Supporting Information for

Different alkyl chains-based fluorescent probes with large Stokes shift for imaging cell membrane and mitochondria in different living cell lines †

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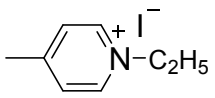
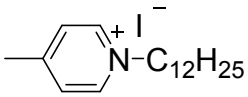
Synthesis



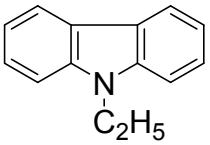
1a

Synthesis of 1a: 4-methylpyridine (0.5mL, 5 mmol) and iodoethane (1.3 mL, 7.5 mmol) were dissolved in the 20 mL of EtOH. Then, the solution mixture was refluxed for 6 h. After cooling and filtrating, the final product was washed with ether, and finally a red viscous liquid was obtained for 3b with a yield of 87%.^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 9.10 (d, J = 8 Hz, 2H), 7.80 (d, J = 8 Hz, 2H), 4.77-4.72 (q, J = 7.4 Hz, 2H), 2.54 (s, 3H), 1.56-1.53 (t, J = 8 Hz, 3H).



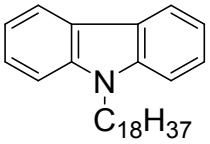
1b

Synthesis of 1b: 4-methylpyridine (0.5mL, 5 mmol) and 1-iodododecane (2.5 mL, 7.5 mmol) were dissolved in the 20 mL of EtOH. Then, the solution mixture was refluxed for 6 h. After cooling and filtrating, the final product was washed with ether, and finally a red viscous liquid was obtained for 3a with a yield of 82%.^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 9.24 (d, J = 6.4 Hz, 2H), 7.93 (d, J = 6.4 Hz, 2H), 4.86 (t, J = 7.4 Hz, 2H), 2.70 (s, 3H), 2.10-1.95 (m, 2H), 1.29 (m, 18H), 0.87 (t, J = 6.9 Hz, 3H).



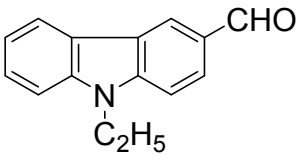
2a

Synthesis of 2a: 9H-carbazole 1 g (6 mmol), bromoethane 1.1mL (9 mmol) and KOH 1g (18 mmol) were dissolved in DMF (10 mL), the mixture were stirred and heated to 50 °C for 12h. After the reaction solution was cooled to room temperature, the product was poured into water. The residue was filtered and washed with water for 3 times, dried under reduced pressure to afford a gray solid with a yield of 85%.^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.14 (d, J = 8 Hz, 2H), 7.55-7.41 (m, 4H), 7.26 (d, J = 6.8 Hz, 2H), 4.41 (q, J = 7.2 Hz, 2H), 1.47 (t, J = 7.2 Hz, 3H).



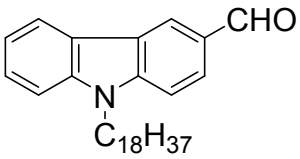
2b

Synthesis of 2b: 9H-carbazole 1 g (6 mmol) , 1-bromooctadecane 3.15mL (9 mmol) and KOH 1g (18 mmol) were dissolved in DMF (10 mL), the mixture were stirred and heated to 50 °C for 12h. After the reaction solution was cooled to room temperature, the product was poured into water. The residue was filtered and washed with water for 3 times, dried under reduced pressure to afford a gray solid with a yield of 80%.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 7.7 Hz, 2H), 7.50-7.36 (m, 4H), 7.22 (d, J = 6.8 Hz, 2H), 4.29 (t, J = 7.3 Hz, 2H), 1.91-1.81 (m, 2H), 1.41-1.19 (m, 30H), 0.87 (t, J = 6.8 Hz, 3H).



3a

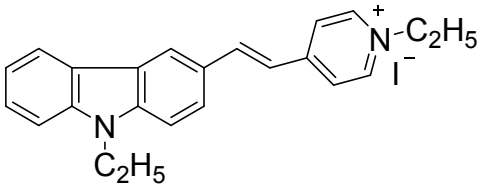
Synthesis of 3a: DMF (2 mL) was added into a drying round-bottom flask and the system was cooled to 0 °C，dropwisely added phosphoryl chloride (2mL, 20mmol) with stirring for 30min, A solution of $CHCl_3$ (10 mL) containing compound 2a 0.5g(2mmol) was then added.The reaction mixture was stirred at room temperature for 1 h and then mixture was warmed at 80 °C under nitrogen for 12 h. After being cooled to room temperature, the reaction mixture was poured into ice-water, neutralized with NaOH solution and then extracted with CH_2Cl_2, the crude product was purified by column chromatography with ethyl acetate/petroleum ether (5:1) as eluent, finally the white solid was obtained with a yield of 80%. H NMR (400 MHz, $CDCl_3$), δ (ppm): 10.08 (s, 1H), 8.59 (s, 1H), 8.14 (d, J = 7.4 Hz, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 7.45 (d, J = 8.5 Hz, 2H), 7.32 (t, J = 7.3 Hz, 1H), 4.39 (m, 2H), 1.45 (t, J = 6.5 Hz, 3H).



