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Influence of alkyl chain lengths on self-assembly of supramolecular hydrogels of naphthalene diimide-capped dipeptides

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Supporting Information

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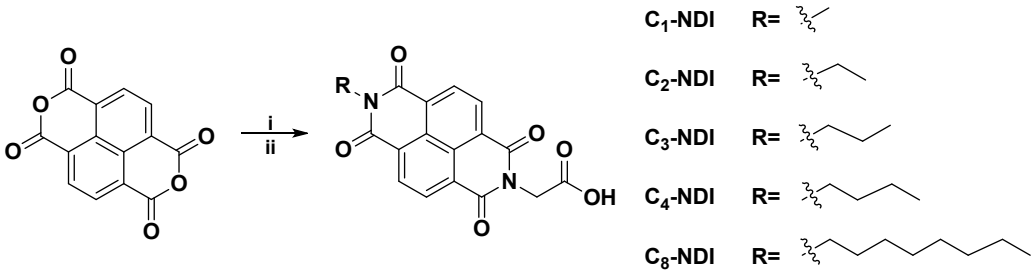


i) $C_nH_{2n+1}NH_2$; ii) NH_2CH_2COOH, DMF

Synthesis of C_1-NDI. A solution of 1,4,5,8-Naphthalenetetracarboxylic dianhydride (2.68 g, 10.0 mmol), methylamine (0.62 mL, 20.0 mmol), and glycine (0.62 g, 20.0 mmol) in DMF (40 mL) was stirred at 120 °C for 4 h. After the mixture had cooled to room temperature, the insoluble solid was filtered off and the solution was poured into water (100 mL). The precipitate was filtered, washed with water (15 mL), methanol (15 mL), and CH_2Cl_2 (15 mL). **C_1-NDI** was obtained as a brown solid (1.39 g, 31 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.47-3.48 (m, 3H), 4.82 (s, 2H), 8.72-8.83 (m, 4H) ;: δ = 27.9, 35.2, 42.4, 126.2, 126.8, 131.2, 131.9, 163.0, 163.5, 169.9; MS [FAB$^-$]: calcd. m/z 338.05, obsvd. 337.7 $[M - H]^-$.

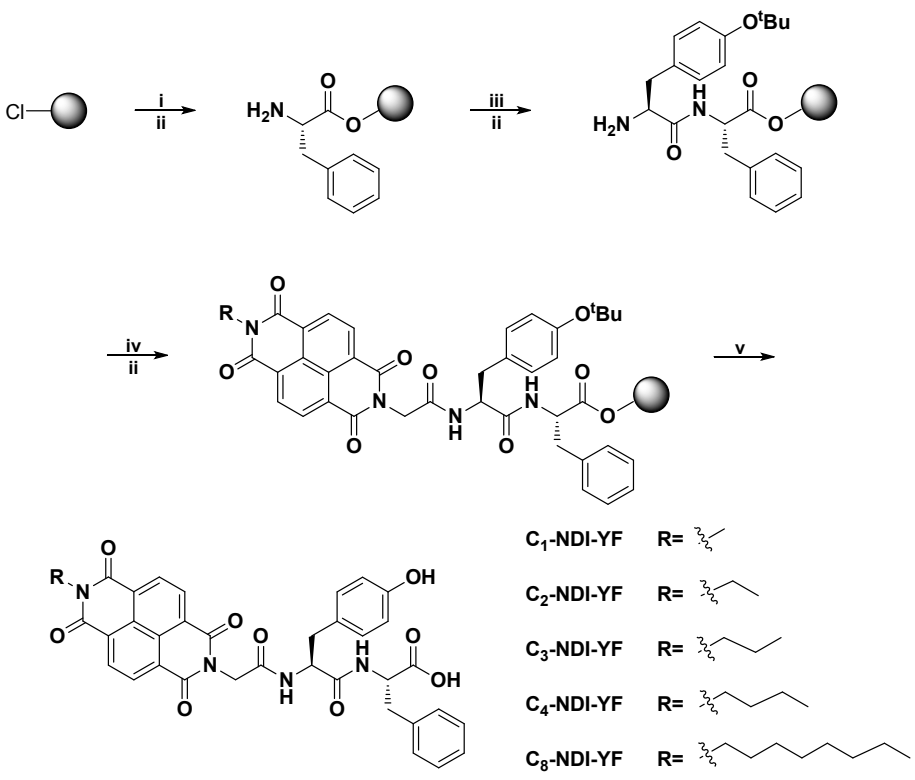
Synthesis of C_2-NDI. A solution of 1,4,5,8-Naphthalenetetracarboxylic dianhydride (2.68 g, 10.0 mmol), ethylamine (0.91 mL, 20.0 mmol), and glycine (0.62 g, 20.0 mmol) in DMF (40 mL) was stirred at 120 °C for 4 h. After the mixture had cooled to room temperature, the insoluble solid was filtered off and the solution was poured into water (100 mL). The precipitate was filtered, washed with water (15 mL), methanol (15 mL), and CH_2Cl_2 (15 mL). **C_2-NDI** was obtained as a red solid (1.42g, 39%). ^{1}H

