Supporting Information

A copper(Ⅱ) rhodamine complex with tripodal ligand as highly selective fluorescence imaging agent for nitric oxide

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Experimental section

Instruments and Materials

All the solvents and reagents were of analytic grade, and used without further purification. Rhodamine B，Tris(2-aminoethyl)amine(Tren) are commercially available (ACROS) and used as received. Compound **RBT** was synthesized according to the reference.[1] The NO aqueous solution was prepared by passing NO gas through a 0.1 M deoxidized phosphate buffer at pH = 7.4 for 3 h.

^{1}H NMR was taken on Varian Inova-400 spectrometer with TMS as an internal standard and $CDCl_3$ as solvent. Mass spectrometric data was obtained with a LCQ-Tof MS spectrometry. And LC-MS was taken by High Performance Liquid Chromatography /Mass selective Detector HP1100. EPR spectra were recoded on EMX-10/12 spectrometer, using a LABOTEC capillary tube. Modulation frequency is 100 kHz. IR spectra were recorded using KBr pellets on a Vector 22 Bruker spectrophotometer in the 4000–400 cm^{-1} regions. Fluorescence spectra were determined with FS920 luminescence spectrometer (Edinburgh Instruments). UV-vis spectra were recorded on a Lambda35 UV-vis spectrophotometer. All pH measurements were made with a Model PHS-3C meter.

Synthesis of [Cu(RBT)Cl] (ClO_4) (CuRBT)

To a methanol solution of **RBT** (57 mg, 0.1 mmol) was added $Cu(ClO_4)_2 \cdot 6H_2O$ (45 mg, 0.12 mmol) and 1 M NaCl (100 μL, 0.1 mmol), and refluxed for 5 h. The resulting mixture was kept at room temperature for a week to obtain blue needlelike crystals, which were filtered, and dried in air. Yield: 65%. Anal. Calc. for $C_{34}H_{46}N_6O_6Cl_2Cu$: H 5.98, C 53.04, N 10.92%. Found: H 5.95, C 53.09, N 10.89%.

Procedures of fluorescence sensing

The stock solution of **CuRBT** (1 mM) was prepared in CH_3CN. The solution of **CuRBT** was then diluted to 10 μM or 100μM with phosphate buffer solution (0.1 M, pH = 7.4) or NO solution. For fluorescence and UV-vis experiments, each time a 2 or 3 mL NO phosphate buffer solution (0.1 M, pH = 7.4) was filled in a quartz optical cell of 1 cm optical path length, and the **RBT** and **CuRBT** solutions were added into the quartz optical cell by using a micro-pippet. Spectral data were recorded from 0 min to 10 min after the addition. In selectivity experiments, all the reactions were carried out with the same **CuRBT** concentration (10 μM) for 2 h at room temperature. Hydrogen peroxide (H_2O_2) was diluted immediately from a stabilized 30% solution. Peroxynitrite was synthesized from sodium nitrite and H_2O_2. After the reaction, the solution was treated with MnO_2 to eliminate the excess H_2O_2.[2] Singlet oxygen was chemically generated from $^-OCl/H_2O_2$ system in buffer. Freshly prepared aqueous solutions of NaClO, $NaNO_2$, and $NaNO_3$ were used as hypochlorite anion (ClO^-), nitrite (NO_2^-), and nitrate (NO_3^-) sources, respectively. For fluorescence measurements, excitation was provided at 510 nm, and emission was collected from 520 nm to 750 nm.

Quantum yield measurement

Fluorescence quantum yield was determined using optically matching solutions of Rhodamine-6G ($\mathbf{\Phi}_f$ = 0.94 in ethanol) as standards at an excitation wavelength of 500 nm and the quantum yield is calculated using equation (1).[3]

$$\phi_{unk} = \phi_{std} \frac{(I_{unk} / A_{unk})}{(I_{std} / A_{std})} \left(\frac{\eta_{unk}}{\eta_{std}} \right)^2 \qquad (1)$$

Where $\mathbf{\Phi}_{unk}$ and $\mathbf{\Phi}_{std}$ are the radiative quantum yields of the sample and standard, $\boldsymbol{I}_{unk}$ and $\boldsymbol{I}_{std}$ are the integrated emission intensities of the corrected spectra for the sample and standard, $\mathbf{A}_{unk}$ and $\mathbf{A}_{std}$ are the absorbance of the sample and standard at the excitation wavelength (500 nm for Rhodamine-6G, 510 nm for **CuRBT**).

