Electronic Supplementary Information

Light-triggered syneresis of a water insoluble peptide-hydrogel effectively removes small molecule waste contaminants

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Experimental

General: H-Cys(Trt)NH_2, Fmoc-Lys(Boc)-OH, HBTU and HOBT were procured from GL Biochem, China. All the other chemicals and solvents used were procured from Sigmaaldrich, TCI or Merck, India. To prepare samples, Milli-Q water with a conductivity of less than 2 $mScm^{-1}$ was used. Chromatographic purifications were performed on a Luna 5 μm (C18, 250 × 4.6 mm) column (Phenomenex) using a Dionex Ultimate 3000 HPLC. UV-Visible spectra were recorded on a PerkinElmer Lambda 750 spectrometer. Standard 10 mm-path quartz cuvettes were used for all spectroscopic measurements. ^{1}H NMR, ^{13}C NMR were recorded using a Bruker Ascend 600 MHz (Bruker, Coventry, UK) spectrometer and referenced to deuterated solvents. ESI-MS were performed with a Q-Tof Micro Quadrupole mass spectrophotometer (Micromass). For FTIR, Nicolate iS 10 FTIR spectrometer was used. Circular Dichroism (CD) experiment was performed by using Jasco J-1500 spectropolarimeter. Powder XRD were recorded on a BRUKER D2 Phaser X-Ray diffractometer (30kV, 10mA).

Preparation of Hydrogel: To prepare the hydrogel, 1.5 mg of **Azo-KC** was added in 100 μL of water along with 30 μl of 1 N NaOH sloution and shaken to completely dissolve the solid. The solution was kept undisturbed at room temperature for 2 h to get the self-supporting hydrogel. Unless otherwise mentioned, all the studies were performed with 1.15 wt% hydrogel at room temperature.

Determination of Sol–Gel Transition Temperature (T_g): The gel was placed in a water bath and the bath was heated at a rate of 0.5 °C/min. The temperature at which gel started flowing by inverting the vial was noted as T_g. The experiments were performed in triplicate.

FESEM: 10 μL of the samples were casted on silicon wafers and dried under ambient condition for 24 hours. Images were taken on a Gemini SEM 300 (Sigma Zeiss) instrument.

Rheology: The viscoelastic properties of the hydrogel were characterized using an AntonPaar MCR 102 rheometer equipped with a 20 mm parallel plate (with 0.5 mm zero gap) measuring system at 25 °C. A strain sweep test was performed to identify the linear viscoelastic region (LVR) over 0.01 to 100% strain at a fixed oscillatory frequency of 1 rad/s. Further, the mechanical strength of the gel was determined from oscillatory test i.e. frequency sweep, which was carried out under an appropriate strain (γ = 0.1 %) selected from the LVR with the frequency ranging from 0.1 to 1000 rad/s at 25 °C. For the injectable behaviour of the gel, time dependent rheology experiment was performed by alternating the applied strain at a fixed angular frequency 1 rad/sec. With the cyclic appearances of the applied strain against time, viscoelastic property was found to be reversible with applied strain (γ = 1000 % to γ = 0.1 %). Injectability behaviour was physically tested by preparing the H-gel in a 1ml syringe. When pressure was applied to the plunger liquid sol was released through the needle which reverted back to the gel state immediately.

Analytical HPLC: Purity of compound **6** and **Azo-KC** was checked using analytical HPLC with luna 5μm (C18) column. Acetonitrile and water with 0.1% TFA was used as the mobile phase. For the time dependent dimerization, a solution of **Azo-KC** (0.0075 wt%) in water (maintaining the basic condition by adding appropriate amount of NaOH solution) was incubated at room temperature and at different time, samples were taken and analysed. To analyse the extent of dimerization in the gel state, a small portion of a 24 h matured hydrogel was taken in minimum amount of DMSO to dissolve it and then diluted in water. This solution was analysed with analytical HPLC and only less than 1% monomer was found in the sample.

Preparation of stock solution of Azo-KC dimer: A 0.5 mM stock solution of **Azo-KC** was prepared in water (containing appropriate amount of NaOH to maintain the basic condition) and incubated for 12 h. This solution was used for UV-Vis and CD experiments.

Circular Dichroism (CD): The CD spectra of all the samples were recorded at room temperature. The data were collected at 0.5 nm intervals with 2 nm band width. All measurements were done in 0.2 cm path length cuvette with 800 μL sample volume. Each CD profile is an average of 3 scans of the same sample collected at a scan rate 100 nm min^{-1}, with a proper baseline correction from the water medium.

UV-Vis spectra: The above mentioned **Azo-KC** dimer was diluted with water to maintain a concentration of 0.01 wt% of **Azo-KC**. The solution was irradiated with UV-light (365 nm) for 120 min and at different time interval, the UV-Vis spectra were recorded at room temperature.

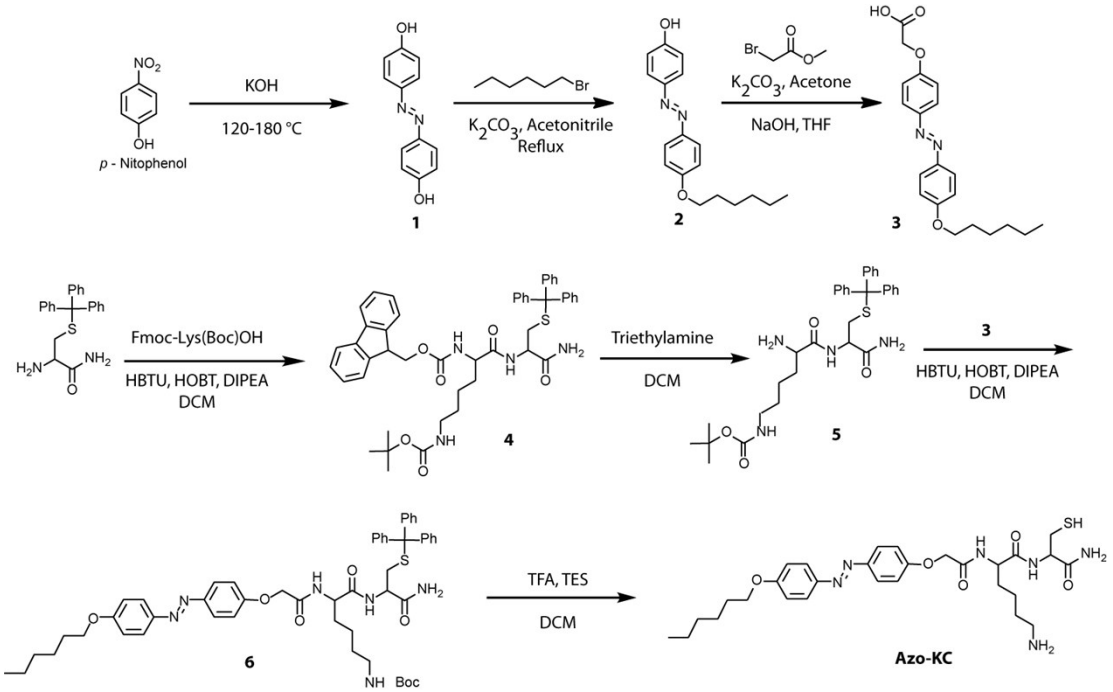
HPLC and ESI-MS analyses of hydrogel samples: Both H- and S-gels were found to be soluble in dimethylformamide (DMF). A small portion of these gels were dissolved in minimum amount of DMF and the solutions were further diluted with acetonitrile (to maintain DMF content less than 1%) before analysing the samples by ESI-MS and HPLC.

Dissolution Study: The dissolution studies were performed following our previously published protocol.[1] A solution of **Azo-KC** was prepared at MGC containing the required amount of NaOH solution and the sample was equally divided in different vials (100 μL each). After 12 h of incubation, the H-gels were formed and to these samples, 500 μL of water (or buffers of different pH) was added and the samples were shaken (100 rpm) at room temperature. At different times, the aliquots from supernatant bulk aqueous medium were taken out and the presence of the **Azo-KC** dimer was monitored using UV-Visible spectroscopy. Finally, the gels were disrupted completely by the treatment of glutathione (GSH) for 100% dissolution. The % dissolutions were calculated using the cumulative absorbance at λ_{max} (330 nm). For complete dissolution of the hydrogels, similar protocol was applied using aqueous solutions of GSH and tris(2-carboxyethyl)phosphine (TCEP). In each of these solutions, the H-gel dissolved completely within 5 min.

Buffers used for the dissolution study: HCl-KCl (pH 1); Glycine-HCl (pH 3); Citric acid-Sodium citrate (pH 5); Tris (hydroxylmethyl) aminomethane - Hydrochloric acid (pH 7 and 9); Sodium hydrogen orthophosphate / Sodium hydroxide (pH 11); Potassium chloride - Sodium hydroxide (pH 13). All buffers were freshly prepared maintaining 20 mM concentration.

