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Is More Better? A Comparison of Tri- and Tetrapeptidic Catalysts

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Supporting Information

Contents

1. General Aspects and Materials

Reagents and materials were of the highest commercially available grade and used without further purification. Reactions were monitored by thin layer chromatography using Merck silica gel 60 F254 aluminium sheets. Visualization of the compounds was achieved by UV or $KMnO_4$. Flash chromatography and plug filtrations were performed using Fluka silica gel 60 (particle size 0.040 – 0.063 mm, 200 – 400 mesh). Solvents for extraction and chromatography were of technical quality and distilled before use. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 400, a Bruker AV III 400 (400 MHz/100 MHz) or a Bruker AV III 600 (600 MHz/150 MHz). All spectra were recorded at 25 °C, unless stated otherwise. Chemical shifts (δ) are reported in parts per million (ppm) relative to the signal of tetramethylsilane (TMS) using the residual solvent signals. HPLC analyses were performed on an analytical Ultimate 3000 HPLC system from Dionex with a diode array detector and chiral stationary phases (Daicel AD-H, Daicel AS-H, AY-H, OD-H or Daicel OJ-H). High-resolution electron ionization (HR-EI) mass spectra were measured on a Waters Micromass AutoSpec Ultima spectrometer. High-resolution MALDI spectra were acquired on a Bruker solariX 94 (ESI/MALDI-FT-ICR) and a Bruker Ultra-Flex II (MALDI-TOF) spectrometer.

2. Synthesis and Analytical Data of Peptides A-I

2.1 General Protocols for Solid Phase Peptide Synthesis

Peptides were prepared on solid phase using Rink Amide resin. The general protocol for Fmoc/*t*Bu peptide synthesis was followed according to the general procedures below.

General procedure for peptide couplings: iPr_2NEt (10 equiv.) was added to a solution of Fmoc-Xaa-OH (5 equiv.) and HCTU (5 equiv.) in DMF. The solution of the activated amino acid (≈ 100 mM) was added to the amino-functionalized resin, preswollen in CH_2Cl_2 and the mixture was agitated for 1 h before washing with DMF (3 ×) and CH_2Cl_2 (3 ×).

General procedure for Fmoc-deprotections: A solution of 20% piperidine in DMF was added to the resin (preswollen in CH_2Cl_2) and the reaction mixture was agitated for 10 min, drained and the piperidine treatment was repeated for 10 min. Finally the resin was washed with DMF (3 ×) and CH_2Cl_2 (3 ×). The couplings as well as the deprotections were monitored by qualitative Kaiser (primary amines),[1] and chloranil tests (secondary amines).[2]

General procedure for side chain deprotection and cleavage of the peptides from the solid support: The peptides were deprotected and cleaved from the resin by stirring in a mixture of TFA/TIS/H_2O (95:2.5:2.5) for 1 h and a second time for 30 min. Pooling of the filtrates and removal of all volatiles under reduced pressure followed by precipitation and thorough washing with Et_2O afforded the peptides as their TFA salts. The peptides were redissolved in MeCN/H_2O 1:1, dried by lyophilisation and used without further purification.

2.2 Analytical Data of Peptides 1-2 and A–I

TFA·H-Pro-Pro-Asp-NH_2 (1-LLL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. The analytical data are in agreement with previously published data.[3]

TFA·H-D-Pro-Pro-Asp-NH_2 (1-DLL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. The analytical data are in agreement with previously published data.[3]

TFA·H-Pro-D-Pro-Asp-NH_2 (1-LDL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. The analytical data are in agreement with previously published data.[3]

TFA·H-D-Pro-D-Pro-Asp-NH_2 (1-DDL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. The analytical data are in agreement with previously published data.[3]

TFA·H-Pro-Pro-Glu-NH_2 (2-LLL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. The analytical data are in agreement with previously published data.[4]

TFA·H-D-Pro-Pro-Glu-NH$_2$ (2-DLL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. The analytical data are in agreement with previously published data.[4]

TFA·H-Pro-D-Pro-Glu-NH$_2$ (2-LDL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. The analytical data are in agreement with previously published data.[4]

TFA·H-D-Pro-D-Pro-Glu-NH$_2$ (2-DDL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. The analytical data are in agreement with previously published data.[4]

TFA·H-Pro-Pro-Asp-Pro-NH$_2$ (A-LLLL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, d^6-DMSO) δ = 12.57 (bs, 1H), 9.56 (bs, 1H), 8.53 – 8.45 (m, 1H), 8.33 (d, J = 8.0 Hz, 1H), 6.99 (s, 1H), 6.97 (s, 1H), 4.78 (q, J = 7.2 Hz, 1H), 4.51 – 4.42 (m, 1H), 4.37 (ddd, J = 13.7, 8.3, 4.3 Hz, 1H), 4.18 (ddd, J = 16.2, 8.5, 3.0 Hz, 1H), 3.69 – 3.33 (m, 4H), 3.15 (s, 2H), 2.88 – 2.67 (m, 1H), 2.46 – 2.33 (m, 1H), 2.19 – 1.63 (m, 12H). ^{13}C NMR (101 MHz, d^6-DMSO) δ = 173.44, 172.11, 170.69, 168.95, 166.61, 59.82, 59.55, 58.31, 47.49, 46.78, 46.53, 45.79, 35.96, 29.25, 29.10, 27.90, 24.45, 24.22, 23.54. HRMS (ESI): *m/z* calcd for $C_{19}H_{30}N_5O_6^+$: 424.2191 $[M + H]^+$; found: 424.2192.

TFA·H-Pro-Pro-Asp-D-Pro-NH$_2$ (A or A-LLLD): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, d^6-DMSO) δ = 12.46 (bs, 1H), 9.65 (bs, 1H), 8.61 (d, J = 7.7 Hz, 1H), 8.46 (bs, 1H), 6.97 (s, 1H), 6.87 – 6.81 (m, 1H), 4.66 (td, J = 8.0, 5.7 Hz, 1H), 4.53 – 4.40 (m, 1H), 4.36 (dd, J = 8.4, 4.9 Hz, 1H), 4.26 – 4.13 (m, 1H), 3.75 – 3.29 (m, 4H), 3.27 – 3.15 (m, 2H), 2.80 (ddd, J = 16.4, 8.3, 4.2 Hz, 1H), 2.47 – 2.32 (m, 1H), 2.18 – 1.65 (m, 12H). ^{13}C NMR (101 MHz, d^6-DMSO) δ = 173.41, 171.85, 171.09, 168.87, 166.77, 60.01, 59.52, 58.13, 47.93, 46.77, 46.77, 45.91, 35.85, 29.18, 29.18, 28.00, 24.40, 24.36, 23.71. HRMS (ESI): *m/z* calcd for $C_{19}H_{30}N_5O_6^+$: 424.2191 $[M + H]^+$; found: 424.2189.

TFA·H-D-Pro-Pro-Asp-Pro-NH$_2$ (A-DLLL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, d^6-DMSO) δ = 12.49 (bs, 1H), 9.59 (s, 1H), 8.54 (bs, 1H), 8.41 (d, J = 7.7 Hz, 1H), 6.98 (s, 1H), 6.97 (s, 1H), 4.77 (q, J = 7.1 Hz, 1H), 4.53 – 4.45 (m, 1H), 4.32 (dt, J = 8.8, 4.5 Hz, 1H), 4.26 – 4.13 (m, 1H), 3.71 – 3.51 (m, 2H), 3.51 – 3.33 (m, 2H), 3.26 – 3.12 (m, 2H), 2.80 (ddd, J = 16.6, 13.2, 6.0 Hz, 1H), 2.48 – 2.32 (m, 1H), 2.19 – 1.63 (m, 12H). ^{13}C NMR (101 MHz, d^6-DMSO) δ = 173.48, 172.12, 170.88, 168.94, 166.38, 59.86, 59.77, 58.36, 47.59, 46.72, 46.54, 45.76, 35.99, 29.39, 29.25, 27.92, 24.23, 24.02, 23.60. HRMS (ESI): *m/z* calcd for $C_{19}H_{30}N_5O_6^+$: 424.2191 $[M + H]^+$; found: 424.2190.

TFA·H-Pro-D-Pro-D-Asp-Pro-NH$_2$ (A-LDDL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing

peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, d^{6}-DMSO) δ = 12.38 (bs, 1H), 9.54 (bs, 1H), 8.59 (d, J = 7.7 Hz, 1H), 8.50 (bs, 1H), 6.99 (s, 1H), 6.93 (s, 1H), 4.71 (td, J = 8.3, 5.6 Hz, 1H), 4.55 – 4.43 (m, 1H), 4.31 (dd, J = 8.9, 2.9 Hz, 1H), 4.20 (dd, J = 8.6, 3.1 Hz, 1H), 3.75 – 3.55 (m, 2H), 3.58 – 3.30 (m, 2H), 3.27 – 3.12 (m, 2H), 2.78 (dt, J = 14.5, 7.3 Hz, 1H), 2.42 – 2.31 (m, 1H), 2.15 – 1.64 (m, 12H).^{13}C NMR (100 MHz, d^{6}-DMSO) δ = 173.57, 171.75, 171.08, 168.81, 166.52, 59.86, 59.69, 58.42, 47.48, 46.79, 46.60, 45.77, 35.82, 29.34, 29.19, 27.78, 24.19, 24.04, 23.54. HRMS (ESI): *m/z* calcd for $C_{19}H_{30}N_5O_6^+$: 424.2191 $[M + H]^+$; found: 424.2193.

TFA·H-Pro-D-Pro-Asp-Pro-NH$_2$ (A-LDLL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, d^{6}-DMSO) δ = 12.45 (bs, 1H), 9.59 (bs, 1H), 8.47 (bs, 1H), 8.41 (d, J = 8.5 Hz, 1H), 7.02 (s, 1H), 6.97 (s, 1H), 4.89 (dt, J = 8.8, 7.1 Hz, 1H), 4.59 – 4.43 (m, 1H), 4.41 – 4.27 (m, 1H), 4.26 – 4.09 (m, 1H), 3.63 (dtt, J = 20.0, 6.5, 3.5 Hz, 2H), 3.54 – 3.34 (m, 2H), 3.21 (d, J = 28.4 Hz, 2H), 2.79 (ddd, J = 29.7, 16.5, 6.4 Hz, 1H), 2.47 – 2.33 (m, 1H), 2.17 – 1.73 (m, 12H). ^{13}C NMR (101 MHz, d^{6}-DMSO) δ = 173.44, 172.20, 170.54, 168.74, 166.33, 59.89, 59.80, 58.44, 47.22, 46.73, 46.58, 45.69, 36.38, 29.64, 29.31, 27.78, 24.15, 23.93, 23.58. HRMS (ESI): *m/z* calcd for $C_{19}H_{30}N_5O_6^+$: 424.2191 $[M + H]^+$; found: 424.2191.

TFA·H-Pro-D-Pro-Asp-D-Pro-NH$_2$ (A-LDLD): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, d^{6}-DMSO) δ = 12.41 (bs, 1H), 9.56 (bs, 1H), 8.57 – 8.38 (m, 2H), 6.93 (s, 1H), 6.86 (s, 1H), 4.88 – 4.72 (m, 1H), 4.54 – 4.43 (m, 1H), 4.32 (dd, J = 8.6, 3.2 Hz, 1H), 4.27 – 4.13 (m, 1H), 3.78 – 3.10 (m, 6H), 2.77 (dt, J = 15.5, 7.7 Hz, 1H), 2.49 – 2.32 (m, 1H), 2.21 – 1.51 (m, 12H). ^{13}C NMR (100 MHz, d^{6}-DMSO) δ = ^{13}C NMR (101 MHz, DMSO) δ 173.47, 171.83, 170.85, 168.87, 166.53, 59.92, 59.80, 58.49, 47.43, 46.74, 46.74, 45.71, 36.09, 29.57, 29.17, 27.77, 24.27, 23.94, 23.59. HRMS (ESI): *m/z* calcd for $C_{19}H_{30}N_5O_6^+$: 424.2191 $[M + H]^+$; found: 424.2192.

TFA·H-Pro-Pro-D-Asp-Pro-NH$_2$ (A-LLDL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, d^{6}-DMSO) δ = ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.46 (bs, 1H), 9.66 (bs, 1H), 8.56 (d, J = 8.1 Hz, 1H), 8.42 (bs, 1H), 6.97 (s, 1H), 6.78 (s, 1H), 4.77 (td, J = 8.1, 5.9 Hz, 1H), 4.48 (dq, J = 8.4, 3.8 Hz, 1H), 4.41 – 4.31 (m, 1H), 4.19 (td, J = 9.8, 8.8, 4.0 Hz, 1H), 3.81 – 3.30 (m, 4H), 3.22 (t, J = 16.7 Hz, 2H), 2.77 (td, J = 16.6, 7.9 Hz, 1H), 2.39 (dq, J = 10.4, 6.0, 4.5 Hz, 1H), 2.16 – 1.66 (m, 12H). ^{13}C NMR (101 MHz, d^{6}-DMSO) δ = 173.46, 171.76, 170.74, 168.97, 166.68, 59.93, 59.57, 58.28, 47.47, 46.79, 46.76, 45.84, 36.17, 29.21, 29.18, 28.00, 24.45, 24.25, 23.52. HRMS (ESI): *m/z* calcd for $C_{19}H_{30}N_5O_6^+$: 424.2191 $[M + H]^+$; found: 424.2190.

TFA·H-Pro-Pro-D-Asp-D-Pro-NH$_2$ (A-LLDD): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, d^{6}-DMSO) δ = 12.50 (bs, 1H), 9.63 (bs, 1H), 8.47 (bs, 1H), 8.40 (d, J = 8.6 Hz, 1H), 7.10 – 7.00 (m, 1H),

7.00 – 6.94 (m, 1H), 4.92 – 4.82 (m, 1H), 4.52 – 4.40 (m, 1H), 4.36 (dd, *J* = 8.4, 4.8 Hz, 1H), 4.17 (dd, *J* = 8.5, 2.9 Hz, 1H), 3.69 – 3.35 (m, 4H), 3.26 – 3.14 (m, 2H), 2.73 (dd, *J* = 16.2, 6.4 Hz, 1H), 2.46 – 2.32 (m, 1H), 2.16 – 1.70 (m, 12H). ^{13}C NMR (101 MHz, d^6-DMSO) δ = 173.45, 172.12, 170.47, 168.70, 166.60, 59.90, 59.51, 58.20, 47.35, 46.78, 46.55, 45.84, 36.39, 29.31, 29.25, 28.02, 24.36, 24.20, 23.47. HRMS (ESI): *m/z* calcd for $C_{19}H_{30}N_5O_6^+$: 424.2191 $[M + H]^+$; found: 424.2191.

TFA·H-Pro-D-Pro-D-Asp-D-Pro-NH$_2$ (B or ent-A-DLLL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, d^6-DMSO) δ = 9.44 (m, 1H), 8.55 (m, 1H), 8.42 (d, *J* = 7.8 Hz, 1H), 6.99 (s, 2H), 4.77 (dm, *J* = 14.3 Hz, 1H), 4.48 (m, 1H), 4.31 (dd, *J* = 8.7 Hz, 2.9 Hz, 1H), 4.17 (m, 1H), 3.62 (m, 2H), 3.43 (m, 2H), 3.20 (m, 2H), 2.81 (m, 1H), 2.40 (m, 1H), 2.21-1.62 (m, 12H). ^{13}C NMR (100 MHz, d^6-DMSO) δ = 173.5, 172.1, 170.8, 168.9, 166.4, 59.8, 59.7, 58.3, 47.5, 46.7, 46.5, 45.7, 35.9, 29.3, 29.2, 27.9, 24.2, 24.0, 23.6. HRMS (ESI): *m/z* calcd for $C_{19}H_{30}N_5O_6^+$: 424.2196 $[M + H]^+$; found: 424.2193.

TFA·H-D-Pro-D-Pro-D-Glu-D-Pro-NH$_2$ (C): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, D_2O) δ = 4.73 (dd, *J* = 9.6, 4.6 Hz, 1H), 4.69 – 4.60 (m, 1H), 4.49 (ddd, *J* = 14.7, 8.3, 6.2 Hz, 1H), 4.45 – 4.35 (m, 1H), 3.89 – 3.71 (m, 2H), 3.71 (dd, *J* = 7.4, 5.9 Hz, 1H), 3.60 (dt, *J* = 10.2, 7.0 Hz, 1H), 3.51 – 3.37 (m, 2H), 2.62 – 2.54 (m, 2H), 2.43 – 2.26 (m, 2H), 2.24 – 1.79 (m, 7H). ^{13}C NMR (101 MHz, D_2O) δ = 177.03, 176.75, 173.56, 171.55, 167.97, 60.34, 60.34, 59.10, 50.68, 47.92, 47.68, 46.59, 29.57, 29.36, 29.31, 28.30, 25.48, 24.62, 24.62, 23.79. HRMS (MALDI): *m/z* calcd for $C_{20}H_{32}N_5O_6^+$: 438.2347 $[M + H]^+$; found: 438.2344.

TFA·H-Pro-Pro-Glu-Pro-NH$_2$ (C-LLLL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (600 MHz, D_2O) δ = 4.82 (dd, *J* = 9.6, 4.7 Hz, 1H), 4.74 (ddd, *J* = 10.4, 6.8, 4.0 Hz, 1H), 4.60 (dd, *J* = 8.4, 6.3 Hz, 1H), 4.49 (dd, *J* = 8.5, 5.7 Hz, 1H), 3.97 – 3.88 (m, 1H), 3.87 – 3.78 (m, 2H), 3.70 (dt, *J* = 10.1, 7.2 Hz, 1H), 3.61 – 3.53 (m, 1H), 3.50 (dt, *J* = 11.5, 6.7 Hz, 1H), 2.67 (t, *J* = 7.6 Hz, 2H), 2.67 – 2.57 (m, 1H), 2.49 – 2.38 (m, 2H), 2.26 (dtd, *J* = 12.7, 8.0, 4.8 Hz, 1H), 2.23 – 1.89 (m, 10H). ^{13}C NMR (151 MHz, D_2O) δ = 177.10, 176.75, 173.57, 171.56, 167.97, 60.35, 60.34, 59.10, 50.69, 47.92, 47.68, 46.58, 29.56, 29.42, 29.31, 28.29, 25.49, 24.62, 24.62, 23.79. HRMS (ESI): *m/z* calcd for $C_{20}H_{32}N_5O_6^+$: 438.2347 $[M + H]^+$; found: 438.2347.

TFA·H-Pro-Pro-Glu-D-Pro-NH$_2$ (C-LLLD): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (600 MHz, D_2O) δ = 4.89 – 4.80 (m, 1H), 4.79 – 4.71 (m, 1H), 4.60 (dd, *J* = 8.4, 6.1 Hz, 1H), 4.51 (dd, *J* = 8.5, 5.7 Hz, 1H), 3.91 (dt, *J* = 10.3, 6.7 Hz, 1H), 3.86 – 3.78 (m, 2H), 3.71 (dt, *J* = 10.1, 7.2 Hz, 1H), 3.63 – 3.43 (m, 2H), 2.75 – 2.53 (m, 3H), 2.45 (dddt, *J* = 31.4, 13.6, 8.6, 6.8 Hz, 2H), 2.27 (dtd, *J* = 14.8, 7.7, 4.6 Hz, 1H), 2.23 – 1.88 (m, 10H). ^{13}C NMR (151 MHz, D_2O) δ = 176.93, 176.84, 173.42, 171.48, 168.03, 60.54, 60.41, 59.07, 50.53, 47.90, 47.68, 46.60, 29.66, 29.55,

29.48, 28.35, 25.71, 24.59, 24.59, 23.86. HRMS (ESI): *m/z* calcd for $C_{20}H_{32}N_5O_6^+$: 438.2347 $[M + H]^+$; found: 438.2349.

