Electronic Supplementary Information

A novel coumarin derivative as sensitive probe for tracing intracellular pH changes

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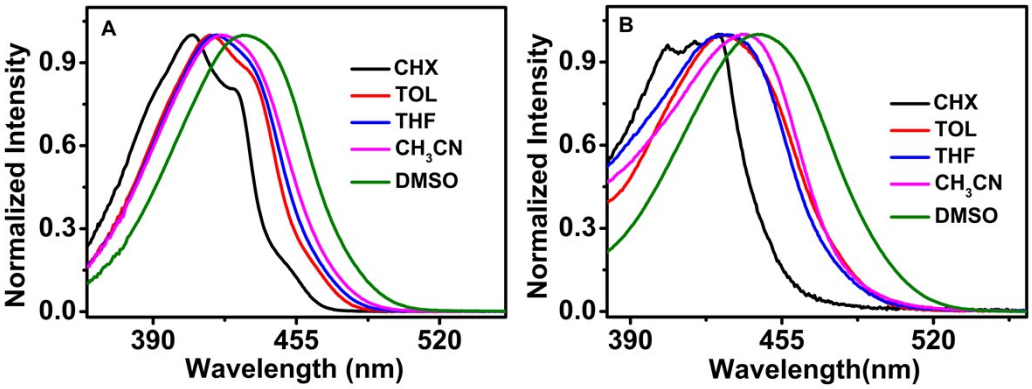


Figure S1 Absorption spectra of **CS** (A) and **CS-P** (B) in different solvents normalized to 1.0.

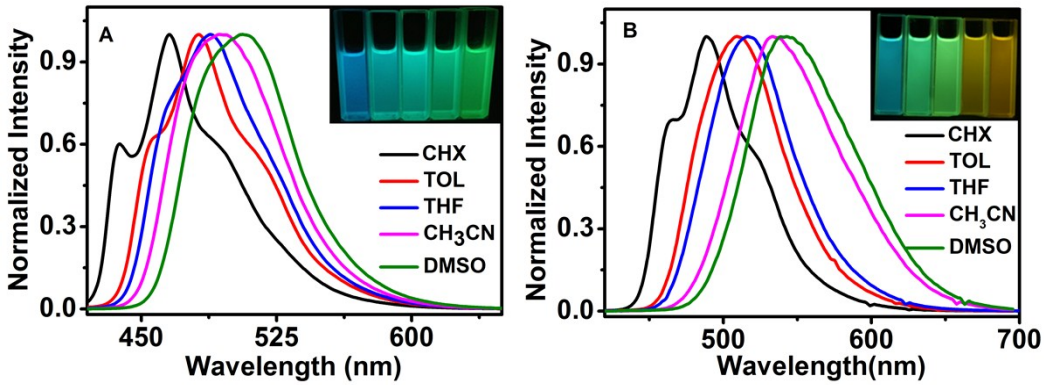


Figure S2 Fluorescence spectra of **CS** (A) and **CS-P** (B) in different organic solvent, the maxima peaks in **CS-P** showed strongly bathochromic-shift.

Table S1 UV-VIS and fluorescence emission data for **CS** and **CS-P** in different solvents.

Dye	Solvent	λ_{max} (nm)	ε_{max}(L mol^{-1} cm^{-1})	λ_{max} (nm)	stokes shift (nm)
CS	cyclohexane	408	7.3×10^4	465	57
	toluene	416	7.2×10^4	481	65
	tetrahydrofuran	420	9.3×10^4	488	68
	acetonitrile	422	7.6×10^4	495	73
	dimethylsulfoxide	433	7.7×10^4	507	74
CS-P	cyclohexane	415	1.1×10^4	489	74
	toluene	428	2.9×10^4	510	82
	tetrahydrofuran	432	2.2×10^4	517	85
	acetonitrile	440	3.0×10^4	533	93
	dimethylsulfoxide	446	6.7×10^4	542	96

