Electronic Supplementary Material (ESI) for Dalton Transactions.


# Ruthenium nitrosyl complex based highly selective colorimetric sensor for biological $H_2S$ and $H_2S$-NO cross talk regulated release of NO

Nancy Sharma[a], Vinod Kumar[a] and D. Amilan Jose*[a]

[a]Department of Chemistry, National Institute of Technology (NIT) Kurukshetra, Kurukshetra -136119, Haryana, INDIA

Email: amilanjosenit@nitkkr.ac.in and amilanjose@gmail.com

## 1. Materials

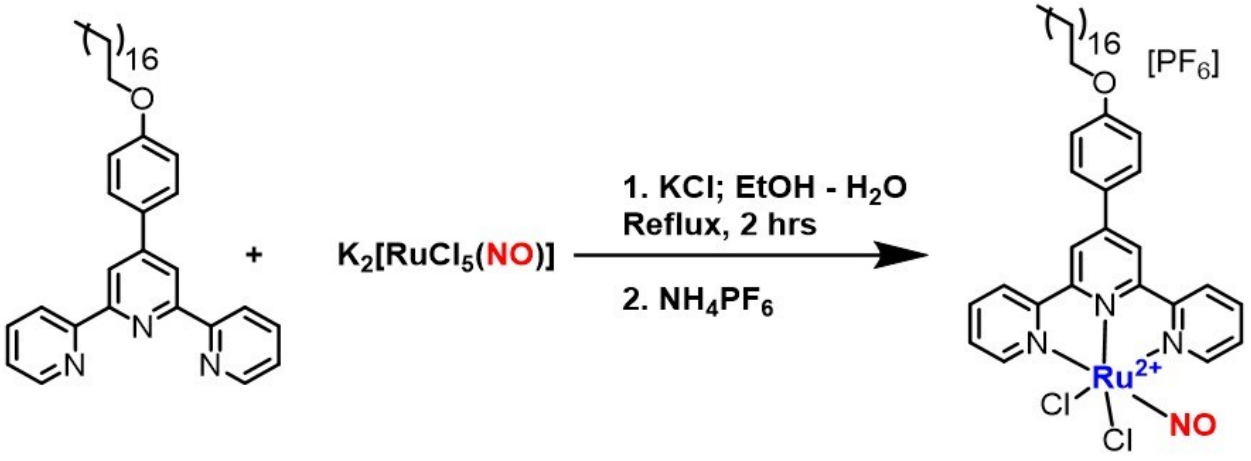
Modified Greiss reagent, ammonium hexafluorophosphate, Sodium Hydrosulfide (NaHS), 1-bromooctadecane, potassium chloride, potassium pentachloronitrosylruthenate(II), Tetrabutylammonium salts of various anions ($F^-$, $Cl^-$, $Br^-$, $I^-$, $CN^-$, $CH_3COO^-$, $NO_3^-$, $HSO_4^-$, $PO_4^{3-}$, PPi), Adenosine-5′-triphosphate disodium salt hydrate, Adenosine-5′-diphosphate sodium salt and Adenosine-5′-monophosphate sodium salt were purchased from Sigma-Aldrich. All the titrations have been performed with the NaHS and it is a source for $H_2S$ in aqueous solution. NaHS and $Na_2S$ are the familiar donors for the instantaneous generation of $H_2S$. Potassium carbonate, Potassium Bromide (IR Spectroscopy), L-Cysteine, Glutathione and Silica Gel (mesh 100-200) were purchased from Loba Chemie Pvt. Ltd. DAF-FM Diacetate NO detection Kit was purchased from Thermo Fischer Scientific. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was purchased from Avanti Polar Lipids. All the solvents purchased were of analytical grade and used without further purification.

## 2. Instrumentation Methods

$^1$H-NMR spectra were performed on Bruker Advance 500 MHz at room temperature using $CD_3CN$ as solvent. Chemical shift values are reported in ppm values using TMS as an internal reference. Waters Q-Tof mass spectrometer was used to record ESI-mass spectrum using positive ion mode. An Evolution 201 UV-Vis spectrophotometer was used to record UV absorption spectra with quartz cells of 1.0 cm path length. For the preparation of vesicles, Mini-Extruder set from Avanti Polar Lipids was used. To record the fluorescence spectra, Cary Eclipse fluorescence spectrophotometer

was used. A zeta sizer Nano ZS instrument equipped with a He-Ne laser in backscattering mode at a scattering angle of 173° was employed for the DLS experiments. SHIMADZU Infrared spectrophotometer was used for performing FT-IR experiments using KBr pellet method.