NMR (300 MHz, DMSO-d_6): δ = 1.28 (t, J = 7.05 Hz, 3H), 4.05-4.13 (m, 2H), 4.80 (s, 2H), 8.65-8.73 (m, 4H) ; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 13.5, 39.2, 42.1, 126.5, 130.9, 131.5, 162.7, 169.5; MS [FAB$^-$]: calcd. m/z 352.07, obsvd. 351.6 [M – H]$^-$.

Synthesis of C_3-NDI. A solution of 1,4,5,8-Naphthalenetetracarboxylic dianhydride (2.68 g, 10.0 mmol), n-propylamine (1.18 mL, 20.0 mmol), and glycine (0.62 g, 20.0 mmol) in DMF (40 mL) was stirred at 120 ^{o}C for 4 h. After the mixture had cooled to room temperature, the insoluble solid was filtered off and the solution was poured into water (100 mL). The precipitate was filtered, washed with water (15 mL), methanol (15 mL), and CH_2Cl_2 (15 mL). **C_3-NDI** was obtained as a brown solid (1.48 g, 41 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.92-1.08 (m, 3H), 1.61-1.78 (m, 2H), 3.95-4.12 (m, 2H) ,4.80 (s, 2H), 8.65-8.74 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 11.9, 21.3, 39.2, 42.0, 126.4, 130.9, 131.5, 162.7,169.5; MS [FAB$^-$]: calcd. m/z 366.1, obsvd. 365.9 [M – H]$^-$.

Synthesis of C_4-NDI. A solution of 1,4,5,8-Naphthalenetetracarboxylic dianhydride (2.68 g, 10.0 mmol), n-butylamine (1.46 mL, 20.0 mmol), and glycine (0.62 g, 20.0 mmol) in DMF (40 mL) was stirred at 120 ^{o}C for 4 h. After the mixture had cooled to room temperature, the insoluble solid was filtered off and the solution was poured into

water (100 mL). The precipitate was filtered, washed with water (15 mL), methanol (15 mL), and CH_2Cl_2 (15 mL). **C_4-NDI** was obtained as a brown solid (1.33 g, 29 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ =0.98 (t, J = 7.05 Hz, 3H), 1.35-1.49 (m, 2H), 1.62-1.73 (m, 2H), 4.07-4.18 (m, 2H), 4.82 (s, 2H), 8.71-8.83 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 13.7, 19.9, 29.5, 34.2, 41.5, 125.8, 130.4, 131.0, 162.1, 169.0; MS [FAB$^-$]: calcd. m/z 380.10, obsvd. 378.6 $[M - H]^-$.



i) Fmoc-L-AA-R_1, DIEA; ii) 20 % piperidine; iii) Fomc-L-AA-R_2; iv) C_n-NDI, HBTU, DIEA; v) TFA:water=9:1

Synthesis of C_1-NDI-YF (1). This peptide derivative was prepared through SPPS using 2-chlorotrityl chloride resin, Fmoc-L-phenylalanine, Fmoc-L-tyrosine and **C_1-NDI**. The resin (1.20 g) was swelled in anhydrous CH_2Cl_2 for 30 min and then Fmoc-