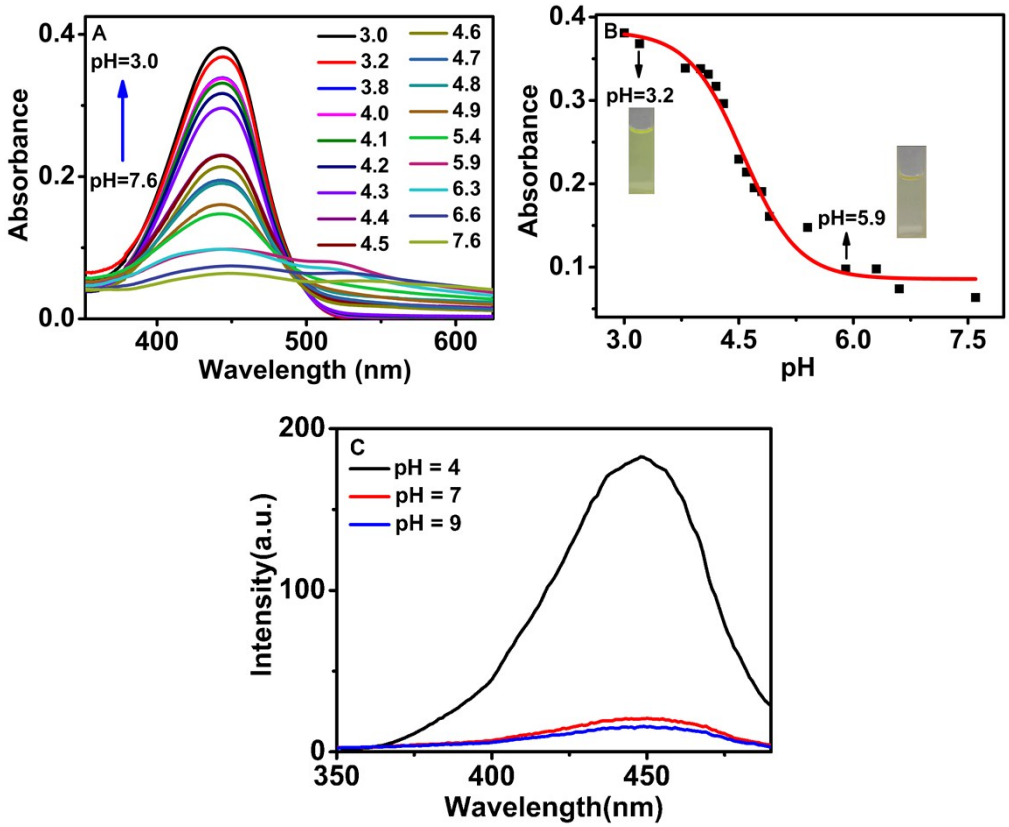


Figure S3 (A) Absorption spectra of **CS-P** (4 μM) in DMSO-water solution (1:1, v/v) at different pH values, **(B)**The absorption curve of **CS-P** at different pH values based on the intensity peaks at 443 nm, (**C**) The excitation spectra of CS-P (4 μM) in DMSO-water solution (1:1, v/v) at different pH values.

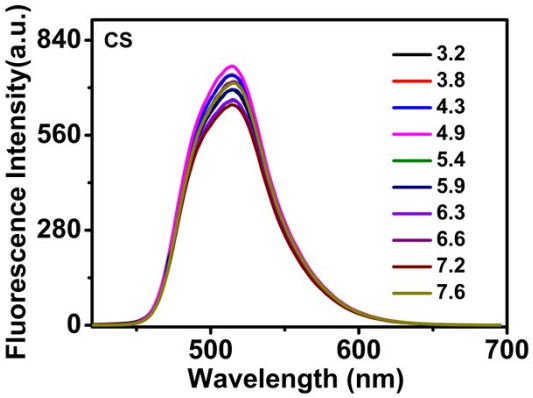


Figure S4. Fluorescence spectra of **CS** (4 μM) in various pH (7.6-3.2) in DMSO-water (1:1, v/v) solution (λ_{ex} = 400 nm).

