SUPPORTING INFORMATION

N-Triethyleneglycol (*N*-TEG) as a substitute for the *N*-methyl group in Sansalvamide A peptide analogs

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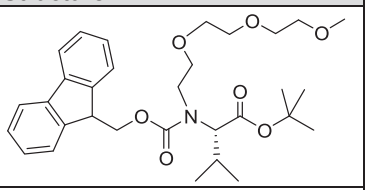
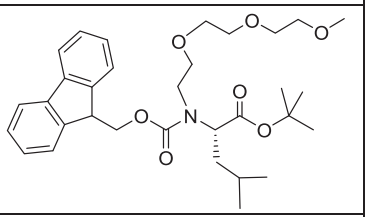
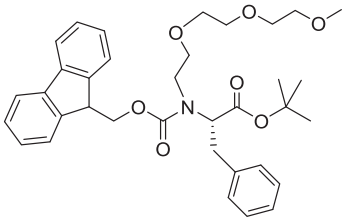
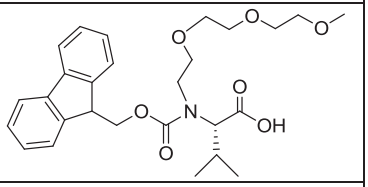
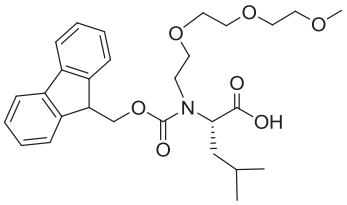
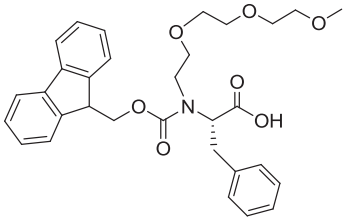
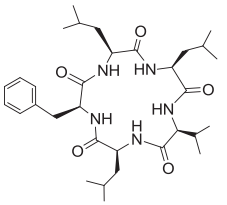
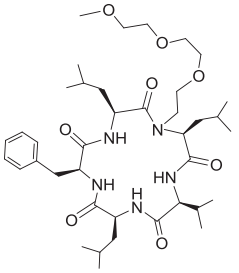
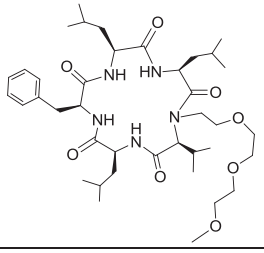
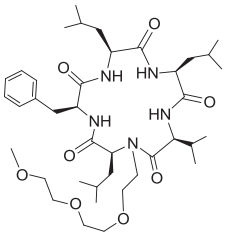
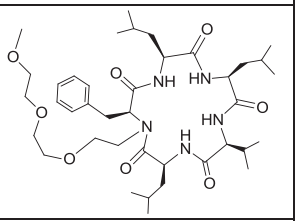
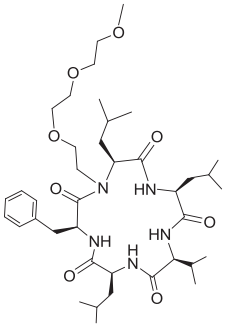
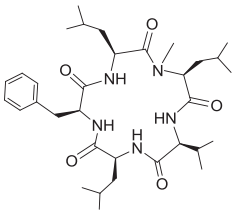
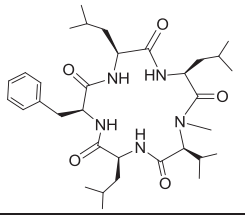
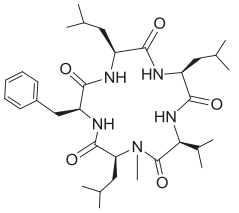
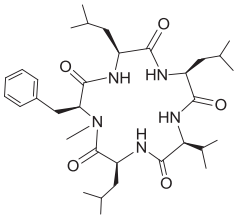
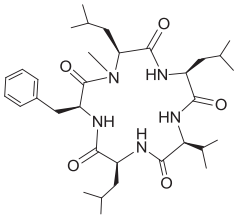
ABBREVIATIONS

4-DMAP, 4-dimethylaminopyridine; ACN, acetonitrile; AcOEt, ethyl acetate; AcOH, acetic acid; BTC, bis(trichloromethyl)carbonate; DIEA, *N*,*N*-diisopropylethylamine; DIPCDI, *N,N′*-diisopropylcarbodiimide; EDC·HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N′*,*N′*-tetramethyluronium hexafluorophosphate; MeOH, methanol; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; *N*-TEG: *N*-triethyleneglycol; OxymaPure, ethyl-2-cyano-2-(hydroxyimino)acetate.

1. TABLE OF COMPOUND STRUCTURES

Compound	Structure
Fmoc-*N*-TEG-Val-O^{t}Bu (**6**)	
Fmoc-*N*-TEG-Leu-O^{t}Bu (**7**)	
Fmoc-*N*-TEG-Phe-O^{t}Bu (**8**)	
Fmoc-*N*-TEG-Val-OH (**9**)	
Fmoc-*N*-TEG-Leu-OH (**10**)	
Fmoc-*N*-TEG-Phe-OH (**11**)	

Compound	Structure
cyclo[Phe-Leu-Leu-Val-Leu] (**1**)	
cyclo[Phe-Leu-(*N*-TEG)Leu-Val-Leu] (**1a**)	
cyclo[Leu-Leu-(*N*-TEG)Val-Leu-Phe] (**2a**)	
cyclo[Leu-Val-(*N*-TEG)Leu-Phe-Leu] (**3a**)	
cyclo[Val-Leu-(*N*-TEG)Phe-Leu-Leu] (**4a**)	
cyclo[Leu-Phe-(*N*-TEG)Leu-Leu-Val] (**5a**)	

cyclo[Phe-Leu-*N*MeLeu-Val-Leu] (**1b**)	
cyclo[Leu-Leu-*N*MeVal-Leu-Phe] (**2b**)	
cyclo[Leu-Val-*N*MeLeu-Phe-Leu] (**3b**)	
cyclo[Val-Leu-*N*MePhe-Leu-Leu] (**4b**)	
cyclo[Leu-Phe-*N*MeLeu-Leu-Val] (**5b**)	

2. COMPOUND SYNTHESIS AND CHARACTERIZATION

Materials and methods

All commercial reagents and solvents were used as received. Fmoc-protected amino acids were purchased from Iris Biotech (Marktredwitz, Germany). *Tert*-butyl amino acid esters were purchased from Sigma-Aldrich (Milwaukee, USA). 2-ClT resin was obtained from Novabiochem (Läufelfingen, Switzerland). OxymaPure was obtained from Luxembourg Industries (Tel Aviv, Israel). TFA was purchased from Scharlau (Barcelona, Spain). Piperidine was obtained from SDS (Peypin, France). The rest of chemicals were purchased from Sigma-Aldrich (Milwaukee, USA). THF and toluene were purchased from Scharlau (Barcelona, Spain). The rest of solvents were purchased from SDS (Peypin, France). Analytical TLC was carried out on Merck Kieselgel 60 F254 plates. For flash chromatography, silica gel (60 mesh, 35-70 μm) was purchased from SDS (Peypin, France).

^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectroscopy was performed on a Varian Mercury 400 MHz instrument. Chemical shifts (δ) are expressed in parts per million downfield from tetramethylsilyl chloride. Coupling constants are expressed in Hertz. The following abbreviations are used to indicate multiplicity: s, singlet; d, doublet, dd, double doublet; t, triplet; dt, double triplet; m, multiplet; and bs, broad signal. IR spectra were recorded with a Termo Nicolet spectrometer and with the Omnic 6.0 program from the Termo Nicolet Corporation. Optical rotation values were measured at 20 ºC with a Perkin-Elmer 241 polarimeter. High resolution mass spectra (HRMS) were obtained with an Agilent 1100 series (LC/MSD trap) spectrometer using the electrospray ionization (ESI-MS) method in positive or negative mode (as indicated). Analytical HPLC was carried out on a Waters instrument comprising a Sunfire™ C18 reversed-phase analytical column, 3.5 μm, 4.6 x 100 mm, a separation module (Waters 2695), an automatic injector, and a photodiode array detector (Waters 2298). Data were managed with Empower 2 software. UV detection was performed at 220 nm, and linear gradients of ACN (+0.036% TFA) into H_2O (+0.045% TFA) were run at a flow rate of 1.0 mL/min over 8 min. HPLC-MS analyses of peptide samples were carried out on a Waters instrument comprising a Sunfire™ C18 reversed-phase analytical column, 3.5 μm, 4.6 x 100 mm, a separation module (Waters 2695), an automatic injector, a photodiode array detector (Waters 2298), and a Waters micromass ZQ unit. Data were managed with MassLynx V4.1 software (Waters). UV detection was performed at 220 nm, and linear gradients of ACN (+0.07% formic acid) into H_2O (+0.1% formic acid) were run at a flow rate of 1.0 mL/min over 8 min. Semipreparative HPLC was carried out on a Waters instrument comprising a Sunfire™ C18 reversed-phase semipreparative column, 5.0 μm, 19 x 100 mm, a separation module (Waters Delta 600), a Waters 600 controller, an automatic injector, and a dual absorbance detector (Waters 2487). UV detection was at 220 nm, and linear gradients of ACN (+0.05% TFA) into H_2O (+0.1% TFA) were run at a flow rate of 15.0 mL/min or with isocratic flow in the conditions specified for each case. Fractions were collected with a Waters Fraction Collector II. Purities of the compounds synthesized were determined by analytical HPLC using the area percentage method on the UV trace recorded at a wavelength of 220 nm.

General SPPS remarks

The solid-phase syntheses were carried out manually on 50 mL polypropylene syringes, which were fitted with a polyethylene porous disc and attached to a vacuum manifold. Solvents and soluble reagents were removed by suction. Washings between deprotection and coupling steps were carried out with DMF (3x1 min) and DCM (3x1 min), unless otherwise stated. Resin loading was determined by quantification of the UV-absorbance of dibenzofulvene-piperidine adduct measured at 290 nm. The Fmoc- group was removed with piperidine/DMF 1:4 (3x5 min, 1x10 min). The progress of the coupling reactions was monitored with Kaiser's ninhydrin test. In the case of couplings to the *N*-substituted residues, the progress of the reaction was monitored with the chloranyl test, which reveals the presence of secondary amines. Peptides were cleaved from the resin with 2% TFA in DCM (3x2 min). After cleavage from the solid support, TFA was removed under reduced pressure, the residue was redissolved in ACN/H_2O 1:1 and liophilized.

Synthesis of the Fmoc-*N*-TEG amino acids

Synthesis of 3,6,9-trioxadecanaldehyde. A solution of oxalyl chloride (222.75 mmol, 25.0 mL) in DCM $_{(anh.)}$ (75 mL) was prepared under N_2 atmosphere and cooled in a dry ice-acetone bath. To this solution was carefully added a solution of DMSO (445.5 mmol, 31.6 mL) in DCM $_{(anh.)}$ (40 mL). The resulting mixture was stirred at -78 ºC for 10 min, and then a solution of triethylene glycol monomethyl ether (148.50 mmol, 25.0 mL) in DCM $_{(anh.)}$ (50 mL) was added slowly. After stirring for 15 min, NEt_3 $_{(anh.)}$ (891.00 mmol, 91.1 mL) was added dropwise over a period of 20 min. The reaction mixture was left for 30 min at -78 ºC and then allowed to reach room temperature. The reaction was quenched with $NaHCO_3$ $_{(sat.)}$ (100 mL). Phases were separated. The organic phase was washed with NaCl $_{(sat.)}$ (2x50 mL). Aqueous phases were combined and further extracted with DCM (2x50 mL). The combination of organic phases was dried over $MgSO_4$ $_{(anh.)}$, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (AcOEt/MeOH 95:5) affording 16.91 g (70%) of the desired aldehyde. ^{1}H-NMR and ^{13}C-NMR spectral data matched literature values (T. Da Ros and M. Prato, *J. Org. Chem.,* 1996, **61**, 9070).

General procedure A: reductive alkylation + Fmoc- protection. To a solution of 3,6,9-trioxadecanaldehyde (11.26 mmol, 1.42 g) and amino acid *tert*-butyl ester hydrochloride (10.23 mmol) in MeOH/AcOH 99:1 (80 mL) was added $NaBH_3CN$ (13.71 mmol, 0.86 g) over a period of 30 min. This mixture was stirred at room temperature for 2.5 h and then poured into 30 mL of $NaHCO_3$ $_{(sat.)}$. The product was extracted with AcOEt (2x50 mL). The combination of organic phases was washed with NaCl $_{(sat.)}$ (2x50 mL), dried over $MgSO_4$ $_{(anh.)}$, filtered and concentrated under reduced pressure. The resulting residue consisted of an unseparable mixture of expected product and *N,N*-dialkylated amino acid. This residue was dissolved in DCM (60 mL) and treated with Fmoc-Cl (12.28 mmol, 3.18 g) in the presence of DIEA (20.46 mmol, 2.93 mL) under N_2 atmosphere. After overnight stirring at room temperature, the solvent was removed under reduced pressure and the residue was

redissolved in AcOEt (100 mL). The organic phase was washed with $HCl_{(aq.)}$ 1 M (2x40 mL), dried over $MgSO_{4\ (anh.)}$, filtered and concentrated under reduced pressure. The crude residue was purified by flash chromatography (Hexane/AcOEt 75:25), affording the desired product.

General procedure B: acidic cleavage of the *tert*-butyl ester. The *tert*-butyl protected amino acid (aprox. 5.0 mmol) was dissolved in DCM (25 mL) and TFA (25 mL) was added. After stirring for 1 h at room temperature, the mixture was concentrated under reduced pressure. The remaining TFA was removed by co-evaporation with toluene at 50 ºC.

Fmoc-*N*-TEG-Val-O^{t}Bu (**6**). Following the general procedure A with valine *tert*-butyl ester hydrochloride (2.50 g, 11.00 mmol), compound **6** (4.09 g, 69%) was obtained as colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.66-0.81 (m, 3H), 0.84-0.99 (m, 3H), 1.41 (s, 9H), 1.98-2.23 (m, 1H), 3.20-3.72 (m, 15H), 3.82-4.14 (m, 1H), 4.23 (m, 1H), 4.28-4.63 (m, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.75 (d, *J* = 7.5 Hz, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 19.3, 20.2, 28.2, 44.7, 47.6, 47.7, 59.2, 66.6, 66.3, 67.2, 67.6, 68.9, 69.3, 70.5, 70.7, 72.1, 81.5, 81.6, 120.2, 125.0, 125.1, 125.2, 127.3, 127.4, 127.9, 141.6, 144.1, 144.2, 156.5, 156.8, 170.0, 170.3; IR (KBr): v = 2965.39, 2928.51, 1730.75, 1703.74, 1451.78, 1416.11, 1368.26, 1279.93, 1140.40, 1129.49, 759.78, 741.14 cm^{-1}; $[\alpha]^{20}_D$ -32.5 (MeOH, 0.01 g/mL); HRMS (ES$^+$): calc. for $[C_{31}H_{43}NO_7 + NH_4]^+$ 559.3378, found 559.3374.

Fmoc-*N*-TEG-Leu-O^{t}Bu (**7**). Following the general procedure A with leucine *tert*-butyl ester hydrochloride (2.29 g, 10.23 mmol), compound **7** (3.41 g, 60%) was obtained as colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.77-0.95 (m, 6H), 1.41 (bs, 9H), 1.45-1.74 (m, 3H), 3.00-3.73 (m, 15H), 4.22 (m, 1H), 4.30-4.62 (m, 3H), 7.31 (t, *J* = 7.4 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.75 (d, *J* = 7.4 Hz, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 21.4, 21.6, 22.8, 22.9, 24.6, 27.8, 38.1, 38.4, 45.2, 45.5, 47.2, 47.3, 58.8, 58.8, 66.7, 67.3, 69.0, 69.3, 70.2, 70.3, 71.7, 81.1, 81.3, 119.8, 124.6, 124.7, 126.9, 127.5, 141.1, 141.2, 141.2, 143.6, 143.7, 143.8, 143.9, 156.3, 170.8; IR (KBr): v = 2955.29, 2870.64, 1732.09, 1704.06, 1451.60, 1413.68, 1367.73, 1277.06, 1244.55, 1145.61, 1111.21, 759.30, 740.88 cm^{-1}; $[\alpha]^{20}_D$ -31.0 (MeOH, 0.01 g/mL); HRMS (ES$^+$): calc. for $[C_{32}H_{45}NO_7 + NH_4]^+$ 573.3534, found 573.3535.

Fmoc-*N*-TEG-Phe-O^{t}Bu (**8**). Following the general procedure A with phenylalanine *tert*-butyl ester hydrochloride (4.57 g, 17.88 mmol), compound **8** (4.22 g, 40%) was obtained as colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.35-1.46 (m, 9H), 2.60-3.15 (m, 2H), 3.21-3.71 (m, 15H), 4.17-4.30 (m, 2H), 4.32-4.43 (m, 1H), 4.48-4.84 (m, 1H), 6.89-7.27 (m, 5H), 7.31 (m, 2H), 7,39 (m, 2H), 7.58 (m, 2H), 7.75 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 28.2, 35.5, 36.0, 47.5, 47.8, 28.0, 48.1, 59.2, 67.1, 67.3, 69.3, 69.4, 70.5, 70.7, 70.7, 72.1, 120.2, 120.2, 120.3, 125.0, 125.1, 125.2, 126.6, 127.3, 127.3, 127.9, 128.0, 128.6, 129.3, 129.4, 129.4, 138.3, 138.6, 141.6, 141.7, 144.0, 144.2, 155.8, 156.3, 169.8, 170.0; IR (KBr): v = 2928.03, 2875.22, 1732.26, 1703.89, 1452.02, 1418.10, 1367.92, 1280.44, 1244.34, 1138.27, 758.43, 740.98 cm^{-1}; $[\alpha]^{20}_D$ -62.7 (MeOH, 0.01 g/mL); HRMS (ES$^+$): calc. for $[C_{35}H_{43}NO_7 + NH_4]^+$ 607.3378, found 607.3378.

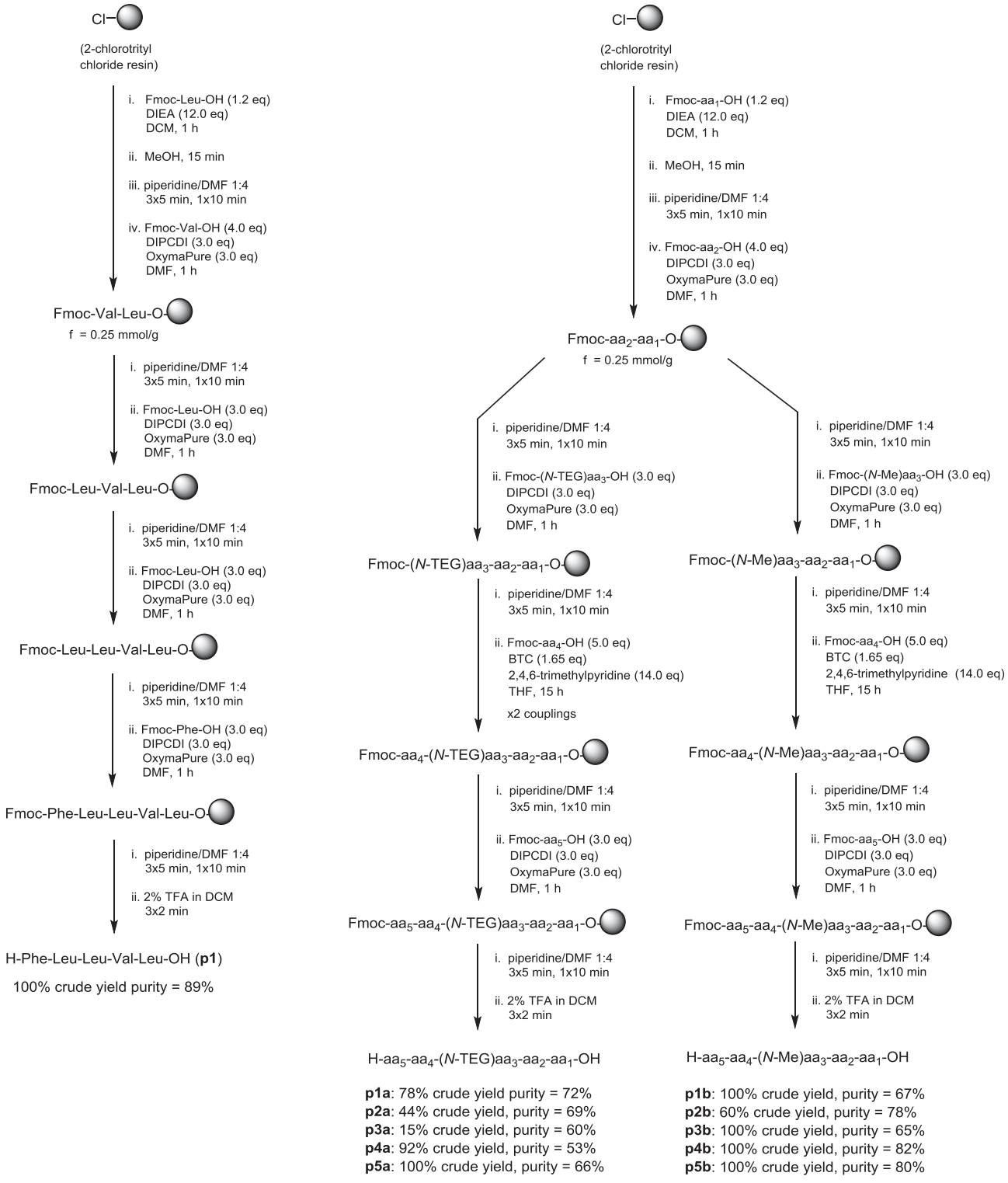
Fmoc-*N*-TEG-Val-OH (**9**). Following the general procedure B with **6** (4.00 g, 7.36 mmol) compound **9** (3.58 g, 100%) was obtained as yellowish oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.67-0.77 (m, 3H), 0.94-1.20 (m, 3H), 2.11-2.39 (m, 1H), 3.00-3.74 (m, 15H), 3.94 (d, *J* = 10.7 Hz, 1H), 4.25 (m, 1H), 4.32-4.70 (m, 2H), 7.30 (t, *J* = 7.4 Hz, 2H), 7.37 (t, *J* = 7.4 Hz, 2H), 7.60 (m, 2H), 7.73 (d, *J* = 7.5 Hz, 2H), 8.8 (bs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 18.6, 18.7, 20.2, 20.5, 27.1, 27.5, 46.7, 47.2, 47.3, 58.6, 58.7, 66.2, 67.0, 67.5, 69.1, 69.2, 69.8, 69.9, 70.1, 70.2, 70.4, 71.6, 119.8, 124.6, 127.1, 127.6, 141.3, 141.3, 141.4, 143.7, 143.8, 143.8, 143.9, 155.9, 156.5, 171.7, 171.1; IR (KBr): v = 3063.88, 2890.62, 1739.90, 1703.63, 1451.94, 1419.21, 1274.55, 1243.43, 1138.12, 1107.59, 758.96, 741.78 cm^{-1}; $[\alpha]^{20}_{D}$ -29.4 (MeOH, 0.01 g/mL); HRMS (ES^+): calc. for $[C_{27}H_{35}NO_7 + Na]^+$ 508.2306, found 508.2303.

