Electronic Supplementary Material (ESI) for Materials Advances.

Supramolecular assemblies of a 1,8-Naphthalimide conjugate and its aggregation-induced emission property

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Experimental Section:

Materials

1,8-Napthalic anhydride, HBTU and tryptophan were purchased from Sigma Chemicals. Concentrated hydrochloric acid, N,N-dimethyl formamide, methanol and other solvents were purchased from Rankem, India.

Instrumentation

HRMS: Mass spectra were recorded on a Q-Tof micro (Waters Corporation) mass spectrometer by a positive-mode electrospray ionization process.

NMR: Nuclear magnetic resonance (NMR) studies were carried out on Bruker DPX500 MHz spectrometer at 300 K temperature. Concentration was in the range of 5−10 mM in DMSO-d_6.

Single Crystal diffraction: The single crystal X-ray diffraction data were collected on a Bruker D8 Quest photon 100 CMOS diffractometer at 150 K, using graphite-monochromatic Mo-Kα radiation (0.71073Å). Data were processed and corrected for Lorentz and polarization effects, solved by standard direct methods and refined by full matrix least squares on F2. All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms are included in the structure factor calculations in geometrically idealized positions with isotropic thermal displacements depending on the parent atom, using a riding model.

UV-Vis Spectroscopy: UV-visible spectra were recorded on a Shimadzu 2401 PC UV-vis spectrophotometer. The samples were prepared in different mixed solvents. During measurement of each different NMI-GW-OMe solution, spectrometer was calibrated using that particular solvent system. During variable temperature UV-Vis spectroscopic measurement, temperature was raised from 30 °C to 90 °C by increasing temperature 10 °C.

Fluorescence and Life time Measurement: The fluorescence emission study completed by using Horiba Photon Technology International 2710 Spectrophotometer instrument with 1.0 cm path length quartz cuvette. All the samples were excited at 343 nm and emission data were collected in between 350 nm to 650 nm wavelength regions. During temperature-dependent solid state fluorescence study, emission spectra were recorded by increasing temperatures from 30-100 °C. The excited-state average lifetimes of the hydrogels were measured by using equation

$$\langle \tau \rangle = \sum_{i=1}^{n} \alpha_i \tau_i$$

where τ_i is the fluorescence lifetime of various fluorescent species and α_i is the normalized pre-exponential factor.

Rheological Study: The rheological experiments of the hydrogels were performed with an advanced rheometer (AR 2000, TA Instrument, USA) using cone plate geometry on a Peltier plate. The diameter of the plate was 40 mm with a cone angle of 4° and a plate gap of 121 μm.

MD Study: The structural feature of the NMI-GW-OMe have been investigated with the help of DFT calculations. The structure of the compound was minimised using B3LYP and 6-311g (d,p) level of theory.

TEM Study: TEM studies were carried out by using a FEI Tecnai G2F30-ST instrument operating at 300 kV. NMI-GW-OMe (3.0 mM) was incubated in 9:1 water/DMSO mixture and for organogel NMI-GW-OMe (8.0 mM) was dissolved in xylene. 5 μL solution was drop-casted on the carbon-coated TEM grids (300 mesh). After 1 minute, the excess solvent was removed by the fine tissue paper. The grids were then allowed to dry in vacuum at 30 °C for two days.

Cell culture: Human adult dermal fibroblast (HADF) cells were purchased from NCCS, Pune, India. Cells were grown in high glucose DMEM supplemented with 10% FBS (Invitrogen, USA) and 0.1% antibiotic-antimycotic (100X) (ThermoFisher Scientific, USA). The cells were maintained at 37 °C and 5% CO_2 condition.

Stock solution of NMI-GW-OMe was prepared by dissolving 3 mg of NMI-GW-OMe in 100μl DMSO and 900 μl doubled distilled water (3mg/ml). Different concentration of NMI-GW-OMe (65, 130, 260 and 325 μM) was obtained by addition of appropriate volume of NMI-GW-OMe from the stock solution in cell culture media before treatment on cells. The total volume of cell culture media used in 96 and 24-well plate was 200μl and 400μl respectively.

