New α,γ-cyclic peptides for large-diameter self-assembling peptide nanotubes

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SUPPORTING INFORMATION

1. General Methods, Instrument Details and Materials

►**General.** Commercially available N-Boc amino acids, O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and *O*-Benzotriazol-1-yl-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (TBTU) were all used as obtained from Novabiochem, Applied Biosystems or Bachem. Deuterated chloroform ($CDCl_3$) was obtained from Aldrich. All other reagents obtained from commercial suppliers were used without further purification unless otherwise noted. Dichloromethane (DCM) and piperidine were dried and distilled over calcium hydride.[1,2] DIEA was dried and distilled over calcium hydride, and then redistilled over nynhidrin.[1,2] Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F_{254} plates. Compounds, which were not UV active, were visualized by dipping the plates in a nynhidrin solution and heating. Silica gel flash chromatography was performed using E. Merck silica gel (type 60SDS, 230-400 mesh). Solvent mixtures for chromatography are reported as v/v ratios. HPLC purification was carried out on Phenomenex Maxsil-10 silica column with CH_2Cl_2/MeOH gradients between 100 and 85:15. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AMX-500 MHz spectrometer. Chemical shifts were reported in parts per million (ppm, δ) relative to tetramethylsilane (δ 0.00). ^{1}H NMR splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q) or pentuplet (p). All first-order splitting patterns were assigned on the basis of the appearance of the multiplet. Splitting patterns that could not be easily interpreted are designated as multiplet (m) or broad (br). Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker AMX-500 MHz spectrometer. Carbon resonances were assigned using distortionless enhancement by polarization transfer (DEPT) spectra obtained with phase angles of 135. Fast Atom Bombardement (FAB) mass spectra were recorded on a Micromass Autospec mass spectrometer. Mass Spectrometry of Laser Desorption/Ionization-Time of Flight (MALDI-TOF) was obtained on a Bruker Autoflex mass spectrometer. FT-IR measurements were made on a JASCO FT/IR-400 spectrophotometer using 5-10 mM in $CHCl_3$ and placed in a NaCl solution IR cell.

►**^{1}H-NMR Assignments of Cyclic Peptides**. The signals of the ^{1}H NMR spectra of the peptides in $CDCl_3$ were identified from the corresponding double-quantum-filled 2D COSY (2QF-COSY), TOCSY and/or NOESY and ROESY spectra acquired at concentration and temperature indicated. Mixing times (~250 ms or 400 ms) were not optimized. Spectra were typically acquired using Bruker standard pulse sequences on 500 MHz apparatuses, and were referenced relative to residual proton resonances in $CDCl_3$ (at 7.26 ppm).

[1] Brown, H. C. "*Organic Synthesis via Boranes"*, Ed. John Wiley & Sons, **1975**.
[2] Perrin, D. D.; Armarego, W. I. F. *"Purification of Laboratory Chemicals"*, Ed. Pergamon Press, **1988**.

2. Cyclic Peptide Synthesis

Linear peptides were prepared following the synthetic strategy previously described.[3]

***cyclo*[(*L*-Leu-*D*-^{Me}N-γ-Acp)$_4$-] (3b). Synthesis from linear octapeptide.** A solution of Boc-[(*L*-Leu-*D*-^{Me}N-γ-Acp)$_4$-]-OFm (22.0 mg, 17.6 μmol) in 20% piperidine in DCM (250 μL) was stirred at rt for 20 min. After removal of the solvent, the residue was dissolved in DCM (5 mL), and this solution was washed with HCl (5 %), dried over Na_2SO_4, filtered and concentrated. The resulting residue was dissolved in 200 μL of TFA/DCM (1:1) and stirred at rt for 15 min. After removal of the solvent, the residue was dried under high vacuum for 3 h and used without further purification. The linear peptide was dissolved in DCM (17.6 mL) and treated with TBTU (6.0 mg, 19.4 μmol), followed (dropwise) by DIEA (12 μL, 70.4 μmol) [an additional 1 equiv. of TBTU (5.6 mg, 17.6 μmol) and 4 equiv. of DIEA (12 μL, 70.4 μmol) were added when the starting material was detected by HPLC, and the resulting mixture was stirred for 3 h at rt to complete the reaction]. After 12 h, the solvent was removed under reduced pressure, and the crude was purified by HPLC, affording 4.3 mg of **3b** as a white solid [27%, R_t = 16 min (Phenomenex Maxsil-10 semipreparative column, 5-15 % MeOH in CH_2Cl_2, 30 min)]. **^{1}H NMR** ($CDCl_3$, 500.13 MHz, δ): 8.33 (d, J = 9.5 Hz, 1H, NH), 5.16 (m, 1H, Hα_{Leu}), 4.84 (m, 1H, Hγ_{Acp}), 3.06 (m, 1H, Hα_{Acp}), 3.03 (s, 3 H, NC$\underline{H}_3$), 2.09-1.14 (m, 9H, 3 x CH_2 γ-Acp + CH_{2Leu} + CH_{Leu}), 0.94 (dd, J_1 = 17.7 Hz, J_2 = 6.3 Hz, 6H, CH_{3Leu}). **^{13}C NMR** ($CDCl_3$, 125.77 MHz, δ): 174.9 (CO), 173.4 (CO), 54.6 (CH), 46.8 (CH), 43.3 (CH), 42.2 (CH_2), 32.8 (CH_2), 29.4 (NCH_3), 27.9 (CH_2), 26.9 (CH_2), 24.7 (CH), 23.4 (CH_3), 22.2 (CH_3). **FTIR** (293 K, $CHCl_3$): 3307 (amide A), 3004, 2960, 1664, 1627 (amide I), 1540 (amide II_{II}) cm^{-1}. **MS (FAB$^+$)** [m/z (%)]: 953 ($[MH]^+$, 25), 477 (8). **HRMS (FAB$^+$) calculated** for $C_{52}H_{89}N_8O_8$ ($[MH]^+$) 953.680338, **found** 953.680045.

***cyclo*[(*L*-Leu-*D*-^{Me}N-γ-Acp)$_4$-] (3b). Synthesis from linear tetrapetide.** A solution of Boc-[(*L*-Leu-*D*-^{Me}N-γ-Acp)$_2$-]-OFm (50.0 mg, 64.8 μmol) in 20% piperidine in DCM (1.25 mL) was stirred at rt for 20 min. After removal of the solvent, the residue was dissolved in DCM (10 mL), and this solution was washed with HCl (5 %), dried over Na_2SO_4, filtered and concentrated. The resulting residue was dissolved in 1 mL of TFA/DCM (1:1) and stirred at rt for 15 min. After removal of the solvent, the residue was dried under high vacuum for 3 h and used without further purification. The linear peptide was dissolved in DCM (13.0 mL) and treated with TBTU (25.0 mg, 77.7 μmol), followed (dropwise) by DIEA (46 μL, 259.1 μmol) [an additional 1 equiv. of TBTU (20.8 mg, 64.8 μmol) and 4 equiv. of DIEA (46 μL, 259.1 μmol) were added when the starting material was detected by HPLC, and the resulting mixture was stirred for 3 h at rt to complete the reaction]. After 12 h, the solvent was removed under reduced pressure, and the crude was purified by HPLC, affording 12.4 mg of **3b** as a white solid [40%, R_t = 16 min (Phenomenex Maxsil-10 semipreparative column, 5-15 %

[3] Brea, R. J.; Amorín, M.; Castedo, L.; Granja, J. R. *Angew. Chem. Int. Ed.* **2005**, *44*, 5710-5713.

