Supplementary information

Exploiting CH-π interactions in supramolecular hydrogels of aromatic carbohydrate amphiphiles

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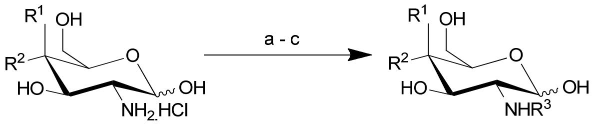
SI 1: Synthesis of aromatic carbohydrate amphiphiles



1: R^1 = OH, R^2 = H, $GalNH_2.HCl$
2: R^1 = H, R^2 = OH, $GlcNH_2.HCl$

Starting material	**Product**	**Reaction conditions**	**Yield (%)**	**R^1**	**R^2**	**R^3**
$GalNH_2.HCl$ **1**	GalNHFmoc **1**	a	82	OH	H	Fmoc
	GalNHFln **3**	b	63	OH	H	Fln
	GalNHNap **5**	c	32	OH	H	Nap
$GlcNH_2.HCl$ **2**	GlcNHFmoc **2**	a	99	H	OH	Fmoc
	GlcNHFln **4**	b	76	H	OH	Fln
	GlcNHNap **6**	c	60	H	OH	Nap

Figure SI 1: Reagents and conditions for the synthesis of compounds **1–6**. a) Fmoc-Cl, $NaHCO_3$, dioxane, water, room temperature, b) 9-Fluorenylacetic acid, DIPEA, TBTU, DMF, room temperature, c) 2-Naphthylacetic acid, DIPEA, TBTU, DMF, room temperature.

SI 1.1 Synthesis of GalNHFmoc (Compound 1)

$NaHCO_3$ (234 mg, 2.78 mmoles) was added to a stirred solution of $GalNH_2.HCl$ (300 mg, 1.39 mmoles) in water (6 mL) under an inert argon atmosphere. The solution was then cooled to 5 °C and Fmoc-Cl (432 mg, 1.67 mmoles) was dissolved in dioxane (3 mL) and added dropwise. Further dioxane (5 mL) and water (3 mL) was added. The resulting mixture was stirred in an ice bath for one hour and allowed to slowly warm to room temperature. After 24 hours, tlc (ethyl acetate : methanol : water, 28:2:1) showed the absence of the starting material (R_f 0.05) and the presence of a major product (R_f 0.50). The reaction mixture was concentrated in *vacuo* and the resulting residue was then diluted with dichloromethane (DCM, 40 mL). A precipitate formed, was washed with DCM (2 x 40 mL) and water (20 mL), collected by vacuum filtration and dried to give GalNHFmoc **1** (456 mg, 82%) as an off-white precipitate.

Tlc analysis (ethyl acetate : methanol : water, 7:2:1) of the isolated product indicated the presence of two major spots (R_f 0.65 and 0.58) – presumably different anomers.

MS (ES+): *m/z* 424 $[M + Na]^+$.

NMR: It is evident from the ^{13}C NMR that there are at least two anomers present: a major anomer 'A' and other anomer(s) 'B' and possibly 'C'. On the basis of the ^1H NMR, the major anomer A exists in a 5:5 ratio where the other anomers (B and C) correspond to the other components of the ^1H NMR. The ^{13}C NMR assignment reflects both A and B anomers whereas the ^1H NMR only shows the signals for anomer A for simplicity.

δ_C [$(CD_3)_2SO$, 125 MHz] 47.2 (CH, Fmoc anomer A), 50.6 (CH, Fmoc anomer B), 52.8 (*C*H, anomer A), 56.0 (*C*H, anomer B), 61.1 (CH_2, anomer B), 61.2 (CH_2, anomer A), 64.3 (C*H*, anomer B), 66.1 (CH_2, Fmoc), 67.5 (*C*H, anomer A), 68.8, 71.0 (2 x *C*H, anomer A), 75.8, 81.7 (2 x *C*H, anomer B), 91.6 (*C*H, anomer A), 96.4 (*C*H, anomer B), 120.4, 120.6, 125.8, 125.9, 126.0, 127.3.127.6, 127.7, 128.2 (8 x *C*H, 8 x Ar-CH), 141.1, 144.4, 144.5, 145.8 (4 x *C*, 4 x Ar-C), 156.8 (*C*, C=O anomer A), 156.9(*C*, C=O anomer B).

