

Electronic Supplementary Information

Iridium(III) Complexes Decorated with Silicane-Modified Rhodamine: Near-Infrared Light-Initiated Photosensitizers for Efficient Deep-Tissue Penetration Photodynamic Therapy

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Experimental Section

Materials and Reagents

The solvents used for synthesis were all in analytical grade. Iridium(III) chloride hydrate, 4,4'-dimethyl-2,2'-bipyridine and Dimethyldichloromethane were purchased from Sigma-Aldrich Chemical Company. 6-bromo-indoline was purchased from Bide Pharmatech Ltd. $[\text{Ir}(\text{mpbqx})_2\text{Cl}]_2$, $[\text{Ir}(\text{dpbqx})_2\text{Cl}]_2$, $[\text{Ir}(\text{dtbqx})_2\text{Cl}]_2$ were all synthesized according to the similar methods with slight modifications our group published before.¹

Physical Measurements and Instrumentation.

The UV-vis absorption spectra at room temperature were collected from Cary 60 UV-vis spectrophotometer. The electronic emission spectra were carried out on an Edinburgh Instruments FLS980 (or FS5) fluorescence spectrometer. NMR measurements were performed on Bruker AVANCE 400 MHz or 500 MHz Fourier transform NMR spectrometer. The chemical shifts for the NMR spectra were relative to tetramethylsilane (TMS). High-resolution mass spectra were conducted on Q-Exactive Hybrid quadrupole-Orbitrap mass spectrometer. The transient absorption and the triplet excited state lifetime was recorded on an Edinburgh LP920 instrument. All the sample for transient absorption and lifetime detections were deaerated thoroughly via a high-vacuum line in a dual-chamber cell consisting of a Pyrex bulb (10-mL in volume) and a quartz cuvette (1-cm in path length) and sealed by a Bibby

Rotaflo HP6 Teflon plug to isolate from the atmosphere. These sample solutions were strictly degassed with five successive freeze-pump-thaw cycles.

Singlet oxygen measurement

Singlet oxygen quantum yield was determined by monitoring the diminution rate of 1,3-diphenylisobenzofuran (DPBF) absorption at 410 nm in air-saturated DMF by UV-vis spectrophotometer. The solution was irradiated with 680 nm Xenon lamp in an interval of 30 seconds or 1 min. The quantum yields were obtained by the comparison of that in **ZnPc** as the reference ($\Phi_{\Delta} = 0.56$ in aerated DMF).² Singlet oxygen emission was recorded on an FLS980 spectrometer in air-saturated DMF which was excited at 680 nm. The absorbances of the Ir(III) samples and the standard **ZnPc** were identical at 680 nm. In order to get rid of high-order diffraction from the visible emission, an 850 nm long-pass filter was placed between the detector and the sample cuvette during the measurements.

Cell culture

4T1 mouse breast cancer cells were incubated in a medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco), 1% penicillin and 1% streptomycin. Cells were incubated at 37 °C in a humidified incubator 5% CO₂.

Cytotoxicity

4T1 cells were seeded at 8×10^3 to 1×10^4 cells per well in 96 well plates and then incubated for 24 h in 5% CO₂ at 37 °C. Solutions of the complexes (1 mM in DMSO) were diluted to the appropriate concentrations with complete medium and added to the cells to give final complex concentrations of 1, 2, 4, 6, 8 and 10 μ M. After incubation for 24 h, the cytotoxicity was determined by CCK8 assay.

Animals and tumor model

Animals received care in accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals. The procedures were approved by the Animal Care and Use Committee (Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences). Six to eight-week-old female BALB/c mice (Vital River Laboratory Animal Technology Co. Ltd., China) were subcutaneously injected with 4T1 cells (1×10^6) in the flank region.

***In vivo* fluorescence imaging**

An IVIS Spectrum imaging system was used to image tumor-bearing animals following in situ treatment with PBS (100 μ L), **Ir-Rho-G2** and **4** (100 μ L, 10 μ M) separately. For one group, the mice were imaged at 570 nm excitation and above 620 nm emission. For the other group, the mice were imaged at 710 nm excitation and above 760 nm emission.

***In vivo* antitumor efficacy studies**

When the tumors had reached an average volume of 50 to 100 mm³, mice (n = 6) were in situ injected with PBS (100 μ L), **Ir-Rho-G2** and **4** (100 μ L, 10 μ M) separately. Led white light source and 808 nm laser system were used to irradiate the tumors for 30 min for two times at 0 and 48 hours after injection. The growth of the tumor was measured with a caliper every 1 or 2 days during the period of treatment (20 days) and the volume was calculated (volume = length \times width² \times 0.5).

Statistical analysis

All the results are reported as mean \pm SD. The differences among groups were determined using one-way ANOVA analysis and student's t-test; *P < 0.05, **P < 0.01.

Synthesis

Synthesis of dimethylbis(1-methylindolin-6-yl) silane

1.724 g 6-bromo-N-methyl-indoline (8.13 mmol) was dissolved in 30 mL anhydrous THF in a flame-dried flask in a glove box with N₂. Then took out the flask from the glove box and cooled it to -78°C. 2 M *n*-BuLi (4.87 mL, 9.76 mmol) was slowly added and stirred for 20 min. At the same temperature, Me₂SiCl₂ (0.51 mL, 4.15 mmol) dissolved in anhydrous THF (10 mL) was slowly added and kept the reaction under this condition for 6 h. The mixture was quenched by slow addition of 1 mL water, and the mixture was stirred for 10 min. Then the solution was extracted with DCM (3 \times 100 mL) by adding a large amount of water. Combined the organic phase and removed the solvent. The residue was purified by silica gel column with dichloromethane and petroleum ether (1:10, v/v). Then colorless oil was obtained with 1.24 g. (yield, 95%).
¹H NMR (400 MHz, CDCl₃-d) δ 7.11 (d, *J* = 7.0 Hz, 2H), 6.89 (dd, *J* = 7.1, 0.6 Hz, 2H), 6.68

(s, 2H), 3.30 (t, $J = 8.1$ Hz, 4H), 2.96 (t, $J = 8.1$ Hz, 4H), 2.77 (s, 6H), 0.53 (s, 6H). ^{13}C NMR (101 MHz, CDCl_3 - d) δ 154.66, 139.09, 133.49, 126.13, 125.72, 114.16, 79.21, 78.90, 78.58, 57.86, 37.99, 30.19. HRMS (ESI): theoretical calc. for $\text{C}_{20}\text{H}_{26}\text{N}_2\text{Si}$ $[\text{M}+\text{H}]^+$ $m/z = 323.19380$, found $[\text{M}+\text{H}]^+$ $m/z = 323.19343$.

Synthesis of **Si-Rho**

A suspension of dimethylbis(1-methylindolin-6-yl) silane (1.0 g, 3.1 mmol), 4'-methyl-[2,2'-bipyridine]-4-carbaldehyde (0.67 g, 3.4 mmol) in 2 M HCl (100 mL) was refluxed overnight. After cooling, the reaction mixture was neutralized by adding saturate NaHCO_3 . The whole was extracted with CH_2Cl_2 (3 \times 100 mL) and then evaporated the solvent. Then dissolved the residue with 60 mL CH_2Cl_2 and followed by addition of sufficient amount of chloranil. The mixture was stirred for 2 h at room temperature. Removed the solvent again and dissolved the residue with 40 mL MeCN with 2 mL concentrated NH_4PF_6 . The mixture was stirred for 2 h and the solvent was evaporated again. Purified the residue by column chromatography on silica gel eluted with MeCN/water (20:1, v/v) to provided green solid (1.02 g, yield 54.1%). ^1H NMR (500 MHz, CD_3CN - d_3) δ (ppm) = 8.93 (d, $J = 4.3$ Hz, 1H), 8.67 (d, $J = 5.0$ Hz, 1H), 8.43 (s, 1H), 8.27 (s, 1H), 7.59 (d, $J = 4.7$ Hz, 1H), 7.45 (d, $J = 4.2$ Hz, 1H), 7.13 (s, 2H), 6.73 (s, 2H), 3.81 (t, $J = 7.9$ Hz, 4H), 3.20 (s, 6H), 2.89 (t, $J = 7.7$ Hz, 4H), 2.60 (s, 3H), 0.58 (s, 6H). ^{13}C NMR (126 MHz, CD_3CN - d_3) δ (ppm) = 160.23, 156.93, 155.02, 151.44, 151.07, 150.82, 150.09, 149.58, 133.46, 132.61, 127.41, 126.65, 125.72, 123.51, 122.08, 115.36, 54.51, 33.12, 25.90, 20.96. HRMS (ESI): theoretical calc. for $\text{C}_{32}\text{H}_{33}\text{N}_4\text{SiPF}_6$ $[\text{M}-\text{PF}_6]^-$ $m/z = 501.24690$, found $[\text{M}-\text{PF}_6]^-$ $m/z = 501.24686$.

General method for **Ir-Si-Rhos**

1 equiv. of the Ir(III) dimer and 2 equiv **Si-Rho** were dissolved in a mixture of DCM/MeCN (1:1, v/v). The solution was refluxed for 8 h in the dark under N_2 atmosphere. Then cooled the solution and removed the solvent. The residue was dissolved in 30 mL MeOH with 2 mL saturated NH_4PF_6 and stirred for 1 h under room temperature. Then removed the solvent and purified the residue by column chromatography on silica gel eluted with MeCN/water (20:1, v/v) to give the pure product as a dark green powder.