MeOH in CH_2Cl_2, 30 min)]. **MS (MALDI-TOF**) [m/z (%)]: 993 ([M +K]$^+$, 7), 975 ([M +Na]$^+$, 100), 953 ([MH]$^+$, 34). **HRMS (MALDI-TOF**) **calculated** for $C_{52}H_{88}N_8O_8Na$ ([M +Na]$^+$) 975.6617, **found** 975.6573.

***cyclo*[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_5$-] (4b).** A solution of Boc-[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_5$-]-OFm (26.8 mg, 18.0 μmol) in 20% piperidine in DCM (250 μL) was stirred at rt for 20 min. After removal of the solvent, the residue was dissolved in DCM (5 mL), and this solution was washed with HCl (5 %), dried over Na_2SO_4, filtered and concentrated. The resulting residue was dissolved in 200 μL of TFA/DCM (1:1) and stirred at rt for 15 min. After removal of the solvent, the residue was dried under high vacuum for 3 h and used without further purification. The linear peptide was dissolved in DCM (18 mL) and treated with TBTU (6.4 mg, 19.8 μmol), followed (dropwise) by DIEA (13 μL, 72.2 μmol) [an additional 1 equiv. of TBTU (5.8 mg, 18.0 μmol) and 4 equiv. of DIEA (13 μL, 72.2 μmol) were added when the starting material was detected by HPLC, and the resulting mixture was stirred for 3 h at rt to complete the reaction]. After 12 h, the solvent was removed under reduced pressure, and the crude was purified by HPLC, affording 9.4 mg of **4b** as a white solid [44%, R_t = 18 min (Phenomenex Maxsil-10 semipreparative column, 8-15 % MeOH in CH_2Cl_2, 30 min)]. **^{1}H NMR** ($CDCl_3$, 500.13 MHz, δ): 8.42 (d, J = 9.1 Hz, 1H, NH), 5.20 (dt, J_1 = 3.7 Hz, J_2 = 9.3 Hz, 1H, Hα$_{Leu}$), 4.92 (m, 1 H, Hγ$_{Acp}$), 3.18 (dt, J_1 = 9.1 Hz, J_2 = 15.0 Hz, 1H, Hα$_{Acp}$), 3.02 (s, 3H, NC$\underline{H}_3$), 1.99 (m, 2H, 2 x CH$_{Acp}$), 1.87-1.67 (m, 4H, 4 x CH$_{Acp}$), 1.61 (dd, J_1 = 7.4 Hz, J_2 = 18.4 Hz, 1H, CH$_{Leu}$), 1.51 (ddd, J_1 = 3.8 Hz, J_2 = 9.8 Hz, J_3 = 13.4 Hz, 1H, 0.5 x CH$_{2Leu}$), 1.36 (ddd, J_1 = 3.7 Hz, J_2 = 9.6 Hz, J_3 = 13.2 Hz, 1H, 0.5 x CH$_{2Leu}$), 0.95 (dd, J_1 = 6.4 Hz, J_2 = 43.9 Hz, 6H, CH$_{3Leu}$). **^{13}C NMR** ($CDCl_3$, 125.77 MHz, δ): 174.9 (CO), 173.3 (CO), 54.2 (CH), 46.8 (CH), 43.9 (CH_2), 42.2 (CH), 31.5 (CH_2), 29.1 (NCH_3), 28.3 (CH_2), 26.2 (CH_2), 24.6 (CH), 23.7 (CH_3), 22.1 (CH_3). **FTIR** (293 K, $CHCl_3$): 3313 (amide A), 3019, 2961, 1665, 1627 (amide I), 1534 (amide II$_{II}$) cm^{-1}. **MS (MALDI-TOF**) [m/z (%)]: 1229 ([M +K]$^+$, 14), 1213 ([M +Na]$^+$, 100), 1191 ([MH]$^+$, 4). **HRMS (MALDI-TOF**) **calculated** for $C_{65}H_{110}N_{10}O_{10}Na$ ([M +Na]$^+$) 1213.8304, **found** 1213.8336.

***cyclo*[(*D*-Phe-*L*-^{Me}N-γ-Acp)$_6$-] (5).** A solution of Boc-[(*D*-Phe-*L*-^{Me}N-γ-Acp)$_6$-]-OFm (40.0 mg, 20.8 μmol) in 20% piperidine in DCM (250 μL) was stirred at rt for 20 min. After removal of the solvent, the residue was dissolved in DCM (5 mL), and this solution was washed with HCl (5 %), dried over Na_2SO_4, filtered and concentrated. The resulting residue was dissolved in 200 μL of TFA/DCM (1:1) and stirred at rt for 15 min. After removal of the solvent, the residue was dried under high vacuum for 3 h and used without further purification. The linear peptide was dissolved in DCM (20.8 mL) and treated with TBTU (8.0 mg, 24.9 μmol), followed (dropwise) by DIEA (15 μL, 83.0 μmol) [an additional 1 equiv. of TBTU (6.7 mg, 20.8 μmol) and 4 equiv. of DIEA (15 μL, 83.0 μmol) were added when the starting material was detected by HPLC, and the resulting mixture was stirred for 3 h at rt to complete the reaction]. After 12 h, the solvent was removed under reduced pressure, and the crude was purified by HPLC, affording 25.4 mg of **5** as a white solid [75%, R_t = 22 min (Phenomenex Maxsil-10 semipreparative column, 5-15 % MeOH in CH_2Cl_2, 30 min)]. **^{1}H NMR** ($CDCl_3$, 500.13 MHz, δ): 8.61 (d, J = 8.9 Hz, 1H, NH), 7.22 (m, 5H, Ar-H$_{Phe}$), 5.36 (dd, J_1 =

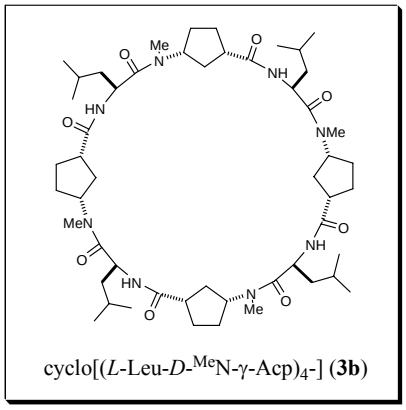
8.4 Hz, J_2 = 15.5 Hz, 1H, Hα_{Phe}), 4.78 (m, 1 H, Hγ_{Acp}), 3.11 (dd, J_1 = 8.9 Hz, J_2 = 17.0 Hz, 1H, Hα_{Acp}), 2.94 (m, 2H, $CH_2\beta_{Phe}$), 2.57 (s, 3H, NC$\underline{H}_3$), 1.93 (c, J = 11.9 Hz, 2H, 2 x CH_{Acp}), 1.78-1.48 (m, 3H, 3 x CH_{Acp}), 1.24 (m, 1H, CH_{Acp}). **^{13}C NMR** ($CDCl_3$, 125.77 MHz, δ): 174.6 (CO), 172.1 (CO), 136.2 (C), 129.5 (CH), 128.4 (CH), 127.0 (CH), 54.6 (CH), 49.9 (CH), 42.0 (CH), 41.1 (CH_2), 30.8 (CH_2), 29.0 (CH_3), 28.1 (CH_2), 26.3 (CH_2). **FTIR** (293 K, $CHCl_3$): 3315 (amide A), 3008, 2967, 1665, 1623 (amide I), 1525 (amide II_{II}) cm^{-1}. **MS (MALDI-TOF)** [m/z (%)]:1673 ([M +K]$^+$, 14), 1656 ([M +Na]$^+$, 100), 1634 ([MH]$^+$, 4). **HRMS (MALDI-TOF) calculated** for $C_{96}H_{120}N_{12}O_{12}Na$ ([M +Na]$^+$) 1655.9041, **found** 1655.9057.

