Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Synthesis and spectroscopic characterization of a fluorescent phenanthrene-rhodamine dyad for ratiometric measurements of acid pH values

Priyanka Srivastava[a]*, Paul Christian Fürstenwerth[a], Jan Felix Witte[b] and Ute Resch-Genger[a]*

[a]BAM Federal Institute for Materials Research and Testing, Richard-Willstaetter-Strasse, 11, 12489 Berlin, Germany

[b] Institute of Chemistry and Biochemistry, Feire University, Kaiserswerther Str. 16-18, 14195 Berlin, Germany.

Experimental

Materials and methods

All chemicals and reagents and solvents were used as received, unless otherwise stated. The solvents DCM, MeOH, DMF, THF, EtOAc, Hex as well as the glacial acetic acid, and anhydrous magnesium sulfate were acquired from Th. Geyer GmbH & Co. KG. Phenanthrene-9 carboxaldehyde (97%), phosphoric acid (85%) and EtOH were procured from Alfa Aesar. The sodium hydrogen carbonate and the aluminum oxide 90 active neutral (activity stage I) for column chromatography 0.063-0.200 mm (70.230 mesh ASTM) were both acquired from Merck KGaA. The copper (II) sulfate pentahydrate was acquired from Carl Roth GmbH & Co. KG. The rhodamine b (≥ 95 %), sodium borohydride (99%), thionyl chloride (≥ 99 %), sodium azide (≥ 99 %), (+)-Sodium L-ascorbate, ethyl diamine (≥ 99 %), propargyl bromide solution in toluene (80%), anhydrous sodium hydroxide (≥ 98 %) and anhydrous potassium carbonate (≥ 99 %) were all acquired from Sigma Aldrich. All aqueous solutions were prepared with pure water (0.055 $\mu S/m$) filtered by Merck, Milli-Q® IQ 700.

Methods

^{1}H NMR and ^{13}C NMR were recorded on Bruker, AVANCE III 500, with CDCl3 and DMSO-d6 (Deutero GmbH) as solvent and tetramethylsilane as an internal reference. For mass spectrometry, samples were measured on an Agilent 6210 ESI-TOF, Agilent Technologies, Santa Clara, CA, USA. All pH measurements were performed with Mettler Toledo pH-Meter Seven Compact Advanced, Gießen, Germany and with the electrode Mettler Toledo InLab® Micro. The pH meter was calibrated by using standard buffers of pH 10.00, 7.01, 4.01, 2.00, 1.00 enabling pH measurements with 98% accuracy.

The UV-vis spectra were recorded on Cary 5000 Spectrophotometer, Agilent Technologies with slit widths of 5 nm. Fluorescence measurements were recorded on FluoroMax-4 Spectrofluorometer, HORIBA Jobin Yvon SAS with 5 nm slit width. Photostability experiments of dyad at pH 7.2 and pH 2.0 were performed with the spectrofluorometer FSP920 from Edinburgh Instruments equipped with a xenon lamp using 315 nm excitation and monochromator slit widths set to 4 nm and 2 nm in excitation and emission, respectively. The spectral data was plotted using Origin Lab Software. The quantum yield measurement at λ_{ex} = 560 nm were performed on a stand-alone Quantaurus Hamamatsu integrating sphere setup (absolute quantum yield measurements) and relatively with the spectrofluorometer Fluoromax from Jobin Yvon using slit widths of the excitation and emission monochromators of 2 nm and 2 nm, respectively, and rhodamine B as a reference (fluorescence quantum yield standard). Quantum yield measurements at λ_{ex} of 315 nm were always done relatively utilizing pyrene as a reference standard with slit widths of the excitation and emission monochromator both set to 3 nm. As the Quantaurus instrument provides only a very low excitation intensity at 315 nm absolute quantum yield measurement were not possible at this excitation wavelength.

The lifetime experiments were performed on a FLS 900 lifetime spectrofluorometer from Edinburgh Instruments equipped with 330 nm and 510 nm EPLEDs. The lifetime experiments at 330 nm excitation were done with a slit width of the excitation and emission monochromators of 0.5 nm and 12 nm, respectively, while at 510 nm excitation, excitation and emission slit widths of 0.5 nm and 6 nm were used. The detector used was a multichannel plate MCP/Visible with 1024 channels. All experimental results were evaluated by the FAST software from the instrument manufacturer using reconvolution and mono- and biexponential fits, thereby also considering the instrument response function (IRF) measured with a scatterer, i.e., LUDOX sample.

For the spectroscopic studies, the dyad solutions were prepared from a 1 mM stock solution of the dyad dissolved in tetrahydrofuran. For the experiments, 20 µL (5 µM) of this stock solution were diluted with a THF-water mixture of a certain pH (see description of the pH studies) to 4 ml. For the selectivity studies, the metals salts $FeCl_2$, $CaCl_2$, $Ba(CH_3COO)_2$, $FeCl_3$, $Hg(ClO_4)_2$, $Cu(CH_3COO)_2$, $NiCl_2$, $Co(ClO_4)_2$, $Cd(CH_3COO)_2$, $Zn(CH_3COO)_2$, $Mg(CH_3COO)_2$, K_2CO_3, and $NaNO_2$ were used. For this purpose, 10^{-1} M stock solution of the metal ions were prepared in Milli Q water. For selectivity studies with anions and selected amino acids, NaBr, NaCl, NaF, NaI, CH_3COONa, Na_2CO_3, NaH_2PO_4, H_2S, KCN, Na_2SO_4, 2-mercaptopropionic acid (for thiol), L-Lycine, L cysteine and reduced glutathione solutions of 10^{-1} M were prepared in milli q water. For 10 equiv addition, 1.5 µl of 10^{-1} M solutions were prepared using in 3 ml of dyad solution (5 µM). The different pH solutions were prepared according to the Britton Robinson (B-R) buffer preparation. The acid solution contains 0.05 M of phosphoric acid, boric acid, and acetic acid and the basic solution 0.05 M sodium hydroxide, respectively. Solutions of different pH were prepared by mixing with different acid and base solutions. All pH solutions are prepared using Milli Q water and then mixed with an equivalent volume of THF to form the final THF-H_2O mixtures (1:1). pH below 2.0 is adjusted by the addition of HCL. Reversibility experiments were also performed by the addition of HCL and NaOH subsequently to adjust pHs 4.0 and 7.0 starting from the solution of dyad at pH 7.0 in THF-H_2O mixture (1:1) in B-R buffer.

Dyad **8** and its protonated form have been optimised at the PBEh-3c level of density functional theory (DFT) using the Turbomole programme package (version 7.3) and COSMO to implicitly include solvation effects. Single point calculations to obtain a proper description of the compounds' electronic structures were conducted at the ωB97X-D3,[1] M06-2X[2], and PBE0-D3(BJ)[3] level employing the def2-TZVP[4] basis set in combination with the CPCM[5] solvation model using the ORCA programme package (version 4.2.0)[6]. ε=78.4 for water was used, because solvent mixtures are inaccessible to standard implicit continuum solvation models. HOMO-LUMO energy differences are, as expected, also quite dependent on the amount of exact exchange used in the functional (TABLE). ωB97X-D3 uses 100% exact exchange in the asymptotic limit of the two-electron distance operator, while M06-2X and PBE0 are not long-range corrected and use 54% and 20%, respectively. Since ωB97X-D3 and M06-2X are usually more well-suited for challenging cases in the framework of calculating excited states using time-dependent DFT (TD-DFT), the most useful values are likely acquired with the PBE0 functional which results in a HOMO-LUMO gap of 4.2 eV for **Deprot** and 3.10 eV for **Prot**. In general, the HOMO-LUMO gap decreases by roughly 1-1.5 eV when protonating the substance.

HOMO-LUMO gaps in eV

	ωB97X-D3	M06-2X	PBE0-D3(BJ)
Dyad 8	7.87	5.94	4.17
Dyad 8+H^+	6.33	4.55	3.10

References

1. ωB97X-D3: 10.1063/1.2834918
2. M06-2X: 10.1007/s00214-007-0310-x
3. PBE0: 10.1063/1.472933 D3: 10.1063/1.3382344 BJ: 10.1002/jcc.21759
4. def2-TZVP: 10.1039/b508541a
5. CPCM: 10.1021/jp9716997
6. ORCA: 10.1002/wcms.1327

The dose/response equation was used to calculate the p*K*a values of the Dyad by sigmoidal curve fitting of the emission intensities with respect to different pHs.

$$\boldsymbol{y = I1 + \frac{I2 - I1}{1 + 10^{(LOGx_0 - x)p}}} \qquad (\mathbf{1})$$

The fluorescence quantum yields were determined from relative measurements using equation (2)

$$\emptyset_s = \emptyset_f * \frac{F_s}{F_f} * \frac{OD_f}{OD_s} * \left(\frac{RI_s}{RI_f}\right)\mathbf{2} \qquad (\mathbf{2})$$

Here ϕ equals the fluorescence quantum yield, F represents the integrated area of fluorescence emission spectra, OD is the optical density and R.I is the refractive index of the solvents used. 's' represents the sample and 'f' indicates the reference or standard used.

Determination of the fluorescence lifetimes from the measured fluorescence decay curves was done according to equations 3 and 4. For a single exponential decay, the time-dependent fluorescence intensity is

$$\boldsymbol{I(t) = \alpha \exp\left(-t/\tau\right)} \qquad (3)$$

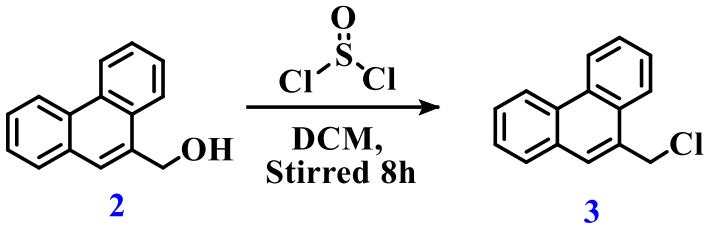
where τ is the decay time and α is the pre-exponential factor or amplitude. For multiexponential decays with n components i, the time-dependent fluorescence intensity is

$$\boldsymbol{I(t) = \sum_{i=1}^{n} \alpha_i \exp\left(-t/\tau_i\right)} \qquad (4)$$

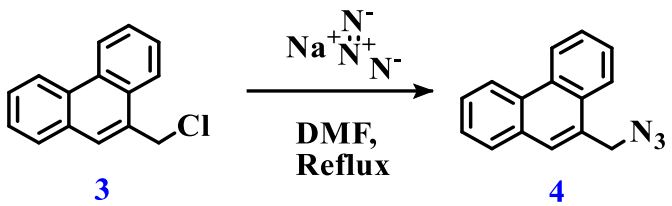
The measured decay curves were fitted with a mono and biexponential decay time function and reconvolution with the instrument response function (IRF) using the software FAST (Edinburgh Instruments).