Synthesis of **1** 37.5 mg, yield 53%. ^1H NMR (400 MHz, $\text{CD}_3\text{CN}-d_3$) δ (ppm) = 8.92 (d, $J = 5.6$ Hz, 1H), 8.72 (d, $J = 5.7$ Hz, 1H), 8.00 – 8.08 (m, 4H), 7.97 – 7.85 (m, 4H), 7.78 – 7.62 (m, 11H), 7.52 (d, $J = 8.8$ Hz, 1H), 7.43 – 7.32 (m, 1H), 7.31 – 7.23 (m, 1H), 7.21 (d, $J = 8.1$ Hz, 2H), 7.13 (s, 1H), 7.07 (s, 1H), 6.88 – 6.56 (m, 6H), 6.47 (s, 1H), 5.99 (s, 1H), 3.89 – 3.83 (m, 2H), 3.75 – 3.69 (m, 2H), 3.18 (d, $J = 26.1$ Hz, 6H), 3.11 – 2.99 (m, 2H), 2.92 – 2.80 (m, 2H), 2.43 (s, 3H), 0.54 (d, $J = 17.0$ Hz, 6H). ^{13}C NMR (101 MHz, $\text{CD}_3\text{CN}-d_3$) δ (ppm) = 163.73, 163.54, 157.24, 156.80, 156.68, 156.15, 154.65, 154.32, 154.17, 153.23, 152.98, 152.94, 152.45, 149.86, 149.80, 148.74, 147.99, 144.84, 144.60, 140.79, 140.69, 140.61, 139.78, 139.73, 135.47, 135.31, 133.54, 133.39, 131.90, 131.65, 131.45, 131.41, 131.37, 130.95, 130.35, 130.06, 129.98, 129.82, 129.25, 129.13, 128.99, 126.77, 125.59, 124.70, 124.49, 124.44, 122.43, 122.34, 118.65, 115.77, 115.73, 54.59, 54.49, 37.42, 33.20, 31.64, 29.47, 26.80, 25.79, , 22.41, 20.34, 17.79, 13.41. HRMS (ESI): theoretical calc. for $\text{C}_{72}\text{H}_{59}\text{IrN}_8\text{SiP}_2\text{F}_{12}$ $[\text{M}-2\text{PF}_6]^{2+}$ $m/z = 628.21251$, found $[\text{M}-2\text{PF}_6]^{2+}$ $m/z = 628.21322$.

Synthesis of **2** 110 mg, yield 74.5%. ^1H NMR (500 MHz, $\text{CD}_3\text{CN}-d_3$) δ 8.77 (d, $J = 5.7$ Hz, 1H), 8.71 (d, $J = 7.9$ Hz, 1H), 8.61 – 8.55 (m, 3H), 8.44 (d, $J = 5.8$ Hz, 1H), 8.35 (s, 1H), 8.12 (d, $J = 8.5$ Hz, 1H), 8.08 (d, $J = 8.5$ Hz, 1H), 7.76 (s, 1H), 7.72 (d, $J = 20$ Hz, 2H), 7.65 – 7.49 (m, 4H), 7.45-7.30 (m, 5H), 7.16 (d, $J = 8.5$ Hz, 1H), 7.06 (d, $J = 3.4$ Hz, 2H), 6.98 (dd, $J = 7.7, 0.9$ Hz, 1H), 6.94 – 6.88 (m, 3H), 6.17 (s, 1H), 5.43 (s, 1H), 3.99-3.91 (m, 2H), 3.80 – 3.74 (m, 1H), 3.69 – 3.64 (m, 1H), 3.51 (s, 3H), 3.40 (s, 3H), 3.22 (s, 3H), 3.21 – 3.16 (m, 1H), 3.15 (s, 1H), 2.91 – 2.81 (m, 1H), 2.51 – 2.42 (m, 1H), 2.29 (s, 3H), 2.28 – 2.22 (m, 1H), 0.54 (s, 3H), 0.45 (s, 3H). ^{13}C NMR (126 MHz, $\text{CD}_3\text{CN}-d_3$) δ 166.48, 166.44, 157.30, 157.09, 156.16, 155.03, 154.95, 154.77, 154.22, 154.03, 152.92, 152.65, 150.21, 150.14, 148.09, 147.89, 145.70, 145.19, 137.55, 137.35, 137.27, 136.36, 136.26, 135.98, 134.05, 133.88, 133.79, 133.63, 133.59, 132.18, 132.11, 131.51, 131.26, 130.05, 129.04, 128.58, 128.53, 128.35, 127.92, 127.87, 127.71, 127.67, 127.46, 127.26, 127.05, 126.96, 125.93, 124.57, 123.82, 123.67, 123.59, 123.06, 116.10, 116.04, 55.12, 54.82, 33.55, 27.81, 27.38, 26.78, 26.26, 20.52. HRMS (ESI): theoretical calc. for $\text{C}_{73}\text{H}_{67}\text{IrN}_8\text{SiP}_2\text{F}_{12}$ $[\text{M}-2\text{PF}_6]^{2+}$ $m/z = 616.21251$, found $[\text{M}-2\text{PF}_6]^{2+}$ $m/z = 616.21246$.

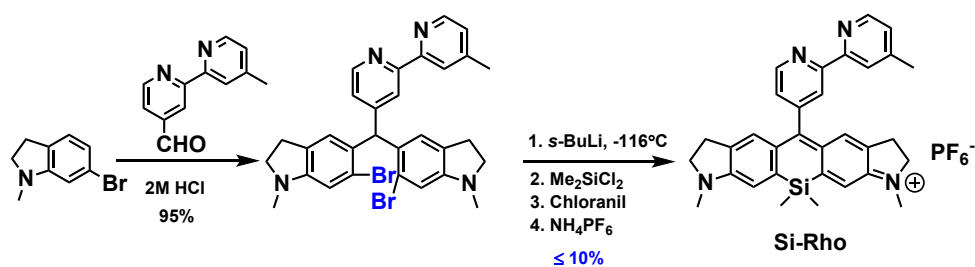
Synthesis of **3** 520 mg, yield 71.2%. ^1H NMR (500 MHz, $\text{CD}_3\text{CN}-d_3$) δ 9.29 (d, $J = 5.5$ Hz, 1H), 9.00 (d, $J = 5.8$ Hz, 1H), 8.70 (d, $J = 13.3$ Hz, 2H), 8.46 (s, 1H), 8.16 (d, $J = 8.4$ Hz, 1H), 8.12 (d, $J = 8.4$ Hz, 1H), 8.07 - 8.06 (m, 2H), 8.02 - 7.96 (m, 3H), 7.92 - 7.90 (m, 3H), 7.88 (s, 1H), 7.84 - 7.79 (m, 3H), 7.73 - 7.70 (m, 3H), 7.64 - 7.55 (m, 2H), 7.52 - 7.48 (m, 3H), 7.38 (d, $J = 8.5$ Hz, 1H), 7.32 (d, $J = 7.7$ Hz, 1H), 7.26 (d, $J = 8.0$ Hz, 1H), 7.07 (s, 1H), 7.02 (s, 1H), 6.89 - 6.66 (m, 6H), 6.28 (s, 1H), 5.63 (s, 1H), 3.83 - 3.59 (m, 4H), 3.16 (s, 3H), 3.13 (s, 3H), 2.45 - 2.39 (m, 1H), 2.38 (s, 3H), 2.30 - 2.27 (m, 1H), 0.55 (s, 3H), 0.46 (s, 3H). ^{13}C NMR (126 MHz, $\text{CD}_3\text{CN}-d_3$) δ 165.72, 165.61, 162.42, 162.13, 161.84, 161.55, 157.55, 157.29, 157.13, 156.98, 155.62, 155.45, 155.14, 155.12, 153.55, 153.24, 150.37, 150.31, 149.52, 148.90, 145.76, 145.36, 140.47, 140.39, 137.97, 137.89, 137.06, 136.33, 136.05, 134.51, 134.43, 134.21, 134.13, 134.07, 133.96, 132.74, 132.36, 131.76, 131.43, 130.75, 130.71, 130.47, 129.87, 129.64, 129.41, 129.15, 129.06, 129.00, 128.94, 128.86, 128.65, 128.23, 127.99, 127.66, 127.24, 126.37, 125.20, 123.78, 123.24, 122.99, 122.86, 120.35, 116.30, 116.14, 115.73, 113.43, 55.02, 33.71, 33.66, 26.24, 20.82. HRMS (ESI): theoretical calc. for $\text{C}_{83}\text{H}_{71}\text{IrN}_8\text{SiP}_2\text{F}_{12} [\text{M}-2\text{PF}_6^-]^{2+}$ $m/z=678.22816$, found $[\text{M}-2\text{PF}_6^-]^{2+}$ $m/z = 678.22876$.

Synthesis of **4** 180 mg, yield 44.2%. ^1H NMR (500 MHz, $\text{CD}_3\text{CN}-d_3$) δ 8.69 (d, $J = 5.7$ Hz, 1H), 8.66 (s, 1H), 8.63 (s, 1H), 8.54 (d, $J = 5.8$ Hz, 1H), 8.17 - 8.03 (m, 5H), 7.96 - 7.93 (m, 2H), 7.89 - 7.76 (m, 4H), 7.62 - 7.53 (m, 3H), 7.52 - 7.42 (m, 4H), 7.39 - 7.31 (m, 2H), 7.17 (d, $J = 8.4$ Hz, 1H), 7.11 (s, 1H), 7.05 (s, 1H), 6.51 (d, $J = 4.9$ Hz, 1H), 6.49 (s, 1H), 6.40 (d, $J = 4.9$ Hz, 1H), 5.72 (s, 1H), 3.78 - 3.69 (m, 4H), 3.18 (s, 3H), 3.15 (s, 3H), 2.64 - 2.54 (m, 1H), 2.49 (s, 3H), 2.44 - 2.39 (m, 2H), 2.12 - 2.08 (m, 1H), 0.56 (s, 3H), 0.49 (s, 3H). ^{13}C NMR (126 MHz, $\text{CD}_3\text{CN}-d_3$) δ 164.02, 163.71, 161.34, 161.15, 159.77, 159.48, 157.02, 156.79, 156.65, 156.47, 155.24, 153.25, 152.83, 149.83, 149.41, 149.05, 148.13, 147.80, 139.63, 139.17, 138.29, 137.99, 137.87, 137.30, 137.02, 136.83, 136.77, 136.75, 134.24, 134.19, 133.65, 133.43, 133.25, 132.95, 132.83, 132.65, 131.99, 131.83, 131.45, 131.23, 130.14, 129.99, 129.19, 128.95, 128.84, 128.52, 128.44, 128.35, 128.15, 127.49, 127.33, 127.23, 127.16, 126.82, 126.75, 126.13, 124.89, 121.02, 120.40, 115.82, 115.69, 115.28, 54.51, 33.16, 25.79,

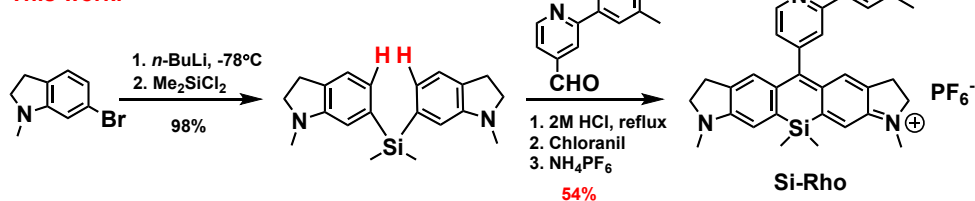
20.40. HRMS (ESI): theoretical calc. for $C_{75}H_{63}IrN_8S_4SiP_2F_{12}$ $[M-2PF_6]^{2+}$ $m/z=690.14100$, found $[M-2PF_6]^{2+}$ $m/z =690.13953$.

