

**Supporting Information for**

**Coumarin-decorated Schiff base hydrolysis as an efficient  
driving force for the fluorescent detection of water in  
organic solvents**

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## Experimental Section

### Synthetic Materials, methods and instrumentations.

All the materials for the synthesis, 4-(Diethylamino)salicylaldehyde (Aldrich), diethyl malonate (Aldrich), piperidine (Aldrich), NaOH (Samchun), acetic acid (Duksan), DMF (J.T.Baker), POCl<sub>3</sub> (Aldrich), 4-nitroaniline (Aldrich), anhydrous ethanol (Alfa Aesar), aniline (Aldrich), p-anisidine (Aldrich) and 2-aminopyridine (Aldrich) were purchased from commercial suppliers and were used without further purification. All the reactions were carried out under nitrogen atmosphere. Silica gel 60 (Merck, 0.063~0.2 mm) was used for column chromatography as a stationary phase. Analytical thin layer chromatography was performed using Merk 60 F254 silica gel (precoated sheets, 0.25 mm thick). The ESI mass spectra were collected using a Shimadzu LC/MS-2020 Series instrument. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded in CDCl<sub>3</sub> on a Varian 300 and 400 MHz spectrometer. All chemical shifts are reported in ppm values using the peak of TMS as an internal reference.

### Spectroscopic measurements.

Stock solutions of probes were prepared in DMSO. All excitation and emission slit widths were set at 5 nm. The concentration of each of the samples was fixed at 10 μM in a total volume of 4 mL.

### Crystallographic Structure Determination

X-ray data was collected on a Bruker SMART APEXII diffractometer equipped with graphite monochromated MoK $\alpha$  radiation ( $\lambda = 0.71073\text{\AA}$ ). Preliminary orientation matrix and cell parameters were determined from three sets of  $\phi$  scans at different starting angles. Data frames were obtained at scan intervals of 0.5° with an exposure time of 10 s per frame. The reflection data were corrected for Lorentz and polarization factors. Absorption corrections were carried out using SADABS. The structures were solved by direct methods and refined by full-matrix least-squares analysis using anisotropic thermal parameters for non-hydrogen atoms with the SHELXTL program.

### LOD and LOQ determination

The limit of detection and quantitation was determined from the linear regression of the fluorescence intensities versus the water content in the 0–1% (v/v) range in DMSO upon excitation at 456 nm.

The limit of detection (LOD) and limit of quantitation (LOQ) are defined in equations S1 and S2, with  $\sigma$  being the standard deviation of the blank and  $m$  the slope of the calibration curve:

$$LOD = \frac{3.3\sigma}{m} \quad (S1)$$

$$LOQ = \frac{10\sigma}{m} \quad (S2)$$

The calibration equation was given by fitting the experimental data:

$$I = 13.322[\text{H}_2\text{O}] + 59.090 \quad (R = 0.9935, [\text{H}_2\text{O}] = 0\text{--}1\%)$$

$$LOQ = 10 * 0.72071 / 13.322 = 0.54$$

$$LOD = 3.3 * 0.72071 / 13.322 = 0.18$$

$m$  = slope of calibration curve (13.322)

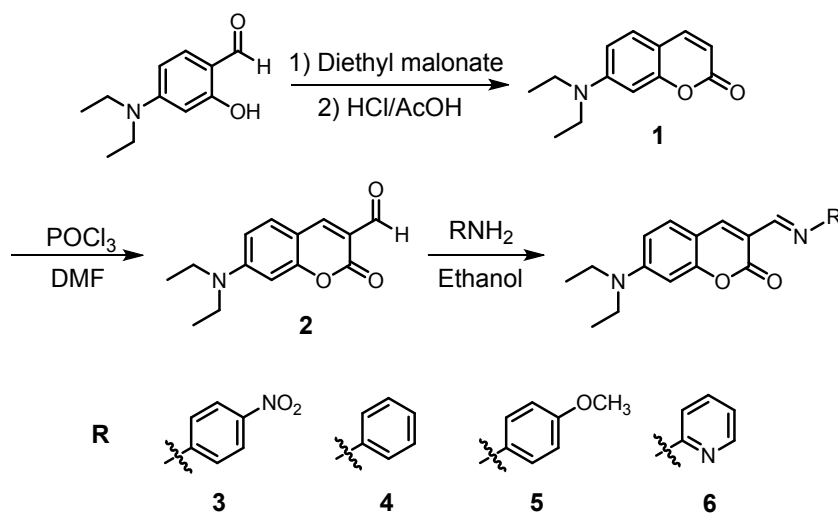
$\sigma$  = standard deviation of blank readings (0.72071)

### Computational section

Density functional theory (DFT) calculations were performed to understand the reaction mechanism and kinetics for the hydrolysis of **4** and **6** with Becke's three parameterized Lee–Yang–Parr (B3LYP)<sup>1</sup> exchange functional and 6-31G(d) basis sets using a suite of Gaussian 09 programs<sup>2</sup>. Polarizable continuum model (PCM) was employed to treat the solvent effect. All the reactant complexes, intermediates, transition states, and product complexes were confirmed to be local minima (no imaginary frequency) or saddle point (only one imaginary frequency) from the vibrational frequency calculations. Furthermore, intrinsic reaction coordinate (IRC) calculations were performed to assure that the reaction pathway from the transition state structure is connected to the reactants and the products. Natural bond orbital (NBO) populations were performed to obtain the atomic charges.

- (1) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.  
 (2) Gaussian 09 (Revision D.01). M. J. Frisch, G. W. T., H. B. Schlegel, G. E. Scuseria, M.; A. Robb, J. R. C., G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H.; Nakatsuji, M. C., X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L.; Sonnenberg, M. H., M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T.; Nakajima, Y. H., O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F.; Ogliaro, M. B., J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R.; Kobayashi, J. N., K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J.; Tomasi, M. C., N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C.; Adamo, J. J., R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C.; Pomelli, J. W. O., R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P.; Salvador, J. J. D., S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V.; Ortiz, J. C., and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.

## Synthesis



Synthetic route of water-sensing probes **3-6**.

### Synthesis of 7-diethylaminocoumarin (**1**)

4-(Diethylamino)salicylaldehyde (1.0 equiv, 1.93 g, 10 mmol) was dissolved in 40.0 mL ethanol. Diethyl malonate (2.0 equiv, 3.12 mL, 20 mmol) and piperidine (1.0 equiv, 1 mL, 10 mmol) were added. The reaction mixture was stirred and heated to reflux overnight. The reaction was monitored by TLC. The solvent was removed under reduced pressure, then concentrated HCl (20.0 mL) and glacial acetic acid (20.0 mL) were added for hydrolysis and stirred for another 6 hours. The solution was cooled and poured into 100 mL ice water. 40% NaOH solution was added to modulate pH of the solution to ~7. 30.0 mL of dichloromethane was added to solution, and the organic layer was washed by water (3 x 10.0 mL), dried over MgSO<sub>4</sub> and then concentrated in vacuum. The crude product was purified by column chromatography ethyl acetate/hexane (1:3) to obtain the product 2.01 g (93%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.54 (d, J = 9.24 Hz, 1H), 7.24 (d, J = 8.80 Hz, 1H), 6.56 (dd, J = 8.80, 2.52

Hz, 1H), 6.49 (d, J = 2.52 Hz, 1H), 6.03 (d, J = 9.24 Hz, 1d), 3.41 (q, J = 7.08 Hz, 4H), 1.21 (t, J = 7.08 Hz, 6H) ppm.

### Synthesis of 7-diethylaminocoumarin-3-aldehyde (**2**)

Dried DMF (3.00 equiv, 2.14 mL, 27.79 mmol) was added dropwise to POCl<sub>3</sub> (2.50 equiv, 2.18 mL, 23.16 mmol) at 50°C with N<sub>2</sub> atmosphere and stirred for 30 minutes to yield a red solution. Compound **1** (1.00 equiv, 2.01 g, 9.26 mmol, dissolved in 10.0 mL DMF) was added dropwise to the solution. The mixture was stirred at 70°C for 12 hours and then poured into 100 mL of ice water. 20% NaOH solution was added to adjust the pH of the mixture to ~7. The crude product was diluted by 30.0 mL of ethyl acetate and the organic layer was washed by water (3 x 10.0 mL), dried over MgSO<sub>4</sub> and then concentrated in vacuum. The crude product was purified by column chromatography ethyl acetate/hexane (1:2) to obtain the product 1.72 g (76%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 10.12 (s, 1H), 8.25 (s, 1H), 7.41 (d, J = 9.04 Hz, 1H), 6.64 (dd, J = 9.04, 2.40 Hz, 1H), 6.48 (d, J = 2.40 Hz, 1H), 3.48 (q, J = 7.14 Hz, 4H), 1.26 (t, J = 7.14 Hz, 6H) ppm

### Synthesis of compound **3**

Compound **2** (1.00 equiv, 150 mg, 0.61 mmol) and 4-nitroaniline (1.20 equiv, 102 mg, 0.74 mmol) were combined in dried ethanol (5.0 mL). The solution was stirred under reflux conditions for 6 hours. The solution was cooled and the precipitate was filtrated, washed with ether and dried ethanol three times to get product 53.9 mg (24.11%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 8.69 (s, 1H), 8.53 (s, 1H), 8.26 (d, J = 9.00 Hz, 2H), 7.43 (d, J = 9.00 Hz, 1H), 7.29 (d, J = 5.76 Hz, 2H), 6.66 (dd, J = 5.76, 2.34 Hz, 1H), 6.52 (d, J = 2.34 Hz, 1H), 3.48 (q, J = 7.16 Hz, 4H), 1.26 (t, J = 7.16 Hz, 6H) ppm.

