

Supporting information for

Facile Synthesis of a Class of Aminochromene-aniliniumion Conjugated Far-Red to Near-Infrared Fluorescent Dyes for Bioimaging

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	Page
1. Calculation of fluorescence quantum yield.....	S2
2. Figures S1-S2.....	S2
3. Figures S3-S4.....	S3
4. Figures S5-S6.....	S4
5. Figures S7-S8.....	S5
6. Table S1-3.....	S6-S7
7. Figures S9.....	S7
8. References.....	S7
8. Figures S10-17.....	S8-S11

Determination of the fluorescence quantum yield

Fluorescence quantum yields for ACA-1~4 were determined by using ICG ($\Phi_f = 0.13$ in DMSO) as a fluorescence standard.¹ The quantum yield was calculated using the following equation:

$$\Phi_{F(X)} = \Phi_{F(S)} (A_S F_X / A_X F_S) (n_X / n_S)^2$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and to the unknown, respectively.

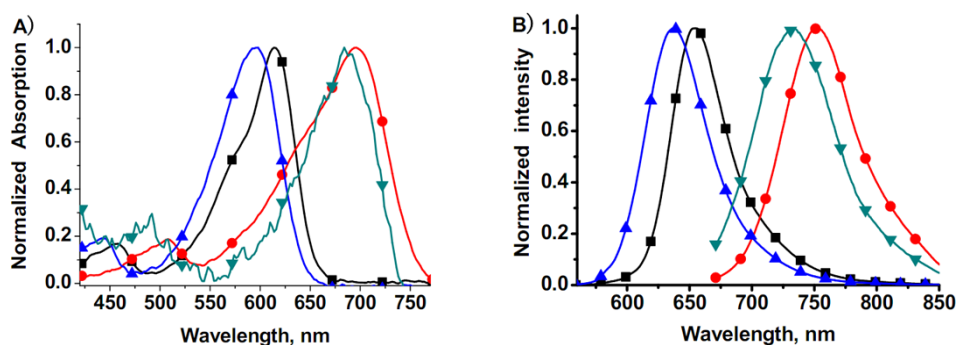


Fig. S1. A) Absorption spectra of the dyes; B) Emission spectra of the dyes; ACA-1 (■), ACA-2 (●), ACA-3 (▲) and ACA-4 (▼) in CH₂Cl₂.

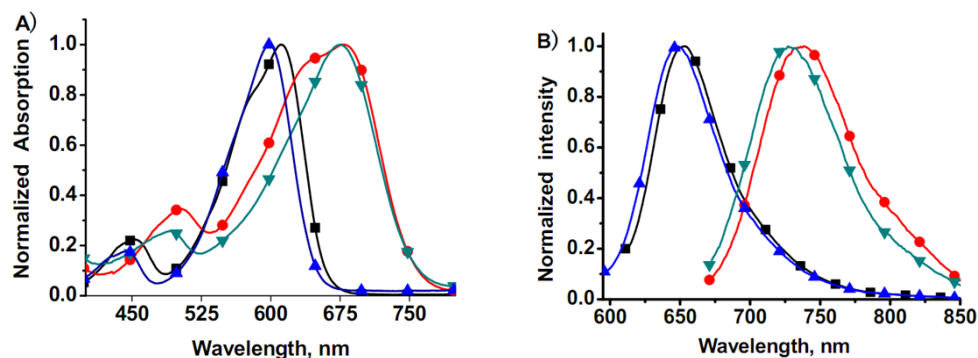


Fig. S2. A) Absorption spectra of the dyes; B) Emission spectra of the dyes; ACA-1 (■), ACA-2 (●), ACA-3 (▲), and ACA-4 (▼) in pH 7.4, 25 mM PBS buffer (containing 0.5 mg/mL BSA).

