

## Electronic Supporting Information

### Ratiometric spiropyran-based fluorescent pH probe

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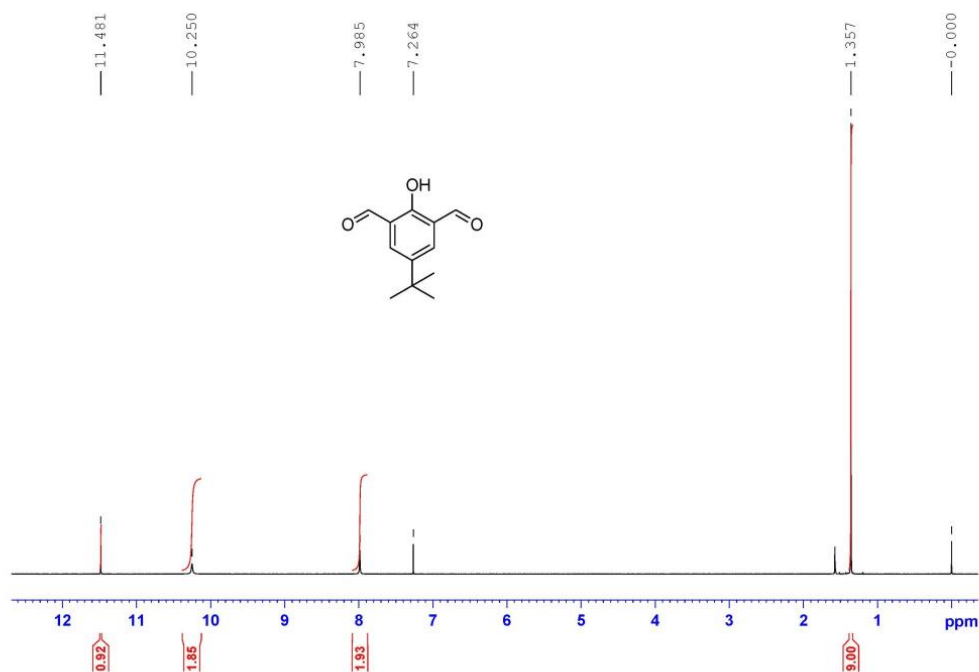
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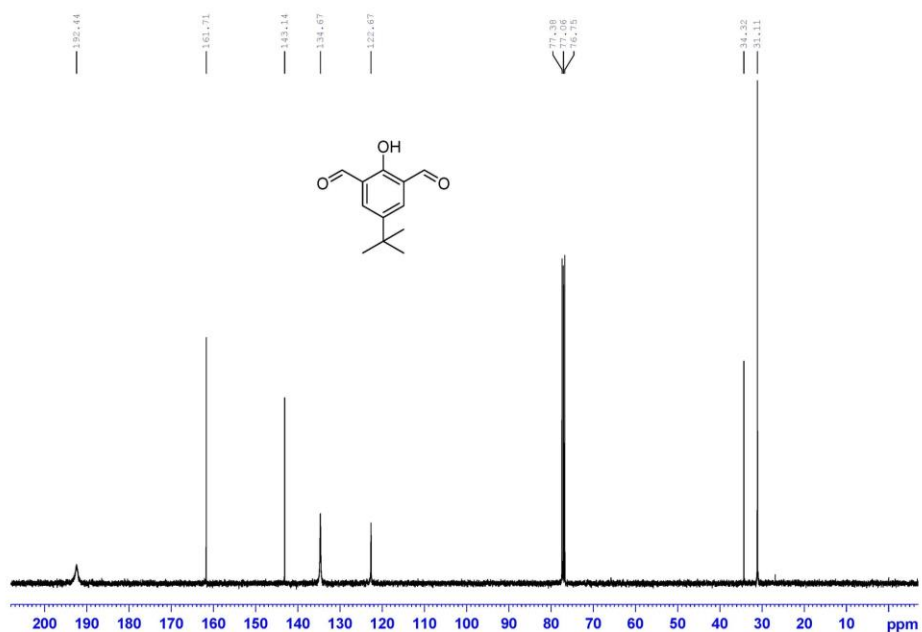
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**Fig. S1** <sup>1</sup>H NMR spectrum of **2**.



**Fig. S2** <sup>13</sup>C NMR spectrum of **2**.

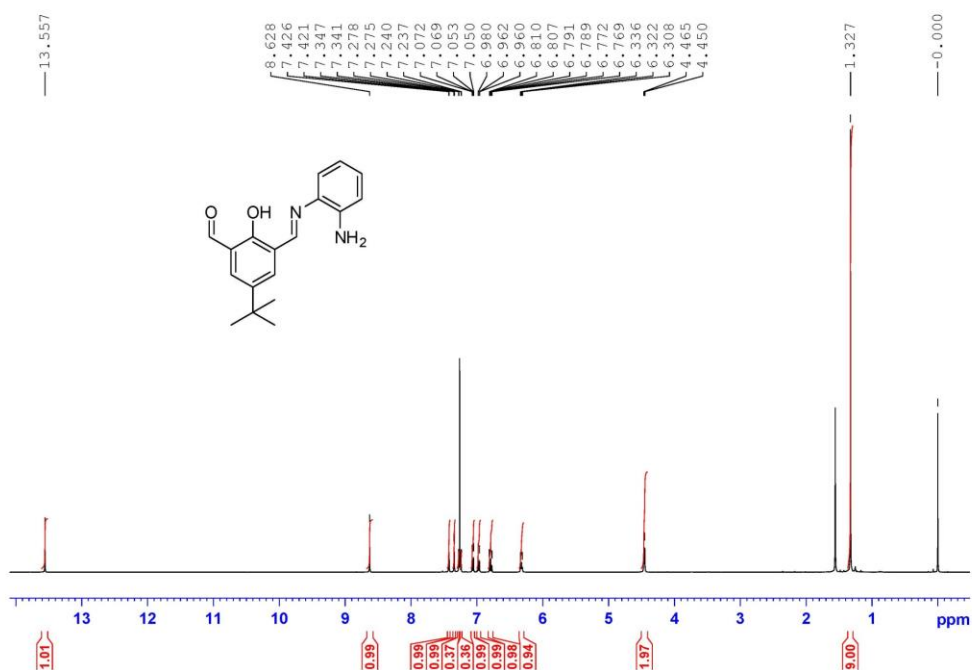


Fig. S3  $^1\text{H}$  NMR spectrum of 3.

