Annealing Multicomponent Supramolecular Gels

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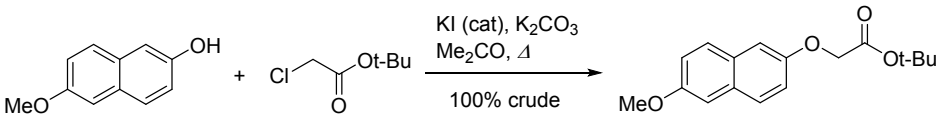
Supporting Information

Materials.

1 was prepared as described previously.[1] **2** was prepared as described in detail below. All other chemicals used were purchased from commercial suppliers and used as received.

Tert-butyl 2-[(6-methoxynaphthalen-2-yl)oxy]acetate (**DL-002**)



DL-002

To a solution of 6-methoxy-2-naphthol (9.47 g, 54.4 mmol) in acetone (250 mL) was added potassium carbonate (1 eq, 7.51 g) and the mixture was stirred at ambient temperature for two hours. Another portion of potassium carbonate (1 eq, 7.51 g), potassium iodide (1 mol%, 90 mg) and *tert*-butyl chloroacetate (1 eq, 7.79 mL) were added and the reaction mixture was heated at reflux (70 °C oil bath temperature) for two days. After this time, TLC (5:95 ethyl acetate/*n*-hexane) indicated the absence of starting naphthol. The reaction mixture was evaporated to dryness and the residue partitioned between dichloromethane and water and stirred until all solids had dissolved. The layers were separated and the aqueous phase was back-extracted with dichloromethane. The combined organics were washed with water, brine, dried ($MgSO_4$), and filtered through a pad of Celite. Evaporation of the solvent afforded the title compound as a brown oil, which solidified on standing (15.9 g, 100 % crude). This was used as is for the next step. A small amount was purified *via* flash chromatography (eluting with dichloromethane) to afford a sample for characterisation.

δ_H (400 MHz, DMSO-d_6) 7.74 (1H, d, *J* 8.88, $\underline{H}_{Ar}$), 7.69 (1H, d, *J* 8.96, $\underline{H}_{Ar}$), 7.27 (1H, d, *J* 2.52, $\underline{H}_{Ar}$), 7.19 (1H, d, *J* 2.52, $\underline{H}_{Ar}$), 7.16 (1H, dd, *J* 8.82, 2.62, $\underline{H}_{Ar}$), 7.12 (1H, dd, *J* 8.96, 2.60, $\underline{H}_{Ar}$), 4.72 (2H, s, $OC\underline{H}_2$), 3.84 (3H, s, $OC\underline{H}_3$), 1.43 (9H, s, $C(C\underline{H}_3)_3$). δ_C (100 MHz, DMSO-d_6) 167.81 ($\underline{C}$=O), 155.84, 153.93, 129.66, 129.12, 128.13, 128.11, 118.81, 118.50, 107.49, and 106.07 ($\underline{C}_{Ar}$), 81.30 ($\underline{C}(CH_3)_3$), 65.15 ($O\underline{C}H_2$), 55.04 ($O\underline{C}H_3$), 27.65 ($C(\underline{C}H_3)_3$). HRMS (ESI) m/z: $[M+Na]^+$ calcd for $C_{17}H_{20}NaO_4$ 311.1254; found 311.1242.

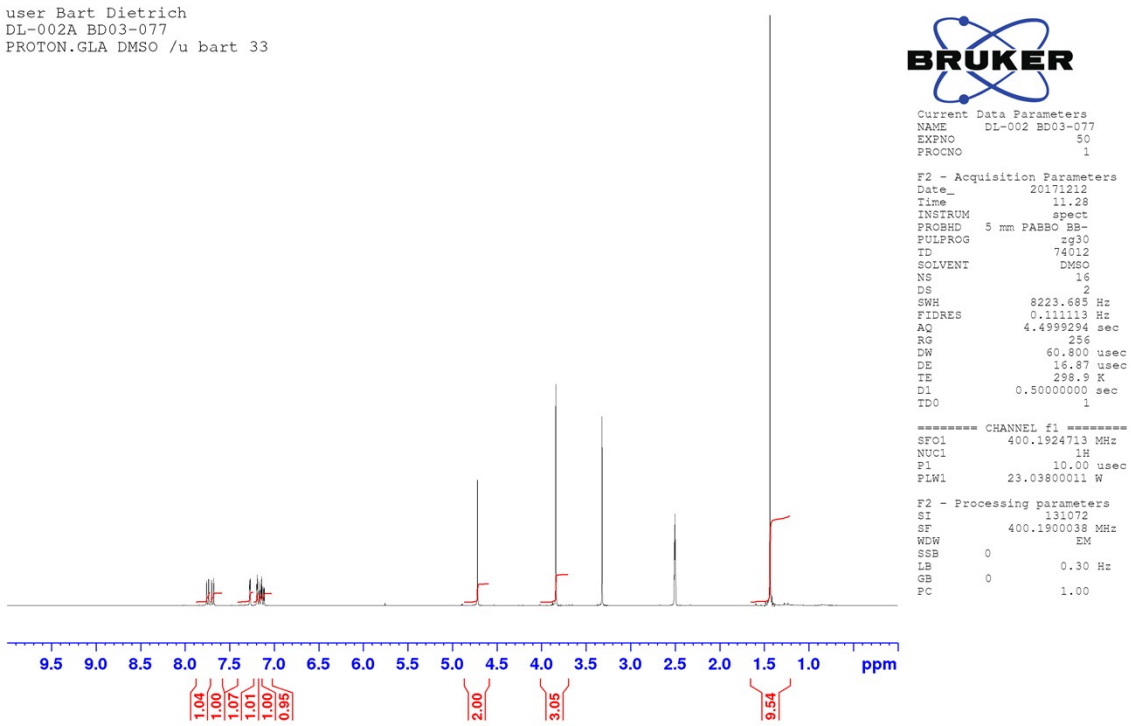


Figure S1. Proton NMR of **DL-002** in d_6-DMSO.

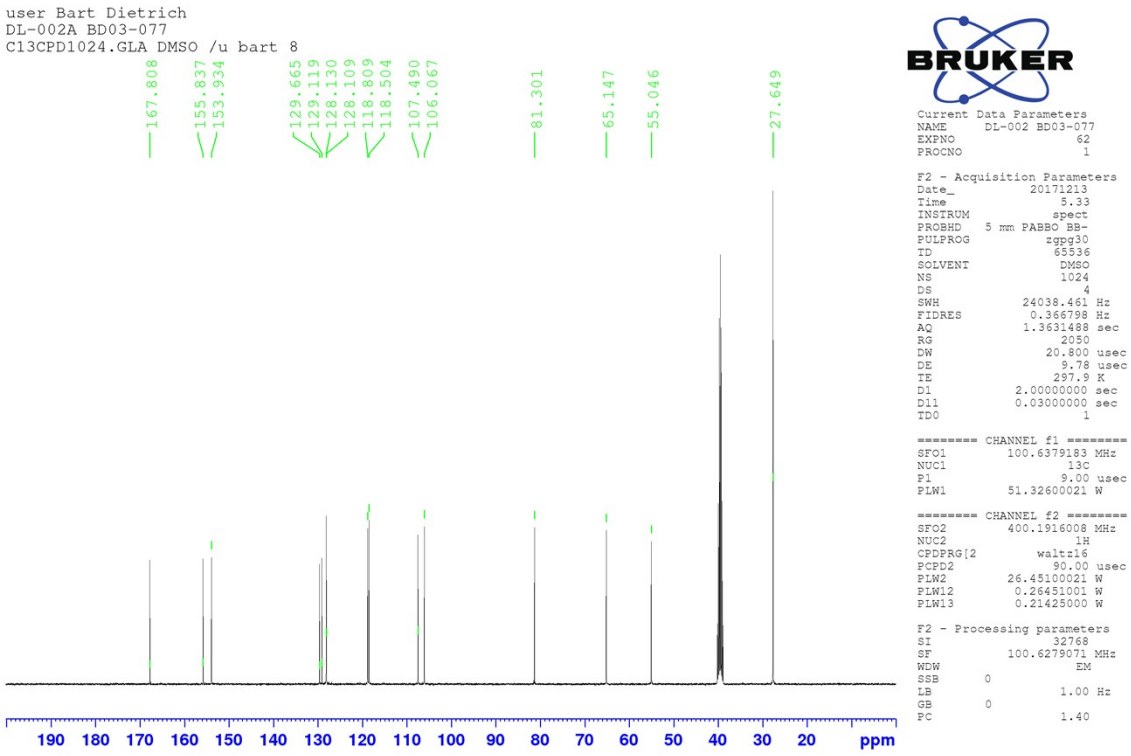
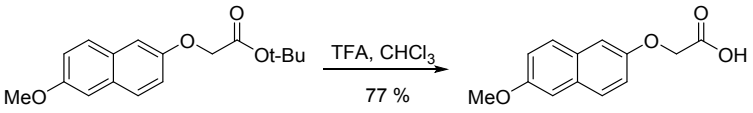


Figure S2. Carbon NMR of **DL-002** in d_6-DMSO.

