Supplementary Information

A Conceptual Framework for the Development of Iridium(III) Complex-based Electrogenerated Chemiluminescence Labels

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Scheme 1. Additional ECL pathways

Synthesis and characterization

- $[Ir(C^N)_2(dm\text{-}bpy)](PF_6)$ and $[Ir(C^N)_2(ptb)](PF_6)$ complexes
- $[Ir(C^N)_2(pt\text{-}TEG)]Cl$ complexes
- pt-TOxT-Sq ligand
- $[Ir(C^N)_2(pt\text{-}TOxT\text{-}Sq)]^+$ ECL labels

Additional data for $Ir(C^N)_2(acac)$, $[Ir(C^N)_2(dm\text{-}bpy)]^+$, $[Ir(C^N)_2(ptb)]^+$, and $[Ir(C^N)_2(pt\text{-}TEG)]^+$

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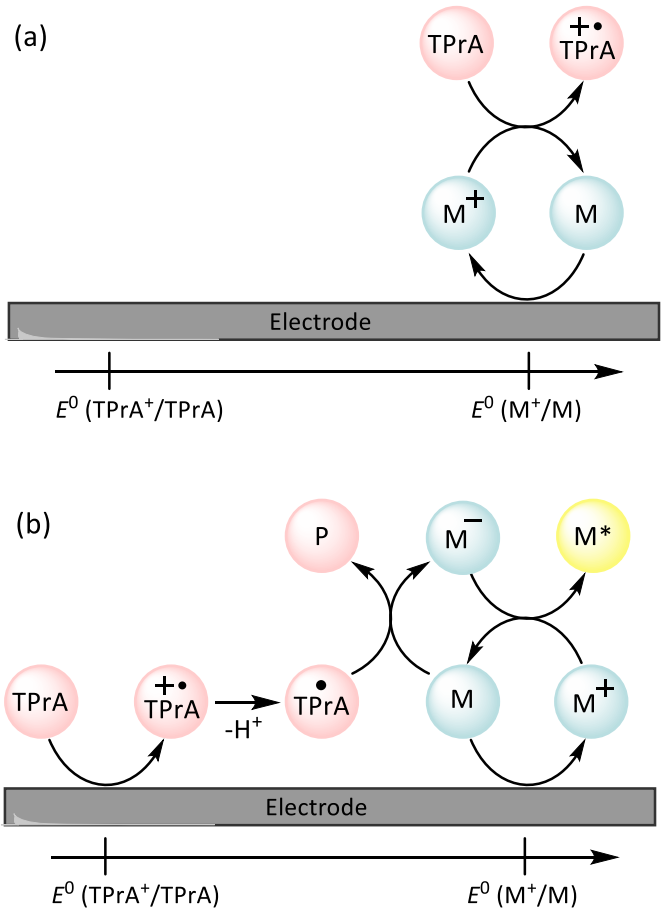
Figure S9. Photoluminescence emission spectra of ECL labels

Table S8. Primers and NASBA amplicon fragment sequences

Figure S10. 3D drawing and photograph of the custom screen-printed electrode (SPE) holder

Figure S11. Photograph of the cell holder

NMR spectra



Scheme S1. (a) The 'catalytic route' of co-reactant ECL involving oxidation of TPrA by M^+, and (b) an alternative ECL pathway[1] in which the excited state is generated from the reaction between M^+ and M^-. The contribution of these pathways to the overall emission intensity is expected to be small when low concentrations of the metal complex are used.

Synthesis of $[Ir(C^N)_2(dm\text{-}bpy)](PF_6)$ and $[Ir(C^N)_2(ptb)](PF_6)$ complexes

Reagents and solvents were purchased from commercial sources and used without further purification. The iridium(III) dimer precursors were purchased from SunaTech (China). NMR spectra were acquired on a Bruker Biospin AV400 spectrometer. ^{1}H NMR spectra were acquired at 400 MHz, and $^{13}C\{^1H\}$ NMR spectra were acquired at 100 MHz. All NMR spectra were recorded at 298 K. Chemical shifts were referenced to residual solvent peaks and are quoted in terms of parts per million (ppm), relative to tetramethylsilane ($Si(CH_3)_4$). Electrospray ionization mass spectra (ESI-MS) were acquired using a Thermo Scientific Exactive Plus Orbitrap Mass Spectrometer.

$[Ir(piq)_2(dm\text{-}bpy)](PF_6)$: The dimer $[Ir(piq)_2(\mu\text{-}Cl)]_2$ (150 mg, 118 μmol) and 4,4′-dimethyl-2,2′-bipyridine (50 mg, 271 μmol) were suspended in a 3:1 mixture of dichloromethane and methanol and heated at reflux whilst stirred in darkness under an inert atmosphere for 16 h. The solution was cooled to ambient temperature and a large excess of KPF_6 was added and the mixture stirred for a further 24 h. The mixture was filtered to remove solid KPF_6 and the filtrate was concentrated under reduced pressure. The residue was redissolved in a minimum amount of acetonitrile and filtered through filter aid (Celite). To this solution was added a saturated aqueous solution of ammonium hexafluorophosphate until precipitation of a brightly colored solid began to occur. The mixture was allowed to stir in darkness for 16 h, and the product then collected by filtration and washed with water, cold ethanol and diethyl ether to yield the product as a dark brown precipitate (114 mg, 52%). ^{1}H NMR (400 MHz; CD_2Cl_2): δ 8.98 (m, 1H), 8.32 (m, 2H), 7.92 (m, 1H), 7.80 (m, 2H), 7.68 (d, *J* = 5.8 Hz, 1H), 7.41 (d, *J* = 6.4 Hz, 1H), 7.34 (d, *J* = 6.4 Hz, 1H), 7.20 (1H, *J* = 5.1 Hz, d), 7.14 (t, *J* = 7.6 Hz 1H), 6.90 (t, *J* = 7.4 Hz, 1H), 6.34 (d, *J* = 8.1 Hz, 1H), 2.57 (s, 3H). ESI-MS (positive ion). Calcd for $C_{42}H_{32}IrN_4$ ($[M]^+$): *m/z* 785.226. Found *m/z* 785.2264.

$[Ir(bt)_2(dm\text{-}bpy)](PF_6)$: The dimer $[Ir(bt)_2(\mu\text{-}Cl)]_2$ (150 mg, 116 μmol) and 4,4′-dimethyl-2,2′-bipyridine (47 mg, 255 μmol) were suspended in a 3:1 mixture of dichloromethane and methanol and heated at reflux whilst stirred in darkness under an inert atmosphere for 16 h. The solution was cooled to ambient temperature and a large excess of KPF_6 was added and the mixture stirred for a further 24 h. The mixture was filtered to remove solid KPF_6 and the filtrate was concentrated under reduced pressure. The crude product was then recrystallized from ethanol to yield the product as an orange solid (119 mg, 54%). ^{1}H NMR (400 MHz; CD_2Cl_2): δ 8.23 (s, 1H), 7.95 (d, *J* = 5.6 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.85 (d, *J* = 7.6 Hz, 1H), 7.37 (t, *J* = 7.4 Hz, 1H), 7.30 (d, *J* = 5.6 Hz, 1H), 7.09-7.14 (m, 2H), 6.88 (td, *J* = 7.5, 1.2 Hz, 1H), 6.41 (d, *J* = 7.6 Hz, 1H), 6.24 (d, *J* = 8.4 Hz, 1H), 2.59 (s, 3H). $^{13}C\{^1H\}$ NMR (100 MHz; CD_2Cl_2): δ 181.8, 156.8, 153, 150.9, 150.7, 149.8, 140.8, 133.9, 132.6, 132.1, 129.6, 128.6, 127.3, 126.5, 125.6, 124, 123.7, 118.3, 21.8. ESI-MS (positive ion). Calcd for $C_{38}H_{28}IrN_4S_2^+$ ($[M]^+$): *m/z* 797.139. Found *m/z* 757.1393.

$[Ir(ppy)_2(dm\text{-}bpy)](PF_6)$: The dimer $[Ir(ppy)_2(\mu\text{-}Cl)]_2$ (150 mg, 140 μmol) and 4,4′-dimethyl-2,2′-bipyridine (57 mg, 309 μmol) were suspended in a 3:1 mixture of dichloromethane and methanol and heated at reflux whilst stirred in darkness under an inert atmosphere for 16 h. The solution was cooled to ambient temperature and a large excess of KPF_6 was added and the mixture stirred for a further 24 h. The mixture was filtered to remove solid

KPF_6 and the filtrate was concentrated under reduced pressure. The crude product was then recrystallized from isopropanol to yield the product as a pale yellow solid (181 mg, 78%). ^{1}H NMR (400 MHz; CD_2Cl_2): δ 8.31 (s, 1H), 7.95 (d, *J* = 8.2 Hz, 1H), 7.82 (d, *J* = 5.6 Hz, 1H), 7.78 (td, *J* = 7.8, 1.5 Hz, 1H), 7.73 (dd, *J* = 7.8, 0.9 Hz, 1H), 7.51 (d, *J* = 5.7 Hz, 1H), 7.23 (d, *J* = 5.5 Hz, 1H), 7.06 (td, *J* = 7.5, 1.1 Hz, 1H), 7.00 (ddd, *J* = 7.4, 5.9, 1.4 Hz, 1H), 6.92 (td, *J* = 7.5, 1.4 Hz, 1H), 6.32 (d, *J* = 7.6 Hz, 1H), 2.58 (s, 3H). ESI-MS (positive ion). Calcd for $C_{34}H_{28}IrN_4$ ($[M]^+$): *m/z* 685.194. Found *m/z* 685.1949.

[Ir(df-ppy)$_2$(dm-bpy)](PF$_6$): The dimer $[Ir(df\text{-}ppy)_2(\mu\text{-}Cl)]_2$ (150 mg, 123 μmol) and 4,4′-dimethyl-2,2′-bipyridine (50 mg, 271 μmol) were suspended in a 3:1 mixture of dichloromethane and methanol and heated at reflux whilst stirred in darkness under an inert atmosphere for 16 h. The solution was cooled to ambient temperature and a large excess of KPF_6 was added and the mixture stirred for a further 24 h. The mixture was filtered to remove solid KPF_6 and the filtrate was concentrated under reduced pressure. The crude product was then recrystallized from isopropanol to yield the product as a pale yellow solid (165 mg, 74%). ^{1}H NMR (400 MHz; CD_2Cl_2): δ 8.37 (s, 1H), 8.32 (d, *J* = 8.4 Hz, 1H), 7.82 (m, 2H), 7.50 (d, *J* = 5.7 Hz, 1H), 7.29 (d, *J* = 5.7 Hz, 1H), 7.05 (m, 1H), 6.62 (ddd, *J* = 12.5, 9.2, 2.4 Hz, 1H), 5.75 (dd, *J* = 8.4, 2.3 Hz, 1H), 2.61 (s, 3H). ESI-MS (positive ion). Calcd for $C_{34}H_{24}F_4IrN_4^+$ ($[M]^+$): *m/z* 757.157. Found *m/z* 757.1575.

[Ir(piq)$_2$(ptb)](PF$_6$): This complex was synthesized according to the previously published procedure.[2] The dimer $[Ir(piq)_2(\mu\text{-}Cl)]_2$ (150 mg, 118 μmol) and 2-(1-(benzyl)-1H-1,2,3-triazol-4-yl)pyridine (56 mg, 236 μmol) were suspended in a 3:1 mixture of dichloromethane and methanol. Starting materials typically solubilized within 1 h. Reactions were stirred in darkness under an inert atmosphere for 16 h. The solvents were then removed and the residue dissolved in acetonitrile and filtered through a filter aid (Celite). The solvent was then removed by evaporation under reduced pressure and the residue redissolved in a minimum amount of ethanol and filtered through filter aid (Celite). To this solution was added a saturated aqueous solution of ammonium hexafluorophosphate until precipitation of a brightly colored solid began to occur. The mixture was allowed to stir in darkness for 16 h, and the product was then collected by filtration and washed with water, cold ethanol, ether, and lastly pentane, and then dried *in vacuo* to yield the product as an orange solid (153 mg, 66 %). ^{1}H NMR spectra was consistent with the literature values. ^{1}H NMR (400 MHz; CD_2Cl_2): δ 9.00 (m, 2H), 8.62 (s, 1H), 8.33 (m, 2H), 8.12 (d, *J* = 7.9 Hz, 1H), 7.91-8.00 (m, 3H), 7.84-7.99 (m, 4H), 7.69 (d, *J* = 5.6 Hz, 1H), 7.60 (d, *J* = 6.4 Hz, 1H), 7.39 (t, *J* = 6.9 Hz, 2H), 7.09-7.33 (m, 9H), 6.93 (t, *J* = 7.4 Hz, 1H), 6.84 (t, *J* = 7.4 Hz, 1H), 6.51 (d, *J* = 7.6 Hz, 1H), 6.32 (d, *J* = 7.6 Hz, 1H), 5.57 (s, 2H). ESI-MS (positive ion). Calcd for $C_{44}H_{32}IrN_6^+$ ($[M]^+$): *m/z* 837.231. Found *m/z* 837.2410.

[Ir(bt)$_2$(ptb)](PF$_6$): The dimer $[Ir(bt)_2(\mu\text{-}Cl)]_2$ (150 mg, 116 μmol) and 2-(1-(benzyl)-1H-1,2,3-triazol-4-yl)pyridine (55 mg, 232 μmol) were suspended in a 3:1 mixture of dichloromethane and methanol. Starting materials typically solubilized within 1 h. Reactions were stirred in darkness under an inert atmosphere for 16 h. The solvents were then removed and the residue dissolved in acetonitrile and filtered through a filter aid (Celite).

The solvent was then removed by evaporation under reduced pressure and the residue redissolved in a minimum amount of ethanol and filtered through filter aid (Celite). To this solution was added a saturated aqueous solution of ammonium hexafluorophosphate until precipitation of a brightly colored solid began to occur. The mixture was allowed to stir in darkness for 16 h, and the product was then collected by filtration and washed with water, cold ethanol, ether, and lastly pentane, and then dried *in vacuo* to yield the product as a dark yellow solid (191 mg, 83%). ^{1}H NMR (400 MHz; CD_2Cl_2): δ 8.67 (s, 1H), 8.06 (dq, *J* = 8.0, 0.8 Hz, 1H), 8.00 (td, *J* = 7.8, 1.6 Hz, 1H), 7.94 (dq, *J* = 5.5, 0.8 Hz, 1H), 7.90 (dq, *J* = 8.1, 0.6 Hz, 1H), 7.87 (dq, *J* = 8.1, 0.6 Hz, 1H), 7.84 (m, 1H), 7.80 (m, 1H), 7.32-7.43 (m, 6H), 7.25-7.29 (m, 2H), 7.11 (td, *J* = 7.6, 1.2 Hz, 1H), 7.03-7.08 (m, 2H), 6.95 (ddd, *J* = 8.5, 7.3, 1.3 Hz, 1H), 6.88 (td, *J* = 7.6, 1.5 Hz, 1H), 6.82 (td, *J* = 7.6, 1.5 Hz, 1H), 6.48 (d, *J* = 7.7 Hz, 1H), 6.40 (d, *J* = 8.4 Hz, 1H), 6.37 (d, *J* = 7.8 Hz, 1H), 6.08 (d, *J* = 8.3 Hz, 1H), 5.65 (d, *J* = 14.8 Hz, 1H), 5.55 (d, *J* = 14.8 Hz, 1H). ^{13}C{^{1}H} NMR (100 MHz; CD_2Cl_2): δ 182.2, 181.3, 151.1, 150.6, 150.2, 150.0, 149.7 (2C), 146.4, 141.3, 140.9, 140.6, 134.2, 134.0 (2C), 132.5, 132.0 (2C), 131.9, 129.9 (2C), 129.8, 128.6 (3C), 128.5, 127.3, 127.2, 126.7, 126.6, 126.4 (2C), 124.0, 123.9, 123.8, 123.5, 123.4, 118.7, 118.0, 56.5. ESI-MS (positive ion). Calcd for $C_{40}H_{28}IrN_6S_2^+$ ([M]$^+$): *m/z* 849.144. Found *m/z* 849.1458.

[Ir(ppy)$_2$(ptb)](PF$_6$): This complex was synthesized according to the previously published procedure.[2] The dimer $[Ir(ppy)_2(\mu\text{-}Cl)]_2$ (150 mg, 140 μmol) and 2-(1-(benzyl)-1H-1,2,3-triazol-4-yl)pyridine (66 mg, 280 μmol) were suspended in a 3:1 mixture of dichloromethane and methanol. Starting materials typically solubilized within 1 h. Reactions were stirred in darkness under an inert atmosphere for 16 h. The solvents were then removed and the residue dissolved in acetonitrile and filtered through a filter aid (Celite). The solvent was then removed by evaporation under reduced pressure and the residue redissolved in a minimum amount of ethanol and filtered through filter aid (Celite). To this solution was added a saturated aqueous solution of ammonium hexafluorophosphate until precipitation of a brightly colored solid began to occur. The mixture was allowed to stir in darkness for 16 h, and the product was then collected by filtration and washed with water, cold ethanol, ether, and lastly pentane, and then dried *in vacuo* to yield the product as a yellow solid (197 mg, 80%). ^{1}H NMR spectra was consistent with the literature values. ^{1}H NMR (400 MHz; CD_2Cl_2): δ 8.62 (s, 1H), 8.11 (d, *J* = 7.9 Hz, 1H), 7.99 (dt, *J* = 7.9, 1.6 Hz, 1H), 7.95 (t, 8.8 Hz, 2H), 7.76-7.84 (m, 3H), 7.69-7.74 (m, 2H), 7.67 (d, *J* = 5.8 Hz, 1H), 7.49 (d, *J* = 5.8 Hz, 1H), 7.38-7.42 (m, 3H), 7.26-7.34 (m, 3H), 7.00-7.09 (m, 3H), 6.98 (ddd, *J* = 7.5, 5.8, 1.4 Hz, 1H), 6.93 (td, *J* = 7.5, 1.3 Hz, 1H), 6.88 (td, *J* = 7.5, 1.3 Hz, 1H), 6.32 (dd, *J* = 7.7, 0.6 Hz, 1H), 6.31 (dd,*J* = 7.6, 0.8 Hz, 1H), 5.62 (m, 2H). ESI-MS (positive ion). Calcd for $C_{36}H_{28}IrN_6^+$ ([M]$^+$): *m/z* 737.200. Found *m/z* 737.2012.

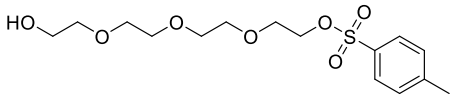
[Ir(df-ppy)$_2$(ptb)](PF$_6$): This complex was synthesized according to the previously published procedure.[2] The dimer $[Ir(df\text{-}ppy)_2(\mu\text{-}Cl)]_2$ (100 mg, 82 μmol) and 2-(1-(benzyl)-1H-1,2,3-triazol-4-yl)pyridine (39 mg, 165 μmol) were suspended in a 3:1 mixture of dichloromethane and methanol. Starting materials typically solubilized within 1 h. Reactions were stirred in darkness under an inert atmosphere for 16 h. The solvents were then removed and the residue dissolved in acetonitrile and filtered through a filter aid (Celite). The solvent was then removed by evaporation under reduced pressure and the residue redissolved in a minimum amount of ethanol and filtered

through filter aid (Celite). To this solution was added a saturated aqueous solution of ammonium hexafluorophosphate until precipitation of a brightly colored solid began to occur. The mixture was allowed to stir in darkness for 16 h, and the product was then collected by filtration and washed with water, cold ethanol, ether, and lastly pentane, and then dried *in vacuo* to yield the product as a yellow solid (126 mg, 80%). ^{1}H NMR spectra was consistent with the literature values. ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.80 (s, 1H), 8.30 (t, *J* = 10.5 Hz, 2H), 8.22 (d, *J* = 7.9 Hz, 1H), 8.00 (td, *J* = 7.9, 1.5 Hz, 1H), 7.79-7.83 (m, 3H), 7.61 (d, *J* = 5.8 Hz, 1H), 7.45 (d, *J* = 5.8 Hz, 1H), 7.36 (s, 5H), 7.32 (m, 1H), 7.11 (ddd, *J* = 7.3, 5.9, 1.3 Hz, 1H), 7.01 (ddd, *J* = 7.3, 5.9, 1.3 Hz, 1H), 6.55 (m, 2H), 5.73 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.68 (dd, *J* = 8.5, 2.3 Hz, 1H), 5.58 (s, 2H). ESI-MS (positive ion). Calcd for $C_{36}H_{24}F_4IrN_6^+$ ($[M]^+$): *m/z* 808.162. Found *m/z* 808.1638.

Synthesis of pt-TEG ligand and [Ir(C^N)$_2$(pt-TEG)]Cl complexes

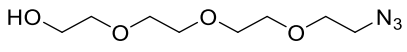
The iridium(III) dimer precursors were purchased from SunaTech (China). The ligand pt-TEG was synthesized according to the previously reported procedure.[3, 4] Nuclear magnetic resonance (NMR) spectra were acquired on a Jeol 400 spectrometer or a Bruker Ascend 500 spectrometer. ^{1}H NMR spectra were acquired at 400 MHz or 500 MHz, ^{13}C{^{1}H} NMR spectra were acquired at 101 MHz or 126 MHz, and ^{19}F NMR acquired at 471 MHz. All NMR spectra were recorded at 298 K. ^{1}H and ^{13}C{^{1}H} chemical shifts are referenced to residual solvent peaks and quoted in ppm relative to TMS. ^{19}F NMR signals are quoted relative to an internal standard of trifluoroacetic acid. HRMS spectra were recorded on an Agilent 6510 ESI-TOF LC/MS Mass Spectrometer.

2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate:



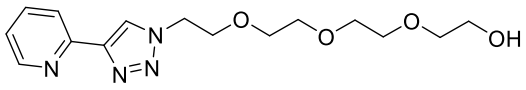
A solution of sodium hydroxide (0.70 g, 17.5 mmol) in H_2O (4 mL) was added to a solution of tetraethylene glycol (20 mL, 116 mmol) in THF (4 mL, 0°C). A mixture of tosyl chloride (2.12 g, 11.1 mmol) in THF (4 mL) was added dropwise over 1 h to the mixture and was stirred for a further 3.5 h at 0°C. The mixture was added to iced water (100 mL) and extracted with DCM (3 × 50 mL). The combined organic extracts were washed (H_2O, 2 × 30 mL) then dried ($MgSO_4$) and the solvent was removed under reduced pressure to afford a slightly yellow oil (3.66g, 91%), ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.81-7.79 (m, 2H, Ar*H*), 7.35-7.33 (m, 2H, Ar*H*), 4.17-4.15 (m, 2H, CH_2), 3.72-3.58 (m, 15H, CH_2), 2.44 (s, 3H, CH_3). ^{13}C{^{1}H} NMR (101 MHz; $CDCl_3$): δ 144.9, 133.0, 129.9, 128.1, 72.5, 70.8, 70.7, 70.6, 70.4, 69.3, 68.8, 61.8, 21.7.

2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-ol:



A mixture of the product above (3.56 g, 9.83 mmol) and sodium azide (1.47 g, 22.6 mmol) in ethanol (60 mL) was stirred at 70°C for 16 h. The solution was let cool to ambient temperature before H_2O (50 mL) was added and the solvent volume was reduced under reduced pressure. The aqueous mixture was extracted with ethyl acetate (3 × 50 mL) and the combined organic extracts were dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue purified by column chromatography (SiO_2, 100% DCM → 5% MeOH) to afford a slightly yellow oil (1.30g, 60%). ^{1}H NMR (400 MHz; $CDCl_3$): δ 3.68 (t, *J* = 4.6 Hz, 2H), 3.64 (m, 10H), 3.57 (t, *J* = 4.6 Hz, 2H), 3.36 (t, *J* = 5.0 Hz, 2H), 2.77 (s, 1H). $^{13}C\{^1H\}$ NMR (101 MHz; $CDCl_3$): δ 72.6, 70.7, 70.7, 70.6, 70.3, 70.0, 61.7, 50.7.

pt-TEG:[3b]



To a solution of the azide (0.57 g, 2.6 mmol) in a 2:1 mixture of DMSO/H_2O (3 mL) was added 2-ethynylpyridine (0.33 g, 3.23 mmol), $CuSO_4.5H_2O$ (15 mg, 2 mol%) and sodium ascorbate (0.10 g, 20 mol%). The mixture was stirred at ambient temperature for 20 h before the reaction was quenched by addition of 1M EDTA in NH_4OH (50 mL). The mixture was extracted with DCM (3 × 50 mL) and the combined organic extracts were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO_2, 100% DCM → 5% MeOH) to afford a yellow oil (0.50 g, 60%). ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.56 (ddd, *J* = 4.9, 1.8, 1.0 Hz, 1H), 8.41 (s, 1H), 8.18 (dt, *J* = 8.0, 1.1 Hz, 1H), 7.81 – 7.75 (m, 1H), 7.23 (ddd, *J* = 7.5, 4.9, 1.2 Hz, 1H), 4.64 – 4.59 (m, 2H), 3.92 (dd, *J* = 5.4, 4.8 Hz, 2H), 3.72 (dd, *J* = 5.3, 3.9 Hz, 2H), 3.67 – 3.57 (m, 10H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 150.3, 149.1, 147.8, 137.5, 123.8, 123.0, 120.6, 72.7, 70.8, 70.7, 70.6, 70.4, 69.6, 61.8, 50.6.