Cell cytotoxicity assay: MTT assay was performed to test the cytotoxicity of NMI-GW-OMe using MTT assay kit purchased from HiMedia, India. HADF cells were seeded at a density of 2×10^4 cells/well in a 96-well plate and incubated with varying concentrations of NMI-GW-OMe (65, 130, 260 and 325 μM) for 24h. After the incubation period, 10 μl of MTT solution was added in 100 μl of serum-free media to each well of the 96-well plate for incubation at 37 °C, 5% CO_2 in dark for 4 h. 100 μl of solubilisation buffer was added into each well to solubilize the purple formazan crystals formed indicating cellular viability. Absorbance of the solution was measured at 570 nm and 670 nm wavelength (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer, Finland). All the experiments were performed in triplicate. The percentage of cell viability was estimated by using the following formula,

$$\% \text{ cell viability} = [(Abs_{test} - Abs_{blank}) / Abs_{control} - Abs_{blank}] \times 100$$

where, Abs_{test}, Abs_{blank} and $Abs_{control}$ signify absorbance of the sample, blank and control at 570 nm.

Cell imaging study: HADF cells were cultured on separate glass coverslips placed in 24-well plate for 24h at 37 °C and 5% CO_2 condition at the same density as in MTT assay. The cells were allowed to incubate with 65, 130, 260 and 325 μM of NMI-GW-OMe for 24 h at 37 °C and 5% CO_2 in separate well plates for performing the intracellular fluorescence study. Fluorescence images of HADF cells were acquired with the help of an inverted fluorescence microscope (Nikon eclipse Tí U, Japan) equipped with an objective lens of 20X magnification. As indicated from the fluorescence spectroscopic studies, the sample showed fluorescence emission at 425 nm and 530 nm. The *in vitro* studies further confirmed fluorescence when cells were excited at the blue region respectively.

Synthesis

(a) **Synthesis of NMI-Gly-OH :** 1,8-Naphthalic anhydride (5.0 mmol, 0.99 g) and glycine (7.0 mmol, 0.52 g) were dissolved in 15 mL of dry dimethyl formamide (DMF) and heated under refluxed condition in N_2 atmosphere for 12 h on a heating mantle[1]. The reaction mixture was cooled to room temperature. The insoluble portion was filtered off and the brown solution was poured into 100 mL of distilled water. The white precipitate was filtered off and washed thoroughly with distilled water. The crude product was air-dried.

Yield: 1.21 g (4.74 mmol, 95.3 %).

(b) Synthesis of NMI-Gly-Trp-OMe: 1.17 g (4.58 mmol) of NMI-Gly-OH was taken in a RB flask and dissolved in 10 mL of dry DMF. The resulting solution was cooled in an ice-salt bath. 1.81 g HBTU (4.58 mmol) and 2.0 mL of triethylamine were added into the reaction mixture. 10.0 mmol H_2N-Trp-OMe was isolated from the corresponding methyl ester hydrochloride salt by neutralization, subsequent extraction with ethyl acetate and concentrated to 10 mL using rotary evaporator. Then, it was added to the reaction mixture. The reaction mixture was stirred for 2 h. After completion of the reaction, the solution was taken into the separating funnel, followed by addition of 50 mL of ethyl acetate and thoroughly washed with 1N HCl (2 × 30 mL), brine (1 × 30 mL), 1M sodium carbonate (2 × 30 mL) and again with brine (1 × 30 mL).The organic layer was collected and dried over anhydrous Na_2SO_4. Ethyl acetate was evaporated in vacuum. A white material was obtained. The product was then purified by column chromatography using silica gel (100-200 mesh) as stationary phase and 3 % MeOH in $CHCl_3$ as eluent.

Yield: 1.26 g (2.77 mmol, 60.5 %).

^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.94 (s, 1H), 8.77-8.76 (d, *J* = 7.5 Hz, 1H), 8.48-8.43 (m, *4*H), 7.87-7.84 (t, *J* = 7.8 Hz, 2H), 7.51-7.50 (d, *J* = 7.5 Hz, 1H), 7.37-7.35 (d, *J* = 8.0 Hz, 1H), 7.20 (s, 1H), 7.10-7.07 (t, *J* = 7.5 Hz, 1H), 7.02-6.99 (m, 1H), 4.73 (s, 2H), 4.59-4.55 (dd, *J* = 7.0 Hz, 1H), 3.59 (s, 3H), 3.21-3.17 (m, 1H), 3.12-3.08 (m, 1H); **^{13}C NMR (125 MHz, DMSO-d_6):** δ 172.1, 166.8, 163.2, 136.0, 134.5, 131.3, 130.8, 127.4, 127.1, 123.7, 121.8, 120.9, 118.4, 117.9, 111.4, 109.2, 53.3, 51.8,

42.1, 27.1; **HRMS (ESI):** m/z calculated for $C_{26}H_{21}N_3O_5$ exact mass 455.1481, found $[M+H]^+$: 456.1070 and $[M+Na]^+$: 478.0864.

