Supporting Information

pH-Activatable Near-Infrared Fluorescent Probes for Detection of Lysosomal pH inside Living Cells

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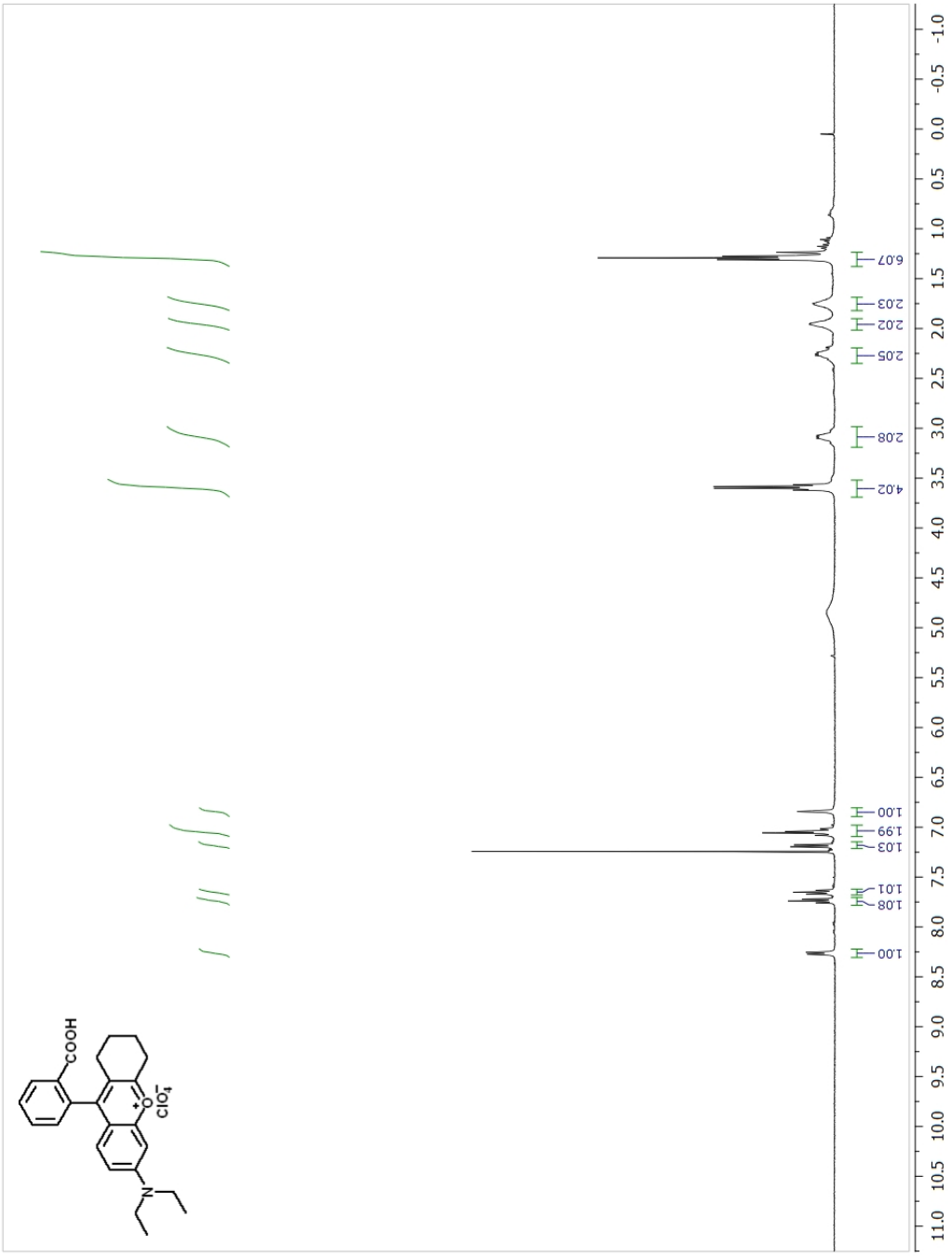


Figure S1: ^{1}H NMR spectrum of compound 3.

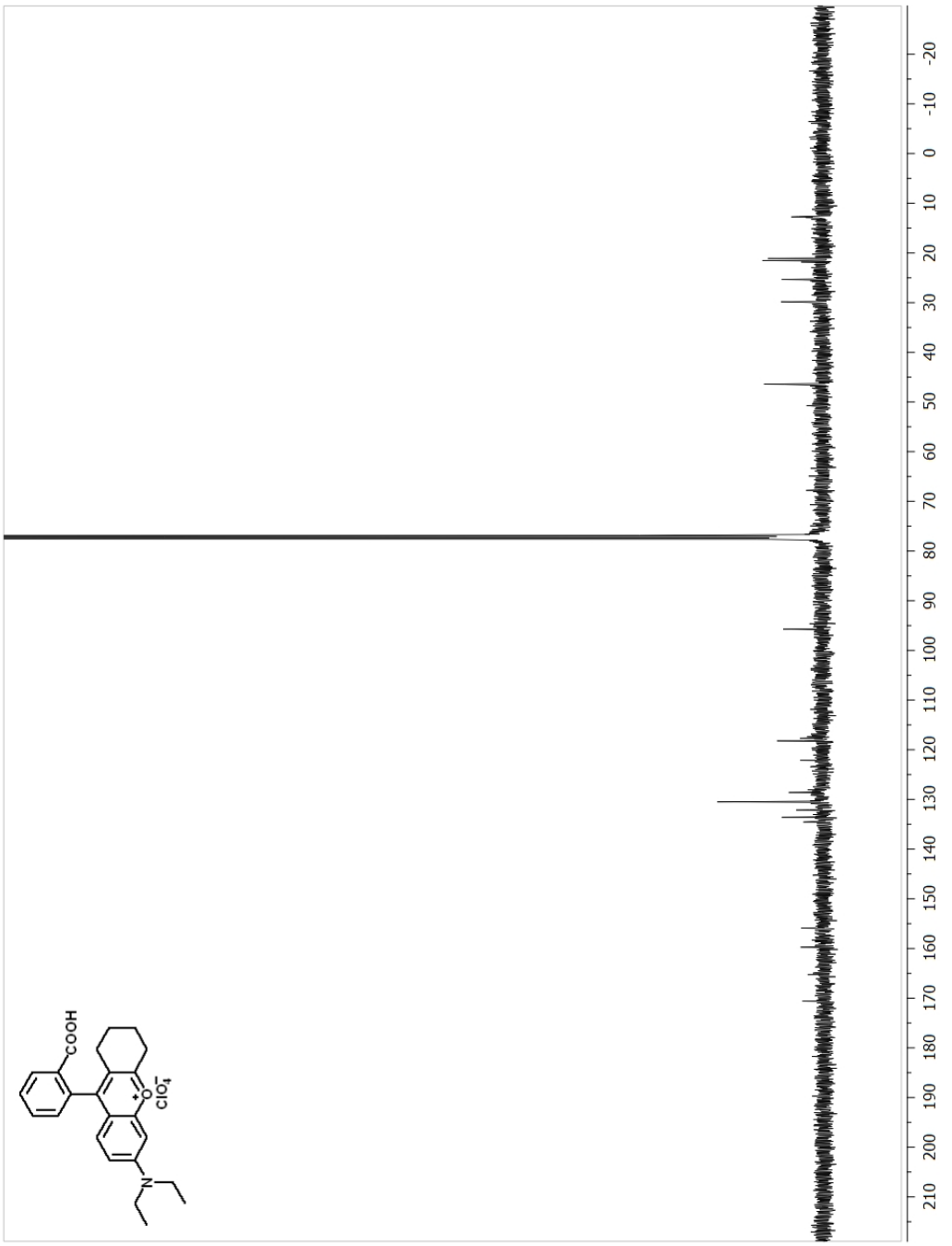


Figure S2: ^{13}C NMR spectrum of compound 3.

