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Supporting Information

Light-mediated control of activity in a photosensitive foldamer that mimics an esterase

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Experimental Procedures

General Methods

High-Performance Liquid Chromatography. The HPLC measurements were performed using an Agilent 1200 apparatus (Palo Alto, CA), equipped with a UV detector at various wavelength and a column Agilent extend-C18 (stationary phase). Eluants: A= 9:1 H_2O/CH_3CN, 0.05 % TFA; B= 1:9 H_2O/CH_3CN, 0.05 % TFA.

Nuclear Magnetic Resonance. ^1H NMR, ^{13}C NMR, and 2D-NMR spectra were recorded at 25°C on Bruker Avance 200 or 400 MHz instruments. ^1H and ^{13}C spectra were referenced relative to the solvent residual peaks and chemical shifts (δ) reported in ppm downfield of tetramethylsilane ($CDCl_3$ δ H: 7.26 ppm, δ C: 77.16 ppm; CD_3CN δ H: 1.94 ppm; DMSO δ H: 2.50 ppm) and *J* values are given in Hz. The multiplicity of a signal is indicated as br, broad; s, singlet; d, doublet; t, triplet; m, multiplet.

Mass Spectrometry. Mass spectra by electrospray ionization (ESI), collected in the positive mode, were performed on Perseptive Biosystem Mariner ESI-ToF5220 spectrometer (Foster City, CA).

Fourier Transform-Infrared Spectroscopy. FT-IR absorption spectra were recorded with a ATi Perkin Elmer Spectra RX1 FT-IR spectrometer. The ῡ maxima for the main absorption bands are given.

UV lamp. Two handheld UV Lamps (Vilber) with bulbs emitting wavelength of 254 nm (6W) or 290-320 nm (8W), 350 nm (8W), 365 nm (15W) and 395-410 nm (10W, MinChen 502B Ultraviolet LED Flashlight Torch) were used in the photoisomerization experiments.

UV-Vis Absorption. The UV-Vis absorption spectra were recorded using a Shimadzu model UV-2501 PC spectrophotometer. A 1-cm path length quartz cell was used.

Melting point. Melting point of the compounds were determined using a Leitz Laborlux 12 microscope equipped with a Mavotherm 32 thermometer (sensor: NiCr-Ni thermocouple; resolution: 0.1 K; inherent deviation: 199°C ± 0.5% meas. val.).

Synthesis and characterization of compounds

Materials

Boc-L-amino acids, Fmoc-L-amino acids, triethylamine (TEA), cyclohexylamine, N,N-diisopropylethylamine (DIPEA), diethylamine (DIEA), N-Ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), trifluoroacetic acid (TFA), 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), Rink-amide resin, 4-Nitrophenyl acetate (*p*-NPA), 4-Nitrophenyl butyrate (*p*-NPB), deuterated chloroform ($CDCl_3$) were obtained from Merck. 1-hydroxy-7-aza-1,2,3-benzotriazole (HOAt) was purchased from GL Biochem (Shanghai). Monomethyl fumarate was synthesized based on the literature.[1] The deuterated solvents dimethyl sulfoxide (DMSO-d_6) and acetonitrile ($CDCN_3$) were purchased from Euriso-Top (France).

Synthesis of Fmoc-Ser(^{t}Bu)-NH-Cy

Fmoc-Ser(^{t}Bu)-OH (3 g, 7.8 mmol) and HOAt (1.06 g, 7.8 mmol) were dissolved in dry CH_3CN (35 ml) and the solution cooled to 0°C. EDC·HCl (1.50 g, 7.8 mmol) was added. After complete dissolution, cyclohexylamine (0.89 ml, 7.8 mmol) was added dropwise to the reaction mixture, and DIPEA was used to reach basic pH. The reaction mixture was diluted with CH_3CN (90 ml) and stirred at r.t. overnight. The solvent was removed under reduced pressure and the residue dissolved in AcOEt. The organic phase was washed with $KHSO_4$(aq) (5%), $NaHCO_3$(aq) (5%) and brine, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was triturated in cold Et_2O.

Yield 3.52 g (97%) of white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H, Ar_{Fmoc}), 7.60 (d, J = 7.5 Hz, 2H, Ar_{Fmoc}), 7.40 (t, J = 7.4 Hz, 2H, Ar_{Fmoc}), 7.31 (t, J = 7.4 Hz, 2H, Ar_{Fmoc}), 6.56 (s, 1H), 5.81 (d, J = 6.0 Hz, 1H), 4.39 (d, J = 7.0 Hz, 2H), 4.23 (t, J = 7.2 Hz, 1H), 4.19 - 4.09 (m, 1H), 3.78 (dt, J = 9.1, 4.4 Hz, 2H), 3.33 (t, J = 8.7 Hz, 1H), 1.90 (dt, J = 12.9, 4.2 Hz, 2H), 1.65 (m, 5H), 1.51 – 1.30 (m, 2H), 1.21 (s, 13H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.3, 144.0, 143.9, 141.4, 141.4, 127.9, 127.2, 125.3, 120.1, 74.3, 62.1, 48.4, 47.3, 33.1, 27.6, 25.7, 24.8. MS (ESI-TOF): $[M+H]^+$ m/z calcd. for $C_{28}H_{36}N_2O_4$, 465.2749; found 465.2752. FT-IR $\tilde{\nu}_{max}$ (cm^{-1}) 3324, 3302, 3064, 1697, 1650, 1602, 1544, 1478.

Synthesis of H_2N-Ser(^{t}Bu)-NH-Cy

Fmoc-Ser(^{t}Bu)-NH-Cy (2.3 g, 4.95 mmol) was dissolved in a solution of diethylamine in DCM (20% v/v). The reaction was stirred at room temperature for 30 min. The solvent was removed under reduced pressure and the residue was redissolved in a solution of diethylamine in DCM (20% v/v) and stirred at r.t. for 30 min. The solvent was then removed under reduced pressure and the residue purified by flash chromatography with a DCM/MeOH eluant mixture, by increasing the polarity from only DCM to 9:1 DCM/MeOH. Yield 1.02 g (83%) of yellow oil.

Synthesis of MeO-Fum-Ser(^{t}Bu)-NH-Cy

Monomethyl fumarate (1.0 g, 7.7 mmol) and HOAt (1.05 g, 7.7 mmol) were dissolved in dry CH_3CN (25 ml) and EDC·HCl (1.48 g, 7.7 mmol) was added. After 10 min, H_2N-Ser(^{t}Bu)-NH-Cy (1.25 g, 5.1 mmol) was to the reaction mixture and DIPEA was used to reach basic pH. The reaction mixture stirred at r.t. overnight. The solvent was removed under reduced pressure and the residue dissolved in AcOEt. The organic phase was washed with $KHSO_{4(aq)}$ (5%), $NaHCO_{3(aq)}$ (5%) and brine, dried over Na_2SO_4, filtered, concentrated to dryness, and precipitated from AcOEt/hexane.

Yield 1.13 g (61%) of white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01-6.92 (m, 2H), 6.82 (d, J = 15.4 Hz, 1H), 6.62 (d, J = 8.0 Hz, 1H), 4.46-4.36 (m, 1H), 3.85 - 3.73 (m, 1H), 3.79 (s, 3H), 3.28 (t, J = 8.8 Hz, 1H), 1.88 (dt, J = 12.4, 4.1 Hz, 2H), 1.77 (s, 3H), 1.73-1.57 (m, 3H), 1.45-1.29 (m, 2H), 1.26 (s, 1H), 1.21 (s, 9H), 1.21-1.09 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.8, 166.0, 163.4, 136.2, 130.6, 74.6, 61.3, 53.1, 52.4, 48.5, 33.1, 33.0, 27.6, 25.6, 24.7, 24.7.

MS (ESI-TOF): $[M+Na]^+$ m/z calcd. for $C_{18}H_{30}N_2O_5$, 377.2047; found 377.2043. FT-IR $\tilde{\nu}_{max}$ (cm^{-1}) 3277, 3080, 1731, 1647, 1635, 1533, 1475.

Synthesis of HO-Fum-Ser(^{t}Bu)-NH-Cy

MeO-Fum-Ser(^{t}Bu)-NH-Cy (586 mg, 1.65 mmol) was dissolved in THF (12 ml) and a solution of LiOH·H_2O (277 mg, 6.6 mmol) in water (12 ml) was added. The solution was stirred at r.t. for 1 h. The mixture was then acidified with solid $KHSO_4$, saturated with brine and extracted with AcOEt. The organic phase was dried over Na_2SO_4, filtered and concentrated to dryness.

Yield 423 mg (75%) of white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (d, J = 8.3 Hz, 1H, NH_{Ser}), 7.87 (d, J = 7.9 Hz, 1H, Cy-NH), 7.13 (d, J = 15.5 Hz, 1H, fumarate =CH-), 6.51 (d, J = 15.5 Hz, 1H, fumarate =CH-), 4.43 (m, 1H, H^{α}_{Ser}), 3.54 (m, 1H, Cy-CH), 3.43 (m, 2H, H^{β}_{Ser}), 1.79-1.61 (m, 4H, Cy), 1.60-1.48 (m, 1H,Cy), 1.10 (m, 14H, ^{t}Bu and Cy). ^{13}C NMR (101 MHz, DMSO-d_6) 168.2, 166.5, 162.9, 137.0, 129.9, 72.8, 62.0, 53.6, 47.6, 32.4, 32.2, 27.2, 25.2, 24.6, 24.5. MS (ESI-TOF): $[M+Na]^+$ m/z calcd. for $C_{17}H_{28}N_2O_5$, 363.1890; found 363.1922. FT-IR $\tilde{\nu}_{max}$ (cm^{-1}) 3283, 3091, 1705, 1644, 1633, 1552, 1476.

General procedure for solid phase peptide synthesis.

Rinke-amide resin (0.65 mmol/g) was added to a vessel. The resin was swelled in DMF and then Fmoc-protecting group was removed with 20% piperidine solution in DMF (40 min). After filtering, the resin was washed with DMF, DCM and DMF. A solution of the amino acid Fmoc-AA-OH (3 eq.), HATU (3 eq.), HOAt (2.7 eq) and DIPEA (5 eq.) in DMF was added to the peptide synthesis vessel and shaken for 1h and 30 minutes. The resin was filtered, and washed with DMF, DCM and DMF. Iterative cycles of Fmoc deprotection and coupling were carried out with 20% piperidine solution in DMF. Unreacted sites were capped by treatment with an 18:4:1 DMF/Ac_2O/DIPEA mixture for 20 minutes. After filtering, the resin was washed with DCM. Then, the resin was treated with DMF and then with 20% piperidine solution in DMF and shaken for 20 minutes twice. After filtering, the resin was washed with DMF, DCM and DMF. For the last coupling of **3,** on the resin a solution of HO-Fum-Ser(^{t}Bu)-NH-Cy (2.3 eq.), HATU (2.3 eq.), HOAt (2 eq) and DIPEA (2.3 eq.) in DMF was added to the peptide synthesis vessel and shaken for 1h and 30 minutes. The resin was filtered, and washed with DMF, DCM and DMF. The resin was acetylated prior to cleavage. Cleavage of the peptide sequence from the resin was performed with a mixture of TFA:H_2O:TIS 95:2.5:2.5 (15 ml) standing for 1 h and washing with DCM; the filtrated was collected and evaporated under reduced pressure. The residue was triturated in cold Et_2O, centrifuged and lyophilized.

(**1**) Yield 370 mg (96%) of white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (s, 1H, $^{Ar_{His}}$), 8.42 (s, 1H, $^{NH_{Aib}}$), 8.27 (s, 1H, $^{NH_{Aib}}$), 8.16 (d, J = 7.7 Hz, 1H, $^{NH_{His}}$), 7.89 (d, J = 7.0 Hz, 1H, $^{NH_{Asp}}$), 7.78 (d, J = 6.2 Hz, 1H, $^{NH_{Ala}}$), 7.62 (d, J = 6.8 Hz, 1H, $^{NH_{Ala}}$), 7.54 (s, 1H, $^{NH_{Aib}}$), 7.21 (s, 1H, $^{Ar_{His}}$), 6.80 (s, 2H, $CONH_2$), 4.46 – 4.29 (m, 2H, H^{α}_{Asp} and H^{α}_{His}), 4.11 (m, 2H, H^{α}_{Ala}), 3.18 (dd, J = 15.2, 4.3 Hz, 1H, H^{β}_{His}), 3.02 (dd, J = 15.2, 9.9 Hz, 1H, H^{β}_{His}), 2.75 (dd, J = 16.7, 4.7 Hz, 1H, H^{β}_{Asp}), 2.64 (dd, J = 16.7, 8.5 Hz, 1H, H^{β}_{Asp}), 1.87 (s, 3H, CH_3-acetamide), 1.40 - 1.15 (m, 24H, Ala and Aib CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 176.3, 174.5, 174.5, 173.4, 172.2, 171.4, 170.7, 170.6, 170.6, 134.1, 55.9, 55.8, 55.7, 49.2, 49.1, 35.0, 25.7, 25.4, 25.2, 24.6, 24.5, 24.4, 23.1, 17.1, 16.6. MS (ESI-TOF): $[M+H]^+$ m/z calcd. for $C_{30}H_{48}N_{10}O_{10}$, 709.3628; found 709.3666. FT-IR $\tilde{\nu}_{max}$ (cm^{-1}) 3314, 3055, 1663, 1540, 1458.

(**2**) Yield 568 mg (92%) of white solid. ^{1}H NMR (400 MHz, DMSO-d_6) 1H NMR (400 MHz, DMSO-d6) δ 8.81 (s, 1H, $^{Ar_{His}}$), 8.55 (s, 1H, $^{NH_{Aib}}$), 8.22 (s, 1H, $^{NH_{Aib}}$), 8.01 (d, J = 6.1 Hz, 1H, $^{NH_{Ser}}$), 7.92 (d, J = 6.4 Hz, 1H, $^{NH_{Ala}}$), 7.84 (d, J = 6.7 Hz, 1H, $^{NH_{Asp}}$), 7.79 – 7.56 (m, 5H, $2 \cdot {}^{NH_{Ala}}$,$2 \cdot {}^{NH_{Aib}}$, $^{NH_{His}}$), 7.32 (s, 1H, $^{Ar_{His}}$), 6.78 (s, 2H, $CONH_2$), 5.10 (bs, 1H, Ser-OH), 4.42 (m, 1H, H^{α}_{His}), 4.34 (m, 1H, H^{α}_{Asp}), 4.18 – 3.96 (m, 4H, H^{α}_{Ala} and H^{α}_{Ser}), 3.78 – 3.62 (m, 2H, H^{β}_{Ser}), 3.21 (dd, J = 15.3, 4.4 Hz, 1H, H^{β}_{His}), 2.99 (dd, J = 15.3, 10.2 Hz, 1H, H^{β}_{His}), 2.79 – 2.59 (m, 2H, H^{β}_{Asp}), 1.89 (s, 3H, CH_3-acetamide), 1.37 – 1.21 (m, 33H, Ala and Aib CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ 176.3, 175.6, 174.6, 173.6, 173.4, 172.0, 171.4, 171.2, 170.9, 170.7, 170.5, 133.9, 116.9, 72.9, 60.4, 57.5, 56.0, 55.9, 55.8, 55.8, 52.6, 50.6, 50.0, 49.6, 49.2, 35.1, 27.2, 26.2, 25.6, 25.4, 25.3, 24.6, 24.1, 24.1, 24.0, 23.1, 17.0, 16.6, 16.4. MS (ESI-TOF): $[M+H]^+$ m/z calcd. for $C_{40}H_{65}N_{13}O_{14}$, 952.4847 m/z; found 952.4841. FT-IR $\tilde{\nu}_{max}$ (cm^{-1}) 3319, 1664, 1541, 1458.

(**3**) Yield 449 mg (92%) of white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.39 (bs, 1H) 8.96 (s, 1H, $^{Ar_{His}}$), 8.74 (d, J = 8.0 Hz, 1H, $^{NH_{His}}$), 8.53 (m, 2H, $^{NH_{Aib}}$ and $^{NH_{Ser}}$), 8.38 (d, J = 5.8 Hz, 1H, $^{NH_{Ala}}$), 7.90 (d, J = 7.3 Hz, 1H, $^{NH_{Asp}}$), 7.79 (d, J = 7.9 Hz, 1H, Cy-NH), 7.69 (s, 1H, $^{NH_{Aib}}$), 7.64 (d, J = 6.6 Hz, 1H, , $^{NH_{Ala}}$), 7.35 (s, 1H, $^{Ar_{His}}$), 7.01 – 6.83 (m, 4H, $CONH_2$ and fumarate =CH-), 4.73 (td, J = 8.1, 5.2 Hz, 1H, $^{H^{\alpha}_{His}}$), 4.36 (m, 2H, $^{H^{\alpha}_{Ser}}$ and $^{H^{\alpha}_{Asp}}$), 4.21 (m, 1H, $^{H^{\alpha}_{Ala}}$), 4.09 (m, 1H, $^{H^{\alpha}_{Ala}}$), 3.53 (m, 3H, Cy-CH and $^{H^{\beta}_{Ser}}$), 3.13 (dd, J = 15.4, 5.1 Hz, 1H, $^{H^{\beta}_{His}}$), 2.99 (dd, J = 15.4, 8.3 Hz, 1H, $^{H^{\beta}_{His}}$), 2.81 – 2.60 (m, 2H, $^{H^{\beta}_{Asp}}$), 1.74 – 1.51 (m, 6H), 1.42 – 0.92 (m, 22H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 176.4, 174.3, 173.3, 172.3, 171.6, 170.7, 169.9, 168.5, 164.0, 163.52,133.8, 133.4, 132.3, 129.2, 116.9, 61.9, 55.9, 55.8, 55.5, 51.6, 50.2, 49.2, 47.6, 35.1, 32.4, 32.3, 27.1, 25.6, 25.3, 25.2, 24.6, 24.6, 24.6, 24.4, 17.14, 17.0. MS (ESI-TOF): $[M+H]^+$ m/z calcd. for $C_{37}H_{57}N_{11}O_{12}$, 848.4261; found, 848.4269. FT-IR $\tilde{\nu}_{max}$ (cm^{-1}) 3306, 3054, 1657, 1535, 1452.

