Electronic Supplementary Material (ESI) for Chemical Communications.

Varying the hydrophobic spacer to influence multicomponent gelation

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Supporting Information

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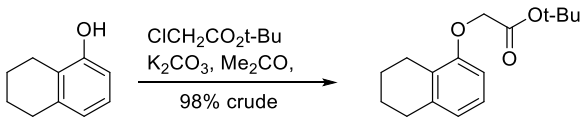
Experimental details

Urease (J61455 Urease, Jack Beans, minimum 45.0 units/mg solid) and urea (ultrapure 99%) were obtained from Alfa Aesar. Compounds **2**-**4** were obtained from Sigma Aldrich and used as received. All other chemicals and solvents used were purchased from commercial suppliers and used as received. Deionised water was used in all experiments.

Compound **1** was synthesized as described below.

tert - Butyl 2 - (5,6,7,8 - tetrahydronaphthalen - 1 - yloxy)acetate (**GG-001**)



GG-001

To a solution of 5,6,7,8-tetrahydronaphth-1-ol (7.69 g, 51.9 mmol) in acetone (150 mL) was added potassium carbonate (4 eq, 28.7 g) and the mixture was heated at 60 °C for 1 hour. *tert*-Butyl chloroacetate (1.05 eq, 7.79 mL) was then added and the mixture was heated to reflux until TLC (1:9 ethyl acetate/*n*-hexane) indicated the absence of starting naphthol (1-2 days). The reaction mixture was then evaporated to dryness under reduced pressure, the residue partitioned between dichloromethane and water, and the mixture stirred until all solids had dissolved. The layers were separated, and the organic phase was washed in turn with water and brine, dried ($MgSO_4$), and evaporated under reduced pressure. Crude **GG-001** was thus obtained as an orange oil (13.4 g, 98% crude) and used in the next step without further purification. A small amount was purified *via* column chromatography (1:9 ethyl acetate/*n*-hexane) to obtain an analytical sample (clear oil).

δ_H (400 MHz, DMSO-d_6) 6.99 (1H, t, *J* 7.88, $\underline{H}_{Ar}$), 6.66 (1H, d, *J* 7.56, $\underline{H}_{Ar}$), 6.57 (1H, d, *J* 8.04, $\underline{H}_{Ar}$), 4.62 (2H, s, $OC\underline{H}_2$), 2.70-2.67 (2H, pseudo-t, $(C\underline{H}_2)_4$), 2.62-2.59 (2H, pseudo-t, $(C\underline{H}_2)_4$), 1.76-1.64 (4H, m, $(C\underline{H}_2)_4$), 1.41 (9H, s, $C(C\underline{H}_3)_3$). δ_C (100 MHz, DMSO-d_6) 167.92 (C=O), 155.37, 137.88, 125.52, 125.24, 121.63, and 108.02 ($\underline{C}_{Ar}$), 81.11 ($\underline{C}(CH_3)_3$), 65.16 ($O\underline{C}H_2$), 28.96 ($(\underline{C}H_2)_4$), 27.62 ($C(\underline{C}H_3)_3$), 22.69, 22.34, and 22.30 ($(\underline{C}H_2)_4$). HRMS (ESI) m/z: $[M+Na]^+$ calcd for $C_{16}H_{22}NaO_3$ 285.1461; found 285.1462.

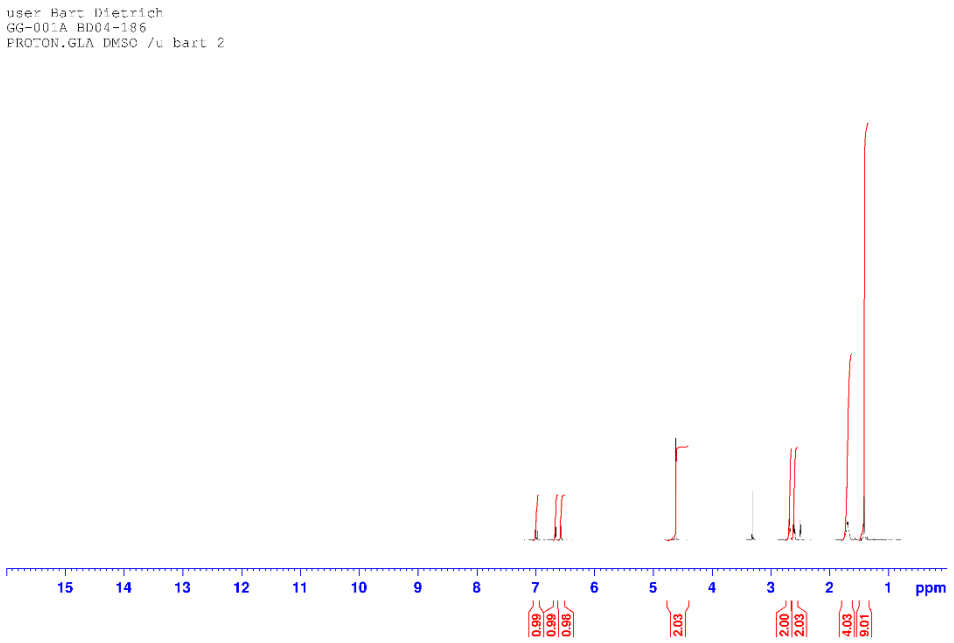


Figure S1. Proton NMR of **GG-001**.

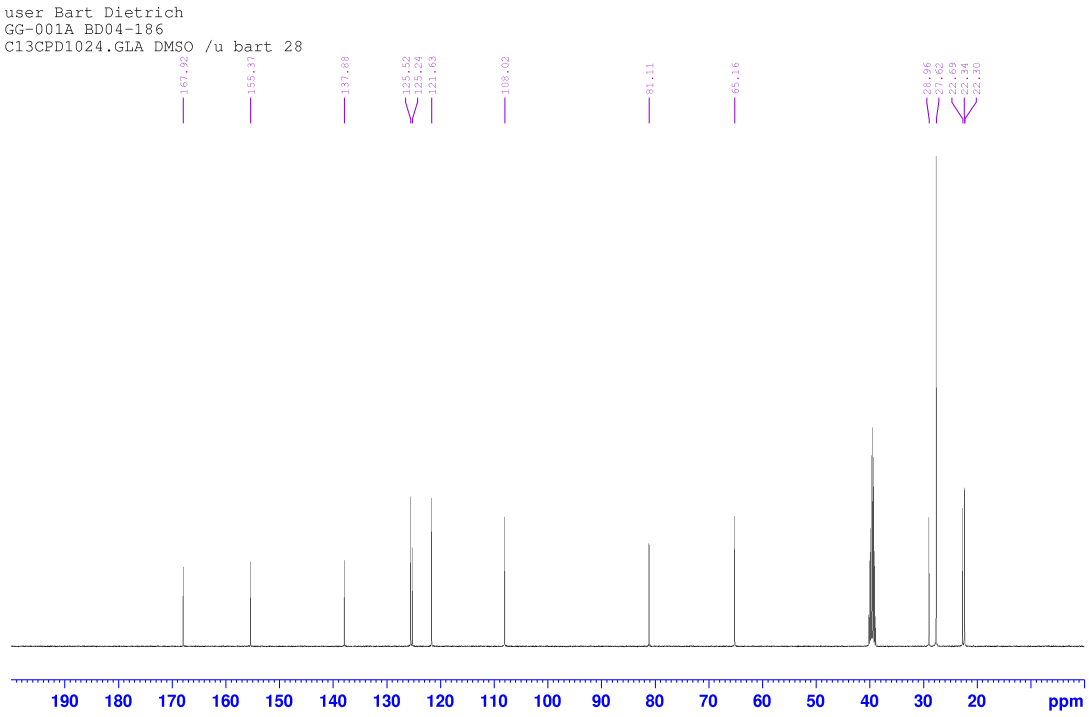
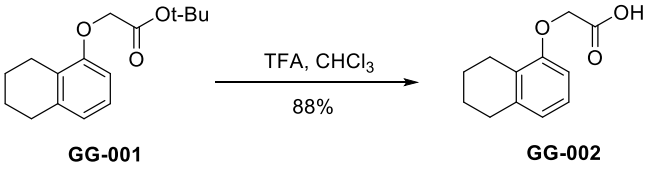


Figure S2. Carbon NMR of **GG-001**.

2-(5,6,7,8-Tetrahydronaphthalen-1-yloxy)acetic acid (**GG-002**)



GG-001 **GG-002**

To a solution of **GG-001** (13.2 g, 50.3 mmol) in chloroform (60 mL) was added trifluoroacetic acid (*ca.* 10 eq, 39 mL) and the mixture was left to stir overnight (a precipitate started forming shortly upon addition of the TFA). After this time, the reaction mixture was concentrated under reduced pressure to remove most of the chloroform. To the resulting thick oil was added excess diethyl ether. During stirring of this solution, a precipitate began forming. This was filtered off after 1 hour, washed with plenty of diethyl ether, and dried under vacuum. The title compound was thus obtained as a white solid (8.61 g). A further crop of product can be obtained by evaporation of the mother liquor, resuspension of the residue in diethyl ether, and filtration. **GG-002** was thus obtained in 88% total yield (9.10 g).