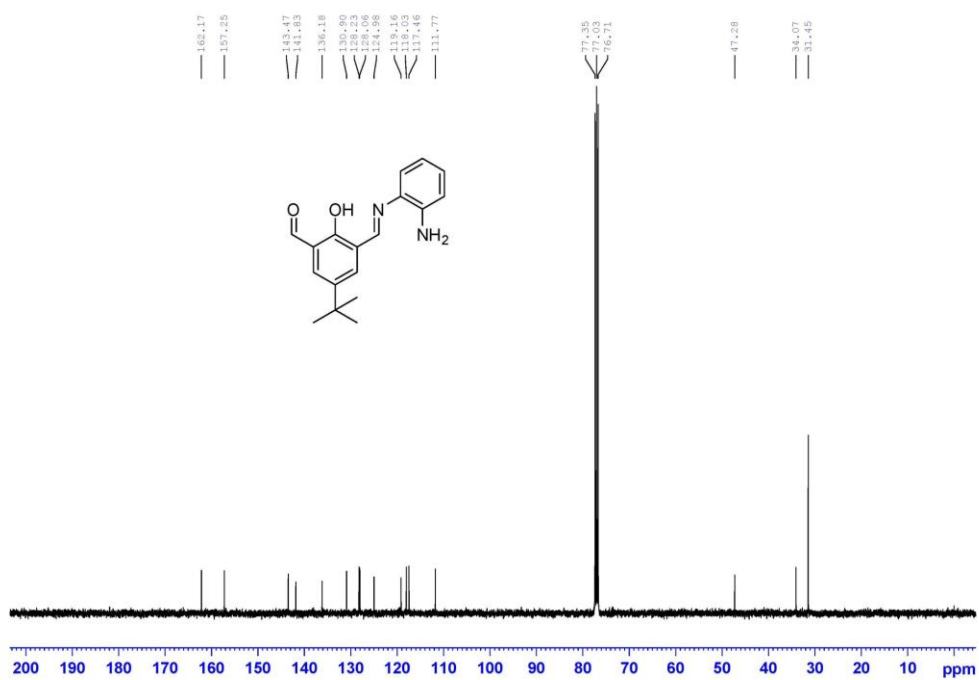


Fig. S4  $^{13}\text{C}$  NMR spectrum of 3.

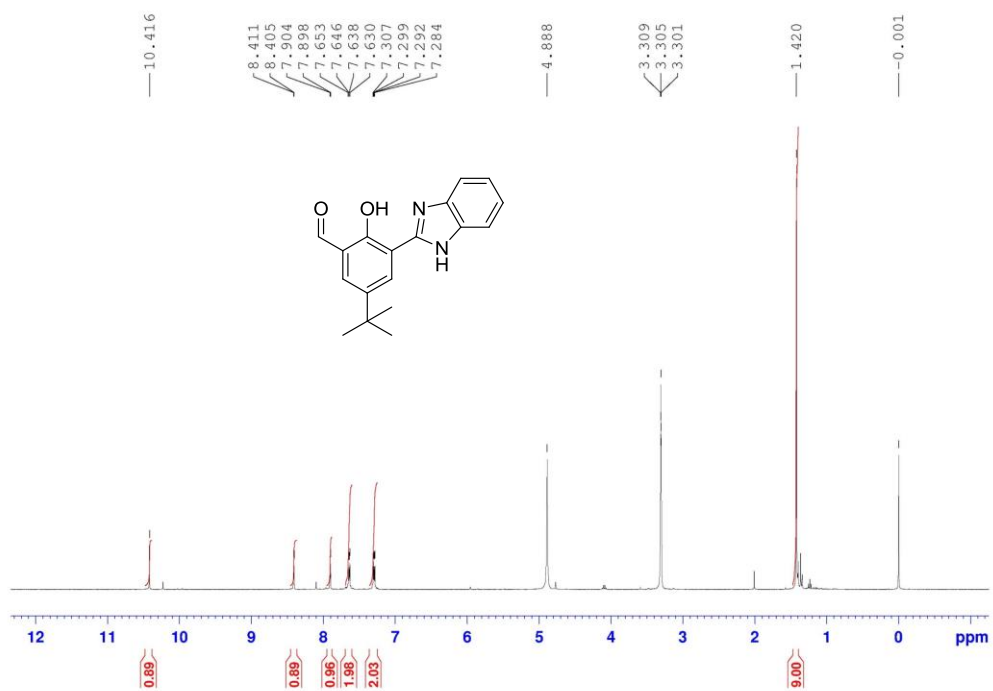


Fig. S5  $^1\text{H}$  NMR spectrum of 4.

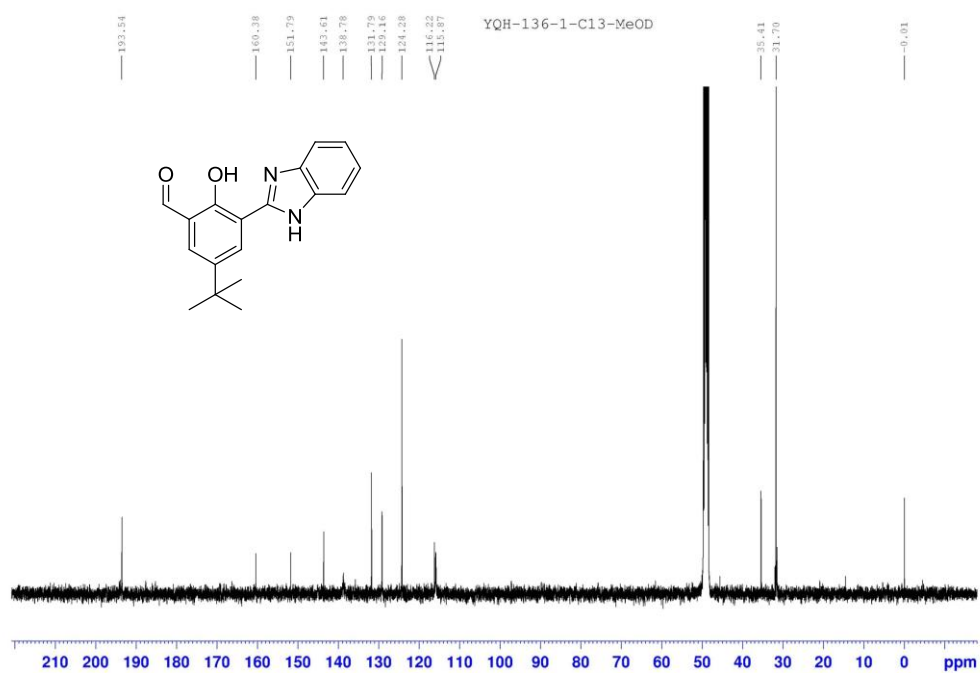


Fig. S6  $^{13}\text{C}$  NMR spectrum of 4.