(**3**) ^{1}H NMR (400 MHz, H_2O/D_2O) δ 8.81 (d, J = 7.4 Hz, 1H, $^{NH_{His}}$), 8.63 (d, J = 6.9 Hz, 1H, $^{NH_{Ser}}$), 8.48 (d, J = 1.5 Hz, 1H, $^{Ar^{C2}_{His}}$), 8.45 – 8.37 (m, 2H, $^{NH_{Aib1}}$ and $^{NH_{Ala1}}$), 8.02 – 7.97 (m, 1H, $^{NH_{Asp}}$), 7.95 (s, 1H, $^{NH_{Aib2}}$), 7.90 (d, J = 8.6 Hz, 1H, Cy-NH), 7.83 (d, J = 6.1 Hz, 1H, $^{NH_{Ala2}}$), 7.21 (s, 1H, $CONH_2$), 7.18 (s, 1H, $^{Ar^{C4}_{His}}$), 6.85 – 6.71 (m, 3H, $CONH_2$ and fumarate =CH-), 4.30 (m, 1H, $^{H^{\alpha}_{Ser}}$), 4.18 – 4.02 (m, 1H, $^{H^{\alpha}_{Ala}}$), 3.77 – 3.65 (m, 2H, $^{H^{\beta}_{Ser}}$), 3.54 – 3.39 (m, 1H, Cy-CH), 3.26 – 3.01 (m, 2H, $^{H^{\beta}_{His}}$), 2.86 – 2.67 (m, 2H, $^{H^{\beta}_{Asp}}$), 1.71 – 1.40 (m, 6H, Cy), 1.34 – 1.28 (m, 12H, Aib CH_3), 1.24 (d, J = 7.2 Hz, 6H, Ala CH_3), 1.21 – 0.96 (m, 4H, Cy).

(**3**) ^{1}H NMR (400 MHz, CD_3OH) δ 8.84 (d, J = 7.9 Hz, 1H, $^{NH_{His}}$), 8.77 (s, 1H, $^{Ar_{His}}$), 8.75 (s, 1H, $^{NH_{Aib}}$), 8.61 – 8.52 (m, 2H, $^{NH_{Ser}}$ and $^{NH_{Ala}}$), 8.27 (s, 1H, $^{NH_{Aib}}$), 8.13 (d, J = 7.3 Hz, 1H, $^{NH_{Asp}}$), 7.99 – 7.89 (m, 2H, $^{NH_{Ala}}$ and Cy-NH), 7.38 – 7.31 (m, 2H,), $^{Ar_{His}}$ and $CONH_2$) 7.04 – 6.85 (m, 3H, fumarate =CH- and $CONH_2$), 4.45 (m, 2H, $^{H^{\alpha}_{Ser}}$ and $^{H^{\alpha}_{Asp}}$), 4.25 (m, 1H, $^{H^{\alpha}_{Ala}}$), 3.79 (d, J = 5.6 Hz, 1H, $^{H^{\beta}_{Ser}}$), 3.72 – 3.59 (m, 1H, Cy-CH), 3.41 – 3.29 (m, 8H, $^{H^{\beta}_{His}}$ and CD_3OH residual peak), 3.24 – 3.13 (m, 1H, $^{H^{\beta}_{His}}$), 3.04 – 2.81 (m, 2H, $^{H^{\beta}_{Asp}}$), 1.93 – 1.57 (m, 6H, Cy), 1.50 – 1.43 (m, 12H, Aib CH_3), 1.42 – 1.36 (m, 6H, Ala CH_3), 1.36 – 1.17 (m, 4H, Cy).

(**3**) ^{1}H NMR (400 MHz, CD_3CN) δ 8.46 (d, J = 1.4 Hz, 1H), 7.91 (d, J = 7.6 Hz, 1H), 7.63 (m, 3H), 7.49 (m, 2H), 7.27 (s, 1H), 7.21 (s, 1H), 6.97 – 6.83 (m, 2H), 6.82 – 6.77 (m, 1H), 6.69 (s, 1H), 5.86 (s, 1H), 4.81 – 4.71 (m, 1H), 4.42 – 4.26 (m, 2H), 4.20 – 4.08 (m, 2H), 3.85 – 3.58 (m, 4H), 3.38 (m, 1H), 3.14 (m, 1H), 3.00 – 2.78 (m, 2H), 1.85 – 1.56 (m), 1.52 – 1.38 (m), 1.38 – 1.24 (m), 1.28 – 1.17 (m).

(**4**) obtained quantitatively from isomerization of (**3**), white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (d, J = 7.2 Hz, 1H, $^{NH_{His}}$), 8.94 (s, 1H, $^{Ar_{His}}$), 8.75 (d, J = 7.8 Hz, 1H, $^{NH_{Ser}}$), 8.54 (d, J = 5.9 Hz, 1H, $^{NH_{Ala}}$), 8.29 (s, 1H, $^{NH_{Aib}}$), 7.91 (d, J = 7.9 Hz, 1H, Cy-NH), 7.81 (d J = 6.9 Hz, 1H, $^{NH_{Asp}}$), 7.60 (m, 2H, $^{NH_{Aib}}$ and $^{NH_{Ala}}$), 7.36 (s, 1H, $^{Ar_{His}}$), 6.88 – 6.79 (m, 1H, $CONH_2$), 6.32 (dd, J = 12.4 Hz, 2H, maleate =CH-), 4.95 (bs, 1H), 4.52 (td, J = 9.2, 7.2, 4.8 Hz, 1H, $^{H^{\alpha}_{His}}$), 4.41 – 4.31 (m, 1H, $^{H^{\alpha}_{Asp}}$), 4.19 – 4.00 (m, 2H, $^{H^{\alpha}_{Ala}}$), 3.62 (d, J = 5.5 Hz, 2H, $^{H^{\beta}_{Ser}}$), 3.55 – 3.31 (m, 27H, Cy-CH and H_2O), 3.22 – 2.95 (m, 2H, $^{H^{\beta}_{His}}$), 2.78 – 2.58 (m, 2H, $^{H^{\beta}_{Asp}}$), 1.65 (m, 5H, Cy), 1.59 – 1.47 (m, 1H, Cy),

1.38 – 1.05 (m, 22H, Ala and Aib CH_3 and Cy). ^{13}C NMR (101 MHz, DMSO-d_6) δ 176.4, 174.6, 173.7, 172.1, 171.6, 171.6, 170.8, 170.5, 168.2, 166.4, 164.8, 133.9, 132.0, 129.5, 129.2, 117.0, 61.7, 56.0, 55.9, 55.9, 55.8, 55.7, 52.5, 50.5, 49.9, 49.3, 47.8, 35.1, 32.4, 32.3, 25.6, 25.2, 25.2, 24.7, 24.7, 24.0, 17.0, 16.7. FT-IR $\tilde{\nu}_{max}$ (cm^{-1}) 3311, 3054, 1657, 1541, 1453.

(**4**) ^1H NMR (400 MHz, H_2O/D_2O) δ 8.82 (d, J = 7.1 Hz, 1H, $^{NH_{His}}$), 8.51 (d, J = 6.9 Hz, 1H, $^{NH_{Ser}}$), 8.49 – 8.45 (m, 2H, $^{Ar^{C2}_{His}}$ and $^{NH_{Ala1}}$), 8.29 (s, 1H, $^{NH_{Aib1}}$), 7.98 – 7.89 (m, 2H, $^{NH_{Asp}}$ and Cy-NH), 7.85 (s, 1H, $^{NH_{Aib2}}$), 7.76 (d, J = 6.0 Hz, 1H, $^{NH_{Ala2}}$) 7.22 (s, 1H, $CONH_2$), 7.17 (s, 1H, $^{Ar^{C4}_{His}}$), 6.75 (s, 1H, $CONH_2$), 6.26 (s, 1H, maleate =CH-), 4.25 (s, 1H, $^{H^{\alpha}_{Ser}}$), 4.15 – 3.99 (m, 2H, $^{H^{\alpha}_{Ala}}$), 3.76 – 3.68 (m, 1H, $^{H^{\beta}_{Ser}}$), 3.51 – 3.39 (m, 1H, Cy-CH), 3.26 – 2.98 (m, 2H, $^{H^{\beta}_{His}}$), 2.86 – 2.65 (m, 2H, $^{H^{\beta}_{Asp}}$), 1.68 – 1.40 (m, 6H, Cy), 1.34 – 1.21 (m, 18H, Ala and Aib CH_3), 1.21 – 0.99 (m, 4H, Cy).

(**4**) ^1H NMR (400 MHz, CD_3OH) δ 9.04 (d, J = 7.1 Hz, 1H, $^{NH_{His}}$), 8.76 (s, 1H, $^{Ar^{C2}_{His}}$), 8.72 – 8.66 (m, 2H, $^{NH_{Ala1}}$ and $^{NH_{Ser}}$), 8.45 (s, 1H, $^{NH_{Aib1}}$), 8.16 – 7.97 (m, 3H, $^{NH_{Asp}}$, Cy-NH and $^{NH_{Aib2}}$), 7.88 (d, J = 5.9 Hz, 1H, $^{NH_{Ala2}}$), 7.36 (s, 1H, $^{Ar^{C4}_{His}}$), 7.26 (s, 1H, $CONH_2$), 6.85 (s, 1H, $CONH_2$), 6.35 (s, 1H, maleate =CH-), 4.48 – 4.37 (m, 2H, $^{H^{\alpha}_{Asp}}$ and $^{H^{\alpha}_{Ser}}$), 4.26 – 4.10 (m, 1H, $^{H^{\alpha}_{Ala}}$), 3.82 (d, J = 5.3 Hz, 2H, $^{H^{\beta}_{Ser}}$), 3.72 – 3.58 (m, 1H, Cy-CH), 3.44 – 3.30 (m, 9H, $^{H^{\beta}_{His}}$ and CD_3OH residual peak), 3.24 – 3.11 (m, 1H, $^{H^{\beta}_{His}}$), 3.01 – 2.81 (m, 2H, $^{H^{\beta}_{Asp}}$), 1.90 – 1.59 (m, 5H, Cy), 1.52 – 1.40 (m, 18H, Ala and Aib CH_3), 1.40 – 1.18 (m, 5H, Cy).

(**4**) ^1H NMR (400 MHz, Acetonitrile-d_3) δ 8.47 (m, 1H, $^{Ar_{His}}$), 8.27 (d, J = 5.4 Hz, 1H, $^{NH_{Ala}}$), 8.22 (d, J = 6.8 Hz, 1H, $^{NH_{His}}$), 7.67 – 7.56 (m, 2H, $^{NH_{Ala}}$ and), 7.57 – 7.52 (m, 2H, $^{NH_{Aib}}$ and), 7.28 (s, 1H), 7.12 (s, 1H), 6.95 (d, J = 8.1 Hz, 1H), 6.70 (s, 1H), 6.33 – 6.25 (m, 2H), 4.65 – 4.55 (m, 1H, $^{H^{\alpha}_{His}}$), 4.40 – 4.26 (m, 2H, $^{H^{\alpha}_{Asp}}$ and $^{H^{\alpha}_{Ser}}$), 4.17 – 3.99 (m, 2H, $^{H^{\alpha}_{Ala}}$), 3.85 – 3.70 (m, 2H, $^{H^{\beta}_{Ser}}$), 3.70 – 3.59 (m, 1H Cy-CH), 3.36 (dd, J = 15.3, 4.5 Hz, 1H, $^{H^{\beta}_{His}}$), 3.11 (dd, J = 15.3, 9.4 Hz, 1H, $^{H^{\beta}_{His}}$), 2.87 (d, J = 6.8 Hz, 2H, $^{H^{\beta}_{Asp}}$), 1.84 – 1.56 (m, 16H, Cy and CD_3CN residual peak), 1.48–1.34 (m, Aib and Ala CH_3),1.28 – 0.99 (m, Cy).

General procedure for hydrolysis experiments

Substrates solutions for hydrolysis experiments were prepared dissolving 2.5 μmol of the selected ester (*p*-NPA or *p*-NPB) in 10 ml of a 3% v/v DMSO/H_2O solution. Experiments were run in a quartz cuvette at pH 8.80 (40 mM Britton−Robinson buffer, titrated to the appropriate pH with NaOH) and followed by UV-Vis spectroscopy at 25°C while stirring, with substrate (*p*-NPA and *p*-NPB) concentration of 250 μM and foldamers concentration of 20 μM (8% mol).

The cuvette was placed in a Shimadzu model UV-2501 PC spectrophotometer and the hydrolysis of the substrates was monitored at 400 nm, acquiring the UV-Vis spectra every 30 seconds under stirring.

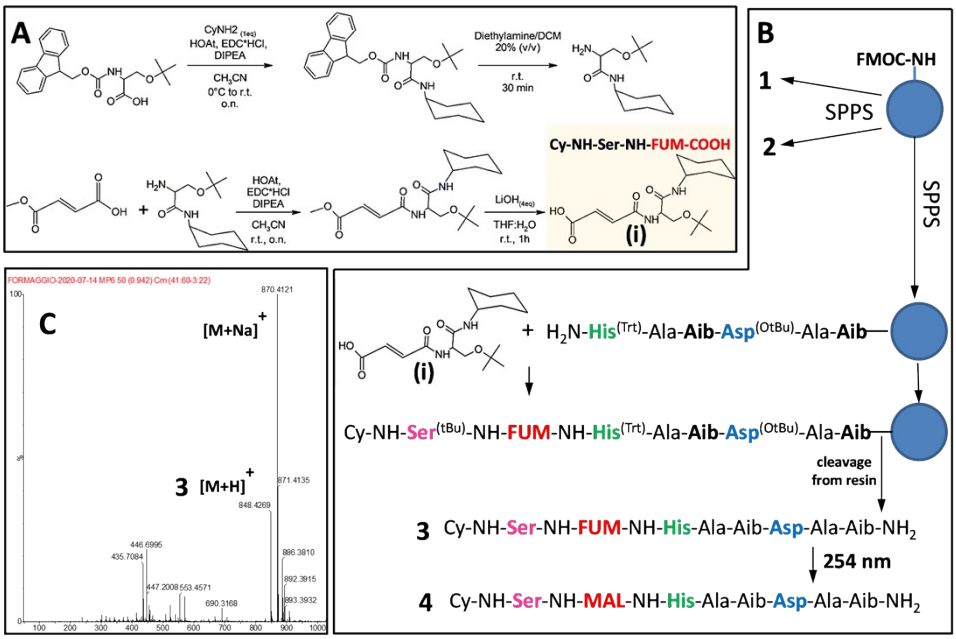

Figure S1: **A** and **B**: synthetic routes for foldamers **1**-**4**. **C**: HRMS spectrum of foldamer **3** after cleavage from the resin.

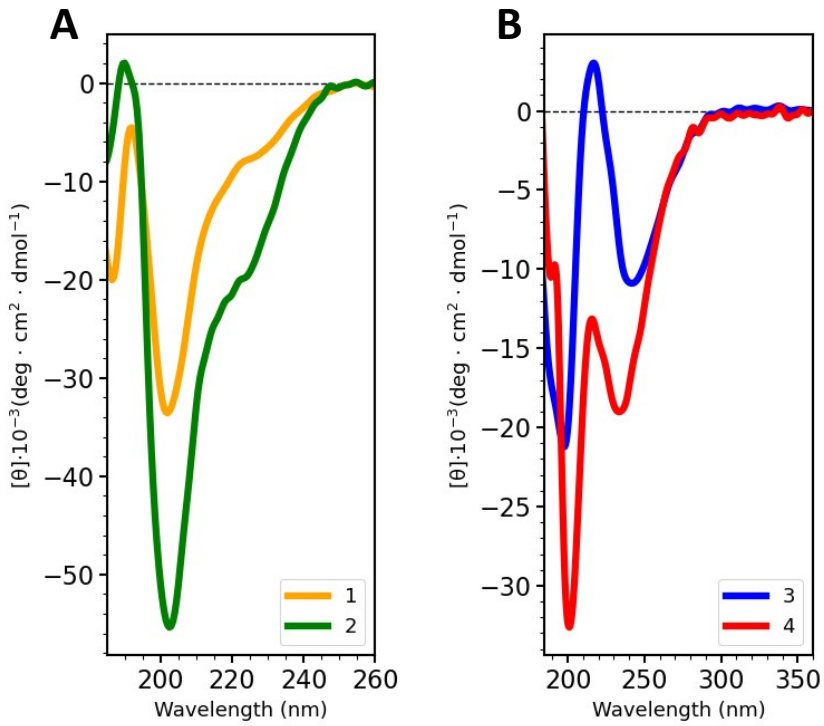

Figure S2. **A:** Comparison of ECD spectra of Compound **1** and **2** in water (0.3 mM). **B:** Comparison of ECD spectra of Compound **3** and **4** in water (0.3 mM).

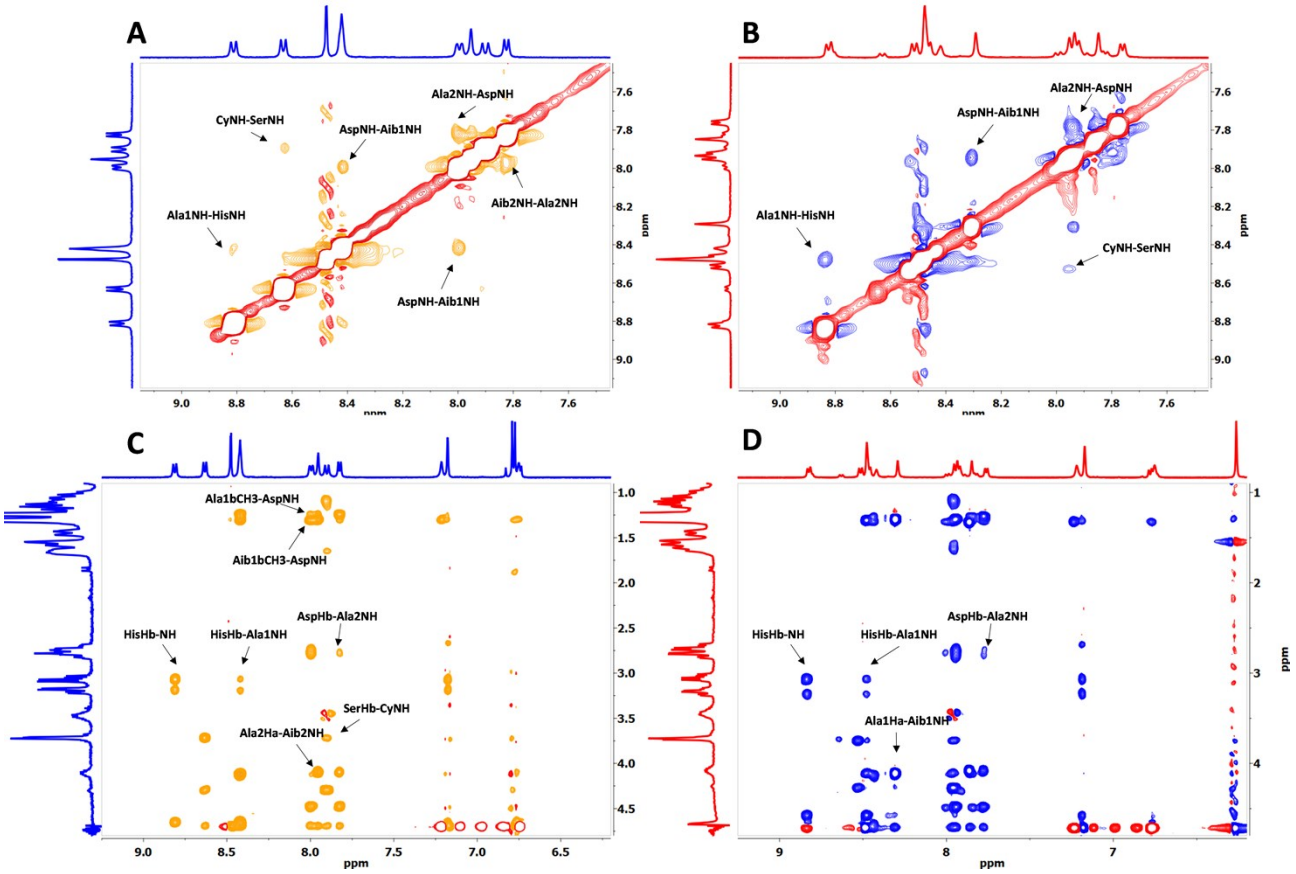

Figure S3. **A:** Series of sequential αNH(i)→ αNH(i + 1) ROESY connectivities for **3**. **B:** Series of sequential αNH(i)→ αNH(i + 1) ROESY connectivities for **4**. **C:** Series of sequential and βCH_3(i) → αNH(i + 1) connectivities for **3**. **D:** Series of sequential and βCH_3(i) → αNH(i + 1) connectivities for **4**.

