Supplementary Information for

Fluorescent Half-Sandwich Iridium Picolinamidate Complexes for In-Cell Visualization

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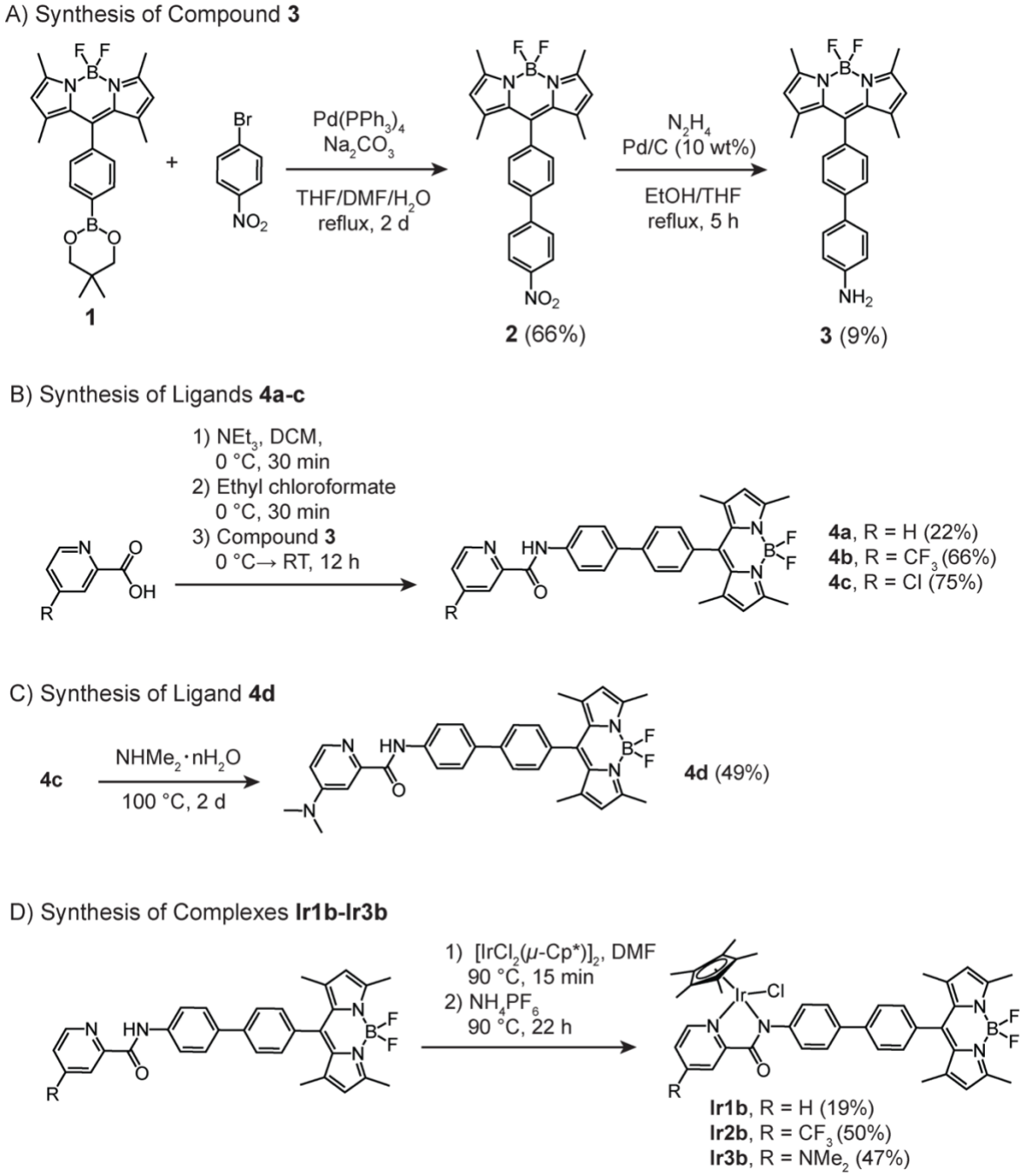
General Procedures

Commercial reagents were used as received without further purification. All air- and water-sensitive manipulations were performed using standard Schlenk techniques or under a nitrogen atmosphere using a glovebox. Anhydrous solvents were obtained from an Innovative Technology solvent drying system saturated with Argon. Iridium complexes **Ir1a**, **Ir2a**, and **Ir3a** were prepared as previously described.[1] NIH-3T3 mouse fibroblast cells was obtained from ATCC.

Physical Methods

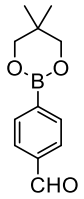
NMR spectra were acquired using JEOL spectrometers (ECA-400, 500, and 600) at room temperature and referenced using residual solvent peaks. A Horiba Fluoromax 4 fluorometer was used to record the emission spectra. Gas chromatography mass spectrometry was performed using an Agilent 7890 GC/5977A MSD instrument equipped with an HP-5MS capillary column. A Varian 810 instrument was used to acquire inductively coupled plasma-mass spectrometry (ICP–MS) analyses. All biological cell images were obtained using Olympus IX-83 inverted microscope equipped with a 100× oil objective.

Synthesis and Characterization



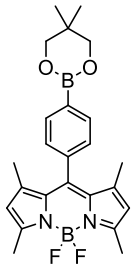
Scheme S1. Synthetic routes for the preparation of complexes **Ir1b**, **Ir2b**, and **Ir3b**.

Preparation of Compound 1:



To a 100 mL round bottom flask, solid phenylboronic acid (750 mg, 5 mmol, 1 equiv.) and solid 2,2-dimethyl-1,3-propanediol (521 mg, 5 mmol, 1 equiv.) was dissolved in 20 mL of dry toluene. The reaction was refluxed for 3 h. After cooling to room temperature, the solvent was then removed by rotary evaporation. Quantitative amount of the crude products was collected as a white solid and was used for the next step without any further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 10.03 (s, 2H), 7.95 (d, *J* = 8.4 Hz, 2H), 7.83 (d, *J* = 9.6 Hz, 2H), 3.78 (s, 4H), 1.02 (s, 6H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 192.91, 137.86, 134.42, 128.73, 125.39, 72.48, 31.96, 21.93 ppm. GC-MS: calc. for $C_{12}H_{15}BO_3$ $[M]^+$ = 218.0, found 218.1. These data match those reported in the literature.[2]

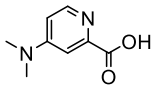
Preparation of Compound 2:



To a 250 mL round bottom flask, compound **1** (654 mg, 3 mmol, 1 equiv.) was dissolved in 100 mL of dry DCM. The reaction mixture was degassed under N_2 for 20 min. 4 drops of TFA were added to the mixture, followed by addition of 2,4-dimethylpyrrole (640 µL, 6.15 mmol, 2.05 equiv.) to the solution via a Luer-lock syringe. The reaction mixture was stirred for 12 h under N_2 in an

ice bath to obtain a red-colored solution. Solid DDQ (1396 mg, 6.15 mmol, 2.05 equiv.) was then added to the solution, giving a dark brown slurry. The mixture was stirred for another 30 min, followed by the addition of triethylamine (6 mL) and $BF_3.Et_2O$ (6 mL) in sequence, and the reaction was stirred for additional 1 h. Water was added to quench the reaction after completion. The mixture was then filtered through a basic alumina plug to remove a dark brown solid using DCM as eluent. Once collected, the brown solute was then concentrated in vacuo until ~ 50 mL of solvent was left. After being washed with an excess amount of water, the organic phase was combined, separated from the aqueous layer, dried over Na_2SO_4, filtered, and evaporated to dryness to give a brown-colored oil. The crude product was purified by silica gel column chromatography using hexanes/DCM as the eluent (10:1 to 1:1, *v/v*), to yield a red solid (385 mg, 30%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.89 (d, *J* = 7.5 Hz, 2H), 7.26 (d, *J* = 6.9 Hz, 2H), 5.95 (s, 2H), 3.79 (s, 4H), 2.54 (s, 6H), 1.35 (s, 6H), 1.05 (s, 6H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 155.44, 143.39, 142.13, 137.31, 134.59, 131.39, 127.24, 121.25, 72.52, 46.94, 32.05, 22.09, 14.56, 8.76 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = -150.76 ppm. CI–MS: calc. for $C_{24}H_{28}B_2N_2O_2F_2$ $[M]^+$ = 436.2305, found 436.2311. These data match those reported in the literature.[3]

Preparation of Compound 3:

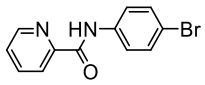


In a 100 mL pressurized tube, solid 4-chloropicolinic acid (945 mg, 6 mmol) and 10 mL of dimethylamine (40% in water) were combined. The tube was then sealed with a PTFE plug equipped with an O-ring and was stirred at 150 °C for 2 d. After cooling to room temperature, the solvent was removed in vacuo to obtain an orange-colored liquid as the crude product. For purification, the mixture was diluted with Et_2O and cooled to -20 °C overnight to precipitate out

the desired product. The precipitated solid was isolated by filtration, rinsed with cold DCM:MeOH (10:1, *v/v*) until it was colorless, and then dried under vacuum to yield the final product as an off-white solid (810 mg, 91%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.38 (s, 1H), 8.02 (d, *J* = 7.5 Hz, 1H), 7.21 (s, 1H), 6.93 (s, 1H), 3.17 (s, 6H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ = 161.04, 157.76, 146.48, 138.64, 107.00, 105.71, 34.00 ppm. ESI–MS(+): calc. for $C_8H_{10}N_2O_2$ $[M+H]^+$ = 167.0815, found 167.0816. These data match those reported in the literature.[4]

General Procedure for the Synthesis of 4a, 4b, and 4c: In a 50 mL round bottom flask, solid picolinic acid (or its derivative) (1.0 equiv.), solid EDC·HCl (1.5 equiv.), solid DMAP (1.5 equiv.), solid HOBt (1.5 equiv.) were combined in 10 mL of anhydrous DMF under N_2. The reaction flask was stirred at RT for 15 min, giving a brown-colored solution. Solid 4-bromoaniline (1 equiv.) was added to the flask, and the reaction mixture was stirred at 50 °C under N_2 for 24 h. When the reaction was complete, excess amounts of water were added to the mixture, followed by the addition of ethyl acetate. The combined organic layer was separated, dried over Na_2SO_4, filtered, and removed in vacuo. The crude mixture was purified by silica gel chromatography using hexanes: ethyl acetate as the eluent.

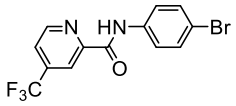
Compound 4a:



Purified using 20% ethyl acetate in hexanes as eluent, 2 mmol scale, white solid, 240 mg, 43%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 10.03 (s, 1H), 8.59 (d, *J* = 4.8 Hz, 1H), 8.27 (d, *J* = 7.7 Hz, 1H), 7.89 (t, *J* = 7.7 Hz, 1H), 7.68 (d, *J* = 8.1 Hz, 2H), 7.48 (t, *J* = 6.0 Hz, 3H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 162.12, 149.56, 148.10, 137.88, 136.95, 132.14, 126.75, 122.55, 121.31, 116.95

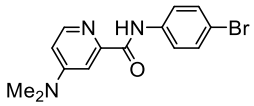
ppm. GC-MS: calc. for $C_{12}H_9BrN_2O$ $[M]^+$ = 275.9, found 276.0. MP: 144.2 – 145.8 ˚C. These data match those reported in the literature.[5]

Compound 4b:



Purified using 10% ethyl acetate in hexanes as eluent, 2 mmol scale, fluffy white solid, 400 mg, 58%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.92 (s, 1H), 8.80 (d, *J* = 5.0 Hz, 1H), 8.51 (s, 1H), 7.72 (d, *J* = 4.9 Hz, 1H), 7.67 (d, *J* = 7.0 Hz, 2H), 7.49 (d, *J* = 6.7 Hz, 1H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 160.67, 151.07, 149.25, 140.62, 140.34, 136.48, 132.28, 123.61, 122.38, 121.41, 118.68, 118.65, 117.50 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = -64.67 ppm. GC-MS: calc. for $C_{13}H_8BrF_3N_2O$ $[M]^+$ = 343.9, found 344.0. IR: *v* = 497, 614, 667, 820, 869, 1131, 1169, 1335, 1525, 1686, 3342 cm^{-1}. MP: 141.7 – 142.6 ˚C.

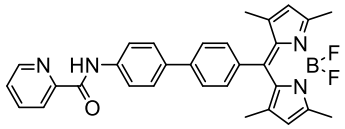
Compound 4c:



Purified using 40% ethyl acetate in hexanes as eluent, 2 mmol scale, off-white solid, 387 mg, 61%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.18 (s, 1H), 8.16 (d, *J* = 5.9 Hz, 1H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.50 (d, *J* = 2.8 Hz, 1H), 7.45 (d, *J* = 8.8 Hz, 2H), 6.56 (dd, *J* = 5.9, 2.8 Hz, 1H), 3.06 (s, 6H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.13, 155.48, 149.59, 148.01, 137.22, 132.04, 121.22, 116.59, 108.45, 105.30, 39.45 ppm. GC-MS: calc. for $C_{14}H_{14}BrN_3O$ $[M]^+$ = 319.0, found 319.0. IR: *v* = 507, 703, 713, 716, 814, 824, 834, 995, 1070, 1225, 1390, 1514, 1676, 3300 cm^{-1}. MP: 222.5 – 224.8 ˚C.

General Procedure for the Synthesis of 5a, 5b, and 5c: In a 50 mL round bottom flask, compound **2** (1.1 equiv.), compound **4** (1 equiv.), Cs_2CO_3 (2 equiv.) were combined in 10 mL of dry THF/PhMe/EtOH (2:2:1). The solvent was degassed under N_2 for at least 30 min before use. The reaction mixture was stirred under N_2 for 10 min. Solutions containing $Pd(OAc)_2$ (0.2 equiv.) and PPh_3 (0.6 equiv.) were prepared in two separate 20 mL screw capped vials using degassed THF/PhMe/EtOH (2:2:1, 2 mL each). These two solutions were sonicated for 5 min and then added into the reaction flask via syringe. The reaction mixture was stirred continuously at 80 °C under N_2 for 2 d to obtain a dark red colored solution. After the reaction was complete, the solvent was removed in vacuo, and the crude product was dissolved into DCM and washed with water. The organic layer was separated, dried over Na_2SO_4, filtered, evaporated to dryness, and purified by silica gel column chromatography, using a hexanes/DCM gradient mixture as eluent, to obtain the final product as red solid.

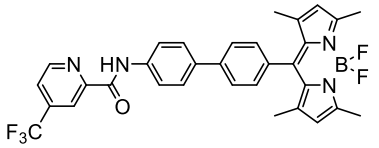
Compound 5a:



Purified using hexanes/DCM (2:3, *v/v*) as eluent, 0.18 mmol scale, red solid, 35 mg, 37%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 10.14 (s, 1H), 8.63 (d, *J* = 5 Hz, 1H), 8.32 (d, *J* = 5 Hz, 1H), 7.95-7.91 (m, 3H), 7.76-7.71 (m, 4H), 7.52-7.50 (m, 1H), 7.33 (d, *J* = 10 Hz, 2H), 5.99 (s, 2H), 2.56 (s, 6H), 1.45 (s, 6H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 162.16, 155.56, 149.77, 148.11, 143.26, 141.56, 141.11, 137.89, 137.68, 135.89, 133.79, 131.56, 128.59, 127.74, 127.38, 126.70, 122.56, 121.32, 120.11, 14.72, 1.13 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = -146.12 (m) ppm. ^{11}B NMR (160 MHz, $CDCl_3$): δ = -0.18 (t, *J* = 33.1 Hz) ppm. ESI–MS(+): calc. for $C_{31}H_{27}BF_2N_4O$ $[M+H]^+$ = 521.2324,

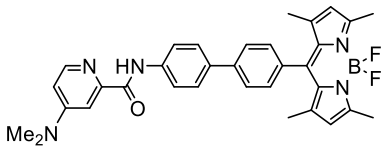
found 521.2246. IR: ν = 415, 803, 813, 1017, 1261, 1507, 1538, 1688, 3319 cm^{-1}. MP: 289.3 – 291.7 °C (decomposed).

Compound 5b:



Purified using hexanes/Et_2O/DCM as eluent (gradient from 7:1:1 to 5:1:1, *v/v*), 0.18 mmol scale, red solid, 30 mg, 32%. ^{1}H NMR (600 MHz, $CDCl_3$): 10.02 (s, 1H), 8.83 (d, J = 6 Hz, 1H), 8.56 (s, 1H), 7.90 (d, J = 6 Hz, 2H), 7.75-7.72 (m, 5H), 7.34 (d, J = 6 Hz, 2H), 5.98 (s, 2H), 2.55 (s, 6H), 1.44 (s, 6H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): 160.68, 155.59, 151.29, 149.21, 143.22, 141.55, 140.97, 137.19, 136.37, 133.93, 131.54, 128.64, 127.82, 127.40, 122.26, 121.33, 120.25, 118.67, 29.80, 14.70 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): -64.66 (s), -146.15 (m) ppm. ^{11}B NMR (160 MHz, $CDCl_3$): -0.17 (t, J = 33.1 Hz) ppm. ESI–MS(+): calc. for $C_{32}H_{26}BF_5N_4O$ $[M+H]^+$ = 589.2198, found 589.2210. IR: ν = 399, 666, 974, 1059, 1143, 1185, 1326, 1507, 1540, 1683, 3340 cm^{-1}. MP: 271.4 – 273.7 °C (decomposed).

Compound 5c:

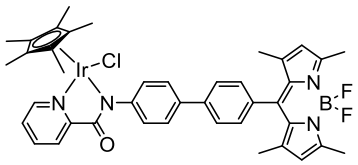


Purified using hexanes/DCM/MeOH as eluent (gradient from 7:4:0 to 1:9:0.1, *v/v*), 0.18 mmol scale, 38 mg, red solid, 37%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 10.18 (s, 1H), 8.20 (d, J = 5.9 Hz, 1H), 7.67 (d, J = 8.8 Hz, 2H), 7.54 (d, J = 2.8 Hz, 1H), 7.47 (d, J = 8.8 Hz, 2H), 7.09 (d, J = 8.4 Hz, 2H), 6.95 (d, J = 8.5 Hz, 2H), 6.60 (dd, J = 5.9, 2.7 Hz, 1H), 5.97 (s, 2H), 3.08 (s, 6H), 2.54

(s, 6H), 1.43 (s, 6H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 163.44, 156.74, 155.55, 155.34, 149.51, 148.12, 143.30, 141.99, 136.98, 132.11, 131.93, 129.42, 126.96, 121.41, 121.23, 116.93, 116.24, 108.55, 105.51, 39.42, 14.68 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = -145.84 (m) ppm. ^{11}B NMR (160 MHz, $CDCl_3$): δ = -0.17 (t, *J* = 33.4 Hz) ppm. ESI–MS(+): calc. for $C_{33}H_{32}BF_2N_5O$ $[M+H]^+$ = 564.2746, found 564.2702. IR: *ν* = 411, 473, 703, 762, 793, 968, 1059, 1151, 1182, 1258, 1302, 1435, 1504, 1522, 1585, 1608, 1610, 1661, 3268 cm^{-1}. MP: 213.1 – 215.0 °C.

General Procedure for the Synthesis of Ir1b, Ir2b, and Ir3b: In a 50 mL round bottom flask, 10 mL of dry DMF was purged with nitrogen for about 30 min. Solid $[IrCl_2(\mu\text{-}Cp^*)]_2$ (1.0 equiv.) and the corresponding picolinamide ligand (2.0 equiv.) were added and stirred for 15 min at 90 °C. The reaction mixture was then treated with ammonium hexafluorophosphate (4.7 equiv.) and stirred for an additional 22 h at 90 °C. After cooling to room temperature, the reaction mixture was diluted with 20 mL of dichloromethane and then washed with water (3×20 mL). The organic phase was separated, dried over Na_2SO_4, filtered, and then evaporated to dryness. The crude product was purified by silica gel column chromatography using hexanes:DCM as the eluent.

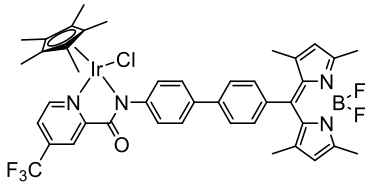
Complex Ir1b:



Purified using hexanes/DCM/MeOH as eluent (gradient from 7:3:0 to 1:9:0.1, *v/v*), 0.01 mmol scale, 5.2 mg, red solid, 59%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.59 (d, *J* = 5 Hz, 1H), 8.17 (d, *J* = 5 Hz, 1H), 7.94 (t, *J* = 5 Hz, 1H), 7.79-7.74 (m, 4H), 7.62 (d, *J* = 10 Hz, 2H), 7.51 (t, *J* = 5 Hz, 1H), 7.31 (d, *J* = 10 Hz, 2H), 5.99 (s, 2H), 2.56 (s, 6H) 1.47-1.45 (m, 21H) ppm. ^{19}F NMR (470

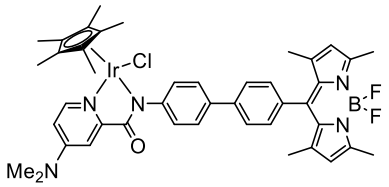
MHz, $CDCl_3$): δ = -146.16 (m) ppm. ^{11}B NMR (160 MHz, $CDCl_3$): δ = -0.15 ppm. ESI–MS(+): calc. for $C_{41}H_{41}BF_2IrN_4O$ $[M\text{-}Cl]^+$ = 847.2971, found 847.3002. IR: *v* = 400, 419, 838, 975, 1154, 1193, 1305, 1364, 1467, 1509, 1540, 1587, 1620 cm^{-1}. MP: 300 °C (decomposed). λ_{max} ($M^{-1}cm^{-1}$, tBuOH/H_2O 1:4) = 267 (27,100), 360 (sh; 9,000), and 500 (31,800) nm. λ_{em} (tBuOH/H_2O 1:4) = 511 nm; Φ = 0.04. *Note: A ^{13}C NMR spectrum of* ***Ir1b*** *could not be obtained due to its poor solubility in typical NMR solvents*.

