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An instantaneous fluorescent probe for detecting hydrogen sulfide in biological systems

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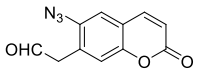
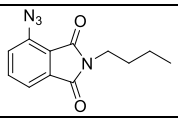
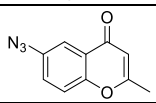
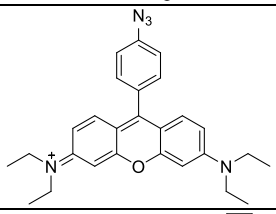
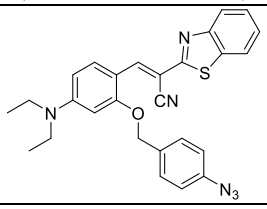
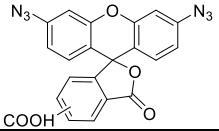
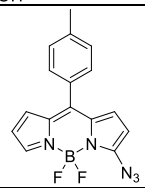
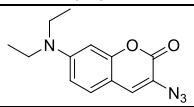
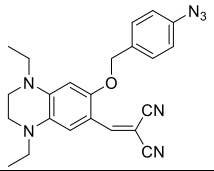
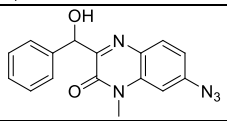
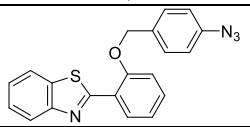
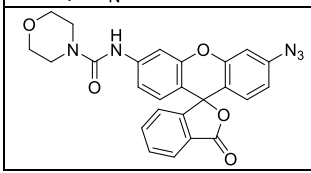
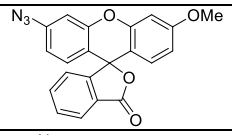
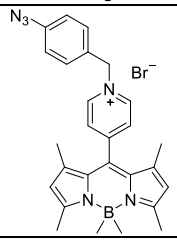
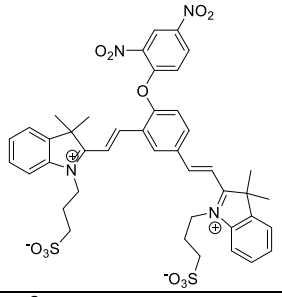
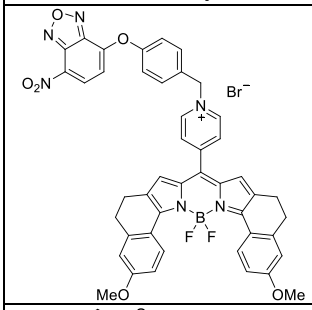
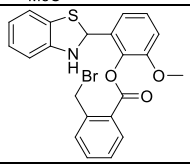
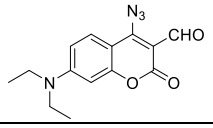
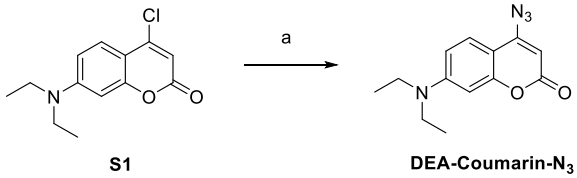
Table S1. Some Fluorescent probes for hydrogen sulfide.

probes	response time	detection limit (μM)	medium	literatures
	>120 min	0.22	In water (containing 5% acetonitrile)	*New J. Chem.*, 2014, **38**, 2770-2773
	40 min	0.0136	In PBS buffer (containing 1.0 mM CTAB)	*RSC Adv.*, 2015, **5**, 98154-98159
	30 min	0.018	In PBS buffer (1:1, v/v)	*Talanta*, 2019, **195**, 850-856
	-	0.25	In DMF/PBS buffer (6:4, v/v, 10 mM)	*New J. Chem.*, 2016, **40**, 6384-6388
	60 min	0.15	In Tris buffer (50 mM, pH = 8.0, containing 50% DMF)	*Talanta*, 2015, **135**, 149-154
	45 min	0.112	In PBS buffer (pH = 7.4)	*Tetrahedron Lett.*, 2013, **54**, 4826-4829
	10 min	0.259	In HEPES buffer (10 mM, pH = 7.4)	*Org. Biomol. Chem.*, 2013, **11**, 8166-8170
	60 min	-	In PBS buffer (pH 7.4, containing 0.05% DMF)	*J. Fluoresc.*, 2013, **23**, 181-186
	30 min	0.0057	In HEPES buffer (containing 1.0 mM CTAB)	*Org. Biomol. Chem.*, 2018, **16**, 1150-1156
	>240 min	1.3	In PBS buffer (pH = 7.4)	*New J. Chem.*, 2017, **41**, 10432-10437
	30 min	2.4	In PBS-CAN buffer (1:1, v/v)	*Talanta*, 2014, **121**, 122-126
	60 min	10	In HEPES buffer (20 mM, pH 7.4)	*J. Am. Chem. Soc.*, 2011, **133**, 10078-10080

	> 60 min	0.086	In PIPES buffer (50 mM, pH 7.4, containing 100 mM KCl)	*J. Am. Chem. Soc.*, 2015, **137**, 10216-10223
	150 min	0.8	In PBS buffer (10 mM, pH 7.4)	*Chem. Lett.*, 2015, **44**, 1524-1526
	2 min	0.011	In PBS buffer (10 mM, pH = 7.2)	*Spectrochim. Acta A*, 2019, **213**, 416-422
	10 min	1.15	In DMSO/PBS buffer (1:1, v/v, 10 mM, pH 7.4)	*Sens. Actuators, B*, 2018, **257**, 1076-1082
	> 60 min	0.5	In HEPES buffer (20 mM, pH 7.4, containing 1 mM CTAB)	*Chin. Chem. Lett.*, 2016, **27**, 1793-1796
	within seconds	0.0035	In acetonitrile/deionized water (3: 7, v/v)	***This work***

Experimental section

Synthesis route of DEA-Coumarin-N_3



Scheme S1. Synthesis of **DEA-Coumarin-N_3**. (a) NaN_3, N-methylpyrrolidone (NMP), room temperature, 2 h, yield 81%.

Synthesis of S1

S1 was synthesized according to the reported method.[1]

Synthesis of DEA-Coumarin-N_3

To a solution of **S1** (0.251 g, 1 mmol) in 1 mL NMP was added sodium azide (0.078 g, 1.2 mmol). The mixture was stirred at room temperature for 2 h and poured into 50 mL H_2O. After filtration, the crude solid was dried under vacuum and purified by silica column chromatography to give product **DEA-Coumarin-N_3** (210 mg, yield 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, *J* = 9.0 Hz, 1H), 6.55 (dd, *J* = 9.0, 2.2 Hz, 1H), 6.47 (d, *J* = 2.1 Hz, 1H), 5.76 (s, 1H), 3.41 (q, *J* = 7.1 Hz, 4H), 1.21 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.08, 156.28, 153.82, 151.68, 124.37, 108.63, 103.51, 97.24, 93.65, 44.84, 12.40. HRMS (EI) m/z: calcd for $C_{13}H_{14}N_4O_2$ $[M+Na]^+$, 281.1009; found, 281.1030.

