

Bicomponent β -sheet assembly of dipeptide-fluorophores of opposite polarity and sensitive detection of nitro-explosives

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Experimental section

1. Materials and methods

Materials. All reagents and solvents were purchased from Sigma Aldrich and used without any further purification unless mentioned. The solvents were brought as HPLC grade from Spectrochem, India. Dulbecco's phosphate-buffered saline (PBS) was purchased from Sigma-Aldrich. All the experiments were performed in 10 mM PBS buffer of pH 7.4.

Absorbance (UV-vis) spectroscopy. UV-vis spectra were recorded on Perkin Elmer Model Lambda 900 spectrophotometer. The spectra were recorded in quartz cuvette of 10 mm path length.

Fluorescence (FL) spectroscopy. Fluorescence spectra were recorded on a PerkinElmer model LS 55 spectrophotometer. All fluorescence emission spectra were recorded with excitation wavelength $\lambda_{\text{ex}} = 350$ nm, and analysed in quartz cuvette of 10 mm path length.

Circular dichroism (CD) spectroscopy. The CD measurements were carried out on a Jasco J-815 spectrometer under nitrogen atmosphere. The CD was recorded in quartz cuvette of 1 mm path length with the speed of 100 nm/min and the spectra represent an average of three scans.

NMR spectroscopy. ^1H and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in $\text{CDCl}_3/\text{DMSO-}d_6$ with tetramethylsilane as internal standard).

Image and microscopy. The digital images of the inverted gel vial were taken in Canon SX50HX digital camera. Fluorescent images were captured using DMi8 Leica Inverted fluorescence microscope). The 1:1 complex (of TGM-82 and TGM-83) at 0.25 mM total concentration was taken in a microscope slide (glass) and covered with a cover slip. FESEM images were acquired using FEI Nova nanoSEM-600 equipped with a field emission gun operating at 15 kV. The samples were prepared by drop-casting solutions on to the silicon (111) surface and dried in air.

Time-correlated single photon counting (TCSPC). Fluorescence decay profiles were performed using FLSP 920 spectrometer, Edinburgh Instrument.

Fourier-transform infrared spectroscopy (FTIR). The FTIR spectra were recorded on a Bruker IFS 66/V spectrometer. The measurements were done for the vacuum-dried solid powders of the derivatives and xerogel at room temperature.

Rheology. The mechanical strength of the hydrogel was measured on MCR 302 rheometer using P-PTD 200/Air measuring and a plate geometry of 1 mm gap.

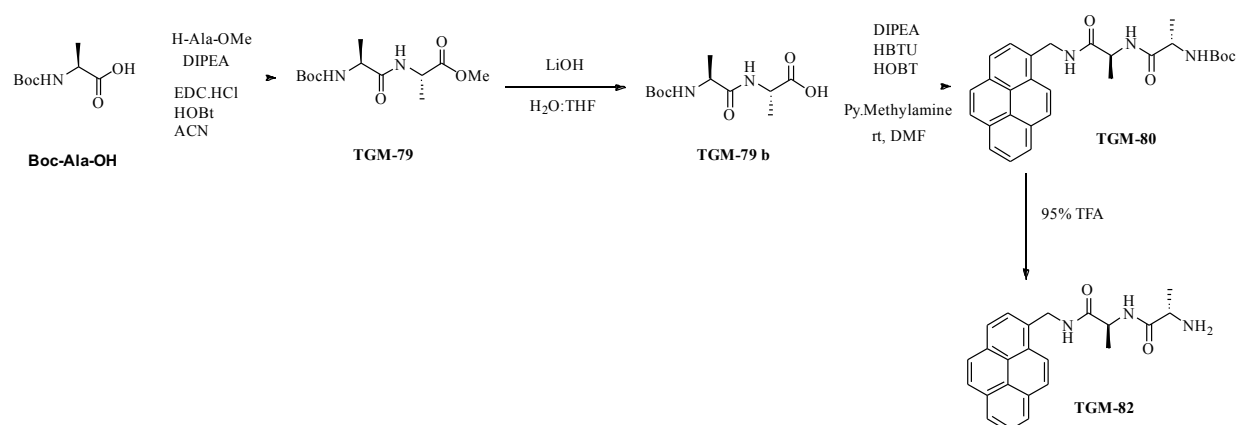
Hydrogel preparation. The dipeptide derivatives (TGM-82 and TGM-83) were taken in calculated amounts and prepared clear stock solutions of 4.0 mM concentration. For hydrogel preparation equivolume solutions of each component were mixed in 10.0 mM PBS buffer (pH 7.4) and sonicated to get clear solution at 25 °C. The final total concentration was maintained at 2.0 mM. After 5 min it formed hydrogel, as confirmed by ‘vial inversion test’ at 25 °C.

Film-based NACs detection. The peptide-coated thin film was developed on quartz plate (almost 5 mm diameter and ~1 μm thickness) following drop-casting method. The fluorescence emission spectra were recorded using front face geometry. For detection, the analytes were dissolved in acetonitrile solvent and drop-casted 5 μL on the films, and the emission spectra were recorded.