## 3. Experimental Section




**1** **1.NO**

### 3.1 Synthesis of [$C_{18}$-Terpy-Ru($Cl_2$)NO](PF6) (1.NO)

According to the procedure reported in our previous work[1], ligand **1** was synthesized. 300 mg of ligand **1** (0.519 mmol) was refluxed in 40 ml solution of EtOH-$H_2O$ (3:1) mixture. 464 mg of KCl (6.228 mmol) and 242 mg of $K_2[RuCl_5(NO)]$ (0.6228 mmol) were added upon dissolving to the solution of **1**. The reaction was allowed to reflux for another 4 h. Upon cooling the reaction mixture was completely dried using rota-evaporator. The solid crude was then dissolved in methanol to remove any unreacted KCl and $K_2[RuCl_5(NO)]$. To the filtrate, aqueous solution of excess $NH_4PF_6$ was added and dried again using rota-evaporator. The precipitates were suspended in water and stirred to dissolve excess $NH_4PF_6$ salt. It was filtered using glass crucible (G4 sintered) to get orangish-brown solid, washed with water and diethyl ether to obtain the expected product **1.NO**. The pure final product was obtained by recrystallization using $CH_3CN$-diethyl ether mixture using diffusion method. Yield: (240 mg, 46%), IR (KBr): $\nu_{max}$ ($cm^{-1}$) 1897 (N-O). $^1$H-NMR ($CD_3CN$, 500 MHz): $\delta$(ppm) 8.79 (dd, J = 5.6 Hz, 0.9 Hz, 2H), 8.70 (s, 2H), 8.69-8.64 (m, 2H), 8.42 (td, J = 7.9 Hz, 1.4 Hz, 2H), 8.09 (d, J = 8.9 Hz, 2H), 7.91-7.87 (m, 2H), 7.23 (d, J = 8.9 Hz, 2H), 4.17 (t, J = 6.6 Hz, 2H), 1.87-1.82 (m, 2H), 1.54-1.49 (m, 2H), 1.30 (s, 28H),

0.90 (t, J = 5.3 Hz, 3H). ESI-MS: *m/z* $[M\text{-}PF_6]^+$ : calcd. 779.83, found 779.27, HR-MS: *m/z* $[M\text{-}PF_6]^+$ : calcd. 779.2433, found 779.2422.

### 3.2 Preparation of nanoscale vesicles using 1.NO and DOPC

5 mol % of the **1.NO** complex and DOPC lipid was used to prepare the unilamellar vesicles by thin-film hydration method using mini-extruder set purchased from Avanti Polar Lipids. For the preparation of vesicles, 5 µmol solution of DOPC lipid in chloroform was taken and 0.5 µmol solution of **1.NO** in $CHCl_3$ was added to it. A thin lipid film was formed upon drying the organic solvent. 5 mL of HPLC grade water was added to the thin film and sonicated for 90 minutes at 60°C to obtain multilamellar lipid suspension. A polycarbonate membrane with a pore size of 0.1 µm was used to homogenize the multilamellar lipid solution by extrusion using the Avanti Polar Lipid Extrusion Set. The orangish-colored vesicles solution was considered to be free of impurities and used without any further treatment. The prepared vesicles **Lip-1.NO** were used within a week and stored at 20°C.

### 3.3 Study of NO release from 1.NO by UV-Vis spectroscopy

5% $CH_3CN\text{-}H_2O$ solution of **1.NO** ($24 \times 10^{-6}$ M, 2 mL) was used for recording the UV-Vis absorption spectra. Each spectrum was recorded after irradiating with light ($\lambda$ = 410 nm, 3W) for 60 min. The quartz cuvette used was of 1.0 cm path length. A distance of 4 cm was maintained between the light source and the quartz cuvette for the irradiation experiments. The changes in the absorbance at 492 nm were monitored against time.