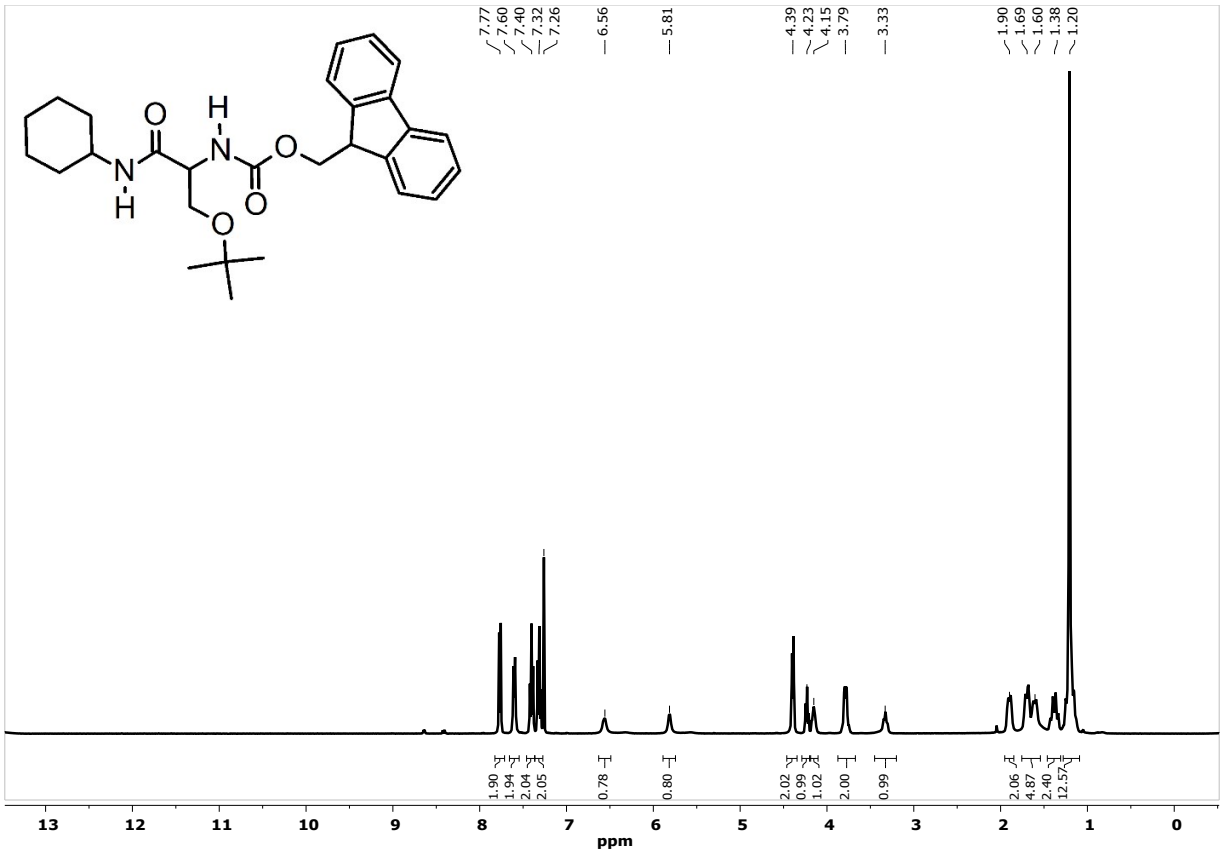

Figure S4: ^{1}H NMR (400 MHz, $CDCl_3$), of **Fmoc-Ser(tBu)-NH-Cy.**

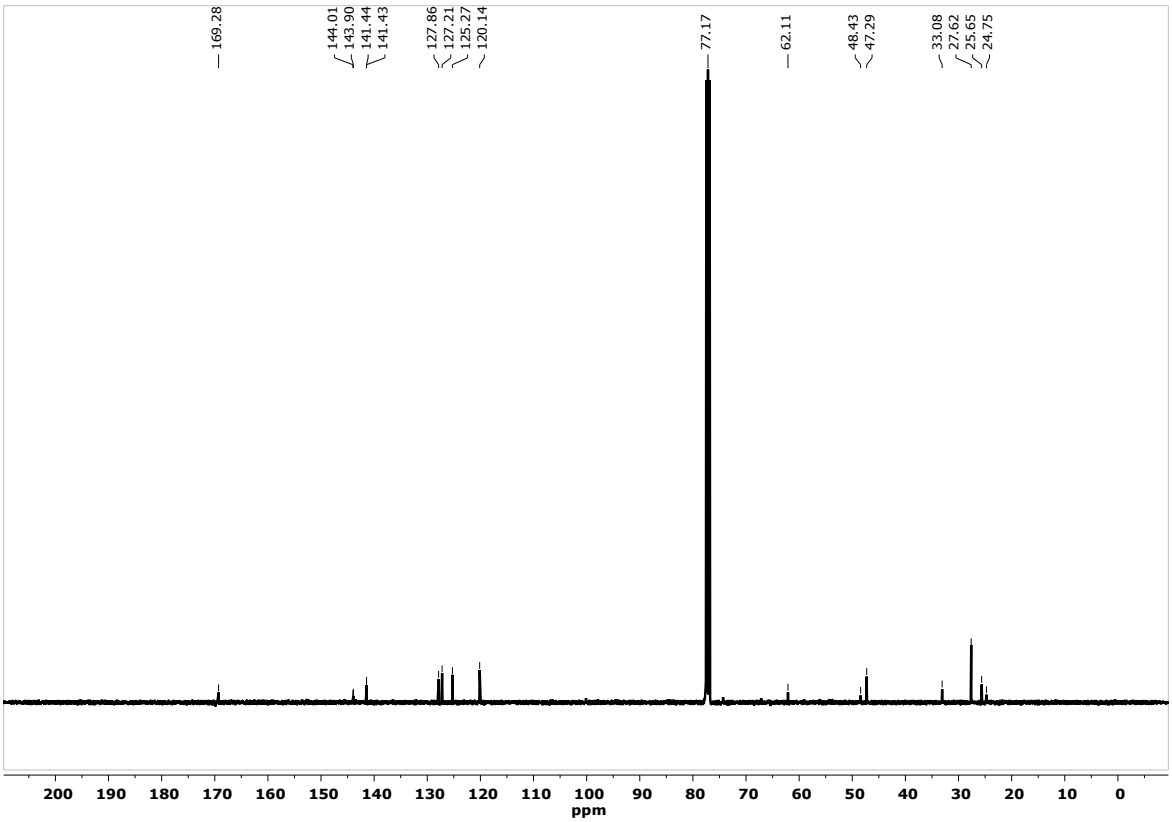

Figure S5: ^{13}C NMR (101 MHz, $CDCl_3$) of **Fmoc-Ser(tBu)-NH-Cy.**

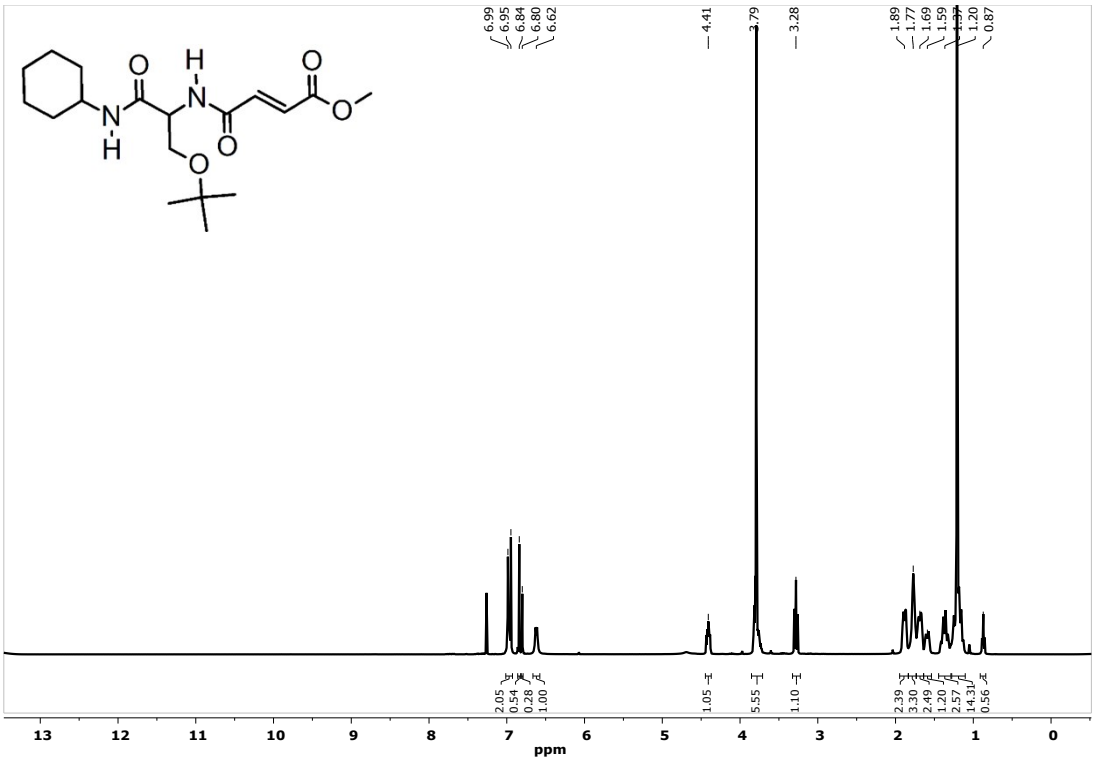

Figure S6: ^{1}H NMR (400 MHz, $CDCl_3$) of **MeO-Fum-Ser(tBu)-NH-Cy.**

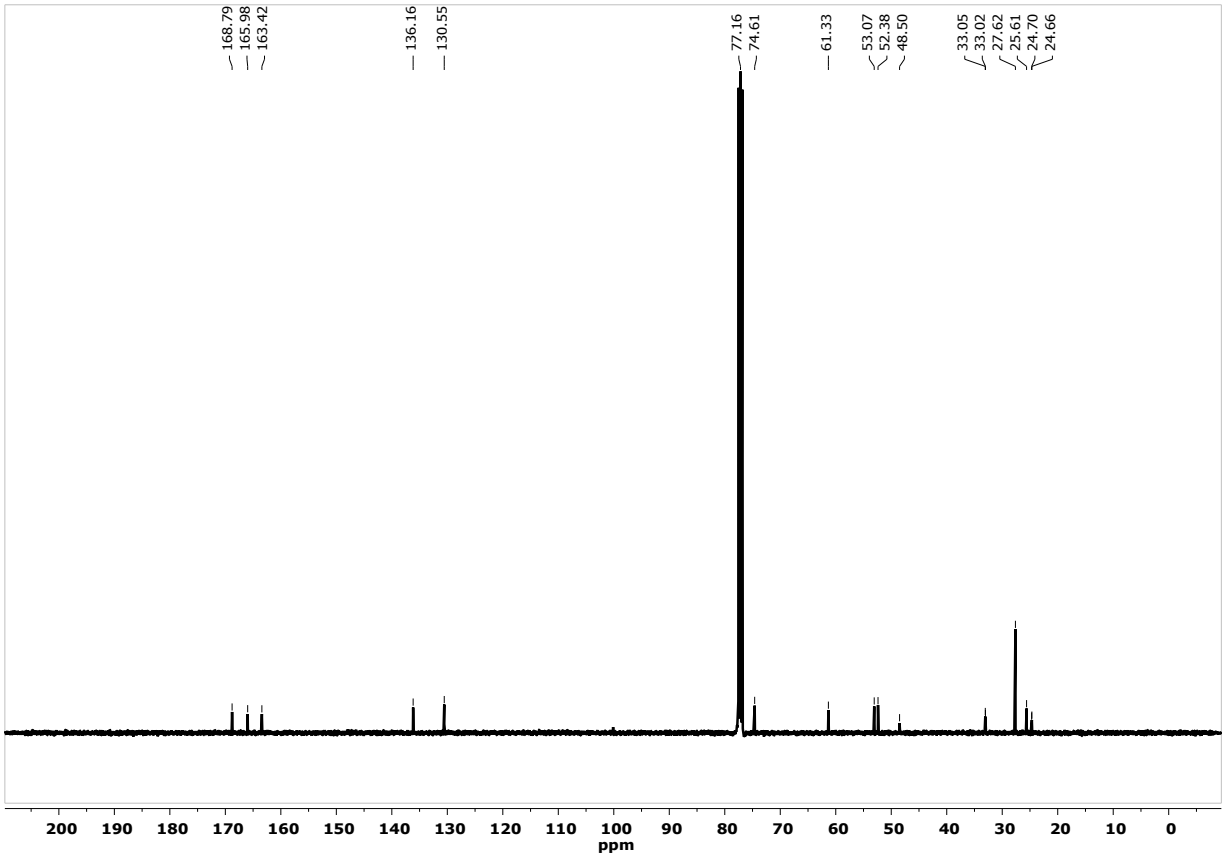

Figure S7: ^{13}C NMR (101 MHz, $CDCl_3$) of **MeO-Fum-Ser(tBu)-NH-Cy.**

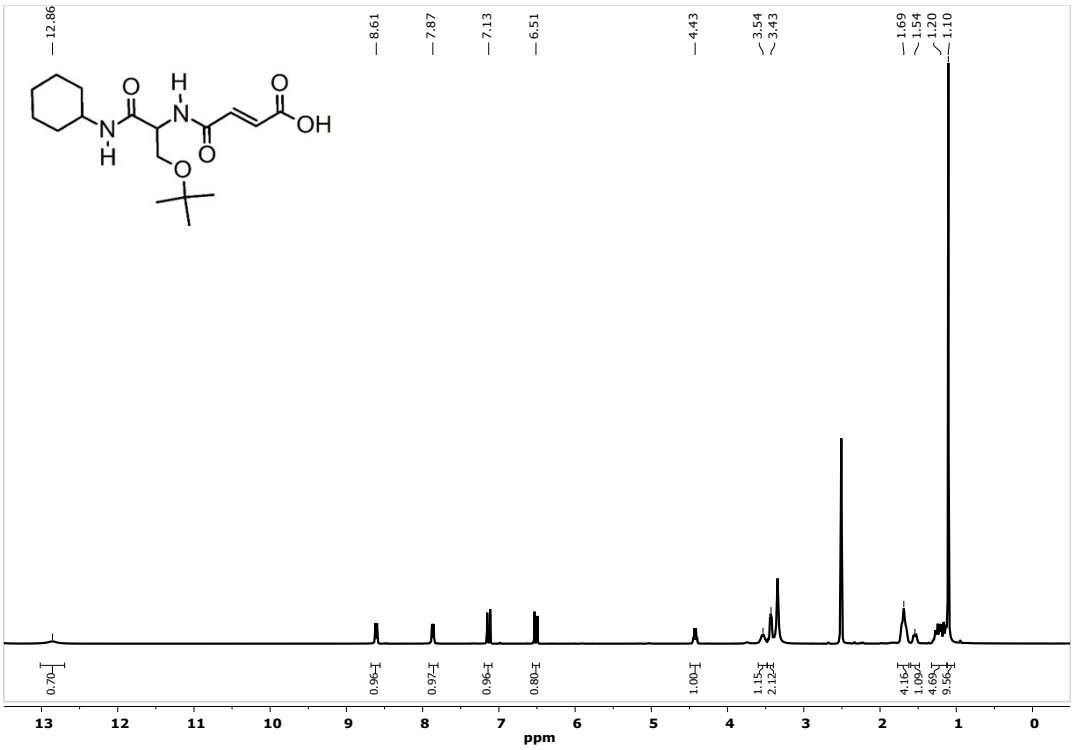

Figure S8: ^{1}H NMR (400 MHz, DMSO-d_6) of **HO-Fum-Ser(tBu)-NH-Cy.**

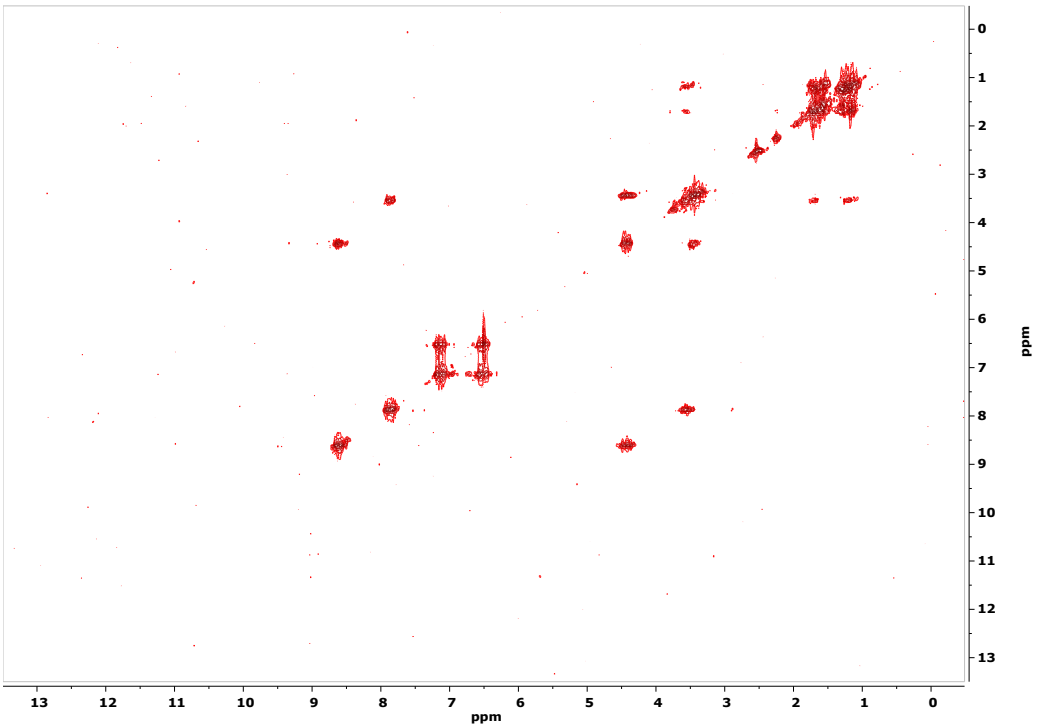

Figure S9: COSY NMR (400 MHz, DMSO-d_6) of **HO-Fum-Ser(tBu)-NH-Cy.**

