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Supporting Information

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Supporting Information

for

Synthesis and Structure–Activity Correlation of a Brunsvicamide-Inspired Cyclopeptide Collection

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Contents

Phosphatase Inhibition Assays

MptpB inhibition assay (pNPP): *Mycobacterium tuberculosis* protein tyrosine phosphatase B was dissolved in 25 mM HEPES / 50 mM NaCl / Na_2*EDTA 2.5 mM / NP-40 0.025 % / DTE 2 nM / 1 % DMSO buffer at a concentration of 50 nM. Kinetic analysis at 37 °C using the substrate 4-nitrophenole phosphate at pH 7.2 and monitoring the increase of pNP (4-Nitrophenole) at 405 nm gave K_M and K_{cat} values of (2.3±0.3) mM and 5 s^{-1} (K_{cat}/K_M = 2300 s^{-1} M^{-1}).

MptpB inhibition assay (DiFUMP): *Mycobacterium tuberculosis* protein tyrosine phosphatase B was dissolved in 25 mM HEPES / 50 mM NaCl / Na_2*EDTA 2.5 mM / NP-40 0.025 % / DTE 2 nM / 1 % DMSO buffer at a concentration of 1.3 nM. Kinetic analysis at 37 °C using the substrate difluoromethylumbelliferyl phosphate at pH 7.2 and monitoring the increase of fluorescence at 455 nm (exitation at 358 nm) gave K_M and K_{cat} values of 38 (±2) µM and 70 s^{-1} (K_{cat}/K_M = 41000 s^{-1} M^{-1}).

General Synthetic Methods and Materials

General methods: ^1H and ^{13}C NMR spectra were recorded on Bruker DRX 500 (500 MHz (^1H) and 126 MHz (^{13}C)), Bruker DRX 400 (400 MHz (^1H) and 101 MHz (^{13}C)) and Varian Mercury 400 (400 MHz (^1H) and 101 MHz (^{13}C)) spectrometers. Chemical shifts are expressed in parts per million (ppm) and the spectra are calibrated to residual solvent signals of $CDCl_3$ (7.26 ppm (^1H) and 77.0 ppm (^{13}C)), DMSO (2.50 ppm (^1H) and 39.43 ppm (^{13}C)) and MeOD (3.31 ppm (^1H) and 49.15 ppm (^{13}C)), respectively. Coupling constants are given in Hertz.

High-resolution mass spectra were recorded on a Jeol SX 102 A (FAB; matrix nitrobenzylalcohol) or on a Thermo Electron LTQ Orbitrap (ESI; source voltage 3.8 kV) spectrometer. Fourier transform infrared spectroscopy (FTIR) spectra were obtained with a Bruker Tensor 27 spectrometer (ATR, neat). Wavenumbers $\tilde{\nu}$ are given in cm^{-1} and the peak intensity is described as w (weak), m (medium), s (strong). Optical

rotations were measured at 589 nm, concentrations c are given in g/100 mL solvent. Melting points were measured with a BÜCHI 540 melting point apparatus and are uncorrected.

Analytical HPLC/MS was carried out with a Hewlett Packard Series 1100/Finnigan LTQ (columns CC 125 Nucleodur C18 Gravity from Macherey-Nagel); detection: 210 and 254 nm: flow rate: 1 mL/min. The following standard gradient was used: (solvent A: water with 0.1 % HCOOH; solvent B: acetonitrile with 0.1 % HCOOH): 0 min: 10 % B; 1 min constant; 10 min: 100 % B; 2 min constant.

Preparative HPLC of the compounds was performed on a Agilent Series HPLC 1100 system with a LC/MSD VL (ESI-MS) mass detector, using a VP 125/21 Nucleodur C18 Gravity 5 µm column (Macherey-Nagel). Eluent: H_2O/MeOH, flow = 25 mL/min, isocratic: 35 % or 40 % H_2O; 25 min. For ESI mass detection 0.1 % of the solvent flow was diluted with 10 mM HCOOH in H_2O/acetonitrile 1:1.

For solid-phase synthesis IRORI MacroKans™, RFT-001 radio frequency tags and an IRORI AccuTag™ 100 system were used.

Materials: Thin layer chromatography (TLC) was carried out on Merck precoated silica gel plates (60F-254) using ultraviolet light irradiation at 254 nm or $KMnO_4$ solution for detection (1 g $KMnO_4$, 6.6 g K_2CO_3, 1.7 mL 5% NaOH solution, 100 mL H_2O). Silica gel chromatography was performed using silica gel from ACROS (particle size 35-70 µm) under approximately 0.5 bar pressure. 2-Chlorotrityl chloride polystyrene resin (copolymer, 1.45 mmol/g, 1 % DVB, 50–100 mesh) was purchased from CBL Patras.

All reactions utilizing dry solvents were performed under argon atmosphere. All solvents, when not purchased in suitable purity or dryness, were distilled using standard methods.[1] Deionized water was used for all experiments. All other reagents were purchased (Acros, Aldrich, Novabiochem, Fluka, IRIS, CBL, GLS) in standard qualities and used without further purification.

Preparation of Urea Building Blocks

***N*$^{\alpha}$-(4-Nitrophenyloxycarbonyl)-L-alanine-*tert*-butyl ester (39): 38** (1.0 g, 6.9 mmol) was placed under argon in a dried flask. Dry dichloromethane (10 mL), and dry pyridine (2.21 mL, 27.6 mmol, 4.0 eq.) were added and the mixture cooled to

[1] Armarego, W. L. F.; Chai, C. L. L. *Purification of Laboratory Chemicals*, 5th Ed, Elsevier, 2002.

0 °C. **37** (1.4 g, 6.9 mmol, 1.0 equiv) was placed under argon in a dried flask and dissolved in dry dichloromethane (30 mL), and dry pyridine (2.21 mL, 4.0 eq.) added. The suspension was added dropwise at 0 °C to the re action mixture. After 1 h the ice bath was removed. After 90 minutes at room temperature the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (100 mL), washed with 5 % $KHSO_4$ solution (3 × 50 mL), water (1 × 50 mL), brine (1 × 50 mL) and dried with $MgSO_4$. Column chromatography (dichloromethane / *n*-pentane 4:1, 150 g silica) yielded 1.09 g of the carbamate **39** as a colourless oil (3.5 mmol, 51 %). R_f = 0.15 (CH_2Cl_2:pentane 4:1, *v/v*); $[\alpha]_D^{20}$: -20 (c = 2.7, $CHCl_3$:MeOH 10:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.24 (d, 2 ArH, *J* = 9.1 Hz), 7.32 (m, 2 ArH), 5.75 (d, 1 H, *J* = 7.2 Hz), 4.31 (qn, 1 H, α-H, *J* = 7.2 Hz), 1.50 (s, 9 H, tBu), 1.47 (d, 3 H, β-H_3, *J* = 7.2 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.6, 155.6, 152.3, 144.8, 125.0, 121.9, 82.5, 50.4, 27.9, 18.6. IR: $\tilde{\nu}$ = 3306 (w), 2161 (w), 1735 (s), 1703 (s), 1617 (w), 1546 (m), 1527 (s), 1493 (m), 1456 (w), 1348 (s), 1260 (w), 1218 (s), 1147 (s), 1066 (w), 1004 (s), 934 (w), 867 (m), 841 (m), 761 (w), 727 (w), 664 (w) cm^{-1}. Anal. for $C_{14}H_{18}N_2O_6$ Calc. C 54.2, H 5.9; N, 9.0. Found: C 54.0, H 5.9, N 8.8.

***N*$^{\alpha}$-(4-Nitrophenyloxycarbonyl)-D-alanine-*tert*-butyl ester (39 B):** Colourless oil; Yield: 1.39 g, 4.5 mmol, 67 %; R_f = 0.46 (cyclohexane / ethyl acetate 2:1, v/v); $[\alpha]_D^{20}$: +22 (c = 3.0, $CHCl_3$:MeOH 10:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.24 (d, 2 ArH, *J* = 9.2 Hz), 7.32 (d, 2 ArH, *J* = 9.2 Hz), 5.75 (d, 1 H, *J* = 7.2 Hz), 4.31 (qn, 1 H, α-H, *J* = 7.2), 1.50 (s, 9 H, tBu), 1.47 (d, 3 H, β-H_3, *J* = 7.2 Hz).

D-Lysineallylester-*N*$^{\varepsilon}$-(9-fluorenylmethoxycarbonyl)-L-alanine-urea (40): *N*$^{\varepsilon}$-(9-fluorenylmethoxycarbonyl)-d-lysineallyl ester (1.6 g, 3.0 mmol) was placed under argon in a dried flask, dry CH_2Cl_2 (30 mL), and dry $EtN(iPr)_2$ (1.05 mL, 6.0 mmol, 2 equiv) were added and cooled to 0 °C. **39** (940 mg, 3.0 mmol, 1 equiv) was dissolved in dry dichloromethane (4 mL) and added dropwise at 0 °C to the reaction mixture. The ice bath was removed after 1 h. After 14 h (TLC control) the solvent was removed, the residue taken up in ethyl acetate (100 mL) and washed with 5 % $KHSO_4$ (3 × 250 mL). The organic layer was concentrated under reduced pressure, the resi-due dissolved in dichloromethane (100 mL), washed with $NaHCO_3$ (aq) (3 × 50 mL), H_2O (1 × 50 mL), brine (aq) (1 × 50 mL) and dried with $MgSO_4$. Column chro-

matography (cyclohexane/ethyl acetate 2:1, 150 g silica) yielded the *tert*-butyl ester as a colourless solid.

The material was dissolved in dichloromethane / trifluoroacetic acid (1:1 *v/v*, 15 mL). After 3 h (TLC control (cyclohexane / ethyl acetate, (2:1)) toluene (75 mL) was added and the volatiles were removed. Dichloromethane (2 x 75 mL) was added and removed. The material was dissolved in trifluoroacetic acid (3 mL), precipitated with water (150 mL) and collected by filtration. Lyophilisation yielded the urea **40** (1.17 g, 2.23 mmol, 74 %) as a colourless solid. R_f = 0.66 (CH_2Cl_2:MeOH 9:1); $[\alpha]_D^{20}$: +2.0 (*c* = 1.5, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$:MeOD (3:1)): δ = 7.57 (d, 2 ArH, *J* = 7.5 Hz), 7.42 (d, 2 ArH, *J* = 7.4 Hz), 7.20 (t, 2 ArH, *J* = 7.4 Hz), 7.12 (td, 2 ArH, *J* = 1.1, 7.4 Hz), 5.76-5.65 (m, 1 H, H_2C=CH), 5.12 (dd, 1 H, H_{trans}HC=CH, *J* = 1.4, 17.2 Hz), 5.03 (dd, 1 H, H_{cis}HC=CH, *J* = 1.0, 10.4 Hz), 4.46-4.36 (m, 2 H, H_2-Allyl), 4.23-4.1 (m, 4 H, Ala-α-H, D-Lys-α-H, CH_2-Fmoc), 4.01 (t, 1 H, *J* = 6.9, CH-Fmoc), 2.99-2.87 (m, 2 H, D-Lys-ε-H_2), 1.68-1.55 (m, 1 H, D-Lys-β-H_2), 1.53-1.41 (m, 1 H, D-Lys-β-H_2), 1.38-1.25 (m, 2 H, D-Lys-δ-H_2), 1.25-1.11 (m, 2 H, D-Lys-γ-H_2), 1.18 (d, 3 H, Ala-β-H_3, *J* = 7.2 Hz); ^{13}C NMR (101 MHz, $CDCl_3$:MeOD (3:1)): δ = 175.4, 172.9, 157.7, 156.9, 143.6, 140.9, 131.3, 127.3, 126.7, 124.6, 119.5, 118.1, 66.2, 65.4, 52.4, 48.2, 46.9, 40.1, 31.9, 28.8, 22.2, 18.1. IR: $\tilde{\nu}$ = 3316 (w), 2950 (w), 2161 (w), 2030 (w), 1733 (m), 1691 (s), 1633 (m), 1537 (s), 1447 (w), 1373 (w), 1333 (w), 1261 (s), 1230 (m), 1182 (m), 1135 (m), 1081 (w), 990 (w), 934 (w), 776 (w), 758 (m), 732 (m) cm^{-1}; M.p.: 152 °C; HR-MS (FAB): $C_{28}H_{33}N_3O_7$ $[M+H]^+$ calc: 524.2391, found: 524.2386.