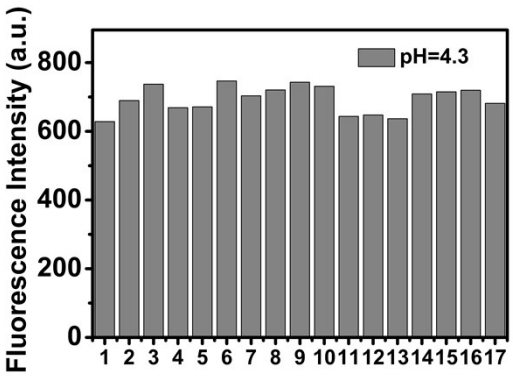


Figure S5 Ion interfering to **CS-P.** 1) H^{+}; 2)Mg^{2+}; 3)Na^{+}; 4)Mn^{2+}; 5)Ba^{2+}; 6)K^{+}; 7)Ca^{2+}; 8)Cu^{2+}; 9)Fe^{3+}; 10)Cr^{3+}; 11)Fe^{2+}; 12)Ni^{2+}; 13)Hg^{2+}; 14)Pb^{2+}; 15)Zn^{2+}; 16)Cys; 17)GSH. The maxima peak value of fluorescence spectra in DMSO-water (1:1, v/v) solution at pH 4.3, which proved that different ions had negligible effect on the fluorescent property of **CS-P**.

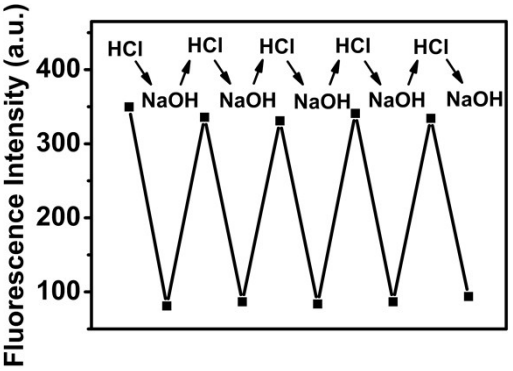


Figure S6 Reversibility of **CS-P**. Fluorescent intensities of **CS-P** in DMSO-water (1:1, v/v) solution with changed pH value by adding HCl or NaOH repeatedly, which proved the reversibility of fluorescence intensity of **CS-P** by changing pH value simply (λ_{ex} = 400 nm).

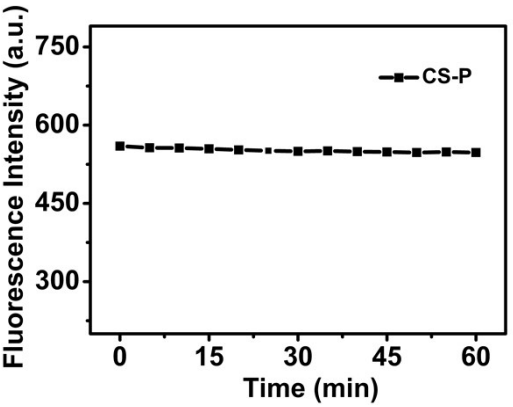


Figure S7 Photostability of **CS-P**. The fluorescent intensities of **CS-P** did not decreased within 60 minutes of continuous excitation, which indicated high photostability of **CS-P** (λ_{ex} = 400 nm and λ_{em} = 515 nm).