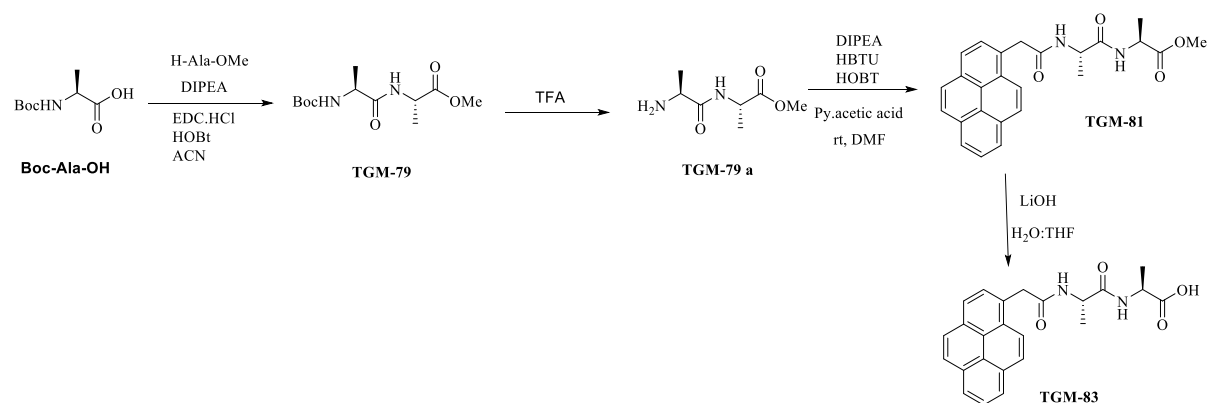
2. Synthesis and characterisation

All the air and moisture sensitive reactions were carried out under argon or nitrogen atmosphere. The syntheses of the two dipeptide derivatives (TGM-82 and TGM-83) is presented in Scheme 1 and 2. All the compounds were thoroughly characterised by NMR and HRMS studies.

Scheme S1 Synthesis of TGM-82



Scheme S2 Synthesis of TGM-83



General experimental procedure for dipeptide derivatives TGM-82 and TGM-83

Synthesis of TGM-79

To the solution of Boc-Ala-OH (0.95 g, 5.0 mmol) in DCM, DIPEA (1.92 mL, 11.0 mmol), EDC. HCl (0.96 g, 5.0 mmol) and HOBt (0.67 g, 5.0 mmol) were added at 0 °C under nitrogen atmosphere. The solution was stirred for 10 min. and a mixture of H-Ala-OMe (0.52 g, 5.0 mmol) and DIPEA (870 μ L, 5.0 mmol) was added. After completion of the reaction (TLC analysis), the reaction mixture was washed with 10% Na₂CO₃ (3 \times 50 mL), 5% citric acid (3 \times 50 mL), water (2 \times 50 mL) and brine solution (1 \times 5 mL). The organic layer was separated, evaporated and the residue was purified through column chromatography to obtain the dipeptide (TGM-79) in 85% yield.

Synthesis of TGM-79a

TGM-79 (1.0 g, 3.65 mmol) was subjected to Boc-deprotection in a round bottom flask by treating with a deprotection-cocktail of TFA, DCM and TIPS (95:3:2), and the reaction mixture was stirred for 3 h. TFA was evaporated under vacuo and triturated with diethylether to obtain TGM-79a in 87% yield.

Synthesis of TGM-79b

TGM-79 (0.27 g, 1.0 mmol) and LiOH (0.05 g, 1.2 mmol) were taken in a round bottom flask containing THF:H₂O (4:6) solvent mixture and stirred at room temperature for 3 h. After completion, THF was evaporated and 10% Na₂CO₃ was added. The reaction mixture was then washed with diethylether (2 \times 50 mL) and neutralised with citric acid to obtained pure

precipitate of TGM-79b. The precipitate was filtered and dried to get solid product in 90% yield.

Synthesis of TGM-80

To a solution of TGM-79b (1.30 g, 5.0 mmol) in DMF, DIPEA (1.92 mL, 11.0 mmol), HBTU (5.0 mmol) and HOBt (0.67 gm, 5.0 mmol) were added under stirring and at nitrogen atmosphere at 0 °C. Pyrenemethylamine hydrochloride (1.34 g, 5.0 mmol) was added to the above reaction mixture and continued for 3 h. The reaction mixture was diluted with water and the precipitate was collected by washing with 10% Na₂CO₃ solution, citric acid, and water. The precipitate was dried and purified in silica gel column chromatography using CHCl₃:CH₃OH (97:3) as eluent to obtain TGM-80 in 80% yield.

Synthesis of TGM-81

Pyrene acetic acid (2.0 g, 7.68 mmol) was dissolved in DMF and DIPEA (1.34 mL, 7.68 mmol), HBTU (2.91 g, 7.68 mmol) and HOBt (1.04 g, 7.68 mmol) were added at 0 °C under nitrogen atmosphere. A neutralised solution of TGM-79a (1.34 g, 7.68 mmol) with DIPEA (1.34 mL, 7.68 mmol) was added to the reaction mixture under stirring condition. After completion (TLC analysis), the reaction mixture was diluted with water to precipitate the product. The precipitate was washed with 10% Na₂CO₃ solution, citric acid, and water. The dried precipice was purified by column chromatography using CHCl₃:CH₃OH (97:3) as eluent to obtain TGM-81 in 78% yield.

