Supporting Information

for

Fluorescent-based detection of nitric oxide in aqueous and methanol media using copper(II) complex

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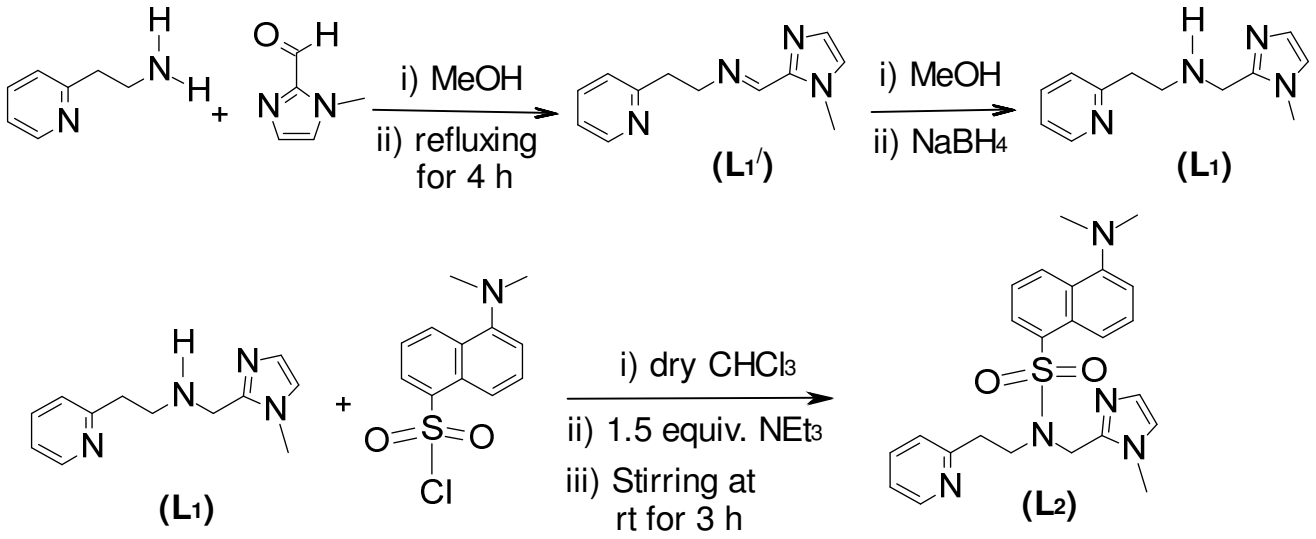
Experimental Section

General

All reagents and solvents were purchased from commercial sources and were of reagent grade. Acetonitrile was distilled from calcium hydride. Deoxygenation of the solvent and solutions were effected by repeated vacuum/purge cycles or bubbling with nitrogen for 30 minutes. The NO gas was purified by passing through KOH column followed by P_2O_5 column. UV-visible spectra were recorded on a Perkin Elmer Lambda-25 spectrophotometer. Fluorescence spectra were recorded on Cary ECLIPSE fluorescence spectrophotometer. FT-IR spectra were taken on a Perkin Elmer spectrophotometer with samples prepared as KBr pellets. Solution electrical conductivity was checked using a Systronic 305 conductivity bridge. ^{1}H- NMR spectra were obtained with a 400 MHz Varian FT spectrometer. Chemical shifts (ppm) were referenced either with an internal standard (Me_4Si) for organic compounds or to the residual solvent peaks for copper complexes. The X-Band Electron Paramagnetic Resonance (EPR) spectra were recorded on a JES-FA 200 EPR spectrometer, at room temperature. Elemental analyses were obtained from a Perkin Elmer Series II Analyzer. The magnetic moment of complex **1** is measured on a Cambridge Magnetic Balance.

Synthesis of the fluorescent ligand

The fluorescent ligand has been synthesized in two steps (scheme S1); the first step is the synthesis of the precursor amine and the second step is the incorporation of the pendant fluorophore into the amine.



Scheme S1. Synthesis of the fluorescent ligand.

(i) Synthesis of the precursor amine

2-pyridine ethylamine (1.46 g, 12 mmol) and one equivalent (1.32g, 12 mmol) of 1-methyl-2-imidazolecarboxaldehyde in 20 ml methanol was heated at 60 °C for 4 hours. The resulting yellow solution was dried under reduced pressure and the dark yellow oil thus obtained was subjected to chromatographic purification using silica gel column to yield the pure Schiff base, L_1' as yellow oil (yield: 1.50 g; ~60%). The Schiff base is then reduced to the corresponding ligand, L_1, using $NaBH_4$ in methanol solution. The pure L_1 was obtained after chromatographic purification using silica gel column (yield: 1g; ~70%). Elemental Analyses: Calcd.(%) for $C_{12}H_{16}N_4$: C, 66.64; H, 7.46; N, 25.90. Found(%): C, 66.66; H, 7.45; N, 25.93. FT-IR: 3504, 1420 cm^{-1}, ^{1}H-NMR: (400 MHz, $CDCl_3$): δ_{ppm}: 2.86(1H, s) δ_{ppm}: 2.95 (2H, d), 3.01 (2H, d), 3.58 (3H, s), 3.83 (2H, s) , 6.76 (1H, s), 6.86 (1H, s) 7.0-7.1 (2H, m), 7.52 (1H, m), 8.47 (1H, d). ^{13}C-

NMR: (100 MHz, $CDCl_3$) δ_{ppm}: 32.47, 38.09, 45.47, 48.77, 121.06, 121.31, 126.64, 126.73, 136.66, 146.30, 149.04, 160.02.