δ_H (400 MHz, DMSO-d_6) 12.92 (1H, br s, COO<u>H</u>), 7.00 (1H, t, *J* 7.89, <u>H</u>$_{Ar}$), 6.66 (1H, d, *J* 7.47, <u>H</u>$_{Ar}$), 6.58 (1H, d, *J* 8.06, <u>H</u>$_{Ar}$), 4.64 (2H, s, OC<u>H</u>$_2$), 2.68 (2H, pseudo-t, *J* 5.78, (C<u>H</u>$_2$)$_4$), 2.60 (2H, pseudo-t, *J* 6.03, (C<u>H</u>$_2$)$_4$), 1.75-1.64 (4H, m, (C<u>H</u>$_2$)$_4$). δ_C (100 MHz, DMSO-d_6) 170.36 (<u>C</u>=O), 155.46, 137.95, 125.61, 125.20, 121.60, and 108.00 (<u>C</u>$_{Ar}$), 64.64 (O<u>C</u>H$_2$), 29.00, 22.77, 22.38, and 22.33 ((<u>C</u>H$_2$)$_4$). HRMS (ESI) m/z: $[M+Na]^+$ calcd for $C_{12}H_{14}NaO_3$ 229.0835; found 229.0834.

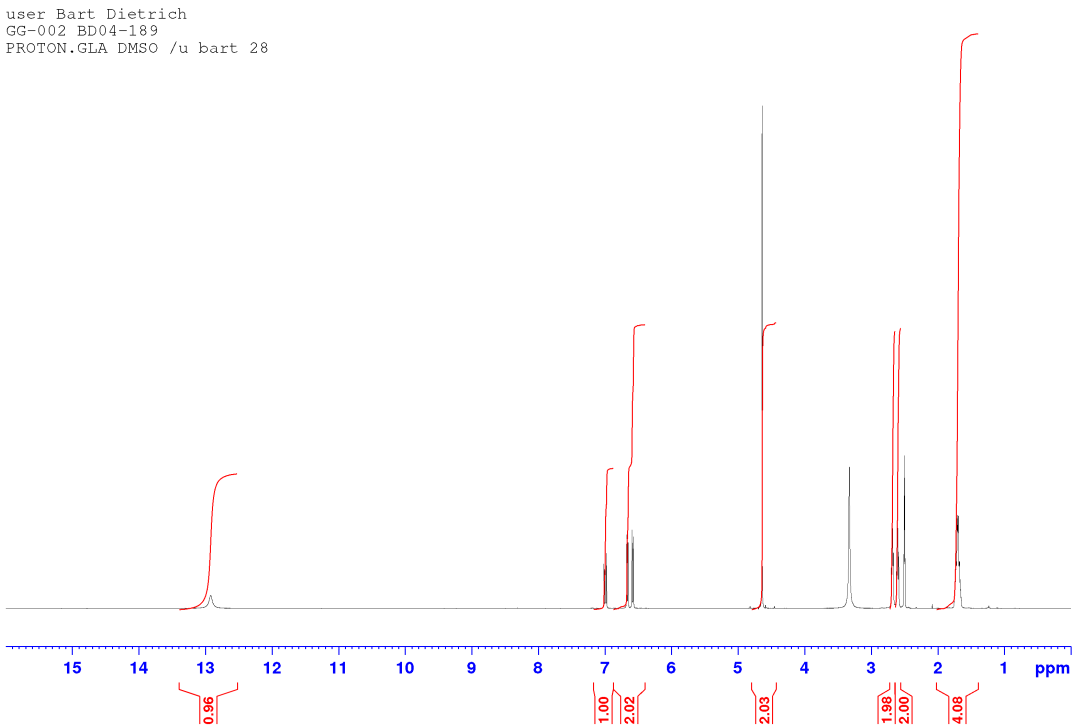


Figure S3. Proton NMR of **GG-002**.

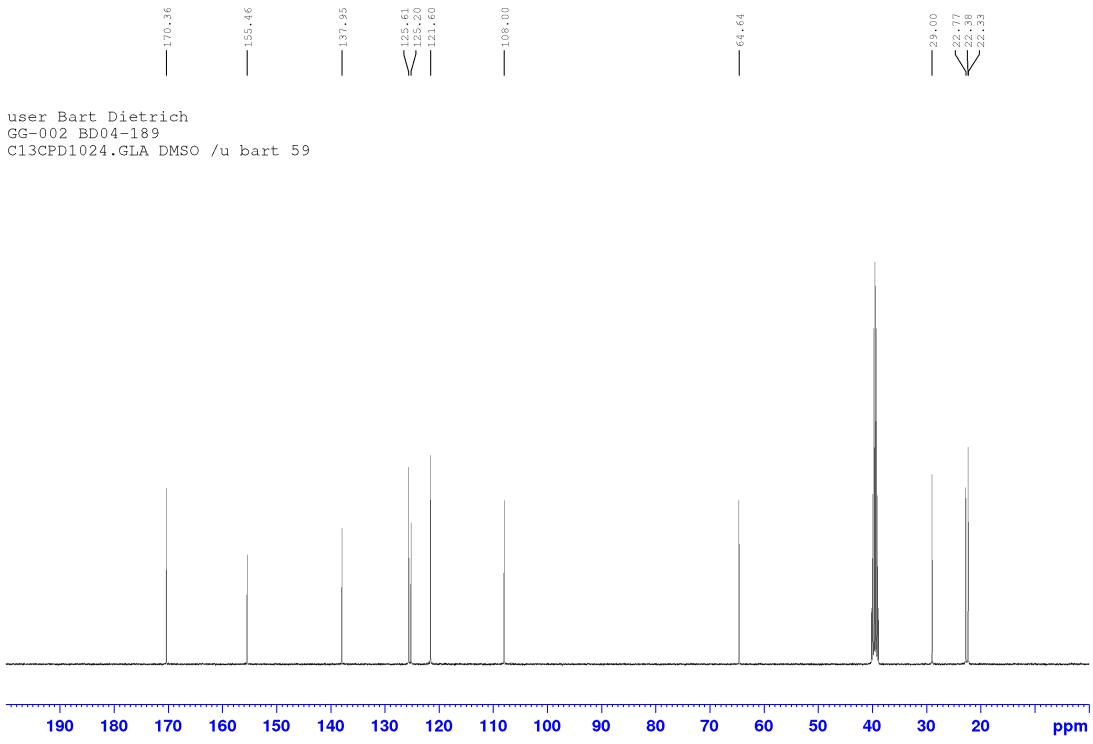
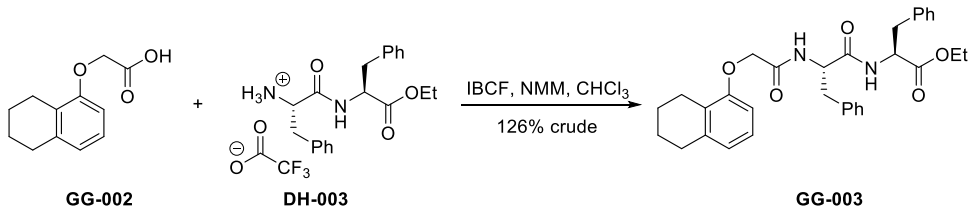


Figure S4. Carbon NMR of **GG-002**.

Ethyl (2*S*)-3-phenyl-2-[(2*S*)-3-phenyl-2-[2-(5,6,7,8-tetrahydronaphthalen-1-yloxy)acetamido]propanamido]propanoate (**GG-003**)



GG-002 **DH-003** **GG-003**

To a solution of **GG-002** (3.95 g, 19.2 mmol) in chloroform (150 mL) cooled in ice/water was added *iso*-butyl chloroformate (1.02 eq, 2.56 mL) followed by *N*-methylmorpholine (1.1 eq, 2.32 mL). After 5 minutes, **DH-003**[1] (1.02 eq, 8.88 g) was added followed by another portion of *N*-methylmorpholine (1.1 eq, 2.32 mL) and the reaction was left to stir overnight. After this time, the mixture was diluted with chloroform, washed in turn with 1M hydrochloric acid, water, and brine, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. Crude **GG-003** was obtained as an off-white solid (12.8 g, 126%) and used without further purification in the next step. A small amount was purified *via* column chromatography (1:9 ethyl acetate/dichloromethane) to obtain an analytical sample. One aliphatic carbon is not resolved in the carbon NMR.

δ_H (400 MHz, DMSO-d_6) 8.62 (1H, d, *J* 7.61, NH), 7.56 (1H, d, *J* 8.49, NH), 7.30-7.18 (10H, m, H_{Ar}), 6.94 (1H, t, *J* 7.84, H_{Ar}), 6.67 (1H, d, *J* 7.61, H_{Ar}), 6.48 (1H, d, *J* 8.14, H_{Ar}), 4.69-4.63 (1H, m, CH*), 4.53-4.47 (1H, m, CH*), 4.40 (1H, d, *J* 14.15, $OCH_2C{=}O$), 4.33 (1H, d, *J* 14.75, OCH_2), 4.06 (2H, q, *J* 7.10, CH_2CH_3), 3.07-2.94 (3H, m, $PhCH_2$), 2.86 (1H, dd, *J* 13.78, 8.92, $PhCH_2$), 2.69-2.66 (2H, m, $(CH_2)_4$), 2.57-2.44 (2H overlapped by DMSO, m, $(CH_2)_4$), 1.75-1.63 (4H, m, $(CH_2)_4$), 1.11 (3H, t, *J* 7.09, CH_2CH_3). δ_C (100 MHz, DMSO-d_6) 171.21, 170.71, and 167.31 (C=O), 155.16, 137.90, 137.09, 136.94, 129.27, 129.08, 128.21, 127.97, 126.54, 126.32, 125.69, 125.09, 121.81, and 108.43 (C_{Ar}), 66.75 ($OCH_2C{=}O$), 60.55 (CH_2CH_3), 53.63 and 52.71 (CH*), 37.58 and 36.73 ($PhCH_2$), 28.97, 22.56, and 22.30 ($(CH_2)_4$), 13.90 (CH_2CH_3). HRMS (ESI) m/z: $[M+Na]^+$ calcd for $C_{32}H_{36}N_2NaO_5$ 551.2516; found 551.2504.