δ_H of anomer A [$(CD_3)_2SO$, 500 MHz] 3.40-3.85 (m, 6H, 4 x C*H* and 1 x CH_2), 4.18-4.30 (m, 3H, C*H* Fmoc and CH_2 Fmoc), 4.93-5.00 (m, 1H, C*H*), 6.30-6.40 (m, 1H), 6.94 (d, 1H, *J* 8.3 Hz), 7.27-7.47 (m, 4H, 4 x Ar-C*H*), 7.65-7.79 (m, 2H, 2 x Ar-C*H*), 7.84-7.93 (m, 2H, 2 x Ar-C*H*).

SI 1.2 Synthesis of GlcNHFmoc (Compound 2)

$NaHCO_3$ (506 mg, 6.02 mmoles) was added to a stirred solution of $GlcNH_2.HCl$ (500 mg, 2.32 mmoles) in water (14 mL) under an inert argon atmosphere. The solution was then cooled to 5 °C and Fmoc-Cl (1.08 g, 4.17 mmoles) was dissolved in dioxane (20 mL) and added dropwise. Further dioxane (10 mL) and water (6 mL) was added. The resulting mixture was stirred in an ice bath for one hour and allowed to slowly warm to room temperature. After 24 hours, tlc (ethyl acetate : methanol : water, 7:2:1) showed the presence of two major products (R_f 0.64 and 0.57) and the absence of the starting material (R_f 0.08). The reaction mixture was concentrated in *vacuo* and the resulting residue was then diluted with DCM (40 mL). A white precipitate was formed, washed with DCM (2 x 20 mL) and water (10 mL), collected by vacuum filtration and dried to give GlcNHFmoc **2** (930 mg, 99%) as an off-white precipitate.

MS (ES+): *m/z* 424 $[M + Na]^+$

NMR: It is evident from the ^{13}C NMR that there are at least two anomers present: a major anomer 'A' and other anomer(s) 'B'. On the basis of the ^1H NMR, the major anomer A exists in a 7:3 ratio where the other anomer(s) correspond to the minor component of the ^1H NMR. The ^{13}C NMR assignment reflects both A and B anomers whereas the ^1H NMR only shows the signals for anomer A for simplicity.

δ_C $[(CD_3)_2SO$, 125 MHz] 47.1 (*C*H, Fmoc), 56.9 (*C*H, anomer B), 59.2 (*C*H, anomer A), 61.7, 61.8 (*C*H_2, anomers A and B), 65.9 (*C*H_2, Fmoc anomer A), 66.1 (*C*H_2, Fmoc anomer B), 70.8 (*C*H, anomer B), 71.5 (*C*H, anomer A), 71.6, 72.6 (2 x *C*H,2 x anomer B), 74.8, 77.3 (2 x *C*H, anomer A), 91.3 (*C*H, anomer B), 96.0 (*C*H, anomer A), 120.6, 125.9, 126.0, 127.6, 128.1 (8 x *C*H, 8 x Ar-CH), 141.1, 144.5 (4 x *C*, 4 x Ar-C), 156.7 (*C*, C=O).

δ_H of anomer A $[(CD_3)_2SO$, 500 MHz] 3.00-3.75 (m, 6H, 4 x C*H* and 1 x C*H*$_2$), 4.18-4.29 (m, 3H, C*H* Fmoc and C*H*$_2$ Fmoc), 4.44 (d, 1H, C*H*, *J* 8.3 Hz), 7.13 (d, 1H, *J* 9.3 Hz), 7.34 (t, 2H, 2 x Ar-C*H*, *J* 9.3 Hz), 7.42 (a-dt, 2H, 2 x Ar-C*H*, *J* 0.8, *J* 7.5 Hz), 7.71-7.77 (m, 2H, 2 x Ar-C*H*), 7.89 (d, 2H, 2 x Ar-C*H*, *J* 7.5 Hz).

SI 1.3 Synthesis of GalNHFln (Compound 3)

DIPEA (715 µL, 4.11 mmoles) was added to a stirred solution of GalNH$_2$.HCl (308 mg, 1.43 mmoles) in DMF (10 mL) under an inert argon atmosphere. After 30 mins, 9-fluorenylacetic acid (343 mg, 1.53 mmoles) and TBTU (715 mg, 2.23 mmoles) were added. After 24 hours, tlc (ethyl acetate : methanol : water, 7:2:1) showed the absence of starting material (R_f 0.08) and the presence of two major products (R_f 0.58 and 0.52). The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene. The resulting residue was diluted with DCM (20 mL) to give a precipitate which was washed with water (3 x 15 mL) and DCM (1 x 20 mL), collected by vacuum filtration and dried to give GalNHFln **3** (346 mg, 63%) as a dark beige precipitate.