Complex Ir2b:



Purified using hexanes/DCM as eluent (gradient from 3:1 to 1:2, *v/v*), 0.01 mmol scale, 4.8 mg, dark red solid, 50%. ^1H NMR (500 MHz, $CDCl_3$): δ = 8.76 (d, *J* = 5 Hz, 1H), 8.42 (s, 1H), 7.76-7.71 (m, 5H), 7.64 (d, *J* = 10 Hz, 2H), 7.32 (d, *J* = 10 Hz, 2H), 5.99 (s, 2H), 2.56 (s, 6H), 1.47 (s, 21H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 167.71, 157.39, 155.49, 150.74, 147.69, 143.32, 141.83, 141.67, 140.72, 140.44, 136.39, 133.54, 131.60, 128.47, 127.49, 127.33, 126.95, 123.01, 121.30, 87.34, 14.78, 8.71 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = -64.69 (s), -146.14 (m) ppm. ^{11}B NMR (160 MHz, $CDCl_3$): δ = -0.16 (t, *J* = 33.2 Hz) ppm. ESI–MS(+): calc. for $C_{42}H_{40}BF_5IrN_4O$ $[M\text{-}Cl]^+$ = 915.0477, found 915.2846. IR: *v* = 399, 477, 666, 972, 1048, 1141, 1185, 1304, 1326, 1405, 1464, 1508, 1541, 1681 cm^{-1}. MP: 113.4 – 115 °C. λ_{max} ($M^{-1}cm^{-1}$) = 271 (27,100), 332 (sh; 13,800), and 500 (57,600) nm. λ_{em} (tBuOH/H_2O 1:4) = 512 nm; Φ = 0.02.

Complex Ir3b:



Purified using hexanes/DCM as eluent (1:1, *v/v*), 0.01 mmol scale, 4.7 mg, dark red amorphous solid, 47%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08 (d, *J* = 8 Hz, 1H), 7.78-7.74 (m, 4H), 7.60 (d, *J* = 8 Hz, 2H), 7.34-7.30 (m, 3H), 6.60-6.57 (m, 1H), 5.99 (s, 2H), 3.14 (s, 6H), 2.56 (s, 6H), 1.47-1.44 (m, 21H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.67, 155.41, 155.23, 154.21, 148.73, 148.67, 143.39, 142.02, 135.50, 133.21, 131.64, 128.34, 127.71, 127.44, 126.57, 121.26, 109.45, 108.70, 85.87, 39.63, 14.80, 8.72, 1.12 ppm. ^{19}F NMR (470 MHz, $CDCl_3$): δ = -146.11 (m) ppm. ^{11}B NMR (160 MHz, $CDCl_3$): δ = -0.18 (t, *J* = 33.2 Hz) ppm. ESI–MS(+): calc. for $C_{43}H_{46}BF_2IrN_5O$ $[M\text{-}Cl]^+$ = 890.34, found 890.33. IR: *v* = 400, 419, 774, 791, 973, 1068, 1161, 1194, 1304, 1435, 1490, 1503, 1522, 1591, 1659 cm^{-1}. MP: 280 °C (decomposed). λ_{max} ($M^{-1}cm^{-1}$) = 276 (37,800), 343 (s; 18,600), and 500 (41,700) nm. λ_{em} (tBuOH/H_2O 1:4) = 512 nm; Φ = 0.02.

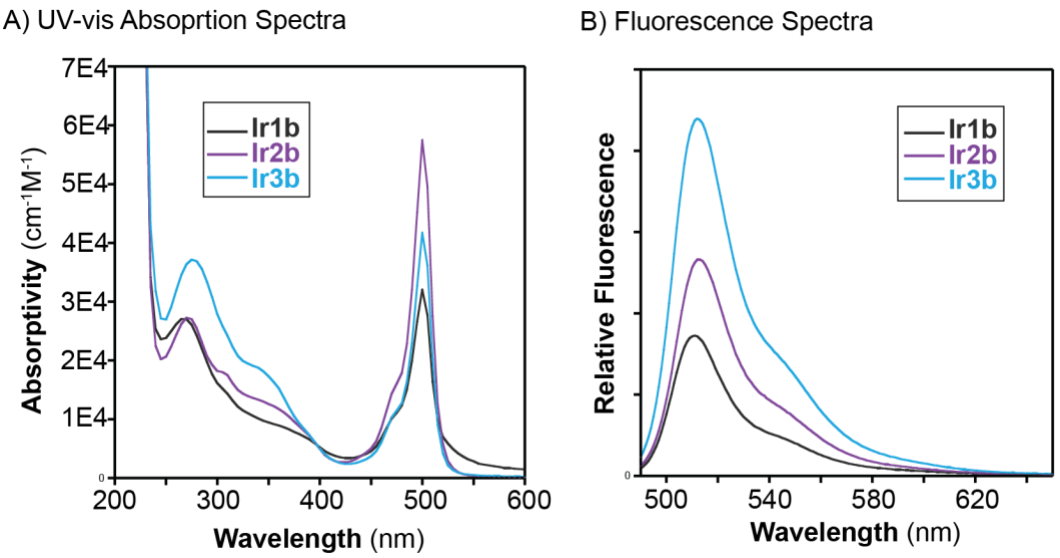
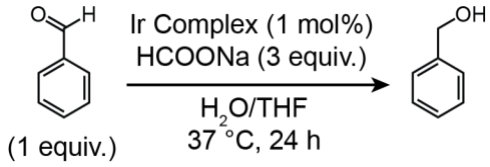


Figure S1. UV-visible absorption (left) and emission spectra of the iridium complexes **Ir1b** (black line), **Ir2b**, (purple line), and **Ir3b** (blue line). The complexes were dissolved in tBuOH/H_2O (1:4) at a concentration of 10 µM.

Table S1. Transfer Hydrogenation Yields Obtained Using Different Ir Complexes[a]



Complex	Yield (%)[e]
Ir1a[b]	85±5
Ir2a[c]	4±1
Ir3a[d]	95±5
Ir1b[b]	68±2
Ir2b[c]	5±1
Ir3b[d]	95±5

[a]Reaction conditions used: benzaldehyde (60 μmol), HCOONa (180 μmol), Ir complex (0.60 μmol), H_2O/THF, 37 °C, 24 h. Slightly different solvent amounts were used due to differences in the catalyst solubility. [b]Solvent = H_2O/THF (2:2.1, 3.5 mL), Ir concentration = 172 μM. [c]Solvent = H_2O/THF (1:1, 4.8 mL), Ir concentration = 125 μM. [d]Solvent = H_2O/THF (1.5:1, 4.2 mL), Ir concentration = 142 μM. [e]The reaction yields were determined by gas chromatography using biphenyl as an internal standard. Yields are average of triplicate runs

Cytotoxicity Studies

NIH-3T3 mouse fibroblast cells (10000 cells/well) were seeded in a 96-well plate and allowed to attach overnight in a 5% CO_2 humidified incubator. The Ir complexes were predissolved in sterile DMSO to give a 5 mM stock solution and then diluted using commercial cell growth media (Dulbecco's Modified Eagle Medium supplemented with 5% fetal bovine serum, FB Essence from VWR) and 1% penicillin streptomycin solution (100×) to give a series of different concentrations (0.1, 1, 25, 50, 100, and 250 μM). The cells were then treated with the iridium solution (6 wells per concentration) for 3 h. The solutions were removed by pipette and the cells were washed with fresh DMEM (2×) before adding DMEM containing 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (2 mL of MTS per 8 mL of DMEM). After 1 h, the absorbance of the 96-well plate was measured at 490 nm to determine the amount of orange formazan product formed. Cell viability was considered to be proportional to the absorbance of the wells. The absorbance of the wells containing solutions of MTS was taken as background and subtracted from the absorbance of wells containing treated and control cells. The cell viability percentage was calculated using the following equation: $(A_{conc}/A_{control})\times 100\%$, where A_{conc} is the absorbance of the test sample and $A_{control}$ is the absorbance of the untreated cells sample. The cell viability data were fit to a single exponential decay function and the IC_{50} value was extracted from this fit at 50% cell viability.

Iridium Accumulation in Live Cells

NIH-3T3 cells were cultured in 100 mm tissue culture plates (Corning) at 37 °C under a 5% CO_2 atmosphere. After 100% confluence was achieved, the cells were incubated with either 5 or 10 μM of iridium complex for 2 h in DMEM supplemented with 5% fetal bovine serum (FB Essence) and 1% penicillin streptomycin solution (100×) (diluted from 5 mM stock solution in sterile DMSO). After 2 h of incubation the catalyst solutions were removed and the cells were rinsed twice with fresh DMEM. The adhered cells were detached by treating with trypsin and counted using a BIO-RAD TC10 automated cell counter. The sample was then centrifuged and the supernatant was removed. The cell pellet was washed an additional time with phosphate buffered saline (PBS), centrifuged, and then the supernatant was removed. The cell pellets were digested with 0.5 mL of 70% HNO_3 at room temperature overnight. Each sample was diluted with 6.5 mL of HPLC grade H_2O to obtain 5% HNO_3 sample solutions. The cloudy solutions were centrifuged and the clear supernatant was separated and used for analysis by ICP-MS. The total iridium content per cell was calculated using the solution volume and cell density from each sample preparation.

Cellular Localization Studies

NIH-3T3 cells were cultured at 37 °C under a 5% CO_2 humidified atmosphere in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FB Essence from VWR) and 1% penicillin streptomycin solution (100×). For live cell imaging, cells were plated in μ-Slide 8-well plates (IBIDI) in 200 μL of DMEM in each well. After overnight cell growth, the medium was removed and cells were treated with 5 μM iridium solution in DMEM (diluted from 5 mM stock in sterile DMSO). After an incubation time of 30 min (37 °C, 5% CO_2), the growth solutions were removed and cells were co-treated with the iridium complex and Mito-ID or Hoechst33342 for another 30 min (37 °C, 5% CO_2). The cells were rinsed with fresh DMEM (1×200 μL each well), followed by phenol red free DMEM (1×200 μL each well), and then visualized immediately using microscopy (Olympus IX83) with a 100× oil-immersion objective. The excitation wavelength used was 488 nm for the iridium complexes (emission filter: 500-550 nm), 405 nm for Hoechst33342 (emission filter: 426-450 nm), and 561 nm for Mito-ID. The images were processed by subtracting background signal and emission from control cells using the program ImageJ. Pearson's Correlation Coefficients (PCC) were calculated using MATLAB.

Table S2. Cytotoxicity and Uptake of Iridium Complexes in NIH-3T3 Cells

Complex	**IC_{50}** (μM)[a]	**Intracellular Conc.** (ng Ir/10^6 cells)[b]
Ir1a	108 ± 30	42 ± 1
Ir2a	31 ± 4	104 ± 29
Ir3a	119 ± 3	21 ± 5
Ir1b	23 ± 2	179 ± 81
Ir2b	67 ± 7	8 ± 1
Ir3b	28 ± 2	111 ± 45

[a]Cells were treated with various concentrations of the iridium complexes for 3 h and then viability was determined using a colorimetric MTS assay. The average IC_{50} values provided were determined from triplicate experiments. [b]Cells were treated with 5 μM of the iridium complexes for 2 h, washed, and then lysed. The cell lysates were analyzed by ICP-MS and the average intracellular iridium concentration was determined from triplicate experiments.

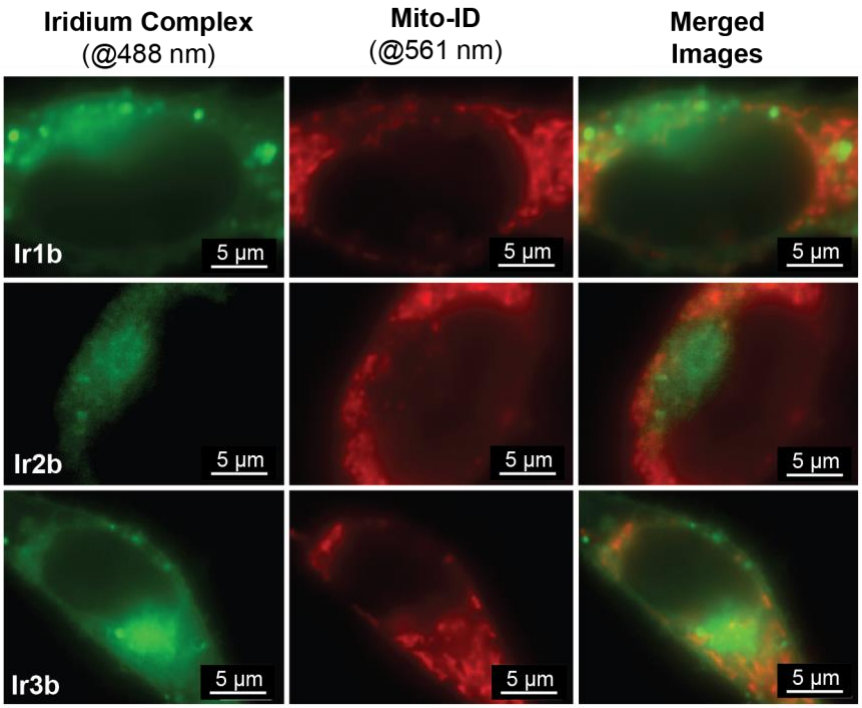


Figure S2. Fluorescence microscope images of NIH-3T3 cells co-treated with iridium complexes (left panel, λ_{ex} = 488 nm) and Mito-ID (middle panel, λ_{ex} = 561 nm). The merged images show the signal overlap between the 488 and 561 nm channels (right panel). These data were acquired using an Olympus IX83 microscope with a 100× oil objective.

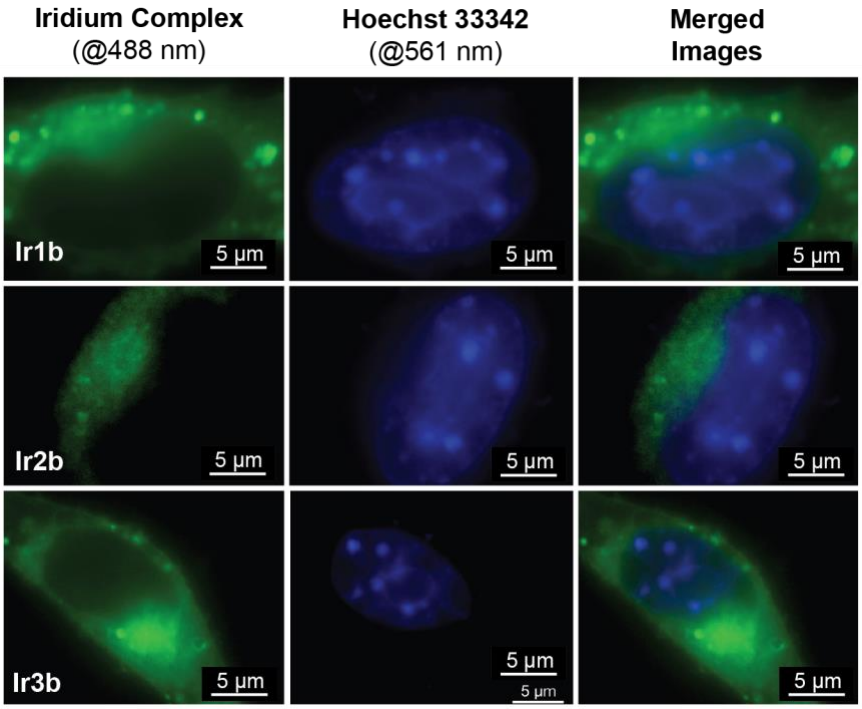


Figure S3. Fluorescence microscope images of NIH-3T3 cells co-treated with iridium complexes (left panel, λ_{ex} = 488 nm) and Hoechst 3342 (middle panel, λ_{ex} = 405 nm). The merged images show the signal overlap between the 405 and 488 nm channels (right panel). These data were acquired using an Olympus IX83 microscope with a 100× oil objective.

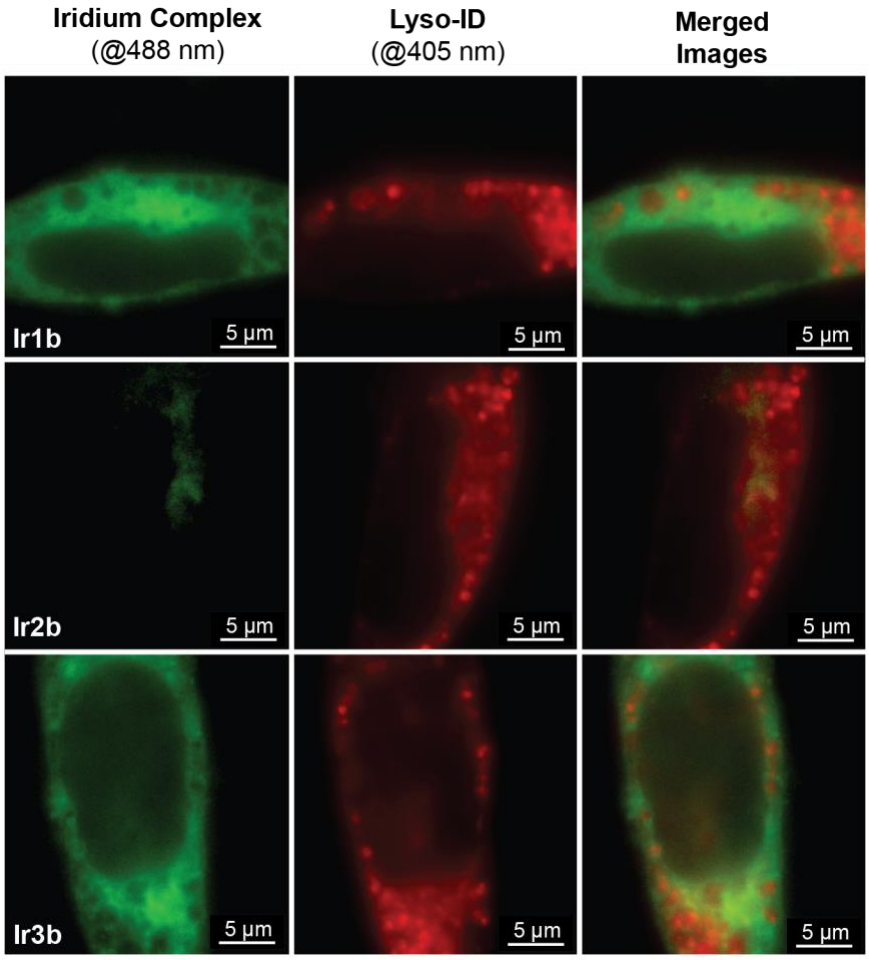


Figure S4. Fluorescence microscope images of NIH-3T3 cells co-treated with iridium complexes (left panel, λ_{ex} = 488 nm) and Lyso-ID (middle panel, λ_{ex} = 561 nm). The merged images show the signal overlap between the 488 and 561 nm channels (right panel). These data were acquired using an Olympus IX83 microscope with a 100× oil objective.