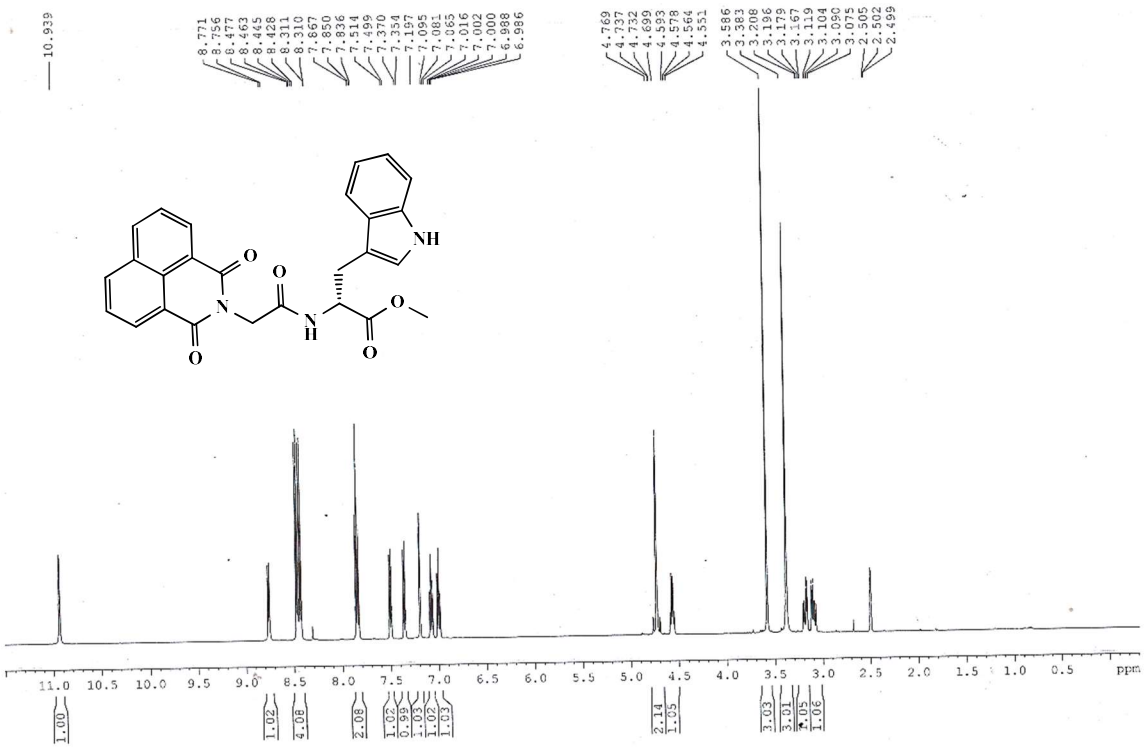

Fig. S1. ^{1}H NMR Spectra (500 MHz) of NMI-GW-OMe in DMSO-d_6 solvent at 27 °C.

