Electronic Supplementary Information

Luminescent oligo(ethylene glycol)-functionalized cyclometalated platinum(II) complexes: cellular characterization and mitochondria-specific localization

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Experimental Section

General Considerations: Solvents for syntheses (analytical grade) were used without further purification and all metalation reactions were performed under a nitrogen atmosphere. Solvents for photophysical measurements were purified according to conventional methods. Autoclaved Milli-Q water was used for the preparation of aqueous solutions. All buffer components were of molecular biology grade and were used as received. Dulbecco's Modified Eagle Medium (DMEM), MitoTracker Green FM, LysoTracker Green DND-26, ER Tracker Green and Hoechst 33342, Fetal Bovine Serum (FBS), Phosphate Buffered Saline (PBS), Trypsin-EDTA, and Penicillin were purchased from Invitrogen. ^{1}H, ^{13}C, ^{31}P and ^{195}Pt NMR spectra were obtained on Bruker DRX 300 and 400 FT-NMR spectrometers (ppm) using Me_4Si as internal standard for ^{1}H and ^{13}C, 85% H_3PO_4 in H_2O as external standard for ^{31}P and K_2PtCl_4 in D_2O as external standard for ^{195}Pt NMR spectroscopy, respectively. ESI mass spectra were measured on a Perkin-Elmer SCIEX API 365 mass spectrometer. Elemental analyses were performed on an Elementar Analysensysteme GmbH Vario EL elemental analyzer. DFT calculations were performed at the B3LYP level with the CEP-31G basis set using the Gaussian 09 program. The lipophilicity (log $P_{o/w}$) of the complexes was determined by reversed-phase HPLC according to the method reported by Minick.[1]

Photophysical Measurement and Instruments: UV-vis absorption spectra were obtained on an Agilent 8453 diode array spectrophotometer. Steady-state emission spectra were recorded on a SPEX FluoroLog 3-TCSPC spectrophotometer equipped with a Hamamatsu R928 PMT detector, and emission lifetime measurements were conducted using NanoLed sources in the fast MCS mode and checked using the

TCSPC mode. Sample and standard solutions were degassed with at least three freeze-pump-thaw cycles. Low-temperature (77 K) emission spectra for glassy samples were recorded in 5-mm diameter quartz tubes which were placed in a liquid nitrogen Dewar equipped with quartz windows. The emission quantum yield was measured by using $[Ru(bpy)_3](PF_6)_2$ in degassed acetonitrile as the standard (Φ_r = 0.062) and calculated by: $\Phi_s = \Phi_r(B_r/B_s)(n_s/n_r)^2(D_s/D_r)$, where the subscripts s and r refer to sample and reference standard solution respectively, n is the refractive index of the solvents, D is the integrated intensity, and Φ is the luminescence quantum yield.[2] The quantity B is calculated by the equation: $B = 1 - 10^{-AL}$; where A is the absorbance at the excitation wavelength and L is the optical path length. Errors for λ (± 1 nm), τ (± 10 %), and Φ (± 10 %) are estimated.

For spectroscopic titration experiments, solutions of platinum(II) complexes (25 μM) were prepared in PBS buffer containing 10% of DMSO. Aliquots of a stock glutathione (GSH) solution (0–1.0 mM in PBS) were then added, followed by aliquots of a stock bovine serum albumin (BSA) solution (0–25 μM in PBS). Emission spectra (λ_{ex} 380 nm) were recorded after equilibration at 20 °C for 5 min.

Cell Culture: HeLa cells were cultured in DMEM, supplemented with 10% FBS and 1% Penicillin. The cells were cultured in a humidified chamber at 37 ^oC under a 5% CO_2 atmosphere.

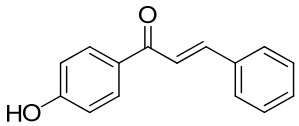
MTT Assays: HeLa cells were plated into 96-well tissue culture plates (10^4 cells/well) in growth medium (100 μL) and incubated at 37 ^oC under a 5% CO_2 atmosphere for 24 h. The platinum(II) complexes and cisplatin (positive control) were then added to the wells, with concentrations ranging from 10^{-7} to 10^{-4} M in a mixture of growth medium/DMSO (99:1, v/v). Wells containing growth medium without cells were used as blank controls. The microplate was incubated at 37 °C under a 5% CO_2 atmosphere for 24 h, and MTT in PBS (5 mg mL^{-1}, 10 μL) was then added to each well, after which incubation was continued for another 2.5 h. Culture medium was removed to reduce interference with the spectrometer reading. A mixture of DMSO and ethanol (1:1; 100 μL) was added to each well and mixed thoroughly by pipetting 10–20 times to dissolve the blue formazan. Absorption was measured at 570 nm by using a microplate reader (PowerWave XS, BioTek). The IC_{50} value of the complex was determined from the dose dependence of surviving cells after exposure to the complex for 24 h relative to the controls.

Cellular Uptake: HeLa cells in growth medium were incubated with the platinum(II) complex (5 μM) at 37 ^oC for 1.5 h. The culture medium was then

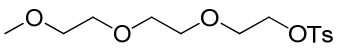
removed and washed throroughly with PBS (1 mL × 5); after which the cells were trypsinized and harvested. The cells, together with the collected PBS, were counted and then digested with 65% HNO_3 at 80 °C. The digested solution was filtered, and the concentration of platinum was determined by ICP-AES (Perkin Elmer Optima 2100DV).

Live-Cell Confocal Microscopy: Images were collected by using a Leica TCS SPE spectral confocal microscope equipped with a diode laser (Leica). Specimens were imaged by using a 405 or 488 nm laser and a 63× objective oil-immersion lens. In the colocalization experiments, after treatment with the platinum(II) complex, HeLa cells were incubated with MitoTracker Green FM, LysoTracker Green DND-26, ER Tracker Green or Hoechst 33342 in PBS buffer for 15–30 min respectively, and the cells were finally washed with PBS (1 mL × 2). The Pearson coefficient (*Rr*) was determined by Leica Application Suite Advanced Fluorescence (LAS AF) software.

Synthetic Methods

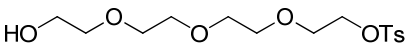


C[1]: 4'-Hydroxylacetophenone (1.5 g, 11.0 mmol) and benzaldehyde (1.6 g, 13.2 mmol) were dissolved in absolute ethanol (11 mL) at room temperature. Toluenesulfonic acid (416 mg, 2.21 mmol) was poured into the solution, and the mixture was heated at reflux for 24 h. The resultant yellow solid was collected by filtration and washed by ethanol (638 mg, 24%). ESI-MS (*m*/*z*): 225.5 $[M + H]^+$. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.02 (d, J = 9.0 Hz, 2H), 7.82 (d, J = 15.8 Hz, 1H), 7.66–7.63 (m, 2H), 7.56 (d, J = 15.8 Hz, 1H), 7.43–7.41 (m, 3H), 6.95 (d, J = 9.0 Hz, 2H), 6.21 (s, 1H) ppm.

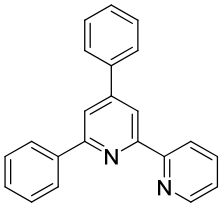


C[2]: Toluenesulfonyl chloride (1.9 g. 10.0 mmol) and triethylene glycol monomethyl ether (1.5 g, 9.2 mmol) were stirred in CH_2Cl_2 (10 mL) at 0 °C in the presence of Et_3N (2.7 g, 27.5 mmol). The resultant mixture was diluted by CH_2Cl_2 (100 mL), then washed with dilute HCl (1 M), brine, and dried over anhydrous $MgSO_4$. The brown oil was purified by chromatography (SiO_2; ethyl acetate/hexane = 1/9) to yield the product as a colorless oil (2.93 g, 92%). ESI-MS (*m*/*z*): 319.3 $[M + H]^+$. ^1H NMR

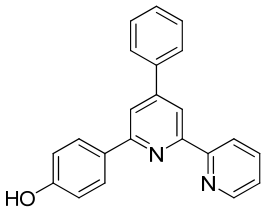
($CDCl_3$, 400 MHz): δ 7.79 (d, J = 8.1 Hz, 2H), 7.34 (d, J = 8.1 Hz, 2H), 4.17–4.14 (m, 2H), 3.69–3.67 (m, 2H), 3.62–3.59 (m, 6H), 3.54–3.51 (m, 2H), 3.67 (s, 3H), 2.44 (s, 3H) ppm.



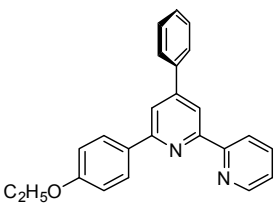
C[3]: The procedure for preparing **C**[2] was employed, except that tetraethylene glycol (6.78 g, 35.0 mmol) and toluenesulfonyl chloride (2.7 g. 14.0 mmol) were used. Yield: 10.8 g, 89%. ESI-MS (m/z): 349.1 $[M + H]^+$. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.79 (d, J = 8.1 Hz, 2H), 7.34 (d, J = 8.1 Hz, 2H), 4.17–4.13 (m, 2H), 3.72–3.59 (m, 15H), 2.44 (s, 3H) ppm.

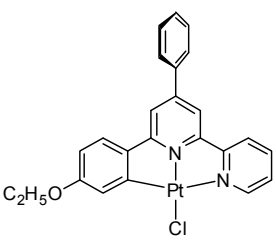


L[1] and $[PtL^1Cl]$ were prepared according to a published method.[3]

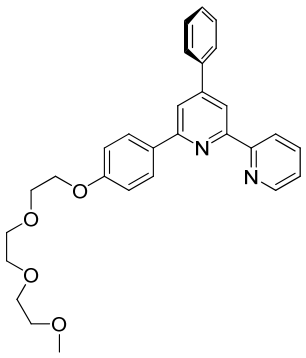


C[4]: 2-Acetylpyridine (136 mg, 1.1 mmol), **C**[1] (253 mg, 1.1 mmol) and NaOH (45 mg, 1.1 mmol) were mixed together by mortar and pestle to give a yellow powder. Ammonium acetate (5.0 g, excess) in methanol (10 mL) was then added and the mixture was refluxed for 12 h. The methanol solvent was removed *in vacuo*, and the crude product was dissolved in CH_2Cl_2. The organic layer was extracted by CH_2Cl_2 and washed by brine and dried over anhydrous Na_2SO_4. The product was purified by chromatography (SiO_2; ethyl acetate/hexane = 1/1) to yield a white solid (150 mg, 41%). ESI-MS (m/z): 325.5 $[M + H]^+$, 347.3 $[M + Na]^+$. ^1H NMR (DMSO-d_6, 300 MHz): δ 9.84 (s, br, 1H), 8.75–8.73 (m, 1H), 8.62 (dd, J = 8.1, 0.9 Hz, 1H), 8.53 (s, 1H), 8.23 (d, J = 8.1 Hz, 2H), 8.18 (s, 1H), 8.04–7.95 (m, 3H), 7.60–7.48 (m, 4H), 6.93 (d, J = 8.1 Hz, 2H) ppm.

