Electronic Supplementary Information for

Sequential and cellular detection of copper and lactic acid by disaggregation and reaggregation of the fluorescent panchromatic fibers of anacylthiourea based sensor

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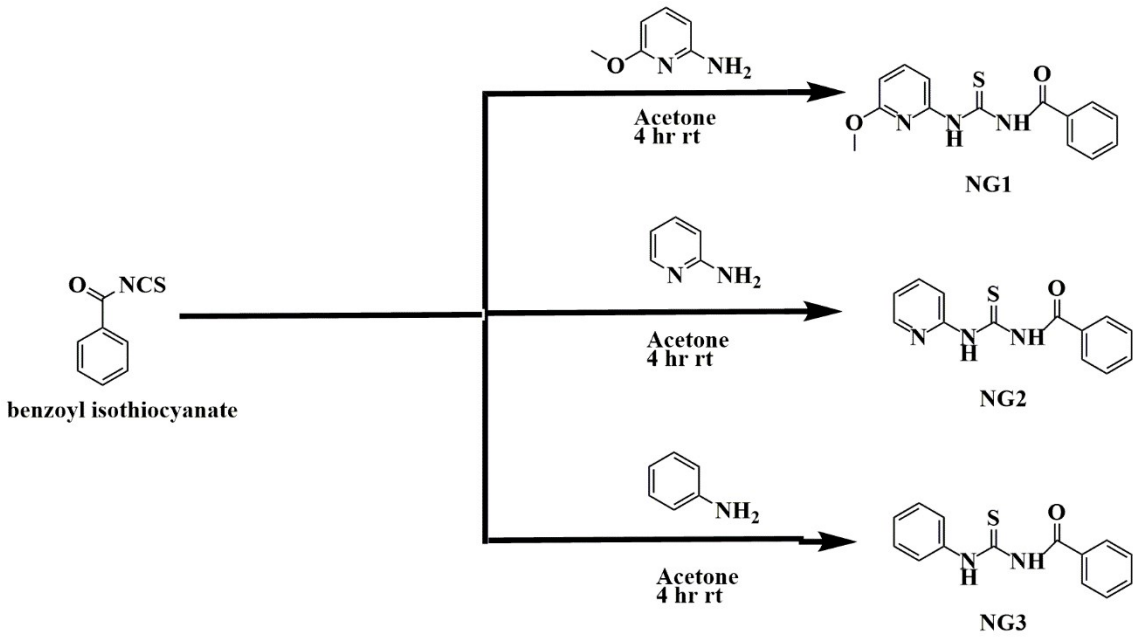
Material and methods:

All the starting materials for **NG1, NG2** and **NG3** synthesis were obtained from commercial suppliers and used as received. 2-Amino-6-methoxypyridine and Benzoyl isothiocyanate were purchased from Combi-Blocks, USA. 2-amino pyridine purchased from TCI chemicals, India, Aniline purchased form spectrochem, India. Acetone, THF, Sodium sulphate was purchased from Sisco Research Laboratories (SRL), India. Moisture sensitive reactions were performed under an atmosphere of dry Nitrogen. All the solvents used for the reactions were distilled prior to use. The Rf was recorded in Analytical TLC Silica Gel $60F_{254}$ purchased from Merck (Germany). The melting point of **NG1, NG2 and NG3** were recorded in Visual Melting Range Apparatus (MR-VIS) provided by LABINDIA. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on an Avance III 400 NMR spectrometer instrument. Proton chemical shifts were reported in parts per million. HPLC was done using the Waters E2695 machine. HPLC was performed using ammonium bicarbonate buffer (ABC) 27 minutes run time and water:ACN as mobile phase in C-18 Column. LC-MS was obtained on a Waters 2690 LC-MS instrument. The method used was Ammonium bicarbonate Buffer (ABC); 7 minutes run time and water: ACN as mobile phase in C-18 Column. UV-visible spectra were recorded on a Shimadzu UV-Vis Spectrophotometer 1900 with 10 mm quartz cell at 25 °C. Fluorescence Spectra were recorded on Horiba Jobin Yvon Fluorolog-3 spectrometer .XRD was recorded in powder mode on D8 DISCOVER (Bruker) Model.

Synthesis of NG1, NG2, and NG3:

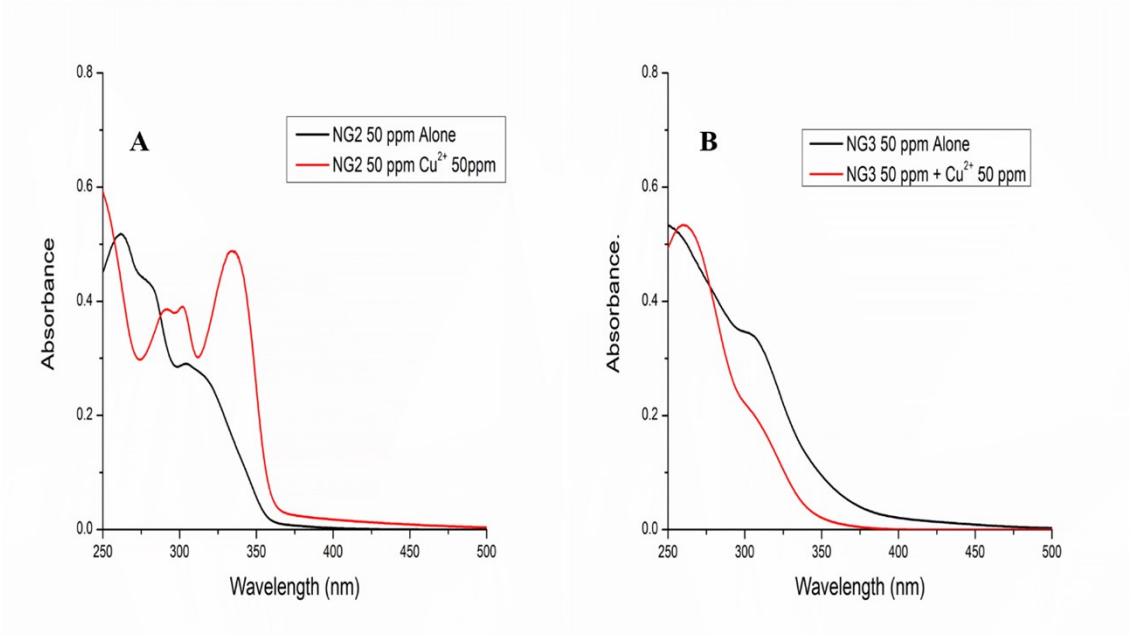


SchemeS1: Synthesis of**NG1**, **NG2**&**NG3**

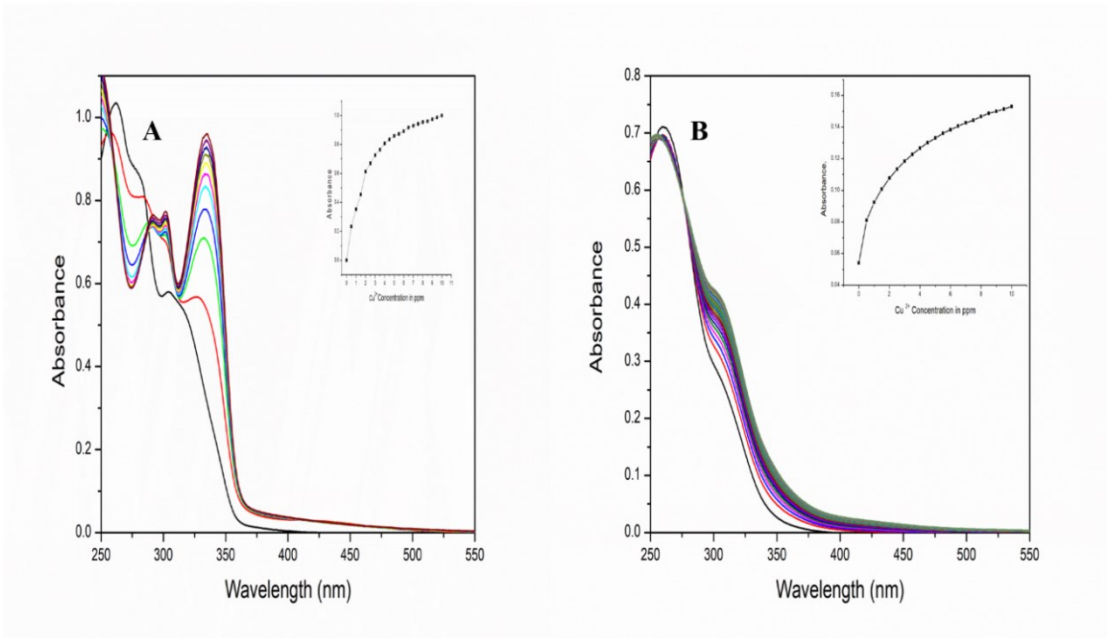
Synthesis of NG1: In a three-necked round bottom flask-fitted with a dropping funnel filled with 50mL of dry acetone- 5.0g (27 mmol) of 2-Amino 6-Methoxy pyridine was placed followed by dropwise addition of dry acetone under N_2 atmosphere during constant stirring of the reaction mixture. Next, 7.2 g (44 mmol) of benzoyl isothiocyanate was added and the reaction mixture was then allowed to stir for another 2h at room temperature. The progress of the reaction was monitored by analytical TLC by using the ethyl acetate-hexane (3:7) solvent mixture. After the completion of the reaction, the reaction mixture was then poured carefully with stirring into 500 mL of cold water and the resulting yellow precipitate of (N-((6-methoxypyridin-2-yl)carbamothioyl)benzamide) is separated by suction filtration followed by washing of precipitate with water(3x100 mL). The filtrate was further purified by vacuum distillation which yielded the desired product (10.0 g, 34mmol, Yields 86 %) as solid lightof white material.M.P.136 ^{0}C R_f 0.53. ^{1}H NMR (400 MHz, DMSO-d_6, 25^{o}C, TMS) δ(ppm) = 13.14 (s, 1H), 11.70 (s, 1H), 8.36 (s, 1H) 7.97 (t, J=8.8 Hz, 2H), 7.80 (t, J=8.0 Hz, 1H), 7.664 (m , 1), 7.53 (t, J=8.0 Hz, 2H), 6.71 (d, J=8.4 Hz, 1H), 3.83 (s, 3H), ^{13}C NMR (100 MHz, DMSO-d_6, 25 ^{o}C, TMS) δ(ppm)= 177.52, 162.54, 140.86, 140.85, 133.23, 128.75,128.44 107.68, 53.32. LCMS: obtained m/z value is 286.8 and, the calculated m/z value is 286.3 for the chemical formula: $C_{14}H_{10}N_2O_3S$.

Synthesis of NG2: In a three-necked round bottom flask-fitted with a dropping funnel filled with 50mL of dry acetone- 5.0g (27 mmol) of 2-Amino pyridine was placed followed by dropwise addition of dry acetone under N_2 atmosphere during constant stirring of the reaction mixture. Next, 6.5 g (42 mmol) of benzoyl isothiocyanate was added and the reaction mixture was then allowed to stir for another 2h at room temperature[1]. The progress of the reaction was monitored by analytical TLC by using the ethyl acetate-hexane (3:7) solvent mixture. After the completion of the reaction, the reaction mixture was then poured carefully with stirring into 500 mL of cold water and the resulting yellow precipitate of N-(pyridine-2-ylcarbamothioyl)benzamide is separated by suction filtration followed by washing of precipitate with water (3x100 mL). The filtrate was further purified by vacuum distillation which yielded the desired product (9.6 g, 34 mmol, Yields 86 %) as solid light yellow material.M.P.136 ^{0}C R_f 0.53.^{1}H NMR (400 MHz, DMSO-d_6, 25^{o}C, TMS) δ(ppm) = 13.28 (s, 1H), 11.71 (s, 1H), 8.78 (s, 1H) 8.43-8.42 (d, J=2.4 Hz, 1H), 7.98-7.89 (m, 3H), 7.67 (d , J=6.0 Hz, 1H), 7.55 (t, J=10.8 Hz, 2H), 7.27 (s, 1H).

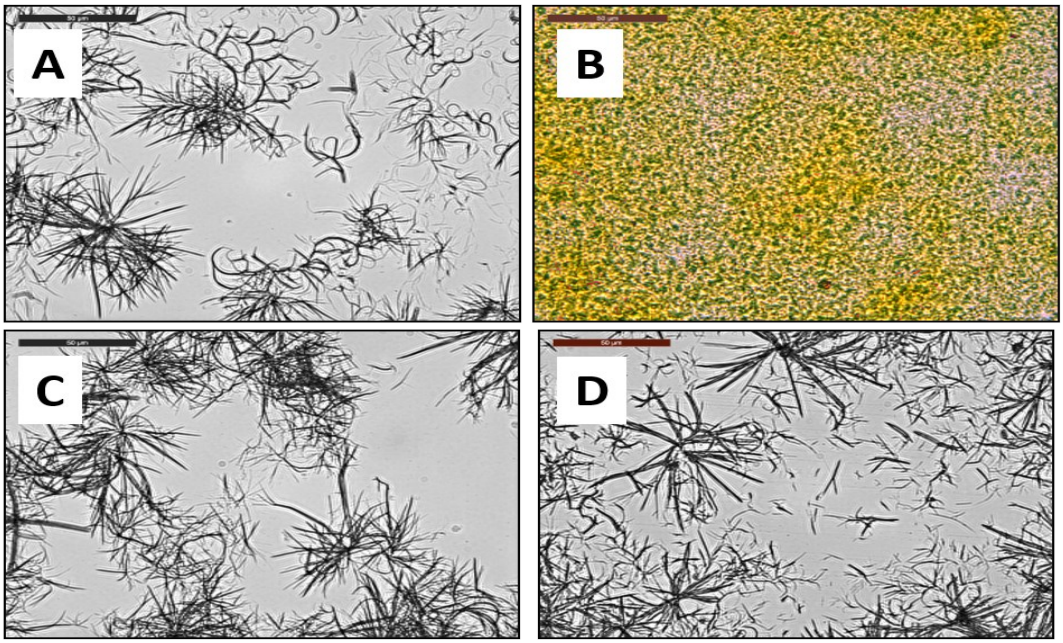
Synthesis of NG3: In a three-necked round bottom flask- fitted with a dropping funnel filled with 50mL of dry acetone- 5.0g (27 mmol) of 2-Amino 6-Methoxy pyridine was placed followed by dropwise addition of dry acetone under N_2 atmosphere during constant stirring of the reaction mixture. Next, 7.2 g (44 mmol) of benzoyl isothiocyanate was added and the reaction mixture was then allowed to stir for another 2h at room temperature[1]. The progress of the reaction was monitored by analytical TLC by using the ethyl acetate-hexane (3:7) solvent mixture. After the completion of the reaction, the reaction mixture was then poured carefully with stirring into 500 mL of cold water and the resulting yellow precipitate of N-(phenylcarbamothioyl)benzamide is separated by suction filtration followed by washing of precipitate with water(3x100 mL). The filtrate was further purified by vacuum distillation which yielded desired product (10.0 g, 34mmol, Yields 86 %) as solid light yellow material.M.P.136 ^{0}C R_f 0.53 (This material was used in next step without any further purification.^{1}H NMR (400 MHz, DMSO-d_6, 25^{o}C, TMS) δ(ppm) = 12.59 (s, 1H), 11.56 (s, 1H), 7.96 (t, J=5.6 Hz, 2H) 7.69-7.64 (m, 3H), 7.53 (t, J=12.4 Hz, 2H), 7.42 (t, J=12.4 Hz, 2H), 7.26 (s, 1H), **^{13}C** NMR (100 MHz, DMSO-d_6, 25 ^{o}C, TMS) δ(ppm)= 179.59, 168.76, 138.48, 133.60, 129.16, 128.92, 126.80, 124.79.