CCDC 2285937, 2285938 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Previous route:

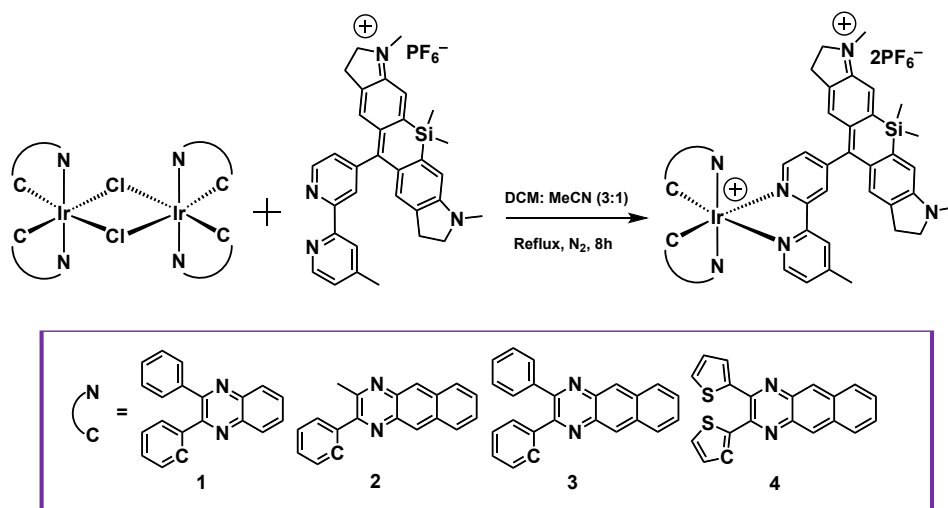


This work:

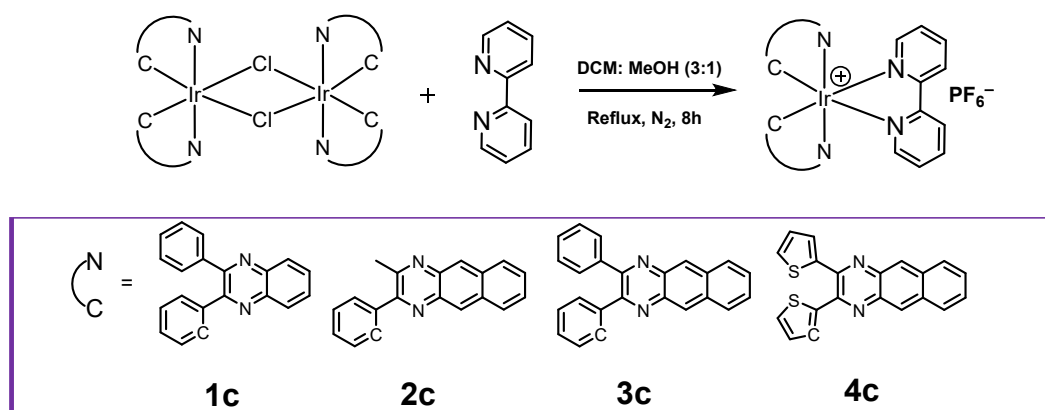


Scheme S1 The synthetic routes of the ligand **Si-Rho**.

The general synthetic routes of the Ir(III) complexes were shown in Scheme S2.



Scheme S2 The synthetic route of the Ir(III) complexes tethered with silicane-modified rhodamine.



Scheme S3. The synthetic route of the rhodamine-free analogues **1c–4c**.

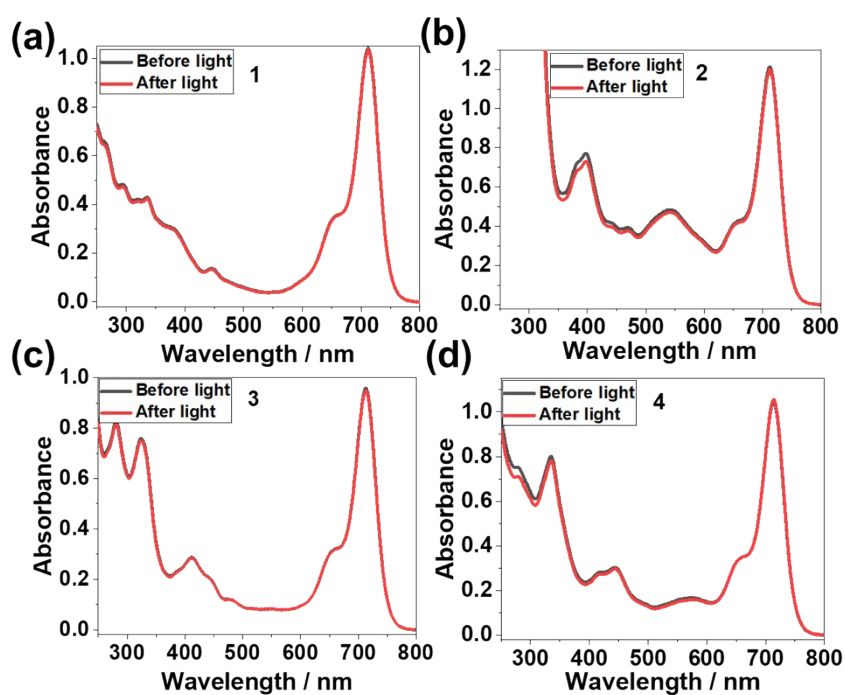


Fig. S1 Photostability study of **1-4** in MeCN under white light irradiation for 30 min, 0.25 W/cm².

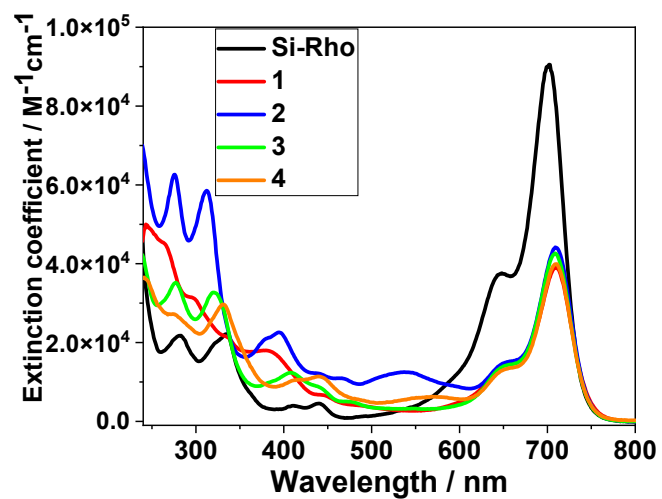


Fig. S2 Molar extinction coefficient of **Si-Rho** and the Ir (III) complexes **Ir-Si-Rhos (1-4)** in HEPES buffer at room temperature, pH=7.4.

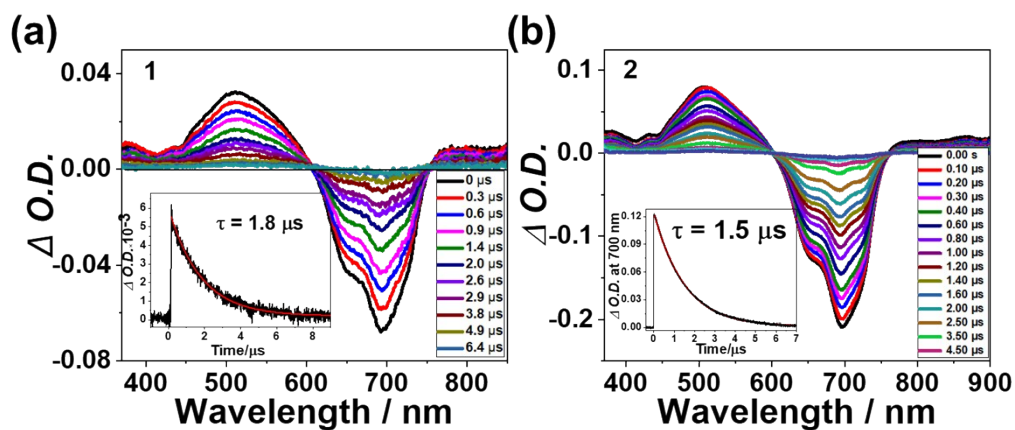


Fig. S3 Transient absorption difference spectra and the triplet excited state lifetime of 1(a), 2(b) in deaerated MeCN at room temperature with laser excitation at 532nm.