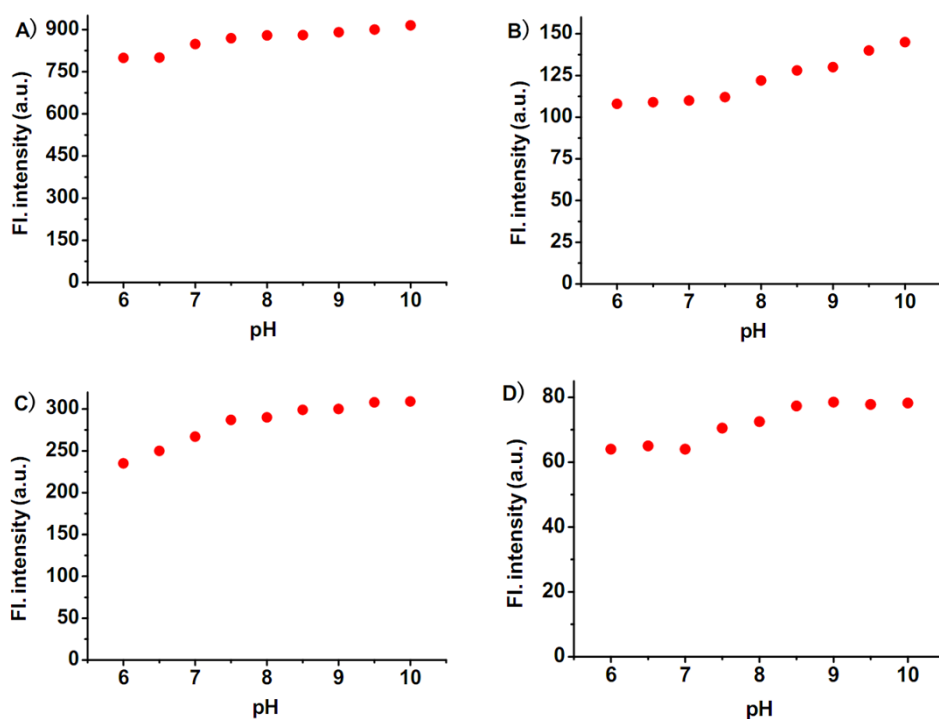


Fig. S3 pH-dependence of the fluorescence intensity of the dyes: A (ACA-1), B (ACA-2), C (ACA-3), and D (ACA-4).

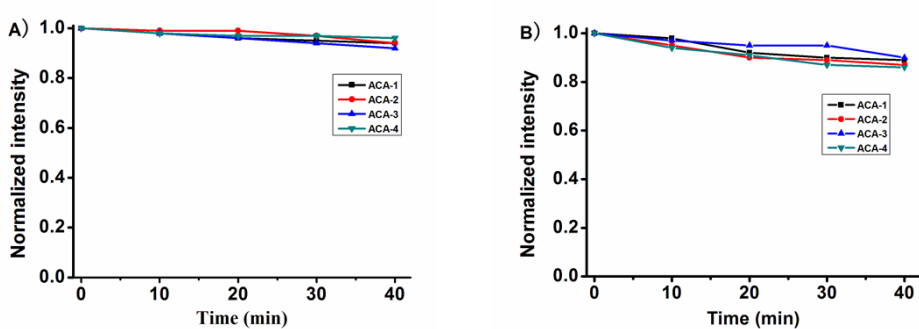


Fig. S4. Photostability of ACA-1 (■), ACA-2 (●), ACA-3 (▲), and ACA-4 (▼) in pH 7.4, 25 mM PBS buffer (containing 0.5 mg/mL BSA). The samples were continuously irradiated by UV light (365 nm) (A) and 500W Xe lamp (B).

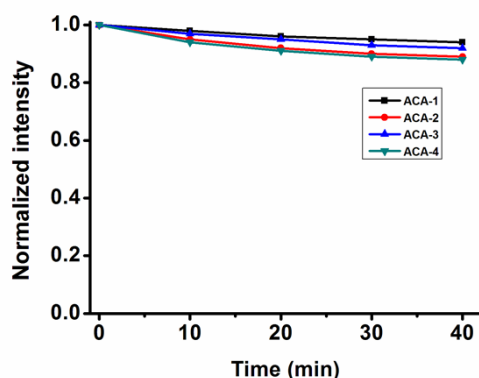


Fig. S5 Photostability of ACA-1 (■), ACA-2 (●), ACA-3 (▲), and ACA-4 (▼) in pH 7.4, 25 mM PBS buffer. The samples were continuously irradiated by UV light (365 nm).

