

Supplementary Information

Synthesis and fluorescence properties of red to near-infrared emitting push-pull dyes based on benzodioxazole scaffolds

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1. Quantum chemistry calculations

Quantum chemistry calculations were performed by Gaussian 16 software conducted using the polarizable continuum model (PCM) with the implicit consideration of the dielectric environment.¹ Geometry optimizations and harmonic vibrational frequency calculations of the ground (S_0) and the lowest singlet and triplet excited (S_1 and T_1) states were optimized by density functional theory (DFT)² and time-dependent density functional theory (TDDFT)³ with B3LYP functional and 6-311+G(d,p) basis set. The vertical ΔE_{ST} values of 2a,3a,3a',4a and 5a were estimated by the vertical energies of S_1 and T_1 . The adiabatic energies of S_1 and T_1 can be obtained by the vertical energies of S_1 and T_1 and their corresponding reorganization energies. Then, the adiabatic ΔE_{ST} values of 2a,3a,3a',4a and 5a were also estimated. Electron-Hole Density Distributions, Transition Dipole Moment, Oscillator Strength and dihedral angle were obtained by the Multiwfn 3.7 program⁴ and the VMD program⁵.

Equation of Transition Dipole Moment and Oscillator Strength

Transition Dipole Moment:

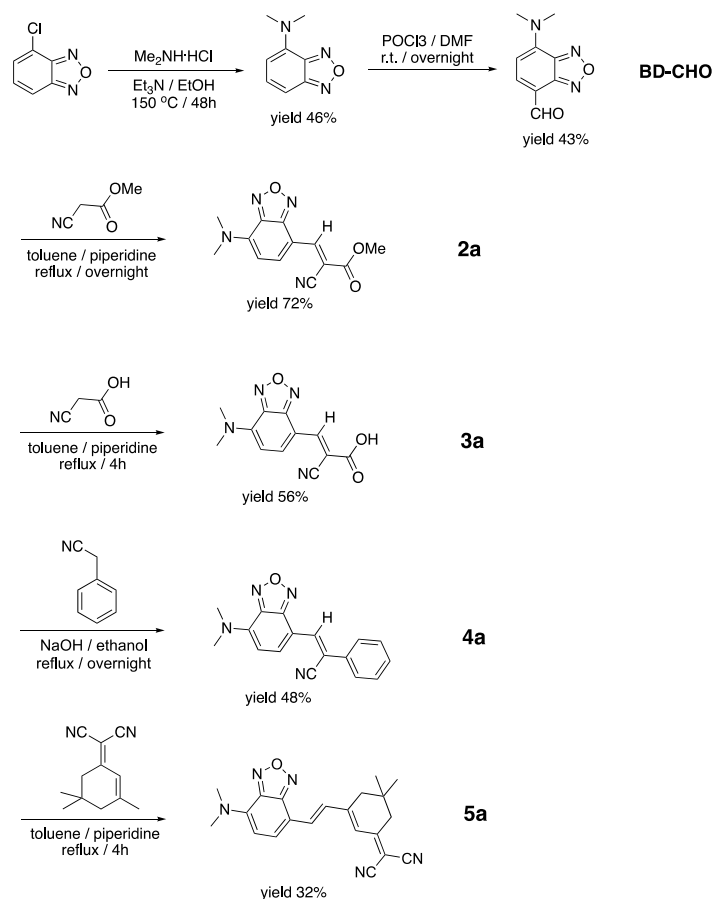
$$\mu_{ij} = \langle i | -r | j \rangle \quad (1)$$

Oscillator Strength:

$$f_{ij} = \frac{2}{3}(E_i - E_j) |\langle i | -r | j \rangle|^2 \quad (2)$$

2. Chemical synthesis and characterization

2.1 Synthesis route of VBDfluors analogues 2a-5a.



Scheme S1. Synthesis route of VBDfluors analogues 2a-5a.

a. Synthesis of N, N-dimethyl-2,1,3-benzoxadiazole

Triethylamine (60 mmol, 6.07 g) was added to the mixture of 4-chlorobenzofuran (10 mmol, 1.54 g) and dimethylamine hydrochloride (50 mmol, 4.07 g) in ethanol (20 mL) in an autoclave and stirred at 150°C for 24 hours. After cooling to room temperature, ethanol was evaporated. 2M HCl (aq) was added to shift the *pH* value of the reaction mixture to neutral. After extraction by ethyl acetate (EA, 20 mL \times 2), the upper layer was collected and dried with Na_2SO_4 . After removing EA under reduced pressure, column chromatography (hexane/EA = 20/1, v/v) was applied for purification and afforded pure compound BD as a yellow solid (756 mg, yield 46%). $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) (ppm): 7.25 (m, 1H), 7.06 (d, $J = 8.8$ Hz, 1H), 6.08 (d, $J = 7.4$ Hz, 1H), 3.33 (s, 6H). $^{13}\text{C NMR}$ (CDCl_3 , 75 MHz) (ppm): 151.02, 145.85, 139.95, 133.55, 105.09, 102.28, 42.16.

b. Synthesis of compound BD-CHO

Phosphorous oxychloride (15 mmol, 2.30 g) was added dropwise to anhydrous DMF

(75 mmol, 5.47g) in ice bath condition with nitrogen protection, followed by stirring for 10 min. Compound BD (3 mmol, 489 mg) in 3 mL anhydrous DMF was added dropwise to the reaction solution in ice bath, then reacted at room temperature for 2 h. After compound BD was totally reacted, ice water was added and then stirred for overnight. Red solid was precipitated. Then 30 mL 10% NaOH (aq) was added and stirred for another 2 h. Followed by collecting red precipitate, the target product BD-CHO was obtained by purification of column chromatography (PE/EA=5/1, v/v) as yellow solid (246 mg, yield 43%). ¹H-NMR (CDCl₃, 400 MHz) (ppm): 10.05 (s, 1H), 7.91 (d, *J* = 8.2 Hz, 1H), 6.17 (d, *J* = 8.2 Hz, 1H), 3.60 (s, 6H). ¹³C NMR (CDCl₃, 75 MHz) (ppm): 186.11, 147.70, 145.15, 144.99, 142.70, 111.35, 102.45, 43.07. ESI-MS: [M+H]⁺ calculated for C₉H₉N₃O₂: 192.07; Found 192.20.