Synthesis of the pH-sensitive tri-color emissive dyad

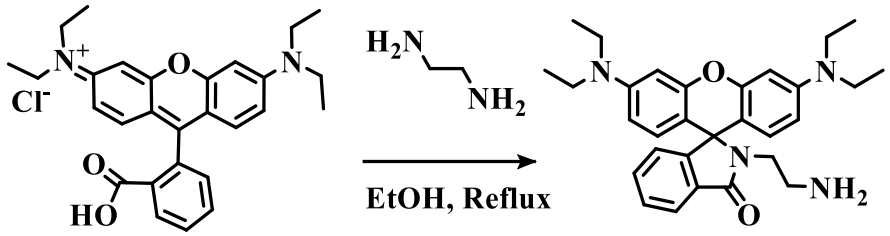
Compound 2. 1.01 g of 9-Phenanthrenecarboxaldehyde (4.9 mmol) was taken in 50 ml DCM and cooled under ice cold condition. 1.85 g of Sodium borohydride (49 mmol) in taken in 25 ml of MeOH and added slowly to the solution and the solution was vigorously stirred for 6h until completion. The solvent was evaporated under rotatory evaporator and the white solid was suspended in water and filtered after few hours and washed with distilled water several times to remove any trace of sodium borohydride left. Yield; 998 mg, 98 %. R_f = 0.55 in solvent system DCM: MeOH; 95:5. ^{1}H NMR (500 MHz, DMSO-d_6) [ppm]: δ = 8.87-8.85 (d, H4, *J = 8 Hz*), 8.81-8.79 (d, H5, *J* = 8 *Hz*), 8.14-8.12 (d, H8, *J = 9 Hz*), 7.99-7.97 (d, H1, *J* = 8.5 *Hz*), 7.88 (s, H9), 7.72-7.62 (m, H2,3,6,7), 5.41 (s, Ha), 5.03 (s, Hb).



Compound 3. 409 mg of compound **1** (2.0 mmol) was dissolved in 25 ml of DCM and cooled under ice cold conditions. To this solution 170 µl thionyl chloride (2.3 mmol) in 25 ml of DCM was added slowly to ice cold solution of compound. The mixture was stirred at RT for 8 h until TLC confirmed a complete reaction. The crude product was distilled for excess thionyl chloride and After complete reaction, excess $SOCl_2$ was removed by distillation. The solvent was evaporated under reduced pressure and washed with hexane several times to obtain pure compound. Compound **2** yielded faintly beige crystals upon recrystallization with DCM (395 mg, 88 %). Rf = 0.58 in the solvent system Hexane: EtOAc; 9:1. ^{1}H NMR (500 MHz, $CDCl_3$) [ppm]: δ = 8.79-8.75 (m, H4), 8.71-8.69 (d, H5, *J* = 9 *Hz*), 8.25-8.22 (m, H8), 7.91-7.89 (d, H1, *J* = 8 *Hz*), 7.84 (s, H9), 7.75-7.69 (m, H3,6,7), 7.65-7.62 (t, H2, *J* = 7,5 *Hz*), 5.13 (s, Ha)

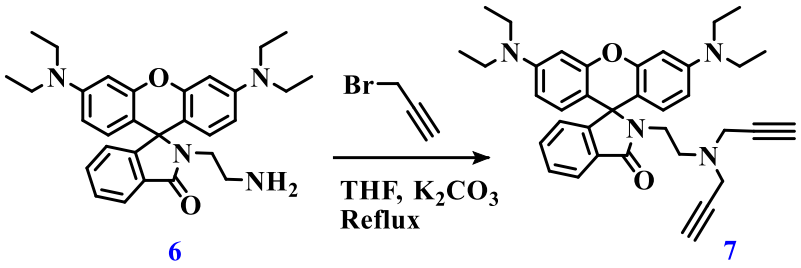


Compound 4. 204 mg of comp **3** (0.87 mmol) were taken in 35 ml of DMF and stirred until dissolution. To this solution 85 mg of Sodium azide (1.31 mmol) was added slowly and stirred until dissolution. The reaction mixture was refluxed at 100 °C for 4 h and then cooled to RT. 200 ml of distilled water was added and the precipitate was collected by filtration, dried and recrystallized in DMF:H_2O (2:1) to yield the pure azide as small cotton balls. (182 mg, 90 %). R_f = 0.48 in the solvent system Hexane: EtOAc; 9:1. ^{1}H NMR (500 MHz, $CDCl_3$) [ppm]: δ = 8.78 (b, H4), 8.71 (b, H5), 8.10 (b, H8), 7.92 (b, H1) 7.78-7.64 (m, H2,3,6,7,9), 4.84 (s, H)



5 6

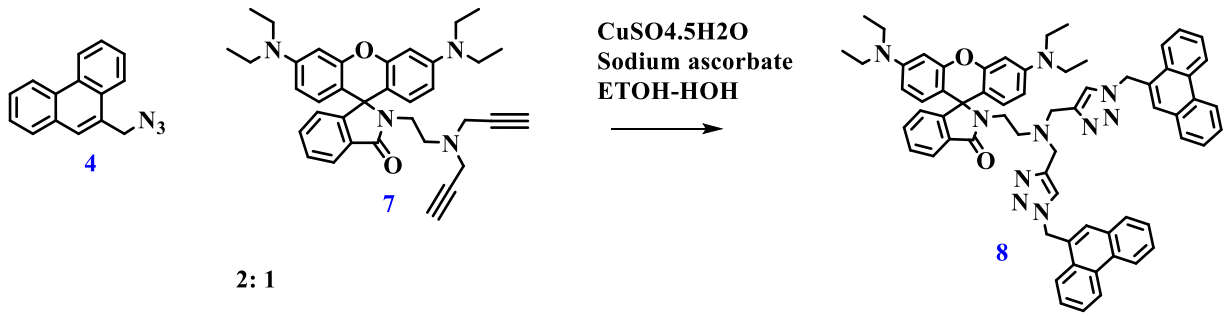
Compound 6. The rhodamine derivatization was started with **5** (rhodamine b), 2.083 g (4.3 mmol) was taken in 50 ml EtOH. To this bright red solution, 5.7 ml ethyl diamine (86.0 mmol) were added and refluxed for 24 h. After removal of the solvent via evaporation the solids were washed with distilled water multiple times until no ethyl diamine was seen in the TLC. The reaction yielded compound **6** as an off-white powder (1.857 g, 88 %). R_f = 0.33 in the solvent system DCM: MeOH; 95:5. ^{1}H NMR (500 MHz, $CDCl_3$) [ppm]: δ = 7.93-7.91 (q, H1, *J* = 2.5 *Hz)*, 7.47-7.45 (b, H2,3), 7.12-7.10 (b, H4), 6.46-6.39 (b, H5,7), 6.30-6.28 (d, H6, *J* = 2.7 *Hz*), 3.37-3.33 (q, Hb, *J* = 7.0 *Hz*), 3.22-3.19 (t, Hc, *J* = 6.7 *Hz*), 2.44-2.42 (t, Hd, *J* = 6.5 *Hz*), 1.20-1.17 (t, Ha, *J* = 7.1 *Hz*)



6 7

Compound 7. 996 mg (2.1 mmol) of compound **6,** was dissolved in 50 ml THF and 624 mg (4.5 mmol) K_2CO_3 was added. 540 µl (4.5 mmol) of propargyl bromide solution was added and refluxed for 18 h under argon atmosphere. After completion the reaction mixture, monitored by tlc, 70 ml of distilled water and 70 ml of DCM were added to the reaction mixture and aqueous layer was extracted with DCM. In a separatory funnel the combined organic phase was washed with distilled water and brine and dried of magnesium sulphate. After evaporation of the solvent the dark red residue was purified by column chromatography using neutral aluminium oxide to give a faintly yellow resin, compound **7** (709 mg, ≈ 60 %). R_f = 0.45 in the solvent system DCM: MeOH; 95:5. ^{1}H NMR (500 MHz, $CDCl_3$) [ppm]: δ = 7.93-7.89 (q, H1, *J* = 3 *Hz*), 7.46-7.43 (b, H2,3), 7.11-7.08 (b, H4), 6.48-6.47 (d, H5, *J* = 8.7 *Hz*), 6.39 (s, H7) 6.31-28 (d, H6, *J* = 9.0 *Hz*), 3.35-3.33 (q, Hb, *J*

= 6.9 *Hz*), 3.29-3.25 (b, Hc,e), 2.30-2.27 (t, Hd, *J* = 7.5 *Hz*), 2.09 (s, Hf), 1.20-1.17 (t, Ha, *J* = 7.3 *Hz*).



Dyad 8. For the final click chemistry reaction, 110 mg of compound **7** (0.2 mmol) and 100 mg of compound **4** (0.4 mmol) were dissolved in 30 ml of EtOH/H_2O 2:1. $CuSO_4.5H_2O$ (10 mol%, 7 mg) and sodium ascorbate (5 mol%, 17 mg) were separately dissolved in 500 µl of distilled water and added to the reaction mixture. The reaction mixture was refluxed at 80 °C for 16 h. After completion and cooling to RT, 10 ml of 5% $NaHCO_3$ were added. The aqueous phase was extracted with three times in a with 50 ml DCM. The combined organic layers were washed with distilled water, brine and dried over magnesium sulfate. The dark orange residue was purified on neutral aluminum oxide column and yielded a transparent off-white compound **8** (90 mg, ≈ 50 %). R_f = 0.26 in the solvent system Hex:EtOAc:MeOH; 47.5:47.5:5. ^{1}H NMR (500 MHz, $CDCl_3$) [ppm]: δ = 8.70-8.64 (b, H4',5'), 8.01-7.99 (d, H8', *J* = 8.2 *Hz*), 7.87-7.86 (d, H1', *J* = 7.8 *Hz*), 7.75-7.73 (b, H1), 7.70-7.67 (b, Hf), 7.64-7.55 (b, H2',3',6',7',9'), 7.42-7.40 (t, H2,3, *J* = 5.5 *Hz*), 7.05-7.03 (d, H4, *J* = 7.5 *Hz*), 6.33-6.32 (d, H5, *J* = 2.5 *Hz*), 6.22-6.20 (d, H7, *J* = 8 *Hz*), 6.06-6.04 (d, H6, *J* = 8.0 *Hz*), 5.98 (s, Ha), 3.49 (s, Hb), 3.23-3.22 (b, Hd,e), 2.07 (b, Hc), 1.09-1.06 (t, Hf, *J* = 6.7 *Hz*). ^{13}C NMR (500 MHz, $CDCl_3$) [ppm]: δ = 153.3, 148.8, 144.6, 132.2, 131.3, 130.8, 129.8, 129.0, 129.8, 127.4, 127.0, 123.8, 123.4, 122.6, 108.0, 105.5, 97.8, 53.5,52.7, 51.4, 47.2, 44.3, 29.8, 12.6. Elemental analysis via ESI-TOF: m/z $[M+H]^+$ calculated for $C_{66}H_{62}N_{10}O_2$ = 1027.5091 m/z, m/z found $[M+H]^+$ = 1027.5113 m/z; second m/z found $[M+Na]^+$ = 1049.4932 m/z.

The protonation of dyad **8** was also confirmed by ESI-MS analysis of **8** in a neutral and acidic solution. For this purpose, the dyad was dissolved in methanol and treated with HCL and the ESI-MS spectra of the dyad solutions were measured. The ESI-MS spectra of dyad **8** showed a mass peak $[8+Na^+]$at 1049.4932 ($C_{66}H_{62}N_{10}NaO_2^+$; 1049.4949) while the protonated form of dyad **8+H$^+$** $[8+Na^++H^+]$ revealed a ms peak at 1051.7987 found ($C_{66}H_{64}N_{10}NaO_2^+$; 1051.5095) clearly confirming dyad protonation.

