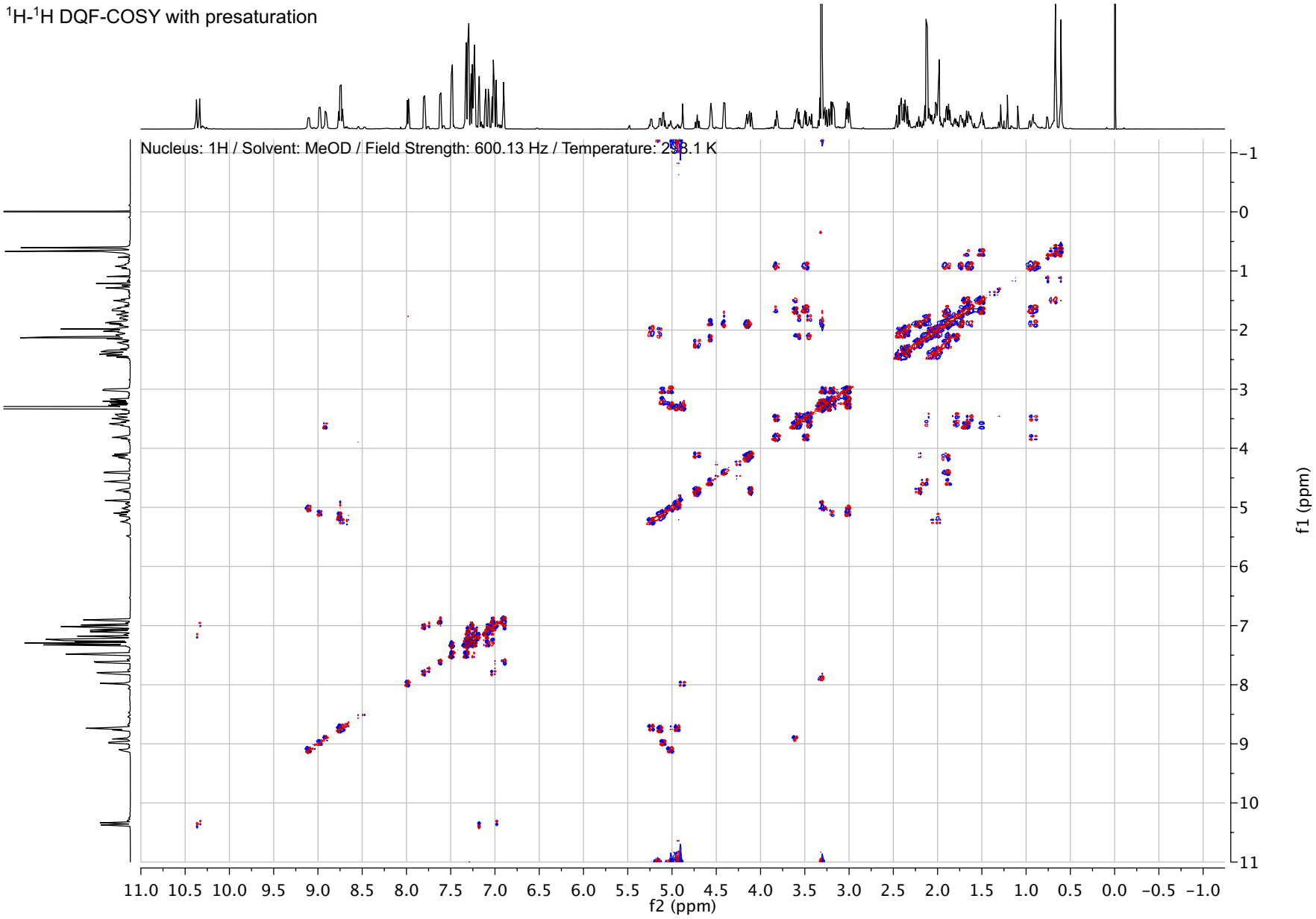

Nucleus: 13C / Solvent: CDCl3 / Field Strength: 100.66 Hz / Temperature: 298.0 K
CDCl3
f1 (ppm)

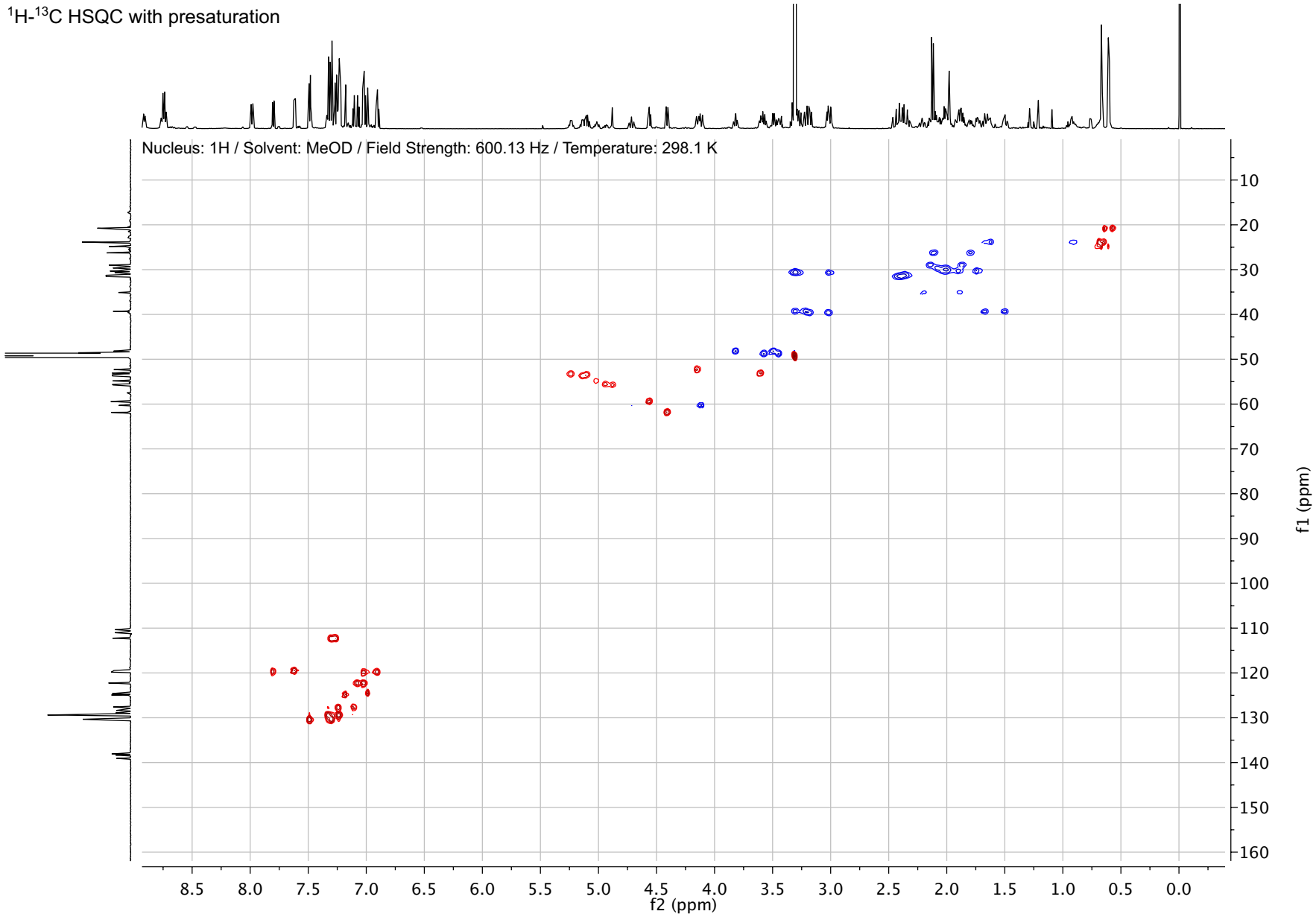
8.6. *depsi*-cyclo(HseWEFpPFEWL) *depsi*-4