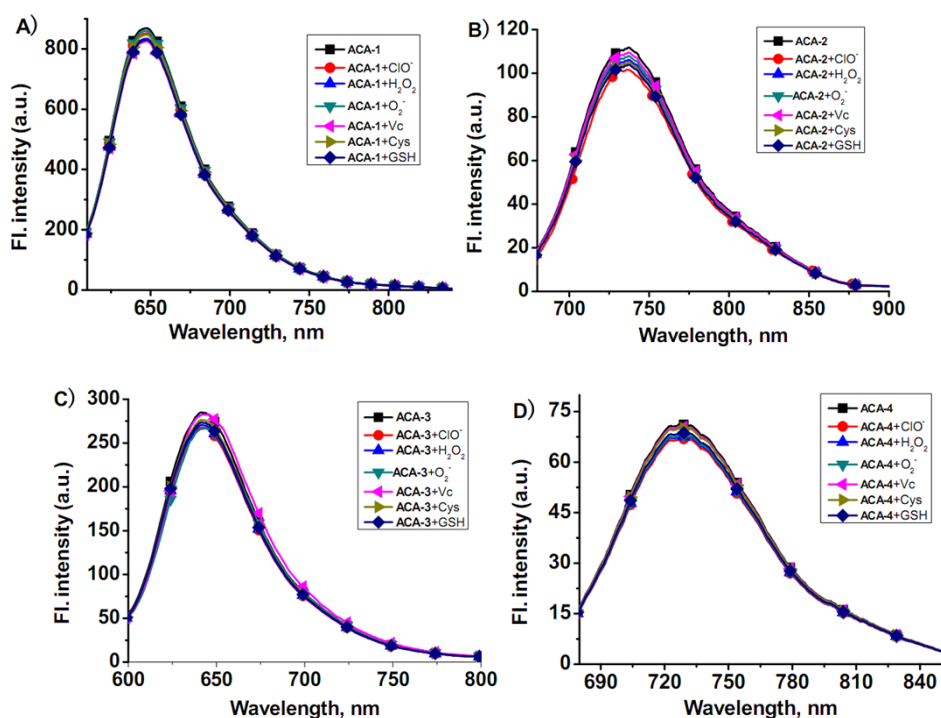


Fig. S6 Fluorescence spectra of a 5 μ M solution of ACA-1~4 before and after reaction with various the representative oxidizing and reducing reagents for 30 min, A (ACA-1), B (ACA-2), C (ACA-3) and D (ACA-4).

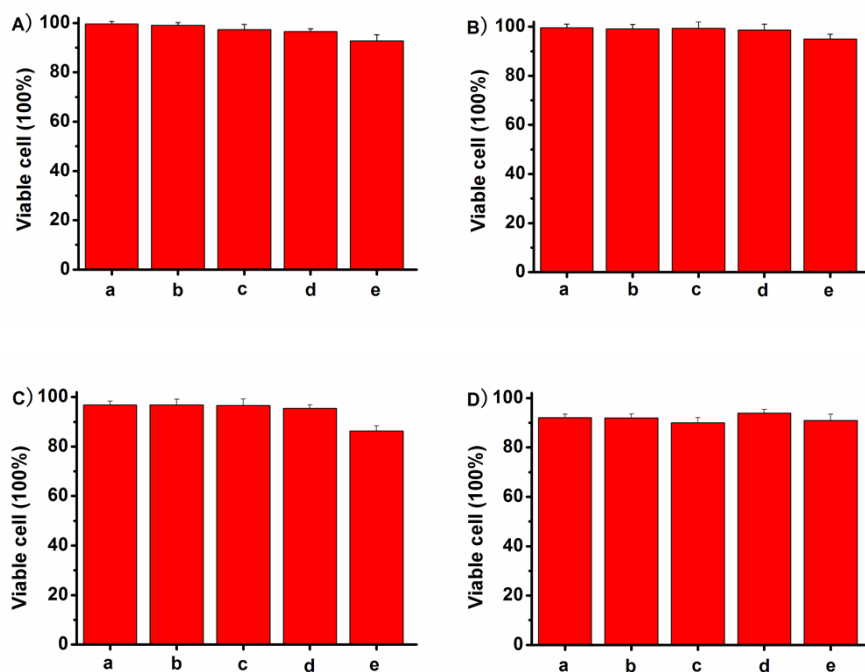


Fig. S7 Cytotoxicity assays of ACA-1~4 at different concentrations (a: 0 μM ; b: 2 μM ; c: 5 μM ; d: 10 μM ; e: 20 μM) for HeLa cells.

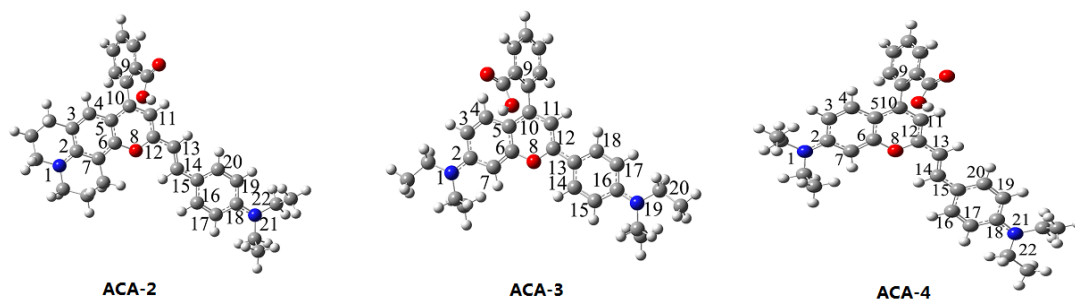


Fig. S8 DFT optimized structure of ACA-2~4. In the ball-and-stick representation, carbon, nitrogen, and oxygen atoms are colored in gray, blue, and red, respectively. H atoms were omitted for clarity.