### 3.4 DAF-FM DA assay of 1.NO and Lip-1.NO

A non-fluorescent probe DAF-FM DA is used to qualitatively detect the NO release from **1.NO**. DAF-FM DA probe (10 µM) in DMSO was added to the 5% $CH_3CN\text{-}H_2O$ solution of **1.NO** (26 µM) and in buffer solution of **Lip-1.NO** (HEPES, 10 mM, pH=7.4), respectively. With the gradual addition of analyte $H_2S$ (0-2 mM), changes in emission intensity were recorded. The increase in fluorescence intensity at 515 nm was monitored to confirm the $H_2S$ induced release of NO from **1.NO**.

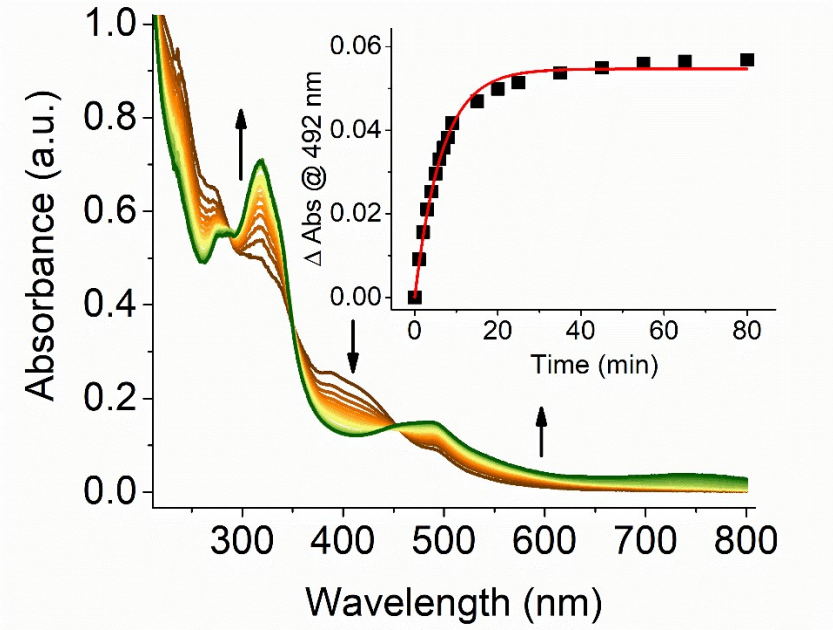



**Figure S1**: Changes in UV-Vis absorption spectra observed for **1.NO** (26 μM) in 5% $CH_3CN$-$H_2O$ when irradiated with Blue light. Repetitive scans were taken at different time intervals for 80 mins. *Inset*: Time dependent change in absorption spectra.