L-phenylalanine (0.78 g, 2.0 mmol) was loaded onto the resin in anhydrous *N*,*N*-dimethylformamide (DMF) and *N*,*N*-diisopropylethylamine (DIEA; 0.83 mL, 5.0 mmol) 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 (2.30 g, 5.0 mmol) was coupled to the free amino group using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluraniumhexafluorophosphate (HBTU) (0.76 g, 2.0 mmol) and *N*,*N*-diisopropylethylamine (DIEA) (0.83 mL, 5.0 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, **C_1-NDI** (1.02 g, 3.0 mmol) was coupled to the free amino group using HBTU (0.76 g, 2.0 mmol) and DIEA (0.83 mL, 5.0 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 under a stream of air and then Et_2O was added to precipitate the target product. The solid was dried under vacuum to remove residual solvent (brown solid: 0.21 g). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.91-3.15 (m, 4H), 3.47 (s, 3H),4.41-4.62 (m, 2H), 4.82 (s, 2H),6.67 (d, *J* = 8.55 Hz, 2H), 7.04 (d, *J* = 8.55 Hz, 2H), 7.23-7.35 (m, 5H), 8.36 (d, *J* = 7.2 Hz, 1H),8.45 (d, *J* = 6.3 Hz, 1H), 8.71-8.82 (m, 4H), 9.19 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 27.1, 34.4, 36.7, 42.6, 53.6, 54.2,

109.7, 114.8, 119.1, 124.6, 126.5, 127.2, 128.3, 129.1, 130.3, 130.8, 137.5, 142.9, 155.8, 162.4, 162.9, 166.0, 171.0, 172.8; MS [ESI$^-$]: calcd. m/z 648.19, obsvd. 647.0 $[M - H]^-$.

Synthesis of C_2-NDI-YF (2). This peptide derivative was prepared through SPPS using 2-chlorotrityl chloride resin, Fmoc-L-phenylalanine, Fmoc-L-tyrosine and **C_2-NDI**. The resin (1.20 g) was swelled in anhydrous CH_2Cl_2 for 30 min and then Fmoc-L-phenylalanine (0.78 g, 2.0 mmol) was loaded onto the resin in anhydrous *N*,*N*-dimethylformamide (DMF) and *N*,*N*-diisopropylethylamine (DIEA; 0.83 mL, 5.0 mmol) 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 (2.30 g, 5.0 mmol) was coupled to the free amino group using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluraniumhexafluorophosphate (HBTU) (0.76 g, 2.0 mmol) and *N*,*N*-diisopropylethylamine (DIEA) (0.83 mL, 5.0 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, **C_2-NDI** (1.06 g, 3.0 mmol) was coupled to the free amino group using HBTU (0.76 g, 2.0 mmol) and DIEA (0.83 mL, 5.0 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 under a stream of air and then Et_2O was added to precipitate the target product. The solid was dried under vacuum to remove residual solvent (red solid: 0.25 g). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.31 (t, J = 5.85 Hz 3H), 2.91-3.02 (m, 2H) , 4.08-4.19 (m, 2H), 4.34-4.51 (m, 2H),4.70 (s, 2H),6.67 (d, J = 8.40 Hz, 2H), 7.04 (d, J = 8.40 Hz, 2H), 7.19-7.38 (m, 5H), 8.25-8.29 (m, 1H),8.47 (d, J = 8.70 Hz, 2H), 8.69-8.78 (m, 4H), 9.22 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 37.3, 42.2, 42.6, 43.1, 46.5, 54.1, 54.6, 115.6, 126.3, 126.8, 127.0, 128.3, 128.8, 129.6, 130.8, 131.0, 131.3, 131.6, 138.0, 156.3, 162.9, 166.4, 171.6, 173.3; MS [FAB$^-$]: calcd. m/z 662.20, obsvd. 661.7 $[M - H]^-$.