3b

Synthesis of 3b: DMF (2 mL) was added into a drying round-bottom flask and the system was cooled to 0 °C，dropwisely added phosphoryl chloride (2mL, 20 mmol) with stirring for 30min, A solution of $CHCl_3$ (10 mL) containing compound 2b 0.9g(2

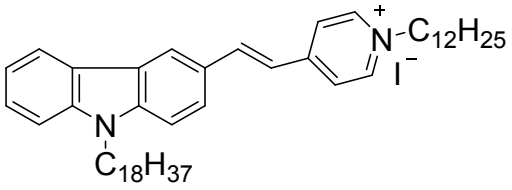
mmol) was then added.The reaction mixture was stirred at room temperature for 1 h and then mixture was warmed at 80 °C under nitrogen for 12 h. After being cooled to room temperature, the reaction mixture was poured into ice-water, neutralized with NaOH solution and then extracted with CH_2Cl_2, the crude product was purified by column chromatography with ethyl acetate/petroleum ether (5:1) as eluent, finally the white solid was obtained with a yield of 80%.^{1}H NMR (400 MHz, $CDCl_3$) , δ (ppm): 10.09 (s, 1H), 8.61 (s, 1H), 8.16 (d, *J* = 7.8 Hz, 1H), 8.01 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.56-7.50 (m, 1H), 7.46 (t, *J* = 8.0 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 1H), 4.33 (t, *J* = 7.3 Hz, 2H), 1.97-1.80 (m, 2H), 1.23 (m, 30H), 0.87 (t, *J* = 6.8 Hz, 3H).



CA-C2

*Synthesis of **CA-C2**:* Compound 3a (0.22 g, 1 mmol) and compound 1a (0.37 g, 1.5 mmol) were dissolved in the 20 mL of EtOH. Then, the solution mixture was refluxed for 12 h. The mixture was poured into water (100 mL) and then extracted by CH_2Cl_2. The organic layer was washed by saturated NaCl solution for several times before being dried with $MgSO_4$. After CH_2Cl_2 was evaporated, the crude product was purified by column chromatography with CH_2Cl_2/CH_3OH (10:1, v/v) as eluent, and finally a red solid was obtained for 4a with a yield of 70%.1H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.84 (d, *J* = 4 Hz, 2H), 8.37 (s, 1H), 8.17 (d, *J* = 8 Hz, 1H), 8.04 (d, *J* = 8 Hz, 2H), 7.92 (d, *J* = 16.1 Hz, 1H), 7.79 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.57-7.47 (m,

1H), 7.44-7.36 (m, 2H), 7.34-7.29 (m, 1H), 7.18 (d, *J* = 16.0 Hz, 1H), 4.68 (q, *J* = 7.4 Hz, 2H), 4.33 (q, *J* = 7.2 Hz, 2H), 1.66 (t, *J* = 7.4 Hz, 3H), 1.44 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*): δ 153.861, 144.133, 142.932, 141.407, 140.548, 126.822, 123.543, 123.190, 122.607, 121.722, 121.017, 120.377, 120.155, 110.335, 110.187, 55.417, 40.636, 16.680, 14.270. HRMS (m/z): $[M-I]^+$calcd for $C_{23}H_{23}N_2$, 327.1861; found: 327.1855.



