Supplementary material for

Electrochemiluminescent chemodosimetric probes for sulfide based on cyclometalated Ir(III) complexes

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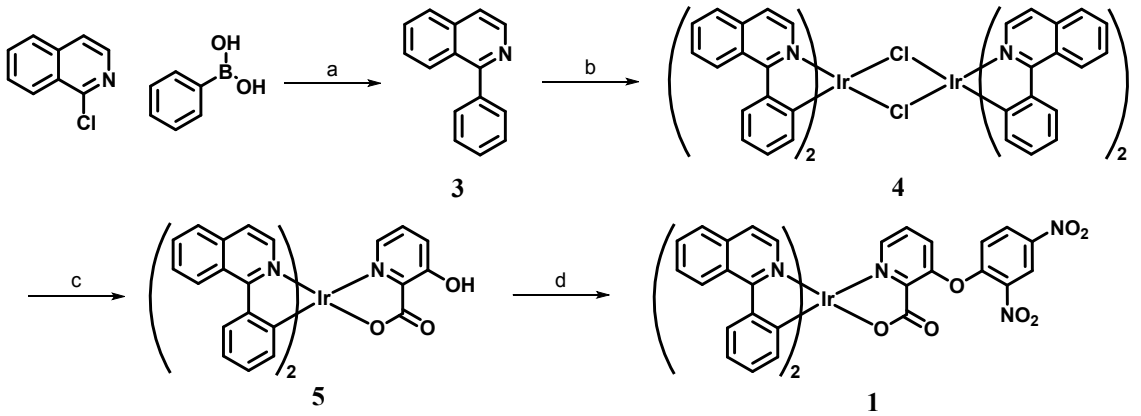
1. Synthesis and characterization

1.1 Instrumentation and materials

All the chemicals were purchased from Sigma-Aldrich (Sigma-Aldrich Corp., MO, USA), TCI (Tokyo Chemical Industry, Tokyo, Japan) or Alfa (Alfa Aesar, MA, USA) and used without further purification. Thin layer chromatography was performed using Merck silica gel 60 F254 on aluminum foil. SiliaFlash® P60 (230-400 mesh) from SILICYCLE was used for stationary phase in chromatographic separation. All the ^{1}H and ^{13}C NMR spectra were obtained from Bruker Advance DPX-300. Chemical shifts (δ) were reported as ppm (in $CDCl_3$ or dimethyl sulfoxide = DMSO). GC-MS (Gas chromatography-mass spectrometry) was performed on JMS 6890 Series, with EI-positive mode. Absorption spectra were measured on a DU 800 Series. Fluorescence emission spectra were obtained on a JASCO FP-6500 spectrometer and the slit width was 5 nm for excitation and emission. The probe **1** and **2** solution for all the photophysical experiments were prepared from 2 mM stock solution in DMSO, diluted with acetonitrile (CH_3CN) and stored in a refrigerator for use. The Na_2S generating sulfide anion was dissolved in 10 % of HEPES in CH_3CN.

1.2 Synthesis of compounds



Scheme S1. a) K_2CO_3, $Pd(PPh_3)_4$, H_2O, THF, reflux; b) $IrCl_3 \cdot xH_2O$, 2-ethoxyethanol, H_2O, reflux; c) 3-hydroxypicolinic acid, Na_2CO_3, 2-ethoxyethanol, 50 °C; d) 1-chloro-2,4-dinitrobenzene, K_2CO_3, DMF, reflux. (THF = tetrahydrofuran, DMF = dimethylformamide)

1.2.1 Synthesis of 3

1-Chloroisoquinoline (1000 mg, 6.1 mmol), phenylboronic acid (968 mg, 7.9 mmol), K_2CO_3 (2533 mg, 18.3 mmol) and $Pd(PPh_3)_4$ (140.98 mg, 0.12 mmol) were dissolved in a mixture of THF (30 mL) and H_2O (30 mL). After stirring overnight at 80°C, the reaction mixture was cooled down to room temperature and extracted with CH_2Cl_2 and water. The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated, and the residue was purified by silica gel column chromatography with hexane/ethyl acetate (5:1 *v/v*) as the eluent to give compound **3** (937 mg, 4.6 mmol, 75 %): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50-7.59 (4H, m), 7.65-7.75 (4H, m), 7.90 (1H, d, *J*=8.2Hz), 8.13 (1H, d, *J*=8.5Hz), 8.64 (1H, d, *J*=5.7Hz).