c. Synthesis of dye 2a

BD-CHO (0.5 mmol, 90 mg) and methyl cyanoacetate (2 mmol, 198 mg) were dissolved in 10 mL toluene, followed by adding piperidine (0.1 mmol, 10 μL) and reflux for overnight. After cooling to room temperature and removing the solvent, the target product was purified by column chromatography (PE/DCM = 6/1). The target product was obtained as solid (98 mg, yield 72%). ¹H-NMR (CD₃CN, 400 MHz) (ppm): 8.61 (d, *J*=8.9 Hz, 1H), 8.57 (s, 1H), 6.42 (d, *J*=8.8 Hz, 1H), 3.88 (s, 3H), 3.57 (s, 6H). ESI-MS: [M+K]⁺ calculated for C₁₃H₁₂N₄O₃: 311.09; Found 311.1.

d. Synthesis of dye 3a

BD-CHO (0.5 mmol, 90mg) and cyanoacetic acid (2 mmol, 170mg) were dissolved in 10 mL toluene, followed by adding piperidine (0.1 mmol, 10 μL) and reflux for 4h. After cooling to room temperature, the solvent was removed, followed by purification of column chromatography (PE/EA = 7/1). The target product was obtained as brown solid (72mg, yield 56%). ¹H-NMR (CD₃CN, 300 MHz) (ppm): 7.50 (m, 2H), 6.35 (d, *J*=16.3 Hz, 1H), 6.16 (d, *J*=8.2 Hz, 1H), 3.43 (s, 6H). ¹³C NMR (CD₃CN, 75 MHz) (ppm): 150.00, 147.24, 147.04, 140.97, 136.07, 120.96, 114.33, 109.27, 104.91, 94.96, 43.29. ESI-MS: [M-H]⁺ calculated for C₁₂H₁₀N₄O₃: 257.08; Found 257.2.

e. Synthesis of dye 4a

BD-CHO (0.5 mmol, 90 mg), 2-phenylacetonitrile (2 mmol, 234 mg) and Sodium

hydroxide (0.2 mmol, 8 mg) were dissolved in 10 mL EtOH, followed reflux for overnight. After cooling to room temperature, solvent was removed. The target product was purified by column chromatography (PE/DCM = 4/1). Yield was calculated to be 48%. ¹H-NMR (CD₃CN, 400 MHz) (ppm): 8.44 (d, *J*=8.0 Hz, 1H), 8.10 (s, 1H), 7.75 (d, *J*=7.6 Hz, 2H), 7.51 (m, 3H), 6.39 (d, *J*=8.5 Hz, 1H), 3.49 (s, 6H). ESI-MS: [M+H]⁺ calculated for C₁₇H₁₄N₄O: 291.12; Found 291.3.

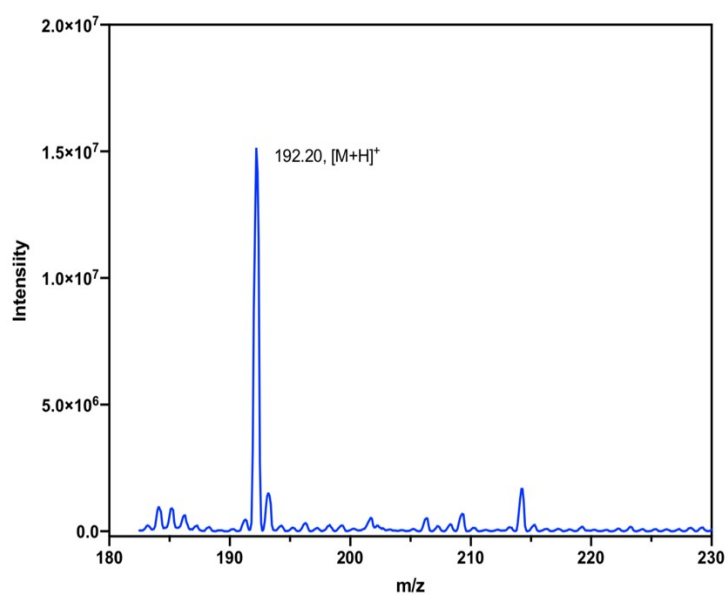
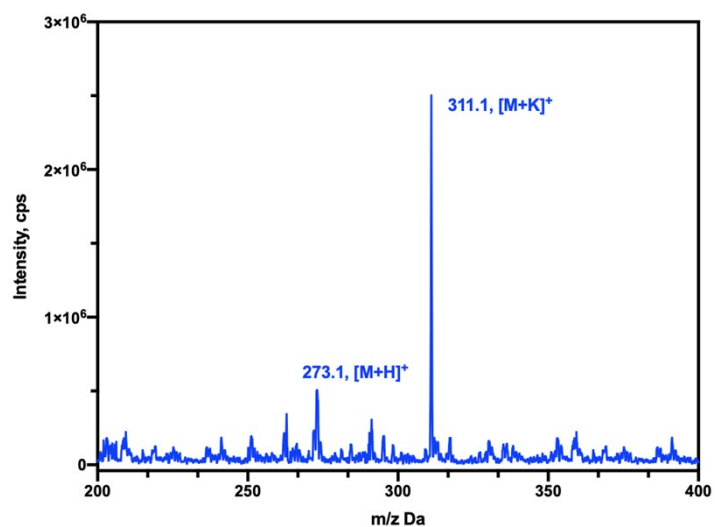
f. Synthesis of dye 5a

BD-CHO (0.5 mmol, 90 mg) and 2-(3,5,5-trimethylcyclohex-2-en-1-ylidene) malononitrile (1 mmol, 186 mg) were dissolved in 10 mL toluene, followed by adding piperidine (0.1 mmol, 10 μL) and reflux for 4 h. After cooling to room temperature and removing the solvent, the target product was purified by column chromatography (PE/DCM = 1/1). The target product was obtained as solid (56 mg, yield 32%). ¹H-NMR (CDCl₃, 400 MHz) (ppm): 7.56 (d, *J*=15.8 Hz, 1H), 7.35 (d, *J*=7.7 Hz, 1H), 7.16 (d, *J*=15.9 Hz, 1H), 6.90 (s, 1H), 6.11 (d, *J*=7.8 Hz, 1H), 3.49 (s, 6H), 2.55 (d, *J*=37.8 Hz, 4H), 1.10 (s, 6H). ¹³C NMR (CD₃CN, 125 MHz) (ppm): 169.16, 155.00, 148.88, 145.70, 140.84, 136.64, 133.07, 129.61, 122.86, 114.20, 113.27, 111.88, 104.61, 43.18, 42.63, 39.39, 32.17, 28.24. ESI-MS: [M] calculated for C₂₁H₂₁N₅O: 359.17; Found 359.5.

