Electronic Supporting Information

for

An Emissive and pH Switchable Hydrazone-Based Hydrogel

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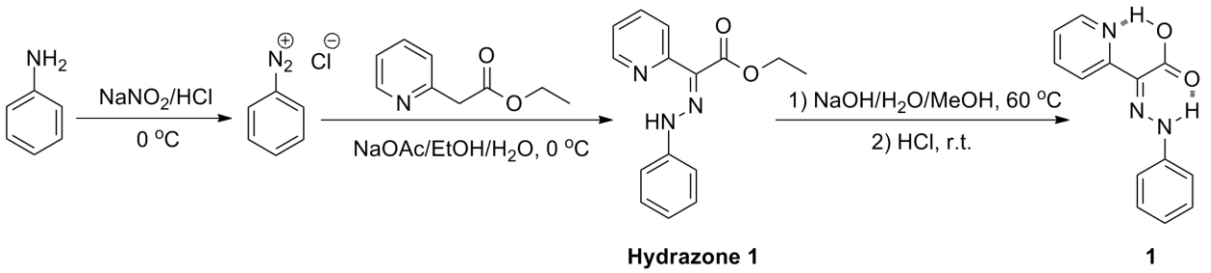
Table of Contents

1 General Information

All reagents and starting materials are commercially available and were used as supplied unless otherwise indicated. All experiments were conducted in air unless otherwise noted. Column chromatography was performed on silica gel (SiliCycle®, 60 A, 230–400 mesh). Deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. and used as received. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 500 MHz NMR spectrometer, with working frequencies of 499.87 MHz for ^{1}H nuclei, and 125.7 MHz for ^{13}C nuclei. Chemical shifts are quoted in ppm relative to tetramethylsilane (TMS), using the residual solvent peak as the reference standard. GC-MS spectra were measured on a Shimadzu Gas Chromatograph/Mass Spectrometer (GCMS-QP2010Plus). Melting points were measured on an Electrothermal Thermo Scientific IA9100X1 digital melting point instrument. UV-Vis spectra were recorded on a Shimadzu UV-1800 UV-Vis spectrophotometer. Fluorescence excitation and emission data in solution were recorded on a JASCO FP-8200 fluorometer, with slit widths kept at 5/5 nm and a scan rate of 500 nm·min^{-1}. Confocal fluorescent images (Parameters: plan fluro 40x, oil, NA 1.3) were obtained using an A1RSi confocal fluorescence microscope from Nikon Company, with λ_{ex} = 405 nm and a collection of λ_{em} at the range of 500 to 550 nm. All spectroscopy samples were taken at room temperature. SEM images were obtained using a XL-30 ESEM-FEG scanning electron microscope from FEI Company, with an accelerating voltage of 15-20 kV.

2 Synthesis

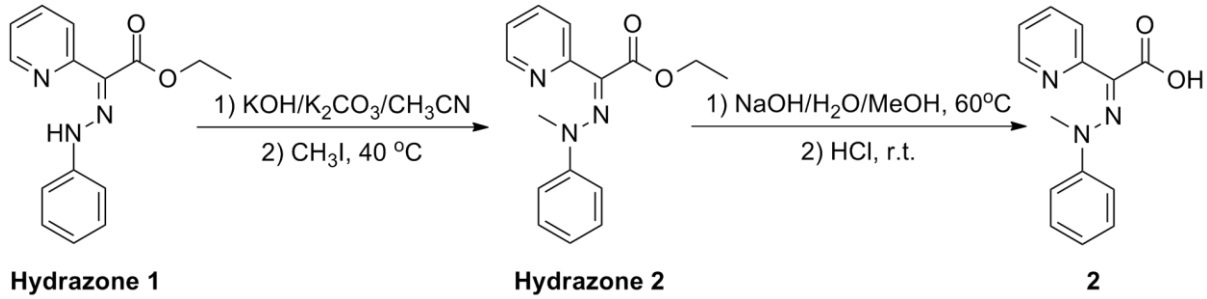


Scheme S1. Synthesis of **Hydrazones 1** and its hydrolyzed product **1**

Hydrazone 1: This compound was synthesized using a modified reported procedure.[S1] Aniline (0.93 g, 0.01 mol) was dissolved in a mixture of 6 mL HCl, and 12 mL of ice cold water, and stirred at 0^{o}C for 15 min. A cold solution (3 mL) of sodium nitrite (1.1 equiv, 0.76 g, 0.011 mol) was then added drop-wise to the aniline solution over a period of 30 min. The resulting light yellow solution was continuously stirred for another hour at 0 ^{o}C. The obtained diazonium salt solution was then added drop-wise to a suspension of ethyl-2-(pyridin-2-yl)acetate (1 equiv, 1.52 ml, 0.01 mol) and sodium acetate (5 equiv, 4.10 g, 0.05 mol) in a cold mixture of ethanol (36 mL) and water (9 mL) over a period of 1 h. The resulting reaction mixture was stirred at room temperature overnight. The precipitate collected by filtration was redissolved in dichloromethane (DCM), washed with saturated sodium bicarbonate solution, then with brine, and finally dried over $MgSO_4$. The crude product was purified by silica gel column chromatography using 1:10 ethyl acetate/hexane as eluent to give **Hydrazone 1** as a yellow powder (2.14 g, 80%). The synthesis of **Hydrazone 1** was confirmed by comparing the obtained ^{1}H NMR spectra with published ones.[S1] ^{1}H NMR (500 MHz, CD_3CN) δ 14.56 (s, 1H), 8.74 (dd, J = 4.9, 1.9, 1H), 8.12 (t, J = 8.3, 1H), 8.03 – 7.80 (m, 1H), 7.41 – 7.36 (m, 5H), 7.08 – 7.01 (m, 1H), 4.34 (q, J = 7.1 Hz, 2H), 1.41 – 1.36 (m, 3H). ppm.

1: This compound was synthesized using a modified procedure.[S2] To a methanol solution (2.0 mL) of **Hydrazone 1** (0.30 g, 1.12 mmol) was added an aqueous solution (2.0 mL) of NaOH (1.5 equiv, 0.067 g, 1.68 mmol). The resulting mixture was stirred at 60 ^{o}C overnight and then cooled to room temperature, followed by the dropwise addition of 1M HCl until the pH

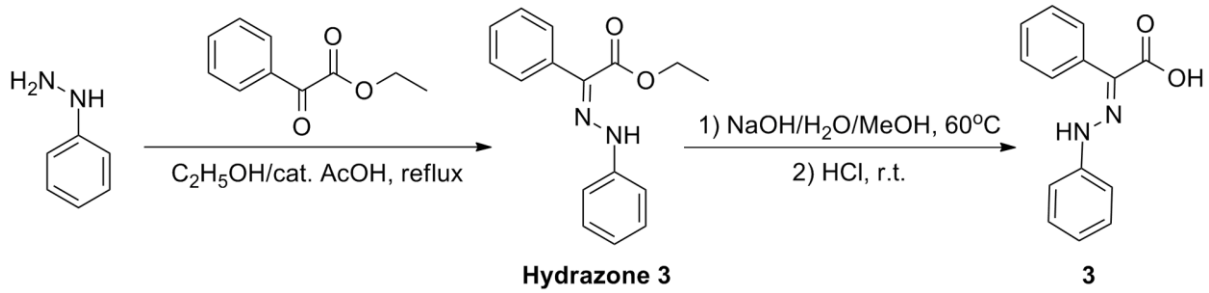
reached to ~6. The crude precipitate was further purified by recrystallization in water to give **1** as a yellow crystalline powder (0.20 g, 74%). m.p. 154.5 – 154.8 ^{o}C; ^{1}H NMR (500 MHz, CD_3CN) δ 18.47 (s, 1H), 13.93 (s, 1H), 8.48 (d, J = 5.2 Hz, 1H), 8.31 (d, J = 8.4 Hz, 1H), 8.07 – 8.00 (m, 1H), 7.49 (d, J = 8.0 Hz, 2H), 7.43 (t, J = 7.9 Hz, 3H), 7.15 (t, J = 7.2 Hz, 1H) ppm; ^{13}C NMR (126 MHz, CD_3CN) δ 143.55, 140.51, 130.12, 124.42, 122.93, 120.44, 115.58 ppm; GC-MS: calcd for $C_{13}H_{11}N_3O_2$ 241.09; m/z (rel. inten.) 197.10 (28%, $[M\text{-}CO_2]^+$), 119.05 (28%), 104.15 (11 %), 92.10 (81%).



Scheme S2. Synthesis of **Hydrazone 2** and its hydrolyzed product **2**

Hydrazone 2: This compound was synthesized following a reported procedure.[S3] The acetonitrile solution (5 mL) of **Hydrazone 1** (0.27 g, 1.0 mmol) was passed through a short plug packed with a mixture of potassium hydroxide and potassium carbonate (1.5 g, KOH/K_2CO_3 (w/w) = 2:1) and then transferred into a flame dried flask, followed by the addition of MeI (3.5 mL, 60 equiv). The resulting mixture was stirred at 50 ^{o}C in a water bath for 2 hrs. The solvent was removed under vacuum, and the residue was dissolved in DCM, then washed with H_2O twice, and dried over $MgSO_4$. The crude product was subjected to silica gel column chromatography using 1:6 ethyl acetate/hexane as eluent to give **Hydrazone 2** as a pale yellow crystalline powder (0.07 g, 25%). m.p. 70.6 – 71.2 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.48 (d, J = 3.9 Hz, 1H), 7.86 (d, J = 8.1 Hz, 1H), 7.62 (t, J = 7.7 Hz, 1H), 7.37 – 7.21 (m, 4H), 7.13 – 7.08 (m, 1H), 7.10 (dd, J = 6.6, 5.5 Hz, 1H), 4.36 (q, J = 7.1 Hz, 2H), 3.52 (s, 3H), 1.32 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.44, 136.50, 129.04, 122.43, 122.25, 119.98, 119.83, 117.20, 116.88, 116.74, 104.14, 100.00, 61.75, 14.08. ppm; GC-MS: calcd for $C_{16}H_{17}N_3O_2$ 283.13; m/z (rel. inten.) 283.10 (2%, M^+), 210.15 (2%), 179.15 (7%), 134.05 (4%), 105.05 (100%), 91.05 (2%), 77.05 (54%).