TFA·H-D-Pro-Pro-Glu-Pro-NH$_2$ (C-DLLL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (600 MHz, D_2O) δ = 4.84 (dd, J = 10.0, 4.5 Hz, 1H), 4.75 (dd, J = 8.8, 7.0 Hz, 1H), 4.59 (dd, J = 8.9, 4.1 Hz, 1H), 4.49 (dd, J = 8.5, 5.8 Hz, 1H), 3.91 (dt, J = 10.4, 6.7 Hz, 1H), 3.87 – 3.78 (m, 2H), 3.72 (dt, J = 10.3, 7.1 Hz, 1H), 3.54 (ddt, J = 38.6, 11.6, 7.0 Hz, 2H), 2.74 – 2.56 (m, 3H), 2.52 – 2.35 (m, 2H), 2.27 (dtd, J = 14.7, 7.8, 4.4 Hz, 1H), 2.23 – 1.92 (m, 10H). ^{13}C NMR (151 MHz, D_2O) δ = 177.11, 176.77, 173.86, 171.50, 167.97, 60.60, 60.36, 59.20, 50.76, 47.93, 47.60, 46.61, 29.56, 29.50, 29.47, 28.08, 25.50, 24.63, 24.23, 23.90. HRMS (ESI): *m/z* calcd for $C_{20}H_{32}N_5O_6^+$: 438.2347 $[M + H]^+$; found: 438.2347.

TFA·H-Pro-D-Pro-D-Glu-Pro-NH$_2$ (C-LDDL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (600 MHz, D_2O) δ = 4.86 (dd, J = 9.6, 4.5 Hz, 1H), 4.75 (dd, J = 8.8, 6.9 Hz, 1H), 4.59 (dd, J = 8.9, 3.9 Hz, 1H), 4.49 (dd, J = 8.8, 4.3 Hz, 1H), 3.93 (dt, J = 10.4, 6.4 Hz, 1H), 3.87 – 3.80 (m, 2H), 3.72 (dt, J = 10.5, 7.3 Hz, 1H), 3.57 (dt, J = 11.6, 7.1 Hz, 1H), 3.51 (dt, J = 11.6, 7.0 Hz, 1H), 2.74 – 2.54 (m, 2H), 2.49 – 2.36 (m, 3H), 2.33 – 1.96 (m, 11H). ^{13}C NMR (151 MHz, D_2O) δ = 177.03, 176.91, 173.89, 171.49, 168.00, 60.63, 60.58, 59.21, 50.86, 47.79, 47.61, 46.63, 29.58, 29.55, 29.43, 28.08, 25.41, 24.25, 24.17, 23.91. HRMS (ESI): *m/z* calcd for $C_{20}H_{32}N_5O_6^+$: 438.2347 $[M + H]^+$; found: 438.2347.

TFA·H-Pro-D-Pro-Glu-Pro-NH$_2$ (C-LDLL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (600 MHz, D_2O) δ = 4.85 (dd, J = 9.7, 4.5 Hz, 1H), 4.75 (dd, J = 8.8, 7.1 Hz, 1H), 4.60 (dd, J = 8.9, 3.5 Hz, 1H), 4.51 (dd, J = 8.5, 5.8 Hz, 1H), 3.92 (dt, J = 10.2, 6.7 Hz, 1H), 3.89 – 3.79 (m, 2H), 3.75 – 3.69 (m, 1H), 3.57 (dt, J = 11.6, 7.0 Hz, 1H), 3.50 (dt, J = 11.5, 7.1 Hz, 1H), 2.73 – 2.64 (m, 1H), 2.64 – 2.56 (m, 2H), 2.55 – 2.37 (m, 2H), 2.33 – 1.85 (m, 11H). ^{13}C NMR (151 MHz, D_2O) δ = 177.21, 176.80, 173.62, 171.42, 168.05, 60.75, 60.39, 59.26, 50.81, 47.90, 47.56, 46.61, 29.79, 29.65, 29.55, 28.01, 25.44, 24.61, 24.13, 23.88. HRMS (ESI): *m/z* calcd for $C_{20}H_{32}N_5O_6^+$: 438.2347 $[M + H]^+$; found: 438.2347.

TFA·H-Pro-D-Pro-Glu-D-Pro-NH$_2$ (C-LDLD): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (600 MHz, D_2O) δ = 4.88 – 4.81 (m, 1H), 4.74 (dd, J = 8.8, 7.2 Hz, 1H), 4.60 (dd, J = 9.0, 3.6 Hz, 1H), 4.48 (dd, J = 8.9, 4.3 Hz, 1H), 3.94 (dt, J = 10.2, 6.3 Hz, 1H), 3.88 – 3.81 (m, 2H), 3.72 (dt, J = 9.6, 6.8 Hz, 1H), 3.56 (dt, J = 8.4, 5.9 Hz, 1H), 3.50 (td, J = 7.1, 4.9 Hz, 1H), 2.73 – 2.39 (m, 5H), 2.33 – 1.95 (m, 11H). ^{13}C NMR (151 MHz, D_2O) δ = 177.03, 176.91, 173.62, 171.37, 168.00, 60.76, 60.58, 59.24, 50.76, 47.81, 47.58, 46.59, 29.81, 29.59, 29.56, 28.02, 25.40, 24.21, 24.17, 23.87. HRMS (ESI): *m/z* calcd for $C_{20}H_{32}N_5O_6^+$: 438.2347 $[M + H]^+$; found: 438.2346.

TFA·H-Pro-Pro-D-Glu-Pro-NH$_2$ (C-LLDL): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (600 MHz, D_2O) δ = 4.85 (dd, J = 9.6, 4.5 Hz, 1H), 4.75 (dd, J = 8.9, 5.7 Hz, 1H), 4.59 (dd, J = 8.4, 6.2 Hz, 1H), 4.47 (dd, J = 8.8, 4.5 Hz, 1H), 3.92 (dt, J = 10.3, 6.4 Hz, 1H), 3.86 – 3.79 (m, 2H), 3.72 (dt, J = 10.1, 7.1 Hz, 1H), 3.58 – 3.48 (m, 2H), 2.74 – 2.51 (m, 3H), 2.49 – 2.37 (m, 2H), 2.33 – 1.88 (m, 11H). ^{13}C NMR (151 MHz, D_2O) δ = 177.02, 176.92, 173.56, 171.43, 168.08, 60.59, 60.54, 59.08, 50.73, 47.79, 47.68, 46.61, 29.60, 29.36, 29.34, 28.32, 25.45, 24.64, 24.21, 23.79. HRMS (ESI): *m/z* calcd for $C_{20}H_{32}N_5O_6^+$: 438.2347 [M + H]$^+$; found: 438.2347.

TFA·H- Pro-Pro-D-Glu-D-Pro-NH$_2$ (C-LLDD): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (600 MHz, D_2O) δ = 4.92 (dd, J = 9.6, 4.3 Hz, 1H), 4.74 (dd, J = 8.8, 6.2 Hz, 1H), 4.61 (dd, J = 8.3, 6.1 Hz, 1H), 4.48 (dd, J = 8.9, 4.2 Hz, 1H), 3.93 (dt, J = 10.1, 6.4 Hz, 1H), 3.82 (dt, J = 10.1, 6.7 Hz, 2H), 3.72 (dt, J = 10.3, 7.0 Hz, 1H), 3.59 – 3.46 (m, 2H), 2.73 – 2.37 (m, 5H), 2.28 – 1.96 (m, 11H). ^{13}C NMR (151 MHz, D_2O) δ = 176.97, 176.92, 173.37, 171.36, 168.04, 60.94, 60.59, 60.59, 59.11, 50.52, 47.79, 47.68, 46.56, 29.64, 29.62, 29.57, 29.47, 28.37, 25.70, 24.63, 24.21, 23.83. HRMS (ESI): *m/z* calcd for $C_{20}H_{32}N_5O_6^+$: 438.2347 [M + H]$^+$; found: 438.2348.

TFA·H-D-Pro-Pro-Asp-Leu-NH$_2$ (D): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, D_2O) δ = 4.73 (t, J = 7.2 Hz, 1H), 4.65 (dd, J = 8.9, 6.8 Hz, 1H), 4.46 (dd, J = 8.8, 3.9 Hz, 1H), 4.39 – 4.29 (m, 1H), 3.74 (ddd, J = 10.1, 7.4, 4.8 Hz, 1H), 3.61 (dt, J = 10.2, 7.3 Hz, 1H), 3.53 – 3.33 (m, 2H), 2.99 (dd, J = 16.9, 7.3 Hz, 1H), 2.90 – 2.76 (m, 1H), 2.56 (ddd, J = 12.9, 8.7, 6.5 Hz, 1H), 2.39 – 2.23 (m, 1H), 2.18 – 1.82 (m, 4H), 1.77 – 1.51 (m, 4H), 1.04 – 0.76 (m, 6H). ^{13}C NMR (101 MHz, D_2O) δ = ^{13}C NMR (101 MHz, D_2O) δ 177.30, 174.13, 173.37, 172.25, 168.01, 60.56, 59.25, 52.11, 50.05, 47.59, 46.60, 39.57, 35.06, 29.58, 28.01, 24.24, 24.14, 23.87, 22.26, 20.23. HRMS (MALDI): *m/z* calcd for $C_{20}H_{34}N_5O_6^+$: 440.2504 [M + H]$^+$; found: 440.2503.

TFA·H-D-Pro-D-Pro-Asp-Leu-NH$_2$ (E): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, D_2O) δ = 4.75 (dd, J = 7.6, 5.4 Hz, 1H), 4.66 (dd, J = 8.9, 6.2 Hz, 1H), 4.48 (dd, J = 8.2, 6.5 Hz, 1H), 4.35 (dd, J = 10.2, 4.3 Hz, 1H), 3.74 (ddd, J = 10.2, 7.2, 5.2 Hz, 1H), 3.62 (dt, J = 10.2, 7.1 Hz, 1H), 3.42 (td, J = 6.9, 2.1 Hz, 2H), 2.97 (dd, J = 17.0, 5.4 Hz, 1H), 2.87 (dd, J = 17.0, 7.6 Hz, 1H), 2.65 – 2.52 (m, 1H), 2.34 (ddt, J = 12.7, 8.2, 6.2 Hz, 1H), 2.21 – 1.85 (m, 7H), 1.84 – 1.47 (m, 3H), 0.95 (d, J = 5.8 Hz, 3H), 0.88 (d, J = 5.9 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ = 177.35, 174.11, 173.81, 172.32, 168.08, 60.72, 58.95, 52.20, 50.20, 47.76, 46.70, 39.54, 35.32, 29.23, 28.34, 24.66, 24.28, 23.89, 22.27, 20.38. HRMS (MALDI): *m/z* calcd for $C_{20}H_{34}N_5O_6^+$: 440.2504 [M + H]$^+$; found: 440.2502.

TFA·H-Pro-Pro-Asp-D-Asp-NH$_2$ (F): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides,

coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, D_2O) δ = 4.73 (dd, J = 7.6, 5.1 Hz, 1H), 4.70 – 4.55 (m, 2H), 4.49 (dd, J = 8.3, 6.4 Hz, 1H), 3.73 (ddd, J = 10.2, 7.0, 5.5 Hz, 1H), 3.63 (dt, J = 10.2, 7.1 Hz, 1H), 3.61 – 3.35 (m, 2H), 2.99 – 2.76 (m, 4H), 2.68 – 2.51 (m, 1H), 2.36 (ddt, J = 12.6, 8.4, 6.2 Hz, 1H), 2.21 – 1.80 (m, 6H). ^{13}C NMR (101 MHz, D_2O) δ = 175.05, 174.61, 173.87, 173.31, 172.33, 168.29, 60.66, 59.08, 50.62, 50.02, 47.72, 46.62, 35.70, 35.60, 29.33, 28.33, 24.62, 23.92. HRMS (MALDI): *m/z* calcd for $C_{18}H_{28}N_5O_8^+$: 442.1932 [M + H]$^+$; found: 442.1932.

TFA·H-Pro-Pro-D-Leu-Glu-NH$_2$ (G): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, D_2O) δ = 4.65 (dd, J = 8.9, 6.5 Hz, 1H), 4.50 (dd, J = 8.2, 6.6 Hz, 1H), 4.43 – 4.35 (m, 2H), 3.74 (ddd, J = 10.2, 7.2, 5.1 Hz, 1H), 3.61 (dt, J = 10.2, 7.2 Hz, 1H), 3.42 (t, J = 6.5 Hz, 2H), 2.69 – 2.52 (m, 1H), 2.52 – 2.42 (m, 2H), 2.42 – 2.27 (m, 1H), 2.20 (dddd, J = 13.6, 8.4, 7.2, 5.0 Hz, 1H), 2.14 – 1.81 (m, 7H), 1.81 – 1.42 (m, 3H), 0.95 (d, J = 5.7 Hz, 4H), 0.90 (d, J = 5.6 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ = 177.00, 175.85, 174.70, 173.90, 167.95, 60.66, 59.04, 52.54, 52.45, 47.73, 46.68, 39.62, 29.99, 29.45, 28.42, 26.17, 24.67, 24.40, 23.98, 22.06, 20.45. HRMS (MALDI): *m/z* calcd for $C_{21}H_{36}N_5O_6^+$: 454.2660 [M + H]$^+$; found: 454.2660.

TFA·H-Pro-Pro-Pro-D-Asp-NH$_2$ (H): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, D_2O) δ = 4.84 – 4.72 (m, 2H), 4.69 – 4.61 (m, 1H), 4.44 (dd, J = 8.4, 5.7 Hz, 1H), 3.85 (dt, J = 10.2, 6.8 Hz, 1H), 3.79 – 3.62 (m, 2H), 3.59 (dt, J = 10.3, 7.2 Hz, 1H), 3.51 – 3.34 (m, 2H), 2.97 (dd, J = 17.0, 4.8 Hz, 1H), 2.84 (dd, J = 17.0, 8.2 Hz, 1H), 2.63 – 2.51 (m, 1H), 2.44 (ddt, J = 12.6, 8.4, 6.5 Hz, 1H), 2.31 (ddt, J = 12.7, 8.3, 6.8 Hz, 1H), 2.17 – 1.99 (m, 7H), 1.93 (tt, J = 13.1, 6.4 Hz, 2H). ^{13}C NMR (101 MHz, D_2O) δ = 174.97, 174.19, 174.06, 171.57, 167.67, 60.57, 59.06, 59.01, 49.65, 47.73, 47.68, 46.61, 35.57, 29.14, 28.22, 28.02, 24.64, 24.53, 23.81. HRMS (MALDI): *m/z* calcd for $C_{19}H_{30}N_5O_6^+$: 424.2191 [M + H]$^+$; found: 424.2191.

TFA·H-Pro-Pro-Pro-His-NH$_2$ (I): The peptide was synthesized according to the general procedures for solid phase peptide synthesis. As expected for Xaa-Pro containing peptides, coexistence of the *trans* and *cis* configured amide bond was observed in the NMR spectra. Only the signals of the major conformer are listed. ^{1}H NMR (400 MHz, D_2O) δ = 8.63 (d, J = 1.4 Hz, 1H), 7.34 (d, J = 1.4 Hz, 1H), 4.83 – 4.78 (m, 1H), 4.70 – 4.61 (m, 2H), 4.42 (dd, J = 8.5, 5.3 Hz, 1H), 3.83 (dt, J = 10.2, 7.0 Hz, 1H), 3.80 – 3.62 (m, 2H), 3.59 (dt, J = 10.2, 7.2 Hz, 1H), 3.43 (qt, J = 11.6, 7.1 Hz, 2H), 3.33 – 3.16 (m, 2H), 2.58 (dtd, J = 12.3, 7.2, 4.4 Hz, 1H), 2.44 (ddt, J = 12.8, 8.3, 6.5 Hz, 1H), 2.37 – 2.23 (m, 1H), 2.17 – 1.98 (m, 7H), 2.01 – 1.79 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ = 174.01, 173.90, 171.68, 167.61, 133.41, 128.36, 117.19, 60.31, 59.04, 58.96, 52.27, 47.70, 47.69, 46.60, 29.24, 28.24, 28.09, 26.27, 24.57, 24.54, 23.83. HRMS (MALDI): *m/z* calcd for $C_{21}H_{32}N_7O_4^+$: 446.2510 [M + H]$^+$; found: 446.2511.

3. Preparation of the Split-and-Mix-Library and Combinatorial Screening Assay

The split-and-mix tetrapeptide library and the dye-labelled ketone was synthesized according to the previously reported procedure.[5] TentaGel S NH_2 from Rapp Polymers was used as a solid support for the library. Also the combinatorial assay was performed as described.[5]

Identified peptide sequences of the assay in DMF (A) and MeOH (B).

A:

AS4	AS3	AS2	AS1
L-Pro	L-Pro	L-Asp	D-Asp
L-Pro	L-Pro	L-Asp	D-Asp
L-Pro	L-Pro	L-Asp	D-Asp
L-Pro	L-Pro	L-Asp	D-Pro
L-Pro	D-Pro	D-Asp	D-Pro
L-Pro	L-Pro	L-Asp	D-Pro
D-Pro	D-Pro	D-Glu	D-Pro
D-Pro	D-Pro	D-Glu	D-Pro
D-Pro	D-Pro	D-Glu	D-Pro
D-Pro	L-Pro	L-Asp	L-Leu
L-Pro	L-Pro	L-Asp	L-Leu
L-Pro	L-Pro	L-Pro	D-Asp
L-Pro	L-Pro	L-Pro	D-Asp
D-Pro	D-Pro	L-Pro	D-Asp
D-Pro	D-Pro	L-Pro	D-Asp
L-Pro	L-Pro	L-Pro	D-Asp
D-Pro	D-Pro	Gly	D-Asp
D-Pro	D-Pro	L-His	D-Asp
D-Pro	L-Pro	D-Leu	L-Glu
D-Pro	L-Pro	D-Leu	L-Glu
L-Pro	L-Pro	L-Asn	L-Glu
L-Pro	L-Pro	L-Pro	L-His
L-Pro	D-Pro	L-Pro	L-His
D-Pro	D-Pro	D-Gln	L-His
D-Pro	D-Pro	D-Glu	D-His

B:

AS4	AS3	AS2	AS1
D-Pro	D-Pro	L-Pro	D-Asp
D-Pro	D-Pro	L-Pro	D-Asp
D-Pro	D-Pro	L-Asp	L-Leu
D-Pro	D-Pro	L-Asp	L-Leu
D-Pro	D-Pro	L-Asp	L-Leu
D-Pro	D-Pro	L-Asp	L-Leu
D-Pro	D-Pro	L-Asp	L-Leu
L-Pro	L-Pro	L-Asp	L-Gln
L-Pro	D-Pro	D-Glu	L-Leu
D-Pro	D-Pro	D-Glu	L-Tyr
D-Pro	D-Pro	D-Glu	L-Glu
L-Pro	L-Pro	Gly	L-Glu
L-Pro	L-Pro	D-Tyr	L-Glu
D-Pro	D-Pro	L-Phe	L-Glu
L-Pro	L-Pro	D-Leu	L-Glu
D-Pro	D-Pro	D-Leu	L-Glu
L-Pro	D-Pro	D-Pro	L-Glu
D-Pro	L-Pro	L-Pro	L-Tyr
L-Pro	L-Pro	D-Gln	L-Tyr

4. General Procedures for Aldol Reaction and 1,4-Addition Reaction

General Procedure – Aldol Reaction (10 mol% catalyst)

The peptide TFA salt (10 mol%, 6.6 μmol) was added to a solution of *N*-methylmorpholine (10 mol%, 0.73 μl), *p*-nitrobenzaldehyde (1 equiv., 66 μmol, 10.0 mg) in acetone (340 μl). The reaction mixture was stirred for 24 h. The conversion was estimated by TLC and the enantiomer excess was determined by chiral stationary phase HPLC (AS-H, hexane/^{i}PrOH 1:1, 0.5 ml/min, 40° C, 12.8 min (*R*), 15.2 min (*S*)).