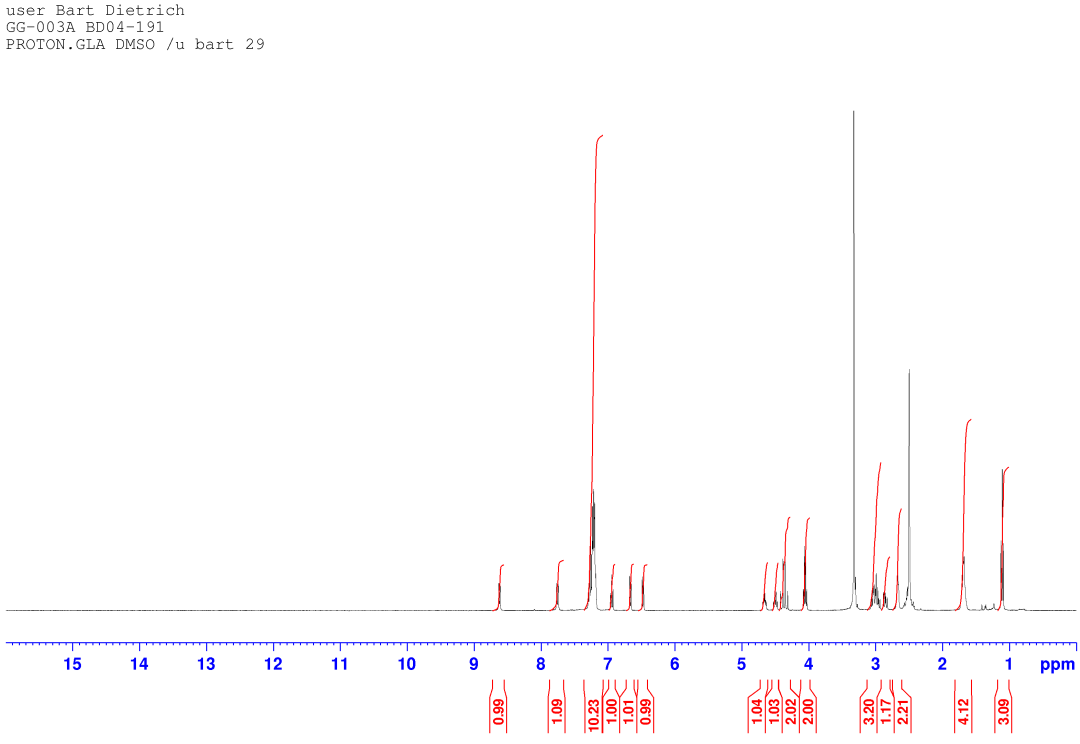


Figure S5. Proton NMR of **GG-003**.

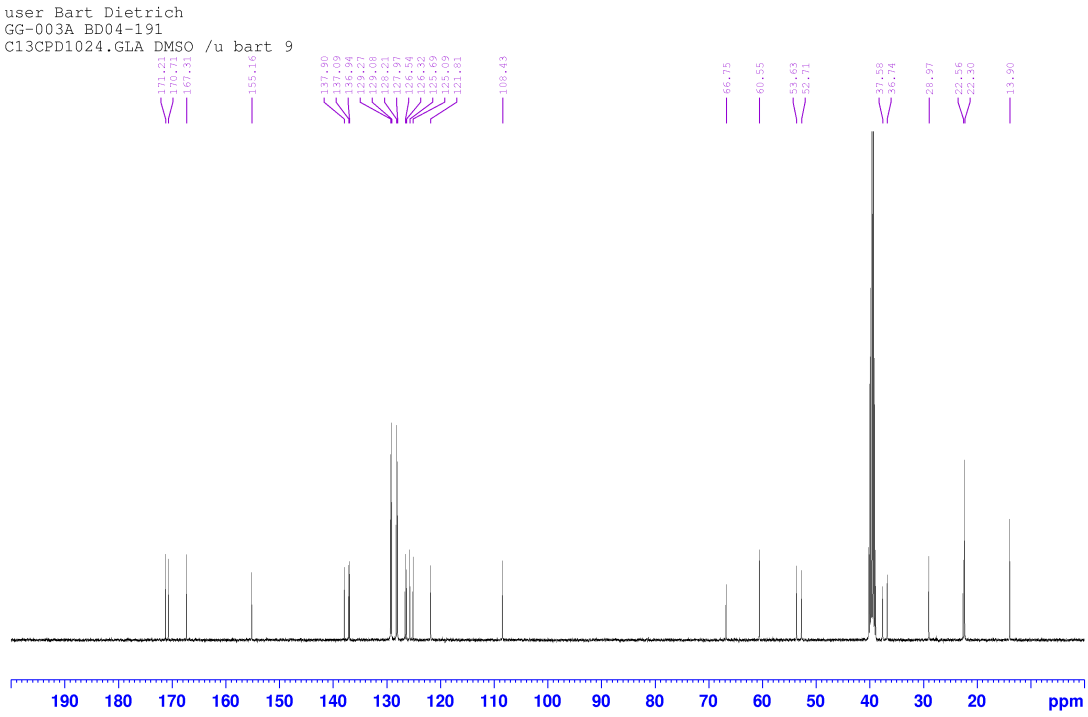
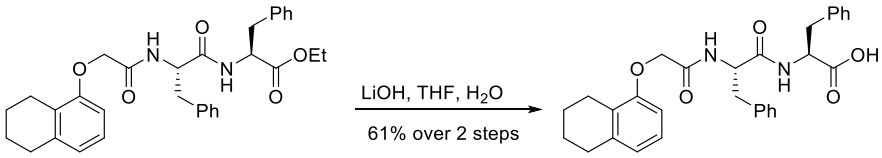


Figure S6. Carbon NMR of **GG-003**.

(2*S*)-3-Phenyl-2-[(2*S*)-3-phenyl-2-[2-(5,6,7,8-tetrahydronaphthalen-1-yloxy)acetamido]propanamido]propanoic acid (**1**)



GG-003 **1**

To a solution of crude **GG-003** (11.2 g, nominally 21.2 mmol) in tetrahydrofuran (100 mL) was added a solution of lithium hydroxide (4 eq, 2.03 g) in water (100 mL) and the mixture was left stirring overnight. After this time, a precipitate had developed in the flask. The flask contents were poured into 1M hydrochloric acid (*ca.* 300 mL) and stirred for 2 hours. The solids were then filtered off, washed with water in the filter, and dried. Recrystallisation from boiling acetonitrile afforded **1** as a white solid (5.90 g, 61% over two steps, < 0.2% acetonitrile). The mother liquor can be concentrated and recrystallised again to obtain further crops of **1**.

δ_H (400 MHz, DMSO-d_6) 12.84 (1H, br s, COOH), 8.46 (1H, d, *J* 7.92, NH), 7.72 (1H, d, *J* 8.51, NH), 7.30-7.17 (10H, m, H_{Ar}), 6.94 (1H, t, *J* 7.88, H_{Ar}), 6.66 (1H, d, *J* 7.59, H_{Ar}), 6.48 (1H, d, *J* 7.99, H_{Ar}), 4.65 (1H, ddd, *J* 8.58, 8.58, 4.40, CH*), 4.47 (1H, ddd, *J* 8.32, 8.32, 5.07, CH*), 4.40 (1H, d, *J* 14.76, OCH_2), 4.32 (1H, d, *J* 14.51, OCH_2), 3.09 (1H, dd, *J* 13.91, 5.20, $PhCH_2$), 3.02 (1H, dd, *J* 13.83, 4.38, $PhCH_2$), 2.93 (1H, dd, *J* 13.92, 8.87, $PhCH_2$), 2.86 (1H, dd, *J* 13.84, 8.76, $PhCH_2$), 2.69-2.64 (2H, m, $(CH_2)_4$), 2.58-2.40 (2H, m, $(CH_2)_4$), 1.73-1.62 (4H, m, $(CH_2)_4$). δ_C (100 MHz, DMSO-d_6) 172.74, 170.59, and 167.31 (C=O), 155.16, 137.94, 137.38, 137.12, 129.36, 129.14, 128.19, 127.99, 126.47, 126.32, 125.74, 125.12, 121.86, and 108.46 (C_{Ar}), 66.77 (OCH_2), 53.48 and 52.74 (CH*), 37.58 and 36.76 ($PhCH_2$), 29.01, 22.59, and 22.34 ((CH_2)$_4$). HRMS (ESI) m/z: $[M+Na]^+$ calcd for $C_{30}H_{32}N_2NaO_5$ 523.2203; found 523.2199.