3. X-ray Crystallographic Determination of the Structure of Dimer D3b

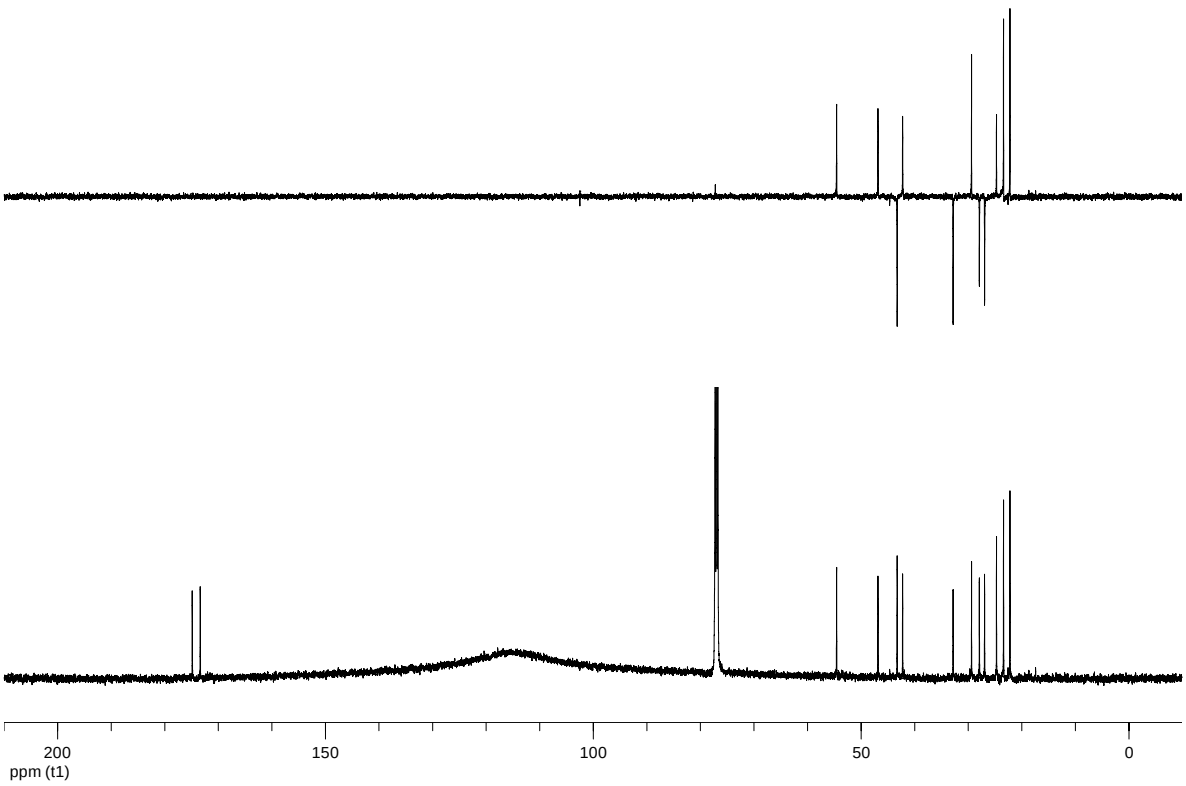
Preparation of Single Crystals for X-ray Analysis. In a typical experiment, 3 mg of HPLC-purified **3b** was dissolved in 0.5 mL of 1,1,2,2-tetrachloroethane and equilibrated by vapour-phase diffusion against 2.5 mL of hexanes. The corresponding dimer crystallized spontaneously within 7-10 days.

X-ray Crystallographic Analysis. Data were collected at low temperature (120 K) in a Bruker Smart X1000 diffractometer using Mo Kα radiation and a graphite monochromator. All calculations were performed on an IBM-compatible PC using the programs COLLECT[4], HKL Denzo and Scalepack[5], SORTAV[6], SHELX-97[7], WinGx[8], SIR2002[9], ORTEP3[10], PLATON (SQUEEZE)[11], and PARST[12]. Supplementary crystallographic data for **D3b** (CIF format) can be obtained free of charge from the Cambridge Crystallographic Data Centre via the Internet at www.ccdc.cam.ac.uk/data_request/cif.

[4] Nonius BV, **1997-2000**.
[5] Otwinowski, Z. and Minor, W. *"Processing of X-ray Diffraction Data Collected in Oscillation Mode"*, Methods in Enzymology, Ed. Carter, C.W. Jr. and Sweet, R. M., Eds., Academic Press, New York; Volume *276*: Macromolecular Crystallography, part A, p. 307-326, **1997**.
[6] Blessing, R. H. *Acta Cryst.* **1995**, A51, 33-38.
[7] G. M. Sheldrick, Institute für Anorganische Chemie, Universitat Gottingen, D-37077 Göttingen, Germany.
[8] Farrugia, L. J. *J. Appl. Cryst.* **1999**, *32*, 837-838.
[9] Burla, M. C.; Camalli, M.; Carrozzini, B.; Cascarano, G. L.; Giacovazzo, C.; Polidori, G and Spagna, R.
[10] Farrugia, L. J. *J. Appl. Cryst.* **1997**, 30, 565.
[11] Spek, A. L., University of Utrecht, The Netherlands, **2001**.
[12] Nardelli, M. *J. Appl. Cryst.* **1995**, *28*, 659.

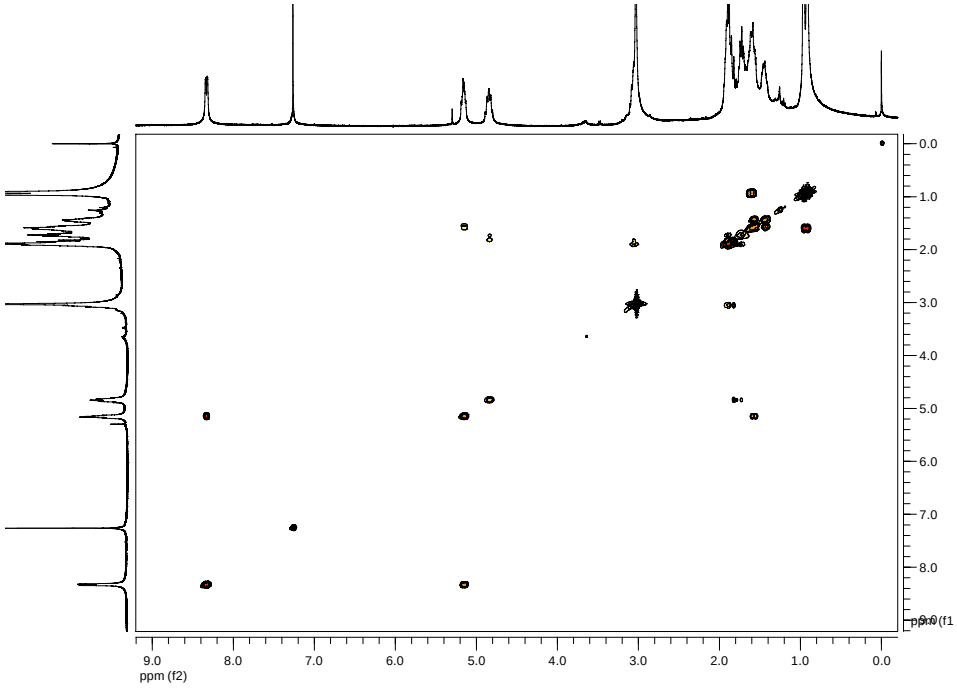


cyclo[(*L*-Leu-*D*-^{Me}N-γ-Acp)$_4$-] (**3b**)

