Electronic Supplementary Information

In situ clicking methylglyoxal for hierarchical self-assembly of nanotubes in supramolecular hydrogel

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Contents:

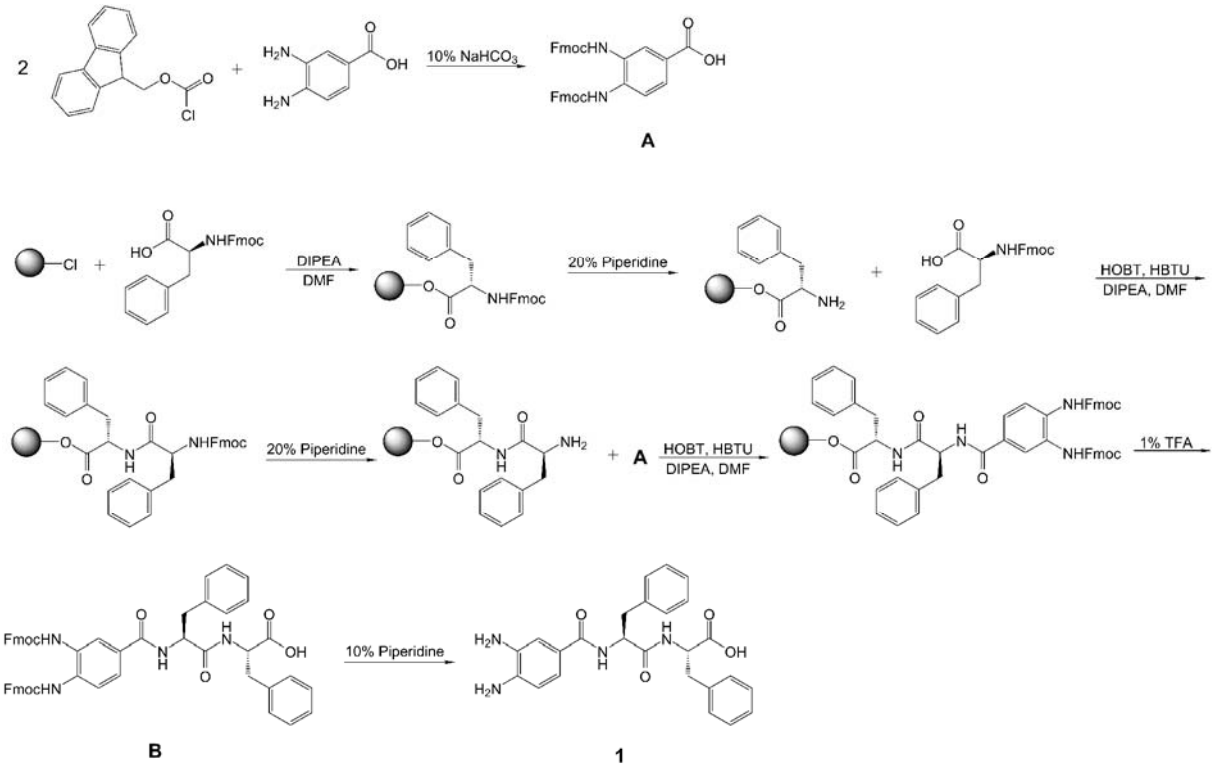
1. General methods

All the starting materials were obtained from Adamas, Sangon Biotech, or Energy Chemical. Commercially available reagents were used without further purification, unless noted otherwise. All other chemicals were reagent grade or better. The spectra of electrospray ionization-mass spectrometry (ESI-MS) were recorded on an Orbitrap XL ETD spectrometer (Thermo Fisher). HPLC analyses were performed on a Shimadzu UFLC system equipped with two LC-20AP pumps and an SPD-20A UV-vis detector using a Shimadzu PRC-ODS column, or on an Agilent 1200 HPLC system equipped with a G1322A pump and an in-line diode array UV detector using an Agilent Zorbax 300SB-C_{18} RP column, with CH_3CN (0.1% of trifluoroacetic acid (TFA)) and ultrapure water (0.1% of TFA) as the eluent. ^{1}H NMR and ^{13}C NMR spectra were obtained on a 400 MHz Bruker AV 400 or a 300 MHz Bruker AV 300. Rheological measurement was performed on an AR2000EX (TA Instruments), with a geometry diameter of 25 mm at the gap of 1 mm. Fluorescence spectra were obtained on a Hitachi FL-4600 fluorescence spectrophotometer. UV-vis spectra were obtained on a PerkinElmer Lambda 25 UV-vis spectrometer. Circular dichroism (CD) spectra were obtained on a Jasco-810-CD. Cryo transmission electron microscopy (cryo-TEM) images were obtained on a Tecnai F20 transmission electron microscope from FEI company, operating at 200 kV.

2. Syntheses and Characterizations

*Preparation and characterization of OPD-Phe-Phe-OH (**1**)*:

Scheme S1. Synthetic route for compound **1**.



*Synthesis of (Fmoc)$_2$-OPD-OH (**A**)*:

3,4-Diaminobenzoic acid (152 mg, 1.00 mmol) was dissolved in a 10% solution of $NaHCO_3$ (8.0 mL). DMF (7.0 mL) was added, and the mixture was stirred in an ice-water bath. 9-Fluorenylmethyloxycarbonyl chloride (621 mg, 2.40 mmol) dissolved in DMF (8 mL) was added dropwise, and stirring was continued at 0 °C for 1 h and then at room temperature for 8 h. The reaction mixture was then poured into water (30 mL) and extracted with diethyl ether (2×). The aqueous solution was cooled in an ice-water bath and acidified with 2 M HCl to pH 2. The aqueous phase was extracted with ethyl acetate (3×). The organic layers were combined, washed with a saturated solution of NaCl (1×), dried with Na_2SO_4 and then concentrated under vacuum. ^{1}H NMR of **A** (300 MHz, DMSO-d_6) δ (ppm): 9.24 (s, 1 H), 9.16

(s, 1 H), 8.13 (s, 1 H), 7.91 (d, J = 7.5 Hz, 4 H), 7.74 (m, 6 H), 7.43 (t, J = 7.4 Hz, 4 H), 7.34 (t, J= 7.4 Hz, 4 H), 4.52 (m, 4 H), 4.34 (m, 2 H) (Figure S1).