Table S3. Pearson's Correlation Coefficient Determined Based on Co-Staining Experiments

Complex	Mitochondria	Nucleus	Lysosome
Ir1b	0.74	0	0.77
Ir2b	0.54	0	0.43
Ir3b	0.69	0	0.68

NMR Spectra

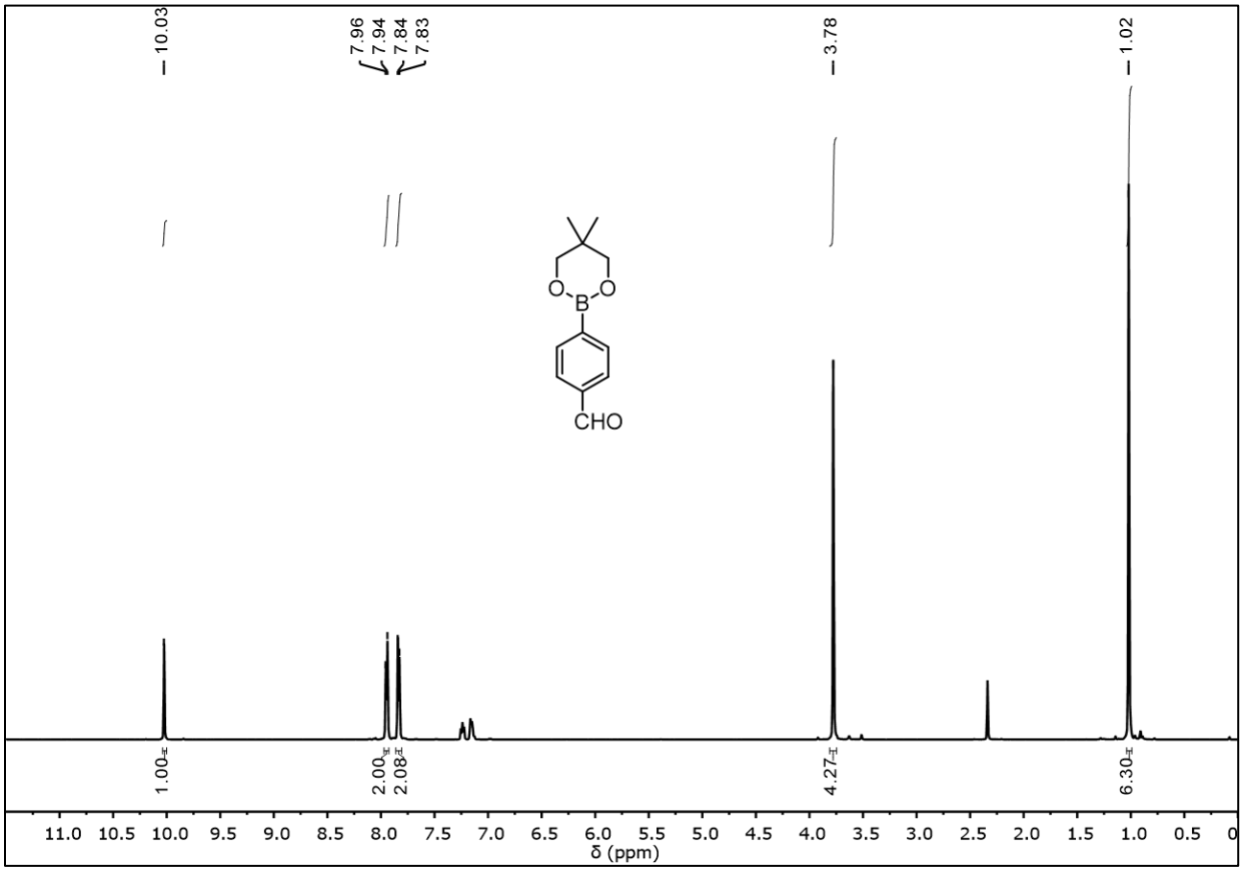


Figure S5. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **1**.

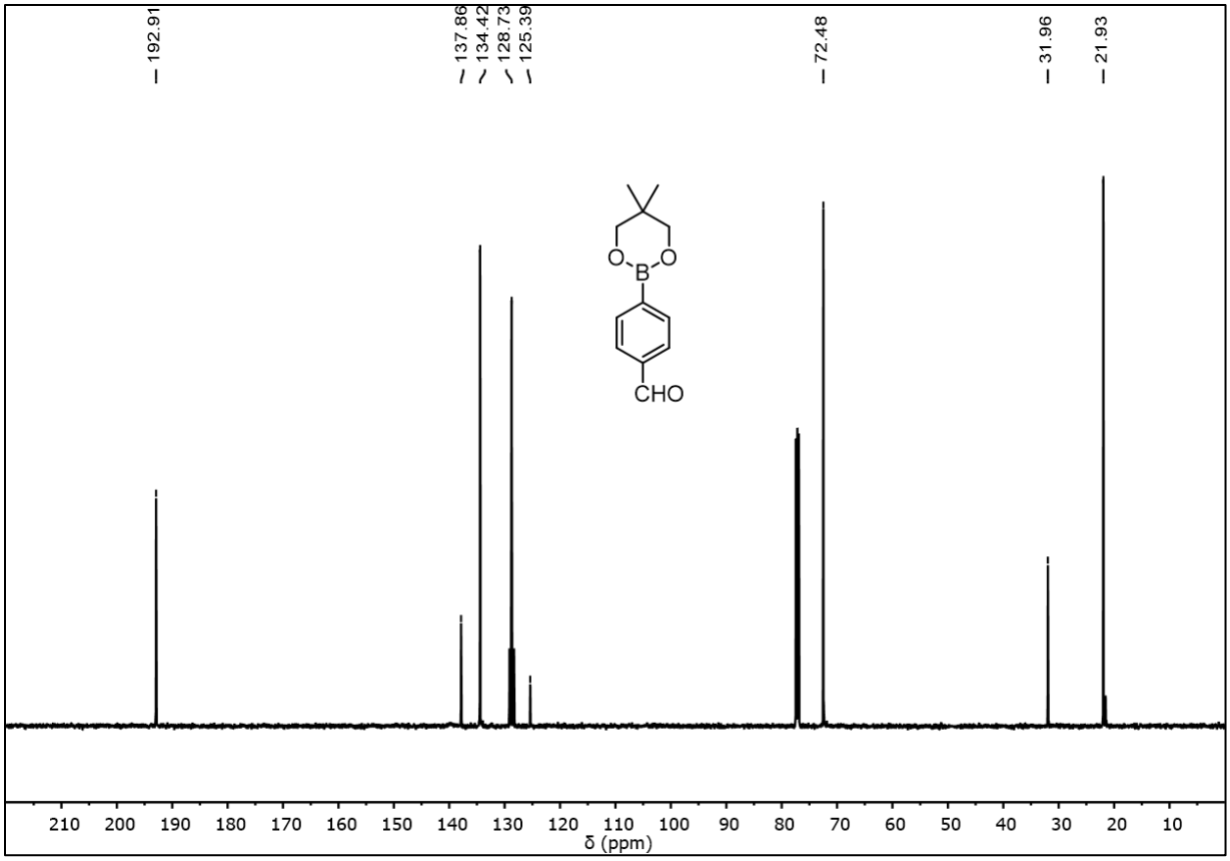


Figure S6. ^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **1**.

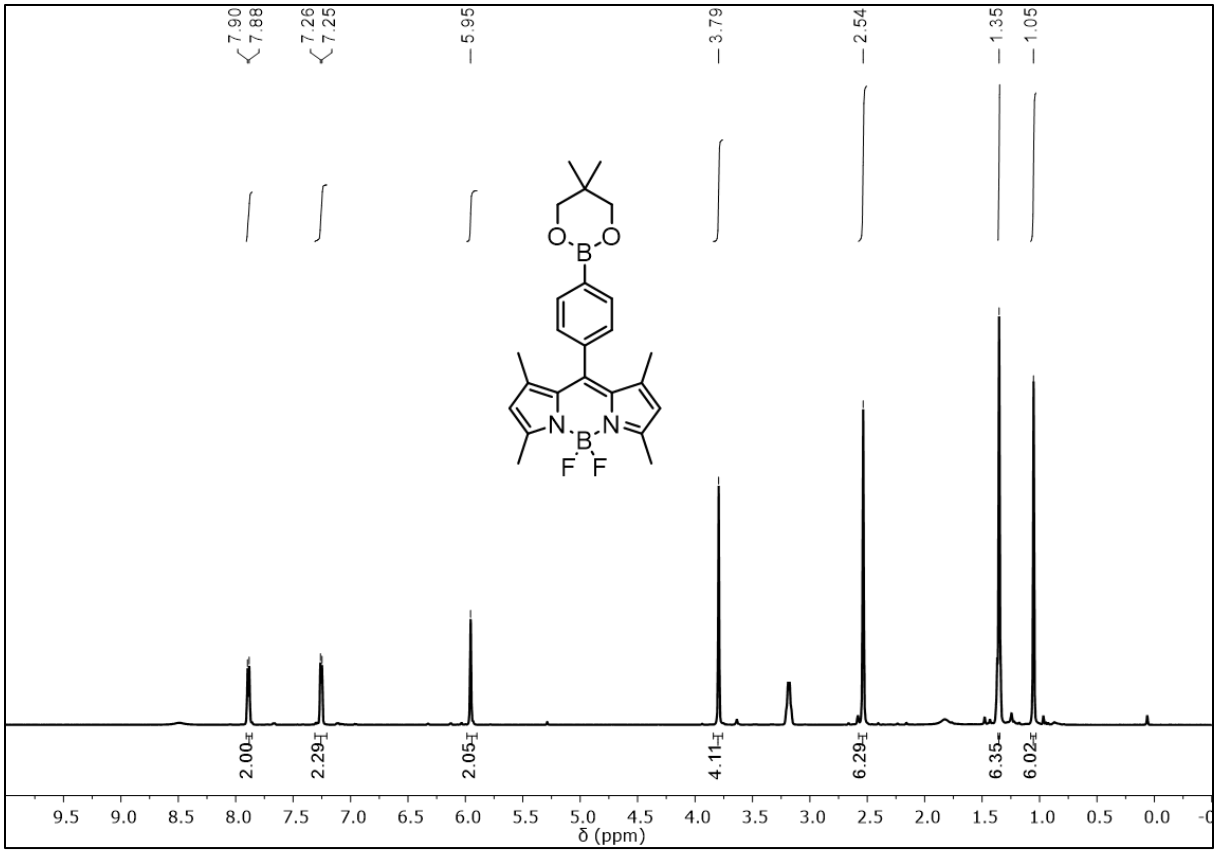


Figure S7. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **2**.

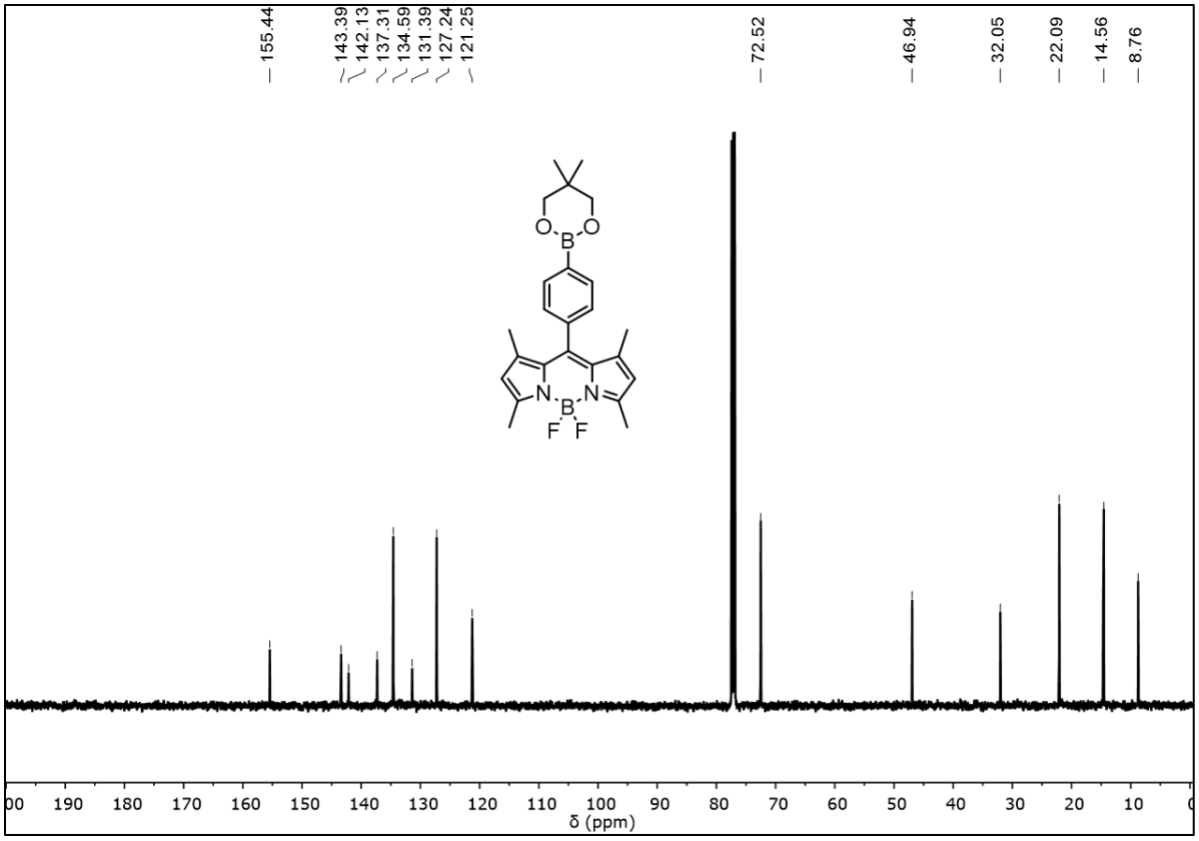


Figure S8. ^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **2**.

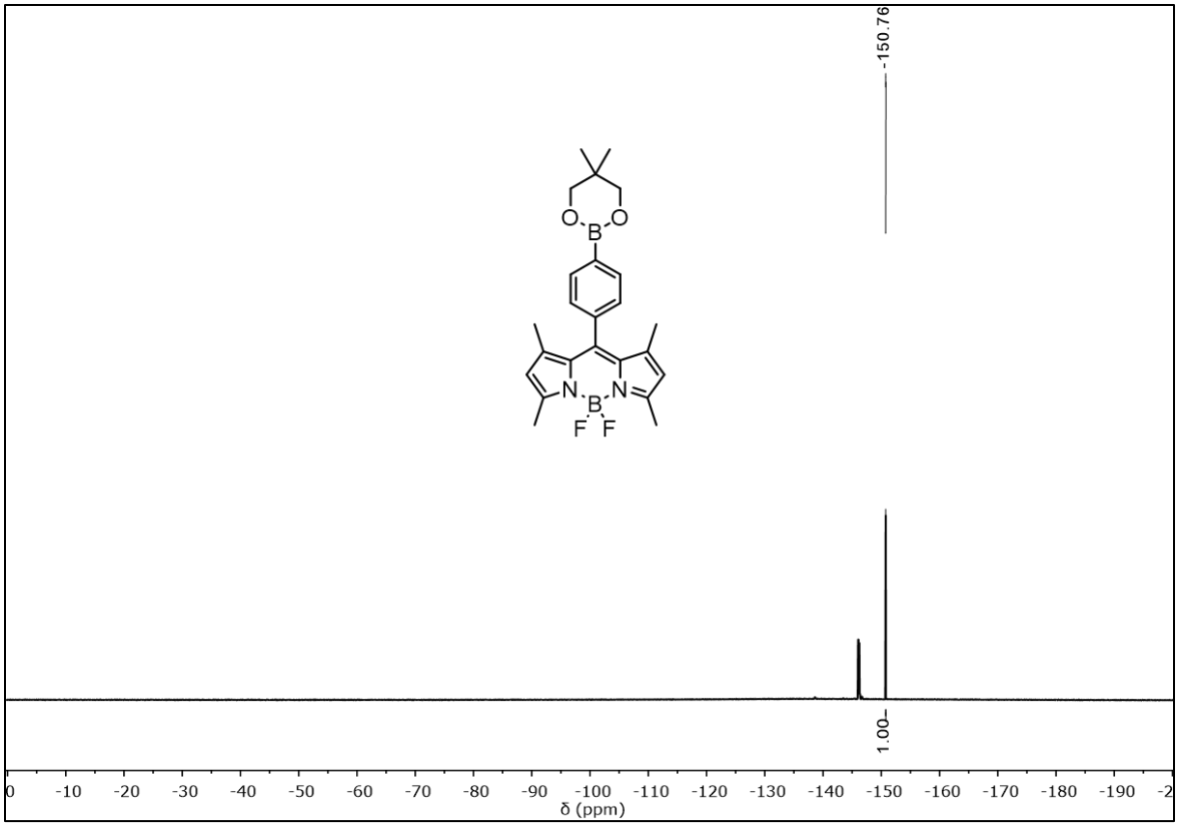


Figure S9. ^{19}F NMR spectrum (470 MHz, $CDCl_3$) of **2**.

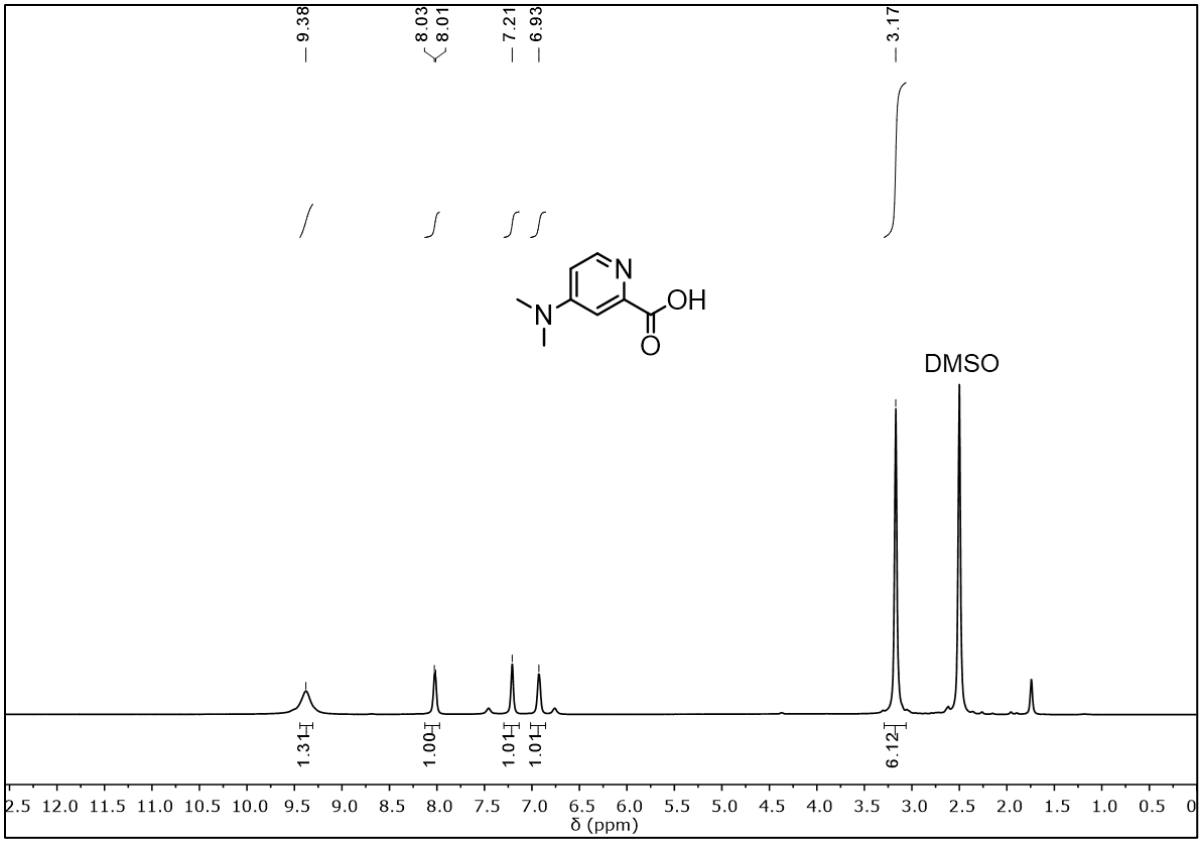


Figure S10. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of **3**.

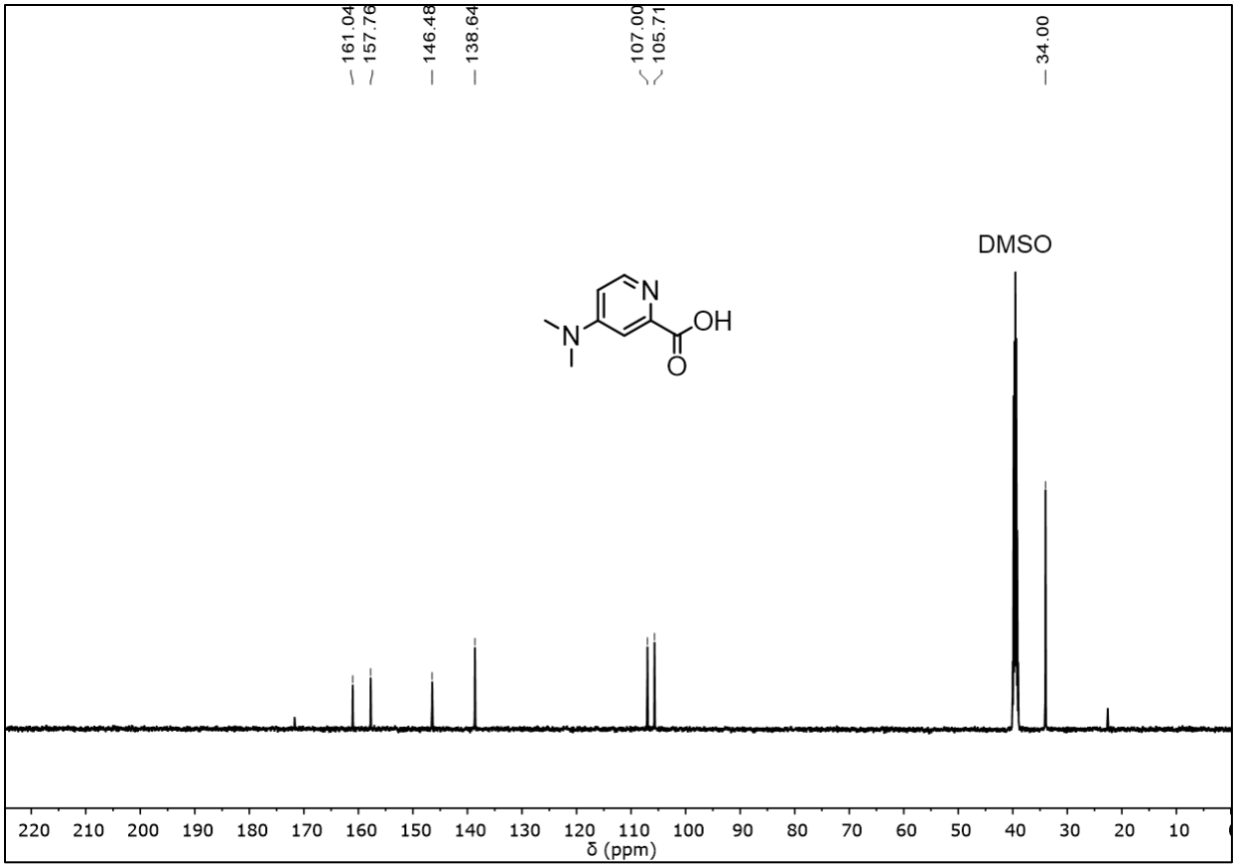


Figure S11. ^{13}C NMR spectrum (126 MHz, DMSO-d_6) of **3**.