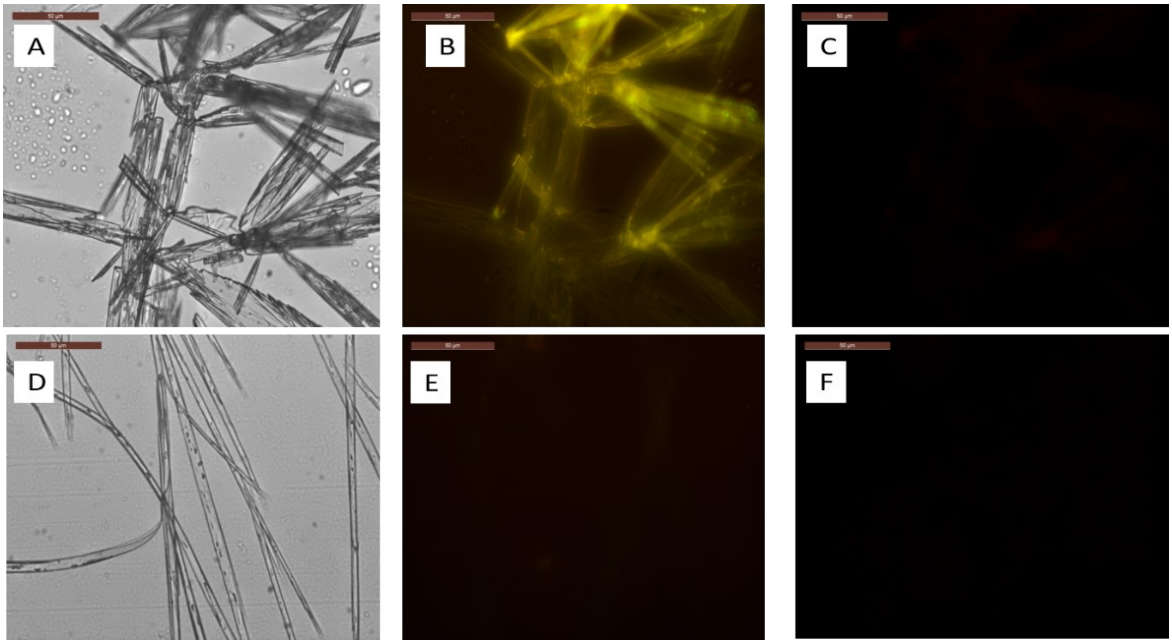
FigureS1 (**A**)UV spectra **NG2** with and without Cu^{2+} 50 ppm;(**B**) UV spectra of **NG3**with and without Cu^{2+} at 50 ppm



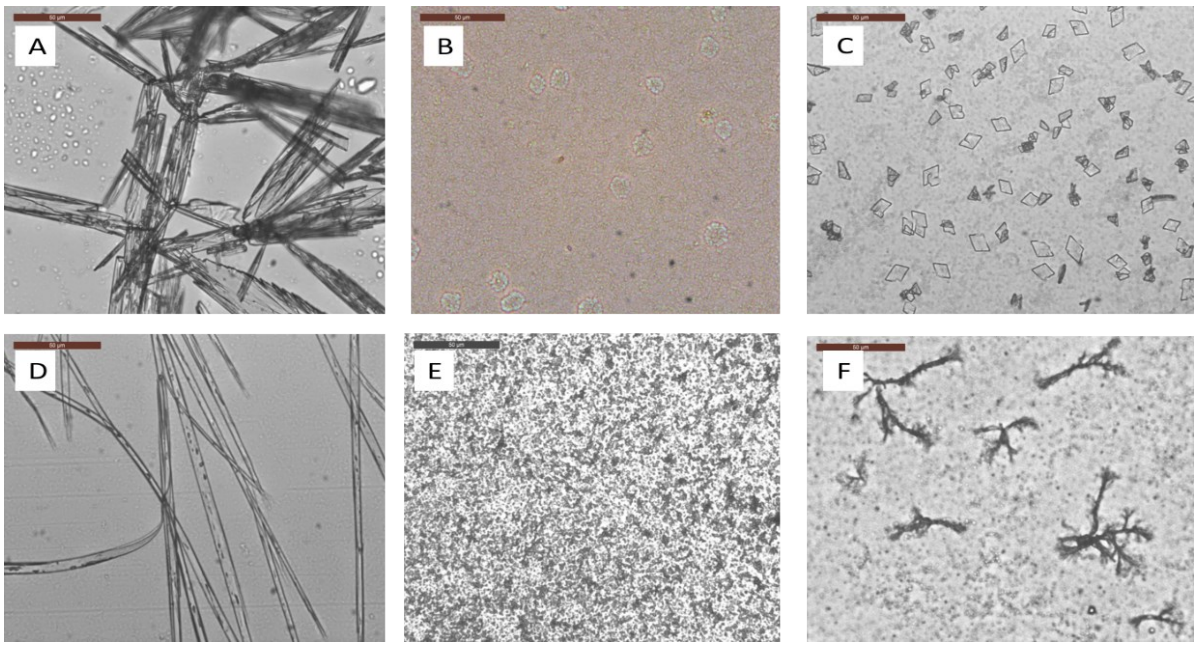
FigureS2(**A**) UV spectra of **NG2** with varying concentration of Cu^{2+} ions from 0 to 50 ppm; (**B**) UV Spectra of **NG3** with varying concentration of Cu^{2+} ionsfrom 0 to 50 ppm



FigureS3 Optical microscopic images of **A**) **NG1**at1 mM concentration in bright field at 40X; **B**) **NG1**+Cu^{2+}complex at 1:1 ratio in bright field at 40X; **C**) **NG1**+Zn^{2+} complex at 1:1ratio in bright field at 40X; **D**) **NG1**+Mg^{2+}complex at 1:1 ratio in bright field at 40X.



FigureS4 Optical microscopic images of **A**) **NG2** at1 mM concentration in bright field at 40X; **B**) **NG2**+Cu^{2+}complex at 1:1 ratio in green filter at 40X; **C**) **NG2**+Cu^{2+} complex at 1:1 red filter at 40X; **D**) **NG3** at1 mM concentration in bright field at 40X; **E**) **NG3**+Cu^{2+}complex at 1:1 ratio in green filter at 40X; **F**) **NG3**+Cu^{2+} complex at 1:1 red filter at 40X.



FigureS5 Optical microscopic images of **A**) **NG2** at1 mM concentration in bright field at 40X; **B**) **NG2**+Cu^{2+}complex at 1:1 ratio in Bright field at 40X; **C**) **NG1**+Cu^{2+}+ Lactic acid

complex at 1:1 Bright field at 40X;**D**) **NG3** at1 mM concentration in bright field at 40X; **B**) **NG3**+Cu^{2+}complex at 1:1 ratio in Bright field at 40X; **C**) **NG3**+Cu^{2+}+ Lactic acid complex at 1:1 Bright field at 40X.

