Electronic Supplementary Material (ESI) for Soft Matter.

Self-Assembly of Single Amino acid-Pyrene Conjugates with Unique Structure-Morphology Relationship

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Supporting Information

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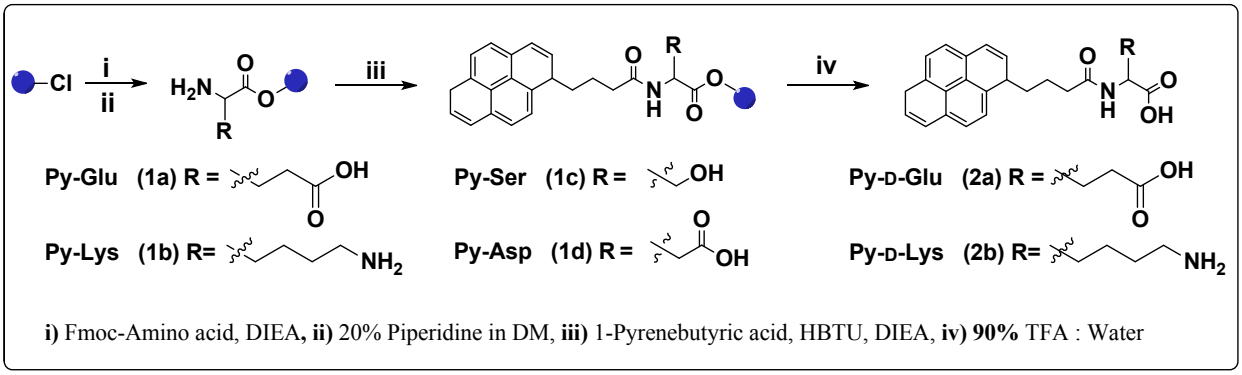

Scheme S1. Synthetic scheme of Py derivatives.

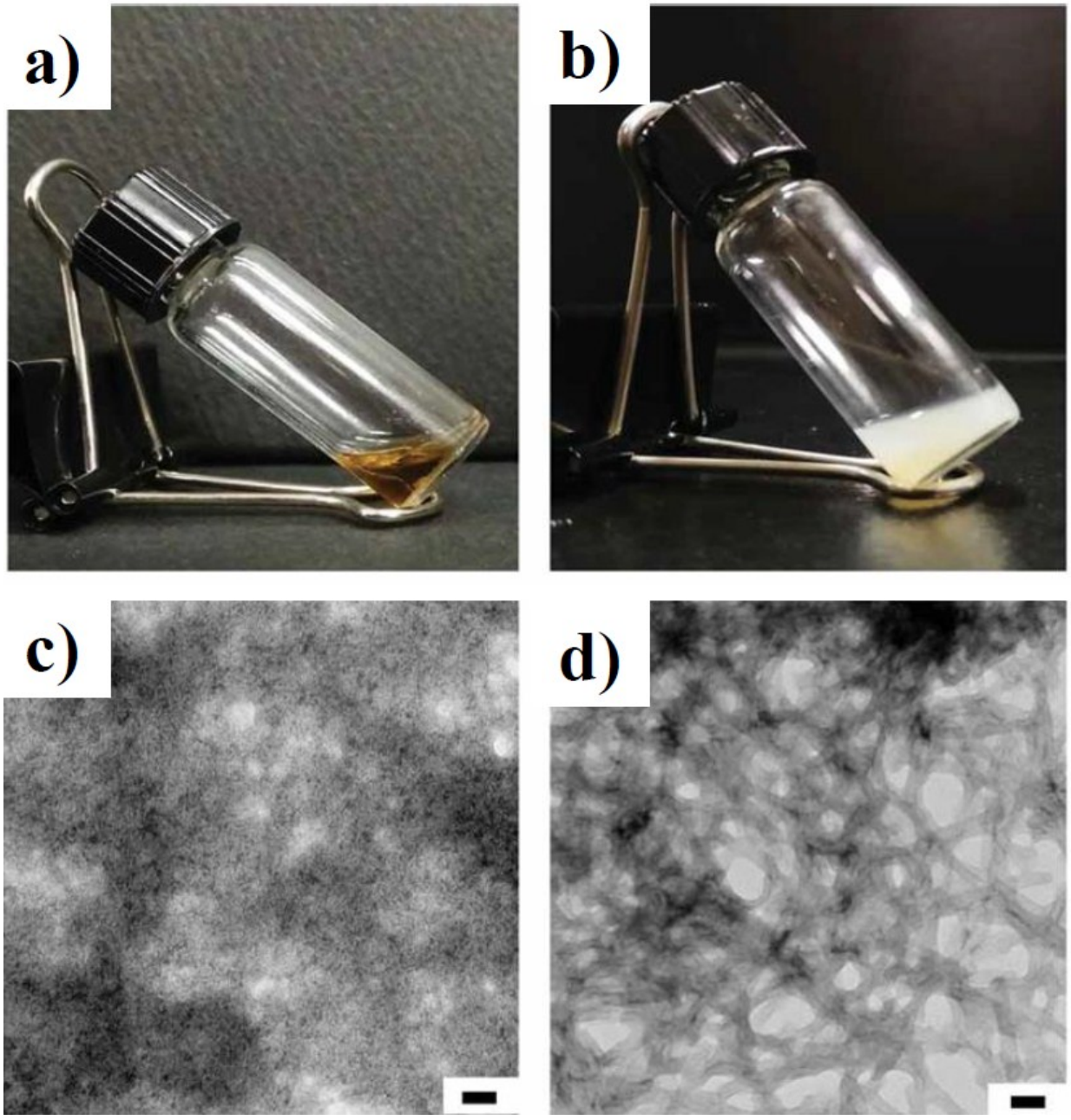

Fig. S1 Optical image of a) **1a**, b) **1b** non-gelation at pH: 7.0 and c) and d) their corresponding TEM images in aqueous media. The scale bar indicates 50 nm.

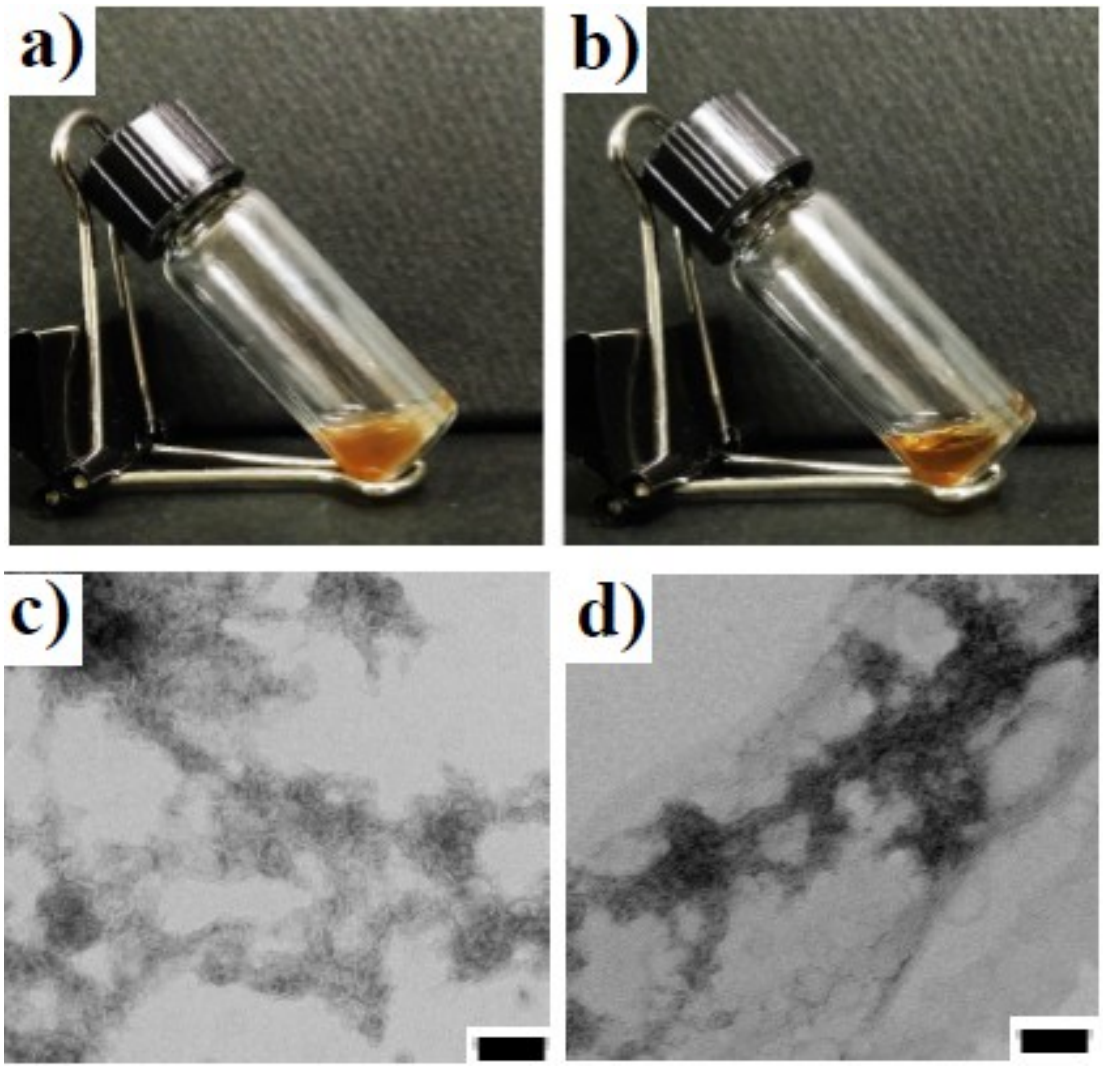

Fig. S2 Optical images of a) **1c**, b) **1d** non-gelation at 3 wt% in aqueous media and c, d) TEM image of **1c** and **1d**. The scale bar indicates 50 nm.