Synthesis of TGM-82

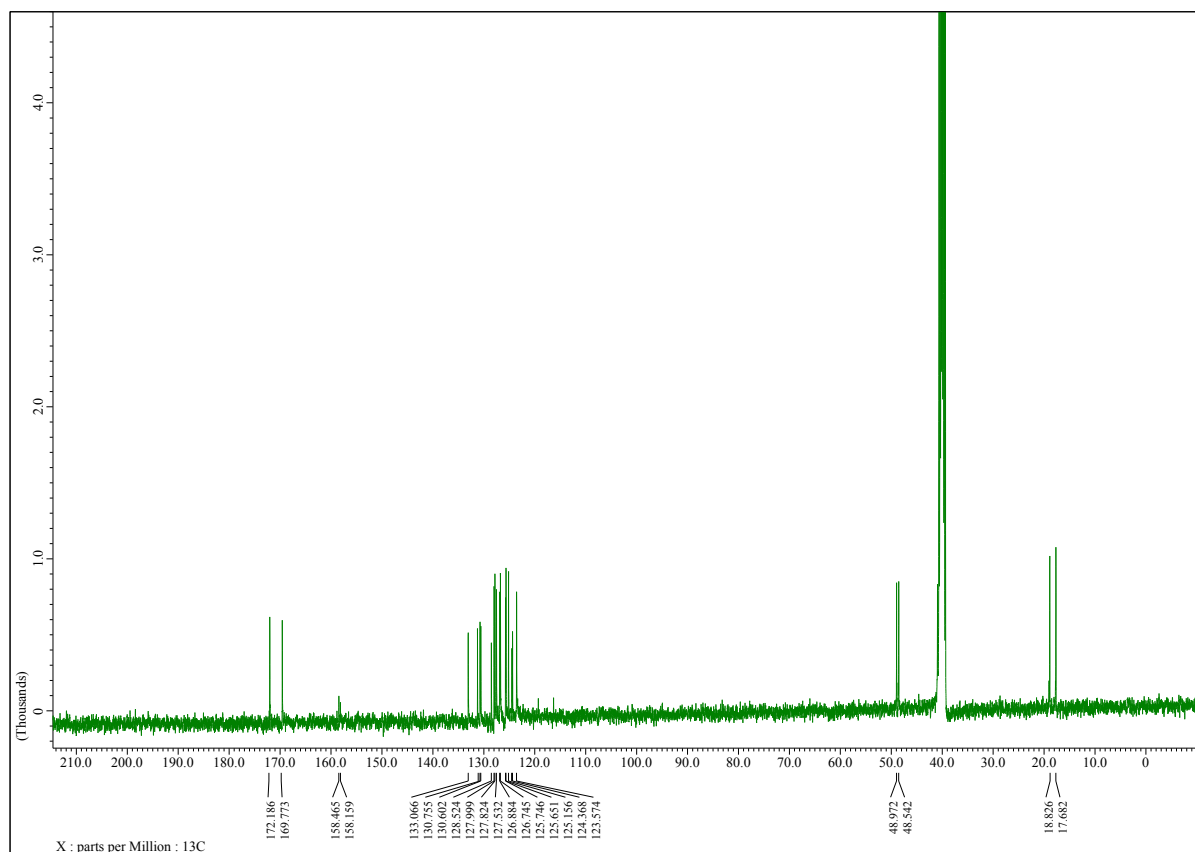
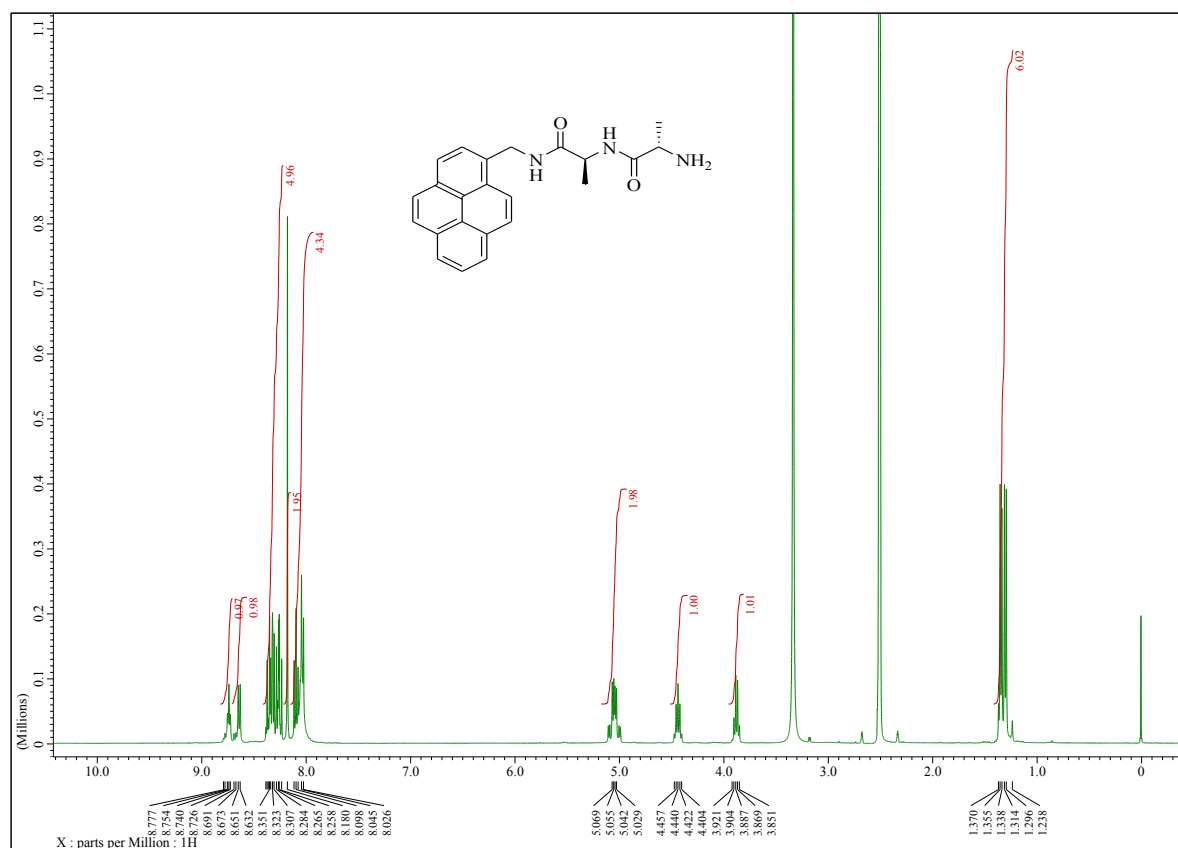
TGM-80 (0.25 g, 0.53 mmol) subjected to BOC-deprotection in a round bottom flask using 2 mL deprotection-cocktail of TFA, DCM and TIPS (95:3:2). After 3 h of stirring, 50 mL diethylether was added to the reaction mixture and the precipitated product was collected by filtration. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.73-8.79 (m, 1H), 8.66 (dd, *J* = 16.3, 7.5 Hz, 1H), 8.23-8.39 (m, 5H), 8.18 (s, 2H), 8.03-8.12 (m, 4H), 5.05 (q, *J* = 5.4 Hz, 2H), 4.40-4.47 (m, 1H), 3.85-3.92 (m, 1H), 1.42-1.23 (6H); ¹³C NMR (100 MHz, DMSO- *d*₆): δ 172.2, 169.8, 158.4, 158.2, 133.1, 131.1, 130.7, 130.6, 128.5, 128.0, 127.8, 127.5, 126.8, 126.7, 125.7, 125.6, 125.2, 124.5, 124.4, 123.6, 48.9, 48.5, 18.8, 17.6; ESI-MS *m/z*: [M+H]⁺ calcd. for C₂₃H₂₃N₃O₂, 374.1863; found 374.1858.

