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Supplementary Information

Hydrogen bond directed self-assembly of cyclic dipeptide derivatives: Gelation and ordered hierarchical architectures

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General experimental procedure

Amino acids were obtained from Novabiochem, all other reagents and solvents of synthesis grade were purchased from Sigma-Aldrich and Fluka and used as such without further purification unless otherwise mentioned. Elemental analysis was carried out on Thermo Scientific FLASH 2000 Organic Element Analyzer. ^{1}H and ^{13}C NMR were recorded on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in $CDCl_3$/DMSO, tetramethylsilane as internal standard). Mass spectra were obtained on Bruker Ultraflex II MALDI/TOF spectrometer. Perkin Elmer model LS 55 spectrophotometer was used to record emission spectra. FESEM imaging was carried out on FESEM, FEI Nova nanoSEM-600.

Synthesis and characterization of compounds

Preperation of Fmoc-lys(Boc)-gly-OMe (3): Fmoc-lys(Boc)-OH (200.0 mg, 0.42 mmol), hydroxybenzotrizole (68.0 mg, 0.51 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (98.0 mg, 0.51 mmol) were dissolved in dichloromethane (8 mL) and cooled to 0 $^{\circ}C$. Glycine methylester hydrochloride (53.0 mg, 0.42 mmol) and diisopropylethylamine (176.0 mg, 1.36 mmol) were added and the reaction mixture was stirred at ice cold temperature for 1 h and then at room temperature for 5 h. The reaction progress was monitored by thin layer chromatography (TLC). Reaction mixture was evaporated to dryness and extracted from dichloromethane, washed with water, dried over anhydrous sodium sulfate and purified using silicagel coloumn chromatography with MeOH/$CHCl_3$ (v/v: 0.8/99.2) as eluent to obtain **3** in quantitative yield (218.0 mg, 92%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.43 (s, 3H), 1.50 (b, 4H), 1.70 (s, 3H), 1.88 (s, 1H), 3.10-3.11 (d, J = 5.2 Hz, 2H), 3.73 (s, 3H), 4.03 (s, 2H), 4.19-4.22 (t, J = 6.4 Hz), 4.40 (s, 2H), 4.65 (s, 2H), 5.52 (s, 1H), 6.68 (s, 1H), 7.28-7.32 (dt, J = 7.6 Hz, J = 0.8 Hz, 2H), 7.37-7.41 (t, J = 7.6 Hz, 2H), 7.58-7.59 (d, J = 7.2 Hz, 2H), 7.75-7.76 (d, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 400

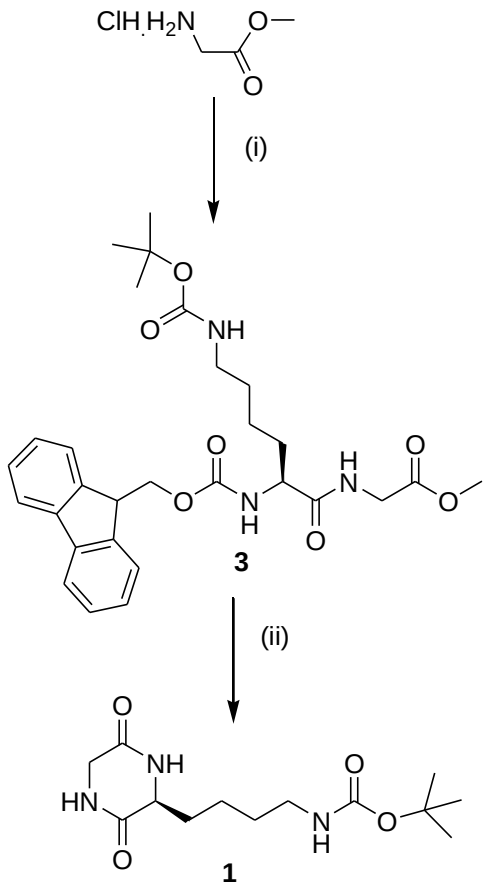
MHz) δ = 22.4, 28.5, 29.7, 32.0, 39.9, 41.2, 47.3, 52.5, 54.8, 67.2, 79.3, 120.1, 125.2, 127.2, 127.8, 141.4, 143.9, 156.3, 170.1, 172.1.

***Preparation of cyclo(lys(Boc)-gly) (1)*:** The linear dipeptide Fmoc-L-lys(Boc)-gly-OMe (**3**) (200.0 mg, 0.37 mmol) was dissolved in DCM-piperidine (v/v: 80/20, 6 mL) and stirred at room temperature for 24 h and the reaction progress was monitored by thin layer chromatography (TLC). Reaction mixture was evaporated to dryness and purified using silicagel coloumn chromatography with MeOH/$CHCl_3$ (v/v: 1.6/98.4) as eluent to obtain **1** in quantitative yield (65.0 mg, 62%).^{1}H NMR (DMSO-*d6*, 400 MHz). δ = 1.20-1.33 (m, 4H), 1.36 (s, 9H), 1.59-168 (m, 2H), 2.86-2.91 (q, *J* = 2 Hz, 2H), 3.62-3.79 (m, 3H), 6.74-6.77 (t, *J* = 4.8 Hz, 1H), 7.97 (s, 1H), 8.14 (s, 1H); ^{13}C NMR (DMSO-*d6*, 400 MHz) δ = 21.3, 28.2, 29.2, 32.4, 44.2, 54.0, 77.32, 166.6, 167.9; MALDI-TOF-MS = m/*z* 288. 39 $[M+3H]^{+}$; Elemental analysis calculated (%) for $C_{13}H_{23}N_3O_4$: C 54.72, H 8.12, N 14.73, O 22.43; found: C 54.75, H 8.15, N 14.75, O 22.35.