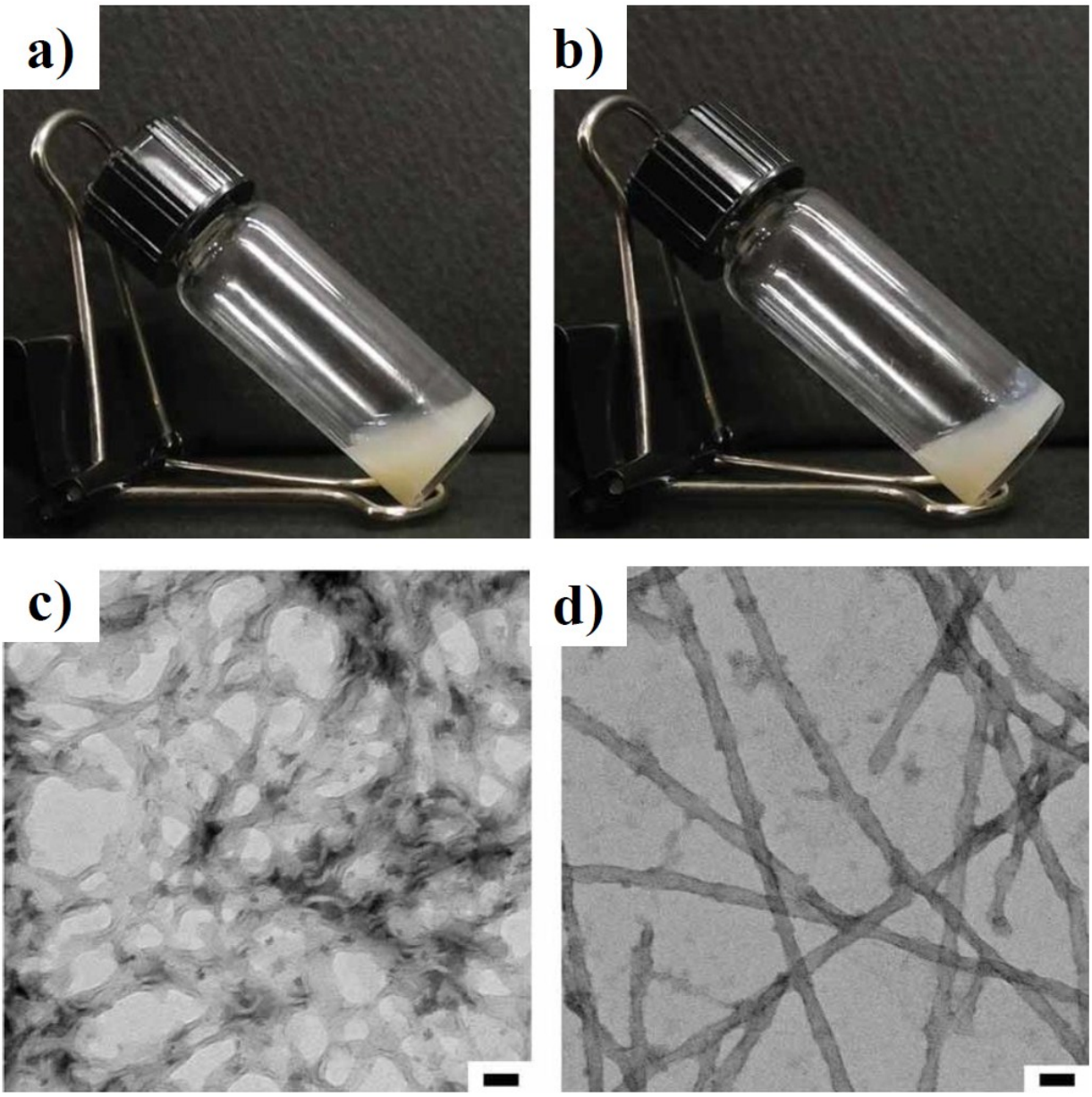

Fig. S3 Optical images of blend (1:9) of a) **1c+1b**, b) **1d+1b** viscous solutions at 3wt% at pH: 7.0 and c), d) their corresponding TEM. The scale bar indicates 50 nm.

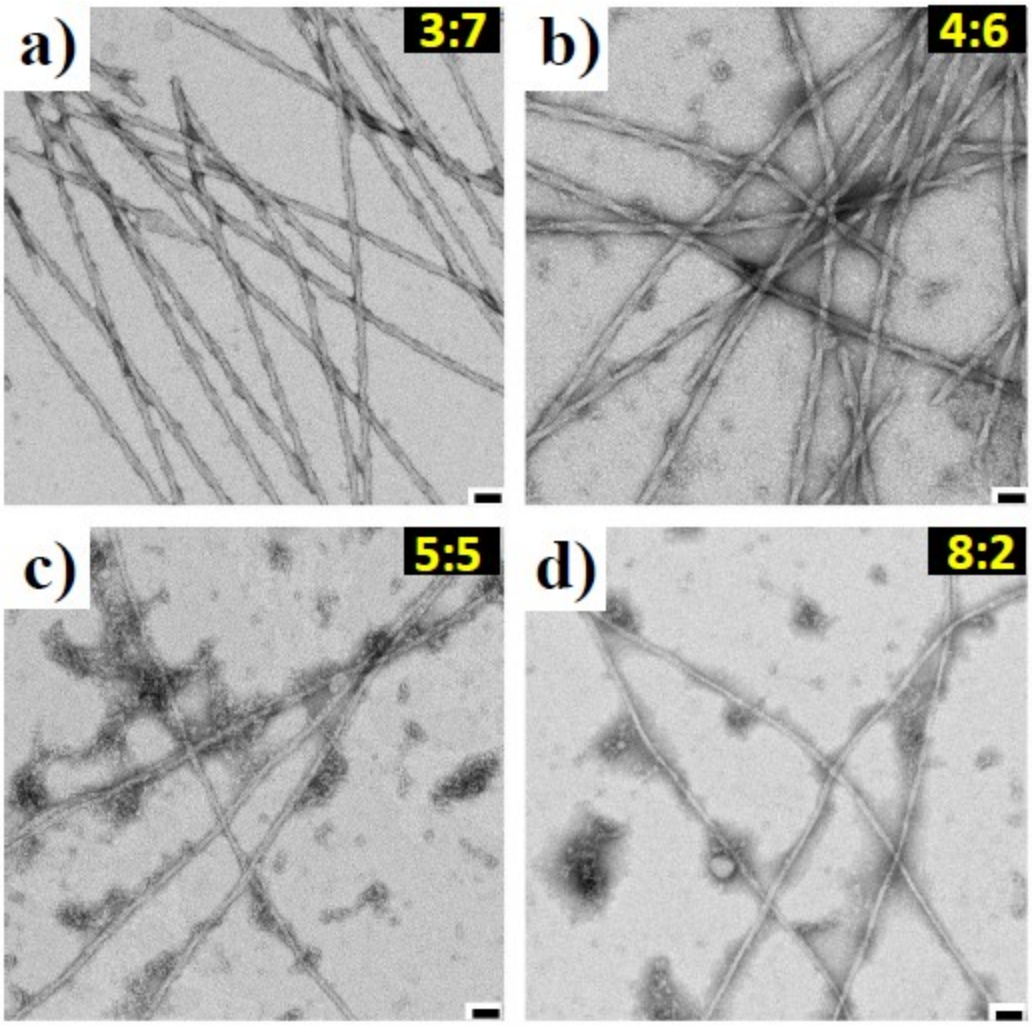

Fig. S4 Optical TEM images of **1a**+**1b**, a) 3:7, b) 4:6, c) 5:5 and d) 8:2 at pH: 7.0. The scale bar indicates 50 nm.

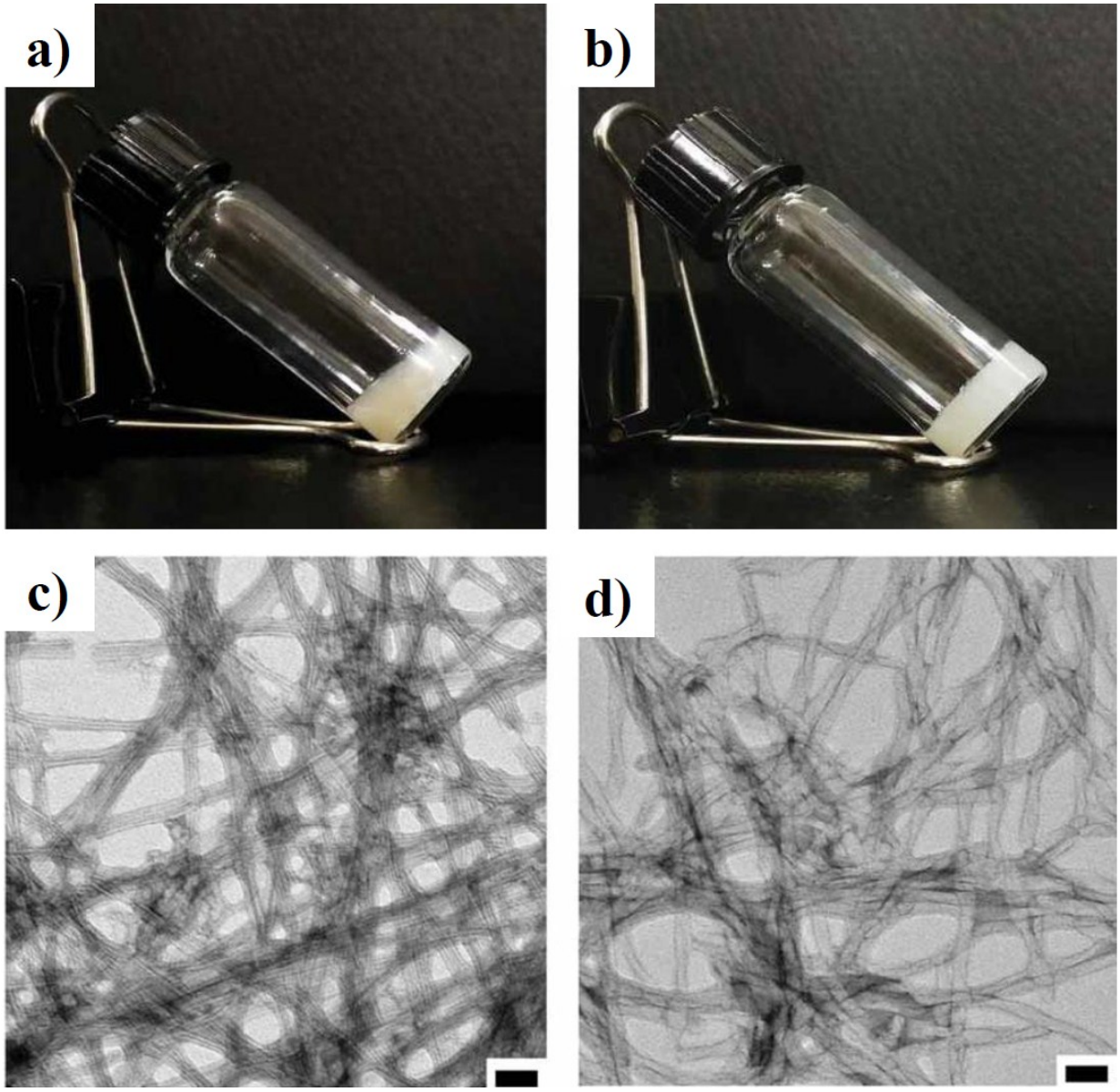

Fig. S5 Optical image of a) **2a**, b) **2b** gels at pH: 5.0 and 9.0 c), d) their corresponding TEM images at 3wt%. The scale bar indicates 50 nm.

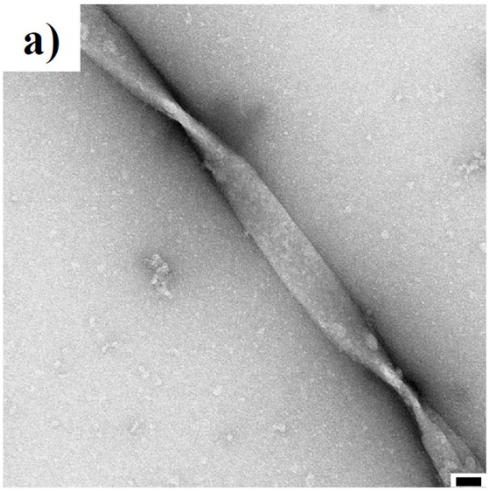

Fig. S6 TEM image of a) **2a**+**2b** (1:9) at pH: 7.0 in aqueous media. The scale bar indicates 50 nm.