[Ir(piq)$_2$(pt-TEG)]Cl: The dimer $[Ir(piq)_2(\mu\text{-}Cl)]_2$ (229 mg, 0.2 mmol) and the ligand pt-TEG (117 mg, 0.4 mmol) were suspended in a stirred solution of dichloromethane and methanol (3:1 v/v, 10 mL). Full dissolution occurred within 1 h and the solution was allowed to stir in the dark for 14 h. The solution was concentrated under reduced pressure and the residue was taken up in a minimum of dichloromethane then diethyl ether was added until the mixture became cloudy. The mixture was stored at -20°C overnight to afford an orange powder. The solid was isolated by filtration, washed with diethyl ether and dried *in vacuo* to afford an orange powder (189 mg, 56%). ^{1}H NMR (400 MHz; $CDCl_3$): δ 10.38 (s, 1H), 9.02 (d, *J* = 7.9 Hz, 1H), 8.95 (ddd, *J* = 10.5, 6.9, 3.4 Hz, 2H), 8.24 (dd, *J* = 10.4, 5.9 Hz, 2H), 8.01 (td, *J* = 7.8, 1.5 Hz, 1H), 7.93-7.87 (m, 2H), 7.79-7.72 (m, 4H), 7.58-7.53 (m, 2H), 7.41-7.37 (m, 2H), 7.32-7.28 (m, 1H), 7.18-7.14 (m, 1H), 7.12-7.08 (m, 1H), 7.06-7.02 (m, 1H), 6.90-6.86 (m, 1H), 6.83-6.79 (m, 1H), 6.39 (dd, *J* = 7.7, 1.1 Hz, 1H), 6.30-6.28 (m, 1H), 4.70-4.61 (m, 2H), 3.94 (ddd, *J* = 13.0, 6.4, 4.2 Hz, 1H), 3.71 (dd, *J* = 6.0, 3.0 Hz, 2H), 3.64-3.45 (m, 11H). $^{13}C\{^1H\}$ NMR (101 MHz; $CDCl_3$): δ 169.6, 168.8, 153.7, 150.2, 150.1, 149.6, 148.4, 145.8, 145.6, 141.4, 140.5, 139.9, 137.2, 137.1, 132.6, 132.5, 131.7, 131.7, 130.8, 130.6, 130.3, 130.1, 129.3, 128.7, 128.6, 127.7, 127.5, 127.2, 126.9, 126.4, 125.9, 124.9, 122.3, 122.0, 121.0, 121.5, 77.4, 72.8, 70.6, 70.6, 70.4, 70.2, 68.4, 61.5, 52.0. ESI-MS (positive ion). Calcd for $C_{45}H_{42}IrN_6O_4^+$ ($[M]^+$): *m/z* 923.29. Found *m/z* 923.2500.

[Ir(bt)$_2$(pt-TEG)]Cl: The dimer [Ir(bt)$_2$(μ-Cl)]$_2$ (247 mg, 0.2 mmol) and the ligand pt-TEG (124 mg, 0.4 mmol) were stirred in a mixture of dichloromethane and methanol (3:1 v/v, 10 mL) and the solution was allowed to stir in the dark for 14 h. Some solid was still present and was removed by centrifugation and the supernatant was decanted off and retained. The supernatant was concentrated under reduced pressure and the residue was taken up in a minimum of dichloromethane then PET spirits (bp 40-60°C) was added until the mixture became cloudy. The mixture was stored at -20°C overnight to afford a yellow crystalline solid. The solid was isolated by filtration, washed with PET spirits and dried *in vacuo* to afford a yellow crystalline powder (154 mg, 42%). ^{1}H NMR (400 MHz; DMSO-d$_6$): δ 9.40 (s, 1H), 8.44 (d, *J* = 8.0 Hz, 1H), 8.25 (m, 3H,), 8.01 (d, *J* = 7.7 Hz, 1H), 7.91 (d, *J* = 7.6 Hz, 1H,), 7.87 (d, *J* = 5.5 Hz, 1H), 7.66 (m, 1H), 7.46 (m, 1H), 7.42 (m, 1H), 7.29 (m, 1H), 7.14 (m, 2H), 7.02 (m, 1H), 6.93 (m, 1H), 6.83 (m, 1H), 6.35 (m, 2H), 6.28 (d, *J* = 7.7 Hz, 1H), 6.08 (d, *J* = 8.4 Hz, 1H), 4.73 (m, 2H), 3.82 (m, 2H), 3.45 (m, 13H,). ^{13}C{^{1}H} NMR (101 MHz; DMSO-d$_6$): δ 181.5, 180.8, 150.0, 149.7, 148.7, 148.5, 148.4, 146.6, 140.8, 140.4, 140.2, 132.9, 132.8, 132.0, 131.3, 131.2 (2C), 131.2, 128.3, 128.0, 127.6, 127.3, 127.0, 126.4, 126.1, 125.9, 124.7, 124.6, 123.2, 122.8, 122.7, 117.5, 116.6, 72.3, 69.7, 69.6, 69.6, 69.6, 68.4, 60.1, 51.9. ESI-MS (positive ion). Calcd for $C_{41}H_{38}IrN_6O_4S_2^+$ ([M]$^+$): *m/z* 935.20. Found *m/z* 935.1620.

[Ir(ppy)$_2$(pt-TEG)]Cl: The chlorido-bridged dimer [Ir(ppy)$_2$(μ-Cl)]$_2$ (132 mg, 123 μmol) and the ligand pt-TEG (80 mg, 247 μmol) were suspended in a stirred solution of dichloromethane and methanol (3:1 v/v, 5 mL). Full dissolution occurred within 1 h and the solution was allowed to stir in the dark for 14 h. The solvent was removed under reduced pressure and the residue redissolved in acetonitrile (3 mL) and filtered through Celite filter aid. The filtrate was evaporated under reduced pressure to dryness and diethyl ether (20 mL) added. The suspension was sonicated for 15 min and the precipitate collected by filtration and copiously washed with diethyl ether and pentane. The crude solid was purified *via* silica gel chromatography with a gradient of methanol (0-10%) in dichloromethane. The collated fractions were evaporated under reduced pressure and to yield the product as a yellow powder (188 mg, 89%). ^{1}H NMR (500 MHz; $CDCl_3$): δ 10.46 (s, 1H), 9.04 (d, *J* = 8.0 Hz, 1H), 8.04 (td, *J* = 7.8, 1.5 Hz, 1H), 7.90 (m, 2H), 7.78-7.74 (m, 3H), 7.70 (dd, *J* = 5.8, 0.7 Hz, 1H), 7.67-7.64 (m, 2H), 7.50 (dd, *J* = 5.8, 0.7 Hz, 1H), 7.22 (ddd, *J* = 7.6, 5.6, 1.3 Hz, 1H), 7.07 (ddd, *J* = 7.3, 5.9, 1.4 Hz, 1H), 7.03 (td, *J* = 7.5, 1.0 Hz, 1H), 7.00-6.97 (m, 2H), 6.91 (td, *J* = 7.4, 1.2 Hz, 1H), 6.87 (td, *J* = 7.4, 1.2 Hz, 1H), 4.77-4.68 (m, 2H), 4.05-3.99 (m, 2H), 3.76-3.66 (m, 4H), 3.68-3.57 (m, 8H). ^{13}C NMR (126 MHz; $CDCl_3$): δ 168.4, 167.6, 150.3, 150.1, 149.5, 149.4, 148.5, 148.4, 146.6, 143.8, 143.6, 139.8, 137.8, 137.7, 131.9, 131.7, 130.6, 130, 129.3, 125.8, 124.7, 124.6, 124.2, 123.3, 122.8, 122.6, 122.1, 119.4, 119.3, 72.6, 70.5 (2C), 70.4, 70.1, 68.3, 61.4, 51.8. ESI-MS (positive ion). Calcd for $C_{37}H_{42}N_6O_4Ir^+$ ([M]$^+$): *m/z* 823.258. Found *m/z* 823.2571.

[Ir(df-ppy)$_2$(pt-TEG)]Cl: The chlorido-bridged dimer [Ir(df-ppy)$_2$(μ-Cl)]$_2$ (340 mg, 280 μmol) and the ligand pt-TEG (182 mg, 564 μmol) were suspended in a stirred solution of dichloromethane and methanol (3:1 v/v, 5 mL). Full dissolution occurred within 1 h and the solution was allowed to stir in the dark for 14 h. The solvent was removed under reduced pressure and the residue redissolved in acetonitrile (3 mL) and filtered through Celite filter aid. The filtrate was evaporated under reduced pressure to dryness and diethyl ether (20 mL) added. The suspension

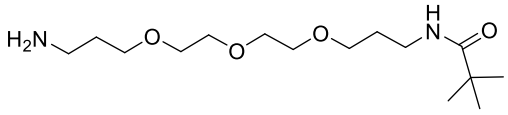
was sonicated for 15 min and the precipitate collected by filtration and copiously washed with diethyl ether and pentane. The solid was dried *in vacuo* to give the complex as a light-yellow powder (380 mg, 73%). ^{1}H NMR (500 MHz; $CDCl_3$): δ 9.16 (s, 1H), 8.40 (d, *J* = 8.0 Hz, 1H), 8.28 (t, *J* = 10.0 Hz, 2H), 8.01 (t, *J* = 7.8 Hz, 1H), 7.80 (m, 3H), 7.65 (d, *J* = 5.7 Hz, 1H), 7.50 (d, *J* = 5.7 Hz, 1H), 7.31 (t, *J* = 6.6 Hz, 1H), 7.15 (t, *J* = 6.7 Hz, 1H), 7.05 (t, *J* = 6.7 Hz, 1H), 6.55 (m, 1H), 6.48 (m, 1H), 5.71 (d, *J* = 7.7 Hz, 1H), 5.66 (d, *J* = 8.0 Hz, 1H), 4.69-4.58 (m, 2H), 3.95-3.89 (m, 2H), 2.69 (broad s, 1H). ^{13}C NMR (101 MHz; $CDCl_3$): □ 165.1 (m, CF), 164.5 (m, CF), 164.2 (m, CF), 162.6 (m, CF), 162.0 (m), 160.0 (m), 153.9 (d), 150.2, 150.0 (d), 149.7, 149.4, 148.7, 148.4, 140.7, 139.1, 139.0, 129.7, 127.9 (m, 2C), 126.4, 125.2, 123.9, 123.7 (d), 123.5 (d), 123.3, 114.2 (m, 2C), 99.1 (m, 2C), 77.4, 72.8, 70.6, 70.5, 70.2, 68.4, 61.4, 52.2. ^{19}F NMR (376 MHz; $CDCl_3$): □ -106.3 (m, 1F), -107.2 (m, 1F), -109.2 (m, 1F), -110.1 (m, 1F). Calcd for $C_{37}H_{34}F_4N_6O_4Ir^+$ ($[M]^+$): *m/z* 895.221. Found *m/z* 895.2184.

Synthesis of the pt-TOxT-Sq ligand

General

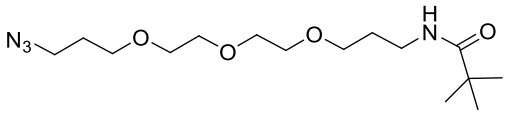
Reagents and solvents were purchased from various commercial sources and used without further purification. NMR spectra were acquired on a Jeol 400 spectrometer or a Bruker Ascend 500 spectrometer. ^{1}H NMR spectra were acquired at 400 MHz or 500 MHz, ^{13}C{^{1}H} NMR spectra were acquired at 101 MHz or 126 MHz, and ^{19}F NMR acquired at 471 MHz. All NMR spectra were recorded at 298 K. Chemical shifts were referenced to residual solvent peaks and are quoted in terms of parts per million (ppm), relative to tetramethylsilane ($Si(CH_3)_4$); ^{19}F NMR signals are quoted relative to an internal standard of trifluoroacetic acid. Electrospray ionization mass spectra (ESI-MS) were acquired using a Thermo Scientific Exactive Plus Orpitrap Mass Spectrometer.

Synthesis of 1-amino,13-(Boc-amino)-4,7,10-trioxatridecane:[3]



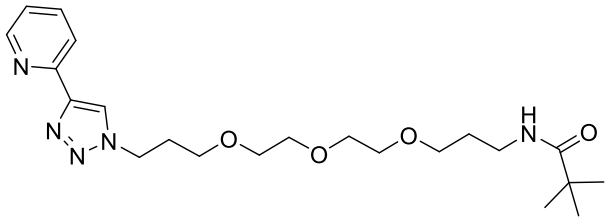
To a solution of 4,7,10-trioxa-1,13-tridecanediamine (18.2 g, 82.5 mmol) in 1,4-dioxane (150 mL) a solution of Boc_2-O (3.00 g, 13.8 mmol) in 1,4-dioxane (80 mL) was added dropwise at ambient temperature over 16 h. The solution was stirred for a further 30 h before the solvent was removed under reduced pressure and the residue taken up in H_2O. The aqueous mixture was extracted with dichloromethane (8 × 80 mL) and the combined organic extracts were washed with brine (2 × 200 mL). The organic phase was then dried ($MgSO_4$), filtered and the solvent removed under reduced and the product isolated as a yellow oil after the residue was purified by silica gel chromatography using a solvent gradient of 100% dichloromethane → 10% methanol/2% aq. ammonia/88% dichloromethane (2.96 g, 9.23 mmol, 67%). ^{1}H NMR (400 MHz; $CDCl_3$): δ 5.17 (s, 1H), 3.63-3.49 (m, 12H), 3.19 (m, 2H), 2.80 (m, 1H), 2.46 (s, 3H), 1.73 (m, 4H), 1.41 (s, 9H).

1-azido, 13-(Boc-amino)-4,7,10-trioxatridecane:[3]



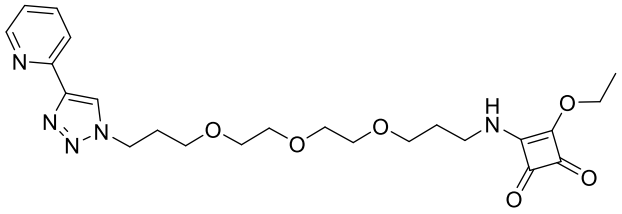
A flask containing sodium azide (6.04 g, 93.0 mmol), H_2O (30 mL) and dichloromethane (75 mL) was cooled to 0°C and triflic anhydride (3 mL, 18.0 mmol) was added dropwise. After 15 min the mixture was stirred vigorously at ambient temperature for 3.5 h. The organic layer was separated and the aqueous layer was back extracted with dichloromethane (2×20mL). The combined organic extracts were washed with sat. Na_2CO_3. The organic phase was then added dropwise to 1-amino,13-(Boc-amino)-4,7,10-trioxatridecane (2.92 g, 9.12 mmol), K_2CO_3 (2.59 g, 18.7 mmol) and $CuSO_4.5H_2O$ (cat.) in a methanol/water (90 mL/60mL) solvent mixture. The mixture was stirred vigorously for 3 days before the organic phase was separated, washed with H_2O (2×40 mL) and the aqueous phases were back extracted with dichloromethane (2×40 mL). The combined organic extracts were dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The residue was loaded onto a silica column and eluted with a gradient of 100% dichloromethane→4%methanol/96% dichloromethane to afford a slightly yellow oil (1.99 g, 5.74 mmol, 63%). ^{1}H NMR (400 MHz; $CDCl_3$): δ 4.98 (s, 1H), 3.65-3.51 (m, 12H), 3.38 (t, *J* = 6.7 Hz, 2H), 3.21 (m, 2H), 1.84 (m, 2H), 1.74 (m, 2H), 1.42 (s, 9H). {^{1}H}^{13}C NMR (101 MHz; $CDCl_3$): δ 156.1, 79.0, 70.7, 70.7, 70.5, 70.3, 69.7, 68.0, 48.5, 38.7, 29.7, 29.2, 28.5.

2-(1-(13-(Boc-amino)-4,7,10-trioxatridecyl)-1H-1,2,3-triazol-4-yl)pyridine:[3]



A mixture of 1-azido,13-(Boc-amino)-4,7,10-trioxatridecane (1.95 g, 5.62 mmol), 2-ethynylpyridine (0.65 g, 6.26 mmol) and sodium ascorbate (20 mol%) was set stirring in DMSO/H_2O (2:1, 3 mL) then $CuSO_4.5H_2O$ (2 mol%) was added. After 5 days H_2O was added (40 mL) and the mixture was extracted with dichloromethane (3×30 mL). The organic phase was washed with brine, dried ($MgSO_4$) and filtered then the solvent was removed under reduced pressure and any remaining DMSO was removed *in vacuo*. The residue was purified by silica gel chromatography using ethyl acetate as the eluent to afford a yellow oil (2.36 g, 5.44 mmol, 97%). ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.57 (ddd, *J* = 4.9, 1.8, 1.0 Hz, 1H), 8.18 (s, 1H), 8.16 (t, *J* = 1.0 Hz, 1H), 7.77 (td, *J* = 7.8, 1.8 Hz, 1H), 7.22 (ddd, *J* = 7.5, 4.9, 1.2 Hz, 1H), 5.01 (br, 1H), 4.55 (t, *J* = 6.8 Hz, 2H), 3.66-3.59 (m, 8H), 3.50 (dt, *J* = 16.0, 5.9 Hz, 4H), 3.21 (q, *J* = 6.1 Hz, 2H), 1.75 (dd, *J* = 12.7, 6.4 Hz, 4H), 1.42 (s, 9H). {^{1}H}^{13}C NMR (101 MHz; $CDCl_3$): δ 156.1, 150.5, 149.5, 148.3, 137.0, 122.9, 122.6, 120.3, 70.7, 70.6, 70.5, 70.3, 69.7, 67.2, 47.4, 38.6, 30.3, 29.8, 29.7, 28.5.

pt-TOxT-Sq:



A mixture of 2-(1-(13-(Boc-amino)-4,7,10-trioxatridecyl)-1H-1,2,3-triazol-4-yl)pyridine (0.656 g, 1.44 mmol) and trifluoroacetic acid (2 mL) in acetonitrile (10 mL) was set stirring at 50°C. After 2.5 h the reaction was cooled to ambient temperature and potassium carbonate was cautiously added to adjust the reaction mixture to pH 7. The reaction mixture was added dropwise to a round bottom flask containing 3,4-diethoxy-3-cyclobutene-1,2-dione (0.737 g, 4.33 mmol) and potassium carbonate (3.43 g, 24.8 mmol) in acetonitrile (25 mL). The reaction mixture was then stirred for 18 h before being filtered through celite and the solvent was removed affording a yellow residue. The residue was washed with dichloromethane, the washings were retained, and the solvent volume reduced before purification by column chromatography (SiO_2, DCM→10%MeOH/DCM) to afford a light-yellow oil (0.625 g, 1.32 mmol, 92%). ^{1}H NMR (500 MHz; CD_3CN): δ 8.56 (d, *J* = 4.6 Hz, 1H), 8.27 (s, 1H), 8.07 (d, *J* = 7.9 Hz, 1H), 7.82 (td, *J* = 7.7, 1.7 Hz, 1H), 7.27 (ddd, *J* = 7.5, 4.9, 1.0 Hz, 1H), 4.65 (m, 2H), 4.50 (t, *J* = 6.9 Hz, 2H), 3.54 (m, 14H), 2.15 (dq, *J* = 13.4, 7.0 Hz, 3H), 1.78 (q, *J* = 6.2 Hz, 2H), 1.38 (t, *J* = 6.5 Hz, 3H). ^{13}C{^{1}H} NMR (126 MHz; CD_3CN): δ 190.2, 151.5, 150.6, 148.8, 137.9, 123.9, 123.8, 123.7, 120.5, 71.1, 70.9, 70.9, 70.1, 69.2, 69.0, 68.0, 48.2, 43.0, 31.0, 16.1.

Synthesis of the $[Ir(C^N)_2(pt\text{-}TOxT\text{-}Sq)]^+$ ECL labels:

General procedure[5]

A mixture of the appropriate iridium precursor and **pt-TOxT-Sq** was set stirring in a solvent mixture of methanol/dichloromethane (3:1). The reaction was stirred overnight at ambient temperature and the solvent was removed after TLC confirmed the reaction was complete. The solvent was removed under reduced pressure. The residue was taken up in a minimum of dichloromethane and diethyl ether was layered on top. A residue settled at the bottom of the flask after storing at -20°C for 72 h. The supernatant was decanted off and the residue was washed with diethyl ether (×3). A solid was isolated by filtration after trituration of the residue with diethyl ether.

$[Ir(ppy)_2(pt\text{-}TOxT\text{-}Sq)]Cl$. The chlorido-bridged dimer $[Ir(ppy)_2(\mu\text{-}Cl)]_2$ was reacted as detailed in the general procedure affording a yellow powder (0.473 g, 0.47 mmol, 75%). ^{1}H NMR (500 MHz; CD_3CN): δ 9.40 (m, 1H), 8.41-8.37 (m, 1H), 8.05 (m, 3H), 7.86-7.74 (m, 6H), 7.63 (d, *J*=5.3 Hz, 1H), 7.34 (ddd, *J* =7.4, 5.8, 1.3 Hz, 1H), 7.10 (ddd, *J* = 7.3, 6.0, 1.3, 1H), 7.04 (m, 2H), 6.92 (m, 2H), 6.81 (td, *J* = 7.4, 1.1 Hz, 1H), 6.26 (m, 2H), 4.64 (m, 2H), 4.51 (m, 2H), 3.45 (m, 15H), 2.08 (q, *J* = 6.2 Hz, 2H), 1.78 (q, *J* = 6.4 Hz, 2H), 1.35 (m, 3H). ^{13}C{^{1}H} NMR (126 MHz; CD_3CN): δ 168.6, 168.2, 151.1, 150.8, 150.7, 150.2, 149.4, 147.7, 145.3, 145.3, 140.7, 139.5, 139.4, 132.8, 132.4, 131.2, 130.6, 128.3, 127.5, 125.8, 125.3, 124.6, 124.3, 124.0, 123.5, 123.1, 120.7, 120.6, 71.0, 70.9, 70.8, 70.8, 70 .0, 69.1, 67.6, 50.4, 30.5, 16.2. ESI-MS (positive ion). Calcd for $C_{45}H_{47}IrN_7O_6$ ($[M]^+$): *m/z* 974.322. Found *m/z* 974.3229.

[Ir(bt)$_2$(pt-TOxT-Sq)]Cl. The chlorido-bridged dimer [Ir(bt)$_2$(μ-Cl)]$_2$ was reacted as detailed in the general procedure affording a yellow powder (0.320 g, 0.29 mmol, 52%). ^{1}H NMR (400 MHz; CD_3CN): δ 9.16 (s, 1H), 8.26 (d, *J* = 7.9 Hz, 1H), 8.06 (m, 3H), 7.95 (m, 2H), 7.84 (m, 1H), 7.40 (m, 3H), 7.27 (m, 1H), 7.11 (m, 2H), 7.03 (td, *J*= 7.5, 1.0 Hz, 1H), 6.89 (td, *J* = 7.5, 1.3 Hz, 1H), 6.80 (td, *J* = 7.5, 1.3 Hz, 1H), 6.55 (d, *J*= 8.3 Hz, 1H), 6.47 (d, *J* = 7.6 Hz, 1H), 6.36 (d, *J* = 7.6 Hz, 1H), 6.16 (d, *J* = 8.4 Hz, 1H), 4.62 (d, *J* = 6.3 Hz, 2H), 4.54 (t, *J* = 6.5 Hz, 2H), 3.37 (m, 14H), 3.02 (s, 1H), 2.03 (q, *J* = 6.1 Hz, 2H), 1.71 (q, *J* = 6.3 Hz, 2H), 1.34 (t, *J* = 14.1 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz; CD_3CN): δ 182.7, 182.1, 151.7, 151.2, 150.9, 150.1, 150.1, 149.8, 147.5, 141.9, 141.5, 141.3, 134.4, 134.4, 132.8, 132.6, 132.6, 132.2, 129.2, 128.9, 128.6, 128.0, 127.7, 127.2, 126.9, 124.9, 124.8, 124.3, 123.9, 123.8, 119.1, 118.3, 70.9, 70.9, 70.8, 70.0, 69.1, 67.2, 50.5, 42.9, 42.6, 31.6, 31.0, 30.6, 18.8, 16.1. ESI-MS (positive ion). Calcd for $C_{49}H_{47}IrN_7O_6S_2^+$ ([M]$^+$): *m/z* 1086.266. Found *m/z* 1086.2673.

[Ir(piq)$_2$(pt-TOxT-Sq)]Cl. The chlorido-bridged dimer [Ir(piq)$_2$(μ-Cl)]$_2$ was reacted as detailed in the general procedure affording an orange powder (0.547 g, 0.49 mmol, 93%). ^{1}H NMR (400 MHz; [CD_3CN]): δ 9.14 (s, 1H), 9.01 (m, 2H), 8.38 (d, *J* = 8.0 Hz, 1H), 8.31 (m, 2H), 8.02 (m, 3H,), 7.83 (m, 4H), 7.70 (m, 1H), 7.67 (d, *J* = 6.4 Hz), 7.54 (d, *J* = 6.4 Hz, 1H), 7.49 (d, *J* = 6.4 Hz, 1H), 7.43 (d, *J* = 6.4 Hz, 1H), 7.31 (ddd, *J* = 7.7, 5.6, 1.3 Hz, 1H), 7.13 (ddd, *J* = 8.2, 7.2, 1.2 Hz, 1H), 7.04 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 6.89 (td, *J* = 7.4, 1.2 Hz, 1H), 6.77 (td, *J* = 7.4, 1.2 Hz, 1H), 6.42 (d, *J* = 7.5 Hz, 1H), 6.28 (dd, *J* = 7.7, 1.1 Hz, 1H), 4.62 (m, 2H), 4.46 (t, *J* = 6.8 Hz, 2H), 3.32 (m, 15H), 1.98 (dd, *J* = 6.5, 5.2 Hz, 2H), 1.73 (q, *J* = 6.4 Hz, 2H), 1.33 (t, *J* = 7.1 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz; [CD_3CN]): δ 169.5, 169.2, 154.3, 151.2, 151.0, 150.4, 149.2, 146.8, 146.7, 142.4, 141.8, 140.7, 138.1, 138.0, 133.3, 133.1, 132.8, 132.8, 131.7, 131.3, 131.2, 130.6, 129.9, 129.8, 128.6, 128.5, 128.1, 127.7, 127.6, 127.1, 127.0, 123.9, 123.3, 122.9, 122.8, 122.6, 70.9, 70.8, 70.7, 70.0, 69.0, 67.3, 50.3, 30.3, 16.1. ESI-MS (positive ion). Calcd for $C_{53}H_{51}IrN_7O_6$ ([M]$^+$): *m/z* 1074.353. Found *m/z* 1074.3534.

