Electronic Supplementary Material (ESI) for New Journal of Chemistry

Supporting Information

pH and salt controlled self assembly of [1]benzothieno[3,2-b][1]-benzothiophene-peptide conjugates in supramolecular hydrogels

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GENERAL METHODS

- Mass Spectrometry

ESI-MS experiments were performed with a ESI-TOF MarinerTM BiospectrometryTM Workstation of Applied Biosystems by flow injection analysis using methanol with formic acid (1 %) as mobile phase.

- NMR Spectroscopy

^{1}H, ^{13}C and 2D NMR were recorded at 289 K on a Bruker Avance III 500 spectrometer using the partially deuterated solvent as internal reference. Deuterated DMSO has been used as solvent (99.9% d_6, Sigma Aldrich). Chemical shifts (δ) are expressed in parts per million (ppm). The multiplicity of a signal is indicated as: s (Singlet), d (Doublet), t (Triplet), dd (Doublet of doublets), dt (Doublet of triplets), td (Triplet of doublets), q (Quartet) and m (Multiplet). The acronym "br" indicates a broadened signal. The spectral width for ^{1}H-NMR is from 0ppm to 14ppm, whereas the ^{13}C-NMR spectral width is from 0 ppm to 210 ppm.

- FT-IR

FT-IR absorption spectrum were recorded with a FT-IR Perkin-Elmer, model 1720X spectrophotometer, in KBr disk, at a nominal resolution of 2 cm^{-1}, averaging 100 scans.

- UV-Vis Spectroscopy

UV-Vis absorption spectra were recorded with a Varian Cary 50 spectrophotometer at 25°C. All spectra are baseline corrected. A rectangular cell with detachable windows (Hellma) and optical path of 0.02 cm (Hellma) was used for the analysis of gelled samples. For non-gelled samples a reduce volume quartz cell with 1 cm or 0.1 cm optical path was used.
General methodology for gel samples: gels were prepared in a glass vial; a small amount was transferred to the sample chamber and the cell was closed with the top window taking care of not forming bubbles.

- Emission Spectroscopy

Emission spectra were recorded in a Varian CaryEclipse spectrophotometer at 25°C. A quartz cell with optical path of 10x4 mm and volume 1400 μL was used for gel samples. A quartz cell with optical path of 10x10 mm and volume 3 mL was used for solutions.
General methodology for gel samples: Gels were prepared in a glass vial and transferred to the cuvette without amendment such as dilution.

- Circular Dichroism Spectroscopy

CD spectra were recorded on a Jasco J-1500 instrument at 25°C and were baseline corrected. The spectra are expressed in terms of total molar ellipticity ($deg \cdot cm^2 \cdot dmol^{-1}$). For non-gelled samples a reduce volume quartz cell with 1 cm or 0.1 cm optical path was used.

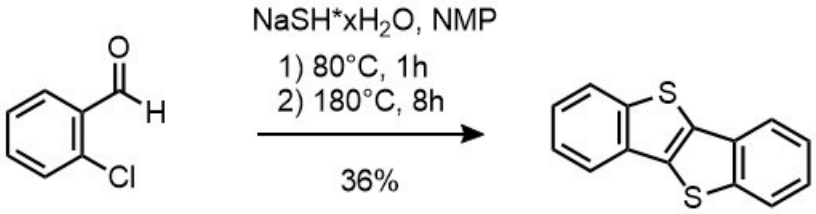
General methodology for gel samples: gels were prepared in a glass vial; a small amount was transferred to the sample chamber and the cell was closed with the top window taking care of not forming bubbles.

- TEM

Transmission electron microscopy (TEM) images were recorded with a Jeol 300PX instrument. A glow discharged carbon coated grid was floated on a small drop of solution and excess was removed by using #50 hardened Whatman filter paper. Samples of the gels were prepared in two different ways: a) by dropping a small amount of gel into a glow discharged carbon coated grid and removing excess of gel with #50 hardened Whatman filter paper; b) gels were diluted prior to analysis, a small amount of each sample has been deposited directly on a glow discharged carbon coated grid and no staining has been used. The excess has been removed by #50 hardened Whatman filter paper. The images obtained have be analysed with ImageJ program.

SYNTHETIC PROCEDURES

Synthesis of BTBT[1]

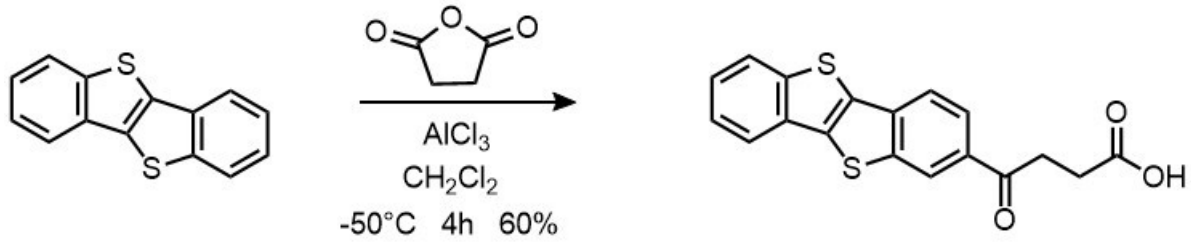


In a 1L roundbottom flask, 2-chlorobenzaldehyde (250.0 g, 1.778 mol) is put under N_2 atmosphere and subsequently dissolved in NMP (450 mL). The solution is heated to 80°C, and sodium hydrosulfide hydrate (267.0 g, 2.902 mol) is slowly added to it. Mixture is stirred for 1 hour, and then temperature is raised to 180°C for 5 hours while distilling water with a Dean-Stark apparatus. During this time, reaction colour turns from red to black. Reaction mixture is allowed to warm, and the obtained precipitate is collected on a fritted funnel and washed with water and methanol (1L). Residual solvent is dried under vacuum to afford the pure product as faint yellow crystals (77.00 g, 36.0% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz): *d* 7.93 (d *J*=7.90 Hz, 2H,), 7.89 (d, *J*=8.1 Hz, 2H), 7.47 (dd, *J*=7.6, 7.3 Hz, 2H), 7.41 (dd, *J*=8.0, 7.3 Hz, 2H).

^{13}C-NMR ($CDCl_3$, 100 MHz): *d* 143.1, 134.3, 134.0, 125.9, 125.8, 124.9 (d, *J*=10.4 Hz), 123.0 (d, *J*=10.6 Hz).

Synthesis of 4-oxo-4-([1]benzothieno[3,2-b][1]benzothienyl)-butyric acid (compound 2)



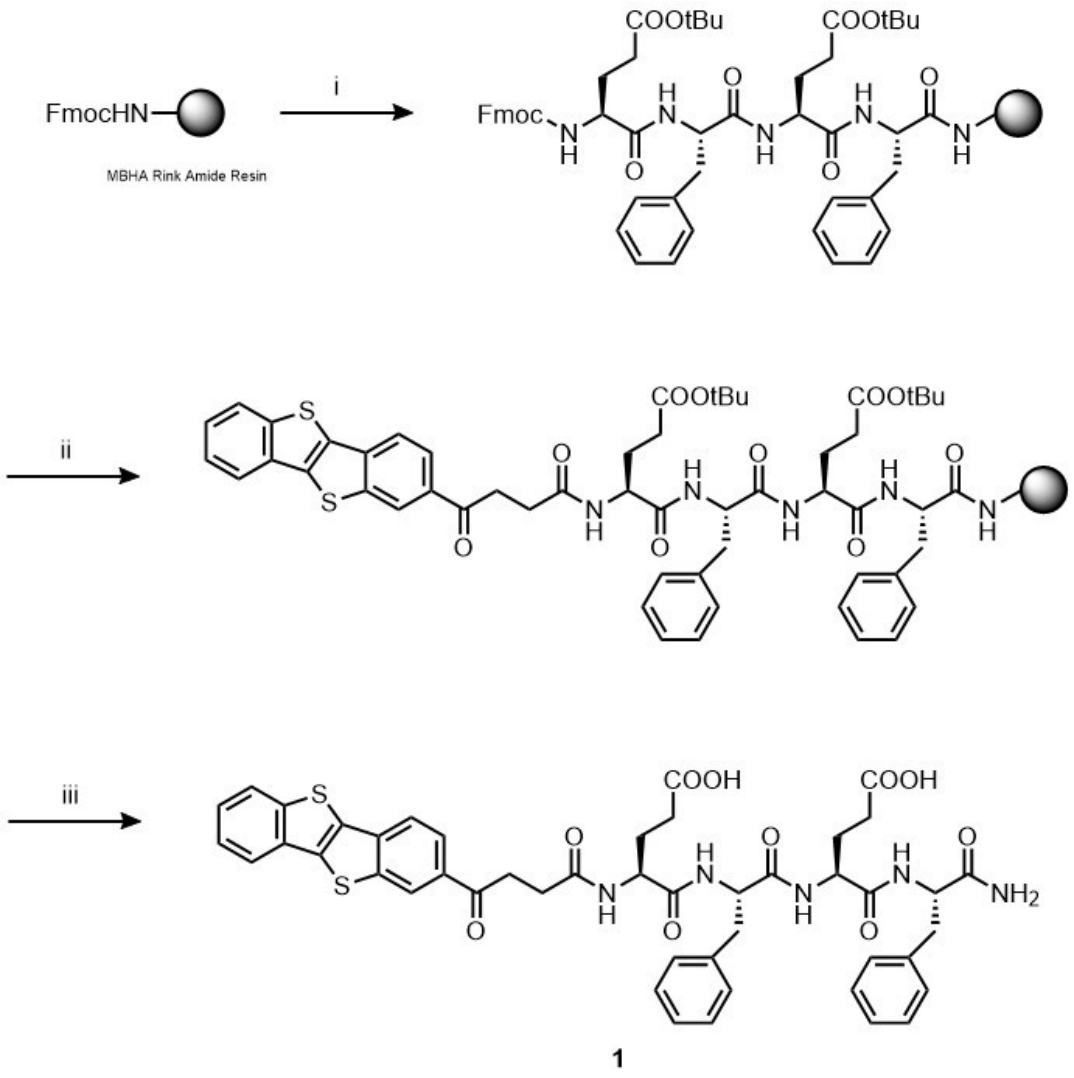
[1]Benzothieno[3,2-b]benzothiophene (5g, 20.08 mmol) was dissolved in dry dichloromethane (500mL), under nitrogen, followed by the addition of aluminium chloride (8.03g, 60.22 mmol) at -50°C. The reaction mixture was stirred for 45 min, until the reaction mixture become dark red. Succinic anhydride was added dropwise (2.3g, 22.98mmol), and the mixture was stirred for 4h at -20°C. The reaction mixture was quenched with ice water (50mL) and methanol (100mL). The with precipitate was filtered and disperse in 200mL of a solution of hydrochloric acid in water (0.1M) and stirred for 4h at room temperature. The white solid was filtered and dry at 100°C under vacuum overnight. The crude product was washed with hot toluene until the toluene was clear. The solid was then recrystallized in tetrahydrofuran with extractive crystallization in

tetrahydrofuran to afford 4-oxo-([1]benzothieno[3,2-b]benzothienyl)-butyric acid (4.100g, 12.04mmol, 60% yield) as crystalline white solid.