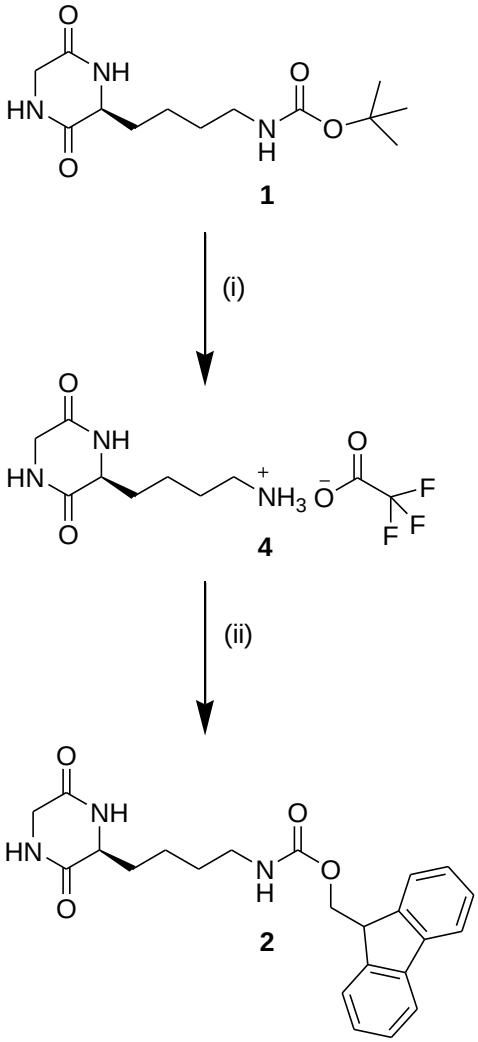
Preparation of cyclo(lys-gly)(4): cyclo(lys(Boc)-gly) (**1**) (100.0 mg, 0.35 mmol) was dissolved in trifluoro aceticacid-water (1:1 6 mL) and catalytic amount of triisopropylsilane (TIPS) was added and the resulting reaction mixture was stirred at room temperature for 4 h. The reaction progress was monitored by thin layer chromatography (TLC). Reaction mixture was evaporated to dryness and co-evaporated with toluene to obtain **4** in quantitative yield (99.0 mg, 95%). ^{1}H NMR(DMSO-*d6*, 400 MHz) δ = 1.27-1.490 (m, 2H), 1.50-1.62 (m, 2H), 1.63-1.74 (m, 2H), 3.66-3.80 (m, 3H), 7.74 (s, 3H), 8.01 (s, 1H), 8.51 (s, 1H); ^{13}C NMR (DMSO-*d6*, 400 MHz) δ = 20.9, 26.7, 32.1, 43.6, 44.2, 53.8, 166.0, 167.8.

Preperation of cyclo(lys(Fmoc)-gly) (2): Sodium hydrogen carbonate (56.0 mg, 0.66 mmol) was added to cyclo(lys-gly) (**4**) (100.0 mg, 0.33 mmol) in water (5 mL) and cooled to ice cold temperature. Fmoc-OSu (N-(9-Fluorenylmethoxycarbonyloxy) succinimide) (135.0 mg, 0.40

mmol) in 1, 4-dioxane (5 mL) was added drop wise to the above solution over a period of 30 min under ice cold conditions. The resultant solution was stirred at 0 °C for 30 min, then at room temperature for 4 h. The reaction progress was monitored by thin layer chromatography (TLC). The resultant solution was washed with ethylacetate thrice. The combined layers of ethylacetate were back extracted with saturated sodiumhydrogen carbonate solution. Combined layers of sodiumhydrogen carbonate, aqueous layers were combined and acidified to pH 1 using 10% HCl. The acidified solution was thrice extracted into ethylacetate, combined layers of ethylacetate were dried over anhydrous sodium sulfate evaporated to dryness and purified using silicagel coloumn chromatography with MeOH/$CHCl_3$ (v/v: 1.8/98.2) as eluent to obtain **2** in quantitative yield (130.0 mg, 96%). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ = 1.23-1.42 (m, 2H), 1.59-1.70 (m, 2H), 2.94-2.99 (q, *J* = 6.4 Hz), 3.64-3.81 (m, 3H), 4.19-4.22 (t, *J* = 6.8 Hz, 1H), 4.28-4.30 (d, *J* = 6.8 Hz, 2H), 7.26-7.29 (t, *J* = 11.2 Hz, 1H), 7.31-7.35 (dt, *J* = 7.6 Hz, J = 1.2Hz, 2H), 7.39-7.43 (t, *J* = 7.6 Hz, 2H), 7.67-7.69 (d, *J* = 7.2 Hz), 7.88-7.89 (d, *J* = 7.6 Hz, 2H), 7.93 (s, 1H), 8.16 (s, 1H); ^{13}C NMR (DMSO-*d6*, 400 MHz) δ = 21.3, 29.0, 32.4, 44.2, 46.7, 54.0, 65.1, 120.1, 125.1, 127.0, 127.5, 140.7, 143.9, 156.0, 166.1, 167.9 ; MALDI-TOF-MS = *m/z* 430.18 $[M + Na]^+$, 446.16 $[M + K]^+$. Elemental analysis calculated (%) for $C_{23}H_{25}N_3O_4$: C 67.80, H 6.18, N 10.31, O 15.71; found: C 67.78, H 6.12, N 10.28, O 15.82.



Scheme S1. Synthesis of CDP **1.** Reagents and conditions: i) Fmoc-Lys(Boc)-OH, EDC.HCl, HOBt, DIPEA, DCM, 0°C- RT, 5 h. ii) 20% piperidine in DCM, RT, 24 h. EDC.HCl = 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, HOBt = 1-hydroxybenzotriazole, DIPEA = Diisopropylethylamine, DCM = dichloromethane.



Scheme S2. Synthesis of CDP **2.** Reagents and conditions: i) TFA-H_2O (1:1), TIPS, RT, 4 h. ii) Fmoc-OSu, $NaHCO_3$, 1, 4-dioxane, H_2O, 0°C- RT, 4 h. TFA = trifluoro aceticacid, Fmoc-OSu = *N*-(9-Fluorenylmethoxycarbonyloxy) succinimide; TIPS = triisopropylsialne.

Gelation experiments. Requisite amount of cyclic dipeptide is weighed into a sample vial containing 1 centimetre cube of solvent and the tube is closed using a cap and heated around 45°C - 50°C until a clear solution is resulted. The obtained clear solution was then cooled to room temperature under the atmospheric conditions. The ability of cyclic dipeptides to form organogels was eventually determined by inverting the vial upside down. Absence of any solvent flow confirmed the formation of gel. During the gelation test the minimal gelation concentrations (MGC) for the two ε-amino derivatives of cyclo(Gly-L-Lys) in different organic solvents were determined.

NMR spectroscopy. ^{1}H-NMR and ^{13}C-NMR spectra for the characterisation of the title compounds and intermediate molecules were recorded at 25°C on a Bruker AV-400 MHz spectrometer. The ^{1}H NMR spectra were acquired with 16 scans per FID and ^{13}C-NMR spectra were acquired with 1024 scans per FID

Field emission scanning electron microscopy (FESEM). Samples for FESEM analysis were prepared by placing a aliquot of the CDP hot clear solution on to a clean one side polished single crystalline silicon surface, dried thoroughly in the air and then under vacuum before submitting for analysis. Microscopic imaging at nanoscale was carried out using FESEM, FEI Nova nanoSEM-600 equipped with field emission gun operating at 5 kV. Sample for CDP **2** in chloroform was prepared by drop casting an aliquot of 100 μM solution using a micropipette and drying in air and then under vacuum.

