Unsymmetrical pentamethine cyanines visualizing physiologic acidities from whole animal to cellular scale with pH-responsive deep-red fluorescence

Yicheng Wang[1a], Zuhai Lei[1a], Cong Wang[a], Chong Cao[a], Jiayi Hu[a], Ling Du*[b], Limei Han*[a] and Cong Li*[a]

[a]Minhang Hospital and Key Laboratory of Smart Drug Delivery, Ministry of Education, School of Pharmacy, Fudan University, Shanghai 201203, China. E-mail: congli@fudan.edu.cn

[b]Key Laboratory of digestive cancer full cycle monitoring and precise intervention of Shanghai Municipal Health Commission, Minhang Hospital, Fudan University, Xingsong Road 170, Shangha 201100, China

[1]These authors contributed equally to this work

Supporting Information

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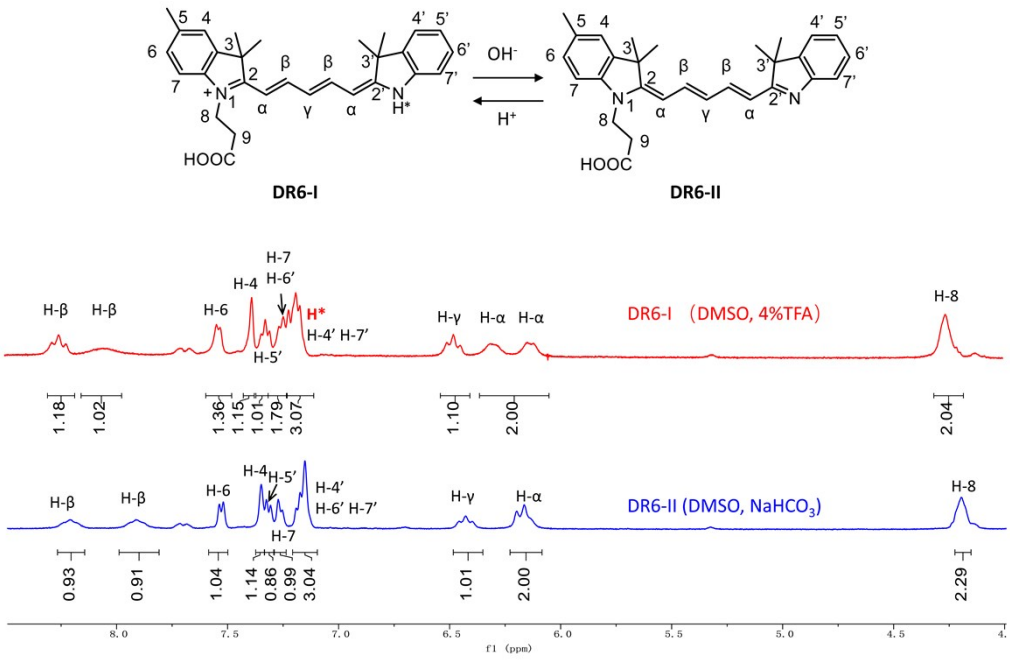


1. Additional Figures and Tables

Figure. S1. ^{1}H-NMR spectra of **DR6** (500 MHz, DMSO-d6) with its protonated with 4% TFA (**DR6-I**) and deprotonated forms with $NaHCO_3$ (**DR6-II**).[1] The significant distinction between protonated form (**DR6-I)** and deprotonated form (**DR6-II**) is the proton in the indole nitrongen atom (H*). The NMR spectra of **DR6-I** and **DR6-II** were shown below (**Figure. RL4**). The NMR peak of H* was only observed in the spectra of **DR6-I**. Furthermore, addition of acid led to downfield shifts of all proton signals, which may attribute to the decrease of electron density. Besides, the chemical shift of H-8 was 4.28 and 4.19 in **DR6-I** and **DR6-II**, respectively, which correlated well with the increased electron-withdrawing ability of quaternary ammonium.

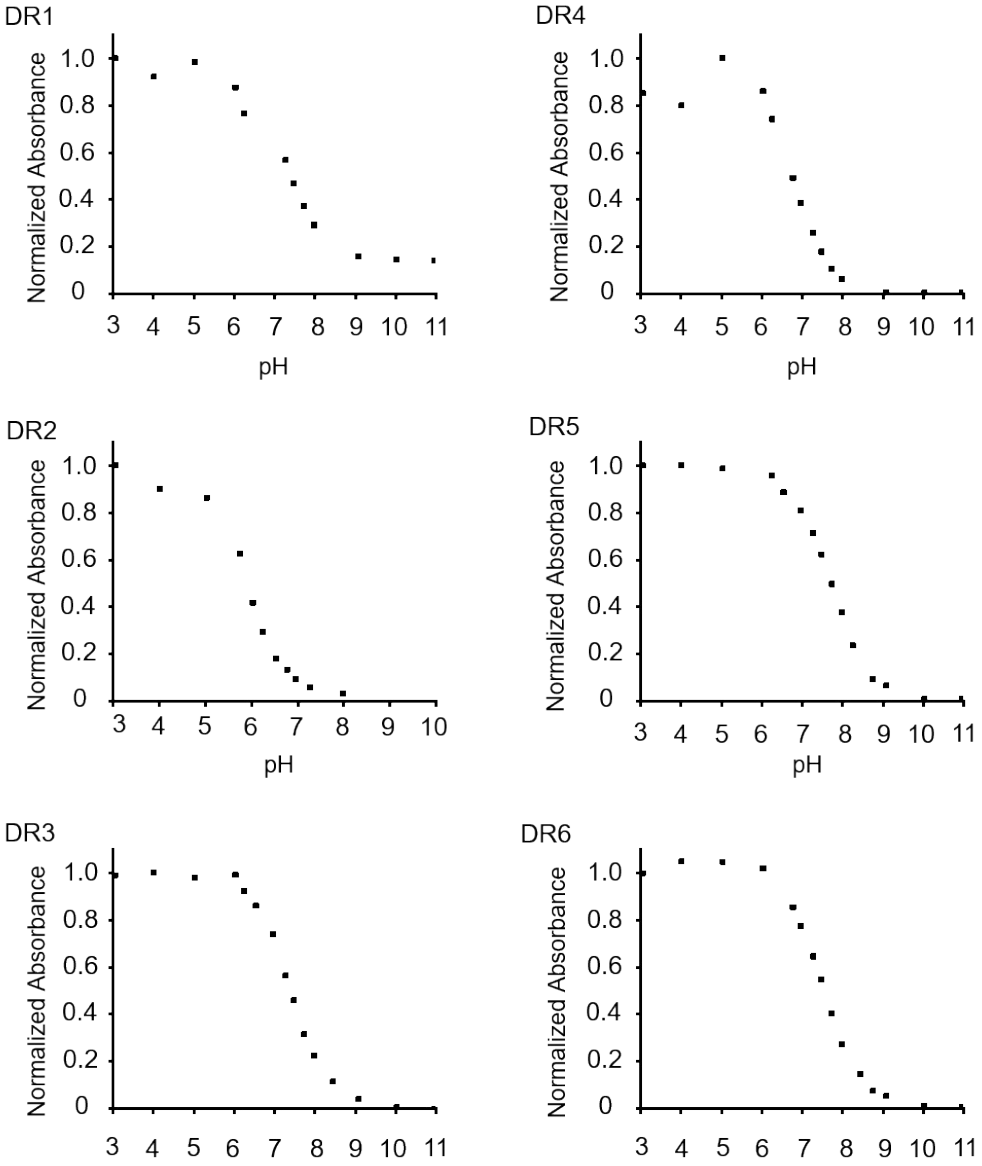


Figure. S2. pH dependence of the normalized absorbance (absorbance/absorbance$_{max}$)

Table S1. TDDFT excitation energies, oscillator strengths, calculated absorption wavelengths and experimental absorption wavelengths of **DR1-6.**

Compounds	Form	ΔE/ eV	f	Theory $\lambda_{Abs\ (max)}$/ nm	Experiment $\lambda_{Abs\ (max)}$/ nm
DR1	protonated	2.47	2.13	525	637
	deprotonated	2.72	1.89	493	486
DR2	protonated	2.46	2.14	525	635
	deprotonated	2.69	1.91	497	463
DR3	protonated	2.51	2.17	512	636
	deprotonated	2.70	1.85	496	488
DR4	protonated	2.49	2.21	526	635
	deprotonated	2.79	2.09	484	475
DR5	protonated	2.38	2.02	548	647
	deprotonated	2.64	1.82	510	500
DR6	protonated	2.44	2.14	532	641
	deprotonated	2.69	1.91	499	497

$\lambda_{Abs\ max}$ is the absorption maxima . ΔE is The excitation HOMO−LUMO energy gap. f represents oscillator strengths.