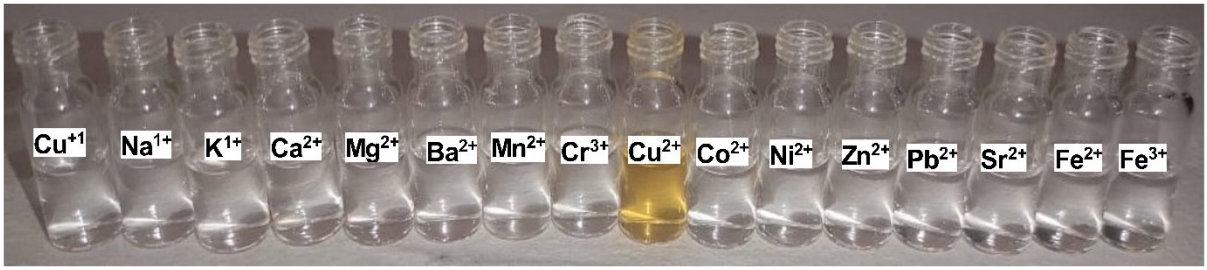
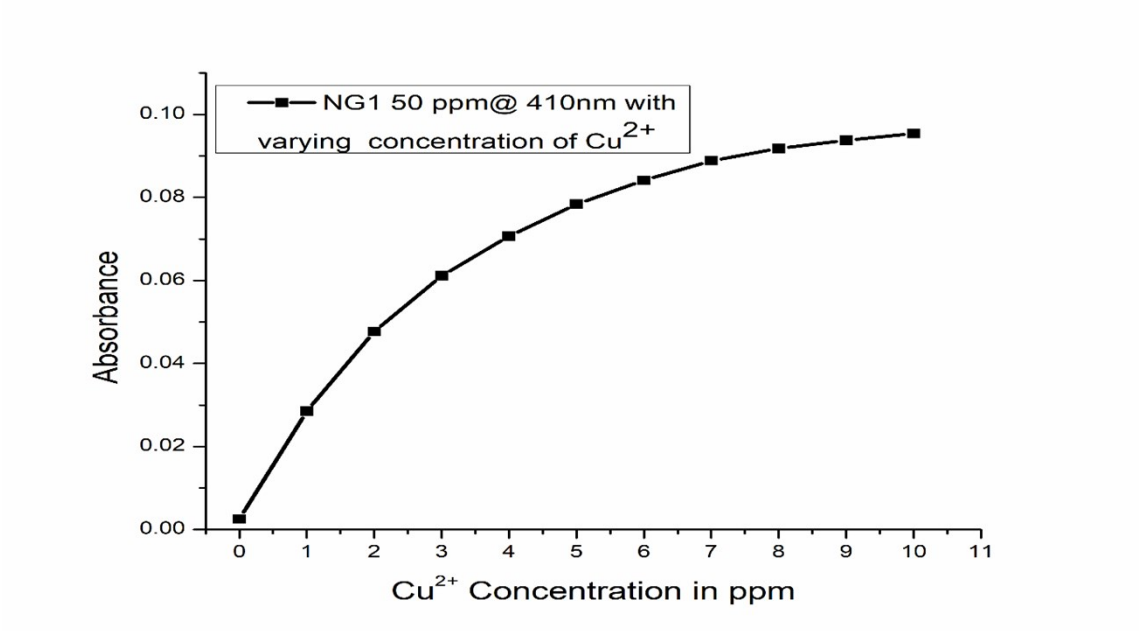
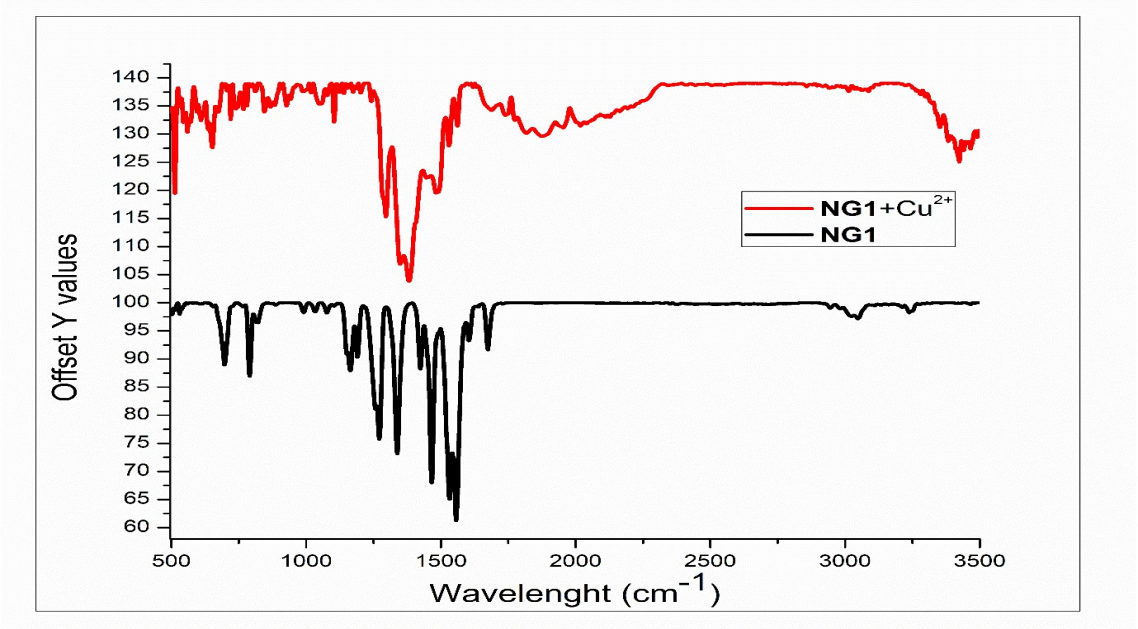


Figure S6 Vial images of **NG1** with Cu^{2+} ions and other metals showing yellow colour and selectivity for Cu^{2+} ions



FigureS7 LOD at the wavelength 410 nm



FigureS8 (**A**) FTIR spectra of Copper nitrate hemihydrate and **NG1**-Cu^{2+} complex.

FTIR spectra of Copper nitrate hydrated alone revealed 1043 and 798 cm^{-1} bands which may be attributed to NO^{3-} bands The main IR bands of **NG1** have been identified to show five absorption bands, namely, ν(N-H), ν(C-H), ν(C=O), ν(C-N), and ν(C=S). The first absorption band can be assigned as the secondary amine N-H which could be observed at 3241.36 cm-1 as a medium intensity absorption band. In most thiourea derivatives, N-H stretching band appears at above 3200 cm^{-1} due to the influence of strong intramolecular hydrogen bond between N-H…O=C which led to the formation of the broad and weak absorption band of N-H stretching. Meanwhile, C-H alkane stretching bands could be observed in the region of 2974 to 3037 cm-1 as aromatic and alkyl type of CH groups were present in their molecular structures. In addition, in a range of 1555 cm^{-1}, the high intensity of ν(C-N) absorption band could be observed. Strong absorption band observed at 1606 to 1675 cm-1 in both spectra may be assigned to C=O absorption band. The C=O stretch in all the synthesized compounds were found at lower wavenumber compared with the expected carbonyl stretching at around 1700 cm-1 as most carbonyl thiourea derivatives were stabilized by the formation of intramolecular hydrogen bond between C=O…H-N which lead to emergence of pseudo-six-membered ring. Consequently, the formation of the intramolecular hydrogen bond interaction led to an increase in its polarity making the double bond character to be weaker and shifting the band to a lower wavenumber region. The absorption band of C=S stretch for all synthesized compounds appear as medium bands within range of 739 to 741 cm-1. The FTIR

spectrum of the **NG1** shows a broad vibration band at 3241 cm^{-1} which corresponds to the N–H group. The most intense band observed at 1675 cm^{-1} was ascribed to the stretching vibration of the carbonyl group. After the complexation reaction, the vibration band of the carbonyl group shifted to lower wavenumber (1675 to 1620 cm-1). The stretching vibration band of the C=S group in the ligand was observed at 1382 cm^{-1}. The shifted vibration band of the C=S group in the complexes cannot be observed because of overlapping with the other bands in that region. Complexation reaction is confirmed by the shifting of the N–H band, C=O, and C=S bands.

	Optimized Energy (Hartree)	Position 1 with Cu (Hartree)	Position 2 with Cu (Hartree)	With Lactic Acid (Hartree)
NG2	-1139.9170	-2780.4292 {-25.06}	-2780.4032 {-8.73}	-3124.1970 {-120.91}
NG3	-1123.8654	-2764.3567 {-11.97}	-2764.3385 {-0.55}	-3108.1511 {-124.46}

The binding energies in kcal/mol are provided in curly braces.

Table 1. Optimized energy (in Hartree) and binding energy (in kcal/mol) of the Complex **NG2 and NG3**

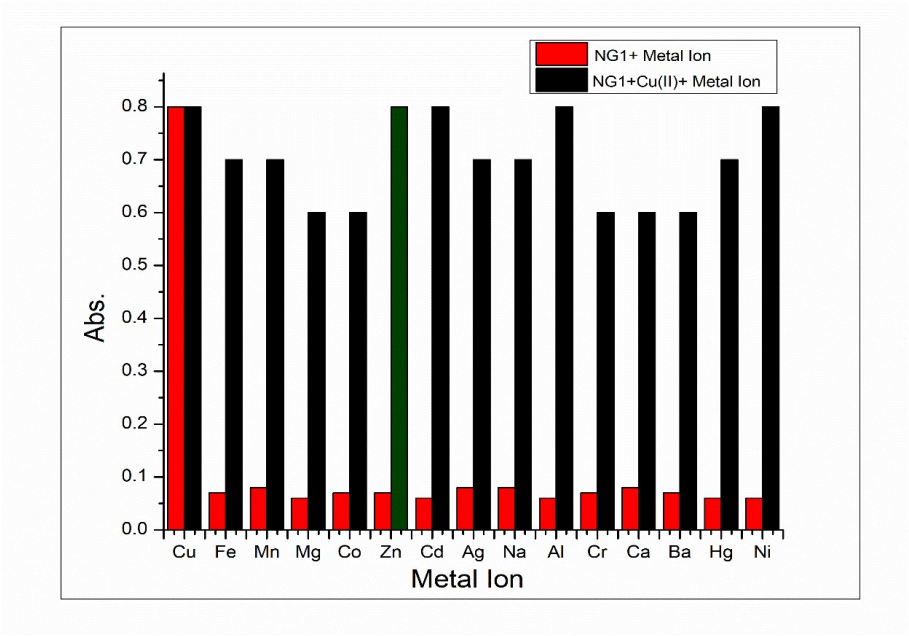


Figure S9. (a) Bar graph representing the change of the relative intensity of **NG1** at 410 nm upon treatment with various metal ions.