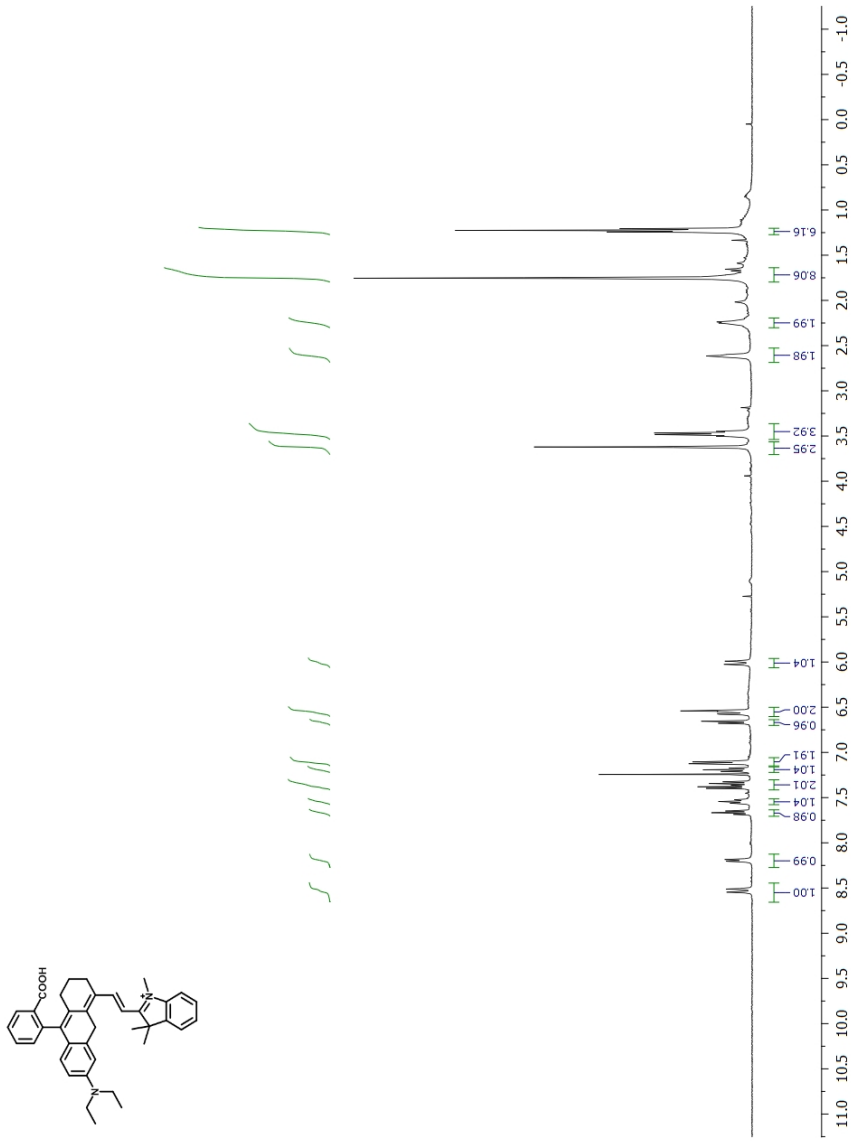


Figure S3: ^{1}H NMR spectrum of compound **5.**

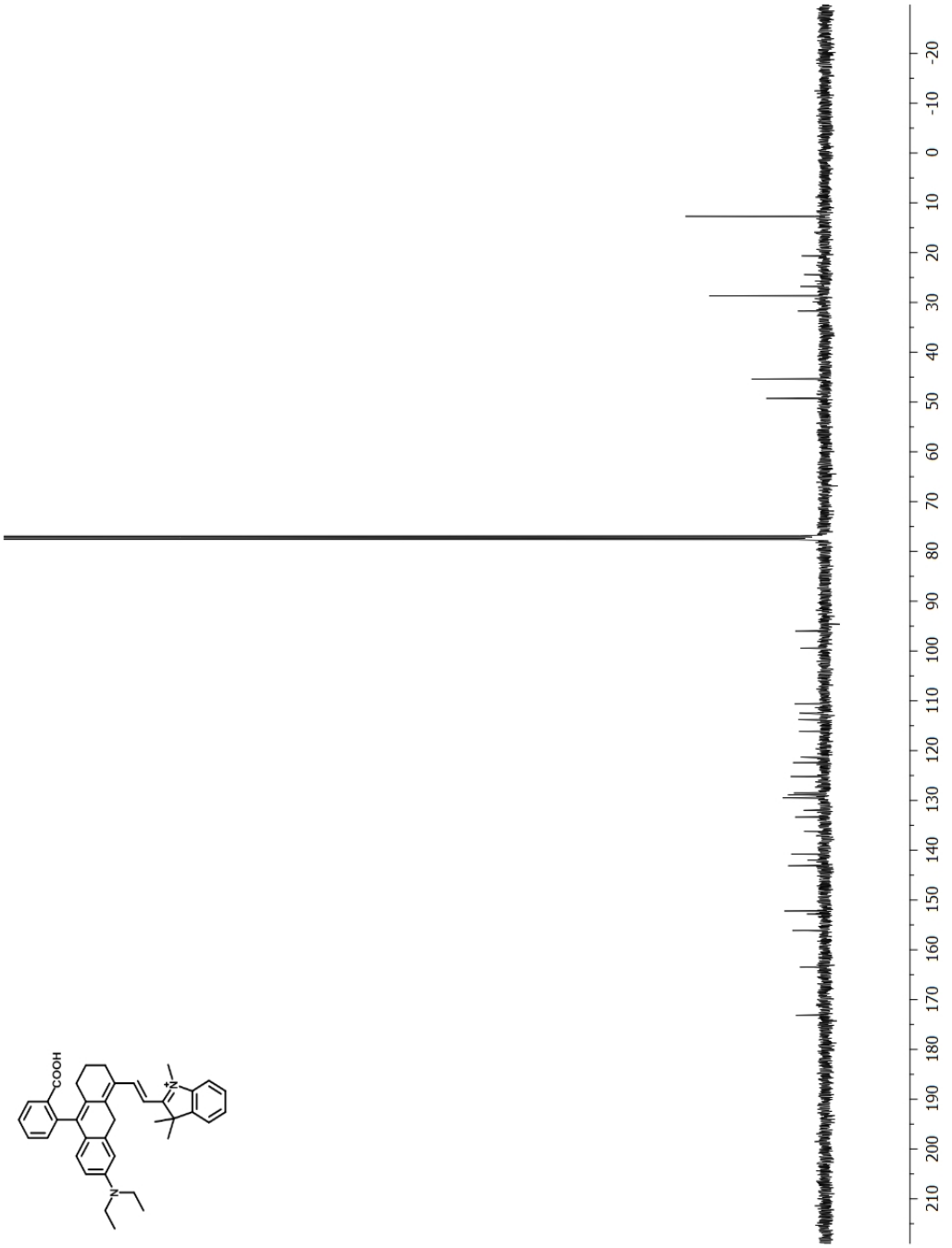


Figure S4: ^{13}C NMR spectrum of compound **5.**

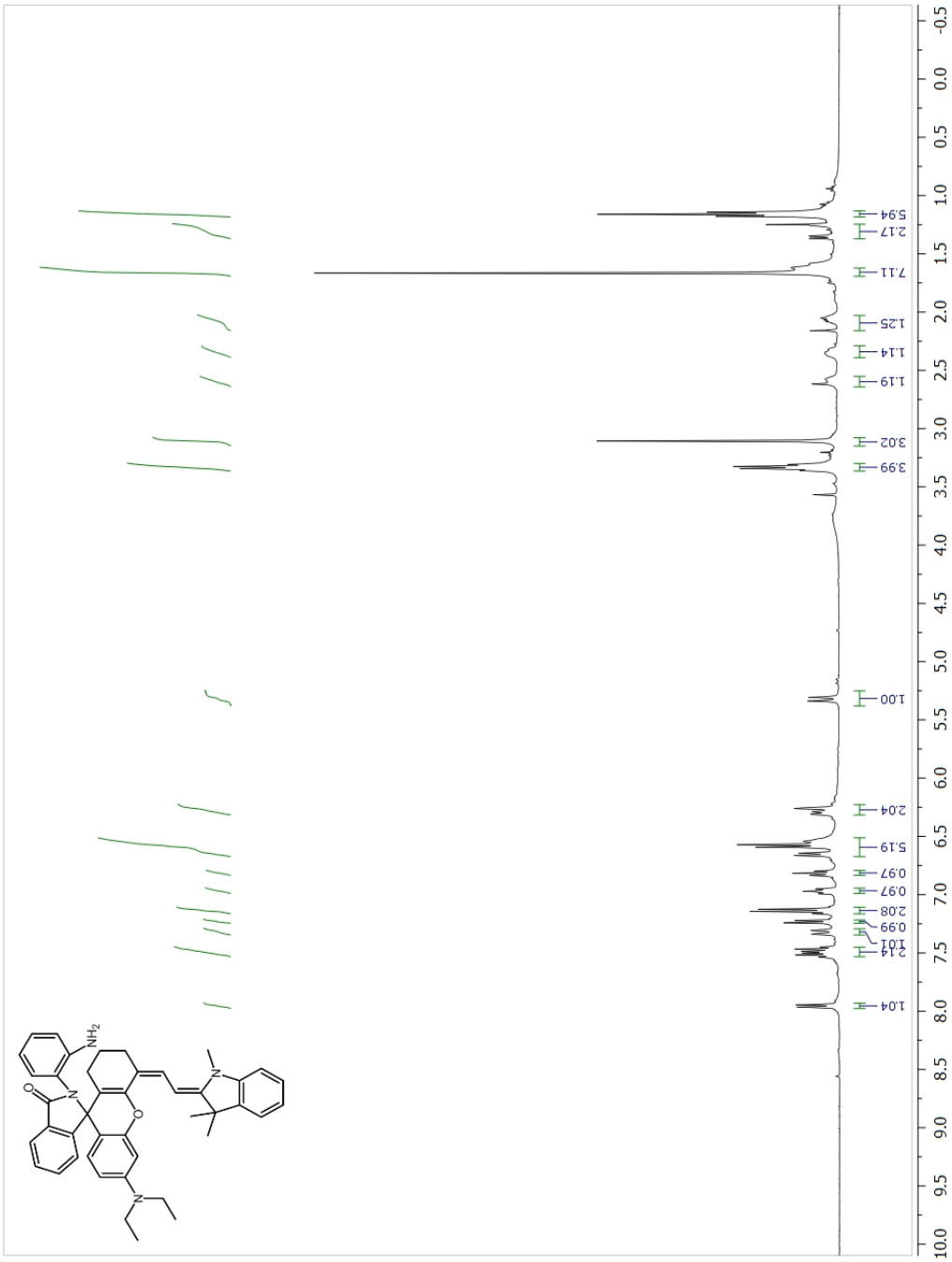


Figure S5: ^{1}H NMR spectrum of fluorescent probe **A.**

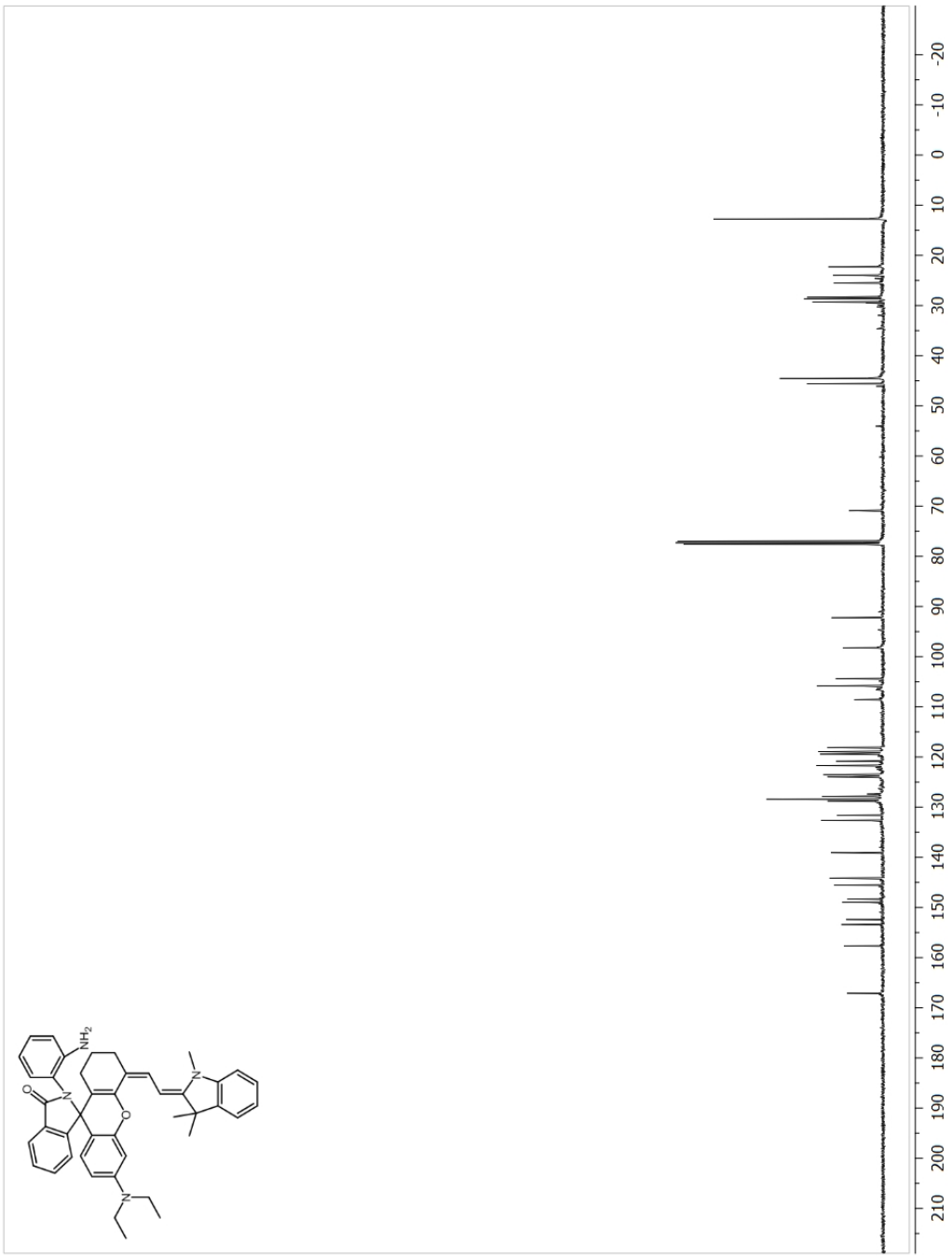


Figure S6: ^{13}C NMR spectrum of fluorescent probe **A.**

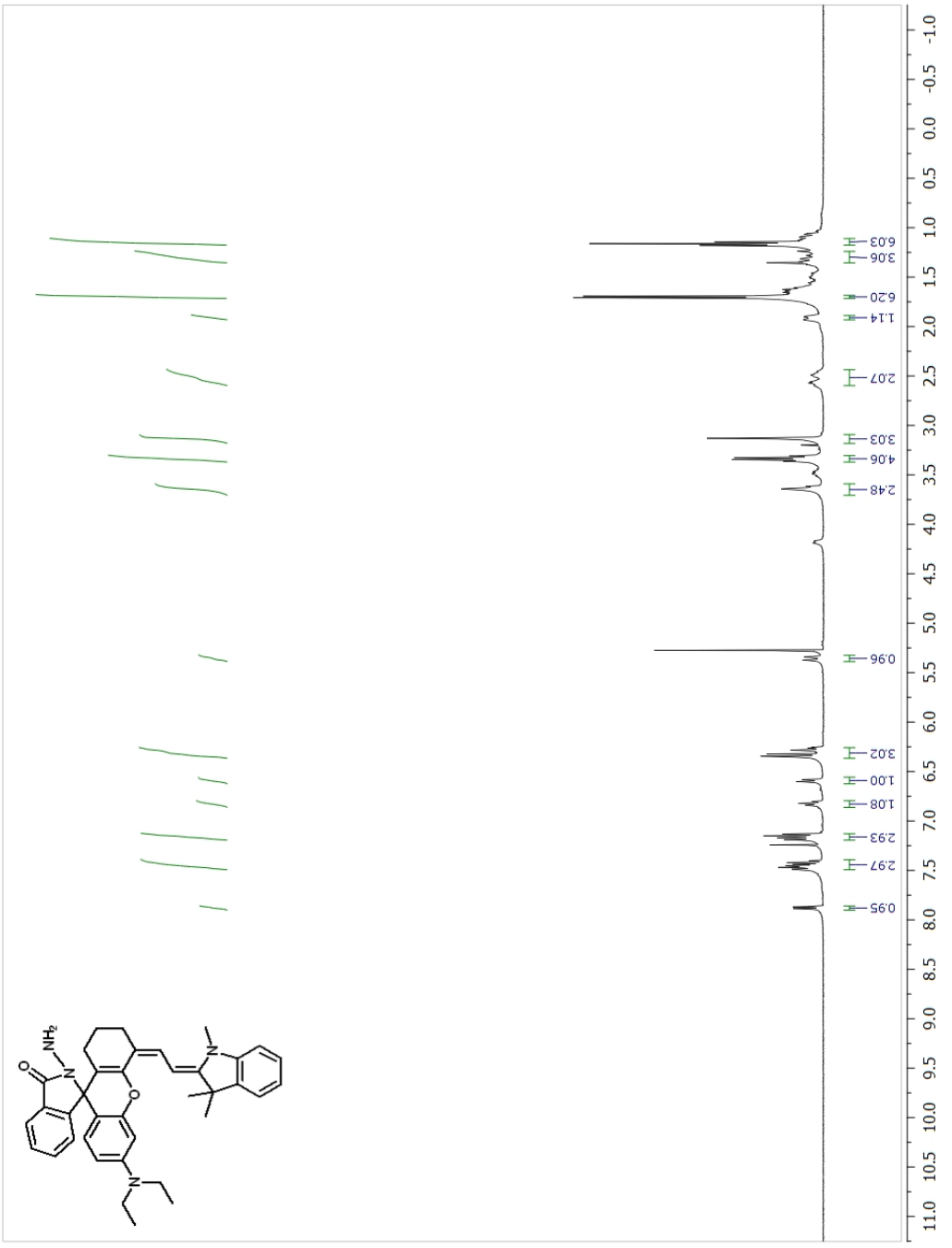


Figure S7: ^{1}H NMR spectrum of fluorescent probe **B.**

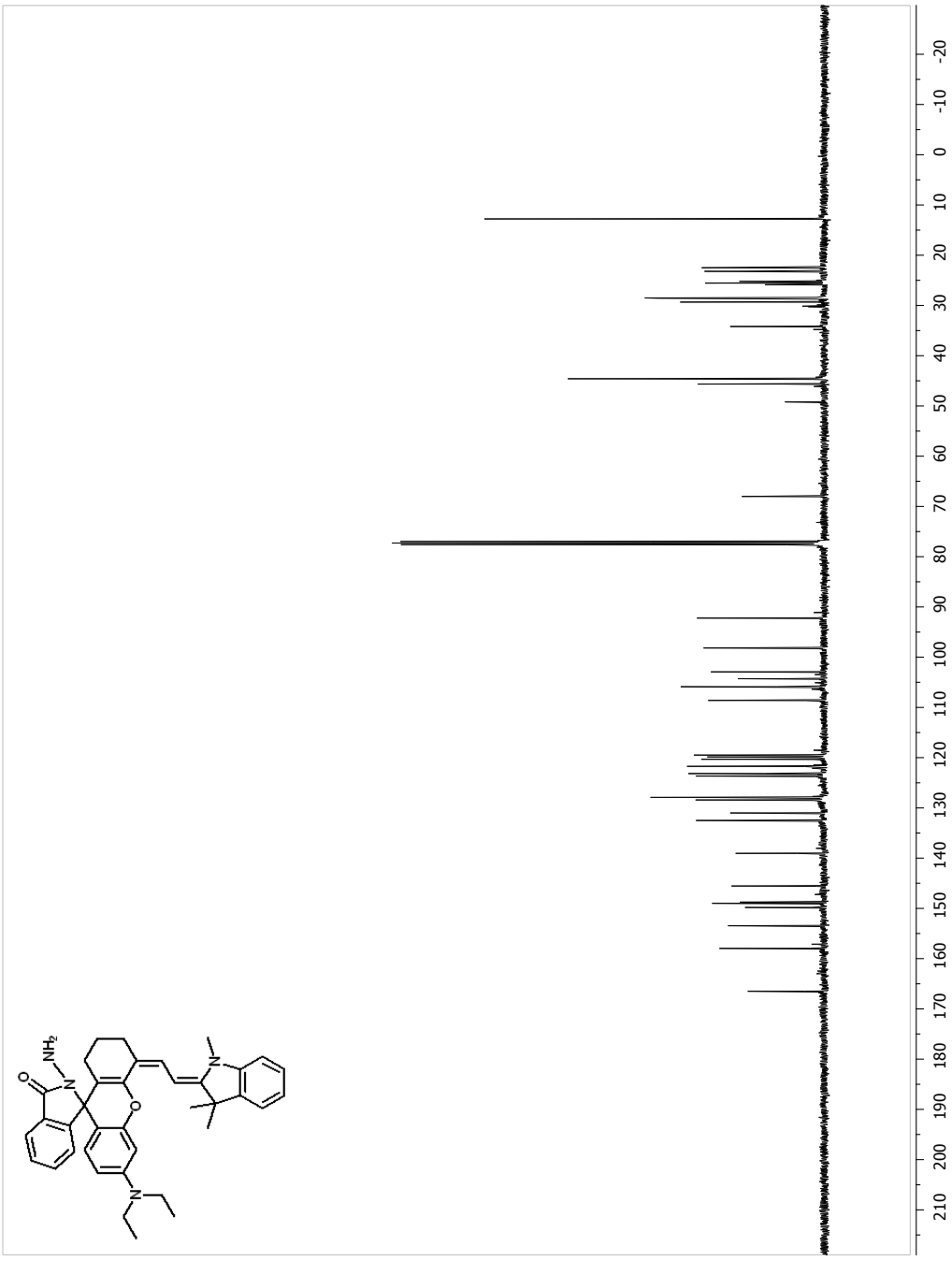


Figure S8: ^{13}C NMR spectrum of fluorescent probe **B.**

