Electronic Supplementary Information for

A novel near-infrared fluorescent light-up probe for tumor imaging and drug-induced liver injury detection

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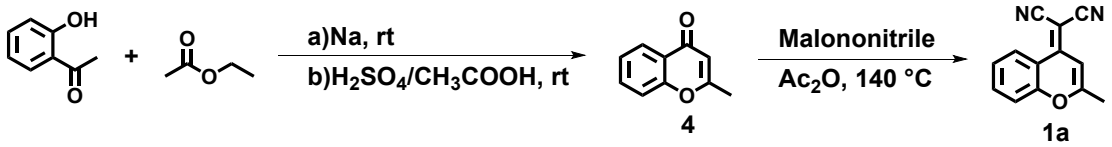
1 General methods

DAPI was purchased from Beyotime Biotechnology and cell-culture products were purchased from Invitrogen Gibco. All solvents were analytical grade purity. Tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone. N, N-Dimethylformamide (DMF) and dichloromethane (CH_2Cl_2) were distilled from calcium hydride. All other standard synthesis reagents were purchased from commercial suppliers (such as Adamas, Aldrich and Energy Chemical) and used without further purification unless otherwise noted. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300), both of which were obtained from the Qingdao Ocean Chemicals. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV400 magnetic resonance spectrometer. Chemical shifts (ppm) were reported relative to internal $CDCl_3$ (^{1}H, 7.26 ppm and ^{13}C, 77.36 ppm). ESI-MS were performed on Finnigan LCQ advantage mass spectrometer. High resolution mass data (HRMS) were obtained with a Thermo LTQ XL Orbitrap instrument. MALDI-TOF-MS were performed on an Applied Biosystems 4700 MALDI TOF mass spectrometer. Preparative high performance liquid chromatography (HPLC) was performed on a Dionex HPLC System with UV-Vis detection a reversed-phase C8 (Thermo, 5 μm, 4.6 × 250 mm) column was used for semi-preparation (mobile phase: water/acetonitrile with 0.06 % TFA). A PerkinElmer Lambda 25 UV-Vis spectrophotometer was used for the absorption measurements. A Hitachi Fluorescence Spectrophotometer F-4600 was utilized for fluorescence spectra detection. Cell images were captured by a Leica-LCS-SP8-STED confocal laser scanning fluorescence microscope (Leica, Germany). *In vivo* and *ex vivo* imaging of the model mice were obtained on a Bruker In Vivo-Xtreme Imaging System (Bruker, Xtreme BI).

2 Synthetic procedures

2.1 Synthesis route of compound **1a**



2.1.1 Synthesis of compound **1a**[1]

Sodium (8 g) was cut into pieces, and then suspended in ethyl acetate (200 mL). 2'-hydroxyacetophenone (10 g, 73.5 mmol) was added into the above suspension at room temperature. A vigorous reaction was observed. After 5 h, TLC analysis indicated the reaction is complete, then quenched by pouring into ice water and further acidified to pH 7 with 2 M HCl solution. The solution was extracted with ethyl acetate and the combined organic layers were washed with brine and dried over magnesium sulfate. The solvent was evaporated to yield the crude product 1, 3-dione.

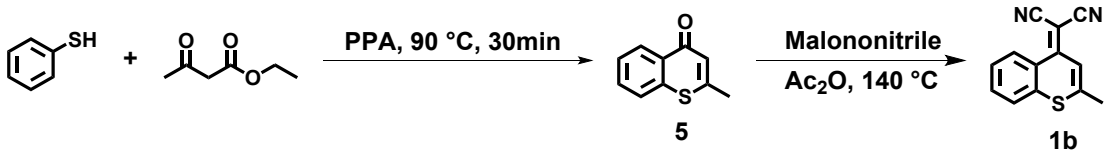
To the solution of the crude 1, 3-dione product in acetic acid (70 mL) was added conc. H_2SO_4 (4.6 mL) at room temperature, and the mixture allowed to reflux for 30 min. TLC analysis indicated the reaction is complete, the cooled mixture was vigorously stirred with ice/water and further basified to pH 7 with saturated K_2CO_3 solution. The solution was extracted with CH_2Cl_2 and the combined organic layers were washed with brine and dried over magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was purified via flash column chromatography (petroleum ether: ethyl acetate = 10:1 v/v) to give the compound **4**.

To a solution of compound **4** (800 mg, 5 mmol) in Ac_2O (5 mL) was added malononitrile (3 g, 6.5 mmol). The resulting mixture was raised to 140 ^oC and stirred at this temperature for 14 h. After that 40 mL methanol was added and stirred at 55 ^oC for another 2 h. Then the solvent was evaporated by vacuum evaporation. The crude product was purified by silica gel chromatography (petroleum ether: ethyl acetate = 20:1 v/v) to give compound **1a** as a yellow solid (703 mg, 18% yield, three steps).

^1H NMR (400 MHz, $CDCl_3$) δ 8.90 (d, J = 8.3 Hz, 1H), 7.75 – 7.68 (m, 1H), 7.45 (dd, J = 12.4, 5.8 Hz, 2H), 6.71 (s, 1H), 2.44 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.0, 153.6, 153.2, 134.9, 126.4, 126.1, 119.0, 117.9, 116.9, 115.8, 105.8, 20.8.

ESI-MS calcd for $C_{13}H_9N_2O^+$ ([M+H]$^+$): 209.07, found: 209.19.

2.2 Synthesis route of compound **1b**



2.2.1 Synthesis of compound **5**[2]

Benzenethiol (1.03 mL, 10 mmol) was added to polyphosphoric acid (12 mL) preheated to 90 °C under mechanical stirring. At this temperature, ethyl 3-oxobutanoate (1.3 mL, 10 mmol) was added very slowly to the mixture and stirring was continued for 30 min after the addition. The cooled mixture was vigorously stirred with ice/water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, and evaporated in vacuo. The crude product was purified by silica gel chromatography (petroleum ether: ethyl acetate = 10:1 v/v) to give compound **5** as a brown solid (564 mg, 32% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 8.1 Hz, 1H), 7.59 – 7.52 (m, 2H), 7.52 – 7.47 (m, 1H), 6.83 (s, 1H), 2.44 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.9, 151.6, 138.0, 131.7, 131.0, 128.9, 127.8, 126.3, 125.2, 23.6.

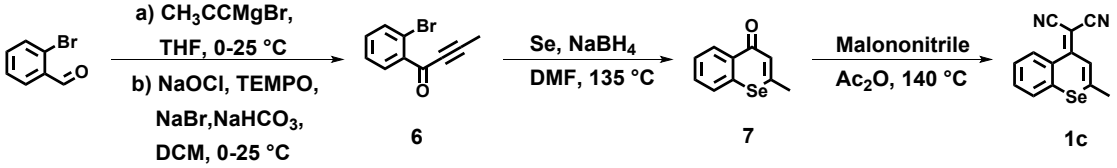
2.2.2 Synthesis of compound **1b**

To a solution of compound **5** (500mg, 3.12mmol) in Ac_2O (10 mL) was added malononitrile (1.87 g, 31.2 mmol). The resulting mixture was raised to 140 °C and stirred at this temperature for 4 h. After that 15 mL methanol was added and stirred at 55 °C for another 2 h. Then the solvent was evaporated by vacuum evaporation. The crude product was purified by silica gel chromatography (petroleum ether: ethyl acetate = 20:1 v/v) to give compound **1b** as a yellow solid (344 mg, 53% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (d, *J* = 7.9 Hz, 1H), 7.66 – 7.55 (m, 3H), 7.41 (s, 1H), 2.53 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.3, 149.2, 136.4, 132.2, 128.8, 128.7, 127.4, 125.3, 121.0, 117.3, 116.0, 23.8.

HRMS calcd for $C_{13}H_8N_2NaS^+$ ([M+Na]$^+$): 247.0300, found: 247.0297.

2.3 Synthesis route of compound **1c**



2.3.1 Synthesis of compound **6**[3]

To a solution of 2-bromobenzaldehyde (712 mg, 3.85 mmol) in anhydrous THF (10 mL), 1-propynylmagnesium bromide (0.5 M in THF, 5 mmol, 10 mL) was added at 0 ^oC. The resulting mixture was stirred at 0 ^oC for 1 h, and then the reaction temperature was raised to room temperature. Until aldehyde disappeared by TLC analysis. The resulting mixture was quenched with a saturated solution of NH_4Cl and extracted with ethyl acetate (15 mL × 3). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4, filtered, and evaporated by vacuum evaporation. The crude product was used for the next step without further purification.

To the crude product in DCM (20 mL) was added sodium bicarbonate (647 mg, 7.7 mmol), sodium bromide (395 mg, 3.85 mmol), TEMPO (30 mg, 0.1925 mmol). The solution was cooled at 0°C and 6 mL sodium hypochlorite (14.5%) was added slowly. Until TLC analysis indicated reaction is complete, the resulting mixture was extracted with dichloromethane (15 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated by vacuum evaporation. The crude product was purified by silica gel chromatography (petroleum ether: ethyl acetate = 10:1 v/v) to give compound **6** as a faint yellow liquid (593 mg, 69% yield, two steps).

^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (dd, J = 7.6, 1.7 Hz, 1H), 7.64 (dd, J = 7.8, 0.9 Hz, 1H), 7.40 (td, J = 7.5, 1.0 Hz, 1H), 7.33 (td, J = 7.7, 1.7 Hz, 1H), 2.11 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.6, 137.2, 134.9, 133.3, 133.0, 127.3, 121.0, 93.9, 80.0, 4.5.

2.3.2 Synthesis of compound **1c**

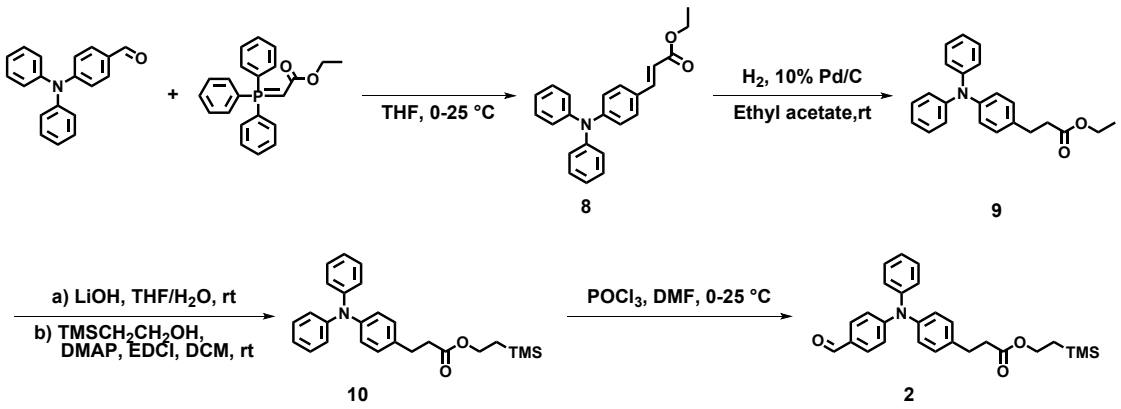
To a solution of $NaBH_4$ (179 mg, 4.63 mmol) in anhydrous DMF (25 mL), Se (440 mg, 5.56 mmol) was added at room temperature. The reaction temperature was raised to 105 ^oC and stirred at this temperature for 1 h. Then the solution of compound **6** (1.03g, 4.63 mmol) in DMF was added dropwise and stirred at 105 ^oC for another 4 h. After the solution was cooled to room temperature, the resulting mixture was extracted with ethyl acetate (15 mL× 3). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4, filtered, and evaporated by vacuum evaporation. The crude product compound **7** was used for the next step without further purification.

To the crude product compound **7** in Ac_2O (30 mL) was added malononitrile (3 g, 46.3 mmol). The solution was raised to 140 ^oC and stirred at this temperature for 4 h. After that 40 mL methanol was added and stirred at 55 ^oC for another 2 h. Then the solvent was evaporated by vacuum evaporation. The crude product was purified by silica gel chromatography (petroleum ether: ethyl acetate = 20:1 v/v) to give a yellow solid compound **1c** (703 mg, 56% yield, two steps).

^1H NMR (400 MHz, $CDCl_3$) δ 8.73 (dd, J = 5.9, 3.6 Hz, 1H), 7.71 (dd, J = 6.0, 3.3 Hz, 1H), 7.54 (dd, J = 6.0, 3.4 Hz, 2H), 7.51 (s, 1H), 2.60 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.9, 151.2, 135.4, 131.6, 129.8, 129.1, 128.2, 126.0, 122.4, 116.6, 115.3, 25.3.

HRMS calcd for $C_{13}H_8N_2NaSe^+$ ($[M+Na]^+$): 294.9745, found: 294.9739.

2.4 Synthesis route of compound **2**



2.4.1 Synthesis of compound **8**

Ethyl (triphenylphosphoranylidene) acetate (7.65 g, 22 mmol) was added to a solution of 4-(N,N-Diphenylamino)benzaldehyde (5 g, 18.3 mmol) in anhydrous tetrahydrofuran (50 mL) under an N_2 atmosphere. The solution was stirred for 48 h at room temperature. Then the reaction mixture was concentrated by vacuum evaporation and the residue was purified by silica gel chromatography (petroleum ether: ethyl acetate = 16:1 v/v) to give a bright yellow oil **8** (6.1 g, 97% yield).

^{1}H NMR (400 MHz, CDCl3) δ 7.63 (d, J = 15.9 Hz, 1H), 7.37 (d, J = 8.6 Hz, 2H), 7.29 (t, J = 7.8 Hz, 4H), 7.16 – 7.05 (m, 6H), 7.01 (d, J = 8.6 Hz, 2H), 6.30 (d, J = 15.9 Hz, 1H), 4.26 (q, J = 7.1 Hz, 2H), 1.34 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7, 150.1, 147.2, 144.5, 129.8, 129.5, 127.8, 125.6, 124.2, 122.0, 115.7, 60.6, 14.7. HRMS calcd for $C_{23}H_{21}NNaO_2^+$ ([M+Na]$^+$): 366.1465, found: 366.1459.

2.4.2 Synthesis of compound **9**

A mixture of compound **8** (6 g, 17.5 mmol) and 10% Pd/C (0.037 g) in ethyl acetate (100 mL) was evacuated and back-filled with H_2. After stirring 24 h at room temperature, the mixture was filtered over a pad of Celite (ethyl acetate eluent) and the solvent was evaporated by vacuum evaporation. The crude product was further purified by silica gel chromatography (petroleum ether: ethyl acetate = 16:1 v/v) to afford compound **9** as a colorless oil (6 g, 99% yield).

^{1}H NMR (400 MHz, CDC_{l3}) δ 7.14 (t, J = 7.9 Hz, 4H), 6.98 (t, J = 7.7 Hz, 6H), 6.91

(dd, *J* = 15.7, 8.0 Hz, 4H), 4.06 (q, *J* = 7.1 Hz, 2H), 2.82 (t, *J* = 7.8 Hz, 2H), 2.53 (t, *J* = 7.8 Hz, 2H), 1.16 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.3, 148.2, 146.3, 135.4, 129.5, 129.4, 124.8, 124.2, 122.8, 60.7, 36.3, 30.7, 14.6.

HRMS calcd for $C_{23}H_{23}NNaO_2^+$ ([M+Na]$^+$): 368.1621, found: 368.1615.

2.4.3 Synthesis of compound **10**

To a solution of compound **9** (5 g, 14.5 mmol) in THF (100 mL), and the resulting solution was chilled to 0-5 ^{o}C in an ice bath. Then a solution of LiOH (0.8678 g, 36.25 mmol) in H_2O (36 mL) was added and the reaction mixture was stirred at 0-5 ^{o}C for 1 h and then warmed to ambient temperature. TLC analysis indicated that the reaction was completed within 12 h. The reaction mixture was acidified to pH 3 with 2 M HCl solution, extracted with ethyl acetate (3×100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was used for the next step without further purification.

To a solution of the acid in CH_2Cl_2 (80 mL) was added 4-dimethylaminopyridine (356 mg, 2.9 mmol), EDCI (4.2 g, 23.2 mmol) and 2-(trimethylsilyl)ethanol (2.74 g, 23.2 mmol). The reaction was stirred at room temperature for 24 h. Then the reaction extracted with DCM (3×100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated by vacuum evaporation. The crude product was purified by silica gel chromatography (petroleum ether: ethyl acetate = 20:1 v/v) afforded compound **10** as a colorless oil (4.72 g, 78% yield, two steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (t, *J* = 7.9 Hz, 4H), 7.17 (t, *J* = 7.4 Hz, 6H), 7.13 – 7.04 (m, 4H), 4.35 – 4.22 (m, 2H), 3.00 (t, *J* = 7.8 Hz, 2H), 2.70 (t, *J* = 7.8 Hz, 2H), 1.14 – 1.03 (m, 2H), 0.15 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4, 148.2, 146.3, 135.4, 129.4, 129.4, 124.8, 124.2, 122.7, 62.9, 36.4, 30.6, 17.6, -1.2.

HRMS calcd for $C_{26}H_{31}NNaO_2Si^+$ ([M+Na]$^+$): 440.2016, found: 440.2010.