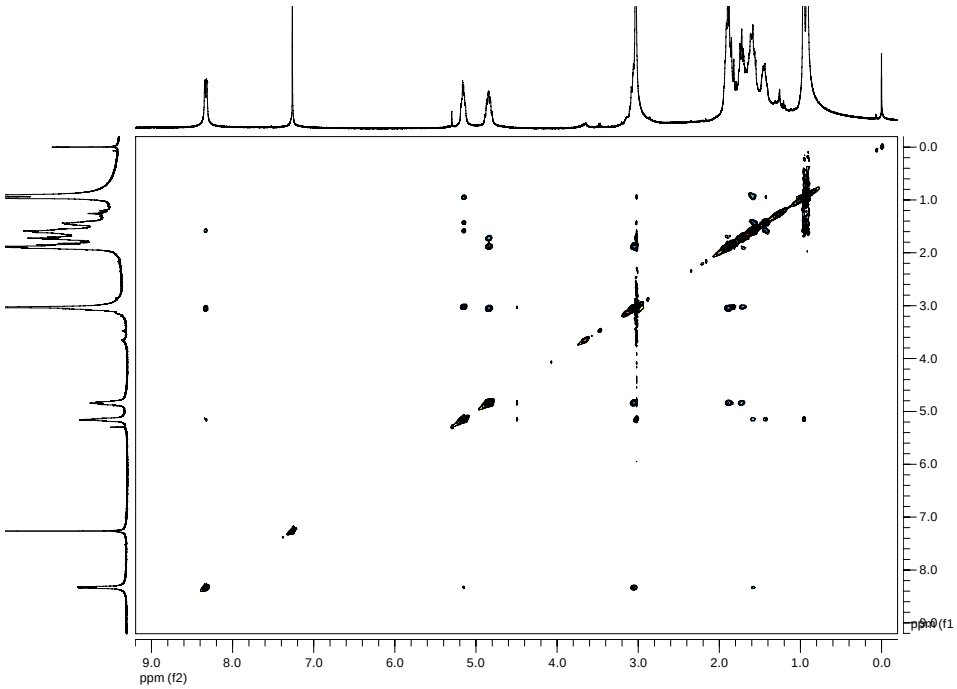


***cyclo*[(*L*-Leu-*D*-^{Me}N-γ-Acp)$_4$-] (3b)**

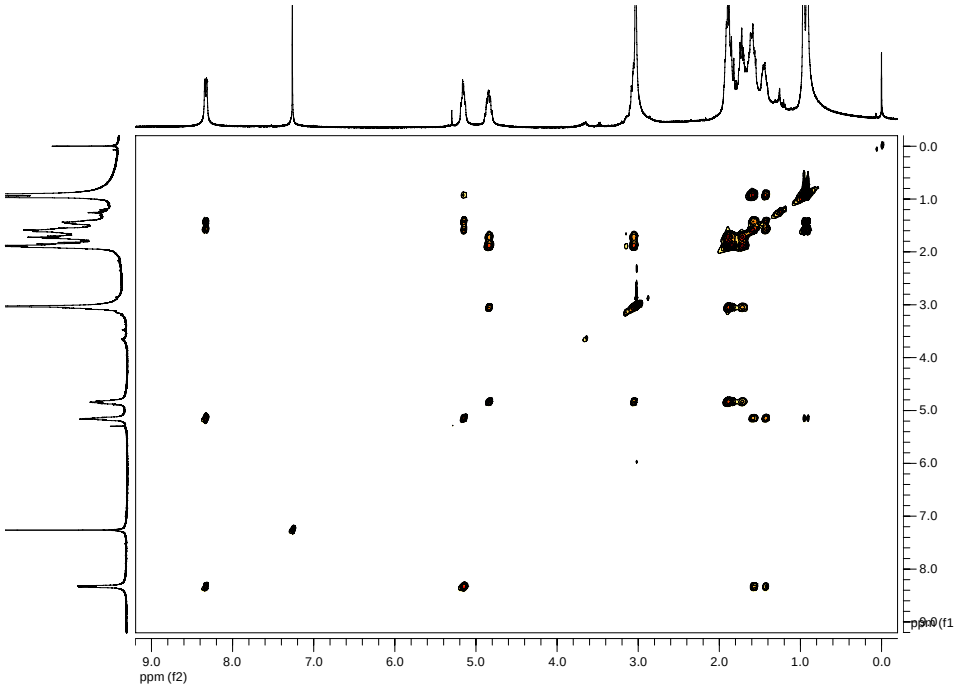
1) COSY [10.02 mM, $CDCl_3$, 298 K (25 ºC), 500.13 MHz]



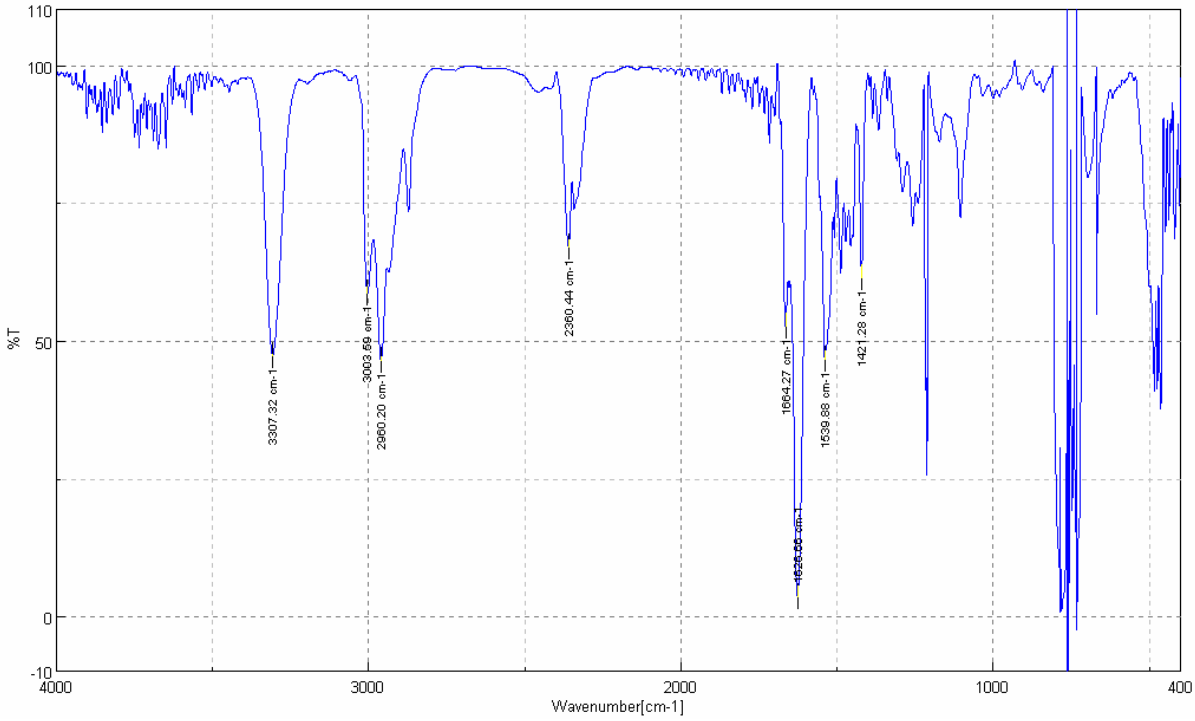
2) ROESY [10.02 mM, $CDCl_3$, 298 K (25 ºC), 500.13 MHz]