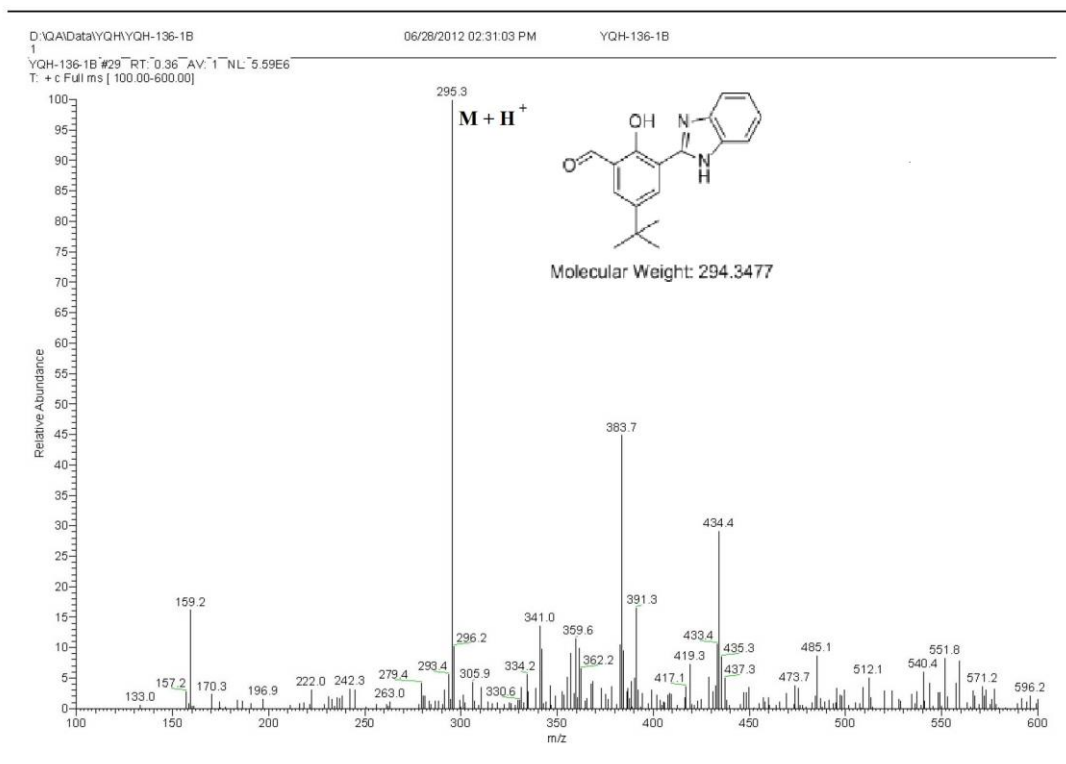


Fig. S7 ESI-MS spectrum of 4.

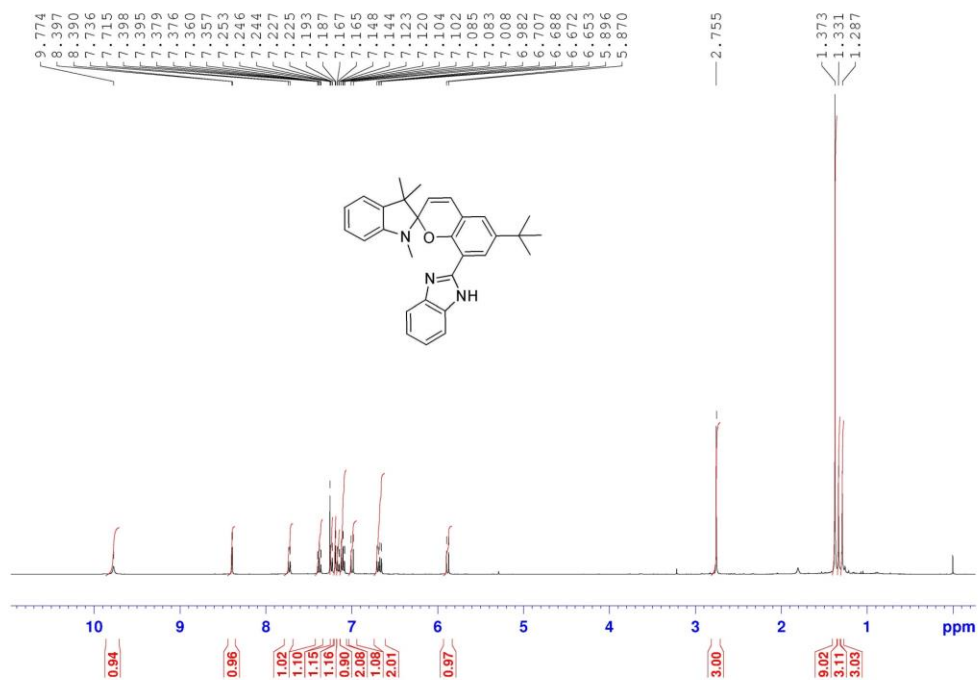


Fig. S8  $^1H$  NMR spectrum of probe 1.

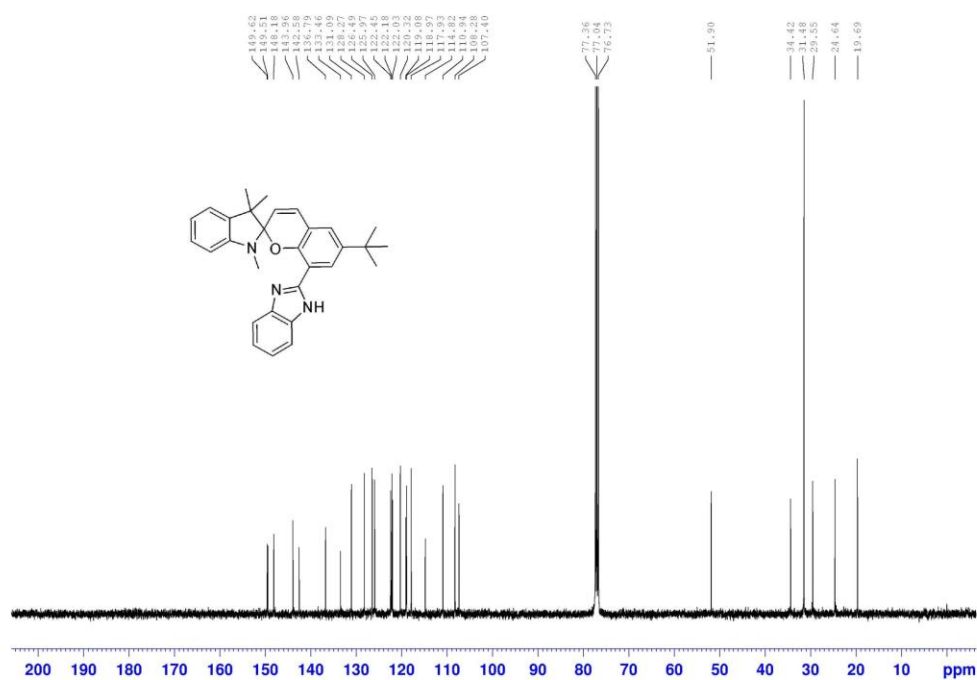


Fig. S9 <sup>13</sup>C NMR spectrum of probe 1.

