Electronic Supplementary Material (ESI) for Dalton Transactions
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Electronic Supplementary Information

A highly sensitive, selective ratiometric fluorescent probe for cobalt (II) and its applications for biological imaging

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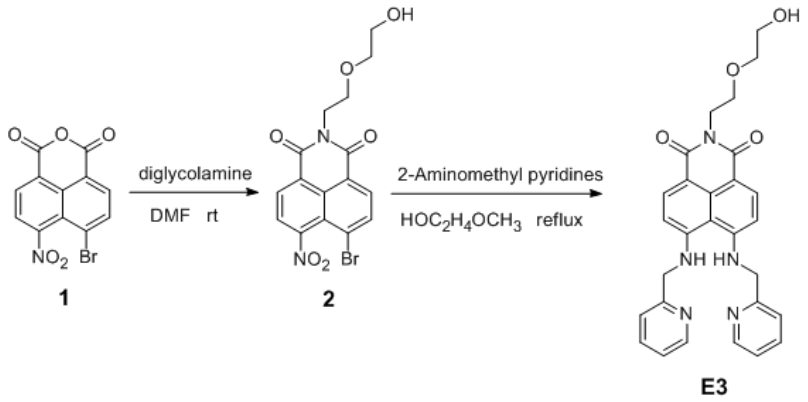
1. Materials and general methods:

N, N'–dimethylformamide (DMF) was distilled from calcium hydride (CaH_2) under anhydrous condition. Other solvents were of analytic grade. All reactions were carried out under a helium atmosphere with analytic grade solvents, unless noted. Mass spectra were measured on a HP 1100 LC-MS spectrometer. Double distilled water was used to prepare all aqueous solutions. All spectroscopic measurements were performed in 50 mM HEPES/EtOH (v/v : 60/40) buffer at pH 7.2. Fluorescence spectra were determined on a VARIAN CARY Eclipse Fluorescence spectrophotometer. Absorption spectra were determined on a VARIAN CARY 100 Bio UV-Visible spectrophotometer. ^{1}H NMR and ^{13}C NMR were measured on a BrukerAV-400 spectrometer with chemical shifts reported in ppm (in $CDCl_3$; TMS as internal standard). All pH measurements were made with a Sartorius basic pH-Meter PB-10.

All reactions were monitored by thin-layer chromatography (TLC) using UV-light (254 nm) and Flu-light (365 nm). Silica gel (300 - 400 mesh) was used for column chromatography.

2. Synthesis:



Scheme 1 Synthesis of fluorescent probe **E3**.

Preparation of 1:

Compounds 1 was prepared according to the reported procedure.[1]

Preparation of 2:

To a solution of compound **1** (500 mg, 1.55 mmol) in EtOH, diglycolamin (163 mg, 1.55 mmol) was added, and the mixture was stirred refluxing for 2 h. Then the mixture cooled to room temperature, and concentrated in vacuo to afford yellow oil crude compounds. The crude product was purified by column chromatography (silica gel: 100 mL, eluent: DCM/MeOH 50/1) to afford yellow powder **2** in 60 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, 20 °C): δ 8.73 (d, J = 8.0 Hz, 1 H), 8.53 (d, J = 8.0 Hz, 1 H), 8.23 (d, J = 8.0 Hz, 1 H), 7.94 (d, J = 8.0 Hz, 1 H), 4.45 (t, J = 5.6 Hz, 5.2 Hz, 2 H), 3.87 (t, J = 5.2 Hz, 5.6 Hz, 2 H), 3.70 – 3.66 (m, 4 H), 2.1 (s, 1H).