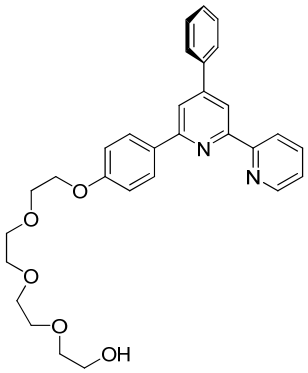




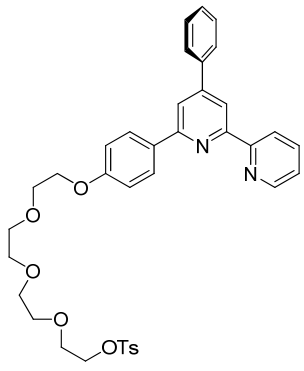
L[2] and $[PtL^2Cl]$ were prepared according to a published method.[4]



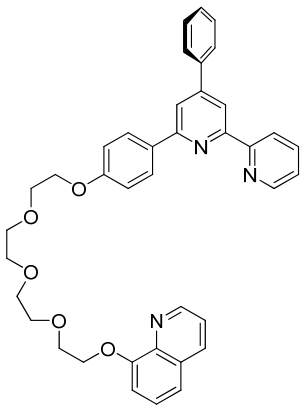
L^{3}: A mixture of **C**^{2} (149 mg, 0.47 mmol), **C**^{4} (152 mg, 0.47 mmol) and potassium carbonate (130 mg, 0.94 mmol) in DMF (10 mL) was stirred for 24 h. The resultant mixture was diluted using diethyl ether, then washed with brine water, and dried over anhydrous $MgSO_4$. The brown oil was purified by chromatography (SiO_2; ethyl acetate/hexane = 1/9) to afford the product as yellow oil (123 mg, 56%). ESI-MS (*m*/*z*): 471.6 $[M + H]^+$. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.72–8.67 (m, 2H), 8.58 (d, J = 1.2 Hz, 1H), 8.18–8.15 (m, 2H), 8.01 (s, 1H), 7.93 (d, J = 1.2 Hz, 1H), 7.90–7.81 (m, 3H), 7.54–7.46 (m, 3H), 7.36–7.33 (m, 1H), 7.08–7.05 (m, 2H), 4.25–4.22 (m, 2H), 3.93–3.89 (m, 2H), 3.78–3.60 (m, 8H), 3.30 (s, 3H) ppm.



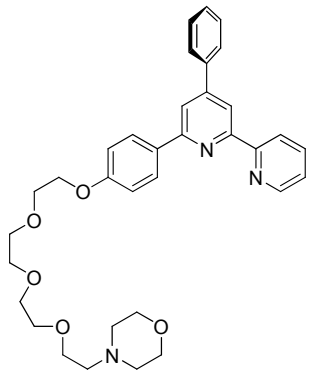
L^{4}: The procedure for preparing **L**^{3} was employed, **C**^{3} (140 mg, 0.4 mmol) and **C**^{4} (130 mg, 0.40 mmol) and K_2CO_3 (167 mg, 1.2 mmol) were used. Yield: 100 mg, 50%. ESI-MS (*m*/*z*): 501.6 $[M + H]^+$, 523.4 $[M + Na]^+$. ^{1}H NMR (CD_3Cl, 300 MHz): δ 8.72–8.70 (m, 1H), 8.67 (d, J = 7.8 Hz, 1H), 8.58 (d, J = 1.2 Hz, 1H), 8.18–8.15 (m, 2H), 8.01 (s, 1H), 7.93 (d, J = 1.2 Hz, 1H), 7.90–7.81 (m, 3H), 7.54–7.46 (m, 3H), 7.36–7.33 (m, 1H), 7.08–7.05 (m, 2H), 4.25–4.22 (m, 2H), 3.93–3.89 (m, 2H), 3.78–3.60 (m, 12H) ppm.



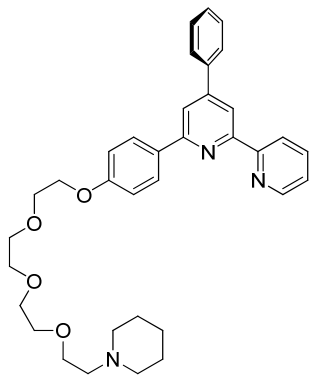
C^{5}: The procedure for preparing **C**^{2} was employed, except that **L**^{4} (250 mg, 0.5 mmol), toluenesulfonyl chloride (88 mg. 0.55 mmol) and Et_3N (1.5 g, 1.5 mmol) were used. Yield: 230 mg, 70%. ESI-MS (*m*/*z*): 655.6 $[M + H]^+$. ^{1}H NMR (CD_3Cl, 300 MHz): δ 8.72–8.70 (m, 1H), 8.67 (d, J = 7.8 Hz, 1H), 8.58 (d, J = 1.5 Hz, 1H), 8.16 (d, J = 8.1 Hz, 2H), 7.93 (d, J = 1.5 Hz, 1H), 7.90–7.77 (m, 5H), 7.52–7.46 (m, 3H), 7.34–7.30 (m, 3H), 7.05 (d, J = 9.3 Hz, 2H), 4.24–4.20 (m, 2H), 4.17–4.14 (m, 2H), 3.92–3.89 (m, 2H), 3.76–3.56 (m, 10H), 2.42 (s, 3H) ppm.



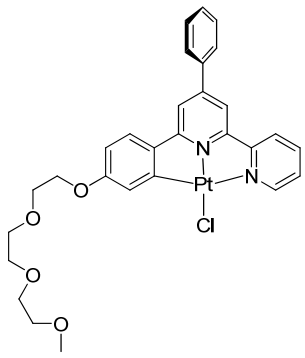
L^{5}: A mixture of **C**^{5} (166 mg, 0.25 mmol), 8-hydroxyquinoline (40 mg, 0.28 mmol) and potassium carbonate (104 mg, 0.75 mmol) in DMF (10 mL) was stirred for 24 h. The resultant mixture was diluted using diethyl ether, then washed with brine, and dried over anhydrous $MgSO_4$. The brown oil was purified by chromatography (SiO_2; ethyl acetate/hexane = 9/1) to afford the product as a white solid (125 mg, 80%). ESI-MS (*m*/*z*): 628.6 $[M + H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.90 (dd, *J* = 4.0, 1.2 Hz, 1H), 8.69 (d, *J* = 4.0 Hz, 1H), 8.65 (d, *J* = 8.0 Hz, 1H), 8.57 (d, *J* = 1.2 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 2H), 8.06 (d, *J* = 8.4 Hz, 1H), 7.89 (s, 1H), 7.84–7.78 (m, 3H), 7.50–7.26 (m, 7H), 7.06 (d, *J* = 8.0 Hz, 1H), 7.02 (d, *J* = 8.8 Hz, 2H), 4.39–4.37 (m, 2H), 4.17–4.15 (m, 2H), 4.06–4.03 (m, 2H), 3.87–3.85 (m, 2H), 3.78–3.76 (m, 2H), 3.71–3.68 (m, 6H) ppm.



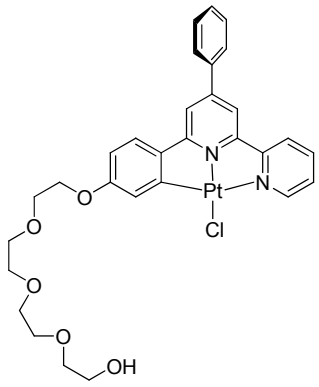
L^{6}: The procedure for preparing **L**^{5} was employed, except that **C**^{5} (200 mg, 0.31 mmol), morpholine (41 mg. 0.47 mmol) and K_2CO_3 (77 mg, 0.56 mmol) were used. Yield: 126 mg, 71%. ESI-MS (*m*/*z*): 570.6 $[M + H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.71 (d, *J* = 3.6 Hz, 1H), 8.67 (d, *J* = 8.0 Hz, 1H), 8.58 (d, *J* = 1.6 Hz, 1H), 8.16 (d, *J* = 8.8 Hz, 2H), 7.92 (d, *J* = 1.6 Hz, 1H), 7.89–7.81 (m, 3H), 7.54–7.44 (m, 3H), 7.35–7.32 (m, 1H), 7.06 (d, *J* = 8.8 Hz, 2H), 4.24–4.22 (m, 2H), 3.92–3.90 (m, 2H), 3.70–3.53 (m, 10H), 2.88–2.75 (m, 4H), 2.53–2.44 (m, 6H) ppm.



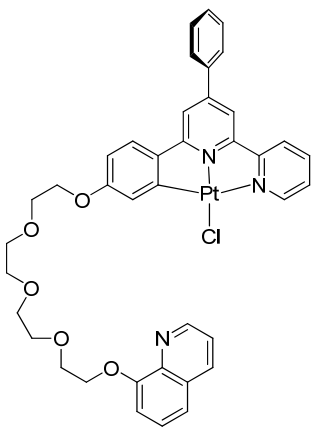
L^{7}: The procedure for preparing **L**^{5} was employed, except that **C**^{5} (275 mg, 0.42 mmol), piperidine (54 mg. 0.63 mmol) and K_2CO_3 (104 mg, 0.76 mmol) were used. Yield: 210 mg, 88%. ESI-MS (*m*/*z*): 568.6 $[M + H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.68–8.63 (m, 2H), 8.56 (d, *J* = 1.2 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 2H), 7.88 (d, *J* = 1.2 Hz, 1H), 7.84–7.77 (m, 3H), 7.50–7.40 (m, 3H), 7.31–7.27 (m, 1H), 7.03 (d, *J* = 8.8 Hz, 2H), 4.19–4.17 (m, 2H), 3.88–3.86 (m, 2H), 3.74–3.56 (m, 10H), 2.53–2.39 (m, 6H), 1.58–1.53 (m, 4H), 1.39–1.38 (m, 2H) ppm.