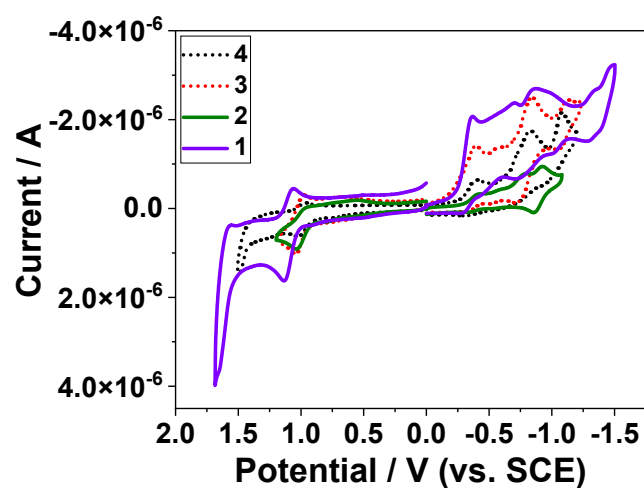


Fig. S4 Cyclic voltammograms of Ir-Si-Rhos in deaerated DCM at room temperature.

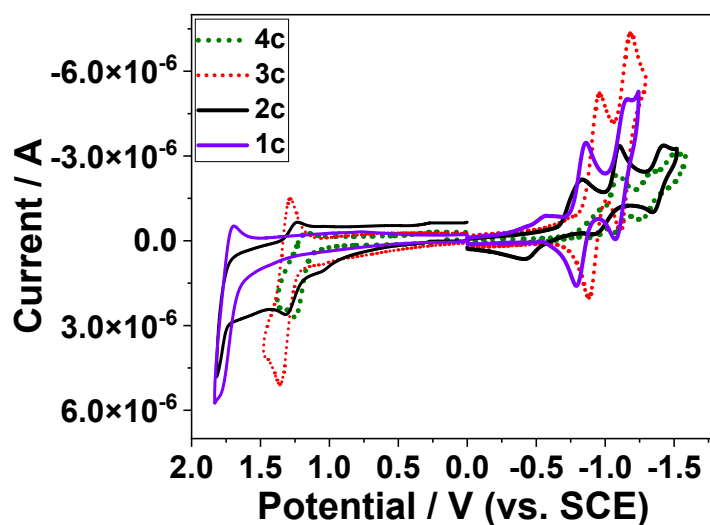


Fig. S5 Cyclic voltammograms of the control complexes in deaerated DCM at room temperature.

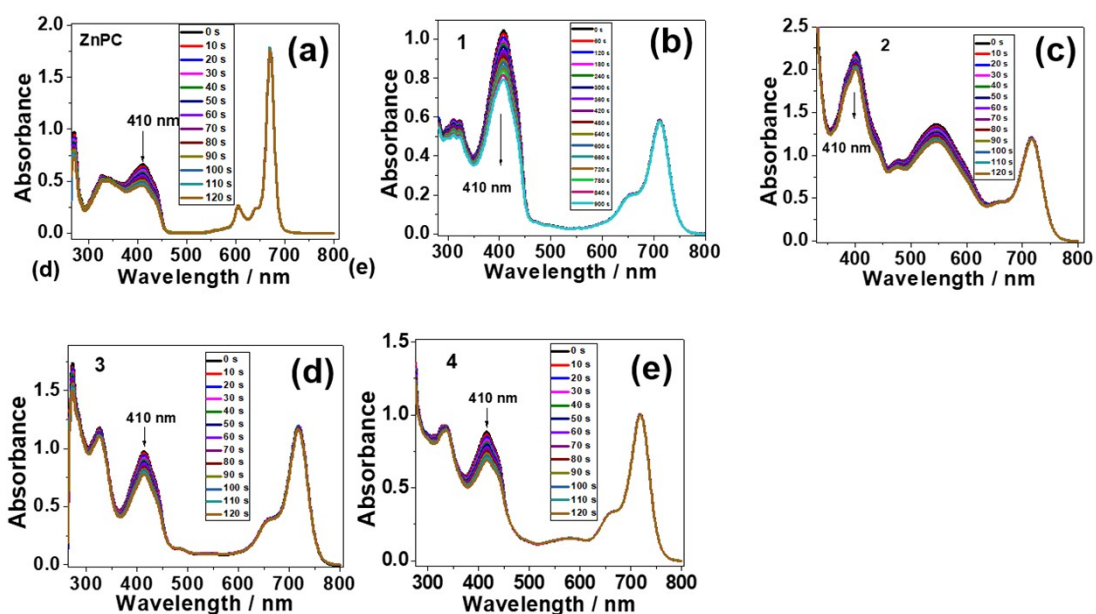


Fig. S6 Singlet oxygen assay using the decreased absorbance of DPBF at 410 nm in the presence of (a) ZnPC (standard), (b) **1**, (c) **2**, (d) **3**, (e) **4** in aerated DMF at room temperature, excited at 680 nm.

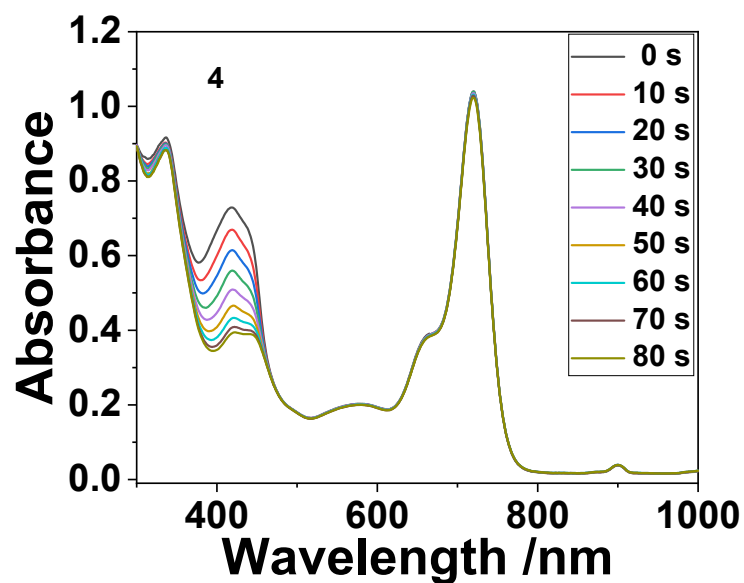


Fig. S7 Singlet oxygen assay using the decreased absorbance of DPBF at 410nm in the presence of **4** in aerated MeOH at room temperature, excited at 808 nm laser, 0.25 W/cm².

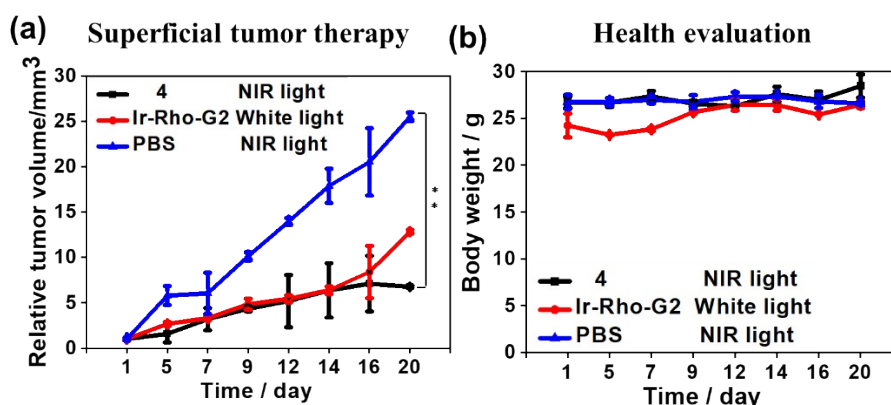


Fig. S8 (a) Relative average tumor volume in the presence of **Ir-Rho-G2** and **4** without covering pork barrier of the mice bearing 4T1 tumors under corresponding light in different groups for 20 days observation period after treatment. (b) Average weights of tumor-bearred mice of **Ir-Rho-G2** and **4** without covering pork barrier under corresponding light during the treatment period. Light sources for **Ir-Rho-G2** and **4** are white light or 808 nm laser, respectively.

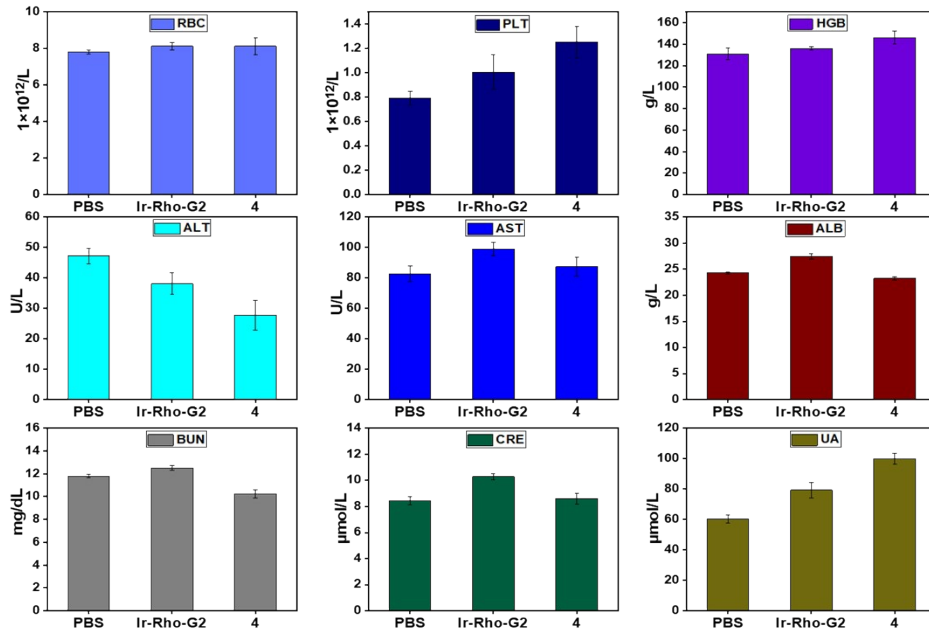


Fig. S9 The liver/kidney function index, including the blood index values and the renal function indicators. The blood and urine were achieved from mice that injected with Ir-Rho-G2 or 4 for 24h, respectively.