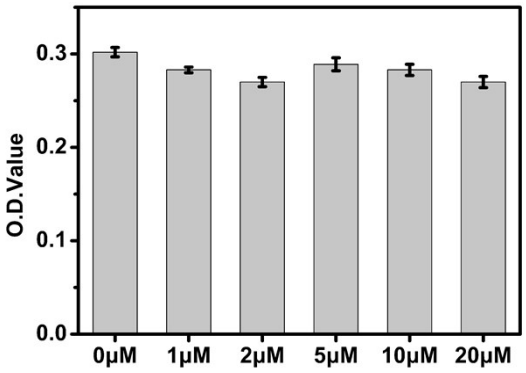
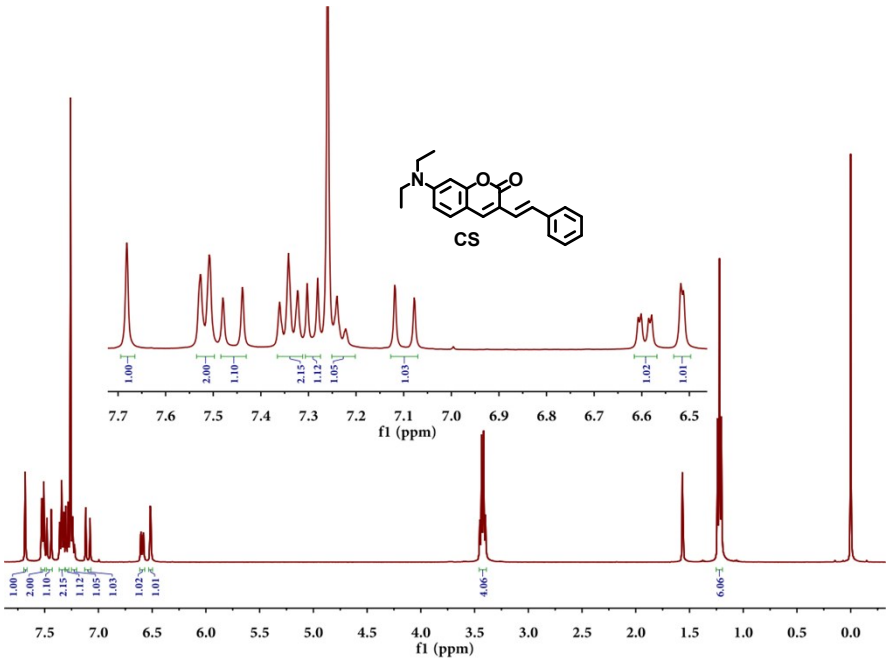
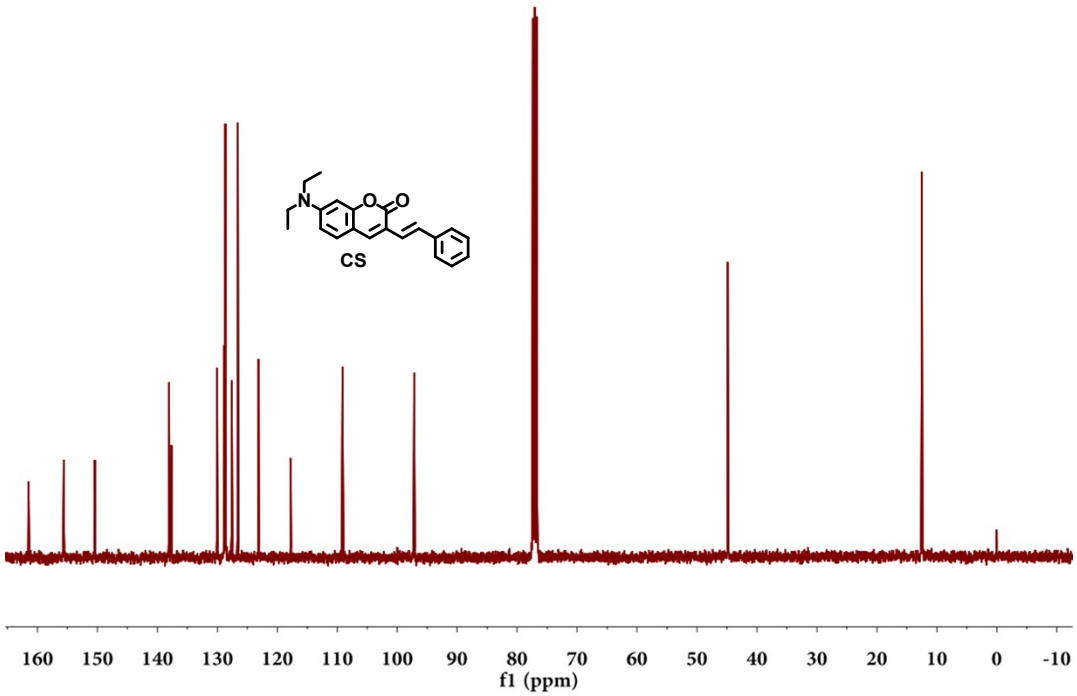