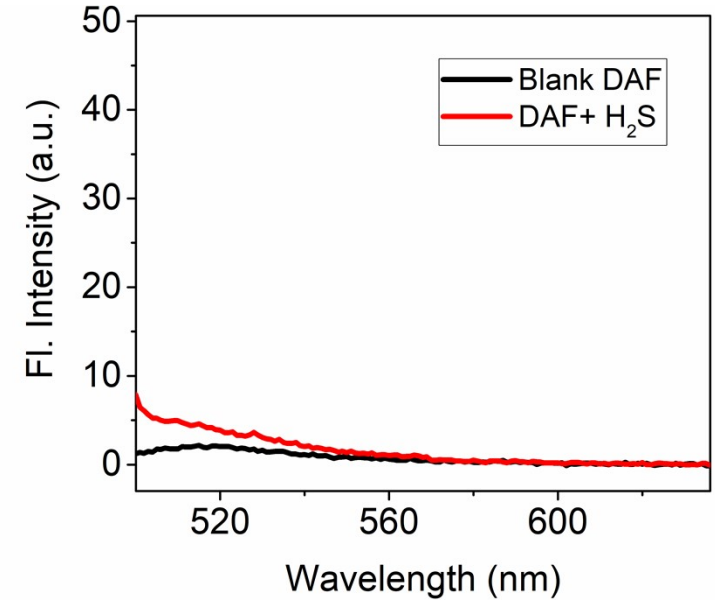



**Figure S2**: Blank DAF-FM DA assay with $H_2S$ (2mM).

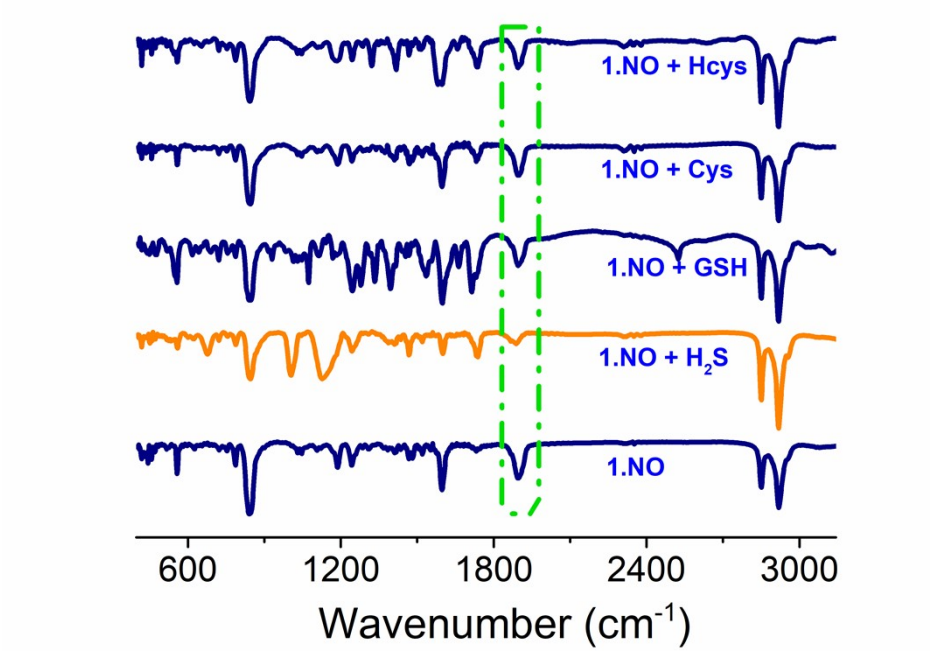