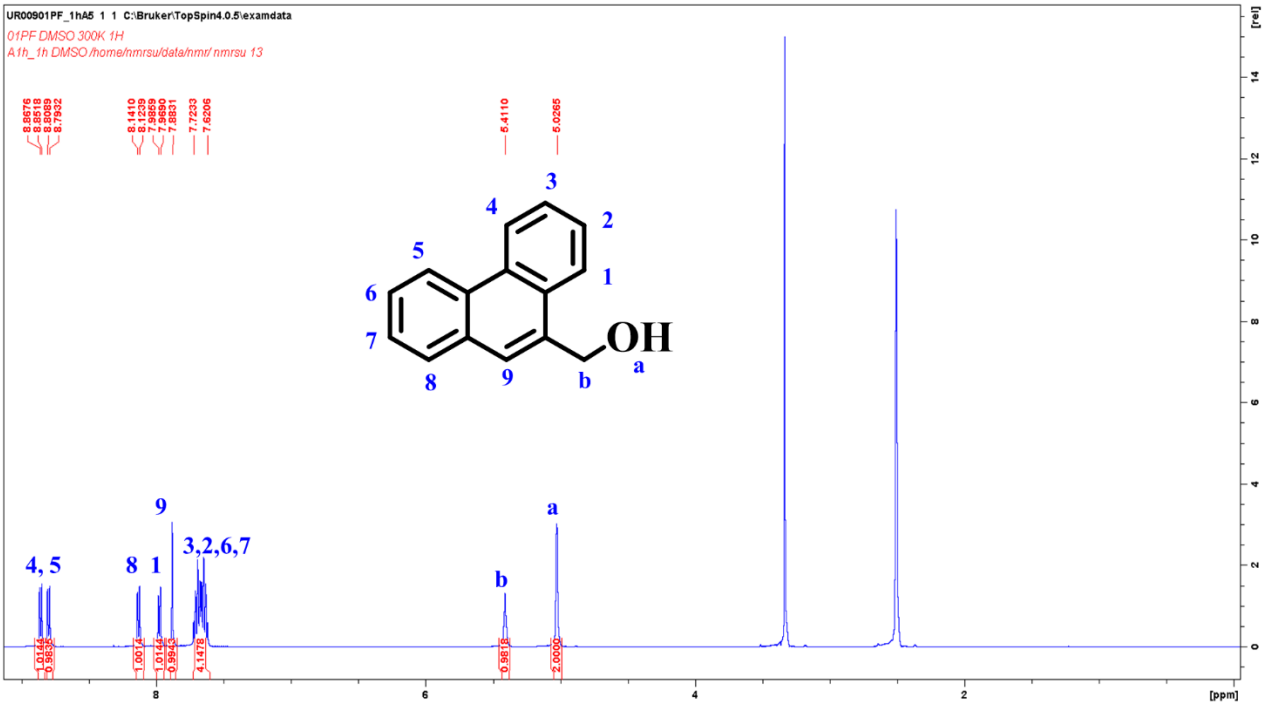


Figure S1. ^{1}H NMR spectra of compound **2** in DMSO-d^{6}.

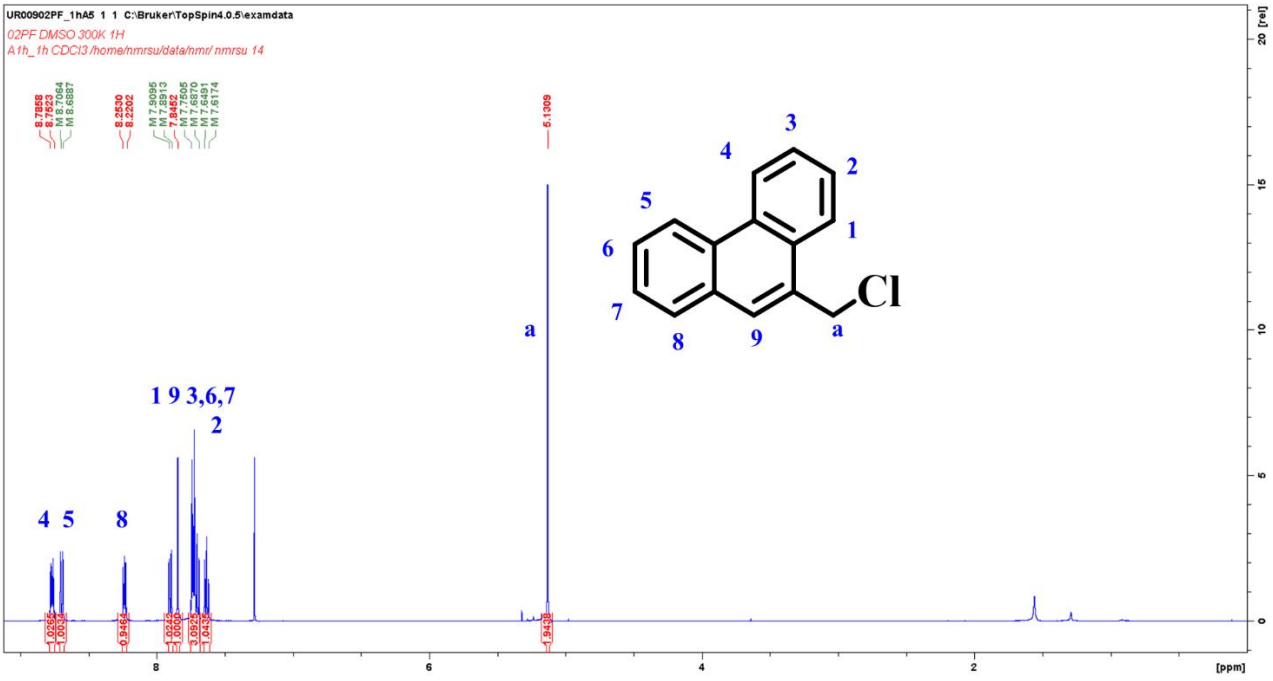


Figure S2. ^{1}H NMR spectra of compound **3** in $CDCl_3$.

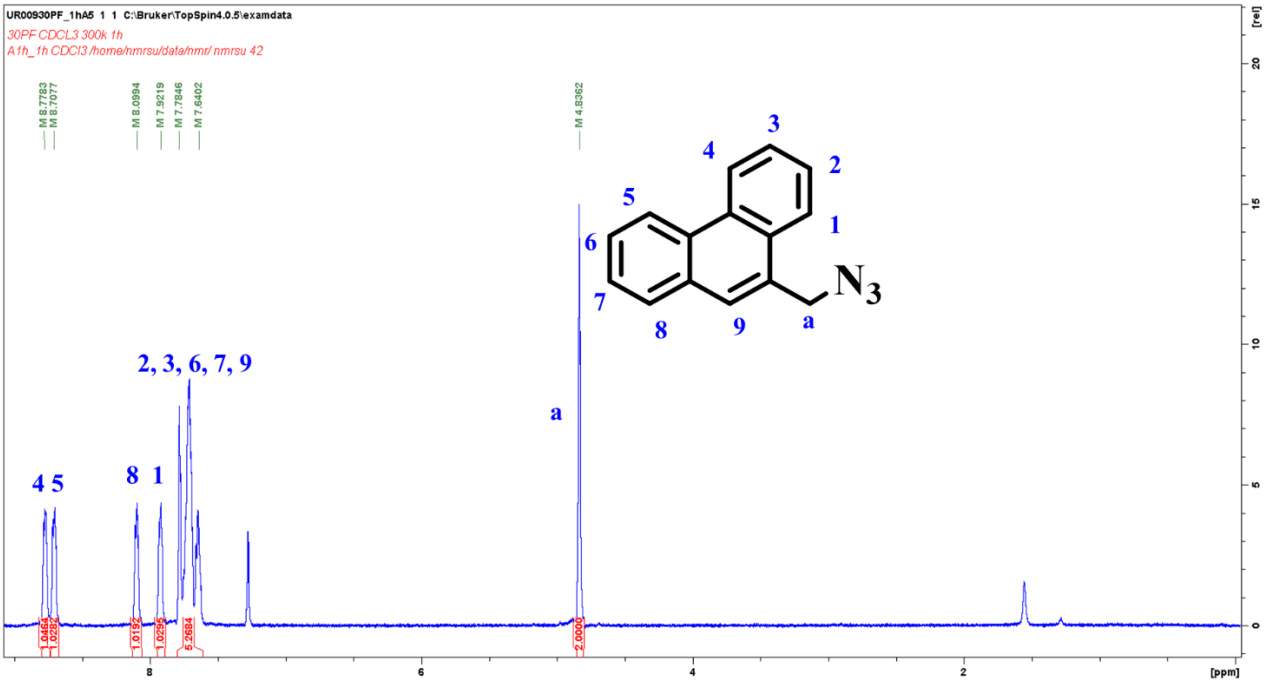


Figure S3. ^{1}H NMR spectra of compound **4** in $CDCl_3$.

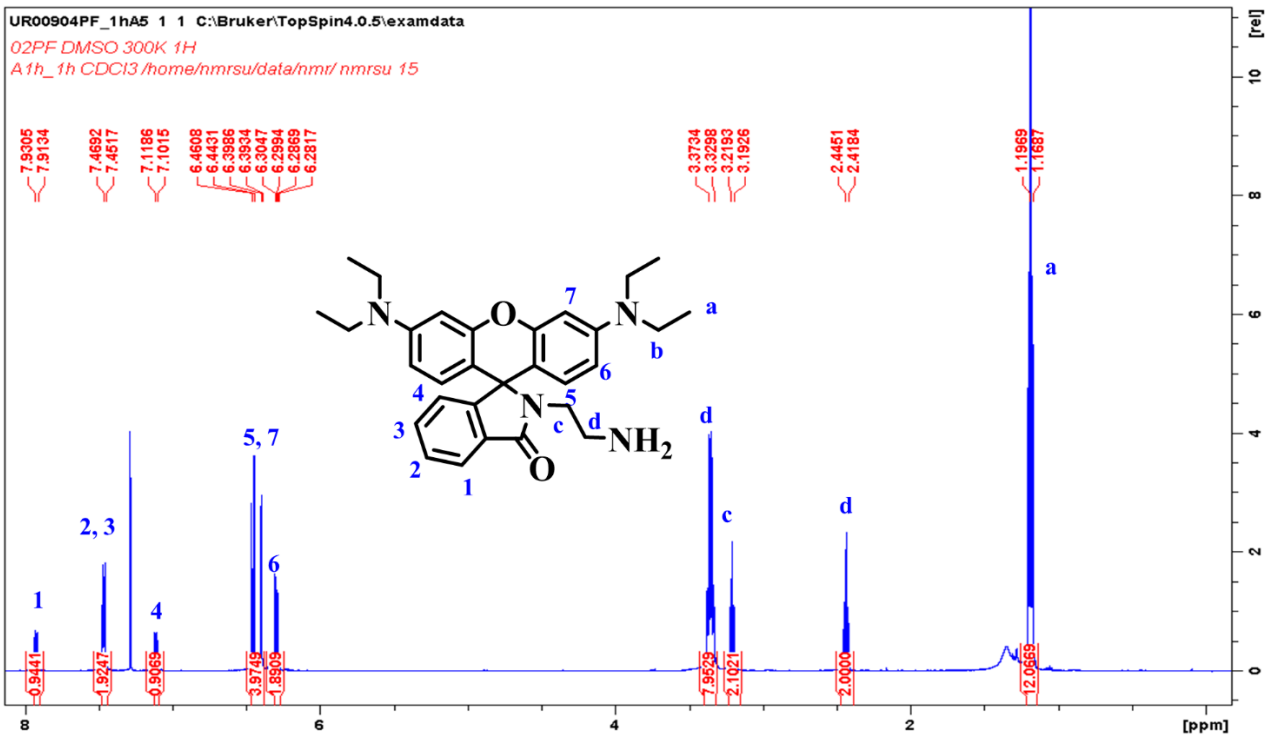


Figure S4. ^{1}H NMR spectra of compound **6** in $CDCl_3$.

