Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

SUPPORTING INFORMATION

Novel Me-blocked Dimeric α,γ-Peptide Nanotubulet Segments: Formation of a Peptide Heterodimer through Backbone-backbone Interactions**

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1. General Methods, Instrument Details and Materials

General. Commercially available N-Boc amino acids, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), 1-hydroxybenzotriazole (HOBt) and *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU) were all used as obtained from Novabiochem, Applied Biosystems or Bachem. *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC.HCl) 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] Tetrahydrofuran (THF) was dried and distilled over sodium/benzophenone.[1,2] DIEA was dried and distilled over calcium hydride, and then redistilled over ninhydrin.[1,2] Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F_{254} plates. Compounds that were not UV-

[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**

active were visualized by dipping the plates in a ninhydrin or cerium ammonium molybdate 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 columns with CH_2Cl_2/MeOH gradients between 100:0 and 90:10. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Varian Inova-750 MHz, Varian Mercury-300 MHz, Bruker AMX-500 MHz or Bruker WM-250 MHz spectrometers. 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 Varian Mercury-300 MHz, Bruker WM-250 MHz or Bruker AMX-500 MHz spectrometers. Carbon resonances were assigned using distortionless enhancement by polarization transfer (DEPT) spectra obtained with phase angles of 135°. Chemical Ionization (CI) mass spectra were recorded on a Finnigan TraceMS mass spectrometer. Fast Atom Bombardement (FAB) mass spectra were recorded on a Micromass Autospec mass spectrometer. Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) mass spectrometry was performed on a Bruker Autoflex mass spectrometer. Crystallographic data were collected in a Bruker Smart X1000 diffractometer. FTIR measurements were made on a JASCO FT/IR-400 spectrophotometer using 5-10 mM solutions in $CHCl_3$ placed in an NaCl 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 the indicated concentrations and temperatures. 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}H NMR spectra were also obtained on a Varian Inova-750 MHz spectometer. Due to conformational averaging on the NMR time scale, monomeric peptides with C_3 sequence symmetry generally had C_3-degenerate ^{1}H NMR spectra, and their dimers D_3-degenerate spectra.

2. Synthesis of (1R,3S)-3-aminocyclopentanecarboxylic acid derivatives

2-Azabicyclo[2.2.1]heptan-3-one.[3] A solution of 2-azabicyclo[2.2.1]hept-5-en-3-one (Vince´s lactam, **4**; 25.00 g, 229.36 mmol) in EtOAc (1 L) was treated with 10% Pd/C (7.32 g, 6.88 mmol), and hydrogenated at balloon pressure for 1-2 days. The insolubles were separated by filtration through Celite, rinsed with EtOAc and concentrated to provide 2-azabicyclo[2.2.1]heptan-3-one as a white solid [25.20 g, 99 %, R_f = 0.20 (EtOAc)]. **^{1}H NMR** ($CDCl_3$, 250.13 MHz, δ): 7.08 (br, 1H), 3.72 (m, 1H), 2.53 (m, 1H), 1.85-1.11 (m, 6H). **^{13}C NMR** ($CDCl_3$, 62.90 MHz, δ): 181.2 (CO), 54.8 (CH), 44.6 (CH), 40.9 (CH_2), 29.6 (CH_2), 23.1 (CH_2). **MS (CI)** [m/z (%)]: 112 ([MH]$^+$, 100), 94 (4), 84 (2).

***cis*-3-Aminocyclopentanecarboxylic acid hydrochloride.**[3] A solution of 2-aza-bicyclo[2.2.1]heptan-3-one (10.50 g, 94.60 mmol) in 500 mL of HCl (10%) was stirred for 2 days at room temperature and then concentrated *in vacuo*. Addition of acetone to the resulting yellow oil gave a white solid, *cis*-3-aminocyclopentanecarboxylic acid hydrochloride, that was filtered out and washed with acetone [14.61 g, 93 %, R_f = 0.32 (MeOH)]. **^{1}H NMR** (D_2O, 250.13 MHz, δ): 3.70 (m, 1H), 2.95 (p, *J* = 8.0 Hz, 1H), 2.35 (m, 1H). **^{13}C NMR** (D_2O, 62.90 MHz, δ): 180.1 (CO_2H), 51.8 (CH), 42.6 (CH), 34.0 (CH_2), 30.2 (CH_2), 28.0 (CH_2). **MS (CI)** [m/z (%)]: 130 ([MH -HCl]$^+$, 93), 112 (100), 95 (11), 84 (15).

(1*R*,3*S*)-3-Amino-N-*t*-butyloxycarbonylcyclopentanecarboxylic acid (*L*-Boc-γ-Acp-OH, 5). To a solution of *cis*-3-aminocyclopentanecarboxylic acid hydrochloride (14.50 g, 87.61 mmol) in water (300 mL) and dioxane (300 mL) were added Boc_2O (28.65 g, 131.42 mmol) and DIEA (45.91 mL, 262.84 mmol). After stirring at rt for 3 h, the solution was acidified to pH 3 by addition of HCl (10%) and extracted with CH_2Cl_2. The combined organic phases were dried (Na_2SO_4), filtered and concentrated, providing a yellow oil that was crystallized from 1:1 $CHCl_3$/hexanes, giving 13.38 g and 3.91 g of *cis*-Boc-3-aminocyclopentanecarboxylic acid in successive crystallizations. This racemic product was resolved by crystallization from 1:1 $CHCl_3$/hexane in the presence of (+)-α-phenylethylanamine (0.7-1 equiv.), and the resulting white crystals were washed with 2:1 $CHCl_3$/hexane, poured into a separation funnel and dissolved in CH_2Cl_2 and washed with 5% citric acid (this operation was repeated 2-3 times). The combined organic layers were dried (Na_2SO_4), filtered, concentrated and the resulting oil crystallized form $CHCl_3$/hexane (1:1) [86 %, R_f = 0.82 (MeOH), white crystals]. **^{1}H NMR** ($CDCl_3$, 250.13 MHz, δ): 6.34 and 5.03 (m, 1H), 4.15-3.73 (m, 1H), 2.81 (m, 1H), 2.18 (m, 1H), 1.39 (s, 9H). **^{13}C NMR** ($CDCl_3$, 62.90 MHz, δ): 181.7 (CO_2H),

[3] Jagt, J. C.; Van Leusen, A. M. *J. Org. Chem.* **1974**, *39*, 565-566.

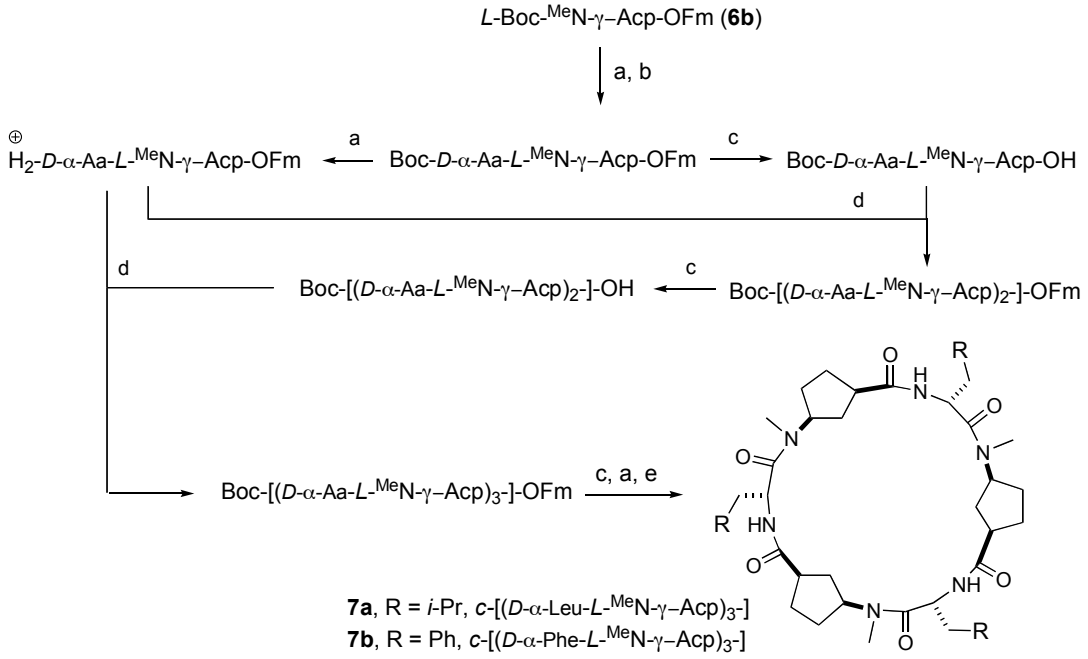
155.4 (CO), 79.2 (C), 51.9 (CH), 41.7 (CH), 36.0 (CH_2), 33.0 (CH_2), 28.3 (CH_3), 27.9 (CH_2). **MS (CI)** [m/z (%)]: 230 ($[MH]^+$, 42), 174 (89), 156 (66), 130 ($[MH - Boc]^+$, 100), 112 (74), 95 (10), 84 (11). **HRMS $[MH]^+$ calculated** for $C_{11}H_{20}NO_4$ 230.139233, **found** 230.140269. $[\alpha]_D$ = -16.8 (c = 1.0, MeOH).

(1*R*,3*S*)-3-Amino-N-*t*-butyloxycarbonyl-N-methylcyclopentanecarboxylic acid (*L*-Boc-^{Me}N-γ-Acp-OH, 6a). A solution of *L*-Boc-γ-Acp-OH (750 mg, 3.27 mmol) in dry THF (25 mL) was treated with NaH (390 g, 60% in mineral oil, 9.82 mmol) and stirred at 0 °C for 30 min. Iodomethane (610 µL, 9.82 mmol) was added and the resulting mixture was stirred overnight at rt. After quenching with water, the THF was removed and the resulting aqueous solution was washed with Et_2O, acidified to pH 3 by addition of 10% HCl, and finally extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure, and crystallization of the residue from Et_2O/hexanes gave *L*-Boc-^{Me}N-γ-Acp-OH as colourless crystals [0.78 g, 98%, R_f = 0.43 (5% MeOH in CH_2Cl_2)]. **^{1}H NMR** ($CDCl_3$, 250.13 MHz, δ): 4.53 (m, 1H), 2.82 (m, 1H), 2.76 (s, 3H), 1.46 (s, 9H). **^{13}C NMR** ($CDCl_3$, 62.90 MHz, δ): 181.2 (CO), 155.8 (CO), 79.6 (C), 55.8 (CH), 41.2 (CH), 31.7 (CH_2), 28.3 (CH_3), 28.2 (CH_3), 27.3 (CH_2), 27.2 (CH_2). **MS (CI)** [m/z (%)]: 244 ($[MH]^+$, 3), 188 (36), 170 (30), 144 ($[MH - Boc]^+$, 100), 126 (85), 112 (9). **HRMS $[MH]^+$ calculated** for $C_{12}H_{22}NO_4$ 244.154883, **found** 244.155279.

(9*H*-Fluoren-9-yl)methyl (1*R*,3*S*)-3-amino-N-*t*-butyloxycarbonyl-N-methylcyclopentane-carboxylate (*L*-Boc-^{Me}N-γ-Acp-OFm, 6b). A solution of *L*-Boc-^{Me}N-γ-Acp-OH (600 mg, 2.47 mmol) in dry CH_2Cl_2 (25 mL) was treated with EDC.HCl (710 mg, 3.70 mmol), HOBt (500 mg, 3.70 mmol), 9-fluorenemethanol (580 mg, 2.96 mmol) and DMAP (405 mg, 3.70 mmol). After 2 h stirring at rt, the mixture was washed with 10% HCl, NH_4Cl (sat) and $NaHCO_3$ (sat). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure, and the resulting crude material was purified by flash chromatography (8-12% EtAcO/hexanes), giving 960 mg of **3** [92%, R_f = 0.87 (50% EtOAc/hexanes), white foam]. **^{1}H NMR** ($CDCl_3$, 250.13 MHz, δ): 7.77 (d, J = 7.4 Hz, 2 H), 7.61 (t, J = 7.2 Hz, 2 H), 7.48-7.27 (m, 4 H), 4.47 (m, 2 H), 4.31-3.99 (m, 2 H), 2.77 (m, 1 H), 2.69 (s, 3 H), 2.13-1.64 (m, 6 H), 1.47 (s, 9 H). **^{13}C NMR** ($CDCl_3$, 62.90 MHz, δ): 174.7 (CO), 154.9 (CO), 143.1 (C), 140.6 (C), 127.0 (CH), 126.3 (CH), 124.2 (CH), 119.3 (CH), 78.5 (C), 65.1 (CH_2), 55.1 (CH), 46.3 (CH), 40.7 (CH), 31.0 (CH_2), 27.8 (CH_3), 27.6 (CH_3), 26.7 (CH_2), 26.6 (CH_2). **MS (CI)** [m/z (%)]: 422 ($[MH]^+$, 22), 350 (24), 322 ($[MH - Boc]^+$, 100), 178 (62), 126 (39). **HRMS $[MH]^+$ calculated** for $C_{26}H_{32}NO_4$ 422.233134, **found** 422.233733.

3. Cyclic Peptide Synthesis

Cyclopeptides **7a** and **7b** were prepared following the synthetic strategy shown in the scheme below, starting from *L*-Boc-^{Me}N-γ-Acp-OFm. The corresponding dipeptides, tetrapeptides and hexapeptides were prepared using standard solution-phase peptide synthesis protocols, and treatment of the unprotected linear hexapeptides with TBTU and DIEA (1 mM in dichloromethane) led to formation of the desired cyclic products in good yields (50-75%).



Scheme. Standard procedure for solution-phase cyclic peptide synthesis.

Boc-[*D*-Leu-*L*-^{Me}N-γ-Acp]-OFm. A solution of **6b** (775 mg, 1.84 mmol) in 18 mL of TFA/DCM (1:1) was stirred at rt for 15 min. After removal of the solvent, the residue was dried under high vacuum for 3 h. The resulting TFA salt was dissolved in dry DCM (18 mL), and *D*-Boc-Leu-OH.H_2O (467 mg, 2.02 mmol), HATU (769 mg, 0.601 mmol) and DIEA (1.29 mL, 7.36 mmol) were successively added. After 1 h stirring at rt, the solution was poured into a separation funnel and washed with HCl (5 %) and $NaHCO_3$ (sat.). The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure, providing a yellow oil that when purified by flash chromatography (15-25 % EtOAc in hexanes) gave 897 mg of the dipeptide as a white foam [91 %, R_f = 0.66 (50 % EtOAc/hexanes)]. **^{1}H NMR** ($CDCl_3$, 250.13 MHz, δ): 7.67 (d, J = 7.3 Hz, 2H), 7.50 (d, J = 7.3 Hz, 2H), 7.27 (td, J_1 = 7.4 Hz, J_2 = 22.2 Hz, 4H), 5.24 (m, 1H), 4.85 (m, 1H), 4.70-4.30 (m, 3H), 4.12 (m, 1H), 2.76 (s, 3H), 2.64 (m, 1H), 2.08-1.05 (m, 18 H), 0.88 (ddd, J_1 = 5.1 Hz, J_2 = 7.7 Hz, J_3 = 10.2 Hz, 6H). **^{13}C NMR** ($CDCl_3$, 62.90 MHz, δ): 175.5 (CO), 173.2 (CO), 155.5 (CO), 143.5 (C), 141.2 (C), 127.7 (CH), 127.0 (CH), 124.7 (CH), 119.9 (CH), 79.3 (C), 65.8 (CH_2),

57.2 (CH), 53.9 (CH), 49.2 (CH), 46.9 (CH), 42.7 (CH_2), 41.5 (CH), 30.9 (CH_2), 29.0 (CH_3), 28.3 (CH_3), 27.5 (CH_2), 27.0 (CH_2), 23.4 (CH_3), 21.7 (CH_3). **MS (FAB$^+$)** [m/z (%)]: 535 ([MH]$^+$, 17), 479 (8), 435 ([MH -Boc]$^+$, 7). **HRMS (FAB$^+$) calculated** for $C_{32}H_{43}N_2O_5$ ([MH]$^+$) 535.317198, **found** 535.318447.