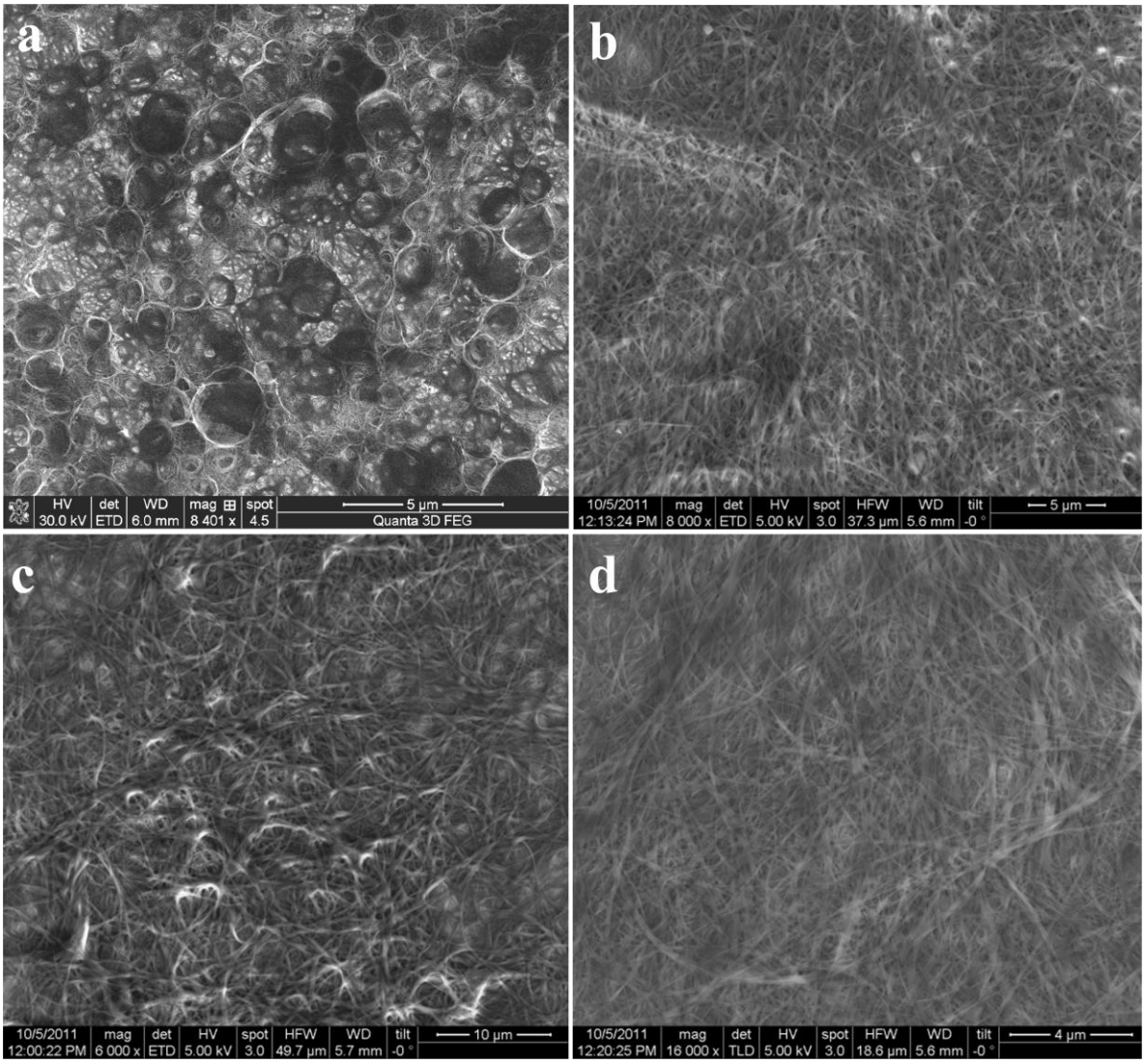


Fig. S1 Large area view FESEM image of **1** xerogels in chloroform (a), carbontetrachloride (b), hexane (c) and in toluene (d).

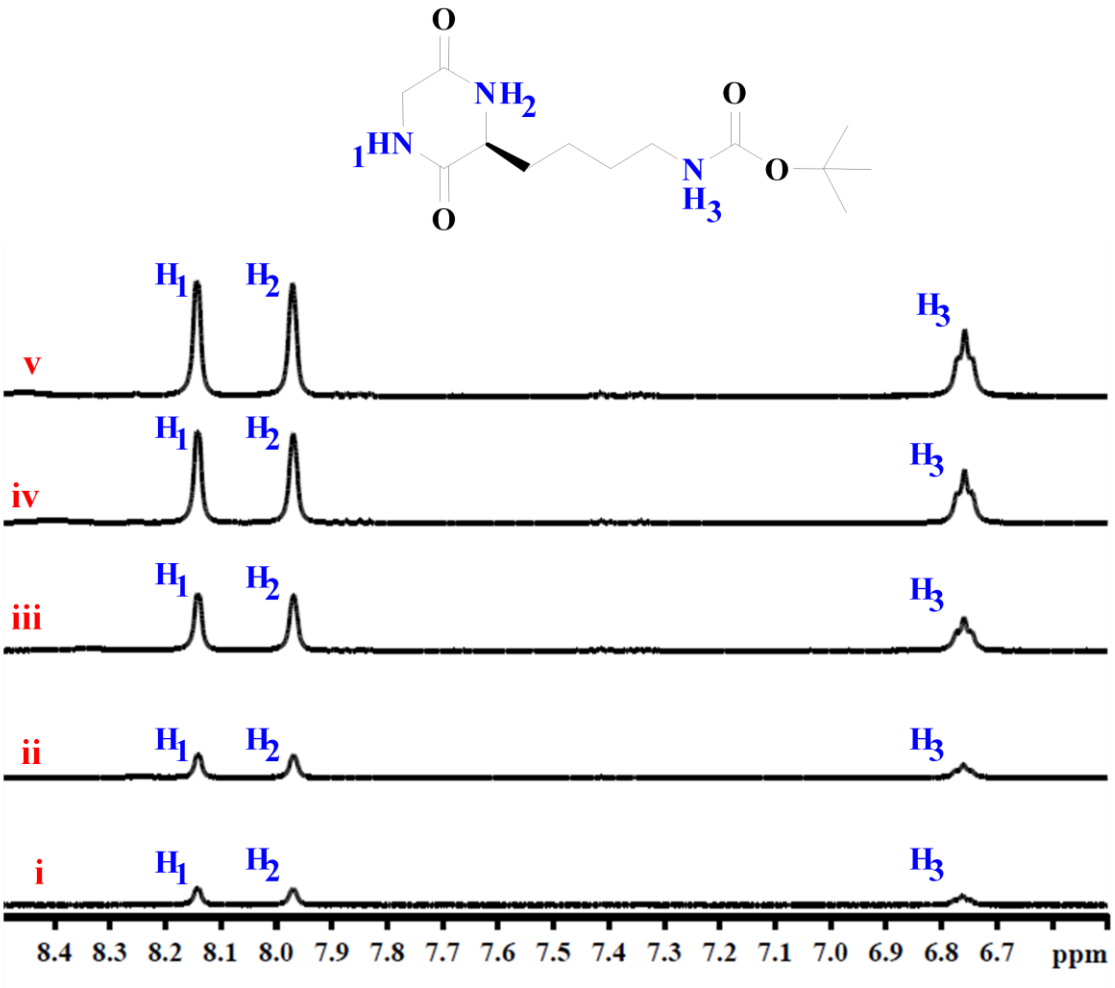


Fig. S2 Concentration dependant ^{1}H NMR spectra of CDP **1** in DMSO-*d*6 at 25°C. Concentration: i: 3.5 mM, ii: 9.8 mM, iii: 21.7 mM, iv: 33.6 mM , v: 56.0 mM.