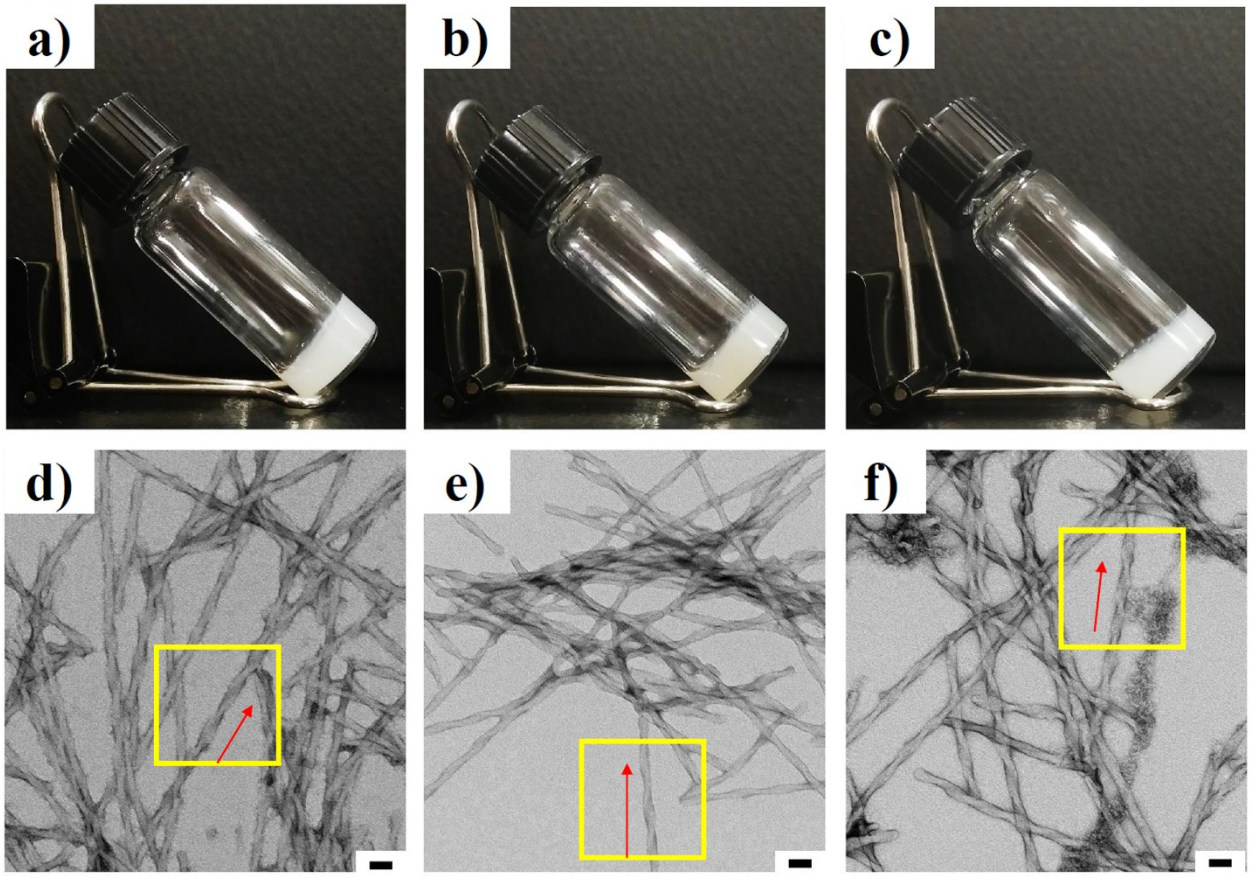

Fig. S7 Optical image of blend gels (1:9) a) **2a**+**2b**, b) **2a**+**1b** and c) **1a**+**2b** at pH: 7.0 and d, e, f) their corresponding TEM images in aqueous media at 3wt%. The scale bar indicates 50 nm.

Table S1. Physical properties of L and D amino acid based Py mixed compounds at 3 wt%.

Entry	pH	Appr.[a]	$T_{gel\text{-}sol}$ (°C)	Fiber width (nm)
1a	7.0	TS	n.d	n.d
1b	7.0	VS	n.d	17±2
1a+1b(5:5)	7.0	TS	n.d	14±2
1a+1b(8:2)	7.0	TS	n.d	12±2
1c+1b(1:9)	7.0	OS	n.d	21±2
1d+1b(1:9)	7.0	O.S	n.d	20±2
2a	4.0-5.0	OG	70	12±2
2b	9.0	OG	75	18±2
1a+2b(1:9)	7.0	OG	85	21±2
2a+1b(1:9)	7.0	OG	82	22±2
2a+2b(1:9)	7.0	OG	82	22±2

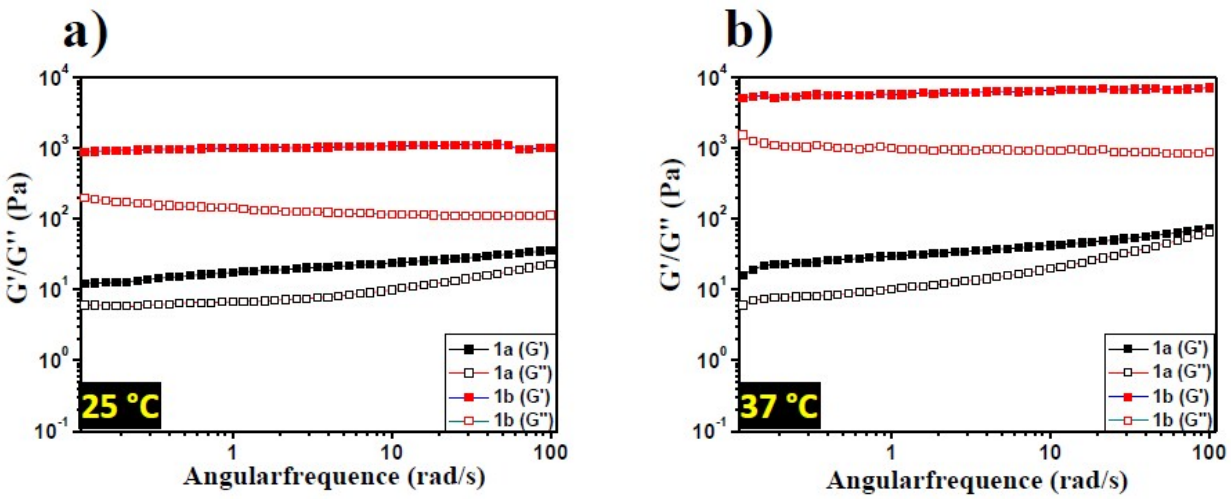

Fig. S8 The frequency sweep of the supramolecular hydrogels of **1a** and **1b**, a) at 25 °C and b) at 37 °C at 3wt% in aqueous media.

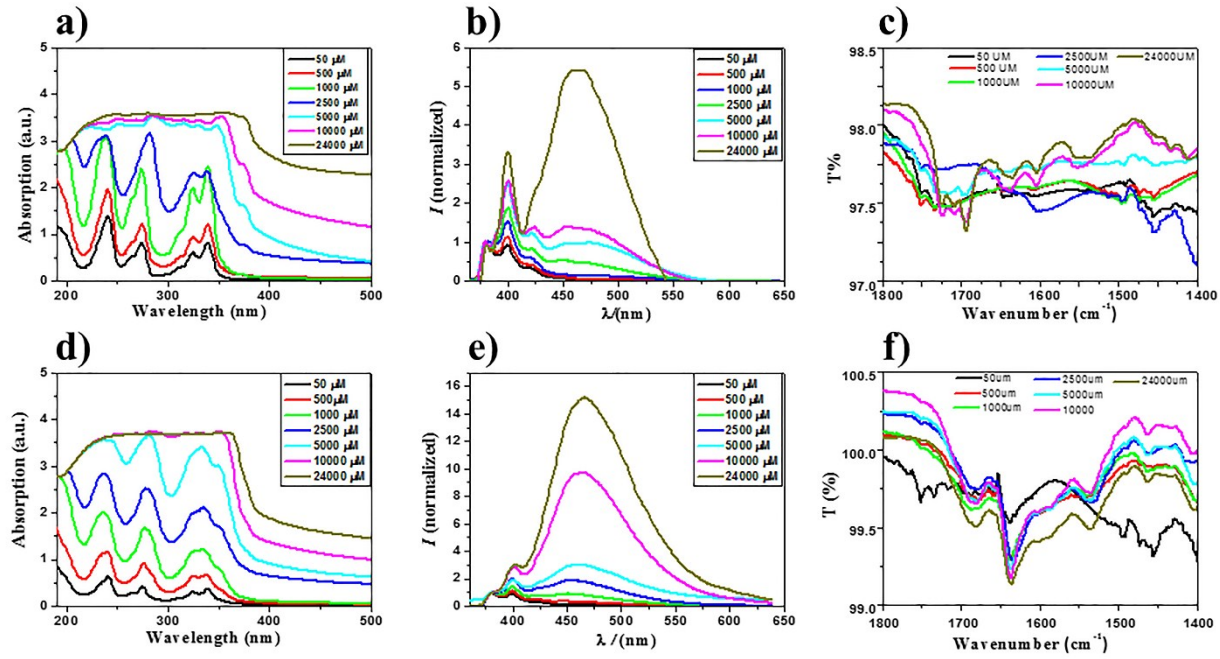

Fig. S9 Concentration dependent a, d) UV-vis b, e) Fluorescent emission (λ_{max} excited at 340 nm) and c, f) FT-IR spectra of **1a** and **1b** in aqueous media.

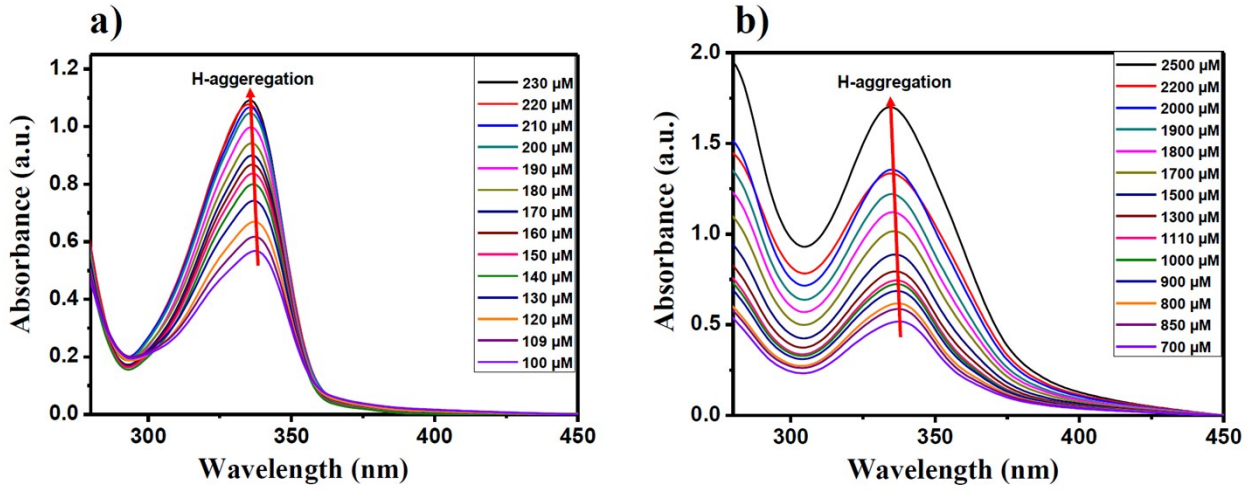

Fig. S10 Concentration dependent UV-vis absorbance spectra of a) **1a** at 230-100 μM and b) **1b** at 2500-700 μM in aqueous media.