2.4.4 Synthesis of compound **2**

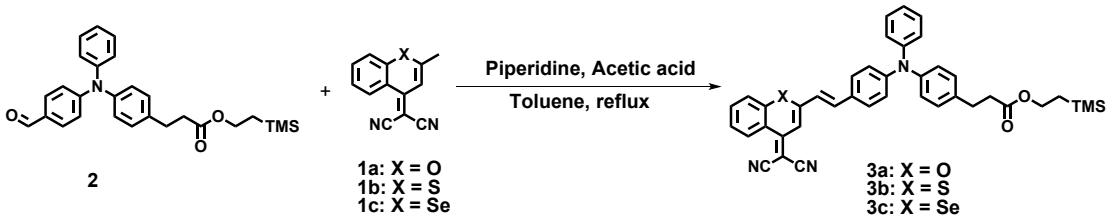
Phosphorus oxychloride (7.81 mL, 83.8 mmol) was added dropwise to DMF (10 mL)

at 0 °C, and the mixture was stirred for 2 h at this temperature. Then the solution of compound **10** (3.5 g, 8.38 mmol) in DMF (10 mL) was added dropwise into the reaction mixture. The reaction mixture was warmed to room temperature and stirred for another 12 h. When TLC analysis indicated that the reaction was finished, the mixture was poured into ice water. Then the reaction extracted with ethyl acetate (3×100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated by vacuum evaporation. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 20:1 v/v) afforded a yellow oil compound **2** (3.5 g, 94% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.78 (s, 1H), 7.65 (d, *J* = 8.6 Hz, 2H), 7.32 (t, *J* = 7.8 Hz, 2H), 7.16 (t, *J* = 7.6 Hz, 5H), 7.08 (d, *J* = 8.3 Hz, 2H), 6.98 (d, *J* = 8.6 Hz, 2H), 4.25 – 4.13 (m, 2H), 2.94 (t, *J* = 7.7 Hz, 2H), 2.62 (t, *J* = 7.8 Hz, 2H), 1.05 – 0.91 (m, 2H), 0.04 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.6, 173.2, 153.6, 146.3, 144.5, 137.9, 131.5, 130.0, 129.9, 129.2, 126.7, 126.5, 125.3, 119.3, 63.0, 36.2, 30.6, 17.6, -1.2.

HRMS calcd for $C_{27}H_{31}NNaO_3Si^+$ ([M+Na]$^+$): 468.1965, found: 468.1960.

2.5 Synthesis route of compound **3a-3c**



2.5.1 Synthesis of compound **3a**

Compound **1a** (208 mg, 1 mmol) and compound **2** (445 mg, 1 mmol) were dissolved in toluene (45 mL) with piperidine (0.5 mL) and acetic acid (0.5 mL) under argon atmosphere at room temperature. Then the mixture was refluxed for 15 h while the solution color changed from orange to red. After the solution was cooled to room temperature, the solvent was evaporated by vacuum evaporation. The crude product

was purified by silica column chromatography (petroleum ether: ethyl acetate = 10:1 v/v) to obtain compound **3a** as a red powder (280 mg), yield 44%.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (d, *J* = 8.3 Hz, 1H), 7.74 – 7.68 (m, 1H), 7.59 – 7.50 (m, 2H), 7.42 (t, *J* = 8.9 Hz, 3H), 7.31 (t, *J* = 7.8 Hz, 2H), 7.18 – 7.11 (m, 5H), 7.08 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 8.7 Hz, 2H), 6.79 (s, 1H), 6.62 (d, *J* = 15.8 Hz, 1H), 4.24 – 4.14 (m, 2H), 2.94 (t, *J* = 7.8 Hz, 2H), 2.63 (t, *J* = 7.8 Hz, 2H), 1.04 – 0.94 (m, 2H), 0.05 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.3, 158.5, 153.1, 152.6, 150.6, 146.9, 145.0, 139.1, 137.3, 134.7, 129.9, 129.8, 129.6, 127.5, 126.2, 126.1, 125.9, 124.6, 121.3, 118.8, 118.2, 117.4, 116.4, 115.8, 106.4, 63.1, 61.7, 36.3, 30.7, 17.6, -1.1.
HRMS calcd for $C_{40}H_{37}N_3NaO_3Si^+$ ([M+Na]$^+$): 658.2496, found: 658.2489.

2.5.2 Synthesis of compound **3b**

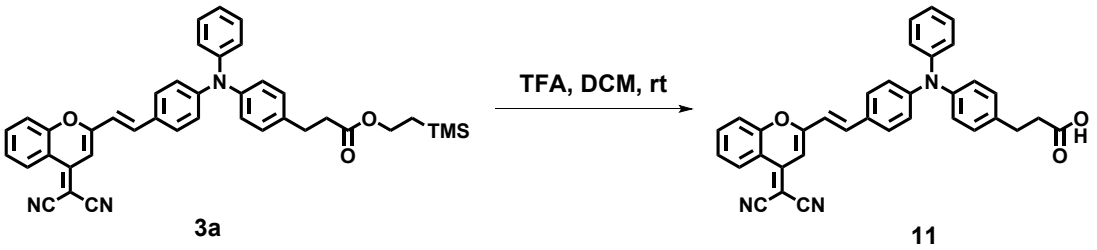
Compound **1b** (78 mg, 0.3482 mmol) and compound **2** (155 mg, 0.3482 mmol) were dissolved in toluene (17 mL) with piperidine (0.17 mL) and acetic acid (0.17 mL) under argon atmosphere at room temperature. Then the mixture was refluxed for 12 h while the solution color changed from orange to dark red. After the solution was cooled to room temperature, the solvent was evaporated by vacuum evaporation. The crude product was purified by silica column chromatography (petroleum ether: ethyl acetate = 10:1 v/v) to obtain compound **3b** as a dark red powder (90 mg), yield 39%.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (d, *J* = 8.2 Hz, 1H), 7.66 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.60 (dd, *J* = 11.0, 4.1 Hz, 1H), 7.57 – 7.52 (m, 1H), 7.49 (s, 1H), 7.38 (d, *J* = 8.7 Hz, 2H), 7.35 – 7.27 (m, 2H), 7.21 (d, *J* = 16.0 Hz, 1H), 7.18 – 7.10 (m, 5H), 7.06 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 8.7 Hz, 2H), 6.95 (d, *J* = 16.0 Hz, 1H), 4.24 – 4.13 (m, 2H), 2.94 (t, *J* = 7.8 Hz, 2H), 2.62 (t, *J* = 7.8 Hz, 2H), 1.05 – 0.93 (m, 2H), 0.05 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4, 156.1, 150.3, 148.4, 147.0, 145.1, 137.5, 137.2, 135.2, 132.1, 129.9, 129.8, 129.3, 128.6, 128.4, 127.8, 127.7, 126.1, 126.0, 125.8, 124.5, 122.6, 121.6, 121.0, 116.5, 67.9, 63.1, 36.3, 30.7, 17.7, -1.1.
HRMS calcd for $C_{40}H_{37}N_3NaO_2SSi^+$ ([M+Na]$^+$): 674.2268, found: 674.2262.

2.5.3 Synthesis of compound **3c**

Compound **1c** (100 mg, 0.3688 mmol), compound **2** (197 mg, 0.4421 mmol) were dissolved in toluene (20 mL) with piperidine (0.2 mL) and acetic acid (0.2 mL) under argon atmosphere at room temperature. Then the mixture was refluxed for 12 h while the solution color changed from orange to deep purple red. After the solution was cooled to room temperature, the solvent was evaporated by vacuum evaporation. The crude product was purified by silica column chromatography (petroleum ether: ethyl acetate = 10:1 v/v) to obtain compound **3c** as a purple red powder (92 mg), yield 35%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 – 8.66 (m, 1H), 7.76 – 7.69 (m, 1H), 7.60 (s, 1H), 7.55 – 7.49 (m, 2H), 7.36 (d, *J* = 8.6 Hz, 2H), 7.30 (t, *J* = 7.8 Hz, 2H), 7.13 (dd, *J* = 13.7, 6.1 Hz, 5H), 7.08 – 7.03 (m, 4H), 7.00 (d, *J* = 8.6 Hz, 2H), 4.24 – 4.14 (m, 2H), 2.94 (t, *J* = 7.8 Hz, 2H), 2.62 (t, *J* = 7.8 Hz, 2H), 1.02 – 0.95 (m, 2H), 0.05 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4, 159.1, 150.4, 150.2, 147.0, 145.1, 138.4, 137.1, 134.1, 131.9, 129.9, 129.9, 129.8, 129.7, 129.2, 128.4, 127.9, 127.4, 126.0, 125.7, 124.8, 124.5, 123.0, 121.6, 116.1, 71.8, 63.1, 36.3, 30.7, 17.6, -1.1.
HRMS calcd for $C_{40}H_{37}N_3NaO_2SeSi^+$ ([M+Na]$^+$): 722.1712, found: 722.1706.

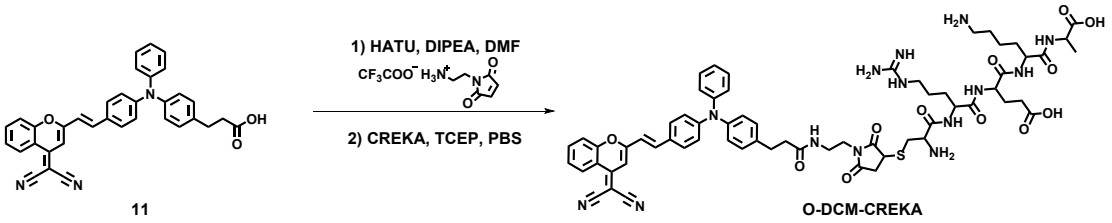
2.6 Synthesis of compound **11**



To a solution of compound **3a** (10 mg, 0.0157 mmol) in DCM (2 mL) was added TFA (1 mL) at 0 °C. The reaction mixture was slowly warmed to room temperature. TLC analysis indicated that the reaction was completed within 6 h. Then the reaction mixture was poured into ice water and filtered to obtain the crude product, which was further purified by silica column chromatography (dichloromethane: methanol = 100: 1, v/v) to obtain the desired product compound **11** as a dark red solid (6 mg), yield 73%.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (d, *J* = 8.3 Hz, 1H), 7.71 (t, *J* = 7.8 Hz, 1H), 7.59 – 7.50 (m, 2H), 7.42 (t, *J* = 8.9 Hz, 3H), 7.31 (t, *J* = 7.7 Hz, 2H), 7.12 (dt, *J* = 20.6, 8.5 Hz, 7H), 7.01 (d, *J* = 8.6 Hz, 2H), 6.79 (s, 1H), 6.62 (d, *J* = 15.8 Hz, 1H), 2.96 (t, *J* = 7.7 Hz, 2H), 2.71 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.5, 153.1, 152.7, 150.6, 146.9, 145.2, 139.1, 136.8, 134.8, 129.9, 129.8, 129.6, 127.6, 126.2, 126.1, 125.9, 124.7, 121.5, 118.9, 118.3, 117.4, 116.4, 115.9, 106.4, 61.8, 35.7, 30.4.
HRMS calcd for $C_{35}H_{25}N_3NaO_3^+$ ([M+Na]$^+$): 558.1788, found: 558.1782.

2.7 Synthesis of **O-DCM-CREKA**



To the solution of compound **11** (1.3 mg, 0.0025 mmol) in anhydrous DMF (900 μl) was added 10 μL DIPEA under N_2 atmosphere. Then *N*-(2-Aminoethyl) maleimide trifluoroacetate salt (0.7611 mg, 0.0030 mmol) and HATU (1.1410 mg, 0.0030 mmol) were added into the solution. Keep reacting 3 h at room temperature. After that, the reaction solution was diluted with PBS. Then the CREKA (1.817 mg, 0.0030 mmol) which was pretreated with TCEP (1eq) in PBS was added into the reaction solution and reacted overnight under N_2 atmosphere. Finally, the crude product was further purified by HPLC to get the final product **O-DCM-CREKA** as a red semi-solid (1.8 mg), yield 57%.

MALDI-TOF-MS calcd for $C_{64}H_{75}N_{14}O_{11}S^+$ ([M+H]$^+$): 1264.5443, found: 1264.3093.

3 Determination of the fluorescence quantum yield

The fluorescence quantum yield measurement was carried out using B-DCM-P (quantum yield was reported as 32.37% in DCM) [1] as the reference. In a typical procedure, a series of solutions of dyes in DCM with absorbance values below 0.10 at 530 nm were prepared respectively. And the 530 nm laser was used as the excitation

source and the emission spectrum in the range of 550 to 900 nm was recorded.

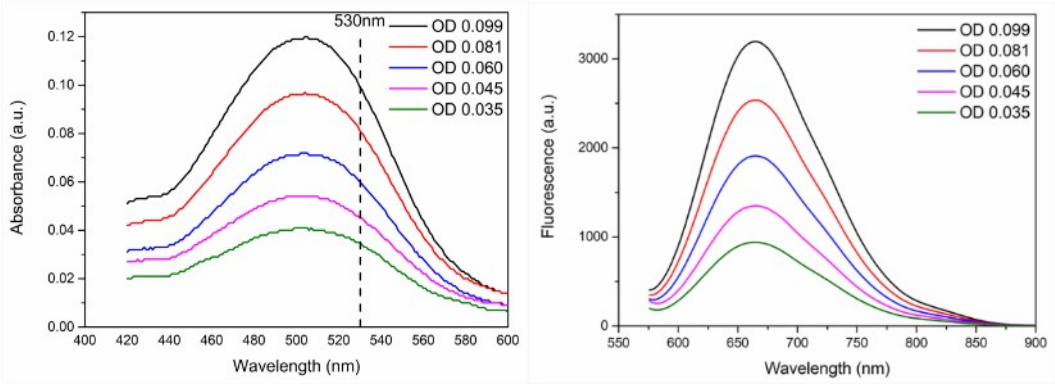


Figure S1. A series of solutions of B-DCM-P in DCM with absorbance values at 530 nm to be ~ 0.10, ~ 0.08, ~ 0.06, ~0.05 and ~0.04 were prepared respectively, and the emission spectrum in the range of 580 to 900 nm was recorded.

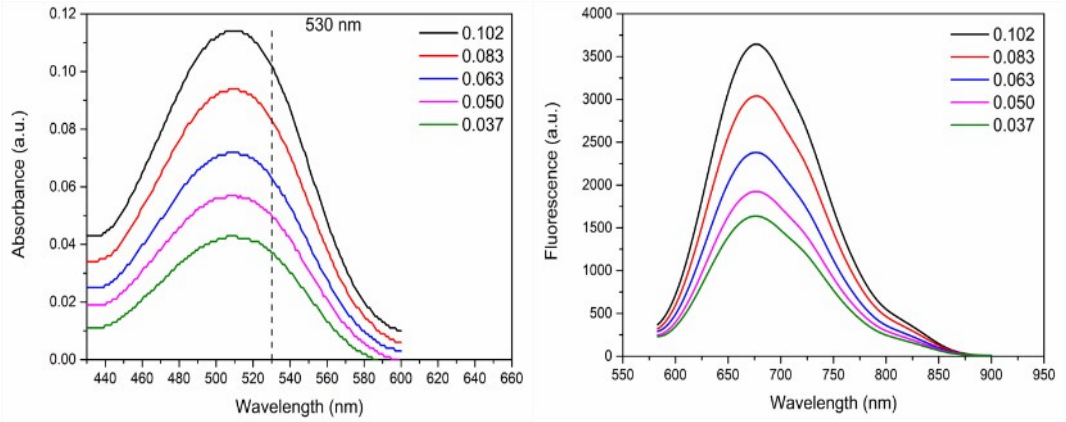


Figure S2. A series of solutions of compound **3a** in DCM with absorbance values at 530 nm to be ~ 0.10, ~ 0.08, ~ 0.06, ~0.05 and ~0.04 were prepared respectively, and the emission spectrum in the range of 580 to 900 nm was recorded.

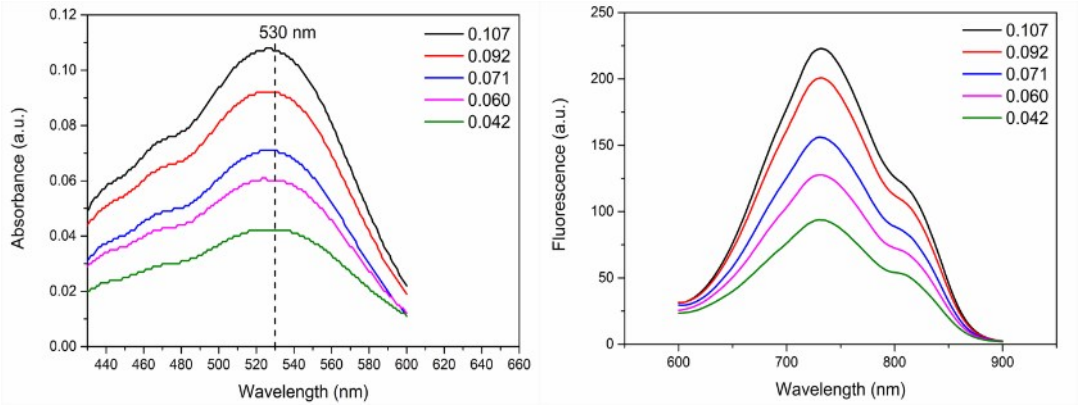


Figure S3. A series of solutions of compound **3b** in DCM with absorbance values at 530 nm to be ~ 0.10, ~ 0.08, ~ 0.06, ~0.05 and ~0.04 were prepared respectively, and the emission spectrum in the range of 580 to 900 nm was recorded.