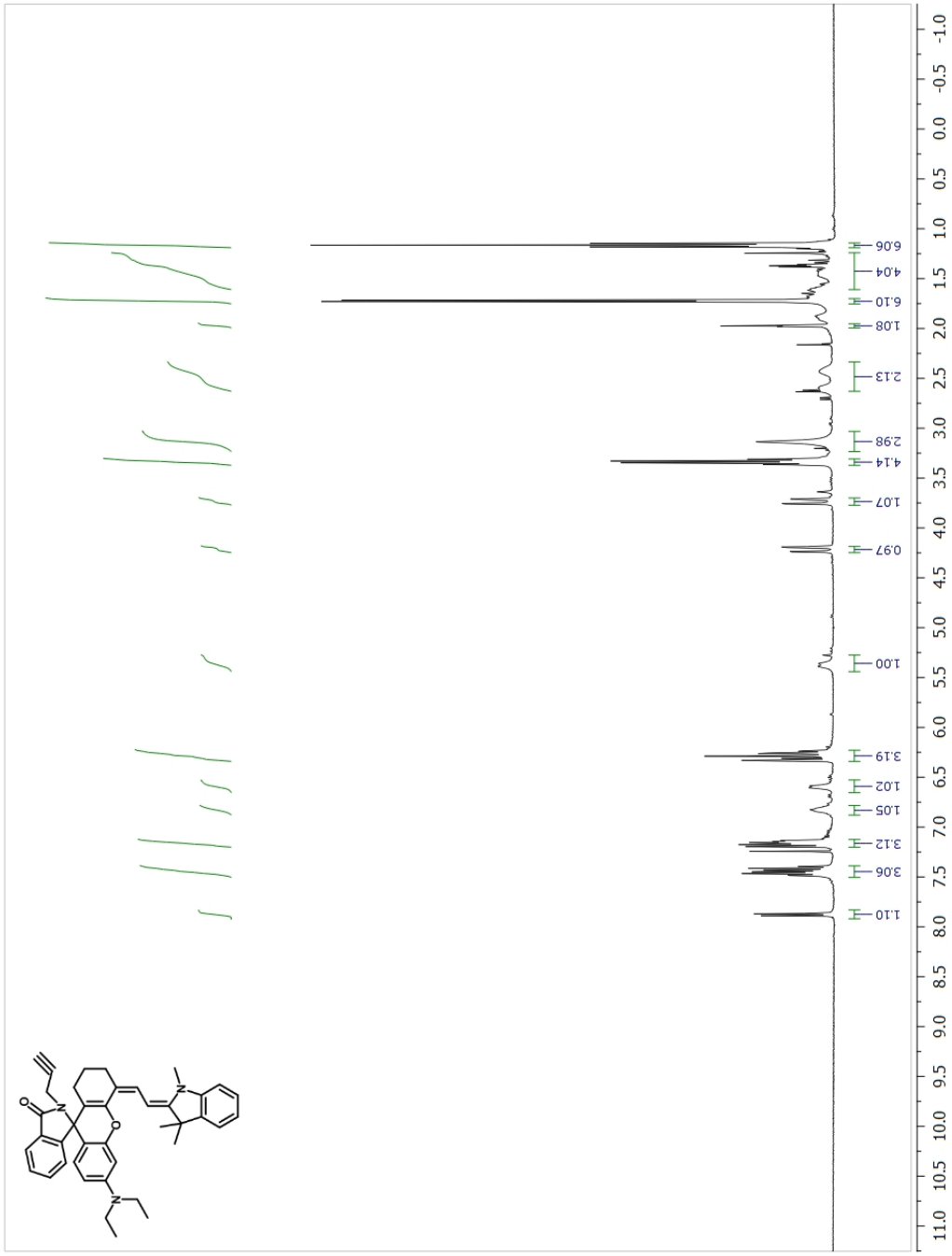


Figure S9: ^{1}H NMR spectrum of fluorescent probe **C.**

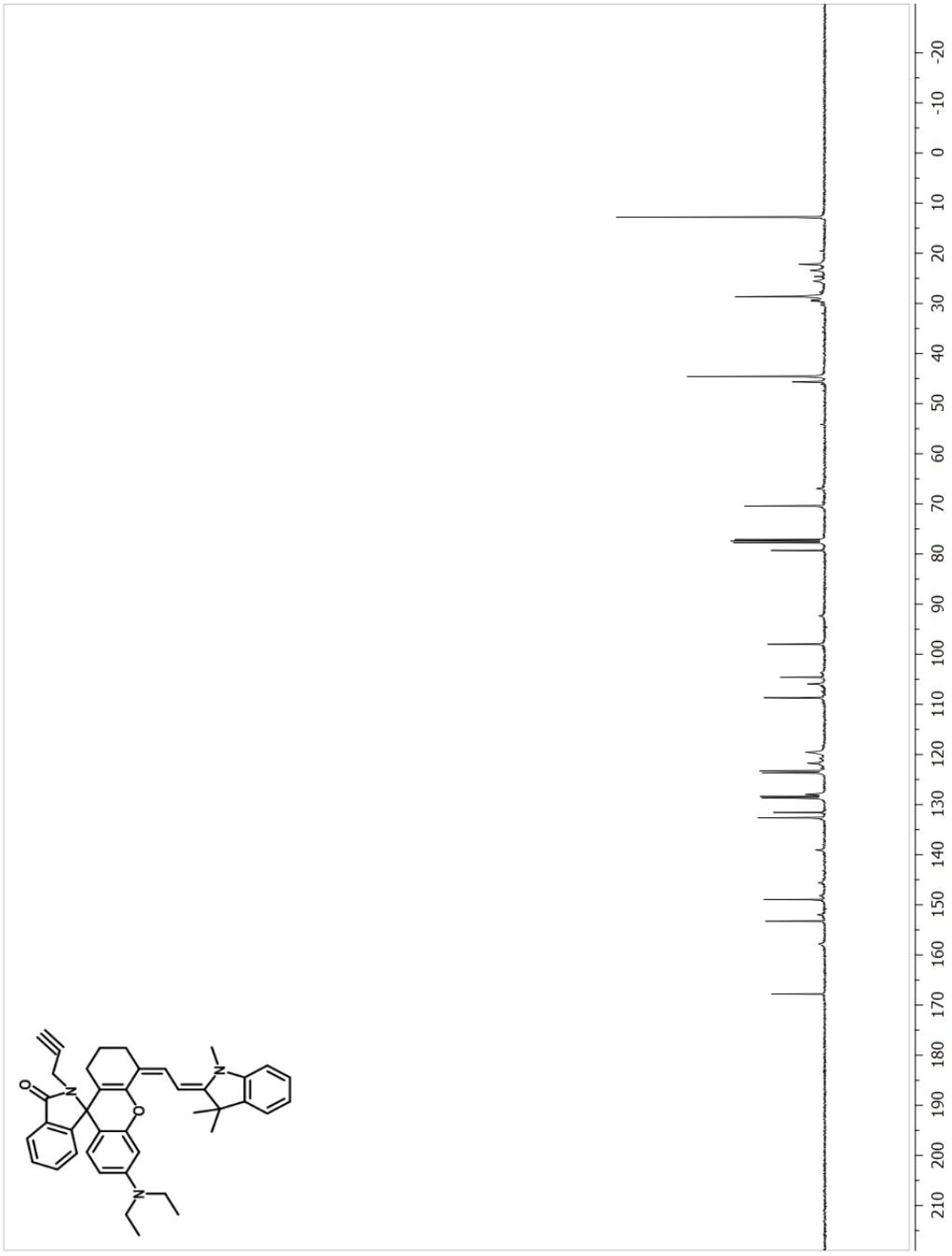


Figure S10: ^{13}C NMR spectrum of fluorescent probe **C.**

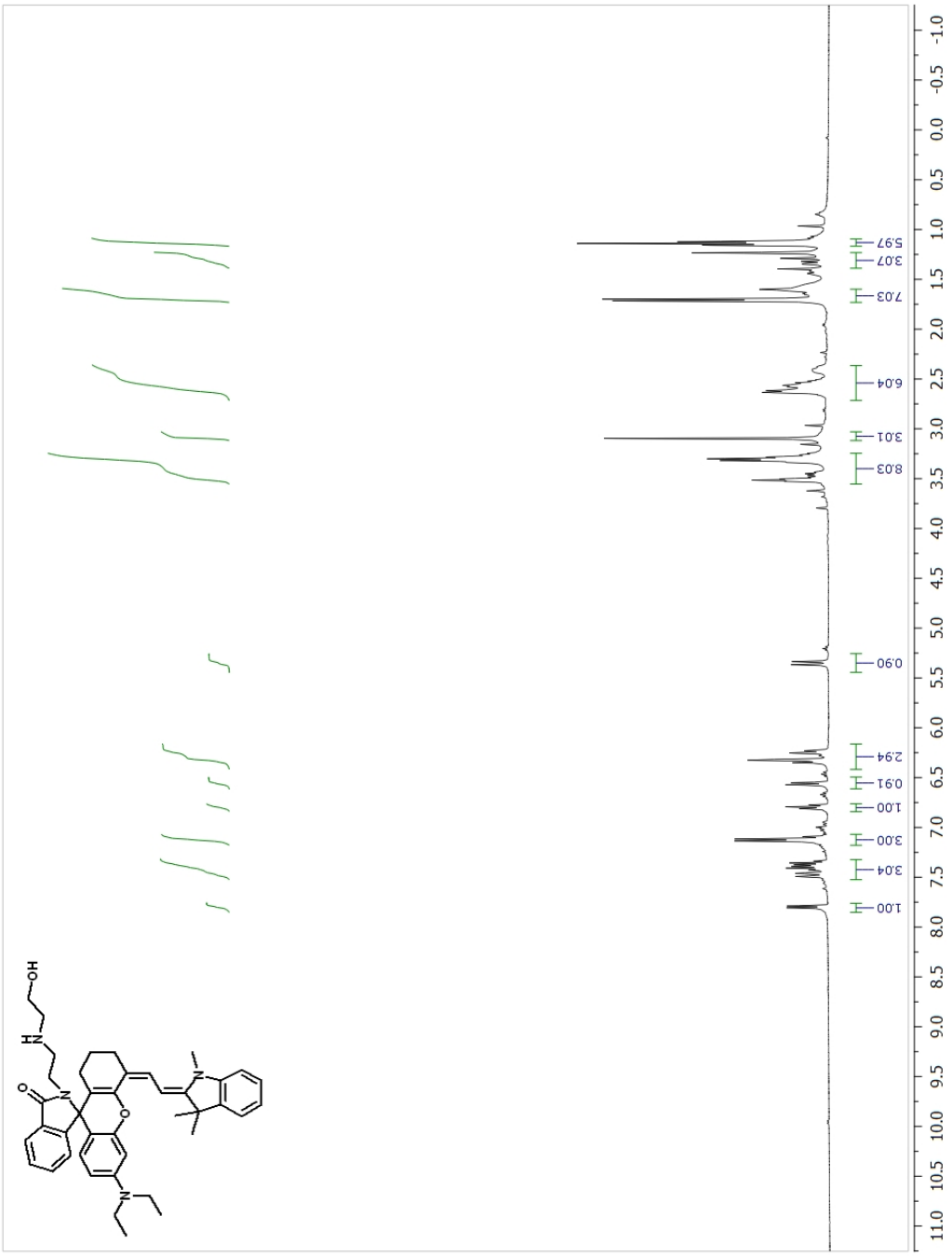


Figure S11: ^{1}H NMR spectrum of fluorescent probe **D**

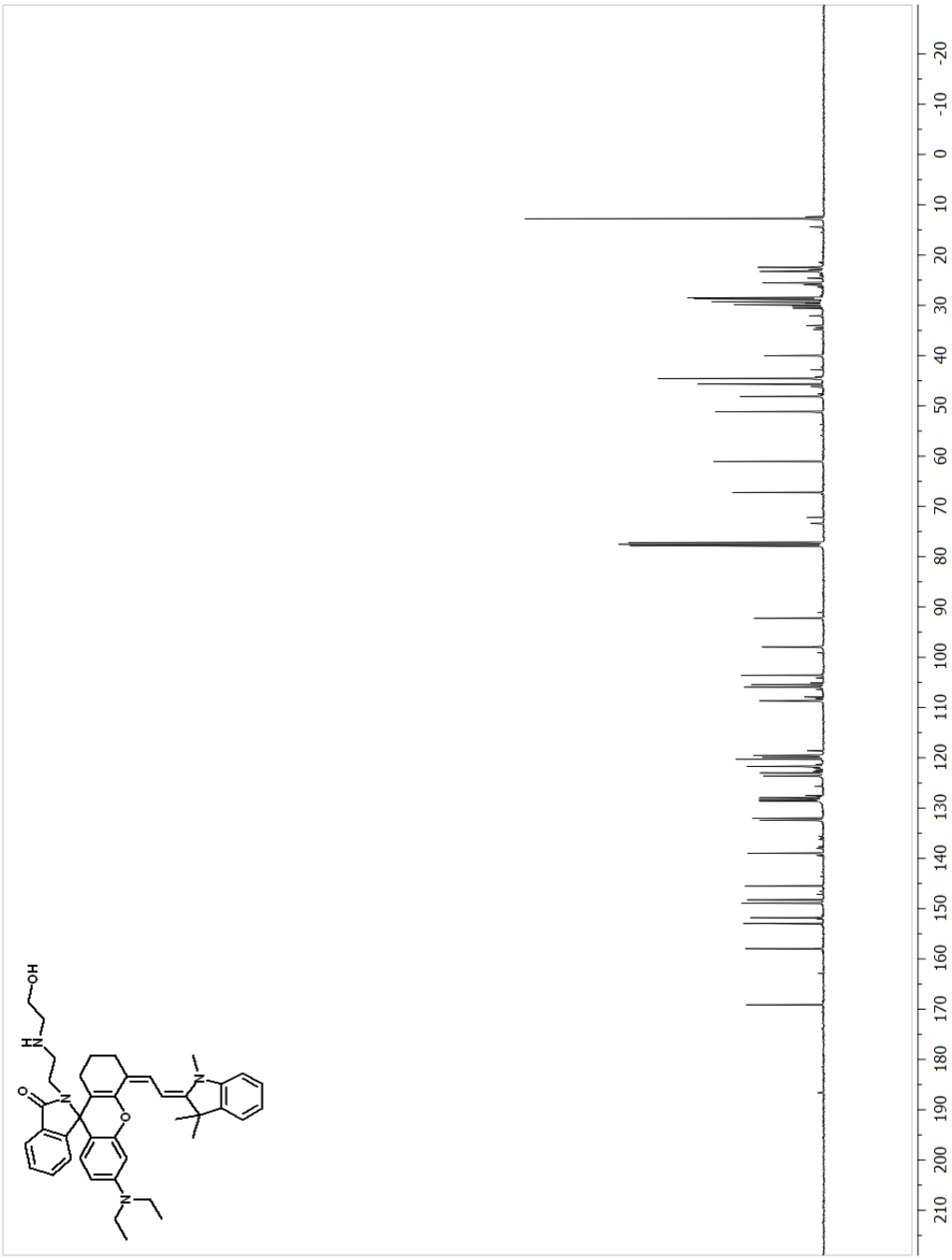


Figure S12: ^{13}C NMR spectrum of fluorescent probe **D**

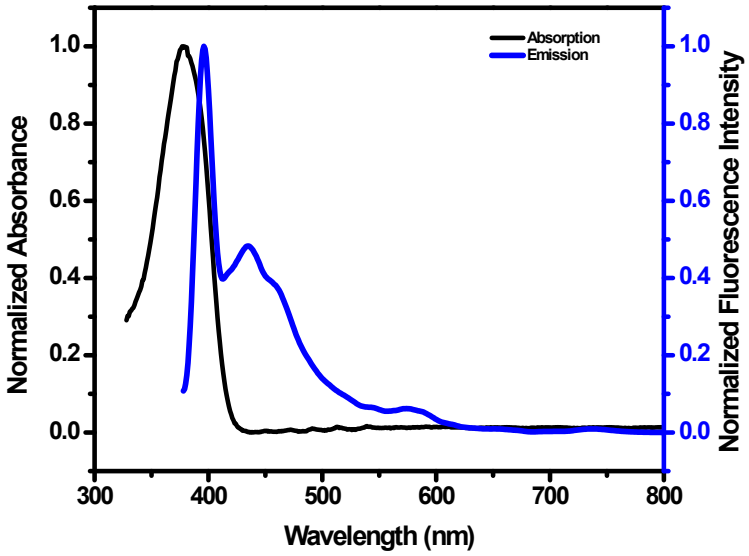


Figure S13: Normalized absorbance and fluorescence spectra of fluorescent probe **A** in EtOH/pH8.0 HEPES Buffer (v/v = 2/3). Excitation wavelength: 350 nm.