^{1}H-NMR (500MHz, DMSO) δ 12.19 (s, 1H), 8.91 (d, J=1.1 Hz, 1H), 8.22 (dd, *J*=6.7, 2.2 Hz, 1H), 8.19 (d, *J*=8.4 Hz, 1H), 8.17-8.15 (m, 1H), 8.10 (dd, *J*=8.3, 1.4 Hz, 1H), 7.61-7.53 (m, 2H), 3.42-3.37 (m, 2H), 2.68-2.63 (m, 2H).

^{13}C-NMR (126 MHz, DMSO) δ 198.95, 175.22, 143.53, 143.01, 137.92, 136.87, 134.52, 133. 72, 133.41, 127.59, 126.87, 126.93, 125.90, 123.56, 122.92, 34.64, 29.36.

Solid phase peptide synthesis (compound 1)



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Compound **1** was synthesized using standard solid phase 9-Fluorenylmethoxycarbonyl (Fmoc) chemistry on Rink amide resin. When not in use the resin was dried and stores in freezer with the amino-terminus Fmoc-

protected. The MBHA Rink Amide Resin was purchased from Irish Biotech (commercial loading 0.68 mmol/g); O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU), 1-Hydroxybennzotriazole hydrate (HOBt), N,N-Diisopropylethylamine, Piperidine, all the amino acids and solvents were purchased from Sigma-Aldrich. SPPS was performed in a standard vessel for manual SPPS equipped with a glass frit and two outlets. The stirring was achieved by bubbling nitrogen from below, thus all the steps have been carried out under N_2 flux. A washing step implies 1 min of stirring and then removal of solvent. In general, 10 mL of solvent must be used for 1 gram of resin. The resin was prepared dumping 1g of resin into the SPPS vessel and then 10mL of DMF were added for resin swelling and stirred gently for 30 minutes. For each amino acid coupling step (i), Fmoc deprotection was performed by mixing the resin in a piperidine/DMF (2:8) solution for 15 minutes (2x), then washing with DMF (x3). For all of the amino acid couplings we used the following protocol: 4.0 eq. (relative to the resin loading) of Fmoc-protected amino acid were activated externally with 3.9 eq. of HBTU, 3.9 eq. of HOBt and 12 eq. of DIPEA in DMF. This mixture was then added to a peptide chamber containing the Rink amide resin and mixed for 3 hours. All coupling and deprotection steps were monitored by performing a Kaiser test on a few resin beads which were removed from the peptide chamber after drying with DCM. If necessary, the coupling step was repeated. The deprotection activation and coupling steps were repeated until the desiderated structure was obtained.

The coupling with BTBT functionalized core (ii) was performed using 1.5 equiv of BTBT, 1.45 equiv of HBTU and HOBt and 4.5 equiv of DIPEA. The reaction was performed for 5 hours. The solvent was removed and the resin was washed with DMF (3 x 10 mL), DCM (3 x 10 mL) and DMF (2 x 10 mL).

Cleavage from the resin and removal of side-chain protecting groups (iii) was accomplished by stirring the resin with 10 mL of TFA, water and TIPS (95:2.5:2.5) for 3 hours. The solvents were collected in a flask and the resin (that eventually turned red) was washed with DCM (3 x 10 mL). Solvents collected were concentrated in rotavapor (a potassium hydroxide trap was used) to the half. DCM was added and the volatiles were evaporated again. The process was repeated 3 times, after which the solvents were evaporated to dryness. The product was precipitated from cold diethyl ether and the precipitated peptide was isolated by centrifugation and lyophilized. A white powder was obtained.

ESI-MS: $[M+Na]^+$ calculated for $C_{46}H_{45}N_5NaO_{10}S_2$ 914.2506, found: 914.3.

^{1}H-NMR (DMSO-d6, 500 MHz): δ 12.09 (bs, COOH), 8.87 (s, 1H, BTBT), 8.23-8.13 (m, 3H), 8.08 (dd, J = 8.4, 1.5 Hz, 1H, BTBT), 8.05 (d, J = 7.7 Hz, 1H, NH), 7.94 (d, J = 7.8 Hz, 1H, NH), 7.83 (d, J = 8.1 Hz, 1H, NH), 7.60-7.50 (m, 2H, BTBT), 7.36 (s, 1H, NH_2), 7.28-7.21 (8H, m, Ar of Phe), 7.19-7.12 (m, 2H, Ar of Phe), 7.10 (s, 1H, NH2), 4.59-4.46 (m, 1H, H_α, Phe), 4.46-4.39 (m, 1H, H_α, Phe), 4.24-4.15 (m, 2H, 2 H_α, Glu), 3.45-3.27 (m, 2H, BTBT, partially covered by H2O signal), 3.07-2.98 (m, 2H, H_β, Phe), 2.86-2.77 (m, 2H, H_β, Phe), 2.67-2.47 (m, 2H, BTBT, partially covered by DMSO signal), 2.25-2.11 (4H, m, H_γ, Glu), 1.89-1.79 (2H, m, H_β, Glu), 1.77-1.65 (4H, m, H_β, Glu) ppm.

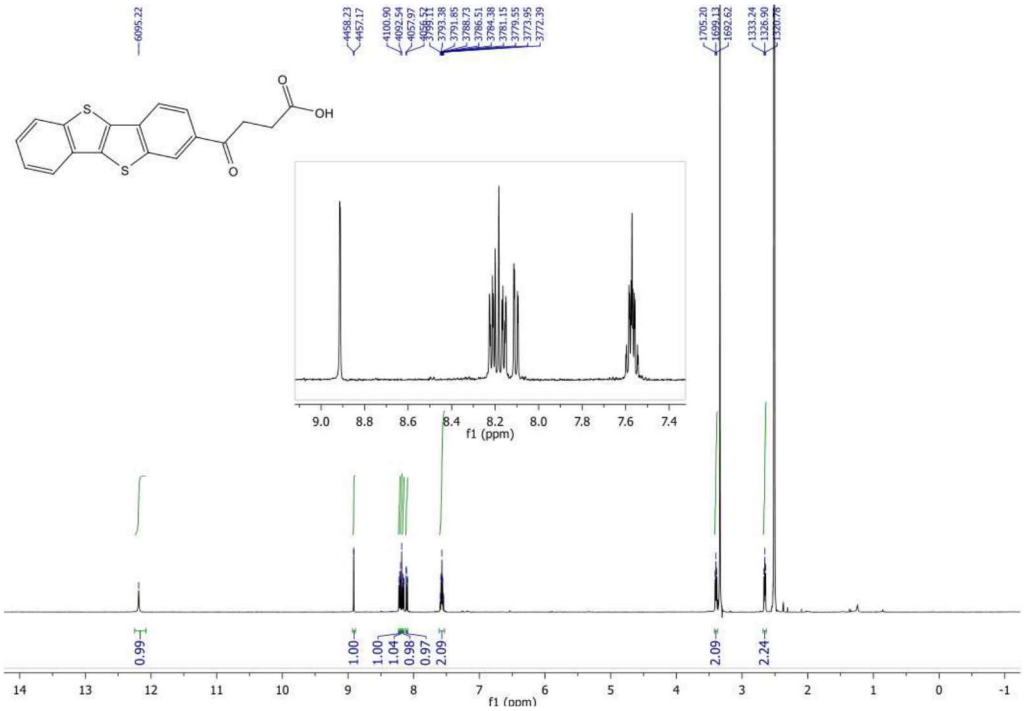
^{13}C-NMR (126 MHz, DMSO-d6): δ 198.28, 174.10, 174.08, 171.80, 171.41, 171.08, 170.67, 142.20, 141.70, 137.75, 136.59, 135.55, 133.35, 132.42, 132.11, 129.18, 129.15, 128.04, 126.32, 126.26, 126.22, 125.61, 125.26, 124.68, 122.29, 121.69, 53.87, 53.70, 52.26, 52.19, 37.49, 37.00, 33.60, 30.15, 30.12, 29.26, 27.27, 27.22 ppm.

FT-IR (KBr): $\tilde{\nu}$ (cm^{-1}) = 3287, 3086, 3060, 3029, 2924, 1725, 1660, 1651, 1615, 1545, 1407, 1199, 1170, 749, 699, 646, 582.