(ii) Synthesis of the ligand, L_2

The fluorescent ligand L_2 has been prepared by the incorporation of the dansyl group into the ligand L_1 (scheme 2). This has been done by stirring an equimolar mixture of the yellow oil, L_1 and dansyl chloride in presence of triethylamine in distilled chloroform for 5 h at room temperature. The volume of the resulting solution was then reduced in rotavapour and the greenish yellow fluorescent mass was subjected to column chromatographic purification to result into the pure greenish yellow fluorescent ligand L_2 (yield: ~70%). Elemental Analyses: Calcd.(%) for $C_{24}H_{27}N_5O_2S$: C, 64.12; H, 6.05; N, 15.58. Found (%): C, 64.09; H, 6.06; N, 15.61. FT-IR: 2927, 2851, 2788, 1322, 1140 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) δ_{ppm}: 2.74 (2H, t), 2.86 (6H, s), 3.58 (2H, t), 3.61 (3H, s), 4.756 (2H, s), 6.8-7.00 (4H, m), 7.12 (1H, d), 7.34-7.52 (3H, m), 8.02 (1H, d), 8.28 (2H, m) 8.48 (1H, d) , ^{13}C-NMR (100 MHz, $CDCl_3$) δ_{ppm}: 29.87, 33.29, 36.59, 44.42, 45.60, 47.54, 76.91, 77.23, 77.54, 115.46, 119.56., 121.40, 122.59, 123.36, 123.44, 127.70,128.08, 128.38, 129.41, 130.30, 130.65, 134.69, 136.25, 142.45, 149.25, 151.99, 158.52.

Preparation of the complex, 1

Copper(II)perchlorate hexahydrate, $[Cu(H_2O)_6](ClO_4)_2$ (370 mg, 1.0 mmol) was dissolved in 20 ml of freshly distilled methanol and to this light blue solution, the ligand L_2 , (449 mg, 1.0

mmol), was added drop wise. The color of the solution was changed to light green. The resulting mixture was stirred for 1 h. Then the solution was dried under reduced pressure and the solid mass thus obtained was washed with diethyl ether to afford complex **1** as light green solid. Yield: 640 mg (~85%). Elemental Analyses: Calcd.(%) for $CuC_{25}H_{30}Cl_2N_5O_{11}S$: C, 40.42; H, 4.03; N, 9.42. Found (%): C, 40.45; H, 4.03; N, 9.38. FT-IR (KBr pellet): υ_{ClO4^-}, 1081 cm^{-1}, 625 cm^{-1}. X-band EPR data: g_{av} = 2.07. Molar conductance: 217 $Scm^{-1}mol^{-1}$. The observed magnetic moment is found to be 1.77 BM.

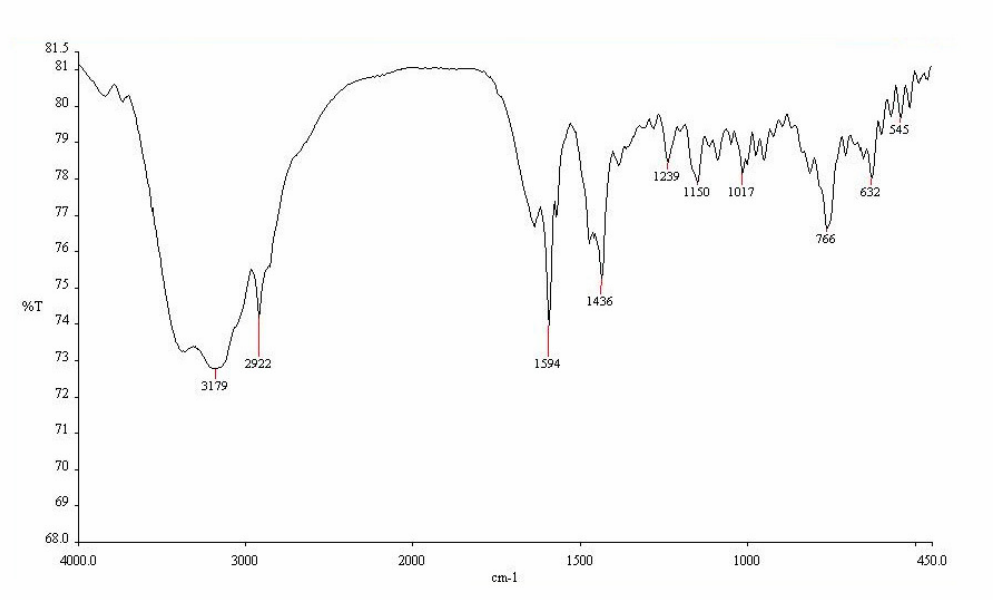


Figure S1: FT-IR spectrum of $\mathbf{L_1}$ in KBr pellet.