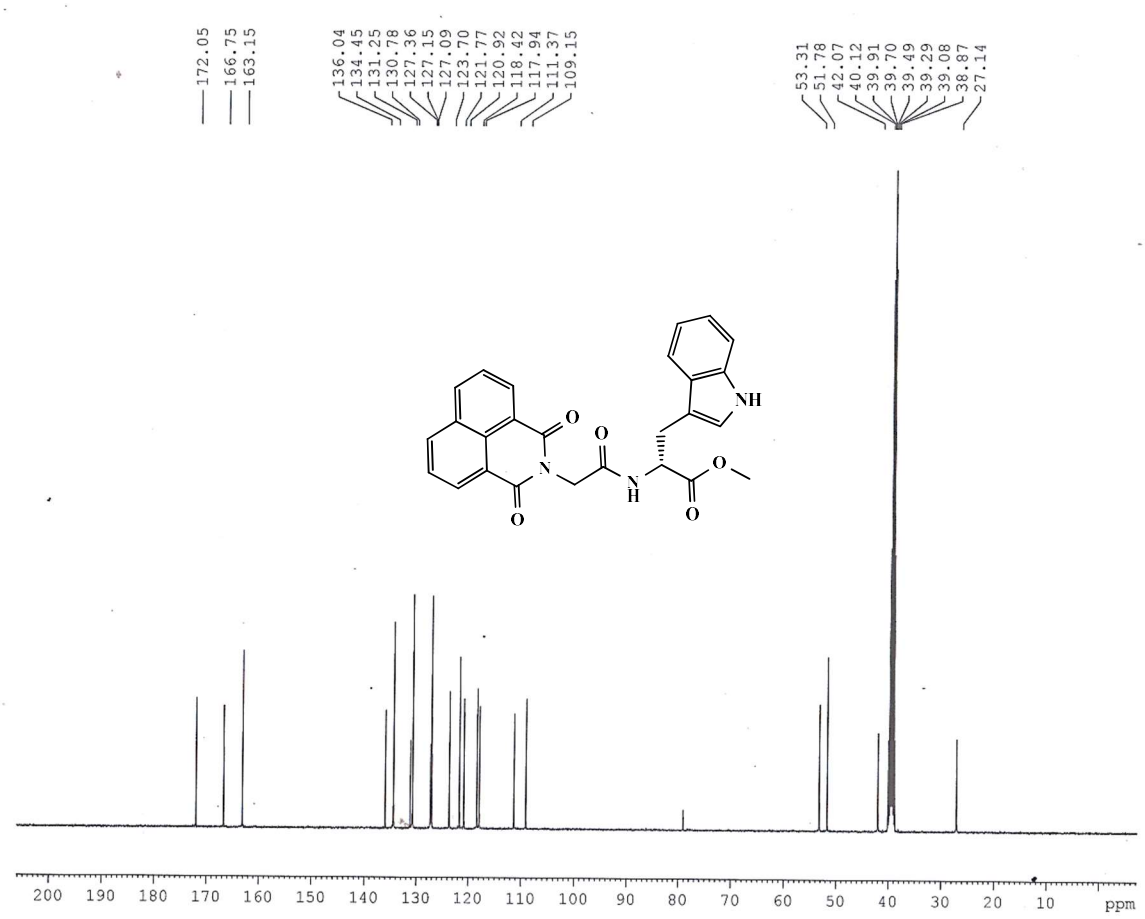

Fig. S2. ^{13}C NMR Spectra (125 MHz) of NMI-GW-OMe in DMSO-d6 solvent at 27 °C.

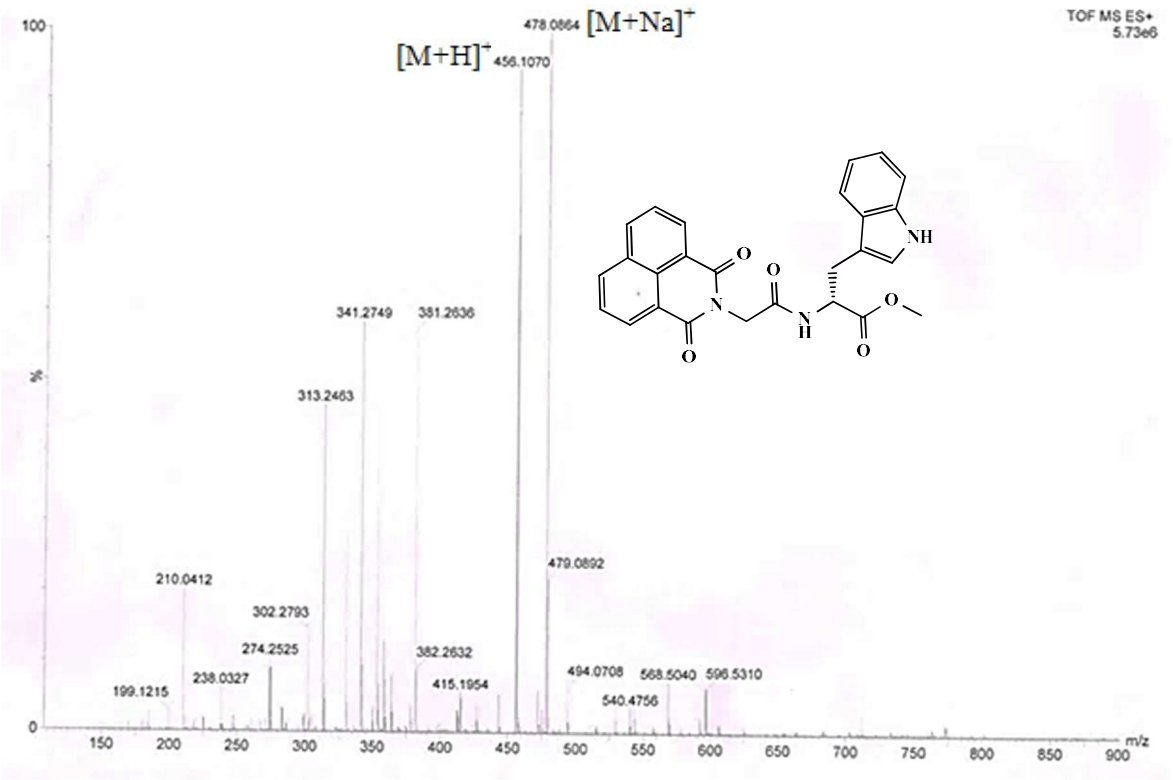

Fig. S3. HRMS spectrum of NMI-GW-OMe.