Determination of extent of water expulsion during syneresis:
Several samples of the H-gel were prepared (600 μL) in vials and the gel samples were irradiated with UV light (365 nm, 12 lamps of 8 watt) for different time intervals. After irradiation with UV light, the expelled water was carefully taken out and the volume was measured. Weight of the vials before and after removing the expelled water were also noted. Each experiment was repeated five times to get an average value.

Model dye removal experiments:
1 mM stock solutions of the model dyes were prepared in water. From these solutions, appropriate amounts were added to the solutions of **Azo-KC** to maintain a final concentration of the dyes at 20 μM. The solutions were incubated for 12 h to form the H-gels containing these dyes. The samples were then irradiated with UV-light as before when the gels undergo syneresis. The concentrations of the dyes in the expelled water were then monitored using UV-Visible spectroscopy. In another set of experiments, the initial dye concentrations were varied to identify dye removal capacity of the gel. Dyes used are, Methyl Orange (MO), Eriochrome Black T (EBT), Methylene Blue (MB), Rhodamine B (Rh-B), Nile Red (NR), and Neutral Red (Neu R).



Scheme 3: Synthetic route for **Azo-KC**.

Synthesis of compound 1

4-Nitrophenol (5 g, 1equiv.) and KOH (25 g, 12.8 equiv.) were taken in a clean 100 ml round bottom flask and was heated at 120 °C for three hours when the mixture turned to yellow. After that, the temperature was gradually increased to 200 °C. The hot brown mixture was cooled at room temperature and the pH was adjusted to 3 by adding dilute HCL. The brown precipitate was thoroughly washed with water and the product was crystallized from methanol (yield = 40%). ESI-MS (m/z): calculated, 215.07 for $C_{12}H_{10}N_2O_2$; found, 215.08 for $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) = 10.18 (s, 2H), 7.74 – 7.69 (d, 4H), 6.96 – 6.88 (d, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm) = 160.44, 145.70, 124.63, 116.26.

Synthesis of compound 2

A mixture of **1** (1.8 g, 1 equiv.) and K_2CO_3 (5.7 g, 5 equiv.) was taken in acetonitrile and stirred for 30 min before hexyl bromide (825 µL, 0.7 equiv.) was added to it. The reaction was refluxed overnight under argon atmosphere. It was then filtered and the solid obtained was thoroughly washed with acetonitrile. The product was purified by column chromatography using ethyl acetate and hexane as mobile phase (yield = 32%). ESI-MS (m/z): calculated, 299.17 for $C_{18}H_{22}N_2O_2$; found, 299.17 for $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) = 7.89 – 7.79 (m, 4H), 7.01 – 6.90 (m, 4H), 5.47 (s, 1H), 4.03 (t, J = 6.6 Hz, 2H), 1.86 – 1.77 (m, 2H), 1.52 – 1.44 (m, 2H), 1.35 (dq, J = 7.3, 3.8 Hz, 4H), 1.26 (s, 1H), 0.94 – 0.88 (m, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm) = 161.26, 124.56, 124.54, 124.38, 124.36, 115.80, 115.77, 114.72, 114.70, 77.27, 77.06, 76.84, 68.36, 31.60, 31.58, 29.19, 25.72, 25.70, 22.63, 22.61, 14.08, 14.06.

Synthesis of compound 3

Compound **2** (0.825 g, 1 equiv.) was dissolved in acetone in presence of KOH (0.775 g, 5 equiv.) and the solution was stirred for 30 min. Bromo-methyl acetate (550 µL, 2 equiv.) was carefully added to the reaction mixture and was refluxed under argon atmosphere overnight. Completion of the reaction was monitored by TLC. The mixture was concentrated under reduced pressure and the yellow solid was dissolved in DCM and washed with brine. DCM layer was concentrated under reduced pressure to get a yellow product which was dissolved in dry THF with an excess amount of NaOH flakes. Once all the ester was hydrolysed, THF was removed and the product was recovered by acidic precipitation. The precipitate was thoroughly washed with water and dried (Yield = 99%). ESI-MS (m/z): calculated, 357.17 for $C_{20}H_{24}N_2O_4$; found, 357.18 for $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) =13.13 (s, 1H), 7.88 – 7.78 (m, 4H), 7.10 (m, 4H), 4.80 (s, 2H), 4.06 (t, *J* = 6.5 Hz, 2H), 1.74 (p, *J* = 6.8 Hz, 2H), 1.43 (t, *J* = 7.6 Hz, 2H), 1.32 (m, 4H), 0.91 – 0.86 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm) = 175.14, 166.22, 165.08, 151.69, 151.24, 129.46, 129.26, 73.15, 69.90,3 6.20, 33.78, 33.37, 27.30, 19.15.

Synthesis of compound 4

Fmoc-Lys(Boc)-OH (2.16 g, 1 equiv.), HOBT (0.68 g, 1.1 equiv.) HBTU (0.97 g, 1.1 equiv.) and DIPEA (0.650g, 1.1 equiv.) were taken in dry DCM under argon atmosphere and stirred for 30 min at 0 - 5 °C. H-Cys(Trt)NH_2 solution (1.82g, 1 equiv.) in dry DCM, was added to the mixture under cold condition and was allowed to come to room temperature. After 24 h, the reaction mixture was extracted with DCM, washed with brine solution, and the organic layer was dried over anhydrous Na_2SO_4. After evaporating the DCM, the crude mixture was purified using column chromatography. The product obtained was a light yellow solid (yield = 65%). MALDI-TOF (m/z): calculated, 812.36 for $C_{48}H_{52}N_4O_6S$; found, 835.43 for $[M+Na]^+$; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) =7.78 (d, J = 7.6 Hz, 2H), 7.59 (dd, J = 7.6, 2.9 Hz, 2H), 7.42 (d, J = 7.8 Hz, 8H), 7.33 – 7.27 (m, 8H), 7.20 (t, J = 7.2 Hz, 3H), 6.66 (s, 1H), 6.35 (s, 1H), 5.83 (s, 1H), 5.57 (s, 1H), 4.39 (d, J = 6.9 Hz, 2H), 4.18 (t, J = 6.9 Hz, 2H), 4.09 (s, 1H), 3.16 – 2.99 (m, 2H), 2.78 (s, 1H), 2.65 – 2.54 (m, 1H), 1.87 (s, 4H), 1.45 (s, 13H), 1.35 (s, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm) =172.24, 171.89, 144.41, 129.63, 128.19, 127.90, 127.89, 127.25, 127.02, 125.18, 120.12, 67.29, 53.57, 52.02, 47.23, 33.20, 29.78, 28.56, 22.27.

Synthesis of compound 5

Compound **4** (2.43 g, 1 equiv.) was dissolved in DCM (25 mL), to which triethyl amine (5 mL, excess) was added and stirred for 36 h at room temperature. The reaction was stopped when the spot of the starting material disappeared on TLC plate. The reaction mixture was extracted with DCM, washed with brine solution, and the organic layer was dried over anhydrous Na_2SO_4. DCM was evaporated and the product was purified using column chromatography to obtain a white solid (yield = 95%). MALDI-TOF (m/z): calculated, 590.29 for $C_{33}H_{42}N_4O_4S$; found, 613.25 for $[M+Na]^+$; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) =7.73 (s, 1H), 7.40 (d, J = 7.8 Hz, 6H), 7.28 (t, J = 7.6 Hz, 6H), 7.21 (t, J = 7.3 Hz, 3H), 6.25 (s, 1H), 5.56 (s, 1H), 4.62 (s, 1H), 3.99 (s, 1H), 3.35 (s, 1H), 3.11 (s, 1H), 3.05 (s, 1H), 2.67 (dd, J = 13.1, 8.1 Hz, 1H), 2.61 (dd, J = 13.0, 5.6 Hz, 1H), 1.76 (s, 1H), 1.42 (s, 13H), 1.37 (t, J = 7.3 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm) = 175.56, 172.24, 144.41, 129.60, 128.06, 126.89, 67.15, 54.91, 51.73, 46.11, 34.46, 33.11, 29.85, 28.45, 22.75.

