Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Water-soluble copper pyrithione complexes with cytotoxic and antibacterial activity

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Supporting Information

Contents

Synthetic Procedures:

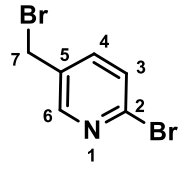
Materials:

Commercially available reagents were purchased from Merck Life-sciences, Fluorochem and Fischer Scientific and were used as received from suppliers. Solvents were laboratory reagent grade and dried using an appropriate drying agent when required. Reactions requiring anhydrous conditions were carried out under an atmosphere of dry nitrogen using Schlenk-line techniques.

NMR spectra (^{1}H, ^{13}C{^{1}H}) were recorded on a Varian VXR-600 spectrometer (^{1}H at 599 MHz, ^{13}C{^{1}H} at 151 MHz). Spectra were recorded at 295 K in commercially available deuterated solvents and referenced internally to the residual solvent proton resonances. Electrospray, Atmospheric Pressure and high-resolution mass spectrometry were performed on an SQD mass spectrometer with Acquity UPLC.

Methods:

2-Bromo-5-bromomethylpyridine (1)



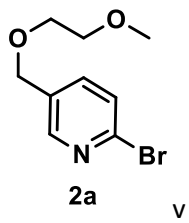
1

2-Bromo-5-methylpyridine (0.33 ml, 2.91 mmol) was dissolved in 20 ml of dry chloroform under N_2 atmosphere. This solution was then charged with *N*-bromosuccinimide (NBS) (570 mg, 3.2 mmol) and benzoyl peroxide (35 mg, 0.15 mmol) and the reaction mixture was left to reflux overnight. After cooling the mixture to room temperature, the solvent was evaporated to obtain a yellow oil as crude product. The crude product was purified by silica gel column chromatography using 90% hexane:10% EtOAc mixture as the eluent to produce *compound **1*** as white solid (316 mg, 44%).

δ_H (599 MHz, Chloroform-*d*) 8.37 (1H, d, $^{4}J_{H\text{-}H}$ 2.5 Hz, H^{6}), 7.58 (1H, dd, $^{3}J_{H\text{-}H}$ 8.2 Hz, $^{4}J_{H\text{-}H}$ 2.5 Hz, H^{4}), 7.47 (1H, dd, $^{3}J_{H\text{-}H}$ 8.2 Hz, $^{5}J_{H\text{-}H}$ 0.7 Hz, H^{3}), 4.40 (2H, s, H^{7}); **δ_C** (151 MHz, Chloroform-*d*) 150.0 (1C, C^{6}), 141.9 (1C, C^{2}), 139.1 (1C, C^{4}), 133.0 (1C, C^{5}), 128.3 (1C, C^{3}),

28.3 (1C, C^7); *m/z* (ESI HRMS$^+$) 249.8872 $[M + H]^+$ ($C_6H_6{}^{79}Br_2N$ requires 249.8867); R_f = 0.5 (silica, 80%Hexane:20% EtOAc).

2-Bromo-5-(2-methoxyethoxy)methyl)pyridine (2a)



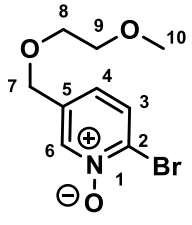
2a

v

2-Bromo-5-(bromomethyl)pyridine (500 mg, 1.99 mmol) was added to a solution of 2-methoxyethanol (0.19 ml, 2.39 mmol) and sodium hydride (96 mg, 2.39 mmol) in dry THF (15 ml) under N_2 atmosphere at 0 °C. The reaction mixture was then stirred at room temperature overnight under N_2 atmosphere. After completion, the reaction mixture was quenched with H_2O and product was extracted in EtOAc. The organic layer was washed with H_2O (3 x 20 ml), dried over $MgSO_4$ and the solvent was evaporated under reduced pressure to produce *compound **2a*** as a yellow oil (350 mg, 71%).

δ_H (599 MHz, Chloroform-*d*) 8.32 (1H, d, $^4J_{H\text{-}H}$ 2.4 Hz, H^6), 7.56 (1H, dd, $^3J_{H\text{-}H}$ 8.1 Hz, $^4J_{H\text{-}H}$ 2.4 Hz, H^3), 7.45 (1H, d, $^3J_{H\text{-}H}$ 8.1 Hz, H^4), 4.53 (2H, s, H^7), 3.67 – 3.61 (2H, m, H^8), 3.60-3.53 (2H, m, H^9), 3.38 (3H, s, H^{10}); **δ_C** (151 MHz, Chloroform-*d*) 149.3 (1C, C^6), 141.3 (1C, C^5), 138.0 (1C, C^4), 133.1 (1C, C^2), 127.9 (1C, C^3), 71.9 (1C, C^9), 69.9 (1C, C^8), 69.9 (1C, C^7), 59.1 (1C, C^{10}); *m/z* (ESI HRMS$^+$) 246.0130 $[M + H]^+$ ($C_9H_{13}{}^{79}BrNO_2$ requires 246.0130).

2-Bromo-5-((2-methoxyethoxy)methyl)pyridine-N-oxide (3a)



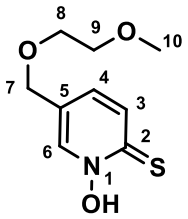
3a

2-Bromo-5-((2-methoxyethoxy)methyl)pyridine (300 mg, 0.85 mmol) was added to chloroform (20 ml) and the mixture was allowed to stir at room temperature. Then 3-chloroperbenzoic acid (444 mg, 2.57 mmol) was added slowly and the reaction mixture was stirred for a further 14 h. The reaction was quenched with the addition of saturated aqueous solution of sodium bicarbonate (approx. 10 ml) and the product was extracted with chloroform (3 x 20 ml). The organic layers were combined, dried over $MgSO_4$, filtered and the solvent was

removed under reduced pressure. The crude yellow solid was purified by column chromatography on silica (95% CH_2Cl_2:5% MeOH) to produce *compound **3a*** as a brown liquid (200 mg, 63%).

δ_H (599 MHz, Chloroform-*d*) 8.39 (1H, s, H^6), 7.59 (1H, d, $^3J_{H\text{-}H}$ 8.3 Hz, H^4), 7.07 (1H, d, $^3J_{H\text{-}H}$ 8.3 Hz, H^3), 4.50 (2H, s, H^7), 3.71 – 3.60 (2H, m, H^9), 3.60-3.53 (2H, m, H^8), 3.38 (3H, s, H^{10}); **δ_C** (151 MHz, Chloroform-*d*) 139.1 (1C, C^6), 136.8 (1C, C^5), 131.5 (1C, C^2), 130.3 (1C, C^4), 124.7 (1C, C^3), 71.8 (1C, C^9), 70.3 (1C, C^8), 69.1 (1C, C^7), 59.1 (1C, C^{10}); *m/z* (ESI $HRMS^+$) 262.0057 $[M + H]^+$ ($C_9H_{13}{}^{79}BrNO_3$ requires 262.0079).

5((2-methoxyethoxy)methyl)pyridine-2-thiol-N-oxide (4a)

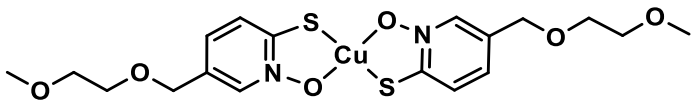


4a

2-Bromo-5-((2-methoxyethoxy)methyl)pyridine-*N*-oxide (300 mg, 1.14 mmol) was dissolved in H_2O (5 ml). A solution of sodium sulfide (446 mg, 5.7 mmol) and sodium hydroxide (137 mg, 3.43 mmol) in H_2O (10 ml) was then added to above solution and the reaction mixture was stirred at 93 °C overnight. After cooling reaction to room temperature, the yellow solution was acidified to pH 1-2 with 1 M HCl and product was extracted with EtOAc (3 x 20 ml). The organic layer was collected, dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was then purified by dissolving in acetone where yellow solid impurity precipitated out. Residue yellow solution was collected through gravity filtration and solvent was evaporated under vacuum to obtain *compound **4a*** as a brown solid (125 mg, 51%).

δ_H (599 MHz, Chloroform-*d*) 8.12 (1H, s, H^6), 7.63 (1H, d, $^3J_{H\text{-}H}$ 8.7 Hz, H^3), 7.22 (1H, d, $^3J_{H\text{-}H}$ 8.7 Hz, H^4), 4.45 (2H, s, H^7), 3.67 – 3.64 (2H, m, H^8), 3.58-3.54 (2H, m, H^9), 3.38 (3H, s, H^{10}); **δ_C** (151 MHz, Chloroform-*d*) 165.9 (1C, C^2), 132.1 (1C, C^4), 131.7 (1C, C^3), 130.0 (1C, C^6), 125.7 (1C, C^5), 71.9 (1C, C^9), 70.1 (1C, C^8), 69.3 (1C, C^7), 59.1 (1C, C^{10}); *m/z* (ESI $HRMS^+$) 216.0696 $[M + H]^+$ ($C_9H_{14}NO_3S$ requires 216.0694).

[Copper(5-(2-methoxyethoxy)methyl)pyridine-2-thiolate-N-oxide)$_2$]

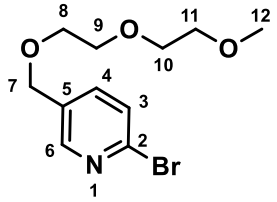


[Cu(PyS1)$_2$]

5((2-methoxyethoxy)methyl)pyridine-2-thiol-*N*-oxide (100 mg, 0.46 mmol) was dissolved in a solution of sodium hydroxide (18 mg, 0.46 mmol) in H_2O (5 ml). A solution of copper chloride dihydrate (39 mg, 0.23 mmol) in H_2O (2 ml) was added dropwise to the reaction mixture and grey solid was observed. After 1 h of stirring the reaction mixture at room temperature, the grey solid were collected through filtration in a sintered funnel. This crude product was further purified through trituration process using dichloromethane as the solvent. The solvent was evaporated under reduced pressure to obtain *[Cu(PyS1)$_2$]* as a dark green solid (110 mg, 43%).

m/z (ESI HRMS$^+$) 492.0434 $[M + H]^+$ ($C_{18}H_{25}{}^{63}CuN_2O_6S_2$ requires 492.0450); Anal. Found (Expected) **C** 43.79 (43.94) **H** 4.92 (4.92) **N** 5.64 (5.69).

2-Bromo-5((2-(2-methoxyethoxy)ethoxy)methyl)pyridine (2b)



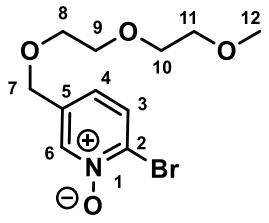
2b

2-Bromo-5-(bromomethyl)pyridine (100 mg, 0.4 mmol) was added to a solution of 2-(2-methoxyethoxy)ethanol (0.06 ml, 0.48 mmol) and sodium hydride (19 mg, 0.48 mmol) in dry THF (10 ml) under N_2 atmosphere at 0 °C. The reaction mixture was then stirred at room temperature overnight under N_2 atmosphere. After completion, the reaction mixture was quenched with H_2O and extracted in EtOAc, dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The crude orange solid was further purified using silica gel column chromatography (80% hexane:20% EtOAc) to produce *compound **2b*** as a brown oil (127 mg, 90%).

δ_H (599 MHz, Chloroform-*d*) 8.26 (1H, d, $^4J_{H\text{-}H}$ 2.4 Hz, H^6), 7.59 – 7.45 (1H, dd, $^3J_{H\text{-}H}$ 8.1 Hz, $^4J_{H\text{-}H}$ 2.4 Hz, H^4), 7.39 (1H, d, $^3J_{H\text{-}H}$ 8.1 Hz, H^3), 4.48 (2H, s, H^7), 3.65 – 3.60 (4H, m, $H^{8,9}$), 3.59 – 3.56 (2H, m, H^{10}), 3.50 – 3.47 (2H, m, H^{11}), 3.31 (3H, s, H^{12}); **δ_C** (151 MHz, Chloroform-

d) 149.3 (1C, C^5), 141.1 (1C, C^2), 138.0 (1C, C^4), 133.2 (1C, C^6), 127.9 (1C, C^3), 71.9 (1C, C^{11}), 70.6 (1C, C^{10}), 70.5 (1C, C^9), 70.0 (1C, C^8), 69.8 (1C, C^7), 59.0 (1C, C^{12}); *m/z* (ESI $HRMS^+$) 290.0413 $[M + H]^+$ ($C_{11}H_{17}{}^{79}BrNO_3$ requires 290.0392); R_f = 0.2 (silica, 80% Hexane:20% EtOAc).

2-Bromo-5((2-(2-methoxyethoxy)ethoxy)methyl)pyridine-N-oxide (3b)

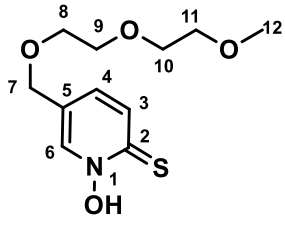


3b

2-Bromo-5((2-(2-methoxyethoxy)ethoxy)methyl)pyridine (100 mg, 0.35 mmol) was added to chloroform (5 ml) and the mixture was allowed to stir at room temperature. 3-chloroperbenzoic acid (179 mg, 1.03 mmol) was added slowly and the reaction mixture was stirred for a further 14 h. The reaction was quenched with the addition of saturated aqueous solution of sodium bicarbonate (approx. 10 ml) and the product was extracted with chloroform (3 x 10 ml). The organic layers were combined, dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude yellow solid was purified by column chromatography on silica (96% CH_2Cl_2:4% MeOH) to produce *compound **3b*** as a red oil (65 mg, 62%).

δ_H (599 MHz, Chloroform-*d*) 8.47 – 8.30 (1H, m, H^6), 7.59 (1H, d, $^3J_{H\text{-}H}$ 8.3 Hz, H^4), 7.08 (1H, ddt, $^3J_{H\text{-}H}$ 8.3 Hz, $^4J_{H\text{-}H}$ 1.7 Hz, $^5J_{H\text{-}H}$ 0.8 Hz, H^3), 4.51 (2H, s, H^7), 3.68 (4H, s, $H^{8, 9}$), 3.65 – 3.62 (2H, m, H^{10}), 3.56 – 3.53 (2H, m, H^{11}), 3.38 (3H, s, H^{12}); **δ_C** (151 MHz, Chloroform-*d*) 139.1 (1C, C^5), 136.9 (1C, C^6), 131.5 (1C, C^2), 130.3 (1C, C^4), 124.8 (1C, C^3), 71.9 (1C, C^{11}), 70.7 (1C, C^{10}), 70.6 (1C, C^9), 70.3 (1C, C^7), 69.1 (1C, C^8), 59.1 (1C, C^{12}); *m/z* (ESI $HRMS^+$) 306.0366 $[M + H]^+$ ($C_{11}H_{17}{}^{79}BrNO_4$ requires 306.0341); R_f = 0.4 (silica, 95% CH_2Cl_2:5% MeOH).