2: The compound was synthesized following the procedure for **1**, while starting from **Hydrazone 2**. The compound was obtained as a yellow powder; yield 41%. m.p. 72.2 – 72.9 ^{o}C; ^{1}H NMR (500 MHz, CD_3CN) δ 8.50 (d, J = 5.0 Hz, 1H), 8.20 (d, J = 8.3 Hz, 1H), 7.93 (t, J = 7.8 Hz, 1H), 7.56 (d, J = 8.3 Hz, 2H), 7.44 (t, J = 7.8 Hz, 2H), 7.41 – 7.36 (m, 1H), 7.15 (t, J = 7.3 Hz, 1H), 3.58 (s, 3H) ppm; ^{13}C NMR (126 MHz, CD_3CN) δ 146.01, 139.09, 129.70, 123.74, 123.18, 121.09, 117.28, 43.27 ppm; GC-MS: calcd for $C_{14}H_{13}N_3O_2$ 255.10; m/z (rel. inten.) 211.20 (6%, $[M\text{-}CO_2]^+$), 133.20 (4%), 119.20 (3%), 105.20 (47%), 79.15 (100%).



Scheme S3. Synthesis of **Hydrazone 3** and its hydrolyzed product **3**

Hydrazone 3: The compound was synthesized using a modified reported procedure.[S4] Phenylhydrazine (0.65 g, 1.2 equiv, 0.006 mol) and several drops of acetic acid were added to an ethanol solution (10 mL) of ethyl phenylglyoxylate (0.89 g, 0.005 mol) under a nitrogen atmosphere. The resulting mixture was refluxed for 3 hrs, and then cooled to room temperature. The solvent was removed under vacuum, and the crude residue was subjected to column chromatography using 12:1 hexanes/ethyl acetate as eluent to give **Hydrazone 3** as an orange crystalline solid (0.93 g, 78 %). The synthesis of **Hydrazone 3** was confirmed by comparing the obtained ^{1}H NMR with a published one.[S4] ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.42 (s, 1H), 7.69 – 7.62 (m, 2H), 7.43 – 7.28 (m, 7H), 7.00 (ddd, J = 8.5, 3.5, 1.7 Hz, 1H), 4.36 (q, J = 7.1 Hz, 2H), 1.37 (t, J = 7.1 Hz, 3H) ppm.

3: The compound was synthesized by following the procedure for **1**, while starting from **Hydrazone 3**. The compound was obtained as a yellow crystalline solid; yield 78%. m.p. 164.3 – 165.0 ^{o}C; ^{1}H NMR (500 MHz, CD_3CN) δ 12.34 (s, 1H), 7.73 – 7.67 (m, 2H), 7.45 – 7.30 (m, 7H), 7.04 (tt, J = 7.2, 1.4 Hz, 1H) ppm; ^{13}C NMR (126 MHz, CD_3CN) δ 164.64,

129.96, 129.09, 128.41, 128.17, 122.99, 114.68 ppm; GC-MS: calcd for $C_{14}H_{12}N_2O_2$ 240.09; m/z (rel. inten.) 196.15 (31%, $[M\text{-}CO_2]^+$), 118.15 (5%), 92.10 (94%).

3 NMR Spectra

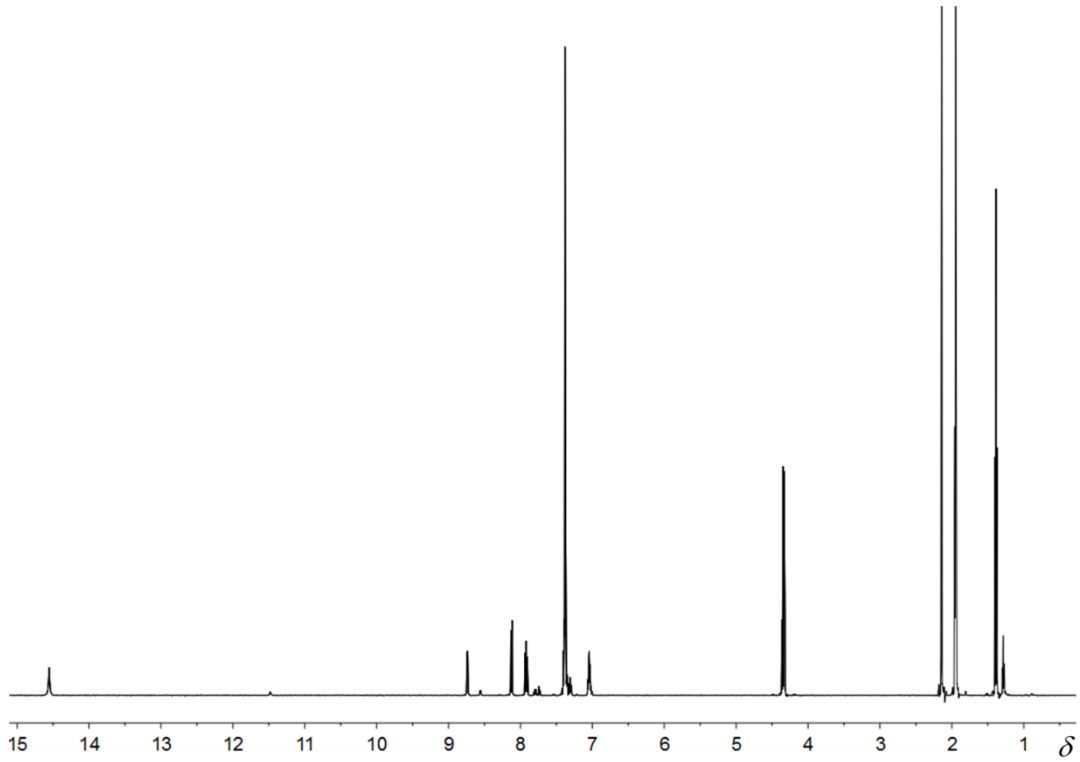


Figure S1. ^{1}H NMR spectrum of the **Hydrazone 1** in CD_3CN at 294 K (the spectra contains a small amount of the *Z* isomer)