Cell incubation and imaging

MCF-7 cells were cultured in 1640 supplemented with 10% FCS (Invitrogen). Cells were seeded in coverglass-bottom dishes. After 12 h, MCF-7 cells were incubated with 40 μM probes (in the culture medium containing 0.5% DMSO) for 30 min at room temperature and then washed with PBS (0.1 M, pH = 7.4) three times. After incubating with NO solution for another 15 min at room temperature, the MCF-7 cells were rinsed with PBS (0.1 M, pH = 7.4) three times again.

Confocal fluorescence imaging of intracellular NO in MCF-7 cells was performed with a Nikon A1R-si laser scanning microscopy and a 20× objective lens. Excitation wavelength of laser was 488 nm. Emission was centered at 585 ± 30 nm (single channel).

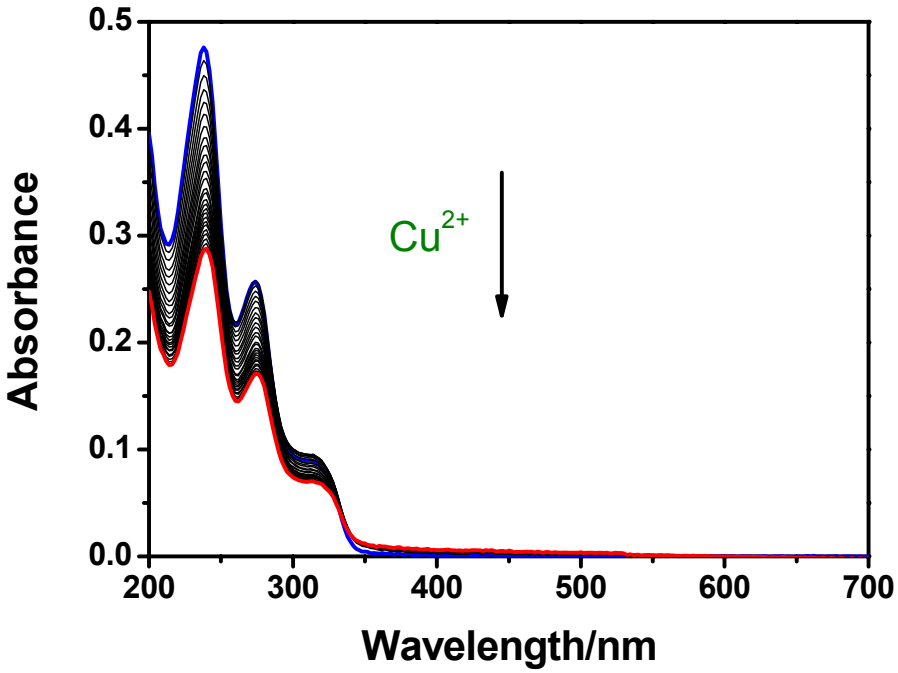
Crystallography

Diffraction data were collected on a Siemens SMART-CCD diffractometer with graphite-monochromated Mo-Kα (λ = 0.71073 Å) using the SMART and SAINT programs. The structures were solved by direct methods and refined on F^2 by full-matrix least-squares methods with SHELXTL version 5.1. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were fixed geometrically at calculated distances and allowed to ride on the parent non-hydrogen atoms with the isotropic displacement being fixed at 1.2 and 1.5 times of the aromatic and methyl carbon atoms they attached, respectively.