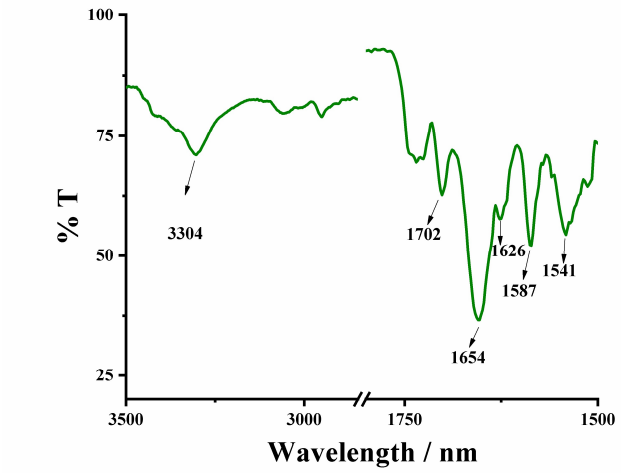

Fig. S4. FTIR spectrum of NMI-GW-OMe.

Table-S1 Crystal data and refinement parameters for NMI-GW-OMe.

Formula	$C_{26}\ H_{21}\ N_3\ O_5$
Formula Weight	455.46
Crystal System	Orthorhombic
Space group	$P2_12_12_1$
a (Å)	4.8611(3)
b(Å)	15.2401(11)
c (Å)	28.170(2)
α(°)	90
β(°)	90
γ(°)	90
V ($Å^3$)	2086.9(3)
Z	4
D(calc) (g/cm^3)	1.450
μ (MoKa) (/mm)	0.102
Temperature (K)	150(2)
Radiation MoKα (Å)	0.71073
Theta Min-Max (°)	2.673, 25.773
Dataset	-5:5; -18:18; -34: 34
Tot., Uniq. Data, R(int)	29297, 3962, 0.0887
Observed data I > 2 σ(I)	3627
Nref, Npar	3962, 312
R, wR2, S	0.0984, 0.2748, 1.189
Max. and Av. Shift/Error	0.000, 0.000
Min. and Max. Resd. Dens. [$e/Å^3$]	-0.483, 0.453
CCDC No.	1991131

Table-S2. Hydrogen bonding distances (Å) and angles (°) present in **NMI-GW-OMe**

Donor (D) H···Acceptor (A)	D–H (Å)	H···A (Å)	D···A (Å)	< D–H···A (°)
N2-H2 ···O3	0.8800	2.1000	2.957(9)	164.00
C5-H5 ···O3	0.9500	2.5900	3.450(12)	151.00
C13-H13A···O2	0.9900	2.3000	2.733(10)	105.00
C15-H15 ···O4	0.91(9)	2.42(9)	3.162(11)	139
C22-H22···N3	0.9500	2.4600	3.398(13)	169.00

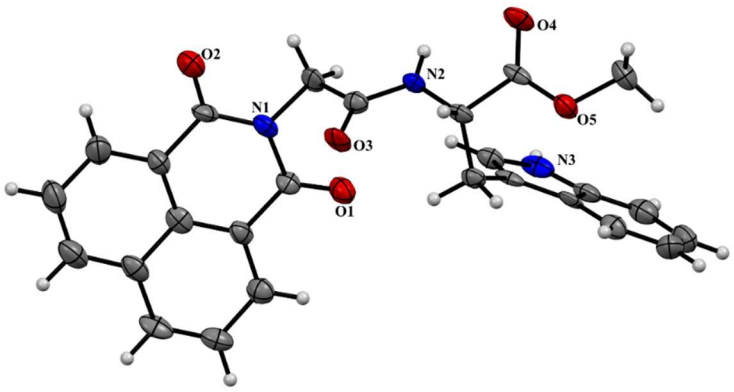

Fig. S5. ORTEP diagram of NMI-GW-OMe.