Table S1. Representative C-C (N or O) Bond Lengths (in pm) of **ACA-2** determined by DFT Calculations.

C-C Bond	Bond Lengths (in pm)	C-C Bond	Bond Lengths (in pm)	C-C/N/O Bond	Bond Lengths (in pm)
C2-C3	144.2	C12-C13	141.8	C2-N1	136.3
C3-C4	137.3	C13-C14	137.5	C6-O8	142.4
C4-C5	141.8	C14-C15	142.9	C12-O8	135.4
C5-C6	141.9	C15-C16	141.8	C18-N21	136.5
C6-C7	138.7	C16-C17	137.9	C22-N21	147.1
C2-C7	142.5	C17-C18	142.7		
C5-C10	141.6	C18-C19	143.1		
C9-C10	149.4	C19-C20	137.7		
C10-C11	139.0	C15-C20	141.9		
C11-C12	139.9				

Table S2. Representative C-C (N or O) Bond Lengths (in pm) of **ACA-3** determined by DFT Calculations.

C-C Bond	Bond Lengths (in pm)	C-C Bond	Bond Lengths (in pm)	C-C/N/O Bond	Bond Lengths (in pm)
C2-C3	143.8	C11-C12	140.0	C2-N1	136.5
C3-C4	137.3	C12-C13	143.4	C6-O8	137.1
C4-C5	141.8	C13-C14	141.8	C12-O8	135.2
C5-C6	141.7	C14-C15	137.6	C16-N19	136.4
C6-C7	138.1	C15-C16	143.0	C20-N19	148.1
C2-C7	142.0	C16-C17	143.0		
C5-C10	142.0	C17-C18	137.8		
C9-C10	149.1	C13-C18	141.7		
C10-C11	139.1				

Table S3. Representative C-C (N or O) Bond Lengths (in pm) of **ACA-4** Determined by DFT Calculations.

C-C Bond	Bond Lengths (in pm)	C-C Bond	Bond Lengths (in pm)	C-C/N/O Bond	Bond Lengths (in pm)
C2-C3	143.6	C12-C13	141.5	C2-N1	136.4
C3-C4	137.3	C13-C14	137.7	C6-O8	137.1
C4-C5	141.8	C14-C15	142.7	C12-O8	135.5
C5-C6	141.8	C15-C16	141.8	C18-N21	136.6
C6-C7	138.2	C16-C17	137.9	C22-N21	147.1
C2-C7	141.9	C17-C18	142.8		
C5-C10	142.2	C18-C19	143.2		
C9-C10	149.2	C19-C20	137.5		
C10-C11	138.8	C15-C20	142.1		
C11-C12	140.2				

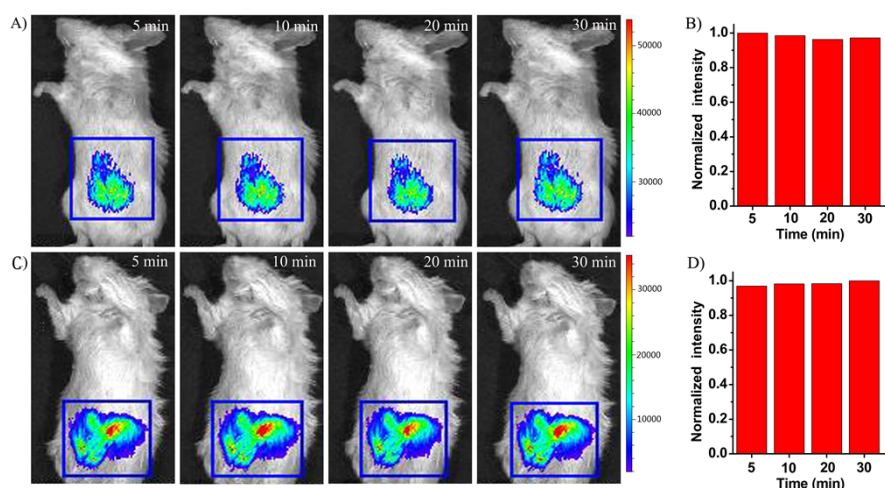


Figure S9. Time-lapsed (5, 10, 20 and 30 min) *in vivo* imaging of the mice with the dyes **ACA-2** (A) and **ACA-4** (C).

Reference:

- (a) R. C. Benson, H. A. Kues. *J. Chem. Eng. Data.*, 1977, **22**, 379-383; (b) D. Oushiki, H. Kojima, T. Terai, M. Arita, K. Hanaoka, Y. Urano, T. Nagano, *J. Am. Chem. Soc.*, 2010, **132**, 2795-2801.

