The Role of Aromatic Side Chains on the Supramolecular Hydrogelation of Naphthaleneimide/Dipeptide Conjugates

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Supporting Information

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Synthesis of NI-Phe -Phe (NI-FF). The peptide/dye conjugate derivative of **1** was prepared through SPPS using 2-chlorotrityl chloride resin, Fmoc-L-phenylalanine, Fmoc-L-phenylalanine and NI (Scheme 1)**.** The resin (2.4 g) was swollen in anhydrous CH_2Cl_2 for 30 min and then Fmoc-L-phenylalanine (1.16 g, 3.000 mmol) was loaded onto the resin in anhydrous *N*,*N*-dimethylformamide and *N*,*N*-diisopropylethylamine (DIEA; 1.24 mL, 7.500mmol) for 1 h. For deprotection of the Fmoc group, piperidine (20% in DMF) was added and the sample left for 20 min; this procedure was repeated twice (each time for 2 min). Fmoc-L-phenylalanine (1.55 g, 4.000 mmol) was coupled to the free amino group using *O*-(benzotriazol-1-yl)- *N*,*N*,*N*′,*N*′-tetramethyluraniumhexafluorophosphate (HBTU) (1.52 g, 4.000 mmol) and *N*,*N*-diisopropylethylamine (DIEA) (1.65 mL, 10.000 mmol) as coupling agents for 30 min. Again, the sample was treated with piperidine (20% in DMF) for 20 min; this procedure was repeated twice (each time for 2 min). Finally, NI (1.53 g, 6.000 mmol) was coupled to the free amino group using HBTU (2.28 g, 6.000 mmol) and DIEA (2.48 mL, 15.000 mmol) as coupling agents. After the reaction mixture had been stirred overnight, the peptide derivative was cleaved through treatment with CF_3CO_2H (90% in DI water) for 3 h. The resulting solution was dried by air and then Et_2O was added to precipitate the target product. The solid was dried under vacuum to remove residual solvent (White solid: 0.35 g).^{1}H NMR (300 MHz, [D_6]DMSO, 25°C): δ = 2.75-3.15 (m, 4H; CH_2),

4.40-4.50 (m, 1H; CH), 4.55-4.65 (m, 1H; CH), 4.688 (s, 2H; CH_2), 7.20-7.35 (m, 10H; CH), 7.90-8.00 (t, J = 7.8 Hz, 2H; CH), 8.38 (d, J = 8.1 Hz, 1H; NH), 8.48 (d, J = 8.4 Hz, 1H; NH), 8.54 (d, J = 7.5 Hz, 4H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 25°C): δ = 37.7, 38.5, 43.1, 54.6, 122.8, 127.1, 127.4, 128.2, 128.4, 128.9, 129.1, 130.0, 130.2, 131.8, 132.3, 135.4, 138.3, 138.5, 164.1, 167.3, 171.8, 173.6; MS [ESI$^-$]: calcd. m/z 549.19, obsvd. 548.1[M – H]$^-$.

Synthesis of NI-Tyr-Phe (NI-YF). The peptide/dye conjugate derivative of **2** was prepared through SPPS using 2-chlorotrityl chloride resin, Fmoc-L-phenylalanine, Fmoc-L-tyrosine and NI (Scheme 1)**.** The resin (2.4 g) was swollen in anhydrous CH_2Cl_2 for 30 min and then Fmoc-L-phenylalanine (1.16 g, 3.000 mmol) was loaded onto the resin in anhydrous *N*,*N*-dimethylformamide and *N*,*N*-diisopropylethylamine (DIEA; 1.24 mL, 7.500mmol) for 1 h. For deprotection of the Fmoc group, piperidine (20% in DMF) was added and the sample left for 20 min; this procedure was repeated twice (each time for 2 min). Fmoc-L-tyrosine (1.84 g, 4.000 mmol) was coupled to the free amino group using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′- tetramethyluranium hexafluorophosphate (HBTU) (1.52 g, 4.000 mmol) and *N*,*N*-diisopropylethylamine (DIEA) (1.65 mL, 10.000 mmol) as coupling agents for 30 min. Again, the sample was treated with piperidine (20% in DMF) for 20 min; this procedure was repeated twice (each time for 2 min). Finally, NI (1.53 g, 6.000 mmol) was coupled to the free amino

group using HBTU (2.28 g, 6.000 mmol) and DIEA (2.48 mL, 15.000 mmol) as coupling agents. After the reaction mixture had been stirred overnight, the peptide derivative was cleaved through treatment with CF_3CO_2H (90% in DI water) for 3 h. The resulting solution was dried by air and then Et_2O was added to precipitate the target product. The solid was dried under vacuum to remove residual solvent (White solid: 1.06 g).^{1}H NMR (300 MHz, [D_6]DMSO, 25°C): δ = 2.60-3.15 (m, 4H; CH_2), 4.40-4.55 (m, 2H; CH), 4.693 (s, 2H; CH_2), 6.68 (d, *J* = 8.1 Hz, 2H; CH), 7.06 (d, *J* = 8.4 Hz, 2H; CH), 7.15-7.40 (m, 5H; CH), 7.3 (t, *J* =7.65 Hz, 2H; CH), 8.33 (d, *J* = 7.5 Hz, 1H; NH), 8.42 (d, *J* = 8.4 Hz, 1H; NH), 8.53 (d, *J* = 8.1 Hz, 4H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 25°C): δ=37.7, 43.1, 54.5, 55.0, 115.8, 122.9, 127.4, 128.2, 128.4, 128.6, 129.2, 130.1, 131.1, 131.8, 132.3, 135.5, 138.4, 156.7, 164.1, 167.3, 171.9, 173.6; MS [ESI$^-$]: calcd. m/z 565.18, obsvd. 564.22[M – H]$^-$.

