CinNapht AIE(E)Gens for selective imaging of lipid droplets

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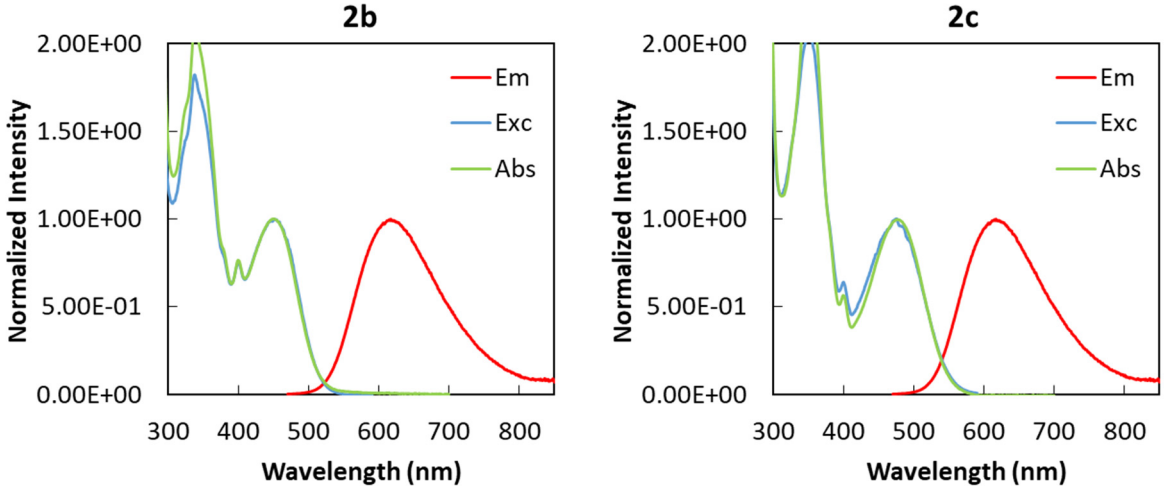
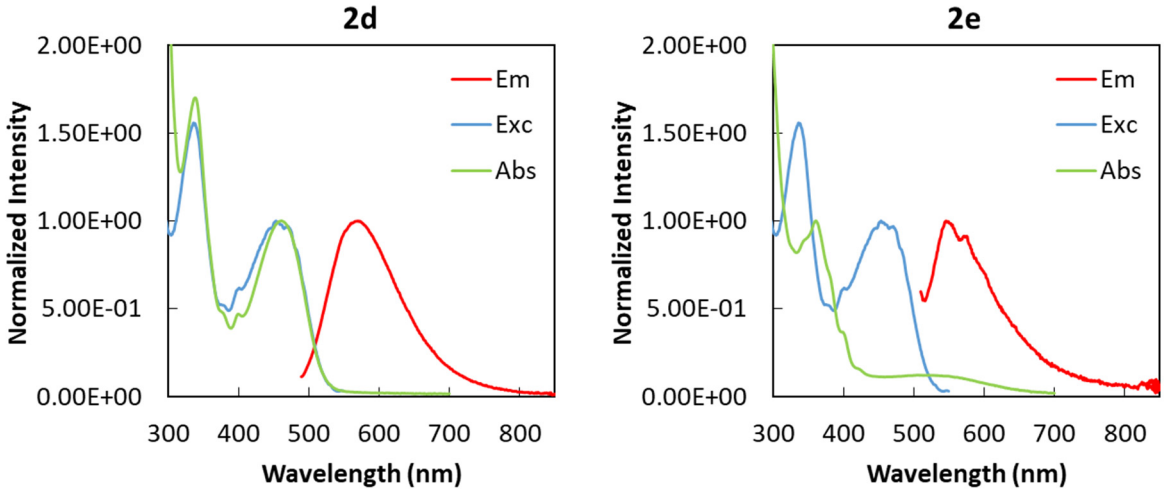
Supporting Information

I. Additional Figures

Fig S1: Absorbance, Emission and Excitation Spectra in $CHCl_3$

*Spectra were recorded at 25°C, at 10^{-5} M in solution in $CHCl_3$. Emission spectrum was recorded using an excitation at 470 nm for CInnapht **2b, 2c, 2d** and 490 nm for CinNapht **2e, 2f**. Spectra of compound 2a can be found in our previous study[1]*





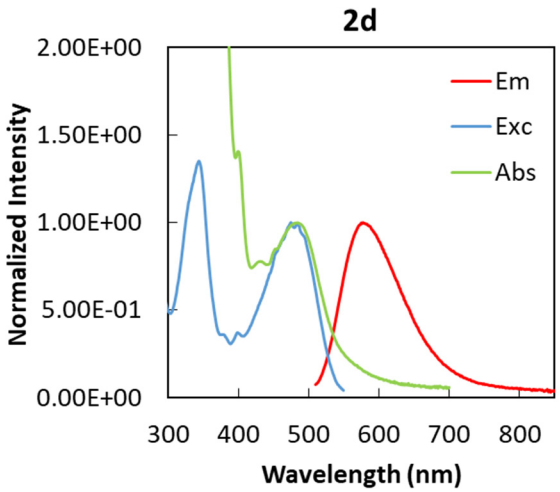


Fig S2. Extinction coefficient (ε) calculation in $CHCl_3$

Absorbance spectra were recorded at 25°C in $CHCl_3$.

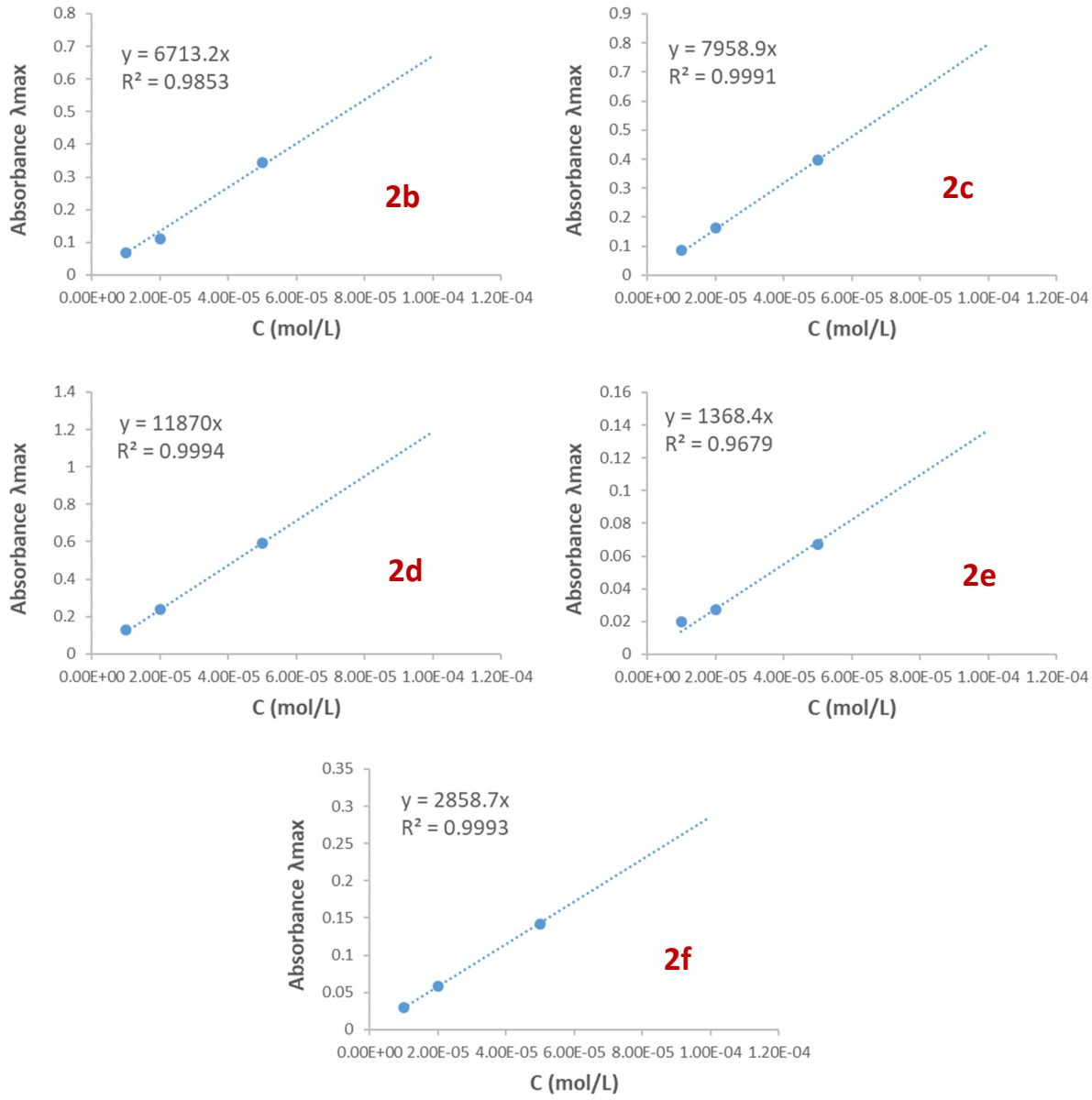


Fig S3. Emission spectra in solid state

*Spectra were recorded at 25°C with λ_{Exc} = 400 nm for CinNaphts **2b** and **2c**, λ_{Exc} = 470 nm for CinNaphts **2a** and **2d**, λ_{Exc} = 490 nm for CinNaphts **2f***

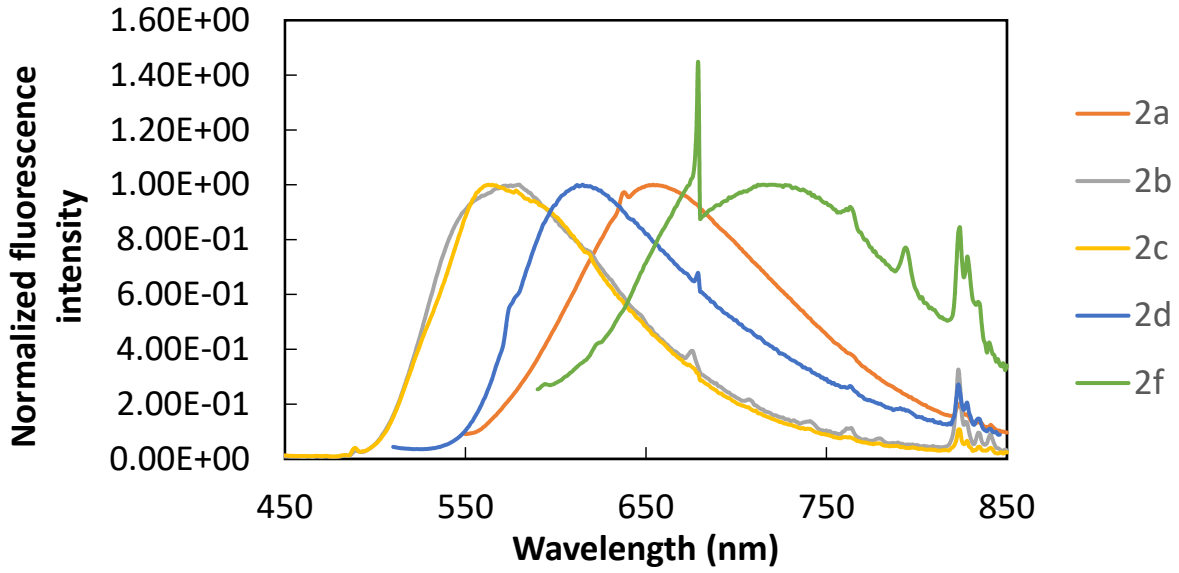


Fig S4. Absolute fluorescence quantum yield measurement

Spectra (in powder state) were recorded at room temperature (approximately 20 °C) using the excitation wavelengths determined by the λAbs (max) value in chloroform solution. The reference emission spectra (without compound) are represented by red lines, while the emission spectra of the powder are depicted by black lines

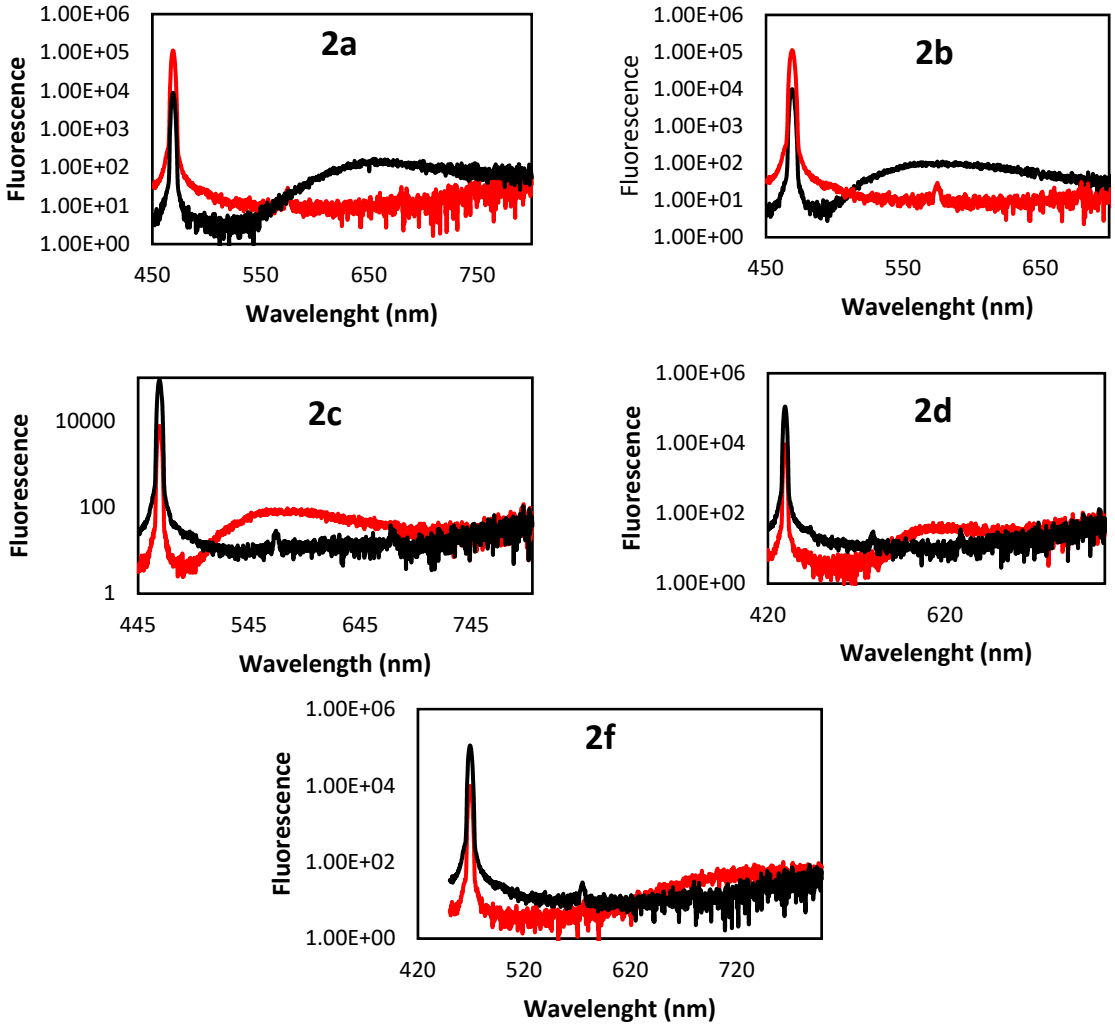


Fig S5. Absorbance, Emission and Excitation Spectra in Canola Oil

Spectra were recorded at 25°C in Canola Oil. Emission spectrum was recorded using an excitation at 470 nm. Excitation spectrum was recorded using an emission at 600 nm.