Synthesis of compound 6

Compound **3** (0.5 g, 1 equiv.), HOBT (0.2 g, 1.1 equiv.), HBTU (0.585 g, 1.1 equiv.) and DIPEA (0.2 g, 1.1 equiv.) were taken in dry DCM, cooled to 0 °C and stirred for 30 min. Compound **5** (0.91 g, 1.1 equiv.) was added to the mixture and the reaction was allowed to come to room temperature and continued to stir overnight. The reaction mixture was then concentrated on a rotory evaporator and the crude mixture was purified by column chromatography using 1% MeOH-DCM as mobile phase (yield = 32%). MALDI-TOF (m/z): calculated, 929.45 for $C_{53}H_{64}N_6O_7S$; found, 929.49 for $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) = 7.92 – 7.86 (m, 4H), 7.43 – 7.38 (m, 6H), 7.31 – 7.25 (m, 7H), 7.25 – 7.16 (m, 4H), 7.05

– 6.98 (m, 4H), 6.91 (s, 1H), 6.17 (s, 1H), 5.70 (s, 1H), 4.80 (s, 1H), 4.54 (d, *J* = 16.1 Hz, 2H), 4.05 (t, *J* = 6.6 Hz, 2H), 3.11 – 2.96 (m, 2H), 2.75 (dd, *J* = 13.1, 7.4 Hz, 1H), 2.60 (dd, *J* = 13.1, 5.5 Hz, 1H), 2.04 (s, 3H), 1.85 – 1.81 (m, 2H), 1.73 – 1.58 (m, 1H), 1.54 – 1.46 (m, 2H), 1.42 (s, 11H), 1.37 (h, *J* = 3.9 Hz, 4H), 1.32 – 1.26 (m, 2H), 0.96 – 0.90 (m, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm) = 171.15, 168.14, 165.77, 161.50, 158.61, 156.14, 147.96, 146.76, 144.28, 129.54, 128.10, 126.95, 124.54, 115.01, 114.70, 68.36, 67.25, 52.65, 52.17, 40.00, 38.63, 33.18, 31.59, 29.18, 28.46, 25.71, 22.62, 22.37, 14.07.

Synthesis of Azo-KC:

Compound **6** was taken in 80% TFA in DCM containing 1% triethylsilane and stirred at room temperature for 1h. The solvent and TFA was removed under reduced pressure and the crude material was slowly added to cold dry diethylether to precipitate **Azo-KC**. The solid was washed several times with diethylether and the purity of the solid was checked by analytical HPLC. Yield = 98%. MALDI-TOF (m/z): calculated, 587.29 for $C_{29}H_{42}N_6O_5S$; found, 587.30 for $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.28 (d, *J* = 8.0 Hz, 2H), 8.13 (d, *J* = 8.1 Hz, 1H), 7.88 – 7.79 (m, 4H), 7.61 (s, 2H), 7.45 (s, 1H), 7.23 (s, 2H), 7.12 (dd, *J* = 13.1, 8.8 Hz, 4H), 4.69 (d, *J* = 9.7 Hz, 2H), 4.39 – 4.33 (m, 2H), 4.07 (t, *J* = 6.7 Hz, 2H), 2.97 (s, 4H), 2.76 – 2.72 (m, 2H), 1.74 (d, *J* = 6.0 Hz, 2H), 1.63 (s, 3H), 1.57 – 1.47 (m, 4H), 1.44 (s, 3H), 1.36 – 1.28 (m, 6H), 1.25 (d, *J* = 10.4 Hz, 2H), 0.92 – 0.87 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.54, 160.30, 147.13, 146.55, 124.67, 124.48, 115.78, 115.47, 115.45, 68.45, 67.43, 65.37, 55.23, 52.72, 52.43, 39.19, 31.69, 31.44, 29.03, 27.05, 26.54, 25.60, 22.56, 22.51, 15.62, 14.35.

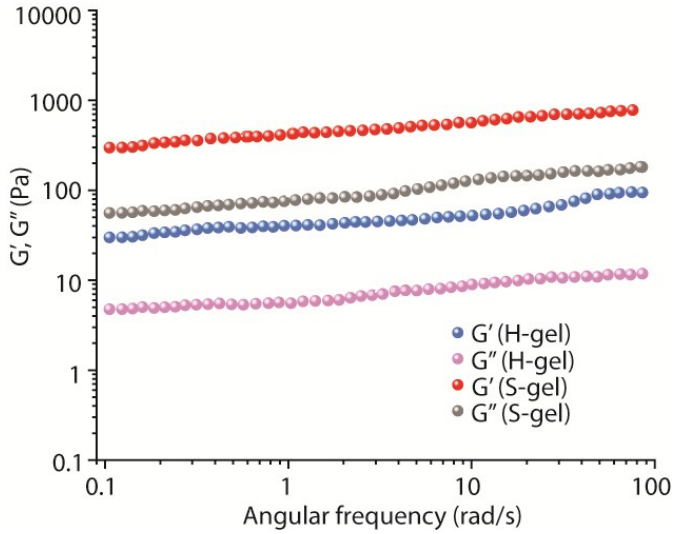


Fig. S1 Changes in storage and loss moduli as a function of angular frequency for H- and S-gels.

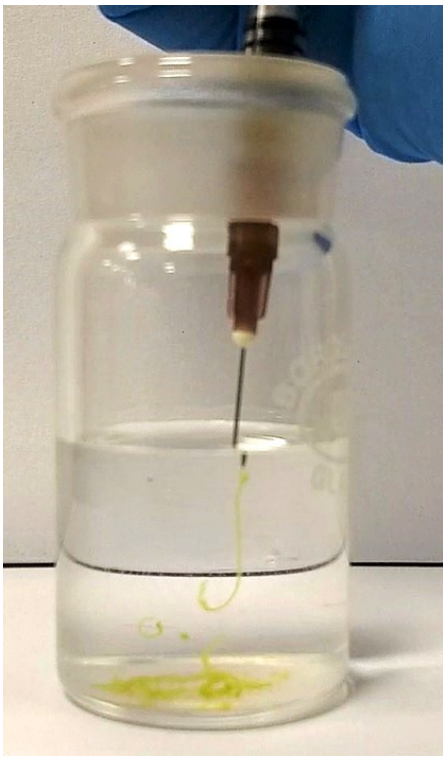


Fig. S2 Photograph of H-gel injected through a syringe into bulk water showing both injectability and insolubility in water.

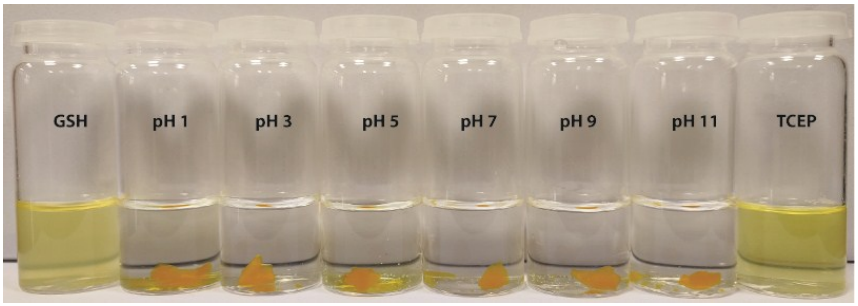


Fig. S3 Photograph of vials containing different aqueous solutions where small portions of the H-gel were immerged and stirred for 10 min at room temperature. Though the gel remained insoluble in buffers, TCEP and GSH solutions could dissolve the gel completely.