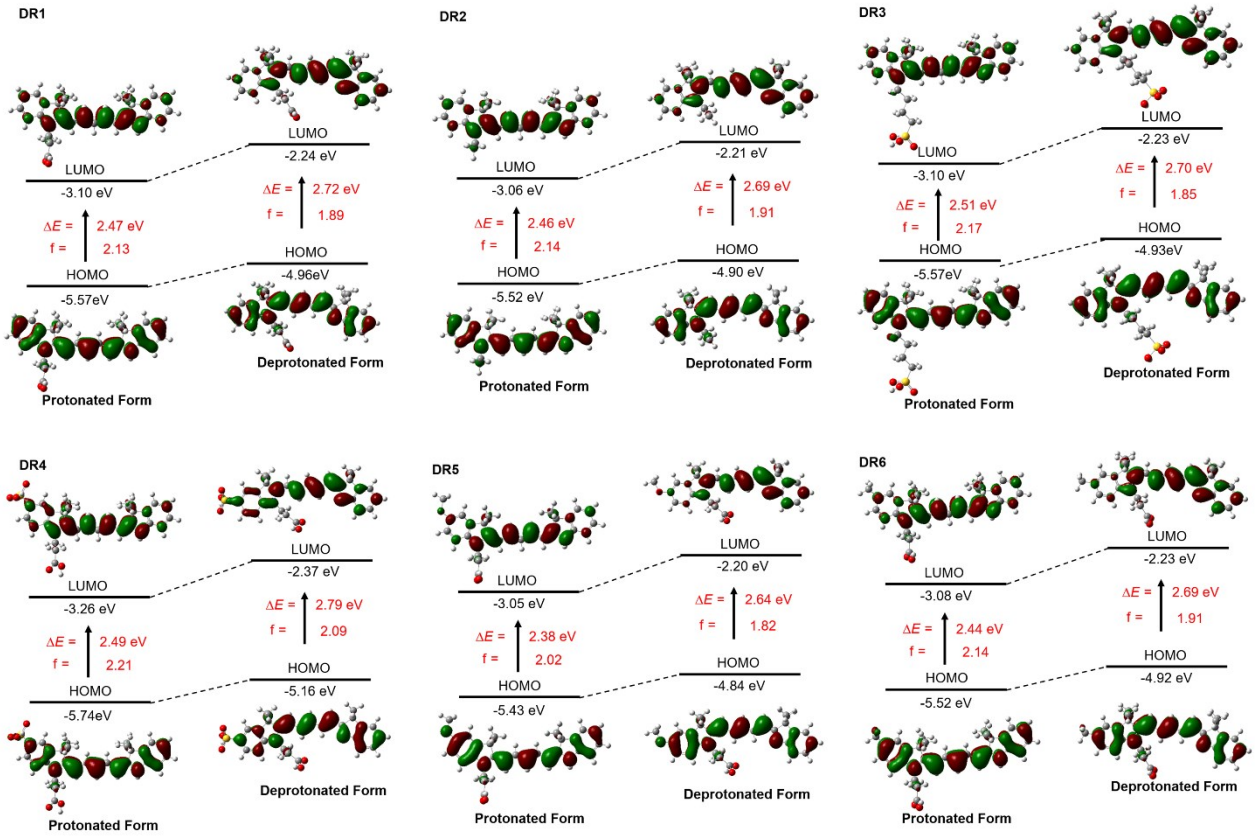


Figure S3. Comparison of the HOMO and LUMO energy levels, excitation energies, and oscillator strengths for **DR1-6**, based on TDDFT calculations at the B3LYP/6–311 G(d,p) level.

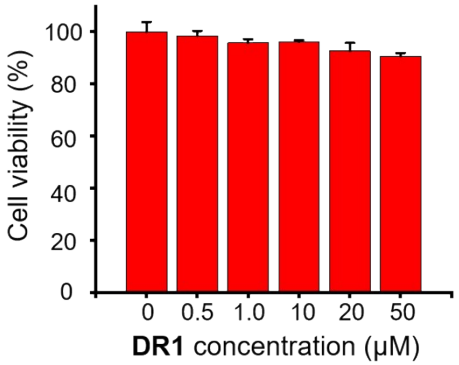


Fig. S4. Cell viability of **DR1** on Hela cells by a standard CCK-8 assay. Data are expressed as mean values ± standard error of the mean of three independent experiments.

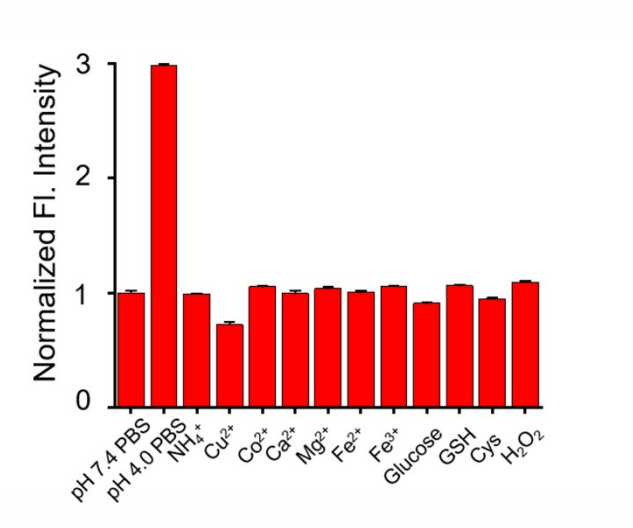


Fig. S5. Fluorescence intensities of **DR1** (1.0 μM) in the presence of endogenous ions and other potential interferents in phosphate buffer (pH 7.4) (200 μM for NH_4^+, Cu^{2+}, Co^{2+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+}, H_2O_2 and 5 mM for Glucose, Glutathione and Cysteine). Data were acquired in with λex= 640 nm. Each experiment was performed in triplicate, and error bars are determined from the mean and standard deviation (SD).