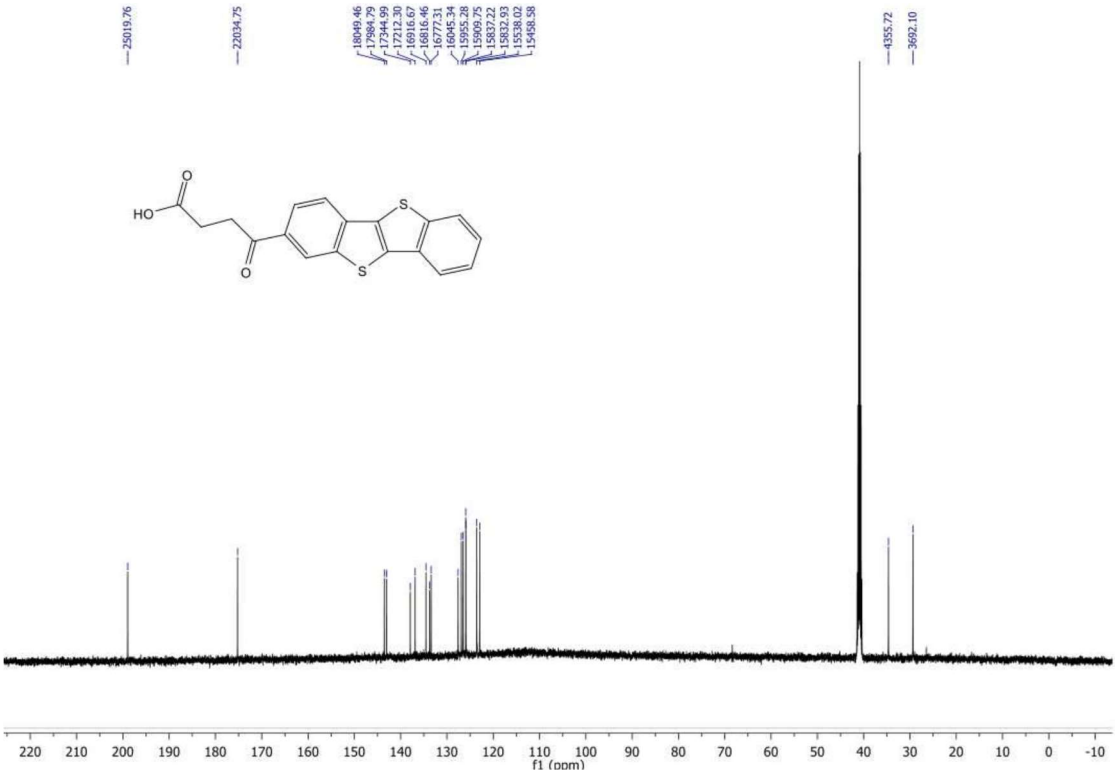
References

1. M. Saito, I. Osaka, E. Miyazaki, K. Takimiya, H. Kuwabara and M. Ikeda, *Tetrahedron Letters*, 2011, **52**, 285-288.

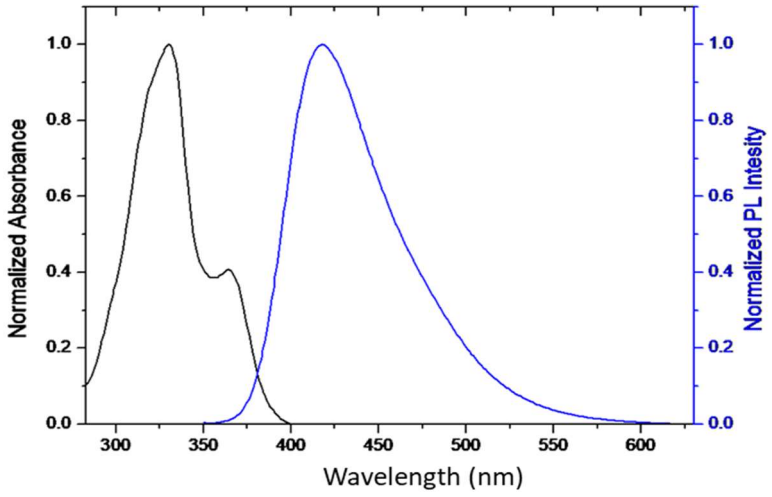
CHARACTERIZATION OF THE NEW COMPOUNDS



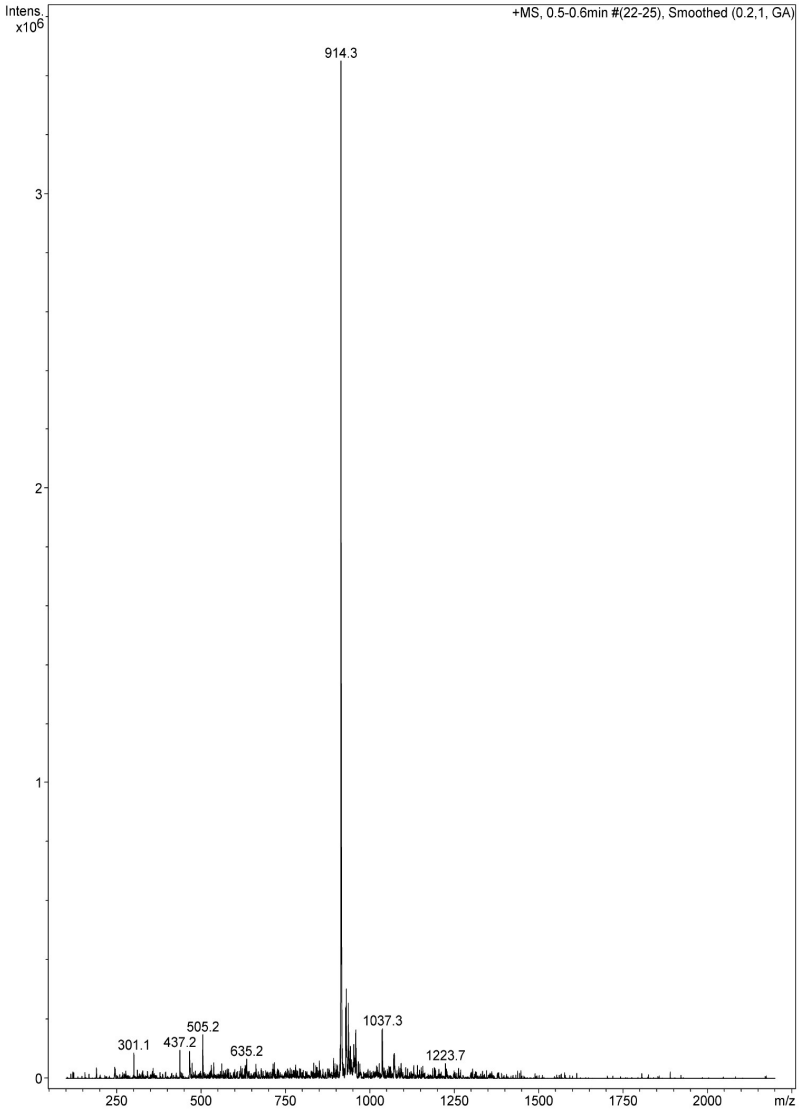
S1. ^{1}H NMR (DMSO-d_6, 500 MHz) of **2**



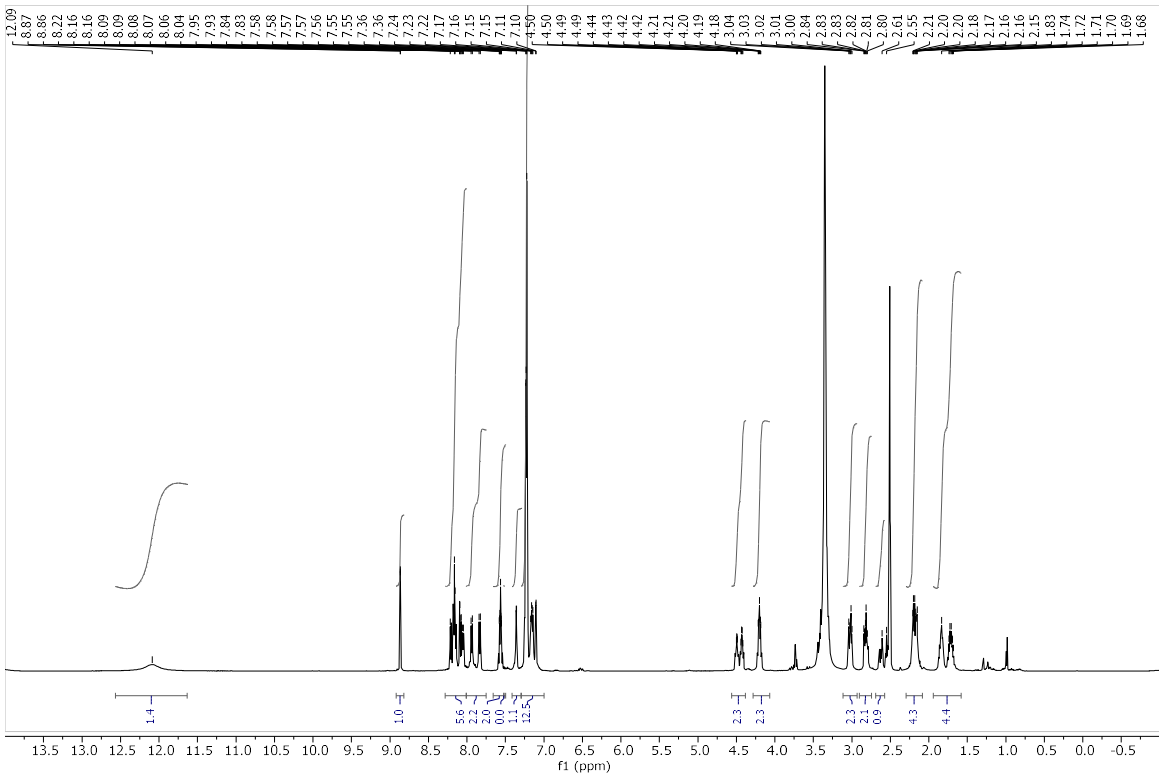
S2. ^{13}C-NMR (DMSO-d_6, 126 MHz) of **2**



