Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Phenylalanine-containing cyclic dipeptides – The lowest molecular weight hydrogelators based on unmodified proteinogenic amino acids

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1 Experimental

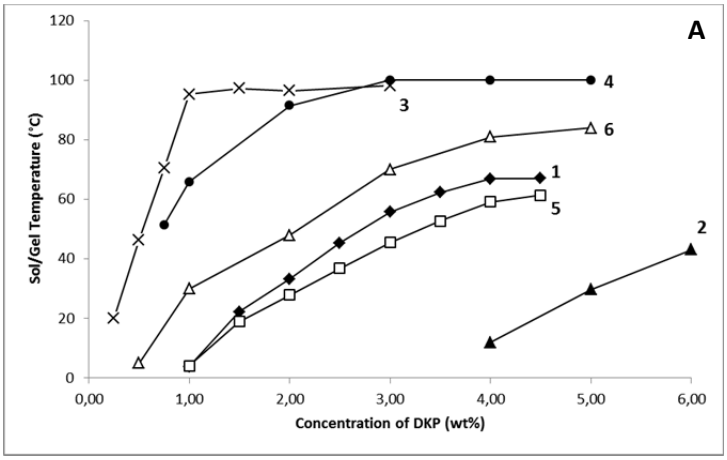
1.1 Materials

1H NMR spectra were recorded on a 400 MHz instrument. Chemical shifts for ^{1}H NMR were reported as δ (parts per million) relative to the signal of DMSO at 2.50 (q) ppm. Chemical shifts for ^{13}C NMR were reported as δ (parts per million) relative to the DMSO septuplet at 39.43 ppm. The following abbreviations were used to describe splitting patterns: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Coupling constants J are given in Hz. Mass spectra were recorded using EI or FAB method with a Finnigan MAT TSQ 70 mass spectrometer. High resolution mass spectra were recorded using ESI method with a Bruker Daltonics Apex II FT-ICR mass analyzer. Infrared spectra were obtained on a Jasco FT/IR-4100 spectrometer. Optical rotations were measured with sodium light on a Jasco P-1020 polarimeter. A Zeiss DSM 940 scanning electron microscope was used to record the images of the diketopiperazine xerogels. The xerogels were coated with a thin layer of platinum using a Balzers sputter coater SCD 050. UV-spectra for the determination of the concentrations for the drug diffusion experiments were measured with a Perkin Elmer UV/VIS Spectrometer Lambda 2. Elemental analysis was carried out on an Elementar Vario MICRO Cube analyzer. Melting points were determined with a Büchi B-540 melting point analyzer. Rheological measurements were carried out using an Anton Paar Physica MCR 501. The geometry was plate-plate with a diameter of 25 mm. All experiments were performed at 25°C and a 0.2 mm gap between the plates. Unless otherwise stated, all chemicals were used as received from a commercial supplier. Flash column chromatography was performed on silica gel (0.040 – 0.063 mm).

1.2 Determination of $T_{sol\text{-}gel}$

The sol-gel phase transition temperatures (T_{sg}) of the diketopiperazines were determined visually by the "tube-inversion method". For this method, a certain amount of the DKP and 0.5 mL of water were introduced to a closed vial with a thermometer attached. The suspension was heated until the DKP dissolved completely. The vial was cooled down by repeated immersion in an ice bath for a few seconds. The T_{sg} was recorded when a homogenous gel was formed exhibiting no gravitational flow upon inversion. Each measurement was repeated ten times and averaged to obtain the mean sol/gel transition temperature.

A



B

DKP	pH
cyclo[L-Phe-Gly] (**1**)	7.0
cyclo[L-Phe-L-Ser] (**2**)	7.0
cyclo[L-Phe-L-Cys] (**3**)	7.0
cyclo[L-Phe-L-Glu] (**4**)	4.0
cyclo[L-Phe-L-His] (**5**)	8.0
cyclo[L-Phe-L-Lys] (**6**)	10.0 - 10.5

Fig S1 A: Sol/gel transition temperatures as a function of concentration for DKPS **1-6**; **B**: pH values of saturated DKP-solutions at room temperature.

1.3 Rheometry

Hydrogel samples of **1**-**6** for rheological measurements were generated in situ by cooling their solutions on the rheometer plate from 75 °C to 4°C. After 10 minutes of cooling at 4 °C, the hydrogels were warmed up to 25 °C followed by another 10 minutes of aging (except noted otherwise). Strain sweep experiments were performed at 10 rad/s to determine the linear viscoelastic regime and the mechanical strength of the hydrogel. Frequency experiments were performed at low strain within the linear viscoelastic region (LVR) of the sample. For regeneration experiments, the samples were exposed to a deformation of 100% for thirty seconds to destroy the supramolecular network, afterwards the regeneration of G' was measured at low strain within the LVR. The time dependent increase of the mechanical strength of the gels was determined by time sweep experiments at low strain (0.05% deformation). Here the DKP solutions were cooled to 4°C for 30 seconds, so that a gel had just formed and the measurement was directly started after room temperature was reached.

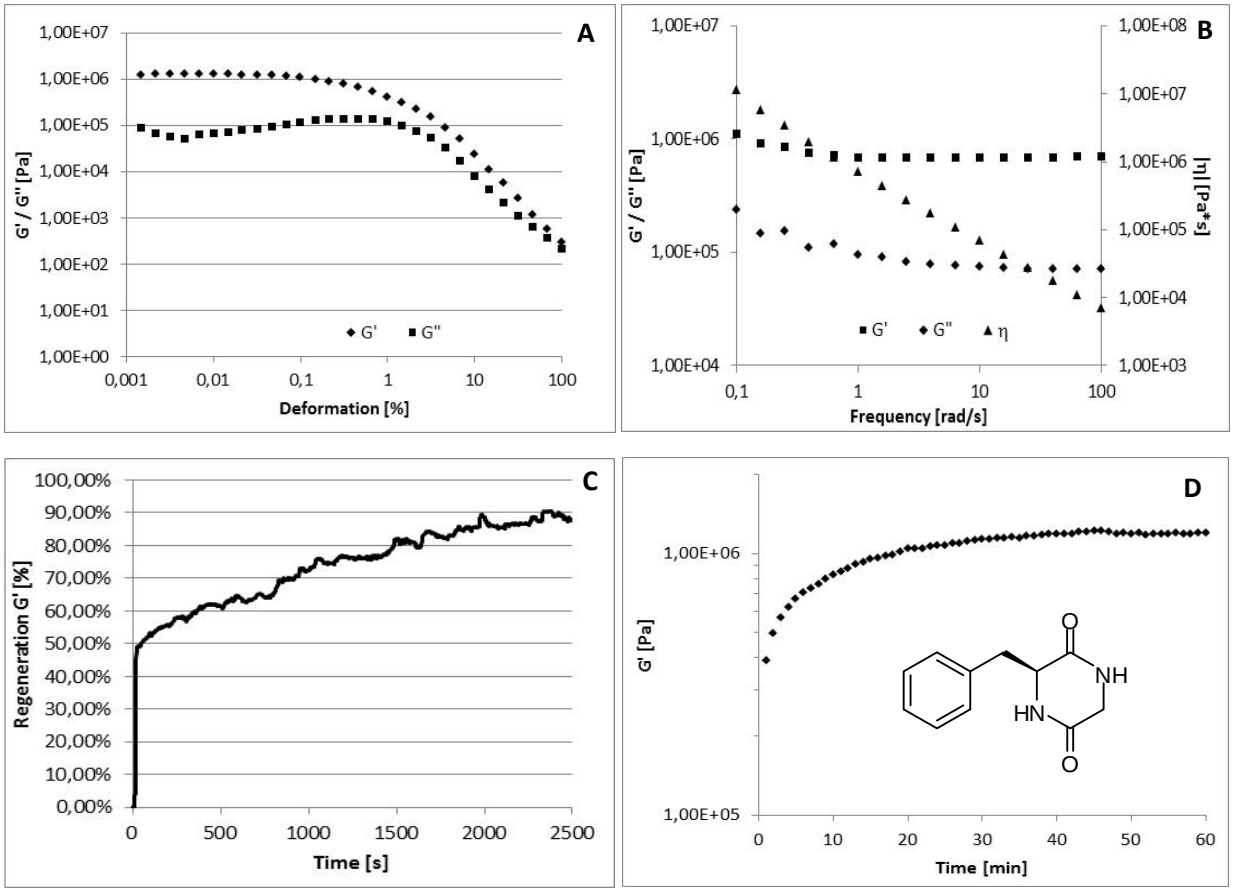


Fig S2 A: Strainsweep experiment of cyclo[L-Phe-Gly] (**1**) (1.5 wt%); **B**: Frequency sweep experiment; **C**: Regeneration of G' after gel was sheared for 30 seconds at 100% deformation; **D**: Time sweep experiment.

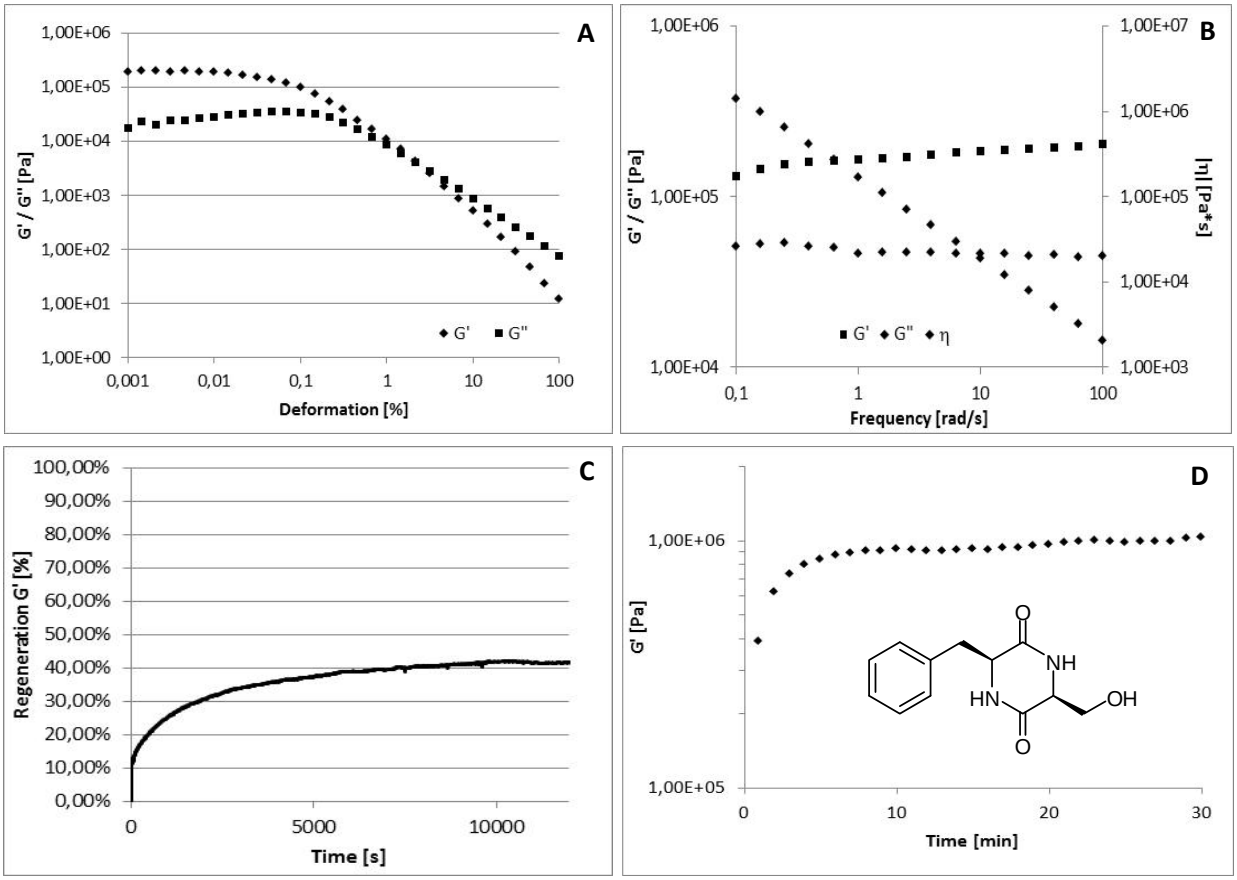


Fig S3 A: Strainsweep experiment of cyclo[L-Phe-L-Ser] (**2**) (5.0 wt%); **B**: Frequency sweep experiment; **C**: Regeneration of G' after the gel was sheared for 30 seconds at 100% deformation; **D**: Time sweep experiment.