Study of the photoinduced electron transfer process (PET)

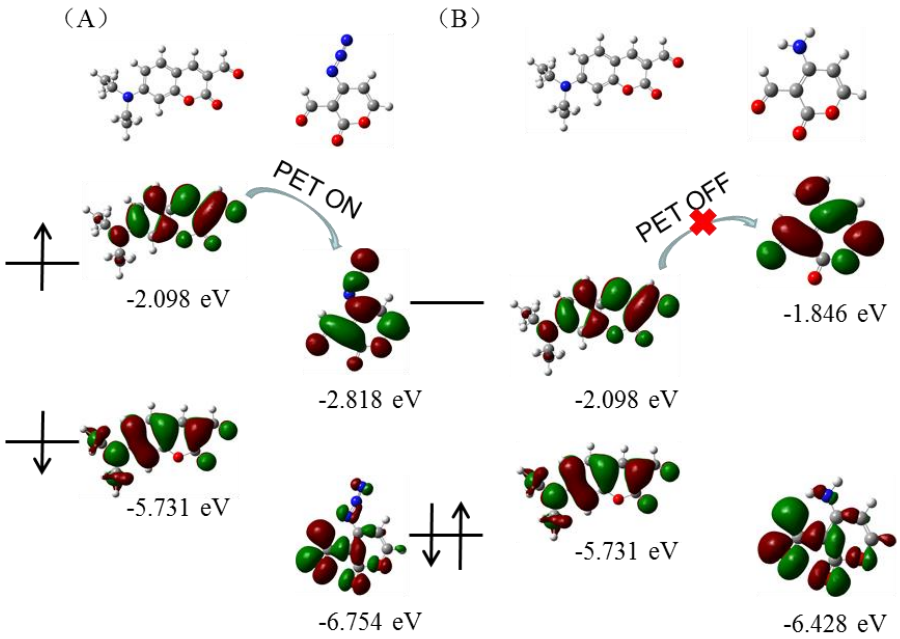
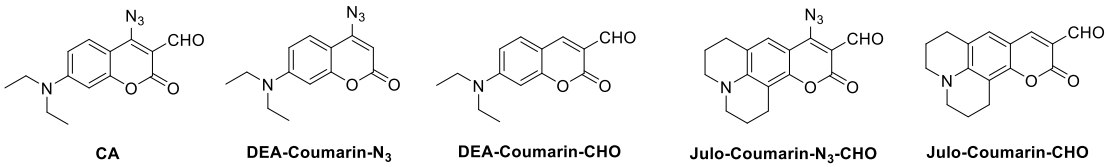


Fig. S1 Orbital energy diagrams of the photoinduced electron transfer process of probe **CA** before (A) and after (B) the reaction with H_2S.

We performed DFT calculation on probe **CA** and the expected product of probe **CA** with H_2S, **CA-NH_2**. As shown in Fig. S1, a strong PET effect in probe **CA** can be triggered by azido group while the PET process is inhibited in **CA-NH_2**. To make a

better explanation, we made compounds **DEA-Coumarin-N$_3$**, **DEA-Coumarin-CHO**, **Julo-Coumarin-N$_3$-CHO**, and **Julo-Coumarin-CHO** (Scheme S2). Both **DEA-Coumarin-N$_3$** (Fig. S2) and **DEA-Coumarin-CHO**[2] exhibited weak fluorescence. But probe **CA** was completely non-fluorescent. Furthermore, TICT (twisted intramolecular charge transfer) process also contributed to the fluorescence quench of probe **CA** (twisted diethylamino group at excited state). Compound **Julo-Coumarin-CHO** containing a rigid julolidine moiety without azido group had been reported to be strongly fluorescent.[2] In contrast, **Julo-Coumarin-N$_3$-CHO** was completely non-emissive and exhibited strong fluorescence after reacting with H_2S (Fig. S3). As a result, it could be confirmed that the fluorescence quench of **Julo-Coumarin-N$_3$-CHO** was mainly caused by the PET process from azido group.



Scheme S2. Chemical structures of **CA**, **DEA-Coumarin-N$_3$**, **DEA-Coumarin-CHO**, **Julo-Coumarin-N$_3$-CHO** and **Julo-Coumarin-CHO**.

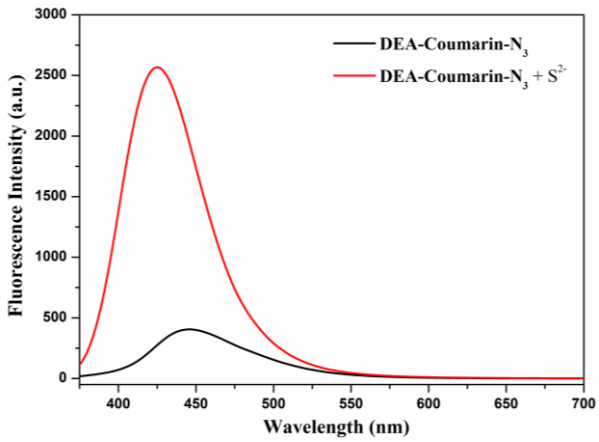


Fig. S2 Fluorescence spectra of compound **DEA-Coumarin-N$_3$** (10.0 μM) in the absence (black) and presence (red) of Na_2S (15 equiv.) in acetonitrile/deionized water (3:7, v/v). Excitation wavelength: 355 nm.

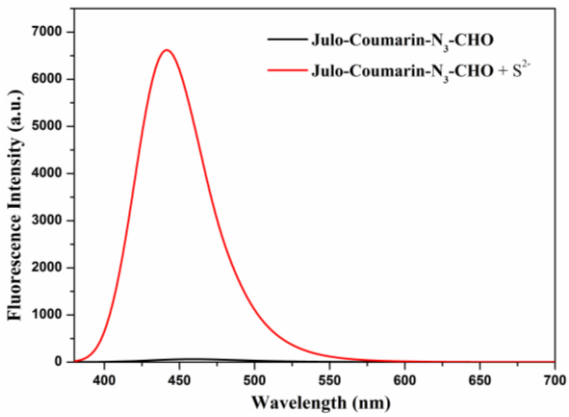


Fig. S3 Fluorescence spectra of **Julo-Coumarin-N_3-CHO** (10.0 μM) in the absence (black) and presence (red) of Na_2S (15 equiv.) in acetonitrile/deionized water (3:7, v/v). Excitation wavelength: 360 nm.

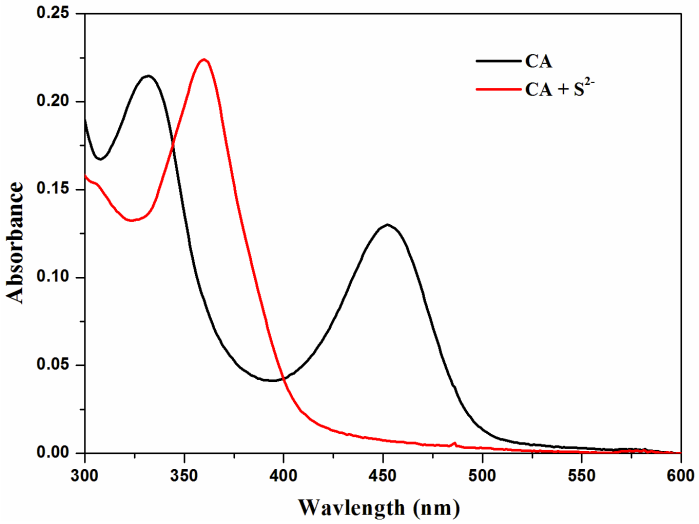


Fig. S4 Absorption spectra of probe **CA** (10.0 μM) in the absence (black) and presence (red) of Na_2S (15.0 equiv.) in acetonitrile/deionized water (3:7, v/v).