[Ir(df-ppy)$_2$(pt-TOXT-Sq)]Cl. The chlorido-bridged dimer [Ir(df-ppy)$_2$(μ-Cl)]$_2$ was reacted as detailed in the general procedure affording a yellow powder (0.276 g, 0.25 mmol, 61%). ^{1}H NMR (500 MHz; CD_3CN): δ 9.50 (m, 1H), 8.44 (m, 1H), 8.30 (m, 2H), 8.10 (m, 1H), 7.89 (m, 3H), 7.76 (m, 1H), 7.66 (m, 1H), 7.39 (m, 1H), 7.13 (m, 2H), 6.66 (m, 2H), 5.73 (ddd, *J* = 24.1, 8.7, 2.3 Hz, 2H), 4.63 (m, 2H), 4.54 (m, 2H), 3.45 (m, 15H), 2.09 (q, *J* = 6.2 Hz, 2H), 1.78 (q, *J* = 6.4 Hz, 2H), 1.36 (m, 3H). ^{13}C{^{1}H} NMR (126 MHz; CD3CN): δ 164.7 (m, CF), 163.4 (m, CF), 162.8 (m, CF), 161.0 (m, CF), 154.9 (m, CF), 151.9 (m, CF), 151.6, 151.0, 150.7, 150.3, 141.3, 140.5, 140.5, 128.4, 128.0, 125.1, 124.8, 124.7, 124.54, 124.5, 124.3, 124.2, 114.8 (m, CF), 99.6 (m, CF), 71.0, 70.9, 70.8, 70.79, 70.0, 69.2, 67.6, 57.9, 50.6, 30.4, 18.8, 16.2. ^{19}F NMR (471 MHz; CD_3CN): δ -106.18 (d, *J* = 10.6, 1F), -107.06 (d, *J* = 9.6, 1F), -108.07 (d, *J* = 10.5, 1F), -108.87 (d, *J* = 10.2, 1F). ESI-MS (positive ion). Calcd for $C_{45}H_{43}F_4IrN_7O_6^+$ ([M]$^+$): *m/z* 1046.284. Found *m/z* 1046.2845.

Additional data

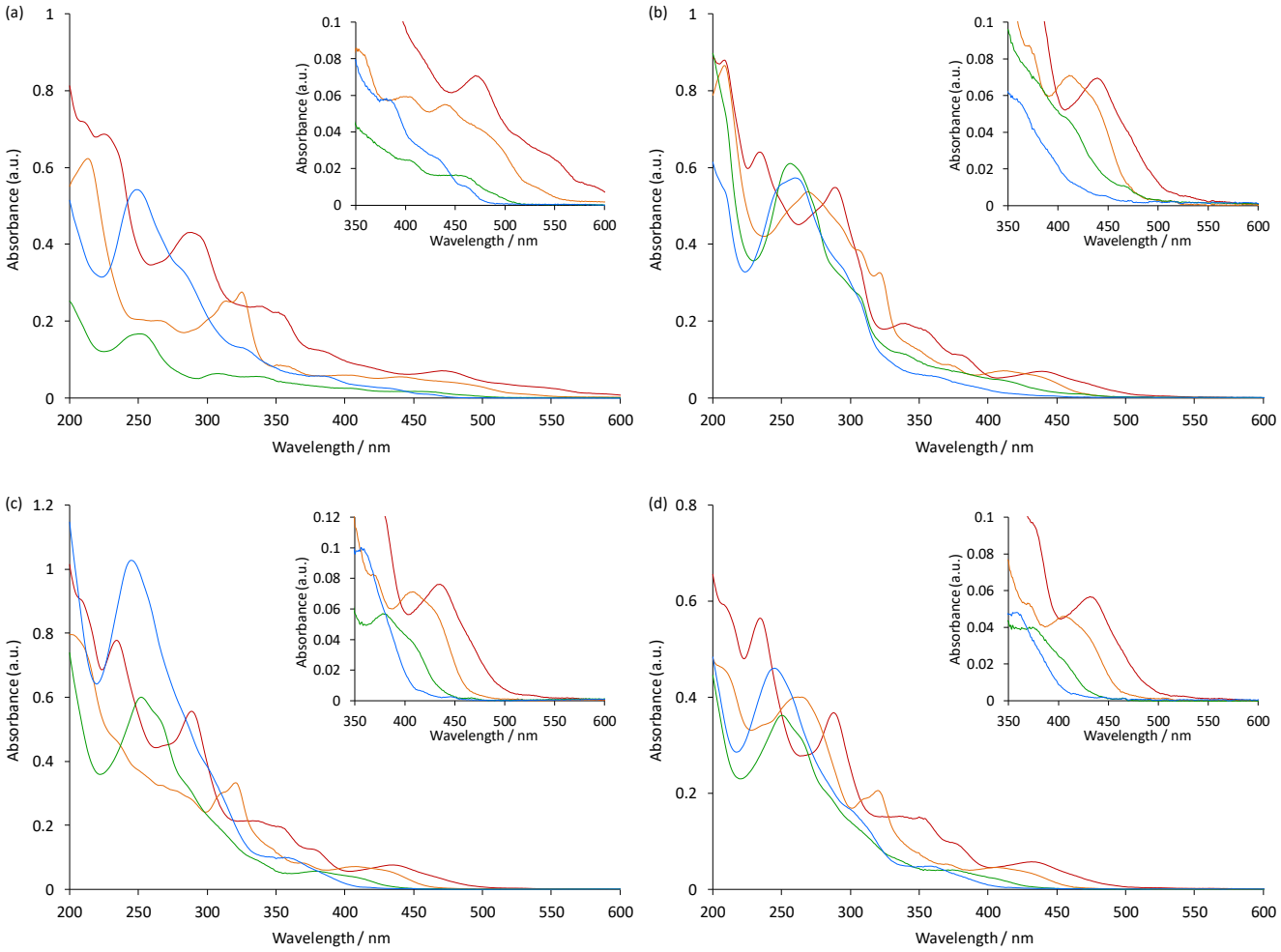


Figure S1. UV-Vis absorption spectra of (a) $Ir(C^N)_2(acac)$, (b) $[Ir(C^N)_2(dm\text{-}bpy)]^+$, (c) $[Ir(C^N)_2(ptb)]^+$, and (d) $[Ir(C^N)_2(pt\text{-}TEG)]^+$, where C^N = piq (red lines), bt (orange lines), ppy (green lines) or df-ppy (blue lines). The metal complexes were prepared at a concentration of 10 μM in acetonitrile (a-c) or water (d). The insets show lowest energy absorption bands.

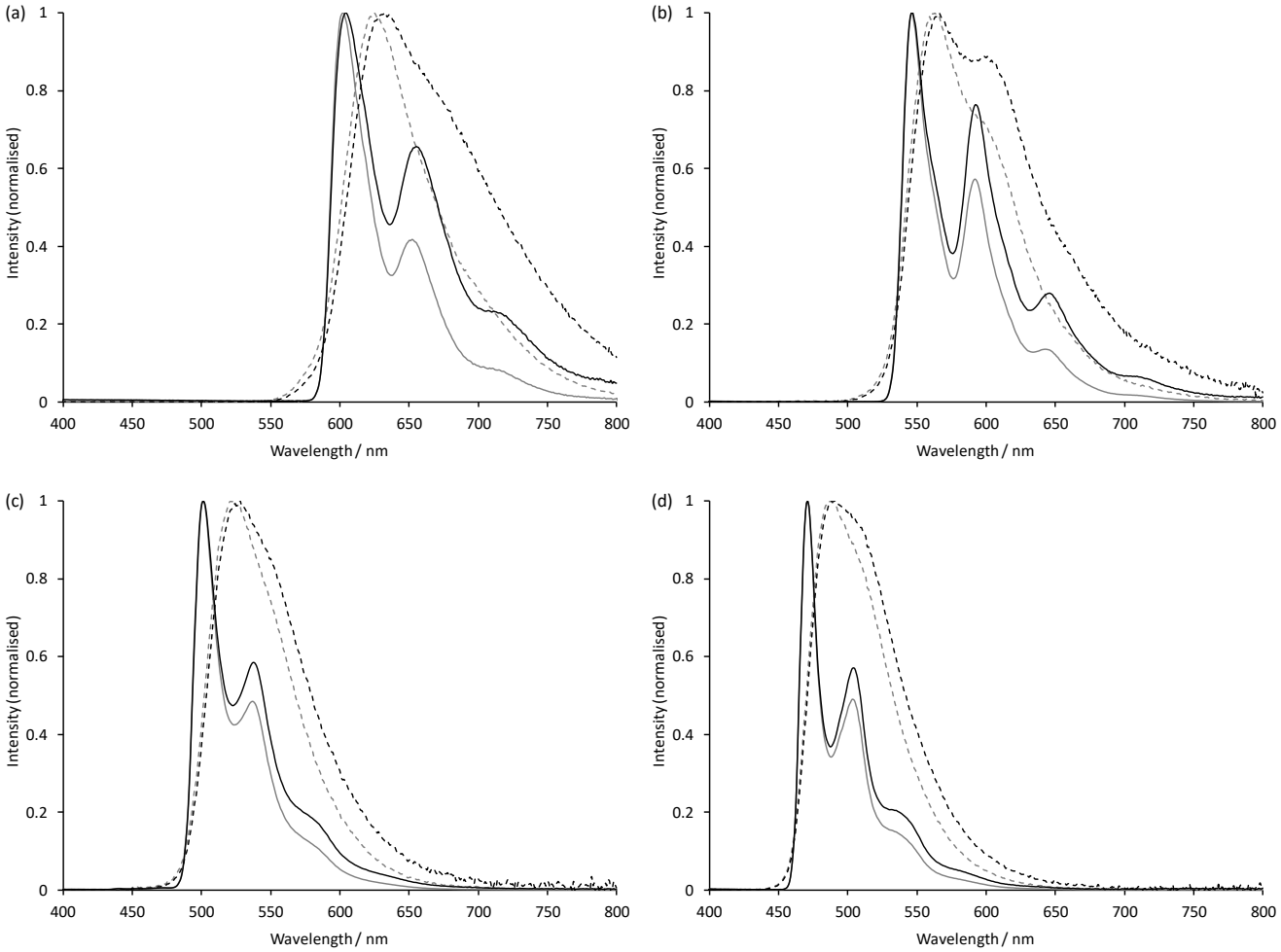


Figure S2. Normalized photoluminescence emission spectra of (a) $Ir(piq)_2(acac)$, (b) $Ir(bt)_2(acac)$, (c) $Ir(ppy)_2(acac)$, and (d) $Ir(df\text{-}ppy)_2(acac)$. Black lines: corrected spectra at 85 K (solid) and room temperature (dashed). Grey lines: uncorrected spectra at 85 K (solid) and room temperature (dashed). Conditions: 85 K spectra: 5 μM in 4:1 (v/v) ethanol:methanol; excitation wavelengths (λ_{ex}) were between 300 and 342 nm. Room temperature spectra: 10 μM in acetonitrile; λ_{ex} = 350 nm.

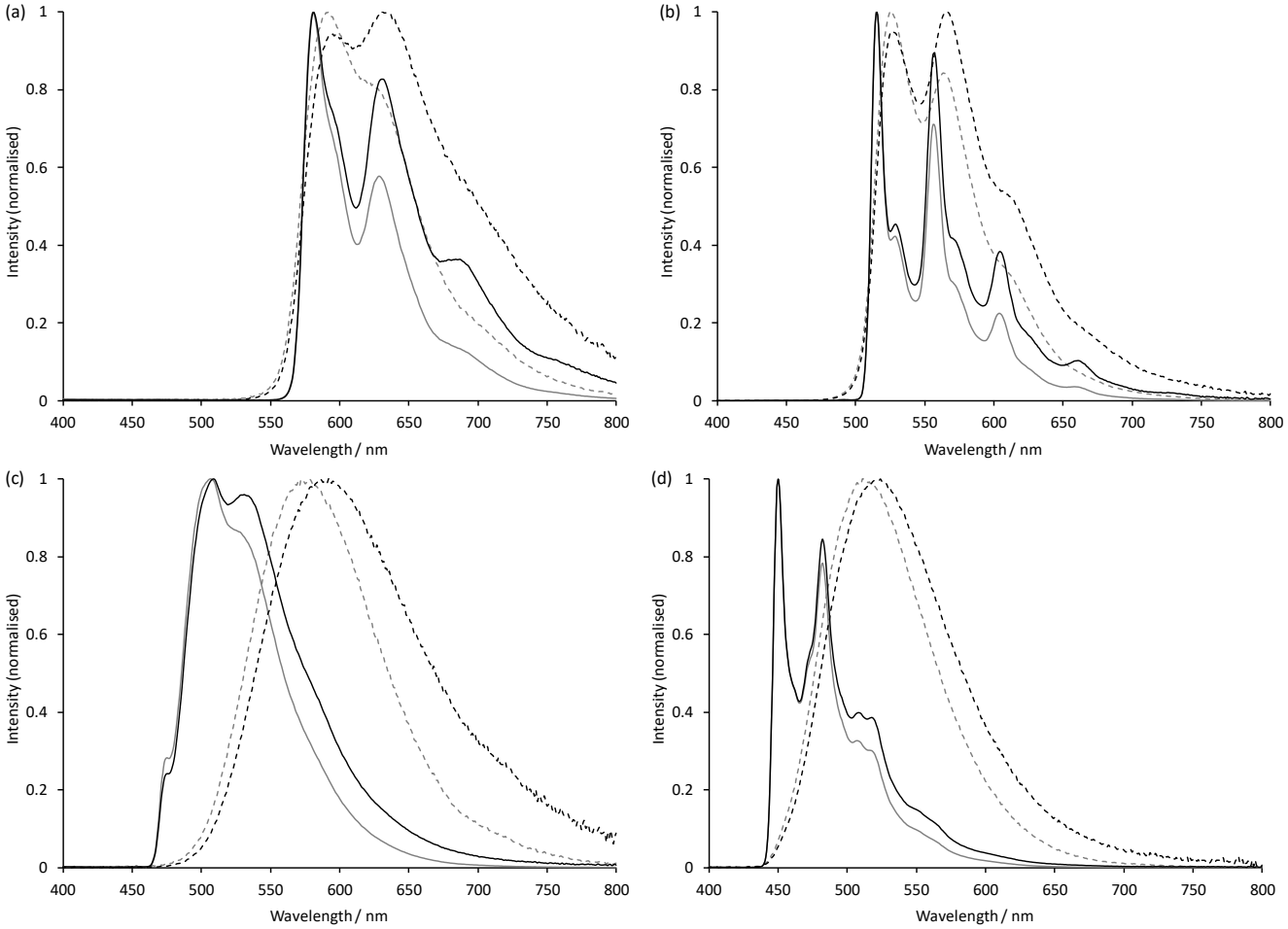


Figure S3. Normalized photoluminescence emission spectra of (a) $[Ir(piq)_2(dm\text{-}bpy)]^+$, (b) $[Ir(bt)_2(dm\text{-}bpy)]^+$, (c) $[Ir(ppy)_2(dm\text{-}bpy)]^+$, and (d) $[Ir(df\text{-}ppy)_2(dm\text{-}bpy)]^+$. Black lines: corrected spectra at 85 K (solid) and room temperature (dashed). Grey lines: uncorrected spectra at 85 K (solid) and room temperature (dashed). Conditions: 85 K spectra: 5 μM in 4:1 (v/v) ethanol:methanol; λ_{ex} = 295-374 nm. Room temperature spectra: 10 μM in acetonitrile; λ_{ex} = 350 nm.

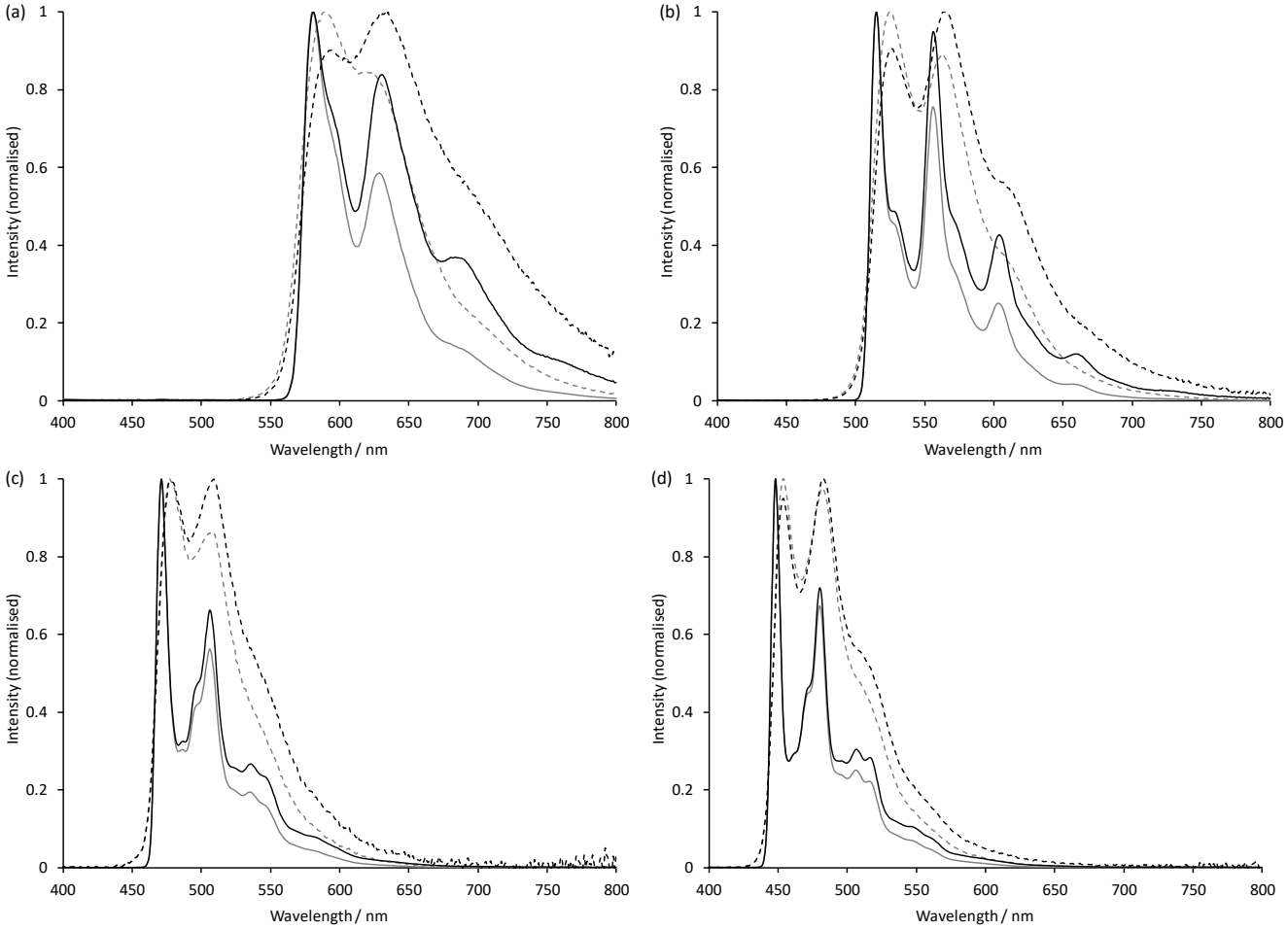


Figure S4. Normalized photoluminescence emission spectra of (a) $[Ir(piq)_2(ptb)]^+$, (b) $[Ir(bt)_2(ptb)]^+$, (c) $[Ir(ppy)_2(ptb)]^+$, and (d) $[Ir(df\text{-}ppy)_2(ptb)]^+$. Black lines: corrected spectra at 85 K (solid) and room temperature (dashed). Grey lines: uncorrected spectra at 85 K (solid) and room temperature (dashed). Conditions: 85 K spectra: 5 μM in 4:1 (v/v) ethanol:methanol; λ_{ex} = 290-324 nm. Room temperature spectra: 10 μM in acetonitrile; λ_{ex} = 350 nm.

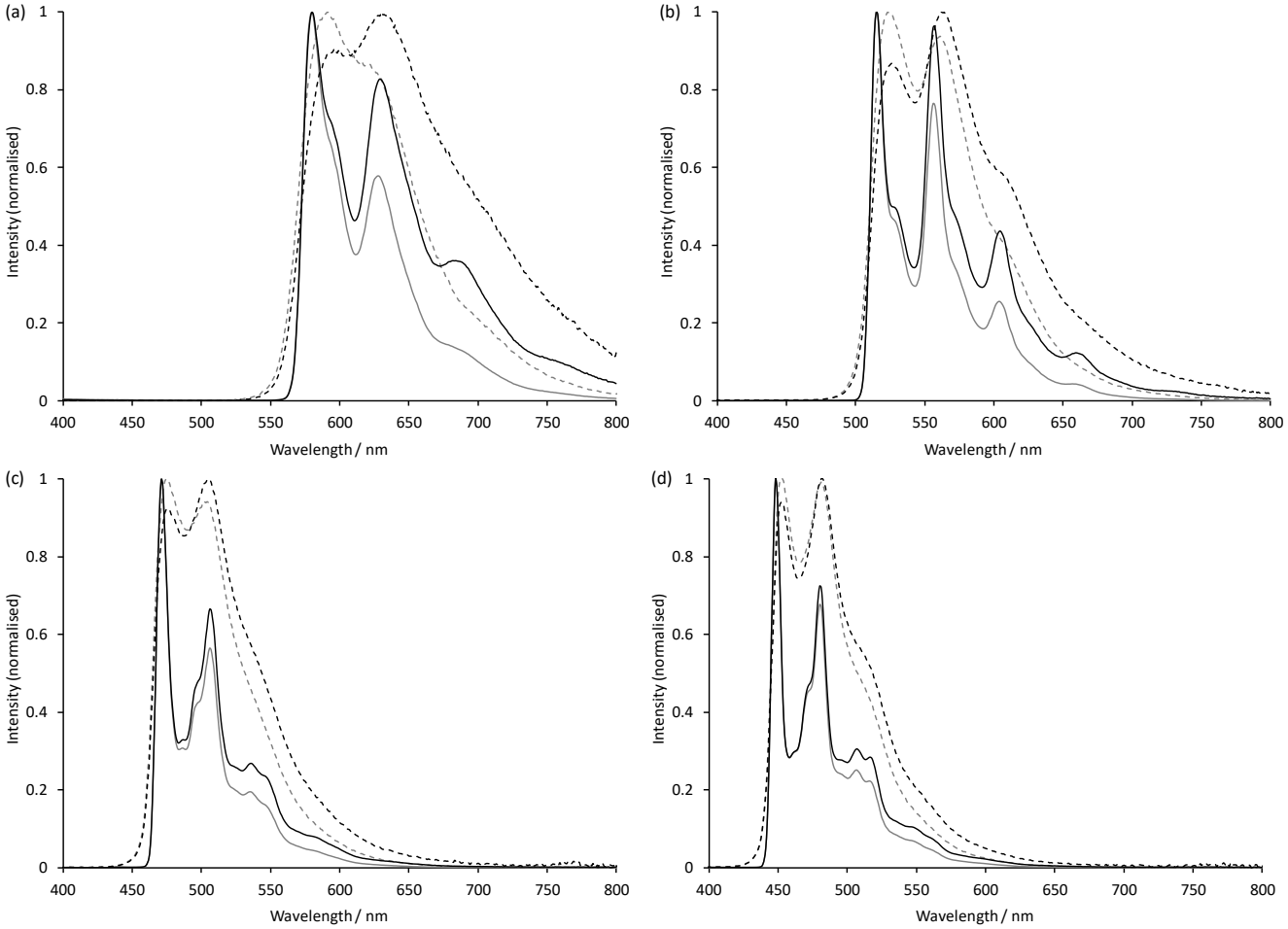


Figure S5. Normalized photoluminescence emission spectra of (a) $[Ir(piq)_2(pt\text{-}TEG)]^+$, (b) $[Ir(bt)_2(pt\text{-}TEG)]^+$, (c) $[Ir(ppy)_2(pt\text{-}TEG)]^+$, and (d) $[Ir(df\text{-}ppy)_2(pt\text{-}TEG)]^+$. Black lines: corrected spectra at 85 K (solid) and room temperature (dashed). Grey lines: uncorrected spectra at 85 K (solid) and room temperature (dashed). Conditions: 85 K spectra: 5 μM in 4:1 (v/v) ethanol:methanol; λ_{ex} = 267-324 nm. Room temperature spectra: 10 μM in water; λ_{ex} = 340 nm.