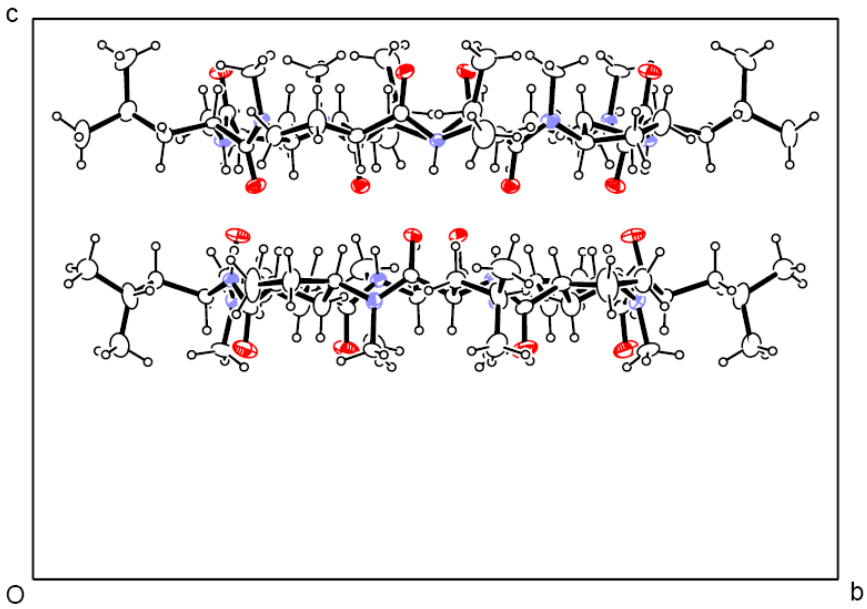
3) TOCSY [10.02 mM, $CDCl_3$, 298 K (25 ºC), 500.13 MHz]

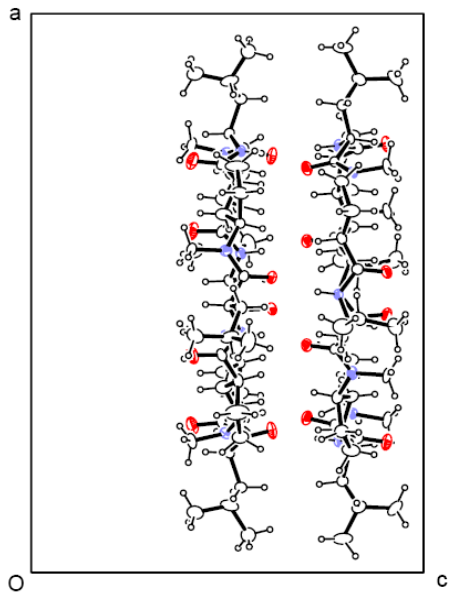


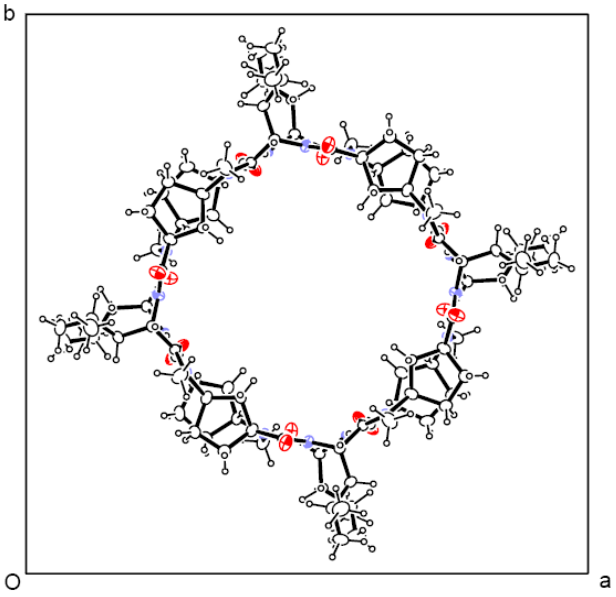
4) FT-IR [$CHCl_3$, 298 K (25 ºC)]