5((2-(2-methoxyethoxy)ethoxy)methyl)pyridine-2-thiol-N-oxide (4b)

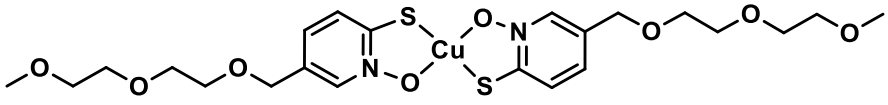


4b

2-Bromo-5((2-(2-methoxyethoxy)ethoxy)methyl)pyridine-*N*-oxide (650 mg, 2.13 mmol) was dissolved in H_2O (15 ml). A solution of sodium sulfide (831 mg, 10.66 mmol) and sodium hydroxide (256 mg, 6.39 mmol) in H_2O (10 ml) was then added to above solution and the reaction mixture was stirred at 95 °C overnight. After cooling the reaction to room temperature, the yellow solution was acidified to pH 1-2 with 1 M HCl and product was extracted with EtOAc (3 x 20 ml). The organic layer was collected, dried over $MgSO_4$, filtered and solvent was removed under reduced pressure. The crude product was then purified by dissolving in acetone where a yellow solid impurity precipitated out. The residue yellow solution was collected through gravity filtration and the solvent was evaporated under vacuum to obtain *compound **4b*** as a brown oil. A small amount of dried product was kept for characterisation purposes and rest of the product was dissolved in aqueous NaOH solution to stabilise the compound and this ligand solution then directly used for the next step of copper complex formation.

δ_H (599 MHz, Chloroform-*d*) 8.15 (1H, dt, $^4J_{H\text{-}H}$ 1.9 Hz, $^5J_{H\text{-}H}$ 0.8 Hz, H^6), 7.62 (1H, d, $^3J_{H\text{-}H}$ 8.8 Hz, H^4), 7.21 (1H, dd, $^3J_{H\text{-}H}$ 8.8 Hz, $^4J_{H\text{-}H}$ 1.9 Hz, H^3), 4.46 (2H, d, $^4J_{H\text{-}H}$ 0.8 Hz, H^7), 3.67 (4H, m, $H^{8,9}$), 3.65 – 3.62 (2H, m, H^{10}), 3.57 – 3.54 (2H, m, H^{11}), 3.38 (3H, s, H^{12}); **δ_C** (151 MHz, Chloroform-*d*) 165.9 (1C, C^2), 132.2 (1C, C^3), 131.7 (1C, C^4), 130.1 (1C, C^5), 125.9 (1C, C^6), 71.9 (1C, C^{11}), 70.6 (1C, C^{10}), 70.5 (1C, C^9), 70.0 (1C, C^8), 69.0 (1C, C^7), 59.0 (1C, C^{12}); *m/z* (ESI $HRMS^+$) 260.0983 $[M + H]^+$ ($C_{11}H_{18}NO_4S$ requires 260.0957).

[Copper(5((2-(2-methoxyethoxy)ethoxy)methyl)pyridine-2-thiolate-N-oxide)$_2$]

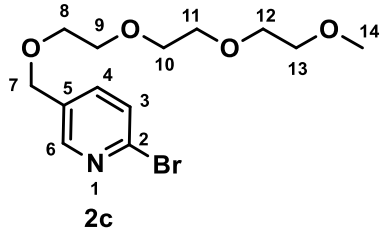


[Cu(PyS2)$_2$]

5((2-(2-methoxyethoxy)ethoxy)methyl)pyridine-2-thiolate-*N*-oxide (80 mg, 0.31 mmol) was dissolved in a solution of sodium hydroxide (12 mg, 0.31 mg) in H_2O (5 ml). A solution of copper chloride dihydrate (27 mg, 0.16 mmol) in H_2O (2 ml) was added dropwise to the reaction mixture and the resulted dark green solution was then allowed to be stirred for 1 h. Mass spectrometry data of reaction mixture suggested formation of desired complex and after evaporation of water, *complex [Cu(PyS2)$_2$]* was obtained as a dark green sticky oil (30 mg, 33%).

M/z (ESI HRMS$^+$) 580.0980 [M + H]$^+$ ($C_{22}H_{33}{}^{63}CuN_2O_8S_2$ requires 580.0974); Anal. Found (Expected) **C** 45.43 (45.55) **H** 5.53 (5.56) **N** 4.89 (4.83).

2-Bromo-5((2-(2-(2-methoxyethoxy)ethoxy)ethoxy)methyl)pyridine (2c)

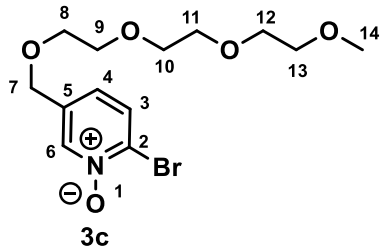


2c

2-Bromo-5-(bromomethyl)pyridine (500 mg, 1.99 mmol) was added to a solution of 2-(2-(2-methoxyethoxy)ethoxy)ethanol (0.4 ml, 2.39 mmol) and sodium hydride (96 mg, 2.39 mmol) in dry THF (15 ml) under N_2 atmosphere at 0 °C. The reaction mixture was then stirred at room temperature overnight under N_2 atmosphere. After completion, the reaction mixture was quenched with H_2O and product was extracted in EtOAc. The organic layer was washed with H_2O (3 x 20 ml), dried over $MgSO_4$ and solvent was evaporated under reduced pressure to produce the *compound **2c*** as yellow oil (460 mg, 69%).

δ_H (599 MHz, Chloroform-*d*) 8.31 (1H, d, $^4J_{H\text{-}H}$ 2.4 Hz, H^6), 7.56 (1H, dd, $^3J_{H\text{-}H}$ 8.2 Hz, $^4J_{H\text{-}H}$ 2.4 Hz, H^3), 7.45 (1H, d, $^3J_{H\text{-}H}$ 8.2 Hz, H^4), 4.53 (2H, s, H^7), 3.68 – 3.62 (10H, m, $H^{8, 9, 10, 11, 12}$), 3.53 (2H, m, H^{13}), 3.36 (3H, s, H^{14}); **δ_C** (151 MHz, Chloroform-*d*) 149.3 (1C, C^6), 141.2 (1C, C^2), 138.0 (1C, C^3), 133.2 (1C, C^5), 127.9 (1C, C^4), 71.9 (1C, C^{13}), 70.7 (1C, C^{11}), 70.6 (2C, $C^{9, 10}$), 70.5 (1C, C^{12}), 70.0 (1C, C^8), 69.8 (1C, C^7), 59.0 (1C, C^{14}); *m/z* (ESI HRMS$^+$) 334.0667 [M + H]$^+$ ($C_{13}H_{21}{}^{79}BrNO_4$ requires 334.0654).

2-Bromo-5((2-(2-(2-methoxyethoxy)ethoxy)ethoxy)methyl)pyridine-N-oxide (3c)



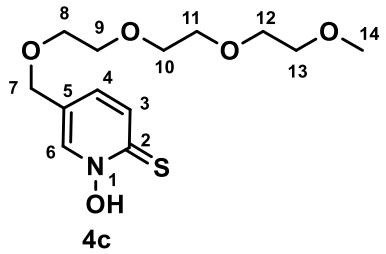
3c

2-Bromo-5((2-(2-(2-methoxyethoxy)ethoxy)ethoxy)methyl)pyridine (450 mg, 1.35 mmol) was added to chloroform (20 ml) and the mixture was allowed to stir at room temperature. Then 3-Chloroperbenzoic acid (700 mg, 4.05 mmol) was added slowly, and the reaction mixture was stirred for a further 16 h. The reaction was quenched with the addition of saturated aqueous solution of sodium bicarbonate (approx. 20 ml) and the product was extracted with chloroform

(3 x 15 ml). The organic layers were combined, dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude yellow solid was purified by column chromatography on silica (95% CH_2Cl_2:5% MeOH) to produce *compound **3c*** as a brown oil (200 mg, 48%).

δ_H (599 MHz, Chloroform-*d*) 8.40 (1H, s, H^6), 7.59 (1H, d, $^3J_{H\text{-}H}$ 8.3 Hz, H^3), 7.09 (1H, dt, $^3J_{H\text{-}H}$ 8.3 Hz, $^4J_{H\text{-}H}$ 1.1 Hz, H^4), 4.50 (2H, s, H^7), 3.69 – 3.61 (10H, m, $H^{8, 9, 10, 11, 12}$), 3.55 – 3.52 (2H, m, H^{13}), 3.36 (3H, s, H^{14}); **δ_C** (151 MHz, Chloroform-*d*) 139.1 (1C, C^6), 136.9 (1C, C^2), 131.5 (1C, C^5), 130.3 (1C, C^3), 124.8 (1C, C^4), 71.9 (1C, C^{13}), 70.7-70.3 (5C, $C^{8, 9, 10, 11, 12}$), 69.0 (1C, C^7), 59.0 (1C, C^{14}); *m/z* (ESI $HRMS^+$) 350.0619 $[M + H]^+$ ($C_{13}H_{21}{}^{79}BrNO_5$ requires 350.0603); R_f = 0.4 (silica, 95% CH_2Cl_2:5% MeOH).

5((2-(2-(2-methoxyethoxy)ethoxy)ethoxy)methyl)pyridine-2-thiol-N-oxide (4c)



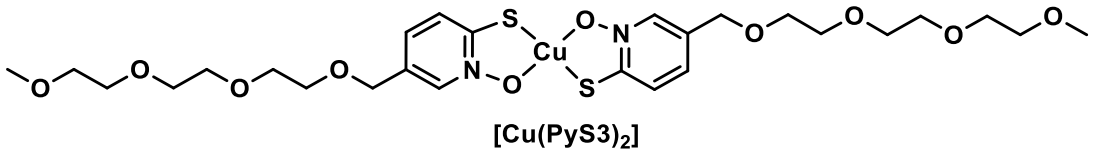
4c

2-Bromo-5((2-(2-(2-methoxyethoxy)ethoxy)ethoxy)methyl)pyridine-*N*-oxide (150 mg, 0.43 mmol) was dissolved in H_2O (5 ml). A solution of sodium sulfide (168 mg, 2.15 mmol) and sodium hydroxide (52 mg, 1.29 mmol) in H_2O (5 ml) was then added to above solution and the reaction mixture was stirred at 93 °C overnight. After cooling the reaction to room temperature, the yellow solution was acidified to pH 1-2 with 1 M HCl and product was extracted with EtOAc (3 x 10 ml). The organic layer was collected, dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was then purified by dissolving in acetone where a yellow solid impurity precipitated out. The residue yellow solution was collected through gravity filtration and the solvent was evaporated under vacuum to obtain *compound **4c*** as a brown oil. A small amount of dried product was kept for characterisation purpose and rest of the product was dissolved in aqueous NaOH solution to stabilise the compound and this ligand solution then directly used for the next step of copper complex formation.

δ_H (599 MHz, D_2O) 7.91 (1H, s, H^6), 7.42 (1H, d, $^3J_{H\text{-}H}$ 8.7 Hz, H^3), 7.06 (1H, d, $^3J_{H\text{-}H}$ 8.7 Hz, H^4), 4.70 (2H, s, H^7), 3.52 – 3.48 (10H, m, $H^{8, 9, 10, 11, 12}$), 3.46 – 3.44 (2H, m, H^{13}), 3.19 (3H, s, H^{14}); **δ_C** (151 MHz, D_2O) 170.7 (1C, C^2), 145.8 (1C, C^6), 148.1 (1C, C^4), 137.2 (1C, C^3),

122.9 (1C, C^5), 70.9 (1C, C^7), 70.9 – 69.3 (5C, $C^{8, 9, 10, 11, 12}$), 68.8 (1C, C^{13}), 58.0 (1C, C^{14}); *m/z* (ESI HRMS$^+$) 304.1230 $[M + H]^+$ ($C_{13}H_{21}NO_5S$ requires 304.1219).

[Copper(5((2-(2-(2-methoxyethoxy)ethoxy)ethoxy)methyl)pyridine-2-thiolate-N-oxide)$_2$]



[Cu(PyS3)$_2$]

5((2-(2-(2-methoxyethoxy)ethoxy)ethoxy)methyl)pyridine-2-thiol-*N*-oxide (300 mg, 0.99 mmol) was dissolved in a solution of sodium hydroxide (40 mg, 0.99 mmol) in H_2O (10 ml). A solution of copper chloride dihydrate (84 mg, 0.49 mmol) in H_2O (5 ml) was added dropwise to the reaction mixture and the resulted dark green solution was then allowed to be stirred for 1 h. Mass spectrometry data of reaction mixture suggested formation of desired complex and after evaporation of water, dark brown oily liquid was obtained. The crude product was further purified by column chromatography on silica (CH_2Cl_2) to produce the *complex [Cu(PyS3)$_2$]* as dark green oil (135 mg, 20%).

m/z (ESI HRMS$^+$) 668.1506 $[M + H]^+$ ($C_{26}H_{41}{}^{63}CuN_2O_{10}S_2$ requires 668.1499); Anal. Found (Expected) **C** 46.63 (46.73) **H** 6.20 (6.03) **N** 4.10 (4.19).

Analytical Data:

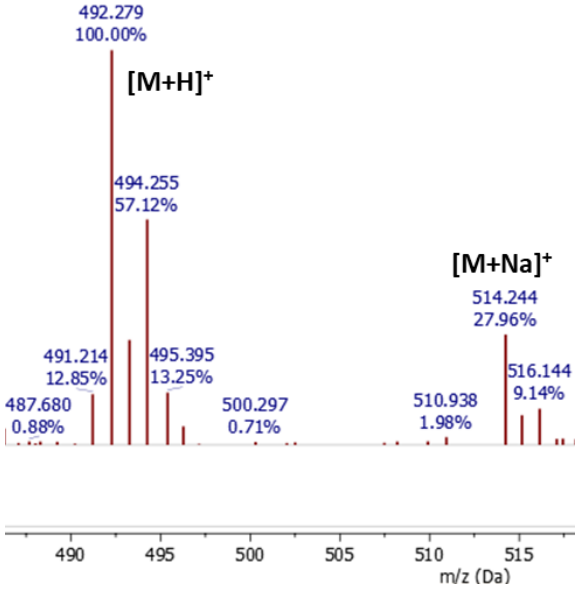


Figure S1: *Mass Spectrometry data for* ***[Cu(PyS1)$_2$]***

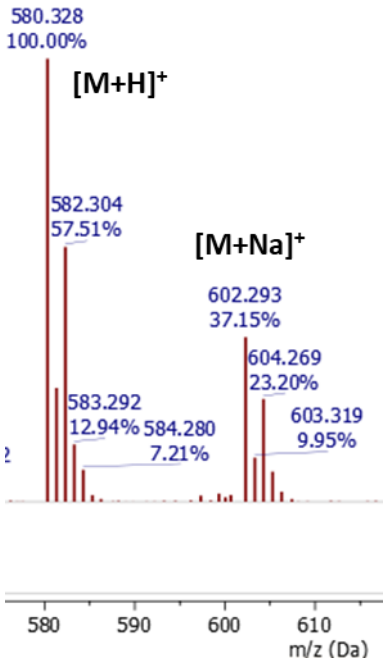


Figure S2: *Mass Spectrometry data for* ***[Cu(PyS2)$_2$]***

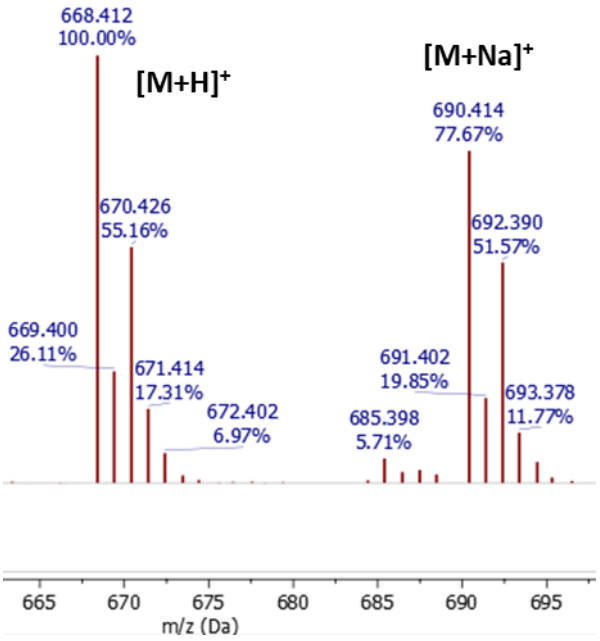


Figure S3: *Mass Spectrometry data for* ***[Cu(PyS3)$_2$]***

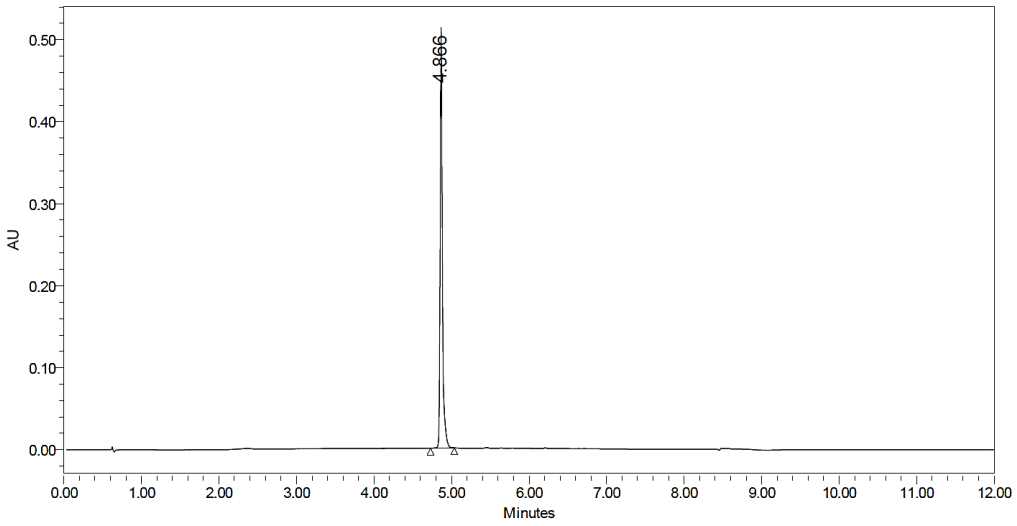


Figure S4: *Purity Check by HPLC data for* ***[Cu(PyS1)$_2$]***

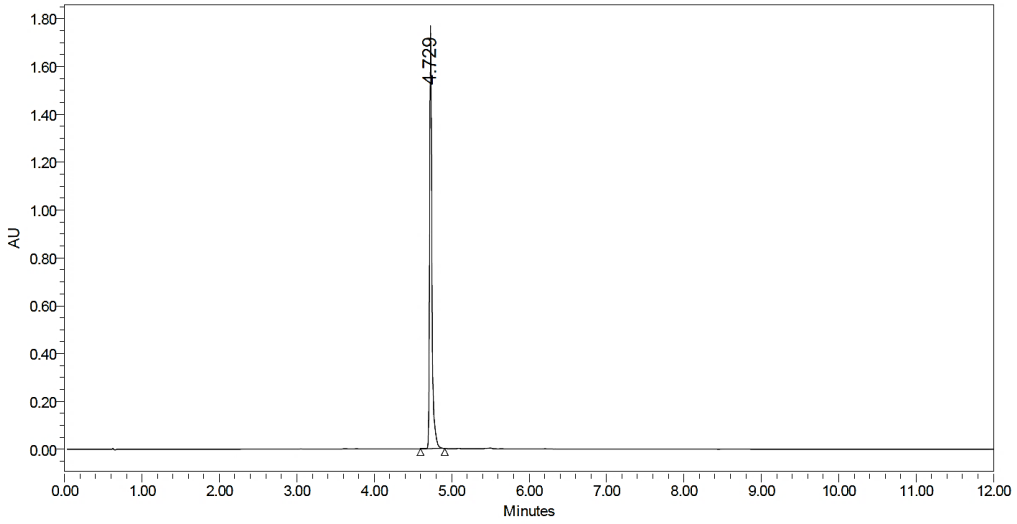


Figure S5: *Purity Check by HPLC data for* ***[Cu(PyS2)$_2$]***

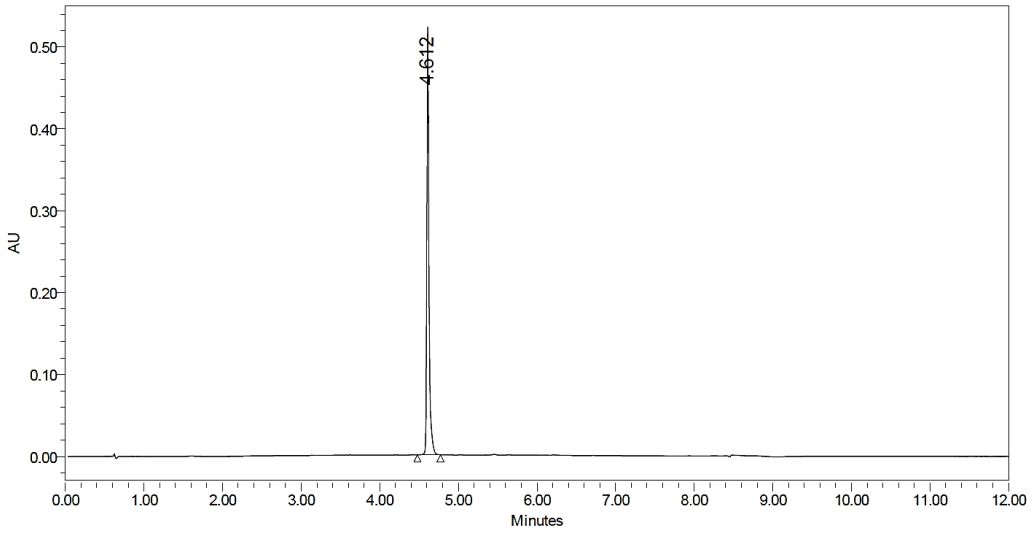


Figure S6: *Purity Check by HPLC data for* ***[Cu(PyS3)$_2$]***

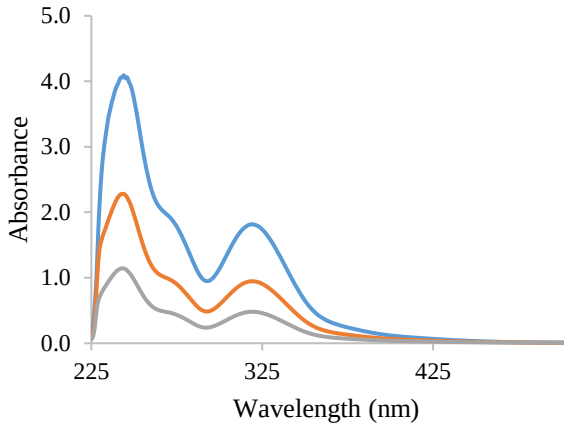
General procedure for log P measurement:

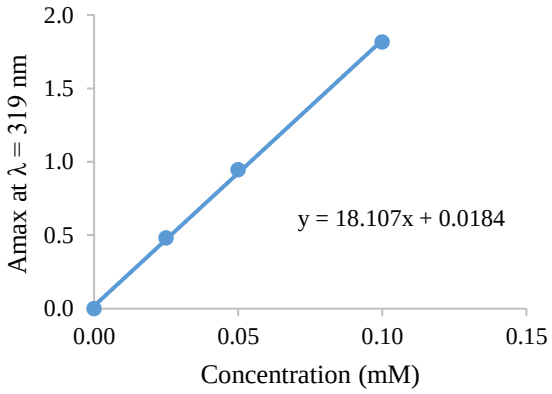
Spectrophotometric determination of log P:

Stock solutions (10 mM) of three PEG chain substituted copper complexes were prepared in dimethyl sulfoxide (DMSO) solvent. Molar absorptivity or extinction coefficient (ε) of copper complexes in both water and *n*-octanol solvents were determined by preparing series of solutions with half dilutions. Water and octanol used in this experiment were saturated with each other to maintain the similar condition as that for later experiments. For both solvents, the starting solution with concentration of 0.1 mM was obtained by adding 100 µl of stock solution in 10 ml of respective solvents. Hereby, the maximum amount of DMSO in the experimental system was kept to 1%. UV-vis absorbance spectra of prepared solutions were recorded in 200-600 nm range at 22 °C temperature and the absorbance maxima (A) around 320 nm have been utilised for calculating ε of the complexes. Absorbance maxima for different concentrations of solutions were plotted in *y* axis against the concentrations at *x* axis to obtain a straight-line plot. Following Beer-Lambert law ($A = \varepsilon cl$, where c = concentration and l = length of light path = 1 cm (constant)), ε were calculated from slope.

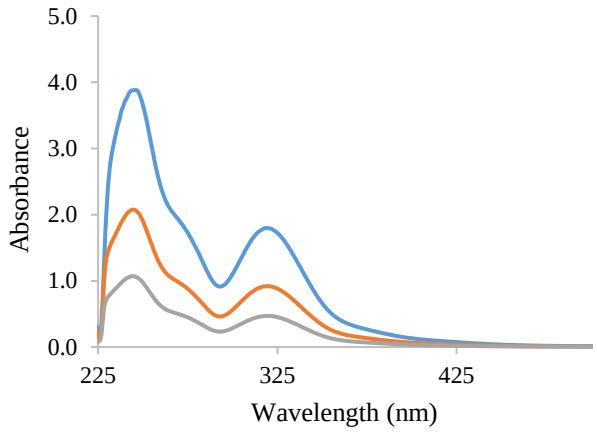
$$\mathbf{A = \varepsilon cl}$$

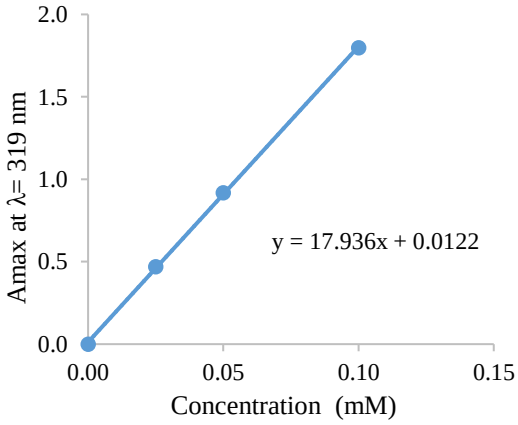
$$\mathbf{\varepsilon = A/cl}$$



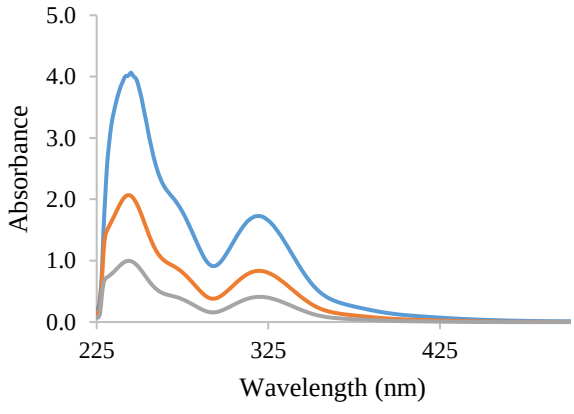


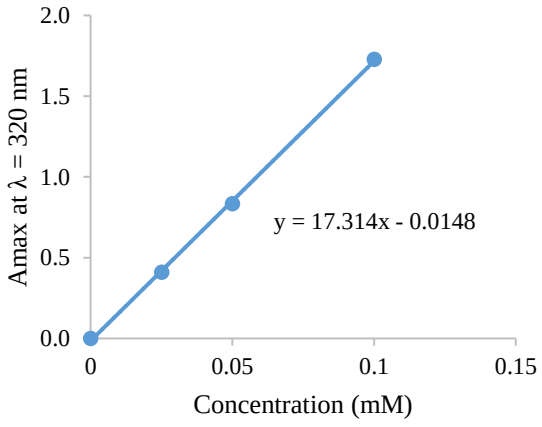
[Cu(PyS1)$_2$]





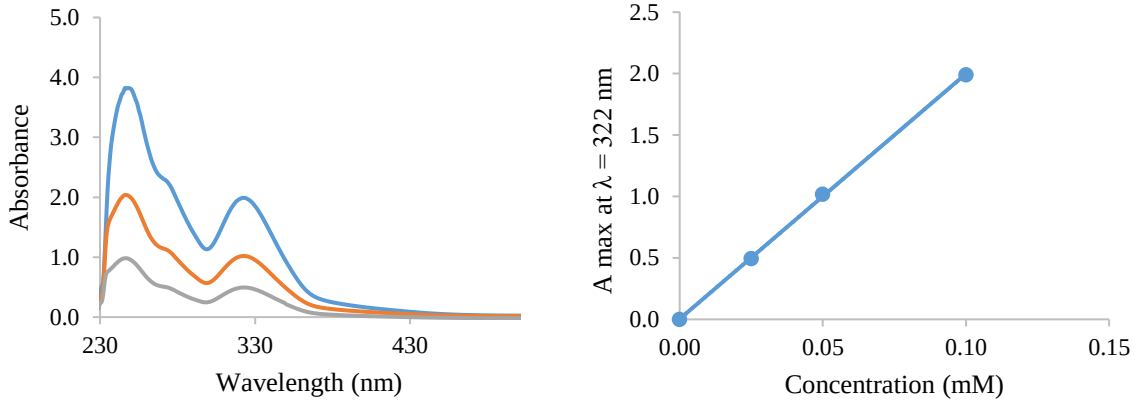
[Cu(PyS2)$_2$]



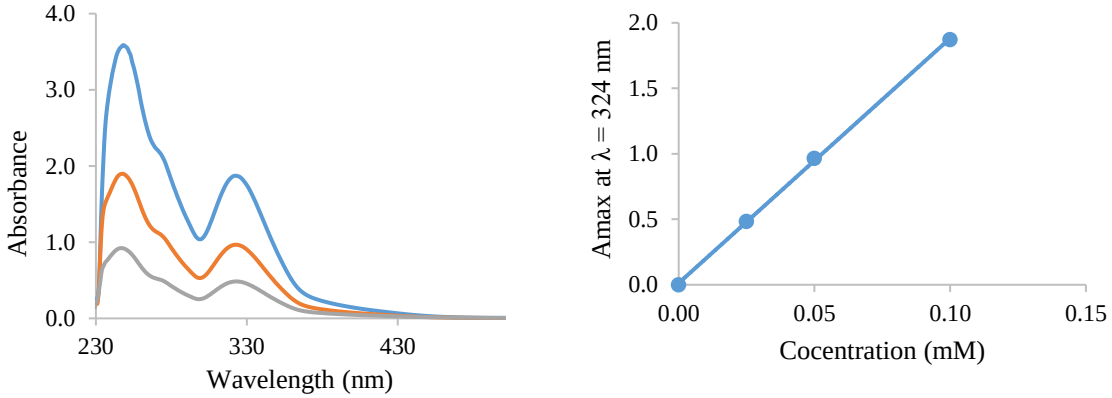


[Cu(PyS3)$_2$]