D-Lysineallylester-*N*$^{\varepsilon}$-(9-fluorenylmethoxycarbonyl)-D-alanine-urea (40 B): Colourless powder; Yield: 1.17 g, 2.23 mmol, 74 %; R_f = 0.66 (CH_2Cl_2:MeOH 9:1); $[\alpha]_D^{20}$: +2.0 (c = 1.5, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$:MeOD (3:1)): δ = 7.57 (d, 2 ArH, *J* = 7.5 Hz), 7.42 (d, 2 ArH, *J* = 7.4 Hz), 7.20 (t, 2 ArH, *J* = 7.4 Hz), 7.12 (td, 2 ArH, *J* = 1.1, 7.4 Hz), 5.76-5.65 (m, 1 H, H_2C=C*H*), 5.12 (dd, 1 H, H_{trans}HC=CH, *J* = 1.4, 17.2 Hz), 5.03 (dd, 1 H, H_{cis}HC=CH, *J* = 1.0, 10.4 Hz), 4.46-4.36 (m, 2 H, H_2-allyl), 4.23-4.1 (m, 4 H, Ala-α-H, D-Lys-α-H, CH_2-Fmoc), 4.01 (t, 1 H, *J* = 6.9, CH-Fmoc), 2.99-2.87 (m, 2 H, D-Lys-ε-H_2), 1.68-1.55 (m, 1 H, D-Lys-β-H_2), 1.53-1.41 (m, 1 H, D-Lys-β-H_2), 1.38-1.25 (m, 2 H, D-Lys-δ-H_2), 1.25-1.11 (m, 2 H, D-Lys-γ-H_2), 1.18 (d, 3 H, Ala-β-H_3, *J* = 7.2 Hz); ^{13}C NMR (101 MHz, $CDCl_3$:MeOD (3:1)): δ = 175.4, 172.9, 157.7, 156.9, 143.6, 140.9, 131.3, 127.3, 126.7, 124.6, 119.5, 118.1, 66.2, 65.4, 52.4, 48.2, 46.9, 40.1, 31.9, 28.8, 22.2, 18.1; IR: $\tilde{\nu}$ = 3316 (w), 2950 (w), 2161 (w), 2030

(w), 1733 (m), 1691 (s), 1633 (m), 1537 (s), 1447 (w), 1373 (w), 1333 (w), 1261 (s), 1230 (m), 1182 (m), 1135 (m), 1081 (w), 990 (w), 934 (w), 776 (w), 758 (m), 732 (m) cm^{-1}; M.p.: 152 °C; HR-MS (FAB): $C_{28}H_{33}N_3O_7$ $[M+H]^+$ calc: 524.2391, found: 524.2386.

Solid-Phase Peptide Synthesis

Loading of 2-chlorotrityl chloride resin: Dry dichloromethane, the urea acid (1.2 equiv, 30 mM) and $EtN(iPr)_2$ (4.8 eq) were placed under argon in a dried flask. The IRORI cans containing the resin (250 mg) were added, covered with additional DCM, and agitated for 16 h at RT. MeOH (10 equiv) was added for 30 min. The solution was removed and the resin covered with dry CH_2Cl_2/MeOH/$EtN(iPr)_2$ (17:2:1, *v/v*) for 30 min. The resin was drained, and washed with dichloromethane (3 x 5 min) and diethyl ether (2 x 5 min). The resin was dried in vacuo over night and the loading determined by analytical Fmoc cleavage.[2]

Coupling of Fmoc-protected amino acids and Fmoc deprotection: The Fmoc-protected amino acid and coupling reagents were dissolved in DMF under argon in a dried flask. The IRORI cans containing the resin were added and covered with additional DMF. After 2 h shaking at RT the solution was removed and the resin washed with DMF (3 x 5 min). Before sorting or test cleavages, additional washing with dichloromethane (3 x 5 min) and diethyl ether (2 x 5 min) was performed. General coupling conditions: Fmoc amino acid (3 equiv, 0.15 M), DIC (3 equiv), and HOBt (3 equiv). Couplings to *N*-methylated amino acids: Fmoc-amino acid (3 equiv, 0.15 M), HATU (3 equiv), HOAt (3 equiv), $EtN(iPr)_2$ (6 equiv). For Fmoc-deprotection the resin was treated with DMF / piperidine (4:1, *v/v*; 15 min), followed by washing with DMF (3 x 5 min).

Allyl ester cleavage: Sodium benzenesulfinate (10 eq) was dissolved in DMF under argon in a dried flask. The IRORI cans containing the dried resin were added and covered with dry DMF. The suspension was degassed and tetrakis triphenylphosphino palladium (0.05 equiv, 2.5 mM) was added. The mixture was shaken for 16 h at RT with protection against light, the liquid was removed and the resin washed with DMF (3 x 5 min), 0.5 % DIPEA, and 0.5 % sodium diethyldithiocarbamate trihydrate in DMF (3 x 5 min), and DMF (3 x 5 min).

[2] I. Coin, M. Beyermann, M. Bienert, *Nat. Protoc.* **2007**, *2*, 3247–3256.

Ring closure: HOBt (6 eq) was dissolved in DMF under argon in a dried flask. The IRORI cans containing the resin were added and covered with additional DMF and DIC (6 equiv, 0.3 M). After 16 h shaking at room temperature the liquid was removed and the resin washed with DMF (3 x 5 min), dichloromethane (3 x 5 min) and diethyl ether (2 x 5 min).

Release: The resin was covered with dichlormethane:trifluoroacetic acid (98:2 *v/v*), shaken at RT (2 x 30 min, 3 x 5 min), and drained.

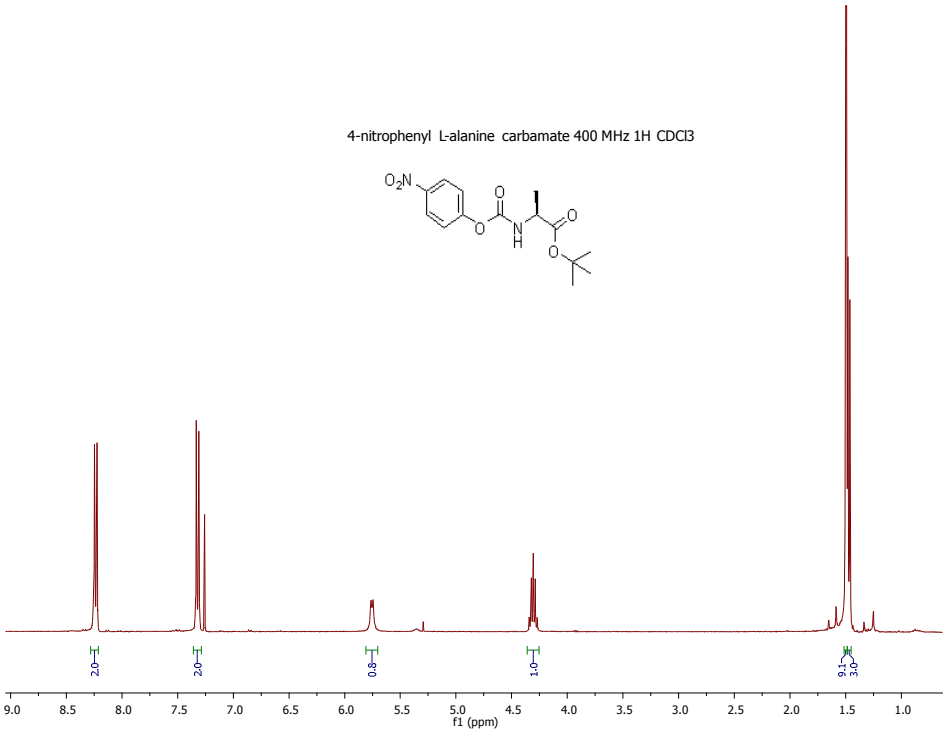
Purification: The cleavage product was dissolved in trifluoroacetic acid (2 mL), precipitated with water (100 mL) and collected by filtration. Silica gel chromatography with (dichloromethane/methanol 9:1 +0.25% formic acid, 10 g silica) was followed by HPLC purification (C18, MeOH/H_2O (65:35)).

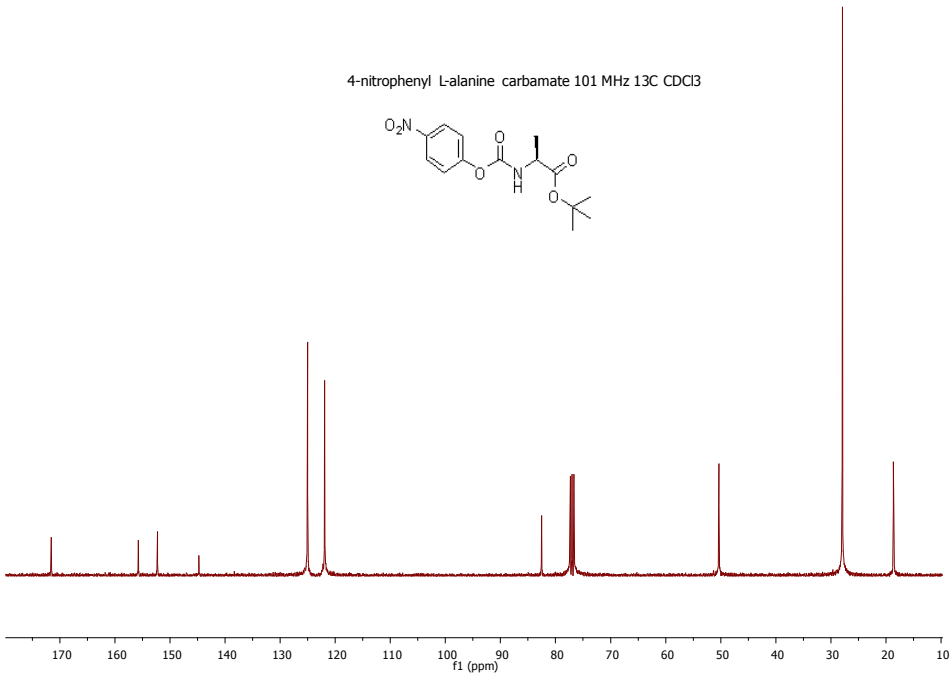
Purification and deprotection of compounds containing serine residues: After silica gel chromatography of the cleavage product (dichloromethane / methanol 9:1 +0.25% formic acid, 10 g silica) the deprotection of the *tert*-butyl protected serine (92.5 % TFA, 5 % TES, 2.5 % H_2O, RT, 1 h) was performed. Toluene (50 mL) was added and all volatiles were removed in vacuo. HPLC purification (C18, MeOH/H_2O (60:40)) gave the pure products.

Yields: All yields of solid phase peptide syntheses were calculated with respect to the amount of resin-loaded first building block.

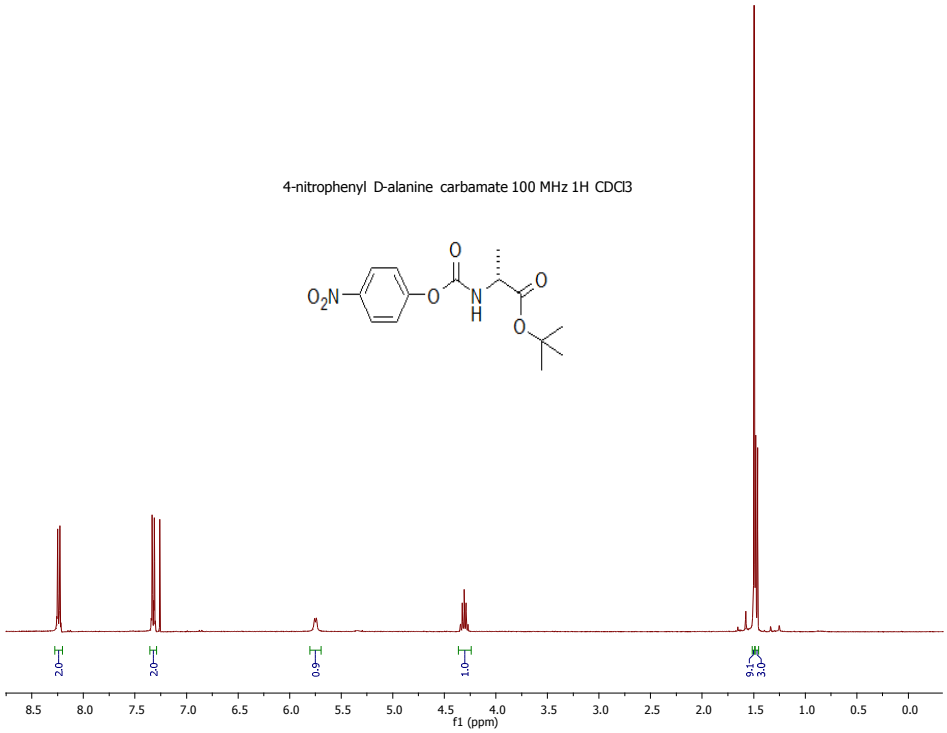
NMR Spectra and HPLC Traces

N^{α}-(4-Nitrophenyloxycarbonyl)-L-alanine-tert-butylester (39):