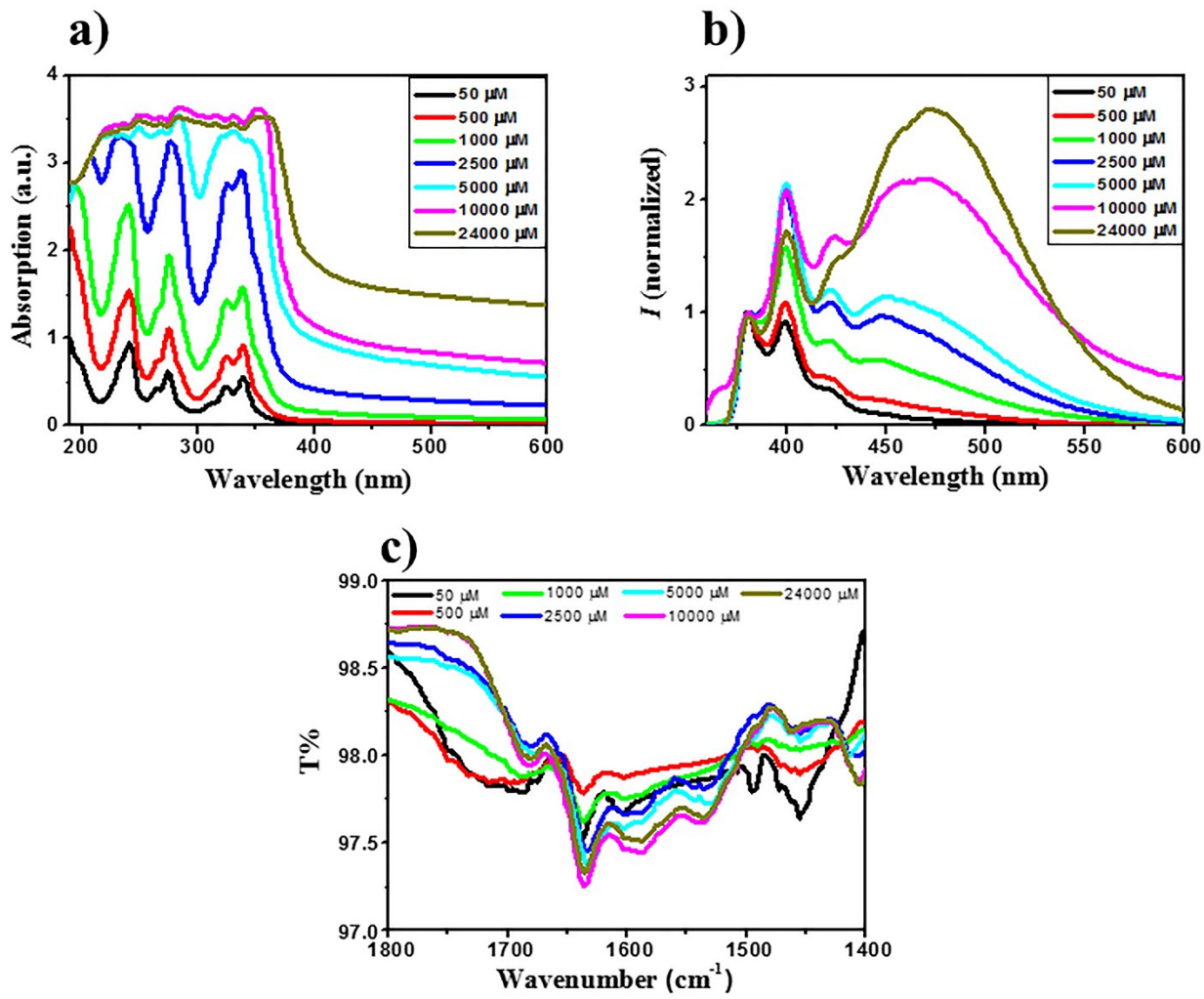

Fig. S11 Concentration dependent a) UV-vis b) Fluorescent emission (λ_{max} excited at 340 nm) and c) FT-IR spectra of **1a+1b** (1:9) in aqueous media.

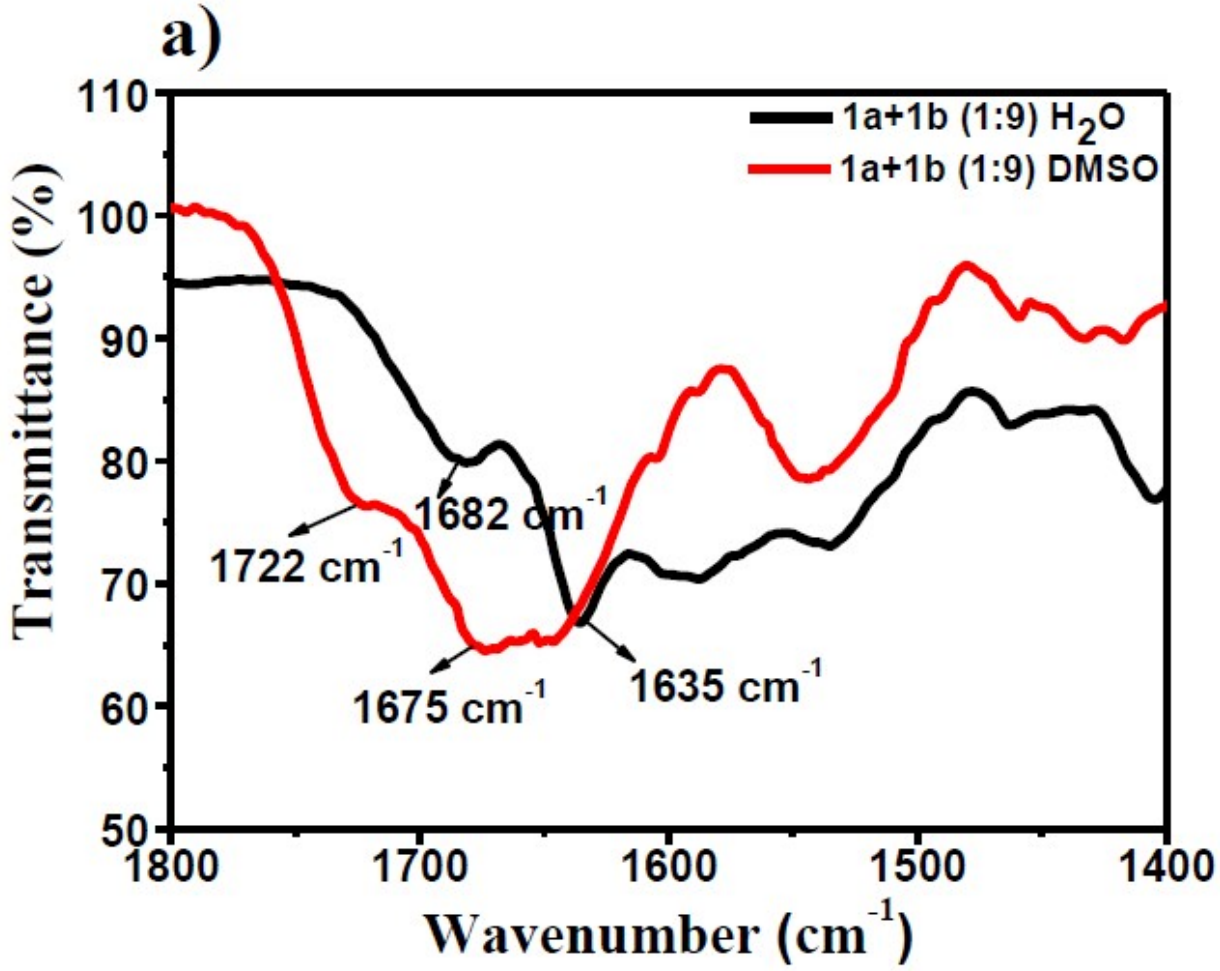

Fig. S12 a) Solvent dependent FT-IR spectra of **1a+1b** (1:9), black line: Water and red line: DMSO systems.