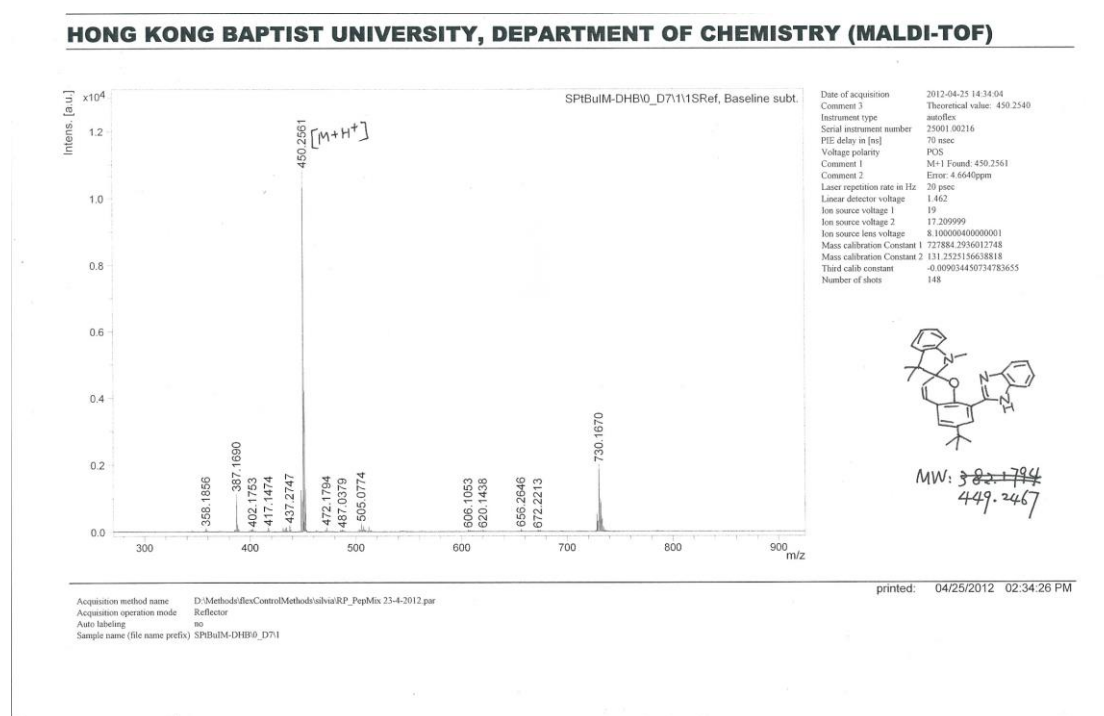
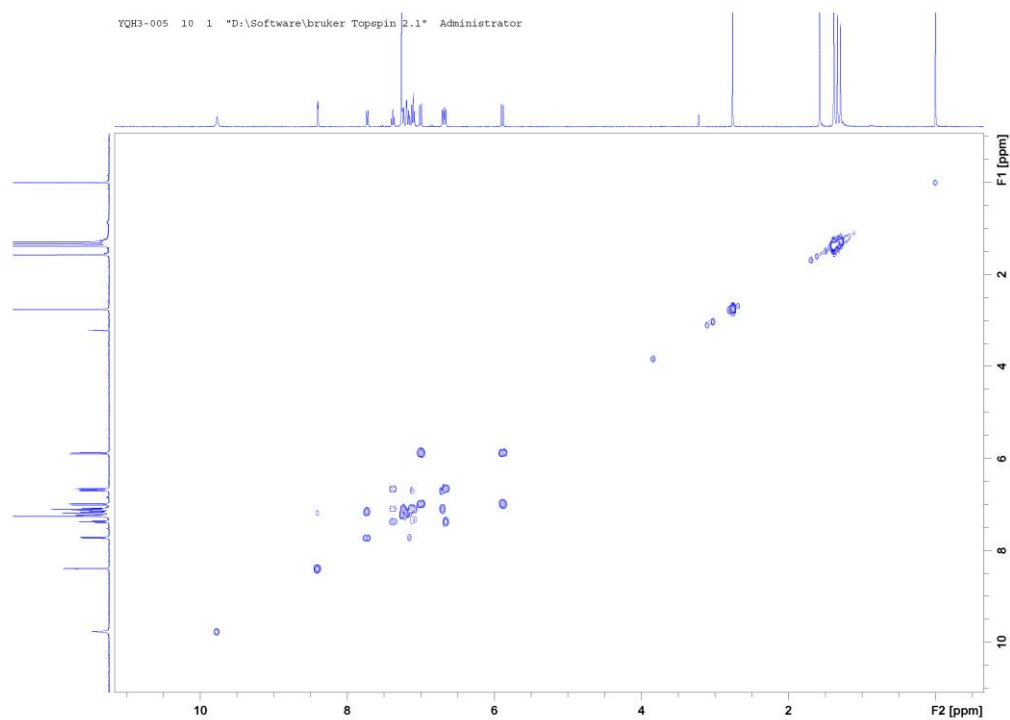
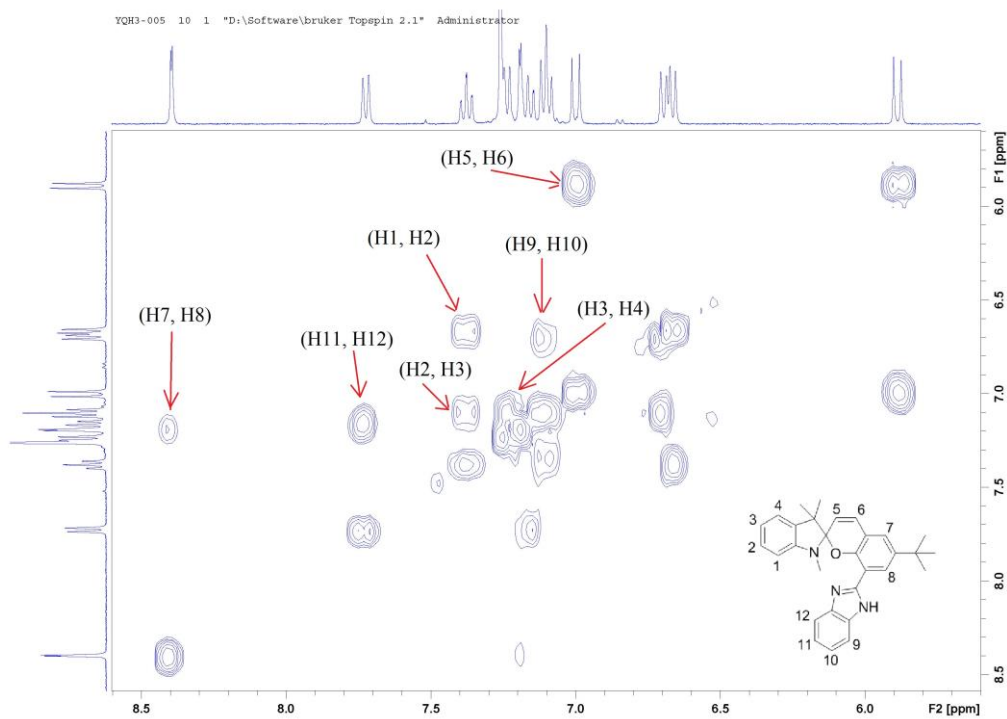


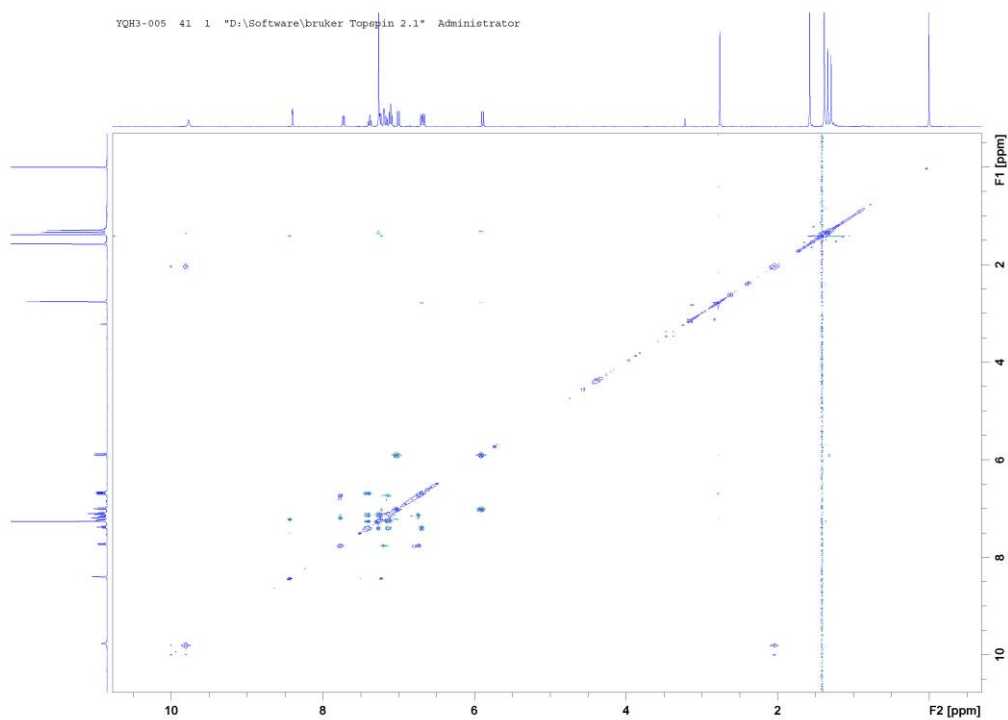
Fig. S10 MALDI-TOF HRMS spectrum of probe 1.