Fmoc-*N*-TEG-Leu-OH (**10**). Following the general procedure B with **7** (2.00 g, 3.60 mmol), compound **10** (1.80 g, 100%) was obtained as yellowish oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.79-0.93 (m, 6H), 1.39-1.84 (m, 3H), 3.00-3.72 (m, 15H), 4.22 (m. 1H), 4.30-4.62 (m, 3H), 7.30 (t, *J* = 7.4 Hz, 2H), 7.37 (m, 2H), 7.58 (m, 2H), 7.73 (d, *J* = 7.4 Hz, 2H), 8.71 (bs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 21.5, 22.9, 24.4, 37.4, 37.8, 45.6, 47.0, 47.1, 58.3, 58.6, 66.8, 67.4, 69.3, 69.7, 69.9, 70.0, 71.6, 119.7, 124.5, 124.5, 124.6, 126.8, 126.9, 127.4, 141.1, 141.1, 143.6, 143.6, 143.7, 156.1, 156.3, 174.0, 174.4; IR (KBr): v = 2955.10, 2871.21, 1738.72, 1703.09, 1451.09, 1415.57, 1285.96, 1232.62, 1146.95, 1110.04, 759.38, 741.11 cm^{-1}; $[\alpha]^{20}_{D}$ -24.9 (MeOH, 0.01 g/mL); HRMS (ES^+): calc. for $[C_{28}H_{37}NO_7 + NH_4]^+$ 517.2908, found 517.2907.

Fmoc-*N*-TEG-Phe-OH (**11**). Following the general procedure B with **8** (3.69 g, 6.26 mmol), compound **11** (3.34 g, 100%) was obtained as yellowish oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.55-2.81 (m, 2H), 3.00-3.70 (m, 15H), 4.15-4.34 (m, 2H), 4.44-4.56 (m, 1H), 4.58-4.72 (m, 1H), 6.82-7.25 (m, 5H), 7.26-7.42 (m, 4H), 7.50 (m, 2H), 7.63 (m, 2H), 7.73 (m, 2H), 9.39 (bs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 34.2, 34.7, 46.7, 46.8, 47.9, 58.1, 58.1, 63.3, 66.6, 66.7, 68.7, 68.8, 69.4, 69.5, 69.6, 71.2, 119.4, 119.5, 124.2, 124.3, 125.9, 126.0, 126.6, 126.7, 127.1, 127.2, 127.9, 128.0, 128.5, 128.6, 137.3, 137.5, 140.8, 140.8, 140.9, 143.2, 143.3, 143.4, 154.5, 155.0, 172.3, 172.6; IR (KBr): v = 2925.17, 2739.77, 1703.16, 1451.45, 1417.01, 1282.94, 1244.52, 1143.83, 1113.31, 759.62, 741.20 cm^{-1}; $[\alpha]^{20}_{D}$ -82.2 (MeOH, 0.01 g/mL); HRMS (ES^+): calc. for $[C_{31}H_{35}NO_7 + Na]^+$ 556.2306, found 556.2300.

SPPS of the linear pentapeptides

Scheme S1. SPPS of the linear pentapeptides **p1**, **p1a-p5a** and **p1b-p5b**.



The pentapeptides **p1**, **p1a-p5a** and **p1b-p5b** were synthesized on the 2-chlorotrityl resin as shown in Scheme S1. For each of the syntheses, the 2-chlorotrityl resin (2.0 g, 1.6 mmol/g) was swollen in DCM for 15 min. A solution of the first amino acid (1.2 equiv., 0.60 mmol) and DIEA (12.0 equiv., 60.0 mmol, 10.5 mL) was poured onto the resin and the mixture was shaken for 1 h. After this time, the free sites of the resin were capped with MeOH (4.0 mL, 15 min) and the resin was washed. The Fmoc- group was removed and the resin loading was determined to be 0.25 mmol/g. Elongation of the peptide chain was accomplished by stepwise assembly of the following Fmoc-protected amino acids. Standard couplings were performed with DIPCDI/OxymaPure following the general procedure described below. Couplings onto the *N*-alkylated amino acids were performed using BTC activation following the general procedure described below. In the case of couplings onto the *N*-TEG residues, two overnight couplings were performed. After cleavage with from the solid support, the crude pentapeptides were lyophilized and obtained in purities between 53% and 89%.

- **General procedure for coupling with DIPCDI/OxymaPure activation.** The Fmoc-aa-OH (3.0 equiv., 1.50 mmol) and OxymaPure (3.0 equiv., 1.50 mmol, 213 mg) were dissoved in DMF. DIPCDI (3.0 equiv., 1.50 mmol, 233 µL) was added and, after 3 min of preactivation, the solution was poured to the resin. The peptidyl-resin was shaken for 1 h. Then, the excess of reagents was filtered off and the resin was washed.

- **General procedure for coupling with BTC activation.** The peptidyl-resin was pre-swollen in THF $_{(anh.)}$ for 15 min. Fmoc-aa-OH (5.00 equiv., 2.50 mmol) and BTC (1.65 equiv., 0.83 mmol, 245 mg) were weighed in a 2 mL eppendorf tube and dissolved in THF $_{(anh.)}$ to a 0.1-0.2 M concentration. This mixture was cooled to 0 ºC. 2,4,6-trimethylpyridine (14.0 equiv., 7.00 mmol, 928 µL) was added slowly and a white precipitate was formed. The suspension was mixed with a Pasteur pipette for 1 min, added to the resin and the mixture was left to react overnight with shaking. The excess of reagents was filtered off and the resin was washed with MeOH (3x1 min), DCM (2x1 min), MeOH (2x1 min), DCM (2x1 min), DMF (2x1 min) and DCM (3x1 min). [Hazard: BTC should be handled in well-ventilated hood with extreme caution.].

H-Phe-Leu-Leu-Val-Leu-OH (p1, 301 mg, 100% crude yield). HPLC (linear gradient from 10% to 90% ACN over 8 min): t_r = 4.98 min, purity = 89%; HPLC-MS (linear gradient from 0% to 60% ACN over 11 min): t_r = 8.09 min, $[M+H]^+$ = 604.48.

H-Phe-Leu-(*N*-TEG)Leu-Val-Leu-OH (p1a, 291 mg, 78% crude yield). HPLC (linear gradient from 10% to 90% ACN over 8 min): t_r = 5.42 min, purity = 72%; HPLC-MS (linear gradient from 0% to 60% ACN over 11 min): t_r = 8.81 min, $[M+H]^+$ = 750.41.

H-Leu-Leu-(*N*-TEG)Val-Leu-Phe-OH (p2a, 165 mg, 44% crude yield). HPLC (linear gradient from 10% to 90% ACN over 8 min): t_r = 5.36 min, purity = 69%; HPLC-MS (linear gradient from 0% to 60% ACN over 11 min): t_r = 8.79 min, $[M+H]^+$ = 750.41.

H-Leu-Val-(*N*-TEG)Leu-Phe-Leu-OH (p3a, 55 mg, 15% crude yield). HPLC (linear gradient from 10% to 90% ACN over 8 min): t_r = 5.56 min, purity = 60%; HPLC-MS (linear gradient from 0% to 60% ACN over 11 min): t_r = 9.09 min, $[M+H]^+$ = 750.41.

H-Val-Leu-(*N*-TEG)Phe-Leu-Leu-OH (p4a, 346 mg, 92% crude yield). HPLC (linear gradient from 10% to 90% ACN over 8 min): t_r = 5.58 min, purity = 53%; HPLC-MS (linear gradient from 0% to 60% ACN over 11 min): t_r = 9.03 min, $[M+H]^+$ = 750.41.

H-Leu-Phe-(*N*-TEG)Leu-Leu-Val-OH (p5a, 438 mg, 100% crude yield). HPLC (linear gradient from 10% to 90% ACN over 8 min): t_r = 5.45 min, purity = 66%; HPLC-MS (linear gradient from 0% to 60% ACN over 11 min): t_r = 9.01 min, $[M+H]^+$ = 750.41.

H-Phe-Leu-*N*MeLeu-Val-Leu-OH (p1b, 497 mg, 100% crude yield). HPLC (linear gradient from 10% to 90% ACN over 8 min): t_r = 5.20 min, purity = 67%; HPLC-MS (linear gradient from 10% to 70% ACN over 11 min): t_r = 5.40 min, $[M+H]^+$ = 618.11.

H-Leu-Leu-*N*MeVal-Leu-Phe-OH (p2b, 541 mg, 60% crude yield). HPLC (linear gradient from 10% to 90% ACN over 8 min): t_r = 5.10 min, purity = 78%. HPLC-MS (linear gradient from 0% to 60% ACN over 11 min): t_r = 8.37 min, $[M+H]^+$ = 618.43.

H-Leu-Val-*N*MeLeu-Phe-Leu-OH (p3b, 541 mg, 100% crude yield). HPLC (linear gradient from 10% to 90% ACN over 8 min): t_r = 5.22 min, purity = 65%; HPLC-MS (linear gradient from 10% to 70% ACN over 11 min): t_r = 5.40 min, $[M+H]^+$ = 618.15.

H-Val-Leu-*N*MePhe-Leu-Leu-OH (p4b, 403 mg, 100% crude yield). HPLC (linear gradient from 10% to 90% ACN over 8 min): t_r = 5.41 min, purity = 82%; HPLC-MS (linear gradient from 10% to 70% ACN over 11 min): t_r = 5.46 min, $[M+H]^+$ = 618.16.

H-Leu-Phe-*N*MeLeu-Leu-Val-OH (p5b, 456 mg, 100% crude yield). HPLC (linear gradient from 10% to 90% ACN over 8 min): t_r = 5.22 min, purity = 80%; HPLC-MS (linear gradient from 10% to 70% ACN over 11 min): t_r = 5.39 min, $[M+H]^+$ = 618.15.

Cyclization of the linear pentapeptides

The crude linear pentapeptide (approx. 0.036 mmol) was dissolved in DCM/DMF 9:1 (72 mL). EDC·HCl (0.36 mmol, 69 mg) and 4-DMAP (0.072 mmol, 9 mg) were added to the solution. After stirring overnight at room temperature, the mixture was concentrated to 25 mL and washed with 2% citric acid (2x15 mL), $Na_2CO_{3\ (sat.)}$ (2x15 mL) and $NaCl_{\ (sat.)}$ (2x15 mL). The organic layer was dried over $MgSO_{4\ (anh.)}$, filtered and concentrated

under reduced pressure at 50 ºC for 30 min to remove the maximum amount of DMF. The residue was purified by HPLC using a Sunfire™ 18 reversed-phase semipreparative column (linear gradient from 30:70 to 60:40 ACN/H_2O in 15 min, flow rate = 15 mL/min; alternatively: isocratic 60:40 ACN/H_2O in 20 min, flow rate = 15 mL/min). Fractions were collected, pooled and liophilized to afford the pure cyclic peptides.

cyclo[Phe-Leu-Leu-Val-Leu] (1). Crude pentapeptide **p1** (22 mg, 89% purity, approx. 0.036 mmol) was cyclized and purified following the procedure described above. This afforded cyclic peptide **1** (12 mg, 58%). HPLC (linear gradient from 40% to 100% ACN over 8 min): t_r = 5.97 min, purity >99%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.78 (d, *J* = 6.5 Hz, 6H), 0.88 (d, *J* = 6.5 Hz, 3H), 0.90-1.01 (m, 15H), 1.26-1.49 (3H), 1.49-1.74 (m, 4H), 1.87 (m, 1H), 2.02 (m, 2H), 2.95 (dd, *J* = 14.0, 8.9 Hz, 1H), 3.16 (dd, *J* = 14.0, 6.0 Hz, 1H), 3.91 (dd, *J* = 14.2, 6.8 Hz, 1H), 4.59-4.80 (m, 3H), 4.66 (dd, *J* = 14.4, 7.7 Hz, 1H), 6.88 (bs, 1H), 7.12 (bs, 1H), 7.15-7.30 (m, 5 H), 7.67 (bs, 1H), 7.80 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 18.6, 19.3, 22.0, 22.2, 22.3, 22.4, 22.6, 22.7, 24.6, 24.9, 25.4, 37.7, 38.6, 39.0, 39.7, 51.7, 53.7, 55.3, 57.9, 58.8, 127.0, 128.6, 129.0, 136.2, 171.2, 172.0, 172.6, 173.2, 173.4; HRMS (ES^+): calc. for $[C_{32}H_{51}N_5O_5 + H]^+$ 586.3963, found 586.3940.

cyclo[Phe-Leu-(*N*-TEG)Leu-Val-Leu] (1a). Crude pentapeptide **p1a** (27 mg, 72% purity, approx. 0.036 mmol) was cyclized and purified following the procedure described above. This afforded cyclic peptide **1a** (14 mg, 53%). HPLC (linear gradient from 40% to 100% ACN over 8 min): t_r = 7.53 min, purity = 95%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.63-1.04 (m, 24H), 1.05-1.74 (m, 7H), 1.87-2.22 (m, 2H), 3.19-3.48 (m, 6H), 3.45-3.90 (m, 12H), 3.91 (m, 1H), 4.01-4.27 (m, 2H), 4.47 (m, 1H), 4.95 (m, 1H), 6.28 (bs, 1H), 6.97 (bs, 1H), 7.11-7.36 (m, 5H), 7.38-7.68 (m, 1H), 7.78 (bs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 19.0, 19.1, 21.9, 22.4, 22.6, 22.7, 22.7, 23.0, 24.5, 24.8, 25.7, 29.7, 31.9, 34.2, 37.2, 39.8, 41.6, 48.6, 50.5, 52.6, 56.1, 58.9, 59.8, 66.4, 68.6, 70.2, 70.7, 71.6, 127.4, 128.8, 129.0, 136.0, 170.2, 172.6, (2C), 173.0 (2C); HRMS (ES^+): calc. for $[C_{39}H_{65}N_5O_8 + H]^+$ 732.4906, found 732.4910.

cyclo[Leu-Leu-(*N*-TEG)Val-Leu-Phe] (2a). Crude pentapeptide **p2a** (27 mg, 69% purity, approx. 0.036 mmol) was cyclized and purified following the procedure described above. This afforded cyclic peptide **2a** (14 mg, 53%). HPLC (linear gradient from 40% to 100% ACN over 8 min): t_r = 7.22 min, purity = 98%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.75 (d, *J* = 6.6 Hz, 3H), 0.64 (d, *J* = 6.4 Hz, 3H), 0.84-0.97 (m, 18H), 1.17-1. 83 (m, 9H), 2.92-3.24 (m, 3H), 3.38 (s, 3 H), 3.50-3.72 (m, 12H), 3.67 (m, 1H), 3.97 (m, 1H), 4.27 (m, 1H), 4.46 (m, 1H), 4.97 (dd, *J* = 16.2 Hz, 7.6 Hz, 1H), 5.74 (bs, 1H), 6.51 (d, *J* = 8.6 Hz, 1H), 7.12-7.33 (m, 6H), 8.15 (d, *J* = 8.9 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 19.1, 20.1, 21.1, 21.9, 22.6, 22.8, 22.9, 23.0, 24.4, 24.7, 24.8, 27.3, 29.7, 31.9, 34.2, 36.8, 39.5, 40.4, 41.3, 48.3, 50.4, 50.9, 53.5, 56.6, 59.0, 66.4, 68.5, 70.3, 70.4, 71.9, 127.1, 128.8, 128.9, 135.9, 170.8, 171.6 (2C), 173.0, 173.7; HRMS (ES^+): calc. for $[C_{39}H_{65}N_5O_8 + H]^+$ 732.4906, found 732.4920.

cyclo[Leu-Val-(*N*-TEG)Leu-Phe-Leu] (3a). Crude pentapeptide **p3a** (27 mg, 60% purity, approx. 0.036 mmol) was cyclized and purified following the procedure described above. This afforded cyclic peptide **3a** (11 mg, 42%). HPLC (linear gradient from 40% to 100% ACN over 8 min): t_r = 6.98 min, purity >99%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.77-1.01 (m, 24H), 1.24-2.02 (m, 8H), 2.02-2.17 (m, 2H), 2.94 (dd, *J* = 13.7, 8.0 Hz, 1H), 3.04-3.24

(m, 1H), 3.30-3.41 (m, 4H), 3.45-3.67 (m, 11H), 3.63-3.83 (m, 1H), 4.13-4.23 (m, 2H), 4.52-4.64 (m, 2H), 6.40 (d, *J* = 5.1 Hz, 1H), 6.45 (d, *J* = 9.5 Hz, 1H), 7.11-7.34 (m, 5H), 6.82 (d, *J* = 7.5 Hz, 1H), 8.42 (d, *J* = 9.4 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 18.1, 19.4, 21.4, 22.2, 22.3, 22.4, 22.7, 22.8, 24.5, 25.2, 25.4, 29.7, 31.1, 36.9, 38.5, 39.8, 40.9, 51.2, 51.9, 53.9, 54.4, 55.4, 59.0, 67.9, 69.0, 70.3, 70.5, 70.6, 71.9, 126.7, 128.5, 129.1, 136.7, 171.5, 171.6, 171.7, 172.0, 173.5; HRMS (ES^+): calc. for $[C_{39}H_{65}N_5O_8 + H]^+$ 732.4906, found 732.4919.

cyclo[Val-Leu-(*N*-TEG)Phe-Leu-Leu] (4a). Crude pentapeptide **p4a** (27 mg, 53% purity, approx. 0.036 mmol) was cyclized and purified following the procedure described above. This afforded cyclic peptide **4a** (14 mg, 53%). HPLC (linear gradient from 40% to 100% ACN over 8 min): t_r = 7.34 min, purity >99%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.88-1.09 (m, 24H), 1.33-1.79 (m, 9H), 2.22-2.39 (m, 1H), 3.05-3.22 (m, 2H), 3.22-3.33 (m, 3H), 3.34-3.44 (m, 4H), 3.49-3.61 (m, 7H), 3.68 (dd, *J* = 13.2, 10.2 Hz, 1H), 3.79 (dd, *J* = 9.9, 6.3 Hz, 1H), 4.13 (dd, *J* = 7.3, 5.8 Hz, 1H), 4.22 (dd, *J* = 14.6, 7.7 Hz, 1H), 4.42 (dd, *J* = 15.7, 7.1 Hz, 1H) , 4.87 (dd, *J* = 15.8, 7.2 Hz, 1H), 6.35 (bs, 1H), 6.65 (d, *J* = 6.8 Hz, 1H), 7.05 (bs, 1H), 7.13-7.29 (m, 5H), 8.10 (bs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 17.8, 19.5, 22.2, 22.2, 22.6, 22.6, 22.8, 22.9, 24.8, 24.8, 24.9, 29.4, 35.2, 39.7, 39.9, 41.5, 48.5, 51.0, 51.5, 52.8, 59.0, 60.2, 68.4, 70.3, 70.8, 71.9, 126.9, 128.5, 129.1, 137.3, 170.0, 171.2, 171.8, 173.5, 173.6; HRMS (ES^+): calc. for $[C_{39}H_{65}N_5O_8 + H]^+$ 732.4906, found 732.4917.

cyclo[Leu-Phe-(*N*-TEG)Leu-Leu-Val] (5a). Crude pentapeptide **p5a** (27 mg, 66% purity, approx. 0.036 mmol) was cyclized and purified following the procedure described above. This afforded cyclic peptide **5a** (11 mg, 42%). HPLC (linear gradient from 40% to 100% ACN over 8 min): t_r = 7.05 min, purity >99%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.83 (d, *J* = 6.6 Hz, 3 H), 0.84-1.01 (m, 21H), 1.20-1.35 (m, 1H), 1.35-1.50 (m, 1H), 1.49-1.69 (m, 4H), 1.92 (m, 1H), 2.01 (m, 2H), 2.10 (bs, 2H), 2.88 (dd, *J* = 13.1, 5.8 Hz, 1H), 3.08 (dd, *J* = 13.1, 9.0 Hz, 1H), 3.17-3.29 (m, 1H), 3.32-3.43 (m, 4 H), 3.45 (m, 1H), 3.45-3.66 (m, 9H), 3.79 (t, *J* = 8.9 Hz, 1H), 4.20 (m, 1H), 4.45 (dd, *J* = 16.3, 7.3 Hz, 1H), 5.10 (td, *J* = 8.9, 6.0 Hz, 1H), 6.37 (bs, 1H), 6.74 (d, *J* = 8.6 Hz, 1H), 7.13 (bs, 1H), 7.14-7.33 (m, 5H), 8.08 (d, *J* = 9.1 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 19.1, 19.1, 21.1, 22.3, 22.3, 22.6, 22.8, 22.9, 24.9, 25.0, 25.3, 29.5, 38.3, 38.5, 40.1, 40.5, 51.2, 51.2, 51.3, 53.6, 58.9, 60.6, 68.0, 68.8, 70.3, 70.4, 70.5, 71.9, 126.6, 128.6, 129.6, 136.8, 171.1, 171.5, 171.7, 172.7, 173.4.HRMS (ES^+): calc. for $[C_{39}H_{65}N_5O_8 + H]^+$ 732.4906, found 732.4908.