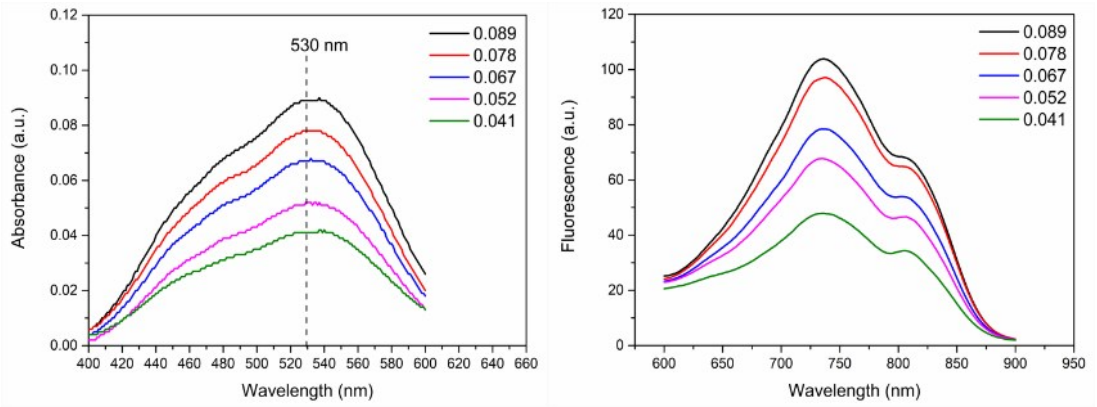


Figure S4. A series of solutions of compound **3c** in DCM with absorbance values at 530 nm to be ~ 0.10, ~ 0.08, ~ 0.06, ~0.05 and ~0.04 were prepared respectively, and the emission spectrum in the range of 580 to 900 nm was recorded.

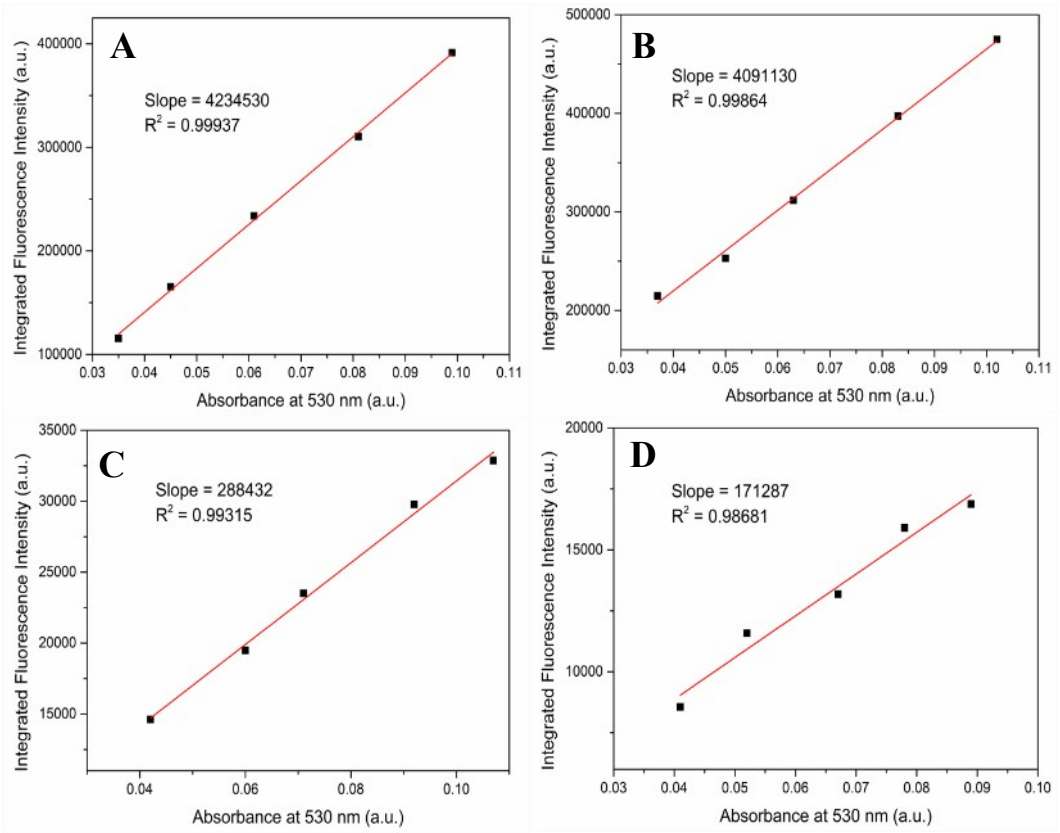


Figure S5. Quantum yield Measurements of **3a** (B), **3b** (C), **3c** (D). In order to measure the quantum yield, a reference B-DCM-P (A) (32.37%) was chosen. Five difference concentrations were measured and the integrated fluorescence was plotted against absorbance. The quantum yield was calculated in the following manner:

$$QY_{sample} = QY_{reference} \times \frac{slope_{sample}}{slope_{reference}} \times \frac{{n_{sample}}^2}{{n_{reference}}^2}$$

4 Absorption and emission spectra of dyes in different solvents

A series of solutions of these three DCM derivatives in six different solvents (hexane, toluene, DCM, THF, acetone, DMSO) were prepared respectively. And the 530 nm laser was used as the excitation source and the emission spectrum in the range of 550 to 900 nm was recorded. A was used for the absorption measurements. A Hitachi Fluorescence Spectrophotometer F-4600 was utilized for fluorescence spectra detection. The absorption spectra were recorded with a PerkinElmer Lambda 25 UV-Vis spectrophotometer. And then the maximum absorption wavelength laser was used as the excitation source, the fluorescence spectra were measured with a Hitachi Fluorescence Spectrophotometer.

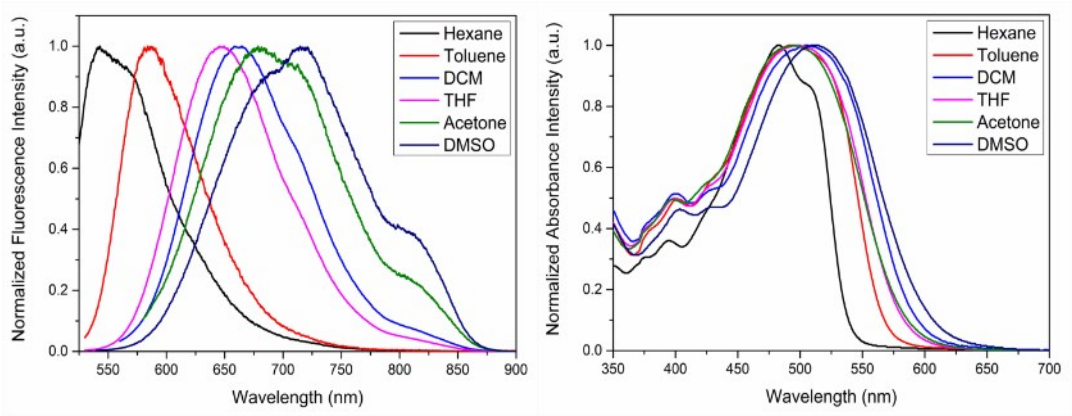


Figure S6. The normalized absorption and emission spectra of **3a** in different solvents.

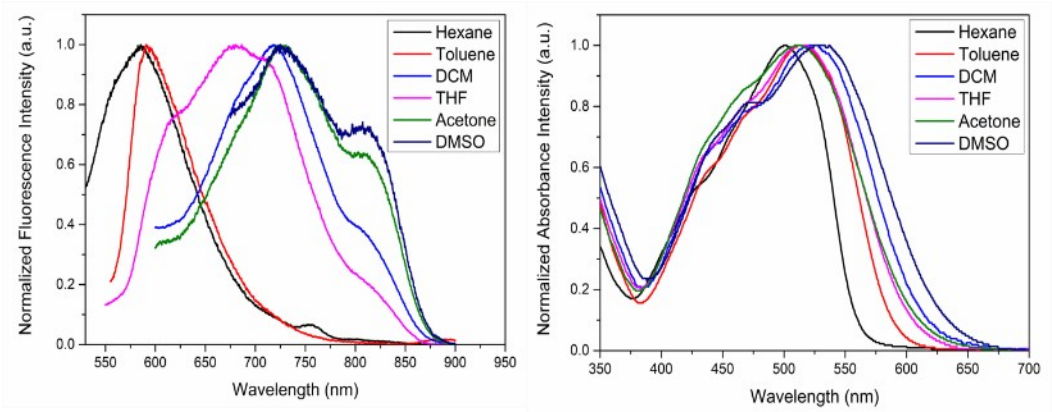


Figure S7. The normalized absorption and emission spectra of **3b** in different solvents.

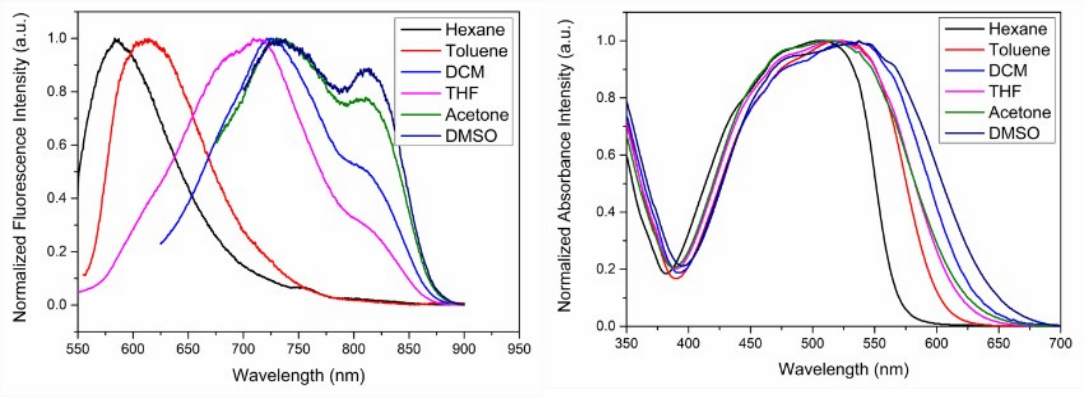
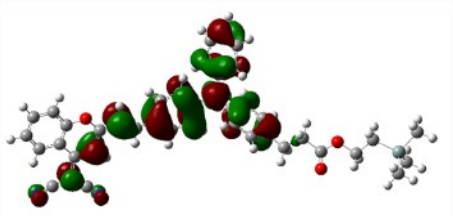
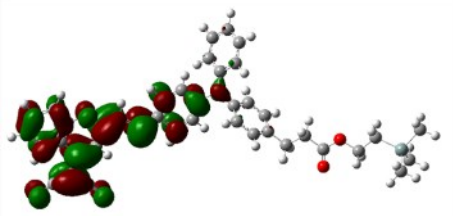
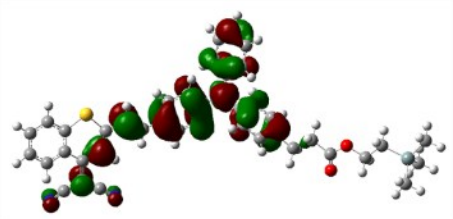
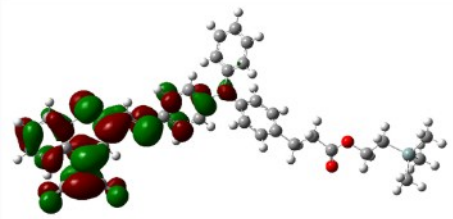
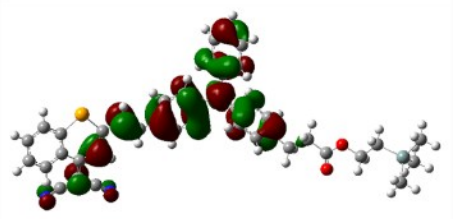
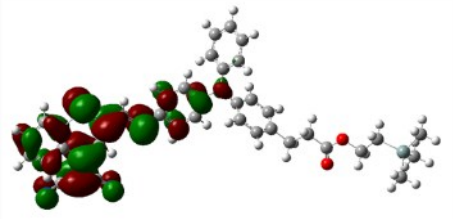


Figure S8. The normalized absorption and emission spectra of **3c** in different solvents.

5 HOMO and LUMO electron distribution

Table S1. Comparison of HOMO and LUMO orbital surfaces of **3a, 3b** and **3c** using DFT B3LYP/6-31G(d) scrf = (cpcm, solvent=dichloromethane) method. E_{gap} =E_{LUMO}-E_{HOMO}.

Dye	HOMO Energy (eV)	LUMO Energy (eV)
3a	 -5.200	 -2.658
3b	 -5.182	 -2.789
3c	 -5.174	 -2.822

6 Investigation of the AIE Property and ICT effect

The AIE properties and ICT effects of **3a**, **3b** and **3c** were investigated by measuring

their fluorescence intensity in water-THF mixtures of various volume fractions. Generally, different solutions were prepared with varying water volume percentages between 0-90%. Next, 60 μg **3a**, **3b** or **3c** was dissolved in 600 μL of each set of water-THF solutions. The fluorescence emissions of these solutions were recorded using 500 nm excitation.

The ICT effect of **O-DCM-CREKA** was investigated by measuring their fluorescence intensity in PBS-THF mixtures of various volume fractions. Generally, different solutions were prepared with varying PBS volume percentages between 0-100%. Next, 60 μL **O-DCM-CREKA** PBS solution (1 μg/μL) was mixed with 540 μL of each set of PBS-THF solutions. The fluorescence emissions of these solutions were recorded using 500 nm excitation.

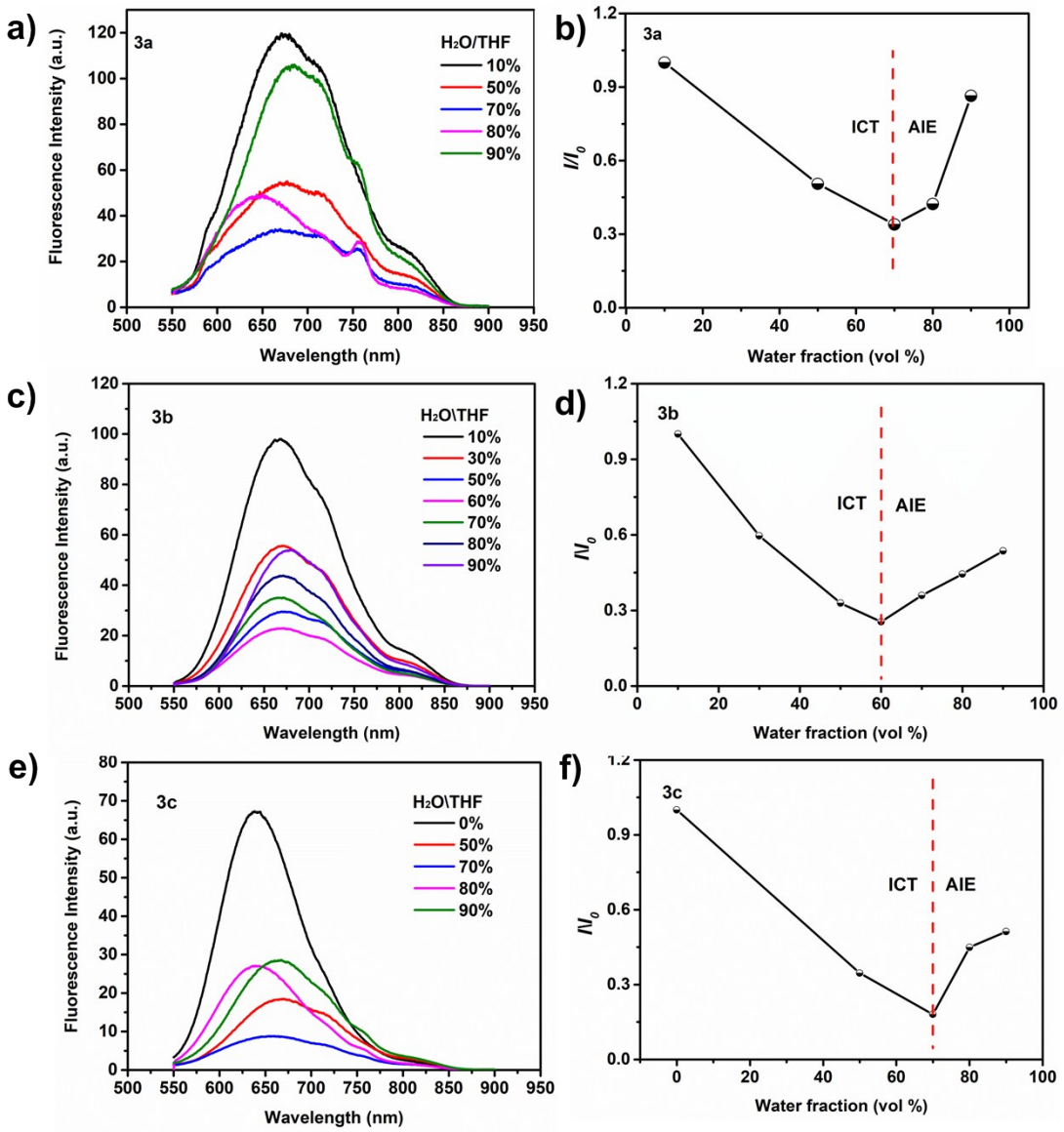


Figure S9. Emission spectra (a, c, e) and relative fluorescence intensity (b, d, f) of **3a** (a, b), **3b** (c, d) and **3c** (e, f) in THF–water mixture (the concentration is 0.1 μg/μL).

