Electronic Supplementary Material (ESI) for ChemComm.


# Optochemical control of Cu(I) homeostasis in mammalian cells

## Contents

## General Methods:

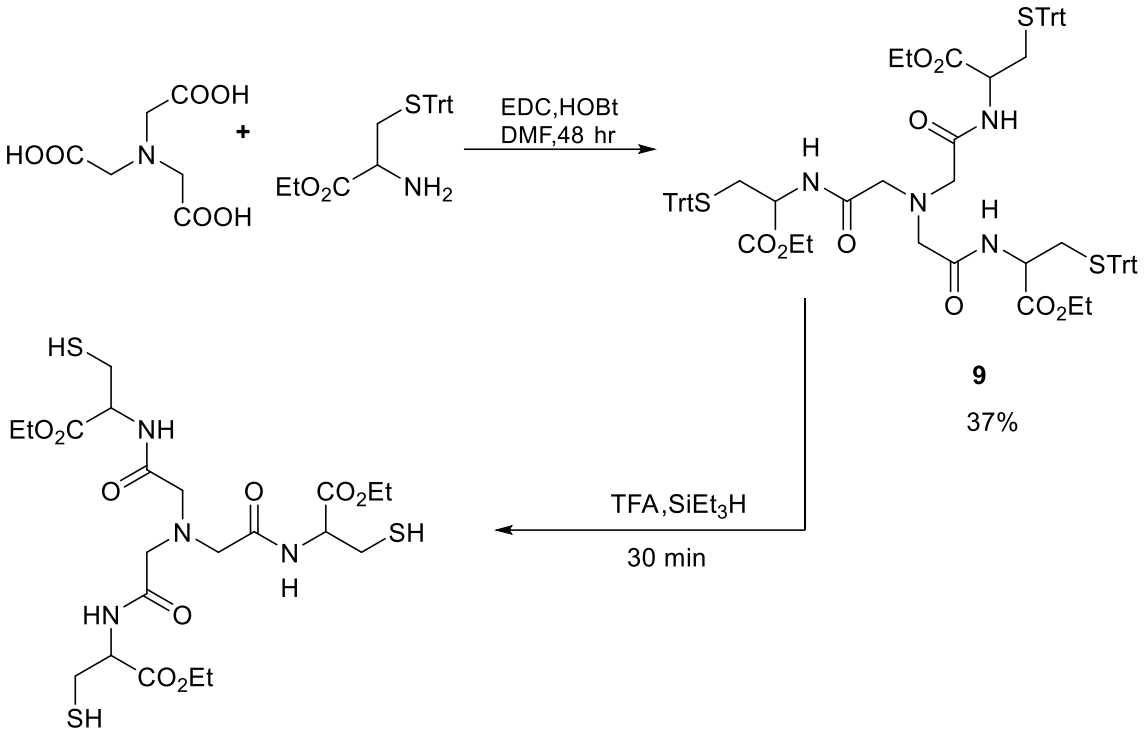
All chemicals were purchased from Sigma-Aldrich, Spectrochem, CDH fine, BLD Pharm, Alfa-aesar, TCI etc and used without further purification. DCM were dried over phosphorus pentoxide and THF was dried with metallic sodium with benzophenone as indicator, anhydrous DMF was purchased from Sigma Aldrich. All the NMR spectra were recorded in Bruker Avance II 400,500 MHz instruments. Mass spectrometry was done in Agilent UPLC-QTOF. Merck F254 TLC plates were used to monitor TLC, Buchi Rotavaps were used to evaporate solvents.

HPLC was performed in Waters Alliance e2695 equipped with Waters 2998 PDA detector where elution system is MilliQ Water (0.1% TFA)/ Acetonitrile (0.1% TFA). Data was analyzed with EMPOWER software by Waters. Stock was made with 10 mM in DMSO, from that it was diluted to 50 μM in Phosphate buffer (50mM, pH = 7.4). The HPLC vial was irradiated with 365 nm wavelength light (5 mW) as close to the solution as possible. The data was taken in different intervals ranging from 30 sec to 40 min. Gradient was given from water/ACN 95:5 to 5:95 for 8 mins and 2 mins to reach again 95:5.