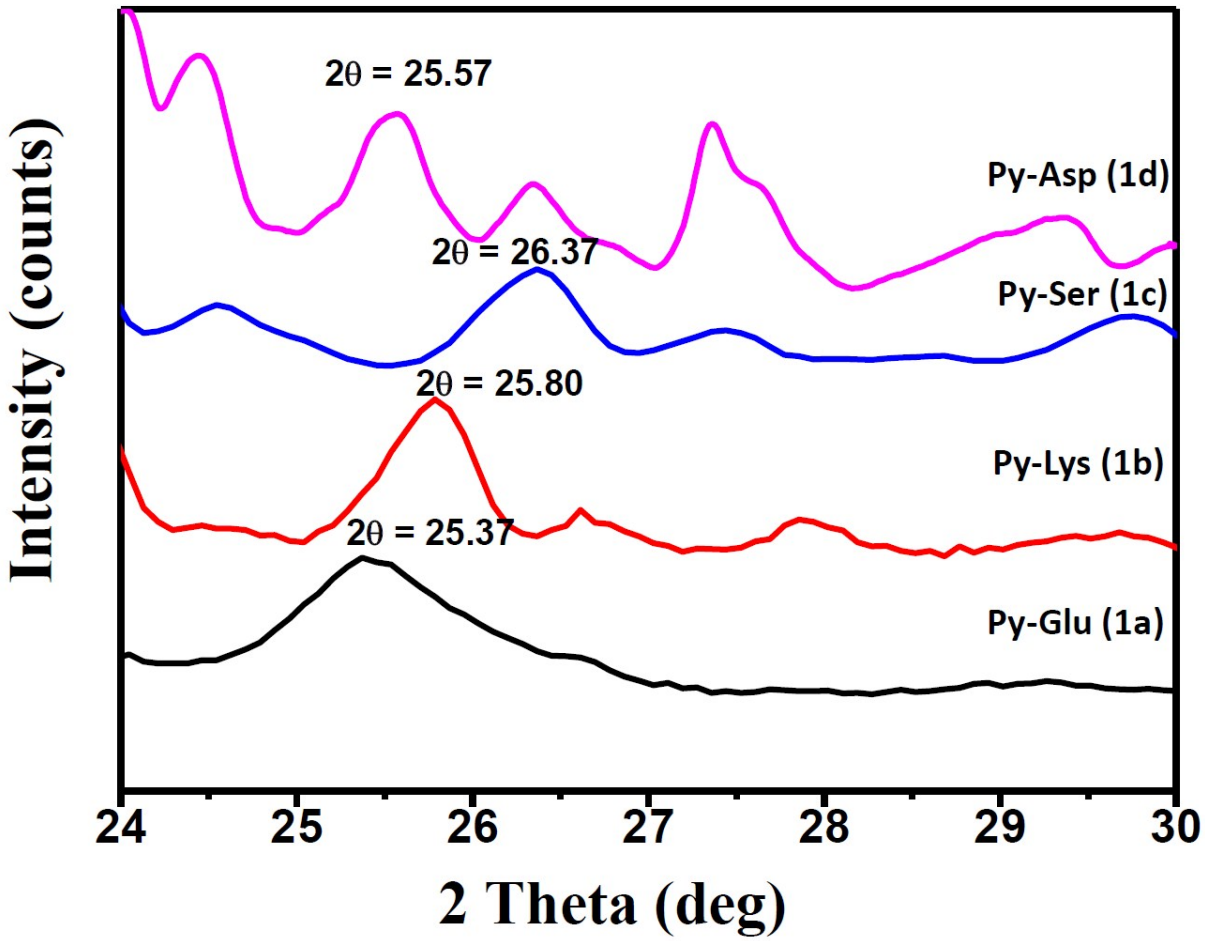

Fig. S13 a) X-ray powder diffraction of the hydrogelators **1a-1d.**

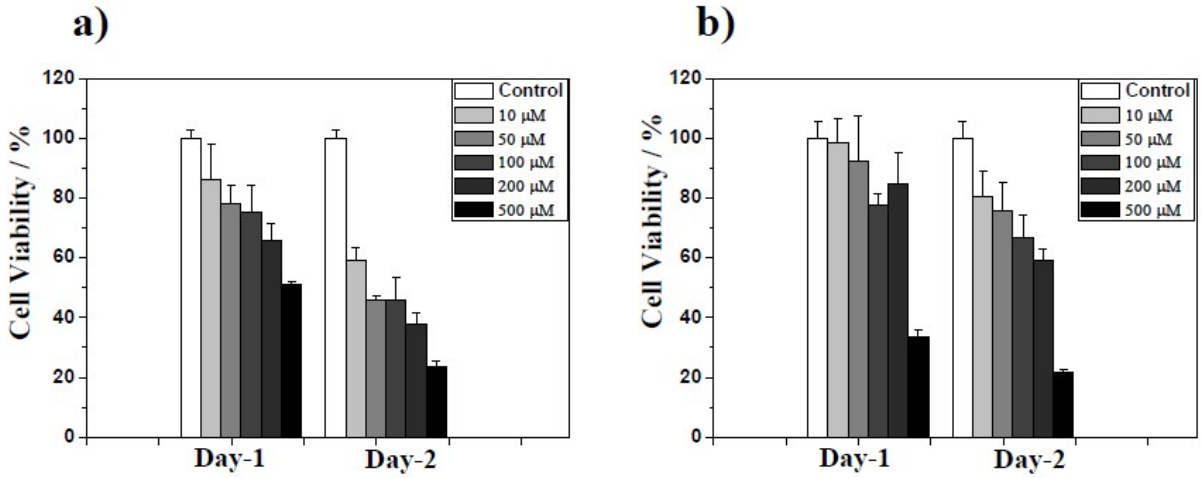

Fig. S14 MCF-7 cell viability in the presence of **1a**+**1b** a) 1:9, b) 2:8 at 0 μM (control), 10 μM, 50 μM, 100 μM, 200 μM and 500 μM, respectively.

Experimental Section:

1. Synthesis of Py-L-Glutamic acid (1a)

All the Pyrene capped peptide derivatives were prepared using solid phase peptide synthesis (SPPS). Firstly, 1.2 g of resin was swollen with anhydrous dichloromethane (DCM) for 30 min under nitrogen atmosphere. Corresponding Fmoc protected amino acid (2 mmol) i.e., Fmoc-L-Glutamic acid 5-tert-butyl ester or N-alpha-Fmoc-Nepsilon-BOC-L-Lysine or O-tert-Butyl-L-serine (2 mmol) or Fmoc-L-aspartic acid 4-tert-butyl ester (0.823 g, 2.000 mmol) or Fmoc-D-Glutamic acid 5-tert-butyl ester or Nα-Fmoc-Nε-Boc-D-lysine in anhydrous N, N-Dimethlyformamide (DMF) and N, N-Diisopropylethylamine (DIEA) (0.850 mL, 5 mmol) were added in to the resin solution and stirred for 1 h at room temperature. After 30 min, the block solution (DCM: MeOH: DIEA) was added followed by the addition of 20% of piperidine for the deprotection of Fmoc group for 30 min and repeated for every 2 min twice. Then 1.080 g of (2.500 mmol) 1-Pyrenebutyric acid (Py) was added in to the above solution and then the free amino group was coupled by using O-(benzotriazol-1-yl)-N, N, N', N',-tetramethyluroniumhexafluorophospate (HBTU) (0.759 g, 2 mmol) and N, N-Diisopropylethylamine (DIEA) (0.83 mL, 5 mmol) as the coupling reagent. The reaction mixture was stirred overnight, followed by the treatment with 90 % trifluoroaceticacid (TFA) in water for 3 h for the cleavage of resin from the peptide derivative. The resultant solution was collected and solid product was precipitated by adding ice cold diethyl ether. The solid was dried under the vacuum to afford the title compound as brown color solid (0.280g). Pyrene derivatives **1b-1d** and **2a**, **2b** were synthesized in a similar manner by the reaction of **1a**. Similarly all pyrene derivatives have been synthesized.

^{1}H NMR (300 MHz, d6-DMSO, 25^{o}C): δ=1.65-1.90 (m, 1H; CH2), 1.90-2.20 (m, 3H; CH2), 2.05 (t, 4H; *J=6.7 Hz,* CH2), 4.20-4.40 (m, 1H; CH), 7.95 (d, *J=7.8 Hz,* 1H; CH), 8.15-8.05 (m, 2H; CH), 8.28-8.20 (m, 6H; CH), 8.44 (d, *J=9.3 Hz,* 1H; NH).). ^{13}C NMR (75 MHz, d6-DMSO, 25^{o}C): δ=27.3, 28.5, 31.1, 33.1, 35.7, 52.1, 124.5, 125.1, 125.2, 125.7, 125.9, 127.1, 127.4, 128.2, 128.4, 128.5, 129.1, 130.2, 131.4, 131.8, 137.6, 173.2, 174.5, 174.7. HRMS [ESI^{-}]: m/z (%): $C_{25}H_{24}NO_5$: Calculated, 418.1649: observed, 418.1657 [M-H]$^{-}$.

2. Synthesis of Py- L-Lysine (1b)

Pyrene derivative (**1b**) was prepared in a similar manner **1a** by the mixture of Nα-Fmoc-Nε-Boc-L-lysine (0.936 g, 2.000 mmol) and anhydrous N, N-Dimethlyformamide (DMF) and N, N-Diisopropylethylamine (DIEA) (0.830 mL, 5.000 mmol) were added in to the resin solution and stirred for 1 h. (0.180 g.)

^{1}H NMR (300 MHz, d6-DMSO, 25^{o}C): δ=1.25-1.45 (m, 2H; CH2), 1.45-1.65 (m, 3H; CH2), 1.65-1.95 (m, 1H; CH2), 1.95-2.20 (m, 2H; CH2), 2.20-2.45 (m, 2H; CH2), 2.65-2.90 (m, 2H; CH2), 4.25-4.40 (m, 1H; CH), 7.70 (s, 2H; NH), 7.95 (d, *J=7.8 Hz,* 1H; CH), 8.15-8.05 (m, 2H;

CH), 8.28-8.20 (m, 6H; CH), 8.44 (d, *J=9.3 Hz,* 1H; NH). ^{13}C NMR (75 MHz, d6-DMSO, 25^{o}C): δ=23.4, 27.4, 28.5, 31.3, 33.0, 35.6, 52.5, 124.4, 125.0, 125.1, 125.7, 125.8, 127.0, 127.4, 128.1, 128.3, 128.4, 129.0, 130.2, 131.3, 131.8, 132.0, 137.5, 173.2, 174.7. HRMS [ESI^{-}]: m/z (%): $C_{26}H_{29}N_2O_3$: Calculated, 417.2173: observed, 417.2183 [M-H]$^{-}$.

3. Synthesis of Py-L-Serine (1c)

Pyrene derivative (**1c**) was prepared in a similar manner **1a** by the mixture of O-tert-Butyl-L-serine (0.950 g, 2.000 mmol) and anhydrous N, N-Dimethlyformamide (DMF) and N, N-Diisopropylethylamine (DIEA) (0.830 mL, 5.000 mmol) were added in to the resin solution and stirred for 1 h. (0.190 g).

^{1}H NMR (300 MHz, d6-DMSO, 25^{o}C): δ=1.95-2.15 (m, 2H; CH2), 2.38 (t, *J=6.4 Hz,* 2H; CH2), 3.55-3.85 (m, 2H; CH2), 4.25-4.40 (m, 1H; CH), 7.97 (d, *J=7.8 Hz,* 1H; CH), 8.15-8.05 (m, 2H; CH), 8.29-8.20 (m, 6H; CH), 8.44 (d, *J=9.3 Hz,* 1H; NH). ^{13}C NMR (75 MHz, d6-DMSO, 25^{o}C): δ=28.6, 33.1, 35.8, 55.7, 62.4, 124.5, 125.1, 125.2, 125.7, 125.9, 127.1, 127.4, 128.1, 128.4, 128.5, 129.1, 130.2, 131.4, 131.8, 137.6, 173.1. HRMS [ESI^{-}]: m/z (%): $C_{23}H_{22}NO_4$: Calculated, 376.1554: observed, 376.1543[M-H]$^{-}$.

4. Synthesis of Py-L -Aspartic acid (1d)

Pyrene derivative (**1d**) was prepared in a similar manner **1a** by the mixture of Fmoc-L-aspartic acid 4-tert-butyl ester (0.823 g, 2.000 mmol) and anhydrous N, N-Dimethlyformamide (DMF) and N, N-Diisopropylethylamine (DIEA) (0.830 mL, 5.000 mmol) were added in to the resin solution and stirred for 1 h. (0.200 g).

^{1}H NMR (300 MHz, d6-DMSO, 25^{o}C): δ=1.95-2.15 (m, 2H; CH2), 2.38 (t, *J=6.4 Hz,* 2H; CH2), 2.55-2.75 (m, 2H; CH2), 4.25-4.40 (m, 1H; CH), 7.97 (d, *J=7.8 Hz,* 1H; CH), 8.15-8.05 (m, 2H; CH), 8.29-8.20 (m, 6H; CH), 8.44 (d, *J=9.3 Hz,* 1H; NH).). ^{13}C NMR (75 MHz, d6-DMSO, 25^{o}C): δ=28.6, 28.9, 33.1, 35.8, 37.5, 49.6, 124.5, 125.1, 125.2, 125.7, 125.9, 127.1, 127.4, 128.2, 128.4, 128.5, 129.1, 130.2, 131.4, 131.8, 137.6, 172.8, 173.1. HRMS [ESI^{-}]: m/z (%): $C_{24}H_{22}NO_5$: Calculated, 404.1492: observed, 404.1503 [M-H]$^{-}$.

5. Synthesis of Py-D-Glutamic acid (2a)

Pyrene derivative (**2a**) was prepared in a similar manner **1a** by the mixture of Fmoc-D-Glutamic acid 5-tert-butyl ester (0.936 g, 2.000 mmol) and anhydrous N, N-Dimethlyformamide (DMF) and N, N-Diisopropylethylamine (DIEA) (0.830 mL, 5.000 mmol) were added in to the resin solution and stirred for 1 h. (0.180 g.)

^{1}H NMR (300 MHz, d6-DMSO, 25^{o}C): δ=1.65-1.90 (m, 1H; CH2), 1.90-2.20 (m, 3H; CH2), 2.20-2.45 (m, 4H; CH2), 3.20-3.55 (m, 2H; CH2),4.20-4.40 (m, 1H; CH), 7.95 (d, *J=7.8 Hz,* 1H; CH), 8.05-8.15 (m, 1H; NH), 8.28-8.20 (m, 7H; CH), 8.44 (d, *J=9.3 Hz,* 1H; NH).). ^{13}C NMR (75 MHz, d6-DMSO, 25^{o}C): δ=27.1, 28.4, 31.0, 33.0, 35.6, 52.0, 124.4, 125.0, 125.1, 125.6, 125.8, 127.0, 127.3, 128.1, 128.3, 128.4, 129.0, 130.1, 131.3, 131.7, 137.4, 173.1, 174.4, 174.6. HRMS [ESI^{-}]: m/z (%): $C_{25}H_{24}NO_5$: Calculated, 417.1517: observed, 416.1503 [M-H]$^{-}$.