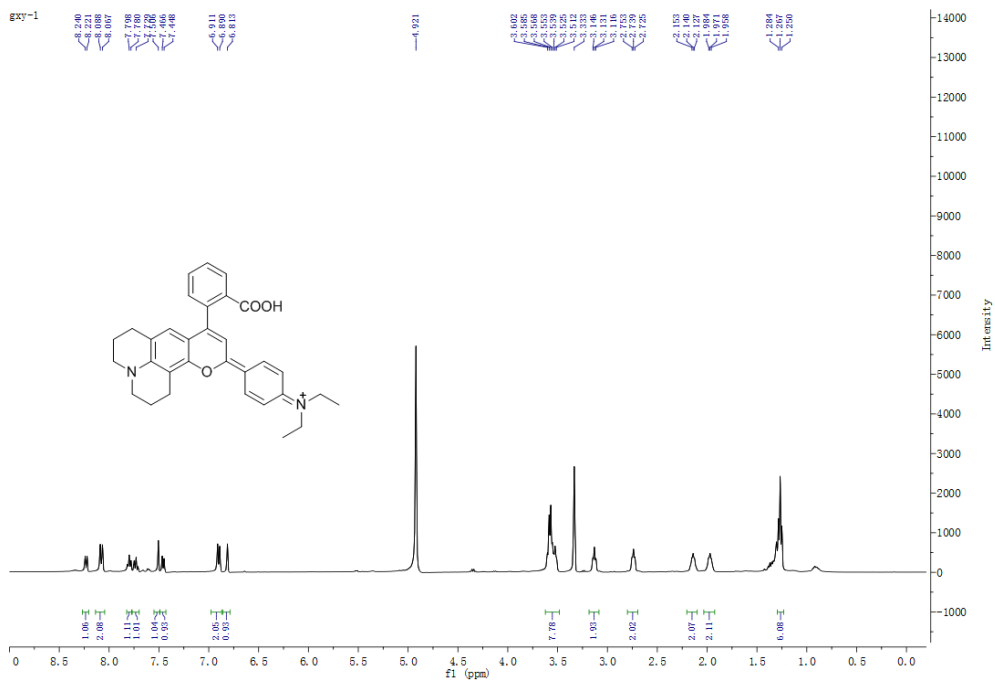


Fig. S10. ¹H NMR spectrum of the compound ACA-1.

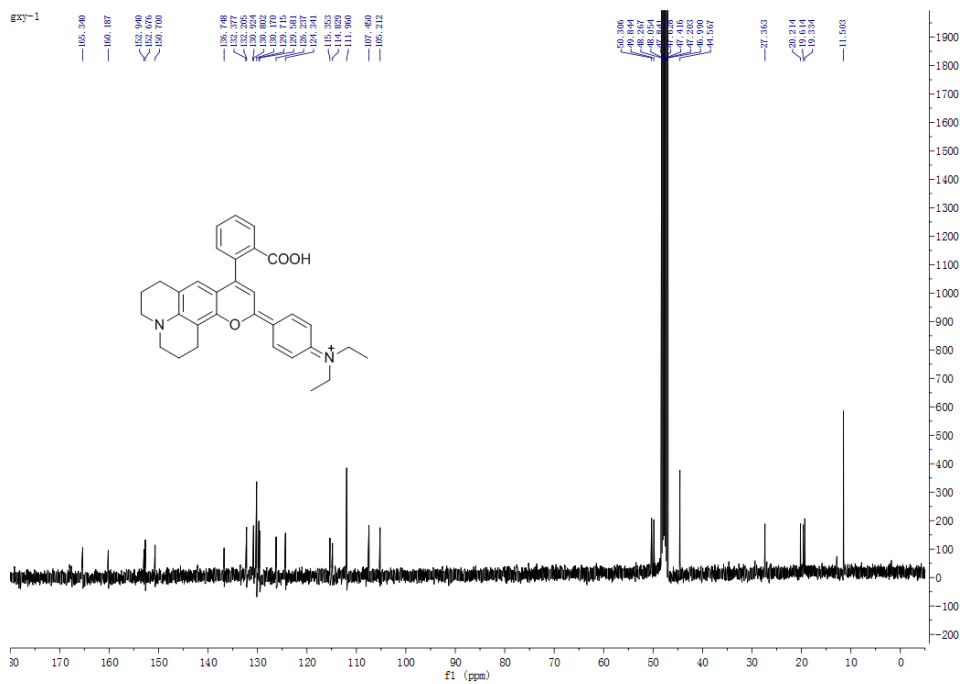


Fig. S11. ¹³C NMR spectrum of the compound ACA-1.