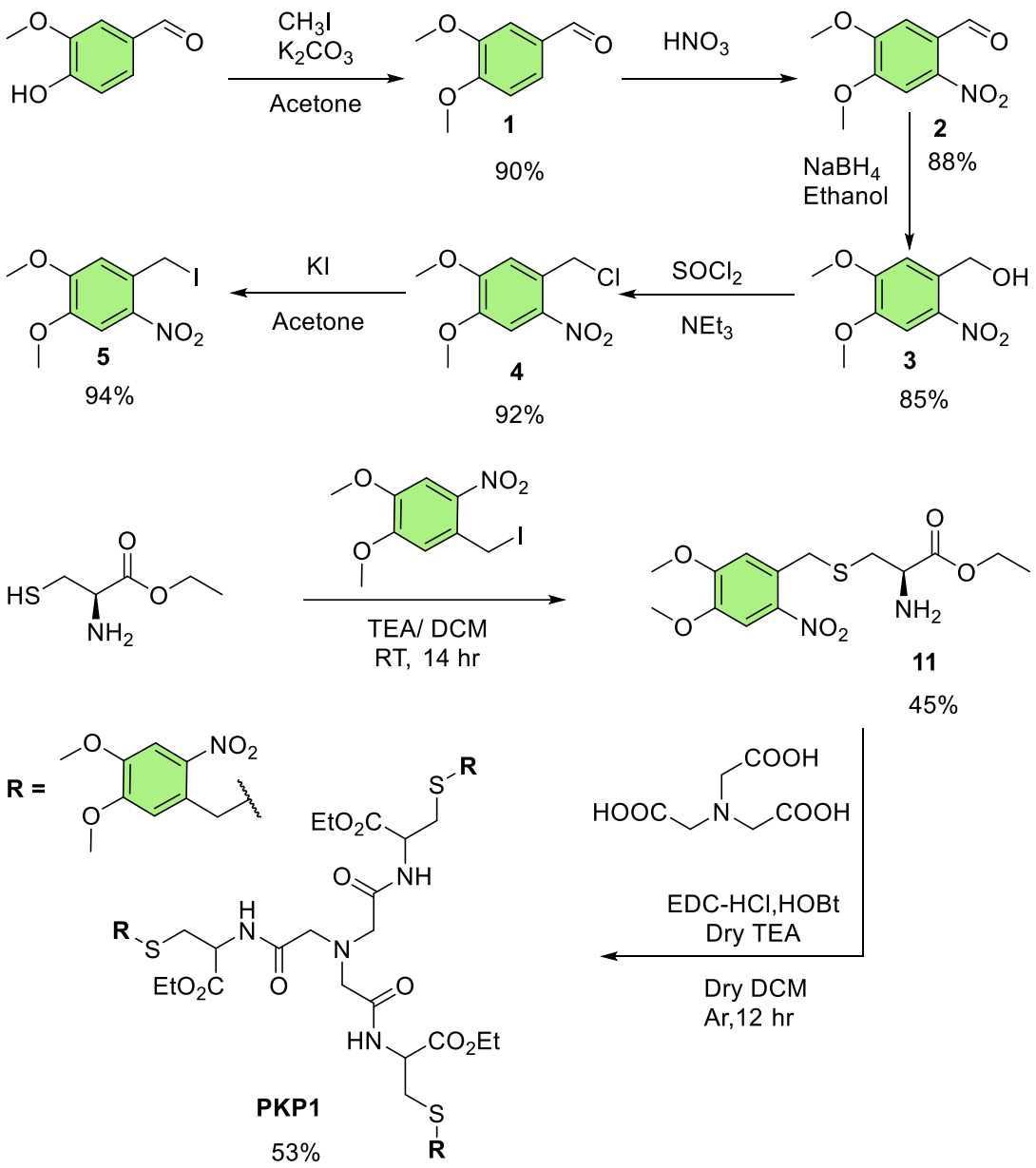
UV experiments were performed in Agilent Cary UV-vis-NIR spectrophotometer with 3.5 ml Quartz cuvette (Starna) in solution mode. BCS, a standard copper chelator was purchased from Sigma Aldrich and Tetrakis(acetonitrile)copper(I) hexafluorophosphate was used as copper salt. Phosphate buffer (50 mM, 7.2 pH) was used for the UV-Vis studies.