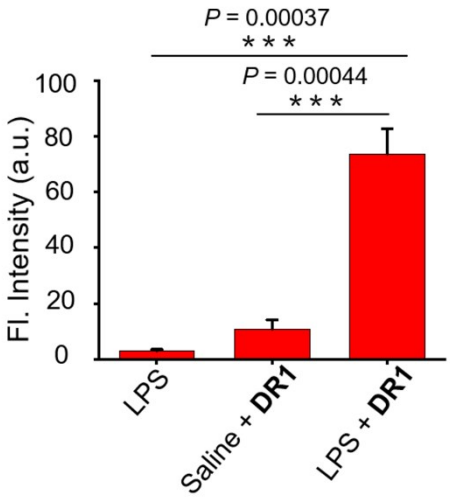
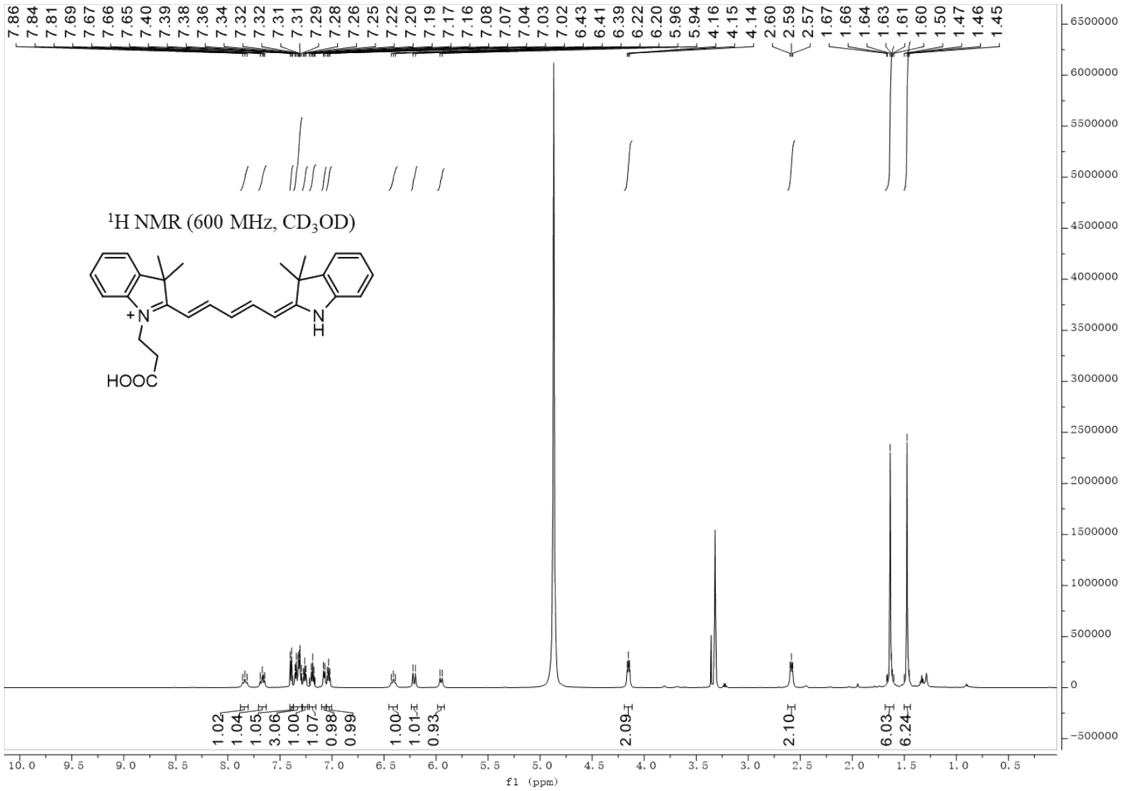
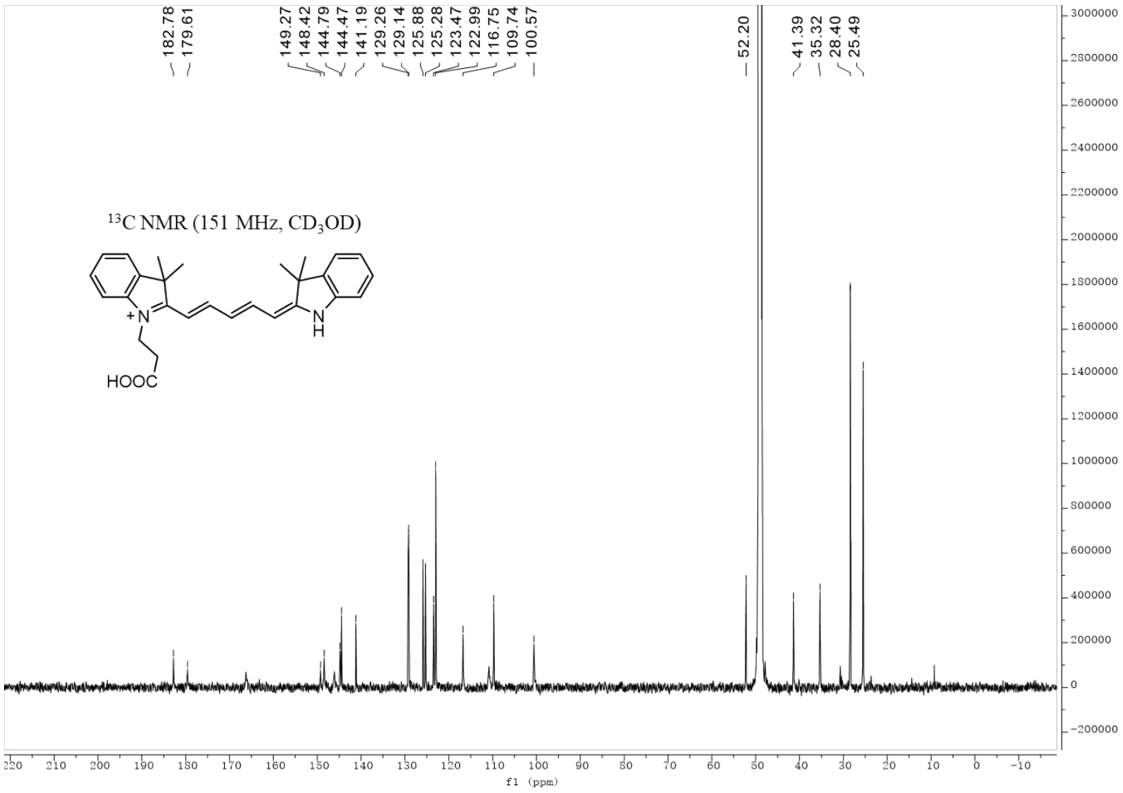


Fig. S6. Mean fluorescence intensity in the abdominal region at 30 min post administration of **DR1** (n = 3). Error bars represent mean deviation (S.D.), *P* values were calculated using two-tailed Student's t-tests.

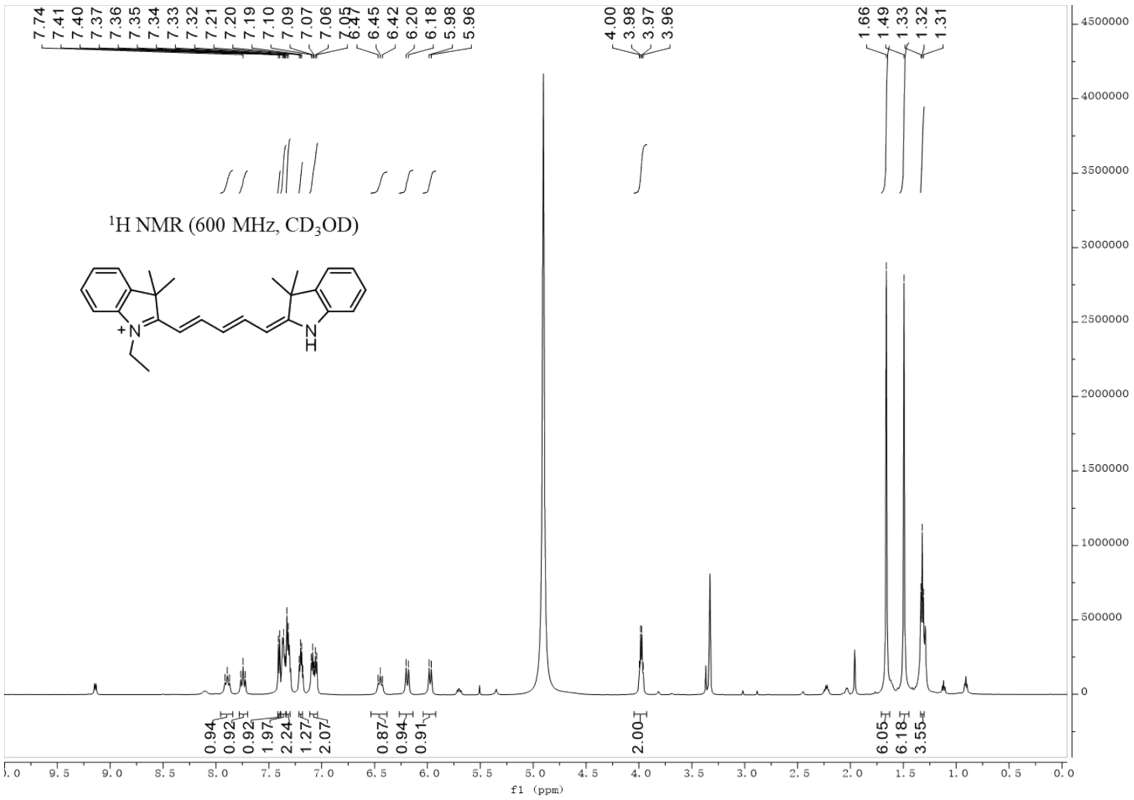
2. NMR spectra of dyes DR1–6.