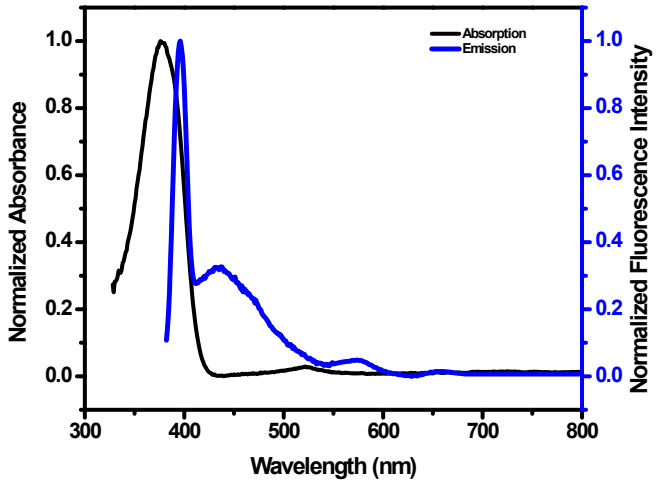


Figure S14: Normalized absorbance and fluorescence spectra of fluorescent probe **B** in EtOH/pH7.0 HEPES Buffer (v/v = 2/3). Excitation wavelength: 350 nm

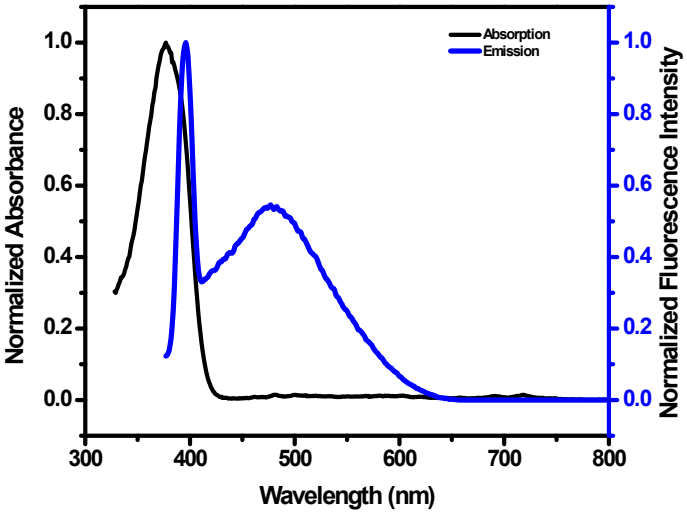


Figure S15: Normalized absorbance and fluorescence spectra of fluorescent probe **C** in EtOH/pH7.0 HEPES Buffer (v/v = 2/3). Excitation wavelength: 350 nm.

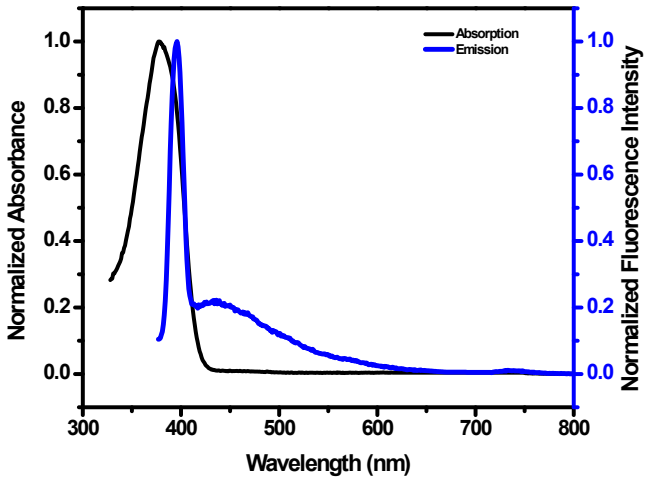