General Procedure – Aldol Reaction (1 mol% catalyst)

The peptide TFA salt (1 mol%, 2 μmol, 1.07 mg) was added to a solution of *N*-methylmorpholine (1 mol%, 0.22 μl), *p*-nitrobenzaldehyde (1 equiv., 200 μmol, 30.0 mg) in acetone (809 μl). The reaction mixture was stirred for 24 h. The conversion was estimated by TLC and the enantiomer excess was determined by chiral stationary phase HPLC (AS-H, hexane/^{i}PrOH 1:1, 0.5 ml/min, 40° C, 254 nm, 12.8 min (*R*), 15.2 min (*S*)).

The analytical data of the product was in agreement with those previously published, e.g. ref. 5.

(Note, no product formation was observed when the reactants were mixed under identical conditions in the absence of peptidic catalysts.)

General Procedure – 1,4-Addition Reaction to Nitrostyrene

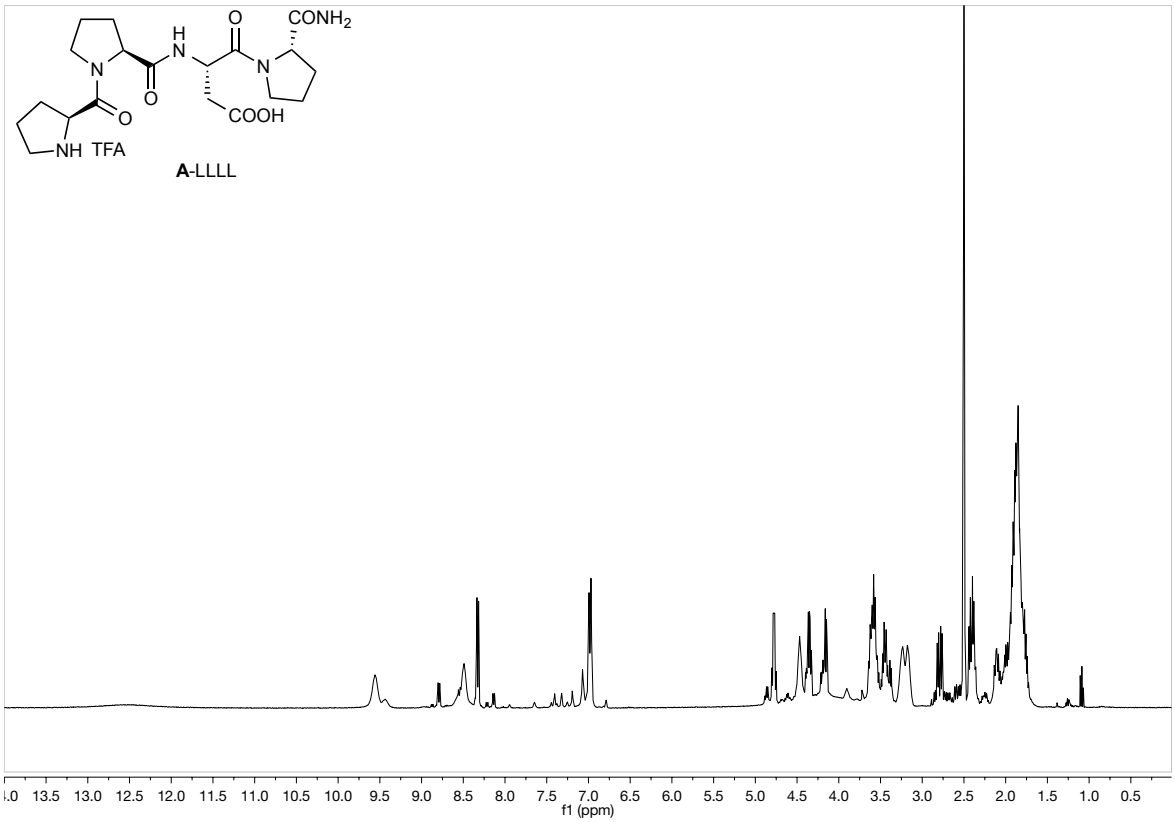
The peptide TFA salt (1 mol%, 5 μmol, 2.76 mg) was added to a solution of *N*-methylmorpholine (1 mol%, 0.55 μl), (*E*)-nitrostyrene (1 equiv., 500 μmol, 74.6 mg) and butanal (1.5 equiv., 750 μmol, 67.6 μl) in $CHCl_3$/^{i}PrOH 9:1 (1.116 ml). The reaction mixture was stirred for 24 h. Conversion and d.r. were determined by ^{1}H NMR of the crude mixture and comparison of the aldehyde signals Ar-C<u>H</u>O signals and the enantiomer excess was determined by chiral stationary phase HPLC (AD-H, hexane/^{i}PrOH 98:2, 0.9 ml/min, 25° C, 254 nm, 19.5 min (*syn,* minor), 22.6 min (*syn,* major)).

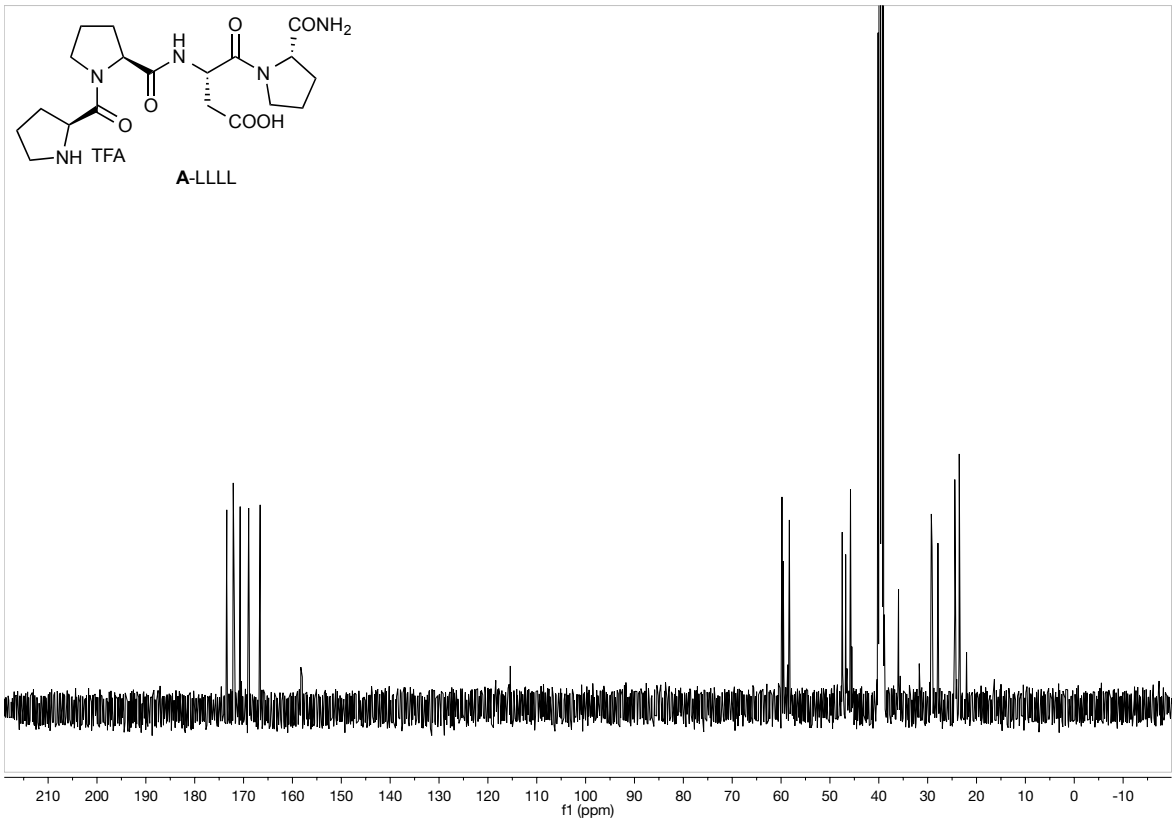
The analytical data of the product was in agreement with those previously published, e.g. ref. 3.

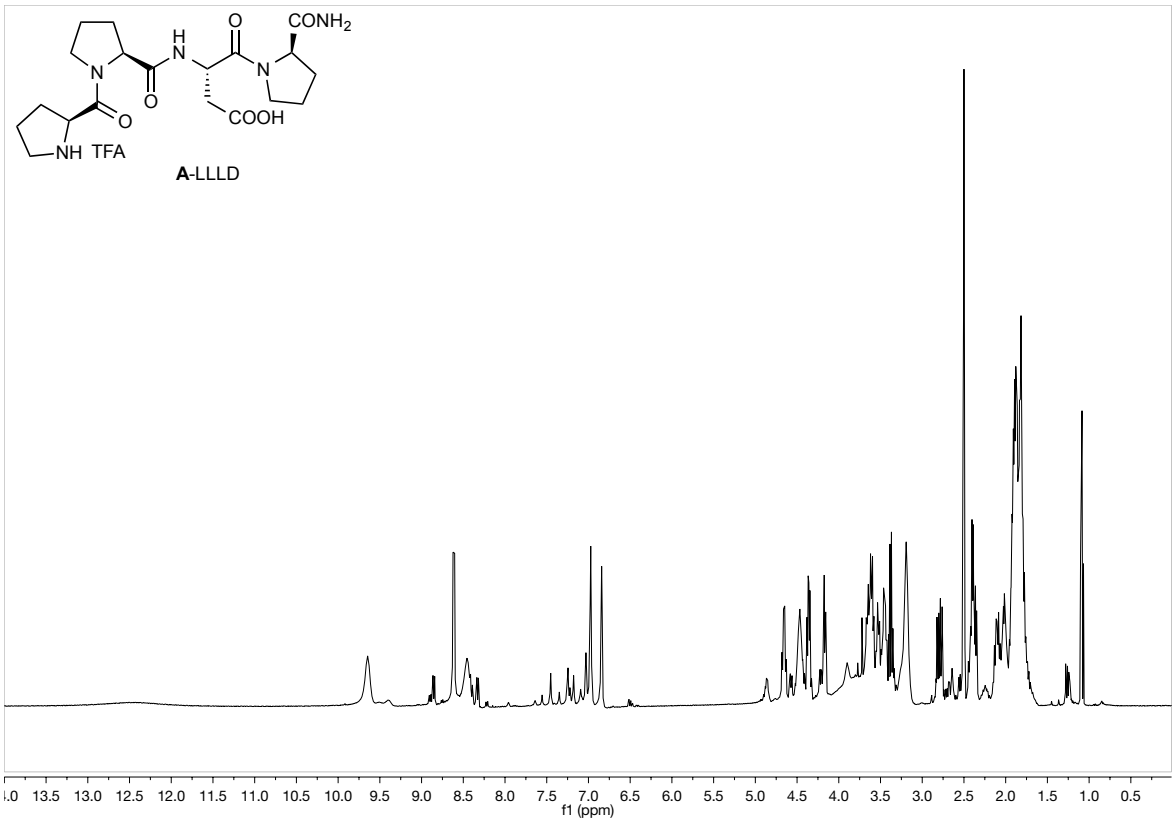
(Note, no product formation was observed when the reactants were mixed under identical conditions in the absence of peptidic catalysts.)

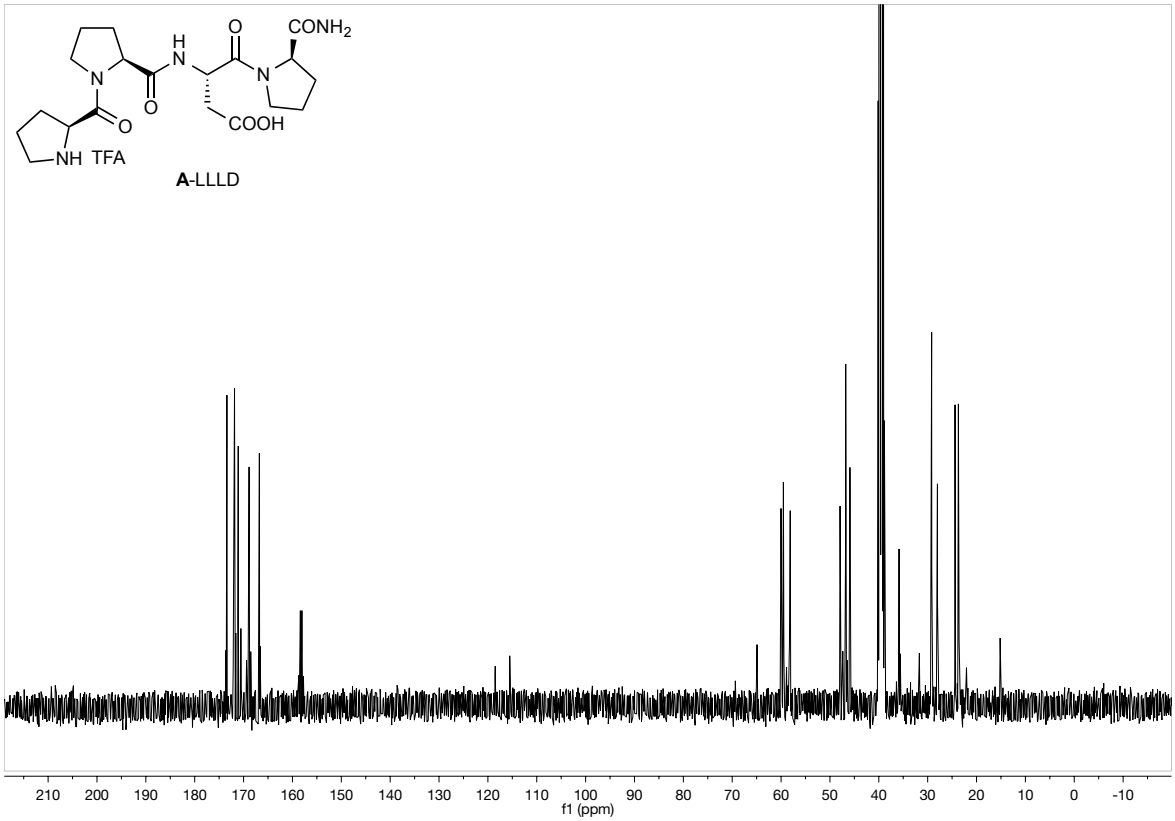
5. NMR Spectra of Peptides A-I

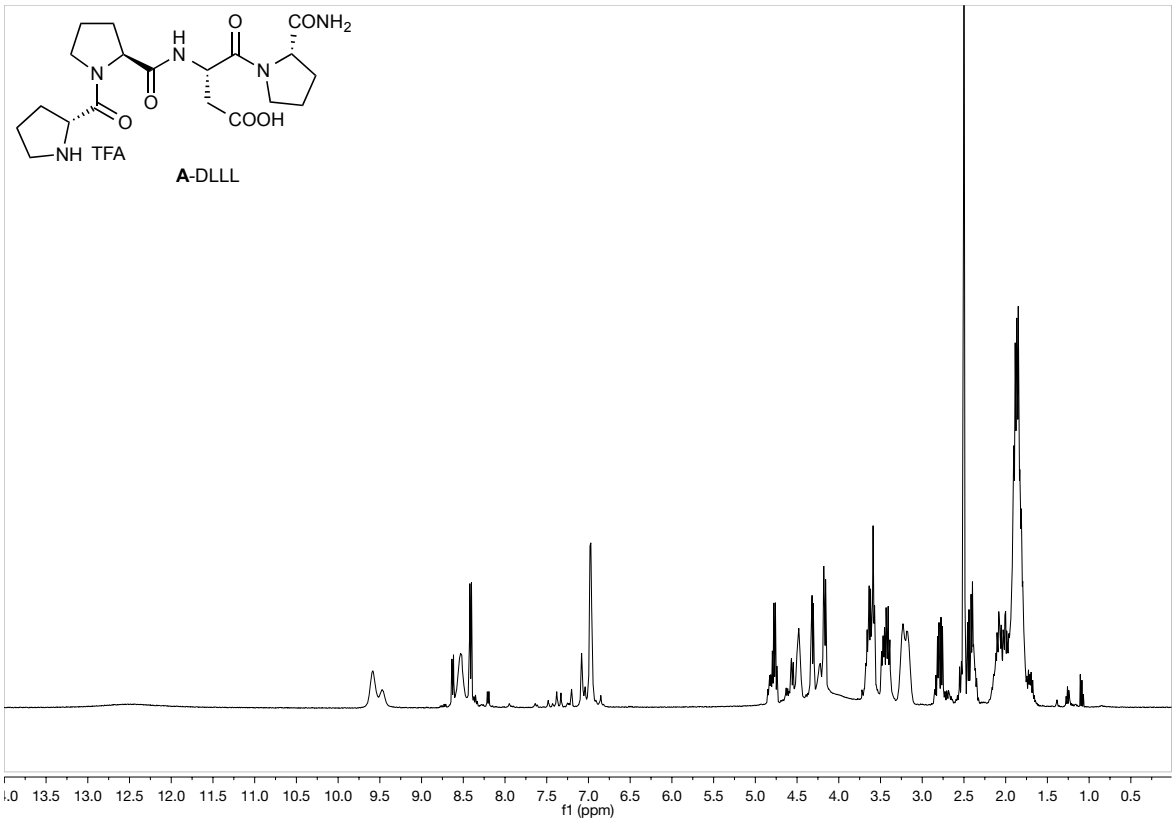
(Spectra of **A**-XXXX were recorded in d6-DMSO, all others in D_2O.)

