

Supporting information

Copper-promoted probe for nitric oxide based on *o*-phenylenediamine: Large blue-shift in absorption and fluorescence enhancement

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1、 Materials and methods

All the solvents were of analytic grade. ¹H-NMR was measured on a Bruker AV-400 spectrometer with chemical shifts reported in ppm (in D₂O, CDCl₃, CD₃OD with TMS as the internal standard). Mass spectra were measured on a HP 1100 LC-MS spectrometer. Fluorescence spectra were determined on a VARIAN CARY Eclipse Fluorescence spectrophotometer. Absorption spectra were determined on a VARIAN CARY 100 Bio UV-Visible spectrophotometer. The excitation and emission slit widths for **CYPs** were all 5 nm.

2、 Synthesis of sensor

4

3

2

FS

FS-1

Scheme S1: Synthesis of FS and FS-1

Compound 4: was synthesized according to the literature.¹ Mp: 233-234 °C.

Compound 3: Under Ar gas, **4** (0.40 g, 1.37 mmol) and quinolin-8-amine (0.256 g, 1.78 mmol) were dissolved in 8 mL anhydrous DMF, then stirred for 24 h at room temperature. Water (20 mL) was next introduced, causing the product to precipitate. The reaction mixture was filtered and the cake was washed with H₂O. The red solid (0.35 g, 73%) so obtained without further purification.

Compound 2: Compound **3** (0.30 g, 0.78 mmol) and SnCl₂·2H₂O (0.88 g, 4.67 mmol) were added into hydrogen chloride (10 mL, 35% concentration). The reaction mixture was stirred for 1 h at 80 °C. After cooling to r. t., the precipitated solid was collected by filtration and washed with water. The crude material was purified by flash chromatography on silica (CH₂Cl₂: CH₃OH, 20 : 1, v/v), which afforded a yellow powder (0.26 g, 93%). Mp: 265-267 °C.

¹H NMR (400 MHz, DMSO-*d*₆): δ 6.21-6.23 (m, 2 H), 7.34-7.36 (m, 2 H), 7.64 (dd, *J* = 8.4 Hz, 8.4 Hz, 1 H), 7.75 (dd, *J* = 8.0 Hz, 4.0 Hz, 1 H), 8.07 (d, *J* = 8.4 Hz, 1 H), 8.17 (d, *J* = 7.2 Hz, 1 H), 8.27 (s, 1 H), 8.55 (d, *J* = 8.0 Hz, 1 H), 9.04 (d, *J* = 4.4 Hz, 1 H); ¹³C NMR (400 MHz, DMSO-*d*₆): δ 108.0, 116.4, 119.4, 122.4, 123.8, 124.5, 127.6, 127.8, 129.5, 131.0, 132.1, 132.9, 133.7, 134.1, 136.6, 138.3, 142.1, 144.8, 148.1, 161.1, 161.6; HRMS (ES+) Calcd for ([M+H])⁺, 356.0957; Found, 356.1034

Compound FS:

5-Amino-2-butyl-6-(quinolin-8-ylamino)-1H-benzo[de]isoquinoline-1,3(2H)-dione

To a solution of compound 2 (0.50 g, 1.41 mmol) in anhydrous ethanol (20 mL) was added butan-1-amine (0.51 g, 7.04 mmol). The solution was heated at reflux for 2 h. After cooling to r. t., the precipitated solid was collected by filtration and washed with ethanol. The crude material was purified by flash chromatography on silica (CH₂Cl₂ : CH₃OH, 50 : 1, v/v), which afforded a red-brown powder (0.30 g, 52%). Mp: 231.0-232.5 °C.

¹H NMR (400 MHz, CDCl₃): δ 1.01 (t, *J*₁ = *J*₂ = 7.6 Hz, 3 H), 1.51-1.45 (m, 2 H), 1.77-1.71 (m, 2 H), 4.21 (t, *J*₁ = *J*₂ = 7.6 Hz, 2 H), 4.42 (s, 2 H), 6.40 (d, *J* = 7.6 Hz, 1 H), 7.32-7.24 (m, 2 H), 7.52 (dd, *J* = 8.4 Hz, 4.4 Hz, 1 H), 7.57 (dd, *J* = 7.6 Hz, 7.6 Hz, 1 H), 8.12 (d, *J* = 8.4 Hz, 1 H), 8.04 (s, 1H), 8.20 (d, *J* = 8.4 Hz, 1 H), 8.25 (s, 1 H), 8.35 (d, *J* = 7.2 Hz, 1 H), 8.92 (d, *J* = 4.0 Hz, 1 H); ¹³C NMR (400 MHz, CDCl₃): δ 13.8, 20.4, 30.3, 40.2, 108.0, 116.8, 121.4, 121.7, 122.5, 123.0, 123.3, 123.6, 127.3, 127.4, 127.5, 127.8, 128.9, 130.8, 136.3, 138.1, 141.0, 142.3, 147.8, 164.0, 164.5; HRMS (ES+) Calcd for ([M+H])⁺, 411.1743; Found, 411.1821.