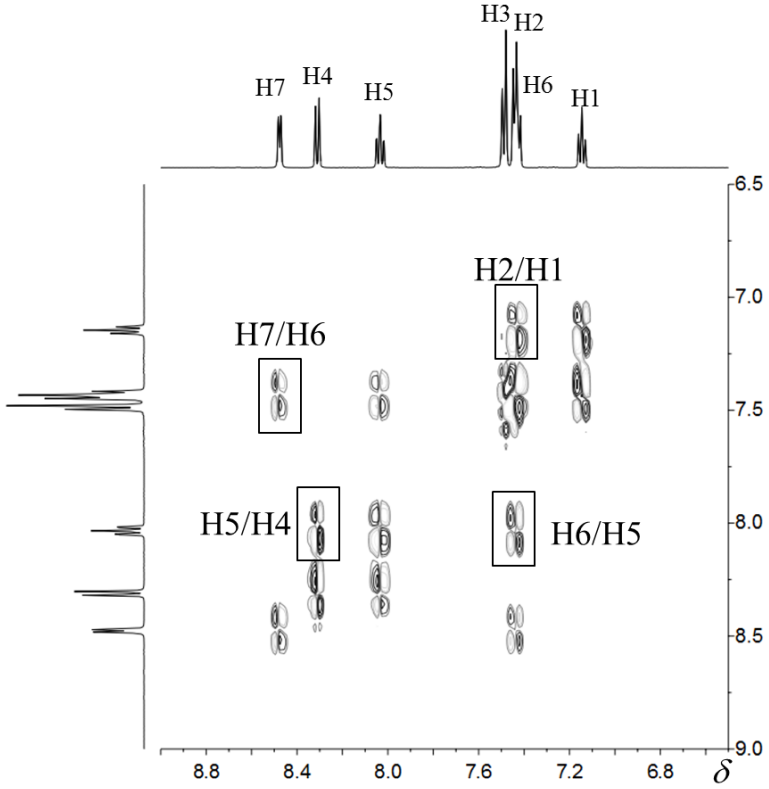


Figure S2. The 2D COSY of **1** in CD_3CN at 294 K

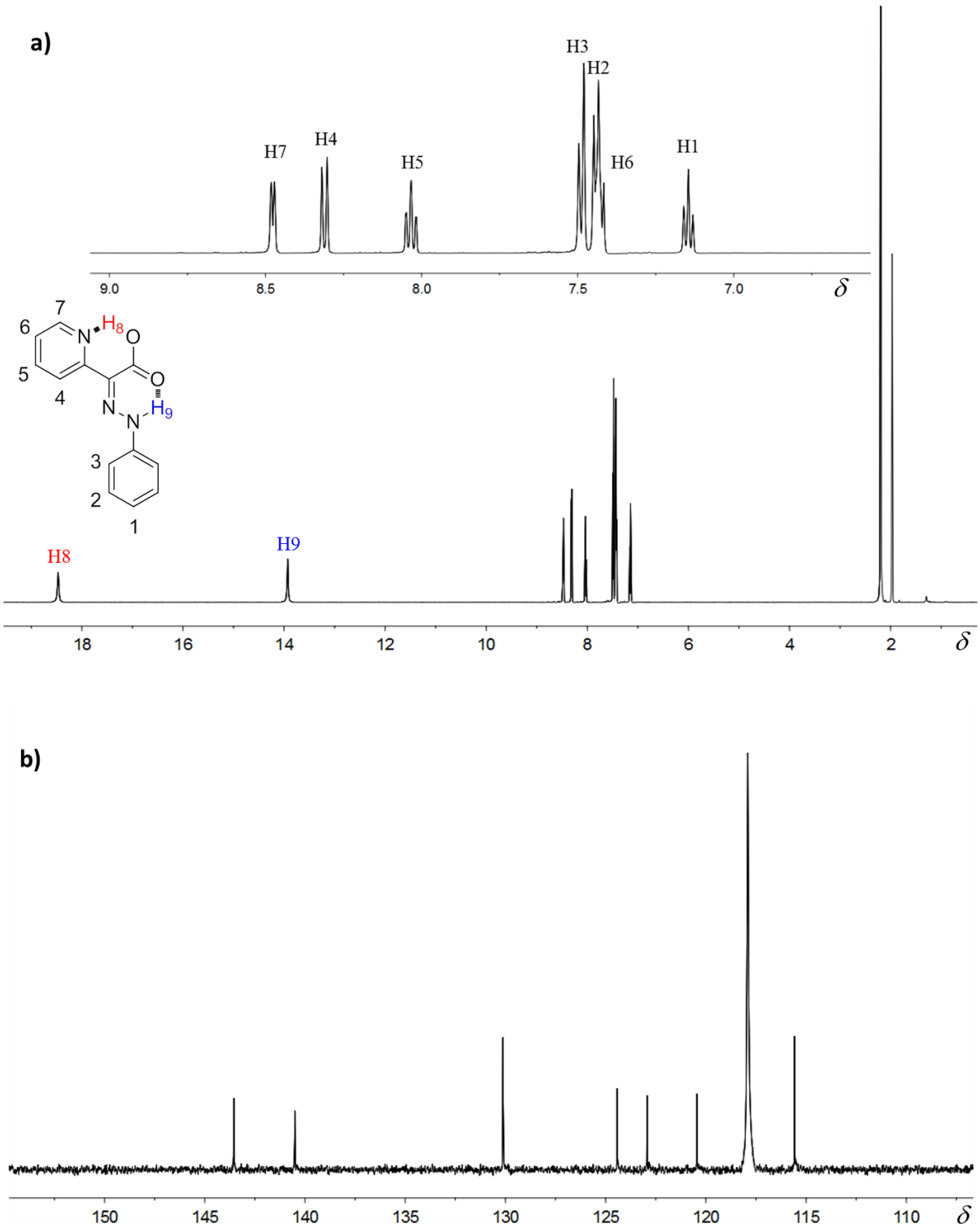


Figure S3. The a) ^{1}H NMR spectrum of **1** with peak assignments and a zoom-in on the aromatic region; and b) ^{13}C NMR spectrum in CD_3CN at 294 K

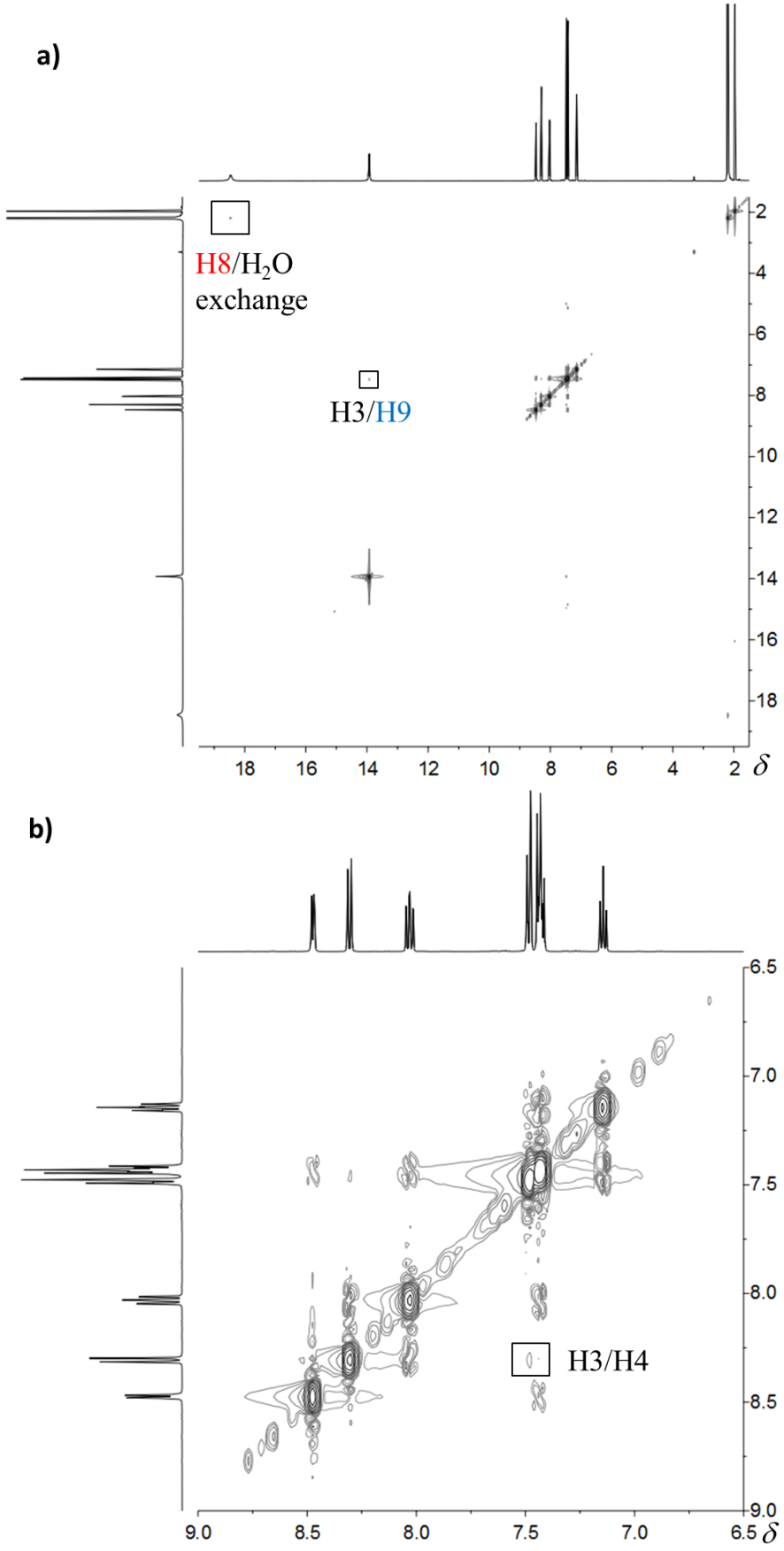


Figure S4. The 2D ROESY of the **1** in CD_3CN at 294 K, a) full and b) a zoom-in spectrum on the aromatic region