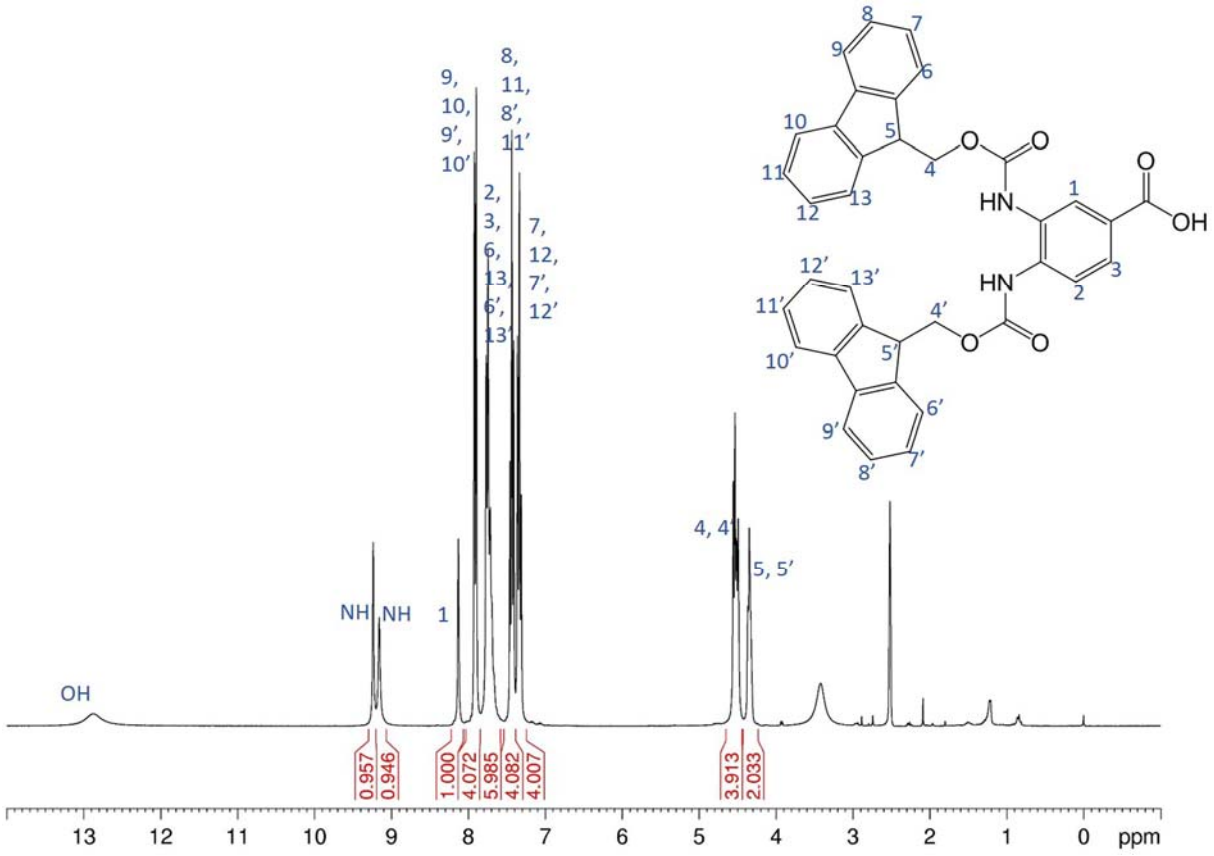


Figure S1. ^{1}H NMR spectrum of **A**.

*Synthesis of (Fmoc)$_2$-OPD-Phe-Phe-OH (**B**)*:

B was synthesized with solid phase peptide synthesis and purified with HPLC. ^{1}H NMR of **B** (300 MHz, DMSO-d_6) δ (ppm): 9.11 (s, 1 H), 9.07 (s, 1 H), 8.48 (d, J = 8.5 Hz, 1 H), 8.31 (d, J = 7.8 Hz, 1 H), 7.90 (d, J = 7.1 Hz, 5 H), 7.73 (d, J = 7.3 Hz, 4 H), 7.57 (s, 2 H), 7.42 (t, J = 7.4 Hz, 4 H), 7.31 (t, J = 7.1 Hz, 6 H), 7.18 (m, 9 H), 4.73 (m, 1 H), 4.48 (m, 5 H), 4.32 (m, 2 H), 3.08 (m, 2 H), 2.94 (m, 2 H) (Figure S2).

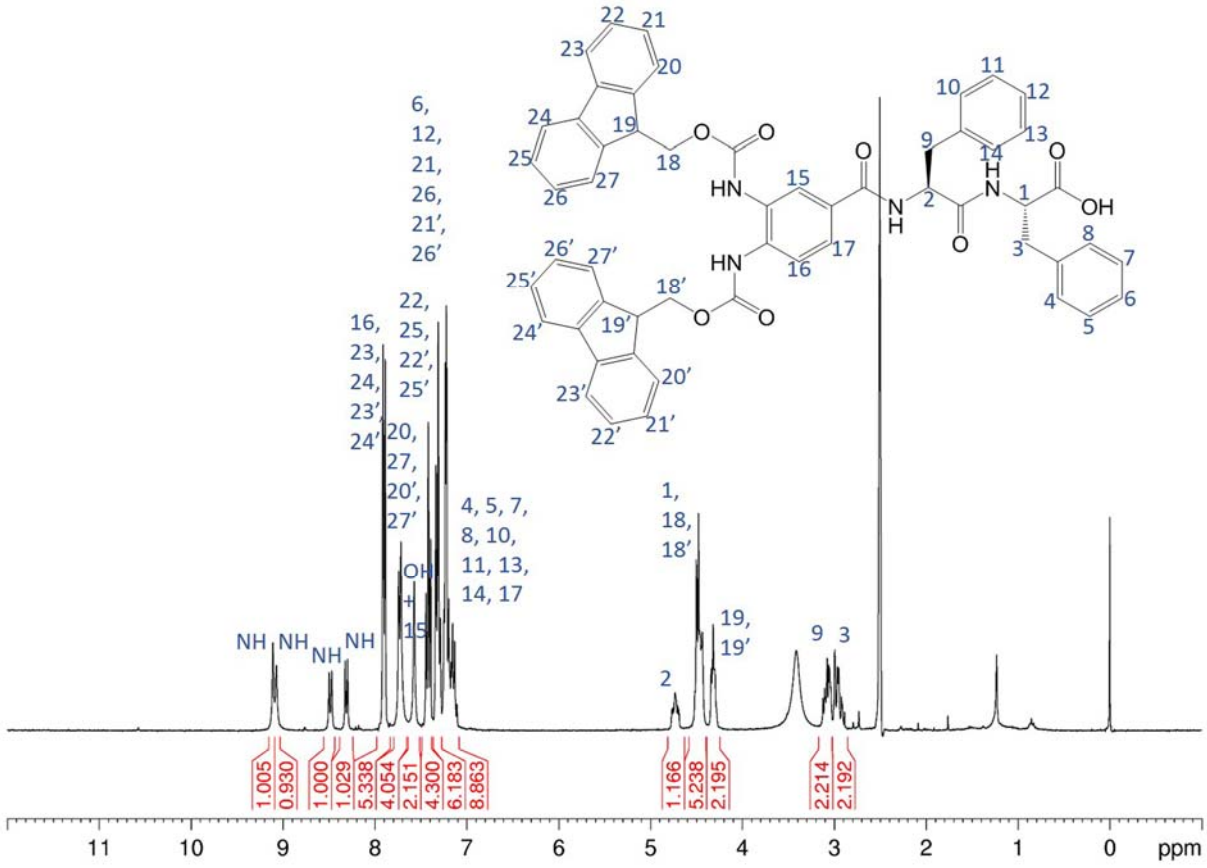


Figure S2. ^{1}H NMR spectrum of **B**.