[PtL^{3}Cl]: **L^3** (135 mg, 0.29 mmol) and K_2PtCl_4 (119 mg, 0.29 mmol) were dissolved in glacial acetic acid (20 mL) and the mixture was refluxed for 24 h. The resultant solution was concentrated in vacuo. The residue was dissolved in acetonitrile (5 mL) and adddition of diethyl ether (20 mL) resulted in the precipitation of an orange solid, which was collected by centrifugation and washed with water (10 mL × 2), ethanol (10 mL × 2) and diethyl ether (10 mL × 2) to afford a yellow solid (131 mg, 65%). ESI-MS (*m*/*z*): 723.3 $[M + Na]^+$. ^{1}H NMR (CD_3CN, 400 MHz): δ 8.72 (d, J = 5.6 Hz, 1H), 8.10–8.06 (m, 2H), 7.86–7.84 (m, 2H), 7.79 (s, 1H), 7.58–7.51 (m, 5H), 7.31 (d, J = 8.8 Hz, 1H), 6.82 (m, 1H), 6.50 (dd, J = 8.4, 2.4 Hz, 1H), 3.96–3.93 (m, 2H), 3.74–3.72 (m, 2H), 3.66–3.57 (m, 6H), 3.49–3.47 (m, 2H), 3.29 (s, 3H) ppm. ^{13}C NMR characterization was hampered by insufficient solubility in CD_3CN and CD_2Cl_2.

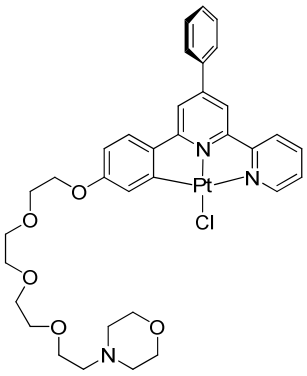


[PtL^{4}Cl]: The procedure for preparing **[PtL^{3}Cl]** was employed, except **L^4** (174 mg, 0.35 mmol) and K_2PtCl_4 (144 mg, 0.35 mmol) were used. Yield: 153 mg, 60%. ESI-MS (*m*/*z*): 730.3 $[M + H]^+$. ^{1}H NMR (CD_3CN, 400 MHz): δ 8.81 (d, J = 4.8 Hz, 1H), 8.18–8.11 (m, 2H), 7.86 (m, 3H), 7.66–7.56 (m, 6H), 7.40 (d, J = 8.4 Hz, 1H), 6.92 (m, 1H), 6.57–6.55 (m, 1H), 4.14–4.11 (m, 2H), 4.02–4.00 (m, 2H), 3.76–3.74 (m, 2H), 3.64–3.58 (m, 10H) ppm. ^{13}C NMR (CD_2Cl_2, 100 MHz): δ 171.1, 165.5, 160.2, 157.6, 154.3, 150.4, 148.5, 145.5, 139.5, 139.1, 137.9, 130.0, 129.4, 127.6, 127.0, 125.9, 123.1, 118.9, 116.0, 115.9, 110.7, 71.1, 71.0, 70.9, 70.0, 69.4, 67.2, 63.9 ppm.

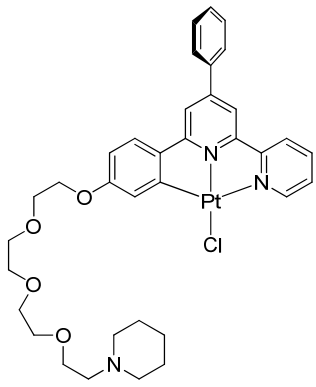


[PtL^{5}Cl]: The procedure for preparing **[PtL^{3}Cl]** was employed, except **L^5** (165 mg, 0.26 mmol) and K_2PtCl_4 (110 mg, 0.26 mmol) were used. Yield: 115 mg, 52%. ESI-MS (*m*/*z*): 857.5 $[M + H]^+$. ^{1}H NMR (CD_3CN, 400 MHz): δ 8.80 (dd, J = 5.2 Hz, 1H), 8.83–8.81 (m, 1H), 8.23–8.16 (m, 3H), 7.93–7.88 (m, 3H), 7.72–7.58 (m, 6H), 7.39–7.38 (m, 3H), 7.09 (dd, J = 5.6, 3.6 Hz, 1H), 6.99 (d, J = 2.8 Hz, 1H), 6.54 (dd, J = 8.4, 2.4 Hz, 1H), 4.28–4.26 (m, 2H), 4.05–4.03 (m, 2H), 3.92–3.89 (m,

2H), 3.79–3.76 (m, 2H), 3.72–3.65 (m, 8H) ppm. ^{13}C NMR (CD_2Cl_2, 100 MHz): δ 170.7, 165.8, 157.6, 154.9, 150.7, 149.3, 148.6, 140.3, 139.5, 139.3, 137.9, 136.3, 130.1, 129.8, 129.5, 127.5, 127.2, 127.1, 126.1, 123.0, 122.0, 120.1, 119.1, 116.0, 110.7, 109.4, 71.1, 71.0, 70.9, 70.0, 69.9, 68.4, 67.3 ppm.



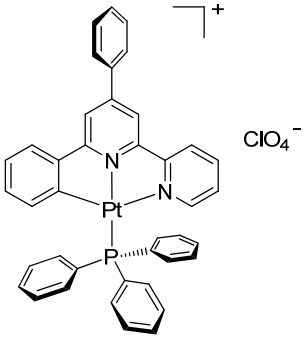
[PtL^{6}Cl]: The procedure for preparing **[PtL^{3}Cl]** was employed, except **L^6** (126 mg, 0.22 mmol) and K_2PtCl_4 (92 mg, 0.22 mmol) were used. Yield: 79 mg, 0.045%. ESI-MS (*m*/*z*): 799.8 $[M + H]^+$. ^{1}H NMR (CD_3CN, 400 MHz): δ 8.76 (d, J = 4.4 Hz, 1H), 8.17–8.10 (m, 2H), 7.89–7.84 (m, 3H), 7.62–7.55 (m, 5H), 7.36 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 2.8 Hz, 1H), 6.52 (dd, J = 8.4, 2.8 Hz, 1H), 3.99–3.96 (m, 2H), 3.75–3.73 (m, 2H), 3.66–3.50 (m, 14H), 2.47–2.44 (t, J = Hz, 2H), 2.39–2.37 (m, 4H) ppm. ^{13}C NMR (CD_2Cl_2, 100 MHz): δ 165.8, 160.4, 157.7, 154.5, 150.8, 148.7, 145.5, 139.5, 139.3, 137.9, 130.1, 129.5, 127.5, 127.2, 126.1, 123.0, 119.1, 116.1, 115.9, 110.8, 71.1, 71.0, 70.7, 70.0, 68.9, 67.3, 67.2, 58.6, 54.5 ppm.



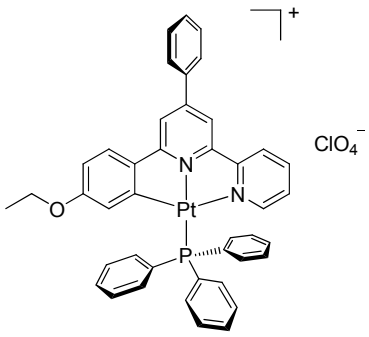
[PtL^{7}Cl]: The procedure for preparing **[PtL^{3}Cl]** was employed, except **L^7** (210 mg, 0.37 mmol) and K_2PtCl_4 (154 mg, 0.37 mmol) were used. Yield: 147 mg, 50%. ESI-MS (*m*/*z*): 797.6 $[M + H]^+$. ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 8.83 (d, J = 4.4 Hz, 1H), 8.04 (td, J = 8.0, 1.6 Hz, 1H), 7.96 (d, J = 8.0 Hz, 1H), 7.79–7.76 (m, 2H), 7.63 (d, J = 1.2 Hz, 1H), 7.56–7.50 (m, 4H), 7.41 (d, J = 1.2 Hz, 1H), 7.27 (d, J = 8.8 Hz, Hz, 1H), 6.97 (d, J = 2.4 Hz, 1H), 6.56 (dd, J = 8.4, 2.4 Hz, 1H), 4.01–3.99 (m, 2H), 3.78–3.41 (m, 12H), 2.80 (m, 6H), 1.75 (m, 4H), 1.49 (m, 2H) ppm. ^{13}C NMR (CD_2Cl_2, 100 MHz): δ 165.5, 160.2, 157.6, 154.3, 150.5, 148.5, 145.5, 139.5, 139.2, 137.8, 130.0, 129.4, 127.6, 127.0, 126.0, 123.1, 118.9, 116.0, 115.9, 110.6, 71.1, 70.9, 70.7, 70.0, 68.6, 67.2, 58.6, 55.1, 25.7, 24.3 ppm.

[Pt(L$^{1-7}$)PPh$_3$]ClO$_4$, (1–7)

Representative procedure: For complex **1**, PPh_3 (45 mg, 0.17 mmol) was added to **[Pt(L^1)Cl]** (89 mg, 0.17 mmol) in CH_3CN/CH_3OH (1:1, 20 mL) and the mixture was stirred for 12 h under a nitrogen atmosphere. The yellow resultant solution was filtered and evaporated to 10 mL. Addition of excess $LiClO_4$ afforded a bright orange solid, which was collected by centrifugation and washed with water (10 mL × 3), ethanol (10 mL × 3) and diethyl ether (10 mL × 3) to give a yellow solid, which was dried *in vacuo*. Yield: 100 mg, 70%.

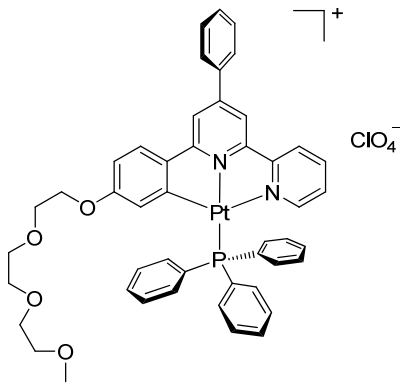


1: ESI-MS (*m*/*z*): 764.3 $[M - ClO_4]^+$. ^{1}H NMR (CD_3CN, 400 MHz): δ 8.39 (d, J = 8.0 Hz, 1H), 8.30 (s, 1H), 8.23 (s, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.01–7.99 (m, 2H), 7.95–7.90 (m, 6H), 7.77 (d, J = 8.0 Hz, 1H), 7.66–7.62 (m, 6H), 7.56–7.52 (m, 6H), 7.14–7.05 (m, 2H), 6.70–6.63 (m, 2H), 6.54–6.38 (m, 1H) ppm. ^{13}C NMR (CD_3CN, 100 MHz): δ 164.7, 159.6, 155.7, 155.1, 151.8, 149.0, 142.1, 139.3, 139.2, 137.3, 136.3, 136.2, 133.1, 131.9, 131.5, 130.4, 130.2, 130.1, 129.9, 129.5, 128.7, 128.0, 126.7, 125.5, 118.9 ppm. ^{31}P{^{1}H} NMR (CD_3CN, 161.8 MHz): δ 25.54 ($^1J_{P,Pt}$ = 4028 Hz) ppm. Anal. Calcd for $C_{40}H_{30}ClN_2O_4PPt$: C, 55.59; H, 3.50; N 3.24. Found C, 55.34; H, 3.31; N, 3.39.