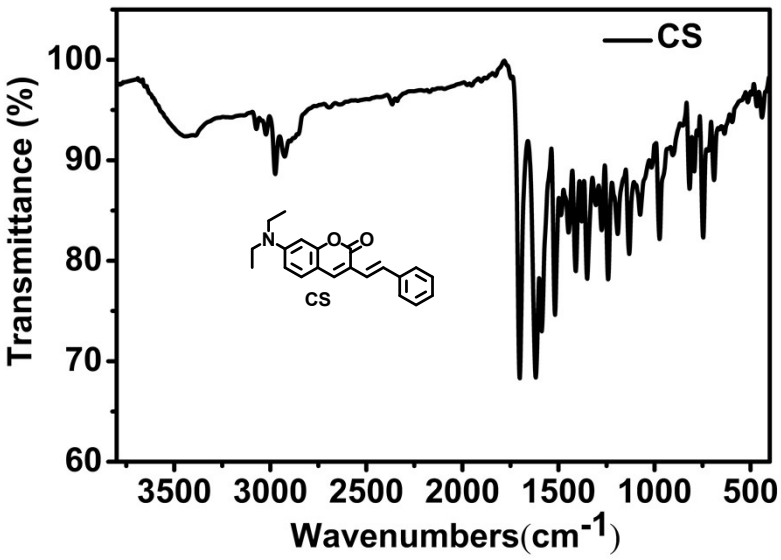
Figure S8 Cytotoxic experiment of CS-P via MTT method. The number of MG63 cells did not decreased after treatment with different concentrations of **CS-P** for 24 hours.