*Synthesis of OPD-Phe-Phe-OH (**1**)*:

The Fmoc protecting groups of **B** were cleaved with 10% piperidine in DMF for 5 min at room temperature to yield compound **1** in good yield after HPLC purification. ^{1}H NMR of **1** (400 MHz, DMSO-d_6) δ (ppm): 8.25 (d, J = 7.8 Hz, 1 H), 8.14 (d, J = 8.4 Hz, 1 H), 7.41 (s, 1 H), 7.38 (d, J = 8.4 Hz, 1 H), 7.30 (d, J = 7.1 Hz, 2 H), 7.18 (m, 8 H), 6.73 (d, J = 8.4 Hz, 1 H), 4.68 (m, 1 H), 4.47 (m, 1 H), 3.06 (m, 2 H), 2.94 (m, 2 H) (Figure S3). ^{13}C NMR of **1** (100 MHz, DMSO-d_6) δ (ppm): 172.63, 171.56, 165.55, 138.37, 137.30, 129.15, 129.13, 128.14, 127.95, 126.40, 126.10, 115.05, 54.33, 53.42, 36.86, 36.71 (Figure S4). MS: calculated for $C_{25}H_{26}N_4O_4$ [$(M+H)^+$] = 447.20323, obsvd. HR-ESI/MS: *m*/*z* 447.20267 (Figure S5).

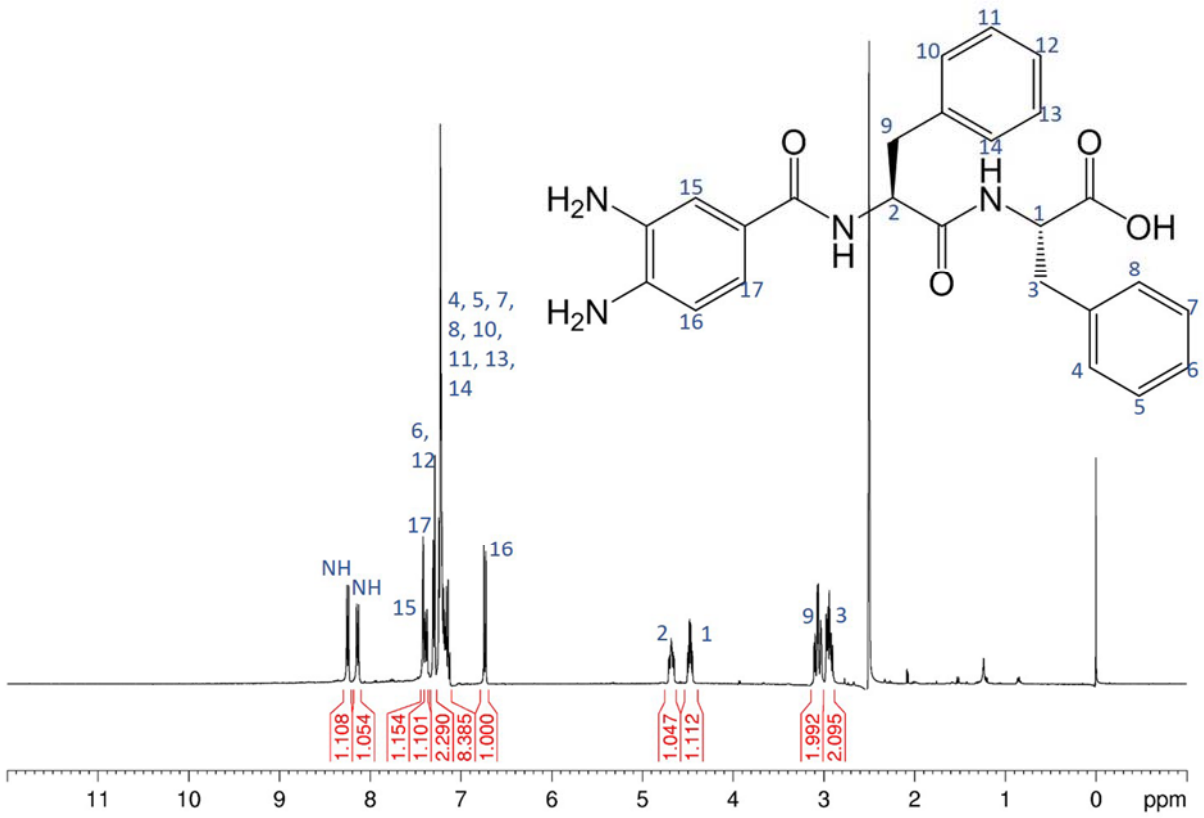


Figure S3. ^{1}H NMR spectrum of **1**.

