

An attractive family of cyclometalated Ir(III) dyes functionalized with tryptophan for potential neuroimaging applications

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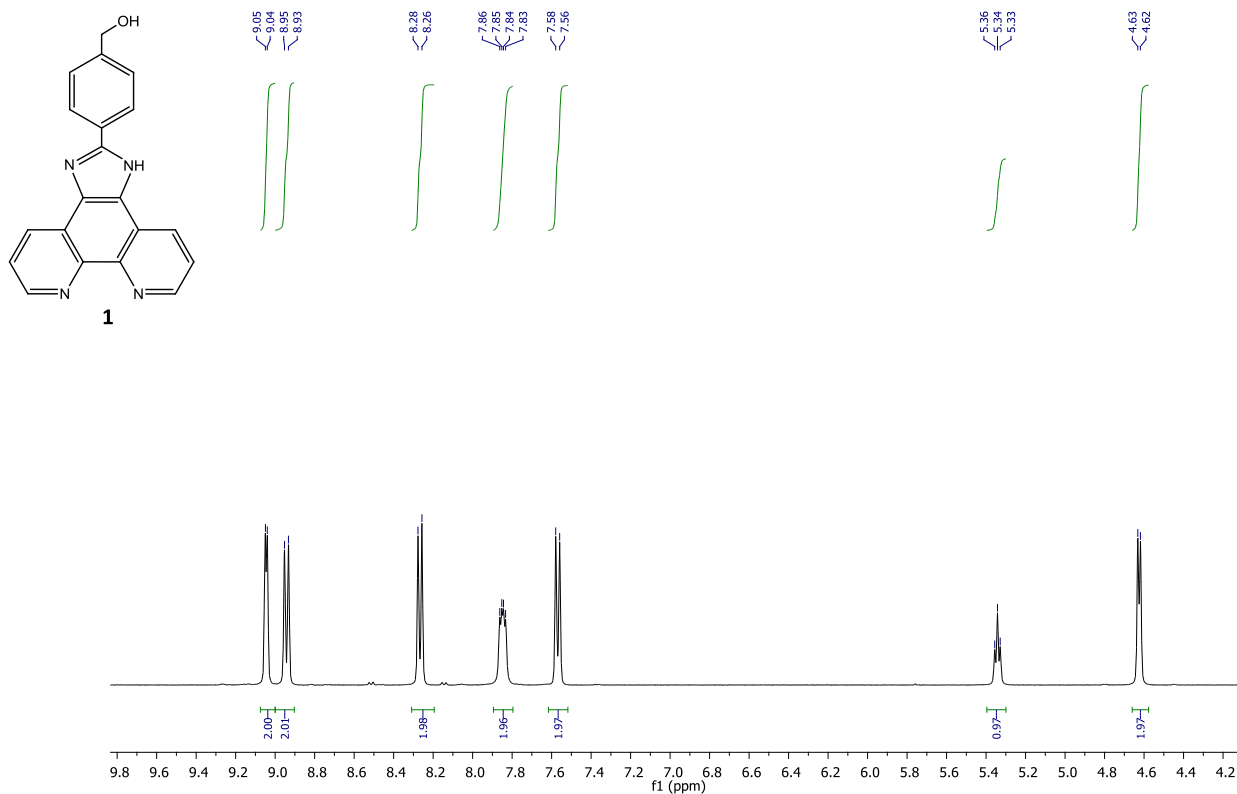
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‡ These authors have contributed equally to this work.

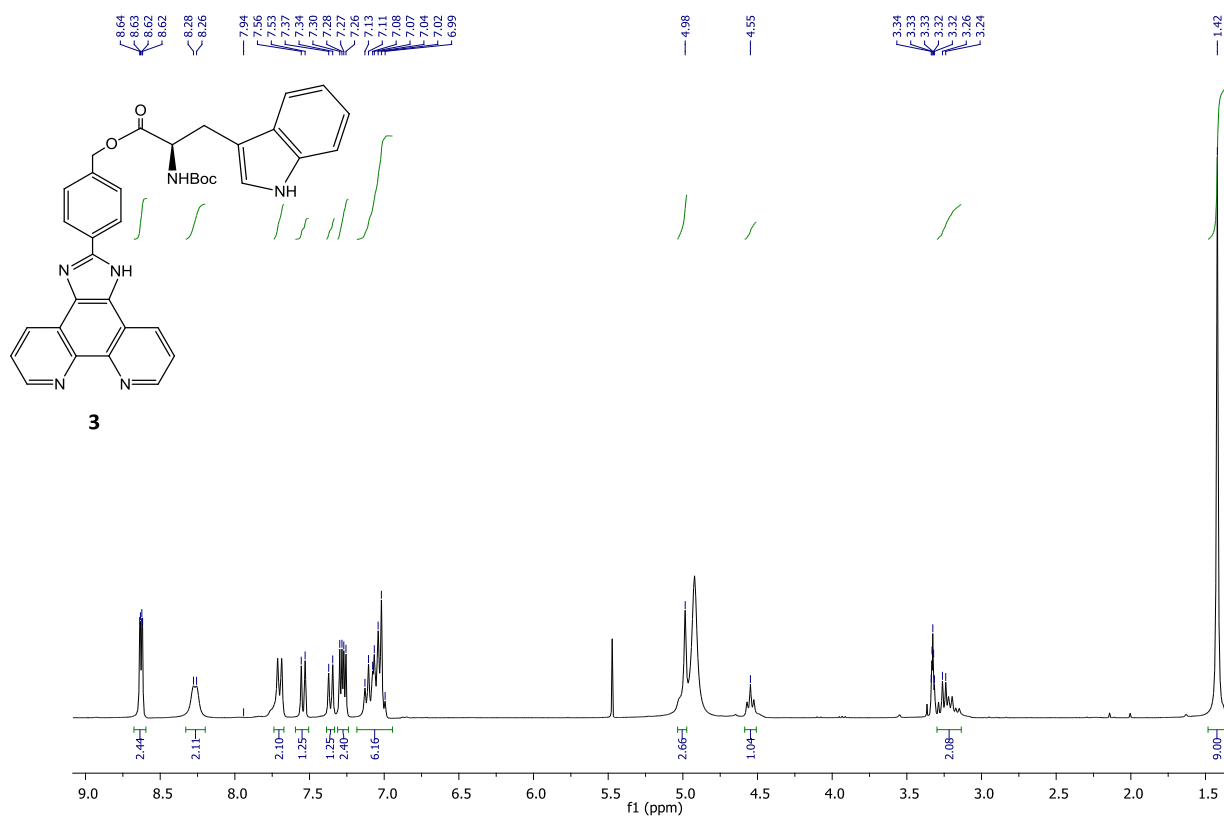
Contents

I. ¹ H, ¹³ C, and ¹⁹ F NMR spectra	S2
II. Photoluminescence properties	S7

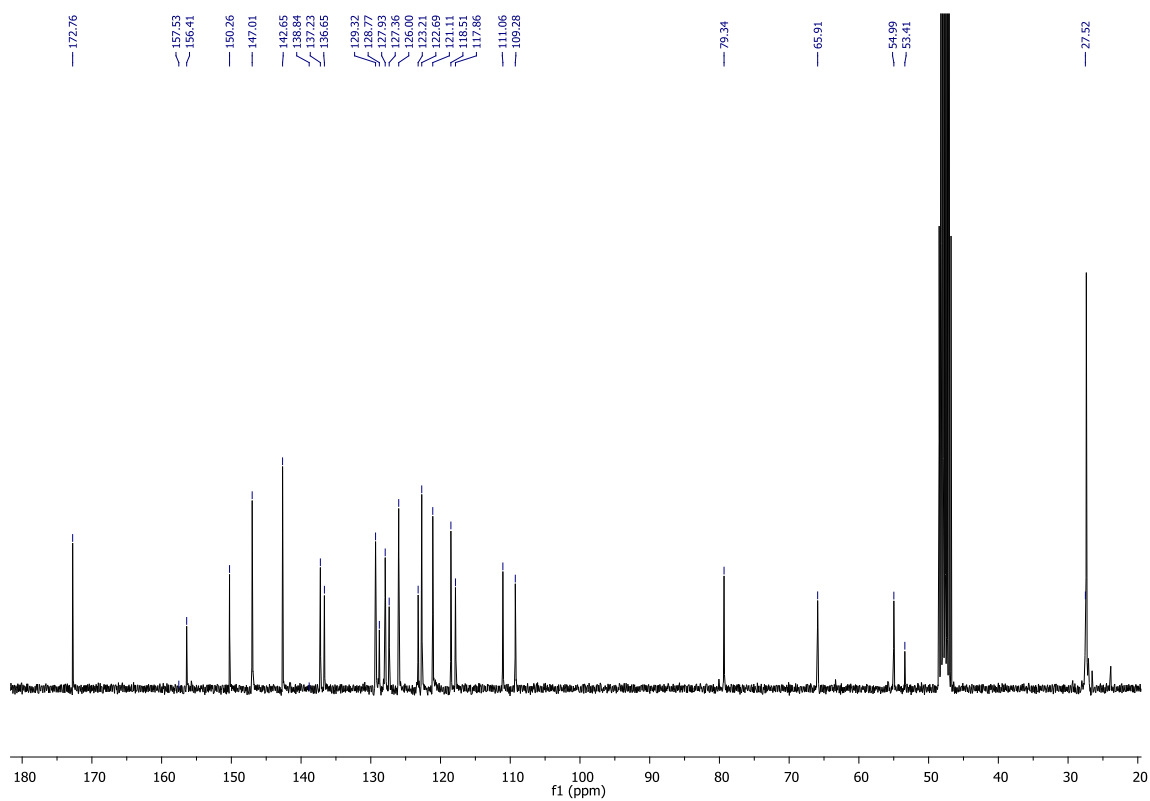
I. ^1H , ^{13}C , and ^{19}F NMR spectra