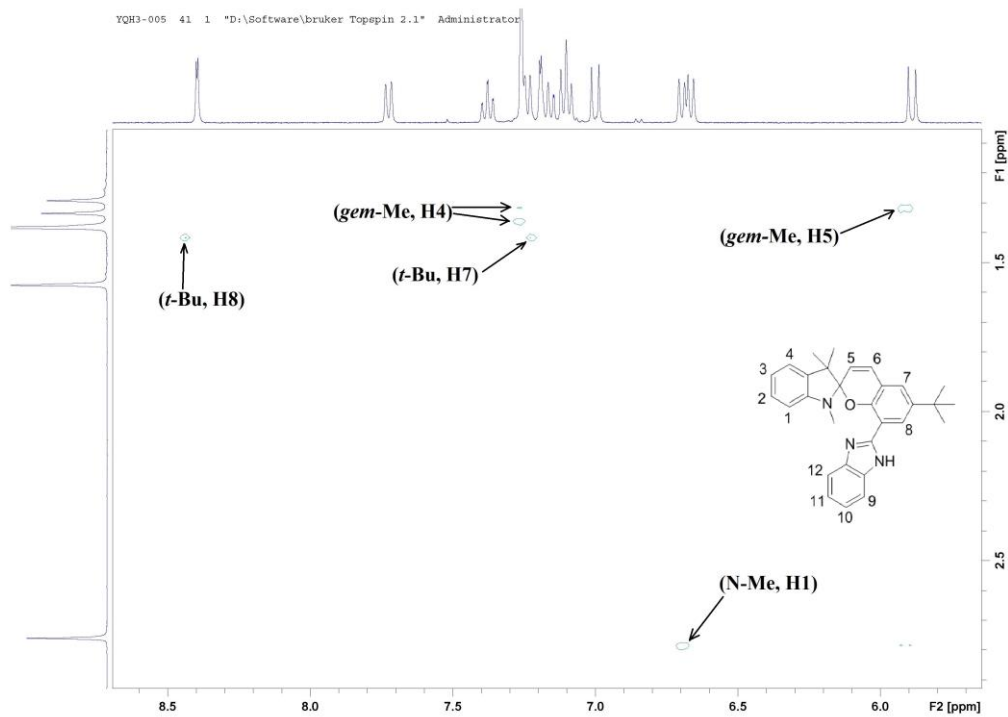
**Fig. S11** H, H-COSY spectrum of probe **1**.