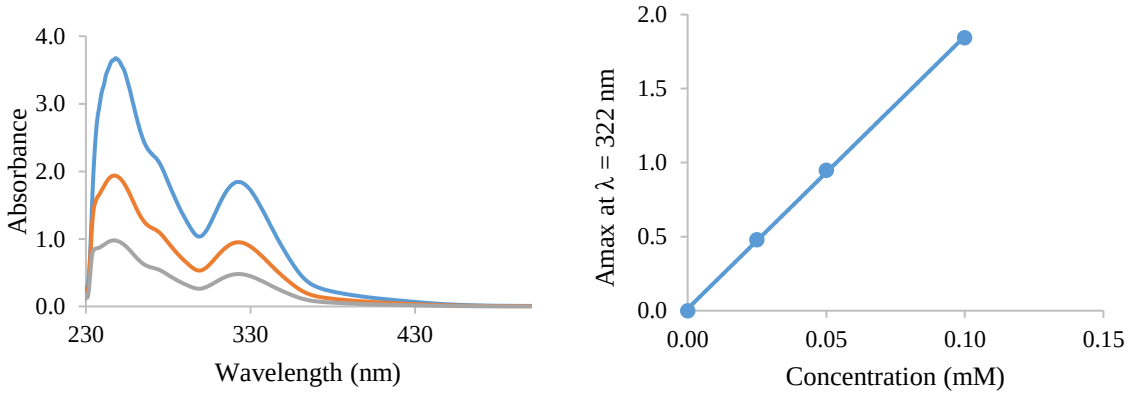
Figure S7: *UV absorbance spectra and corresponding concentration graph for calculating extinction coefficient of PEG chain (n =1, 2, 3) substituted copper pyrithione complex in water solvent*



[Cu(PyS1)$_2$]



[Cu(PyS2)$_2$]



[Cu(PyS3)$_2$]

Figure S8: *UV absorbance spectra and corresponding concentration graph for calculating extinction coefficient of PEG chain (n =1, 2, 3) substituted copper pyrithione complex in Octanol solvent*

Table S1: *Extinction Coefficient (ε) of PEG chain complexes in Octanol (319 nm) and Water (322 nm) solvent*

	ε ($M^{-1}cm^{-1}$)	
	Octanol	**H_2O**
[Cu(PyS1)$_2$]	20000	19800
[Cu(PyS2)$_2$]	18900	18200
[Cu(PyS3)$_2$]	18600	17100

Next, log P values for [Cu(PyS1)$_2$], [Cu(PyS2)$_2$] and [Cu(PyS3)$_2$] were determined using shake-flask method. In this process, for each complex, 60 µl of the stock solution was added to a mixture of 3 ml water and 3 ml *n*-octanol. The solution was shaken by hand and vortex. The two layers were collected separately and UV-absorbance spectra recorded at 200-600nm. Previously determined extinction coefficients were utilised to determine the concentrations of copper complex in each layer following Beer-Lambert law ($c = A/\varepsilon l$) and considering absorbance maxima around 320 nm. Further calculations were done to determine partition coefficient (P) and respective log P value according to the following equation.

$$\log P = \log\left(\frac{[octanol]}{[water]}\right)$$

Determination of log P value using ICP-OES technique:

As non-substituted copper pyrithione complex has very poor solubility in water, determining the extinction coefficient of this complex in water is highly inefficient. Therefore, another technique, ICP-OES analysis was utilised to measure concentrations of copper in each layer. In this process, sample preparation for each complex followed everything similar as that for shake-flask method until UV measurement. After separating the two solvent layers, samples for measuring copper concentrations in octanol and water layer were prepared differently. For the octanol layer, 100 µl was collected in a vial and the solvent was evaporated under high vacuum. Next, the residue complex was dissolved in 100 µl methanol and further diluted with 10 ml of 5% aqueous nitric acid (HNO_3) solution for digestion. For the water layer, 1 ml of solution was diluted with 9 ml of 5% aqueous HNO_3 solution. After analysis through ICP-OES technique, the amount of copper in each sample was determined in ppm. Thereafter, the concentrations of copper in starting solution of each layer was calculated considering dilution methods to determine the subsequent P and log P values.

General procedure for solubility measurement:

Solubility of PEG chain substituted copper pyrithione complexes in water was measured using UV-vis spectroscopy. First, each copper complex was dissolved in 1 ml of 1% DMSO/water solvent system until the solution reaches saturation. Then, the saturated solutions were filtered using small pipette filtration and further diluted several times using 1% DMSO/water to measure UV absorbance data at different concentrations. Absorbance maxima at 319 nm were considered for further calculations using Beer-Lambert law to obtain concentration at different dilution. Consequently, this calculation led to concentration of the solution prepared initially in 1 ml solvent and could be concluded as the solubility (mg/ml or g/l) of respective copper complex.

Material and methods for Anticancer Study:

Growth media and Reagents:

Gicbo™ high glucose Dublecco's Modified Eagle Medium (DMEM) and Gicbo™ Roswell Park Memorial Institute (RPMI-1640) medium supplemented with sodium pyruvate (1 mM) and L-glutamine (2 mM), Fetal bovine serum (FBS), phosphate buffer solution (PBS) and Trypsin-EDTA (0.25%) were all purchased from Fisher Scientific. The MIA PaCa-2, 143B and ARPE-19 cell lines were kindly gifted or purchased from the University of Bradford's Institute of Cancer Therapeutics, Dr Darrell Green (UEA, School of Medicine) and Dr Simon Allison (University of Huddersfield, School of Applied Sciences) respectively.

Cell viability assay:

All assays were conducted using human cell lines: pancreatic carcinoma (MIA PaCa-2), bone osteosarcoma (143B) and normal epithelial retinal (ARPE-19), and cell lines were routinely maintained as monolayer cultures in appropriate complete medium (MIA PaCa-2 and ARPE-19 in high glucose DMEM complete medium (+ 10% FBS); 143B in RPMI-1640 complete medium (+ 10% FBS)) and grown in either T-25 or T-75 flasks at 37 °C and 5% CO_2. Prior to chemosensitivity studies, cell monolayers were passaged using Trypsin-EDTA (0.25%) and diluted to a concentration of 1 x 10^4 cells/ml. All assays were conducted using 96-well plates, in which 100 µl of the cell suspension was added to columns 3-11 (column 2 = media blank), and columns 1 and 12, and rows A and H were filled with 200 µl PBS to prevent media evaporation. The plates were then incubated for 48 h at 37 °C and 5% CO_2. After 48 h, 100 µl of compound/media dilutions were added to the plates in columns 4-11 in a series of half

dilutions (column 3 = 100% cells spiked with 0.1% DMSO). All compound stocks were made using DMSO at 100 mM prior to dilution. The plates were incubated for 24 h at 37 °C and 5% CO_2, then 20 µl MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 5 mg/ml) was added to each well and incubated for a further 3 h at 37°C and 5% CO_2. All solutions were then removed via pipette and 150 µl of DMSO added to each well, mixed using a pipette and the absorbance of each well measured at 540 nm using a ClarioStar spectrophotometer microplate reader. Results were plotted on a logarithmic scale, and the half maximal inhibitory concentration (IC_{50}) determined from triplicate of triplicate repeats and reported as an IC_{50} ± Standard Deviation (SD).

Cell Uptake Assay:

Cell uptake assays were conducted using 143B cells and these were maintained as described above. All assays were conducted in 6-well plates and cell concentrations of 1 x 10^6 cells/ well in 2 ml of complete cell media. These cell suspensions were added to the wells and incubated at 37°C and 5% CO_2 for 24 h. The media was then removed and 2 ml of media/copper compound was added to each well. For each compound, a concentration of 1 µM of the copper complexes were used. The cells were incubated with the complexes for 4 h, before removing and discarding the media. The cell monolayers were then individually washed with PBS (3 x 1 ml) and all waste discarded. 0.25% Trypsin-EDTA (0.5 ml) was then added until the cells were removed from the surface and then diluted with media (1.5 ml). The contents of each well were transferred to separate Falcon tubes and centrifuged at 1500 rpm for 5 min. The supernatant was removed, the cell pellets were resuspended in PBS (1 ml) and centrifuged again at 1500 rpm for 5 min (this was completed 3 times). On the final time, the cells were counted before the last centrifuge and then pelleted and store in the freezer for ICP-OES analysis. All assays were conducted in triplicate. Pellets were digested in ARISTAR 69% nitric acid (0.5 ml) for 16 h, before being diluted with ultrapure H_2O (2.5 ml). Samples were analysed via ICP-OES to determine ppm Cu content, which was converted to ng of Cu / 10^6 cells (Table 2).

Material and methods for the Antibacterial study:

Growth media and Reagents:

Mueller-Hinton cation supplemented broth II (MHBII) and Mueller-Hinton Agar (MHA) were purchased from Merck Life-Sciences. Meropenem and Ertapenem were obtained from Melford Laboratories.

Bacterial strains:

All copper pyrithione complexes along with copper chloride and levofloxacin were screened against ESKAPE pathogen panel, including *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *K. pneumoniae* BAA-1705, *A. baumannii* BAA-1605, *P. aeruginosa* ATCC 27853 and *Enterococcus* sp. These strains were procured from Biodefense and Emerging Infections Research Resources Repository/American Type Culture Collection (BEI/ATCC, USA) and routinely cultivated on MHA and MHBII. Before starting the experiment, a single colony was picked from MHA plate, inoculated in MHBII and incubated overnight at 37 °C with shaking for 18–24 h to obtain the starter culture.

All copper pyrithione complexes were also screened against a β-lactam resistant strain of *E. coli* bacteria that carries the pSU2718::$bla_{\text{NDM-1}}$ plasmid expressing the New Delhi metallo-β-lactamase 1. This strain has been described previously (refer to the main article) and it is routinely cultivated in ampicillin containing MHBII and agar media at 37 °C. Before starting the experiments, single colonies were picked up from media plate and was inoculated in MHBII.

Antibiotic susceptibility testing:

For screening of ESKAPE, antibiotic susceptibility tests were conducted according to the CLSI guidelines using the broth microdilution assay. Briefly, 10 mg/ml stock solutions of test compounds were prepared in DMSO. Bacterial cultures (~10^6 CFU/ml, 0.1 ml total) were prepared in MHBII in 96-well round bottom microtiter plates. The compounds were tested from 0.5 – 64 mg/l in two-fold serial diluted fashion. The plates were incubated at 37 °C for 18-24 h without shaking, following which the minimum inhibitory concentration (MIC) was determined. The MIC is defined as the lowest concentration of the compound at which there is absence of visible growth. For each test compound, MIC determinations were carried out independently three times using duplicate samples.

Antibiotic susceptibility testing against β-lactam resistant strain of *E. coli* was conducted as above but using 0.2 ml of bacterial cultures and 0 – 100 µM of copper(II) compounds.

Antibiotic synergy study:

Antibiotic synergy study was again conducted following CLSI guidelines using the checkerboard assay method. Bacterial cultures were (~10^6 CFU/ml, 0.2 ml total) were prepared in MHBII in flat-bottomed, 96-well microtiter plates. Copper complexes (0 – 100 µM) and/or ertapenem (0 – 12 µM) or meropenem (0 – 4 µM) were tested in two-fold serial diluted fashion.

Turbidity in each well was measured using a microtiter plate reader after incubation at 37 °C for 18-24 h. The MIC was defined as the lowest concentration of agent that completely inhibited bacterial growth.

Crystallography data:

The X-ray single crystal data have been collected for **[Cu(PyS1)$_2$]** (CCDC-2184348) using λMoKα radiation (λ =0.71073Å) on an Bruker D8 Venture (Photon III MM C7 CPAD detector, IμS micro-source, focusing mirrors) 3-circle diffractometer equipped with a Cryostream-700 (Oxford Cryosystems) open-flow nitrogen cryostat at the temperature 130.0 (2)K. The structure was solved by direct method and refined by full-matrix least squares on F^2 for all data using Olex2[1] and SHELXTL [2] software. All non-hydrogen atoms were refined in anisotropic approximation, hydrogen atoms were placed in the calculated positions and refined in riding mode. Crystal data and parameters of refinement are listed in Table S3. Some views of crystal packing are given in Figure S9.

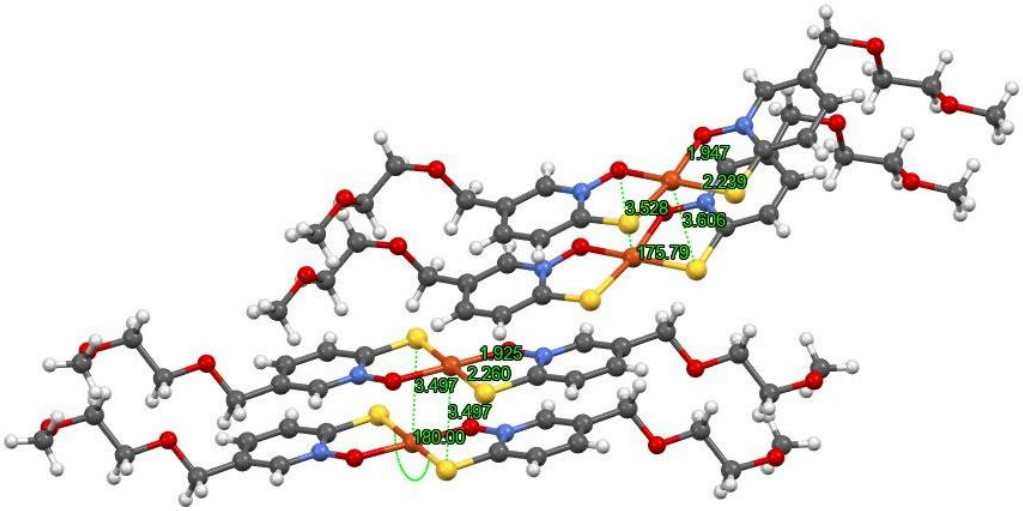
1.O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann, J. *Appl. Cryst.* (2009), **42**, 339-341.