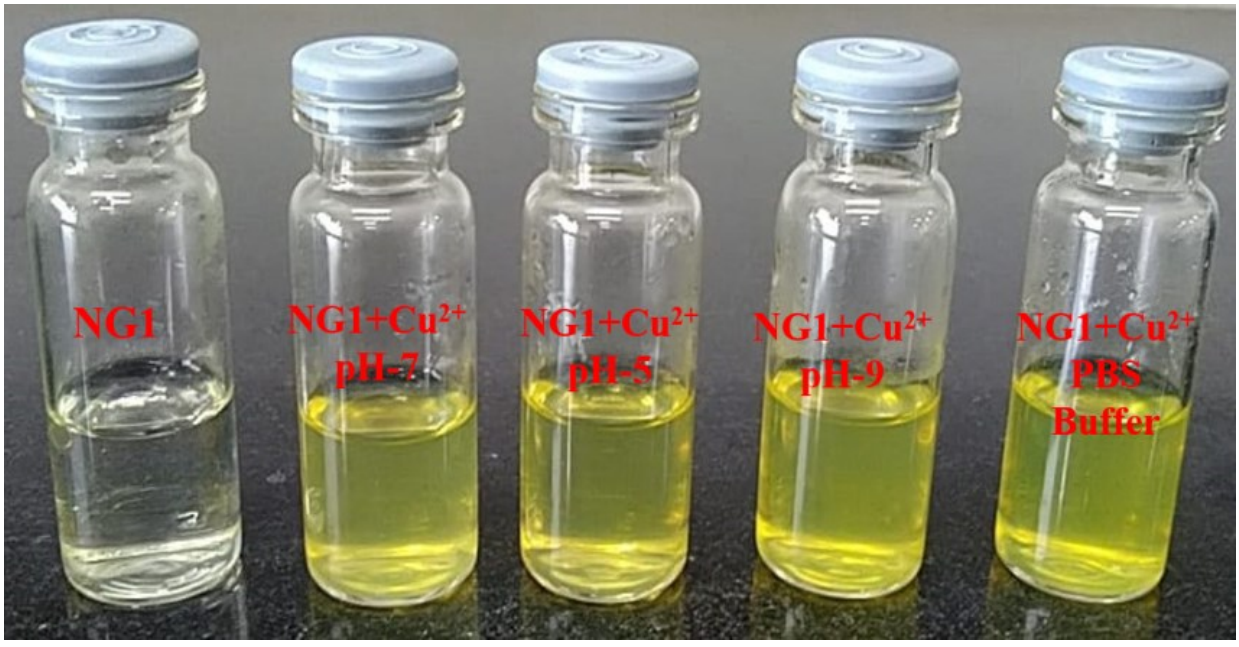
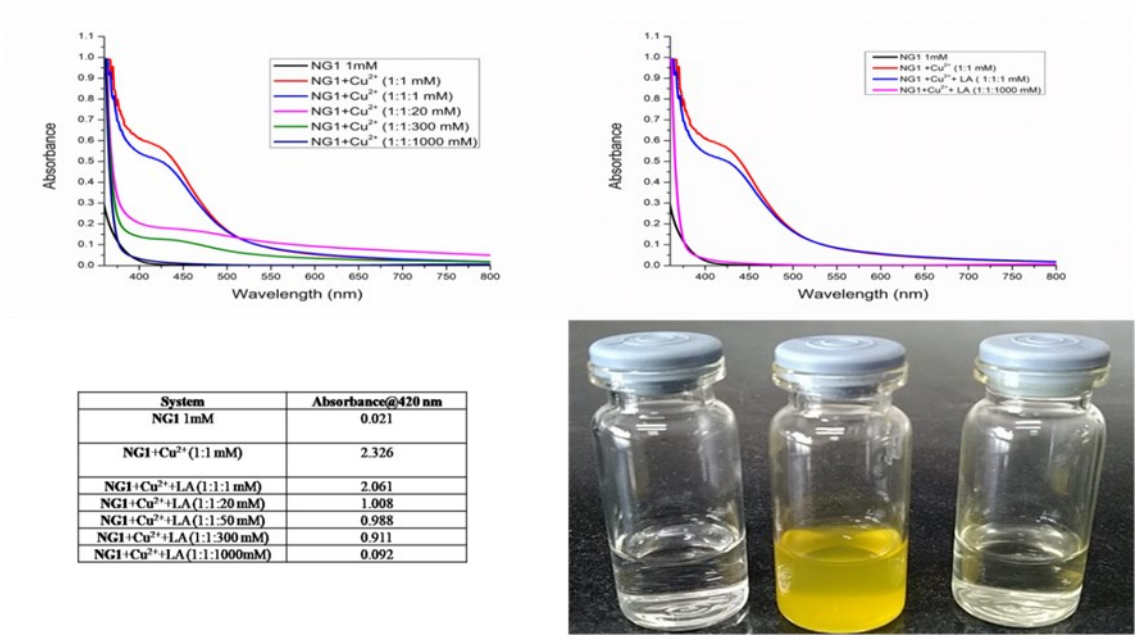


Figure S10. Image of vials capturing**NG1** at different pH. **NG1** pH was made acidic till pH 5.0 and also basic pH 9.0. Also, **NG1** was dissolved in PBS buffer pH 7.4 and subsequently Cu^{2+} was added. In all of these vials **NG1** was able to sense Cu^{2+} and produced yellow colour, indicating pH does not affect the sensing properties.



System	Absorbance@420 nm
NG1 1mM	0.021
NG1+Cu^{2+} (1:1 mM)	2.326
NG1+Cu^{2+}+LA (1:1:1 mM)	2.061
NG1+Cu^{2+}+LA (1:1:20 mM)	1.008
NG1+Cu^{2+}+LA (1:1:50 mM)	0.988
NG1+Cu^{2+}+LA (1:1:300 mM)	0.911
NG1+Cu^{2+}+LA (1:1:1000mM)	0.092

Figure S11. UV visible spectra of colour change on sequential addition of Cu^{2+} and Lactic acid.

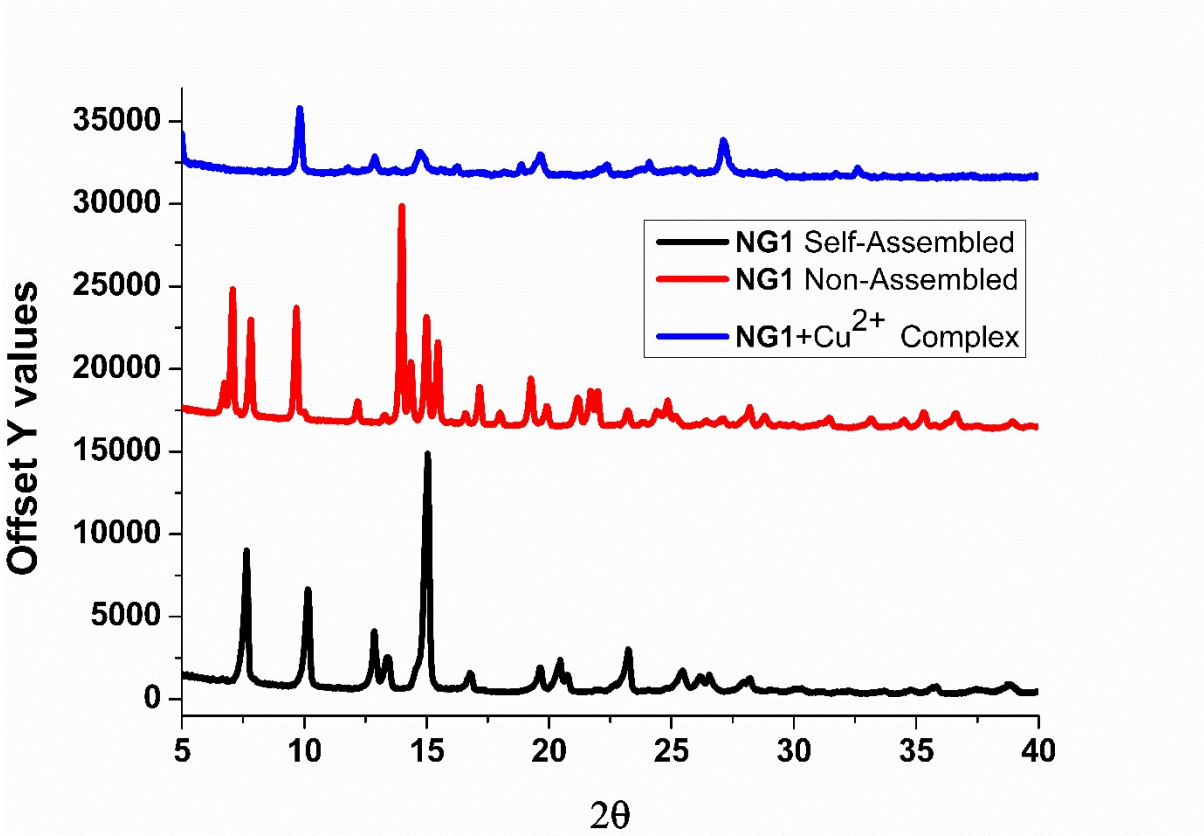
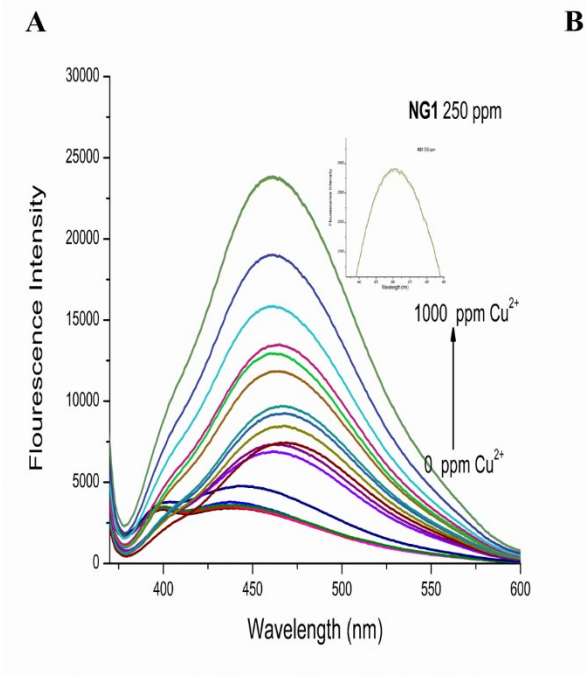


