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SUPPORTING MATERIAL

Self-assembling Venturi-like peptide nanotubes

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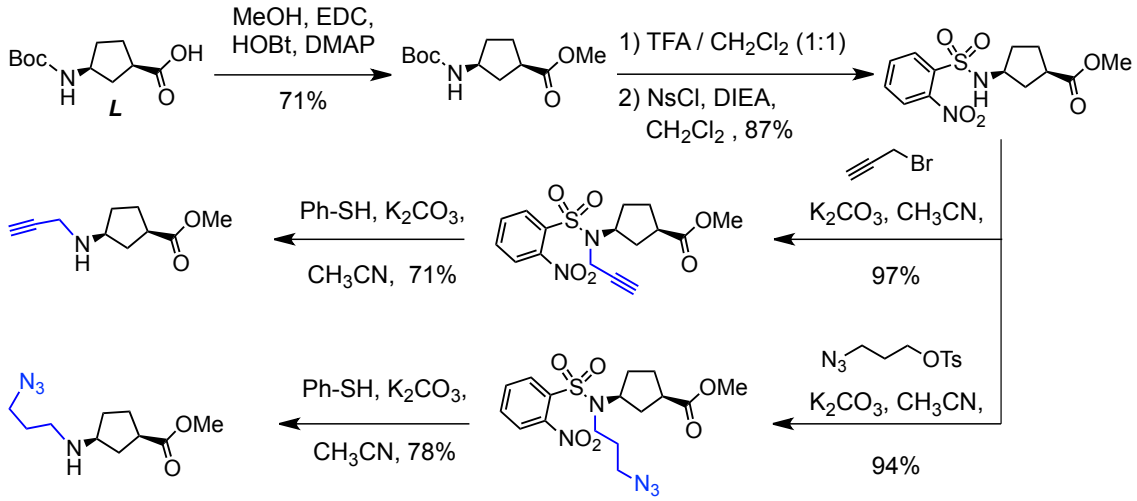
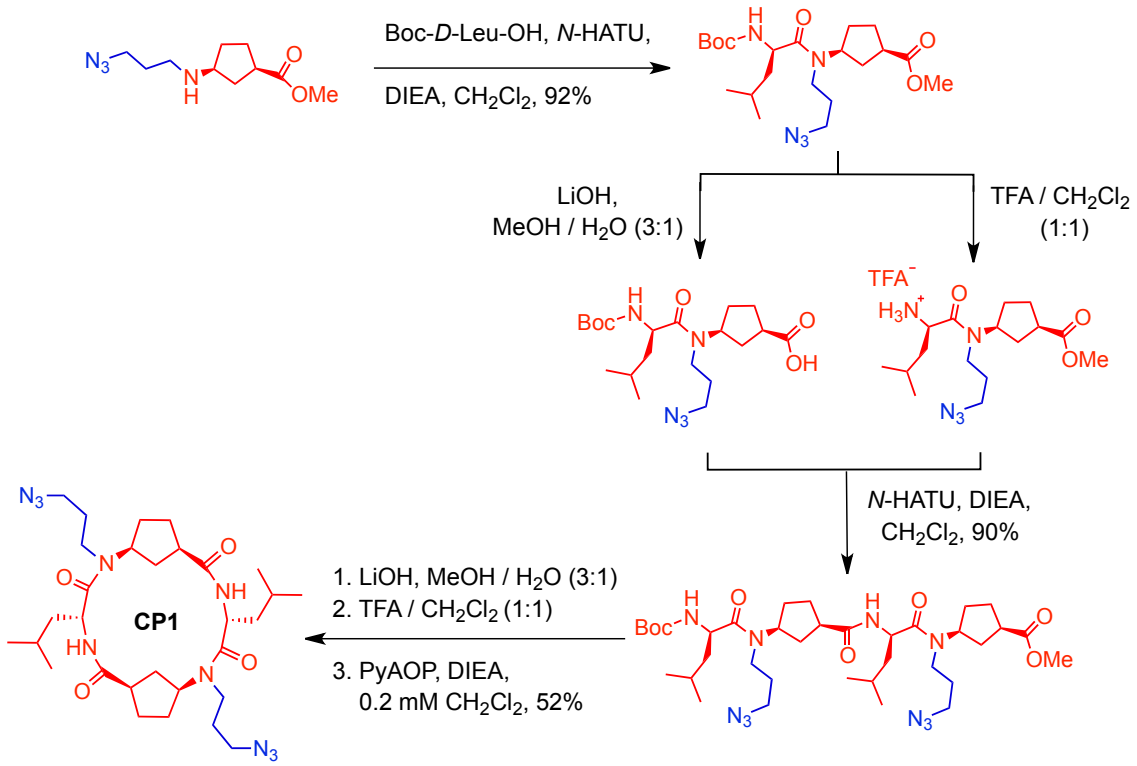
Index

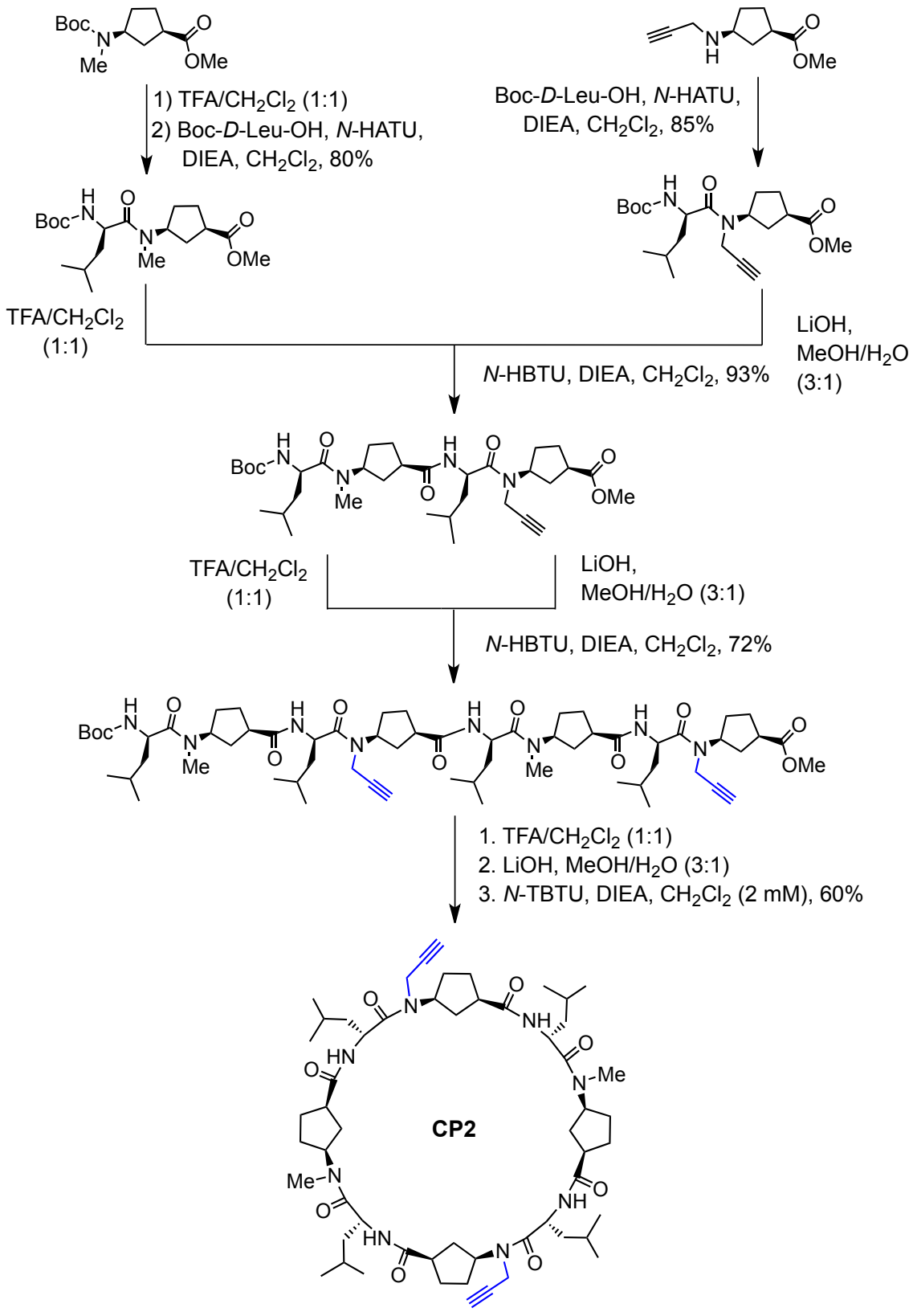


Scheme 1SI. Preparation of *N*-functionalized *L*-Acp derivatives using Fukuyama's strategy.[1]



Scheme 2SI. Preparation of cyclic tetrapeptide *c*-[(*D*-Leu-^{C3N3}*N*-*L*-γ-Acp-)$_2$] (**CP1**) bearing propyl-azide groups in solution.

[1] a) T. Fukuyama, C. K. Jow, M. Cheung, *Tetrahedron Lett.* 1995, **36**, 6373–6374; b) T. Fukuyama, M. Cheung, C. K. Jow, Y. Hidai, T. Kan, *Tetrahedron Lett.* 1997, **38**, 5831–5834.



Scheme 3SI. Synthetic route followed to obtain the cyclic octapeptide *c*-[(*D*-Leu-^{Me}*N*-*L*-γ-Acp-*D*-Leu-^{prg}*N*-*L*-γ-Acp-)$_2$] (**CP2**) bearing two propargyl groups.

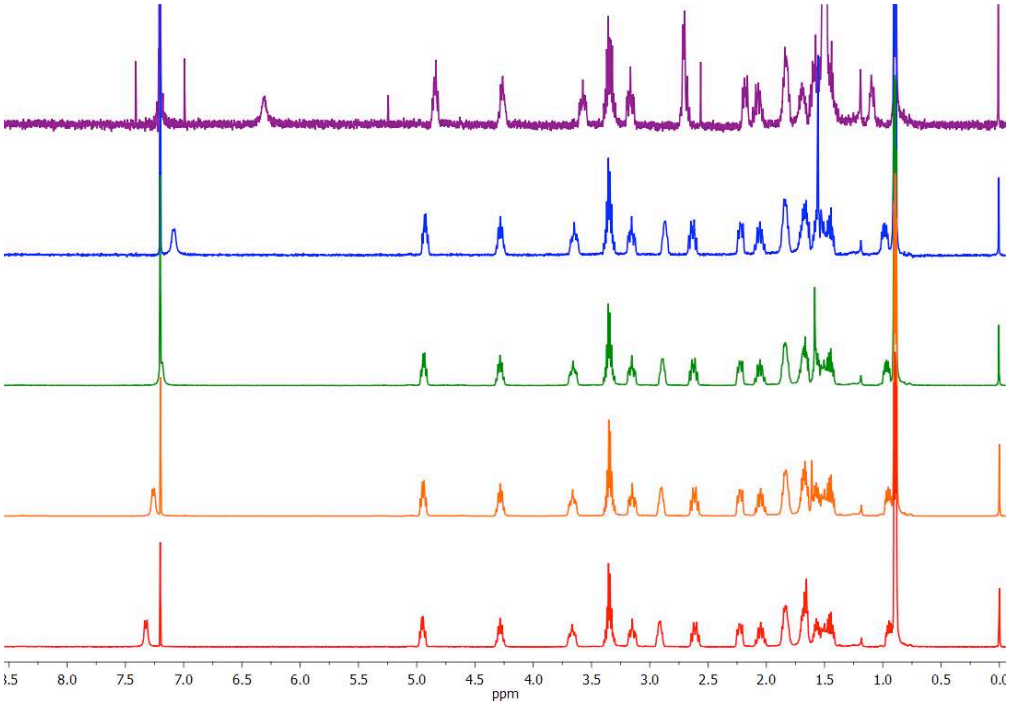
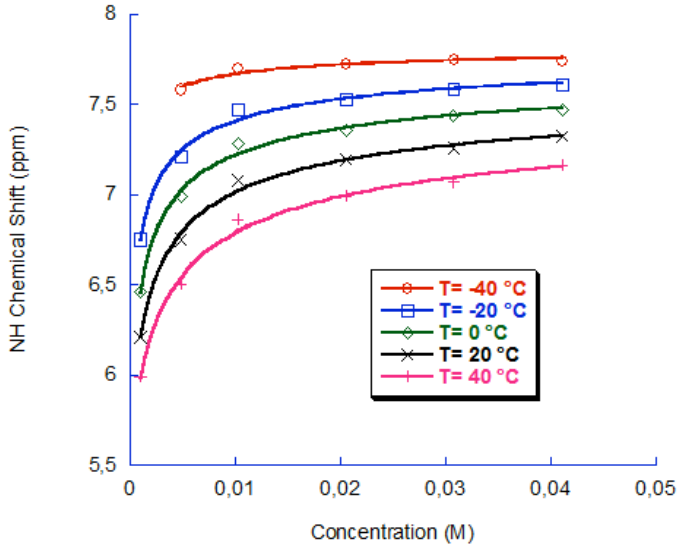


Fig. 1SI. Set of different ^{1}H NMR spectra recorded for **CP1** at different concentrations (from top to bottom 1, 10, 20, 30 and 40 mM) in $CDCl_3$ at room temperature.



$$\delta_{obs} = \delta_{dim} + \frac{(\delta_{mon} - \delta_{dim})(-1 + \sqrt{1 + 8K_a C}}{4K_a C} \quad (1)$$

$$-Log(K_a) = \left(\frac{\Delta H}{2{,}3R}\right) \cdot \left(\frac{1}{T}\right) - \left(\frac{\Delta S}{2{,}3R}\right) \quad (2)$$

Fig. 2SI. Top: Plot obtained for the chemical shift of the NH signal vs the concentration of the sample at different temperatures. Bottom: Chemical formulas utilized in order to obtain (1) the association constant K_a at different temperatures and (2) the thermodynamic data for the dimerization process.

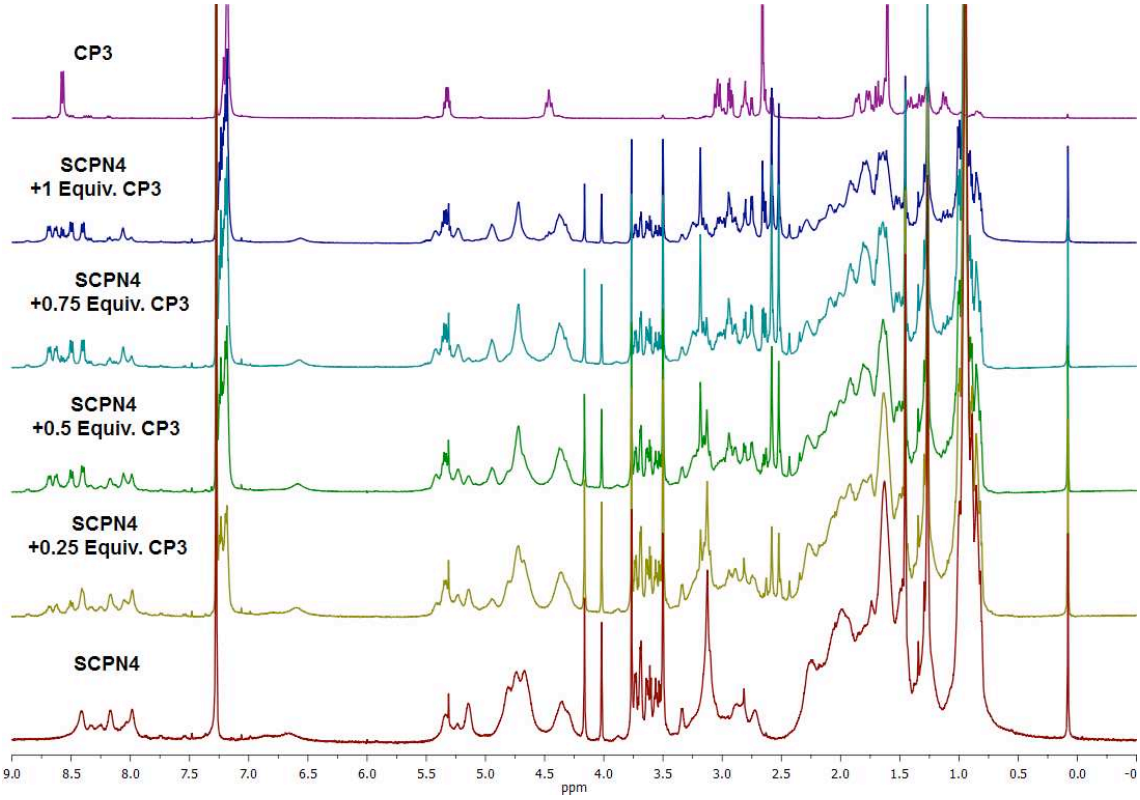


Fig. 3SI. ^{1}H NMR studies of the **D(D3-4)** formation by the addition of different amounts of **CP3** [0.0 (red), 0.25 (yellow), 0.5 (green), 0.75 (cyan) and 1.0 equiv. (dark blue) per dimer] to a solution of **CP4** in $CDCl_3$ (7.0 mM, $CDCl_3$ at 298 K).

Dimerization analysis of *c*-[(*D*-Leu-^{C3N3}*N*-*L*-γ-Acp-)$_2$] (CP1).

Different samples containing solutions of **CP1** in $CDCl_3$ were prepared at concentrations ranging from 1 to 40 mM. For each sample, ^{1}H NMR spectra were recorded at various temperatures; from -40 to +40 ^oC (ΔT = 20 ^oC). For each set of temperatures, the chemical shift of the NH protons was accurately determined using the chemical shift of $CHCl_3$ (7.26 ppm) as reference and plotted versus the concentration of the sample (Fig 2SI, bottom).

Then, using a non-linear equation (**1**) proposed by Laplanche and co-workers,[2] it was possible to determine the association constant (Ka) for each temperature (Fig 2SI, top).

K_a was found to be 329 M^{-1} at 25 ^oC, being in good agreement with previously calculated association constants for similar systems (N-methylated γ-amino acids).[3] The Van't Hoff plot (**2**) was used to calculate the thermodynamic parameters driving this dimerization process. I was found that the assembling process was spontaneous at 20 ^oC, with ΔG= -14.4 kJ/mol, where there was a entropic penalty (ΔS = -26.0 J mol^{-1} K^{-1}) derived from the increased order in the system that was opposed by a favorable enthalpic factor (ΔH = -22.1 kcal/mol) caused by the formation of hydrogen bonds.

For the systems like **D3** and the different heterodimeric aggregates, despite the fact that a full analysis was not possible due to the reduced range of concentrations that we could study, it was possible to make the qualitative approximation that the association constant through this small **CP1** was not affected by the fact that it was bound to another unit of **CP2** after the click, maintaining similar chemical shifts for every concentration/temperature measured.

Diffusion Ordered Spectroscopy (DOSY) measurements.

The samples were prepared by dissolving previously purified (HPLC) samples in either pure $CDCl_3$ or a 1% MeOH-d_3 solution in $CDCl_3$, a solvent system which allowed the disruption of higher aggregates in certain samples without having a remarkable influence on the viscosity of the medium, which is a critical factor in this technique. Also, due to our interest in quantifying apparent diffusion radii, the samples included tetrakis(trimethylsilyl)silane (TMSS) as internal standard. The concentration of TMSS was different for each sample, but a constant ratio of [TMSS] = 1.2 [Sample] was maintained throughout the whole study. It is necessary to keep this ratio as long as the decay of TMSS, because of its smaller size, is much more accused along the gradient as compared to the larger cyclic peptide adducts that are studied in this work. Also, we considered that the peptides and the internal standard have independent diffusion rates, as long as no interactions were observed in NOESY experiments. DOSY NMR experiments were performed in a Varian Inova 500 MHz spectrometer, at 25 °C and without spinning the sample. The default Dbppste sequence was used and the gradients were calibrated right before the

[2] L. A. LaPlanche, H. B. Thompson, M. T. Rogers, *J. Phys. Chem.* 1965, **69**, 1482-1488.
[3] a) M. Amorín, R. J. Brea, L. Castedo, J. R. Granja, *Org. Lett.* 2005, **7**, 4681–4684; b) C. Reiriz, M. Amorín, R. García-Fandiño, L. Castedo, J. R. Granja, *Org. Biomol. Chem.* 2009, **7**, 4358–4361; c) M. Amorín, R. J. Brea, L. Castedo, J. R. Granja, *Heterocyclic* 2006, **67**, 575–583.

measurement to ascertain for the trustworthiness of the diffusivity data obtained. Parameters such as diffusion delay were optimized for each sample, ranging from 20-40 ms, in order to obtain a signal decay of 90 to 95% of the initial maximum peak intensity (vide infra) along the set of increments in the gradient values (30-80 increments were used). Each sample was measured twice or three times in order to check the reproducibility of the measurement and to obtain an average of the diffusion data used to determine hydrodynamic radii.