2. G.M. Sheldrick, *Acta Cryst.* (2008), **A64**, 112-122

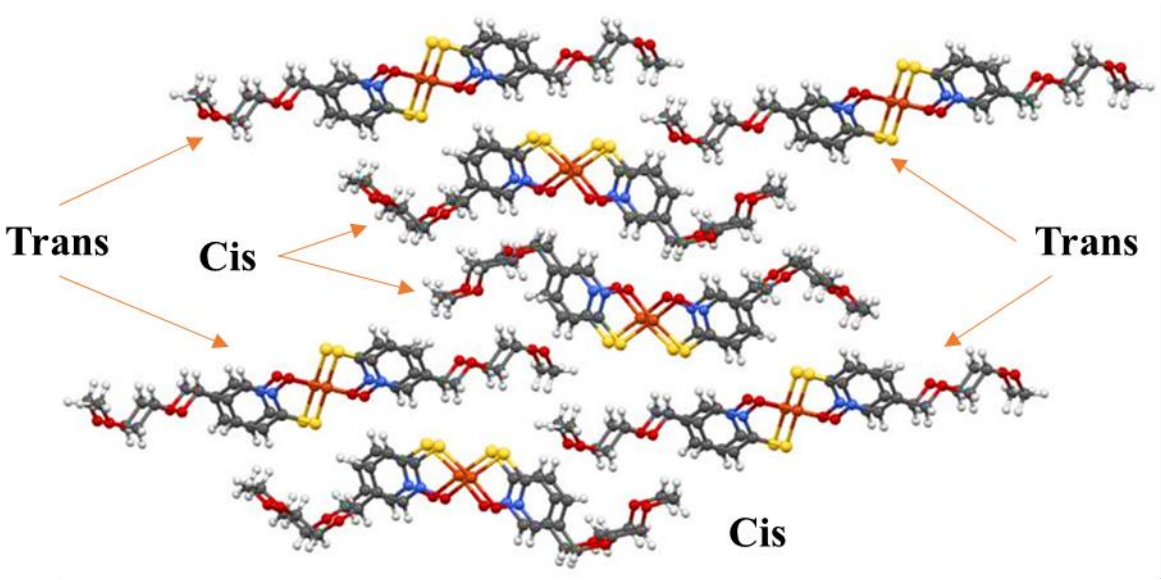
Table S3: *Crystal data and structure refinement for* ***[Cu(PyS1)$_2$]***

Empirical formula	**$C_{18}H_{24}CuN_2O_6S_2$**
Formula weight	492.05
Temperature/K	130.0
Crystal system	triclinic
Space group	P-1
a/Å	4.2745(2)
b/Å	16.1974(7)
c/Å	23.4885(11)
α/°	106.784(2)
β/°	92.454(2)
γ/°	95.446(2)
Volume/Å^3	1545.73(12)
Z	3

ρ_{calc}g/cm^3	1.586
μ/mm^{-1}	1.300
F(000)	765.0
Crystal size/mm^3	0.17 × 0.1 × 0.02
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	3.62 to 59.996
Index ranges	$-6 \le h \le 6$, $-22 \le k \le 22$, $-33 \le l \le 33$
Reflections collected	26528
Independent reflections	8894 [R_{int} = 0.0375, R_{sigma} = 0.0478]
Data/restraints/parameters	8894/0/397
Goodness-of-fit on F^2	1.040
Final R indexes [I>=2σ (I)]	R_1 = 0.0399, wR_2 = 0.0694
Final R indexes [all data]	R_1 = 0.0557, wR_2 = 0.0740
Largest diff. peak/hole / e Å^{-3}	0.40/-0.58



A



B

Figure S9: *Crystal packing structures for* ***[Cu(PyS1)$_2$]***

NMR Spectra

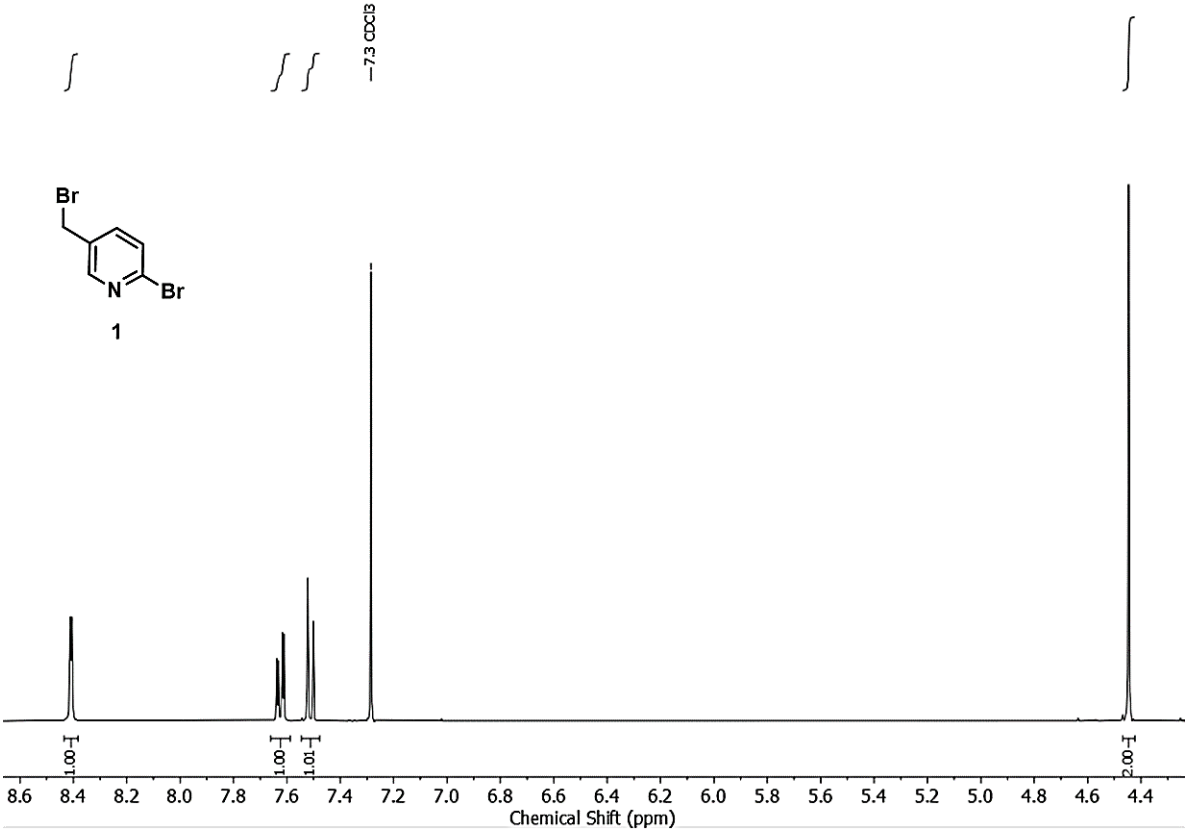


Figure S7: *^{1}H NMR spectra of compound* ***1*** *in chloroform-d (599 MHz) at 295 K*

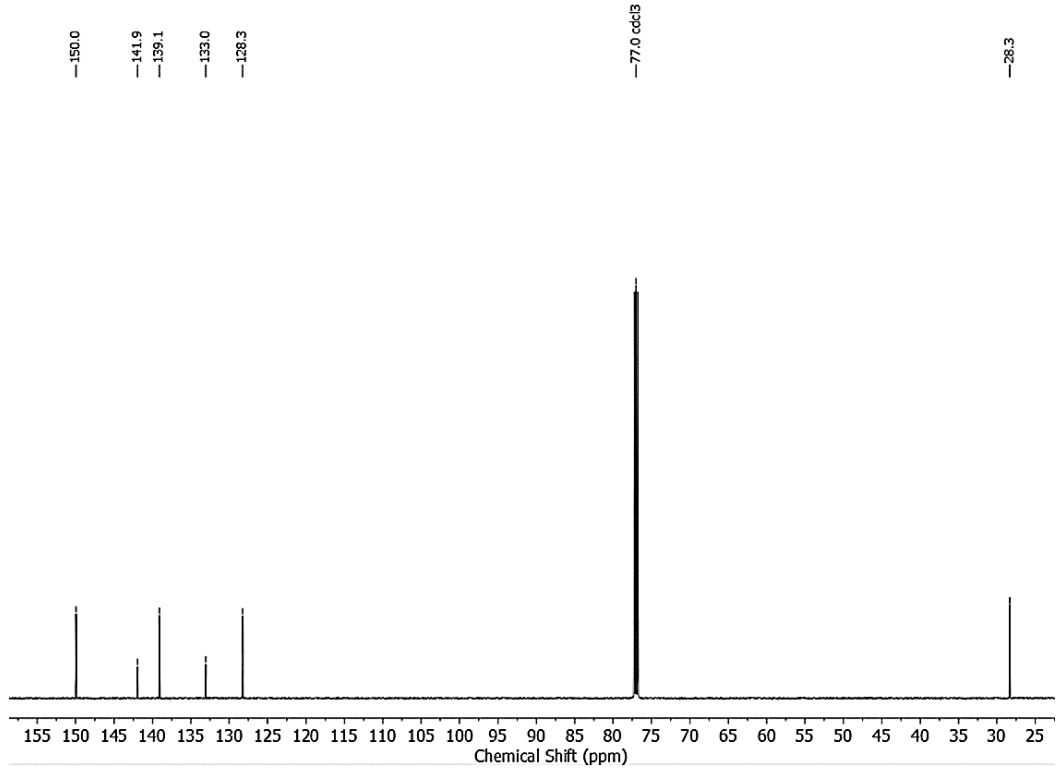


Figure S8: *^{13}C NMR spectra of compound **1** in chloroform-d (151 MHz) at 295 K*

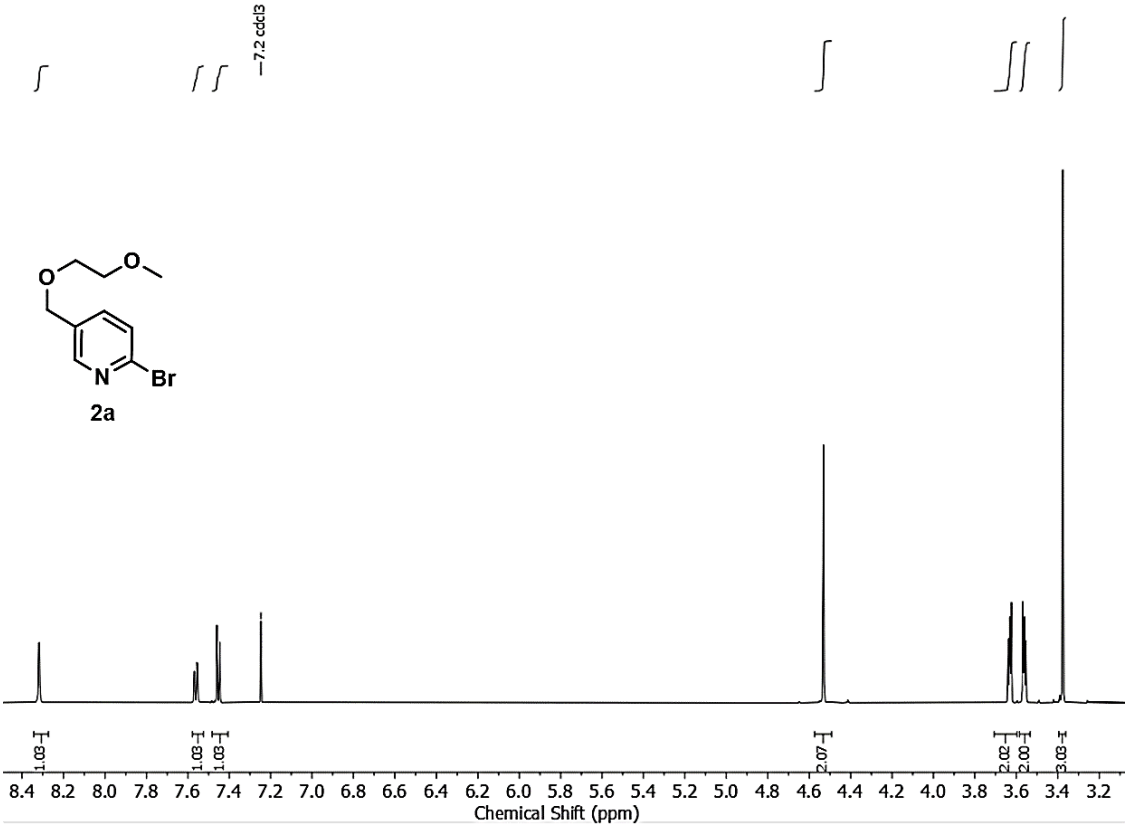


Figure S9: *^{1}H NMR spectra of compound **2a** in chloroform-d (599 MHz) at 295 K*

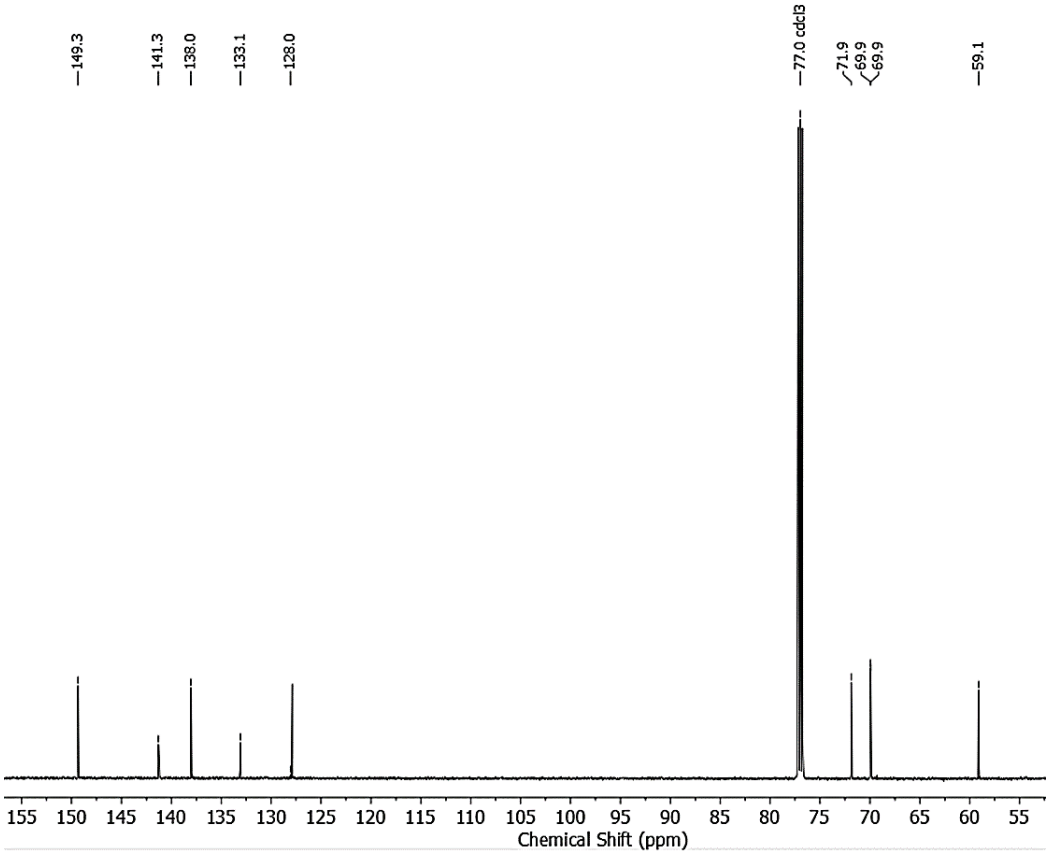


Figure S10: *^{13}C NMR spectra of compound **2a** in chloroform-d (151 MHz) at 295 K*