**Fig. S12** Partial H, H-COSY spectrum of probe **1**.

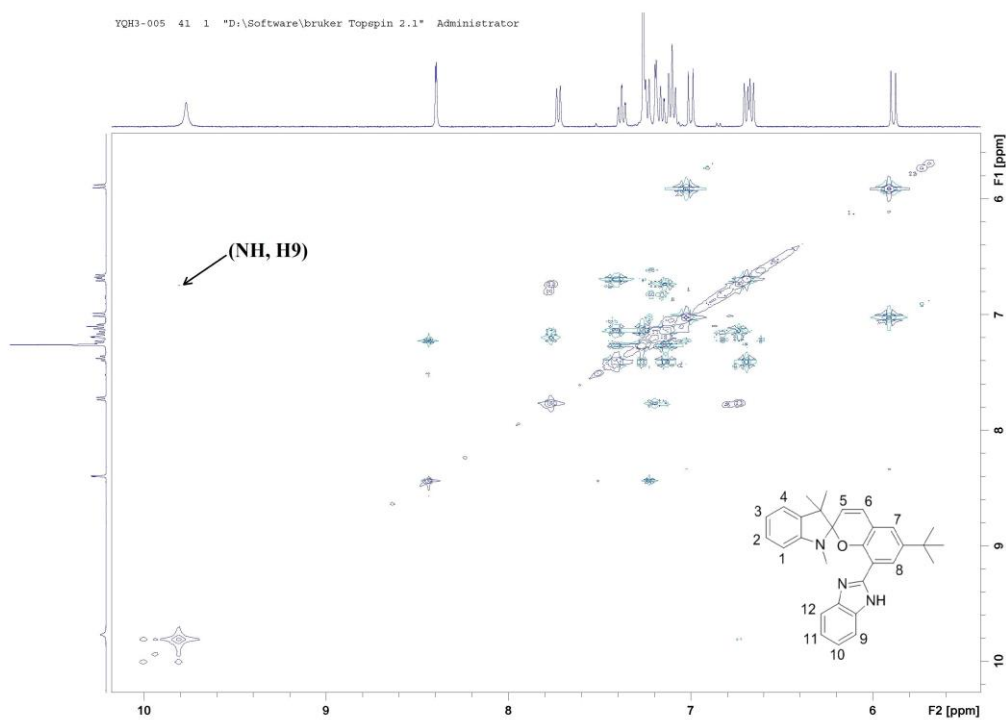


**Fig. S13** 2D-NOESY spectrum of probe 1.

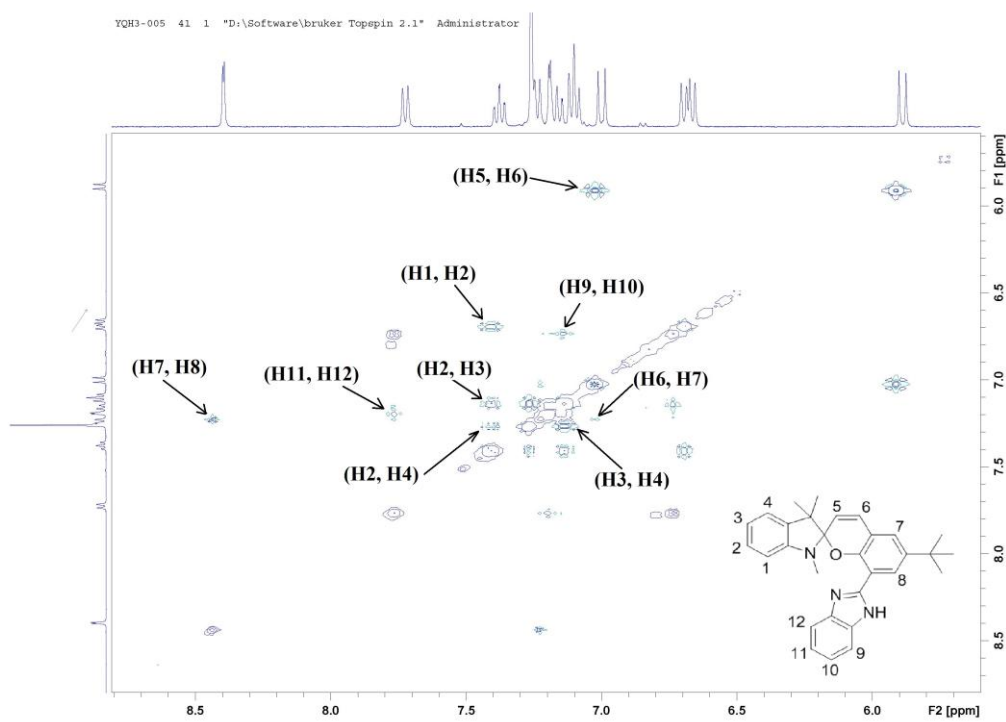


**Fig. S14** Partial 2D-NOESY spectrum of probe 1.





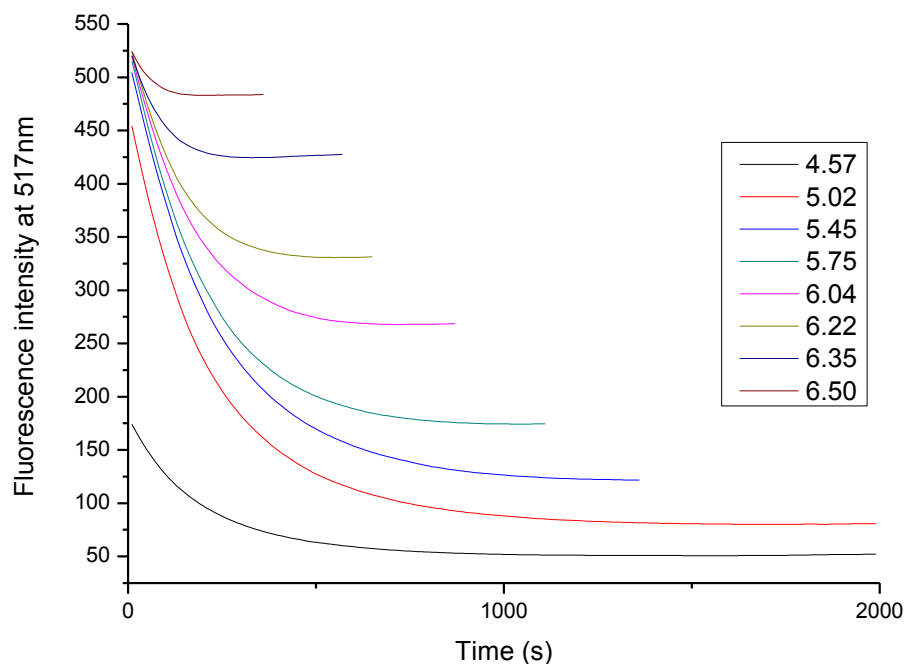
**Fig. S15** Partial 2D-NOESY spectrum of probe 1.



**Fig. S16** Partial 2D-NOESY spectrum of probe 1.



**Fig. S17** Photo of probe **1** (50  $\mu\text{M}$ ) in ACN-phosphate buffer (20 mM, 1:1, v/v) at various pH.



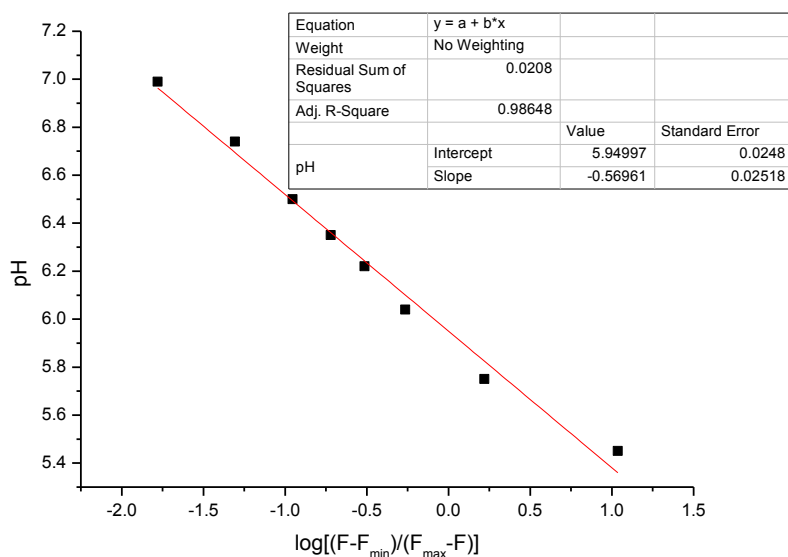
**Fig. S18** Time course of fluorescence intensity at 517 nm of **1** (50  $\mu\text{M}$ ) in ACN-phosphate buffer (20 mM, 1:1, v/v) at various pH.

## Determination of $pK_a$ from fluorimetric titration

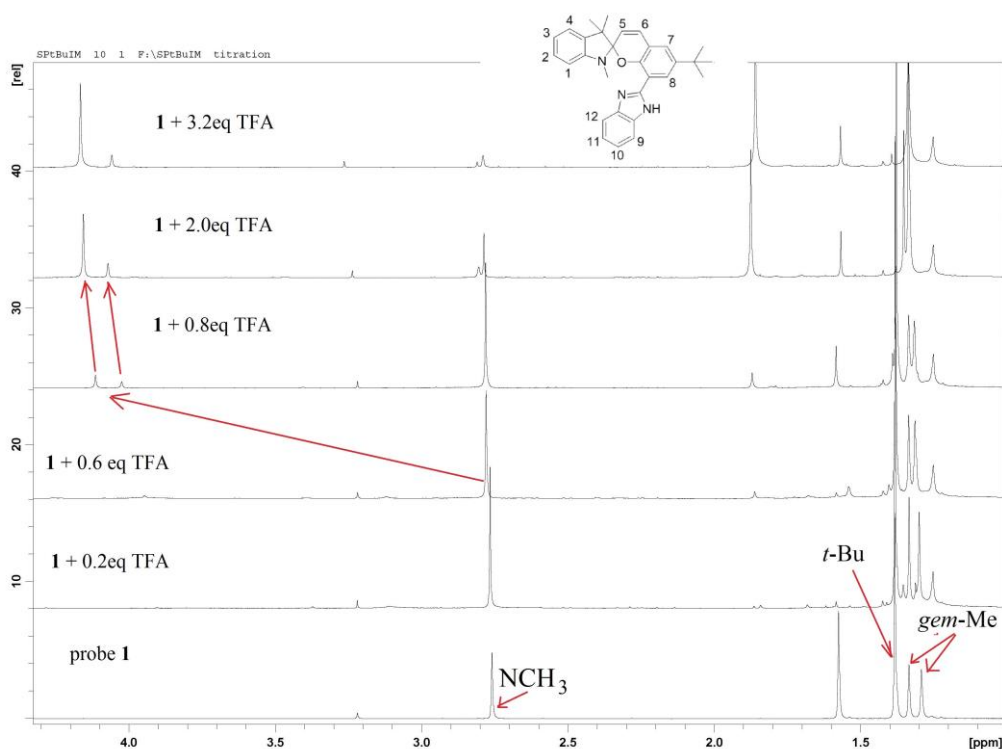
The constants  $K_a$  of probe **1** was determined in aqueous buffered solution by fluorimetric titration as a function of pH using the fluorescence emission spectra. The expression of the steady-state fluorescence signal  $F$  as a function of the  $\text{H}^+$  concentration has been derived for the case of a  $n$ :1 complex between  $\text{H}^+$  and probe **1**,<sup>1-4</sup>

$$\text{equation 1: } F = \frac{F_{\max}[\text{H}^+]^n + F_{\min}K_a}{K_a + [\text{H}^+]^n} \quad (1)$$