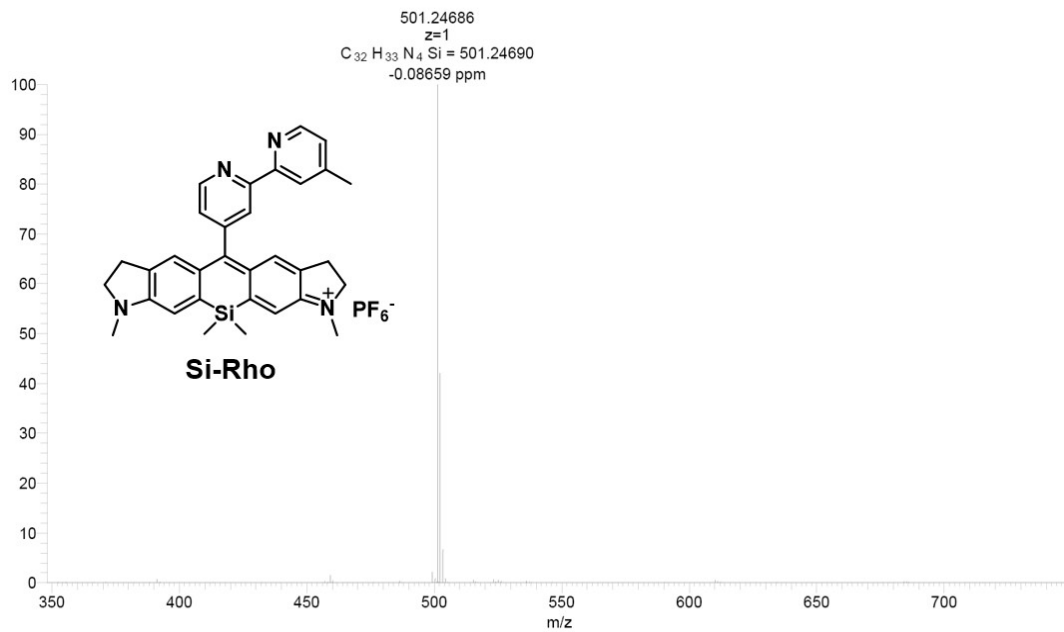


Fig. S10 HRMS spectrum of Si-Rho.

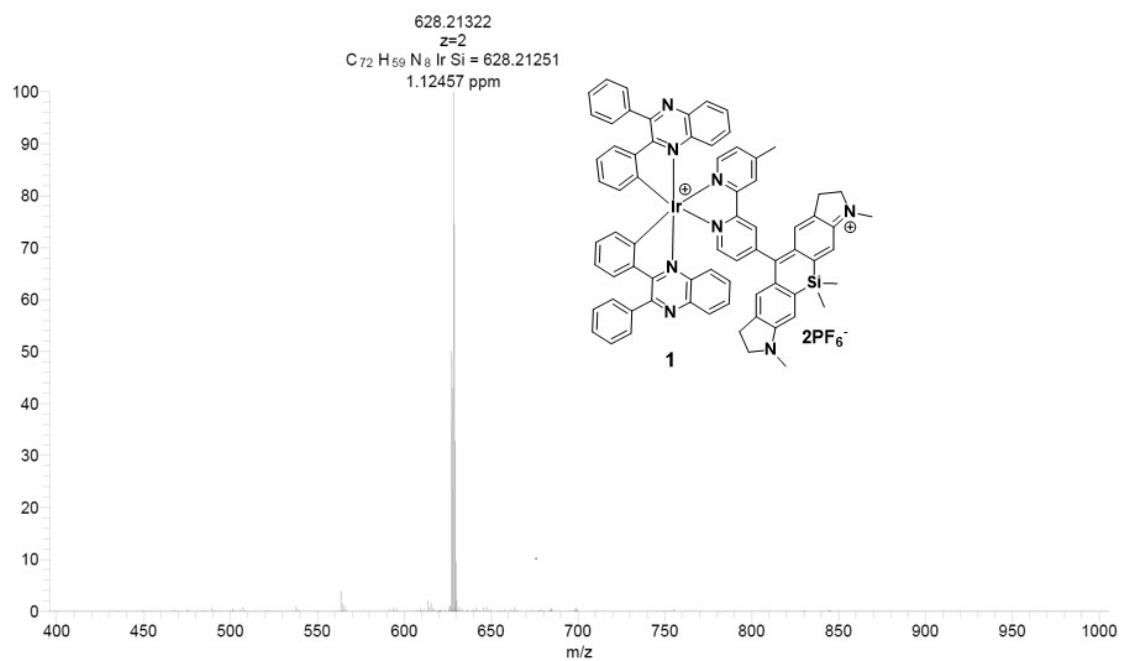


Fig. S11 HRMS spectrum of 1.

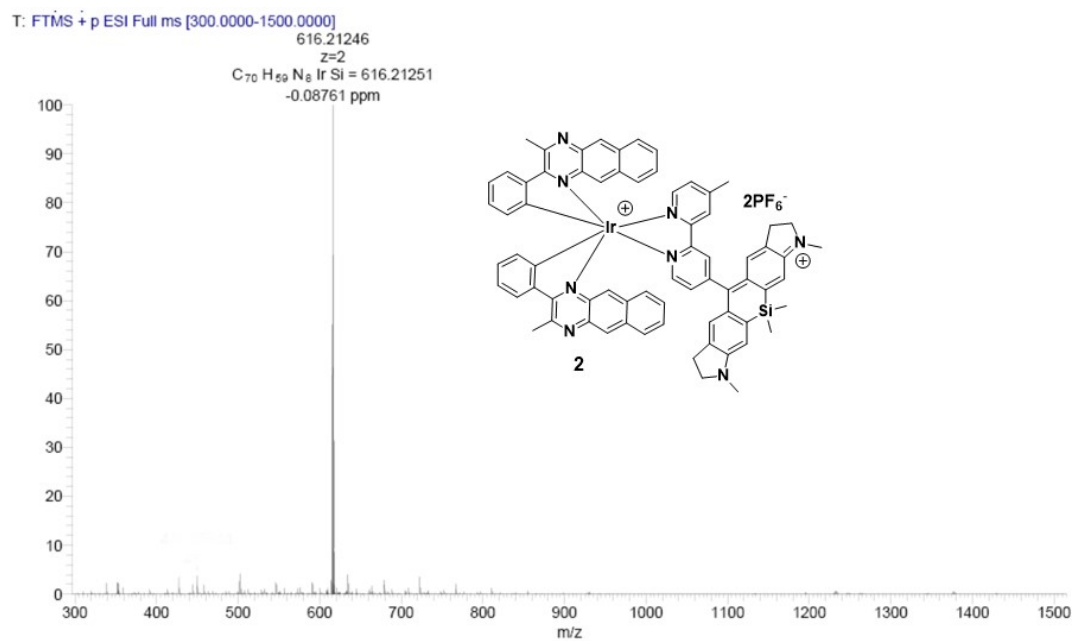


Fig. S12 HRMS spectrum of 2.

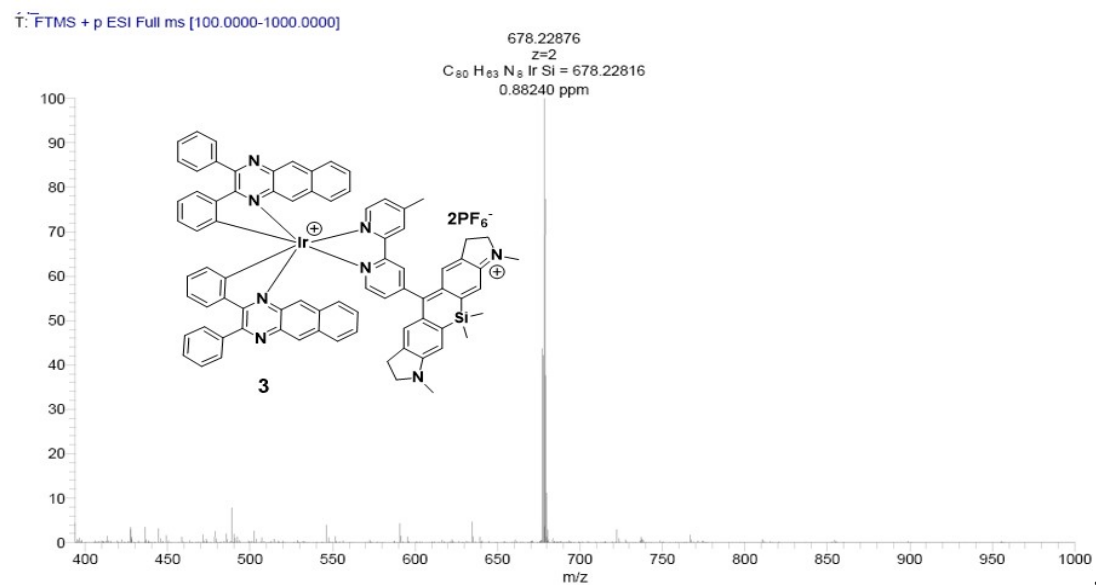


Fig. S13. HRMS spectrum of 3.