Preparation of E3:

To a solution of compound **2** (205 mg, 0.50 mmol) in ethylene glycol monomethyl ether, 2-Aminomethyl pyridine (542 mg, 5.01 mmol) was added, and the mixture was stirred at reflux for 6

[1] Y. F. Xu, F. Lu, Z. C. Xu, T. Y. Cheng and X. H. Qian, *Sci. china ser. B-chem.* 2009, **52**, 771.

h. Then the mixture cooled to room temperature, and concentrated in vacuo to afford yellow oil crude compounds. The crude product was purified by column chromatography (silica gel: 100 mL, eluent: DCM/MeOH 20/1) to afford yellow powder **E3** in 42 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, 20℃): δ 8.44 (d, J = 8.4 Hz, 2 H), 8.31 (d, J = 4.8 Hz, 2 H), 7.73 – 7.67 (m, 4 H), 7.41 (d, J = 7.6 Hz, 2 H), 7.21 – 7.18 (m, 2 H), 6.80 (d, J = 8.8 Hz, 2 H), 4.68 (s, 2 H), 4.66 (s 2 H), 4.42 (t, J = 5.6 Hz, 5.6 Hz, 2 H), 3.86 (t, J = 5.6 Hz, 5.6 Hz, 2H), 3.76 (s, 1 H), 3.72 – 3.68 (m, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$, 20℃): δ 170.74, 169.34, 164.96, 156.01, 152.06, 149.04, 136.84, 134.06, 122.52, 121.86, 111.71, 107.08, 72.23, 68.84, 61.91, 49.15, 39.08. HRMS (ESI$^+$): m/z calcd for: $C_{28}H_{28}N_5O_4^+$: 498.2141, found: $[M+H]^+$ 498.2147.

3. Supplementary data:

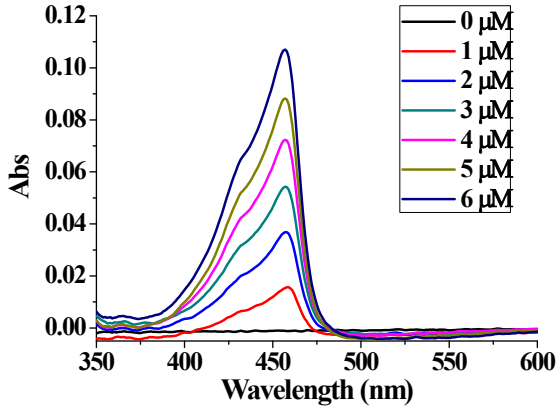


Figure S1. The absorbtion spectra of **E3** with different concentrations in 50 mM HEPES/EtOH (v/v : 60/40) buffer at pH 7.2.

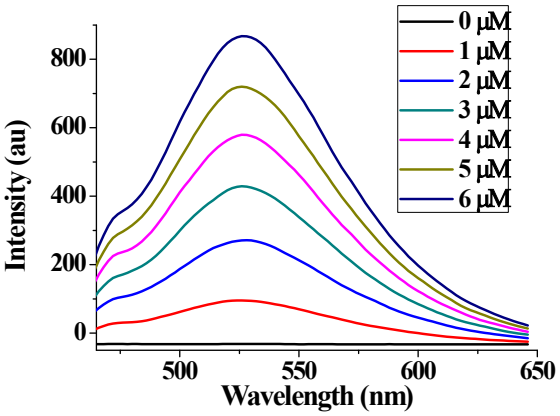


Figure S2. The fluorescence emission spectra of **E3** with different concentrations in 50 mM HEPES/EtOH (v/v : 60/40) buffer at pH 7.2.

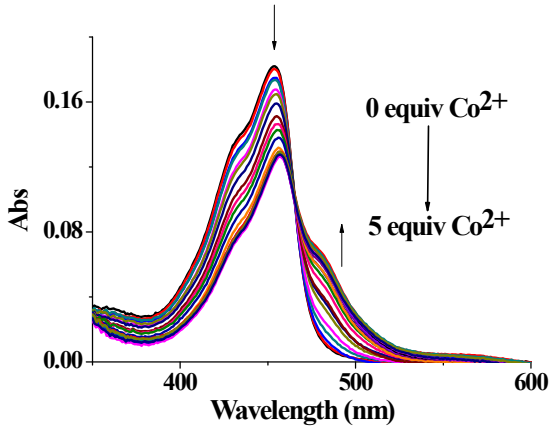


Figure S3. The changes in the absorbtion spectra of **E3** (10×10^{-6} M in 50 mM HEPES/EtOH (v/v : 60/40) buffer, pH 7.2) upon titration with $Co(ClO_4)_2$ from 1.0×10^{-6} M to 50×10^{-6} M.