MS (ES+): *m/z* 408 $[M + Na]^+$

NMR: It is evident from the ^{13}C NMR that there are at least two anomers present: a major anomer 'A' and other anomer(s) 'B'. On the basis of the ^1H NMR, the major anomer A exists in a 6:4 ratio where the other anomer(s) B correspond to the minor component of the ^1H NMR. The ^{13}C NMR assignment reflects both A and B anomers whereas the ^1H NMR only shows the signals for anomer A for simplicity.

δ_C $[(CD_3)_2SO$, 125 MHz] 39.9 (*C*H_2, fluorenyl), 44.2 (*C*H, fluorenyl), 51.0 (*C*H, anomer A), 54.6 (*C*H, anomer B), 61.1 (*C*H_2, anomer B) 61.2 (*C*H_2, anomer A), 68.1 (*C*H, anomer A), 68.2 (*C*H, anomer B), 69.0, 71.0 (2 x *C*H, anomer A), 72.3, 75.6 (2 x *C*H, anomer B), 91.6 (*C*H, anomer A), 96.5 (*C*H, anomer B), 120.3, 120.4, 125.3, 125.5, 125.5, 125.6, 127.6, 127.7 (8 x *C*H, 8 x Ar-CH), 140.5, 140.6, 140.6, 147.3, 147.3, 147.4, 147.4 (4 x *C*, 4 x Ar-C), 171.8 (*C*, C=O anomer A), 172.0(*C*, C=O anomer B).

δ_H of anomer A [$(CD_3)_2SO$, 500 MHz] 2.44-2.66 (m, 2H, C*H*$_2$ fluorenyl), 3.43-3.90(m, 5H, 3 x C*H* and 1 x C*H*$_2$), 4.17-4.26 (m, 1H, C*H*), 4.33-4.41 (m, 1H, C*H* fluorenyl), 4.50-4.57 (m, 1H), 5.10 (t, 1H, C*H*, *J* 3.9 Hz), 6.42 (dd, 1H, *J* 3.3, *J* 4.4 Hz), 7.20-8.19 (m, 8H, 8 x Ar-C*H*).

SI 1.4 Synthesis of GlcNHFln (Compound 4)

DIPEA (698 µL, 4.01 mmoles) was added to a stirred solution of GlcNH$_2$.HCl (300 mg, 1.39 mmoles) in DMF (10 mL) under an inert argon atmosphere. After 1 hour, 9-fluorenylacetic acid (343 mg, 1.53 mmoles) and TBTU (715 mg, 2.23 mmoles) were added. After 24 hours, tlc (ethyl acetate : methanol : water, 7:2:1) showed the presence of a major product (R_f 0.61) and absence of the starting material (R_f 0.08). The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene. The resulting residue was diluted with DCM (20 mL) to give a precipitate which was washed with DCM (2 x 20 mL) and water (3 x 10 mL), collected by vacuum filtration and dried to give GlcNHFln **4** (405 mg, 76%) as a dark beige precipitate.

MS (ES+): *m/z* 408 $[M + Na]^+$

NMR: It is evident from the ^{13}C NMR that there are at least two anomers present: a major anomer 'A' and other anomer(s) 'B'. On the basis of the ^{1}H NMR, the major anomer A exists in a 7:3 ratio where the other anomer(s) B correspond to the minor component of the ^{1}H NMR. The ^{13}C NMR assignment reflects both A and B anomers whereas the ^{1}H NMR only shows the signals for anomer A for simplicity.

δ_C [$(CD_3)_2SO$, 125 MHz] 39.9 (*C*H$_2$, fluorenyl), 44.2 (*C*H, fluorenyl), 55.0 (*C*H, anomer A), 57.7 (*C*H, anomer B), 61.7 (*C*H$_2$, anomers A and B), 71.1 (*C*H, anomer A), 71.6 (*C*H, anomer B), 71.8, 72.6 (2 x *C*H, anomer A), 75.0, 77.4 (2 x *C*H, anomer B), 91.3 (*C*H, anomer A), 96.1 (*C*H, anomer B), 120.3, 120.4, 125.3, 125.4, 127.6, 127.7 (8 x *C*H, 8 x Ar-CH), 140.5, 140.6, 147.3, 147.4 (4 x *C*, 4 x Ar-C), 171.5 (*C*, C=O).

δ_H of anomer A [$(CD_3)_2SO$, 500 MHz] 2.41-2.70 (m, 2H, C*H*$_2$ fluorenyl), 3.08-3.25 (m, 1H, C*H*), 3.44-3.86 (m, 5H, 3 x C*H* and 1 x C*H*$_2$), 4.37 (t, 1H, C*H* fluorenyl, *J* 7.5 Hz), 4.40-4.48 (m, 1H, C*H*), 4.66 (d, 1H, *J* 5.9 Hz), 4.92 (d, 1H, *J* 5.5 Hz), 5.10 (t, 1H, C*H*, *J* 3.9 Hz), 6.51 (m, 1H), 7.24-7.95 (m, 8H, 8 x Ar-C*H*).