For the each sample, a certain peak in the ^{1}H NMR spectra was used in order to obtain the ratio between the observed diffusion of TMSS and the target supramolecular assemblies. The selection of the signal was not arbitrary, but chosen to ensure the highest signal-to-noise ratio in order to avoid incorrect weightings. For each systems the selected signals are as follows:

a) TMSS (constant throughout all experiments): Singlet at 0.22 ppm
b) **D3** (both in $CDCl_3$ and 1% MeOH d_3/$CDCl_3$): Singlet at 3.13 ppm (N-Me of **CP3**)
c) Heterodimers **D3**-**CP4** (all stoichiometries): Singlet at 3.06 ppm (N-Me of **CP3**)

In this systems the rate of equilibration is higher than the time scale of the diffusion experiments, thus, a single horizontal row (Diffusion scale) is observed for the supramolecular systems, which is a weighted average of the individual contributions of monomer, dimers, trimers, etc. that are present in solution (see Fig. 3SI).

In order to analyze the diffusion data, we decided to take into account the considerations established for by Maccioni et al.,[4] as long as this analysis is not straightforward. For that, we first considered an approximation to the Einstein-Stokes ecuation (**3**), in which two correction factors are included to the original expression; $\mathbf{c_s}$ wich responds to the fact that our aggregates do not present an apparent r_h larger than 20 Å and also the $\mathbf{f_s}$ factor, which accounts for the asymmetry in the shape of some of our tubular aggregates (note that these equations consider spherical particles which are much larger than the solvent). Hence, in our particular case:

- c_s= 5.85, estimating an average size of 10-15 Å and following Chen plots.[5]
- f_s= 1.00 for **D4**, **D3** (1.4 mM to 2.6 mM) and **D3-4** 1:1 and 1:2; 1.05 for **D3** (3.5 mM) and **D3-4** 1:3, considering a prolate (cylinder-like) aggregate is being formed.[6]

Both these factors are known for TMSS, thus it is possible to establish a ratio (4) between the apparent diffusion of TMSS and the sample as follows, obtaining the parameter of interest r_{sample}:

$$D_t = \frac{kT}{c(r_{sol}, r_H) f_s(a,b) \pi \eta r_H} \quad (3) \quad \rightarrow \quad \frac{D_{sample}}{D_{TMSS}} = \frac{c(TMSS) f_s(TMSS)\, r_{TMSS}}{c(sample) f_s(sample)\, r_{sample}} \quad (4)$$

[4] A. Macchioni, G. Ciancaleoni, C. Zuccaccia, D. Zuccaccia, *Chem. Soc. Rev*. 2008, **37**, 479-489.
[5] H.-C. Chen, S.-H. Chen, *J. Phys. Chem*. 1984, **88**, 5118-5121.
[6] F. Perrin, *J. Phys. Radium.* 1936, **7**, 1-11.

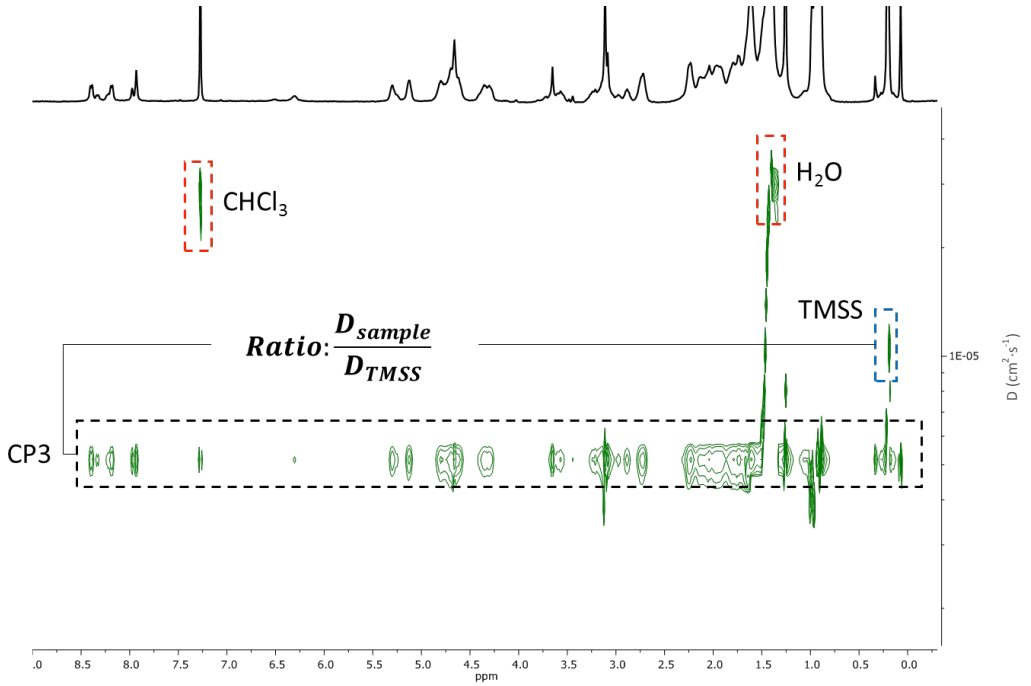


Figure 4SI. Representative DOSY NMR experiment of **CP3** in a 1% solution of MeOH-d_3 in $CDCl_3$, in which the different diffusion bands can be observed.

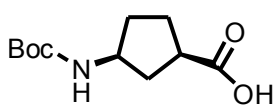
Materials and Methods

General considerations:

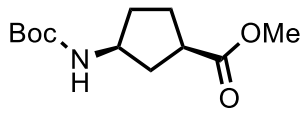
1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo-[4,5-b]pyridinium hexafluorophosphate 3-oxide (*N*-HATU), 1-[bis(dimethylamino)methylene]-1H-benzotriazolium hexafluorophosphate 3-oxide (*N*-HBTU), 1-[bis(dimethylamino)methylene]-1H-benzotriazolium tetrafluoroborate 3-oxide (*N*-TBTU), (7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyAOP), 1-N-(3-dimethylaminopropyl)-N´-ethylcarbodiimide hydrochloride (EDC·HCl), 1-hydroxybenzotriazole (HOBt), 4-dimethylaminopyridine (DMAP), methyl iodide (MeI), potassium carbonate (K_2CO_3), tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA), tetrakis-acetonitrilehexafluorophosphate copper (I) ($[Cu(MeCN)_4PF_6]$), tetrakis(trimethylsilyl)silane (TMSS) and Boc-*D*-Leu-OH were purchased from Iris Biotech, Fischer Scientific, Alfa Aesar, Aldrich or from Global Sales Manager, GL Biochem (Shanghai) Ltd, China. All reagents and solvents were used as purchased unless otherwise stated. CH_2Cl_2 and THF were distilled from CaH_2 and Na/benzophenone over argon, respectively, immediately prior to their use. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F_{254} plates. In case of not been UV active (254/365 nm), the compounds were visualized by dipping the plates in certain revealing solutions, such as: nynhidrin (2% in EtOH), Ce/Mo (200 mg $Ce(NH_4)_2(NO_3)_6$ + 9.6 g $(NH_4)_2MoO_4$ + 11.2 mL H_2SO_4 + 200 mL H_2O) or phosphomolibdic acid (3 g $H_3[P(Mo_3O_{10})_4]$ in 100 mL EtOH), followed by heating. Silica-gel flash chromatography was performed using E. Merck silica gel (type 60SDS, 230-400 mesh). Mixtures for chromatography are reported as v/v ratios of the solvents noted for each compound. HPLC purification was carried out on a HITACHI D-7000 or a JASCO LC-4000 using a Phenomenex Luna 5µ Silica 100 Å column with the CH_2Cl_2/MeOH gradients detailed for each compound. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Inova 500 MHz or Varian Mercury 300 MHz. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (δ = 0.00 ppm) or to the deuterated solvent in which the spectrum was recorded. ^{1}H NMR splitting patterns are designated as singlet (s), doublet (d), triplet (t) or quartet (q). All first-order splitting patterns were assigned based on the appearance of the multiplet. Non-easily interpreted signals are designated as multiplet (m) or broad (br). Carbon resonances were assigned using either Distortionless Enhancement by Polarization Transfer (DEPT) spectra obtained with phase angles of 135° or by Heteronuclear Single Quantum Coherence experiments (HSQC).

^{1}H NMR Assignments of Cyclic Peptides (CPs) and their adducts: The signals of the ^{1}H NMR spectra of the peptides were identified from the corresponding double-quantum-filled 2D: COSY, TOCSY and NOESY and/or ROESY spectra acquired at a concentration between 2 and 100 mM and at room temperature unless otherwise specified (Mixing times for NOESY and/or ROESY were not optimized). Electrospray (ESI) mass spectra were recorded on a Bruker BIOTOF II mass spectrometer, and are reported as mass-per-charge ratio *m/z* (intensity in %, assignation). Accurate mass determination (HRMS) using ESI-MS were performed on a Sciex QSTAR Pulsar spectrometer. FT-IR measurements were made on a JASCO FT/IR-400 spectrophotometer placing the sample on a CaF_2 pellet or on a Perkin Elmer Spectrum Two ATR-FTIR, directly depositing the sample as a thin film over its diamond plate.

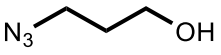
Synthesized Compounds:



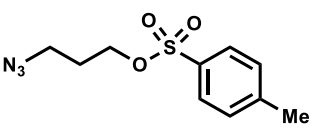
(1*R*,3*S*)-3-amino-*N*-(*tert*-butoxycarbonyl)cyclopentanecarboxylic acid (Boc-*L*-γ-Acp-OH). This γ-amino acid was obtained following the route previously developed in our research group using Vince's Lactam as commercial source.[7]



Methyl (1*R*,3*S*)-3-amino-*N*-(*tert*-butoxycarbonyl)cyclopentane-carboxylate (Boc-*L*-γ-Acp-OMe). Amino acid ***L*-Boc-γ-Acp-OH** (2.05 g, 8.73 mmol) was dissolved in MeOH (44 mL) and EDC·HCl (2.51 g, 13.1 mmol), HOBt (1.77 g, 13.1 mmol) and DMAP (1.60 g, 13.1 mmol) were added. The resulting solution was stirred for 2 h under argon atmosphere. After this time, it was concentrated on rotary evaporator and redissolved in CH_2Cl_2. The resulting solution was washed with aqueous HCl (5%, 3x50 mL) and with saturated aqueous $NaHCO_3$ (3x50 mL). The resulting solution was dried over anhydrous $MgSO_4$, filtered and concentrated, and the solid was purified by flash chromatography (5-20% AcOEt/Hexane) to afford the desired product as a white solid [1.61 g, 76%, R_f = 0.78 (5% MeOH/CH_2Cl_2)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 4.93 and 4.45 (br, 1H), 4.04 (br, 1H), 3.67 (2s, 3H), 2.85-2.79 (br, 1H), 2.24-1.49 (m, 6H), 1.43 (s, 9H). **^{13}C NMR** ($CDCl_3$, 75.4 MHz, δ): 177.2 (CO), 155.3 (CO), 78.9 (C), 51.9 (CH_3), 51.8 (CH), 41.6 (CH), 36.4 (CH_2), 32.0 (CH_2), 28.3 (CH_3), 27.8 (CH_2). **MS (ESI)** [m/z (%)]: 266 ([M+Na]$^+$, 100), 267 ([M+Na]$^+$, 25), 228 ([M-Me]$^+$, 1). **HRMS (ESI)** Calculated for $C_{12}H_{21}NO_4$: 243.1471, found**:** 243.1473.



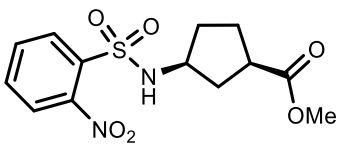
3-azido-1-propanol. Following a previously described procedure,[8] 3-bromo-1-propanol (2.5 mL, 28.6 mmol) was dissolved in EtOH/H_2O (52 mL, 50 % v/v) mixture. Then, NaN_3 (7.436 g, 114 mmol) was added at room temperature and the mixture stirred for 24 h at 70°C. Ethanol was evaporated under reduced pressure and the aqueous layer was extracted with CH_2Cl_2 (3×20 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo to give a colorless oil [2.699 g, 92%, R_f = 0.31 (25% AcOEt/Hexane)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 3.69 (m, 2H), 3.40 (t, *J* = 6.6 Hz, 2H), 2.42 (br, 1H), 1.79 (m, *J* = 6.3, 2H). **^{13}C RMN** (75 MHz, $CDCl_3$, δ): 59.7 (CH_2), 48.4 (CH_2), 31.4 (CH_2).



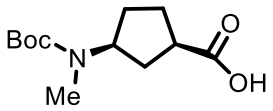
3-azidopropyl-*p*-toluenesulphonate.[9] A solution of 3-azido-1-propanol (0.909 g, 9 mmol) in CH_2Cl_2 (36 mL) was treated with Et_3N (2.5 mL, 18 mmol) and *p*-toluenesulfonyl chloride (1.80 g, 9.45 mmol). The mixture was stirred overnight and then washed with H_2O (milli-Q grade, 2x25 mL), dried over anhydrous $MgSO_4$, filtered and concentrated. The resulting oil was purified by flash chromatography (0-35% AcOEt/Hexane) to yield the desired product as a colorless oil [2.135 g, 93%, R_f = 0.51 (25% AcOEt/Hexane)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 7.78 (d, *J* = 8.3 Hz, 2H), 7.35 (d, *J* = 7.9 Hz, 2H), 4.09 (t, *J* = 6.0 Hz, 2H), 3.36 (t, *J* = 6.5

[7] R. J. Brea, M. Amorín, L. Castedo, J. R. Granja, *Angew. Chem. Int. Ed.* 2005, **44**, 5710-5713.
[8] M. Bertoldo, G. Zampano, F. La Terra, V. Villari, V. Castelvetro, *Biomacromol.* 2011, **12**, 388-398.
[9] Y. Yuan, C.-J. Zhang, M. Gao, R. Zhang, B. Z. Tang, B. Liu, Bin, *Angew. Chem. Int. Ed*. 2015, **54**, 1780-1786.

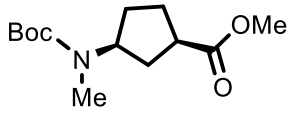
Hz, 2H), 2.44 (s, 3H), 1.93-1.82 (m, *J* = 6.2 Hz, 2H). **^{13}C NMR** (75 MHz, $CDCl_3$, δ): 145.0 (Ar), 132.7 (Ar), 129.9 (Ar), 127.9 (Ar), 67.0 (CH_2), 47.3 (CH_2), 28.4 (CH_2), 21.6 (CH_3).



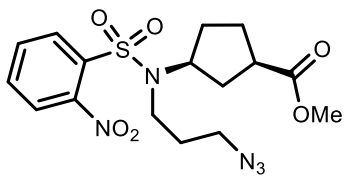
Methyl (1*R*,3*S*)-3-amino-*N*-(2-nitrobenzenesulfonyl) cyclopentanecarboxylate (Ns-*L*-γ-Acp-OMe). A solution of **Boc-*L*-γ-Acp-OMe** (2.62 g, 10.7 mmol) in CH_2Cl_2 (27 mL) was treated with TFA (27 mL). After 15 minutes, the solution was concentrated under vacuum and further dried at high vacuum for 2 h. The resulting solid was dissolved in dry CH_2Cl_2 (53 mL) and DIEA (5.8 mL, 32.9 mmol) was added, followed by *o*-nitrobenzenesulfonyl chloride (2.56 g, 16.1 mmol). The mixture was stirred for 22 h under argon atmosphere. The solution was washed with aqueous HCl (5%, 2x50 mL) and saturated aqueous solution of $NaHCO_3$ (2x50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum. The resulting residue was purified by flash chromatography (15-50% AcOEt/Hexane) and ***o*-Ns-*L*-γ-Acp-OMe** was obtained as a yellow solid [3.05 g, 87%, R_f = 0.68 (50% AcOEt/Hexane)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 8.29 (d, *J* = 8.8 Hz, 2H), 8.02 (d, *J* = 8.8 Hz, 2H), 5.84 and 5.33 (2d, *J* = 6.9 and 7.2 Hz, 1H), 3.61 and 3.57 (2s, 3H), 3.78 (br, 1H, H_γ), 2.76 (m, 1H). **^{13}C RMN** ($CDCl_3$, 75 MHz, δ): 176.6 (C=O), 150.0 (Ar), 133.7 (Ar), 133.4 (Ar), 132.8 (Ar), 130.7 (Ar), 125.3 (Ar), 55.5 (CH_3), 52.8 (CH), 41.6 (CH), 36.4 (CH_2), 33.2 (CH_2), 27.9 (CH_2). **MS (ESI)** [m/z (%)]: 328 ([M+H]$^+$, 100). **HRMS (ESI)** Calculated for $C_{13}H_{16}N_2NaO_6S$: 351.0621, found: 351.0608.



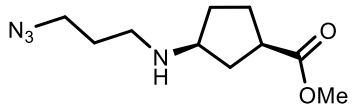
(1*R*,3*S*)-3-amino-*N*-(*tert*-butoxycarbonyl)-*N*-methyl-cyclopentanecarboxylic acid (Boc-^{Me}*N*-*L*-γ-Acp-OH). This compound was prepared following a previously described protocol.[7]



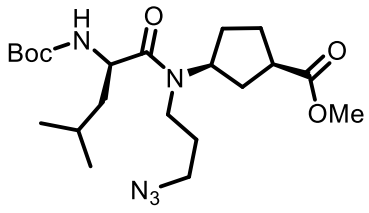
Methyl (1*R*,3*S*)-3-amino-*N*-(*tert*-butoxycarbonyl)-*N*-methyl-cyclopentanecarboxylate (Boc-^{Me}*N*-*L*-γ-Acp-OMe). A solution of Boc-^{Me}*N*-*L*-γ-Acp-OH (0.780 g, 3.21 mmol), EDC·HCl (0.923 g, 4.81 mmol), HOBt (0.650 g, 4.81 mmol) and DMAP (0.587 g, 4.81 mmol) in MeOH (16 mL) was stirred at r.t. for 1 h. After this time the solution was concentrated to dryness and the resulting residue dissolved in CH_2Cl_2 (20 mL) and washed with aqueous solutions of $NaHCO_3$ (sat., 2x15 mL) and HCl (5%, 2x15 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The resulting residue was purified via flash chromatography (5-20% AcOEt/Hexane) to afford **Boc-^{Me}*N*-*L*-γ-Acp-OMe** as a colorless oil [0.607 g; 74%; R_f = 0.57 (5% MeOH/CH_2Cl_2)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 4.52 (br, 1H), 3.78-3.63 (s, 3H), 2.73 (m, 4H), 2.19-1.54 (m, 6H), 1.52-1.40 (m, 9H). **^{13}C NMR** (75 MHz, $CDCl_3$, δ): 176.1 (C=O), 155.5 (C=O), 79.1 (Q), 69.5 (CH_3), 51.5 (CH_2), 41.1 (CH_2), 28.2 (CH_2), 28.0 (CH_2), 27.1 (CH_3). **MS (ESI)** [m/z (%)]: 280 ([M+Na]$^+$, 100). **HRMS (ESI)** Calculated for $C_{13}H_{23}NNaO_4$: 280.1519, found: 280.1520.



Methyl (1*R*,3*S*)-3-amino-*N*-(2-nitrobenzenesulfonyl)-*N*-(3-azidopropyl)cyclopentanecarboxylate (Ns-^{C3N3}*N*-*L*-γ-Acp-OMe). Protected amino acid Ns-*L*-γ-Acp-OMe (0.53 g, 1.6 mmol) was dissolved in MeCN (8.1 mL) and treated with K_2CO_3 (1.790 g, 13.0 mmol) and 3-azidopropyl-*p*-toluenesulphonate (2.480 g, 9.72 mmol). The mixture was stirred for 24 h under argon atmosphere and then MeCN was evaporated under reduced pressure. The residue was suspended in CH_2Cl_2 and washed with H_2O (2x15 mL) and brine (2x15 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated on rotary evaporator. Purification was carried out via flash chromatography (10-20% AcOEt/Hexane) and the entitled product was obtained as a colorless viscous oil [0.64 g, 97%, R_f = 0.33 (50% AcOEt/Hexane)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 7.95 (m, 1H), 7.75-7.52 (m, 3H), 4.18 (br, 1H), 3.59 (2s, 3H), 3.47-3.09 (m, 4H), 3.09-2.64 (m, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$, δ): 175.9 (C=O), 148.1 (Ar), 133.8 (Ar), 133.2 (Ar), 131.8 (Ar), 130.5 (Ar), 124.1 (Ar), 58.7 (CH_3), 51.9 (CH), 48.9 (CH_2), 41.6 (CH_2), 40.5 (CH), 32.4 (CH_2), 30.7 (CH_2), 28.5 (CH_2), 26.8 (CH_2). **MS (ESI)** [m/z (%)]: 434 ([M+Na]$^+$, 100), 412 ([M+H]$^+$, 60). **HRMS (ESI)** Calculated for $C_{16}H_{21}N_5O_6S$: 411.1214, found 411.1216.