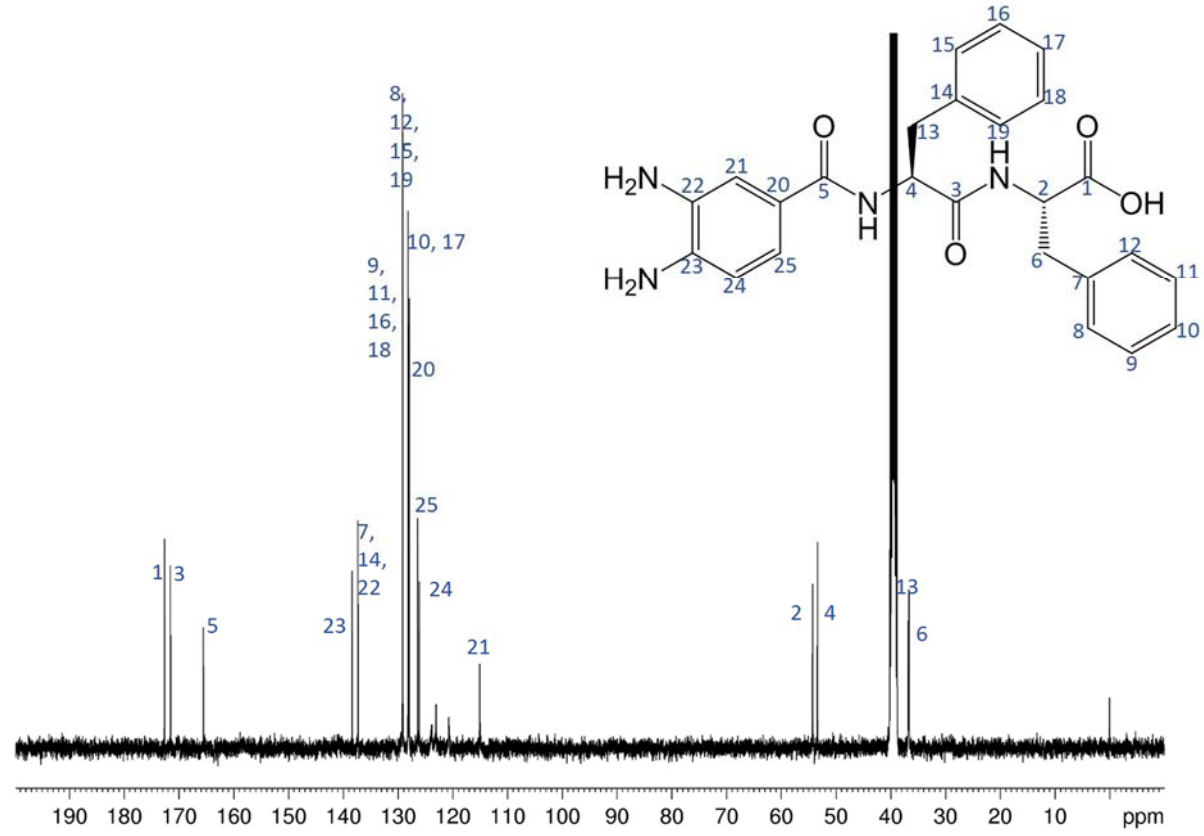


Figure S4. ^{13}C NMR spectrum of **1**.

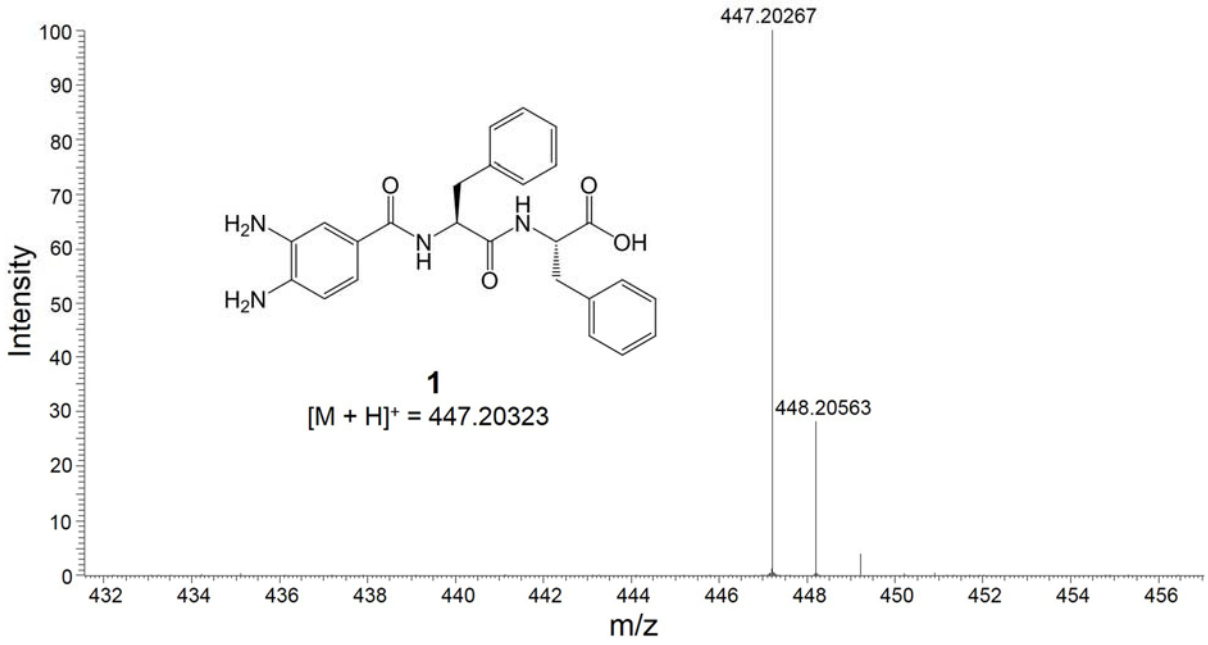
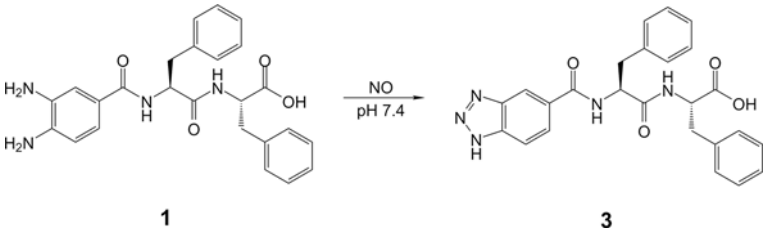


Figure S5. HR-ESI/MS of **1**.

Scheme S2. Preparation of compound **3** in Sol III.



3. Supporting figures and tables

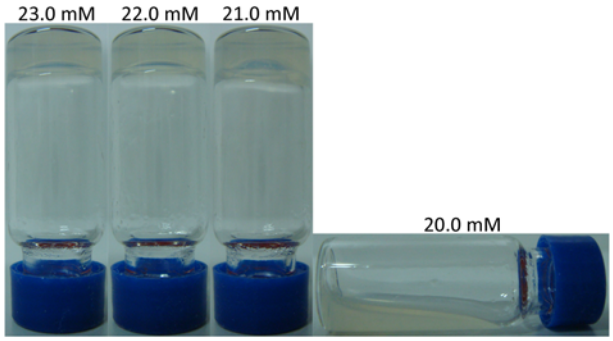


Figure S6. Critical gelation concentration (CGC) determination. Inverted tube test indicated that the CGC for Gel II was 20.5 ± 0.5 mM.

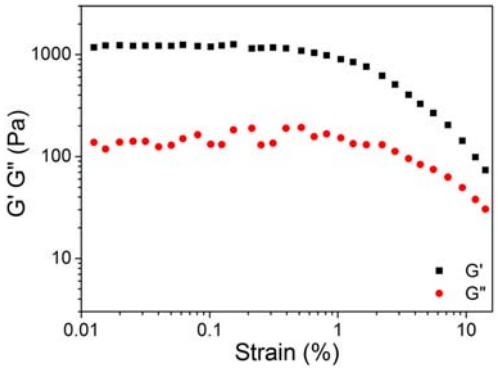


Figure S7. Dynamic strain of storage modulus (G') and the loss modulus (G") of Gel II at the frequency of 1 Hz. Condition: [**1**] = [MGO] = 25.0 mM, pH 7.4, room temperature.