Boc-[*D*-Phe-*L*-^{Me}N-γ-Acp]-OFm. Prepared in the same way as Boc-[*D*-Leu-*L*-^{Me}N-γ-Acp-]-OFm. Yield 329 mg (98%), white foam [R_f = 0.40 (30 % EtOAc in hexanes)]. **^{1}H NMR** ($CDCl_3$, 250.13 MHz, δ): 7.65 (t, *J* = 6.5 Hz, 2H), 7.46 (d, *J* = 7.2 Hz, 2H), 7.36-7.02 (m, 9H), 5.41 (d, *J* = 8.7 Hz, 1H), 4.75 (m, 1H), 4.38 (m, 2H), 4.17-3.74 (m, 2H), 2.87 (m, 2H), 2.73-2.19 (m, 4H), 2.15-0.83 (m, 15 H). **^{13}C NMR** ($CDCl_3$, 62.90 MHz, δ): 175.4 (CO), 171.8 (CO), 154.9 (CO), 143.5 (C), 141.2 (C), 136.3 (C), 129.3 (CH), 128.3 (CH), 127.6 (CH), 126.9 (CH), 126.7 (CH), 124.7 (CH), 119.8 (CH), 79.5 (C), 65.7 (CH_2), 53.7 (CH), 51.9 (CH), 46.8 (CH), 41.2 (CH), 40.3 (CH_2), 30.8 (CH_2), 28.7 (CH_3), 28.2 (CH_3), 27.8 (CH_2), 26.6 (CH_2). **MS (FAB$^+$)** [m/z (%)]: 569 ([MH]$^+$, 33), 513 (14), 469 ([MH -Boc]$^+$, 37). **HRMS [MH]$^+$ calculated** for $C_{35}H_{41}N_2O_5$ 569.301548, **found** 569.301296.

Boc-[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_2$-]-OFm. A solution of the dipeptide Boc-[*D*-Leu-*L*-^{Me}N-γ-Acp-]-OFm (225 mg, 0.42 mmol) in 1:4 piperidine/DCM (5 mL) was stirred at rt for 20 min, the solvent was removed *in vacuo*, and the residue was dissolved in DCM (10 mL). This solution was washed with HCl (5%), dried over Na_2SO_4, filtered and concentrated, giving Boc-[*D*-Leu-*L*-^{Me}N-γ-Acp-]-OH which was used without further purification.

A solution of Boc-[*D*-Leu-*L*-^{Me}N-γ-Acp]-OFm (225 mg, 0.42 mmol) in 4 mL of TFA/DCM (1:1) was stirred at rt for 15 min. After removal of solvent, the residue was dried under high vacuum for 3 h and dissolved in dry DCM (4 mL), and Boc-[*D*-Leu-*L*-^{Me}N-γ-Acp-]-OH, HBTU (176 mg, 0.46 mmol) and DIEA (294 μL, 1.68 mmol) were successively added. After 1 h stirring at rt, the solution was washed with HCl (5%) and $NaHCO_3$ (sat.), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography (0-3 % MeOH in CH_2Cl_2), giving 270 mg of Boc-[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_2$-]-OFm as a white foam [83 %, R_f = 0.28 (3 % MeOH in DCM)]. **MS (FAB$^+$)** [m/z (%)]: 773 ([MH]$^+$, 100), 673 ([MH-Boc]$^+$, 14), 560 (22), 452 (48). **HRMS [MH]$^+$ calculated** for $C_{45}H_{65}N_4O_7$ 773.485326, **found** 773.485750.

Boc-[(*D*-Phe-*L*-^{Me}N-γ-Acp)$_2$-]-OFm. Prepared in the same way as dipeptide Boc-[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_2$-]-OFm. Yield 153 mg (94%), white foam [R_f = 0.32 (3 % MeOH in DCM)]. **MS (FAB$^+$)** [m/z (%)]: 841 ([MH]$^+$, 100), 741 ([MH-Boc]$^+$, 36), 594 (52), 520 (60), 420 (90). **HRMS [MH]$^+$ calculated** for $C_{51}H_{61}N_4O_7$ 841.454026, **found** 841.453716.

Boc-[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_3$-]-OFm. A solution of the tetrapeptide Boc-[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_2$-]-OFm (40 mg, 52 μmol) in 1:4 piperidine/DCM (500 μL) was stirred at rt for 20 min, the solvent was removed *in vacuo* and the residue was dissolved in DCM (10 mL). This solution was washed with HCl (5%), dried over Na_2SO_4, filtered and concentrated, giving Boc-[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_2$-]-OH, which was used without further purification.

A solution of Boc-[*D*-Leu-*L*-^{Me}N-γ-Acp-]-OFm (28 mg, 52 μmol) in 500 μL of TFA/DCM (1:1) was stirred at rt for 15 min. After removal of solvent, the residue was dried under high vacuum for 3 h and dissolved in dry DCM (500 μL), and Boc-[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_2$-]-OH, HBTU (22 mg, 57 μmol) and DIEA (36 μL, 207 μmol) were successively added. After 1 h stirring at rt, the solution was washed with HCl (5%) and $NaHCO_3$ (sat.), dried over Na_2SO_4, and concentrated under reduced pressure, and the residue was purified by flash chromatography (0-5% MeOH in CH_2Cl_2), giving 45 mg of Boc-[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_3$-]-OFm as a white foam [87 %, R_f = 0.51 (10% MeOH in DCM)]. **MS (FAB$^+$)** [m/z (%)]: 1011 ([MH]$^+$, 36), 911 ([MH-Boc]$^+$, 13), 798 (6), 560 (100), 452 (11). **HRMS [MH]$^+$ calculated** for $C_{58}H_{87}N_6O_9$ 1011.653454, **found** 1011.654554.

Boc-[(*D*-Phe-*L*-^{Me}N-γ-Acp)$_3$-]-OFm. Prepared in the same way as hexapeptide Boc-[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_3$-]-OFm from Boc-[(*D*-Phe-*L*-^{Me}N-γ-Acp)$_2$-]-OFm (148 mg, 0.481 mmol) and Boc-[*D*-Phe-*L*-^{Me}N-γ-Acp-]-OFm (100 mg, 176 μmol). Yield 186 mg (95%), white foam [R_f = 0.53 (10 % MeOH in DCM)]. **MS (FAB$^+$)** [m/z (%)]: 1113 ([MH]$^+$, 13), 1013 ([MH-Boc]$^+$, 3), 594 (14). **HRMS (FAB$^+$) calculated** for $C_{67}H_{81}N_6O_9$ ([MH]$^+$) 1113.606504, **found** 1113.606564.

***cyclo*[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_3$-] (7a)**. A solution of Boc-[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_3$-]-OFm (45 mg, 45 μmol) in 20% piperidine in DCM (500 μL) 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 (3 x 5 mL), dried over Na_2SO_4, filtered and concentrated. The resulting residue was dissolved in 500 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 (45 mL) and treated with TBTU (17 mg, 54 μmol), followed (dropwise) by DIEA (32 μL, 178 μmol) [an additional 1 equiv. of TBTU (14 mg, 45 μmol) and 4 equiv. of DIEA (32 mL, 178 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 19 mg of **7a** as a white solid (60%). **^{1}H NMR** ($CDCl_3$, 500.13 MHz, δ): 8.23 (d, J = 9.4 Hz, 1H, NH), 5.18 (td, J_1 = 6.9 Hz, J_2 = 13.8 Hz, 1 H, Hα_{Leu}), 4.80 (qd, J_1 = 8.2 Hz, J_2 = 16.3 Hz, 1 H, Hγ_{Acp}), 3.07 (s, 3 H, NC$\underline{H}_3$), 2.94 (qd, J_1 = 7.6 Hz, J_2 = 15.4 Hz, 1 H, Hα_{Acp}), 2.29 (m, 1 H, C2-H$_{Acp}$), 2.10 (m, 1H, C2-H$_{Acp}$), 1.93-1.50 (m, 7 H),

0.93 (t, J = 6.0 Hz, 6 H, CH_{3Leu}). **^{13}C NMR** ($CDCl_3$, 125.77 MHz, δ): 175.2 (C=O), 173.4 (C=O), 54.8 (CH), 47.0 (CH), 42.5 (CH), 42.4 (CH_2), 35.7 (CH_2), 29.9 (NCH_3), 29.7 (CH_2), 27.3 (CH_2), 24.8 (CH), 23.0 (CH_3), 22.5 (CH_3). **FTIR** (293 K, $CHCl_3$): 3587, 3304 (amide A), 3030, 2928, 1661, 1626 (amide I), 1534 (amide II_{II}) cm^{-1}. **MS (FAB$^+$)** [m/z (%)]: 1429 ($[2MH]^+$, 3), 737 ([M +Na]$^+$, 3), 715 ([MH]$^+$, 100), 477 (6). **HRMS (FAB$^+$) calculated** for $C_{39}H_{67}N_6O_6$ ([MH]$^+$) 715.512210, **found** 715.511861.

***cyclo*[(*D*-Phe-*L*-^{Me}N-γ-Acp)$_3$-] (7b)**. Prepared in the same way as **7a** from Boc-[(*D*-Phe-*L*-^{Me}N-γ-Acp)$_3$-]-OFm (80 mg, 72 μmol). Yield after HPLC purification, 45 mg (77%); white solid. **^{1}H NMR** ($CDCl_3$, 750 MHz, δ): 8.56 (d, J = 9.2 Hz, 1 H, NH), 7.22 (m, 5 H, Ar), 5.32 (dt, J_1 = 6.4 Hz, J_2 = 9.3 Hz, 1 H, $H\alpha_{Phe}$), 4.77 (td, J_1 = 8.5 Hz, J_2 = 17.0 Hz, 1 H, $H\gamma_{Acp}$), 3.14 (dd, J_1 = 9.3 Hz, J_2 = 13.1 Hz, 2 H, $CH_2\beta_{Phe}$), 3.06 (dd, J_1 = 6.1 Hz, J_2 = 13.1 Hz, 1 H, $H\alpha_{Acp}$), 2.66 (s, 3H, $NC\underline{H}_3$), 2.34 (m, 1 H, C2-H_{Acp}), 2.01 (td, J_1 = 8.0 Hz, J_2 = 17.3 Hz, 1 H, CH_{Acp}), 1.74 (dt, J_1 = 7.6 Hz, J_2 = 14.0 Hz, 1H, CH_{Acp}), 1.61 (m, 1 H, CH_{Acp}), 1.47 (dd, J_1 = 10.9 Hz, J_2 = 23.6 Hz, 1 H, CH_{Acp}). **^{13}C NMR** ($CDCl_3$, 75.47 MHz, δ): 174.9 (C=O), 172.4 (C=O), 136.8 (C), 129.1 (CH), 128.3 (CH), 126.8 (CH), 54.5 (CH), 50.3 (CH), 42.7 (CH), 40.0 (CH_2), 36.1 (CH_2), 29.7 (CH_3), 26.9 (CH_2), 26.8 (CH_2). **FTIR** (293 K, $CHCl_3$): 3303 (amide A), 3007, 1664, 1625 (amide I), 1530 (amide II_{II}) cm^{-1}. **MS (MALDI-TOF)** [m/z]: 839.5 ([M +Na]$^+$), 817.5 ([MH]$^+$). **HRMS (MALDI-TOF) calculated** for $C_{48}H_{61}N_6O_6$ ([MH]$^+$) 817.465260, **found** 817.464710.

4. Solution FT-IR Characterization of Cyclic Peptide Dimers[4]

HPLC-purified peptides were dissolved at concentrations of 5-10 mM in $CHCl_3$ and placed in an NaCl IR cell. Spectra were acquired in transmission mode using a JASCO FT/IR-400 spectrophotometer with a step size of 4 cm^{-1}.

Entry	Dimer	IR (cm^{-1})		
		Amide $I_\perp$ [a]	Amide II_{II}	Amide A
1	**8_{7a}**	1626 (1661)	1534	3304
2	**8_{7b}**	1625 (1664)	1530	3304
3	**10**	1625 (1665)	1527	3309
4	**11_{7a-9}**	1625 (1660)	1531	3300
5	**11_{7b-9}**	1625 (1663)	1531	3300

Table 1. IR amide bands of compounds **8_{7a}**, **8_{7b}**, **10**, **11_{7a-9}**, **11_{7b-9}**. [a] Values in parentheses correspond to bands tentatively identified as the amide $I_\perp$ bands of *N*-alkylated linkages.

[4] a) P. I. Haris, D. Chapman, *Biopolymers (Peptide Sci.)* **1995**, *37*, 251-263; b) S. Krimm, J. Bandekar, In *Advances in Protein Chemistry*; Anfinsen, C. B.; Edsall, J. T., Richards, F. M., Eds.; Academic Press: Orlando, FL, 1986; pp 181-364; c) J. Bandekar, *Biochim. Biophys. Acta* **1992**, *1120*, 123-143.

5. X-ray Crystallographic Determination of the Structures of Heterodimer $11_{7a\text{-}9}$ and Homodimers 8_{7a} and 10

Preparation of Single Crystals for X-ray Analysis. In a typical experiment, 2 mg of HPLC-purified $\mathbf{8}_{7a}$ or **10,** or **2 mg** of a 1:1 mixture of HPLC-purified **7a** and **9,** was dissolved in 0.5 mL of $CHCl_3$ and equilibrated by vapour-phase diffusion against 2.5 mL of hexanes. The corresponding homodimer or heterodimer crystallized spontaneously within 1-3 days.

X-ray Crystallographic Analysis. Data were collected at 120 K in a Bruker Smart X1000 diffractometer using Mo Kα radiation and a graphite monochromator. All calculations were performed on an IBM-PC-compatible computer, using the programs COLLECT,[5] HKL Denzo and Scalepack,[6] SORTAV,[7] SHELX-97,[8] WinGx,[9] SIR2002,[10] ORTEP3,[11] PLATON (SQUEEZE),[12] and PARST[13]. Supplementary crystallographic data for $\mathbf{11}_{7a\text{-}9}$ (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.

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[6] Otwinowski, Z. and Minor, W. "*Processing of X-ray Diffraction Data Collected in Oscillation Mode*", Methods in Enzymology, Volume *276*: Macromolecular Crystallography, part A, p. 307-326, **1997**. Ed. Carter, C.W. Jr. and Sweet, R. M., Eds., Academic Press. New York.

[7] Blessing, R. H. *Acta Cryst.* **1995**, A51, 33-38.7

[8] G. M. Sheldrick, Institute für Anorganische Chemie, Universitat Gottingen, D-37077 Göttingen, Germany.

[9] Farrugia, L. J. *J. Appl. Cryst.*, **1999**, *32*, 837-838.

[10] Burla, M. C.; Camalli, M.; Carrozzini, B.; Cascarano, G. L.; Giacovazzo, C.; Polidori, G and Spagna, R. *J. Appl. Cryst.* ***2003****, 36, 1103*.

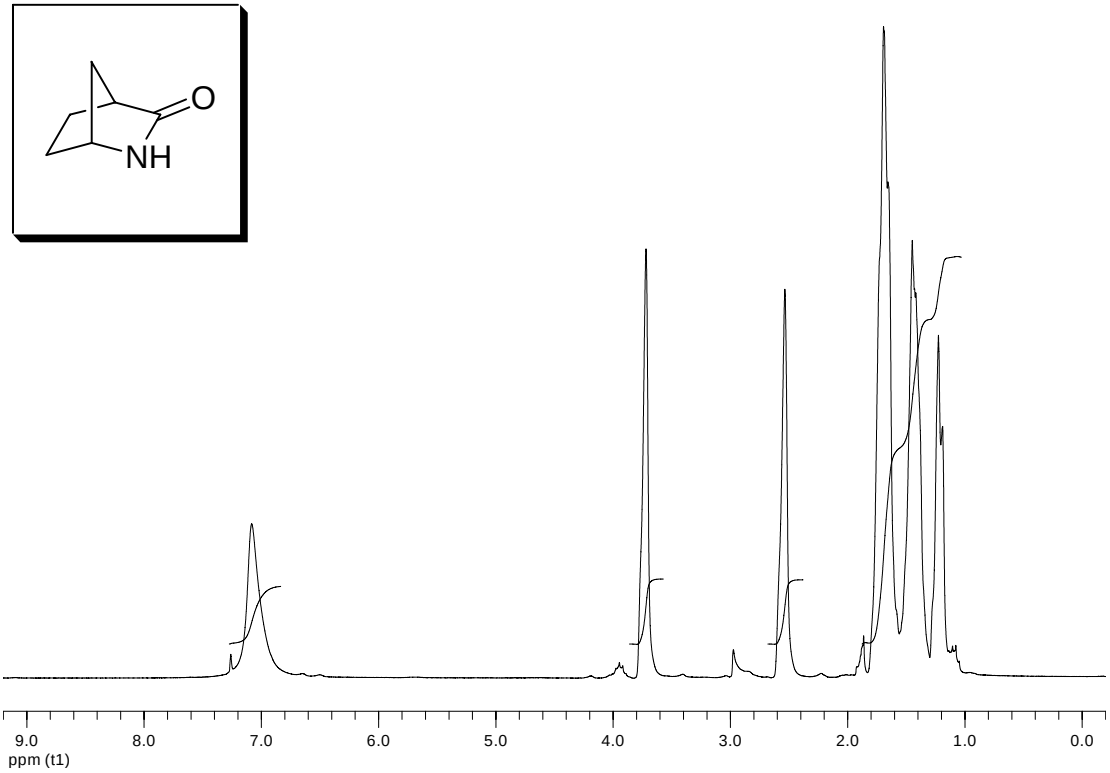
[11] Farrugia, L. J. *J. Appl. Cryst.*, **1997**, 30, 565.