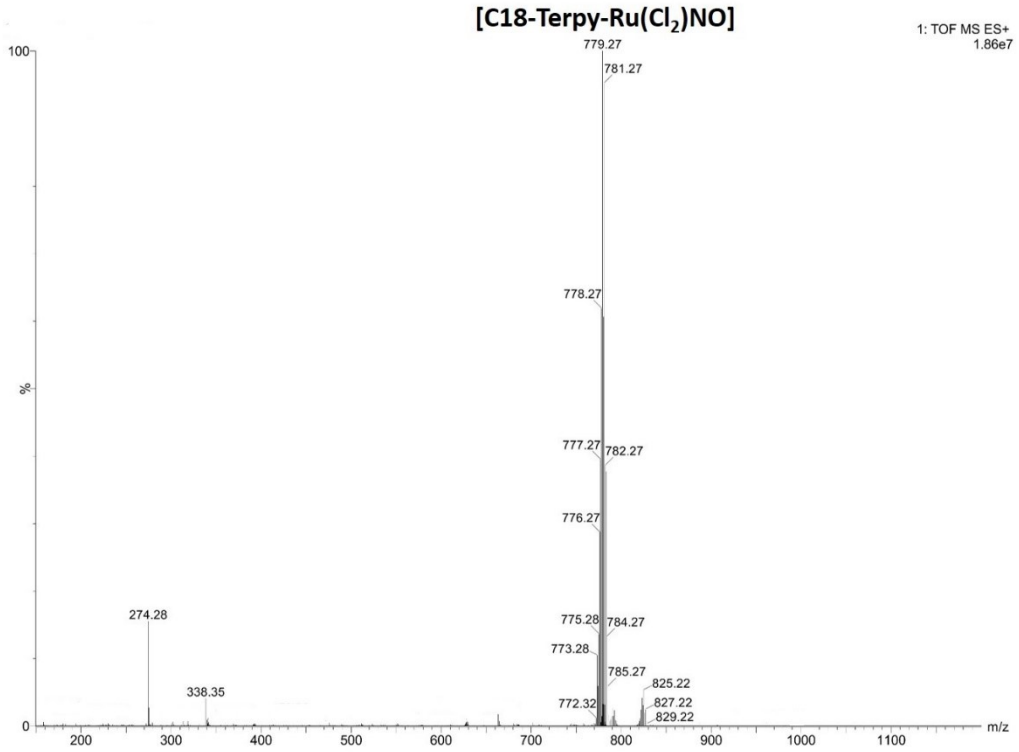
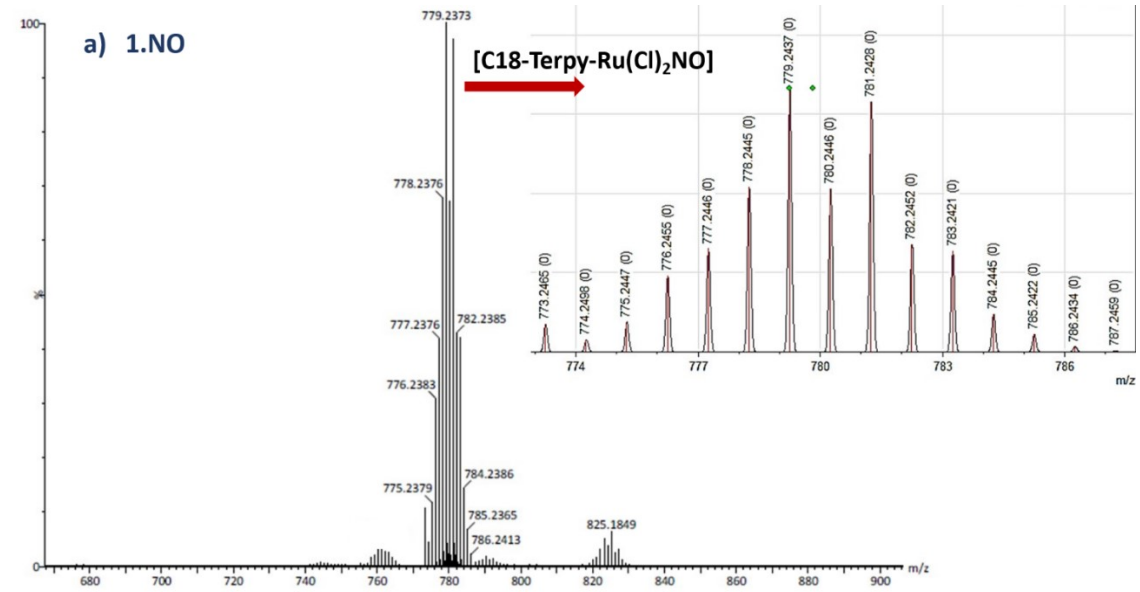
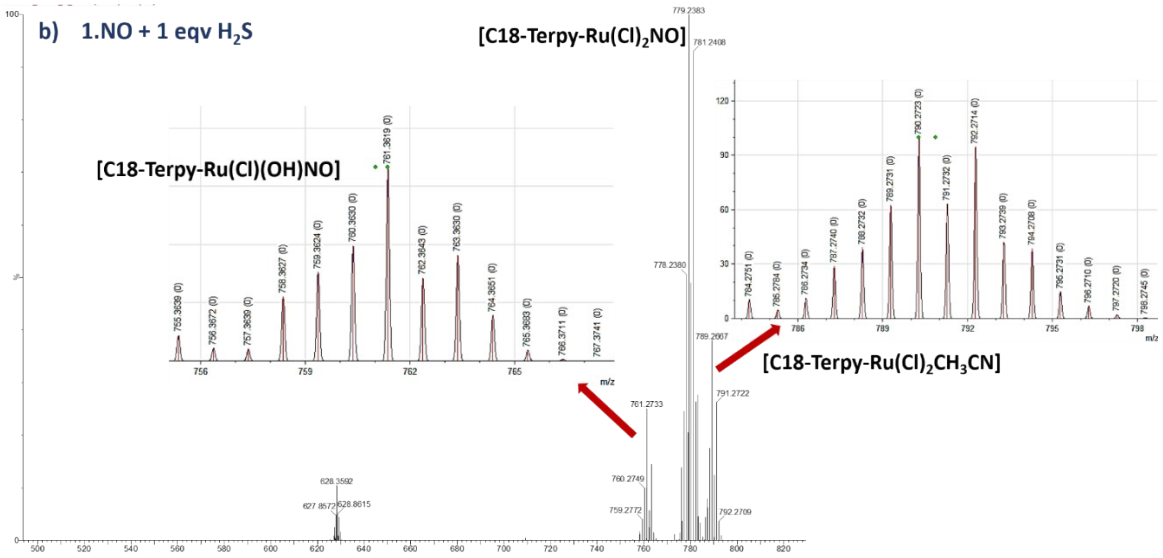