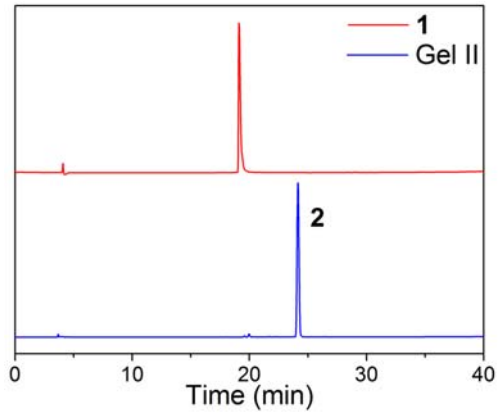


Figure S8. HPLC traces of **1** (red) and Gel II (blue). Absorbance wavelength: 254 nm.

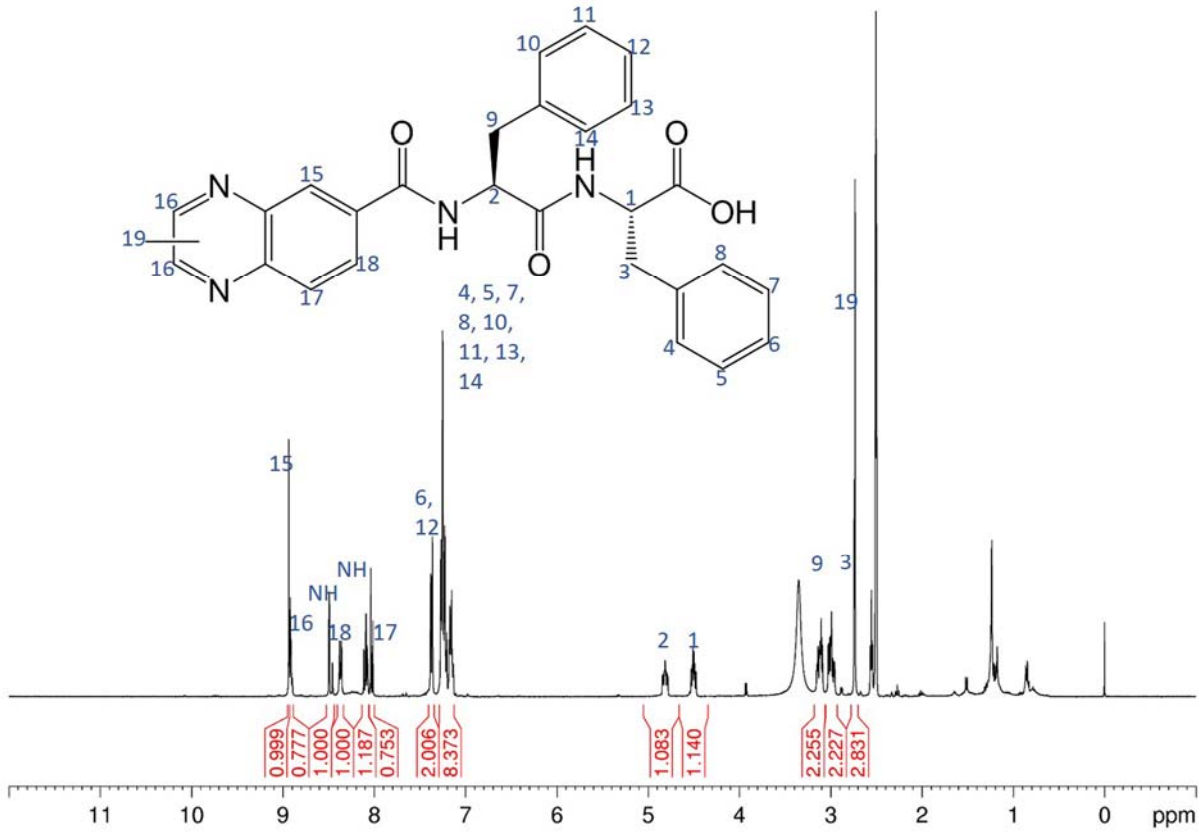


Figure S9. ^{1}H NMR spectrum of **2** (400 MHz, DMSO-d_6) δ (ppm): 8.93 (s, 1 H), 8.91 (m, 1 H), 8.49 (m, 1 H), 8.37 (d, J = 7.8 Hz, 1 H), 8.09 (m, 1 H), 8.02 (d, J = 8.7 Hz, 1 H), 7.37 (d, J = 7.3 Hz, 2 H), 7.20 (m, 8 H), 4.81 (m, 1 H), 4.50 (m, 1 H), 3.12 (m, 2 H), 2.99 (m, 2 H), 2.74 (d, J = 3.4 Hz, 3 H).