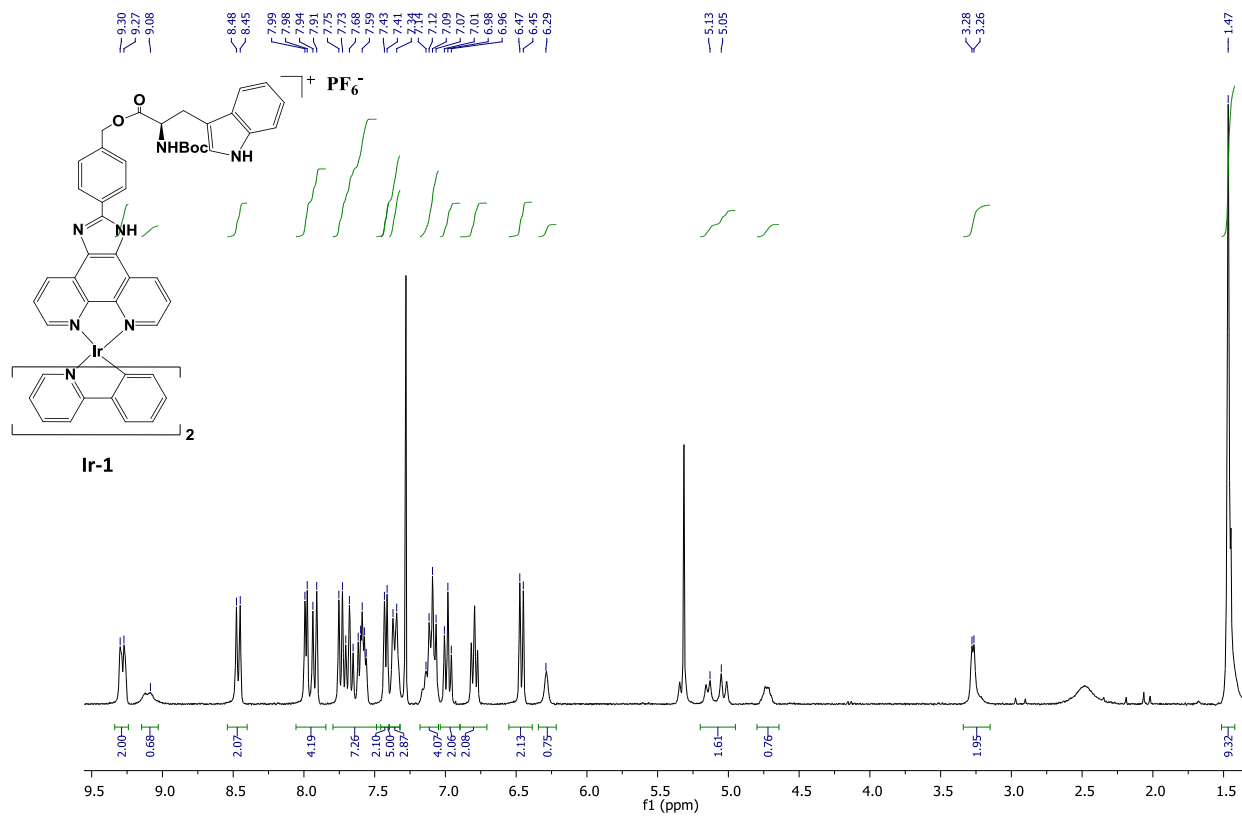
^1H NMR spectrum (DMSO- d_6 , 400 MHz) of compound **1**



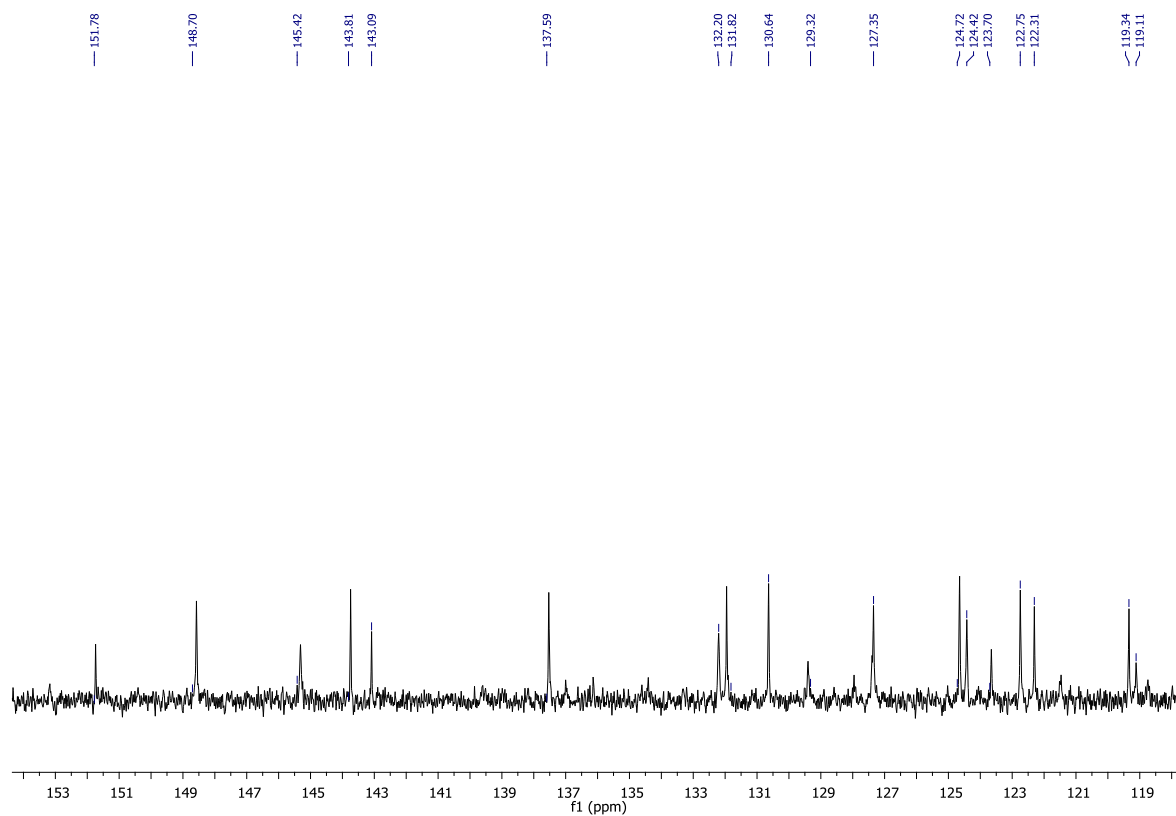
^1H NMR spectrum (MeOD, 300 MHz) of compound **3**



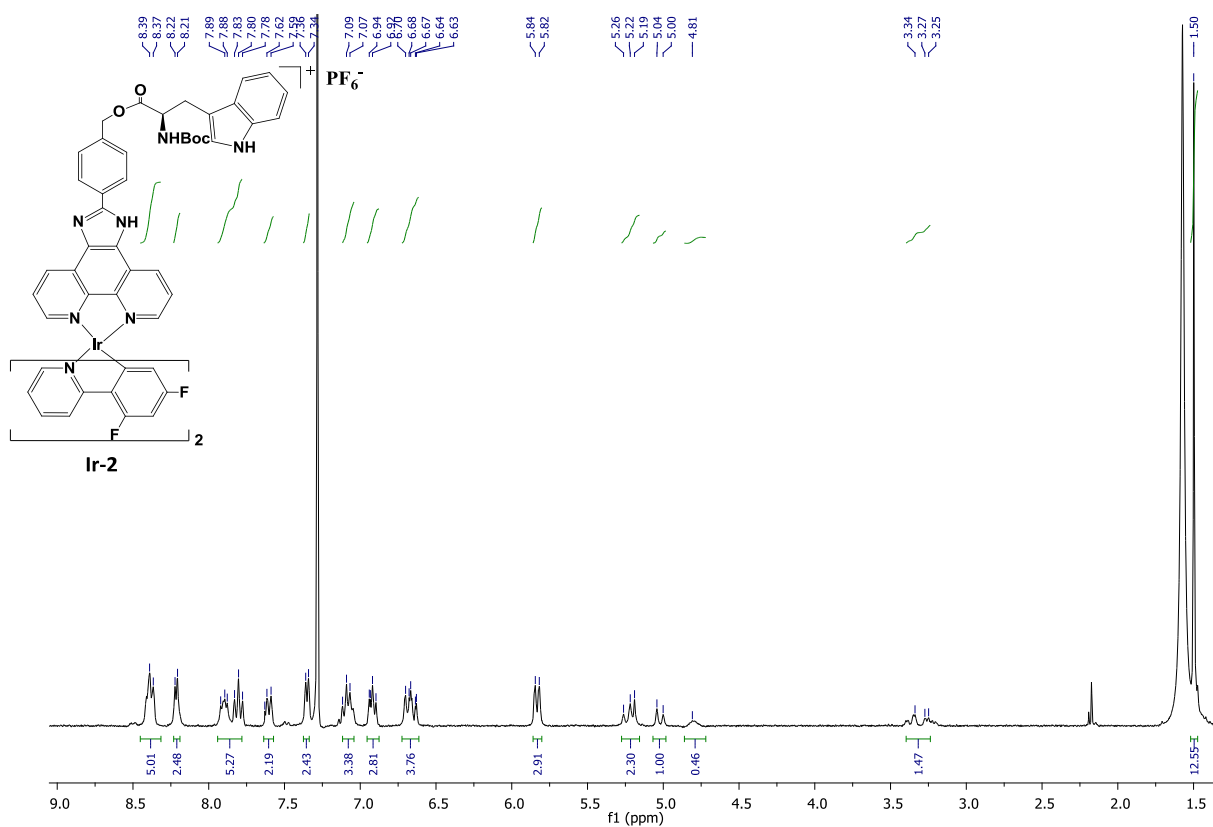
$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum (MeOD, 75 MHz) of compound **3**