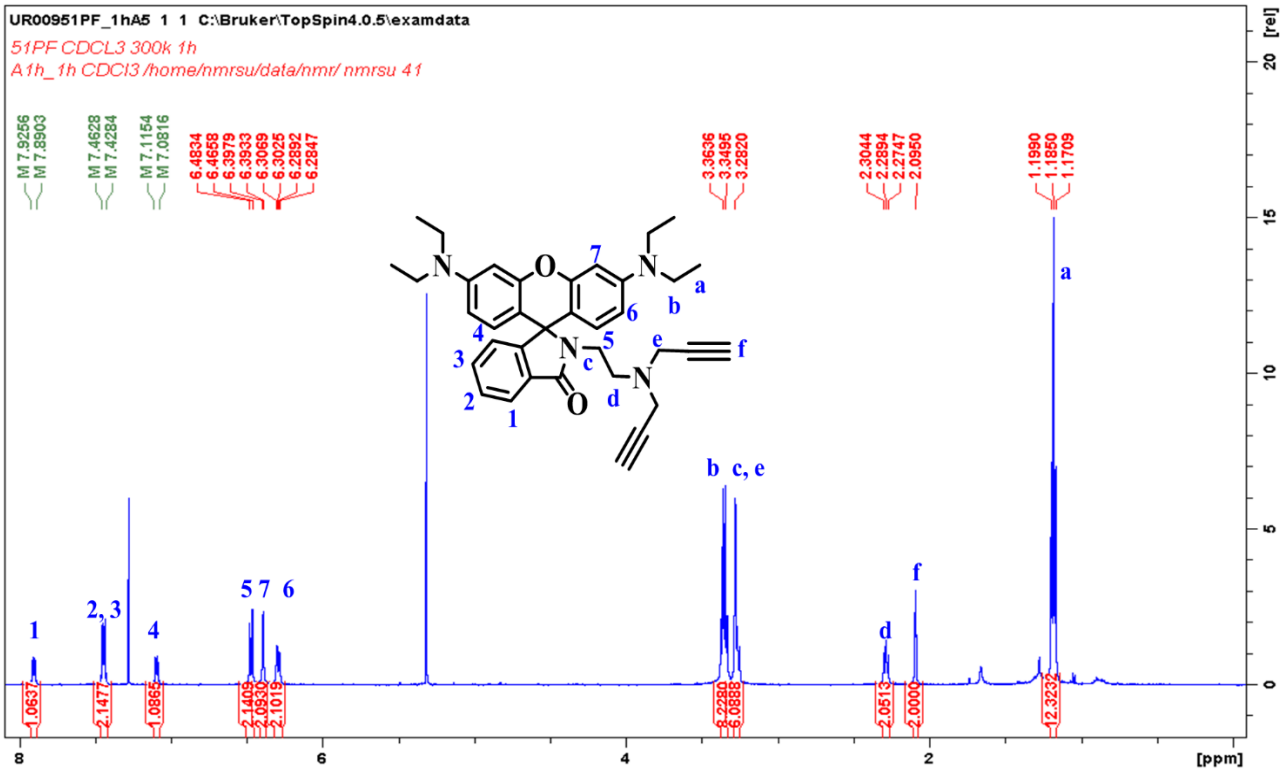


Figure S5. ^{1}H NMR spectra of compound **7** in $CDCl_3$.

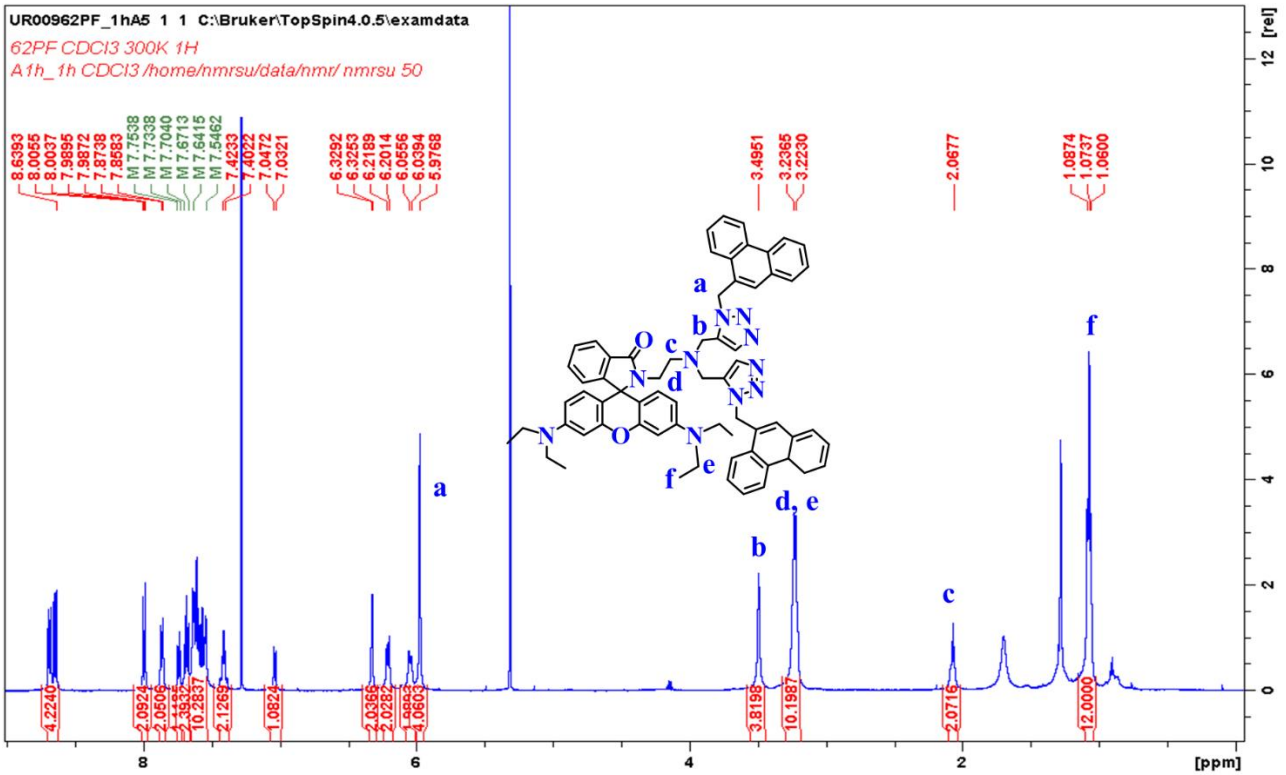


Figure S6. ^{1}H NMR spectra of compound **8** in $CDCl_3$.

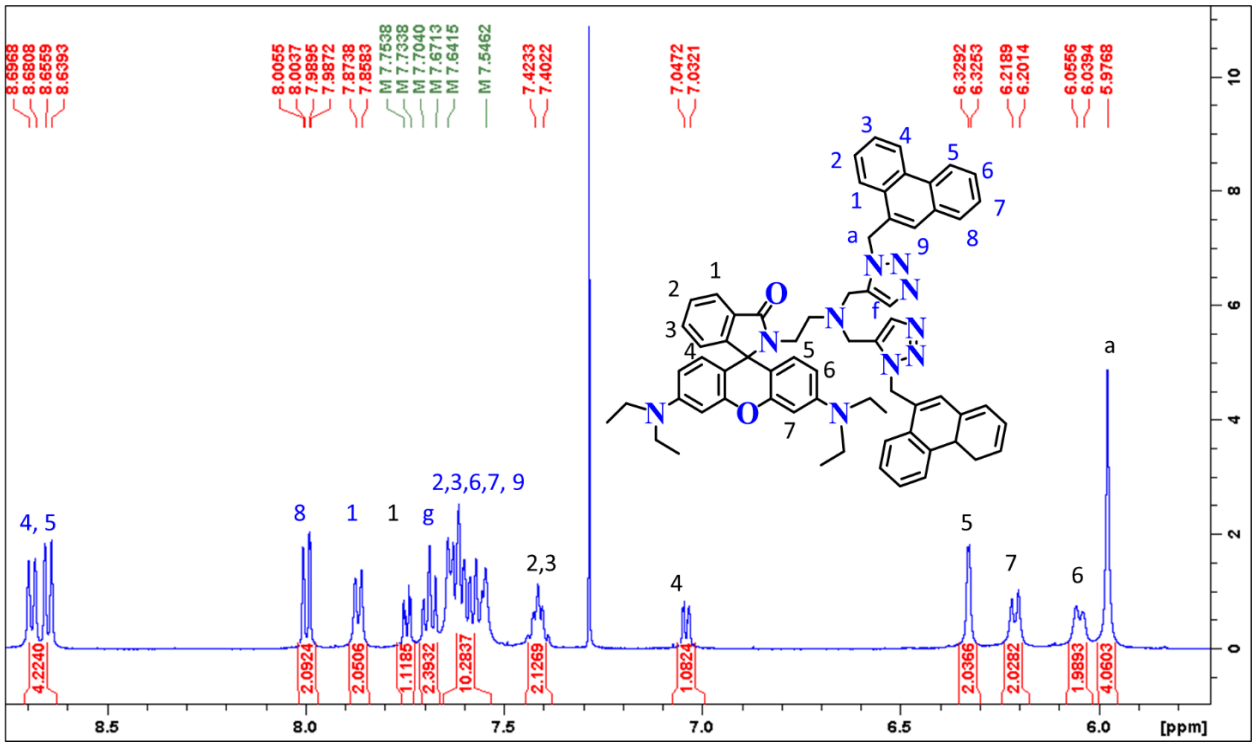


Figure S7. Magnified aromatic region of ^{1}H NMR spectra of compound **8** in $CDCl_3$.