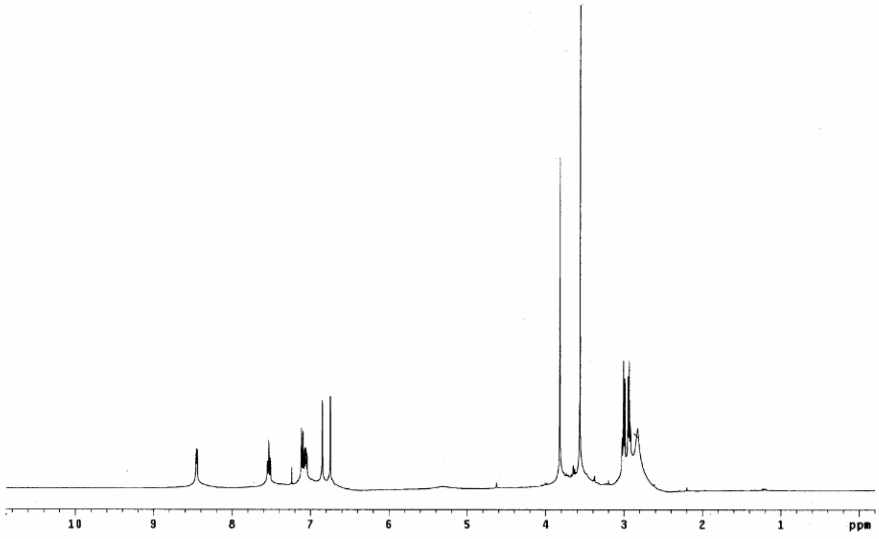


Figure S2: ^{1}H-NMR Spectrum of $\mathbf{L_1}$ in $CDCl_3$.

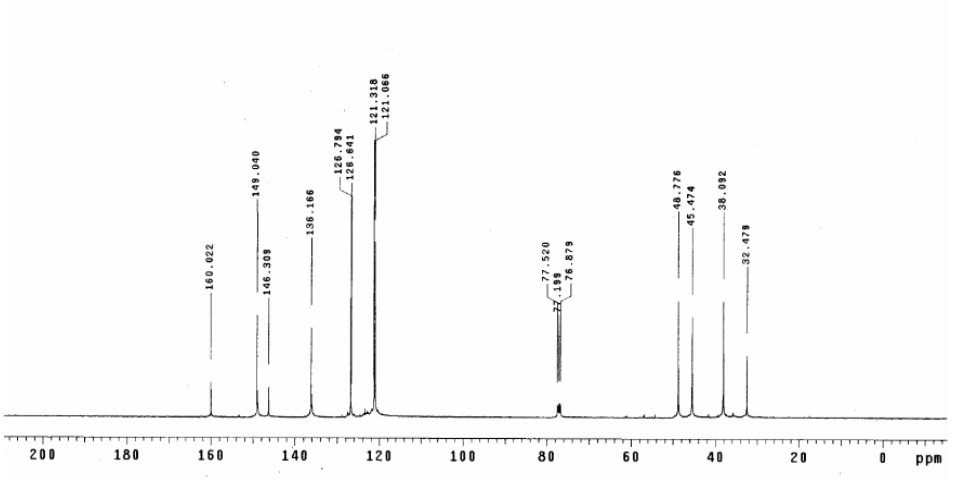


Figure S3: ^{13}C-NMR spectrum of $\mathbf{L_1}$ in $CDCl_3$.

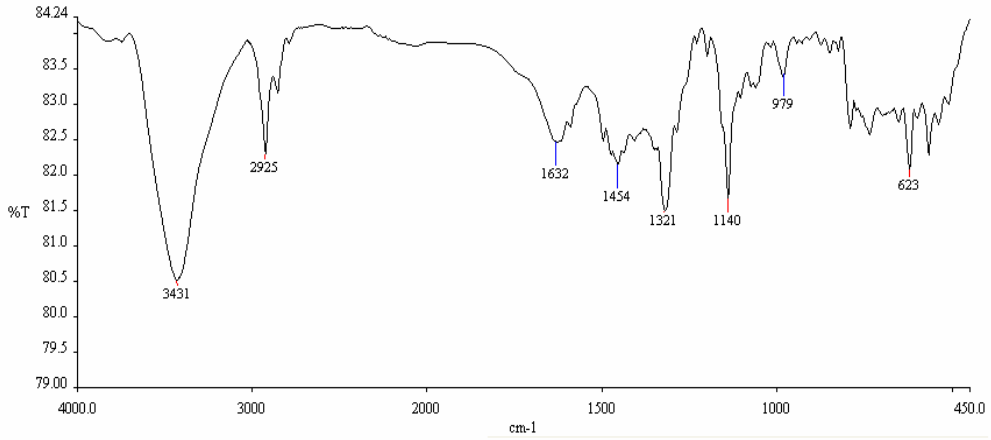


Figure S4: FT-IR spectrum of $\mathbf{L_2}$ in KBr pellet.