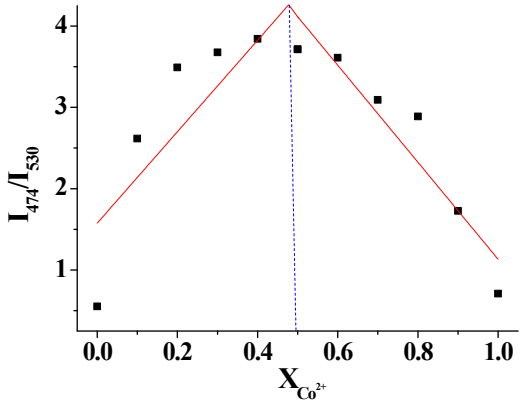


Figure S4. Job plot analysis of **E3** and Co^{2+} in 50 mM HEPES/EtOH (v/v : 60/40) buffer at pH 7.2; The total molar concentration of **E3** and Co^{2+} is 1.0×10^{-5} M.

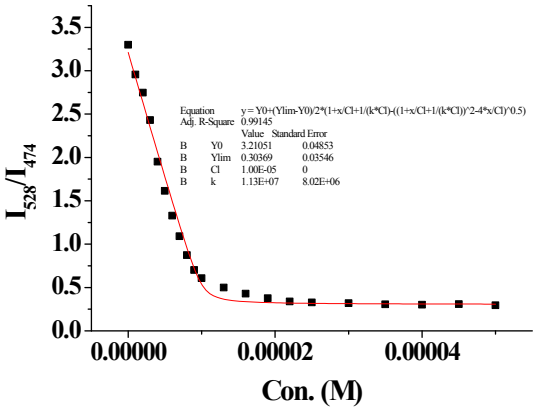
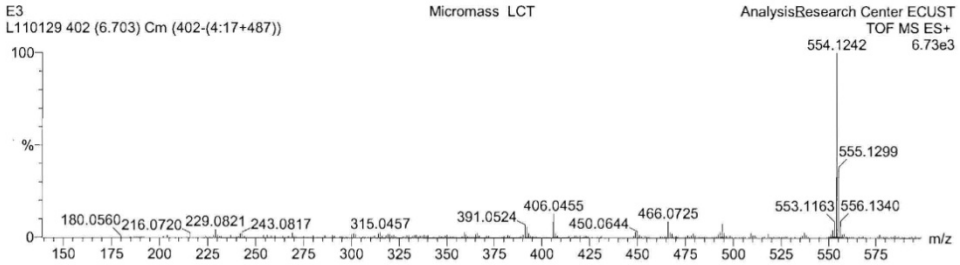


Figure S5. The fitting curve of fluorescence intensity at I_{474} nm/I_{528} nm of **E3** versus increasing concentrations of Co^{2+} in water/EtOH solution (v/v : 60/40, 50 mM HEPES buffer, pH 7.2). The concentration of **E3** was 10×10^{-6} M.

Elemental Composition Report **Page 1**

Multiple Mass Analysis: 2 mass(es) processed
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
20 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)



Minimum:	20.00				-1.5		
Maximum:	100.00		5.0	5.0	100.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	Score	Formula
554.1242	100.00	554.1239	0.3	0.6	19.0	n/a	12C28 1H25 14N5 16O4 59Co
555.1299	38.10	555.1272	2.7	4.9	19.0	n/a	12C27 13C 1H25 14N5 16O4 59Co