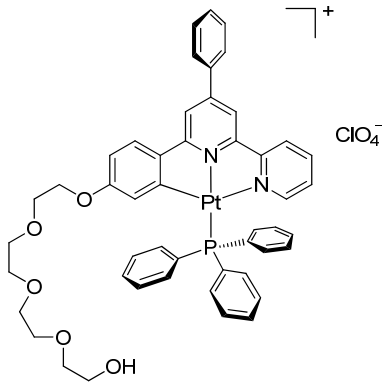


2: Yield: 135 mg, 77%. ESI-MS (*m*/*z*): 808.3 $[M - ClO_4]^+$. ^{1}H NMR (CD_3CN, 400 MHz): δ 8.35 (d, J = 8.0 Hz, 1H), 8.19 (s, 1H), 8.12–8.07 (m, 2H), 7.98–7.90 (m, 8H),

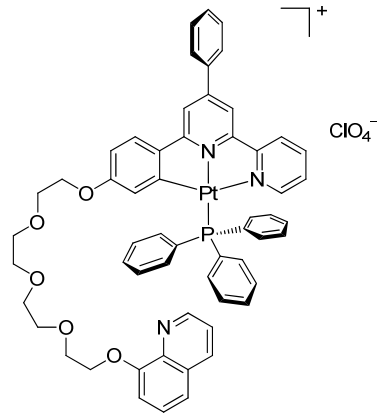
7.68–7.53 (m, 13H), 7.12–7.08 (m, 1H), 6.66 (d, J = 5.2 Hz, 1H), 6.58 (dd, J = 8.8, 2.8 Hz, 1H), 5.98 (m, 1H), 3.23 (q, J = 7.2 Hz, 2H), 0.92 (t, J = 7.2 Hz, 3H) ppm. ^{13}C NMR (CD_3CN, 100 MHz): δ 164.7, 161.0, 159.7, 155.4, 154.8, 151.8, 142.0, 141.0, 137.4, 136.3, 136.2, 133.2, 131.8, 130.3, 130.2, 130.1, 129.9, 129.5, 128.6, 128.2, 127.9, 125.4, 124.8, 124.7, 117.6, 113.4, 64.0, 14.5 ppm. ^{31}P{^{1}H} NMR (CD_3CN, 161.8 MHz): δ 26.04 ($^1J_{P,Pt}$ = 4025 Hz) ppm. Anal. Calcd for $C_{42}H_{34}ClN_2O_5PPt$: C, 55.54; H, 3.77; N 3.08. Found C, 55.66; H, 3.85; N, 2.94.



3: Yield: 220 mg, 68%. ESI-MS (m/z): 926.3 $[M - ClO_4]^+$. ^{1}H NMR (CD_3CN, 400 MHz): δ 8.31 (d, J = 8.0 Hz, 1H), 8.16 (s, 1H), 8.07 (td, J = 8.0, 1.2 Hz, 1H), 8.03 (s, 1H), 7.95–7.89 (m, 8H), 7.67–7.54 (m, 13H), 7.11–7.07 (m, 1H), 6.65 (d, J = 5.2 Hz, 1H), 6.56 (dd, J = 8.0, 2.4 Hz, 1H), 5.95 (m, 1H), 3.53–3.50 (m, 2H), 3.49–3.44 (m, 4H), 3.41–3.39 (m, 2H), 3.33–3.30 (m, 2H), 3.28 (s, 3H), 3.27–3.24 (m, 2H) ppm. ^{13}C NMR (CD_3CN, 100 MHz): δ 164.4, 160.6, 159.4, 155.0, 154.6, 151.6, 141.9, 141.0, 136.9, 136.2, 136.1, 133.2, 133.1, 131.7, 130.2, 130.1, 129.8, 129.2, 128.4, 128.0, 127.8, 125.3, 124.5, 124.4, 113.3, 72.5, 71.1, 70.9, 69.3, 67.9, 58.8 ppm. ^{31}P{^{1}H} NMR (CD_3CN, 161.8 MHz): δ 25.62 ($^1J_{P,Pt}$ = 4001 Hz) ppm. Anal. Calcd for $C_{47}H_{44}ClN_2O_8PPt$: C, 55.00; H, 4.32; N 2.73. Found C, 54.93; H, 4.44; N, 2.65.

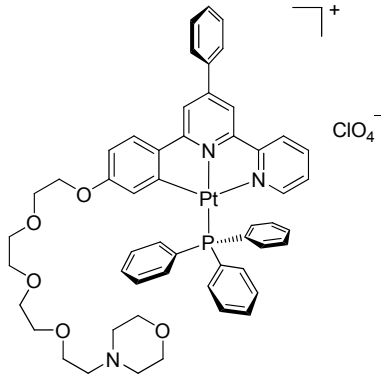


4: Yield: 115 mg, 65%. ESI-MS (*m*/*z*): 956.4 $[M - ClO_4]^+$. ^{1}H NMR (CD_3CN, 400 MHz): δ 8.31 (d, J = 8.0 Hz, 1H), 8.16 (s, 1H), 8.07 (td, J = 8.0, 1.2 Hz, 1H), 8.03 (s, 1H), 7.94–7.89 (m, 8H), 7.67–7.54 (m, 13H), 7.10–7.07 (m, 1H), 6.65 (d, J = 6.4 Hz, 1H), 6.56 (dd, J = 8.0, 2.4 Hz, 1H), 5.95 (m, 1H), 4.13–4.10 (m, 2H), 3.61–3.59 (m, 2H), 3.56–3.51 (m, 4H), 3.49–3.47 (m, 2H), 3.41–3.40 (m, 2H), 3.33–3.31 (m, 2H), 3.26–3.24 (m, 2H) ppm. ^{13}C NMR (CD_3CN, 100 MHz): δ 171.5, 164.4, 160.6, 159.4, 154.9, 154.6, 151.6, 141.9, 141.0, 137.3, 137.2, 136.9, 136.2, 136.0, 133.1, 131.7, 130.2, 130.2, 129.8, 129.2, 128.4, 128.0, 127.8, 125.3, 124.5, 117.6, 117.3, 113.2, 71.1, 71.0, 69.5, 69.3, 67.9, 64.2 ppm. ^{31}P{^{1}H} NMR (CD_3CN, 161.8 MHz): δ 25.57 ($^1J_{P,Pt}$ = 3998 Hz) ppm. Anal. Calcd for $C_{48}H_{46}ClN_2O_9PPt$: C, 54.57; H, 4.39; N 2.65. Found C, 54.85; H, 4.59; N, 2.54.

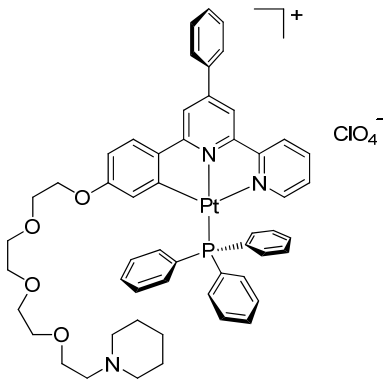


5: Yield: 210 mg, 70%. ESI-MS (*m*/*z*): 1083.3 $[M - ClO_4]^+$. ^{1}H NMR (CD_3CN, 400 MHz): δ 8.74–8.73 (m, 1H), 8.32 (d, J = 8.0 Hz, 1H), 8.15–8.07 (m, 3H), 8.00 (s, 1H), 7.96–7.87 (m, 7H), 7.64–7.52 (m, 14H), 7.39–7.35 (m, 3H), 7.12–7.07 (m, 2H), 6.63 (d, J = 5.2 Hz, 1H), 6.52 (dd, J = 8.4, 2.4 Hz, 1H), 5.92 (m, 1H), 4.23–4.21 (m, 2H), 3.85–3.82 (m, 2H), 3.68–3.66 (m, 2H), 3.62–3.60 (m, 2H), 3.55–3.53 (m, 2H), 3.45–3.43 (m, 2H), 3.35–3.33 (m, 2H), 3.25–3.23 (m, 2H) ppm. ^{13}C NMR (CD_3CN,

100 MHz): δ 155.0, 154.6, 154.1, 151.5, 150.0, 141.9, 141.1, 137.4, 136.1, 133.1, 131.7, 130.2, 130.0, 129.8, 129.2, 128.4, 127.9, 127.9, 127.8, 125.3, 122.9, 121.2, 113.1, 110.7, 70.7, 70.6, 70.4, 69.3, 69.2, 68.4, 67.8 ppm. $^{31}P\{^{1}H\}$ NMR (CD_3CN, 161.8 MHz): δ 25.47 ($^{1}J_{P,Pt}$ = 4008 Hz) ppm. Anal. Calcd for $C_{57}H_{51}ClN_3O_9PPt$: C, 57.84; H, 4.34; N 3.55. Found C, 57.94; H, 4.36; N, 3.35.