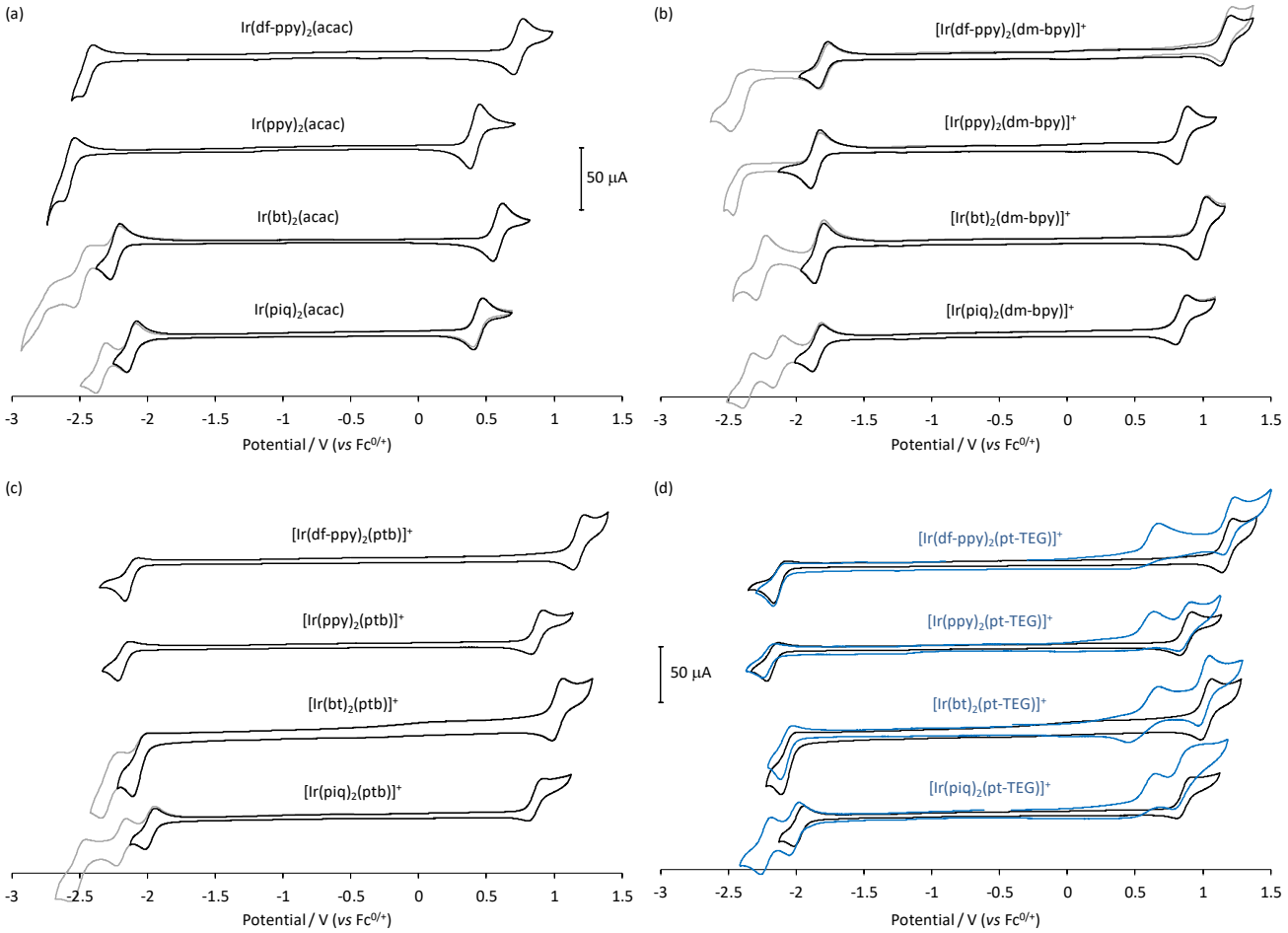


Figure S6. Cyclic voltammograms for (a) $Ir(C^N)_2(acac)$, (b) $[Ir(C^N)_2(dm\text{-}bpy)]^+$, (c) $[Ir(C^N)_2(ptb)]^+$, and (d) $[Ir(C^N)_2(pt\text{-}TEG)]^+$ (blue lines) overlaid on those for $[Ir(C^N)_2(ptb)]^+$ (black lines). The grey plots show selected further reduction peaks. Metal complexes were prepared at a concentration of 0.25 mM in acetonitrile with 0.1 M $TBAPF_6$ supporting electrolyte. The additional peaks at ~0.65 V in the CVs for the $[Ir(C^N)_2(pt\text{-}TEG)]^+$ complexes are due to their chloride counter ions.

Table S1. Selected properties of $Ir(C^N)_2(acac)$, $[Ir(C^N)_2(dm\text{-}bpy)]^+$, and $[Ir(C^N)_2(ptb)]^+$ complexes in acetonitrile.

	Photoluminescence			Electrochemical potentials (*vs* $Fc^{+/0}$)				ECL
	λ_{max}/nm[a,b]	λ_{max} (85 K)/nm[b,c]	$E_{0\text{-}0}$/eV[d]	E_{ox}/V[e]	E_{red}/V[e]	E_{ox}*/V[f]	E_{red}*/V[f]	I_{ECL}[g]
$[Ru(bpy)_3]^{2+}$	**620**	**581**, 629, 685(sh)	2.13	0.89	−1.73, −1.92, −2.15	−1.24	0.40	1
$Ir(piq)_2(acac)$	**633**	**604**, 655, 716	2.05	0.43	−2.12, −2.34	−1.62	−0.07	2.05
$Ir(bt)_2(acac)$	**565**, 605	**546**, 592, 645	2.27	0.58	−2.24	−1.69	0.03	0.81
$Ir(ppy)_2(acac)$	**525**	**501**, 537	2.48	0.42	−2.59	−2.06	−0.11	0.015
$Ir(df\text{-}ppy)_2(acac)$	**491**	**471**, 504, 542(sh)	2.63	0.73	−2.44, −2.68	−1.90	0.19	0.17
$[Ir(piq)_2(dm\text{-}bpy)]^+$	595, **631**	**581**, 594(sh), 631, 687, 758(sh)	2.13	0.84	−1.85, −2.14, −2.36	−1.29	0.28	0.76
$[Ir(bt)_2(dm\text{-}bpy)]^+$	526, **566**	**515**, 529, 557, 573(sh), 605, 626(sh), 661	2.41	0.99	−1.83, −2.26	−1.42	0.58	0.66
$[Ir(ppy)_2(dm\text{-}bpy)]^+$	**592**	475(sh),[h] **509**, 531	2.44	0.85	−1.86, −2.46	−1.59	0.58	0.27
$[Ir(df\text{-}ppy)_2(dm\text{-}bpy)]^+$	**524**	**450**, 482, 508, 518, 549(sh), 564(sh)	2.76	1.16	−1.80, −2.48	−1.60	0.96	0.89
$[Ir(piq)_2(ptb)]^+$	594, **633**	**581**, 594(sh), 631, 687, 758(sh)	2.13	0.86	−1.98, −2.19, −2.51	−1.27	0.15	0.43
$[Ir(bt)_2(ptb)]^+$	526, **565**	**515**, 528(sh), 556, 572(sh), 604, 659	2.41	1.02	−2.06, −2.27	−1.39	0.35	0.34
$[Ir(ppy)_2(ptb)]^+$	**477**, **509**	**471**, 487, 495(sh), 506, 536, 549(sh), 585(sh)	2.63	0.87	−2.18	−1.74	0.43	0.10
$[Ir(df\text{-}ppy)_2(ptb)]^+$	454, **482**	**448**, 480, 506, 516	2.77	1.18	−2.12	−1.49	0.55	0.18

[a]Metal complexes at 10 μM in acetonitrile at ambient temperature. [b]Corrected for the change in instrument sensitivity over the wavelength range. [c]Metal complexes at 5 μM in ethanol:methanol (4:1) at 85 K (sh = shoulder). [d]Energy gap between the zeroth vibrational levels of the ground and excited states, estimated from the highest energy peak of the low-temperature emission spectrum. [e]Metal complexes at 0.25 mM in acetonitrile with 0.1 M $TBAPF_6$ supporting electrolyte; scan rate: 0.1 V/s. [f]Excited state potentials based on the ground state potentials and $E_{0\text{-}0}$.[6] [g]ECL intensities relative to $[Ru(bpy)_3]^{2+}$ (10 μM metal complex, 10 mM TPrA, 0.1 M $TBAPF_6$ supporting electrolyte; 10 s pulse, 10 Hz). The co-reactant ECL intensities of Ir(III) complexes relative to $[Ru(bpy)_3]^{2+}$ are highly dependent on instrumental and chemical conditions, such as co-reactant concentration, the applied potential pulse time, and sensitivity of the photodetector in different regions of the spectrum.[7] The conditions used in this study were based on those previously employed[7] that provide a conservative evaluation for iridium complexes. [h]Shoulder arising from the characteristic rigidochromic blueshift of $[Ir(ppy)_2(N^N)]^+$ complexes (where N^N = bpy or a derivative) involving contribution to the emission from a higher energy excited state.[8]

Table S2. Calculated MO energies (eV; BP86/def2-TZVP) for Ir(C^N)$_2$(acac) complexes in acetonitrile.

	Ir(piq)$_2$(acac)	Ir(bt)$_2$(acac)	Ir(ppy)$_2$(acac)	Ir(df-ppy)$_2$(acac)
LUMO+3	-1.92	-1.56	-1.97	-1.99
LUMO+2	-2.01	-2.04	-1.98	-2.05
LUMO+1	-2.78	-2.73	-2.40	-2.48
LUMO	-2.87	-2.73	-2.41	-2.49
HOMO	-4.63	-4.80	-4.63	-4.88
HOMO-1	-4.91	-4.99	-4.91	-5.06
HOMO-2	-5.22	-5.37	-5.26	-5.45
HOMO-3	-5.50	-5.61	-5.69	-5.76
HOMO-LUMO GAP	1.76	2.07	2.22	2.39

Table S3. Calculated MO energies (eV; BP86/def2-TZVP) for [Ir(C^N)$_2$(dm-bpy)]$^+$ complexes in acetonitrile.

	[Ir(piq)$_2$(dm-bpy)]$^+$	[Ir(bt)$_2$(dm-bpy)]$^+$	[Ir(ppy)$_2$(dm-bpy)]$^+$	[Ir(df-ppy)$_2$(dm-bpy)]$^+$
LUMO+3	-2.29	-2.22	-2.30	-2.34
LUMO+2	-2.86	-2.85	-2.47	-2.55
LUMO+1	-2.98	-2.92	-2.56	-2.63
LUMO	-3.06	-3.04	-3.06	-3.12
HOMO	-5.06	-5.13	-5.09	-5.31
HOMO-1	-5.29	-5.53	-5.31	-5.52
HOMO-2	-5.48	-5.62	-5.56	-5.72
HOMO-3	-5.75	-5.97	-5.95	-5.94
HOMO-LUMO GAP	2.00	2.09	2.03	2.19

Table S4. Calculated MO energies (eV; BP86/def2-TZVP) for [Ir(C^N)$_2$(ptb)]$^+$ complexes in acetonitrile.

	[Ir(piq)$_2$(ptb)]$^+$	[Ir(bt)$_2$(ptb)]$^+$	[Ir(ppy)$_2$(ptb)]$^+$	[Ir(df-ppy)$_2$(ptb)]$^+$
LUMO+3	-2.27	-2.23	-2.31	-2.34
LUMO+2	-2.74	-2.77	-2.53	-2.62
LUMO+1	-2.91	-2.87	-2.60	-2.66
LUMO	-3.06	-2.92	-2.76	-2.82
HOMO	-5.04	-5.18	-5.07	-5.31
HOMO-1	-5.50	-5.58	-5.57	-5.75
HOMO-2	-5.51	-5.69	-5.63	-5.78
HOMO-3	-5.85	-6.00	-5.90	-6.01
HOMO-LUMO GAP	1.98	2.26	2.31	2.49

Table S5. Calculated MO energies (BP86/def2-TZVP) for $[Ir(C^N)_2(pt\text{-}TEG)]^+$ complexes in water.

	$[Ir(piq)_2(pt\text{-}TEG)]^+$	$[Ir(bt)_2(pt\text{-}TEG)]^+$	$[Ir(ppy)_2(pt\text{-}TEG)]^+$	$[Ir(df\text{-}ppy)_2(pt\text{-}TEG)]^+$
LUMO+3	-2.20	-2.17	-2.25	-2.27
LUMO+2	-2.70	-2.72	-2.51	-2.58
LUMO+1	-2.94	-2.84	-2.57	-2.63
LUMO	-2.98	-2.90	-2.71	-2.76
HOMO	-5.03	-5.15	-5.04	-5.26
HOMO-1	-5.40	-5.53	-5.54	-5.70
HOMO-2	-5.52	-5.65	-5.58	-5.71
HOMO-3	-5.80	-5.97	-5.88	-5.95
HOMO-LUMO GAP	2.05	2.25	2.33	2.50

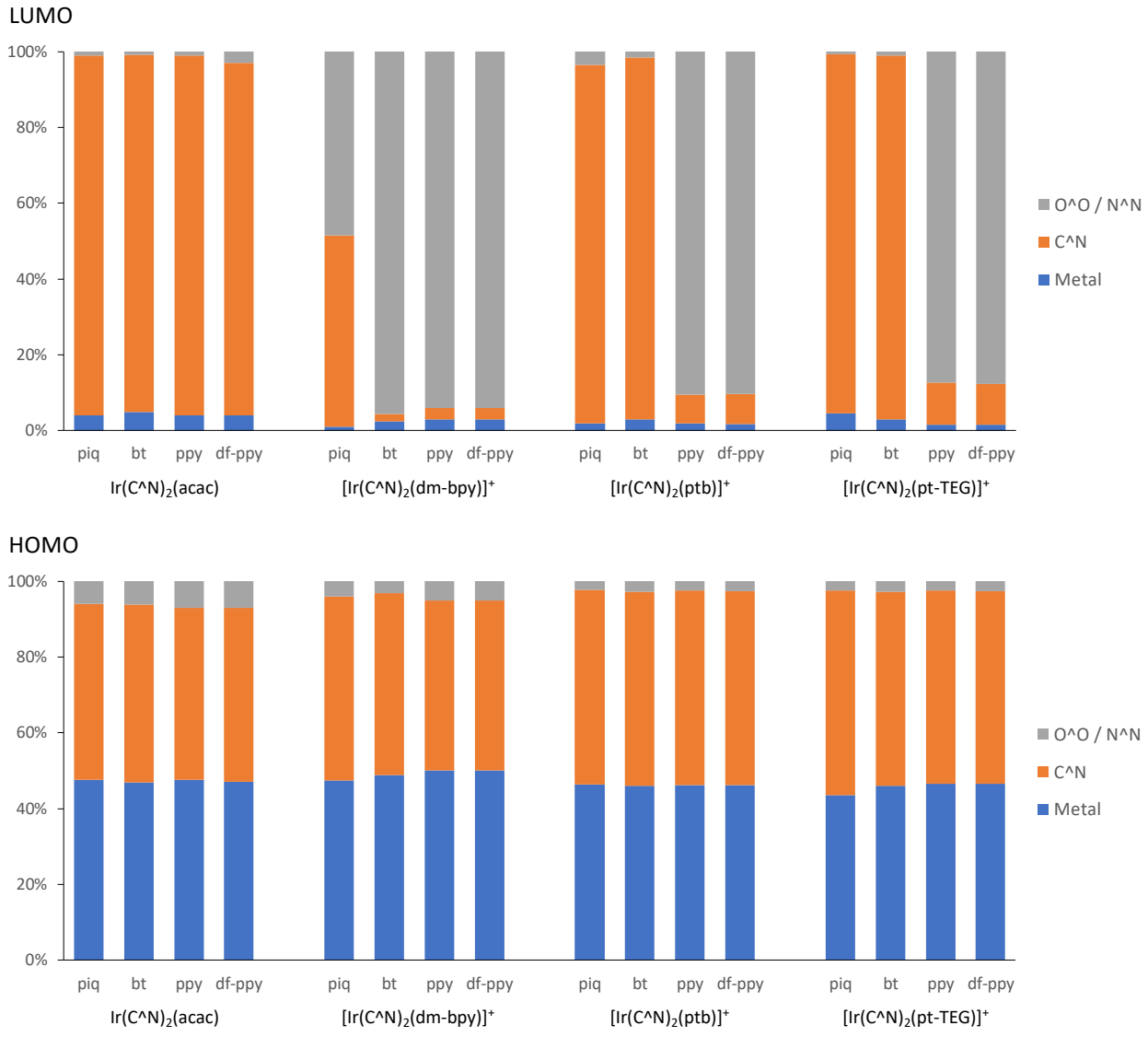
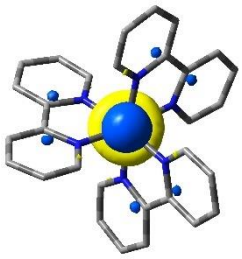
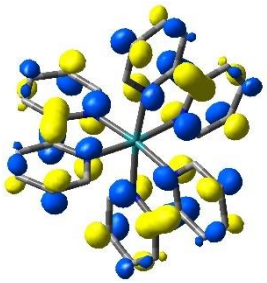
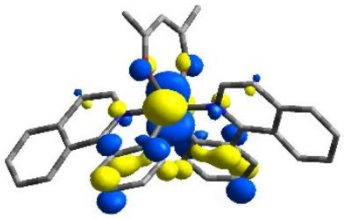
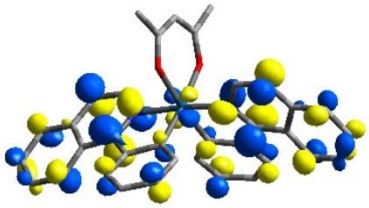
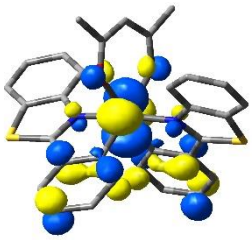
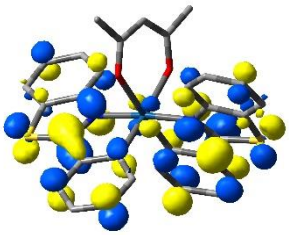
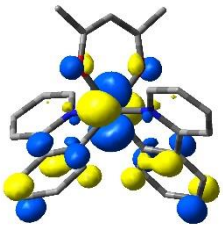
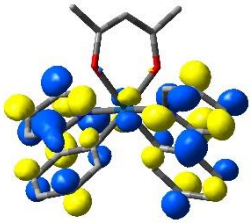
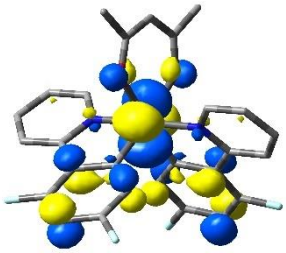
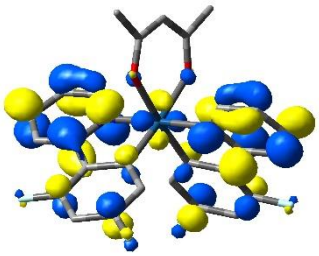
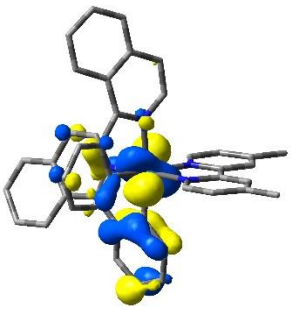
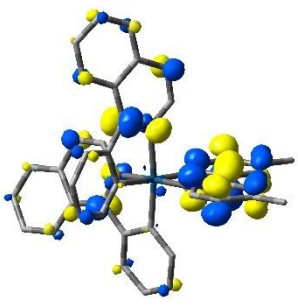
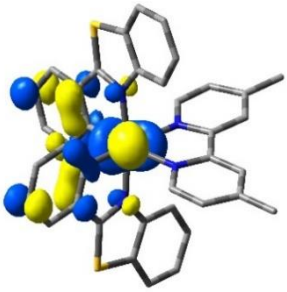
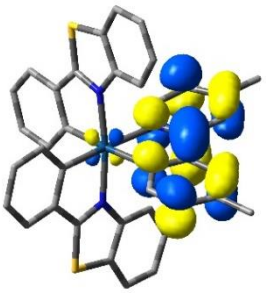
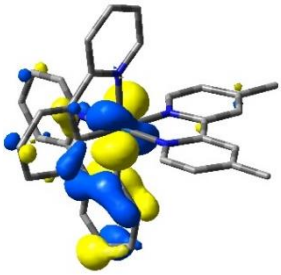
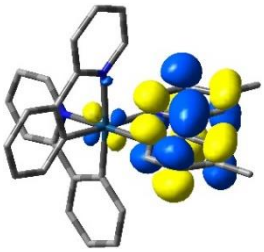
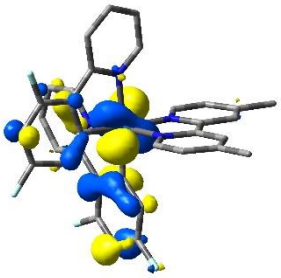
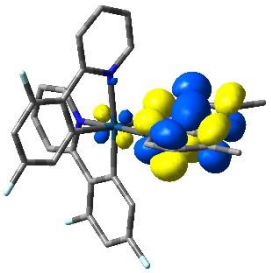
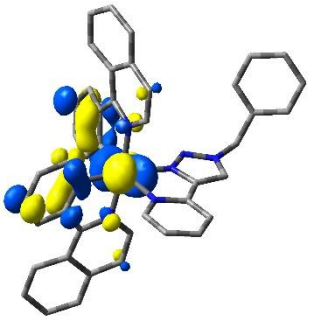
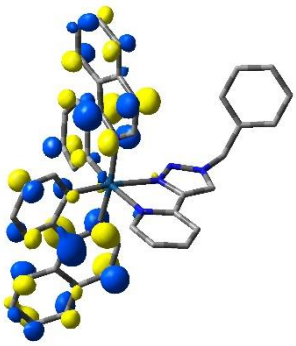
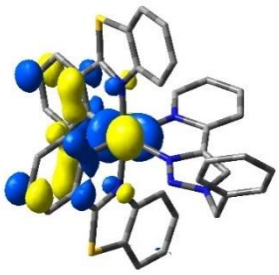
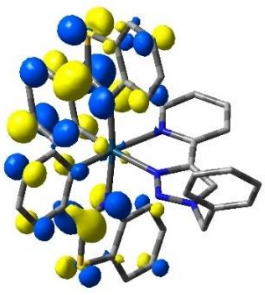


Figure S7. Contribution to the respective MOs (BP86/def2-TZVP) of the metal and ligands in $Ir(C^N)_2(acac)$, $[Ir(C^N)_2(dm\text{-}bpy)]^+$, $[Ir(C^N)_2(ptb)]^+$, and $[Ir(C^N)_2(pt\text{-}TEG)]^+$ complexes, where C^N = piq, bt, ppy, or df-ppy.

Table S6. Contour plots for $[Ru(bpy)_3]^{2+}$, $Ir(C^N)_2(acac)$, $[Ir(C^N)_2(dm\text{-}bpy)]^+$ and $[Ir(C^N)_2(ptb)]^+$ complexes in acetonitrile.

	HOMO	LUMO
$[Ru(bpy)_3]^{2+}$		
$Ir(piq)_2(acac)$		
$Ir(bt)_2(acac)$		
$Ir(ppy)_2(acac)$		
$Ir(df\text{-}ppy)_2(acac)$		
$[Ir(piq)_2(dm\text{-}bpy)]^+$		

$[Ir(bt)_2(dm\text{-}bpy)]^+$		
$[Ir(ppy)_2(dm\text{-}bpy)]^+$		
$[Ir(df\text{-}ppy)_2(dm\text{-}bpy)]^+$		
$[Ir(piq)_2(ptb)]^+$		
$[Ir(bt)_2(ptb)]^+$		

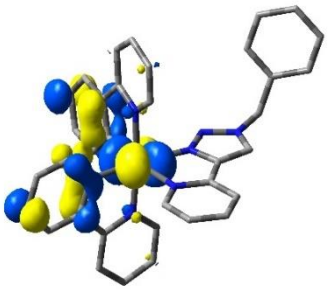
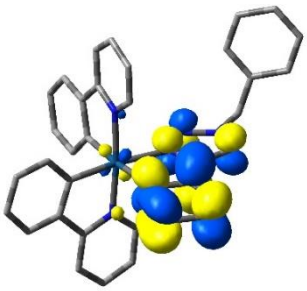
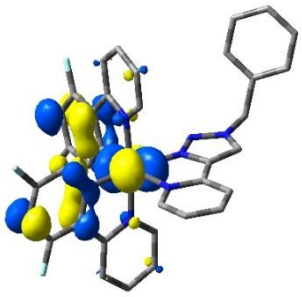
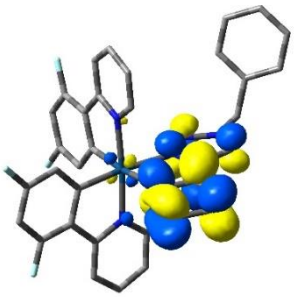
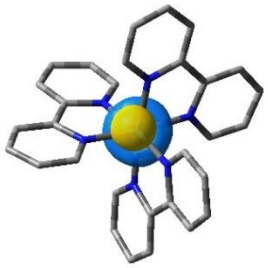
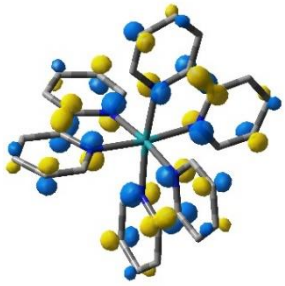
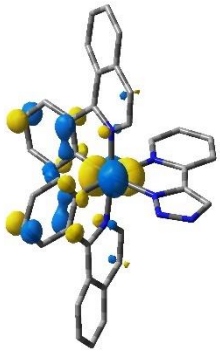
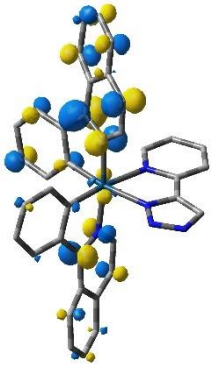
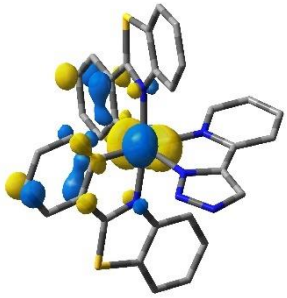
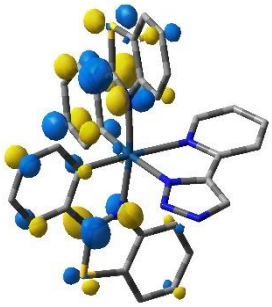
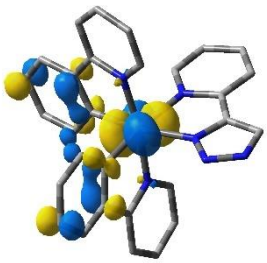
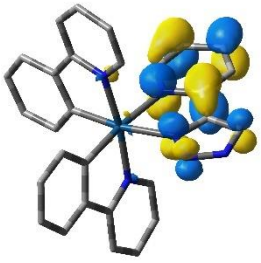
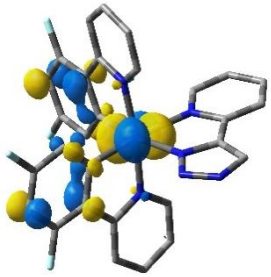
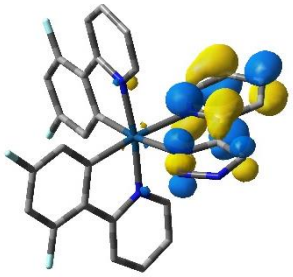
$[Ir(ppy)_2(ptb)]^+$		
$[Ir(df\text{-}ppy)_2(ptb)]^+$		

Table S7. Contour plots for $[Ru(bpy)_3]^{2+}$ and the $[Ir(C^N)_2(pt\text{-}TEG)]^+$ complexes in water. The hydrogen atoms and TEG chain have been omitted for clarity.