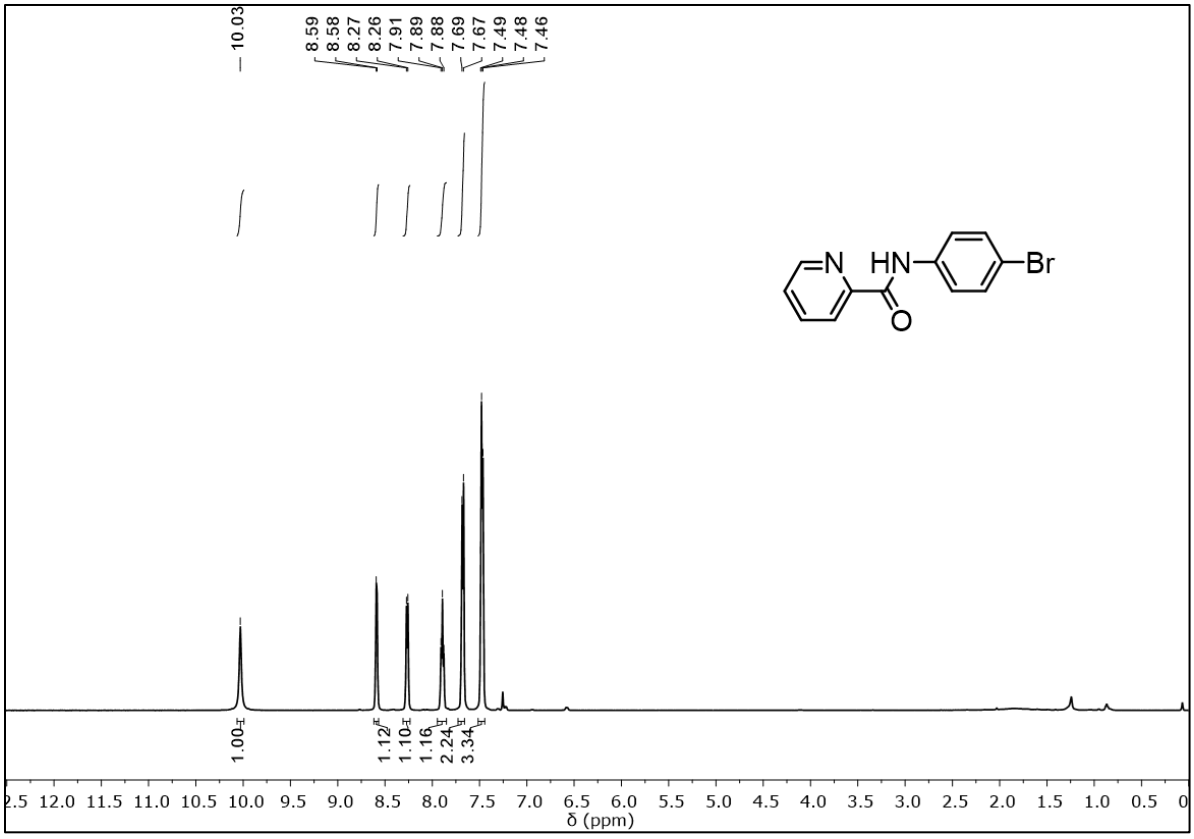


Figure S12. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **4a**.

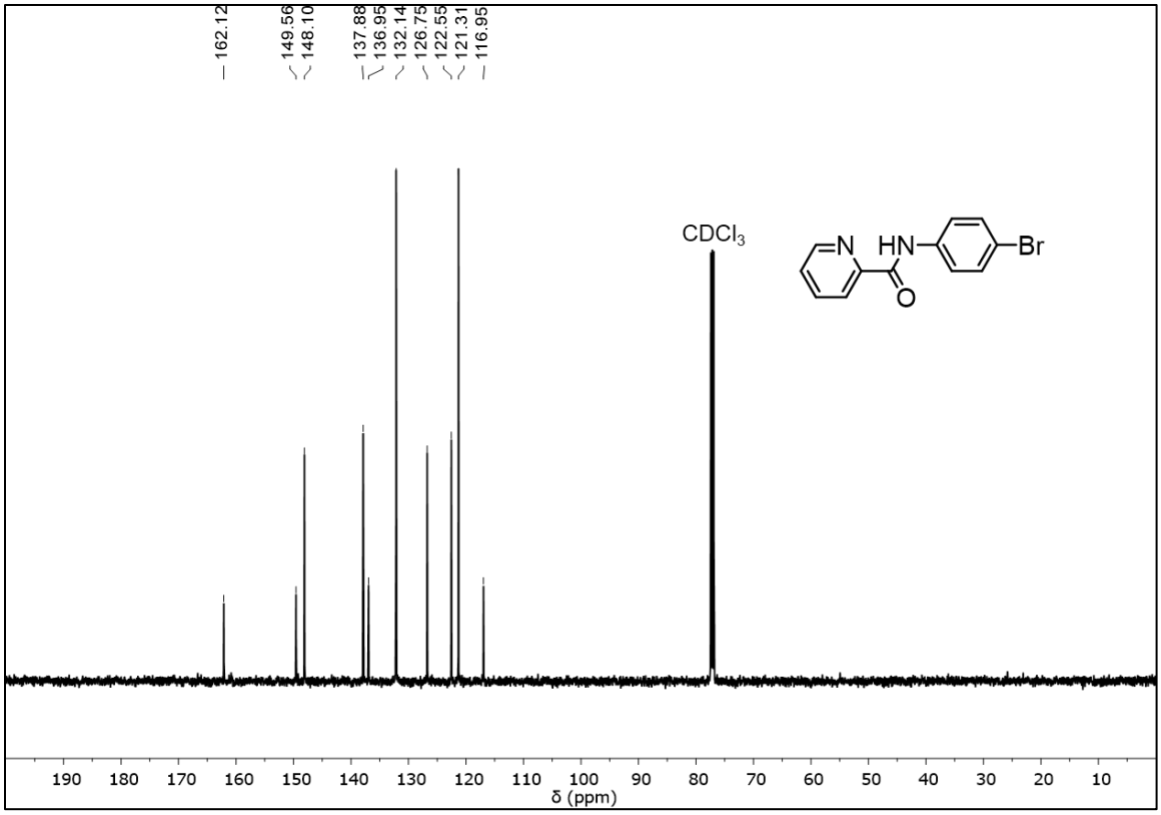


Figure S13. ^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **4a**.

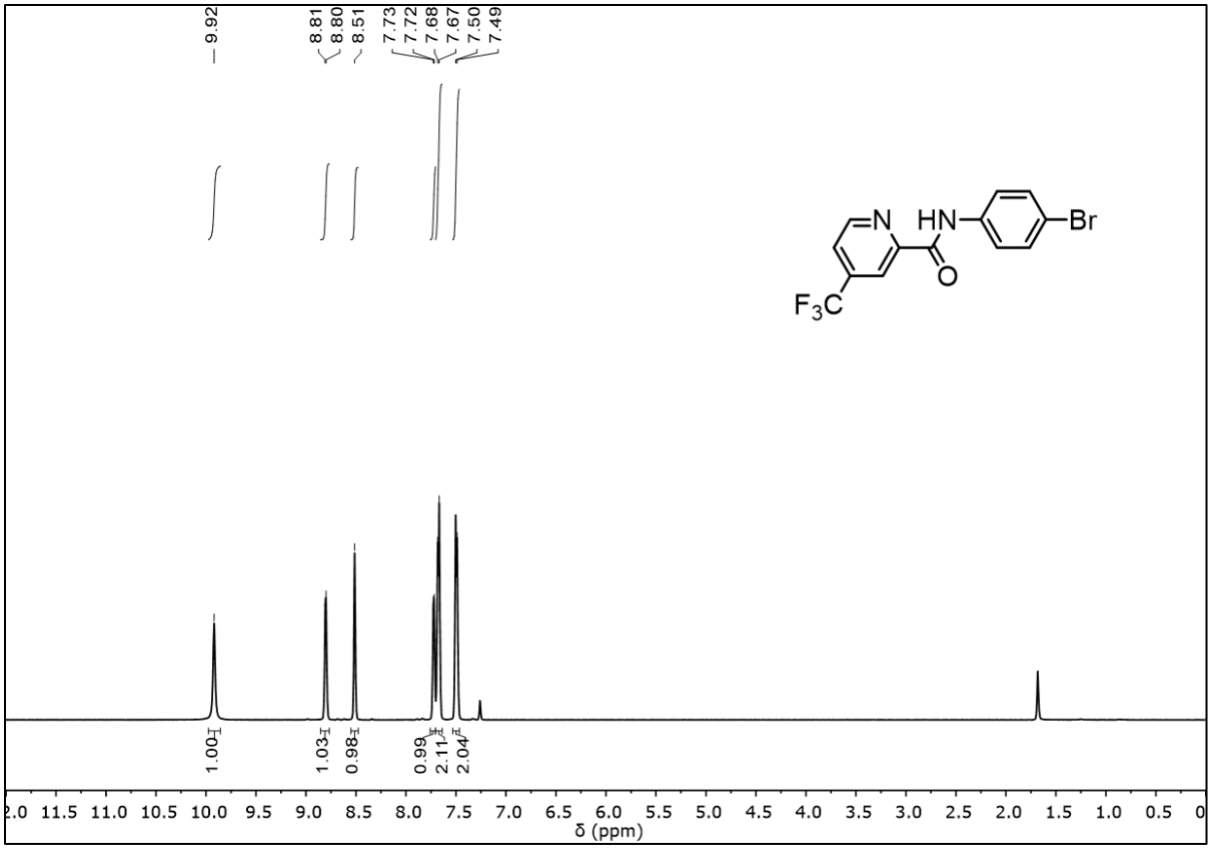


Figure S14. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **4b**.

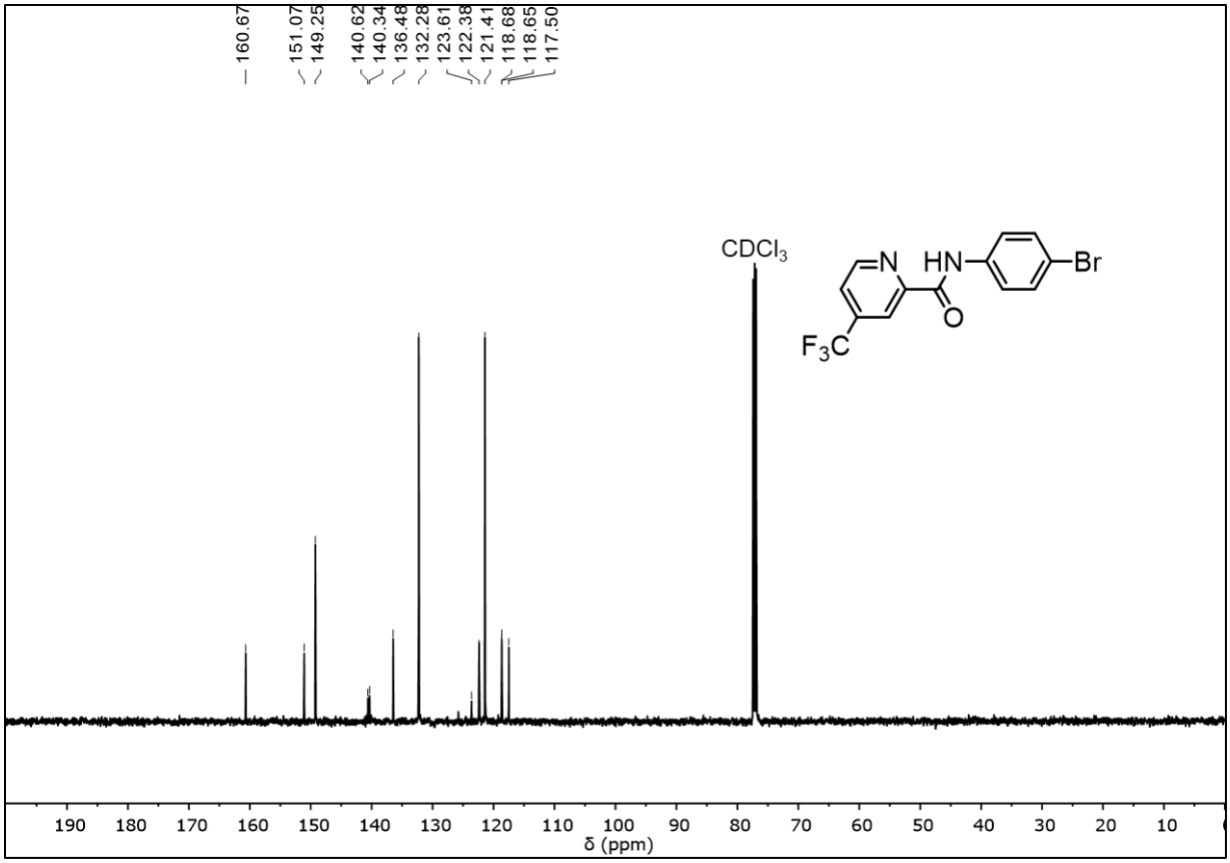


Figure S15. ^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **4b**.

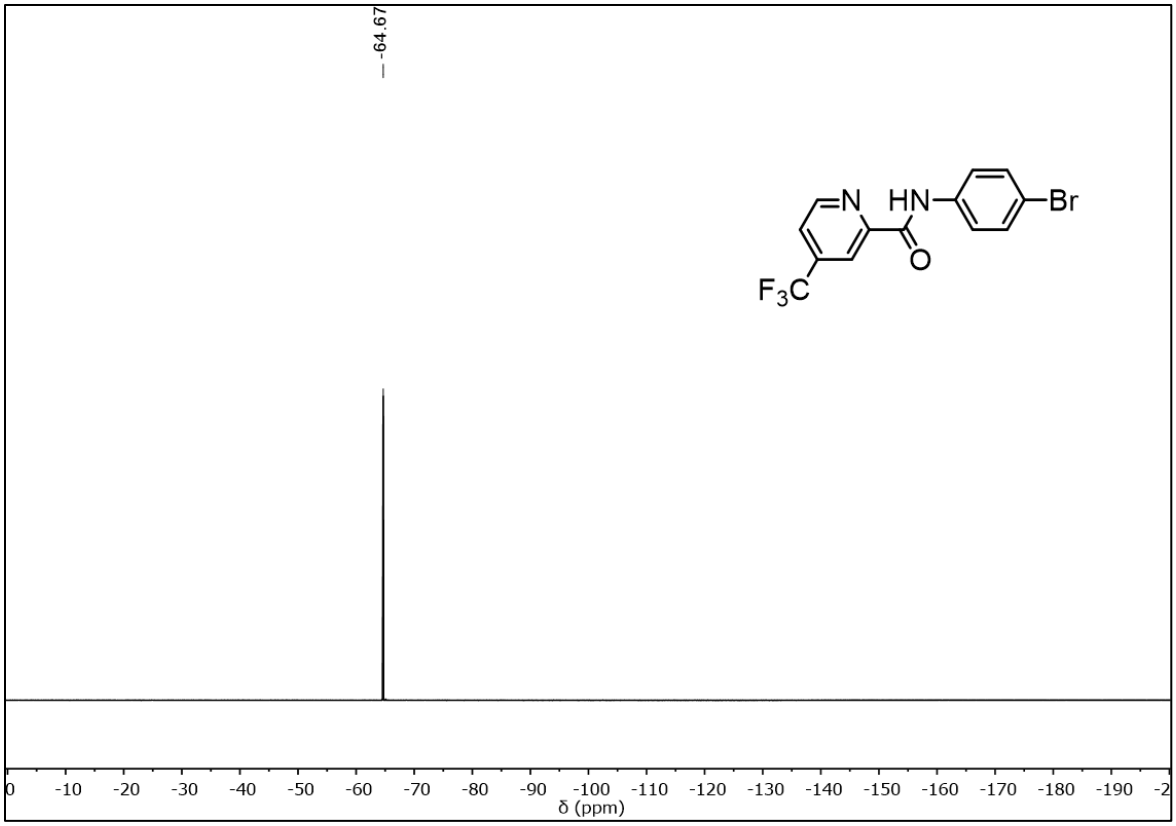


Figure S16. ^{19}F NMR spectrum (470 MHz, $CDCl_3$) of **4b**.

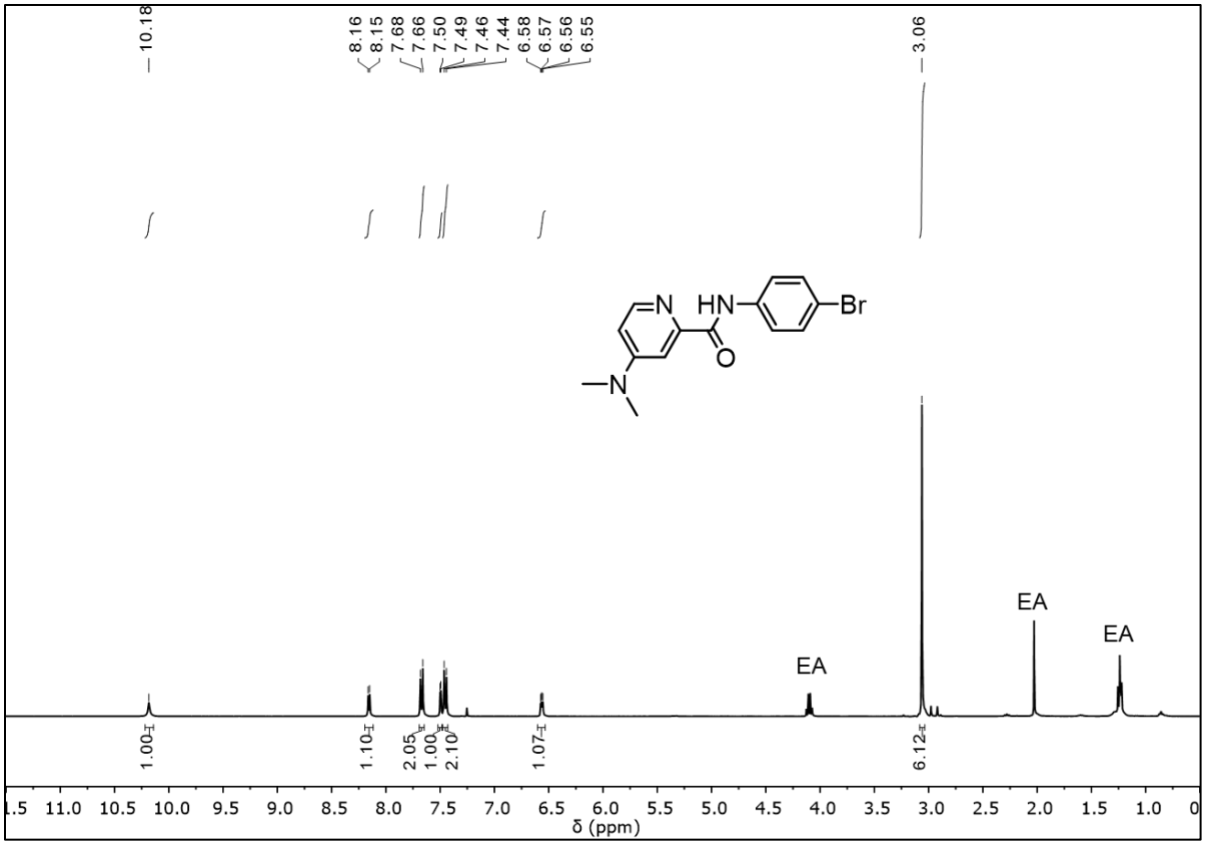


Figure S17. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **4c**.