Synthesis of TGM-83

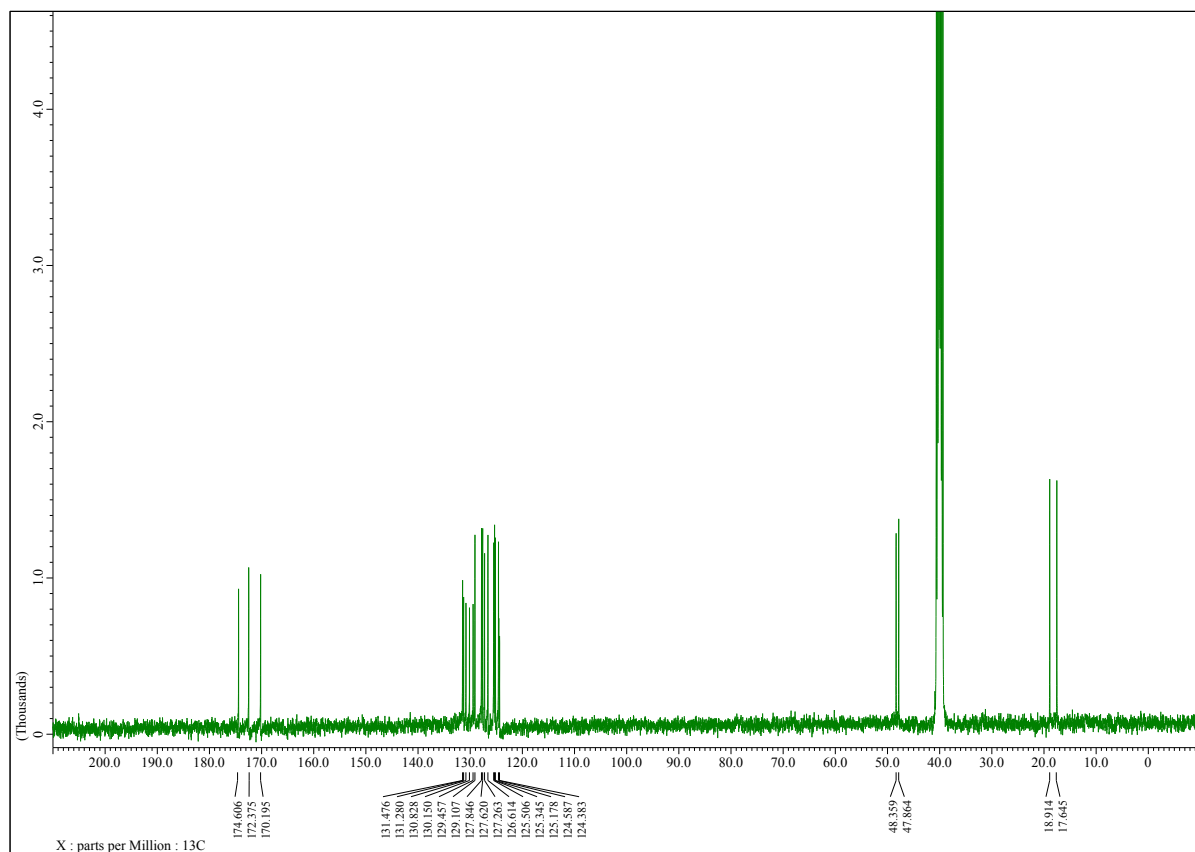
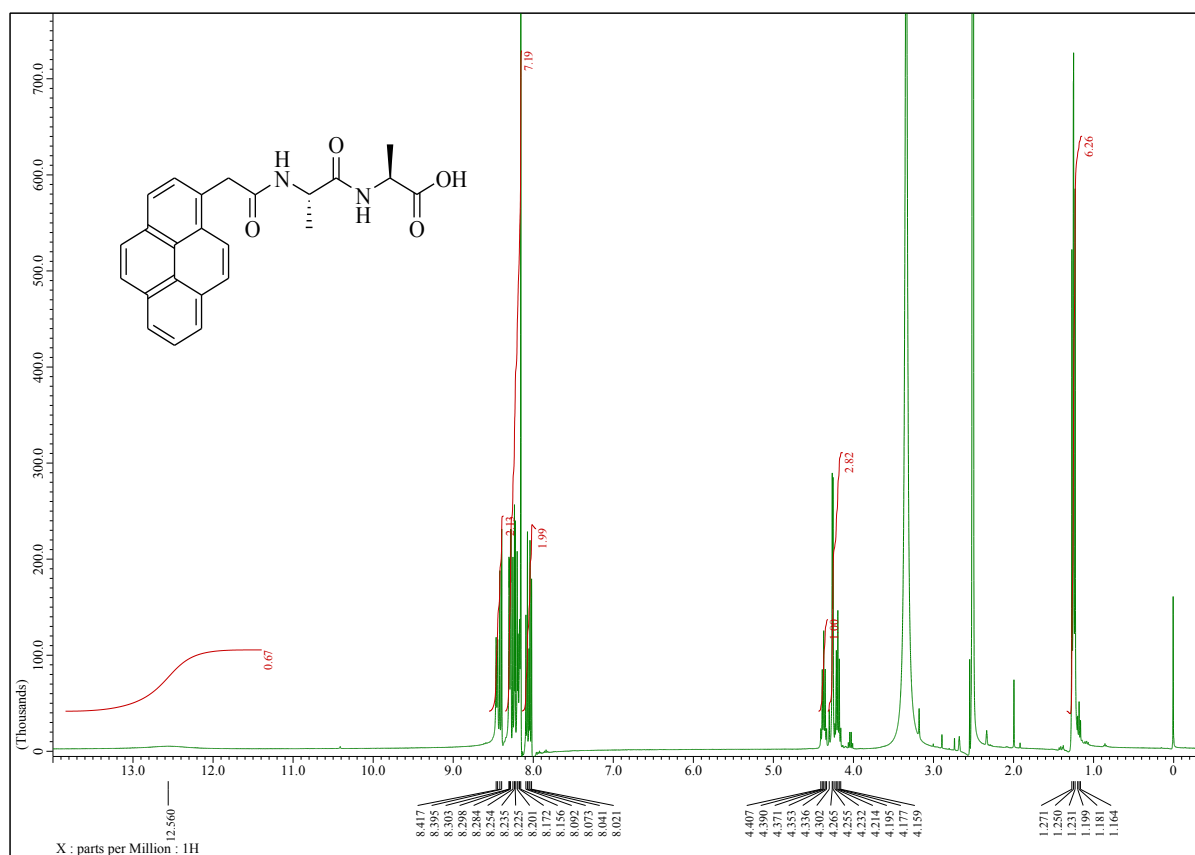
TGM-81 (0.39 g, 1.0 mmol) was taken in a round bottom flask, LiOH (50 mg, 1.2 mmol) was added in THF:H₂O (4:6) solvent mixture and stirred at room temperature for 3 h. After