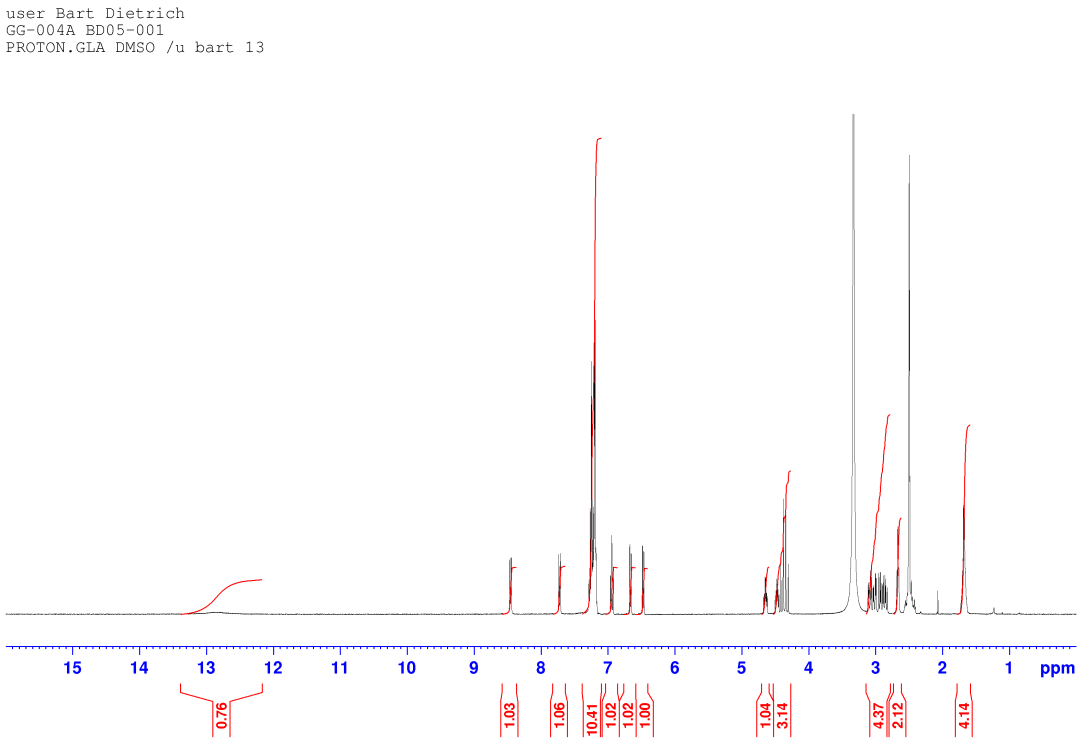


Figure S7. Proton NMR of **1**.

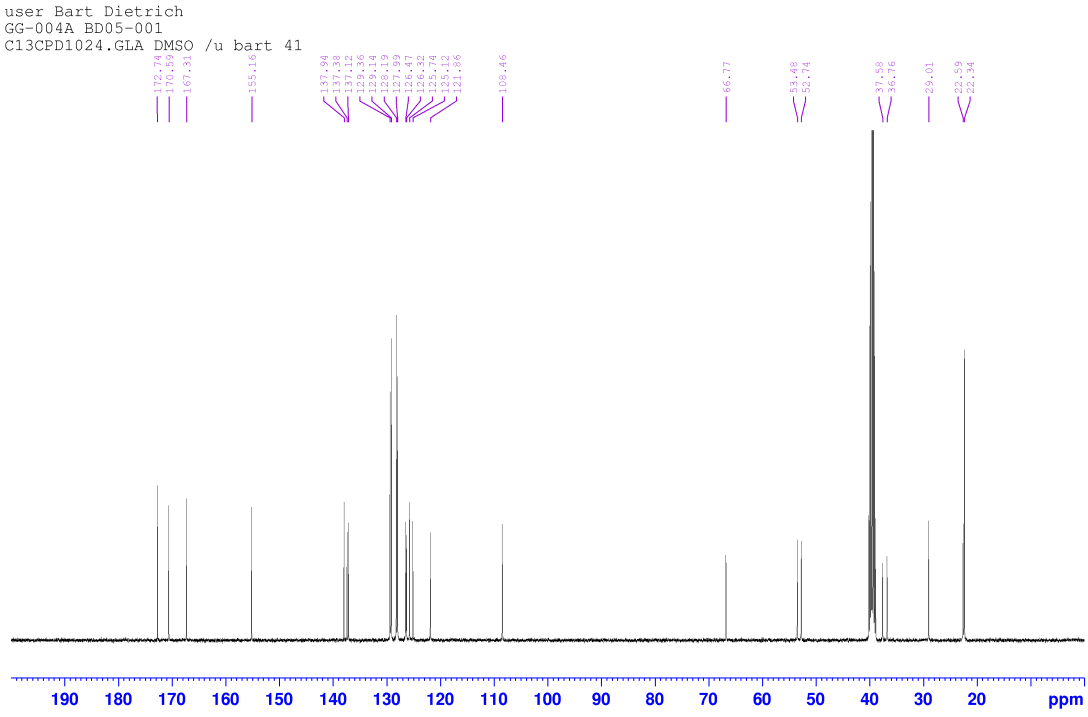


Figure S8. Carbon NMR of **1**.

Preparation of solutions: Stock solutions of **1** (in DMSO, 10 mg/mL), **2**-**4** (in water, 4 mg/mL), urea (in water, 4 M), and urease (in water, 5 mg/mL) were prepared by stirring the solids in the appropriate solvent until complete dissolution. The enzyme concentration in the stock solution was determined from the mass (in mg) dissolved in a known volume of H_2O. Solutions of all the components were prepared freshly before each experiment. A stock solution of NaOH was prepared at a concentration of 0.1 M in H_2O.

Preparation of gels: Hydrogels of **1** were prepared by adding 1.60 mL of H_2O to 0.4 mL of the DMSO solution of **1** in one aliquot. Therefore, the ratio of DMSO and water was 20:80 and the concentration of **1** was 2 mg/mL.
The multicomponent hydrogels were prepared from a mixture of **1** and **2** in DMSO/H_2O (20/80, v/v) under different conditions. To prepare the gels at low pH, 0.40 mL of the solution of **1** was transferred into a 7 mL Sterilin vial. To this solution, a mixture of 0.6 mL of H_2O and 1 mL of solution of **2** was added in one aliquot. To prepare the multicomponent gels at high pH, a mixture of 0.4 mL of H_2O and 1 mL of solution of **2** was transferred to the vial containing a mixture of 0.4 mL of **1** and 0.2 mL of NaOH. A similar procedure was followed to prepare the multicomponent gels of **1** with **3** and **4**. The pH of the gels prepared in absence and presence of NaOH was recorded to be pH 3.1-3.3 and pH 10.2-10.4, respectively. Therefore, in the respective gels, initial concentrations of **1**-**4** were 2 mg/mL and concentration of NaOH was 0.01 M. In all cases, the ratio of DMSO and water was 20:80.
To prepare gels involving the enzymatic reaction, a common procedure was followed. For these experiments, a mixture of the Fmoc-amphiphile (**2**-**4** as required) and urease was prepared by diluting urease solution with water followed by addition of the Fmoc-salt. The mixture was then immediately transferred to the solution of **1** or a mixture of **1** and urea. Initially, the multicomponent gels of **1** and **2** were prepared at low pH in presence of urease but in absence of urea by diluting 0.4 mL of DMSO solution of **1** with a mixture of 0.4 mL of H_2O, 0.2 mL of solution of urease and 1 mL of solution of **2**. Therefore, the concentrations of **1** and **2** were 2 mg/mL and concentration of urease was 0.5 mg/mL. A similar procedure was followed to prepare the multicomponent gels of **1** with **3** and **4** in presence of urease. The pH of the gels was recorded to be 3.3-3.4.
To prepare the gels in presence of both urease and urea, a mixture of 0.35 mL of H_2O, 1 mL of solution of **2** (or **3** and **4**) and 0.2 mL of solution of urease was transferred to the vial containing 0.40 mL of **1** and 50 µL of urea. Therefore, in the respective gels, initial concentrations of **1- 4** were 2 mg/mL, concentration of urease was 0.5 mg/mL and initial concentration of urea was 0.1 M.
In all cases, the gels were prepared in a volume of 2 mL in which the ratio of DMSO and H_2O was maintained at 20:80. All samples were left overnight before measurements were carried out.

pH measurements: pH measurements were carried out using a FC200 pH probe (Hanna) with a 6×10 mm conical tip with an accuracy of ±0.1 pH units. For the urea-urease reaction involving compounds **1**-**4**, the reaction mixtures were prepared as described above at a 2 mL volume in a 7 mL Sterilin vial. The vials were kept in a circulating water bath at 25 °C while the pH was measured over time.
pK_as were determined through titration of individual solutions of **1-4** (2 mg/mL in 20% DMSO/H_2O) containing 1 molar equivalent of NaOH (0.1 M). The titrant was 0.1 M of HCl, and pH of the solutions was recorded after each addition of the titrant. The solutions were stirred to avoid gel formation, and were kept at 25 °C.

Rheological measurements: An Anton Paar Physica MCR 301 rheometer was used for the rheology experiments. All rheological measurements were conducted at 25 °C using a vane and cup geometry. All samples were prepared at 2 mL volume in 7 mL Sterilin vials following the same methodology as described above and were left to age ~16 hours before measurement. Strain sweeps were performed at 10 rad/s from 0.01% to 1000% strain. Frequency sweeps were performed at 0.5 % strain from 1 rad/s to 100 rad/s. Time sweeps were performed at an angular frequency of 10 rad/s and with a strain of 0.5%.