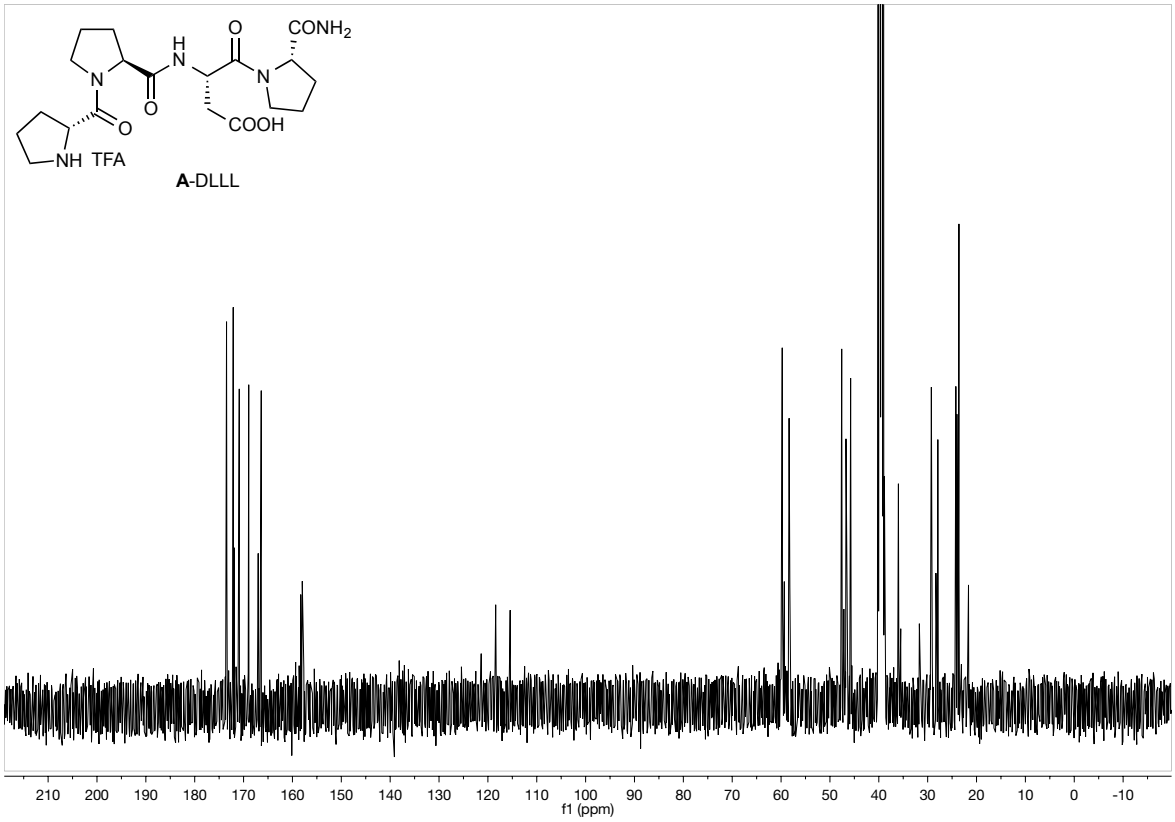





CONH2
H
N
O
N
N
O
O
COOH
NH TFA
A-LLLD
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)


CONH2
H
N
O
N
N
O
O
COOH
NH TFA
A-LLLD
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)


CONH2
O
H
N
N
N
O
O
COOH
O
NH TFA
A-DLLL
13.5 13.0 12.5 12.0 11.5 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
f1 (ppm)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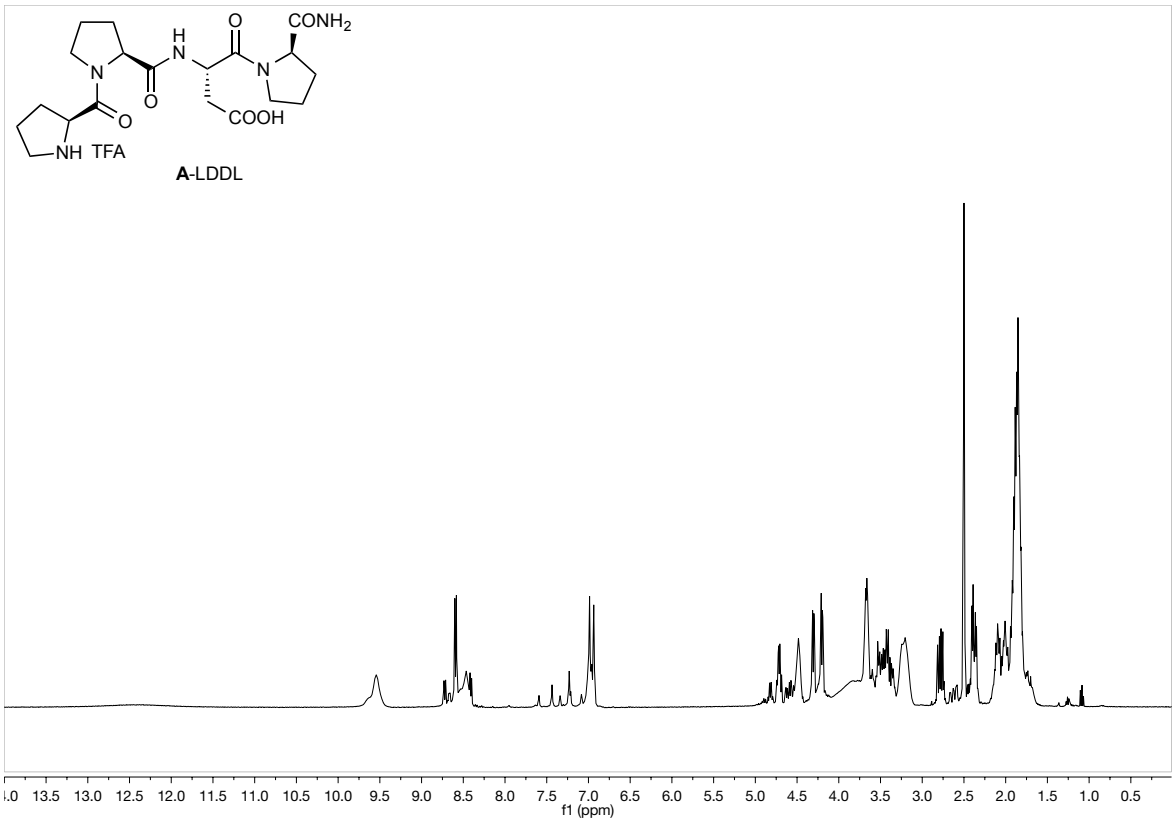
O
H
N
N
N
O
O
COOH
O
NH TFA
A-DLLL
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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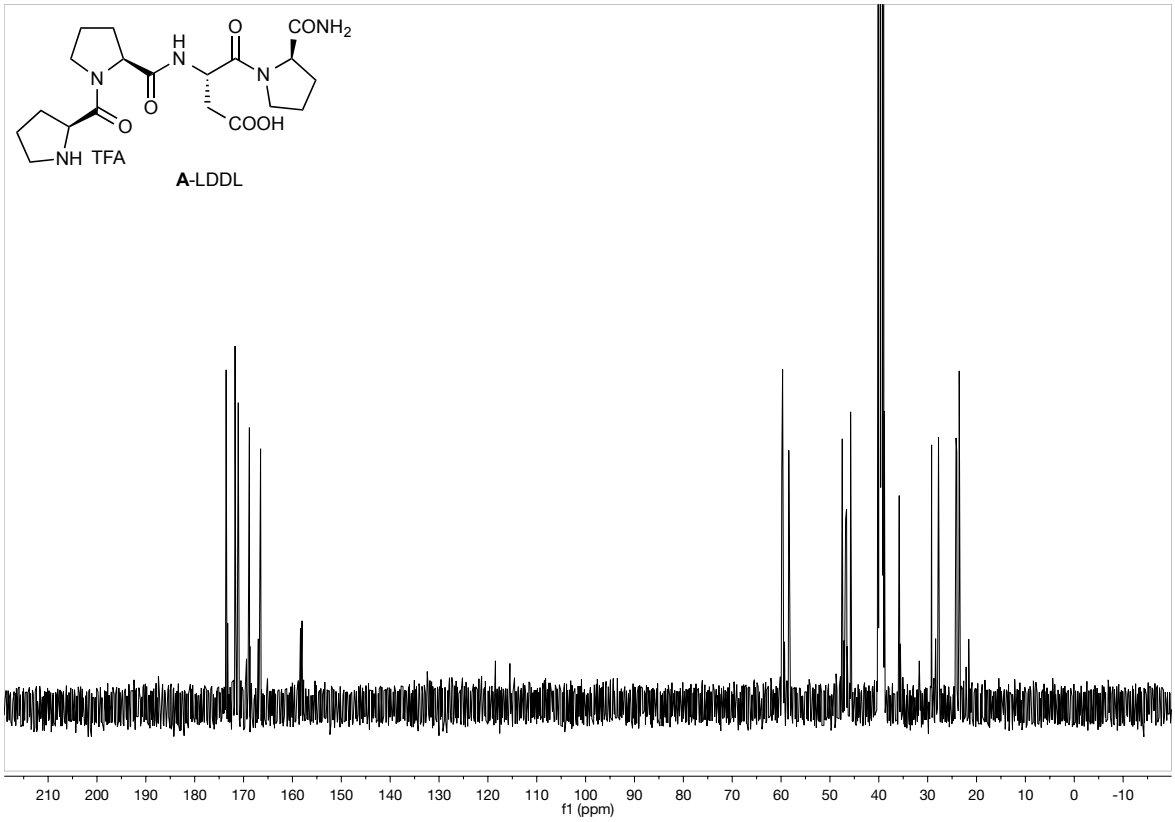
O
N
N
O
O
COOH
O
NH TFA
A-LDDL
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)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O
N
N
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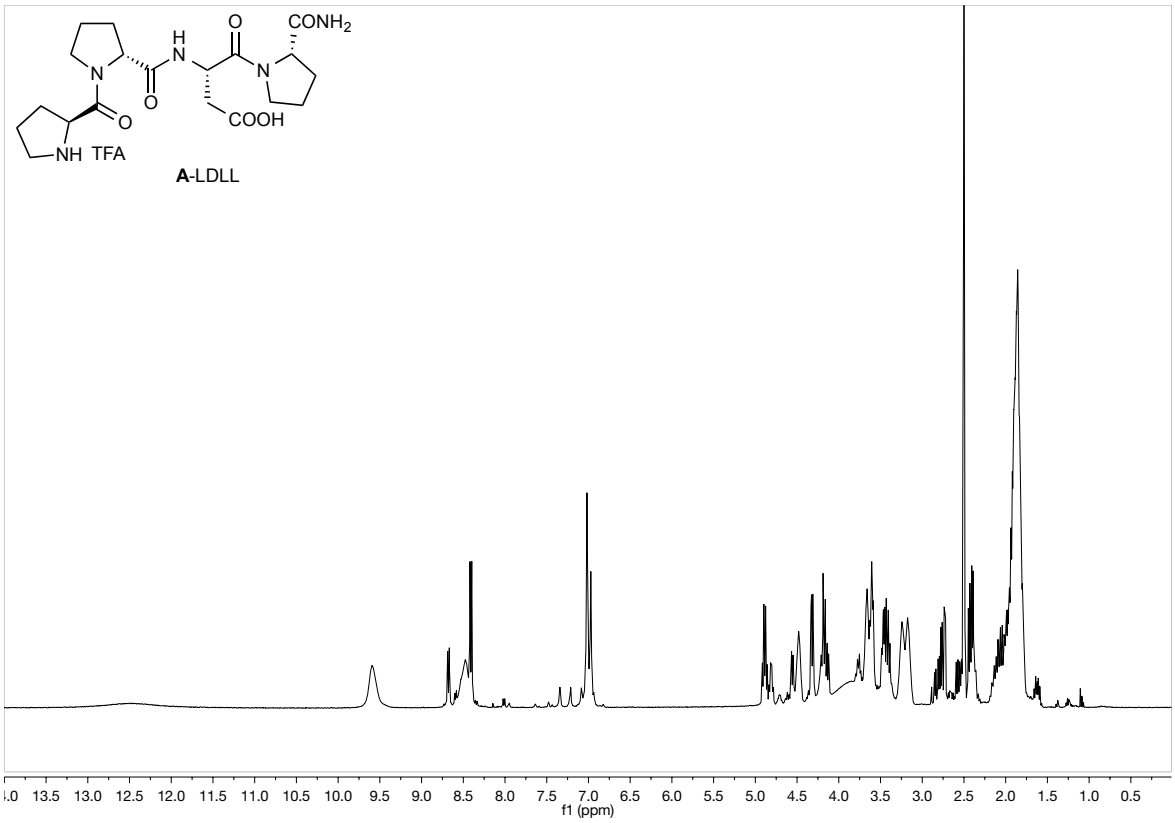
O
COOH
O
NH TFA
A-LDDL
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


CONH2
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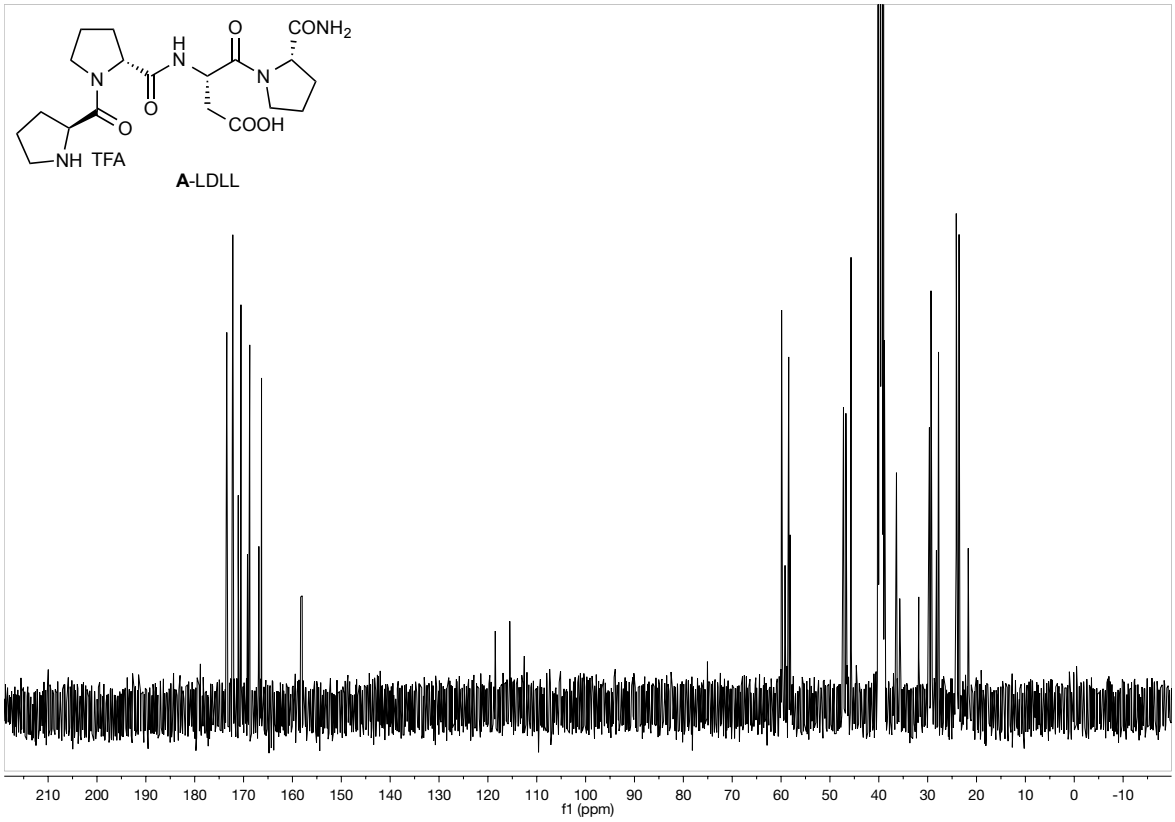
O
N
N
O
O
COOH
NH TFA
A-LDLL
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)


CONH2
H
N
O
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N
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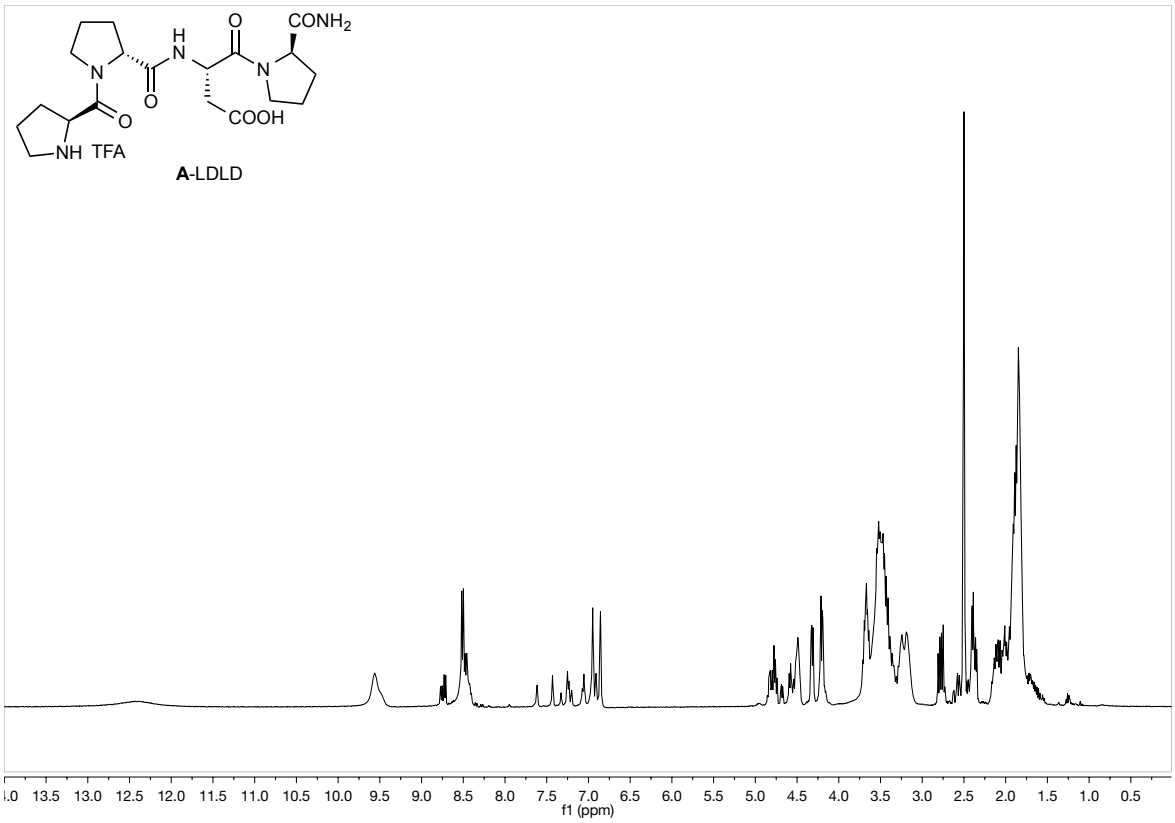
COOH
NH TFA
A-LDLL
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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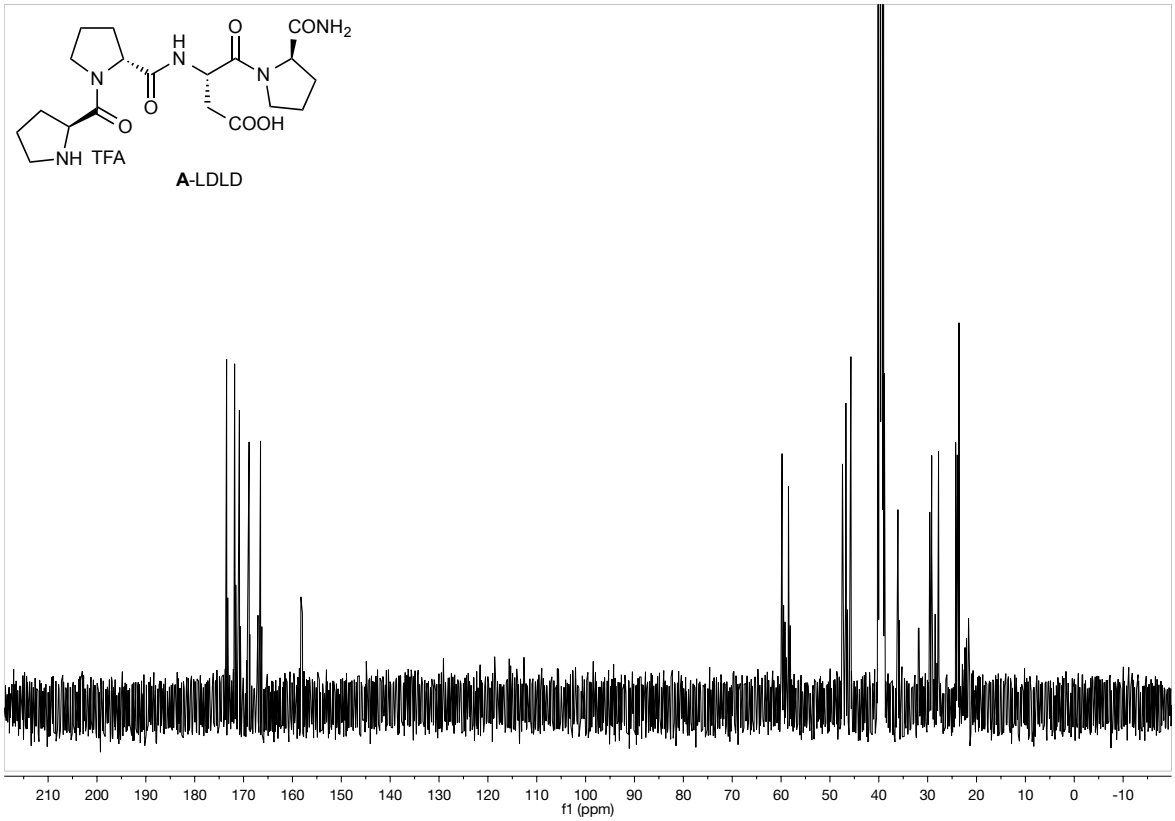
O
N
N
O
O
COOH
O
NH TFA
A-LDLD
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)