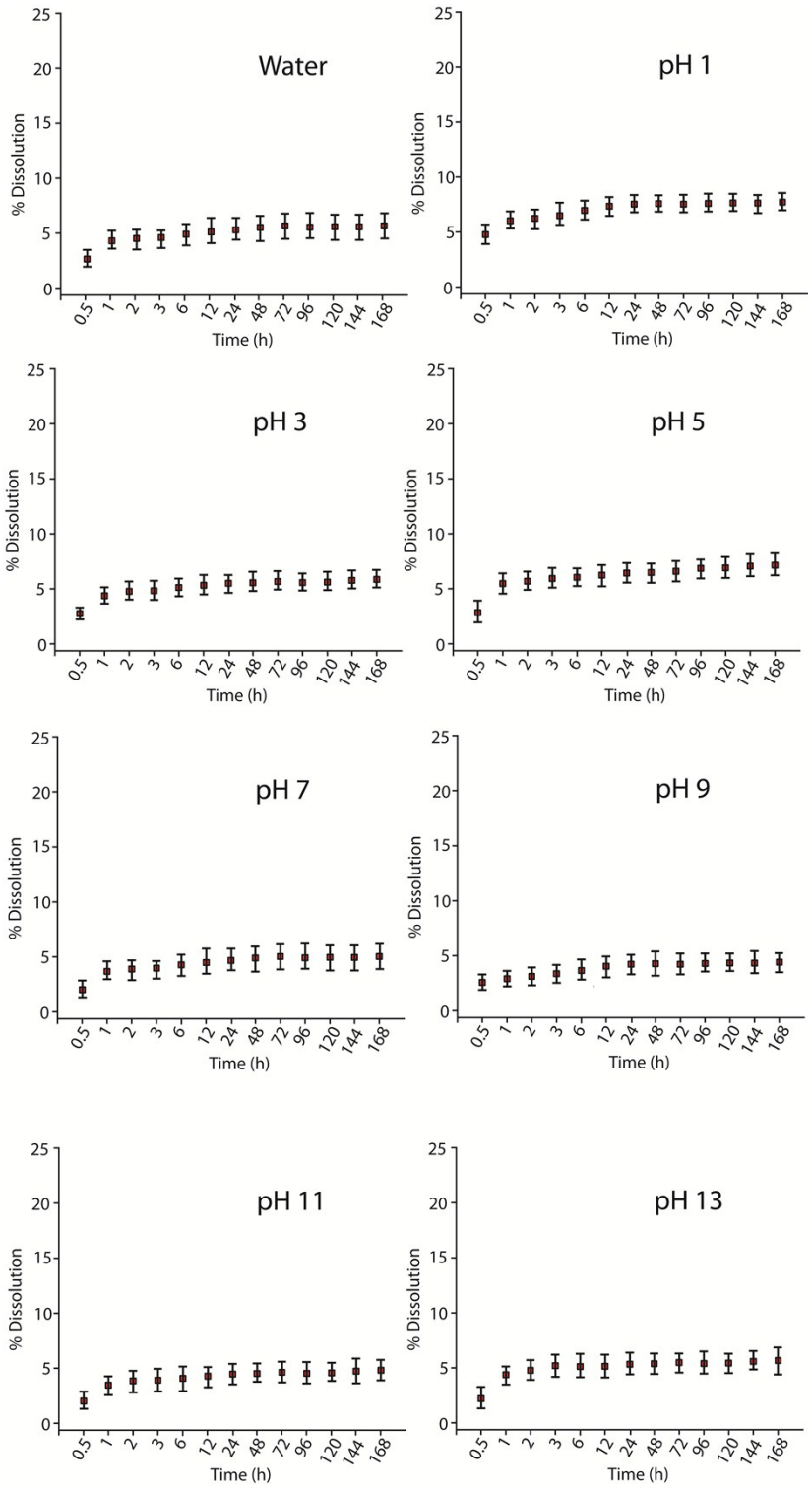


Fig. S4 Dissolution of H-gel (at MGC) when placed in bulk water and buffers of different pH (1-13).

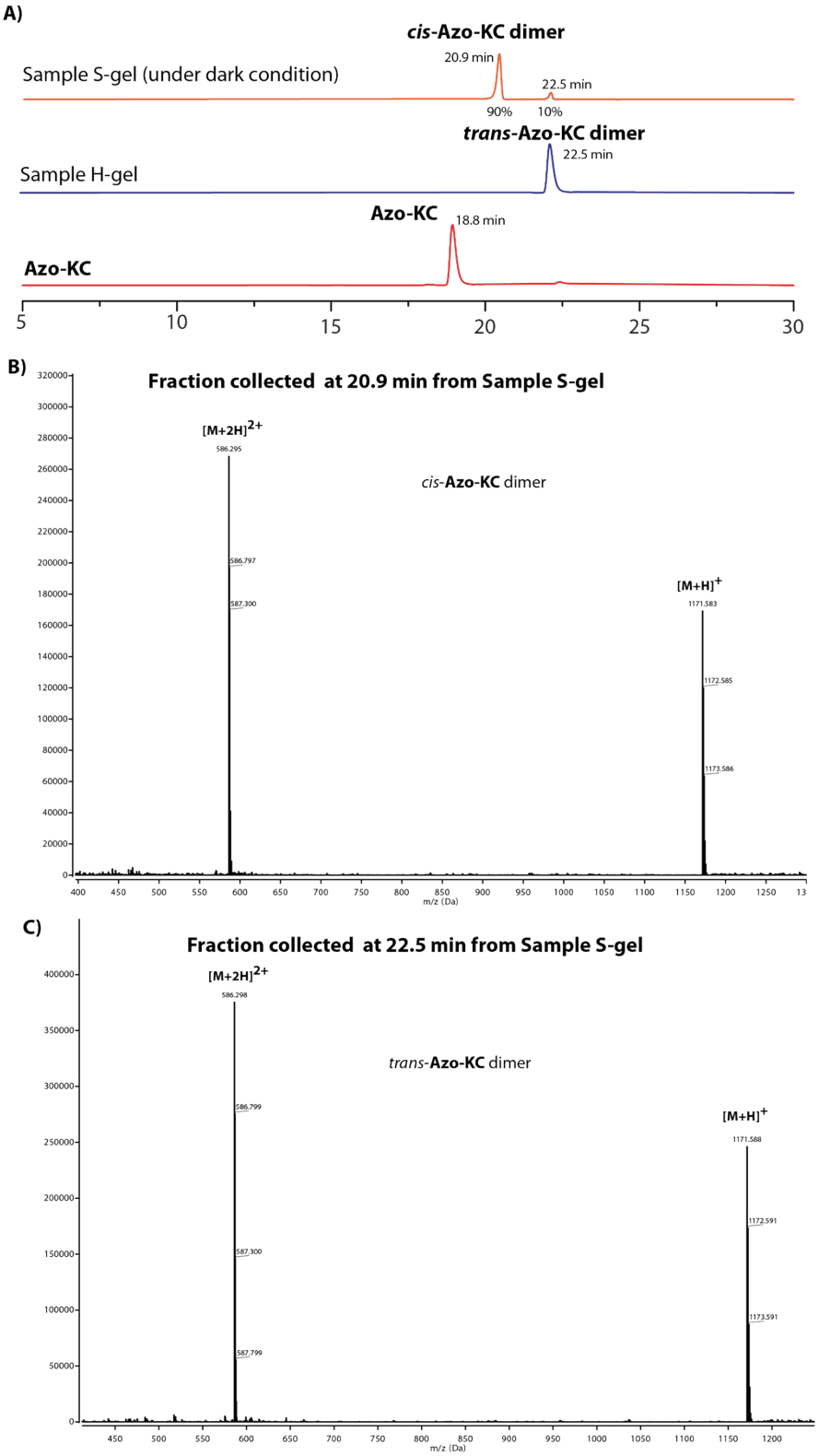


Fig. S5 A) Chromatographic analysis of **Azo-KC**, H-gel and S-gel to determine the extent of dimers in the gel states. B) and C) ESI-MS of the fractions collected from the peaks corresponding to R_T = 20.9 and 22.5 min respectively from the chromatogram of Sample S-gel.

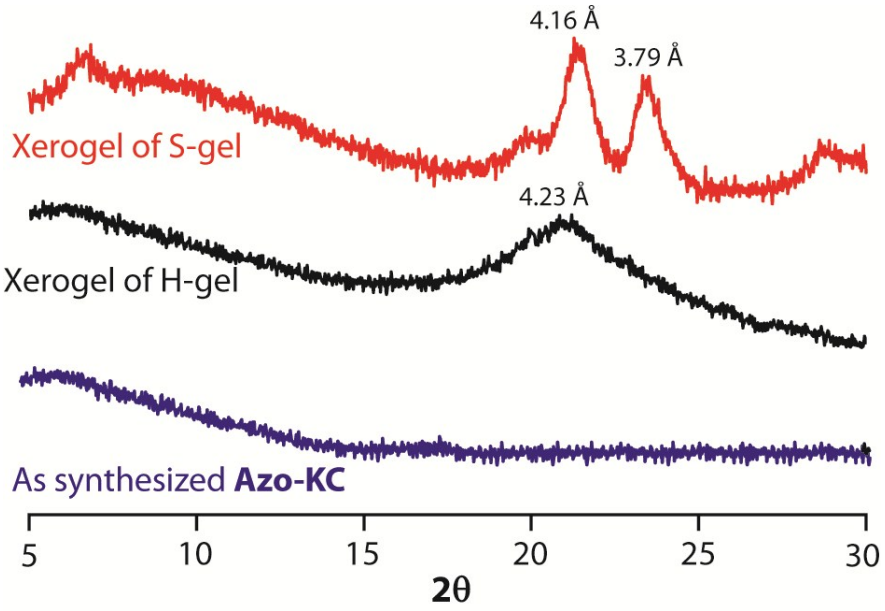


Fig. S6 PXRD of the as synthesized **Azo-KC** and xerogels prepared from H-gel and S-gel showing different $\pi-\pi$ interactions in the two gel states.