	HOMO	LUMO
$[Ru(bpy)_3]^{2+}$		
$[Ir(piq)_2(pt\text{-}TEG)]^+$		
$[Ir(bt)_2(pt\text{-}TEG)]^+$		
$[Ir(ppy)_2(p\text{-}TEG)]^+$		
$[Ir(df\text{-}ppy)_2(p\text{-}TEG)]^+$		

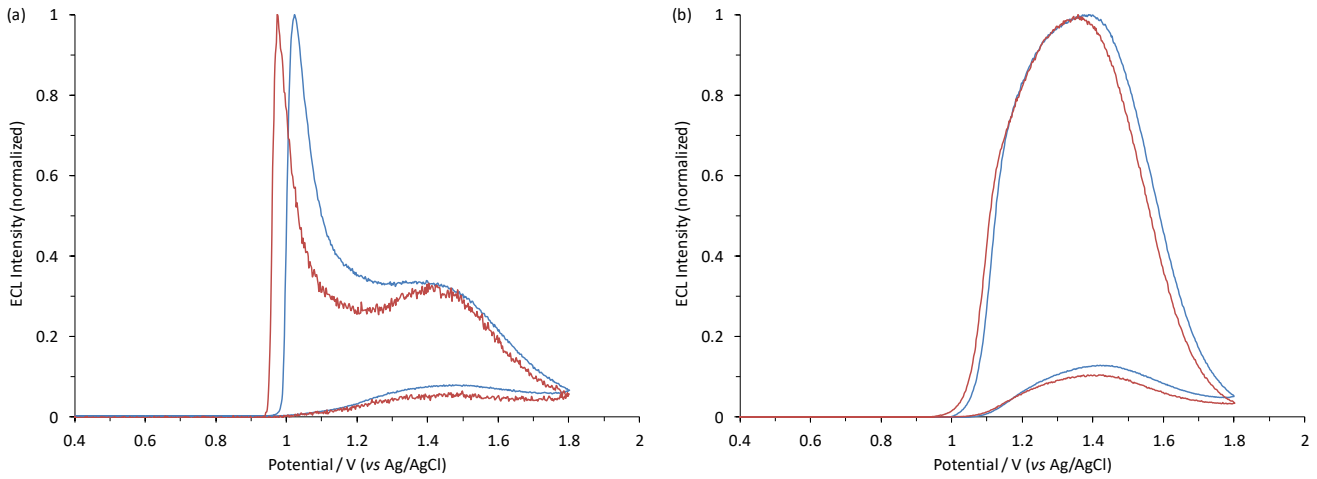


Figure S8. Normalized ECL intensity during an applied potential sweep from 0 V to 1.8 V and back to 0 V (*vs* Ag/AgCl) for (a) $[Ir(piq)_2(pt\text{-}TEG)]^+$ and (b) $[Ir(ppy)_2(pt\text{-}TEG)]^+$ at 1 μM (blue lines) and 0.1 μM (red lines) in (ProCell) phosphate buffer solution containing TPrA as a co-reactant.

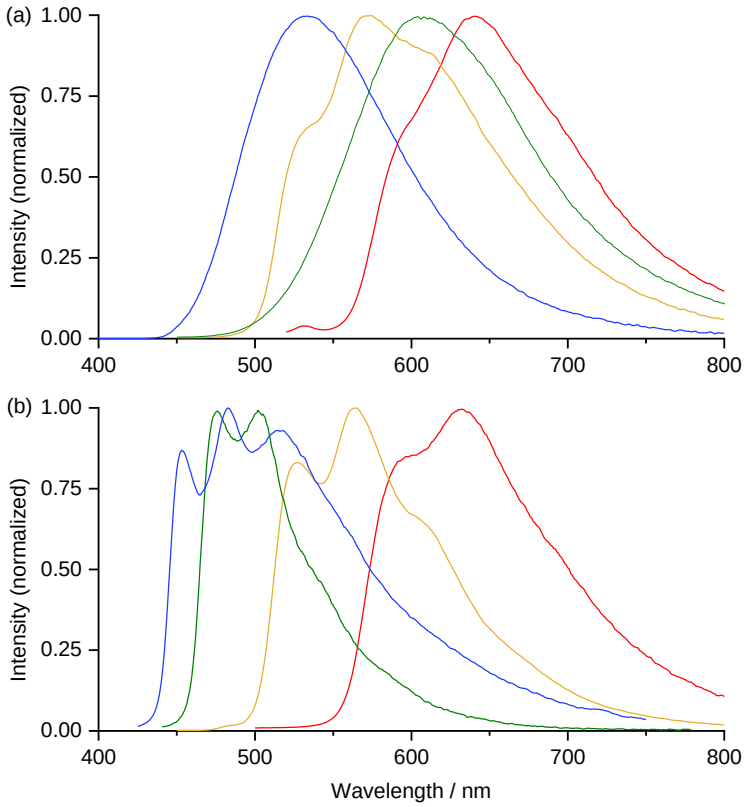


Figure S9. Photoluminescence emission spectra (corrected) of (a) $[Ir(C^N)_2(mbpy\text{-}COOH)]^+$, and (b) $[Ir(C^N)_2(pt\text{-}TOxT\text{-}Sq)]^+$ labels, where C^N = df-ppy (blue lines), ppy (green lines), bt (yellow lines), or piq (red lines), at a concentration of 10 μM in phosphate buffer.

Table S8. Primers and NASBA amplicon fragment sequences

	Sequences (5' - 3' orientation)
P1[a]	***AAT TTC TAA TAC GAC TCA CTA TAG GGA A***GT GCC ATC CGA TAA CAG
P2[b]	***GAT GCA AGG TCG CAT ATG AG***A GCC TTA CCG TAG TGT ACT A
Detection probe	Amino-C6-GAT GCA AGG TCG CAT ATG AG
Capture probe	GAC AAT TTC GGG TGG GTT CC-Biotin TEG
NASBA amplicon (176 nt)	GGGAAGUGCC AUCCGAUAAC AGGACGAUCG CACGGAACCC ACCCGAAAUU GUCGGUGGUACUUAUCGUCC AGGUGUAUCG AAAGUGCGUG AAUAAAUACG CUUUUGCUAG CGAGGGAGCUAAUGCUGCCC UGGAGUUAGU ACACUACGGU AAGGCU***CUCA UAUGCGACCU UGCAUC***

[a]Primer 1 consists of a 3’ terminal sequence that is target specific (underlined) and a 5’ terminal T7 promoter sequence (bold) that can be recognized by T7 RNA polymerase. [b]Primer 2 consists of a 3’ terminal target specific sequence (underlined) and 5’ terminal ECL detection probe sequence (bold).

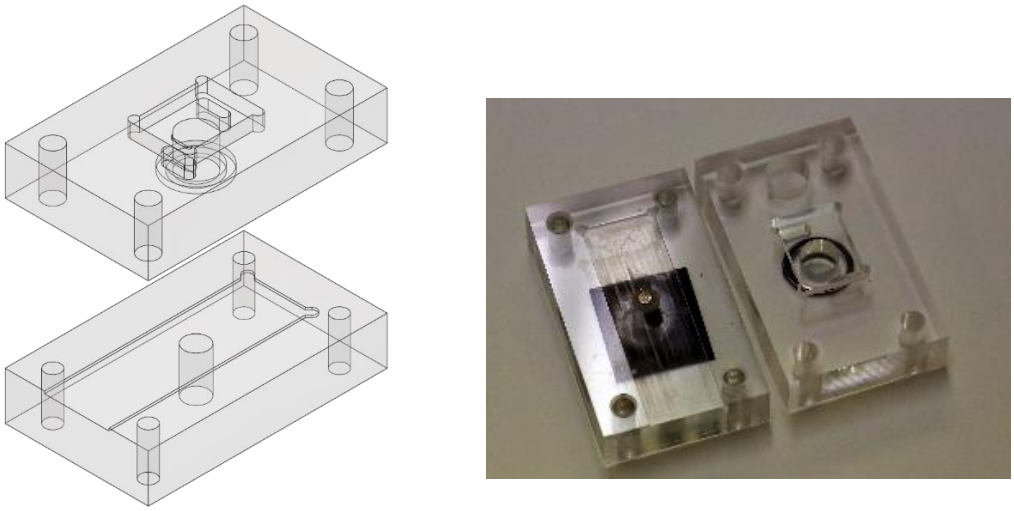


Figure S10. 3D drawing and photograph of the custom screen-printed electrode (SPE) holder, which was designed using SolidWorks 2015 CAD package (Dassault Systems, France). G-code CNC toolpaths were created using Siemens NX 10 CAD/CAM package (Siemens, Germany). The holders were machined from 10 mm thick cast poly(methylmethacrylate) (PMMA) sheets (Resi-Plex, Australia) using a Datron M7HP CNC mill (Datron AG, Germany). They were designed to house Zensor TE-100 SPE (eDAQ, Australia) which consist of carbon ink working (3 mm diameter) and counter electrodes, and an Ag|AgCl based ink reference electrode. These electrodes have been shown to have characteristics well suited for ECL-based analytical methods.[9] To perform ECL assays using paramagnetic particles, the holders incorporated a 3 × 4 mm diameter rod shaped N42 rare earth magnet (Aussie Magnets, Australia) beneath the working electrode.

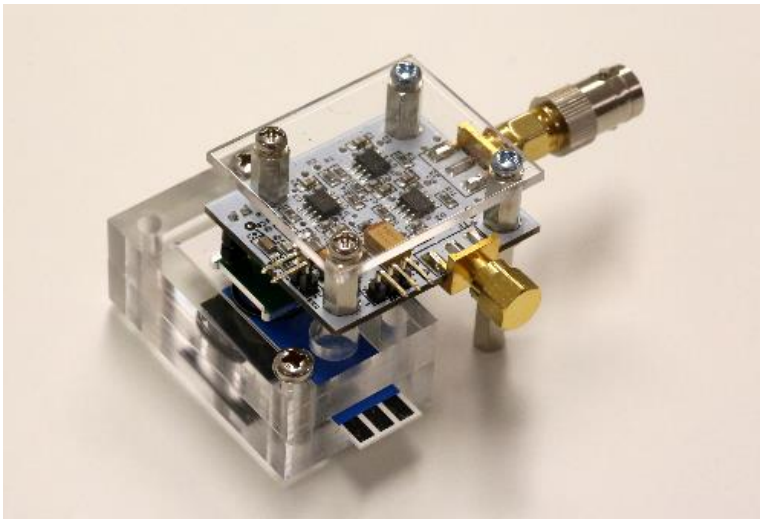
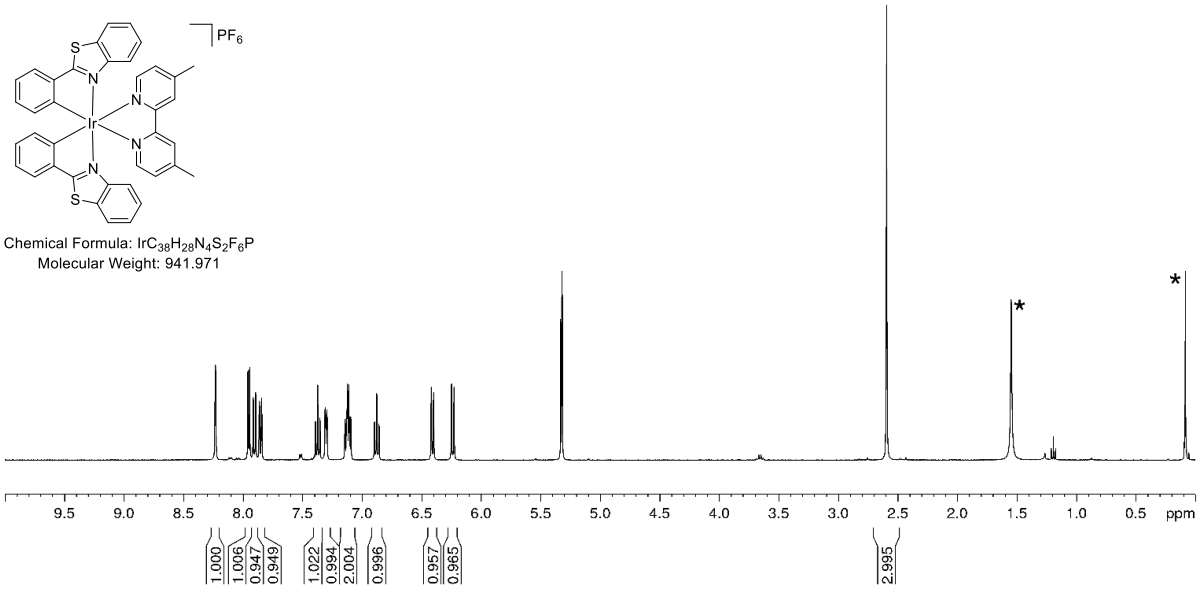


Figure S11. Photograph of the cell holder containing magnet and Zensor SPE, coupled with the silicon photomultiplier detector (ASD-RGB3S-P; AdvanSiD, Italy) and ASD-EP-EB-N amplifier board (AdvanSiD).

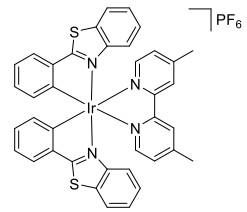
NMR spectra of $[Ir(C^N)_2(dm\text{-}bpy)](PF_6)$ and $[Ir(C^N)_2(ptb)](PF_6)$ complexes

^{1}H NMR spectrum of $[Ir(bt)_2(dm\text{-}bpy)](PF_6)$



^{1}H NMR (400 MHz; CD_2Cl_2): δ 8.23 (s, 1H), 7.95 (d, *J* = 5.6 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.85 (d, *J* = 7.6 Hz, 1H), 7.37 (t, *J* = 7.4 Hz, 1H), 7.30 (d, *J* = 5.6 Hz, 1H), 7.09-7.14 (m, 2H), 6.88 (td, *J* = 7.5, 1.2 Hz, 1H), 6.41 (d, *J* = 7.6 Hz, 1H), 6.24 (d, *J* = 8.4 Hz, 1H), 2.59 (s, 3H).

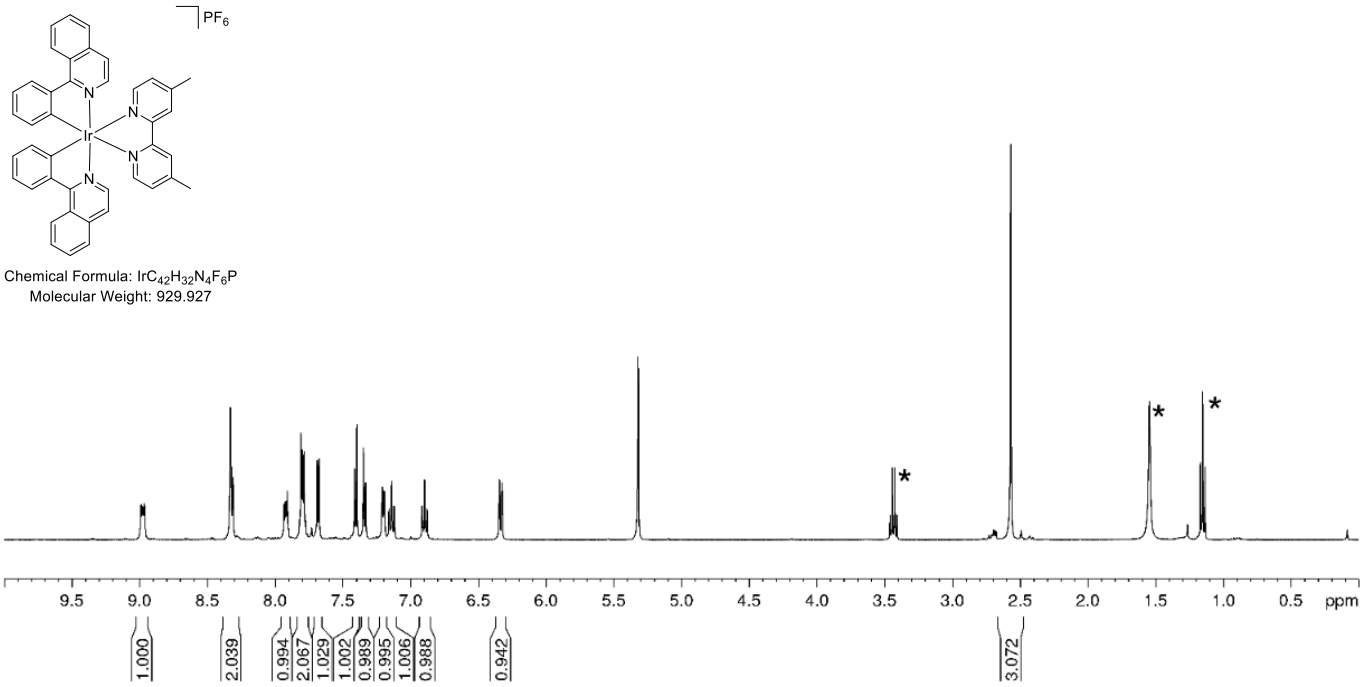
$^{13}C\{^{1}H\}$ NMR spectrum of $[Ir(bt)_2(dm\text{-}bpy)](PF_6)$



Chemical Formula: $IrC_{38}H_{28}N_4S_2F_6P$
Molecular Weight: 941.971

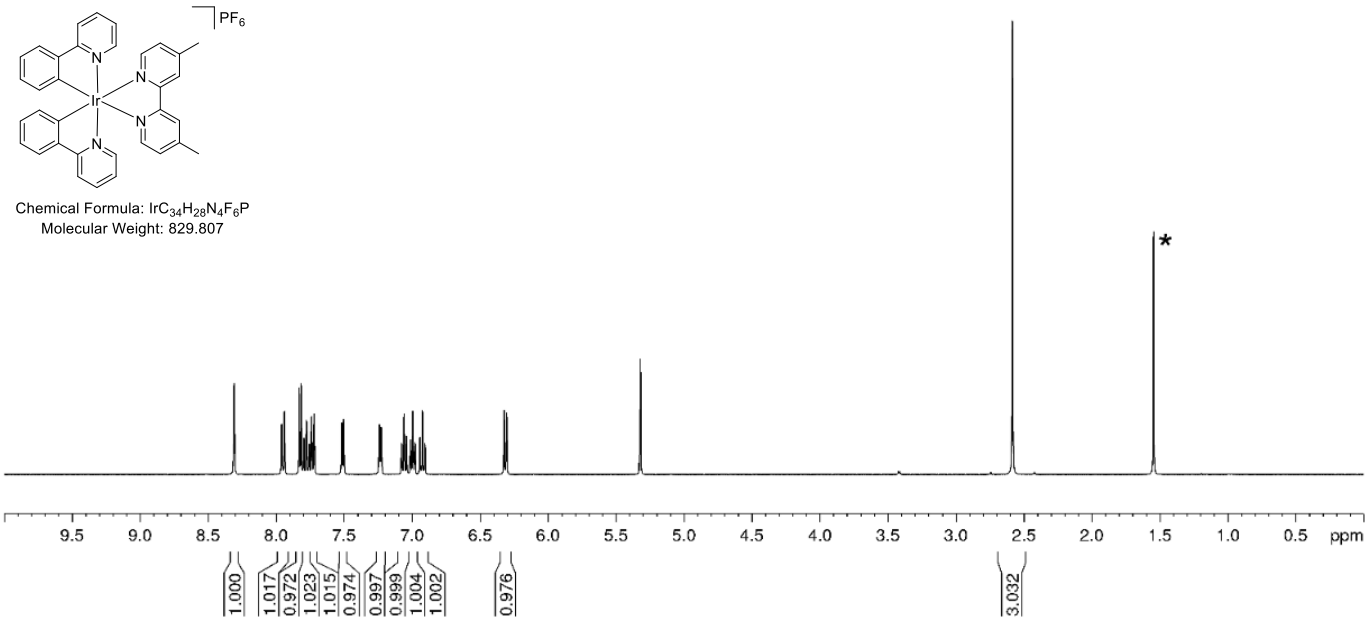
$^{13}C\{^{1}H\}$ NMR (100 MHz; CD_2Cl_2): δ 181.8, 156.8, 153.0, 150.9, 150.7, 149.8, 140.8, 133.9, 132.6, 132.1, 129.6, 128.6, 127.3, 126.5, 125.6, 124.0, 123.7, 118.3, 21.8.

^{1}H NMR spectrum of [Ir(piq)$_2$(dm-bpy)](PF$_6$)



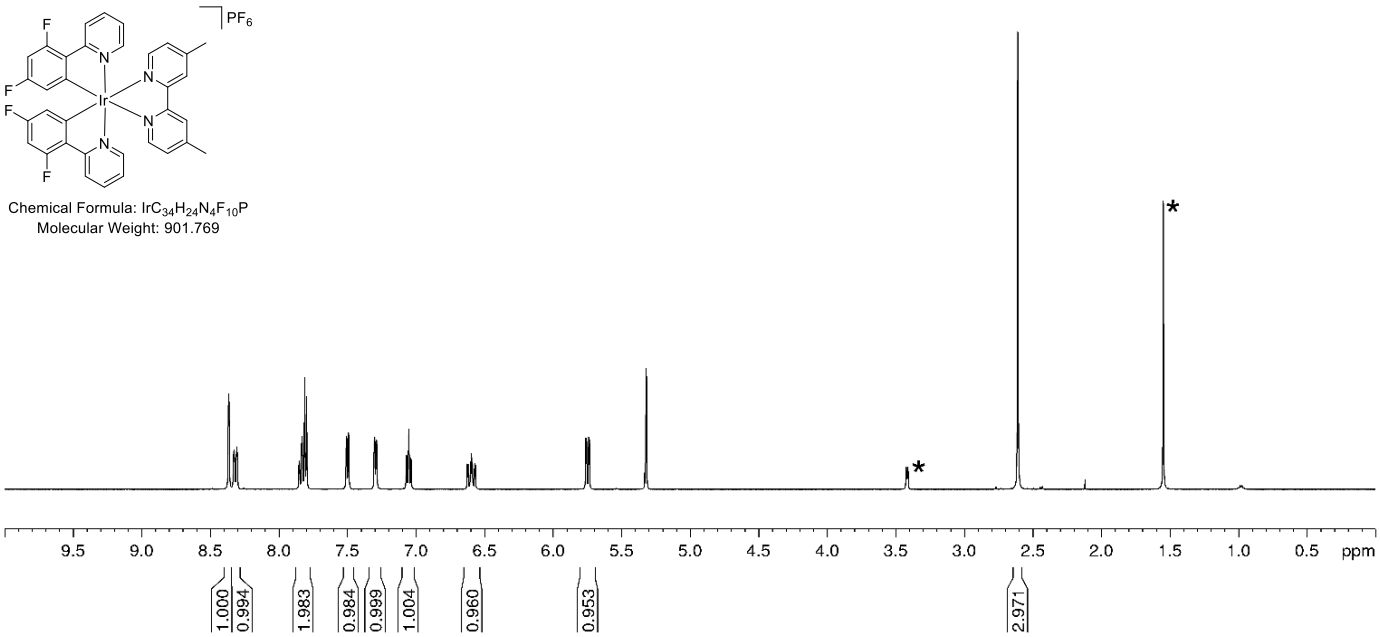
^{1}H NMR (400 MHz; CD_2Cl_2): δ 8.98 (m, 1H), 8.32 (m, 2H), 7.92 (m, 1H), 7.80 (m, 2H), 7.68 (d, *J* = 5.8 Hz, 1H), 7.41 (d, *J* = 6.4 Hz, 1H), 7.34 (d, *J* = 6.4 Hz, 1H), 7.20 (1H, *J* = 5.1 Hz, d), 7.14 (t, *J* = 7.6 Hz 1H), 6.90 (t, *J* = 7.4 Hz, 1H), 6.34 (d, *J* = 8.1 Hz, 1H), 2.57 (s, 3H).