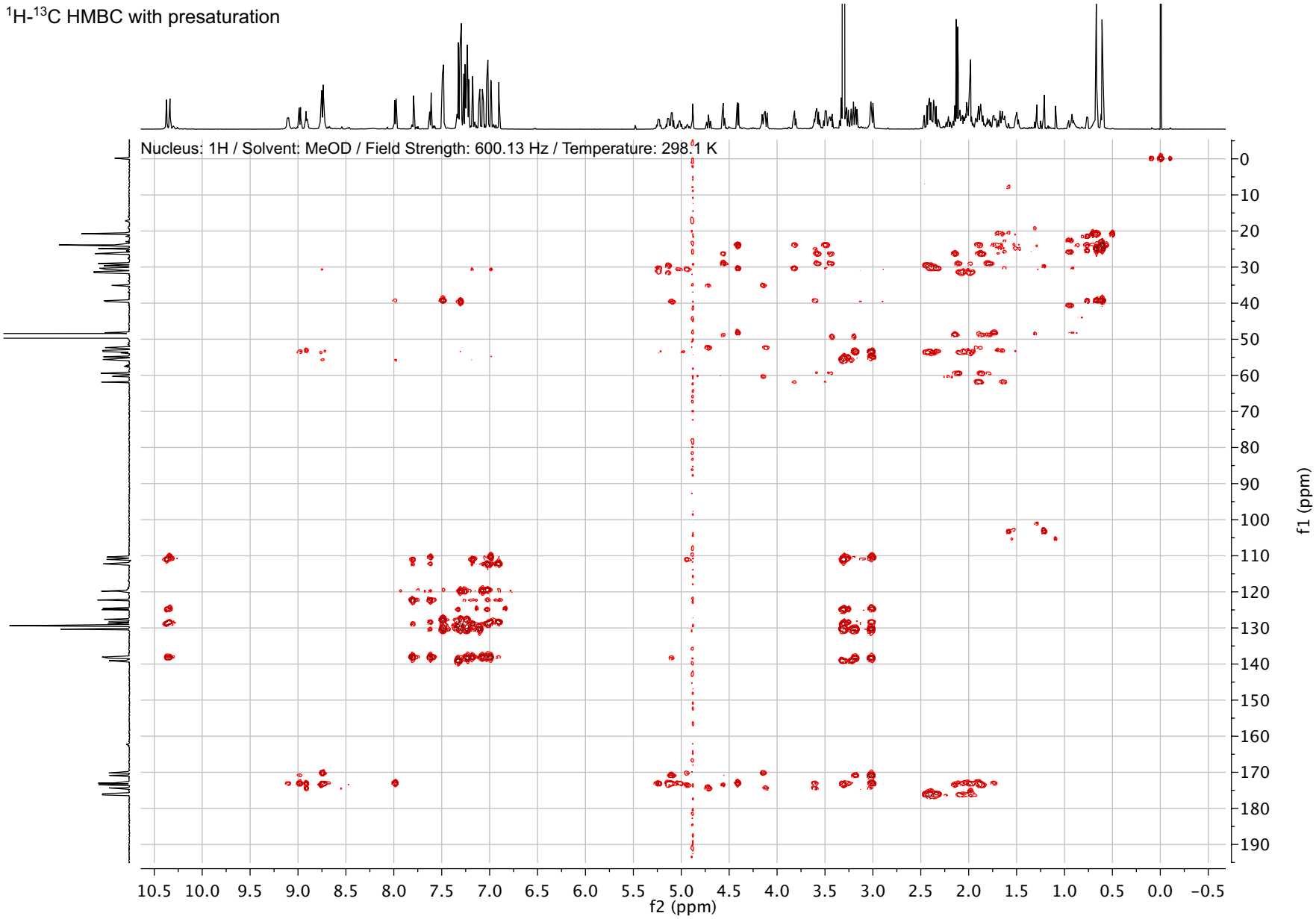


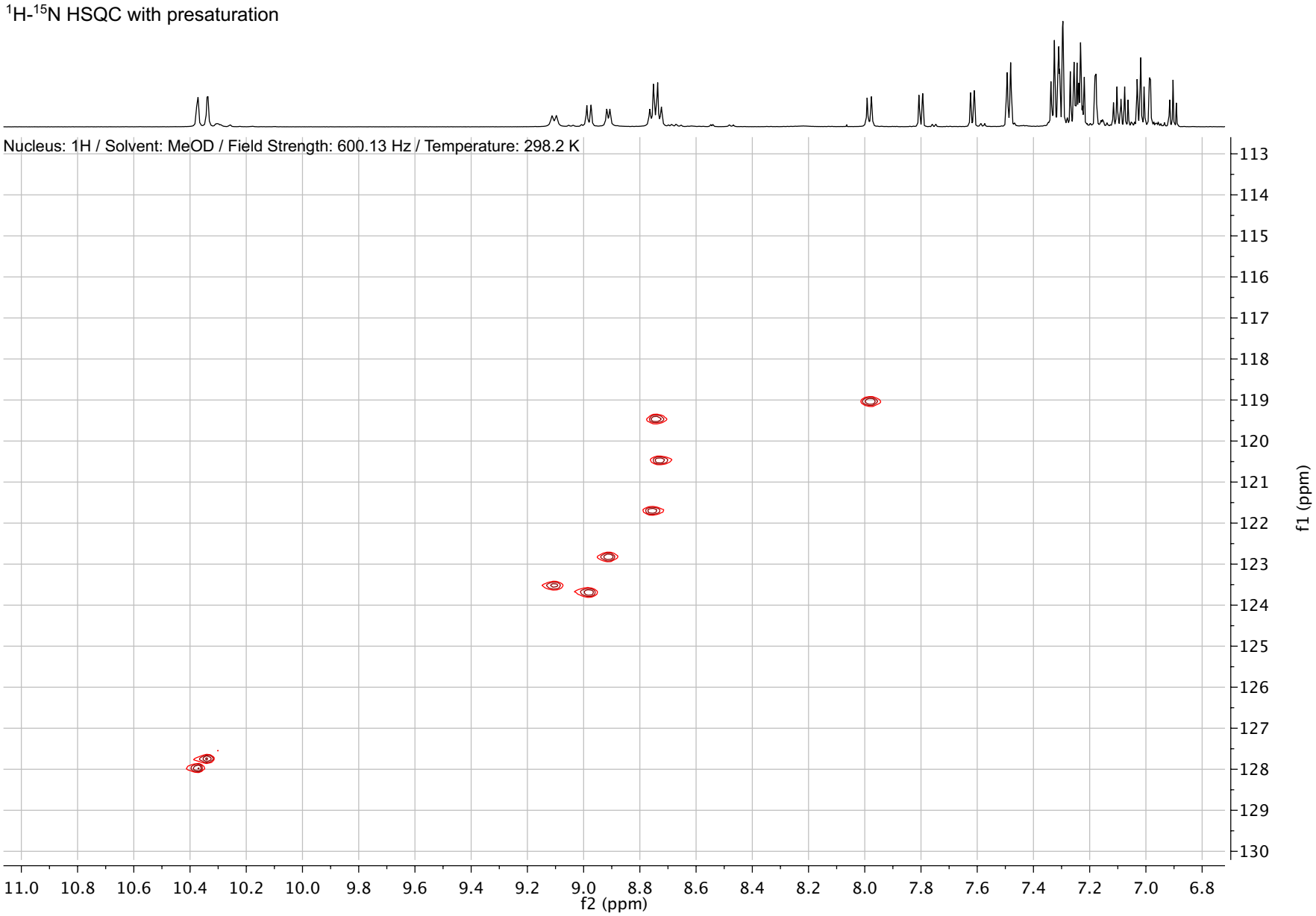

Nucleus: 13C / Solvent: MeOD / Field Strength: 150.93 Hz / Temperature: 298.1 K
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