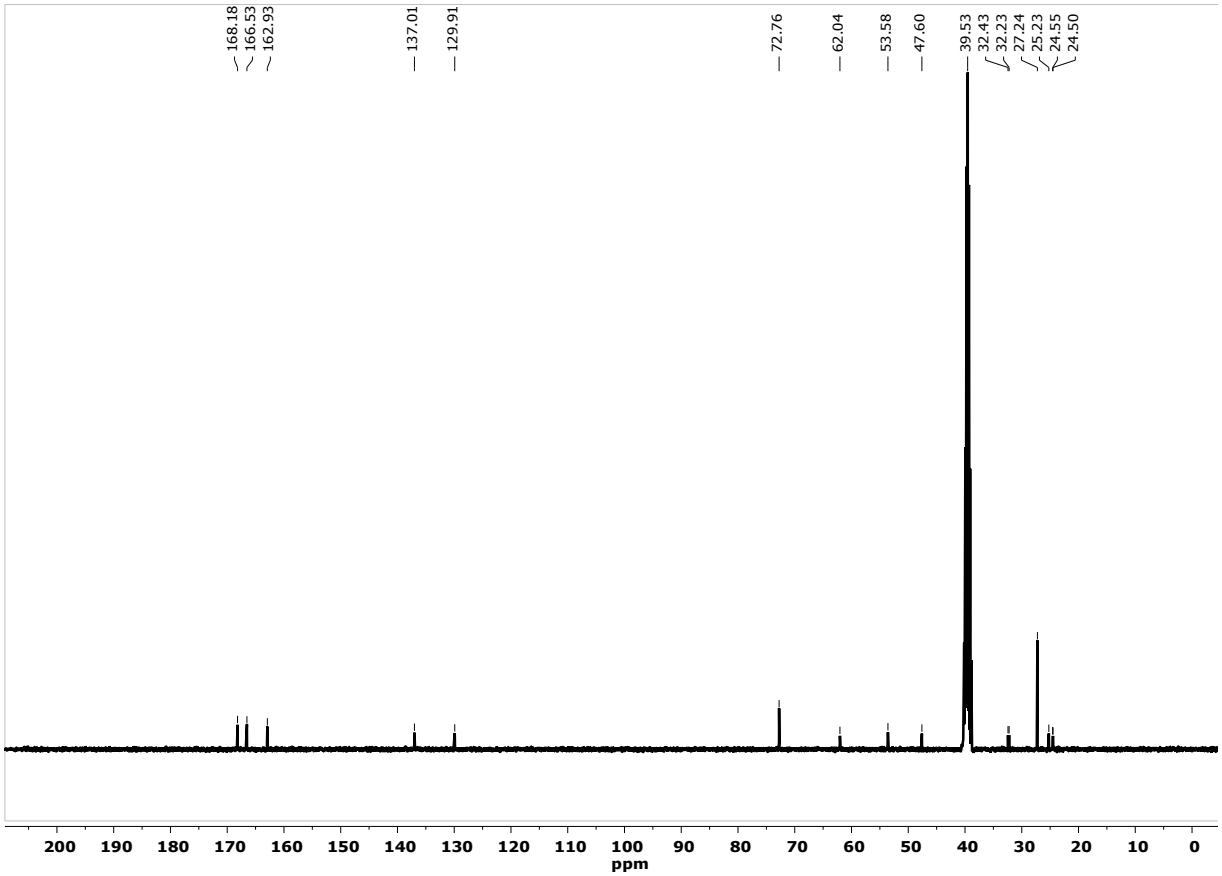

Figure S10: ^{13}C NMR (101 MHz, DMSO-d_6) of **HO-Fum-Ser(tBu)-NH-Cy.**

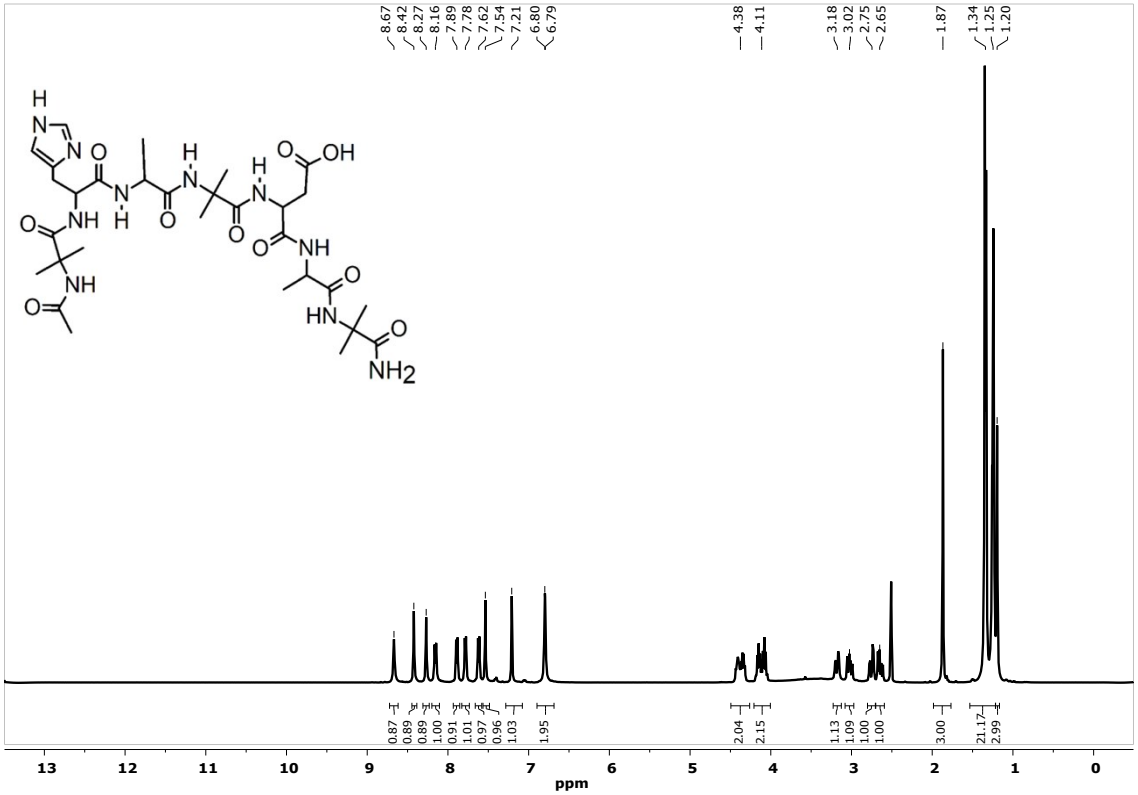

Figure S11: ^{1}H NMR (400 MHz, DMSO-d_6) of **1.**

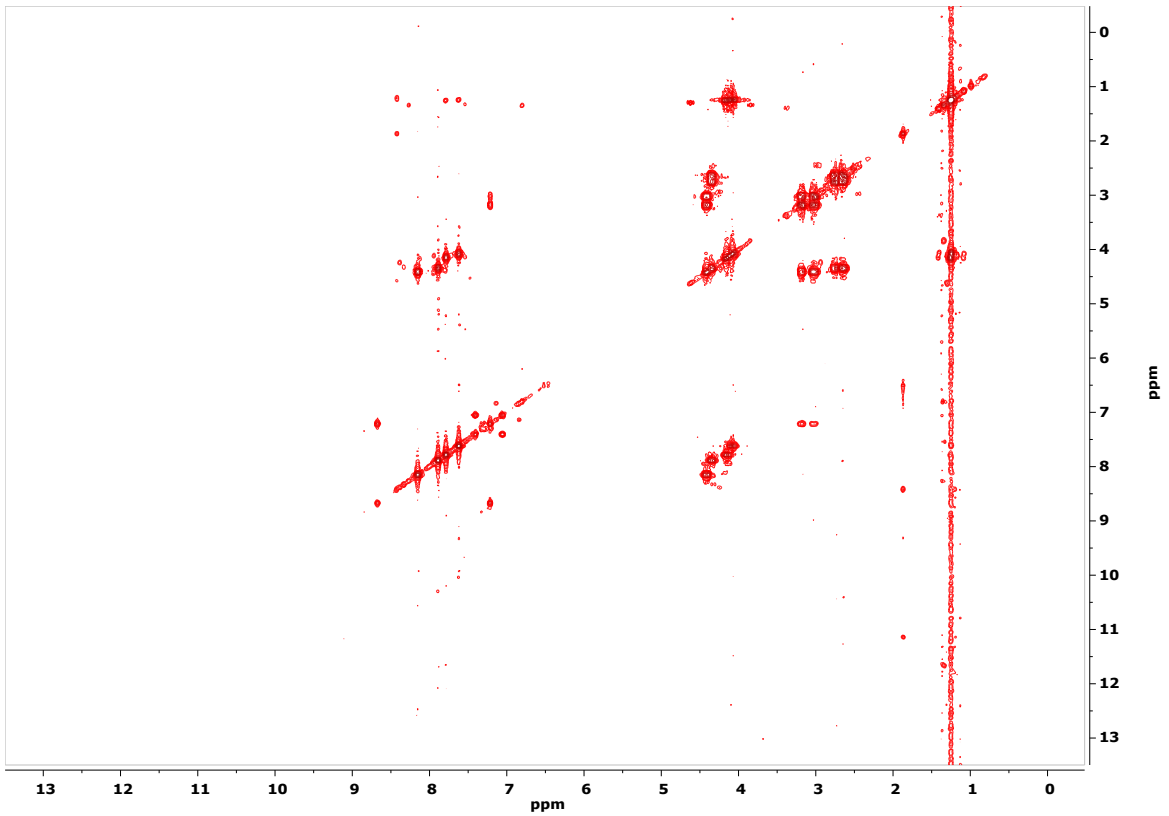

Figure S12: COSY NMR (400 MHz, DMSO-d_6) of **1.**

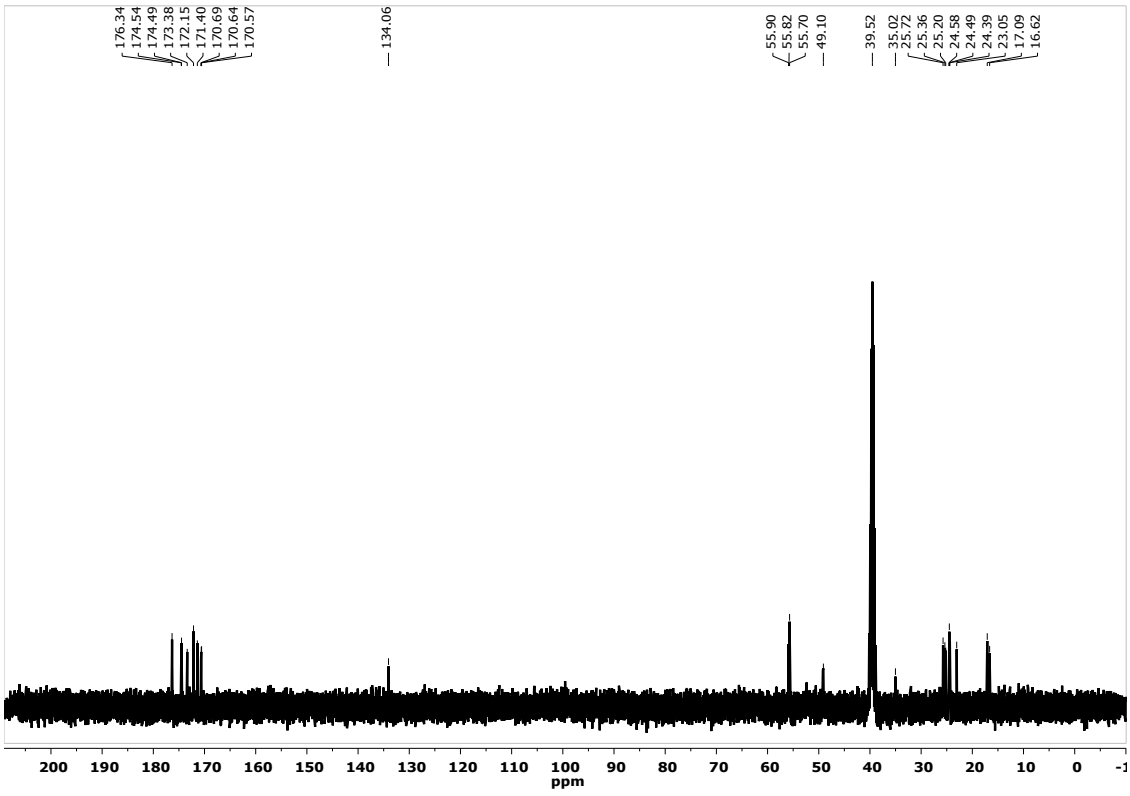

Figure S13: ^{13}C NMR (101 MHz, DMSO-d_6) of **1.**

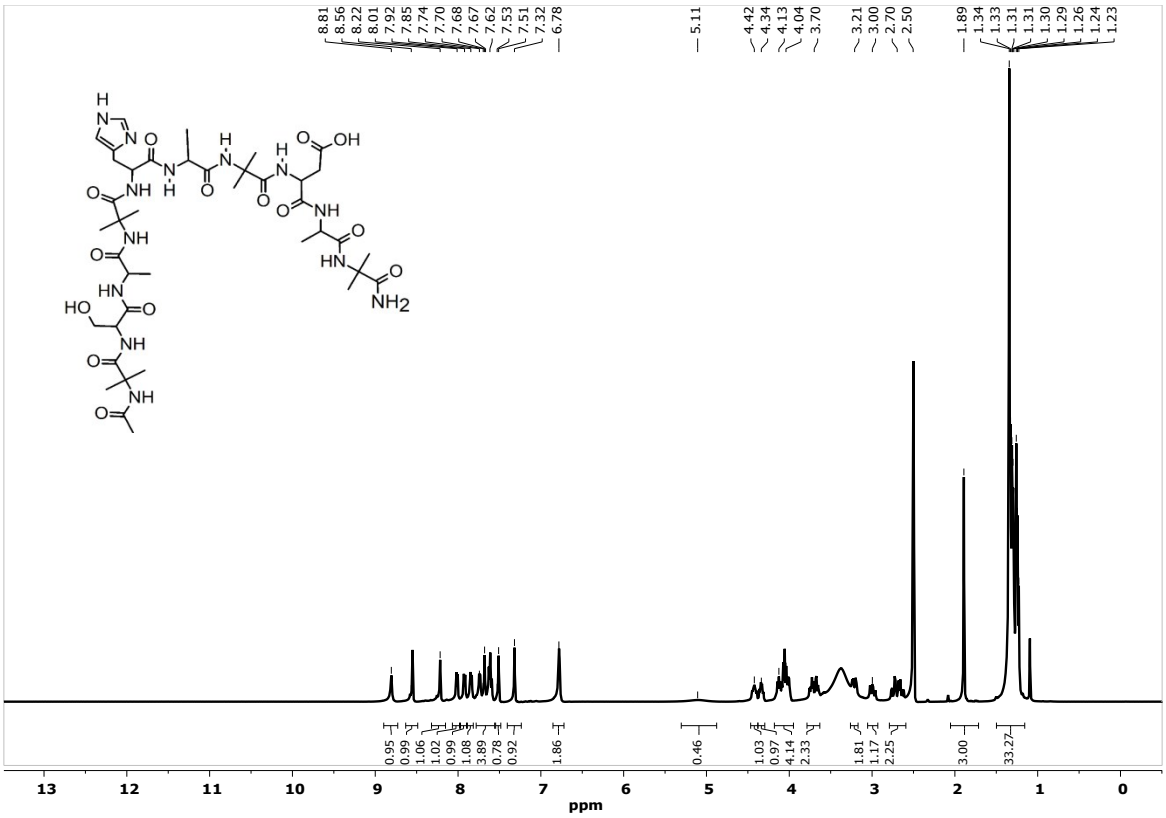

Figure S14: ^{1}H NMR (400 MHz, DMSO-d_6) of **2.**

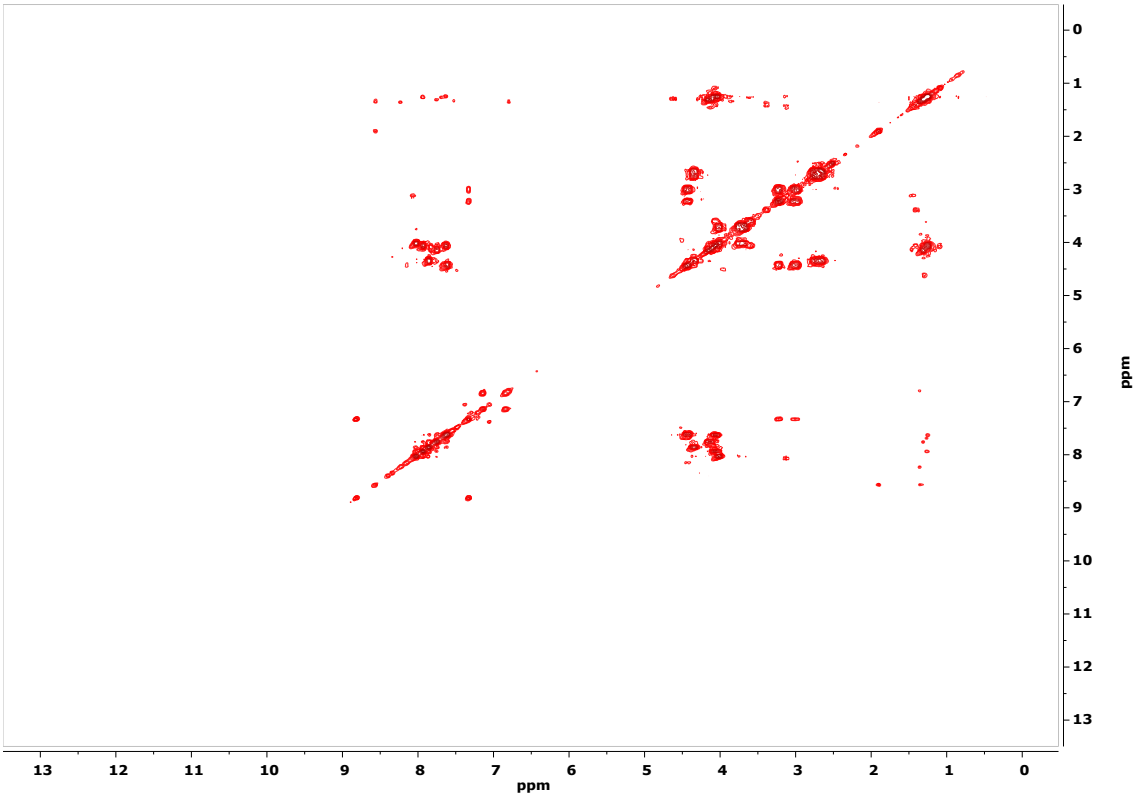

Figure S15: COSY NMR (400 MHz, DMSO-d_6) of **2.**

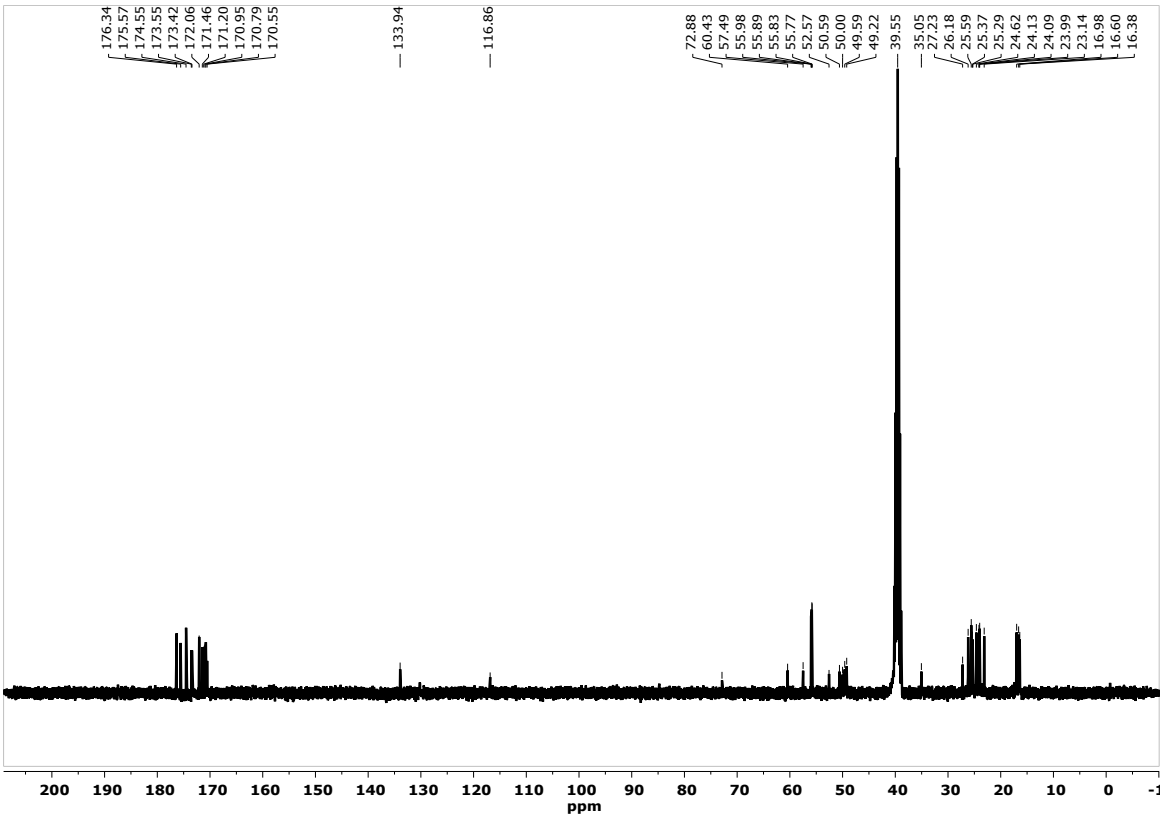

Figure S16: ^{13}C NMR (101 MHz, DMSO-d_6) of **2.**