Compound FS-1:

5-Butyl-10-(quinolin-8-yl)benzo[de][1,2,3]triazolo[4,5-g]isoquinoline-4,6(5H,10H)-dione

An aqueous solution of NaNO₂ (34 mg, 0.48 mmol) was added to a suspension of FS (200mg, 0.48 mmol) in 15 mL of 25% HCl at 0 °C. The mixture was stirred at 0 °C for 30 min. The resulting mixture was filtered and the cake was washed with H₂O. The crude material was purified by flash chromatography on silica (CH₂Cl₂ : CH₃OH, 10 : 1, v/v), which afforded a light-yellow powder (0.18 g, 88%). Mp > 300 °C.

¹H NMR (400 MHz, DMSO-*d*₆): δ 0.95 (t, *J*₁ = *J*₂ = 7.2 Hz, 3 H), 1.42-1.37 (m, 2 H), 1.69-1.63 (m, 2 H), 4.10 (t, *J*₁ = *J*₂ = 7.2 Hz, 2 H), 7.31 (d, *J* = 8.0 Hz, 1 H), 7.60 (dd, *J* = 8.0 Hz, 8.0 Hz, 1 H), 7.70 (dd, *J* = 8.4 Hz, 4.0 Hz, 1 H), 8.04 (dd, *J* = 8.0 Hz, 8.0 Hz, 1 H), 8.42 (d, *J* = 7.2, 1H), 8.48 (d, *J* = 7.2 Hz, 1 H), 8.53 (d, *J* = 8.4 Hz, 1 H), 8.68 (d, *J* = 3.6 Hz, 1 H), 8.72 (d, *J* = 8.4 Hz, 1 H), 9.19 (s, 1 H); ¹³C NMR (400 MHz, CDCl₃): δ 13.8, 20.4, 30.2, 40.6, 118.7, 120.5, 122.8, 123.6, 125.9, 126.5, 127.1, 127.8, 128.1, 128.9, 129.2, 130.6, 131.5, 134.3, 134.5, 136.4, 143.4, 152.1, 163.7, 164.0; HRMS (ES+) Calcd for ([M+H])⁺, 422.1539 ; Found, 422.1617.

3、 Determination of quantum yield

The quantum yield of Cu²⁺[FS] and FS-1 were determined according to the literature.²

$$\phi_1 = \frac{\phi_B I_1 A_B \lambda_{exB} \eta_1}{I_B A_1 \lambda_{ex1} \eta_B}$$

Where Φ is quantum yield; *I* is integrated area under the corrected emission spectra; *A* is absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; η is the refractive index of the solution; the S and B refer to the sample and the standard, respectively. We chose Quinine sulfate with 0.1 M H₂SO₄ as standard, which has the quantum yield of 0.58. The quantum yield of Cu²⁺[FS] and FS-1 are 0.022 and 0.075, respectively.

4、 Supplementary spectra data

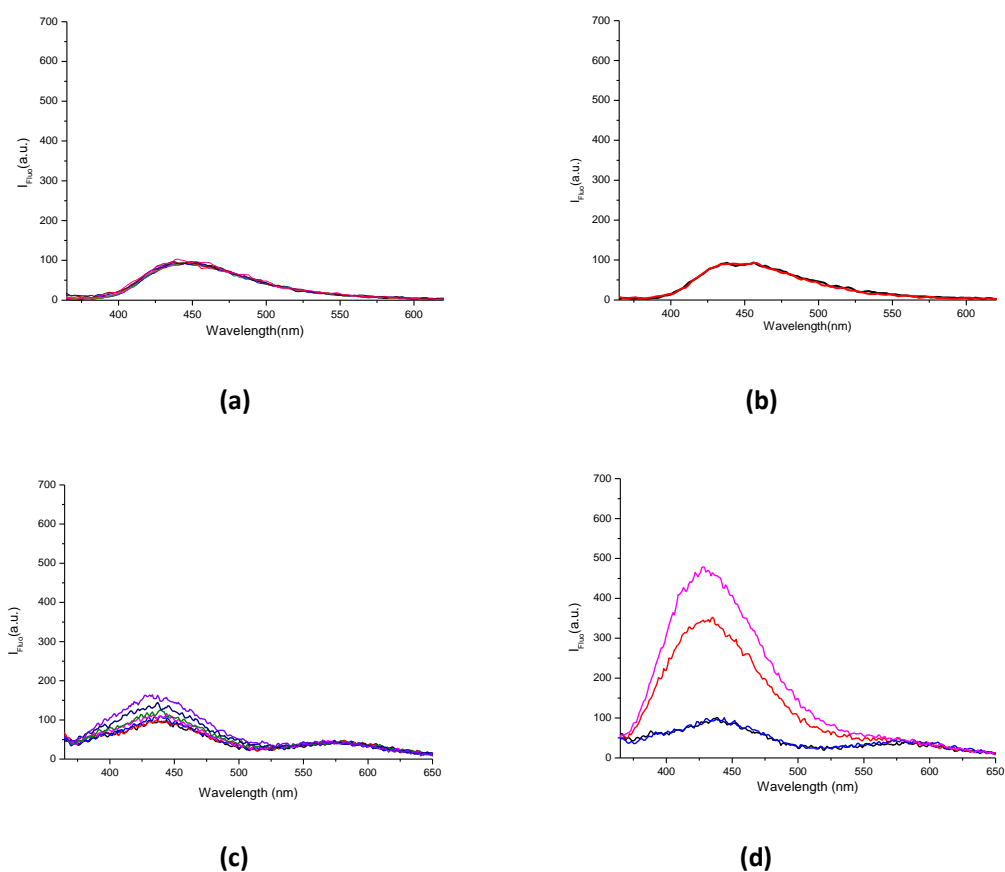
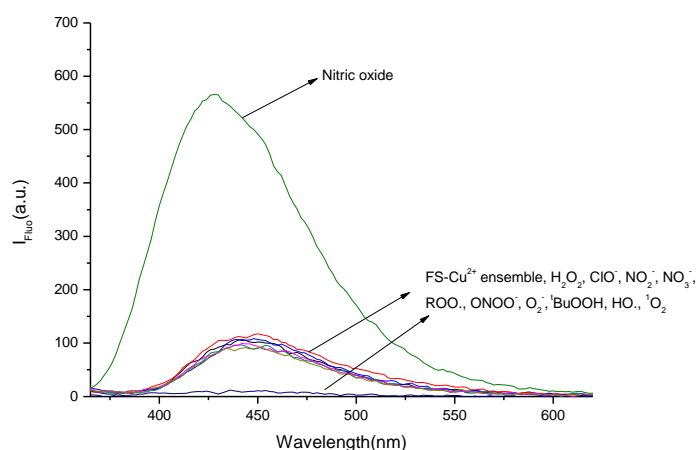