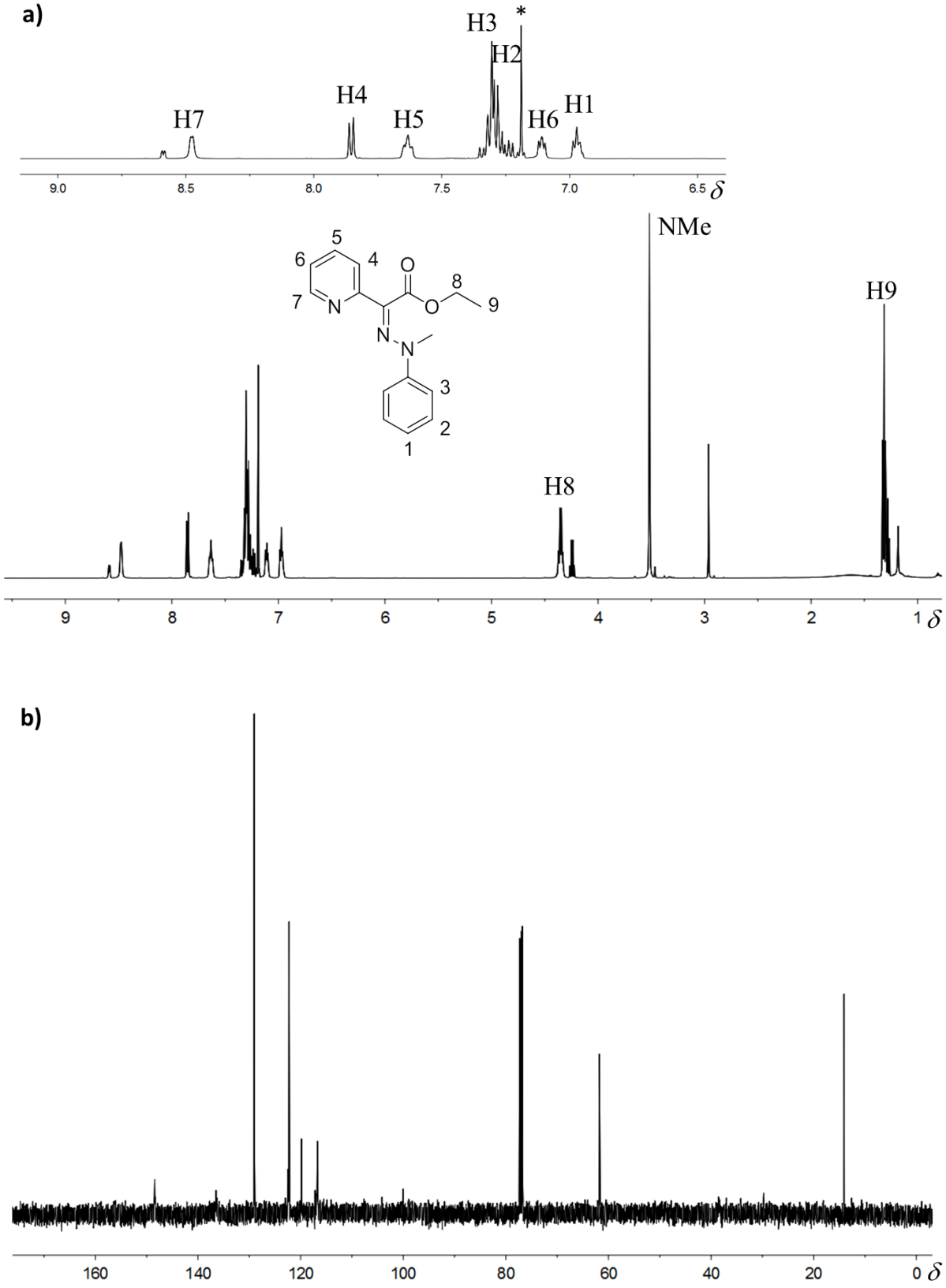


Figure S5. The a) ^{1}H NMR spectrum of **Hydrazone 2** with peak assignments and a zoom-in on the aromatic region; and b) ^{13}C NMR spectrum in $CDCl_3$ at 294 K. The star indicates the chloroform peak. Both *Z* and *E* configurations **Hydrazone 2** are evident.

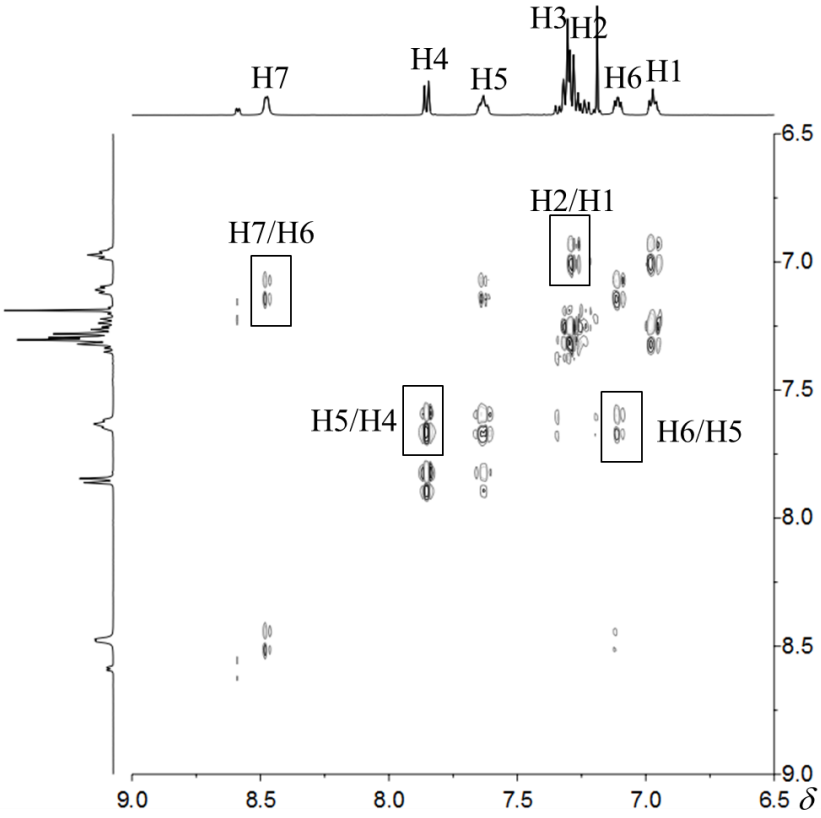


Figure S6. The 2D COSY of **Hydrazone 2** in $CDCl_3$ at 294 K

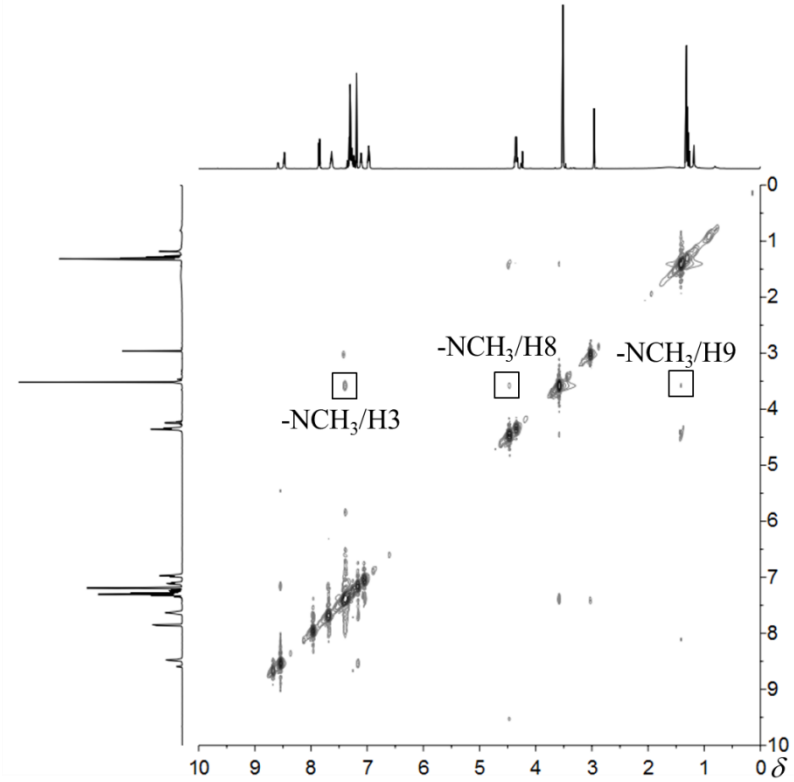


Figure S7. The 2D NOESY of **Hydrazone 2** in $CDCl_3$ at 294 K

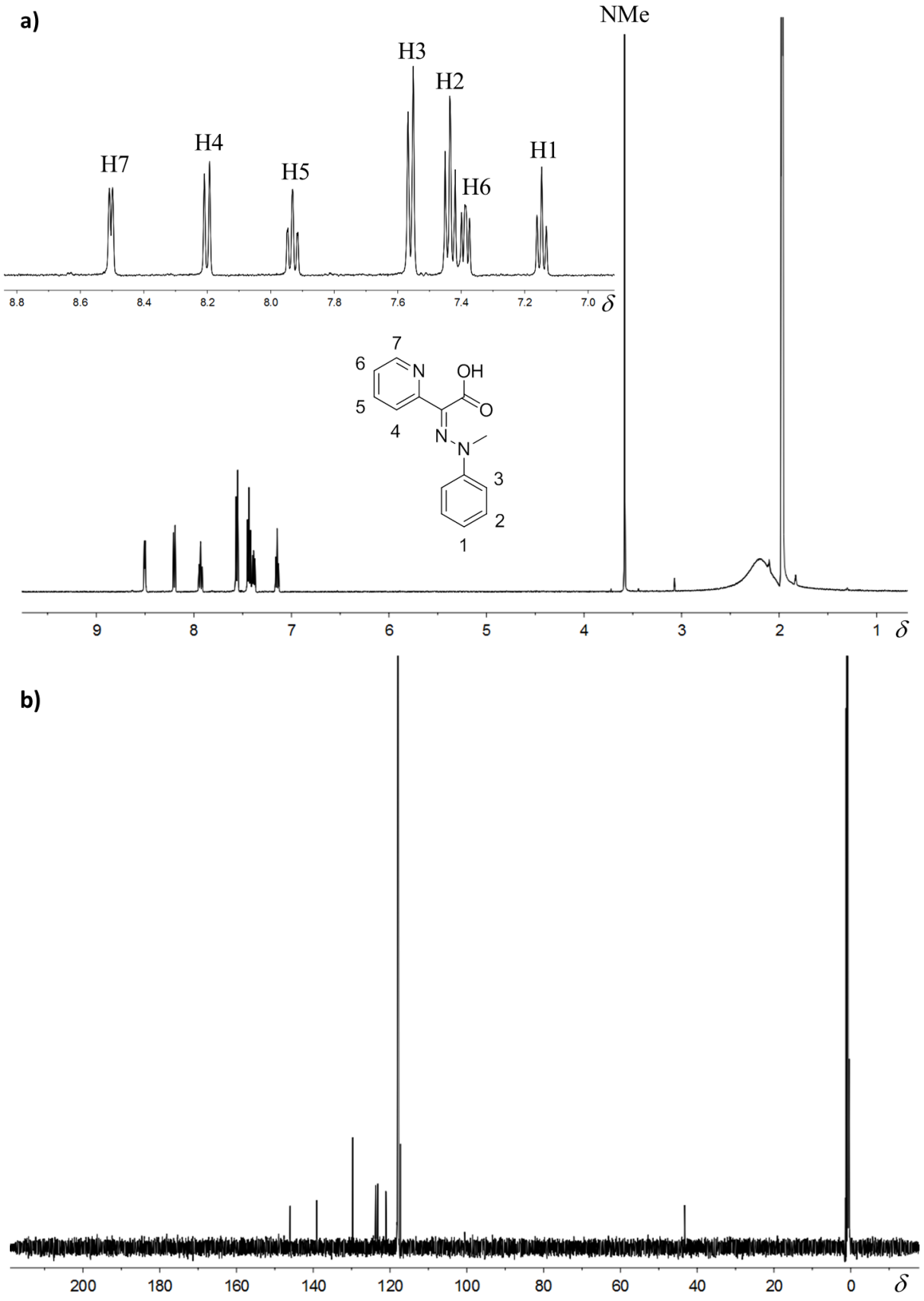


Figure S8. The a) ^{1}H NMR spectrum of **2** with peak assignments and a zoom-in on the aromatic region; and b) ^{13}C NMR spectrum in CD_3CN at 294 K