Synthesis of C_3-NDI-YF (3). This peptide derivative was prepared through SPPS using 2-chlorotrityl chloride resin, Fmoc-L-phenylalanine, Fmoc-L-tyrosine and **C_3-NDI**. The resin (1.20 g) was swelled in anhydrous CH_2Cl_2 for 30 min and then Fmoc-L-phenylalanine (0.78 g, 2.0 mmol) was loaded onto the resin in anhydrous *N*,*N*-dimethylformamide (DMF) and *N*,*N*-diisopropylethylamine (DIEA; 0.83 mL, 5.0 mmol) 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 (2.30 g, 5.0 mmol) was coupled to the free amino group

using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluraniumhexafluorophosphate (HBTU) (0.76 g, 2.0 mmol) and *N*,*N*-diisopropylethylamine (DIEA) (0.83 mL, 5.0 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, **C_3-NDI** (1.10 g, 3.0 mmol) was coupled to the free amino group using HBTU (0.76 g, 2.0 mmol) and DIEA (0.83 mL, 5.0 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 under a stream of air and then Et_2O was added to precipitate the target product. The solid was dried under vacuum to remove residual solvent (brown solid: 0.18 g). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.99 (t, *J* =7.35 Hz, 3H), 1.63-1.77 (m, 2H),2.91-3.02 (m, 4H), 4.02-4.18 (m, 2H), 4.49-4.53 (m, 2H), 4.70 (s, 2H), 6.67 (d, *J* = 8.40 Hz, 2H), 7.05 (d, *J* = 8.40 Hz, 2H), 7.30-7.33 (m, 5H), 8.37 (d, *J* = 7.5 Hz, 1H), 8.47 (d, *J* = 8.70 Hz, 1H), 8.69-8.78 (m, 4H), 9.21 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 11.9, 21.3, 37.3, 39.2, 40.9, 43.0, 54.1, 54.6, 115.4, 126.3, 126.8, 127.0, 127.1, 128.1, 128.8, 129.6, 130.7, 131.0, 131.2, 137.9, 156.3, 162.8, 163.1, 166.5, 171.5, 173.2; MS [FAB$^-$]: calcd. m/z 676.22, obsvd. 675.9 [M – H]$^-$.

Synthesis of C_4-NDI-YF (4). This peptide derivative was prepared through SPPS using 2-chlorotrityl chloride resin, Fmoc-L-phenylalanine, Fmoc-L-tyrosine and **C_4-NDI**. The resin (1.20 g) was swelled in anhydrous CH_2Cl_2 for 30 min and then Fmoc-L-phenylalanine (0.78 g, 2.0 mmol) was loaded onto the resin in anhydrous *N*,*N*-dimethylformamide (DMF) and *N*,*N*-diisopropylethylamine (DIEA; 0.83 mL, 5.0 mmol) 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 (2.30 g, 5.0 mmol) was coupled to the free amino group using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluraniumhexafluorophosphate (HBTU) (0.76 g, 2.0 mmol) and *N*,*N*-diisopropylethylamine (DIEA) (0.83 mL, 5.0 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, **C_4-NDI** (1.14 g, 3.0 mmol) was coupled to the free amino group using HBTU (0.76 g, 2.0 mmol) and DIEA (0.83 mL, 5.0 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 under a stream of air and then Et_2O was added to precipitate the target product. The solid was dried under vacuum to remove residual solvent (brown solid: 0.15 g). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.95-1.17 (m, 3H),1.35-1.49 (m, 2H), δ

= 1.62-1.31 (m, 2H), 2.89-3.02 (m, 4H), 4.01-4.13 (m, 2H), 4.41-4.51 (m, 2H), 4.72 (s, 2H), 6.73 (d, J = 7.95 Hz, 2H), 7.17 (d, J = 7.95 Hz, 2H), 7.26-7.38 (m, 5H), 8.42 (d, J =7.80 Hz, 1H),8.48-8.52 (m,1 H), 8.62-8.72 (m, 4H), 9.20 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 14.3, 20.3, 30.1, 39.2, 39.5, 40.9, 43.1, 54.1, 54.6, 115.4, 126.5, 126.6, 127.1, 128.1, 128.2, 128.8, 129.5, 130.7, 131.0, 131.2, 137.9, 156.3, 162.6, 166.7, 171.5, 173.2.