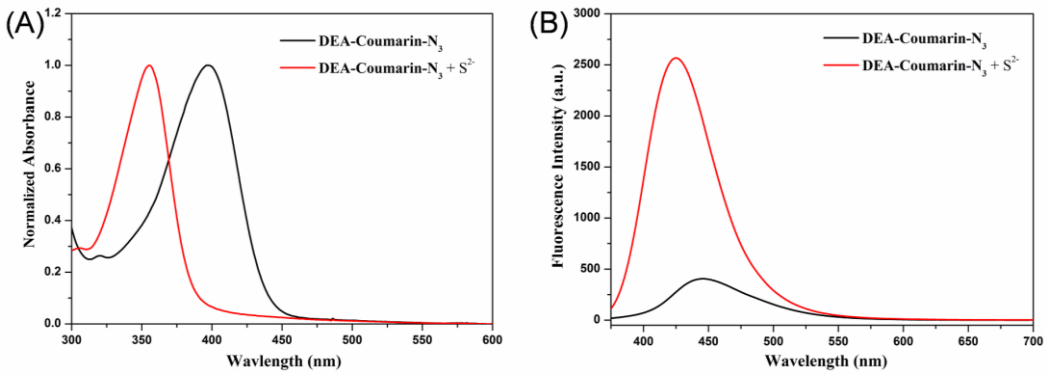


Fig. S5 Normalized absorbance spectra (A) and emission spectra (B) of **DEA-Coumarin-N_3** (10.0 μM) in the absence (black) and presence (red) of Na_2S (15.0 equiv.) in acetonitrile/deionized water (3:7, v/v). Excitation wavelength: 355 nm. slit widths: 5.0 nm/5.0 nm.

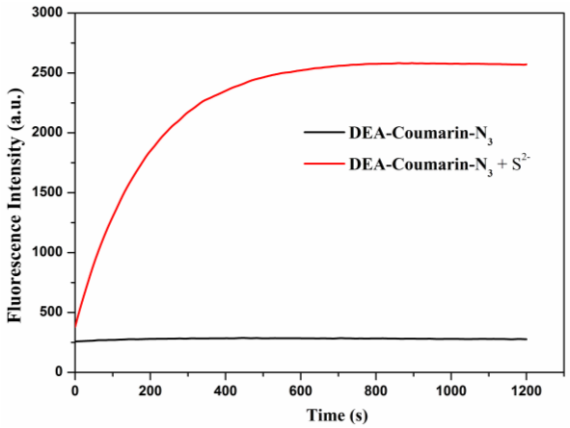


Fig. S6 Plot of fluorescence intensity of **DEA-Coumarin-N_3** (10.0 μM) with the addition of Na_2S (15.0 equiv.) in acetonitrile/deionized water (3:7, v/v) versus time. Excitation wavelength: 355 nm. slit widths: 5.0 nm/5.0 nm.

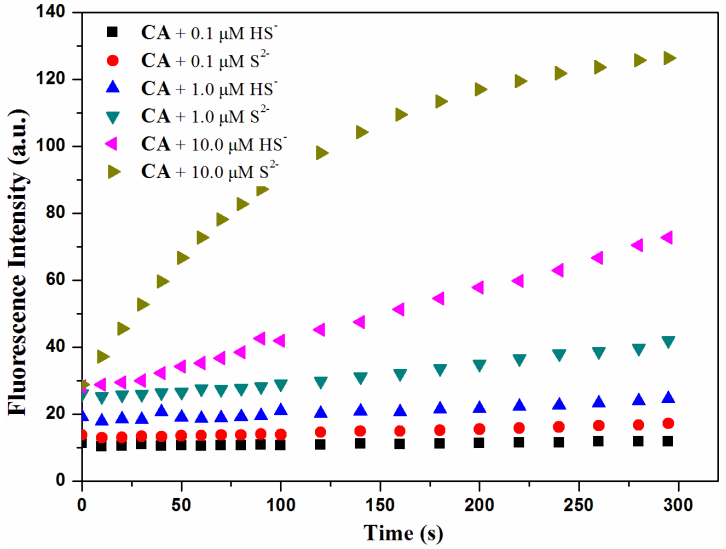


Fig. S7 Plot of fluorescence intensity of probe **CA** (10.0 μM) with the addition of Na_2S and NaHS in different concentrations (0.1 μM, 1 μM and 10 μM) in acetonitrile/deionized water (3:7, v/v) versus time.

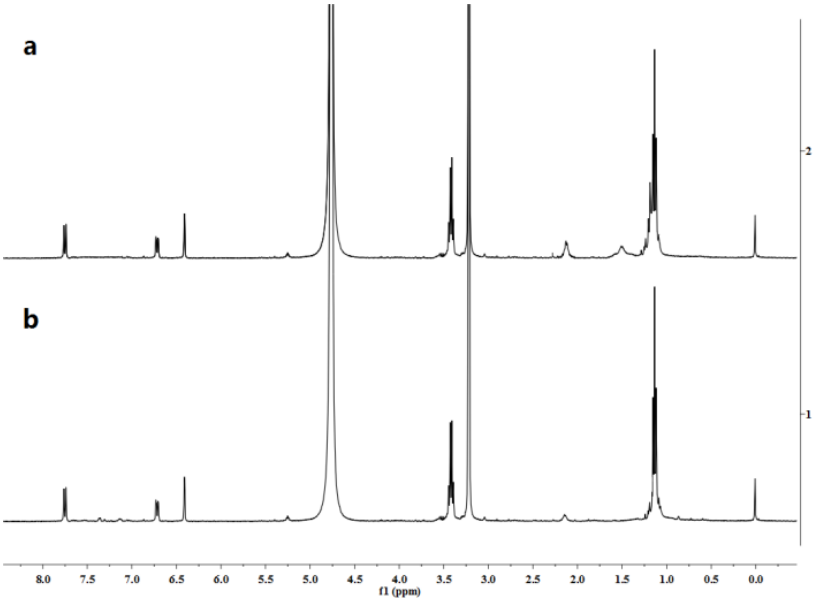


Fig. S8 ^{1}H NMR (400 MHz) spectra of dye **CA-NH$_2$** (a) and the isolated product of probe **CA** with Na_2S (b) in CD_3OD.

