

Supporting Information

Facile fluorescent labeling of a polyacrylamide-based hydrogel film via radical initiation enables selective and reversible detection of Al³⁺

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Instrumentation

Proton nuclear magnetic resonance (^1H NMR) spectra were recorded using Bruker 300 MHz NMR spectrometers at 25 °C. Proton chemical shift are expressed in part per million (ppm, δ scale) and are referenced to tetramethylsilane ($(\text{CH}_3)_4\text{Si}$ 0.00 ppm) or to residual protium in the solvent (CDCl_3 , δ 7.26 ppm, DMSO, δ 2.51 ppm, and D_2O , δ 4.70 ppm). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances, br = broad peak), integration.

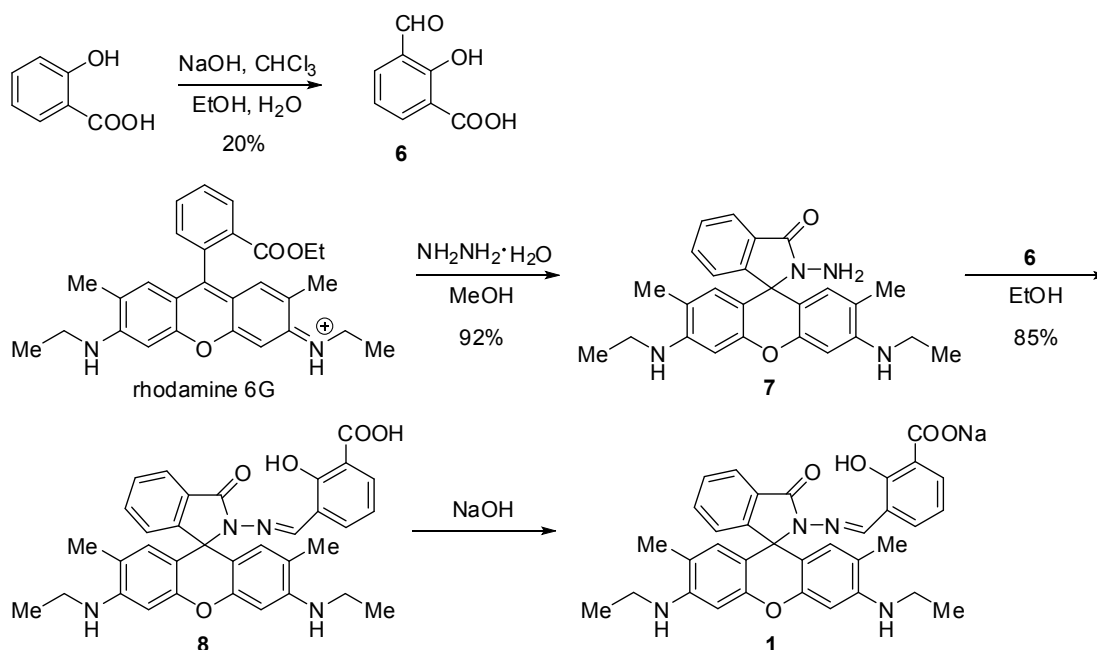
For the tensile test, as-prepared hydrogel films were cut using a laser cutter. The films were glued between two PMMA clamps with a superglue following a similar manner previously reported.¹ The resulting specimens have a size of 10 mm \times 5 mm \times 1 mm (length \times width \times thickness). Uniaxial tensile tests were performed using Instron 5543 universal testing machine (UTM) with a 1000-N load cell at 25 °C in air. The specimens were stretched at a rate of 5 mm/min until the samples were broken. Each hydrogel film sample was triplicated during the measurement. The stress-strain curve was recorded. The ultimate tensile strength was determined as the stress at the breaking point. The ultimate strain was determined as the strain at the breaking point. Young's modulus was obtained from the initial slope of the stress-strain curve in the strain range of 10–30%. Toughness was taken from the area under the stress-strain curve.

Dynamic rheological experiments were carried out on a TA Instruments AR 2000 rheometer using 20-mm cone and plate configuration. The specimen was tested in frequency sweep mode (from 0.1 rad/s to 100 rad/s).

Fluorescence spectra were measured using Shimadzu RF-5301PC spectrofluorometer. All fluorescence spectra were obtained under excitation at 500 nm.

Micromorphology of dried hydrogel film was observed using a Carl Zeiss SUPRA 55VP scanning electron microscope (SEM) at an accelerating voltage of 2 kV. Before the measurement, the sample was dried using a lyophilizer for 3 d and coated with a thin platinum layer.