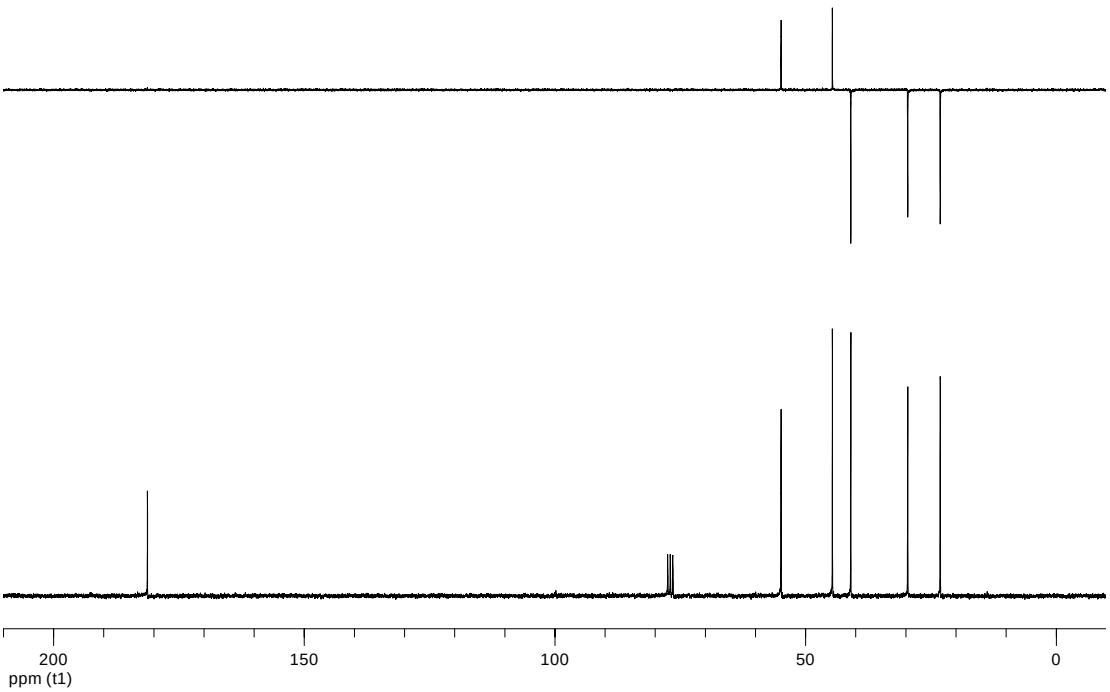
[12] Spek, A. L., University of Utrecht, The Netherlands, **2001**.

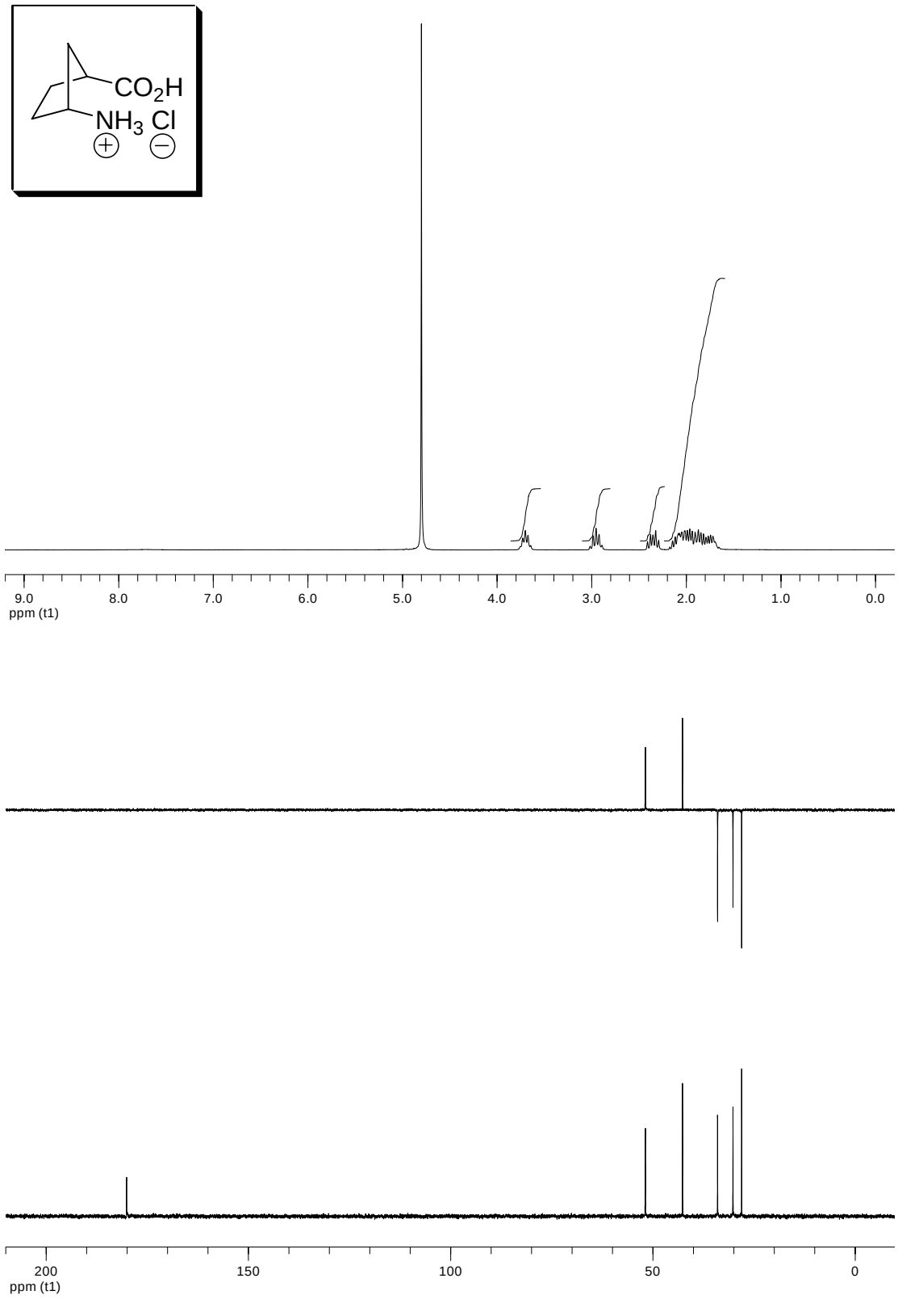
[13] Nardelli, M. *J. Appl. Cryst.*, **1995**, *28*, 659.

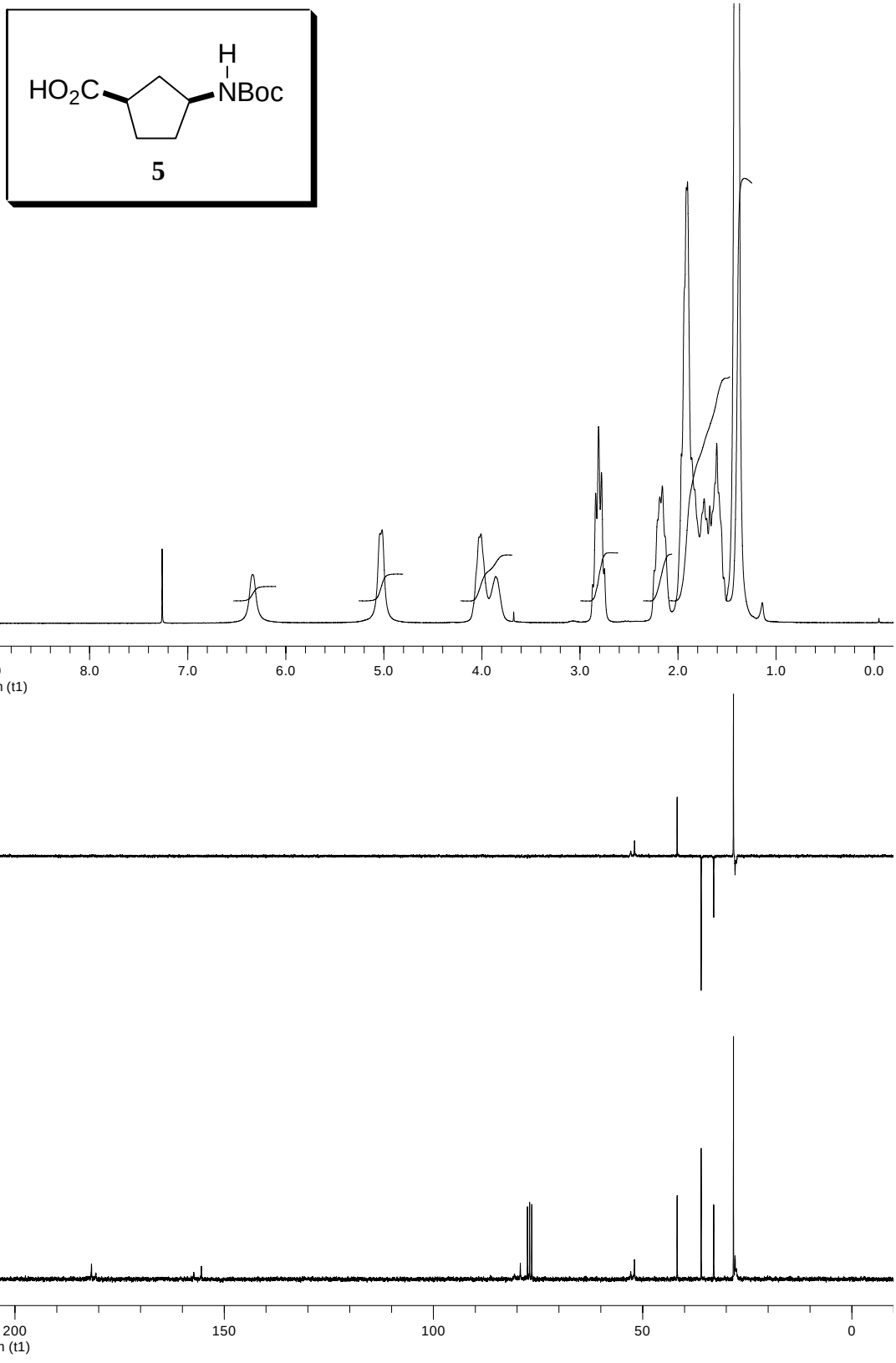
Dimer	^{Me}N···C=O	$C_{H\gamma}$···$C_{H\alpha}$	$C_{H\alpha}$···$C_{H\gamma}$	O=C···N^{Me}	HN···O=C	$C_{H\alpha}$···$C_{H\alpha}$	C=O···NH
	5.65	4.49	4.74	5.85	2.90	5.09	2.88
8_{7a}	5.74	4.61	4.45	5.71	2.88	5.06	2.91
	5.62	4.42	4.50	5.63	2.87	5.14	2.98
	5.71	4.56	4.51	5.58	2.83	5.05	2.93
10	5.84	4.79	4.85	5.86	2.91	4.88	2.97
	5.62	4.61	4.63	5.67	3.00	5.02	2.89
	5.68	4.58	4.39	5.61	2.93	5.07	2.90
11_{7a-9}	5.48	4.18	4.48	5.66	2.97	5.15	2.82
	5.49	4.19	4.59	5.71	2.83	5.08	2.87

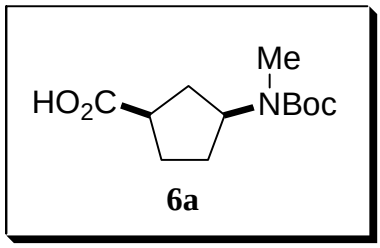
Table 2. Backbone-backbone distances within-dimers in the crystal structures of **8_{7a}**, **10** and **11_{7a-9}** (distances are in Å)


O
NH
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)


200
150
100
50
0
ppm (t1)

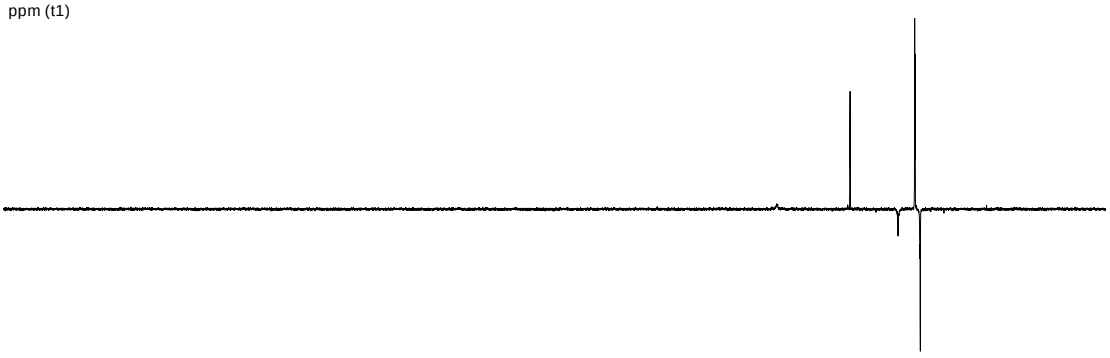

CO2H
NH3 Cl
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)
200
150
100
50
0
ppm (t1)

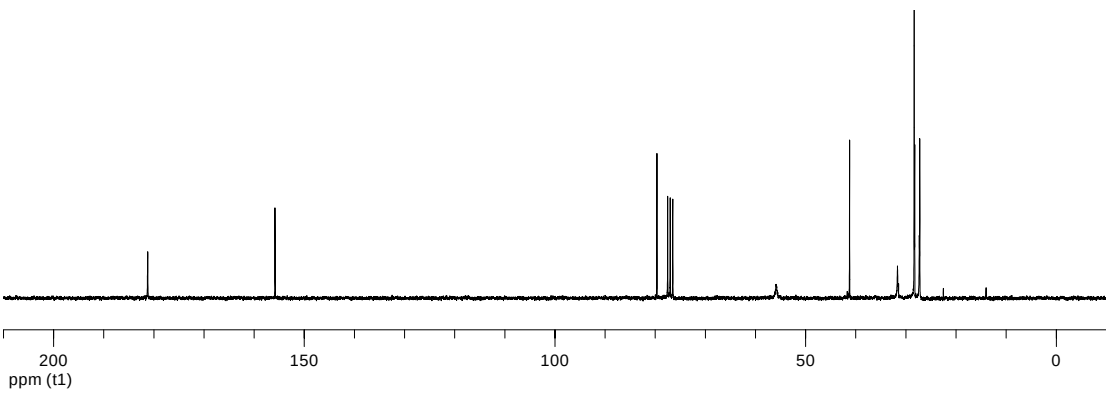

H
HO2C
NBoc
5
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)
200
150
100
50
0
ppm (t1)