completion of the reaction, THF was evaporated and 10% Na₂CO₃ was added. The reaction mixture was washed with diethylether (2× 50 mL) and neutralised with 5% HCl solution to precipitate the product. The precipitate was filtered and dried to obtain TGM-83 in 75% yield. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.56 (b) 8.39-8.46 (m, 2H), 8.16-8.30 (m, 7H), 8.02-8.09 (m, 2H), 4.34-4.41 (q, *J* = 7.1 Hz, 1H), 4.16-4.30 (m, 3H), 1.16-1.27 (m, 6H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 174.6, 172.4, 170.2, 131.5, 131.3, 130.8, 130.1, 129.5, 129.1, 127.8, 127.6, 127.3, 126.6, 125.5, 125.3, 125.2, 124.6, 124.4, 48.4, 47.9, 18.9, 17.6; ESI-MS *m/z*: [M] calcd. for C₂₄H₂₂N₂O₄, 402.1580; found 403.1651.

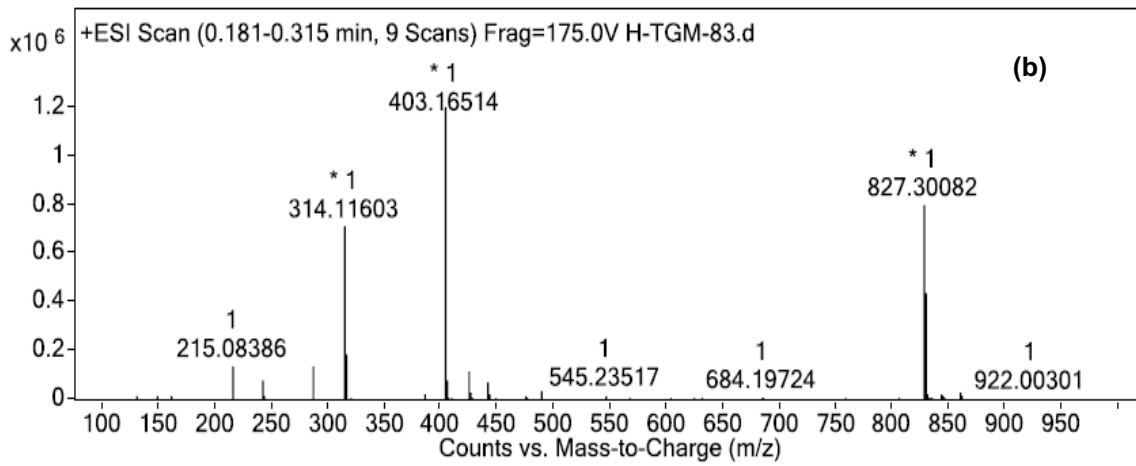
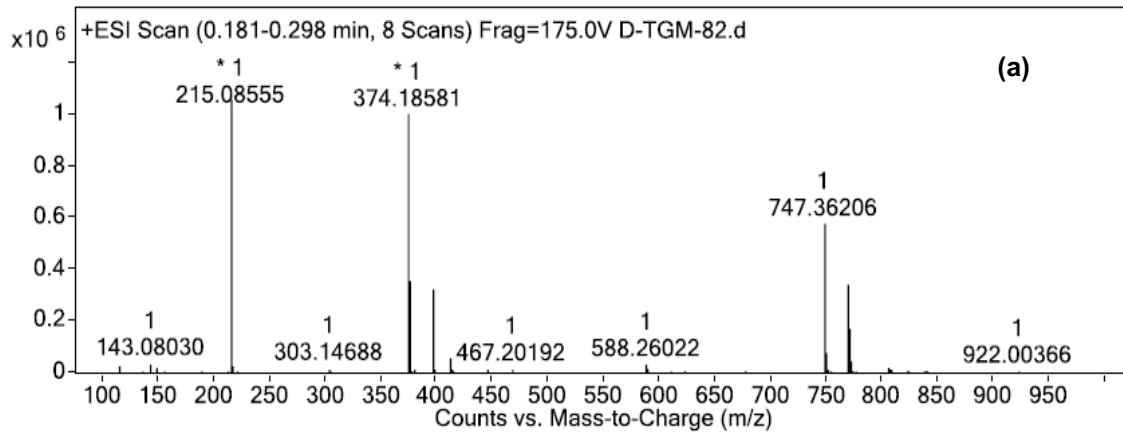
¹H and ¹³C NMR spectra of TGM-82