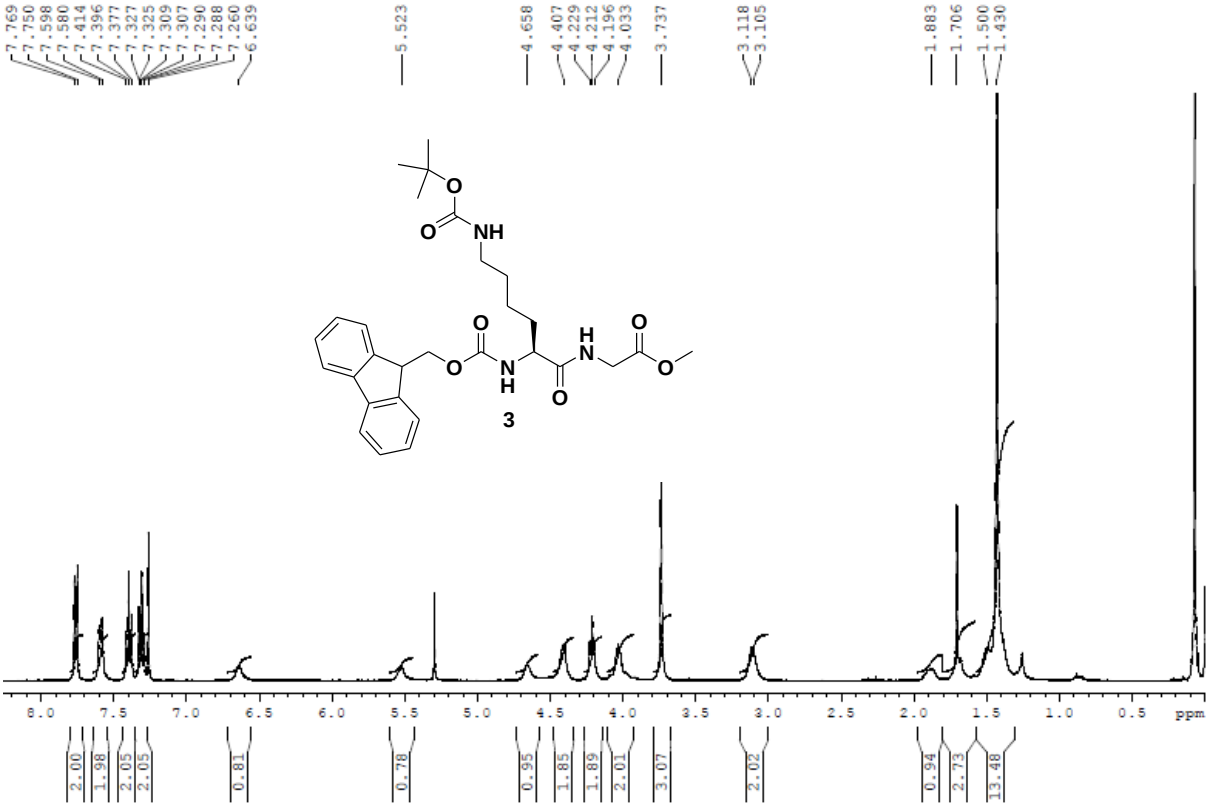


Fig. S3 ^{1}H NMR spectra of Fmoc-lys(Boc)-gly-OMe (**3**) in $CDCl_3$.

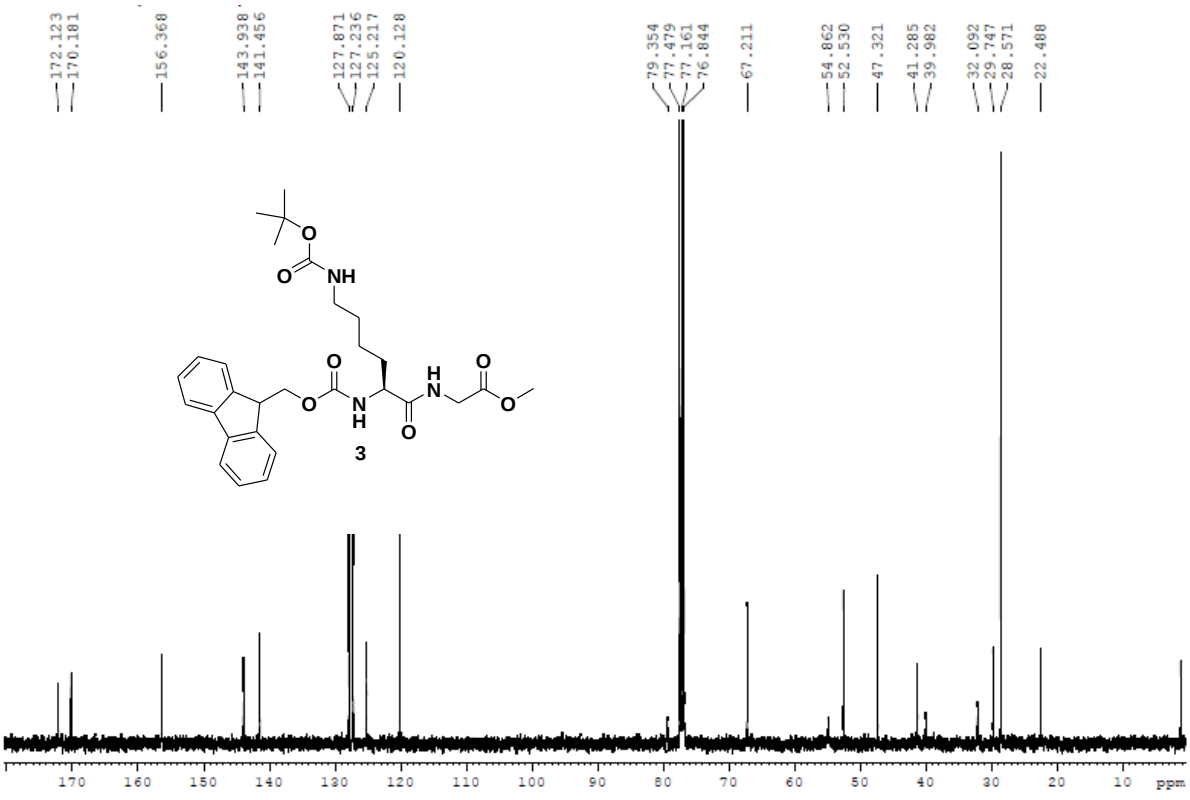


Fig. S4 ^{13}C NMR spectra of Fmoc-lys(Boc)-gly-OMe (**3**) in $CDCl_3$.