Table S1. Transition Dipole Moment and Oscillator Strength of 2a-5a

VBDfluors	Trans. Dip. x ¹	Trans. Dip. y ¹	Trans. Dip. z ¹	Dip. S. ²	Oscil.str. ³
2a	2.762369	0.026609	0.000050	7.631390	0.486070
3a	2.821430	-0.227163	0.022736	8.012586	0.514770
3a'	2.207190	-0.279304	0.000502	4.949700	0.303250
4a	2.711938	-0.131641	-0.051501	7.374591	0.433960
5a	-4.502951	0.201146	-0.036904	20.318393	1.088170

1. Trans. Dip. x/y/z: transition dipole moment at x/y/z directions; 2. Dip. S.: square of transition dipole moment $|\langle i | -r | j \rangle|^2$; 3. Oscil.str.: Oscillator Strength.

**Figure S1.** ESI mass spectrum of dye BD-CHO.**Figure S2.** ESI mass spectrum of dye 2a.

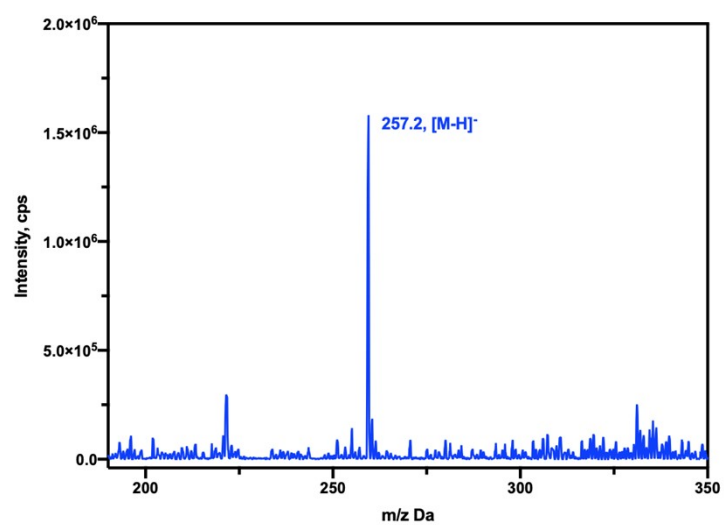


Figure S3. ESI mass spectrum of dye 3a.

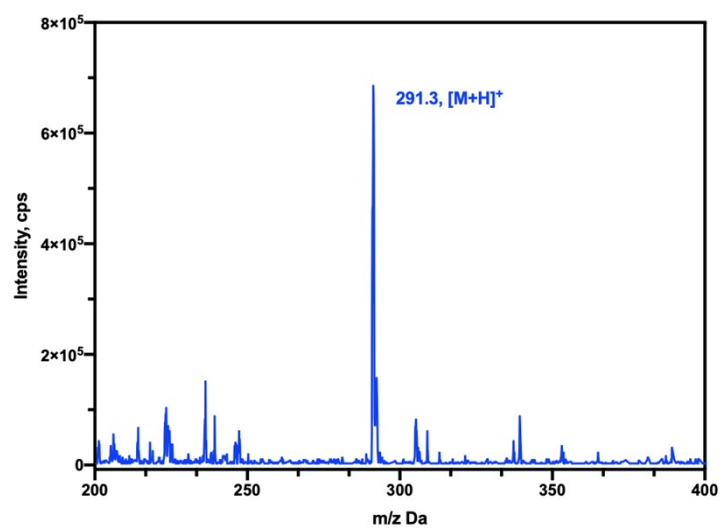


Figure S4. ESI mass spectrum of dye 4a.

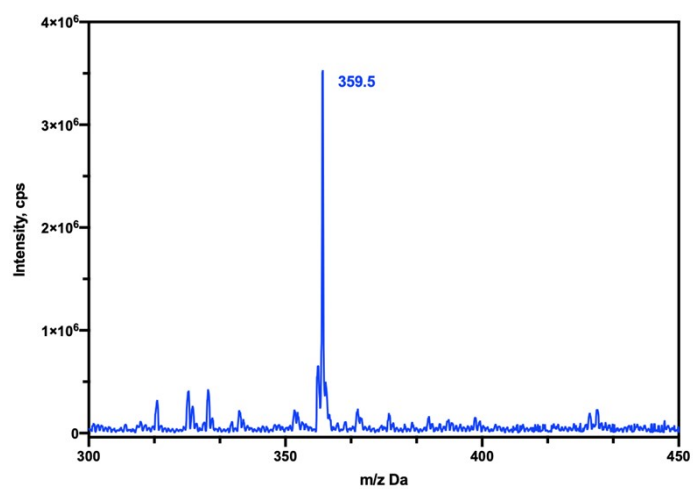


Figure S5. ESI mass spectrum of dye 5a.

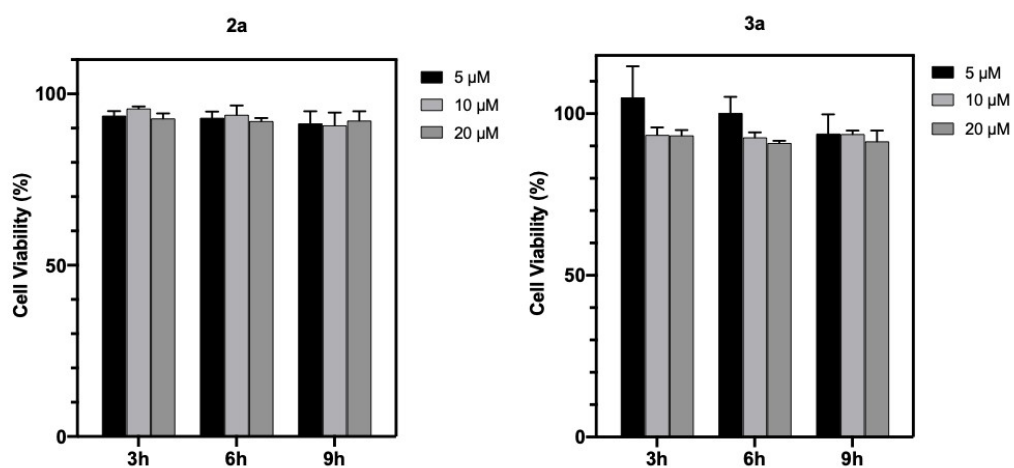


Figure S6. Cytotoxicity of 2a and 3a in HeLa cells (incubation for 3/6/9 h).