^{1}H NMR spectrum of [Ir(ppy)$_2$(dm-bpy)](PF$_6$)



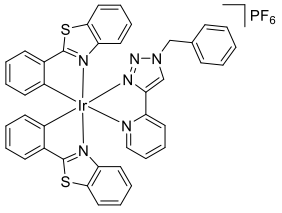
^{1}H NMR (400 MHz; CD_2Cl_2): δ 8.31 (s, 1H), 7.95 (d, *J* = 8.2 Hz, 1H), 7.82 (d, *J* = 5.6 Hz, 1H), 7.78 (td, *J* = 7.8, 1.5 Hz, 1H), 7.73 (dd, *J* = 7.8, 0.9 Hz, 1H), 7.51 (d, *J* = 5.7 Hz, 1H), 7.23 (d, *J* = 5.5 Hz, 1H), 7.06 (td, *J* = 7.5, 1.1 Hz, 1H), 7.00 (ddd, *J* = 7.4, 5.9, 1.4 Hz, 1H), 6.92 (td, *J* = 7.5, 1.4 Hz, 1H), 6.32 (d, *J* = 7.6 Hz, 1H), 2.58 (s, 3H).

^{1}H NMR spectrum of [Ir(df-ppy)$_2$(dm-bpy)](PF$_6$)



^{1}H NMR (400 MHz; CD_2Cl_2): δ 8.37 (s, 1H), 8.32 (d, *J* = 8.4 Hz, 1H), 7.82 (m, 2H), 7.50 (d, *J* = 5.7 Hz, 1H), 7.29 (d, *J* = 5.7 Hz, 1H), 7.05 (m, 1H), 6.62 (ddd, *J* = 12.5, 9.2, 2.4 Hz, 1H), 5.75 (dd, *J* = 8.4, 2.3 Hz, 1H), 2.61 (s, 3H).

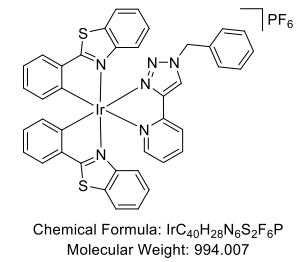
^{1}H NMR spectrum of [Ir(bt)$_2$(ptb)](PF$_6$)



Chemical Formula: $IrC_{40}H_{28}N_6S_2F_6P$
Molecular Weight: 994.007

^{1}H NMR (400 MHz; CD_2Cl_2): δ 8.67 (s, 1H), 8.06 (dq, *J* = 8.0, 0.8 Hz, 1H), 8.00 (td, *J* = 7.8, 1.6 Hz, 1H), 7.94 (dq, *J* = 5.5, 0.8 Hz, 1H), 7.90 (dq, *J* = 8.1, 0.6 Hz, 1H), 7.87 (dq, *J* = 8.1, 0.6 Hz, 1H), 7.84 (m, 1H), 7.80 (m, 1H), 7.32-7.43 (m, 6H), 7.25-7.29 (m, 2H), 7.11 (td, *J* = 7.6, 1.2 Hz, 1H), 7.03-7.08 (m, 2H), 6.95 (ddd, *J* = 8.5, 7.3, 1.3 Hz, 1H), 6.88 (td, *J* = 7.6, 1.5 Hz, 1H), 6.82 (td, *J* = 7.6, 1.5 Hz, 1H), 6.48 (d, *J* = 7.7 Hz, 1H), 6.40 (d, *J* = 8.4 Hz, 1H), 6.37 (d, *J* = 7.8 Hz, 1H), 6.08 (d, *J* = 8.3 Hz, 1H), 5.65 (d, *J* = 14.8 Hz, 1H), 5.55 (d, *J* = 14.8 Hz, 1H).

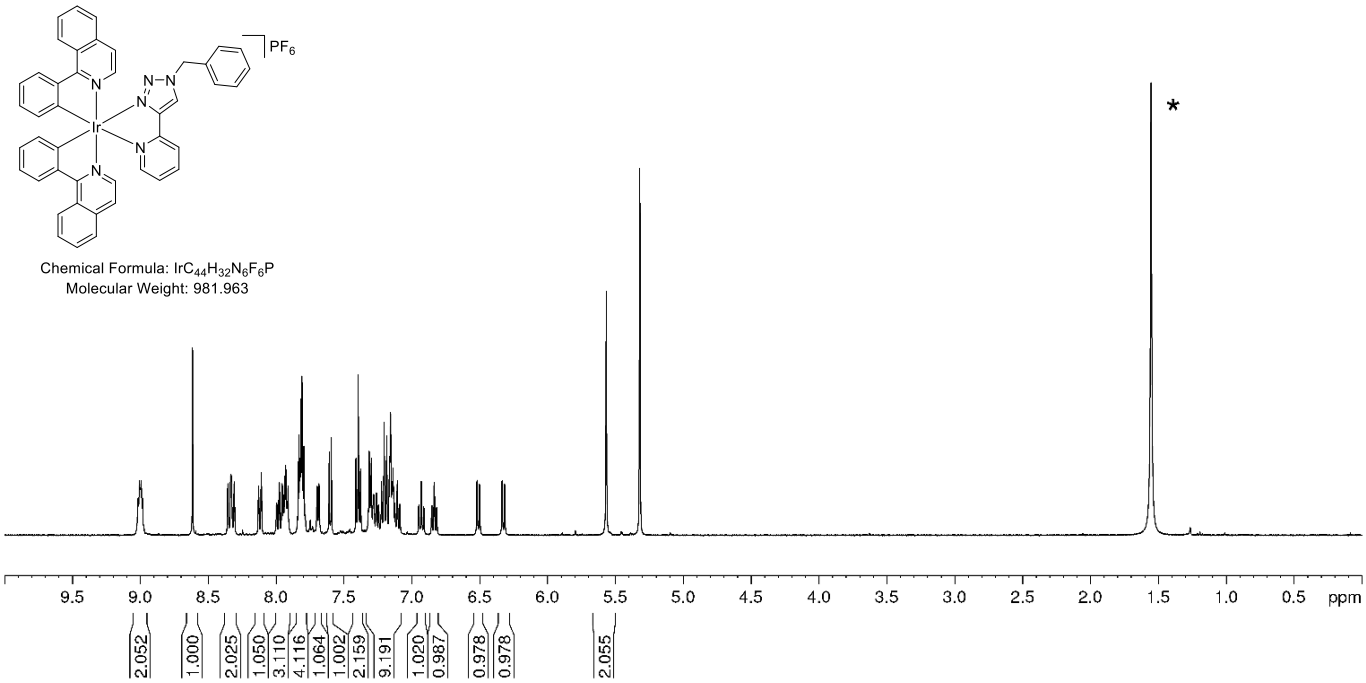
$^{13}C\{^{1}H\}$ NMR spectrum of $[Ir(bt)_2(ptb)](PF_6)$



Chemical Formula: $IrC_{40}H_{28}N_6S_2F_6P$
Molecular Weight: 994.007

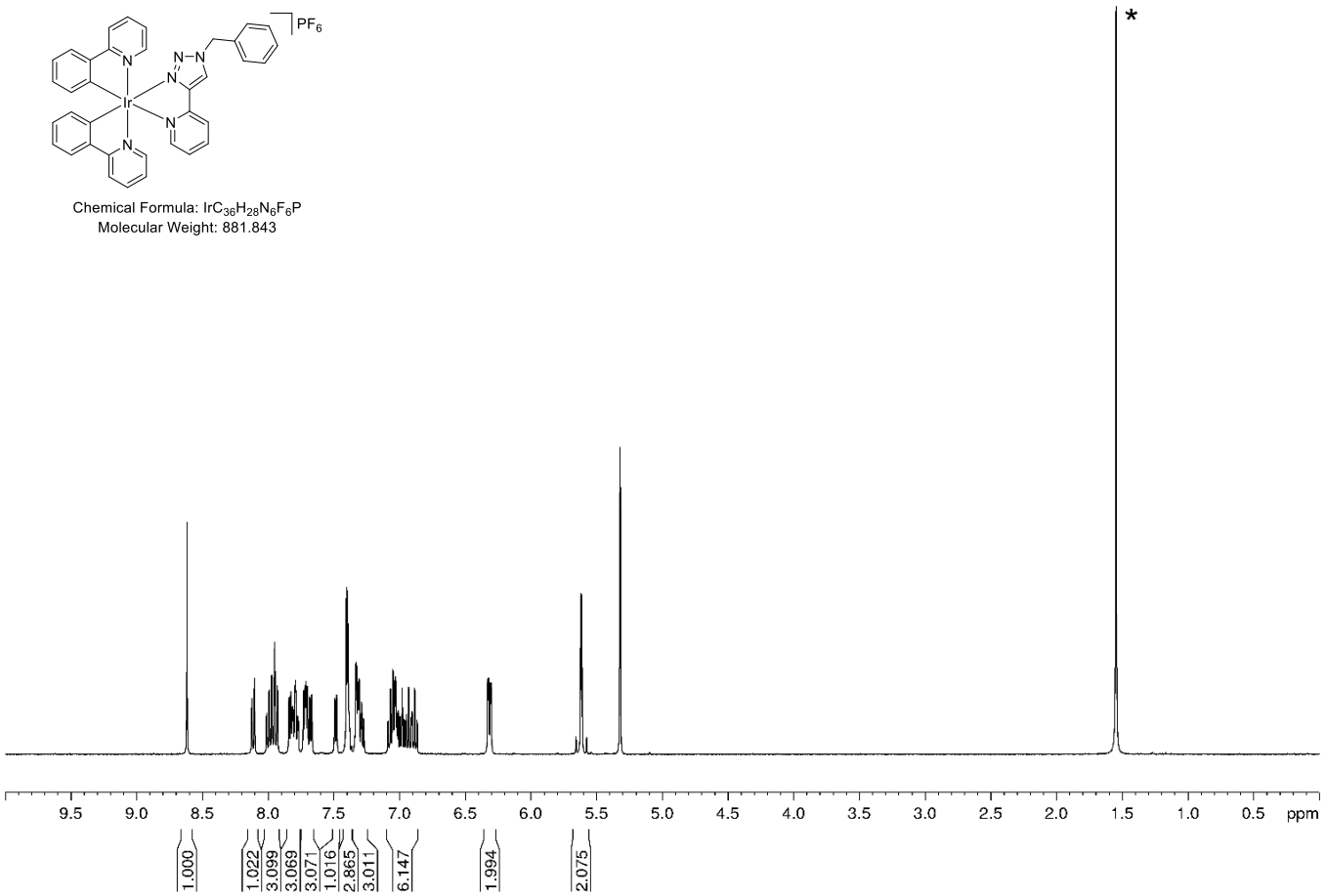
$^{13}C\{^{1}H\}$ NMR (100 MHz; CD_2Cl_2): δ 182.2, 181.3, 151.1, 150.6, 150.2, 150, 149.7 (2C), 146.4, 141.3, 140.9, 140.6, 134.2, 134.0 (2C), 132.5, 132.0 (2C), 131.9, 129.9 (2C), 129.8, 128.6 (3C), 128.5, 127.3, 127.2, 126.7, 126.6, 126.4 (2C), 124, 123.9, 123.8, 123.5, 123.4, 118.7, 118, 56.5.

^{1}H NMR spectrum of [Ir(piq)$_2$(ptb)](PF$_6$)



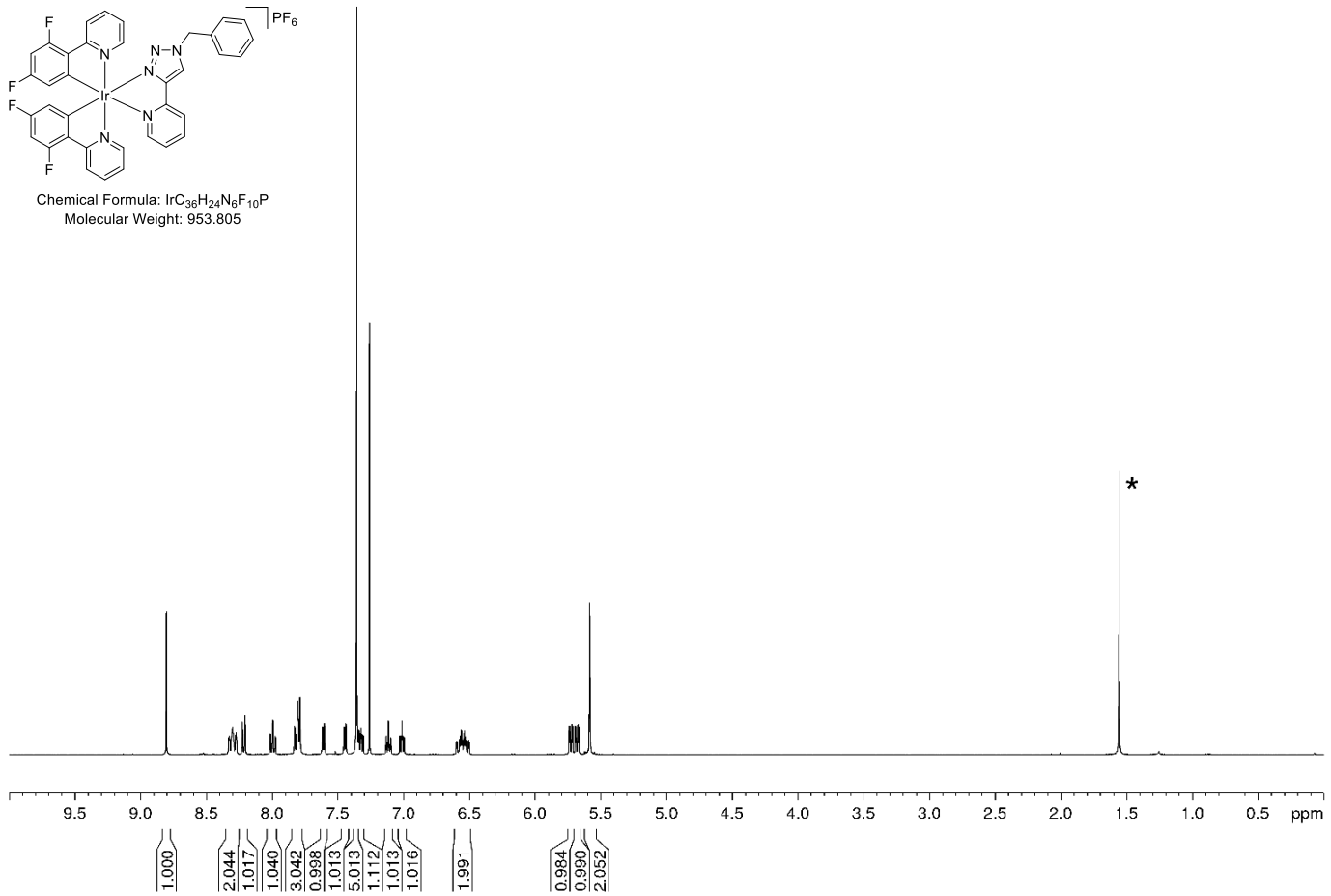
^{1}H NMR spectra was consistent with the literature values. ^{1}H NMR (400 MHz; CD_2Cl_2): δ 9.00 (m, 2H), 8.62 (s, 1H), 8.33 (m, 2H), 8.12 (d, *J* = 7.9 Hz, 1H), 7.91-8.00 (m, 3H), 7.84-7.99 (m, 4H), 7.69 (d, *J* = 5.6 Hz, 1H), 7.60 (d, *J* = 6.4 Hz, 1H), 7.39 (t, *J* = 6.9 Hz, 2H), 7.09-7.33 (m, 9H), 6.93 (t, *J* = 7.4 Hz, 1H), 6.84 (t, *J* = 7.4 Hz, 1H), 6.51 (d, *J* = 7.6 Hz, 1H), 6.32 (d, *J* = 7.6 Hz, 1H), 5.57 (s, 2H).

^{1}H NMR spectrum of [Ir(ppy)$_2$(ptb)](PF$_6$)



^{1}H NMR (400 MHz; CD_2Cl_2): δ 8.62 (s, 1H), 8.11 (d, *J* = 7.9 Hz, 1H), 7.99 (dt, *J* = 7.9, 1.6 Hz, 1H), 7.95 (t, 8.8 Hz, 2H), 7.76-7.84 (m, 3H), 7.69-7.74 (m, 2H), 7.67 (d, *J* = 5.8 Hz, 1H), 7.49 (d, *J* = 5.8 Hz, 1H), 7.38-7.42 (m, 3H), 7.26-7.34 (m, 3H), 7.00-7.09 (m, 3H), 6.98 (ddd, *J* = 7.5, 5.8, 1.4 Hz, 1H), 6.93 (td, *J* = 7.5, 1.3 Hz, 1H), 6.88 (td, *J* = 7.5, 1.3 Hz, 1H), 6.32 (dd, *J* = 7.7, 0.6 Hz, 1H), 6.31 (dd,*J* = 7.6, 0.8 Hz, 1H), 5.62 (m, 2H).

^{1}H NMR spectrum of [Ir(df-ppy)$_2$(ptb)](PF$_6$)

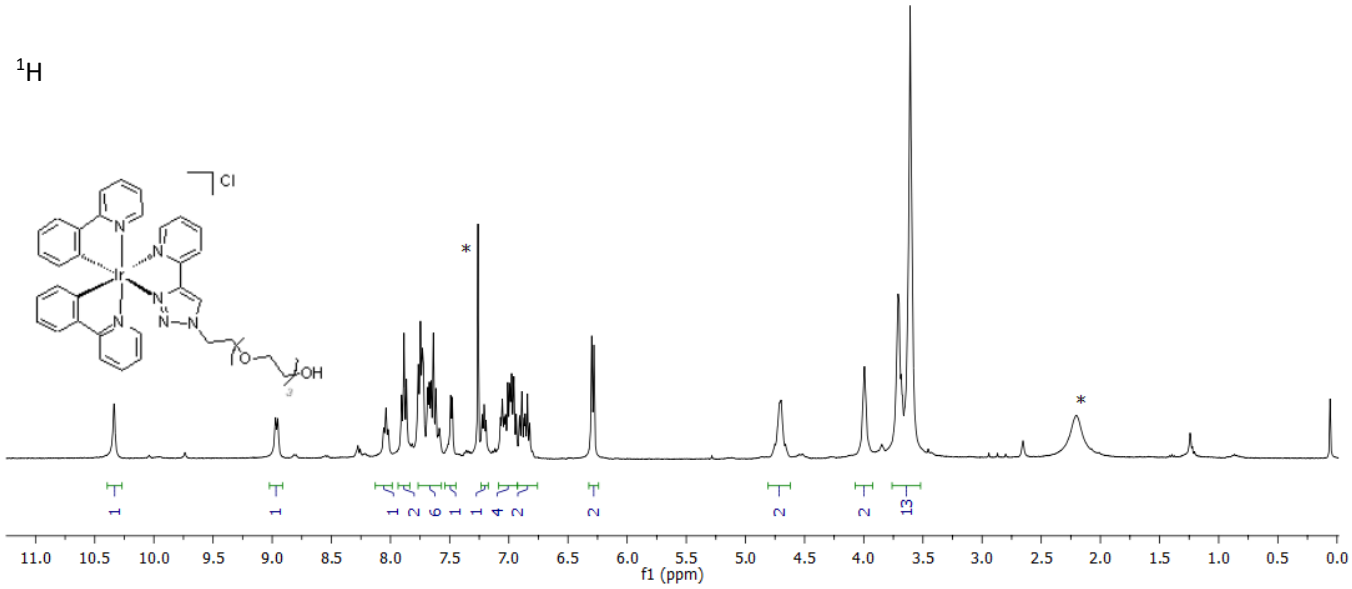


^{1}H NMR (400 MHz; $CDCl_3$): δ 8.80 (s, 1H), 8.30 (t, *J* = 10.5 Hz, 2H), 8.22 (d, *J* = 7.9 Hz, 1H), 8.00 (td, *J* = 7.9, 1.5 Hz, 1H), 7.79-7.83 (m, 3H), 7.61 (d, *J* = 5.8 Hz, 1H), 7.45 (d, *J* = 5.8 Hz, 1H), 7.36 (s, 5H), 7.32 (m, 1H), 7.11 (ddd, *J* = 7.3, 5.9, 1.3 Hz, 1H), 7.01 (ddd, *J* = 7.3, 5.9, 1.3 Hz, 1H), 6.55 (m, 2H), 5.73 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.68 (dd, *J* = 8.5, 2.3 Hz, 1H), 5.58 (s, 2H).

NMR spectra of [Ir(C^N)$_2$(pt-TEG)]Cl complexes

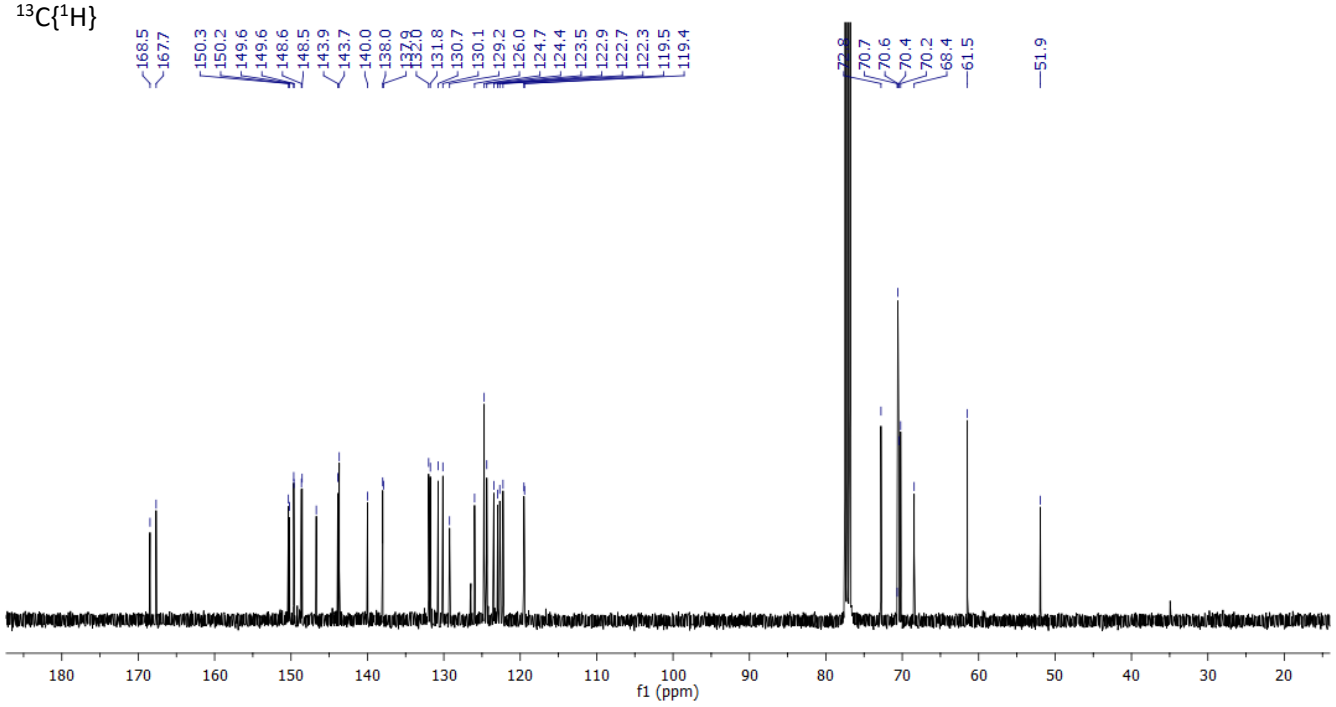
[Ir(ppy)$_2$(pt-TEG)]Cl, $CDCl_3$. Residual solvent marked with an asterisk.

^{1}H



^{1}H NMR (500 MHz; $CDCl_3$): δ 10.46 (s, 1H), 9.04 (d, *J* = 8.0 Hz, 1H), 8.04 (td, *J* = 7.8, 1.5 Hz, 1H), 7.90 (m, 2H), 7.78-7.74 (m, 3H), 7.70 (dd, *J* = 5.8, 0.7 Hz, 1H), 7.67-7.64 (m, 2H), 7.50 (dd, *J* = 5.8, 0.7 Hz, 1H), 7.22 (ddd, *J* = 7.6, 5.6, 1.3 Hz, 1H), 7.07 (ddd, *J* = 7.3, 5.9, 1.4 Hz, 1H), 7.03 (td, *J* = 7.5, 1.0 Hz, 1H), 7.00-6.97 (m, 2H), 6.91 (td, *J* = 7.4, 1.2 Hz, 1H), 6.87 (td, *J* = 7.4, 1.2 Hz, 1H), 4.77-4.68 (m, 2H), 4.05-3.99 (m, 2H), 3.76-3.66 (m, 4H), 3.68-3.57 (m, 8H).