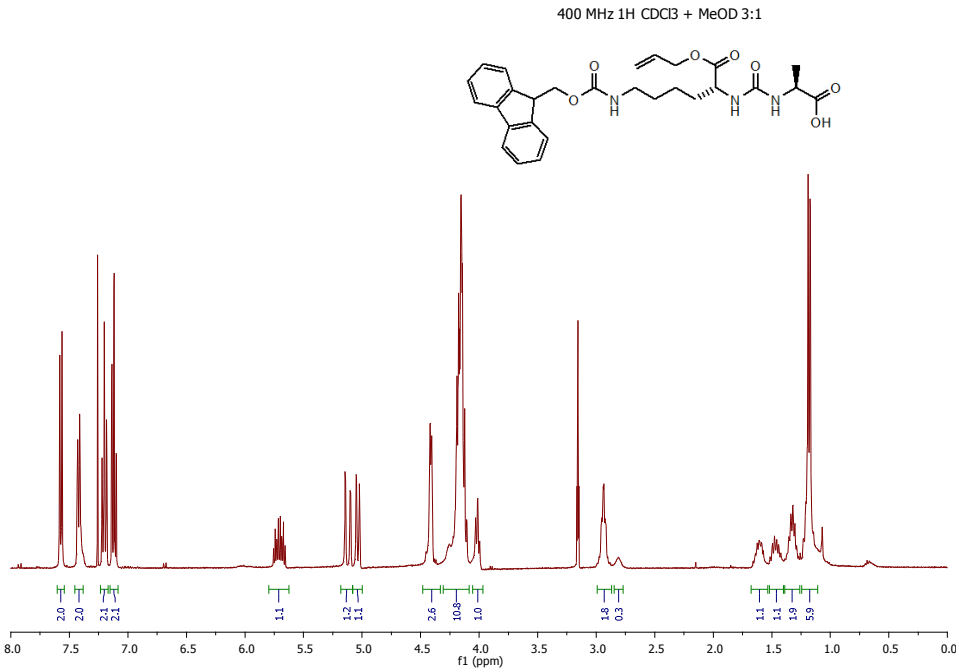


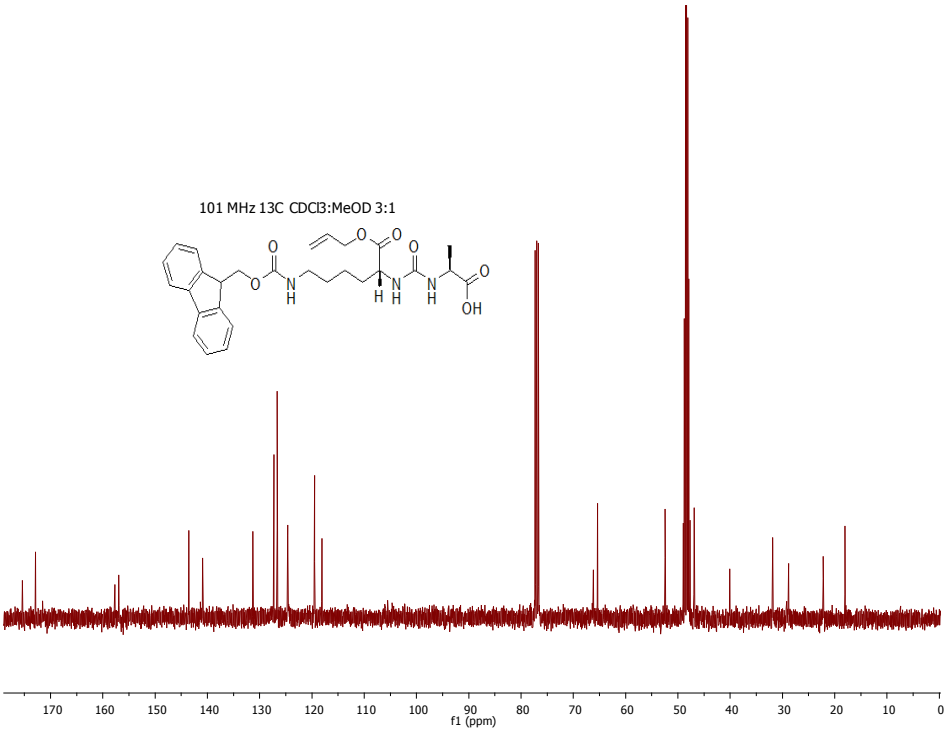


Nα-(4-Nitrophenyloxycarbonyl)-D-alanine-tert-butylester (39 B):



D-Lysineallylester-N^{ε}-(9-fluorenylmethoxycarbonyl)-L-alanine-urea (40):

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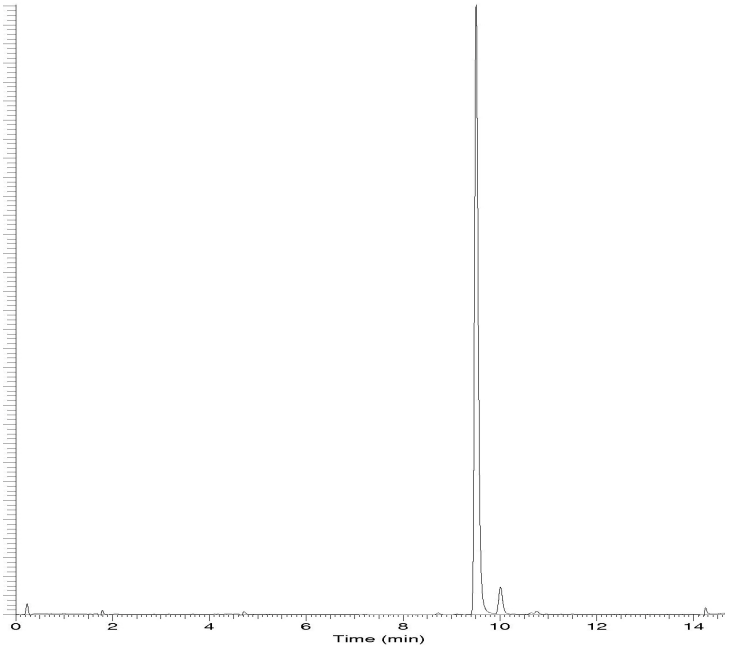
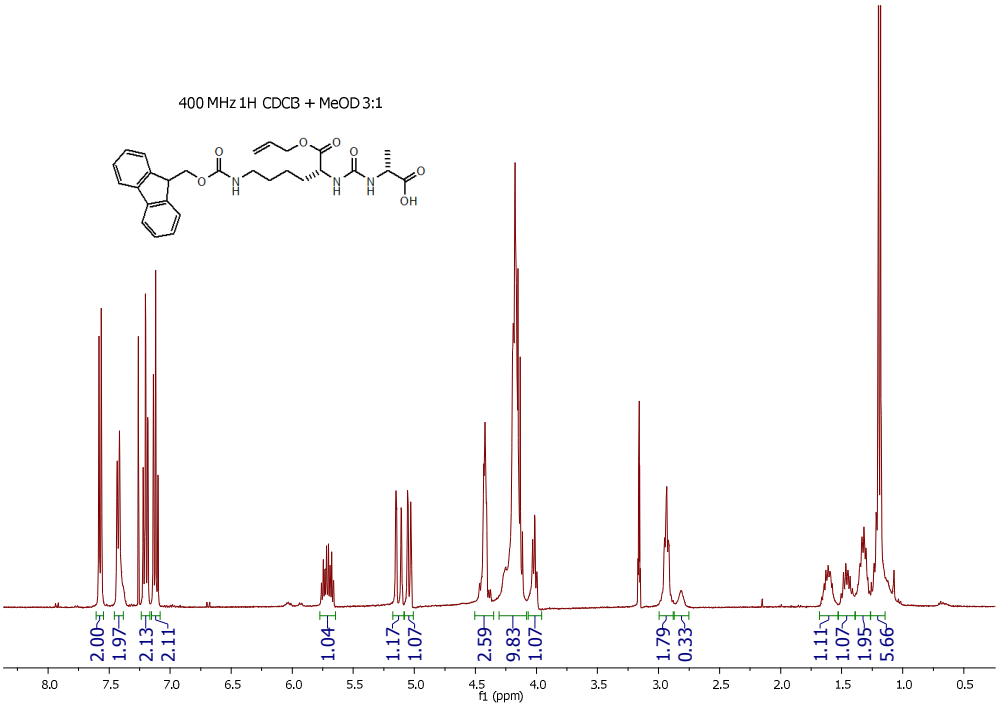
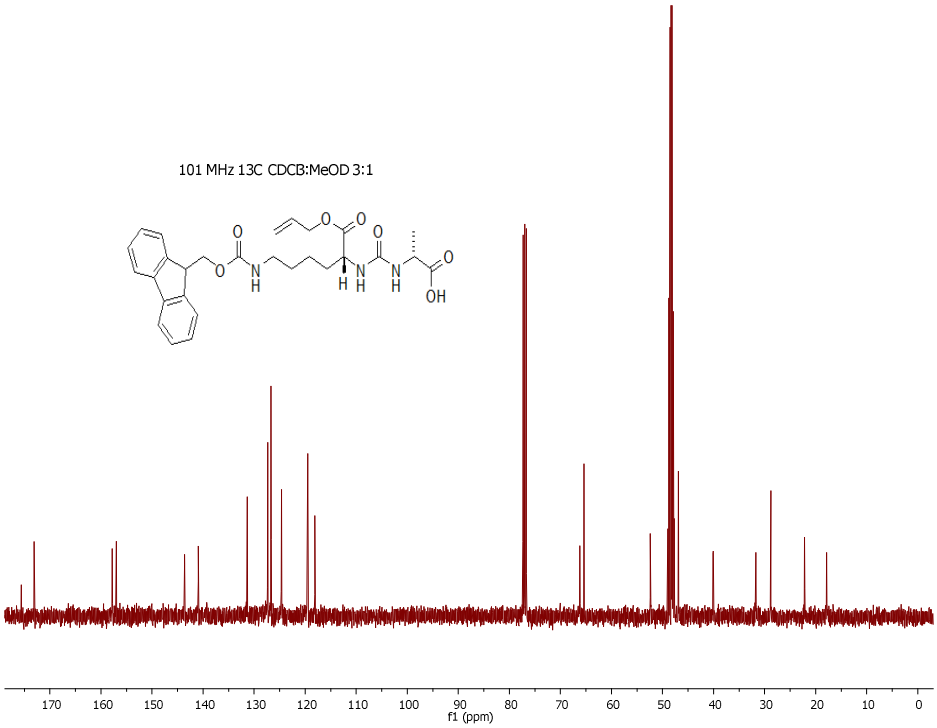
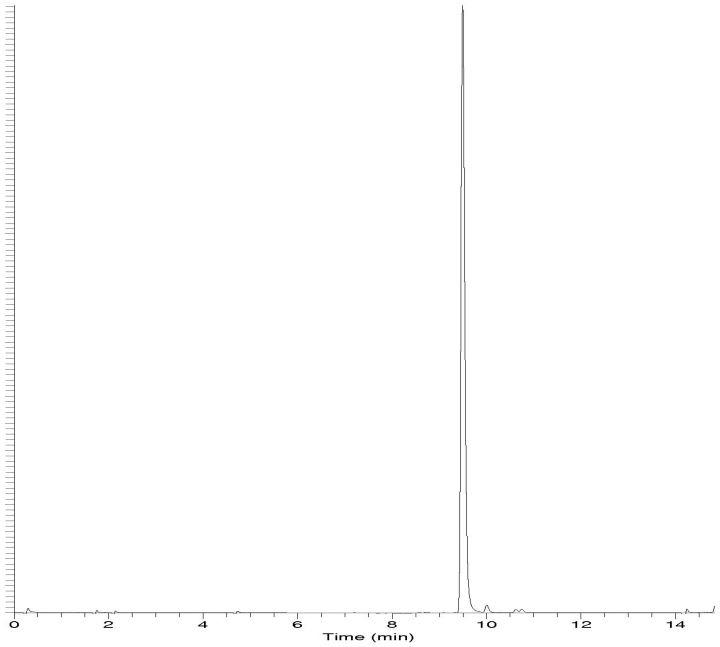
f1 (ppm)