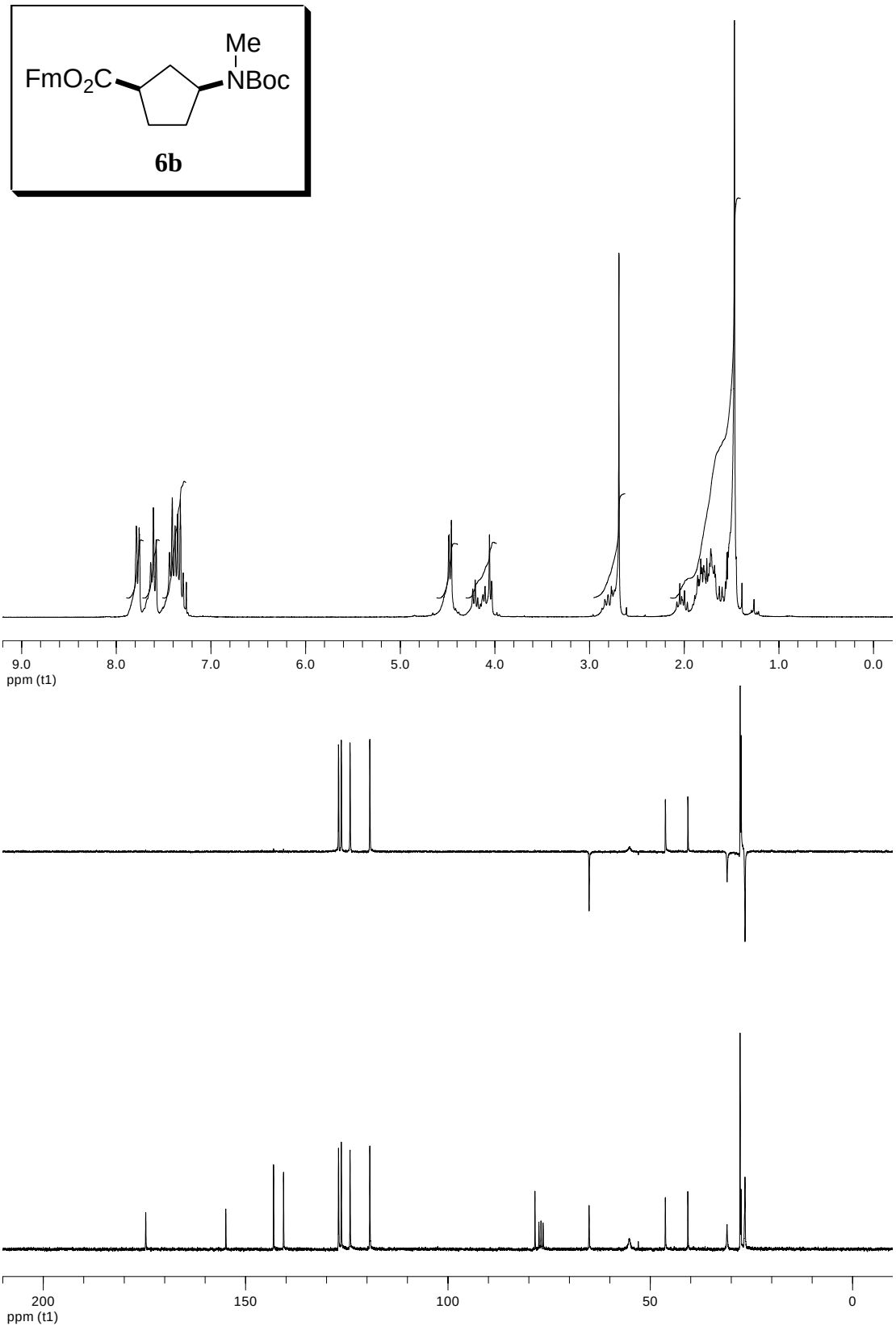

Me
HO2C
NBoc

6a

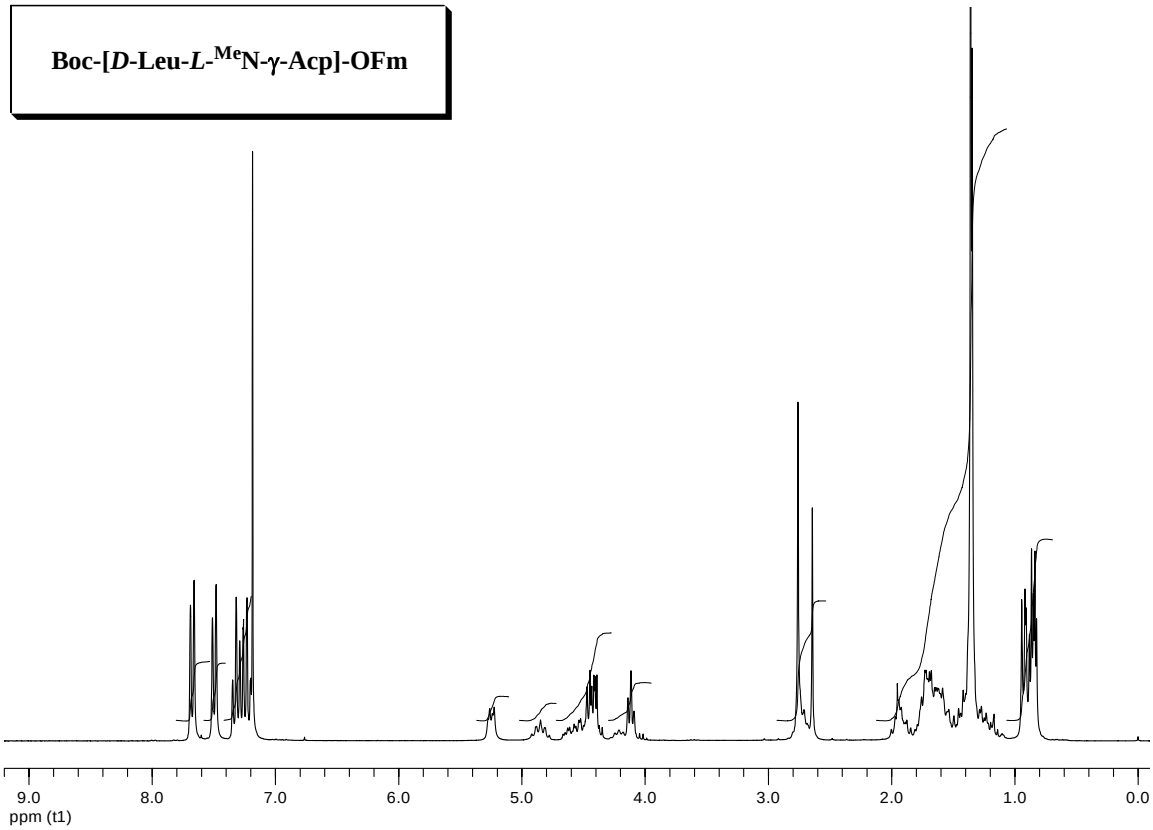
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

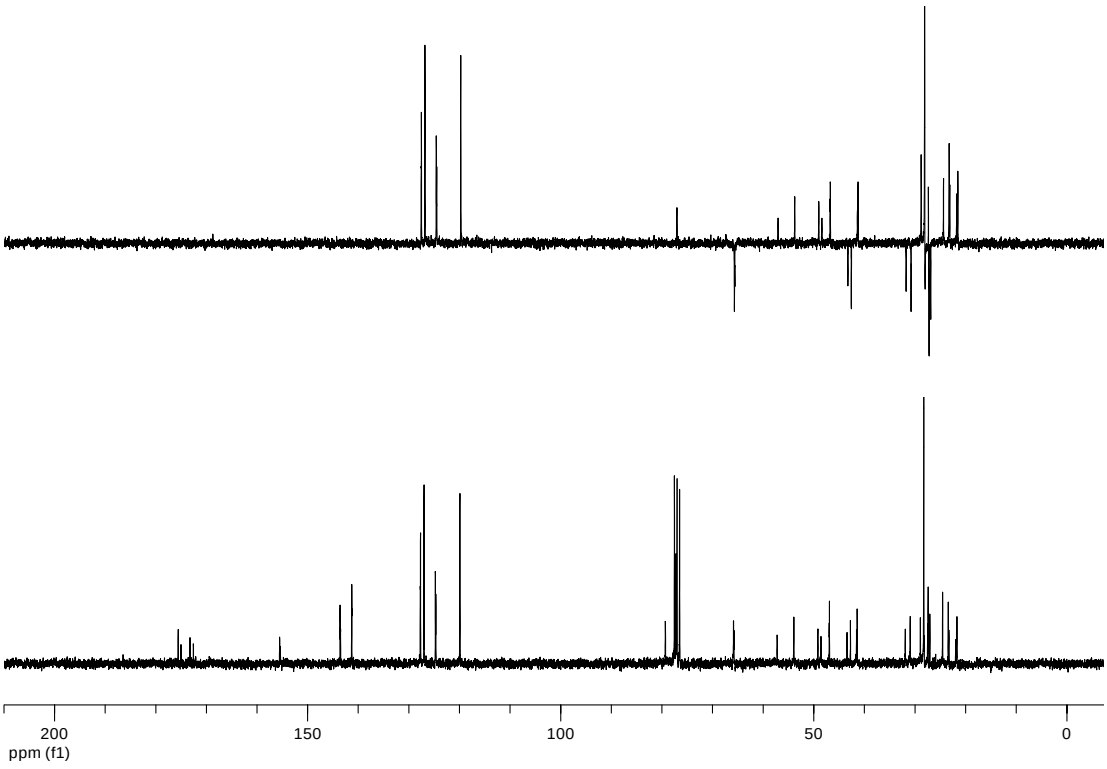



200
150
100
50
0
ppm (t1)

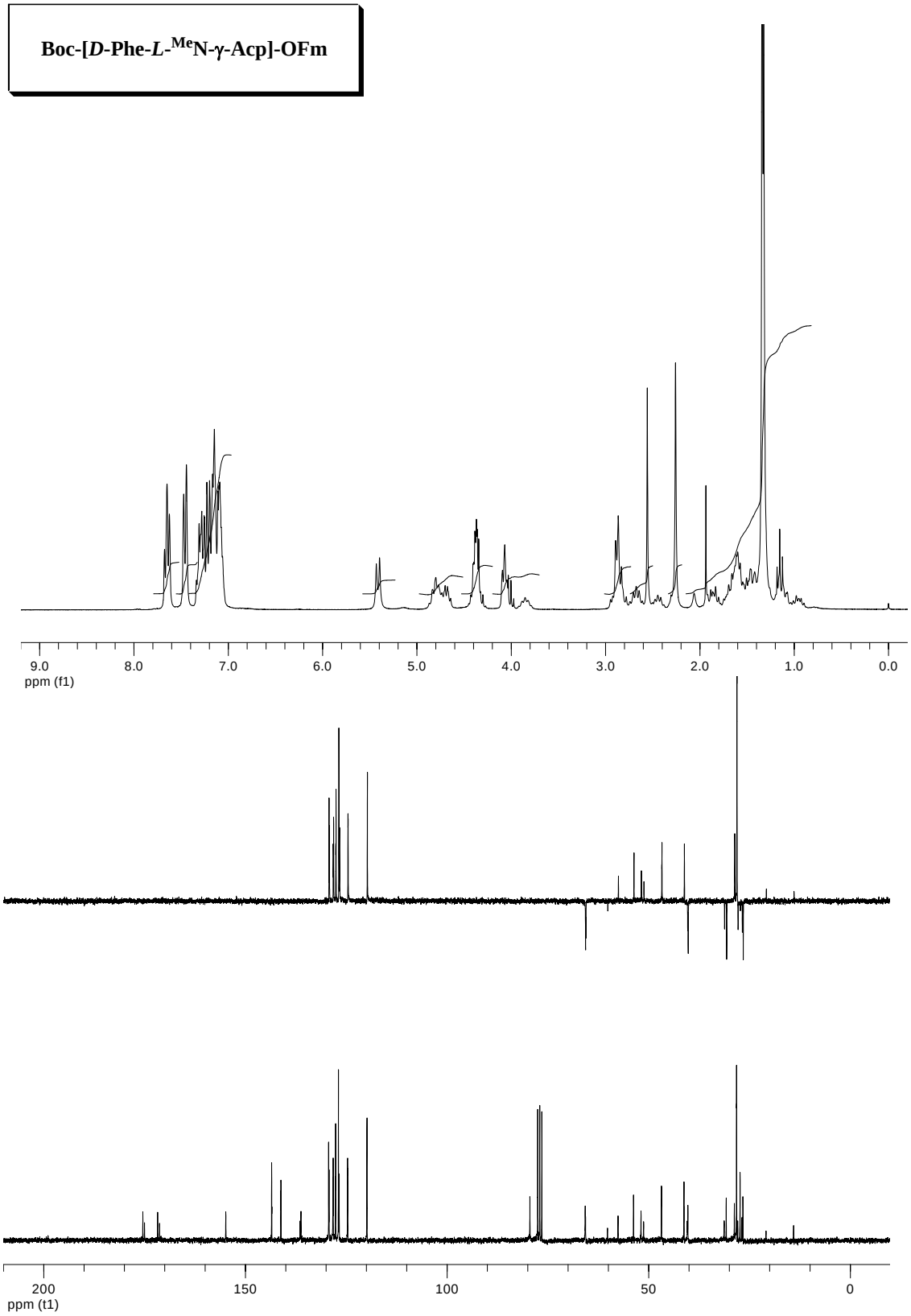

Me
FmO2C
NBoc
6b
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)
200
150
100
50
0
ppm (t1)