Synthesis of Rhodamine Spirolactam Probe



Scheme S1. Synthetic routes to the probe 1.

3-formyl-2-hydroxybenzoic acid (**6**):

To a round-bottom flask was added salicylic acid (4.1 g, 30 mmol, 1.0 equiv) and ethanol (18 mL). Chloroform (9 mL, 110 mmol, 3.7 equiv) and 50% sodium hydroxide aqueous

solution (45 mL) were added in sequence. After stirring at 55 °C for 24 h, The mixture was acidified with sulfuric acid. The crude product was recrystallized form ethanol to obtain desired product as a white solid (1.0 g, 6.0 mmol, 20%). ¹H NMR (300 MHz, DMSO): δ 9.97 (s, 1H), 8.44 (s, 1H), 8.08 (d, 1H), 7.22 (d, 1H). The NMR spectrum (Figure S6) matched with the previous result.²

2-amino-3',6'-bis(ethylamino)-2',7'-dimethylspiro[isoindoline-1,9'-xanthen]-3-one

(7):

Rhodamine 6G (3.0 g, 6.3 mmol, 1.0 equiv) was dissolved in methanol (20mL). Then hydrazine monohydrate (1.0 mL, 19 mmol, 3.0 equiv) was added. The solution was refluxed for 6h and diluted with ethyl acetate (400 mL). The solution was washed with water (100 mL) and 1 N sodium hydroxide (100 mL). The organic layer was dried over MgSO₄, filtered, and concentrated. After purifying by column chromatography on silica-gel (elution with 10:2:1 *n*-hexane/DCM/MeOH), the desired product was obtained as a light pink solid (2.5 g, 5.8 mmol, 92%). ¹H NMR (300 MHz, CDCl₃): δ 7.97–7.94 (m, 1H), 7.45 (m, 2H), 7.07–7.05 (t, 1H), 6.43–6.38 (d, 2H), 6.26 (d, 2H), 3.57–3.52 (d, 3H), 3.22-3.20 (q, 4H), 1.91 (s, 6H), 1.34-1.29 (t, 6H). The NMR spectrum (Figure S7) matched with the previous result.³

(E)-3-((3',6'-bis(ethylamino)-2',7'-dimethyl-3-oxospiro[isoindoline-1,9'-xanthen]-2-ylimino)methyl)-2-hydroxybenzoic acid (8):

To compound 7 (1.3 g, 3.0 mmol, 1.0 equiv) in ethanol (30 mL) was added compound 6 (0.5 g, 3.0 mmol, 1.0 equiv) and a few drops of glacial acetic acid were added into the

reaction mixture. After stirring at reflux for 2 h, the reaction mixture was cooled and filtered. The crude product was purified by recrystallization from ethanol to afford the desired product as a pink solid (1.5 g, 2.6 mmol, 87%). ¹H NMR (300 MHz, DMSO): δ 8.94 (s, 1H), 7.91 (d, 1H), 7.89–7.69 (m, 2H), 7.59–7.55 (m, 2H), 7.02 (d, 1H), 6.85 (t, 1H), 6.33 (s, 2H), 6.17 (s, 2H), 3.12 (d, 4H), 1.84 (s, 6H), 1.20 (t, 6H). The NMR spectrum (Figure S8) matched with the previous result.⁴

(E)-3-((3',6'-bis(ethylamino)-2',7'-dimethyl-3-oxospiro[isoindoline-1,9'-xanthene]-2-ylimino)methyl)-2-hydroxybenzoate (1):

To enhance the solubility in water, we introduced sodium carboxylate to compound **8** using a NaOH solution as following a same manner previously reported.⁴

Model Reaction for Redox-Initiated Polymerization

Acrylamide **2** (1.0 g, 14 mmol, 1.0 equiv) and *N*-methylaniline **4** (40 mg, 0.28 mmol, 0.02 equiv) were dissolved in water (8 mL). After the mixture solution was sonicated for 30 min and degassed by bubbling with N₂, a solution of potassium persulfate (76 mg, 0.28 mmol, 0.02 equiv) in water (2 mL) was added to the mixture dropwise by syringe. Then, the solution mixture was left at rt for 6h for polymerization. The resulting product was isolated by precipitation in methanol. The polymer **5** was purified by redissolving in water and reprecipitating by addition of methanol, and obtained as a white powder. Yield, 75%.