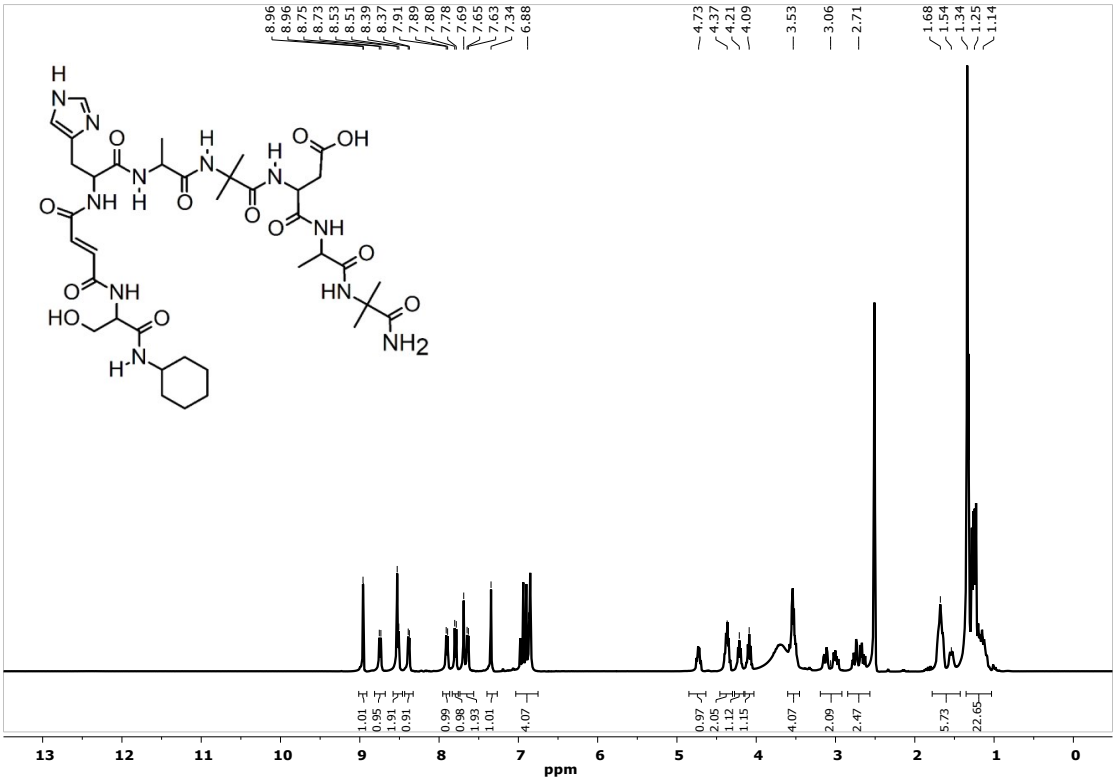

Figure S17: ^{1}H NMR (400 MHz, DMSO-d_6) of **3.**

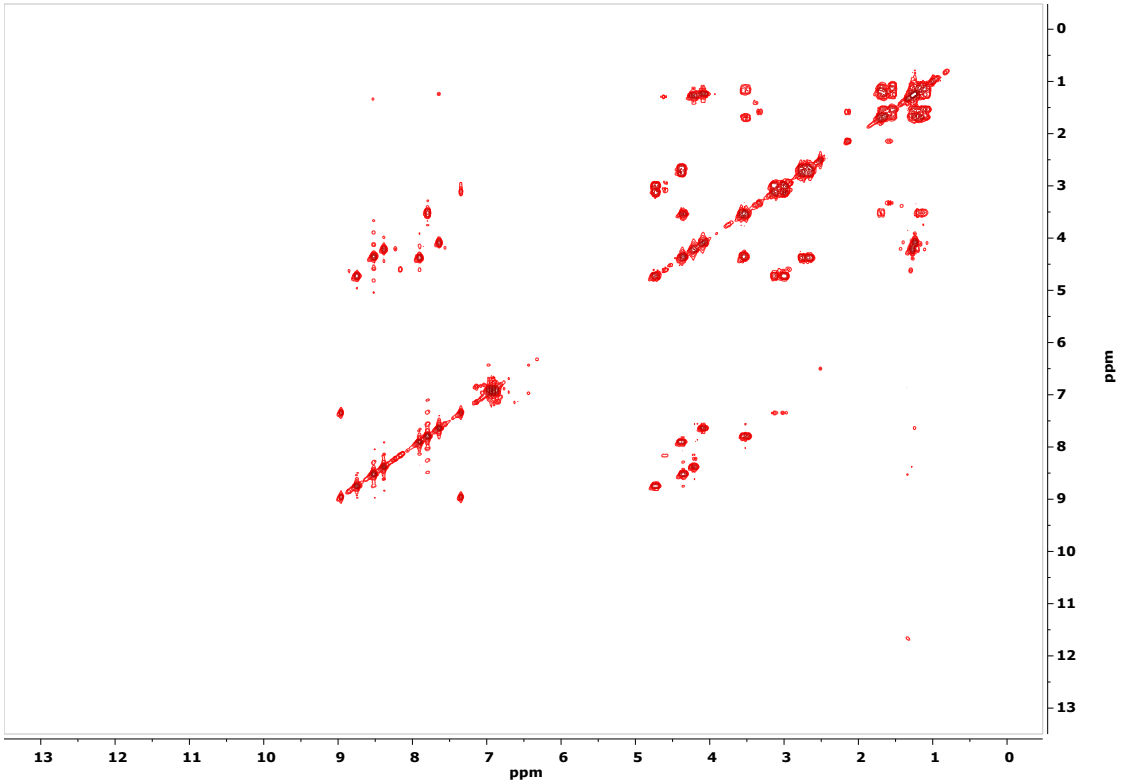

Figure S18: COSY NMR (400 MHz, DMSO-d_6) of **3.**

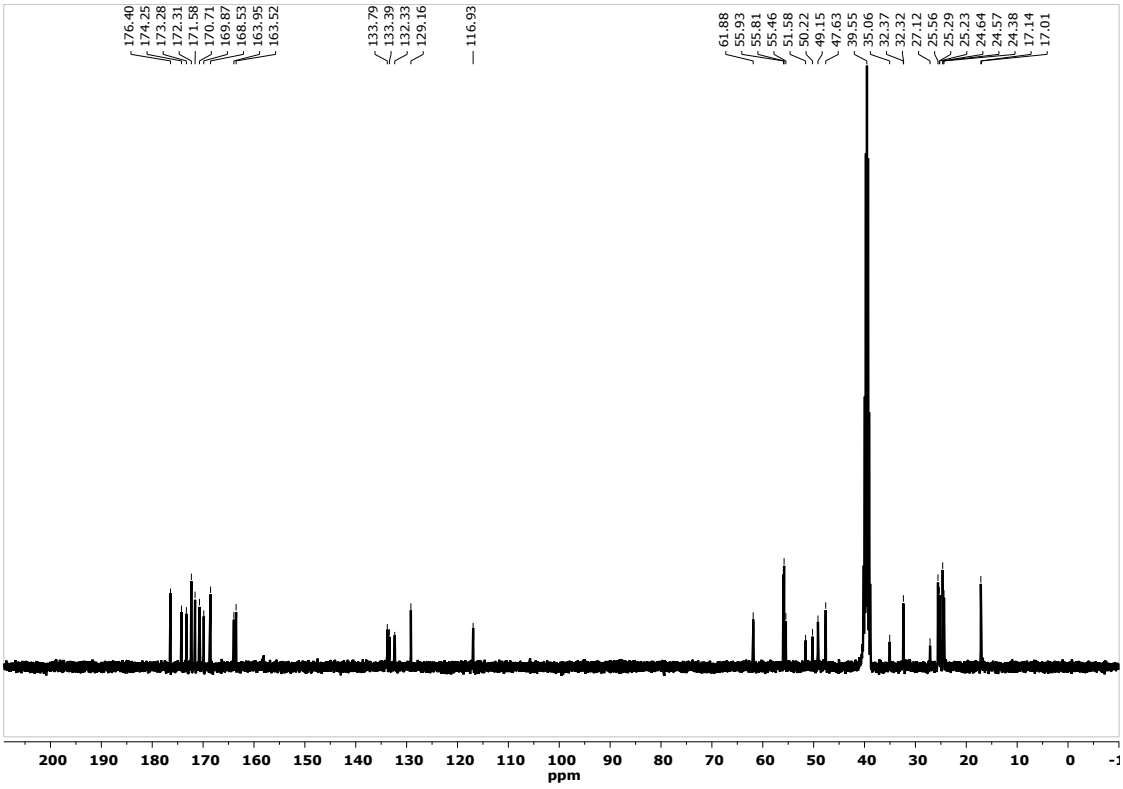

Figure S19: ^{13}C NMR (101 MHz, DMSO-d_6) of **3.**

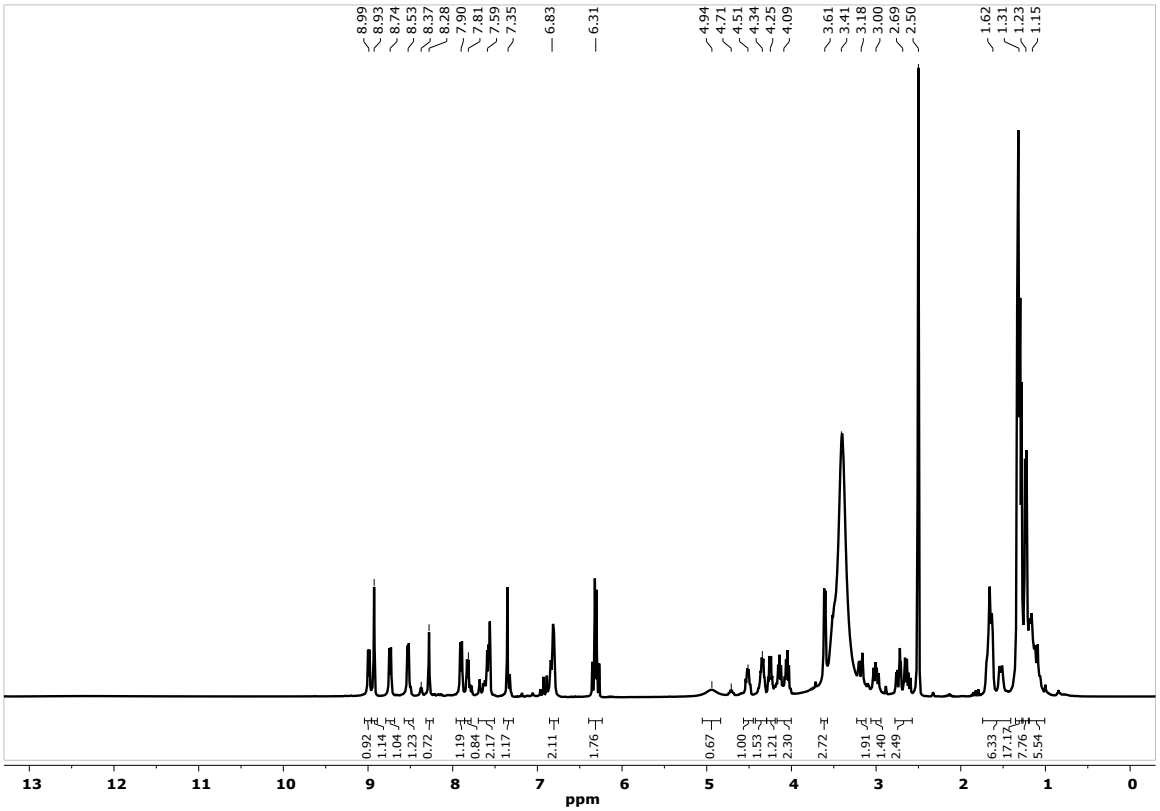

Figure S20: ^{1}H NMR (400 MHz, DMSO-d_6) of **4.**

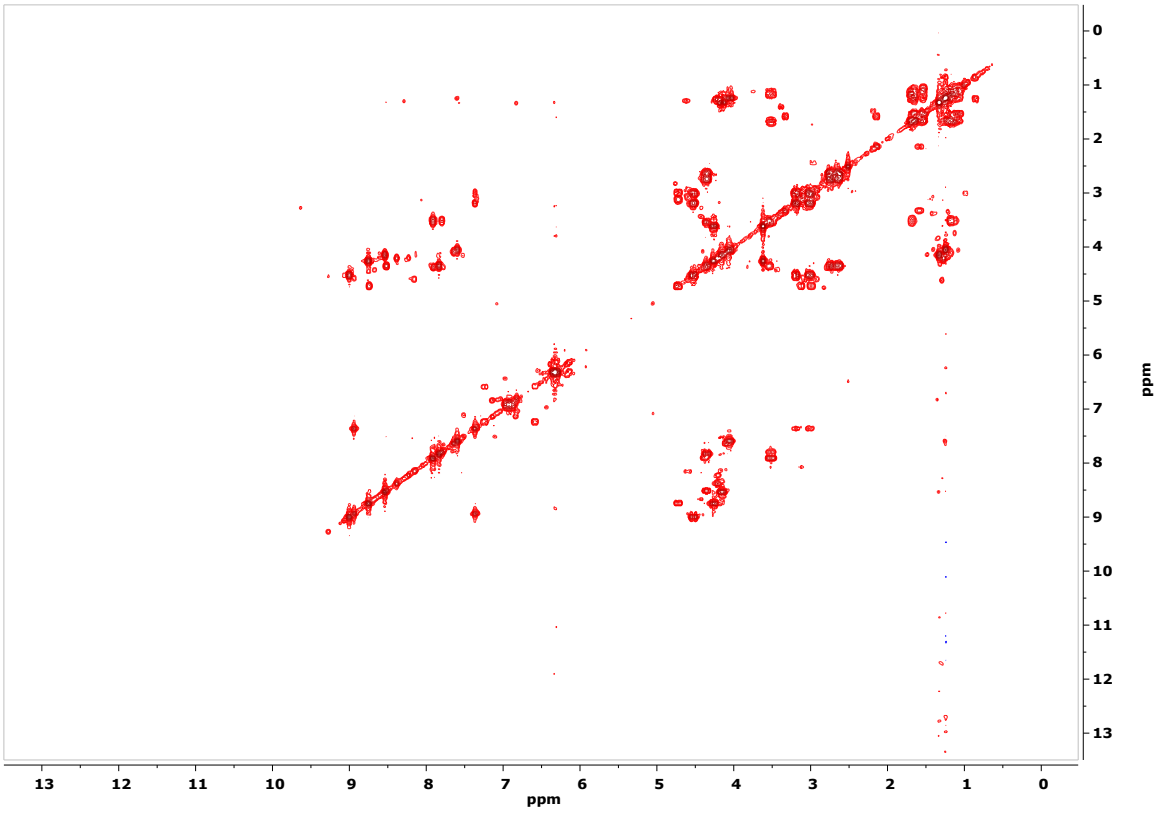

Figure S21: COSY NMR (400 MHz, DMSO-d_6) of **4.**

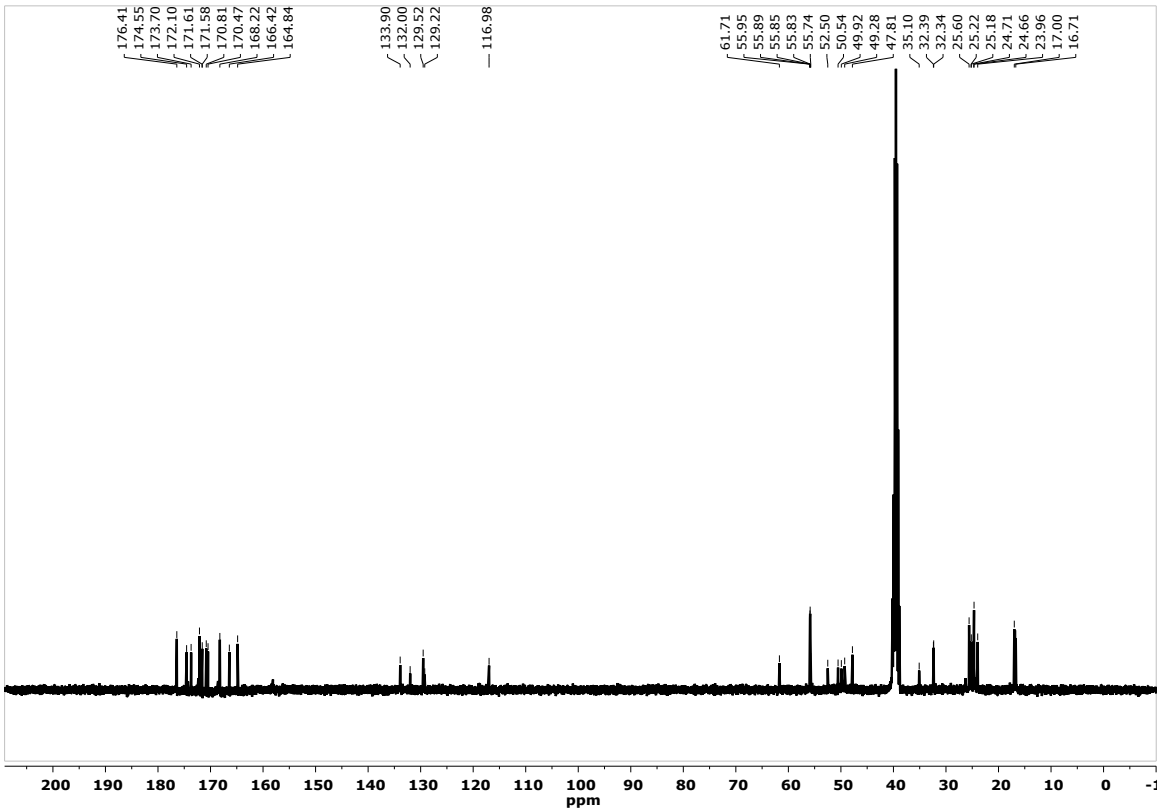

Figure S22: ^{13}C NMR (101 MHz, DMSO-d_6) of **4.**

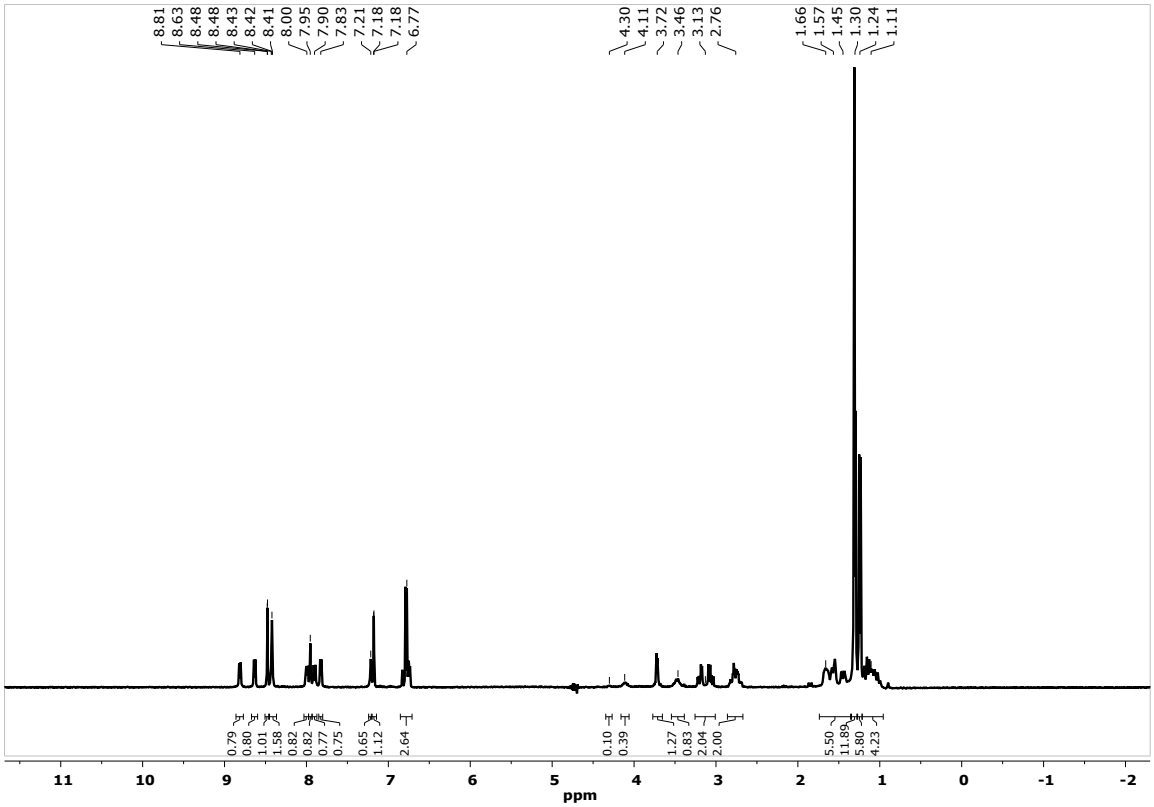

Figure S23: ^{1}H NMR (400 MHz, H_2O/D_2O) of **3.**