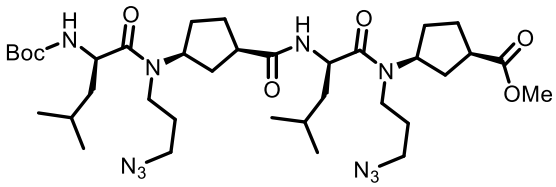


Methyl (1*R*,3*S*)-3-amino-*N*-(3-azidopropyl)cyclopentanecarboxylate (^{C3N3}***N*-*L*-γ-Acp-OMe)**. Protected amino acid Ns-^{C3N3}*N*-*L*-γ-Acp-OMe (0.715 g, 1.7 mmol) was dissolved in MeCN (9.0 mL) and treated with K_2CO_3 (1.20 g, 8.7 mmol) and PhSH (0.71 mL, 7.0 mmol). The mixture was stirred overnight and then acetonitrile was evaporated under vacuum. The resulting product was dissolved in CH_2Cl_2 and washed with H_2O (2x25 mL) and brine (2x25 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue purified by flash chromatography (0-8% MeOH/CH_2Cl_2) to finally affords the desired product as white solid [0.321 g; 81%; R_f = 0.13 (5% MeOH/CH_2Cl_2)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 7.11 (br, 1H), 3.60 (2s, 3H), 3.45 (t, *J* = 6.5 Hz, 2H), 3.35-3.22 (m, 1H), 2.84 (t, *J* = 7.2 Hz, 2H), 2.72 (m, 1H), 2.25 (m, *J* = 7.2 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 75 MHz, δ): 175.7 (C=O), 59.1 (CH_3), 51.9 (CH), 48.9 (CH_2), 44.7 (CH_2), 42.0 (CH), 34.3 (CH_2), 29.9 (CH_2), 27.3 (CH_2), 27.2 (CH_2). **MS (ESI)** [m/z (%)]: 227 ([M+H]$^+$, 100), 156 ([M-$C_2H_4N_3$]$^+$, 10). **HRMS (ESI)** Calculated for $C_{10}H_{18}N_4O_2$: 226.1430, found 226.1431.

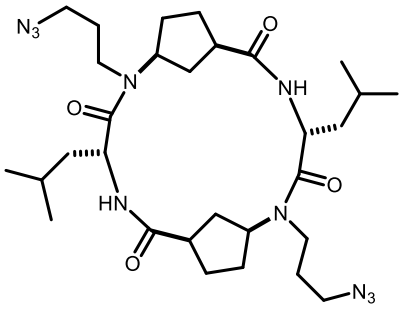


Boc-*D*-Leu-^{C3N3}*N*-*L*-γ-Acp-OMe. A solution of ^{C3N3}*N*-*L*-γ-Acp-OMe (0.320g, 1.4 mmol) in dry CH_2Cl_2 (5 mL) was treated with DIEA (0.50 mL, 2.8mmol). In another round bottom flask, Boc-*D*-Leu-OH (0.346 g, 1.5 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and then succesively treated with *N*-HATU (0.820 g, 2 mmol) and DIEA (0.67 mL, 3.6 mmol). Then both solutions were combined and stirred during 4 h under argon atmosphere. After this time, the mixture was washed with saturated aqueous solution of $NaHCO_3$ (3x20 mL) and aqueous HCl (5%, 3x20 mL). The resulting organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (15-20% AcOEt/Hexane) to afford mentioned product as a transparent oil [0.552 g, 92%, R_f = 0.68 (5% MeOH/CH_2Cl_2)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 5.23 (d, *J* = 9.1 Hz, 0.65H), 5.12 (d, *J* = 9.3 Hz, 0.35H), 4.69-4.40 (m, 1.35H), 4.29-4.13 (m, 0.65H), 3.65 (2s, 3H), 3.44-3.09 (m, 4H), 2.90-2.63 (m, 1H), 2.26-1.26 (m, 11H), 0.99-0.83 (m, 6H). **^{13}C NMR** ($CDCl_3$, 75MHz, δ): 175.7 (C=O), 172.9

(C=O), 155.5 (C=O), 79.4 (Q), 57.7 (CH_3), 51.8 (CH), 51.7 (CH), 49.47 (CH_2), 48.8 (CH_2), 43.3 (CH), 40.9 (CH_2), 39.5 (CH_2), 32.9 (CH_2), 29.0 (CH_2), 28.3 (CH_3), 26.9 (CH_2), 24.6 (CH), 23.4 (CH_3). **MS (ESI)** [m/z (%)]: 440 ([M+H]$^+$, 100), 462 ([M+Na]$^+$, 15). **HRMS (ESI)** Calculated for $C_{21}H_{37}N_5O_5Na$: 462.2687, found 462.2687.

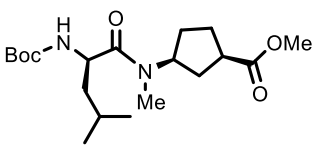


Boc-(*D*-Leu-^{C3N3}*N*-*L*-γ-Acp)$_2$-OMe. Previously obtained dipeptide Boc-*D*-Leu-^{C3N3}*N*-*L*-γ-Acp-OMe was divided into two portions. The first fraction (0.280 g, 0.6 mmol) was dissolved in a MeOH/H_2O mixture (3:1, 13 mL) and then LiOH (0.077 g, 3.2 mmol) was added. After stirring for 1 h, MeOH was evaporated under reduced pressure and the aqueous phase was acidified with aqueous HCl (5%) to pH 2. Then, it was extracted with CH_2Cl_2 (4x15 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to yield a white foam. The other portion of the same dipeptide (0.280 g, 0.6 mmol) was dissolved in CH_2Cl_2 (3.5 mL) and treated with TFA (3.5 mL). After stirring for 15 min the mixture was concentrated in the rotary evaporator, diluted in CH_2Cl_2 and concentrated for 3 times and finally dried under high vacuum for 3 h. After this time, the residue was dissolved in dry CH_2Cl_2 (3 mL) and DIEA (0.224 mL, 1.3 mmol) was added. Subsequently, the first portion residue was dissolved again in dry CH_2Cl_2 (5.5 mL) and subsequently treated with DIEA (0.447 mL, 2.6 mmol), and *N*-HATU (0.293 g, 0.77 mmol). Finally, both mixtures were combined and stirred for 3 h under argon atmosphere. The resulting solution was washed saturated aqueous solution of $NaHCO_3$ (3x20 mL) and aqueous HCl (5%, 3x20 mL). Then the organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under vacuum. The resulting foam was purified by flash chromatography (30-50% AcOEt/Hexane) to yield the desired product as a white foam [0.475 g, 99%, R_f = 0.30 (50% AcOEt/Hexane)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 6.49-6.03 (m, 2H), 5.29-4.82 (m, 2H), 4.82-4.41 (m, 4H), 4.36-4.14 (m, 2H), 3.77-3.57 (m, 3H), 3.52-3.11 (m, 4H), 2.72-2.55 (m, 1H), 2.29-1.18 (m, 22H), 1.13-0.84 (m, 12H). **MS (ESI)** [m/z (%)] 769 ([M+Na]$^+$, 100), 747 ([M+H]$^+$, 30). **HRMS (ESI)** Calculated for $C_{36}H_{62}N_{10}NaO_7$: 769.4695, found: 769.4661.

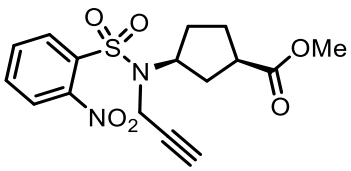


***c*-[(*D*-Leu-^{C3N3}*N*-*L*-γ-Acp-)$_2$]** (**CP1**). Linear tetrapeptide Boc-(*D*-Leu-^{C3N3}*N*-*L*-γ-Acp)$_2$-OMe (0.383 g, 0.52 mmol) was dissolved in a MeOH/H_2O mixture (3:1, 10.8 mL) and then LiOH (62 mg, 2.6 mmol) was added. After stirring for 1 h, MeOH was evaporated under vacuum and the aqueous mixture was acidified with aqueous HCl (5%) to pH 2. Then, the aqueous solution was extracted with CH_2Cl_2 (4x15 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum to yield white foam. A fraction of the resulting residue (97 mg, 0.13 mmol) was dissolved in CH_2Cl_2 (1.3 mL) and treated with TFA (1.3 mL). After stirring during 15 minutes, mixture was concentrated in the rotary evaporator, diluted in CH_2Cl_2 and concentrated (3 times) and dried under high vacuum for 3 h. Finally, the residue was dissolved in CH_2Cl_2 (660 mL) and DIEA (140 µL, 0.81 mmol) and PyAOP (65 mg, 0.13 mmol) were added. The mixture was stirred under argon atmosphere and after 14 h DIEA (0.046 mL, 0.27 mmol) and PyAOP (0.033 g, 0.067 mmol) were added. 5 h after the addition, the solvent was reduced until 5% of its initial volume and then

washed with an aqueous solutions of NH_4Cl (sat., 2x20 mL) and $NaHCO_3$ (sat., 2x20 mL). The resulting organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated. The solid was purified via HPLC (silica gel, 4-6% MeOH/CH_2Cl_2 over 30 min) to yield the desired cyclic tetrapeptide **CP1** as a white solid [166.1 mg, 52%, R_f = 0.34 (5% MeOH/CH_2Cl_2)]. **^{1}H NMR** ($CDCl_3$, 500 MHz, δ): 7.32 (m, 2H, NH), 4.95 (m, 2H), 4.28 (m, 2H), 3.67 (m, 2H), 3.35 (m, 4H), 3.15 (m, 2H), 2.91 (br, 2H), 2.61 (m, 2H), 2.23 (m, 2H), 2.05 (m, 2H), 1.84 (m, 4H), 1.67 (m, 4H), 1.47 (m, 6H), 0.92 (m, 12H). **^{13}C NMR** ($CDCl_3$, 125 MHz, δ): 177.1 (C=O), 174.6 (C=O), 56.4 (CH), 48.8 (CH), 48.1 (CH), 41.6 (CH_2), 41.2 (CH_2), 40.8 (CH_2), 31.1 (CH_2), 28.5 (CH_2), 25.8 (CH_2), 24.9 (CH_2), 23.3 (CH), 22.2 (CH_3). **FT-IR** (293 K, $CHCl_3$) 3301 (amide A), 2100 (azide), 1668 (amide I_a), 1624 (amide I_b), 1528 (amide II) cm^{-1}. **HRMS (ESI)** Calculated for $C_{30}H_{51}N_{10}O_4$: 615.4087, found 615.4089.

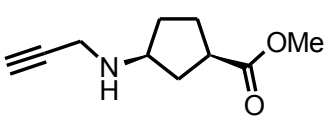


Boc-*D*-Leu-^{Me}*N*-*L*-γ-Acp-OMe. Compound Boc-^{Me}*N*-*L*-γ-Acp-OMe (0.317 g, 1.2 mmol) was dissolved in a 1:1 mixture of CH_2Cl_2 and TFA (12.5 mL). The solution was stirred for 10 minutes and then mixture was concentrated on the rotary evaporator, diluted in CH_2Cl_2 and concentrated (3 times) and finally dried under high vacuum for 3 h. The resulting residue was dissolved in dry CH_2Cl_2 (12.4 mL) and successively treated with DIEA (1.4 mL, 7.4 mmol), Boc-*D*-Leu-OH (0.305 g, 1.32 mmol) and *N*-HATU (0.547 g, 1.44 mmol). The resulting solution was stirred for 2 h under argon atmosphere and then washed with aqueous solutions of HCl (5%, 2x30 mL) and $NaHCO_3$ (sat., 2x30 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum. The residue was purified via flash chromatography (20-40% AcOEt/Hexane) to give the desired dipeptide **Boc-*D*-Leu-^{Me}*N*-*L*-γ-Acp-OMe** as a white foam [0.260 g, 60%, R_f = 0.77 (10% MeOH/CH_2Cl_2)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 5.49-5.22 (m, 1H), 5.08-4.67 (m, 1H), 4.66-4.22 (m, 1H), 3.73-3.63 (m, 3H), 3.01-2.74 (m, 4H), 2.20-1.18 (m, 17H), 1.13-0.83 (m, 6H). **^{13}C NMR** ($CDCl_3$, 75 MHz, δ): 175.7 (C=O), 173.1 (C=O), 155.5 (C=O), 79.4 (Q), 56.7 (CH_3), 54.5 (CH), 52.00 (CH), 49.3 (CH), 43.5(CH_3), 41.7 (CH_2), 35.9 (CH), 31.8 (CH_3), 30.1 (CH_2), 28.8 (CH_2), 23.4 (CH_2), 21.8 (CH_3). **HRMS (ESI)** Calculated for $C_{19}H_{34}N_2NaO_5$ 393.2360, found: 393.2360.

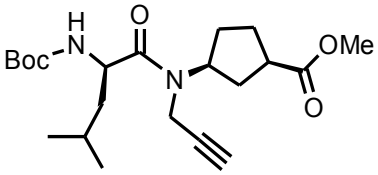


Methyl (1*R*,3*S*)-3-amino-*N*-(2-nitrobenzenesulfonyl)-*N*-propargylcyclopentanecarboxylate (Ns-^{prg}*N*-*L*-γ-Acp-OMe). Protected amino acid Ns-*L*-γ-Acp-OMe (1.259 g, 3.8 mmol) was dissolved in MeCN (19 mL) and treated with K_2CO_3 (2.10 g, 15.2 mmol) and propargyl bromide (80%, 1.978 g, 13.3 mmol). The mixture was stirred for 5 h under argon atmosphere and then acetonitrile was evaporated under reduced pressure. The residue was suspended in CH_2Cl_2 (25 mL) and washed with H_2O (milli-Q grade, 3x25 mL) and aqueous solution of NaCl (sat., 3x25 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated on rotary evaporator. The residue was purified by flash chromatography (20-30% AcOEt/Hexane) and the title product was obtained as a yellow viscous oil [1.31 g, 97%, R*f* = 0.65 (50% AcOEt/Hexane)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 8.18-8.06 (m, 1H), 7.76-7.53 (m, 3H), 4.41-4.19 (m, 1H), 4.17 (2d 2H), 3.74-3.56 (s, 3H), 2.95-2.64 (m, 1H), 2.24-1.79 (m, 7H). **^{13}C NMR** ($CDCl_3$, 75 MHz, δ): 176.0 (C=O), 148.0 (Q), 133.8 (CH), 133.5 (Q), 131.8 (CH), 131.2 (CH), 124.2 (CH), 79.4 (Q), 73.1 (CH), 58.8 (CH), 58.3 (CH_3), 51.9 (CH), 40.7 (CH), 32.9 (CH_2), 32.6 (CH_2), 28.5 (CH_2), 26.9 (CH_2). **MS (ESI)** [m/z (%)]: 240

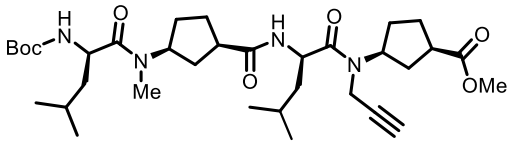
([MH]$^+$-Ns-^{prg}N, 60), 367 ([MH]$^+$, 35). **HRMS (ESI)** Calculated for $C_{16}H_{18}N_2NaO_6S_1$: 389.0778, found: 389.0776.



Methyl (1*R*,3*S*)-3-amino-*N*-propargylcyclopentane carboxylate (^{prg}***N*-*L*-γ-Acp-OMe)**. A solution of Ns-^{prg}*N*-*L*-γ-Acp-OMe (1.120 g, 3.1 mmol) in MeCN (15 mL) was treated with K_2CO_3 (2.142 g, 15.5 mmol) and PhSH (1.25 mL, 12.4 mmol). The resulting mixture was stirred for 18 h and then the solvent was removed under vacuu. The residue was suspended in CH_2Cl_2 and washed with H_2O (milli-Q grade, 2x15 mL) and aqueous solution of NaCl (sat., 2x15 mL). Finally the organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum. The resulting residue was purified by flash chromatography (25-40% AcOEt/Hexane) to afford the desired N-functionalized **^{prg}*N*-*L*-γ-Acp-OMe** [0.390 g, 69%, R_f = 0.26 (75% AcOEt/Hexane)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 4.14-3.86 (m, 1H), 3.56 (s, 3H), 3.43-3.13 (m, 3H), 2.69 (m, 1H), 2.21-1.13 (m, 7H). **^{13}C NMR** ($CDCl_3$, 75 MHz, δ): 176.3 (C=O), 82.2 (Q), 71.1 (CH), 57.5 (CH_3), 51.6 (CH), 42.1 (CH), 36.5 (CH_2), 36.2 (CH_2), 32.1 (CH_2), 27.4 (CH_2). **MS (ESI)** [m/z (%)]: 182 ([MH]$^+$, 100). **HRMS (ESI)** Calculated for $C_{10}H_{16}N_1O_2$: 182.1176, found: 182.1175.



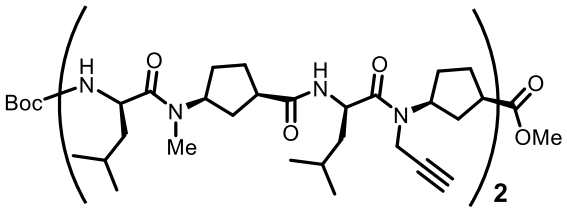
Boc-*D*-Leu-^{prg}*N*-*L*-γ-Acp-OMe. A solution of ^{prg}*N*-*L*-γ-Acp-OMe (0.390 g, 2.2 mmol) in dry CH_2Cl_2 (22 mL) was successively treated with DIEA (1.5 mL, 8.6 mmol), Boc-*D*-Leu-OH (0.548 g, 2.4 mmol) and *N*-HATU (0.980 g, 2.58 mmol). The resulting solution was stirred under argon for 1 h and then washed with aqueous solutions of HCl (5%, 2x20 mL) and $NaHCO_3$ (sat., 2x20 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by flash chromatography (7-30% AcOEt/Hexane) to give the desired dipeptide **Boc-*D*-Leu-^{prg}*N*-*L*-γ-Acp-OMe** as a colorless oil [0.688 g, 82%; R_f = 0.80 (50% AcOEt/Hexane)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 5.49 and 5.11 (2d, *J* = 8.92, 1H), 4.88-4.57 (m, 1H), 4.40 (m, 1H), 4.21-3.83 (m, 2H), 3.67 (d, *J* = 6.5 Hz, 3H), 3.06-2.71 (m, 1H), 2.43-1.07 (m, 19H), 1.05-0.81 (m, 6H). **^{13}C NMR** ($CDCl_3$, 75 MHz, δ): 175.8 (C=O), 172.5 (C=O), 155.3 (C=O), 80.4 (Q), 72.7 (Q), 57.6 (CH_3), 51.7 (CH), 48.6 (CH), 42.0 (CH_2), 41.0 (CH), 32.6 (CH_2), 29.6 (CH_2) 28.2 (CH_3), 27.8 (CH_2), 27.4 (CH_2) 24.5 (CH_3), 23.4 (CH), 21.6 (CH). **HRMS (ESI)** Calculated for $C_{21}H_{34}N_2O_5Na$: 417.2360, found: 417.2362.



Boc-*D*-Leu-^{Me}*N*-*L*-γ-Acp-*D*-Leu-^{prg}*N*-*L*-γ-Acp-OMe. A solution of Boc-*D*-Leu-^{Me}*N*-*L*-γ-Acp-OMe (0.217 g, 0.55 mmol) in a MeOH/H_2O mixture (3:1, 11 mL) was treated with LiOH (0.066 g, 2.8 mmol). After stirring for 1 h, MeOH was removed under reduced pressure and the aqueous phase was acidified with aqueous solution of HCl (5%) to pH 2. The resulting aqueous solution was extracted with CH_2Cl_2 (4x15 mL), and combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated to yield a white foam that was dried under vacuum.

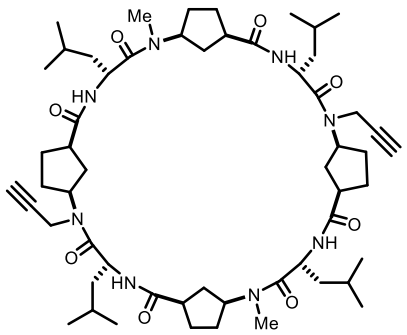
A solution of Boc-*D*-Leu-^{prg}*N*-*L*-γ-Acp-OMe (0.200 g, 0.54 mmol) in a 1:1 mixture of CH_2Cl_2 and TFA (5.4 mL) was stirred for 15 min and then concentrated in the rotary evaporator. The

residue was dissolved in CH_2Cl_2 and concentrated (3 times) and finally dried under high vacuum for 3 h. After this time, the residue was dissolved in dry CH_2Cl_2 (2.7 mL) and DIEA (0.38 mL, 2.2 mmol) was added. Subsequently, the previous portion was also dissolved in dry CH_2Cl_2 (2.7 mL) and then DIEA (0.19 mL, mmol) and *N*-HBTU (0.246 g, 0.64 mmol) were added in this order. Finally, both mixtures were combined and stirred for 3 h under argon atmosphere. After this time, the solution was washed with aqueous solutions of $NaHCO_3$ (sat, 2x15 mL) and HCl (5%, 2x15 mL), and then dried over anhydrous $MgSO_4$, filtered and evaporated under vacuum. The resulting residue was purified by column chromatography (30-50% AcOEt/Hexane) to yield the desired product as a foam [0.340 g, 96%, R_f = 0.20 (50% AcOEt/Hexane)]. **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 6.49-6.04 (m, 1H), 5.38-3.85 (m, 5H), 3.81-3.60 (m, 3H), 3.02-2.57 (m, 7H), 2.26-1.31 (m, 20H), 1.10-0.78 (m, 12H). **MS (ESI)** [m/z (%)]: 633 ([M+H]$^+$, 100), 655 ([M+Na]$^+$, 60). **HRMS (ESI)** Calculated for $C_{34}H_{56}N_4O_7Na$: 655.4041, found 655.4038.