cyclo[Phe-Leu-*N*MeLeu-Val-Leu] (1b). Crude pentapeptide **p1b** (22 mg, 67% purity, approx. 0.036 mmol) was cyclized and purified following the procedure described above. This afforded cyclic peptide **1b** (13 mg, 60%). HPLC (linear gradient from 40% to 100% ACN over 8 min): t_r = 7.36 min, purity = 95%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.63-1.04 (m, 24H), 1.05-1.74 (m, 7H), 1.87-2.22 (m, 2H), 3.19-3.48 (m, 6H), 3.45-3.90 (m, 12H), 3.91 (m, 1H), 4.01-4.27 (m, 2H), 4.47 (m, 1H), 4.95 (m, 1H), 6.28 (bs, 1H), 6.97 (bs, 1H), 7.11-7.36 (m, 5H), 7.38-7.68 (m, 1H), 7.78 (bs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 19.0, 19.1, 21.9, 22.4, 22.6, 22.7, 22.7, 23.0, 24.5, 24.8, 25.7, 29.7, 31.9, 34.2, 37.2, 39.8, 41.6, 48.6, 50.5, 52.6, 56.1, 58.9, 59.8, 66.4, 68.6, 70.2, 70.7, 71.6, 127.4, 128.8, 129.0, 136.0, 170.2, 172.6, (2C), 173.0 (2C); HRMS (ES^+): calc. for $[C_{33}H_{53}N_5O_5 + H]^+$ 600.4119, found 600.4131.

cyclo[Leu-Leu-*N*MeVal-Leu-Phe] (2b). Crude pentapeptide **p2b** (22 mg, 78% purity, approx. 0.036 mmol) was cyclized and purified following the procedure described above. This afforded cyclic peptide **2b** (10 mg, 46%). HPLC (linear gradient from 40% to 100% ACN over 8 min): t_r = 7.07 min, purity >99%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.62 (d, *J* = 5.0 Hz, 3 H), 0.73 (m, 3H), 0.84-0.99 (m, 18H), 1.20-1.34 (m, 2H), 1.34-1.51 (m, 2H), 1.45-1.79 (m, 5H), 2.84-3.14 (m, 6 H), 3.68 (m, 1H), 3.97 (m, 1H), 4.20 (m, 1H), 4.47 (m, 1H), 4.85 (d, *J* = 6.8 Hz, 1H), 5.71 (bs. 1H), 6.87 (bs, 1H), 7.04-7.31 (m, 5H), 8.04 (bs, 1H), 8.14 (bs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 19.1, 19.9, 21.0, 21.9, 22.6, 22.6, 22.7, 23.1, 24.2, 24.6, 25.0, 27.4, 29.7, 31.9, 34.2, 36.5, 39.3, 40.3, 40.9, 48.3, 51.5, 53.2, 57.5, 65.1, 127.1, 128.8, 128.9, 136.0, 170.6, 171.6, 172.2, 172.4, 174.3; HRMS (ES^+): calc. for $[C_{33}H_{53}N_5O_5 + H]^+$ 600.4119, found 600.4112.

cyclo[Leu-Val-*N*MeLeu-Phe-Leu] (3b). Crude pentapeptide **p3b** (22 mg, 65% purity, approx. 0.036 mmol) was cyclized and purified following the procedure described above. This afforded cyclic peptide **3b** (11 mg, 51%). HPLC (linear gradient from 40% to 100% ACN over 8 min): t_r = 6.82 min, purity >99%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.77 (d, *J* = 6.3 Hz, 3H), 0.84 (d, *J* = 6.4 Hz, 6H), 0.95-0.82 (m, 12H), 0.97 (d, *J* = 6.2 Hz, 3H), 1.22-1.36 (m, 2H), 1.36-1.51 (m, 2H), 1.51-1.90 (m, 4H) 2.14 (m, 2H), 2.92-3.02 (m, 1H), 3.02-3.13 (m, 1H), 3.07 (s, 3H), 3.44 (t, *J* = 7.6 Hz, 1H), 4.05-4.27 (m, 2H), 4.49 (t, *J* = 9.4 Hz, 1H), 4.58 (dd, *J* = 16.7, 8.5 Hz, 1H), 6.54 (bs, 1H), 6.73 (bs, 1H), 7.03 (bs, 1H), 7.07-7.58 (m, 5H), 8.13 (bs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 18.1, 19.6,21.3, 21.9, 22.0, 22.5, 22.7, 22.8, 24.4, 25.2, 25.5, 29.7, 30.6, 31.9, 34.2, 36.7, 37.7, 39.8, 40.6, 52.1, 54.3, 54.6, 55.3, 68.9, 126.8, 128.5, 129.1, 136.6, 171.9, 172.1, 172.4, 172.6, 174.3; HRMS (ES^+): calc. for $[C_{33}H_{53}N_5O_5 + H]^+$ 600.4119, found 600.4109.

cyclo[Val-Leu-*N*MePhe-Leu-Leu] (4b). Crude pentapeptide **p4b** (22 mg, 83% purity, approx. 0.036 mmol) was cyclized and purified following the procedure described above. This afforded cyclic peptide **4b** (11 mg, 51%). HPLC (linear gradient from 40% to 100% ACN over 8 min): t_r = 6.97 min, purity >99%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.84 (d, *J* = 6.6 Hz, 6 H), 0.87-0.99 (m, 18 H), 1.27-1.73 (m, 9H), 2.33 (m, 1H), 2.74 (s, 3 H), 3.22 (dd, *J* = 11.6, 7.3 Hz, 1H), 3.69 (m, 1H), 3.70 (m, 1H), 4.11 (m, 1H), 4.24 (d, *J* = 6.9 Hz, 1H), 4.45 (dd, *J* = 15.5 Hz, 7.3 Hz, 1H), 4.73 (dd, *J* = 15.3 Hz, 7.2 Hz, 1H), 6.38 (bs, 1H), 6.85 (d, *J* = 8.3 Hz, 1H), 7.05-7.35 (m, 6H), 8.04 (bs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 17.6, 19.5, 22.2, 22.3, 22.4, 22.5, 22.5, 22.7, 24.6, 24.8, 24.9, 29.1, 29.7, 34.9, 39.6, 40.0, 41.2, 48.3, 51.7, 53.0, 60.2, 72.1, 127.0, 128.6, 128.8, 137.0, 170.1, 171.3, 172.6, 172.8, 173.6; HRMS (ES^+): calc. for $[C_{33}H_{53}N_5O_5 + H]^+$ 600.4119, found 600.4130.

cyclo[Leu-Phe-*N*MeLeu-Leu-Val] (5b). Crude pentapeptide **p5b** (22 mg, 80% purity, approx. 0.036 mmol) was cyclized and purified following the general procedure described above. This afforded cyclic peptide **5b** (10 mg, 46%). HPLC (linear gradient from 40 to 100% ACN over 8 min): t_r = 6.86 min, purity >99%; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 0.77 (d, *J* = 6.6 Hz, 3H), 0.81 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.0 Hz, 3H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.93 (d, *J* = 5.8 Hz, 3H), 0.94 (d, *J* = 6.2 Hz, 1H), 0.96 (d, *J* = 6.1 Hz, 3H), 1.01 (d, *J* = 6.6 Hz, 3H), 1.15 (m, 1H), 1.36 (m, 1H), 1.51-1.73 (m, 5H), 2.01 (m, 1H), 2.23 (m, 1H), 2.92 (s, 3 H), 2.85 (dd, *J* = 13.3, 6.1 Hz, 1H), 3.14 (dd, *J* = 13.3, 8.8 Hz, 1H), 3.43 (dd, *J* = 9.6, 6.7 Hz, 1H), 3.69 (dd, *J* = 9.8, 7.9 Hz, 1H), 4.21 (m, 1H), 4.46 (dd, *J* = 17.0, 7.5 Hz, 1H), 5.05 (ddd, *J* = 9.2, 8.8, 6.1 Hz, 1H), 6.14 (d, *J* = 6.5 Hz, 1H), 6.75 (d, *J* = 9.2 Hz, 1H), 7.18-

7.28 (m, 5H), 6.93 (d, *J* = 7.4 Hz, 1H), 8.07 (d, *J* = 9.7 Hz, 1H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 19.1, 19.2, 20.9, 21.6, 22.3, 22.5, 23.0, 23.0, 25.0, 25.1, 25.2, 29.3, 38.0, 38.1, 39.7, 40.4, 51.2, 51.5, 53.4, 61.2, 70.0, 126.6, 128.3, 129.6, 136.9, 170.9, 171.6, 171.9, 171.9, 173.8; HRMS (ES^+): calc. for $[C_{33}H_{53}N_5O_5 + H]^+$ 600.4119, found 600.4125.

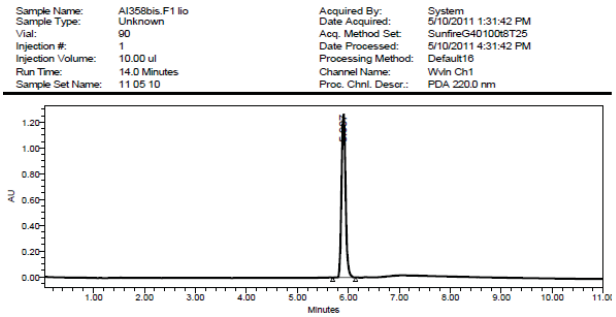
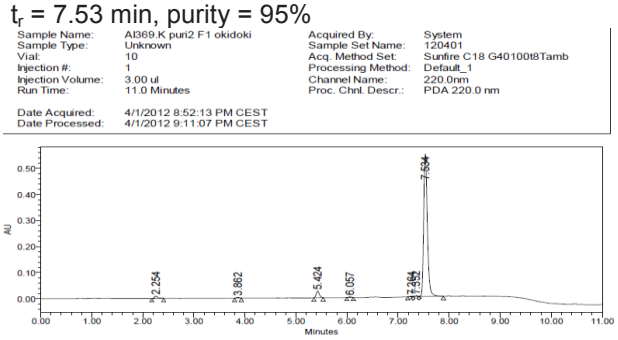
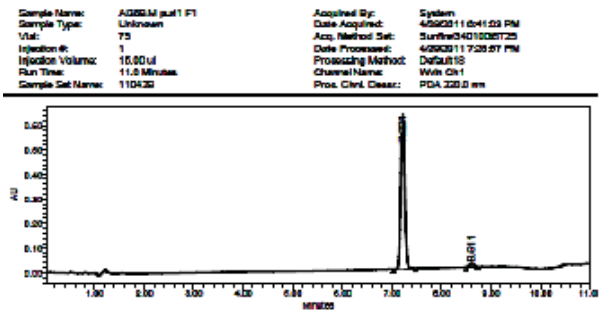
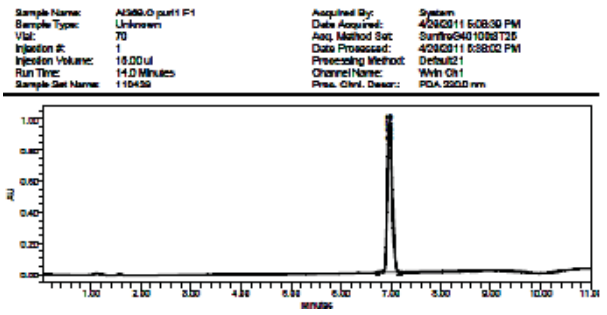
3. BIOLOGICAL ACTIVITY EVALUATION

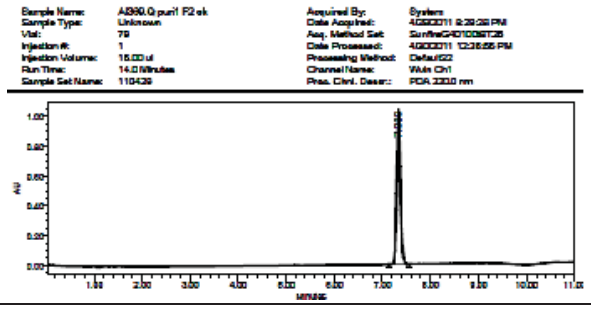
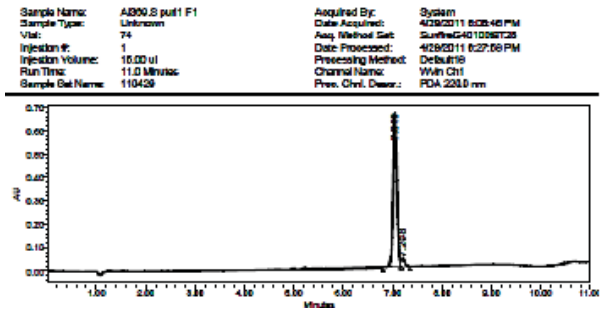
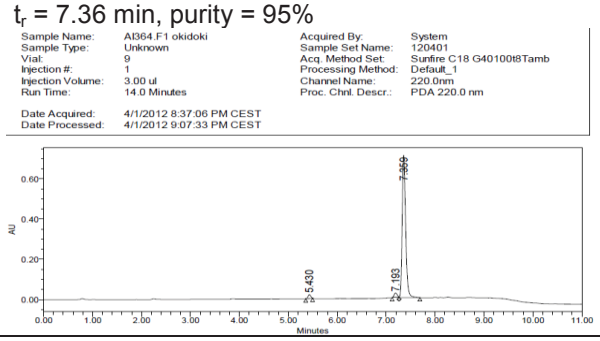
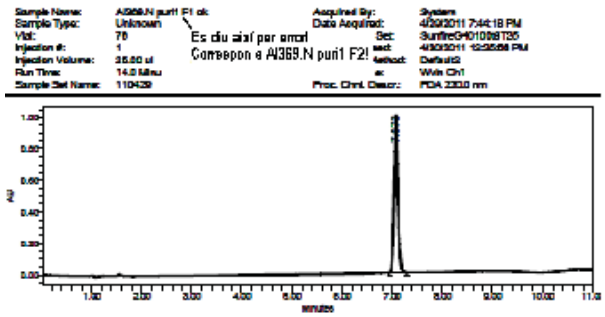
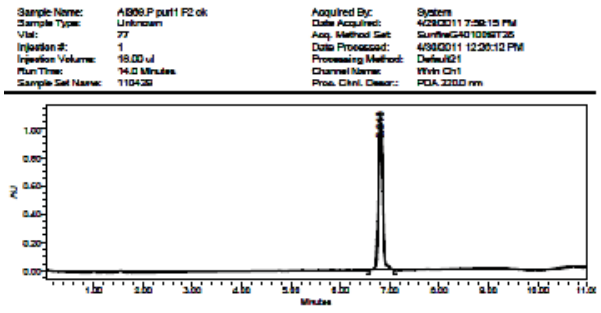
Cancer cell lines. The cancer cell lines that have been used in this study are GLC-4 (small cell lung cancer) and MDA-MB-231 (breast cancer). Both proceeded from the American Type Culture Collection (ATCC).

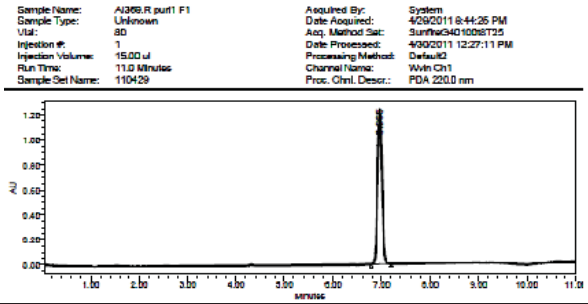
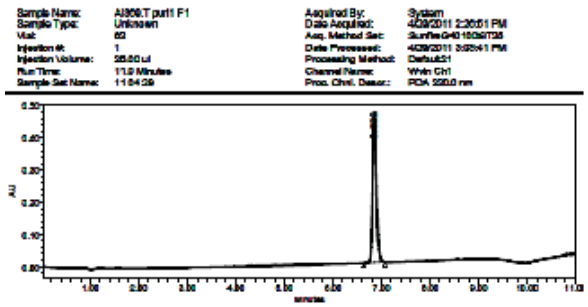
Growing of cancer cell lines. GLC-4 cancer cells were grown in suspension and they were regularly seeded into 25 cm^2 or 75 cm^2 flasks. MDA-MB-231 cancer cells were grown as an adherent culture and they were regularly seeded into 60 mm or 100 mm plates. The medium was RPMI 1640 (Roswell Park Memorial Institute) for GLC-4 cancer cells and DMEM (Dubelco's modified Eagle's medium) for MDA-MB-231 cancer cells. The medium was supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 u/mL penicillin and 100 µg/mL streptamicin. Cells were maintained at 37 ºC in humidified 5% CO_2 and the medium was changed every few days. Cell growth was monitorized under a phase-contrast microscope and, when an 80% confluence was reached, cells were split. In case of adherent cell cultures, they were digested with trypsin prior to split.

Cytotoxicity assay. The ability of our compounds to induce cell cytotoxicity was tested on 3 cancer cell lines. GLC-4 cells were seeded into 96-well plates at a concentration of 20000 cells/well. MDA-MB-231 cells were seeded into 96-well plates at a concentration of 5000 cells/well and incubated at 37 ºC during 24 h prior to drug addition. The different compounds were added at a 50 µM concentration, the final volume in each well being 100 µL. The DMSO concentration was kept constant at 1% in all wells. Cells were cultured with and without the compounds for 72 h. After this time, the % of cell viability was determined through MTT assay. To each well, 10 µL of MTT reagent (i.e. a 5 mg/mL solution of MTT in PBS) were added and the plate was incubated for 4 h. Then, the purple formazan crystals were dissolved by adding 100 µL of isopropanol/$HCl_{(aq.)}$ 1 M 24:1. The absorbance at 570 nm was measured in an ELISA plate reader. 100% cell viability is given to the net absorbance of the untreated cells, which are used as a control, and the % of cell viability is expressed as a percentage of the untreated control cells. Each experiment was performed three times, seeding the plates at different times and, for each plate, all test conditions were assayed in triplicate wells. Results were averaged and expressed with the corresponding standard deviation.

4. HPLC SPECTRA OF THE CYCLIC PENTAPEPTIDES

Compound	**HPLC analysis** (linear gradient from 40 to 10% ACN over 8 min)
cyclo[Phe-Leu-Leu-Val-Leu] (**1**)	t_r = 5.97 min, purity >99% 
cyclo[Phe-Leu-(*N*-TEG)Leu-Val-Leu] (**1a**)	t_r = 7.53 min, purity = 95% 
cyclo[Leu-Leu-(*N*-TEG)Val Leu-Phe] (**2a**)	t_r = 7.22 min, purity = 98% 
cyclo[Leu-Val-(*N*-TEG)Leu-Phe-Leu] (**3a**)	t_r = 6.98 min, purity >99% 

cyclo[Val-Leu-(*N*-TEG)Phe-Leu-Leu] (**4a**)	t_r = 7.34 min, purity >99% 
cyclo[Leu-Phe-(*N*-TEG)Leu-Leu-Val] (**5a**)	t_r = 7.05 min, purity >99% 
cyclo[Phe-Leu-*N*MeLeu-Val-Leu] (**1b**)	t_r = 7.36 min, purity = 95% 
cyclo[Leu-Leu-*N*MeVal-Leu-Phe] **(2b)**	t_r = 7.07 min, purity >99% 
cyclo[Leu-Val-*N*MeLeu-Phe-Leu] **(3b)**	t_r = 6.81 min, purity >99% 

cyclo[Val-Leu-*N*MePhe-Leu-Leu] **(4b)**	t_r = 6.97 min, purity >99% 
cyclo[Leu-Phe-*N*MeLeu-Leu-Val] **(5b)**	t_r = 6.86 min, purity >99% 

5. NMR DATA

Comparison of the C^{α}-chemical shifts of the cyclic pentapeptides

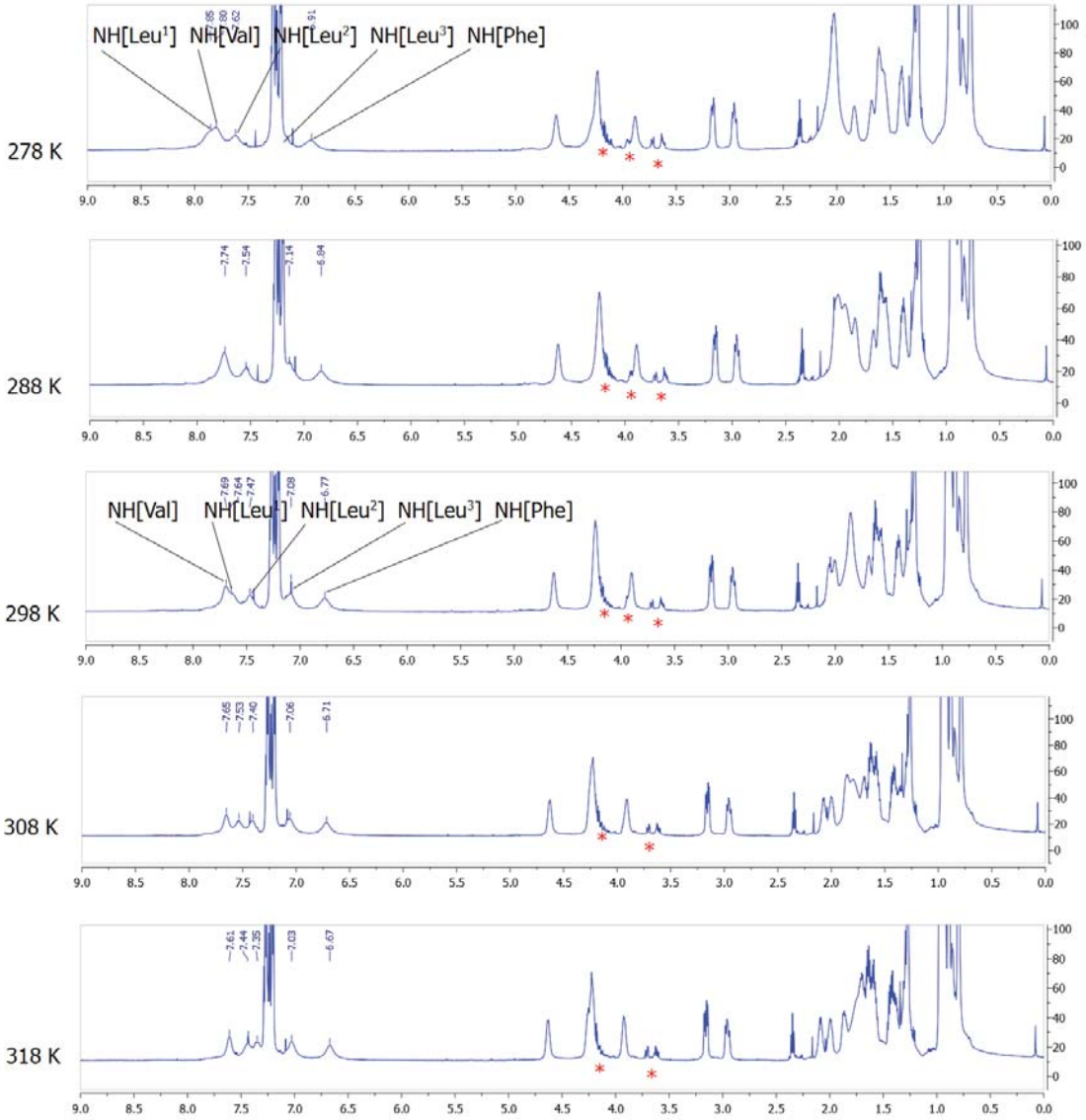
Table S1. C^{α}-chemical shifts (ppm) of **1**, **1a-5a** and **1b-5b** in $CDCl_3$.