2-[(6-Methoxynaphthalen-2-yl)oxy]acetic acid (**DL-003**)



DL-002 **DL-003**

To a solution of **DL-002** (15.6 g, 54.2 mmol) in chloroform (60 mL) was added trifluoroacetic acid (30 mL, *ca.* 7 eq.) and the mixture was stirred overnight. A precipitate was found in the flask after this time. The entire reaction mixture was poured into diethyl ether (500 mL), stirred for three hours, then filtered and the solid in the filter washed with diethyl ether. After drying under reduced pressure, the title compound was obtained as a white solid (9.69 g, 77 %). One carbon NMR signal is not resolved.

δ_H (400 MHz, DMSO-d_6) 13.02 (1H, br s, COO<u>H</u>), 7.74 (1H, d, *J* 9.00, <u>H</u>$_{Ar}$), 7.70 (1H, d, *J* 9.00, <u>H</u>$_{Ar}$), 7.27 (1H, d, *J* 2.48, <u>H</u>$_{Ar}$), 7.21 (1H, d, *J* 2.56, <u>H</u>$_{Ar}$), 7.16 (1H, dd, *J* 8.90, 2.62, <u>H</u>$_{Ar}$), 7.12 (1H, dd, *J* 8.90, 2.58, <u>H</u>$_{Ar}$), 4.75 (2H, s, C<u>H</u>$_2$), 3.84 (3H, s, OC<u>H</u>$_3$). δ_C (100 MHz, DMSO-d_6) 170.27 (<u>C</u>=O), 155.86, 154.10, 129.70, 129.22, 128.22, 118.87, 118.68, 107.33, and 106.11 (<u>C</u>$_{Ar}$), 64.61 (<u>C</u>H$_2$), 55.11 (O<u>C</u>H$_3$). HRMS (ESI) m/z: $[M+Na]^+$ calcd for $C_{13}H_{12}NaO_4$ 255.0628; found 255.0623.

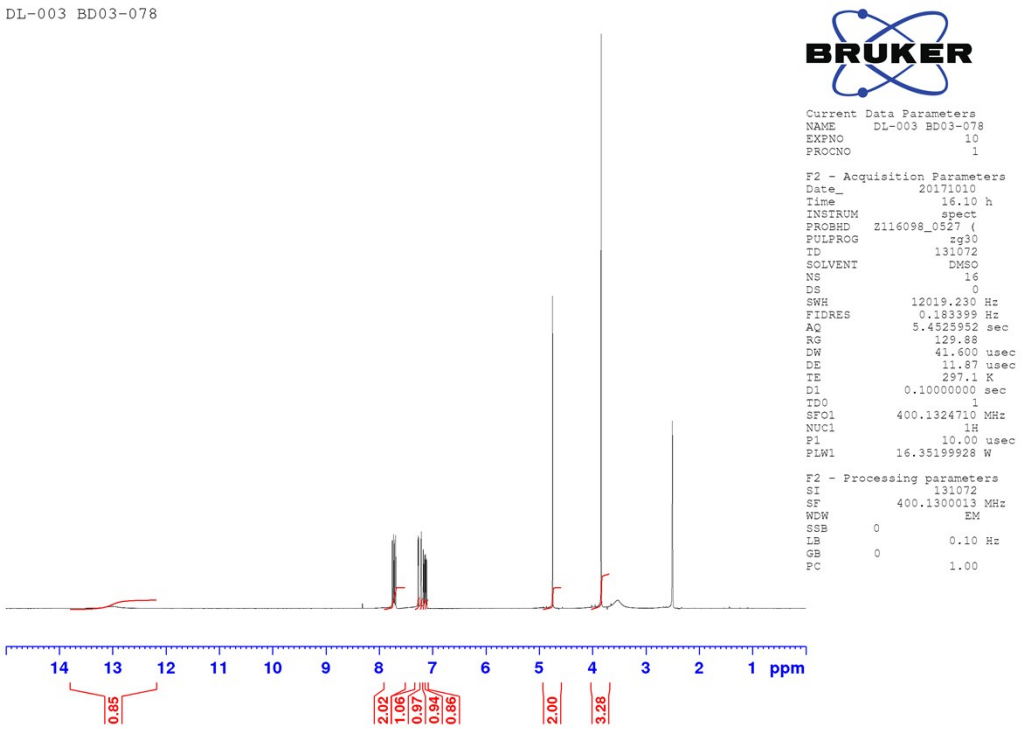


Figure S3 Proton NMR of **DL-003** in d_6-DMSO.

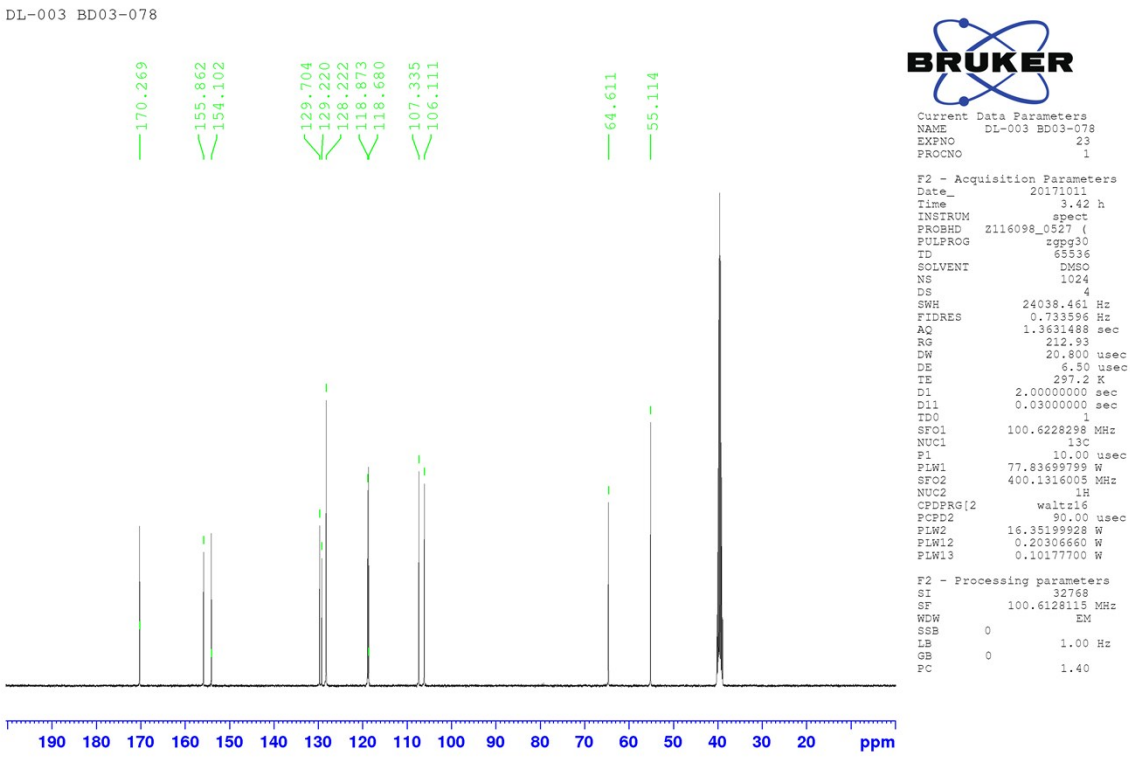
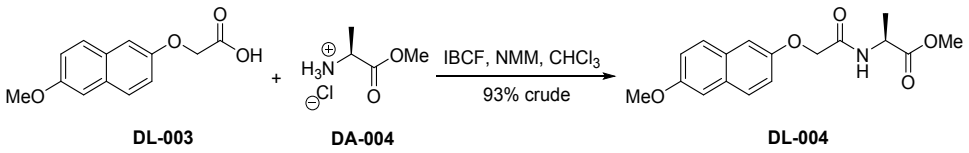


Figure S4. Carbon NMR of **DL-003** in d_6-DMSO.

Methyl (2*S*)-2-{2-[(6-methoxynaphthalen-2-yl)oxy]acetamido}propanoate (**DL-004**)



To a suspension of **DL-003** (4.34 g, 18.7 mmol) in chloroform (100 mL) was added *N*-methylmorpholine (1 eq., 2.06 mL) followed by *iso*butyl chloroformate (1 eq., 2.42 mL) and the mixture was stirred for two hours. Another portion of *N*-methylmorpholine (1 eq., 2.06 mL) and **DA-004** (*L*-alanine methyl ester hydrochloride, prepared from *L*-alanine, methanol and acetyl chloride) was added and the mixture stirred overnight. After this time, it was diluted with chloroform, washed with 1M hydrochloric acid, water, brine, dried ($MgSO_4$), and evaporated under reduced pressure. The title compound was thus obtained as a grey solid (5.53 g, 93% crude) and used as is for the next step. A small amount was purified *via* flash chromatography to yield a sample for characterisation.

δ_H (400 MHz, DMSO-d_6) 8.56 (1H, d, *J* 7.40, NH), 7.76 (1H, d, *J* 8.88, H_{Ar}), 7.70 (1H, d, *J* 9.00, H_{Ar}), 7.28 (1H, d, *J* 2.52, H_{Ar}), 7.25-7.21 (2H, m, H_{Ar}), 7.14 (1H, dd, *J* 8.92, 2.60, H_{Ar}), 4.62 (1H, d, *J* 14.73, CH_aH_b), 4.58 (1H, d, *J* 14.73, CH_aH_b), 4.41 (1H, pseudo-quintet, *J* 7.29, CH*), 3.84 (3H, s, $C_{Ar}OCH_3$), 3.62 (3H, s, CO_2CH_3), 1.34 (3H, d, *J* 7.28, CH^*CH_3). δ_C (100 MHz,

DMSO-d_6) 172.74 and 167.66 (C=O), 155.87, 154.03, 129.74, 129.14, 128.15, 128.08, 118.82, 118.79, 107.76, and 106.12 (C_{Ar}), 66.84 (OCH_2), 55.08 ($C_{Ar}OCH_3$), 51.92 (CO_2CH_3), 47.30 (CH^*), 16.85 (CH^*CH_3). HRMS (ESI) m/z: $[M+Na]^+$ calcd for $C_{17}H_{19}NNaO_5$ 340.1155; found 340.1149.