¹H and ¹³C NMR spectra of TGM-83



HR-MS (ESI) spectra of (a) TGM-82 and (b) TGM-83



3. Experimental results

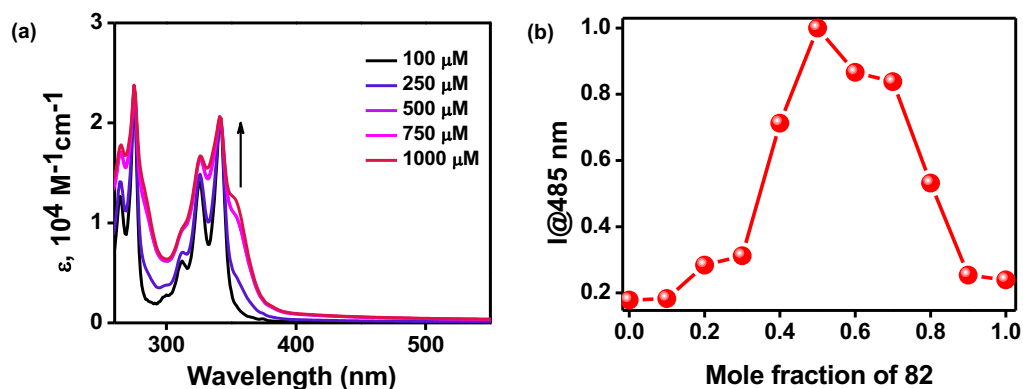


Fig. S1 (a) Concentration dependent absorbance spectra of 1:1 complex (of TGM-82 and TGM-83) at pH 7.4 and at 25 °C; (b) Job's plot was derived from the intensity of excimer formation at 0.25 mM total concentration at pH 7.4 and at 25 °C.

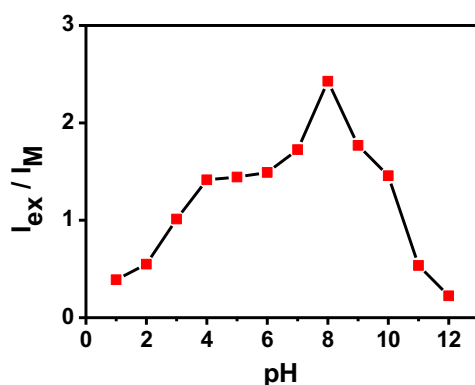


Fig. S2 The pH dependent FL study was carried out with the 1:1 complex (of TGM-82 and TGM-83) at 0.25 mM total concentration and at 25 °C.

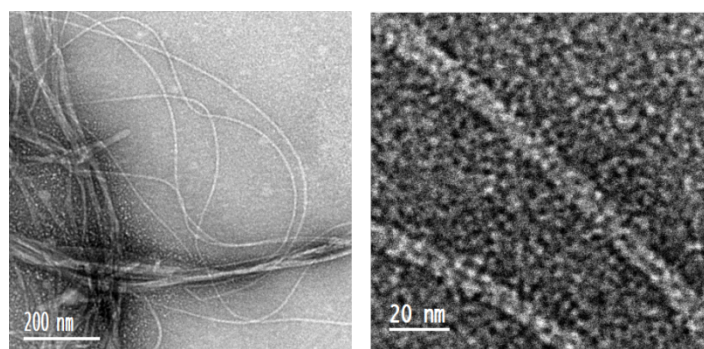


Fig. S3 TEM images of the 1D nanofibers of 1:1 complex (of TGM-82 and TGM-83) prepared at 0.25 mM total concentration, at 25 °C.

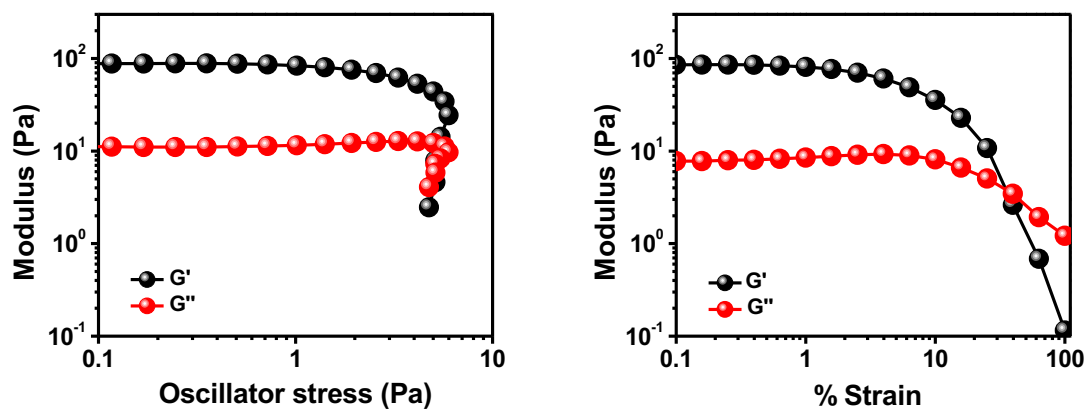


Fig. S4 Rheology experiment for oscillation stress and %strain determination of the bicomponent hydrogel at 2.0 mM total concentration at 25 °C.

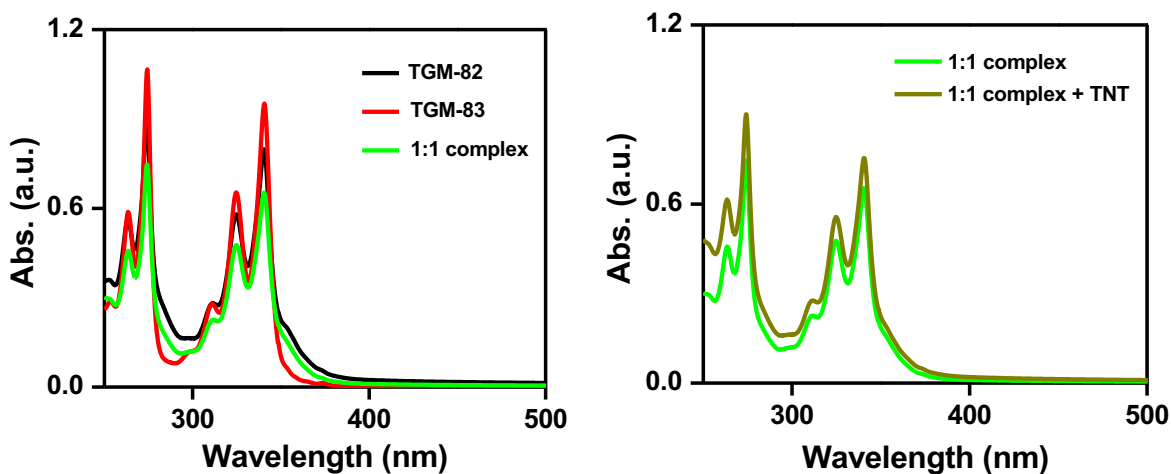


Fig. S5 The absorbance study was carried out with the 1:1 complex (of TGM-82 and TGM-83) at 0.25 mM total concentration and in the presence of 100 μ M TNT at 25 °C.