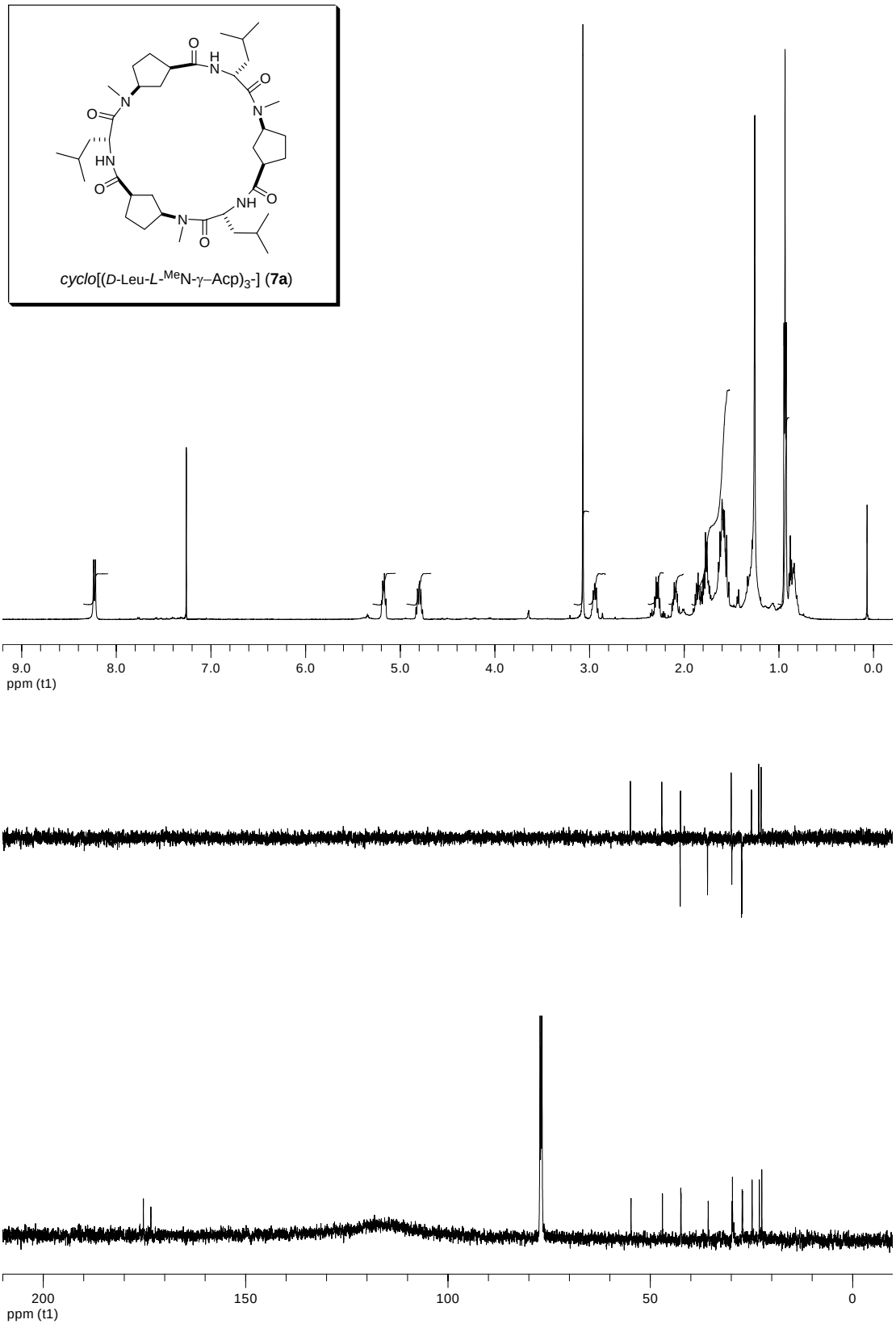
Boc-[*D*-Leu-*L*-^{Me}N-γ-Acp]-OFm





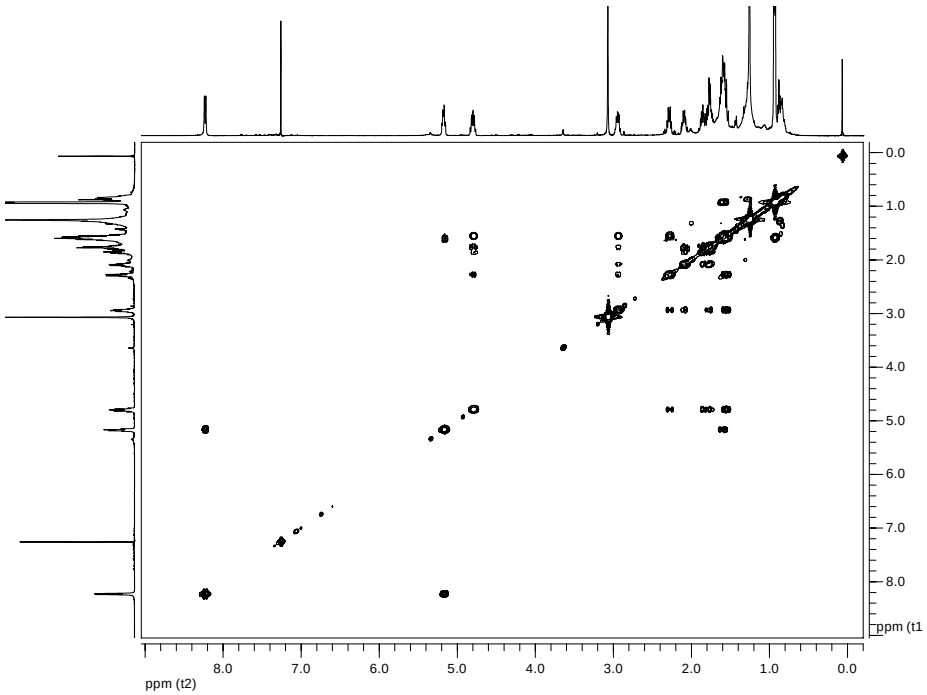
Boc-[*D*-Phe-*L*-^{Me}N-γ-Acp]-OFm



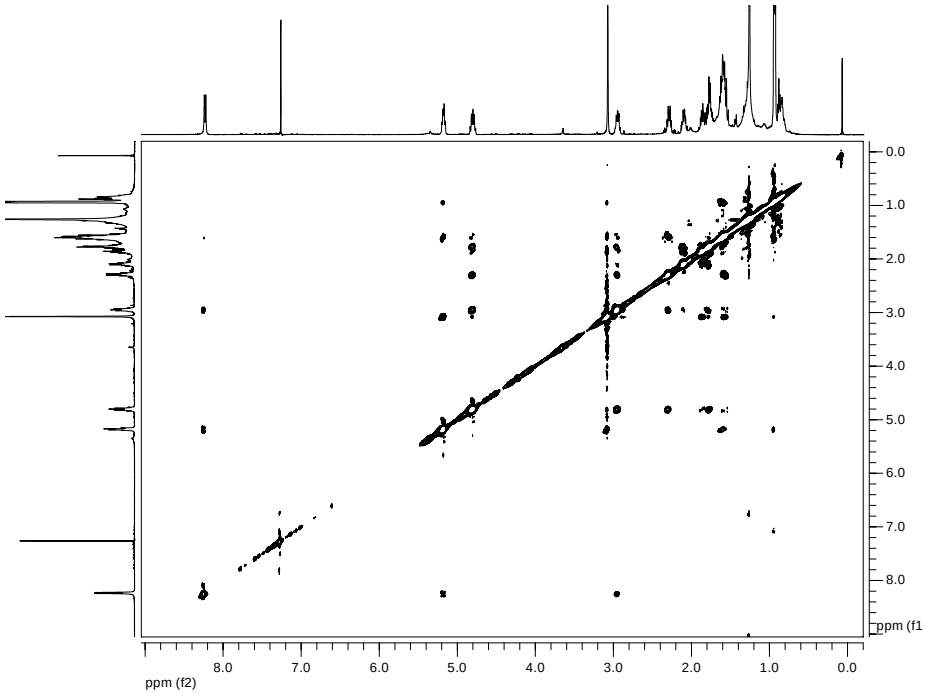

cyclo[(D-Leu-L-MeN-γ–Acp)3-] (7a)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)
200
150
100
50
0
ppm (t1)

***cyclo*[(*D*-Leu-*L*-^{Me}N-γ-Acp)$_3$-] (7a)**

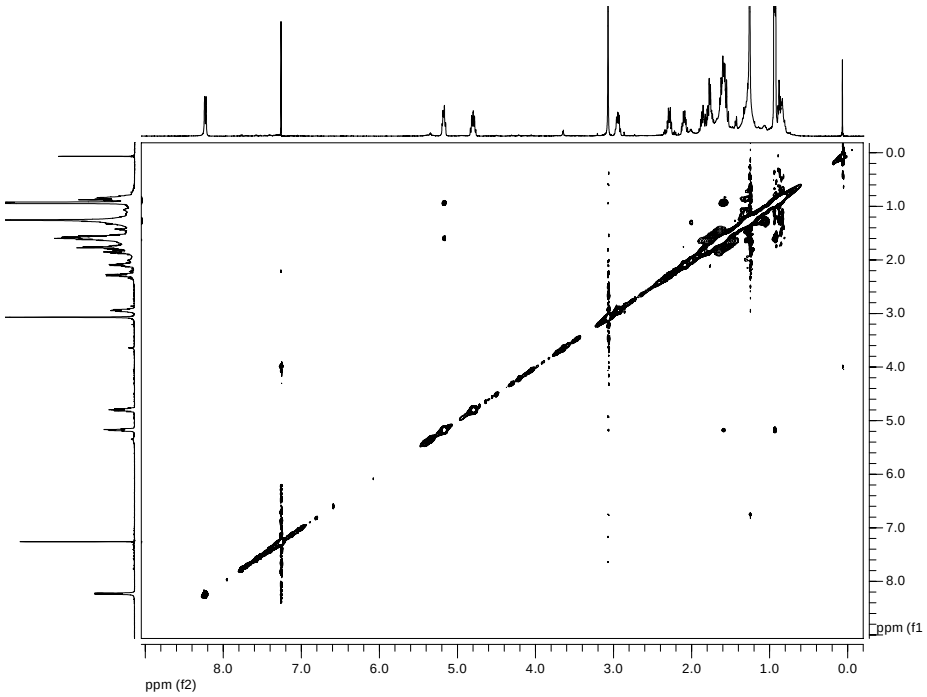
1) COSY [14.01 mM, $CDCl_3$, 298 K (25 º C), 500.13 MHz]



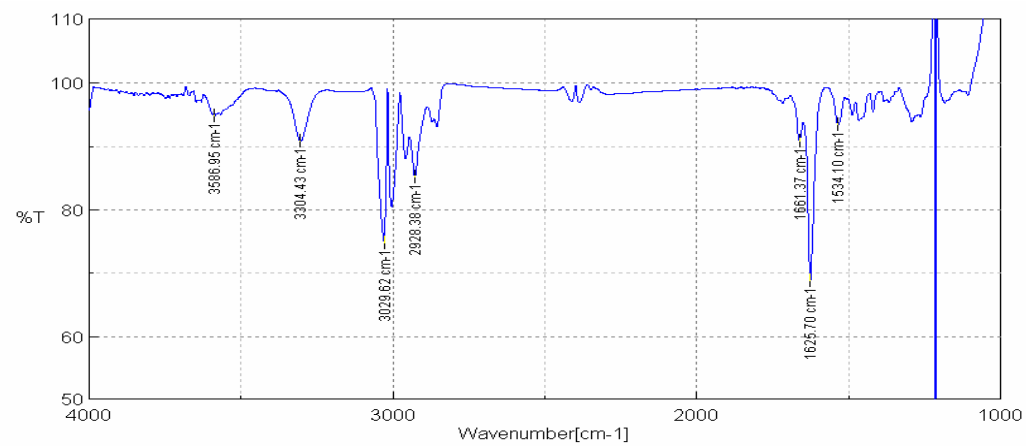
2) ROESY [14.01 mM, $CDCl_3$, 298 K (25 º C), 500.13 MHz]

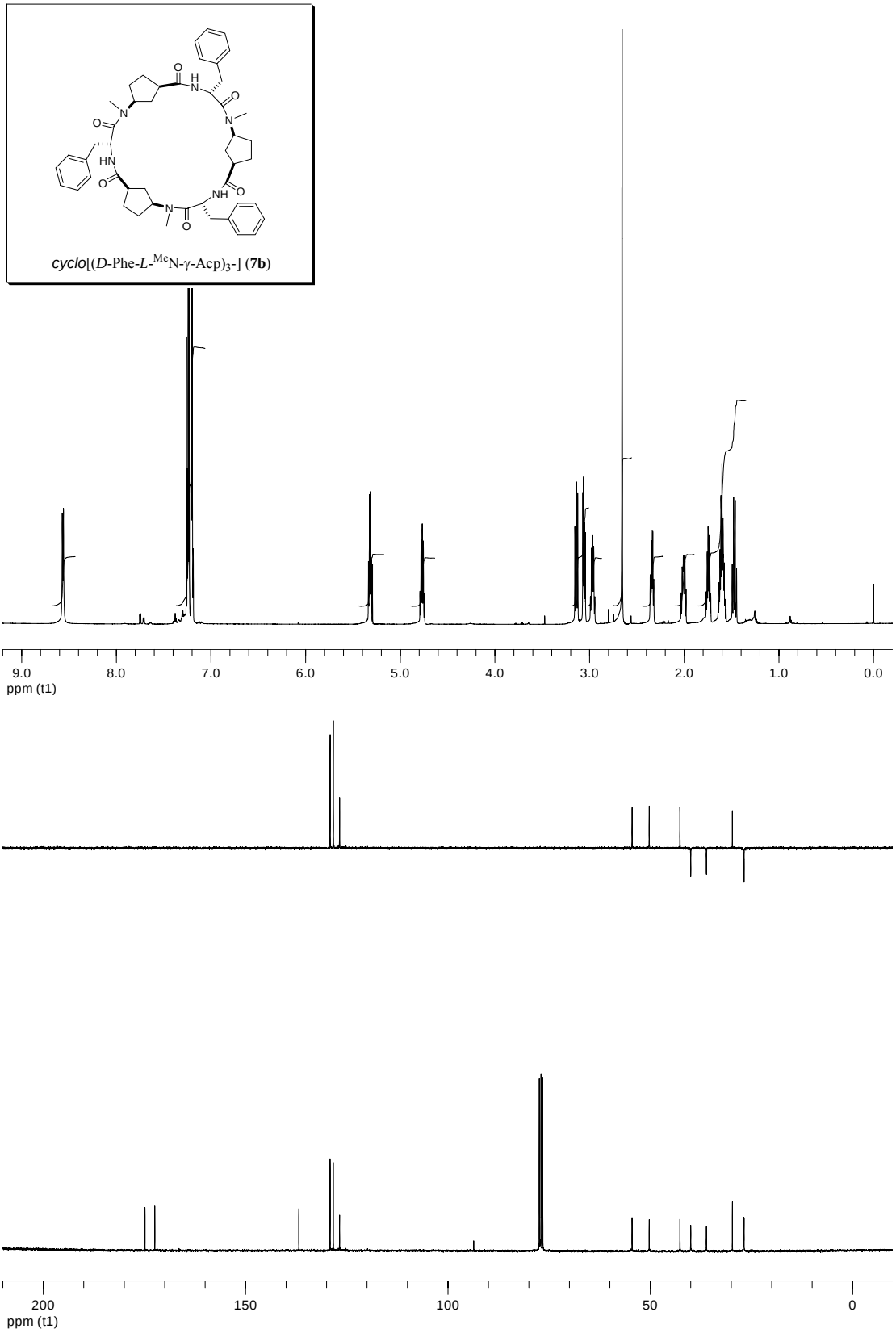


3) NOESY [14.01 mM, $CDCl_3$, 298 K (25 ° C), 500.13 MHz]



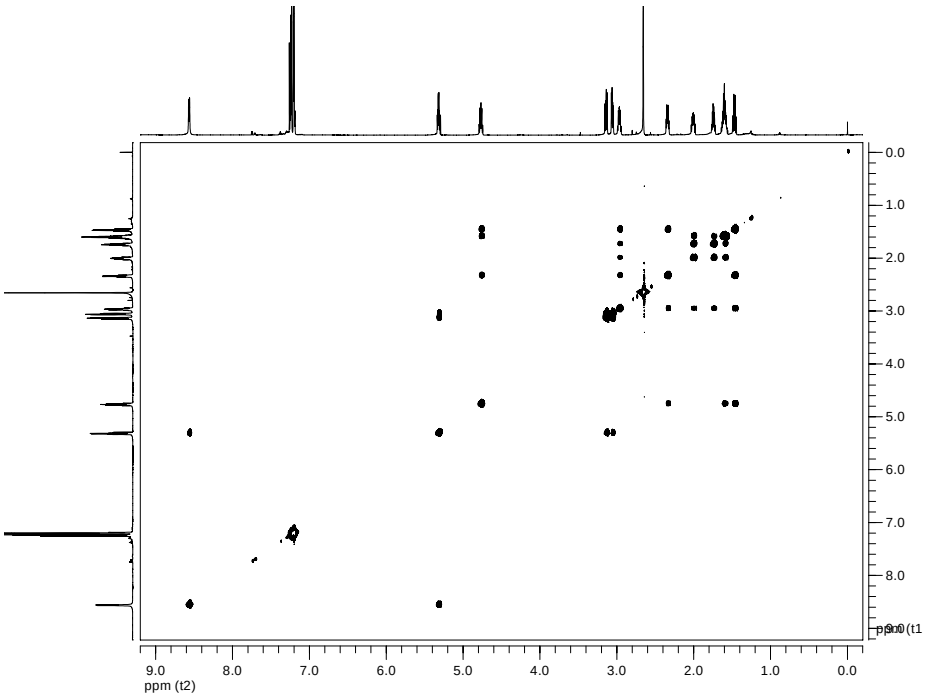
4) FT-IR [$CHCl_3$, 298 K (25 ° C)]