CA-C12

Synthesis of ***CA-C12****:* Compound 3b (0.23 g, 0.5 mmol) and compound 1b (0.3 g, 0.75 mmol) were dissolved in the 20 mL of EtOH. Then, the solution mixture was refluxed for 12 h. The mixture was poured into water (100 mL) and then extracted by CH_2Cl_2. The organic layer was washed by saturated NaCl solution for several times before being dried with $MgSO_4$. After CH_2Cl_2 was evaporated, the crude product was purified by column chromatography with CH_2Cl_2/CH_3OH (10:1, v/v) as eluent, and finally a yellow solid was obtained for 4a with a yield of 70%.^{1}H NMR (400 MHz, $CDCl_3$) , δ (ppm): 8.86 (d, *J* = 6.5 Hz, 2H), 8.33 (s, 1H), 8.14 (d, *J* = 8 Hz, 1H), 8.00 (d, *J* = 6.8 Hz, 2H), 7.89-7.85 (d, *J* = 16.0 Hz,1H), 7.76 (d, *J* = 8.7 Hz, 1H), 7.55-7.47 (m, 1H), 7.44-7.35 (m, 2H), 7.31 (t, *J* = 7.4 Hz, 1H), 7.24 (s, 1H), 4.60 (t, *J* = 7.3 Hz, 2H), 4.24 (t, *J* = 7.2 Hz, 2H), 1.68 (s, 2H), 1.59 (s, 4H), 1.4-1.24 (m, 46H), 0.9-0.83 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.129, 148.990, 148.281, 144.008, 143.439, 142.258, 140.995, 127.926, 126.612, 125.533, 123.438, 122.665, 120.328,

118.588, 60.955, 43.370, 42.574, 31.894, 29.668, 27.276, 22.679, 14.104. HRMS (m/z): $[M-I]^+$calcd for $C_{49}H_{75}N_2$, 691.5930; found: 691.5934.

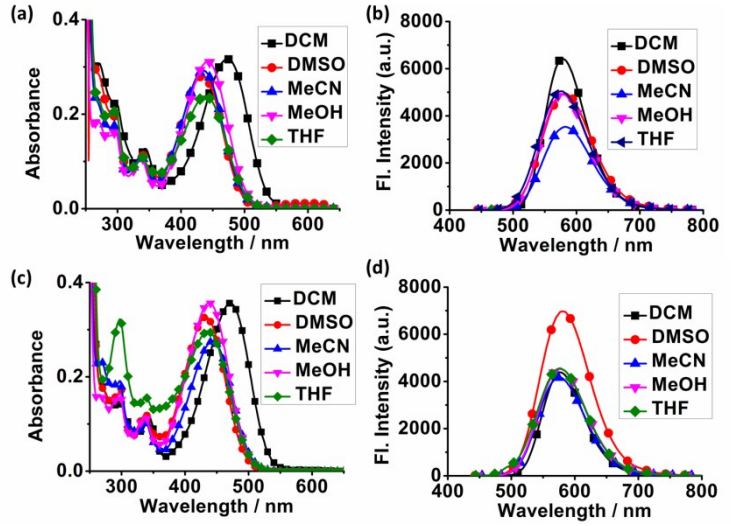


Fig. S1 Absorption and fluorescence spectra of **CA-C12** (a, b) and **CA-C2** (c, d) in another five solvents. DCM(■), DMSO(●), MeCN(▲), MeOH(▼), THF(◆). [**CA-C12**] = [**CA-C2**] =10 μM.

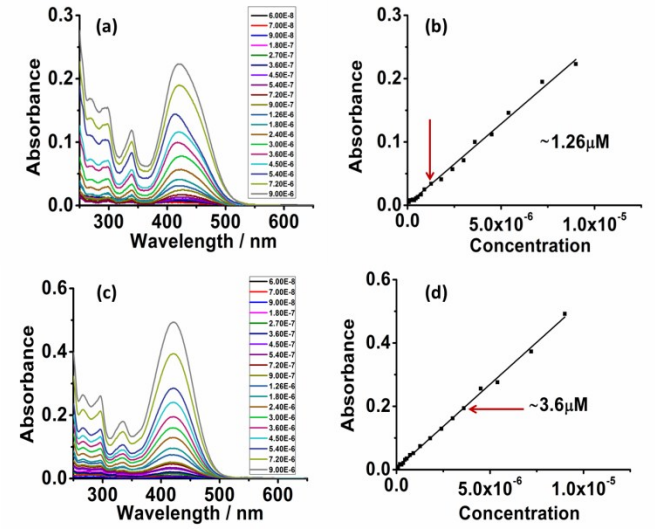


Fig. S2. Absorption spectra and plot of intensity against dye concentration for **CA-C12** (a, b) and **CA-C2** (c, d).