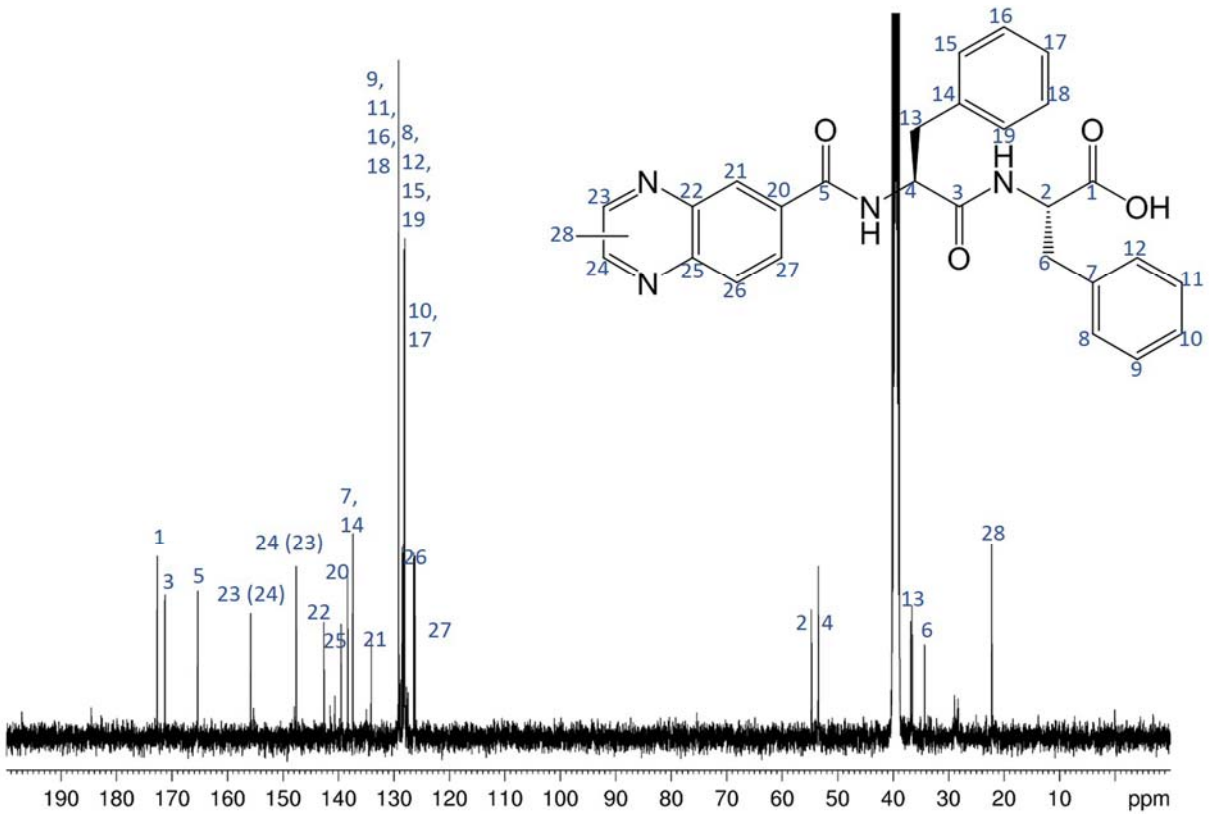


Figure S10. ^{13}C NMR spectrum of **2** (100 MHz, DMSO-d_6) δ (ppm): 172.66, 171.26, 165.34, 155.80, 147.59, 142.58, 139.50, 138.32, 137.40, 134.05, 129.14, 128.14, 128.02, 126.39, 126.19, 54.75, 53.52, 36.74, 34.36, 22.24.

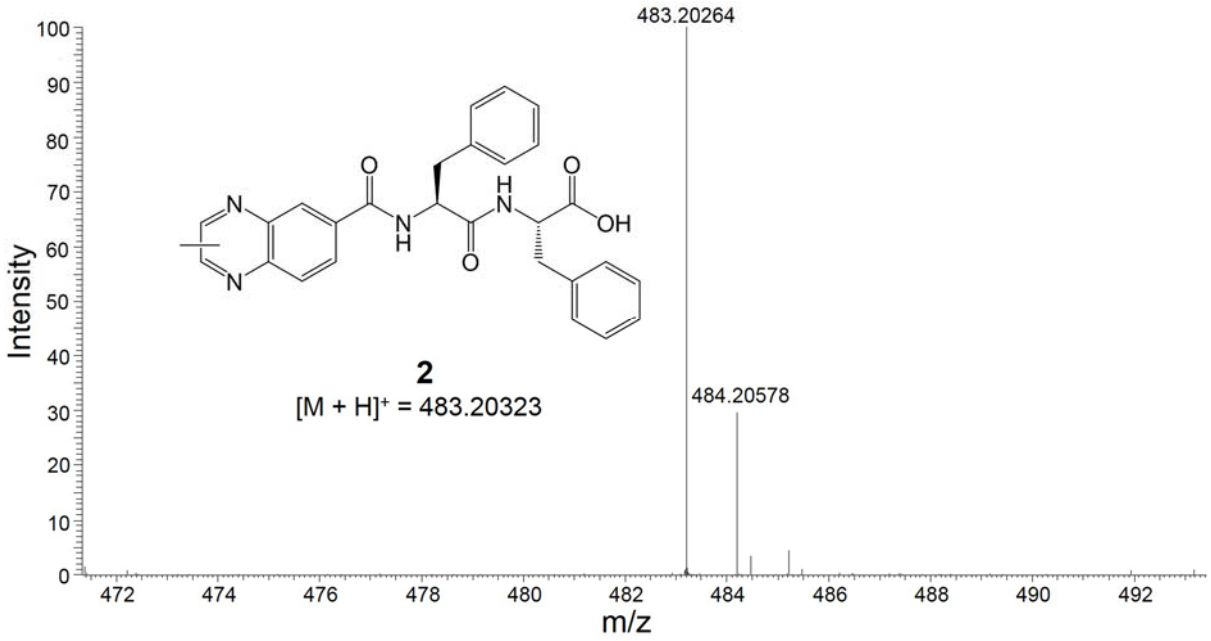


Figure S11. HR-ESI/MS of **2**. Calculated for $C_{28}H_{26}N_4O_4$ $[(M+H)^+]$ = 483.20323, obsvd. HR-ESI/MS: *m*/*z* 483.20264.

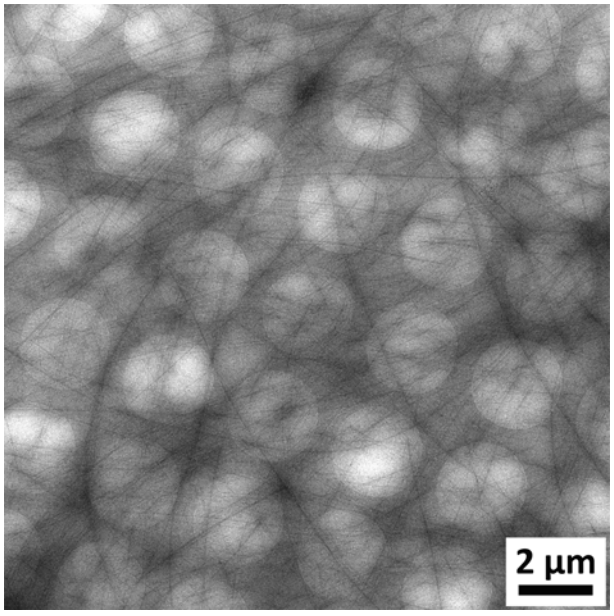


Figure S12. Cryo-TEM image of Gel II at low magnification.

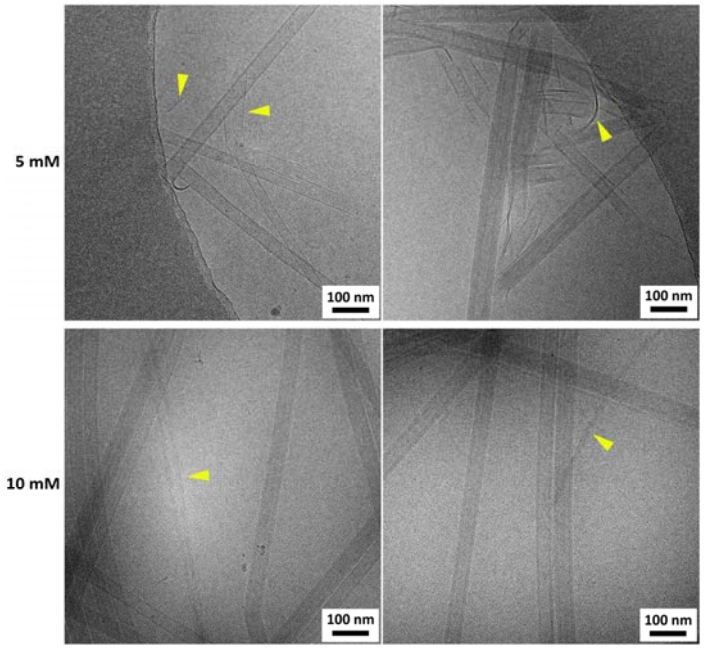


Figure S13. Cryo-TEM images of the reaction mixtures of **1** and equivalent MGO at different concentrations. Yellow arrow heads indicate the nanofibers for self-assembling into the nanotubes.