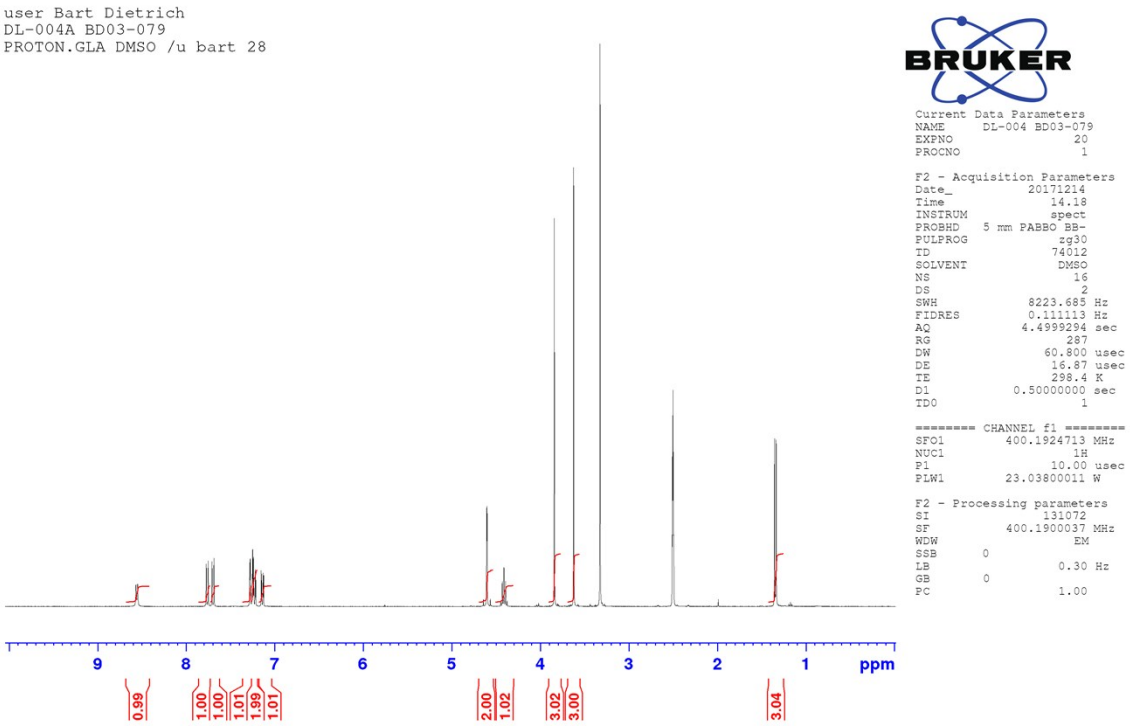


Figure S5. Proton NMR of **DL-004** in d_6-DMSO.

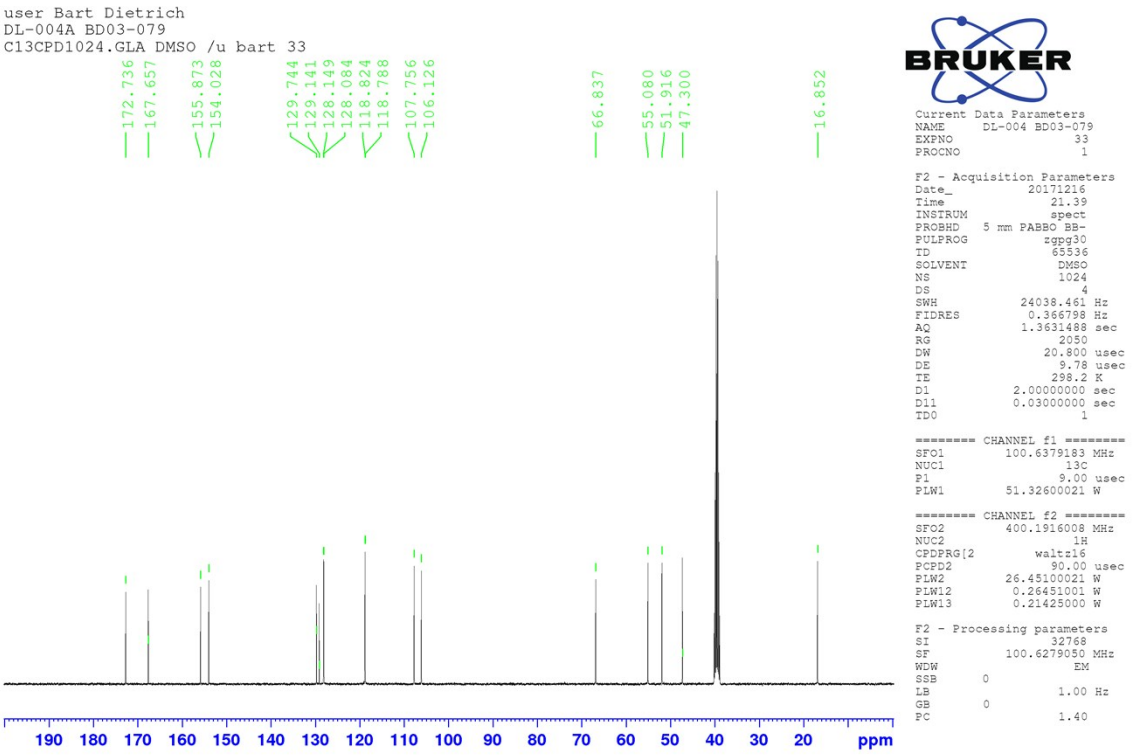
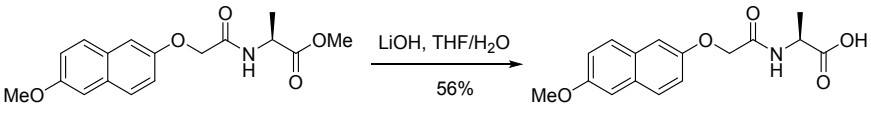


Figure S6. Carbon NMR of **DL-004** in d_6-DMSO.

(2*S*)-2-{2-[(6-Methoxynaphthalen-2-yl)oxy]acetamido}propanoic acid (**DL-005, Gelator 2**)



DL-004 **DL-005**

To a solution of **DL-004** (5.29 g, 16.7 mmol) in tetrahydrofuran (80 mL) was added a solution of lithium hydroxide (4 eq., 1.60 g) in water (80 mL) and the mixture was stirred overnight. After this time, it was poured into 1M hydrochloric acid (*ca.* 500 mL), stirred for one hour, and filtered. The solid in the filter was washed with water, then recrystallized from boiling methanol, affording the title compound as a white wool-like solid (2.29 g). A second crop of title compound (536 mg) was obtained by filtration of the mother liquor, which had developed a precipitate upon standing. Total yield 2.83 g (56 %). Evaporation of the mother liquor and repeated recrystallization did not yield any more **DL-005** of acceptable purity. One carbon NMR signal is not resolved.

δ_H (400 MHz, DMSO-d_6) 12.68 (1H, br s, COOH), 8.40 (1H, d, *J* 7.56, NH), 7.76 (1H, d, *J* 8.96, H_{Ar}), 7.69 (1H, d, *J* 9.00, H_{Ar}), 7.27 (2H, dd, *J* 4.56, 2.64, H_{Ar}), 7.22 (1H, dd, *J* 8.86, 2.58, H_{Ar}), 7.13 (1H, dd, *J* 8.90, 2.54, H_{Ar}), 4.62 (1H, d, *J* 14.65, OCH_aH_b), 4.57 (1H, d, *J* 14.65, OCH_aH_b), 4.33 (1H, dq, *J* 7.32, 7.38, CH^*), 3.84 (3H, s, OCH_3), 1.34 (3H, d, *J* 7.32, CH^*CH_3). δ_C (100 MHz, DMSO-d_6) 173.88 and 167.52 (C=O), 155.91, 154.08, 129.76, 129.20, 128.21, 128.12,

118.83, 107.81, and 106.14 ($\underline{C}_{Ar}$), 66.92 (O$\underline{C}$H$_2$), 55.11 (O$\underline{C}$H$_3$), 47.29 ($\underline{C}$H*), 17.14 (CH$^*\underline{C}$H$_3$). HRMS (ESI) m/z: [M+Na]$^+$ calcd for $C_{16}H_{17}NNaO_5$ 326.0999; found 326.0988.