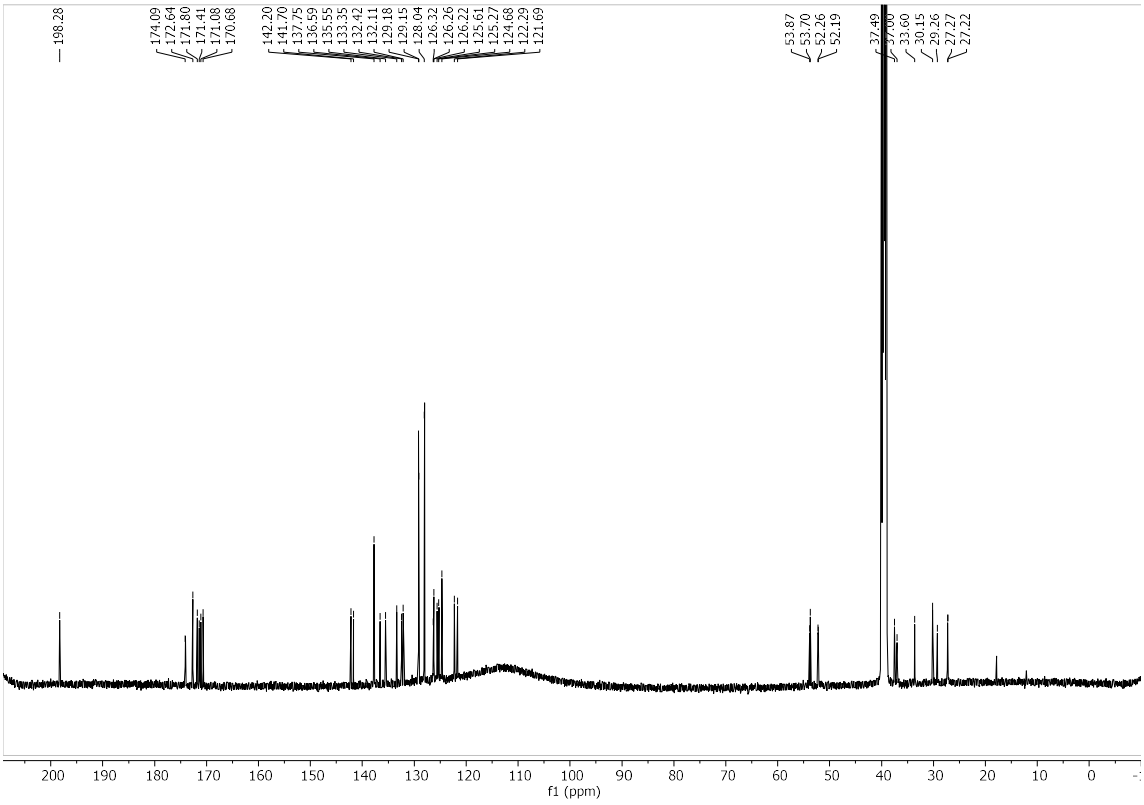
S3. Normalized absorption (black line) and emission (blue line) spectra of **2** in dichloroethane.



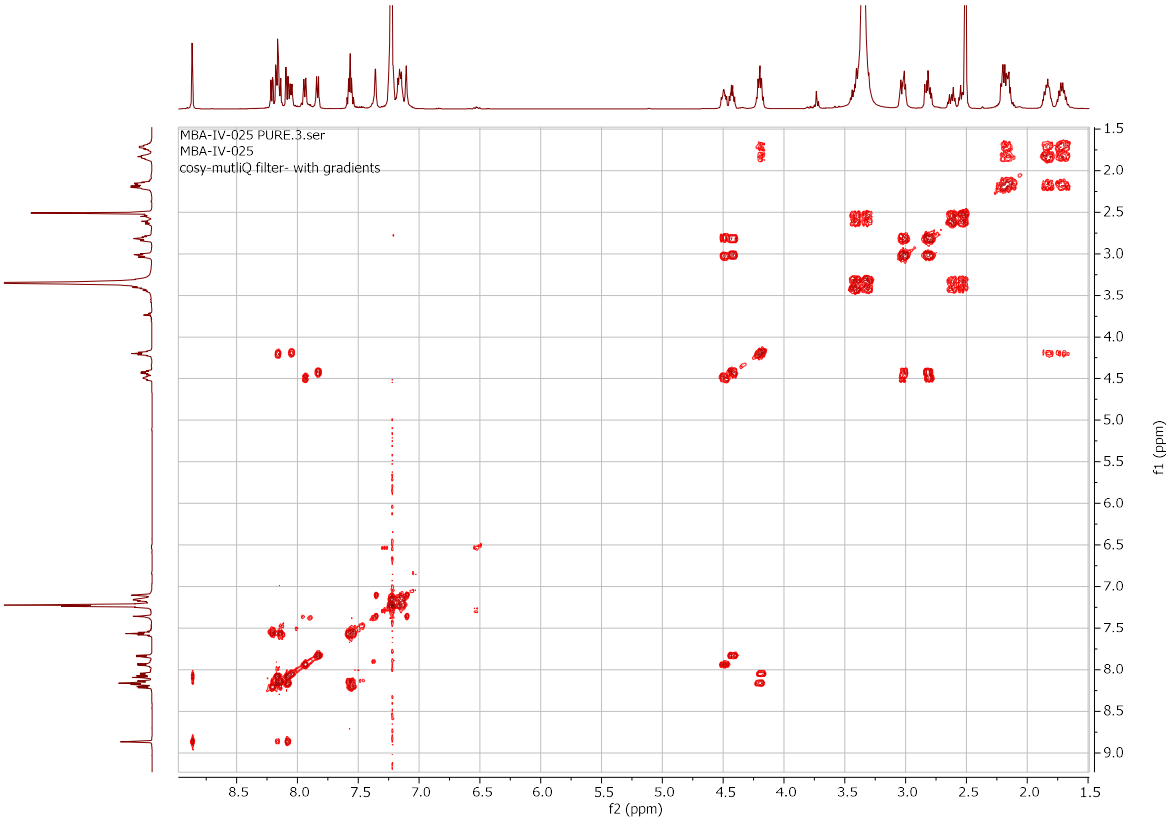
S4. ESI-MS compound **1** in MeOH



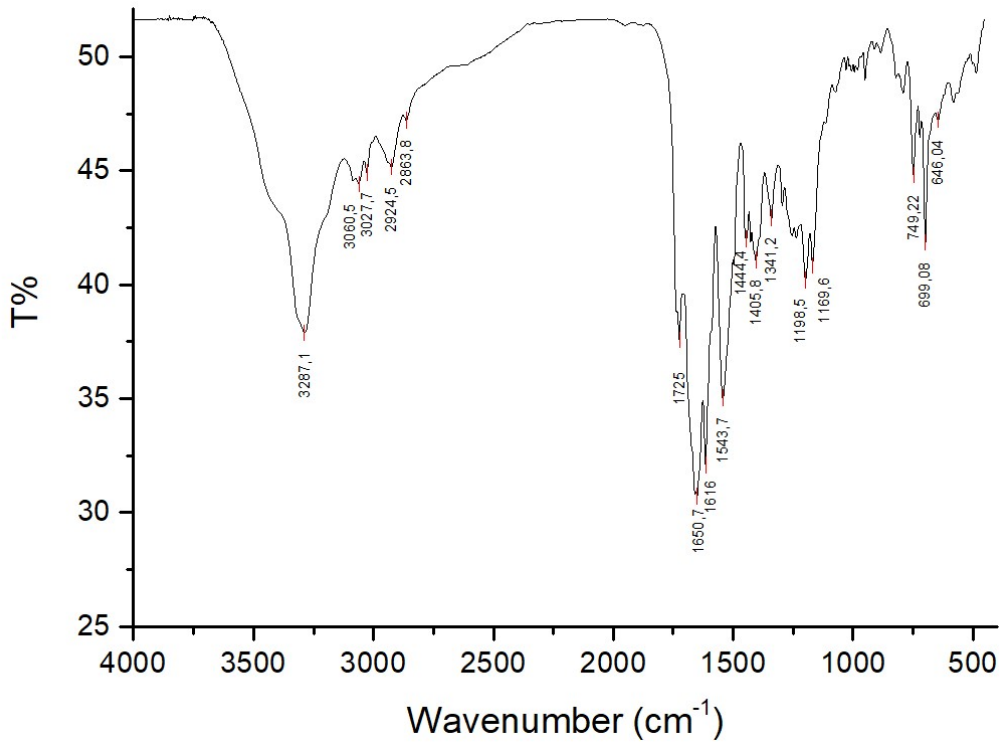
S5. ^{1}H NMR (DMSO-d_6, 500 MHz) of **1**



S6. ^{13}C-NMR (DMSO-d_6, 126 MHz) of **1**



S7. 2D COSY NMR of **1** DMSO-d_6.

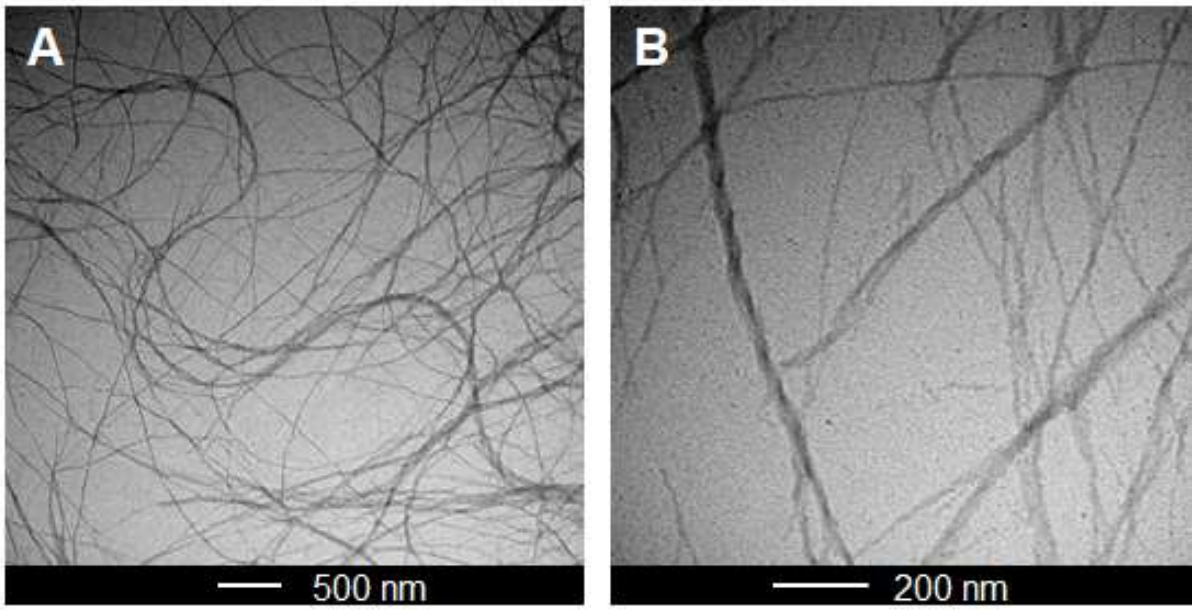


S8. FT-IR spectrum of **1** (KBr disk).