Confocal microscopy: Confocal imaging was carried out on a Zeiss LSM710 microscope equipped with a Zeiss LD EC Epiplan NEUFLUAR 50X, 0.55 DIC objective. Samples were stained with Nile blue (2 µL/mL of a 0.1 wt % solution) and prepared in 35 mm diameter CELLview culture dishes. Samples were excited at 633 nm using a He-Ne laser and the images were captured using Zeiss ZEN 2011 v7.0.3.286 software.

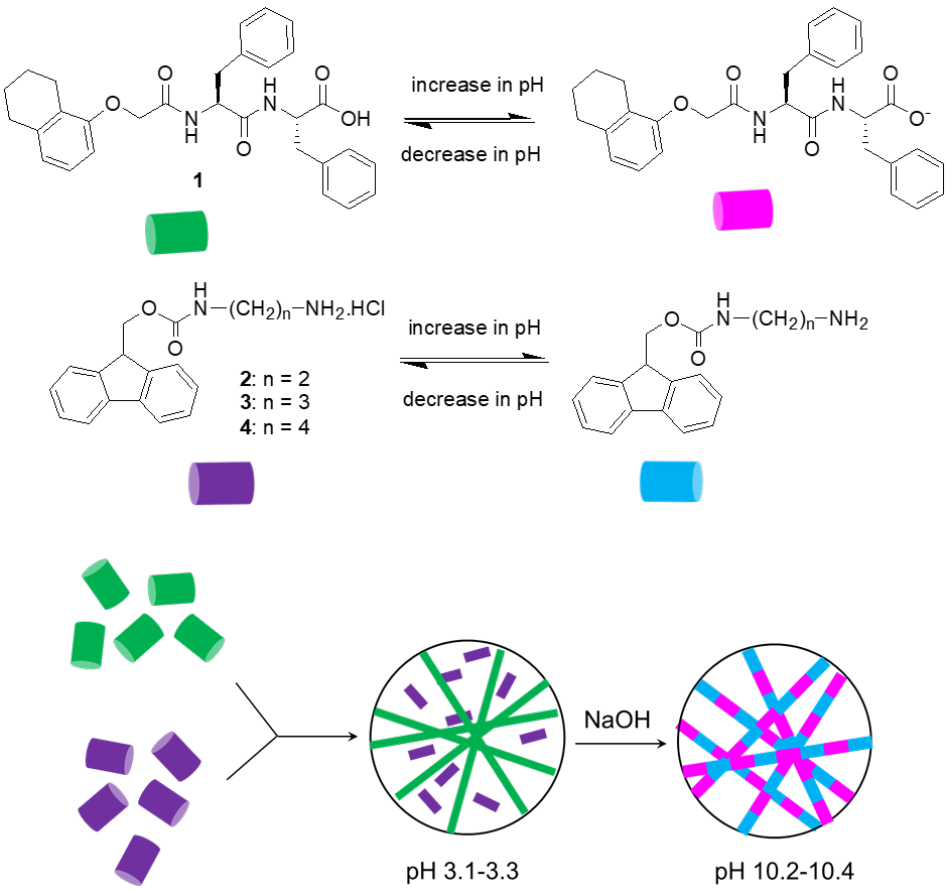
UV-Vis measurements: Gel samples were prepared in Sterilin vials as described earlier and left overnight before measurements were carried out. Small amounts of the gels were then transferred to a

0.01 mm path length quartz cuvette and measured on an Agilent Technologies Cary 60 UV-vis spectrometer.

Fluorescence spectroscopy: Fluorescence measurements were conducted on an Agilent Technologies Cary Eclipse fluorescence spectrometer. Samples were prepared in PMMA cuvettes with a path length of 1 cm by following the same procedure as mentioned before. All gels were left overnight before measurements were undertaken. Time variable data for the gels were recorded after 1 min, 2 mins, 5 mins, 10 mins, 15 mins, 30 mins, 1 h, 1.5 h, 2 h and then after each hour until 16 h after addition of the components. In all cases, the excitation wavelength was 300 nm. Both the excitation and emission slit widths were 5 nm.

FTIR spectroscopy: All gel samples were prepared using DMSO-d_6, D_2O, and NaOD following the same methodology as described above. Small amounts of the gels were placed onto the ATR crystal for recording the spectra using an Agilent Cary 630 FTIR spectrometer with ATR attachment. The background for solid (amorphous) samples was the empty ATR crystal, while for gels a 20 % DMSO-d_6 in D_2O solution was used.

Supplementary Figures



Scheme S1. Schematic showing pH dependent aggregations of **1** with **2**-**4** in DMSO/H_2O (20/80, v/v).

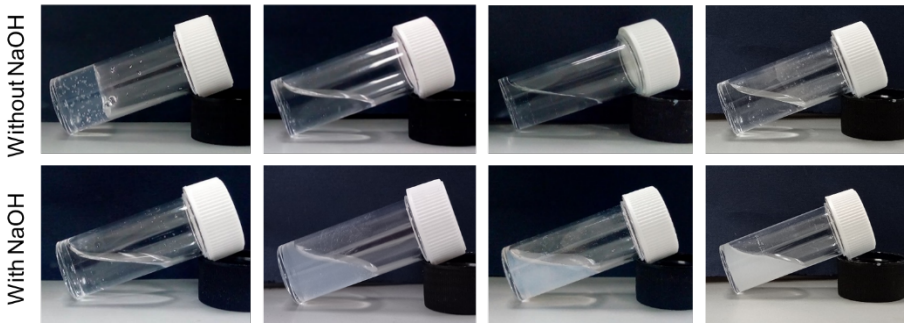


Figure S9. Photographs showing the phase change of **1**-**4** (from left to right) in absence and presence of 0.01 M of NaOH in 20:80 DMSO/water (v/v). In all cases, concentrations of **1**-**4** are 2 mg/mL.

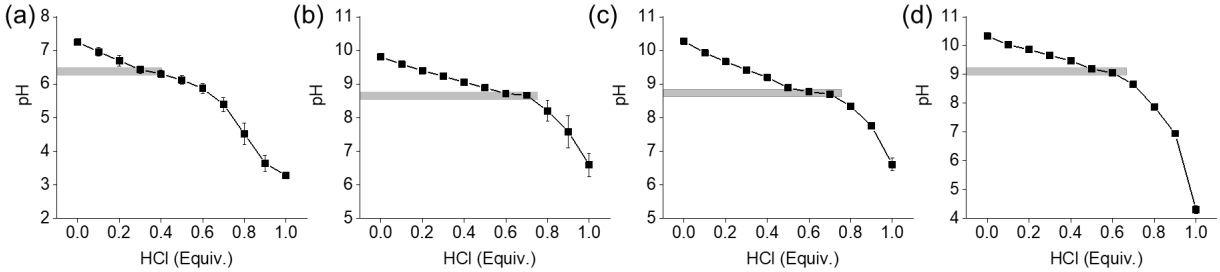


Figure S10. (a-d) Determination of apparent pK_a of **1**-**4** (2 mg/mL) in 20:80 DMSO/water (v/v) respectively.[2, 3] The plateau is taken to represent the apparent pK_a value, shown by the horizontal shading.

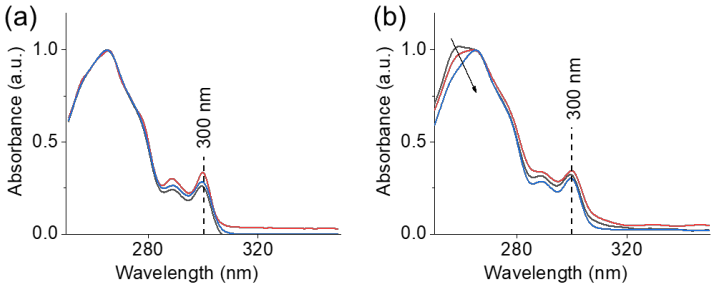


Figure S11. Normalized UV-vis spectra of **2** (black), **3** (red), and **4** (blue) (a) in absence and (b) presence of 0.01 M of NaOH. In all cases, concentrations of **2**-**4** are 2 mg/mL, solvent is 20:80 DMSO/water (v/v).

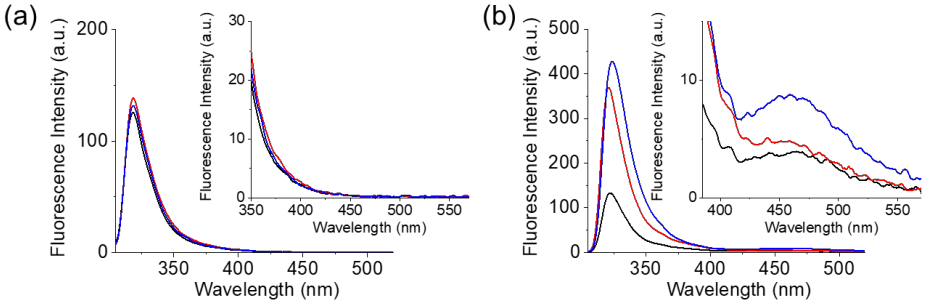


Figure S12. Emission spectra of **2** (black), **3** (red), and **4** (blue) (a) in absence and (b) presence of 0.01 M of NaOH. In all cases, concentrations of **2**-**4** are 2 mg/mL, solvent is 20:80 DMSO/water (v/v). Inset represents expanded section of the corresponding graph.