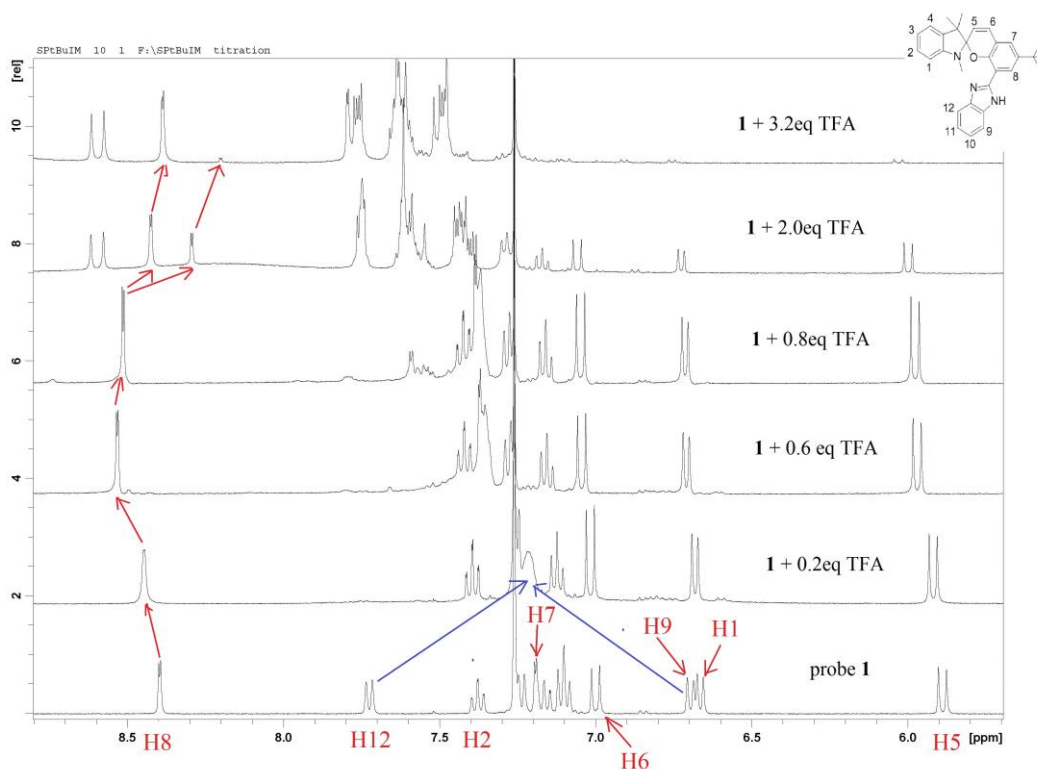
In our experiments, probe **1** showed a ratiometric response to  $\text{H}^+$  concentration in the range 7.0 to 5.5, thus, the fluorescence signals  $F_{\min}$  and  $F_{\max}$  were considered as the ratio of  $I_{690 \text{ nm}}/I_{517 \text{ nm}}$  at minimal and maximal  $\text{H}^+$  concentration, respectively, and  $n$  was considered as 1 (the stoichiometry of  $\text{H}^+$  for spiropyran ring opening). Then we get a  $pK_a$  of 5.9.



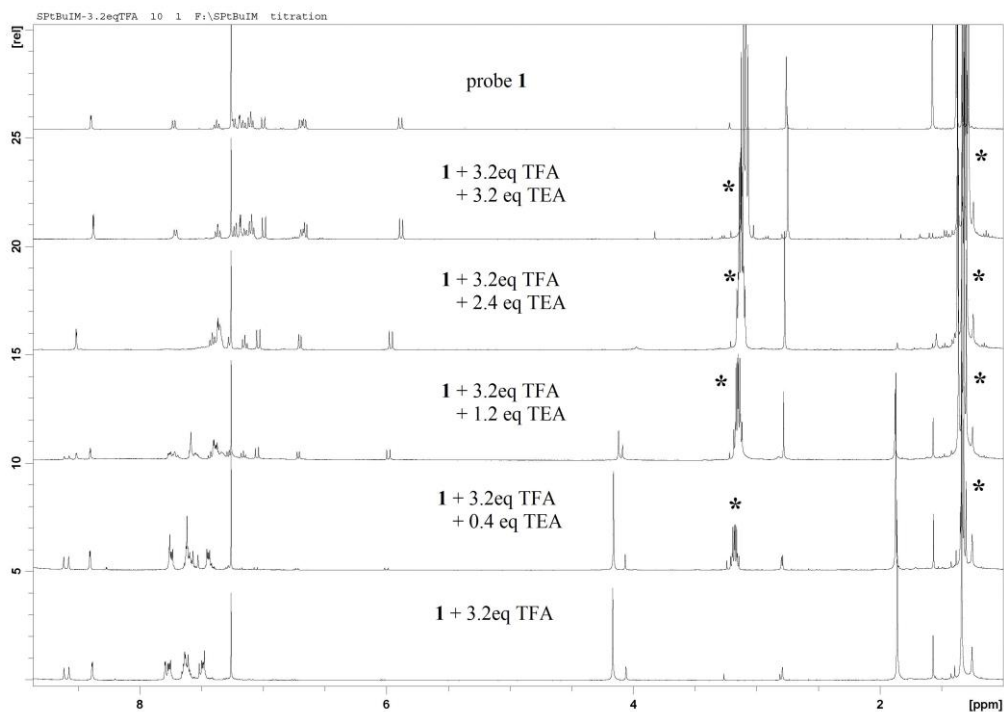
**Fig. S19** The calculation of  $pK_a$  based on ratiometric response of probe **1** to  $H^+$  concentration in pH range from 7.0 to 5.5.



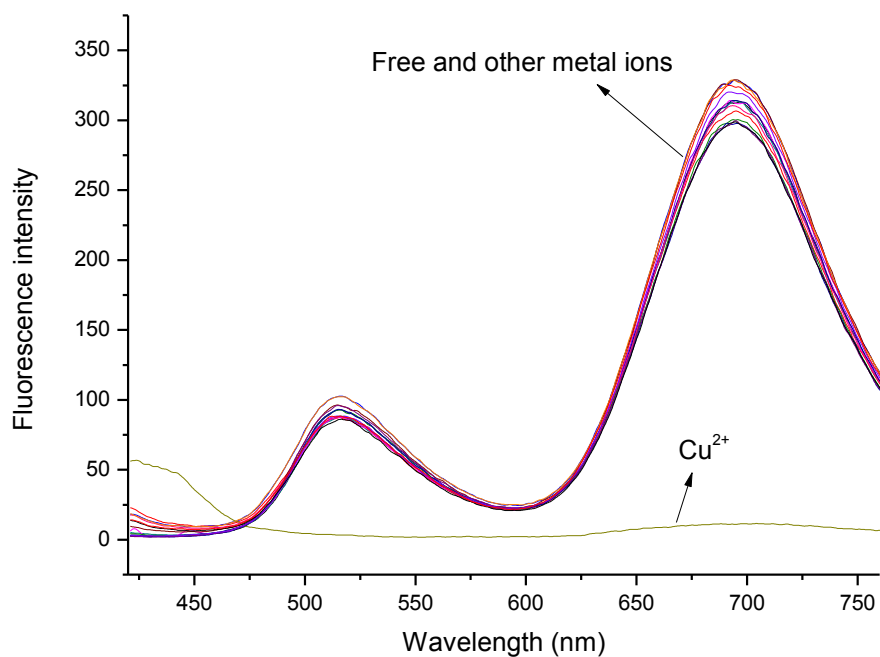
**Fig. S20** Partial  $^1H$  NMR titration spectra (1.0 – 4.4 ppm) of probe **1** with stepwise addition of TFA (0 – 3.2 equiv).