### Synthesis of compound **4**

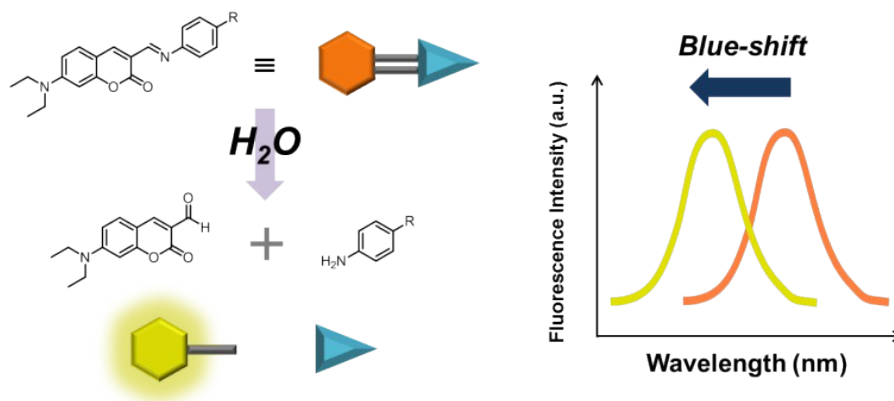
Compound **2** (1.00 equiv, 153 mg, 0.62 mmol) and aniline (1.20 equiv, 67.6 μL, 0.74 mmol) were combined in dried ethanol (5.0 mL). The solution was stirred under reflux conditions for 6 hours. The solution was cooled and the precipitate was filtrated, washed with ether and dry ethanol three times to get the product 104.4 mg (52.15%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 8.72 (s, 1H), 8.52 (s, 1H), 7.39 (dd, J = 14.40, 8.50 Hz, 3H), 7.24 (d, J = 8.50 Hz, 3H), 6.63 (dd, J = 8.50, 2.52 Hz, 1H), 6.52 (d, J = 2.52 Hz, 1H), 3.46 (q, J = 7.12 Hz, 4H), 1.25 (t, J = 7.12 Hz, 6H) ppm.

### Synthesis of compound 5

Compound **2** (1.00 equiv, 143 mg, 0.58 mmol) and p-anisidine (1.26 equiv, 90.4 mg, 0.73 mmol) were combined in dried ethanol (5.0 mL). The solution was stirred under reflux conditions for 6 hours. The solution was cooled and the precipitate was filtrated, washed with ether and dry ethanol three times to get the product 131.9 mg (64.56%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 8.72 (s, 1H), 8.53 (s, 1H), 7.40 (d, J = 8.84 Hz, 1H), 7.26 (d, J = 8.84 Hz, 2H), 6.92 (d, J = 8.84 Hz, 2H), 6.62 (d, J = 8.84 Hz, 1H), 6.52 (s, 1H), 3.45 (q, J = 7.20 Hz, 4H), 1.24 (t, J = 7.20 Hz, 6H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 162.6, 158.5, 157.5, 152.7, 151.9, 144.8, 140.6, 130.9, 122.6, 115.7, 114.5, 109.7, 108.9, 97.3, 45.1, 12.6 ppm. ESI-MS: m/z calcd for C<sub>21</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> [M+H<sup>+</sup>], 351.16; found 351.3.

### Synthesis of compound 6

Compound **2** (1.00 equiv, 549.9 mg, 2.24 mmol) and 2-aminopyridine (1.00 equiv, 207.0 mg, 2.20 mmol) were combined in dried ethanol (20.0 mL). The solution was stirred under reflux conditions for 6 hours. The solution was cooled and the precipitate was filtrated, washed with hexane and 2-propanol three times to get the product 123.2 mg (17.1%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 9.25 (s, 1H), 8.60 (s, 1H), 8.49 (ddd, J = 4.83, 1.89, 0.78 Hz, 1H), 7.73 (dt, J = 7.92, 1.89 Hz, 1H), 7.41 (d, J = 7.92 Hz, 1H), 7.24 (s, 1H), 7.15 (dd, J = 7.92, 4.83, 1.05 Hz, 1H), 6.62 (dd, J = 7.92, 2.49 Hz, 1H), 6.51 (d, J = 2.49 Hz, 1H), 3.46 (q, J = 7.14 Hz, 4H), 1.25 (t, J = 7.14 Hz, 6H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 162.2, 161.9, 158.2, 158.1, 152.4, 149.2, 142.1, 138.2, 131.3, 121.6, 118.4, 115.0, 109.8, 108.9, 97.3, 45.2, 12.6 ppm. ESI-MS: m/z calcd for C<sub>19</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub> [M+H<sup>+</sup>], 322.25; found 322.25.