0 2 4 6 8 10 12 14
Time (min)

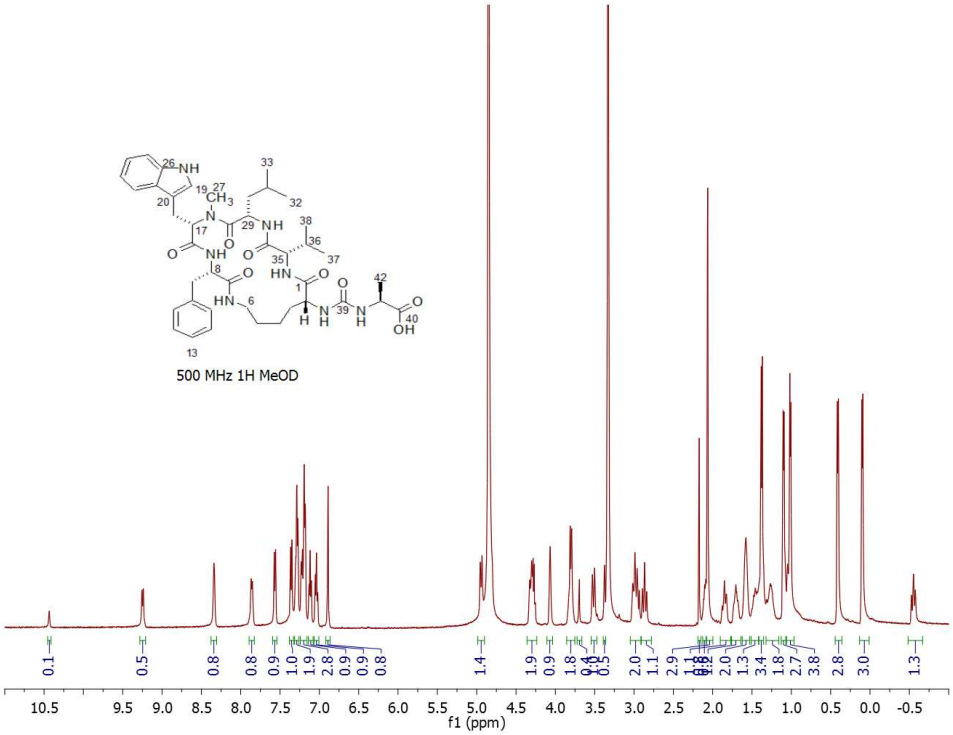
D-Lysineallylester-N$^{\varepsilon}$-(9-fluorenylmethoxycarbonyl)- D -alanine-urea (40 B):

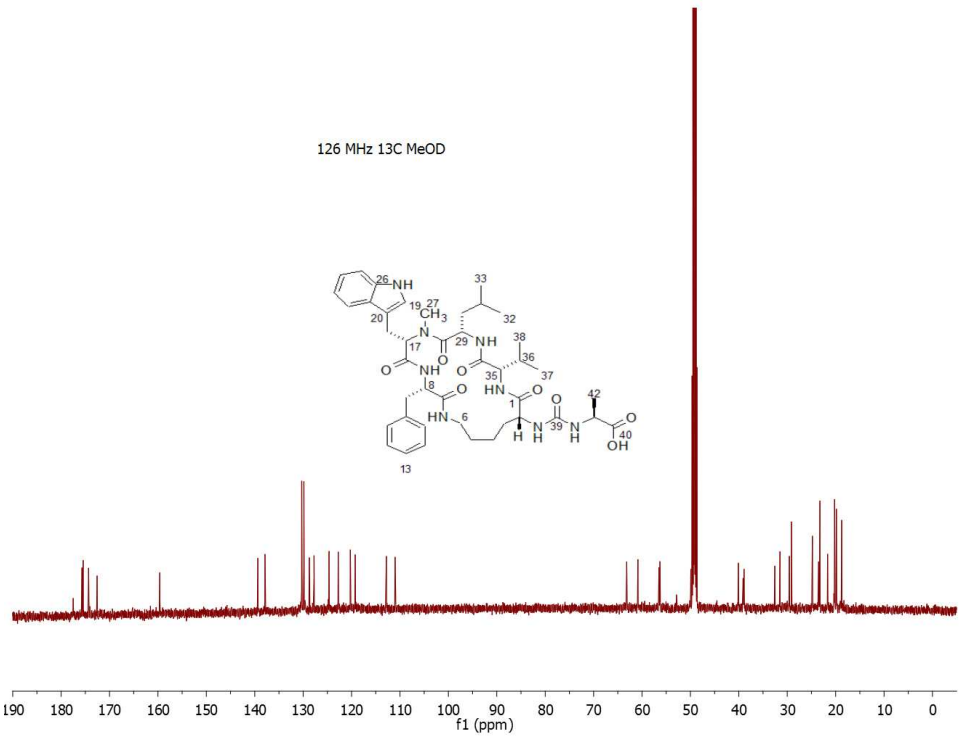






1,5-anhydro(D-lysyl-(N^{α}-oxamido-L-alanyl)-L-valinyl-L-leucyl-L-*N*-methyl-tryptophyl-L-phenylalanine) (54):

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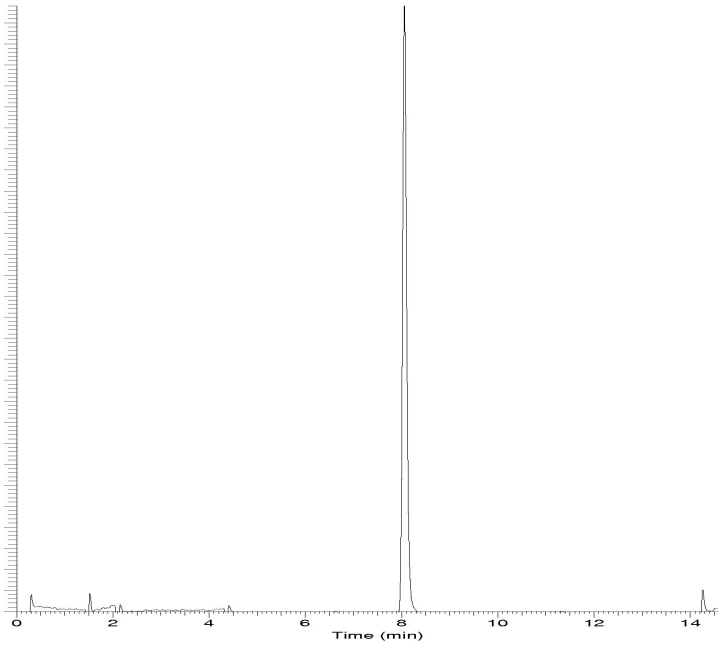
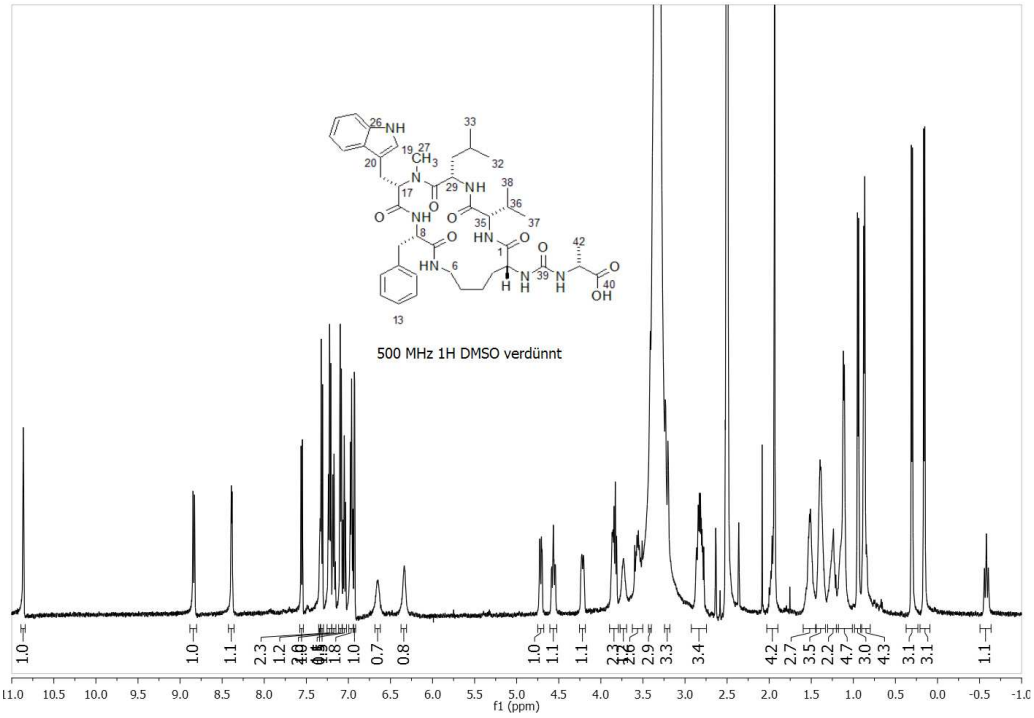
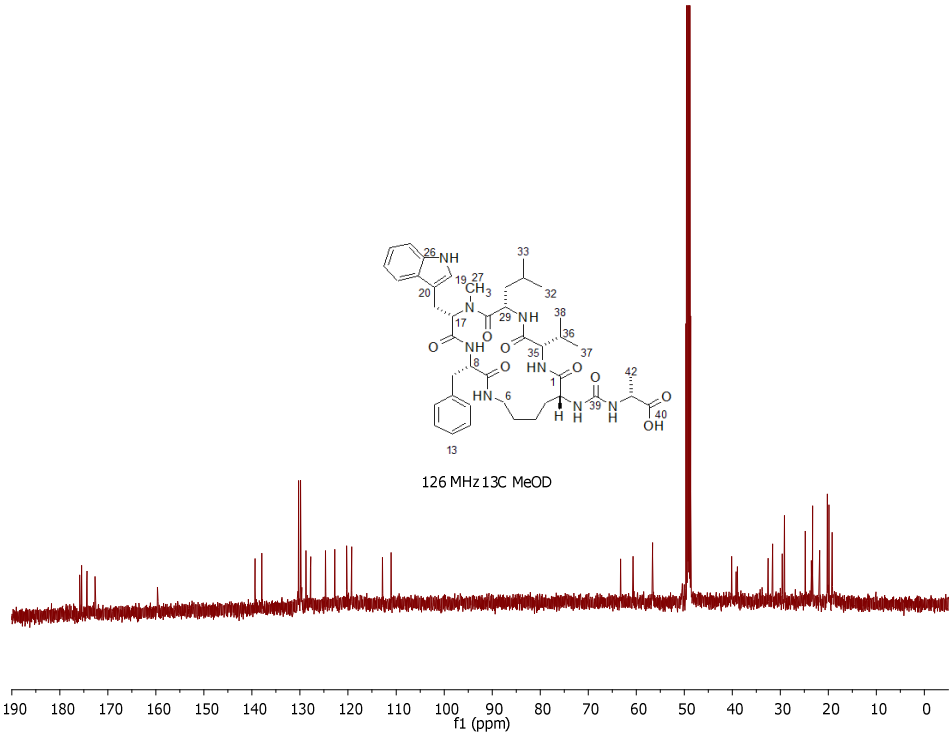
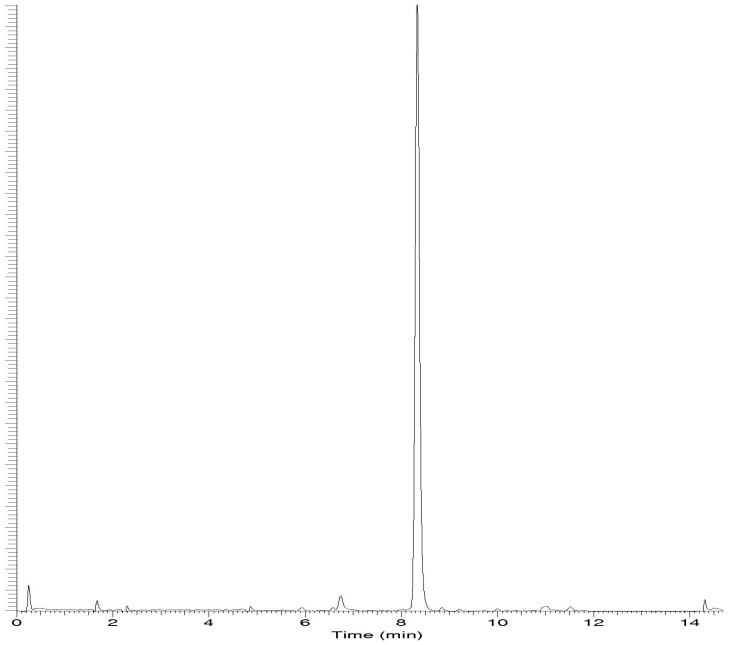
f1 (ppm)