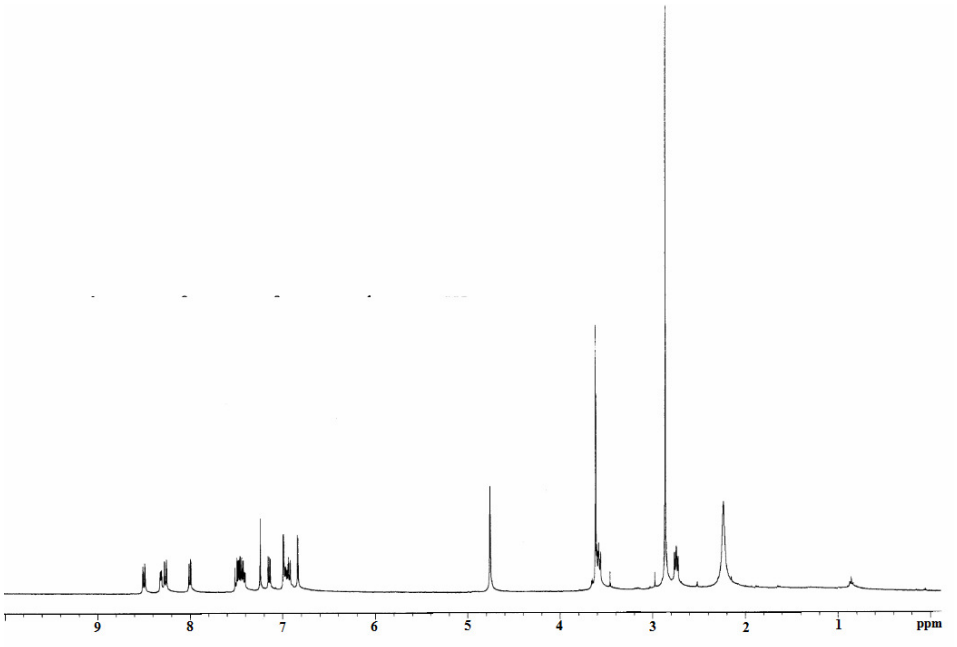


Figure S5: ^{1}H-NMR spectrum of the **L_2** in $CDCl_3$.

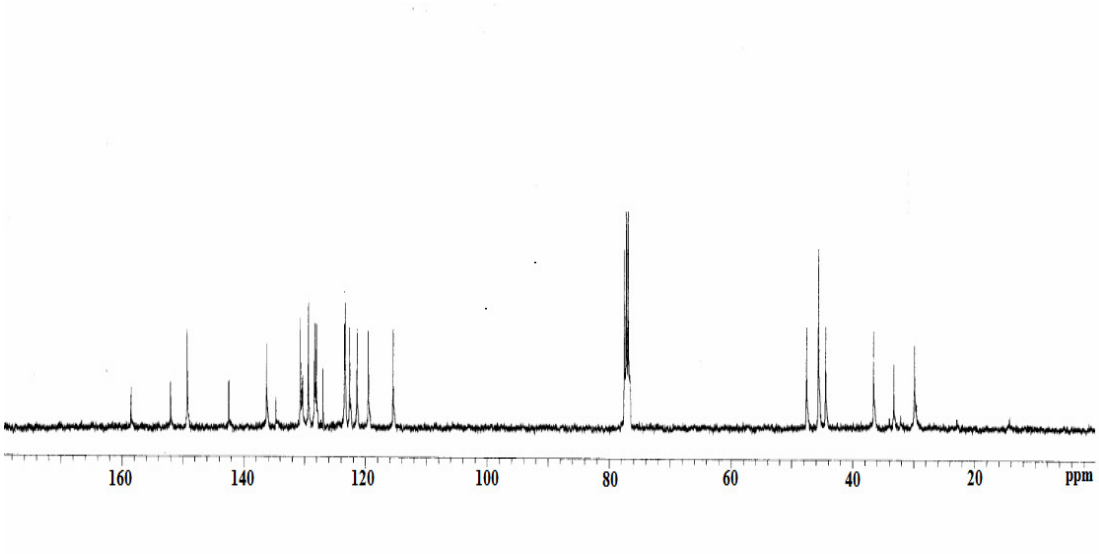


Figure S6: ^{13}C-NMR spectrum of the **L_2** in $CDCl_3$.