Control Polymerization Initiated by Rhodamine 6G

Acrylamide **2** (0.5 g, 7.03 mmol) and rhodamine 6G (0.9 mg, 1.8 μmol) were dissolved in water (5 mL). After degassing with N_2 bubbles for 1 h, potassium persulfate (KPS; 1 mg, 3.7 μmol) were added to the solution. After incubation for 2 h at rt, the storage and loss moduli of the polymerizing solution were measured by an oscillatory rheometer.

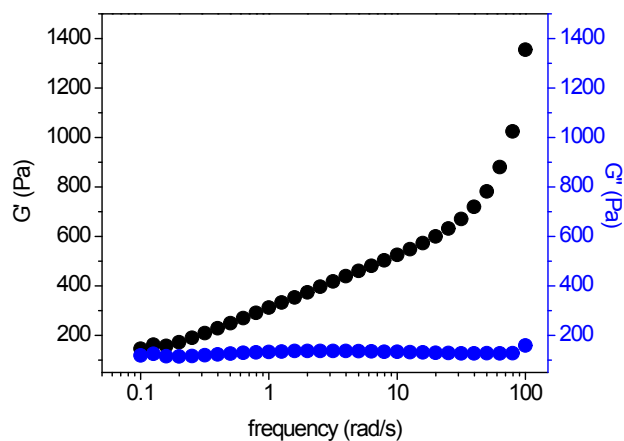


Figure S1. Dynamic frequency sweep data for an aqueous solution of **2** in the presence of rhodamine 6G and KPS, measured by an oscillatory rheometer at rt. Storage (black) and loss (blue) moduli were measured in the sweep profile.

Table S1. Average Young's modulus and toughness that correspond to Fig. 4b.

cross-linker (wt%)	Young's modulus (kPa)					Toughness (MJ/m^3)				
	1st try	2nd try	3rd try	Avg.	Std.	1st try	2nd try	3rd try	Avg.	Std.
1	1.5	2.5	2.7	2.2	0.5	0.094	0.104	0.113	0.104	0.008
2	3.2	3.5	5.7	4.1	1.1	0.021	0.045	0.023	0.030	0.011
3	6.8	5.9	8.5	7.1	1.1	0.017	0.014	0.019	0.017	0.002
4	7.3	6.3	7.2	6.9	0.4	0.015	0.013	0.013	0.014	0.001
8	14.9	16.8	14.8	15.5	0.9	0.006	0.001	0.001	0.003	0.002

Effect of Cross-Linker on Mechanical Strength

The hydrogel films containing different amounts of **3** were prepared by following the procedure reported in the experimental section in the main text (for the 2 wt% film) except for using different amounts of **3**. *Quantities of 3 required for the fabrication of hydrogel films:* For the 1 wt% film, 10 mg (0.039 mmol) of **3** was incorporated. For the 3 wt% film, 30 mg (0.117 mmol); for the 4 wt% film, 40 mg (0.156 mmol); for the 8 wt% film, 80 mg (0.312 mmol).

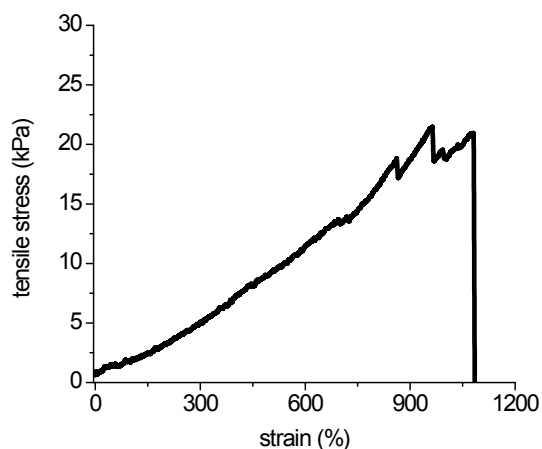


Figure S2. Representative tensile stress–strain curve for the hydrogel film containing 1 wt% of **3**.

Fluorescent Responses from Hydrogel Film

Fluorescence measurement upon the addition of Al³⁺ ions:

For test, hydrogel films were cut into the same size (width × length × thickness, 10 mm × 10 mm × 1 mm). After washing with water for 30 min, the films were immersed for 30 min into solutions of Al³⁺ in an aqueous ethanol (1:1, v/v) having different concentrations. After rinsing with the ethanol solution, the emission spectra were measured under

excitation at 500 nm. The limit of detection was calculated as $3 \times (SD/m)$ where SD means standard deviation of the blank and m means the slope of the initial linear region.