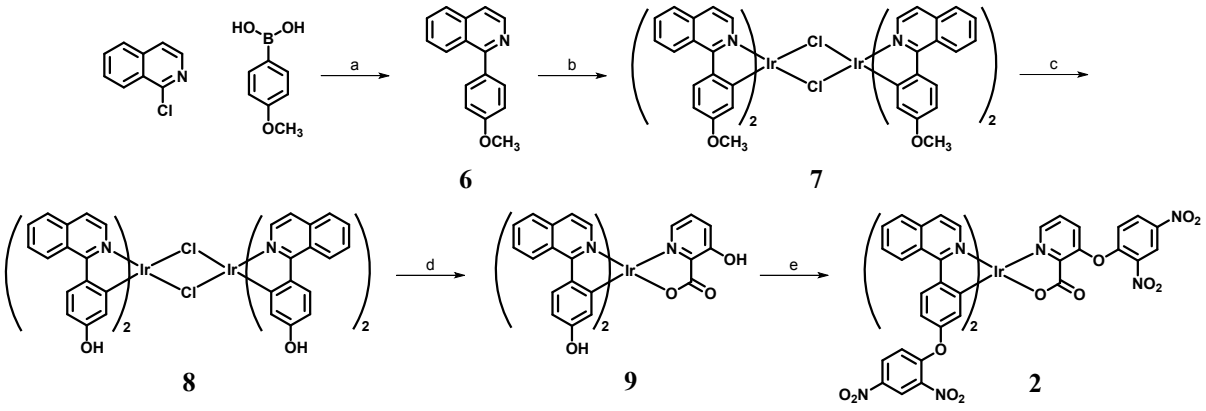
1.2.2 Synthesis of 5

Iridium(Ⅲ) chloride hydrate $IrCl_3 \cdot xH_2O$ (173.2 mg, 0.6 mmol) and compound **3** (298.6 mg, 1.5 mmol) were dissolved in a mixture of 2-ethoxyethanol (15 mL) and H_2O (5 mL). The mixture was heated at reflux for 24 h. The resulting solution was cooled to room temperature, and water (100 mL) added. The red precipitate was filtered, washed with water, and the dried under IR lamp. The dimeric precursor was isolated as a red powder (209 mg, 0.16 mmol, 55 %). Dimer **4** (40.7 mg, 0.03 mmol), 3-hydroxypicolinic acid (21 mg, 0.15 mmol) and Na_2CO_3 (16 mg, 0.15 mmol) were dissolved in 2-ethoxyethanol (3 mL) and heated at 50°C for 45 min. After cooling to room temperature, the solvent was evaporated under reduced pressure. The residue was re-dissolved in CH_2Cl_2 and washed with water. The crude compound was purified by silica gel column chromatography with CH_2Cl_2/methyl alcohol (50:1 *v/v*) as the eluent to afford compound **5** (41 mg, 0.055 mmol, 92 %): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.24 (1H, d, J=7.5Hz), 6.50 (1H, d, J=7.6Hz), 6.73 (1H, t, J=7.3Hz), 6.80 (1H, t, J=7.4Hz), 6.96 (1H, t, J=7.3Hz), 7.03 (1H, t, J=7.4Hz), 7.46 (1H, d, J=6.4Hz), 7.53 (1H, d, J=6.4Hz), 7.73-7.77 (4H, m), 7.88-7.97 (2H, m), 8.21 (1H, d, J=8.0Hz), 8.27 (1H, d, J=8.0Hz), 8.66 (1H, d, J=6.4Hz), 8.96-8.99 (2H, m), 13.8 (1H, s)

1.2.3 Synthesis of 1

Compound **5** (56 mg, 0.076 mmol), 1-chloro-2,4-dinitrobenzene (46 mg, 0.23 mmol) and K_2CO_3 (31 mg, 0.23 mmol) were dissolved in 3 mL of DMF, and the mixture was heated at reflux for 1.5 h. The

reaction mixture was cooled to room temperature, concentrated *in vacuo* to remove all volatiles, diluted with CH_2Cl_2, added H_2O, and extracted with CH_2Cl_2 twice. The combined organic extracts were dried over Na_2SO_4, filtered, and evaporated. The crude compound was purified by silica gel column chromatography with $CHCl_3$/acetone (15:1 *v/v*) as the eluent to give compound **1** (53 mg, 0.059 mmol, 77 %): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.14 (1H, d, J=7.6Hz), 6.56 (1H, d, J=7.5Hz), 6.70 (1H, t, 7.3Hz), 6.76 (1H, d, J=9.2Hz), 6.83 (1H, t, J=7.2Hz), 6.94 (1H, t, J=7.3Hz), 7.06 (1H, t, J=7.2Hz), 7.36 (1H, d, J=6.4Hz), 7.42-7.46 (2H, m), 7.55 (1H, d, J=6.4Hz), 7.68-7.80 (6H, m), 7.91-8.01 (2H, m), 8.18 (1H, d, J=8.0Hz), 8.24 (1H, dd, J=9.2Hz, 2.7Hz), 8.30 (1H, d, J=8.0Hz), 8.68 (1H, d, J=6.4Hz), 8.90 (1H, d, J=2.6Hz), 8.93-9.02 (2H, m). GC-MS (FAB$^+$) [M=$C_{42}H_{26}IrN_5O_7$], calculated 905.1462, found 905.1464.