0 2 4 6 8 10 12 14
Time (min)

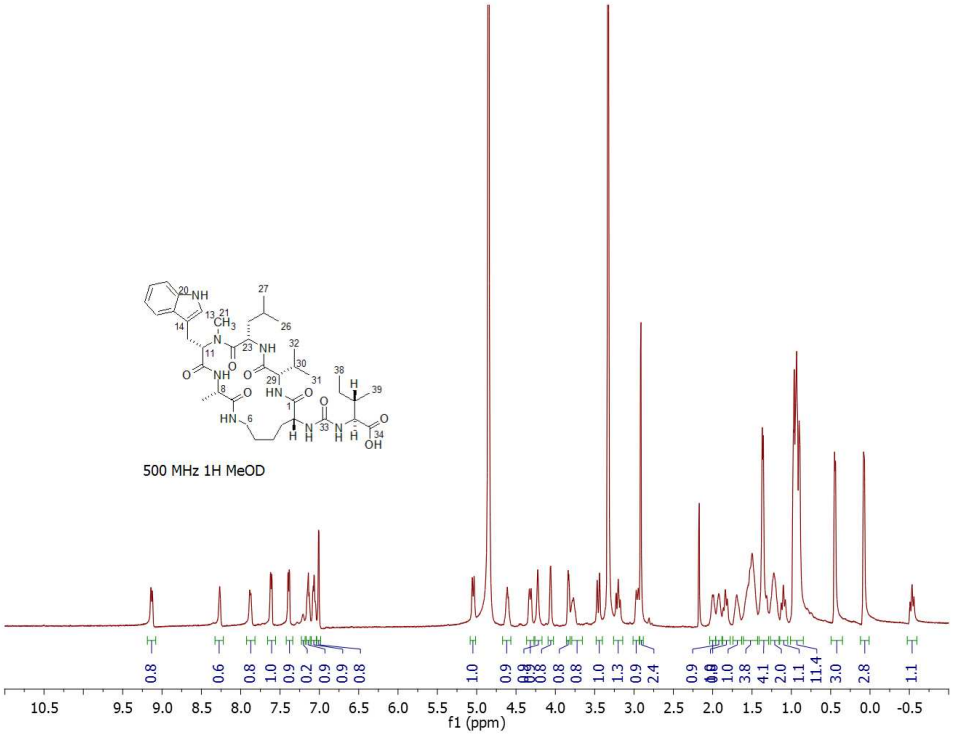
1,5-anhydro(D-lysyl-(N^{α}-oxamido-D-alanyl)-L-valinyl-L-leucyl-L-*N*-methyl-tryptophyl-L-phenylalanine) (55):

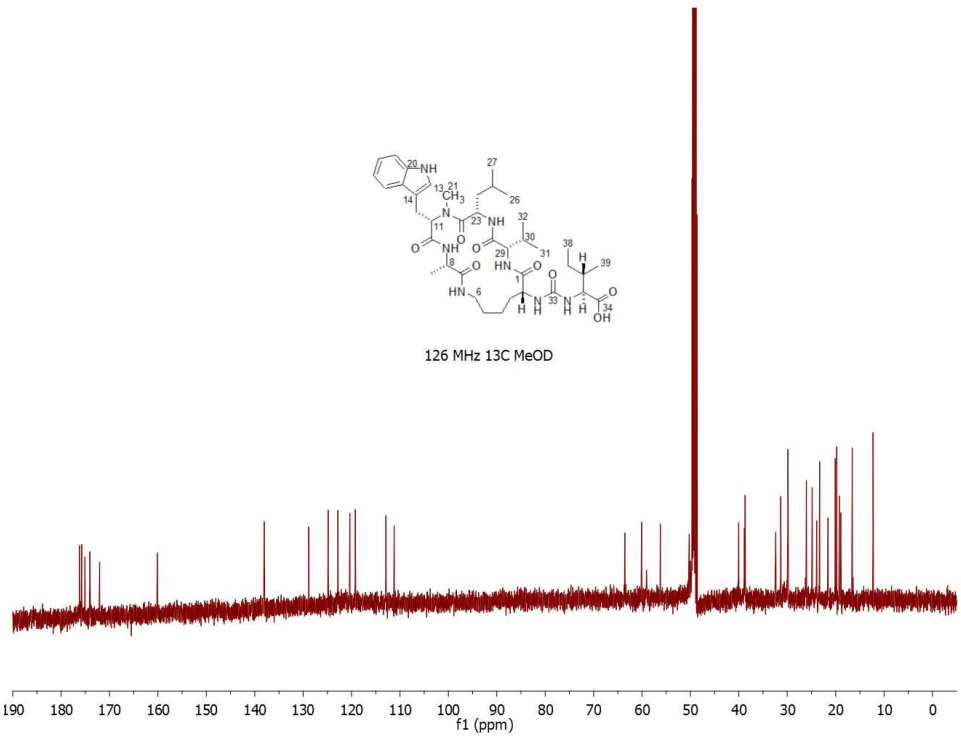


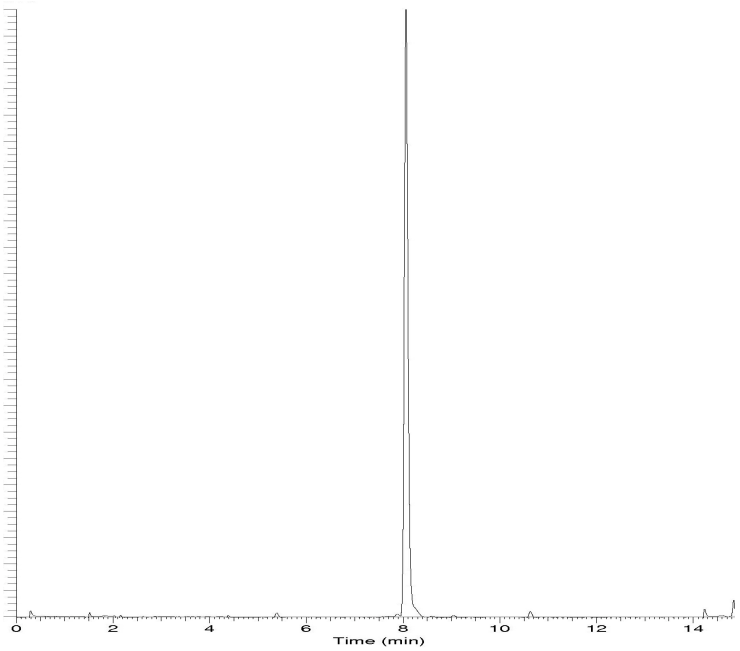




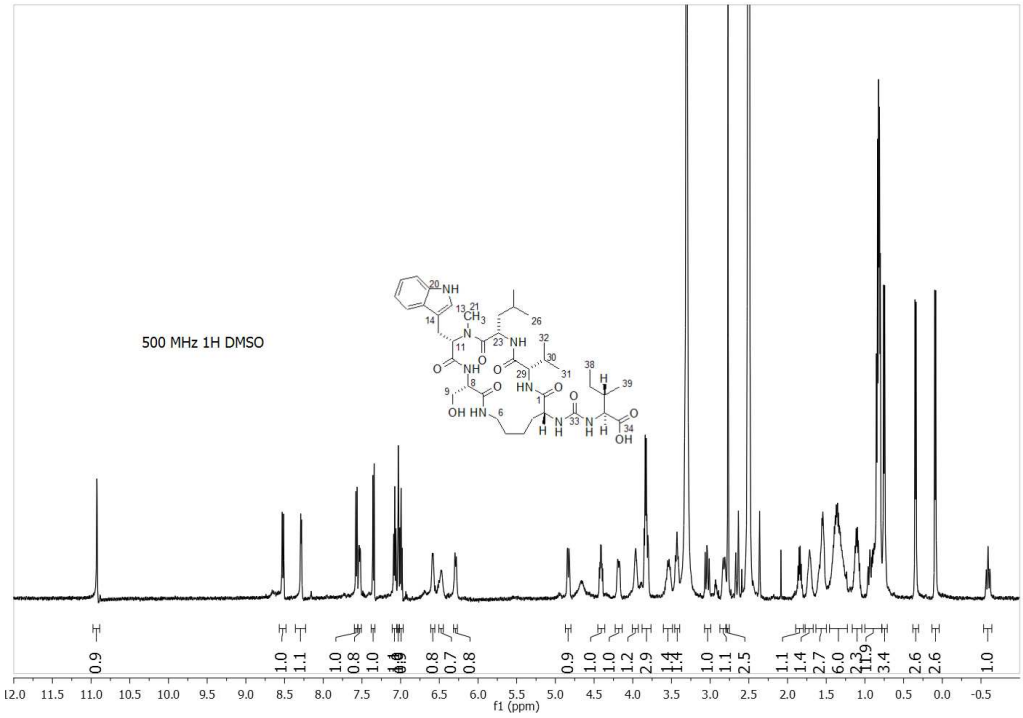
1,5-anhydro(D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-valinyl-L-leucyl-L-*N*-methyl-tryptophyl-L-alanine) (56):

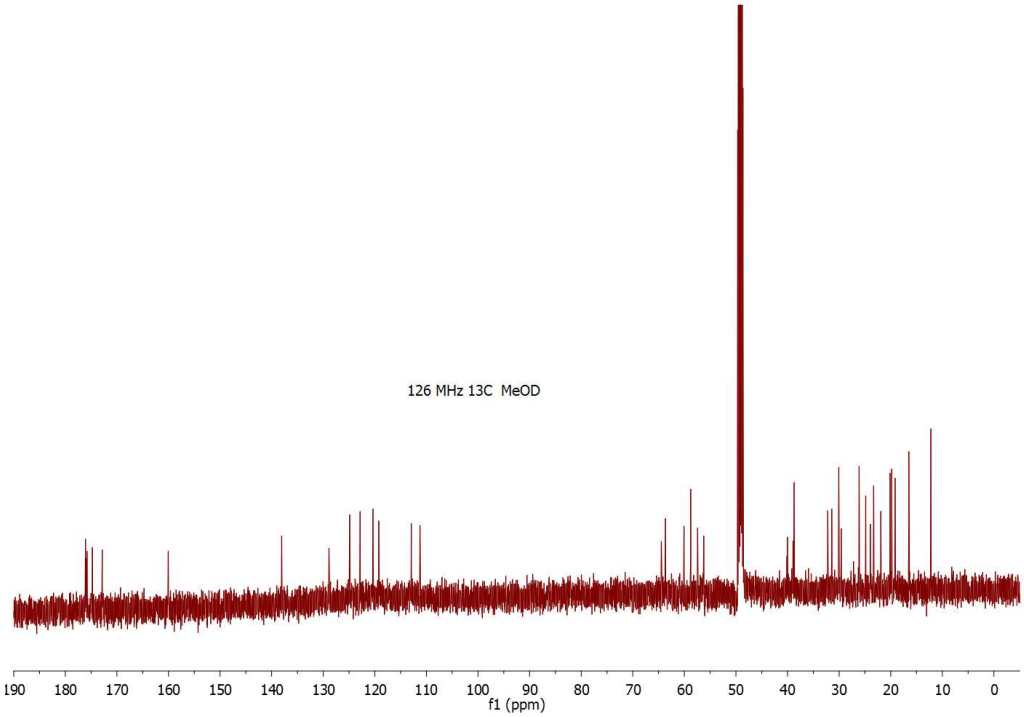


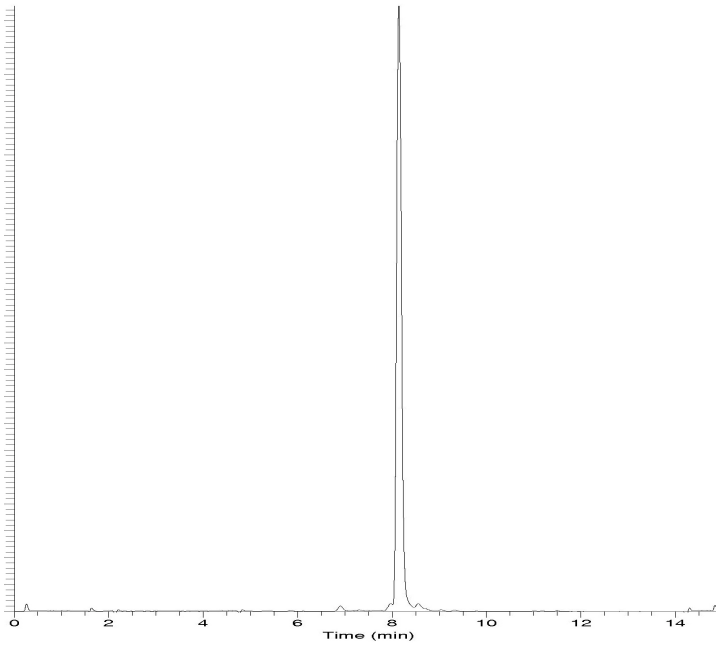

126 MHz 13C MeOD
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


0 2 4 6 8 10 12 14
Time (min)