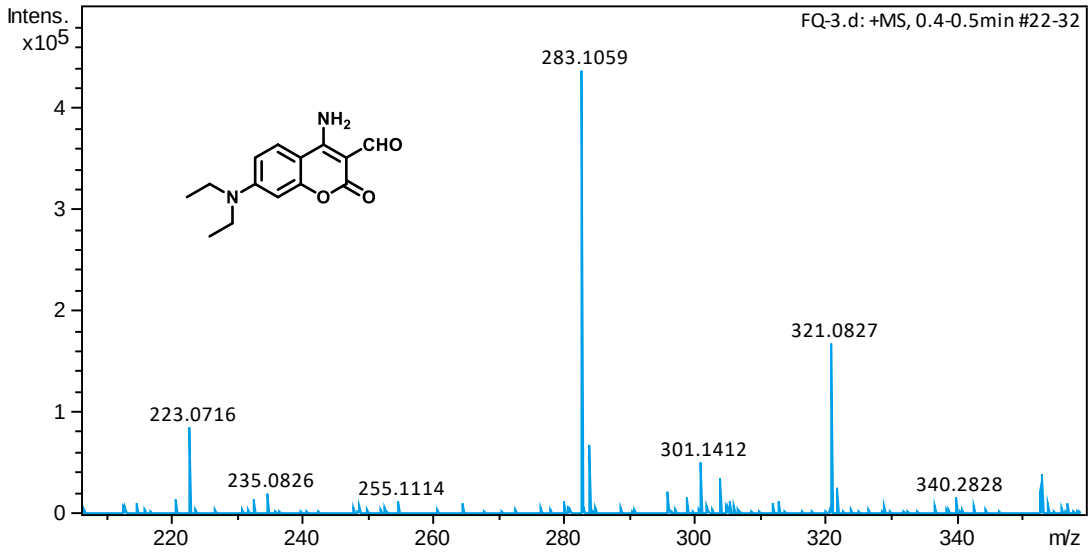


Fig. S9 HRMS spectrum of the reaction product from probe **CA** with Na_2S.

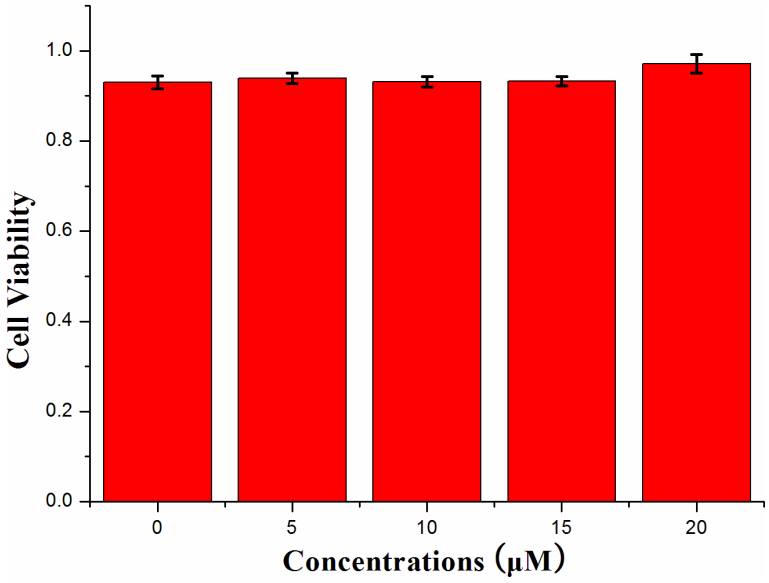


Fig. S10 Cytotoxicity assay of probe **CA** at different concentrations for HeLa cells.

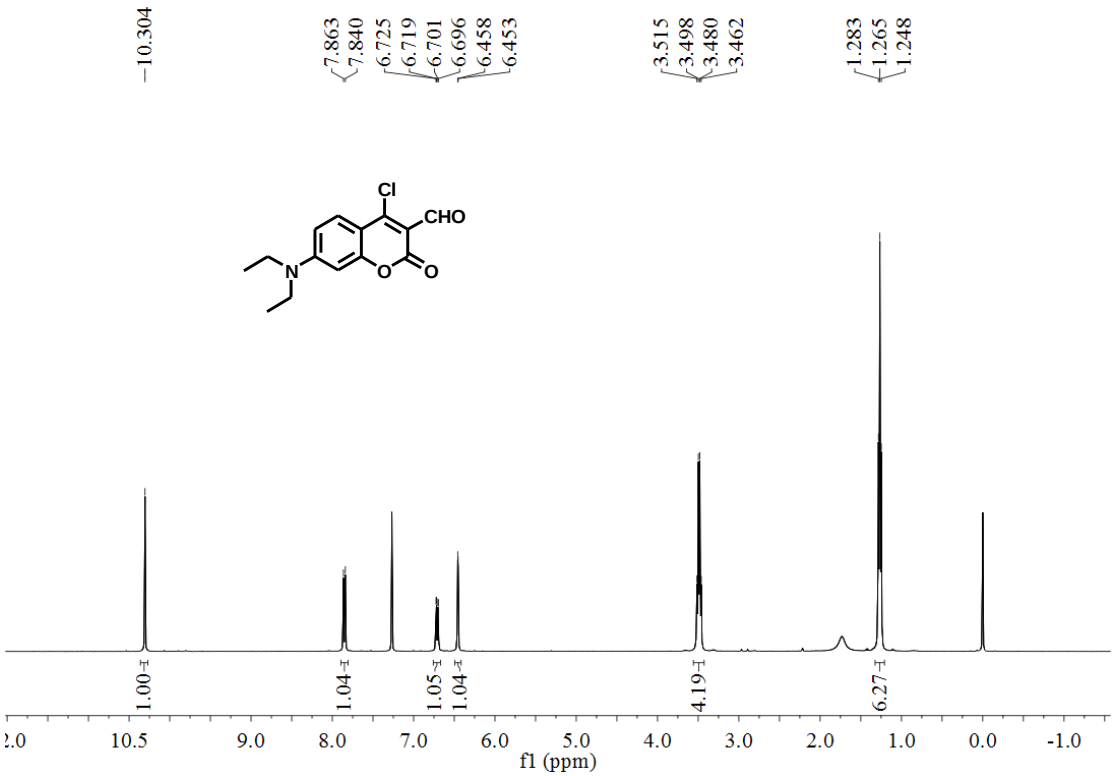


Fig. S11 ^{1}H NMR spectrum of compound **2** in $CDCl_3$.