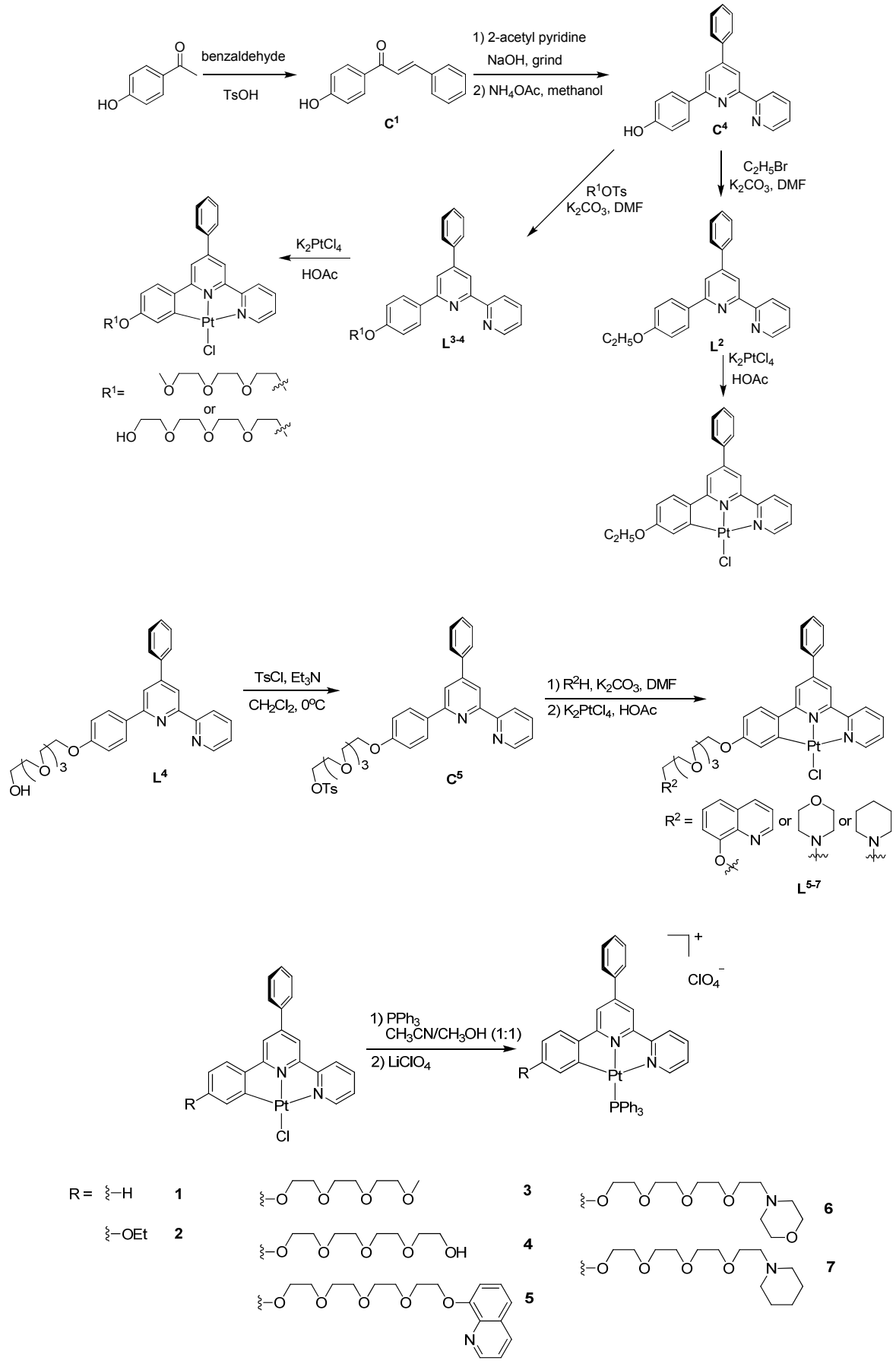


6: Yield: 110 mg, 68%. ESI-MS (*m*/*z*): 1026.0 $[M - ClO_4]^+$. ^{1}H NMR (CD_3CN, 400 MHz): δ 8.35 (d, J = 8.0 Hz, 1H), 8.19 (s, 1H), 8.09 (t, J = 8.0 Hz, 1H), 8.07 (s, 1H), 7.97–7.83 (m, 8H), 7.69–7.54 (m, 13H), 7.10 (t, J = 5.6 Hz, 1H), 6.67 (d, J = 5.6 Hz, 1H), 6.60 (dd, J = 8.0, 2.0 Hz, 1H), 5.96 (m, 1H), 3.60 (t, J = 4.4 Hz, 4H), 3.56 (t, J = 5.6 Hz, 2H), 3.51–3.47 (m, 6H), 3.41–3.38 (m, 2H), 3.32–3.31 (m, 2H), 3.28–3.26 (m, 2H), 2.57–2.52 (m, 6H) ppm. ^{13}C NMR (CD_3CN, 100 MHz): δ 160.6, 154.7, 151.7, 142.0, 136.1, 130.3, 129.9, 128.1, 125.3, 113.3, 71.0, 69.4, 68.3, 68.0, 66.8, 58.6, 54.5 ppm. $^{31}P\{^{1}H\}$ NMR (CD_3CN, 161.8 MHz): δ 25.56 ($^{1}J_{P,Pt}$ = 4000 Hz) ppm. Anal. Calcd for $C_{52}H_{53}ClN_3O_9PPt$: C, 55.49; H, 4.75; N 3.73. Found C, 55.71; H, 4.66; N, 3.56.



7: Yield: 213 mg, 77%. ESI-MS (*m*/*z*): 1024.4 $[M - ClO_4]^+$. ^{1}H NMR (CD_3CN, 400 MHz): δ 8.34 (d, J = 8.0 Hz, 1H), 8.19 (s, 1H), 8.09 (t, J = 8.0 Hz, 1H), 8.06 (s, 1H),

7.97–7.90 (m, 8H), 7.68–7.54 (m, 13H), 7.10 (t, J = 6.0 Hz, 1H), 6.66 (d, J = 5.2 Hz, 1H), 6.59 (dd, J = 8.4, 2.4 Hz, 1H), 5.96 (m, 1H), 3.64 (t, J = 5.6 Hz, 2H), 3.58–3.53 (m, 4H), 3.51–3.49 (m, 2H), 3.42–3.40 (m, 2H), 3.34–3.32 (m, 2H), 3.27–3.24 (m, 2H), 2.91–2.87 (m, 6H), 1.69–1.63 (m, 4H), 1.50–1.48 (m, 2H) ppm. ^{13}C NMR (CD_3CN, 100 MHz): δ 163.9, 160.0, 158.9, 156.5, 154.6, 154.2, 151.1, 141.4, 136.4, 135.7, 135.6, 132.6, 131.2, 129.7, 129.6, 129.3, 128.7, 127.9, 127.6, 127.3, 124.8, 117.7, 117.2, 116.9, 112.8, 70.8, 70.4, 70.3, 68.8, 67.4, 65.9, 57.4, 54.3, 24.2, 22.7 ppm. ^{31}P{^{1}H} NMR (DMSO-d_6, 161.8 MHz): δ 25.53 ($^1J_{P,Pt}$ = 4000 Hz) ppm. ^{195}Pt NMR (DMSO-d_6, 85.6 MHz): δ –4143.3 ($^1J_{Pt,P}$ = 4022 Hz) ppm. Anal. Calcd for $C_{53}H_{55}ClN_3O_8PPt$: C, 56.66; H, 4.93; N 3.74. Found C, 56.71; H, 4.86; N, 3.58.



Scheme S1 Synthesis of complexes **1**–**7**.

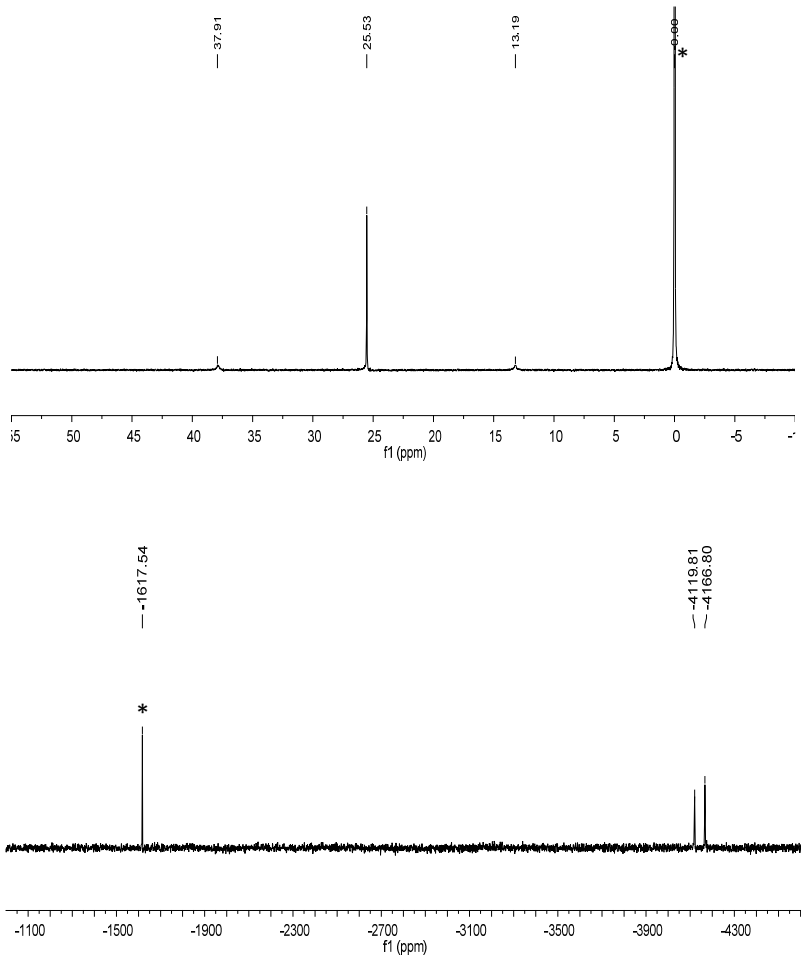


Fig. S1 ^{31}P NMR (top; 162 MHz) and ^{195}Pt NMR (bottom; 86 MHz) spectra of **7** in DMSO-d_6 at 298 K (* = 85% H_3PO_4 in H_2O and K_2PtCl_4 in D_2O respectively as external standards).

Table S1 Photophysical Data for **1**–**7**.

Complex	λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) [a]	λ_{ex}/nm	Fluid 298 K: [a] λ_{max}/nm (τ/μs); Φ	Fluid 77 K: [b] λ_{max}/nm (τ/μs)
1	291 (34980), 326 (20540), 354 (15040), 408 (930)	430	534 (0.30); 0.007	504 (max; 13.1), 542
2	267 (30800), 277 (31730), 301 (34850), 337 (24100), 443 (1280)	430	586 (1.41); 0.023	535 (max; 12.8), 573
3	267 (28710), 277 (29710), 300 (33350), 338 (22920), 451 (1120)	430	579 (1.68); 0.036	536 (max; 16.0), 573
4	267 (36970), 303 (48480), 337 (26980), 339 441 (1450)	430	580 (1.73); 0.023	537 (max; 12.0), 575
5	270 (30030), 277 (30530), 299 (33590), 339 (21300), 451 (1092)	430	582 (1.70); 0.034	536 (max; 15.0), 586
6	268 (36780), 277 (37510), 300 (41230), 340 (27830), 449 (1400)	430	580 (1.64); 0.010	538 (max; 14.4), 576
7	268 (36180), 276 (36830), 300 (40410), 339 (27660), 440 (1470)	430	582 (1.38); 0.010	538 (max; 12.5), 571

[a] In CH_3CN. [b] In ^{n}BuCN

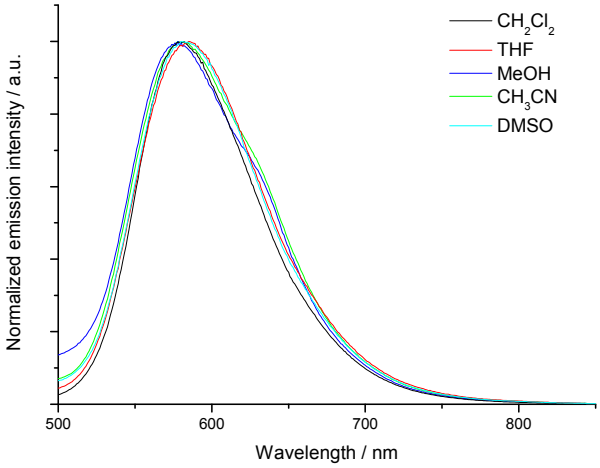


Fig. S2 Normalized emission spectra for **3** (10^{-5} M; λ_{ex} = 400 nm) in different solvents at 298 K.