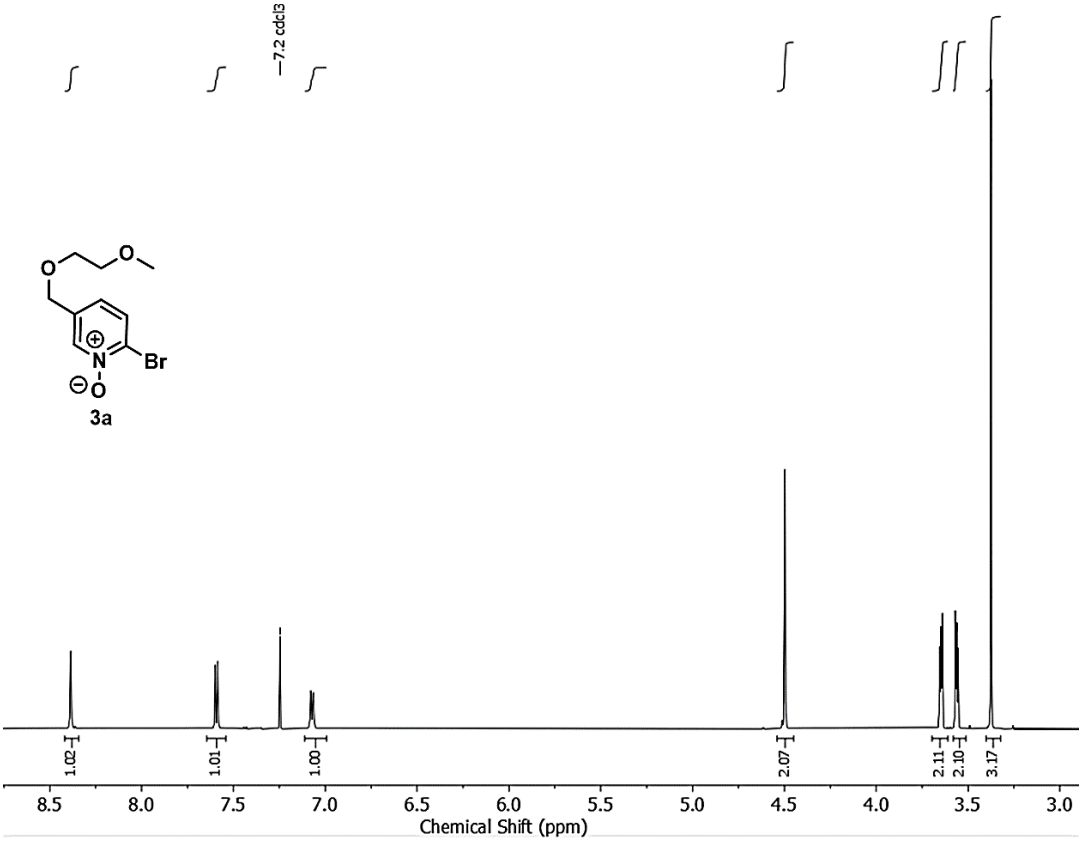


Figure S11: *^{1}H NMR spectra of compound **3a** in chloroform-d (599 MHz) at 295 K*

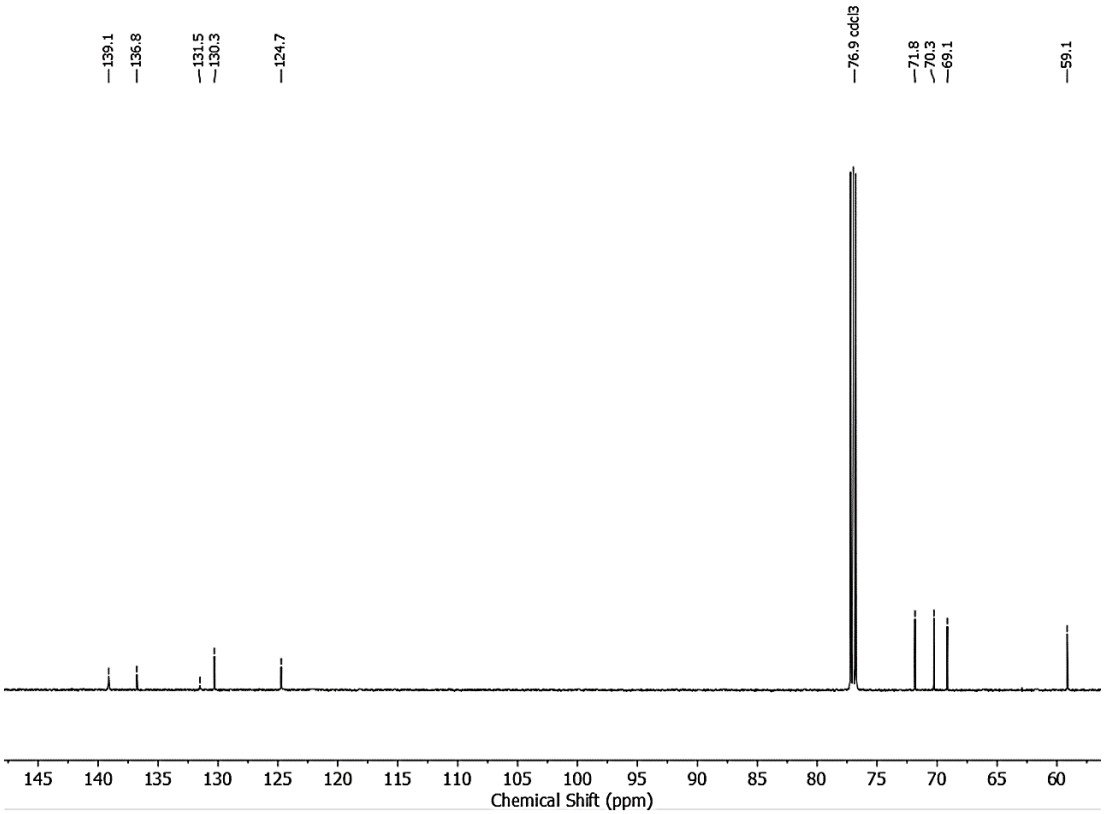


Figure S12: *^{13}C NMR spectra of compound **3a** in chloroform-d (151 MHz) at 295 K*

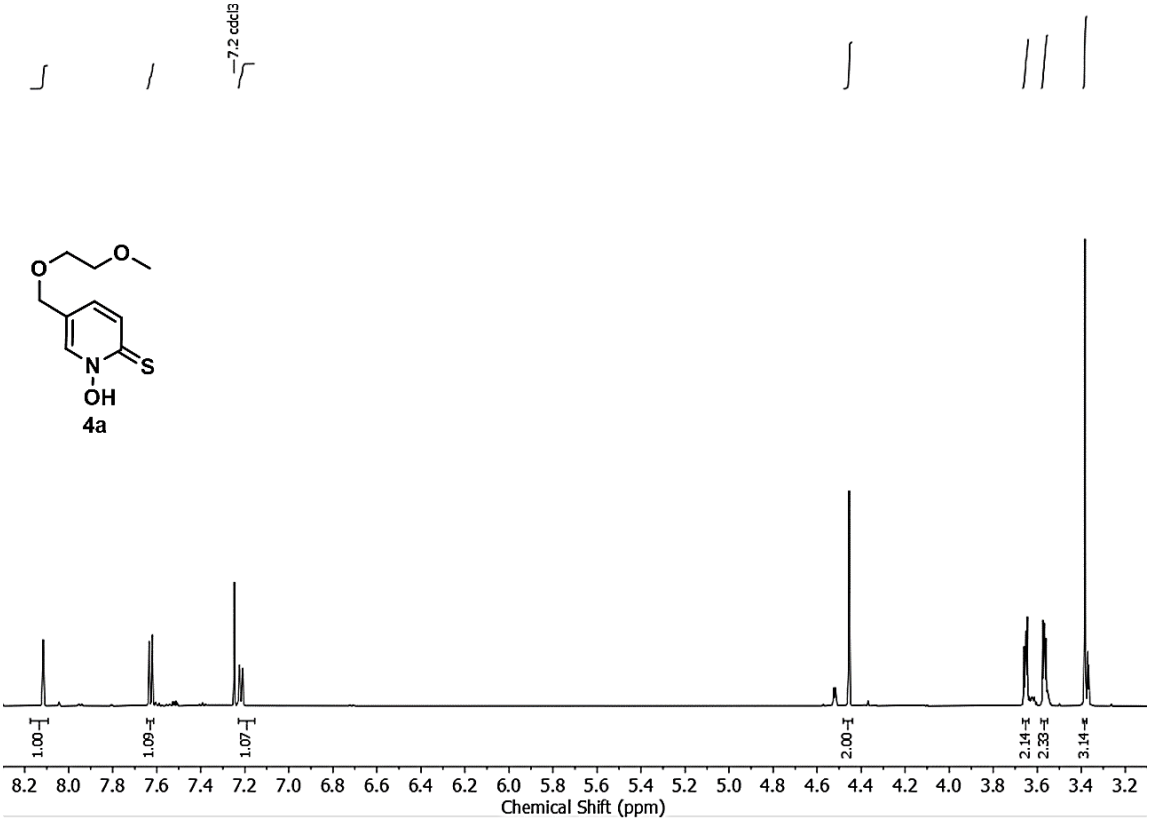


Figure S13: *^{1}H NMR spectra of compound **4a** in chloroform-d (599 MHz) at 295 K*

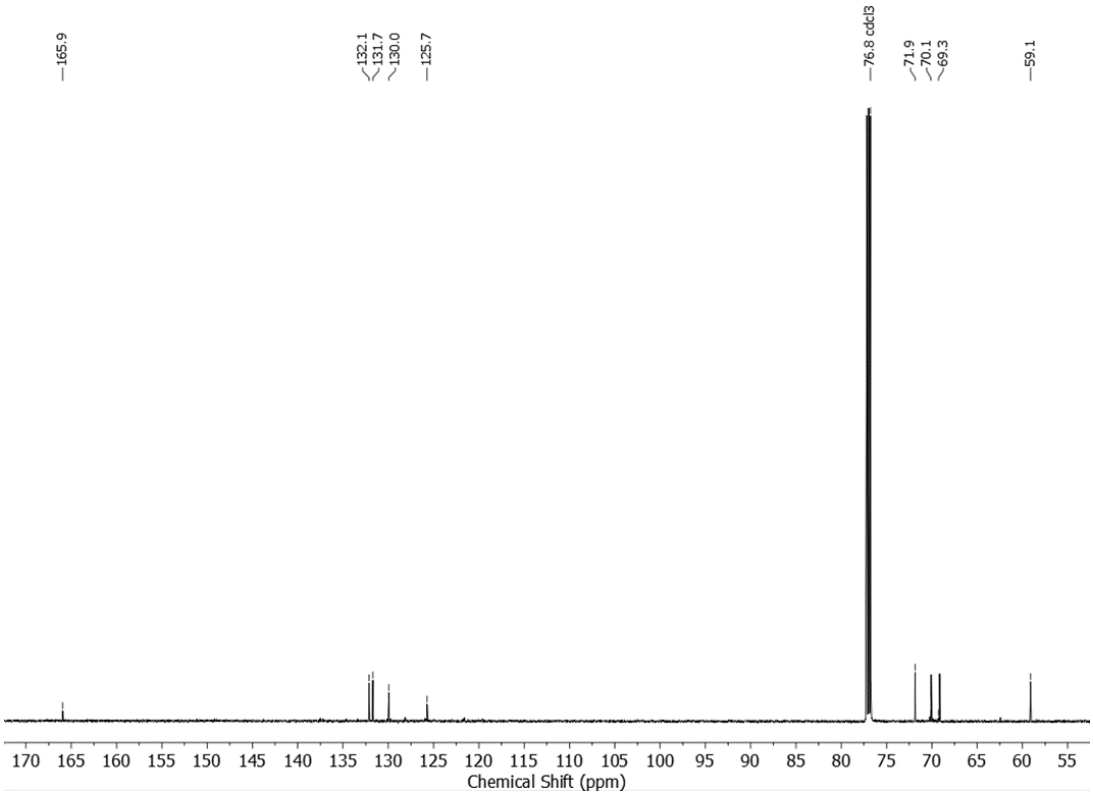


Figure S14: *^{13}C NMR spectra of compound **4a** in chloroform-d (151 MHz) at 295 K*

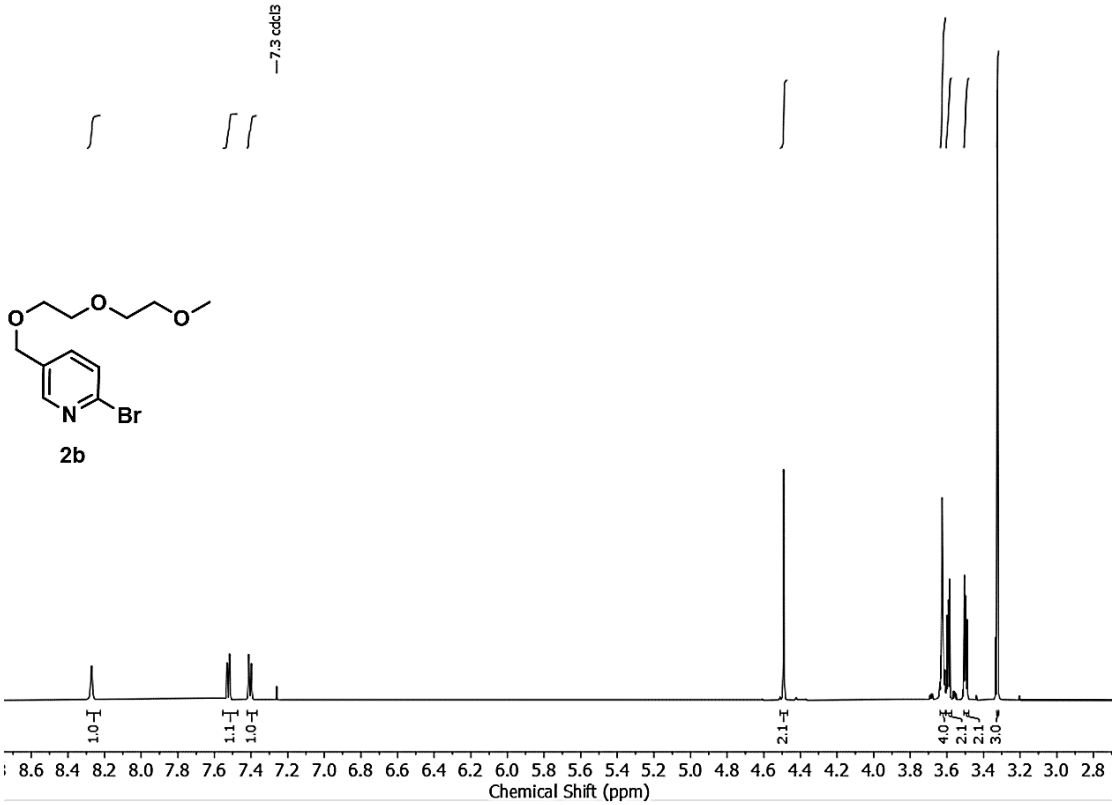


Figure S15: *^{1}H NMR spectra of compound **2b** in chloroform-d (599 MHz) at 295 K*