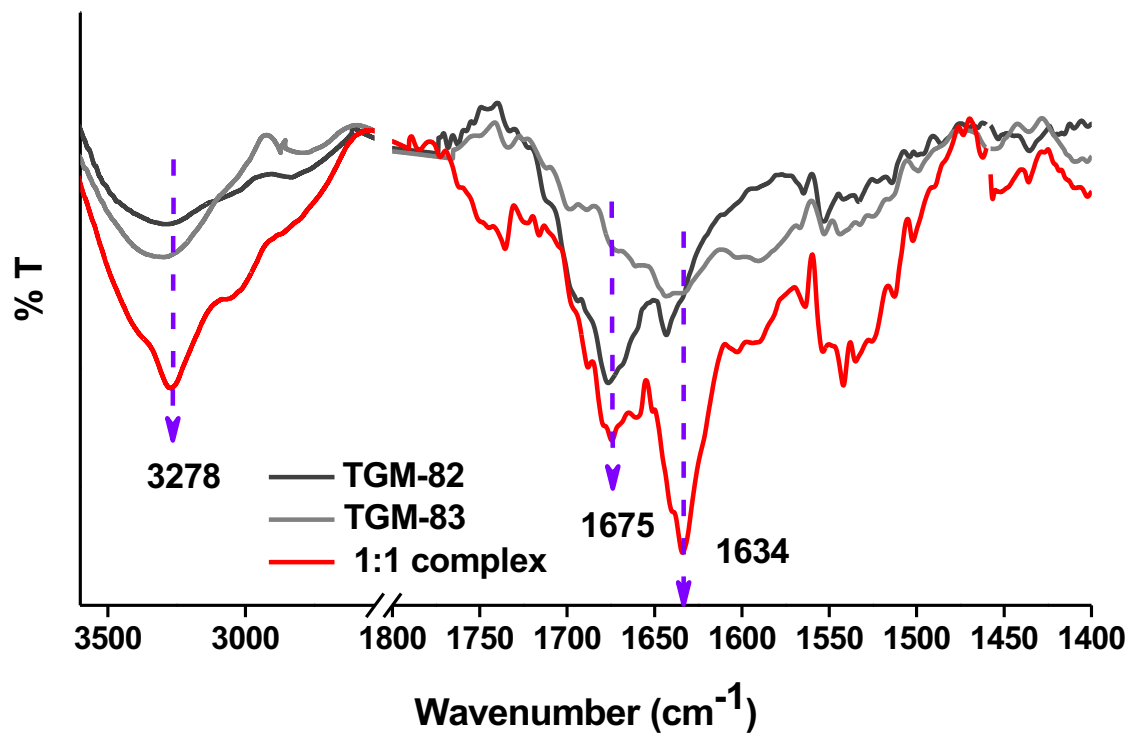


Fig. S6 FTIR spectra of the individual components TGM-82 and TGM-83, and their hydrogel in the dried state (xerogel).

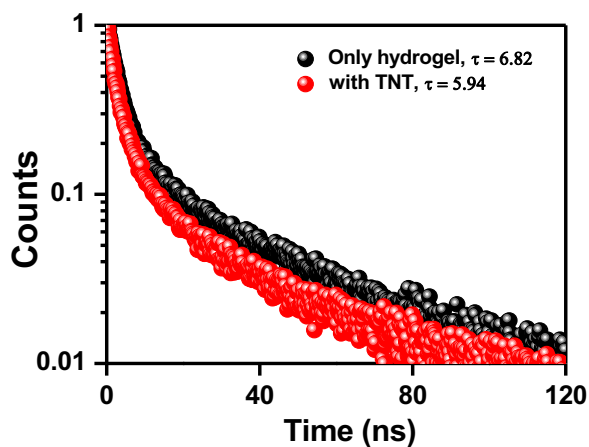


Fig. S7 Time-correlated Single Photon Counting (TCSPC) study of the hydrogel in presence and absence of TNT.

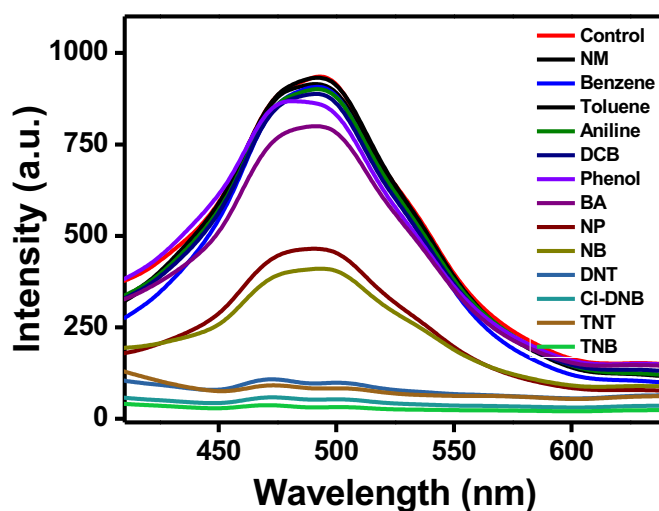


Fig. S8 The change in fluorescence intensity of 1:1 complex (250 μM) in the presence of different NACs (100 μM).