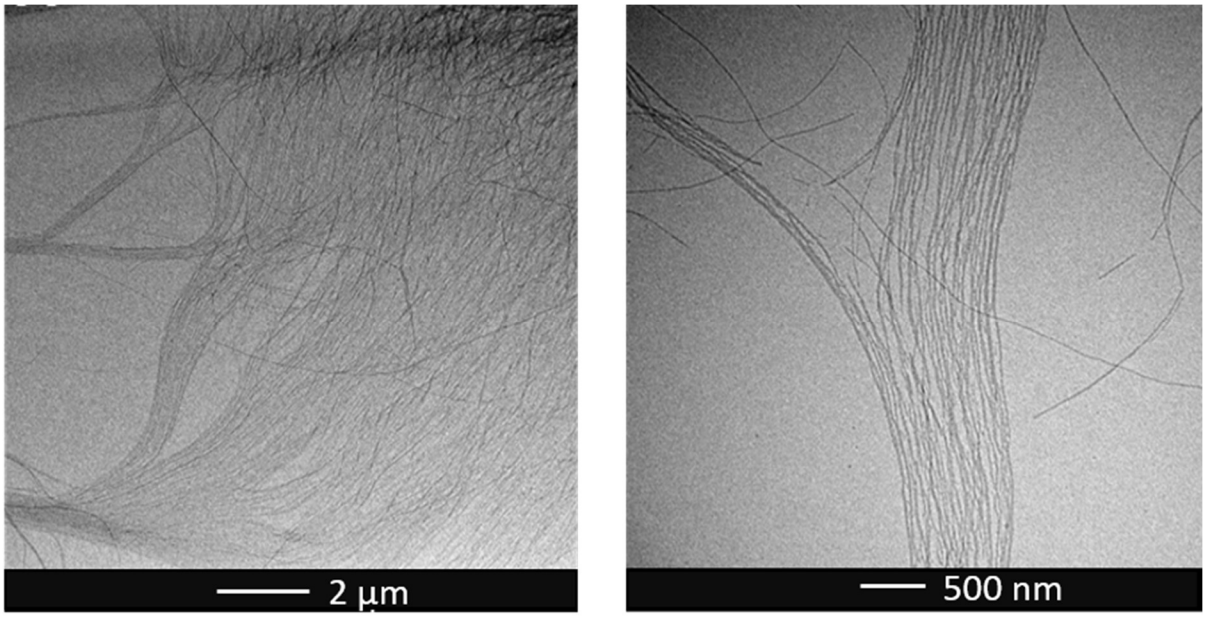
PREPARATION AND CHARACTERIZATION OF GELS

pH triggered gelation. A known amount of 1 was introduced in a 4 mL vial. Then 900 μL of milliQ water were added. NaOH 1N was added in portions of 10 μL till a clear solution was obtained (sonication and/or vortex) followed by addition of water to a final volume of 1 mL. Then HCl 1N was added in portions of 10 μL (vortex was avoided). Gel formation was assessed by the vial inversion test.

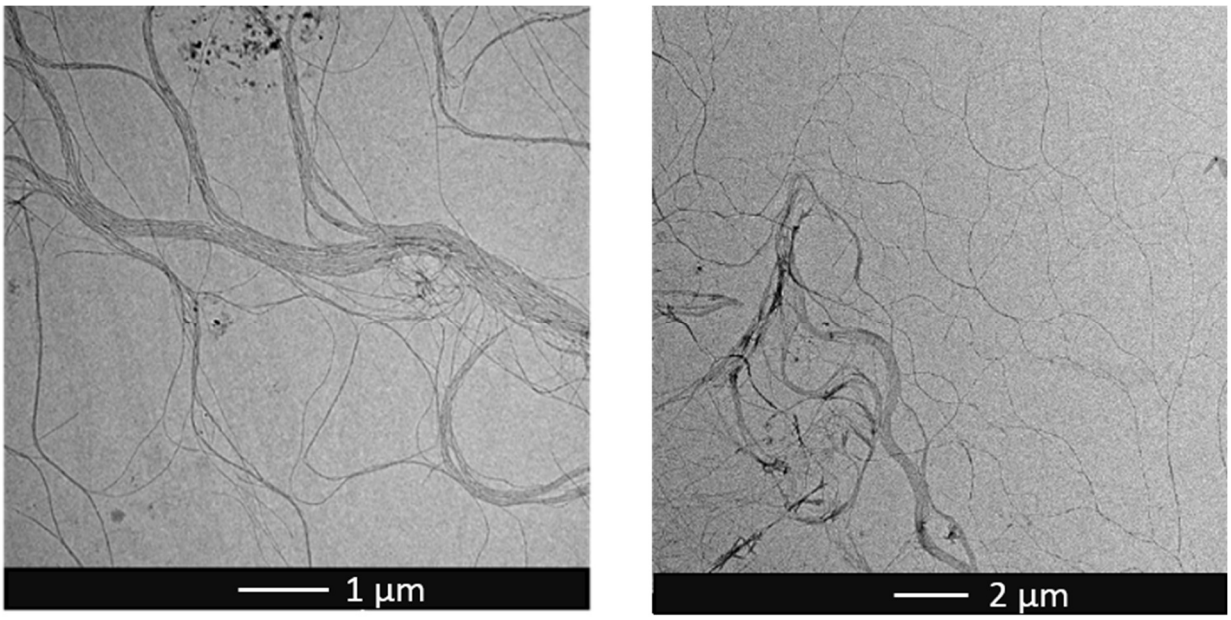
Salt triggered gelation. A known amount of 1 was introduced in a 4 mL vial. Then 900 μL of milliQ water were added. NaOH 1N was added in portions of 10 μL till a clear solution was obtained (sonication and/or vortex) followed by addition of water to a final volume of 1 mL. Then a solution of salt 1N was added in portions of 10 μL till gel formation was observed. Gel formation was assessed by the vial inversion test.



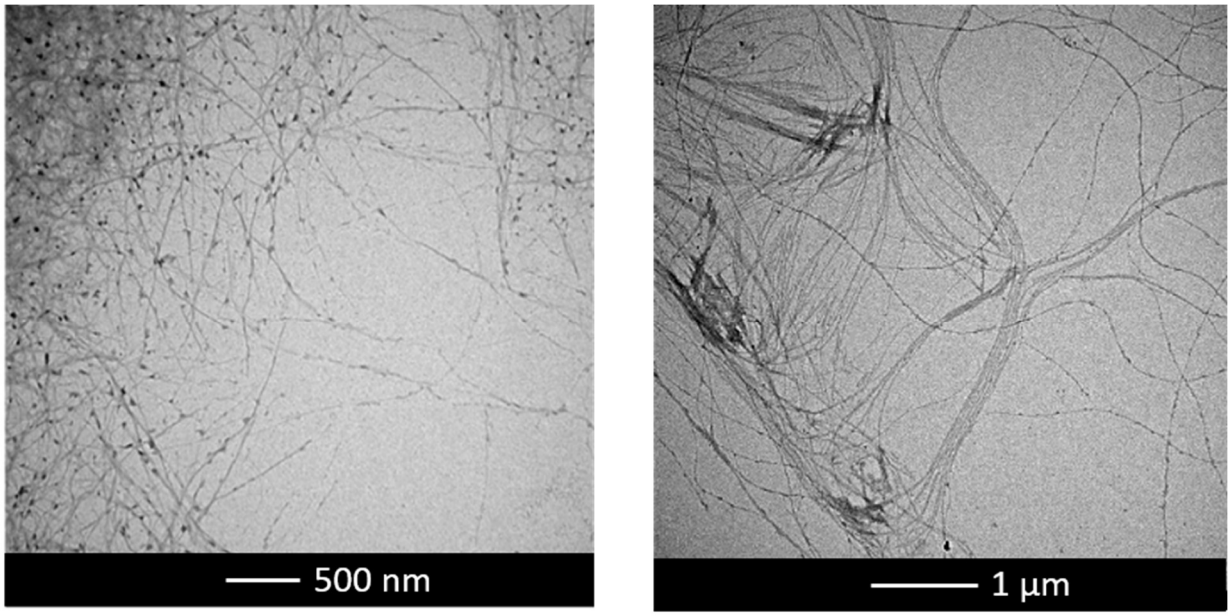
S9. TEM images of HCl-gel



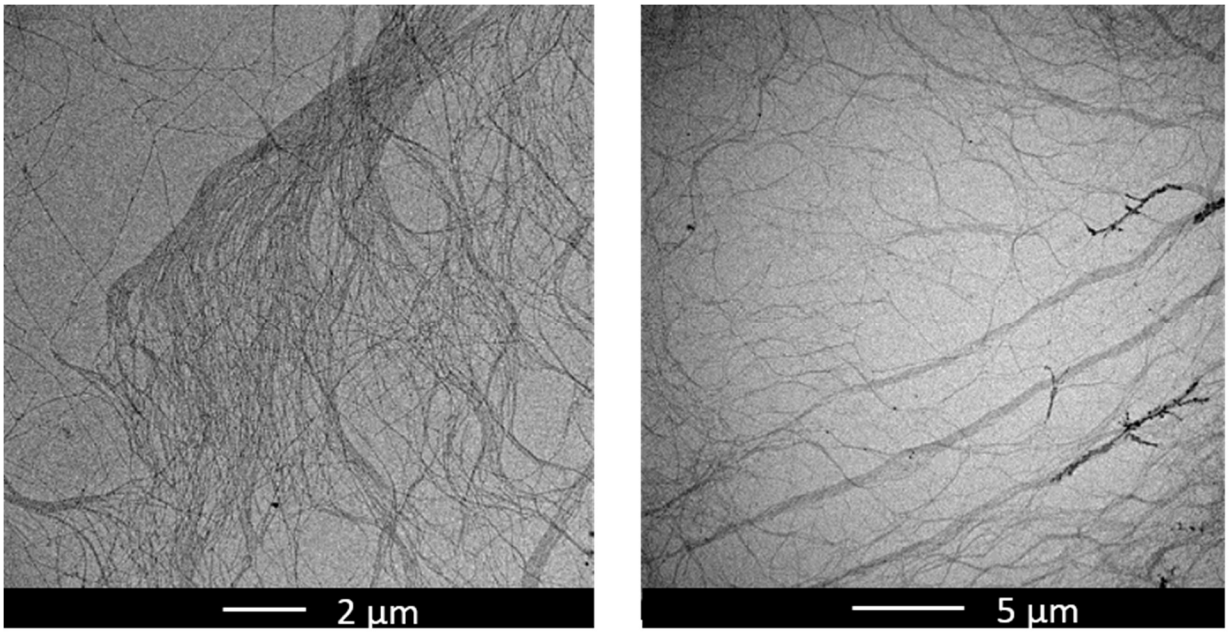
S10. TEM images of NaOH-gel.