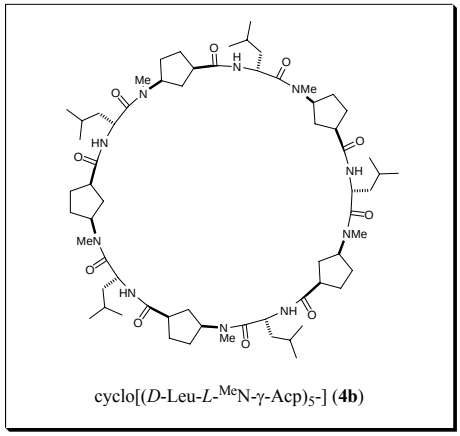


5) X-Ray ORTEP diagrams of **D3b**

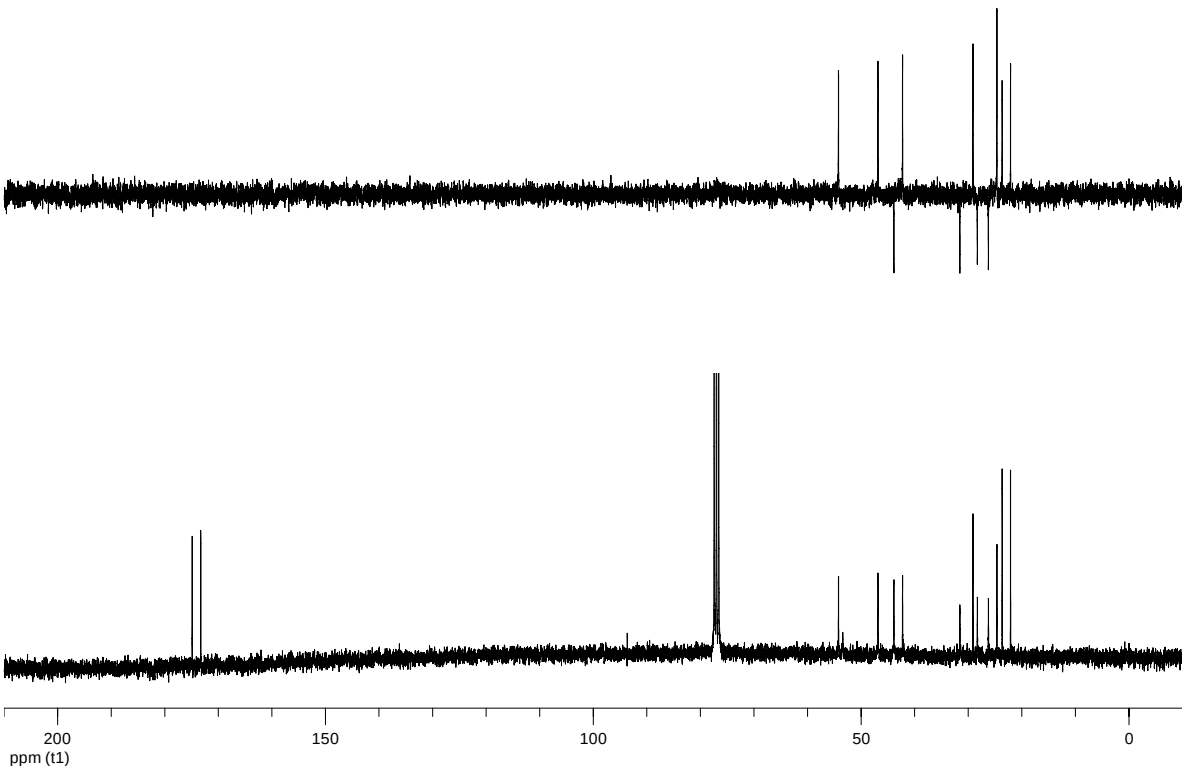






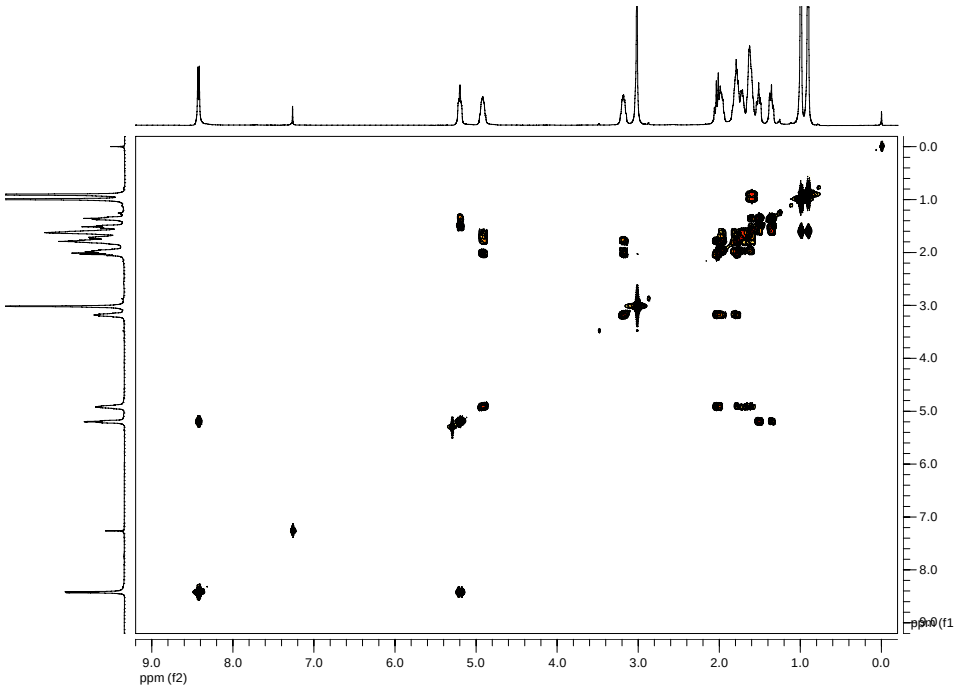


cyclo[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_5$-] (**4b**)