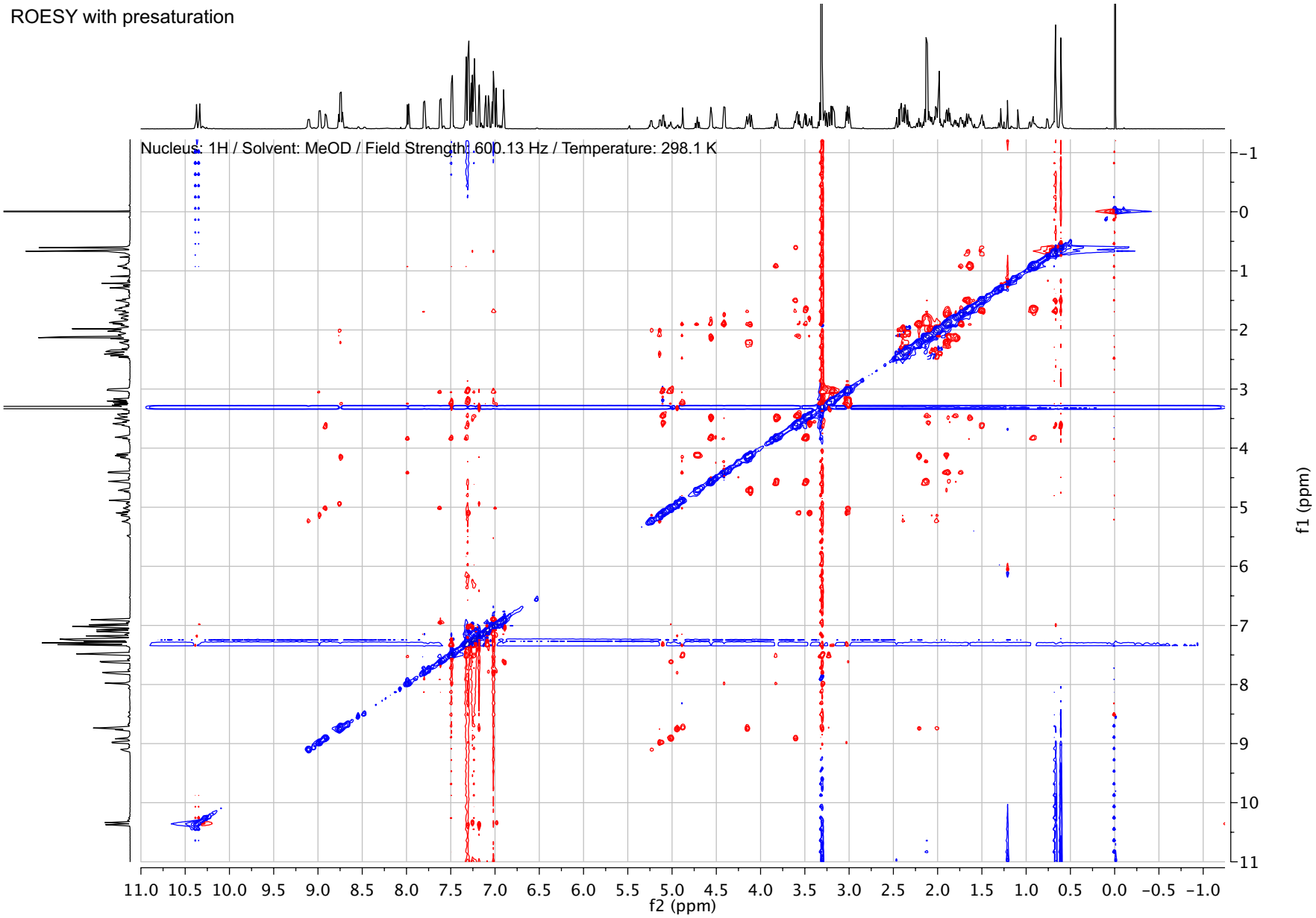

^{1}H-^{1}H TOCSY with presaturation
Nucleus: 1H / Solvent: MeOD / Field Strength: 600.13 Hz / Temperature: 298.1 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f2 (ppm)
-1 0 1 2 3 4 5 6 7 8 9 10 11
f1 (ppm)


^{1}H-^{1}H DQF-COSY with presaturation
Nucleus: 1H / Solvent: MeOD / Field Strength: 600.13 Hz / Temperature: 298.1 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f2 (ppm)
-1 0 1 2 3 4 5 6 7 8 9 10 11
f1 (ppm)

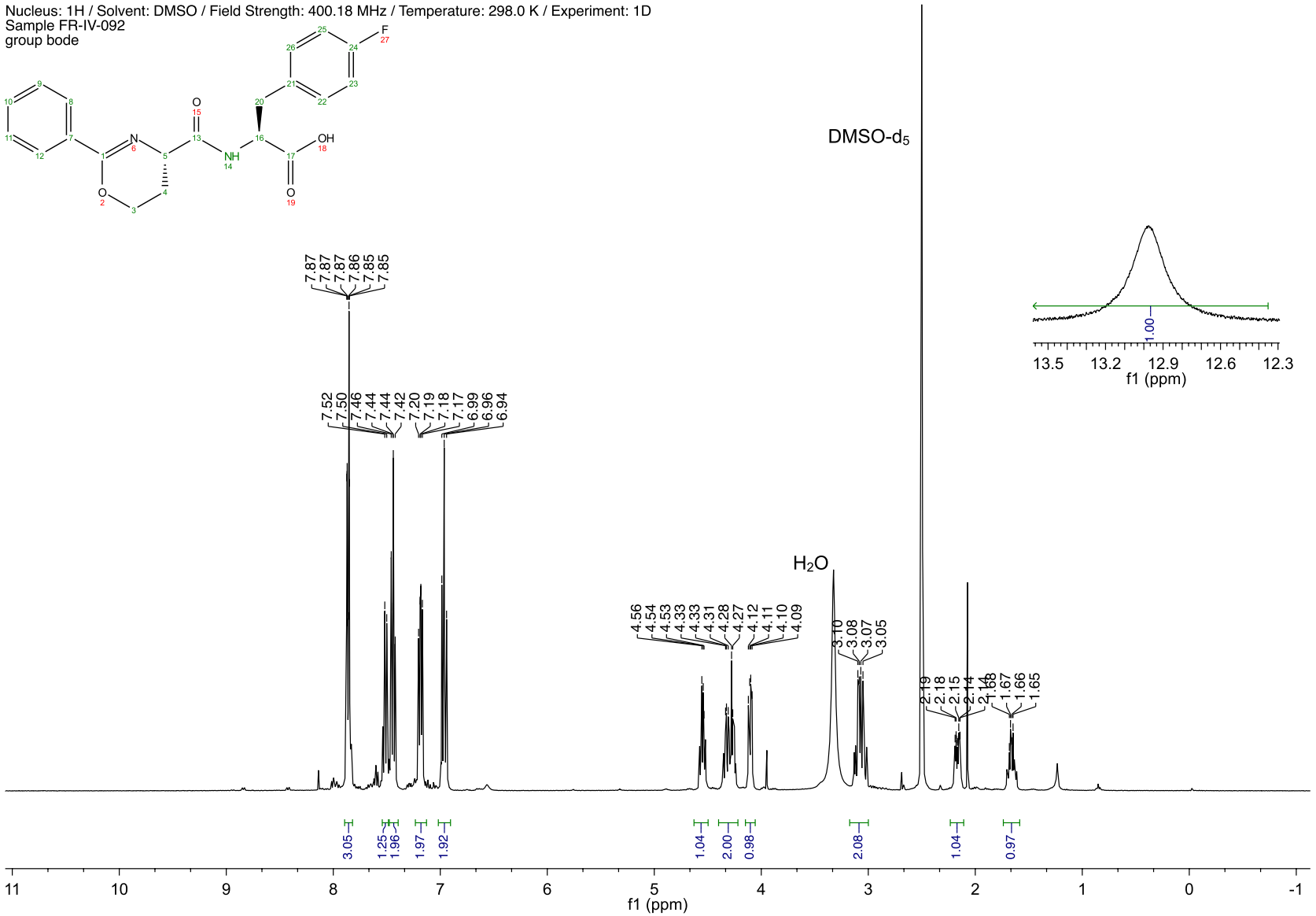

^{1}H-^{13}C HSQC with presaturation
Nucleus: 1H / Solvent: MeOD / Field Strength: 600.13 Hz / Temperature: 298.1 K
f2 (ppm)
f1 (ppm)