Scheme S2. a) $Pd(PPh_3)_4$, Na_2CO_3, toluene, H_2O, ethyl alcohol, reflux; b) $IrCl_3{\cdot}xH_2O$, 2-ethoxyethanol, H_2O, reflux; c) BBr_3, DCM, 0 °C → r.t; d) 3-hydroxypicolinic acid, Na_2CO_3, 2-ethoxyethanol, 50 °C; e) 1-chloro-2,4-dinitrobenzene, K_2CO_3, DMF, reflux. (DCM = dichloromethane)

1.2.4 Synthesis of 6

A two-neck round bottom flask was charged with a 1-chloroisoquinoline (200 mg, 1.2 mmol), 4-methoxyphenylboronic acid (241 mg, 1.6 mmol), Na_2CO_3 (905 mg, 8.5 mmol) and $Pd(PPh_3)_4$ (42.3 mg, 0.04 mmol). Toluene (5 mL), H_2O (5 mL), and ethyl alcohol (1 mL) were sequentially added. The reaction mixture was refluxed for 8 h, and then cooled to room temperature. To the reaction mixture was added aqueous NH_4Cl (10 mL), extracted by ethyl acetate for three times, dried over anhydrous Na_2SO_4, filtered, and evaporated in vacuum to afford the crude product. The

crude product was purified by silica gel column chromatography with hexane/ethyl acetate (5:1 *v/v*) as the eluent to give compound **6** (234 mg, 1 mmol, 82 %): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.93 (1H, s), 7.10 (1H, d, *J*=8.6Hz), 7.56 (1H, t, *J*=7.1Hz), 7.64 (1H, d, *J*=5.7Hz), 7.68-7.74 (3H, m), 7.90 (1H, d, *J*= 8.2Hz), 8.17 (1H, d, *J*=8.5Hz), 8.61 (1H, d, *J*=5.7Hz).

1.2.5 Synthesis of 9

Iridium(III) chloride hydrate $IrCl_3 \cdot xH_2O$ (119 mg, 0.4 mmol) and compound **6** (234 mg, 1 mmol) were dissolved in a mixture of 2-ethoxyethanol (9 mL) and H_2O (3 mL). The mixture was heated at reflux for 24 h. The resulting solution was cooled to room temperature, and water (100 mL) added. The precipitate was filtered, washed with water, and dried under IR lamp. The crude product **7** was used for the next step without further purification. To a stirred solution of **7** (633 mg, 0.45 mmol) in DCM (7 mL) at 0 °C, BBr_3 (2.7 mL, 1.0 M solution in CH_2Cl_2) was added dropwise via syringe. The reaction mixture was stirred at room temperature overnight. The reaction was quenched slowly with MeOH (10 mL), diluted with CH_2Cl_2, and neutralized with aqueous saturated $NaHCO_3$ solution. All volatiles were removed under vacuum, and then the residue was re-dissolved in CH_2Cl_2 and H_2O (100 mL) added with stirring. The precipitate was collected by filtration and used without further purification. The formation of compound **8** was determined by ^{1}H NMR in DMSO-d_6, which showed disappearance of a singlet peak of methoxy hydrogens (δ 3.93, 3H, s). Compound **8** (609 mg, 0.5 mmol), 3-hydroxypicolinic acid (200.5 mg, 1.4 mmol) and Na_2CO_3 (152.6 mg, 1.4 mmol) were dissolved in 2-ethoxyethanol (15 mL) and heated at 50°C for 1.5 h. After cooling to room temperature, the solvent was evaporated under reduced pressure. The residue was re-dissolved in CH_2Cl_2 and washed with water. The crude compound was purified by silica gel column chromatography with CH_2Cl_2/methyl alcohol (50:1 *v/v*) as the eluent to afford compound **9** (177 mg, 0.23 mmol, 10 %): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.67 (1H, d, J=2.5Hz), 5.92 (1H, d, J=2.6Hz), 6.50 (1H, dd, J=8.7Hz, 2.6Hz), 6.58 (1H, dd, J=8.7Hz, 2.6Hz), 7.16-7.20 (2H, m), 7.24 (1H, d, J=6.5Hz), 7.33-7.40 (2H, m), 7.44 (1H, d, J=6.4), 7.71-7.75 (4H, m), 7.85-7.88 (1H, m), 7.90-7.93 (1H, m), 8.14 (1H, d, J=8.8Hz), 8.20 (1H, d, J=8.8Hz), 8.56 (1H, d, J=6.4Hz), 8.88-8.90 (2H, m), 13.78 (1H, s)