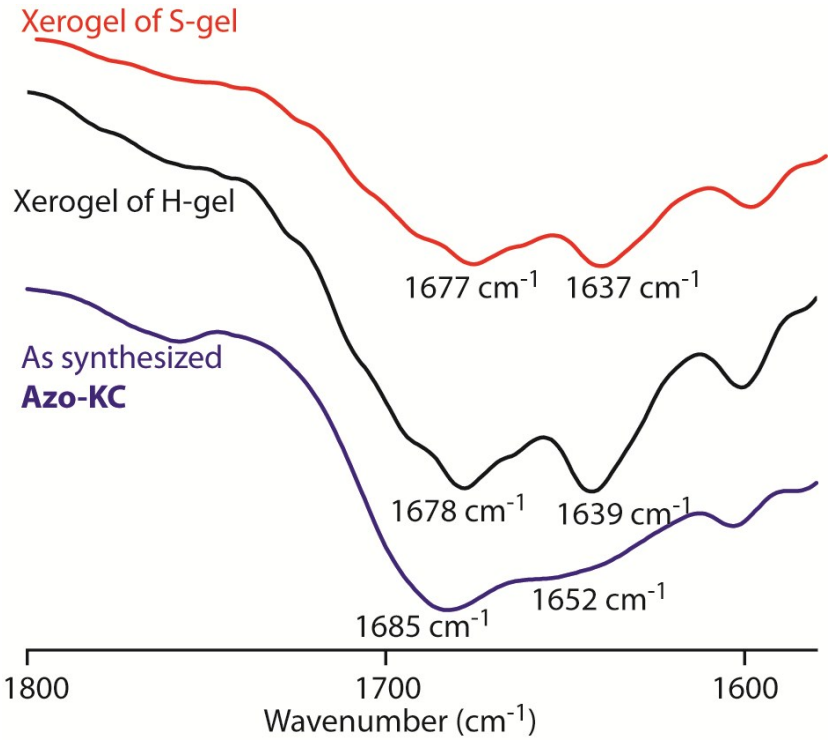


Fig. S7 Relevant region of the FTIR spectra of as synthesized **Azo-KC** and xerogels prepared from H-gel and S-gel showing the involvement of hydrogen bonding in the gel state.

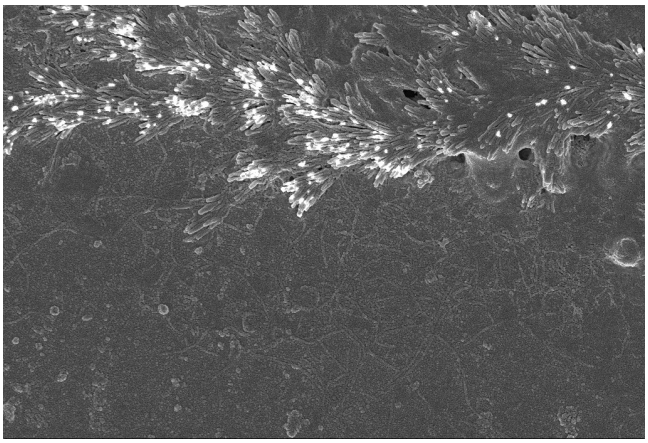


Fig. S8 FESEM image of H-gel after 30 min of UV-light (365 nm) irradiation showing presence of both rod and fibers, which represents the intermediate state during the syneresis process.

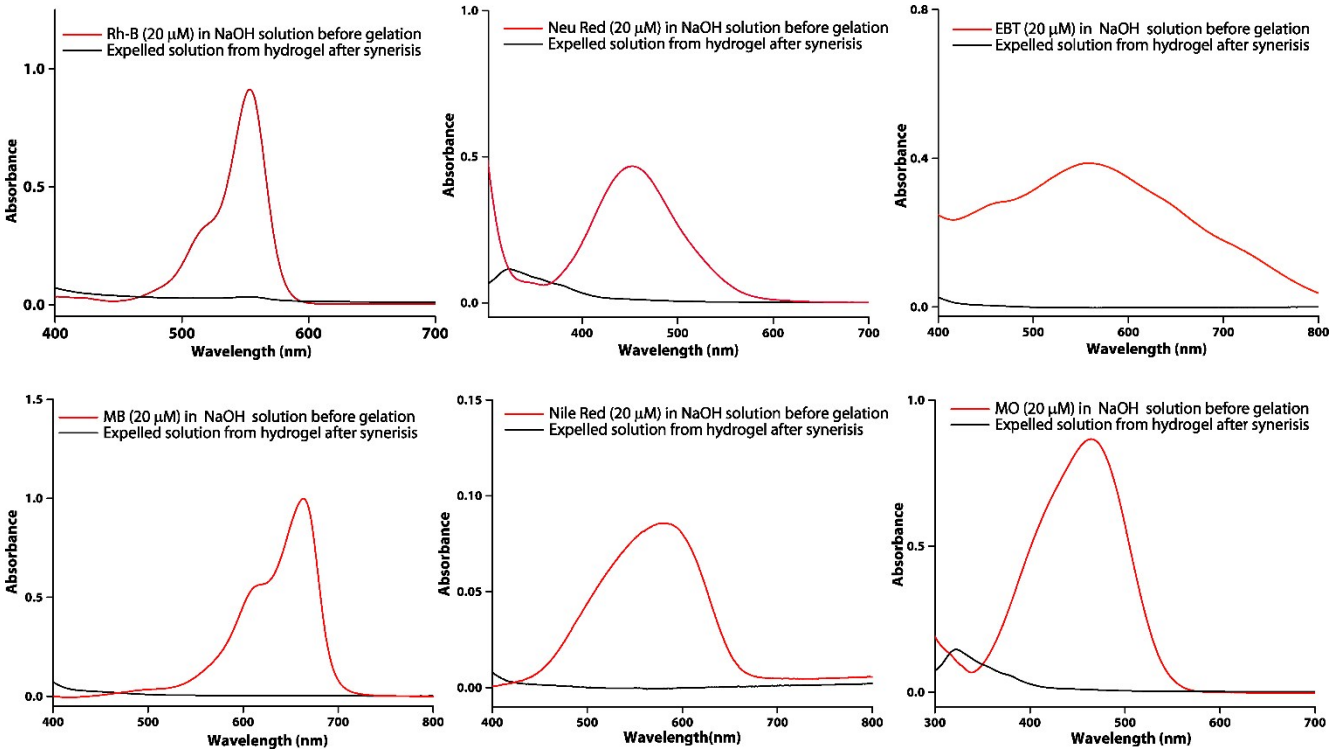


Fig. S9 The UV-visible spectra of aqueous (containing NaOH) solutions of various dyes (20 μM) and the expelled water after syneresis of H-gels prepared using 20 μM solutions of the respective dyes. All experiments were carried out at room temperature.