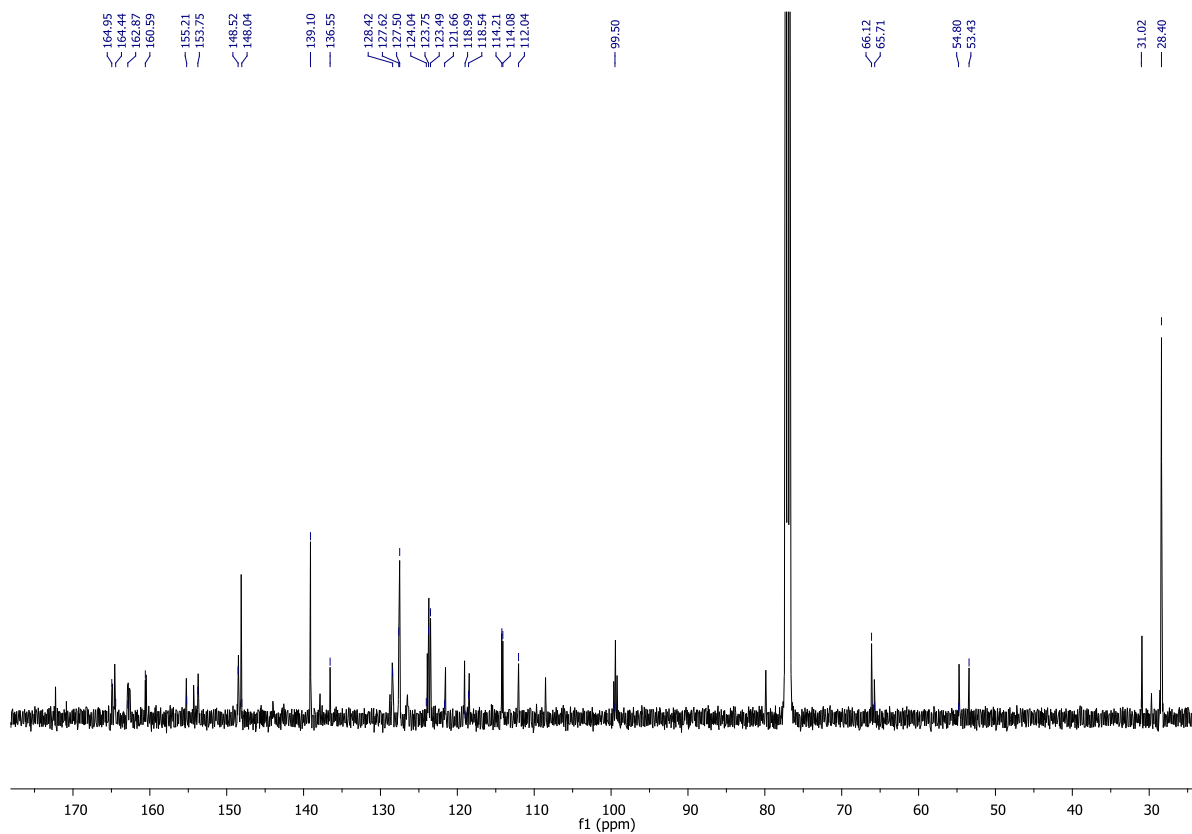
^1H NMR spectrum (CDCl₃, 300 MHz) of compound **Ir-1**



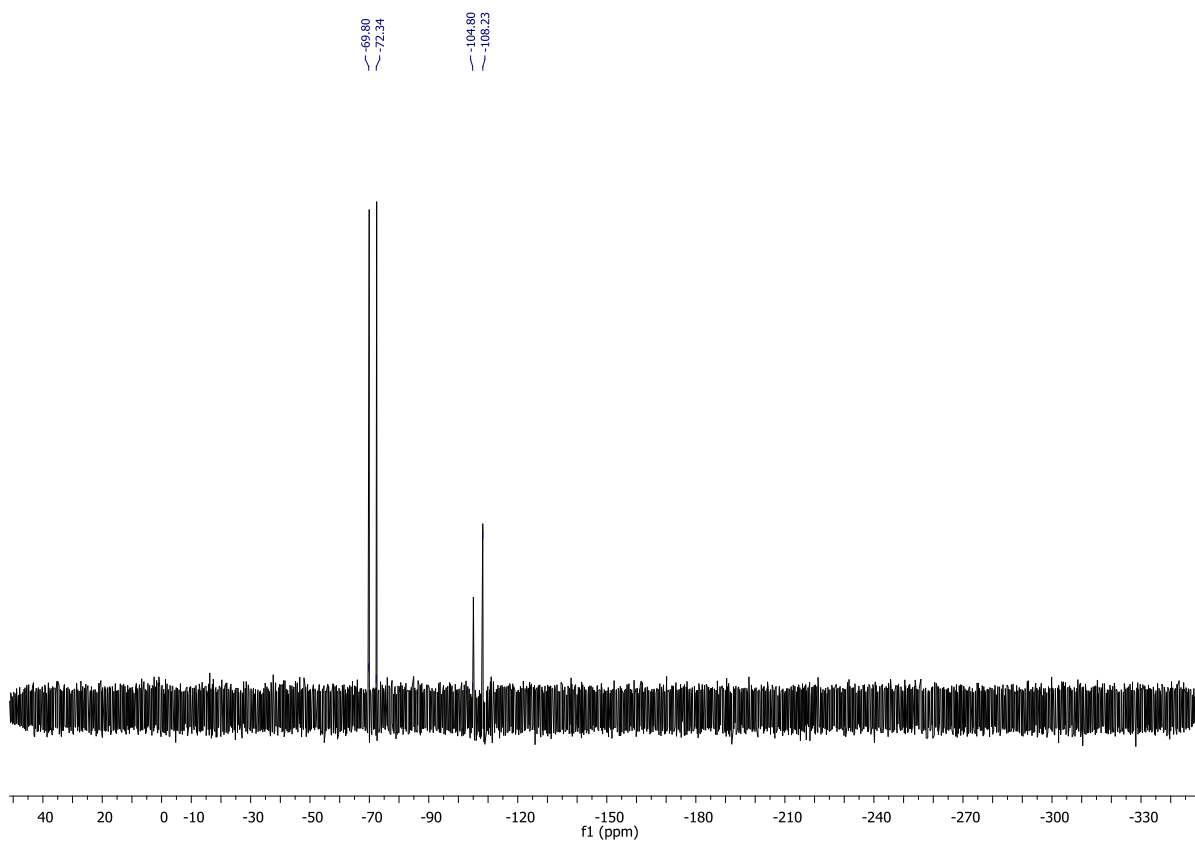
$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum (CDCl_3 , 75 MHz) of compound **Ir-1**



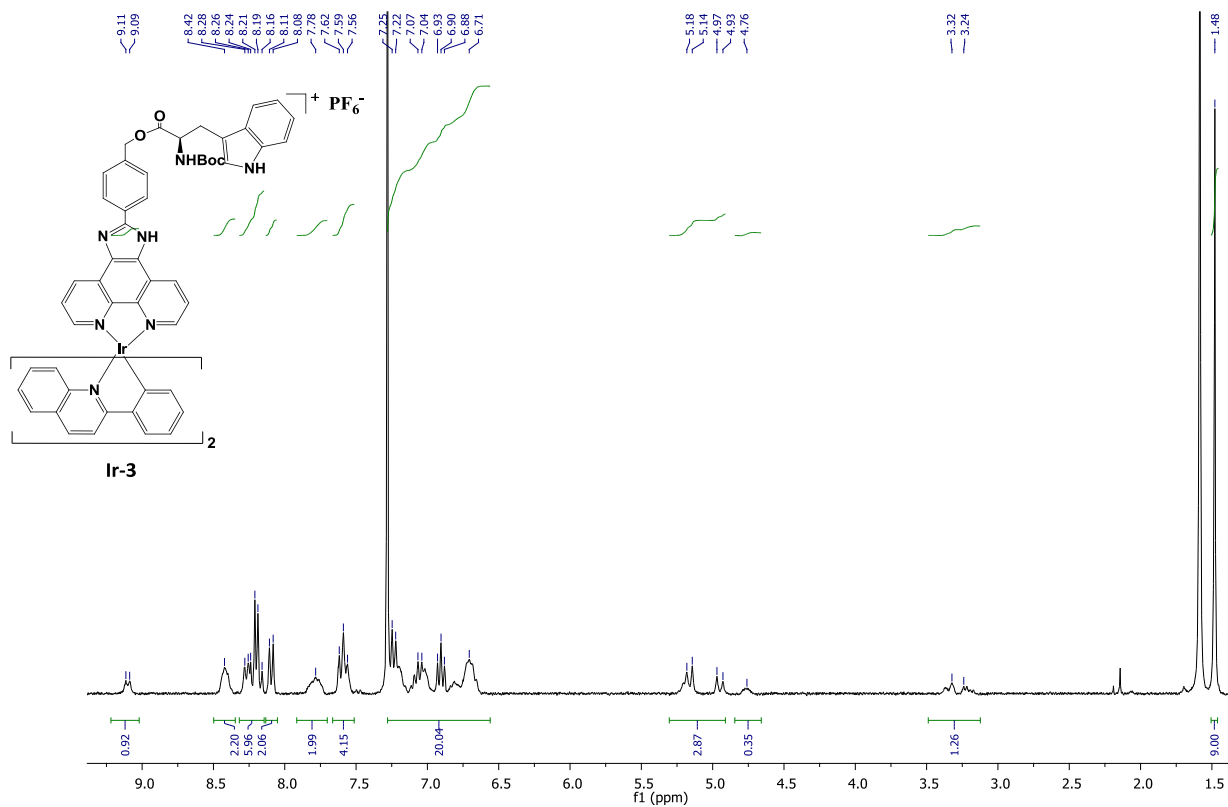
^1H NMR spectrum (CDCl_3 , 300 MHz) of compound **Ir-2**



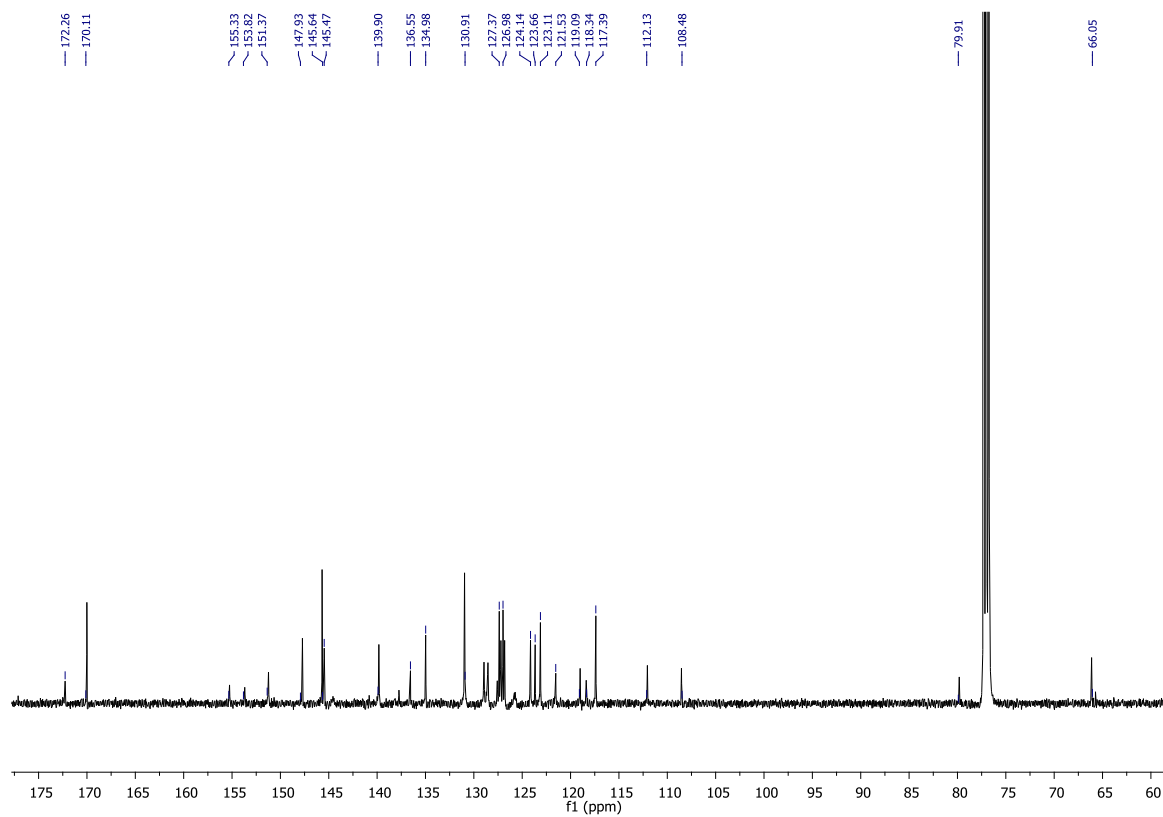
$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum (CDCl_3 , 126 MHz) of compound **Ir-2**



$^{19}\text{F}\{^1\text{H}\}$ NMR spectrum (CDCl_3 , 282.36 MHz) of compound **Ir-2**



¹H NMR spectrum (CDCl₃, 300 MHz) of compound Ir-3



¹³C{¹H} NMR spectrum (CDCl₃, 126 MHz) of compound Ir-3

II Photoluminescence properties

General informations

Electronic absorption spectra of the tryptophan conjugated ligand (**L-trp**) and the three complexes in solution were obtained with a UV-3600i Plus UV-VIS-NIR Spectrophotometer (Shimadzu Italia S.r.l., Milan, Italy).