1.2.6 Synthesis of 2

Compound **9** (177 mg, 0.23 mmol), 1-chloro-2,4-dinitrobenzene (140 mg, 0.7 mmol) and K_2CO_3 (95.4 mg, 0.7 mmol) were dissolved in 10 mL of DMF, and the mixture was heated at reflux for 1.5 h. The reaction mixture was cooled at room temperature, concentrated *in vacuo* to remove all volatiles, diluted with CH_2Cl_2, added H_2O, and extracted with CH_2Cl_2 twice. The combined organic extracts were dried over Na_2SO_4, filtered, and evaporated. The crude compound was purified by silica gel column chromatography with hexane/ethyl acetate (2:1 *v/v* to only ethyl acetate) as the eluent to give compound **2** (56 mg, 0.04 mmol, 19 %): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.76 (1H, dd, J=8.7Hz, 2.5Hz), 6.80 (1H, d, J=9.2Hz), 6.88 (1H, dd, J=8.7Hz, 2.5Hz), 6.95 (1H, d, J=9.2Hz), 7.04 (1H, d, J=9.2Hz), 7.34 (2H, s), 7.44 (1H, d, J=6.4Hz), 7.53-7.58 (1H, m), 7.69-7.86 (9H, m), 8.04 (1H, dd, J=9.2Hz, 2.7Hz), 8.13 (1H, dd, J=9.2Hz, 2.7Hz), 8.18 (1H, d, J=8.8Hz), 8.25 (1H, dd, J=9.1Hz, 2.7Hz), 8.34 (1H, d, J=8.8Hz), 8.50 (1H, d, J=2.7Hz), 8.53 (1H, d, J=6.4Hz), 8.63 (1H, d, J=2.7Hz), 8.72-8.75 (1H, m), 8.85 (1H, d, J=2.5Hz). GC-MS (FAB$^+$) [M = $C_{54}H_{31}IrN_9O_{17}$], calculated 1270.1467, found 1270.1472.

2. Electrochemical and electrochemiluminescent (ECL) measurements

Electrochemical study was performed with a CH Instruments 660 Electrochemical Analyzer (CH Instruments, Inc., TX, USA). In the electrochemical study, cyclic voltammetry (CV) was applied to individual solutions in order to investigate electrochemical oxidative and reductive behaviors. Particularly, a CH Instruments 650B Electrochemical Analyzer was used in ECL experiments to apply potential sweeps. The ECL intensity profile was obtained using a low-voltage photomultiplier tube module (H-6780, Hamamatsu photonics K.K., Tokyo, Japan) operated at 1.0 V. A 25 μL volume ECL cell was directly mounted PMT module with home-made mounting support during the experiments. All the ECL data were collected by the simultaneous cyclic voltammetry in the solution. The ECL solutions commonly contained 10 mM TPA (tripropylamine, Sigma-Aldrich, MO, USA) coreactant and 0.1 M tetrabutylammonium hexafluorophosphate ($TBAPF_6$, TCI) supporting electrolyte in acetonitrile (CH_3CN, spectroscopy grade, ACROS). Especially, TPA was selected and used as an ECL coreactant as it has been widely studied and known on its electrochemical properties. All the electrochemical and ECL experiments were referenced with respect to an Ag/Ag^+ reference electrode. All potential values were calibrated against the saturated calomel electrode (SCE) by measuring the oxidation potential of 1 mM ferrocene (vs Ag/Ag^+) as a standard ($E^{o'}_{ox}(Fc/Fc^+)$ = 0.424 V vs SCE). Cyclic voltammetry for ECL experiments was applied to the solutions at the scan rate of 0.1 V/s. The electrochemical and ECL solutions were freshly prepared in each experiment, and Pt working electrode was polished with 0.05 M alumina (Buehler, IL, USA) on a felt pad. Then the electrode was blown by ultra-pure N_2 gas for 1 min. A single solution was only used for one experiment, and discarded after collecting data. The reported ECL values were obtained by averaging the values from at least three experiments with a good reliability.