Synthesis of NI-Phe-Tyr (NI-FY). The peptide/dye conjugate derivative of **3** was prepared through SPPS using 2-chlorotrityl chloride resin, Fmoc-L-tyrosine, Fmoc-L-phenylalanine and NI (Scheme 1)**.** The resin (2.4 g) was swollen in anhydrous CH_2Cl_2 for 30 min and then Fmoc-L-tyrosine (1.38 g, 3.000 mmol) was loaded onto the resin in anhydrous *N*,*N*-dimethylformamide and *N*,*N*-diisopropylethylamine (DIEA; 1.24 mL, 7.500mmol) for 1 h. For deprotection of the Fmoc group, piperidine (20% in DMF) was added and the sample left for 20 min; this procedure was repeated twice (each time

for 2 min). Fmoc-L-phenylalanine (1.55 g, 4.000 mmol) was coupled to the free amino group using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluranium hexafluorophosphate (HBTU) (1.52 g, 4.000 mmol) and *N*,*N*-diisopropylethylamine (DIEA) (1.65 mL, 10.000 mmol) as coupling agents for 30 min. Again, the sample was treated with piperidine (20% in DMF) for 20 min; this procedure was repeated twice (each time for 2 min). Finally, NI (1.53 g, 6.000 mmol) was coupled to the free amino group using HBTU (2.28 g, 6.000 mmol) and DIEA (2.48 mL, 15.000 mmol) as coupling agents. After the reaction mixture had been stirred overnight, the peptide derivative was cleaved through treatment with CF_3CO_2H (90% in DI water) for 3 h. The resulting solution was dried by air and then Et_2O was added to precipitate the target product. The solid was dried under vacuum to remove residual solvent (White solid: 1.15 g).^{1}H NMR (300 MHz, [D_6]DMSO, 25°C): δ = 2.75-3.10 (m, 4H; CH_2), 4.35-4.45 (m, 1H; CH), 4.55-4.65 (m, 1H; CH), 4.694 (s, 2H; CH_2), 6.70 (d, *J* = 8.4 Hz, 2H; CH), 7.06 (d, *J* = 8.4 Hz, 2H; CH), 7.20-7.35 (m, 5H; CH), 7.92 (t, *J* =7.65 Hz, 2H; CH), 8.29 (d, *J* = 7.5 Hz, 1H; NH), 8.45-8.55 (m, 5H; CH, NH); ^{13}C NMR (75 MHz, [D_6]DMSO, 25°C): δ =40.0, 38.5, 43.1, 54.6, 54.9, 116.0, 122.8, 127.1, 128.2, 128.3, 128.4, 128.9, 130.2, 131.0, 131.8, 132.3, 135.5, 138.6, 156.9, 164.1, 167.4, 171.8, 173.7; MS [ESI$^-$]: calcd. m/z 565.18, obsvd. 564.2[M – H]$^-$.

Synthesis of NI-Tyr-Tyr (NI-YY). The peptide/dye conjugate derivative of **4** was prepared through SPPS using 2-chlorotrityl chloride resin, Fmoc-L-tyrosine, Fmoc-L-tyrosine and NI (Scheme 1)**.** The resin (1.2 g) was swollen in anhydrous CH_2Cl_2 for 30 min and then Fmoc-L-tyrosine (0.919 g, 2.000 mmol) was loaded onto the resin in anhydrous *N*,*N*-dimethylformamide and *N*,*N*-diisopropylethylamine (DIEA; 0.830 mL, 5.000mmol) for 1 h. For deprotection of the Fmoc group, piperidine (20% in DMF) was added and the sample left for 20 min; this procedure was repeated twice (each time for 2 min). Fmoc-L-tyrosine (0.919 g, 2.000 mmol) was coupled to the free amino group using *O*-(benzotriazol-1-yl) -*N*,*N*,*N*′,*N*′-tetramethyluraniumhexafluorophosphate (HBTU) (0.758 g, 2.000 mmol) and *N*,*N*-diisopropylethylamine (DIEA) (0.83 mL, 5.000 mmol) as coupling agents for 30 min. Again, the sample was treated with piperidine (20% in DMF) for 20 min; this procedure was repeated twice (each time for 2 min). Finally, NI (0.510 g, 2.000 mmol) was coupled to the free amino group using HBTU (0.758 g, 2.000 mmol) and DIEA (0.830 mL, 5.000 mmol)as coupling agents. After the reaction mixture had been stirred overnight, the peptide derivative was cleaved through treatment with CF_3CO_2H (90% in DI water) for 3 h. The resulting solution was dried by air and then Et_2O was added to precipitate the target product. The solid was dried under vacuum to remove residual solvent (light-yellow solid: 0.32 g).^{1}H NMR (300 MHz, [D_6]DMSO, 25°C): δ=2.60-3.15 (m, 4H; CH_2), 4.30-4.40 (m, 1H;

CH), 4.40-4.55 (m, 1H; CH), 2.70-2.90 (m, 1H; CH_2), 4.697 (s, 2H; CH_2), 6.60-6.75 (m, 4H; CH), 7.00-7.15(m, 4H; CH), 7.94 (t, *J* =7.8 Hz, 2H; CH), 8.21 (d, *J* =6.9 Hz, 1H; NH), 8.43 (d, *J* =8.4 Hz, 1H; NH), 8.54 (d, *J* =7.8 Hz, 4H; CH); ^{13}C NMR (75 MHz, [D_6]DMSO, 25°C): δ=^{13}C NMR (300 MHz, DMSO-d6): δ = 37.0, 37.7, 43.2, 55.0, 115.8, 116.0, 122.9, 128.2, 128.4, 128.7, 131.0, 131.2, 131.8, 132.3, 135.5, 156.7, 156.9, 164.2, 167.3, 171.9, 173.8; MS [ESI^-]: calcd. m/z 581.1, obsvd. 580.5[$M - H]^-$.