Figure S16: Normalized absorbance and fluorescence spectra of fluorescent probe **D** in EtOH/pH8.0 HEPES Buffer (v/v = 2/3). Excitation wavelength: 350 nm

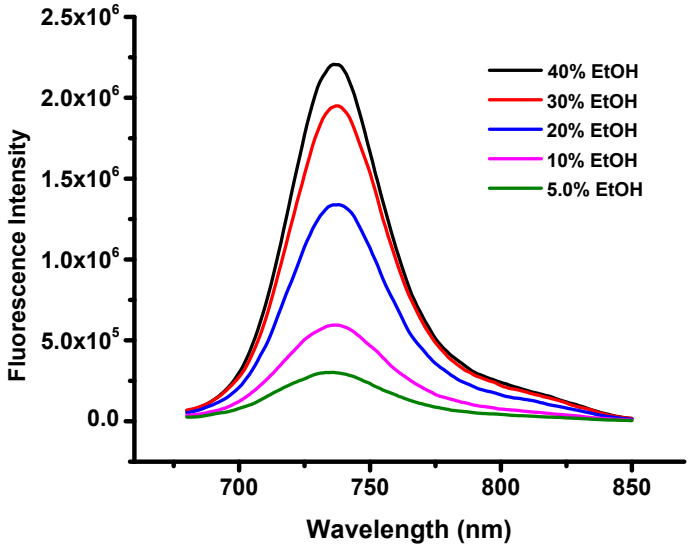


Figure S17. Fluorescent spectra of 5 μM fluorescent probe **A** in 40 mM citrate-phosphate buffer solution at pH 4 containing different concentrations of ethanol.

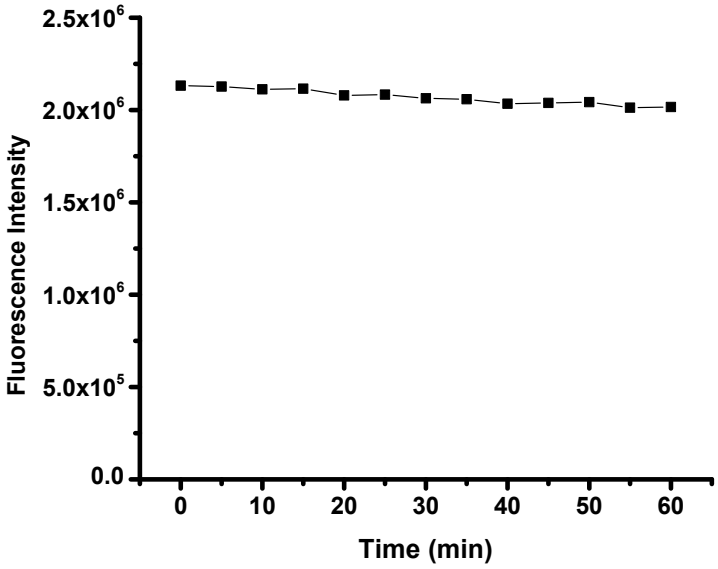


Figure S18. Photostability of 5 μM fluorescent probe **A** at pH 4.1 in 40% ethanol solution. Sample was exposed under respective optimal excitation wavelength and fluorescence intensities were measured at 5-min intervals.