Figure S12 Powder XRD Offset graph of Non-assembled **NG1**, Assembled **NG1** and **NG1**+Cu^{2+} complex.

A



B

Cu2+ Concentration	Copeer citrate	Copper lactate
0.001	3402.788	3402.788
0.01	3433.675	3403.225
0.1	3775.450 (LOD)	3418.363
1	4727.056	3437.394
10	5935.344	3441.824
100	13764.67	3445.794
1000	21281.342	3459.444

Figure S13. Fluorescence spectra of **NG1, NG1**+Cu^{+2} recorded using excitation wavelngth 353nm; A) Fluorescence spectra with increasing concentration of Cu^{2+} from 0 to 100 ppm; B) Fluorescence intensity of Copper citrate complex and copper lactate with increasing concentration of Cu^{2+} ions.

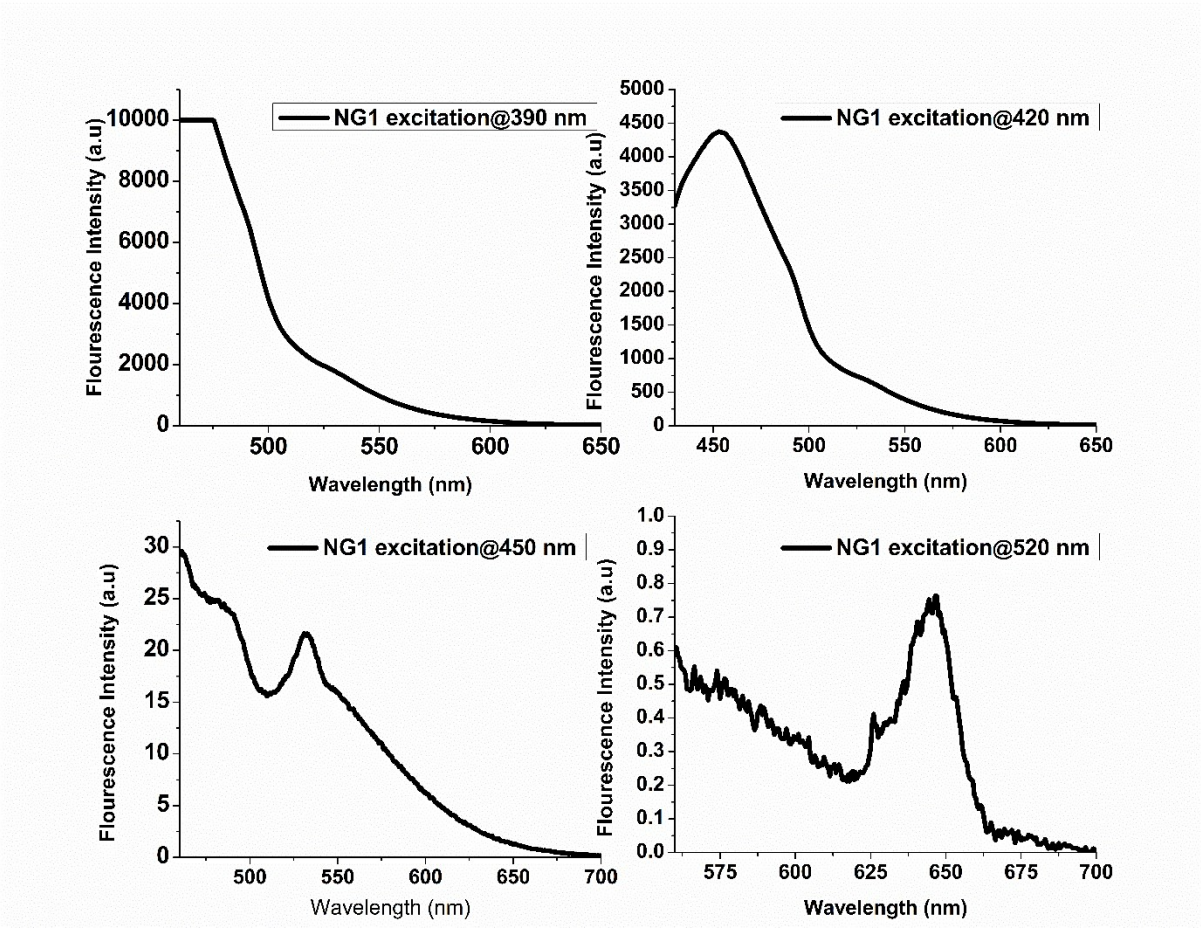
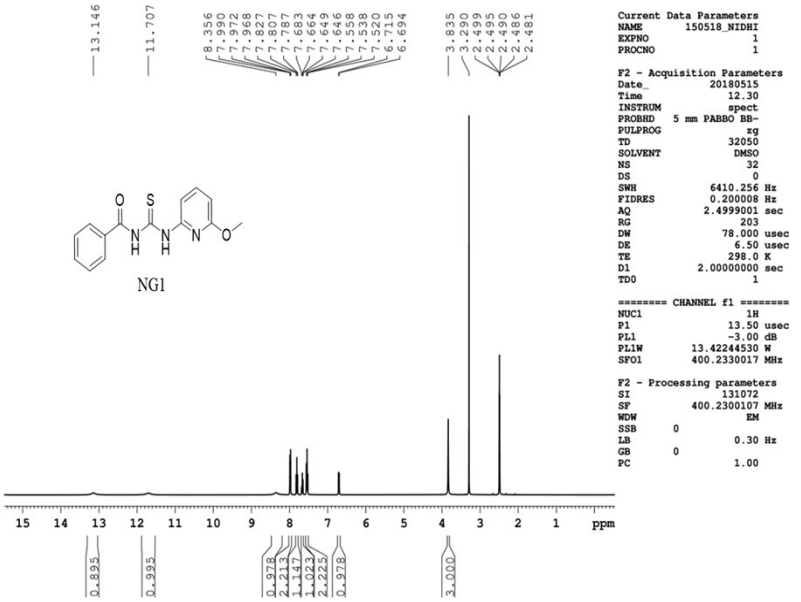
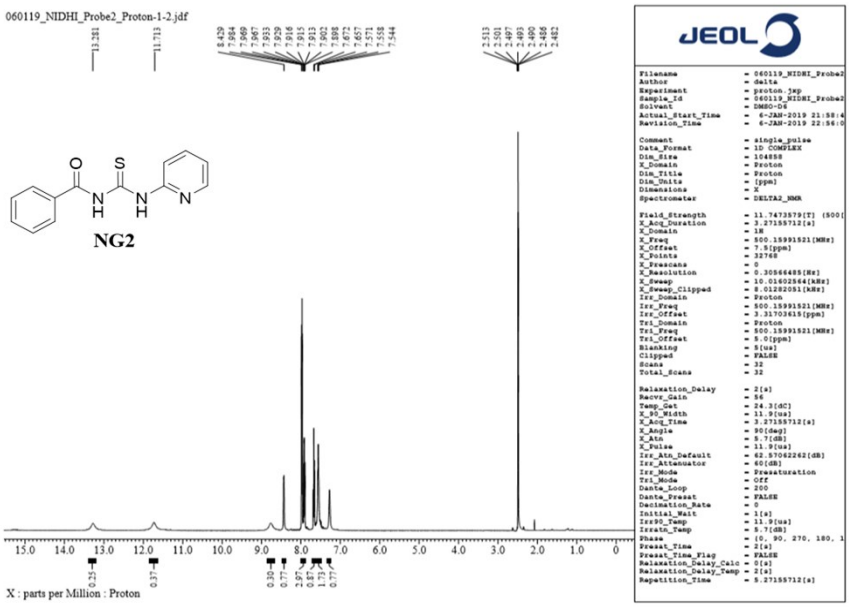