6. Synthesis of Py-D-Lysine (2b)

Pyrene derivative (**2b**) was prepared in a similar manner **1a** by the mixture of Nα-Fmoc-Nε-Boc-D-lysine (0.936 g, 2.000 mmol) and anhydrous N, N-Dimethlyformamide (DMF) and N, N-Diisopropylethylamine (DIEA) (0.830 mL, 5.000 mmol) were added in to the resin solution and stirred for 1 h. (0.180 g.)

^{1}H NMR (300 MHz, d6-DMSO and d6-D_2O, 25^{o}C): δ=1.25-1.45 (m, 2H; CH2), 1.45-1.85 (m, 4H; CH2), 1.95-2.10 (m, 2H; CH2), 2.20-2.40 (m, 2H; CH2), 2.65-2.85 (m, 2H; CH2), 3.20-3.40 (m, 2H; CH2) 4.10-4.20 (m, 1H; CH), 7.95 (d, *J=7.8 Hz,* 1H; CH), 7.95-8.10 (m, 3H; CH), 8.10-8.25 (m, 4H; CH), 8.44 (d, *J=9.3 Hz,* 1H; CH). ^{13}C NMR (75 MHz, d6-DMSO, 25^{o}C): δ=23.3, 27.1, 28.5, 31.0, 33.0, 35.7, 52.6, 124.3, 125.0, 125.1, 125.7, 125.9, 127.1, 127.4, 128.1, 128.3, 128.5, 129.0, 130.2, 131.3, 131.7, 137.4, 174.0, 174.7. HRMS [ESI^{+}]: m/z (%): $C_{26}H_{29}N_2O_3$: Calculated, 416.1571: observed, 417.2174 [M-H]$^{-}$.

[1]H<u>NMR and Mass Spectra:</u>

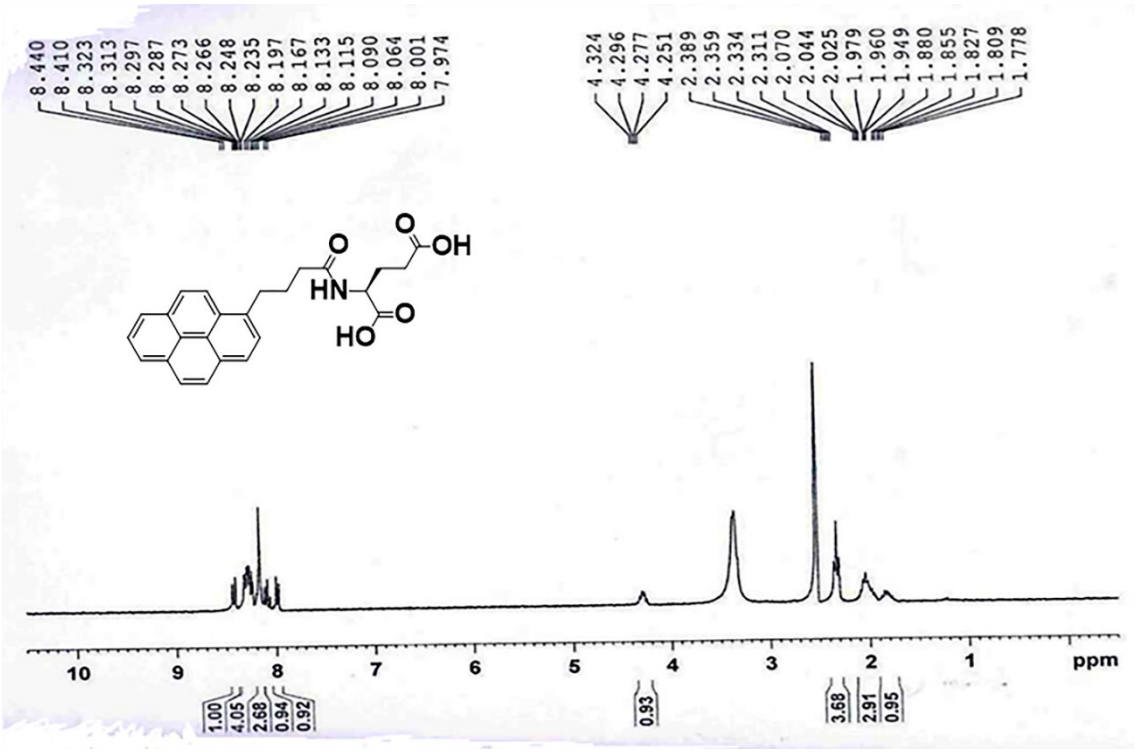

Fig. S15. [1]H NMR spectrum of **1a** in d_6-DMSO.

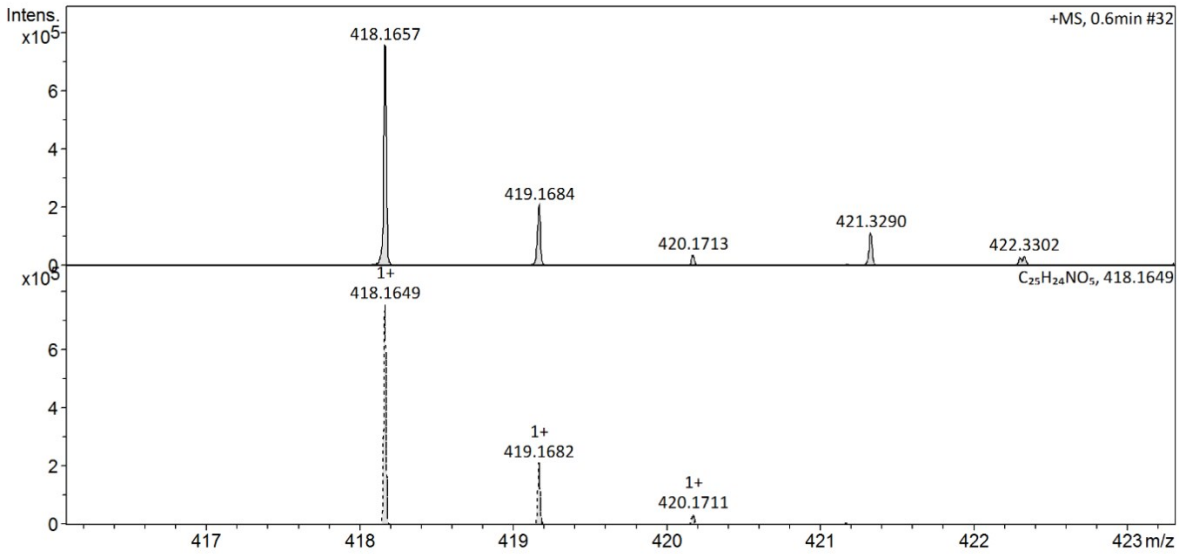

Fig. S16. ESI-MS spectrum of **1a**.

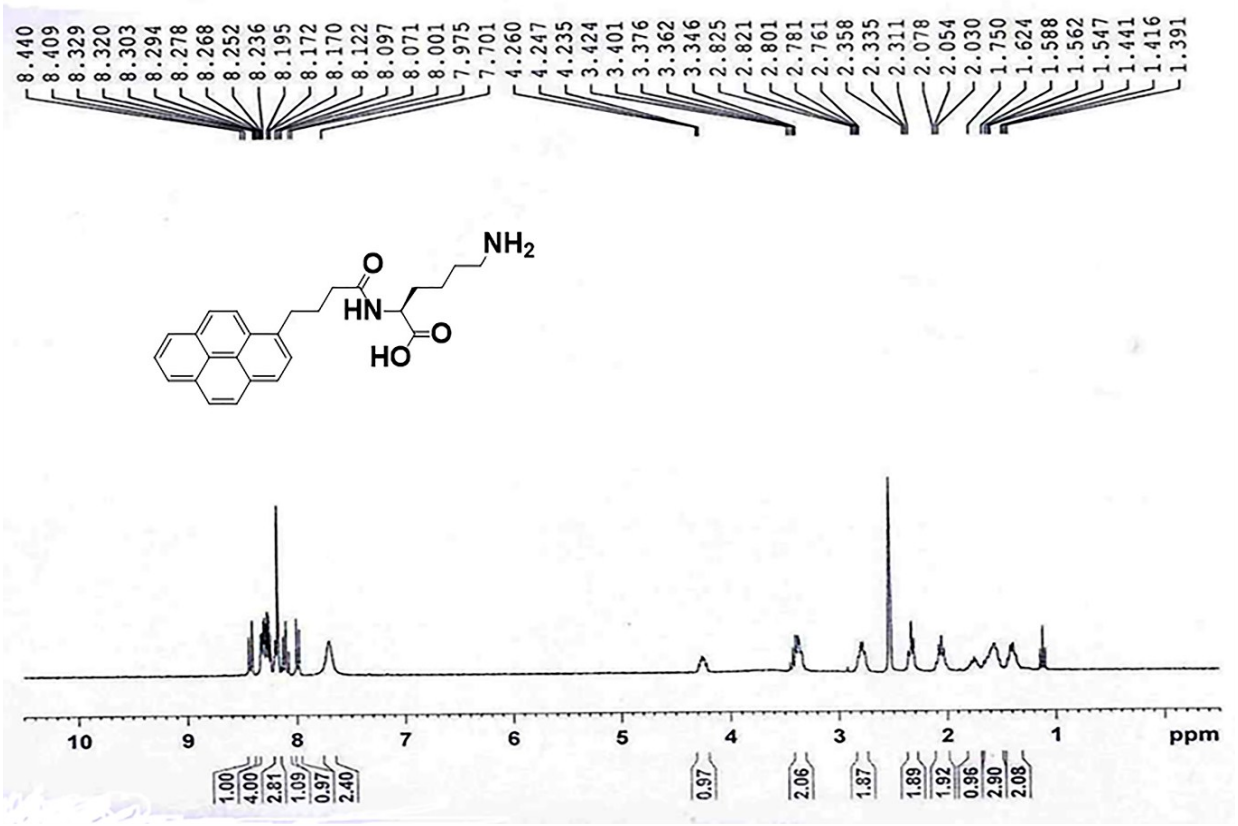

Fig. S17. ^{1}H NMR spectrum of **1b** in d_6-DMSO.