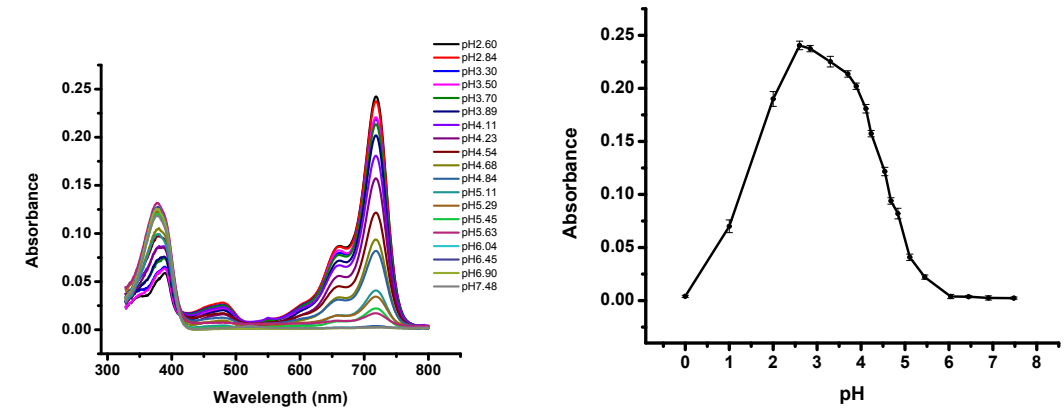
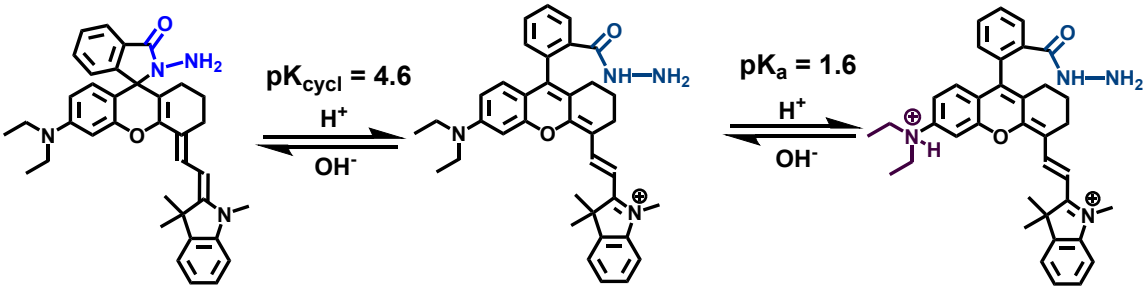


Figure S19: Absorption spectra of 5 μM fluorescent probe **B (with three repetitions/error bars)** at different pH values (left) and effect of pH on absorbance of the fluorescent probe **B** at 718 nm (right).



Fluorescent probe B

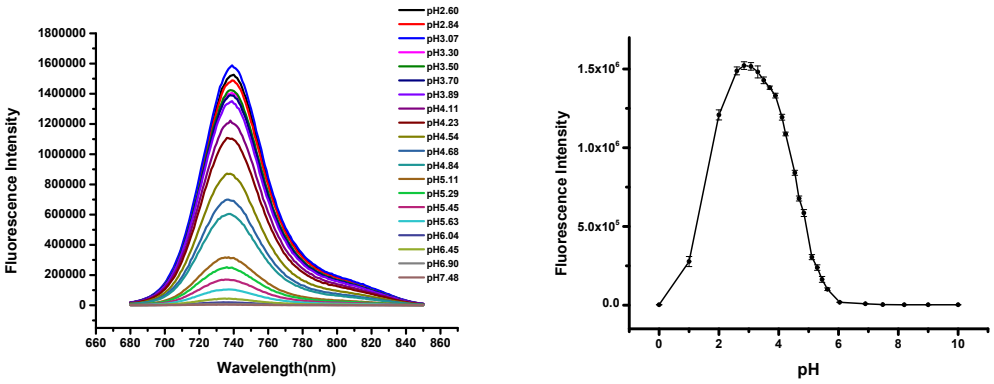


Figure S20: Fluorescence spectra of 5 μM fluorescent probe **B (with three repetitions/error bars)** at different pH values (left) and effect of pH on fluorescence of the fluorescent probe **B** at 743 nm(right). Excitation wavelength: 670 nm

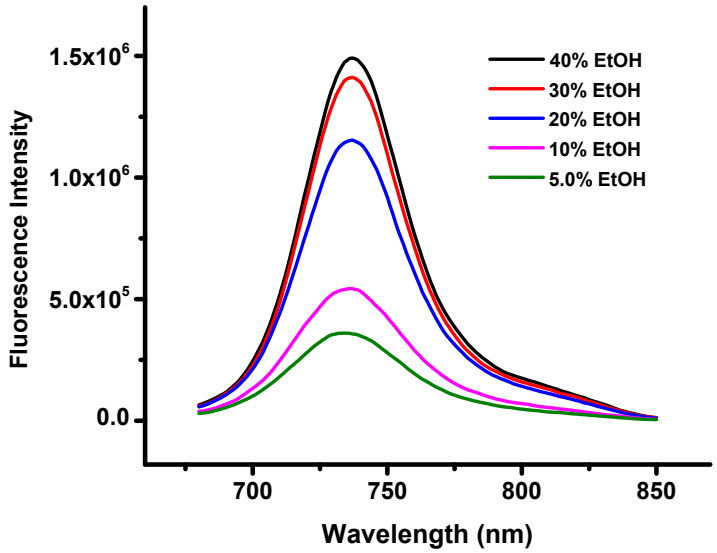


Figure S21. Fluorescent spectra of 5 μM fluorescent probe **B** in 40 mM citrate-phosphate buffer solution at pH 4 containing different concentrations of ethanol.

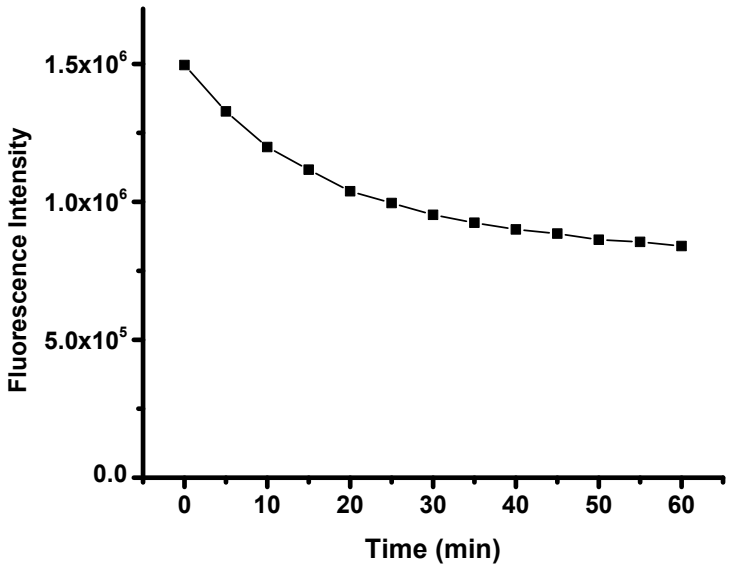


Figure S22. Photostability of 5 μM fluorescent probe **B** at pH 4.1 in 40% ethanol solution. Sample was exposed under respective optimal excitation wavelength and fluorescence intensities were measured at 5-min intervals.

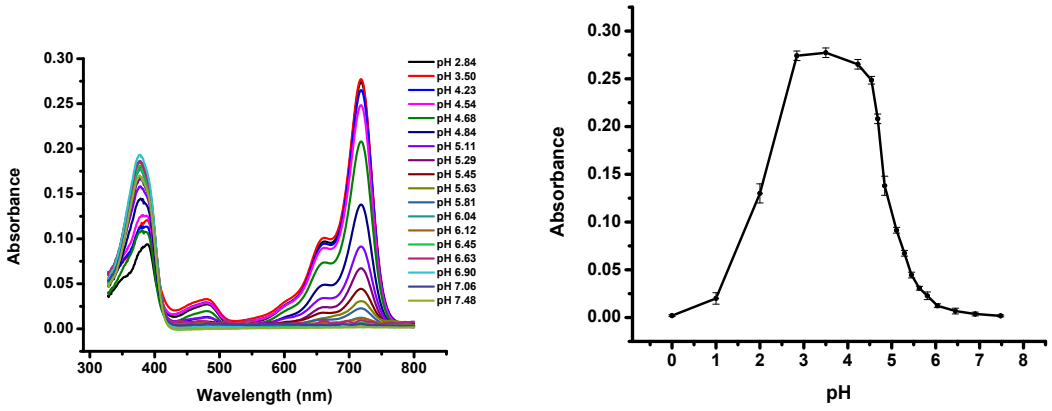


Figure S23: Absorption spectra of 5 μM fluorescent probe **C** (with three repetitions/error bars) at different pH values (left) and effect of pH on absorbance of the fluorescent probe **C** at 718 nm (right).

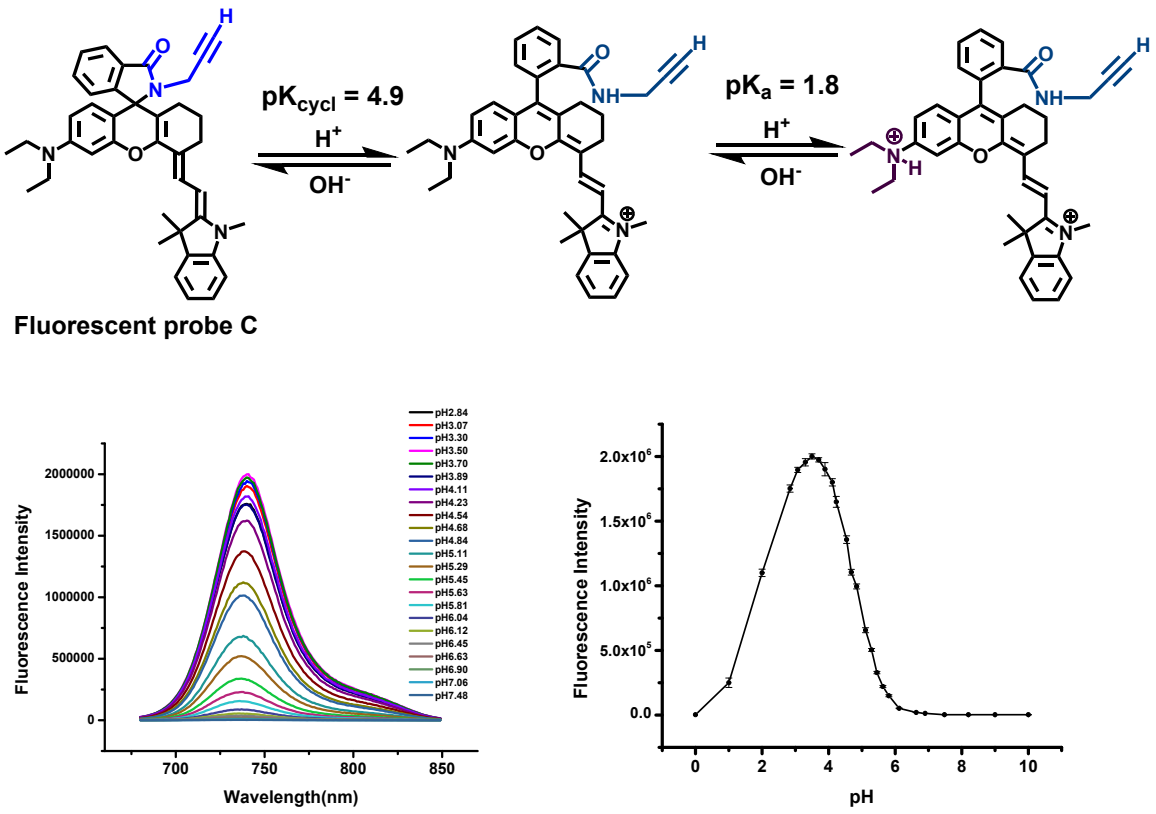


Figure S24: Fluorescence spectra of 5 μM fluorescent probe **C** with three repeated measurements at different pH values (left) and effect of pH on fluorescence of the fluorescent probe **C** at 743 nm(right). Excitation wavelength: 670 nm.