Luminescence measurements of the three complexes were carried out in CH₂Cl₂ solution after three freeze-pump-thaw cycles in order to remove dissolved oxygen; while the **L-trp** in aerated dichloromethane solution.

Absolute photoluminescence quantum yield, Φ , was measured using a C11347 Quantaaurus Hamamatsu Photonics K.K spectrometer. A description of the experimental setup and measurement method can be found in the article of K. Suzuki et al.¹ Φ was calculated through Equation:

$$\Phi = \frac{PN(Em)}{PN(Abs)} = \frac{\int \frac{\lambda}{hc} [I_{em}^{sample}(\lambda) - I_{em}^{reference}(\lambda)] d\lambda}{\int \frac{\lambda}{hc} [I_{exc}^{sample}(\lambda) - I_{exc}^{reference}(\lambda)] d\lambda}$$

where PN(Em) is the number of emitted photons, PN(Abs) the number of absorbed photons, λ the wavelength, h the Planck's constant, c the speed of light, I_{em}^{sample} and $I_{em}^{reference}$ the photoluminescence intensities of the sample solution and reference in CH₂Cl₂, I_{exc}^{sample} and $I_{exc}^{reference}$ the excitation light intensities of the sample solution and reference in CH₂Cl₂. PN(Em) is calculated in the wavelength interval $[\lambda_i, \lambda_f]$, where λ_i is taken 10 nm above the excitation wavelength, while λ_f is the upper end wavelength in the emission spectrum. The error made was estimated at around 5%.

Steady state and time-resolved fluorescence data were obtained using a FLS980 spectrofluorimeter (Edinburgh Instrument Ltd). For diluted solutions, quartz cuvettes with 1 cm optical path length were used in a right-angle configuration between the excitation beam and the detection system; while, for concentrated solutions, quartz cuvettes of 1 mm optical path length were used in a configuration front-face with an angle of 45° or less between the excitation beam and the detection system. Emission spectra were corrected for background intensity and quantum efficiency of the photomultiplier tube. Excitation spectra were corrected for the intensity fluctuation of a 450 W Xenon arc lamp. Moreover, in order to remove inner filter effects that appear in a concentrated solution, the luminescence signal was optimized by varying the angle between the excitation beam

and the detection system, while the excitation and emission wavelength were fixed at the maximum absorption and maximum emission wavelength, respectively.

Time-resolved fluorescence measurements were performed by either the time-correlated single photon counting technique with an Edinburgh Picosecond Pulsed Diode Laser (emitted wavelength 300, 374 nm and 404 nm) or Multi-Channel Scaling technique with a microsecond pulsed Xenon flashlamp. In addition, time-resolved fluorescence curves were fitted using a multi-exponential function:

$$I(\lambda, t) = \sum_{i=1}^n \alpha_i(\lambda) \exp\left(\frac{-t}{\tau_i}\right)$$

where n is the number of exponentials, $\alpha_i(\lambda)$ is the amplitude at wavelength λ and τ_i is the lifetime of the component i . The quality of the fit was evaluated through the reduced χ^2 values.

Photoluminescence investigations of the complexes

[Ir-1]:

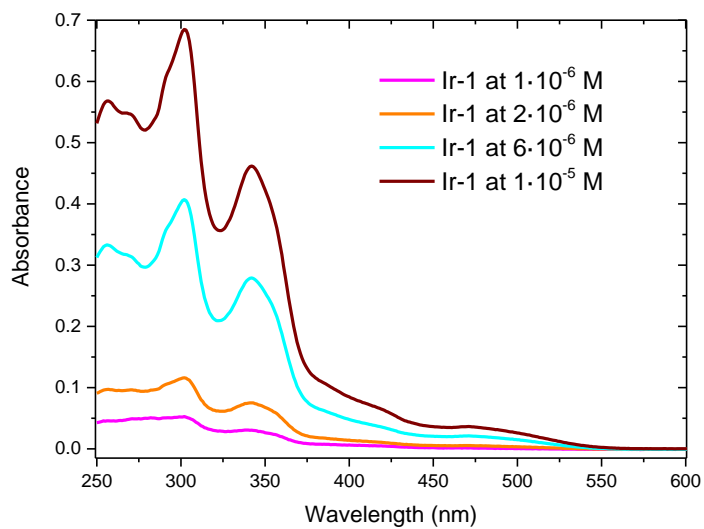


Figure S1. Absorption spectra at different concentrations of **Ir-1** in CH₂Cl₂ at 298 K.

Concentration (M)	Before FPT (%)	After FPT (%)
$2 \cdot 10^{-6}$	5.2	51.2
$6 \cdot 10^{-6}$	2.8	16.9
$1 \cdot 10^{-5}$	2.4	13.8
$2 \cdot 10^{-4}$	< 0.1	3.2

Table S1. Absolute Quantum Yield (%) of **Ir-1** in dichloromethane at different concentrations, before and after the Freeze-Pump-Thaw (FPT) cycles.

- Measurements at the concentration of $2 \cdot 10^{-6}$ M:

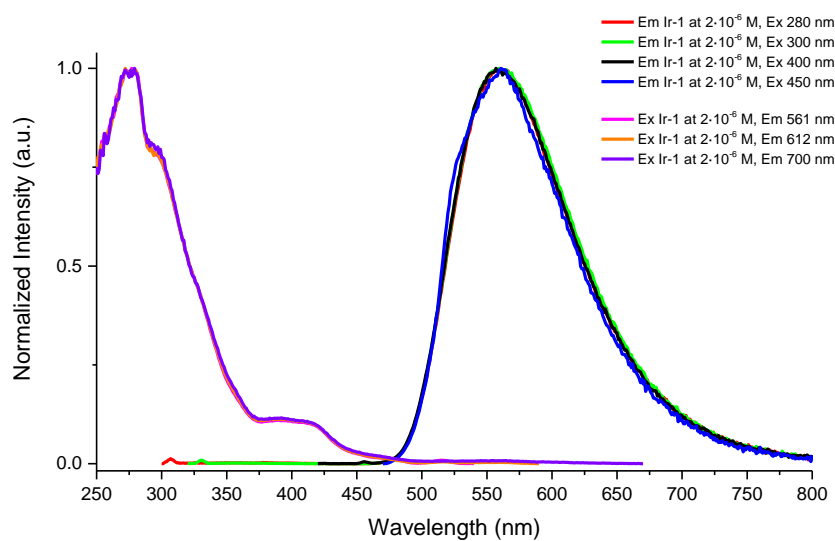


Figure S2. Normalized excitation and emission spectra of a diluted solution ($2 \cdot 10^{-6}$ M) of **Ir-1** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles.

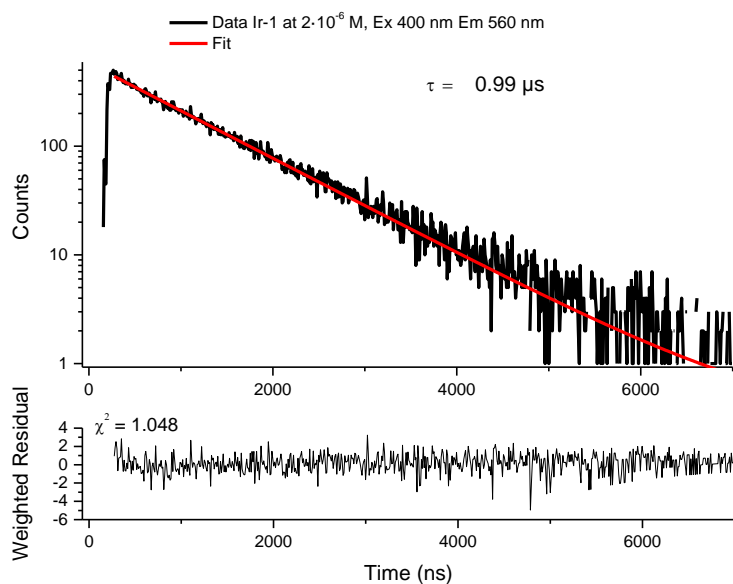


Figure S3. Lifetime measurement of a diluted solution ($2 \cdot 10^{-6}$ M) of **Ir-1** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles. Excitation 400 nm, Emission 560 nm.

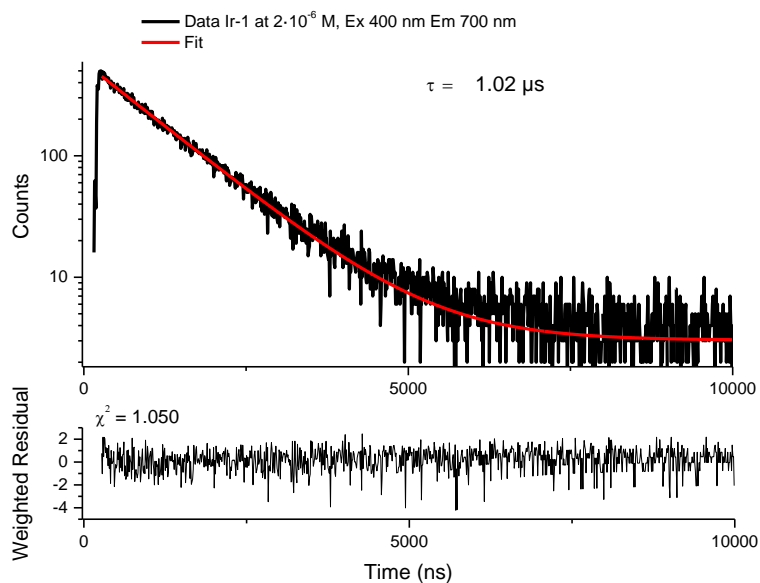


Figure S4. Lifetime measurement of a diluted solution ($2 \cdot 10^{-6}$ M) of **Ir-1** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles. Excitation 400 nm, Emission 700 nm.