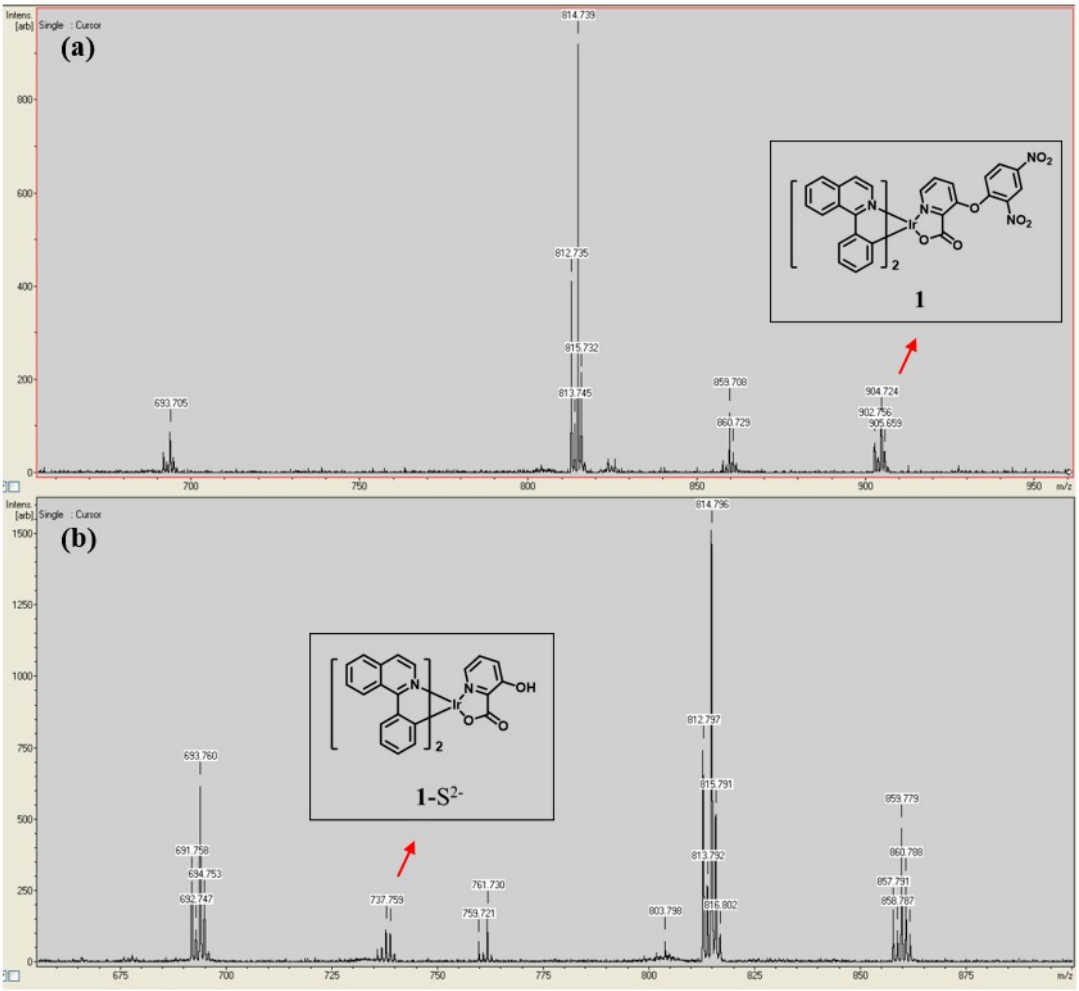


Fig S1. MALDI-TOF mass spectrum of **1** (a) before and (b) after the addition of Na_2S (10 eq).

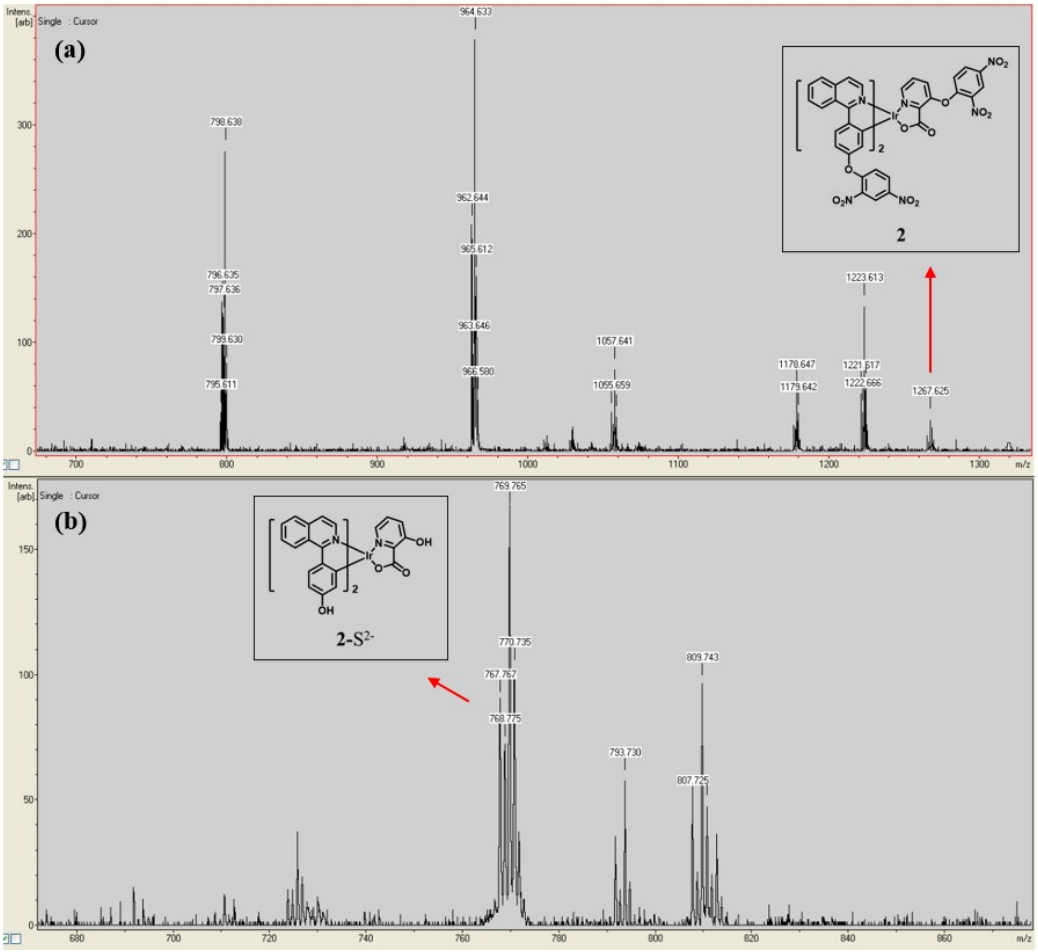


Fig S2. MALDI-TOF mass spectrum of **2** (a) before and (b) after the addition of Na_2S (20 eq).