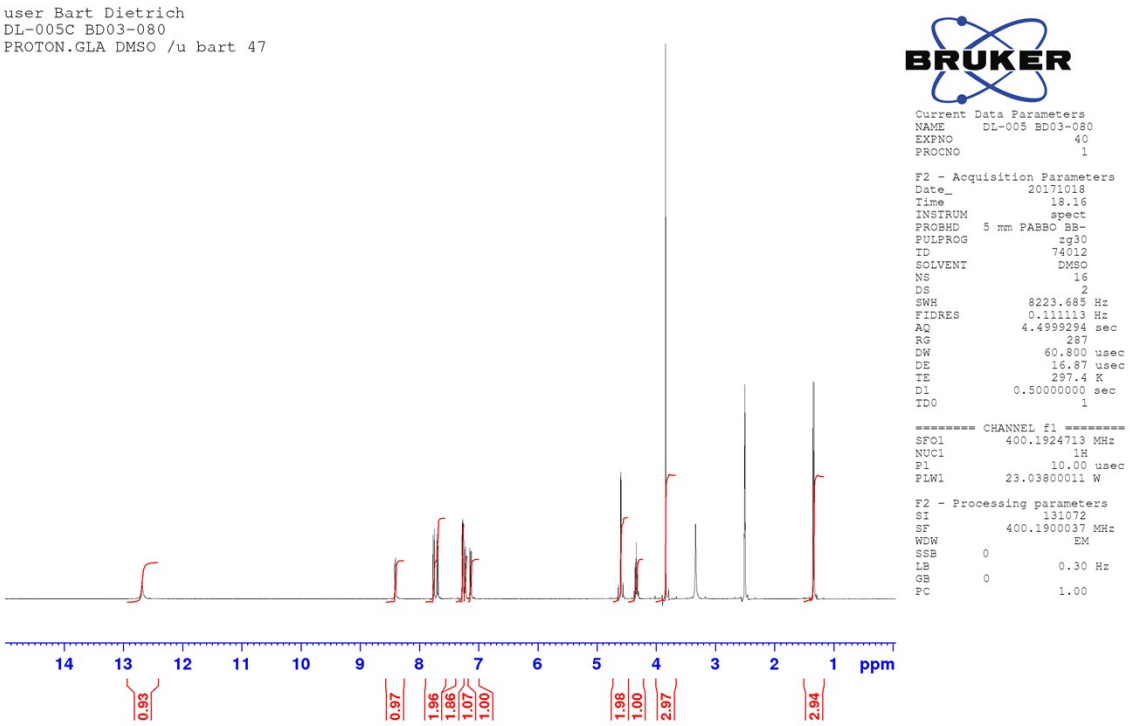


Figure S7. Proton NMR of **DL-005 (Gelator 2)** in d_6-DMSO.

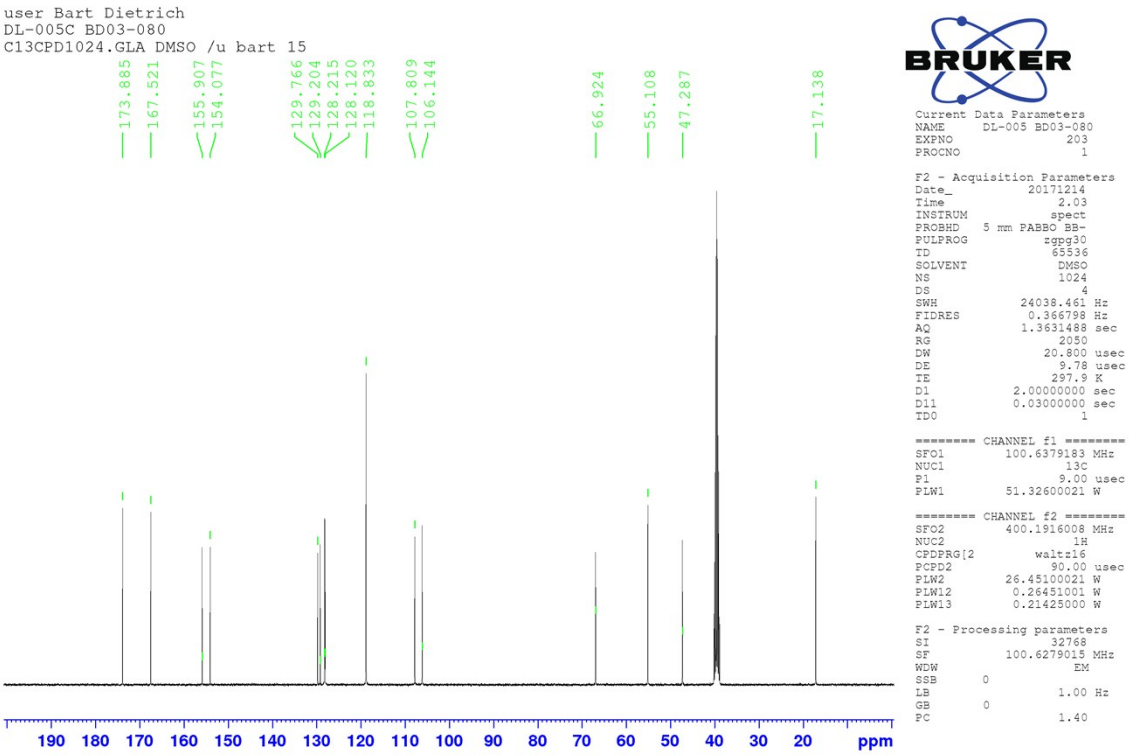


Figure S8. Carbon NMR of **DL-005 (Gelator 2)** in d_6-DMSO.

Experimental Details

Gel Formation. For the single component gels, either **1** or **2** was dissolved in DMSO. Water was then added in one aliquot to either solution such that the final ratio of DMSO:water was 3:7 (final volume of 2 mL) and the concentrations were 4 mg/mL (1) or 8 mg/mL (2). Typically, these gels are at pH 3.9 - 4.3 and are stable. For the mixed gels, a solution containing both **1** and **2** was prepared in DMSO, and water was added to this in one aliquot. Annealing was carried out by heating and cooling the gels typically at 1ºC/min.

Rheology. Dynamic rheological measurements were carried out using Anton Paar Physica MCR 101 and MCR 301 rheometers. A cup and vane system was used to perform the strain, frequency and temperature sweeps. 2mL gels were prepared in 7 mL Sterilin vials and metal rheology cups were used for the temperature sweeps, then left overnight (~ 18 hours) at room temperature to gel before measurements. Strain and frequency sweeps were performed at 25 °C for samples before annealing and at 15 °C for those after annealing. The strain sweeps were carried out from 0.1 % to 1000 % strain at a frequency of 10 $rad{\cdot}s^{-1}$. The viscoelastic region was determined as the region where G' and G'' remain constant up to a strain amplitude at which the gel breaks (γ_c) and G' deviates from linearity. Frequency sweeps were carried out from an angular frequency of 1-100 $rad{\cdot}s^{-1}$ at a constant strain of 0.5 %, value below the critical strain γ_c. Temperature sweep measurements were performed at a strain of 0.5 % and frequency of 10 $rad{\cdot}s^{-1}$ over a heating-cooling cycle between 15 °C and 90 °C at a rate of 1 $°C{\cdot}min^{-1}$.

pH Measurement. A calibrated HI2020-01 pH probe from Hanna instruments was used for measuring the pH of the gels. The stated accuracy of the pH measurements is ±0.1.

Confocal Microscopy. A Zeiss LSM 710 confocal microscope was used to take the confocal images. The objective used was a LD EC Epiplan NEUFLUAR 50x (0,55 DIC). The samples were stained with 2 $\mu L.mL^{-1}$ of a 0.1 w% Nile Blue solution and excited at 634 nm using a He-Ne laser. All the samples were prepared in-situ using the same methodology as described above where **1** or **2** or (**1**+**2**) was dissolved in DMSO and then water was added such the final ratio of DMSO:water was 3:7 (final volume 2 mL) and then left overnight to gel. For all of them,

thin sections were cut from the central bulk region of each gel using a scalpel to avoid any surface effect. Each cut section was placed in a concave glass slide from Pearl with a cover slip from Menzel-Gläser on the top, and then sealed with nail polish to avoid evaporation. To acquire data after annealing, the samples were first heated in an oven to 90 °C and then gently left to cool down by switching off the oven to ensure a slow cooling rate. Multiple parts of the gel were imaged to ensure a representative structure.

NMR Spectrocopy. ^{1}H NMR spectroscopy was used to investigate the gel-sol transition when heating and cooling the samples. The ^{1}H NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer. Gels were prepared as above in an NMR tube, replacing DMSO and H_2O with DMSO-d_6 and D_2O. A mixture of 10% DMSO-d_6, 1% poly(dimethylsiloxane) and 89% of tetrachloroethylene was added in a capillary as the internal standard.

SAXS. All X-ray scattering was performed on a SAXSLAB Ganesha 300XL instrument in a Q range of 0.007-0.25Å^{-1}, with an exposure time of 7200 seconds per sample. Six samples were performed in total, three before annealing and the other three after annealing. The samples before and after annealing were prepared as explained before and a thin section was cut using a scalpel and then transferred directly to a flat mica cell for measurements. Background corrections were made using both an empty cell and one filled with water. Sample thickness corrections were made using a cell half filled with water and comparing the beam intensities. The data were fitted in the SasView software package.[2]

UV-Vis. Data were collected on an Agilent Cary 60 spectrophotometer. Samples were prepared in 1 mm pathlength quartz cuvettes purchased from Starna. Firstly, water was added into the cuvette and then DMSO solution and mixed with the help of a needle. Absorbance was measured over a period of 120 min at a wavelength of 600 nm.