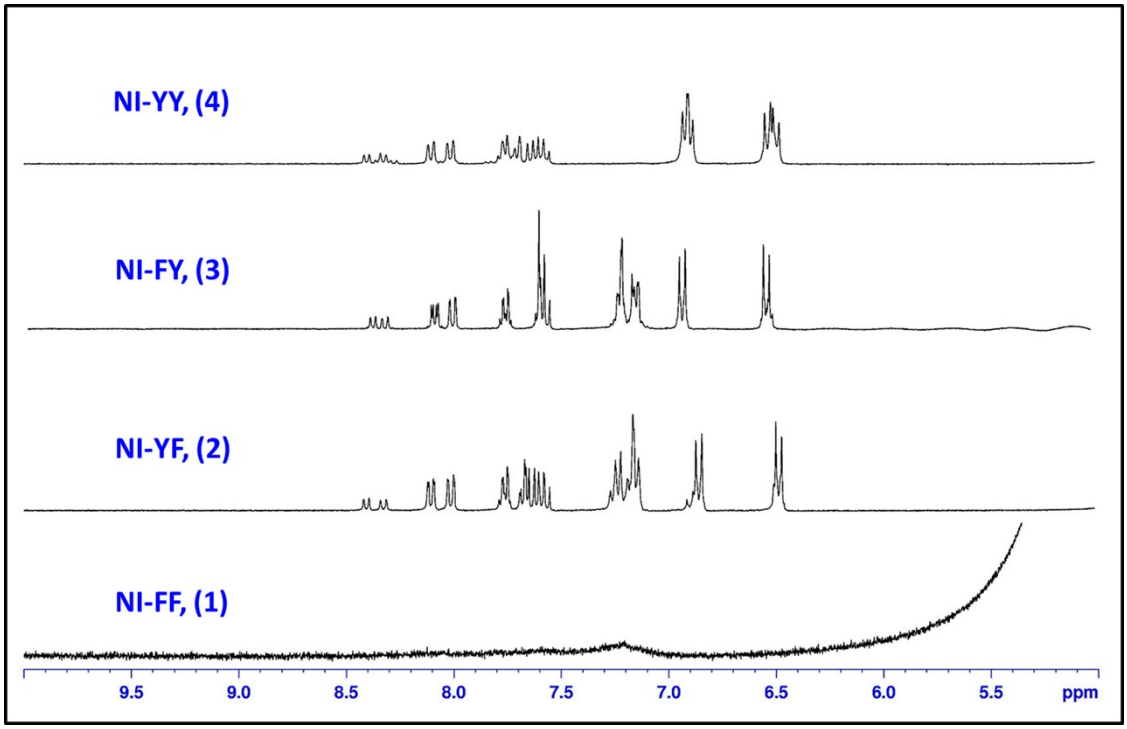

Fig. S1. Partial ^{1}H NMR spectra (300 MHz, D_2O + NaOD, pD = 11) of the hydrogelators **1-4.**

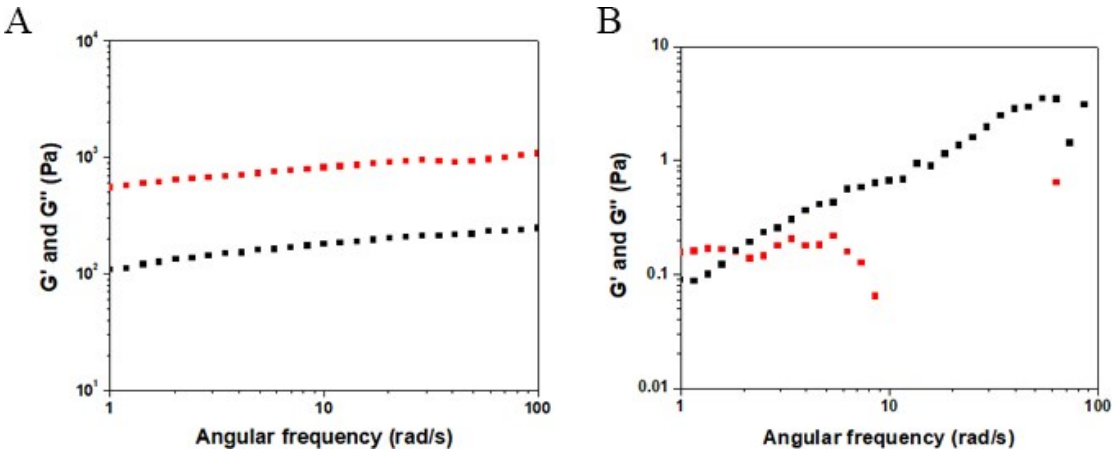

Fig. S2. Frequency sweep rheological data of hydrogelator **1** measured twice.