Figure S1. (a) Fluorescence spectra ($\lambda_{ex} = 350$ nm) of FS (50 μ M) with varied concentrations of NO (1eq, 2eq, 3eq, 4eq) in 10 mM Tris-HCl buffer (pH 7.30, containing 40% ethanol); (b) Fluorescence spectra ($\lambda_{ex} = 350$ nm) of FS and FS + 2eq Cu^{2+} in 10 mM Tris-HCl buffer (pH 7.30, containing 40% ethanol); (c) Fluorescence spectra ($\lambda_{ex} = 350$ nm) of FS (red line), FS + 2 eq Zn^{2+} (black line) and subsequent with varied concentrations of NO (0.25 eq, 0.75 eq, 1.25 eq, 1.5 eq, 2.0 eq) in 10 mM Tris-HCl buffer (pH 7.30, containing 40% ethanol); (d) Fluorescence spectra ($\lambda_{ex} = 350$ nm) of FS (black line), FS + 2 eq Zn^{2+} + 2 eq Cu^{2+} (blue line) and subsequent with varied concentrations of NO (1.0 eq, 2.0 eq) in 10 mM Tris-HCl buffer (pH 7.30, containing 40% ethanol).



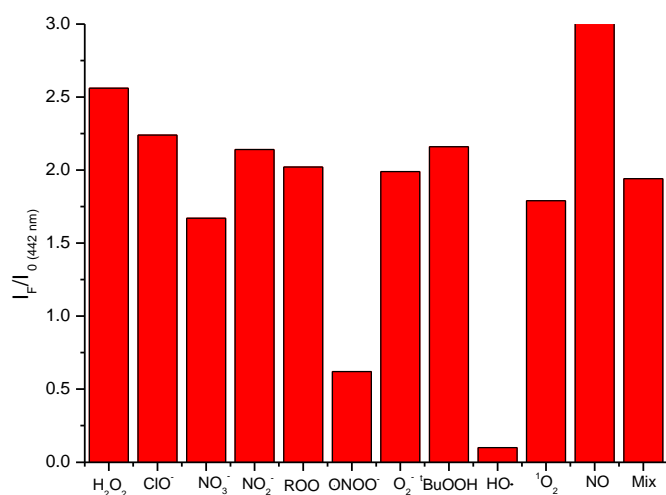


Figure S2. (a) Fluorescence responses of Cu²⁺[FS] (50 μM FS and 100 μM Cu²⁺) to 100 μM NO aqueous solution (2 mM for saturated NO aqueous solution) and 500 μM other various ROS and RNS species. (b) Fluorescence responses of Cu²⁺[FS] (50 μM FS and 100 μM Cu²⁺) to other various 100 μM ROS and RNS species, followed by 50 μM NO aqueous solution. Spectra were acquired in 10 mM Tris buffer solution (pH 7.30, containing 40.0% ethanol), at 25 °C for 2 min.