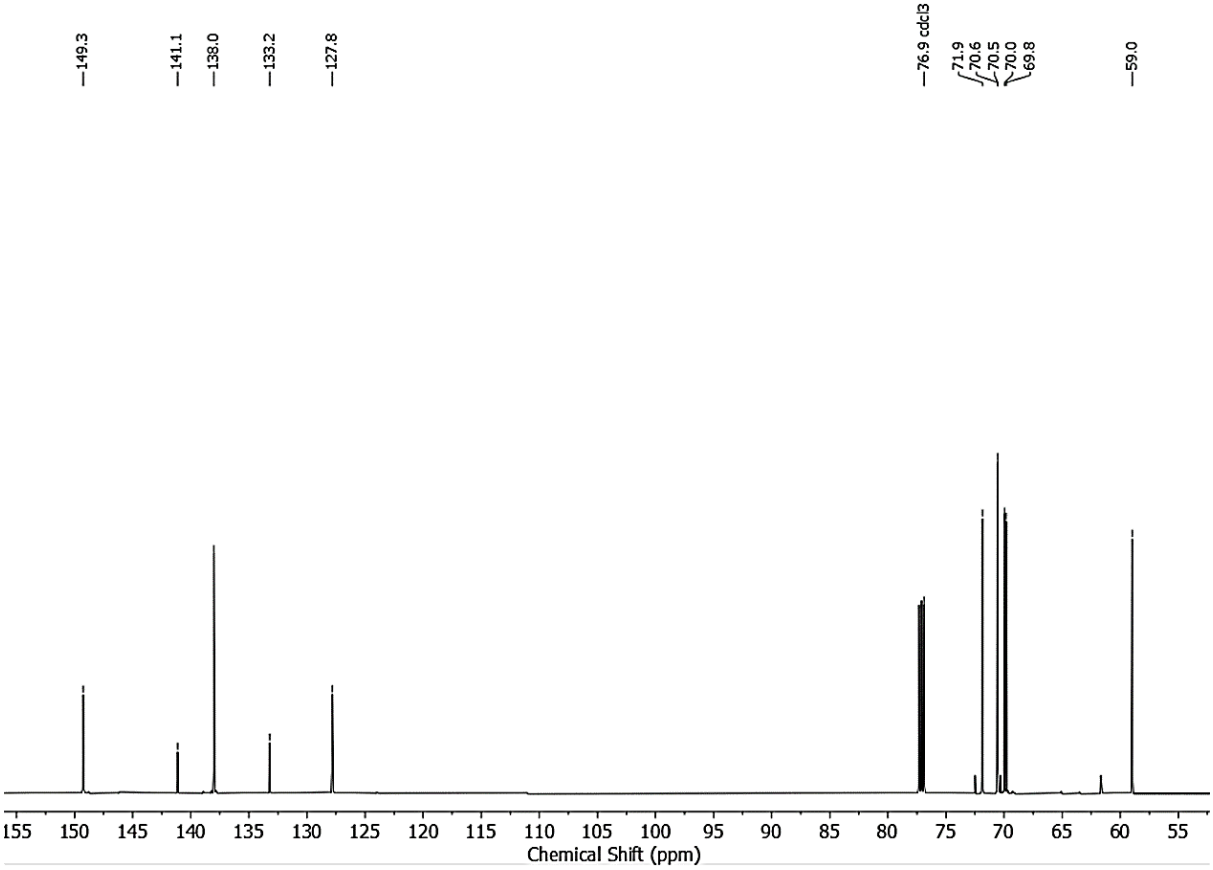


Figure S16: *^{13}C NMR spectra of compound **2b** in chloroform-d (151 MHz) at 295 K*

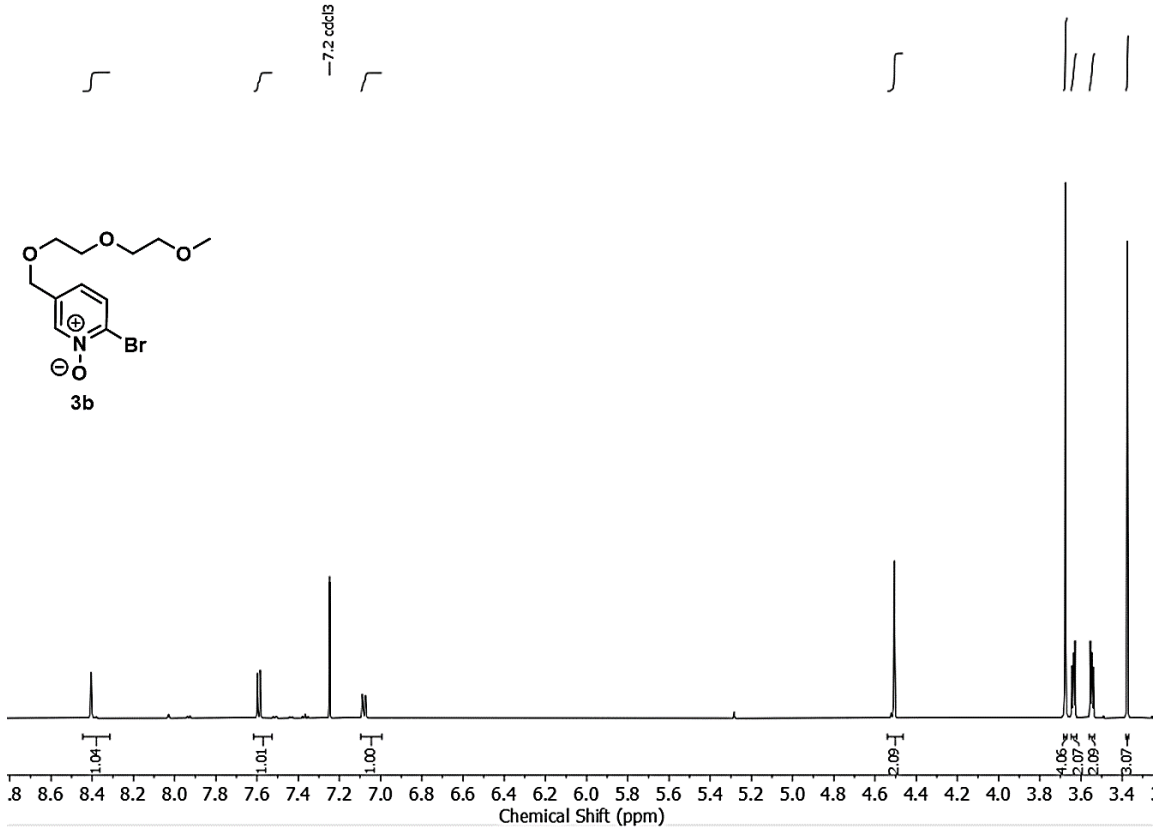


Figure S17: *^{1}H NMR spectra of compound **3b** in chloroform-d (599 MHz) at 295 K*

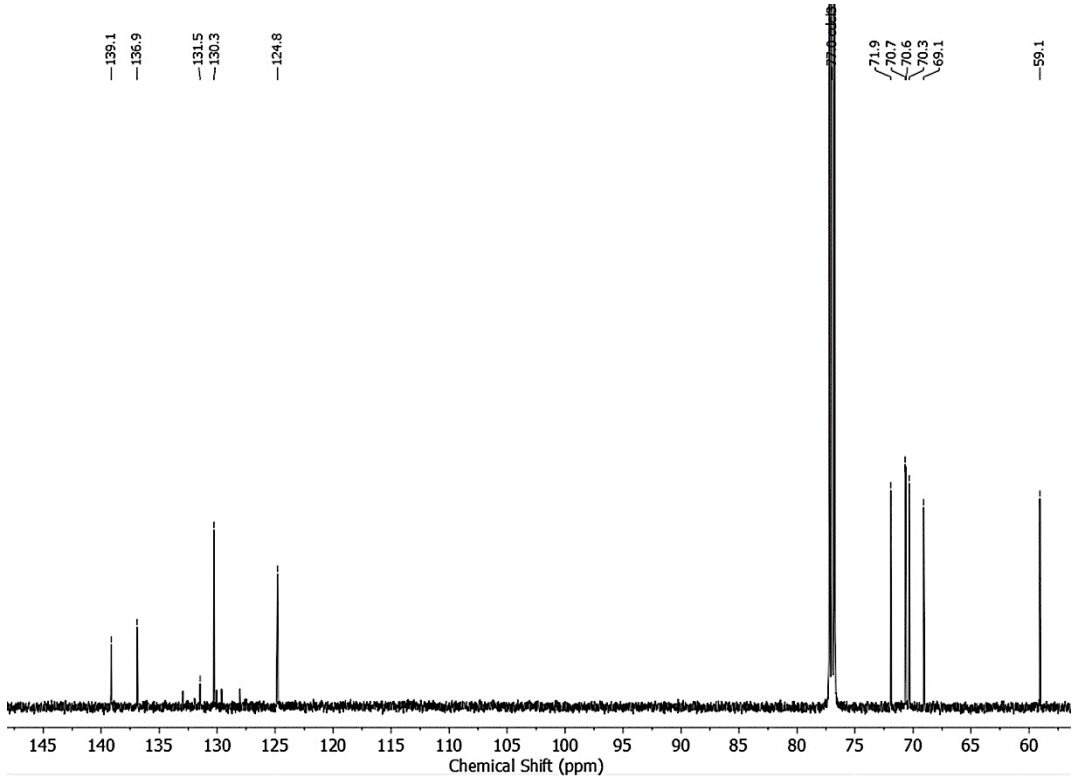


Figure S18: *^{13}C NMR spectra of compound **3b** in chloroform-d (151 MHz) at 295 K*

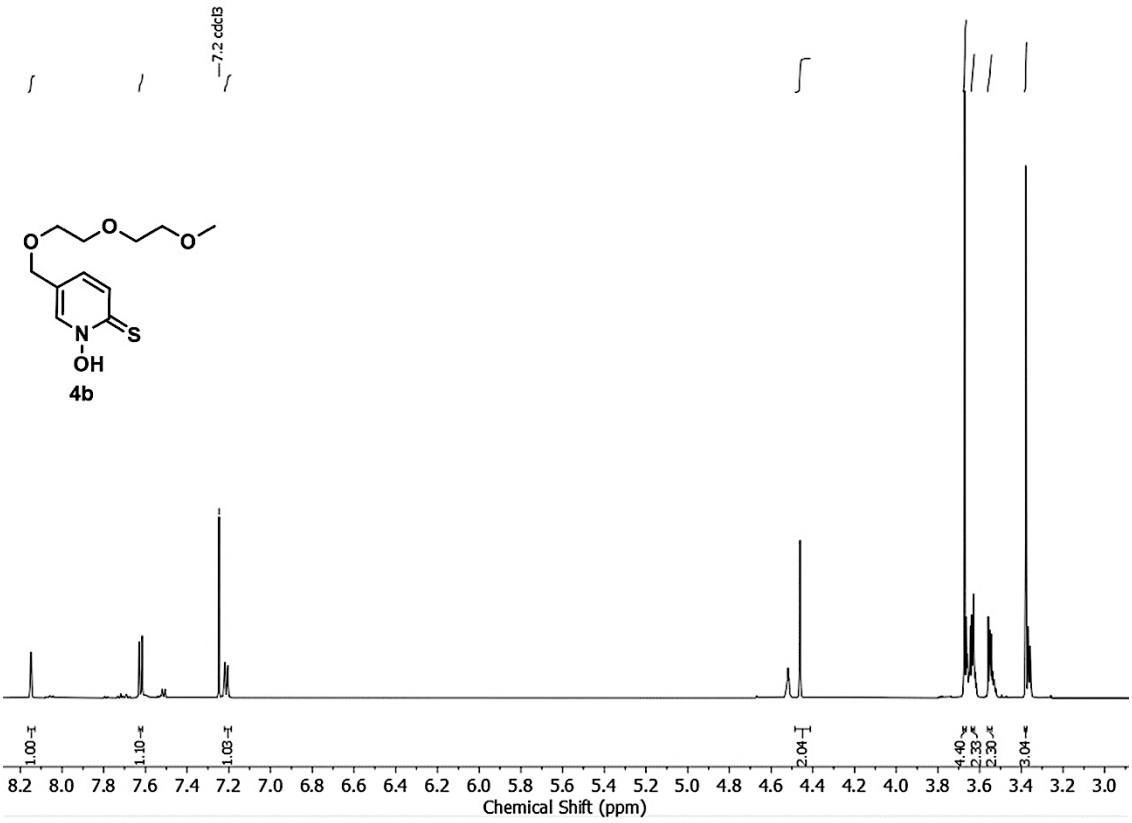


Figure S19: *^{1}H NMR spectra of compound **4b** in chloroform-d (599 MHz) at 295 K*

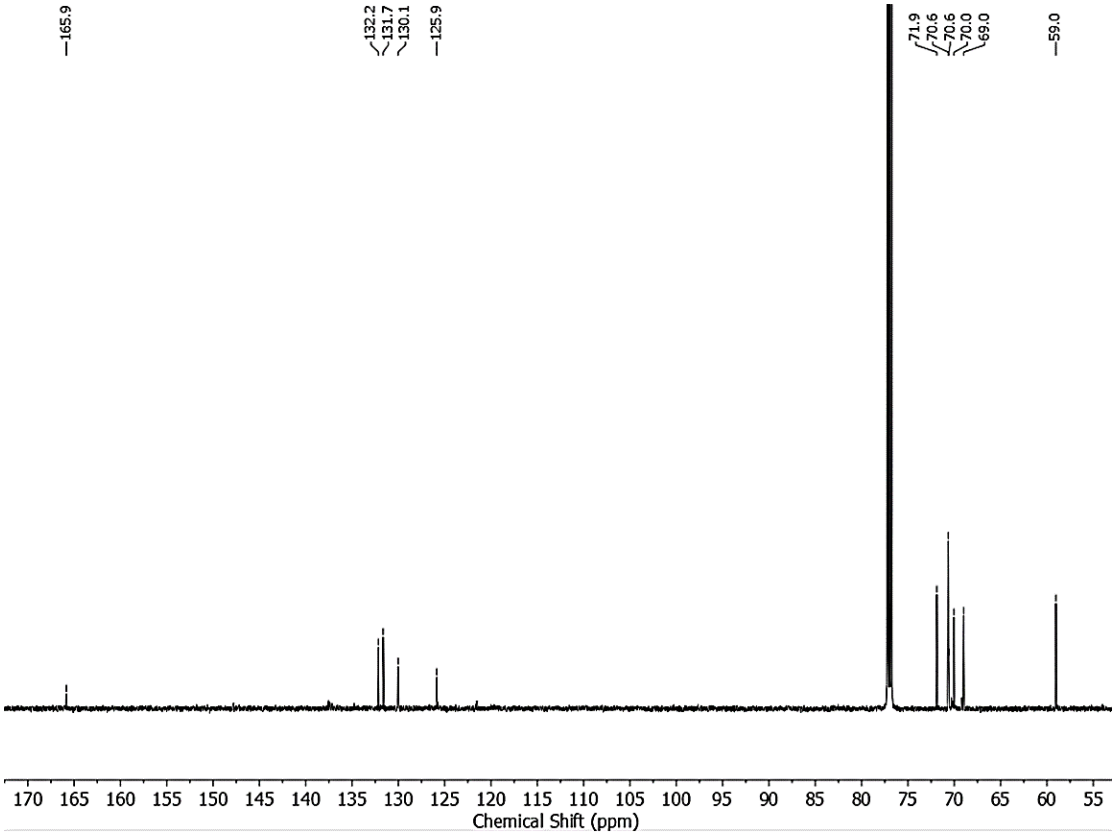


Figure S20: *^{13}C NMR spectra of compound **3b** in chloroform-d (151 MHz) at 295 K*