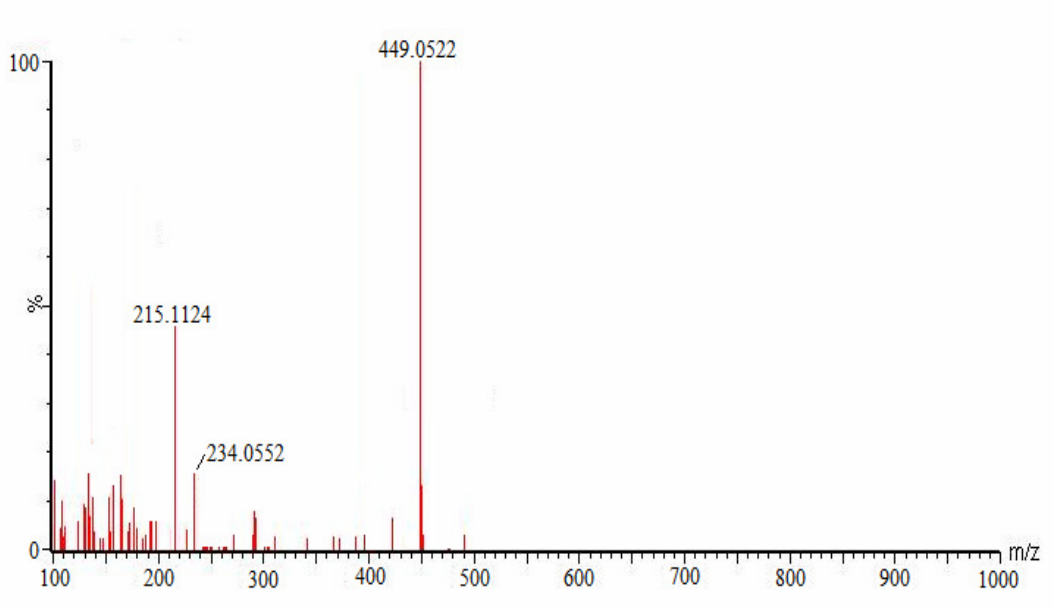


Figure S7: ESI-Mass spectrum for L_2.

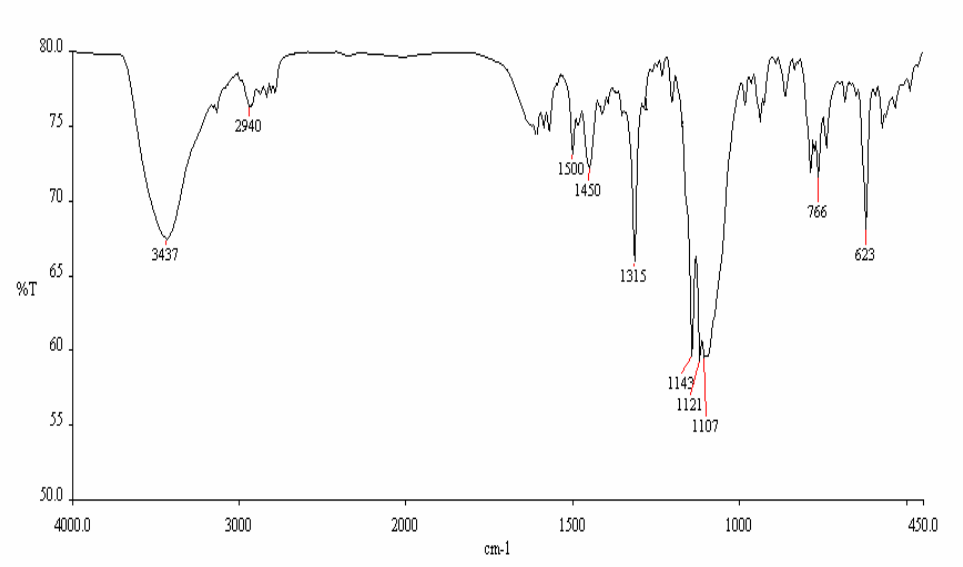


Figure S8: FT-IR Spectrum of complex **1** in KBr pellet.

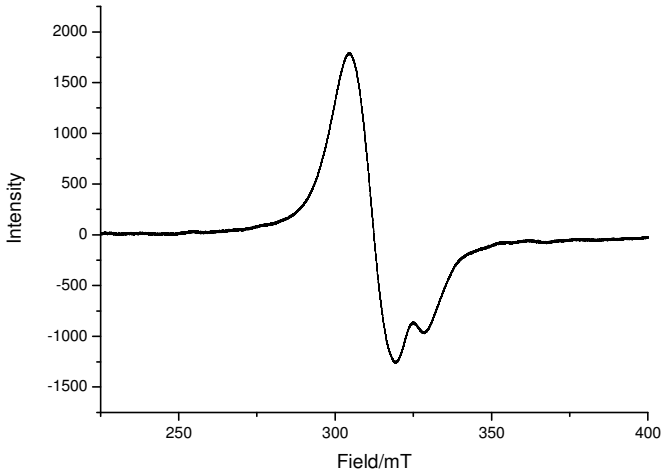


Figure S9: X-band EPR spectrum of complex **1** in MeOH at room temperature.