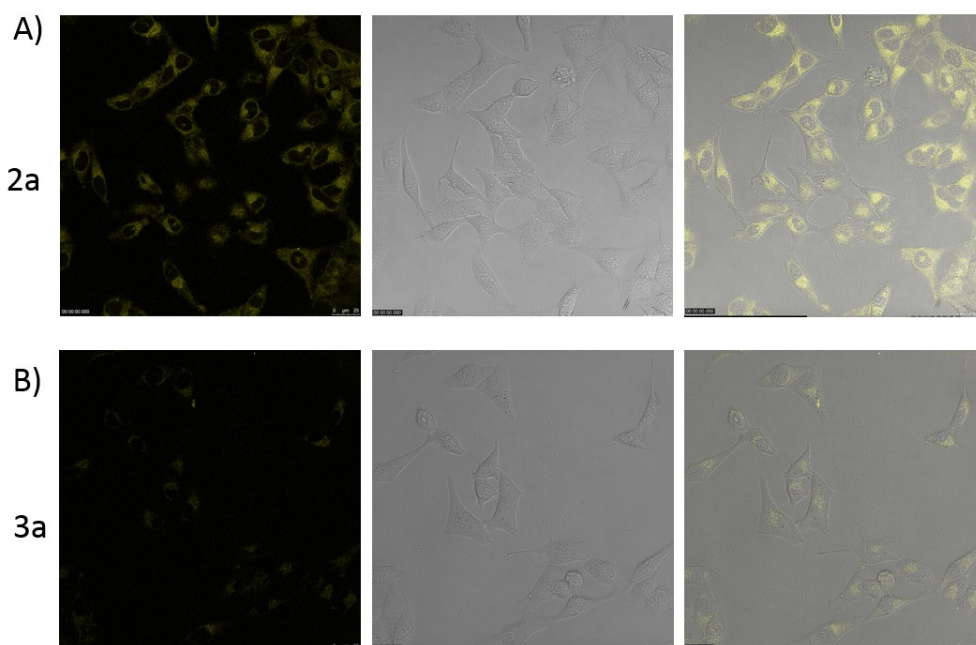
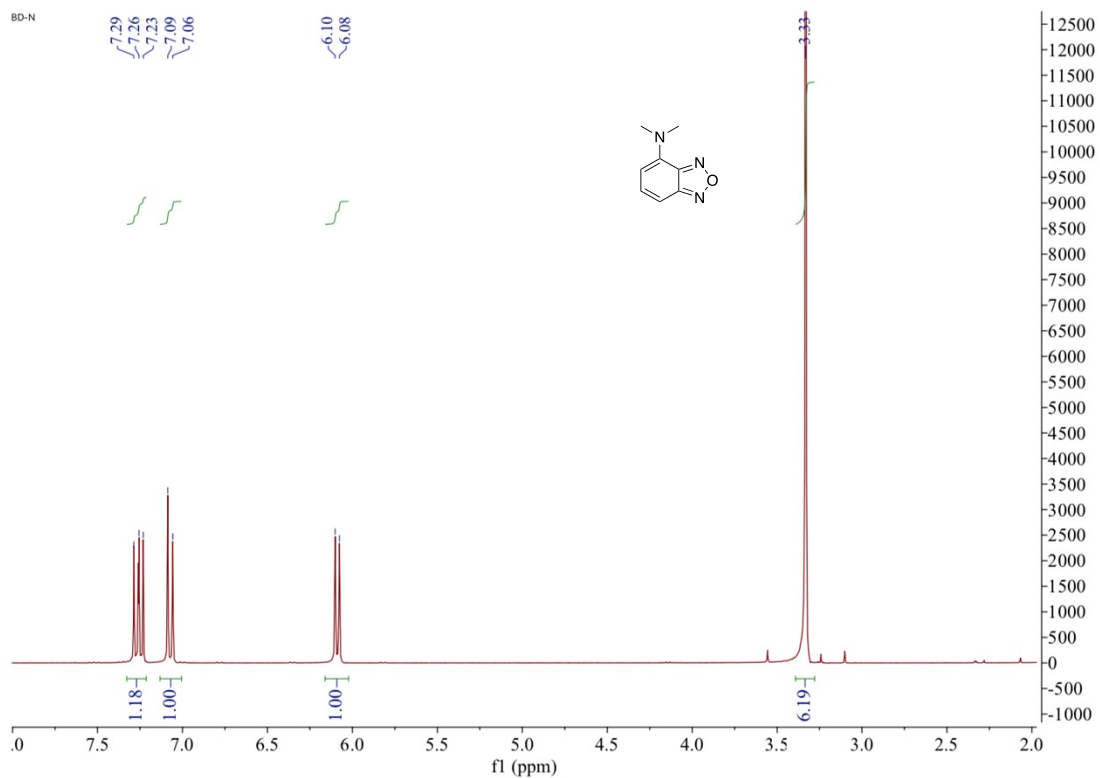
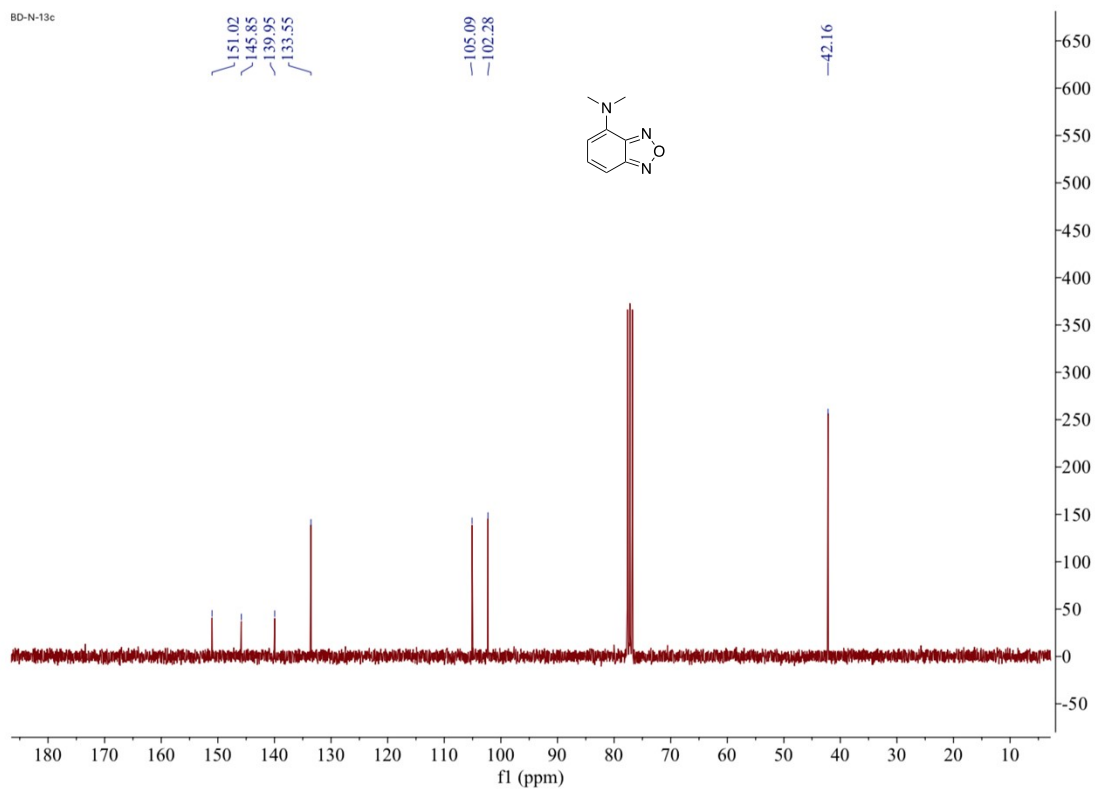