Table S1. Relative ECL efficiencies of Ir(III) complexes

$Ru(bpy)_3^{2+}$	**1**	**1**-S^{2-}	**2**	**2**-S^{2-}
1[a]	0.77	1.16	0.035	0.13

[a] Taking the ECL efficiency of $Ru(bpy)_3^{2+}$ as 1

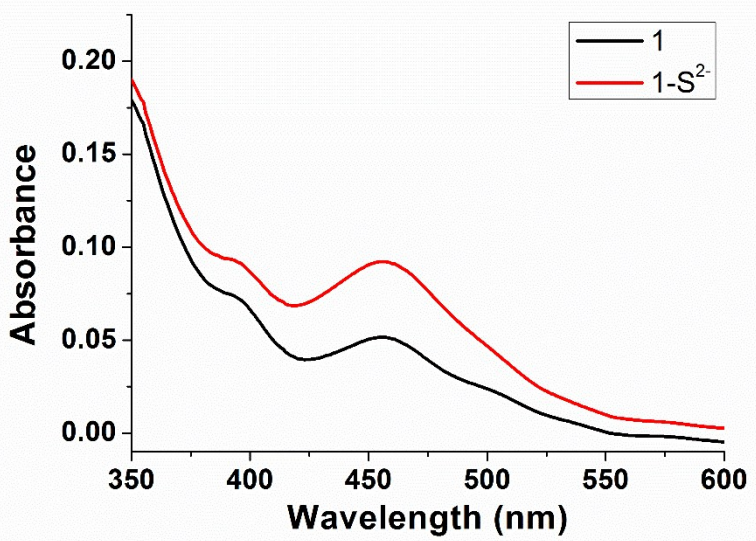


Fig S3. UV-vis absorption of **1** (10 μM) before (black) and after (red) the addition of sulfide (100 μM) in CH_3CN.

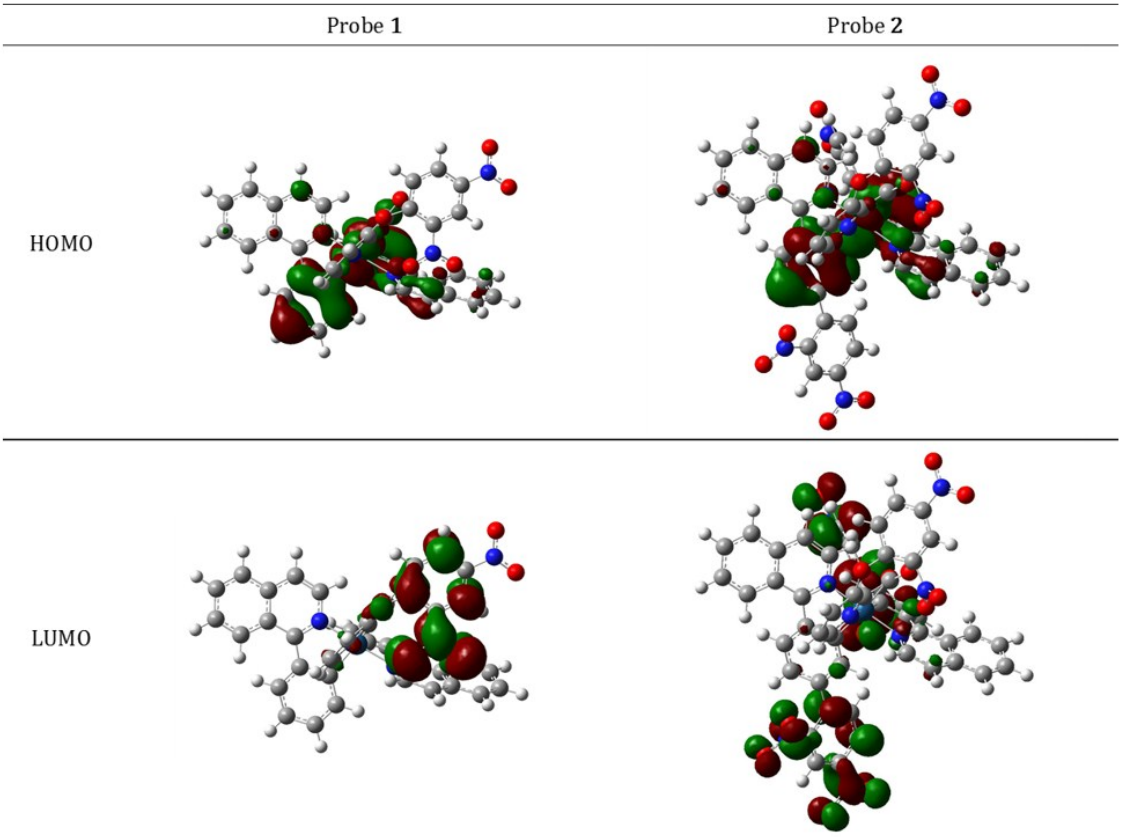


Fig S4. Electronic distributions of probes **1** and **2** from DFT calculations.