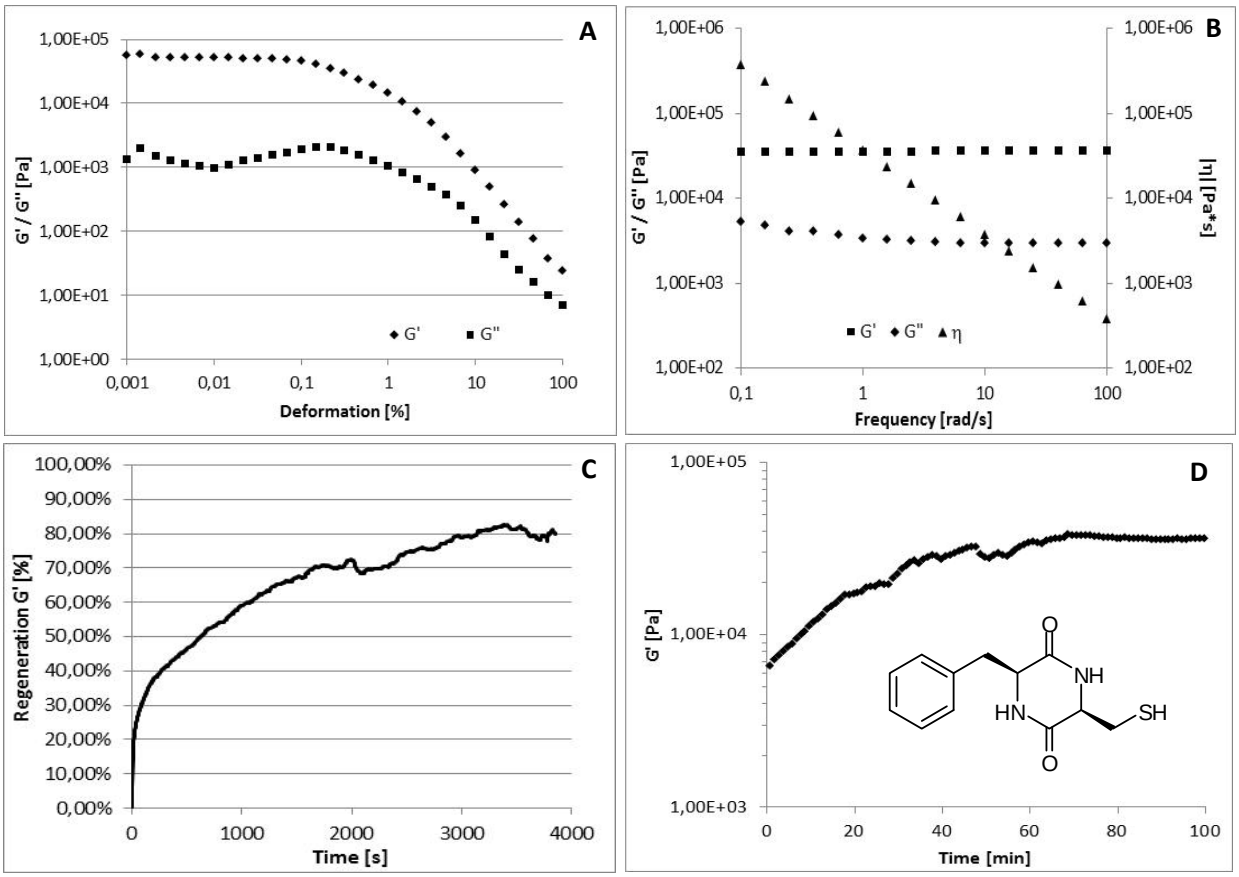


Fig S4 A: Strain sweep experiment of cyclo[L-Phe-L-Cys] (**3**) (0.5 wt%); **B**: Frequency sweep experiment; **C**: Regeneration of G' after the gel was sheared for 30 seconds at 100% deformation; **D**: Time sweep experiment.

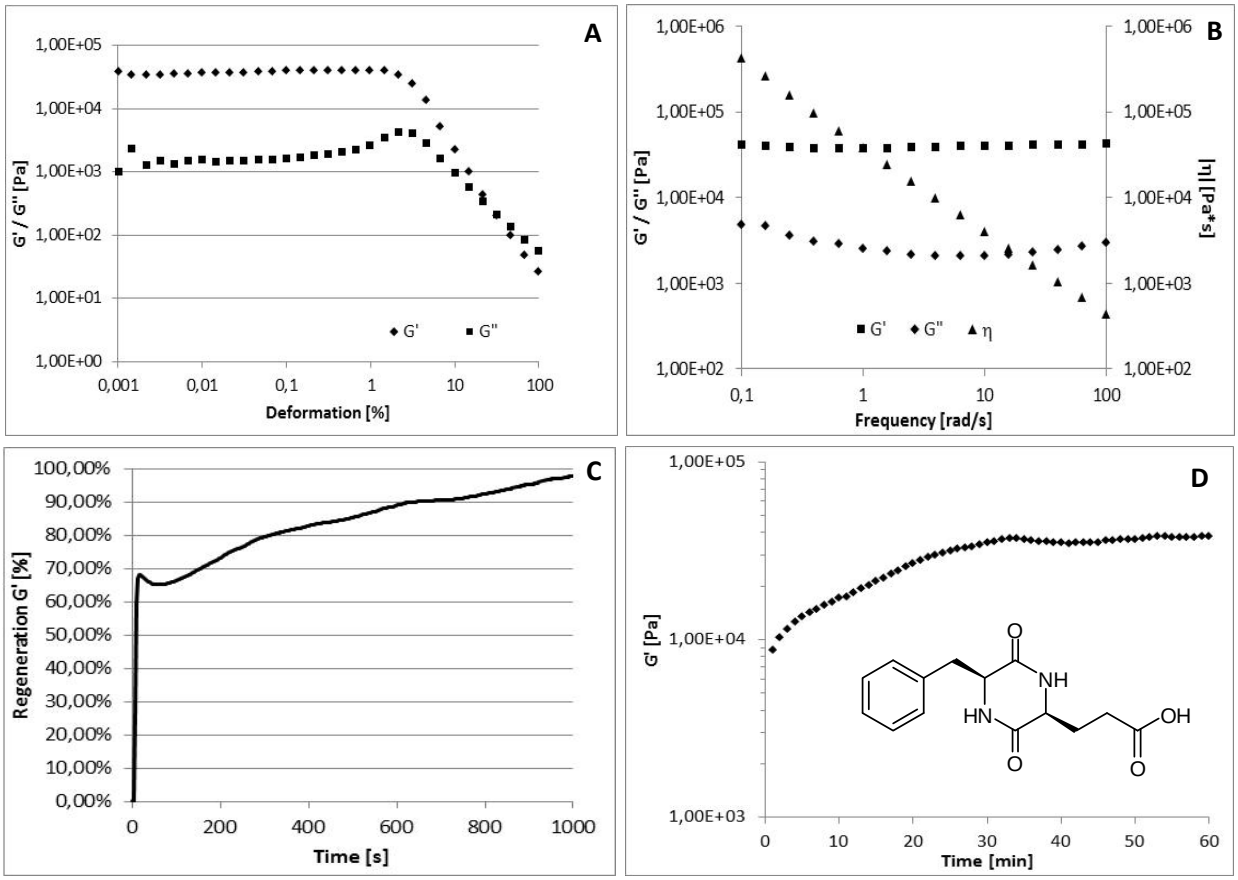


Fig S5 A: Strain sweep experiment of cyclo[L-Phe-L-Glu] (**4**) (4.0 wt%) in phosphate buffer at pH 6.0; **B**: Frequency sweep experiment; **C**: Regeneration of G' after the gel was sheared for 30 seconds at 100% deformation; **D**: Time sweep experiment.

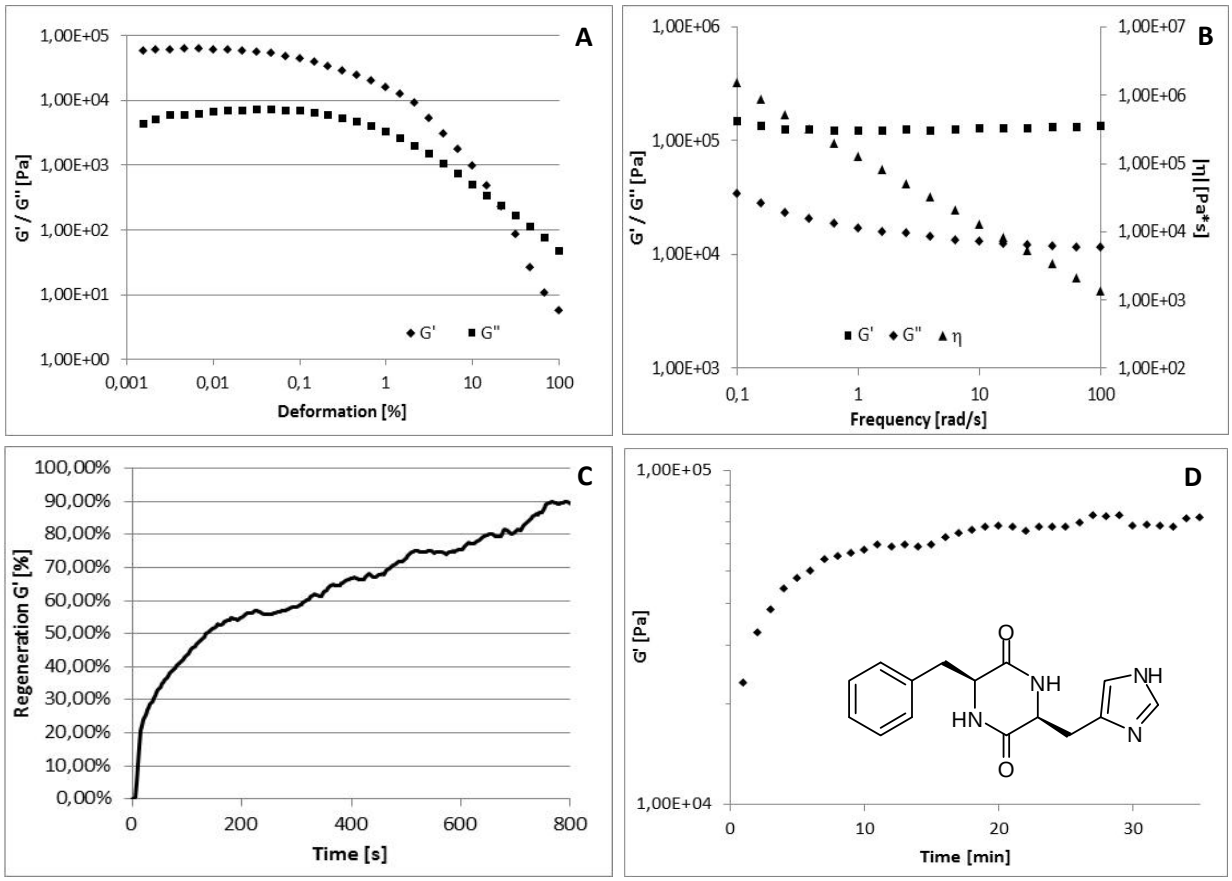


Fig S6 A: Strains weep experiment of cyclo[L-Phe-L-His] (**5**) (1.5 wt%); **B**: Frequency sweep experiment; **C**: Regeneration of G' after the gel was sheared for 30 seconds at 100% deformation; **D**: Time sweep experiment.