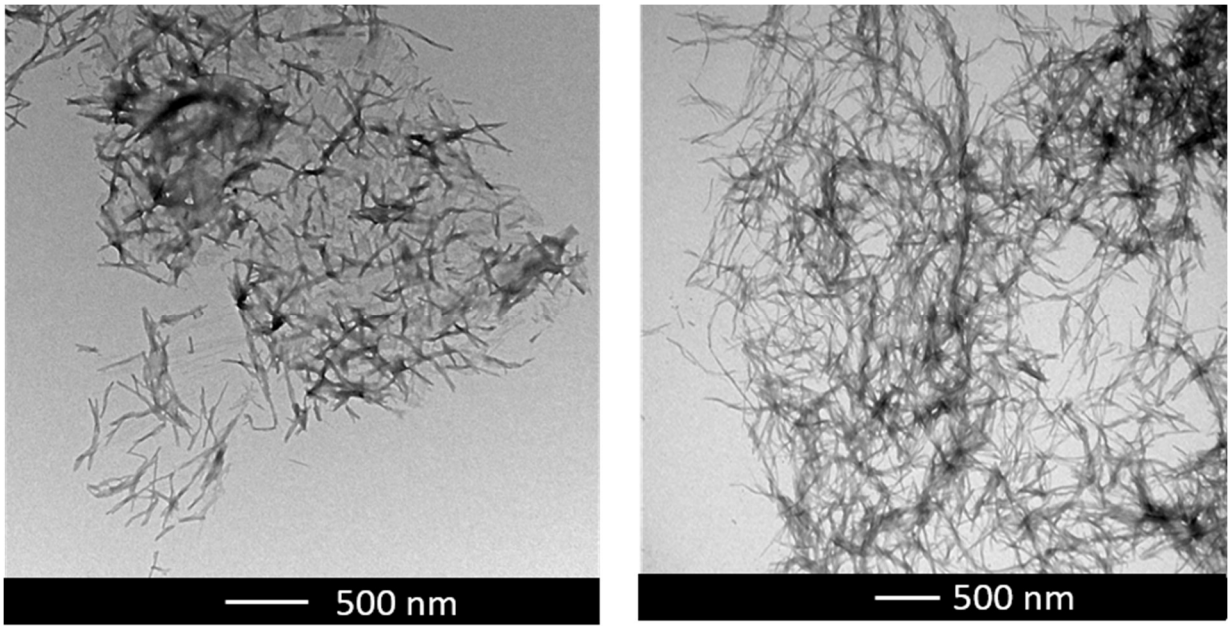
S11. TEM images of LiCl-gel.



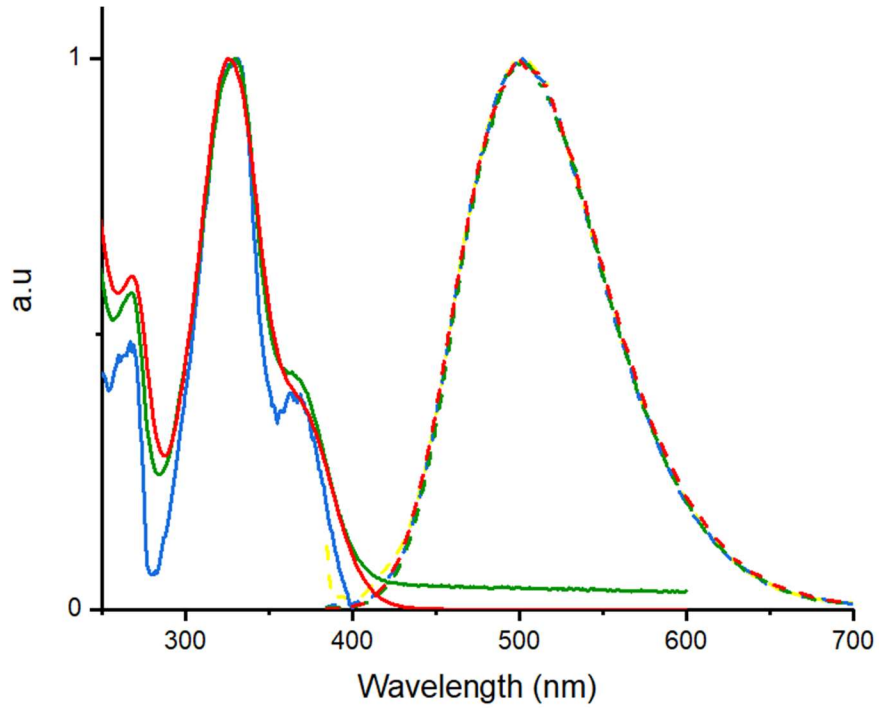
S12. TEM images of NaCl-gel.



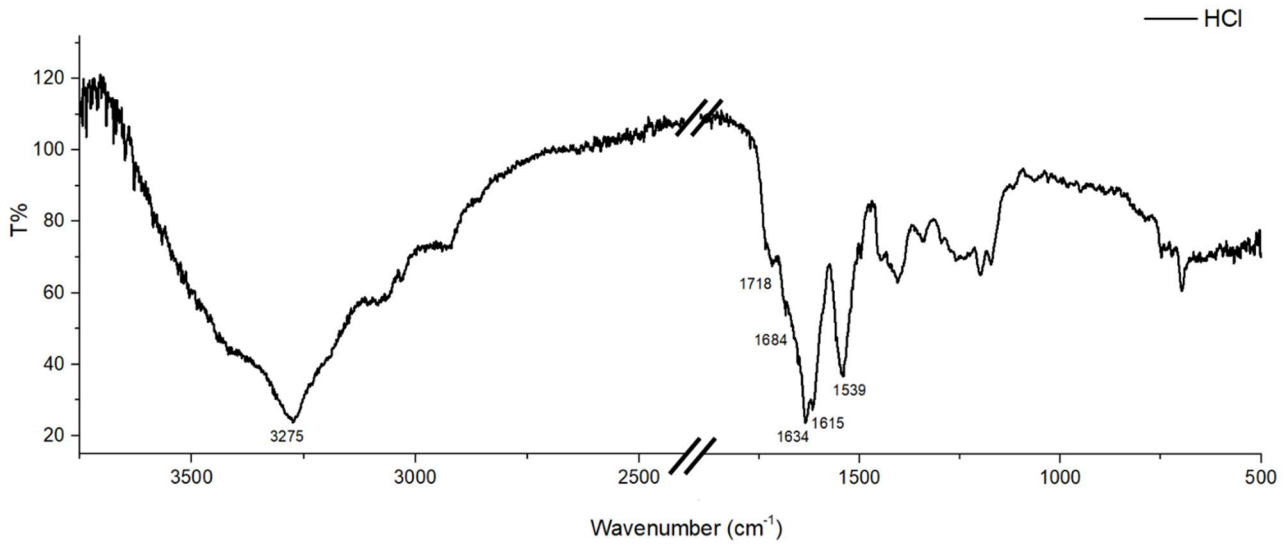
S13. TEM images of KCl.



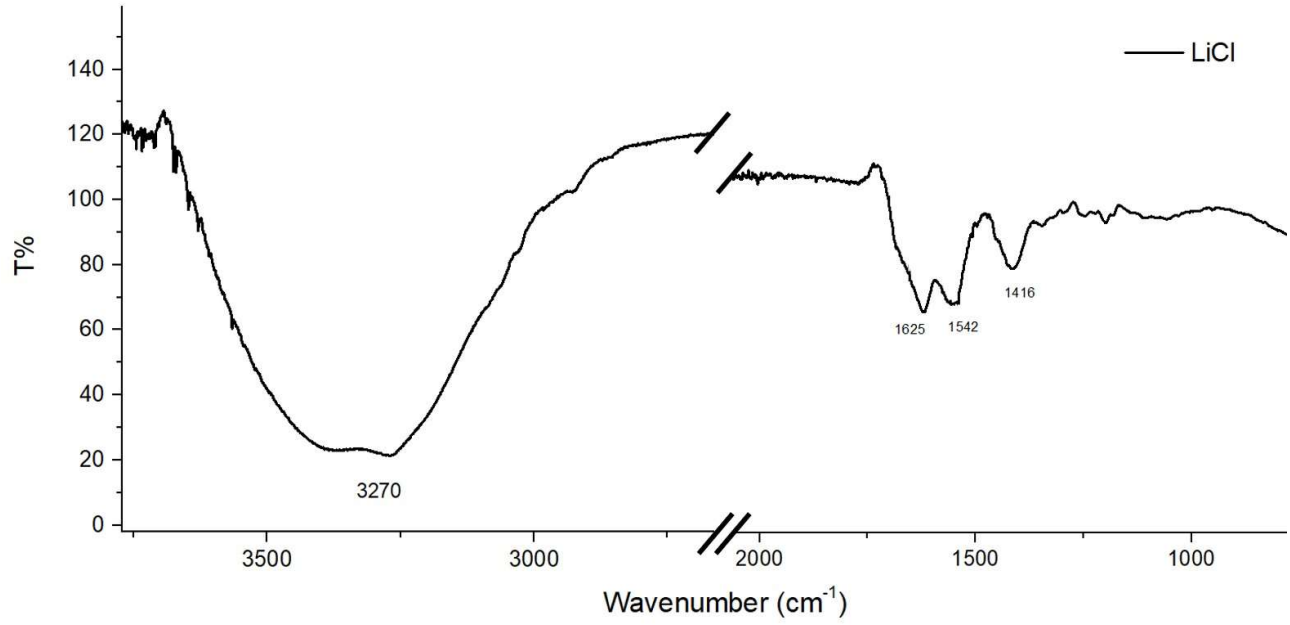
S14. TEM images of $CaCl_2$-gel.