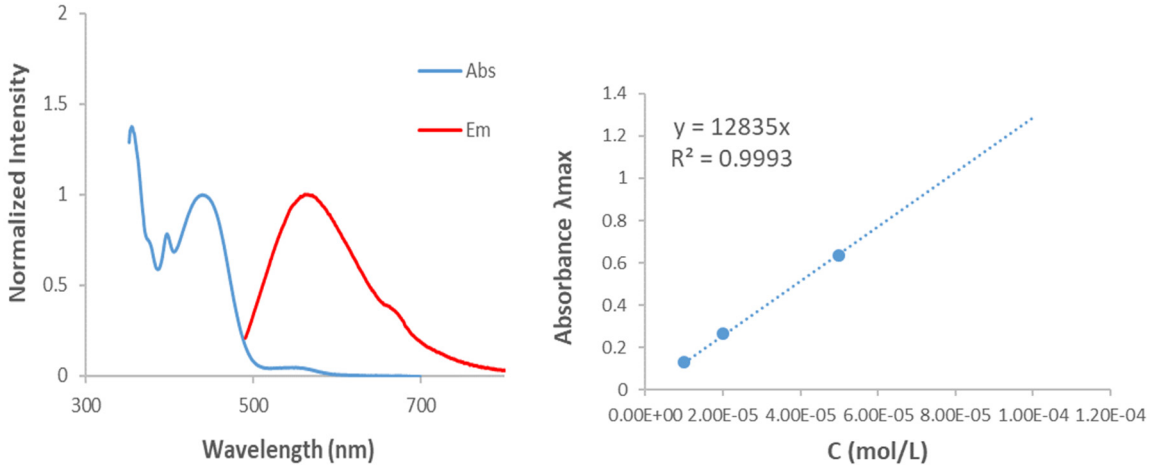
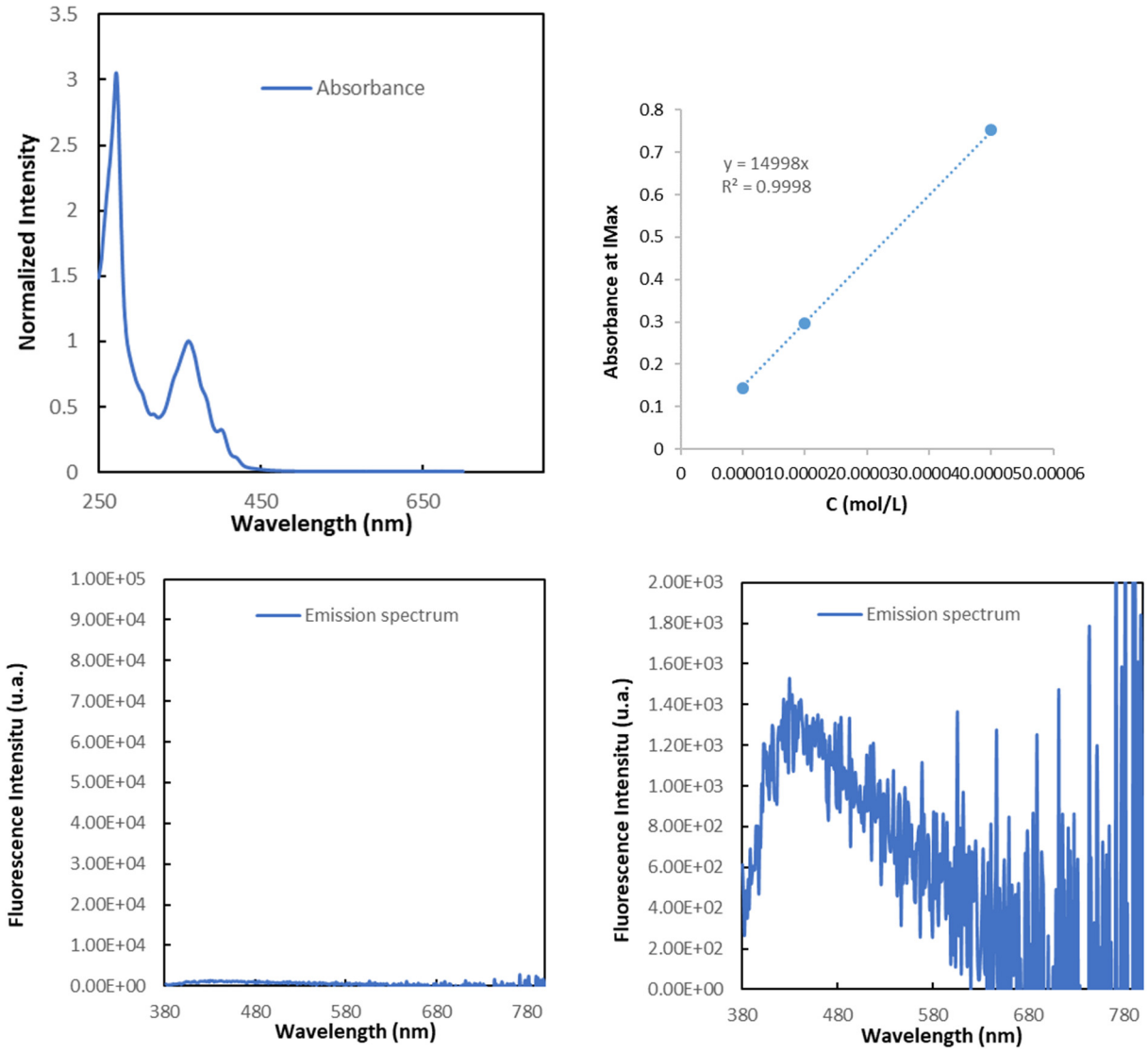


Fig S6. Photophysical properties of CinNapht 3 in $CHCl_3$

Spectra were recorded at 25°C at 10^{-5}M in solution in $CHCl_3$. Emission spectrum was recorded using an excitation at 360 nm showing the complete absence of emission. Emission spectra of CinNapht 3 was recorded at 25 °C for an excitation at 360 nm.



II. Experimental Section

Experimental section

Abbreviations

The following abbreviations are used throughout the text of the ESI file. Abs: Absorption; aq: aqueous; Ar, argon; $CHCl_3$: chloroform; DCM: dichloromethane; DMF: dimethylformamide; DMSO: dimethylsulfoxyde; Em: emission; EtOH: ethanol; EtOAc: ethyl acetate; Exc: excitation; H_2O: water; Hept: heptane; HRMS: High-Resolution Mass Spectrum; IR: infrared; MeOH: methanol; min: minutes; NMR: Nuclear Magnetic Resonance; PBS: phosphate buffered saline; MS: mass spectrometry; RT: room temperature; TLC: Thin Layer Chromatography; UV: ultraviolet; Vis: Visible.

General

Unless otherwise noted, all commercially available reagents and solvents were used without further purification. TLC were carried out on silica gel aluminum plates with F-254 indicator. The spots were directly visualized or through illumination with UV lamp (λ = 254/365 nm). Flash-column chromatography purifications were performed on silica gel cartridges (50 µm) from Interchim. Organic solvents for spectroscopy were purchased from Acros Organics, Carlo Ebra or Sigma Aldrich. Colza oil (mainly composed of unsaturated lipids ~93% from Lesieur, Asnières-sur-Seine, France).

Instruments and methods

Proton NMR (^{1}H) spectra were recorded on a Bruker Advance 500 or 300 MHz and proton-decoupled carbon ^{13}C NMR spectra were recorded at 125 MHz. NMR experiments were carried out in deuterated solvents and chemical shifts are expressed in parts per million (ppm) from the residual signals of partially deuterated solvent summarized in 2010 by Fulmer et al.[2] The following abbreviations are used for the multiplicities: s: singlet; d: doublet; t: triplet; q: quadruplet; qt: quintuplet, sx: sextuplet; m: multiplet or overlap of non-equivalent resonance; br s: broad singlet; coupling constants (J) are reported in Hertz (Hz).

High resolution mass spectra were determined on an AEI MS-9 using electrospray ionization (ESI) and a time offlight (TOF) analyser. IR spectra were recorded with a PerkinElmer Spectrum BX FT-IR spectrometer directly from the substance via attenuated total reflectance (ATR-IR) and bond vibration frequencies are expressed in reciprocal centimeters (cm^{-1}). HPLC-MS analyses were performed on an Alliance W2690 system (Waters, USA). UV-Visible absorption spectra were recorded on a Varian Cary 60 Spectrophotometer using a 10 mm path quartz cell. Emission spectra experiments were performed on an Edimburgh FS-5 spectrofluorimeter with a SC-20 module. A right angle configuration was used. The relative fluorescence quantum yields were determined using DCM (4-Dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran) as reference (ϕ_{FL} = 0.43 in EtOH).

Photophysical characterization

UV-Vis absorption measurements (scan mode) were conducted on a Cary UV 60 spectrophotometer (Varian) using rectangular 10 mm path length quartz cuvettes at 25°C. Fluorescence spectroscopic studies (scan mode) were performed with a spectrofluorimeter FS-5 (Edimburgh) at 25°C, with rectangular 10 mm path length quartz cuvettes from Hellma. The emission and excitation spectra were recorded with a diluted solution lower than 0.1 absorbance values. For the excitation and emission spectra the slits width were adjusted between 2-5 nm. All fluorescence spectra were corrected from

the lamp fluctuations and the apparatus response fluctuations. Fluorescence quantum yields were measured at 25°C, using three diluted solutions (A<0.1) and three diluted solution of DCM (QY = 0.43 in EtOH). For each QY measurements, slit width, excitation wavelength, scan rate, integration time and emission range were kept the same for the reference and the sample. The fluorescence quantum yield was determined thanks to the following formula:

$$\varphi_{FL}(x) = \varphi_{FL}(0)\frac{1-10^{-A_0}}{1-10^{-A_x}}\frac{S_x}{S_0}\left(\frac{n_x}{n_0}\right)^2$$

Where S is the slope of the linear plot of the integrated fluorescence intensity in function of the absorbance value, n is the refractive index of the solvents (at 25°C) used in measurements, and the subscripts s and x represent standard and unknown, respectively.

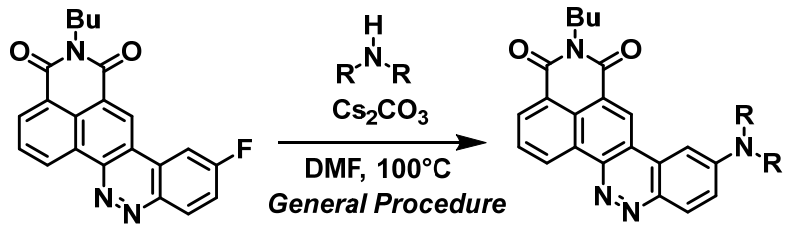
Cell culture and Confocal Microscopy

Cell lines were obtained from the American type Culture Collection (Rockville, USA) and were cultured according to the supplier's instructions. A549 cells were grown in RPMI 1640 containing 10 % FCS and 1% glutamine. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2 and were split every 3 or 4 days at a time when enough confluence was obtained. An Ibidi® µ-Slide 8 Well High Glass Bottom plate was seeded with 15 000 cells/well and then maintained at 37°C in a humidified atmosphere containing 5% CO_2 for 24h to 48h. After adequate confluence was obtained, the medium was removed and replaced by a solution of fluorophore at desired concentration in the appropriate medium, prepared from a stock solution of fluorophore in DMSO, final concentration in DMSO < 1.0% (please note that no solubility issue was noted). The cells were incubated for appropriate amount of time at 37°C in a humidified atmosphere containing 5% CO_2. The fluorophore solution was then removed and the cells were washed twice with pre-warmed PBS (1X). Finally, RPMI medium was added before imaging.

Fluorescence images were acquired using a Leica SP8-X inverted confocal microscope with a 63x oil immersion objective (HC PL APO CS2 Leica). Excitation was performed using a white laser pulsed at 80 MHz set at the desired excitation wavelength or with a diode 405 nm (Leica, 50 mV). Detection was carried out by using PMT detector (Hamamatsu 6357) or GaAsP Hybrid (Hamamatsu) collecting photons over the appropriate emission wavelength window.

Synthesis protocols

General procedure :

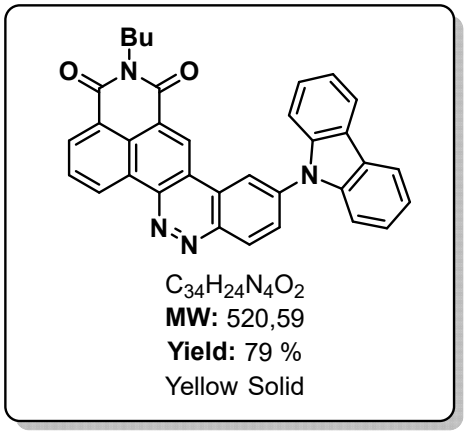


CinNapht-F

CinNapht-F (20.0 mg, 0.05 mmol, 1.00 eq), Cs_2CO_3 (70.0 mg, 0.21 mmol, 4.00 eq) and the corresponding amine (0.11 mmol, 2.00 eq) were dissolved in DMF (0.50 mL, 0.1 M) and stirred at 100 °C overnight. After the reaction was completed, the mixture was cooled down to room temperature and diluted in DCM (50.0 mL). The mixture was washed with aqueous NaCl saturated solution (2 x 50.0 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel or on preparative TLC plate using an appropriate eluting solvent mixture to afford the desired pure product.

CinNapht Carbazole (2b)

Compound **2b** was prepared according to the general procedure using carbazole (18.0 mg, 0.11 mmol). The crude product was purified by flash chromatography on silica gel (DCM/AcOEt 99:1 v/v) to afford compound **2b** (22.0 mg, 79 %) as a yellow solid.