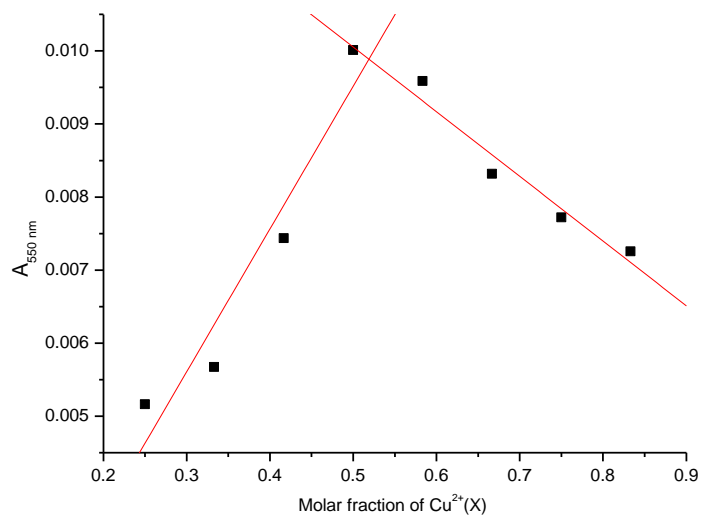


Figure S3. Job's plot showing the 1:1 binding of FS with Cu²⁺. The total concentration of the probe and Cu²⁺ is 20 μM in 10 mM Tris-HCl solution (pH 7.30, containing 40.0% ethanol).

5、 Determination of the detection limit

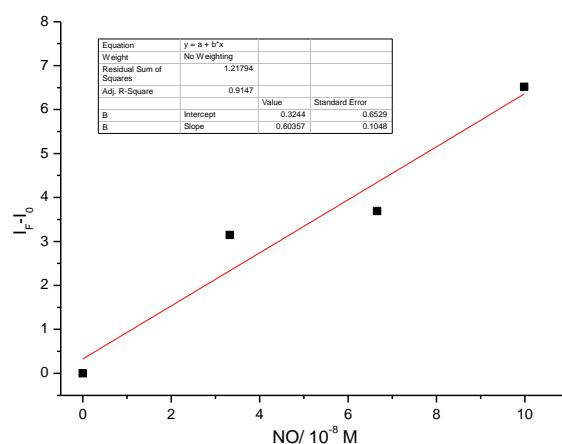


Figure S4. Determination of the detection limit between 0 – 100 nM of NO.

6. Preparation of ROS and RNS

Various ROS and RNS including H_2O_2 , $^1\text{O}_2$, $\text{ROO}\cdot$, NO , $\text{O}_2^{\cdot-}$, OCI^- , $\cdot\text{OH}$, NO_2^- , NO_3^- , $^t\text{BuOOH}$ and ONOO^- were prepared according to the following methods:

Preparation of $^1\text{O}_2$: $^1\text{O}_2$ was generated by the reaction of H_2O_2 with NaClO , which was prepared according to the literature report.³

Preparation of $\text{ROO}\cdot$: $\text{ROO}\cdot$ was generated from 2,2'-azobis (2-amidinopropane) dihydrochloride. AAPH (2,2'-azobis(2-amidinopropane) dihydrochloride) in deionizer water was added, then stirred at 25 °C for 30 min.

Preparation of NO : Nitric oxide (NO) was prepared by treating 3.6 M sulfuric acid solution with 7.3 M sodium nitrite solution and its stock solution (2.0 mM) was prepared by bubbling NO into deoxygenated de-ionized water for 30 min.

Preparation of $\text{O}_2^{\cdot-}$: Superoxide was generated from KO_2 with a saturated solution of KO_2 in DMSO (~1 mM).⁴

Preparation of $\cdot\text{HO}$: Hydroxyl radical was generated by Fenton reaction. To prepare $\cdot\text{OH}$ solution, ferrous chloride was added in the presence of 10 equiv of H_2O_2 .⁵

Preparation of peroxyxynitrite: Peroxyxynitrite solution was synthesized as reported.⁶ The peroxyxynitrite concentration was estimated by using an extinction coefficient of $1670 \pm 50 \text{ cm}^{-1} (\text{mol/L})^{-1}$ at 302 nm.

Preparation of HOCl : Commercial bleach was the source of NaOCl . The concentration of HOCl was determined by titration with $\text{S}_2\text{O}_3^{2-}$.

All other chemicals were from commercial sources and of analytical reagent grade, unless indicated otherwise.