Figure S6. The ESI-MS assay on detection of Co^{2+} with probe **E3**

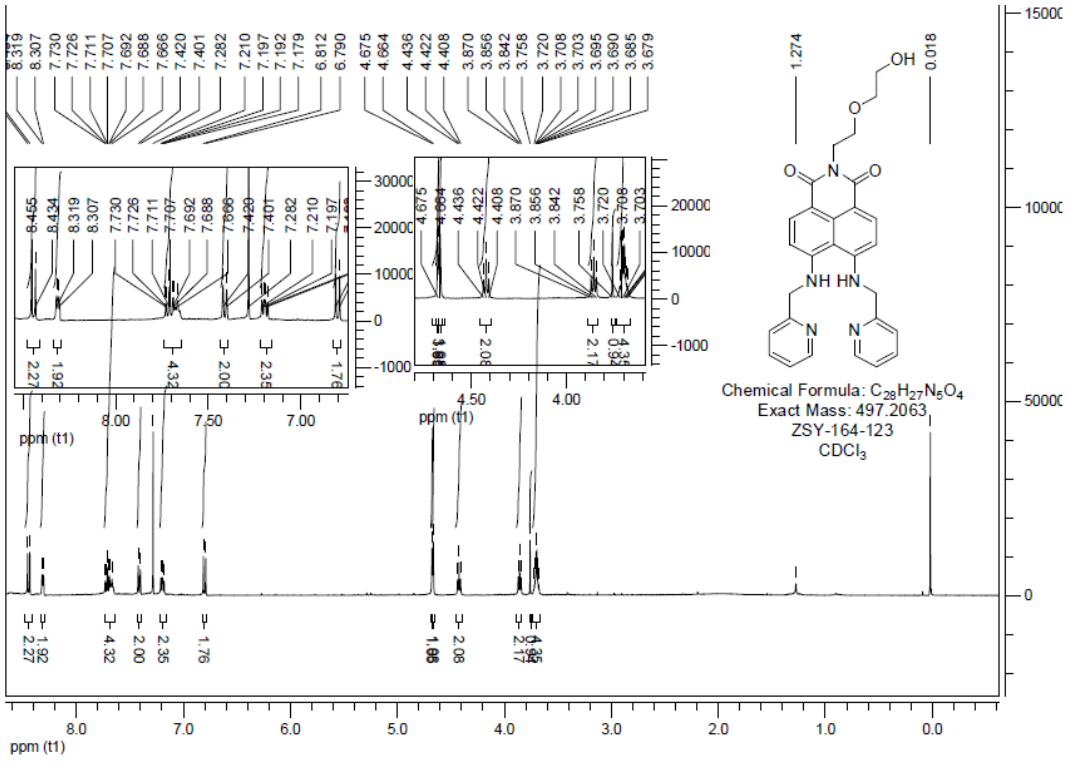


Figure S7. ^{1}H NMR spectrum of **E3**