T: FTMS + p ESI Full ms [300.0000-1000.0000]

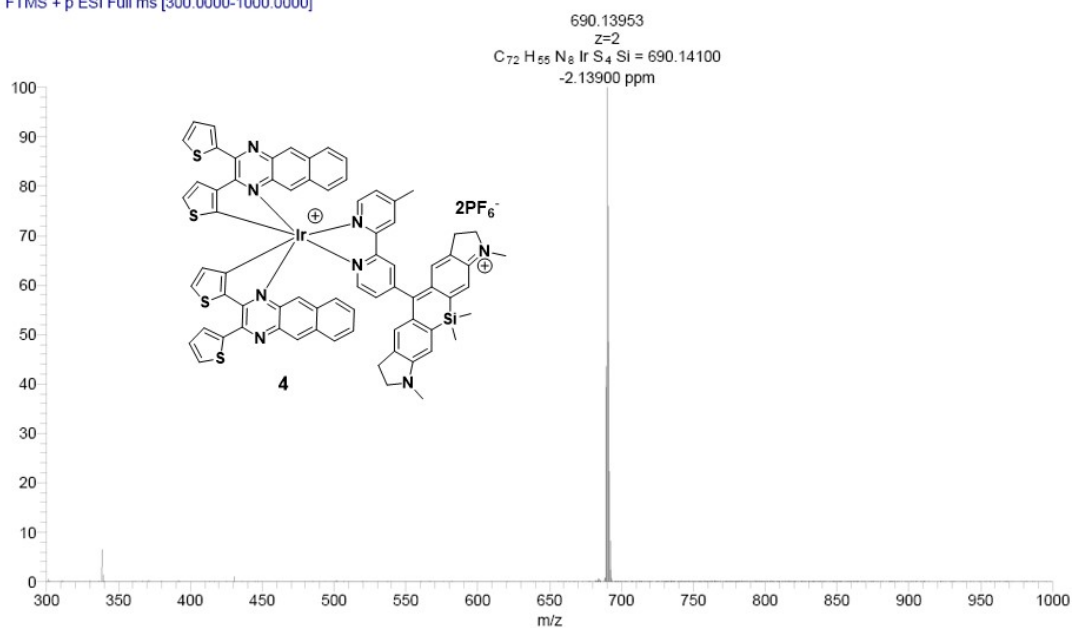


Fig. S14 HRMS spectrum of 4.

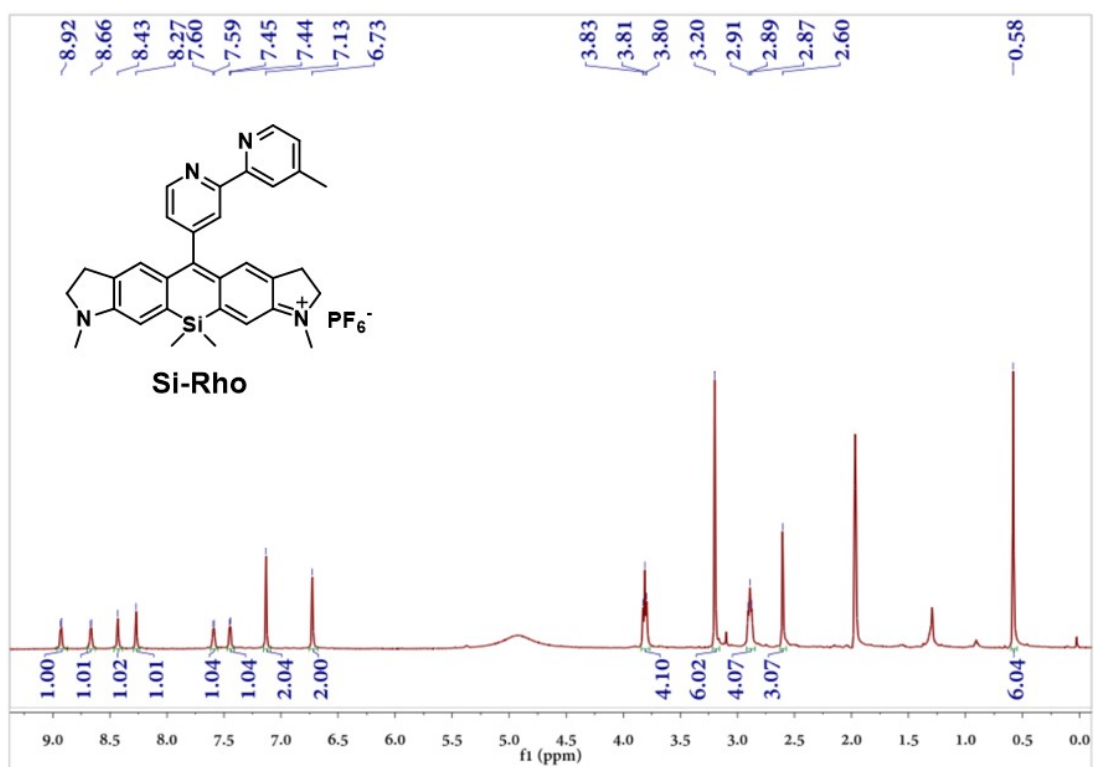


Fig. S15 ¹H NMR spectrum of Si-Rho in MeCN-*d*₃.

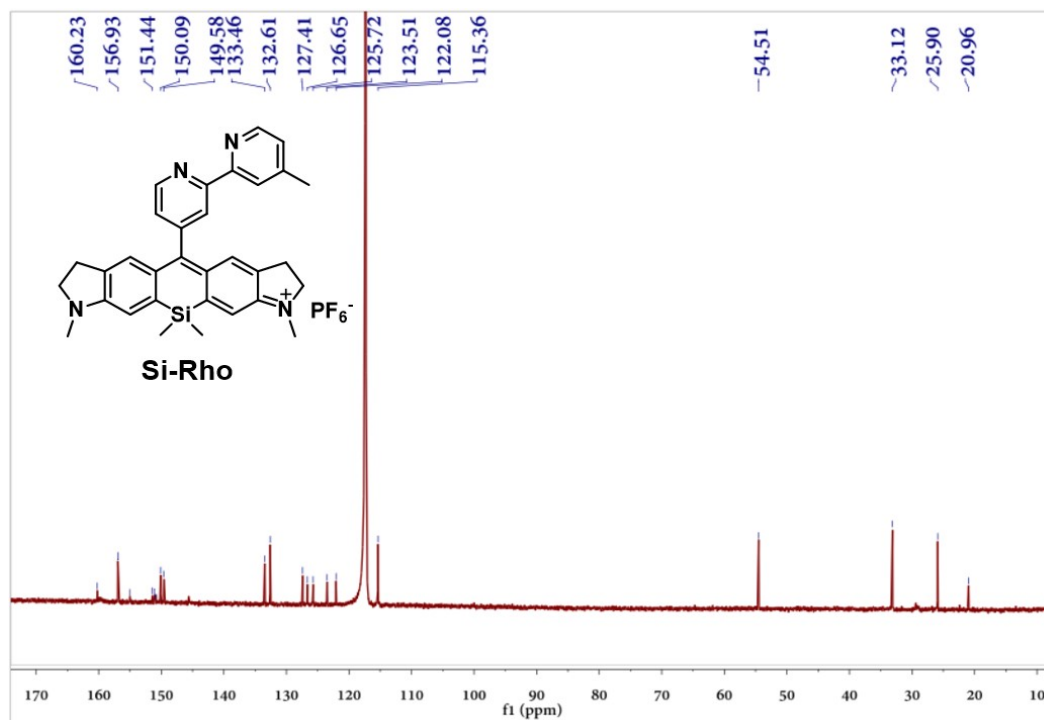


Fig. S16 ^{13}C NMR spectrum of **Si-Rho** in $\text{MeCN-}d_3$.

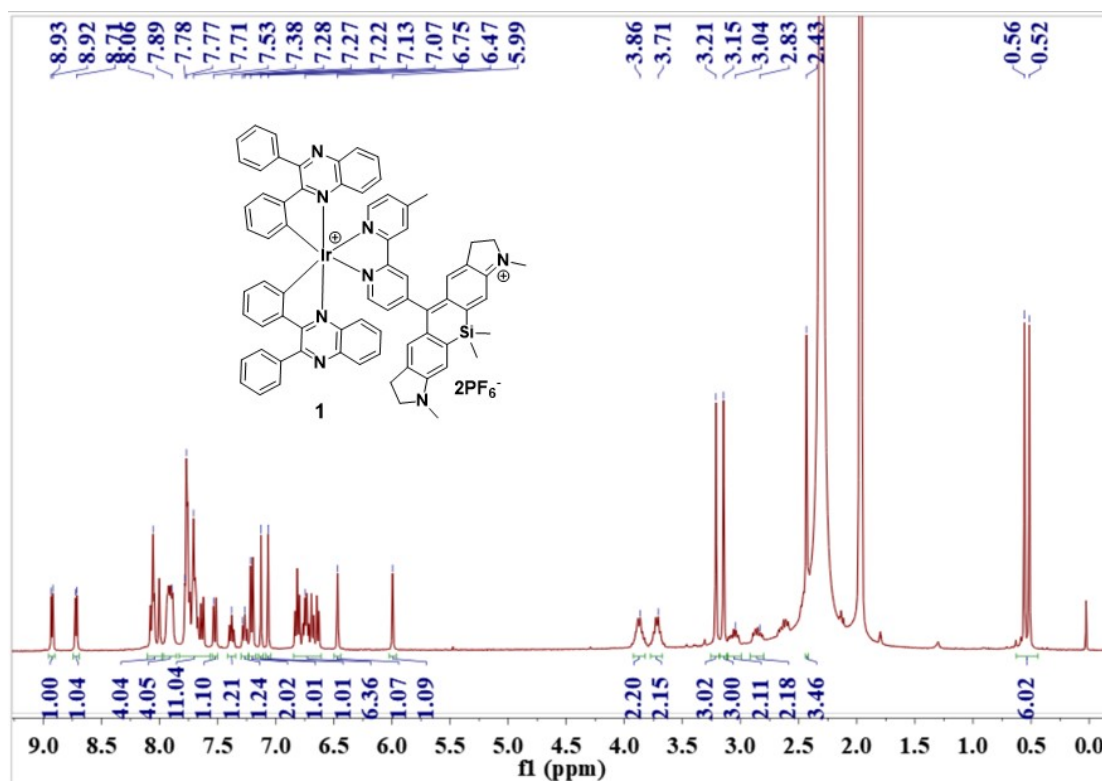


Fig. S17 ^1H NMR spectrum of **1** in $\text{MeCN-}d_3$.