1	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b
51.7	48.6	49.2	48.3	48.3	51.9	52.1	48.5	48.3	51.2	51.2
53.7	52.6	55.0	50.4	51.5	53.9	54.3	51.5	51.7	51.4	51.5
55.3	56.1	56.0	53.5	53.2	54.4	54.6	52.8	53.0	53.6	53.4
57.9	59.8	59.1	56.6	57.5	55.4	55.3	60.2	60.2	60.7	61.3
58.8	66.4	65.0	66.4	65.1	67.9	68.9	70.8	72.1	68.4	70.0

NMR analysis of peptides 1, 5a and 5b

Variable temperature ^{1}H-NMR experiments. For peptides **1**, **5a** and **5b**, ^{1}H-NMR spectra were acquired in the range from 278 K to 318 K with a step size of 10 K. All spectra were recorded on a Bruker Digital Avance 600 MHz spectrometer equipped with a TCI cryoprobe. Measurements were done with 5-20 mM sample solutions in $CDCl_3$. Calibration was performed with reference to the residual solvent signal (^{1}H, 7.26 ppm). Under these conditions, a single set of H^N- and H^α-signals was observed for each peptide and no evidence of conformational equilibria was found (see Figures S1, S2 and S3).

Figure S1. ^{1}H-NMR spectra (in $CDCl_3$) of **1** acquired at the indicated temperatures.



[* --> signals associated to an impuritiy. 2D-NMR spectra indicate no correlation between these signals and those associated to Sansalvamide A peptide (**1**).]

Figure S2. ^{1}H-NMR spectra (in $CDCl_3$) of **5a** acquired at the indicated temperatures.

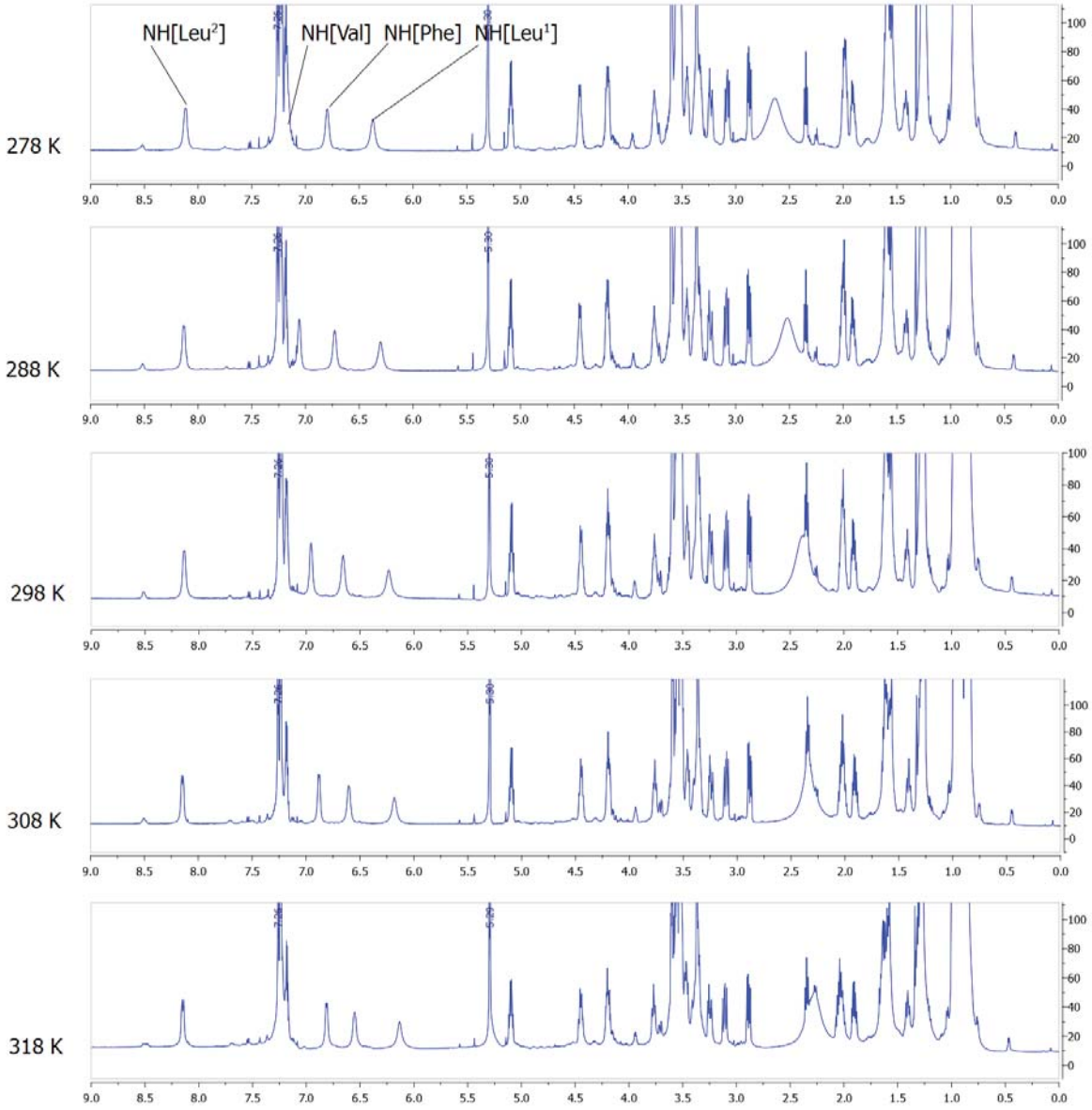
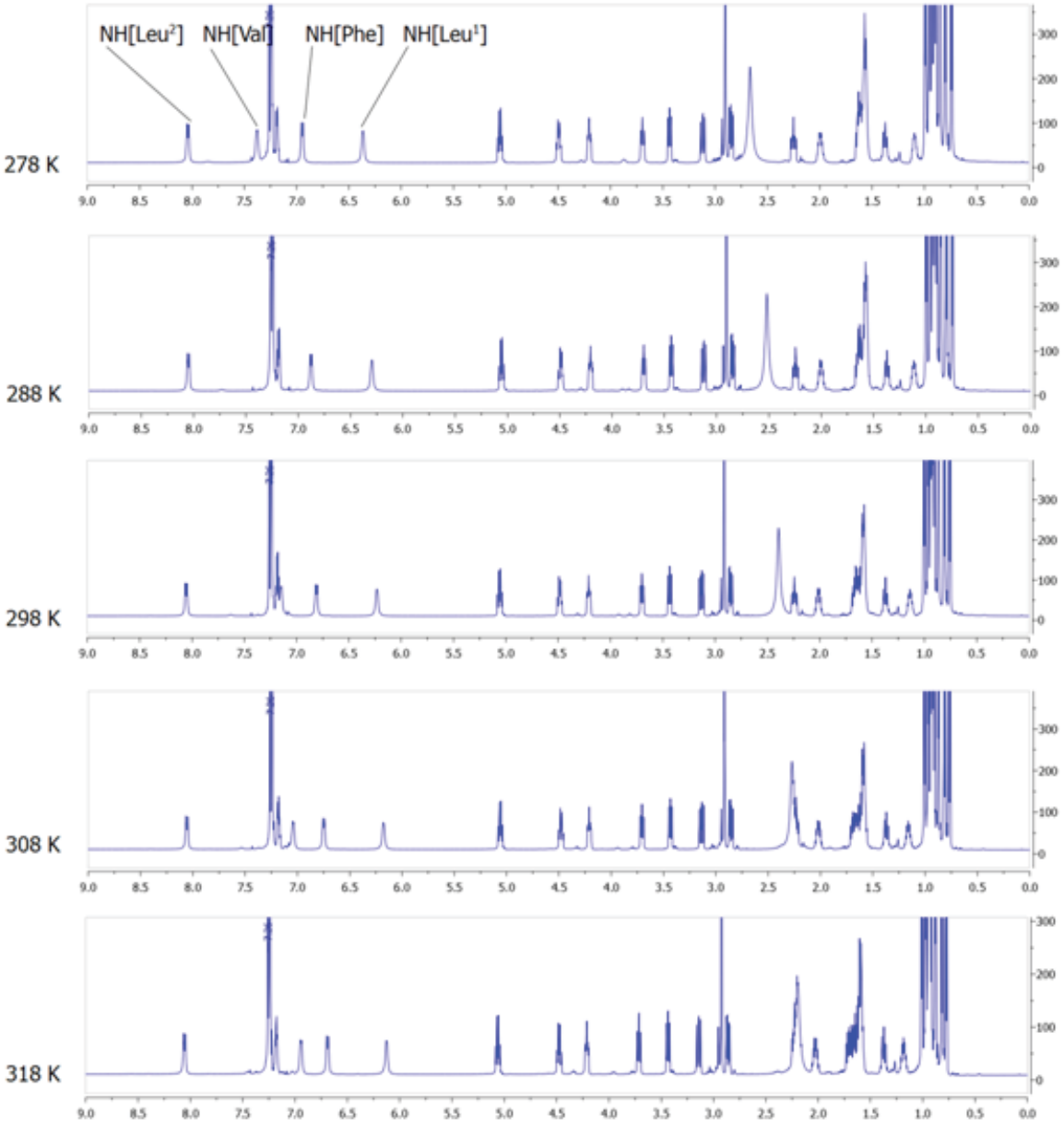


Figure S3. ^{1}H-NMR spectra (in $CDCl_3$) of **5b** acquired at the indicated temperatures.



Assignment of the H^N-, H^α- and C^α-signals. For peptides **1**, **5a** and **5b**, two-dimensional NMR experiments (COSY, HSQC, TOCSY and NOESY) were carried out. All spectra were recorded at 298 K on a Bruker Digital Avance 600 MHz spectrometer. Measurements were done with 5-20 mM sample solutions in $CDCl_3$. Calibration was performed with reference to the residual solvent signal (^1H, 7.26 ppm; ^{13}C, 77.0 ppm). TOCSY spectra were recorded with a mixing time of 70 ms, and NOESY spectra with a mixing time of 500 ms. The assignment of the H^N-, H^α- and C^α-ressonances was accomplished using by through-bond connectivities from the two-dimensional spectra. In particular, we used the $(H^\alpha)_i$-$(H^N)_{i+1}$ cross-peaks from the NOESY spectra, the $(H^\alpha)_i$-$(H^\beta)_i$ cross-peaks from the COSY spectra, the $(H^\alpha)_i$-$(H^\beta)_i$-$(H^\beta)_i$-$(H^N)_i$ the cross-peaks from the TOCSY spectra, and the $(H^\alpha)_i$-$(C^\alpha)_i$ cross-peaks from the HSQC spectra. All the signals could be unequivocally assigned. The assignment of the H^N- and H^α-resonances is given in Table S2, the assignment of the C^α-resonances is given in Table S3, and the analysis of the NOE data for the amide protons is shown in Table S4.

Table S2. H^N- and H^α-chemical shifts (ppm) of **1**, **5a** and **5b** in $CDCl_3$.

Peptide	NH [Leu^3]	NH [Leu^2]	NH [Val]	NH [Phe]	NH [Leu^1]	H^α [Phe]	H^α [Leu^2]	H^α [Leu^1]	H^α [Val]	**1:** H^α [Leu^3] **5a**: H^α [(*N*-TEG)Leu] **5b**: H^α [*N*MeLeu]
cyclo[Leu^1-Phe-Leu^3-Leu^2-Val] (**1**)	7.12	7.51	7.70	6.81	7.65	4.63	3.90	4.24	4.22	4.24
cyclo[Leu^1-Phe-(*N*-TEG)Leu-Leu^2-Val] (**5a**)	-	8.15	6.96	6.66	6.23	5.09	4.45	4.20	3.76	3.55
cyclo[Leu^1-Phe-*N*MeLeu-Leu^2-Val] (**5b**)	-	8.07	6.93	6.75	6.14	5.06	4.46	4.20	3.69	3.43

Table S3. C^α-chemical shifts (ppm) of **1**, **5a** and **5b** in $CDCl_3$.

Peptide	**1:** C^α [Leu^3] **5a**: C^α [(*N*-TEG)Leu] **5b**: C^α [*N*MeLeu]	C^α [Val]	C^α [Leu^1]	C^α [Phe]	C^α [Leu^2]
cyclo[Leu^1-Phe-Leu^3-Leu^2-Val] (**1**)	51.8 or 53.2[a]	58.7	51.8 or 53.2[a]	55.4	57.7
cyclo[Leu^1-Phe-(*N*-TEG)Leu-Leu^2-Val] (**5a**)	68.4	60.7	53.6	51.4	51.2
cyclo[Leu^1-Phe-*N*MeLeu-Leu^2-Val] (**5b**)	70.0	61.3	53.4	51.5	51.2

[a] For Sansalvamide A peptide (**1**), the ^{13}C-NMR signals at 51.8 and 53.2 ppm could not be unambiguosly assigned to C^α [Leu^3] and C^α [Leu^1], since the ^1H-NMR signals associated to H^α [Leu^3] and H^α [Leu^1] overlap at 4.24 ppm. The HSQC spectra shows cross-peaks at (4.24, 51.8) and (4.24, 53.2) ppm.

Table S4. Interproton NOEs observed for the amide protons of **1**, **5a** and **5b** in $CDCl_3$.

Peptide	Significant NOEs
cyclo[Leu^1-Phe-Leu^3-Leu^2-Val] (**1**)	NH [Phe] - H_α [Leu^1] NH [Leu^1] - H_α [Val] NH [Val] - H_α [Leu^2] NH [Leu^2] -H_α [Leu^3] NH [Leu^3] - H_α [Phe]
cyclo[Leu^1-Phe-(*N*-TEG)Leu-Leu^2-Val] (**5a**)	NH [Phe] - H_α [Leu^1] NH [Leu^1] - H_α [Val] NH [Val] - H_α [Leu^2] NH [Leu^2] - H_α [(*N*-TEG)Leu] NH [Phe] - NH [Leu^2] NH [Phe] - NH [Leu^1] NH [Val] - NH [Leu^2]
cyclo[Leu^1-Phe-*N*MeLeu-Leu^2-Val] (**5b**)	NH [Phe] - H_α [Leu^1] NH [Leu^1] - H_α [Val] NH [Val] - H_α [Leu^2] NH [Leu^2] - H_α [*N*MeLeu] NH [Phe] - NH [Leu^2] NH [Phe] - NH [Leu^1] NH [Val] - NH [Leu^2]

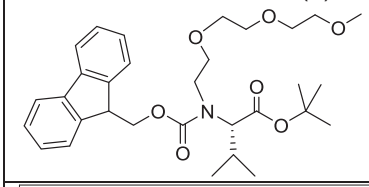
Determination of temperature coefficients for the amide protons. For peptides **1**, **5a** and **5b**, the temperature coefficients for the amide protons were determined from the variable temperature ^{1}H-NMR experiments (see Table S5). The H^N-chemical shifts were plotted versus the acquisition temperature (278 K, 288 K, 298 K, 308 K, 318 K) and the data points were adjusted to a linear fit using an Excel software package. In all cases, a good linear correlation (R^2 >0.98) was observed.

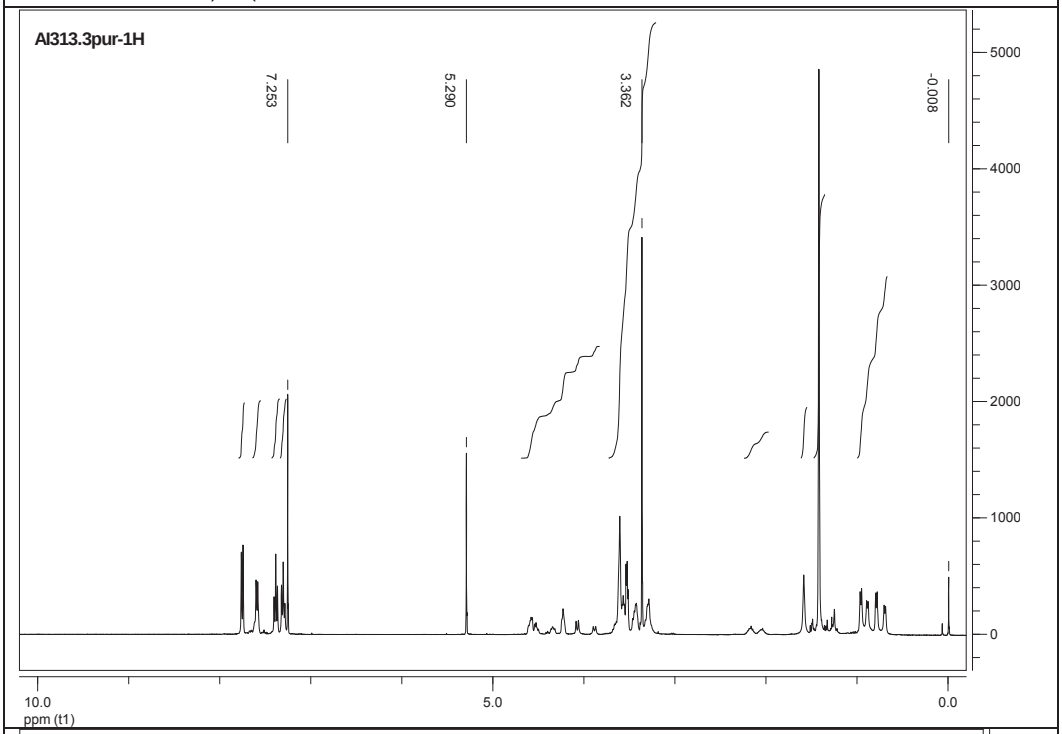
Table S5. Temperature coefficients (ppb K^{-1}) for the amide protons of **1**, **5a** and **5b** in $CDCl_3$.