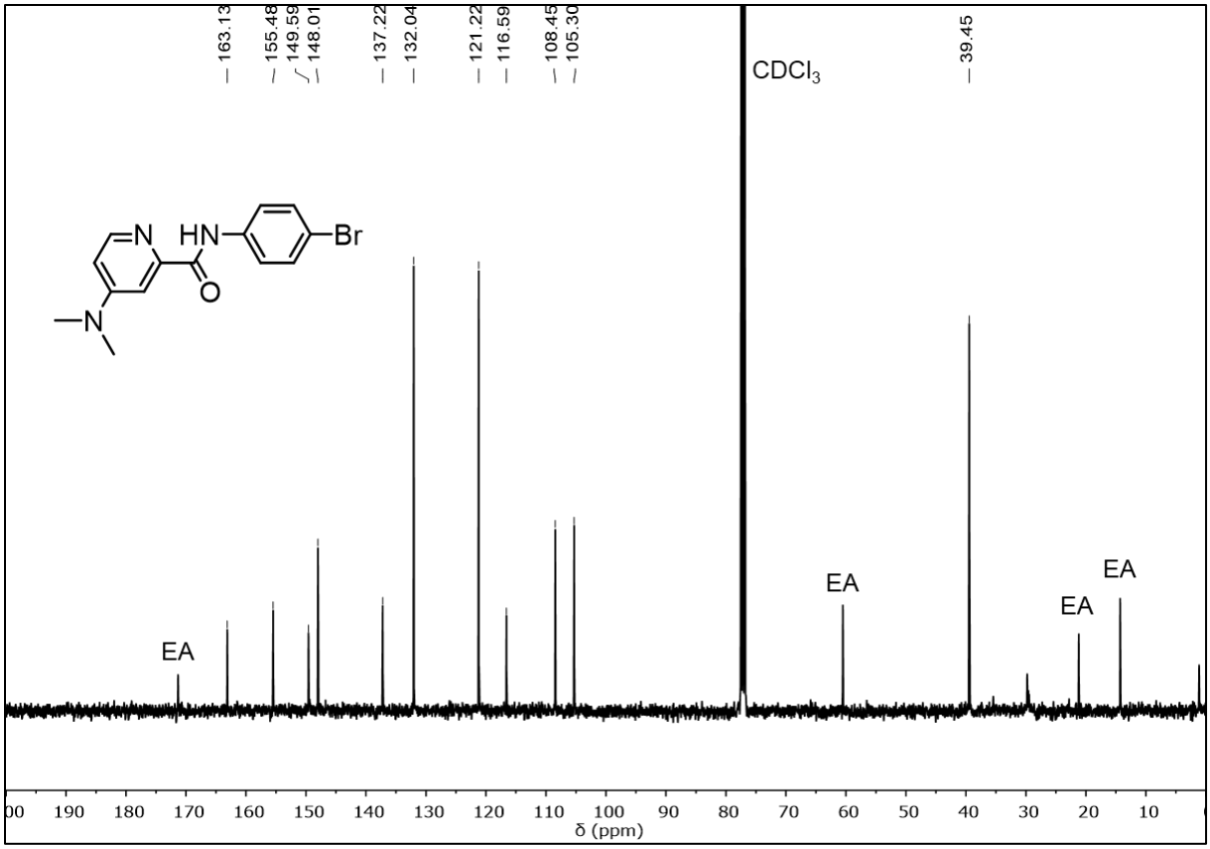


Figure S18. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **4c**.

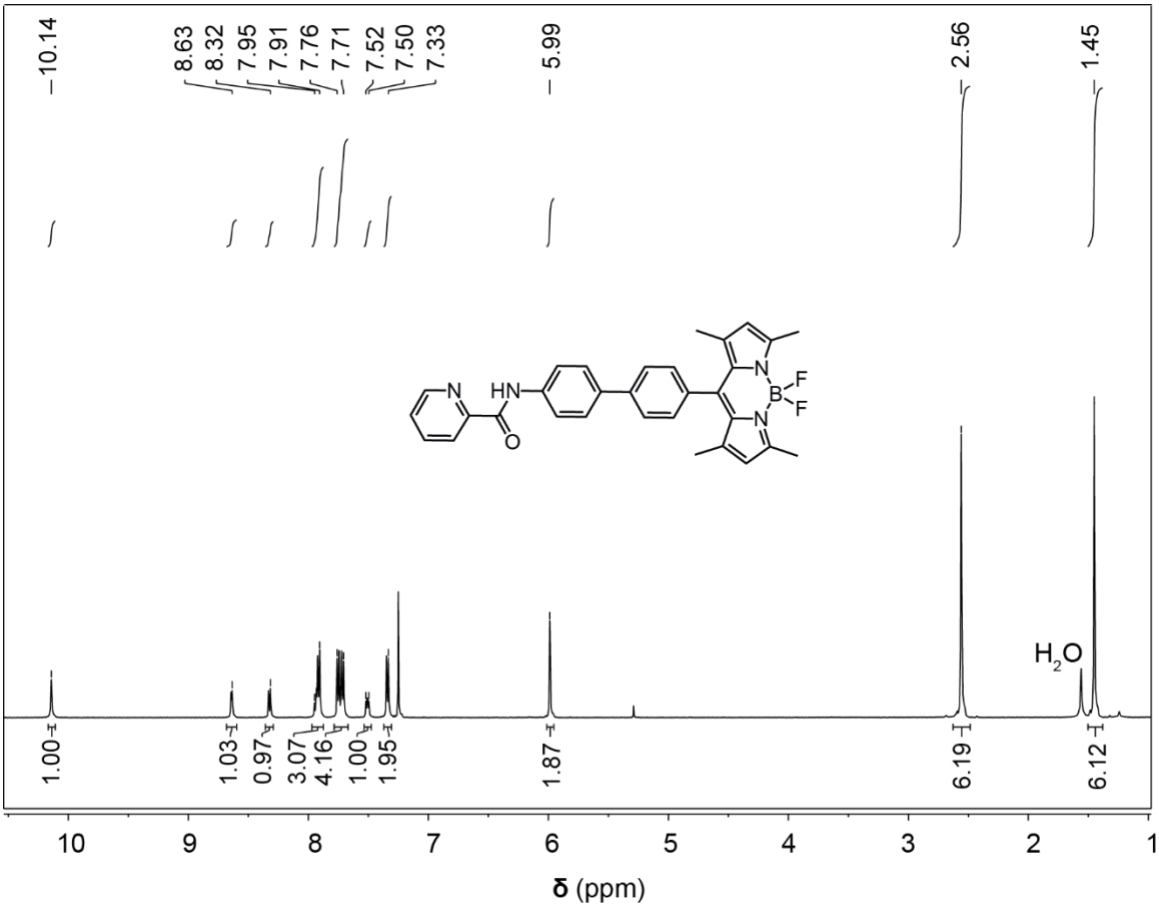


Figure S19. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **5a.**

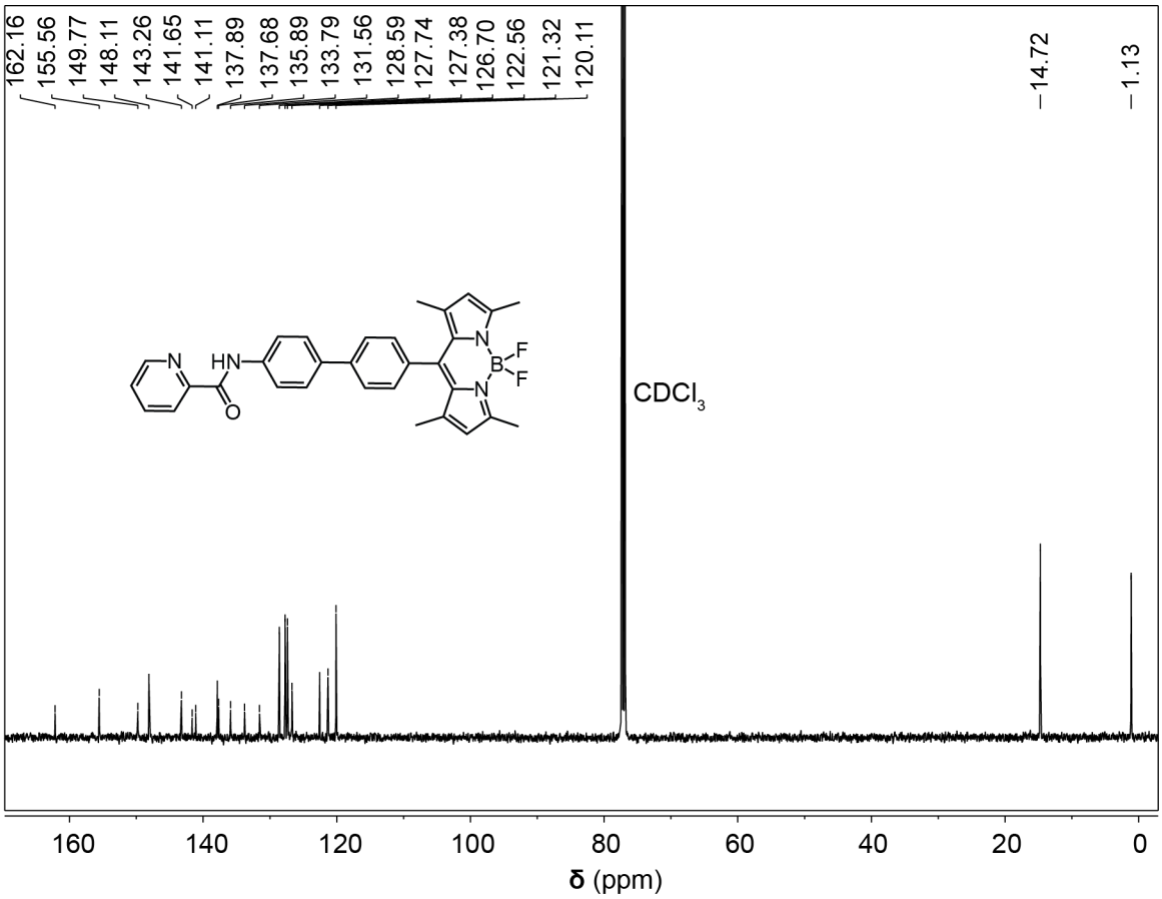


Figure S20. ^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **5a**.

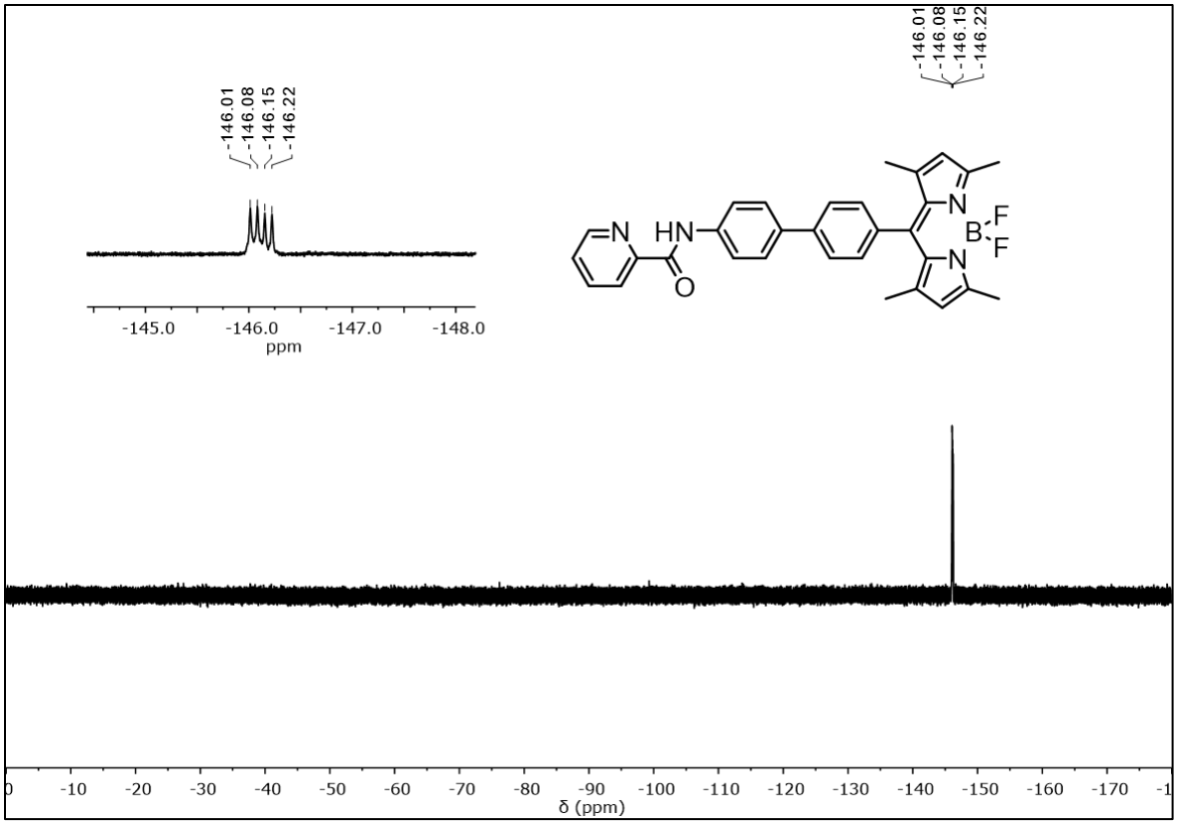


Figure S21. ^{19}F NMR spectrum (470 MHz, $CDCl_3$) of **5a**.

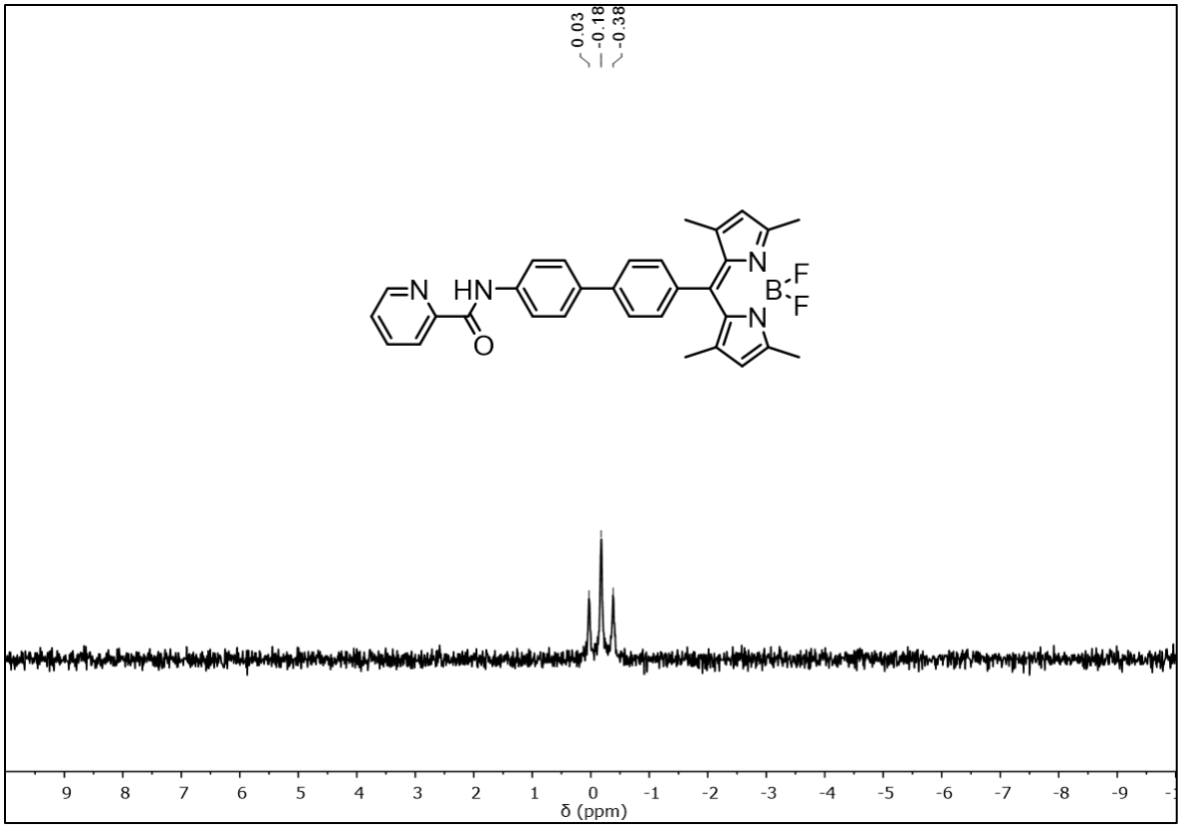


Figure S22. ^{11}B NMR spectrum (160 MHz, $CDCl_3$) of **5a**.

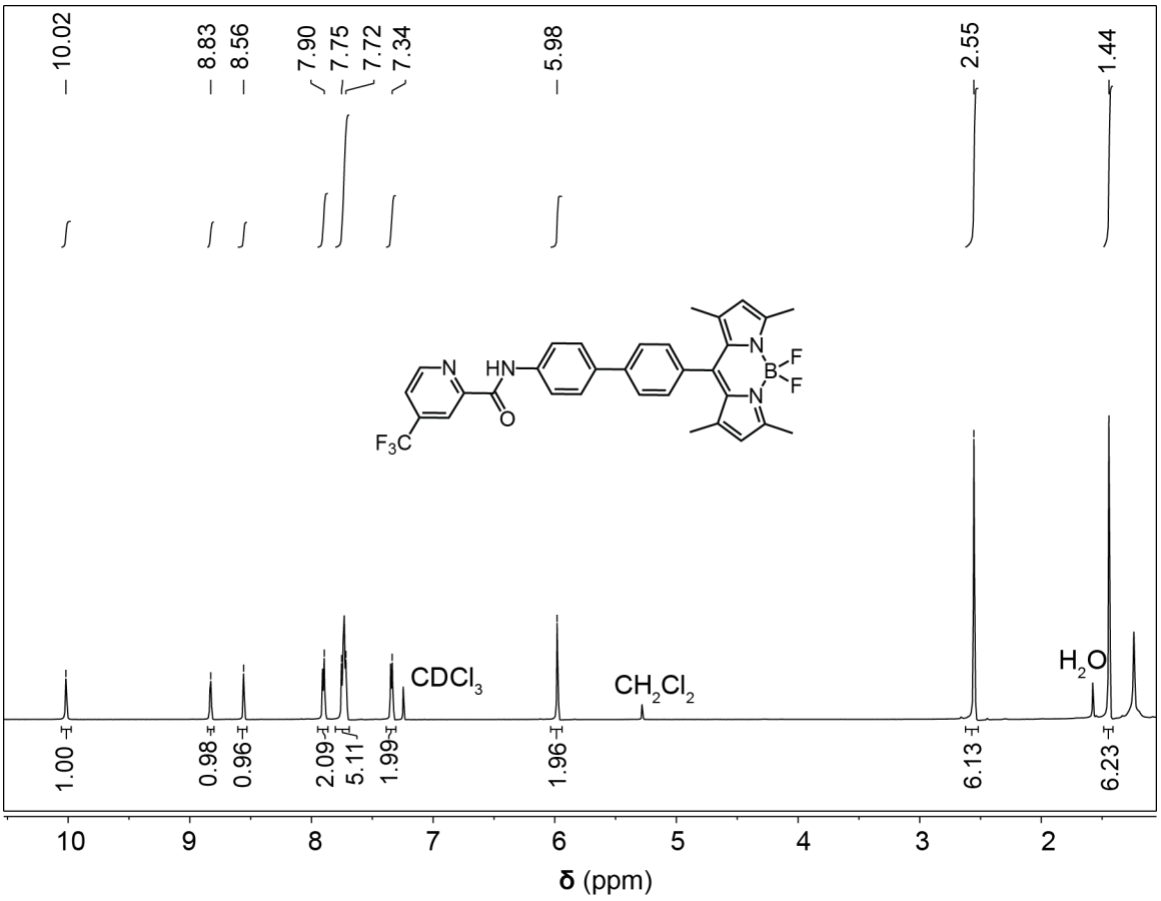


Figure S23. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **5b**.

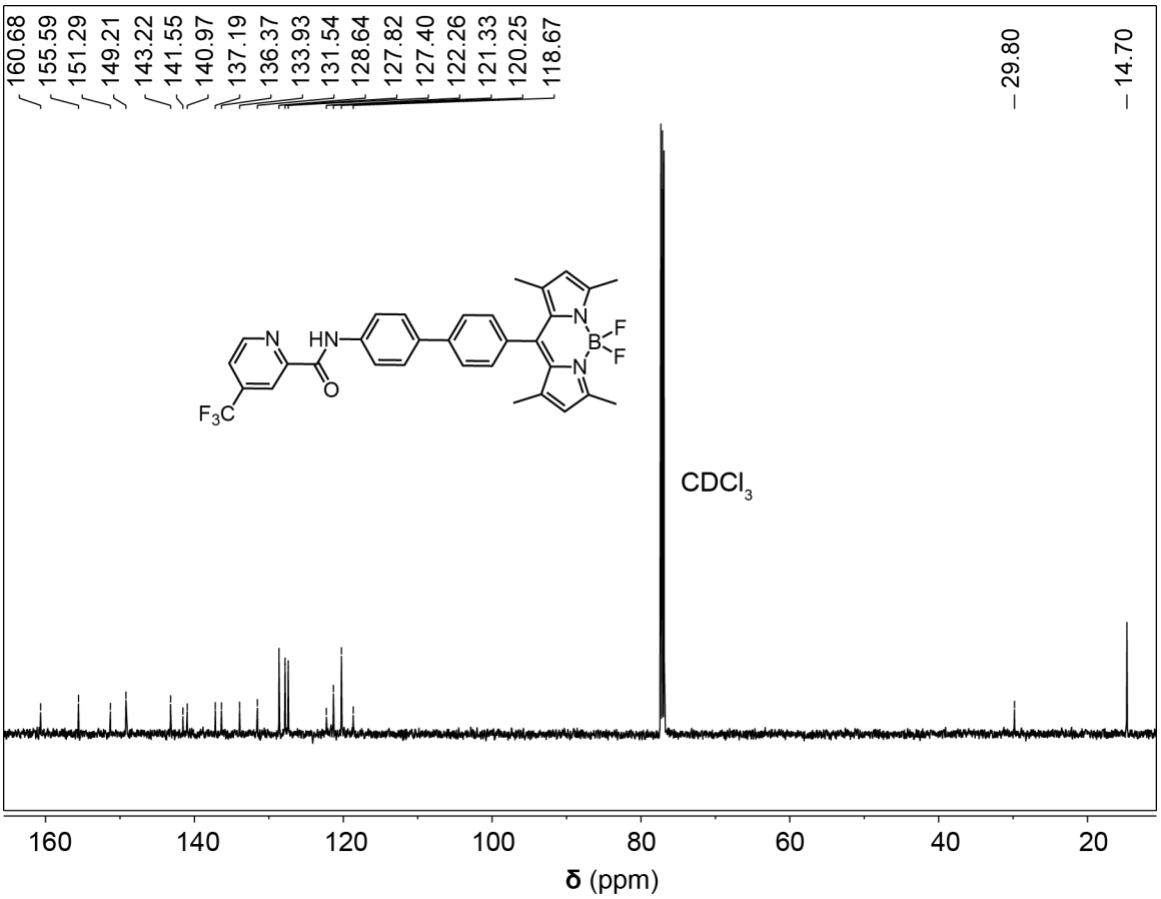


Figure S24 ^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **5b**.