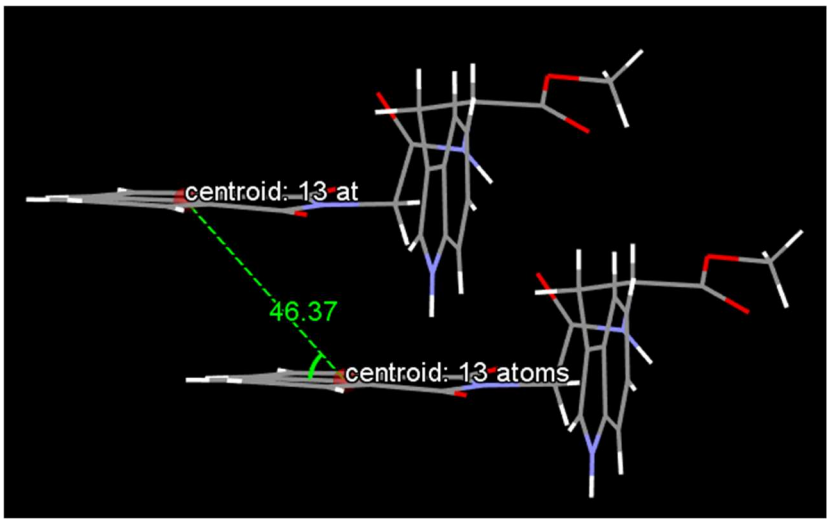

Figure S6. Measurement of the slippage angle between the two napthalimide units.

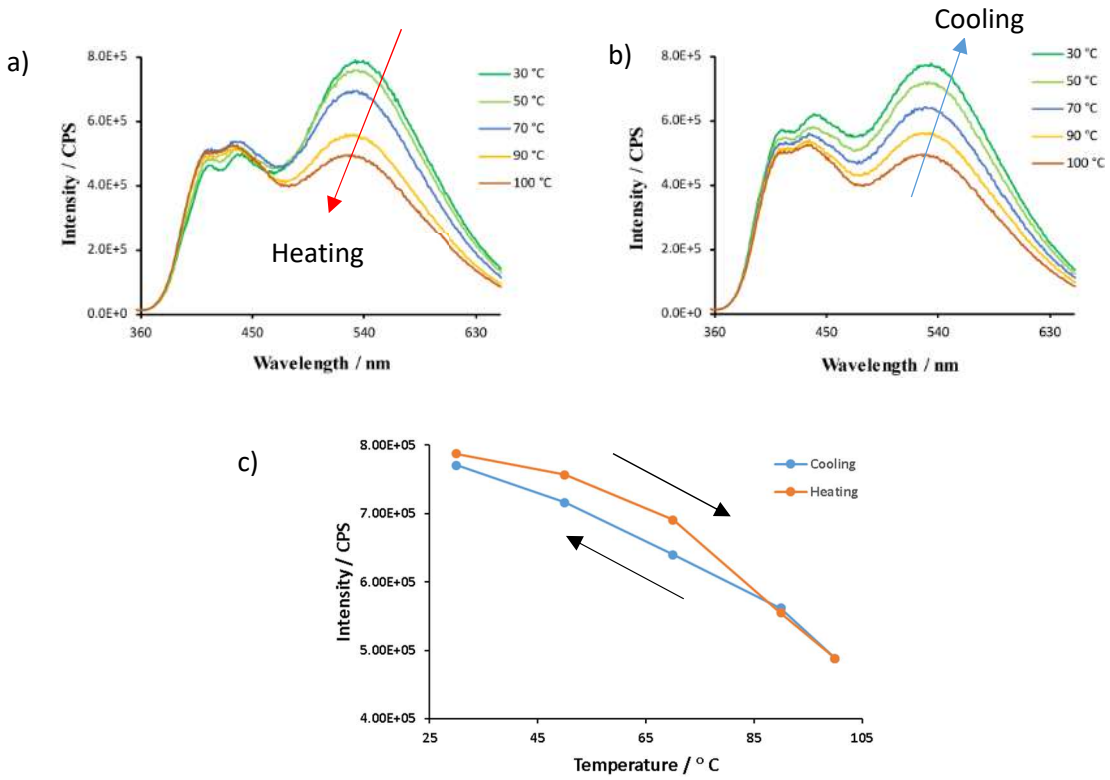

Figure S7. Temperature dependent solid state fluorescence spectra during a) heating and b) cooling. c) The variation of the fluorescence intensity at 535 nm during heating and cooling process indicating the thermochromic nature of NMI-GW-OMe crystal.