7. The characterization data of compounds $\text{Cu}^{2+}[\text{FS}]$ and FS-1

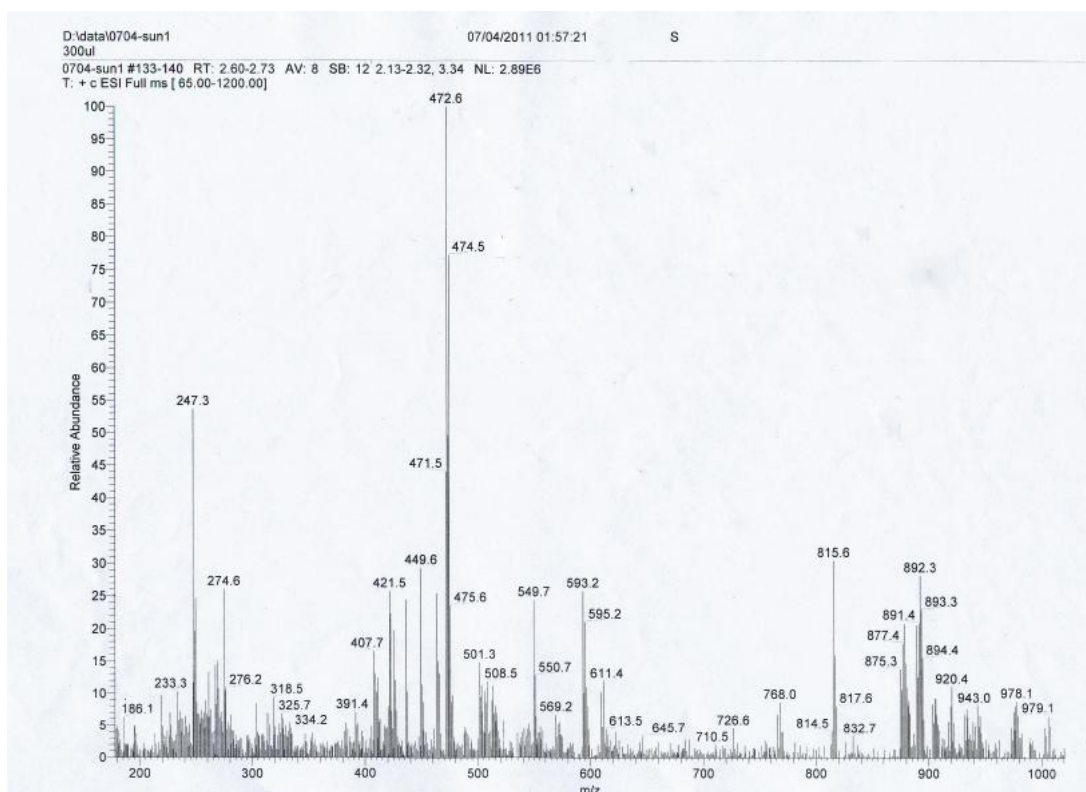


Figure S5. ESI-MS spectrum of FS (50 μM) with Cu^{2+} (100 μM) in methanol. The peak at $m/z = 472.6$ corresponds to $[\text{FS} + \text{Cu}^{2+} - \text{H}]^+$.