Synthesis of C_8-NDI-YF (5). This peptide derivative was prepared through SPPS using 2-chlorotrityl chloride resin, Fmoc-L-phenylalanine, Fmoc-L-tyrosine and **C_8-NDI**. The resin (1.20 g) was swelled in anhydrous CH_2Cl_2 for 30 min and then Fmoc-L-phenylalanine (0.78 g, 2.0 mmol) was loaded onto the resin in anhydrous *N*,*N*-dimethylformamide (DMF) and *N*,*N*-diisopropylethylamine (DIEA; 0.83 mL, 5.0 mmol) 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 (2.30 g, 5.0 mmol) was coupled to the free amino group using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluraniumhexafluorophosphate (HBTU) (0.76 g, 2.0 mmol) and *N*,*N*-diisopropylethylamine (DIEA) (0.83 mL, 5.0 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, **C_8-NDI** (1.31 g, 3.0 mmol) was coupled to the free amino group using HBTU (0.76 g, 2.0 mmol) and DIEA (0.83 mL, 5.0 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 under a stream of air and then Et_2O was added to precipitate the target product. The solid was dried under vacuum to remove residual solvent (brown solid: 0.28 g). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.88 (m, 3H),1.27-1.48 (m, 10H),1.62-1.79 (m, 2H), 2.85-3.23 (m, 4H), 4.05-4.15 (m, 2H), 4.35-4.57 (m, 2H),4.70 (s, 2H),6.66 (d, *J* = 8.25 Hz, 2H), 7.04 (d, *J* = 8.25 Hz, 2H), 7.20-7.40 (m, 5H), 8.29-8.31 (m, 1H), 8.47 (d, *J* = 8.70 Hz, 1H), 8.70-8.85 (m, 4H), 9.20 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 14.9, 23.0, 27.4, 28.3, 29.5, 29.6, 32.2, 37.7, 43.4, 54.5, 55.0, 115.8, 126.8, 127.0, 127.1, 127.4, 127.5, 128.5, 129.1, 130.0, 131.1, 131.3, 131.6, 138.4, 156.7, 163.2, 163.5, 166.9, 171.9, 173.6; MS [FAB$^-$]: calcd. m/z 746.3, obsvd. 745.6 [M – H]$^-$.

Inverted Tube Method: Gelation was performed by weighing a compound (2.0 mg) in a screw-capped 2-mL vial (diameter: 10 mm). Sodium hydroxide solution was added to the suspension to adjust pH; alternating vortex and ultrasonication were applied until a clear solution was obtained. This solution was then neutralized by a

dropwise addition of hydrochloric acid for gelation.

Transmission Electron Microscopy: Images were obtained with a Hitachi HT7700 transmission electron microscope at an accelerating voltage of 100 kV. Hydrogels were applied directly onto 200 mesh carbon-coated copper grids. Excess amount of the hydrogel was carefully removed by capillary action (filter paper), and the grids were then immediately stained with uranyl acetate for 30 s. Excess stain was removed by capillary action, and the grids were allowed to air dry.

Rheological tests: Rheological tests were conducted using an Anton Paar rheometer and a 25-mm parallel plate. The hydrogel sample (400μL, 1 wt %) was placed on the parallel plate for the angular frequency sweep test (test range: 0.25 to 100 $rads^{-1}$; 13 points per decade; sweep mode, "log"; temperature, 25 °C) and oscillatory strain (test range: 0.1% to 15%; frequency, 1 $rads^{-1}$; 21 points per decade; sweep mode, "log"; temperature, 25 °C).