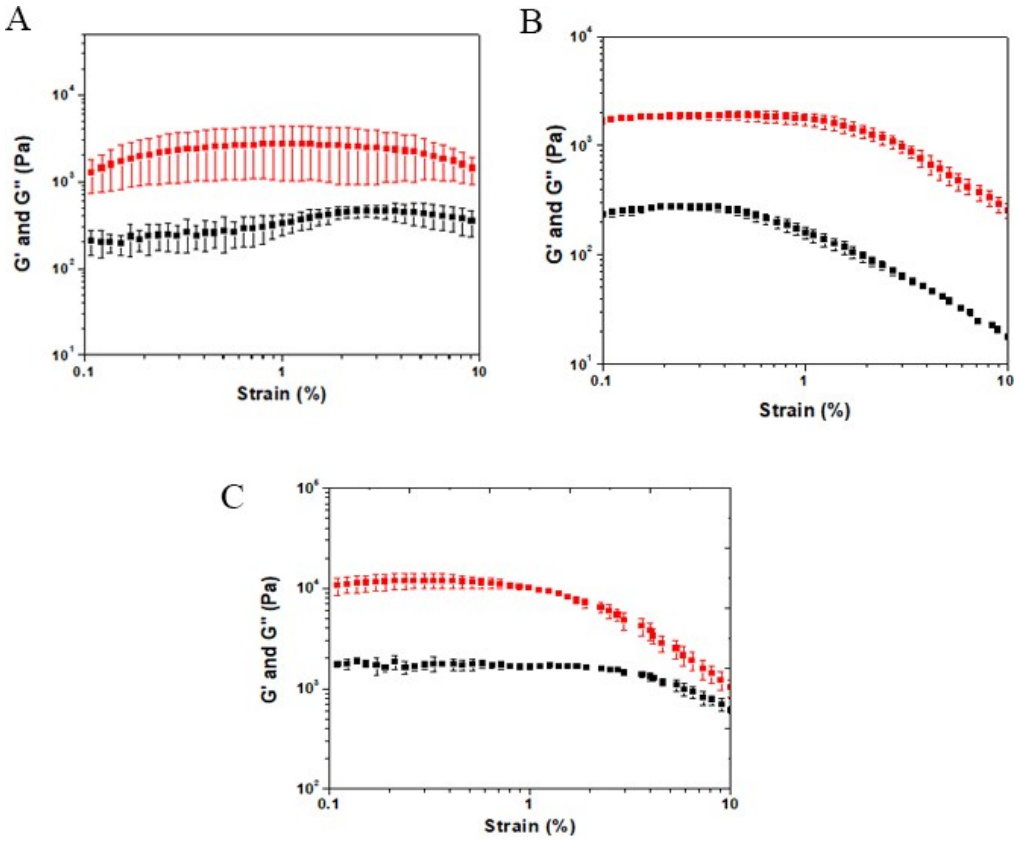

Fig. S3. Strain sweep rheological data of hydrogelators **2-4.**

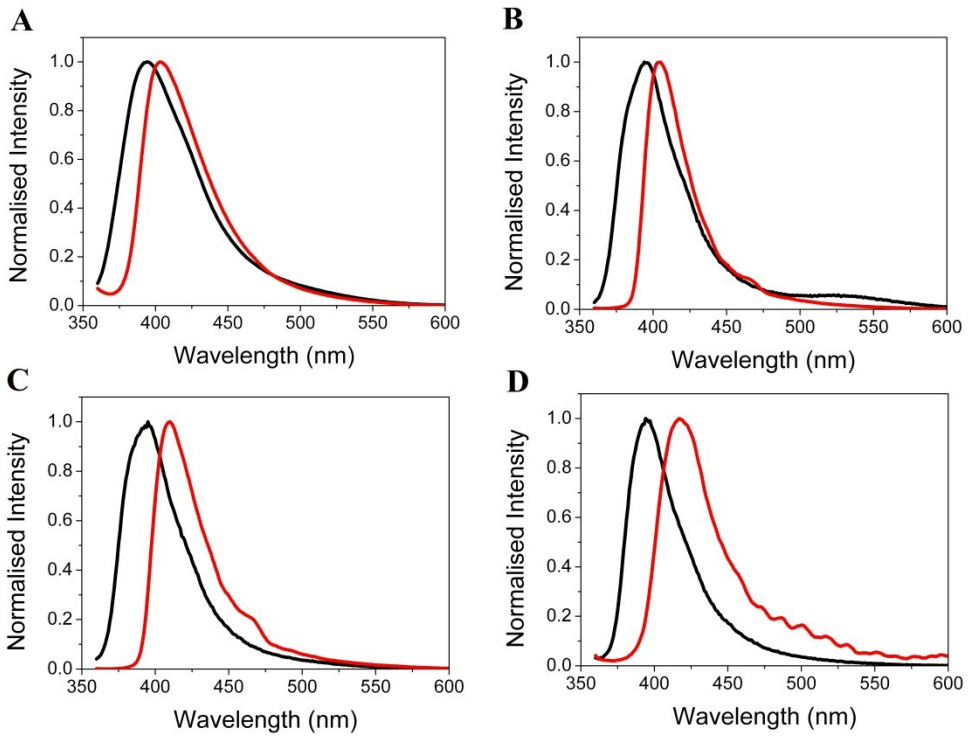

Fig. S4. Emission spectra of hydrogelators (A) **1**, (B) **2**, (C) **3** and (D) **4** at 0.05wt% (solution, black line) and 1wt% (gel, red line).