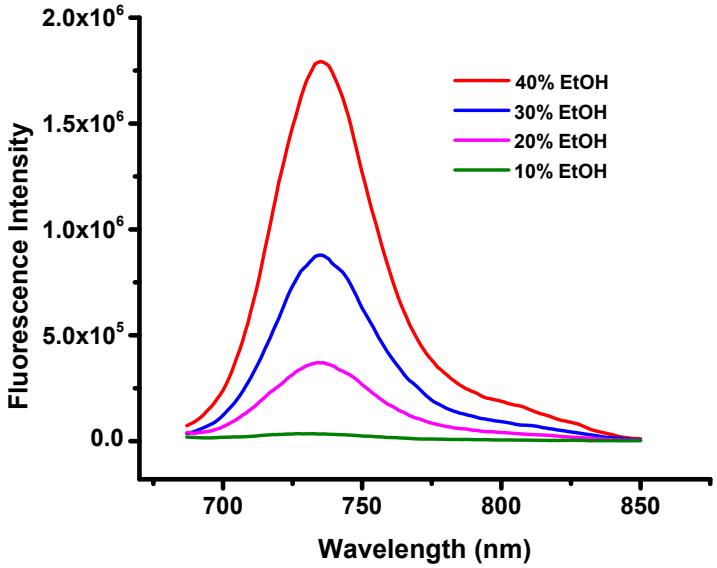


Figure S25. Fluorescent spectra of 5 μM fluorescent probe **C** in 40 mM citrate-phosphate buffer solution at pH 4 containing different concentrations of ethanol.

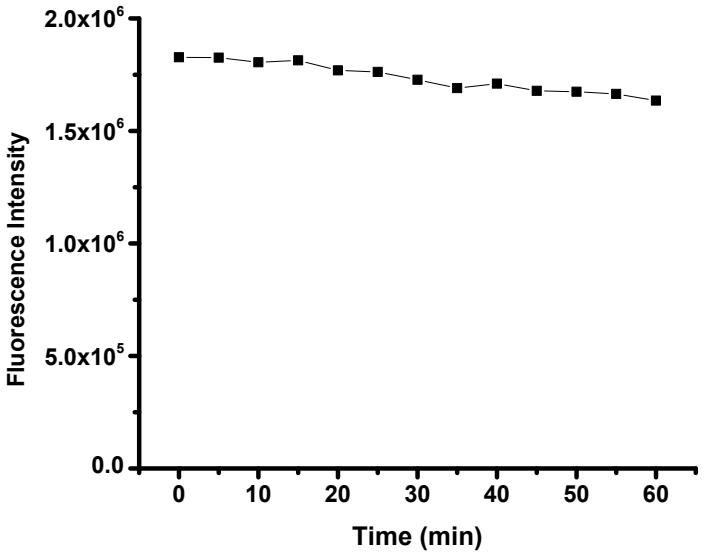


Figure S26. Photostability of 5 μM fluorescent probe **C** at pH 4.1 in 40% ethanol solution. Sample was exposed under respective optimal excitation wavelength and fluorescence intensities were measured at 5-min intervals.

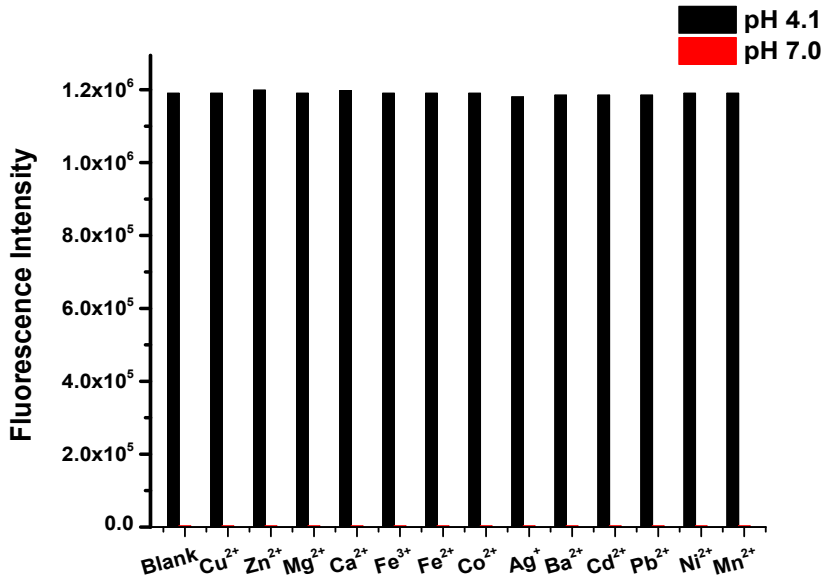


Figure S27. Fluorescent responses of 5 μM fluorescent probe **B** to different metal ions (200 μM) at pH 4.1 and 7.0. Excitation wavelength: 670 nm.

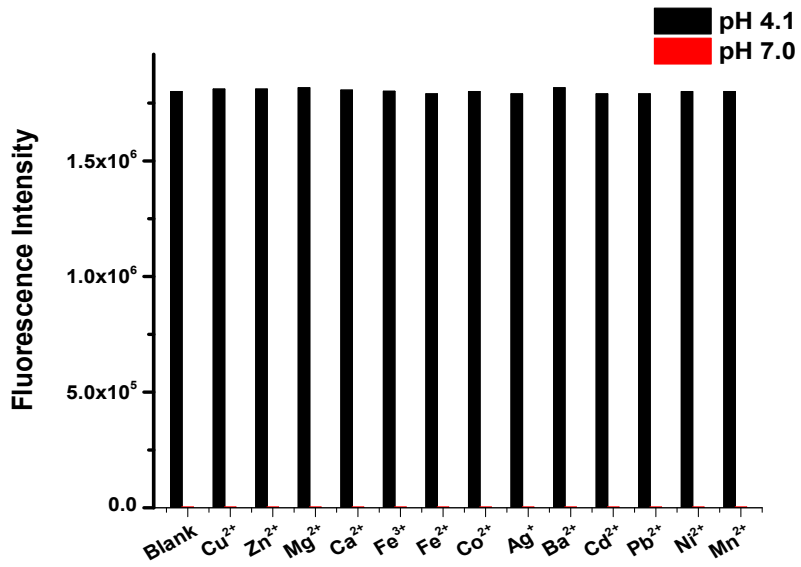


Figure S28. Fluorescent responses of 5μM fluorescent probe **C** to different metal ions (200 μM) at pH 4.1 and 7.0. Excitation wavelength: 670 nm.

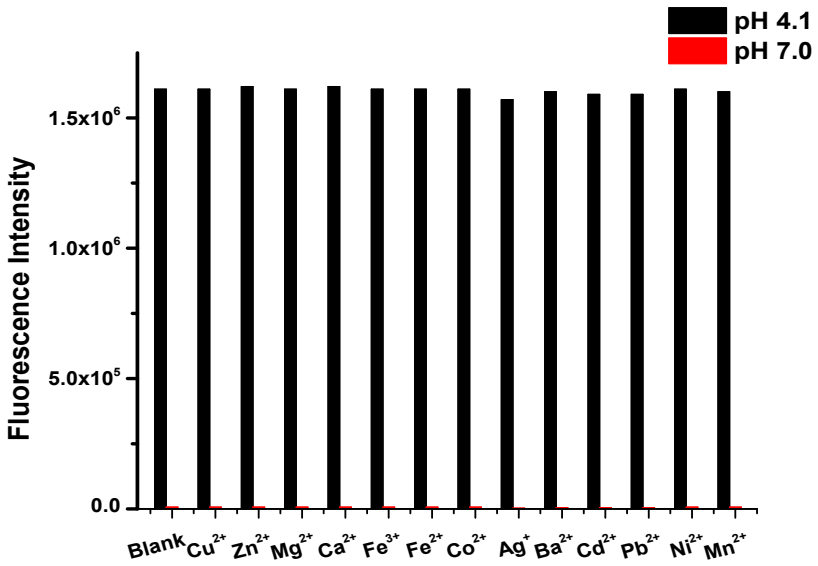


Figure S29. Fluorescent responses of 5 μM fluorescent probe **D** to different metal ions (200 μM) at pH 4.1 and 7.0. Excitation wavelength: 670 nm.

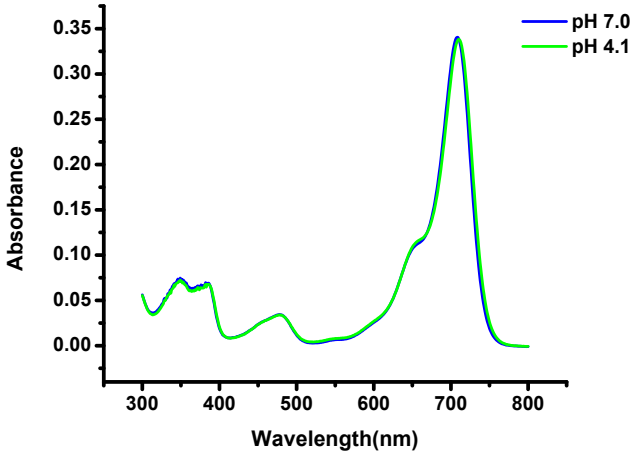


Figure S30. Absorbance spectra of compound **5** at pH 4.1 and 7.0.

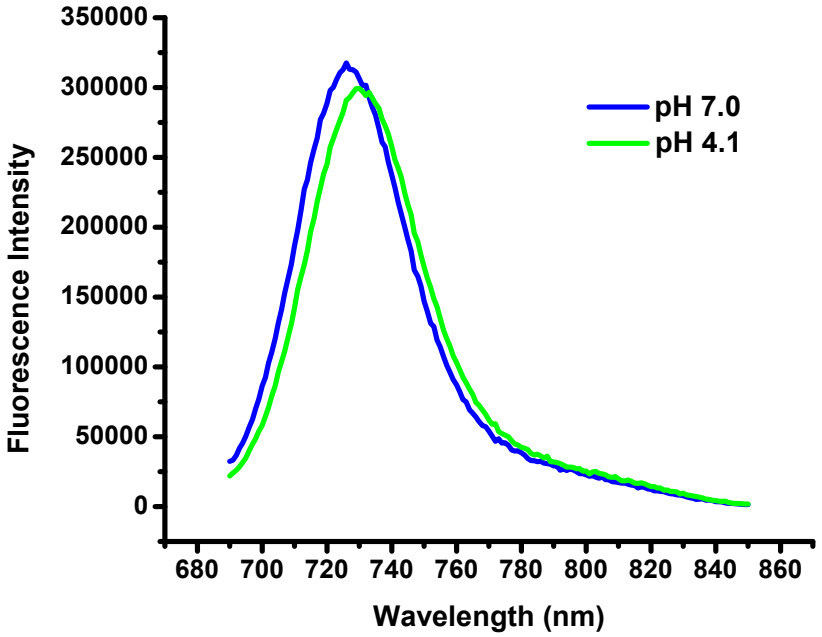


Figure S31. Fluorescence spectra of compound **5** at pH 4.1 and 7.0.