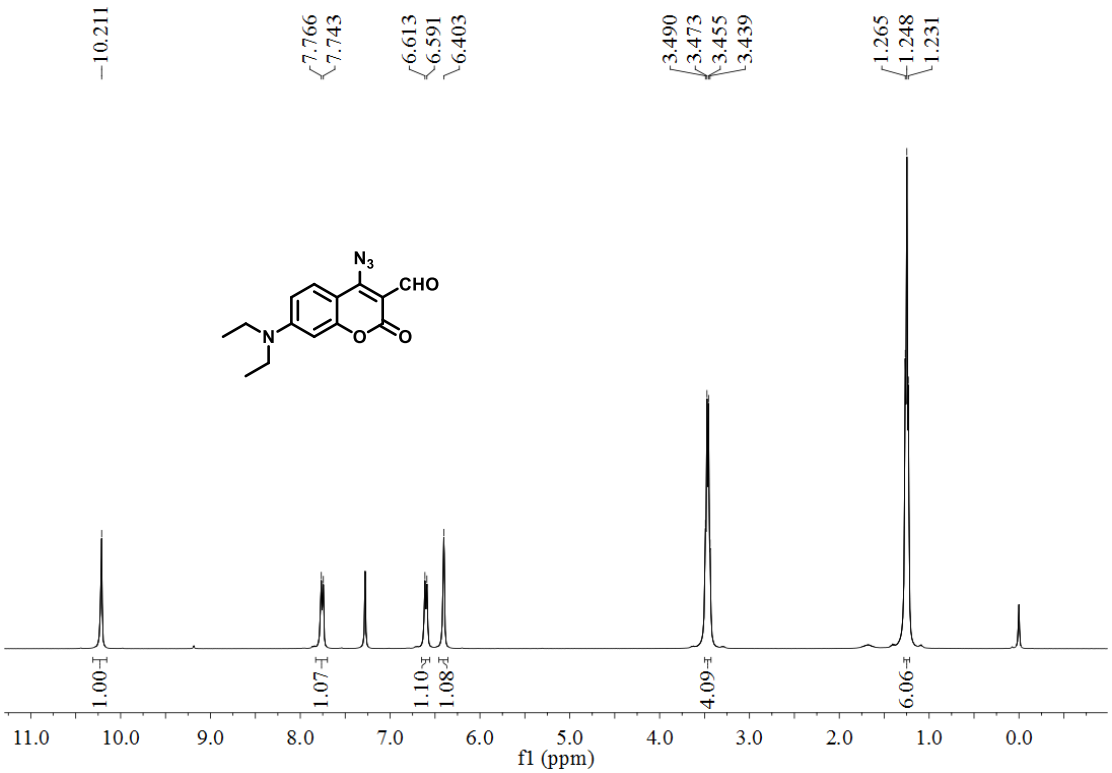


Fig. S12 ^{1}H NMR spectrum of probe **CA** in $CDCl_3$.

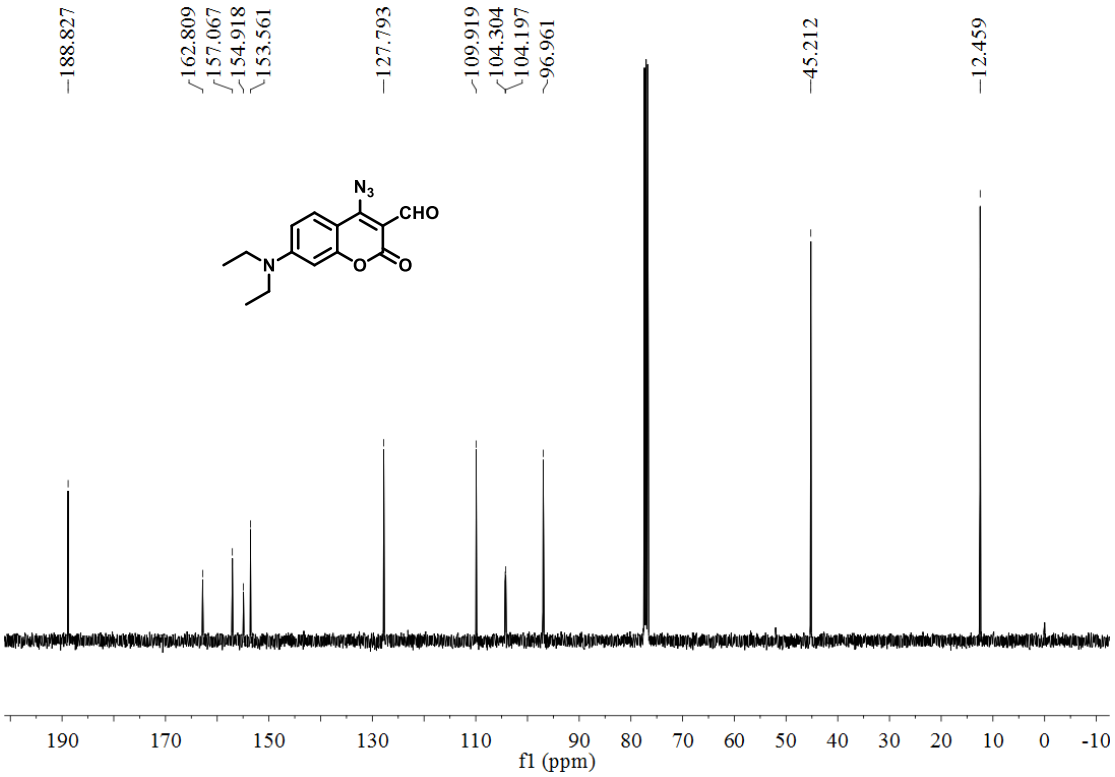


Fig. S13 ^{13}C NMR spectrum of probe **CA** in $CDCl_3$.

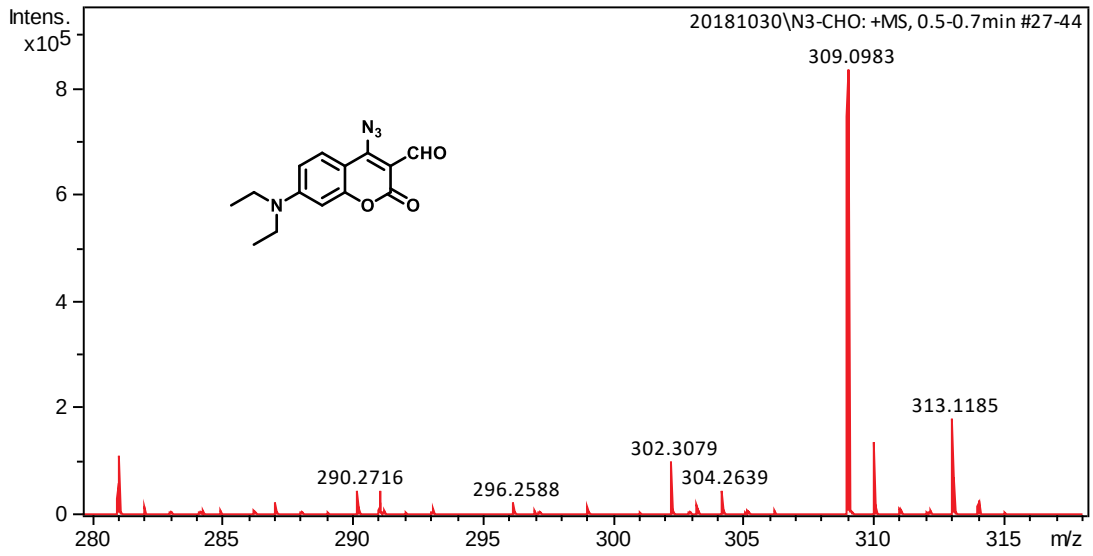


Fig. S14 HRMS spectrum of probe **CA**.