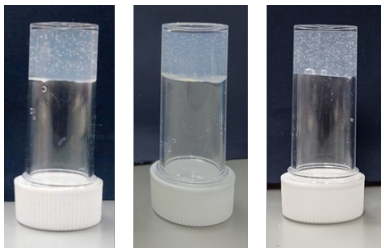


Figure S13. Photographs of multicomponent gels of **1** with **2**-**4** (from left to right) in DMSO/H_2O (20/80, v/v). In all cases, concentrations of **1**-**4** are 2 mg/mL.

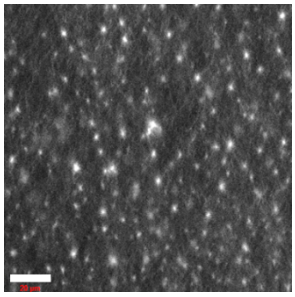


Figure S14. Confocal fluorescence microscopy image (scale bars represent 20 μm) of the hydrogel gel of **1** (2 mg/mL) prepared in DMSO/H_2O (20/80, v/v).

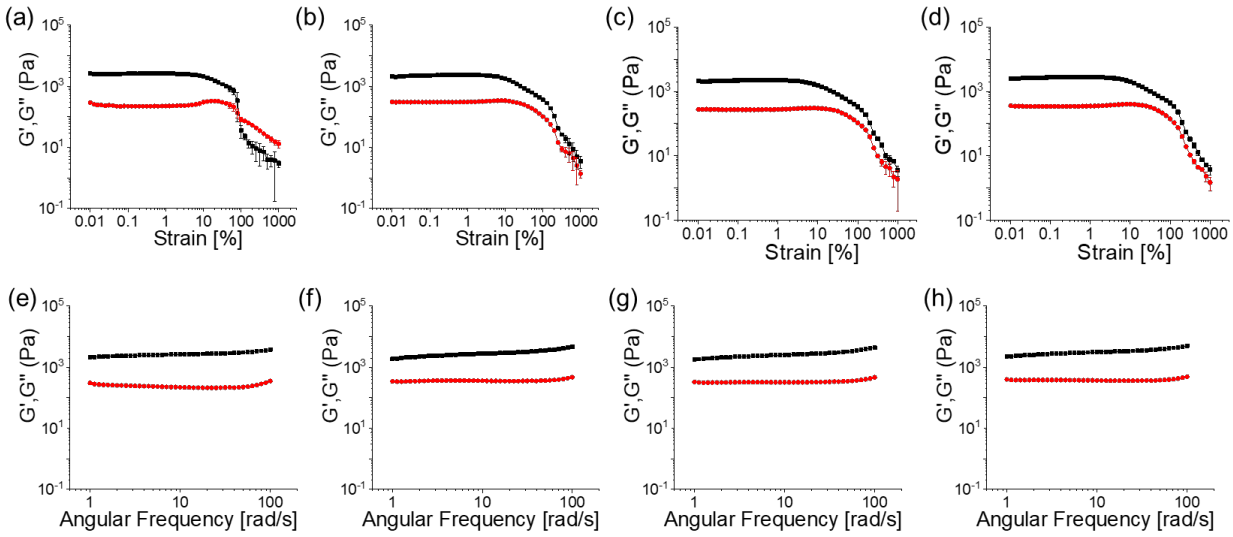


Figure S15. Strain (a-d) and frequency (e-h) sweeps for the hydrogel of **1** (a, e) and multicomponent gels of **1** with **2**-**4** (b-d and f-h, respectively). In all cases, concentrations of **1**-**4** are 2 mg/mL, solvent is 20:80 DMSO/water (v/v). The black symbols represent G′, the red symbols G″.

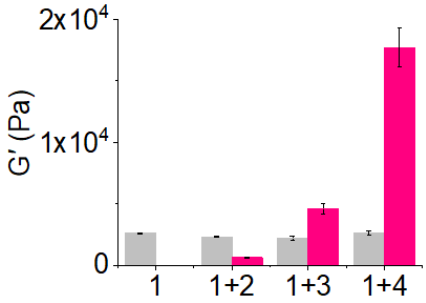


Figure S16. Bar graph representing the stiffness (G', calculated at 0.5% strain from strain sweeps) of the single and multicomponent hydrogels obtained at low pH (grey) and high pH (pink). In all cases, concentrations of **1**-**4** are 2 mg/mL, concentration of NaOH is 0.01 M, solvent is DMSO/H_2O (20/80, v/v).

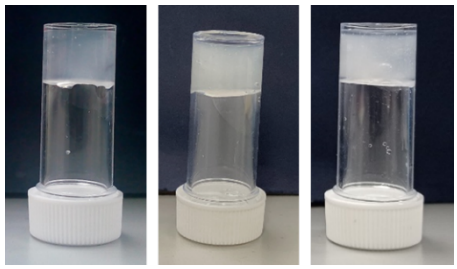


Figure S17. Photographs of multicomponent gels of **1** with **2**-**4** (from left to right) in DMSO/H_2O (20/80, v/v) obtained in presence of 0.01 M of NaOH. In all cases, concentrations of **1**-**4** are 2 mg/mL.

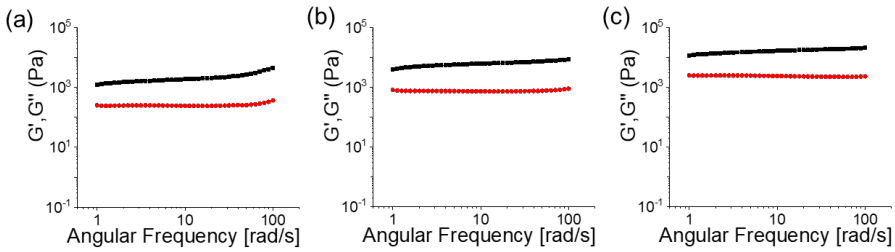


Figure S18. Frequency sweep experiments of the multicomponent gels of (a) (**1**+**2**), (b) (**1**+**3**) and (c) (**1**+**4**) prepared at pH 10.2-10.4. In all cases, concentrations of **1**-**4** are 2 mg/mL, concentration of NaOH is 0.01 M, solvent is DMSO/H_2O (20/80, v/v).

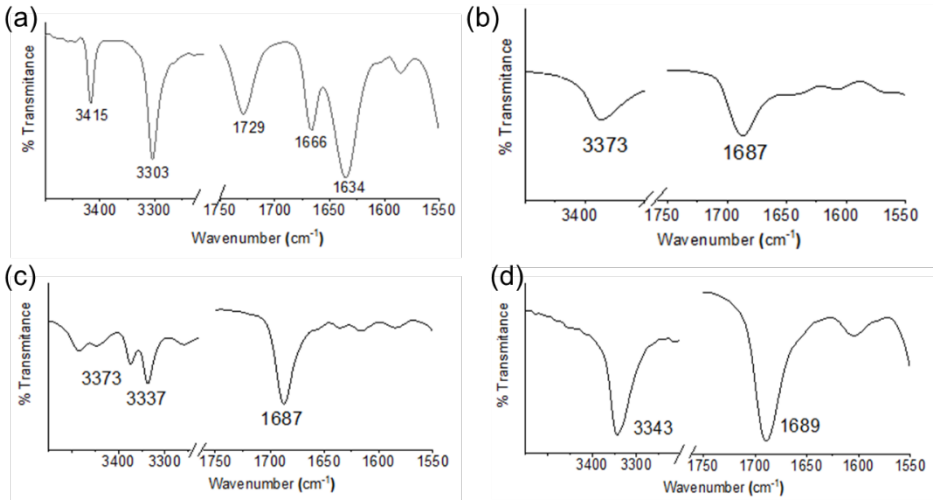


Figure S19. (a)-(d) show partial FTIR spectra of **1-4,** respectively, in their amorphous (solid) states.

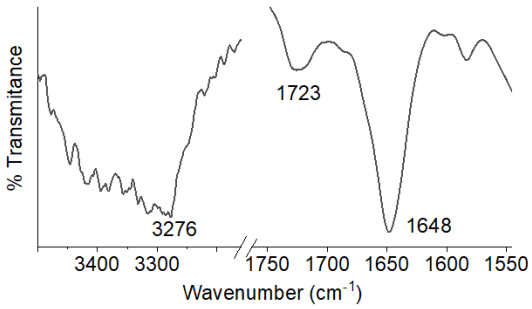


Figure S20. Partial FTIR spectrum of the hydrogel of **1** (2 mg/mL).[3]

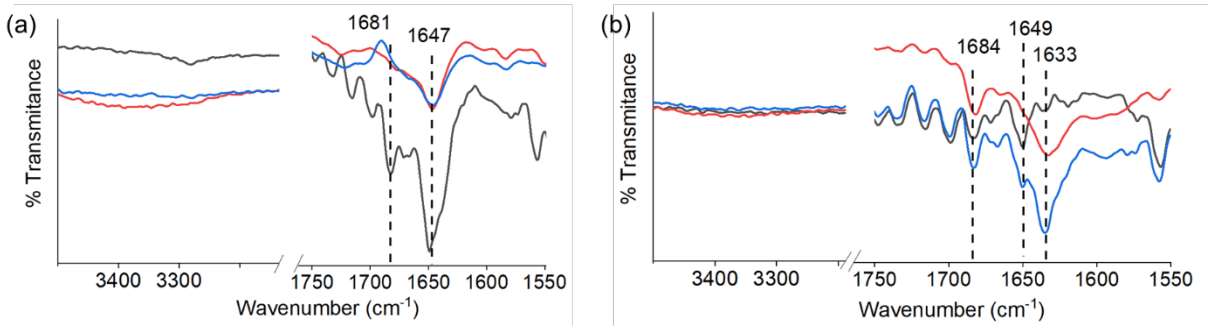


Figure S21. Partial FTIR spectra of the multicomponent gels of **1** with (**2**-**4**) at (a) pH 3.1-3.3 and (b) pH 10.2-10.4 pH. The black, red and blue data represent hydrogels of (**1**+**2**), (**1**+**3**) and (**1**+**4**) respectively.