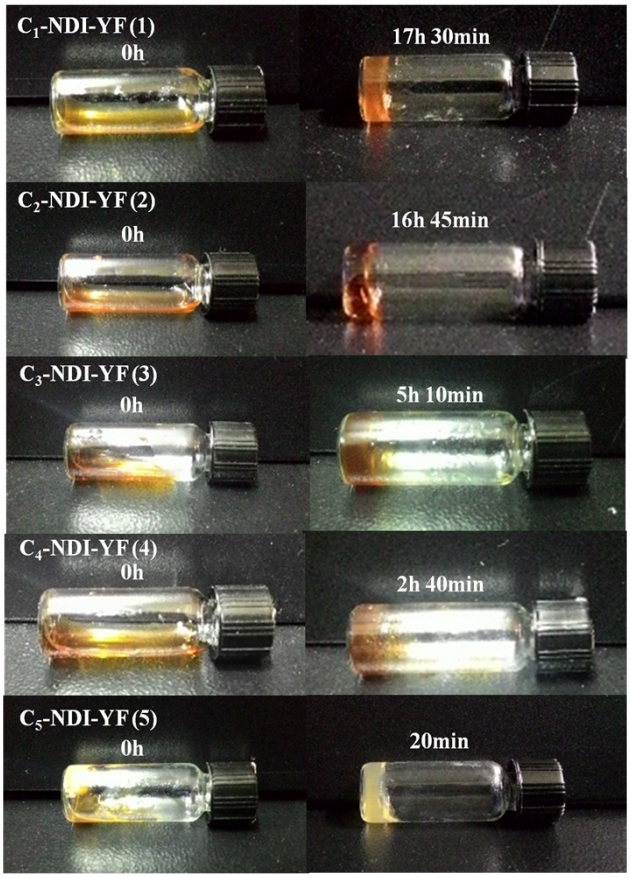


Fig. S1. The kinetics of gelation for **1**-**5**.

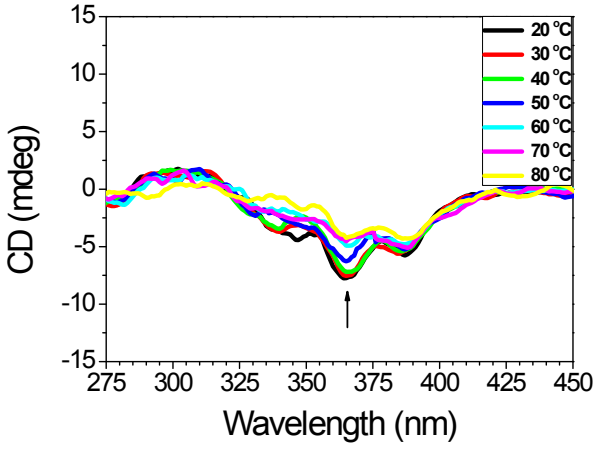
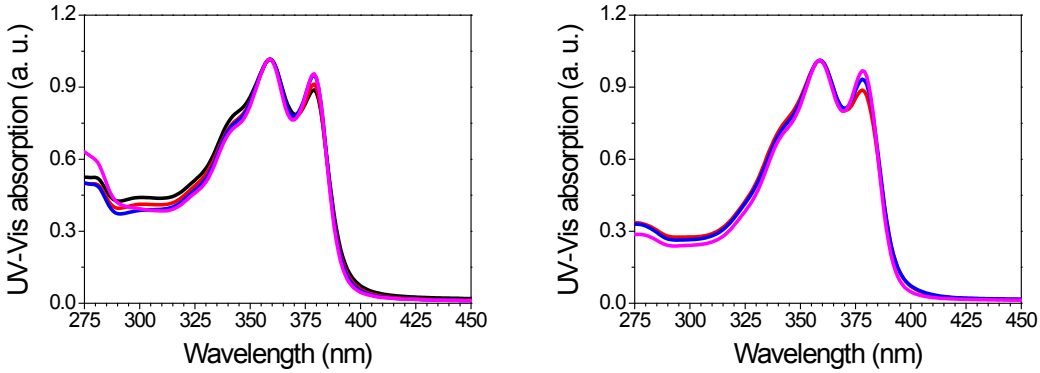


Fig. S2. Temperature-dependent CD spectra of **5** at 2000 μM in water.

(a) (b)



(c) (d)

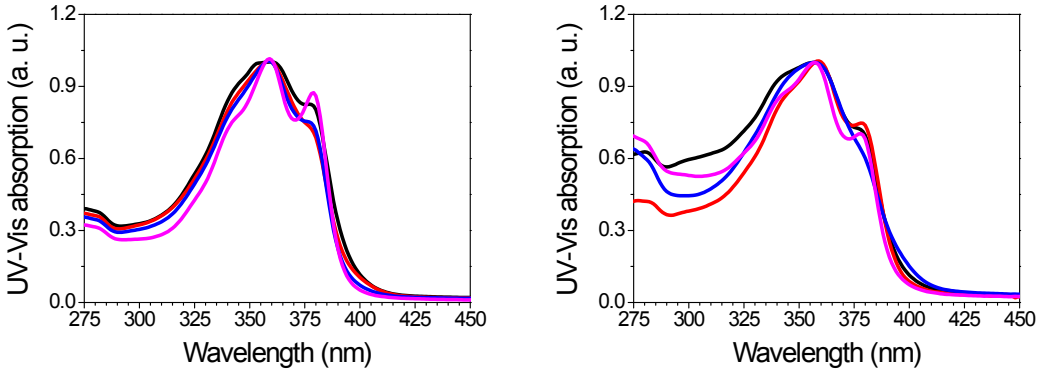


Fig. S3. Concentration-dependent UV-Vis absorption spectra of (a) **1**, (b) **2**, (c) **3** and (d) **4** in water. (500 μM: magenta; 2000 μM: blue; 3000 μM: red; 5000 μM: black).