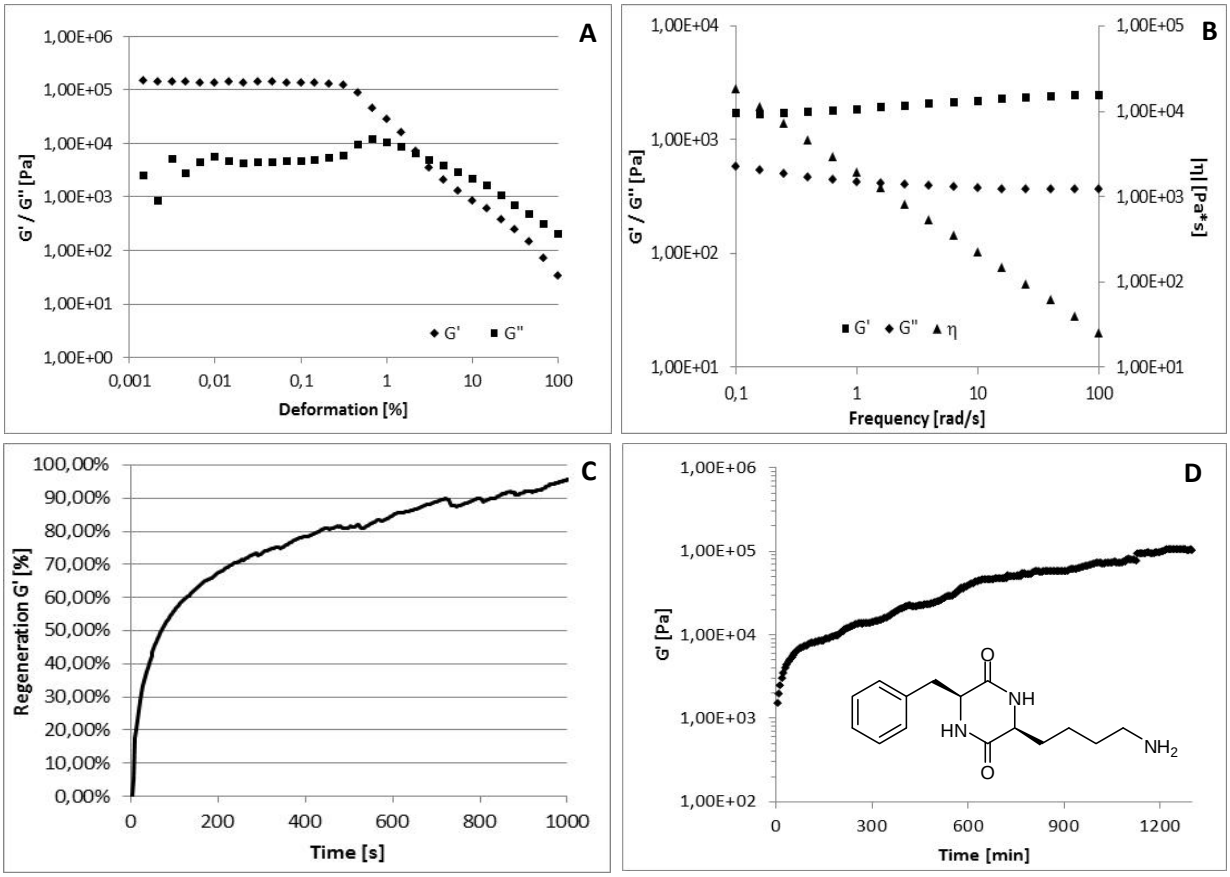


Fig S7 A: Strain sweep experiment of cyclo[L-Phe-L-Lys] (**6**) (1.5 wt%, 20 hours aged); **B**: Frequency sweep experiment (1 hour aged); **C**: Regeneration of G' after the gel was sheared for 30 seconds at 100% deformation (20 hours aged); **D**: Time sweep experiment.

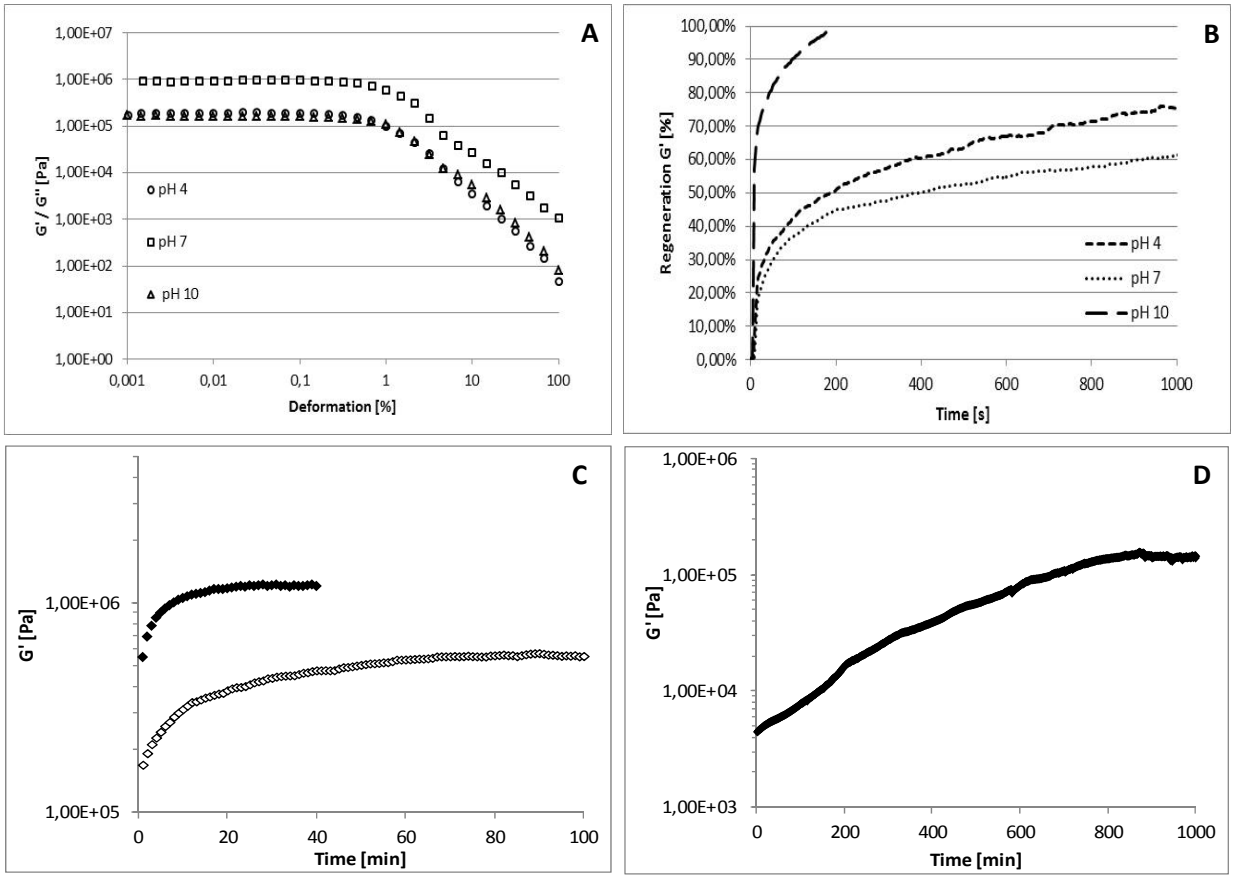


Fig S8 A: Strainsweep experiment of 1:1 mixtures (2.0 wt%) of cyclo[L-Phe-L-Glu] (**4**) and cyclo[L-Phe-L-Lys] (**6**) at pH 4, 7 and 10 (20 hours aged) in 1 M phosphate buffer; **B**: Regeneration of G' after the gels were sheared for 30 seconds at 100% deformation at different pH-values; **C**: Time sweep experiment at pH 4 (hollow diamonds) and pH 7 (filled diamonds); **D**: Time sweep experiment at pH 10.

1.4 SEM-Images of 1:1 mixtures of 4 and 6 at different pH values

1:1 mixtures of DKP **1** and **6** were dissolved in hot water and the pH was adjusted with acetic acid and triethylamine. The solutions with varying pH were dropped on aluminum sheets and cooled at 4 °C for 5 minutes. The hydrogel samples were subsequently lyophilized to generate the corresponding xerogels which were coated with a thin layer of platinum.