- Measurements at the concentration of $1 \cdot 10^{-5}$ M:

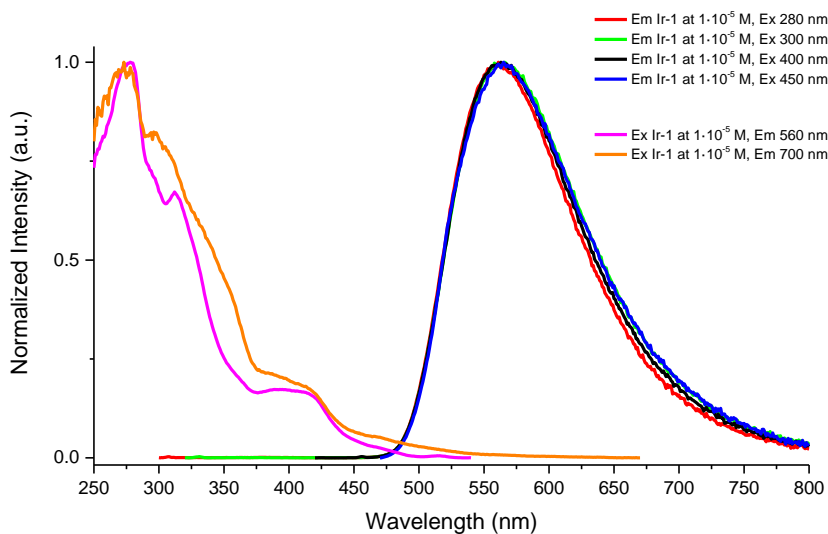


Figure S5. Normalized excitation and emission spectra of a solution ($1 \cdot 10^{-5}$ M) of **Ir-1** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles.

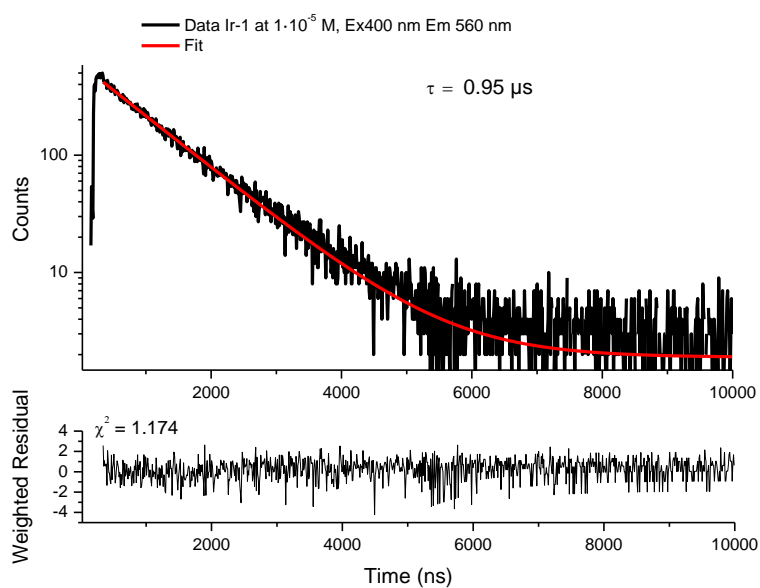


Figure S6. Lifetime measurement of a solution ($1 \cdot 10^{-5}$ M) of **Ir-1** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles. Excitation 400 nm, Emission 560 nm.

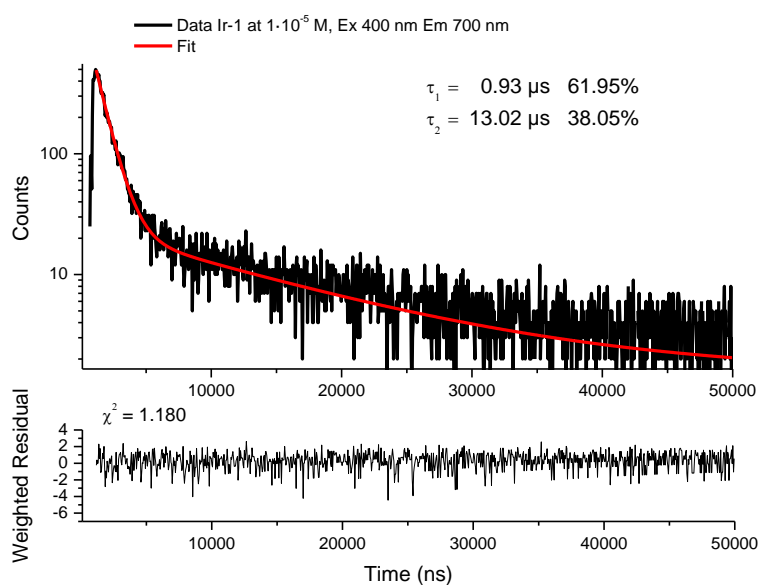


Figure S7. Lifetime measurement of a solution ($1 \cdot 10^{-5}$ M) of **Ir-1** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles. Excitation 400 nm, Emission 700 nm.

- Measurements at the concentration of $2 \cdot 10^{-4}$ M:

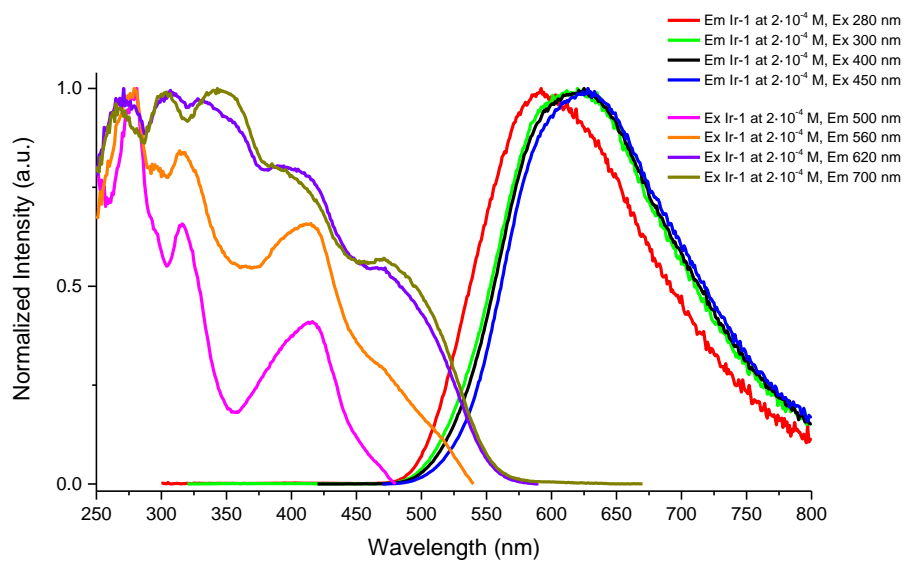


Figure S8. Normalized excitation and emission spectra of a concentrated solution ($2 \cdot 10^{-4}$ M) of **Ir-1** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles.

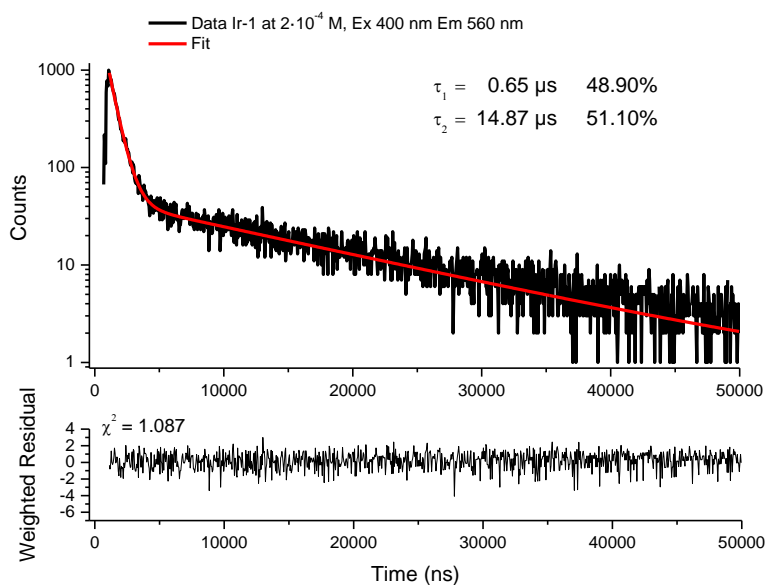


Figure S9. Lifetime measurement of a concentrated solution ($2 \cdot 10^{-4}$ M) of **Ir-1** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles. Excitation 400 nm, Emission 560 nm.

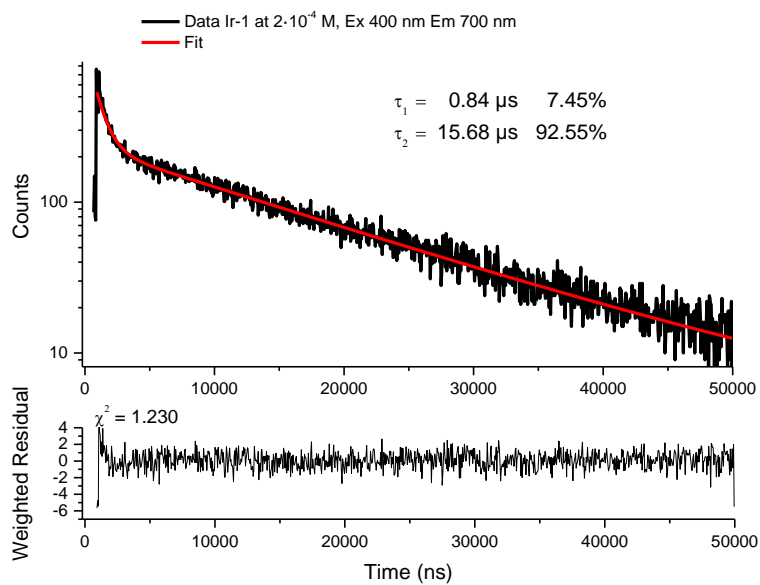


Figure S10. Lifetime measurement of a concentrated solution ($2 \cdot 10^{-4}$ M) of **Ir-1** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles. Excitation 400 nm, Emission 700 nm.