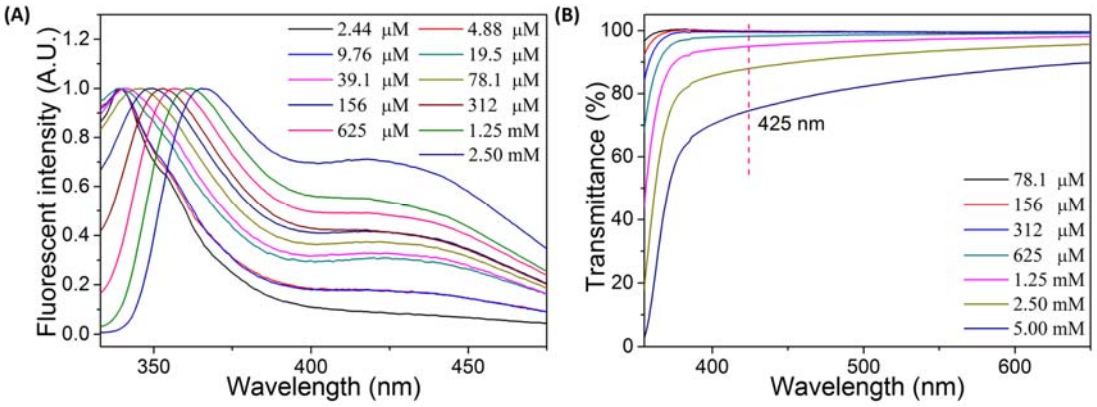


Figure S14. (A) Normalized fluorescence spectra of dilutions of Gel II at different concentrations. Excitation: 280 nm. (B) Transmittance of dilutions of Gel II at different concentrations.

Figure S15. Photograph of Sol III.

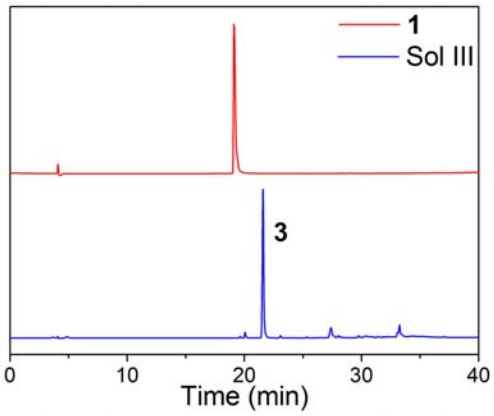


Figure S16. HPLC traces of **1** (red) and Sol III (blue). Absorbance wavelength: 254 nm.

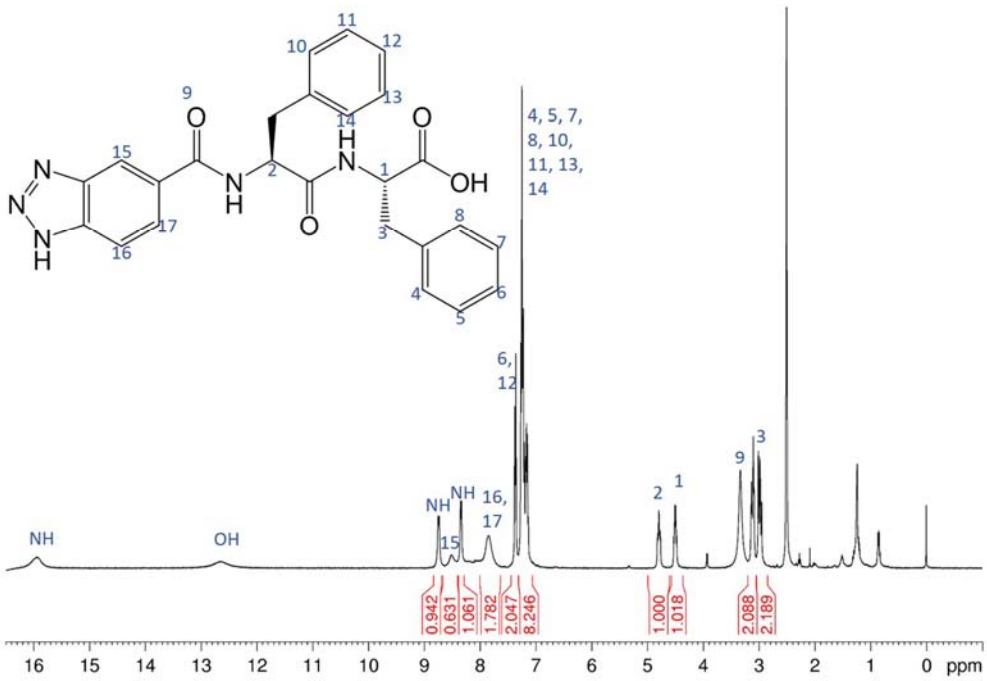


Figure S17. ^{1}H NMR spectrum of **3** (400 MHz, DMSO-d_6) δ (ppm): 8.74 (d, J = 7.6 Hz, 1 H), 8.51 (s, 1 H), 8.34 (d, J = 7.5 Hz, 1 H), 7.84 (s, 2 H), 7.36 (d, J = 7.2 Hz, 2 H), 7.20 (m, 8 H), 4.79 (m, 1 H), 4.50 (m, 1 H), 3.11 (m, 2 H), 2.98 (m, 2 H).