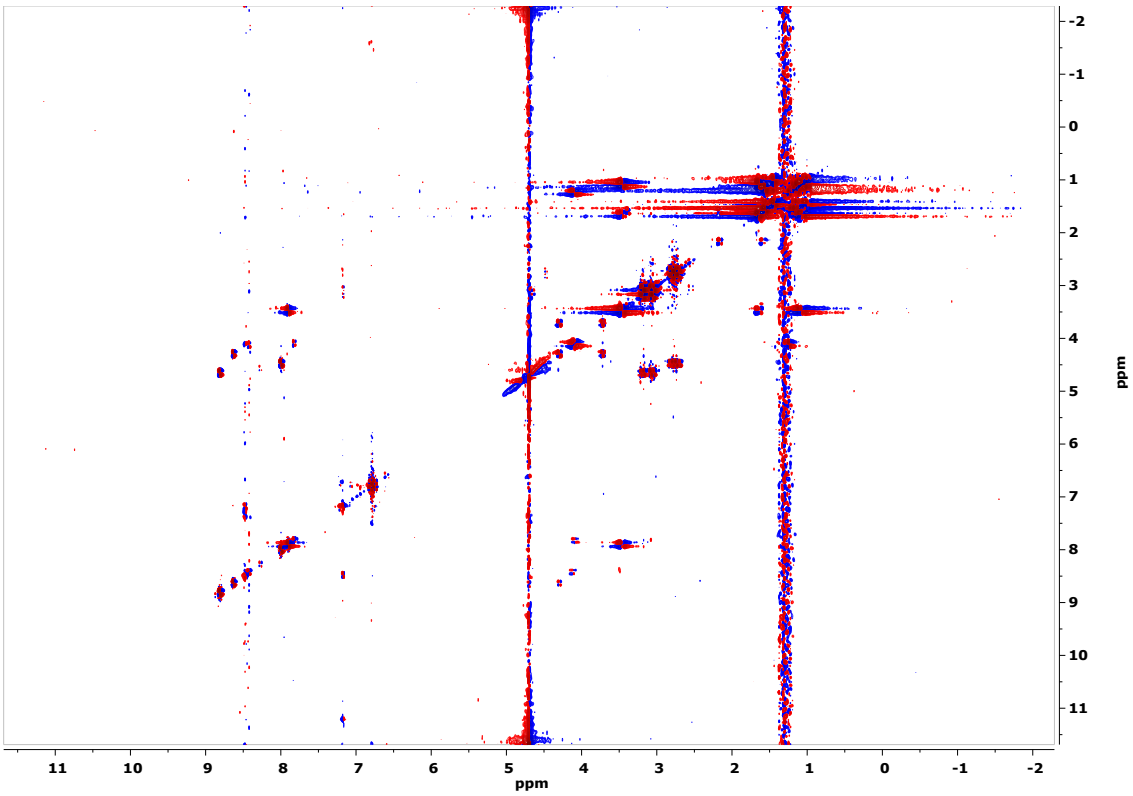

Figure S24: DFQ-COSY NMR (400 MHz, H_2O/D_2O) of **3.**

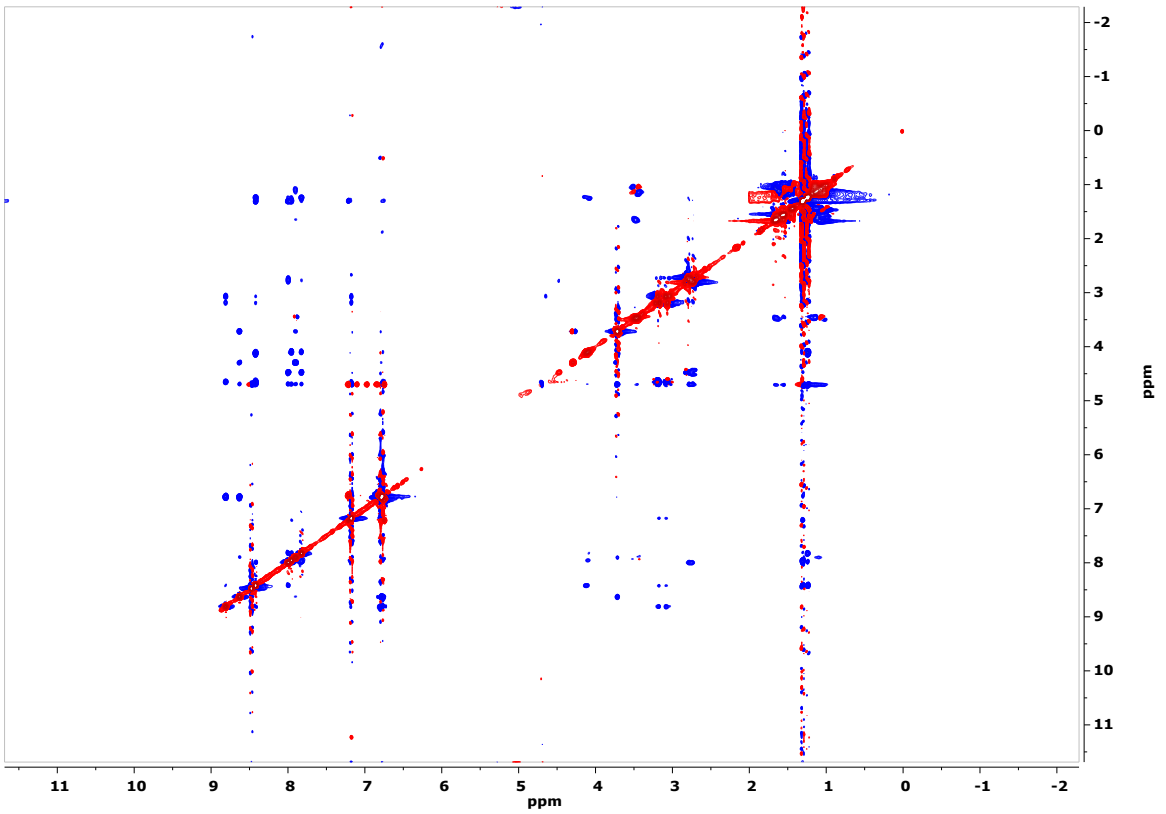

Figure S25: ROESY NMR (400 MHz, H_2O/D_2O) of **3.**

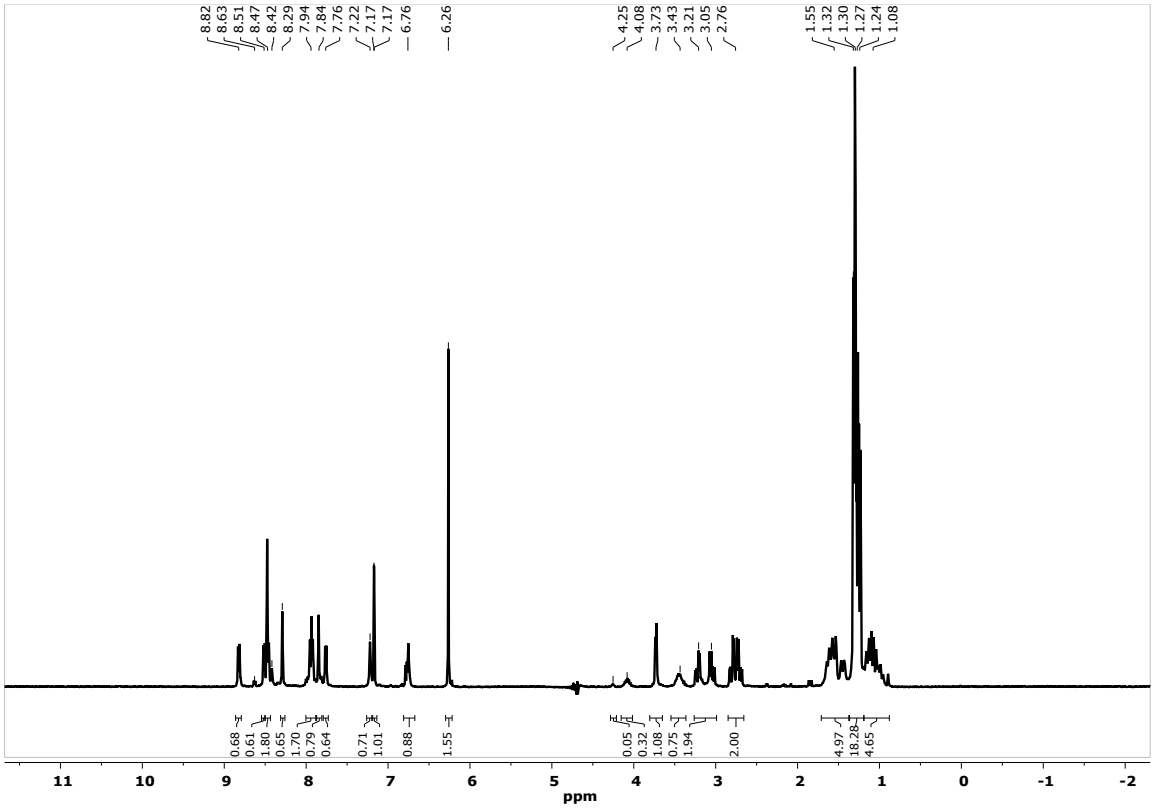

Figure S26: ^{1}H NMR (400 MHz, H_2O/D_2O) of **4.**

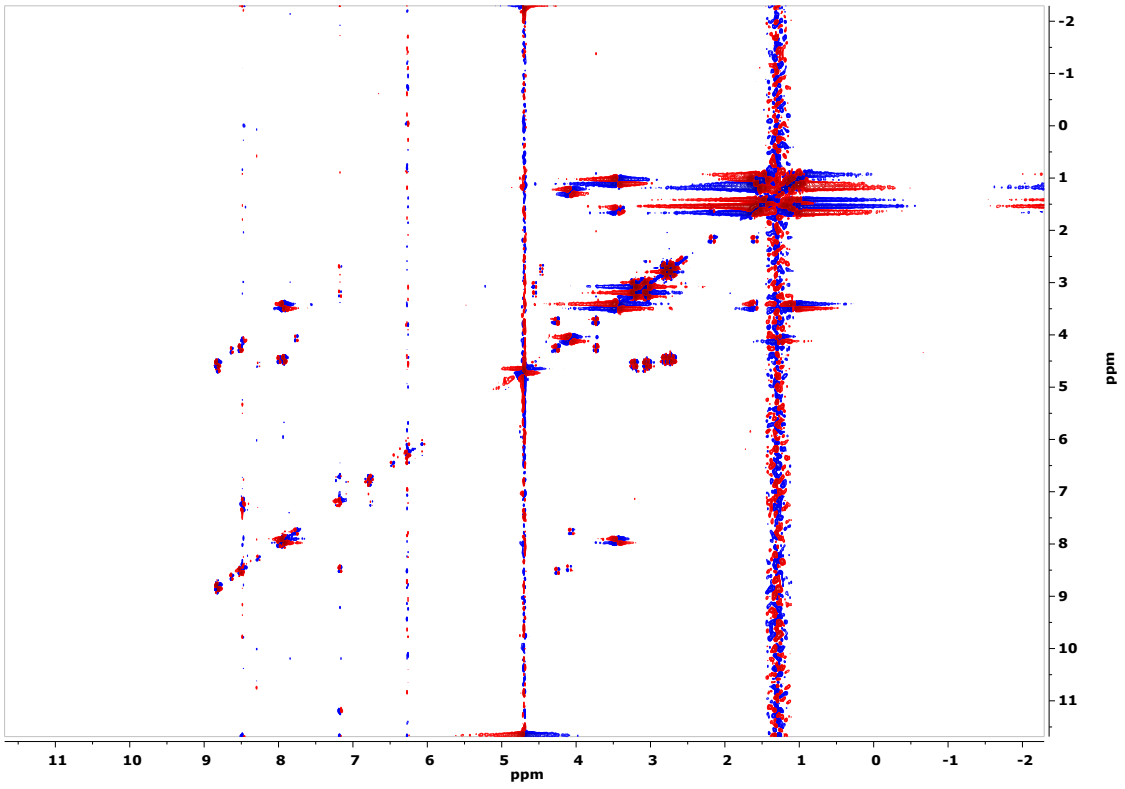

Figure S27: DFQ-COSY NMR (400 MHz, H_2O/D_2O) of **4.**

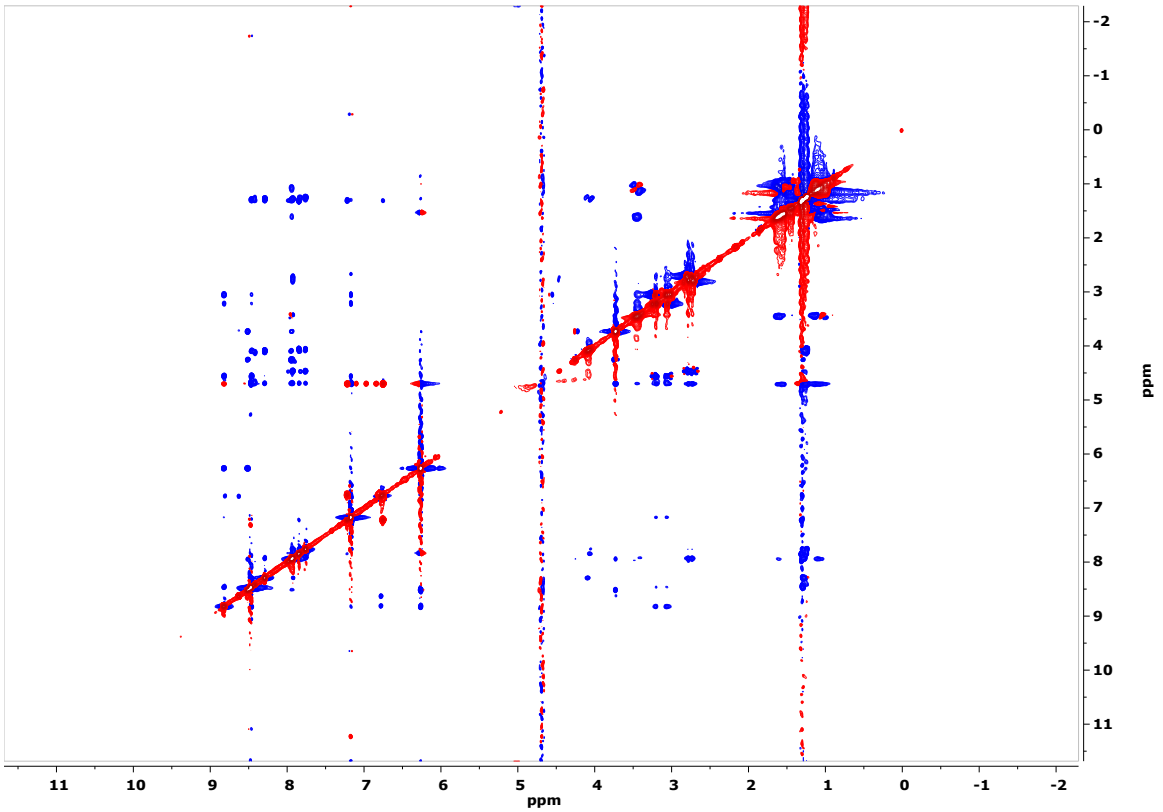

Figure S28: ROESY NMR (400 MHz, H_2O/D_2O) of **4.**

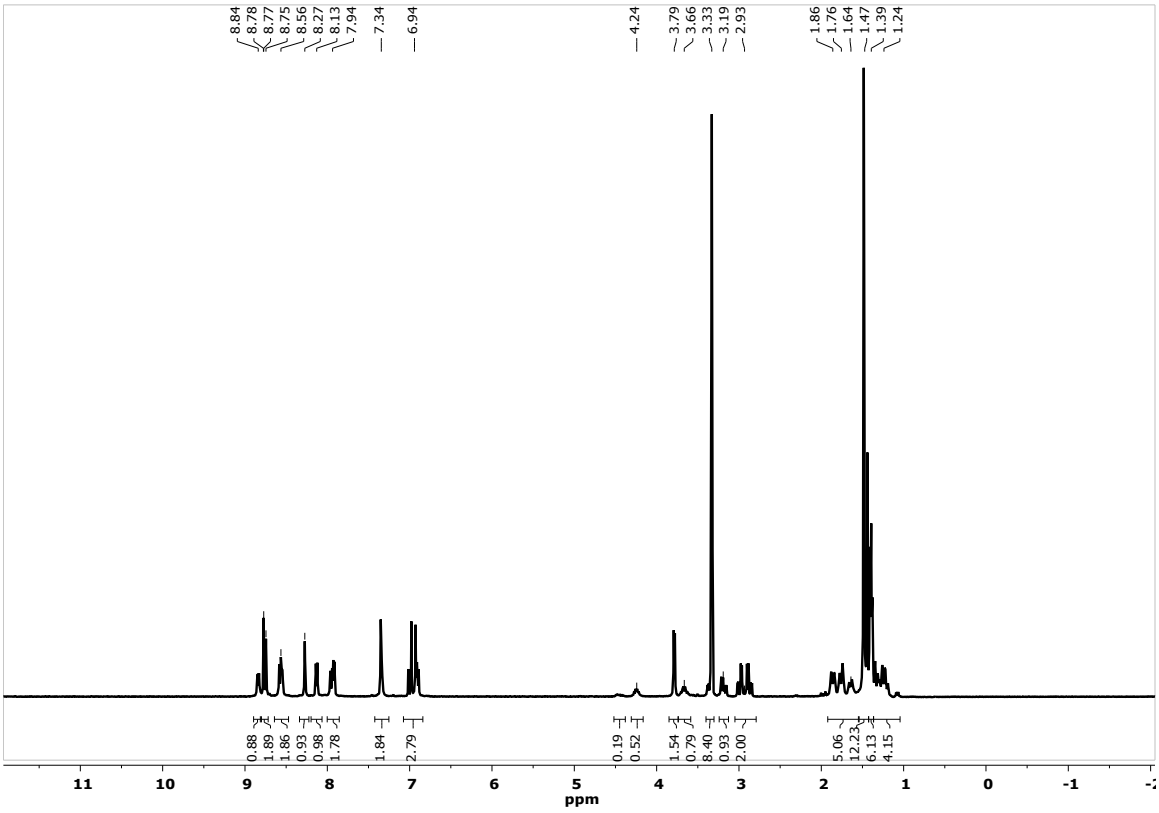

Figure S29: ^1H NMR (400 MHz, CD_3OH) of **3.**

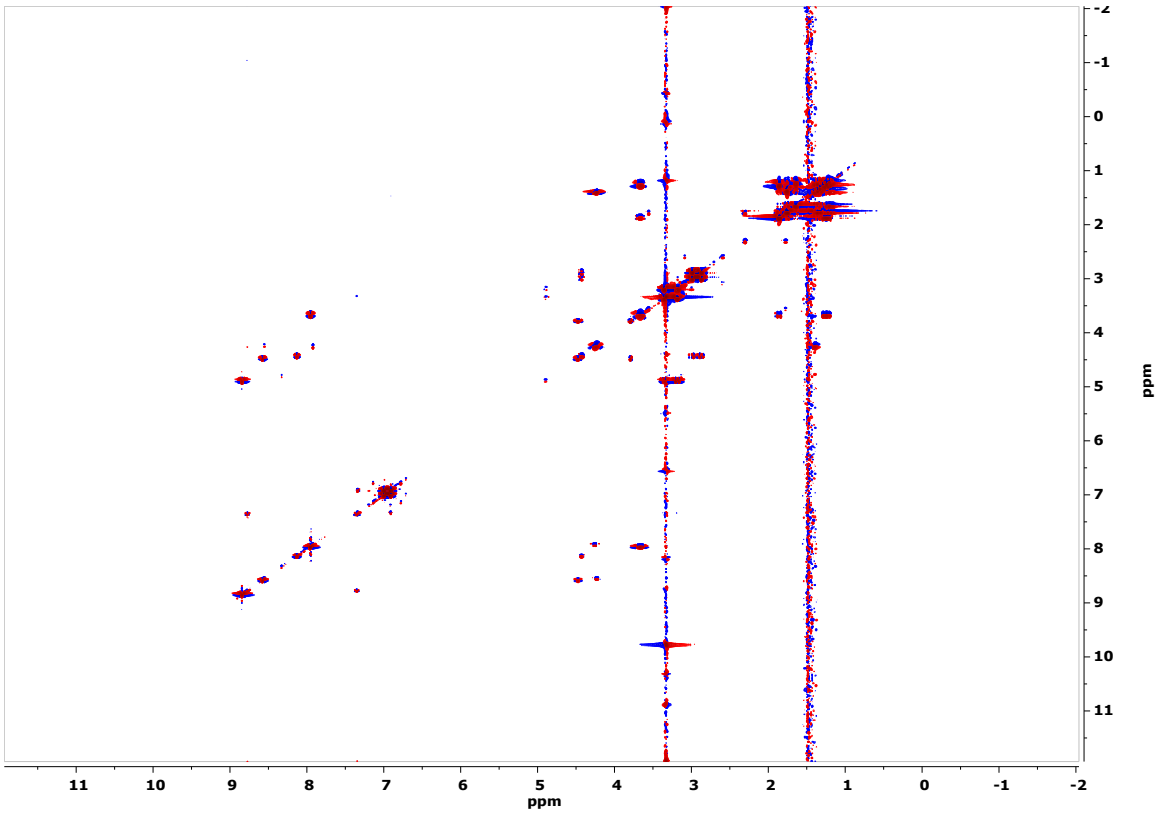

Figure S30: DFQ-COSY NMR (400 MHz, CD_3OH) of **3.**