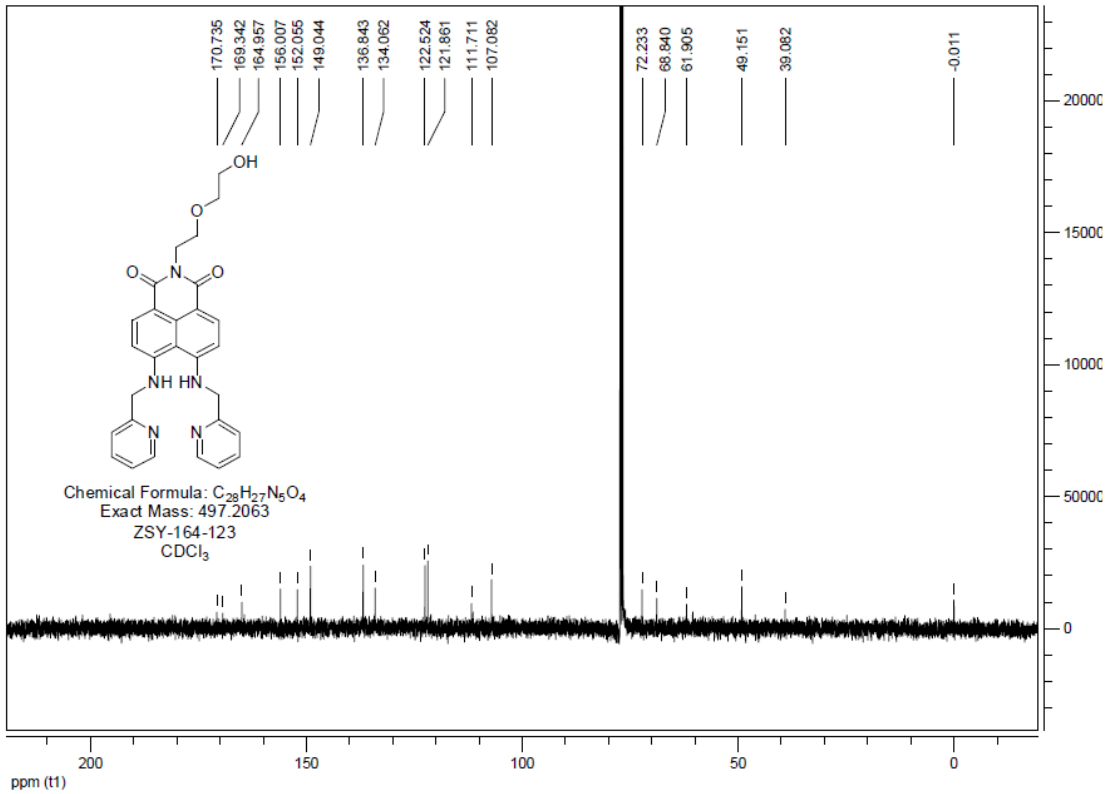


Figure S8. ^{13}C NMR spectrum of **E3**

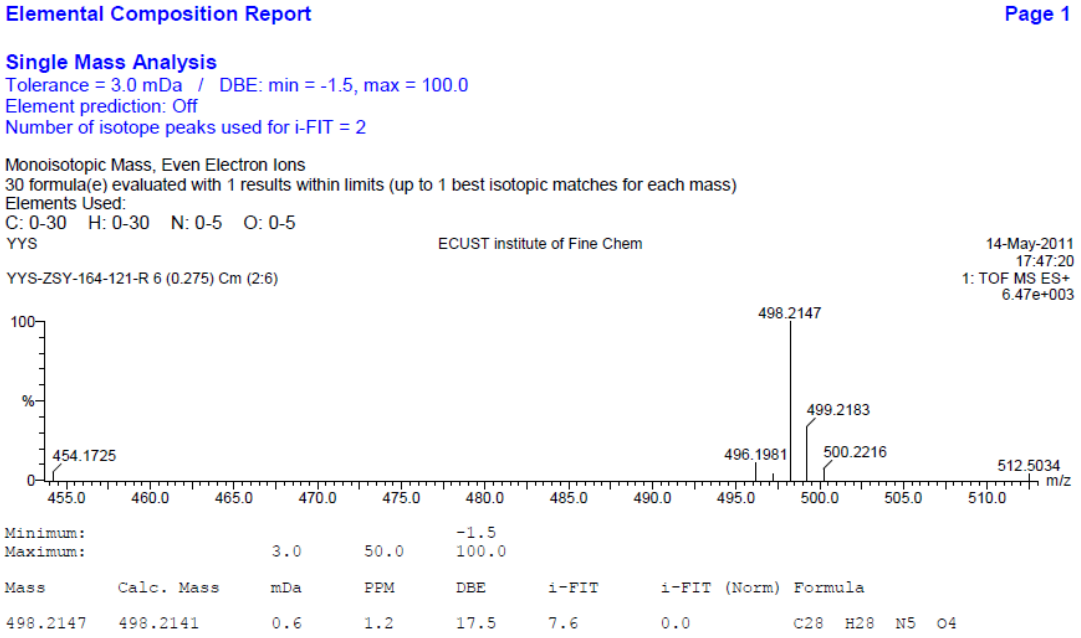


Figure S9. HRMS spectrum of **E3**