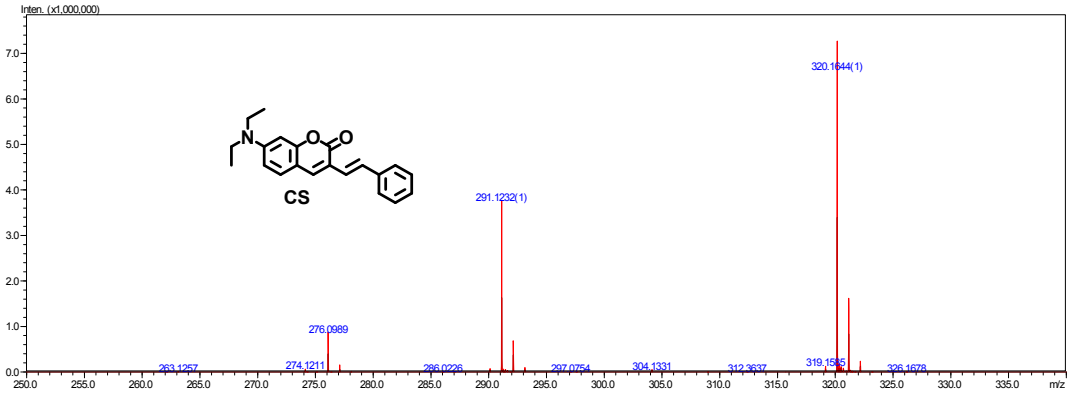
^{1}H NMR spectrum of **CS**



^{13}C NMR spectrum of **CS**

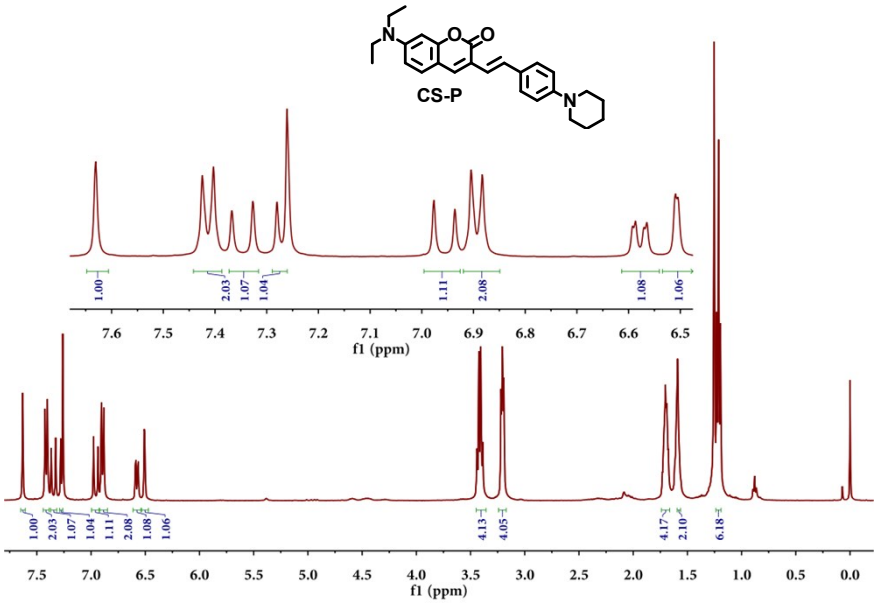


FT-IR spectrum of **CS**

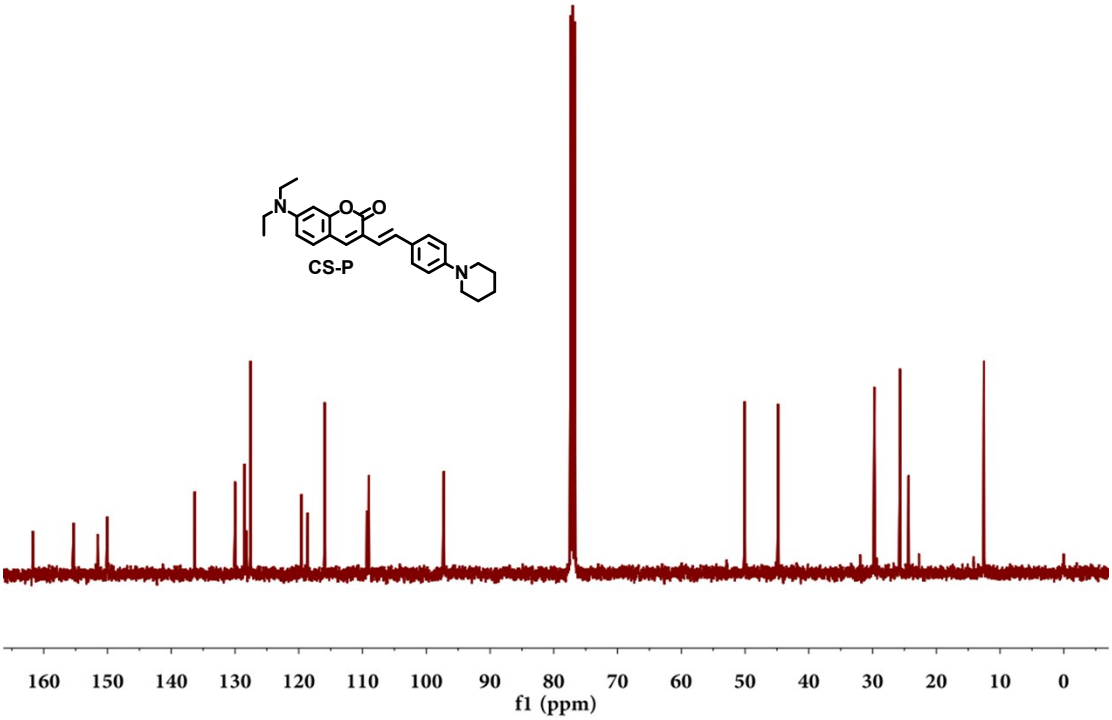


HR-ESI-MS spectrum of **CS**

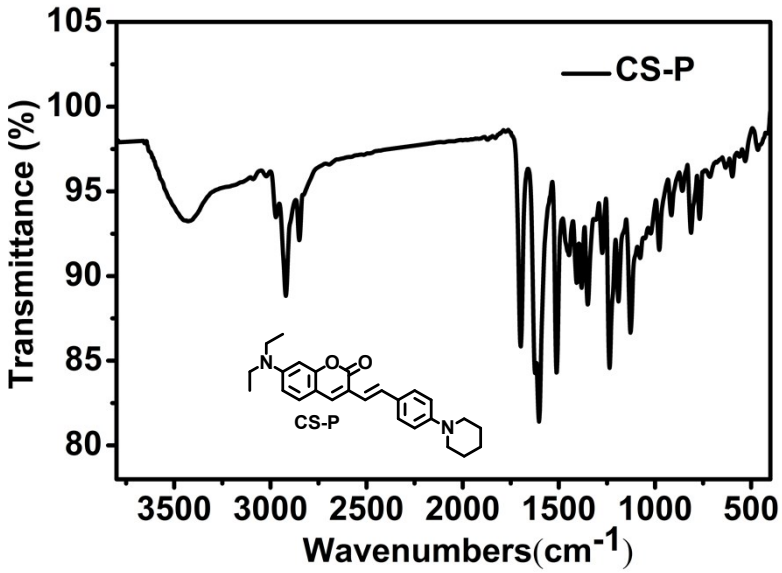