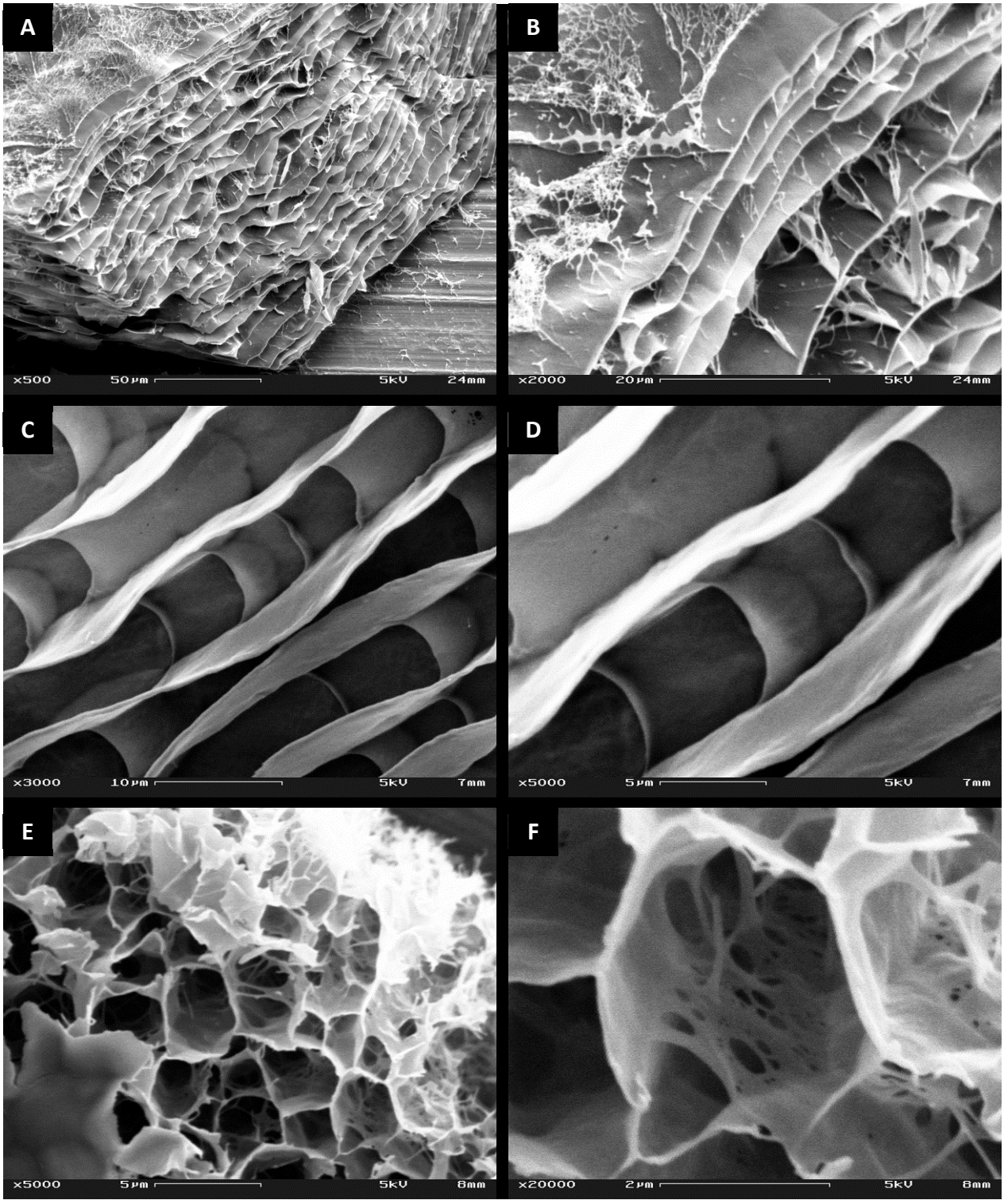


Fig S9 SEM image of 1:1 mixtures (4.0 wt%) of cyclo[L-Phe-L-Glu] (**4**) and cyclo[L-Phe-L-Lys] (**6**); **A** + **B**: pH 4.0; **C** + **D**: pH 7.0; **E** + **F**: pH 10.0.

1.5 Drug diffusion

The samples for the sustained drug diffusion experiments were prepared as follows: The DKP was dissolved in 400 µL of PBS by heating in a closed vial. The solution was cooled to 40°C and 100 µL solution of the drug molecule in PBS was added to the warm DKP-solution. The mixture was cooled to 4 °C and the corresponding drug loaded hydrogel was matured for 24 h. A volume of 500 µL PBS was added on top of the hydrogel, a sample of 400 µL was taken for each measuring point and the same volume of PBS was directly refilled. The concentration of each sample was determined by UV-Vis spectroscopy. Bovine Serum Albumin concentrations were determined at 280 nm and tetracycline concentrations were determined at 366 nm.

1.6 Synthesis

Boc-Lys(Boc)-OH was synthesized according to literature procedure by Ivanov and co-workers.[1]

Boc-His(Boc)-OH was synthesized following the procedure by Castro and co-workers.[2]

Diketopiperazines **1**-**6** were synthesized by a modification of the Boc-solid phase peptide synthesis described by D.-X. Wang and co-workers.[3] The bromoacyl-functionalized polystyrene resin **I** was synthesized by a Friedel-Crafts acylation of polystyrene beads (poly(styrene-divinylbenzene) (2% cross-linked, 200-400 mesh)).[4] Two equivalents of a N^{α}-Boc protected amino acid and 2.2 equivalents of triethylamine in DMF were added to the bromoacyl polystyrene resin and the mixture was stirred for 24 hours. The resin was washed extensively with DMF (×3), 95% EtOH (×5), EtOAc (×2) and DCM (×1). Boc-deprotection was performed by suspending resin **II** in 3.5 N hydrogen chloride in ethyl acetate for 30 minutes and washing with EtOAc (x5). A threefold excess of the second amino acid, HBTU (2.85 eq.) and DIPEA (4.5 eq.) in DMF were stirred for 2 minutes, added to resin and the reaction mixture was stirred for 4-6 hours. Resin **III** was filtered, washed with DMF (x3), MeOH (x5) and DCM (x2) and deprotected with 3.5 N hydrogen chloride in ethyl acetate for 30 minutes. Neutralization was performed with 10% DIPEA in EtOAc/EtOH (1 : 1) (5 min) followed by washing with 95% EtOH (3 x 1 min). After filtration, resin **IV** was mixed with 5% Et_3N in THF/H_2O (8:1) for 24 hours. The solution was filtered off and concentrated under reduced pressure, yielding the corresponding DKP **1**-**6**.

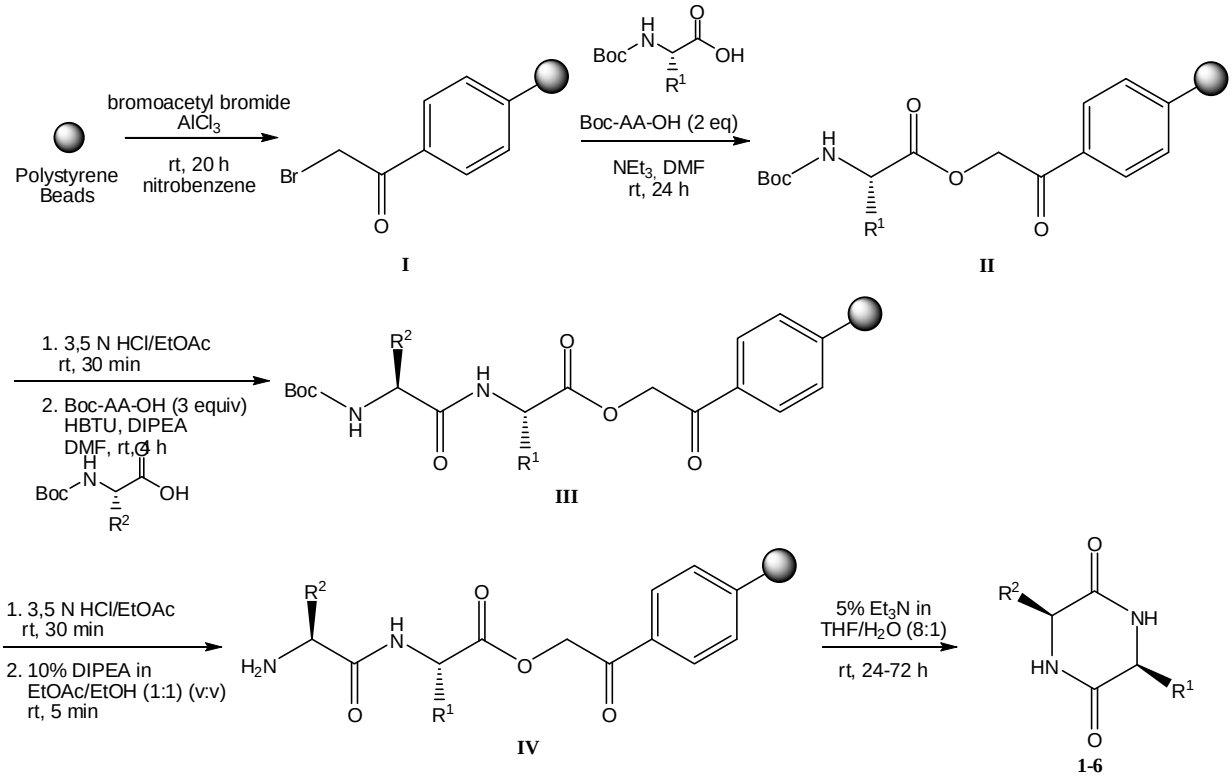
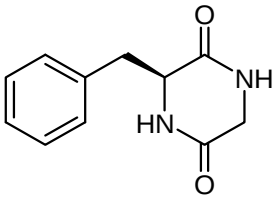


Fig S12 Solid phase synthesis of DKPs **1**-**6**.

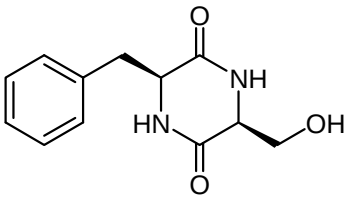
cyclo[L-Phe-L-Gly] (**1**)



Compound **1** was synthesized according to the general procedure using Boc-Phe-OH (4.62 g, 17.4 mmol, 2 eq.) and Boc-Gly-OH (4.57 g, 26.1 mmol, 3 eq.). The crude product was recrystallized from water to yield a white solid (930 mg, 4.55 mmol, 52%).

1H NMR (400 MHz, DMSO-d_6, ppm): δ 8.16 (1H, s (br)), 7.89 (1H, s (br)), 7.31-7.14 (m, 5H), 4.06 (s (br), 1H), 3.35 (d, *J* = 17.3 Hz, 1H), 3.10 (dd, *J* = 4.3 Hz, 13.3 Hz, 1H), 2.88 (dd, *J* = 3.7 Hz, 13.3 Hz, 1H), 2.76 (d, *J* = 17.3 Hz, 1H). ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 167.0, 165.6; 135.9, 130.0 (2x), 128.0 (2x), 126.7, 55.4, 43.6, 38.7. EI-MS calculated for $C_{11}H_{12}N_2O_2$ $[M]^+$: m/z 204.1, found: 204.1. FT-IR (cm^{-1}): 3189.7 (m), 3047.9 (m), 2981.4 (m), 2924.5 (m), 2883.1 (m), 1666.2 (vs), 1461.8 (s), 1335.5 (s), 1088.6 (m), 1004.7 (m), 859.1 (s), 841.8 (s), 795.5 (s), 755.0 (s), 702.0 (vs). Anal. Calcd. for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72; found: C, 64.47; H, 5.72; N, 13.79. $[a]_D^{20}$ = + 53.3 (*c*=0.95, DMSO) {+ 26.6 (c=0.95, DMSO)[5]}. Mp 269-271 °C (decomp.) {266-268 °C[5].

cyclo[L-Phe-L-Ser] (**2**)



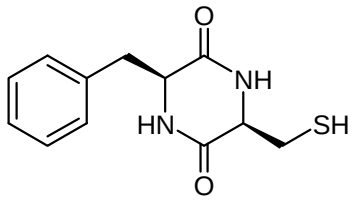
Cyclo[L-Phe-L-Ser(OBzl)] was synthesized according to the general procedure using Boc-Phe-OH (10.61 g, 40.0 mmol, 2 eq.) and Boc-Ser(OBzl)-OH (17.72 g, 60.0 mmol, 3 eq.). The crude product was recrystallized from methanol and dried under reduced pressure. A white solid was received (3.75 g, 11.56 mmol, 58%).