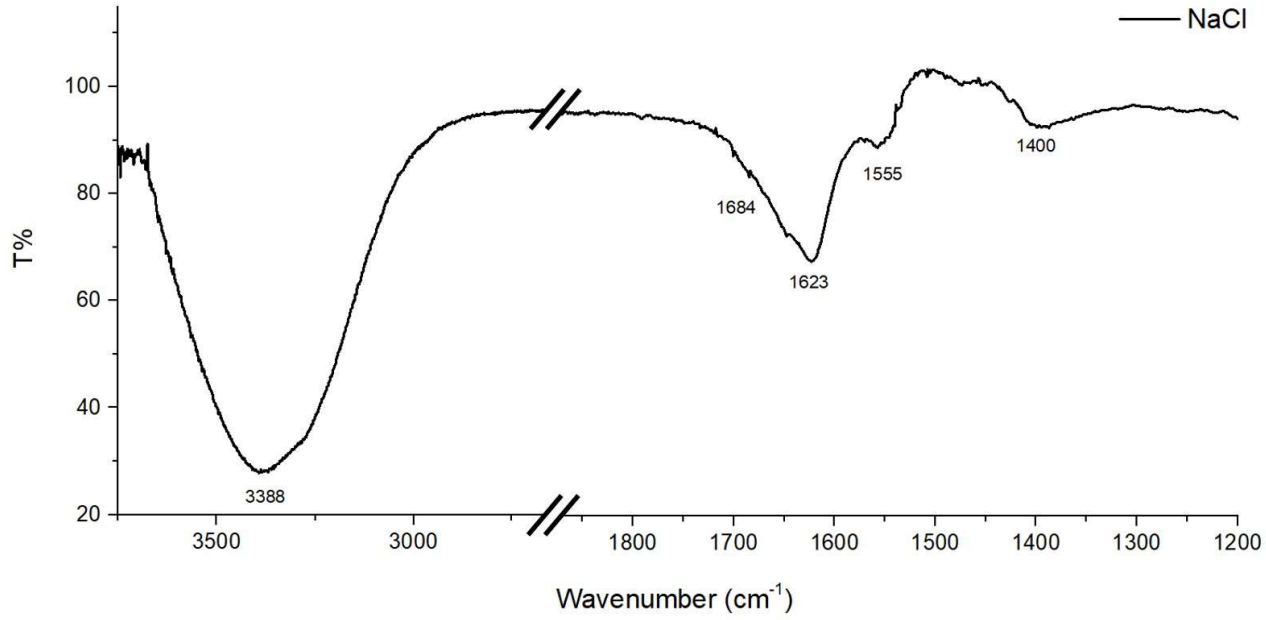
S15. Normalized absorption (solid line) and emission spectra (dashed line) of aqueous basic solutions of **1** at 10^{-6} M (yellow), 10^{-5} M (blue), 10^{-4} M (green), 10^{-3} M (red) concentration. The absorption at 10^{-6} M concentration was too weak to be recorded in the same conditions.



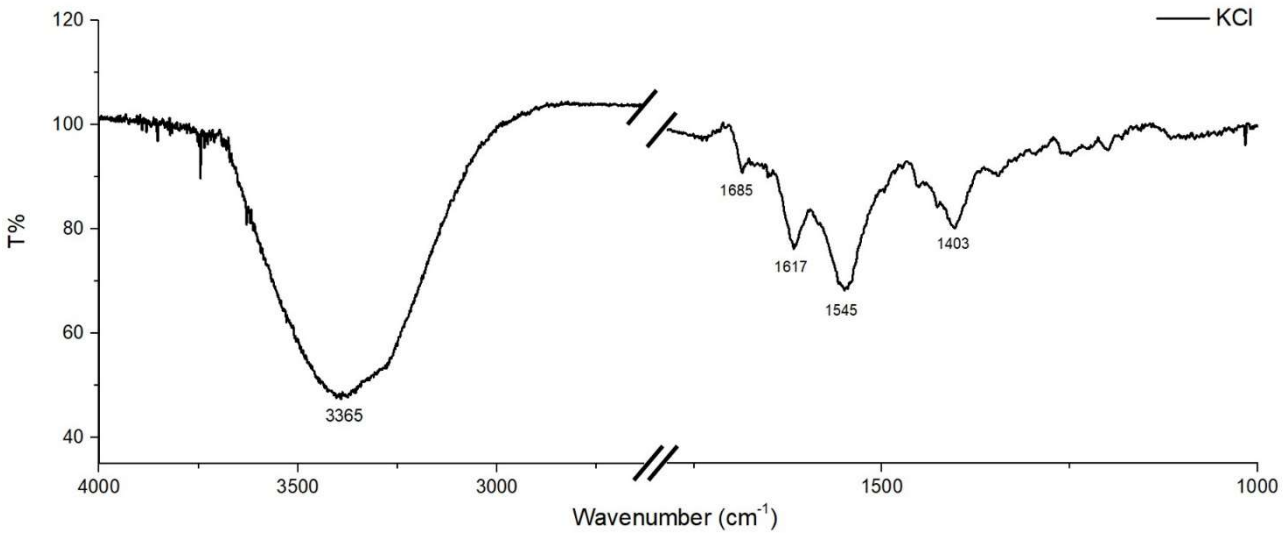
S16. ATR-FTIR spectrum of HCl-gel.



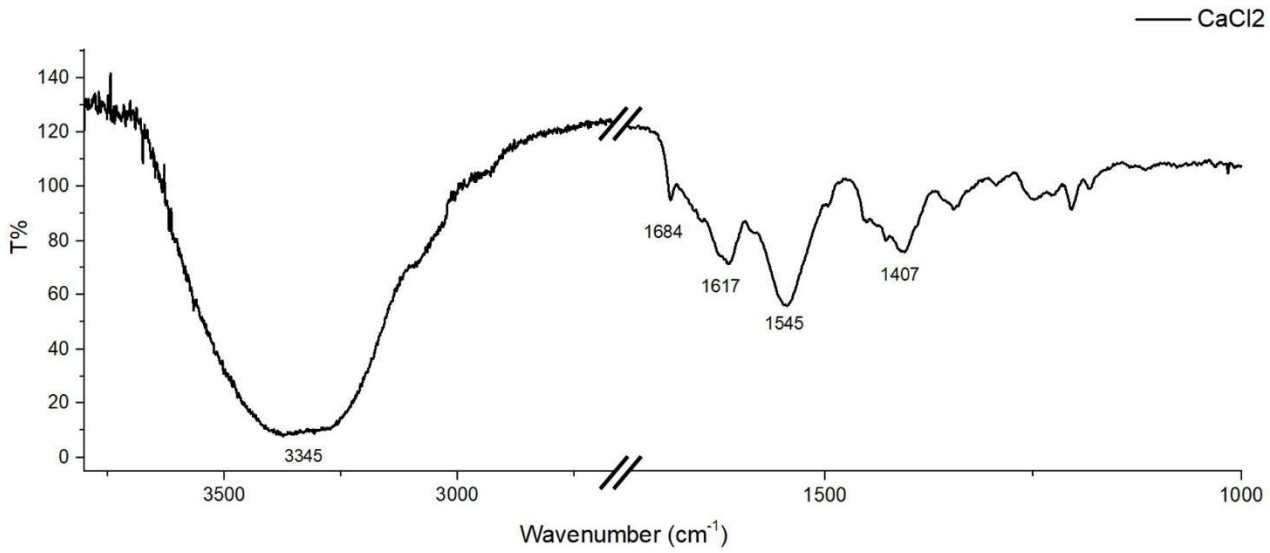
S17. ATR-FTIR spectrum of LiCl-gel.



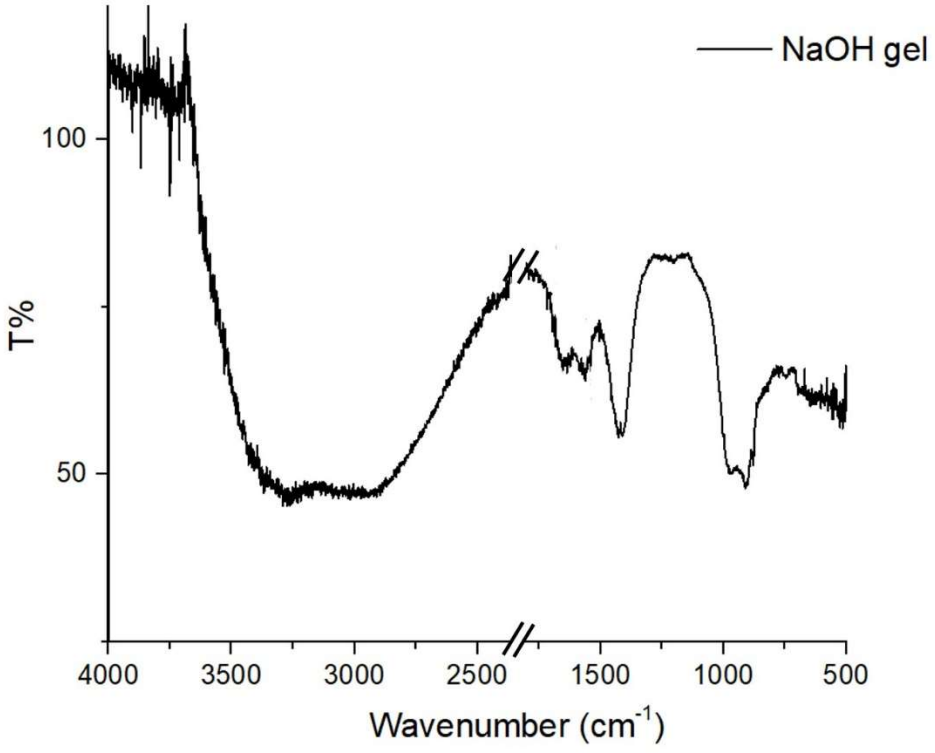
S18. ATR-FTIR spectrum of NaCl-gel.