Table S1. Crystal data and structure refinement for **CuRBT**

Empirical formula	$C_{34}H_{46}Cl_2CuN_6O_6$
Formula weight	769.21
Crystal size	0.2 x 0.08 x 0.07 mm^3
Temperature (K)	200
Wavelength (Å)	0.71073 Å
Crystal system	Triclinic
Space group	*P*-1
a (Å)	8.3705(6)
b (Å)	12.5806(10)
c (Å)	18.6470(13)
α(deg)	70.858(5)
β (deg)	88.773(5)
γ(deg)	75.729(5)
V ($Å^3$)	1794.0(2)
Z	2
D_c ($g \cdot cm^{-3}$)	1.424
μ (mm^{-1})	0.81
F (000)	806
Theta range for data collection	1.76 to 25.00 deg.
Reflections collected	15408
Independent reflections	6286 (R_{int} = 0.0578)
Completeness to theta = 25.00	99.8 %
Absorption correction	none
Refinement method	Full–matrix least–squares on F^2
Goodness–of–fit on F^2	1.007
Final *R* indices [$I>2\sigma(I)$]	R_1 = 0.0544, wR_2 = 0.1222
R indices (all data)	R_1 = 0.0937, wR_2 = 0.1361
Largest peak and hole ($e \cdot Å^{-3}$)	0.609 and –0.415

$R_1 = \Sigma\,(|F_0|-|F_C|) / \Sigma\,|F_0|$; $wR_2 = [\Sigma\, w\,(|F_0|-|F_C|)^2 / \Sigma\, w\, F_0^2]^{1/2}$.



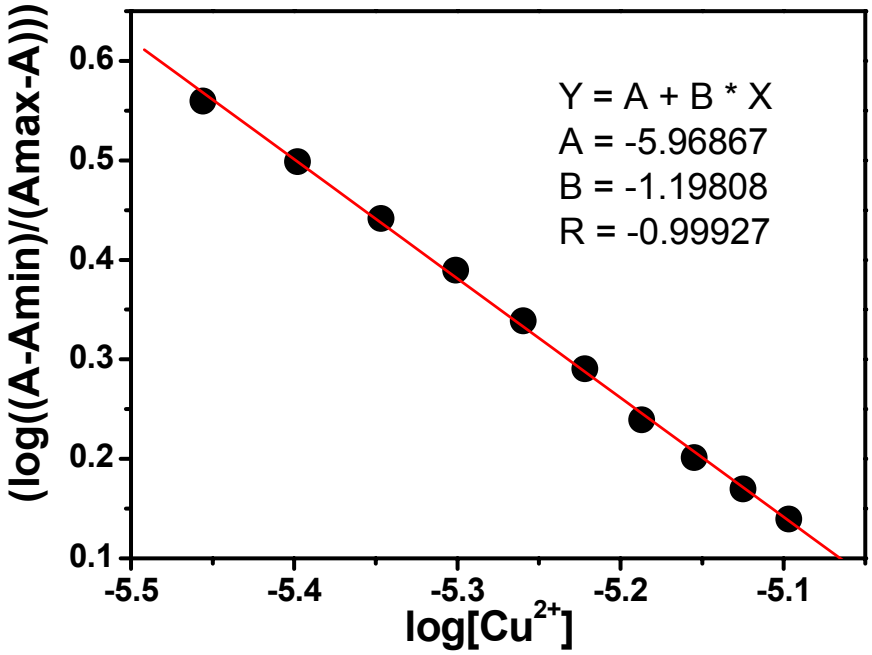


Fig. S1 Absorption spectra of **RBT** (10 μM) for Cu^{2+} ions (0 to 30 μM) in phosphate buffer solution (1 mM, pH = 7.4; NaCl, 100 μM), and the curve of log ((A-Amin)/ (Amax-A)) *vs.* log [Cu^{2+}] (A presents absorption of **RBT** at 238 nm).