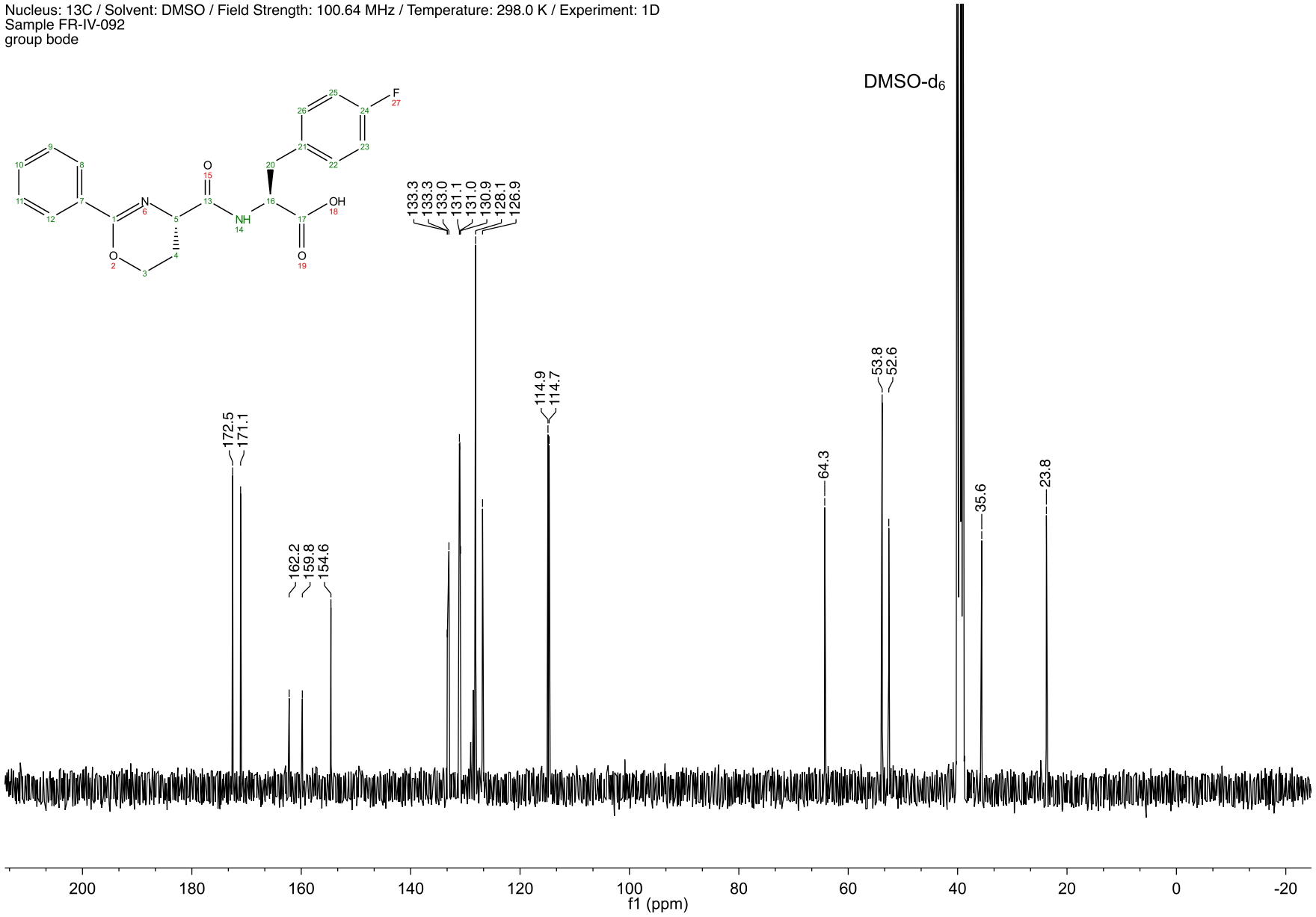

^{1}H-^{13}C HMBC with presaturation
Nucleus: 1H / Solvent: MeOD / Field Strength: 600.13 Hz / Temperature: 298.1 K
f2 (ppm)
f1 (ppm)

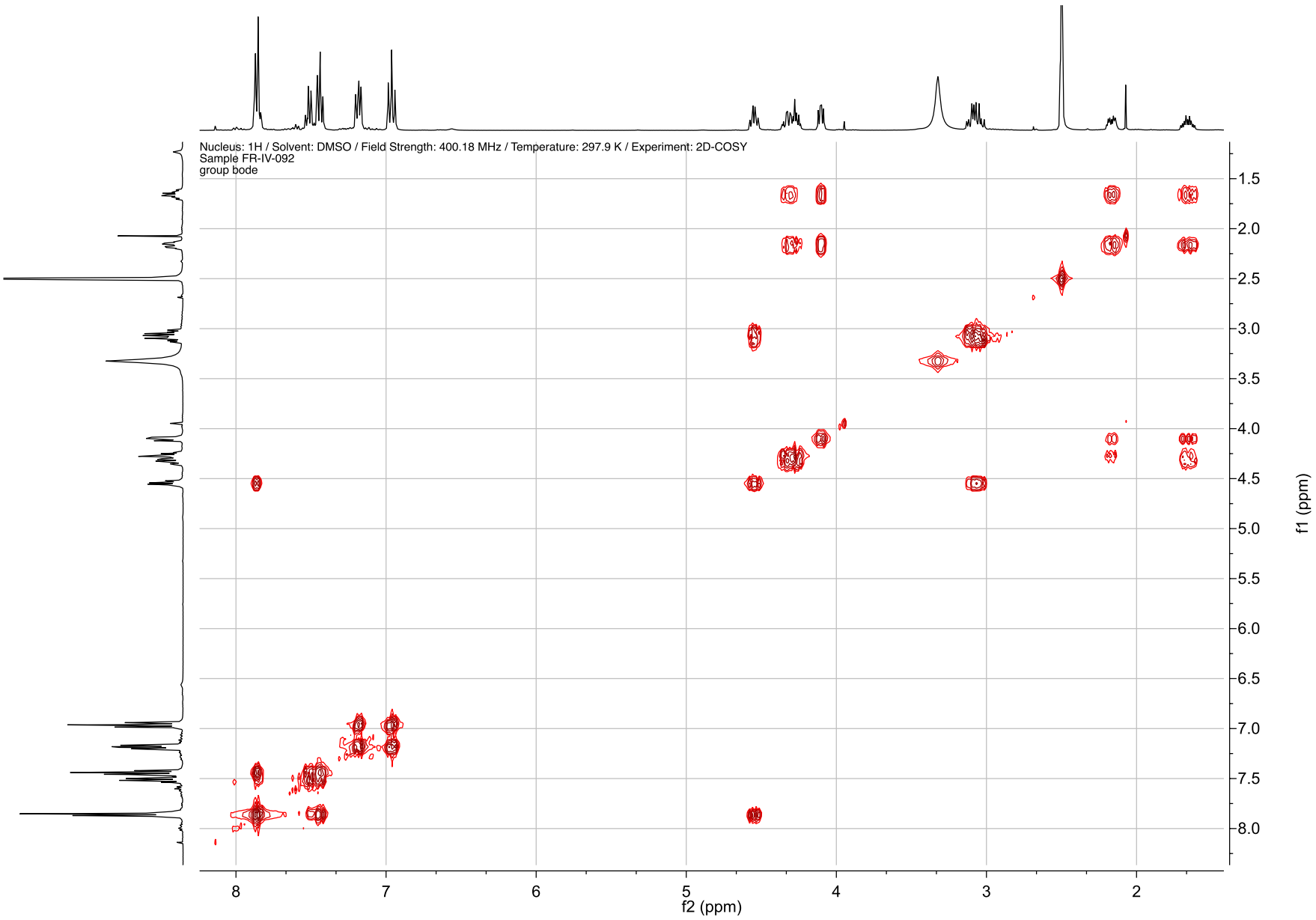

^{1}H-^{15}N HSQC with presaturation
Nucleus: 1H / Solvent: MeOD / Field Strength: 600.13 Hz / Temperature: 298.2 K
f2 (ppm)
f1 (ppm)