Fluorescence of remaining Al^{3+} solution (3.00 mM) after exposure of film:

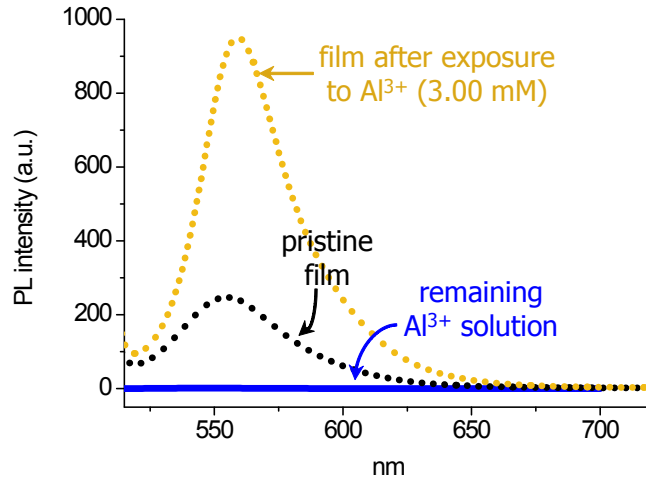


Figure S3. Fluorescence emission spectrum from the remaining Al^{3+} solution (3.00 mM) after immersing the hydrogel film for 30 min (blue) under excitation at 500 nm. The emission spectra from the film before (dotted black) and after exposure to Al^{3+} (dotted yellow) were shown for comparison.

Benesi-Hilderbrand Plot:

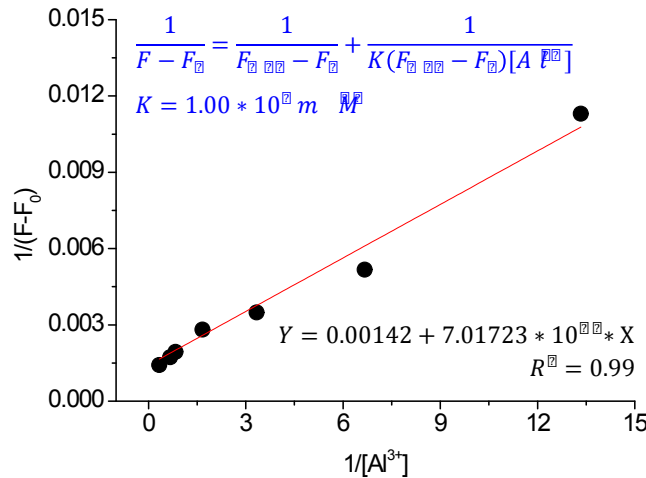


Figure S4. Benesi-Hilderbrand plot obtained from the fluorescence data of the hydrogel film when exposed to different concentrations of Al^{3+} ions. The equation for the plot indicated 1:1 stoichiometric complexation (blue) where F means the measured intensity of fluorescence, F_0 the initial intensity, and F_{max} the maximum intensity; from which the binding constant, K , was also calculated. The linear regression (red) and R-squared value also were shown.

Time-dependent fluorescence enhancement when the film was immersed in 1.5 mM of Al³⁺ solution:

The six hydrogel films were cut into the same size (width × length × thickness, 10 mm × 10 mm × 1 mm). After washing with water for 30 min, the films were immersed in 1.5 mM Al³⁺ in an aqueous ethanol (1:1, v/v) and taken for the measurement at 10-min time interval over 50 min. After rinsing with the ethanolic solution, the emission spectra of samples were measured under excitation at 500 nm.

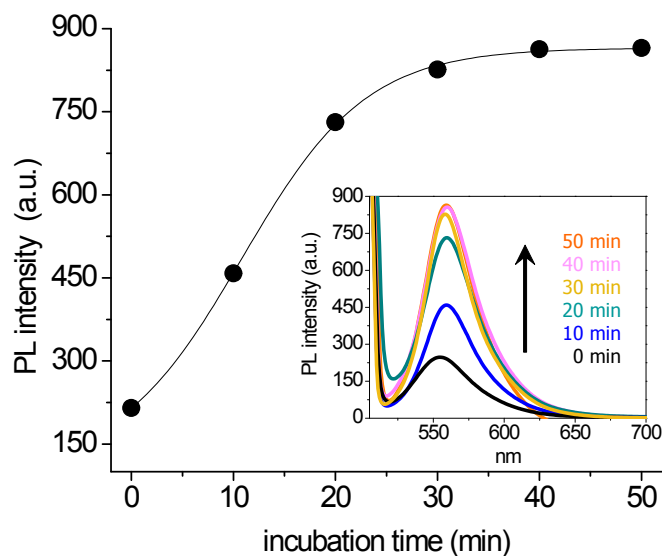


Figure S5. Change in the intensity of fluorescence monitored from the hydrogel film over the course of the exposure time for 50 min to 1.5 mM Al³⁺ ions. The overlapped spectra, measured at each time interval, were shown in inset.