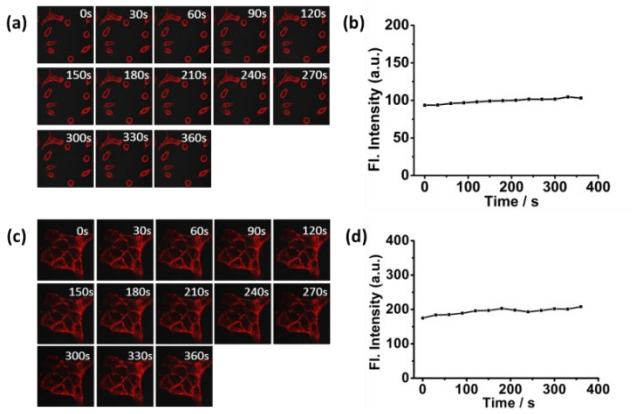


Fig. S3 (a and c) Fluorescence images (the red channel) of A549 and 4T-1 cells incubated with **CA-C12** acquired at different times under successive excitation. (b and d) Mean intensities of the cells incubated with **CA-C12** (10 μM) in the red channel under successive excitation at different times. Excitation wavelength: 405 nm.

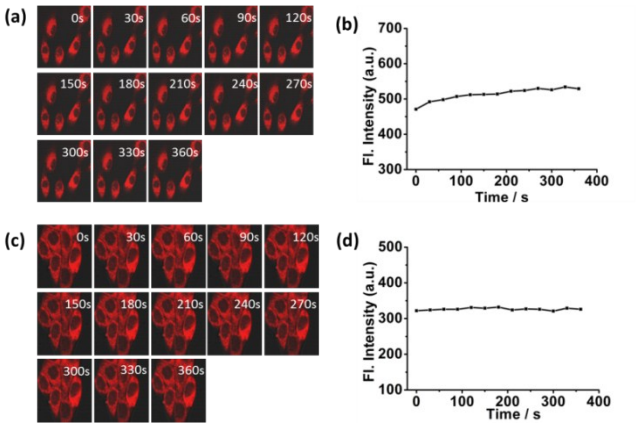


Fig. S4 (a and c) Fluorescence images (the red channel) of A549 and 4T-1 cells incubated with **CA-C2** acquired at different times under successive excitation. (b and d) Mean intensities of the cells incubated with **CA-C2** (10 μM) in the red channel under successive excitation at different times. Excitation wavelength: 405 nm.

Table S1. Cytotoxicity Data (HeLa, 8h) of the probes **CA-C2** and **CA-C12**.

Incubate concentration(μM)	0	1	5	10	20	30
CA-C2 (% cell survival)	100±5	100±5	97±5	92±5	85±5	82±5
CA-C12 (% cell survival)	100±5	96±5	92±5	87±5	82±5	76±5

Cell viability was quantified by the MTT assays (mean ± SD).

Spectral Characterization

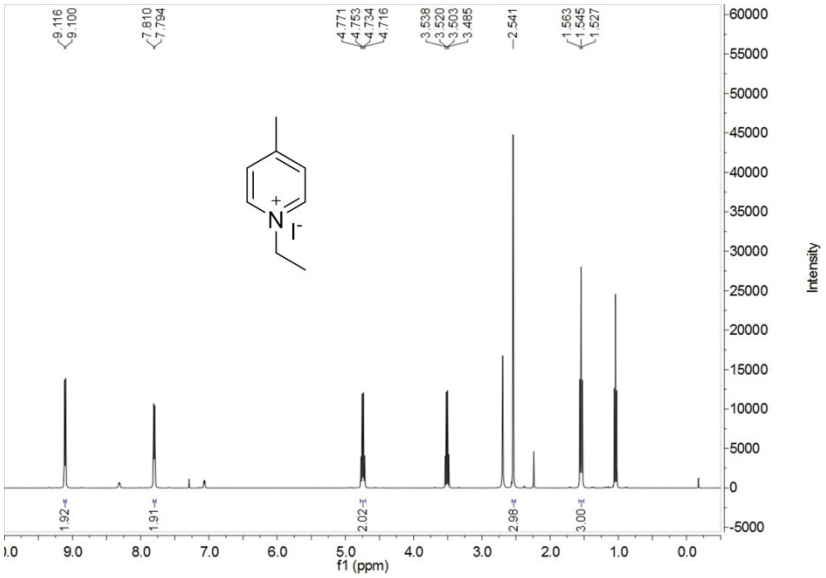


Fig. S5. ^{1}H NMR spectrum of compound **1a** in CD_3Cl.