[Ir-2]:

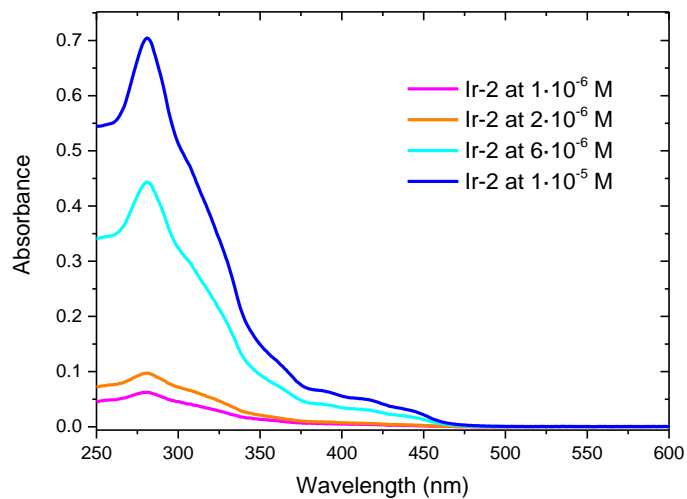


Figure S11. Absorption spectra at different concentrations of **Ir-2** in CH_2Cl_2 at 298 K.

Concentration (M)	Before FPT (%)	After FPT (%)
$2 \cdot 10^{-6}$	6.6	33.3
$6 \cdot 10^{-6}$	4.3	24.2
$1 \cdot 10^{-5}$	2.4	22.5
$2 \cdot 10^{-4}$	1.1	3.4

Table S2. Absolute Quantum Yield (%) of **Ir-2** in dichloromethane at different concentrations, before and after the Freeze-Pump-Thaw (FPT) cycles.

- Measurements at the concentration of $2 \cdot 10^{-6}$ M:

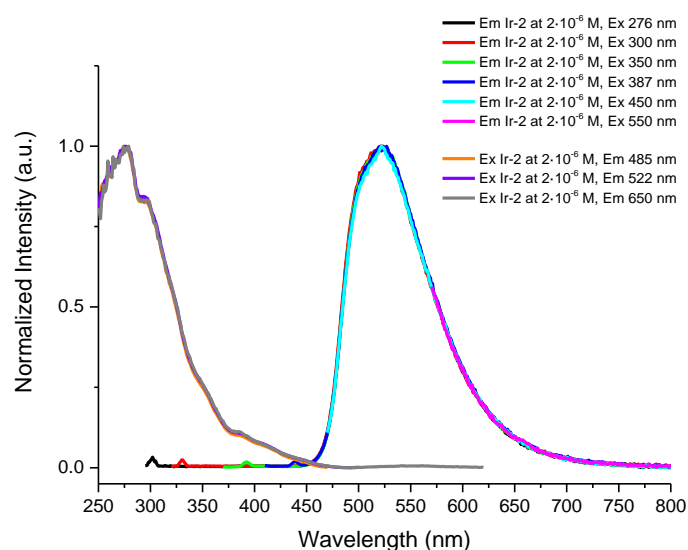


Figure S12. Normalized excitation and emission spectra of a diluted solution ($2 \cdot 10^{-6}$ M) of **Ir-2** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles.

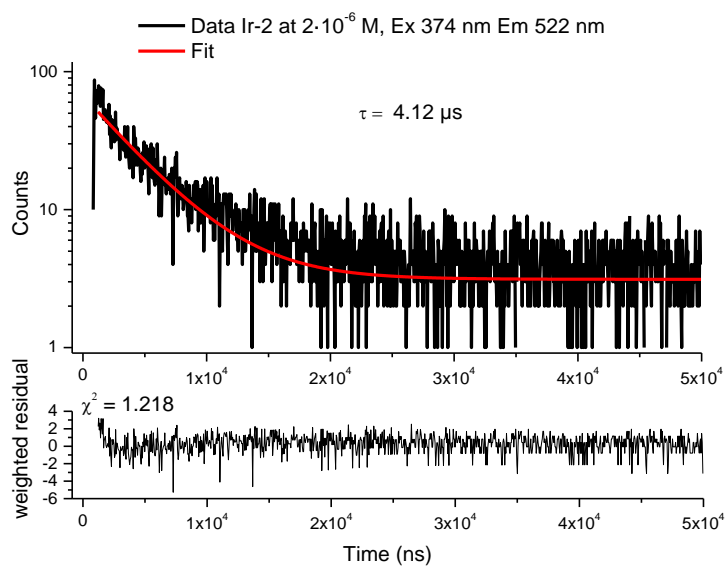


Figure S13. Lifetime measurement of a diluted solution ($2 \cdot 10^{-6}$ M) of **Ir-2** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles. Excitation 374 nm, Emission 522 nm.

- Measurements at the concentration of $1 \cdot 10^{-5}$ M:

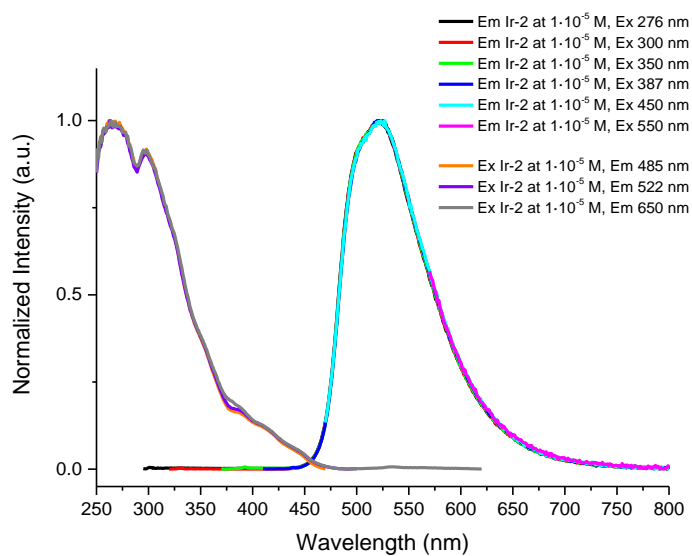


Figure S14. Normalized excitation and emission spectra of a solution ($1 \cdot 10^{-5}$ M) of **Ir-2** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles.

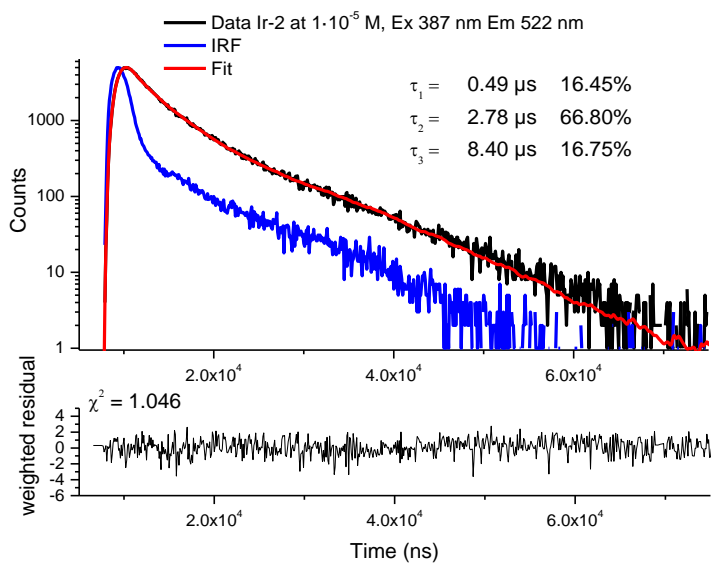


Figure S15. Lifetime measurement of a solution ($1 \cdot 10^{-5}$ M) of **Ir-2** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles. Excitation 387 nm, Emission 522 nm.

- Measurements at the concentration of $2 \cdot 10^{-4}$ M:

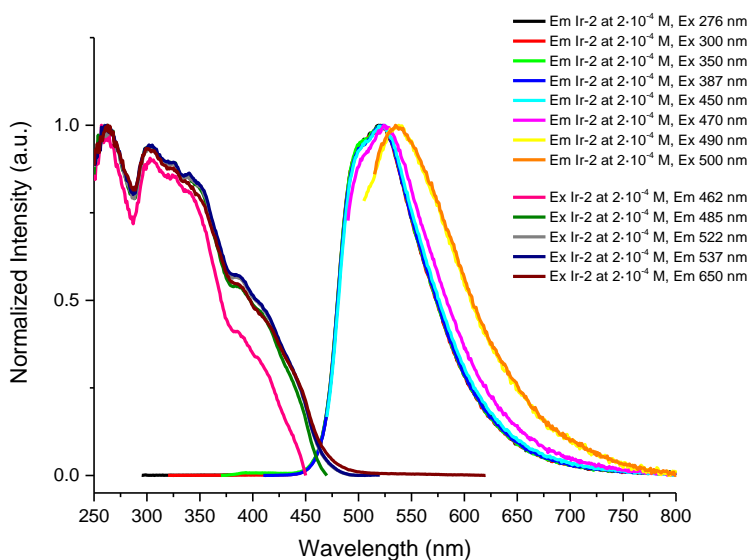


Figure S16. Normalized excitation and emission spectra of a concentrated solution ($2 \cdot 10^{-4}$ M) of **Ir-2** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles.

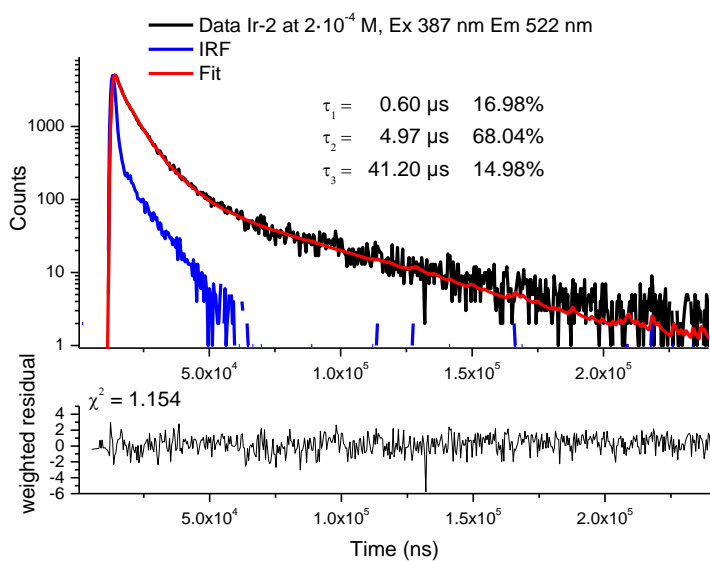


Figure S17. Lifetime measurement of a concentrated solution ($2 \cdot 10^{-4}$ M) of **Ir-2** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles. Excitation 387 nm, Emission 522 nm.