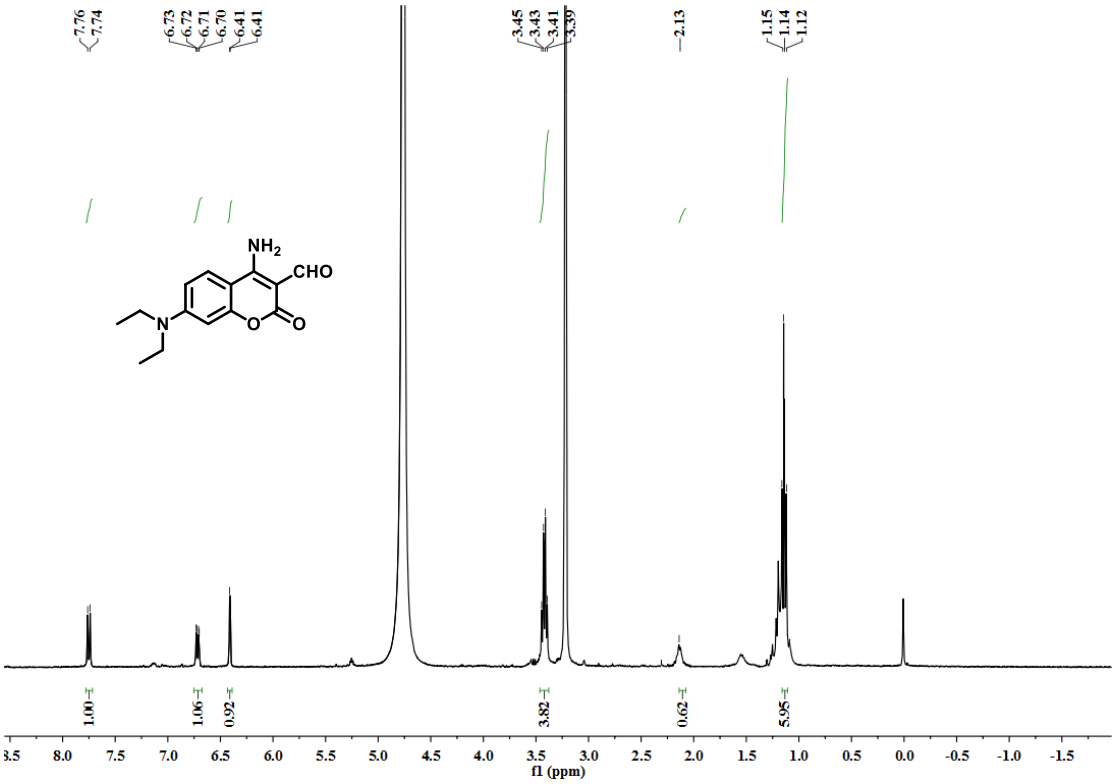


Fig. S15 ^{1}H NMR spectrum of dye **CA-NH$_2$** in CD_3OD.

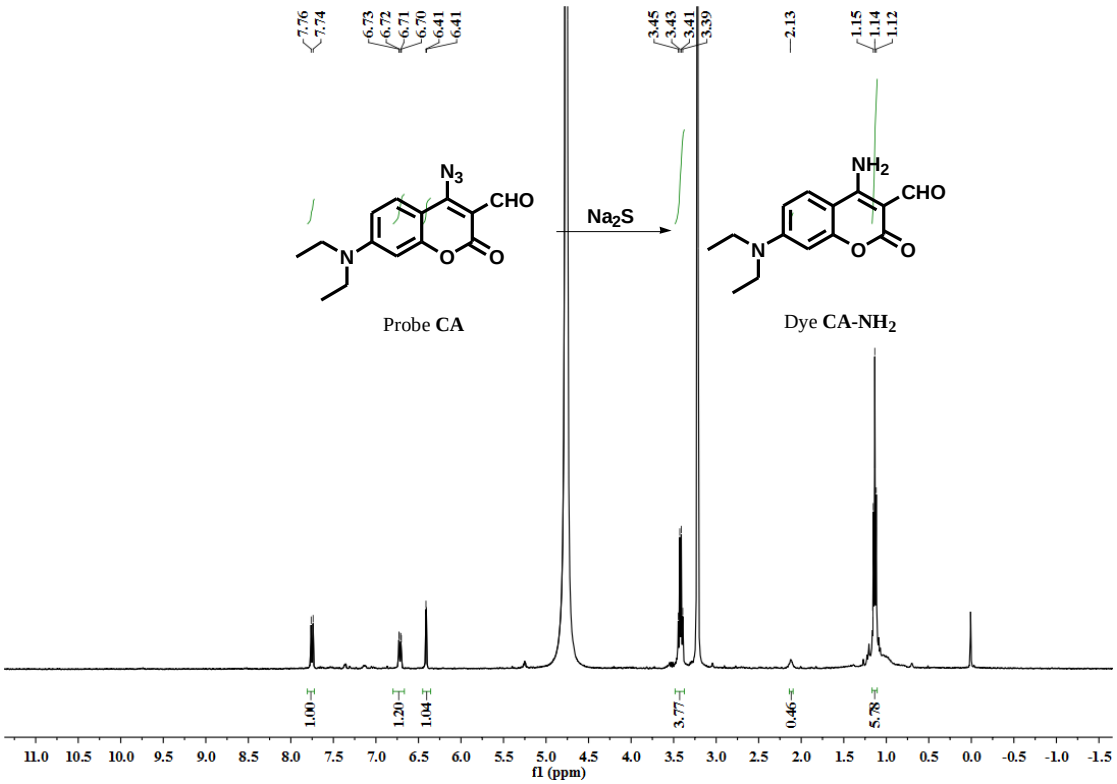


Fig. S16 ^{1}H NMR spectrum of the reaction product of probe **CA** with Na_2S in CD_3OD.