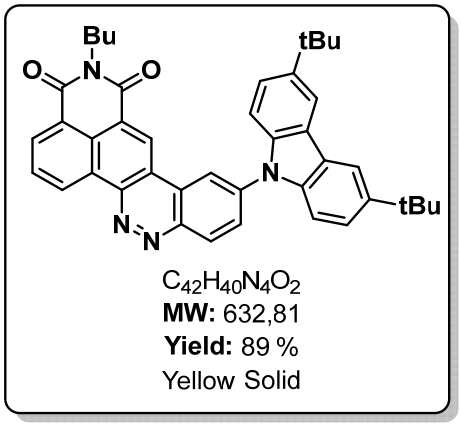


$C_{34}H_{24}N_4O_2$
MW: 520,59
Yield: 79 %
Yellow Solid

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.07 (d, *J* = 8.2 Hz, 1H), 9.53 (s, 1H), 9.03 (d, *J* = 8.7 Hz, 1H), 8.96 (s, 1H), 8.79 (d, *J* = 7.3 Hz, 1H), 8.26 (d, *J* = 8.7 Hz, 1H), 8.15 (t, *J* = 7.8 Hz, 1H), 8.06 (d, *J* = 7.7 Hz, 2H), 7.59 (d, *J* = 8.2 Hz, 2H), 7.41 (t, *J* = 7.6 Hz, 2H), 7.30 (t, *J* = 7.4 Hz, 2H), 4.20 (t, *J* = 7.5 Hz, 2H), 1.80 – 1.71 (p, 2H), 1.52 – 1.40 (sx, *J* = 7.3 Hz, 2H), 0.99 (t, *J* = 7.3 Hz, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 164.0, 163.3, 146.1, 142.2, 142.1, 140.2 (2C), 133.9, 132.1, 130.9, 129.9, 129.6, 129.2, 127.8, 126.8 (2C), 125.6 (2C), 124.4, 123.0 (2C), 121.5 (2C), 120.8 (2C), 118.8, 118.5 (2C), 109.6 (2C), 40.8, 30.4, 20.5, 14.0.
Rf (DCM/AcOEt 8:2) = 0.6
IR (neat): ν = 3676, 2959, 2916, 1735, 1699, 1657, 1612, 1491, 1444, 1407, 1394, 1380, 1334, 1230, 1066, 859, 787, 749, 724, 698, 663
ESI-HRMS calculated for $C_{34}H_{25}N_4O_2$ $[M+H]^+$ 521.1959, found 521.1962

CinNapht Carbazole-di*t*Bu (2c)

Compound **2c** was prepared according to the general procedure using 3,6-Di-*tert*-butylcarbazole (30.0 mg, 0.11 mmol). The crude product was purified by flash chromatography on silica gel (Hept/AcOEt 10:0 to 7:3 v/v) to afford compound **2c** (30.0 mg, 89 %) as a yellow solid.



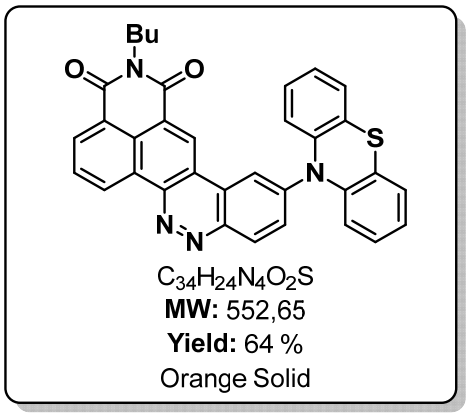
$C_{42}H_{40}N_4O_2$
MW: 632,81
Yield: 89 %
Yellow Solid

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.12 (d, *J* = 8.2 Hz, 1H), 9.60 (s, 1H), 9.07 (d, *J* = 8.8 Hz, 1H), 8.98 (d, *J* = 1.3 Hz, 1H), 8.82 (d, *J* = 7.2 Hz, 1H), 8.34 (dd, *J* = 8.8, 1.6 Hz, 1H), 8.21 (m, 2H), 8.17 (t, *J* = 7.9 Hz, 1H), 7.64 (d, *J* = 8.6 Hz, 2H), 7.56 (dd, *J* = 8.6, 1.4 Hz, 2H), 4.25 (t, *J* = 7.3 Hz, 2H), 1.84 – 1.70 (p, *J* = 7.4 Hz, 2H), 1.52 – 1.47 (m, 20H), 1.00 (t, *J* = 7.4 Hz, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 164.0, 163.4, 146.0, 144.8 (2C), 142.7, 142.1, 138.5 (2C), 133.8, 132.0, 130.9 (2C), 129.9, 129.5, 128.9, 127.8, 125.8, 125.5, 124.6, 124.5 (2C), 123.0, 122.9, 118.8, 117.4, 116.9 (2C), 109.3 (2C), 40.8, 35.0 (2C), 32.1 (6C), 30.4, 20.5, 14.0
Rf (DCM/AcOEt 8:2) = 0.7

IR (neat): ν = 3673, 2958, 2926, 2871, 1705, 1661, 1615, 1555, 1482, 1467, 1453, 1433, 1416, 1405, 1393, 1364, 1333, 1295, 1263, 1230, 1192, 1158, 1136, 1096, 1084, 1066, 938, 911, 879, 846, 809, 786, 752, 710, 691, 655
ESI-HRMS calculated for $C_{42}H_{41}N_4O_2$ $[M+H]^+$ 633.3230, found 633.3222

CinNapht Phenothiazine (2d)

Compound **2d** was prepared according to the general procedure using phenothiazine (21.0 mg, 0.11 mmol). The crude product was purified by flash chromatography on silica gel (DCM/AcOEt 98:2 v/v) to afford compound **2d** (19.0 mg, 64 %) as an orange solid.



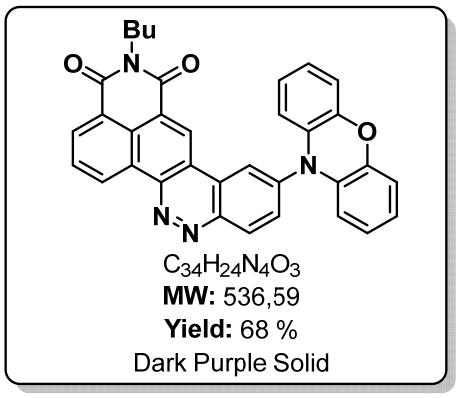
$C_{34}H_{24}N_4O_2S$
MW: 552,65
Yield: 64 %
Orange Solid

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.98 (dd, *J* = 8.3, 1.0 Hz, 1H), 9.23 (s, 1H), 8.73 (dd, *J* = 7.4, 1.0 Hz, 1H), 8.60 (d, *J* = 9.3 Hz, 1H), 8.12 (d, *J* = 2.5 Hz, 1H), 8.10 – 7.99 (m, 1H), 7.73 (dd, *J* = 9.3, 2.5 Hz, 1H), 7.68 – 7.56 (m, 4H), 7.52 (td, *J* = 7.7 Hz, 1.4 Hz, 2H), 7.36 (td, *J* = 7.6, 1.2 Hz, 2H), 4.30 – 4.09 (t, *J* = 7.5 Hz, 2H), 1.87 – 1.70 (m, 2H), 1.55 – 1.42 (sx, *J* = 7.4 Hz, 2H), 1.00 (t, *J* = 7.3 Hz, 3H).
^{13}C NMR (75 MHz, $CDCl_3$) δ 164.1, 163.5, 149.1, 144.7 (2C), 142.0, 140.8, 134.7, 133.2, 131.6, 130.9, 129.8, 129.6 (2C), 129.0, 127.9 (2C), 127.5, 127.2 (2C), 127.0 (2C), 126.1, 124.0, 123.6, 122.7, 120.5 (2C), 118.6, 102.7, 40.7, 30.4, 20.6, 14.0
Rf (DCM/MeOH 95:5) = 0.6

IR (neat): ν = 3775, 2967, 2916, 2850, 1710, 1663, 1616, 1481, 1387, 1256, 1229, 1103, 1066, 823, 783, 763, 740, 689
ESI-HRMS calculated for $C_{34}H_{25}N_4O_2S$ $[M+H]^+$ 553.1698, found 553.1708

CinNapht Phenoxazine (2e)

Compound **2e** was prepared according to the general procedure using phenoxazine (20.0 mg, 0.11 mmol). The crude product was purified by preparative TLC (DCM/MeOH 9:1, v/v) to afford compound **2e** (20.0 mg, 68 %) as a dark purple solid.



$C_{34}H_{24}N_4O_3$
MW: 536,59
Yield: 68 %
Dark Purple Solid

^{1}H NMR (300 MHz, $CDCl_3$) δ 10.08 (dd, *J* = 8.3, 1.2 Hz, 1H), 9.58 (s, 1H), 9.06 (d, *J* = 8.7 Hz, 1H), 8.86 (d, *J* = 2.0 Hz, 1H), 8.80 (dd, *J* = 7.4, 1.2 Hz, 1H), 8.16 (dd, *J* = 8.3, 7.5 Hz, 1H), 7.99 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.76 – 6.65 (m, 4H), 6.63 – 6.52 (m, 2H), 6.05 (dd, *J* = 7.6, 0.8 Hz, 2H), 4.27 – 4.13 (m, 2H), 1.77 – 1.70 (m, 2H), 1.45 (sx, 7.3 Hz, 2H), 0.98 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 163.5, 162.8, 146.1, 144.0 (2C), 143.3, 141.5, 134.7 (2C), 133.0, 132.4, 131.6, 130.4, 129.5, 129.2, 127.4, 125.4, 125.2, 123.7, 123.1 (2C), 123.0, 122.6, 122.4 (2C), 118.4, 115.8 (2C), 113.5 (2C), 40.4, 29.9, 20.1, 13.5

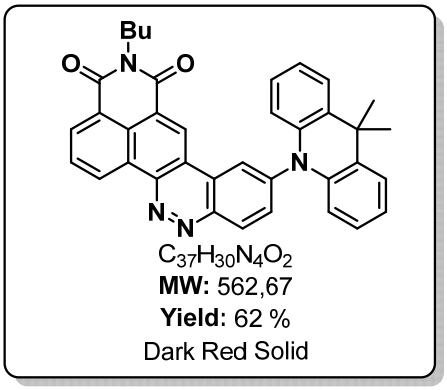
Rf (DCM/MeOH 95:5) = 0.7

IR (neat): ν = 2958, 2926, 2872, 1704, 1659, 1602, 1555, 1486, 1463, 1434, 1417, 1403, 1383, 1363, 1334, 1292, 1273, 1229, 1191, 1150, 1132, 1090, 1066, 1044, 994, 936, 864, 844, 786, 742, 699, 667

ESI-HRMS calculated for $C_{34}H_{25}N_4O_3$ $[M+H]^+$ 537.1927, found 537.1915

CinNapht Dimethylacridine (2f)

Compound **2f** was prepared according to the general procedure using 9,10-Dihydro-9,9-dimethylacridine (22.0 mg, 0.11 mmol). The crude product was purified by flash chromatography on silica gel (Hept/AcOEt 1000 to 7:3 v/v) to afford compound **2f** (19.0 mg, 62 %) as a dark red solid.



$C_{37}H_{30}N_4O_2$
MW: 562,67
Yield: 62 %
Dark Red Solid

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.10 (d, *J* = 8.2 Hz, 1H), 9.52 (s, 1H), 9.06 (d, *J* = 8.8 Hz, 1H), 8.88 – 8.72 (m, 2H), 8.15 (t, *J* = 7.9 Hz, 1H), 8.05 (dd, *J* = 8.8, 1.7 Hz, 1H), 7.58 – 7.52 (m, 2H), 7.07 (dd, *J* = 5.8, 3.4 Hz, 4H), 6.59 – 6.50 (m, 2H), 4.29 – 4.17 (t, *J* = 7.4 Hz, 2H), 1.79 – 1.71 (m, 8H), 1.51 – 1.40 (sx, *J* = 7.4 Hz, 2H), 0.99 (t, *J* = 7.4 Hz, 3H).

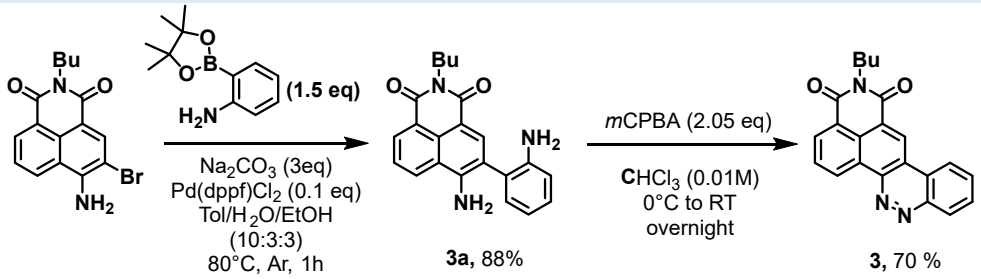
^{13}C NMR (126 MHz, $CDCl_3$) δ 164.0, 163.3, 146.3, 141.9, 140.6, 134.6, 133.2, 132.0, 131.7, 130.9, 129.9, 129.5, 127.8, 126.7 (2C), 125.9, 125.7 (2C), 125.6, 123.6, 123.0, 122.6 (2C), 120.7, 118.8, 116.3 (2C), 77.4, 77.2, 76.9, 40.8, 36.7, 30.7 (2C), 30.4, 20.5, 14.0

Rf (DCM/MeOH 95:5) = 0.6

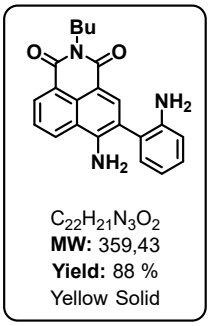
IR (neat): ν = 3661, 2960, 2921, 1704, 1661, 1618, 1599, 1554, 1474, 1448, 1416, 1404, 1383, 1363, 1330, 1266, 1229, 1190, 1131, 1107, 1088, 1076, 1048, 1066, 931, 892, 846, 786, 746, 702, 667

ESI-HRMS calculated for $C_{37}H_{31}N_4O_2$ $[M+H]^+$ 563.2447, found 563.2468

Synthetic route to unsubstituted CinNapht 3



6-amino-5-(2-aminophenyl)-2-butyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (3a)

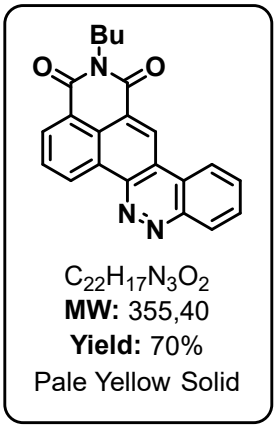


$C_{22}H_{21}N_3O_2$
MW: 359,43
Yield: 88 %
Yellow Solid

6-amino-5-bromo-2-butyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (200 mg, 0.58 mmol, 1.00 eq), 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (189 mg, 0.86 mmol, 1.50 eq) and Na_2CO_3 (183 mg, 1.73 mmol, 3.00 eq) were dissolved in a mixture of H_2O, EtOH and toluene (2.6 mL, 3:3:10 v/v/v) in a Schlenk tube. The resulting mixture was desoxygenated with freeze-pump-thaw cycling before addition of $Pd(dppf)Cl_2$ (42.0 mg, 0.058 mmol, 0.10 eq). The reaction was heated for 1h at 80°C with constant stirring under argon. After cooling to room temperature and evaporating the solvents, the crude mixture was purified by flash chromatography on silica gel (DCM/EtOAc 9:1 v/v) to afford compound **3a** (183 mg, 88%) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.61 (dd, *J* = 7.3, 1.0 Hz, 1H), 8.42 (s, 1H), 8.17 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.69 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.28 (m, 1.6 Hz, 1H), 7.18 (dd, *J* = 7.6, 1.6 Hz, 1H), 6.90 (td, *J* = 7.5, 1.2 Hz, 1H), 6.86 (dd, *J* = 8.1, 1.1 Hz, 1H), 5.05 (s, 2H), 4.17 (m, 2H), 3.70 (s, 2H), 1.71 (m, 2H), 1.44 (sx, *J* = 7.5 Hz, 2H), 0.97 (t, *J* = 7.4 Hz, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 164.4, 164.1, 146.5, 144.3, 135.9, 131.5, 131.4, 129.9, 129.4, 127.2, 125.5, 123.4, 122.4, 120.4, 119.4, 119.2, 116.1, 112.2, 40.2, 30.5, 20.6, 14.0.
Rf (DCM/EtOAc 8:2) = 0.5
IR (neat): ν = 3460, 3354, 3233, 2958, 2827; 2858, 2246, 1680, 1627, 1611, 1583, 1518, 1493, 1464, 1450, 1429, 1392, 1363, 1337, 1289, 1263, 1226, 1206, 1158, 1137, 1075, 935, 907, 847, 777, 751, 730, 695 cm^{-1}
ESI-HRMS calculated for $C_{22}H_{22}N_3O_2$ $[M+H]^+$ 360.1712 found 360.1700