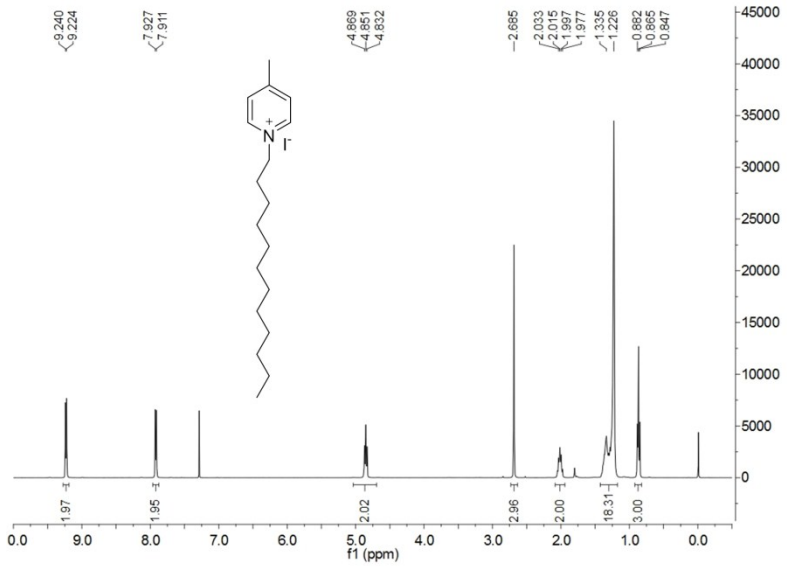


Fig. S6. ^{1}H NMR spectrum of compound **1b** in CD_3Cl.

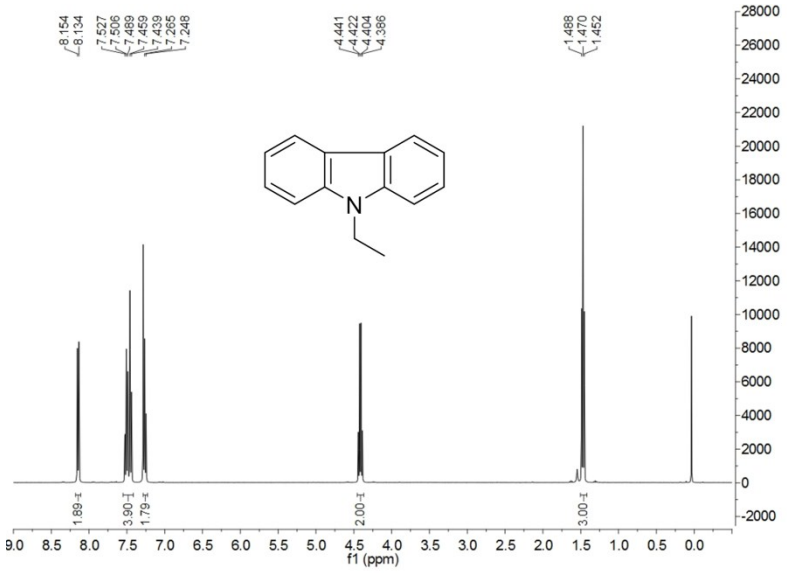


Fig. S7. ^{1}H NMR spectrum of compound **2a** in CD_3Cl.

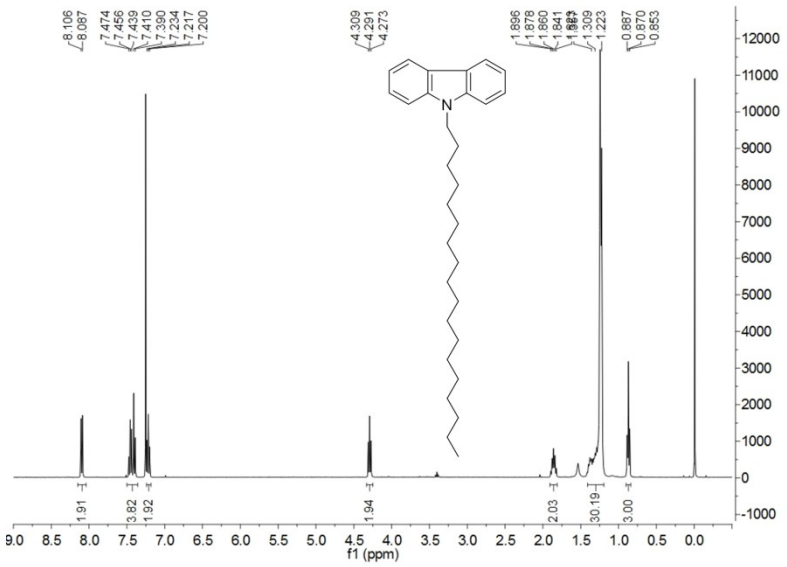


Fig. S8. ^{1}H NMR spectrum of compound **2b** in CD_3Cl.