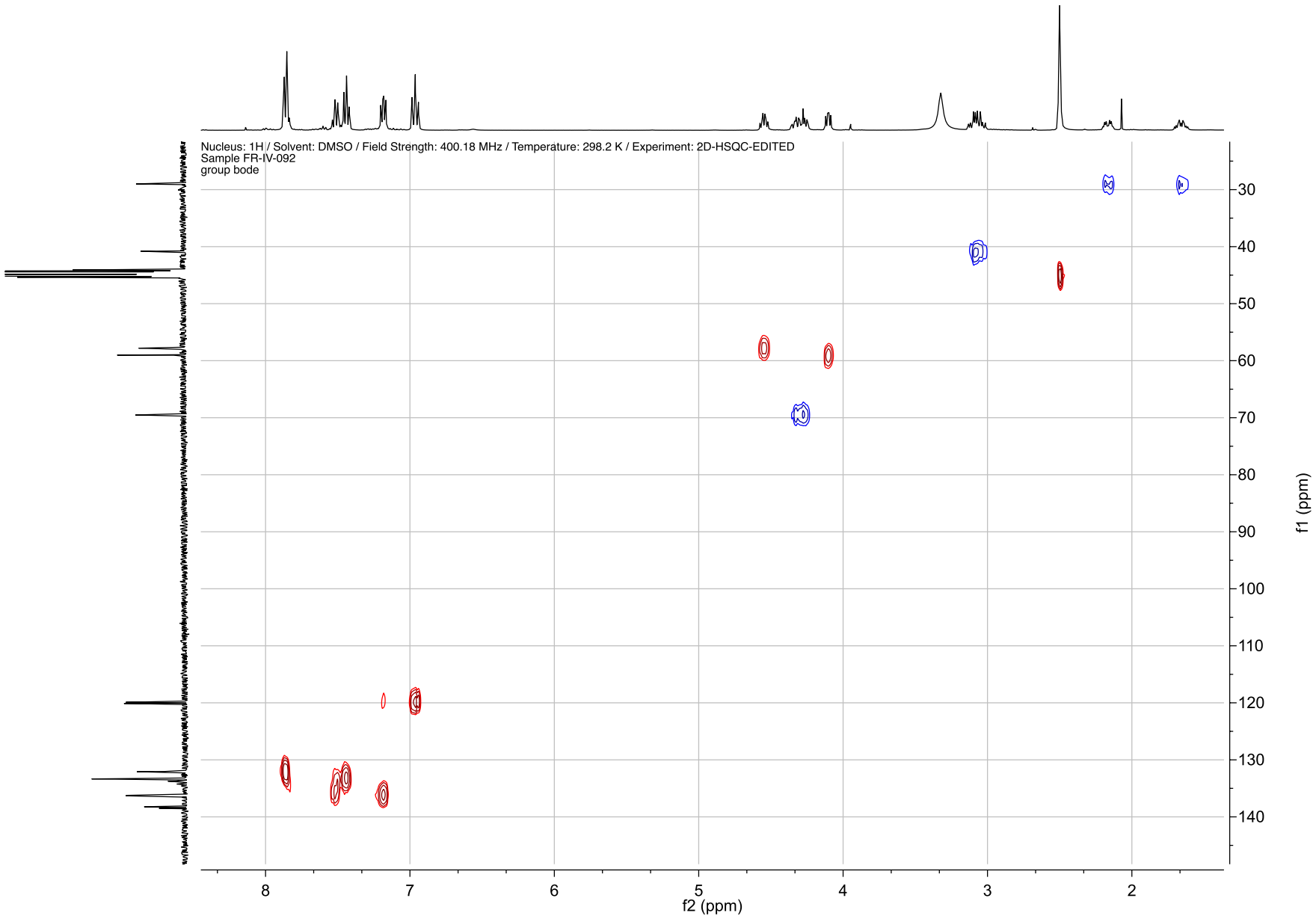

ROESY with presaturation
Nucleus: 1H / Solvent: MeOD / Field Strength: 600.13 Hz / Temperature: 298.1 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f2 (ppm)
-1 0 1 2 3 4 5 6 7 8 9 10 11
f1 (ppm)

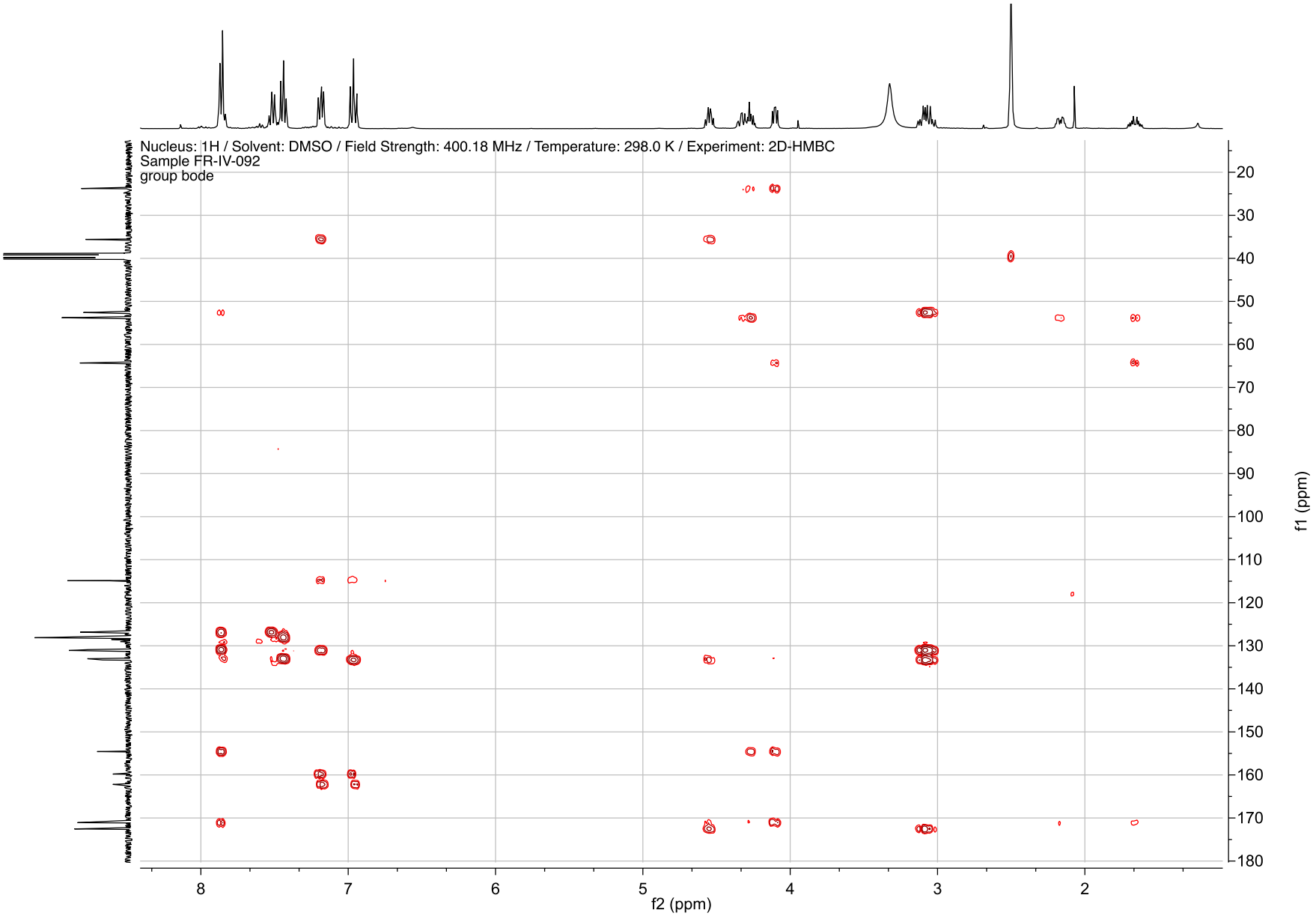
8.7. Iminoether 24




Nucleus: 13C / Solvent: DMSO / Field Strength: 100.64 MHz / Temperature: 298.0 K / Experiment: 1D
Sample FR-IV-092
group bode
DMSO-d6
133.3
133.3
133.0
131.1
131.0
130.9
128.1
126.9
114.9
114.7
53.8
52.6
172.5
171.1
162.2
159.8
154.6
64.3
35.6
23.8
200
180
160
140
120
100
80
60
40
20
0
-20
f1 (ppm)


Nucleus: 1H / Solvent: DMSO / Field Strength: 400.18 MHz / Temperature: 297.9 K / Experiment: 2D-COSY
Sample FR-IV-092
group bode
f2 (ppm)
f1 (ppm)


Nucleus: 1H / Solvent: DMSO / Field Strength: 400.18 MHz / Temperature: 298.2 K / Experiment: 2D-HSQC-EDITED
Sample FR-IV-092
group bode
f2 (ppm)
f1 (ppm)


Nucleus: 1H / Solvent: DMSO / Field Strength: 400.18 MHz / Temperature: 298.0 K / Experiment: 2D-HMBC
Sample FR-IV-092
group bode
f2 (ppm)
f1 (ppm)

9. Chiral GC/MS chromatograms

The following chromatograms belong to **Table S7**.

Chiral GC/MS chromatogram of entry 1

Area Percent Report

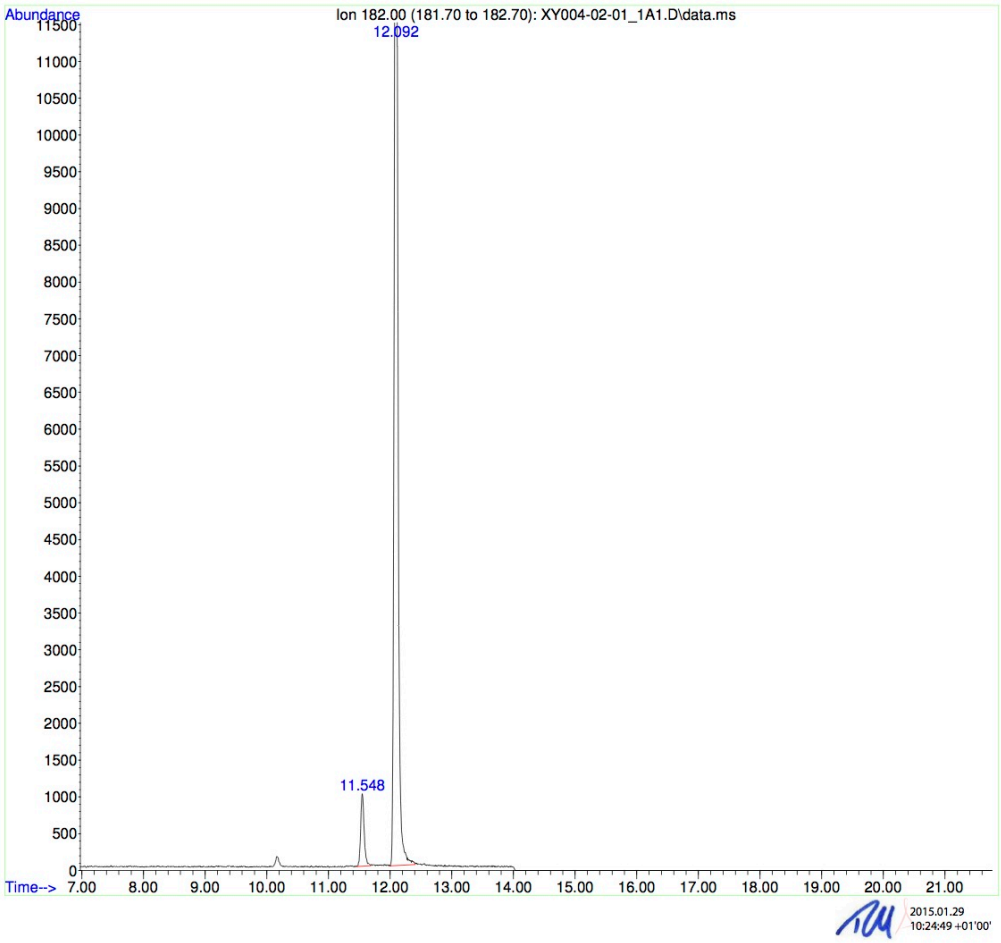
Data Path : F:\Analytik\ANALYSEN15\xy\004\02\
Data File : XY004-02-01_1A1.D
Sample : FR-IV-012-depsi, 1
Acq On : 26 Jan 2015 17:15
Misc : TM
Integrator: ChemStation
Operator : HP1 / S1192
ALS Vial : 52
DataAcq Meth:ET_AAA_SIM_2B.M