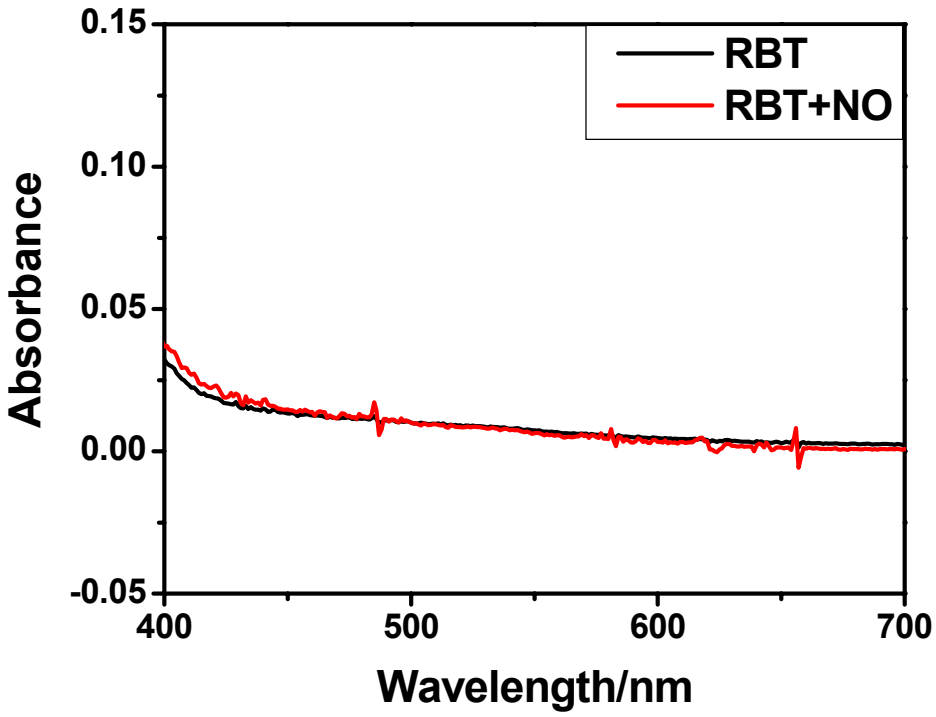


Fig. S2 Absorbance spectra of **RBT** (10 μM) in the absence and presence of NO in 0.1 M phosphate buffer solution (pH = 7.4).

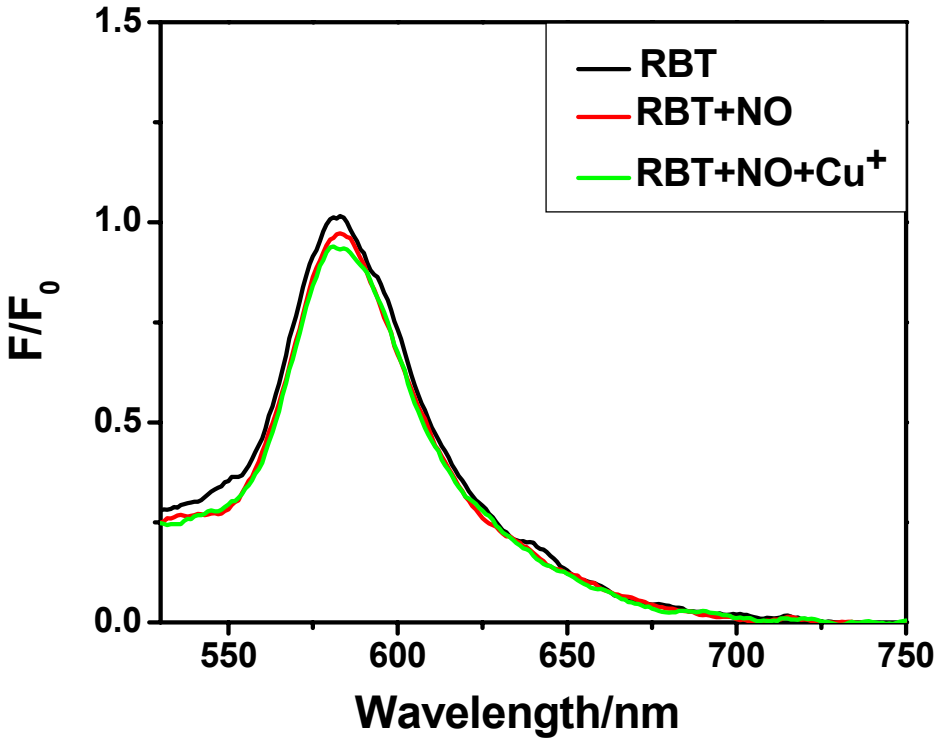


Fig. S3 Fluorescence responses of **RBT** (10 μM) in phosphate buffer solution (0.1 M, pH = 7.4) upon addition of NO, and response of the above solution by further addition of $Cu(CH_3CN)_4(ClO_4)$ (100 μM). Excitation at 510 nm.