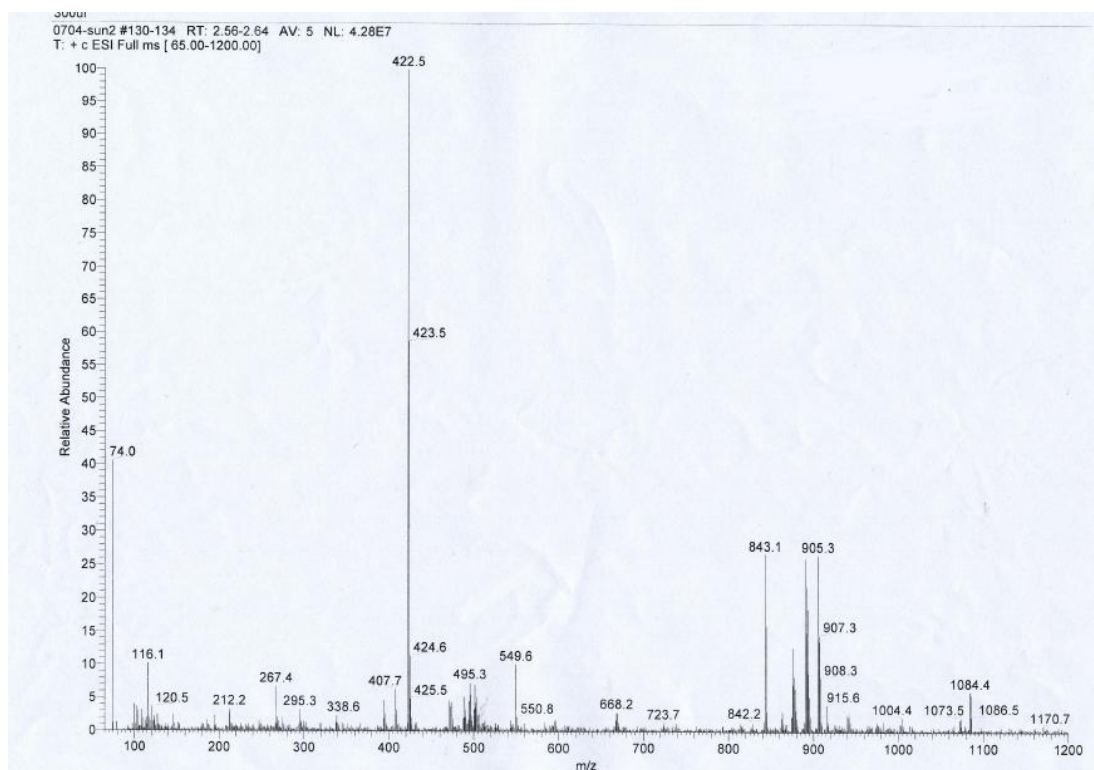
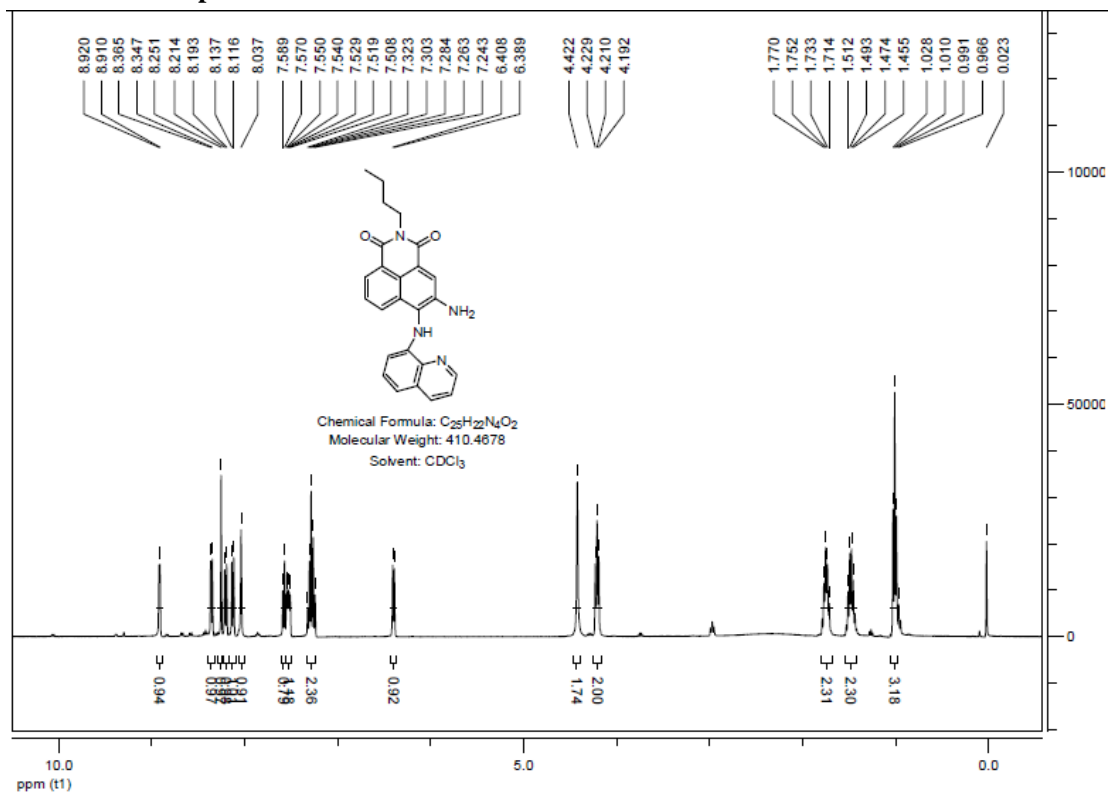


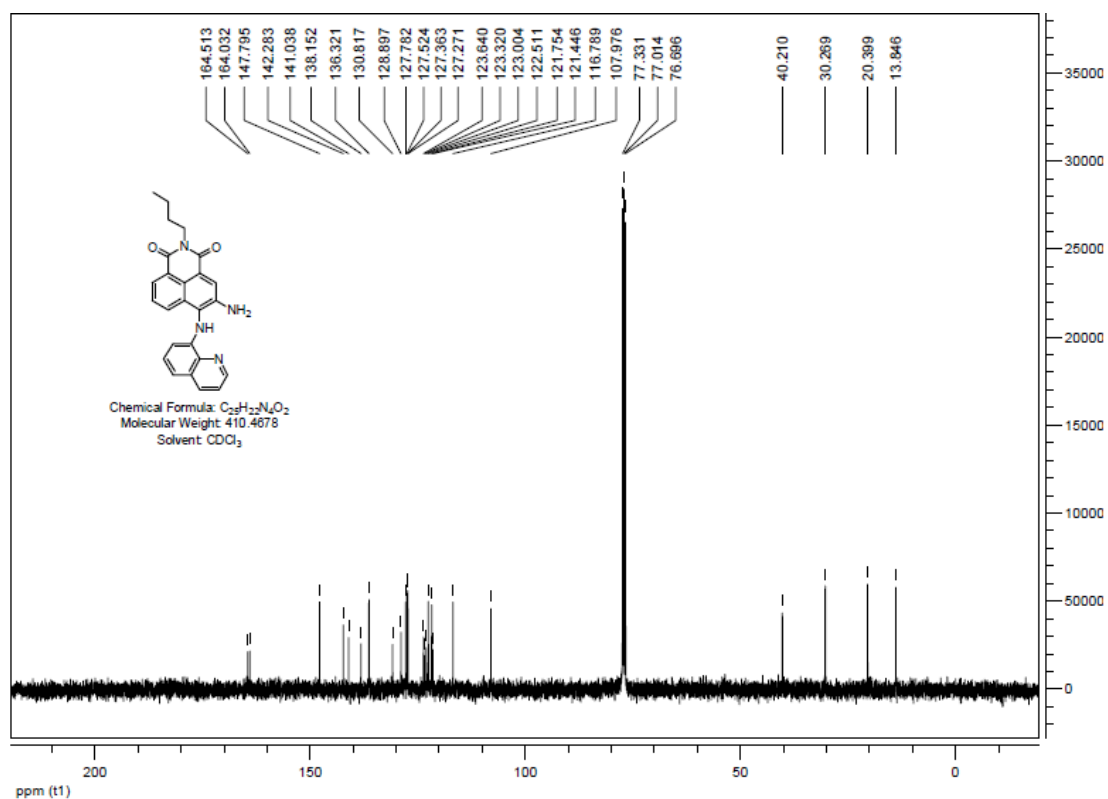
Figure S6. ESI-MS spectrum of FS (50 μM) + Cu^{2+} (100 μM) + NO (100 μM) in methanol. The peak at $m/z = 422.5$ corresponds to FS-1.