CONH2
H
N
O
N
N
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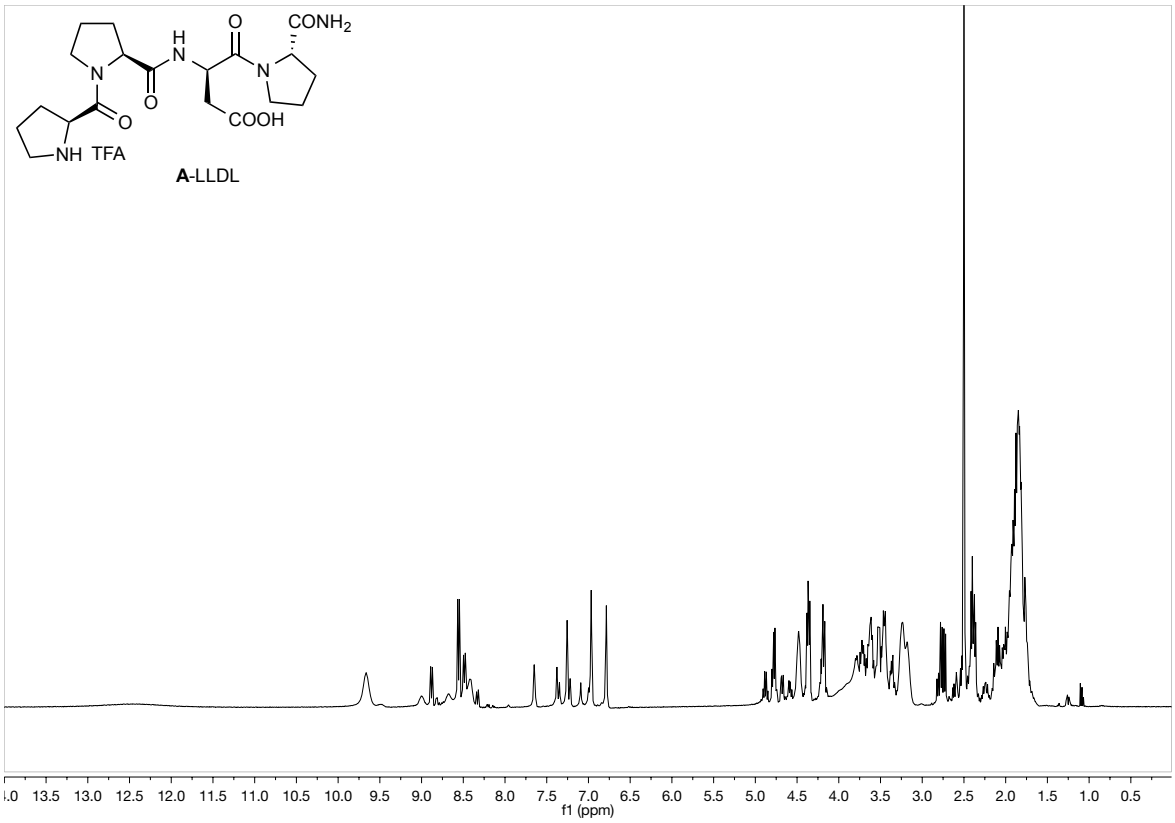
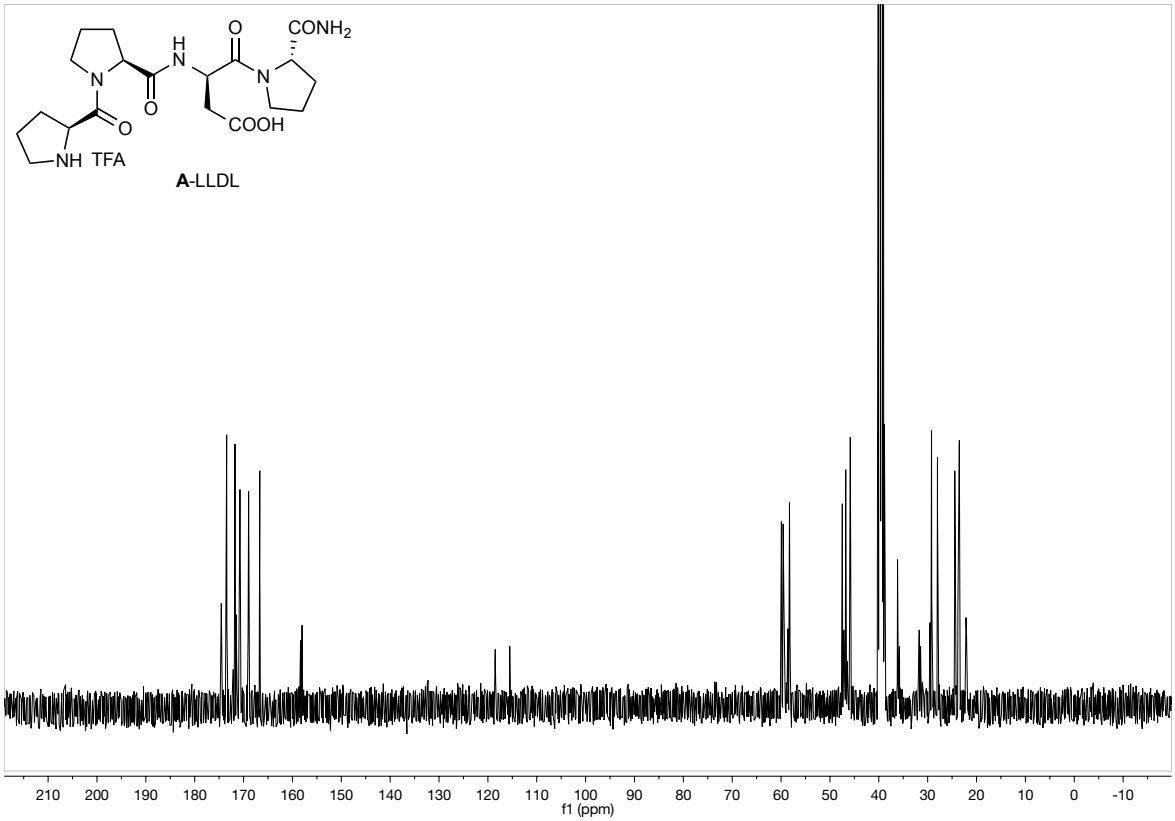
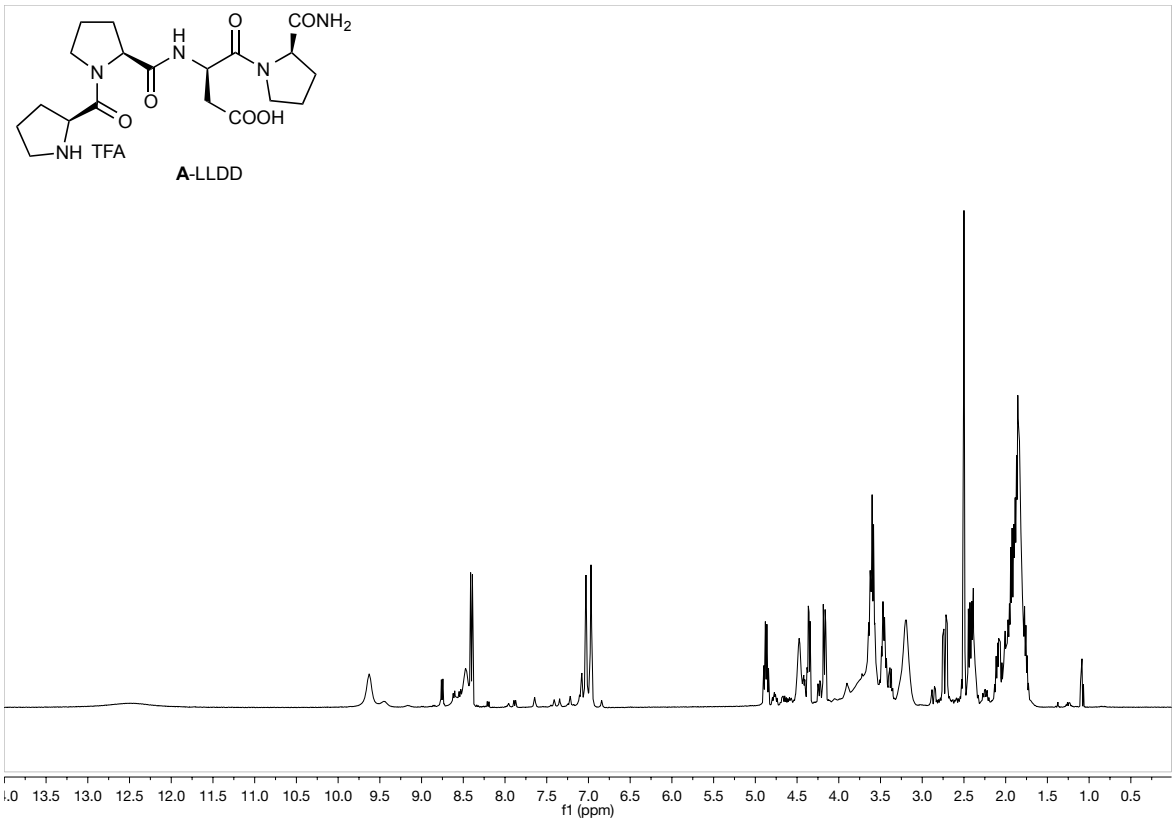
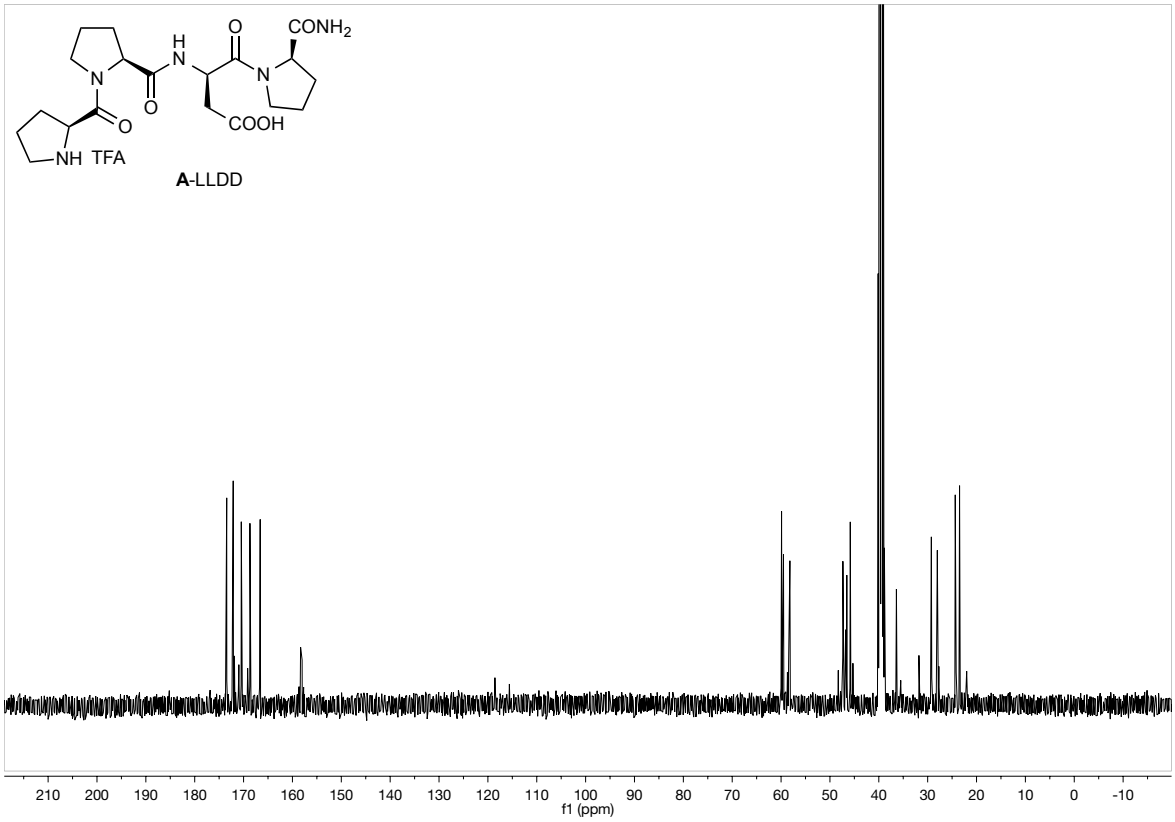
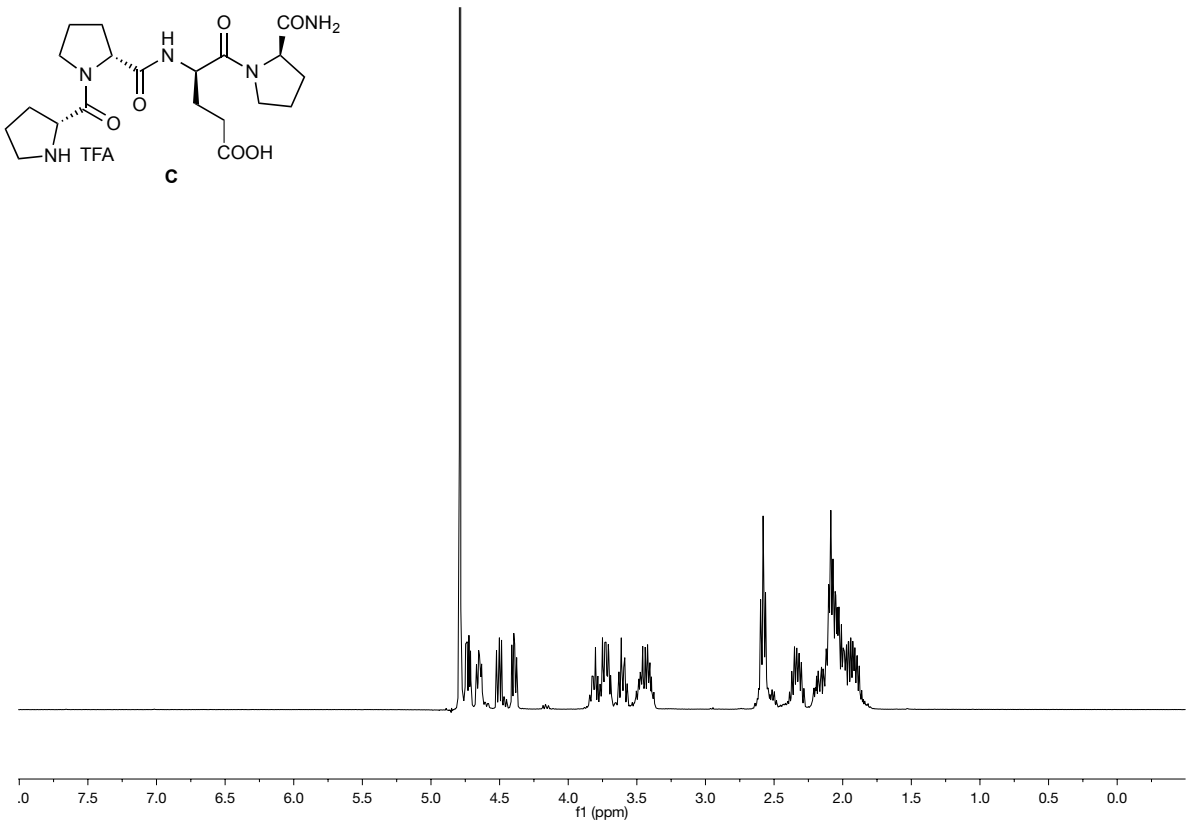
O
COOH
O
NH TFA
A-LDLD
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


CONH2
H
N
O
N
N
O
O
COOH
NH TFA
A-LLDL
13.5 13.0 12.5 12.0 11.5 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
f1 (ppm)

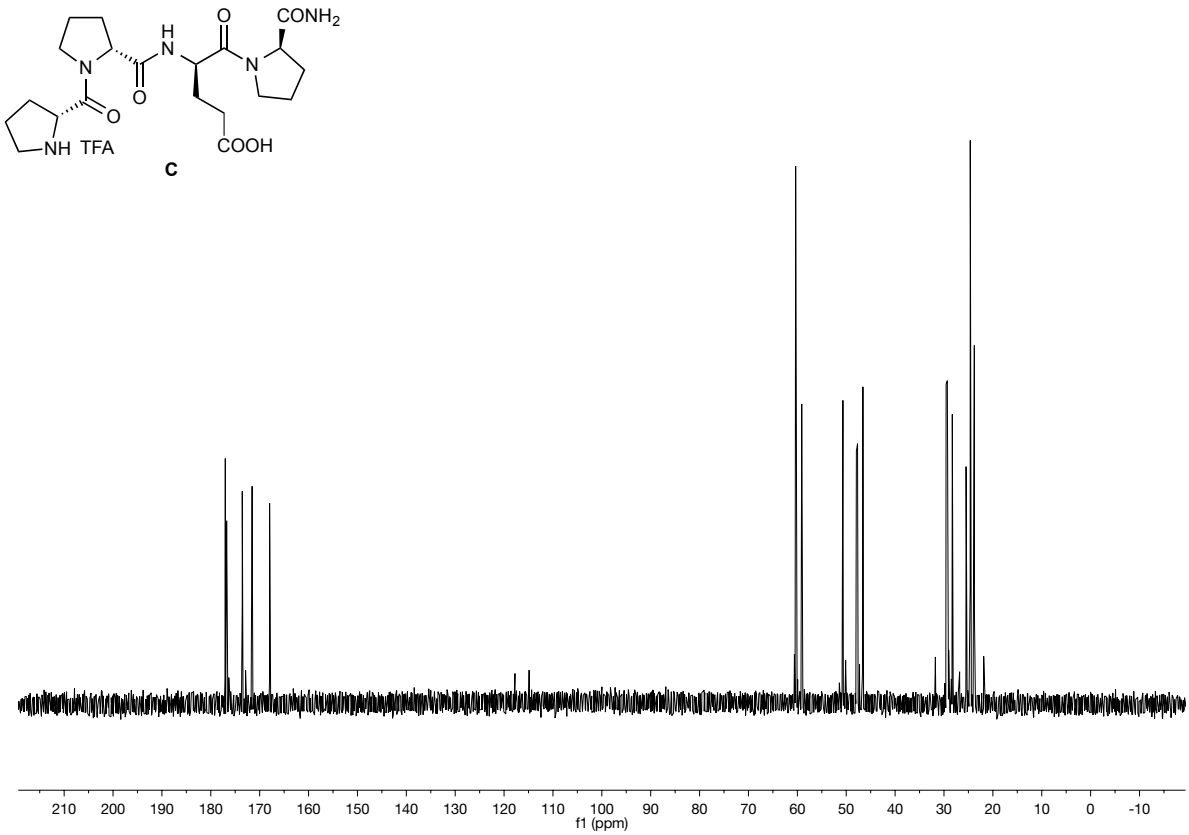

CONH2
H
N
O
N
N
O
O
COOH
NH TFA
A-LLDL
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


CONH2
H
N
O
N
N
O
O
COOH
NH TFA
A-LLDD
13.5 13.0 12.5 12.0 11.5 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
f1 (ppm)

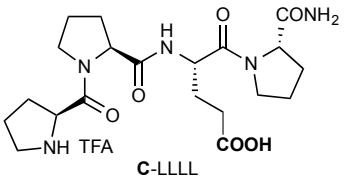

CONH2
H
N
O
N
N
O
O
COOH
NH TFA
A-LLDD
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


CONH2
H
N
O
N
N
O
O
NH TFA
COOH
.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
f1 (ppm)