**Figure S3**: FT-IR assay of **1.NO** with the thiols.




a) 1.NO
[C18-Terpy-Ru(Cl)2NO]
779.2373
778.2376
777.2376
782.2385
776.2383
784.2386
775.2379
785.2365
786.2413
825.1849
m/z


b) 1.NO + 1 eqv H2S
[C18-Terpy-Ru(Cl)2NO]
[C18-Terpy-Ru(Cl)(OH)NO]
[C18-Terpy-Ru(Cl)2CH3CN]
m/z

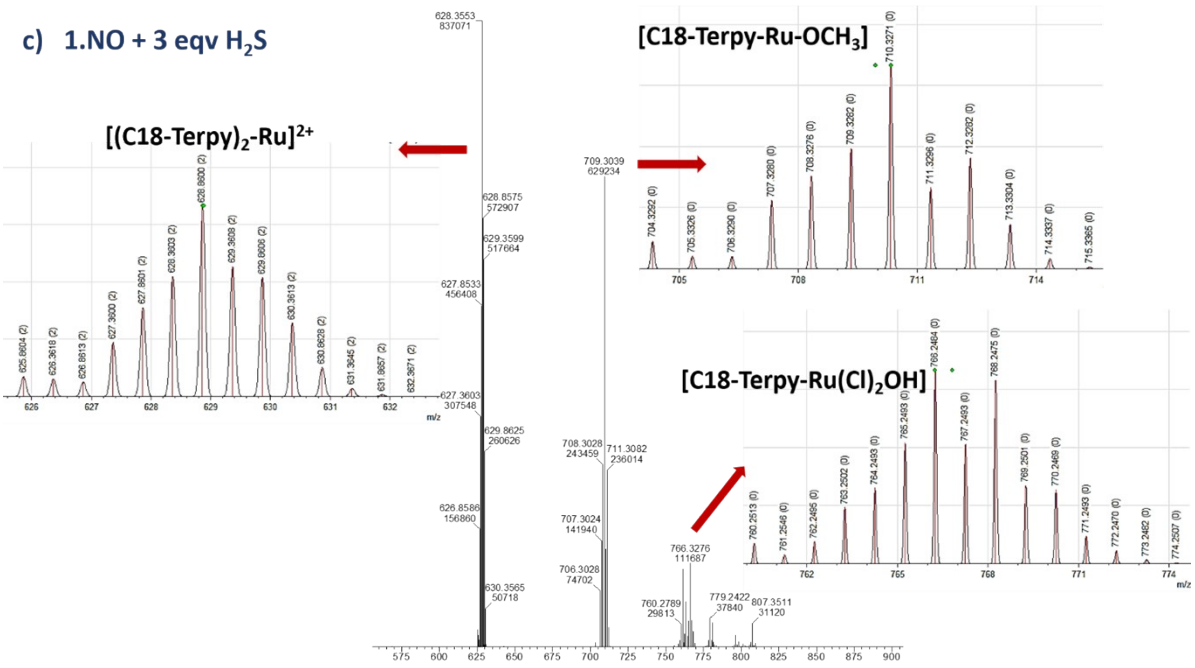



**Figure S4:** ESI-Mass (+ve mode) spectrum of (a) **1.NO**, (b) upon addition of 1 eqv $H_2S$ and (c) 3 eqv $H_2S$ in $CH_3CN$-water mixture.