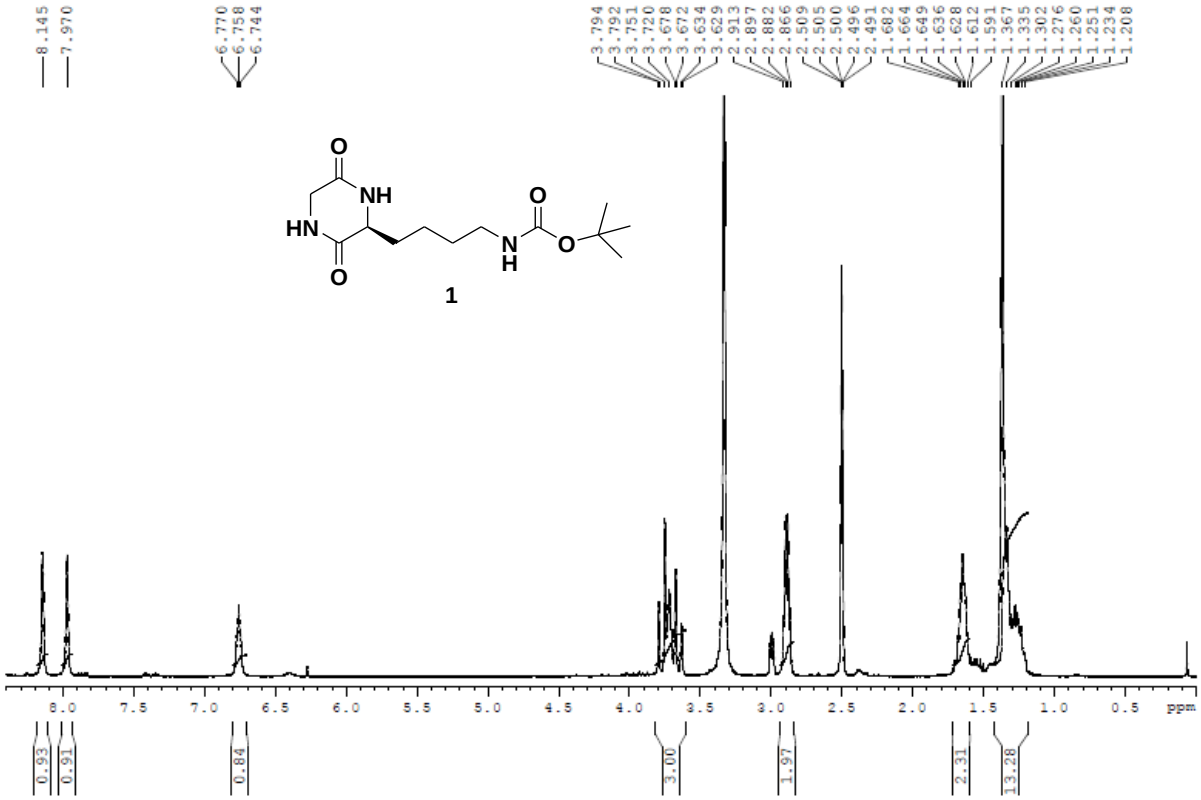


Fig. S5 ^{1}H NMR spectra of cyclo(lys(Boc)-gly) (**1**) in DMSO-*d6*.

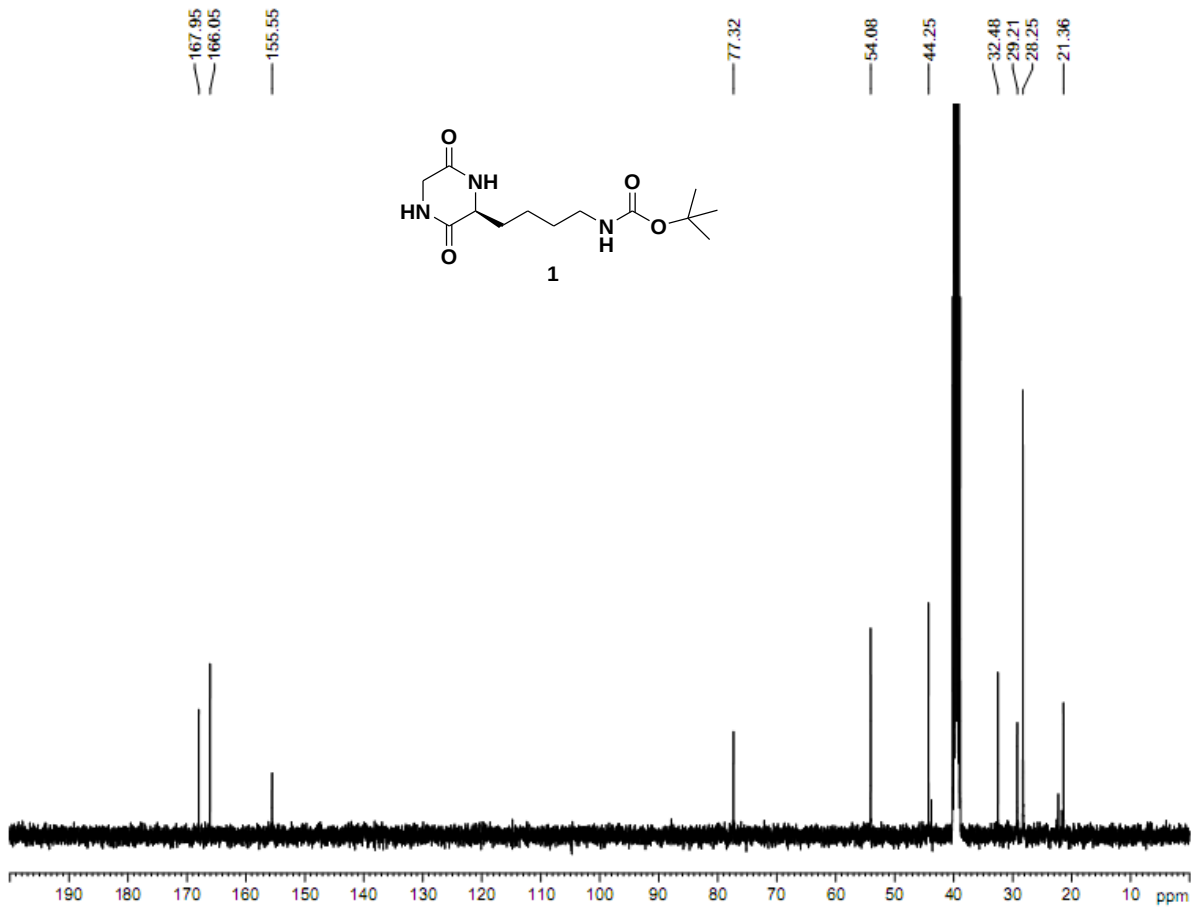


Fig. S6 ^{13}C NMR spectra of cyclo(lys(Boc)-gly) (**1**) in DMSO-*d6*.