**Scheme S1** Conceptual illustration of the water-sensing probe.

# H-NMR, <sup>13</sup>C-NMR and MS Analyses:

SpinWorks 2.5: Std proton

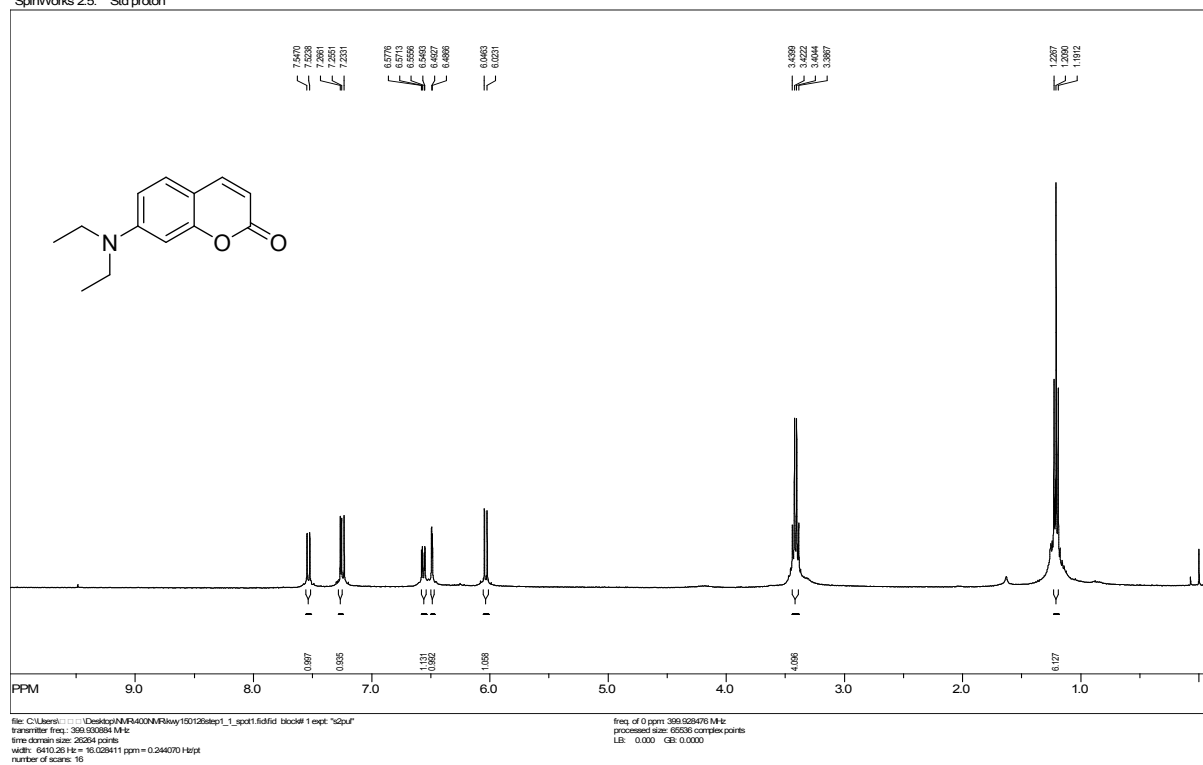


Fig. S1 <sup>1</sup>H NMR spectra (400 MHz) of 1 in CDCl<sub>3</sub>

SpinWorks 2.5: Std proton

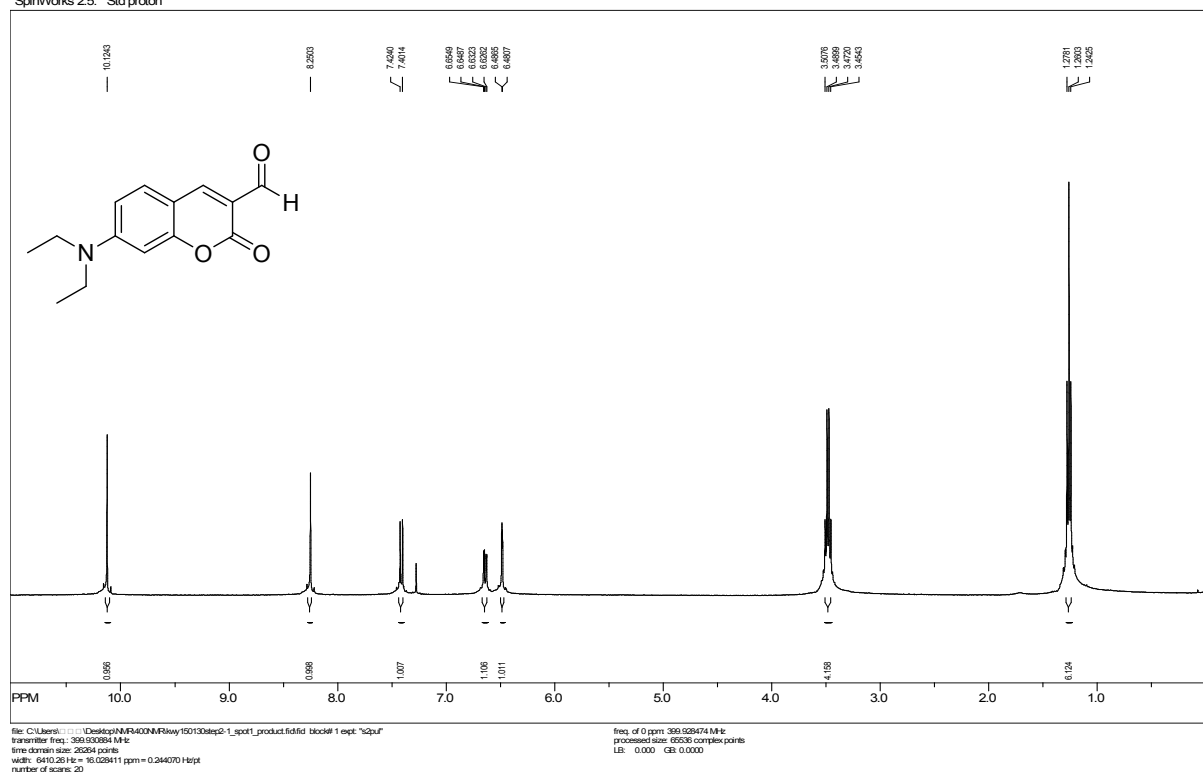


Fig. S2 <sup>1</sup>H NMR spectra (400 MHz) of 2 in CDCl<sub>3</sub>

SpinWorks 2.5: Std proton

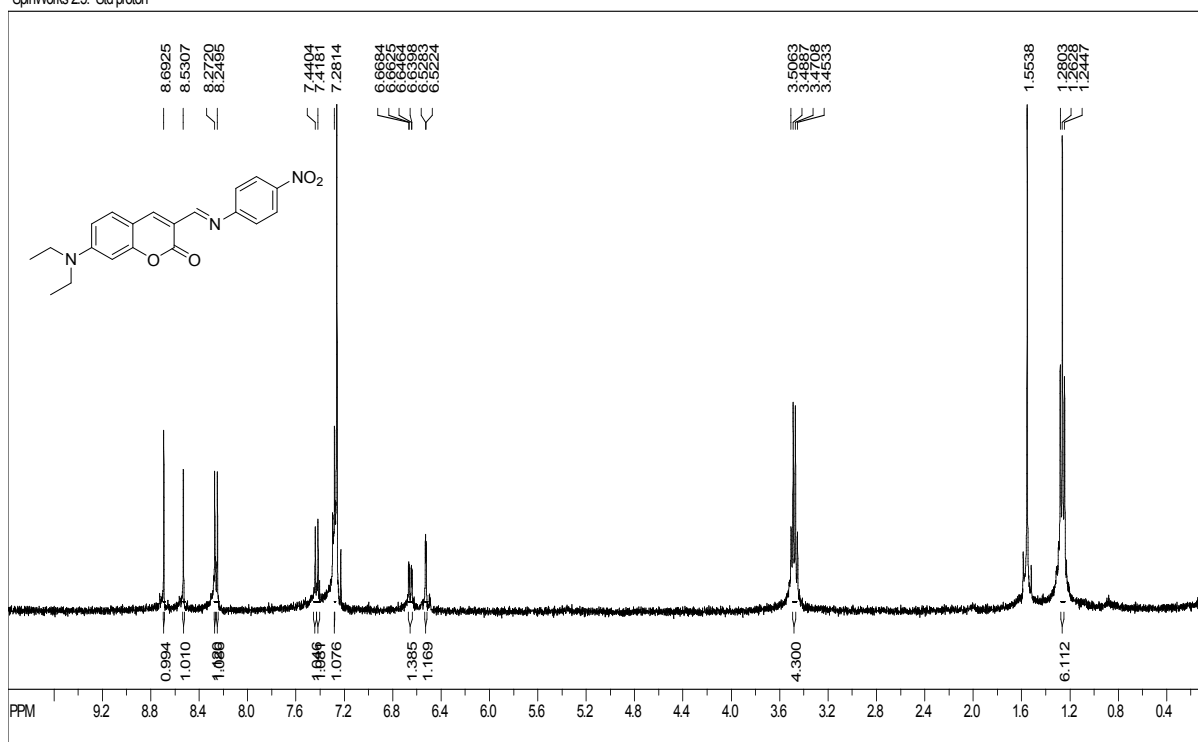


Fig. S3 <sup>1</sup>H NMR spectra (400 MHz) of **3** in CDCl<sub>3</sub>