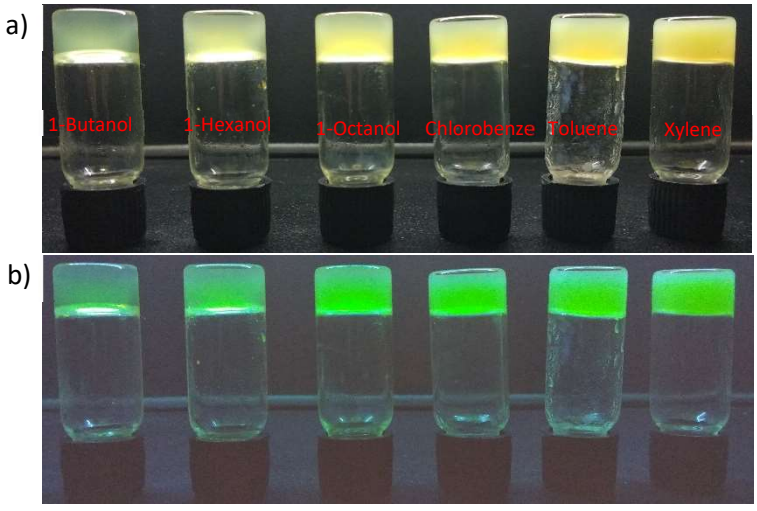

Figure S8. Inverted glass vial image of NMI-GW-OMe in different organic solvents under a) normal day light and b) UV light (365 nm).

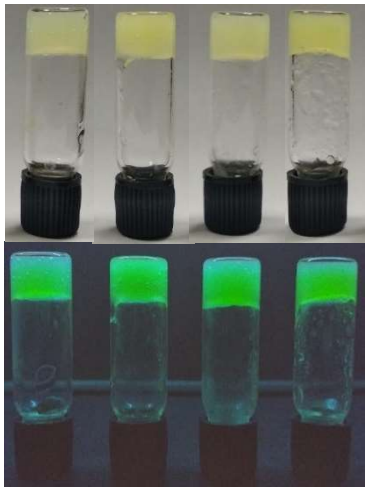

Figure S9. Inverted glass vial image of NMI-GW-OMe in different mixed solvents under a) normal day light and b) UV light (365 nm). Left to right solvent compositions are respectively water-acetonitrile, water-DMF, water-DMSO and water-THF. In each vial water content is 70 %.

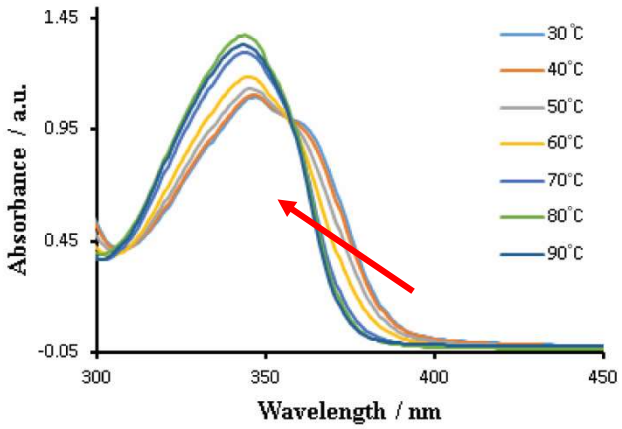

Figure S10. Temperature-dependent UV-Vis spectra of NMI-GW-OMe (50 μM) in 90% water in DMSO solvent.

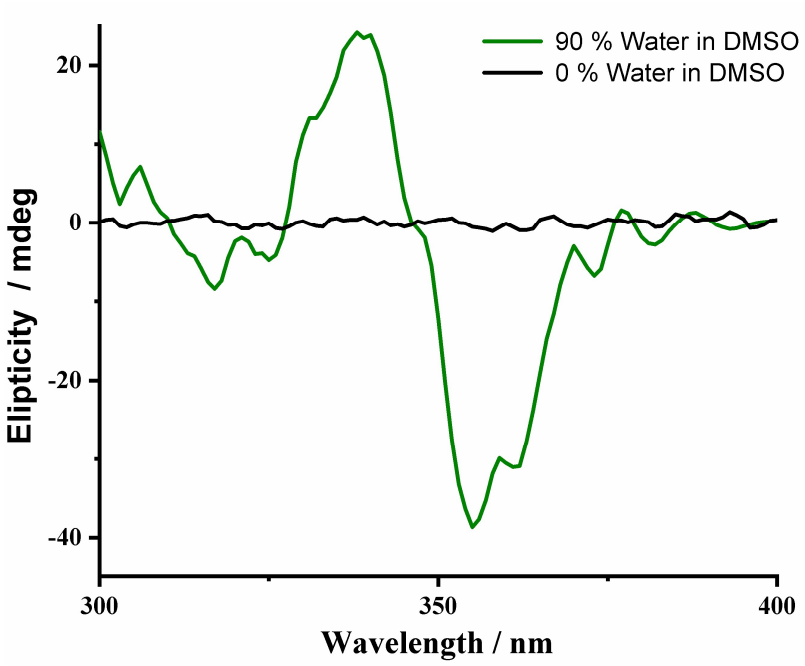

Figure S11. Circular dichroism spectra of NMI-GW-OMe (40 μM) in pure DMSO and 90 % water in DMSO solvent systems.