7 In vitro binding of O-DCM-CREKA to fibrin-fibronectin complexes

Fibrin-fibronectin complexes were generated in situ using fresh frozen plasma from bovine plasma (FFP). 100 μL of FFP was diluted with 660 μL PBS and then mixed with 20 μL of 0.4 M $CaCl_2$ and 20 μL of thrombin (0.1 U/ml in PBS). The mixture was incubated at room temperature for 10 min. After that 100 μL **O-DCM-CREKA** in PBS (1 μg/μL) or 100 μL **3a** in PBS/DMF (v/v, 1:1, 1 μg/μL) was added and then incubation at room temperature for an additional 30 min. The solution in which 100 μL **O-DCM-CREKA** in PBS (1 μg/μL) or 100 μL **3a** in PBS/DMF (v/v, 1:1, 1 μg/μL) was diluted with 800 μL PBS or 800 μL bovine serum albumin (BSA) solution (10 μg/μL) were used as the control group. The fluorescence emissions of these solutions were recorded using 520 nm excitation. Fluorescence spectra were processed on an Origin program for normalization to achieve same intensity of **O-DCM-CREKA** and **3a**, the areas under the curve of **O-DCM-CREKA** and **3a** were both calculated into 1 for fluorescence enhancement comparison.

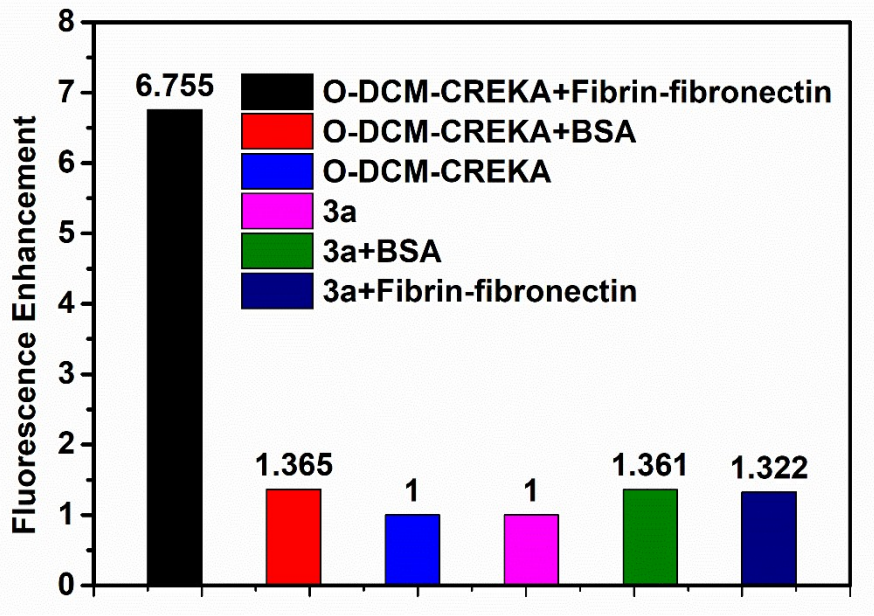


Figure S10. Fluorescence enhancement comparison of **O-DCM-CREKA** and 3a in fibrin-fibronectin complexes and BSA solution. The fluorescence enhancement value of **O-DCM-CREKA** in fibrin-fibronectin complexes (6.755) is higher than that of **O-DCM-CREKA** in BSA

(1.365) or **3a** in fibrin-fibronectin complexes (1.322) and BSA (1.361), suggesting **O-DCM-CREKA** has very high selectivity toward fibrin-fibronectin complexes.

8 Cell experiments

Cell culture

Human lung adenocarcinoma cell line A549 cell was obtained from the China Center for Type Culture Collection (CCTCC). A549 cells were cultured in Low Glucose Dulbecco's Modified Eagle Medium (DMEM) medium (Gibco) supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, 100 μg/mL streptomycin at 37 ℃ in a humidified atmosphere containing 5% CO_2.

Cytotoxicity assay

In vitro cytotoxicity studies of **O-DCM-CREKA** on A549 cells were performed by using a MTT cytotoxicity assay. Cells were cultured for 12 h in a 96-well plate (5×10^3 cells per well) to allow cell attachment. **O-DCM-CREKA** of different concentrations in fresh medium was added into the wells and incubated for 24 h. Then the medium was substituted with 100 μL fresh medium contained MTT (0.05 mg/mL). After 4 h incubation at 37 ℃, the MTT solution was replaced with DMSO (150 μL per well) to dissolve the precipitated formazan violet crystals at 37 °C for 15 min. The absorbance in each well was measured at 492 nm by an automatic microplate reader KHB ST-360 from Shanghai Zhihua Medical Instrument Ltd. Cell viability was calculated using the following formula: cell viability = (mean absorbance of treatment wells)/ (mean absorbance of medium control wells) × 100%.

Cell imaging

A549 cells ($1.5*10^4$ cells) were seeded on a 35 mm × 12 mm style cell confocal dish (φ20 mm glass bottom) and cultured for 12 h in the standard culture atmosphere. After removing the medium, cells were incubated with 1 mL of fresh medium containing **O-DCM-CREKA** (20 μM) and cultured for 6 h. Prior to imaging, cells were washed with PBS for three times and fixed by paraformaldehyde for 30 min at room temperature. Then, cells were washed with PBS for three times and DAPI (100 μL) was added into the cell plate for 5 min at room temperature in a dark. Lastly, cells were washed with

PBS for three times and stored in the PBS under 4 °C before imaging. And then the cells were imaged under a Leica-LCS-SP8-STED confocal microscope.

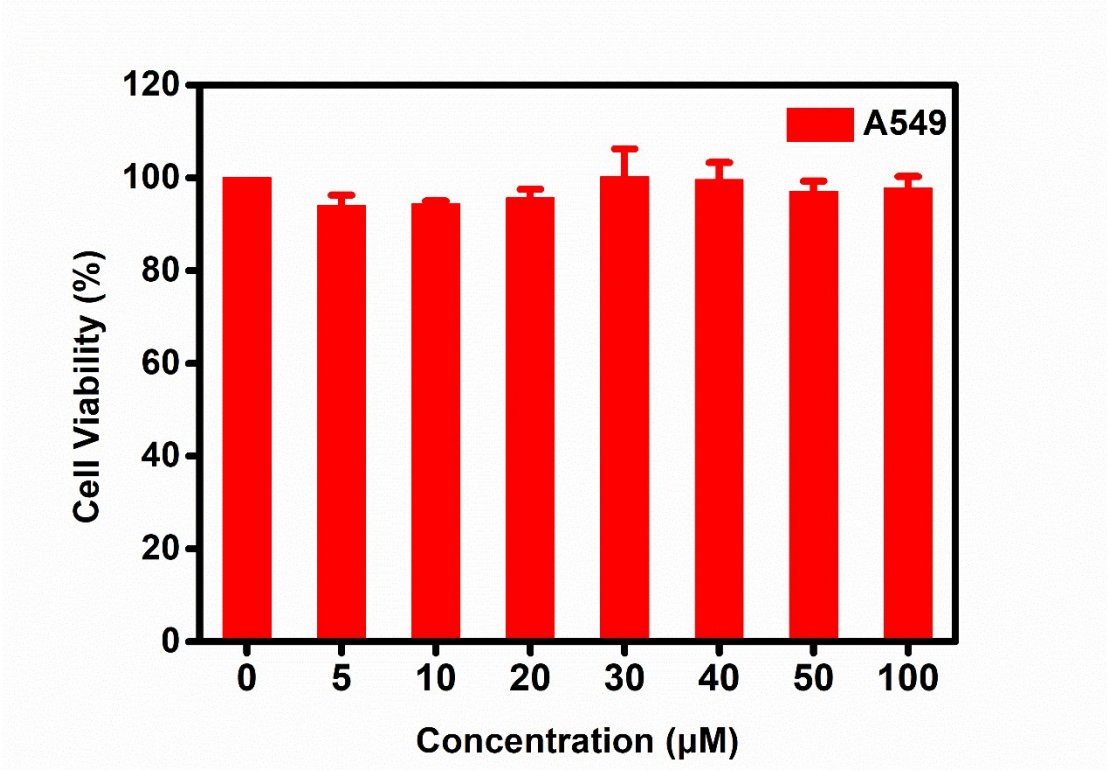


Figure S11. Viability of A549 cells at various **O-DCM-CREKA** concentrations (n = 3).

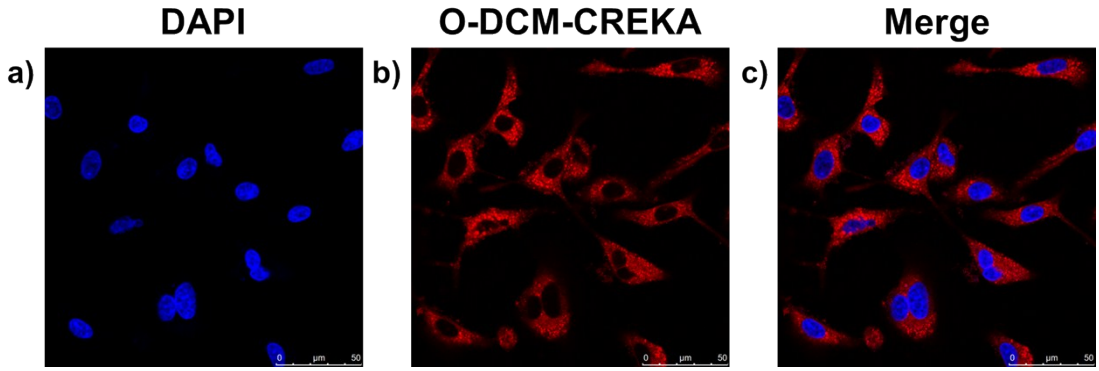


Figure S12. (a-c) Confocal laser scanning microscopy fluorescence images of **O-DCM-CREKA** in A549 cells. (a) DAPI stain, λex = 405 nm, blue channel collected at 411-489 nm. (b) **O-DCM-CREKA** (20 μM) stain, λex = 561 nm, red channel collected 590-790 nm. (c) Merged image of a and b.

9 Animal Models and Fluorescent Imaging *in vivo* and *ex vivo*

All animal experiments were performed according to the Chinese Regulations for the Administration of Affairs Concerning Experimental Animals and approved by the

Institutional Animal Care and Use Committee of Wuhan University.

The A549 mice tumor model was established by subcutaneous injection of A549 cells (roughly 2×10^6 in 50 μL of Low Glucose DMEM medium) into the fore right leg of the 6-week-old female BALB/c nude mice (SPF, Beijing Biotechnology Co., Ltd.). The tumor was allowed to reach ~8 mm in diameter and the mice were subjected to imaging studies.

Carbon tetrachloride (CCl_4) was chosen to establish liver injury mice model. The 6-week-old female BALB/c nude mice (SPF, Beijing Biotechnology Co., Ltd.) were randomly divided into the normal control group (n = 3) and the CCl_4 induced liver injury model group (n = 3). The CCl_4 induced liver injury model group mice received intraperitoneal injection of 15 ml/kg CCl_4 in olive oil (3%, v/v). And the control group received intraperitoneal injections of the same volume of physiological saline at the same time. After 24 h, these mice subjected to imaging studies.

The model mice were administered by tail intravenous injection of **O-DCM-CREKA** (100 μg per mice). NIR fluorescence images were collected using a Bruker in Vivo-Xtreme Imaging System (Bruker, Xtreme BI). During injection and imaging, the mice were anesthetized using a 2 L/min oxygen flow with 2% Isoflurane. *Ex vivo* fluorescence imaging of organs and tissues was performed with the same system. After 24 h, A549 mice were sacrificed, the major organs and tissues were collected. Then the NIR fluorescent signal of each subject was recorded. After 12 h, CCl_4 induced liver injury model group and control group mice were sacrificed, the major organs and tissues were collected. Then the NIR fluorescent signal of each subject was recorded.

10 HPLC purity and MOLDI-TOF-MS of O-DCM-CREKA

O-DCM-CREKA purity analysis was performed using a Dionex HPLC System with UV-Vis detection (254 nm) and a reversed-phase C8 column (Sepax, 5 μm, 4.6 × 250 mm). The data collect and analysis was carried out using the Chromeleon software. The oven temperature was maintained at 25 ^oC, the injection volume was 20 μL and the flow rate was 1.0 mL/min. The separation program of gradient elution was as follows: 0 min: 5% B;

7 min: 95% B;

13 min: 95% B;

15 min: 5% B;

where solvent A was water with 0.06% TFA and solvent B was acetonitrile.

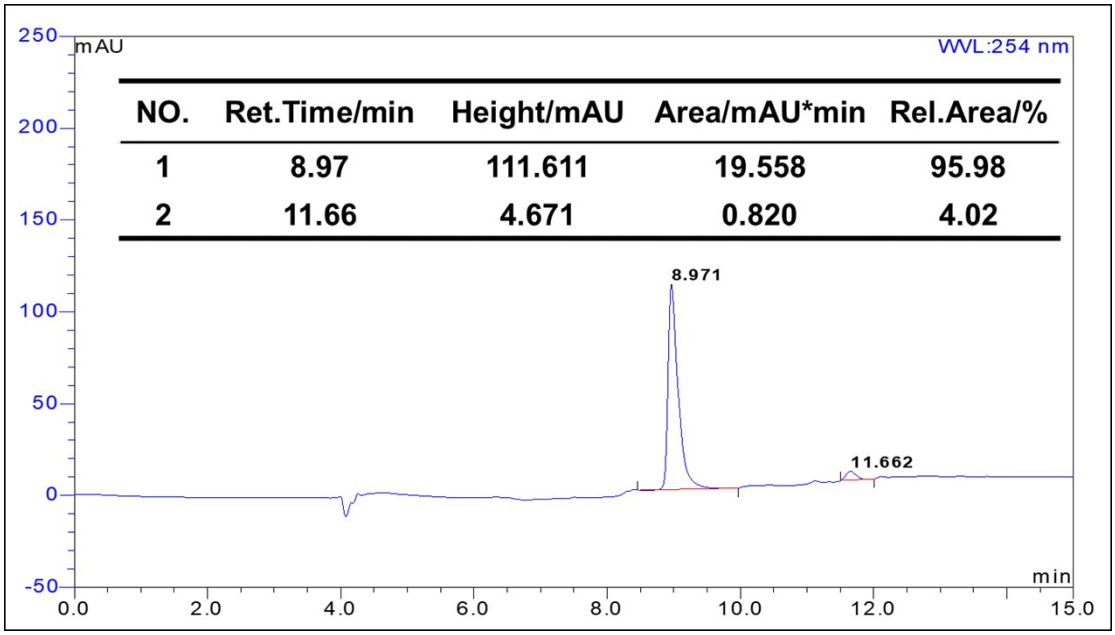


Figure S13. HPLC purity characterization of **O-DCM-CREKA** (retention time = 8.971 min).