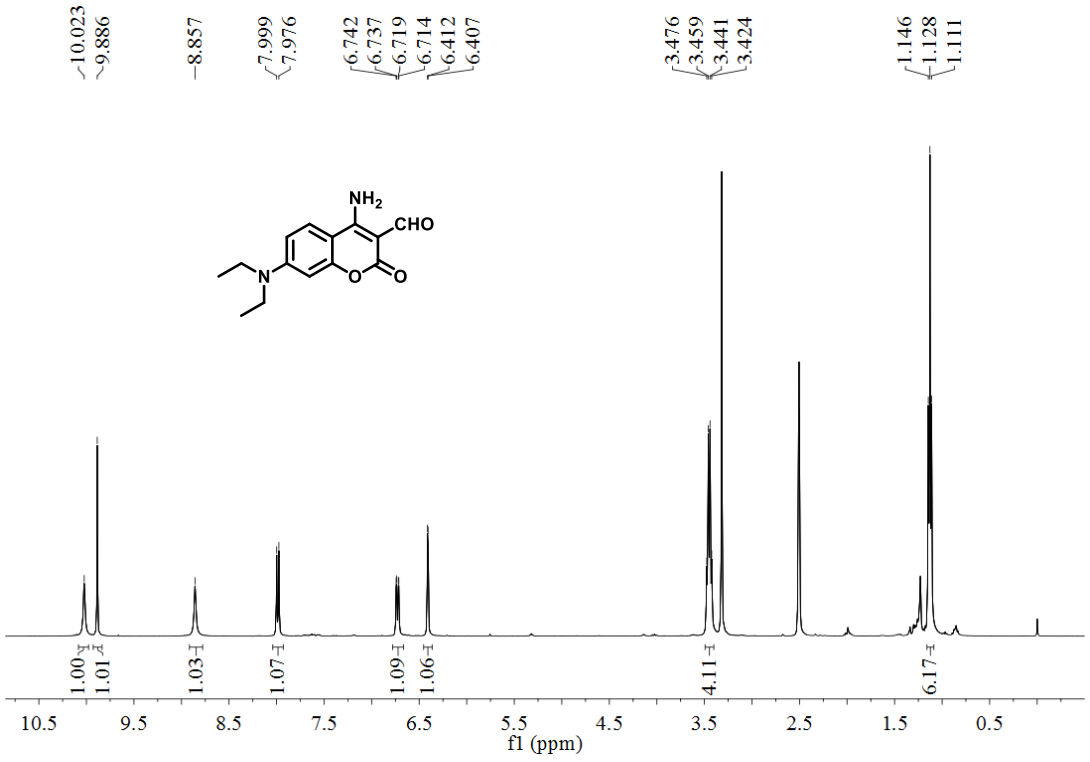


Fig. S17 ^{1}H NMR spectrum of dye **CA-NH$_2$** in DSMO-d_6.

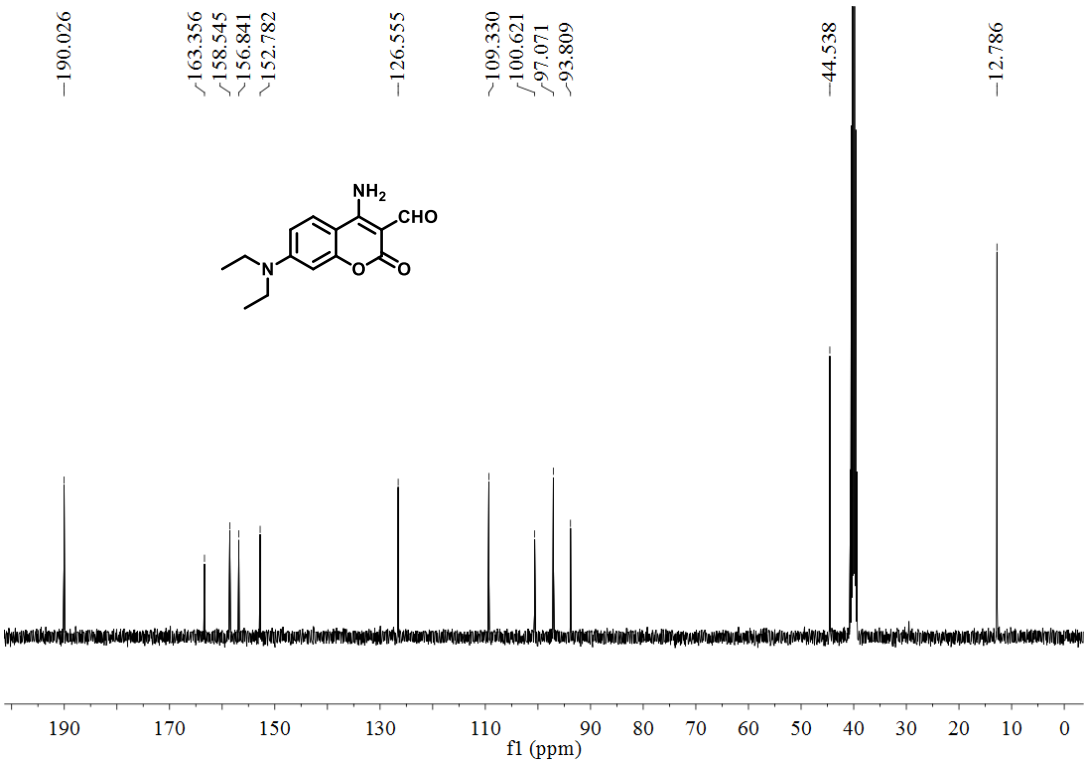


Fig. S18 ^{13}C NMR spectrum of dye **CA-NH$_2$** in DSMO-*d*$_6$.

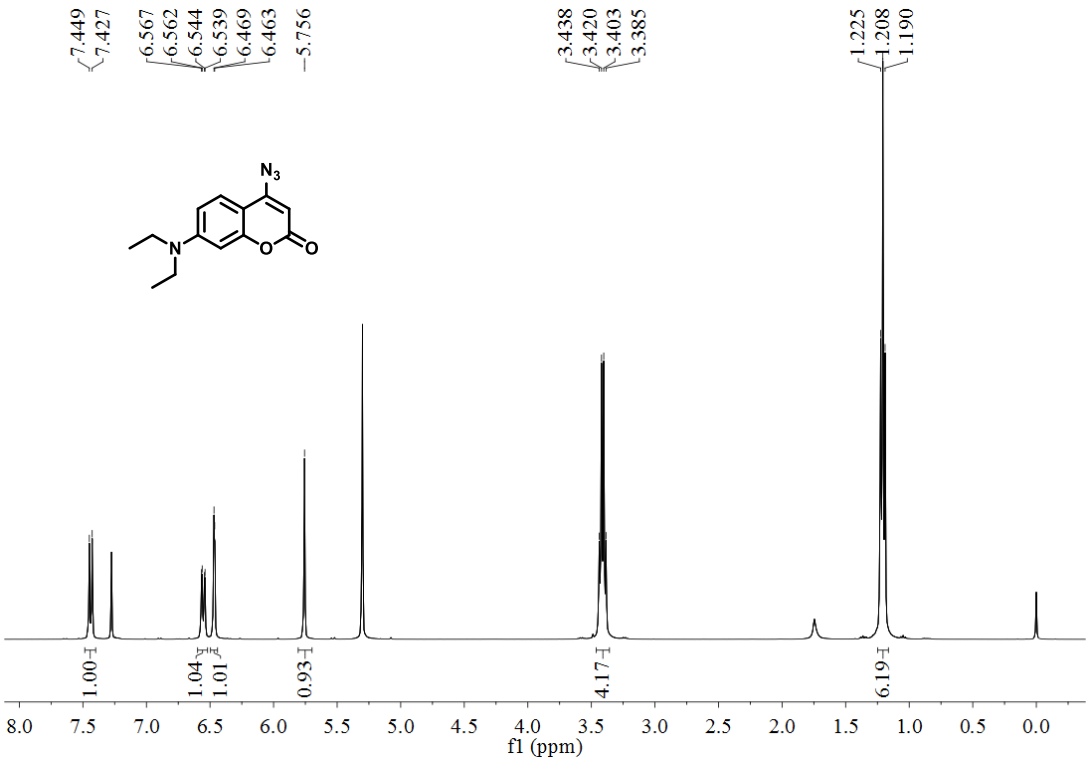


Fig. S19 ^{1}H NMR spectrum of **DEA-Coumarin-N$_3$** in $CDCl_3$.