Signal : EIC Ion 182.00 (181.70 to 182.70): XY004-02-01_1A1.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total	
1	11.548	957	967	983	M2	988	38754	5.92%	5.587%	D Leu
2	12.092	1018	1029	1065	M	17100	654871	100.00%	94.413%	L Leu

Sum of corrected areas: 693625

DEFAULT.M Thu Jan 29 10:24:17 2015



Chiral GC/MS chromatogram of entry 2

Area Percent Report

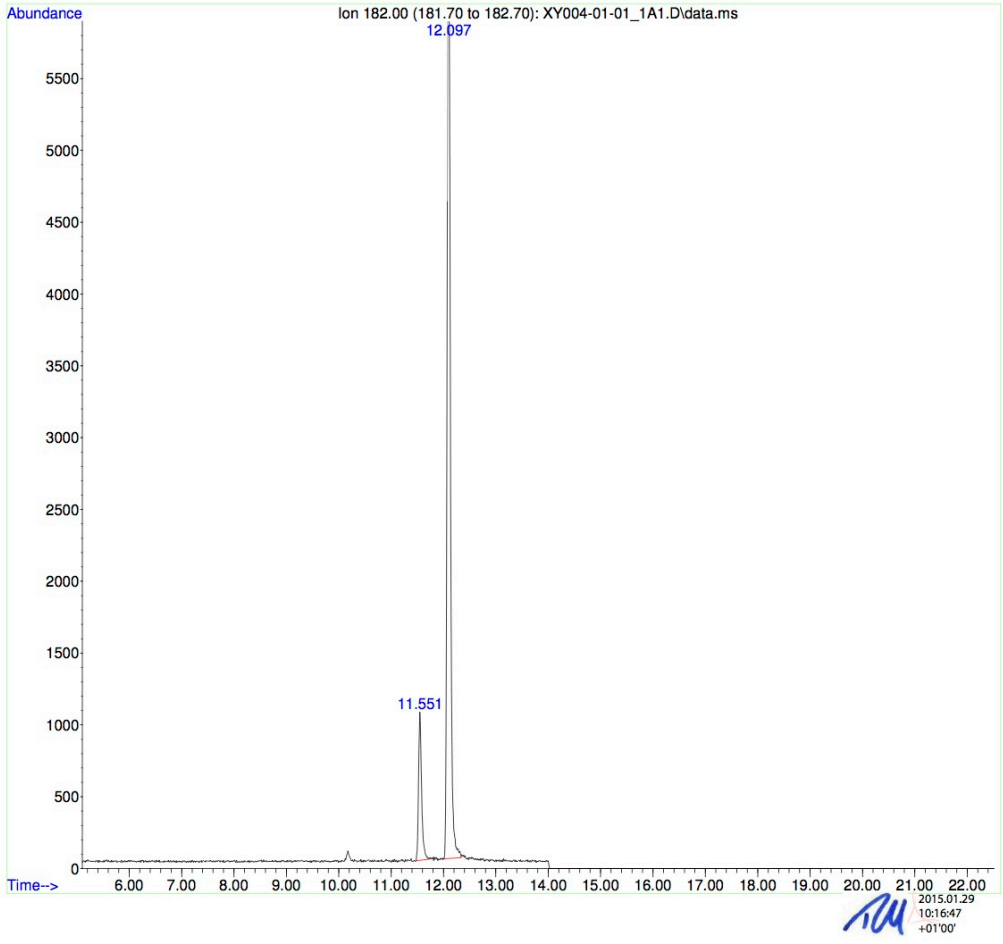
Data Path : F:\Analytik\ANALYSEN15\xy\004\01\
Data File : XY004-01-01_1A1.D
Sample : FR-IV-012-amide, 1
Acq On : 26 Jan 2015 16:25
Misc : TM
Integrator: ChemStation
Operator : HP1 / S1192
ALS Vial : 51
DataAcq Meth:ET_AAA_SIM_2B.M

Signal : EIC Ion 182.00 (181.70 to 182.70): XY004-01-01_1A1.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total	
1	11.551	958	967	987	M2	1037	41981	14.84%	12.922%	D Leu
2	12.097	1018	1030	1057	M2	7170	282895	100.00%	87.078%	L Leu

Sum of corrected areas: 324876

DEFAULT.M Thu Jan 29 10:16:11 2015



Chiral GC/MS chromatogram of entry 3

Area Percent Report

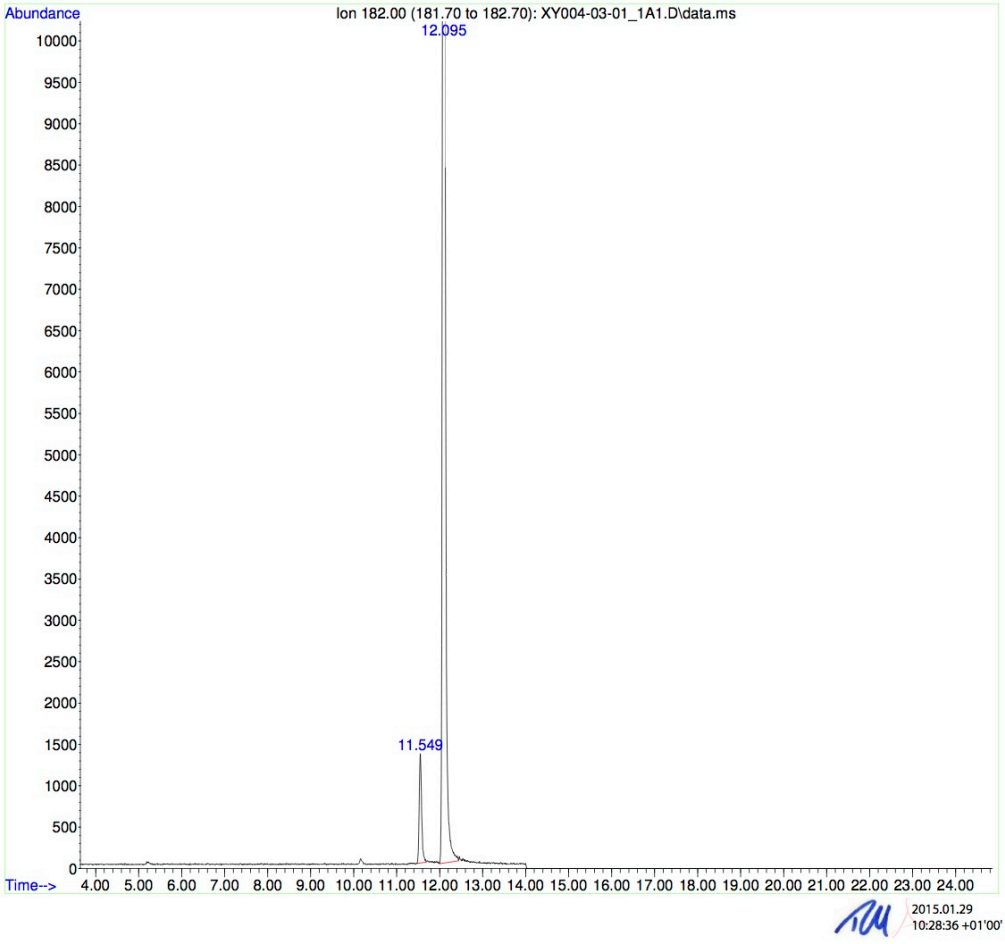
Data Path : F:\Analytik\ANALYSEN15\xy\004\03\
Data File : XY004-03-01_1A1.D
Sample : FR-IV-0123-amide, 1
Acq On : 26 Jan 2015 18:04
Misc : TM
Integrator: ChemStation
Operator : HP1 / S1192
ALS Vial : 53
DataAcq Meth:ET_AAA_SIM_2B.M