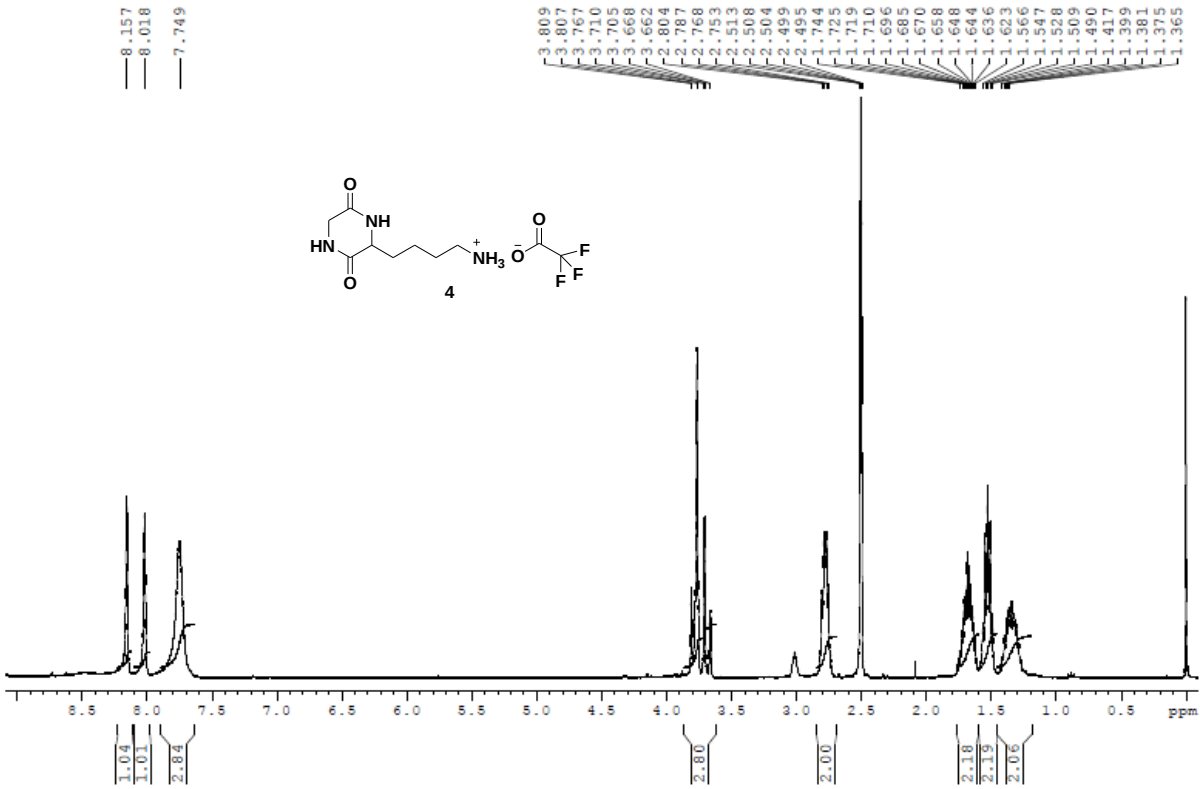


Fig. S7 ^{1}H NMR spectra cyclo(lys-gly) (**4**) in DMSO-*d6*.

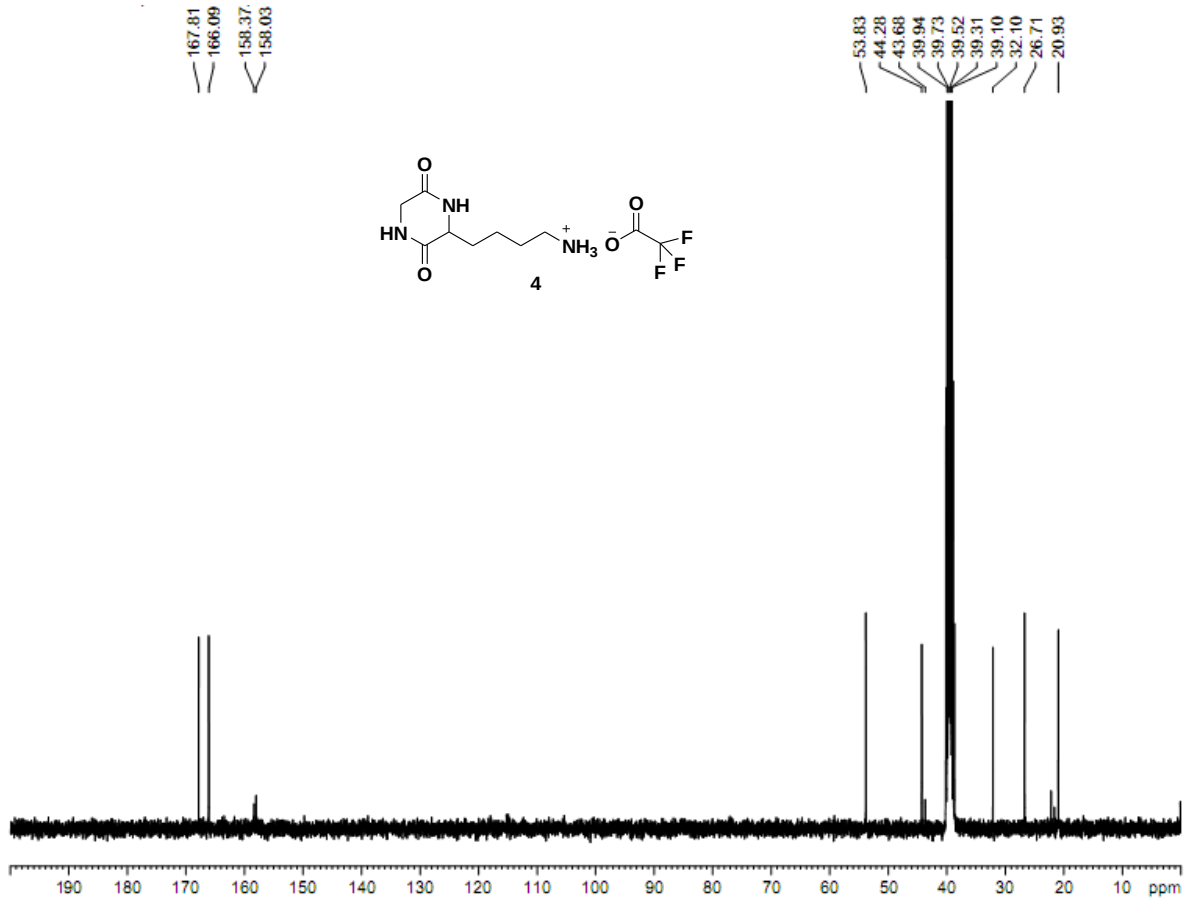


Fig. S8 ^{13}C NMR spectra of cyclo(lys-gly) (**4**) in DMSO-*d6*.