Single Crystal X-Ray Diffraction. Single Crystal X-Ray diffraction data were collected at 150K using a Bruker D8 VENTURE diffractometer equipped with a Photon II CMOS detector, with an Oxford Cryosystems N-Helix device mounted on an IμS 3.0 (dual Cu and Mo) microfocus sealed tube generator at the University of Glasgow.

Supplementary Figures.

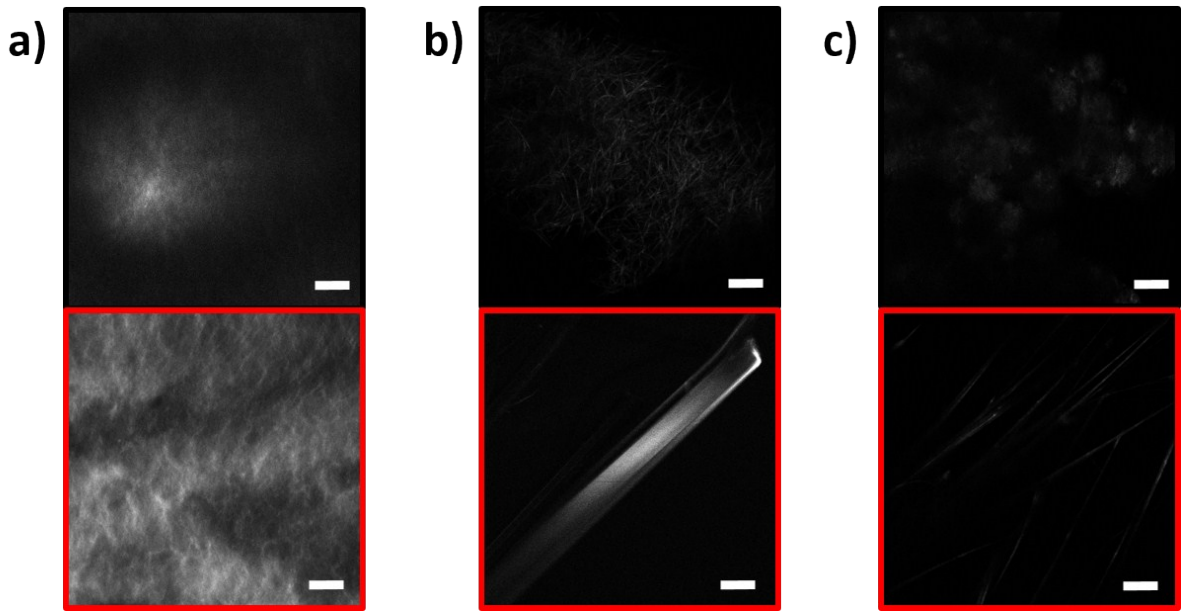


Figure S9. Original confocal images for (a) **1**, (b) **2** and (c) (**1+2**). The black border refers to the structures before annealing and red borders to the structures after annealing. All scale bars represent 20 μm.

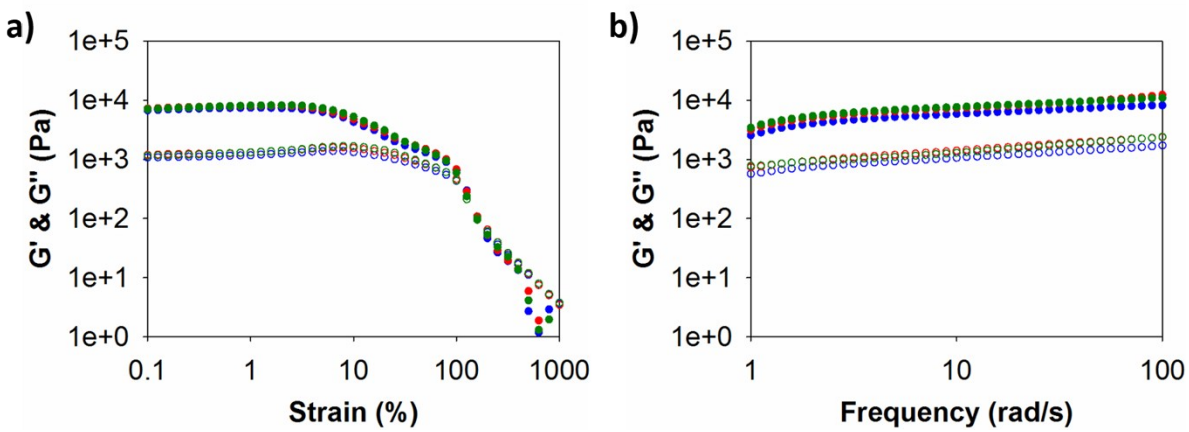


Figure S10. (a) Overlay of three repeat strain sweeps for a gel of **1** to demonstrate reproducibility. (b) Overlay of three repeat frequency sweeps for a gel of **1** to demonstrate reproducibility.

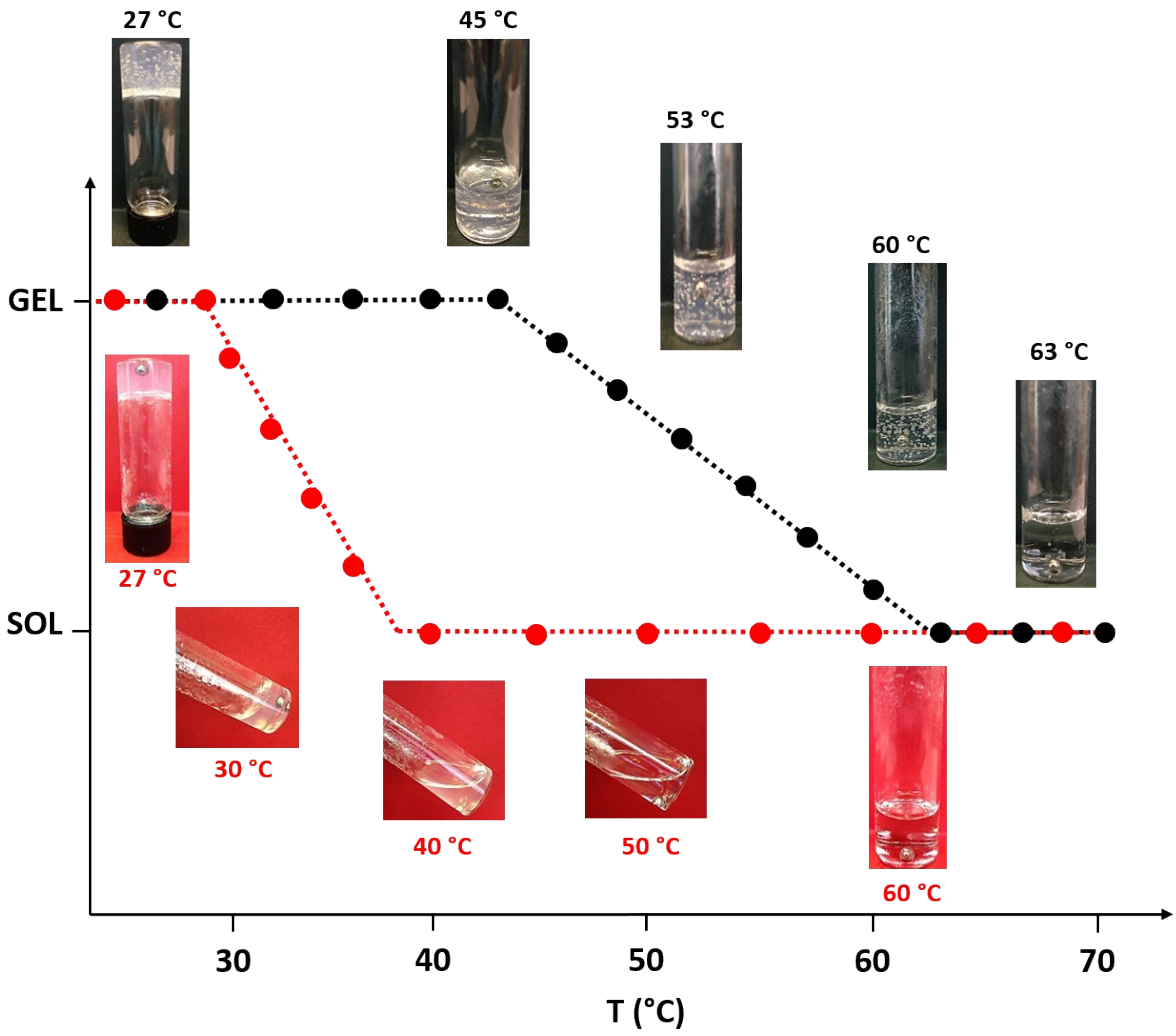


Figure S11. Visual determination of the melting temperature and gel re-formation temperature for a gel of **1**. The black data are for heating and the red data for cooling. A ball bearing has been included at the top of the gel, that slowly falls to the bottom. On re-gelling, this ball bearing is trapped in the network.