Figure S14: Fluorescence spectra of **NG1** recorded at different excitation wavelength

FigureS15: 1H-NMR spectra of **NG1**, **NG2** and **NG3**

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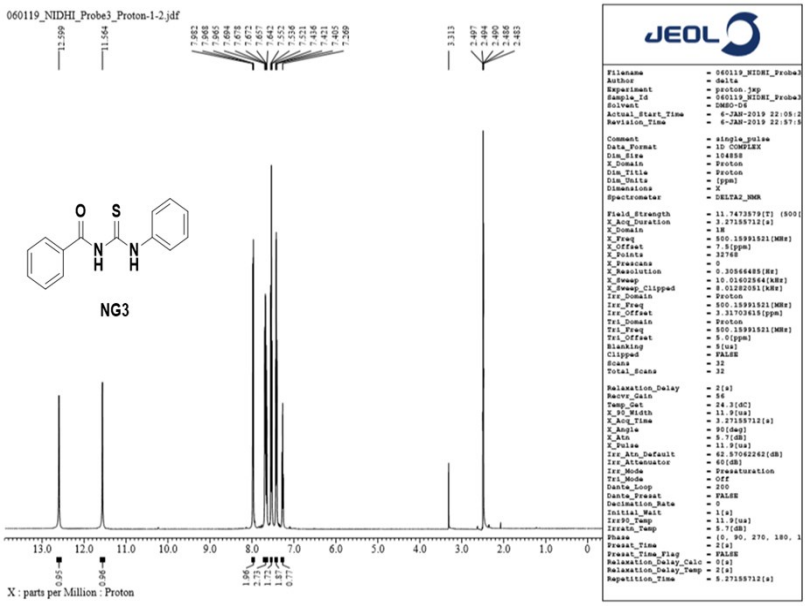
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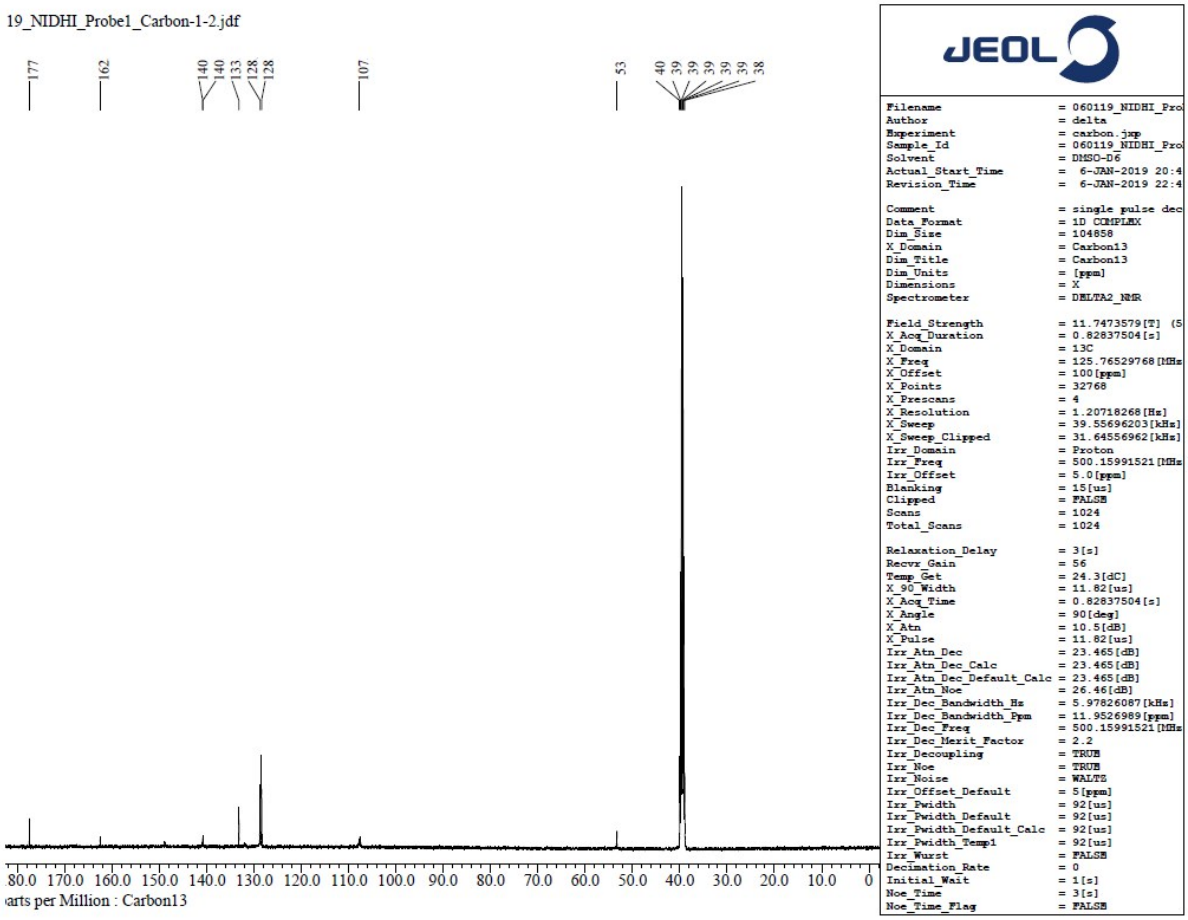
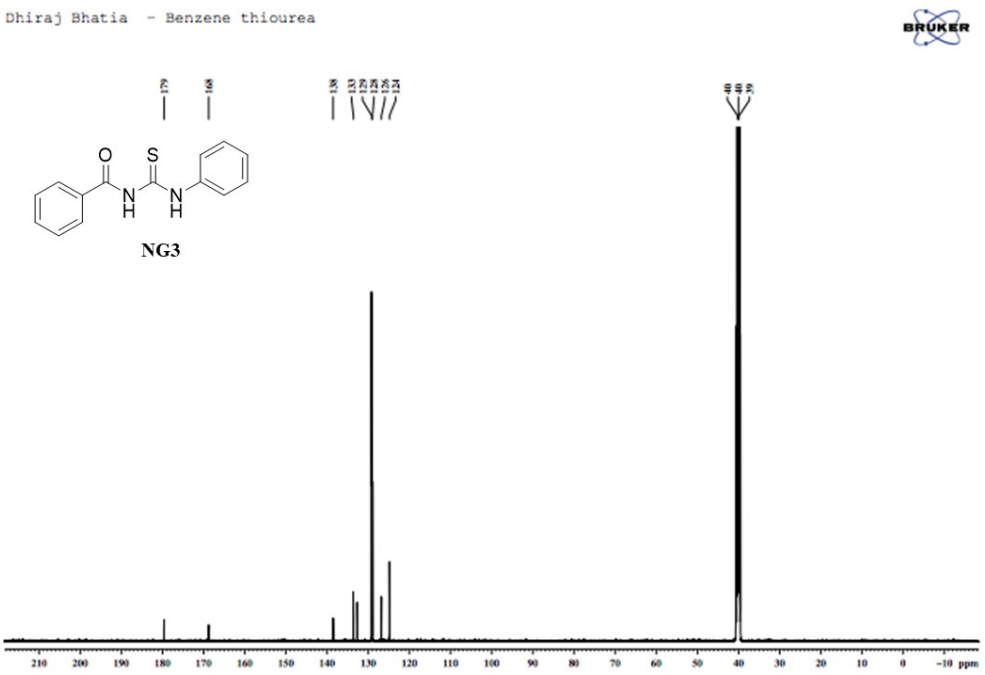
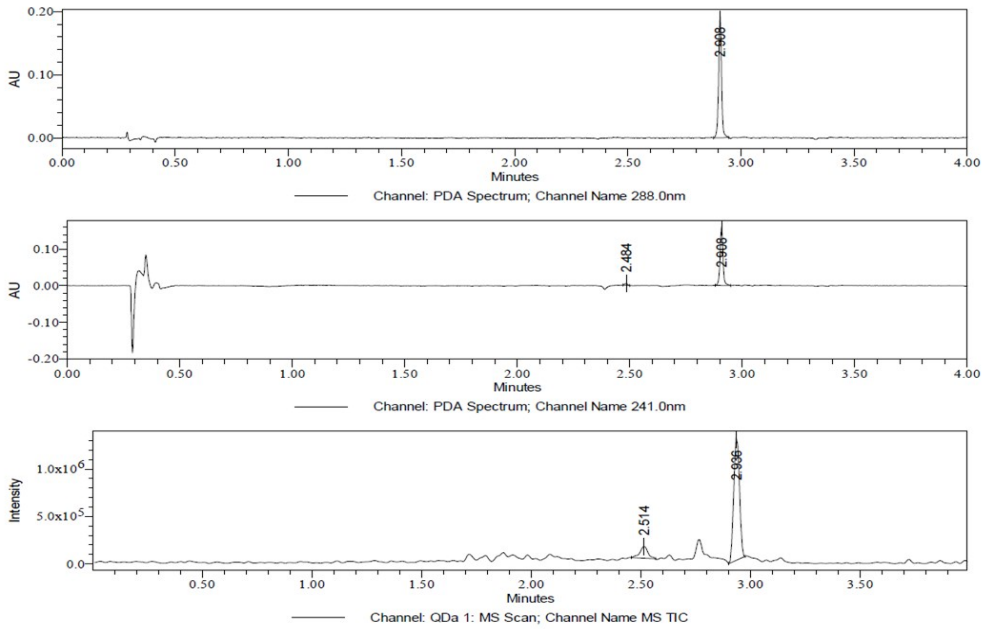
X_Atn = 5.7[dB]
X_Pulse = 11.9[us]
Irr_Atn_Default = 62.57062262[dB]
Irr_Attenuator = 60[dB]
Irr_Mode = Presaturation
Tri_Mode = Off
Dante_Loop = 200
Dante_Preset = FALSE
Decimation_Rate = 0
Initial_Wait = 1[s]
Irr90_Temp = 11.9[us]
Irratn_Temp = 5.7[dB]
Phase = (0, 90, 270, 180, 1
Preset_Time = 2[s]
Preset_Time_Flag = FALSE
Relaxation_Delay_Calc = 0[s]
Relaxation_Delay_Temp = 2[s]
Repetition_Time = 5.27155712[s]