SpinWorks 2.5: Std proton

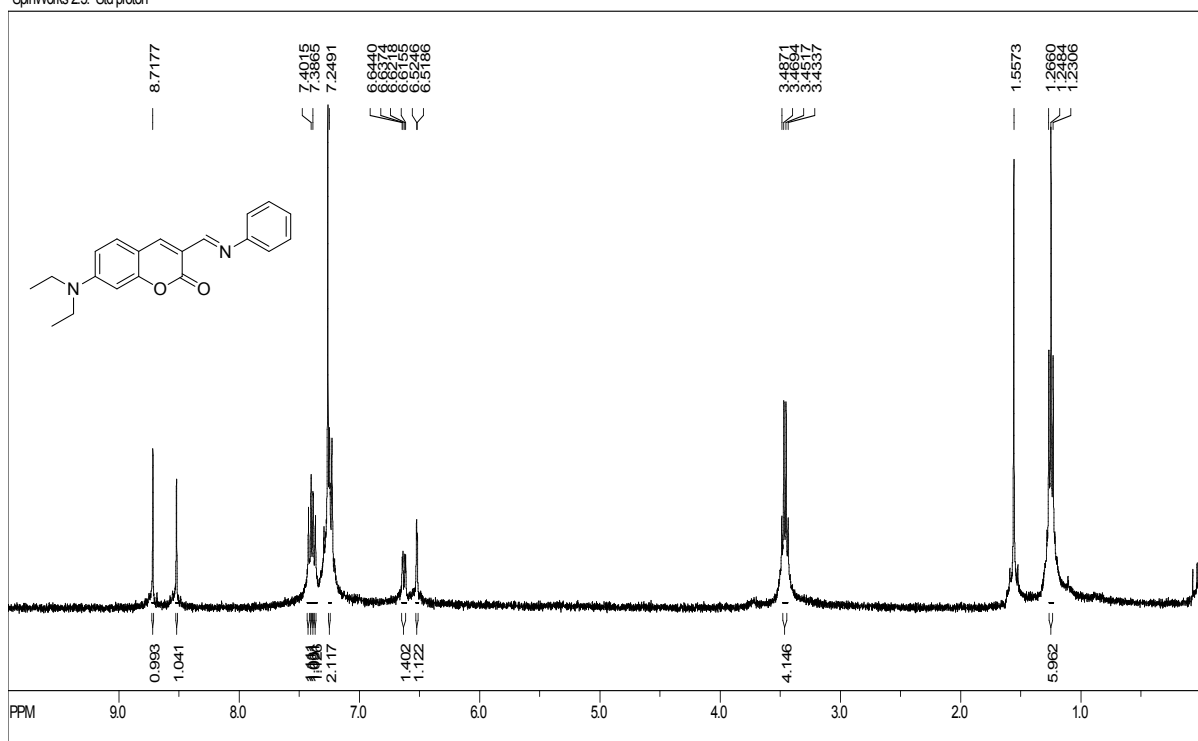


Fig. S4 <sup>1</sup>H NMR spectra (400 MHz) of **4** in CDCl<sub>3</sub>



SpinWorks 2.5: Std proton

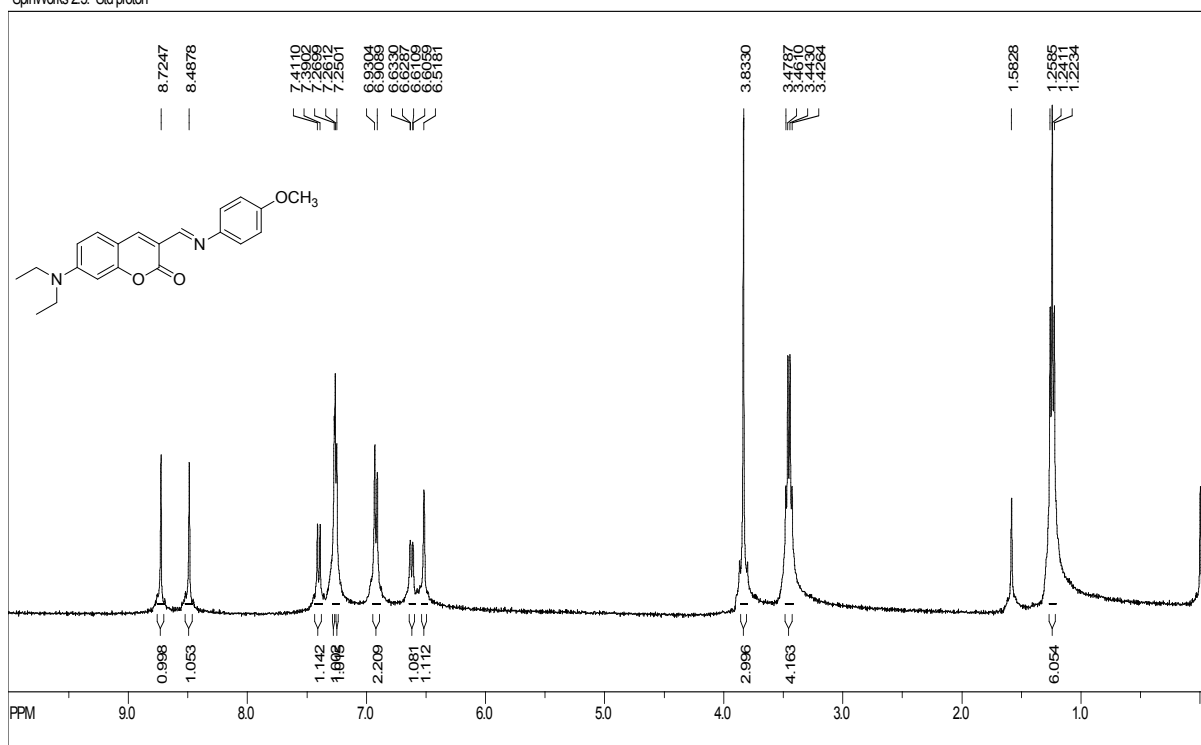


Fig. S5  $^1\text{H}$  NMR spectra (400 MHz) of **5** in  $\text{CDCl}_3$

SpinWorks 2.5: Std proton

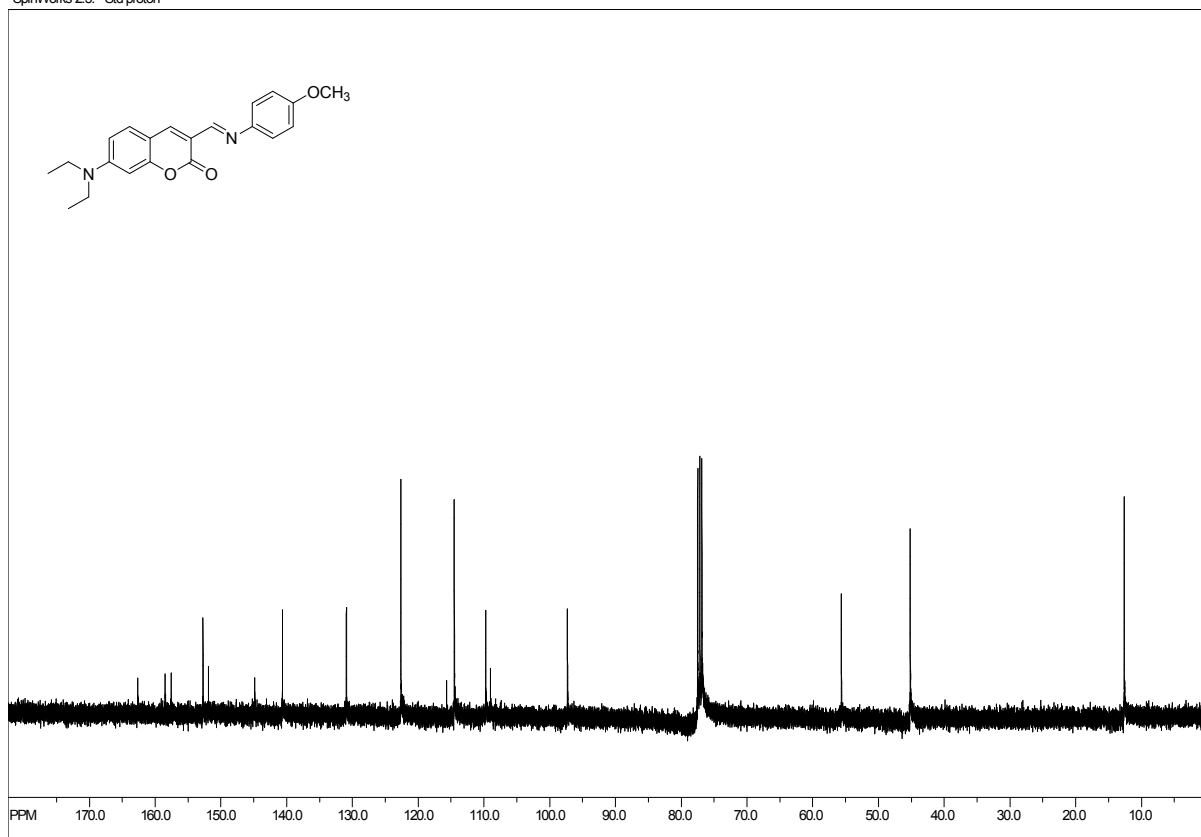


Fig. S6  $^{13}\text{C}$  NMR spectra (100 MHz) of **5** in  $\text{CDCl}_3$

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 BG Mode:None Segment 1 - Event 1