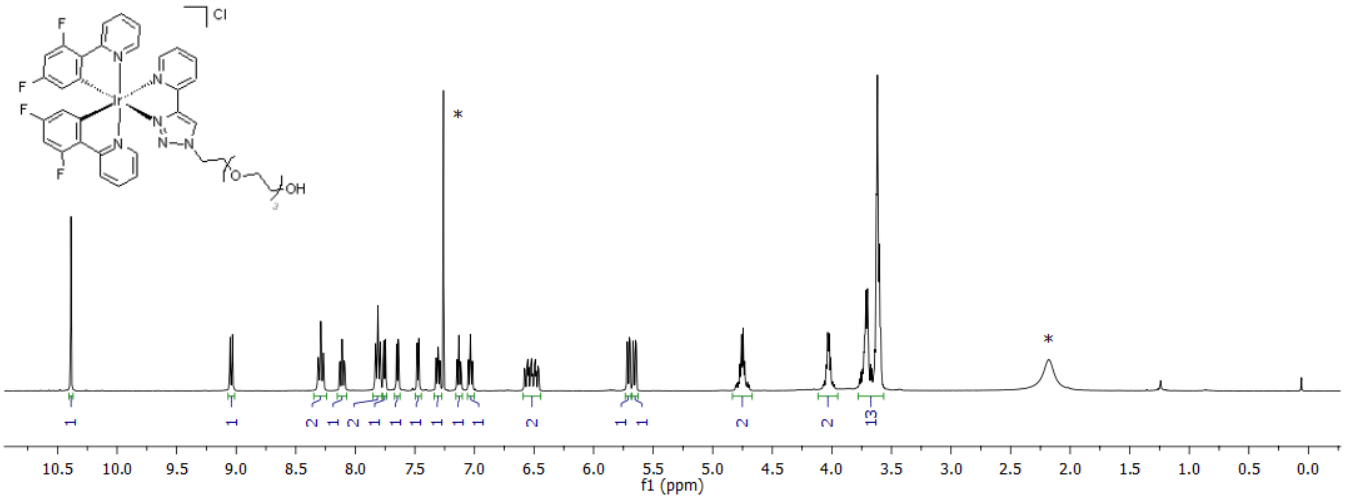
^{13}C{^{1}H}



^{13}C NMR (126 MHz; $CDCl_3$): δ 168.4, 167.6, 150.3, 150.1, 149.5, 149.4, 148.5, 148.4, 146.6, 143.8, 143.6, 139.8, 137.8, 137.7, 131.9, 131.7, 130.6, 130, 129.3, 125.8, 124.7, 124.6, 124.2, 123.3, 122.8, 122.6, 122.1, 119.4, 119.3, 72.6, 70.5 (2C), 70.4, 70.1, 68.3, 61.4, 51.8.

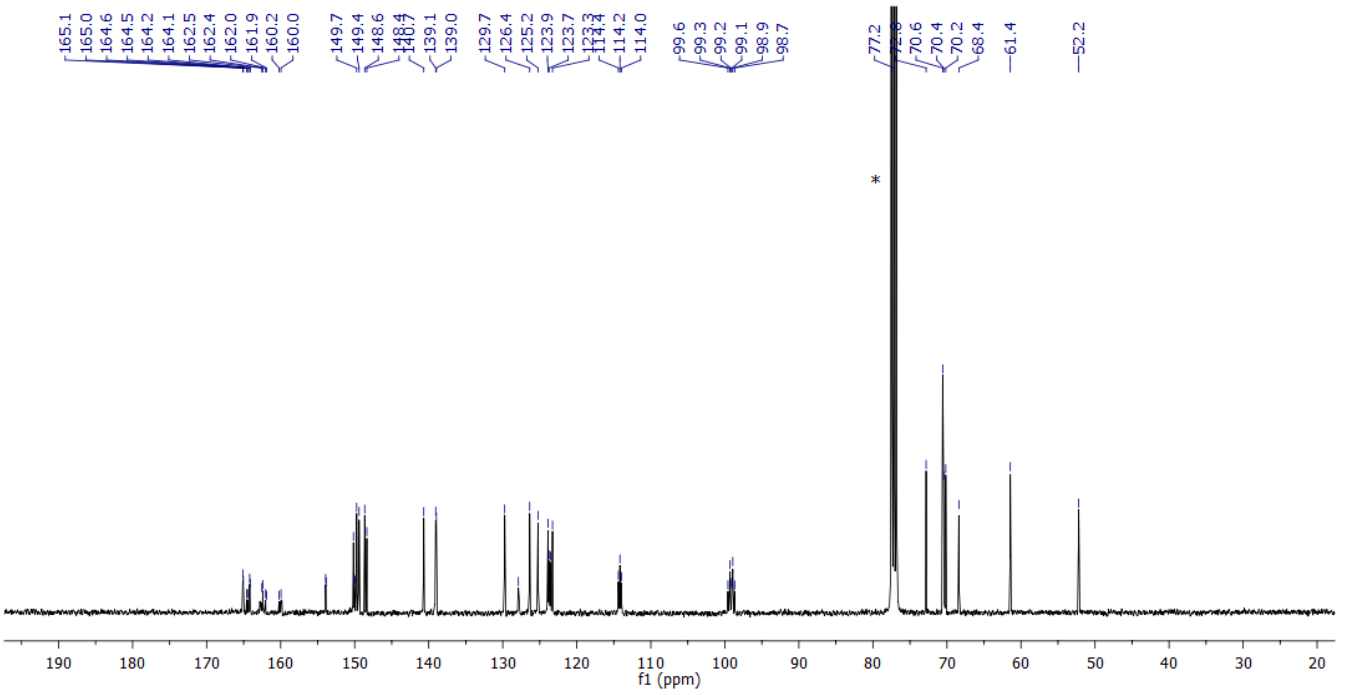
[Ir(df-ppy)$_2$(pt-TEG)]Cl, $CDCl_3$. Residual solvent marked with an asterisk.

^{1}H



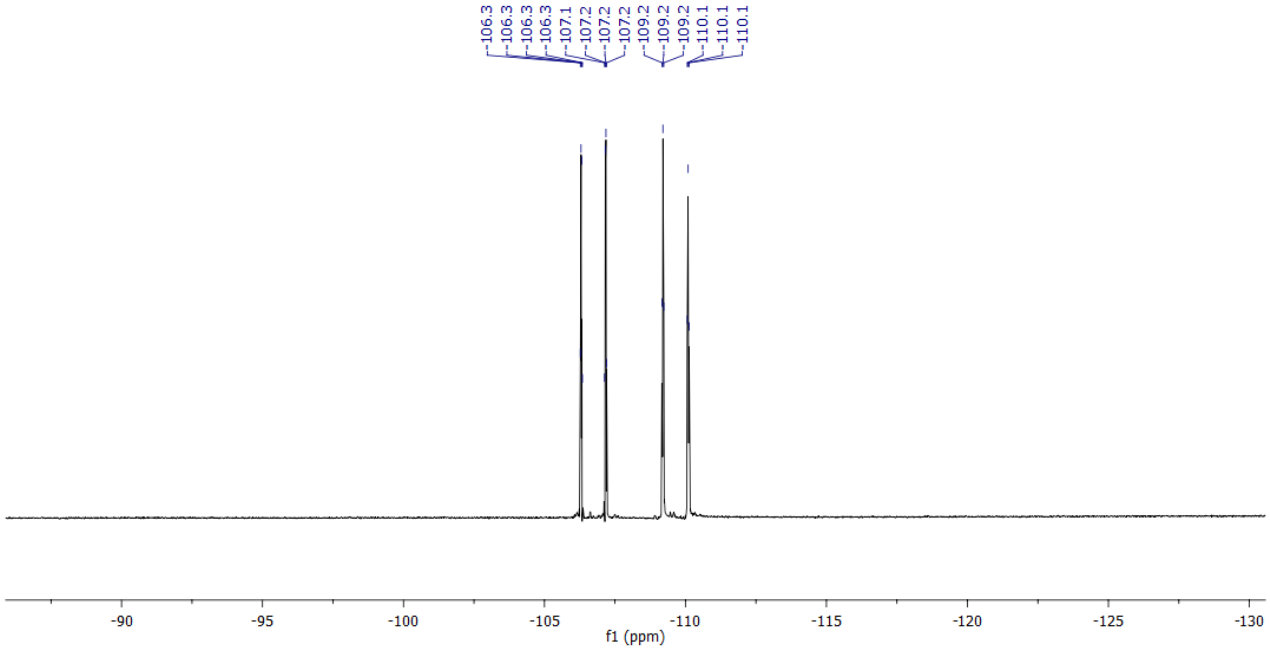
^{1}H NMR (500 MHz; $CDCl_3$): δ 9.16 (s, 1H), 8.40 (d, *J* = 8.0 Hz, 1H), 8.28 (t, *J* = 10.0 Hz, 2H), 8.01 (t, *J* = 7.8 Hz, 1H), 7.80 (m, 3H), 7.65 (d, *J* = 5.7 Hz, 1H), 7.50 (d, *J* = 5.7 Hz, 1H), 7.31 (t, *J* = 6.6 Hz, 1H), 7.15 (t, *J* = 6.7 Hz, 1H), 7.05 (t, *J* = 6.7 Hz, 1H), 6.55 (m, 1H), 6.48 (m, 1H), 5.71 (d, *J* = 7.7 Hz, 1H), 5.66 (d, *J* = 8.0 Hz, 1H), 4.69-4.58 (m, 2H), 3.95-3.89 (m, 2H), 2.69 (broad s, 1H).

$^{13}C\{^{1}H\}$



^{13}C NMR (101 MHz; $CDCl_3$): δ 165.1 (m, CF), 164.5 (m, CF), 164.2 (m, CF), 162.6 (m, CF), 162.0 (m), 160.0 (m), 153.9 (d), 150.2, 150.0 (d), 149.7, 149.4, 148.7, 148.4, 140.7, 139.1, 139.0, 129.7, 127.9 (m, 2C), 126.4, 125.2, 123.9, 123.7 (d), 123.5 (d), 123.3, 114.2 (m, 2C), 99.1 (m, 2C), 77.4, 72.8, 70.6, 70.5, 70.2, 68.4, 61.4, 52.2.

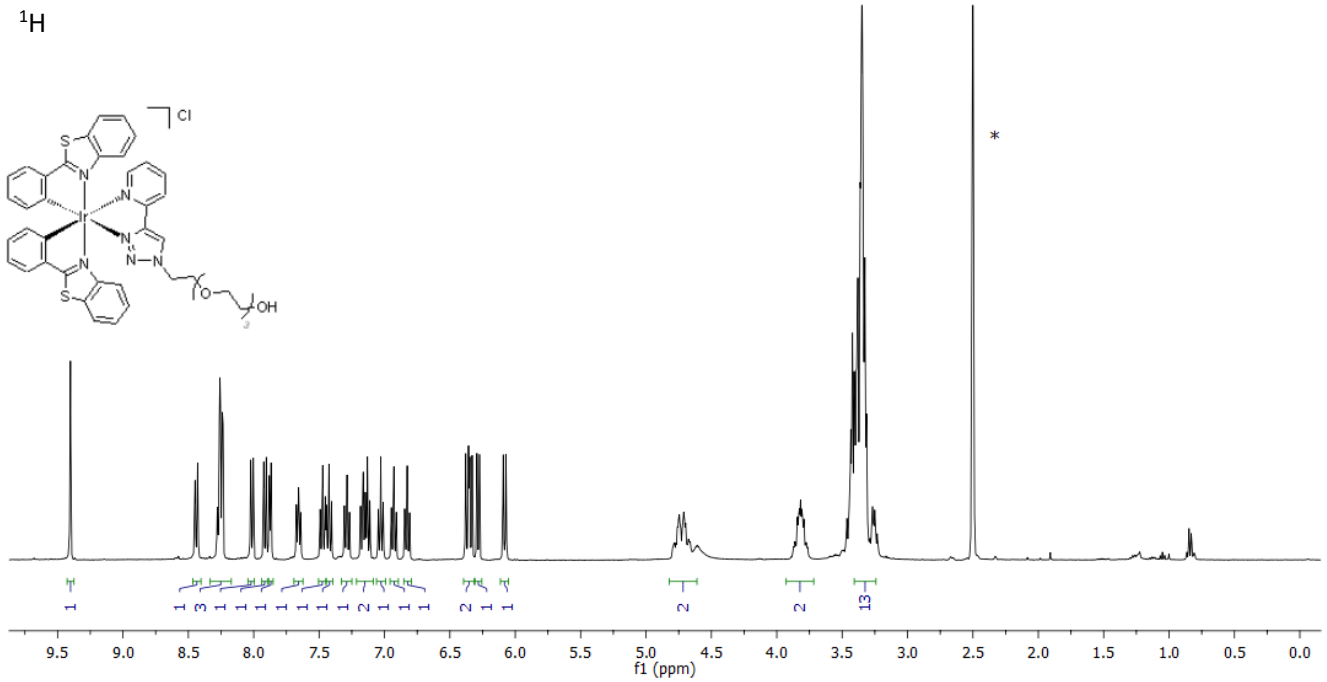
^{19}F



^{19}F NMR (376 MHz; $CDCl_3$): δ -106.3 (m, 1F), -107.2 (m, 1F), -109.2 (m, 1F), -110.1 (m, 1F).

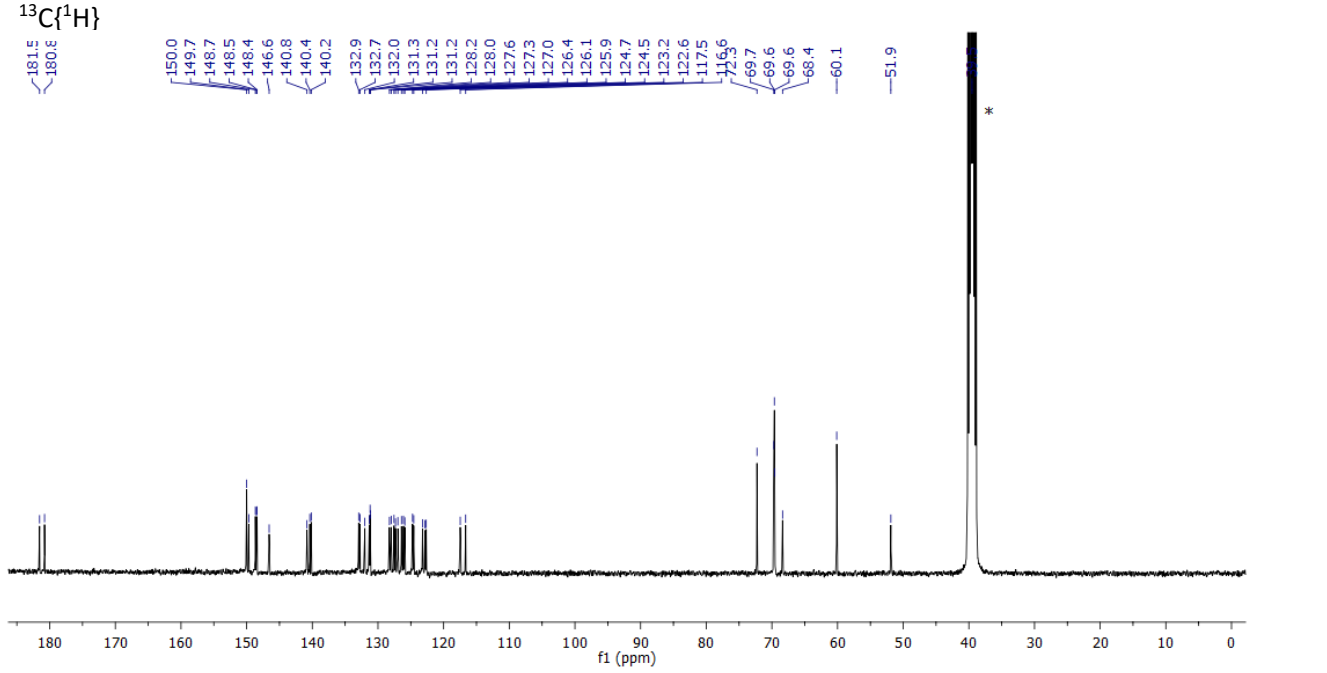
$[Ir(bt)_2(pt\text{-}TEG)]Cl$, d_6-DMSO. Residual solvent marked with an asterisk.

^1H



^1H NMR (400 MHz; DMSO-d_6): δ 9.40 (s, 1H), 8.44 (d, *J* = 8.0, 1H), 8.25 (m, 3H,), 8.01 (d, *J* = 7.7 Hz, 1H), 7.91 (d, *J* = 7.6 Hz, 1H,), 7.87 (d, *J* = 5.5 Hz, 1H), 7.66 (m, 1H), 7.46 (m, 1H), 7.42 (m, 1H), 7.29 (m, 1H), 7.14 (m, 2H), 7.02 (m, 1H), 6.93 (m, 1H), 6.83 (m, 1H), 6.35 (m, 2H), 6.28 (d, *J* = 7.7, 1H), 6.08 (d, *J* = 8.4, 1H), 4.73 (m, 2H), 3.82 (m, 2H), 3.45 (m, 13H).

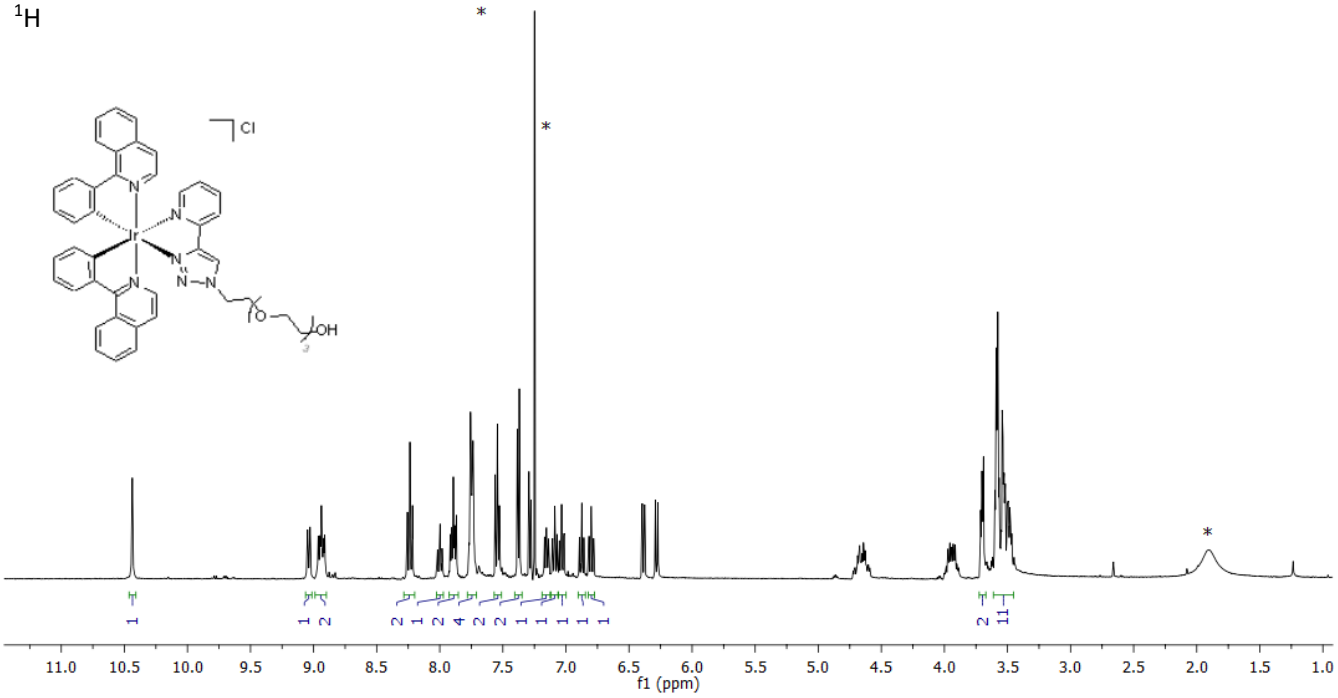
$^{13}C\{^1H\}$



$^{13}C\{^1H\}$ NMR (101 MHz; DMSO-d_6): δ 181.5, 180.8, 150.0, 149.7, 148.7, 148.5, 148.4, 146.6, 140.8, 140.4, 140.2, 132.9, 132.8, 132.0, 131.3, 131.2 (2C), 131.2, 128.3, 128.0, 127.6, 127.3, 127.0, 126.4, 126.1, 125.9, 124.7, 124.6, 123.2, 122.8, 122.7, 117.5, 116.6, 72.3, 69.7, 69.6, 69.6, 69.6, 68.4, 60.1, 51.9.

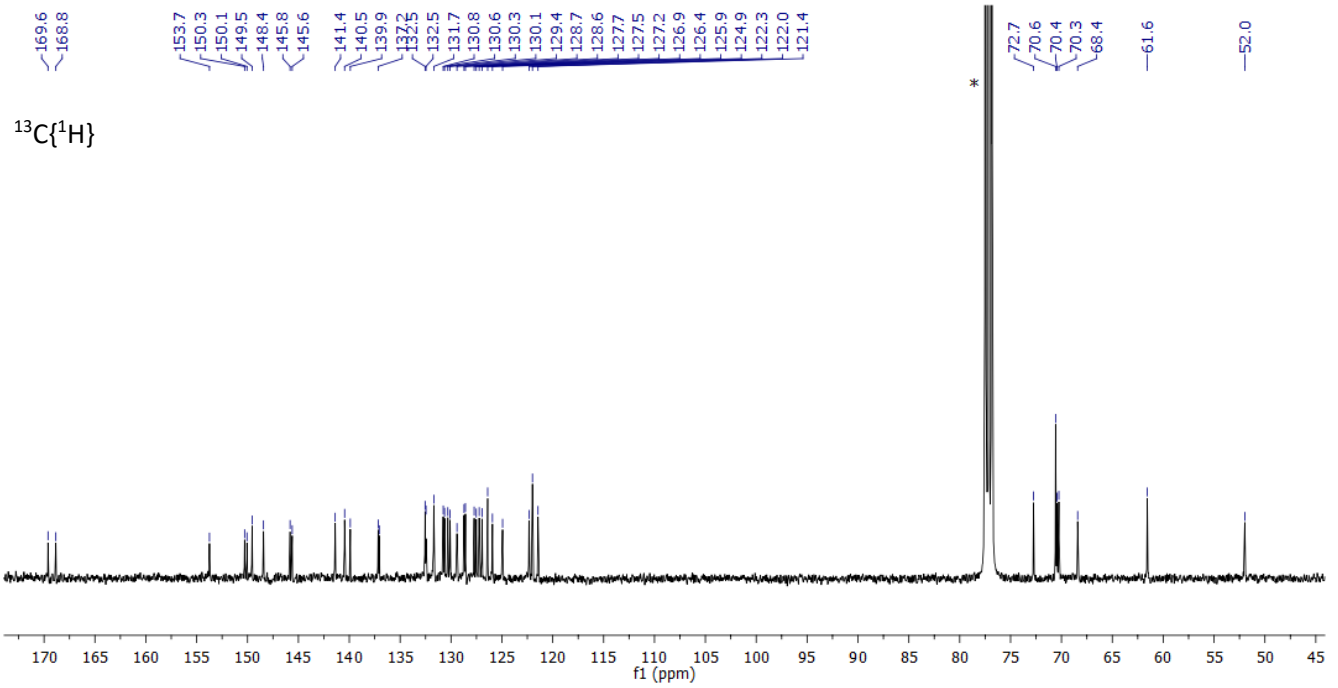
$Ir(piq)_2(pt\text{-}TEG)]Cl$, $CDCl_3$. Residual solvent marked with an asterisk.

^{1}H



^{1}H NMR (400 MHz; $CDCl_3$): δ 10.38 (s, 1H), 9.02 (d, *J* = 7.9, 1H), 8.95 (ddd, *J* = 10.5, 6.9, 3.4, 2H), 8.24 (dd, *J* = 10.4, 5.9, 2H), 8.01 (td, *J* = 7.8, 1.5, 1H), 7.93-7.87 (m, 2H), 7.79-7.72 (m, 4H), 7.58-7.53 (m, 2H), 7.41-7.37 (m, 2H), 7.32-7.28 (m, 1H), 7.18-7.14 (m, 1H), 7.12-7.08 (m, 1H), 7.06-7.02 (m, 1H), 6.90-6.86 (m, 1H), 6.83-6.79 (m, 1H), 6.39 (dd, *J* = 7.7, 1.1, 1H), 6.30-6.28 (m, 1H), 4.70-4.61 (m, 2H), 3.94 (ddd, *J* = 13.0, 6.4, 4.2, 1H), 3.71 (dd, *J* = 6.0, 3.0, 2H), 3.64-3.45 (m, 11H).