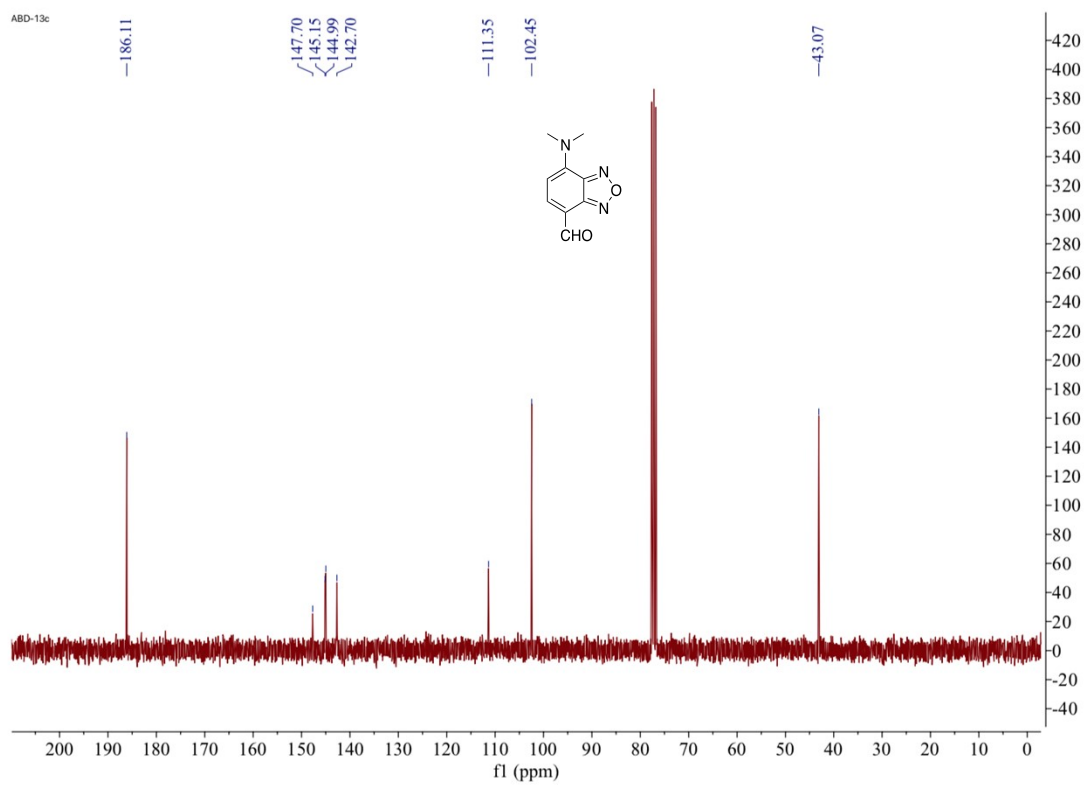
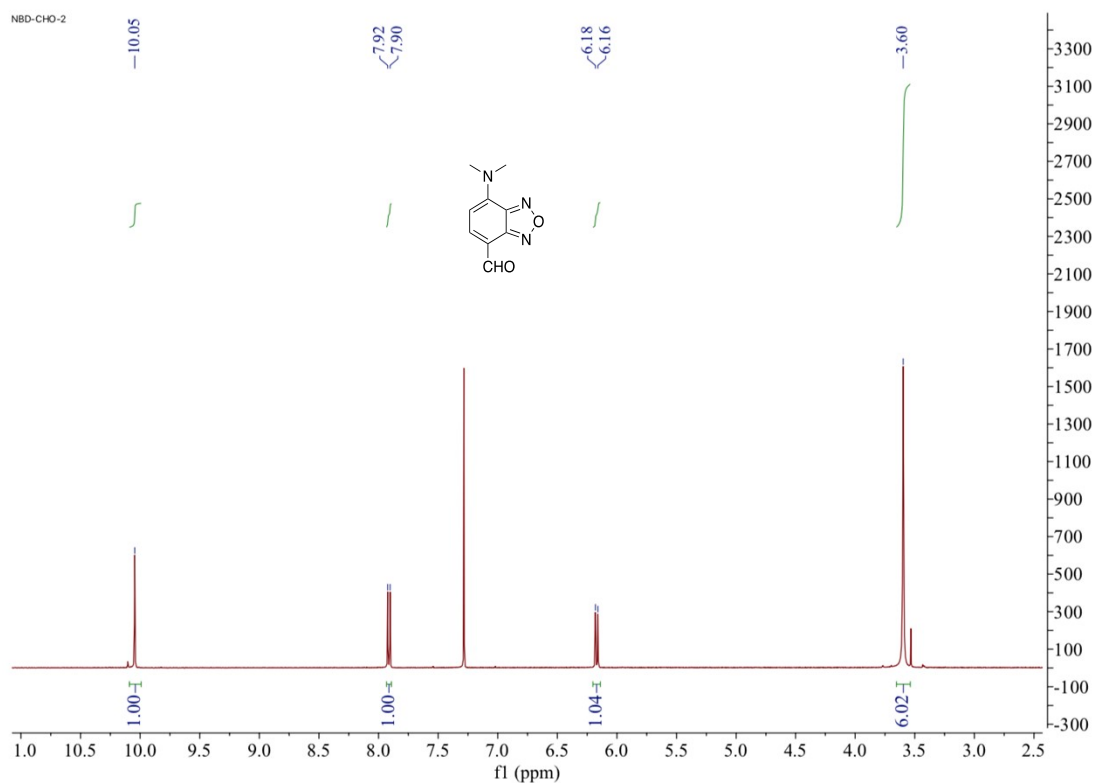
Figure S7. cellular uptake of 2a and 3a. Scale bar: 10 μm. Probe: 10 μM. Incubation time: 30 min.