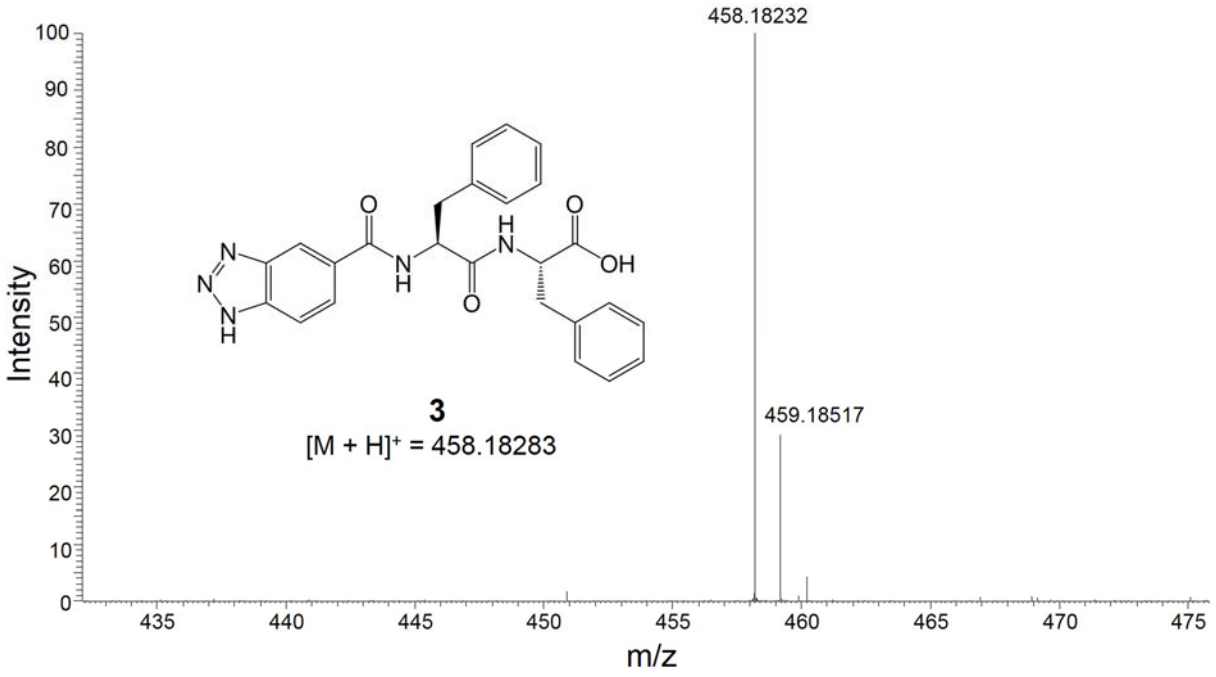


Figure S18. HR-ESI/MS of **3**. Calculated for $C_{25}H_{23}N_5O_4$ $[(M+H)^+]$ = 458.18283, obsvd. HR-ESI-MS: *m*/*z* 458.18232.

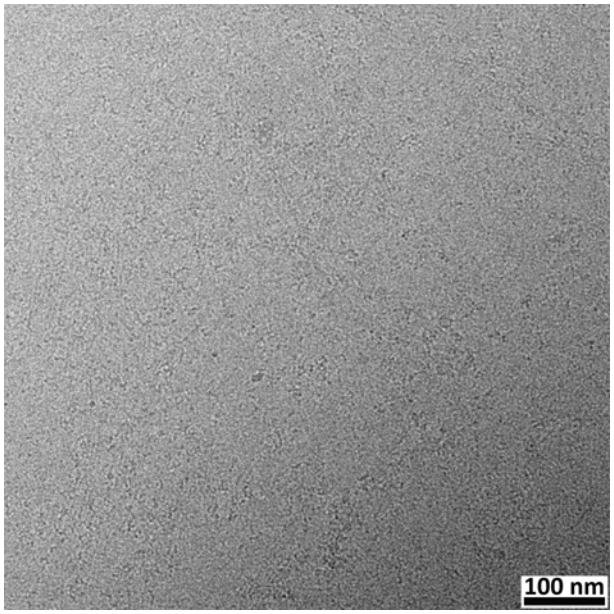


Figure S19. Cryo-TEM image of Sol III.

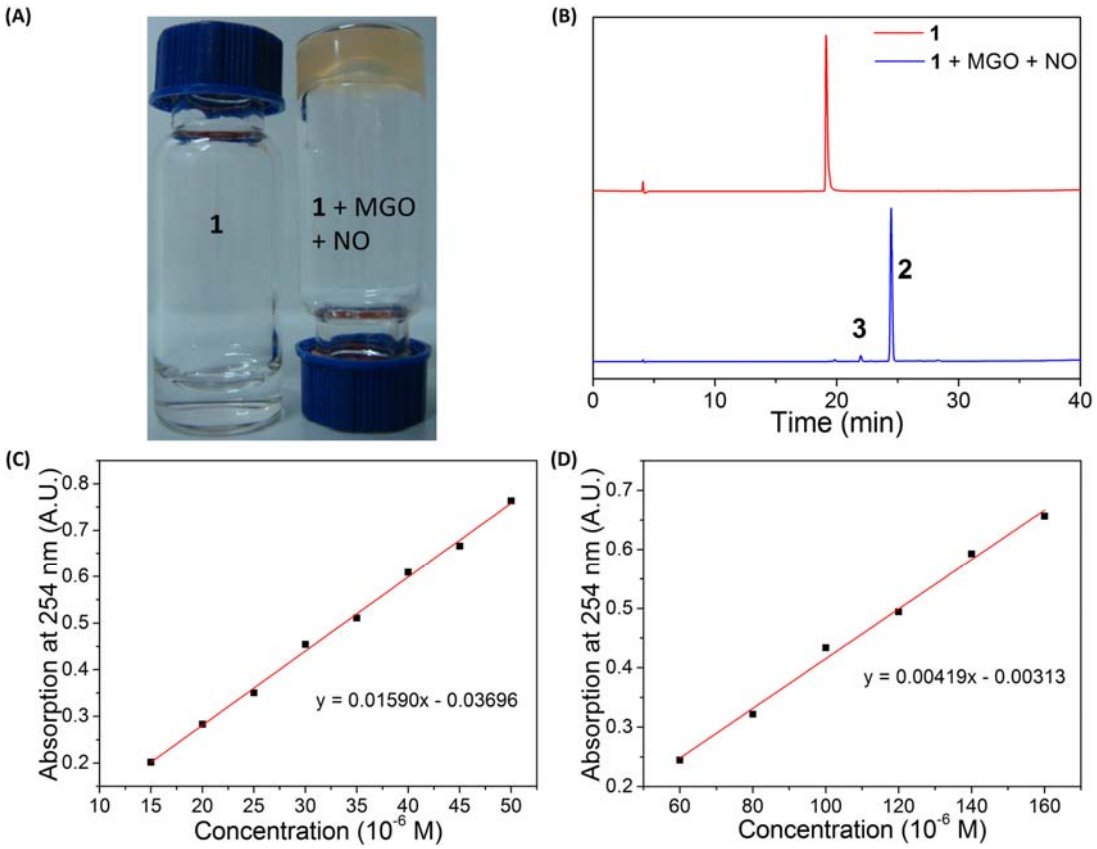


Figure S20. (A) Photographs of solution of **1** at 25.0 mM in buffer (left) and the hydrogel formed from the reaction mixture of 25.0 mM **1**, MGO, and NO (right). (B) HPLC traces of **1** (red) and the reaction mixture of 25.0 mM **1**, MGO, and NO (blue). Absorbance wavelength: 254 nm. (C) Concentration-dependent absorbance of **2** at 254 nm. (D) Concentration-dependent absorbance of **3** at 254 nm.

Table S1. HPLC condition for the purifications of **1**, **2**, **3**, and analyses in Figures S8, 16 & 20B.

Time (min)	Flow (mL/min)	H_2O % (0.1 % TFA)	CH_3CN % (0.1 % TFA)
0	3.0	90	10
3	3.0	90	10
35	3.0	30	70
37	3.0	30	70
38	3.0	90	10
40	3.0	90	10