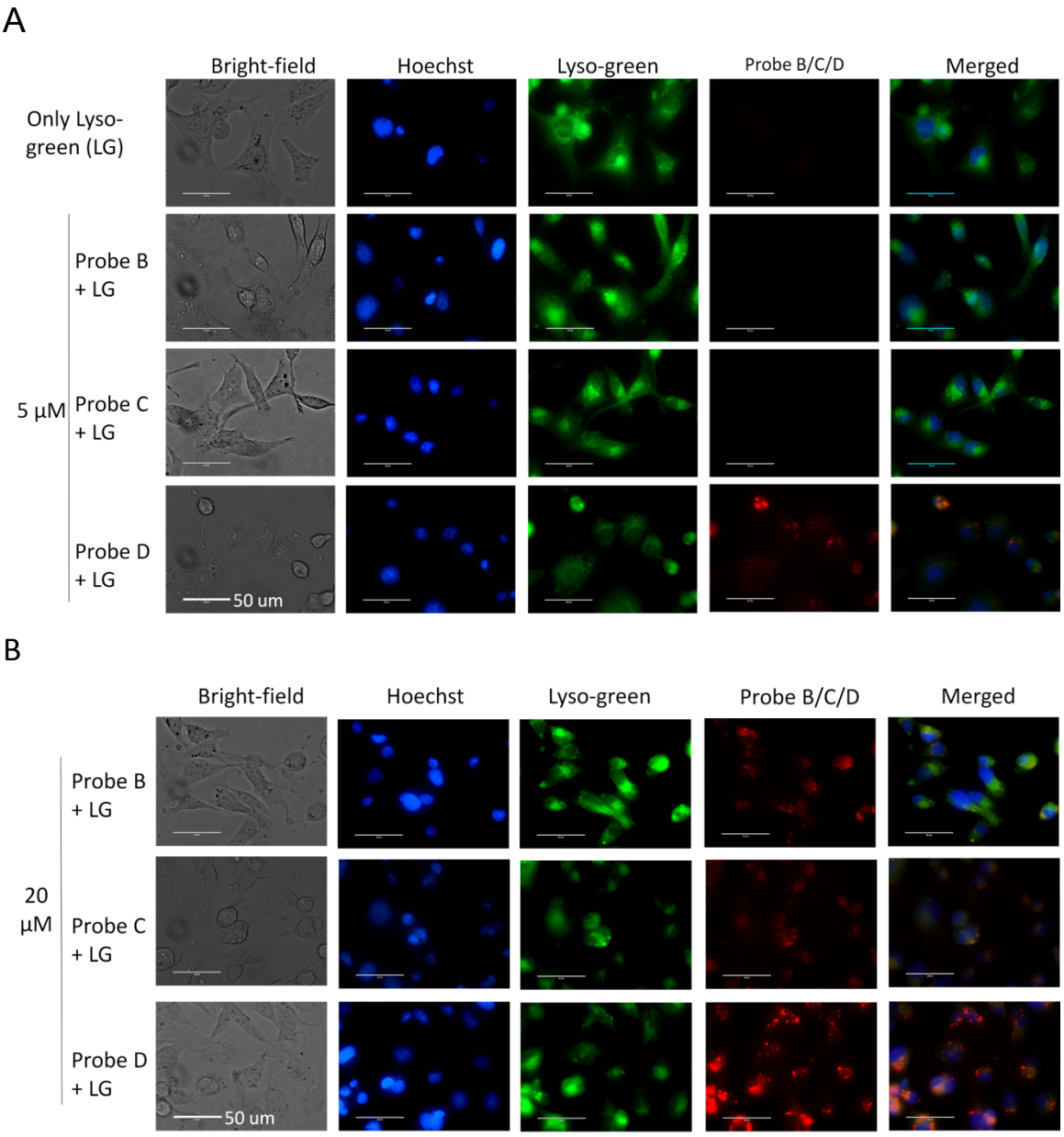


Figure S32. Fluorescence images of MDA-MB-231 cells incubated with fluorescent probes **B**, **C**, and **D**. Cells were incubated with 5 µM **(A)** or 20 µM **(B)** of fluorescent probes **B**, **C**, and **D** for 2 h and imaged for co-localization in presence of 5 µM LysoSensor Green, a lysosomal stain and Hoechst, a nuclear stain. The images were acquired using inverted fluorescence microscope at 60X magnification.

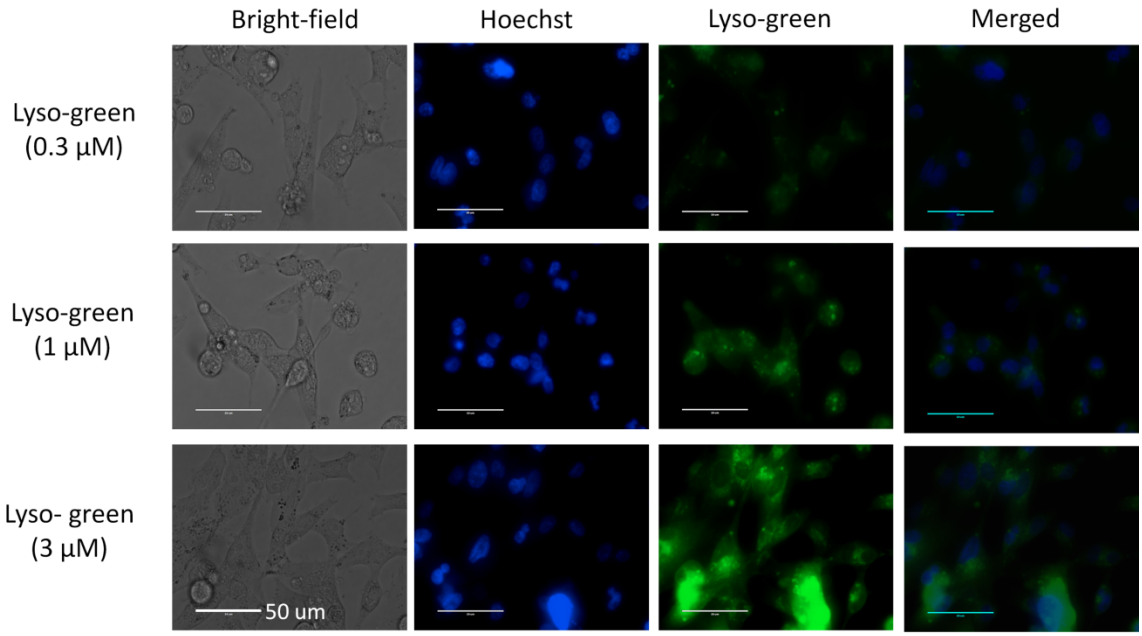


Figure S33. Fluorescence images of MDA-MB-231 cells incubated with 0.3, 1.0, and 3.0 µM of Lysosensor-green dye and 1.0 µg/ml of Hoechst33342 dye for 2 h. The images were acquired using inverted fluorescence microscope at 60X magnification.

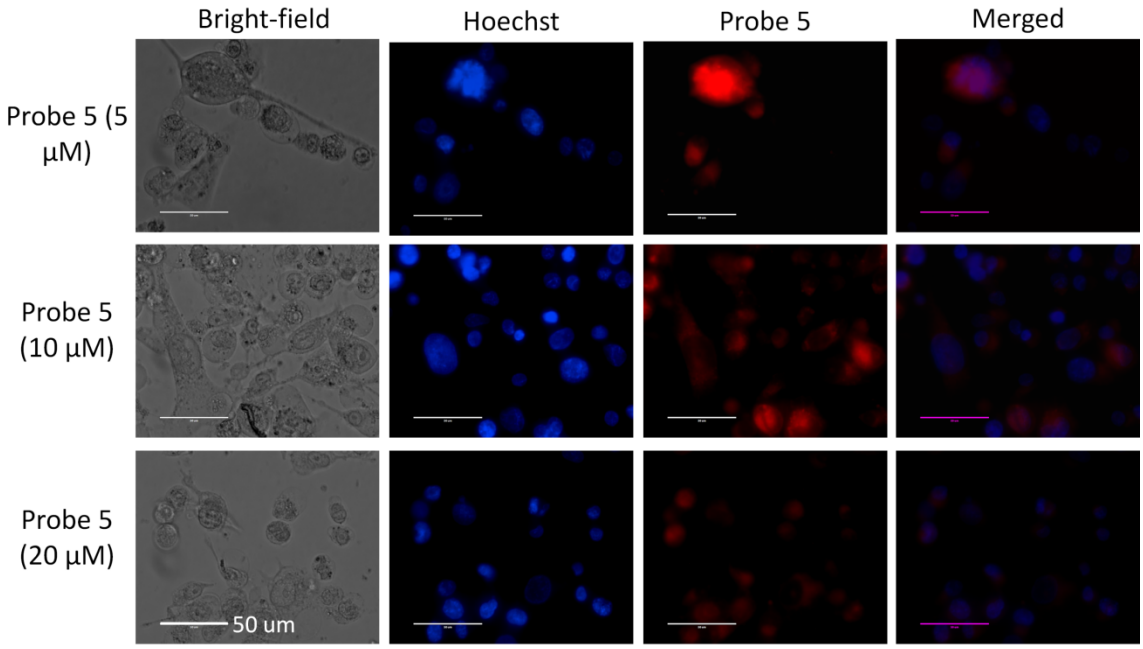


Figure S34. Fluorescence images of MDA-MB-231 cells incubated for 2 h with 5, 10, and 20 µM concentrations of probe 5 and 1 µM of Hoechst33342 dye. The images were acquired using inverted fluorescence microscope at 60X magnification.

Table 1: Absorption coefficients, fluorescent quantum yields, and pK_a values of the probes

Compound	Absorption coefficient (EtOH)	pK_a, pK_{cycl} (Citrate-phosphate buffer with 40%EtOH)	Quantum Yield at pH 4.1 (Citrate-phosphate buffer with 40%EtOH)	Quantum Yield at pH 7.4 (Citrate-phosphate buffer with 40%EtOH)
A	9.8×10^4	1.56, 5.80	0.48	0
B	5.2×10^4	1.55, 4.60	0.57	0
C	6.2×10^4	1.81, 4.86	0.37	0
D	1.1×10^5	1.56, 5.38	0.46	0
5	8.8×10^4			

Determination of the fluorescence quantum yield :

Fluorescence quantum yields for **A**, **B**, **C**, **D** were determined using Rodamin 6G (Φf = 0.95 in ethanol) as fluorescence standard. The equation used for calculating is as follows:

$$\Phi f_{(x)} = \Phi_{f(s)} \, (A_s F_x / A_x F_s) \, (n_x / n_s)^2$$

Where Φ_f is the fluorescence quantum yield, **A** is the absorbance at the excitation wavelength (670nm), **F** is the integration of the emission curve (area under the curve), and **n** is the refractive index of the solvents used. Subscripts **s** and **x** refer to the standard and unknown respectively.