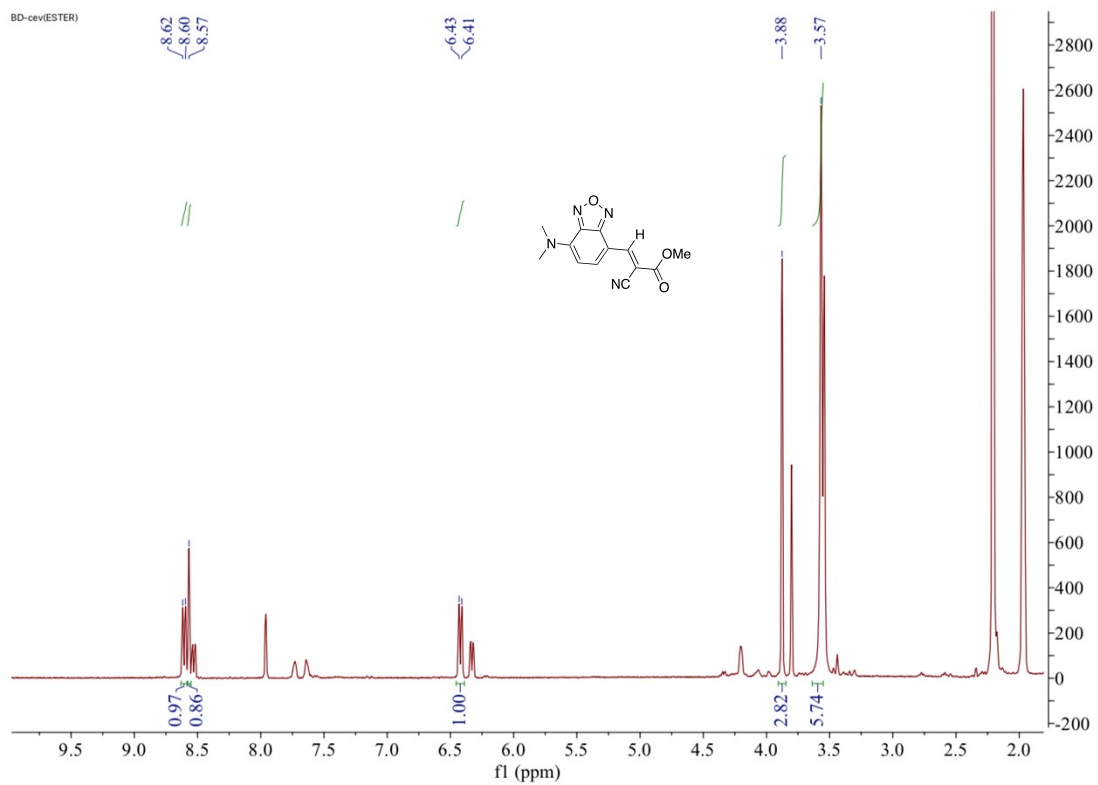


^1H NMR spectrum of compound N, N-dimethyl-2,1,3-benzoxadiazole in CDCl_3 .