Signal : EIC Ion 182.00 (181.70 to 182.70): XY004-03-01_1A1.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total	
1	11.549	958	967	983	M2	1328	51971	5.07%	4.824%	D Leu
2	12.095	1018	1030	1069	M	26482	1025266	100.00%	95.176%	L Leu

Sum of corrected areas: 1077236

DEFAULT.M Thu Jan 29 10:28:12 2015



Chiral GC/MS chromatogram of entry 4

Area Percent Report

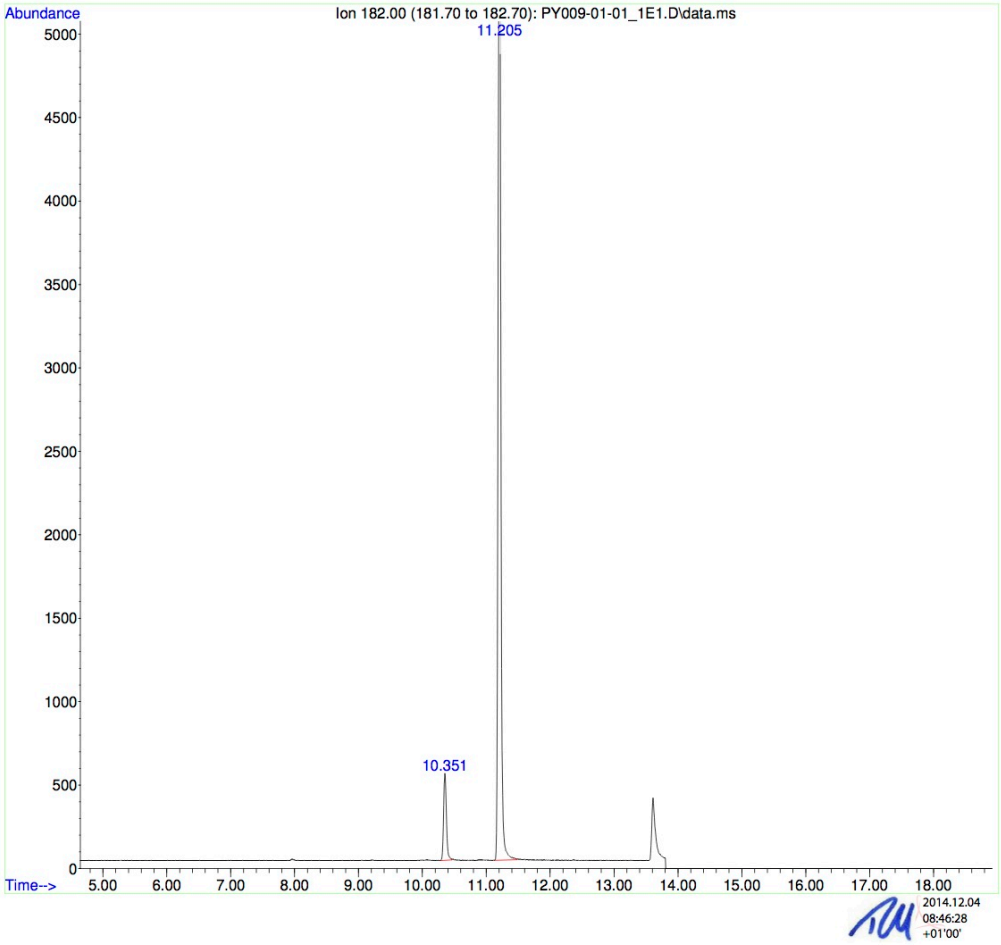
Data Path : F:\Analytik\ANALYSEN14\PY\009\01\
Data File : PY009-01-01_1E1.D
Sample : P0226806-6
Acq On : 3 Dec 2014 9:56
Misc : TM
Integrator: ChemStation
Operator : HP5 / S1194
ALS Vial : 57
DataAcq Meth:ET_AAA_SIM_2A.M

Signal : EIC Ion 182.00 (181.70 to 182.70): PY009-01-01_1E1.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total	
1	10.351	970	979	993	M	524	15697	8.80%	8.092%	D Leu
2	11.205	1084	1094	1132	M	6468	178297	100.00%	91.908%	L Leu

Sum of corrected areas: 193994

DEFAULT.M Thu Dec 04 08:45:48 2014



2014.12.04
08:46:28
+01'00'

Chiral GC/MS chromatogram of entry 5

Area Percent Report

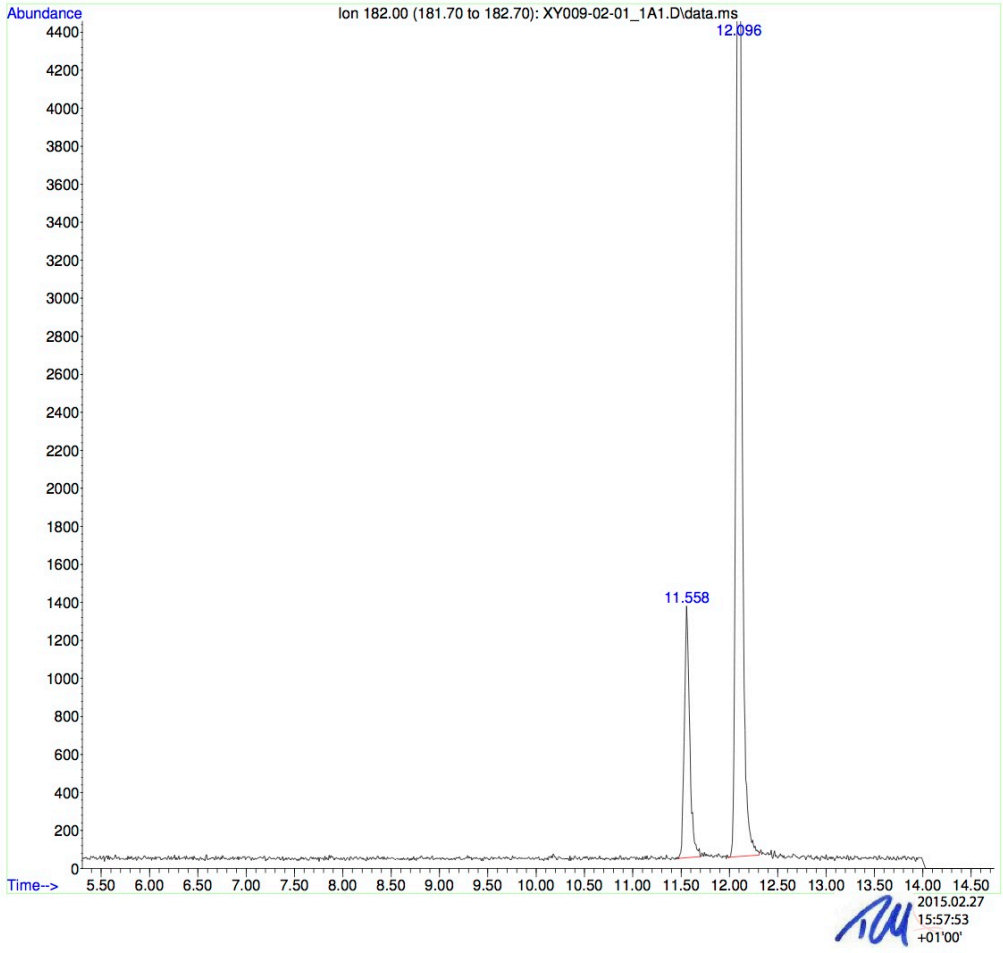
Data Path : F:\Analytik\ANALYSEN15\xy\009\02\
Data File : XY009-02-01_1A1.D
Sample : FR-IV-030-A, 1
Acq On : 27 Feb 2015 14:14
Misc : TM
Integrator: ChemStation
Operator : HP1 / S1192
ALS Vial : 69
DataAcq Meth:ET_AAA_SIM_2B.M

Signal : EIC Ion 182.00 (181.70 to 182.70): XY009-02-01_1A1.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total	
1	11.558	959	968	985	M	1326	50360	21.41%	17.638%	D Leu
2	12.096	1018	1030	1054	M	6159	235165	100.00%	82.362%	L Leu