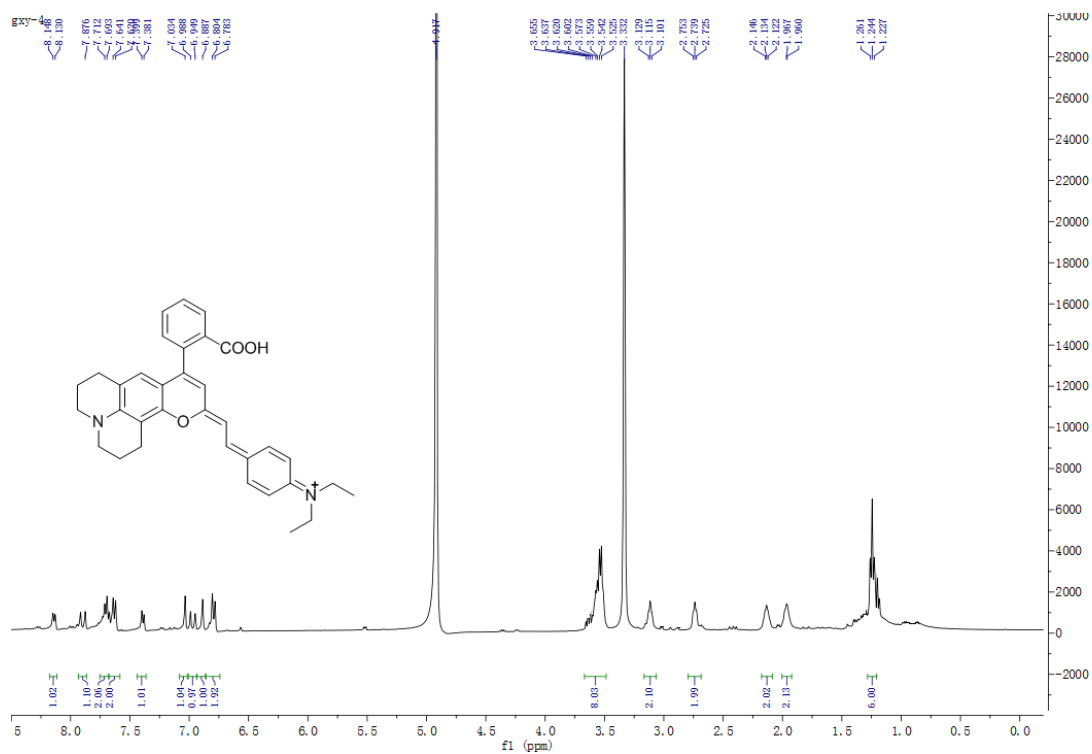


Fig. S12. ^1H NMR spectrum of the compound ACA-2.

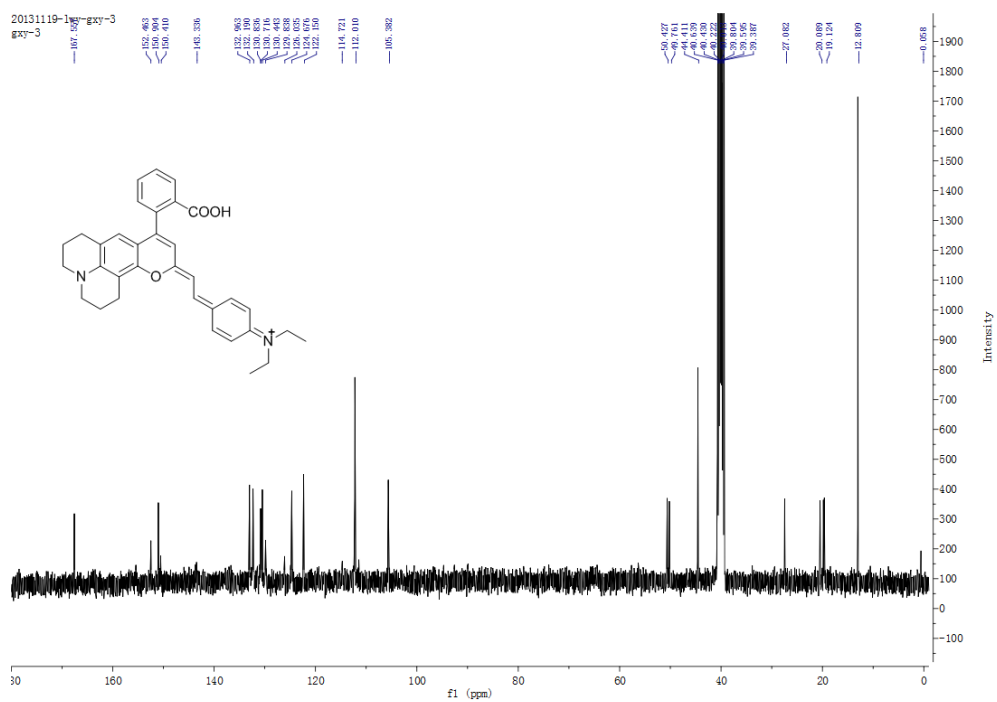


Fig. S13. ^{13}C NMR spectrum of the compound ACA-2.