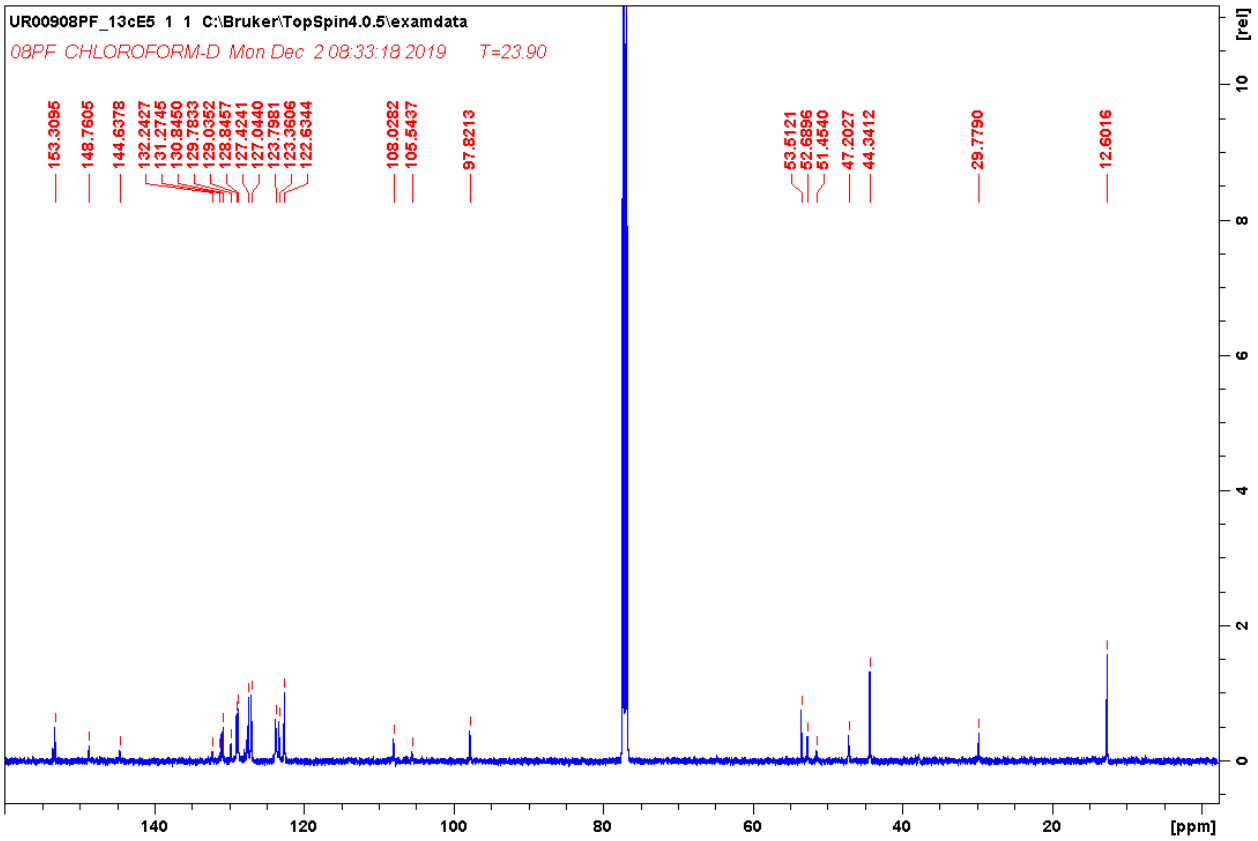


Figure S8. ^{13}C NMR spectra of compound **8** in $CDCl_3$.

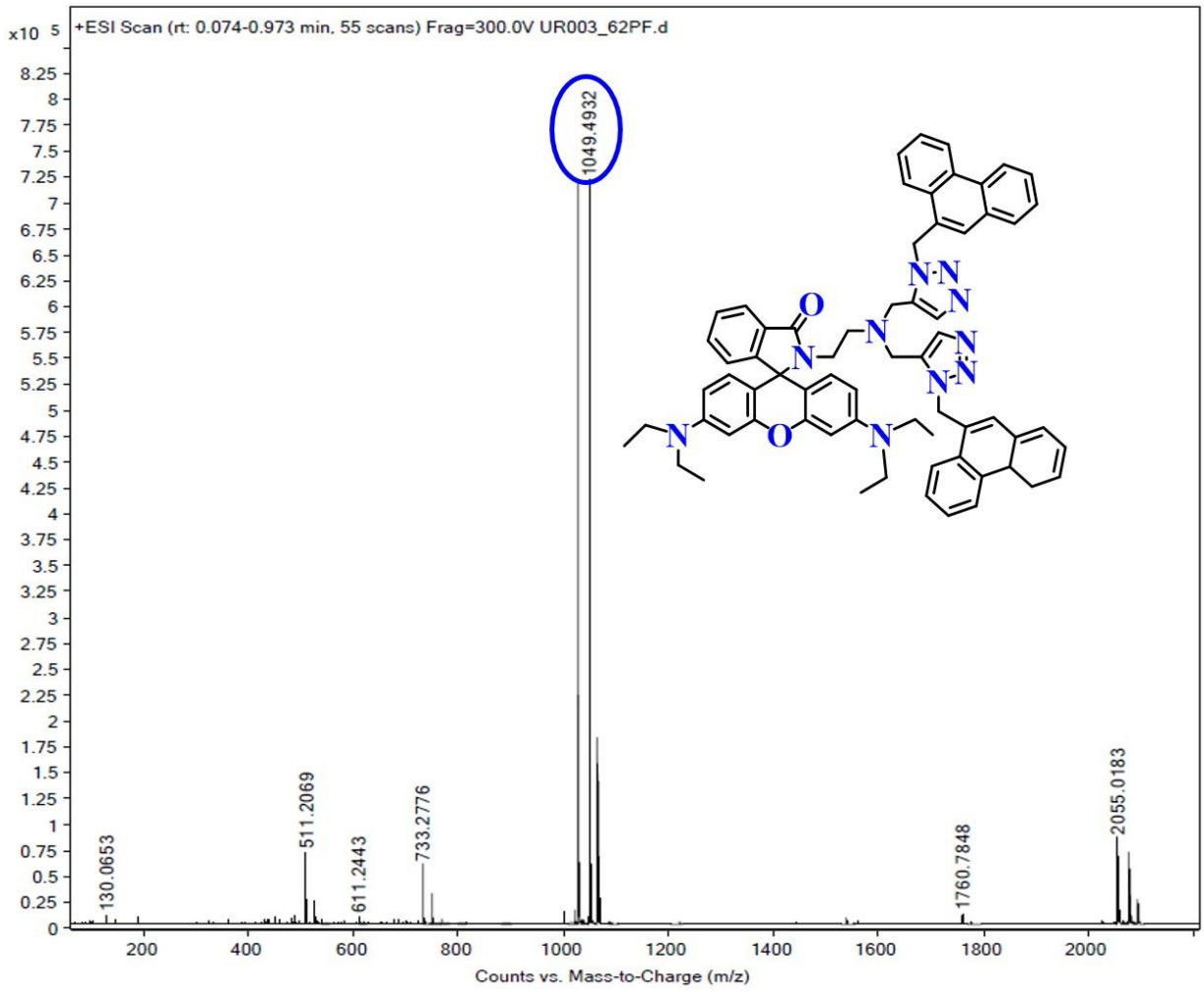


Figure S9. ESI-MS spectra of dyad **8** [8+Na^+] in methanol.

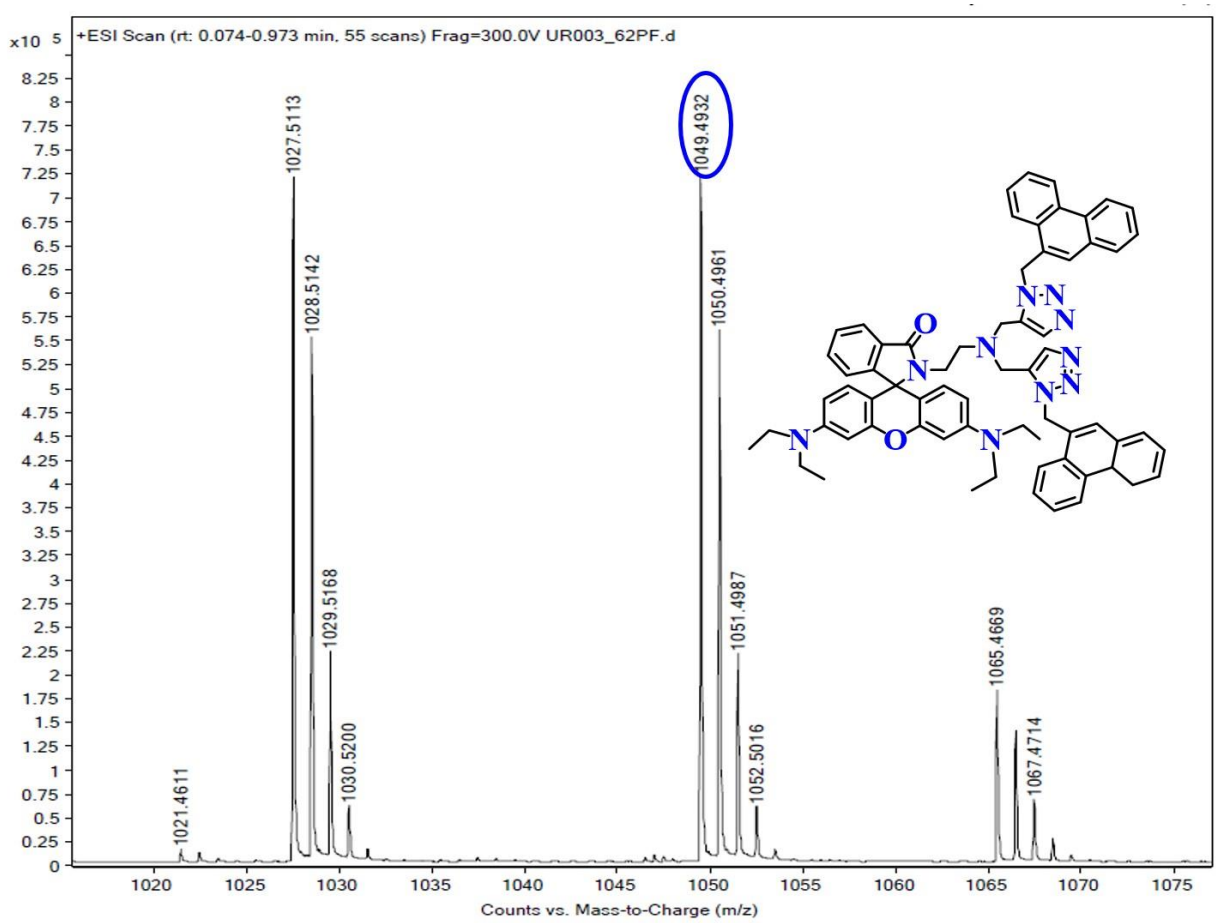


Figure S10. Magnified ESI-MS spectra of dyad **8** [8+Na^+] in methanol.

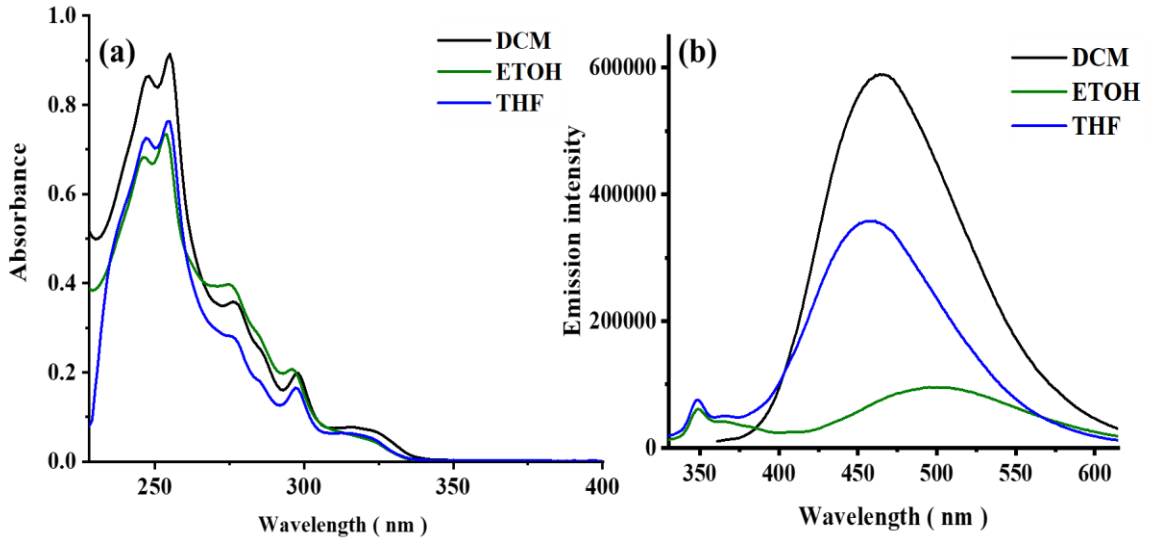


Figure S11. (a) Absorption spectra and (b) emission spectra of dyad **8** (5 µM) in different solvents (DCM, ETOH, and THF).