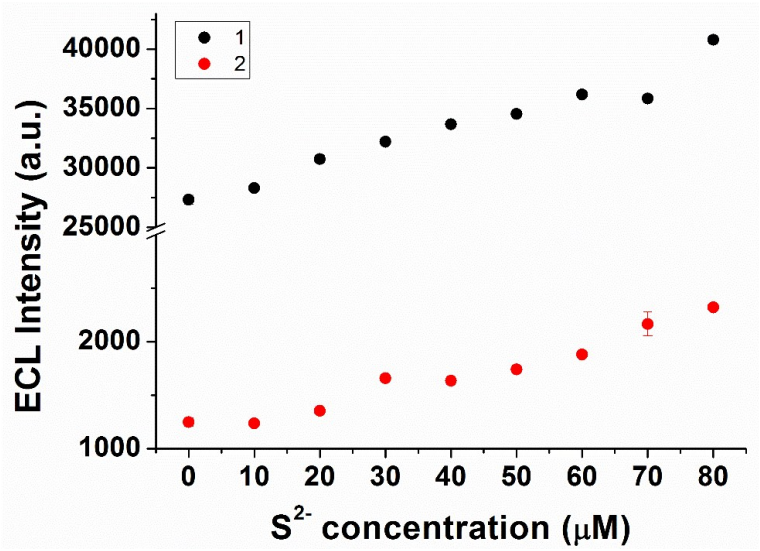


Fig S5. ECL intensity of 10 μM **1** and **2** upon the addition of sulfide in CH_3CN (10 mM TPA, 0.1 M $TBAPF_6$ as the supporting electrolyte, and the potential is swept at a Pt disk electrode (diameter: 2 mm) vs Ag/Ag+, scan rate: 0.1 V/s)

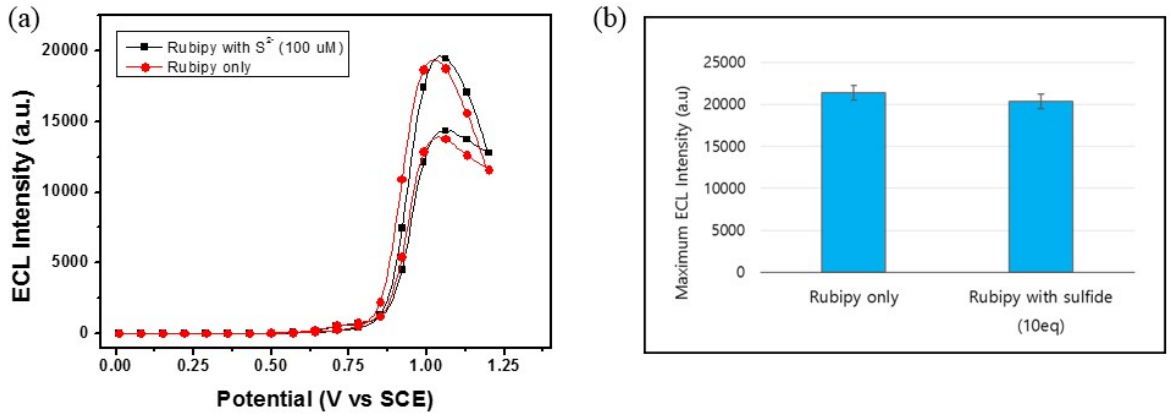


Fig S6. (a) ECL intensity of 10 μM $Ru(bpy)_3^{2+}$ in the absence (red line) and presence of sulfide (100 μM, 10 equiv., black line) in CH_3CN (10 mM TPA, 0.1 M $TBAPF_6$ as the supporting electrolyte, and the potential is swept at a Pt disk electrode (diameter: 2 mm), scan rate: 0.1 V/s). (b) Comparison of average maximum ECL intensity between $Ru(bpy)_3^{2+}$ itself and $Ru(bpy)_3^{2+}$ with excess sulfide (100 μM, 10 equiv.) in CH_3CN (10 mM TPA, 0.1 M $TBAPF_6$ as the supporting electrolyte, and the potential is swept at a Pt disk electrode (diameter: 2 mm), scan rate: 0.1 V/s, n = 5).