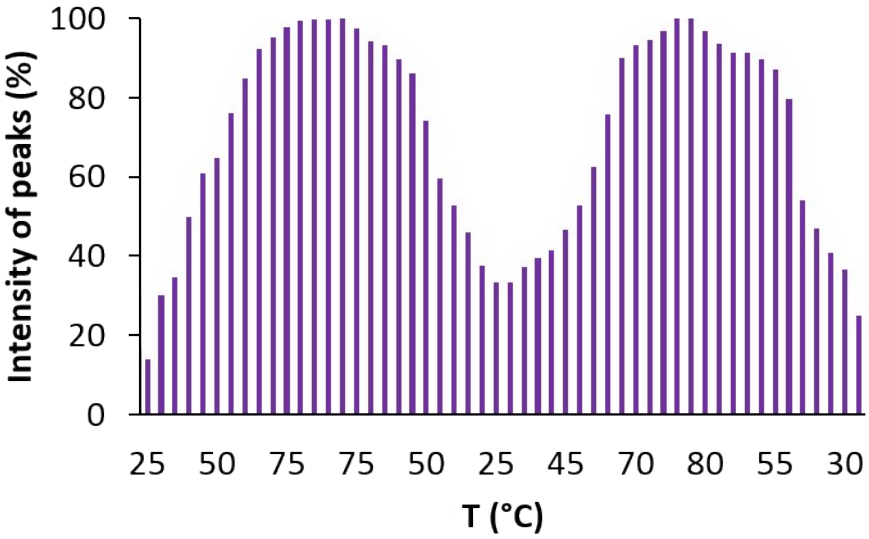


Figure S12. Plot of integrals for **1** against a standard as determined by ^{1}H NMR for **1** in a mixture of DMSO and D_2O (3/7) on heating and cooling. The sample was heated to the stated temperature, and an NMR spectrum recorded after 1 minute, before being heated or cooled to the required next temperature. Two sequential heat/cool cycles on the same sample are shown.

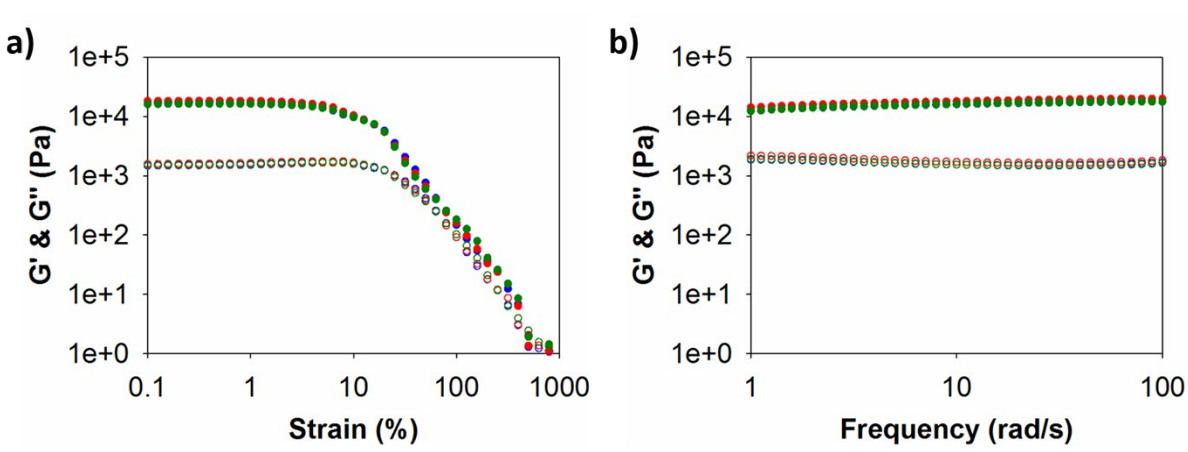


Figure S13. (a) Overlay of three repeat strain sweeps for a gel of **1** after annealing to demonstrate reproducibility. (b) Overlay of three repeat frequency sweeps for a gel of **1** after annealing to demonstrate reproducibility.

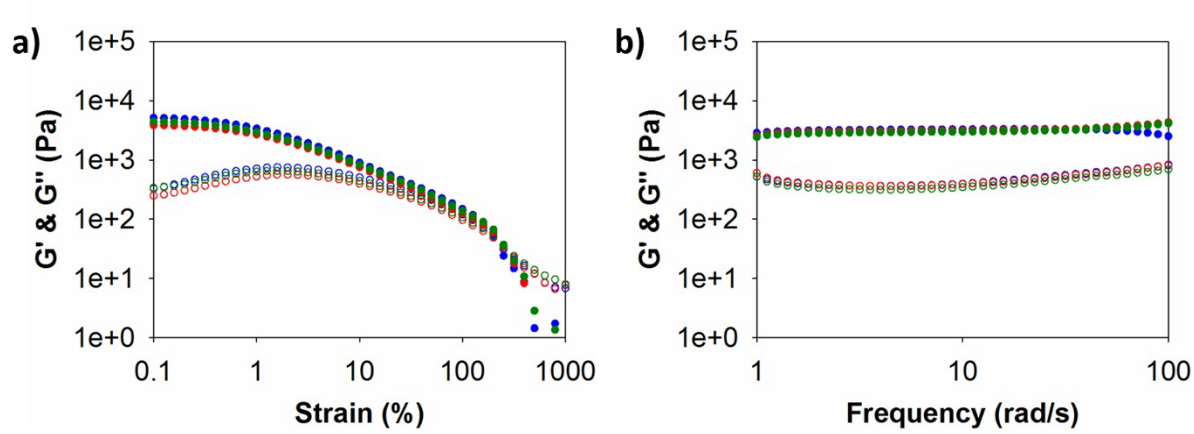


Figure S14. (a) Overlay of three repeat strain sweeps for a gel of **2** to demonstrate reproducibility. (b) Overlay of three repeat frequency sweeps for a gel of **2** to demonstrate reproducibility.

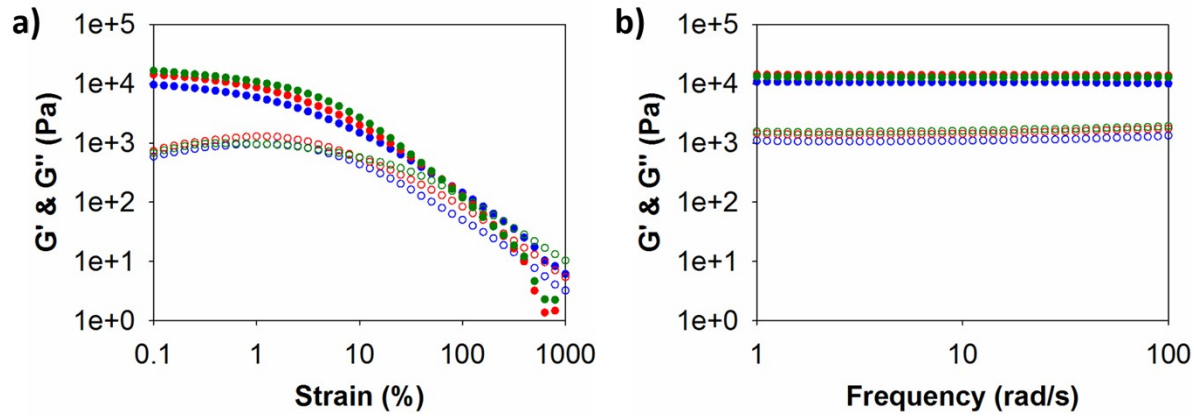


Figure S15. (a) Overlay of three repeat strain sweeps for a gel of **2** after annealing to demonstrate reproducibility. (b) Overlay of three repeat frequency sweeps for a gel of **2** after annealing to demonstrate reproducibility.

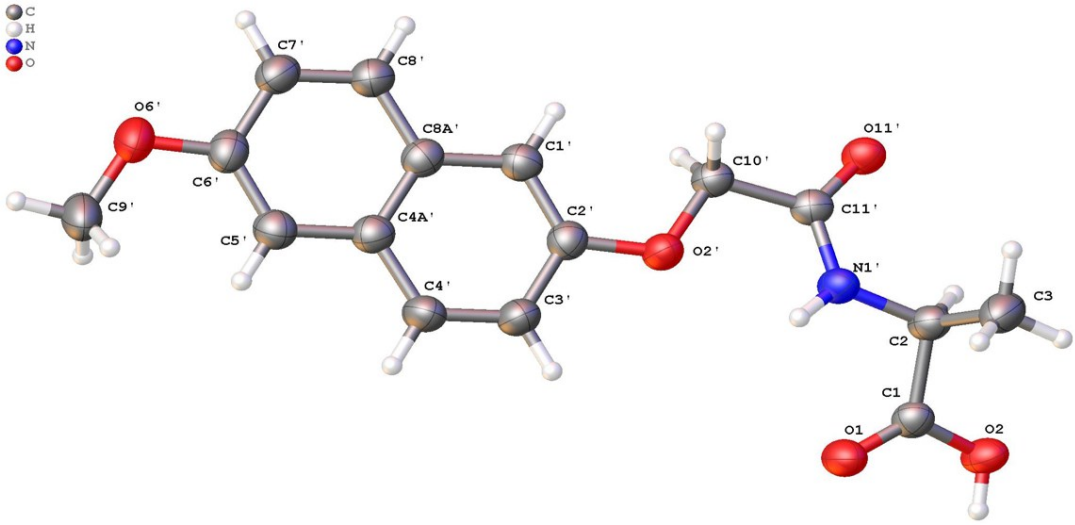


Figure S16. Structure of **2**; crystals grown by a slow cool of a gel of 2. Atomic displacement ellipsoids drawn at 50% probability level. Crystal data. $C_{16}H_{17}NO_5$, M = 303.30, orthorhombic, a = 4.9683 (3), b = 13.7813 (7), c = 21.8629 (13) Å, V = 1496.94 (15) Å^3, T = 150 K, I = 1.54178 Å, space group $P2_12_12_1$ (no.19), Z = 4, 8891 reflections measured, 2719 unique (R_{int} = 0.082), which were used in all calculations. The final w$R(F^2)$ was 0.130 (all data). Flack x, -0.05 (17), determined using 841 quotients [(I+)-(I-)]/[(I+)+(I-)].[3]