SI 1.5 Synthesis of GalNHNap (Compound 5)

DIPEA (745 µL, 4.28 mmoles) was added to a stirred solution of GalNH$_2$.HCl (302 mg, 1.40 mmoles) in DMF (10 mL) under an inert argon atmosphere. After 1 hour, 2-napthylacetic acid (285 mg, 1.53 mmoles) and TBTU (715 mg, 2.23 mmoles) were added. After 25 hours, tlc (ethyl acetate : methanol : water, 7:2:1) showed the presence of two major products (R_f 0.55 and 0.49) and the absence of the starting material (R_f 0.08). The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene. The resulting residue was diluted with DCM (20 mL) to give a precipitate which was washed with water (3 x 20 mL) and DCM (20 mL), collected by vacuum filtration and dried to give GalNHNap **5** (153 mg, 32%) as a beige coloured precipitate.

MS (ES+): *m/z* 370 $[M + Na]^+$

NMR: It is evident from the ^{13}C NMR that there are at least three anomers present: a major anomer 'A' and other anomers 'B' and 'C' where the population of anomers is approximately A>B>C based on the intensity of the ^{13}C signals. It is noted that B and C may represent more than one anomer. On the basis of the ^{1}H NMR, the major anomer A exists in a 4:6 ratio where the other anomers (B and C), although are the major component of the spectrum, results in numerous ^{1}H signals for multiple anomers. The ^{13}C NMR assignment reflects both A and B anomers (and where reliable, signals for anomer C) whereas the ^{1}H NMR only shows the CH and CH_2 signals for anomer A due to the complexity of the ^{1}H NMR spectrum.

δ_C [$(CD_3)_2SO$, 125 MHz] 42.9 (*C*H$_2$, napthyl anomer A), 43.2 (*C*H$_2$, napthyl anomer B), 50.7 (*C*H, anomer C), 51.0 (*C*H, anomer A), 54.7 (*C*H, anomer B), 61.0 (*C*H$_2$, anomer B), 61.1 (*C*H$_2$, anomers A), 67.9 (*C*H, anomer A), 68.1 (*C*H, anomer B), 68.9, 71.0 (2 x *C*H, anomer A), 72.2 (*C*H, anomer B), 75.2 (*C*H, anomer C), 75.6 (*C*H, anomer B), 91.5 (*C*H, anomer A), 91.6 (*C*H, anomer C), 96.4 (*C*H, anomer B), 125.9, 126.3, 126.5, 126.7, 127.8, 127.9, 128.0, 128.0, 128.3, 128.4 (7 x *C*H, 7 x Ar-CH), 132.2, 133.5, 134.8, 135.0 (3 x *C*, 3 x Ar-C), 170.9 (*C*, C=O anomer A), 171.3 (*C*, C=O anomer B), 171.8 (*C*, C=O anomer C).

δ_H of anomer A [$(CD_3)_2SO$, 500 MHz] 3.37-4.25 (m, 8H, C*H*$_2$ napthyl, C*H*$_2$ and 4 x C*H*), 4.93-5.02 (m, 1H, C*H*), 7.30-8.35 (m, 7H, 7 x Ar-C*H*).

SI 1.6 Synthesis of GlcNHNap (Compound 6)

DIPEA (698 µL, 4.01 mmoles) was added to a stirred solution of $GlcNH_2$.HCl (300 mg, 1.39 mmoles) in DMF (10 mL) under an inert argon atmosphere. After 1 hour, 2-napthylacetic acid (285 mg, 1.53 mmoles) and TBTU (715 mg, 2.23 mmoles) were added. After 24 hours, t.l.c. (ethyl acetate : methanol : water, 7:2:1) showed the presence of a major product (R_f 0.57) and absence of the starting material (R_f 0.08). The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene. The resulting residue was diluted with DCM (20 mL) to give a precipitate which was washed with DCM (2 x 20 mL) and water (3 x15 mL), collected by vacuum filtration and dried to give GlcNHNap **6** (292 mg, 60%) as a beige coloured precipitate.

MS (ES+): *m/z* 370 $[M + Na]^+$

NMR: It is evident from the ^{13}C NMR that there are at least two anomers present: a major anomer 'A' and other anomer(s) 'B'. On the basis of the ^{1}H NMR, the major anomer A exists in a 6:4 ratio where the other anomer(s) B correspond to the minor component of the ^{1}H NMR. The ^{13}C NMR assignment reflects both A and B anomers whereas the ^{1}H NMR only shows the signals for anomer A for simplicity.