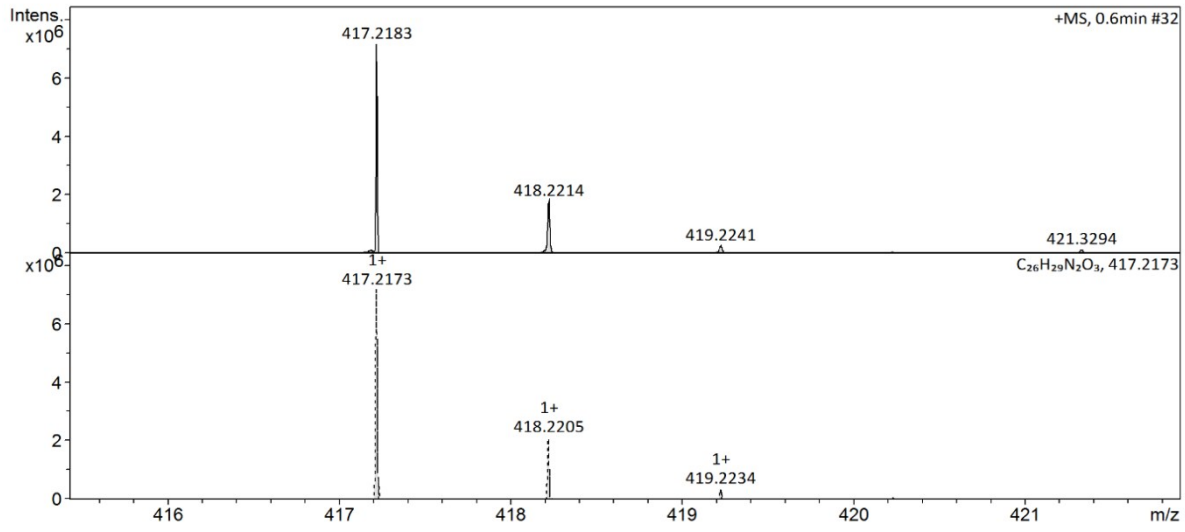

Fig. S18. ESI-MS spectrum of **1b**.

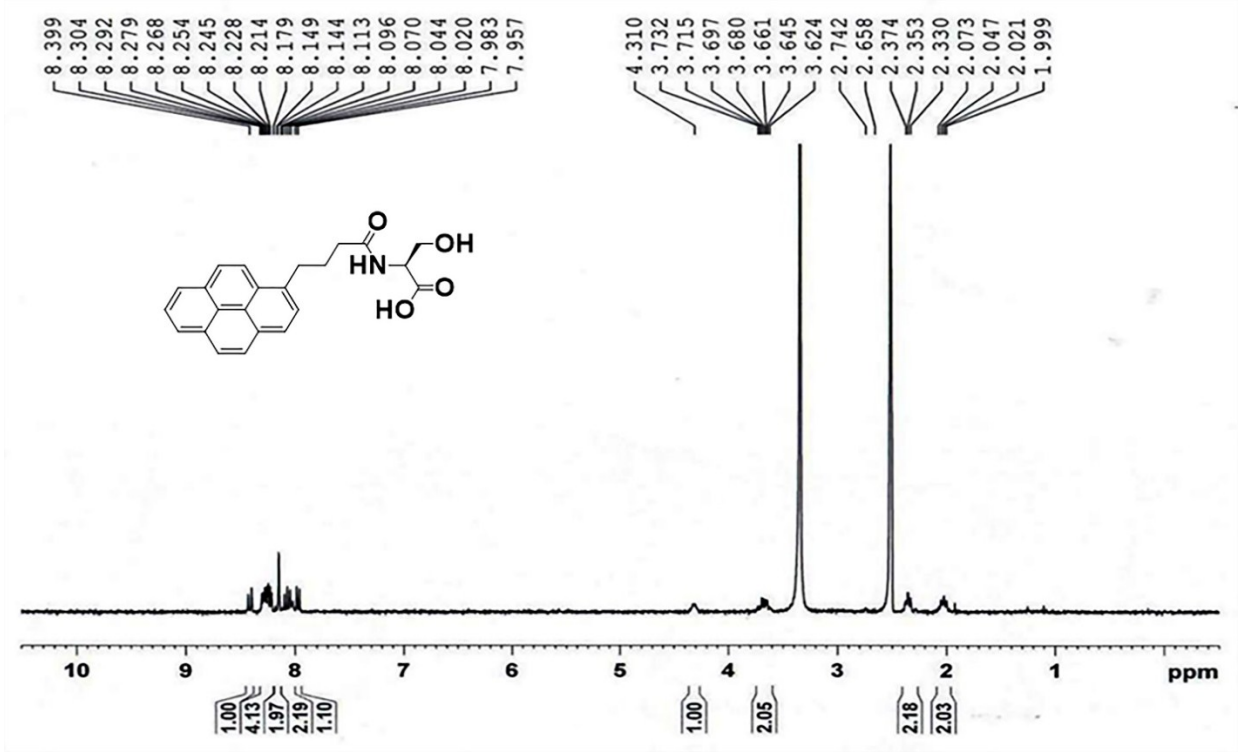

Fig. S19. ^{1}H NMR spectrum of **1c** in d_6-DMSO.

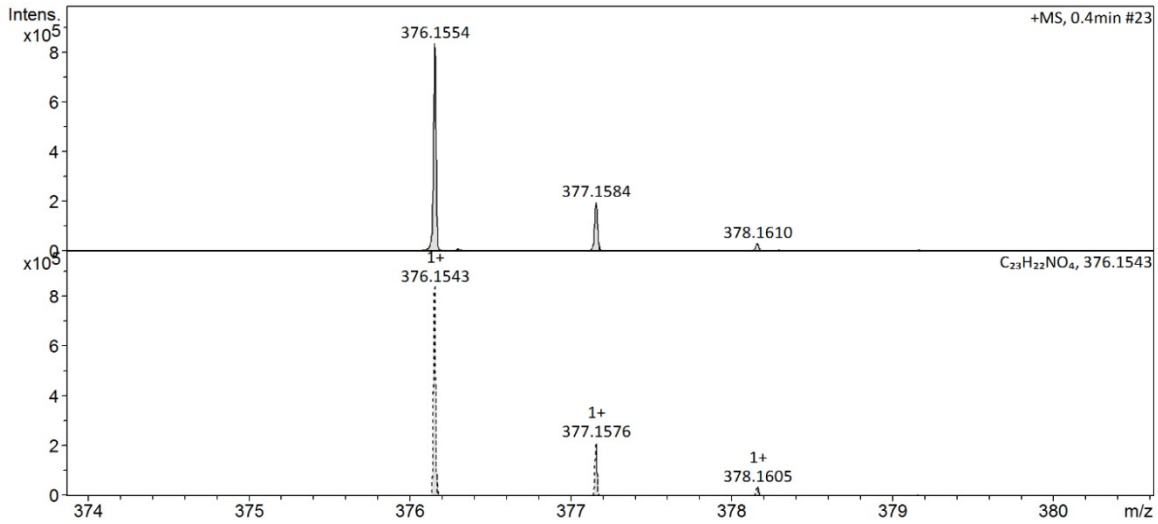

Fig. S20. ESI-MS spectrum of **1c**.

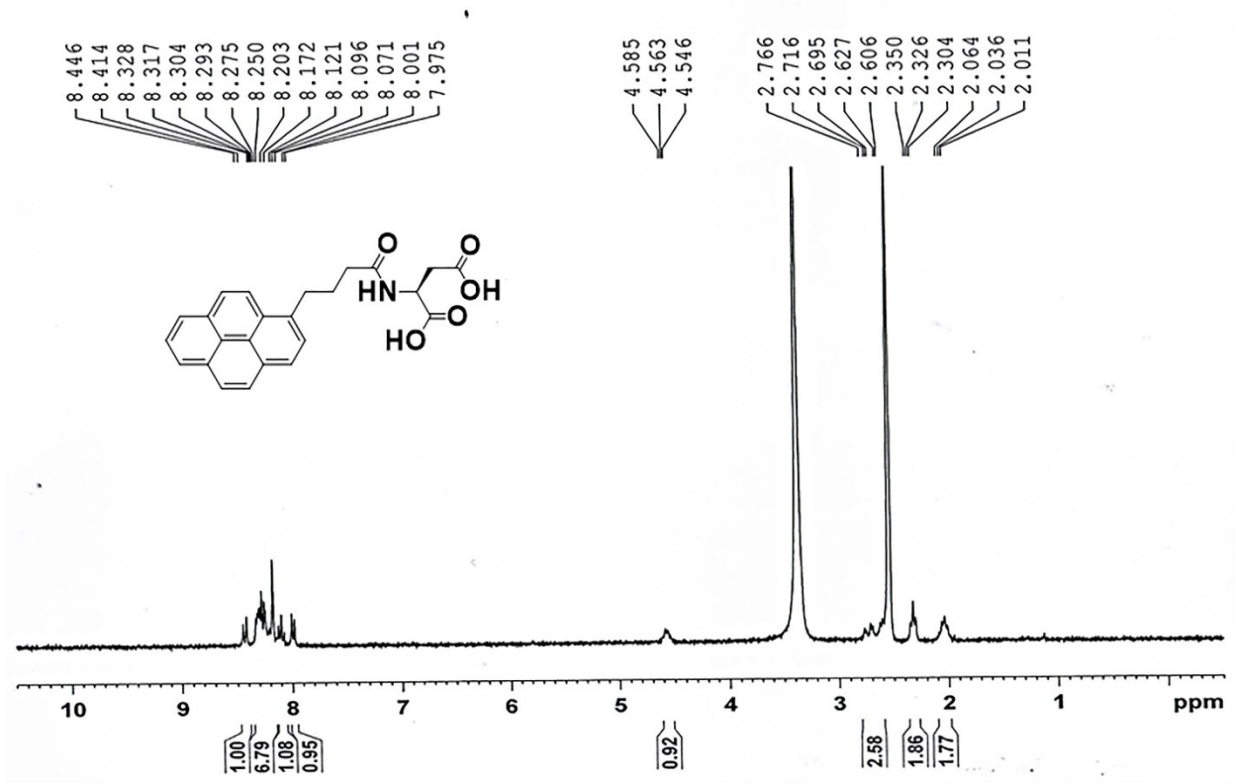

Fig. S21. ^{1}H NMR spectrum of **1d** in d_6-DMSO.

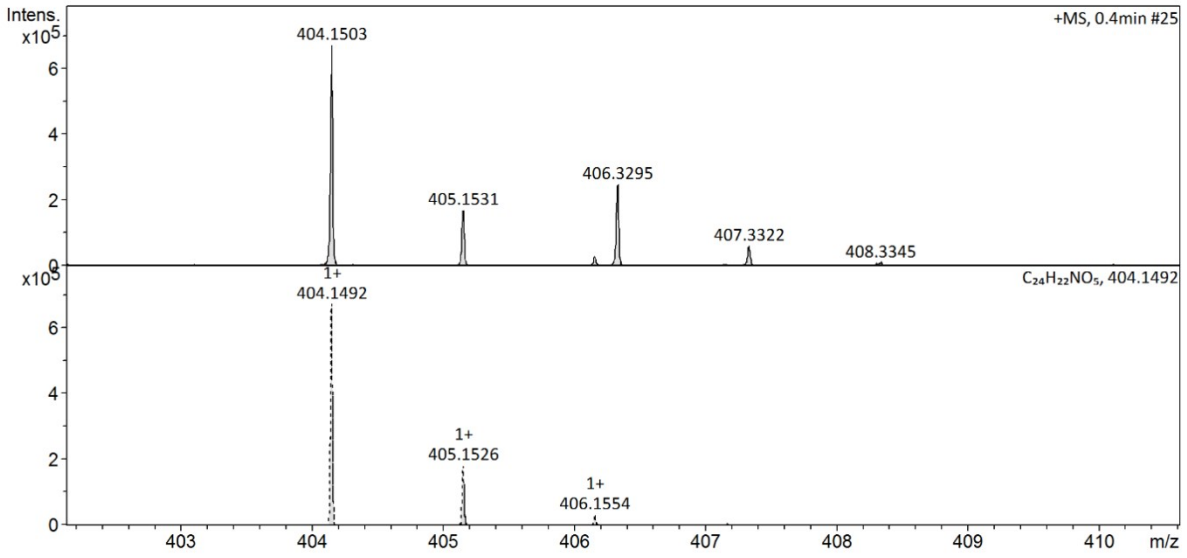

Fig. S22. ESI-MS spectrum of **1d**.