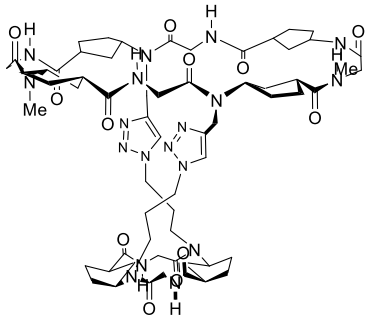


Boc-(*D*-Leu-^{Me}*N*-*L*-γ-Acp-*D*-Leu-^{prg}*N*-*L*-γ-Acp)$_2$-OMe. A solution of Boc-*D*-Leu-^{Me}*N*-*L*-γ-Acp-*D*-Leu-^{prg}*N*-*L*-γ-Acp-OMe (0.165 g, 0.26 mmol) in a mixture MeOH/H_2O (3:1, 4.2 mL) and treated with LiOH (0.031 g, 1.31 mmol) and stirred at r.t. for 1 h. After this time, MeOH was evaporated under reduced pressure and the resulting aqueous solution was acidified with an aqueous solution of HCl (5%) to pH 2. The acidic aqueous solution was extracted with CH_2Cl_2 (4x10 mL) and the combined organic phases were dried over anhydrous $MgSO_4$, filtered and concentrated under vacuo. A second fraction of the same linear tetrapeptide (0.160 g, 0.26 mmol) was dissolved in a 1:1 mixture of CH_2Cl_2 and TFA (5.4 mL), then it was stirred for 15 minutes at ambient temperature and concentrated in the rotary evaporator. The residue was dissolved in CH_2Cl_2 and concentrated (3 times) and finally dried under high vacuum for 3 h. Finally the residue was dissolved in dry CH_2Cl_2 (2.2 mL) and treated with DIEA (0.15 mL, 0.70 mmol).

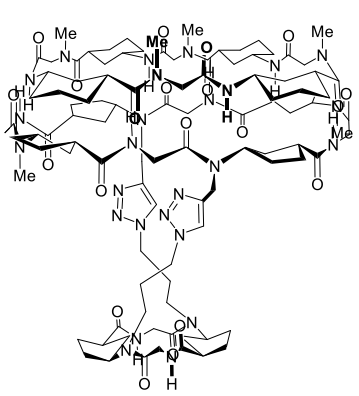
The fraction with the free carboxy group was dissolved in freshly distilled CH_2Cl_2 (2 mL) and then DIEA (0.070 mL, 0.35 mmol) and *N*-HBTU (0.097 g, 0.25 mmol) were added. After two min this solution was poured onto the other mixture and the resulting solution was stirred under argon for 2 h at r.t. After that time, the mixture was washed with aqueous solutions of HCl (5%, 2x15 mL) and $NaHCO_3$ (sat., 2x15 mL). The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated at reduced pressure. The residue was purified by flash chromatography (0-5% MeOH/CH_2Cl_2) to give the title compound as a foam [0.213 g, 85%, R_f = 0.30 (5% MeOH/CH_2Cl_2)]. **^{1}H-NMR** ($CDCl_3$, 300 MHz, δ): 7.53-7.30 (m, 1H), 6.57-6.12 (m, 2H), 5.39-3.89 (m, 9H), 3.83-3.58 (m, 3H), 3.12-2.51 (m, 14H), 2.43-1.09 (m, 31H), 1.09-0.74 (m, 24H). **MS (ESI)** [m/z (%)]: 567 ([M+Na]$^{2+}$, 100), 1133 ([M+Na]$^+$, 55). **HRMS (ESI)** Calculated for $C_{62}H_{101}N_8O_{11}$: 1132.7512, found 1132.7516.



***c*-[(*D*-Leu-^{Me}*N*-*L*-γ-Acp-*D*-Leu-^{prg}*N*-*L*-γ-Acp-)$_2$]** (**CP2**). A solution of linear octapeptide Boc-(*D*-Leu-^{Me}*N*-*L*-γ-Acp-*D*-Leu-^{prg}*N*-*L*-γ-Acp)$_2$-OMe (0.186 g, 0.16 mmol) in a mixture MeOH/H_2O (3:1, 3.2 mL) was treated with LiOH (0.020 g, 0.82 mmol). After stirring for 1 h at room temperature the MeOH was removed on the rotary evaporator y and the resulting solution was acidified with an aqueous solution of HCl (5%) to pH 2. This solution was extracted with CH_2Cl_2 (4x10 mL) y and the combined organic extracts were dried, filtered and concentrated in vacuo. The resulting foam was dissolved in a 1:1 mixture of CH_2Cl_2 (1.5 mL) and TFA (3 mL) and stirred for 15 min. The solution was concentrated and hhe residue was dissolved in CH_2Cl_2 and concentrated (3 times) and finally dried under high vacuum for 3 h. This residue was suspended in dry CH_2Cl_2 (50 mL) and DIEA (0.16 mL, 0.30 mmol) and *N*-TBTU (0.096 g, 0.30 mmol) were added. This mixture was left to stir during 15 h and additional DIEA (0.16 mL, 0.30 mmol) and *N*-TBTU (0.036 g, 0.12 mmol) were added. After 6 h, the solution was washed with aqueous solutions of HCl (5%, 2x10 mL) and $NaHCO_3$ (2x10 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography (0-4% MeOH/CH_2Cl_2) to give the **CP2** as a white solid [0.099 g; 60%; R_f = 0.47 (5% MeOH/CH_2Cl_2)]. **^{1}H RMN** ($CDCl_3$, 500 MHz, δ): 8.35 (d, *J* = 9.5 Hz, 1.2 H), 8.26 (d, *J* = 9.5 Hz, 0.8 H), 8.20 (d, *J* = 9.5 Hz, 0.9 H), 8.14 (d, *J* = 9.5 Hz, 1.1 H), 5.10 (m, 4H), 4.80-4.55 (m, 6H), 3.82 (td, *J* = 13.7 and *J* = 2.2 Hz), 3.08 (m and 2s, 10H), 2.21 (t, *J* = 2.4 Hz, 2H), 0.95-0.78 (m, 24H). **^{13}C RMN** ($CDCl_3$, 125 MHz, δ): 175.4 (C=O), 174.7(C=O), 174.1(C=O), 173.3 (C=O), 80.2 (Q), 72.5 (Q), 55.4 (CH), 54.6 (CH), 47.0 (CH), 46.9 (CH), 43.3 (CH), 42.2 (CH), 41.5 (CH_2), 34.3 (CH_2), 32.6 (CH_2), 31.4 (CH_2), 29.4 (CH_2), 28.3 (CH_2), 28.2 (CH_3), 28.0 (CH_2), 27.7(CH_2), 26.9 (CH_2), 26.7 (CH), 24.8 (CH), 23.5 (CH_3), 22.1 (CH_3). **FT-IR** (293 K, $CHCl_3$) 3308 (amide A), 1673 (amide I_a), 1632 (amide I_b), 1540 (amide II) cm^{-1}. **HRMS (ESI)** Calculated for $C_{56}H_{89}N_8O_8$: 1001.6798, found: 1001.6794.



CP4. Both cyclic peptides *c*-[(*D*-Leu-^{Me}*N*-*L*-γ-Acp-*D*-Leu-^{prg}*N*-*L*-γ-Acp-)$_2$] (6.0 mg, 0.006 mmol) and *c*-[(*D*-Leu-^{3CN3}*N*-*L*-γ-Acp-)$_2$] (4.3 mg, 0.007 mmol) were dissolved in freshly distilled and degassed (Ar flow, 20 min) CH_2Cl_2 (6 mL) and then a solution of DIEA (65 µL, 0.285 M), TBTA (64 µL, 0.005 M) and [$Cu(MeCN)_4PF_6$] (64 µL, 0.012 M) in freshly distilled and degassed CH_2Cl_2 was added. The resulting mixture was stirred overnight under argon atmosphere. The turbid solution was concentrated under reduced pressure and re-suspended in a 10% MeOH in $CHCl_3$. This solution was filtered and the filtrate was purified by semi-preparative HPLC (silica gel, 5-8% MeOH over 30 min) to give the adduct product as a white solid [4.8 mg, 48%, t_R = 15 min]. **^{1}H RMN** ($CDCl_3$, 500 MHz, δ): 8.38 (d, *J* = 9.0 Hz, 1.45 H), 8.32 (d, *J* = 9.3 Hz, 0.45), 8.21 (d, *J* = 8.7 Hz, 0.55H), 8.17 (d, *J* = 8.8 Hz, 1.55H), 8.02-7.89 (2s, 2H), 6.60 (brm, 0.55H), 6.37 (brs, 1.55H) 5.35-5.21 (m, 2H), 5.19-5.06 (m, 2H), 4.87-4.53 (m, 14H), 4.45-4.22 (m, 2H), 3.62-3.49 (m, 2H), 3.32-2.64 (m, 22H), 2.31-1.08 (m, 58H), 1.03-0.83 (m, 36H). **FT-IR** (293 K, $CHCl_3$) 3304 (amide A), 1679 (amide I_a), 1620 (amide I_b), 1531 (amide II) cm^{-1}. **HRMS (ESI)** Calculated for $C_{86}H_{139}N_{18}O_{12}$: 1616.0811, found: 1616.0814.



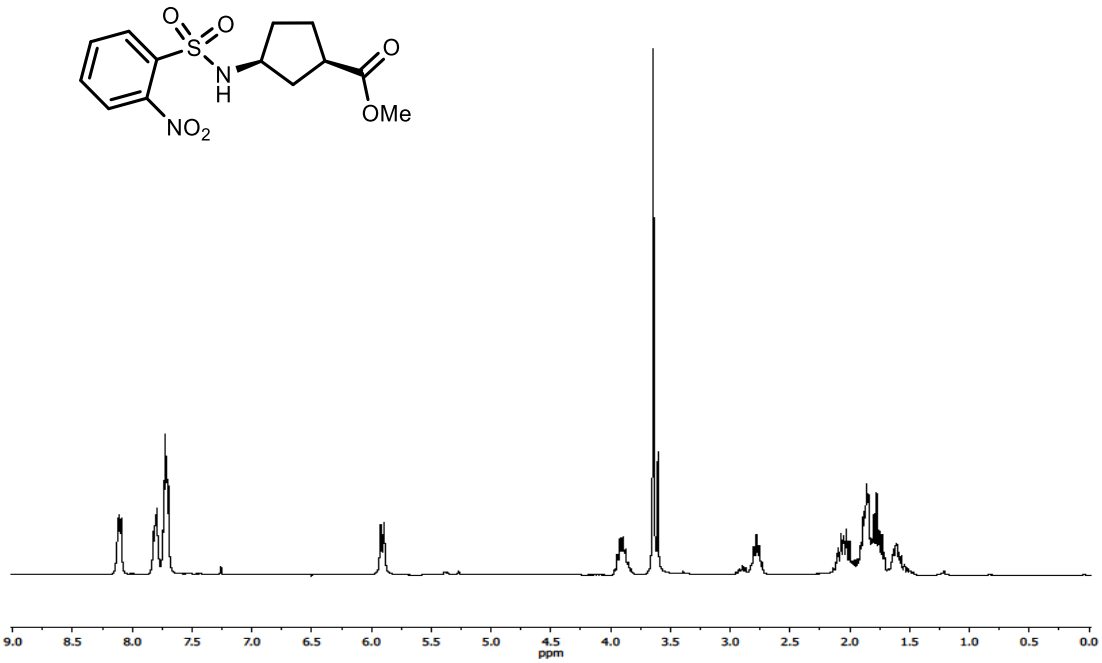
Heterodimer of *c*-[(*D*-Phe-*L*-^{Me}*N*-Ach-)$_4$] (**CP3**) with **CP4** (**D3-4**). To an NMR tube containing a solution of **CP4** (4.5 mg, 2.78·10^{3} mmol) in $CDCl_3$ (400 µL) were successive added portions of a stock solution of **CP3**[10] (7.3 mg, 6.38·10^{3} mmol) in $CDCl_3$ (230 µL). Hence, samples containing a ratio of 0.25, 0.5, 0.75 and 1.0 equivalents of **CP3** to **CP4** were prepared in the same experimental run. In case of any excess *c*-[(*D*-Phe-*L*-^{Me}N-Ach-)$_4$] being added, it was possible to purify the mixture to separate the heterodimer to the excess of any cyclic octapeptide by HPLC (silica gel, 3-7.5% MeOH/CH_2Cl_2 over 30 min, t_R = 24 min for the **heterodimer D3-4** and t_R = 20 min for the **CP4**). **^{1}H RMN** (5% MeOH-d_3/$CDCl_3$, 500 MHz, δ): 8.64-8.57 (m, 4H), 8.47-8.42 (m, 4H), 7.85 (s, 2H), 7.18-7.00 (m, 20H), 6.58 (d, *J* = 7.9 Hz, 2H), 5.34-4.96 (m, 8H), 4.92-4.69 (m, 4H), 4.69-4.44 (m, 8H), 4.37-4.19 (m, 8H), 0.89-0.77 (m, 36H). **FT-IR** (293 K, $CHCl_3$): 3298 (amide A) 1669 (amide I_a), 1618 (amide I_b), 1530 (amide II) cm^{-1}. **HRMS (ESI)** Calculated for $C_{182}H_{223}N_{16}O_8$: 2760.7529, found 2760.7535.

[10] **CP3** was prepared using the protocol described in: M. Amorín, L. Castedo, J. R. Granja *Chem.–Eur. J.* 2005, **11**, 6543-6551.

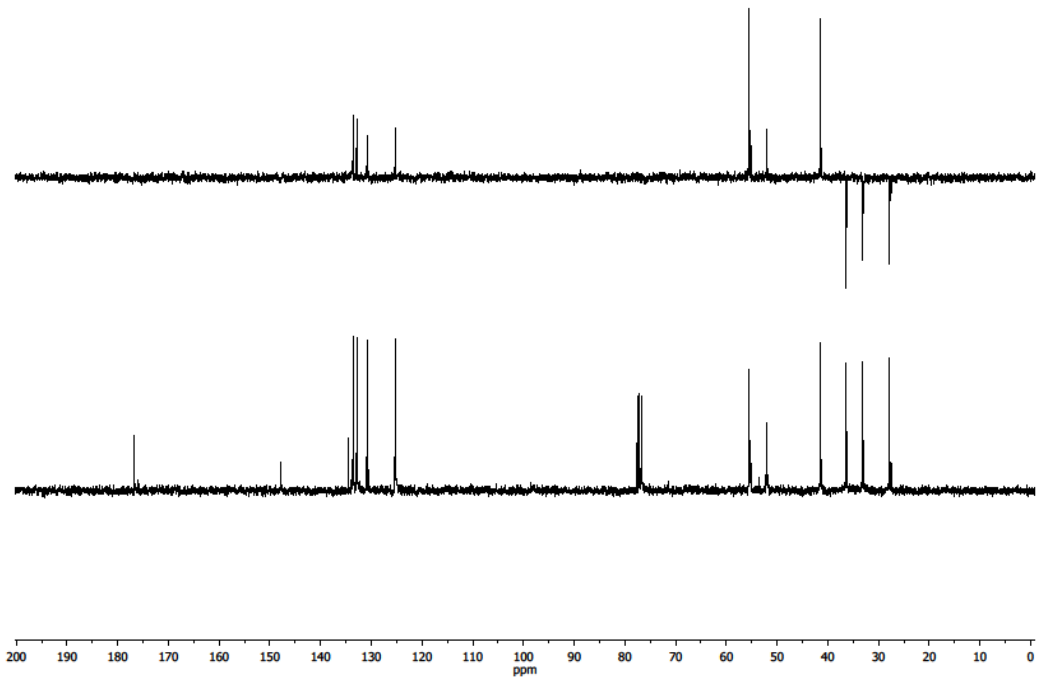
NMR & FTIR spectra

Ns-*L*-γ-Acp-OMe

^{1}H NMR (50 mM in $CDCl_3$, 298K, 300 MHz)

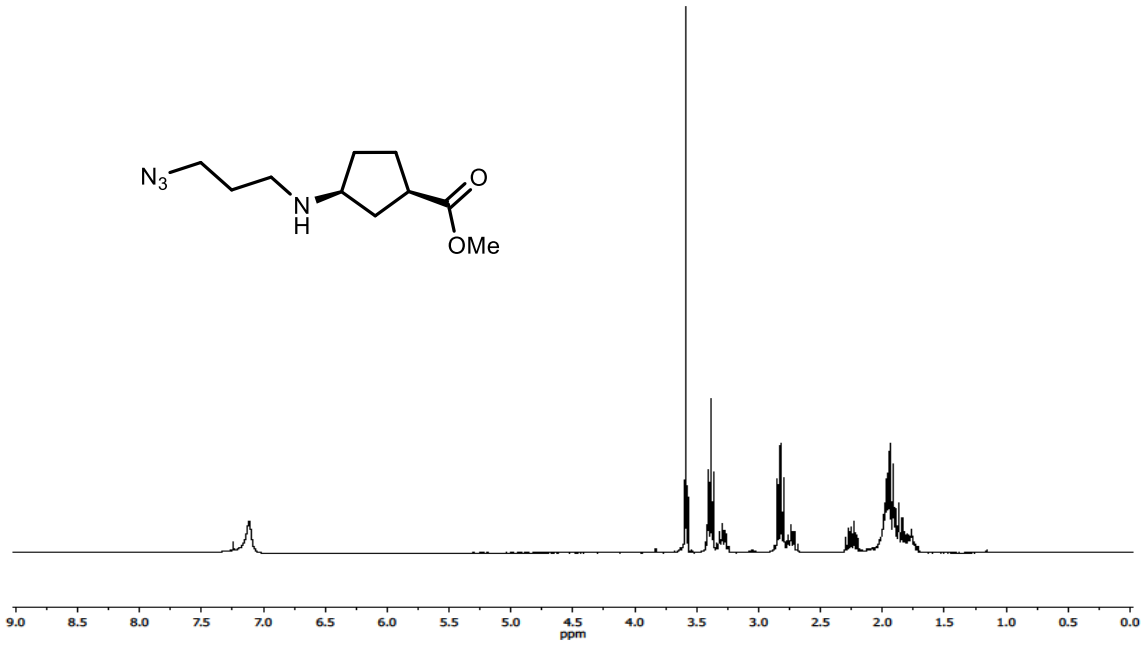


^{13}C & DEPT NMR (50 mM in $CDCl_3$, 298K, 75 MHz)

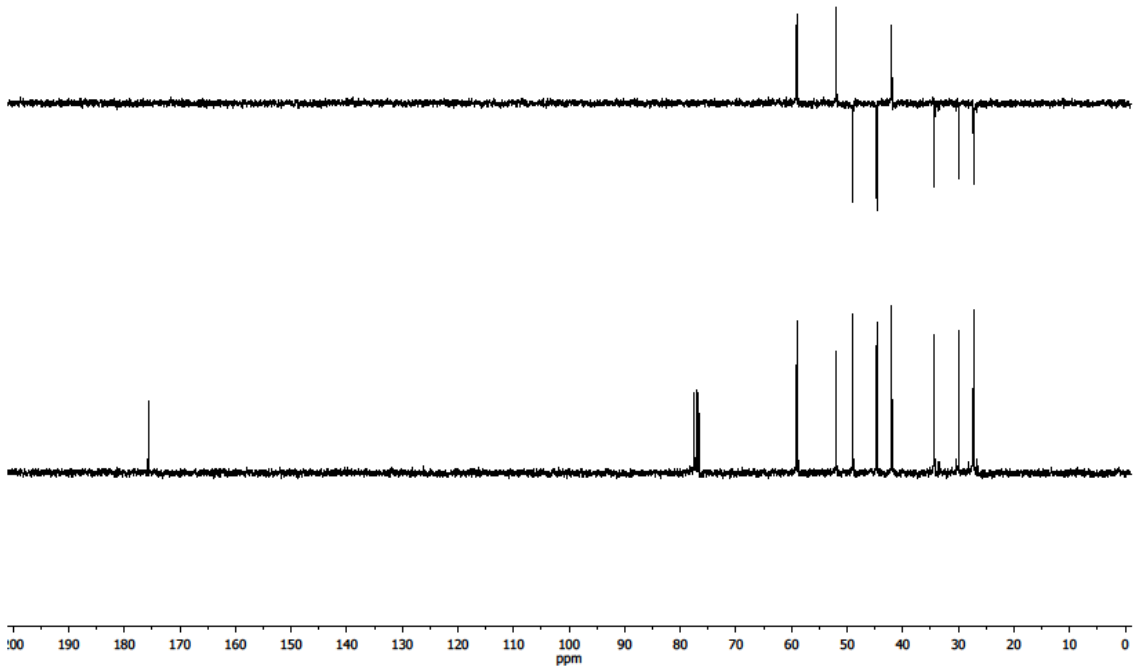


^{C3N3}*N*-*L*-γ-Acp-OMe

^{1}H NMR (50 mM in $CDCl_3$, 298K, 300 MHz)