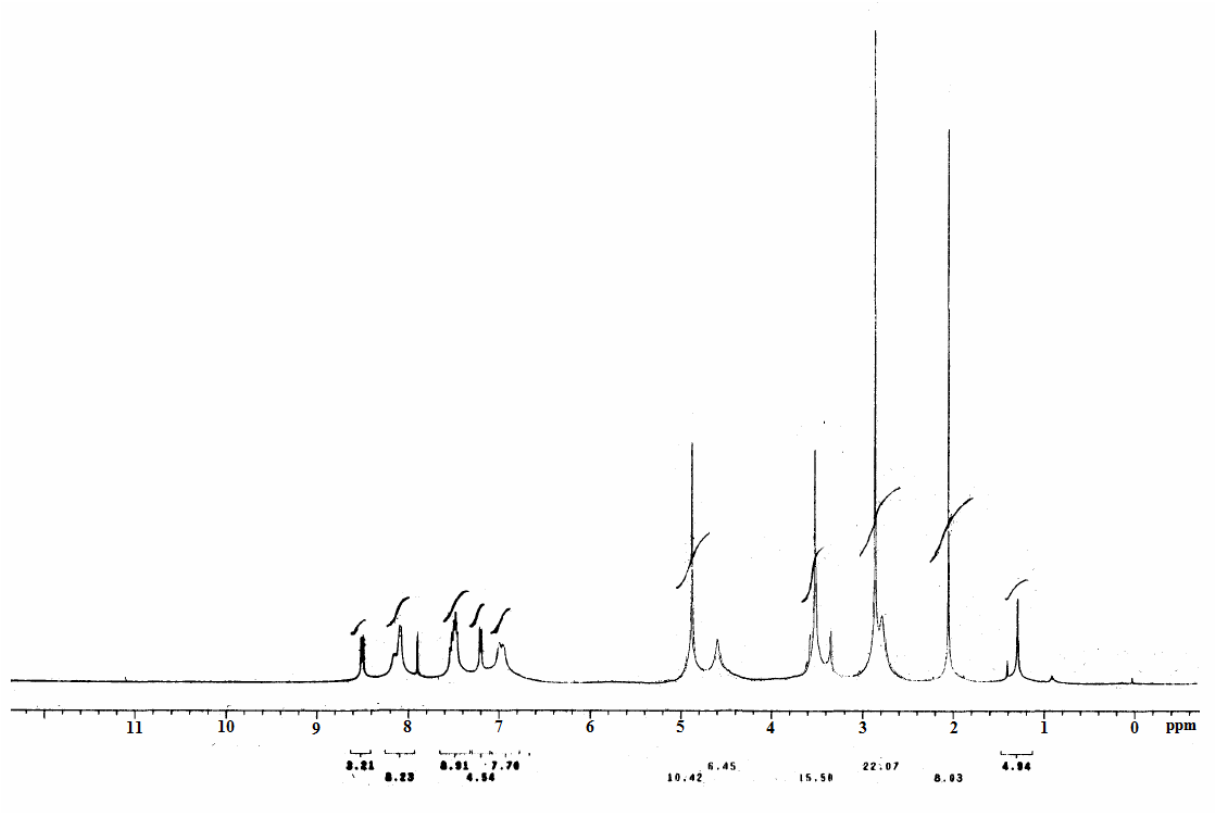


Figure S10: ^{1}H-NMR spectrum of the complex **1** after reaction with nitric oxide at CD_3OD.

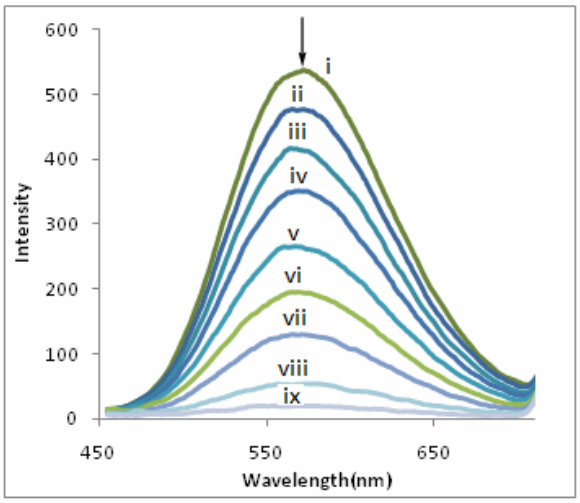


Figure S11: Fluorescence responses in aqueous solution buffered at pH 7.2 of (i) the free L_2 and of that after addition of (ii) 0.1 equivalent, (iii) 0.3 equivalent, (iv) 0.4 equivalent, (v) 0.5 equivalent, (vi) 0.6 equivalent, (vii) 0.7 equivalent, (viii) 0.9 equivalent and (ix) 1.0 equivalent of $[Cu(H_2O)_6]^{2+}$.