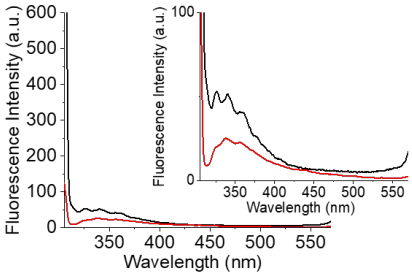


Figure S22. Emission spectra of hydrogel of **1** (black) and the sol of **1** in presence of 0.01 M NaOH (red). In both cases, initial concentration of **1** is 2 mg/mL, solvent is DMSO/H_2O (20/80, v/v). Inset represent expanded section of the graph.

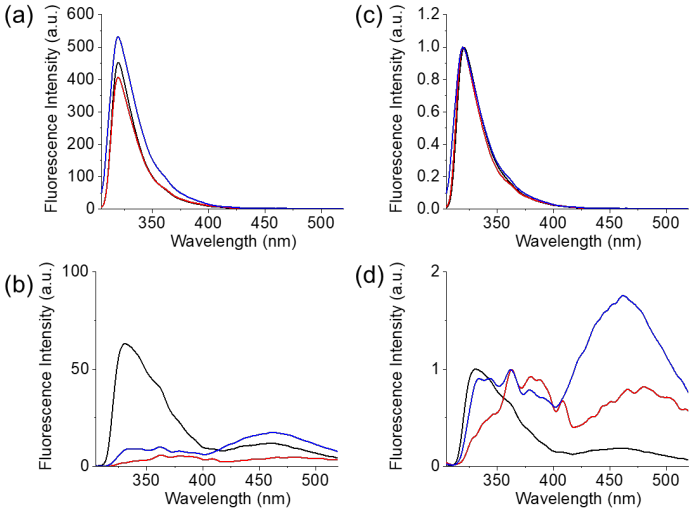


Figure S23. Emission spectra of the multicomponent gels of **1** with (**2**-**4**) at (a) pH 3.1-3.3 and (b) pH 10.2-10.4 pH. The black, red and blue data represent hydrogels of (**1**+**2**), (**1**+**3**), and (**1**+**4**), respectively. (c) and (d) represent the normalized graph of (a) and (b), respectively. In all cases, concentrations of **1**-**4** are 2 mg/mL, concentration of NaOH is 0.01 M, solvent is DMSO/H_2O (20/80, v/v).

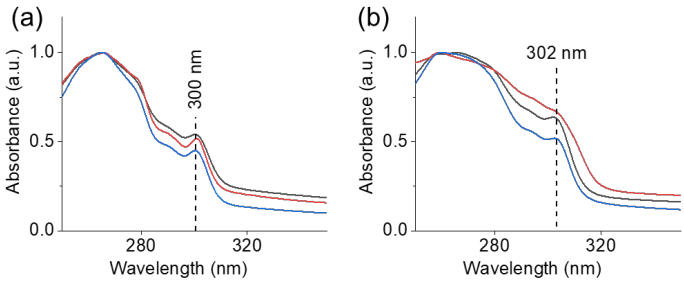


Figure S24. UV-vis spectra of the multicomponent gels of **1** with (**2**-**4**) at (a) pH 3.1-3.3 and (b) pH 10.2-10.4. The black, red and blue data represent hydrogels of (**1**+**2**), (**1**+**3**), and (**1**+**4**), respectively. In all cases, concentrations of **1**-**4** are 2 mg/mL, concentration of NaOH is 0.01 M, solvent is DMSO/H_2O (20/80, v/v).

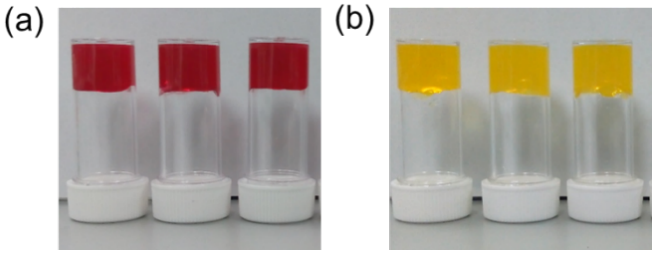


Figure S25. Photographs of the multicomponent gels obtained from the urea-urease reaction (a) after 30 sec, (b) after 16h. In each photograph, from left to right, the vials represent hydrogels of (**1**+**2**), (**1**+**3**), and (**1**+**4**). In all cases, initial concentrations of **1**-**4** are 2 mg/mL, [urease]=0.5 mg/mL, [urea]=0.1 M, solvent is DMSO/H_2O (20/80, v/v). Methyl red (0.05 mg/mL) is used to stain the gels.

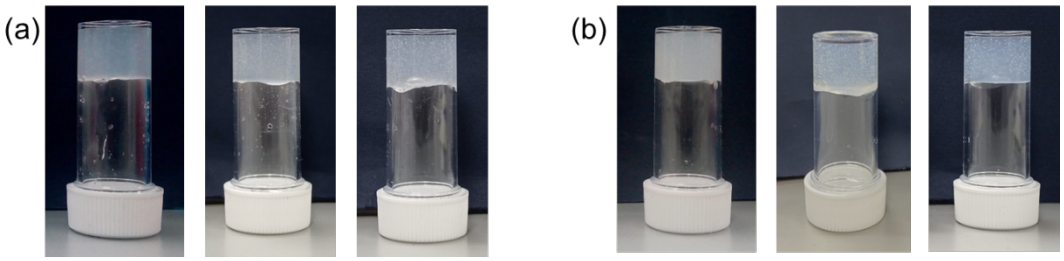


Figure S26. (a) Photographs of the multicomponent gels of **1** with **2**-**4** (from left to right, respectively) obtained in presence of urease. (b) Photographs of the multicomponent gels of **1** with **2**-**4** (from left to right, respectively) obtained from the urease-urea reaction. For (a) and (b), initial concentrations of **1**-**4** are 2 mg/mL, [urease]=0.5 mg/mL. For (b) initial [urea]=0.1 M. In all cases, solvent is DMSO/H_2O (20/80, v/v).

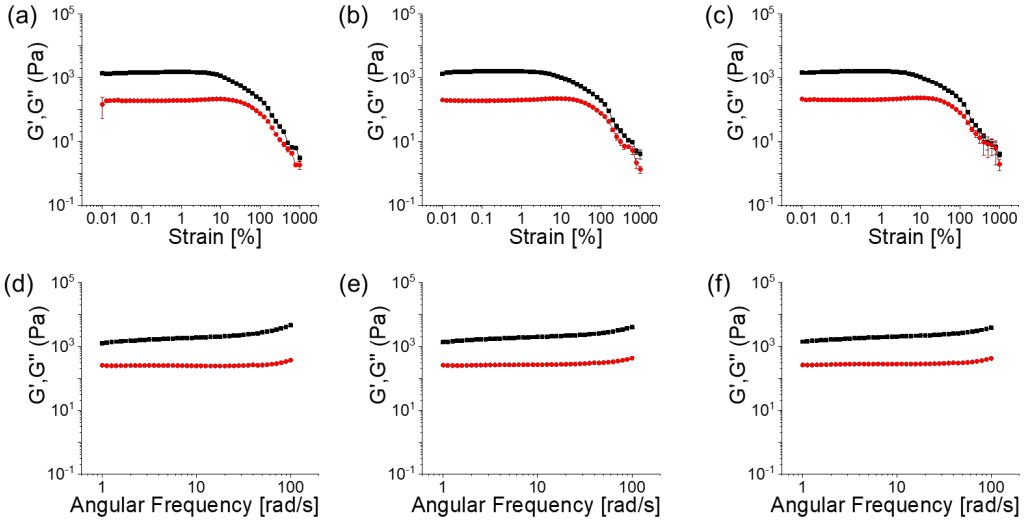


Figure S27. Strain (a-c) and frequency (d-f) sweeps for the multicomponent gels of **1** with **2**-**4** (a-c and d-f, respectively) prepared in presence of urease. In all cases, concentrations of **1**-**4** are 2 mg/mL, [urease]=0.5 mg/mL, solvent is 20:80 DMSO/water (v/v). The black symbols represent G′, the red symbols G″.