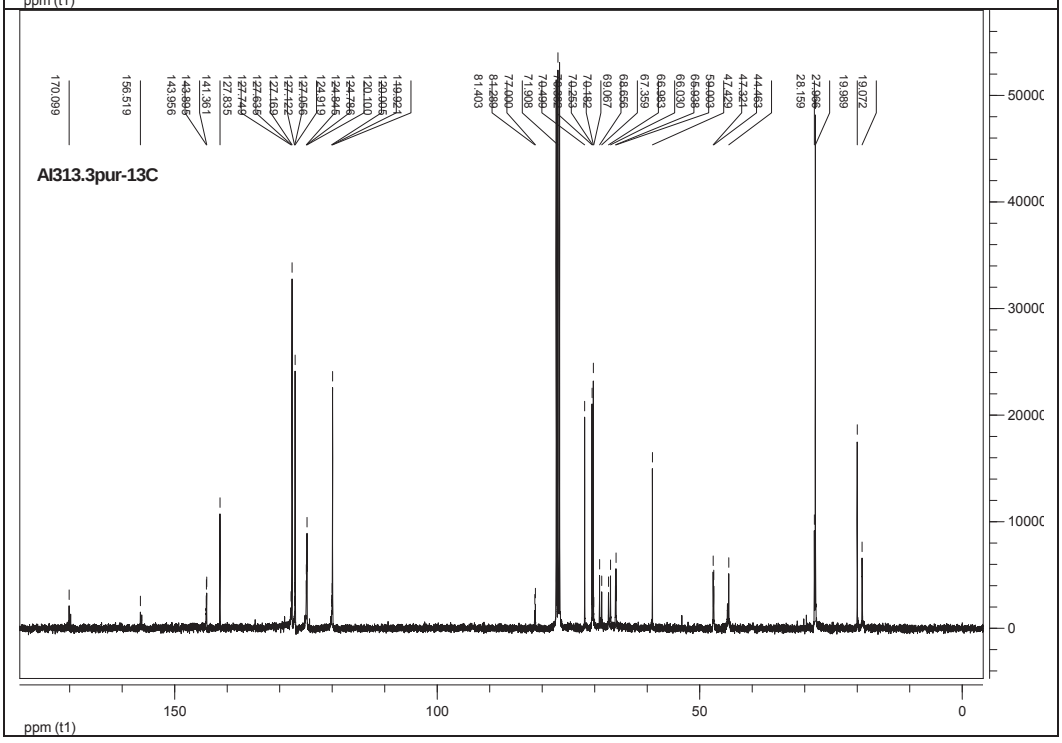
Peptide	Δδ/ΔT [ppb K^{-1}]				
	NH [Leu^3]	NH [Leu^2]	NH [Val]	NH [Phe]	NH [Leu^1]
cyclo[Leu^1-Phe-Leu^3-Leu^2-Val] (**1**)	-3.5	-6.8	-4.8	-6.1	-10.3
cyclo[Leu^1-Phe-(*N*-TEG)Leu-Leu^2-Val] (**5a**)	-	<0.1	-8.7	-6.3	-6.0
cyclo[Leu^1-Phe-*N*MeLeu-Leu^2-Val] (**5b**)	-	<0.1	-10.6	-6.3	-6.0

6. COPIES OF THE ^{1}H- and ^{13}C-NMR SPECTRA

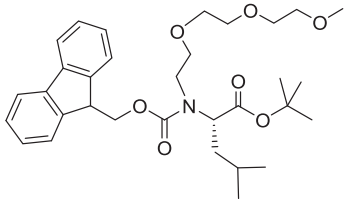
Fmoc-*N*-TEG-Val-O^{t}Bu (6)

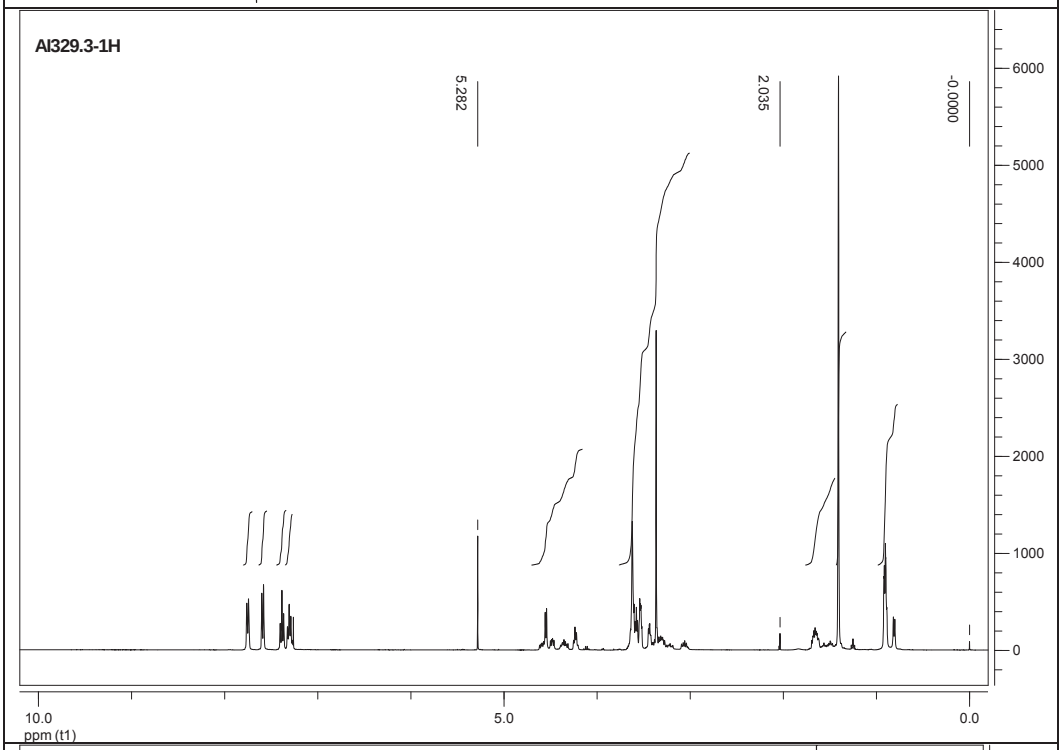


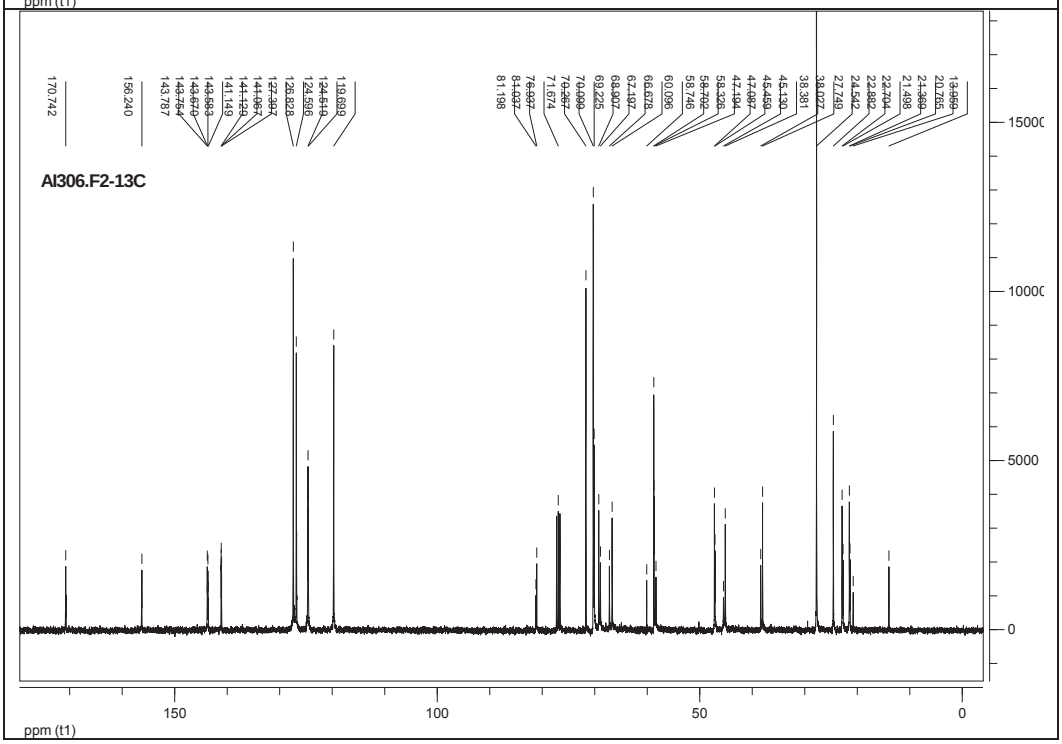




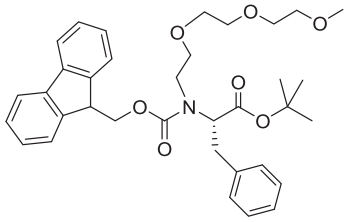
Fmoc-*N*-TEG-Leu-O^{t}Bu (7)

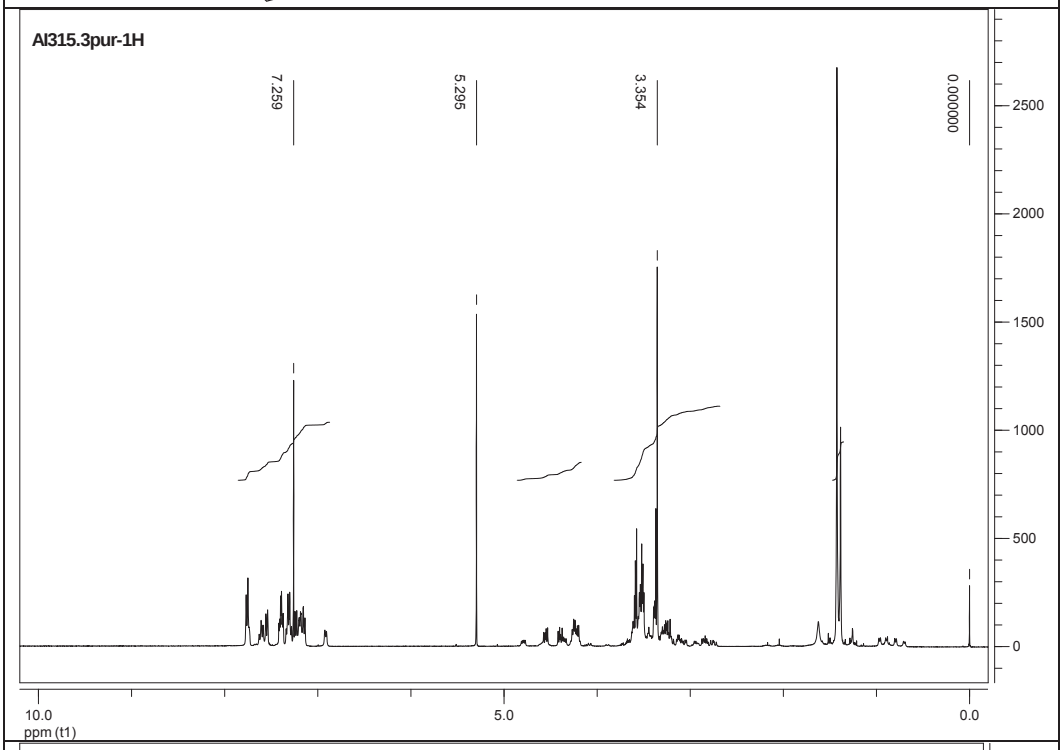


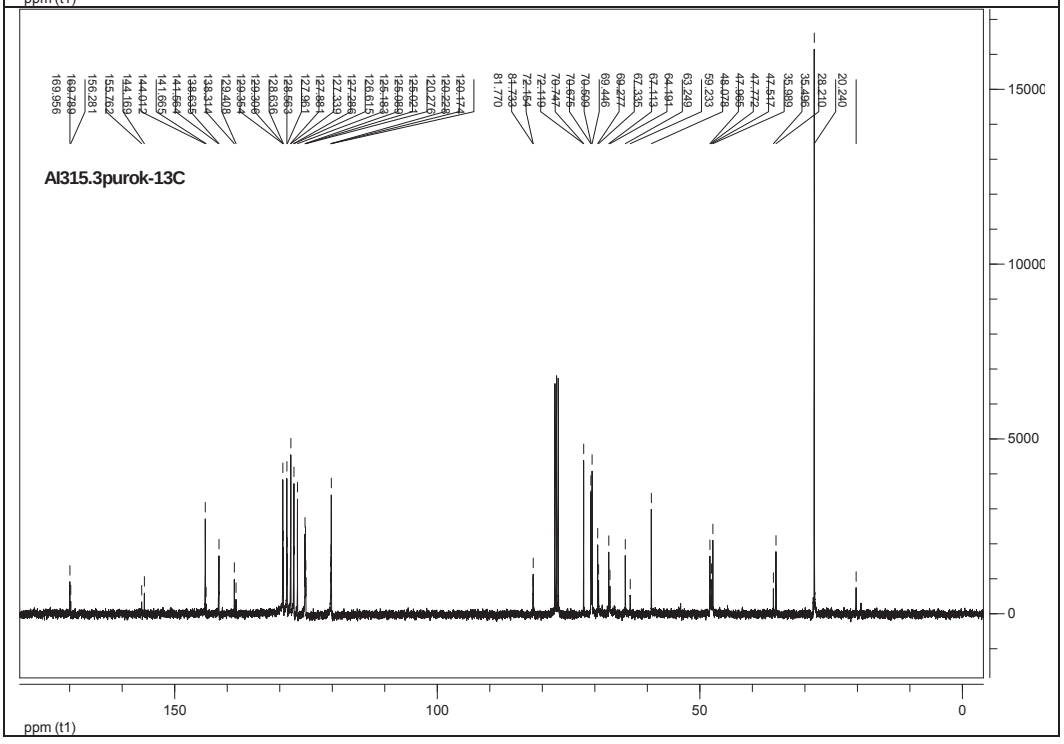




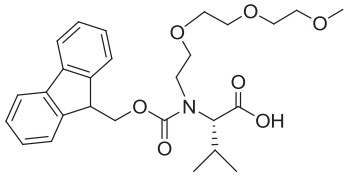
Fmoc-*N*-TEG-Phe-O^{t}Bu (8)

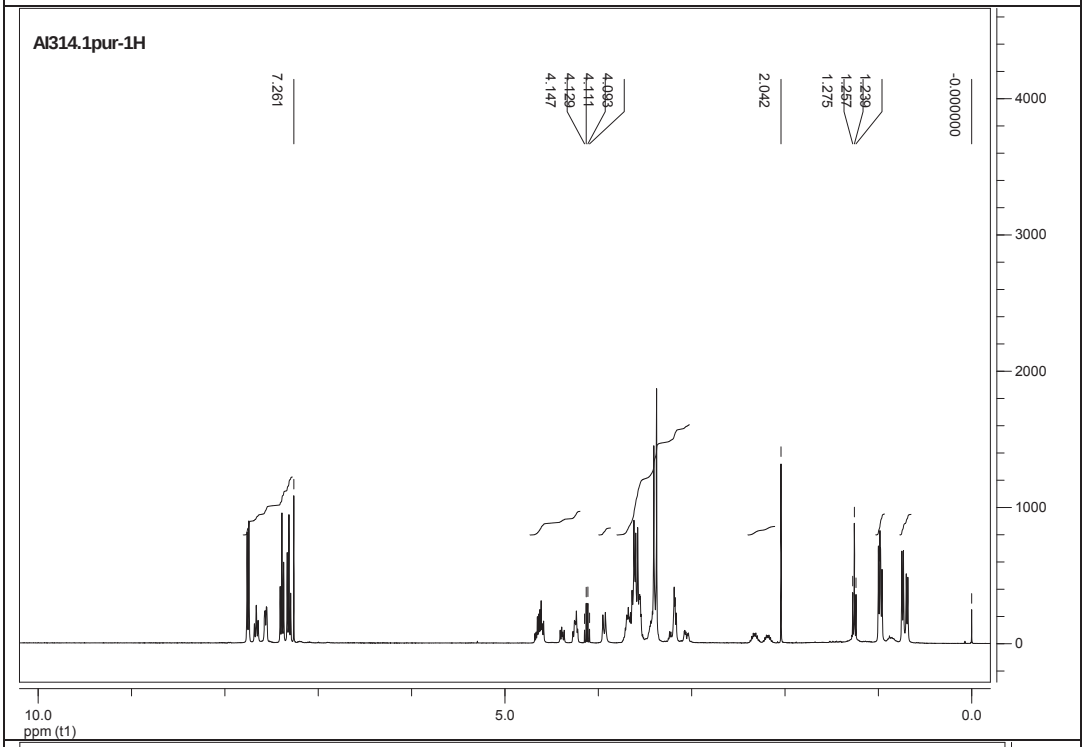


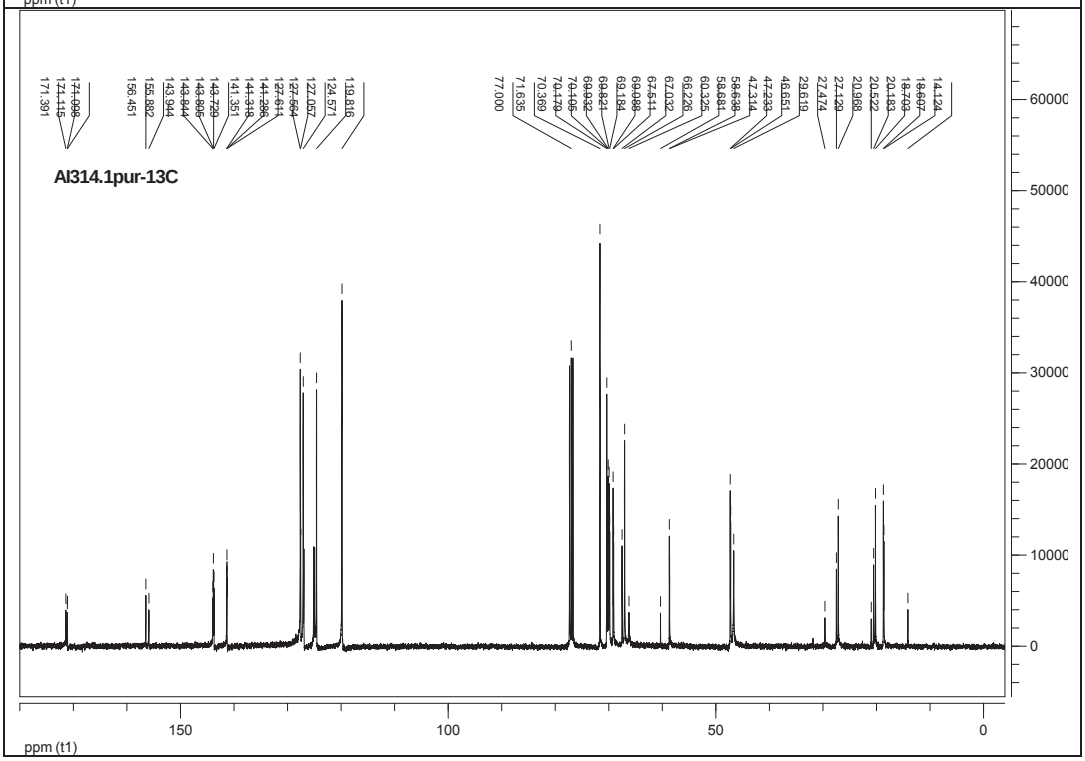




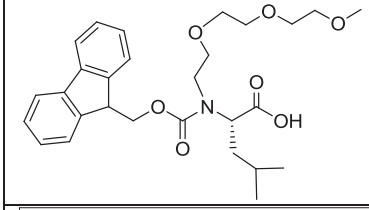
Fmoc-*N*-TEG-Val-OH (9)

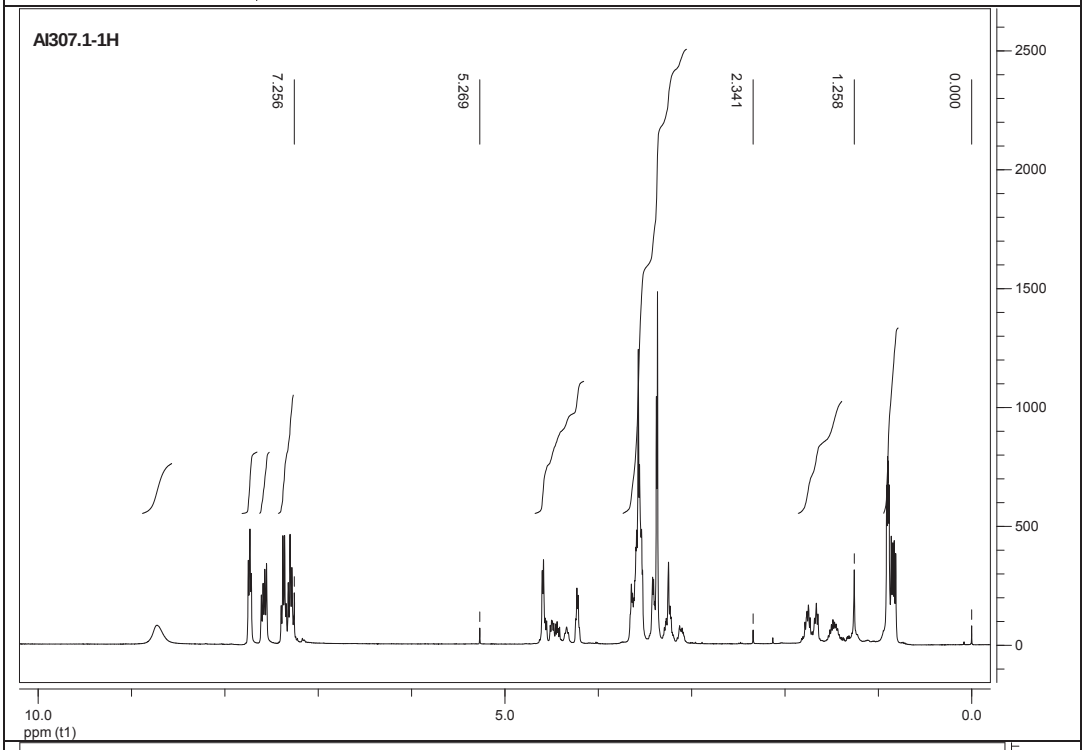


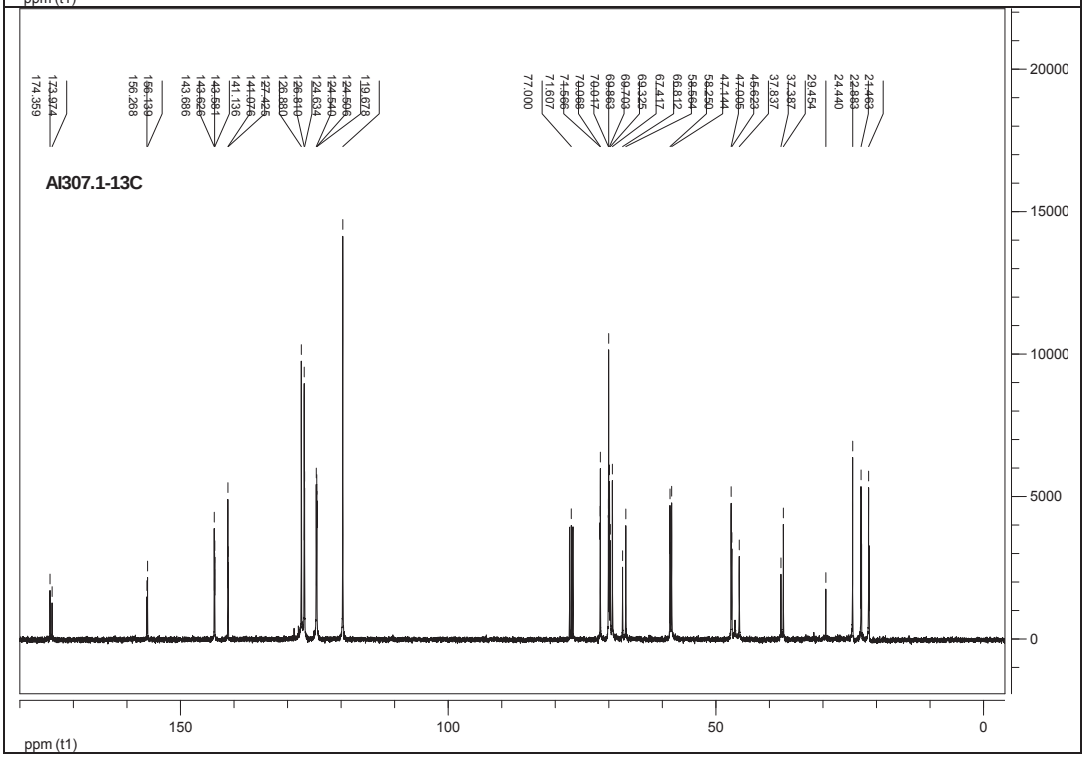




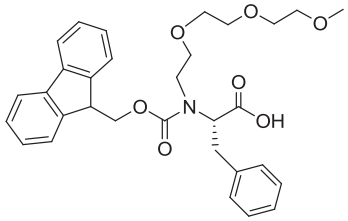
Fmoc-*N*-TEG-Leu-OH (10)