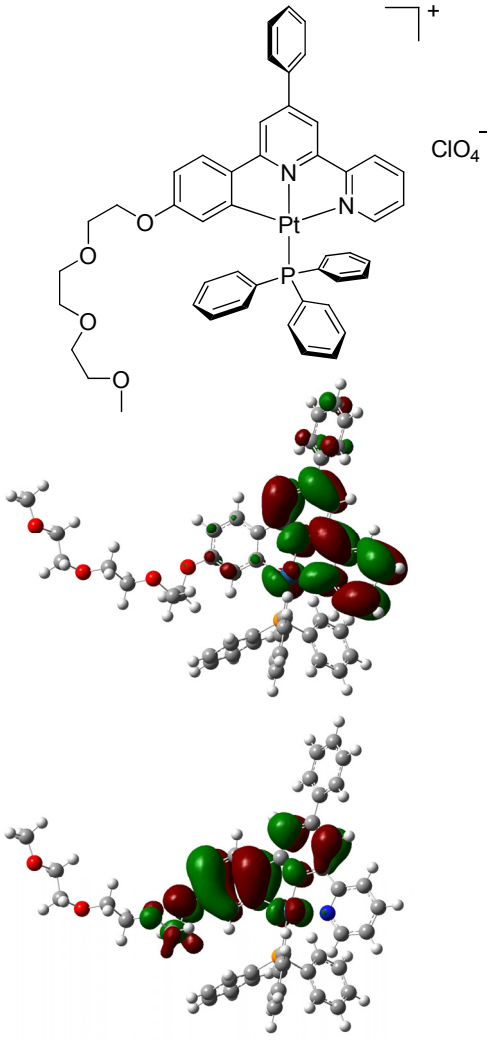


Fig. S3 Highest occupied (bottom) and lowest unoccupied (top) molecular orbitals from energy-minimized calculated (Gaussian) structure of **3**.

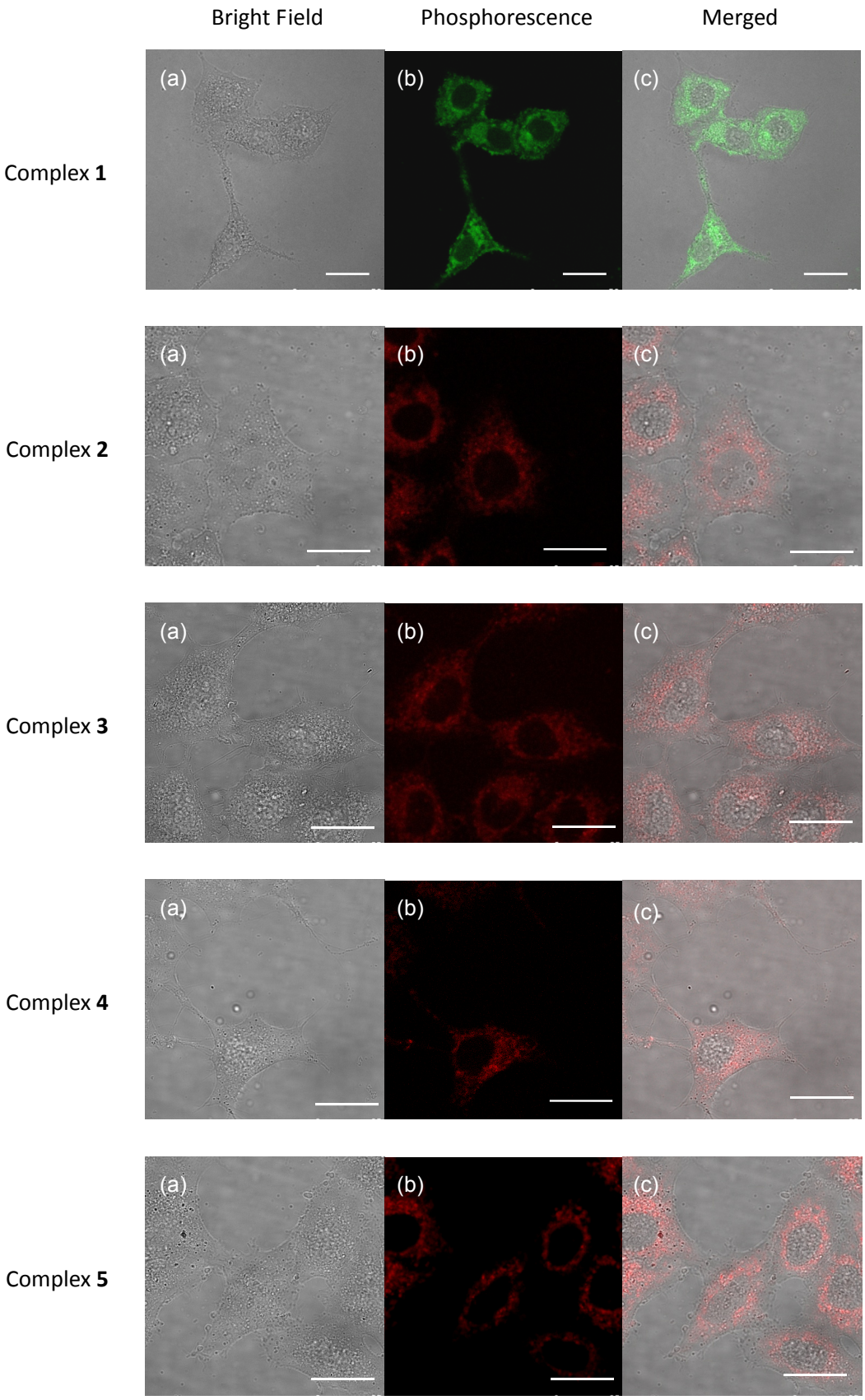


Fig. S4 Confocal microscopy images of Hela cells incubated with **1**–**5** (1 h at 37 ^{o}C): (*a*) bright field; (*b*) phosphorescence (λ_{ex} = 405 nm; λ_{em} = 530–750 nm for **1**, λ_{em} = 600–750 nm for **2**–**5**); (*c*) merged images. Scale bar = 25.0 μm.

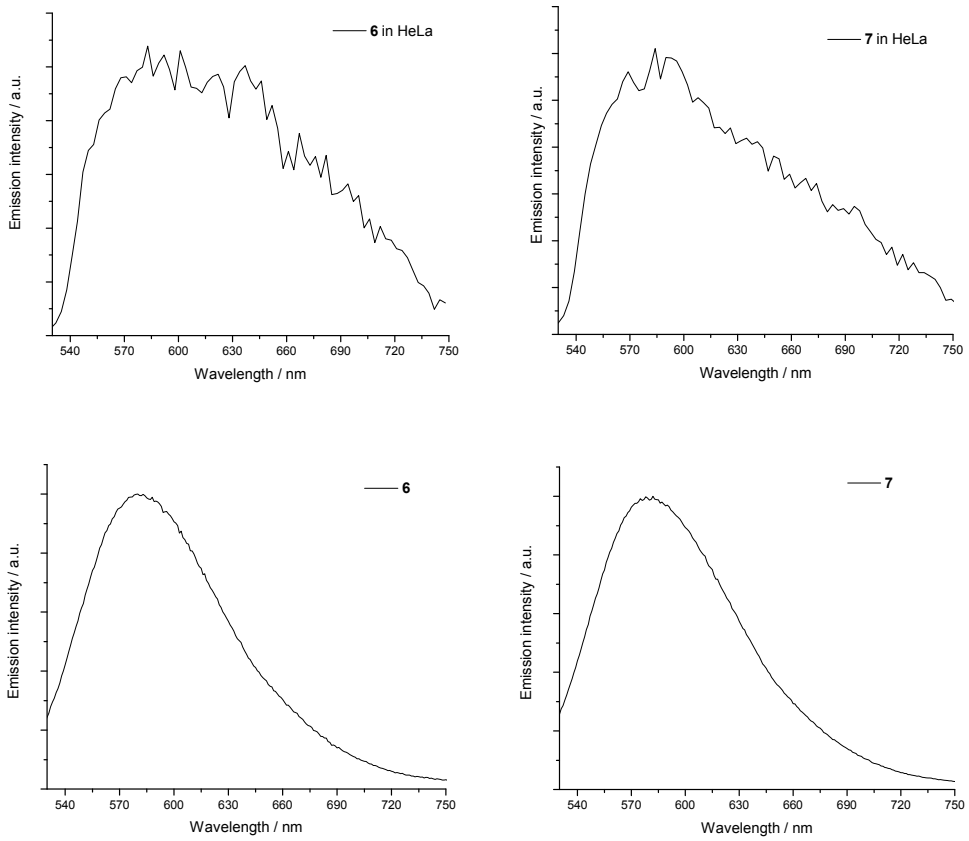


Fig. S5 Top: Emission spectra of HeLa cells upon incubation (5 μM at 37 ^{o}C for 1 h) with **6** (left) and **7** (right). Bottom: Emission spectra of **6** (left) and **7** (right) in CH_3CN at 298 K.