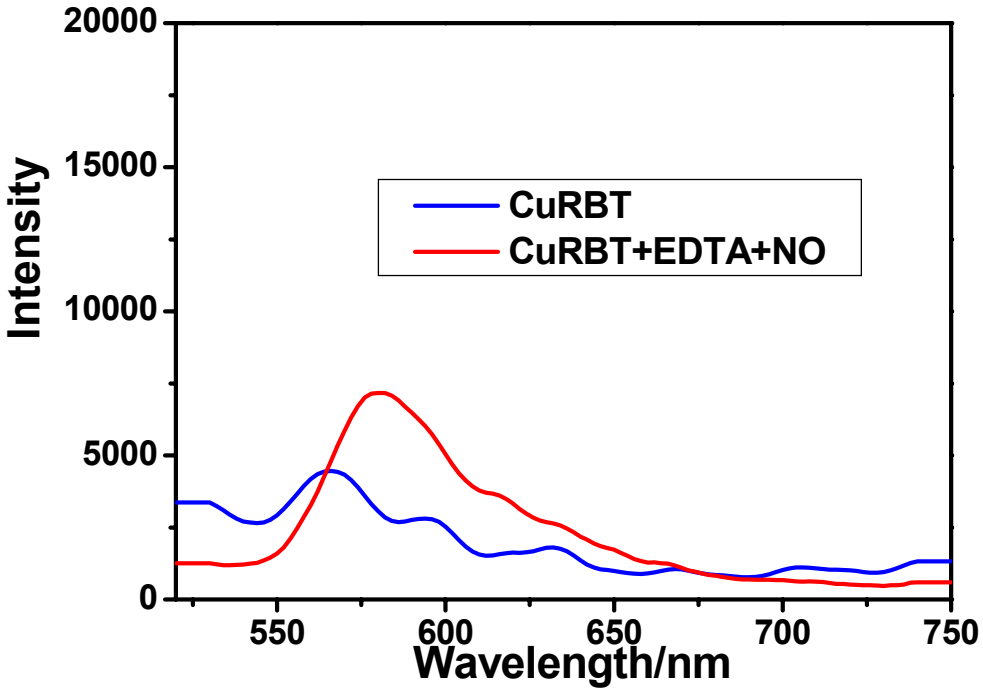


Fig. S4 Fluorescence responses of **CuRBT** (10 μM) in phosphate buffer solution (0.1 M, pH = 7.4), and upon addition of EDTA (100 μM) and NO solution. Excitation at 510 nm.

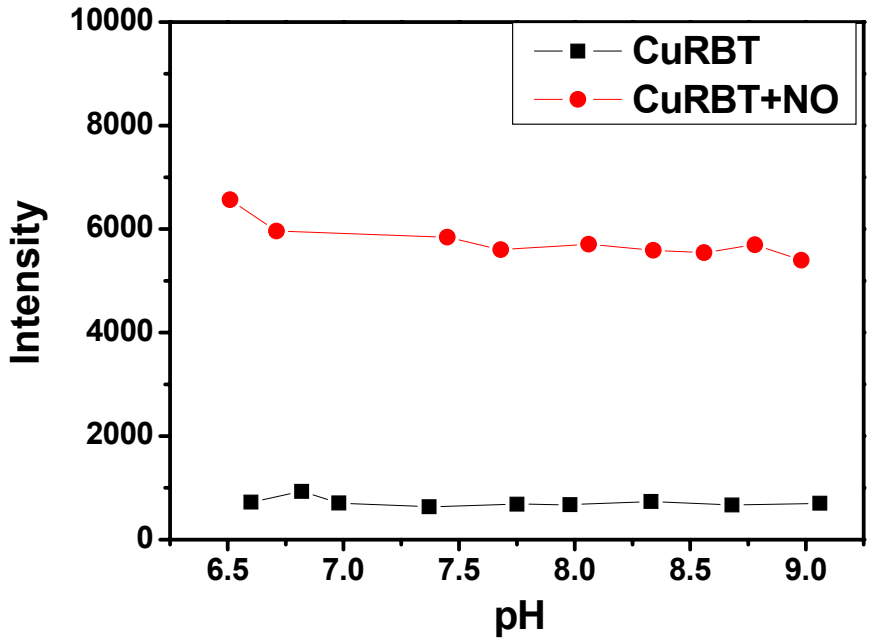


Fig. S5 Black line: The changes of fluorescence intensity of **CuRBT** (20 μM) with different pH values. Red line: The changes of fluorescence intensity of **CuRBT** (20 μM) with different pH values after treated with NO solution. Excitation at 510 nm.