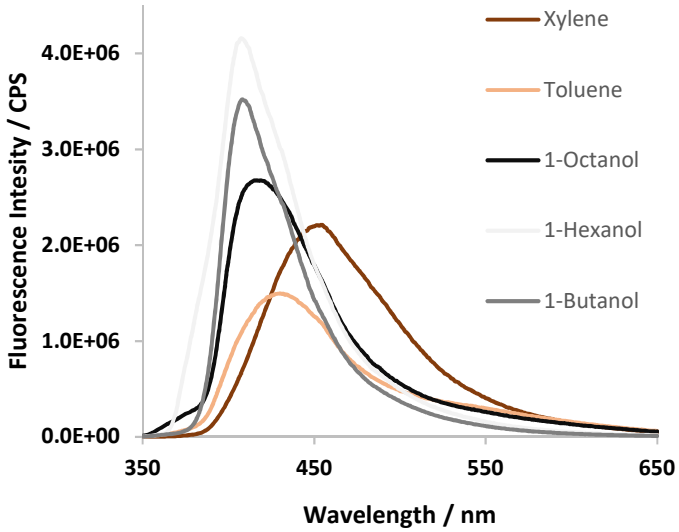

Figure S12. Fluorescence emission spectra of NMI-GW-OMe in different organic solvents at their minimum gelation concentration.

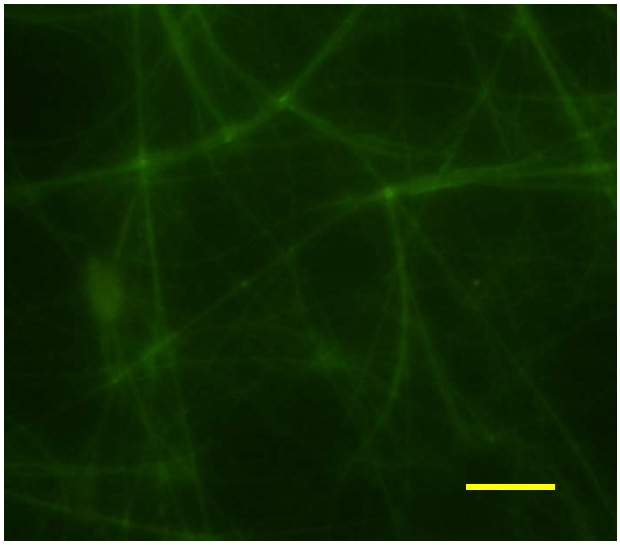

Figure S13. Fluorescence microscopic image of xerogel. The scale bar is 50 µm.

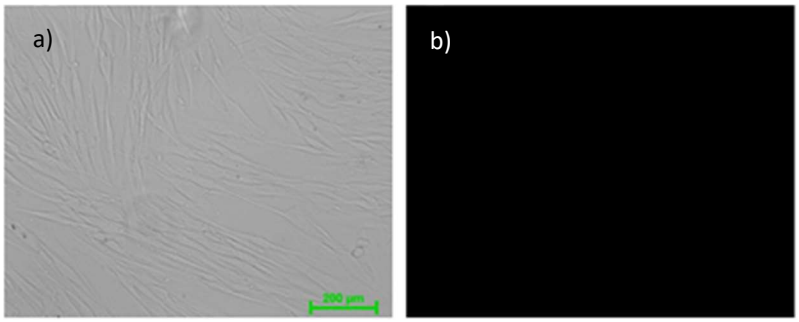

Figure S14. Controlled experiments for HADF cell imaging a) under bright field and b) under fluorescence.

1. S.-M. Hsu, F.-Y. Wu, H. Cheng, Y.-T. Huang, Y.-R. Hsieh, D. T.-H. Tseng, M.-Y. Yeh, S.-C. Hung and H.-C. Lin, *Advanced Healthcare Materials*, 2016, **5**, 2406-2412.