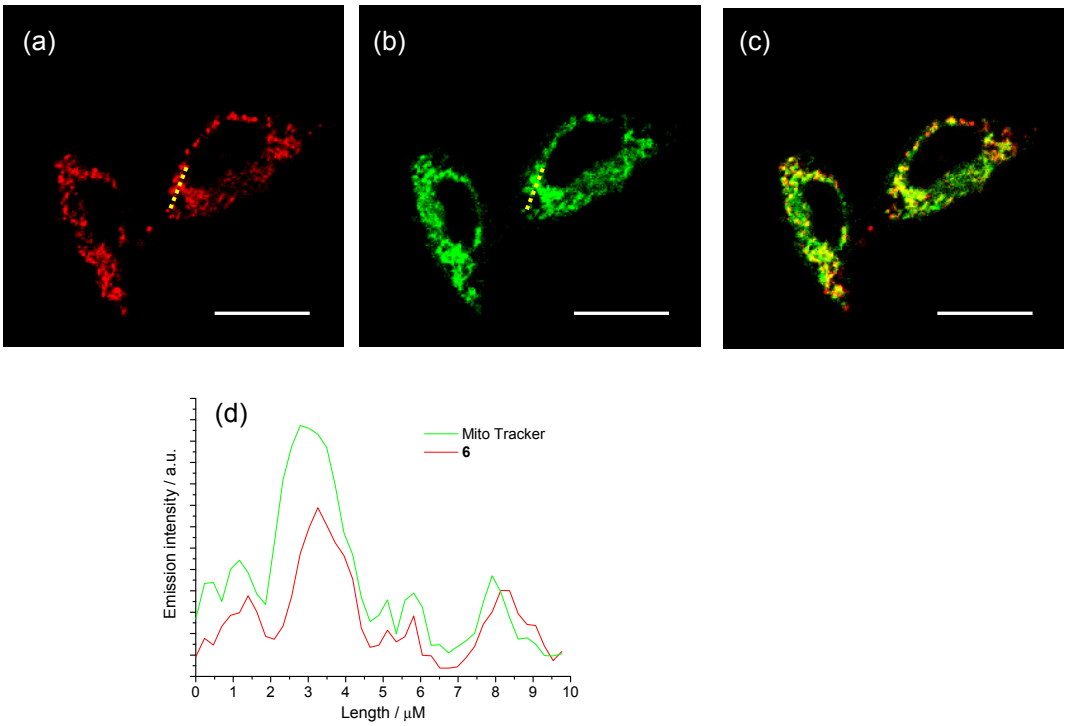


Fig. S6 Confocal microscopy images of Hela cells incubated with (*a*) **6** (2.5 μM, 1 h at 37 °C; λ_{ex} = 405 nm, λ_{em} = 580–750 nm) and (*b*) *MitoTracker Green FM* (25 nM, 20 min, λ_{ex} = 488 nm, λ_{em} = 520–560 nm); (*c*) merged images of (*a*) and (*b*). Scale bar = 25.0 μm. (*d*) Intensity profile of regions of interest (ROI; yellow dash) across HeLa cells.

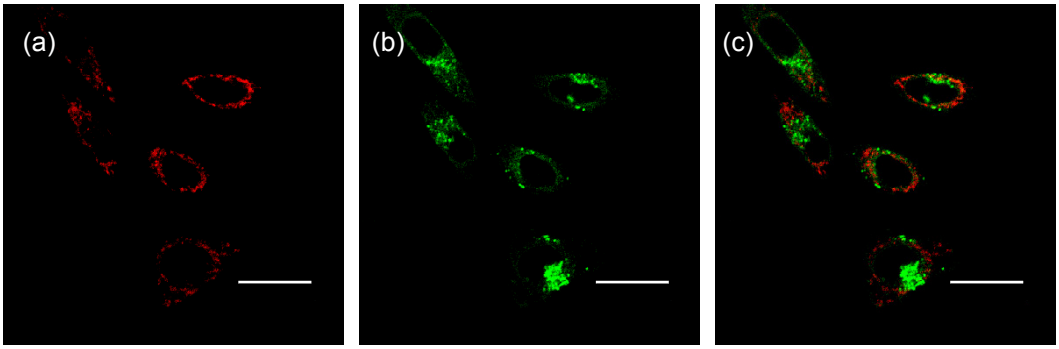
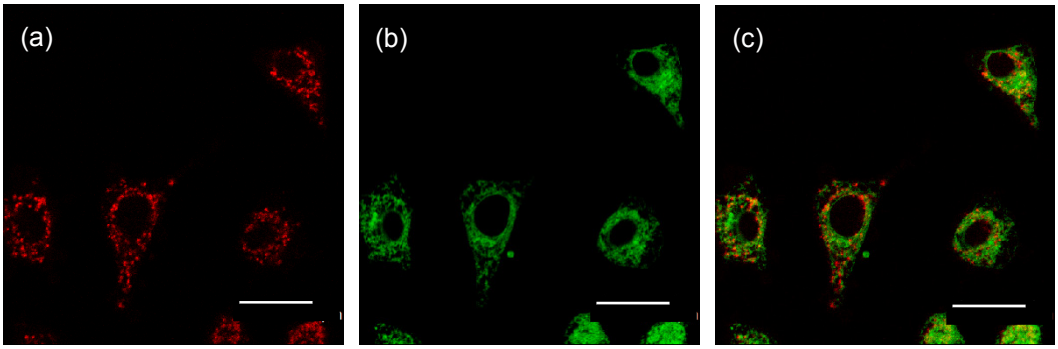
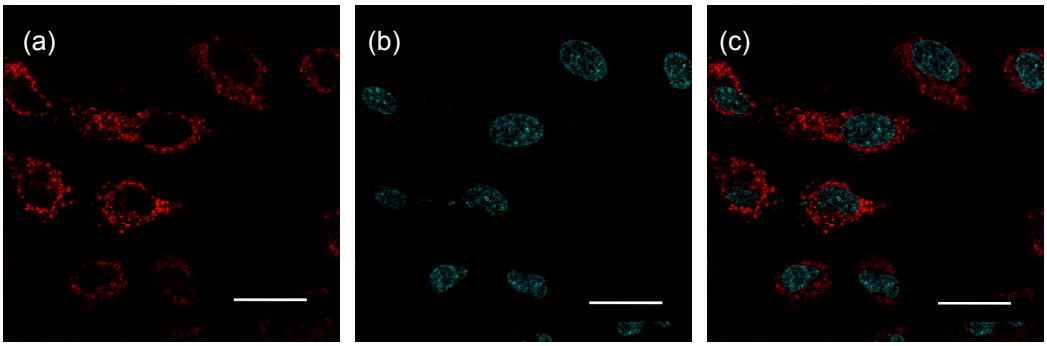


Fig. S7 (*i*) Confocal microscopy images of Hela cells incubated with (*a*) **6** (2.5 μM, 1 h at 37 °C; λ_{ex} = 405 nm, λ_{em} = 580–750 nm) and (*b*) *LysoTracker Green DND-26* (50 nM, 30 min, λ_{ex} = 488 nm, λ_{em} = 520–560 nm); (*c*) merged images of (*a*) and (*b*). Scale bar = 25.0 μm.



(*ii*) Confocal microscopy images of Hela cells incubated with (*a*) **6** (2.5 μM, 1 h at 37 °C; λ_{ex} = 405 nm, λ_{em} = 580–750 nm) and (*b*) *ER Tracker Green* (2 μM, 20 min, λ_{ex} = 488 nm, λ_{em} = 520–560 nm); (*c*) merged images of (*a*) and (*b*). Scale bar = 25.0 μm.



(*iii*) Confocal microscopy images of Hela cells incubated with (*a*) **6** (2.5 μM, 1 h at 37 °C; λ_{ex} = 488 nm, λ_{em} = 600–750 nm) and (*b*) *Hoechst 33342* (0.2 μg/mL, 20 min, λ_{ex} = 405 nm, λ_{em} = 480–500 nm); (*c*) merged images of (*a*) and (*b*). Scale bar = 25.0 μm.

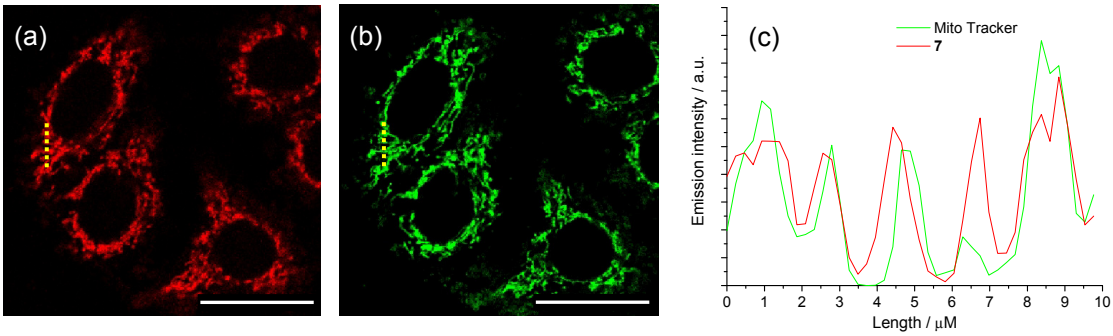


Fig. S8 Confocal microscopy images of Hela cells incubated with (*a*) **7** (2.5 μM, 1 h at 37 °C; λ_{ex} = 405 nm, λ_{em} = 580–750 nm) and (*b*) *MitoTracker Green FM* (25 nM, 20 min, λ_{ex} = 488 nm, λ_{em} = 520–560 nm). Scale bar = 25.0 μm. (*c*) Intensity profile of regions of interest (ROI; yellow dash) across HeLa cells.