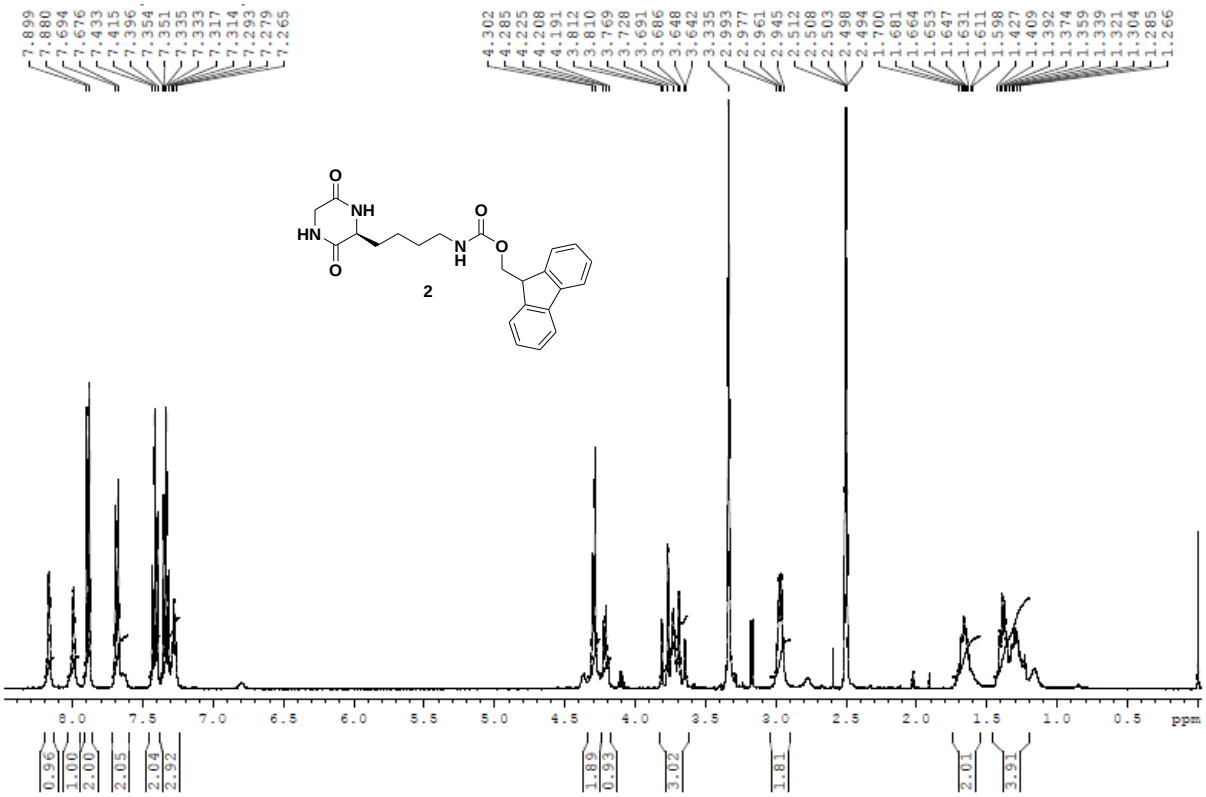


Fig. S9 ^{1}H NMR spectra of cyclo(lys(Fmoc)-gly) (**2**) in DMSO-*d6*.

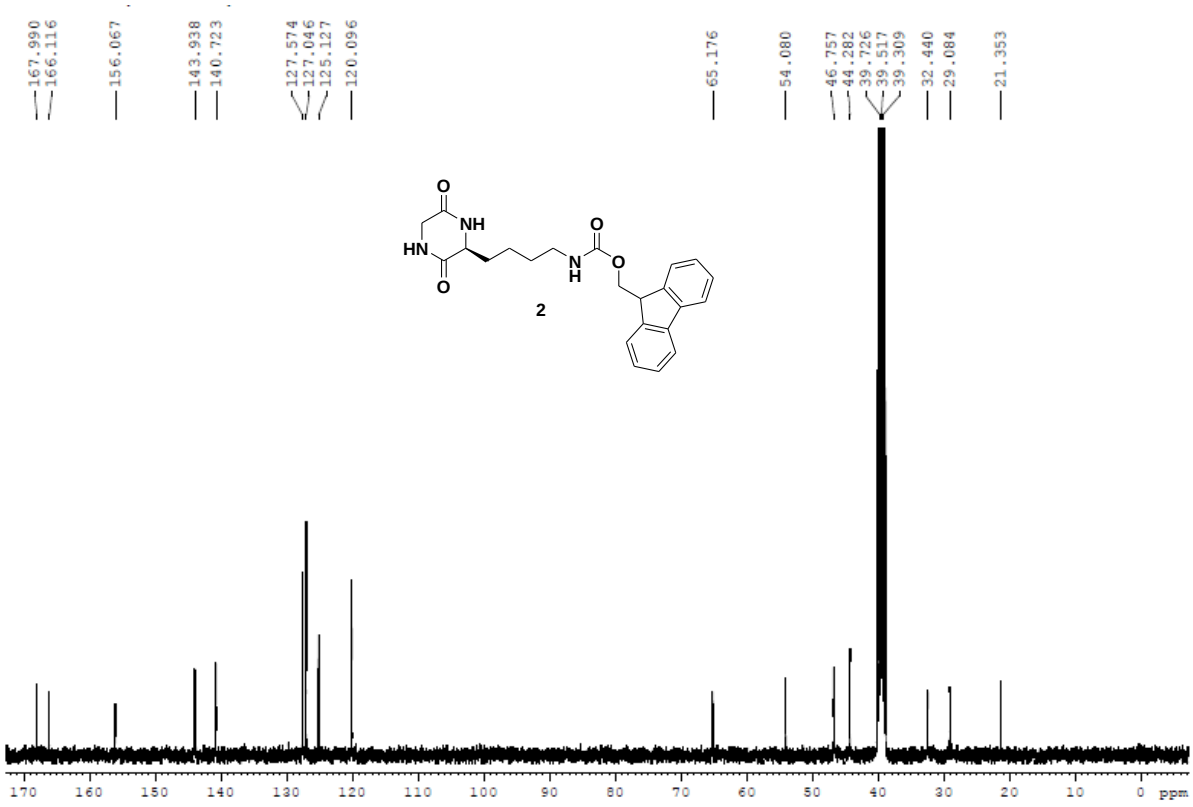


Fig. S10 ^{1}H NMR spectra of cyclo(lys(Fmoc)-gly) (**2**) in DMSO-*d6*.