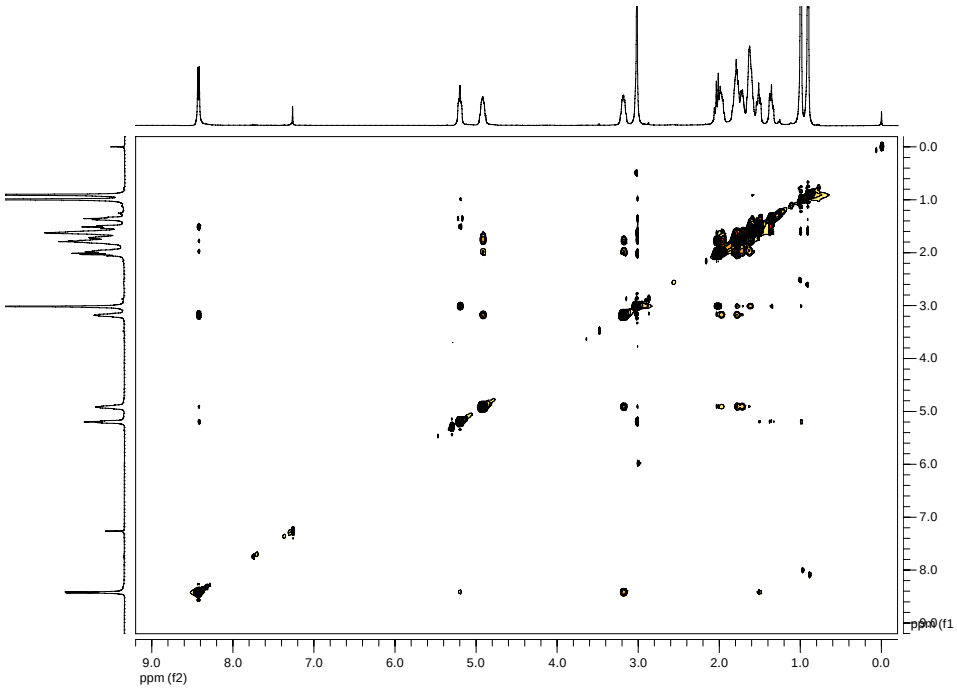


***cyclo*[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_5$-] (4b)**

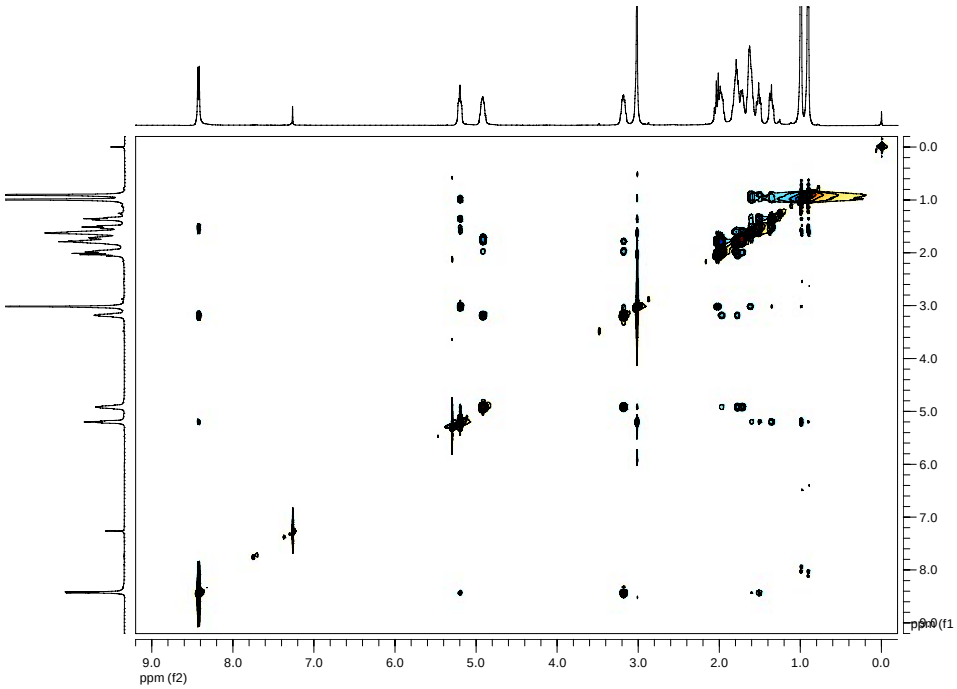
1) COSY [13.17 mM, $CDCl_3$, 298 K (25 ºC), 500.13 MHz]



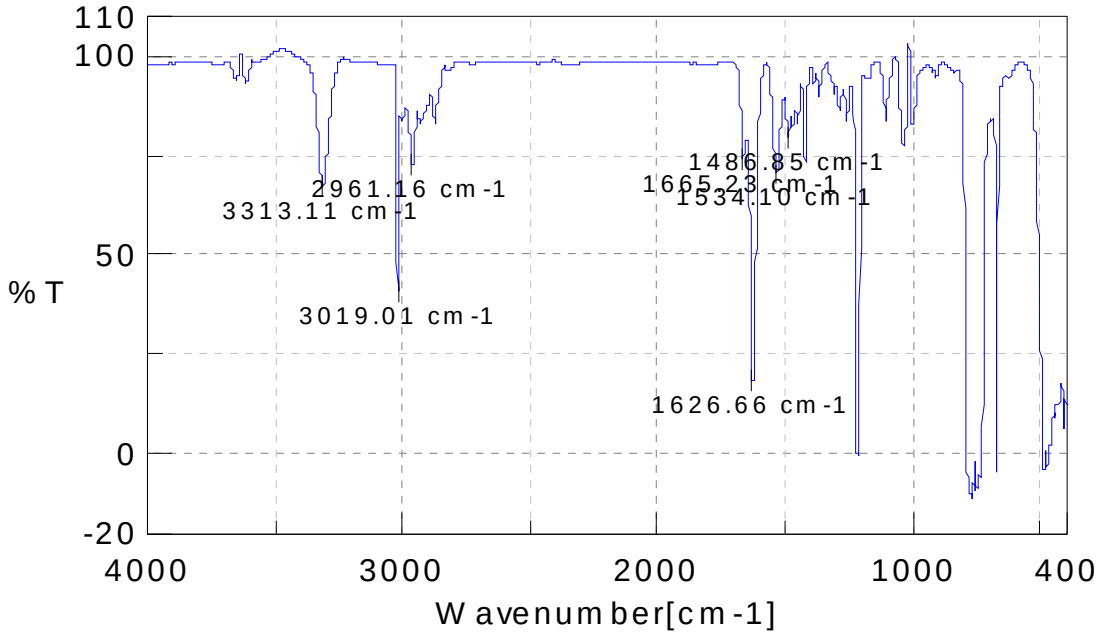
2) NOESY [13.17 mM, $CDCl_3$, 298 K (25 ºC), 500.13 MHz]

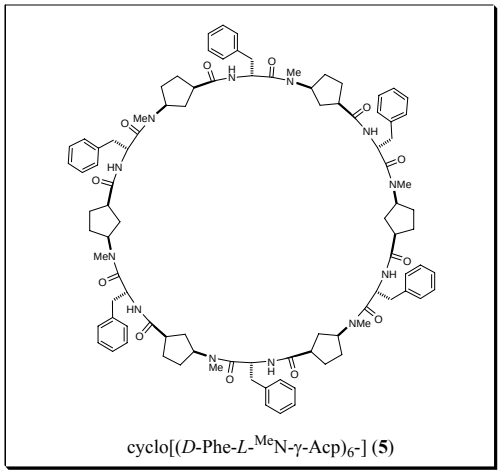


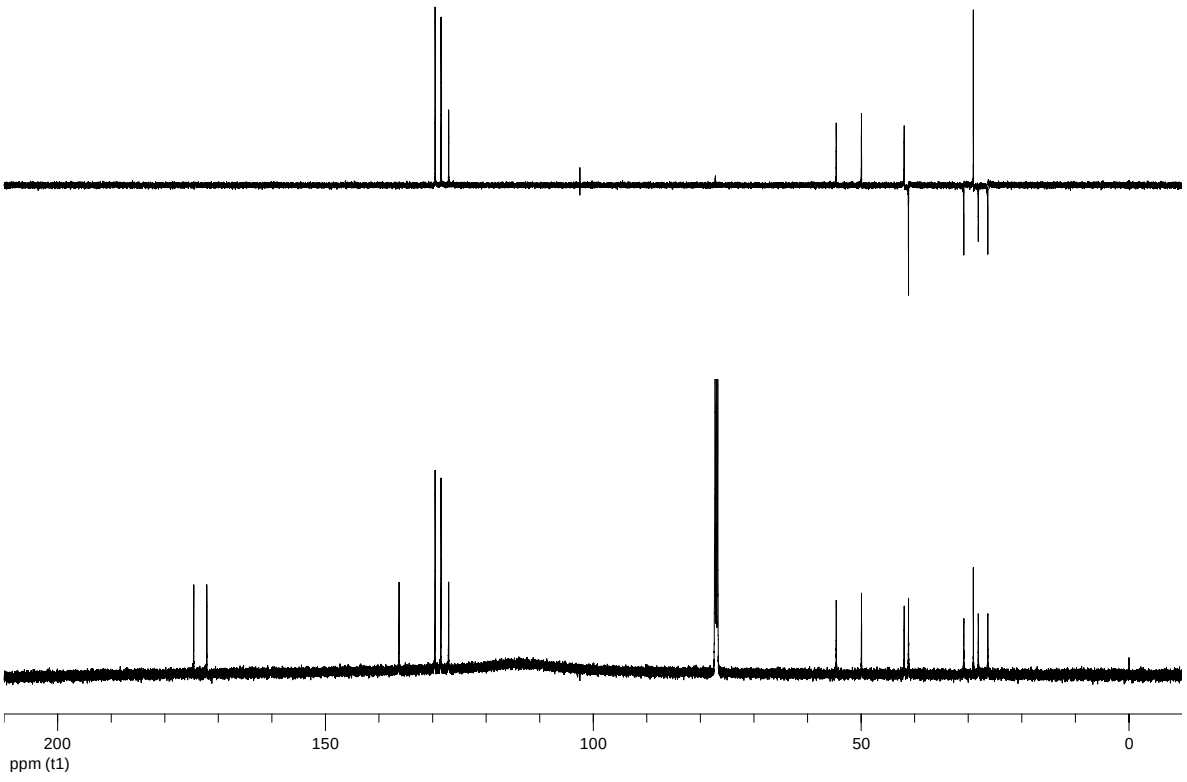
3) ROESY [13.17 mM, $CDCl_3$, 298 K (25 ºC), 500.13 MHz]