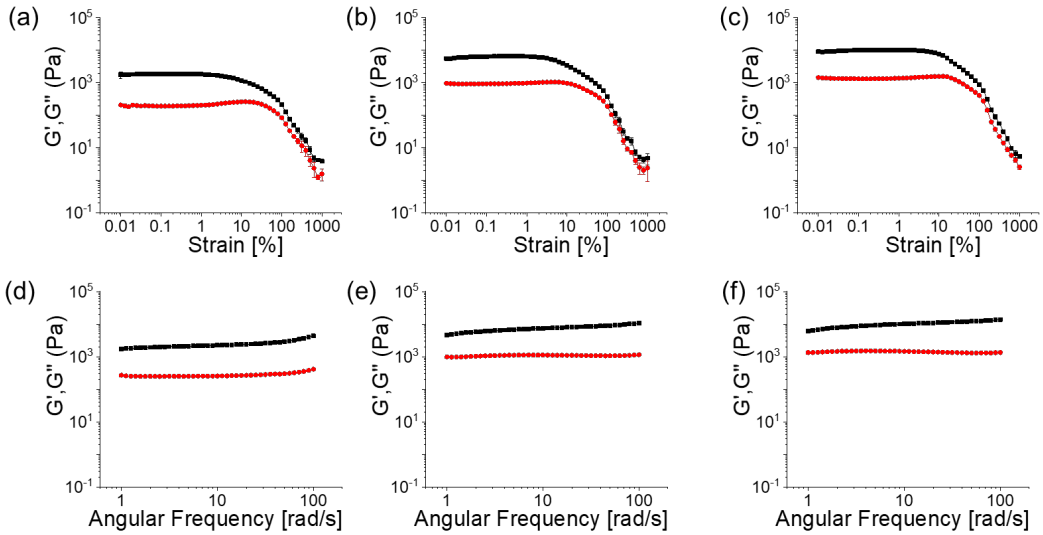


Figure S28. Strain (a-c) and frequency (d-f) sweeps for the multicomponent gels of **1** with **2**-**4** (a-c and d-f, respectively) obtained in presence of urease-urea reaction. In all cases, concentrations of **1**-**4** are 2 mg/mL, [urease]=0.5 mg/mL, initial [urea]=0.1 M, solvent is 20:80 DMSO/water (v/v). The black symbols represent G′, the red symbols G″.

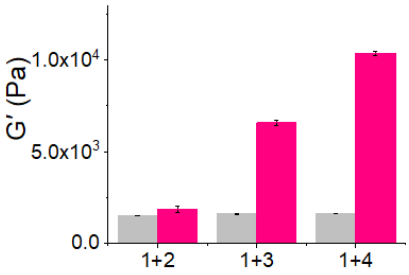


Figure S29. Bar graph representing the stiffness (G', calculated at 0.5% strain from strain sweeps) of the multicomponent hydrogels obtained at pH 3.3-3.4 (grey) and pH 9.3 (pink) involving the enzymatic reaction. In all cases, concentrations of **1**-**4** are 2 mg/mL, [urease]=0.5 mg/mL. For pink data initial [urea]=0.1 M. In all cases, solvent is 20:80 DMSO/water (v/v).

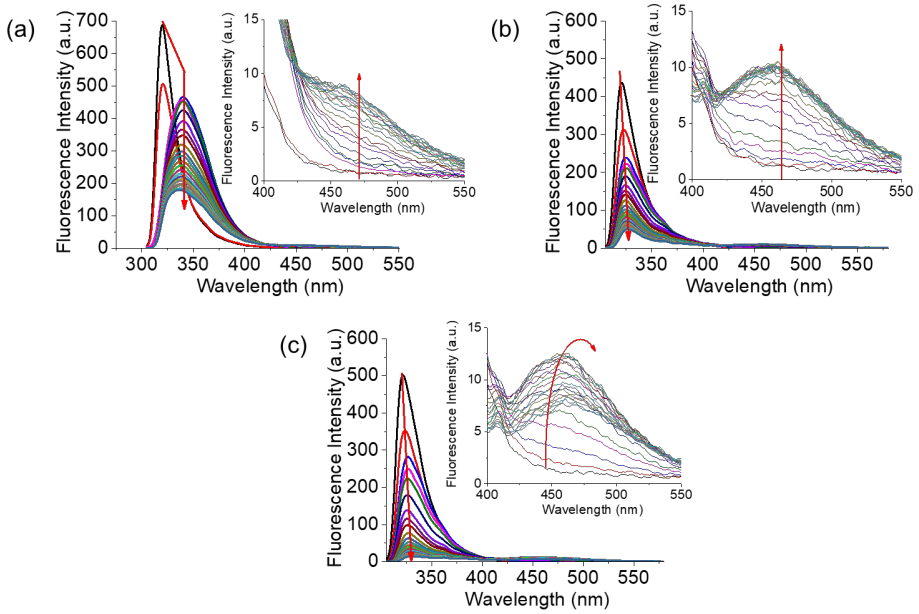


Figure S30. Time variable changes in the emission spectra for the multicomponent systems (a) (**1**+**2**), (b) (**1**+**3**) and (c) (**1**+**4**) in presence of urease-urea reaction. In all cases, initial concentrations of **1**-**4** are 2 mg/mL, [urease]=0.5 mg/mL, initial [urea]=0.1 M, solvent is 20:80 DMSO/water (v/v).

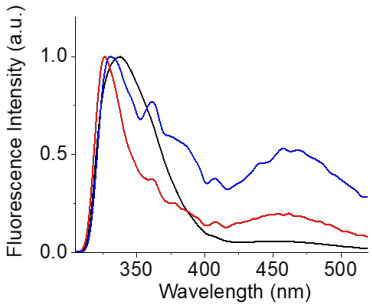


Figure S31. Normalized emission spectra for the multicomponent systems (black) (**1**+**2**), (red) (**1**+**3**) and (blue) (**1**+**4**) obtained from the enzymatic reaction. In all cases, initial concentrations of **1**-**4** are 2 mg/mL, [urease]=0.5 mg/mL, initial [urea]=0.1 M, solvent is 20:80 DMSO/water (v/v).

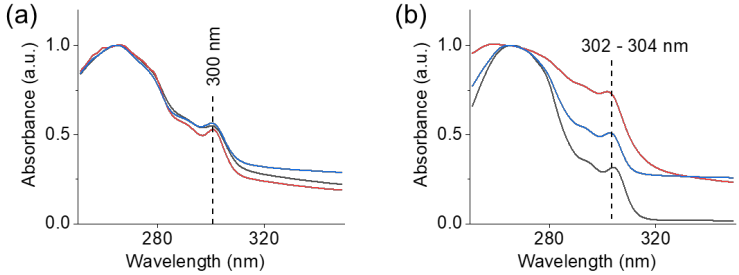


Figure S32. Normalized absorption spectra for the multicomponent systems (black) (**1**+**2**), (red) (**1**+**3**) and (blue) (**1**+**4**) obtained from the enzymatic reaction. For (a, b), initial concentrations of **1**-**4** are 2 mg/mL, [urease]=0.5 mg/mL. For (b) initial [urea]=0.1 M. In all cases, solvent is 20:80 DMSO/water (v/v).

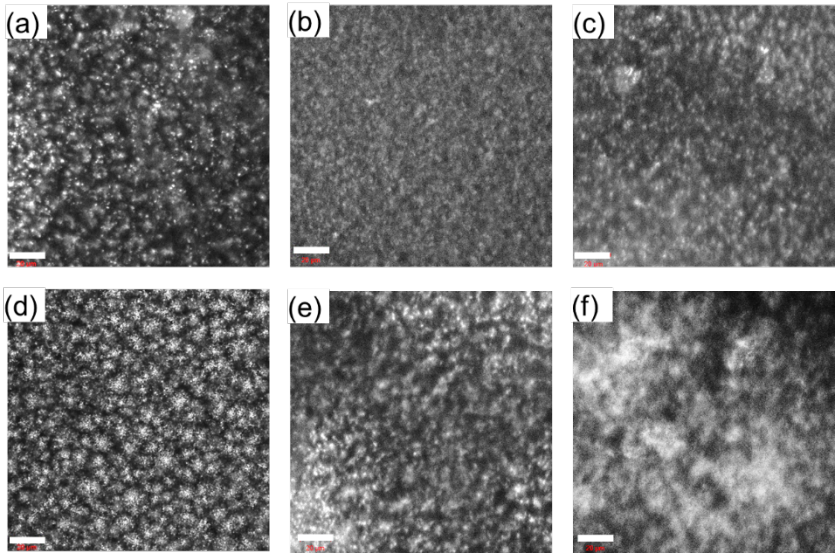


Figure S33. Confocal fluorescence microscopy images (scale bars represent 20 μm) of the multicomponent gel of (**1**+**2**) for (a, d), (**1**+**3**) for (b, e) and (**1**+**4**) for (c, f) at pH 3.3-3.4 (a-c) and pH 9.3 (d-f). For (a-f), initial concentrations of **1**-**4** are 2 mg/mL, [urease]=0.5 mg/mL. For (d-f), initial [urea]=0.1 M. In all cases, solvent is 20:80 DMSO/water (v/v).

References

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2. S. Panja, A. M. Fuentes-Caparrós, E. R. Cross, L. Cavalcanti and D. J. Adams, *Chem. Mater.,* 2020, **32**, 5264-5271.
3. S. Panja, B. Dietrich, O. Shebanova, A. J. Smith and D. J. Adams, *Angew. Chem. Int. Ed.,*, 2021, **60**, 9973-9977.