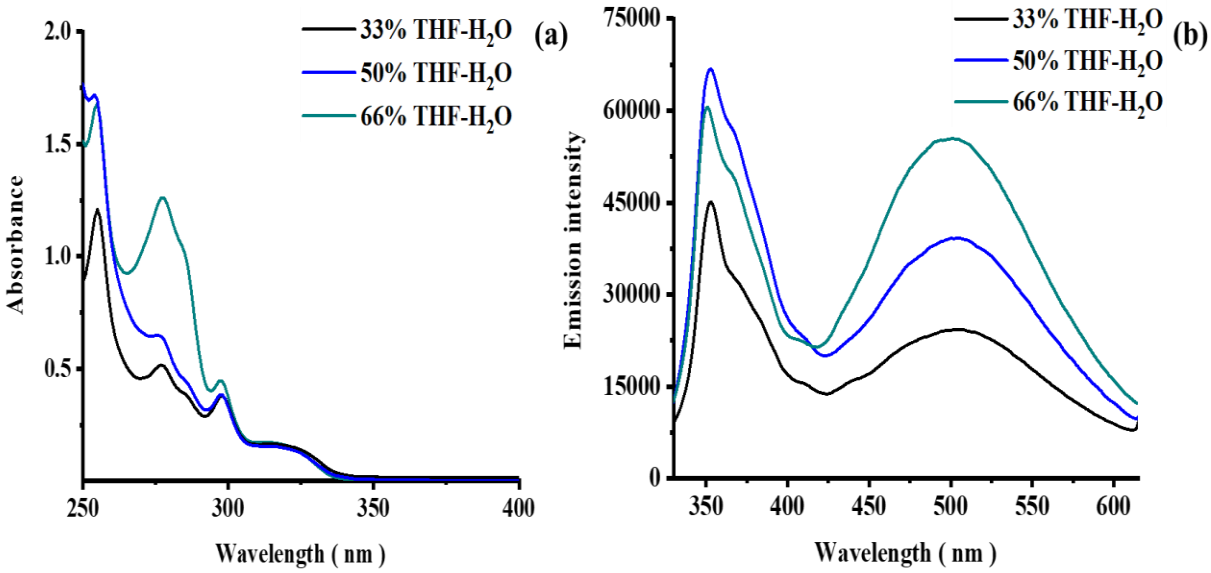


Figure S12. (a) Absorption and (b) emission spectra of dyad **8** (5 µM) in different THF-water mixtures in a B-R buffer (25 mM).

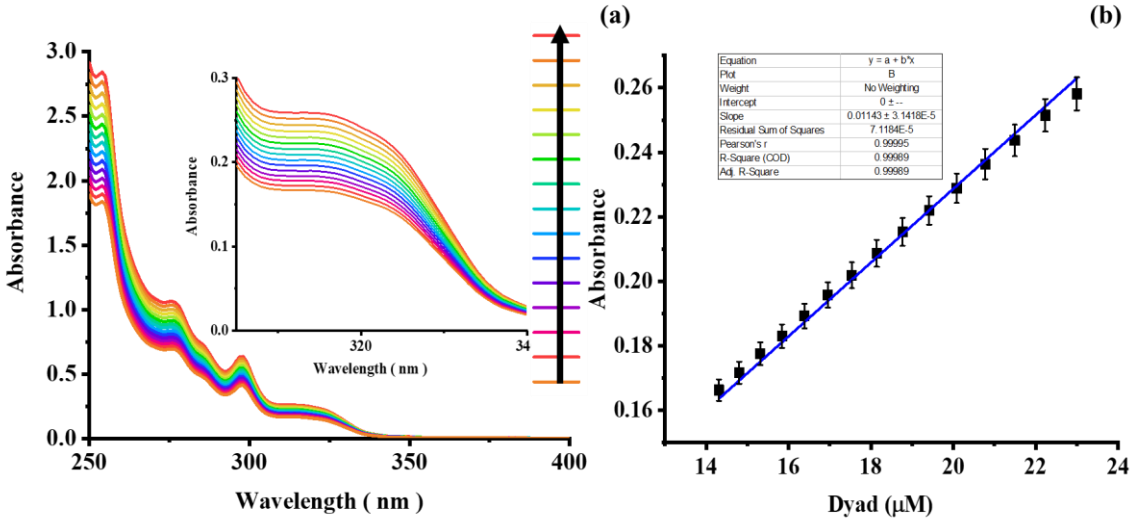


Figure S13. (a) Absorption spectra and (b) linear plot of the absorbance versus concentration of dyad **8** for calibration studies in a THF-H_2O mixture (1:1) in a B-R buffer (25 mM).

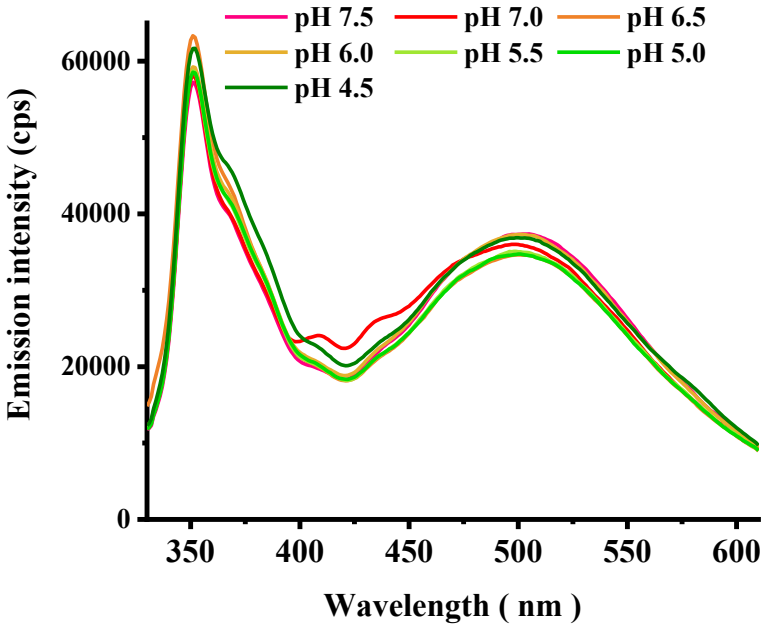


Figure S14. pH interaction studies of dyad; **8** (5 μM) at λ_{ex} 315 in a THF-H_2O mixture (1:1) in B-R buffer (25 mM).

Table S1. Absolute quantum yield measurement data of dyad **8** and comp **6** at pH 2.0 at 560 nm excitation.

Sample	Solvent	pH	$\lambda_{max,,\ Abs}$ /nm	$\lambda_{max,\ Em}$ /nm	Φ (corrected)
Dyad **8**	THF-water (1:1)	2.0	560	580	0.428
Comp **6**	THF-water (1:1)	2.0	560	580	0.472

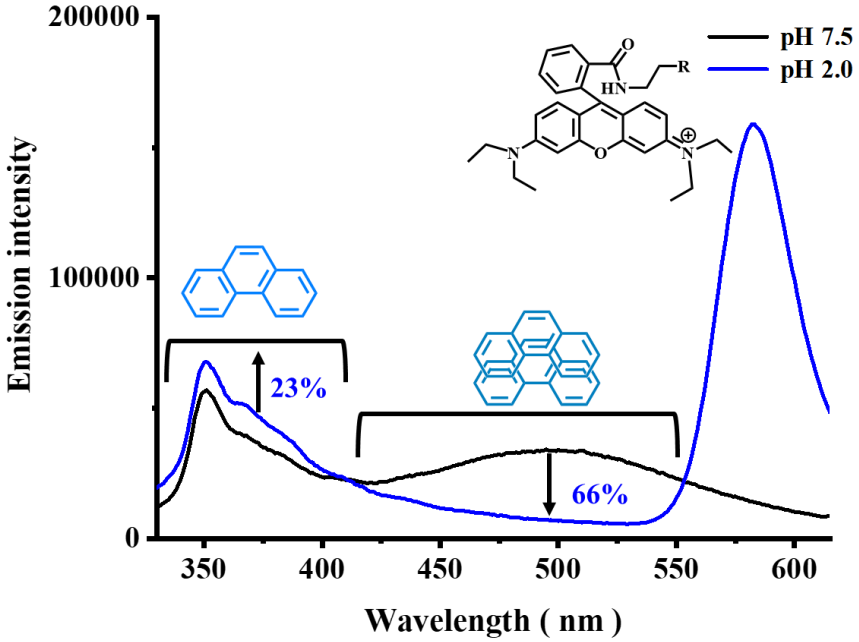


Figure S15. Emission spectra of dyad **8** at pH 7.5 and pH 2.0 in a THF-H_2O mixture (1:1) in B-R buffer (25 mM).

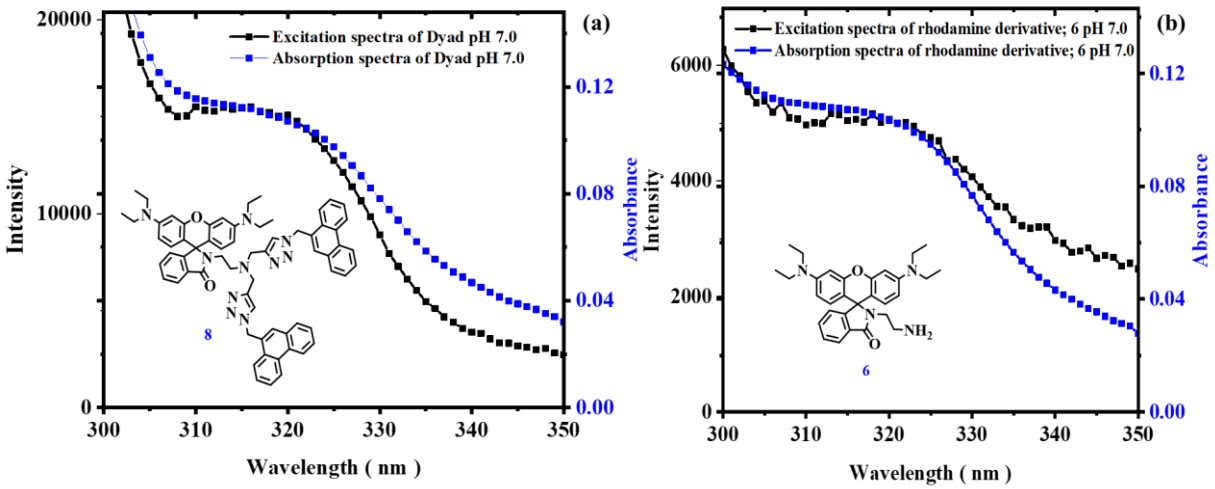


Figure S16. Overlay of the spectrally uncorrected fluorescence excitation spectra recorded at λ_{em} = 580 nm and the corresponding absorption spectra at pH 7.0 (8.5 µM) of (a) dyad **8** and (b) compound **6** in a THF- H_2O mixture (1:1) in B-R buffer (25 mM). The reasonably good match between the absorption and excitation spectra underline the expected excitation wavelength independence of the fluorescence quantum yield of **8** and **6**.