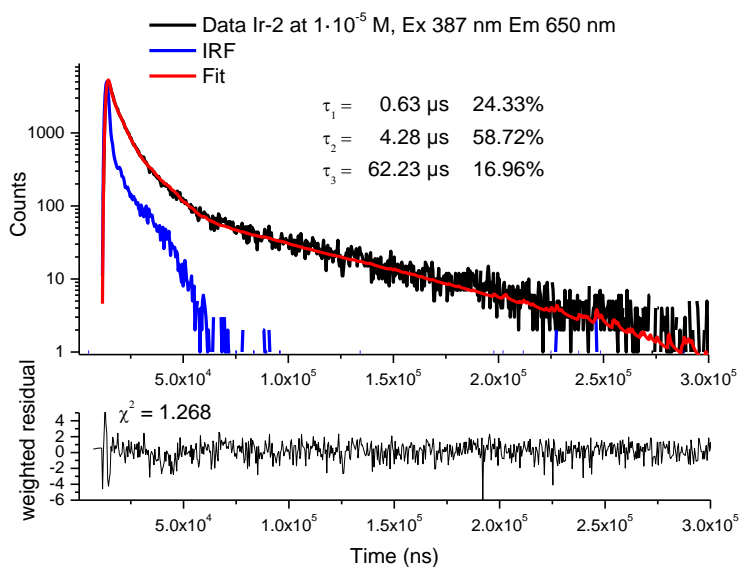


Figure S18. Lifetime measurement of a concentrated solution ($2 \cdot 10^{-4}$ M) of **Ir-2** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles. Excitation 387 nm, Emission 650 nm.

[Ir-3]:

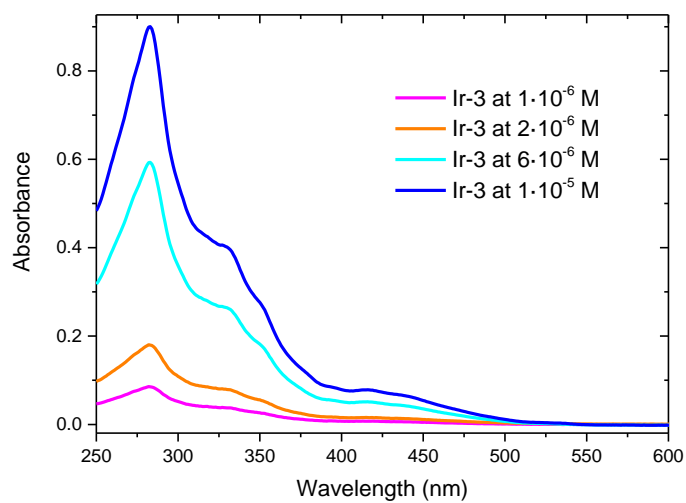


Figure S19. Absorption spectra at different concentrations of **Ir-3** in CH_2Cl_2 at 298 K.

Concentration (M)	Before FPT (%)	After FPT (%)
$2 \cdot 10^{-6}$	43.0	98.1
$6 \cdot 10^{-6}$	23.1	96.9
$1 \cdot 10^{-5}$	22.8	94.2
$2 \cdot 10^{-4}$	19.7	71.4

Table S3. Absolute Quantum Yield (%) of **Ir-3** in dichloromethane at different concentrations, before and after the Freeze-Pump-Thaw (FPT) cycles.

- Measurements at the concentration of $2 \cdot 10^{-6}$ M:

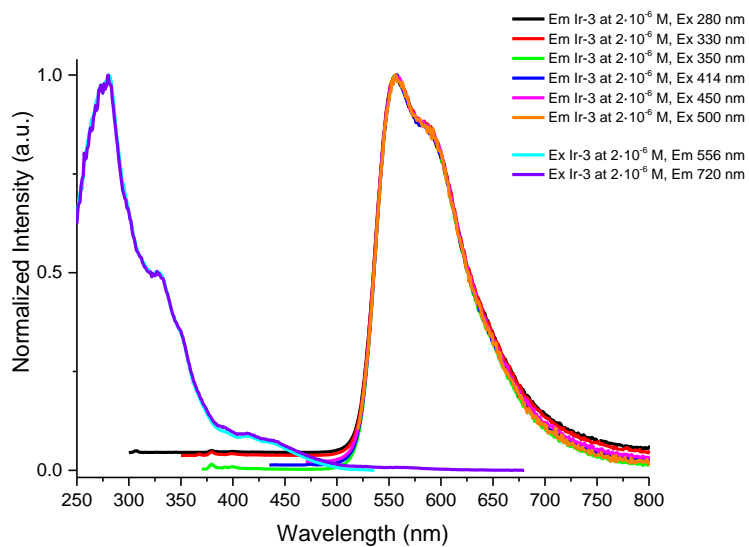


Figure S20. Normalized excitation and emission spectra of a diluted solution ($2 \cdot 10^{-6}$ M) of **Ir-3** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles.

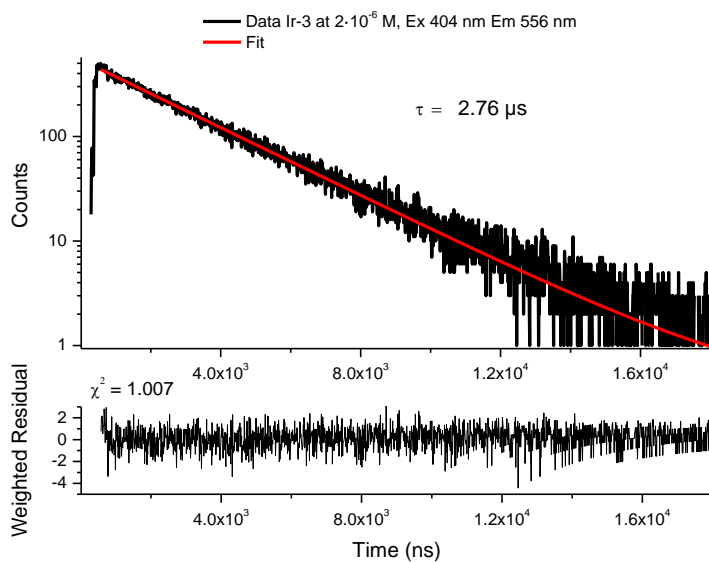


Figure S21. Lifetime measurement of a diluted solution ($2 \cdot 10^{-6}$ M) of **Ir-3** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles. Excitation 404 nm, Emission 556 nm.

- Measurements at the concentration of $1 \cdot 10^{-5}$ M:

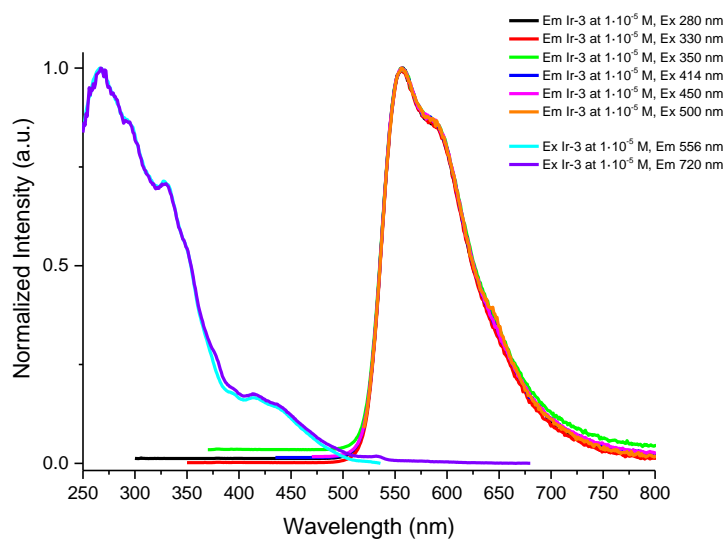


Figure S22. Normalized excitation and emission spectra of a solution ($1 \cdot 10^{-5}$ M) of **Ir-3** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles.

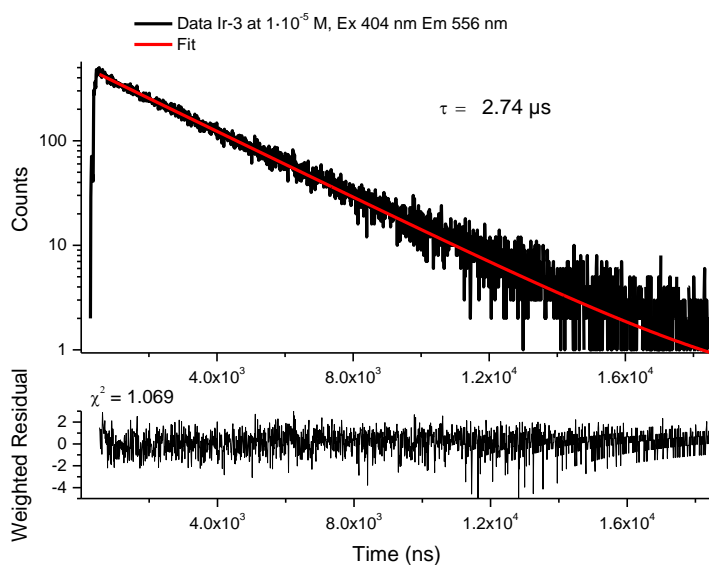


Figure S23. Lifetime measurement of a solution ($1 \cdot 10^{-5}$ M) of **Ir-3** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles. Excitation 404 nm, Emission 556 nm.

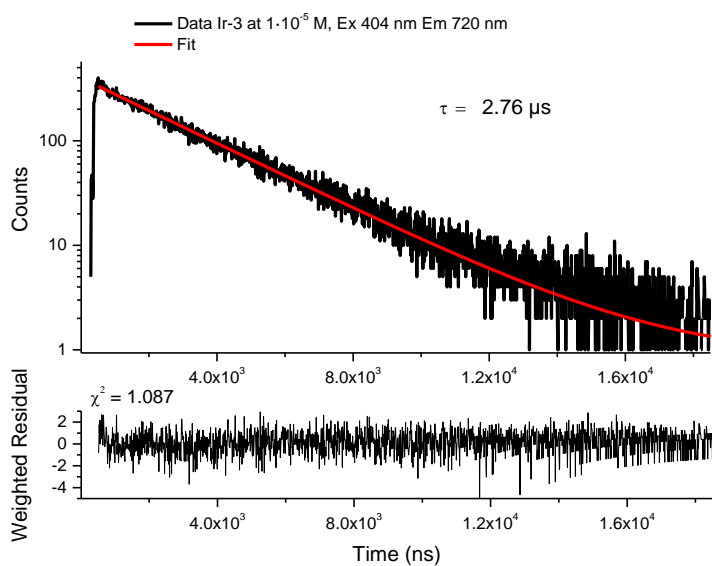


Figure S24. Lifetime measurement of a solution ($1 \cdot 10^{-5}$ M) of **Ir-3** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles. Excitation 404 nm, Emission 720 nm.