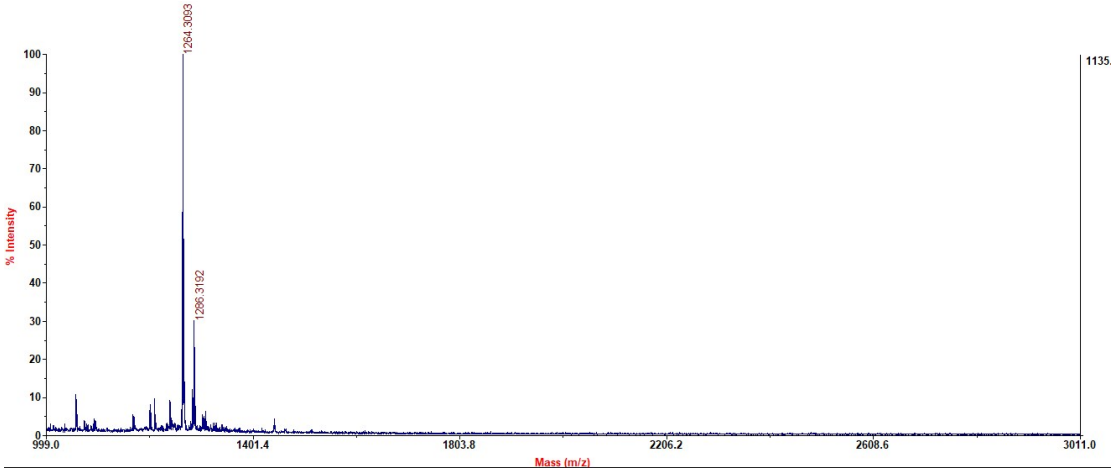
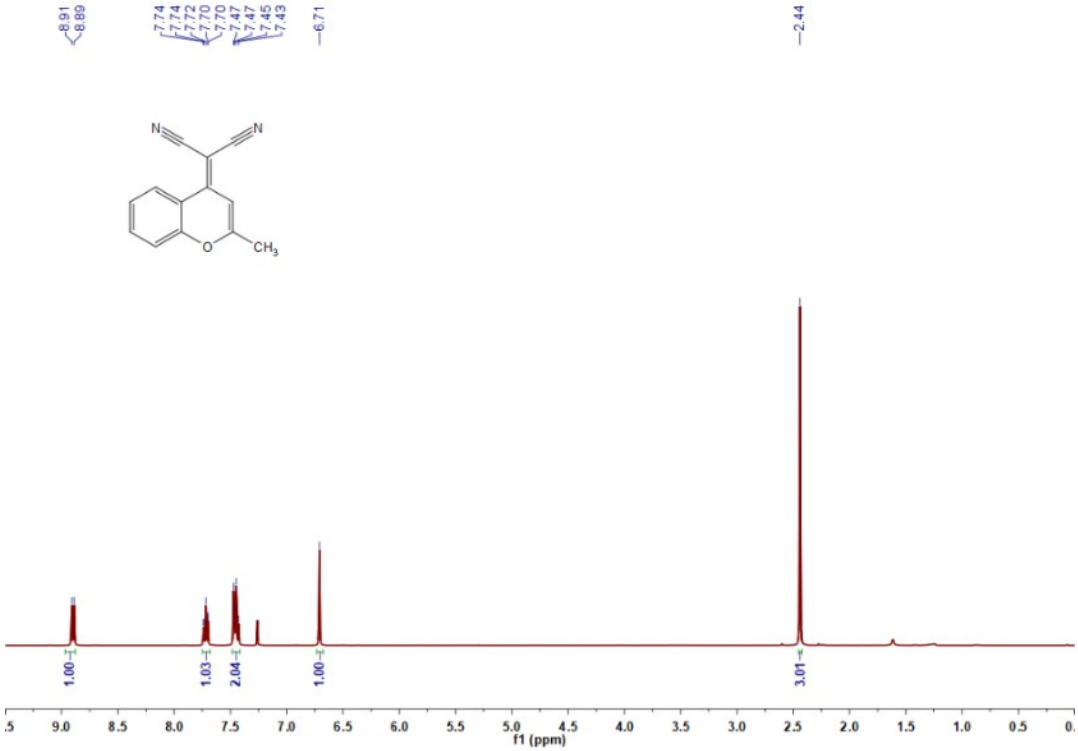
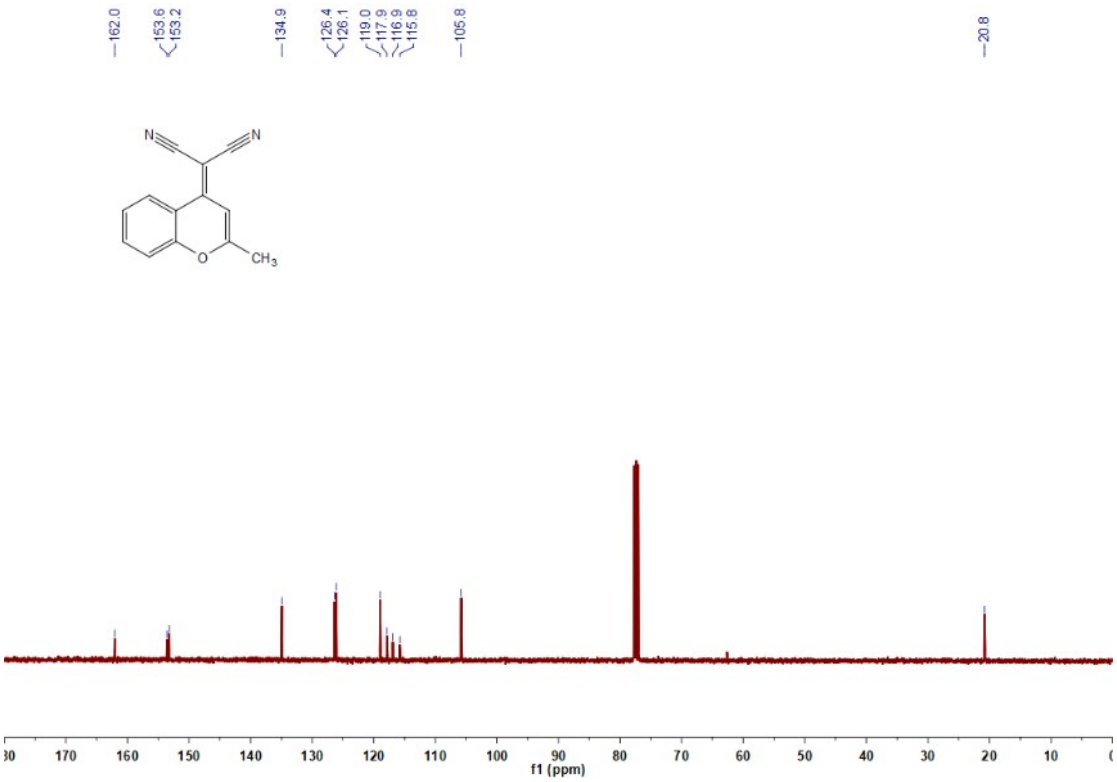


Figure S14. MOLDI-TOF-MS of **O-DCM-CREKA**

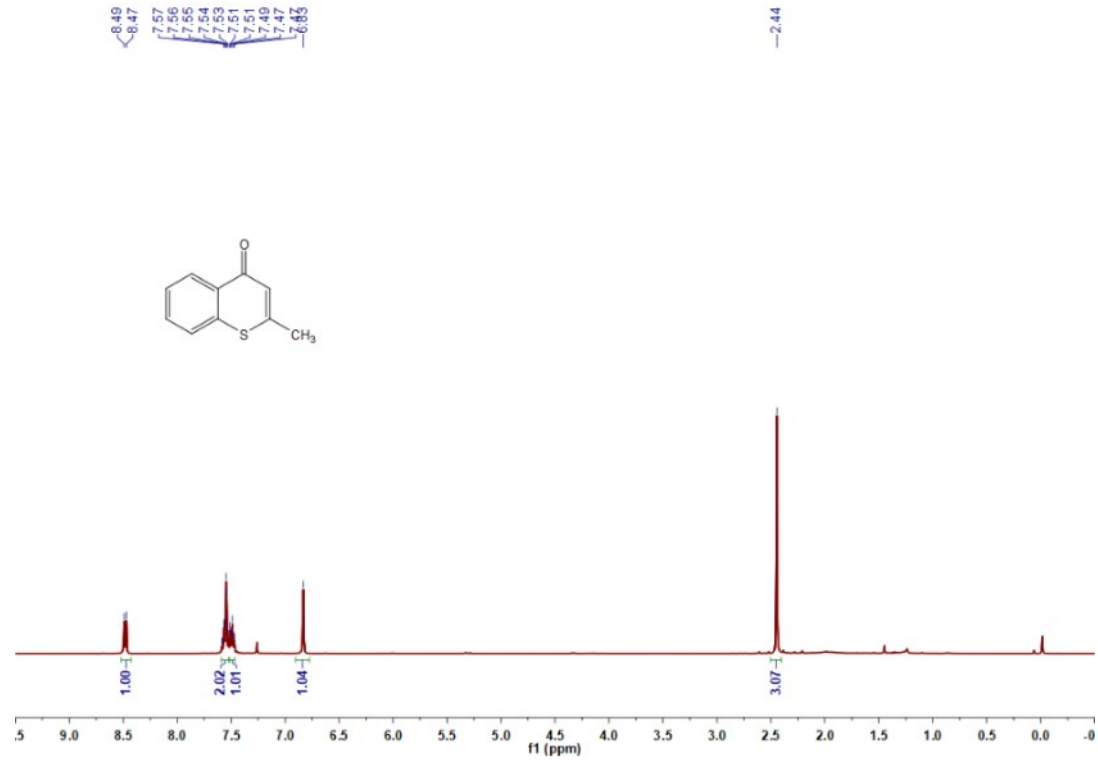
11 ^{1}H and ^{13}C NMR spectra

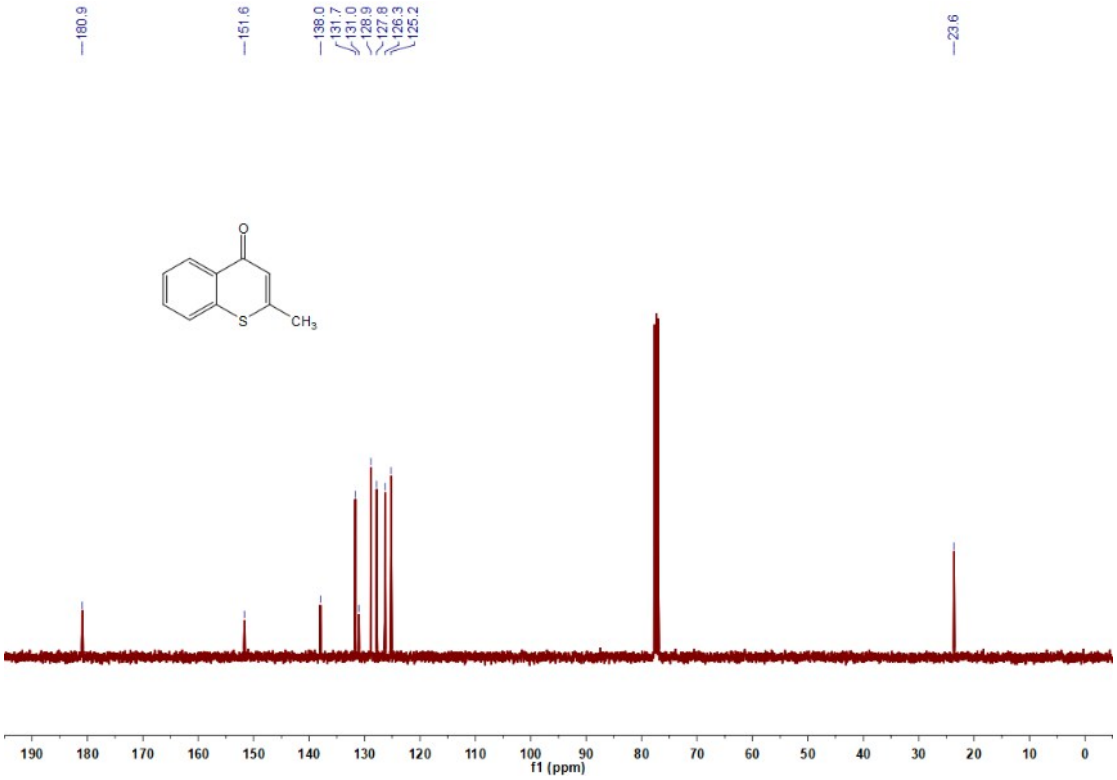
11.1 ^{1}H and ^{13}C NMR for compound 1a



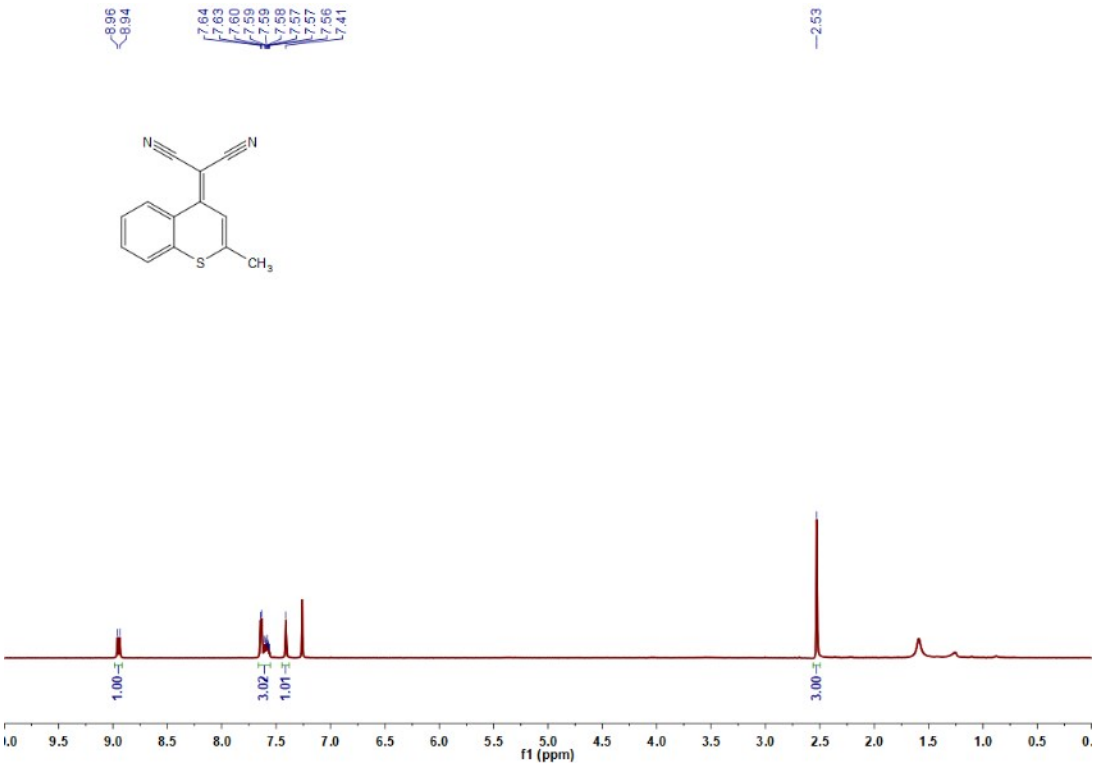


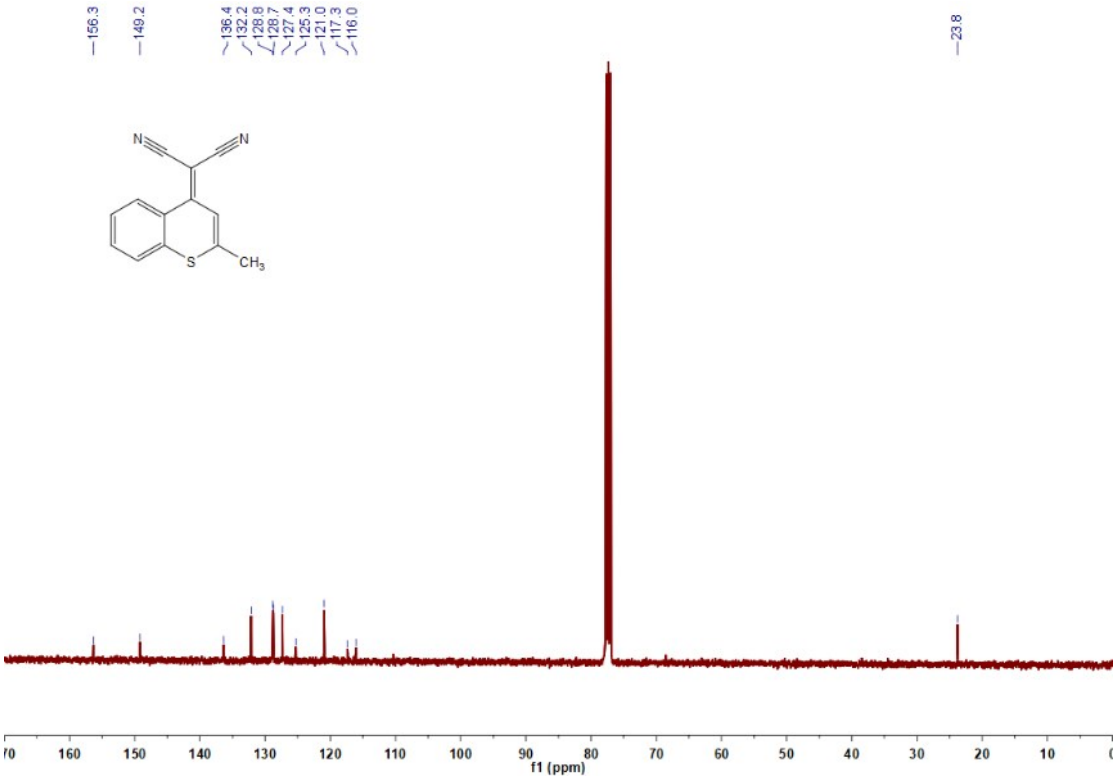
11.2 ^{1}H and ^{13}C NMR for compound 5