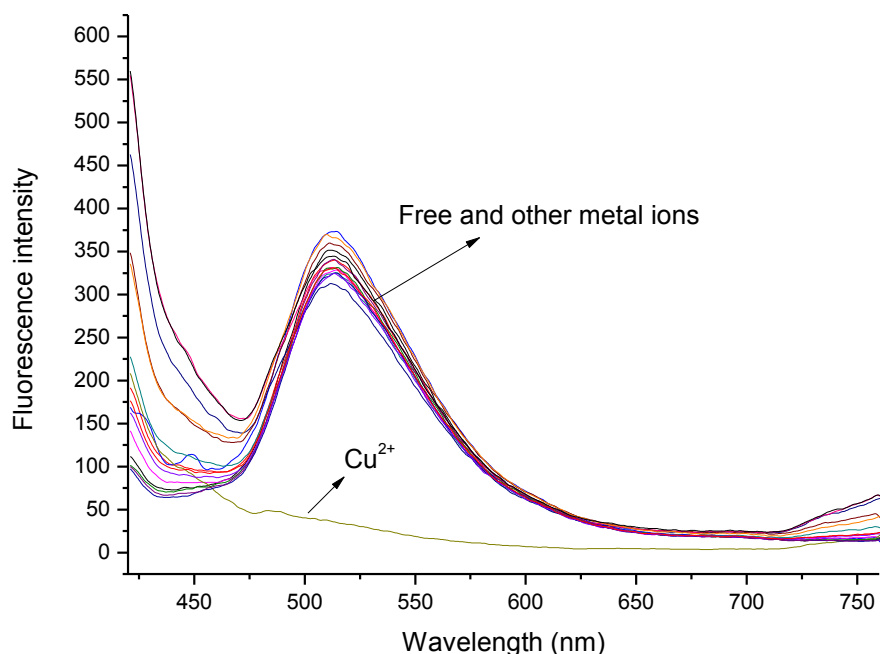
**Fig. S21** Partial <sup>1</sup>H NMR titration spectra (5.5 - 9.0 ppm) of probe **1** with stepwise addition of TFA (0 – 3.2 equiv).



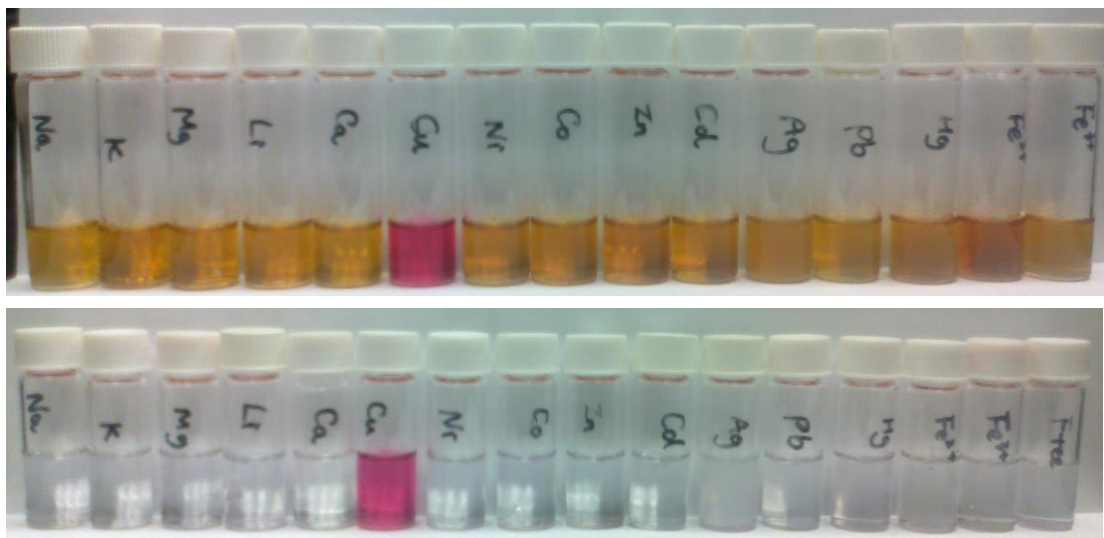
**Fig. S22** <sup>1</sup>H NMR titration spectra of probe **1** with TFA (3.2 equiv) upon the stepwise addition of TEA (0 – 3.2 equiv). Peaks \* were attributed to the protons of TEA.



**Fig. S23** Fluorescence spectra of **1** (50  $\mu\text{M}$ ) in ACN-phosphate buffer (20 mM, pH 4.0, 1:1, v/v) upon addition of various metal ions:  $\text{Na}^+$  (150 mM),  $\text{K}^+$  (150 mM),  $\text{Ca}^{2+}$  (3 mM),  $\text{Mg}^{2+}$  (3 mM),  $\text{Li}^+$  (50  $\mu\text{M}$ ),  $\text{Ag}^+$  (50  $\mu\text{M}$ ),  $\text{Cu}^{2+}$  (50  $\mu\text{M}$ ),  $\text{Fe}^{2+}$  (50  $\mu\text{M}$ ),  $\text{Fe}^{3+}$  (50  $\mu\text{M}$ ),  $\text{Zn}^{2+}$  (50  $\mu\text{M}$ ),  $\text{Co}^{2+}$  (50  $\mu\text{M}$ ),  $\text{Ni}^{2+}$  (50  $\mu\text{M}$ ),  $\text{Cd}^{2+}$  (50  $\mu\text{M}$ ),  $\text{Hg}^{2+}$  (50  $\mu\text{M}$ ),  $\text{Pb}^{2+}$  (50  $\mu\text{M}$ ).



**Fig. S24** Fluorescence spectra of **1** (50  $\mu\text{M}$ ) in ACN-phosphate buffer (20 mM, pH 7.0, 1:1, v/v) upon addition of various metal ions:  $\text{Na}^+$  (150 mM),  $\text{K}^+$  (150 mM),  $\text{Ca}^{2+}$  (3 mM),  $\text{Mg}^{2+}$  (3 mM),  $\text{Li}^+$  (50  $\mu\text{M}$ ),  $\text{Ag}^+$  (50  $\mu\text{M}$ ),  $\text{Cu}^{2+}$  (50  $\mu\text{M}$ ),  $\text{Fe}^{2+}$  (50  $\mu\text{M}$ ),  $\text{Fe}^{3+}$  (50  $\mu\text{M}$ ),  $\text{Zn}^{2+}$  (50  $\mu\text{M}$ ),  $\text{Co}^{2+}$  (50  $\mu\text{M}$ ),  $\text{Ni}^{2+}$  (50  $\mu\text{M}$ ),  $\text{Cd}^{2+}$  (50  $\mu\text{M}$ ),  $\text{Hg}^{2+}$  (50  $\mu\text{M}$ ),  $\text{Pb}^{2+}$  (50  $\mu\text{M}$ ).



**Fig. S25** Photos of probe **1** (50  $\mu$ M) in ACN-phosphate buffer (20 mM, 1:1, v/v) at pH 4.0 (upper) and 7.0 (lower) upon addition of various metal ions (5 equiv).

## References:

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