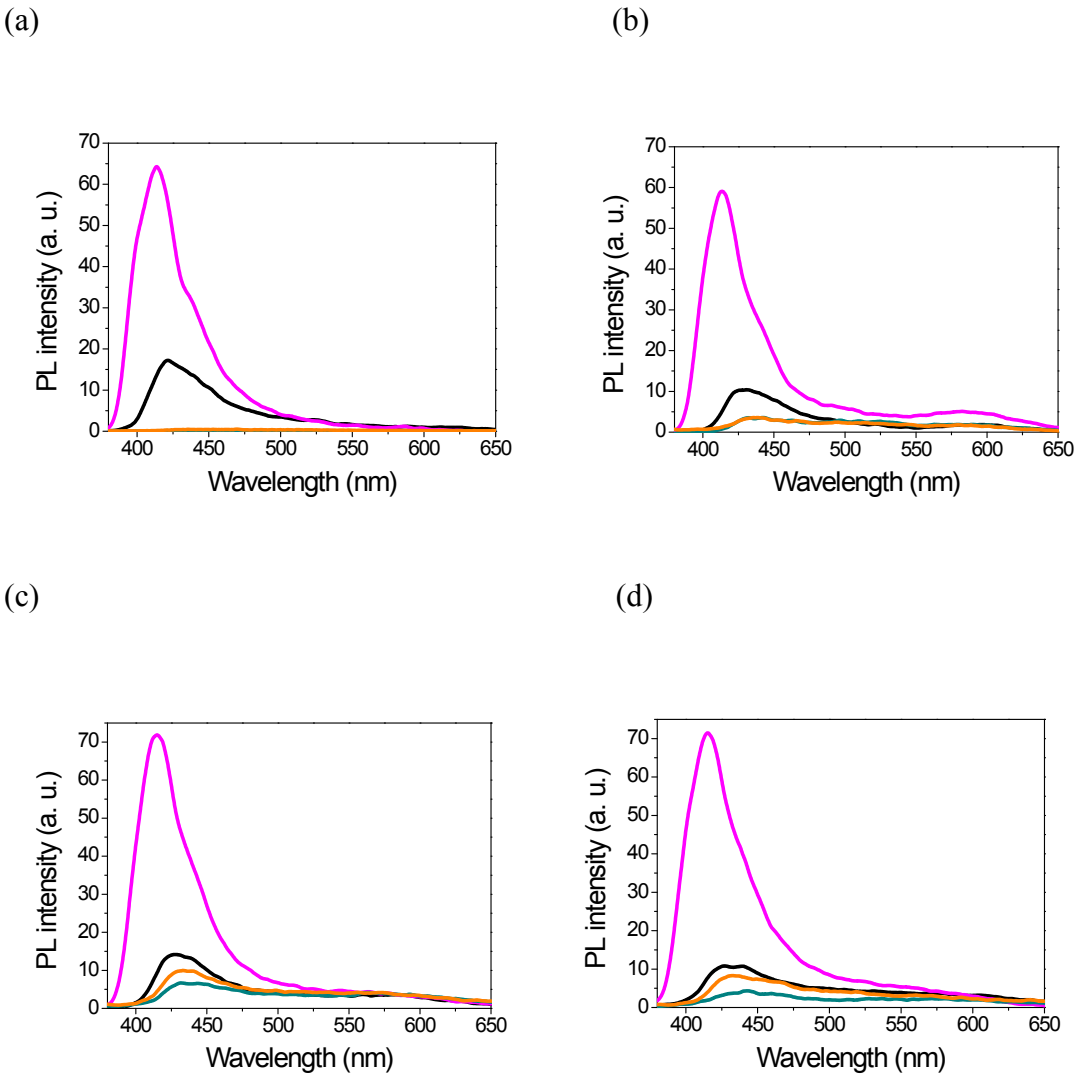


Fig. S4. Concentration-dependent emission spectra of (a) **1**, (b) **2**, (c) **3** and (d) **4** in water. (500 μM: magenta; 5000 μM: black ; 10000 μM: orange; 15000 μM: cyan).