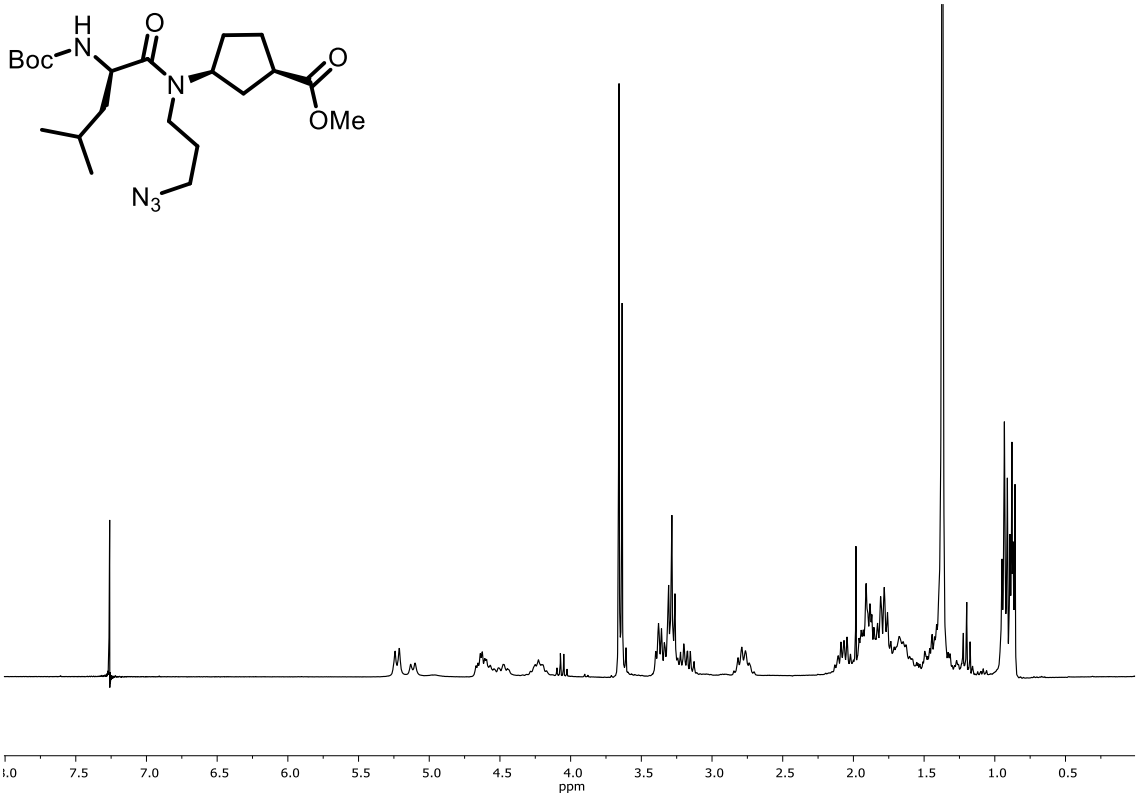


^{13}C & DEPT NMR (50 mM in $CDCl_3$, 298K, 75 MHz)

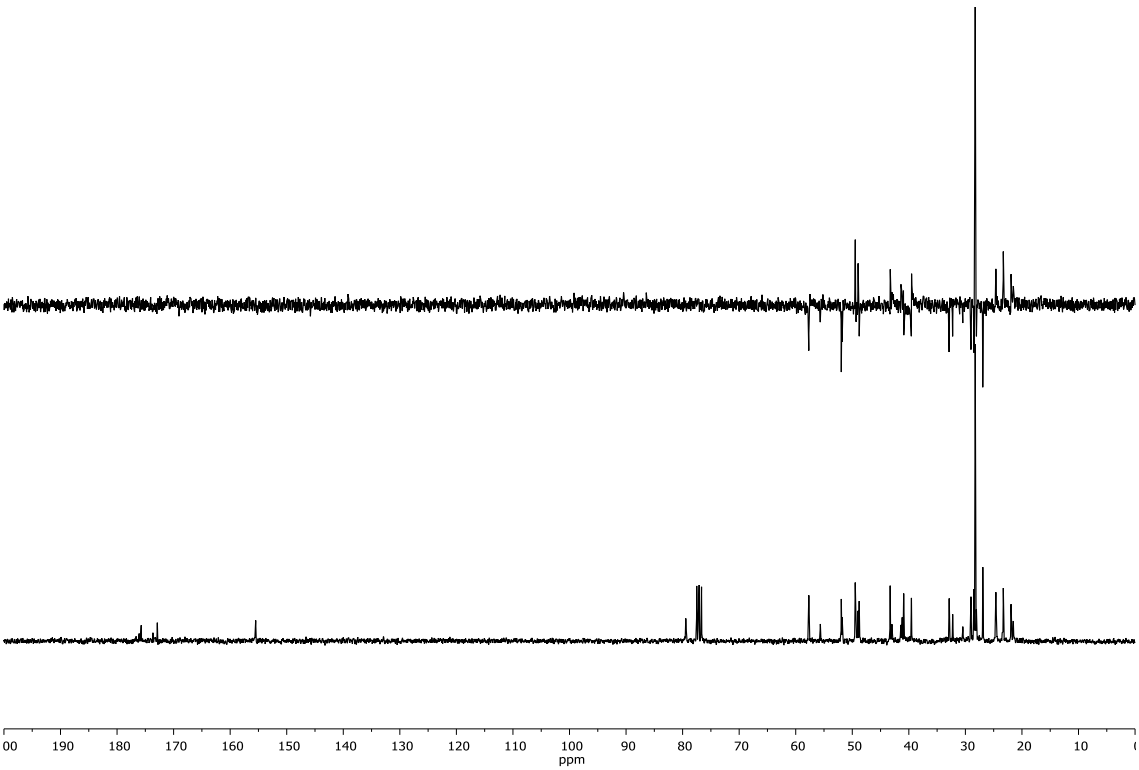


Boc-D-Leu-^{C3N3}*N*-*L*-γ-Acp-OMe

^{1}H NMR (10 mM in $CDCl_3$, 298K, 300 MHz)

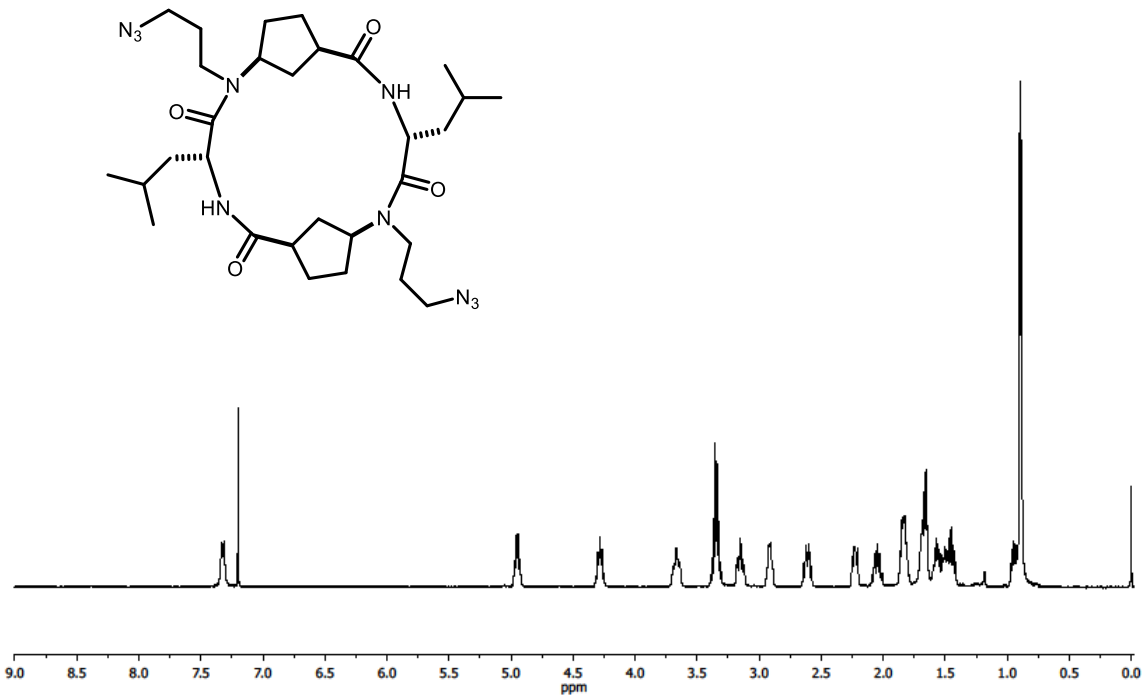


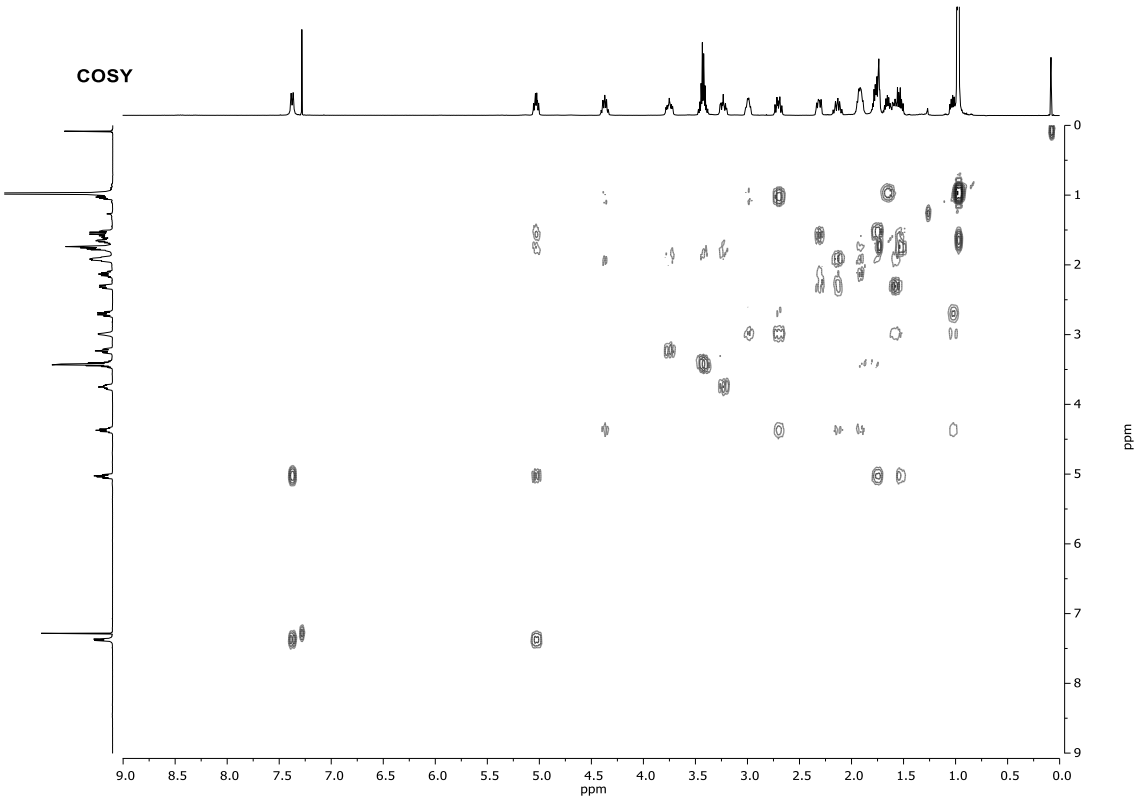
^{13}C & DEPT NMR (10 mM in $CDCl_3$, 298K, 75 MHz)



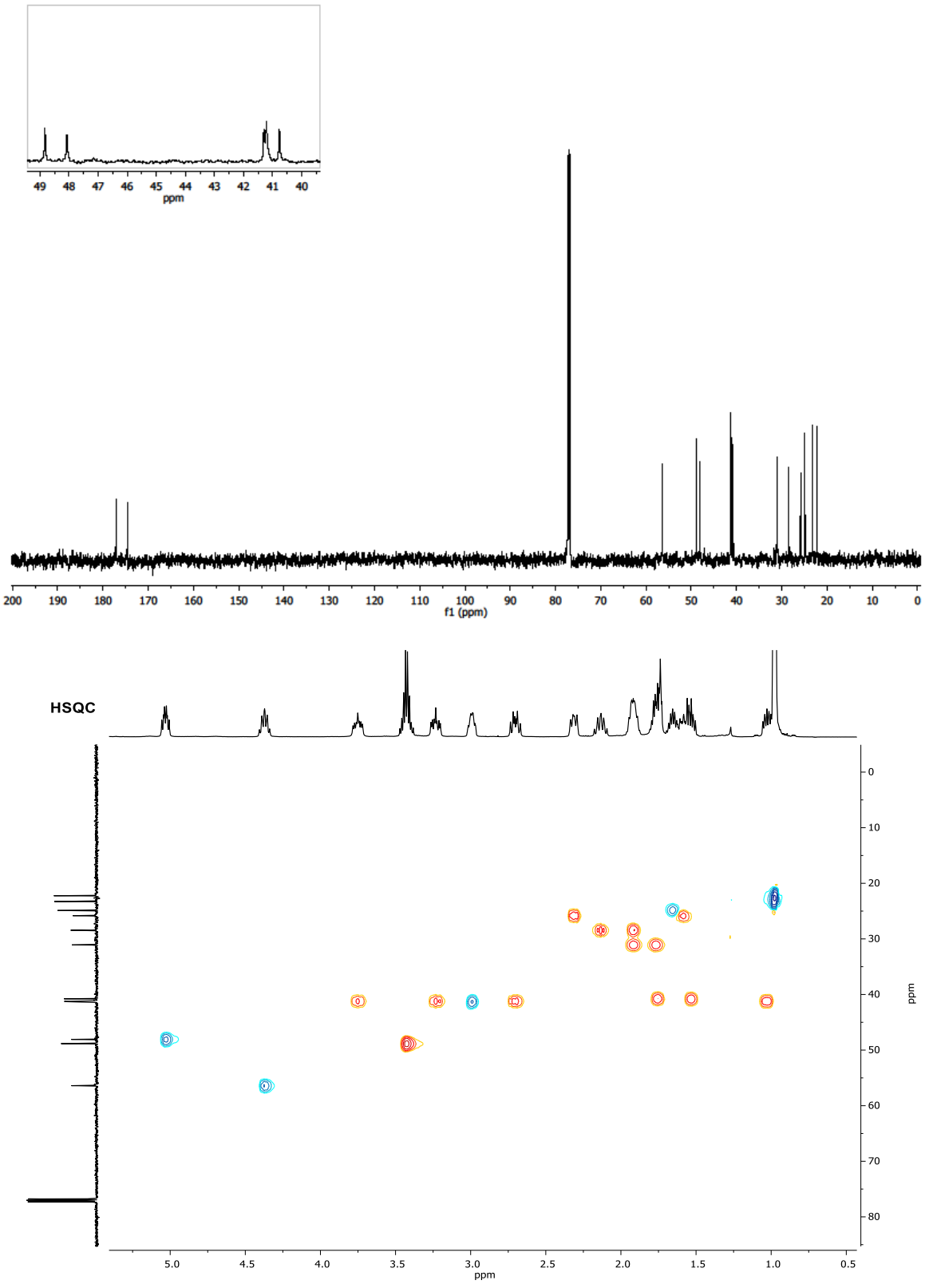
***c*-[(D-Leu-^{C3N3}*N*-*L*-γ-Acp-)$_2$] CP1**

^{1}H & COSY NMR (25 mM in $CDCl_3$, 298K, 500 MHz)

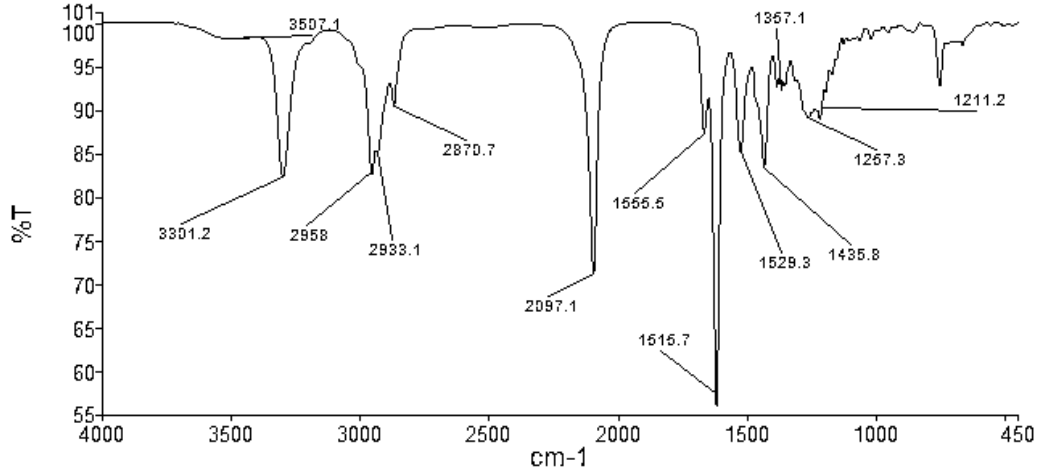




^{13}C & HSQC NMR (25 mM in $CDCl_3$, 298K, 125 MHz)

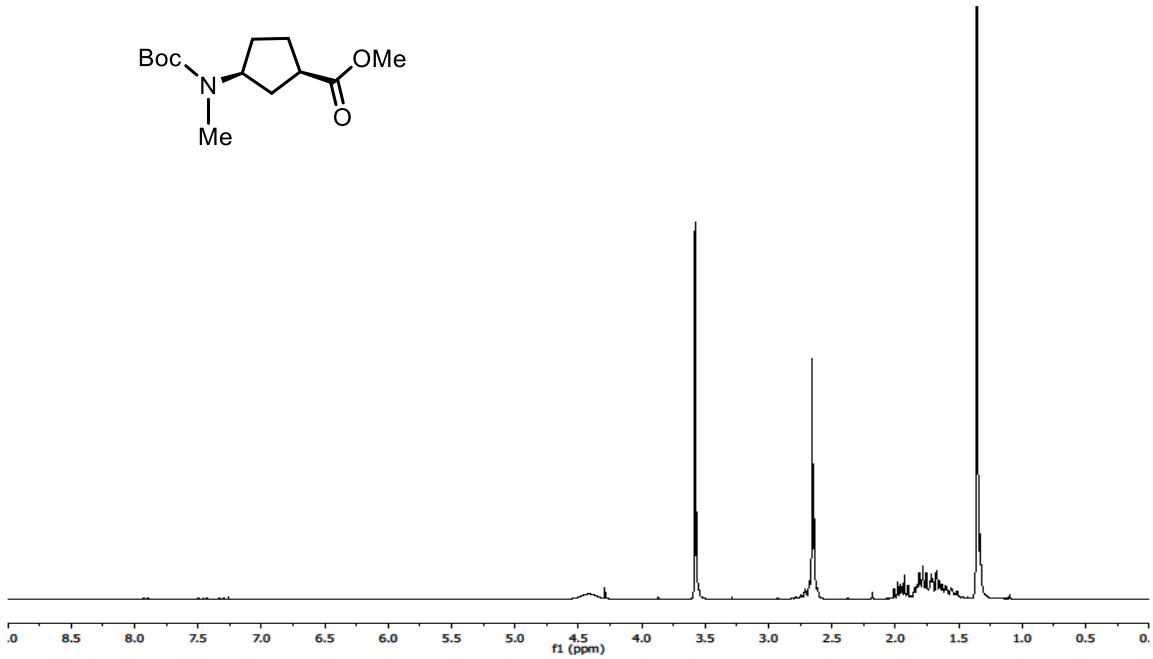


FT-IR (Neat, 298 K)

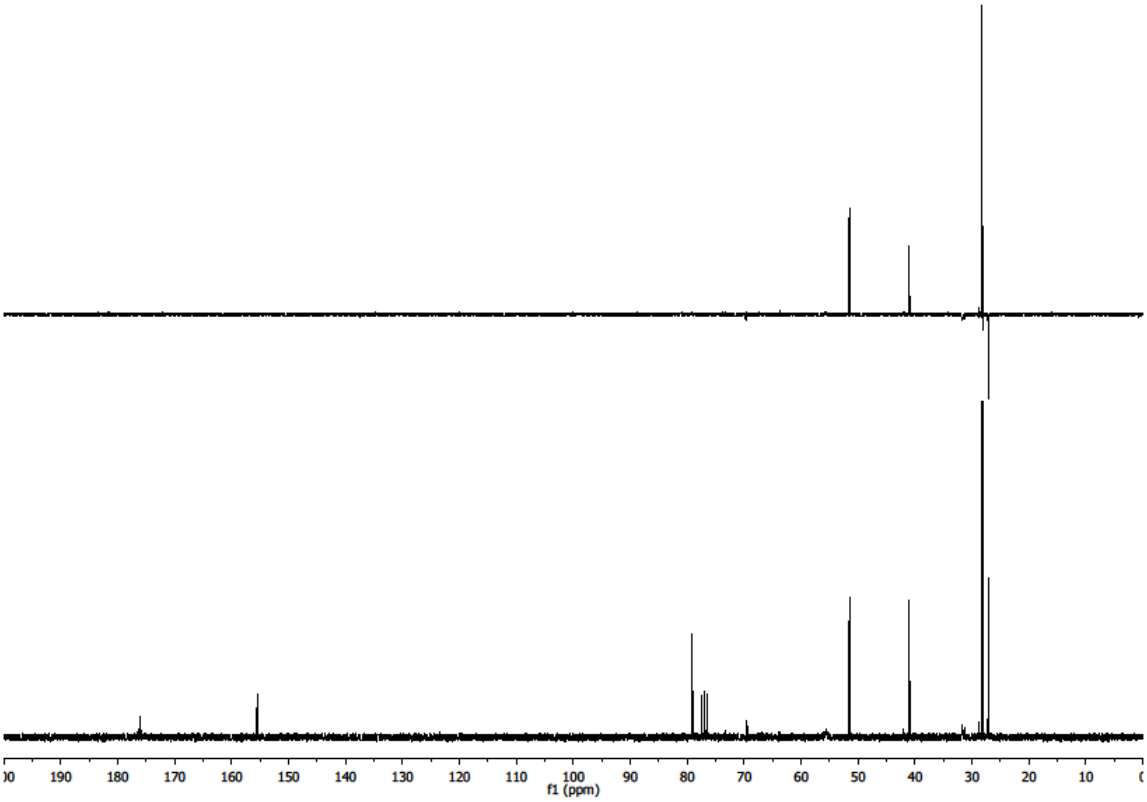


Boc-^{Me}*N*-*L*-γ-Acp-OMe

^{1}H NMR (100 mM in $CDCl_3$, 298K, 300 MHz)