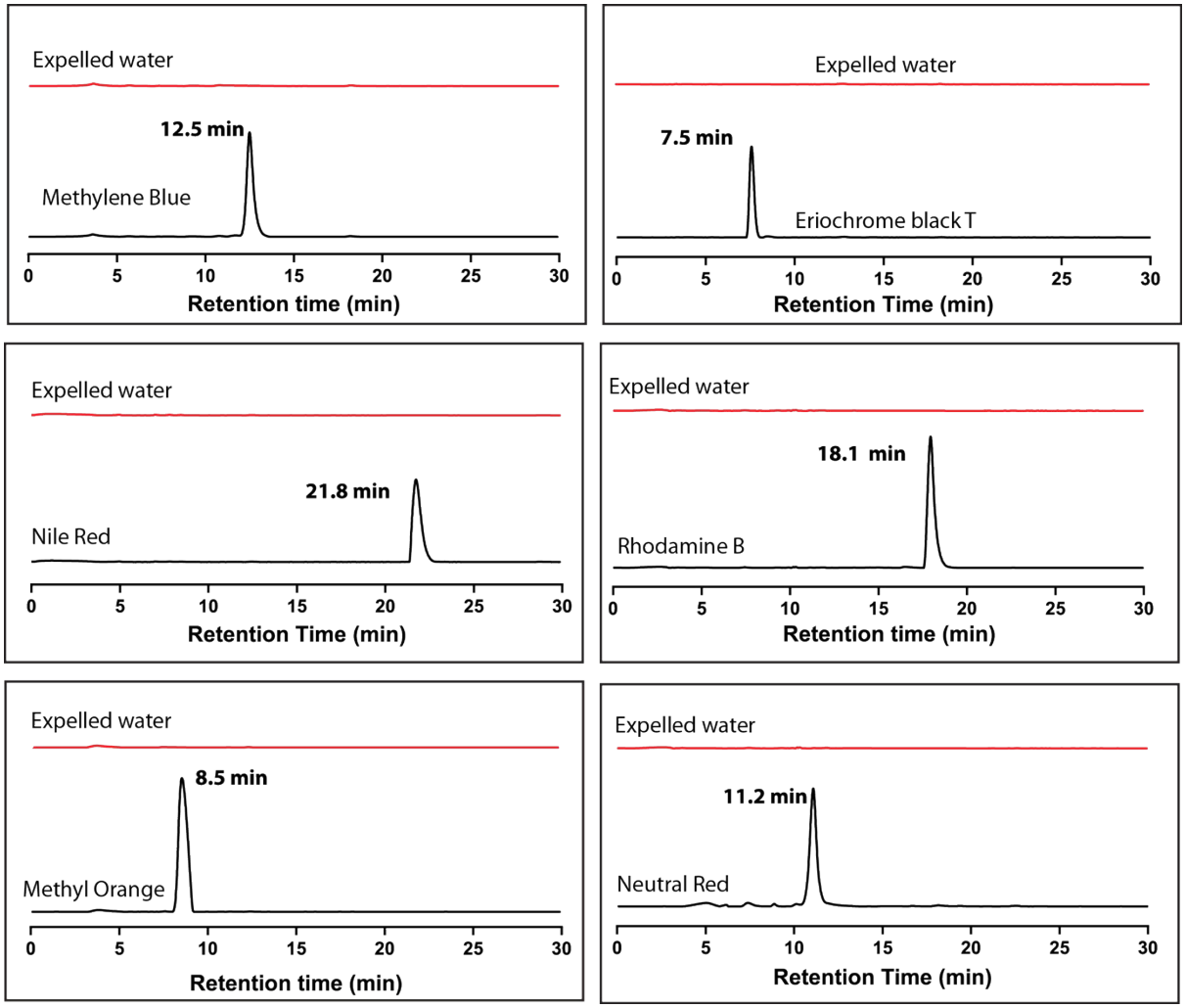


Fig. S10 The chromatograms of aqueous (containing NaOH) solutions of various dyes (black lines) and the expelled water after syneresis of H-gels prepared using solutions of the respective dyes (red lines, at the highest concentrations as per Fig. 4C of the manuscript). All experiments were carried out at room temperature.

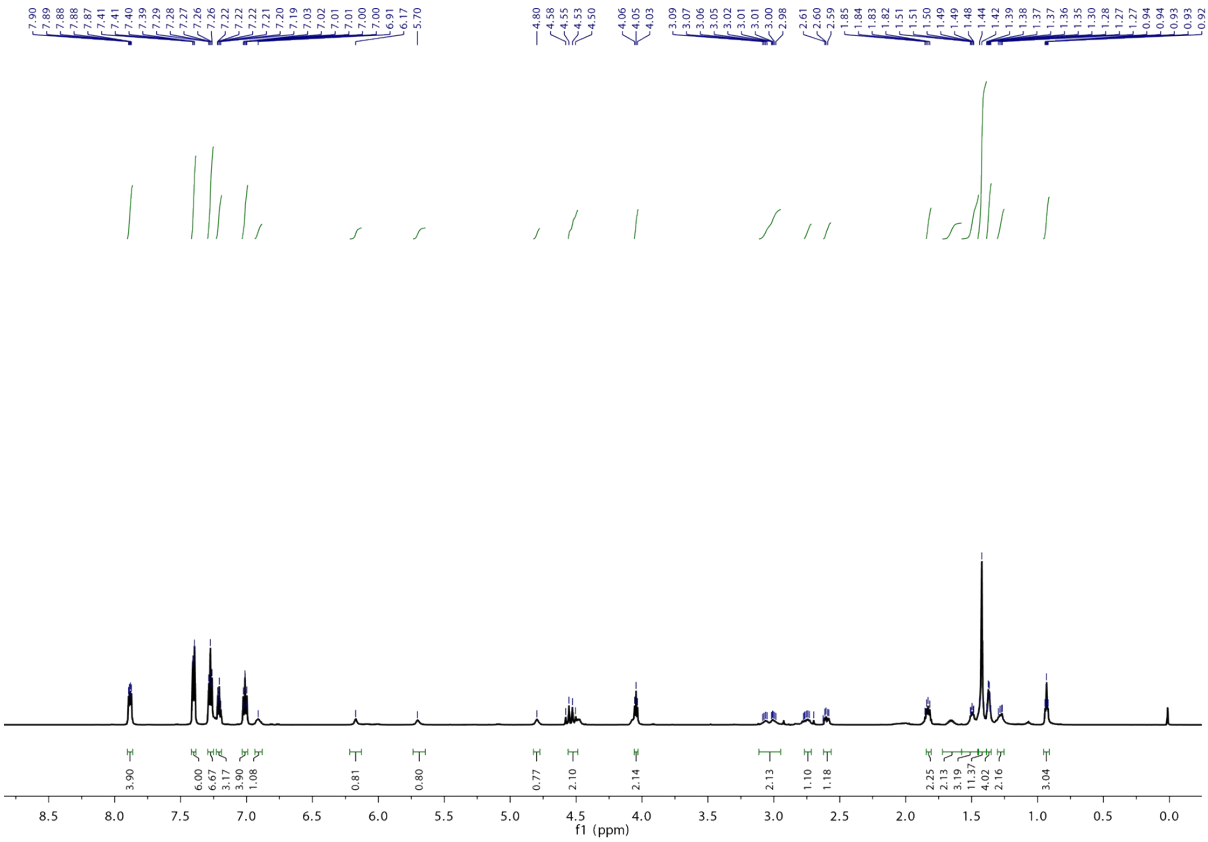


Fig. S10 ^{1}H NMR spectrum of compound **6** in $CDCl_3$ measured at room temperature.

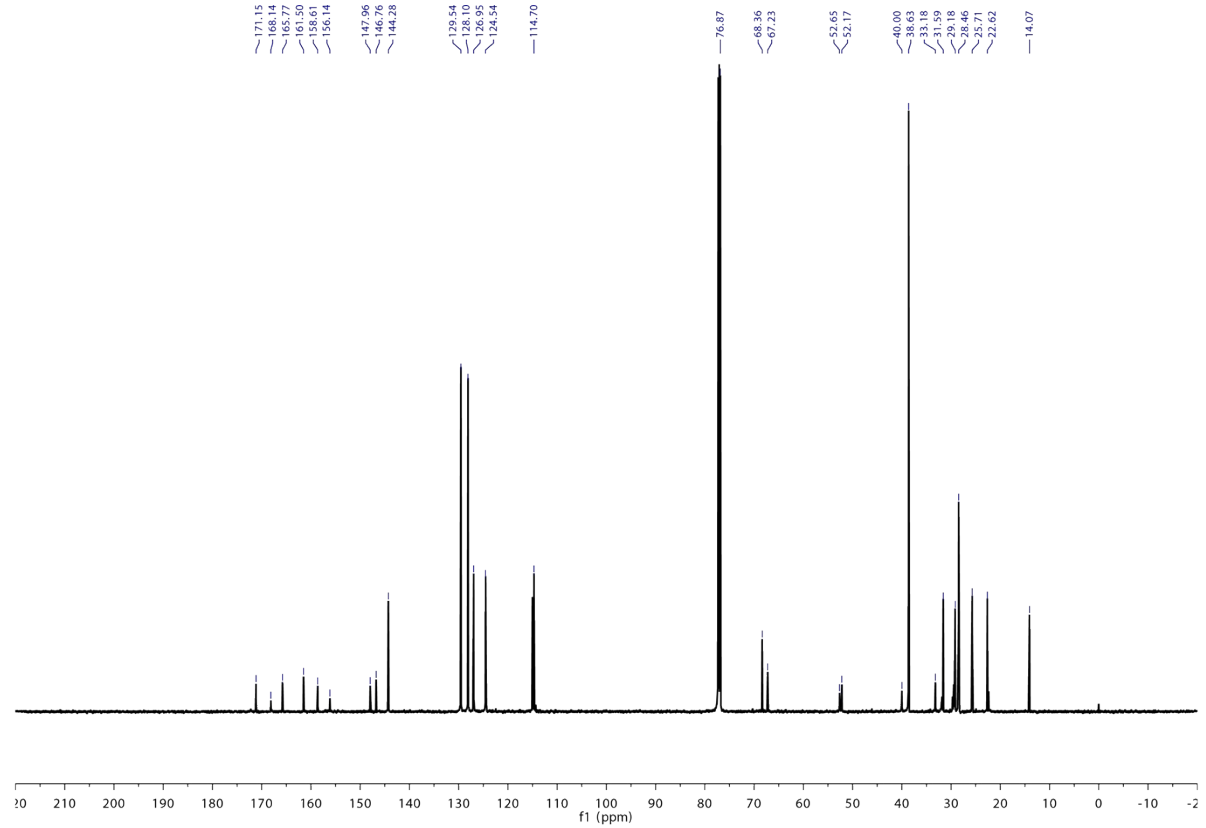


Fig. S11 ^{13}C NMR spectrum of compound **6** in $CDCl_3$ measured at room temperature.