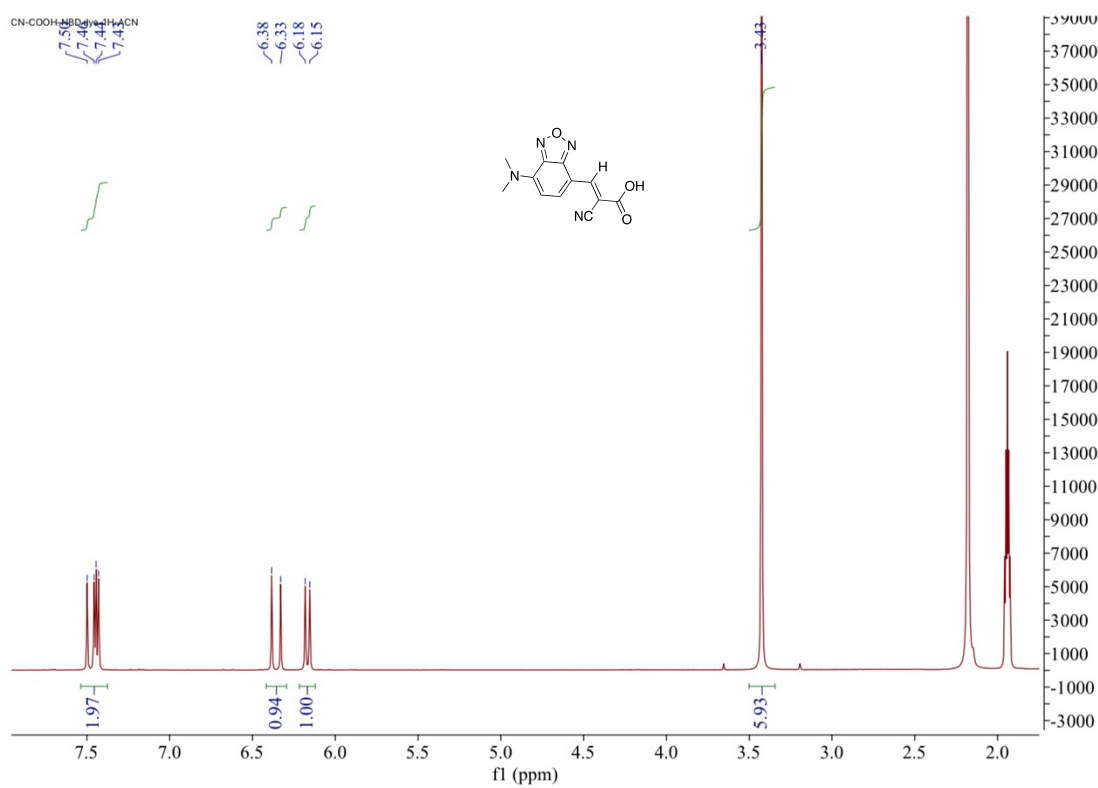


^{13}C NMR spectrum of compound N, N-dimethyl-2,1,3-benzoxadiazole in CDCl_3 .

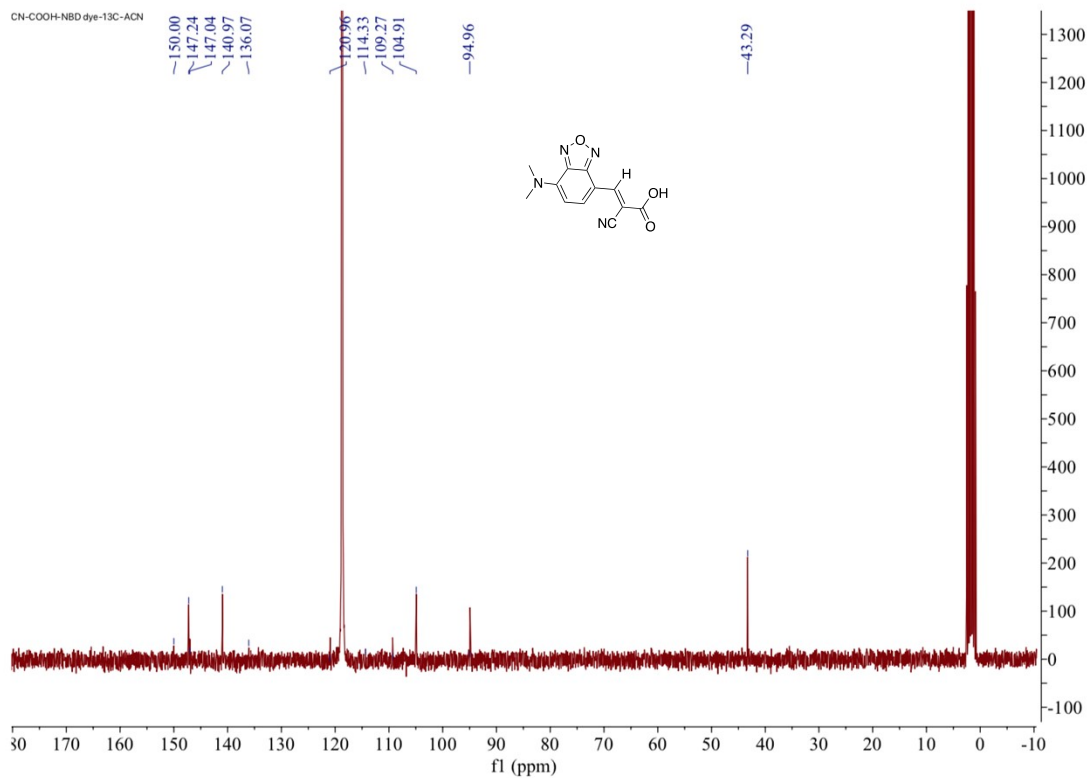




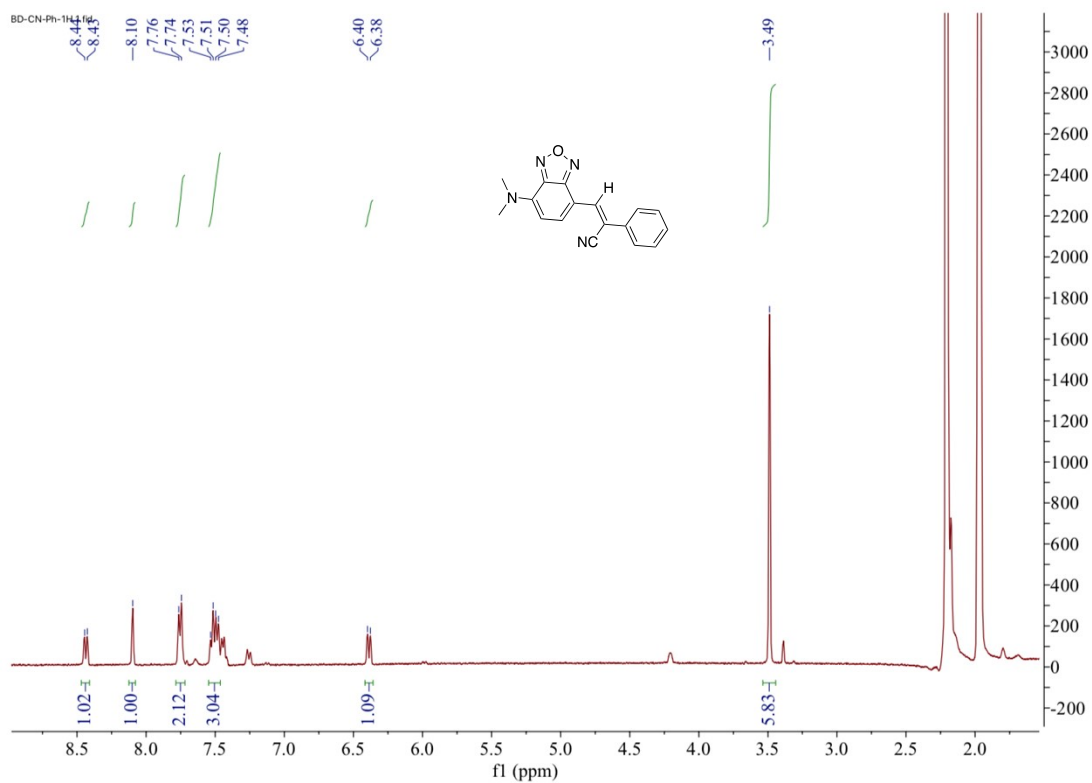
^1H NMR spectrum of dye 2a in CD_3CN .