- Measurements at the concentration of $2 \cdot 10^{-4}$ M:

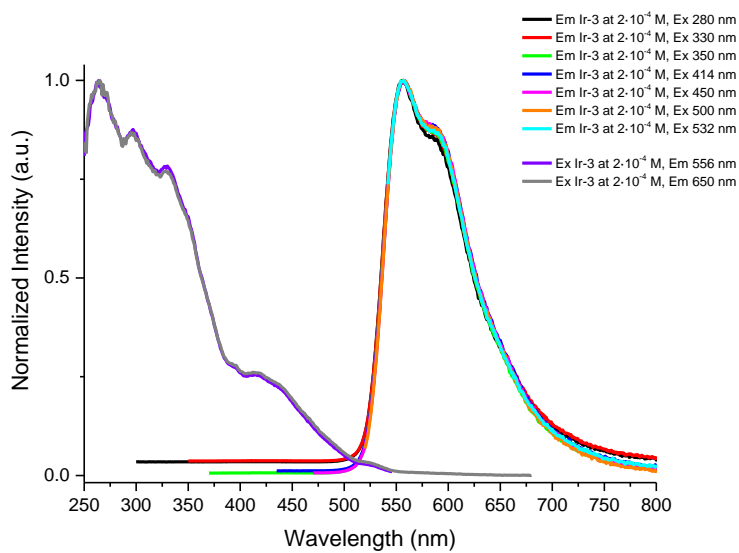


Figure S25. Normalized excitation and emission spectra of a concentrated solution ($2 \cdot 10^{-4}$ M) of **Ir-3** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles.

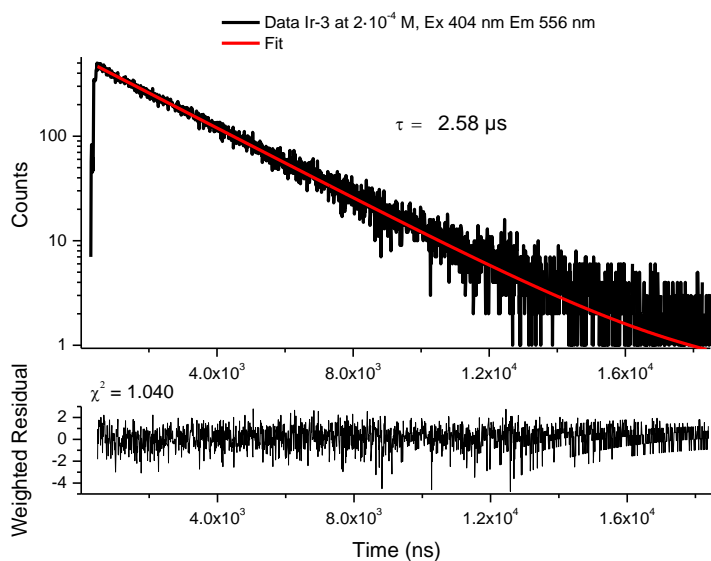


Figure S26. Lifetime measurement of a concentrated solution ($2 \cdot 10^{-4}$ M) of **Ir-3** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles. Excitation 404 nm, Emission 556 nm.

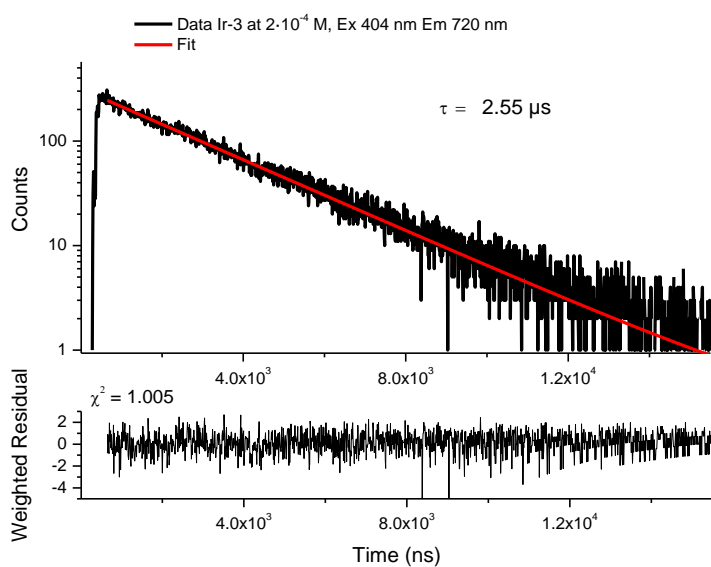


Figure S27. Lifetime measurement of a concentrated solution ($2 \cdot 10^{-4}$ M) of **Ir-3** at room temperature in dichloromethane, after the freeze-pump-thaw (FPT) cycles. Excitation 404 nm, Emission 720 nm.

[L-trp]:

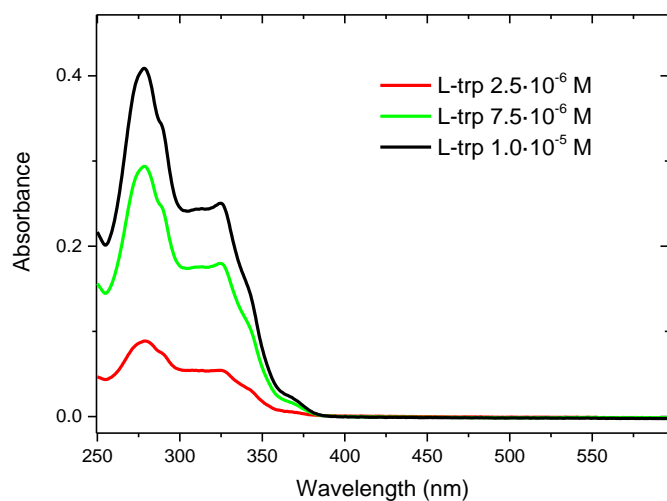


Figure S28. Absorption spectra at different concentrations of **L-trp** in CH₂Cl₂ at 298 K.

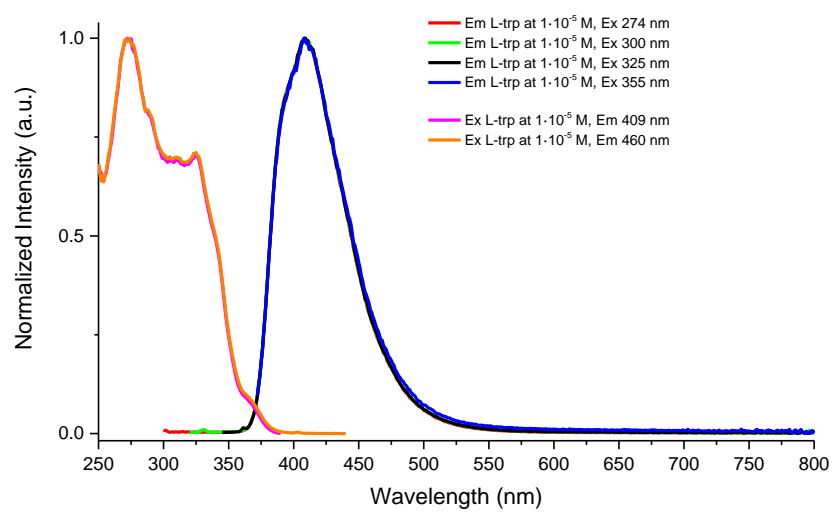


Figure S29. Normalized excitation and emission spectra of a solution (1 · 10⁻⁵ M) of **L-trp** at room temperature in aerated dichloromethane.

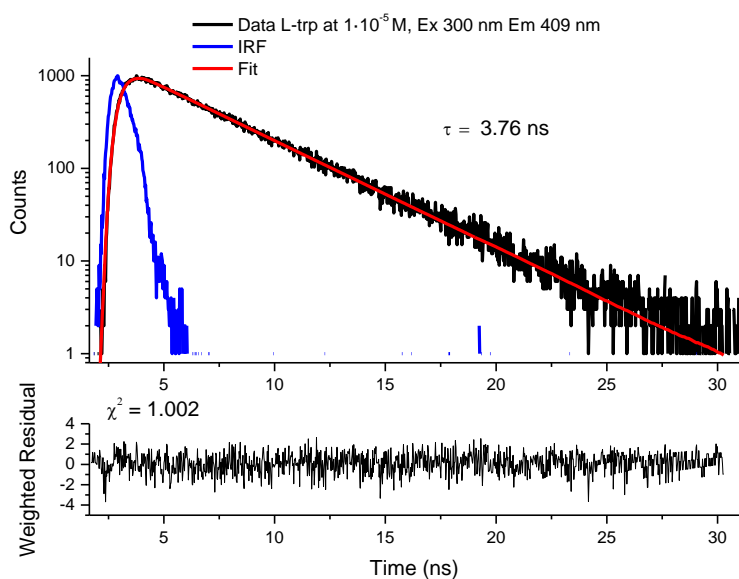


Figure S30. Lifetime measurement of a solution ($1 \cdot 10^{-5}$ M) of **L-trp** at room temperature in aerated dichloromethane. Excitation 300 nm, Emission 409 nm.

Complex	k_r / s^{-1}	k_{nr} / s^{-1}
[Ir-1]	$5.2 \cdot 10^5$	$4.9 \cdot 10^5$
[Ir-2]	$8.1 \cdot 10^4$	$1.6 \cdot 10^5$
[Ir-3]	$2.6 \cdot 10^5$	$5.3 \cdot 10^3$

Table S4. Radiative (Φ_p/τ) and nonradiative ($(1-\Phi_p)/\tau$) rate constants of the **Ir-1**, **Ir-2** and **Ir-3** complexes.

References

1. K. Suzuki, A. Kobayashi, S. Kaneko, K. Takehira, T. Yoshihara, H. Ishida, Y. Shiina, S. Oishic, S. Tobita, "Reevaluation of absolute luminescence quantum yields of standard solutions using a spectrometer with an integrating sphere and a back-thinned CCD detector", *Phys. Chem. Chem. Phys.*, **2009**, 11, 9850–9860. DOI: 10.1039/b912178a