^{13}C{^{1}H}

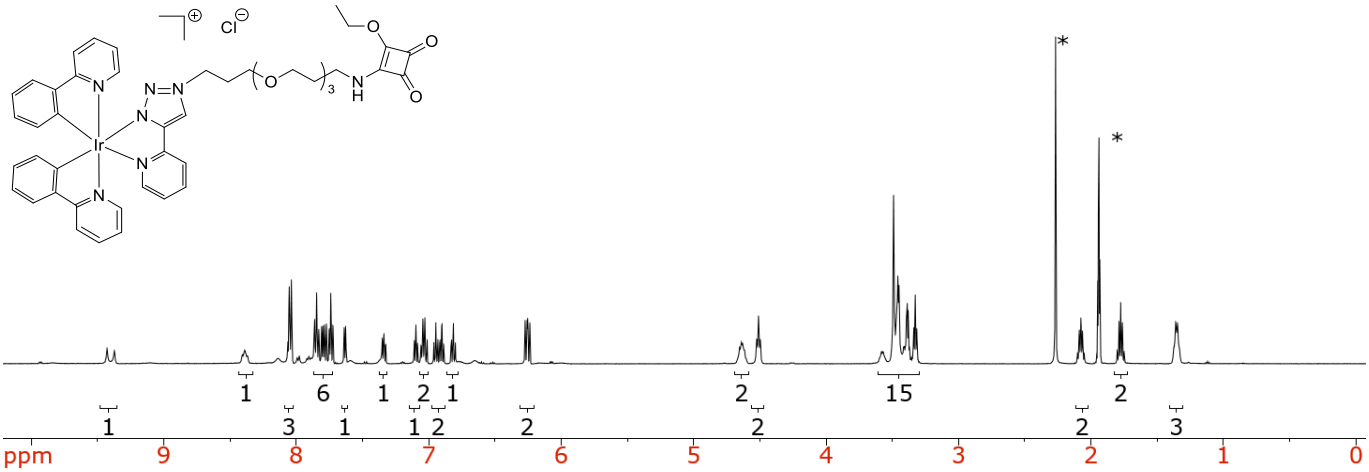


^{13}C{^{1}H} NMR (101 MHz; $CDCl_3$): δ 169.6, 168.8, 153.7, 150.2, 150.1, 149.6, 148.4, 145.8, 145.6, 141.4, 140.5, 139.9, 137.2, 137.1, 132.6, 132.5, 131.7, 131.7, 130.8, 130.6, 130.3, 130.1, 129.3, 128.7, 128.6, 127.7, 127.5, 127.2, 126.9, 126.4, 125.9, 124.9, 122.3, 122.0, 121.0, 121.5, 77.4, 72.8, 70.6, 70.6, 70.4, 70.2, 68.4, 61.5, 52.0.

NMR spectra of [Ir(C^N)$_2$(pt-TOxT-Sq)]Cl ECL labels

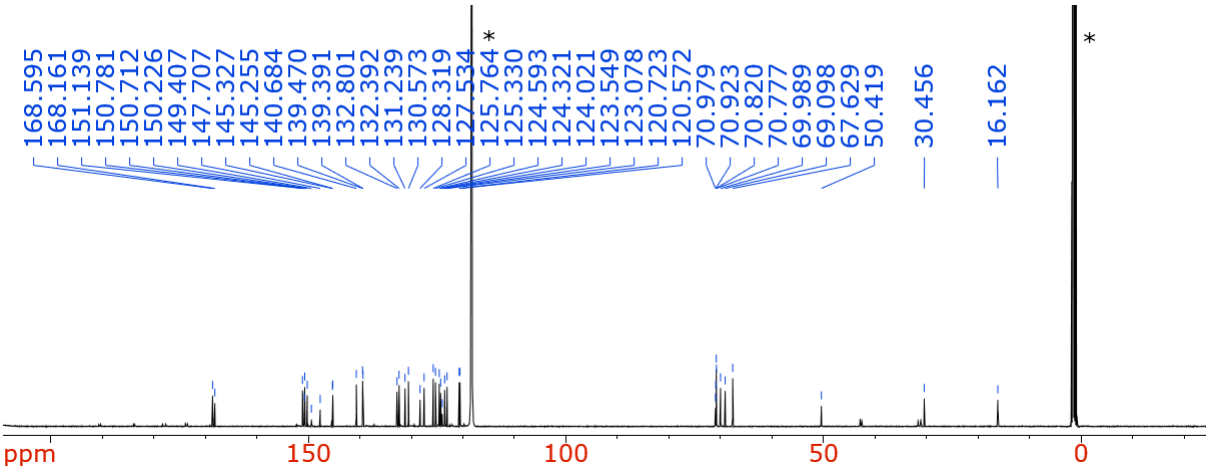
[Ir(ppy)$_2$(pt-TOxT-Sq)]Cl, CD_3CN. Residual solvent marked with an asterisk.

^{1}H



^{1}H NMR (500 MHz; CD_3CN): δ 9.40 (m, 1H), 8.41-8.37 (m, 1H), 8.05 (m, 3H), 7.86-7.74 (m, 6H), 7.63 (d, *J*=5.3 Hz, 1H), 7.34 (ddd, *J* =7.4, 5.8, 1.3 Hz, 1H), 7.10 (ddd, *J* = 7.3, 6.0, 1.3, 1H), 7.04 (m, 2H), 6.92 (m, 2H), 6.81 (td, *J* = 7.4, 1.1 Hz, 1H), 6.26 (m, 2H), 4.64 (m, 2H), 4.51 (m, 2H), 3.45 (m, 15H), 2.08 (q, *J* = 6.2, 2H), 1.78 (q, *J* = 6.4, 2H), 1.35 (m, 3H).

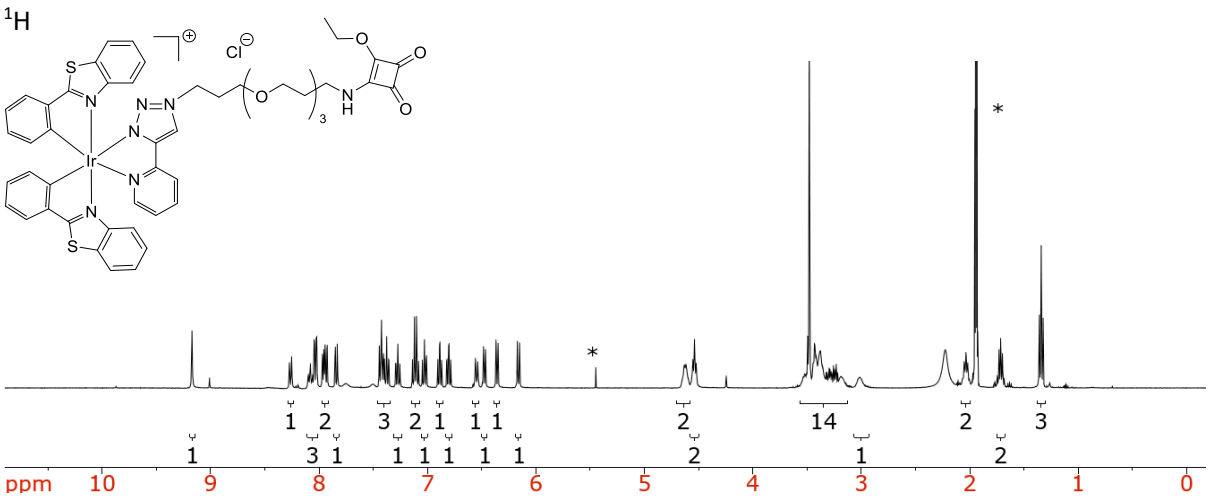
$^{13}C\{^{1}H\}$



$^{13}C\{^{1}H\}$ NMR (126 MHz; CD_3CN): δ 168.6, 168.2, 151.1, 150.8, 150.7, 150.2, 149.4, 147.7, 145.3, 145.3, 140.7, 139.5, 139.4, 132.8, 132.4, 131.2, 130.6, 128.3, 127.5, 125.8, 125.3, 124.6, 124.3, 124.0, 123.5, 123.1, 120.7, 120.6, 71.0, 70.9, 70.8, 70.8, 70 .0, 69.1, 67.6, 50.4, 30.5, 16.2.

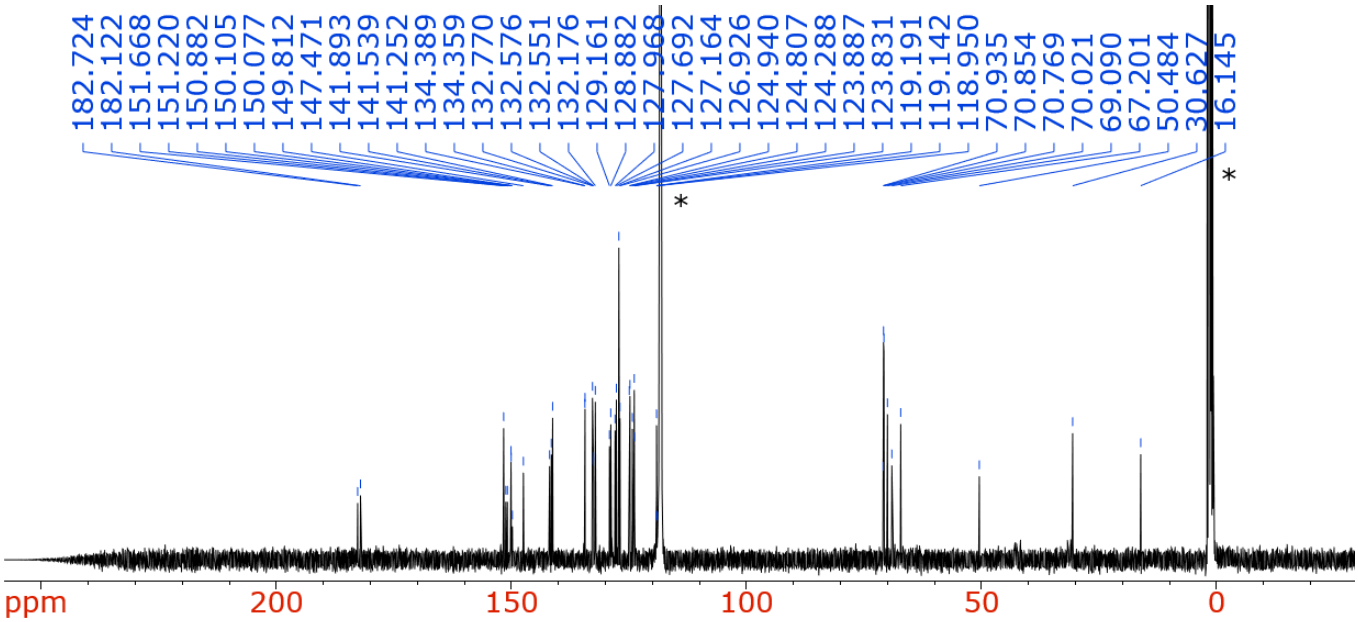
[Ir(bt)$_2$(pt-TOxT-Sq)]Cl, CD_3CN. Residual solvent marked with an asterisk.

^1H



^1H NMR (400 MHz; CD_3CN): δ 9.16 (s, 1H), 8.26 (d, *J* = 7.9 Hz, 1H), 8.06 (m, 3H), 7.95 (m, 2H), 7.84 (m, 1H), 7.40 (m, 3H), 7.27 (m, 1H), 7.11 (m, 2H), 7.03 (td, *J*= 7.5, 1.0 Hz, 1H), 6.89 (td, *J* = 7.5, 1.3 Hz, 1H), 6.80 (td, *J* = 7.5, 1.3 Hz, 1H), 6.55 (d, *J*= 8.3 Hz, 1H), 6.47 (d, *J* = 7.6 Hz, 1H), 6.36 (d, *J* = 7.6 Hz, 1H), 6.16 (d, *J* = 8.4 Hz, 1H), 4.62 (d, *J* = 6.3 Hz, 2H), 4.54 (t, *J* = 6.5 Hz, 2H), 3.37 (m, 14H), 3.02 (s, 1H), 2.03 (q, *J* = 6.1, 2H), 1.71 (q, *J* = 6.3, 2H), 1.34 (t, *J* = 14.1, 3H).

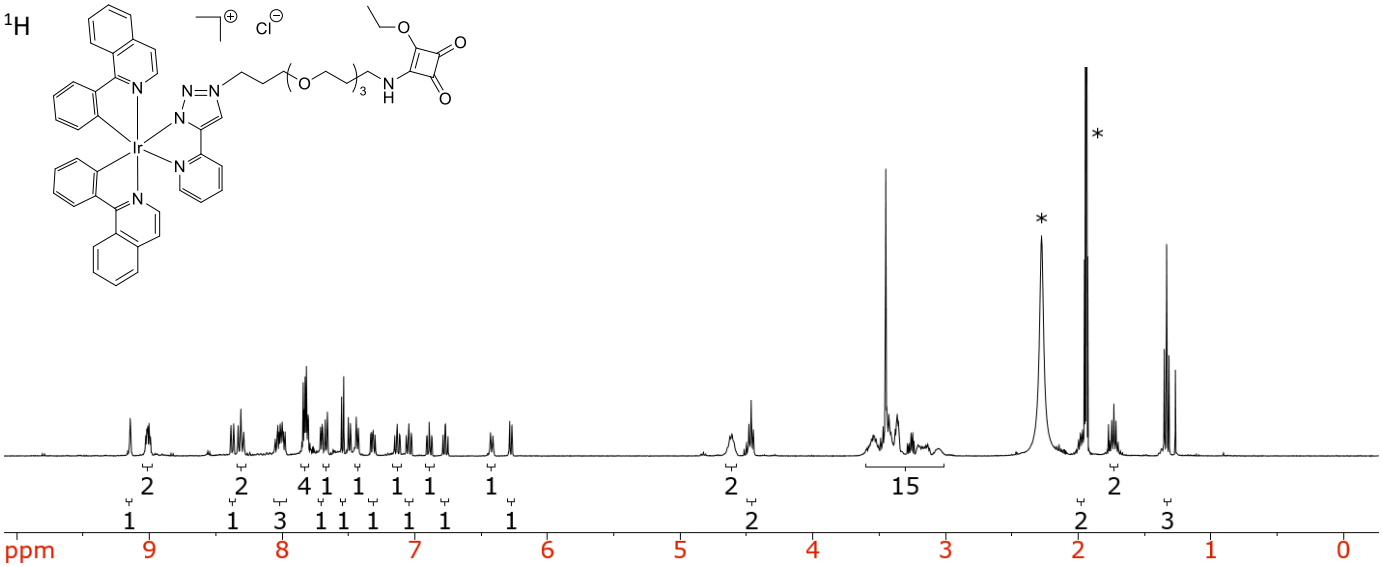
$^{13}C\{^1H\}$



$^{13}C\{^1H\}$ NMR (101 MHz; CD_3CN): δ 182.7, 182.1, 151.7, 151.2, 150.9, 150.1, 150.1, 149.8, 147.5, 141.9, 141.5, 141.3, 134.4, 134.4, 132.8, 132.6, 132.6, 132.2, 129.2, 128.9, 128.6, 128.0, 127.7, 127.2, 126.9, 124.9, 124.8, 124.3, 123.9, 123.8, 119.1, 118.3, 70.9, 70.9, 70.8, 70.0, 69.1, 67.2, 50.5, 42.9, 42.6, 31.6, 31.0, 30.6, 18.8, 16.1.

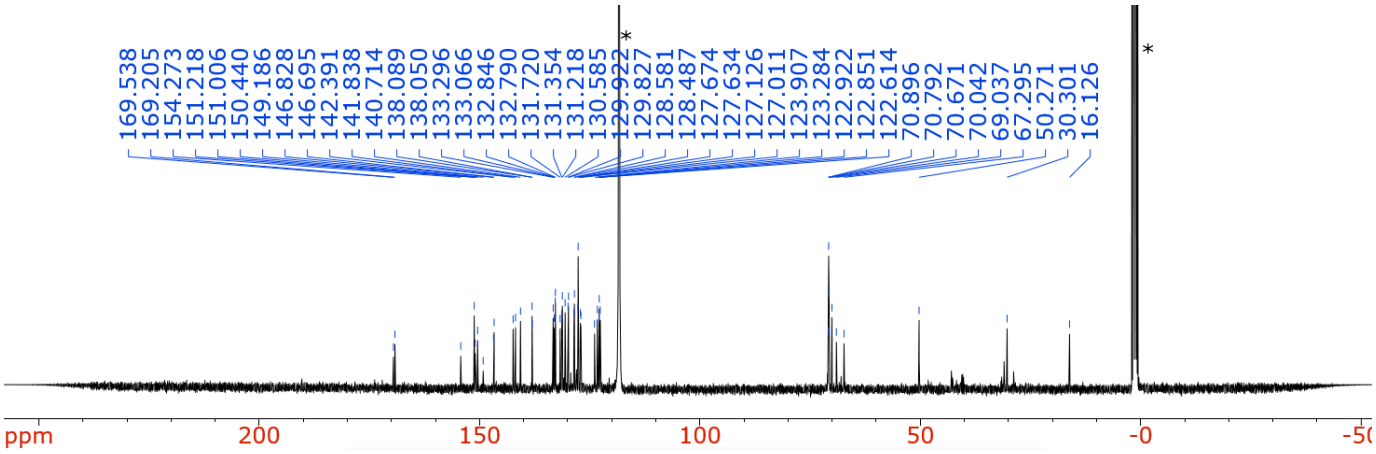
[Ir(piq)$_2$(pt-TOxT-Sq)]Cl, CD_3CN. Residual solvent marked with an asterisk.

^{1}H



^{1}H NMR (400 MHz; [CD_3CN]): δ 9.14 (s, 1H), 9.01 (m, 2H), 8.38 (d, *J* = 8.0 Hz, 1H), 8.31 (m, 2H), 8.02 (m, 3H,), 7.83 (m, 4H), 7.70 (m, 1H), 7.67 (d, *J* = 6.4 Hz), 7.54 (d, *J* = 6.4 Hz, 1H), 7.49 (d, *J* = 6.4, 1H), 7.43 (d, *J* = 6.4 Hz, 1H), 7.31 (ddd, *J* = 7.7, 5.6, 1.3 Hz, 1H), 7.13 (ddd, *J* = 8.2, 7.2, 1.2 Hz, 1H), 7.04 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 6.89 (td, *J* = 7.4, 1.2 Hz, 1H), 6.77 (td, *J* = 7.4, 1.2 Hz, 1H), 6.42 (d, *J* = 7.5 Hz, 1H), 6.28 (dd, *J* = 7.7, 1.1 Hz, 1H), 4.62 (m, 2H), 4.46 (t, *J* = 6.8 Hz, 2H), 3.32 (m, 15H), 1.98 (dd, *J* = 6.5, 5.2 Hz, 2H), 1.73 (q, *J* = 6.4 Hz, 2H), 1.33 (t, *J* = 7.1 Hz, 3H).

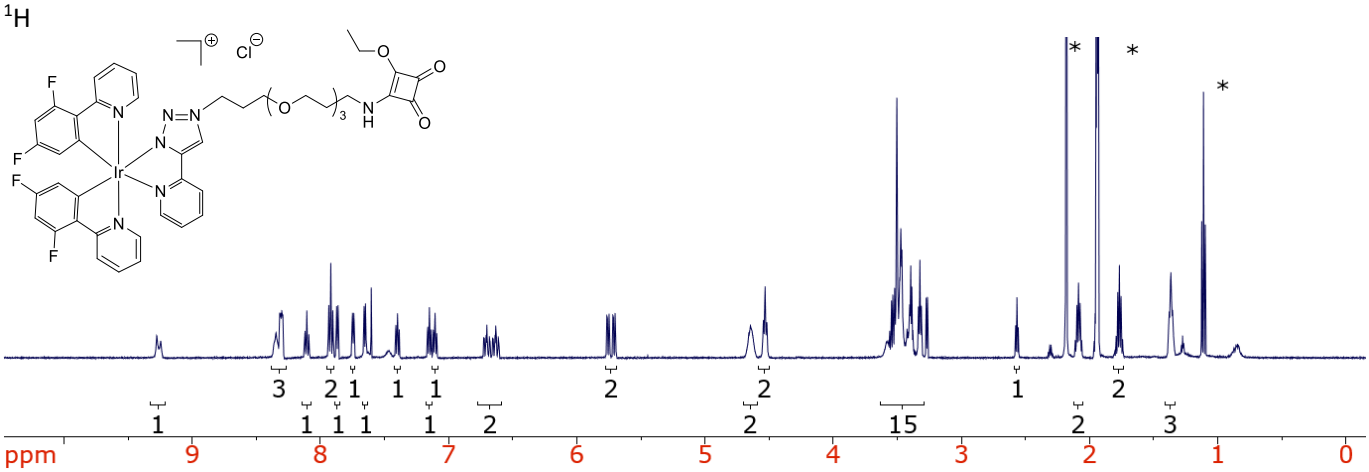
^{13}C{^{1}H}



^{13}C{^{1}H} NMR (101 MHz; [CD_3CN]): δ 169.5, 169.2, 154.3, 151.2, 151.0, 150.4, 149.2, 146.8, 146.7, 142.4, 141.8, 140.7, 138.1, 138.0, 133.3, 133.1, 132.8, 132.8, 131.7, 131.3, 131.2, 130.6, 129.9, 129.8, 128.6, 128.5, 128.1, 127.7, 127.6, 127.1, 127.0, 123.9, 123.3, 122.9, 122.8, 122.6, 70.9, 70.8, 70.7, 70.0, 69.0, 67.3, 50.3, 30.3, 16.1.

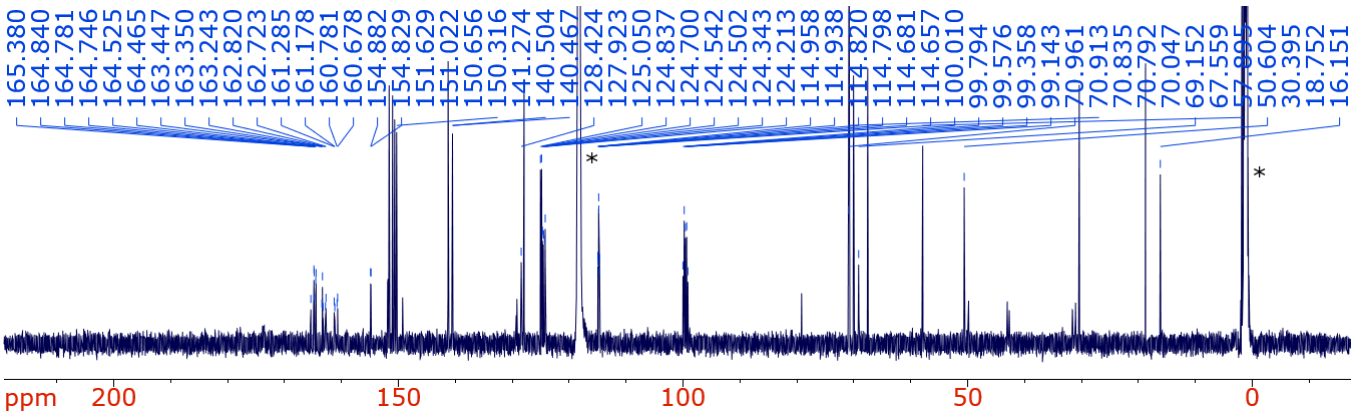
[Ir(df-ppy)$_2$(pt-TOxT-Sq)]Cl, CD_3CN. Residual solvent marked with an asterisk.

^1H



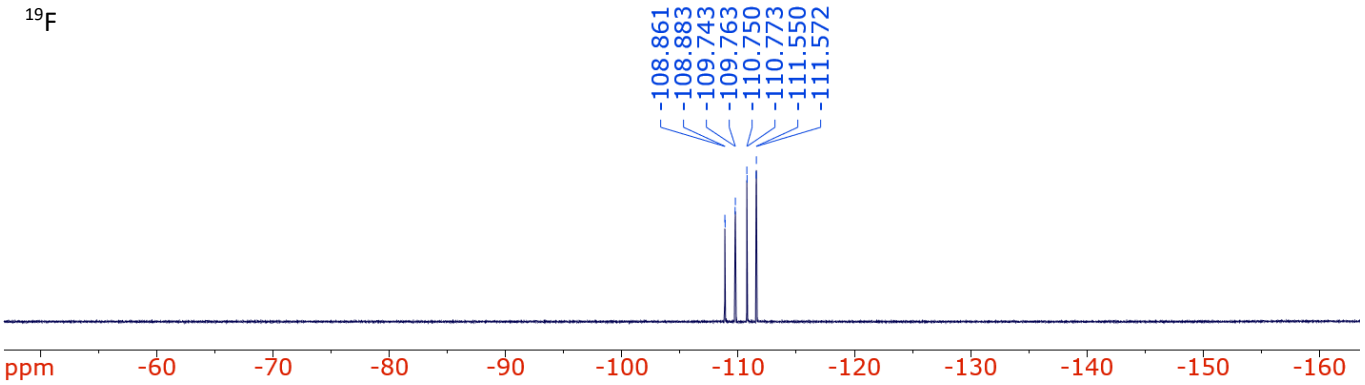
^1H NMR (500 MHz; CD_3CN): δ 9.50 (m, 1H), 8.44 (m, 1H), 8.30 (m, 2H), 8.10 (m, 1H), 7.89 (m, 3H), 7.76 (m, 1H), 7.66 (m, 1H), 7.39 (m, 1H), 7.13 (m, 2H), 6.66 (m, 2H), 5.73 (ddd, *J* = 24.1, 8.7, 2.3 Hz, 2H), 4.63 (m, 2H), 4.54 (m, 2H), 3.45 (m, 15H), 2.09 (q, *J* = 6.2 Hz, 2H), 1.78 (q, *J* = 6.4 Hz, 2H), 1.36 (m, 3H).

$^{13}C\{^1H\}$



$^{13}C\{^1H\}$ NMR (126 MHz; CD3CN): δ 164.7 (m, CF), 163.4 (m, CF), 162.8 (m,CF), 161.0 (m, CF), 154.9 (m, CF), 151.9 (m, CF), 151.6, 151.0, 150.7, 150.3, 141.3, 140.5, 140.5, 128.4, 128.0, 125.1, 124.8, 124.7, 124.54, 124.5, 124.3, 124.2, 114.8 (m, CF), 99.6 (m, CF), 71.0, 70.9, 70.8, 70.79, 70.0, 69.2, 67.6, 57.9, 50.6, 30.4, 18.8, 16.2.

^{19}F



^{19}F NMR (471 MHz; CD_3CN): δ -106.18 (d, *J* = 10.6, 1F), -107.06 (d, *J* = 9.6, 1F), -108.07 (d, *J* = 10.5, 1F), -108.87 (d, *J* = 10.2, 1F).

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