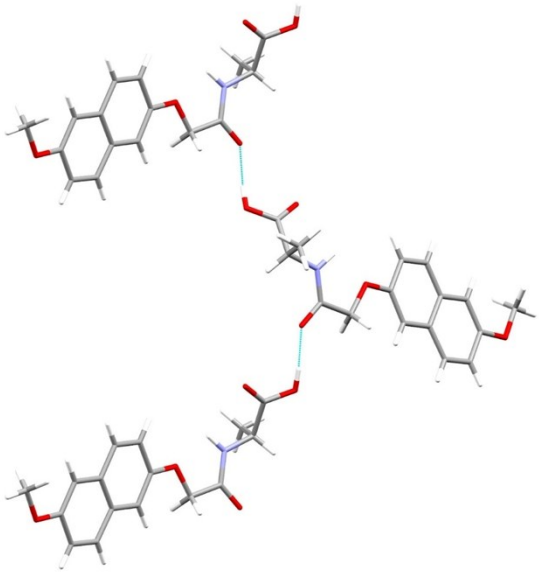


Figure S17. View of structure of **2** showing the hydrogen bonding chain viewed along the c-axis; O2—H2···O11^{i} where (i) -x+3, y-1/2, -z+3/2. O2…O11^{i} = 2.604(3) Å and angle O2—H2···O11^{i} =175º.

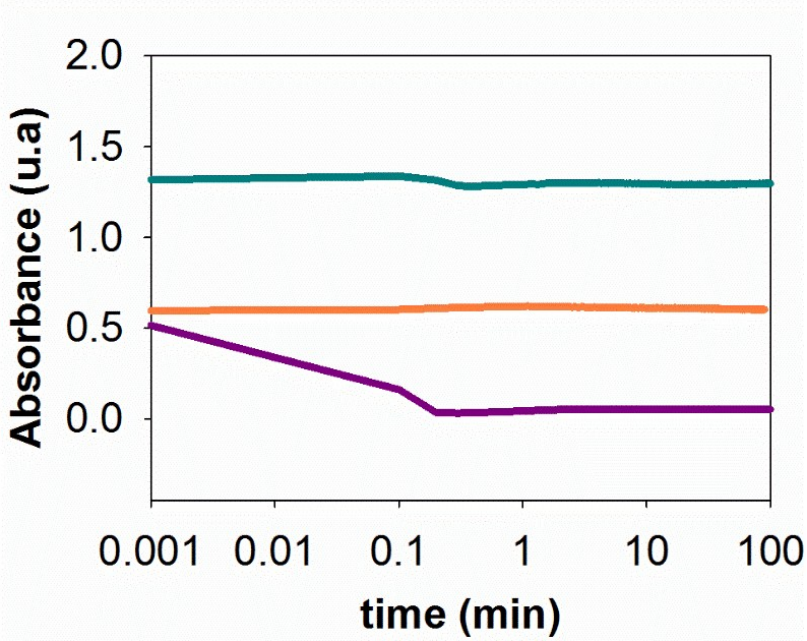


Figure S18. Changes in absorbance at 600 nm with time after adding water to a solution of either **1** (purple), **2** (orange), or **1+2** (cyan blue) in DMSO.

	1 (before annealing)[a]	**1** (after annealing)[a]	**2** (before annealing)[b]	**2** after annealing [c]	**(1+2)** (before annealing)[b]	**(1+2)** (after annealing)[a]
Background (1/cm)	0.0672 ± 0.021	0.0681 ± 0.000	0.075 ± 0.0005	0.002 ± 0.0003	0.0612 ± 0.0004	0.0644 ± 0.0003
Power Law			4.30 ± 0.02	4.04 ± 0.00	3.45 ± 0.01	
Scale			1.55×10^{-8} ± 1.45×10^{-9}	2.00×10^{-7} ± 3.21×10^{-9}	3.66×10^{-6} ± 1.23×10^{-7}	
Length / nm	>1000	>1000		>400		>1000
Kuhn Length / nm	6.77 ± 0.21	6.00 ± 0.61				7.64 ± 0.48
Radius / nm	3.7 ± 0.04	3.9 ± 0.06		3.6 ± 0.02		4.3 ± 0.07
Scale	0.0011 ± 0.0000	0.0008 ± 0.0000		0.0003 ± 0.0000		0.0008 ± 0.0000
Chi squared	1.214	1.2173	2.0139	5.7811	2.2483	1.3035

Table S1. Fits to the SAXS data for the different gels. [a] fits to a flexible cylinder (0.00704<Q<0.217); [b] fits to power law (0.00704<Q<0.217); [c] fits to power law and cylinder (fit to a power law does not fit well at low Q, and this fit results in a chi squared value of >50).

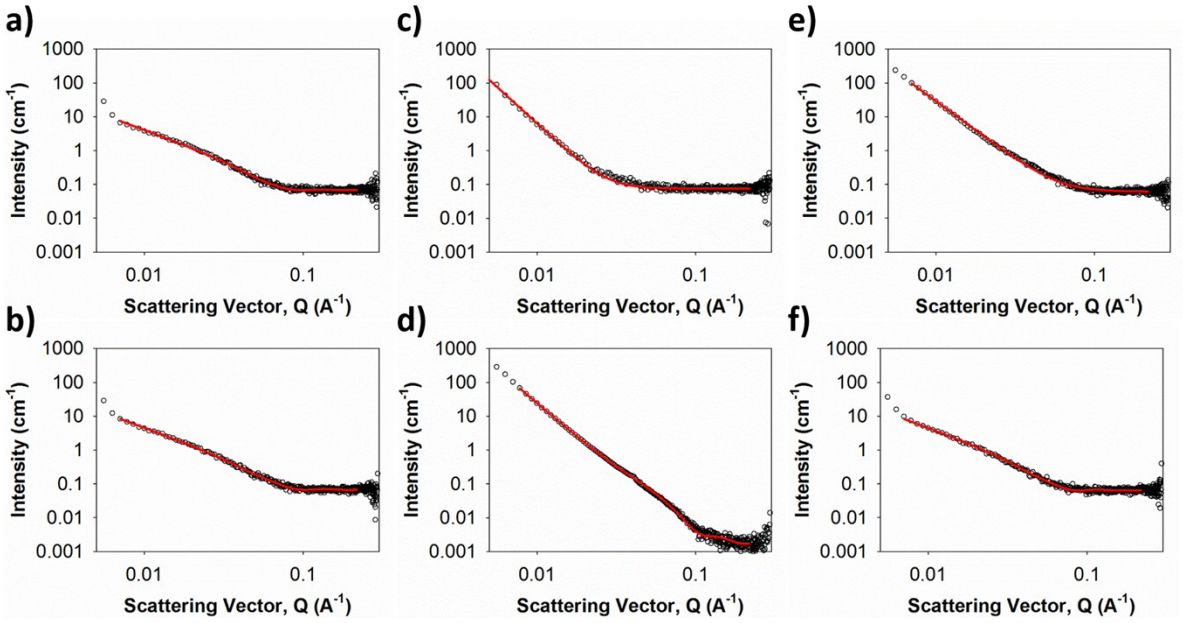


Figure S19. SAXS data and fits for gels of (a) **1** before annealing; (b) **1** after annealing; (c) **2** before annealing; (d) **2** after annealing; (e) (**1**+**2**) before annealing; (f) (**1**+**2**) after annealing. In all cases the data are in open circles and the fits (as described in Table S1) are shown as red lines.

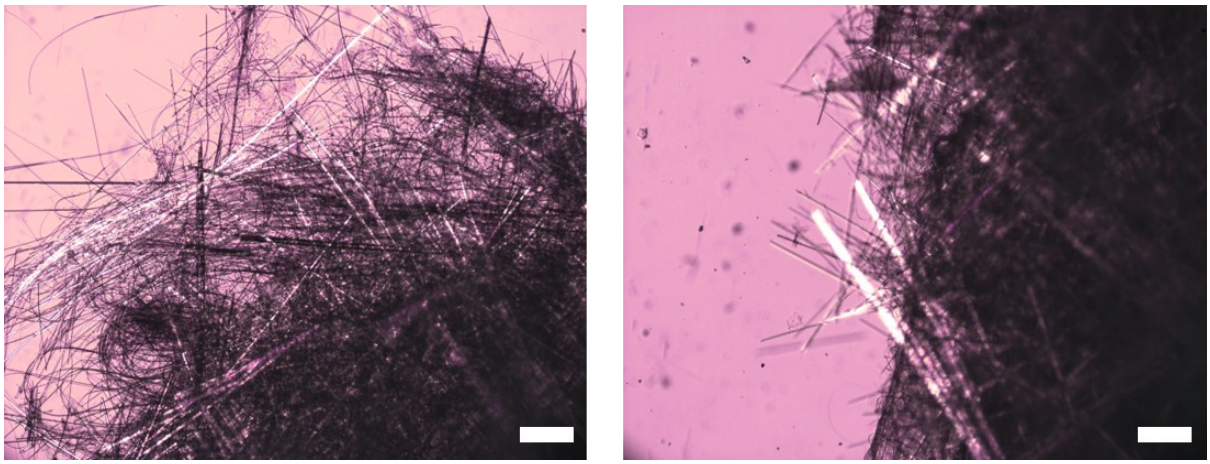


Figure S20. Further images of **(1+2)** after annealing under polarized light. Scale bars represent 200 μm.