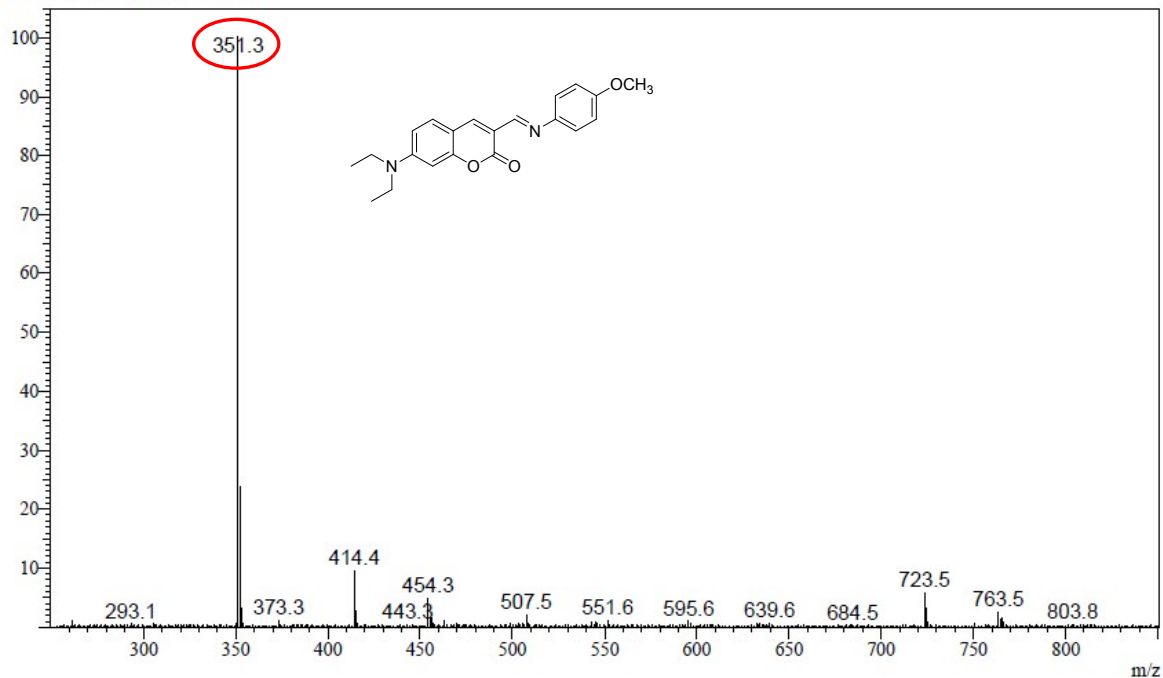


Fig. S7 ESI-MS spectra of 5

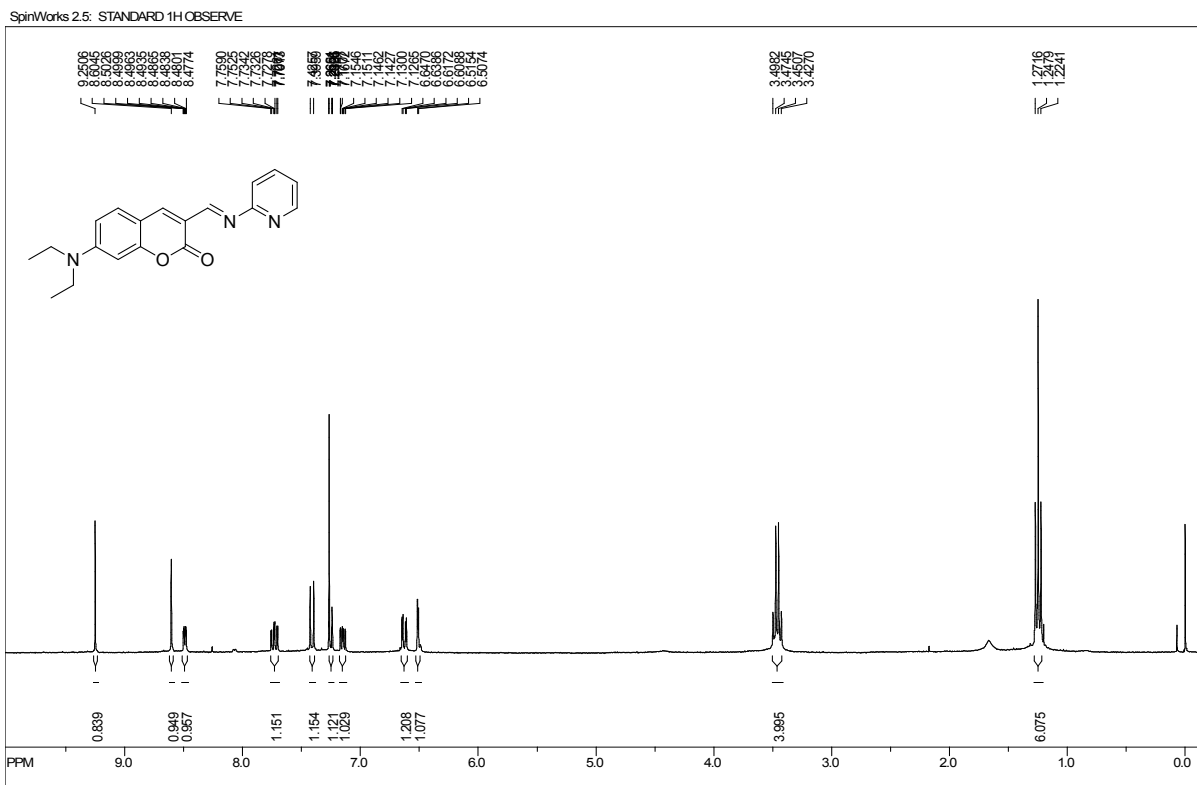


Fig. S8 <sup>1</sup>H NMR spectra (300 MHz) of 6 in CDCl<sub>3</sub>

SpinWorks 2.5: Std proton

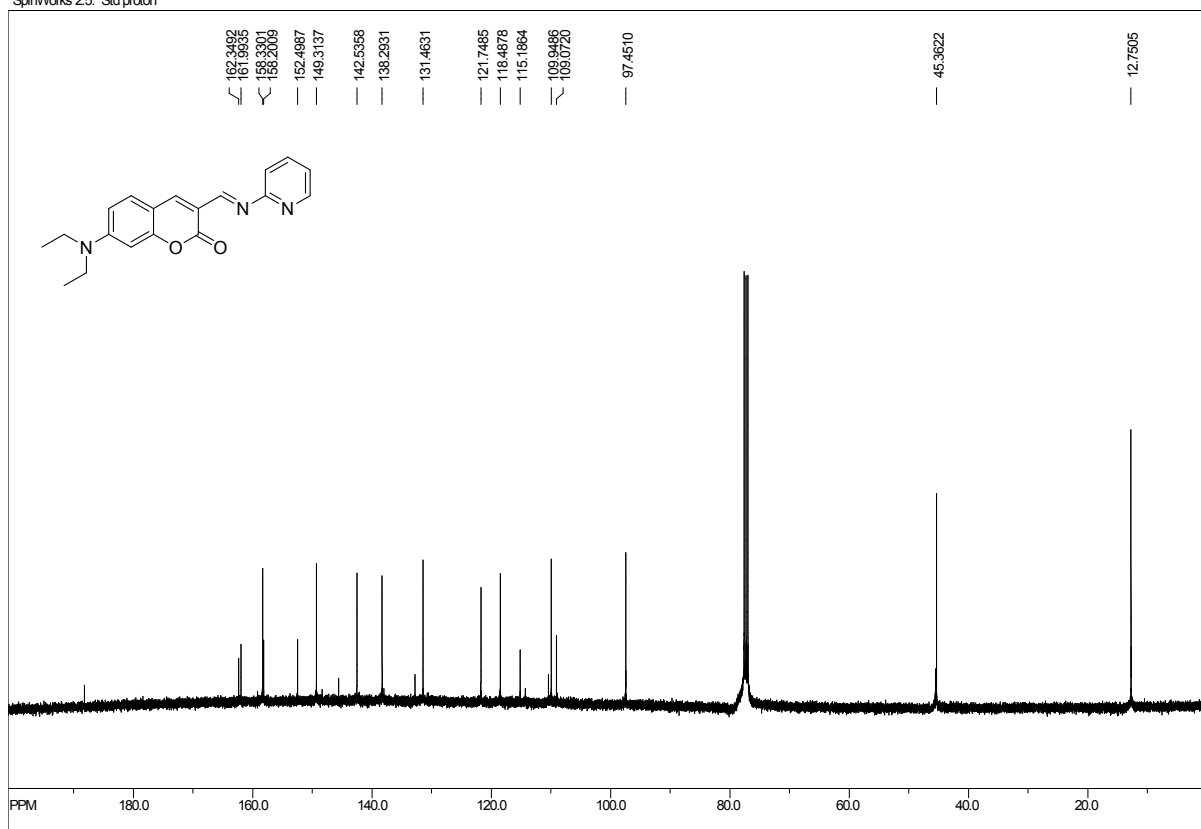


Fig. S9  $^{13}\text{C}$  NMR spectra (100 MHz) of **6** in  $\text{CDCl}_3$

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RawMode:Averaged 0.383-0.733(47-89) BasePeak:322.25(52913)

BG Mode:None Segment 1 - Event 1

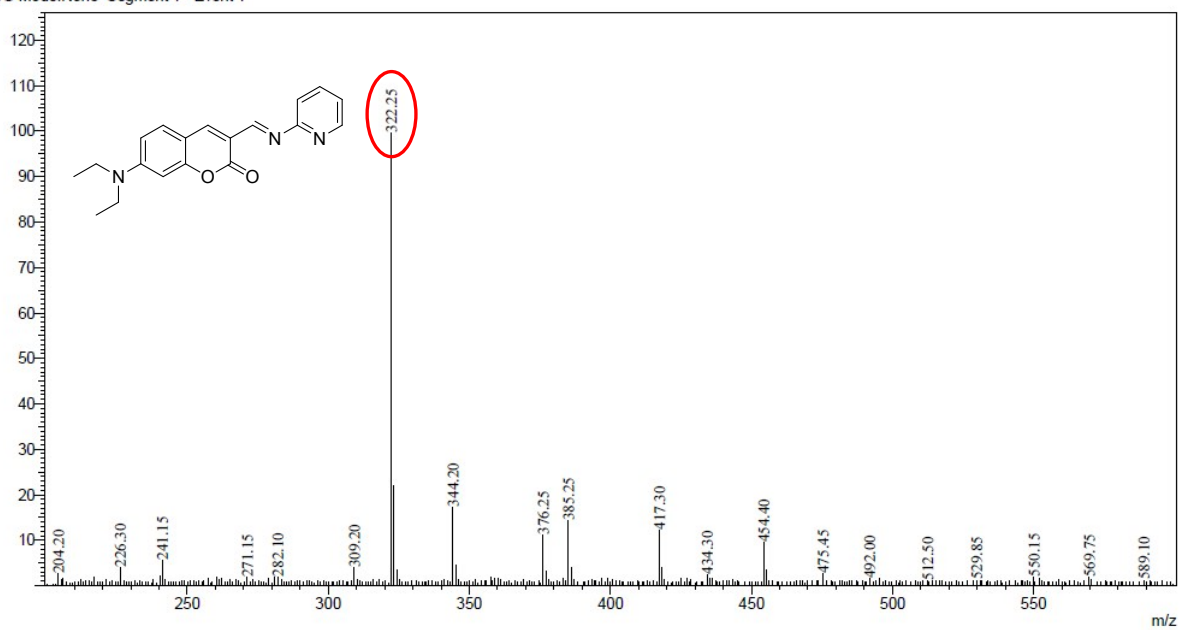
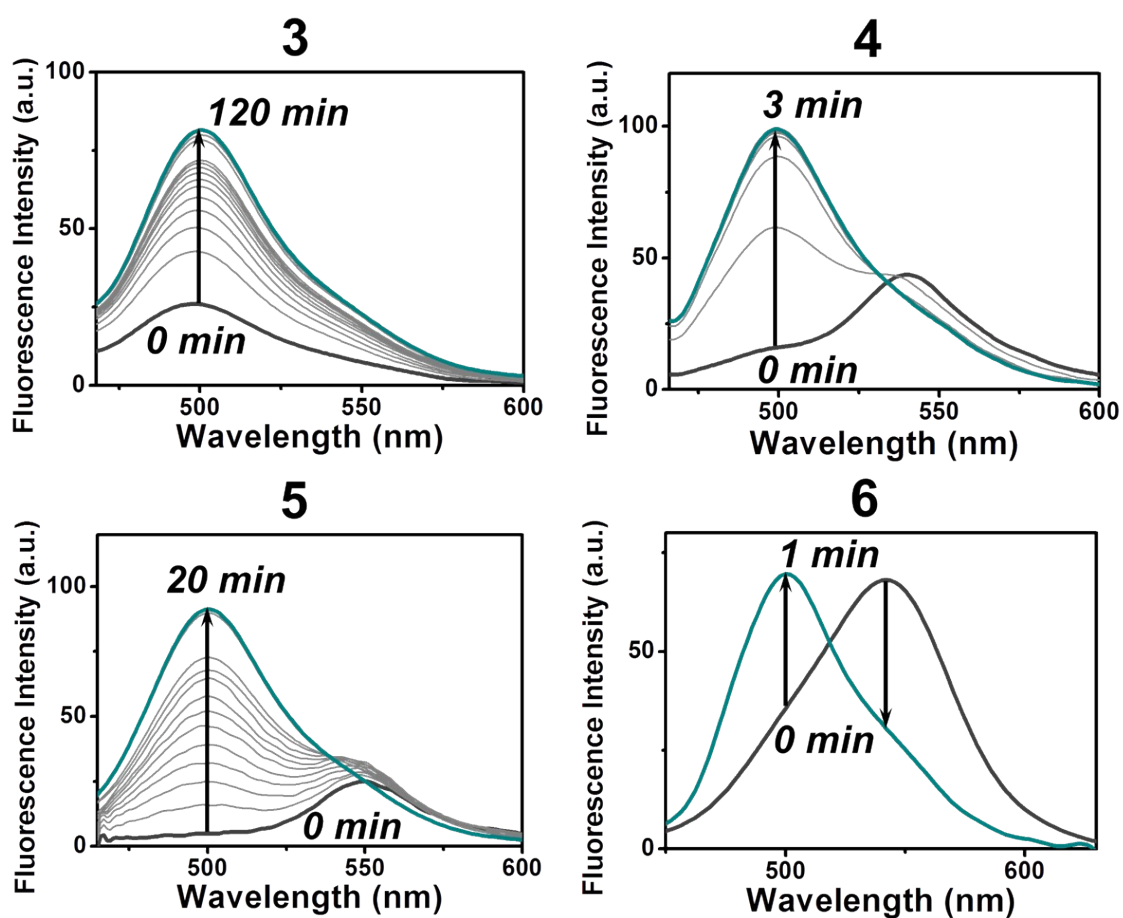