Selective, reversible responses of the film:

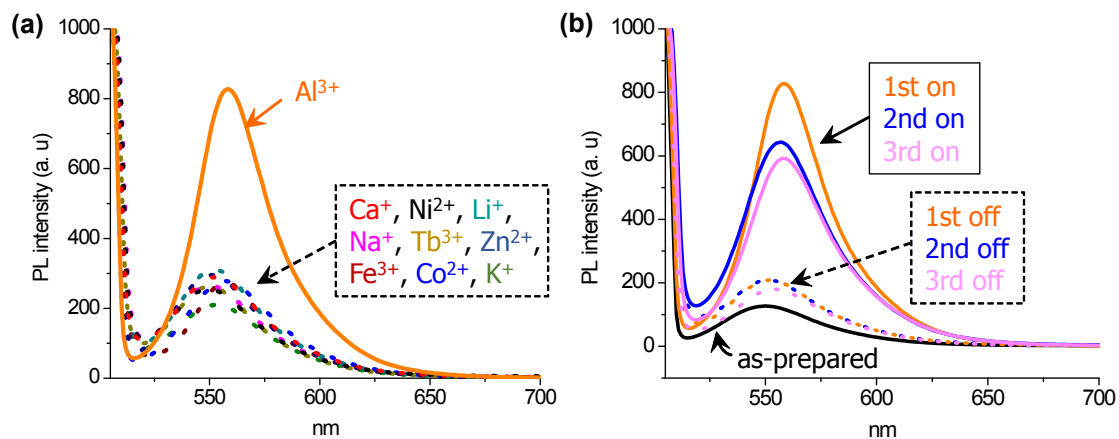


Figure S6. The obtained fluorescence spectra of the hydrogel films (a) when exposed to the solutions of different metal ions (1.5 mM) for 30 min (for the selectivity test), and (b) when repeatedly exposed to Al^{3+} ions (1.5 mM) and EDTA (20 mM) solutions for 30 min each (for the on–off switching test).

References

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4. Y. Fu, X. J. Jiang, Y. Y. Zhu, B. J. Zhou, S. Q. Zang, M. S. Tang, H. Y. Zhang and T. C. W. Mak, A new fluorescent probe for Al³⁺ based on rhodamine 6G and its application to bioimaging, *Dalton Trans.*, 2014, **43**, 12624–12632.

NMR Spectra

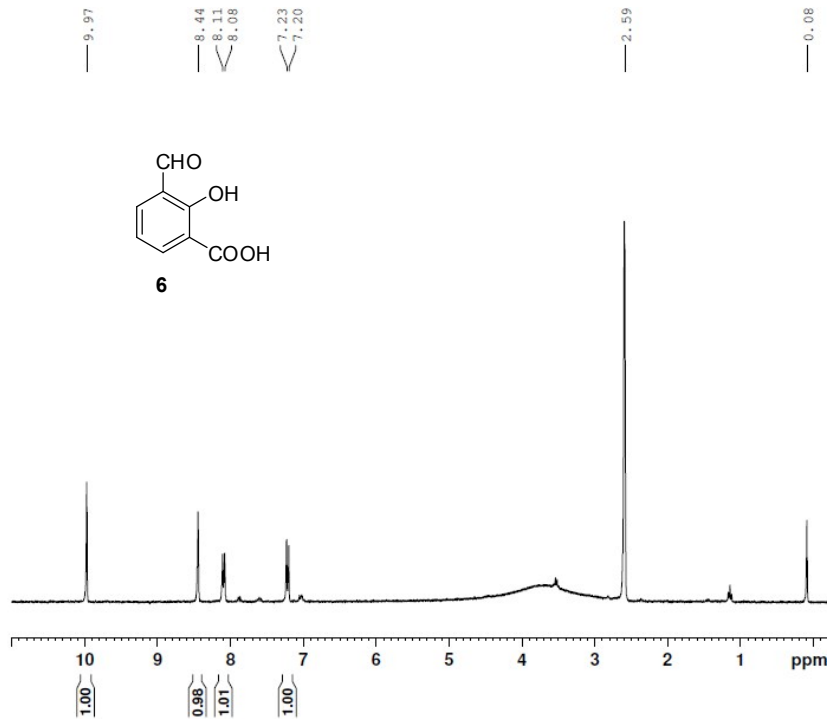


Figure S7. ¹H NMR spectrum of **6**.