δ_C [$(CD_3)_2SO$, 125 MHz] 42.8 (*C*H_2, napthyl anomer A), 43.2 (*C*H_2, napthyl anomer B), 55.0 (*C*H, anomer A), 57.8 (*C*H, anomer B), 61.6 (*C*H_2, anomer A), 61.7 (*C*H_2, anomer B), 71.1 (*C*H, anomer A), 71.5 (*C*H, anomer B), 71.7, 72.6 (2 x *C*H, anomer A), 74.9, 77.4(2 x *C*H, anomer B), 91.1 (*C*H, anomer A), 96.0 (*C*H, anomer B), 125.9, 126.5, 127.8, 127.9, 127.9, 128.4 (7 x *C*H, 7 x Ar-CH), 132.2, 133.5, 134.8, 134.9 (3 x *C*, 3 x Ar-C), 170.7 (*C*, C=O anomer A), 170.8 (*C*, C=O anomer B).

δ_H of anomer A [$(CD_3)_2SO$, 500 MHz] 3.06-3.16 (m, 1H, C*H*), 3.36-3.72 (m, 7H, C*H*$_2$ napthyl, CH_2 and 3 x C*H*), 4.41 (dd, 1H, *J* 5.3, *J* 6.4 Hz), 4.68 (d, 1H, *J* 5.7 Hz), 4.85-4.92 (m, 1H), 4.92-4.97 (m, 1H, C*H*), 6.48 (dd, 1H, *J* 3.3, *J* 4.5 Hz), 7.42-8.07 (m, 7H, 7 x Ar-C*H*).

SI 2: Atomic force microscopy imaging of hydrogels formed from compounds 1 and 2

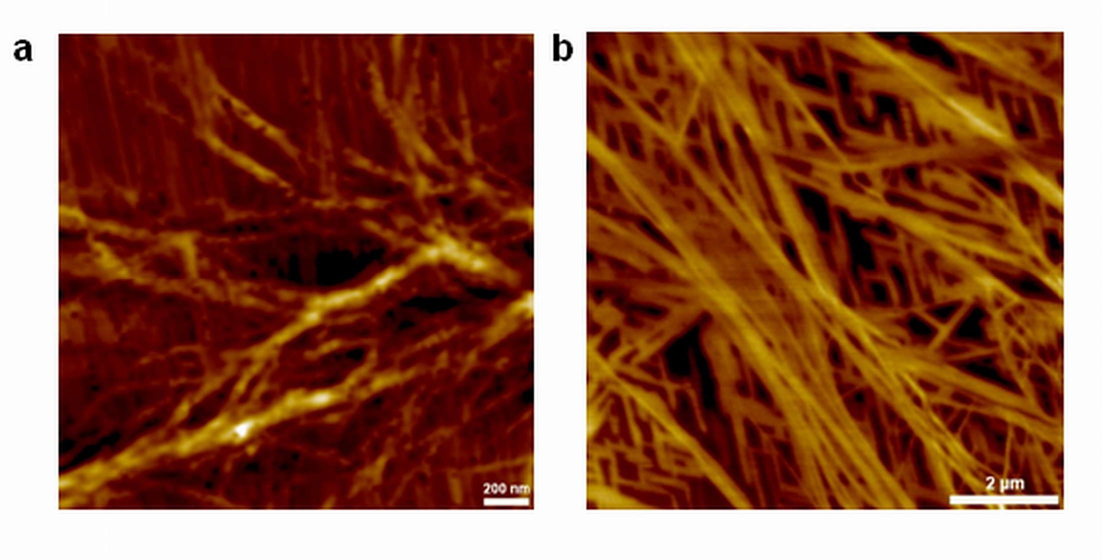


Figure SI 2: AFM images of dried samples of hydrogels formed from compounds **1** (a) and **2** (b).

SI 3: Rheology of hydrogels formed from 1 and 2 in phosphate buffer

Hydrogels of **1** and **2** were formed by suspending the compound (3 mg) in PBS (1 mL, pH 7.4, 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride), raising the temperature to 75 °C and then allowing the resulting solution to cool to room temperature. Hydrogels of **1** had a G' value of 12 kPa, whilst, hydrogels of **2** in a larger value of 29 kPa (Figure SI 3).