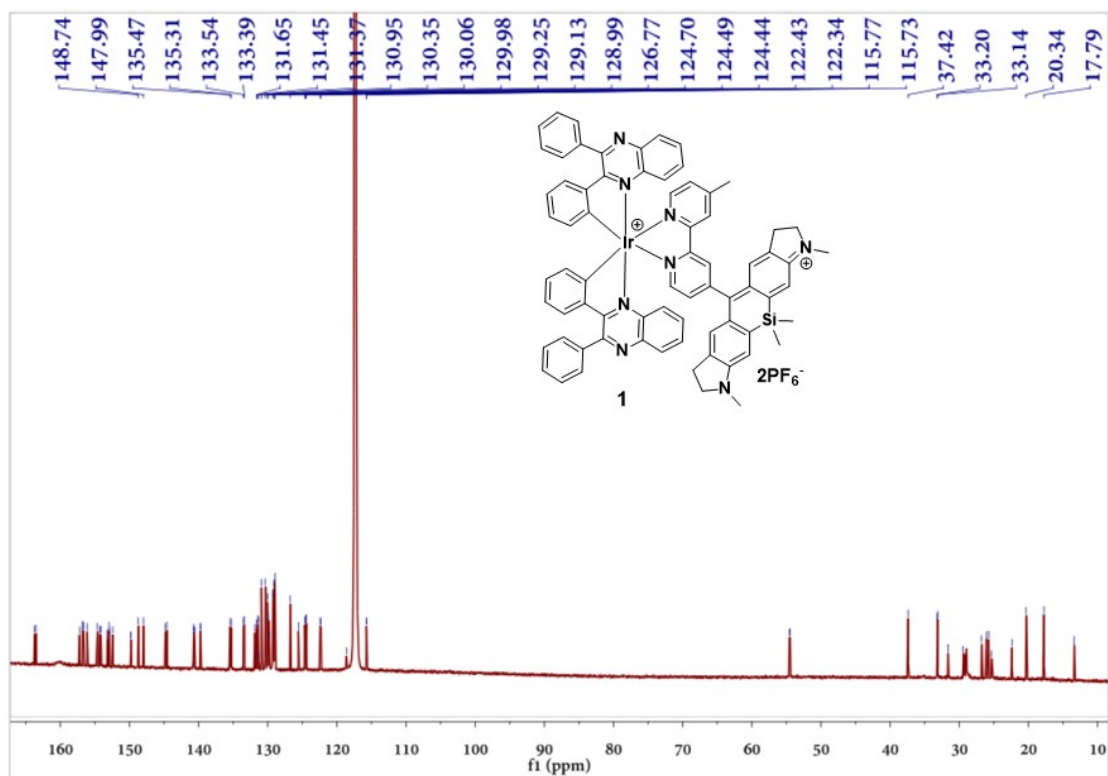


Fig. S18 ^{13}C NMR spectrum of **1** in $\text{MeCN-}d_3$.

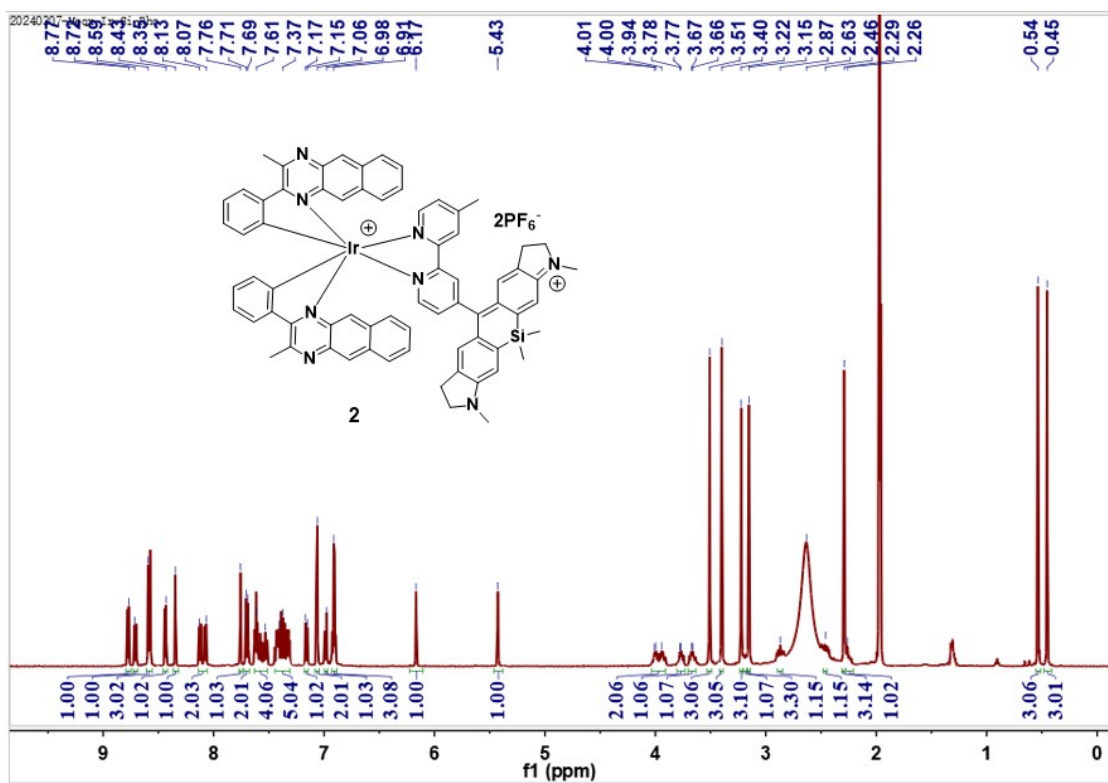


Fig. S19 ^1H NMR spectrum of **2** in $\text{MeCN-}d_3$.

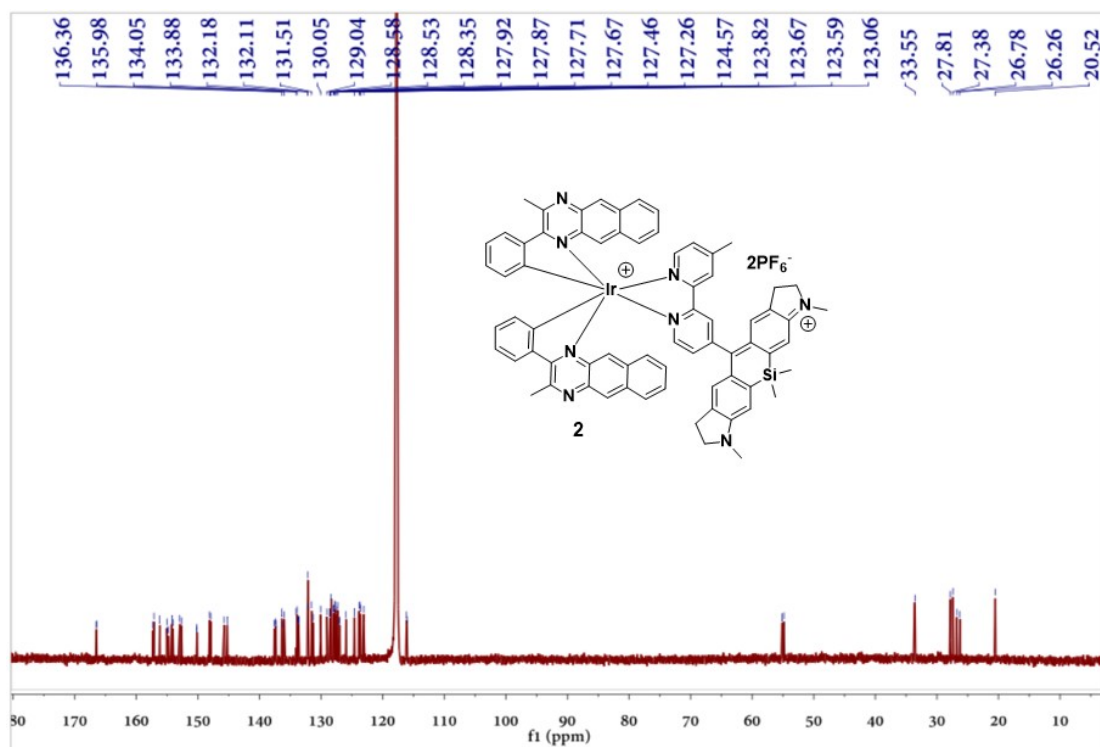


Fig. S20 ^{13}C NMR spectrum of **2** in $\text{MeCN-}d_3$.

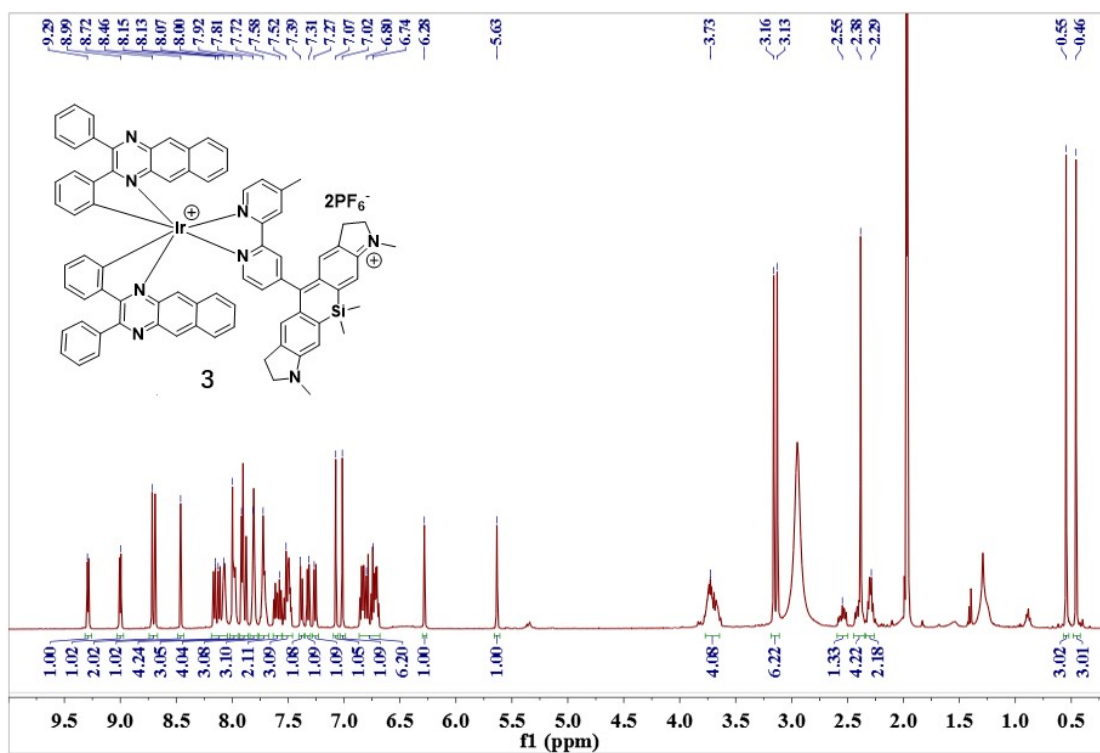


Fig. S21 ^1H NMR spectrum of **3** in $\text{MeCN-}d_3$.