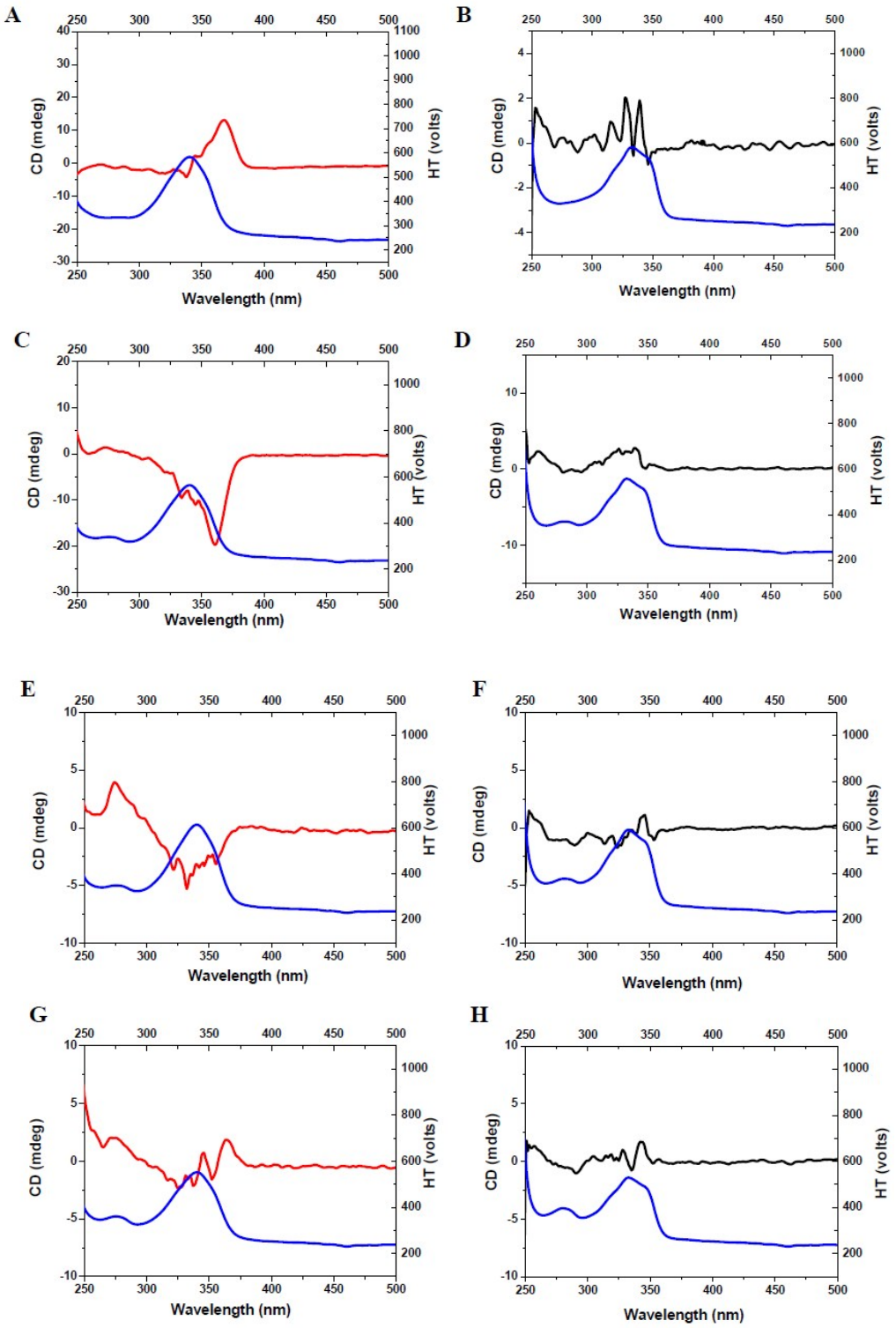

Fig. S5. CD spectra of hydrogelators **1** (A, B), **2** (C, D), **3** (E, F) and **4** (G, H) with HT signals (blue line) at 1600 μM in water (red line) and DMSO (black line)

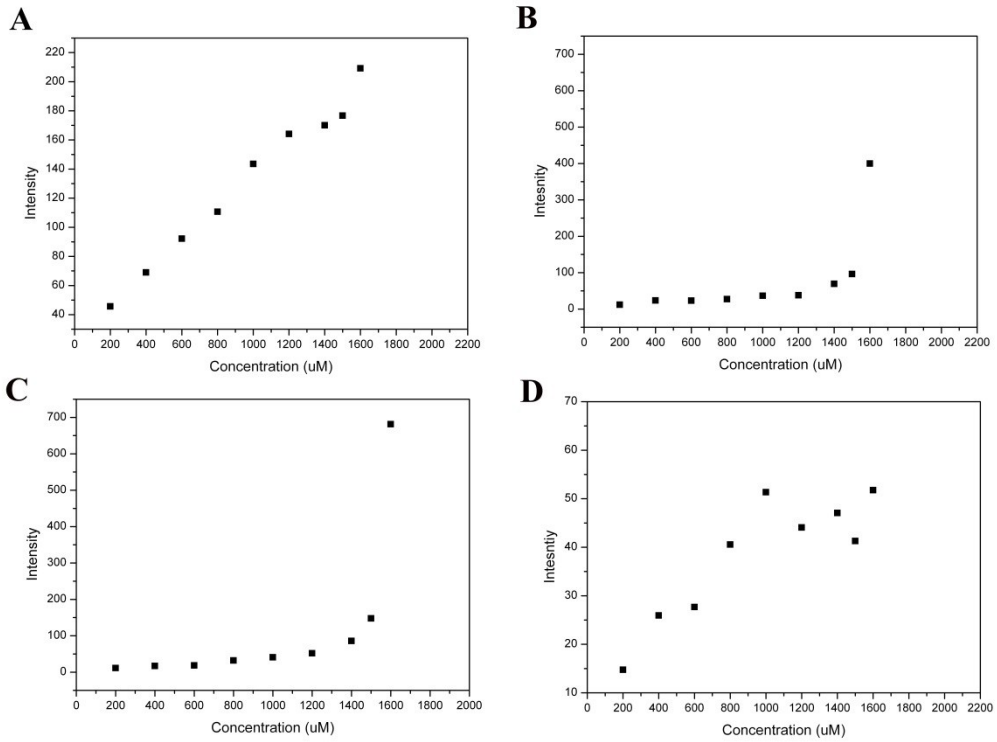

Fig. S6. Emission spectra of hydrogelators (A) **1**, (B) **2**, (C) **3** and (D) **4** at different concentrations with ThT (20 μM); excitation at 440 nm and emission maximum at 480 nm.