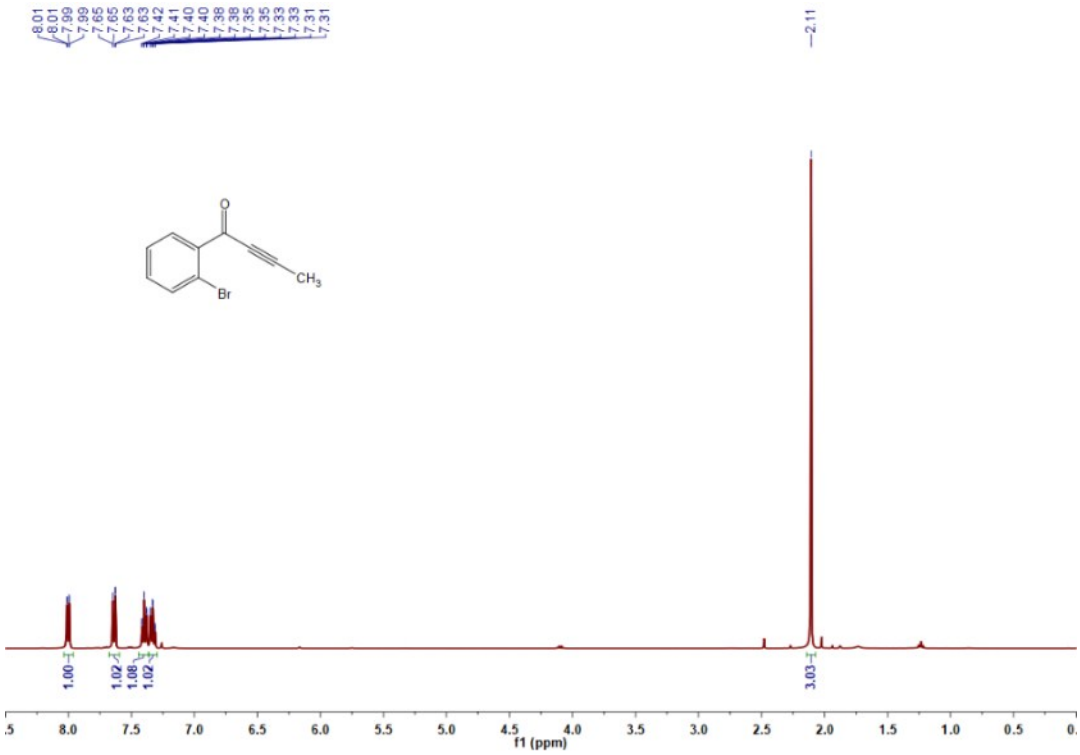


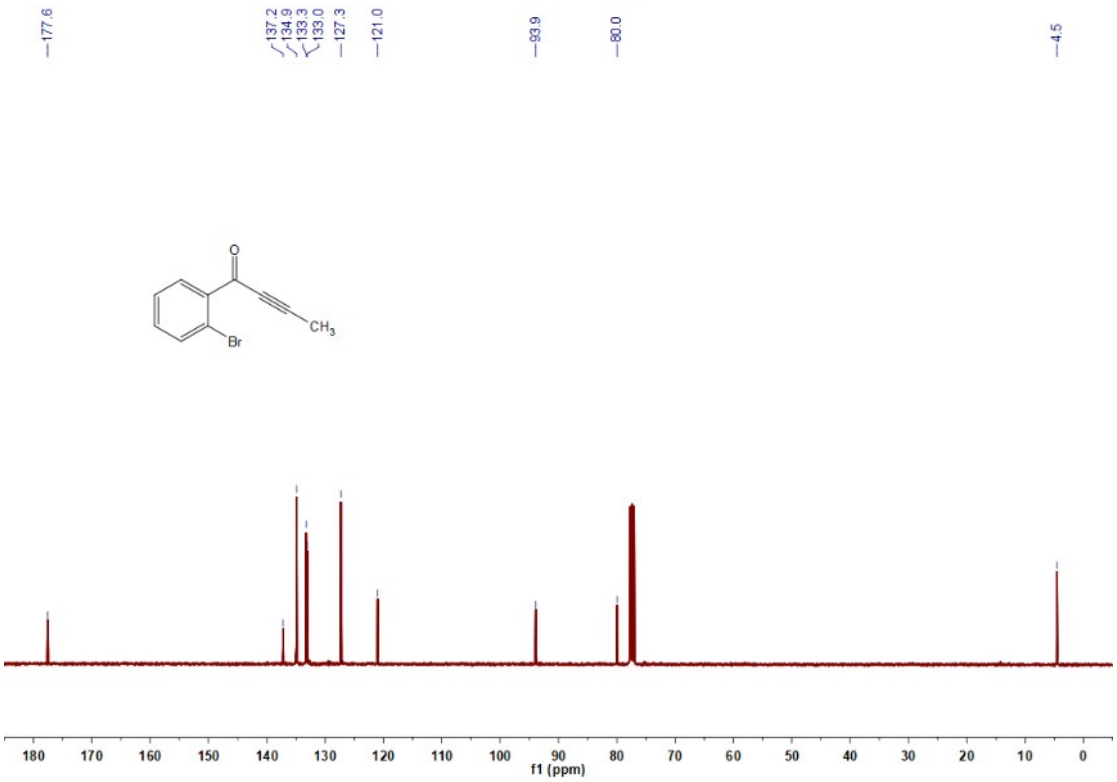
11.3 ^{1}H NMR and ^{13}C NMR for compound 1b



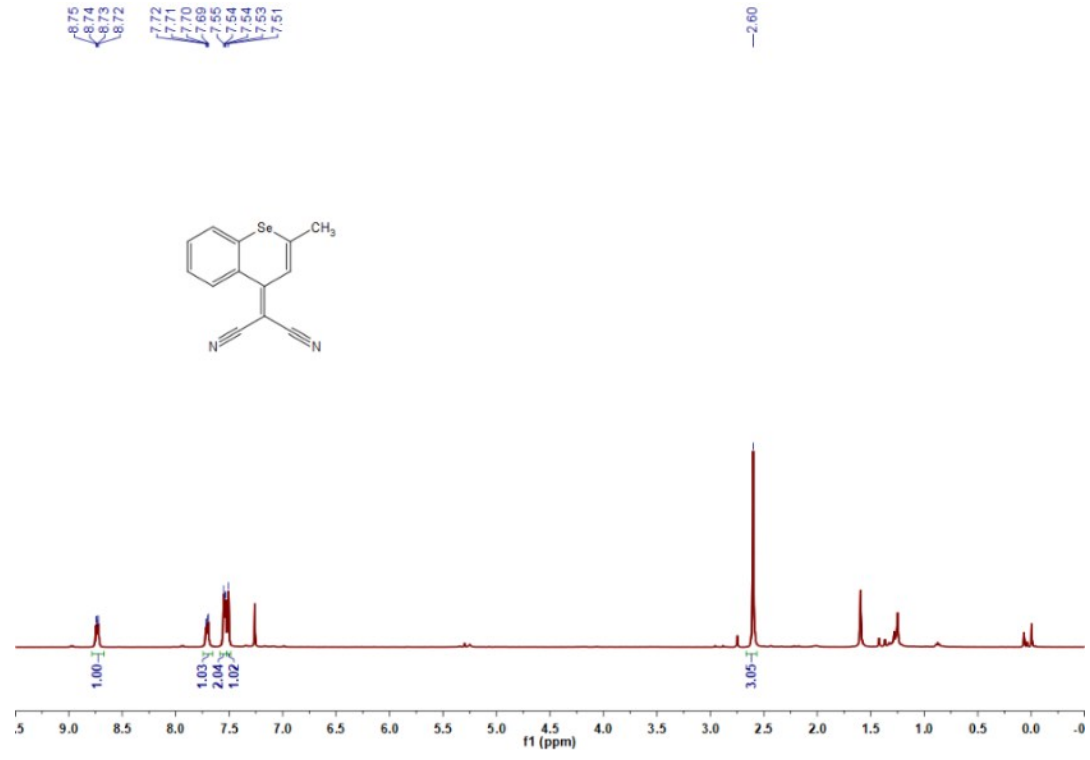


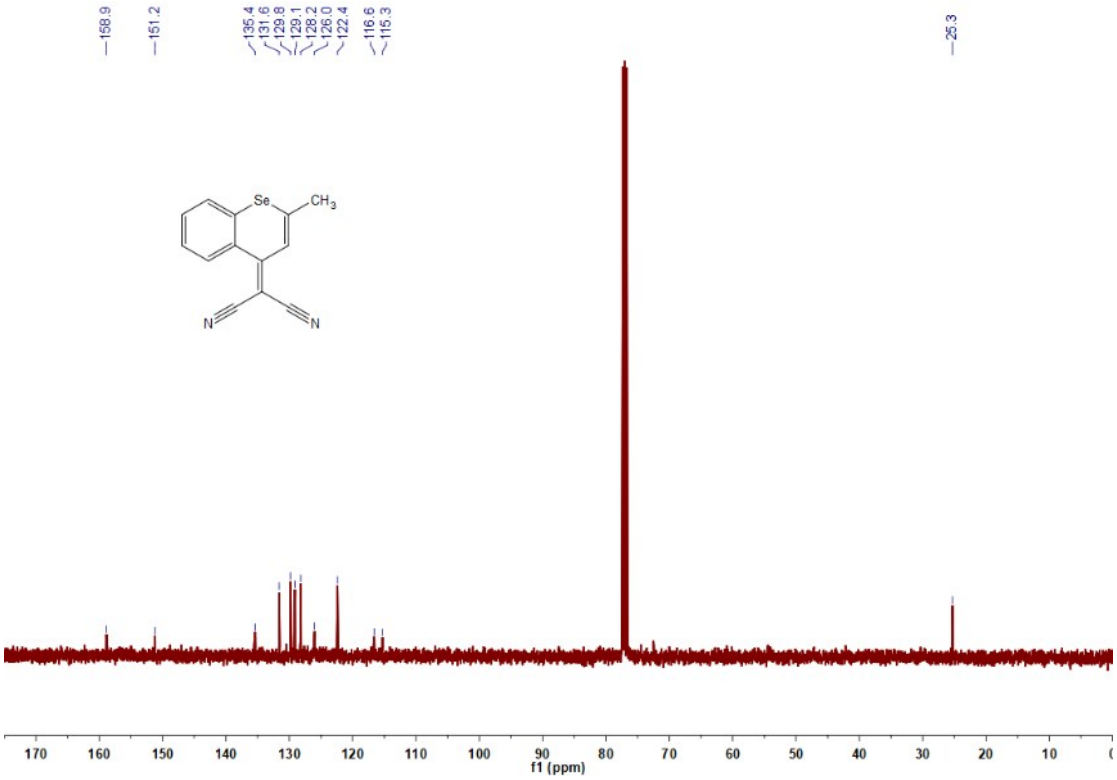
11.4 ^{1}H and ^{13}C NMR for compound 6



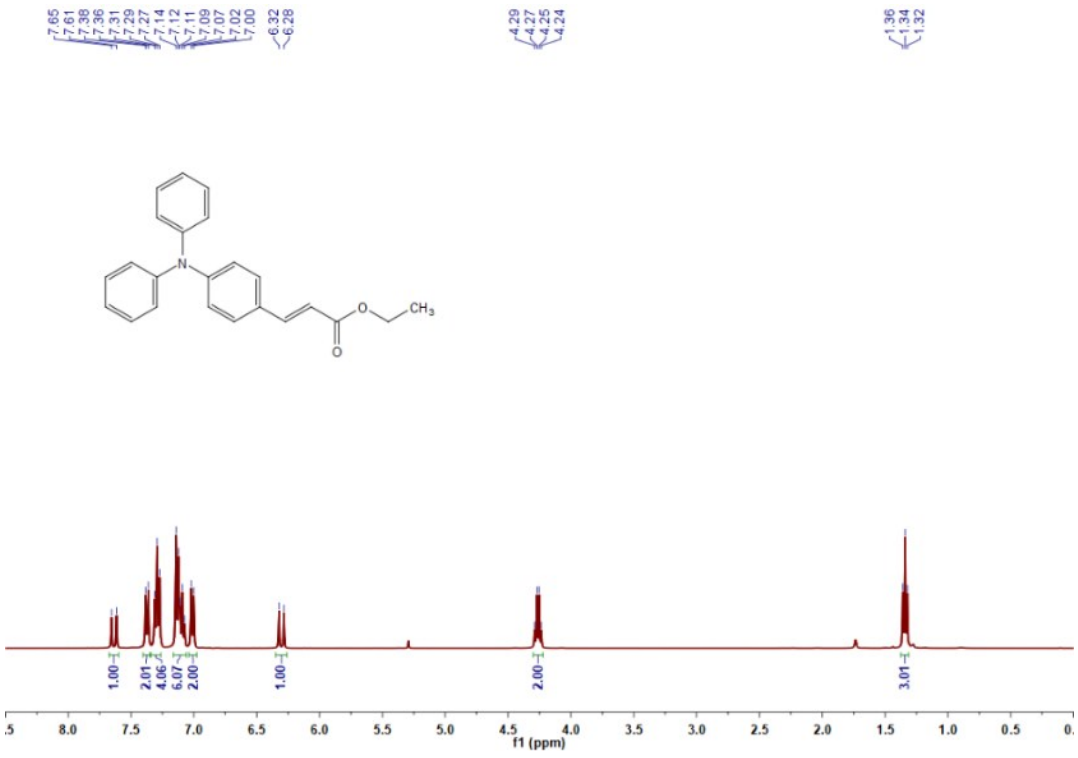


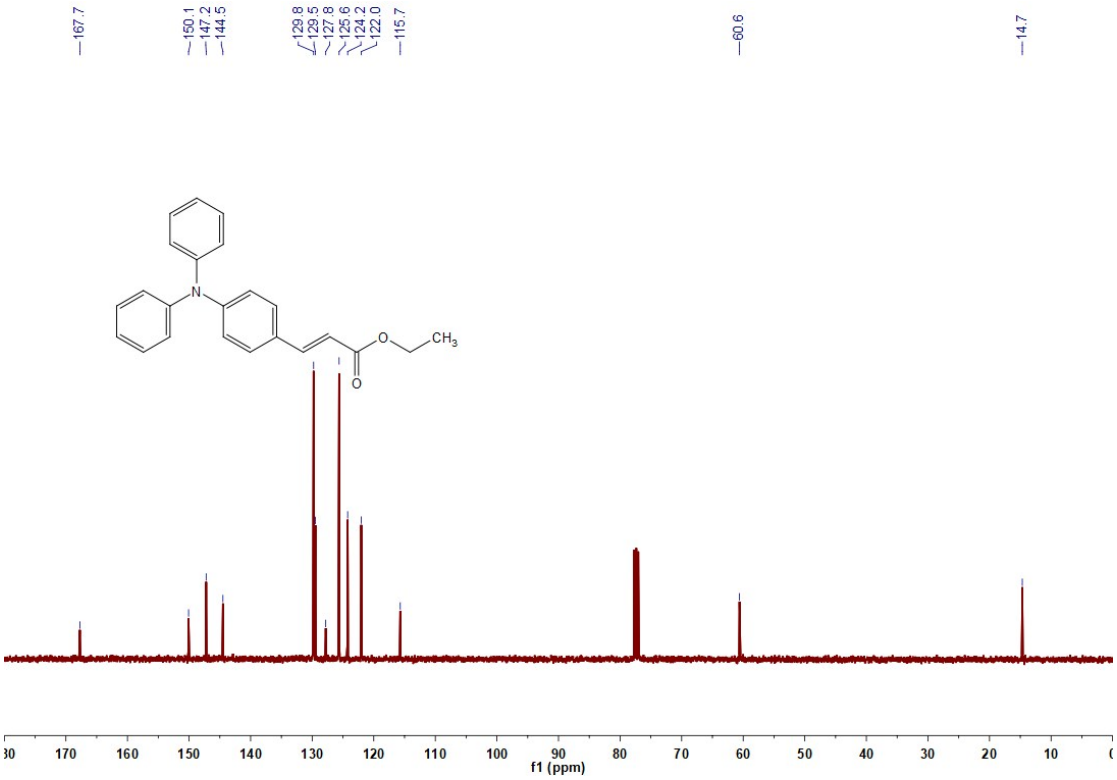
11.5 ^{1}H and ^{13}C NMR for compound 1c



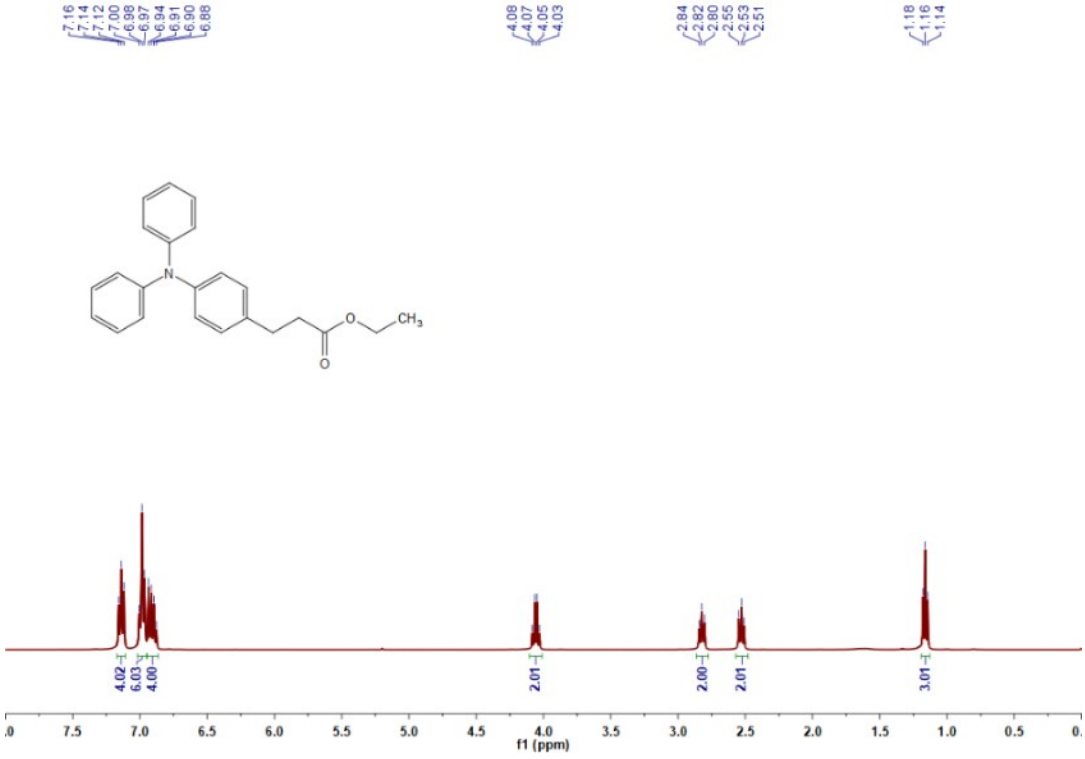


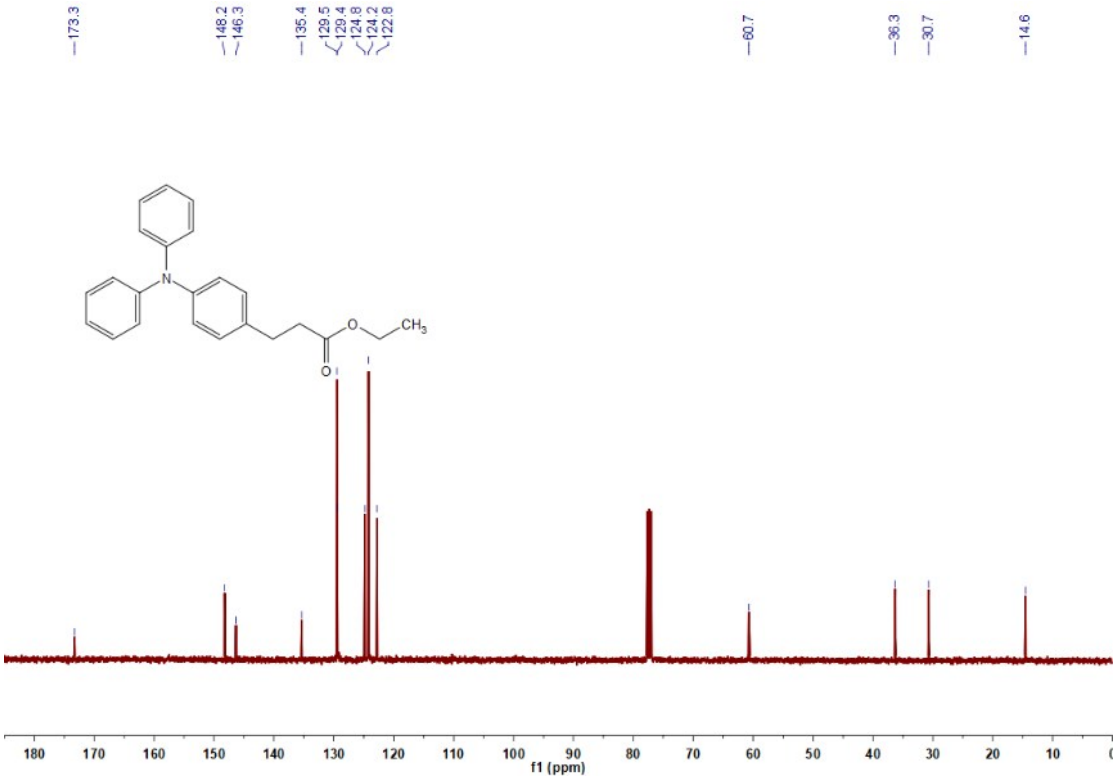
11.6 ^{1}H and ^{13}C NMR for compound 8