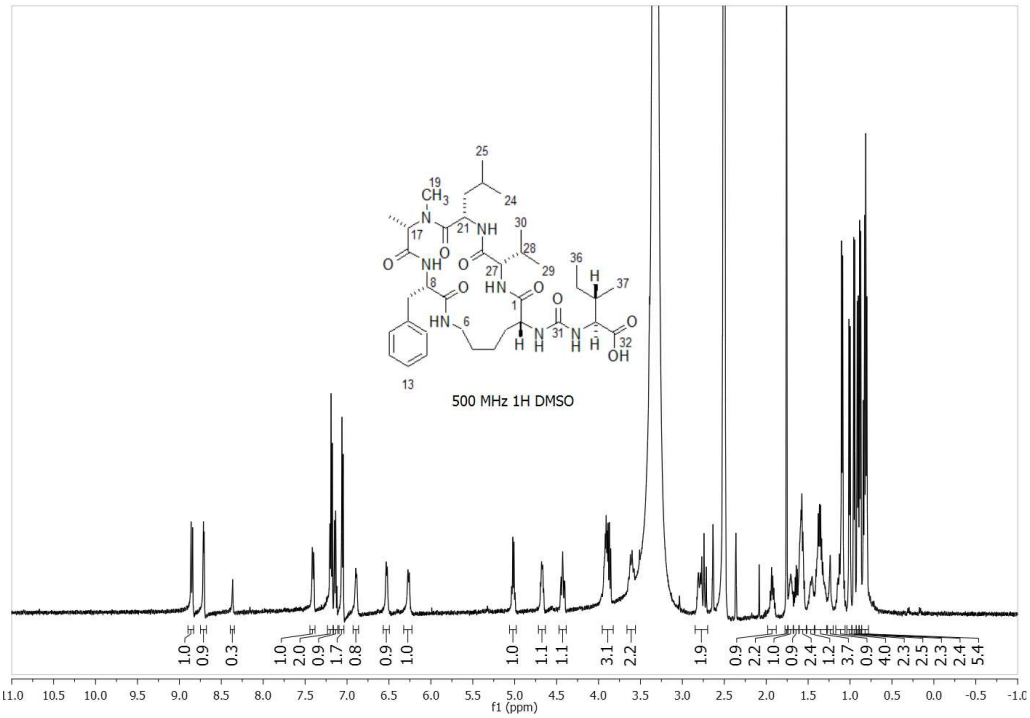
1,5-anhydro(D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-valinyl-L-leucyl-L-*N*-methyl-tryptophyl-L-serine) (57):

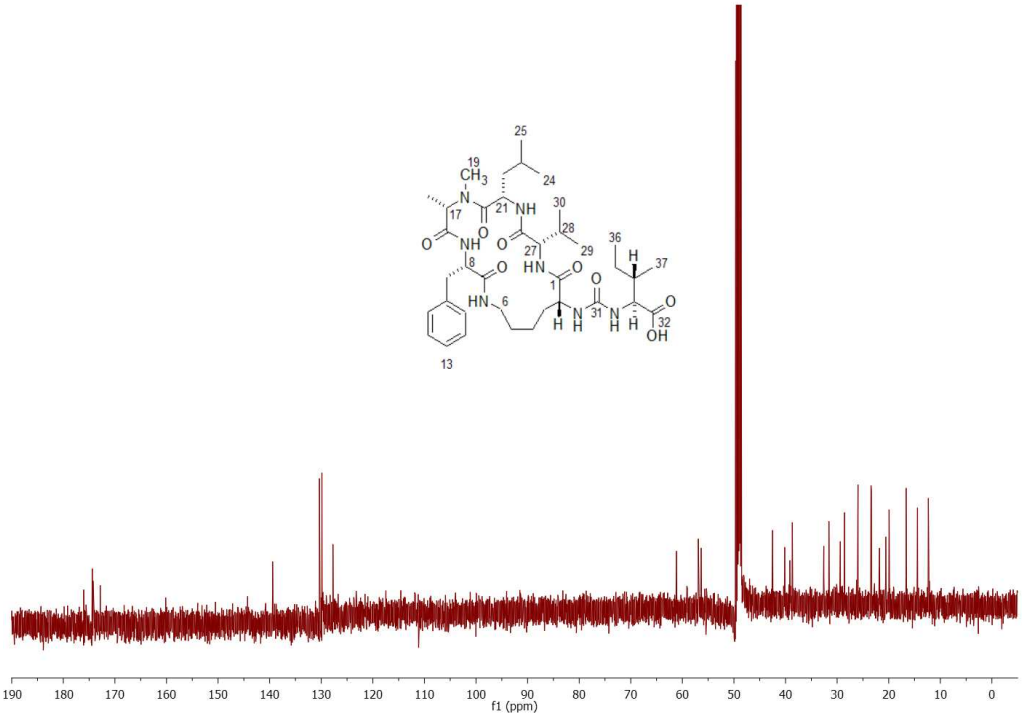


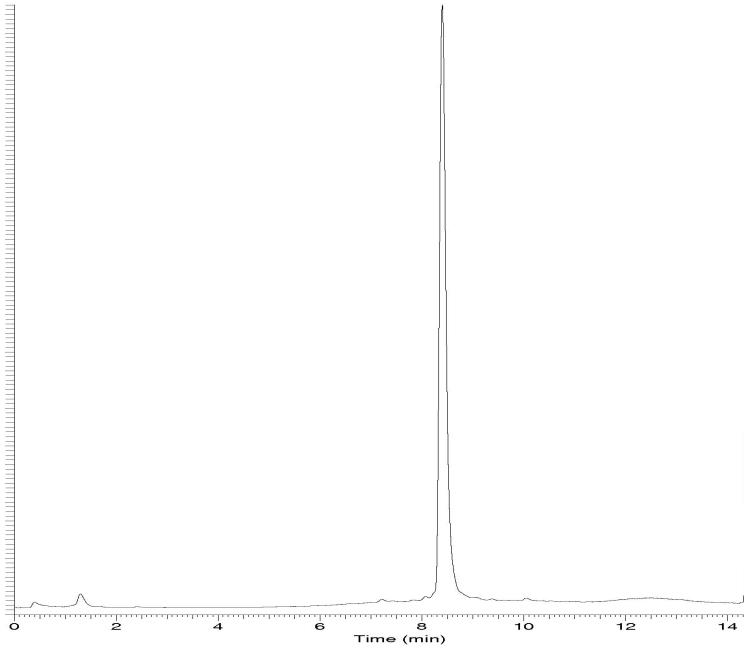




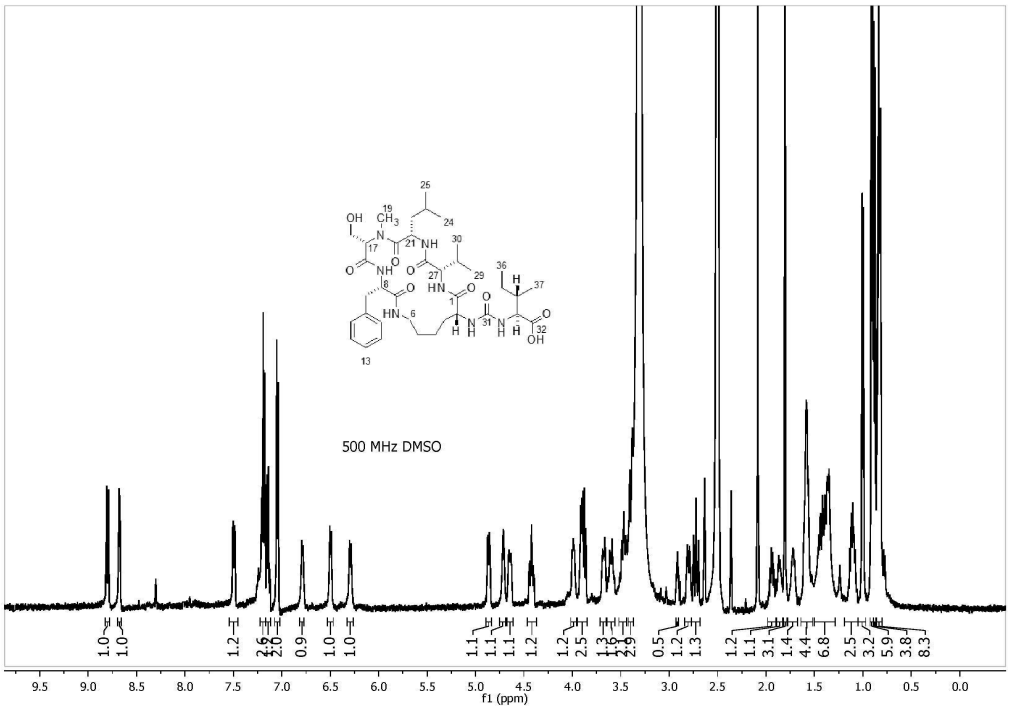
1,5-anhydro(D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-valinyl-L-leucyl-L-*N*-methyl-alanyl-L-phenylalanine) (58):

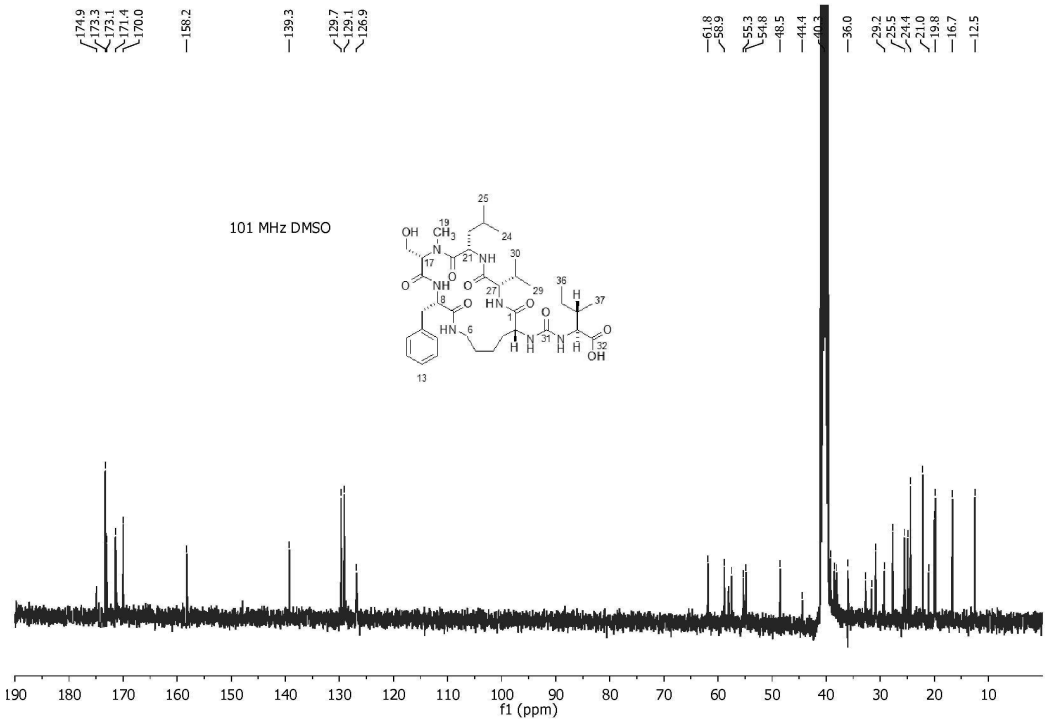


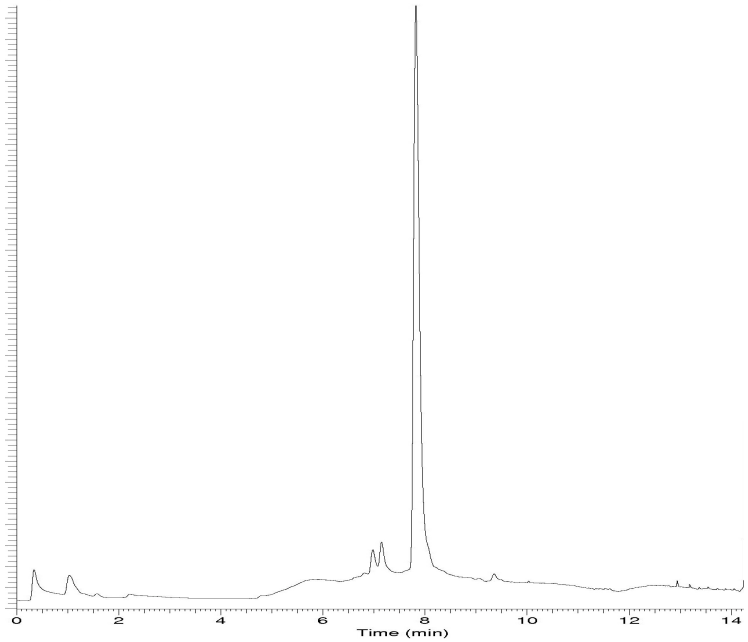

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


0 2 4 6 8 10 12 14
Time (min)