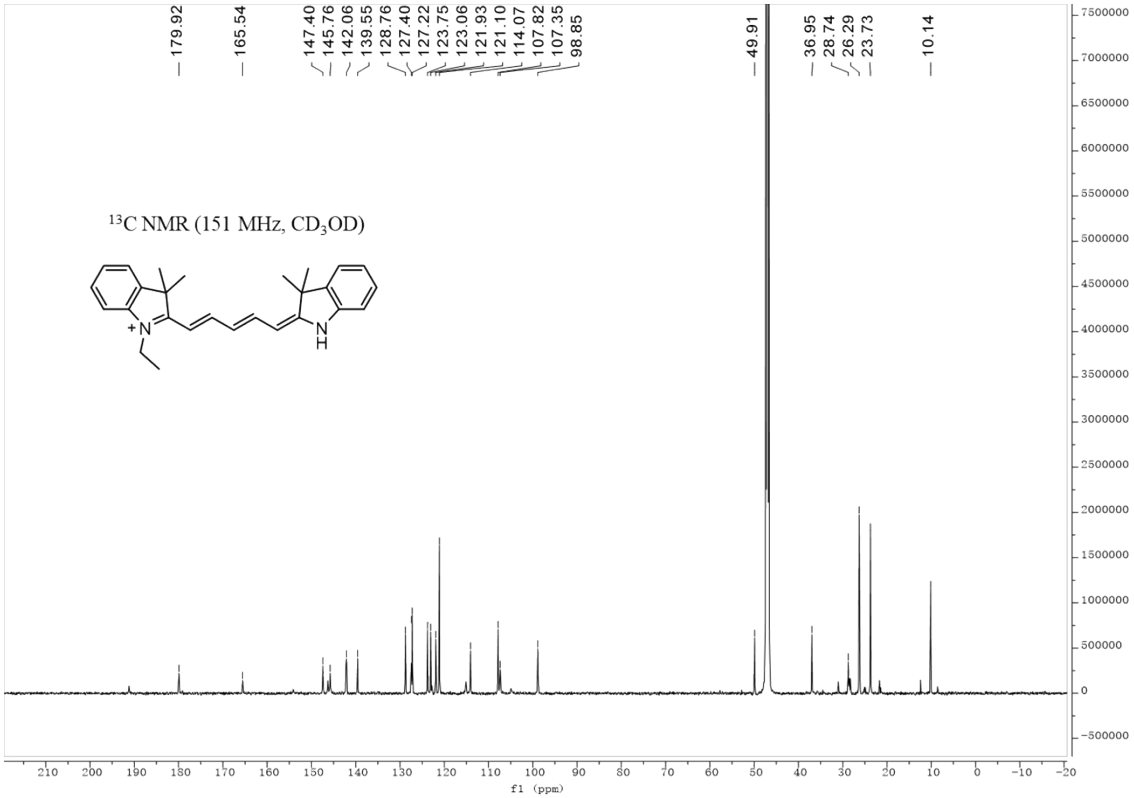
^{1}H NMR spectra of **DR1**



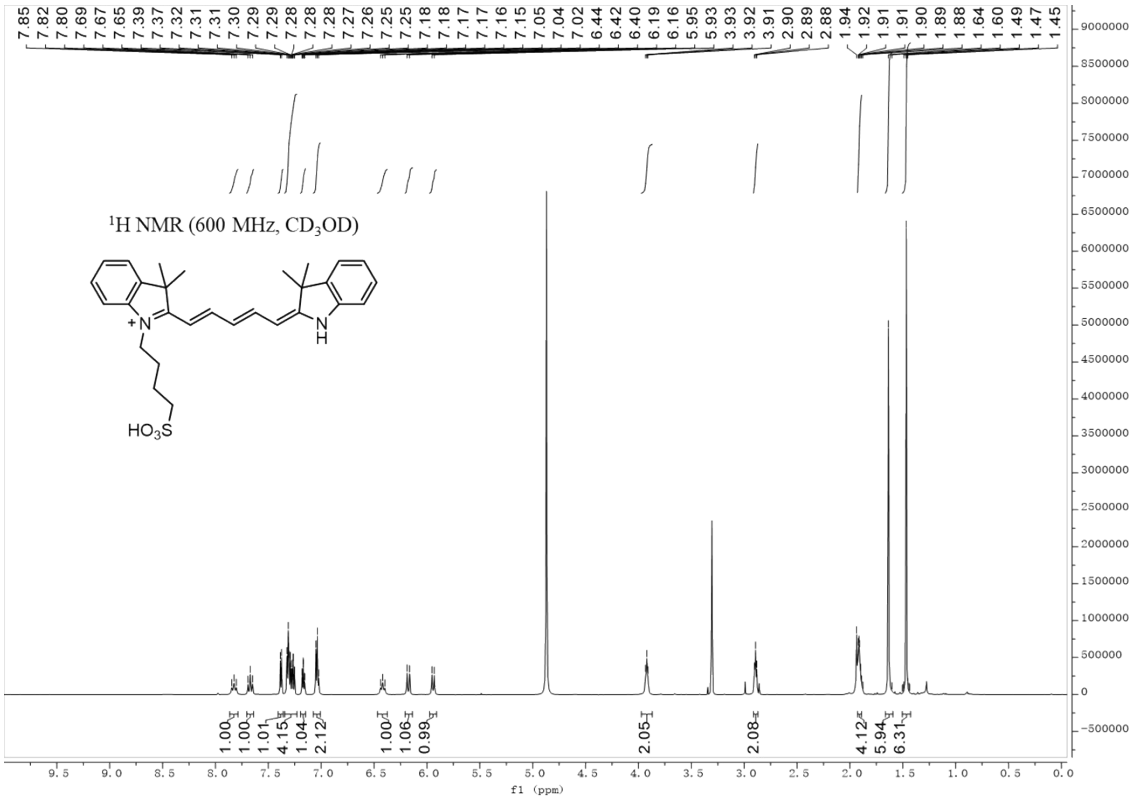
^{13}C NMR spectra of **DR1**



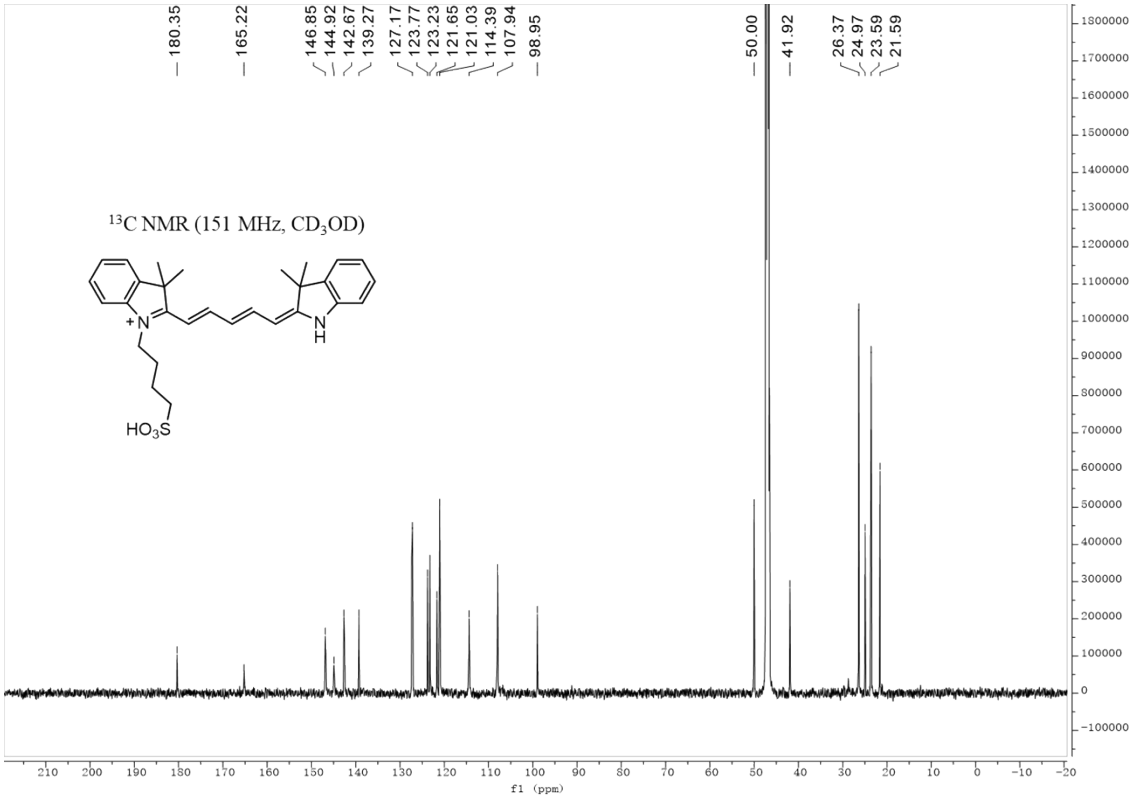
^{1}H NMR spectra of **DR2**



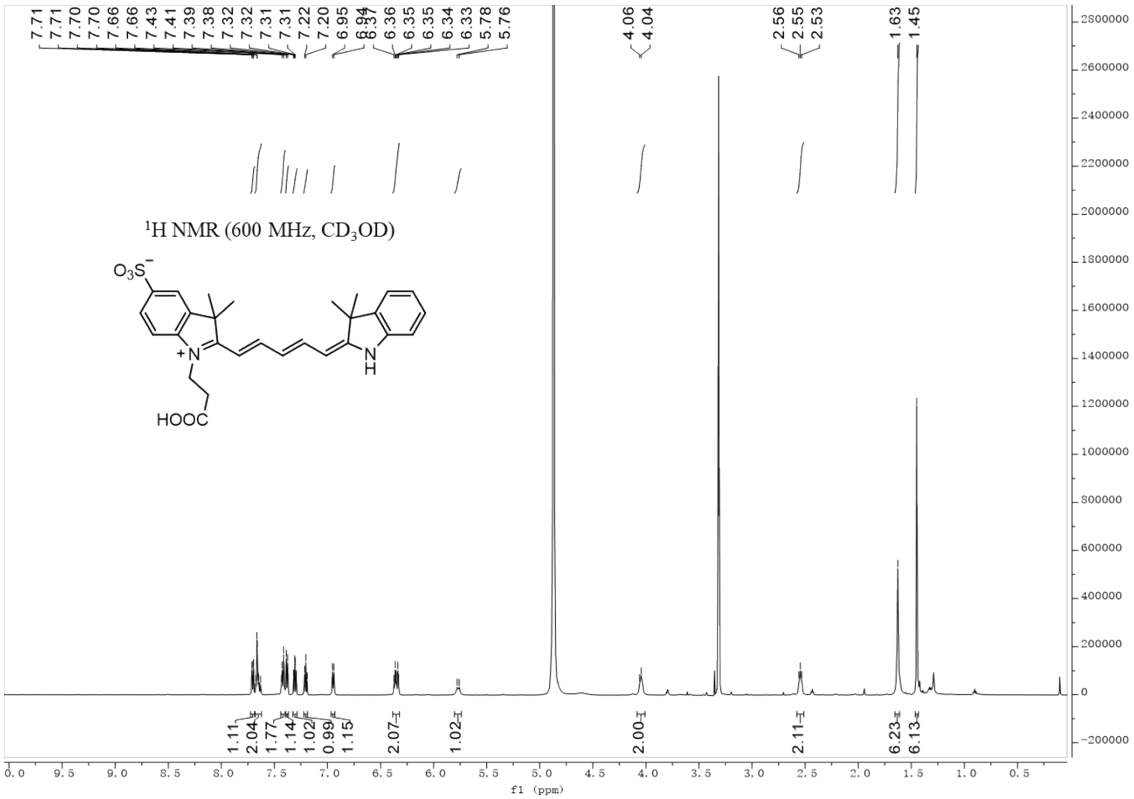
^{13}C NMR spectra of **DR2**



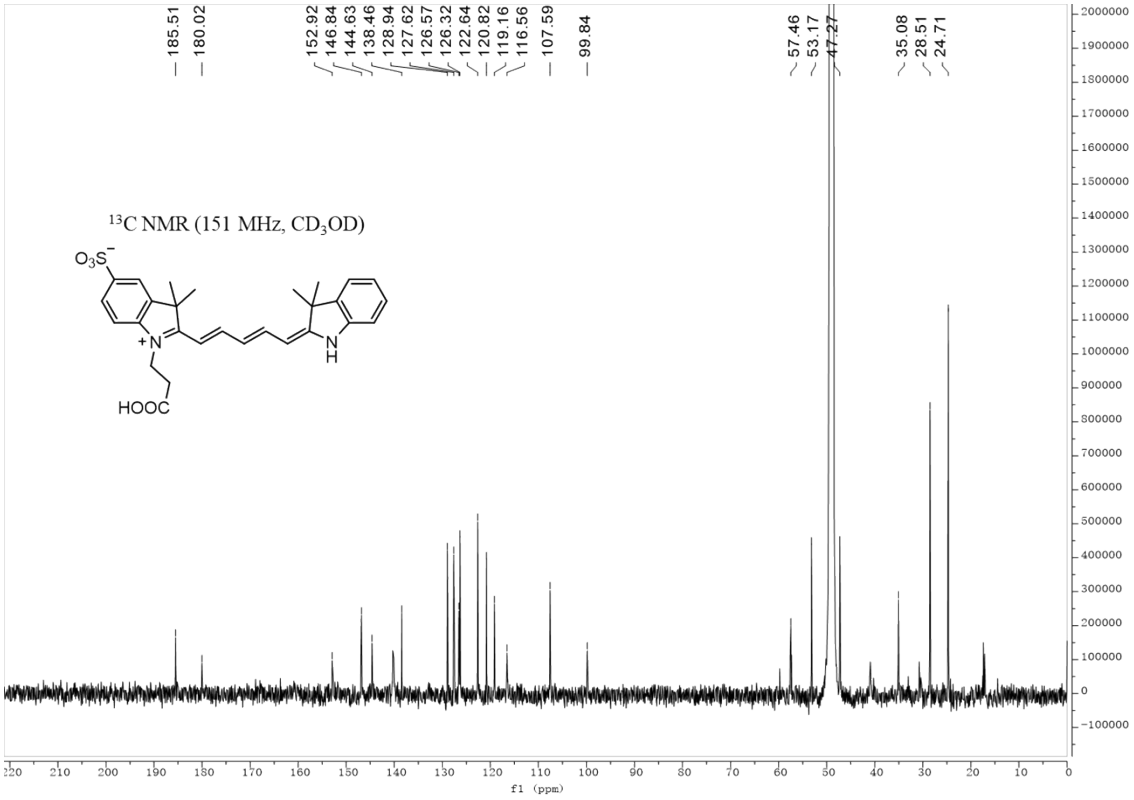
^{1}H NMR spectra of **DR3**



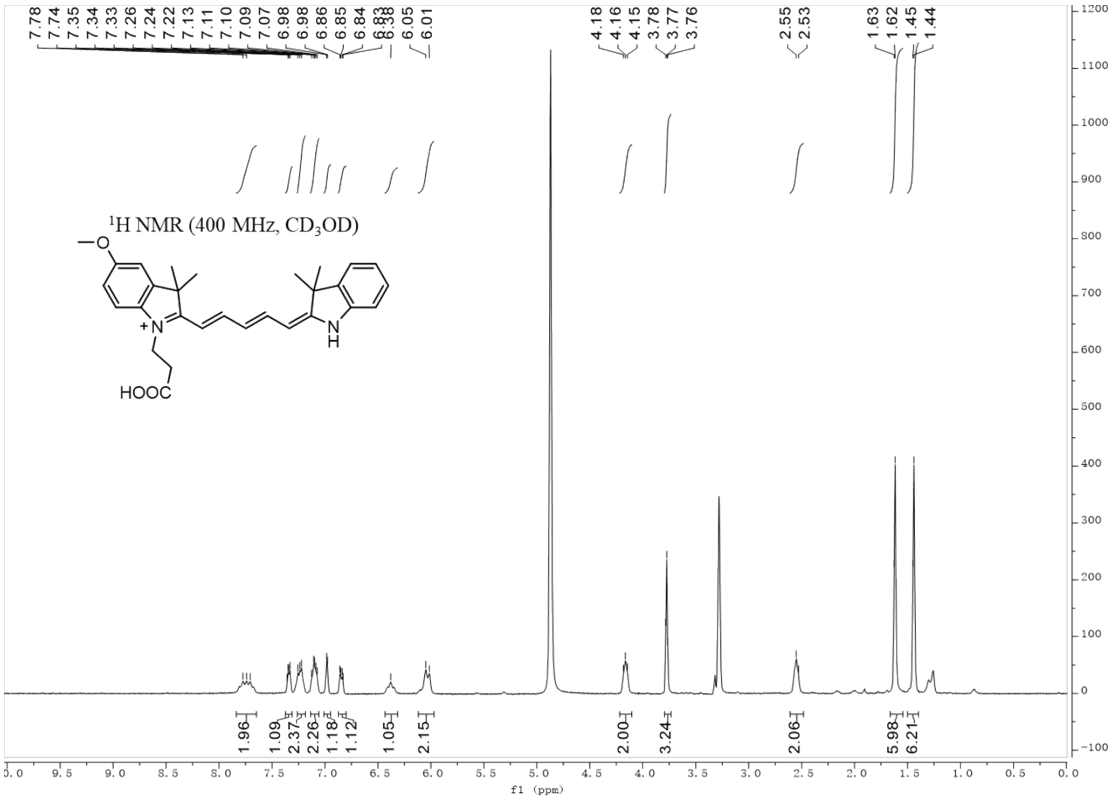
^{13}C NMR spectra of **DR3**