8、 ^1H NMR, ^{13}C NMR and ESI-MS spectra

¹H NMR of compound FS



¹³C NMR of compound FS



HRMS spectrum of compound FS

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 3.0 mDa / DBE: min = -1.5, max = 100.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions

27 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)

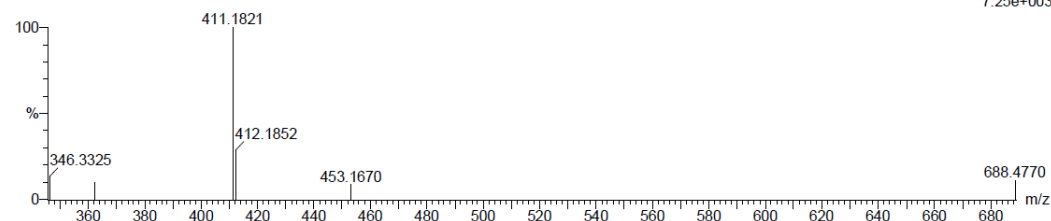
Elements Used:

C: 0-30 H: 0-30 N: 0-4 O: 0-2

YY5

YY5-SXL-000 33 (1.111) Cm (30:33)

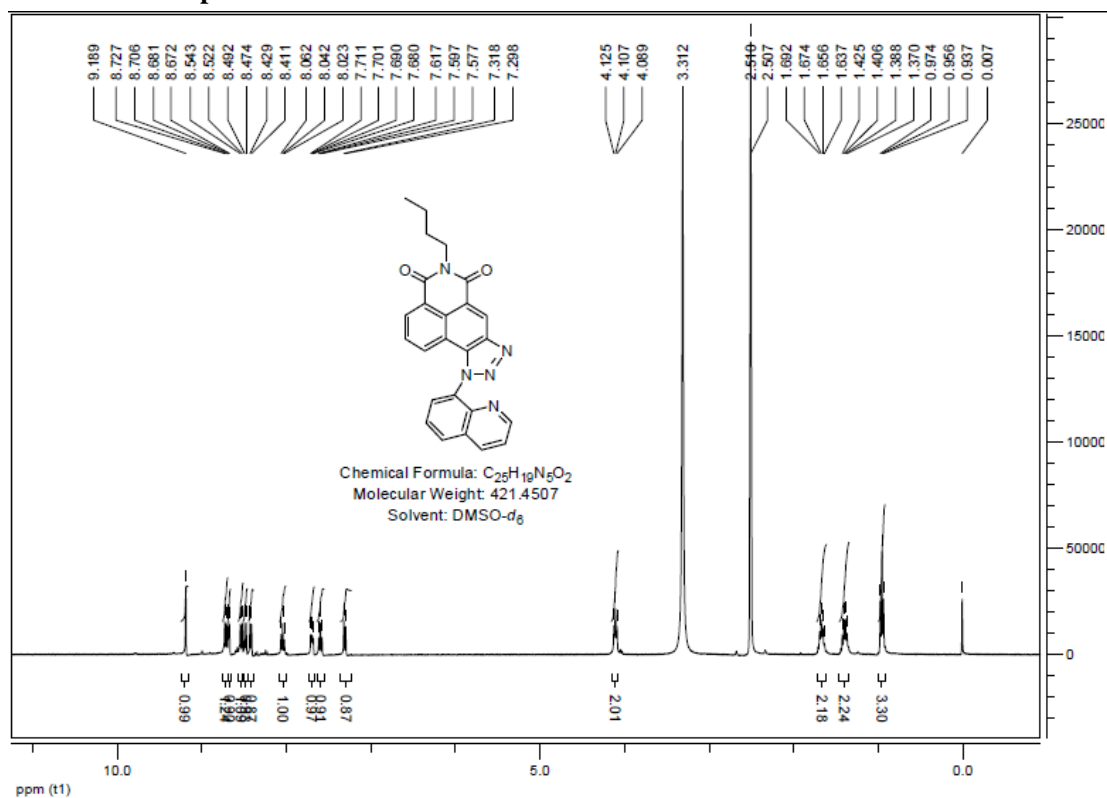
1: TOF MS ES+
7.25e+003



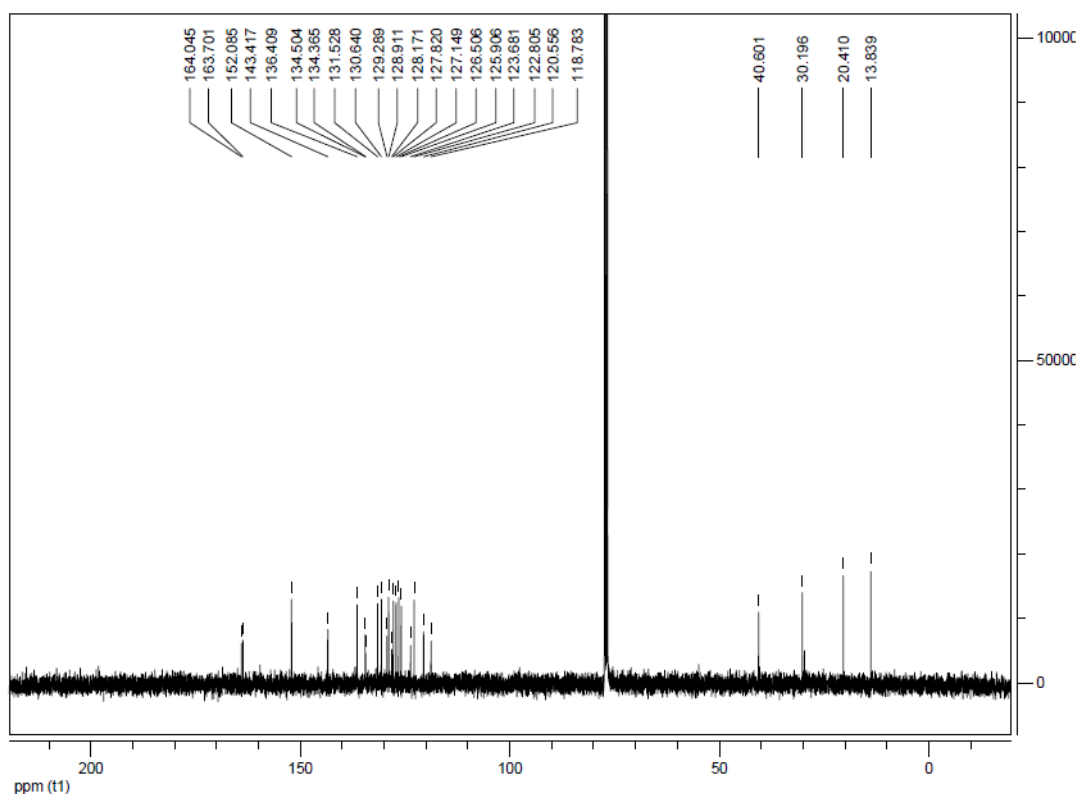
Minimum: -1.5
Maximum: 100.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
411.1821	411.1821	0.0	0.0	16.5	7.4	0.0	C25 H23 N4 O2

¹H NMR of compound FS-1



¹³C NMR of compound FS-1



HRMS spectrum of compound FS-1