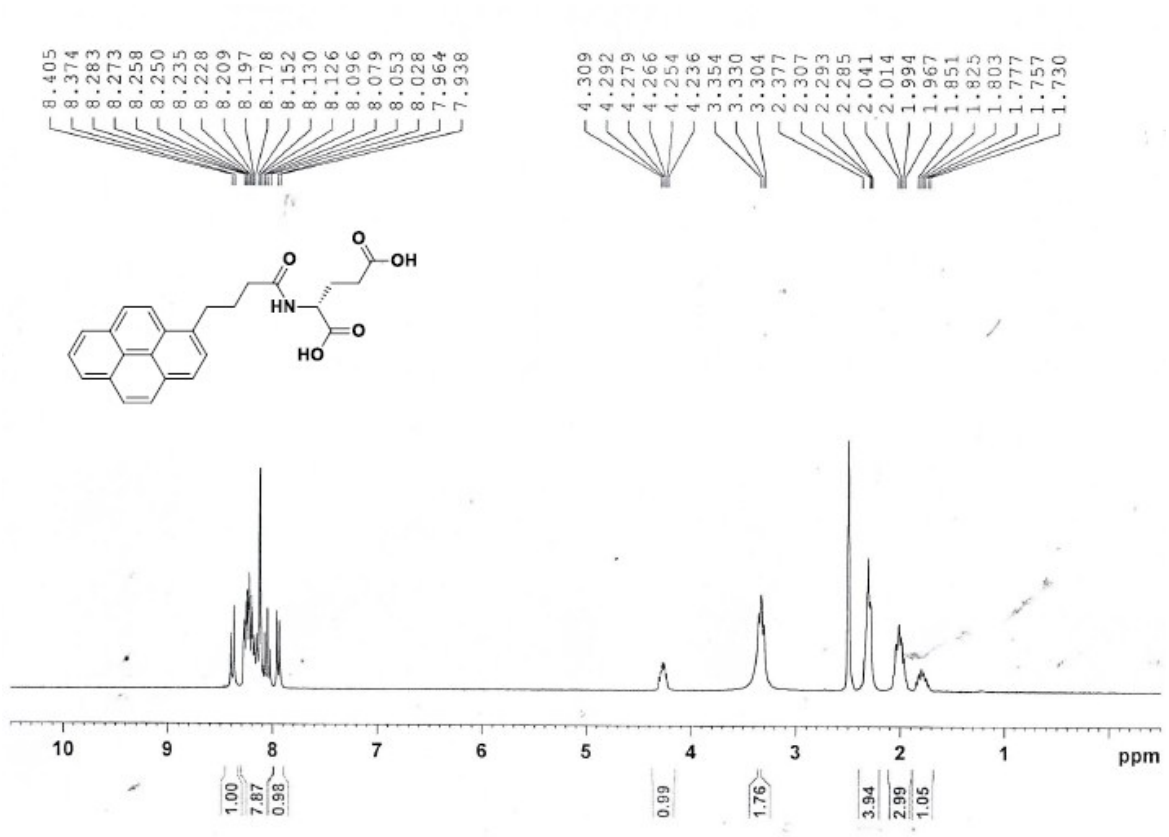

Fig. S23. 1H NMR spectrum of **2a** in d6-DMSO.

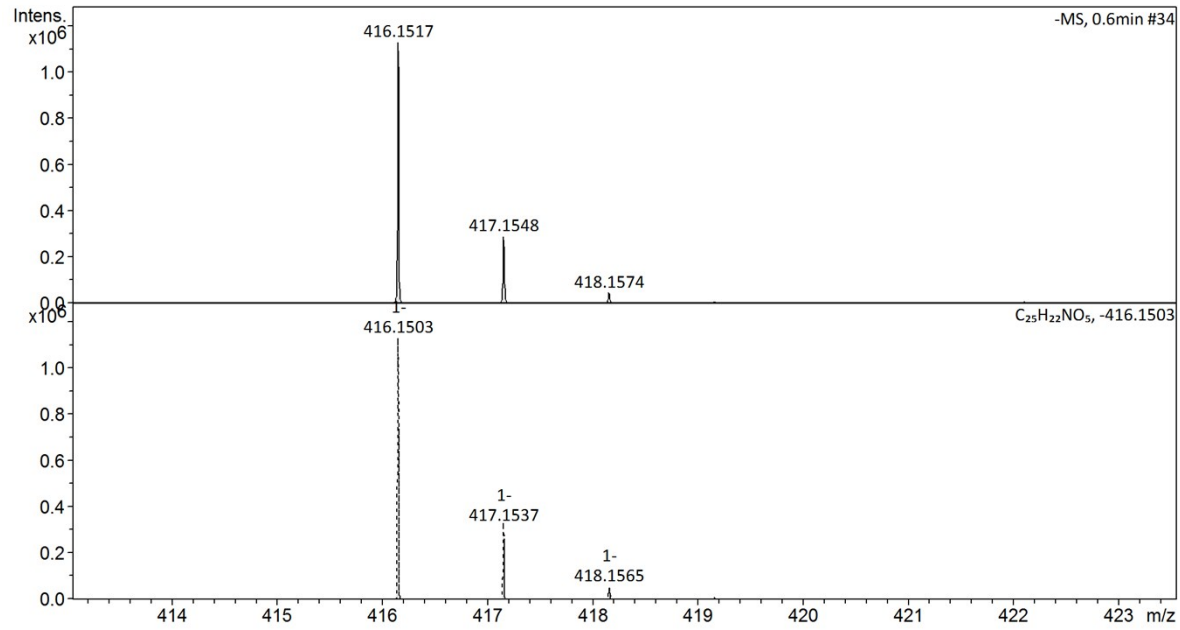

Fig. S24. ESI-MS spectrum of **2a**.

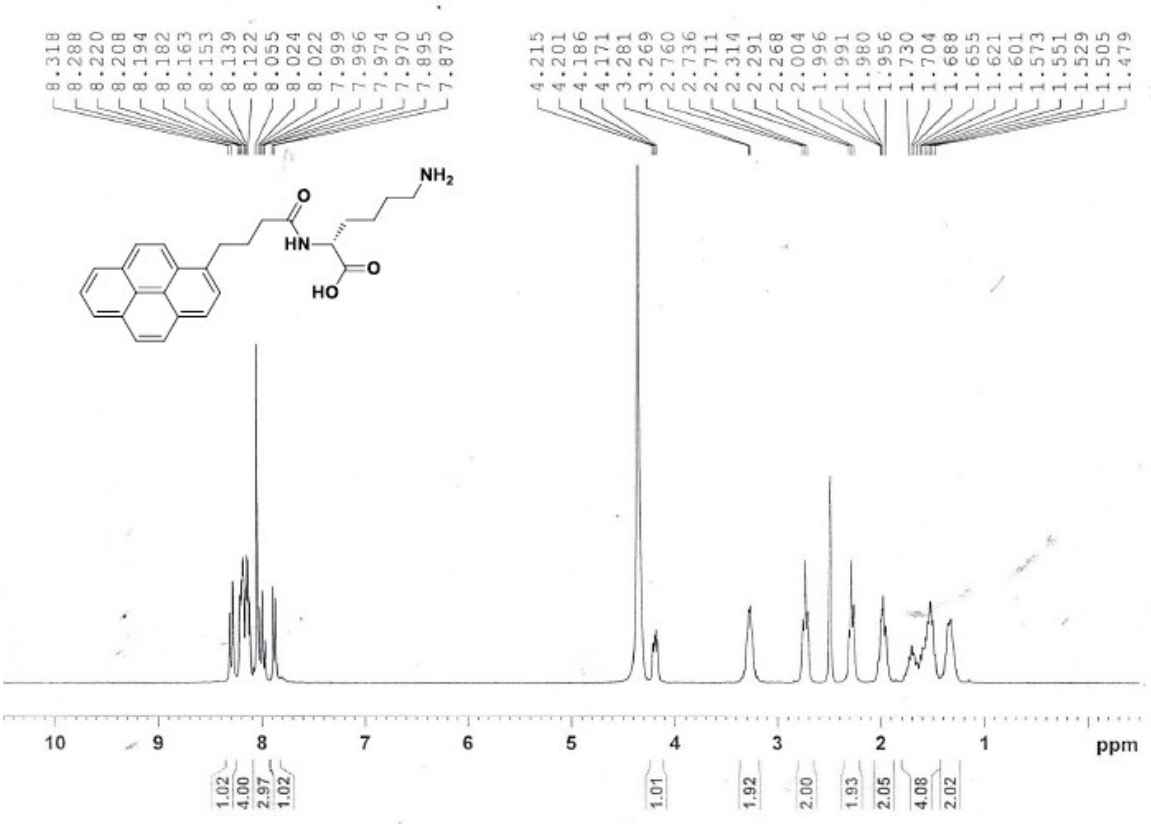

Fig. S25. 1H NMR spectrum of **2b** in d6-DMSO-D_2O.

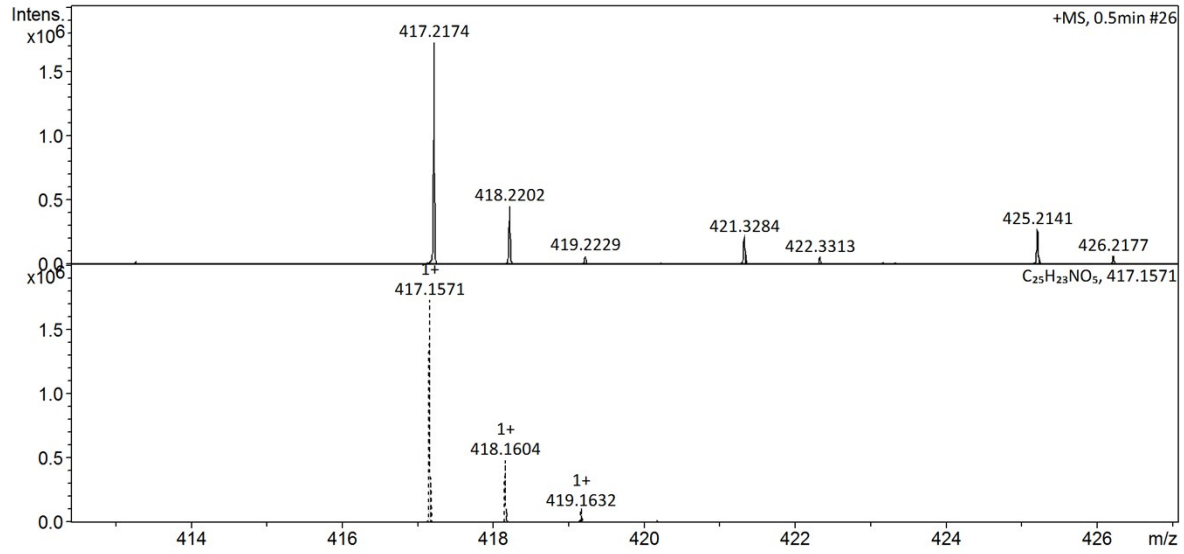

Fig. S26. ESI-MS spectrum of **2b**.