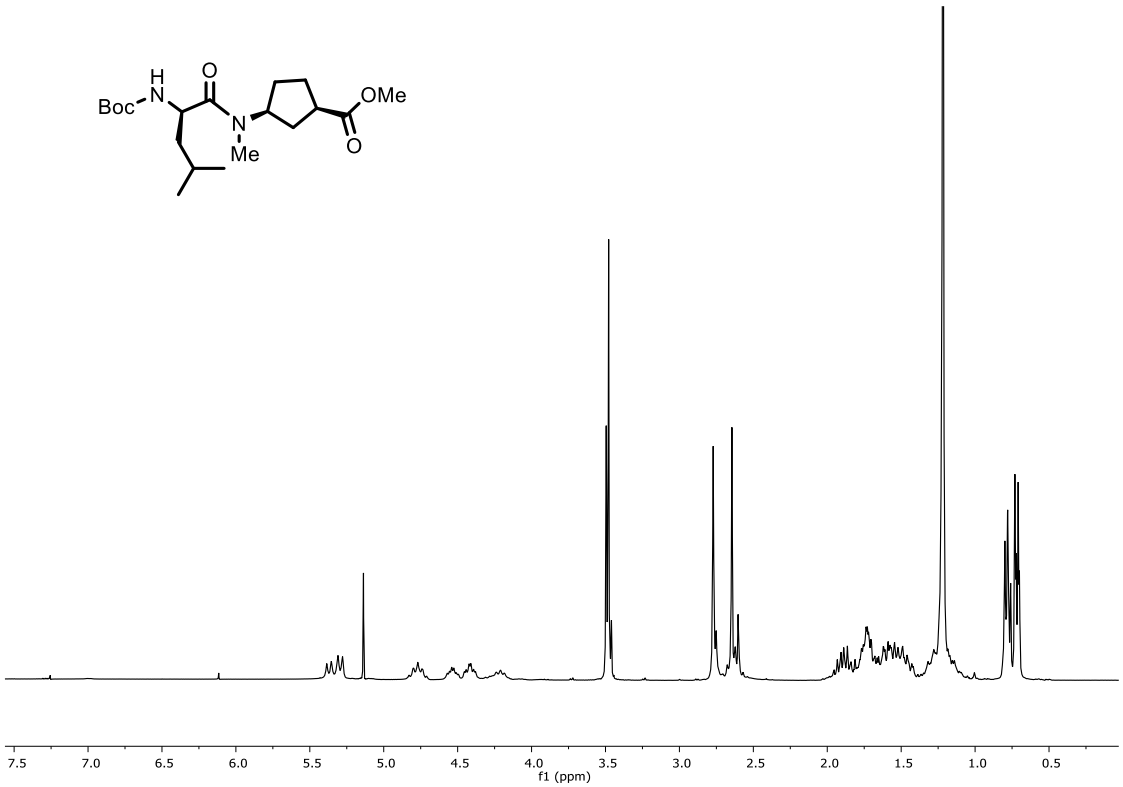


^{13}C & DEPT NMR (100 mM in $CDCl_3$, 298K, 75 MHz)

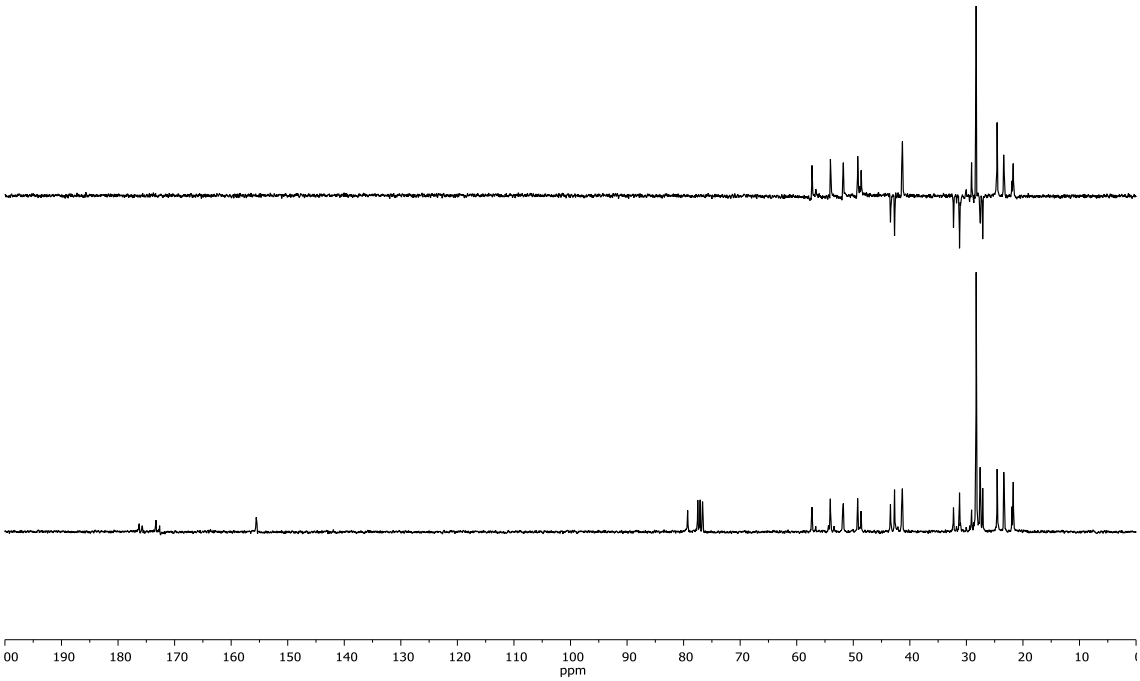


Boc-*D*-Leu-^{Me}*N*-*L*-γ-Acp-OMe

^{1}H NMR (65 mM in $CDCl_3$, 298K, 300 MHz)

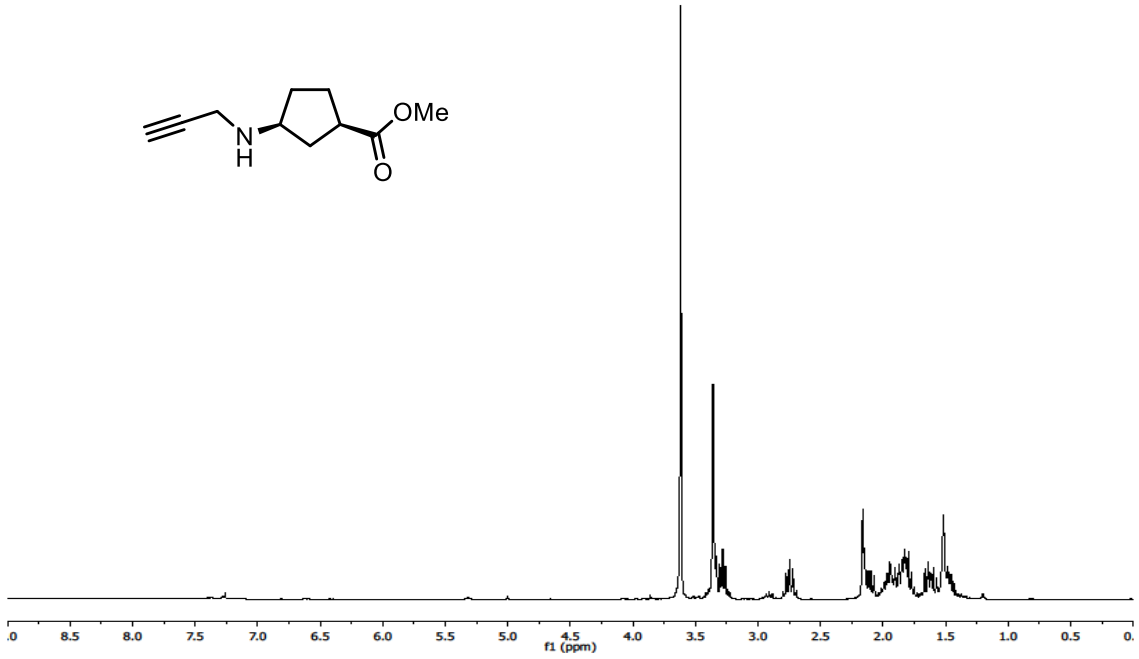


^{13}C & DEPT NMR (65 mM in $CDCl_3$, 298K, 75 MHz)

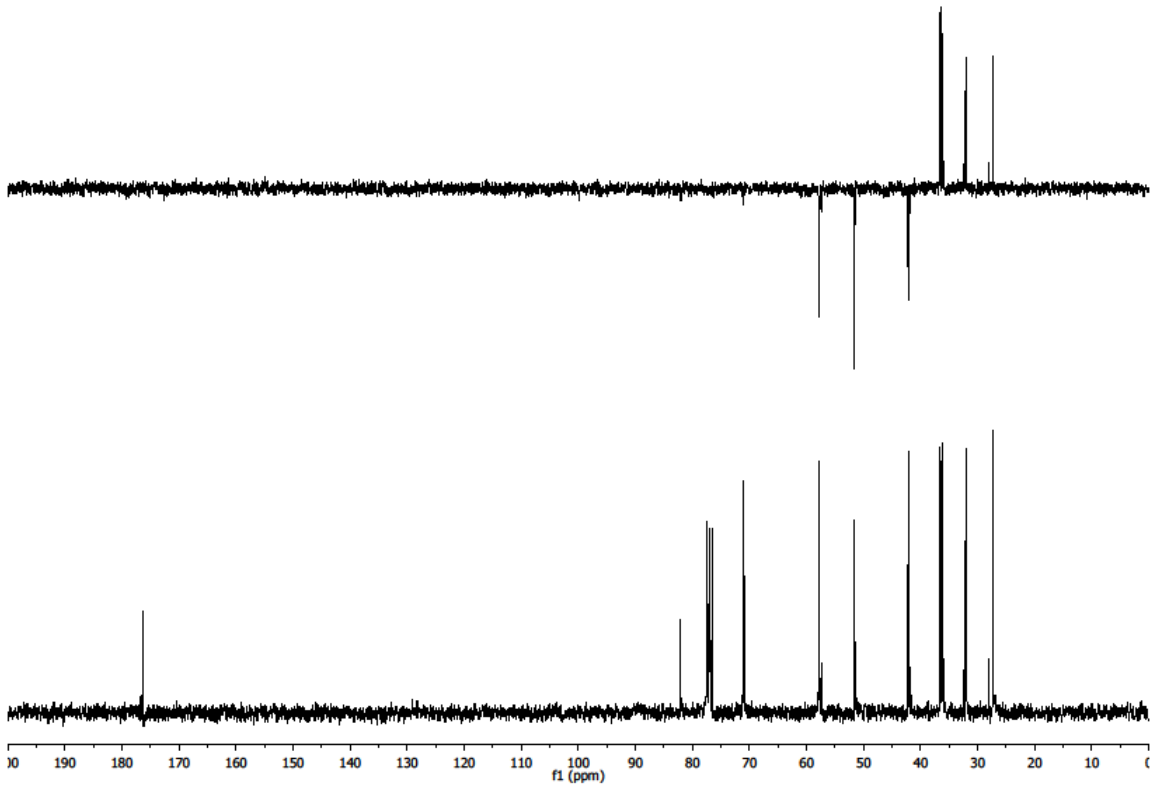


^{prg}*N*-*L*-γ-Acp-OMe

^{1}H NMR (90 mM in $CDCl_3$, 298K, 300 MHz)

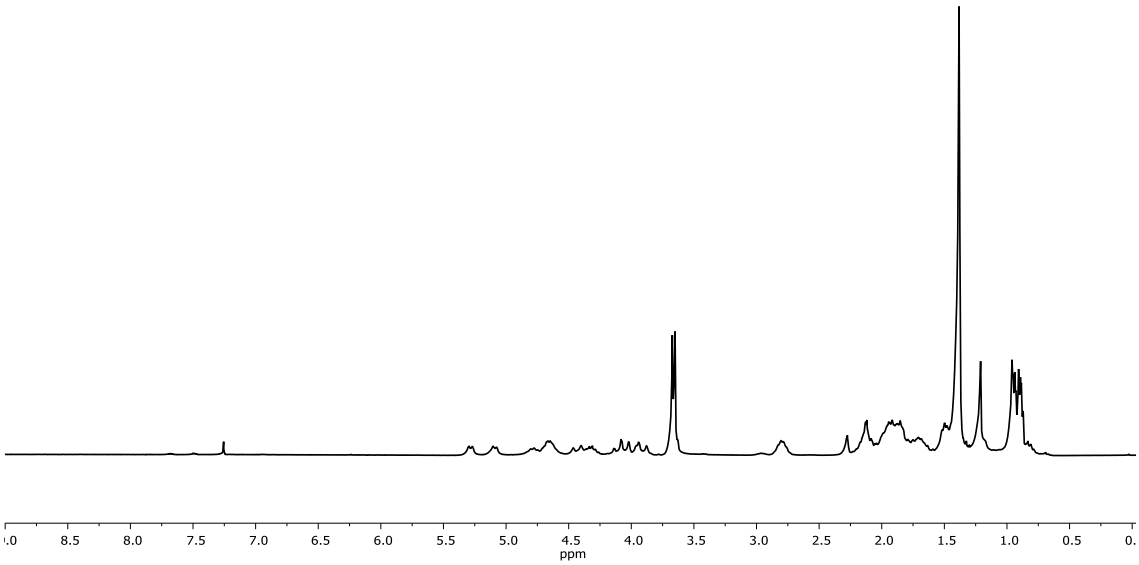


^{13}C & DEPT NMR (65 mM in $CDCl_3$, 298K, 75 MHz)

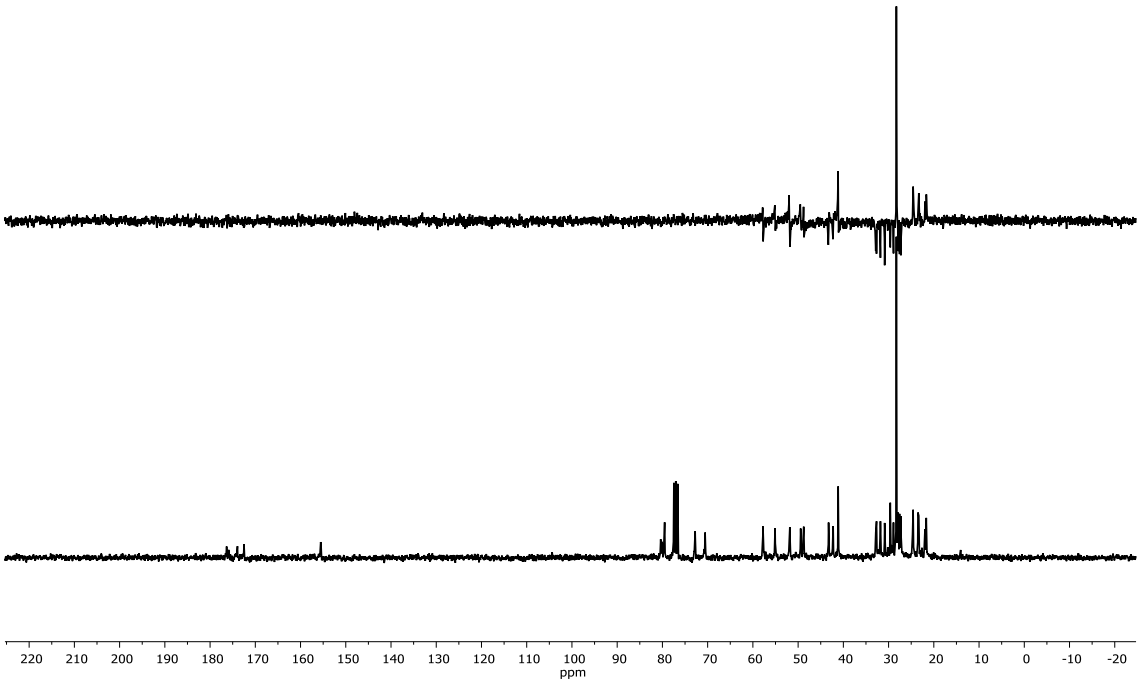


Boc-D-Leu-^{prg}*N*-*L*-γ-Acp-OMe

^{1}H NMR (80 mM in $CDCl_3$, 298K, 300 MHz)

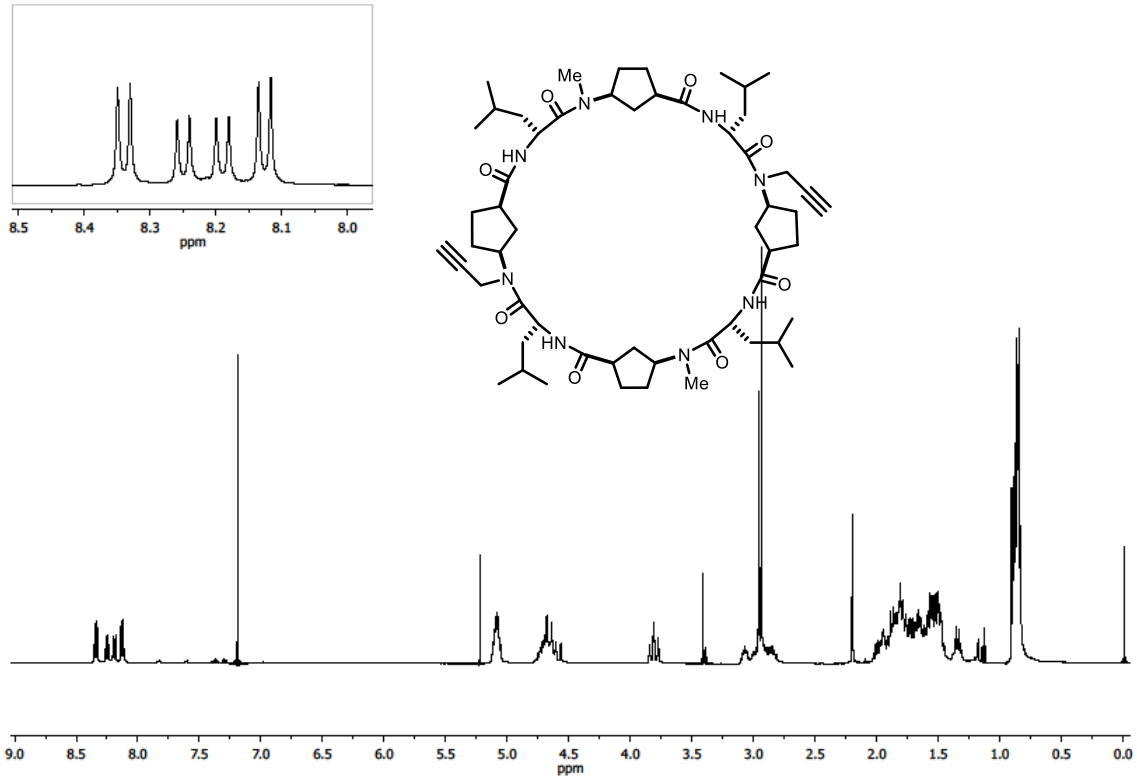


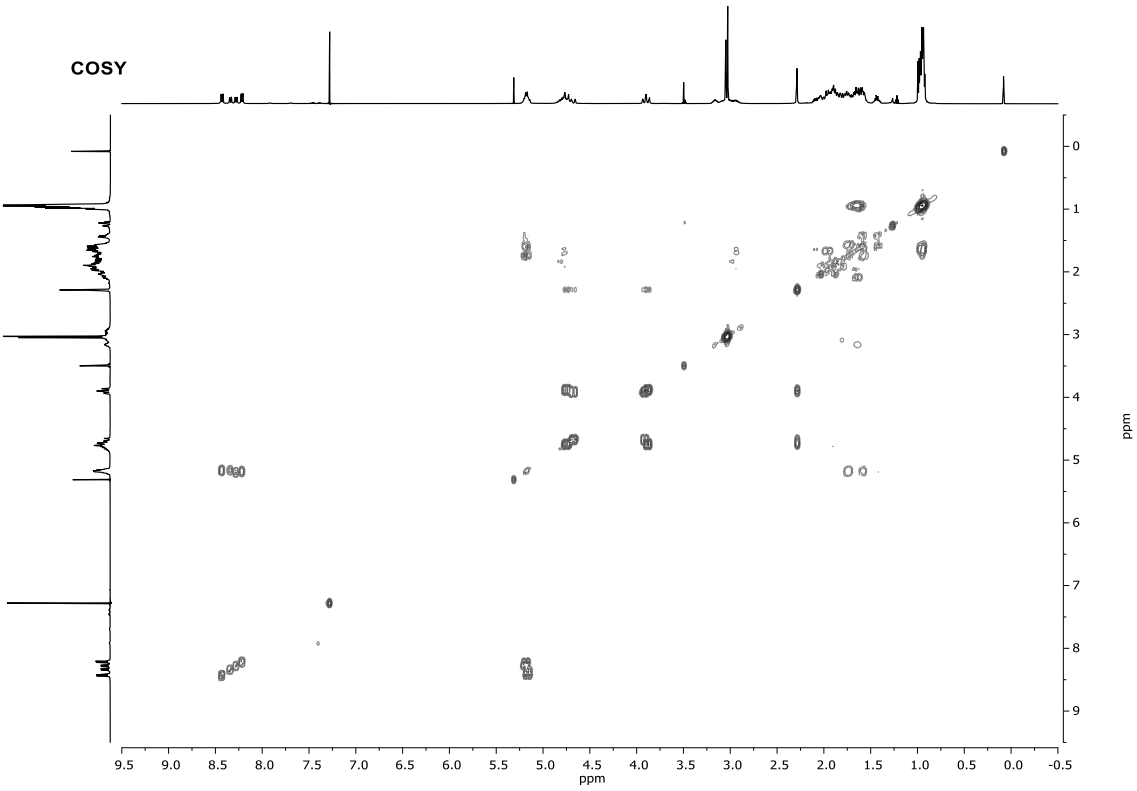
^{13}C & DEPT NMR (65 mM in $CDCl_3$, 298K, 75 MHz)