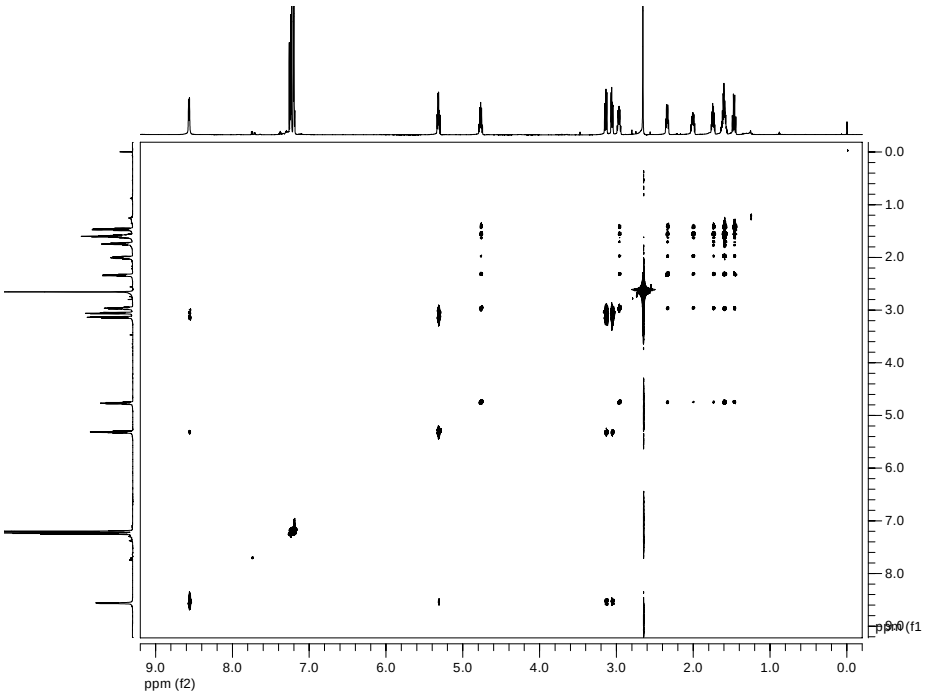

cyclo[(D-Phe-L-MeN-γ-Acp)3-] (7b)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)
200
150
100
50
0
ppm (t1)

***cyclo*[(*D*-Phe-*L*-^{Me}N-γ-Acp)$_3$-] (7b)**

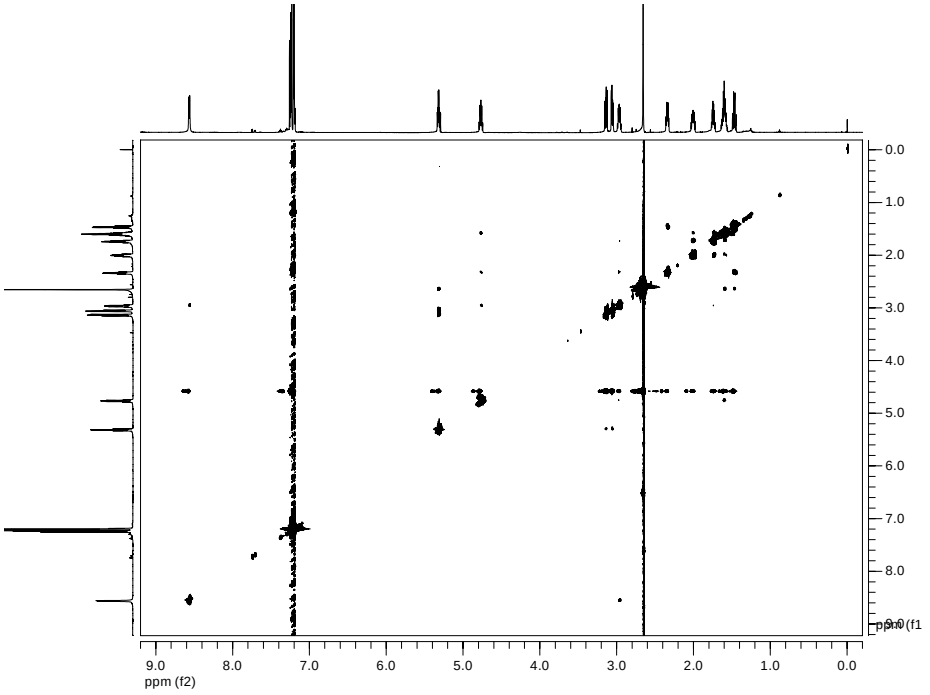
1) COSY [46.57 mM, $CDCl_3$, 298 K (25 º C), 750 MHz]



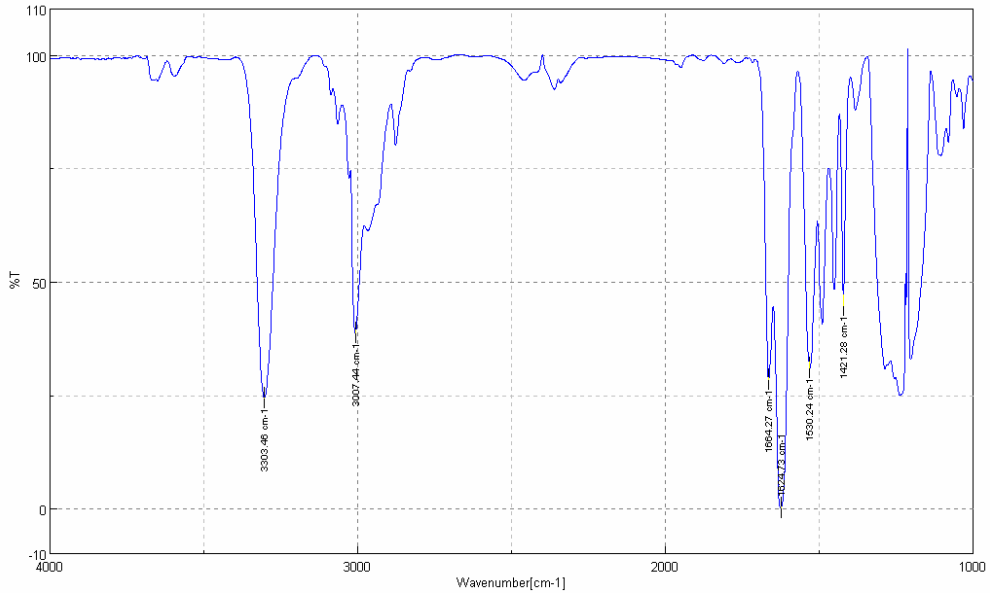
2) TOCSY [46.57 mM, $CDCl_3$, 298 K (25 º C), 750 MHz]



3) NOESY [46.57 mM, $CDCl_3$, 298 K (25 ° C), 750 MHz]

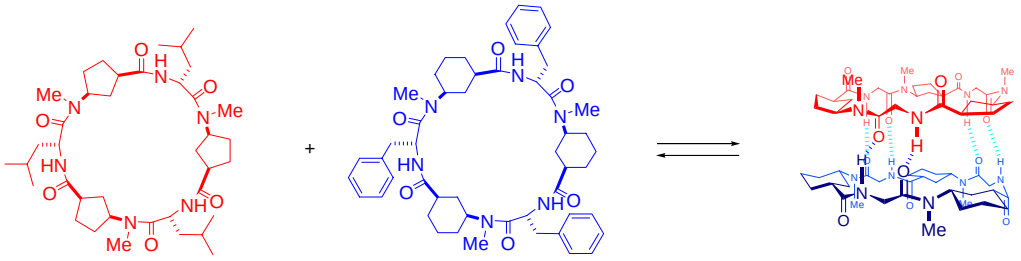


4) FT-IR [$CHCl_3$, 298 K (25 ° C)]

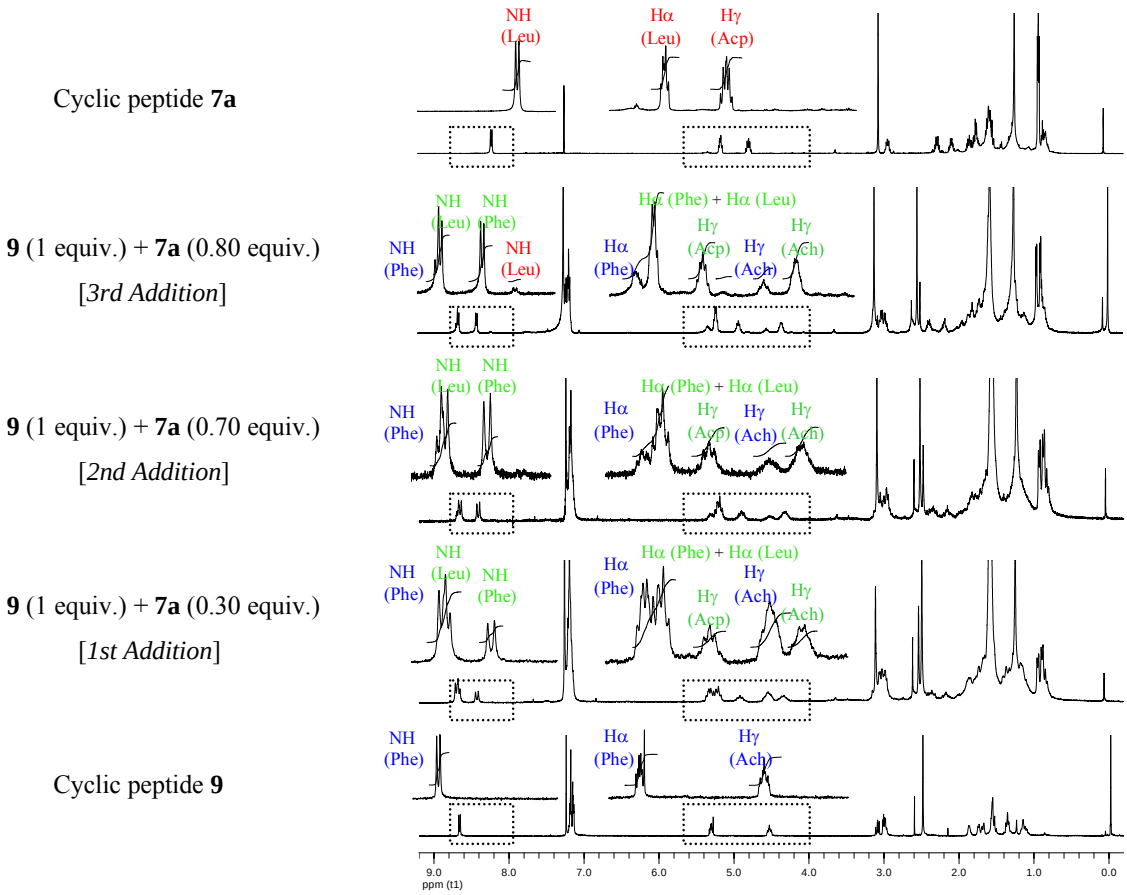


Heterodimer ***11*** $_{7a\text{-}9}$

► Addition of **7a** (2.10 mM solution in $CDCl_3$) to **9** (2.10 mM solution in $CDCl_3$)



Cyclic Peptide 7a ("Homodimer 8_{7a}") + Cyclic Peptide 9 ("Homodimer 10") ⇌ Heterodimer $11_{7a\text{-}9}$



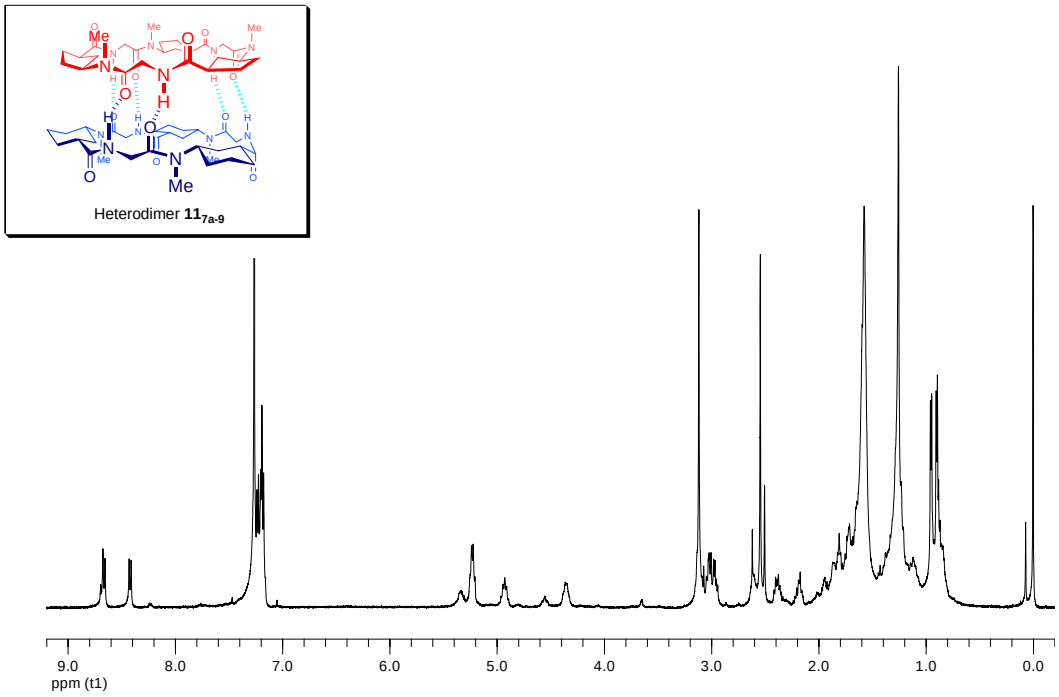
Signals that correspond to the Homodimer 8_{7a}

Signals that correspond to the Homodimer **10**

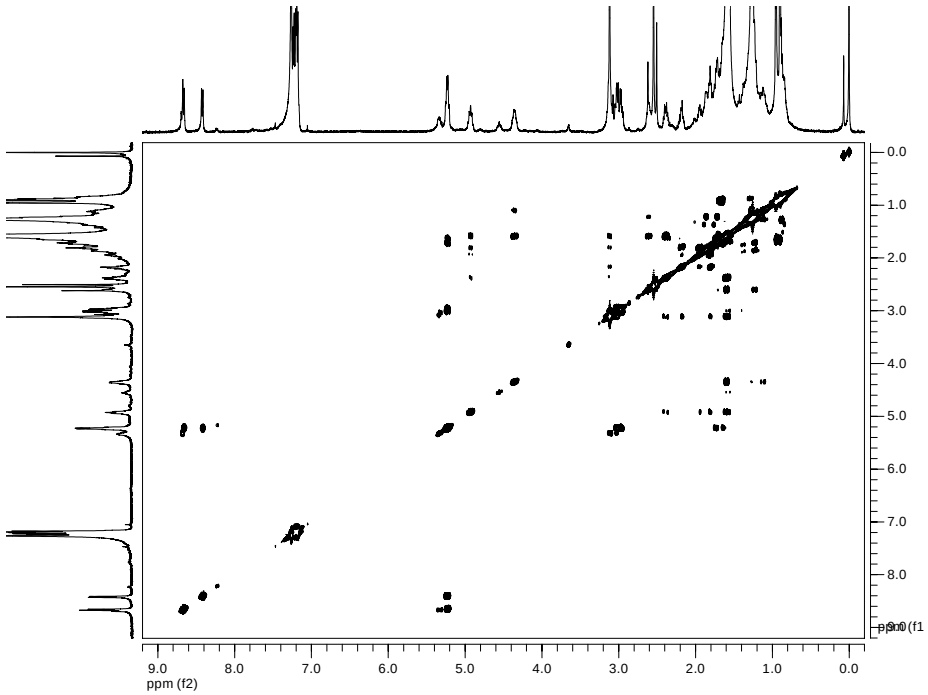
Signals that correspond to the Heterodimer $11_{7a\text{-}9}$

Heterodimer ***11***$_{\mathbf{7a\text{-}9}}$

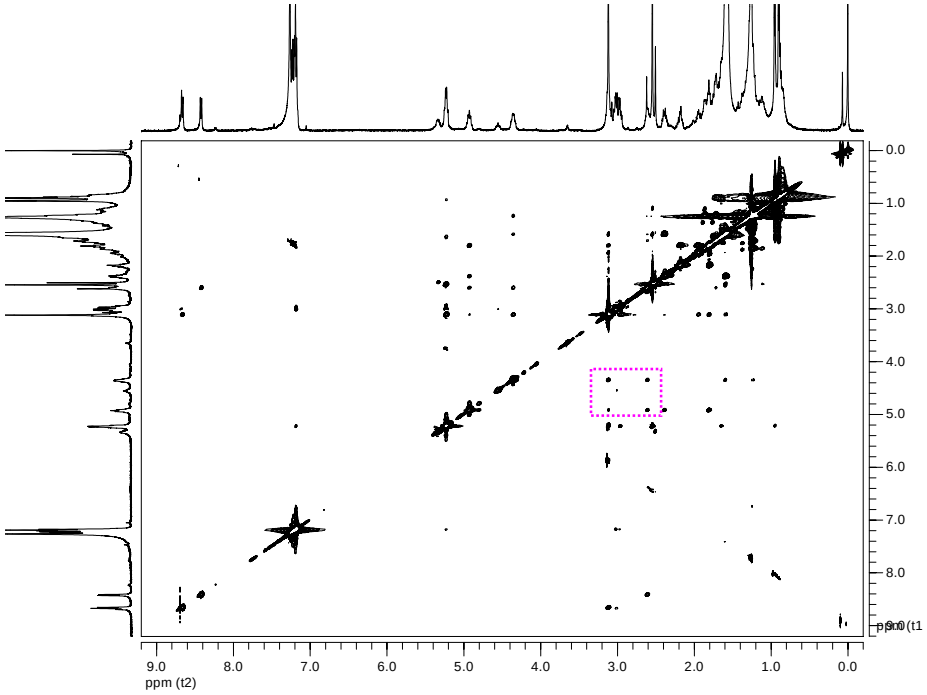
1) ^{1}H-RMN [*3rd Addition*, $CDCl_3$, 298 K (25 º C), 500.13 MHz]