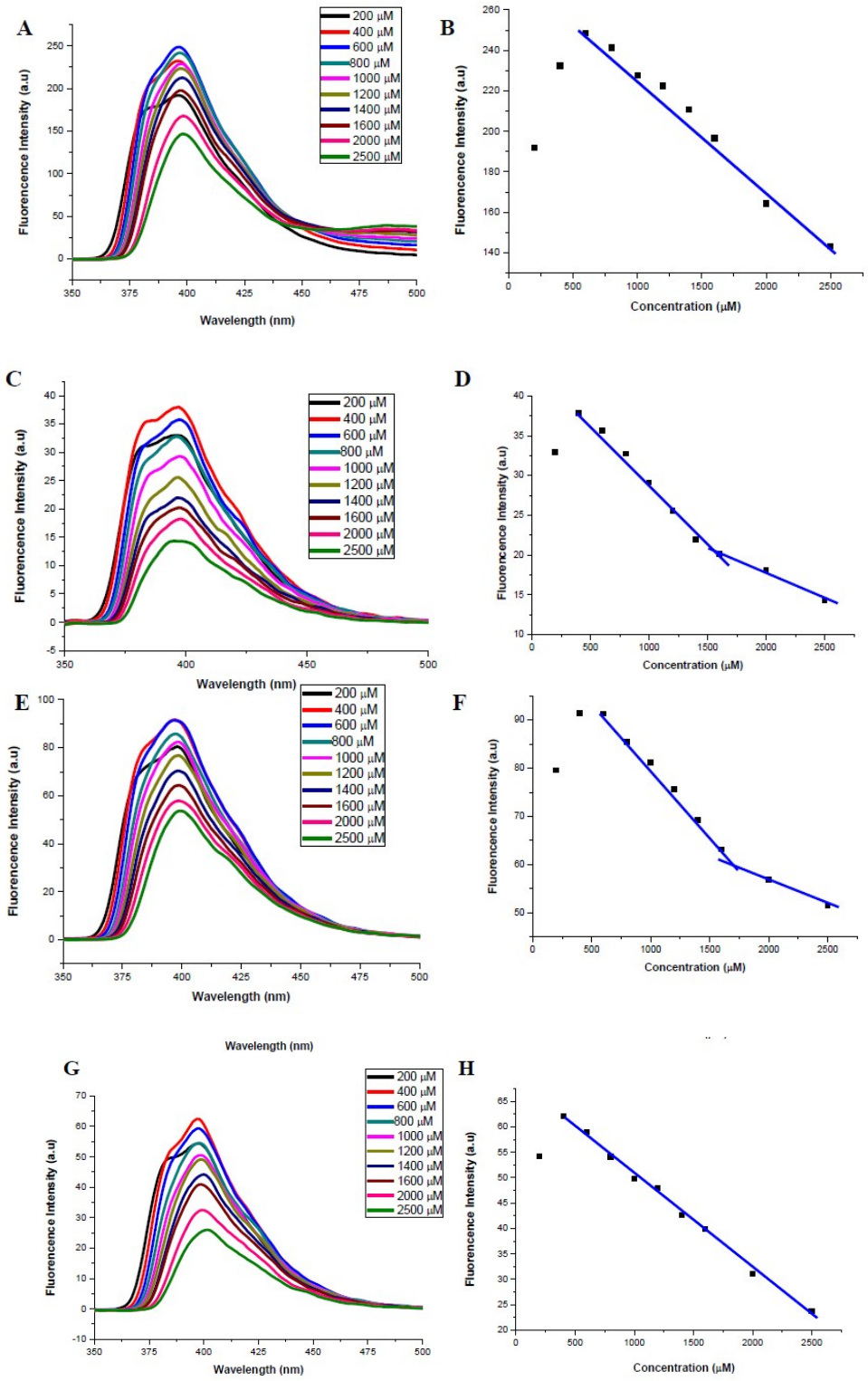

Fig. S7. Emission spectra of hydrogelators **1** (A, B), **2** (C, D), **3** (E, F) and **4** (G, H) at different concentrations; Figures B, D, F and H are the plot of fluorescence emission intensity (at 396 nm) versus the concentration of the **1-4**.

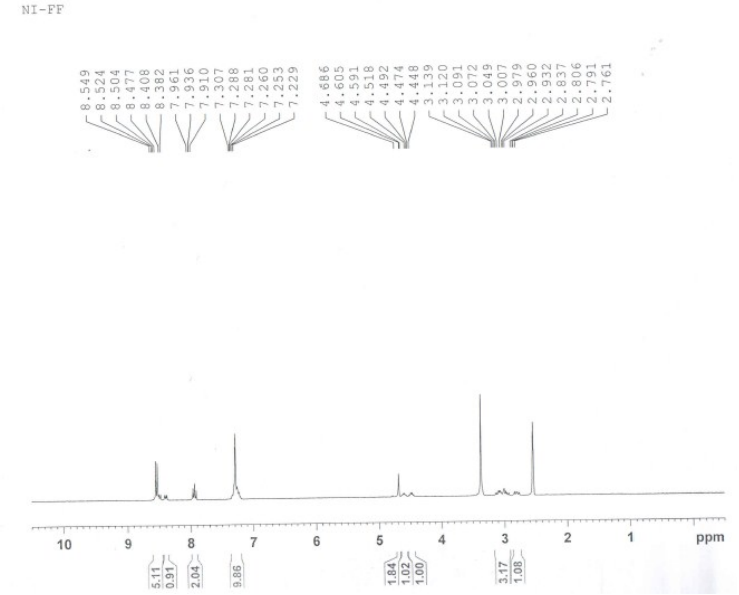

Fig. S8. ^{1}H NMR spectrum of **1** in [D_6]DMSO.