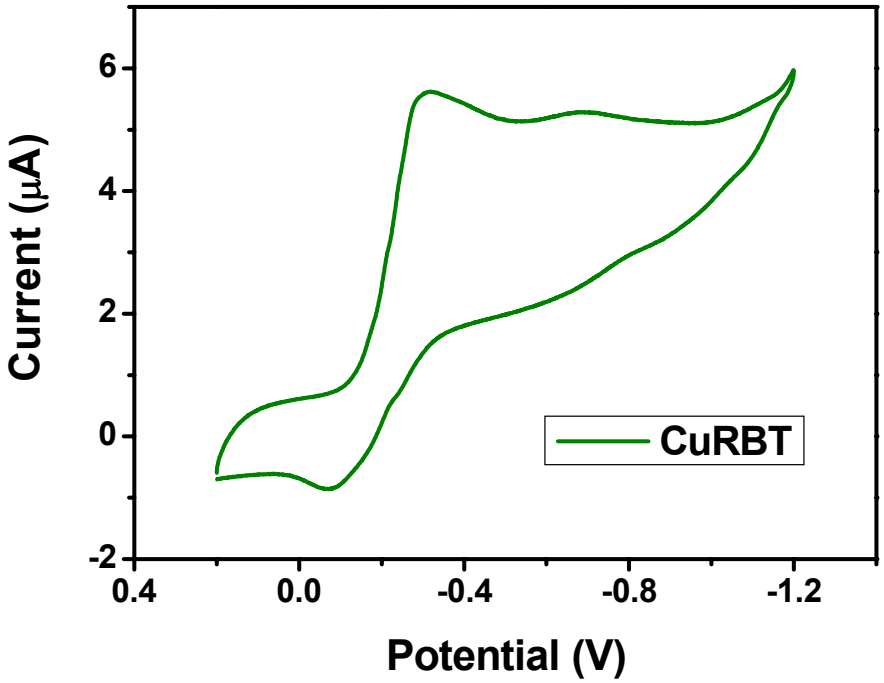


Fig. S6 Cyclic voltammgrams of **CuRBT** in phosphate buffer solution (0.1 M, pH = 7.4) with the scan rate of 0.05 V/s.