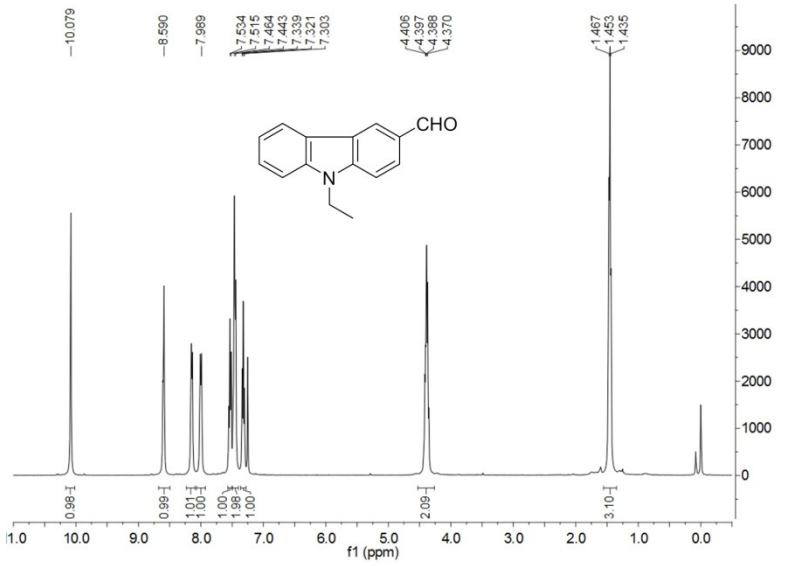


Fig. S9. ^{1}H NMR spectrum of compound **3a** in CD_3Cl.

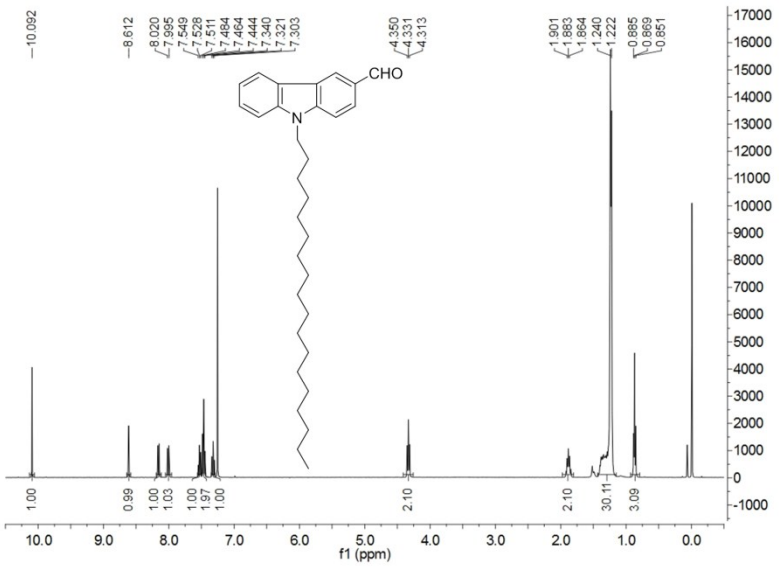


Fig. S10. ^{1}H NMR spectrum of compound **3b** in CD_3Cl.