1H NMR (400 MHz, DMSO-d_6, ppm): δ 8.10 (s (br), 2H), 7.37-7.06 (m, 10H), 4.32 (s, 2H), 4.12-4.08 (m, 1H), 3.86-3.82 (m, 1H), 3.28 (dd, *J* = 3.0 Hz, 9.5 Hz, 1H), 3.05 (dd, *J* = 5.7 Hz, 13.6 Hz), 2.91 (dd, *J* = 4.9 Hz, 13.6 Hz), 2.66 (dd, *J* = 6.3 Hz, 9.5 Hz, 1H). 13C NMR (100 MHz, DMSO-d_6, ppm): δ 166.3, 164.9, 137.8, 136.4, 129.9 (2x), 128.1 (2x), 128.0 (2x), 127.5 (2x), 127.4, 126.4, 72.1, 71.4, 55.3, 54.9, 39.3. FAB-MS calculated for $C_{19}H_{21}N_2O_3$ $[M+H]^+$: m/z 325.1, found: 325.1.

Cyclo[L-Phe-L-Ser(OBzl)] was deprotected with trimethylsilyl iodide according to literature procedure.[6] Trimethylsilyl chloride (3.26 g, 16.3 mmol) was added neat to a suspension of cyclo[L-Phe-L-Ser(OBzl)] (3.77 g, 11.62 mmol) in dry dichloromethane. The reaction mixture was stirred over night and an excess of methanol was added. The volatile components were removed at reduced pressure. The crude product was purified by flash chromatography (DCM : MeOH = 9 : 1 + 0.5% Et_3N) and compound **2** was received as a white solid (2.33 g, 9.94 mmol, 86%).

1H NMR (400 MHz, DMSO-d_6, ppm): δ 8.00 (s (br), 1H), 7.89 (s (br), 1H), 7.31-7.15 (m, 5H), 4.86 (t, *J* = 5.7 Hz, 1H), 4.08-4.03 (m, 1H), 3.68-3.63 (m, 1H), 3.32-3.26 (m, 1H), 3.10 (dd, *J* = 6.1 Hz, 13.5 Hz, 1H), 2.98 (dd, *J* = 4.9 Hz, 13.5 Hz), 2.89-2.82 (m, 1H). 13C NMR (100 MHz, DMSO-d_6, ppm): δ 166.4, 165.6, 136.6, 129.9 (2x), 128.0 (2x), 126.4, 63.0, 57.0, 55.4, 39.8. FAB-MS calculated for $C_{12}H_{15}N_2O_3$ $[M+H]^+$: m/z 235.1, found: 235.2. FT-IR (cm^{-1}): 3319.9, 3189.7, 3083.6, 2964.1, 2920.7, 2878.2, 1665.2, 1454.1, 1368.3, 1334.5, 1066.4, 1037.5, 821.5, 756.0, 697.1, 626.8. Anal. Calcd for $C_{12}H_{14}N_2O_3$: C, 61.53; H, 6.02; N, 11.96; found: C, 61.70; H, 5.40; N, 12.11. $[a]_D^{20}$ = - 130.4 (c=0.36, MeOH) {- 124.6 (c 0.36 MeOH)[7]}. Mp 237-239 °C (decomp.).

cyclo[L-Phe-L-Cys] (**3**)



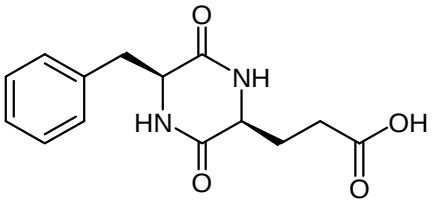
Cyclo[L-Phe-L-Cys(4-MeOBzl)] was synthesized according to the general procedure using Boc-Phe-OH (10.61 g, 40.0 mmol, 2 eq.) and Boc-Cys(4-MeOBzl)-OH (20.49 g, 60.0 mmol, 3 eq.). The crude product was recrystallized from water/ethanol and dried under reduced pressure. Cyclo[L-Phe-L-Cys(4-MeOBzl)] was received as a white solid (3.05 g, 8.2 mmol, 41%).

1H NMR (400 MHz, DMSO-d_6, ppm): δ 8.20 (s (br), 1H), 8.01 (s (br), 1H), 7.29-7.14 (m, 7H), 6.85 (d, *J* = 8.6 Hz, 2H), 4.21-4.17 (m, 1H), 3.85-3.80 (m, 1H), 3.73 (s, 3H), 3.51 (s, 2H), 3.14 (dd, *J* = 4.7 Hz, 13.6 Hz, 1H), 2.94 (dd, *J* = 4.9 Hz, 13.6 Hz), 2.31 (dd, *J* = 3.9 Hz, 13.7 Hz, 1H), 1.52 (dd, *J* = 7.4 Hz, 13. 7 Hz). 13C NMR (100 MHz, DMSO-d_6, ppm): 166.1, 165.8, 158.0, 136.2, 130.1 (2x), 130.0, 129.9 (2x), 128.0 (2x), 126.5, 113.6 (2x), 55.3, 54.9, 53.6, 35.1, 34.6. FAB-MS calculated for $C_{20}H_{23}N_2O_3S$ $[M+H]^+$: m/z 371.1, found 371.2.

The product was dissolved in a mixture of TFA/H_2O/phenol (90 : 5 : 5) and refluxed for one hour. The reaction mixture was cooled to 4°C and diethylether was added. The precipitate was filtered, washed three times with diethylether and finally triturated in water. Compound **3** was received as a white solid (1.77 g, 7.08 mmol, 86%).

1H NMR (400 MHz, DMSO-d_6, ppm): δ 8.21 (s (br), 1H), 8.04 (s (br), 1H), 7.29-7.16 (m, 5H), 4.23-4.19 (m, 1H), 3.92-3.88 (m, 1H), 3.15 (dd, *J* = 4.3 Hz, 13.6 Hz, 1H), 2.92 (dd, *J* = 5.0 Hz, 13.6 Hz, 1H), 2.36-2.29 (m, 1H), 2.01-1.93 (m, 1H), 1.74 (t, *J* = 8.5 Hz). 13C NMR (100 MHz, DMSO-d_6, ppm): δ 166.4, 165.4, 136.2, 130.2 (2x), 128.0 (2x), 126.5, 55.8, 55.1, 38.1, 27.3. HRMS (ESI) calculated for $C_{12}H_{14}N_2O_2SNa$ $[M+Na]^+$: m/z 273.066819, found: 273.066946. FT-IR (cm^{-1}): 3182.0, 3084.6, 3045.1, 2965.0, 2926.5, 2882.1, 1660.4, 1450.2, 1334.5, 1239.0, 1187.0, 1097.3, 846.6, 810.9, 762.7, 747.3, 701.0. $[a]_D^{22}$ = - 54.3 (*c*=1.0, DMSO). Mp 246-248 °C (decomp.).

Cyclo[L-Phe-L-Glu] (**4**)



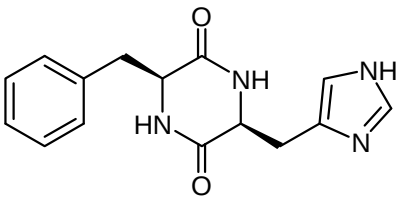
Cyclo[L-Phe-L-Glu(OBzl)] was synthesized according to the general procedure using Boc-Phe-OH (6.82 g, 25.70 mmol, 2 eq.) and Boc-Glu(OBzl)-OH (13.01 g, 38.55 mmol, 3 eq.). The crude product was triturated in water, recrystallized from methanol and dried under reduced pressure. Cyclo[L-Phe-L-Glu(OBzl)] was received as a white solid (1.68 g, 4.75 mmol, 36%).

1H NMR (400 MHz, DMSO-d_6, ppm): δ 8.24 (s (br), 1H), 8.09 (s (br), 1H), 7.44-7.00 (m, 10H), 5.02 (s, 2H), 4.22-4.17 (m, 1H), 3.72-3.67 (m, 1H), 3.15 (d, *J* =2.4 Hz, 13.3 Hz, 1H), 2.82 (dd, *J* = 4.4 Hz, 13.3 Hz, 1H), 1.76-1.66 (m, 2H), 1.32-1.21 (m, 1H), 1.13-1.02 (m, 1H). 13C NMR (100 MHz, DMSO-d_6, ppm): δ 171.9, 166.3, 166.1, 136.2, 135.8, 130.2 (2x), 128.3 (2x), 127.9 (3x), 127.8 (2x), 126.7, 65.2, 55.2, 52.8, 37.9, 28.3, 28.2. EI-MS calculated for $C_{14}H_{15}N_2O_4$ $[M]^+$: m/z 366.2, found 366.2.

Cyclo[L-Phe-L-Glu(OBzl)] (1.40 g, 3.83 mmol) was suspended in 50 mL methanol and Pd/C (10%, 150 mg) was added. The reaction mixture was stirred for 1.5 hours under hydrogen atmosphere, filtered through Celite® and the solvent was removed under reduced pressure. The raw product was further recrystallized from methanol and compound **4** was received as a white solid (950 mg, 3.44 mmol, 91%).

1H NMR (400 MHz, DMSO-d_6, ppm): δ 11.92 (s (br), 1H), 8.19 (s (br), 1H), 8.08 (s (br), 1H), 7.29-7.14 (m, 5H), 4.21-4.16 (m, 1H), 3.71-3.66 (m, 1H), 3.13 (dd, *J* = 3.8 Hz, 13.4 Hz, 1H), 2.85 (dd, *J* = 4.9 Hz, 13.4 Hz, 1H), 1.70-1.63 (m, 1H), 1.31-1.21 (m, 1H), 1.11-1.01 (m, 1H). 13C NMR (100 MHz, DMSO-d_6, ppm): δ 173.8, 166.5, 166.2, 135.9, 130.2 (2x), 127.9 (2x), 126.7, 55.2, 52.9, 37.9, 28.5. HRMS (ESI) calculated for $C_{14}H_{16}N_2O_4Na$ $[M+Na]^+$: m/z 299.100228, found: 299.100375. FT-IR (cm^{-1}): 3207.0, 3172.3, 3151.1, 3078.8, 3030.6, 2921.6, 2886.0, 1736.6, 1663.3, 1470.5, 1310.4, 1271.8, 1173.5, 1098.3, 854.3, 699.1, 638.3. Anal. Calcd for $C_{14}H_{16}N_2O_4$: C, 60.86; H, 5.84; N, 10.14; found: C, 60.92; H, 5.85; N, 10.05. $[a]_D^{22}$ = - 28.4 (*c*=1.0, DMSO). Mp 241-243 °C (decomp.).

cyclo[L-Phe-L-His] (**5**)

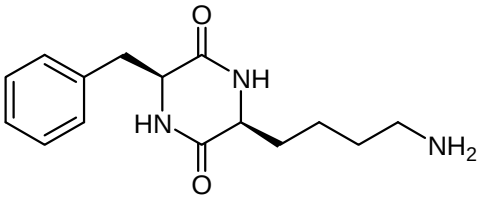


Compound **5** was synthesized according to the general procedure using Boc-Phe-OH (6.40 g, 24.10 mmol, 2 eq.) and Boc-His(Boc)-OH (12.85 g, 36.15 mmol, 3 eq.). The crude product was recrystallized from water and dried under reduced pressure. A white solid was received (1.33 g, 4.7 mmol, 39%).