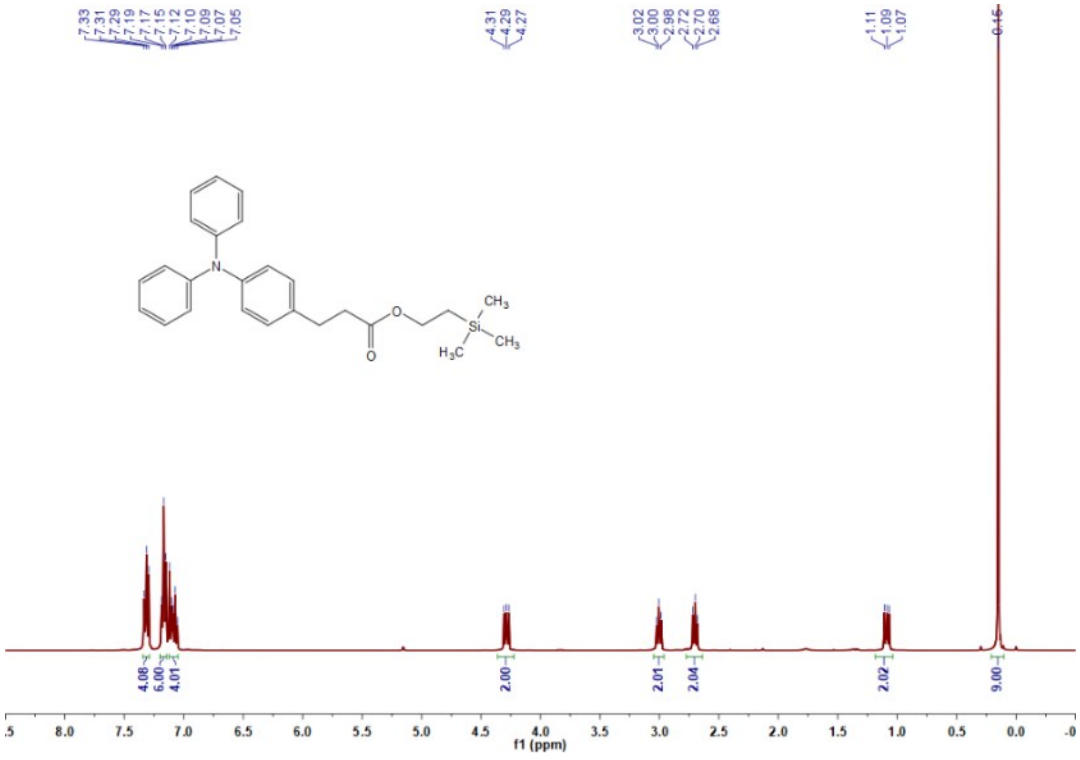


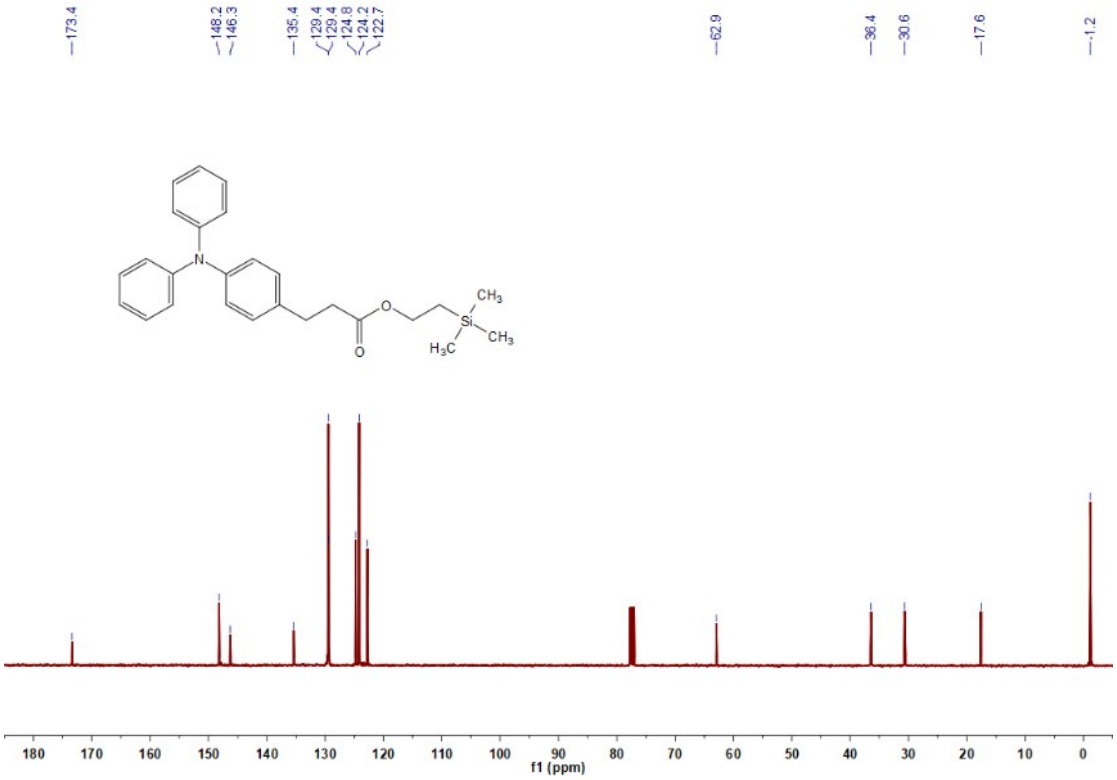
11.7 ^{1}H and ^{13}C NMR for compound 9



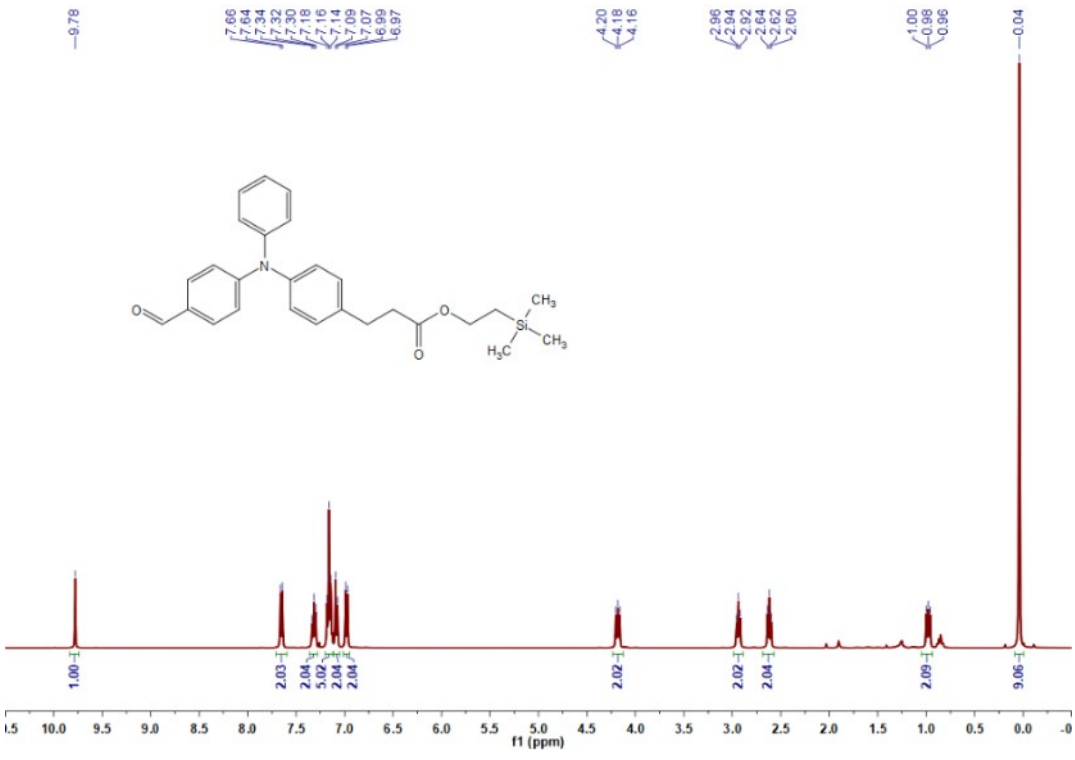


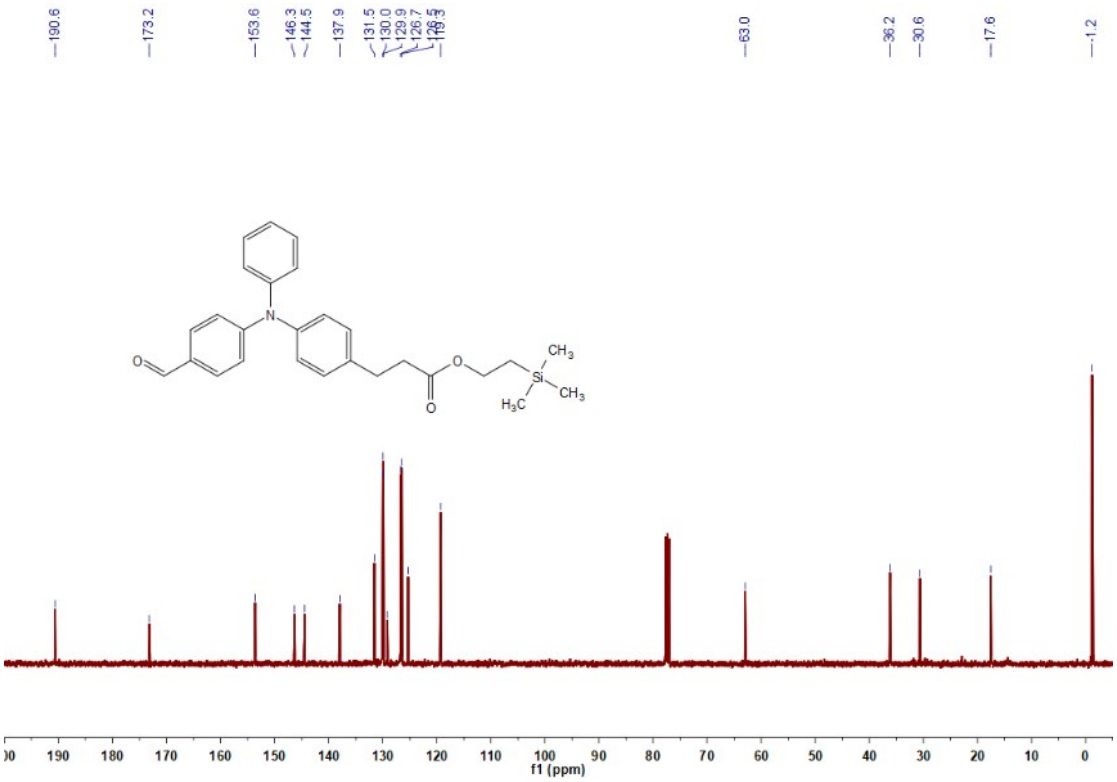
11.8 ^{1}H and ^{13}C NMR for compound 10



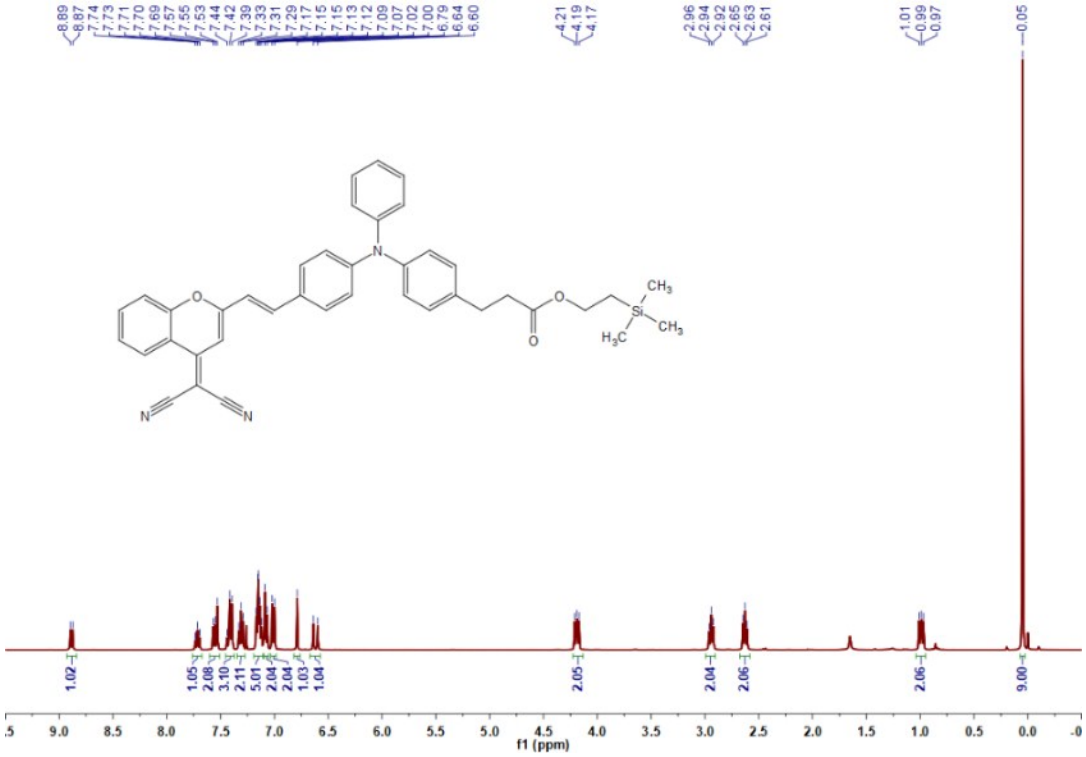


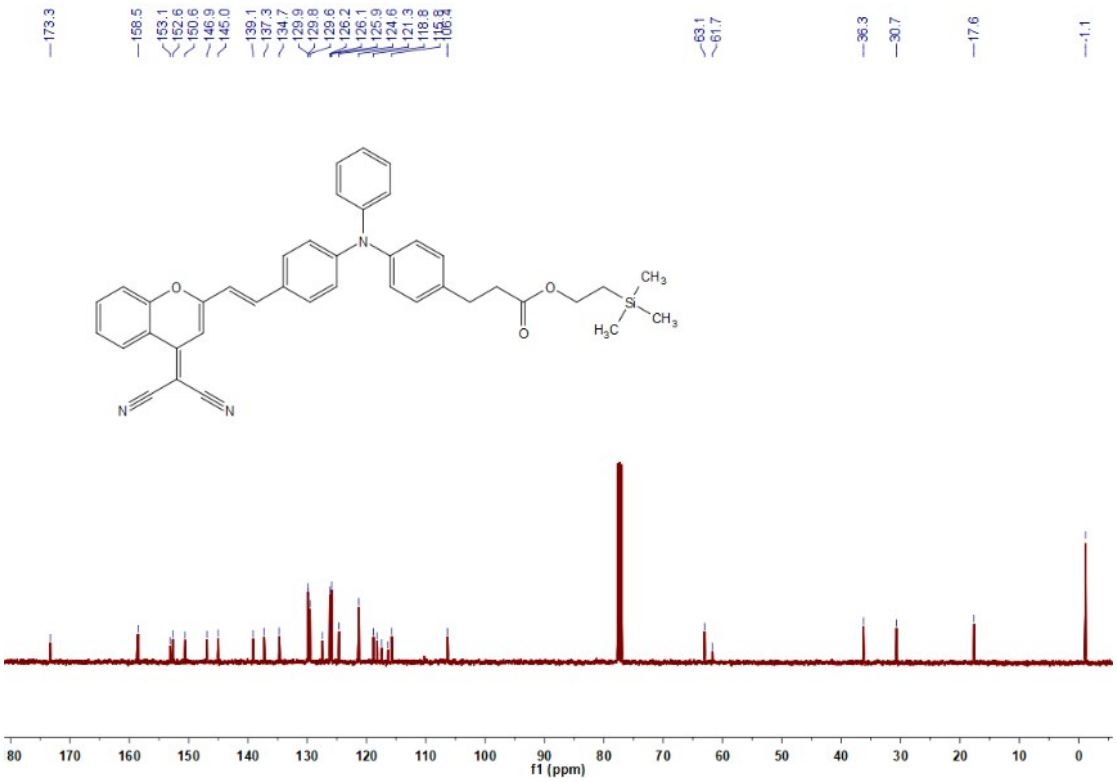
11.9 ^{1}H and ^{13}C NMR for compound 2