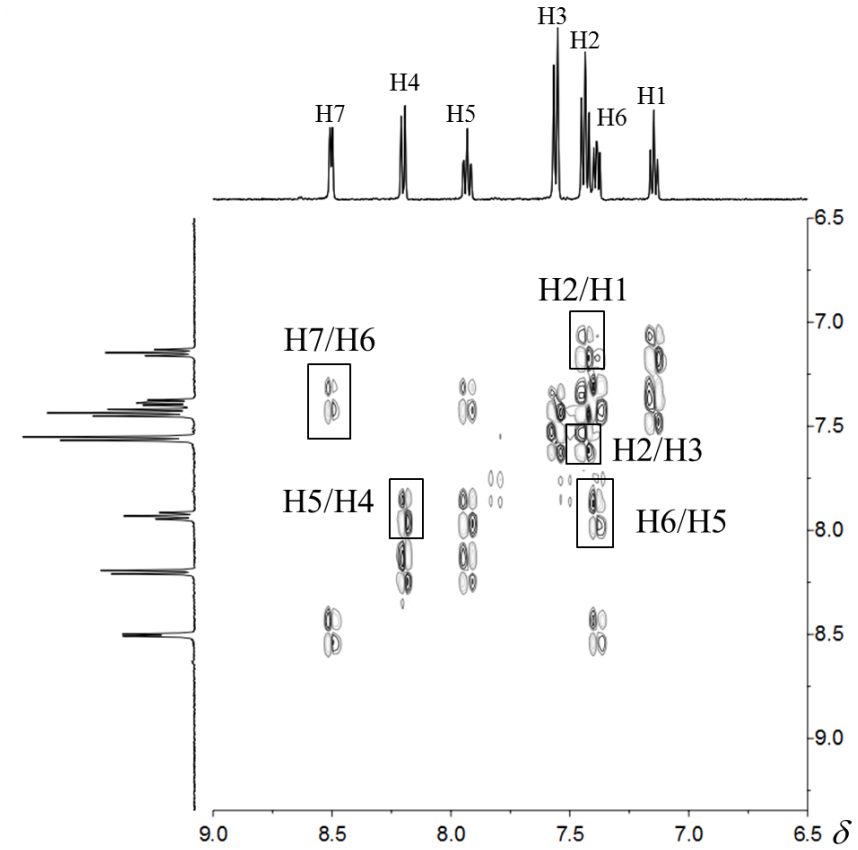


Figure S9. The 2D COSY of **2** in CD_3CN at 294 K

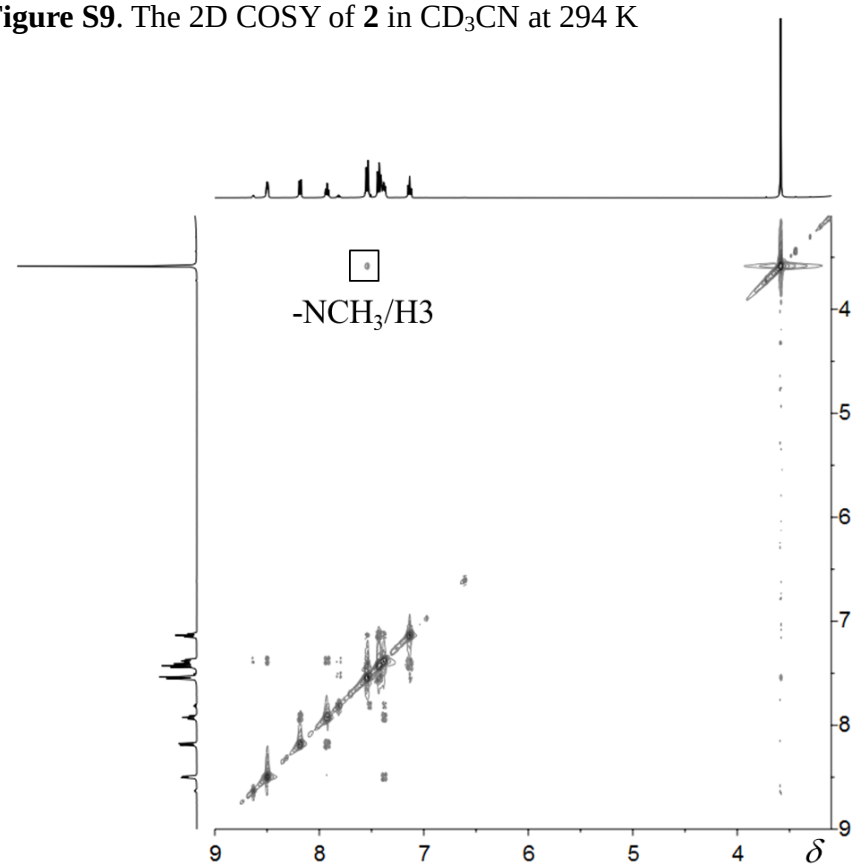


Figure S10. The 2D ROESY of **2** in CD_3CN at 294 K, showing the only correlation between the NCH_3 protons and proton H3.

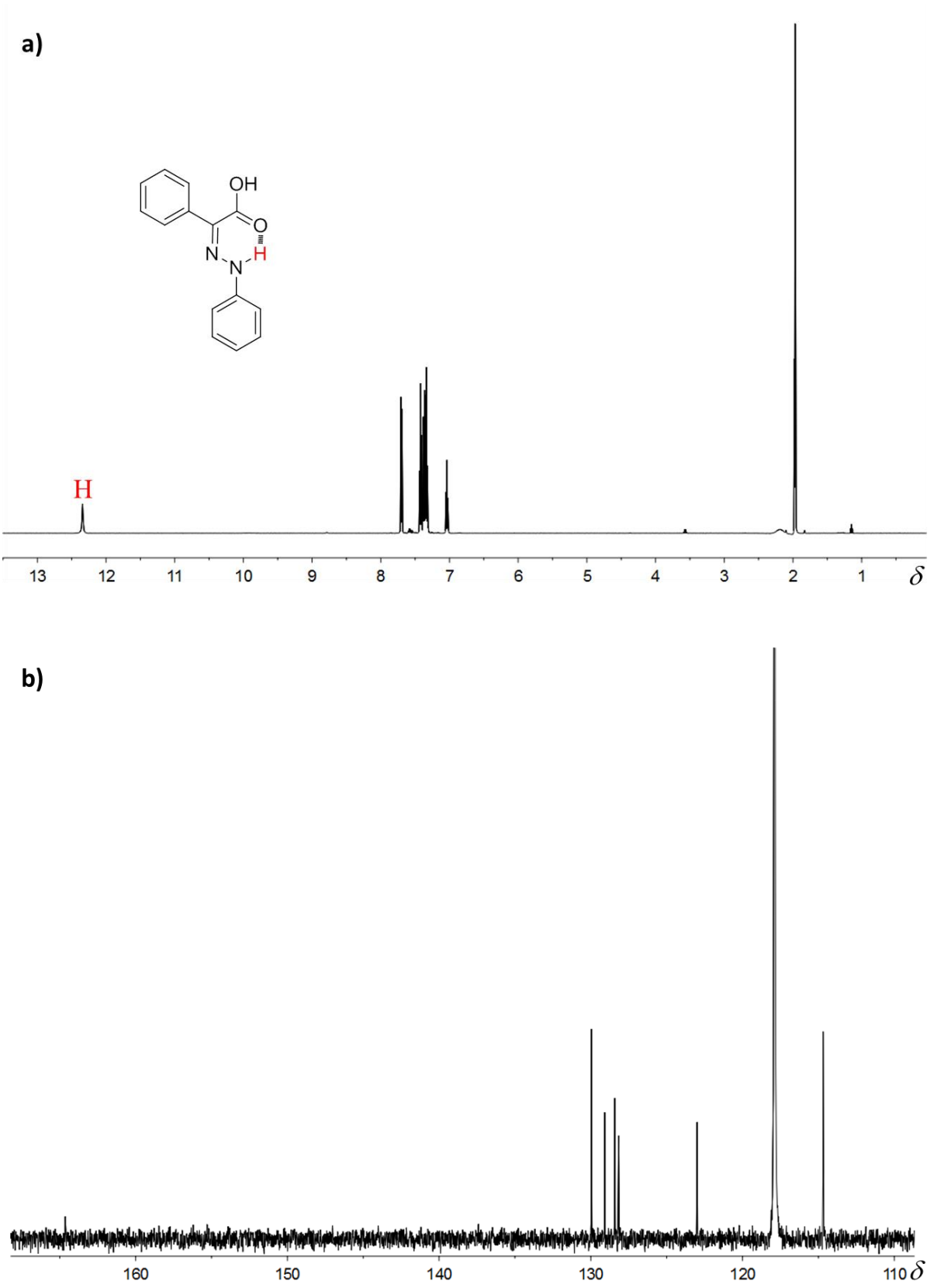


Figure S11. The a) ^{1}H NMR spectrum and b) ^{13}C NMR spectrum of **3** (*Z*) in CD_3CN at 294 K