11-butyl-10H-benzo[c]isoquinolino[4,5-gh]cinnoline-10,12(11H)-dione (3)



$C_{22}H_{17}N_3O_2$
MW: 355,40
Yield: 70%
Pale Yellow Solid

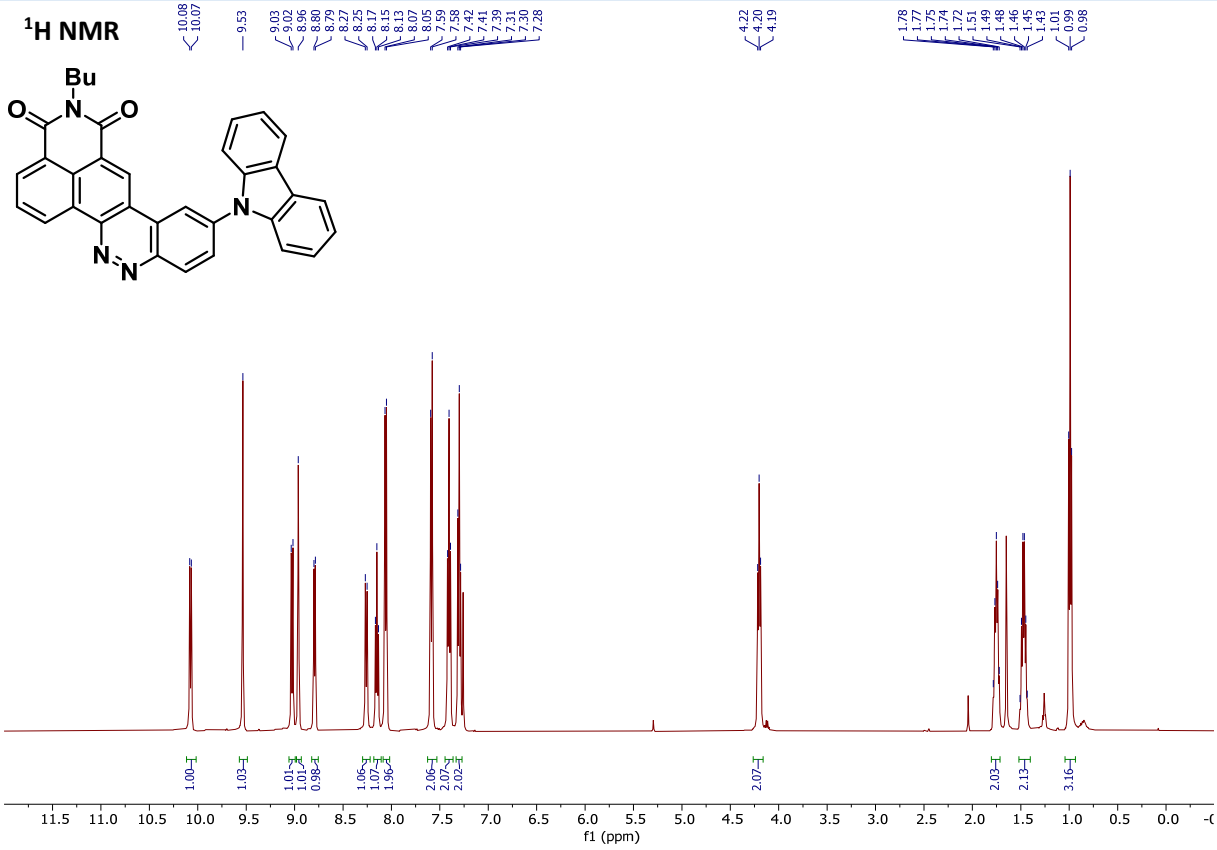
A solution of compound **3a** (150 mg, 0.42 mmol, 1.00 eq.) in $CHCl_3$ was cooled in an ice bath to 0 °C. *m*CPBA (148 mg, 0.86 mmol, 2.05 eq.) was added and the reacting mixture was kept at 0 °C under stirring for 2h after what it was allowed to warm to room temperature. The mixture was then stirred under argon at room temperature overnight. After the reaction was completed (monitored by TLC) the mixture was quenche with a 10% aqueous $Na_2S_2O_3$ solution. The phases were separated and the aqueous layer was extracted with DCM. The organic phase was then washed with a saturated solution of $NaHCO_3$ (3 x 50.0 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (DCM/EtOAc 95:5 v/v) to afford compound 3 (104 mg, 70 %) as a pale yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.09 (dd, *J* = 8.3, 1.2 Hz, 1H), 9.68 (s, 1H), 8.91 – 8.88 (m, 1H), 8.82 (m, 2H), 8.15 (m, 1H), 8.11 – 8.05 (m, 2H), 4.29 – 4.25 (m, 2H), 1.82 – 1.76 (m, 2H), 1.53 (sx, *J* = 7.5 Hz, 2H), 1.02 (t, *J* = 7.4 Hz, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 164.4, 164.1, 147.9, 142.1, 133.3, 132.1, 131.1, 130.9, 130.3, 129.8, 127.9, 126.2, 125.7, 123.1, 122.4, 121.6, 119.5, 117.7, 41.0, 30.6, 20.8, 14.3.
Rf (DCM/EtOAc 9:1) = 0.8
IR (neat): ν = 2957, 2930, 2871, 1702, 1659, 1618, 1600, 1498, 1449, 1434, 1410, 1380, 1335, 1264, 1229, 1156, 1127, 1091, 1080, 1066, 963, 937, 907, 857, 784, 766, 752, 735, 687 cm^{-1}
ESI-HRMS calculated for $C_{22}H_{18}N_3O_2$ $[M+H]^+$ 356.1399 found 356.1411

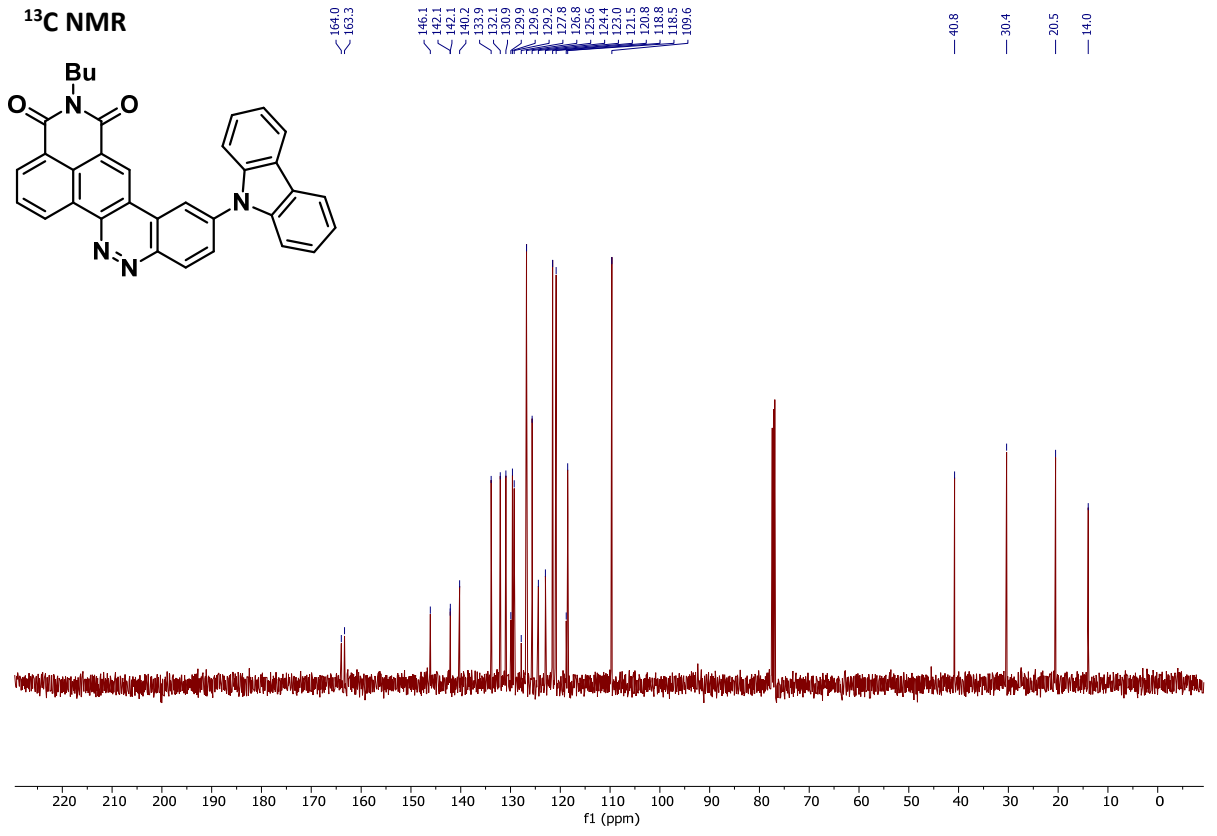
NMR and MS Spectra

Compound 2a:

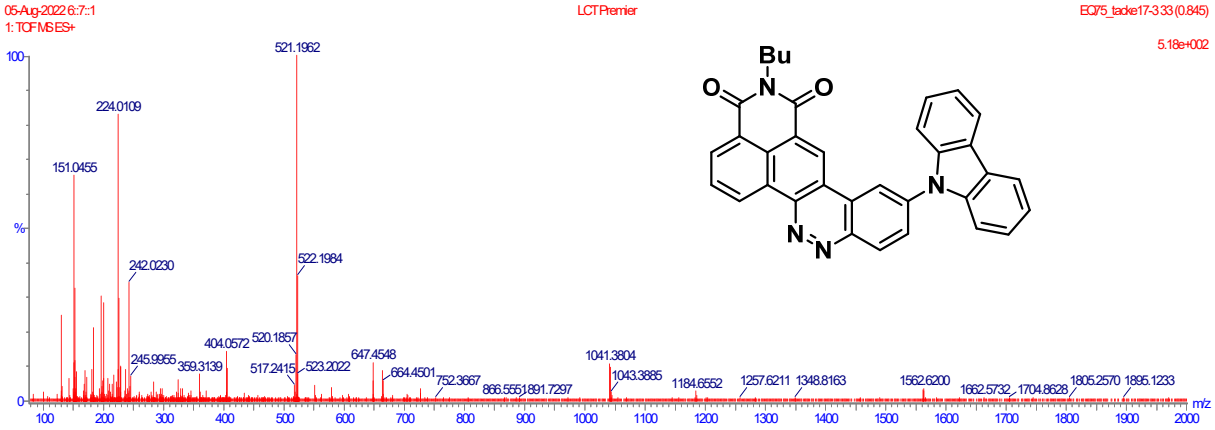
^{1}H NMR



^{13}C NMR



HRMS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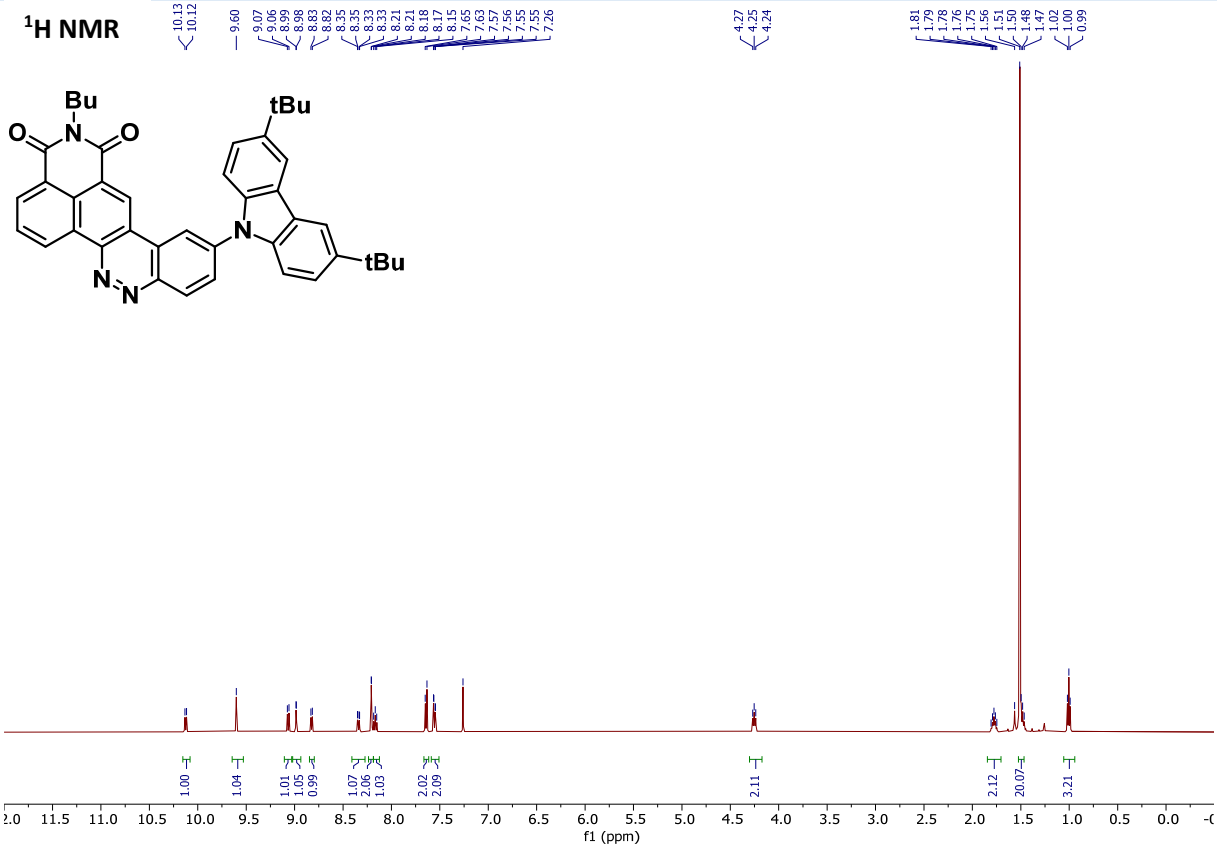
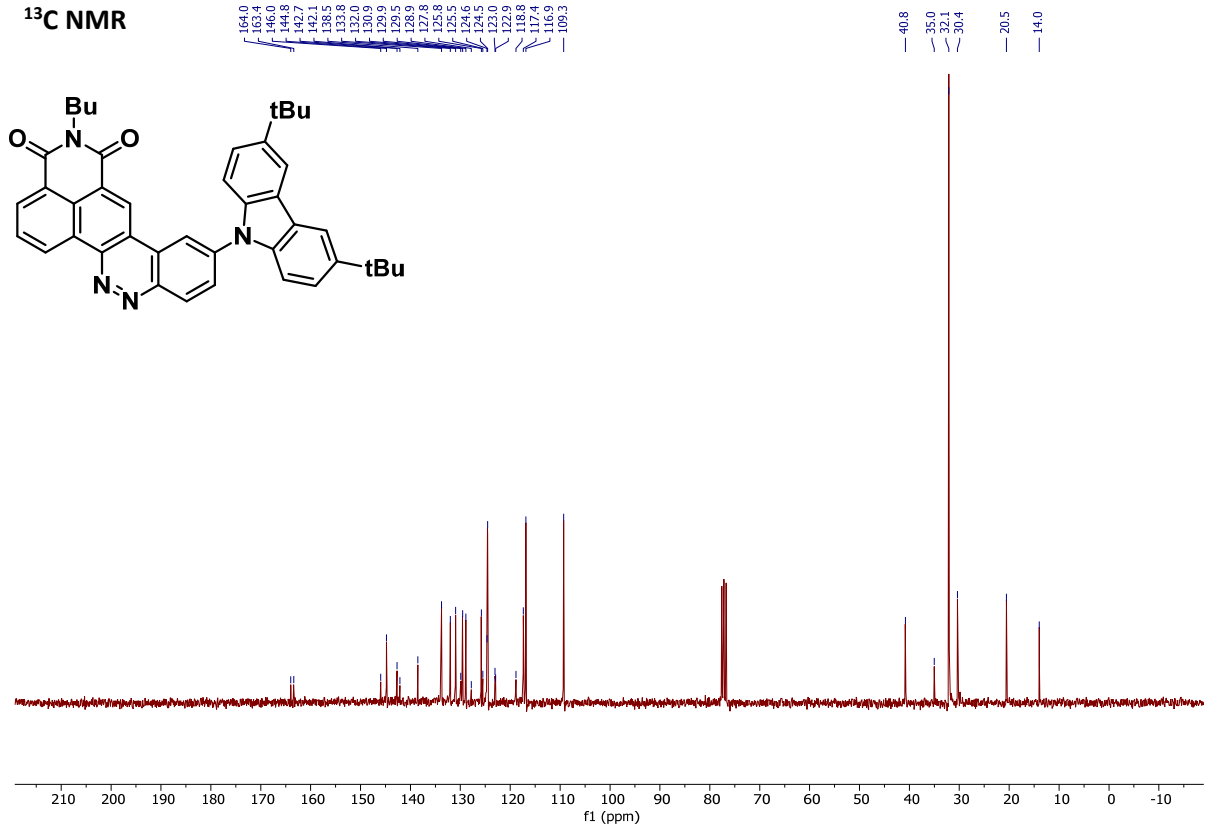
224.0109
151.0455
242.0230
522.1984
404.0572
520.1857
245.9955
359.3139
517.2415
523.2022
647.4548
664.4501
752.3667
866.5551
891.7297
1041.3804
1043.3885
1184.6552
1257.6211
1348.8163
1562.6200
1662.5732
1704.8628
1805.2570
1895.1233
%
m/z
Bu
O
N
O
N
N
N