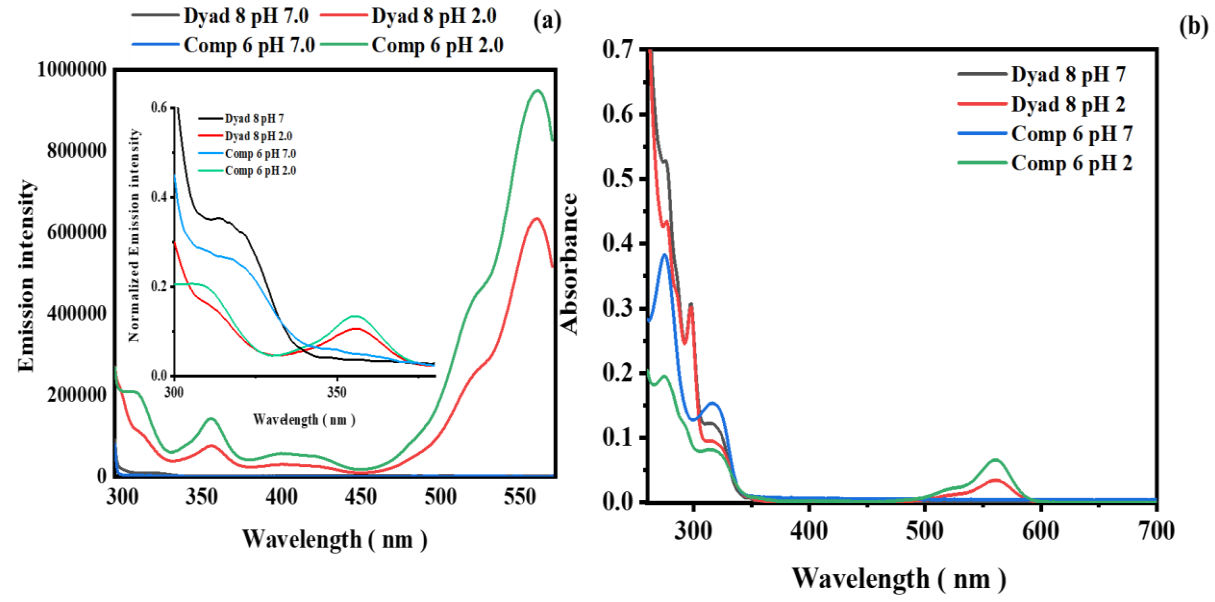
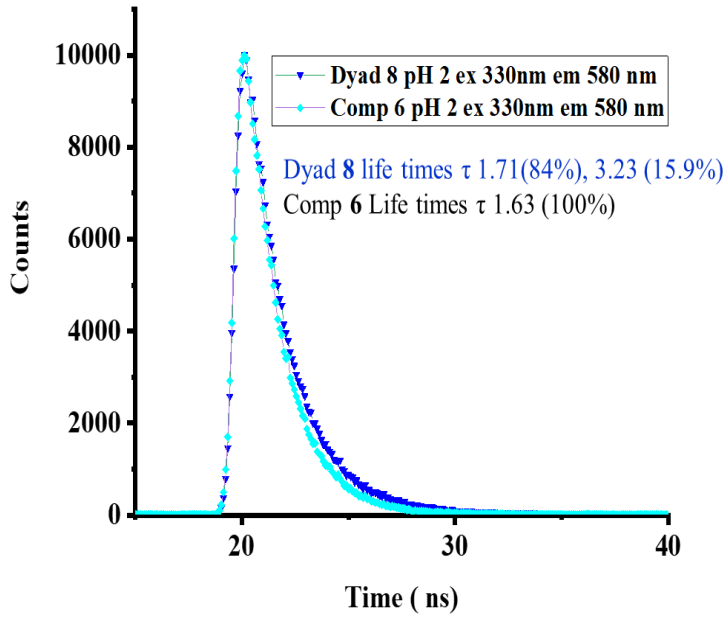


Figure S17. (a) Fluorescence excitation spectra of dyad **8** and comp **6** (3.3 µM) recorded at λ_{em} = 580 nm revealing the contribution of the different fluorophores to the emission detected at this wavelength. Inset: Magnified view of the normalized excitation spectra in the wavelength region

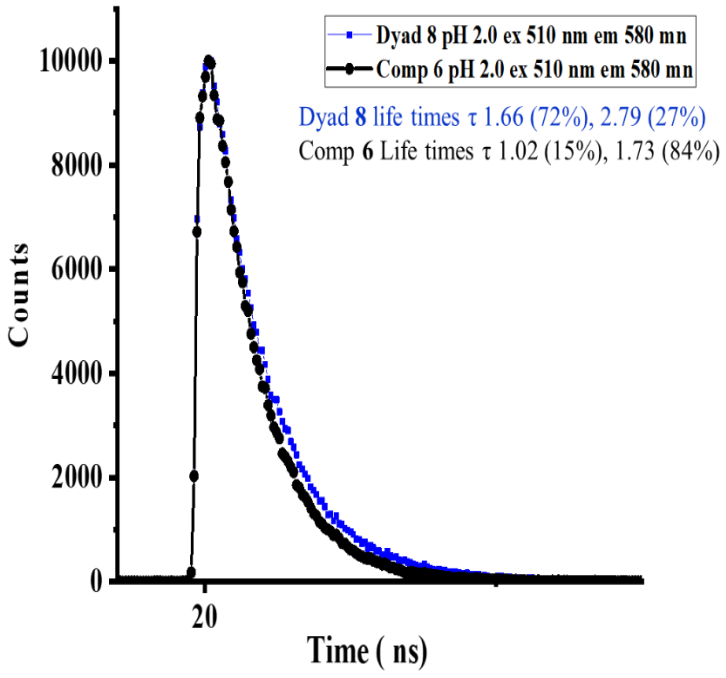
of 300 nm to 380 nm and (b) absorption spectra of dyad **8** and comp **6** (10 µM) at pH 7.0 and 2.0 in a THF-H_2O mixture (1:1) in B-R buffer (25 mM).



Dyad 8 at pH 2.0 excitation 330 nm and emission 580 nm						
Exponential Components Analysis (Reconvolution)						
Fitting Range	[206; 600] channels					
Chi Squared	1.408					
	B	B (Error)	f (%)	f (Error) (%)	Tau (ns)	Tau (Error) (ns)
1	0.1796	0.005	84.029	2.851	1.719	0.01
2	0.0181	0.0051	15.971	4.561	3.238	0.02
Shift	0.058 ns (±0.176 ns)					
Decay Background	6.856 (±0.316)					
IRF Background	0.3					

Comp 6 at pH 2.0 excitation 330 nm and emission 580 nm						
Exponential Components Analysis (Reconvolution)						
Fitting Range	[206; 500] channels					
Chi Squared	1.211					
	B	B (Error)	f (%)	f (Error) (%)	Tau (ns)	Tau (Error) (ns)
1	0.2057	0.0021	100	1.117	1.637	0.002
Shift	0.096 ns (±0.215 ns)					
Decay Background	10.195 (±0.321)					
IRF Background	0.2					

Figure 18. Fluorescence decay curves of the rhodamine moiety of dyad **8** and compound **6** at 2.0 excited at 330 nm and recorded at 580 nm in a THF-H_2O mixture (1:1) in B-R buffer (25 mM).



Dyad 8 at pH 2.0 excitation 510 nm and emission 580 nm							
Exponential Components Analysis (Reconvolution)							
Fitting Range	[207; 500] channels						
Chi Squared	1.25						
	B	B (Error)	f (%)	f (Error) (%)	Tau (ns)	Tau (Error) (ns)	Tau (Error) (ns)
1	0.1759	0.0129	72.79	6.155	1.668	0.019	0.019
2	0.0392	0.0116	27.21	8.239	2.796	0.021	0.021
Shift	-0.490 ns (±0.512 ns)						
Decay Background	2.050 (±0.384)						
IRF Background	0.5						
Comp 6 at pH 2.0 excitation 510 nm and emission 580 nm							
Exponential Components Analysis (Reconvolution)							
Fitting Range	[206; 400] channels						
Chi Squared	1.263						
	B	B (Error)	f (%)	f (Error) (%)	Tau (ns)	Tau (Error) (ns)	
1	0.0542	0.0192	15.917	8.693	1.023	0.197	
2	0.1687	0.021	84.083	11.141	1.737	0.014	
Shift	-0.368 ns (±0.218 ns)						
Decay Background	1.814 (±0.464)						
IRF Background	0.5						

Figure 19. Fluorescence decay curves of dyad **8** and compound **6** at 2.0 excited at 510 nm and detected at 580 nm in a THF-H_2O mixture (1:1) in B-R buffer (25 mM).

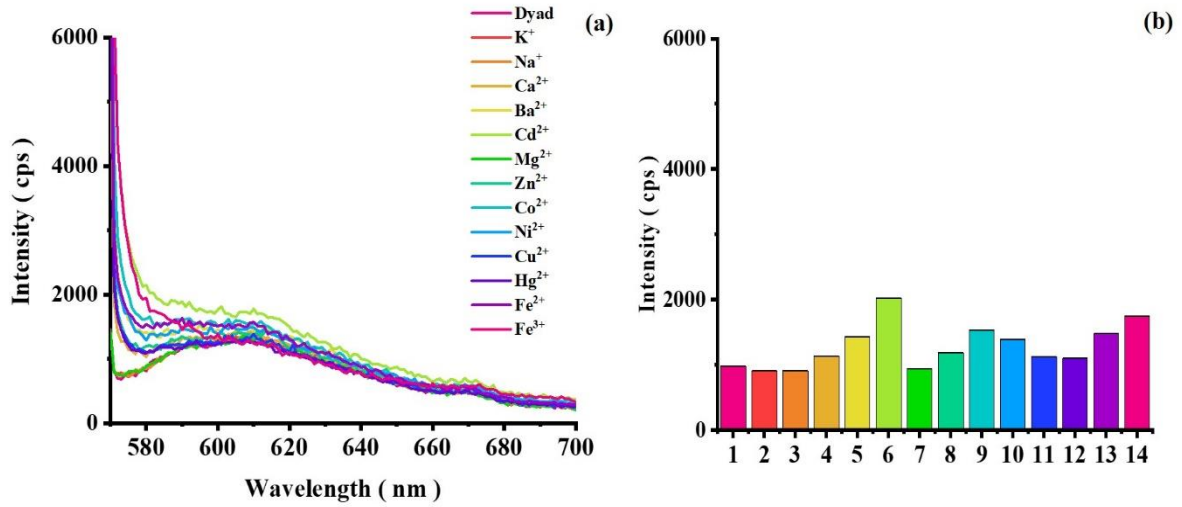


Figure S20. (a) Emission spectra and (b) bar diagram of selectivity studies using 10 equiv (10-fold excess) of K^+, Na^+, Ca^{2+}, Ba^{2+}, Mg^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+}, Fe^{2+}, and Fe^{3+} each added separately to dyad **8** (5 µM) in a THF-H_2O mixture (1:1) in B-R buffer (25 mM). Excitation was at λ_{ex} = 560 nm (rhodamine absorption band).