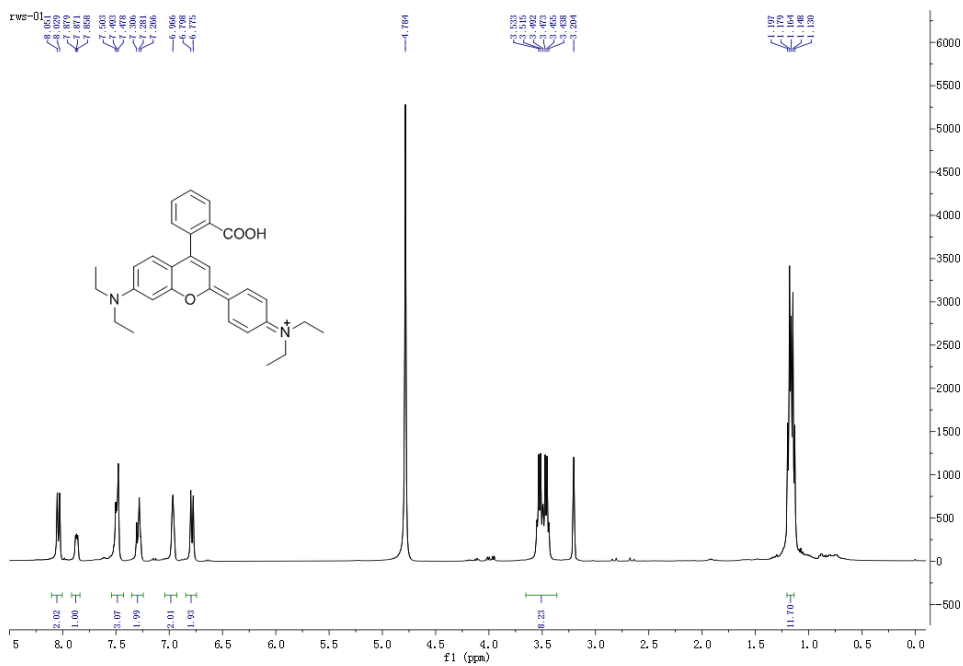


Fig.S14. ^1H NMR spectrum of the compound ACA-3.

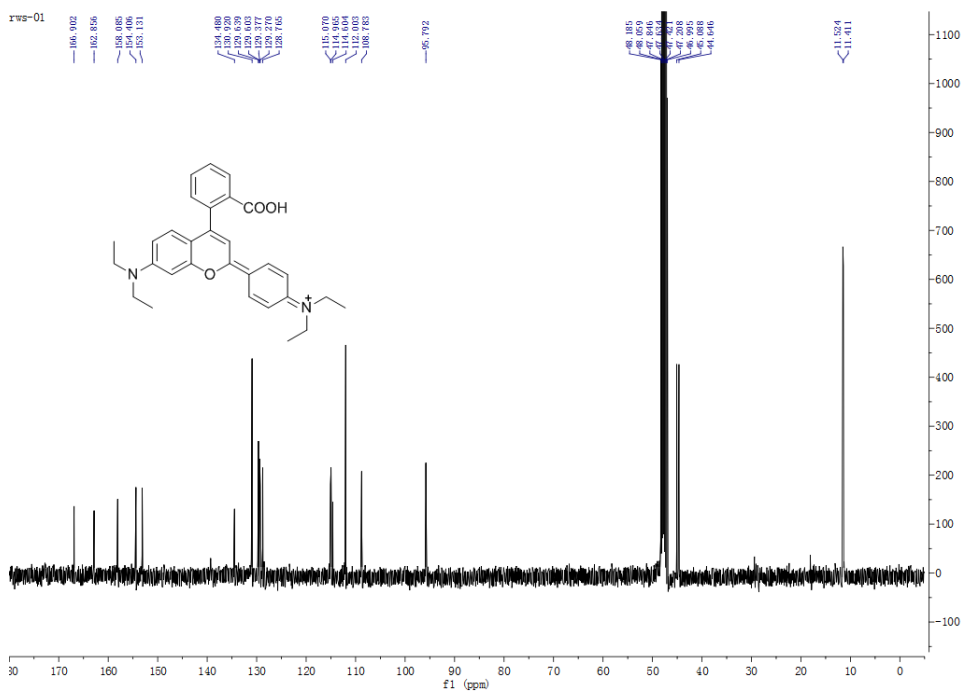


Fig.S15. ^{13}C NMR spectrum of the compound ACA-3.