Emission spectra were recorded by Fluorolog Spectrometer HORIBA (Jovin Yvon) and FluorEssence V3.9 software was used. STARNA 3.5ml Quartz Cuvettes were used to perform the measurements. Stock solutions were prepared using Spectroscopic Grade solvents, MilliQ water was used for preparing buffer solutions.

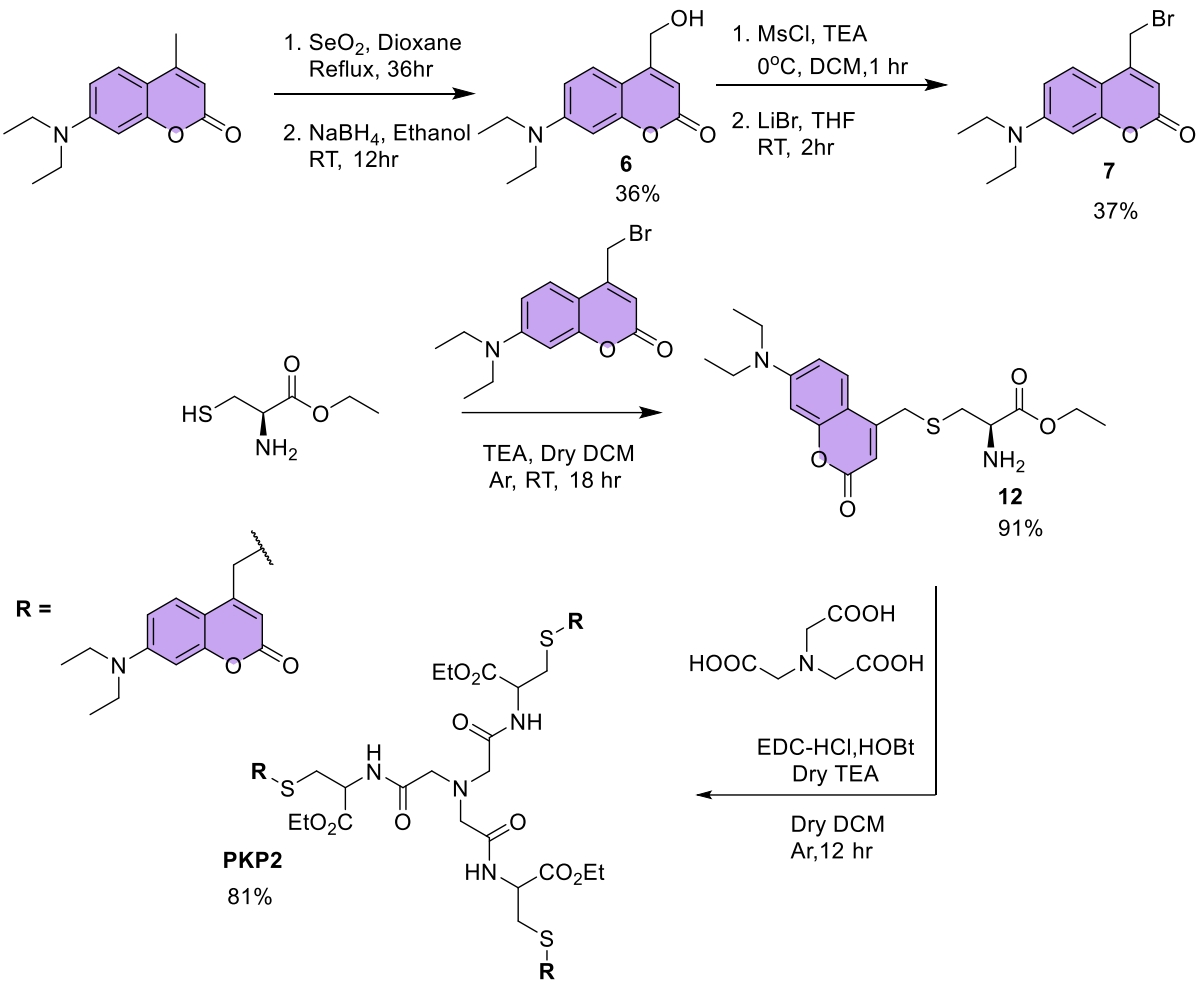
## Reaction Schemes



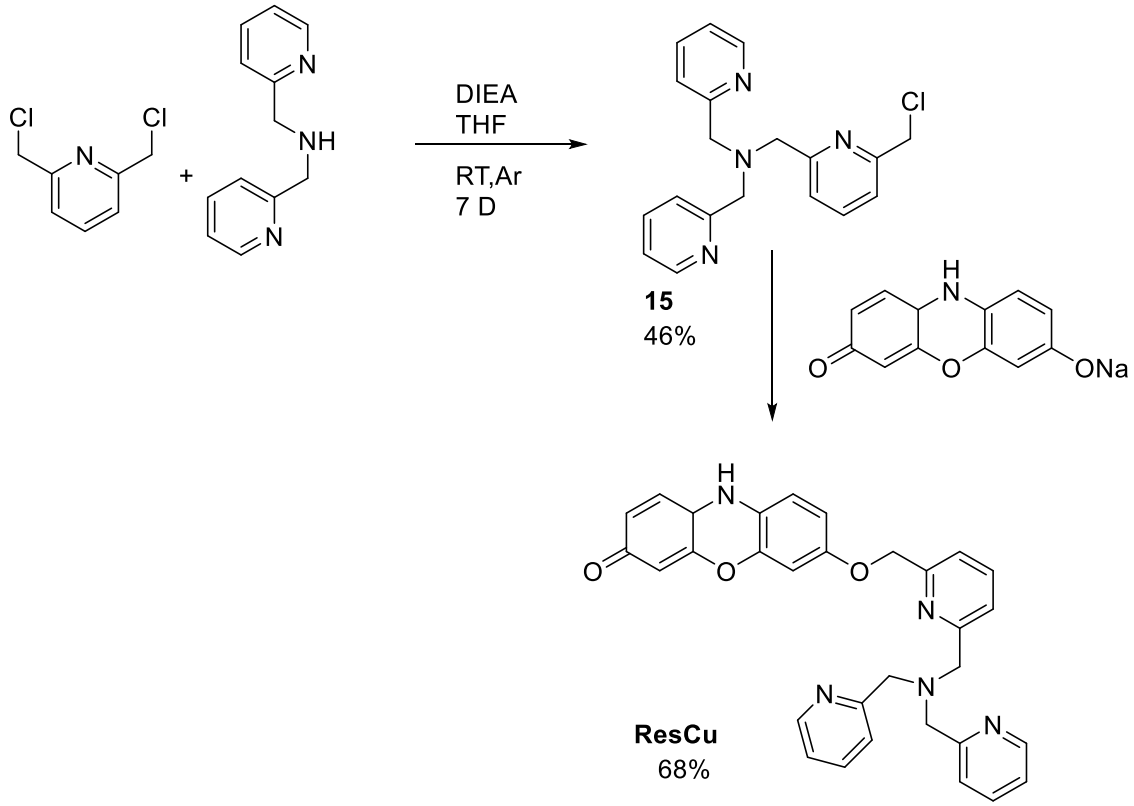

**Scheme S1**: Synthesis of tripodal NTASH Chelator[1]




**Scheme S2**: Synthesis of NVOC- based pro-chelator **PKP1**[2]

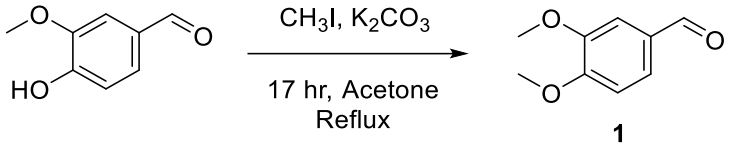



**Scheme S3**: Synthesis of coumarin based pro-chelator **PKP2**[3]




**Scheme S4**: Synthesis of copper specific fluorophore **ResCu**[4]

## Reaction Procedure:

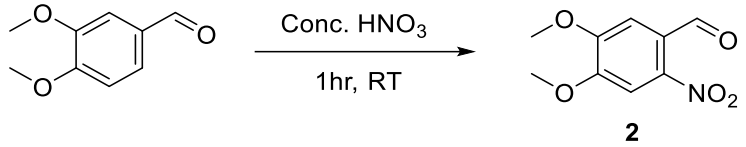



**1**

**Synthesis of compound 1**: To a 100ml round bottom flask vanillin (2.1 g, 13 mmol) and potassium carbonate (2.3 g, 16 mmol) were added and dissolved in 50 ml acetone. Methyl Iodide (2.28 ml, 28 mmol) was added dropwise in the stirring solution. The reaction mixture was heated to reflux