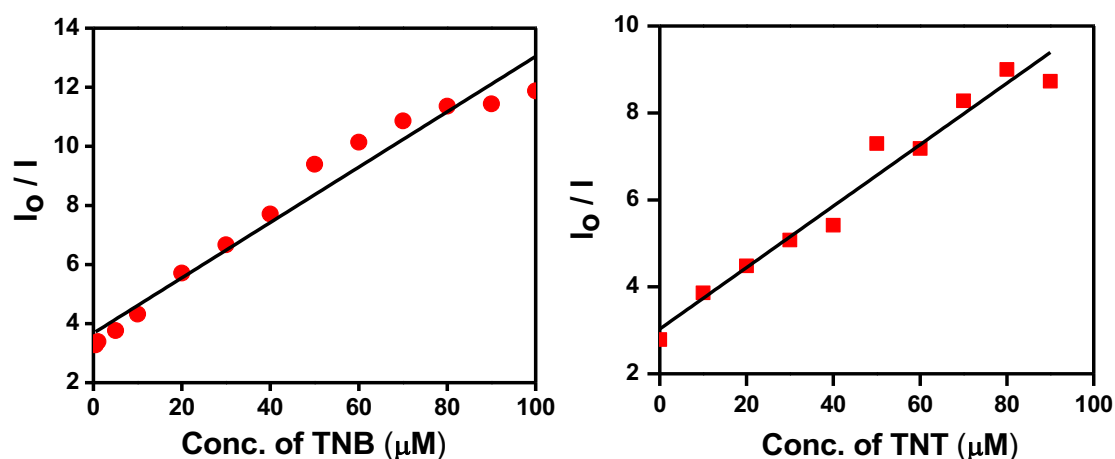


Fig. S9 Detections of TNB (13.4 μM) and TNT (17.8 μM) in the solution of the 1:1 complex (of TGM-82 and TGM-83) at 0.25 mM and 25 $^{\circ}\text{C}$ (see the cali).

Determination of the detection limit¹

The calibration curve was obtained from the plot of fluorescence intensity ratio of complex (H) in complex-quencher (HQ) system in absence and presence of TNB/TNT. The regression curve equation was then obtained as-

The limit of detection (LOD) = $3 \times \text{S.D.} / k$

Where S.D. represents the standard deviation of H system in the absence of Q and 'k' is the slope of the curve.

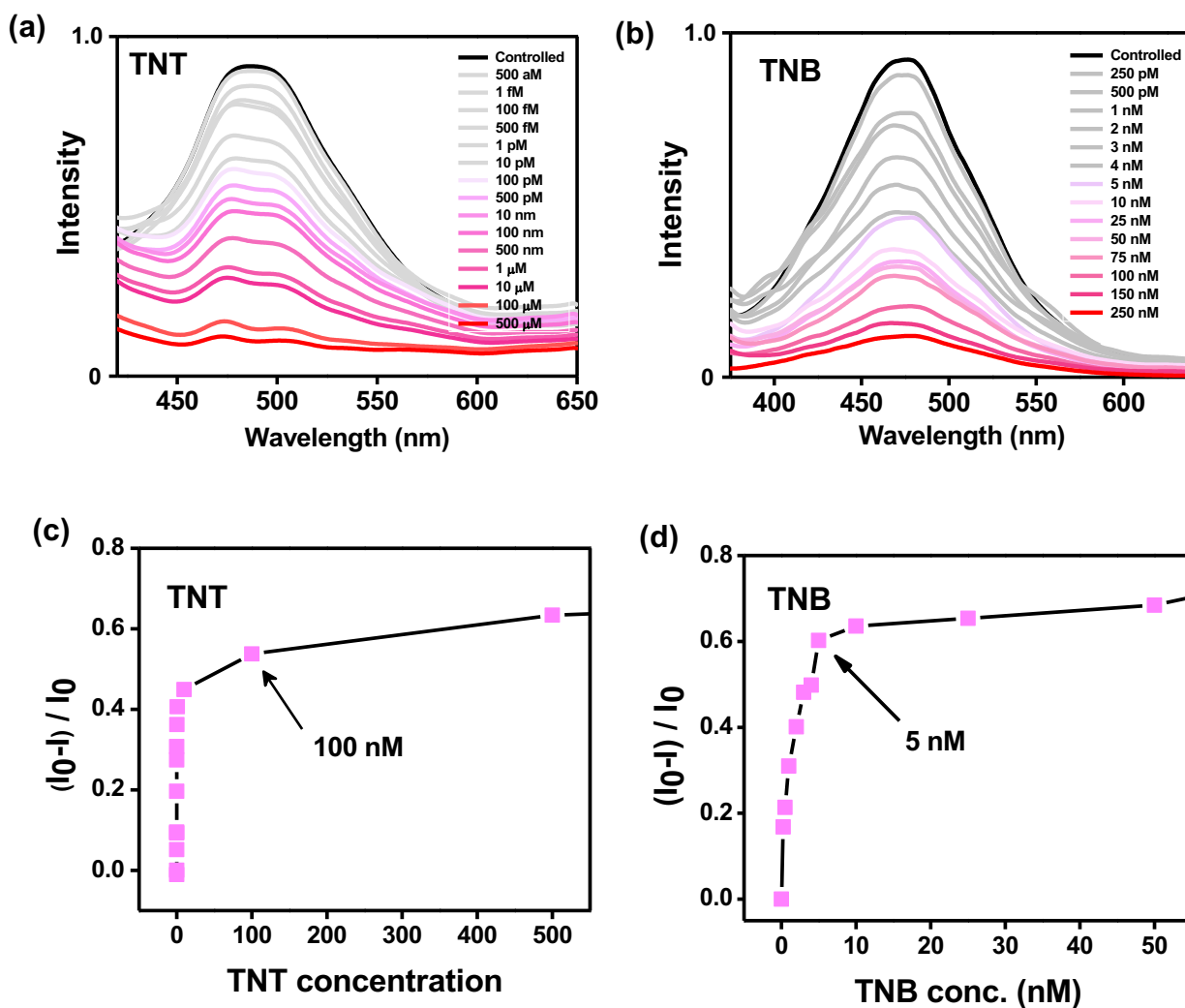


Fig. S10 Film-based detections of TNT and TNB at 25 °C: (a-b) FL intensity change with the addition of NACs (TNT and TNB); (c-d) the relative intensity changes with the NACs (TNT and TNB) concentrations (the marked points signify >5 fold relative intensity change).

Reference

1. B. Ma, F. Zeng, F. Zheng, and S. Wu, *Chem. Eur. J.*, 2011, **17**, 14844.