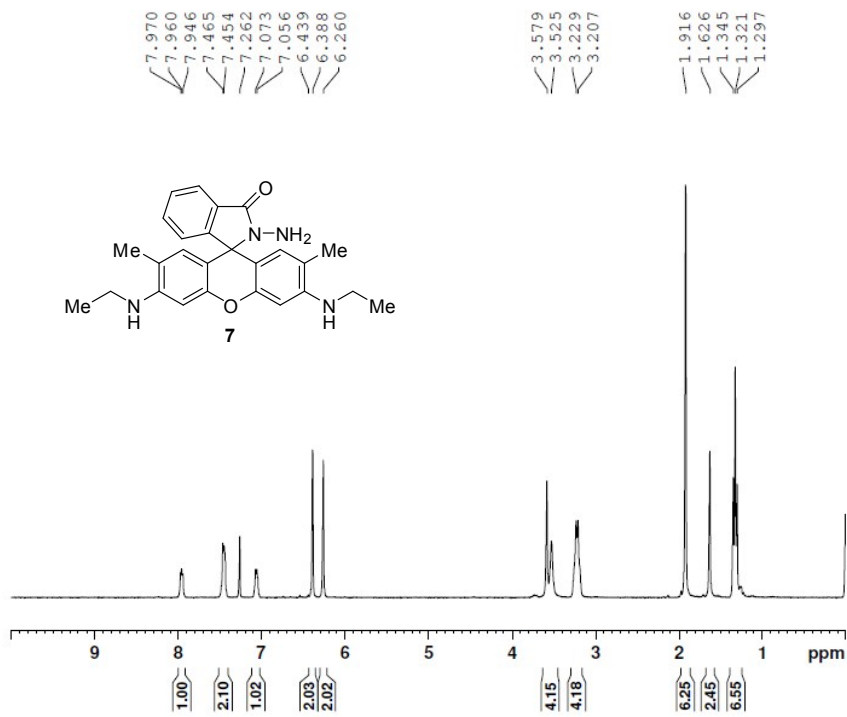


Figure S8. ¹H NMR spectrum of **7**.

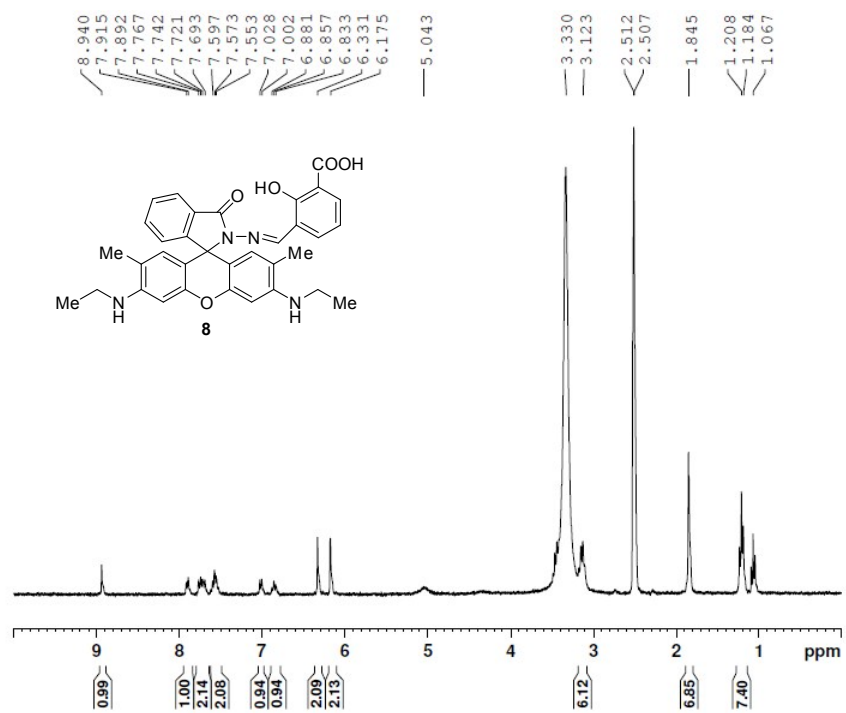


Figure S9. ¹H NMR spectrum of **8**.