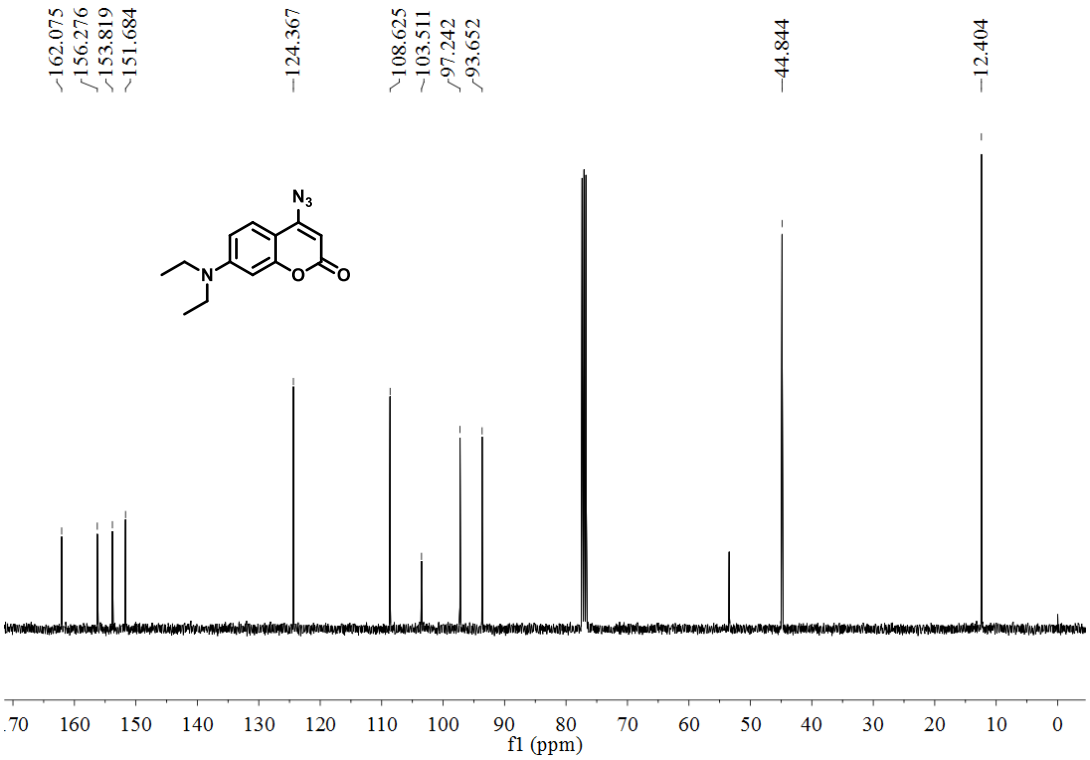


Fig. S20 ^{13}C NMR spectrum of **DEA-Coumarin-N$_3$** in $CDCl_3$.

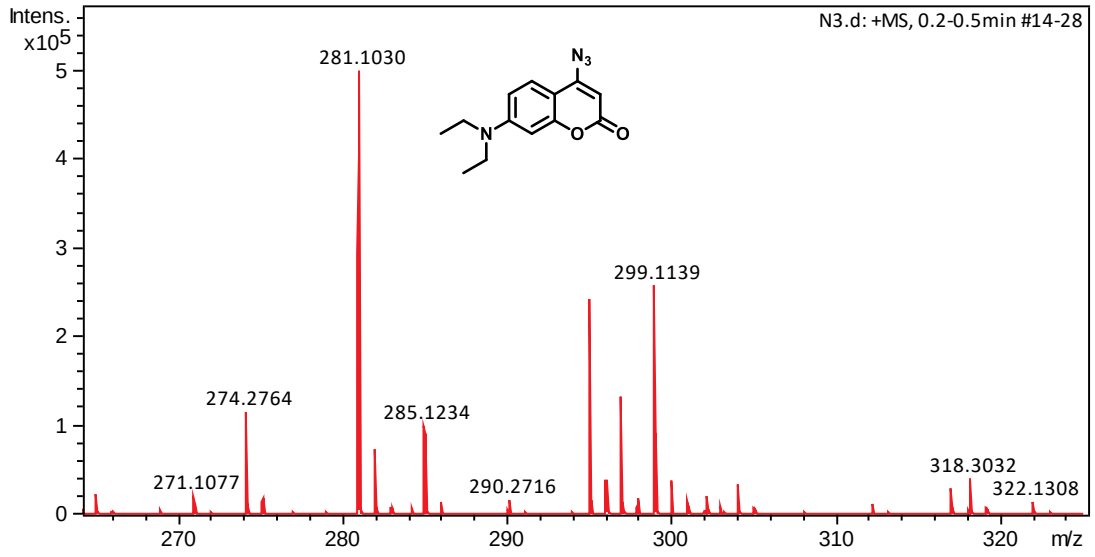


Fig. S21 HRMS spectrum of **DEA-Coumarin-N$_3$**.

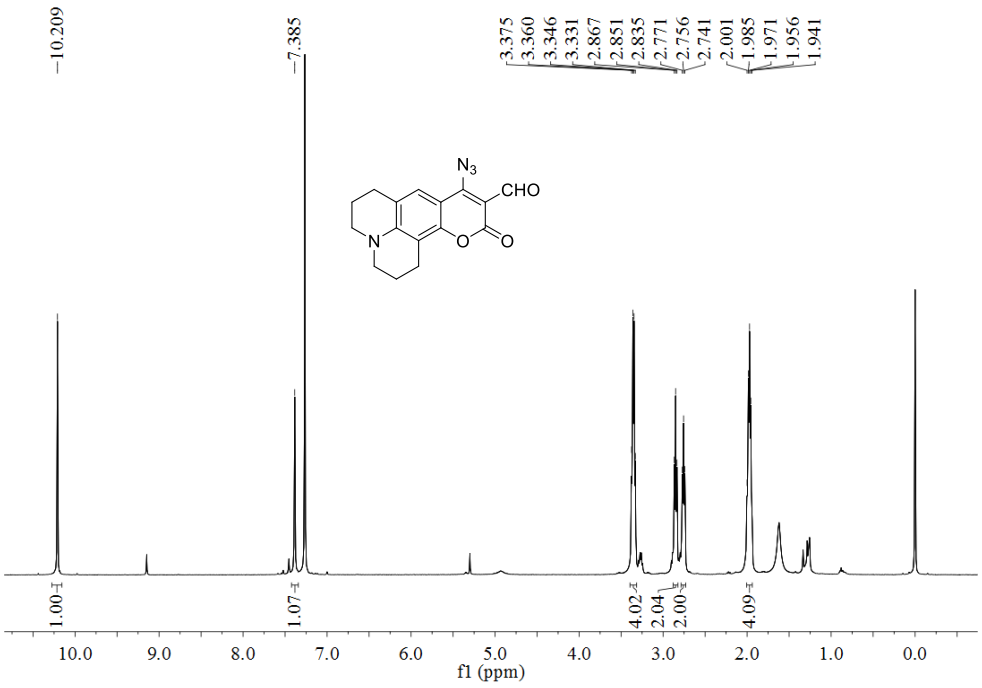


Fig. S22 ^{1}H NMR spectrum of **Julo-Coumarin-N_3-CHO** in $CDCl_3$.

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