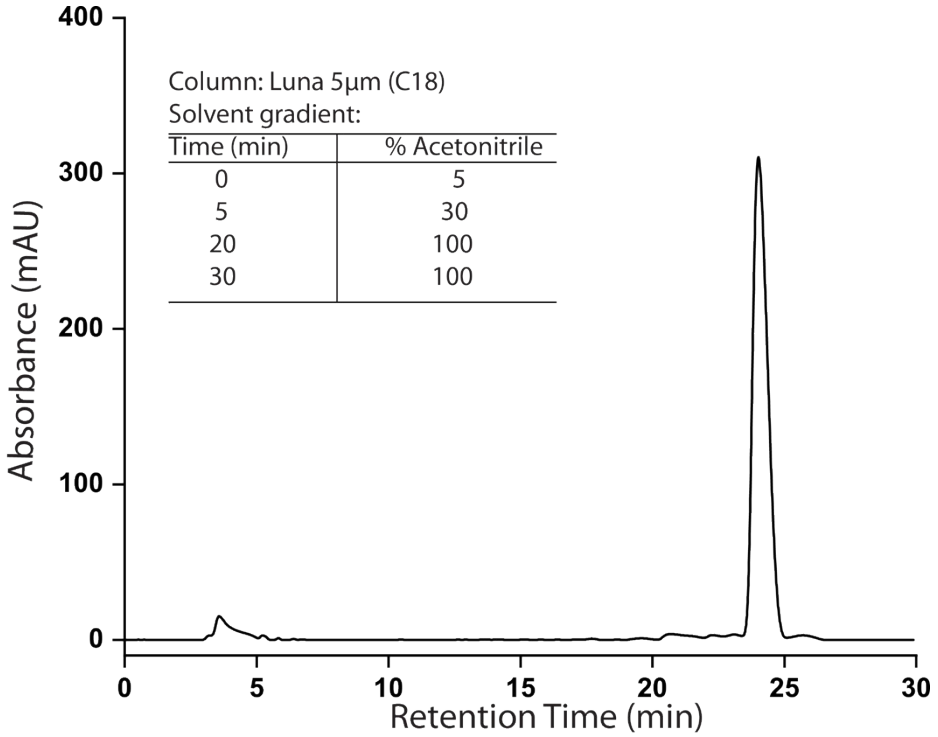


Fig. S12 Chromatogram of compound **6** measured at room temperature.

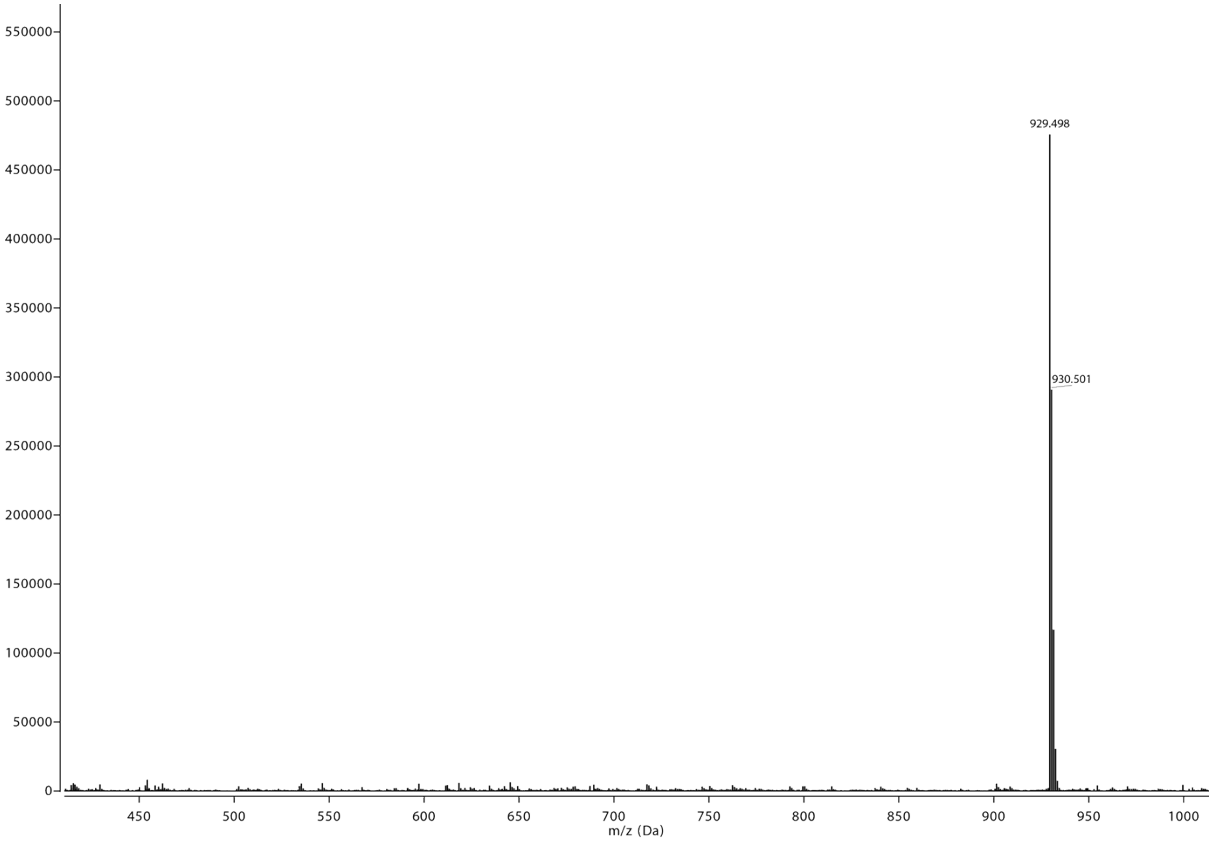


Fig. S13 ESI-MS of compound **6**.

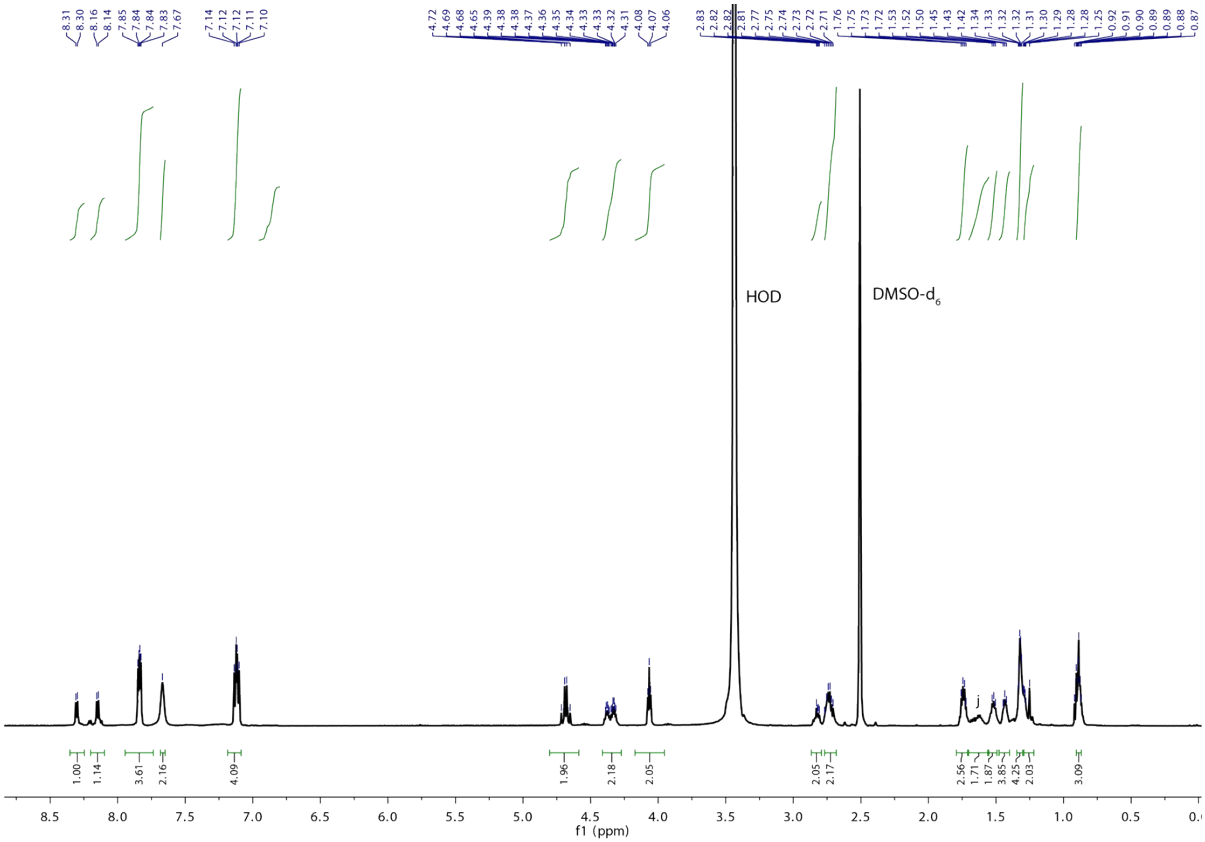


Fig. S14 ^{1}H NMR spectrum of compound **Azo-KC** in DMSO-d_6 measured at room temperature.