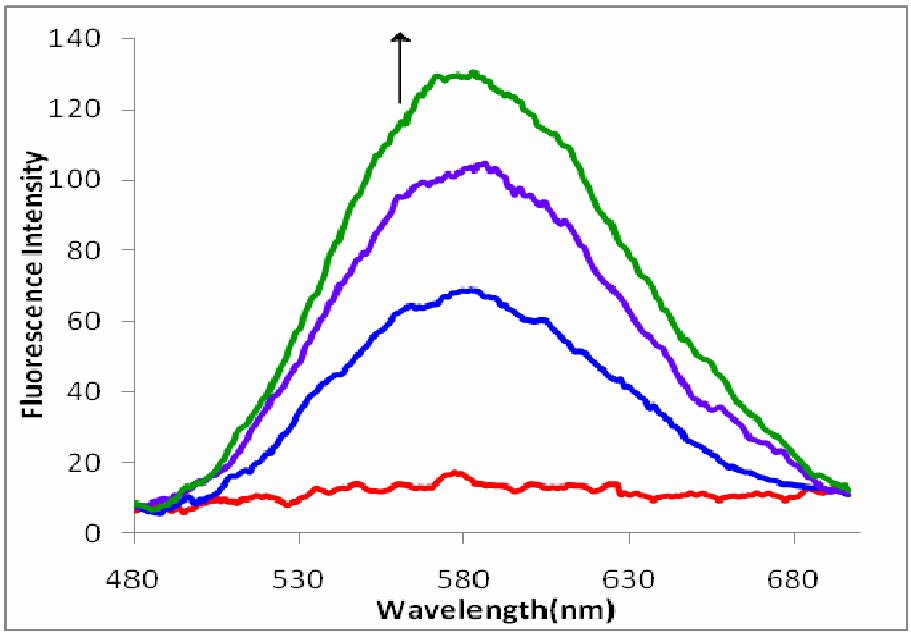


Figure S12. Fluorescence responses (λ_{ex} = 342 nm) for 25 µM solution of complex **1** in TRIS-HCl buffer at pH 7.2(red trace) and after the addition of two equivalent NO at 5, 10, and 20 min (blue, violet and green trace, respectively) at 298K.

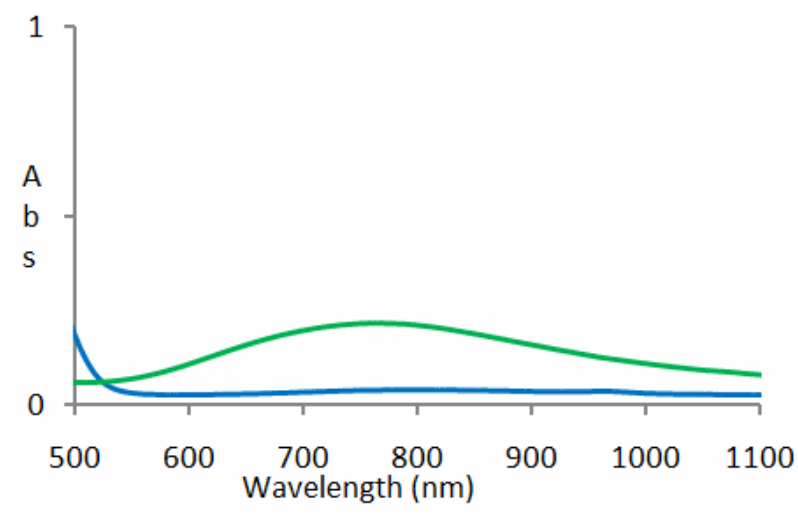


Figure S13: UV-visible spectra of complex **1** before (green line) and after (blue line) purging nitric oxide in aqueous medium buffered at pH 7.2.