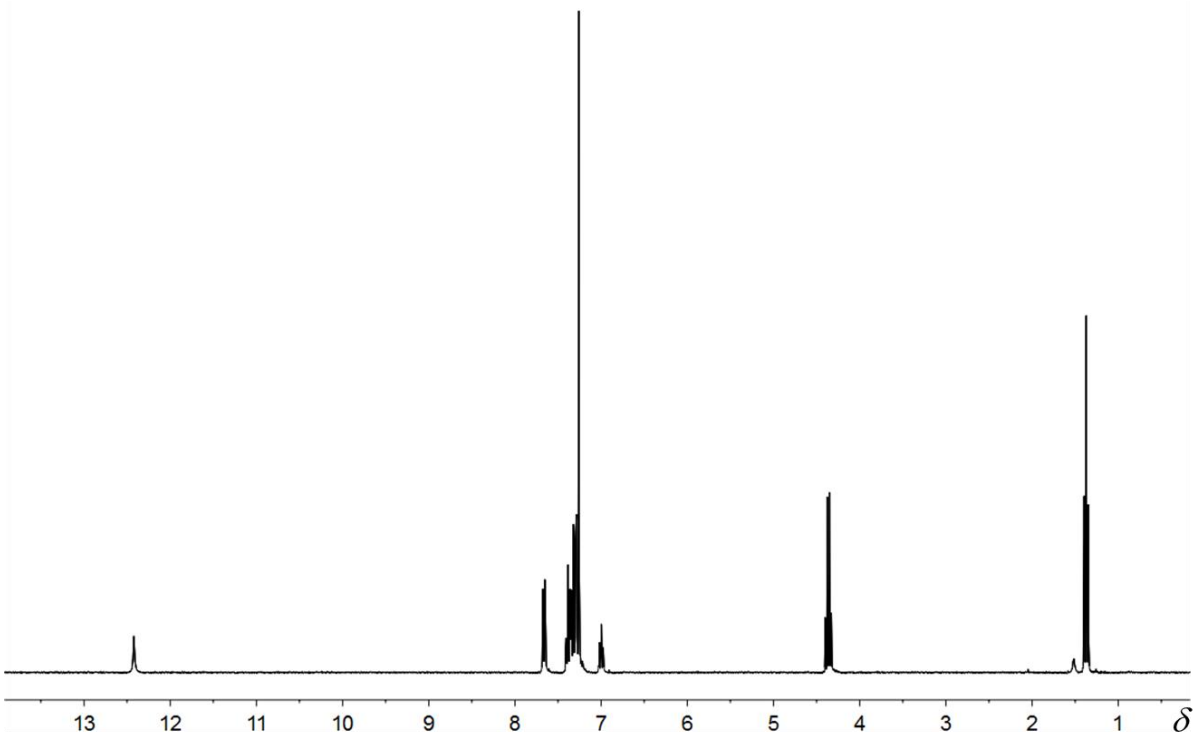


Figure S12. ^{1}H NMR spectrum of the **Hydrazone 3** in $CDCl_3$ at 294 K

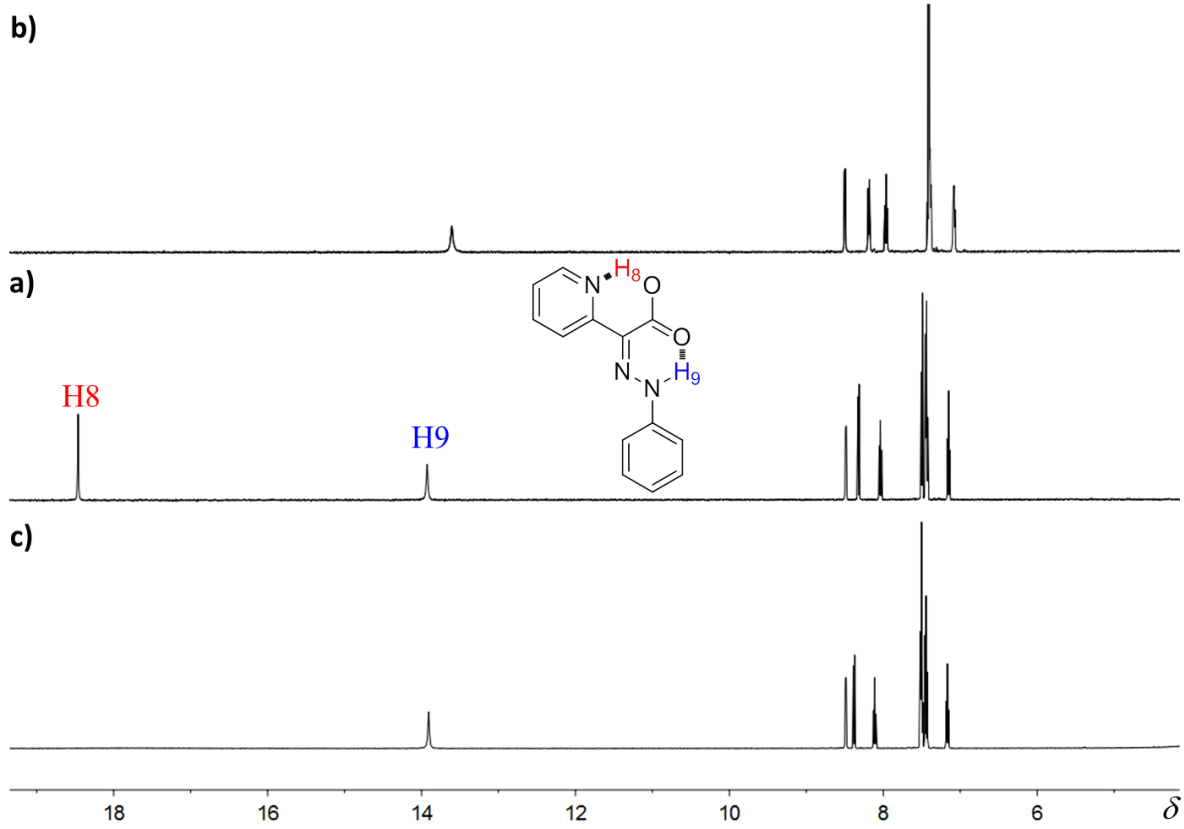


Figure S13. ^{1}H NMR spectra of compound **1** (a) in the presence of b) 1 equiv of Et_3N and c) 1 equiv of CF_3COOH, in CD_3CN at 294 K

4 Gelation Properties

A weighed amount of the potential gelators (**1**–**3**; 0.1 *wt*%) and a measured amount of liquid were placed into a screw-capped vial (ca. 13 mm inner diamer). The vials were heated using a heating gun until the solid was dissolved, and then cooled rapidly in an ice-water bath. Finally the vials were turned upside down to observe whether the solution could flow or not (Table S1).

The gel-to-sol transition temperature (T_{gel}) was determined using the conventional inverse flow method.[S6] Screw-capped vials containing gel were inverted and attached to a thermometer near the glass bulb end. This assembly was immersed in a stirred water bath at room temperature. The temperature of water bath was slowly increased at a rate of 1 ^{o}C/min. Two specific temperatures were recorded: a) one when the first liquid drop fell from the gel and b) the end point when the whole mass fell down. The average of these two temperatures was taken as the T_{gel} of the gel.

A Zicome® Silicone 26 Alphabet Letter Ice Mold was purchased from amazon.com, and used for making self-supporting hydrogels. A 2 mL hot solution of compound **1** (0.5 *wt*%) was poured into the letter D mold, and then cooled down in a fridge. The obtained D-shaped gel was placed in a petri dish and its stability under ambient conditions was monitored (Figure S14).

Table S1. Gelation properties of **1**, **2** and **3**

Solvents	**1**[a]	**2**[a]	**3**[a]
Water	G[b]	S	P
Hexanes	P	P	P
Toluene	S	S	S
Chloroform	S	S	S
Dichloromethane	S	S	S
Ethanol	S	S	S
Methanol	S	S	S
Acetonitrile	S	S	S
DMSO	S	S	S
DMF	S	S	S
Acetone	S	S	S

[a] G, S, and P denote gelation, solution, and precipitation, respectively.
[b] T_{gel} = 49.5 ^{o}C

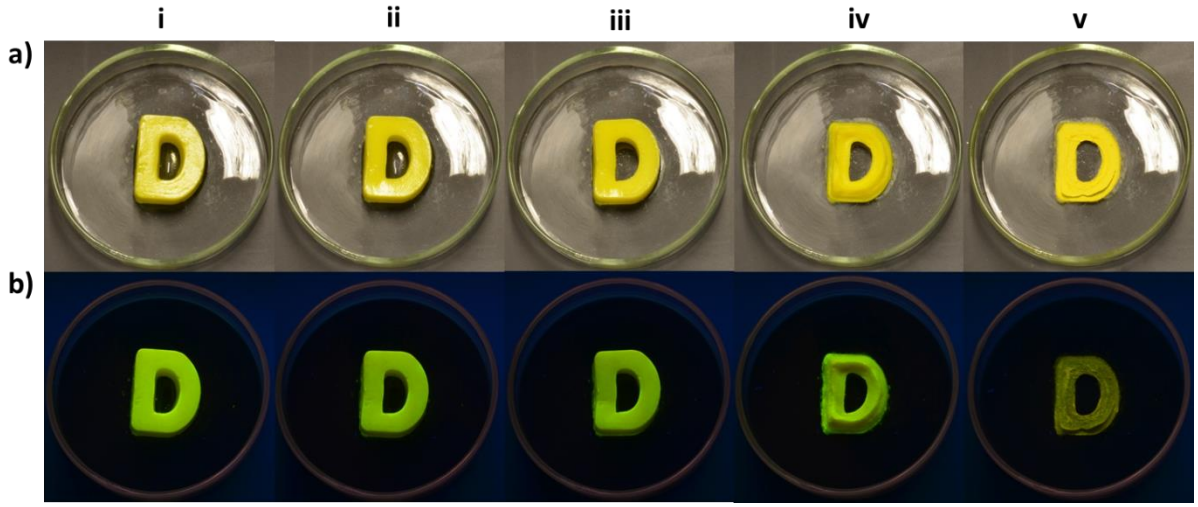


Figure S14. Photographs of a D-shaped hydrogel of compound **1** under a) ambient light or b) UV-light after (i) 0.25, (ii) 2, (iii) 4, (iv) 16, and (v) 28 hrs.