1H NMR (400 Hz, DMSO-d_6, ppm): δ 11.80 (s (br), 1H), 8.08 (s (br), 1H), 7.77 (s (br), 1H), 7.52 (s, 1H), 7.33-7.13 (m, 5H), 6.60 (s, 1H), 4.16-4.11 (m, 1H), 3.87-3.81 (m, 1H), 2.86 (dd, *J* = 4.7 Hz, 13.6 Hz, 1H), 2.81 (dd, *J* = 4.9 Hz, 13.6 Hz, 1H), 2.56 (dd, *J* = 3.4 Hz, 14.4 Hz, 1H), 1.59 (dd, *J* = 9.1 Hz, 14.4 Hz, 1H).

13C NMR (100 MHz, DMSO-d_6, ppm, 120°C): δ 166.3, 165.8, 136.0, 134.1, 133.2, 129.2 (2x), 127.3 (2x), 125.7, 116.0, 54.8, 54.0, 38.1, 30.3. FAB-MS calculated for $C_{15}H_{17}N_4O_2$ $[M+H]^+$: m/z 285.1, found: 285.2. FT-IR (cm^{-1}): 3184.9, 3055.7, 2967.9, 2884.0, 2620.8, 1664.3, 1454.1, 1334.5, 1096.3, 820.6, 746.3, 697.1, 657.6, 626.8. Anal. Calcd for $C_{15}H_{16}N_4O_2$: C, 63.37; H, 5.67; N, 19.71; found: C, 62.77; H, 5.31; N, 19.69. $[a]_D^{20}$ = - 67.4 (*c*=2.0, acetic acid) {- 72.0 (c 2.0 acetic acid)[8]}. Mp 248-250 °C (decomp.).

*cyclo[*L-*Phe*-L-*Lys]* (**6**)

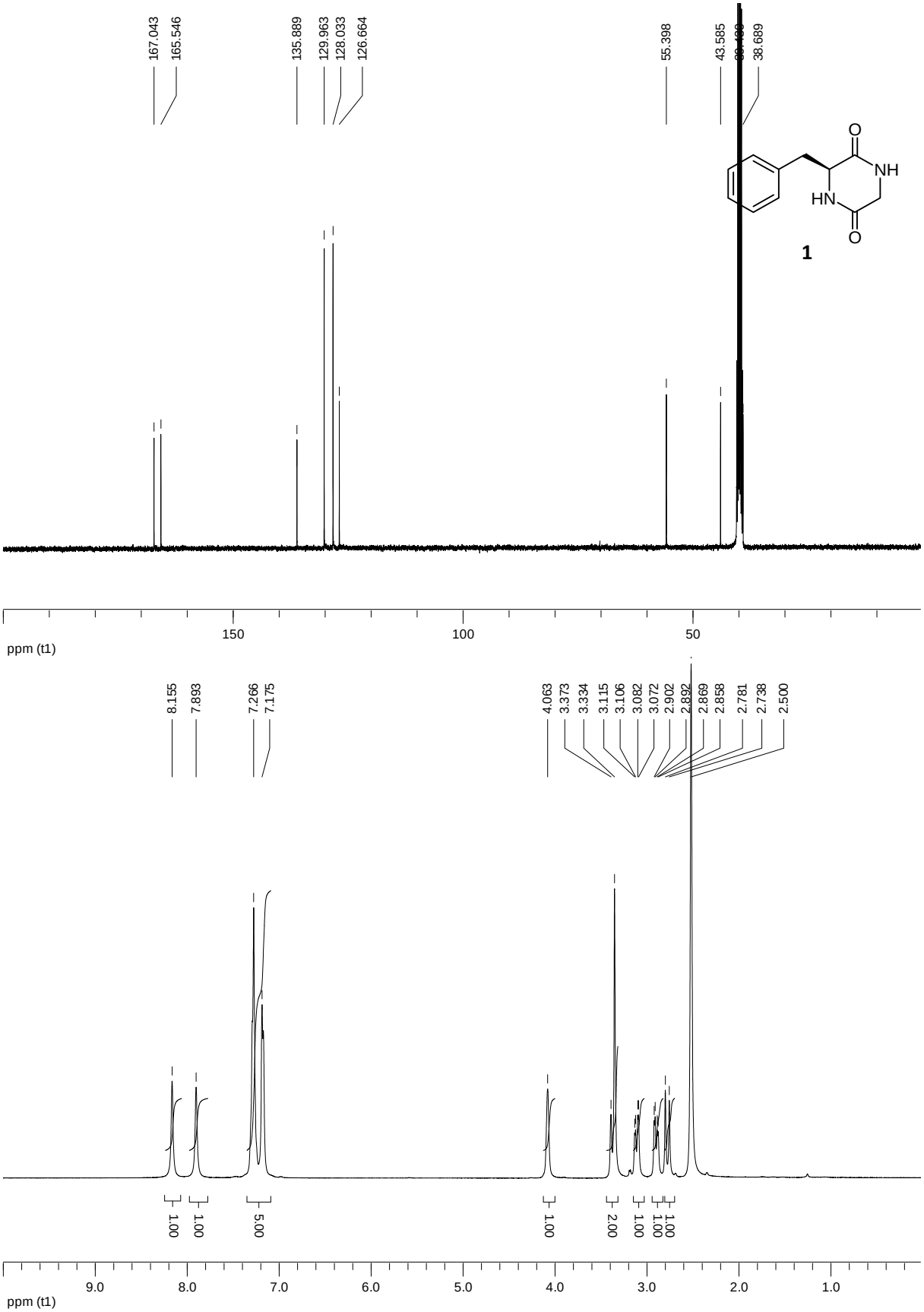


Compound **6** was synthesized according to the general procedure using Boc-Phe-OH (6.40 g, 24.10 mmol, 2 eq.) and Boc-Lys(Boc)-OH (12.52 g, 36.15 mmol, 3 eq.). The crude product was triturated in ethanol, dissolved in water and washed three times with ethyl acetate. The aqueous phase was passed through a column of strong basic ion exchange resin (Ambersep® 900(OH)) and lyophilized. A white solid was received (2.03 g, 6.51 mmol, 54%).

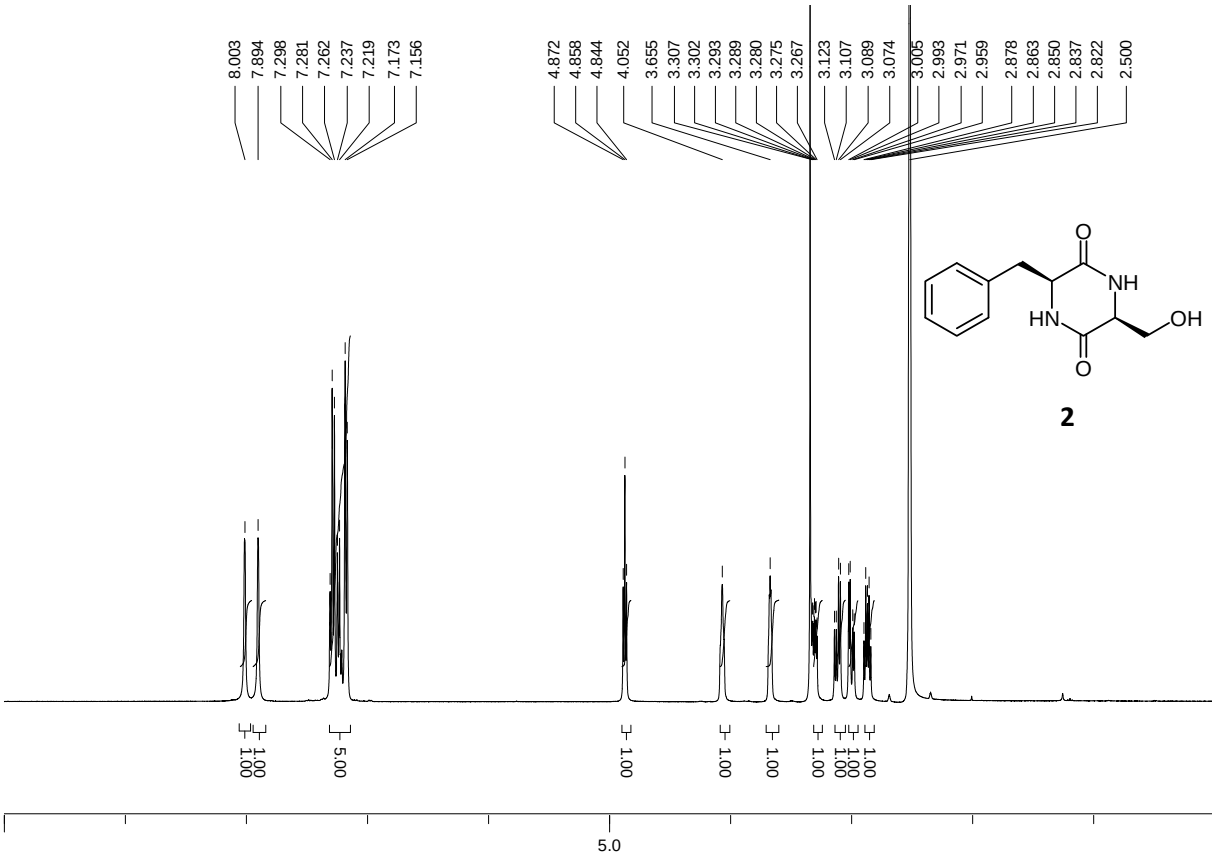
1H NMR (400 MHz, DMSO-d_6, ppm): δ 8.12 (s (br), 1H), 8.03 (s (br), 1H), 7.28-7.13 (m, 5H), 4.20-4.16 (m, 1H), 3.56-3.53 (m, 1H), 3.14 (dd, *J* = 3.7 Hz, 13.4 Hz, 1H), 2.96 (s (br), 2H), 2.83 (dd, *J* = 4.9 Hz, 13.4 Hz, 1H), 2.32 (t, *J* = 7.0 Hz, 2H), 1.09-0.99 (m, 3H), 0.79-0.68 (m, 2H), 0.67-0.59 (m, 1H). 13C NMR (100 MHz, DMSO-d_6, ppm): δ 166.9, 166.0, 136.0, 130.3 (2x), 127.8 (2x), 126.6, 55.2, 53.8, 41.3, 39.4, 33.1, 32.9, 20.9. HRMS (ESI) calculated for $C_{15}H_{22}N_3O_2$ $[M+H]^+$: m/z 276.170653, found: 276.170541. FT-IR (cm^{-1}): 3185.8 (m), 3047.0 (m), 2926.5 (m), 2895.6 (m), 2863.8 (m), 1660.4 (vs), 1454.1 (s), 1339.3 (m), 1101.2 (w), 841.8 (m), 754.0 (m), 697.1 (s). Anal. Calcd for $C_{15}H_{21}N_3O_2$: C, 65.43; H, 7.69; N, 15.26; found: C, 65.12; H, 7.45; N, 15.19. $[a]_D^{22}$ = - 24.2 (*c*=1.0, DMSO). Mp 199-201 °C.