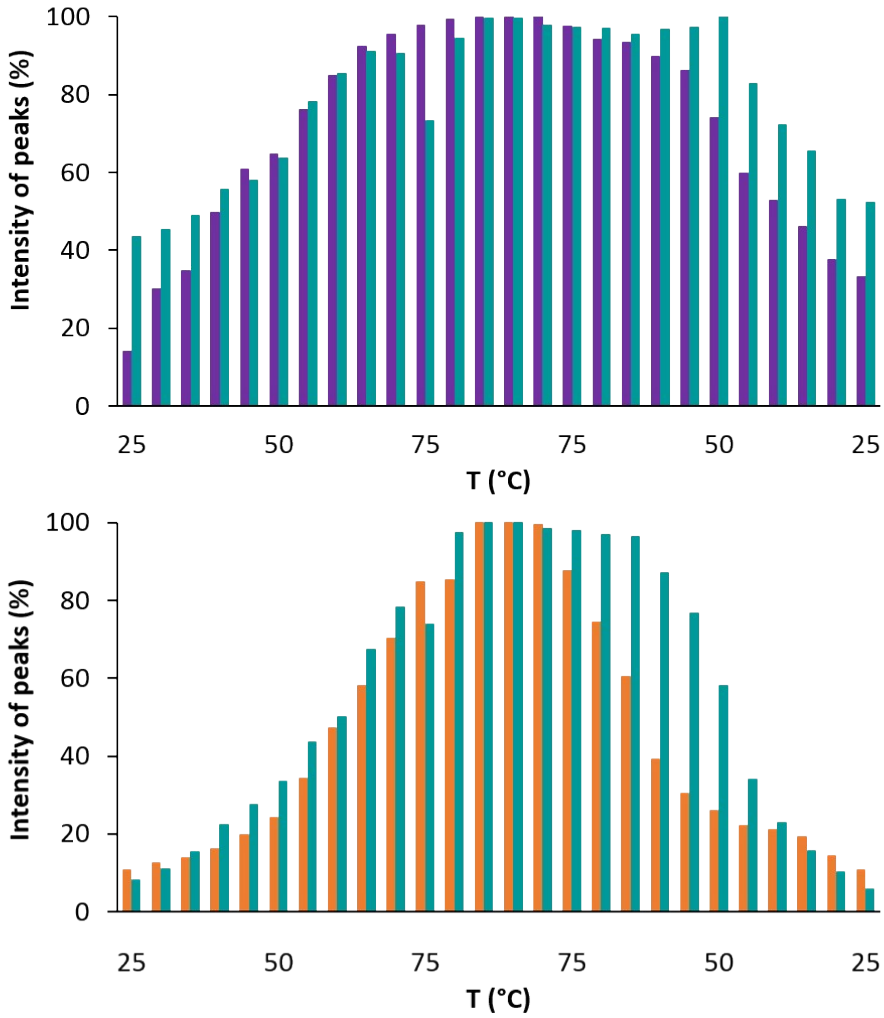


Figure S21. Plot of (top) integrals for **1** against a standard as determined by ^{1}H NMR for **1** alone (purple data) in a mixture of DMSO and D_2O (3/7) on heating and cooling as compared to the integral of **1** in the mixed (blue cyan data) (**1**+**2**) gel; (bottom) integrals for **2** against a standard as determined by ^{1}H NMR for **2** alone (orange data) in a mixture of DMSO and D_2O (3/7) on heating and cooling as compared to the integral of **2** in the mixed (cyan data) (**1**+**2**) gel.

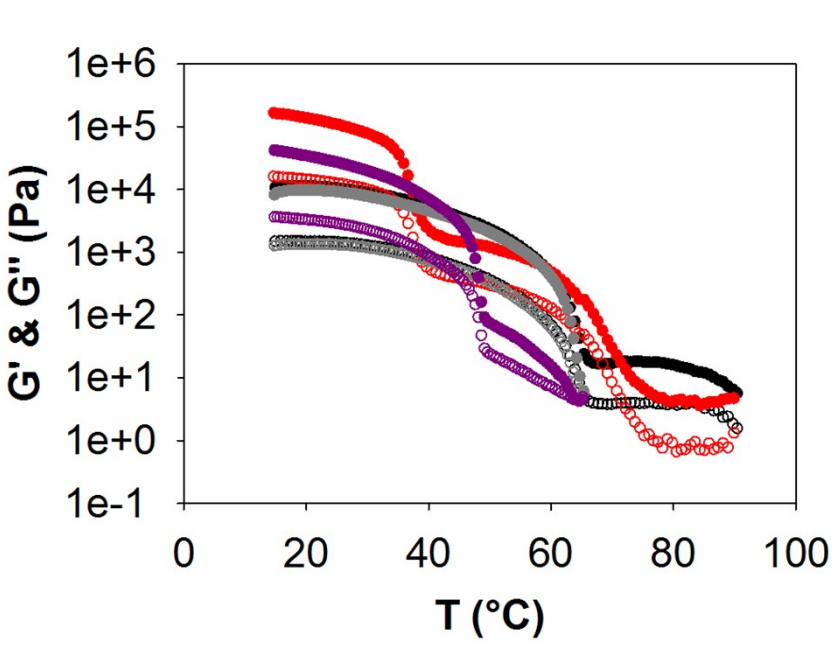


Figure S22. Rheological data for (**1**+**2**) on heating and cooling at a rate of 1°C/min over a range of 15-90 °C and 15-65 °C. Closed symbols represent G′ and open symbols represent G″. The black and red data correspond to the heating and cooling cycle respectively in the range of 15-90 °C, and the grey and purple data correspond to the heating and cooling cycle respectively in the range of 15-65 °C.

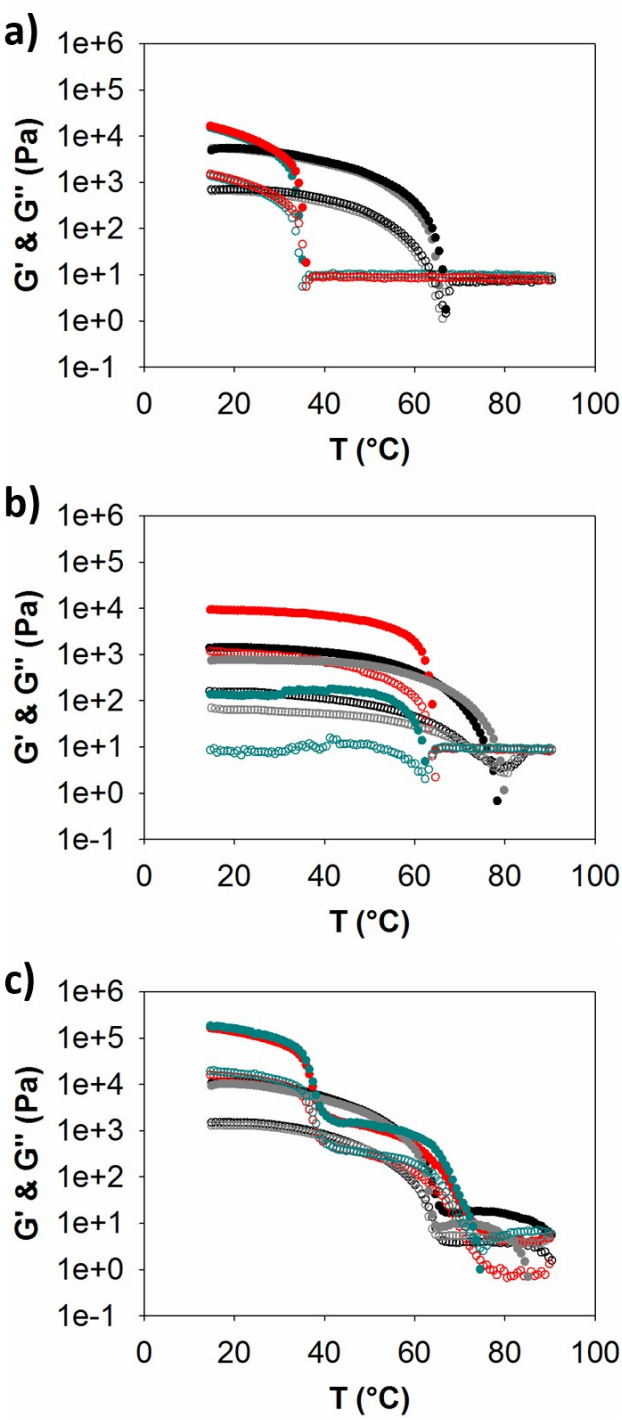


Figure S23. Rheological heating and cooling data for (a) **1**; (b) **2**; (c) (**1**+**2**) at a rate of 1°C/min over a range of 15-90 °C. Closed symbols represent G′ and open symbols represent G″. The black and red data corresponds to the heating and cooling cycle respectively for Day 1, and the grey and cyan data corresponds to the heating and cooling cycle respectively for Day 5 after gel formation.

References

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