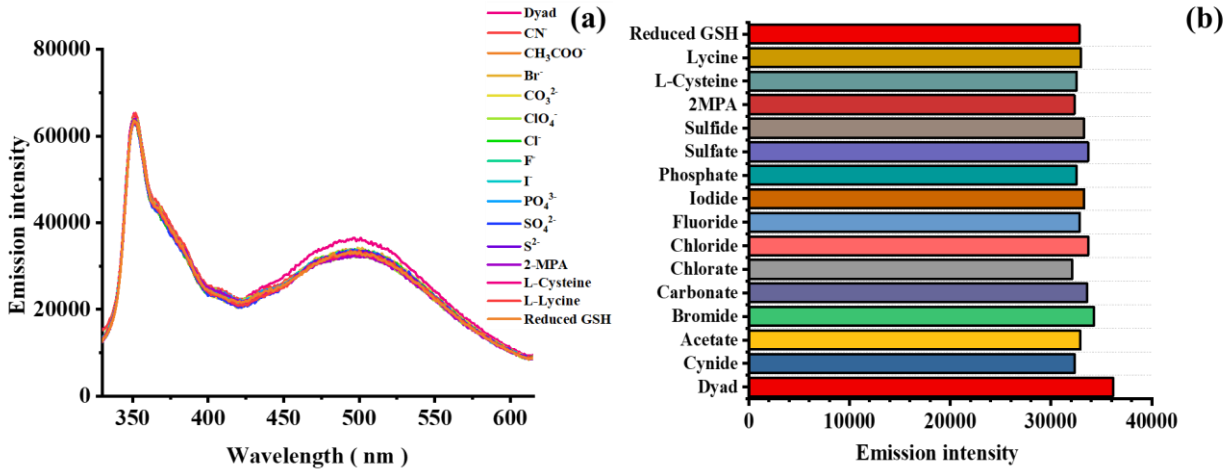


Figure S21. (a) Emission spectra and (b) Selectivity studies of dyad **8** with 10 equiv of different anions and selected amino acids (NaBr, NaCl, NaF, NaI, CH_3COONa, Na_2CO_3, NaH_2PO_4, H_2S, KCN, Na_2SO_4, 2-mercaptopropionic acid (2-MPA; for thiol), L-Lycine, L cysteine and reduced glutathione) in THF- H_2O mixture (1:1) in B-R buffer (pH 7.0; 25 mM). Excitation was at λ_{Ex} of 315 nm (phenanthrene).

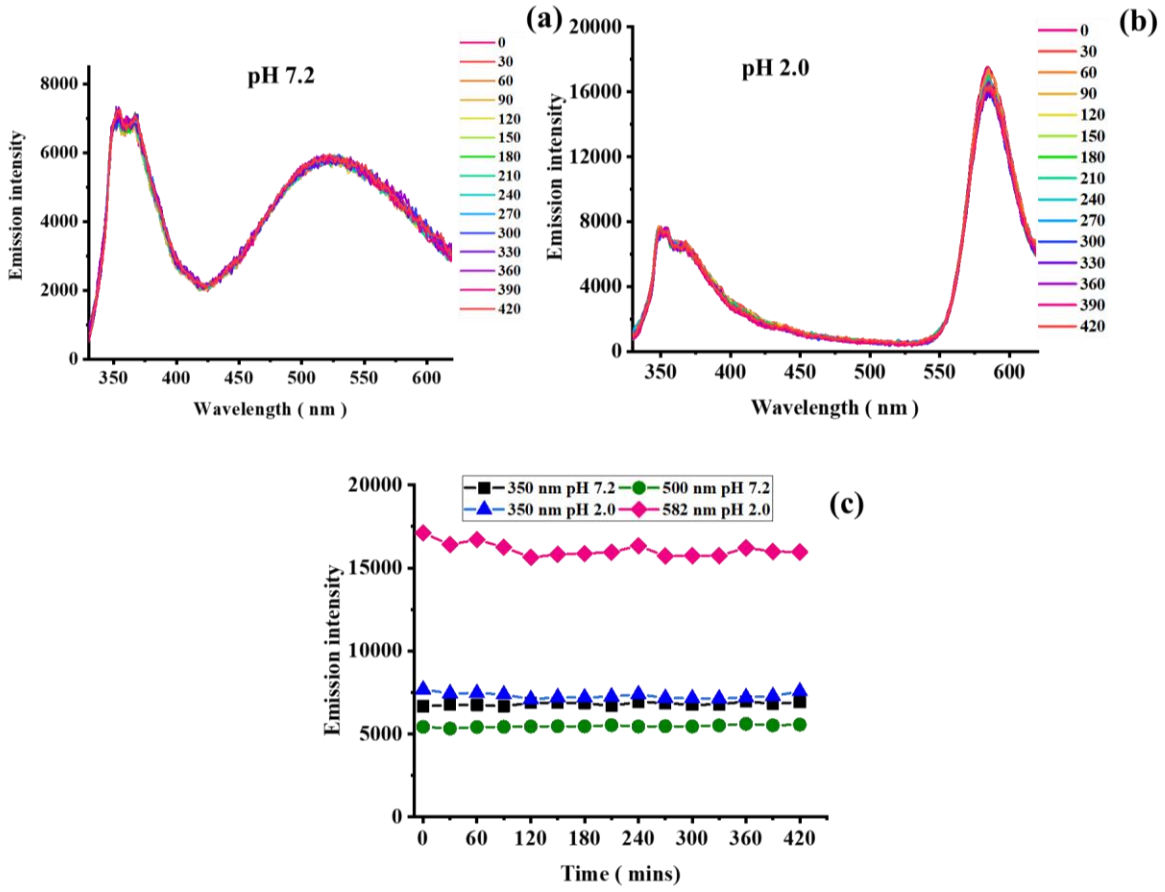


Figure S22. Photostability studies of dyad **8** (5 μM) in a THF-H_2O mixture (1:1) in B-R buffer (25 mM) using pH values of 2.0 and 7.2 illuminated with a spectrofluorometer using 315 nm excitation for up to 420 minutes; (a) pH 7.2 and (b) pH 2.0. (c) Plot of the emission intensity as a function of illumination time recorded at the monomer maximum of 350 nm and the excimer maximum of 500 nm at pH 7.2 and at the monomer maximum of 350 nm and the rhodamine emission maximum of 582 nm at pH 2.0, respectively. For the illumination studies, monochromator slit widths of 4 nm nd 2 nm in excitation and emission, respectively, were used.

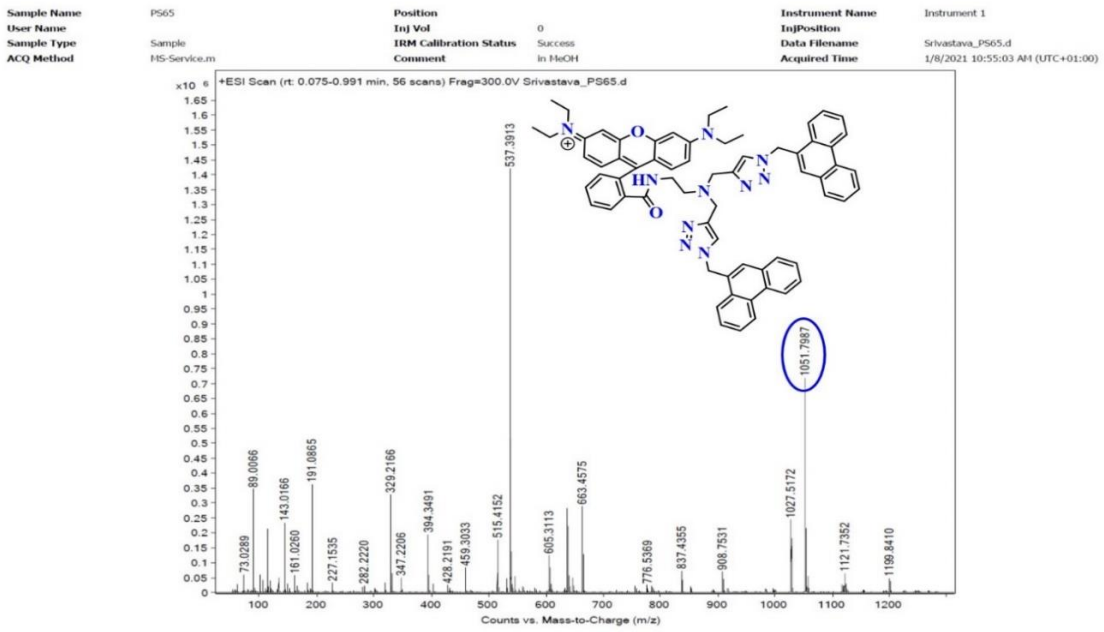


Figure S23. ESI-MS spectra of dyad **[8 +Na^++H^+]** in methanol.

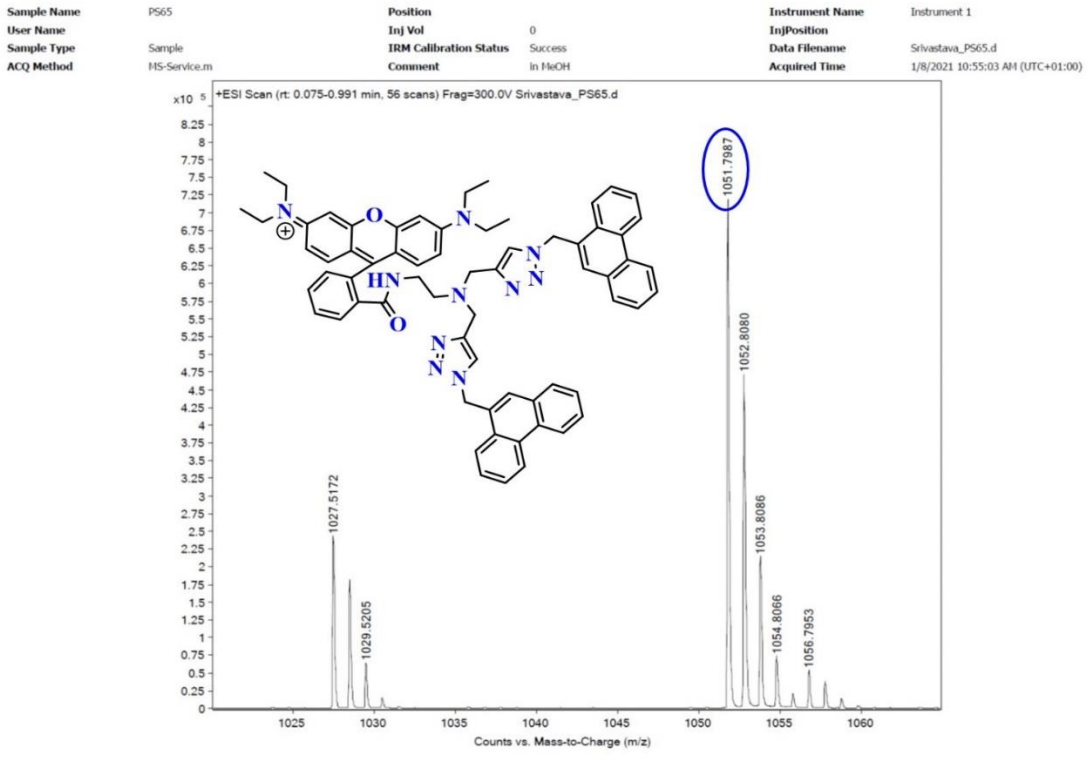


Figure S24. Magnified ESI-MS spectra of dyad **[8 +Na^++H^+]** in methanol.