for 17 hours; the undissolved potassium carbonate was filtered and washed with acetone. Acetone was evaporated and work-up was done with DCM and water; dried with sodium sulphate, and solvent was evaporated to give white solid (1.97 g, 90% yield).

**$^{1}$H NMR** (500 MHz, $CDCl_3$) δ = 9.88 (s, 1H), 7.48 (dd, *J*=8.2, 1.9, 1H), 7.44 (d, *J*=1.9, 1H), 7.00 (d, *J*=8.2, 1H), 3.99 (s, 3H), 3.97 (s, 3H).

**$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 190.88, 154.49, 149.63, 130.15, 126.84, 110.41, 108.96, 56.16, 55.99.

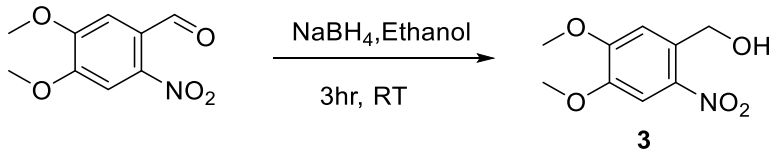



**2**

**Synthesis of compound 2**: To a 100 ml R.B. Flask **1** (1.9 g, 11.4 mmol) was taken, in ice-cold temperature cold Conc. $HNO_3$ (15 ml) was added slowly to the RB. Ice-cold condition was maintained until all the starting material were dissolved. Then it was stirred in room temperature for 1 hour. In a beaker of 100 ml of ice-cold water, the contents of the RB were added. Bright yellow solids appeared, filtered through sintered funnel, residue was collected and work-up was done with DCM/Water. Solvent was removed under high vacuum to give bright yellow solid (2.1g, 88% yield).