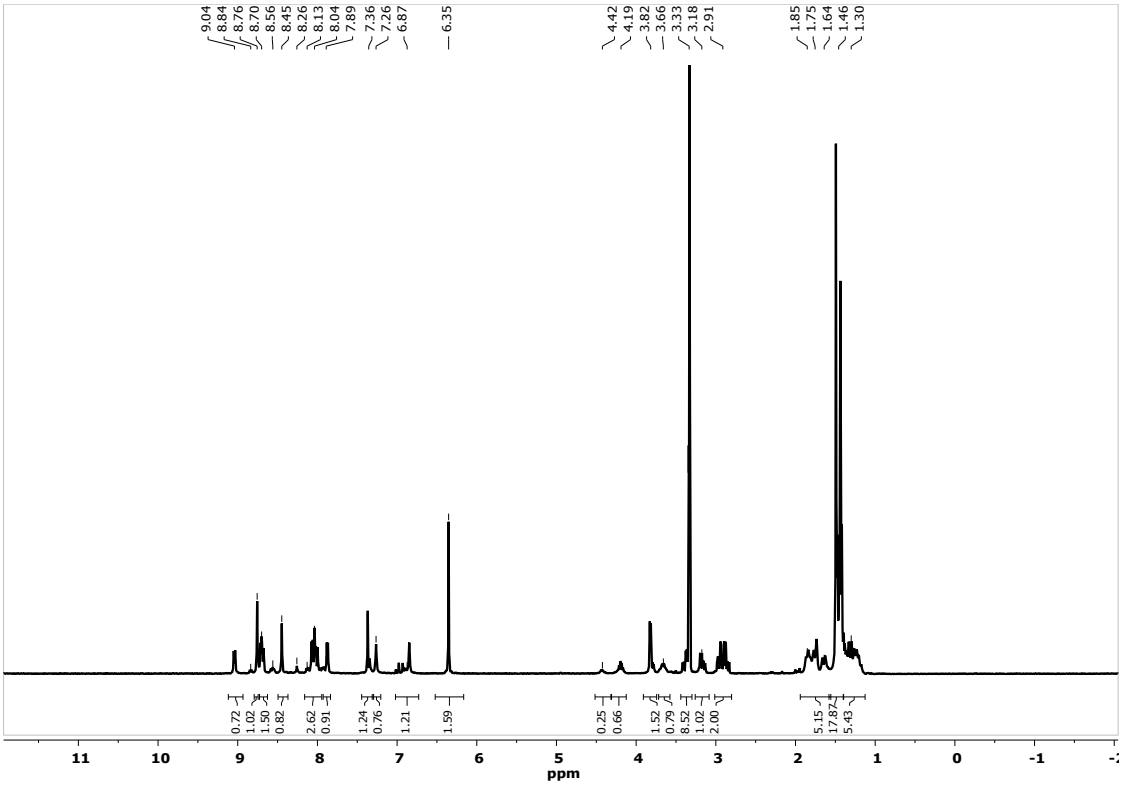

Figure S31: ^1H NMR (400 MHz, CD_3OH) of **4.**

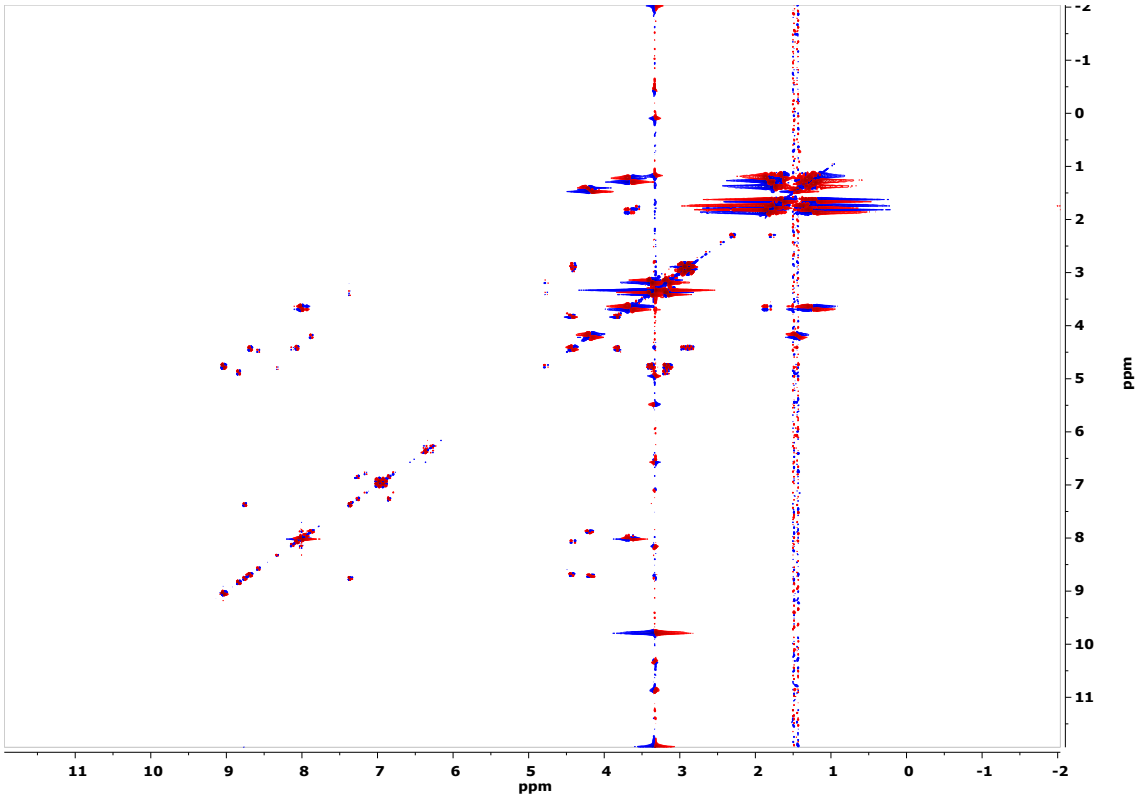

Figure S32: DFQ-COSY NMR (400 MHz, CD_3OH) of **4.**

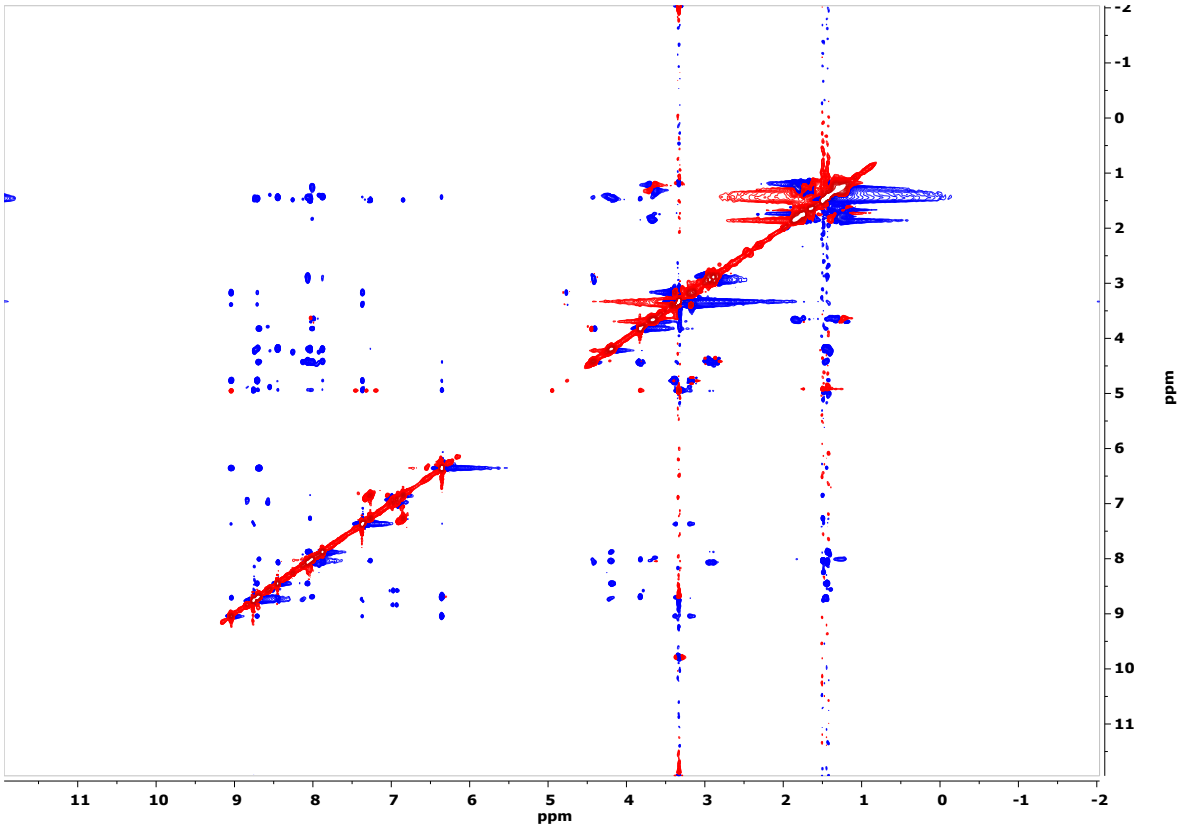

Figure S33: ROESY NMR (400 MHz, CD_3OH) of **4.**

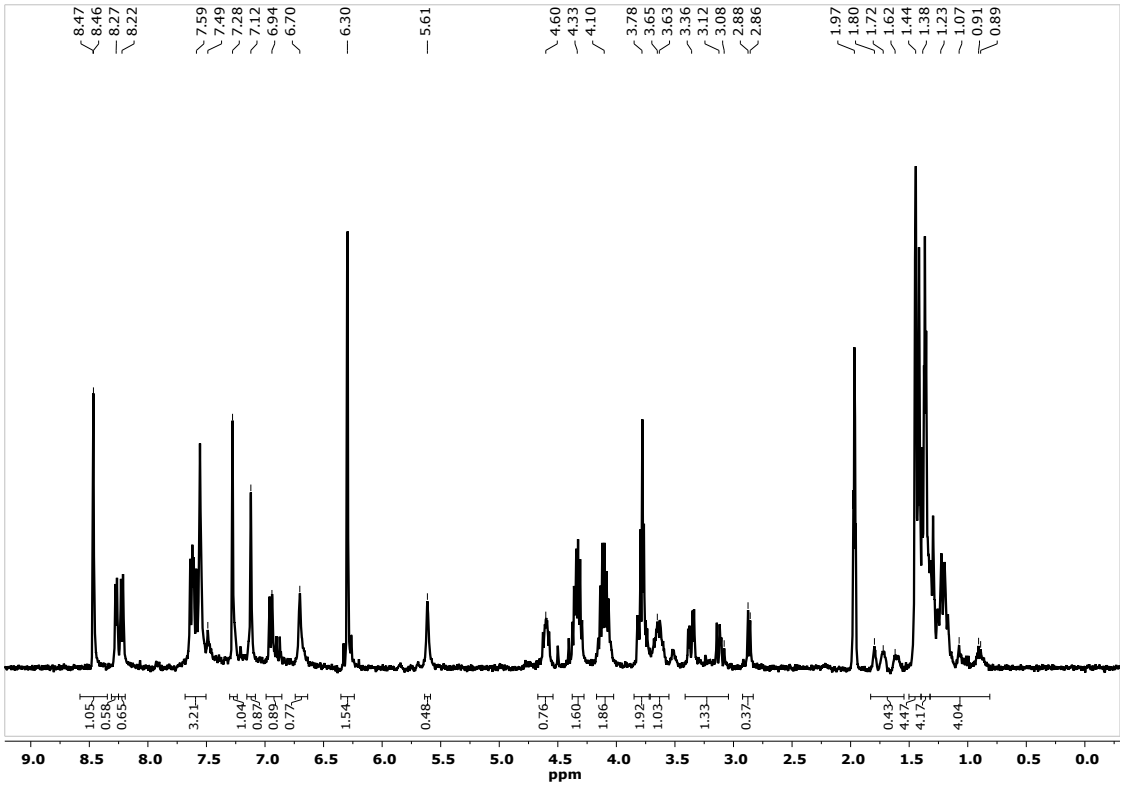

Figure S34: ^{1}H NMR (400 MHz, CD_3CN) of **3.**

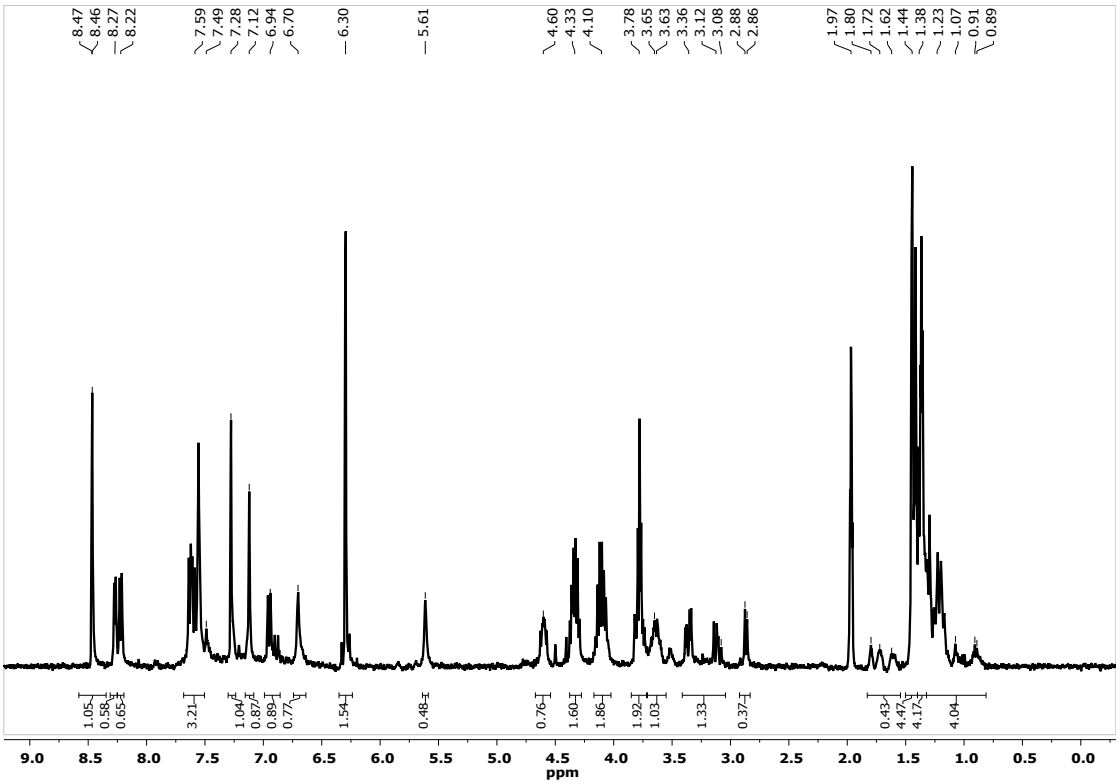

Figure S35: ^{1}H NMR (400 MHz, CD_3CN) of **4.**

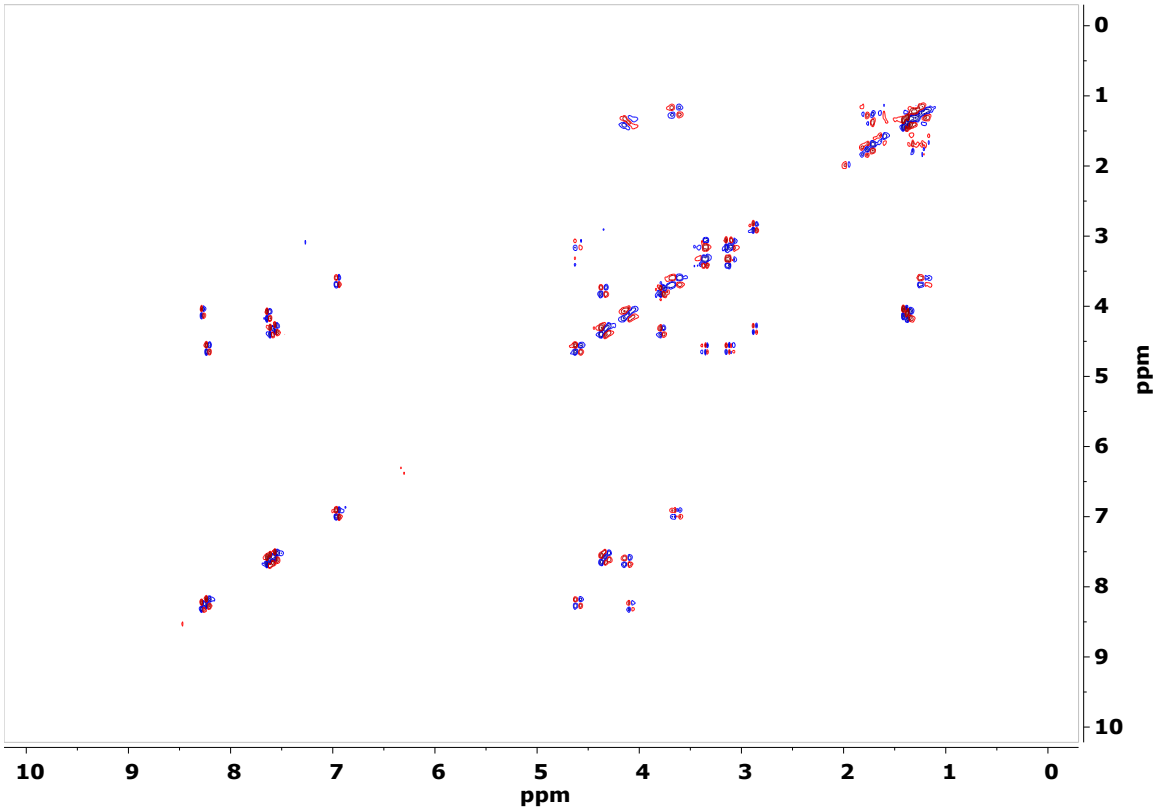

Figure S36: DFQ-COSY NMR (400 MHz, CD_3CN) of **4.**

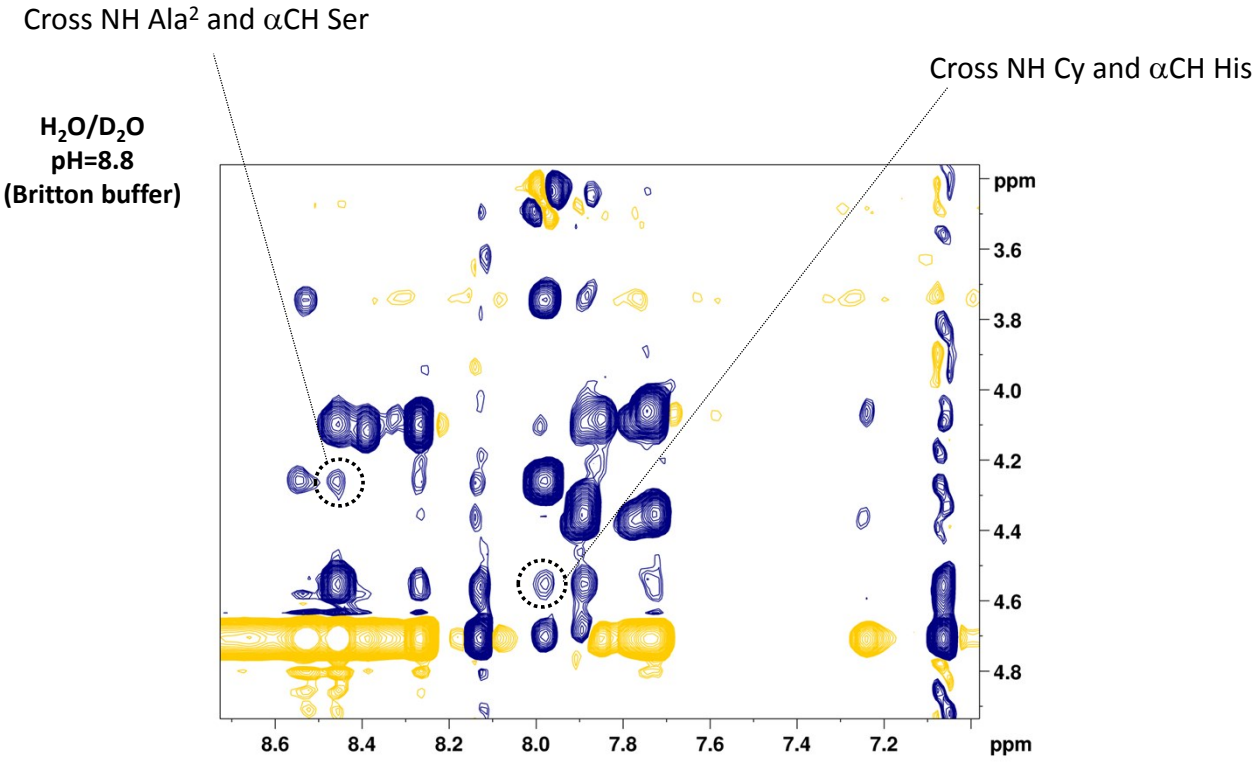