Compound 2c:

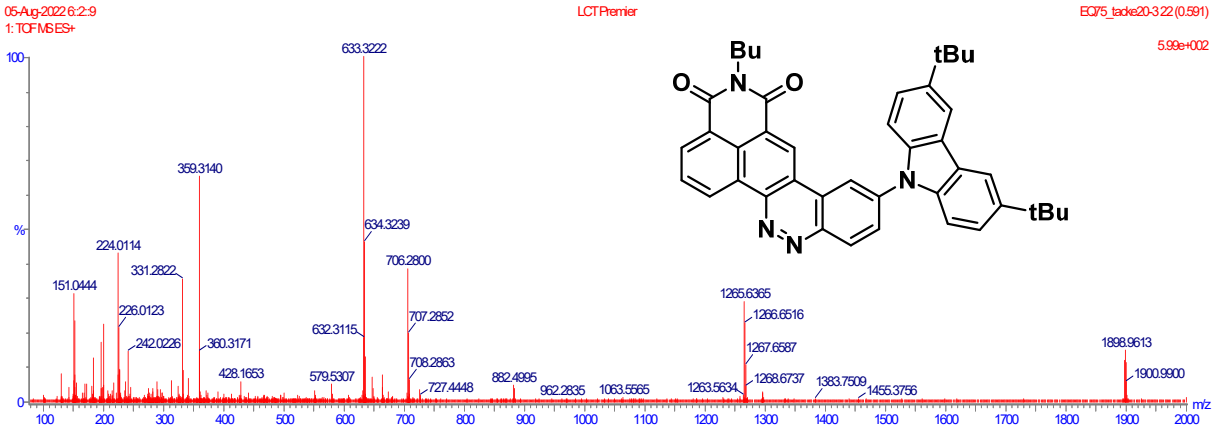
^{1}H NMR



^{13}C NMR

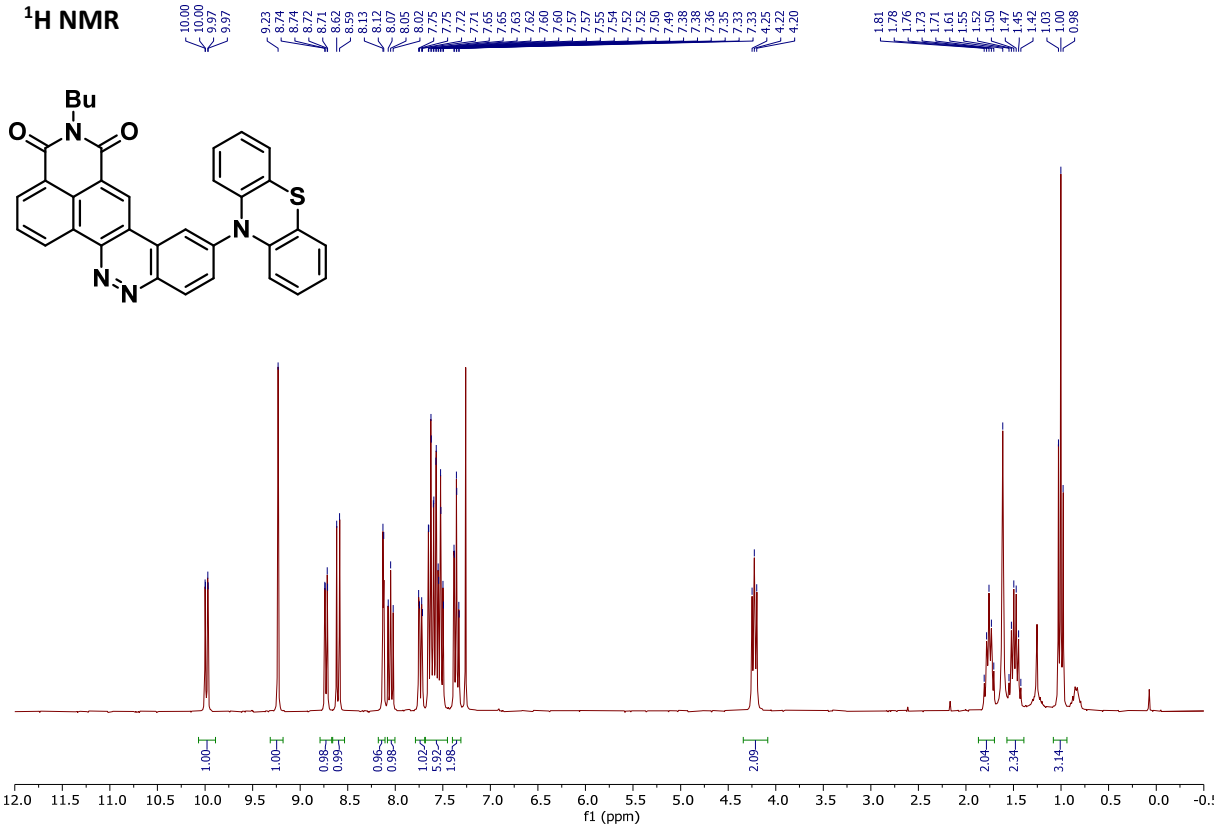


HRMS

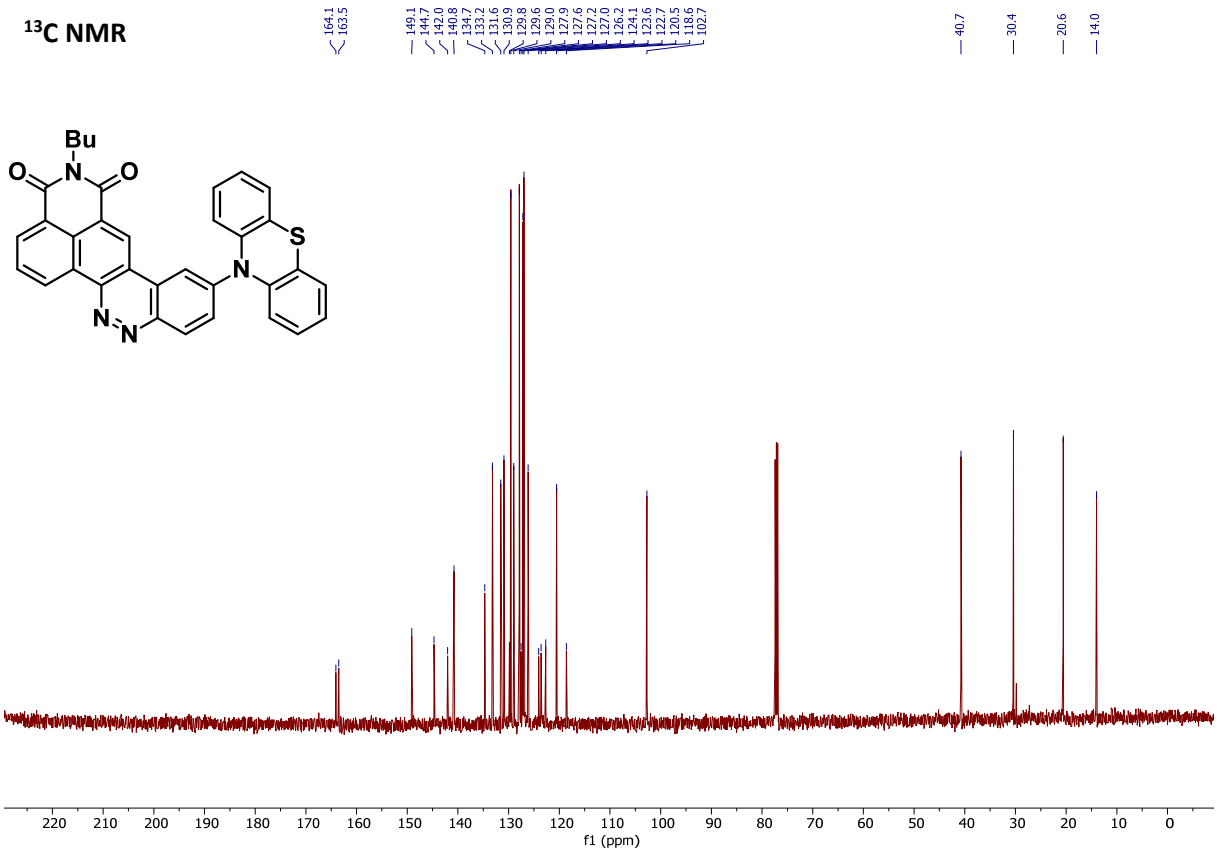

05-Aug-2022 6:2:9
1: TOF MS ES+
LCT Premier
EQ75_tacke20-3 22 (0.591)
5.99e+002
100
%
0
633.3222
359.3140
634.3239
224.0114
706.2800
331.2822
151.0444
707.2852
226.0123
632.3115
242.0226
360.3171
708.2863
428.1653
579.5307
727.4448
882.4995
962.2835
1063.5565
1265.6365
1266.6516
1267.6587
1263.5634
1268.6737
1383.7509
1455.3756
1898.9613
1900.9900
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
m/z
Bu
O
N
O
tBu
N
N
N
tBu

Compound 2d:

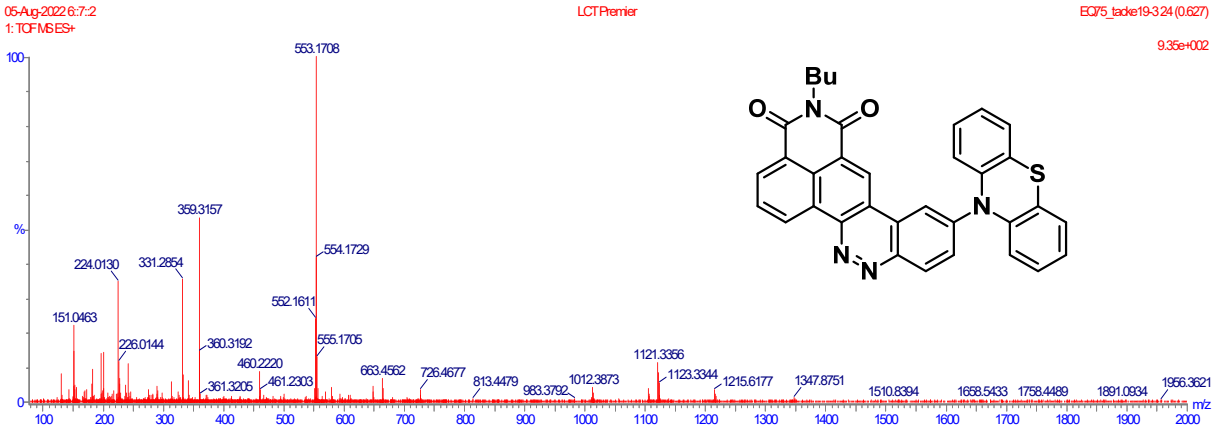
^{1}H NMR



^{13}C NMR



HRMS


05-Aug-2022 6:7:2
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LCT Premier
EQ75_tadke19-3 24 (0.627)
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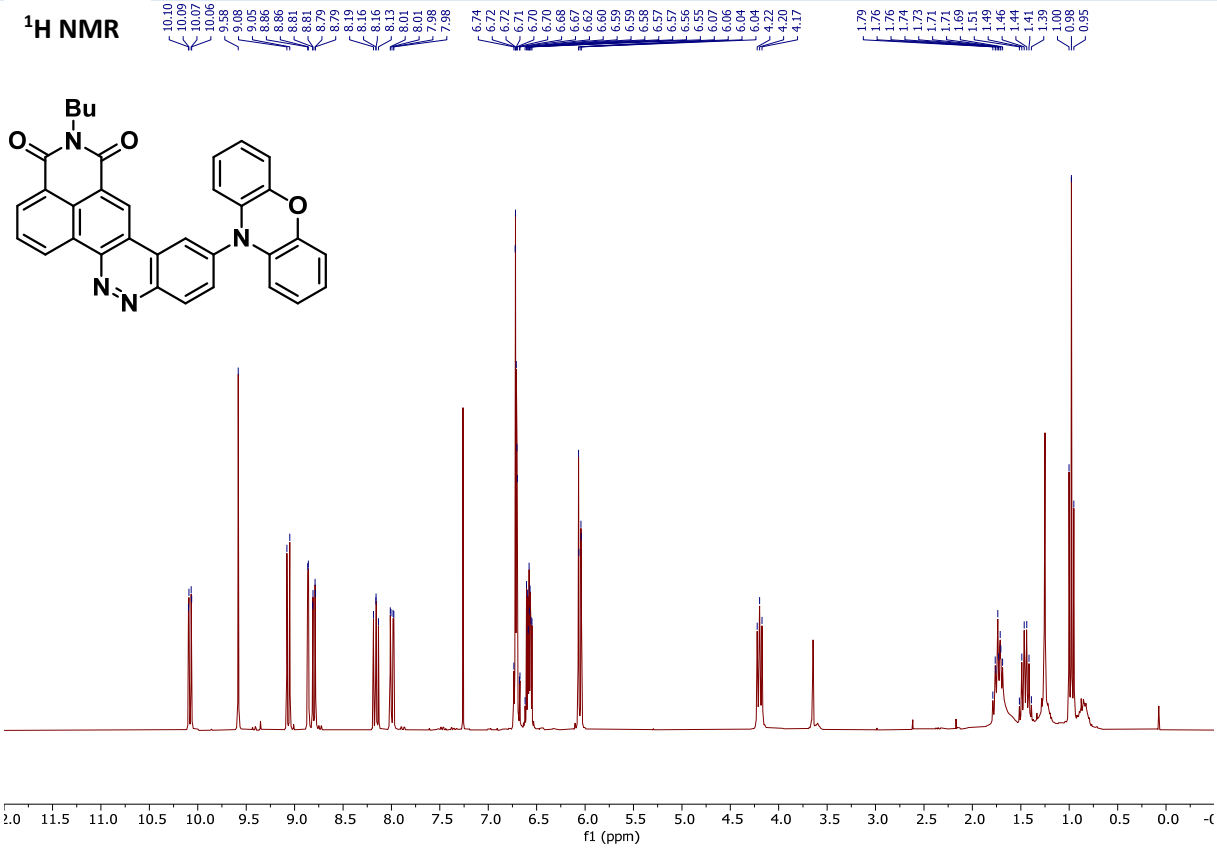
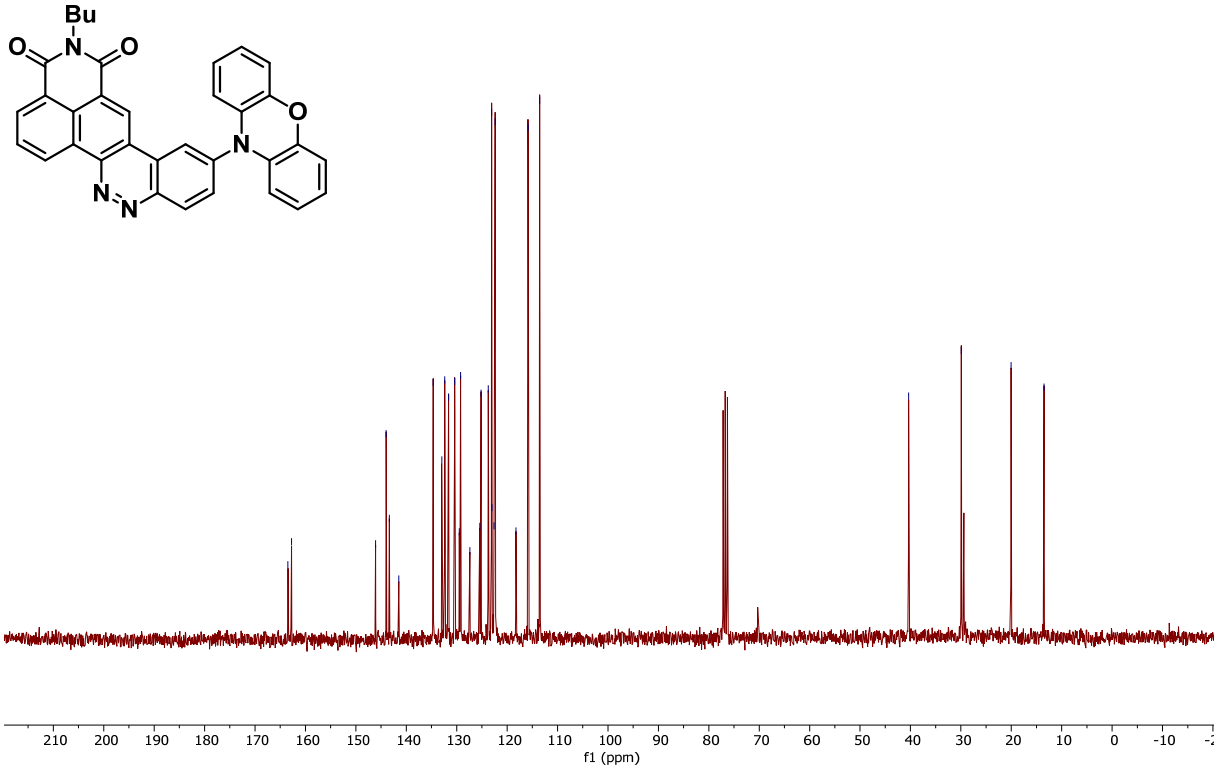
359.3157
554.1729
224.0130
331.2854
552.1611
151.0463
555.1705
226.0144
360.3192
460.2220
361.3205
461.2303
663.4562
726.4677
813.4479
983.3792
1012.3873
1121.3356
1123.3344
1215.6177
1347.8751
1510.8394
1658.5433
1758.4489
1891.0934
1956.3621
Bu
O
N
O
S
N
N
N
%
m/z

Compound 2e:

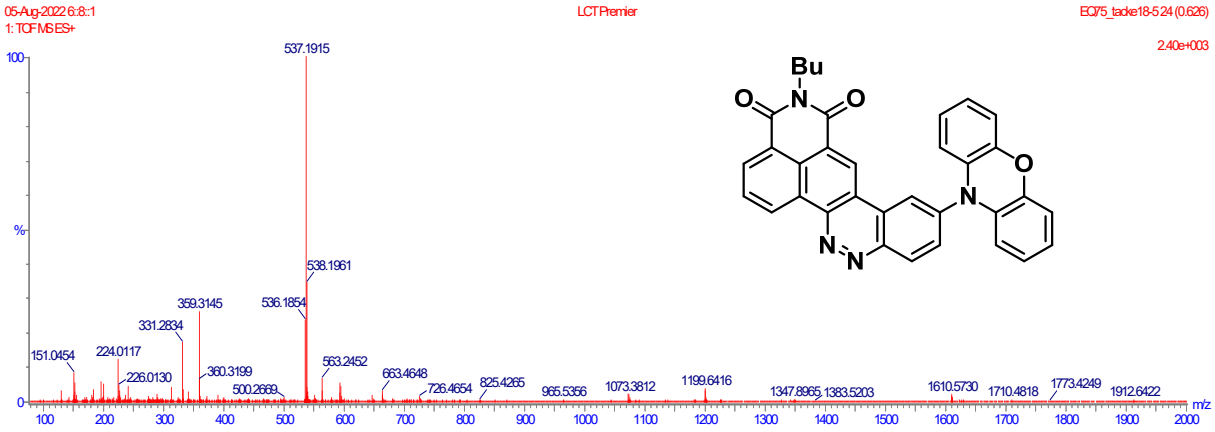
^{1}H NMR



^{13}C NMR



HRMS


05-Aug-2022 6:8::1
1: TOF MS ES+
LCT Premier
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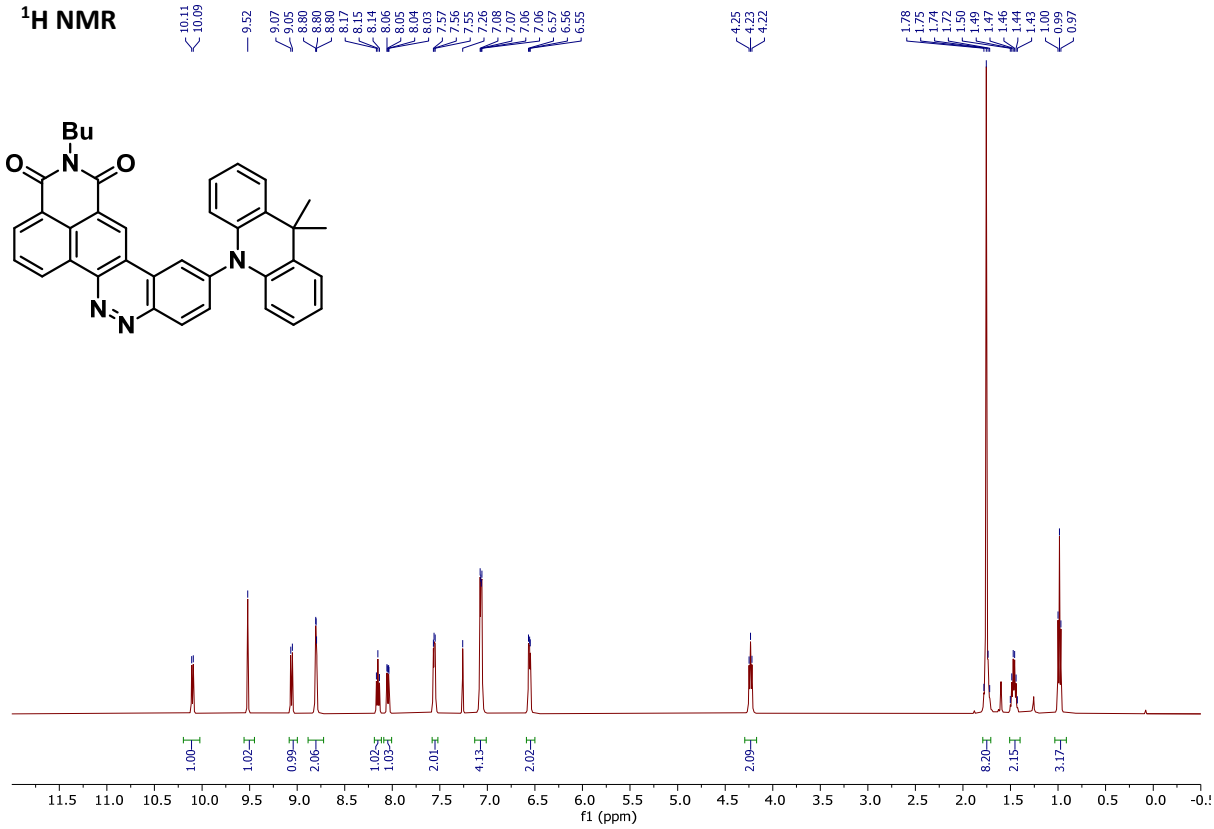
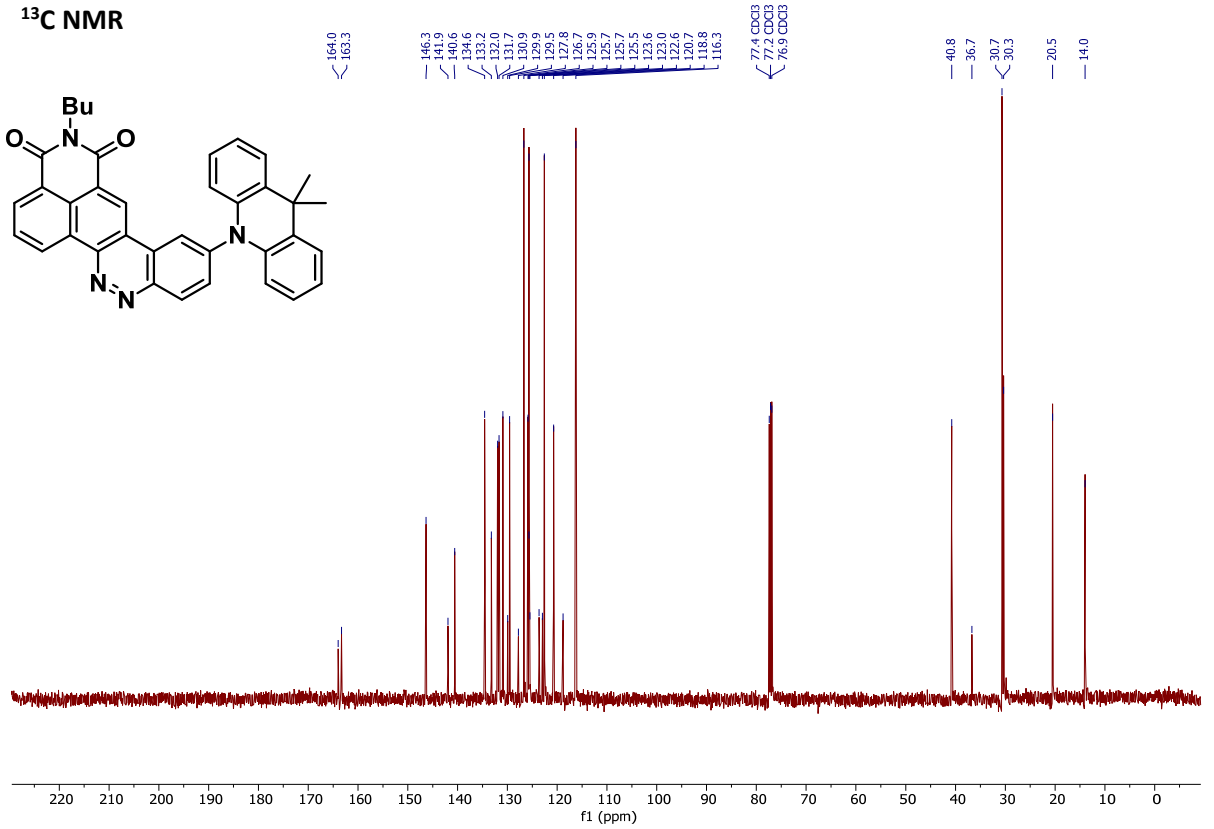
2.40e+003
100
%
0
537.1915
538.1961
536.1854
359.3145
331.2834
151.0454
224.0117
226.0130
360.3199
500.2669
563.2452
663.4648
726.4654
825.4265
965.5356
1073.3812
1199.6416
1347.8965
1383.5203
1610.5730
1710.4818
1773.4249
1912.6422
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
m/z
Bu
O
N
O
N
N
O

Compound 2f:

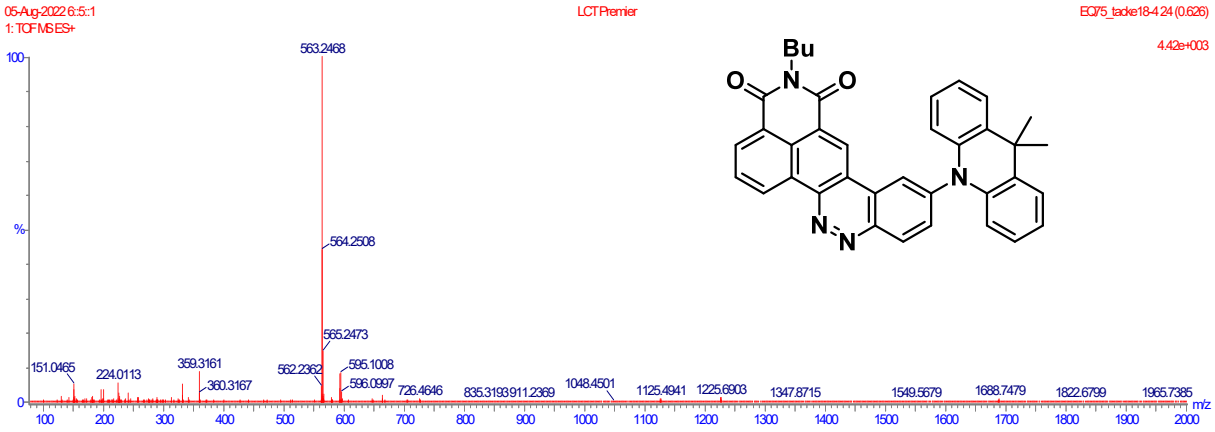
^{1}H NMR



^{13}C NMR



HRMS


05-Aug-2022 6::5::1
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151.0465
224.0113
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360.3167
562.2362
595.1008
596.0997
726.4646
835.3193
911.2369
1048.4501
1125.4941
1225.6903
1347.8715
1549.5679
1688.7479
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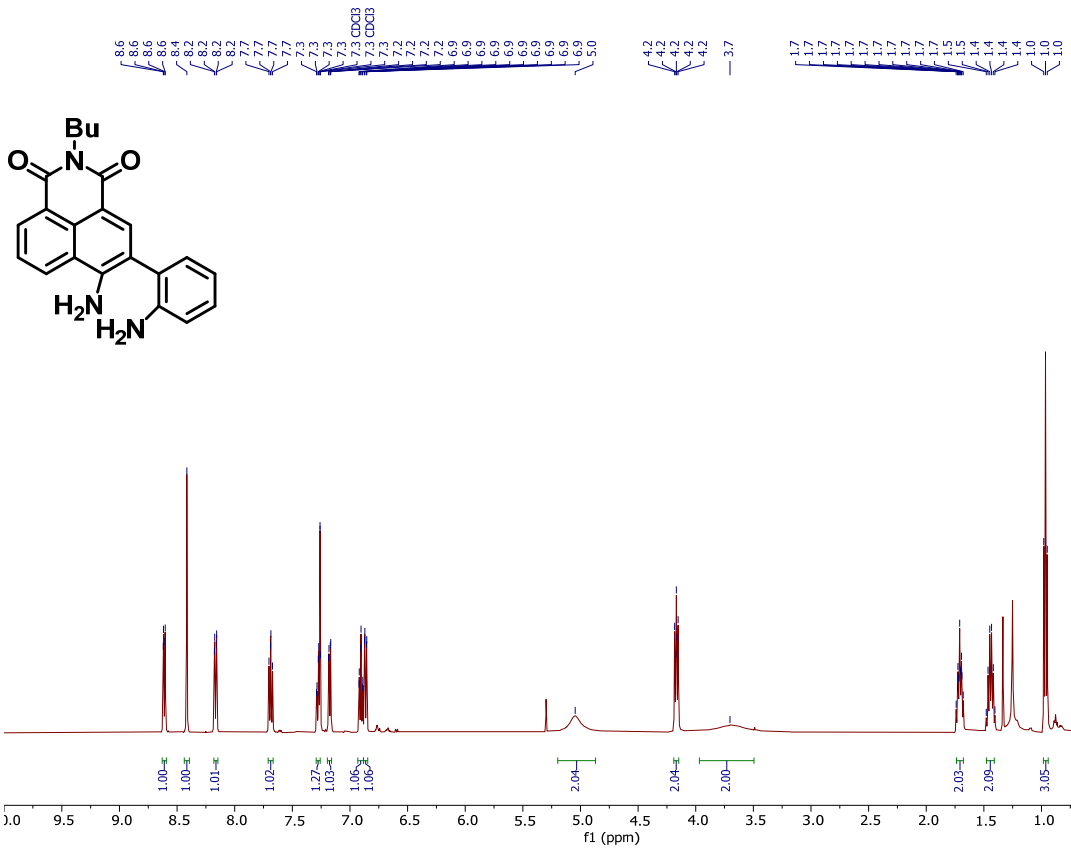
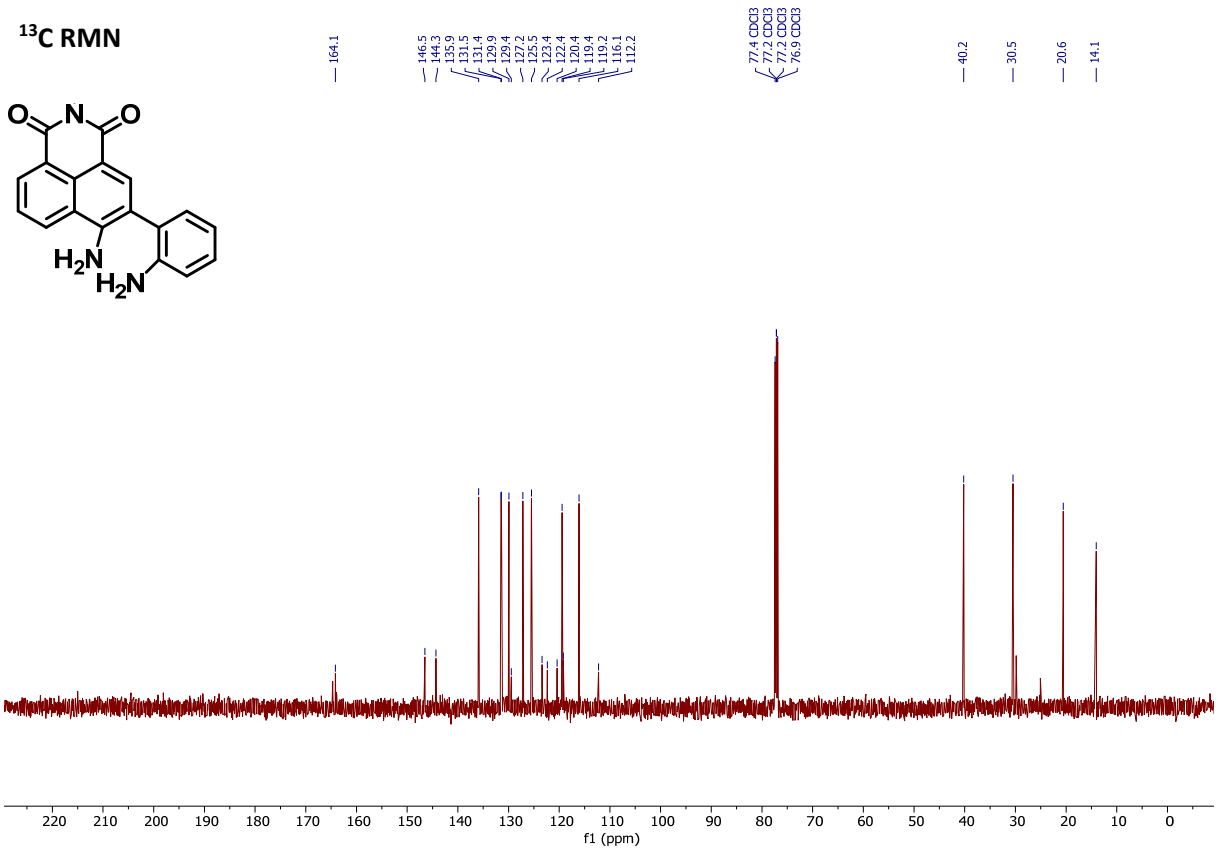
1965.7385
Bu
O
N
O
N
N
N
m/z

Compound 3a

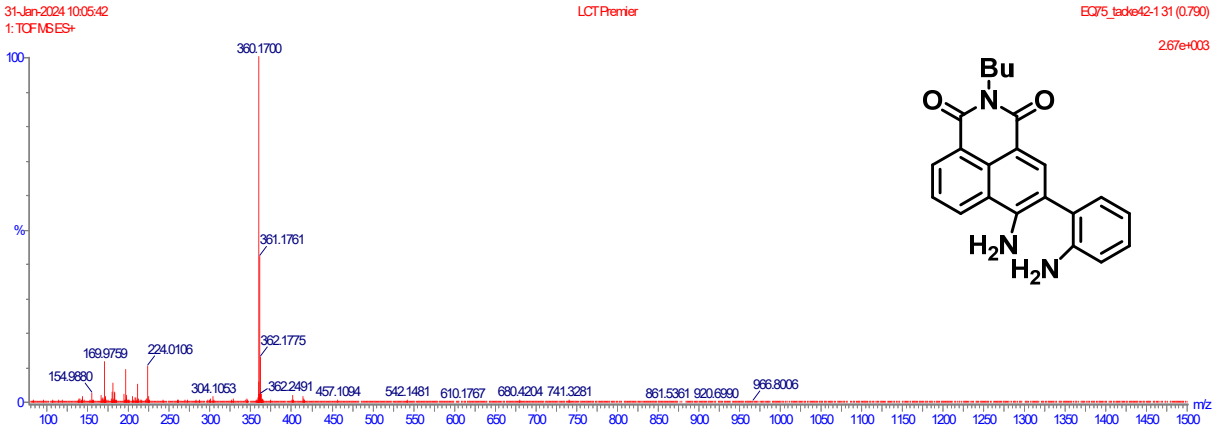
^{1}H RMN



^{13}C RMN



HRMS


31-Jan-2024 10:05:42
1: TOF MS ES+
LCT Premier
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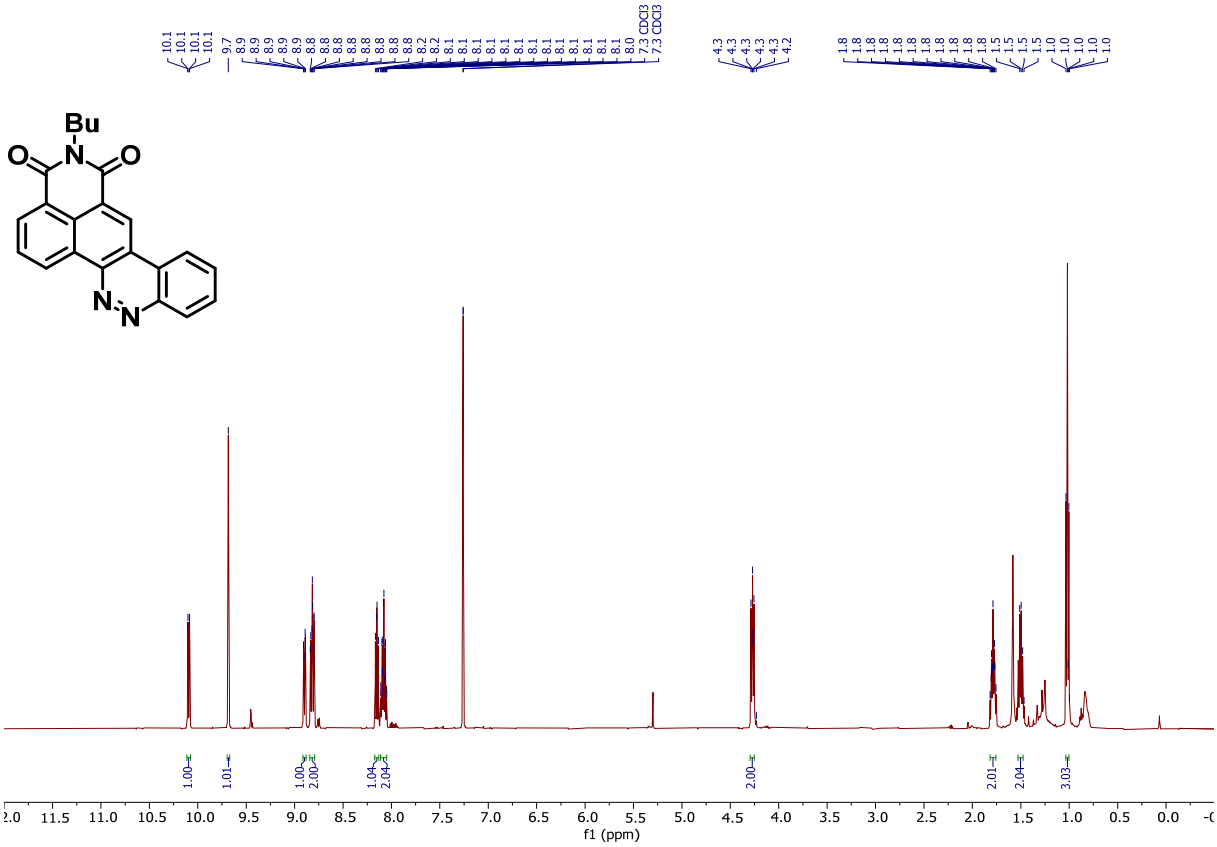
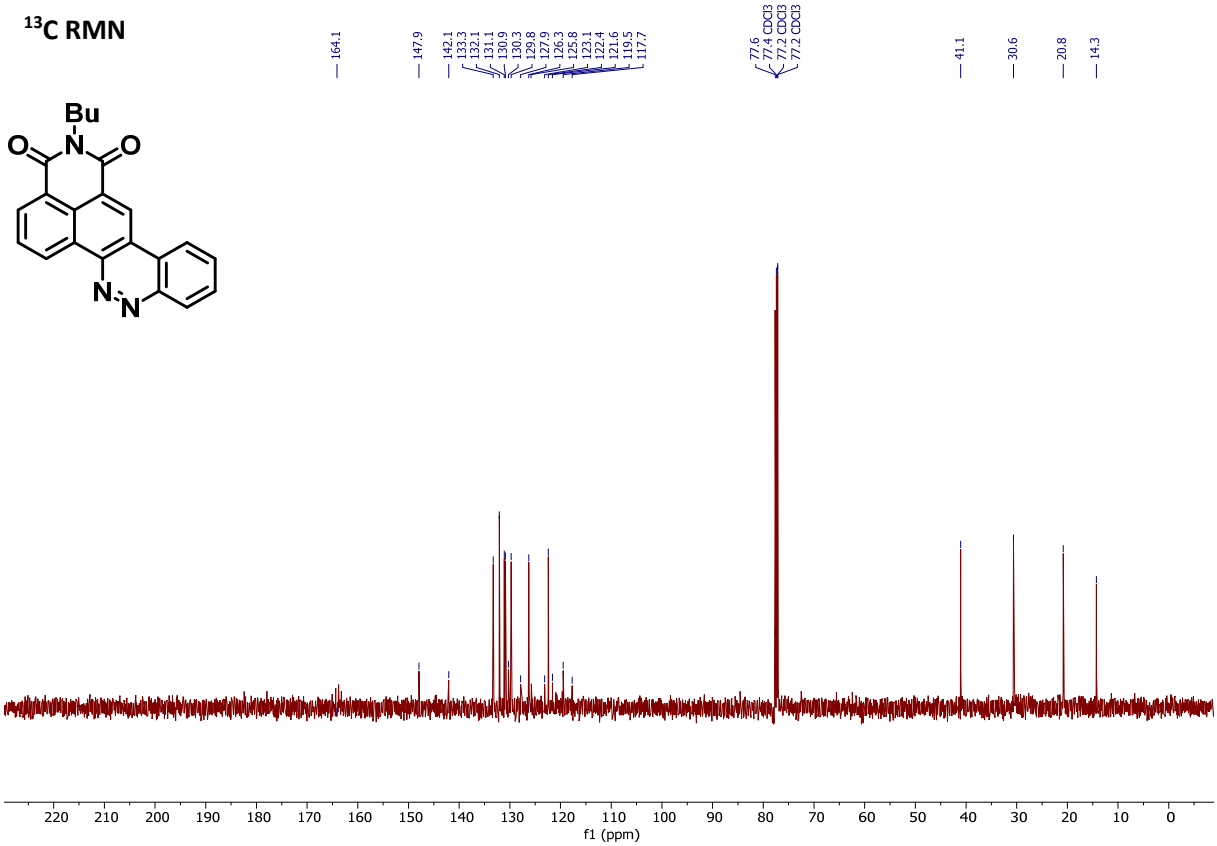
100
%
0
360.1700
361.1761
362.1775
169.9759
224.0106
154.9880
304.1053
362.2491
457.1094
542.1481
610.1767
680.4204
741.3281
861.5361
920.6990
966.8006
100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 950 1000 1050 1100 1150 1200 1250 1300 1350 1400 1450 1500
m/z
Bu
O
N
O
H2N
H2N