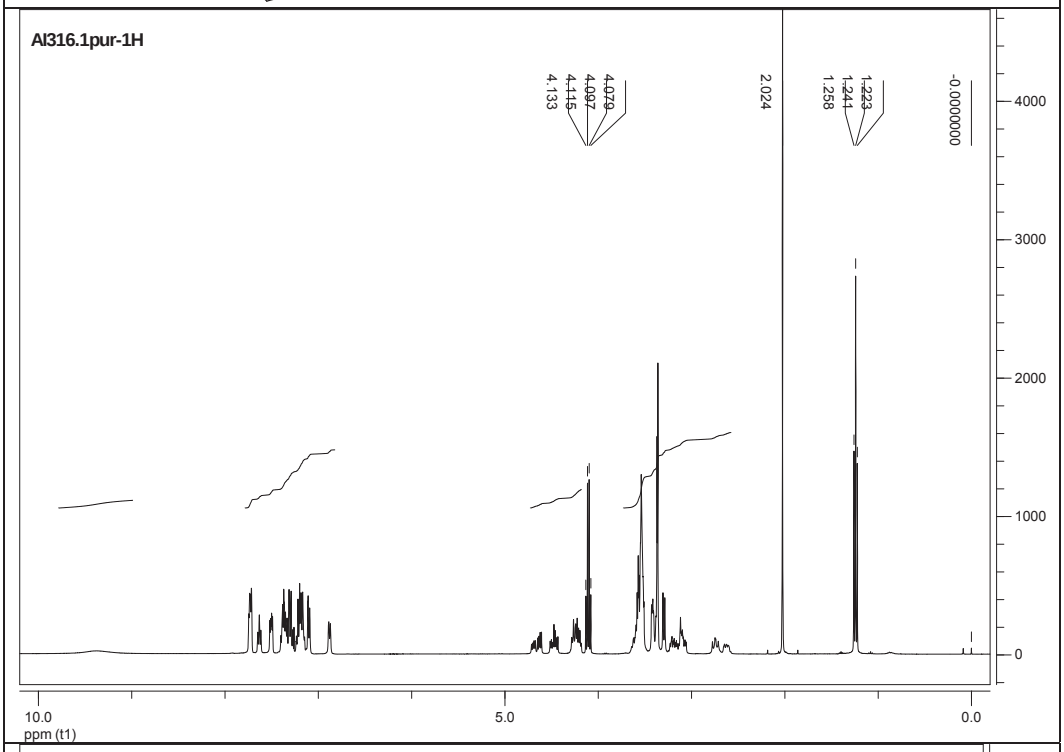


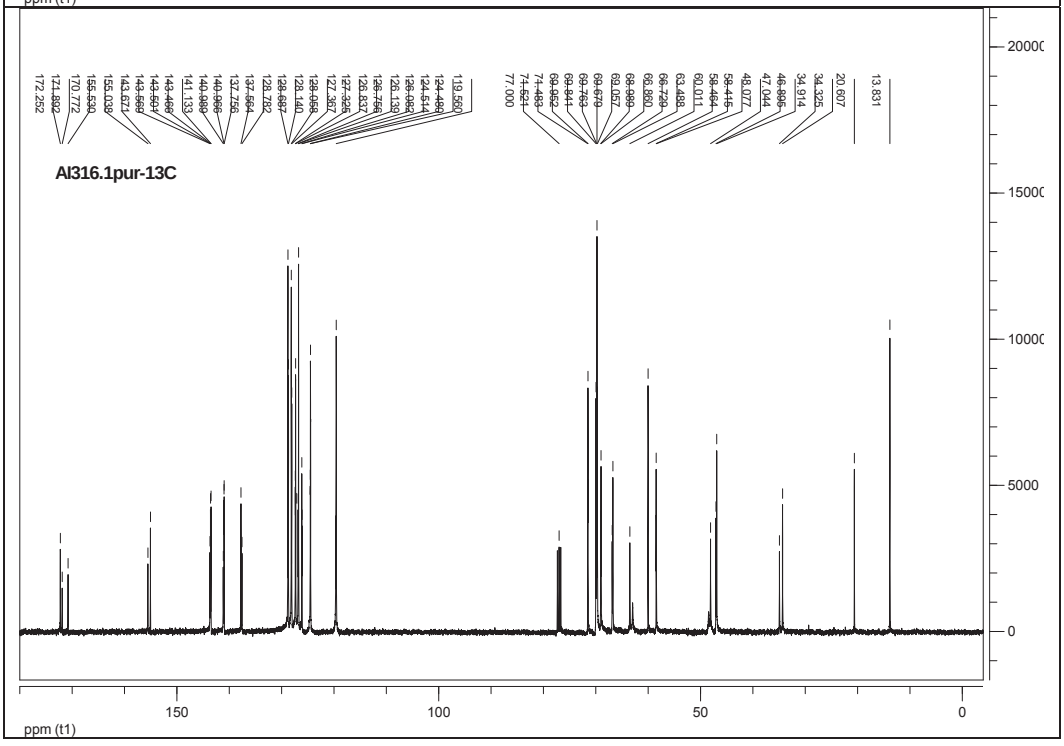




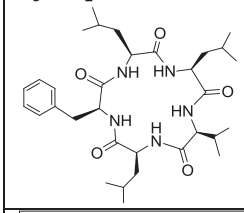
Fmoc-*N*-TEG-Phe-O^{t}Bu (11)

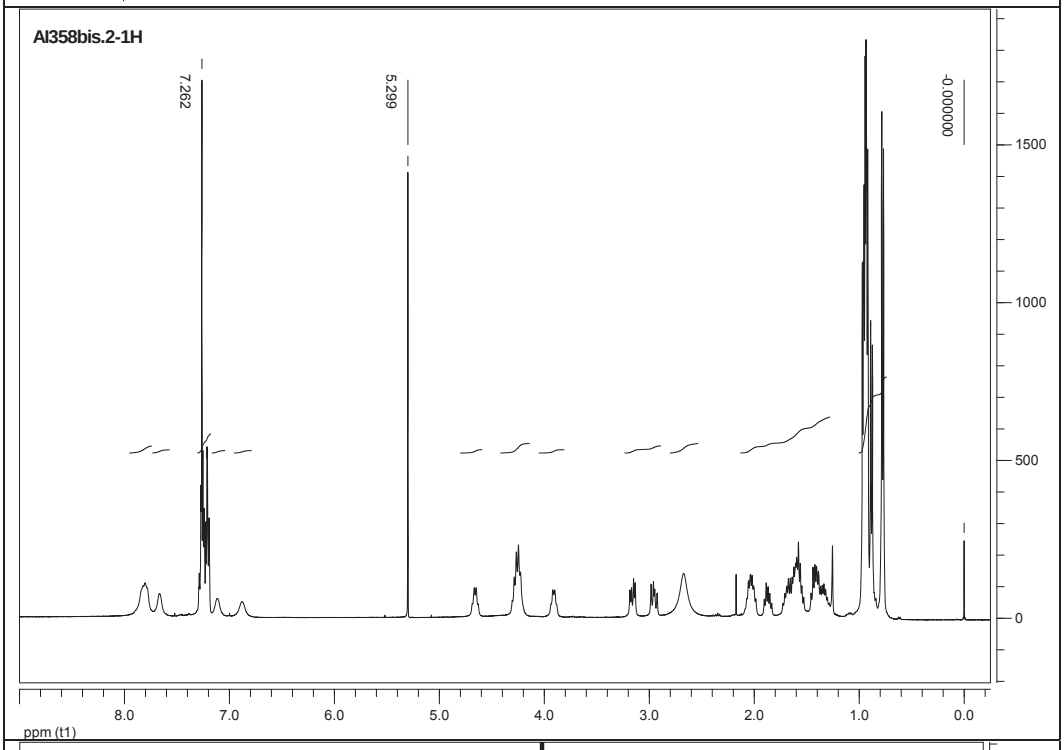


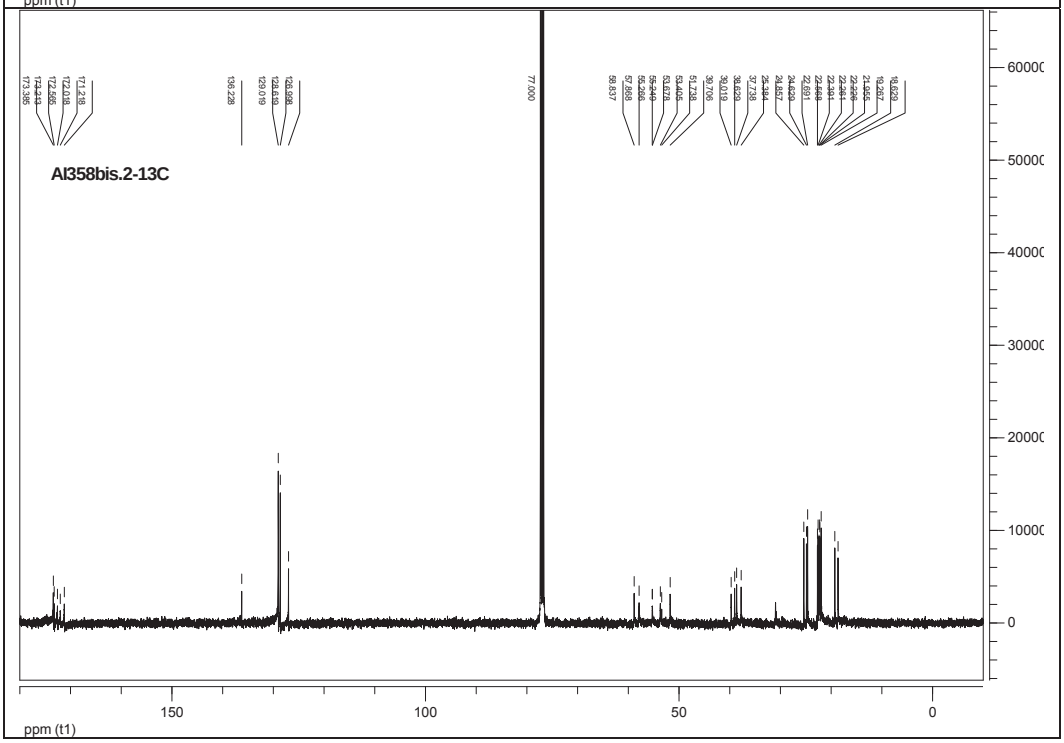




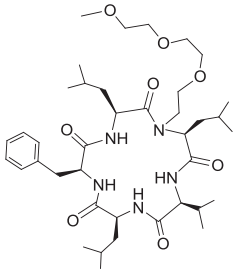
cyclo[Phe-Leu-Leu-Val-Leu] (1)

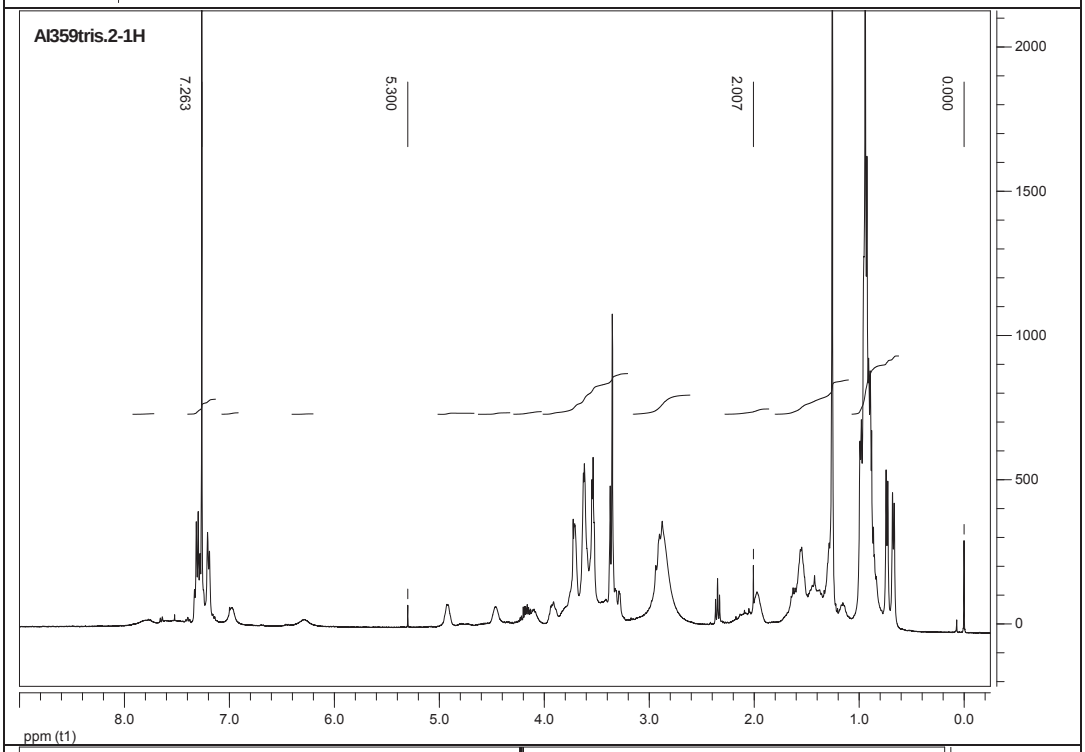


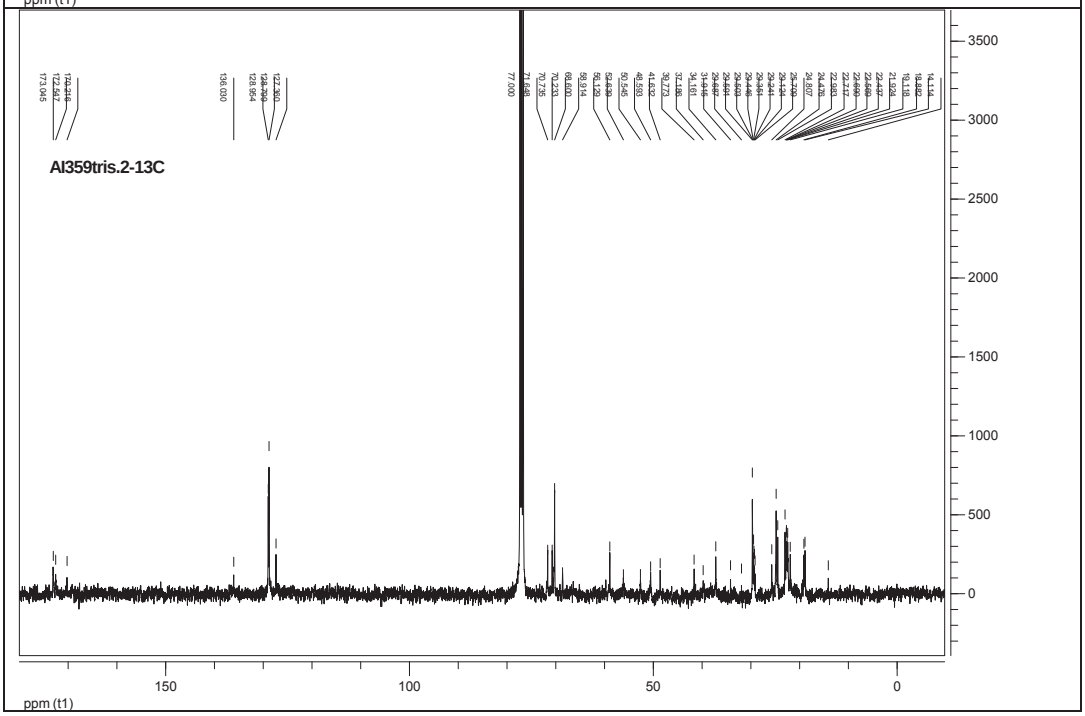




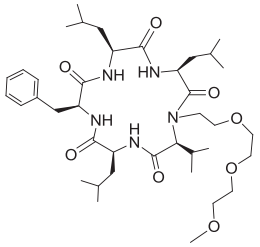
cyclo[Phe-Leu-(*N*-TEG)Leu-Val-Leu] (1a)

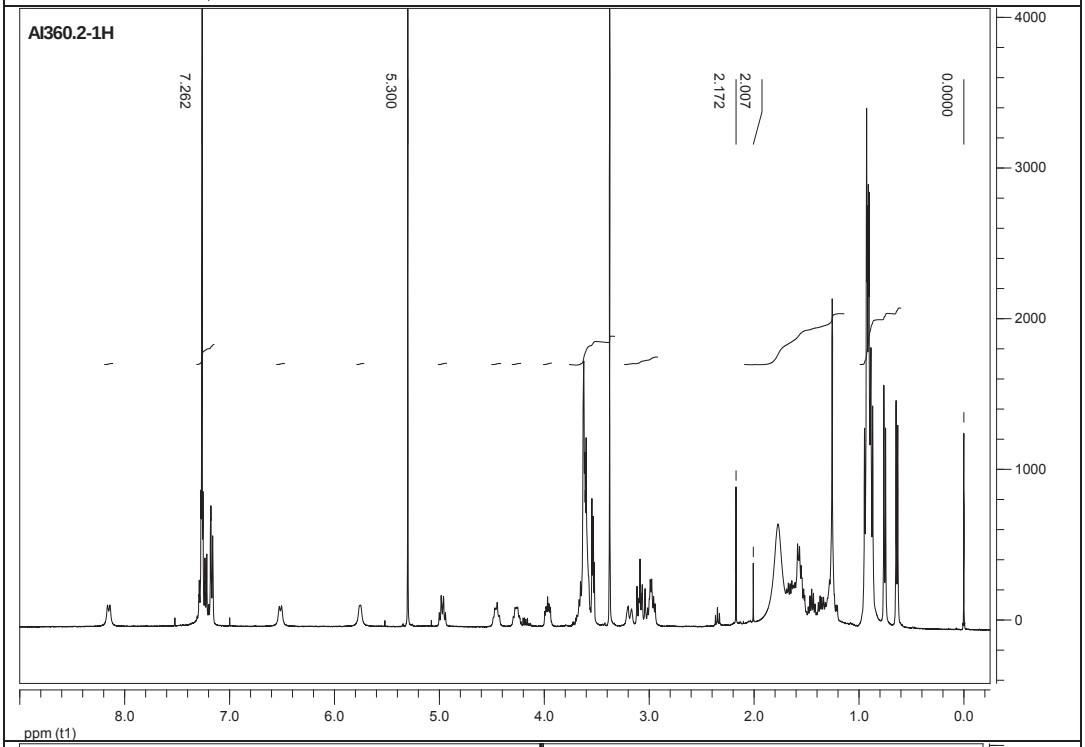


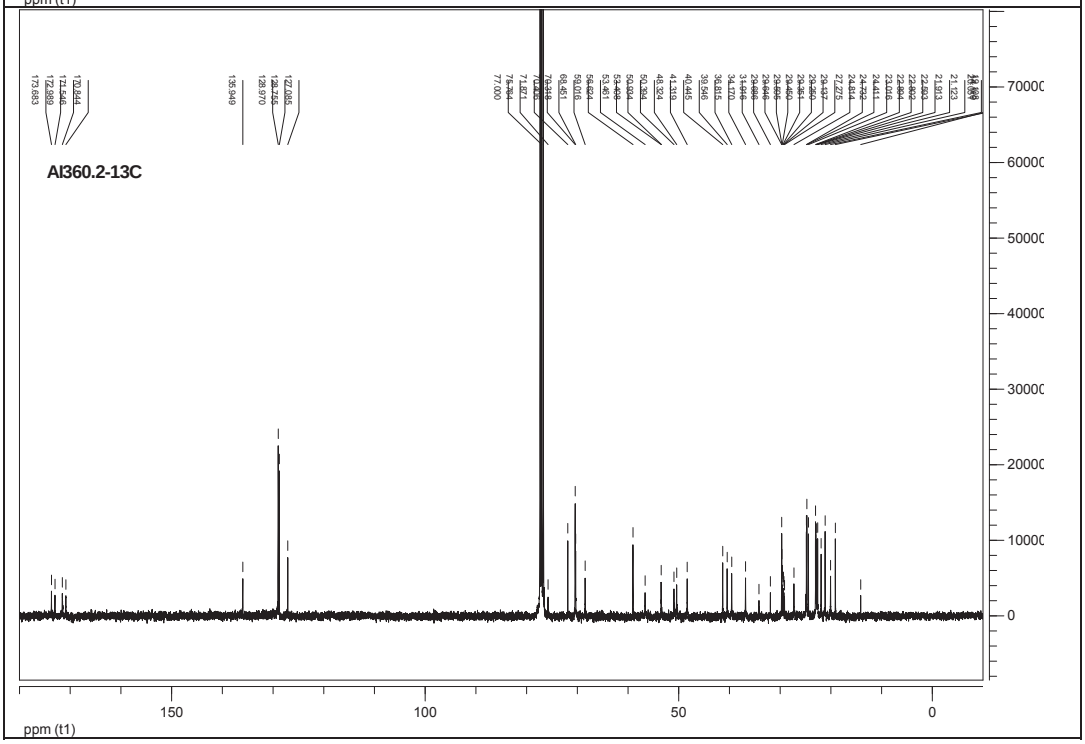




cyclo[Leu-Leu-(*N*-TEG)-Val-Leu-Phe] (2a)