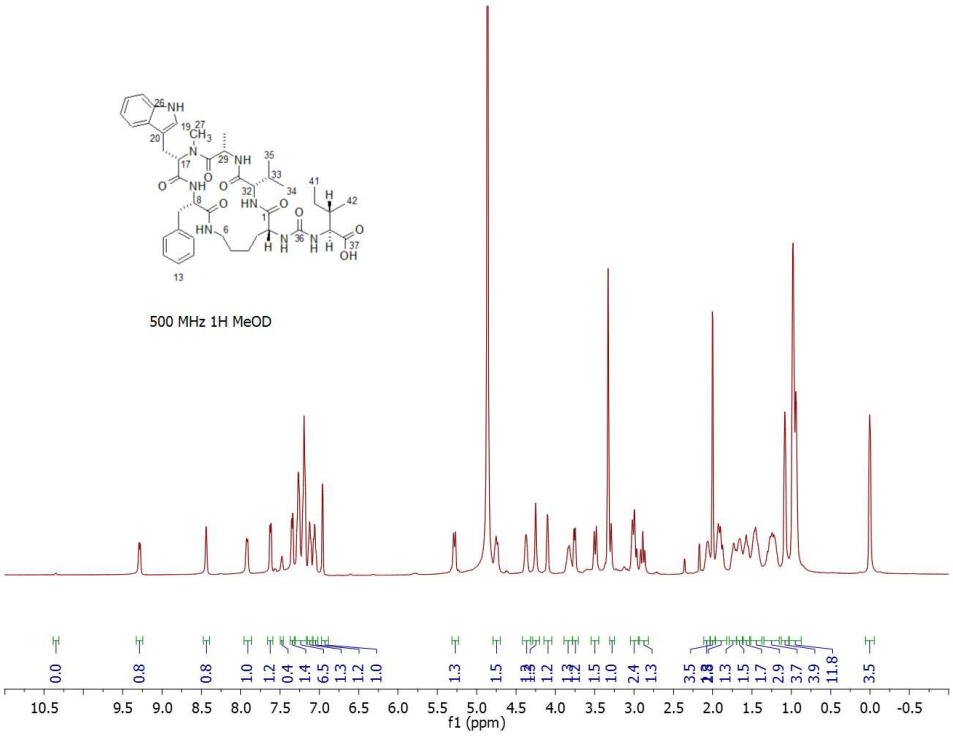
1,5-anhydro(D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-valinyl-L-leucyl-L-*N*-methyl-serinyl-L-phenylalanine) (59):

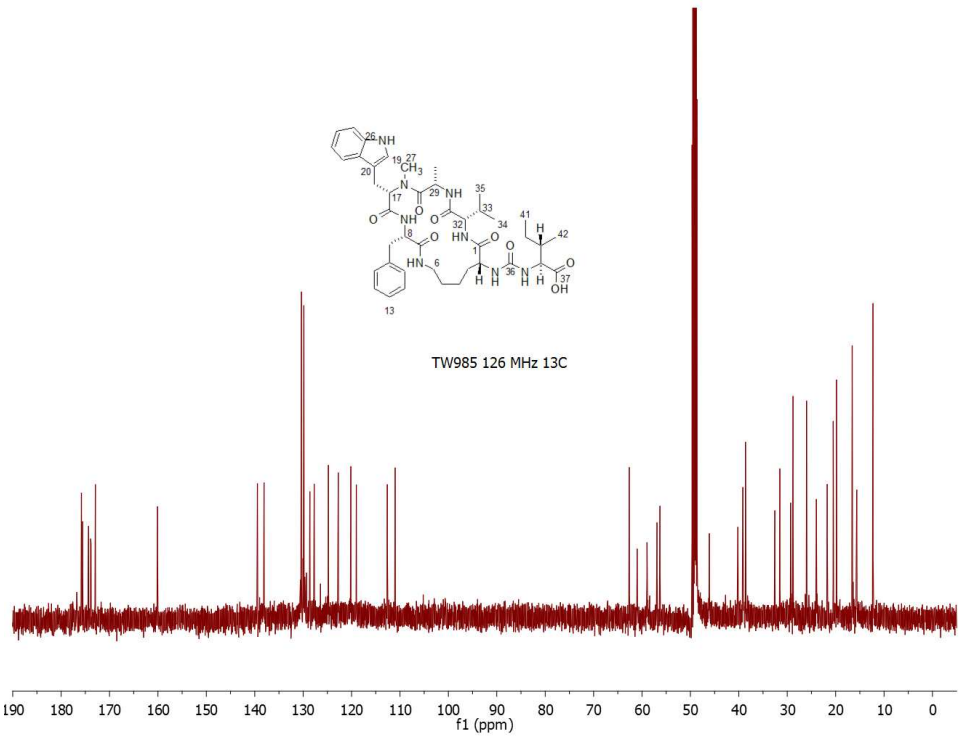


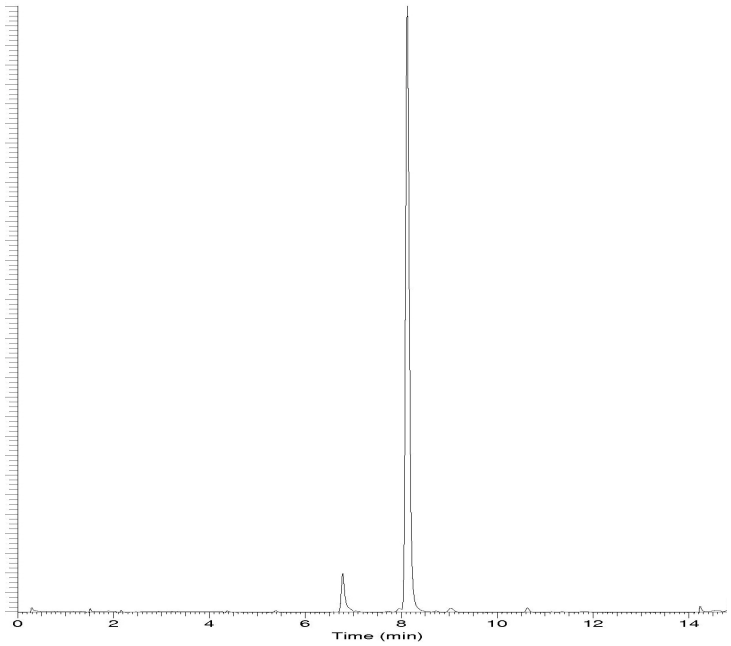




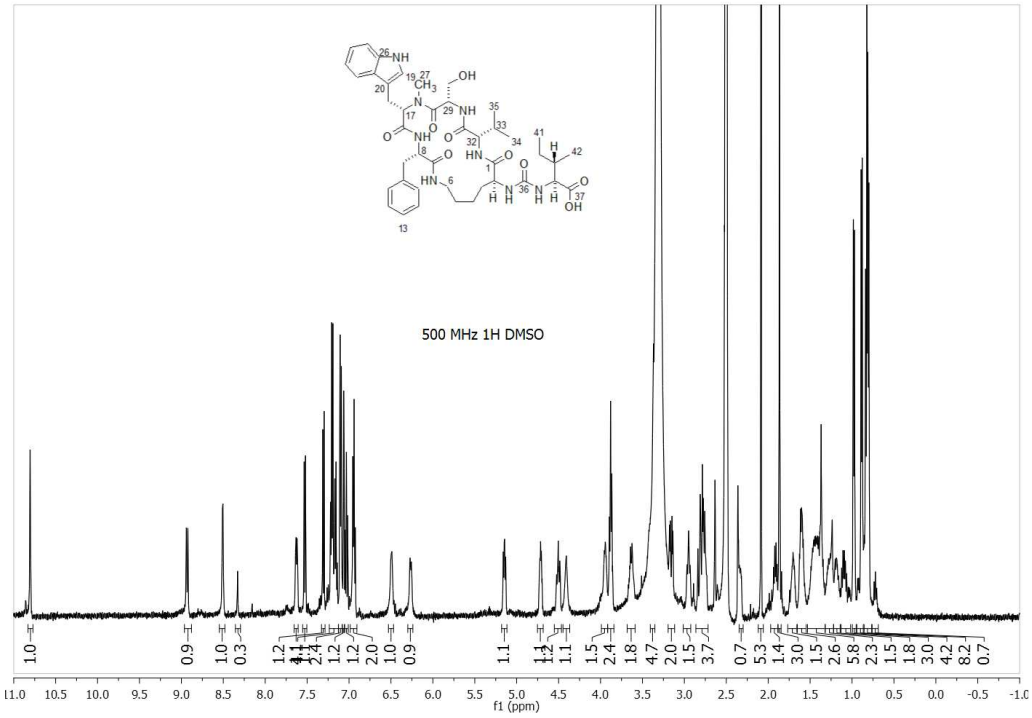
1,5-anhydro(D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-valinyl-L-alanyl-L-*N*-methyl-tryptophyl-L-phenylalanine) (60):

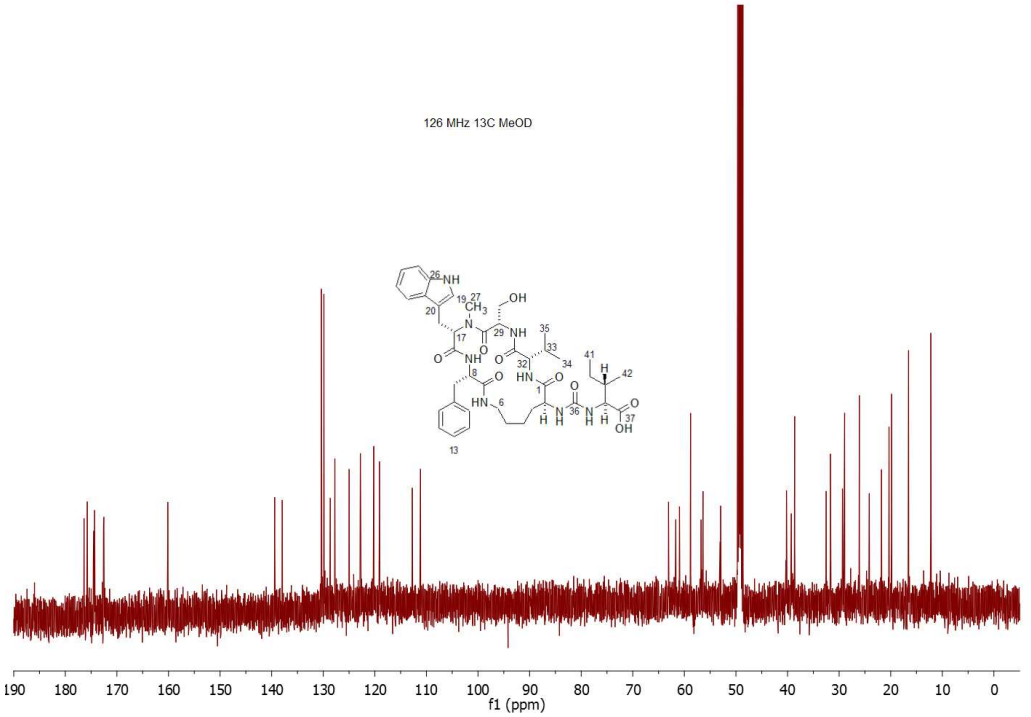


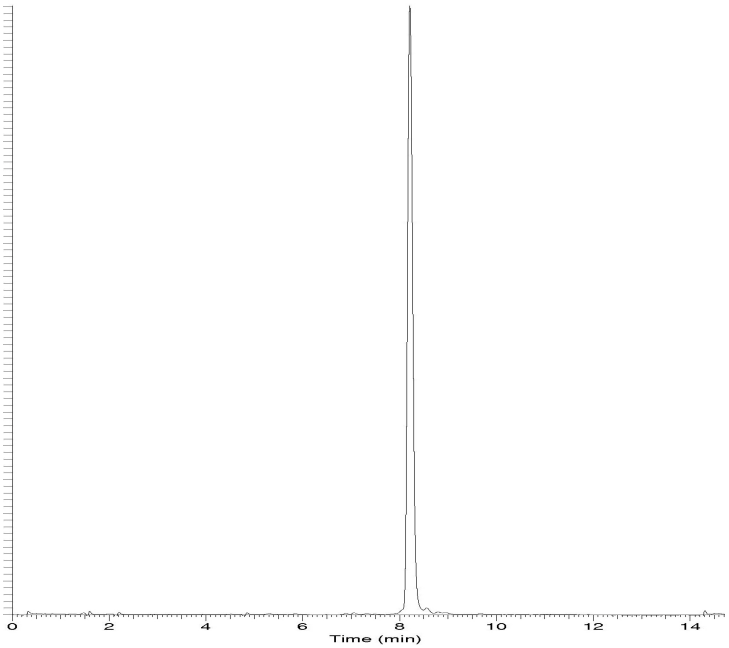

TW985 126 MHz 13C
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


0 2 4 6 8 10 12 14
Time (min)