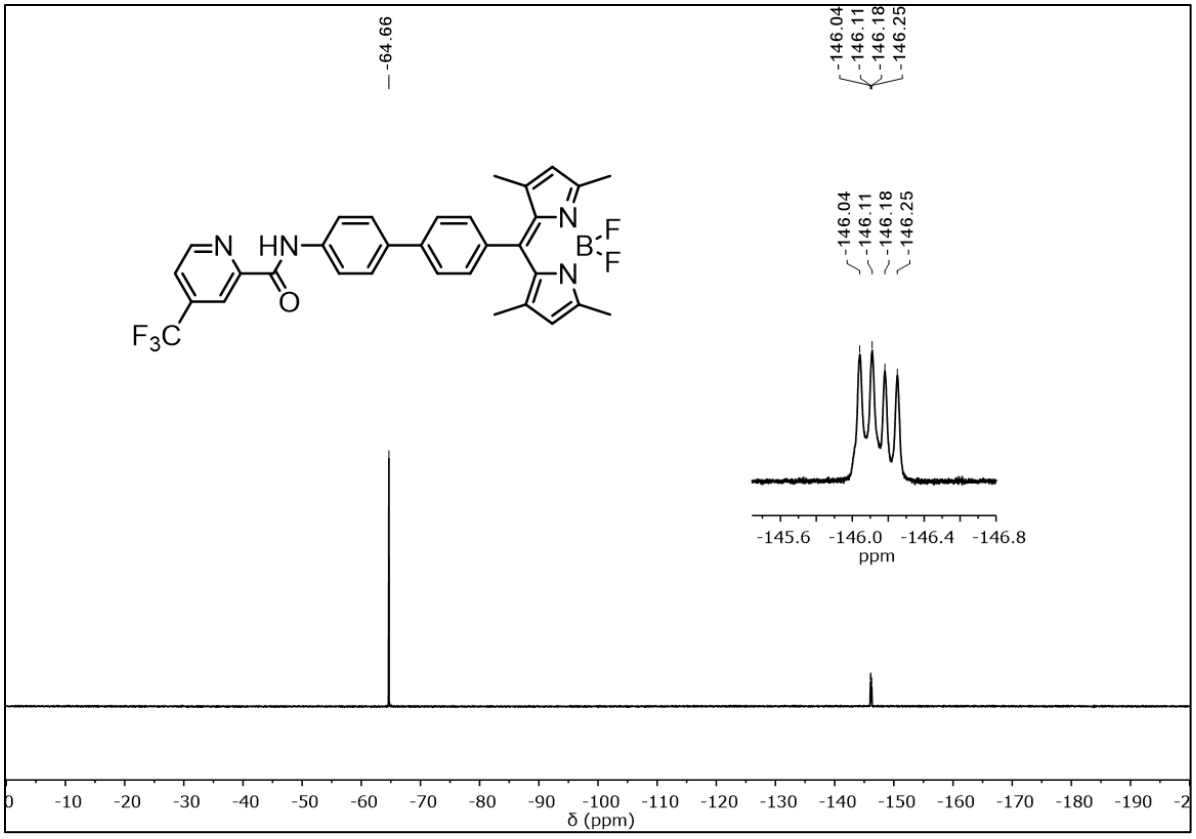


Figure S25. ^{19}F NMR spectrum (470 MHz, $CDCl_3$) of **5b**.

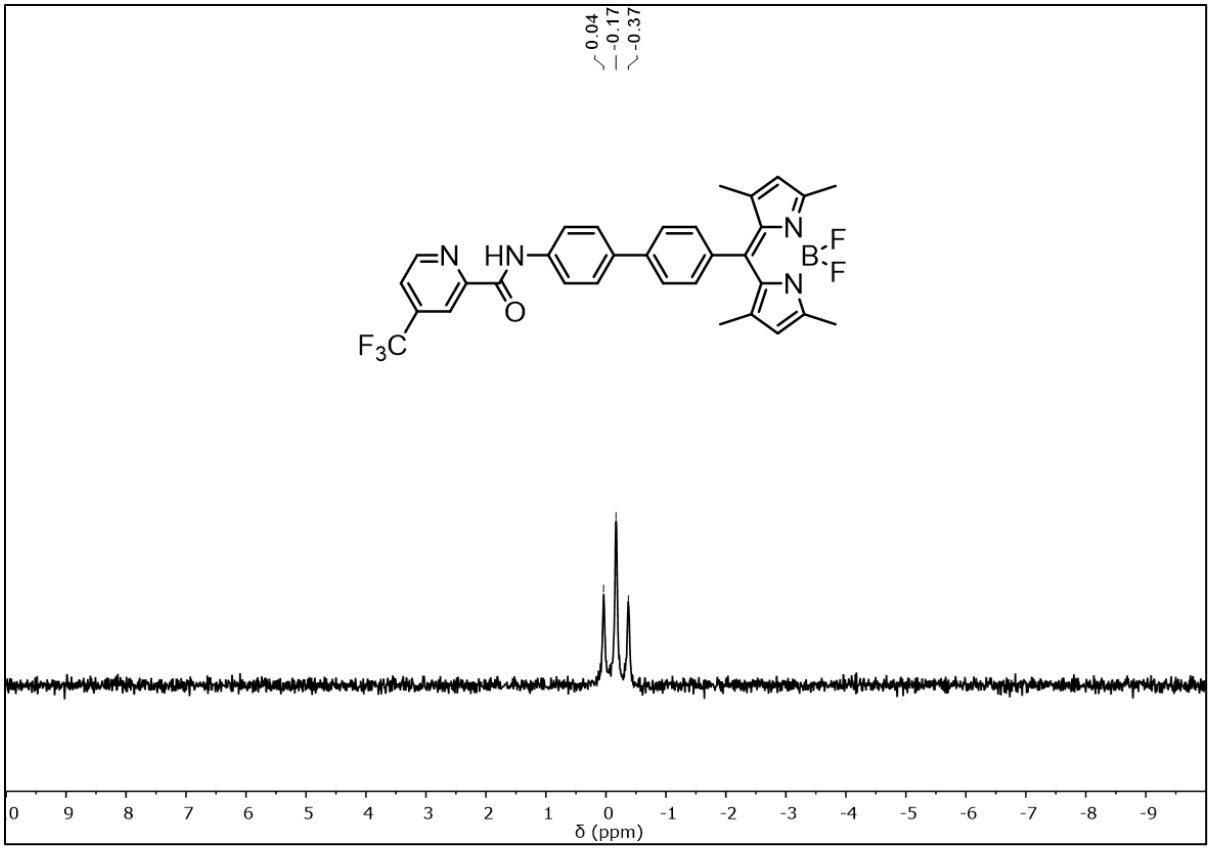


Figure S26. ^{11}B NMR spectrum (160 MHz, $CDCl_3$) of **5b**.

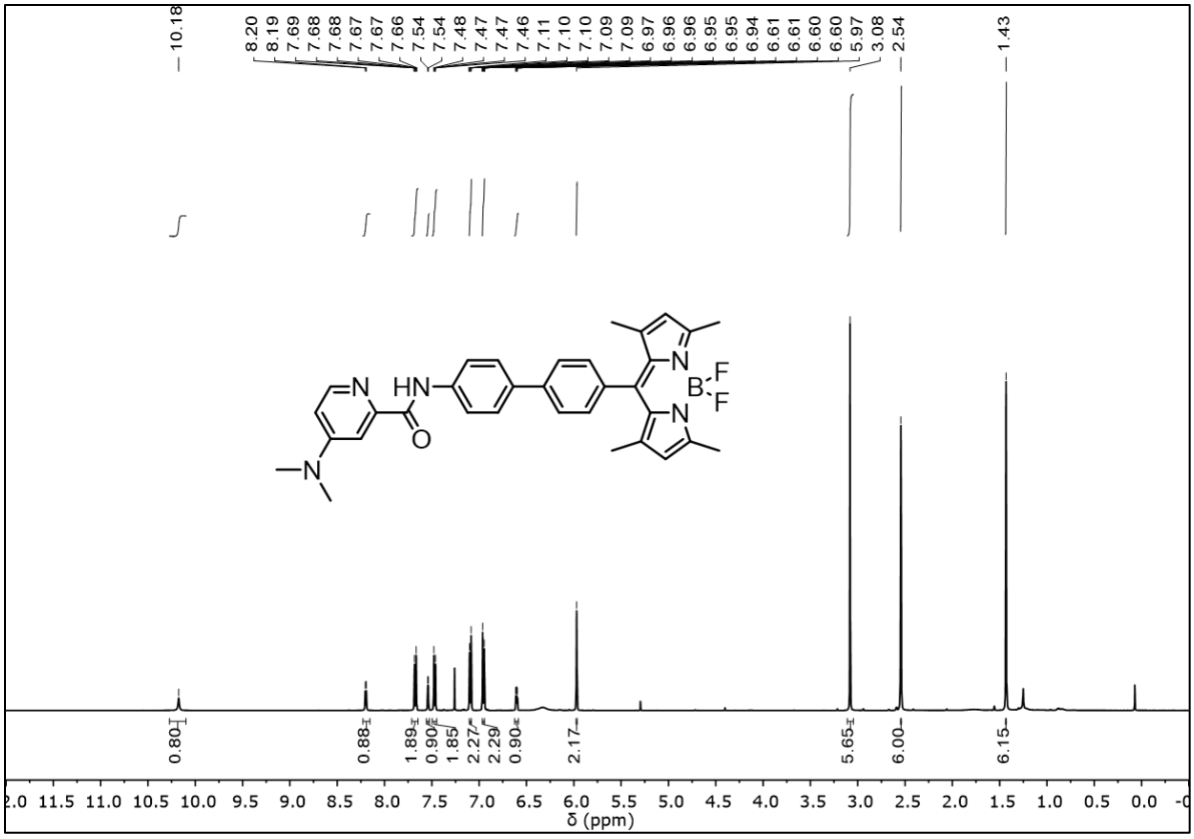


Figure S27. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **5c**.

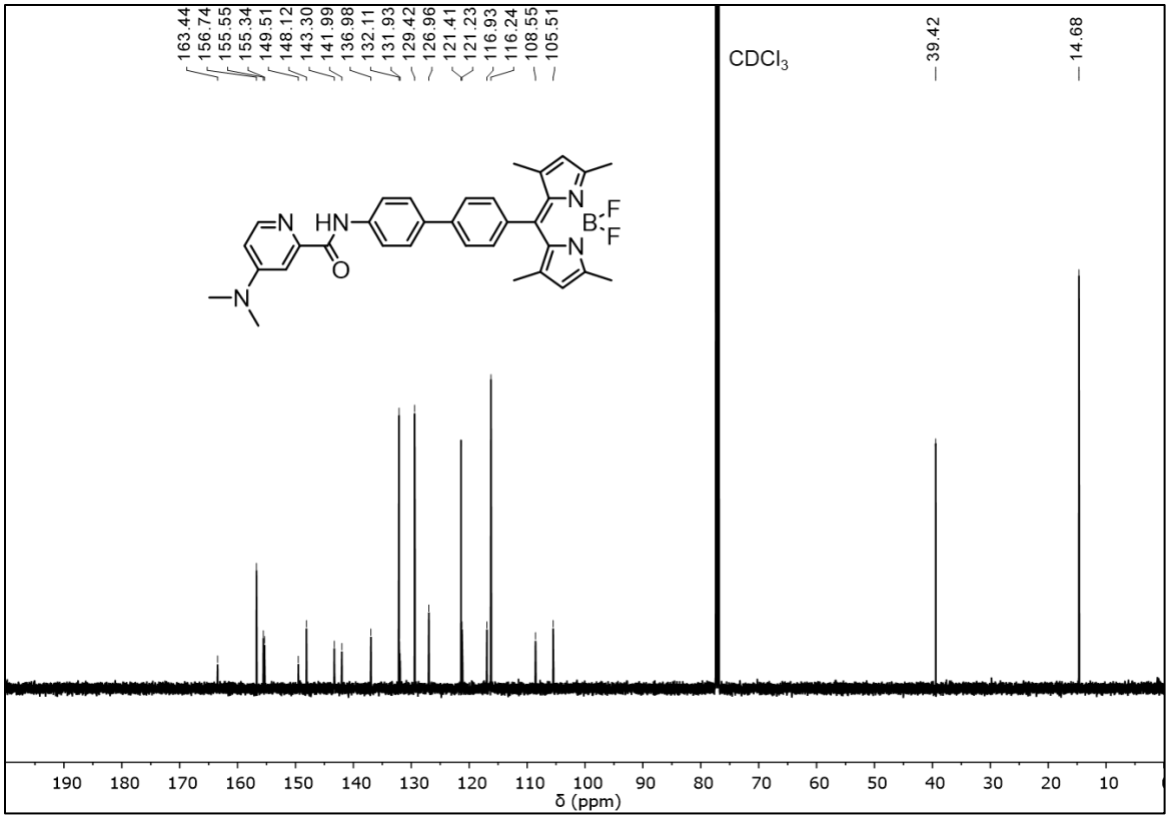


Figure S28. ^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **5c**.

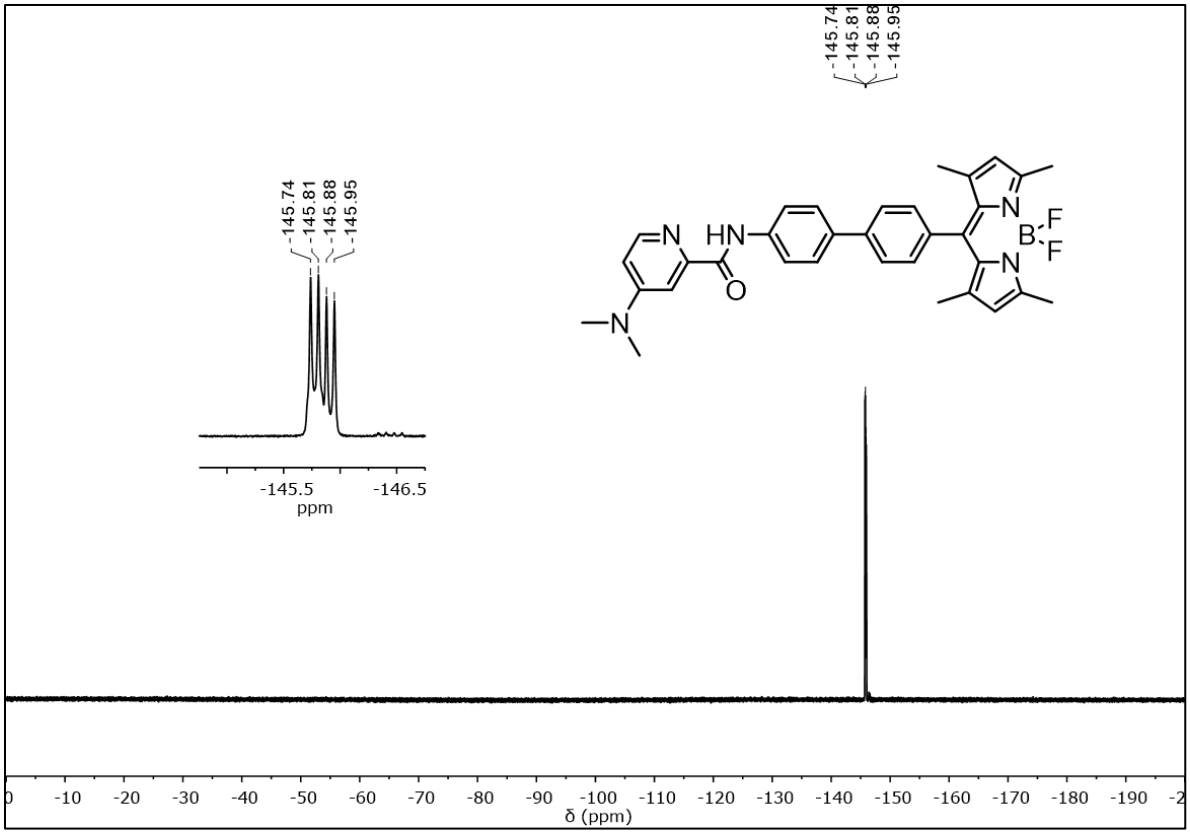


Figure S29. ^{19}F NMR spectrum (470 MHz, $CDCl_3$) of **5c**.

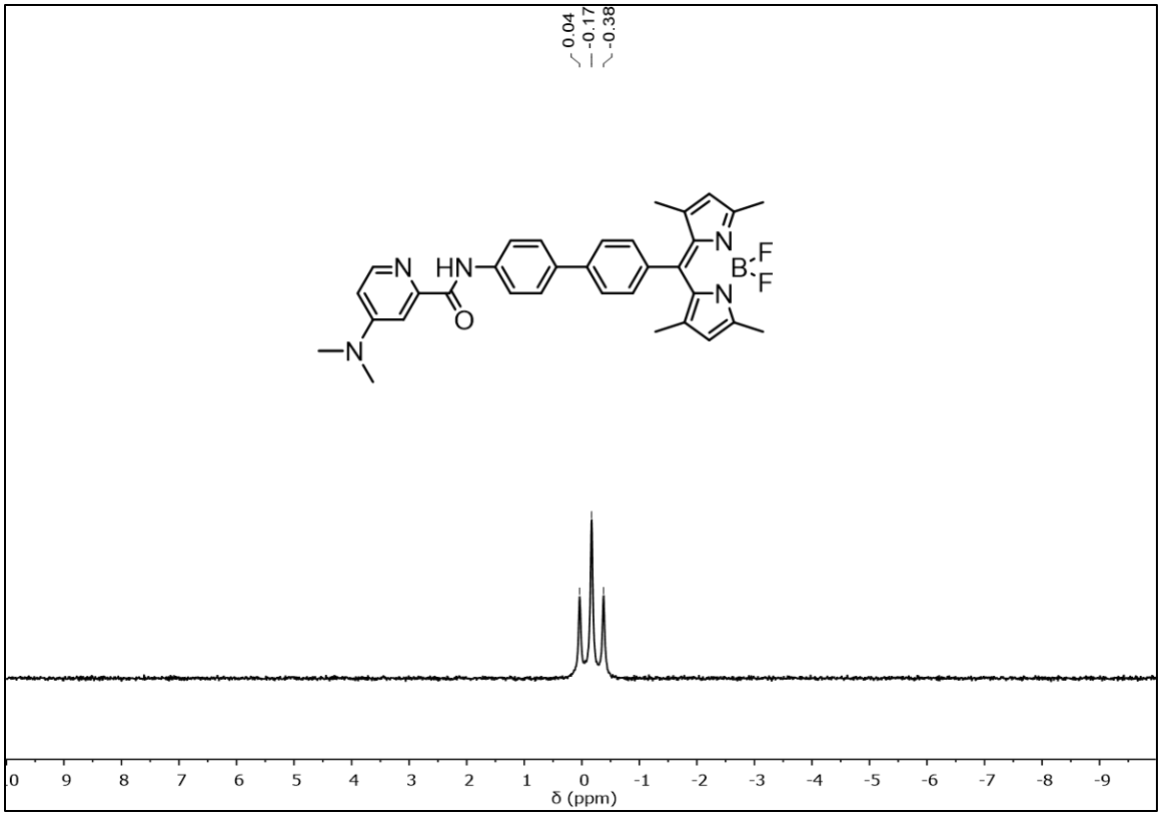


Figure S30. ^{11}B NMR spectrum (160 MHz, $CDCl_3$) of **5c**.