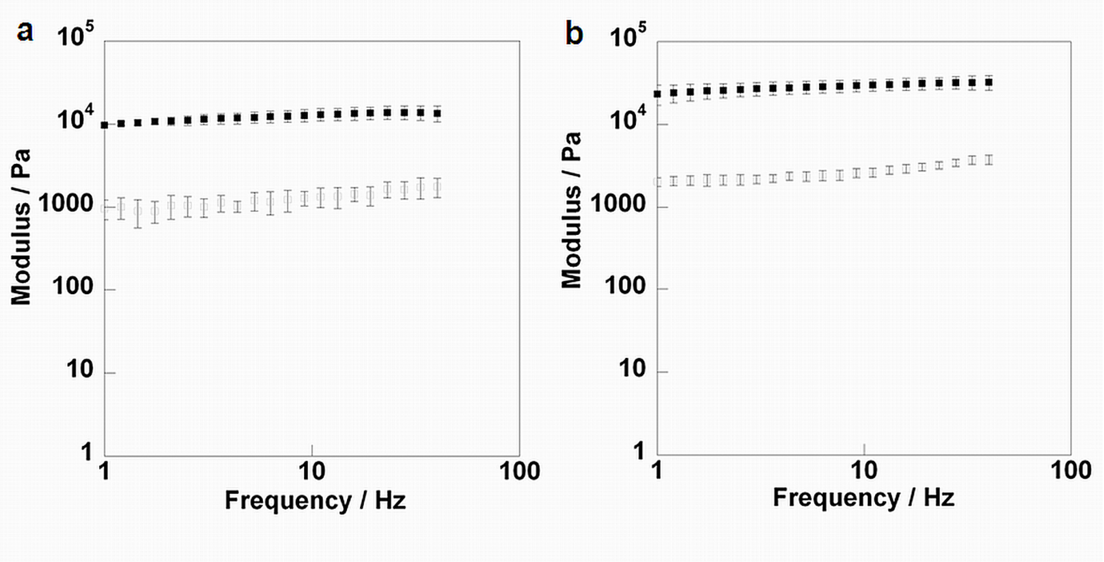


Figure SI 3: Rheology data for hydrogels formed from compounds **1** and **2** in phosphate buffer. G' (■) and G'' (□).

SI 4: Fluorescence emission spectroscopy data for the determination of the mode of self-assembly

SI 4.1 Fluorescence emission spectroscopy of compounds 1 and 2 in solvents with different polarities

The fluorescence emission spectra of compounds **1** and **2** (0.05 mM, *i.e.* well below the critical gelation concentration) in ethyl acetate, ethanol, methanol and water was recorded at 20 °C and 50 °C (Figure SI 4.1 a and b). Varying the temperature (from 20 °C to 50 °C) resulted in a very small change in maximum wavelength of emission (up to 1 nm), and slightly changed the fluorescence intensity (up to 64 AU upon cooling) – data not shown. For both compounds, a decrease in solvent polarity resulted in a decrease in fluorescence intensity (Figure SI 4.1 c and d).