Fig. S10 ESI-MS spectra of **6**

No.	Absorbance (nm)	Path length (cm)	Concentration (mol·L <sup>-1</sup> )	$\epsilon$ (L·cm <sup>-1</sup> ·mol <sup>-1</sup> )
3	0.1598 (405)	1	0.00001	15980
4	0.3607 (448)	1	0.00001	36070
5	0.3563 (454)	1	0.00001	35630
6	0.4084 (447)	1	0.00001	40840

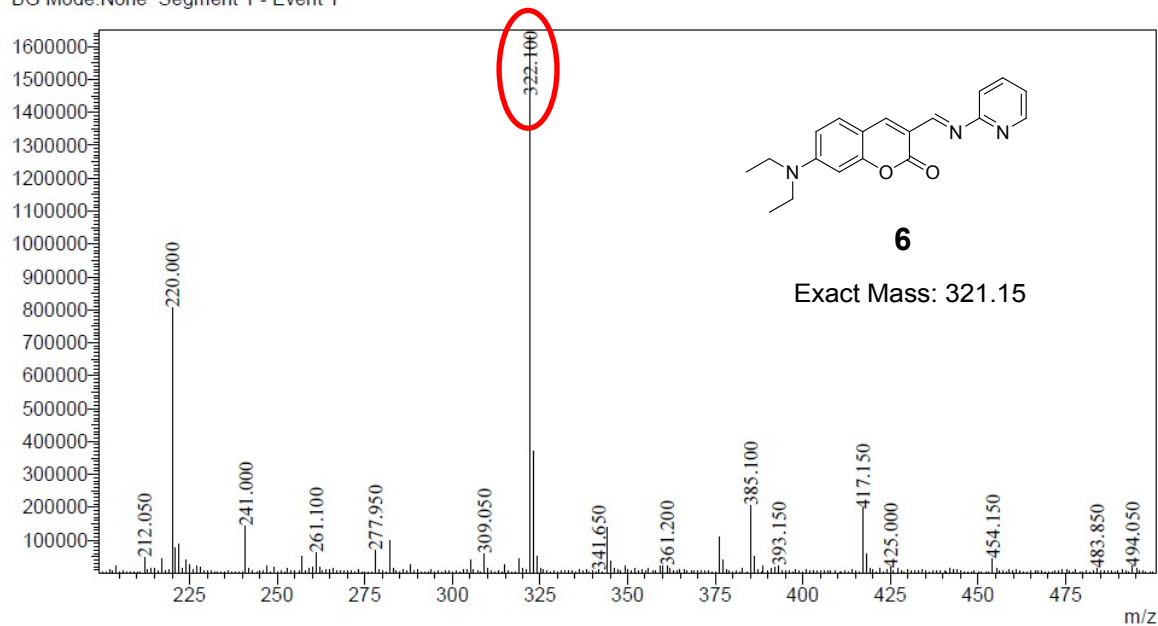
**Table S1.** Determination of the molar absorption coefficients of compounds 3-6 at their maximum absorbance wavelength.



**Fig. S11** Fluorescence emission spectra of probes (10  $\mu$ M) in water solution (DMSO 2%) at rt.  $\lambda_{\text{ex}}$  = 402 nm (3); 456 nm (4); 456 nm (5); 447 nm (6).

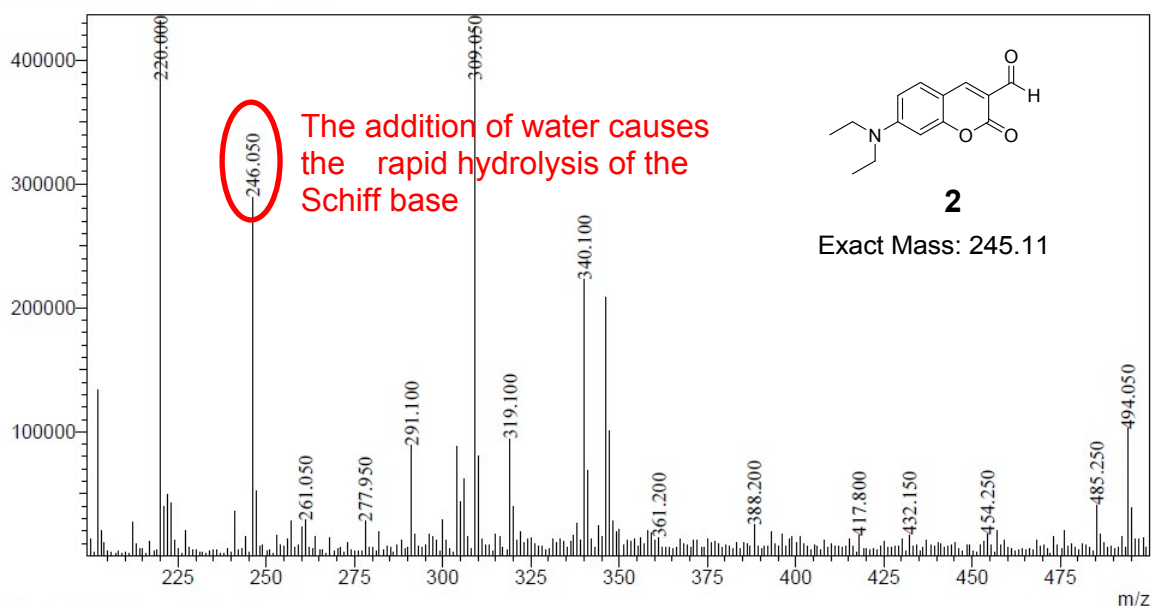
(a)

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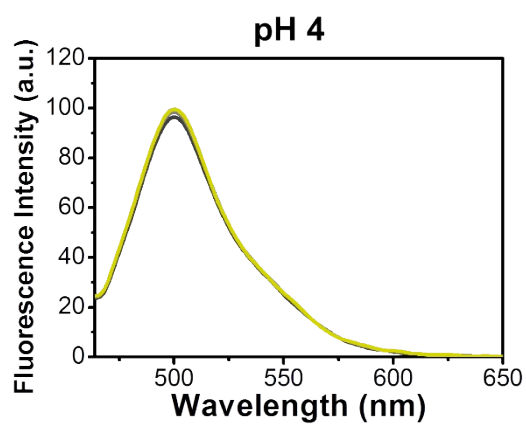
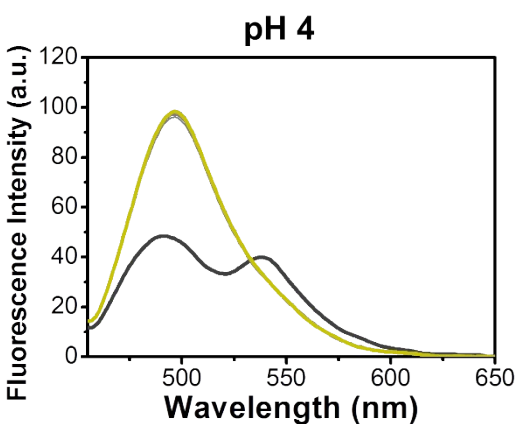
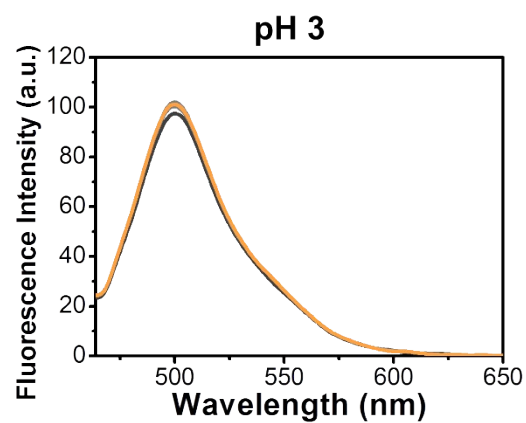
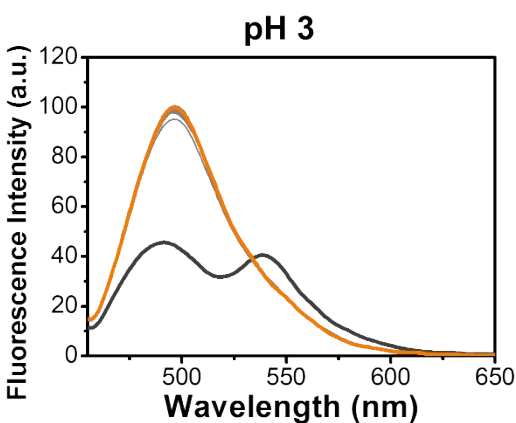
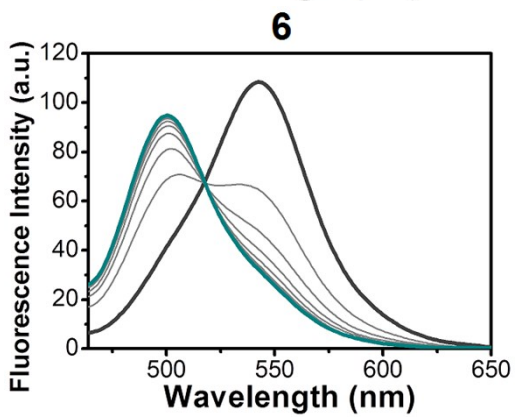
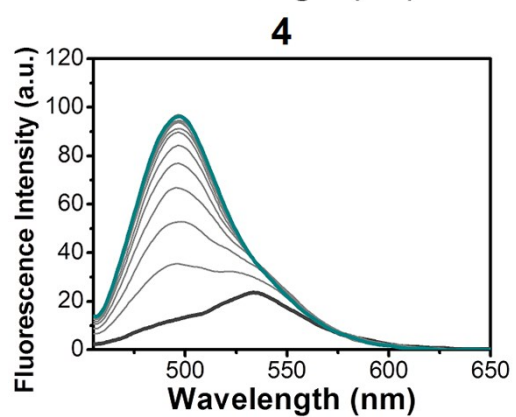
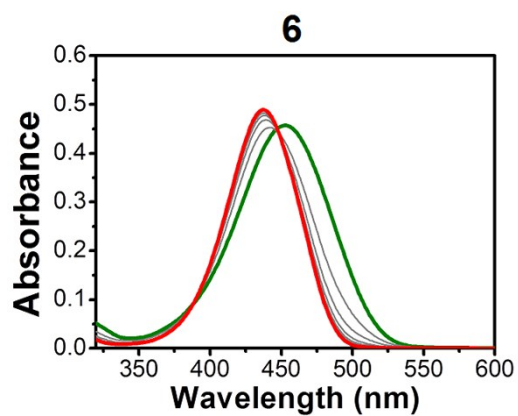
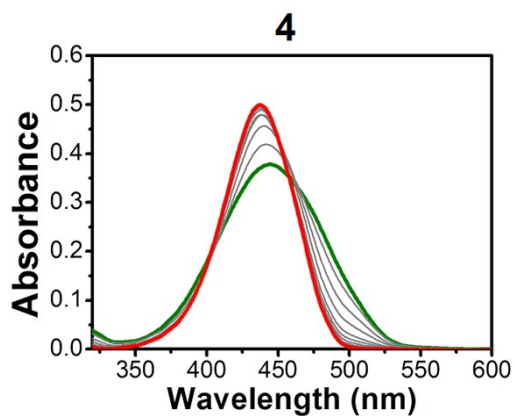