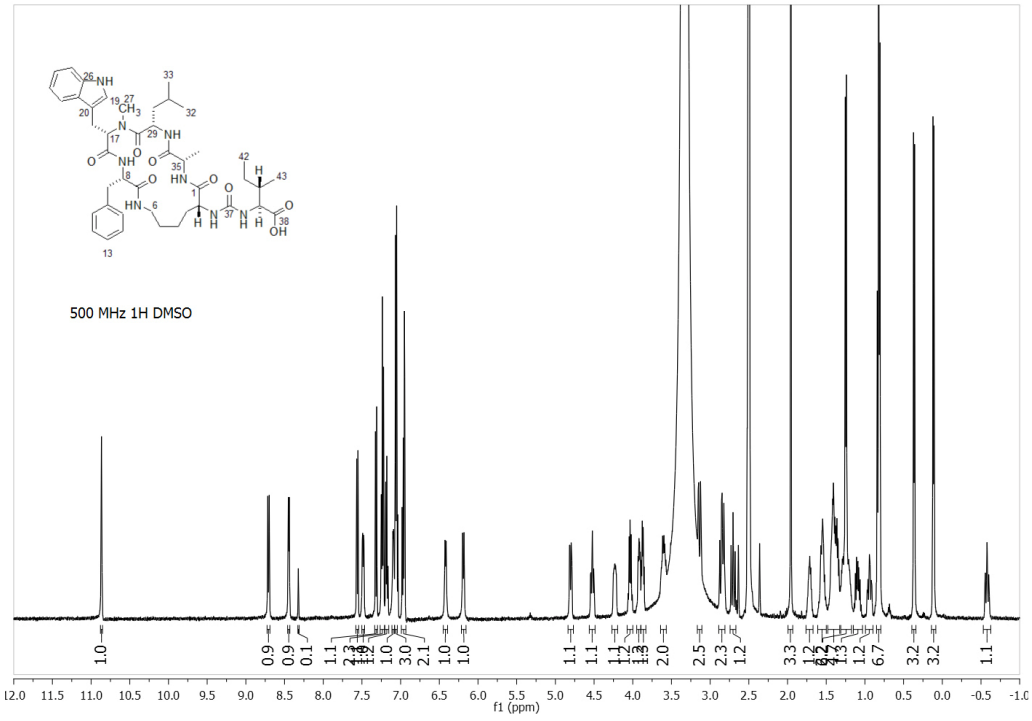
1,5-anhydro(D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-valinyl-L-serinyl-L-*N*-methyl-tryptophyl-L-phenylalanine) (61):

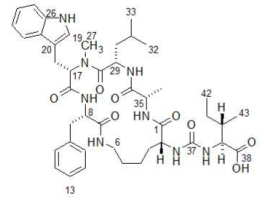




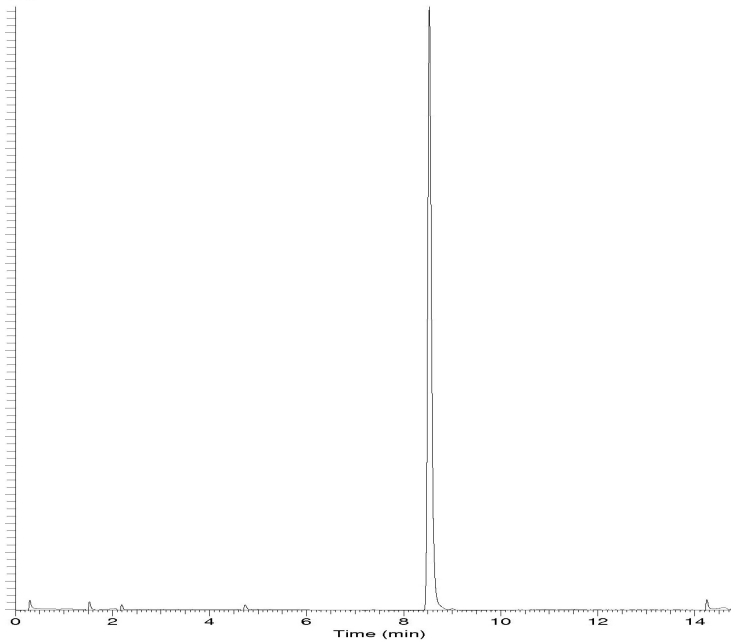


1,5-anhydro(D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-alanyl-L-leucyl-L-*N*-methyl-tryptophyl-L-phenylalanine) (62):

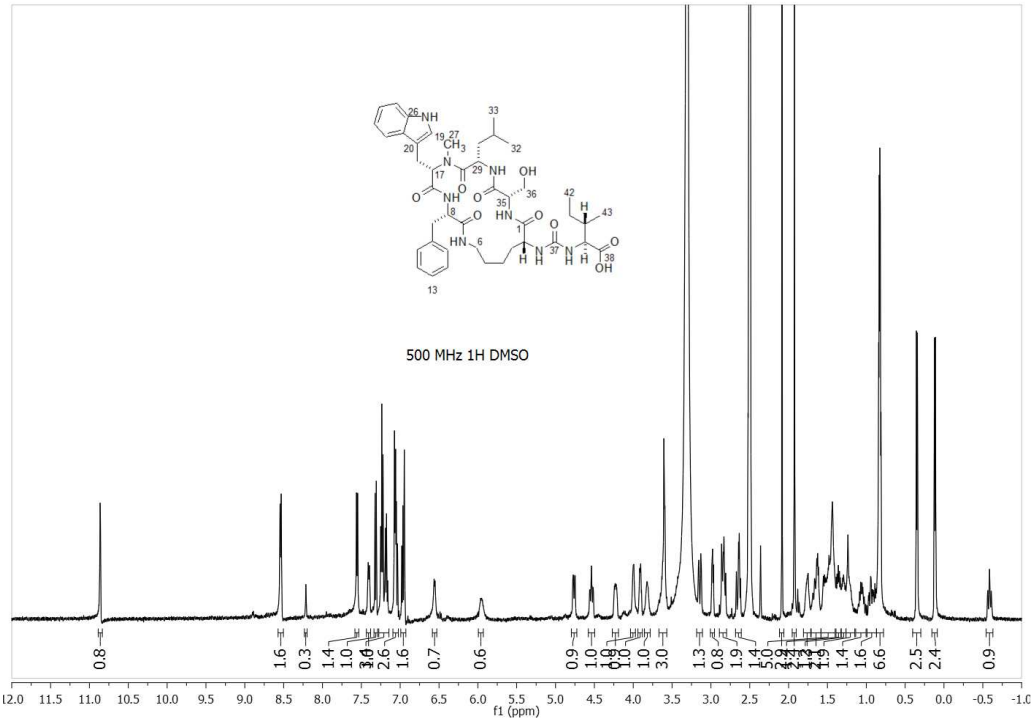


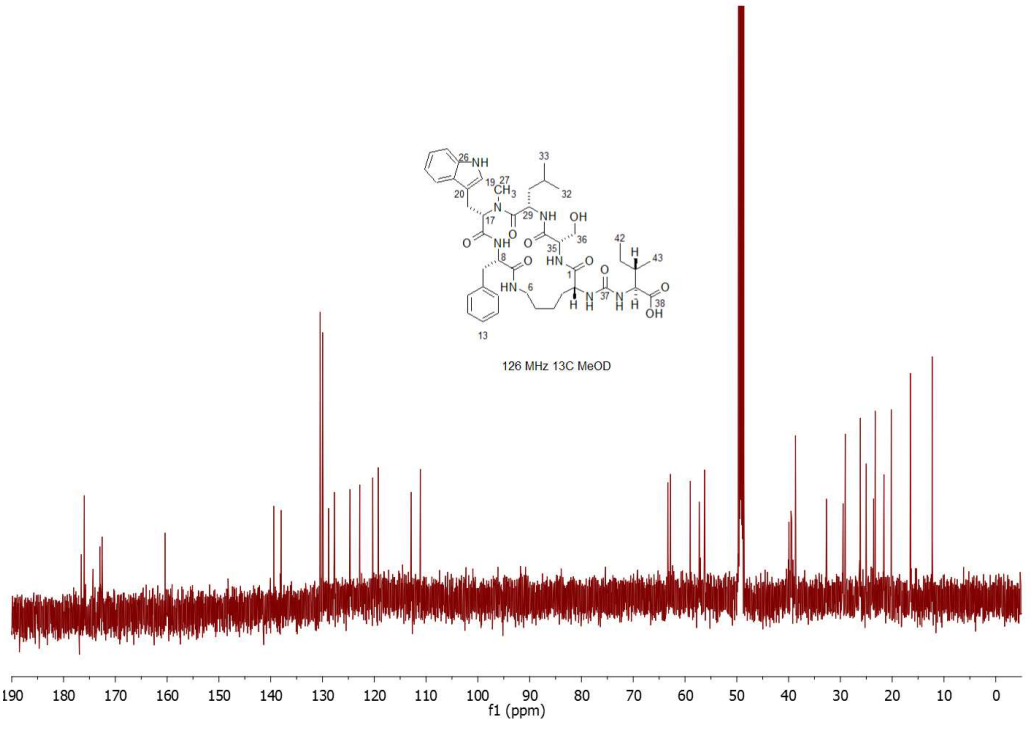


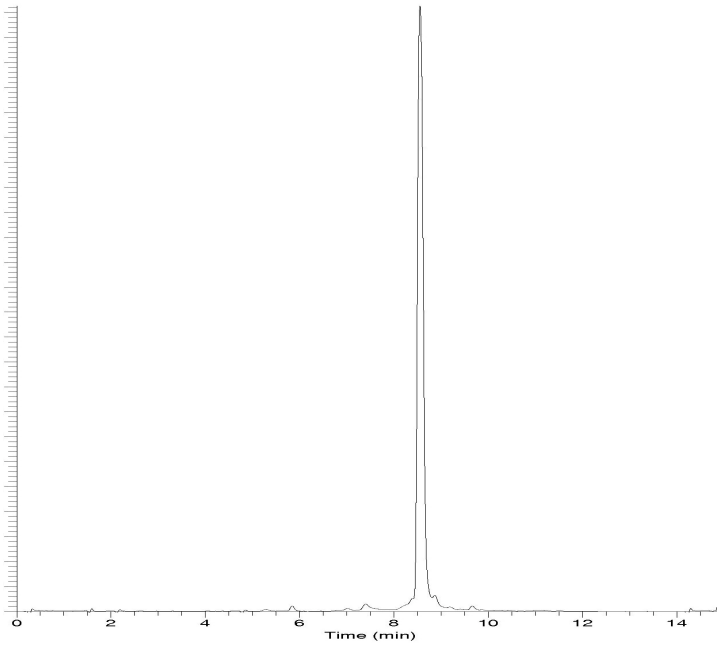
126 MHz 13C MeOD



1,5-anhydro(D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-serinyl-L-leucyl-L-*N*-methyl-tryptophyl-L-phenylalanine) (63):






0
2
4
6
8
10
12
14
Time (min)