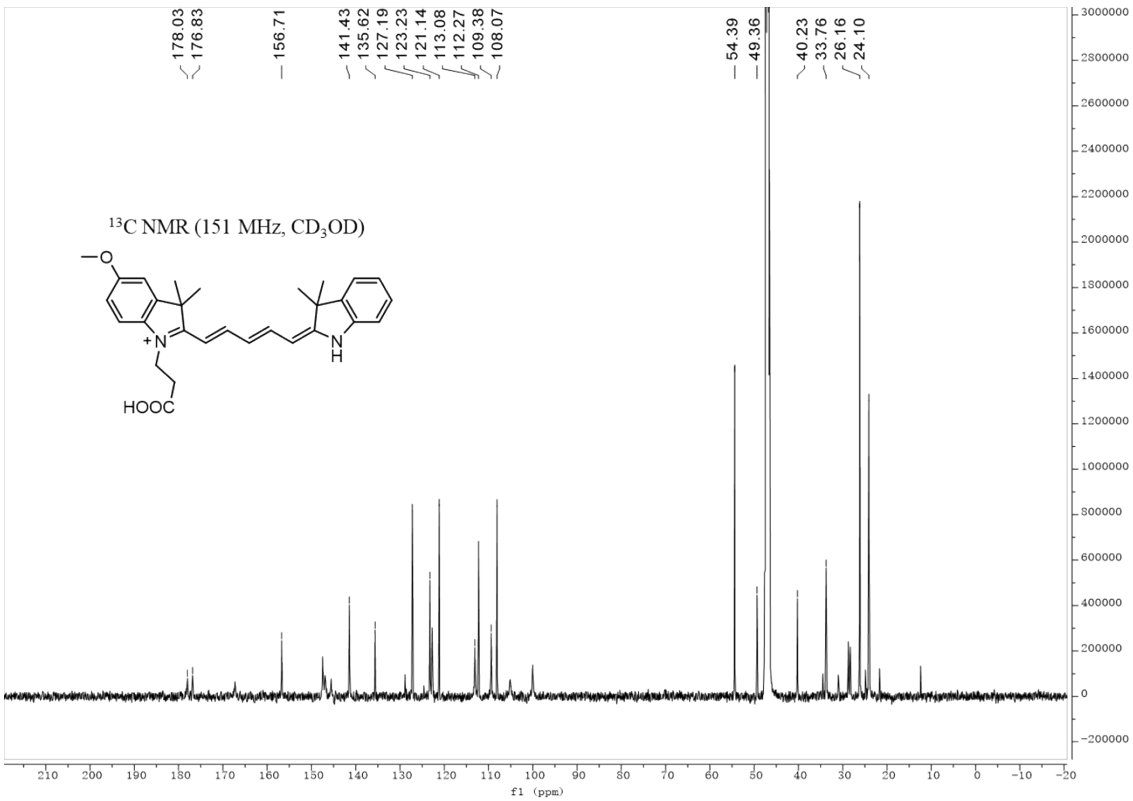
^{1}H NMR spectra of **DR4**



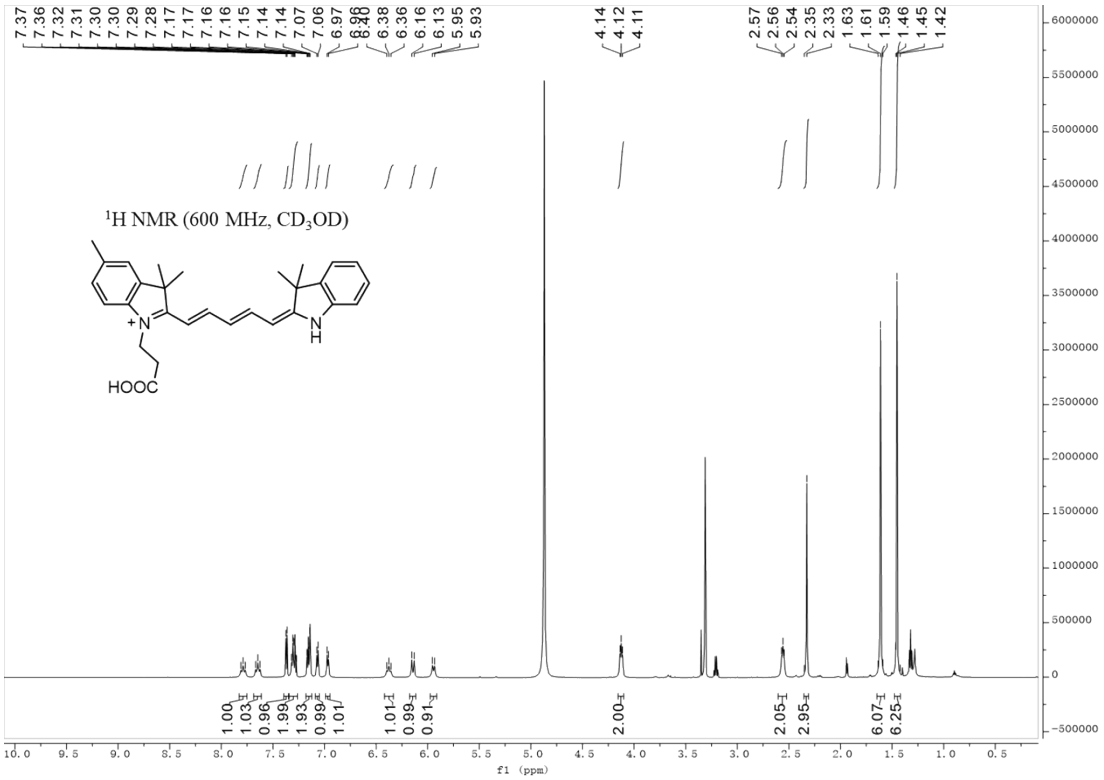
^{13}C NMR spectra of **DR4**



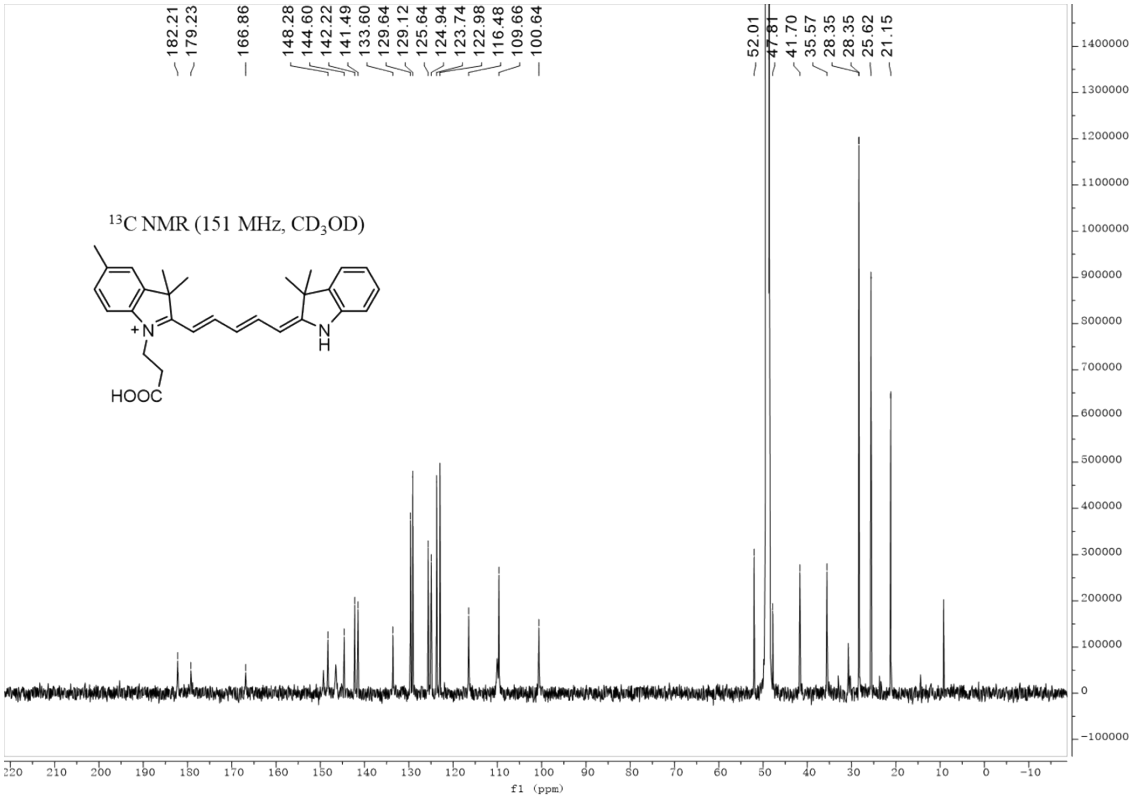
^{1}H NMR spectra of **DR5**



^{13}C NMR spectra of **DR5**

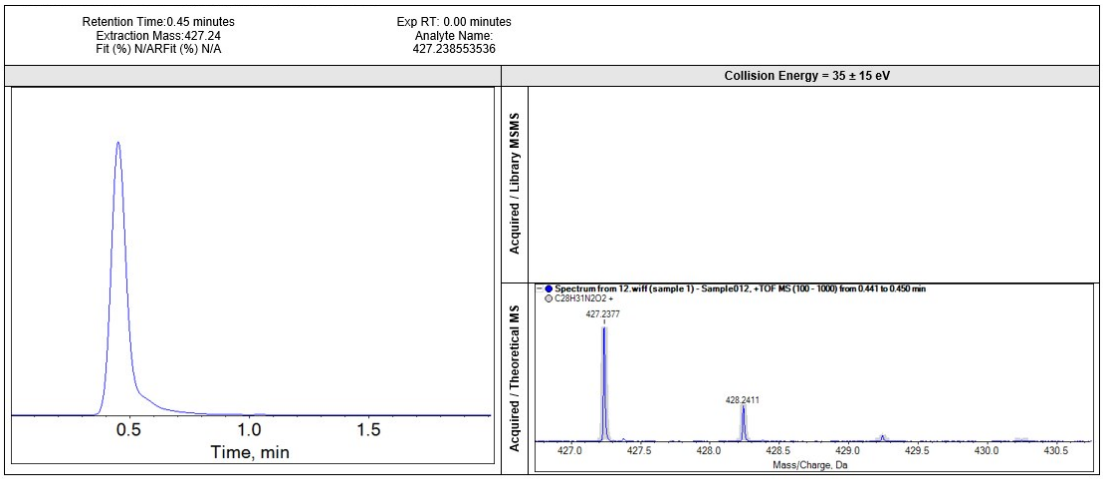


^{1}H NMR spectra of **DR6**



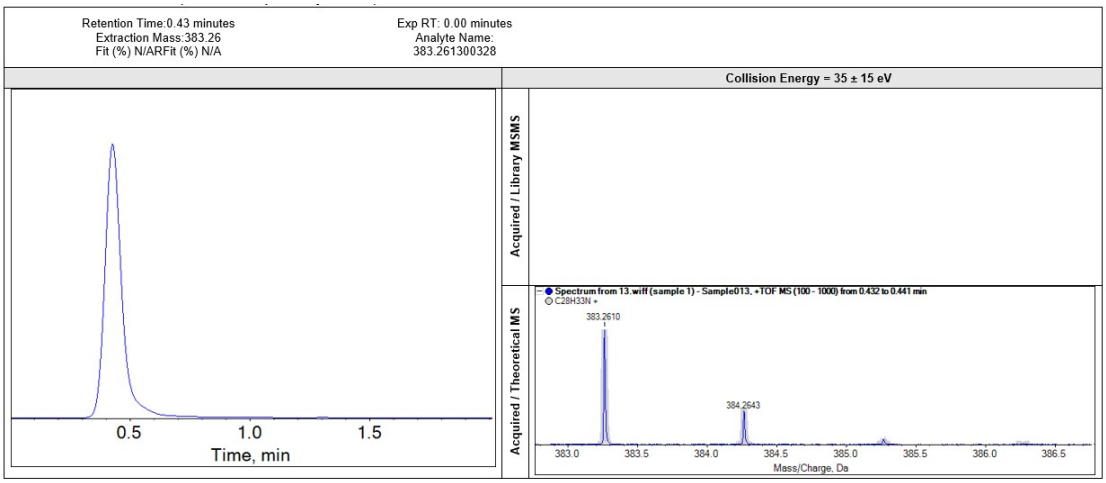
^{13}C NMR spectra of **DR6**

3. High-resolution mass spectra of dyes DR1–6.