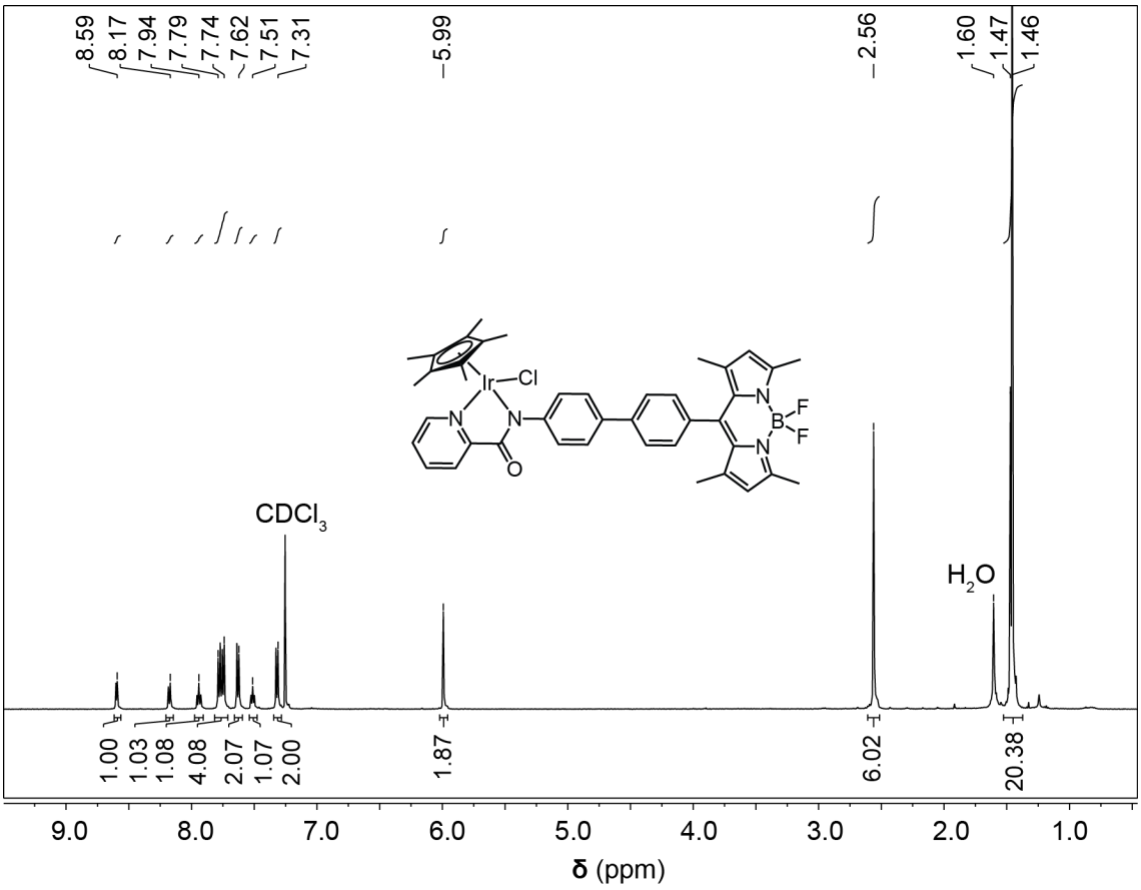


Figure S31. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of complex **Ir1b**.

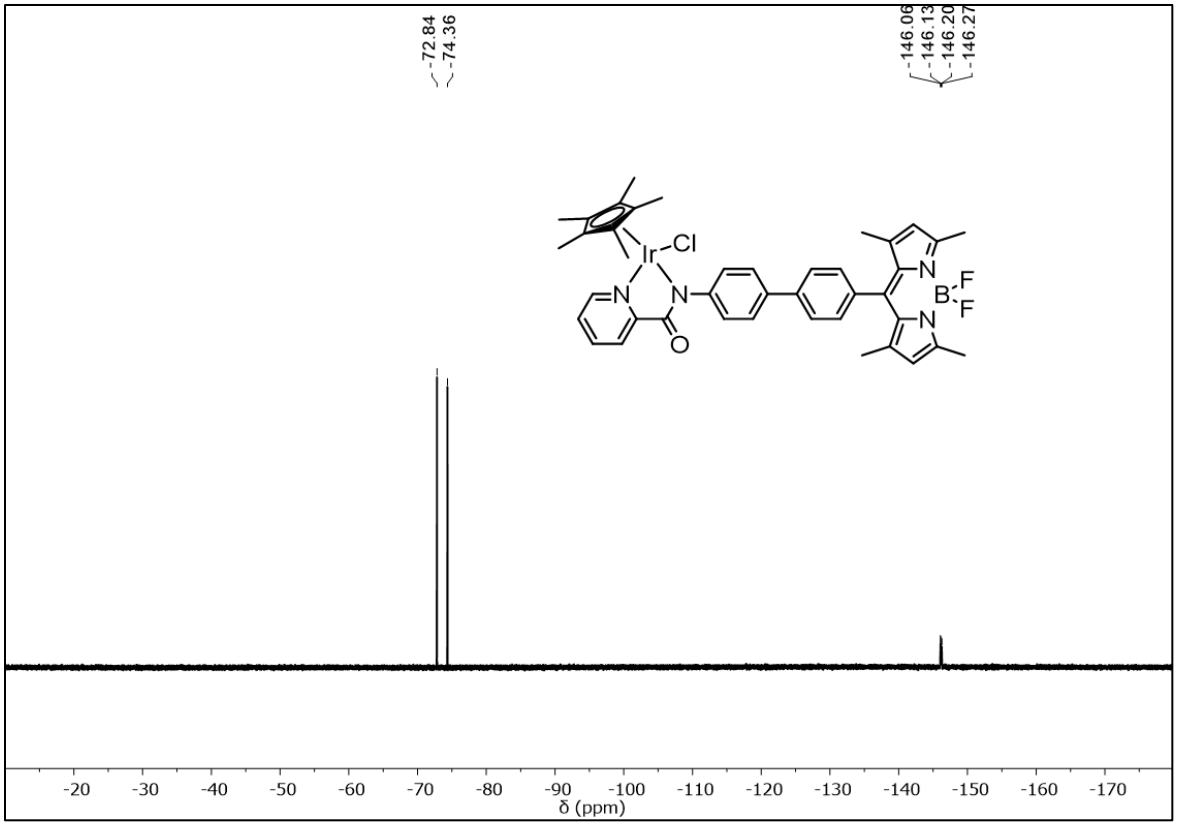


Figure S32. ^{19}F NMR spectrum (470 MHz, $CDCl_3$) of complex **Ir1b**.

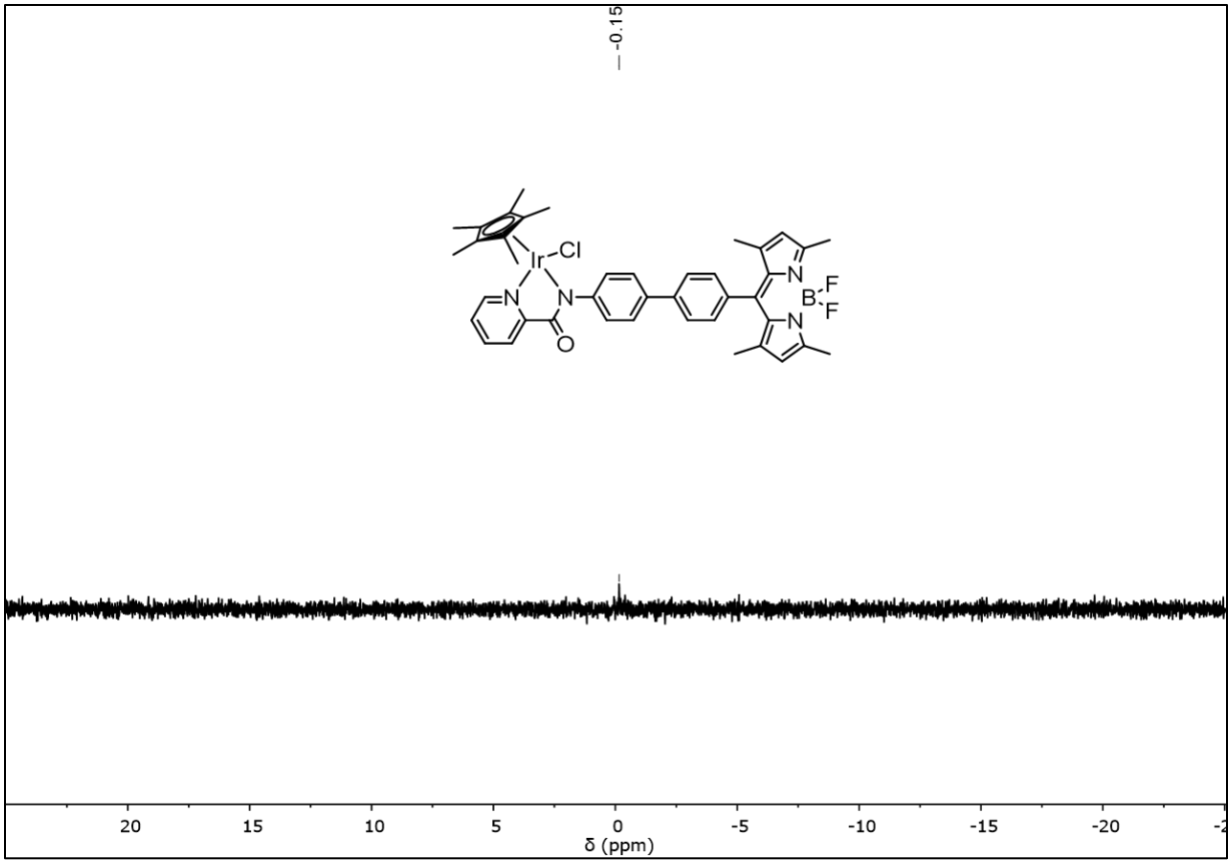


Figure S33. ^{11}B NMR spectrum (160 MHz, $CDCl_3$) of complex **Ir1b**.

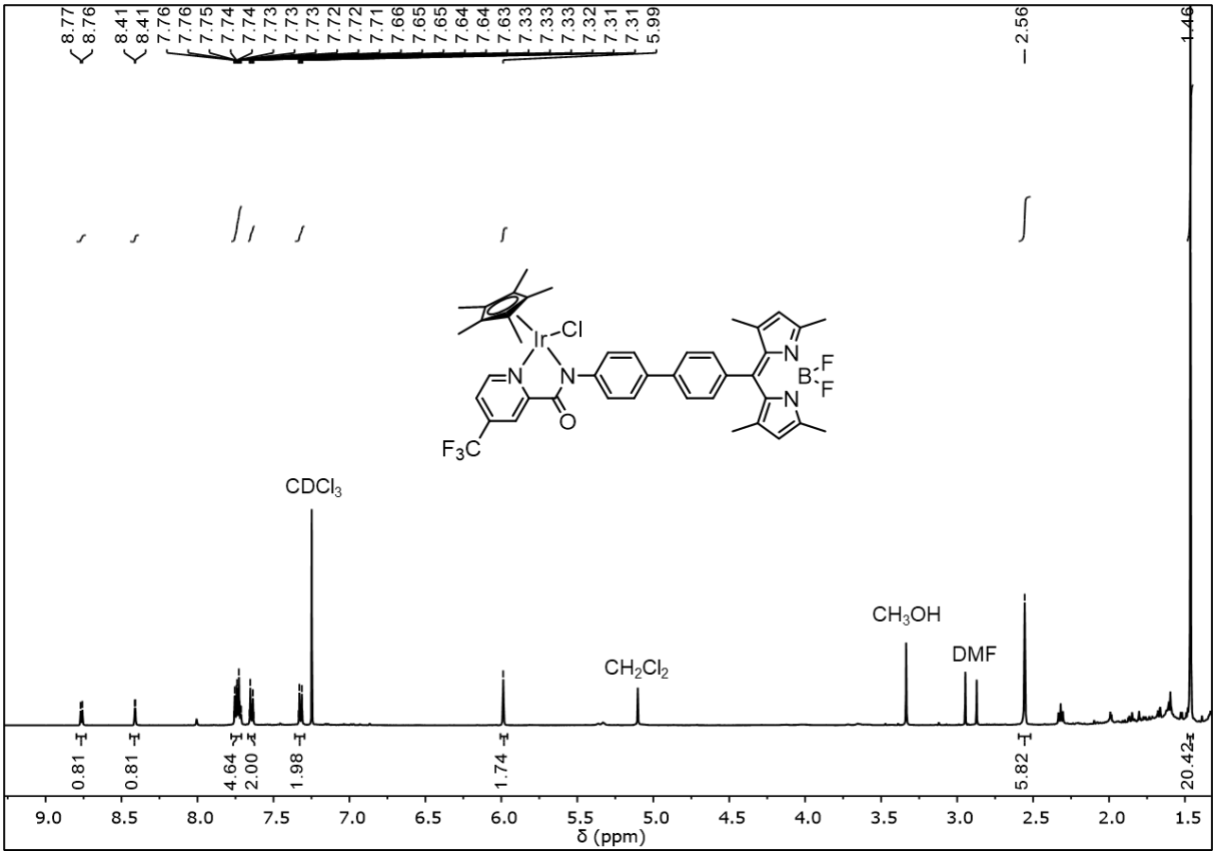


Figure S34. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of complex **Ir2b**.

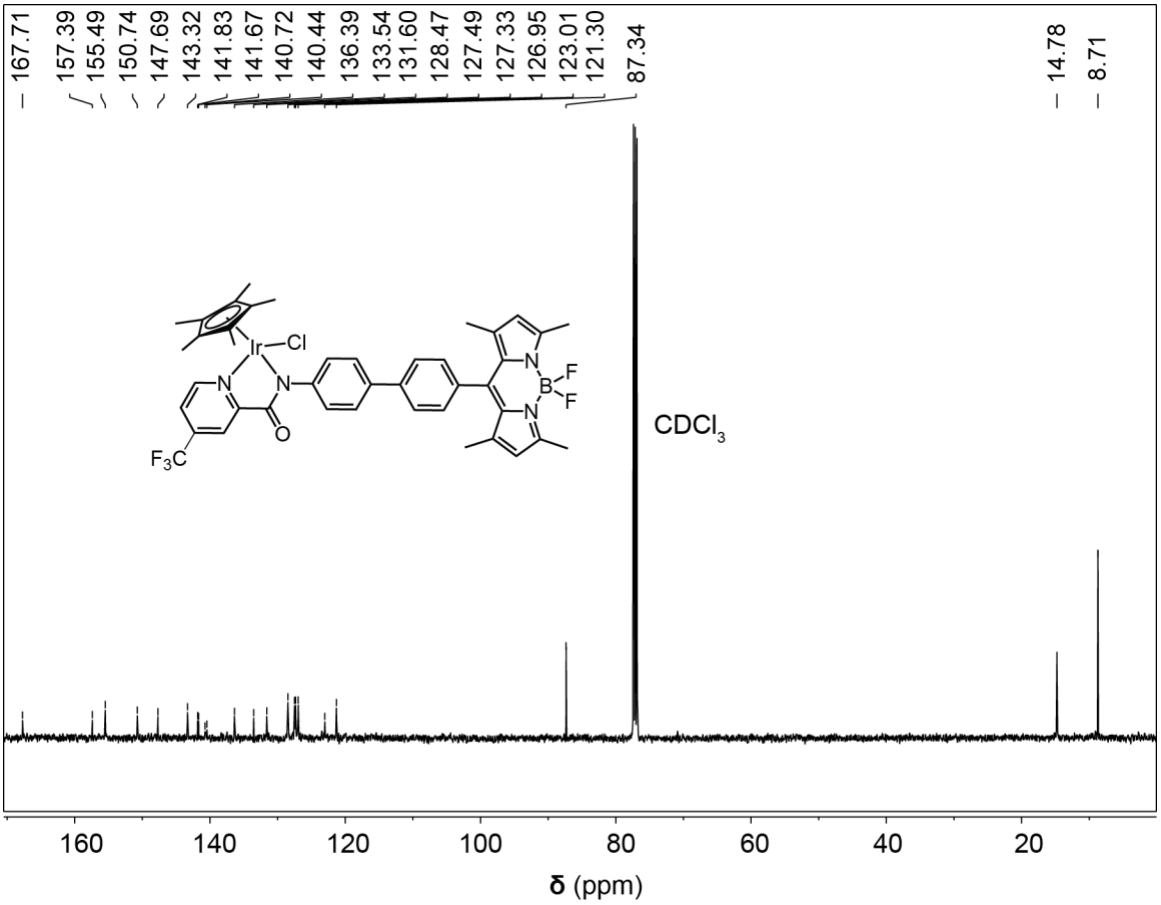


Figure S35. ^{13}C NMR spectrum (126 MHz, $CDCl_3$) of complex **Ir2b**.

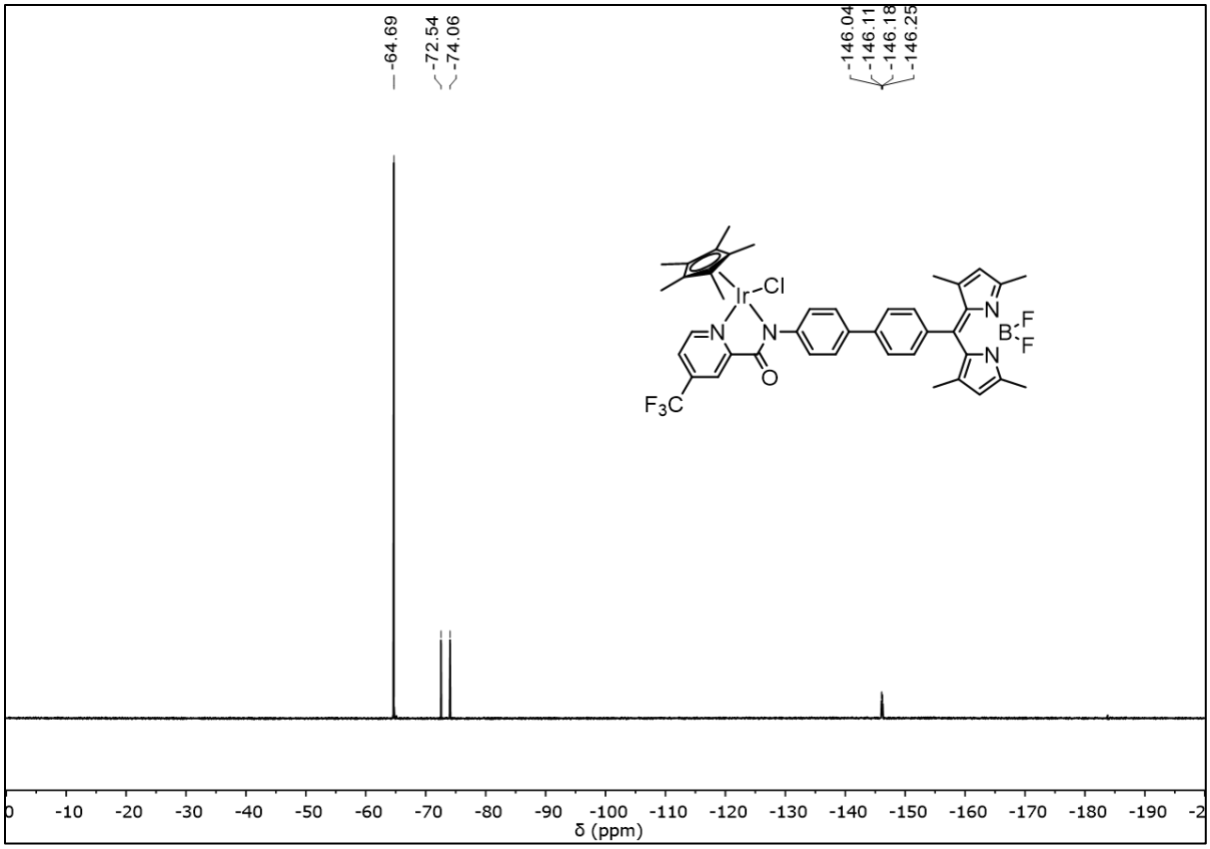


Figure S36. ^{19}F NMR spectrum (470 MHz, $CDCl_3$) of complex **Ir2b**.

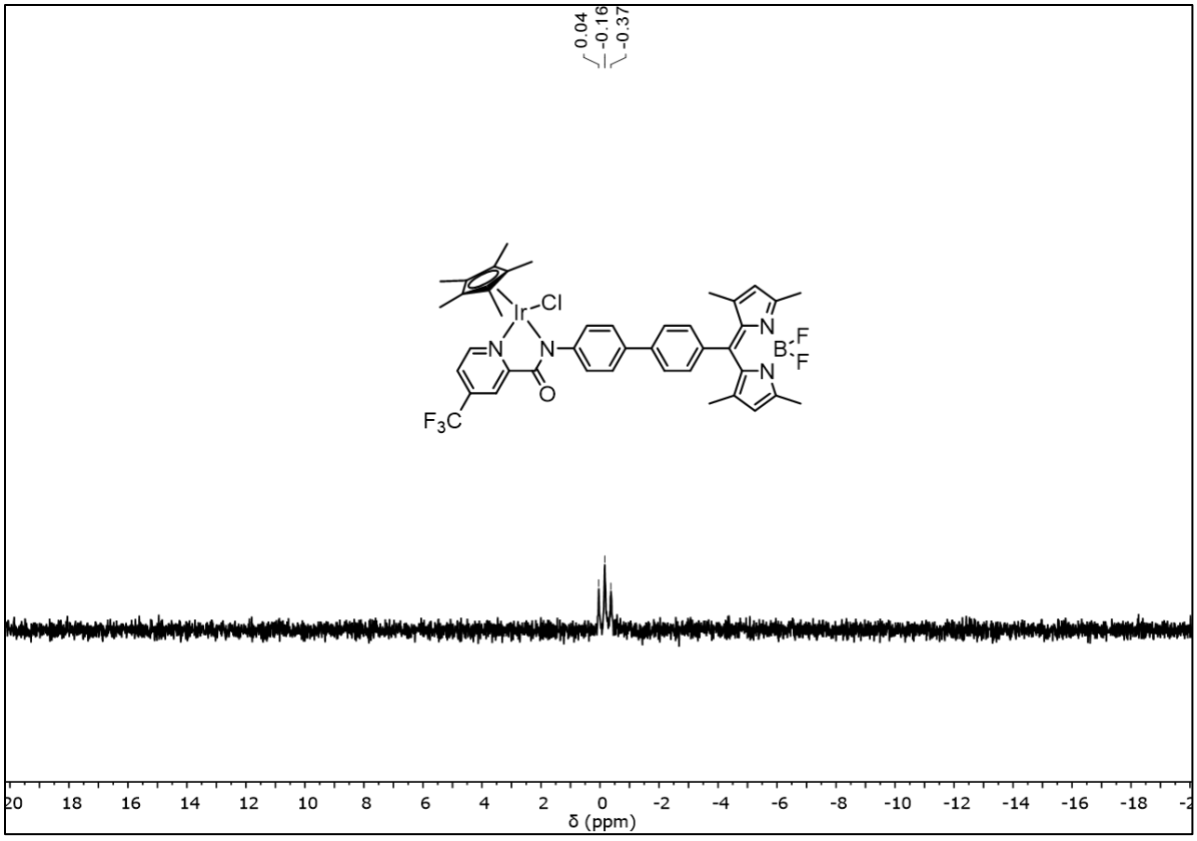


Figure S37. ^{11}B NMR spectrum (160 MHz, $CDCl_3$) of complex **Ir2b**.