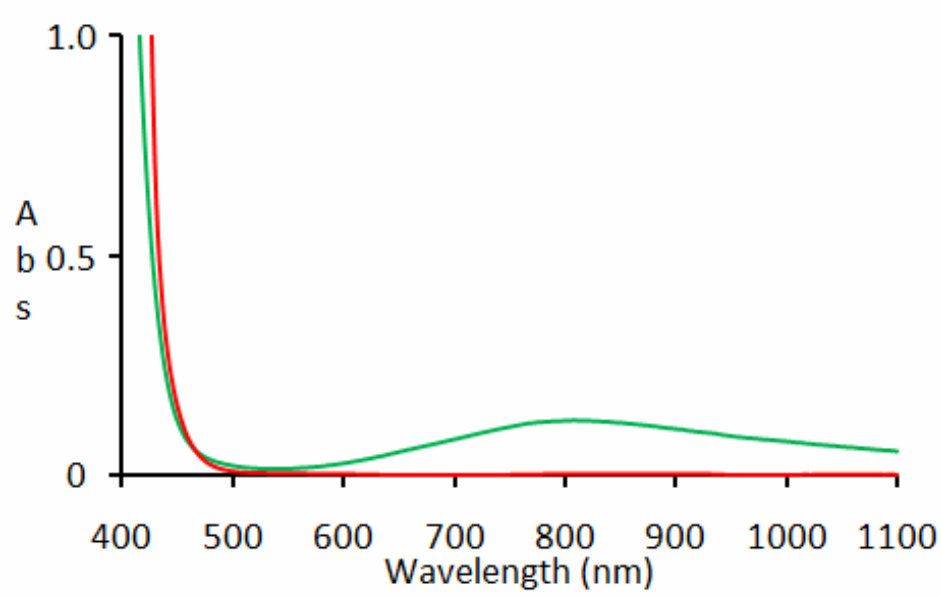


Figure S14. UV-visible spectra of complex **1**(green line) and after its reaction with NO (red line) in methanol solution.

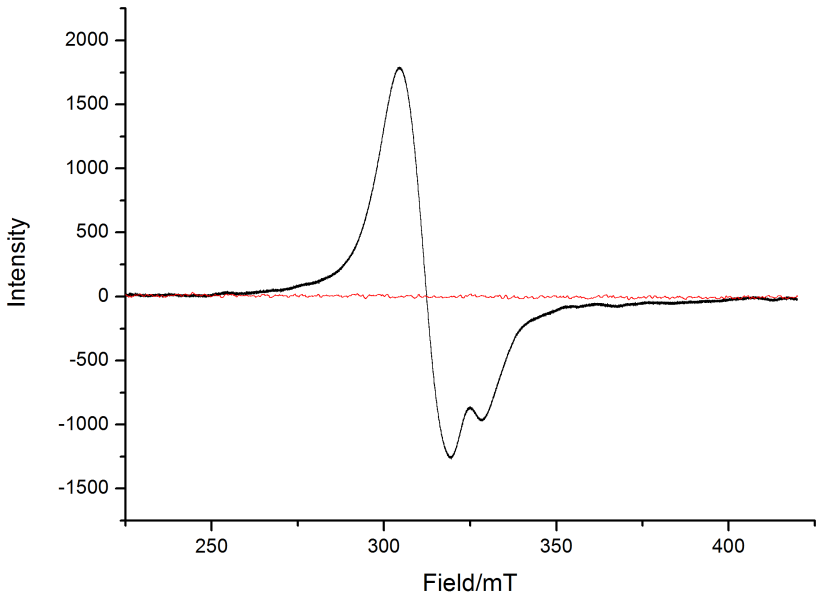


Figure S15. X-band EPR spectra of complex **1** (black trace) and after its reaction with NO (red trace) in methanol at room temperature.

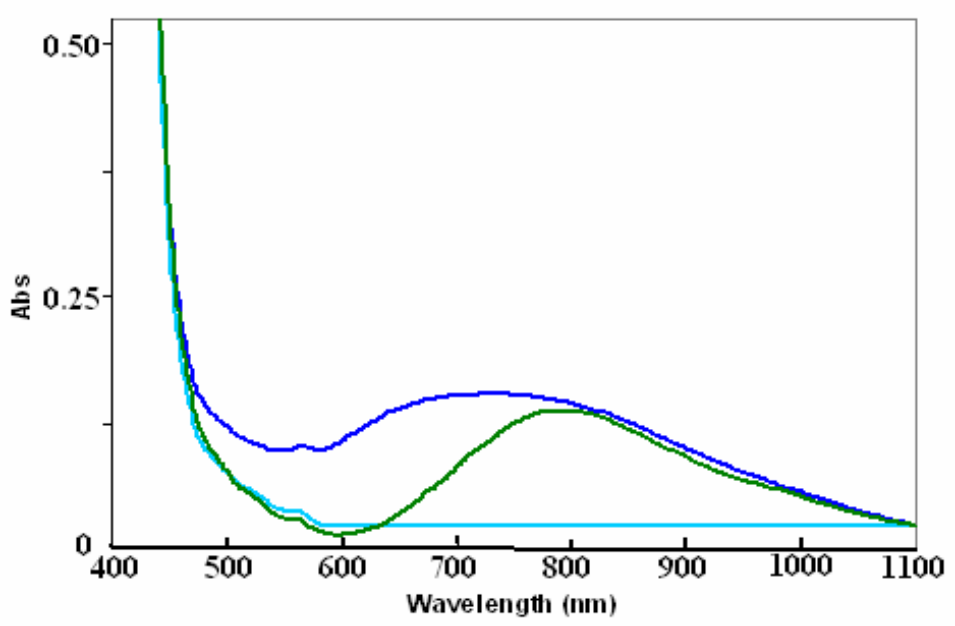


Figure S16: UV-visible spectra of complex **1** (dark blue), immediately after its reaction with NO (light blue) in methanol. The green trace is that of the solution obtained after keeping the reduced species for 2 h at open air and at room temperature.