C

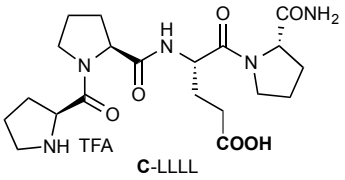

CONH2
H
N
O
N
N
O
O
NH TFA
COOH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

C

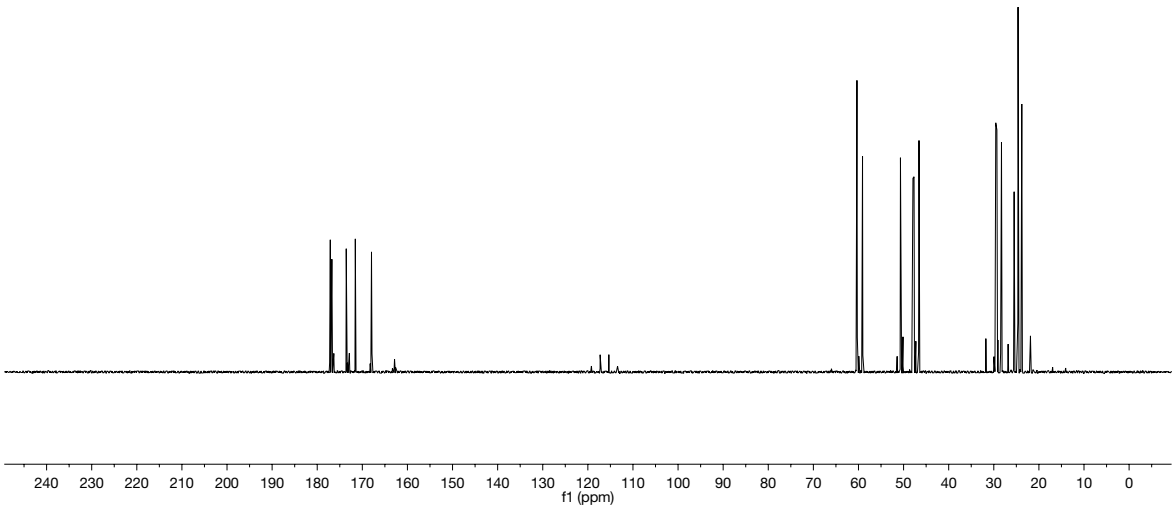

CONH2
H
N
O
N
O
N
O
NH TFA
COOH

C-LLLL

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
f1 (ppm)


CONH2
H
N
O
N
O
N
O
NH TFA
COOH

C-LLLL


240
230
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200
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180
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150
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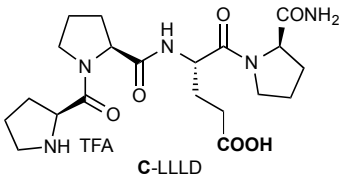
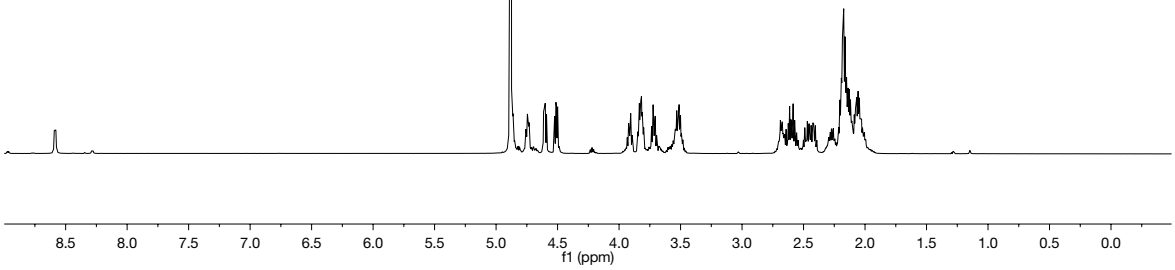
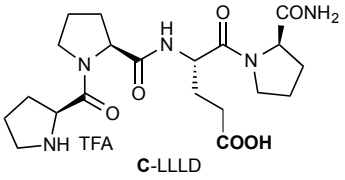
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


CONH2
H
N
O
N
O
N
O
NH TFA
COOH

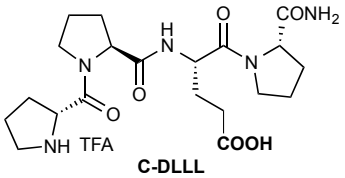
C-LLLD


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
f1 (ppm)


CONH2
H
N
O
N
O
N
O
NH TFA
COOH

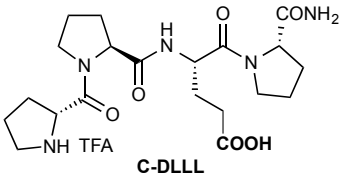
C-LLLD

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)


CONH2
H
N
O
N
O
N
O
NH TFA
COOH

C-DLLL

.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
f1 (ppm)


CONH2
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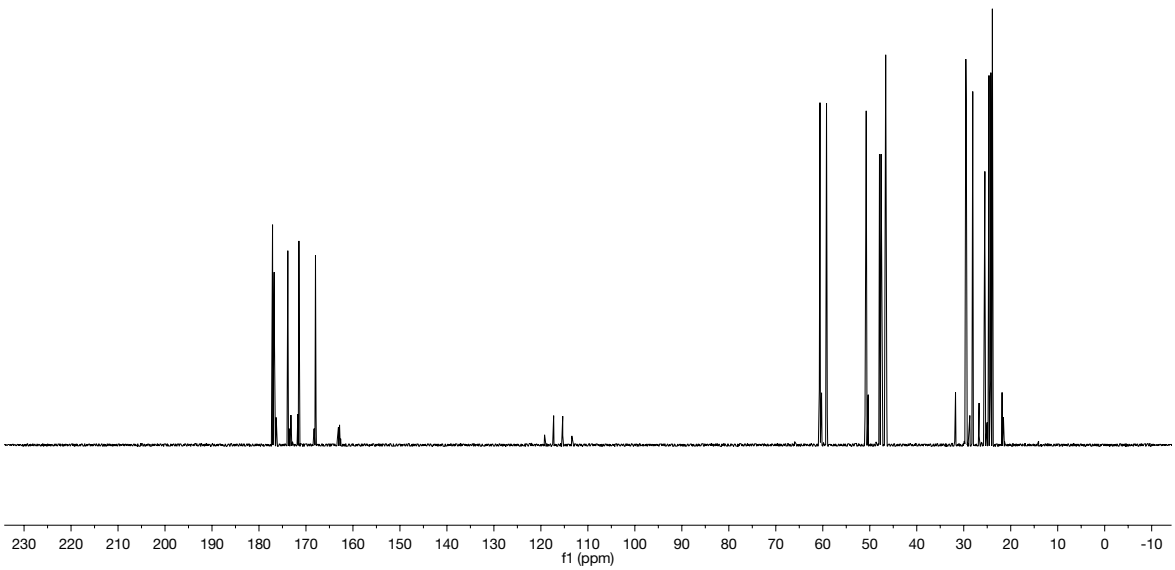
O
N
O
N
O
NH TFA
COOH

C-DLLL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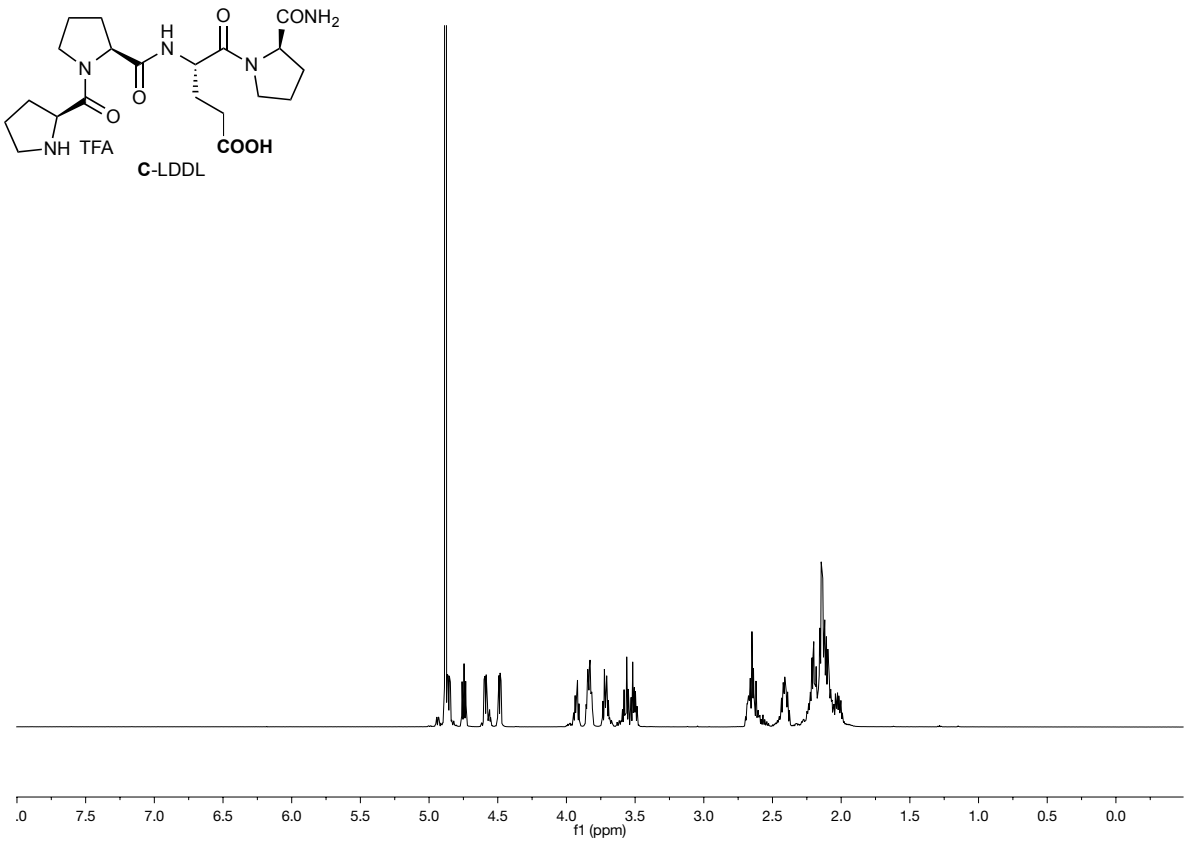
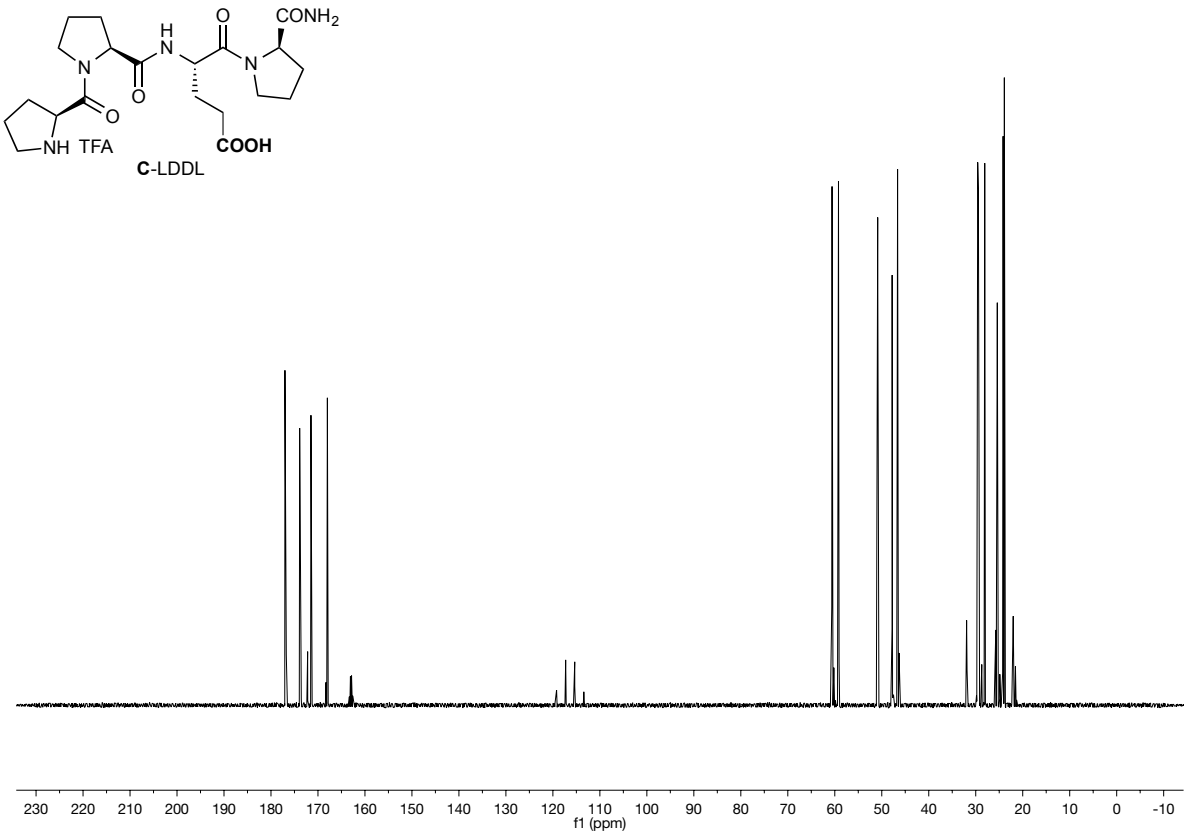
f1 (ppm)


CONH2
H
N
O
N
N
O
O
NH TFA
COOH
C-LDDL
.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
f1 (ppm)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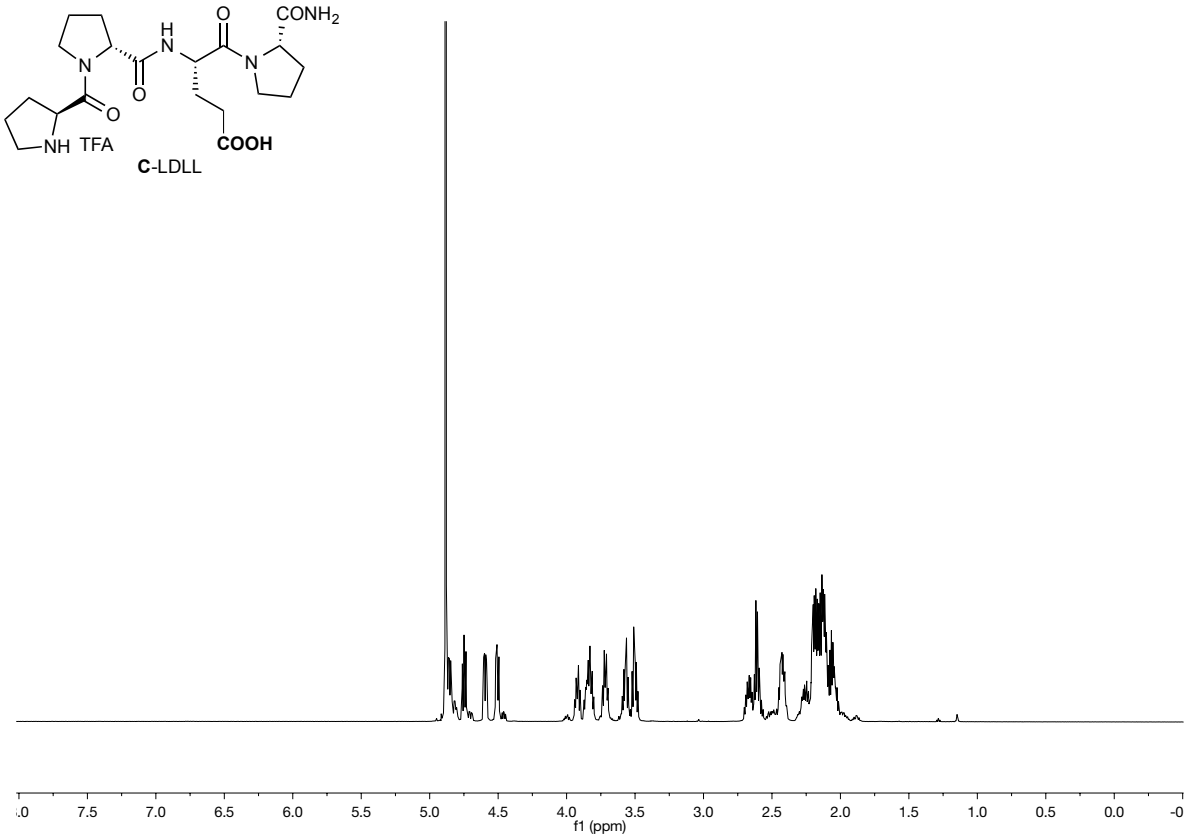
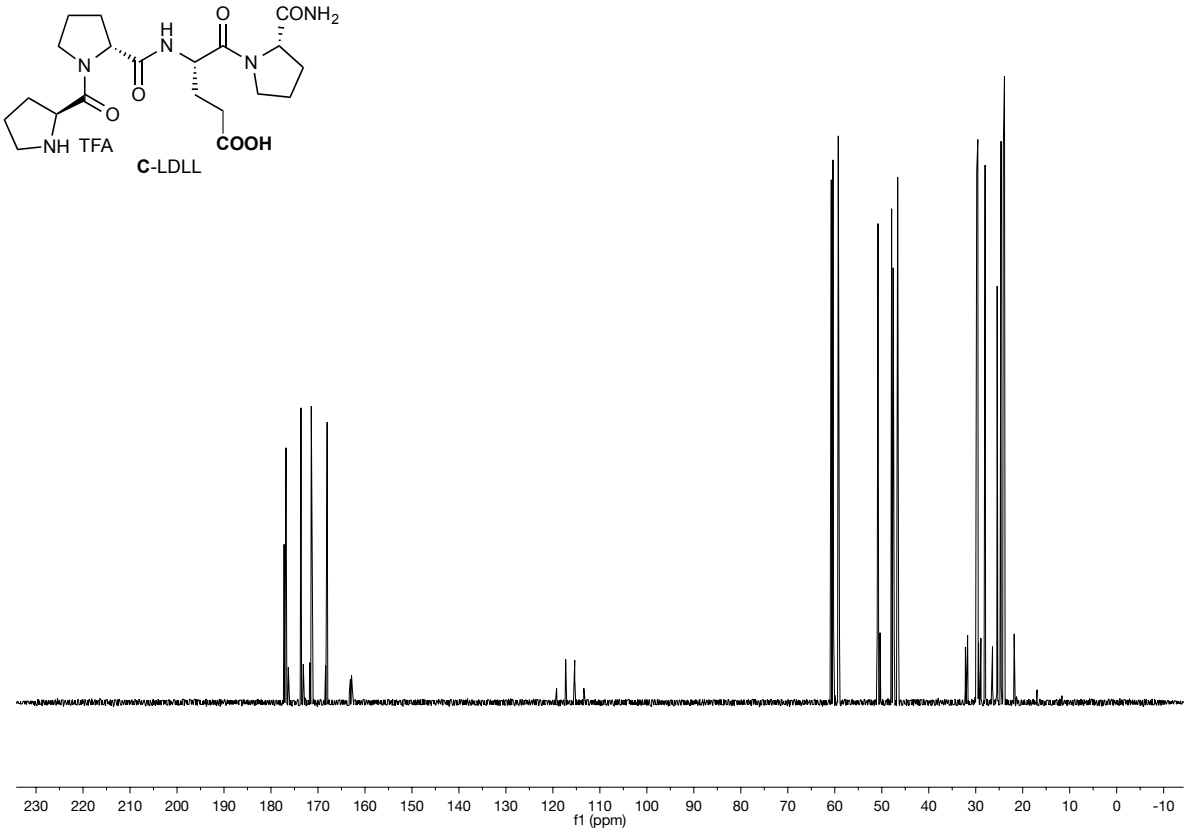
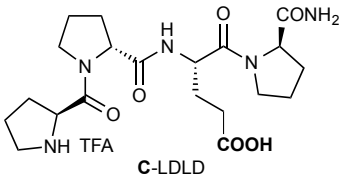
H
N
O
N
N
O
O
NH TFA
COOH
C-LDDL
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