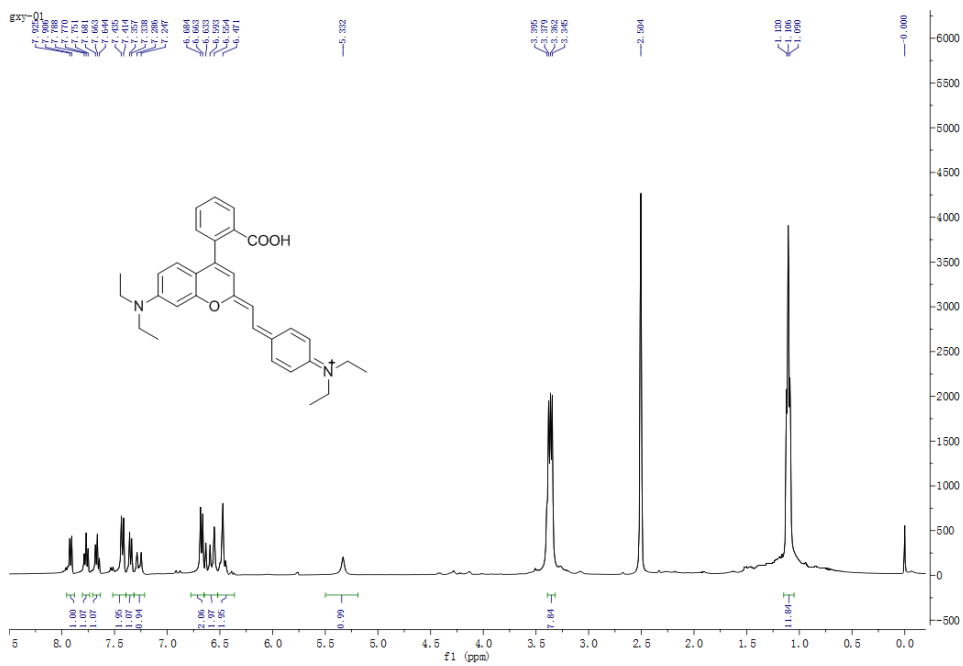


Fig. S16. ¹H NMR spectrum of the compound ACA-4.

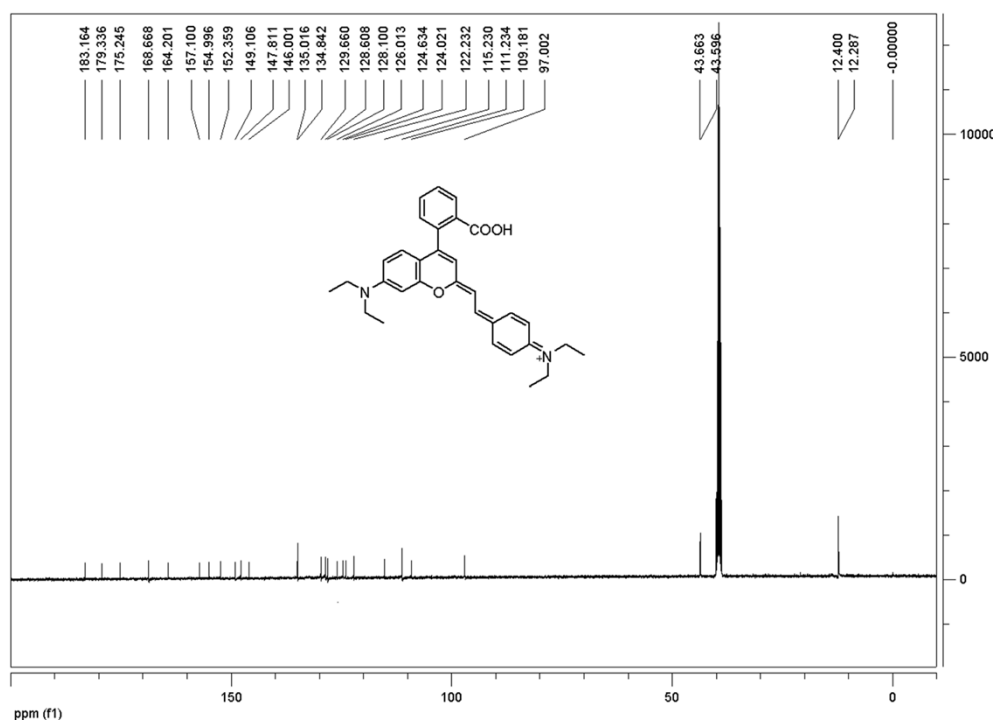


Fig. S17. ¹³C NMR spectrum of the compound ACA-4.