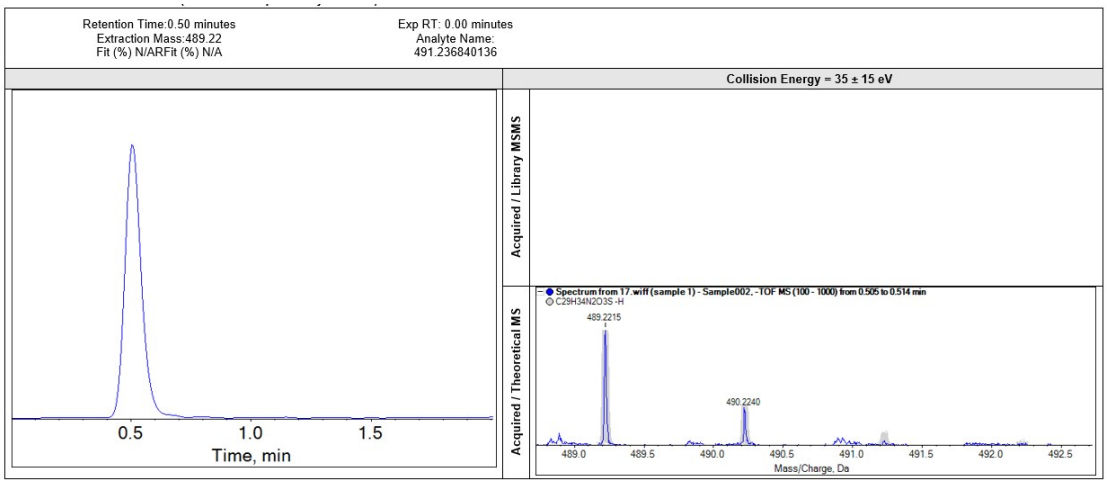
	Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score(%)
✓ ● ✓ ● ●	427.238553536(CY5-COOH)	97%	C28H31N2O2	492797	5	427.2380	427.2377	-0.8	0.00	0.45	0.45	1.2%	N/A

High-resolution mass spectra of **DR1**



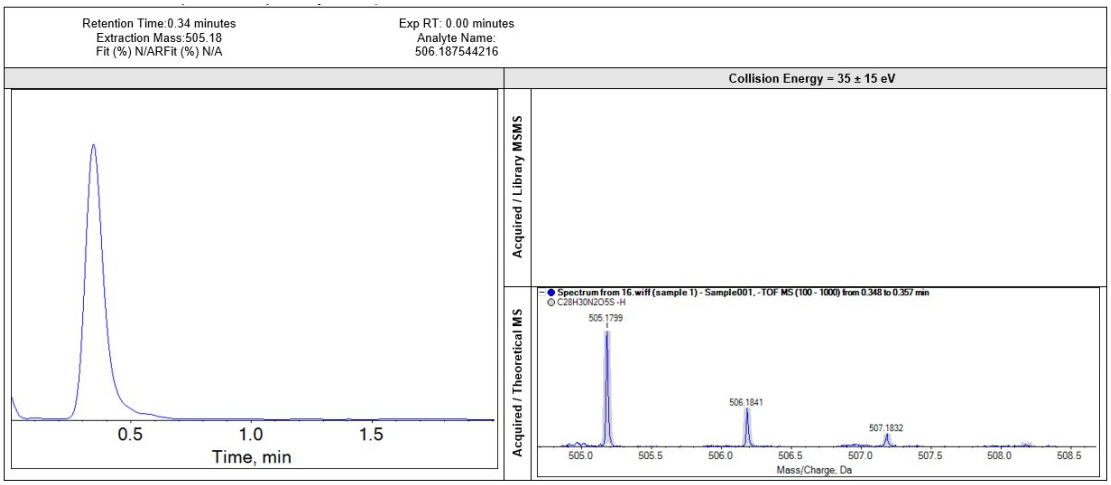
	Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score(%)
✓ ● ✓ ● ●	383.261300328(CY5-Et)	96%	C28H33N	496196	5	383.2608	383.2610	0.6	0.00	0.43	0.43	2.8%	N/A