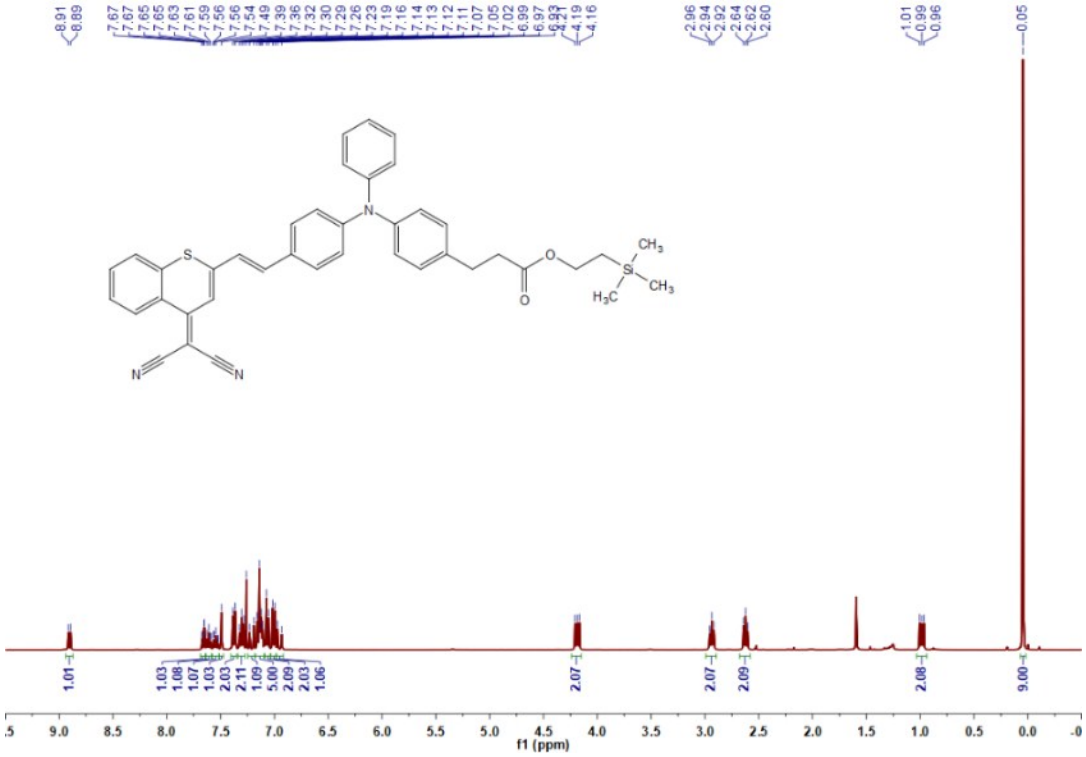


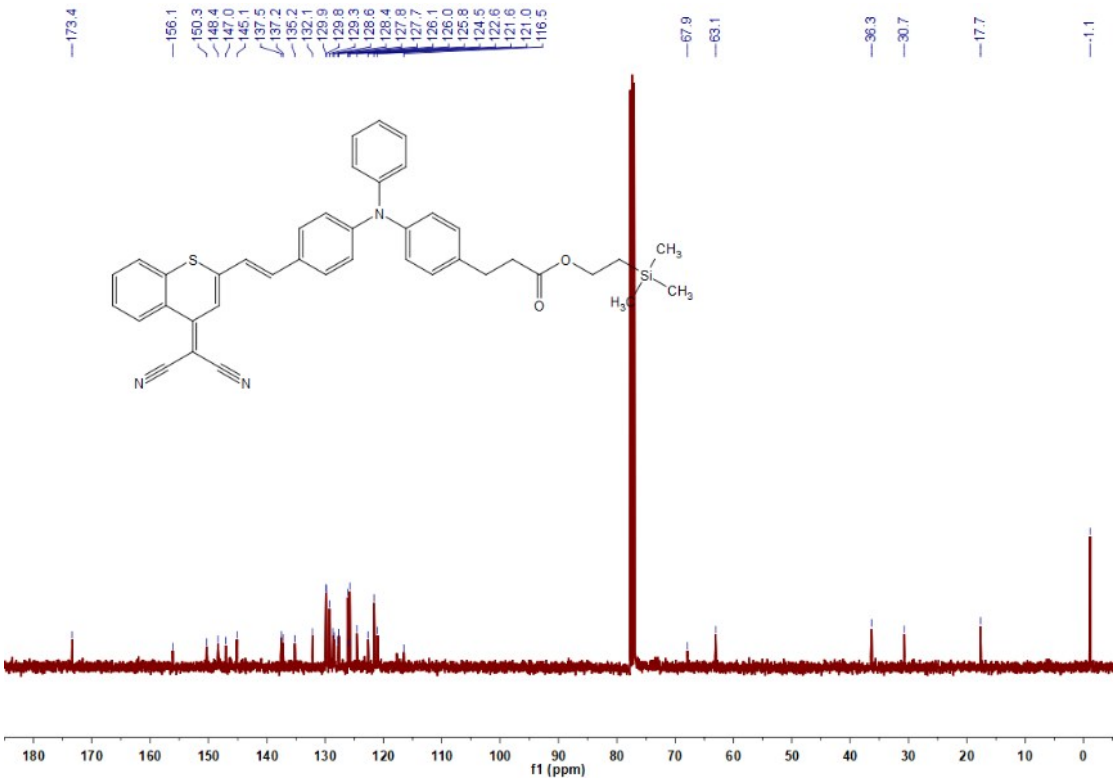
11.10 ^{1}H and ^{13}C for compound 3a



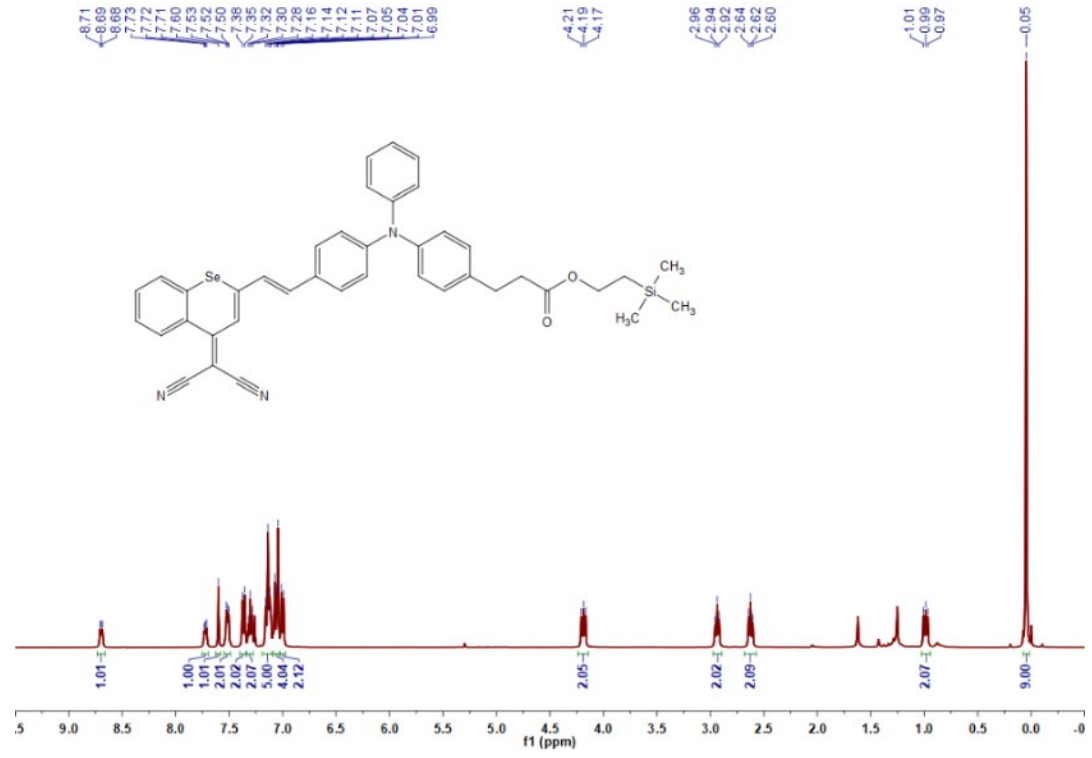


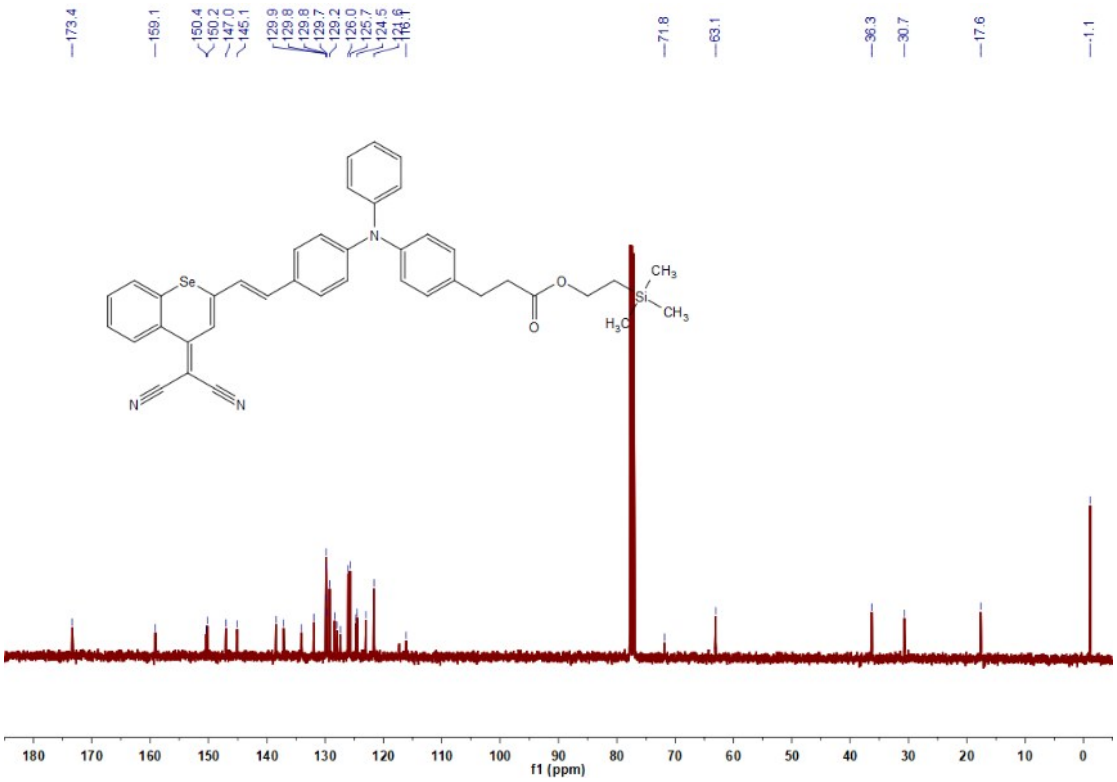
11.11 ^{1}H and ^{13}C NMR for compound 3b



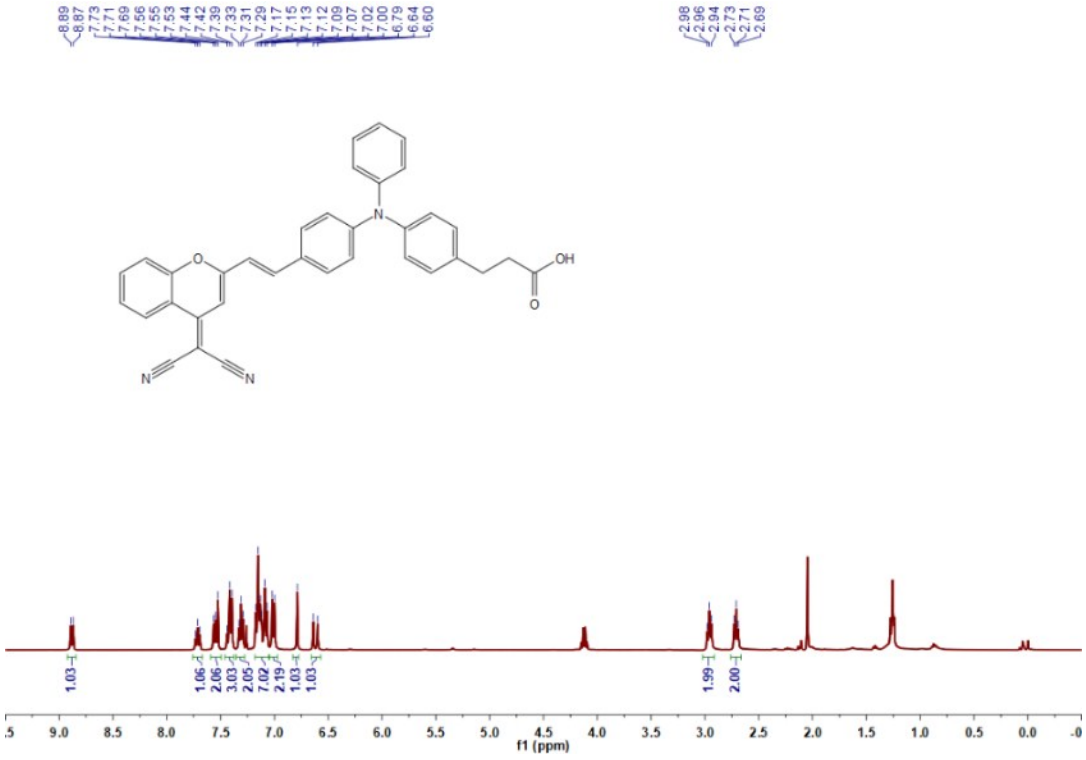


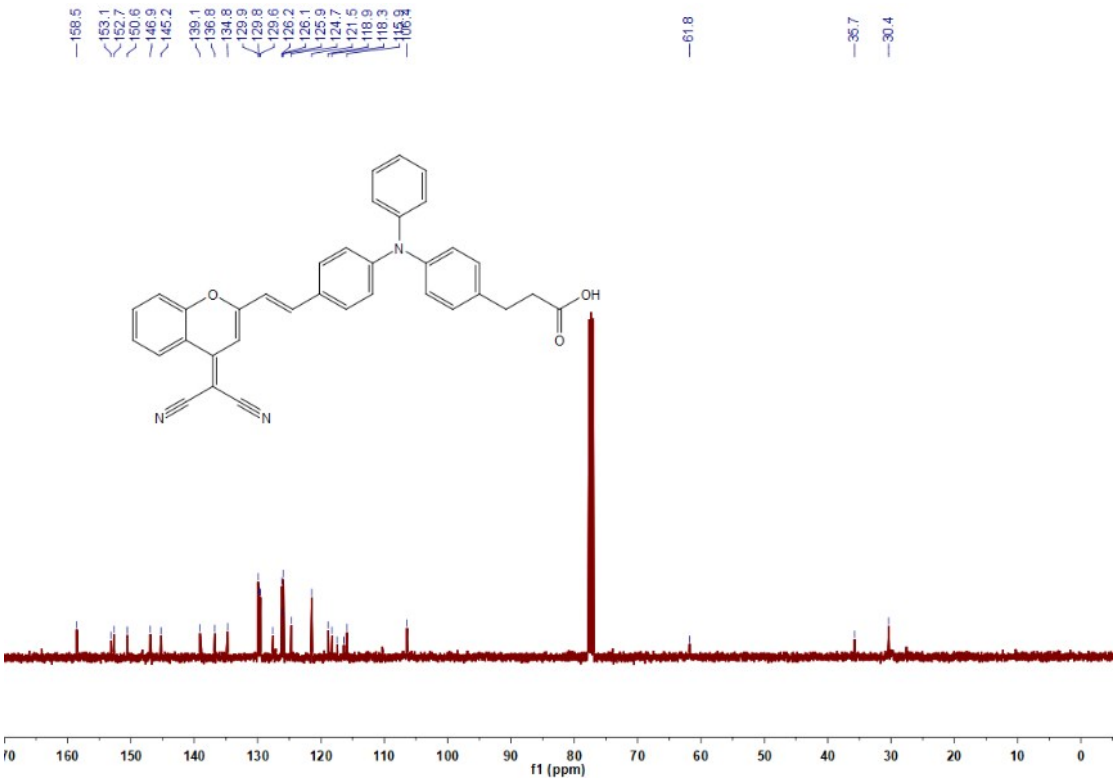
11.12 ^{1}H and ^{13}C NMR for compound 3c





11.13 ^{1}H and ^{13}C NMR for compound 11





12 Reference

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