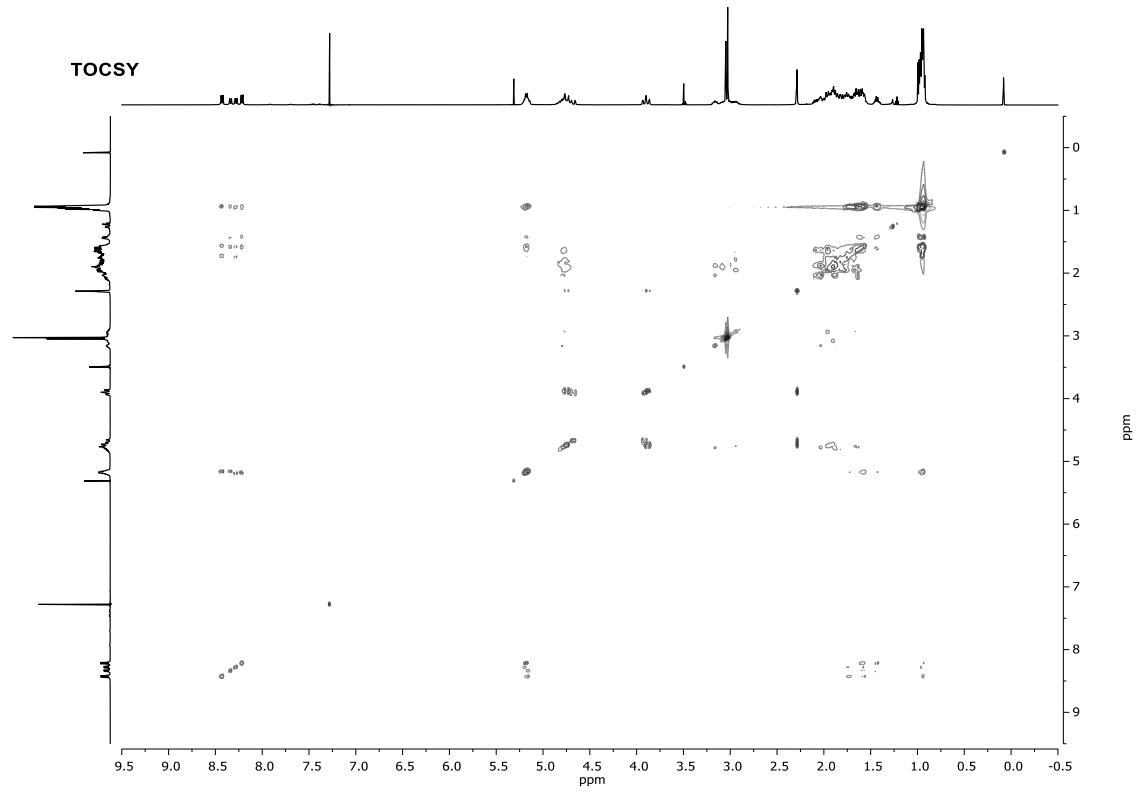


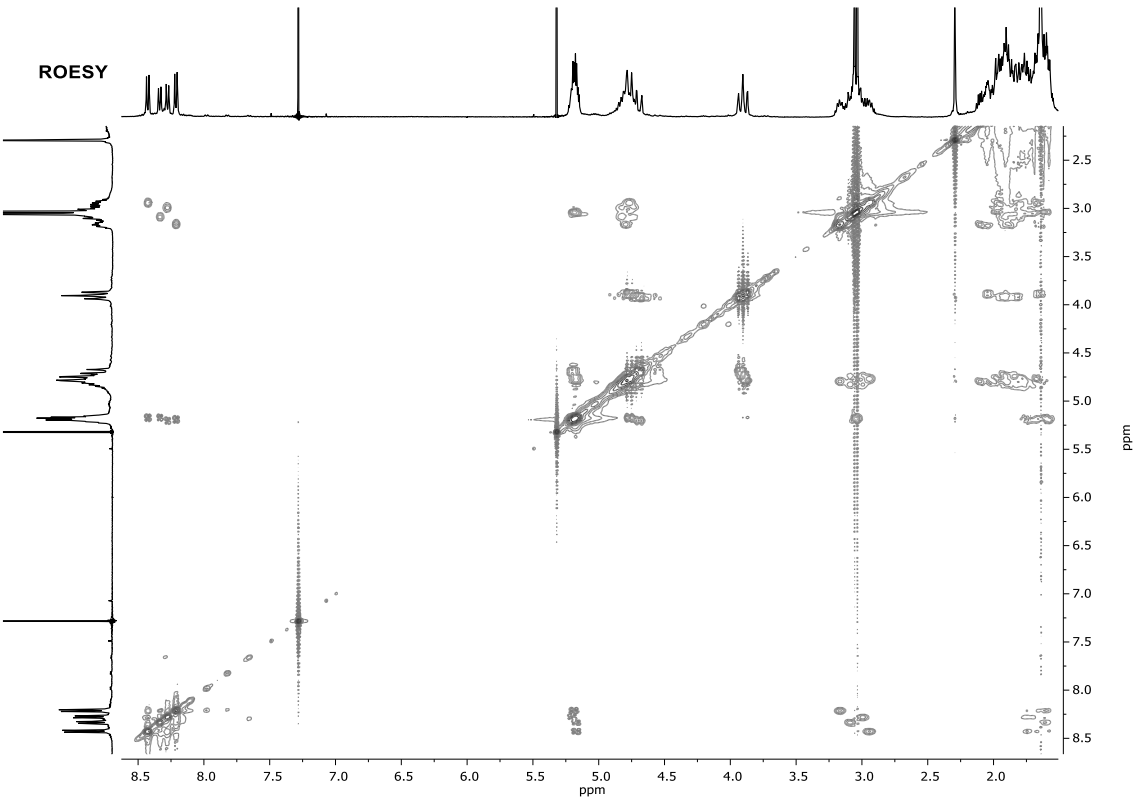
***c*-[(*D*-Leu-^{Me}*N*-*L*-γ-Acp-*D*-Leu-^{prg}*N*-*L*-γ-Acp-)$_2$] CP2**

^{1}H, COSY, TOCSY & ROESY NMR (15 mM in $CDCl_3$, 298K, 500 MHz)

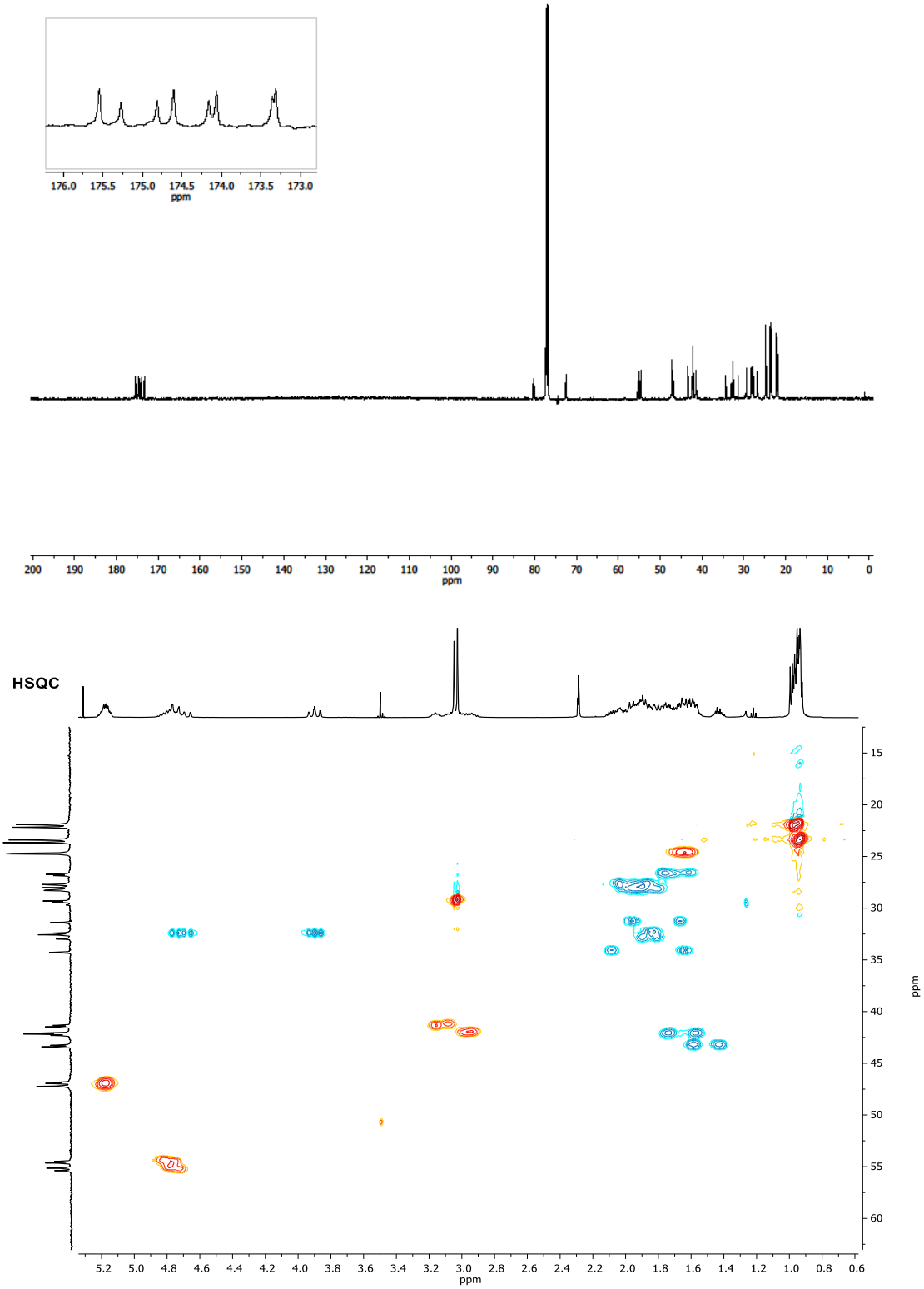




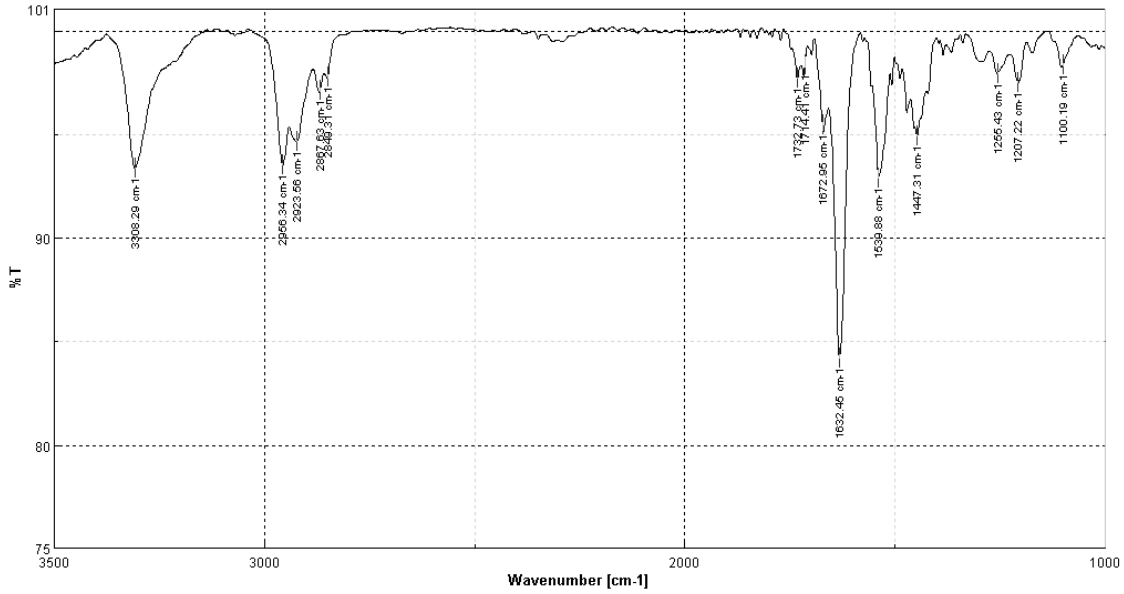

TOCSY
ppm
ppm


ROESY
ppm
ppm

^{13}C & HSQC NMR (25 mM in $CDCl_3$, 298K, 125 MHz)

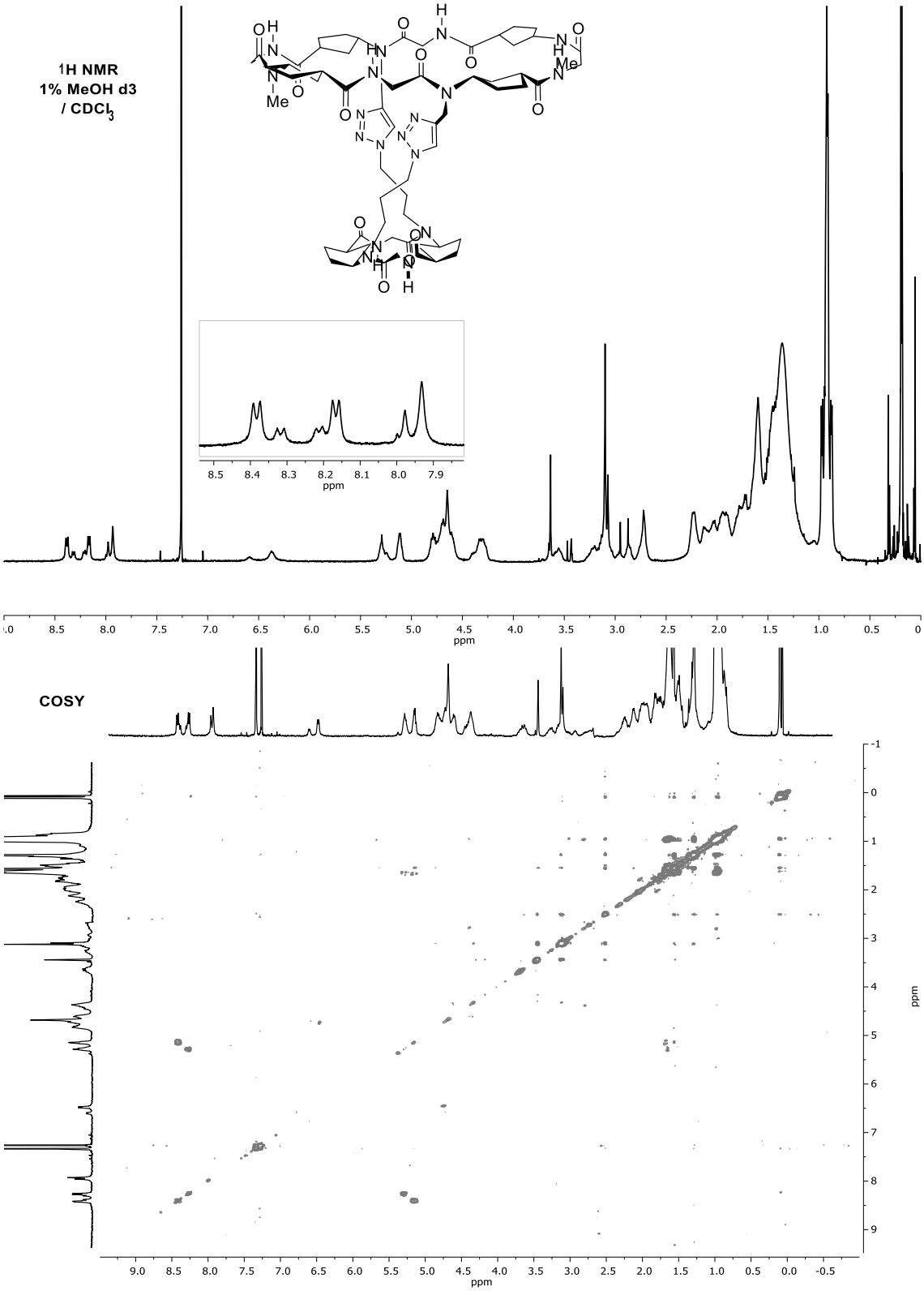


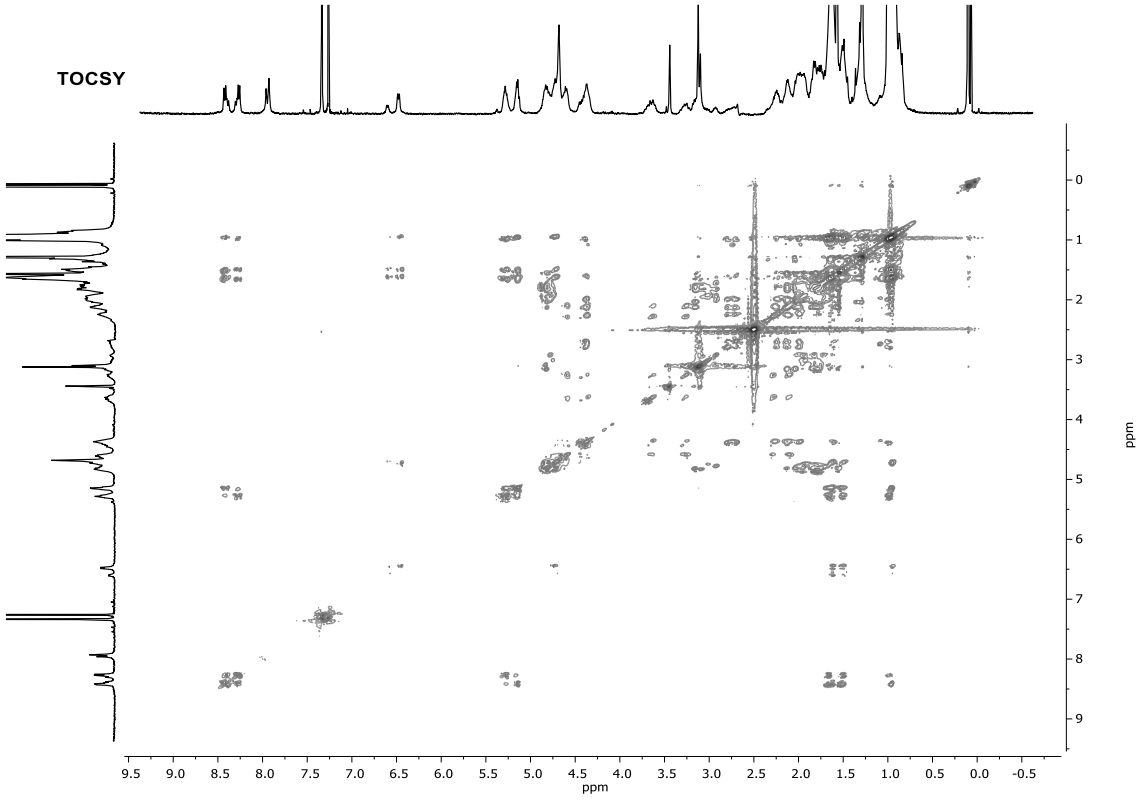
FT-IR (neat, 298 K)