**$^{1}$H NMR** (400 MHz, $CDCl_3$) δ = 10.48 (s, 1H), 7.64 (s, 1H), 7.45 (s, 1H), 4.06 (d, 16H).

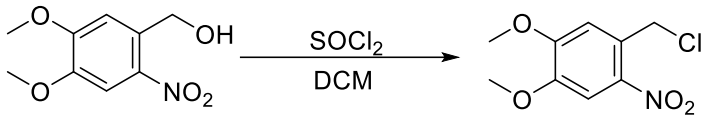
**$^{13}$C NMR** (101 MHz, $CDCl_3$) δ = 192.20, 153.85, 152.44, 125.96, 117.81, 109.80, 107.21, 60.42.




**3**

**Synthesis of compound 3**: To a 100 ml R.B. Flask **2** (300 mg, 1.42 mmol, 1 eq) was taken and dissolved in absolute ethanol/DCM (90:10), under ice-cold condition $NaBH_4$ (120 mg, 3.17 mmol, 2.25 eq) was added slowly. After 12 hours the ethanol was evaporated and work-up was done with ethyl acetate/water. The compound was purified by column chromatography using hexane/ethyl acetate (40:60) as mobile phase. Reddish yellow solid was found as product (256 mg, 85% yield).

**$^{1}$H NMR** (500 MHz, $CDCl_3$) δ = 7.73 (s, 1H), 7.20 (s, 1H), 4.98 (d, *J*=6.1, 2H), 4.03 (s, 3H), 3.98 (s, 3H), 2.63 (s, 1H).
**$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 153.92, 148.02, 139.83, 132.25, 111.07, 108.17, 62.87, 56.50, 56.42.