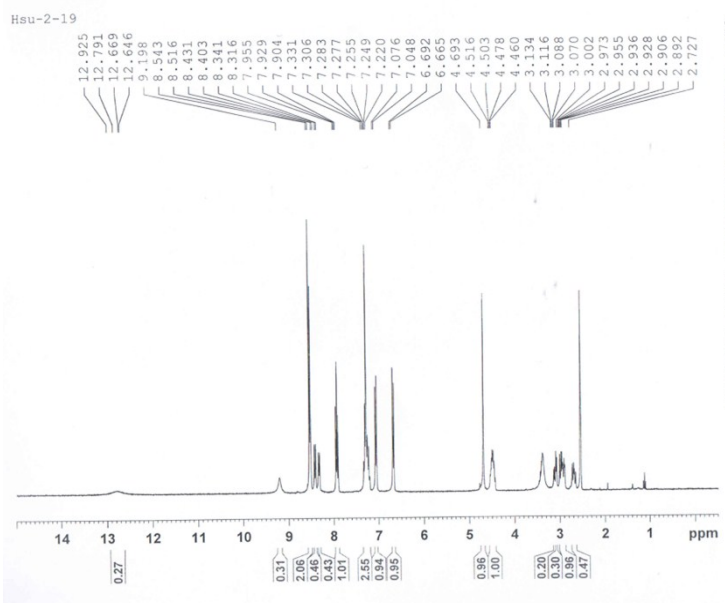

Fig. S9. ^{1}H NMR spectrum of **2** in [D_6]DMSO.

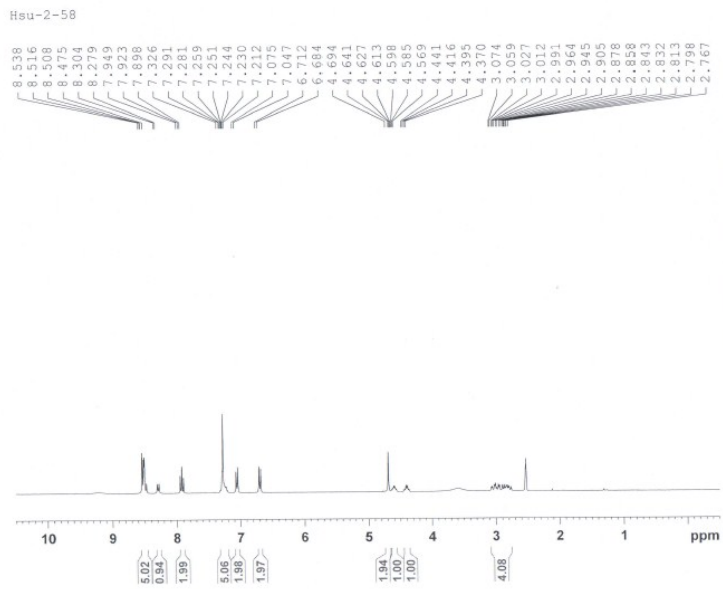

Fig. S10. ^{1}H NMR spectrum of **3** in [D_6]DMSO.

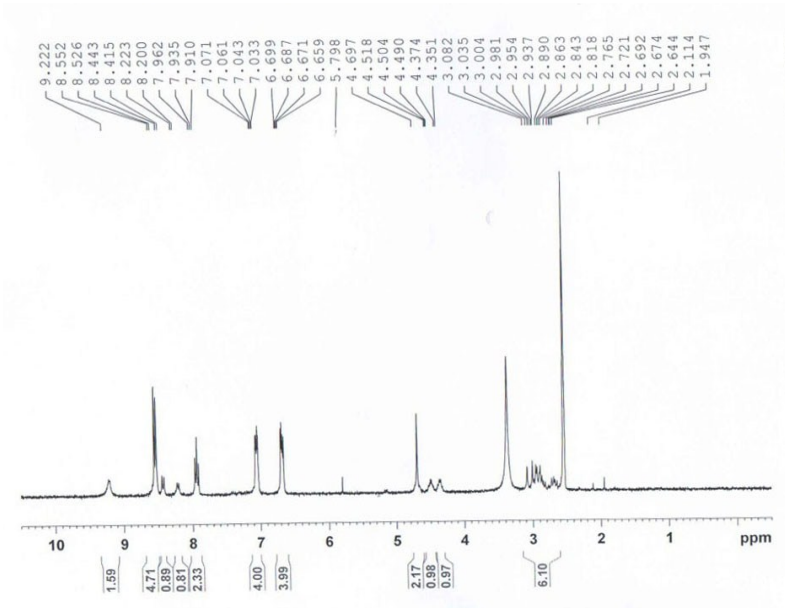

Fig. S11. ^{1}H NMR spectrum of **4** in [D_6]DMSO.