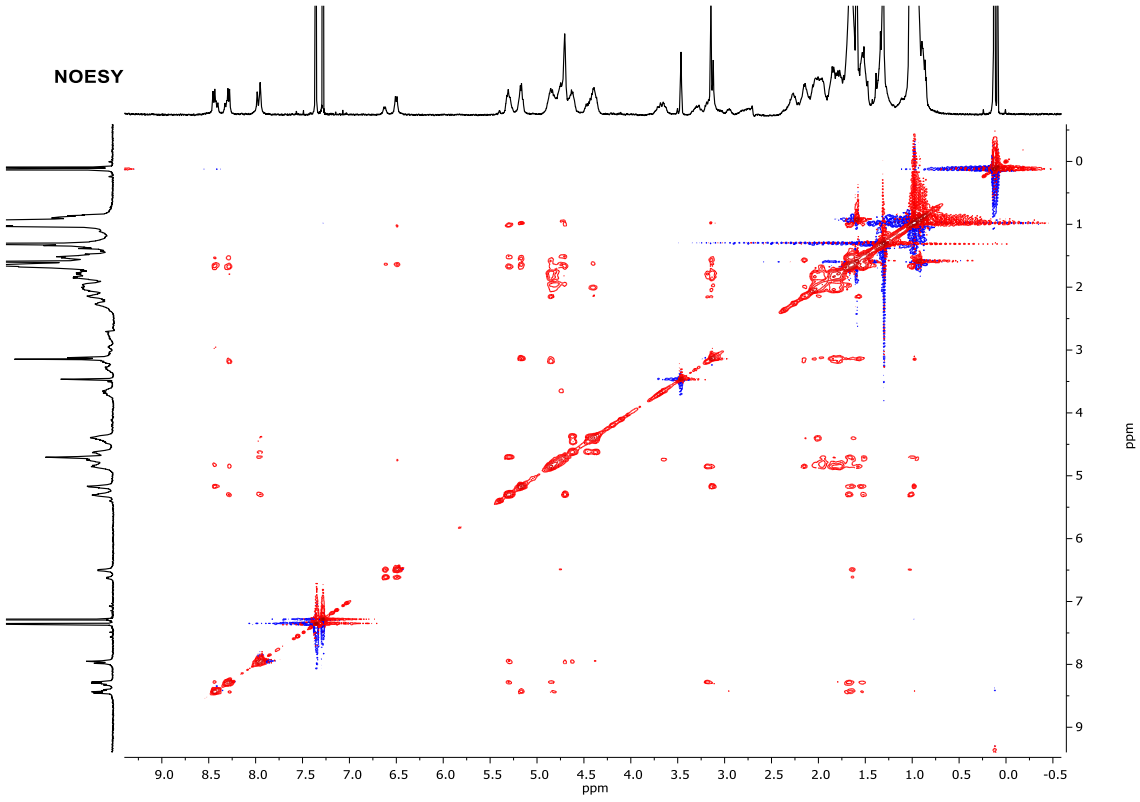


Bis-cyclic peptide CP4

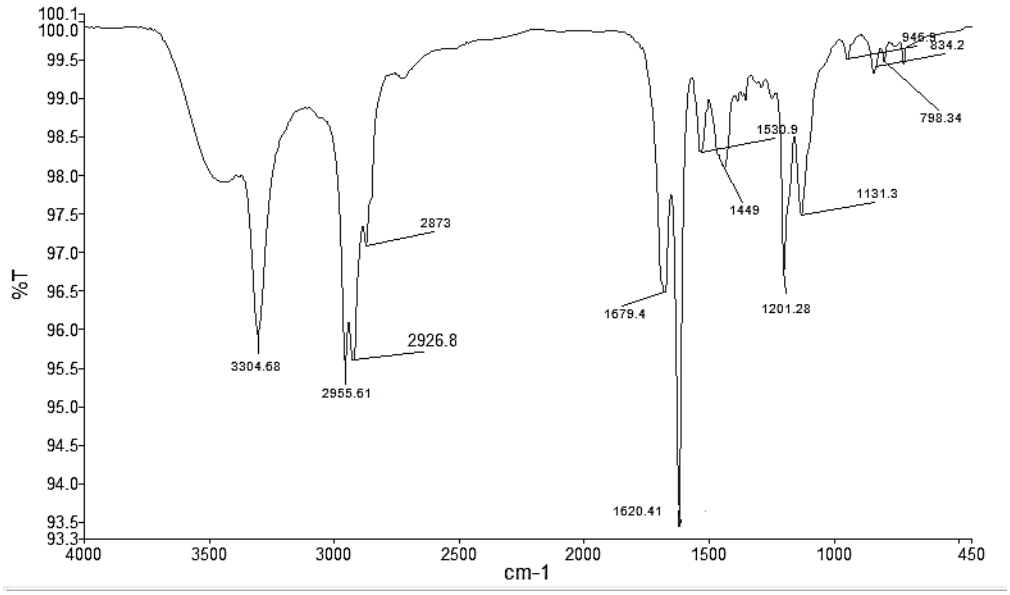
^{1}H, COSY, TOCSY & NOESY NMR (5 mM in 1% MeOH d_3/$CDCl_3$, 298K, 500 MHz)

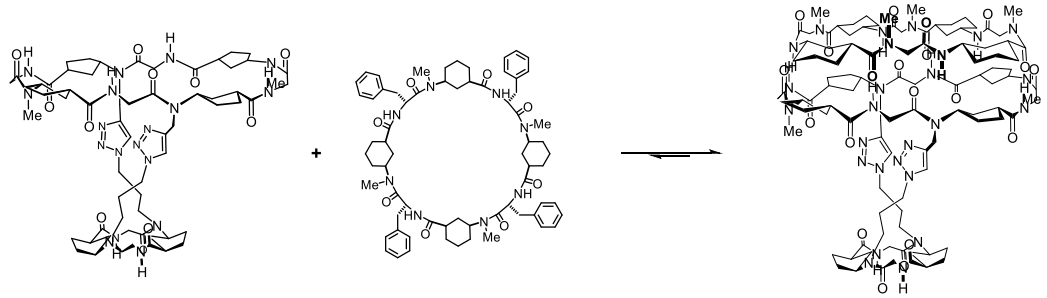


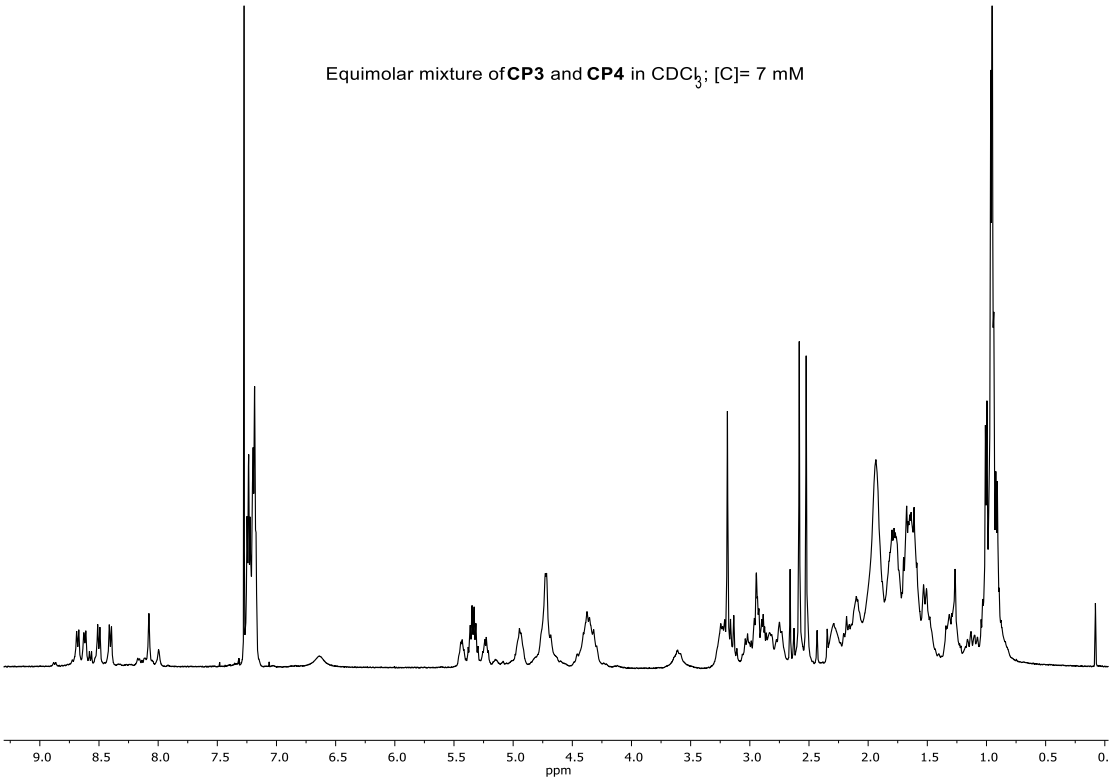

TOCSY
ppm
ppm

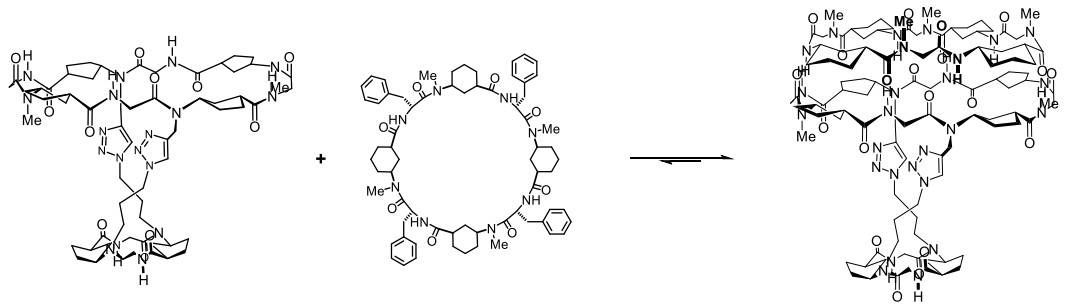

NOESY
ppm
ppm

FT-IR (CaF_2, 298 K)

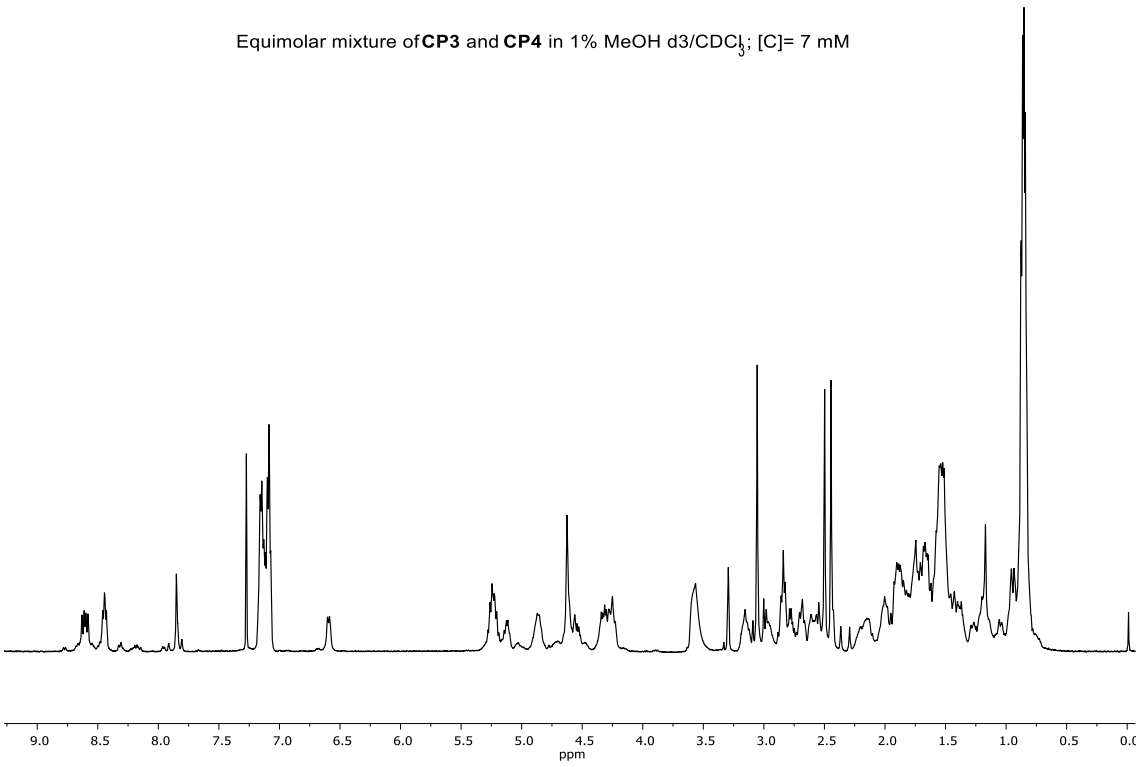


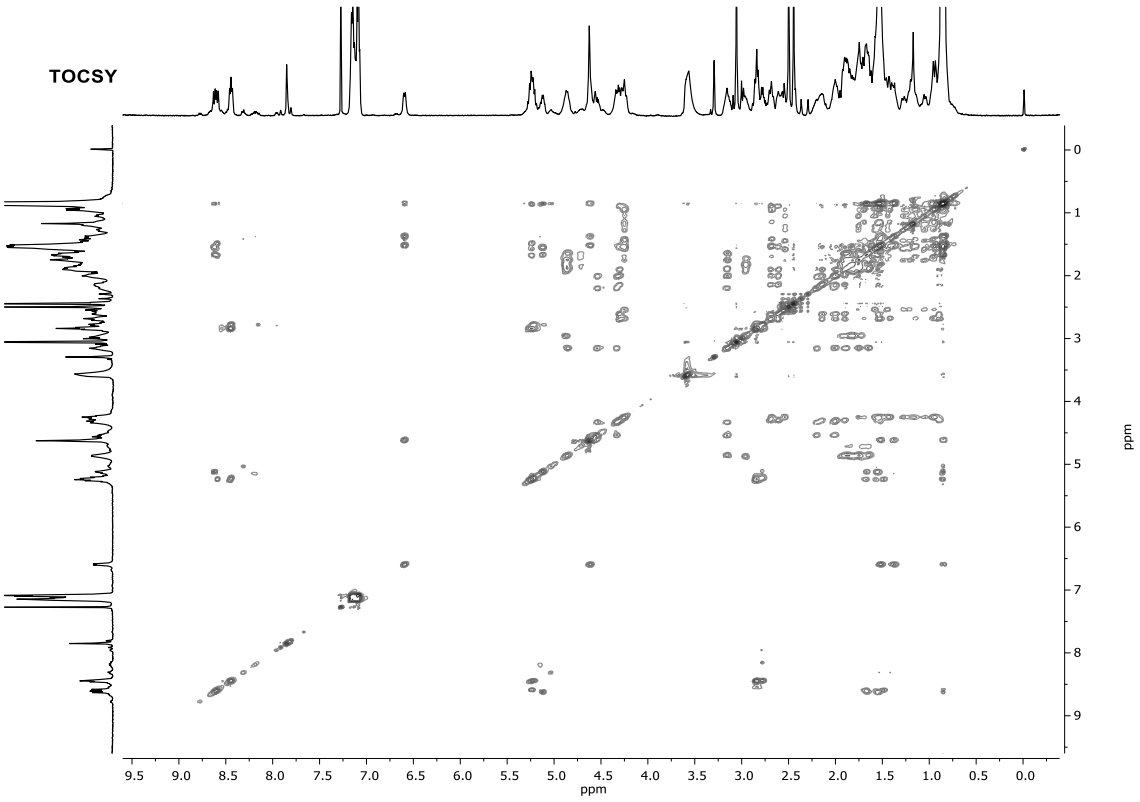


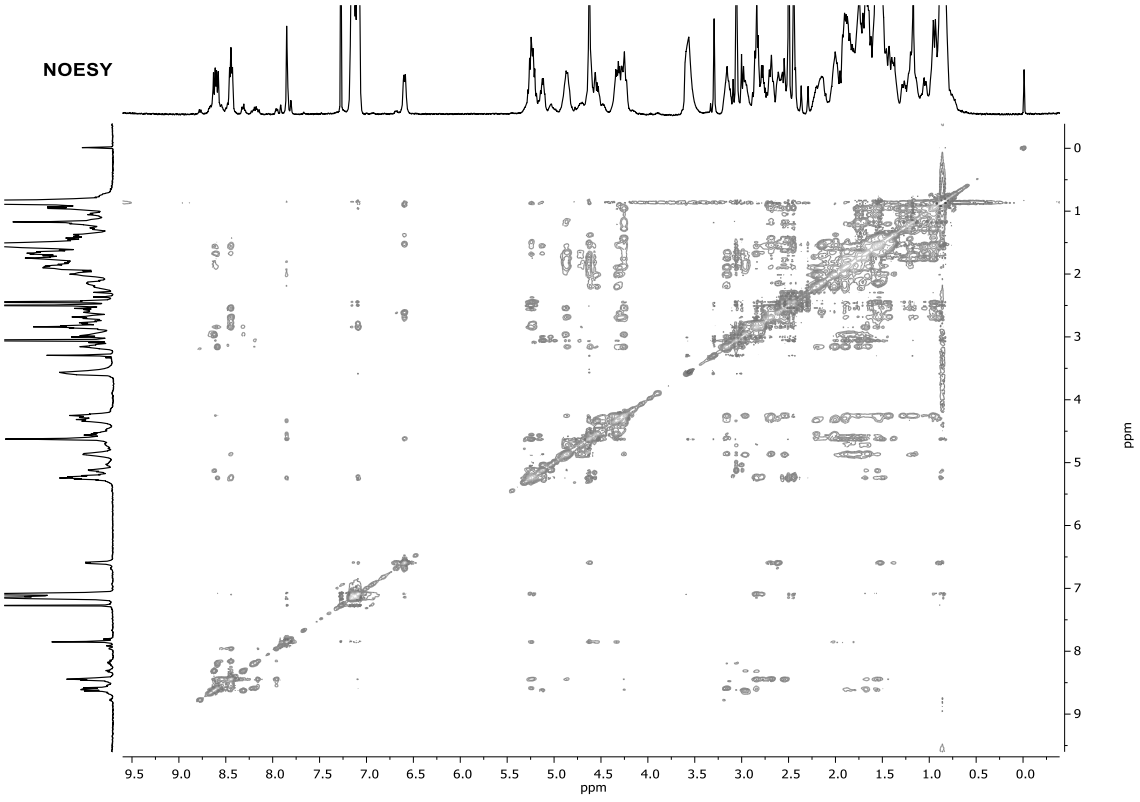

Equimolar mixture of **CP3** and **CP4** in $CDCl_3$; [C]= 7 mM
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
ppm

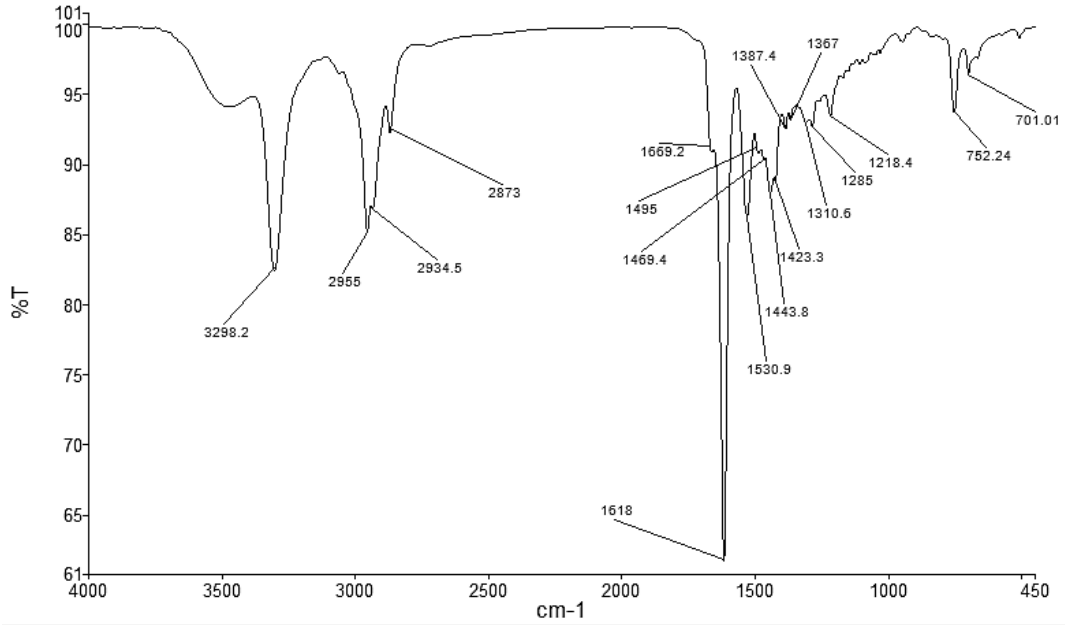


Equimolar mixture of **CP3** and **CP4** in 1% MeOH d3/$CDCl_3$; [C]= 7 mM




TOCSY
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
ppm
0 1 2 3 4 5 6 7 8 9
ppm


NOESY
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
ppm
0 1 2 3 4 5 6 7 8 9
ppm


101
100
95
90
85
80
75
70
65
61
%T
4000
3500
3000
2500
2000
1500
1000
450
cm-1
3298.2
2955
2934.5
2873
1669.2
1618
1495
1469.4
1387.4
1367
1530.9
1443.8
1423.3
1310.6
1285
1218.4
752.24
701.01