Compound 3:

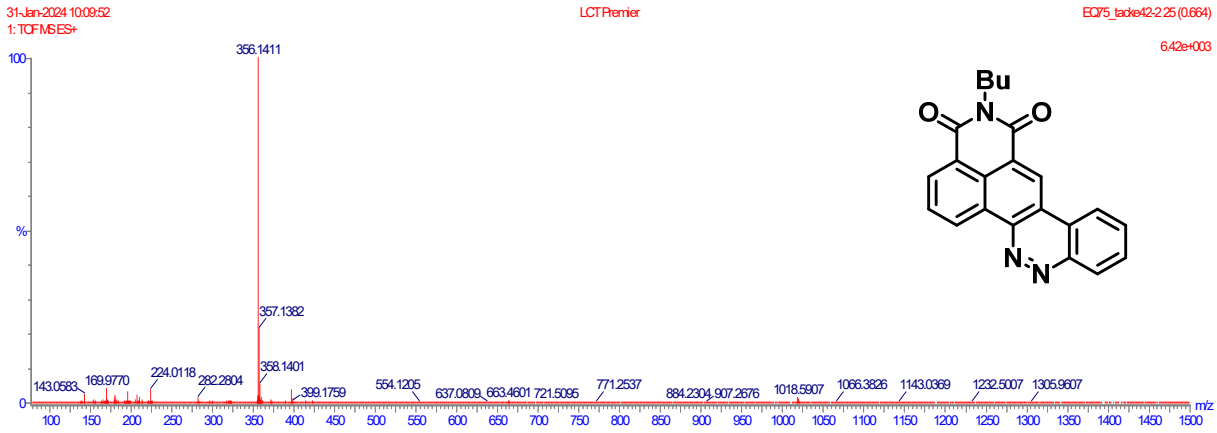
^{1}H RMN



^{13}C RMN


164.1
147.9
142.1
133.3
132.1
131.1
130.9
130.3
129.8
127.9
126.3
125.8
123.1
122.4
121.6
119.5
117.7
77.6
77.4 CDCl3
77.2 CDCl3
77.2 CDCl3
41.1
30.6
20.8
14.3
Bu
O
N
O
N
N
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

HRMS


31-Jan-2024 10:09:52
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LCT Premier
EQ75_tacke42-2 25 (0.664)
6.42e+003
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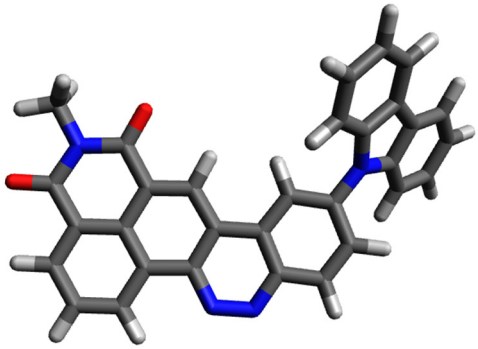
357.1382
358.1401
143.0583
169.9770
224.0118
282.2804
399.1759
554.1205
637.0809
663.4601
721.5095
771.2537
884.2304
907.2676
1018.5907
1066.3826
1143.0369
1232.5007
1305.9607
100
%
0
m/z
100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 950 1000 1050 1100 1150 1200 1250 1300 1350 1400 1450 1500
Bu
O
N
O
N
N

Theoretical calculations:

The ground state geometry of compound **2b'** (for the sake of simplification, the ethyl group was substituted by a methyl) was initially optimized using the B3LYP-D3(BJ)/6-311G(d,p) level of calculation, followed by a frequency calculation to verify convergence to a local minimum. Subsequently, TD-DFT calculations were conducted at the PBE1PBE/6-311G(d,p) level of theory, incorporating an IEF-PCM solvation model for $CHCl_3$. The absorption energies and oscillator strengths of the first 24 vertical excitations were computed and utilized to simulate the UV spectra (as depicted below). As anticipated:

1) The frontier orbital distributions for 2b' tend to affirm the charge transfer character of the first excited state, with the HOMO coefficients situated on the electron-donor moiety (in this case, the carbazole), while the LUMO coefficients are primarily located on the naphthalimide substructure.
2) The first excitation predominantly entails a HOMO–LUMO π–π* transition, contributing 99% to the overall process.

Cartesian coordinates of the optimized geometrie of 2b' (in Å):



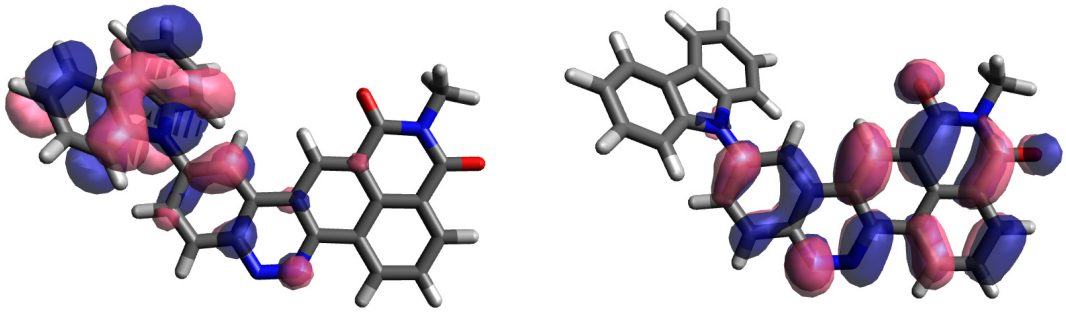
C	6.40147	-0.86900	0.03138
C	6.05311	-2.19516	-0.26352
C	4.72840	-2.56500	-0.38977
C	3.70410	-1.61047	-0.22247
C	4.05705	-0.27092	0.07490
C	5.41527	0.08716	0.19928
C	2.30695	-1.94323	-0.34228
C	1.32314	-0.95281	-0.16085
C	1.71666	0.37959	0.13241

C	3.03917	0.71054	0.24584
N	2.00768	-3.25170	-0.63966
N	0.78661	-3.63578	-0.76484
C	-0.24330	-2.74192	-0.59929
C	-0.04072	-1.37272	-0.29372
C	-1.55608	-3.24177	-0.75110
C	-2.63483	-2.40888	-0.60683
C	-2.43798	-1.04175	-0.28559
C	-1.16020	-0.53679	-0.12658
C	3.40123	2.11895	0.55077
N	4.76091	2.41000	0.66542
C	5.80678	1.48333	0.50946
O	6.96776	1.82355	0.62493
O	2.56685	2.99236	0.69769
C	5.10168	3.80414	0.97055
N	-3.55496	-0.19779	-0.14700
C	-3.71283	1.06183	-0.74784
C	-4.97343	1.58610	-0.37429
C	-5.60086	0.60949	0.49158
C	-4.70150	-0.47683	0.61258
C	-6.81546	0.57839	1.17833
C	-7.11263	-0.51996	1.97573
C	-6.20109	-1.57705	2.10255
C	-4.98310	-1.56972	1.42999
C	-2.86856	1.74525	-1.62048
C	-3.29439	2.98084	-2.09739
C	-4.53142	3.52268	-1.72248
C	-5.37725	2.82805	-0.86691
H	7.43589	-0.56713	0.13276
H	6.83300	-2.93556	-0.39335
H	4.44557	-3.58351	-0.61723

H	0.98499	1.16433	0.27077
H	-1.66904	-4.28877	-1.00185
H	-3.64222	-2.77341	-0.75611
H	-1.03679	0.50130	0.14678
H	4.72077	4.45620	0.18439
H	4.63943	4.09677	1.91328
H	6.18240	3.87324	1.03520
H	-7.51268	1.40390	1.09514
H	-8.05229	-0.55794	2.51323
H	-6.44394	-2.41699	2.74249
H	-4.27900	-2.38159	1.55324
H	-1.91777	1.33218	-1.92890
H	-2.65313	3.53274	-2.77420
H	-4.83017	4.48927	-2.10939
H	-6.33987	3.24067	-0.58829

FMOs distributions for 2b'

Isosurfaces (isovalue 0.02) of HOMO (left) and LUMO (right) calculated for compound **2b'**.

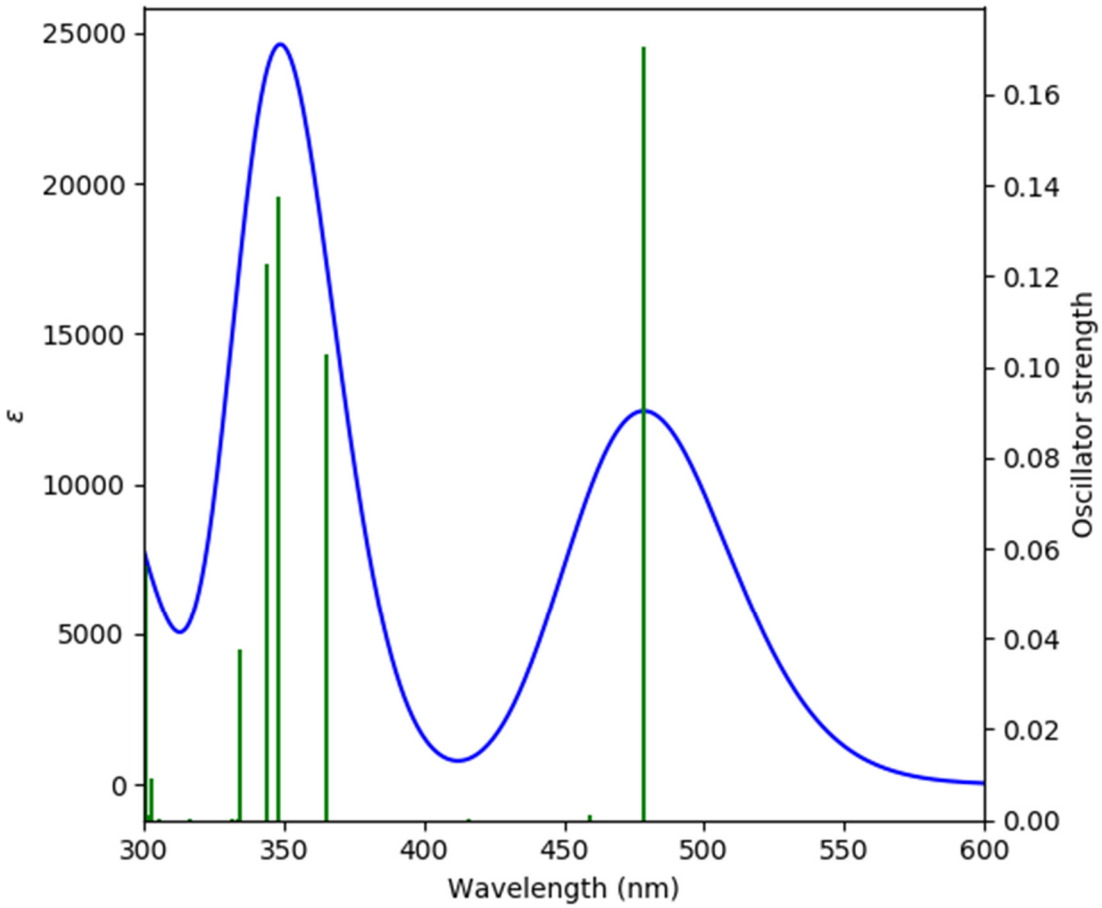


Simulated UV data (excitation number, energy, wavelength, oscilator strenghs and transition involved)

No.	Energy (cm-1)	Wavelength (nm)	Osc. Strength	Major contribs
1	20891.37282	478.666485261	0.1707	HOMO->LUMO (99%)
2	21791.4875627	458.894785003	0.0013	H-2->LUMO (89%)
3	24042.5809741	415.92872291	0.0001	H-1->LUMO (99%)
4	27386.5556367	365.142668273	0.1027	H-3->LUMO (59%), HOMO->L+1 (23%)
5	28717.3704445	348.221297605	0.1376	H-3->LUMO (13%), HOMO->L+1 (71%)

6	29060.9626313	344.104224174	0.1226	H-4->LUMO (39%), H-3->LUMO (22%), HOMO->L+2 (29%)
7	29941.7200678	333.982148566	0.0377	H-4->LUMO (36%), HOMO->L+2 (55%)
8	29988.5002246	333.461157613	0.0004	H-6->LUMO (18%), H-2->L+1 (69%)
9	30161.9094269	331.543996717	0.0005	H-6->LUMO (69%), H-2->L+1 (17%)
10	31594.3500928	316.512286869	0.0001	H-1->L+1 (96%)
11	32766.2736781	305.191859722	0.0005	H-5->LUMO (93%)
12	33083.2495687	302.267767839	0.0091	H-7->LUMO (85%)
13	33172.7771103	301.451999835	0.001	H-1->L+2 (94%)
14	33299.4061557	300.3056557	0.0562	HOMO->L+3 (89%)
15	33841.410732	295.495955508	0.0002	H-2->L+2 (86%)
16	34218.0716503	292.243236328	0.0	H-10->LUMO (75%)
17	34804.4367202	287.319690889	0.0306	H-8->LUMO (62%), H-3->L+1 (23%)
18	35346.4412965	282.913912496	0.0543	H-8->LUMO (22%), H-3->L+1 (50%)
19	36204.615209	276.207879639	0.0359	H-4->L+1 (61%)
20	37240.2310958	268.526797653	0.2918	H-3->L+2 (11%), HOMO->L+4 (72%)
21	37295.8833514	268.126106728	0.1648	H-1->L+3 (69%), HOMO->L+6 (18%)
22	38245.1979143	261.470734768	0.5088	H-9->LUMO (19%), H-4->L+1 (16%), H-3->L+2 (33%), HOMO->L+4 (11%)
23	38651.7013465	258.720823447	0.1207	H-11->LUMO (11%), H-9->LUMO (52%), H-3->L+2 (20%)
24	39555.8488614	252.80711419	0.0103	H-11->LUMO (54%), H-9->LUMO (27%)

Simulated UV spectra of 2b’



1. E. Tacke, M.-D. Hoang, K. Tatoueix, B. Keromnes, E. Van Eslande, P. Durand, G. Pieters and A. Chevalier, *Chem. Sci.*, 2023, **14**, 6000-6010.
2. G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg, *Organometallics*, 2010, **29**, 2176-2179.