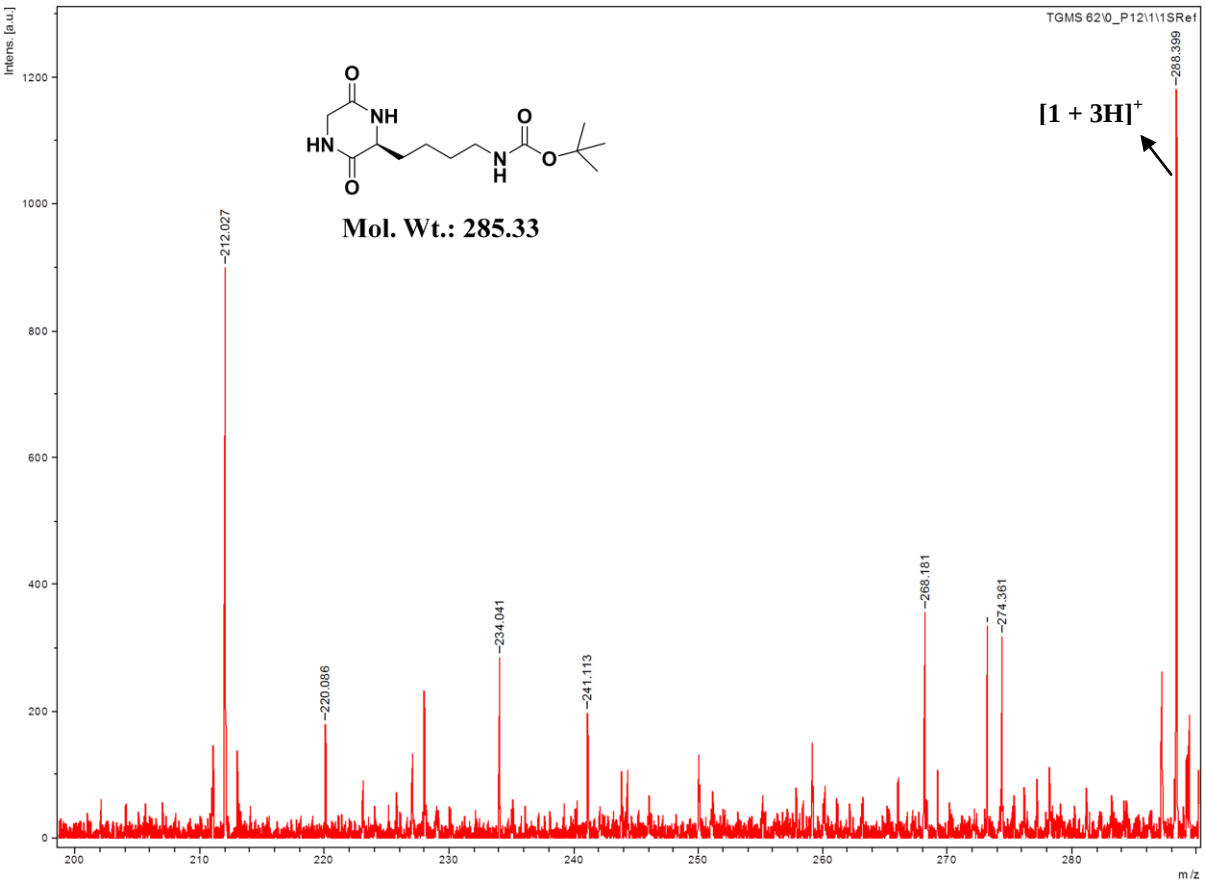


Fig. S11 MALDI-TOF mass spectra of cyclo(lys(Boc)-gly) (**1**).

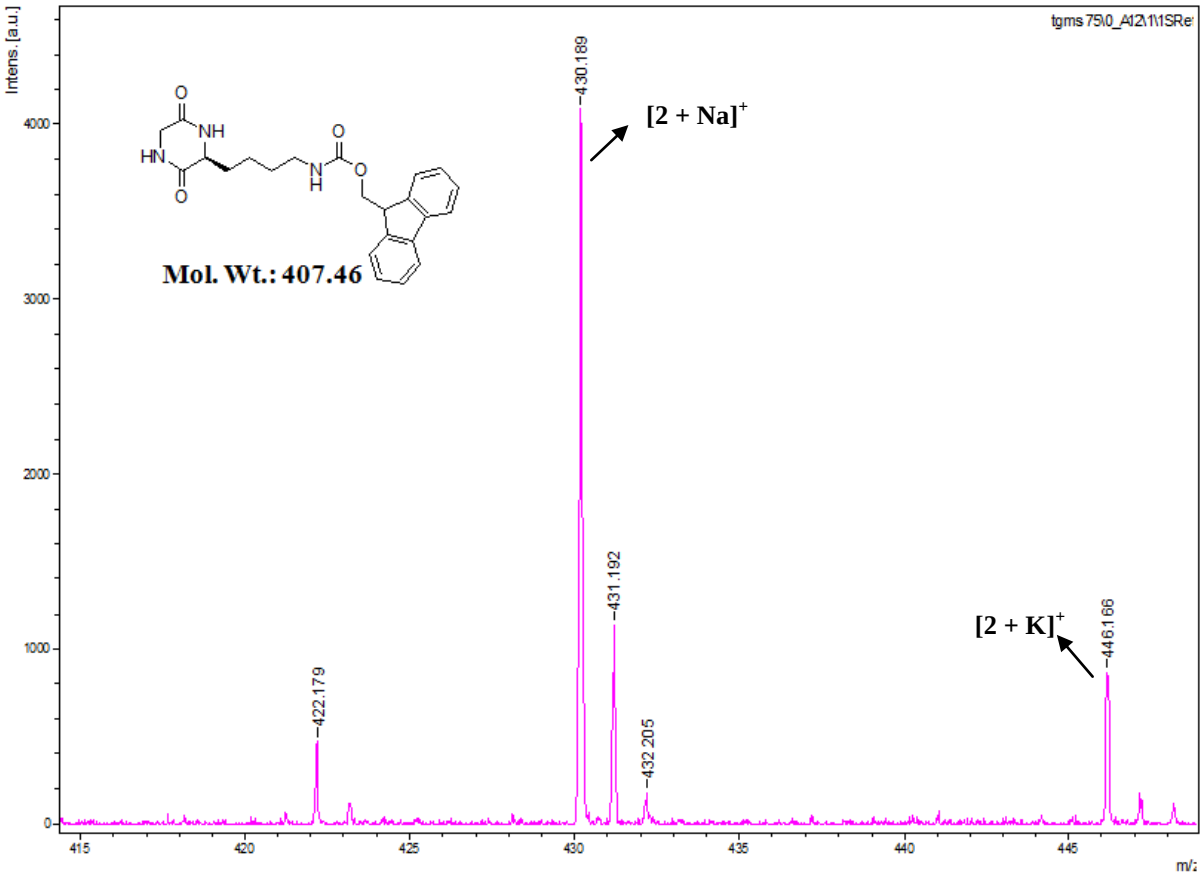


Fig. S12 MALDI-TOF mass spectra of cyclo(lys(Fmoc)-gly) (**2**).