CONH2
H
N
O
N
N
O
O
NH TFA
COOH
C-LDLL
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
f1 (ppm)


CONH2
H
N
O
N
N
O
O
NH TFA
COOH
C-LDLL
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)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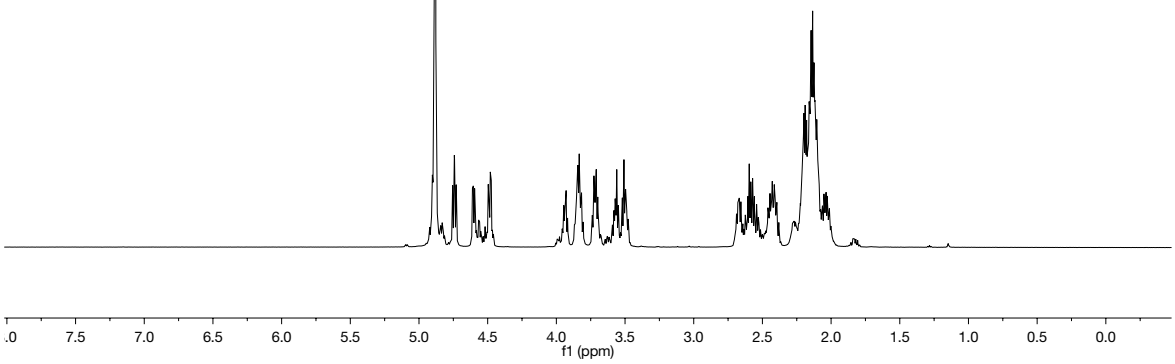
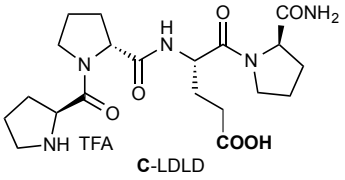
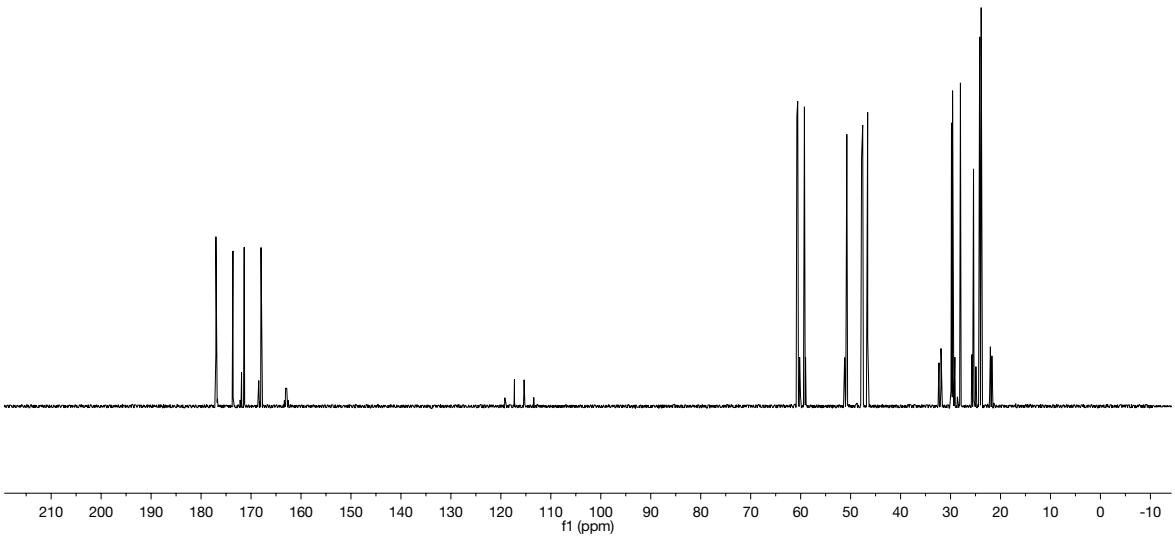
N
O
N
O
N
O
NH TFA
COOH

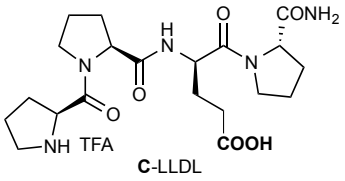
C-LDLD


.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
f1 (ppm)


CONH2
H
N
O
N
O
N
O
NH TFA
COOH

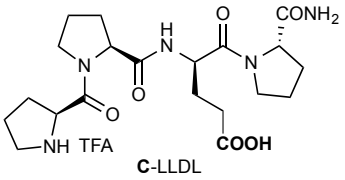
C-LDLD


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)


CONH2
H
N
O
N
N
O
O
NH TFA
COOH

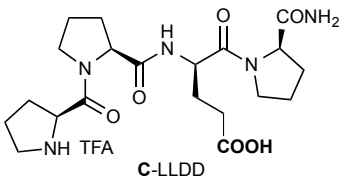
C-LLDL

.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.
f1 (ppm)


CONH2
H
N
O
N
N
O
O
NH TFA
COOH

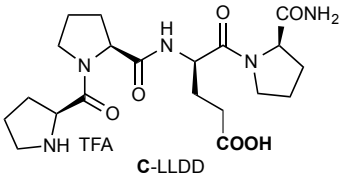
C-LLDL

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)

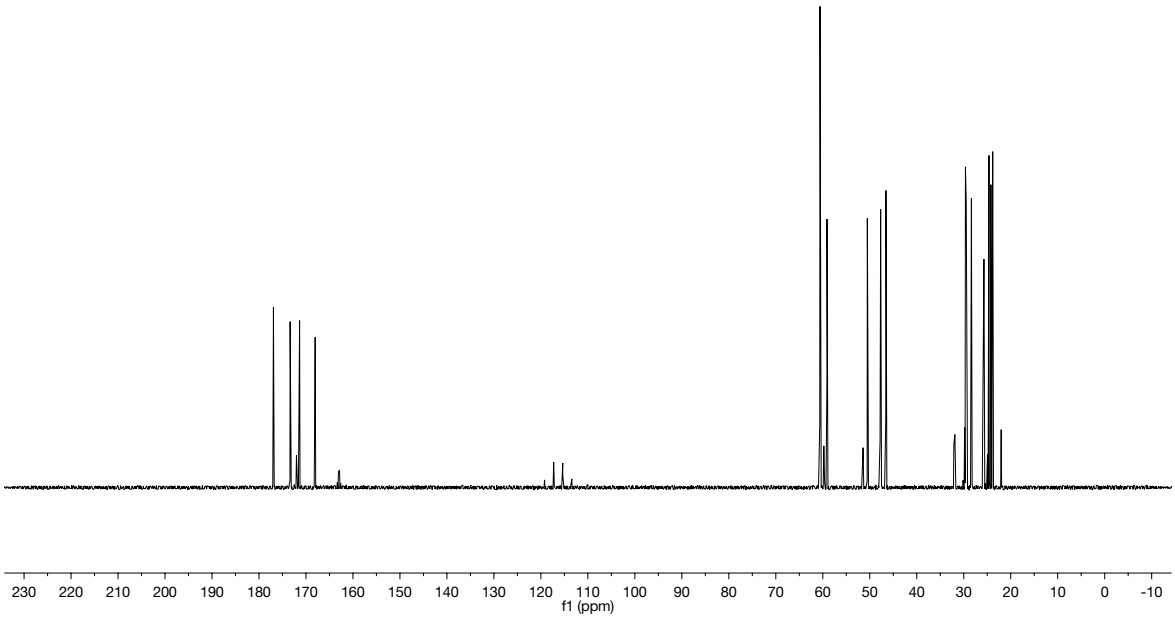

CONH2
O
H
N
N
N
O
O
NH TFA
COOH

C-LLDD

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
f1 (ppm)


CONH2
O
H
N
N
N
O
O
NH TFA
COOH

C-LLDD


230
220
210
200
190
180
170
160
150
140
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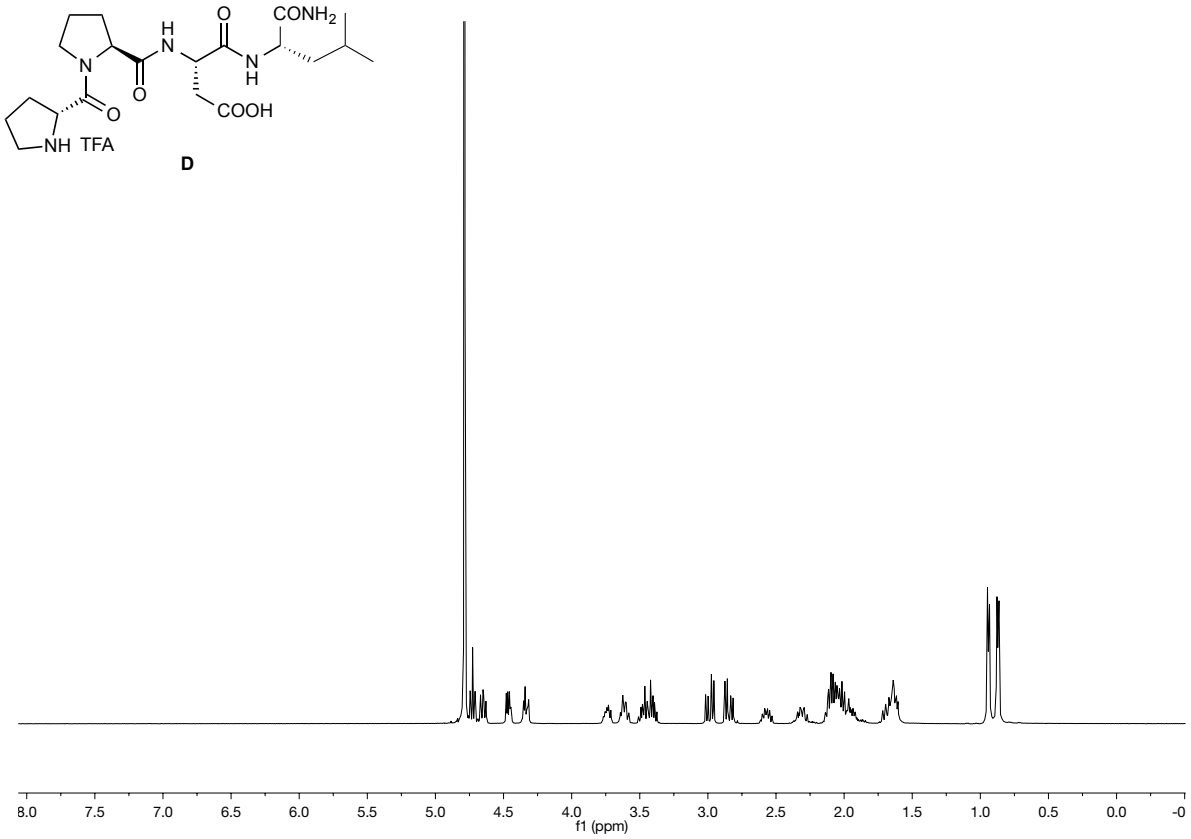
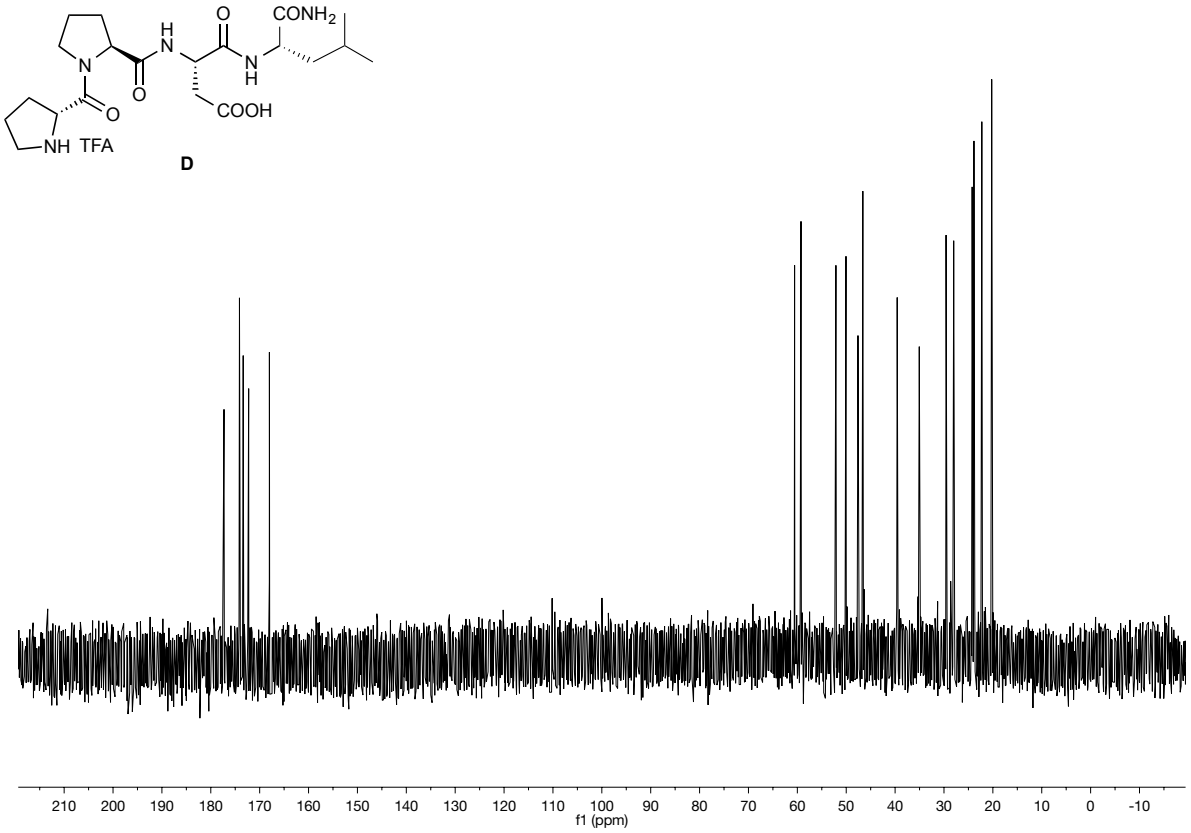
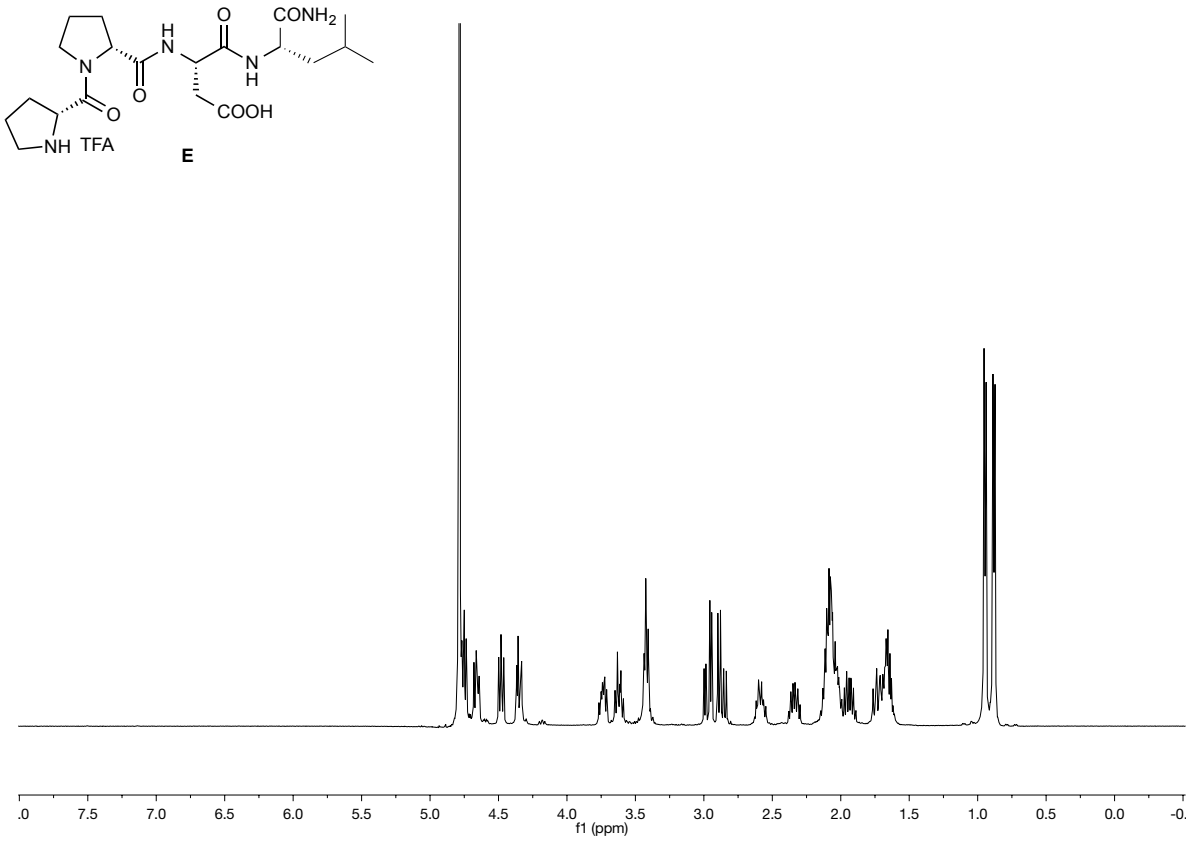
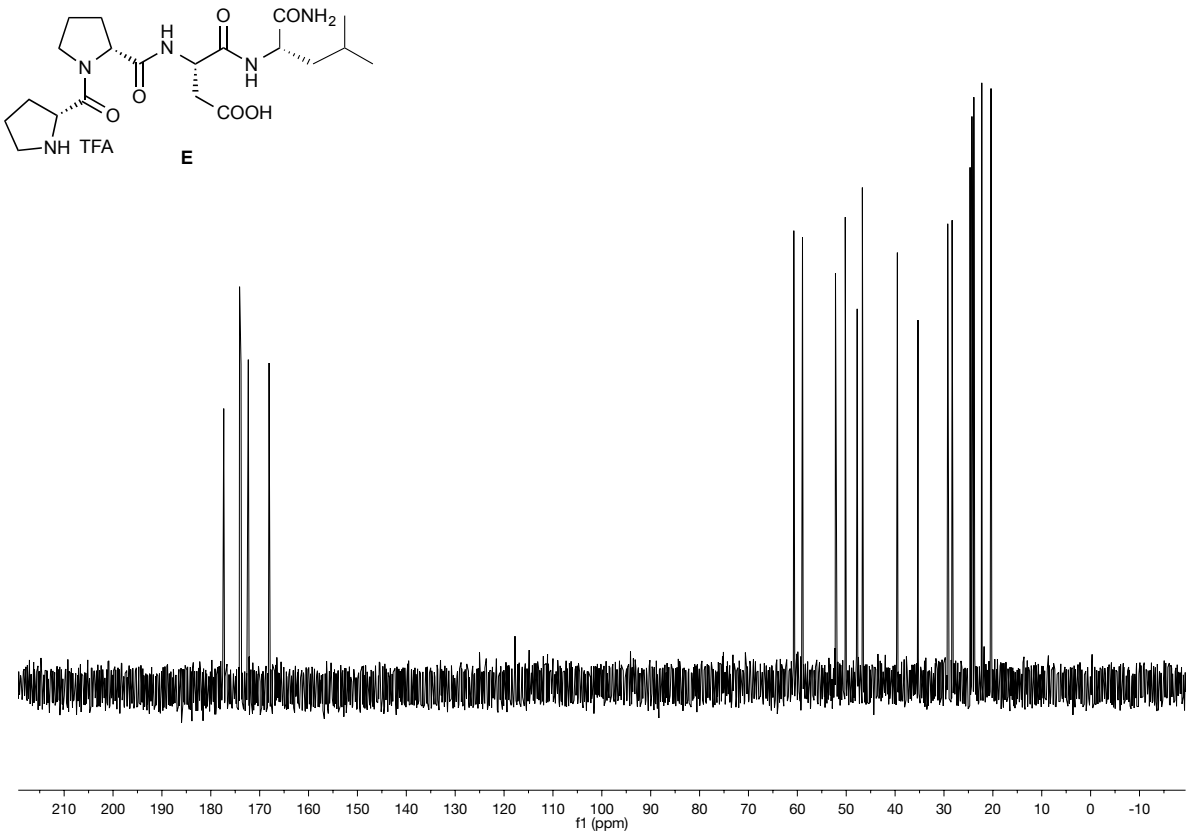
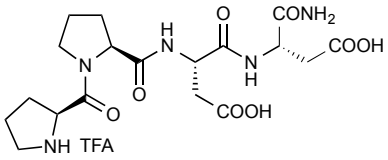
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