Sum of corrected areas: 285525

DEFAULT.M Fri Feb 27 15:57:18 2015



2015.02.27 15:57:53 +01'00'

Chiral GC/MS chromatogram of entry 6

Area Percent Report

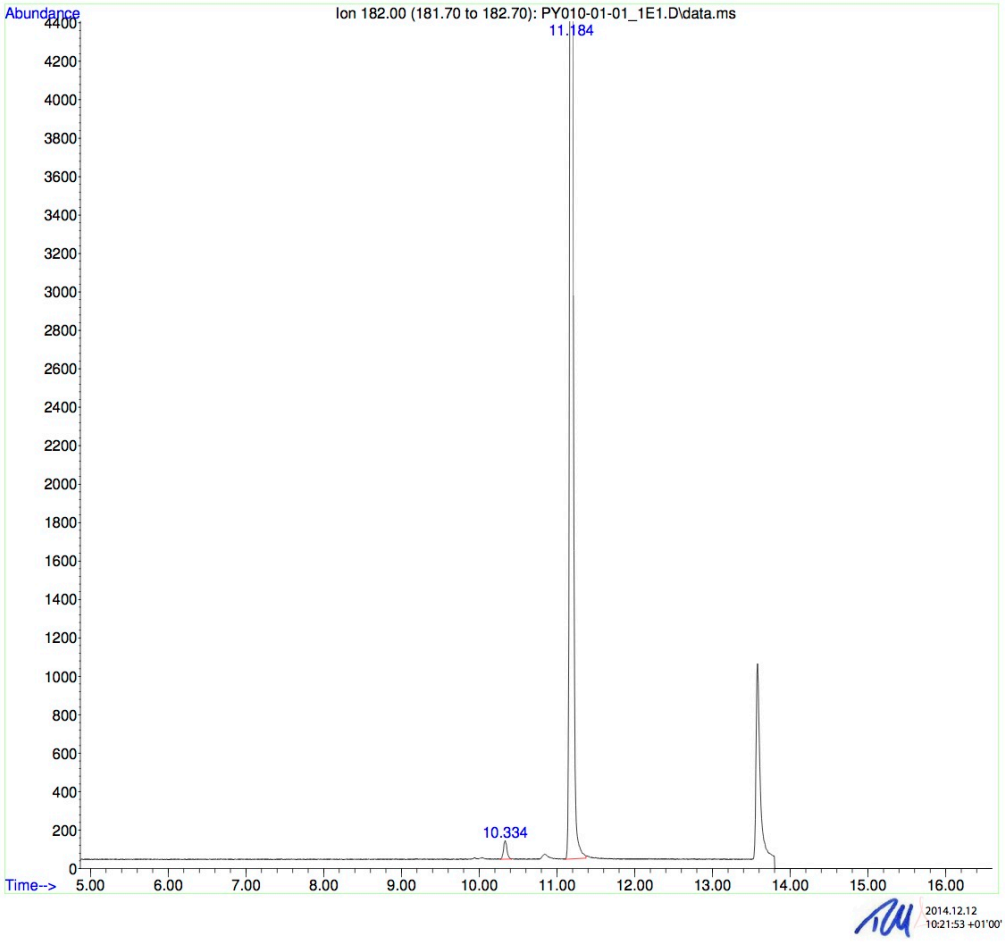
Data Path : F:\Analytik\ANALYSEN14\PY\010\01\
Data File : PY010-01-01_1E1.D
Sample : P0226806-5
Acq On : 10 Dec 2014 11:53
Misc : TM
Integrator: ChemStation
Operator : HP5 / S1194
ALS Vial : 73
DataAcq Meth:ET_AAA_SIM_2A.M

Signal : EIC Ion 182.00 (181.70 to 182.70): PY010-01-01_1E1.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total	
1	10.334	969	977	988	M3	98	2996	1.28%	1.260%	D Leu
2	11.184	1081	1091	1117	M	8859	234709	100.00%	98.740%	L Leu

Sum of corrected areas: 237705

DEFAULT.M Fri Dec 12 10:21:04 2014



Chiral GC/MS chromatogram of entry 7

Area Percent Report

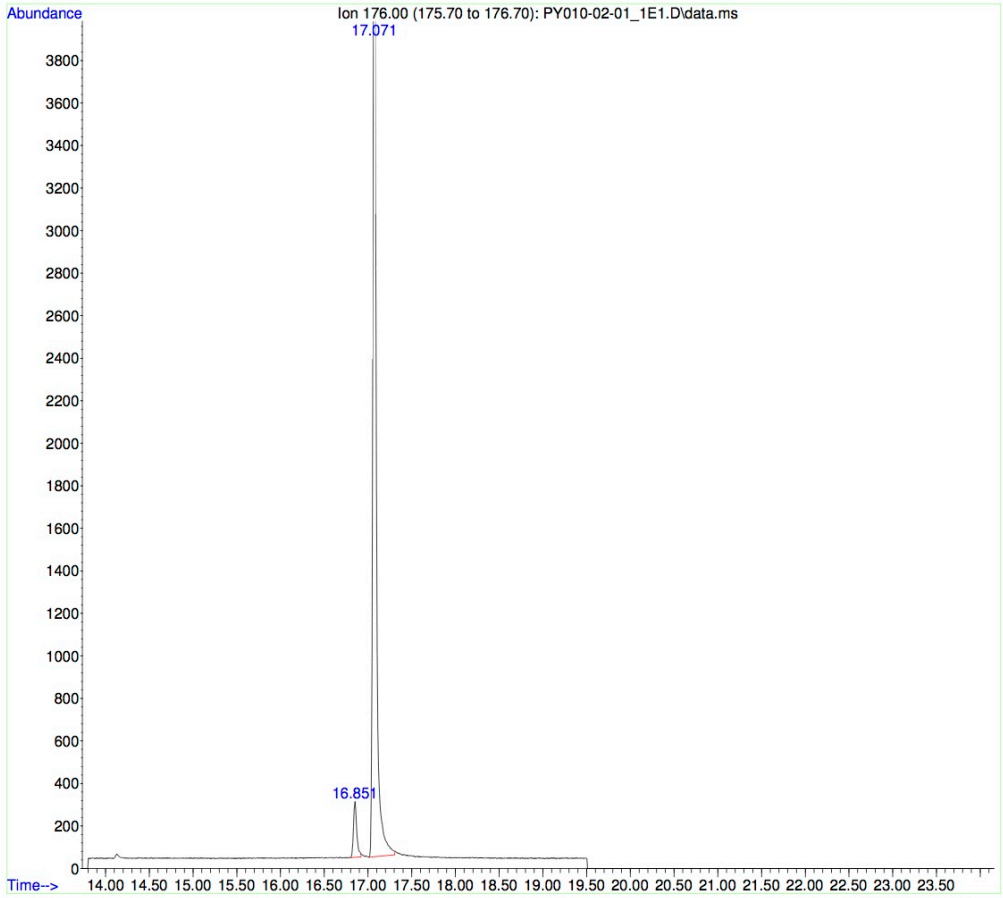
Data Path : F:\Analytik\ANALYSEN14\PY\010\02\
Data File : PY010-02-01_1E1.D
Sample : P0238335-1
Acq On : 10 Dec 2014 12:32
Misc : TM
Integrator: ChemStation
Operator : HP5 / S1194
ALS Vial : 74
DataAcq Meth:ET_AAA_SIM_2A.M

Signal : EIC Ion 176.00 (175.70 to 176.70): PY010-02-01_1E1.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total	
1	16.851	1799	1805	1813	M	263	6858	5.11%	4.864%	D Phe
2	17.071	1824	1831	1859	M	5392	134117	100.00%	95.136%	L Phe

Sum of corrected areas: 140974

DEFAULT.M Fri Dec 12 10:27:59 2014



2014.12.12
10:28:21
+01'00'

Chiral GC/MS chromatogram of entry 8

Area Percent Report

Data Path : F:\Analytik\ANALYSEN14\PY\009\02\
Data File : PY009-02-01_1E1.D
Sample : P0238335-2
Acq On : 3 Dec 2014 10:35
Misc : TM
Integrator: ChemStation
Operator : HP5 / S1194
ALS Vial : 58
DataAcq Meth:ET_AAA_SIM_2A.M

Signal : EIC Ion 176.00 (175.70 to 176.70): PY009-02-01_1E1.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total	
1	16.853	1798	1805	1823	M	3171	75912	10.80%	9.747%	D Phe
2	17.078	1825	1832	1864	M	33211	702926	100.00%	90.253%	L Phe

Sum of corrected areas: 778838

DEFAULT.M Thu Dec 04 08:49:36 2014