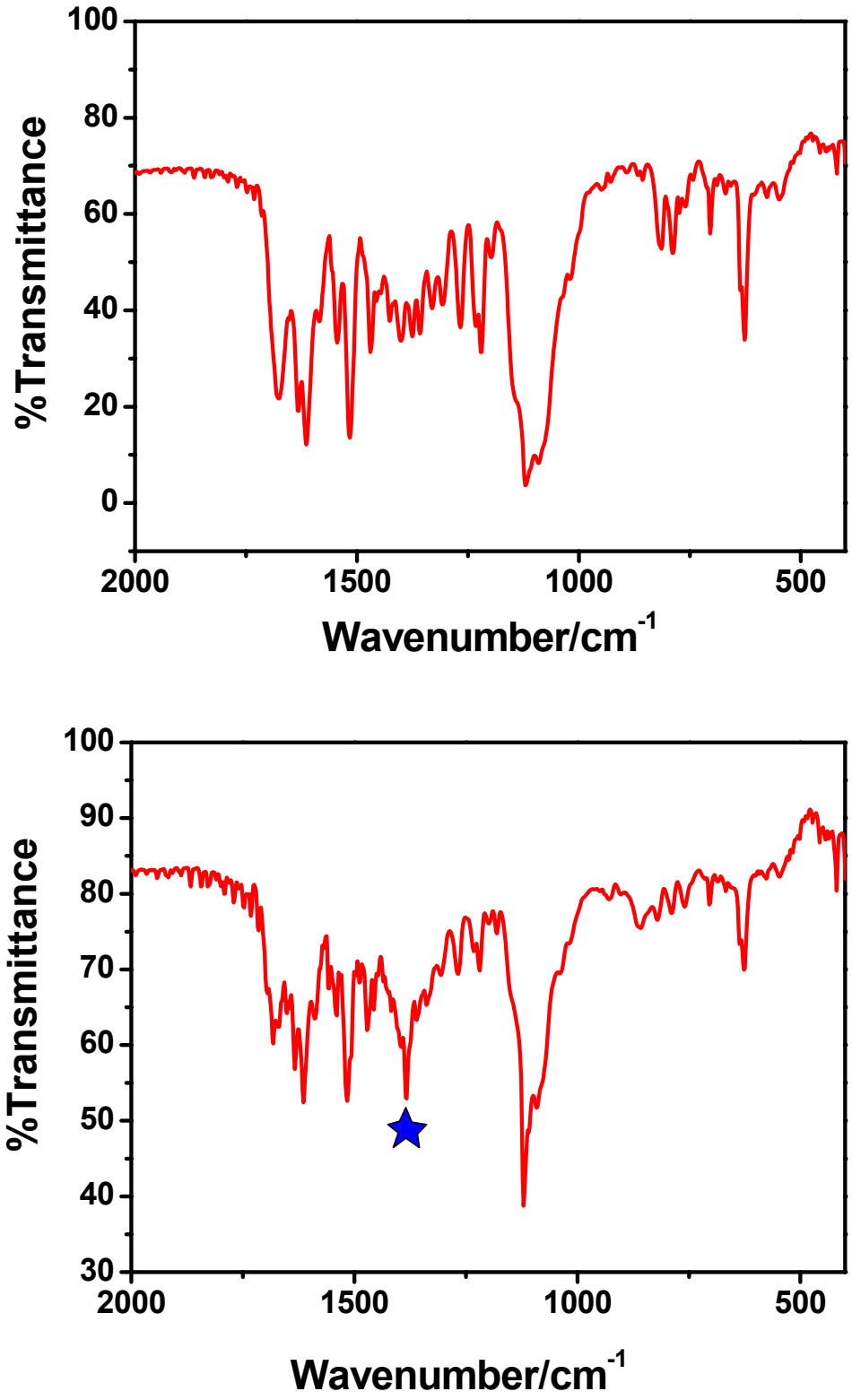


Fig. S7 IR spectra of **CuRBT** (top) and **CuRBT** treated with NO (bottom) in KBr.

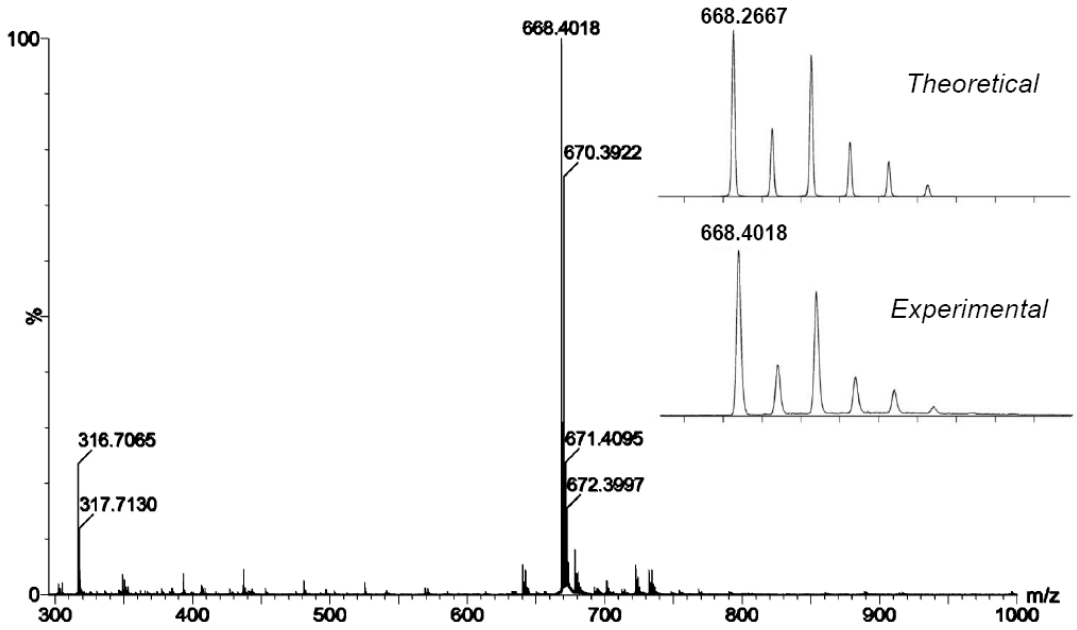


Fig. S8 ESI-MS spectrum of the complex **CuRBT**.