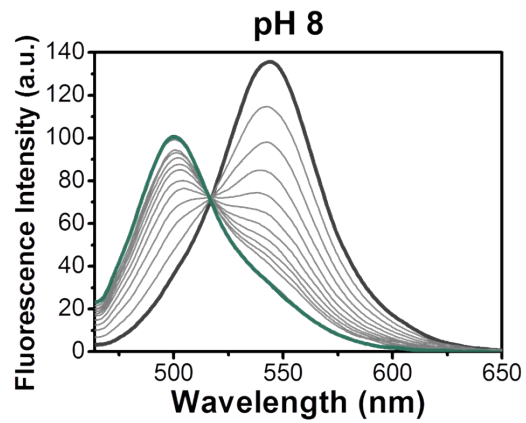
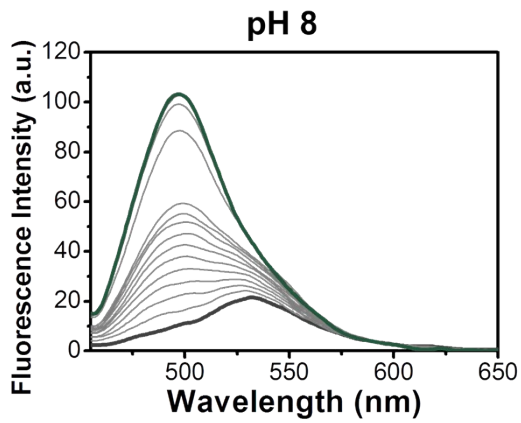
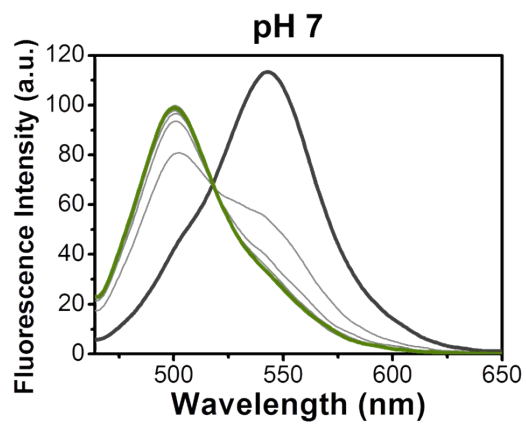
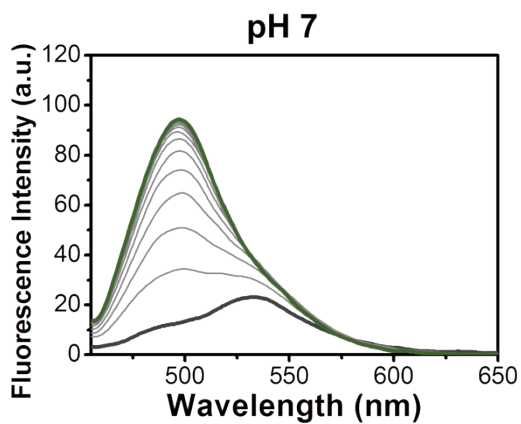
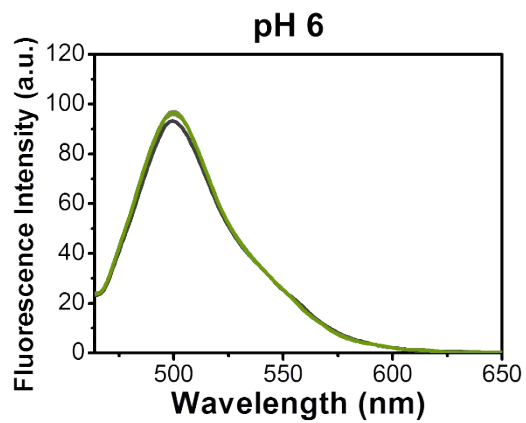
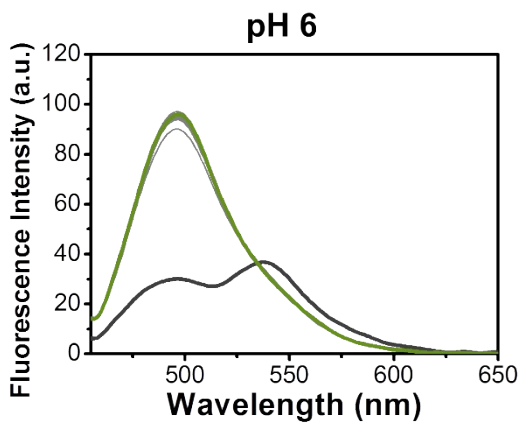
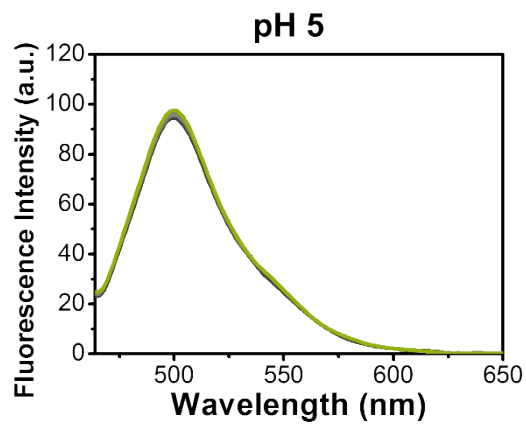
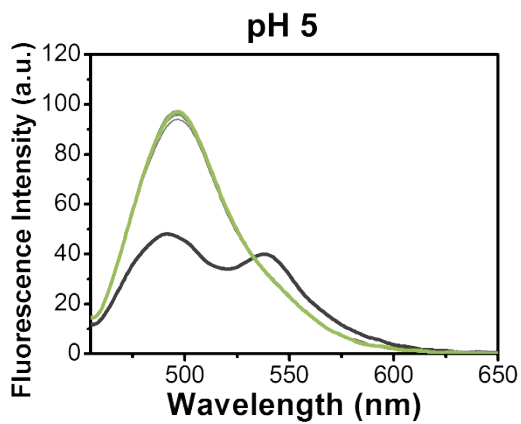
(b)

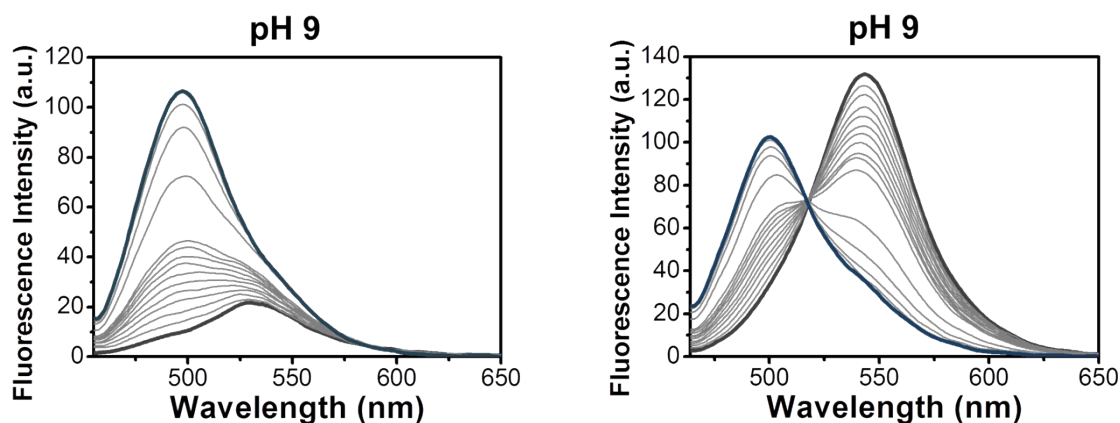
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BG Mode:None Segment 1 - Event 1



**Fig. S12** ESI-MS spectra demonstrating the hydrolysis of **6**. (a) ESI-MS spectrum of **6** prior to the addition of water and (b) ESI-MS spectrum after the addition of water to the sample.