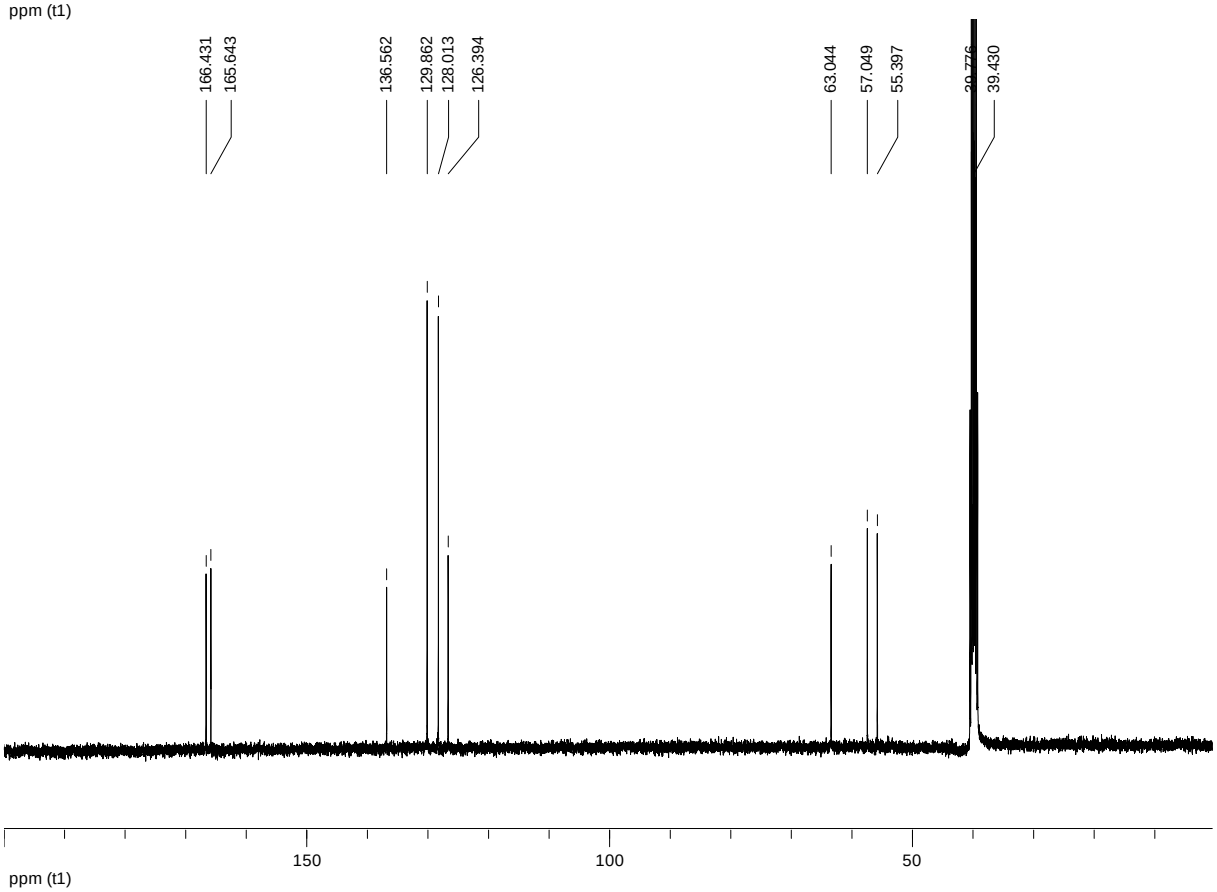
2 NMR spectra

^{1}H- and ^{13}C-NMR spectra of *cyclo*[L-Phe-L-Gly] (**1**) in DMSO-d_6.

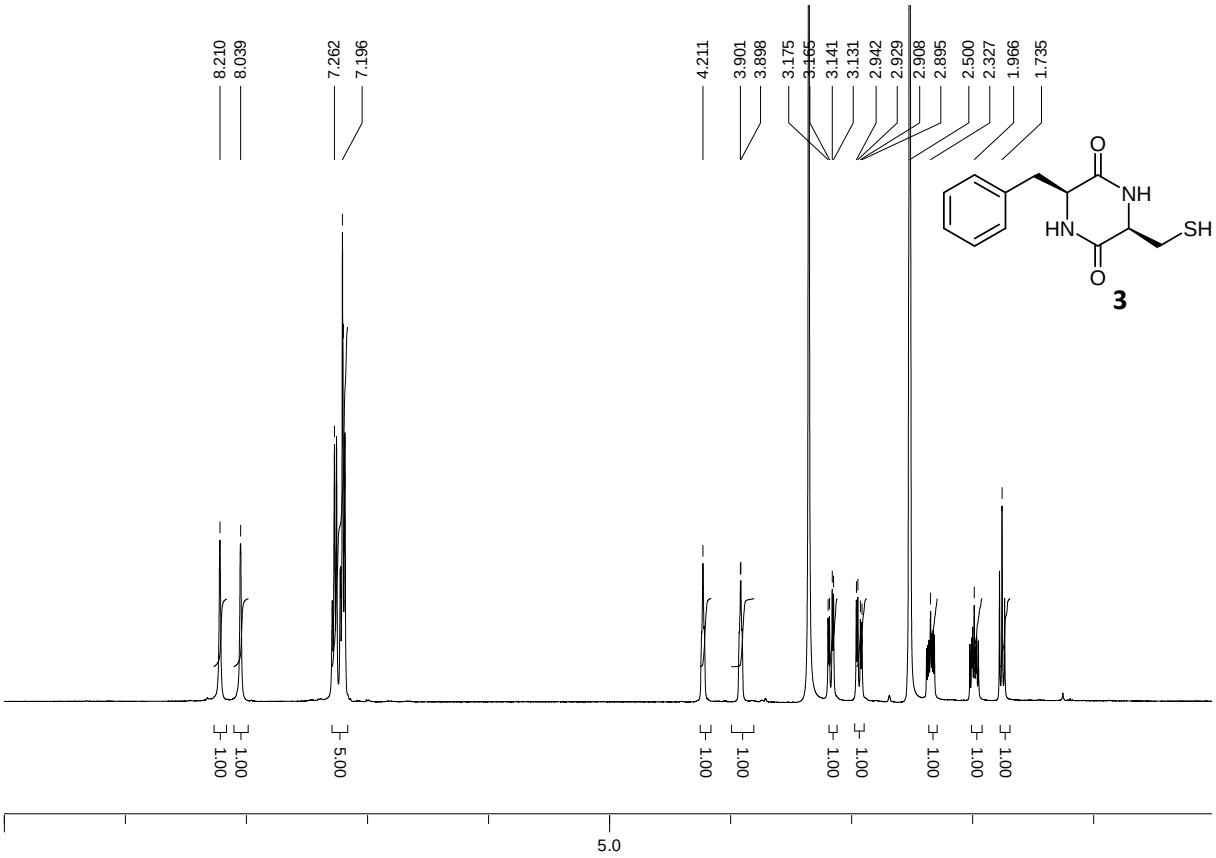


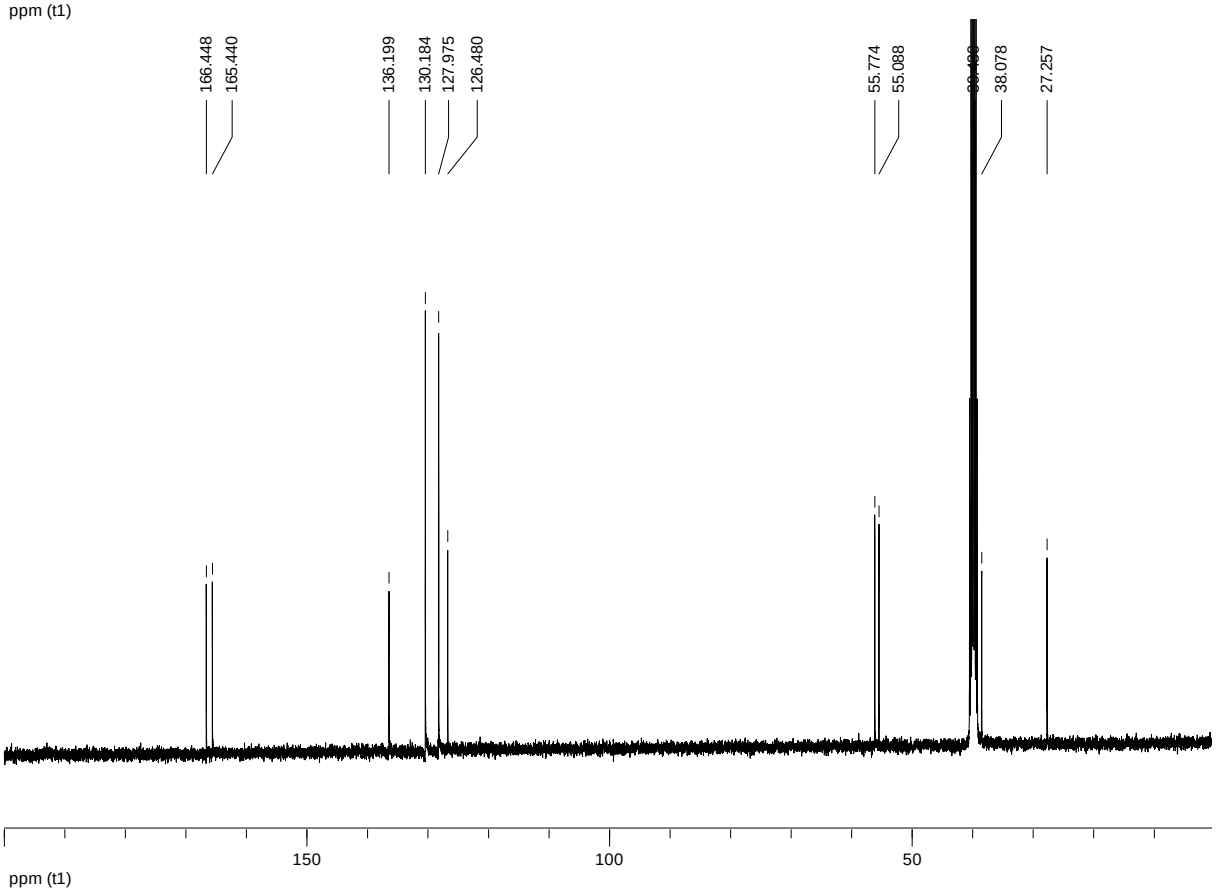
^{1}H- and ^{13}C-NMR spectra of *cyclo*[L-Phe-L-Ser] (**2**) in DMSO-d_6.