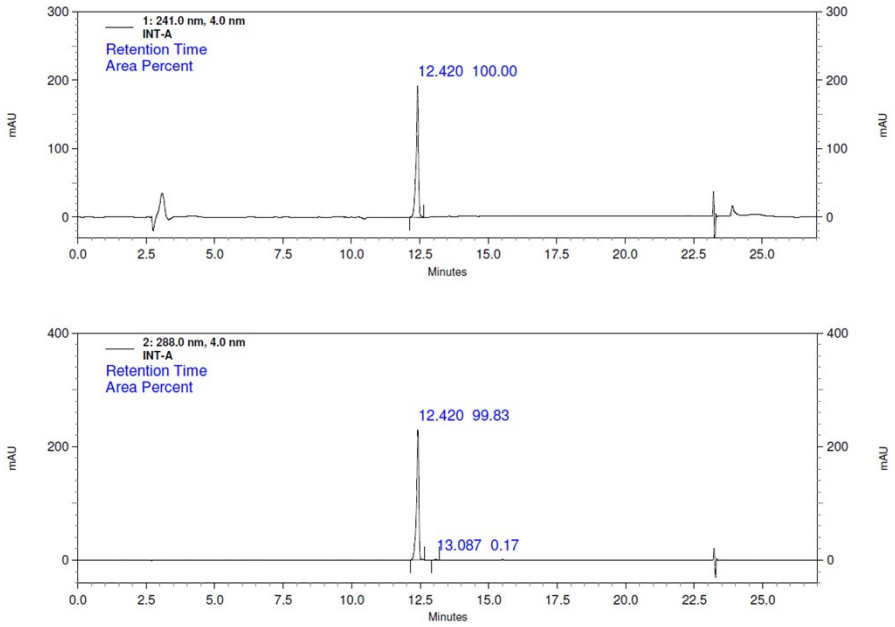
FigureS16: C-13 NMR spectra **NG1**



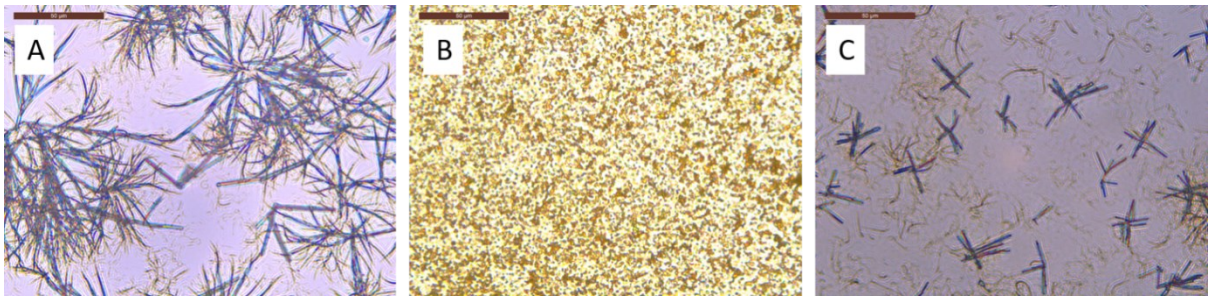
FigureS17 C-13 NMR spectra **NG3**



FigureS18 LCMS spectra of **NG1**



FigureS19:HPLC spectra of **NG1**



FigureS20 Optical microscopic images of **NG1** A) **NG1**alone; B) **NG1**+Cu^{2+}; C) **NG1**+Cu^{2+}+EDTA

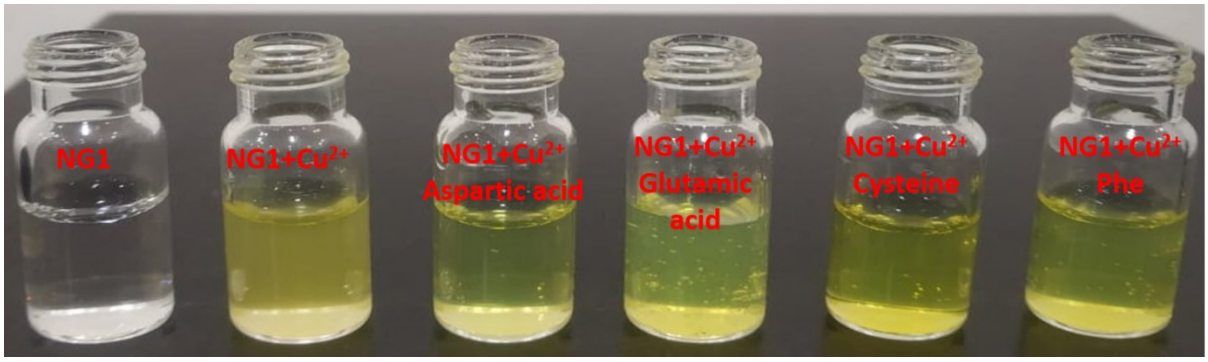


Figure S21depicts the **NG1**:Cu^{2+}complex does notshows any colour changes effects in presence of Aspartic acid, Glutamicacid, Cysteine, and Phenylalanine.