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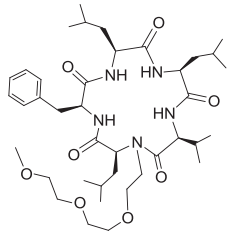
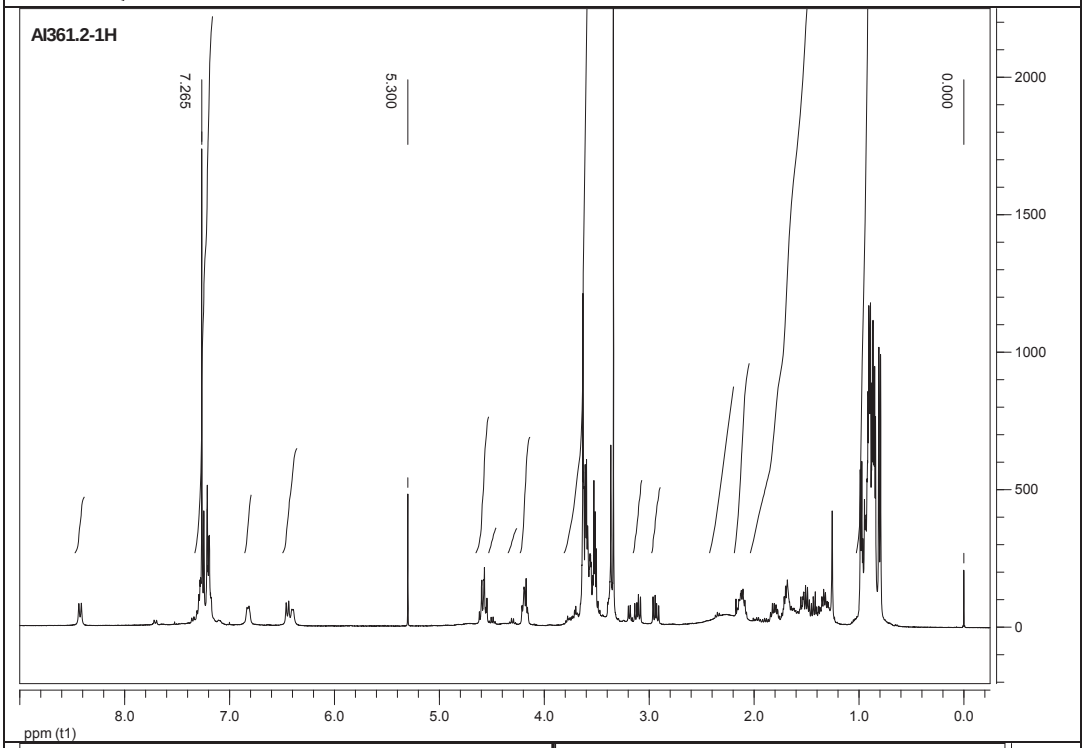
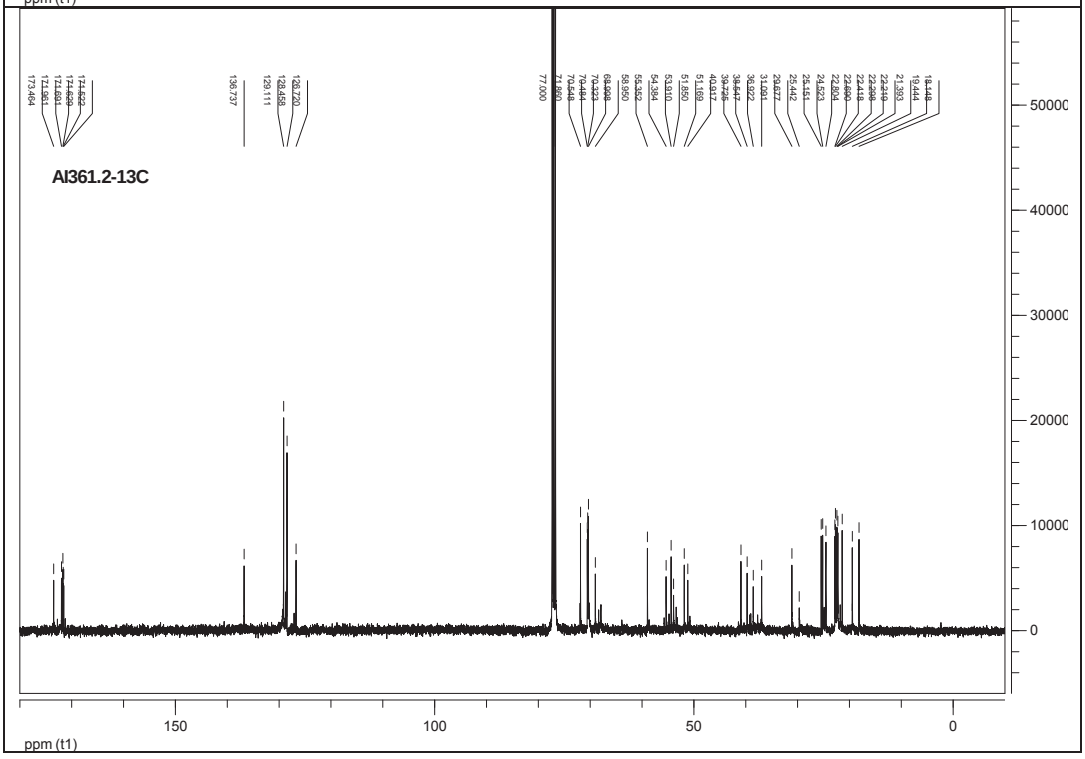
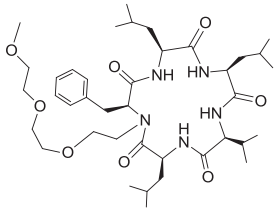
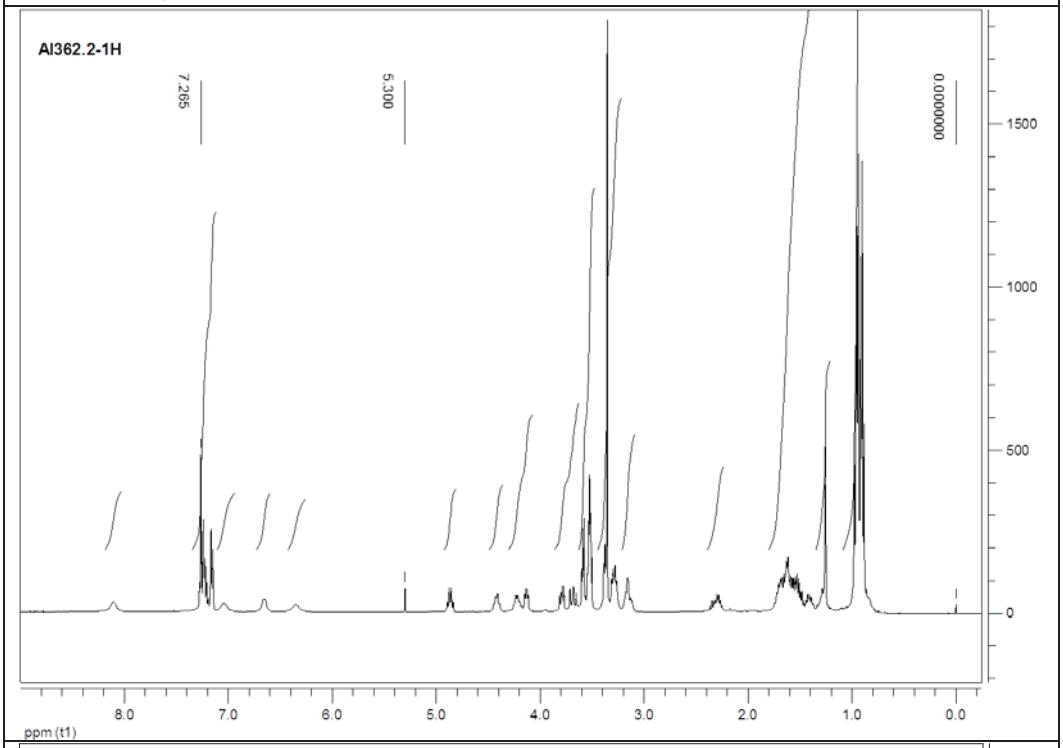


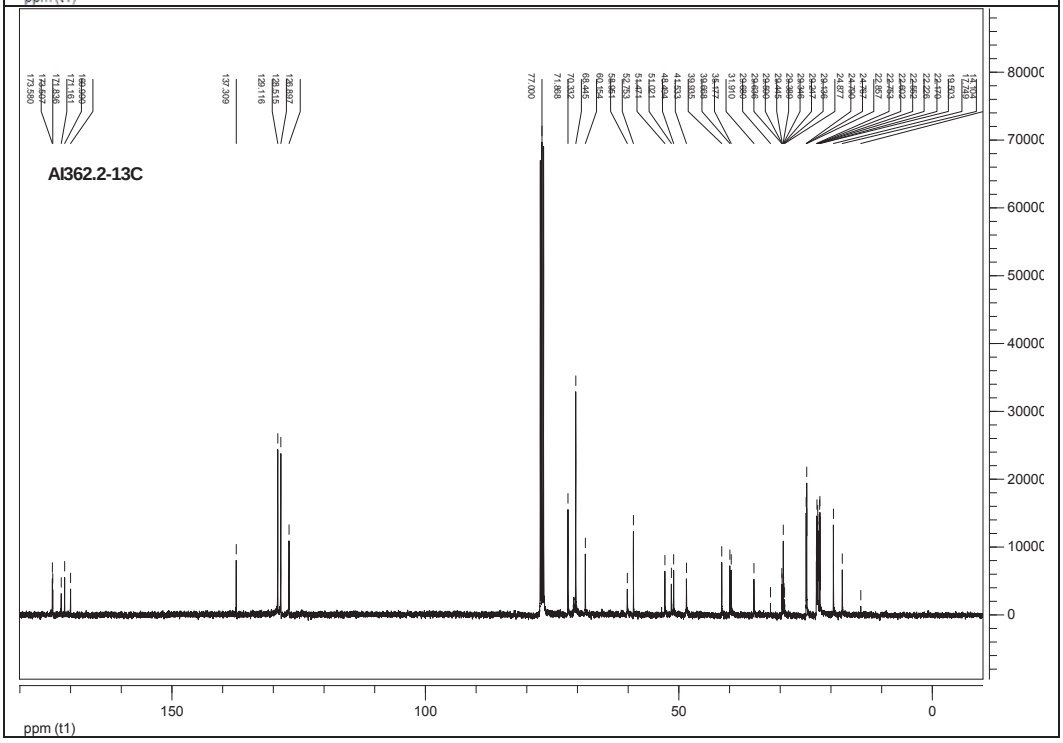




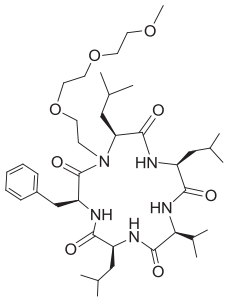
cyclo[Val-Leu-(*N*-TEG)Phe-Leu-Leu] (4a)

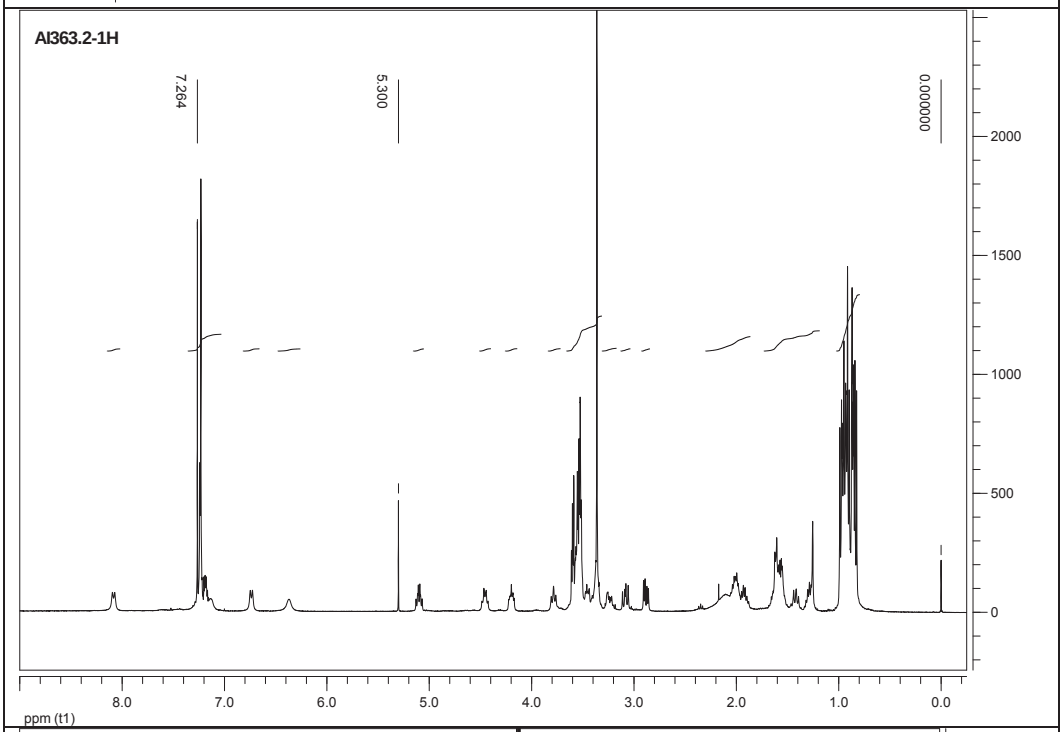


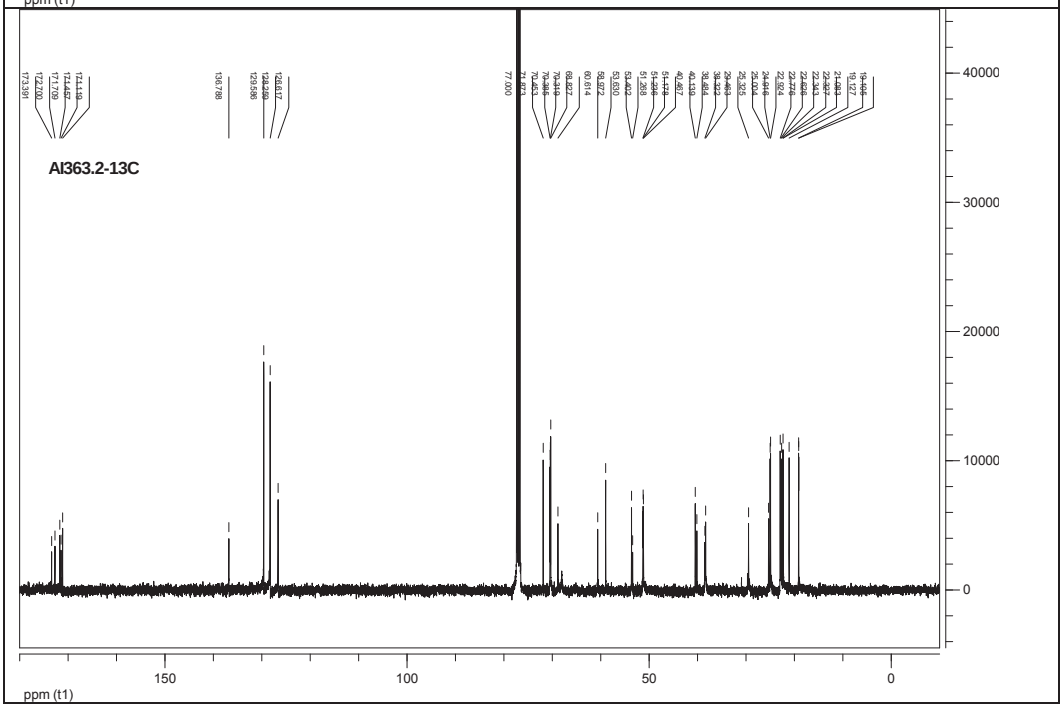




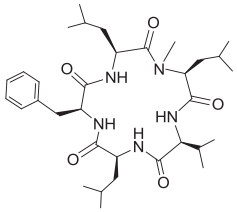
cyclo[Leu-Phe-(*N*-TEG)Leu-Leu-Val] (5a)

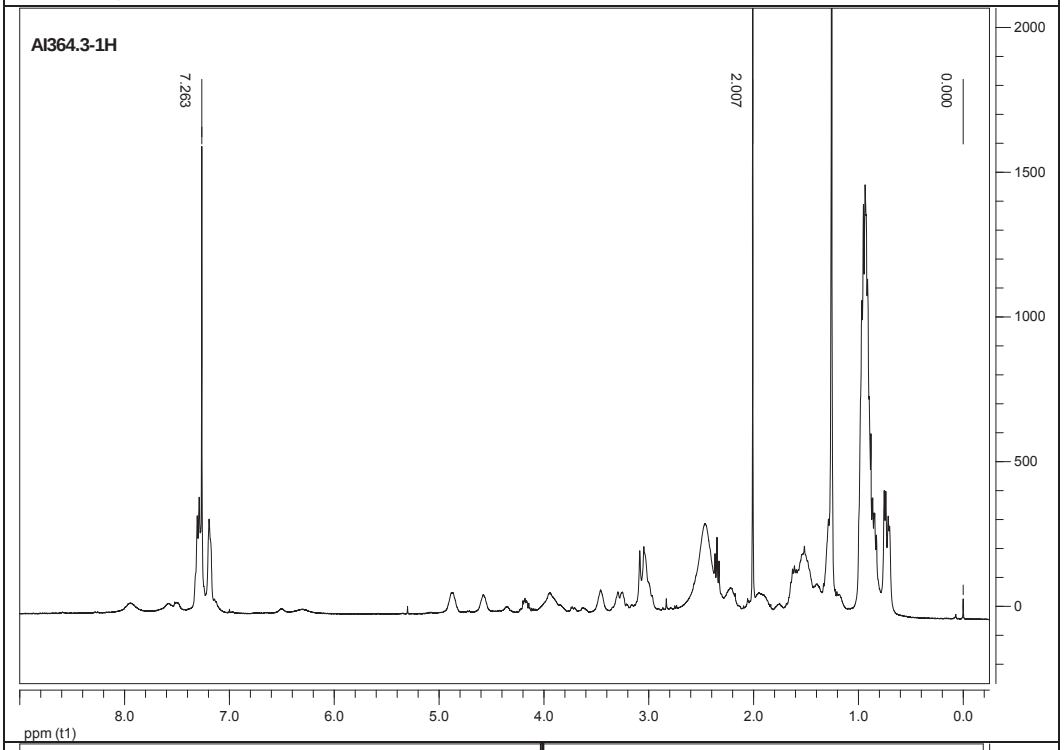


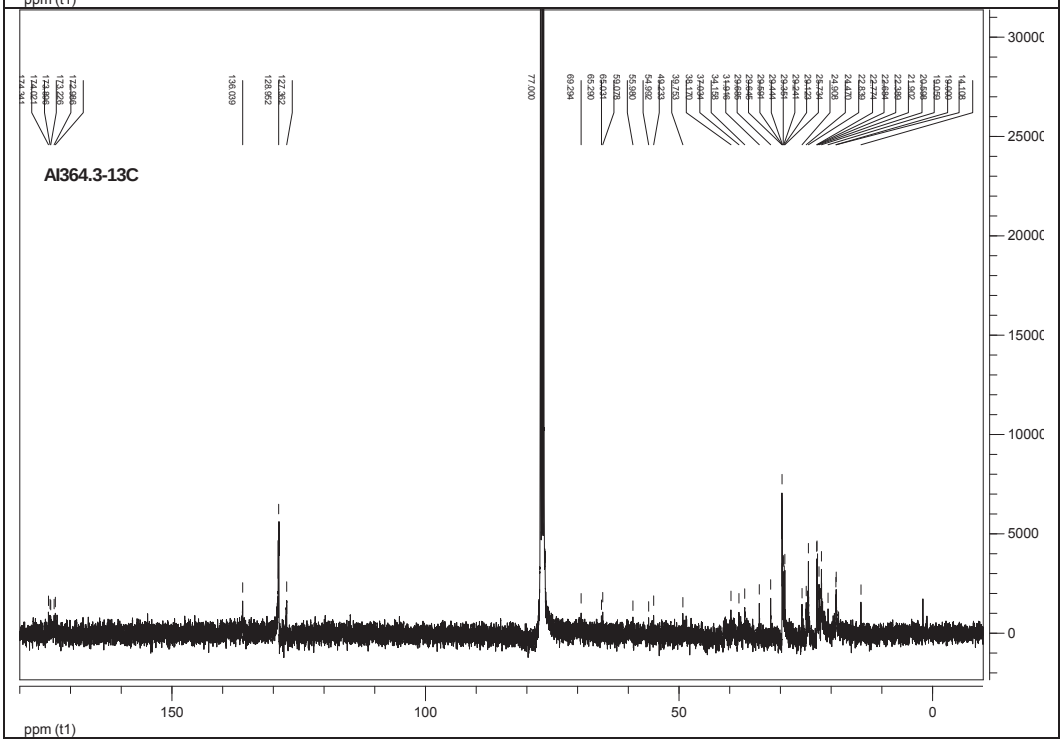




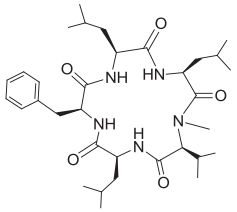
cyclo[Phe-Leu-*N*MeLeu-Val-Leu] (1b)