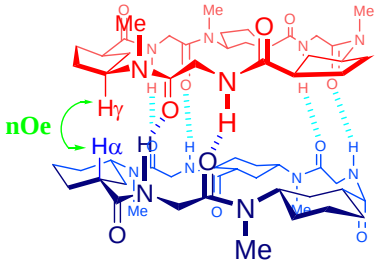


2) COSY [*3rd Addition*, $CDCl_3$, 298 K (25 º C), 500.13 MHz]



3) ROESY [*3rd Addition*, $CDCl_3$, 298 K (25 º C), 500.13 MHz]

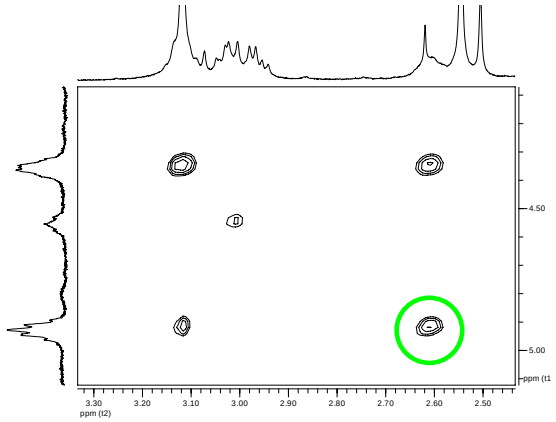




Hα (Acp) Heterodimer **11**$_{7a-9}$

Hα (Ach) Homodimer **9**

Hα (Ach) Heterodimer **11**$_{7a-9}$

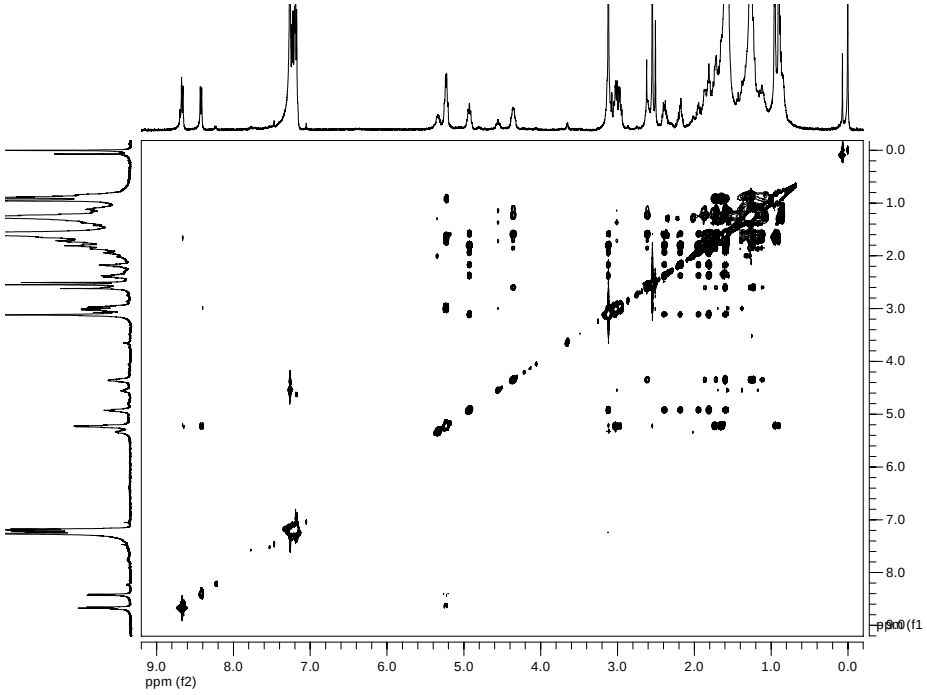


Hγ (Ach) Heterodimer **11**$_{7a-9}$

Hγ (Ach) Homodimer **9**

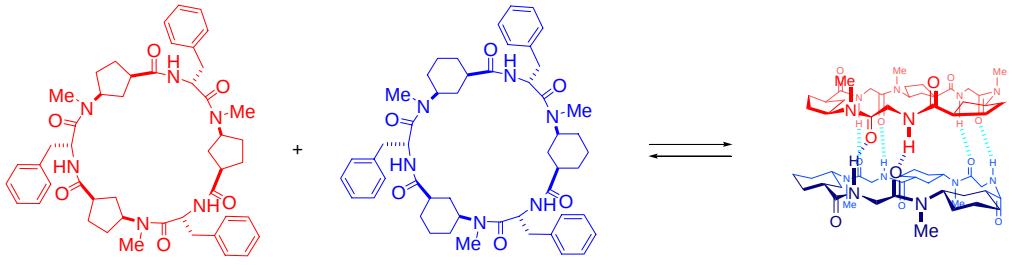
Hγ (Acp) Heterodimer **11**$_{7a-9}$

4) TOCSY [*3rd Addition*, $CDCl_3$, 298 K (25 º C), 500.13 MHz]

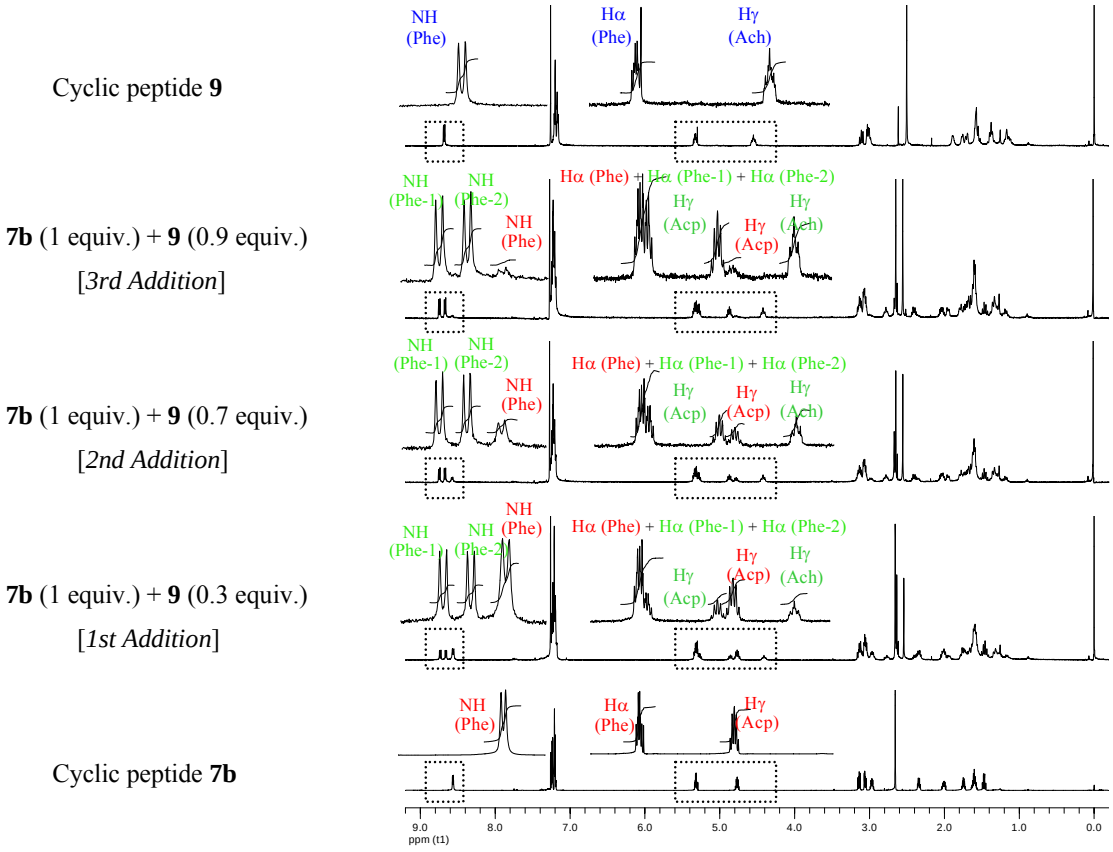


Heterodimer ***11*** $_{7b\text{-}9}$

► Addition of **9** (4.66 mM solution in $CDCl_3$) to **7b** (4.66 mM solution in $CDCl_3$)



Cyclic Peptide 7b ("Homodimer 8_{7b}") + Cyclic Peptide 9 ("Homodimer 10") ⇌ Heterodimer $11_{7b\text{-}9}$



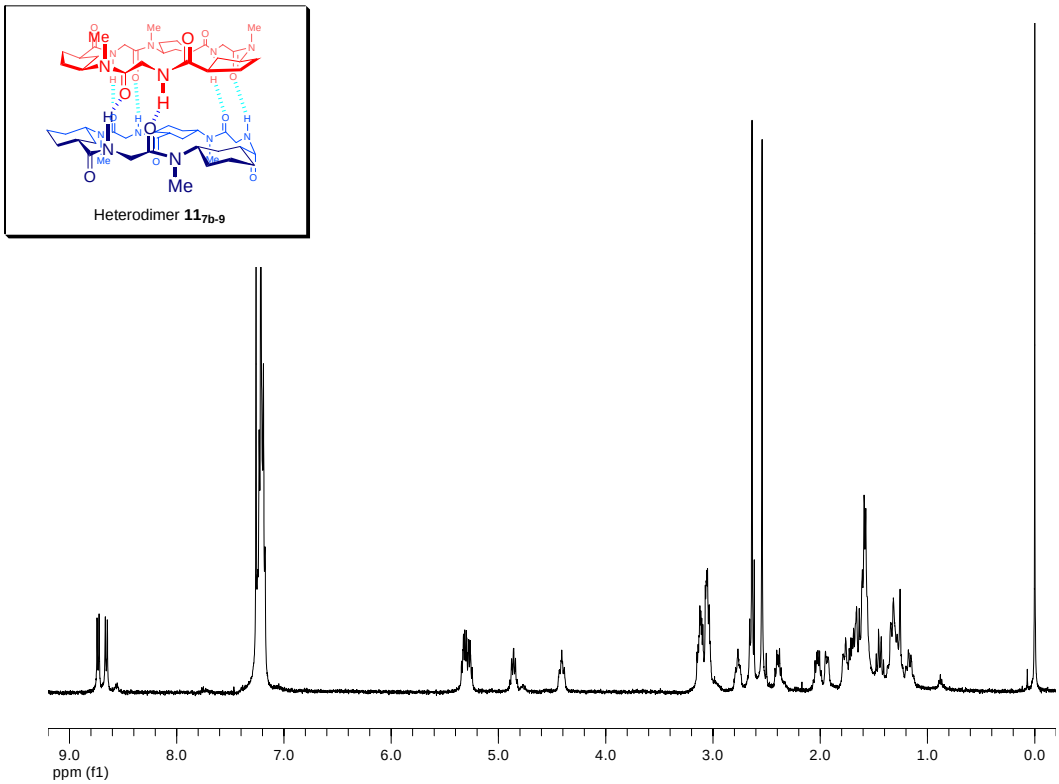
Signals that correspond to the Homodimer $\mathbf{8}_{7b}$

Signals that correspond to the Homodimer **10**

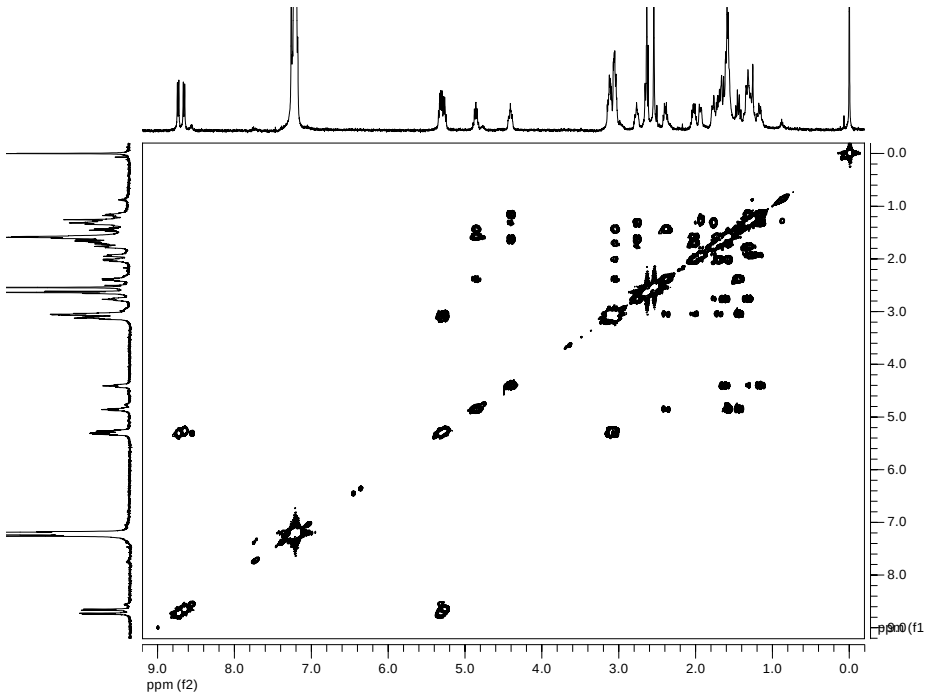
Signals that correspond to the Heterodimer $\mathbf{11}_{7b\text{-}9}$ (Phe-1: Phe of **7b**; Phe-2: Phe of **9**)

Heterodimer ***11***$_{\mathbf{7b\text{-}9}}$

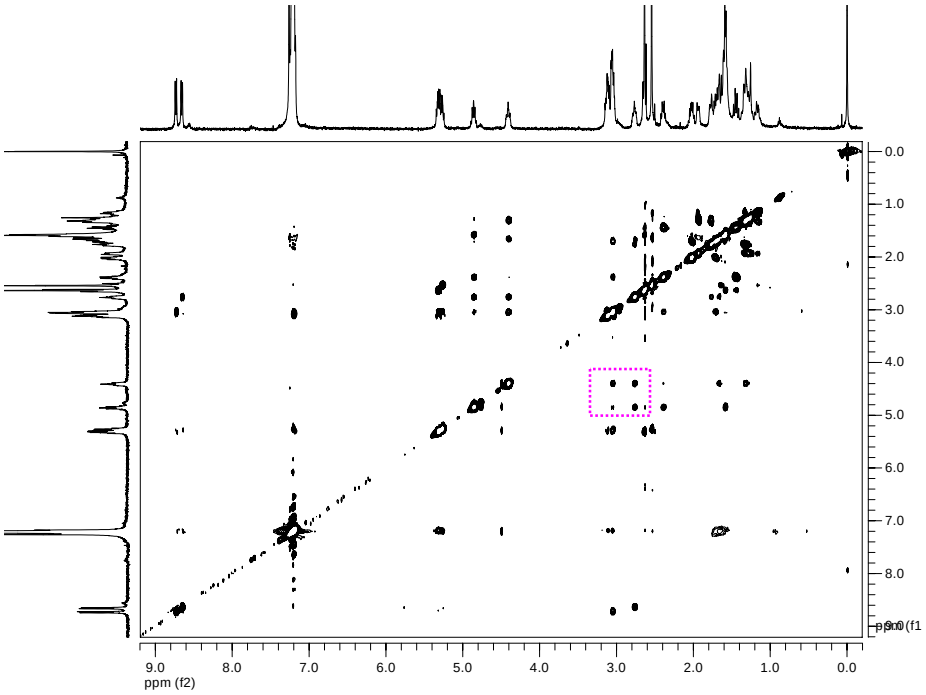
1) ^{1}H-RMN [*3rd Addition*, $CDCl_3$, 298 K (25 º C), 500.13 MHz]