CONH2
H
N
O
N
H
N
O
O
COOH
NH TFA
D
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
f1 (ppm)


CONH2
H
N
O
N
H
N
O
O
COOH
NH TFA
D
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)

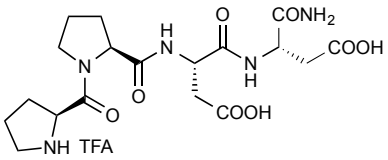

CONH2
H
N
O
N
H
N
O
O
COOH
NH TFA
E
.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.
f1 (ppm)


CONH2
H
N
O
N
H
N
O
O
COOH
NH TFA
E
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)

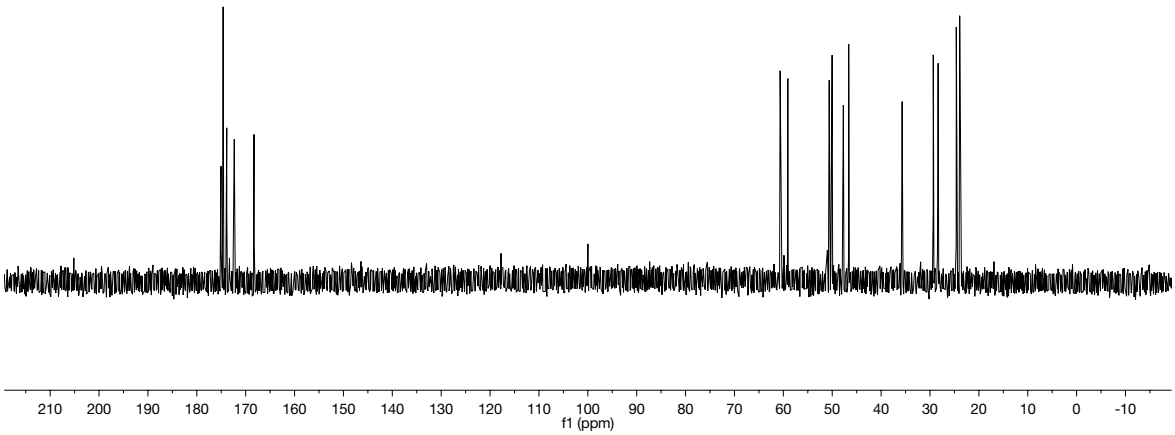

O
CONH2
H
N
N
H
COOH
N
O
O
COOH
O
NH TFA

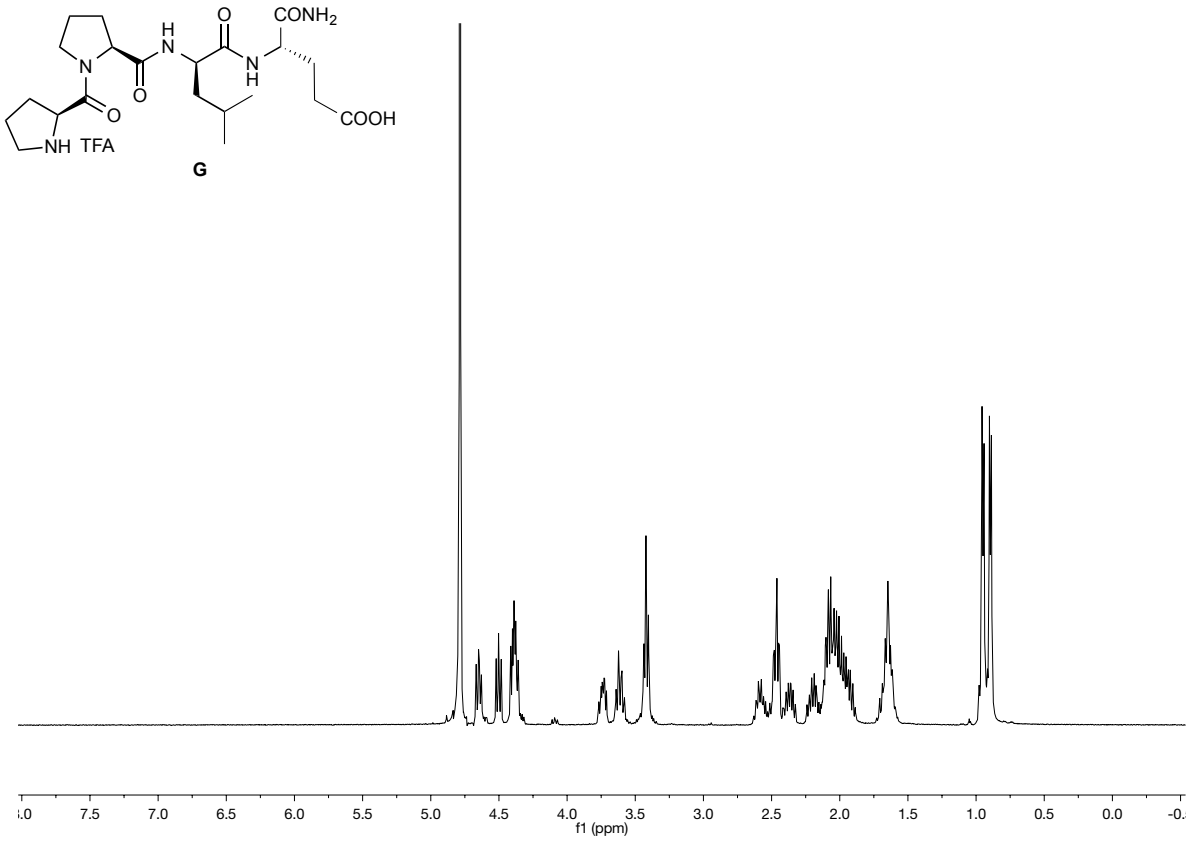
F

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
f1 (ppm)

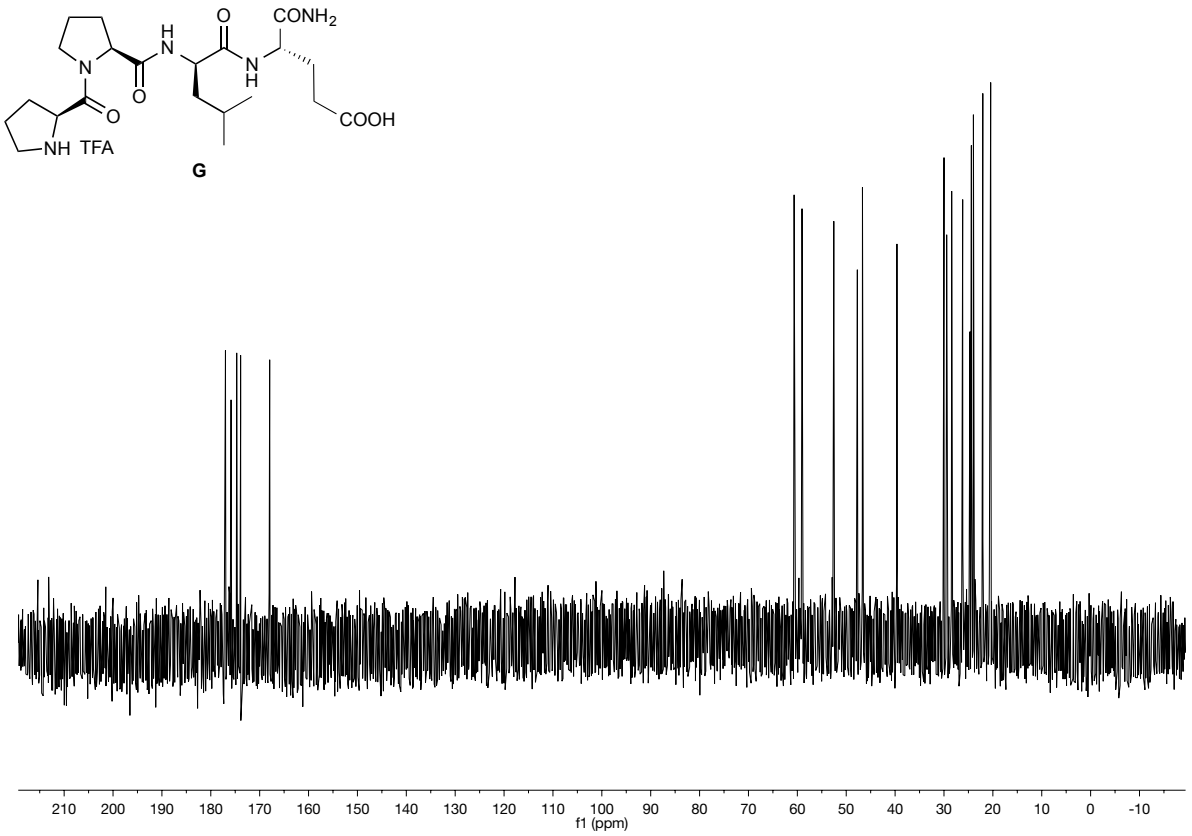

O
CONH2
H
N
N
H
COOH
N
O
O
COOH
O
NH TFA

F

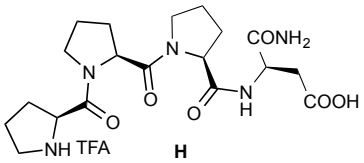

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)



G

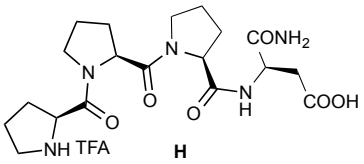


G

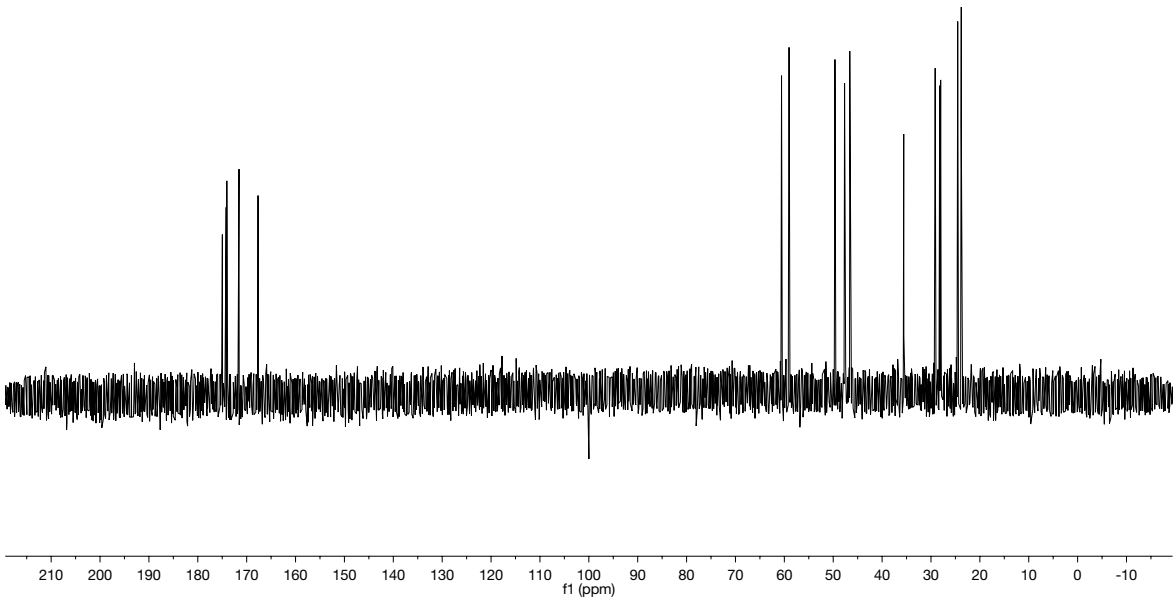

CONH2
N
N
O
O
N
H
O
COOH
NH TFA

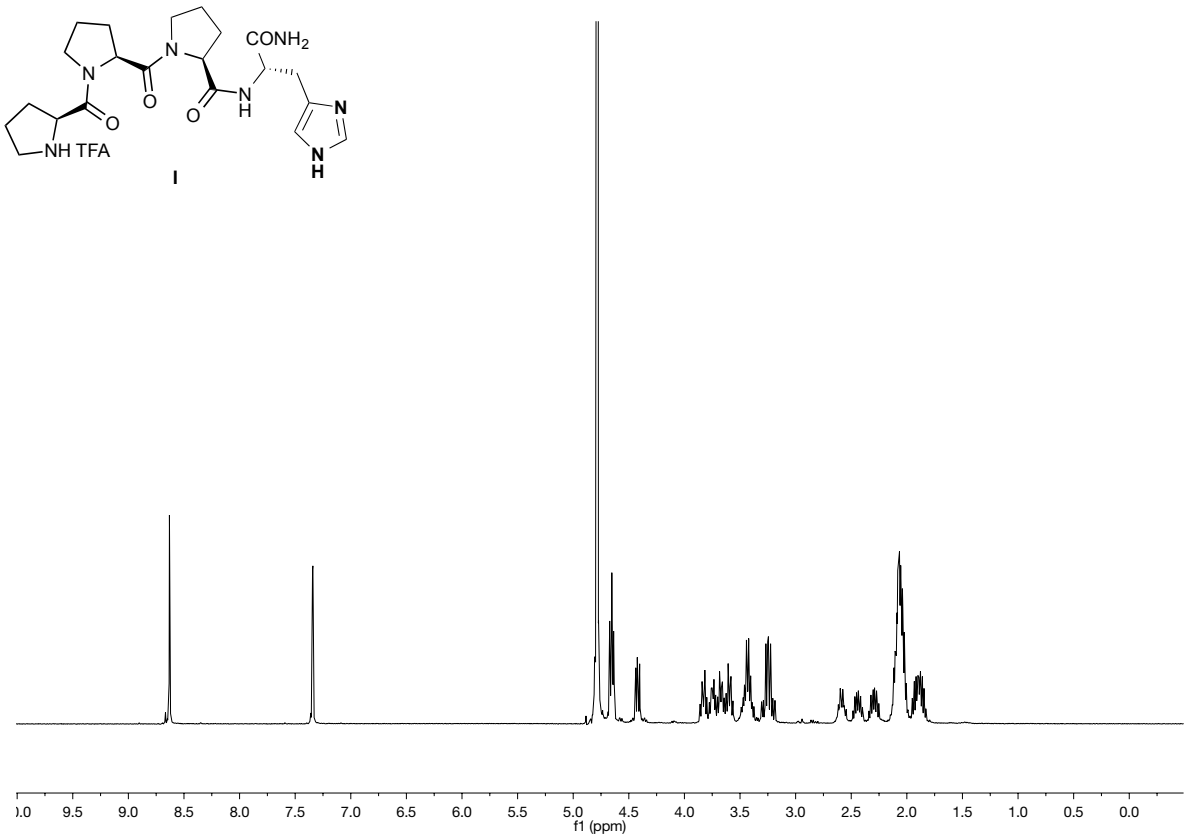
H

3.0
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
f1 (ppm)

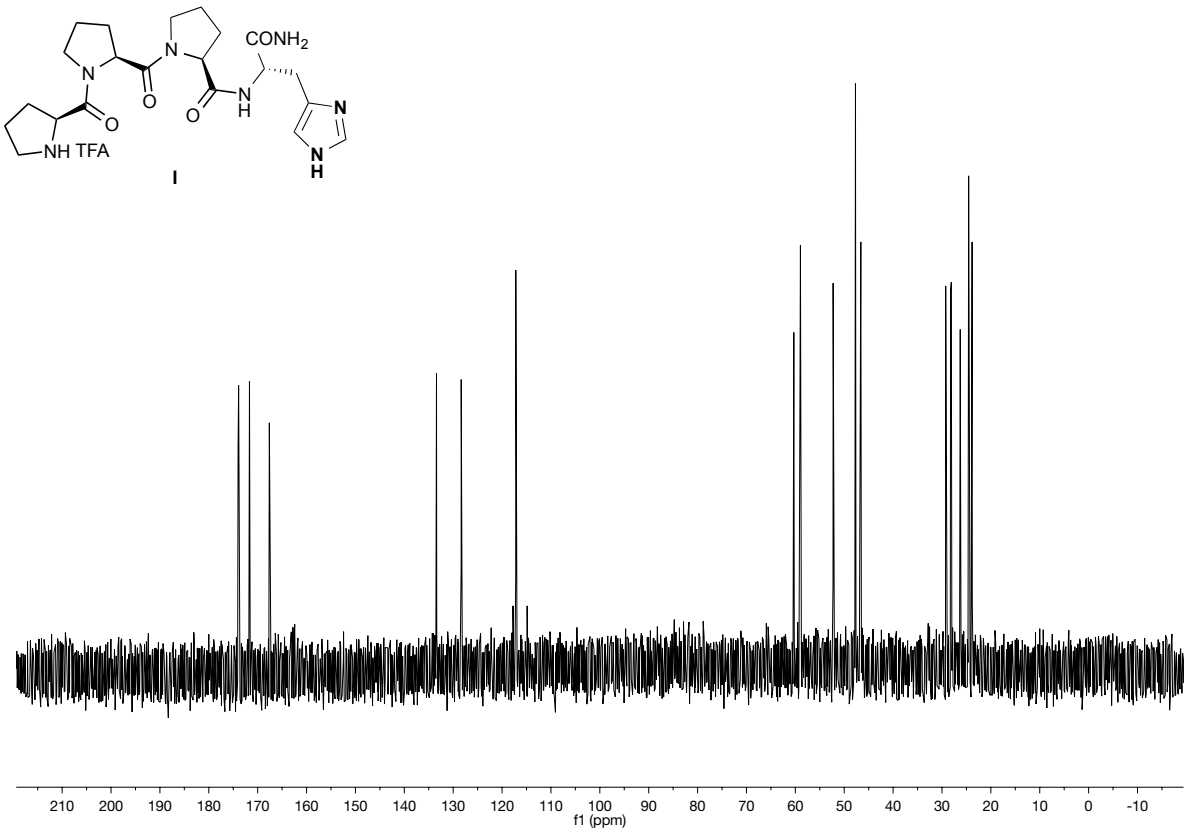

CONH2
N
N
O
O
N
H
O
COOH
NH TFA

H


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)



I



I

6. References

1. E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, *Anal. Biochem* **1970**, 34, 595.
2. T. Vojkovsky, *Peptide Res.* **1995**, 8, 236.
3. M. Wiesner, J. D. Revell, H. Wennemers, *Angew. Chem. Int. Ed.* **2008**, 47, 1871.
4. J. Duschmalé, H. Wennemers, *Chem. Eur. J.* **2012**, 18, 1111.
5. P. Krattiger, R. Kovasy, J. D. Revell, H. Wennemers, *QSAR Comb. Sci.* **2005**, 24, 1158.