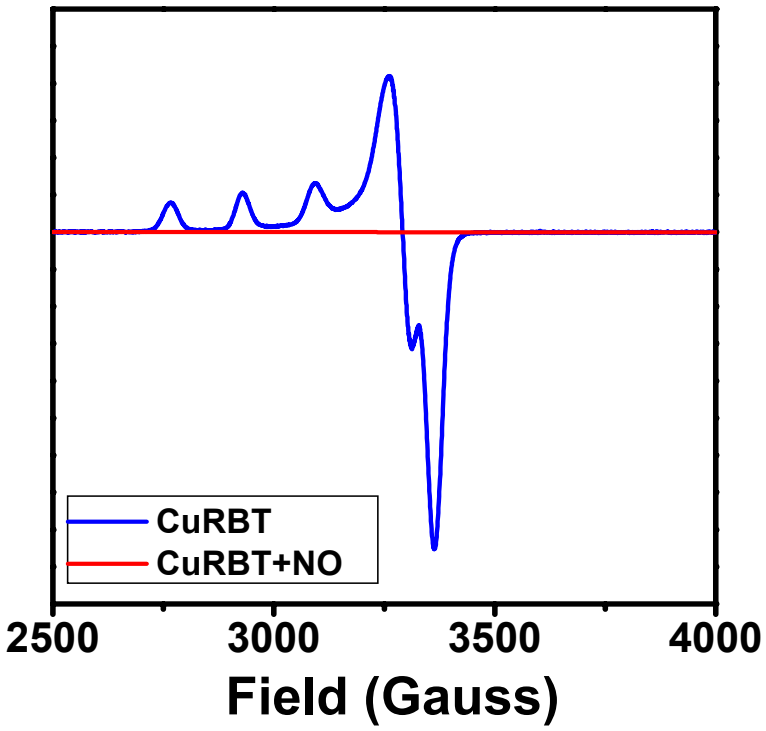


Fig. S9 EPR spectra of **CuRBT** (blue) and **CuRBT** treated with NO (red).

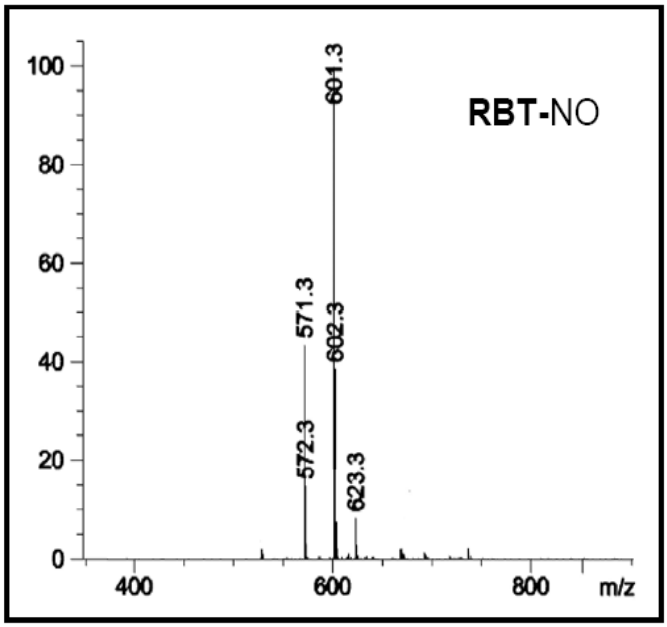


Fig. S10 LC-MS spectrum of **RBT**-NO.

Reference

1. Lee, M. H.; Kim, H. J.; Yoon, S. W.; Park, N.; Kim, J. S. *Org. Lett.* **2008**, *10*, 213.
2. Zhang, R.; Ye, Z. Q.; Wang, G. L.; Zhang, W. Z.; Yuan, J. L. *Chem. Eur. J.* **2010**, *16*, 6884.
3. Fischer, M.; Georges, J. *Chem. Phys. Lett.* **1996**, *260*, 115.