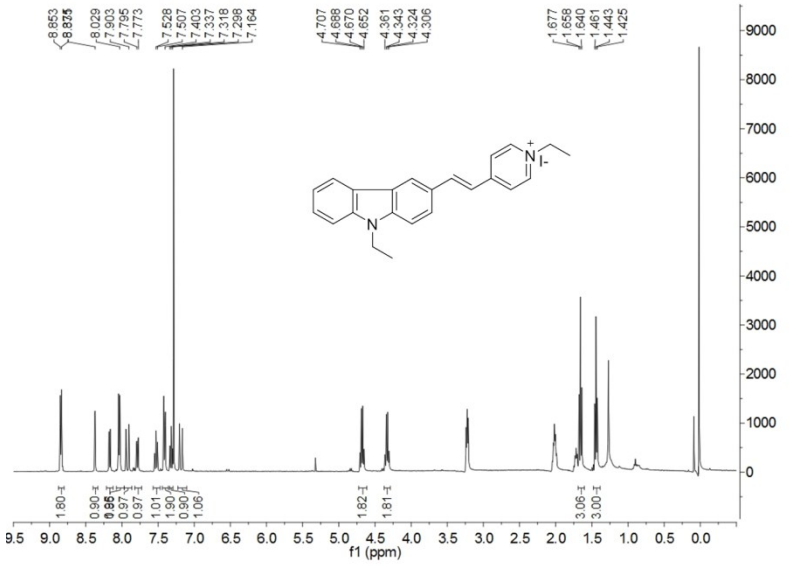


Fig. S11. ^{1}H NMR spectrum of compound **CA-C2** in CD_3Cl

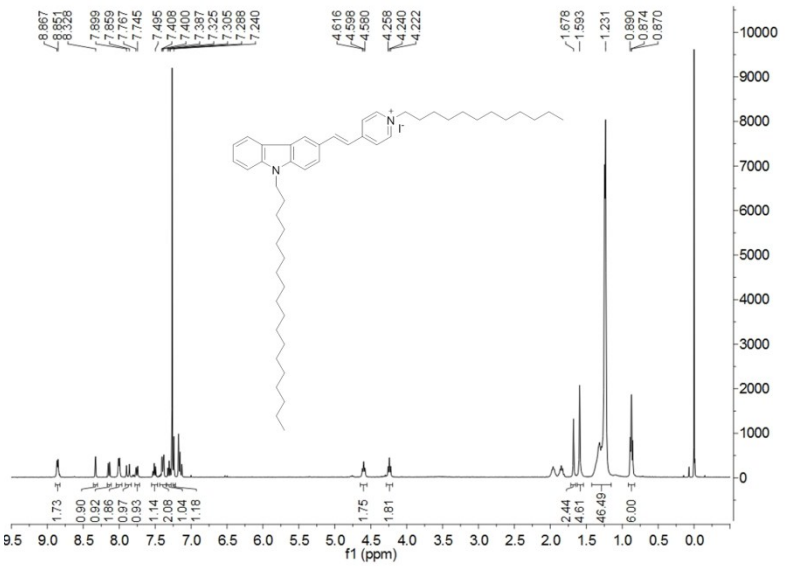


Fig. S12. ^{1}H NMR spectrum of compound **CA-C12** in CD_3Cl

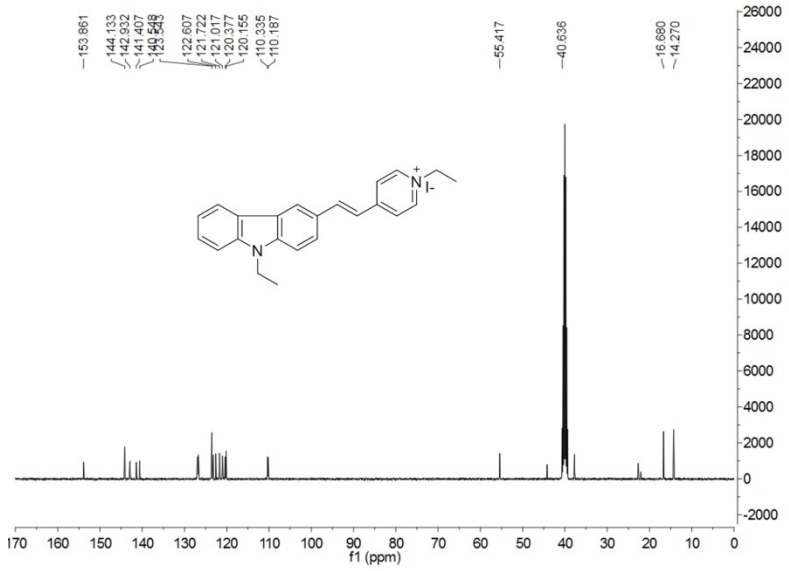


Fig. S13. ^{13}C NMR spectrum of compound **CA-C2**