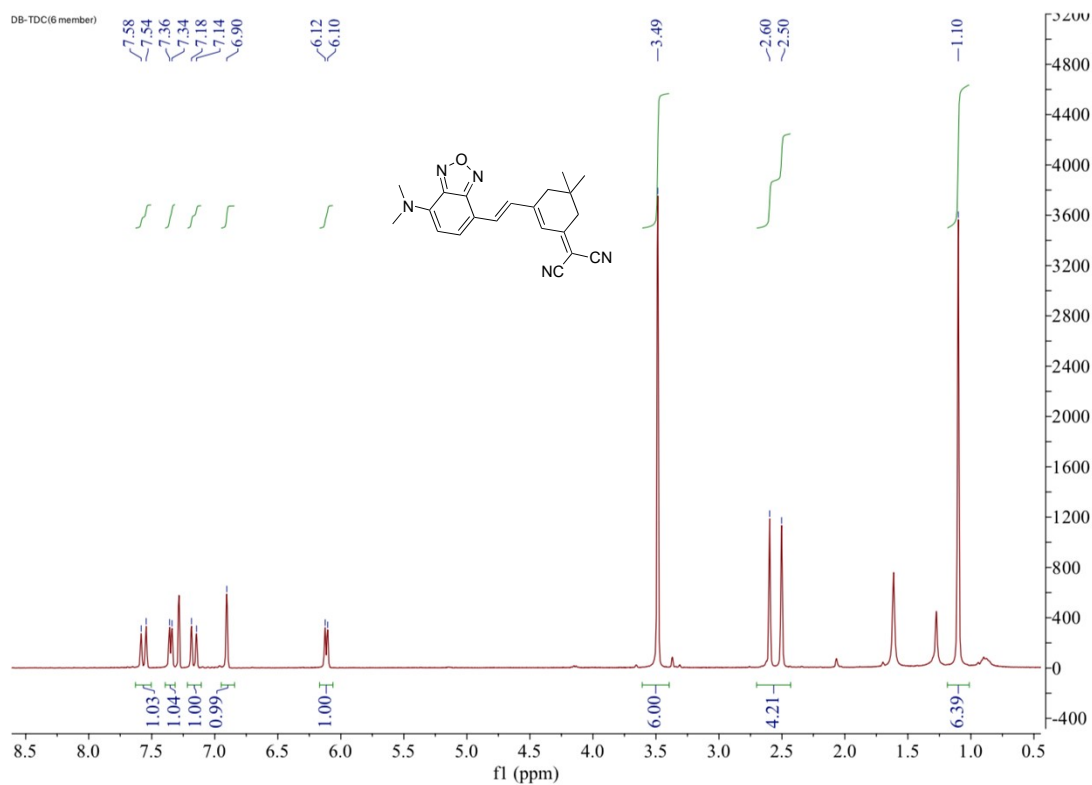
^1H NMR spectrum of dye 3a in CD_3CN .



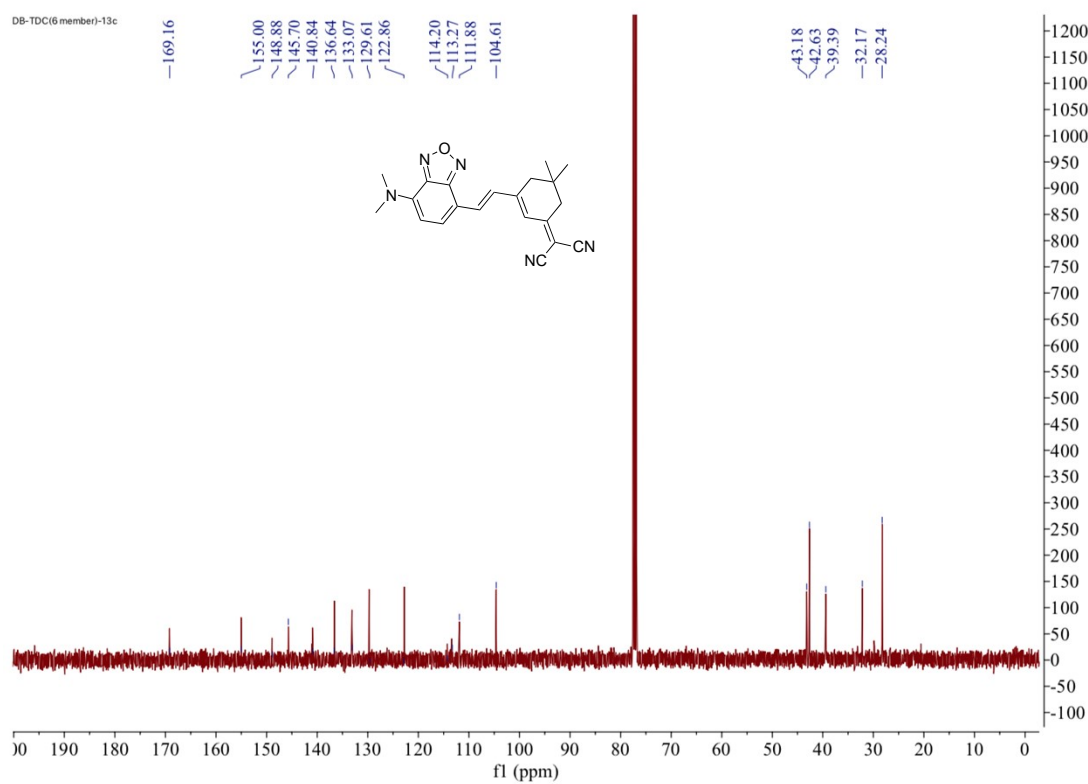
^{13}C NMR spectrum of dye 3a in CD_3CN .



^1H NMR spectrum of dye 4a in CD_3CN .



¹H NMR spectrum of dye 5a in CDCl₃.



¹³C NMR spectrum of dye 5a in CDCl₃.

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