Figure S37: Comparison of ROESY NMR for **3** (orange) and **4** (blue) (400 MHz, H_2O/D_2O pH 8.8, Britton-Robinson buffer) showing cross-peaks nearby the catalytic site occurring only for **4.**

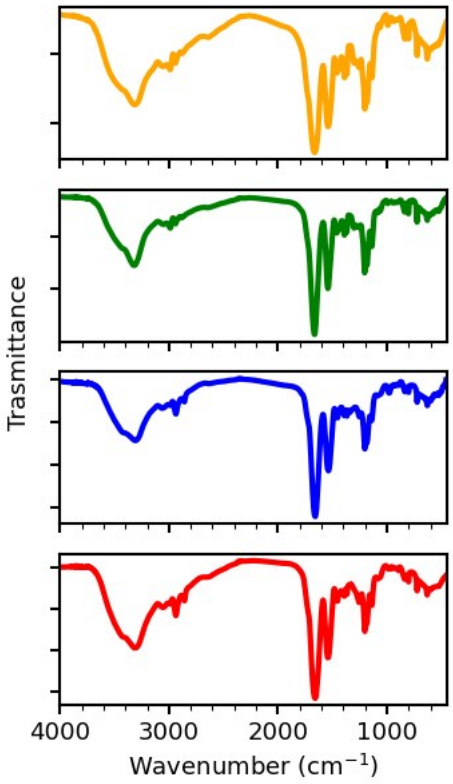

Figure S38: FTIR spectra (KBr pressling) of **1** (orange), **2** (green), **3** (blue) and **4** (red).

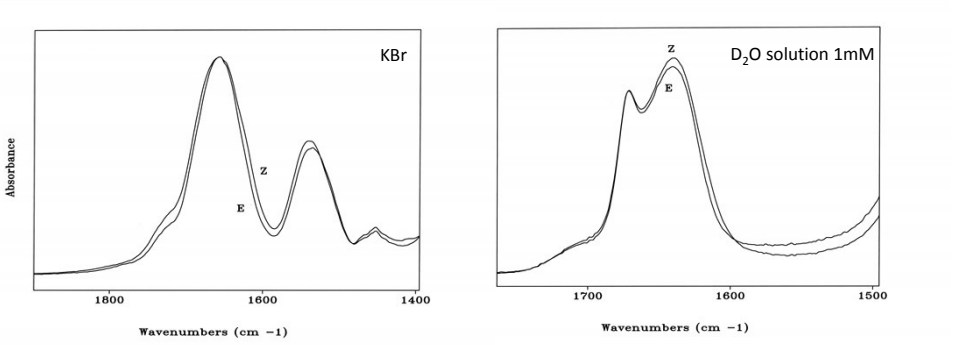

Figure S39: FTIR spectra (KBr pressling) of **3** and **4**. FT-IR spectra, taken in D_2O solution, that highlight the lack of difference between foldamers **3** and **4** in the C=O and NH amide regions.

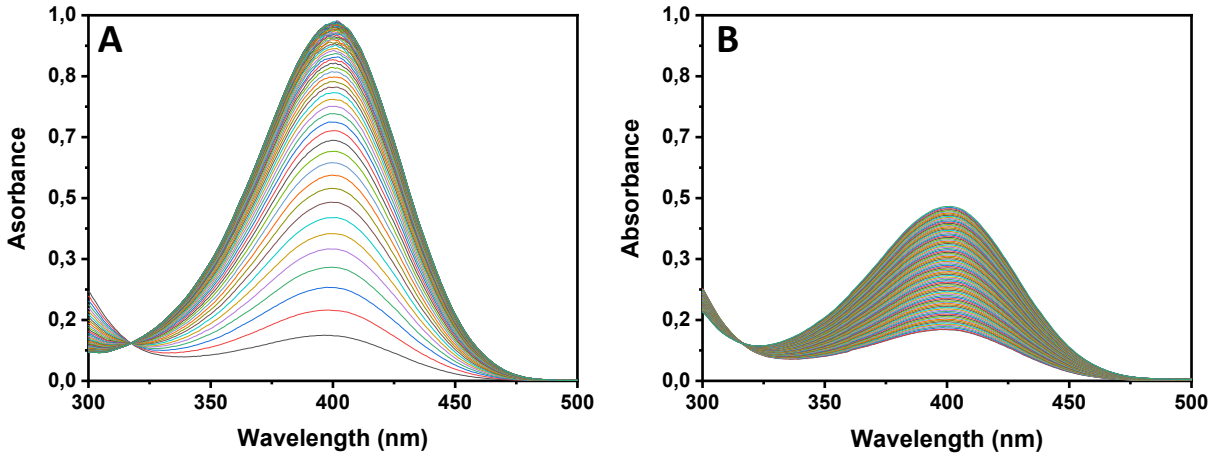

Figure S40: Example of UV-Vis spectra recorded during hydrolytic experiments (A. with foldamer **4**; B: with foldamer **3**)