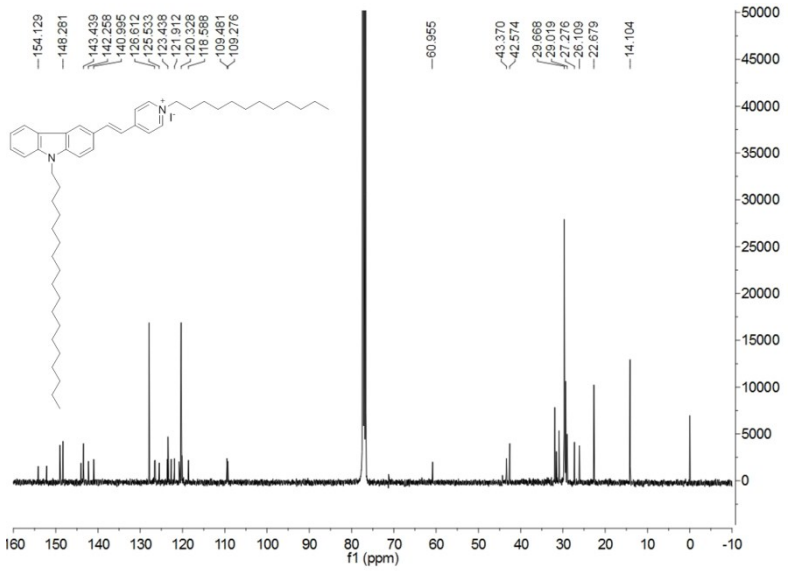


Fig. S14. ^{13}C NMR spectrum of compound **CA-C12**

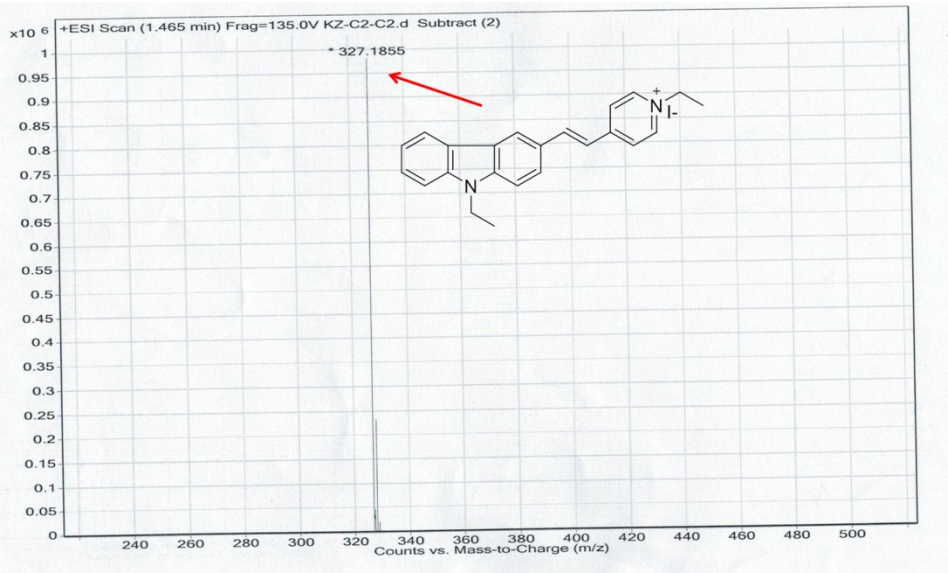


Fig. S15. HRMS spectrum of the compound **CA-C2**

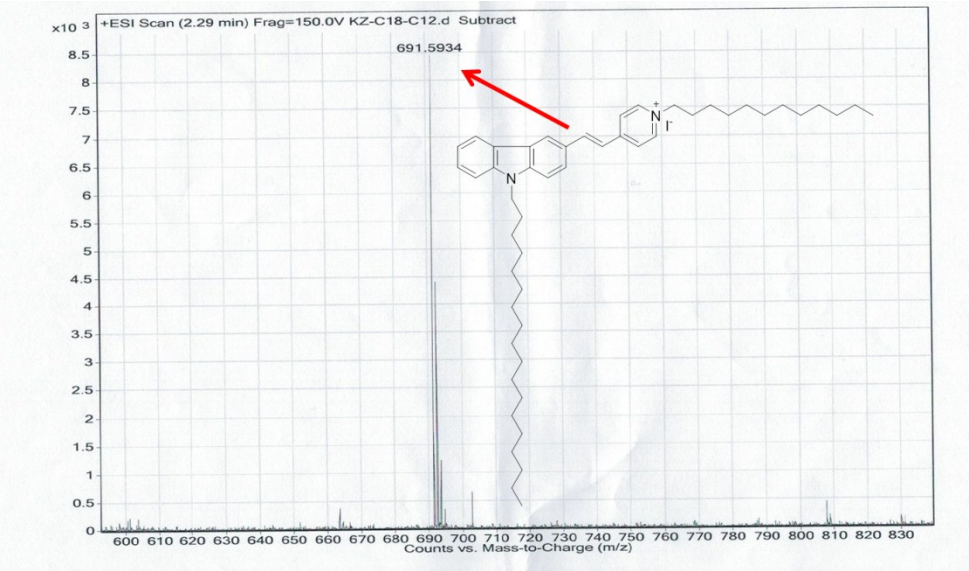


Fig. S16. HRMS spectrum of the compound **CA-C12**