High-resolution mass spectra of **DR2**



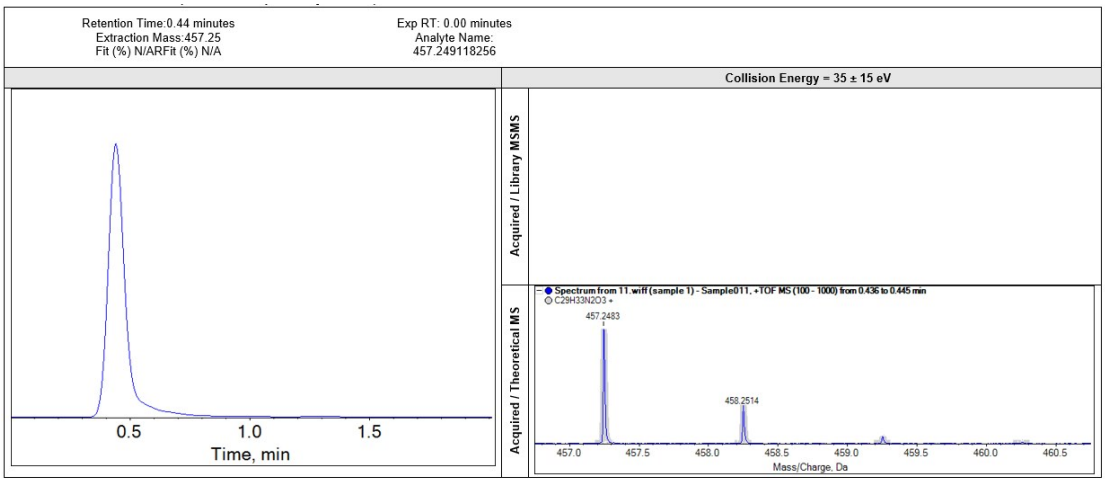
Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score(%)
491.236840136(CY5-N-SO3)	94%	C29H34N2O3S	2446	5	489.2217	489.2215	-0.4	0.00	0.50	0.50	7.4%	N/A

High-resolution mass spectra of **DR3**



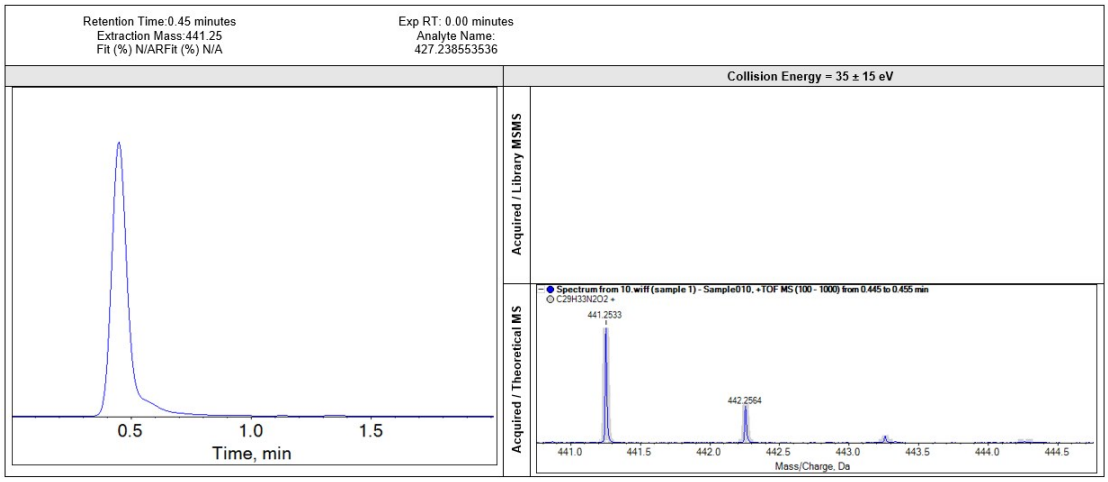
Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score(%)
506.187544216(CY5-SO3)	97%	C28H30N2O5S	3864	5	505.1803	505.1799	-0.7	0.00	0.34	0.34	0.5%	N/A

High-resolution mass spectra of **DR4**



	Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score(%)
✓●✓●●	457.249118256(CY5-MeO)	98%	C29H33N2O3	1362402	5	457.2486	457.2483	-0.6	0.00	0.44	0.44	0.9%	N/A

High-resolution mass spectra of **DR5**



	Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score(%)
✓●✓●●	(CY5-Me)	96%	C29H33N2O2	308292	5	441.2537	441.2533	-0.8	0.00	0.45	0.45	1.9%	N/A

High-resolution mass spectra of **DR6**