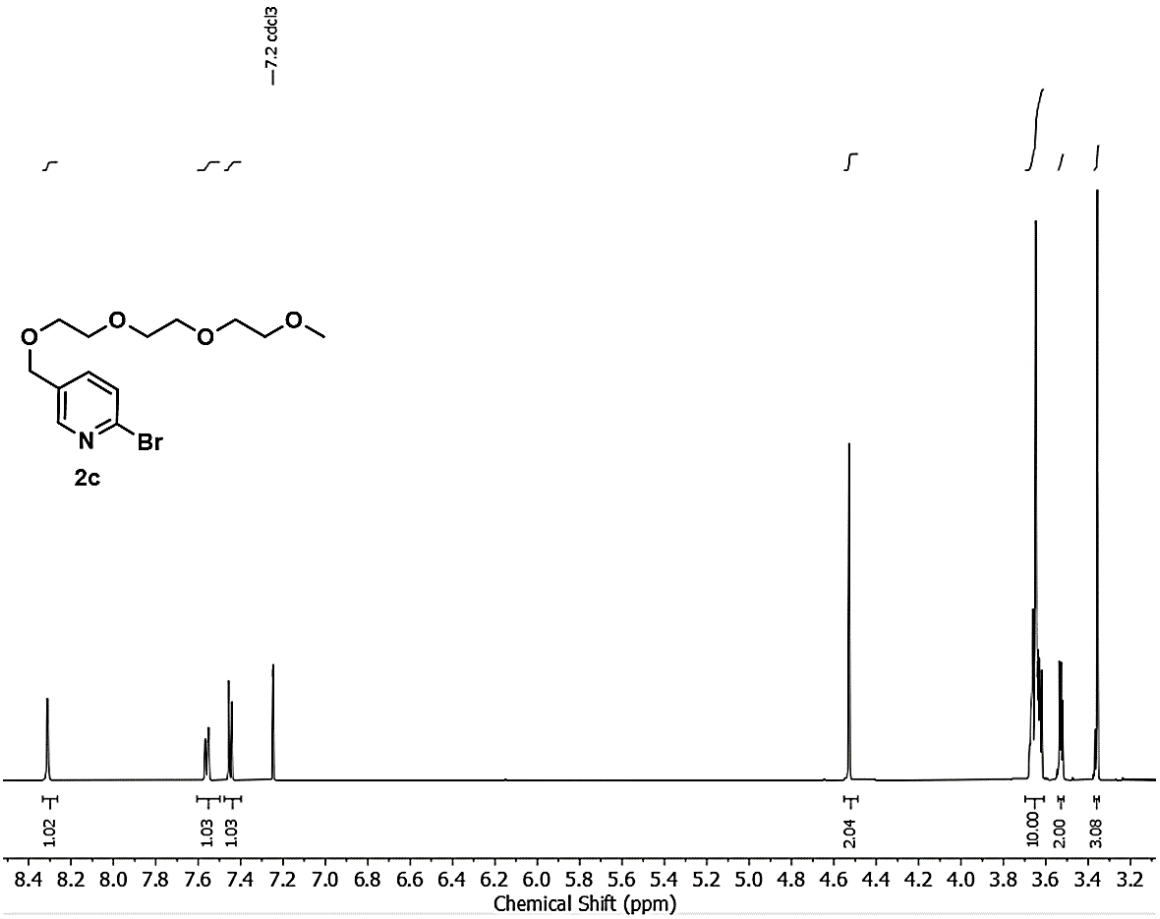


Figure S21: *[1]H NMR spectra of compound **2c** in chloroform-d (599 MHz) at 295 K*

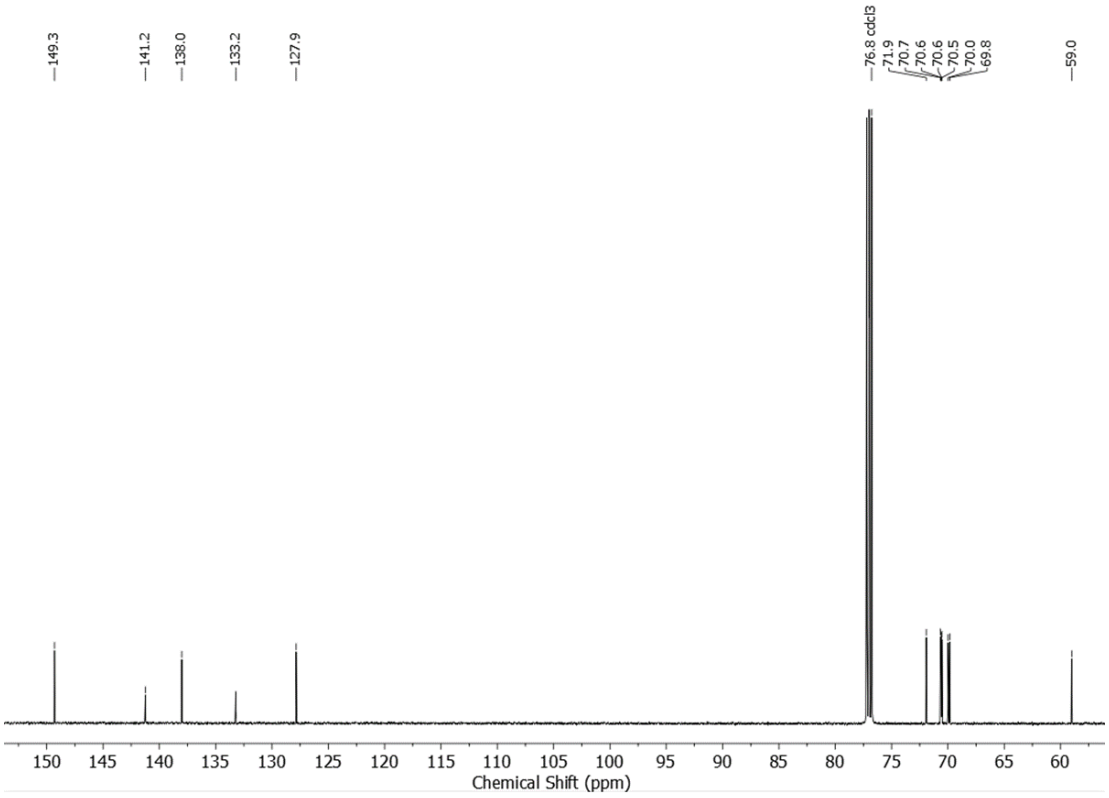


Figure S22: *[13]C NMR spectra of compound **2c** in chloroform-d (151 MHz) at 295 K*

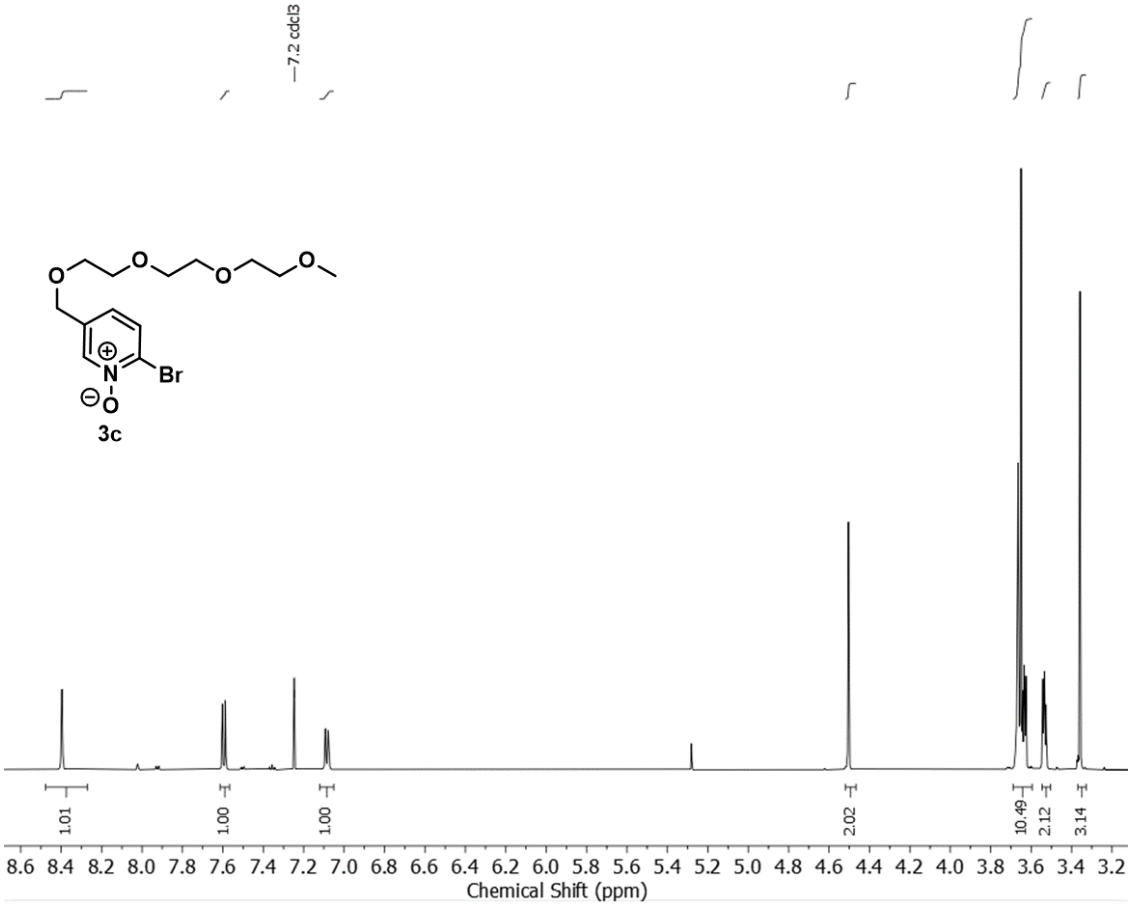


Figure S23: *[1]H NMR spectra of compound **3c** in chloroform-d (599 MHz) at 295 K*

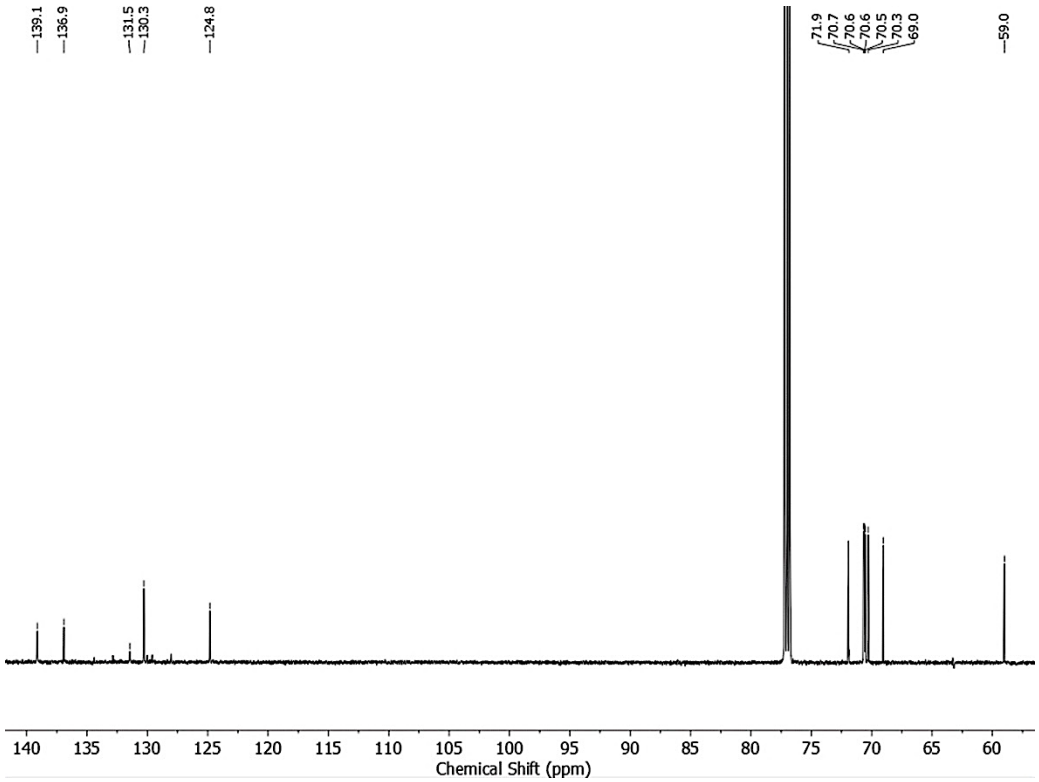


Figure S24: *[13]C NMR spectra of compound **3c** in chloroform-d (151 MHz) at 295 K*

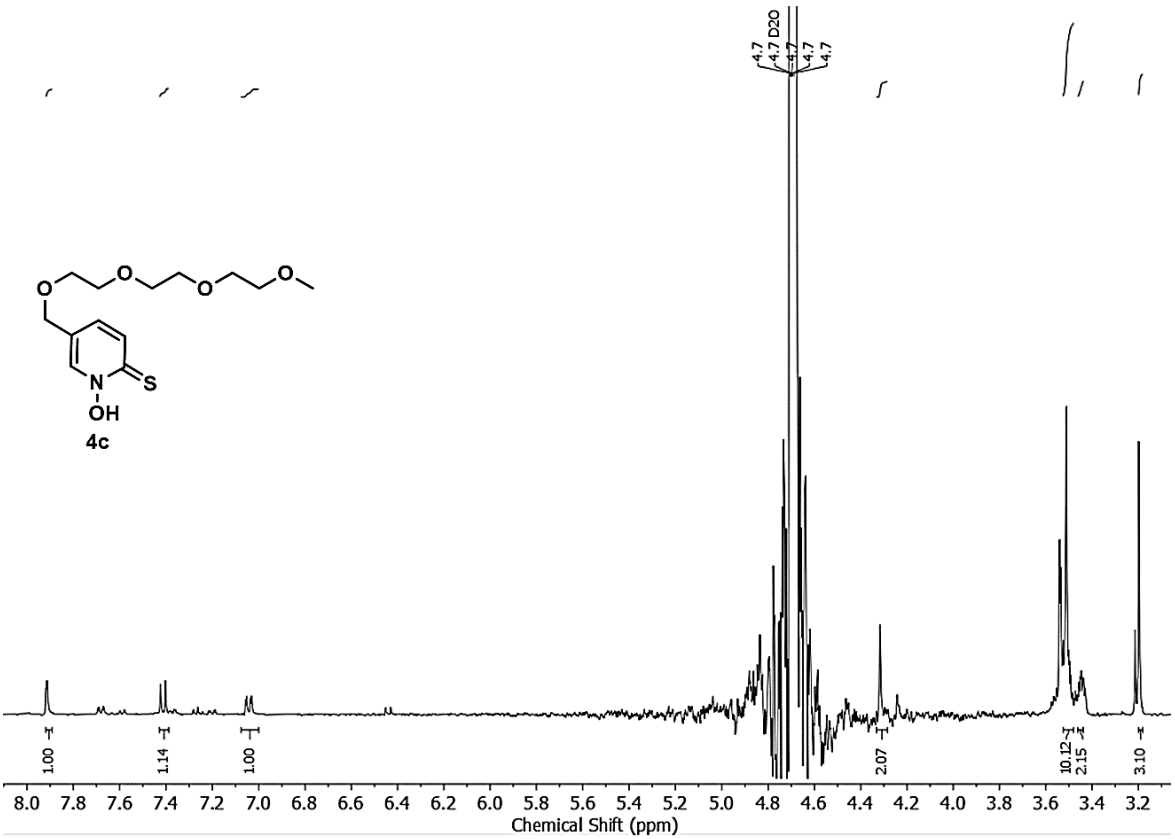


Figure S25: *^{1}H NMR spectra of compound **4c** in D_2O (599 MHz) at 295 K*