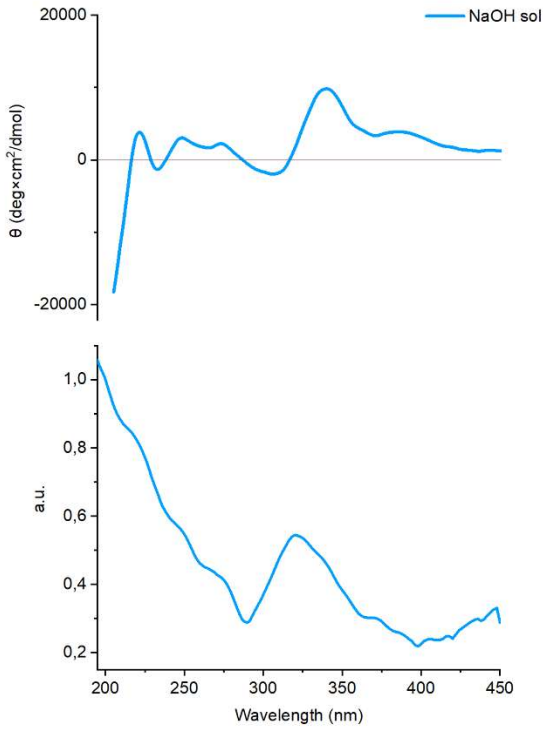
S19. ATR-FTIR spectrum of KCl-gel.



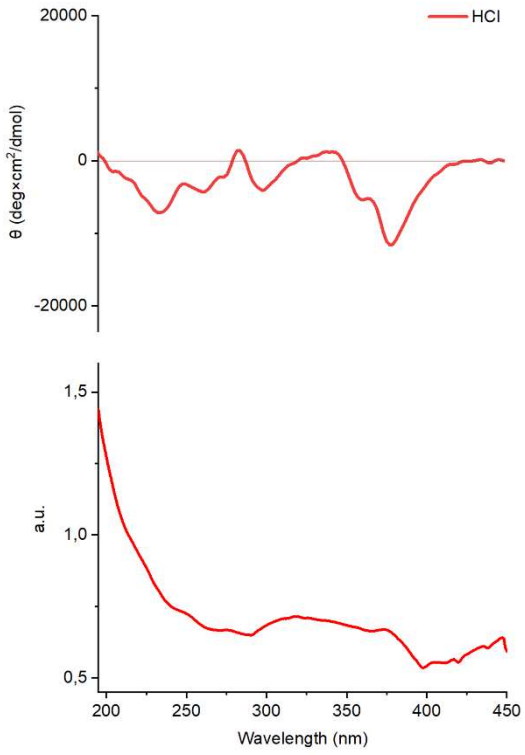
S20. ATR-FTIR spectrum of $CaCl_2$-gel.



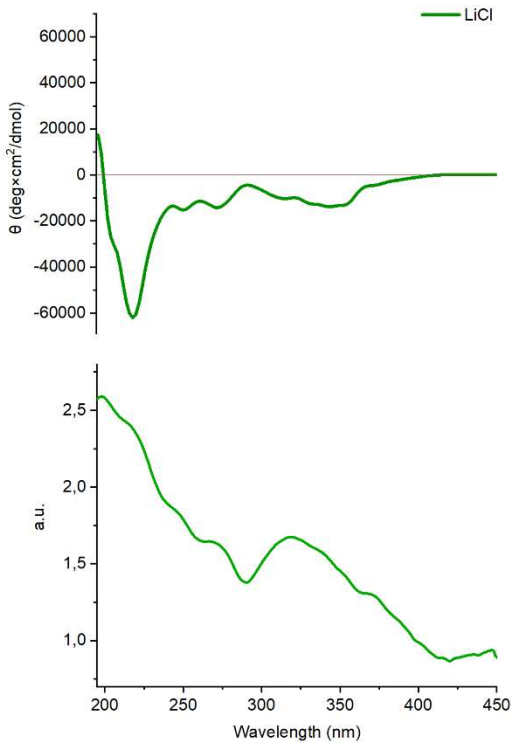
S21. ATR-FTIR spectrum of NaOH-gel.



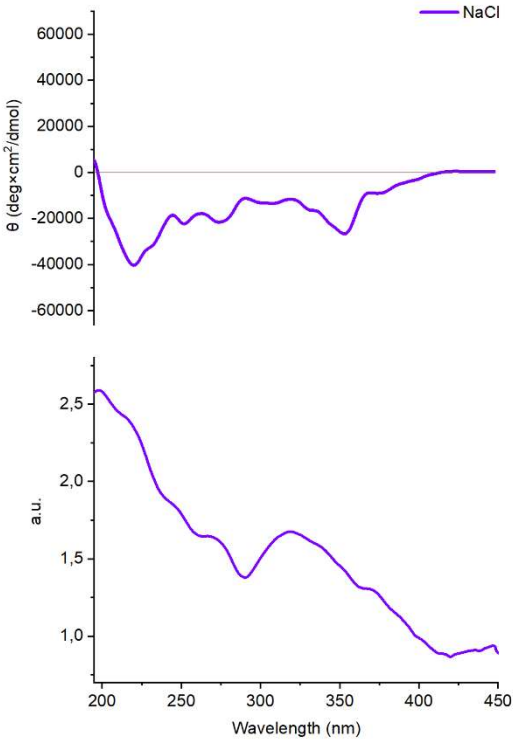
S22: CD spectrum of NaOH solution and corresponding absorption spectrum.



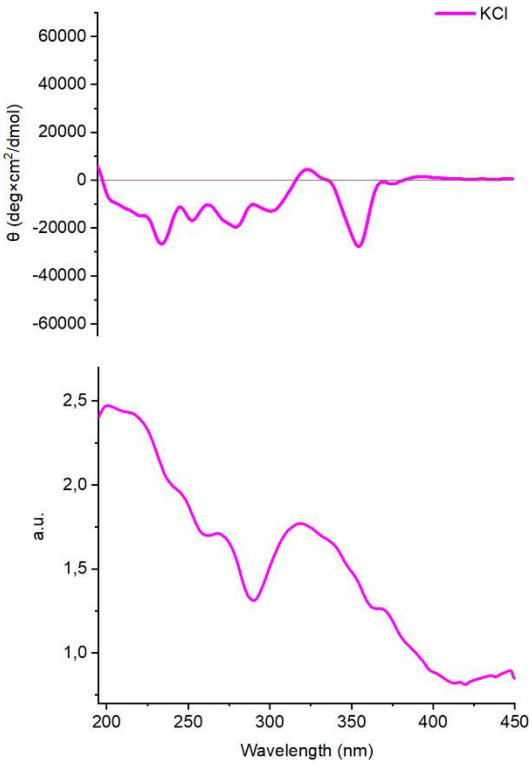
S23: CD spectrum of HCl gel and corresponding absorption spectrum.



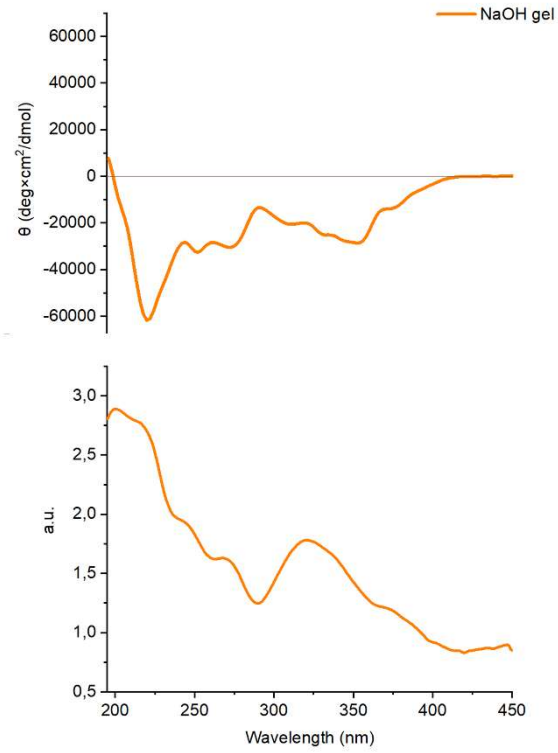
S24: CD spectrum of LiCl gel and corresponding absorption spectrum.



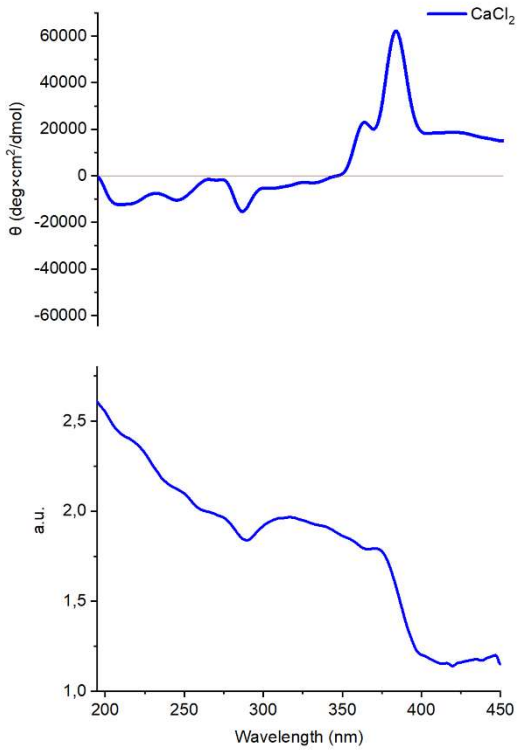
S25: CD spectrum of NaCl gel and corresponding absorption spectrum.



S26: CD spectrum of KCl gel and corresponding absorption spectrum.



S27: CD spectrum of NaOH gel and corresponding absorption spectrum.



S28: CD spectrum of $CaCl_2$ gel and corresponding absorption spectrum