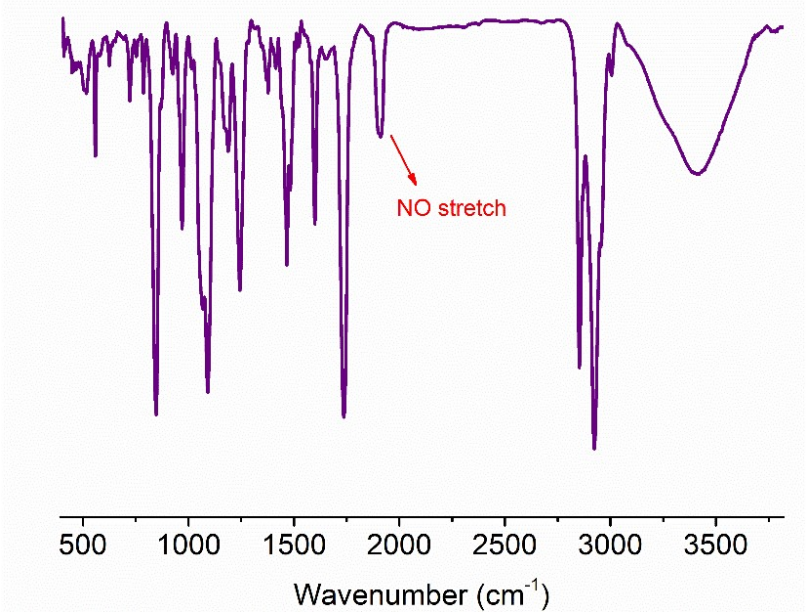



**Figure S5**: FT-IR spectrum of **Lip-1.NO**

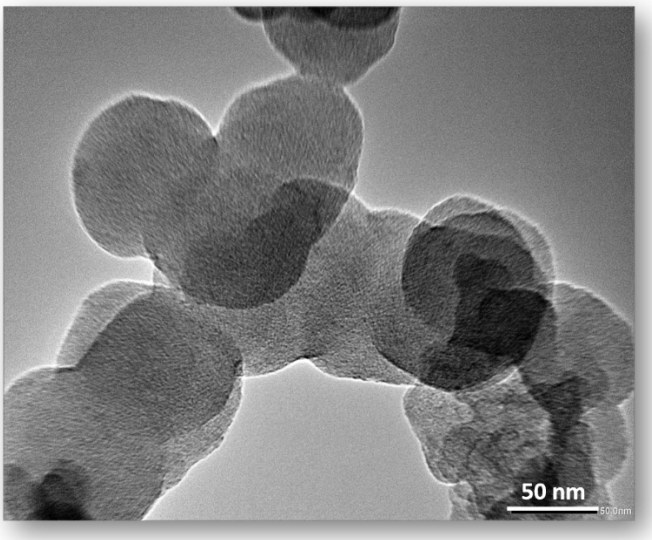



**Figure S6**: HR-TEM image of **Lip-1.NO**[2]

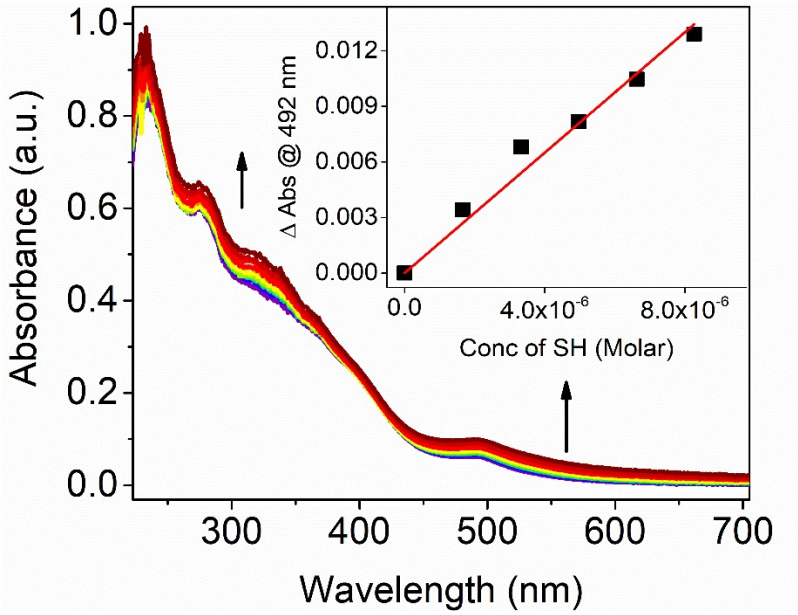
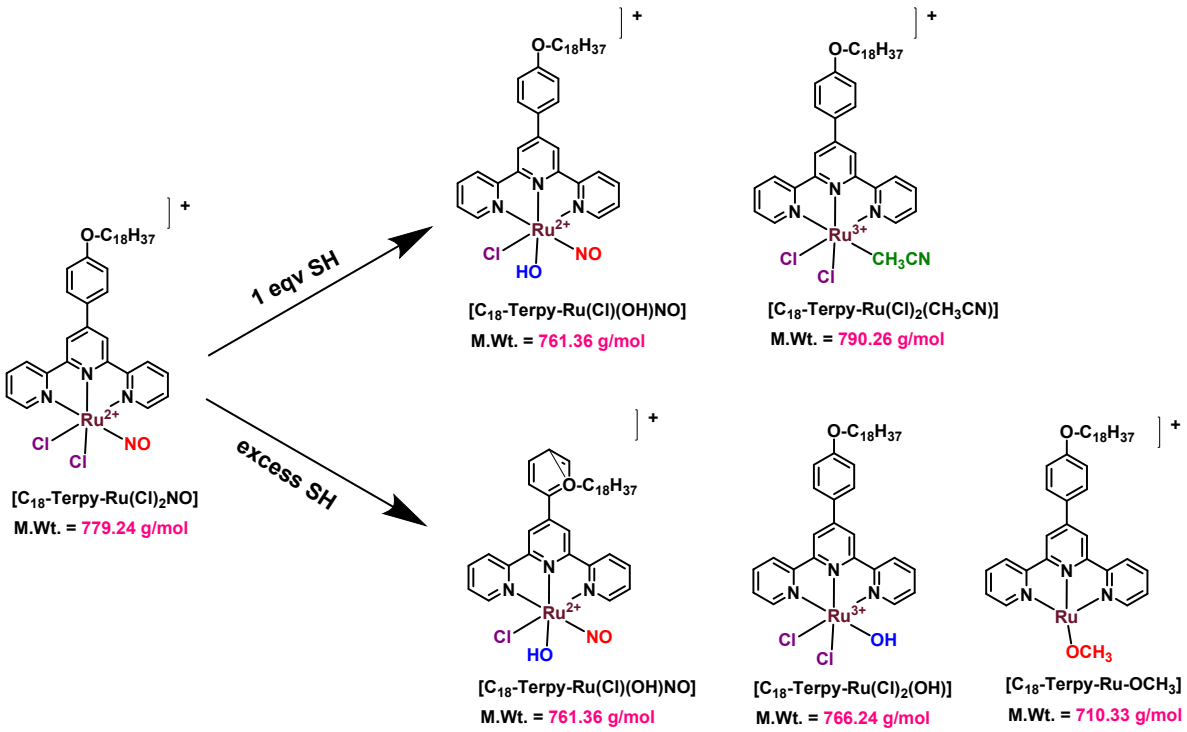



**Figure S7:** The absorption spectra of **Lip-1.NO** (24 μM) with different concentrations of $H_2S$ (0–165 μM).




**Scheme S1:** Formation various possible by products upon interaction of **1.NO** with $H_2S$ predicted by mass analysis.

## Reference:


1. N. Sharma, P. Arjunan, S. Marepally, N. Jain, A. R. Naziruddin, A. Ghosh, C. R. Mariappan and D. A. Jose, *J. Photochem. Photobiol. A: Chem*, 2022, **425**, 113703.
2. The detailed characterization of **Lip-1.NO** is described by us elsewhere in an another work published in N. Sharma, D. A. Jose, N. Jain, S. Parmar, A. Srivastav, J. Chawla, A. R. Naziruddin and C. R. Mariappan, *Langmuir*, 2022, 44, 13602–13612