5 SEM Characterization

Samples for high vacuum mode SEM were prepared by the slow evaporation of 0.1 *wt*% solutions under ambient conditions. The dried SEM images of the samples were obtained using a XL-30 ESEM-FEG scanning electron microscope from FEI Company, with an accelerating voltage of 15 kV and a spot size of 2.0. Before imaging, all the samples were sputter-coated with gold.

For low vacuum mode (H_2O mode), a small piece of the hydrogel (0.1 *wt*%) was placed on the sample holder, and immediately measured without gold sputter-coating. SEM images of wet samples were obtained using the same microscope, with an accelerating voltage of 20 kV and a spot size of 3.0.

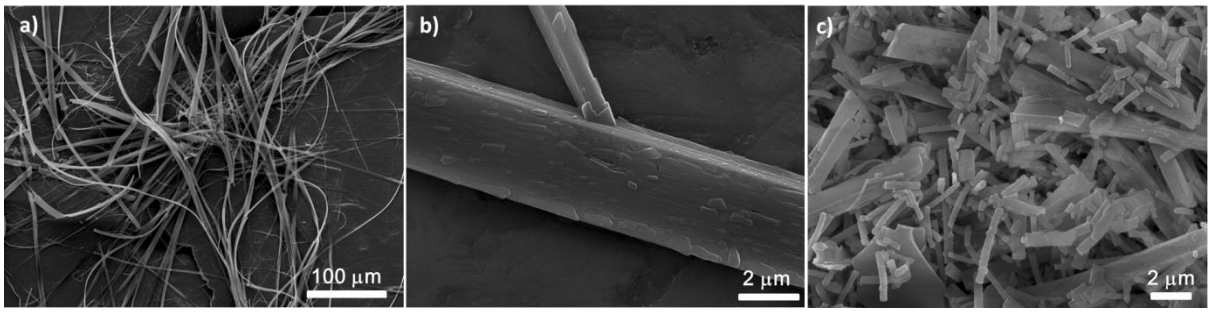


Figure S15. SEM images of 0.1 *wt*% of dried hydrogel **1** in the absence (a, b) and presence (c) of 1 equiv of NaOH.

6 Crystallography

Data were collected using a Bruker CCD (charge coupled device) based diffractometer equipped with an Oxford Cryostream low-temperature apparatus operating at 173 K. Data were measured using omega and phi scans of 0.5° per frame for 30 s. The total number of images was based on results from the program COSMO[S7] where redundancy was expected to be 4.0 and completeness of 100% out to 0.83 Å. Cell parameters were retrieved using APEX II software[S8] and refined using SAINT on all observed reflections. Data reduction was performed using the SAINT software[S9] which corrects for Lp. Scaling and absorption corrections were applied using SADABS[S10] multi-scan technique, supplied by George Sheldrick. The structures are solved by the direct method using the SHELXS-97 program and refined by least squares method on F^2, SHELXL- 97,[S11] which are incorporated in OLEX2.[S12] All non-hydrogen atoms are refined anisotropically. Hydrogens were calculated by geometrical methods and refined as a riding model.

1 (10 mg) was dissolved in 5 mL CH_3CN, and then filtrated. The filtrate was allowed over 3 days evaporation to give yellow chunk crystals. Crystal structure of **1** was solved in the space group Pbcn (# 60). Water was modeled at 0.25 occupancy over two sites in asymmetric cell, so that there is 1/2 a water molecule per molecule of interest.

3 (10 mg) was dissolved in 3 mL hot water, and then filtrated while hot. The hot filtrate was allowed to slowly cool to room temperature. Yellow needle crystals crashed out and then were collected by filtration. Crystal Structure of **3** was solved in the space group Pī (#2). The top phenyl ring was disordered over two orientations, and parted with 50% fixed occupancy. The crystal used for the diffraction study showed no decomposition during data collection.

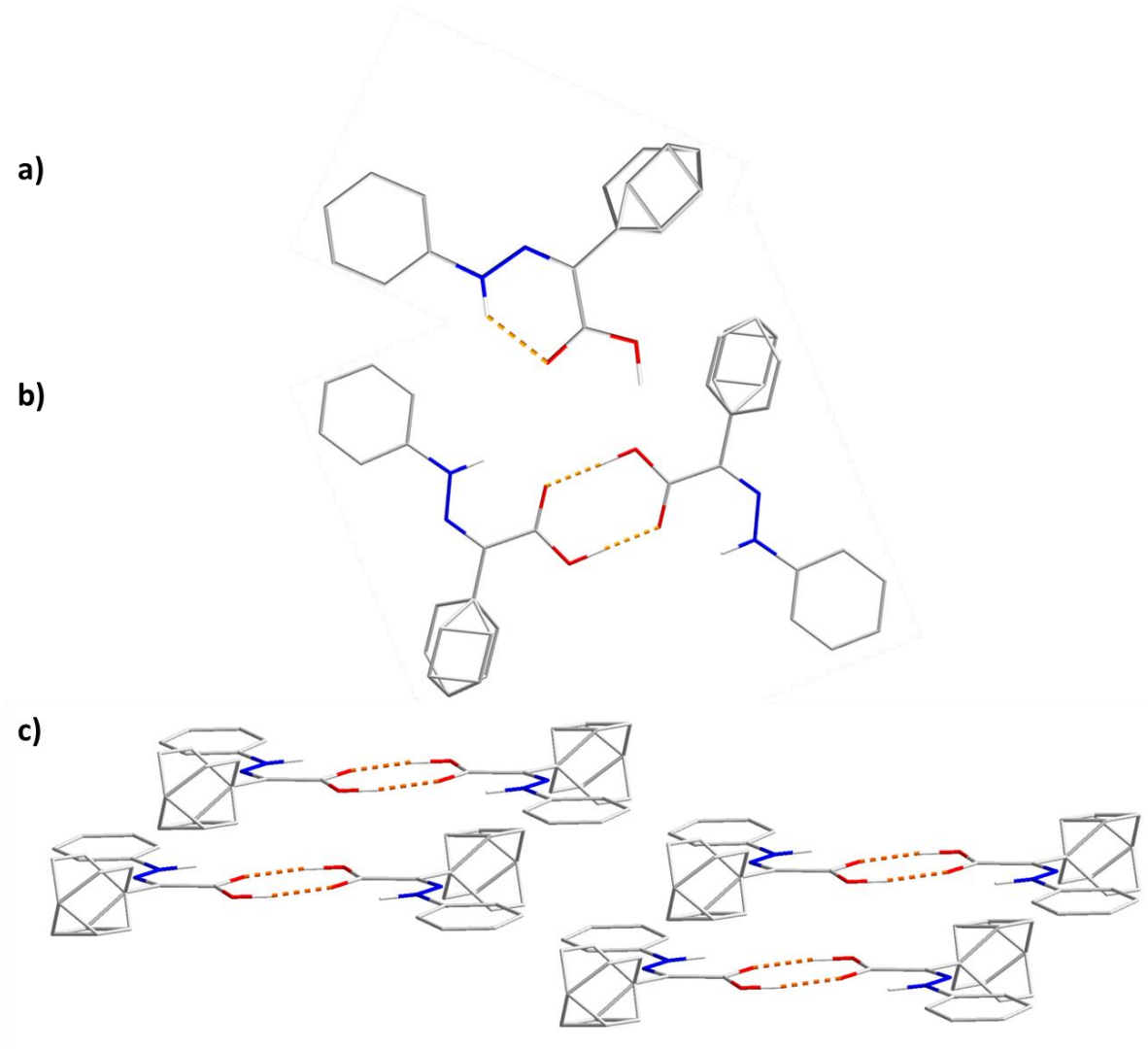


Figure S16. Wire drawings of the a) monomer, b) dimer, and c) crystal packing of **3**. The orange dashed lines indicate hydrogen bonds. Hydrogen atoms on the phenyl ring have been omitted for clarity. The crystal structure shows the modeled disorder of one phenyl ring. Occupancies for both conformations were fixed at 50%.