Figure S9 ^{1}H NMR, ^{13}C NMR, FT-IR and HR-ESI-MS spectrum of **CS**



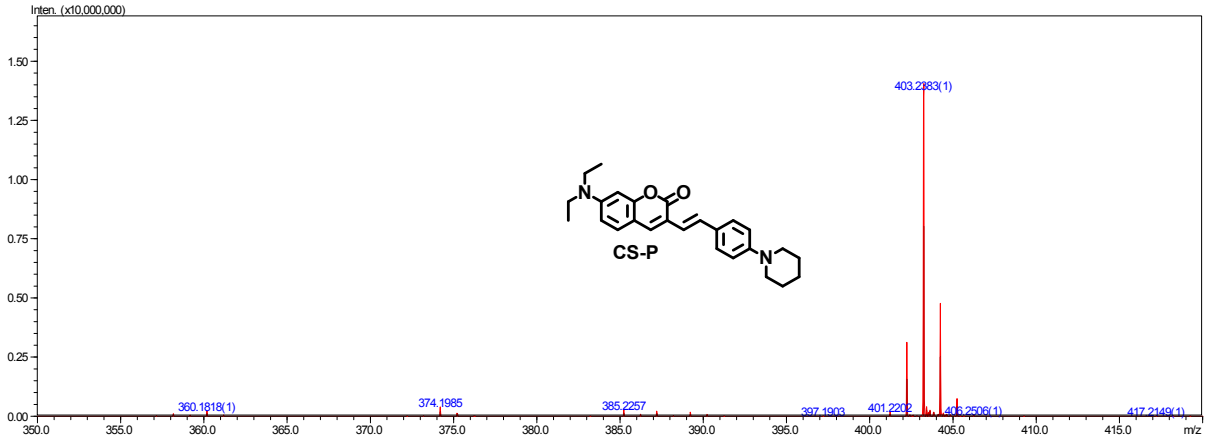
^{1}H NMR spectrum of **CS-P**



^{13}C NMR spectrum of **CS-P**



FT-IR spectrum of **CS-P**



HR-ESI-MS spectrum of **CS-P**

Figure S10 ^{1}H NMR, ^{13}C NMR, FT-IR and HR-ESI-MS spectrum of **CS-P**