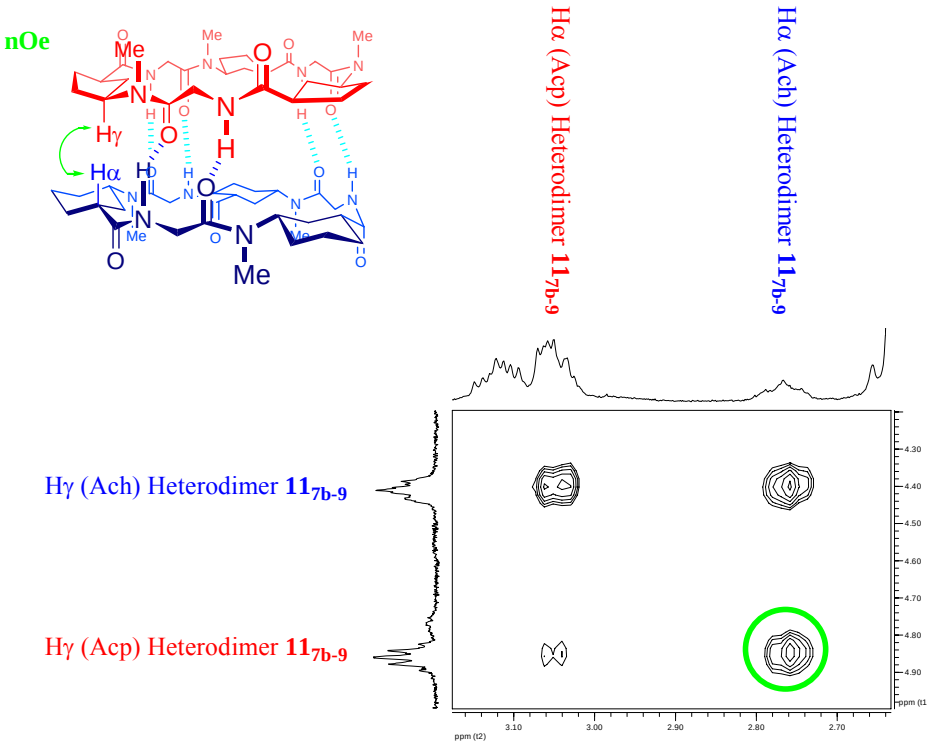


2) COSY [*3rd Addition*, $CDCl_3$, 298 K (25 º C), 500.13 MHz]

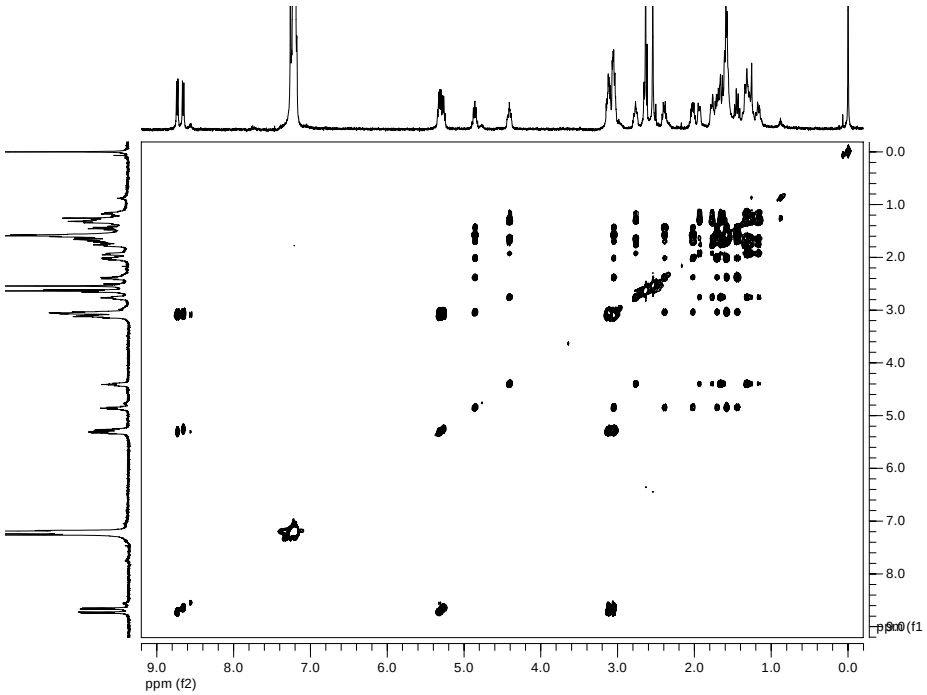


3) ROESY [*3rd Addition*, $CDCl_3$, 298 K (25 º C), 500.13 MHz]



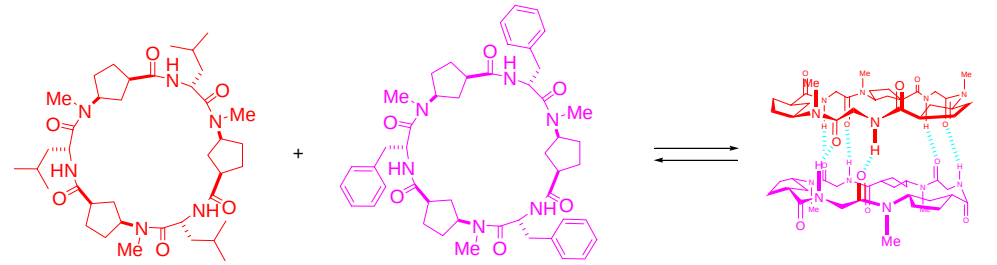


4) TOCSY [*3rd Addition*, $CDCl_3$, 298 K (25 º C), 500.13 MHz]

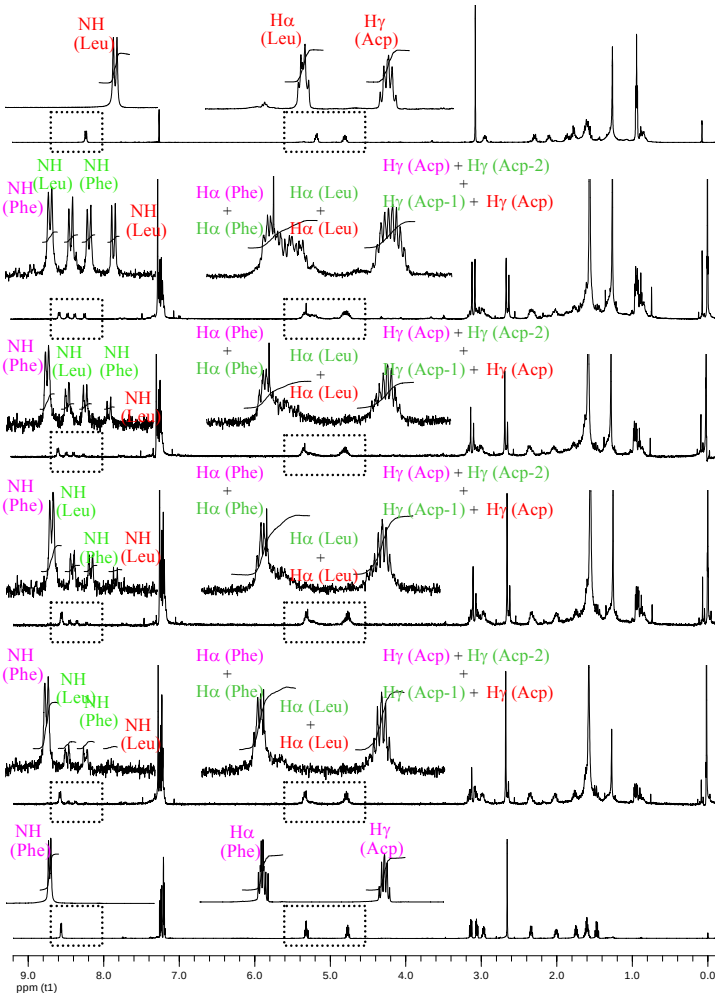


Heterodimer $\boldsymbol{8_{7a\text{-}7b}}$

► Addition of **7a** (1.96 mM solution in $CDCl_3$) to **7b** (1.96 mM solution in $CDCl_3$)



Cyclic Peptide 7a ("Homodimer $\boldsymbol{8_{7a}}$") | Cyclic Peptide 7b ("Homodimer 8_{7b}") | Heterodimer $\boldsymbol{8_{7a\text{-}7b}}$



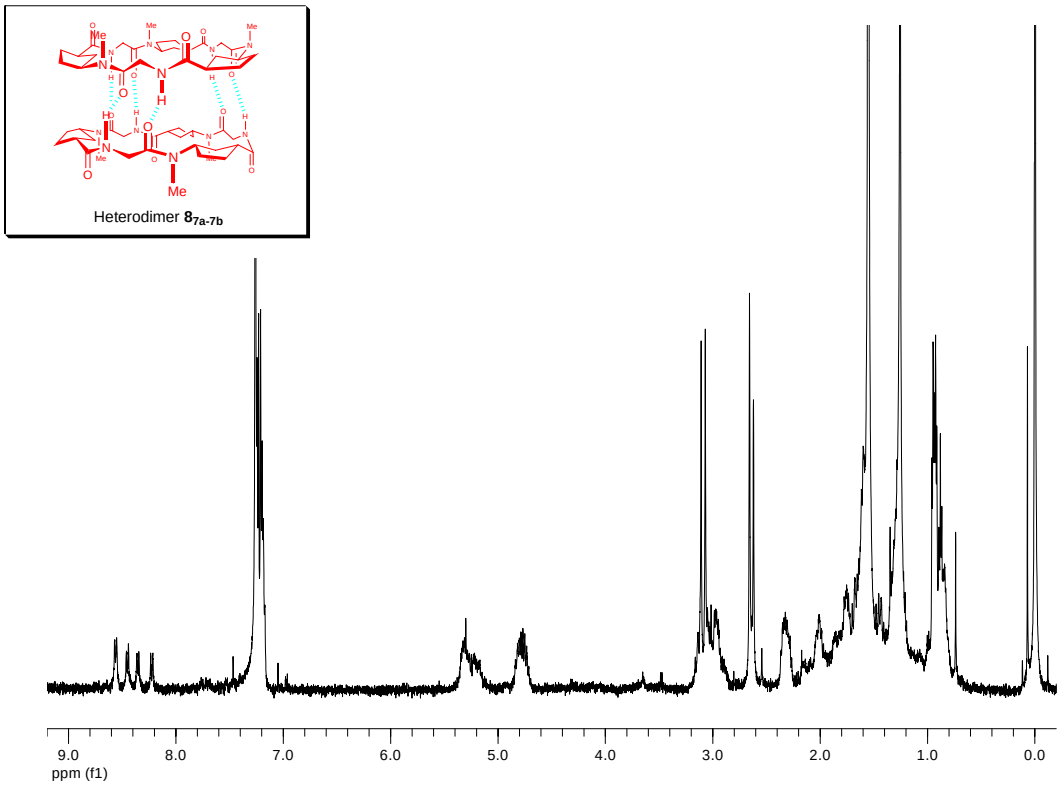
——Signals that correspond to the Homodimer $\boldsymbol{8_{7a}}$

——Signals that correspond to the Homodimer $\boldsymbol{8_{7b}}$

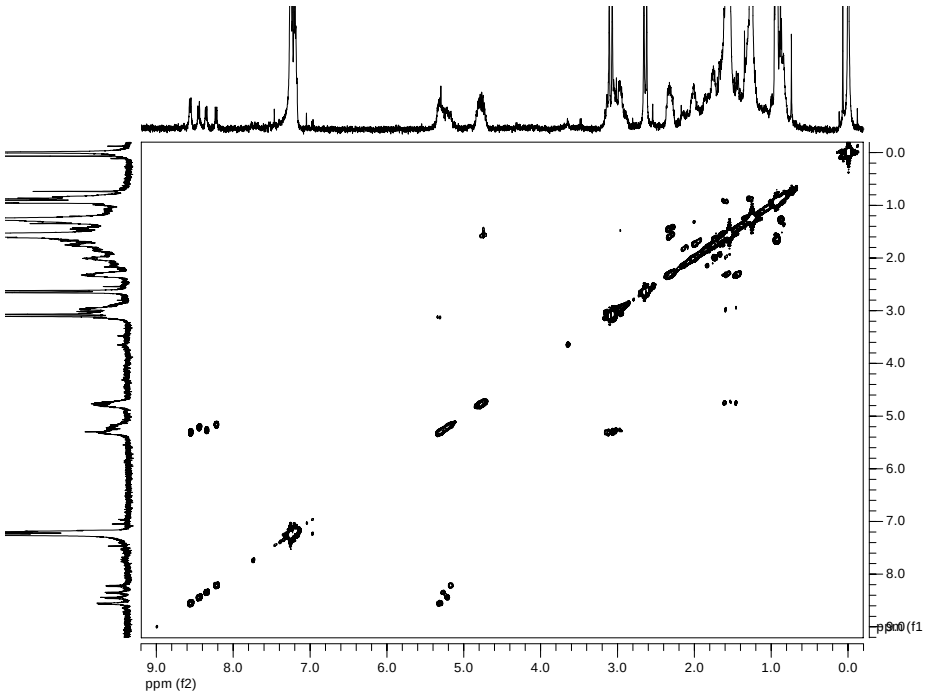
——Signals that correspond to the Heterodimer $\boldsymbol{8_{7a\text{-}7b}}$ (Acp-1: Acp of **7a**; Acp-2: Acp of **7b**)

Heterodimer $\boldsymbol{8_{7a\text{-}7b}}$

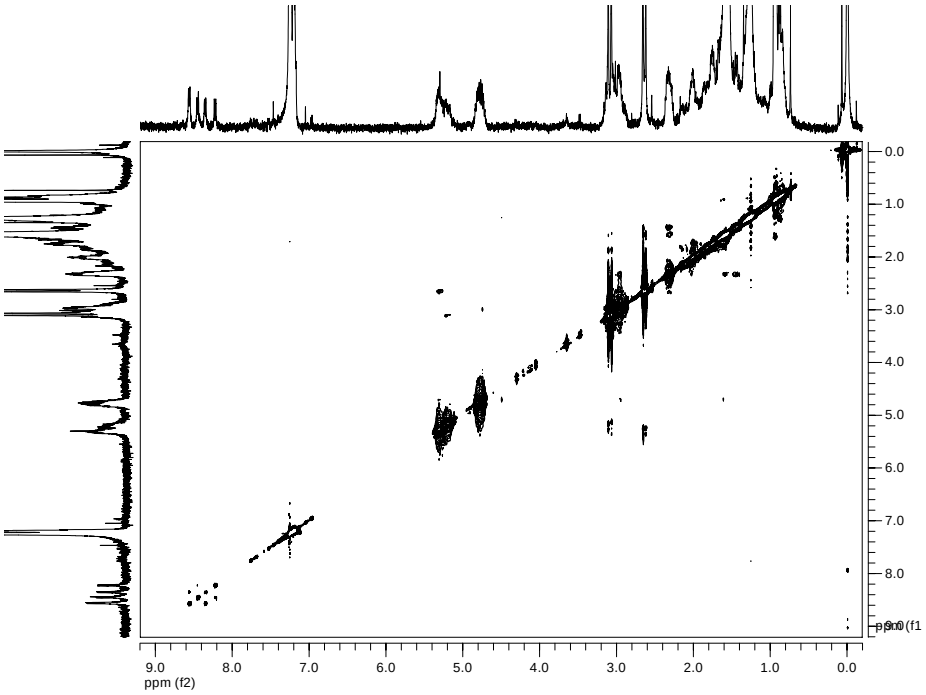
1) ^{1}H-RMN [*3rd Addition*, $CDCl_3$, 298 K (25 º C), 500.13 MHz]



2) COSY [*3rd Addition*, $CDCl_3$, 298 K (25 º C), 500.13 MHz]



3) ROESY [*3rd Addition*, $CDCl_3$, 298 K (25 º C), 500.13 MHz]



4) TOCSY [*3rd Addition*, $CDCl_3$, 298 K (25 º C), 500.13 MHz]