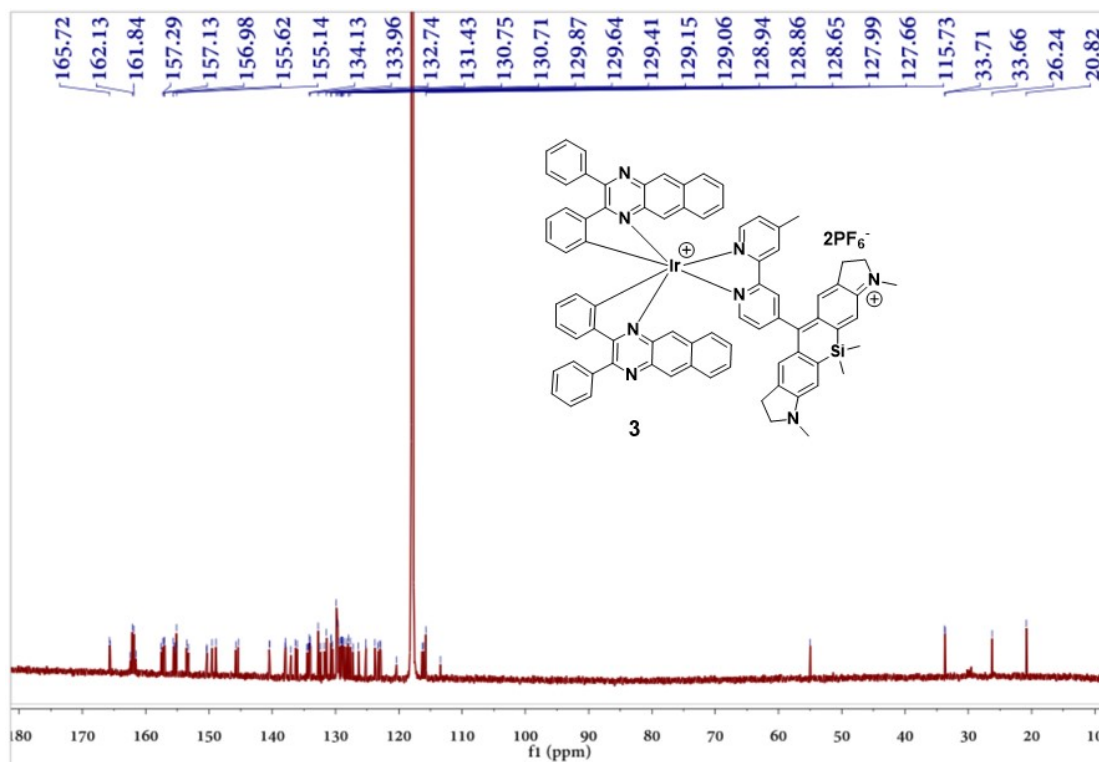


Fig. S22 ¹³C NMR spectrum of **3** in MeCN-*d*₃.

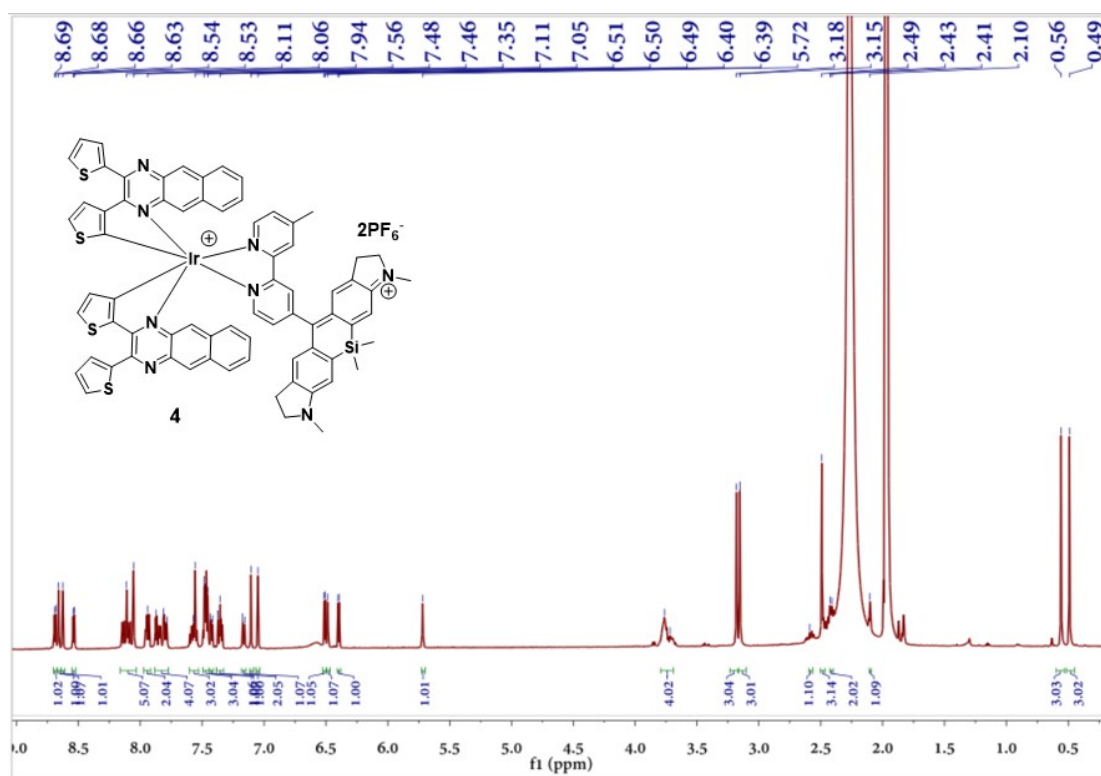


Fig. S23 ¹H NMR spectrum of **4** in MeCN-*d*₃.

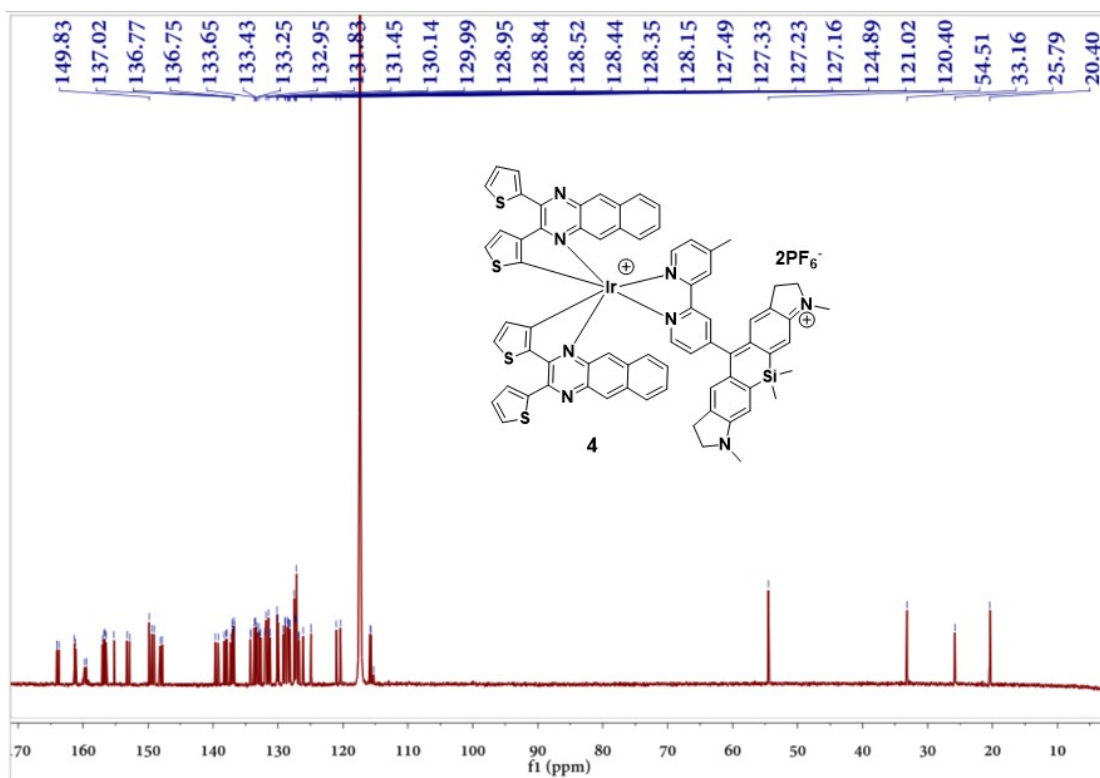


Fig. S24 ^{13}C NMR spectrum of **4** in $\text{MeCN-}d_3$.

Reference

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