4) FT-IR [$CHCl_3$, 298 K (25 ºC)]

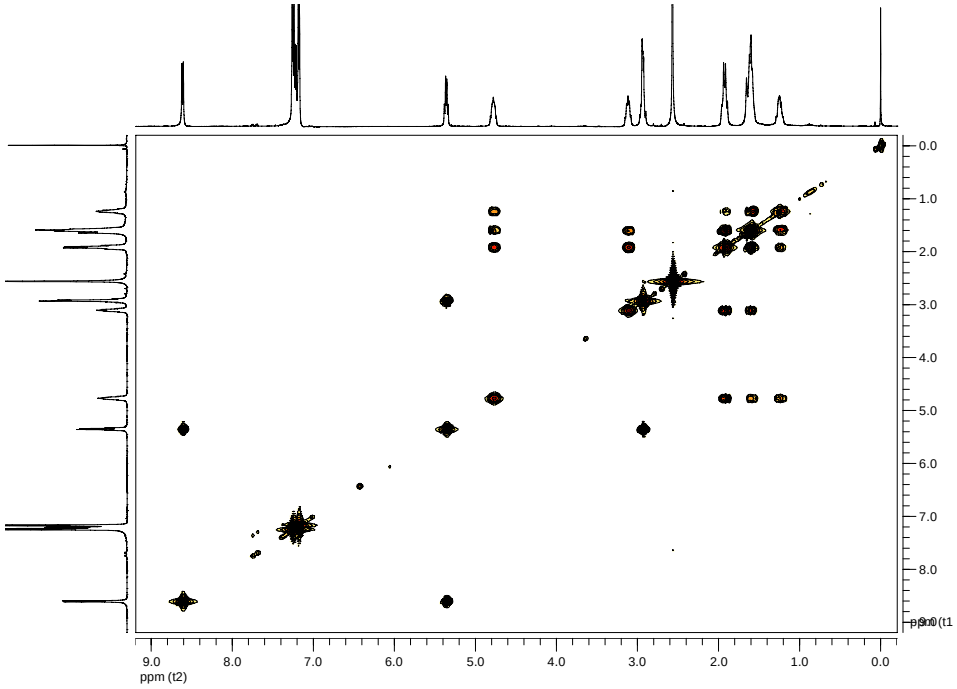




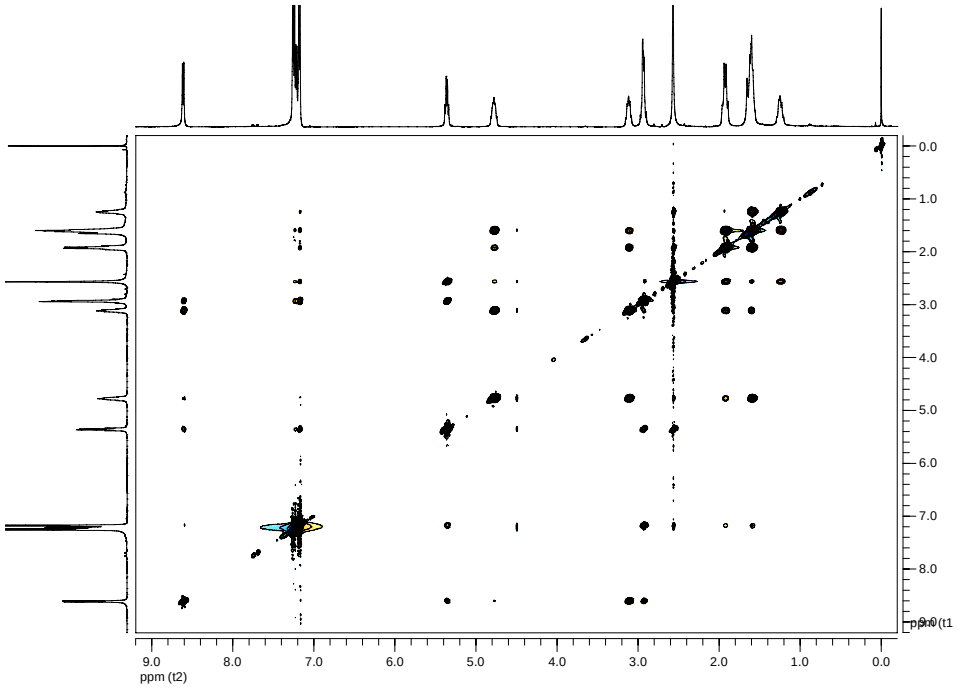


***cyclo*[(*D*-Phe-*L*-^{Me}N-γ-Acp)$_6$-] (5)**

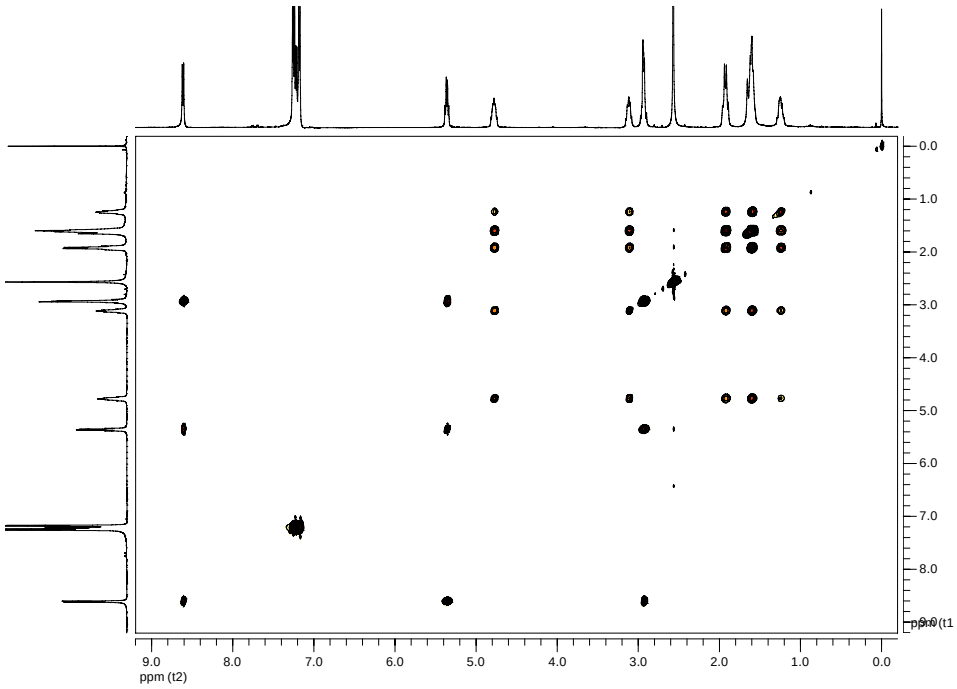
1) COSY [15.32 mM, $CDCl_3$, 298 K (25 ºC), 500.13 MHz]



2) ROESY [15.32 mM, $CDCl_3$, 298 K (25 ºC), 500.13 MHz]



3) TOCSY [15.32 mM, $CDCl_3$, 298 K (25 ºC), 500.13 MHz]



4) FT-IR [$CHCl_3$, 298 K (25 ºC)]