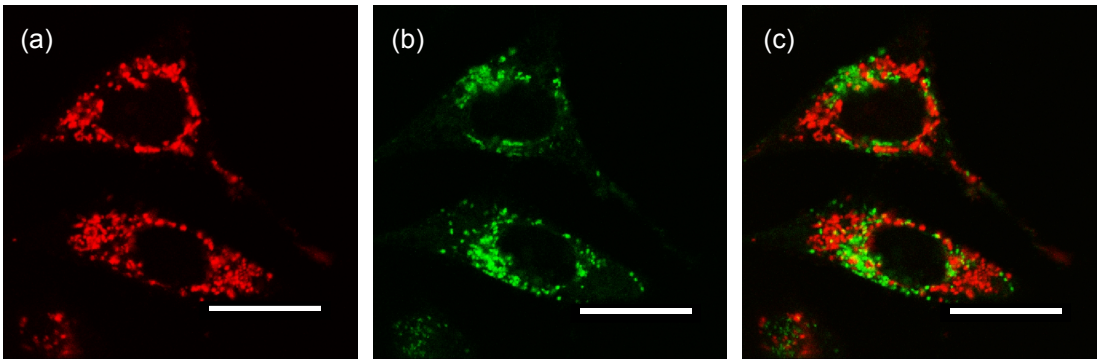
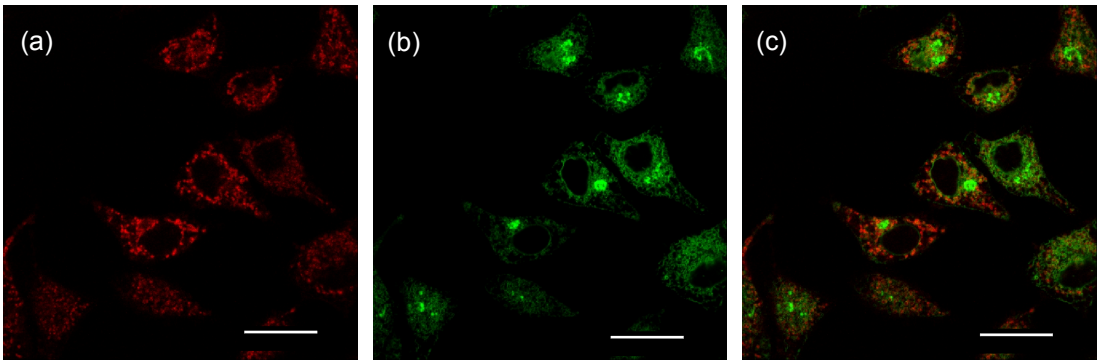
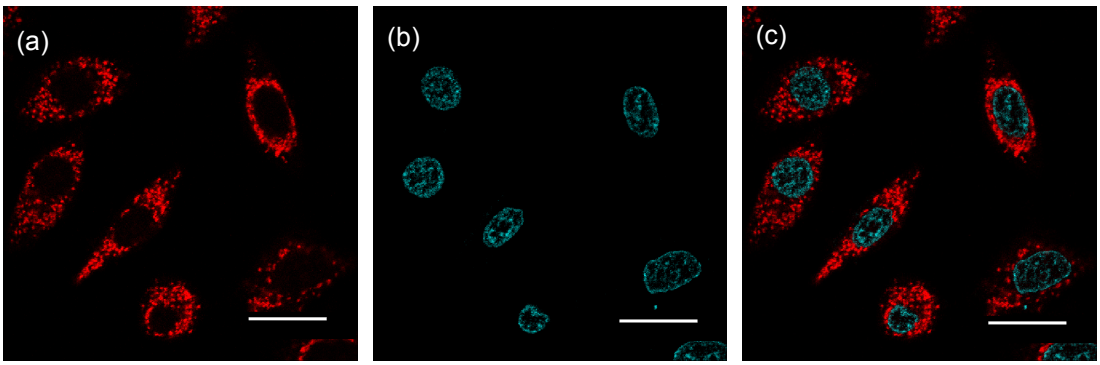


Fig. S9 (*i*) Confocal microscopy images of Hela cells incubated with (*a*) **7** (2.5 μM, 1 h at 37 °C; λ_{ex} = 405 nm, λ_{em} = 580–750 nm) and (*b*) *LysoTracker Green DND-26* (50 nM, 30 min, λ_{ex} = 488 nm, λ_{em} = 520–560 nm); (*c*) merged images of (*a*) and (*b*). Scale bar = 25.0 μm.



(*ii*) Confocal microscopy images of Hela cells incubated with (*a*) **7** (2.5 μM, 1 h at 37 °C; λ_{ex} = 405 nm, λ_{em} = 580–750 nm) and (*b*) *ER Tracker Green* (2 μM, 20 min) λ_{ex} = 488 nm, λ_{em} = 520–560 nm); (*c*) merged images of (*a*) and (*b*). Scale bar = 25.0 μm.



(*iii*) Confocal microscopy images of Hela cells incubated with (*a*) **7** (2.5 μM, 1 h at 37 °C; λ_{ex} = 488 nm, λ_{em} = 600–750 nm) and (*b*) *Hoechst 33342* (0.2 μg/mL, 20 min, λ_{ex} = 405 nm, λ_{em} = 480–500 nm); (*c*) merged images of (*a*) and (*b*). Scale bar = 25.0 μm.

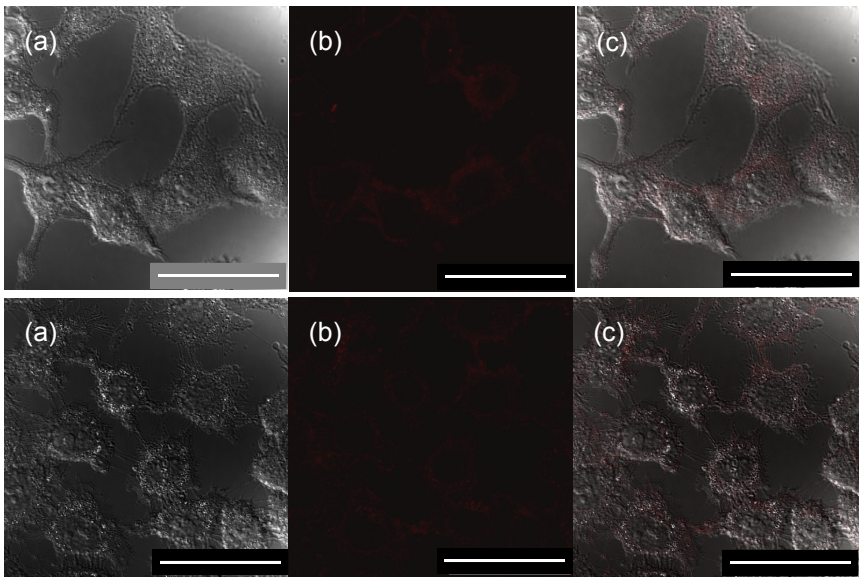


Fig. S10 Emission spectrum of HeLa cell upon incubation with complex **6** (top; 2.5 μM) and **7** (bottom; 2.5 μM) at 4 °C for 1 h: (*a*) bright field; (*b*) phosphorescence (λ_{ex} = 405 nm; λ_{em} = 600–750 nm); (*c*) merged images. Scale bar = 50.0 μM.

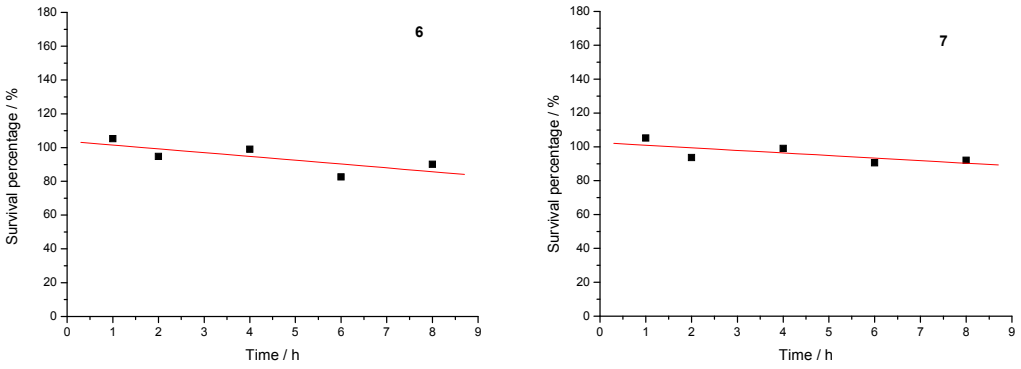


Fig. S11 Time-dependent survival percentage of Hela cells in the presence of **6** (left) and **7** (right) at dose concentration of 2.5 μM.

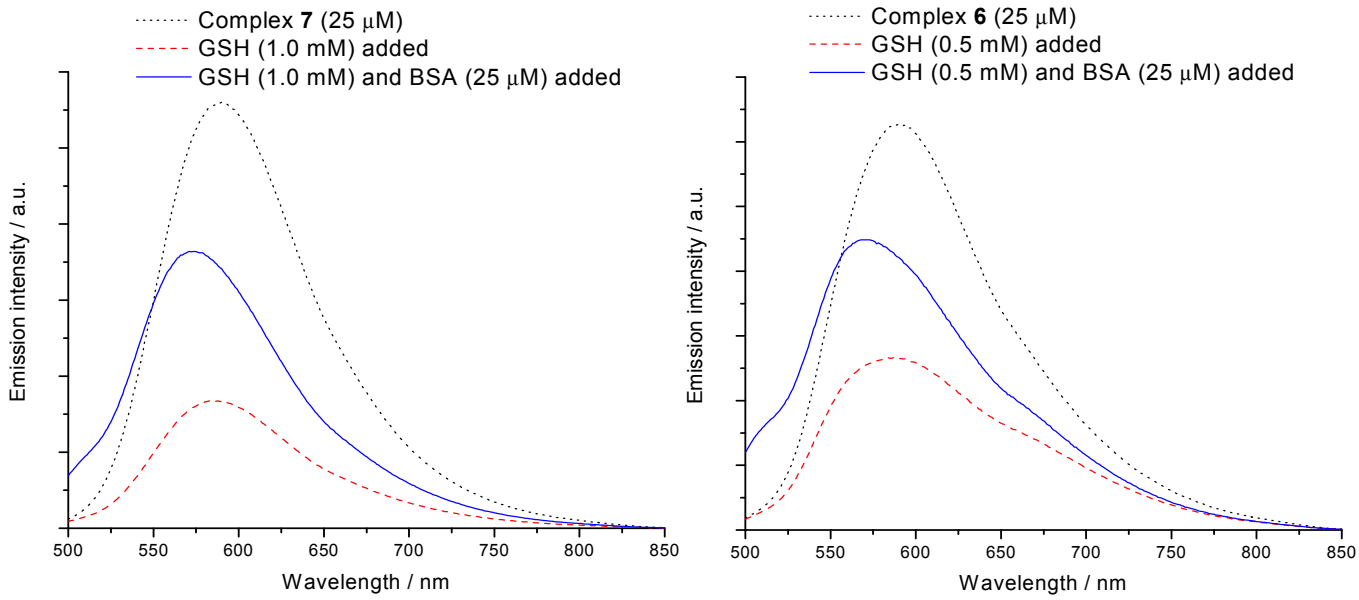


Fig. S12 Emission spectra of **6** and **7** (25 μM, λ_{ex} 380 nm) in PBS/DMSO (9/1), and subsequent spectral changes upon addition of glutathione (GSH) and bovine serum albumin (BSA). For **7** (left), the emission is quenched upon addition of GSH (1.0 mM) to *ca*. 30% of the original intensity (by peak area), but the emission is significantly restored to *ca*. 70% of original peak area (with a minor blue shift) when BSA (25 μM) is subsequently added. For **6** (right), at a lower GSH concentration of 0.5 mM, the emission is quenched to *ca*. 45% of the original peak area, but the emission is significantly restored to *ca*. 80% of original peak area upon subsequent addition of BSA (25 μM).

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