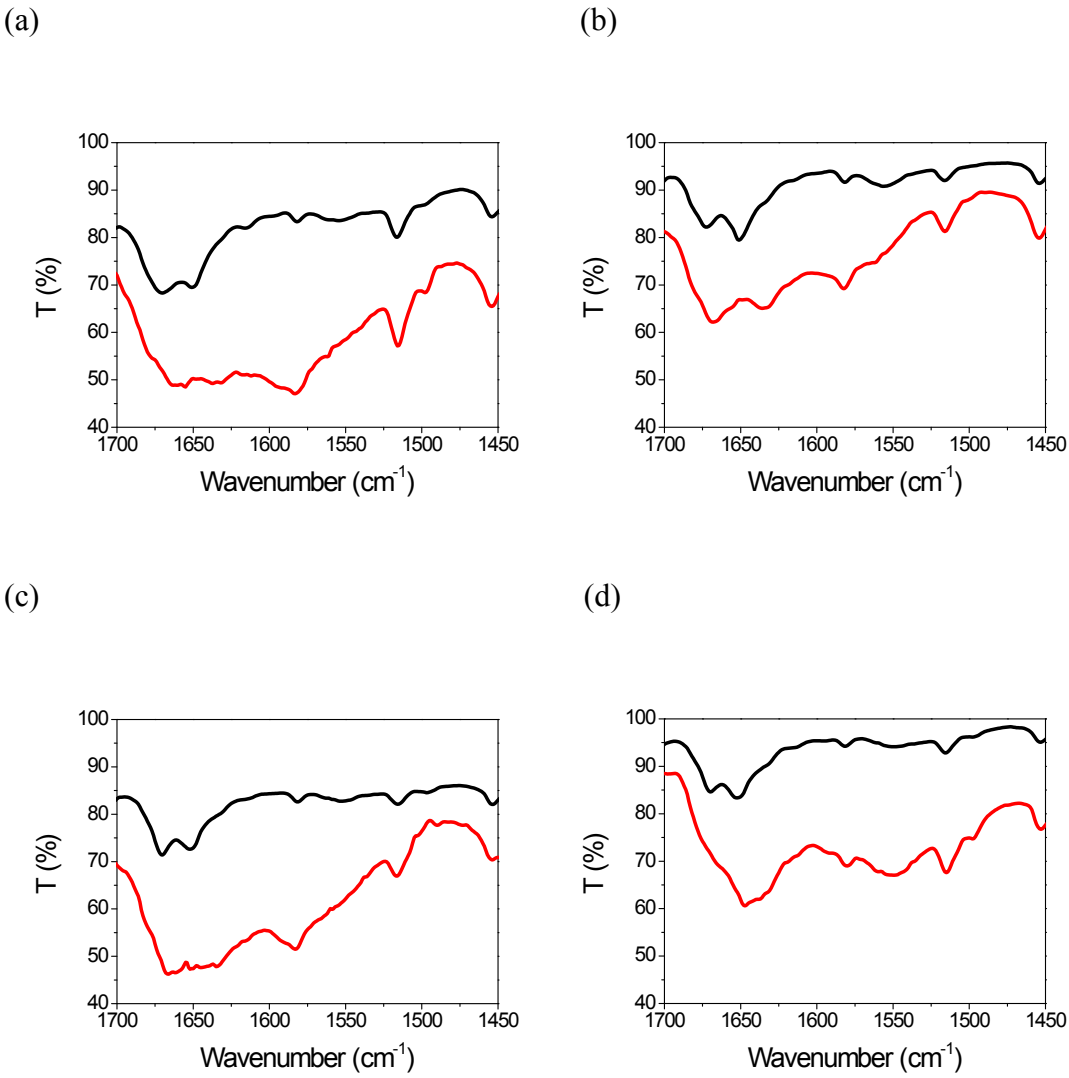


Fig. S5. FT-IR spectra of (a) **1**, (b) **2**, (c) **3** and (d) **4** at 1 wt% in water (red) and MeOH (black).