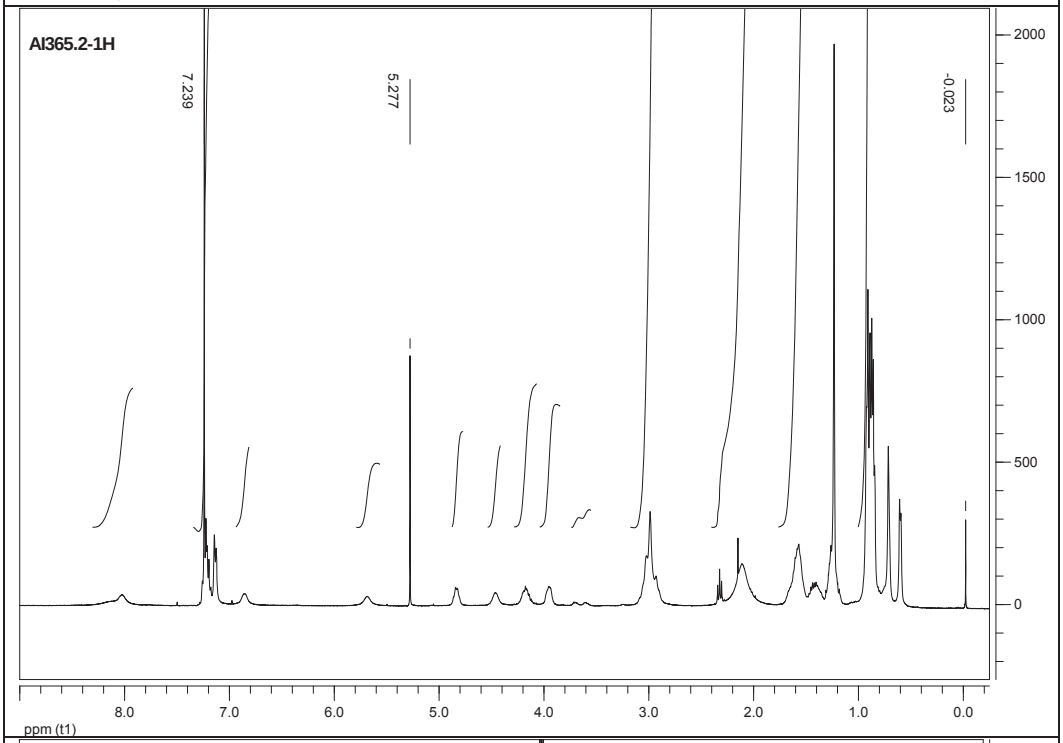


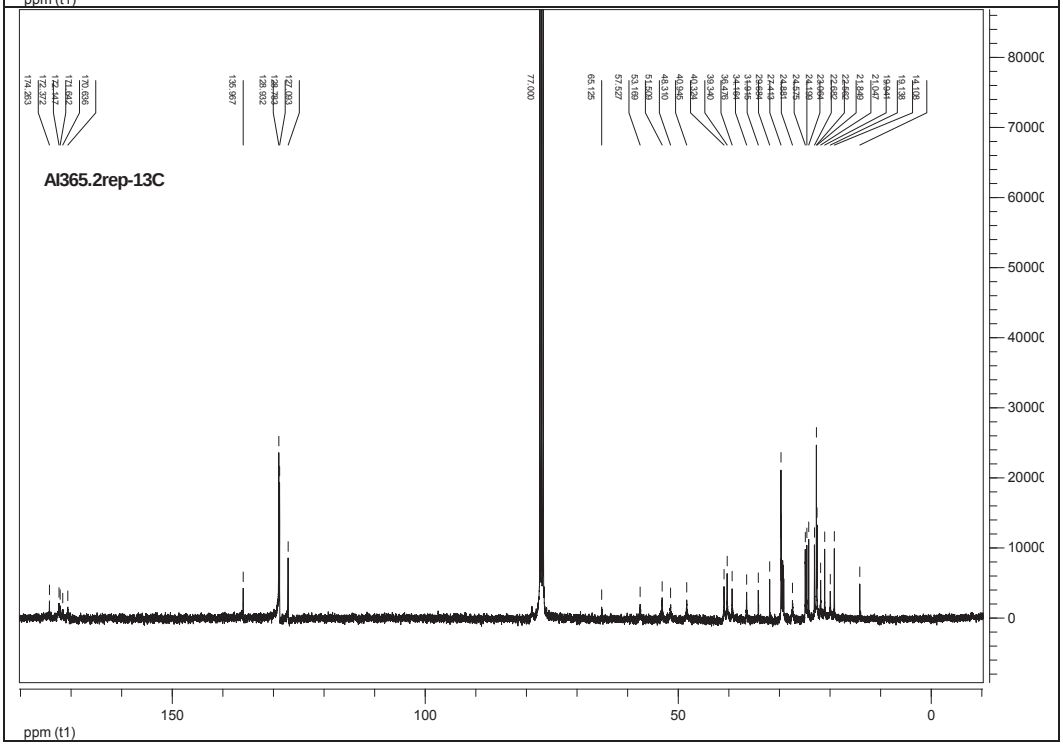




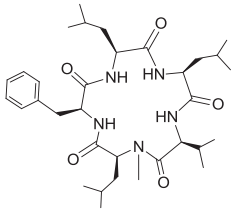
cyclo[Leu-Leu-*N*MeVal-Leu-Phe] (2b)

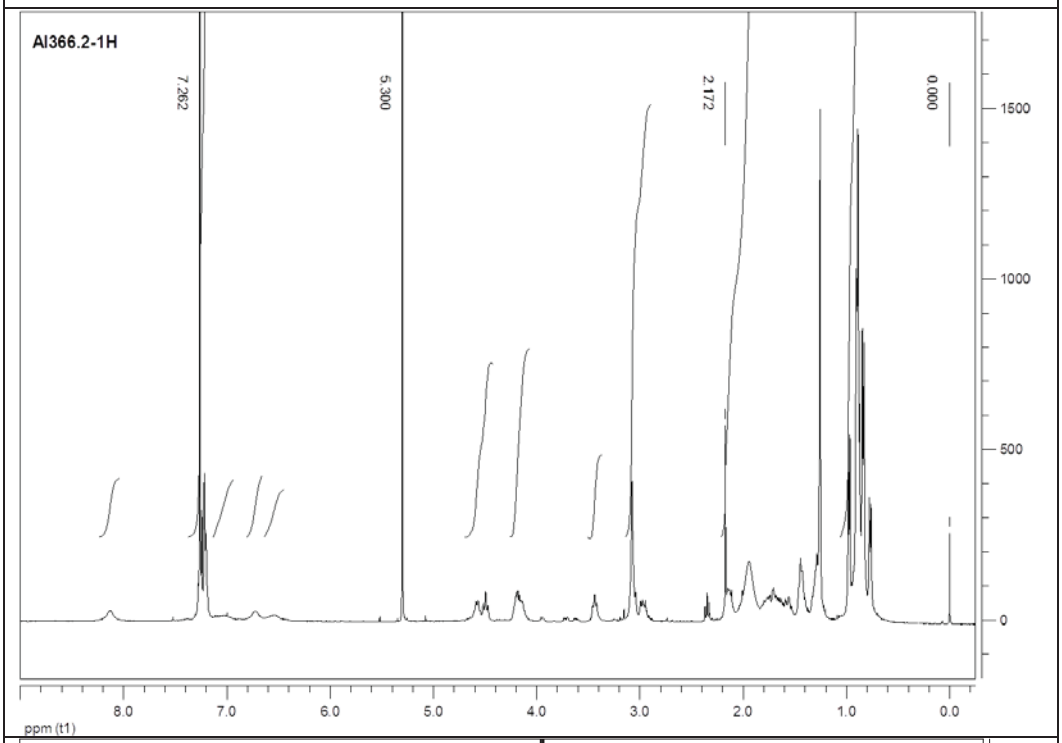


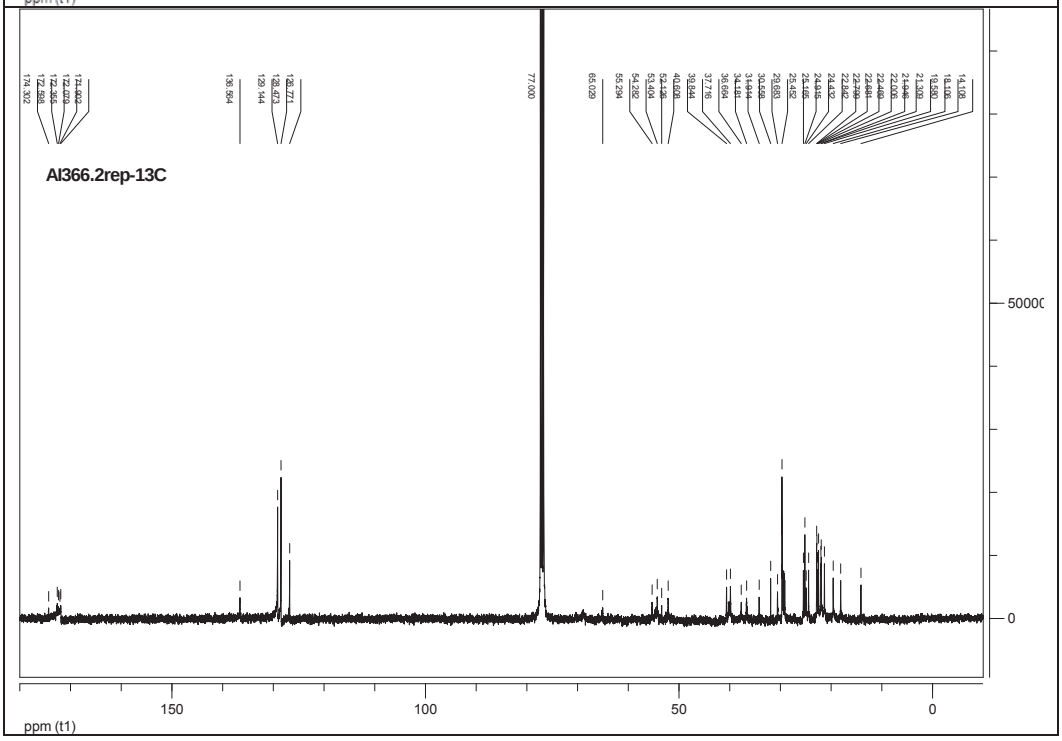




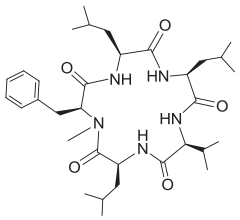
cyclo[Leu-Val-*N*MeLeu-Phe-Leu] (3b)

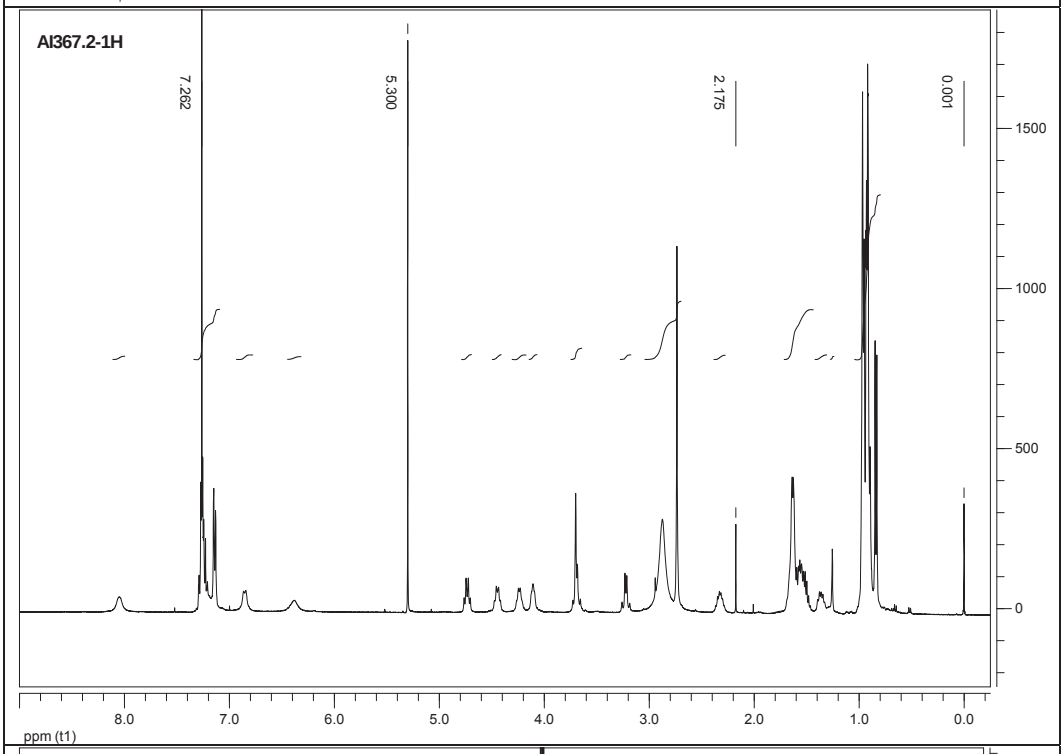


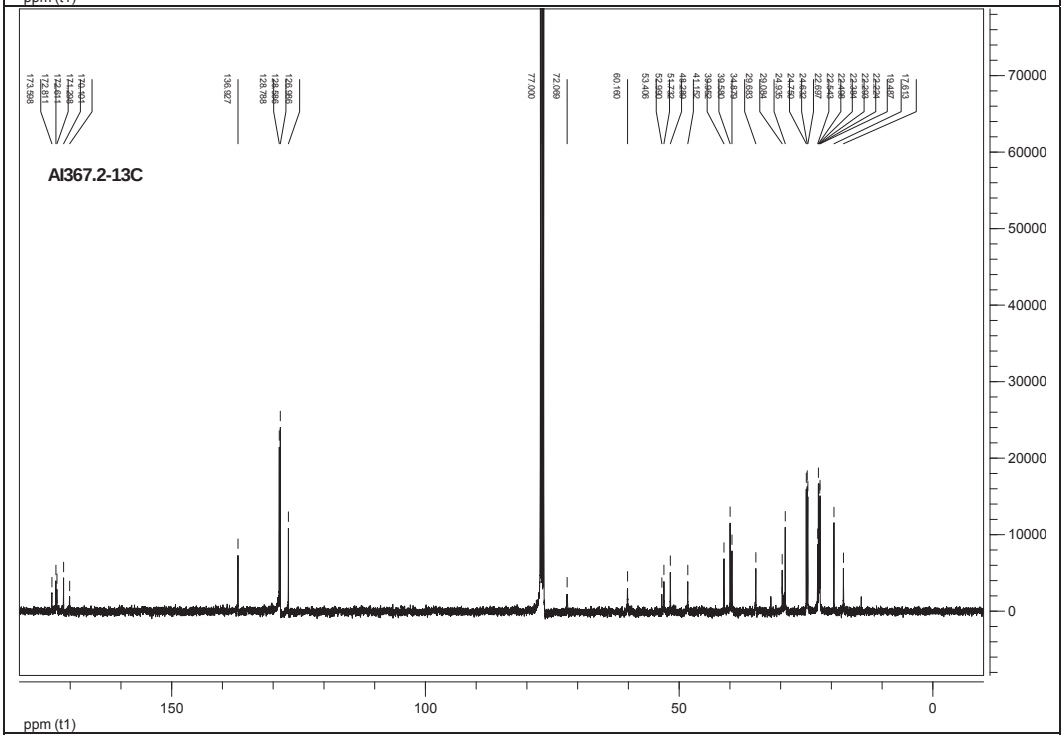




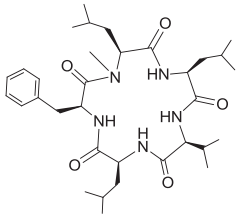
cyclo[Val-Leu-*N*MePhe-Leu-Leu] (4b)

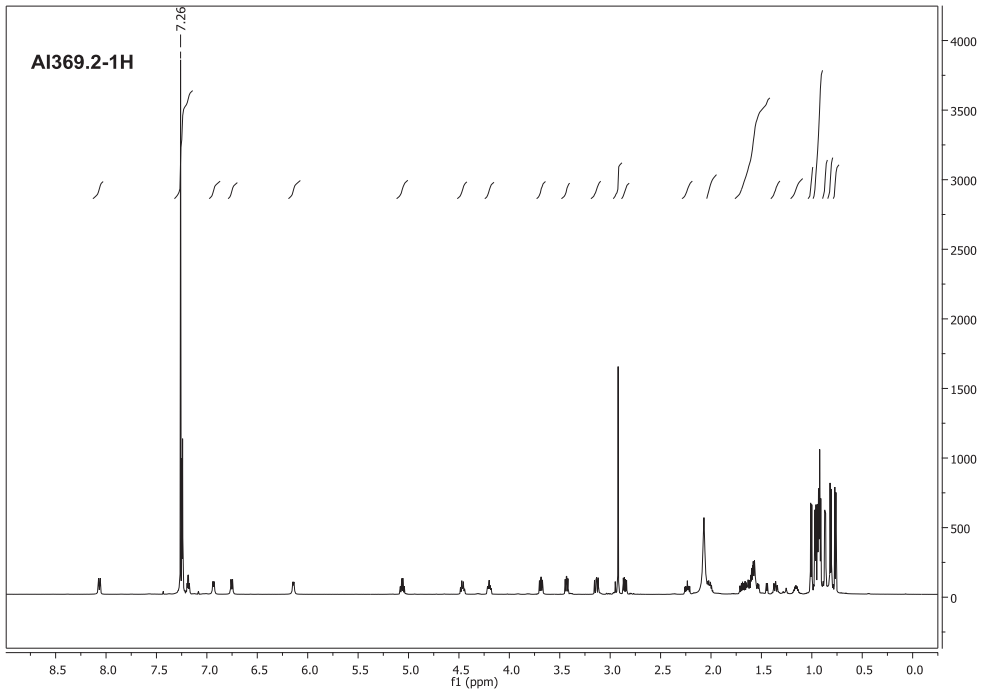


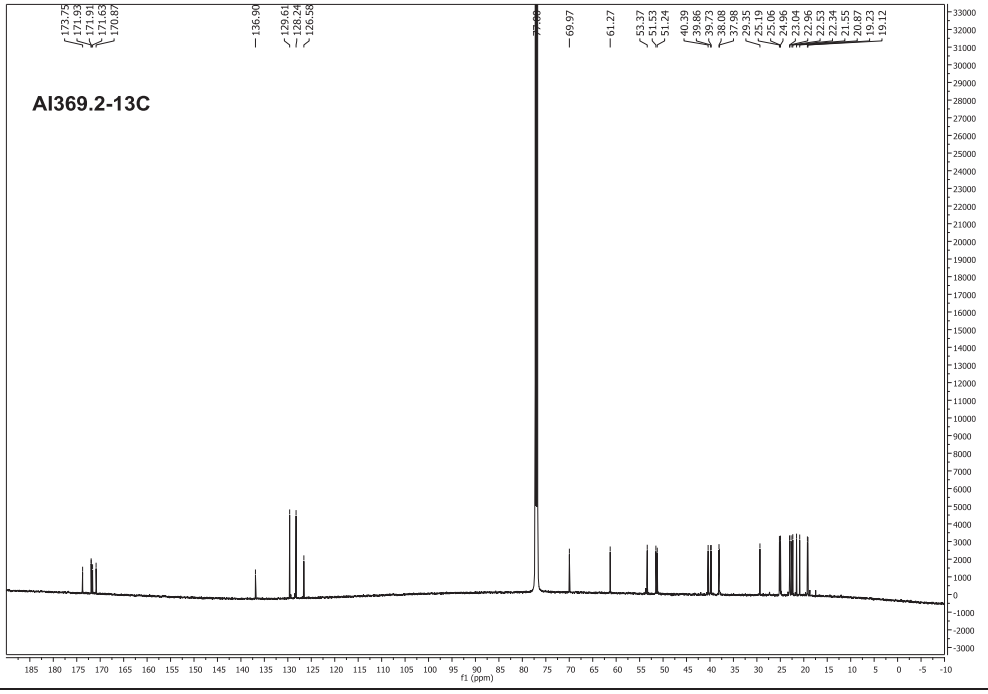




cyclo[Leu-Phe-*N*MeLeu-Leu-Val] (5b)







7. ACKNOWLEGMENTS

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