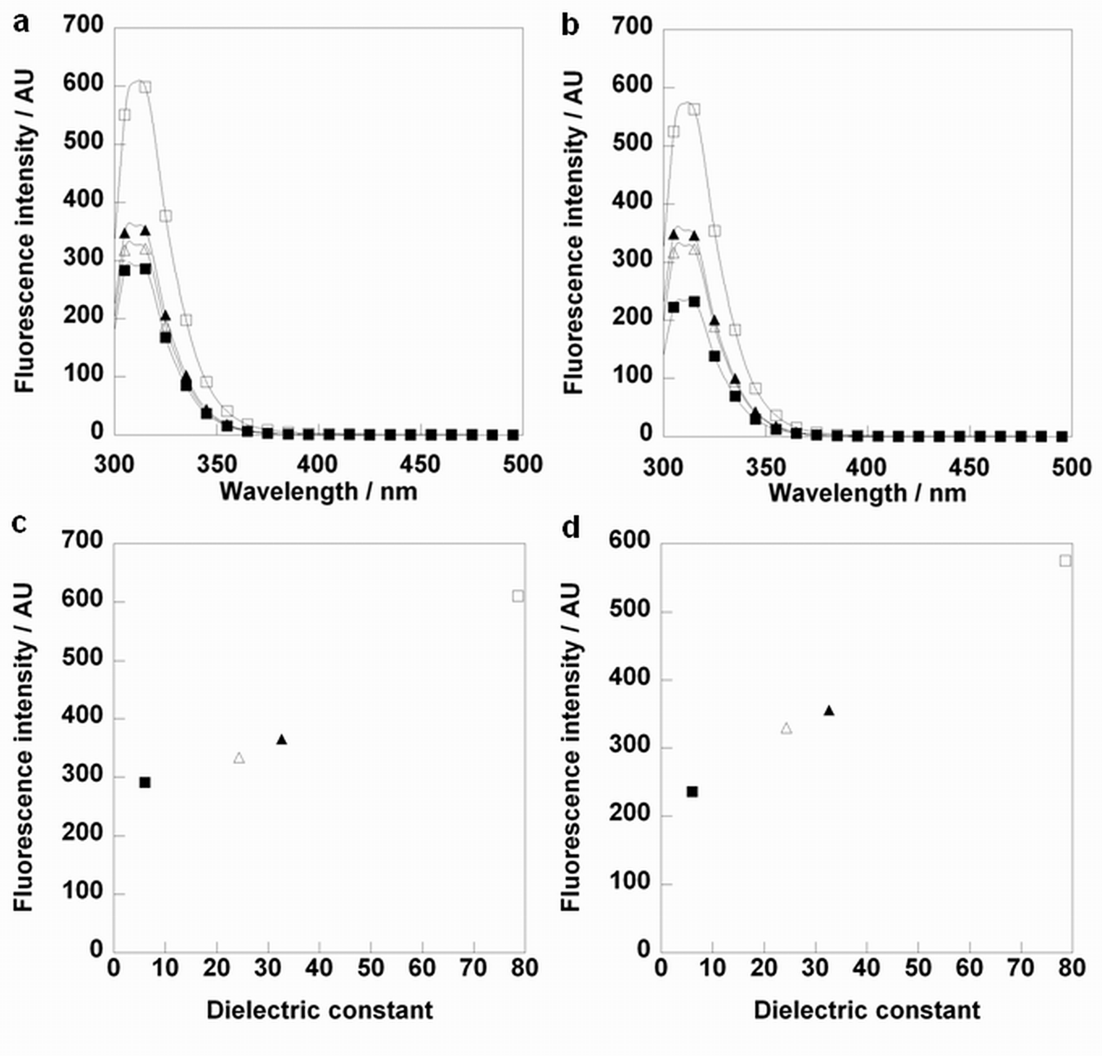


Figure SI 4.1: Fluorescence emission spectra for **1** (a) and **2** (b) in water (□), methanol (▲), ethanol (△) and ethyl acetate (■) at 20 °C. Plots of the variation in fluorescence intensity with changing dielectric constant (*i.e.* solvent polarity) for compounds **1** (c) and **2** (d) at 20 °C.

SI 4.2 Fluorescence emission spectroscopy of Fmoc-YL-OH

Fmoc-YL-OH is an Fmoc-diamino acid which is known to self-assemble to form supramolecular hydrogels.[1] Samples were prepared in phosphate buffer (pH 8, 100 mM). The fluorescence emission spectra were recorded at 20 °C and 75 °C for concentrations of 1 mM (*i.e.* below the critical gelation concentration, solution at both temperatures) and 20 mM (*i.e.* above the critical gelation concentration, solution at high temperature, gel at low temperature). For the 1 mM solution (Figure SI 4.2 a) there was only a 1 nm blue shift in maximum emission wavelength upon cooling, and an increase in intensity. However, for the 20 mM sample (Figure SI 4.2 b) there was a 6 nm red shift in maximum wavelength of emission, which is attributed to excimer formation (*i.e.* π-π stacking interactions) accompanied by a decrease in intensity (*i.e.* quenching) upon gelation. There was also a broad peak at ~ 450 nm indicating the long ranging interactions of stacked fluorenyl groups.