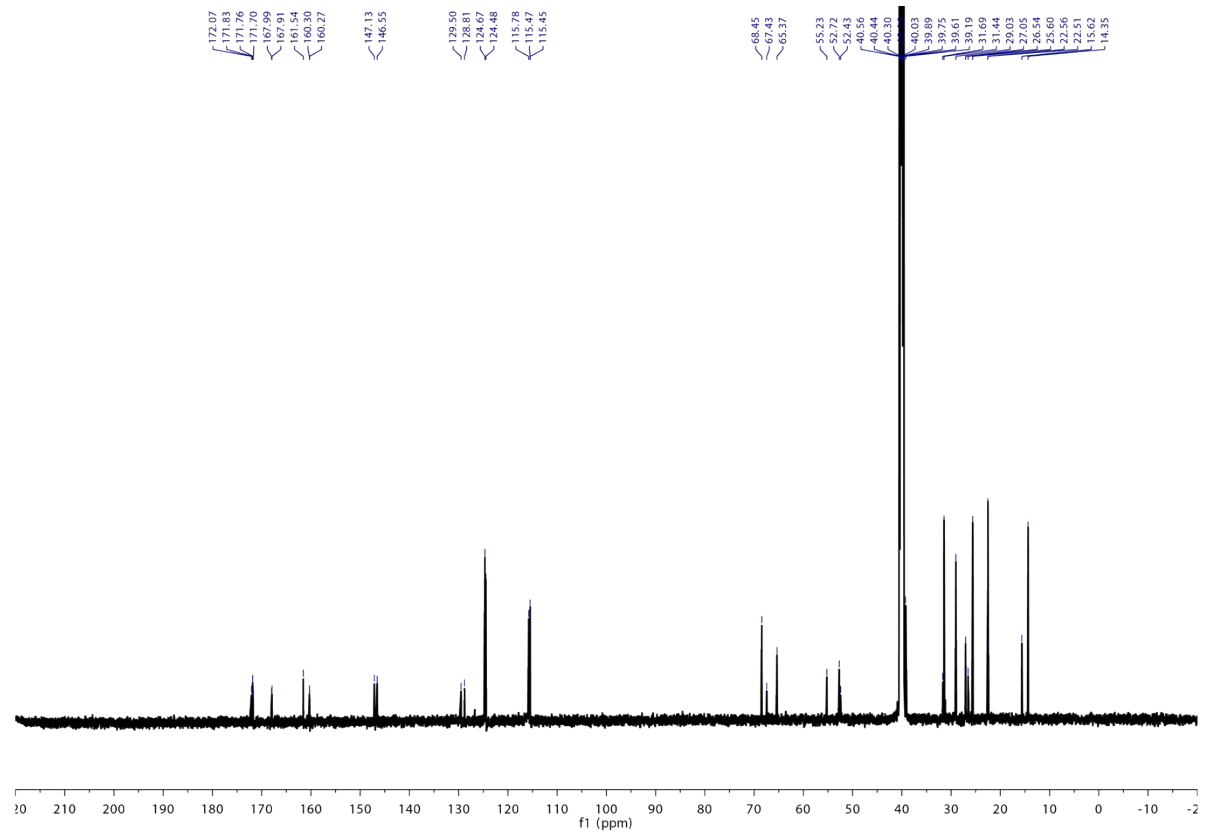


Fig. S15 ^{13}C NMR spectrum of compound **Azo-KC** in DMSO-d_6 measured at room temperature.

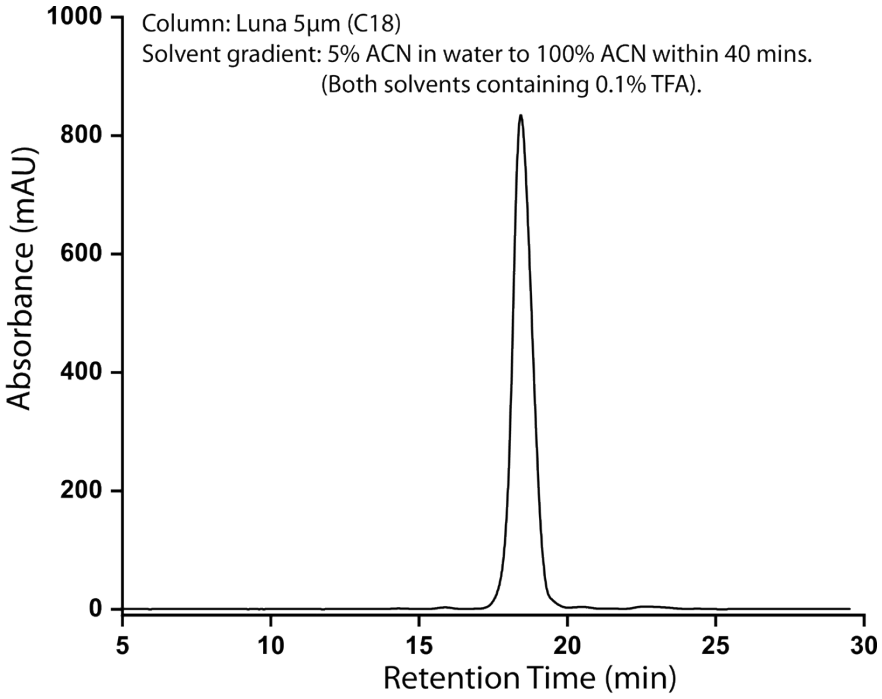


Fig. S16 Chromatogram of compound **Azo-KC** measured at room temperature.

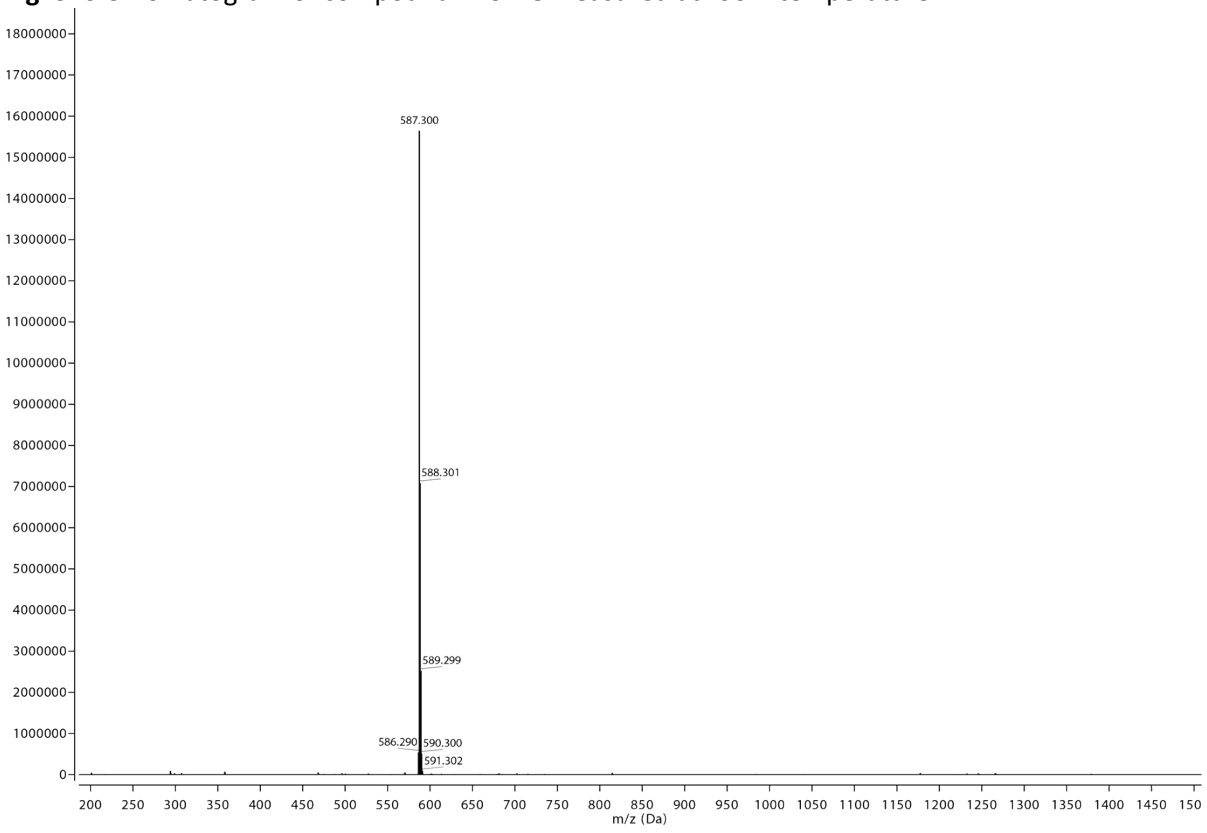


Fig. S17 ESI-MS of **Azo-KC**.

References:

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