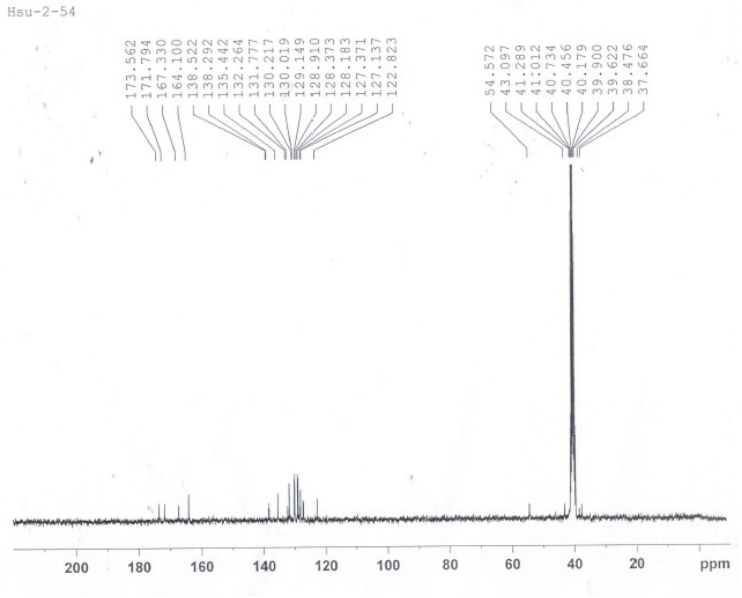

Fig. S12. ^{13}C NMR spectrum of **1** in [D_6]DMSO.

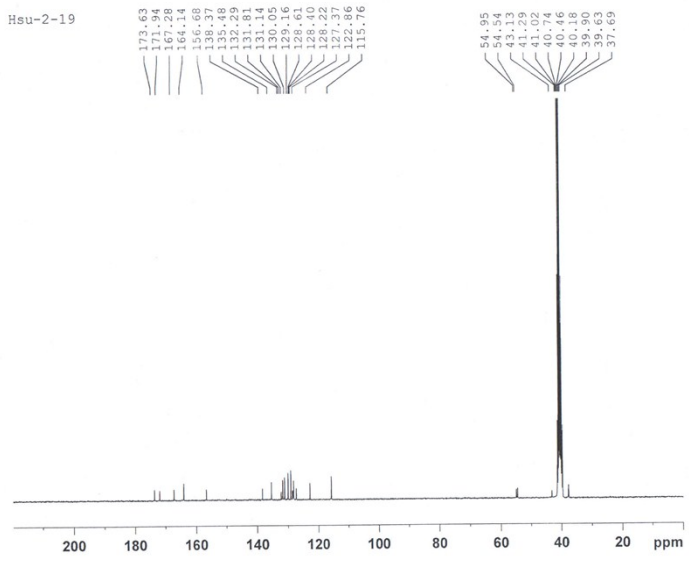

Fig. S13. ^{13}C NMR spectrum of **2** in [D_6]DMSO.

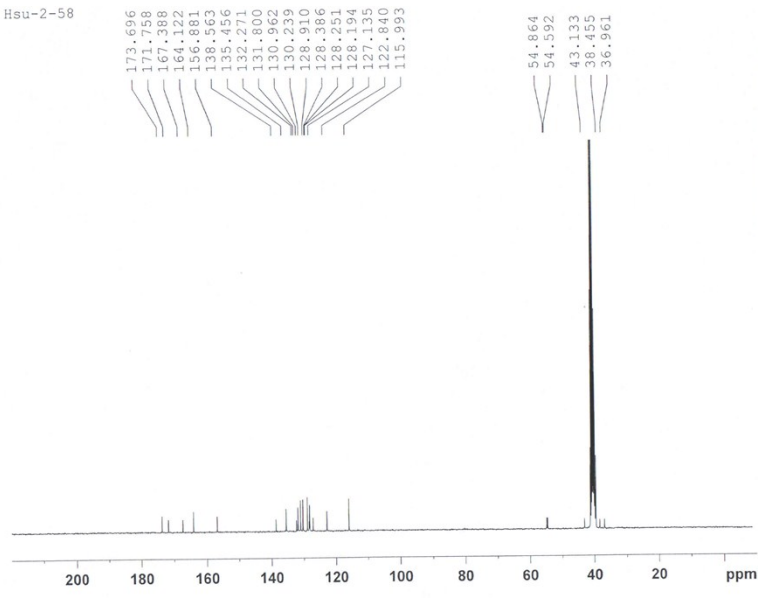

Fig. S14. ^{13}C NMR spectrum of **3** in [D_6]DMSO.

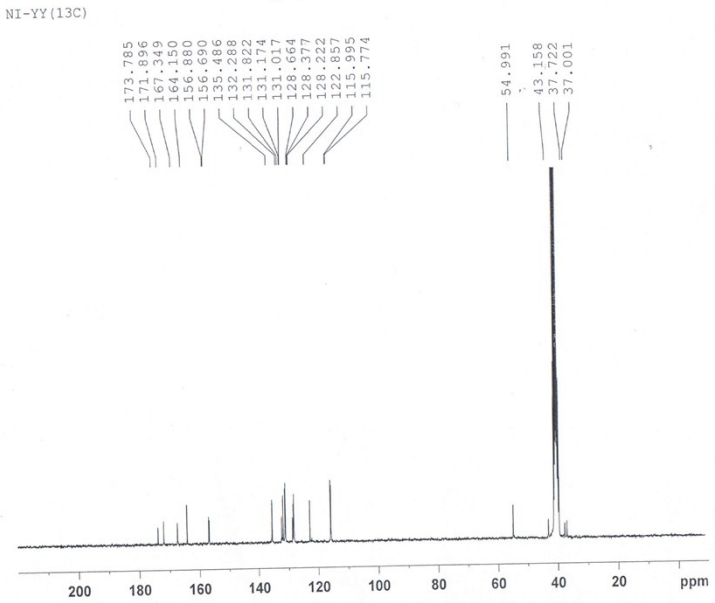

Fig. S15. ^{13}C NMR spectrum of **4** in [D_6]DMSO.