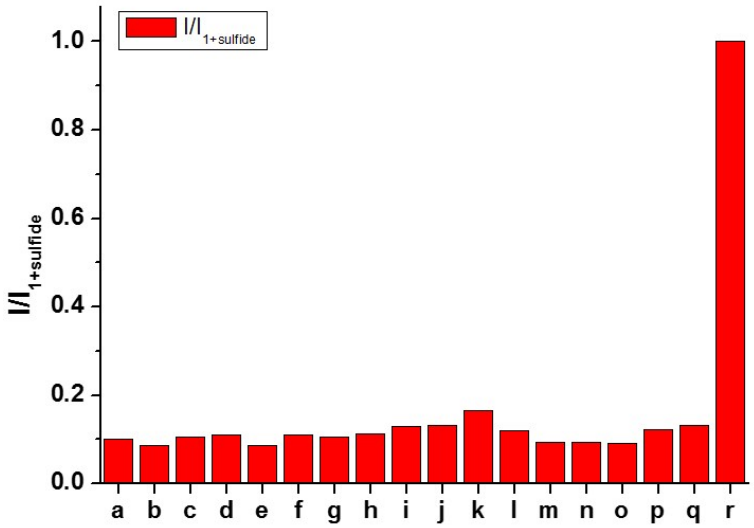


Fig S7. PL responses of **1** (10 μM) in the presence of various analytes (100 μM) in CH_3CN. (25 mM TPA, and 0.1 M $TBAPF_6$ as the supporting electrolyte) (a) probe only, (b) F^-, (c) Cl^-, (d) Br^-, (e) I^-, (f) HCO_3^-, (g) CO_3^{2-}, (h) $C_2O_4^{2-}$, (i) SO_4^{2-}, (j) NO_3^-, (k) N_3^-, (l) AcO^-, (m) SCN^-, (n) CN^-, (o) Cys, (p) Hcy, (q) GSH, (r) S^{2-}.

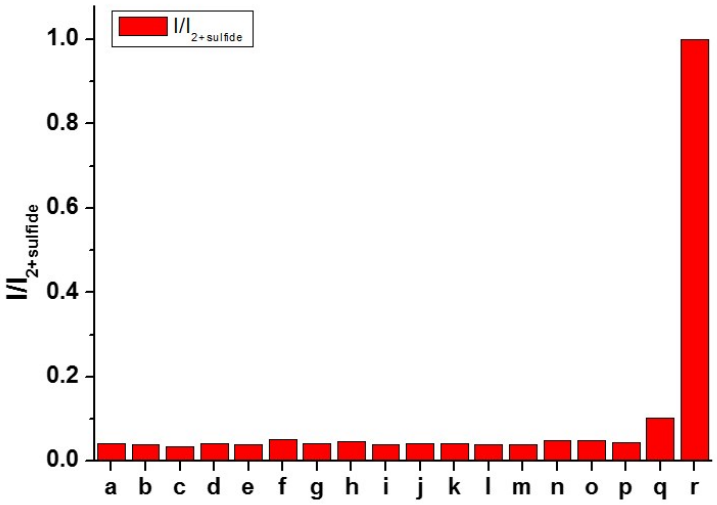


Fig S8. PL responses of **2** (10 μM) in the presence of various analytes (100 μM) in CH_3CN. (25 mM TPA, and 0.1 M $TBAPF_6$ as the supporting electrolyte) (a) probe only, (b) F^-, (c) Cl^-, (d) Br^-, (e) I^-, (f) HCO_3^-, (g) CO_3^{2-}, (h) $C_2O_4^{2-}$, (i) SO_4^{2-}, (j) NO_3^-, (k) N_3^-, (l) AcO^-, (m) SCN^-, (n) CN^-, (o) Cys, (p) Hcy, (q) GSH, (r) S^{2-}.

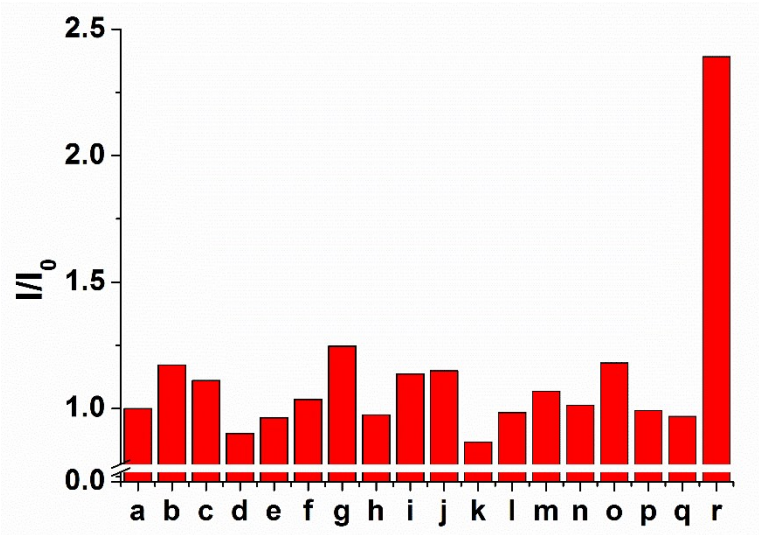


Fig S9. ECL responses of **1** (10 μM) in the presence of various analytes (100 μM) in CH_3CN. (25 mM TPA, and 0.1 M $TBAPF_6$ as the supporting electrolyte) (a) probe only, (b) F^-, (c) Cl^-, (d) Br^-, (e) I^-, (f) HCO_3^-, (g) CO_3^{2-}, (h) $C_2O_4^{2-}$, (i) SO_4^{2-}, (j) NO_3^-, (k) N_3^-, (l) AcO^-, (m) SCN^-, (n) CN^-, (o) Cys, (p) Hcy, (q) GSH, (r) S^{2-}.