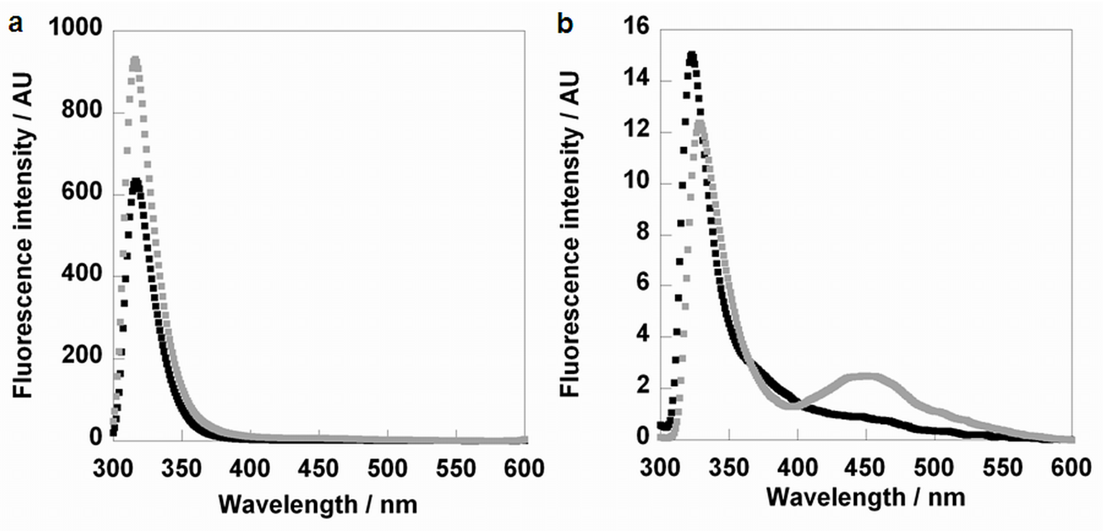


Figure SI 4.2: Fluorescence emission spectra for Fmoc-YL-OH at 1 mM (a) and 20 mM (b) in phosphate buffer, at 75 °C (■) and 20 °C (■).

SI 4.3 Fluorescence emission spectroscopy of compounds 3-6

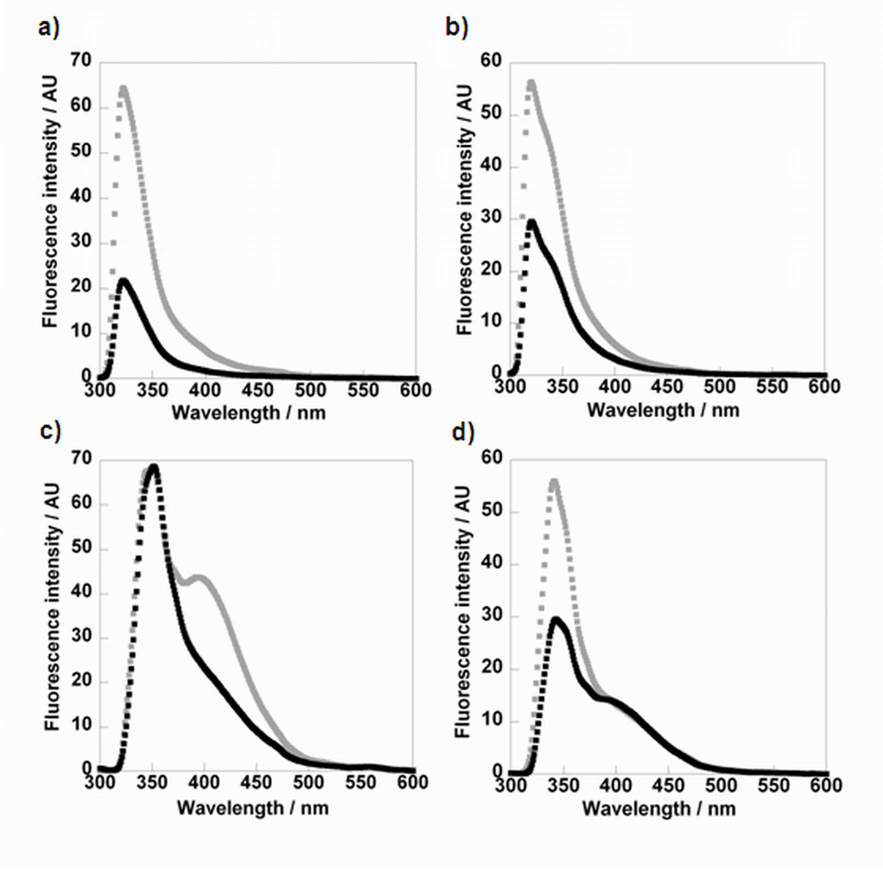


Figure SI 4.3: Fluorescence emission spectra for compounds **3** (a), **4** (b), **5** (c) and **6** (d) at 75 °C (■) and 20 °C (■).

Figure SI 4.3 shows the fluorescence emission spectra for **3**–**6**. For compounds **3** and **4** the intensity increased upon cooling the sample and formed a precipitate. For compounds **3** and **4** at 20 °C and **5** and **6** at both temperatures, the spectra should be viewed with caution as the presence of a precipitate will cause scattering of the emission. In the crystal form (*i.e.* precipitate) there is evidence of aromatic interactions (shoulder peaks *etc.*), these interactions are currently under further investigation.

1. A. R. Hirst, S. Roy, M. Arora, A. K. Das, N. Hodson, P. Murray, S. Marshall, N. Javid, J. Sefcik, J. Boekhoven, J. H. van Esch, S. Santabarbara, N. T. Hunt and R. V. Ulijn, *Nat. Chem.*, 2010, **2**, 1089-1094.