**Fig. S13** UV/Vis spectra and fluorescence spectra of probes (**4** & **6**) (10  $\mu$ M) in various pH buffer solution at rt.  $\lambda_{em} = 499$  nm.

	<b>4</b>	<b>6</b>
formula	$C_{20}H_{20}N_2O_2$	$C_{19}H_{19}N_3O_2$
Mr	320.38	321.37
T (K)	296(2)	296(2)
crystal system	monoclinic	monoclinic
space group	P2(1)/n	P2(1)/c
a ( $\text{\AA}$ )	9.7970(2)	7.9685(9)
b ( $\text{\AA}$ )	9.3477(2)	10.0372(13)
c ( $\text{\AA}$ )	19.3037(5)	20.628(3)
$\alpha$ ( $^\circ$ )	90	90
$\beta$ ( $^\circ$ )	103.3850(10)	91.778(8)
$\gamma$ ( $^\circ$ )	90	90
V ( $\text{\AA}^3$ )	1719.80(7)	1649.1(4)
Z	4	4
$\rho_{calc}$ ( $\text{g cm}^{-3}$ )	1.237	1.294
$\mu$ ( $\text{mm}^{-1}$ )	0.081	0.086
F(000)	680	680
total reflections	4279	4128
GOF	1.043	0.886
R1 <sup>[a]</sup> ( $I \geq 2\sigma(I)$ )	0.0507	0.0658
wR2 <sup>[b]</sup> ( $I \geq 2\sigma(I)$ )	0.1422	0.1540

<sup>a</sup>R1 =  $\sum ||F_o| - |F_c|| / \sum |F_c|$ , <sup>b</sup>wR2 =  $[\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2]^{1/2}$ .

**Table S2.** Crystal data and structure refinement for **4** & **6**



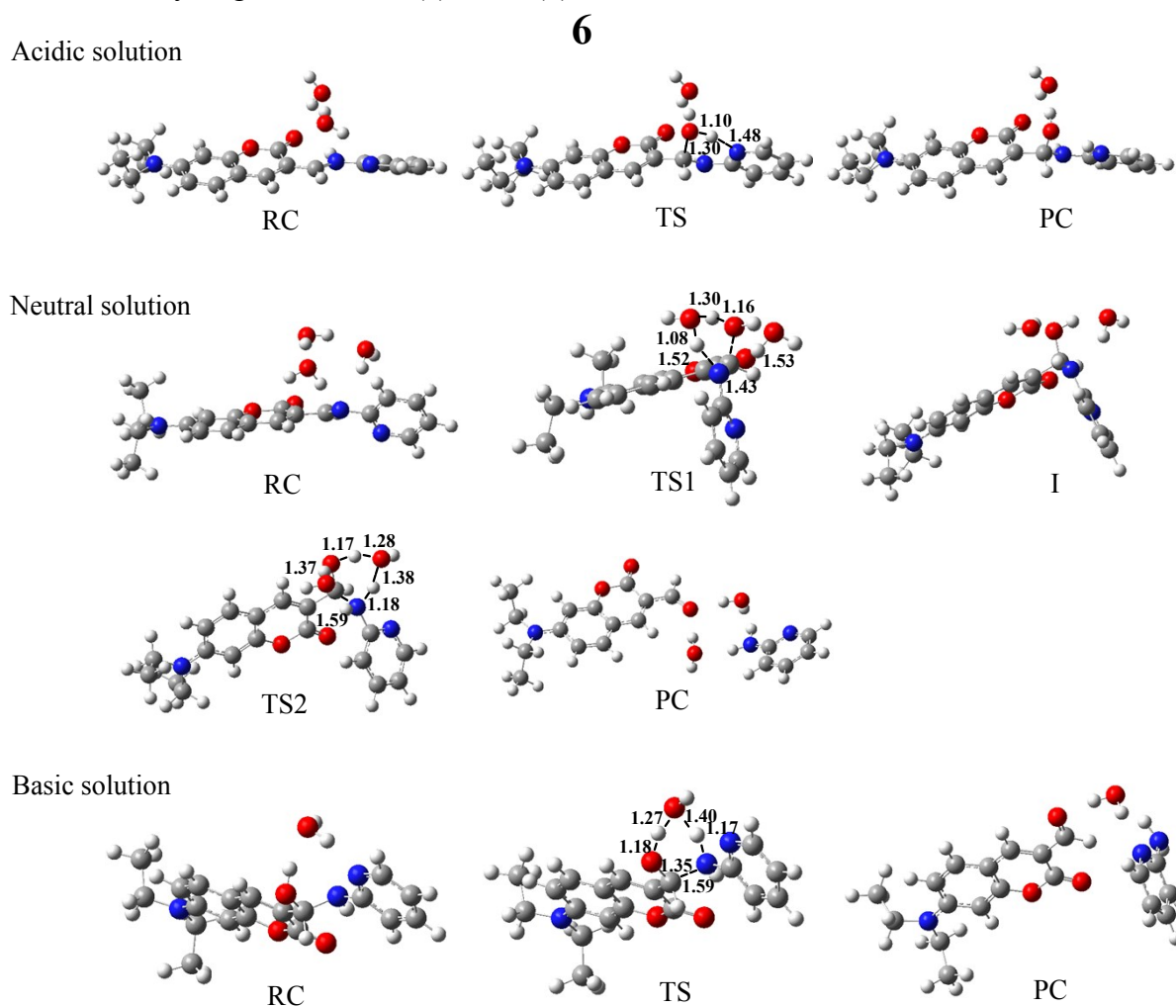
(a)

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
C(7)-H(7)...C(3)	0.93	2.78	2.963(3)	92.0

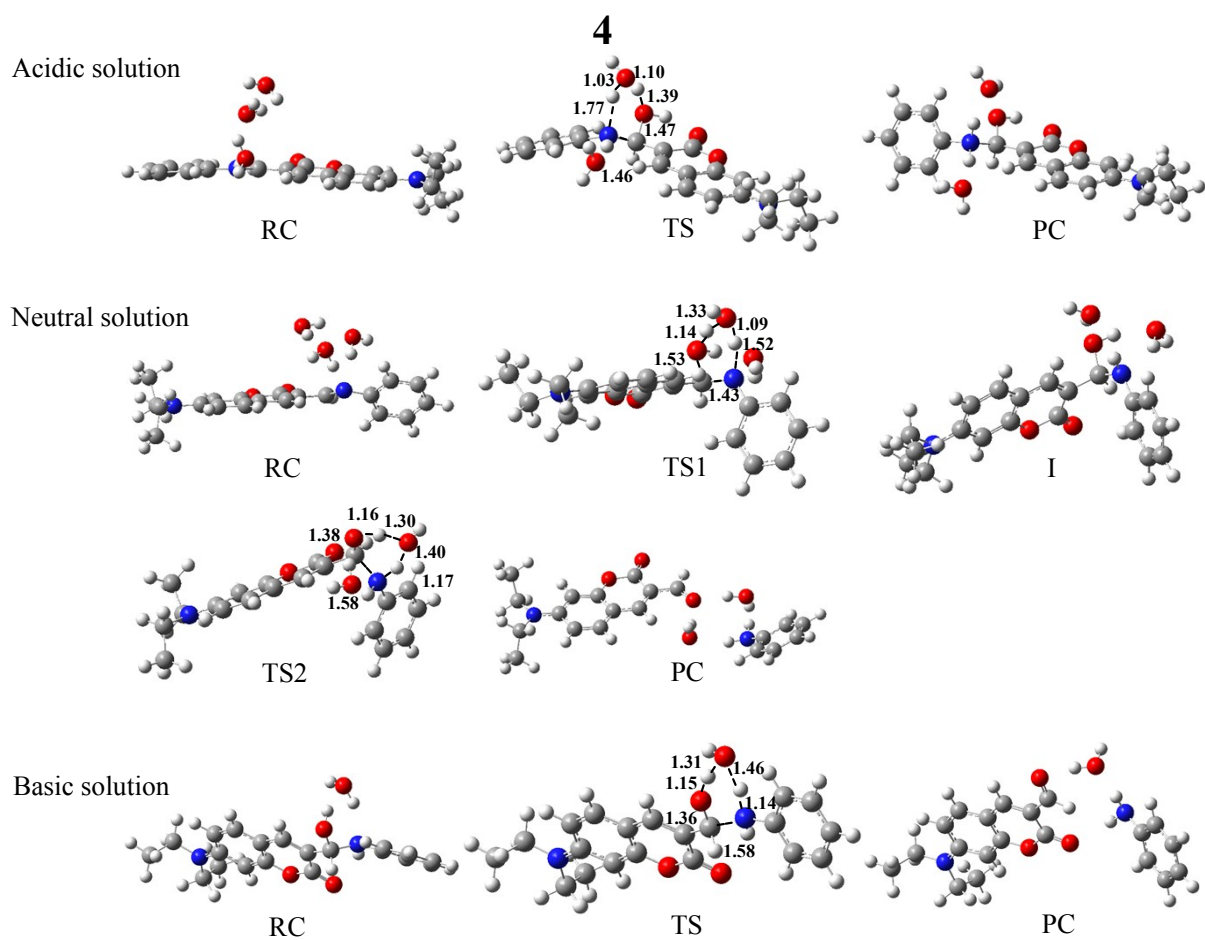
(b)

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
C(6)-H(11)...N(1)	0.93	2.34	2.712(3)	103.2

**Table S3.** Hydrogen bonds for (a) **4** and (b) **6**.



**Fig. S14** Structures of reactant complexes, transition states, and production complexes for the hydrolysis of **6** and **4** in acidic, neutral, and basic solution.



**Fig. S14** (Continued)