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 50.0 mDa / DBE: min = -1.5, max = 100.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions

288 formula(e) evaluated with 36 results within limits (up to 1 closest results for each mass)

Elements Used:

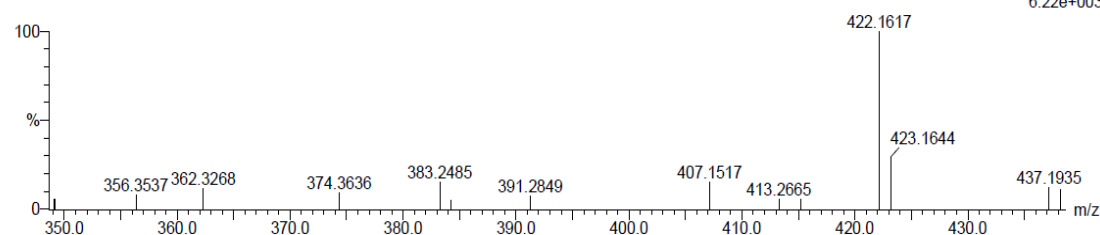
C: 0-30 H: 0-50 N: 0-5 O: 0-5 Se: 0-1

YAO-YS

ECUST institute of Fine Chem

YYS-SXL-002 9 (0.374) Cm (3:9)

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 16:14:26
 1: TOF MS ES+
 6.22e+003



Minimum: -1.5
 Maximum: 50.0 50.0 100.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
422.1617	422.1617	0.0	0.0	18.5	7.2	0.0	C25 H20 N5 O2

9、References

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5. Foote, C. S., S. Wexler., *J. Am. Chem. Soc.*, 1968,**90**, 975-981.
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