Table S2. Crystal data and structure refinement for compounds 1 and 3

	1	**3**
CCDC	1056712	1056713
Empirical formula	$C_{13}H_{12}N_3O_{2.5}$	$C_{14}H_{12}N_2O_2$
Formula weight	250.26	240.26
Temperature	172.99(2) K	173 K
Crystal system	Orthorhombic	Triclinic
Space group	Pbcn	P-1
Unit cell dimensions	23.2307(11)	5.0320(2)
	11.1515(5)	9.7091(4)
	9.2568(4)	12.6168(6)
	90.00	89.600(4)
	90.00	89.102(4)
	90.00	79.669(4)
Volume	2398.04(19) $Å^3$	606.34(5) $Å^3$
Z	8	2
Density (Calcd.)	1.386 mg/mm^3	1.316 mg/mm^3
Absorption coefficient	0.819 mm^{-1}	0.732 mm^{-1}
$\boldsymbol{F}_{000}$	1048.0	252.0
Crystal size	0.294 × 0.228 × 0.116 mm^3	0.495 ×0.074 ×0.048 mm^3
2θ range for data collection	7.61 to 146.328°	7.006 to 144.792°
Index ranges	$-28 \le h \le 28$	$-6 \le h \le 6$
	$-12 \le k \le 6$	$-11 \le k \le 11$
	$-11 \le l \le 10$	$-15 \le l \le 15$
Reflections collected	11703	8490
Independent reflections	2305 [R(int) = 0.0462]	2310 [R(int) = 0.0455]
Data / restraints / parameters	2305 / 0 / 171	2310 / 0 / 231
Goodness-of-fit on F^2	1.108	1.037
Final R indexes [$I \ge 2\sigma(I)$]	R_1 = 0.0837, ωR_2 = 0.2561	R_1 = 0.0589, ωR_2 = 0.1532
Final R indexes [all data]	R_1 = 0.0976, ωR_2 = 0.2690	R_1 = 0.0872, ωR_2 = 0.1732
Largest diff. peak and hole	0.83 and -0.21 e $Å^{-3}$	0.51 and -0.23 e $Å^{-3}$

7 Photophysical Properties

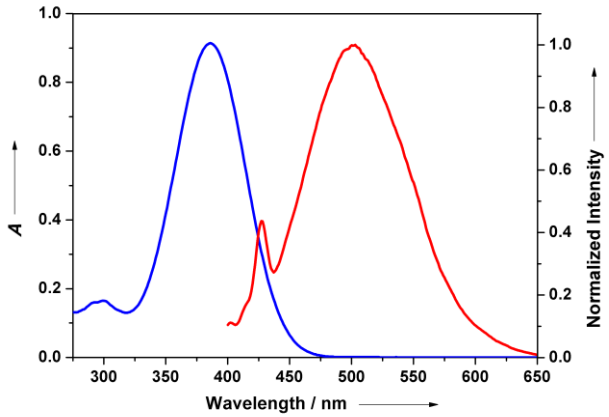


Figure S17. UV-Vis (blue) and fluorescence (red) spectra of compound **1** in CH_3CN (5×10^{-5} M) at room temperature

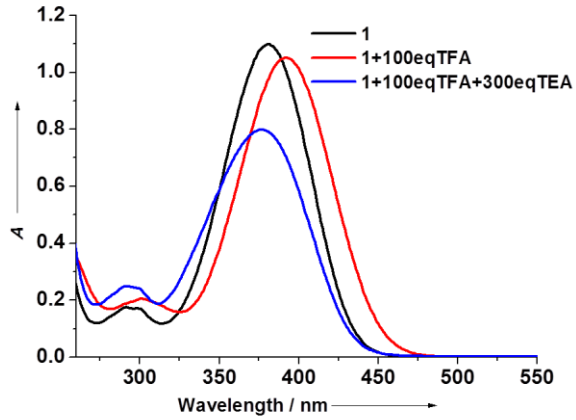


Figure S18. UV-Vis spectra of compound **1** in CH_3CN (5×10^{-5} M) in the presence of acid and base

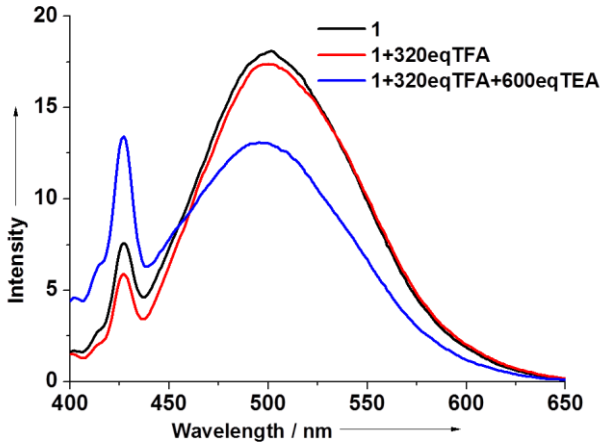


Figure S19. Fluorescence spectra of compound **1** in CH_3CN (5×10^{-5} M) in the presence of acid and base

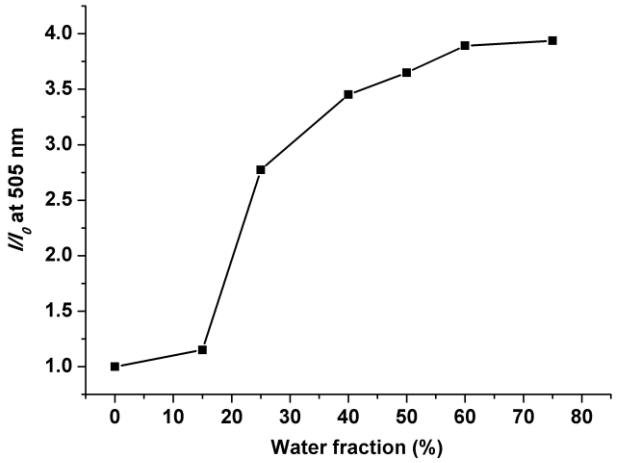


Figure S20. Fluorescence Intensity change as a function of water fraction (%) in THF. This graph indicates that the rigidification of **1** through the formation of aggregates increases its emission.

8 Sensing Organic Amines and Food Spoilage

Fresh cod meat was purchased from the local supermarket. 1 g of freshly ground cod was stored at 37 ^{o}C in a VWR Digital Heatblock to induce spoilage. The remaining fresh cod meat was stored at -5 ^{o}C in a refrigerator and used as control. After 14 days, 0.1 mL deionized water was added to both spoiled and frozen cods. 3 μL of each sample was then added on top of a gel pad prepared from compound **1**.

The effect of different amines on gelation and emission was also studied (Figure S20). While most amines quenched emission and collapsed the gel, pyridine and aniline, which are not basic enough to completely deprotonate the system, only resulted in gel collapse.

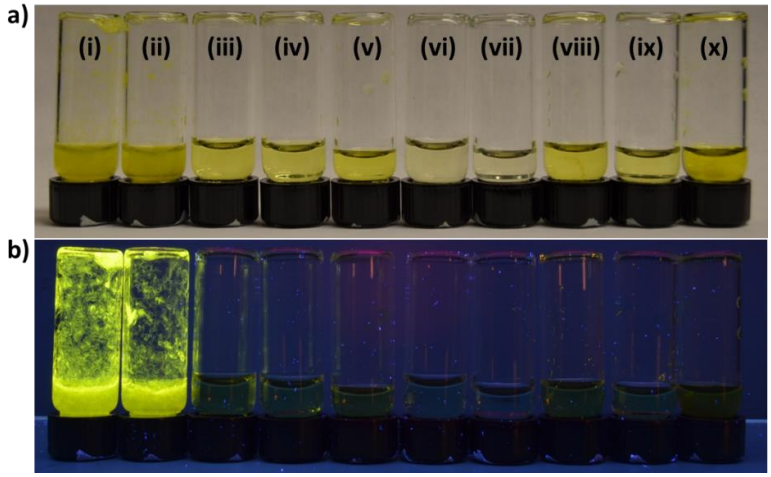


Figure S21. Photographs of hydrogels of compound **1** under a) ambient light or b) UV-light in the presence of 0.75 equiv of (i) pyridine, (ii) aniline, (iii) Et_3N, (iv) *i*-Pr_2NH, (v) *n*-Bu_3N, (vi) putrescine, (vii) cadaverine, (viii) histamine, (ix) tyramine, and (x) 100 equiv HCl.

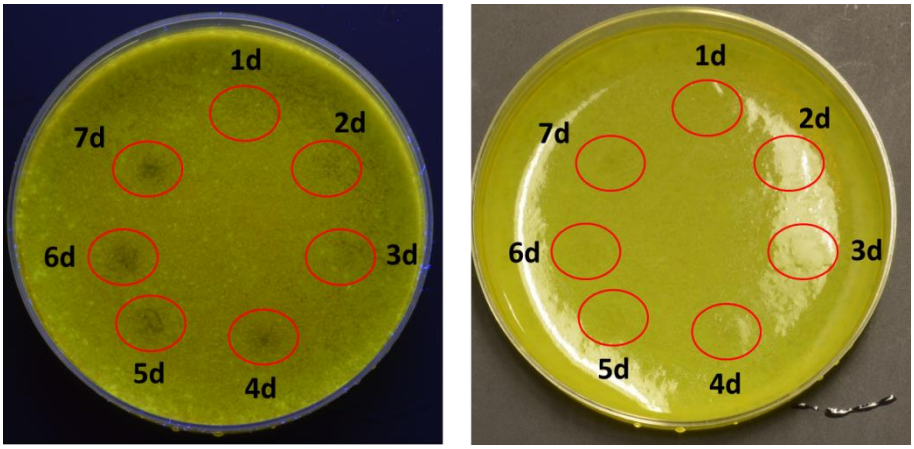


Figure S22. Photographs of a gel pad of compound **1** (under UV (left) and ambient (right) light) used for monitoring the day-to-day spoilage process of cod fish. The quenching of the emission becomes apparent to the naked eye at day 4, which we assume is before the meat decomposes to an unacceptable extent.

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