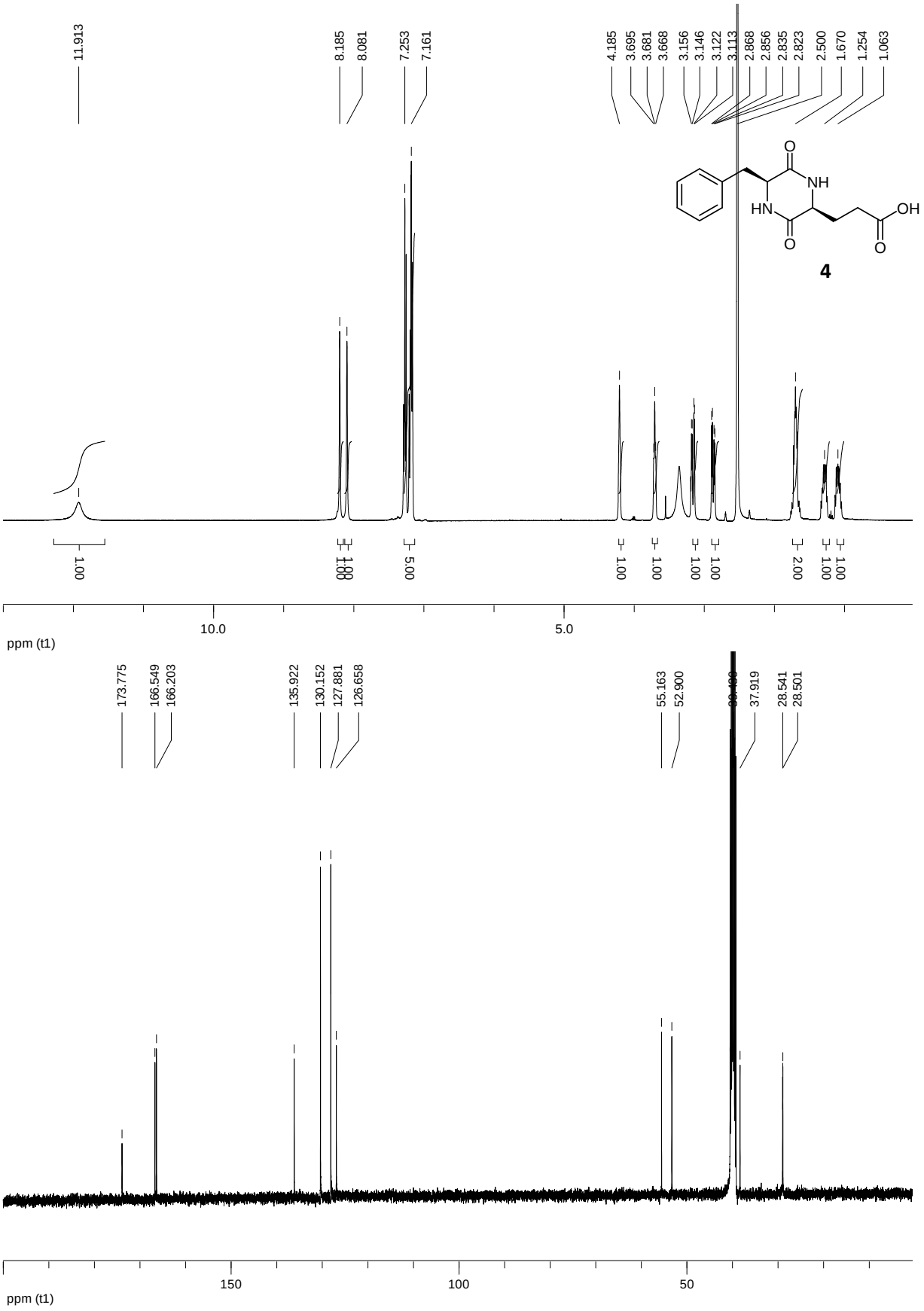


^{1}H- and ^{13}C-NMR spectra of *cyclo*[L-Phe-L-Cys] (**3**) in DMSO-d_6.

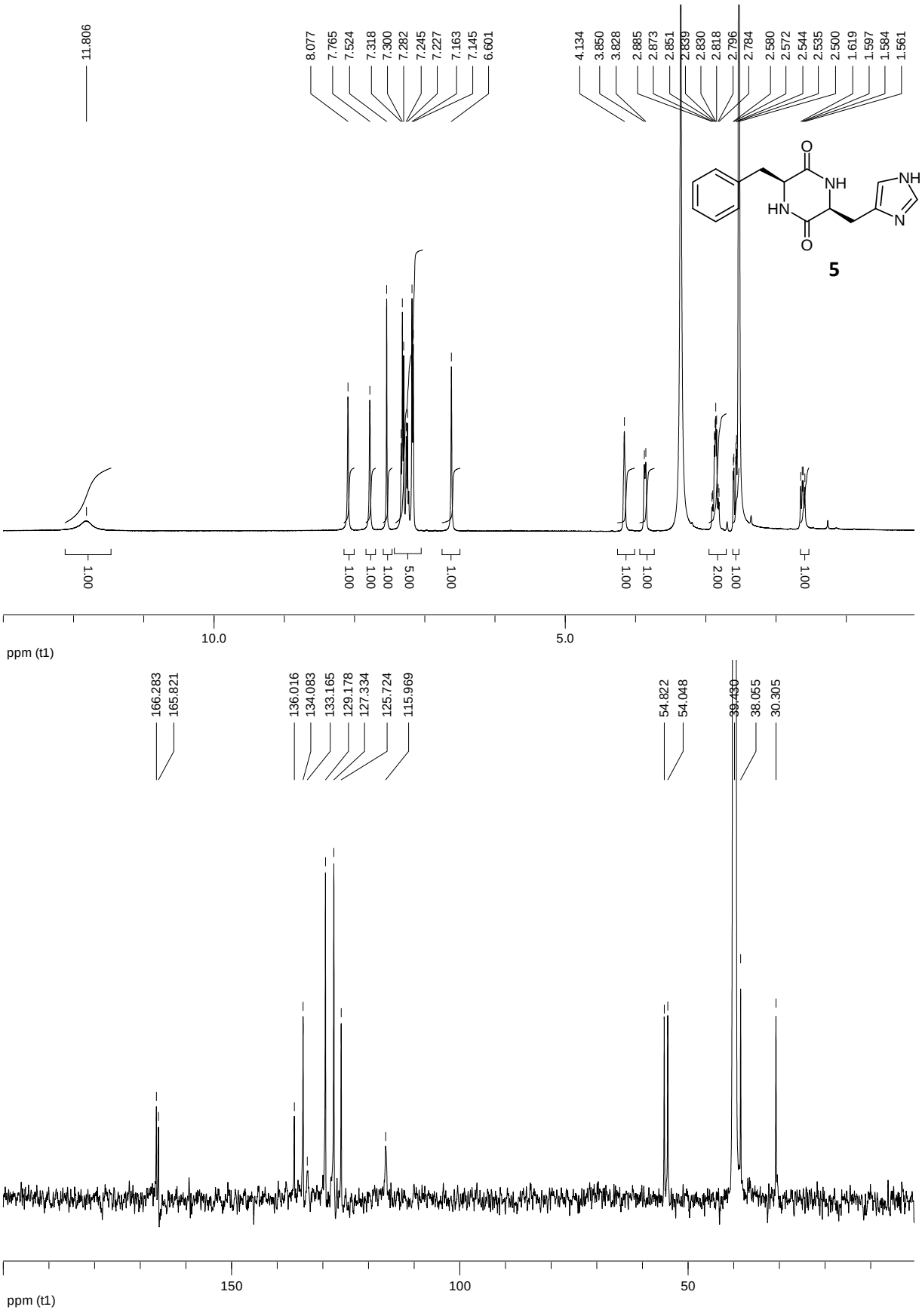




^{1}H- and ^{13}C-NMR spectra of *cyclo*[L-Phe-L-Glu] (**4**) in DMSO-d_6.

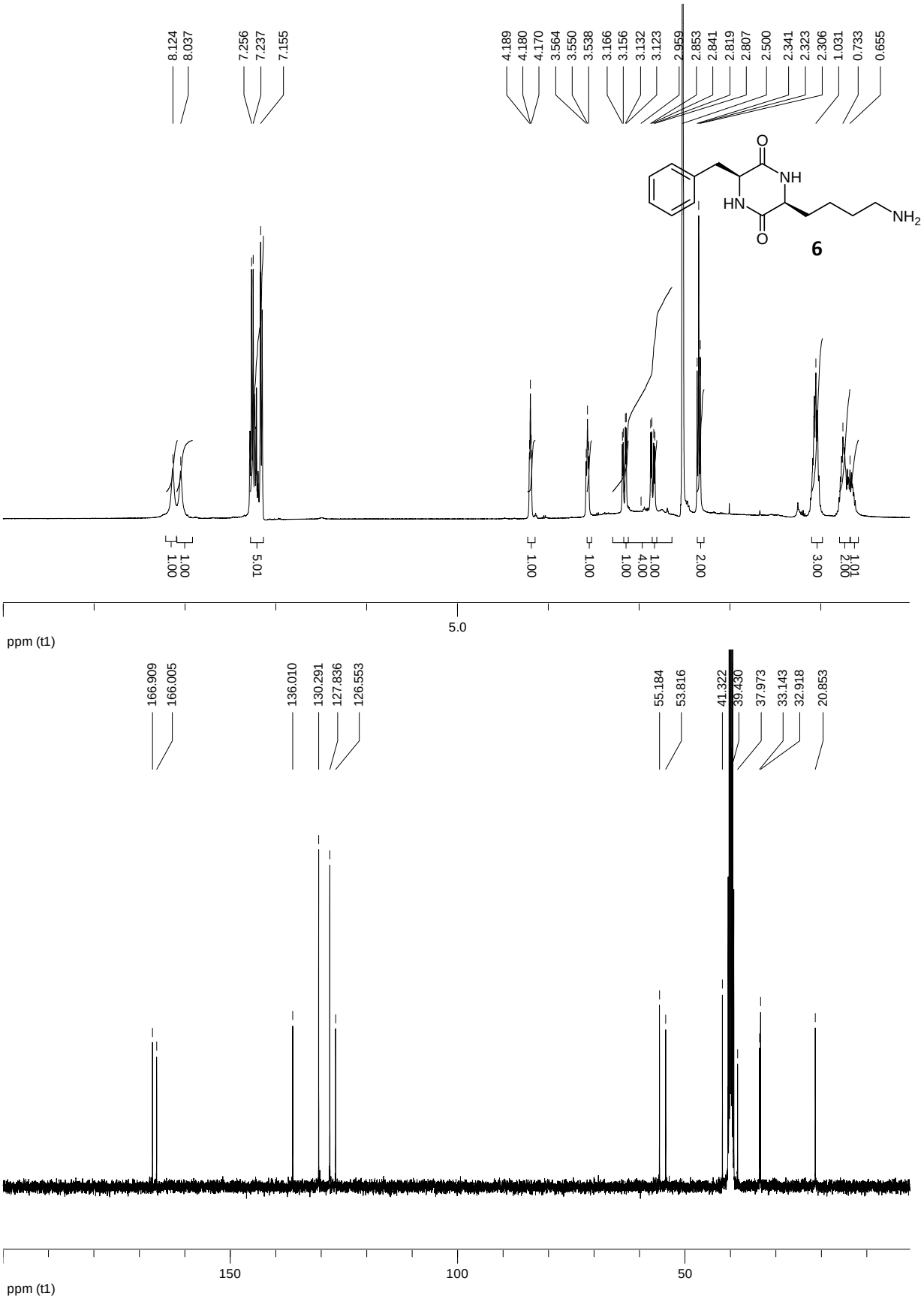


^{1}H- and ^{13}C-NMR spectra of *cyclo*[L-Phe-L-His] (**5**) in DMSO-d_6. It was necessary to measure the ^{13}C-NMR at 120 °C to increase the exchange rate and achieve more narrow signals at 133.2 and 116.0 ppm.



5

^{1}H- and ^{13}C-NMR spectra of *cyclo*[L-Phe-L-Lys] (**6**) in DMSO-d_6.



3 References

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