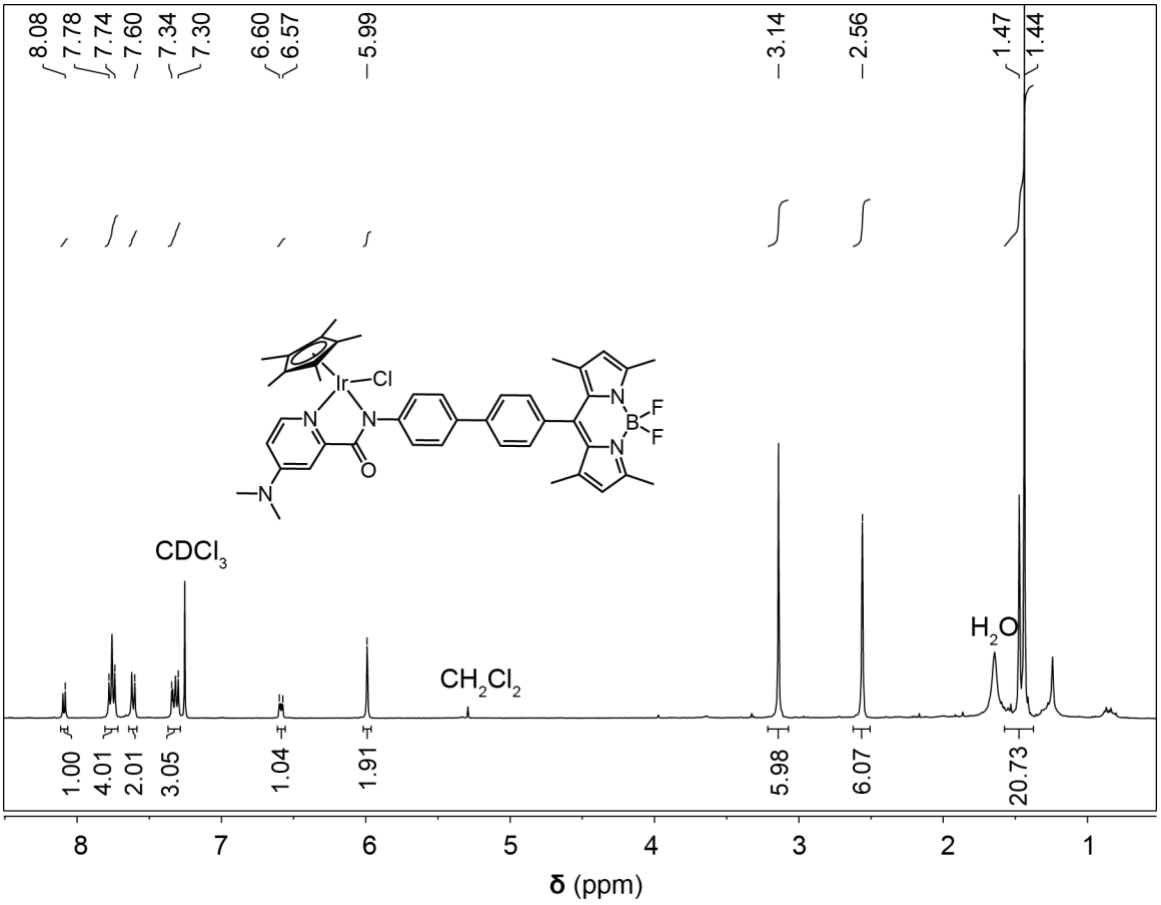


Figure S38. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of complex **Ir3b**.

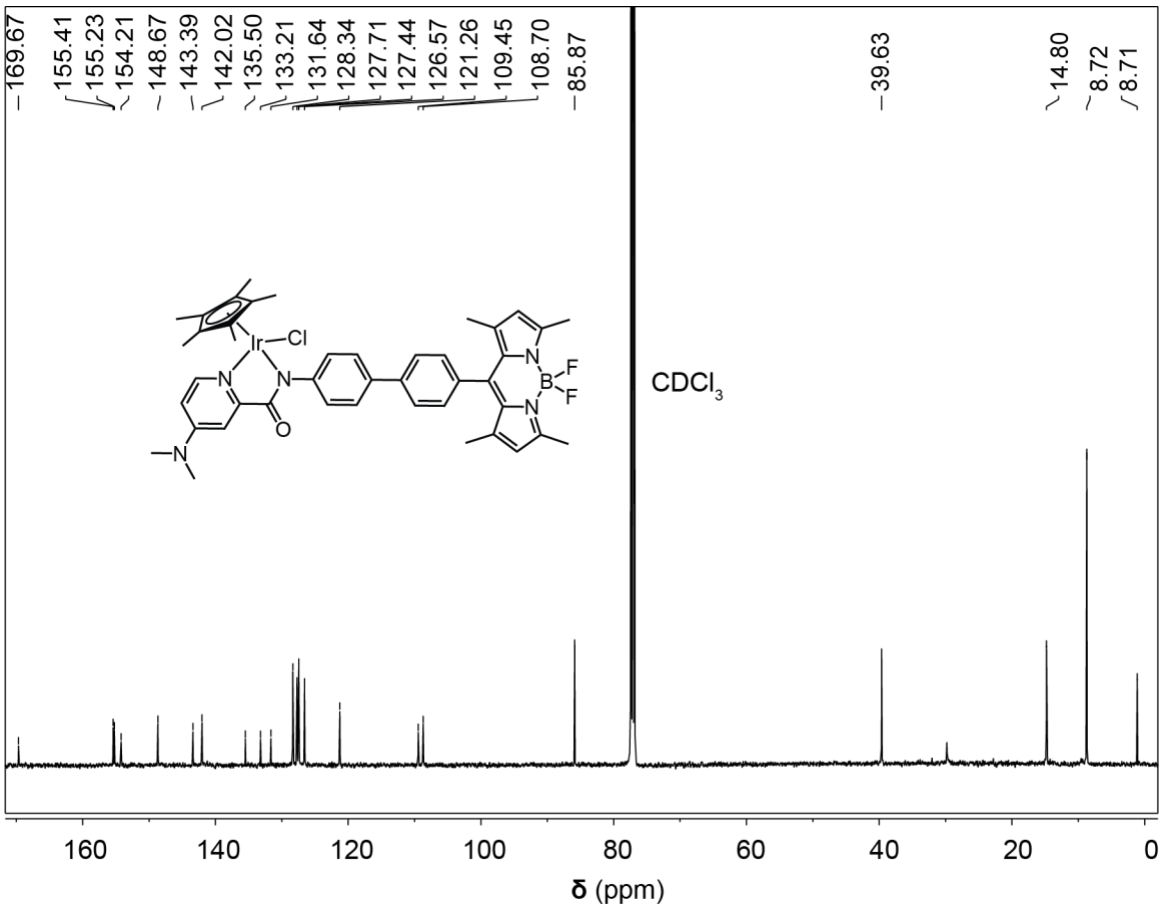


Figure S39. ^{13}C NMR spectrum (126 MHz, $CDCl_3$) of complex **Ir3b**.

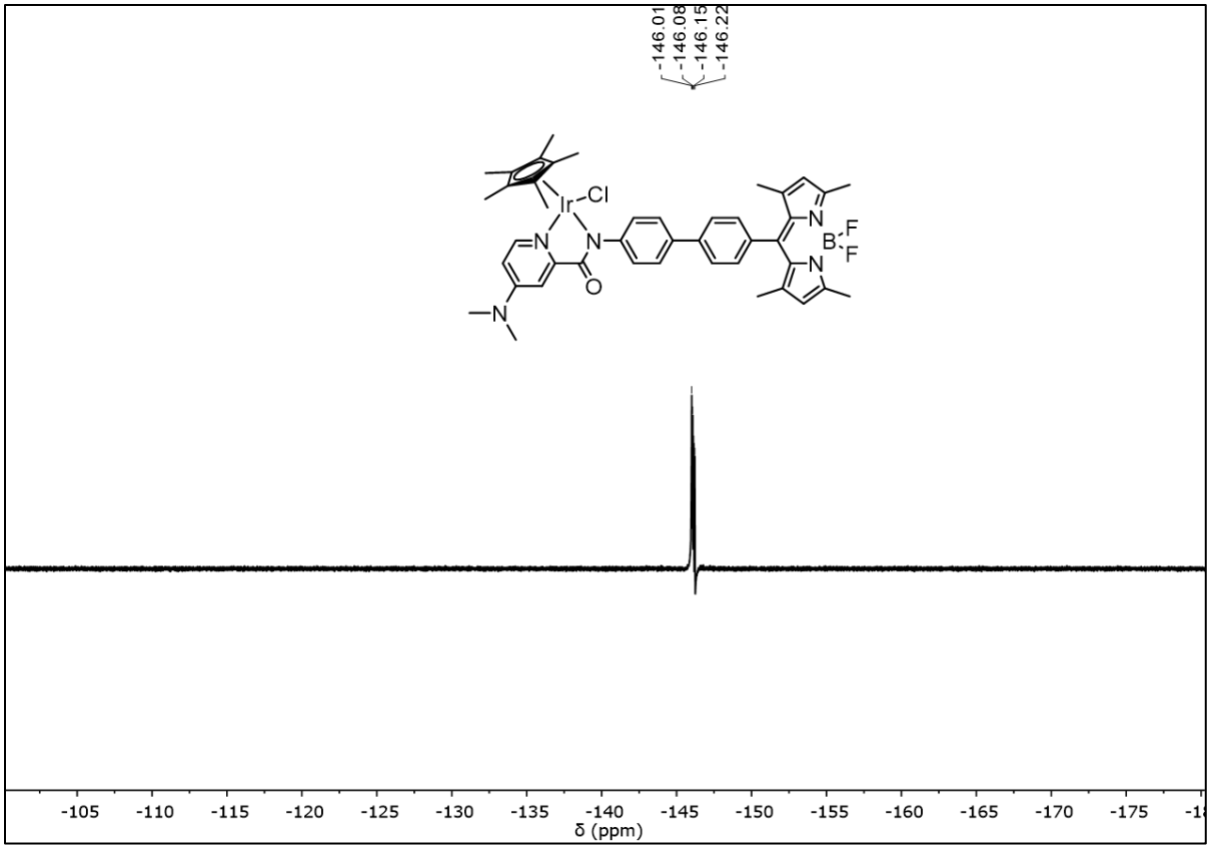


Figure S40. ^{19}F NMR spectrum (470 MHz, $CDCl_3$) of complex **Ir3b**.

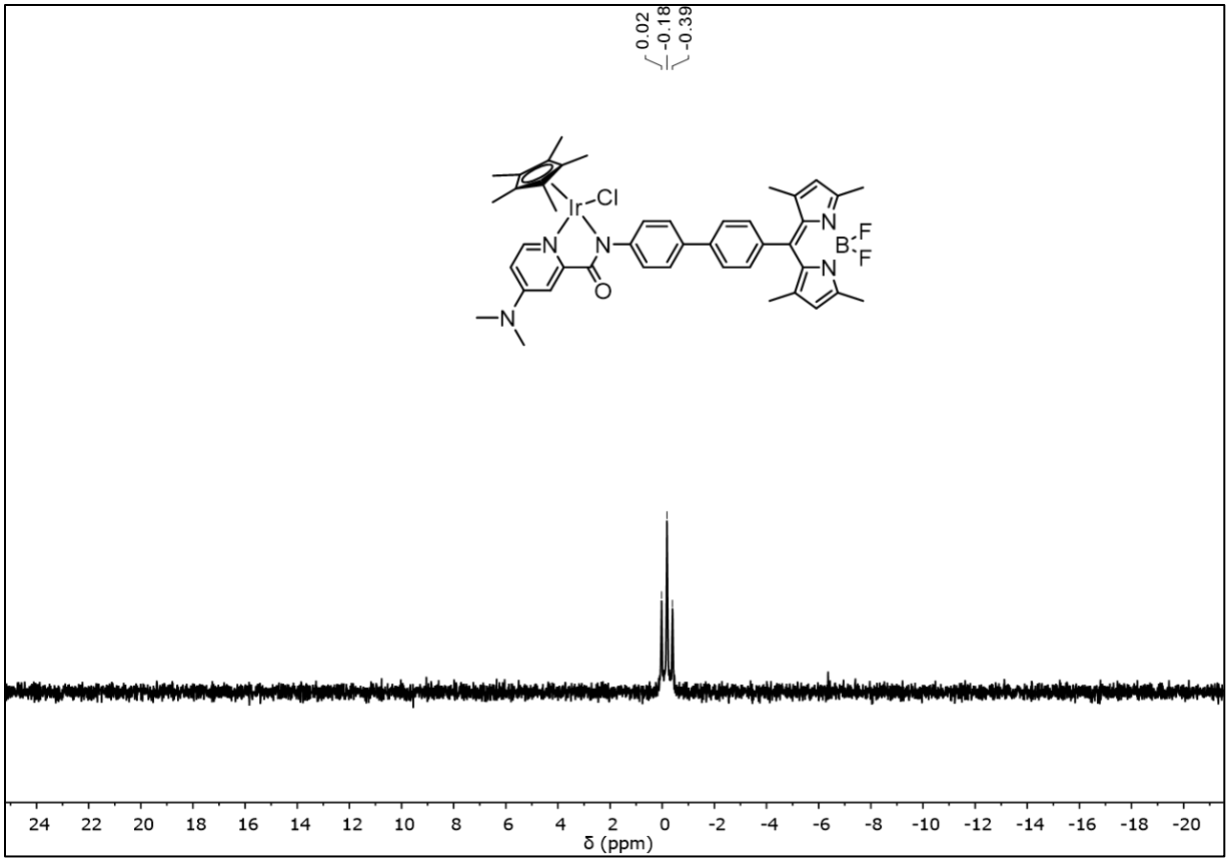


Figure S41. ^{11}B NMR spectrum (160 MHz, $CDCl_3$) of complex **Ir3b**.

Evaluating the Effects of Biomolecules on Fluorescence

A stock solution of **Ir3b** (5 mM) was prepared in DMSO. Stock solutions of *L*-glutamine (100 mM), glycine (100 mM), *L*-lysine (100 mM), *L*-arginine (100 mM), *D*-glucose (100 mM), *L*-glutathione reduced (100 mM) were prepared in millipore water. The appropriate volumes of the stock solutions of interest were combined in a 1.5-dram vial. Additional *t*BuOH and water were added to maintain a constant co-solvent concentration of 20% of *t*BuOH in 3 mL of water. The solutions were stirred for 30 min at 37 °C before transferring to the cuvette for fluorescence measurements.

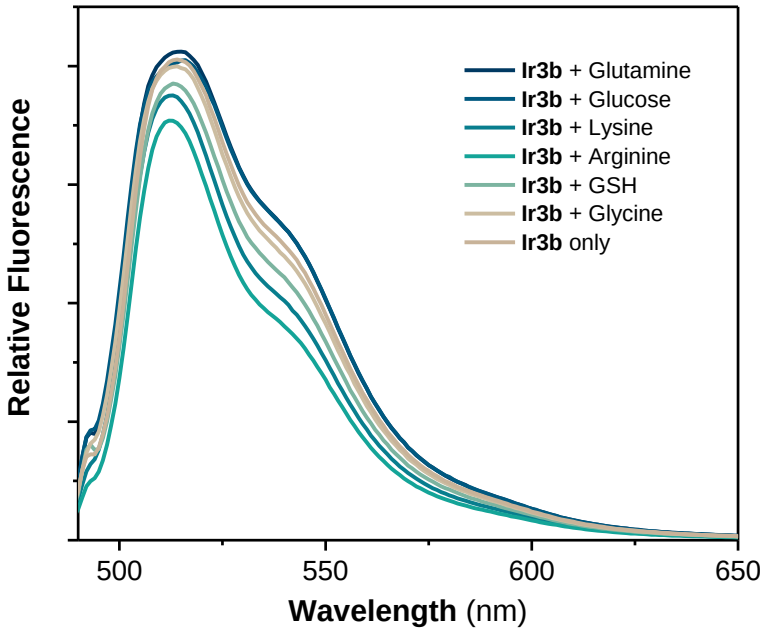


Figure S42. Emission spectra of complex **Ir3b** in the presence of different biomolecules. The complex was dissolved in *t*BuOH/H_2O (1:4) at a concentration of 20 µM. The concentration of biomolecules used correspond to their relative amounts present in commercial DMEM high glucose solutions: *L*-glutamine (4.0 mM), *D*-glucose (25 mM), *L*-lysine (1.0 mM), *L*-arginine (0.2 mM), glycine (0.5 mM), *L*-glutathione reduced (20 µM).

Mass Spectrometry Data

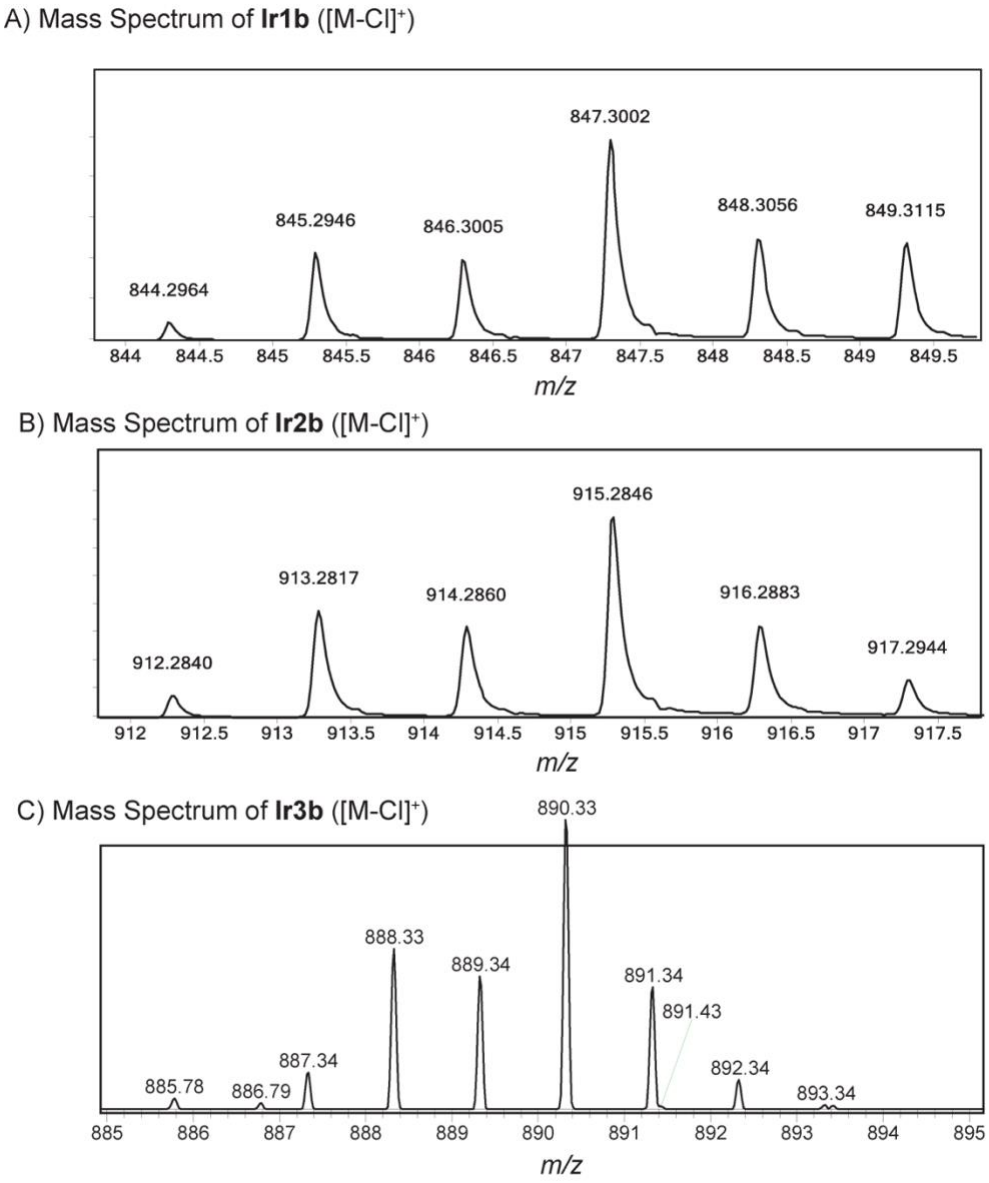


Figure S43. Mass spectrometry data (ESI-MS, positive mode) for **Ir1b** (A), **Ir2b** (B), and **Ir3b** (C) showing the *m*/*z* values for their corresponding $[M\text{-}Cl]^+$ ions.

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