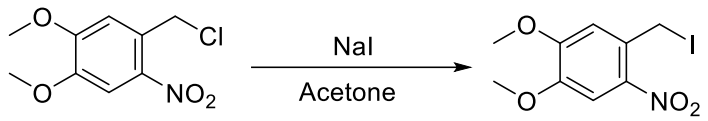



**4**

**Synthesis compound 4**: In a 50 ml R.B. flask compound 3 (1.65 g, 7.7 mmol, 1 eq) was taken and dissolved in 15 ml DCM, under ice-cold condition thionyl chloride (0.6 ml, 8.5 mmol, 1.1 eq) was added slowly. After 6 hr the solvent was evaporated and work-up was done in DCM/Water. Dark yellow solid (1.68 g, 94% yield) was found as a product.

**$^{1}$H NMR** (400 MHz, $CDCl_3$) δ = 7.71 (s, 1H), 7.13 (s, 1H), 5.04 (s, 2H), 4.03 (s, 3H), 3.99 (s, 3H).

**$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 153.36, 148.71, 127.23, 112.74, 108.37, 56.55, 56.49, 43.58.

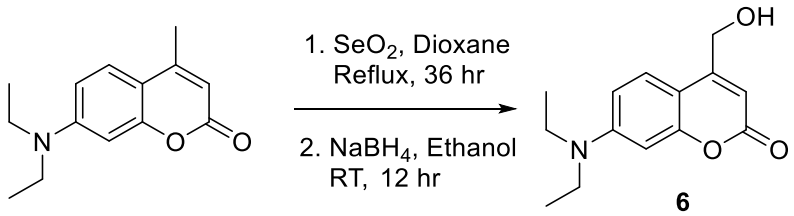



**5**

**Synthesis of compound 5**: In a 100 ml R.B. flask **4** (1.6 g, 6.92 mmol, 1 eq) was taken and dissolved in 20 ml acetone, under ice-cold condition sodium iodide (1.5 g, 10 mmol, 1.4 eq) was added pinch wise. After overnight reaction a thick precipitate was observed, acetone was evaporated and work-up was done in DCM/Water. Pale yellow solids (2.1 g, 92% yield) were found as product.

**$^{1}$H NMR** (500 MHz, $CDCl_3$) δ = 7.68 (s, 1H), 6.87 (s, 1H), 4.84 (s, 2H), 4.00 (s, 3H), 3.97 (s, 3H).

**$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 153.35, 148.63, 129.66, 113.10, 108.77, 56.55, 56.46.

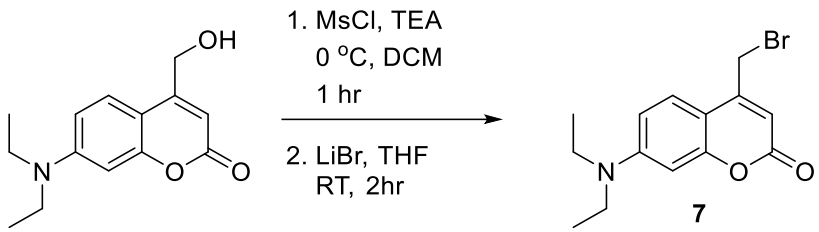



**6**

**Synthesis of compound 6**: In a 250 ml R.B. 7-(diethylamino)-4-methyl-2H-chromen-2-one (2 g, 8.6 mmol, 1eq) and selenium dioxide (2.1 g, 19 mmol, 2.2 eq) were taken, dissolved in 50 ml dioxane, refluxed. After 24 hours it was cooled to room temperature and passed through celite and celite layer was washed with ethyl acetate multiple times. The solvent was evaporated and dried under vacuum. Then dissolved in 50 ml DCM/ethanol, under ice-cold temperature $NaBH_4$ (200 mg, 5.02 mmol, 0.8 eq) was added slowly. The reaction was stirred overnight at RT, ethanol was evaporated, work-up was done with ethyl acetate/water. The compound was purified by column chromatography using DCM/acetone as mobile phase. Dark reddish solid (700 mg, 36% yield) was found.

**$^{1}$H NMR** (500 MHz, $CDCl_3$) δ = 7.33 (d, *J*=9.0, 1H), 6.57 (dd, *J*=9.0, 2.6, 1H), 6.49 (d, *J*=2.5, 1H), 6.28 (d, *J*=1.4, 1H), 4.84 (s, 2H), 3.41 (q, *J*=7.1, 4H), 1.21 (t, *J*=7.1, 6H).

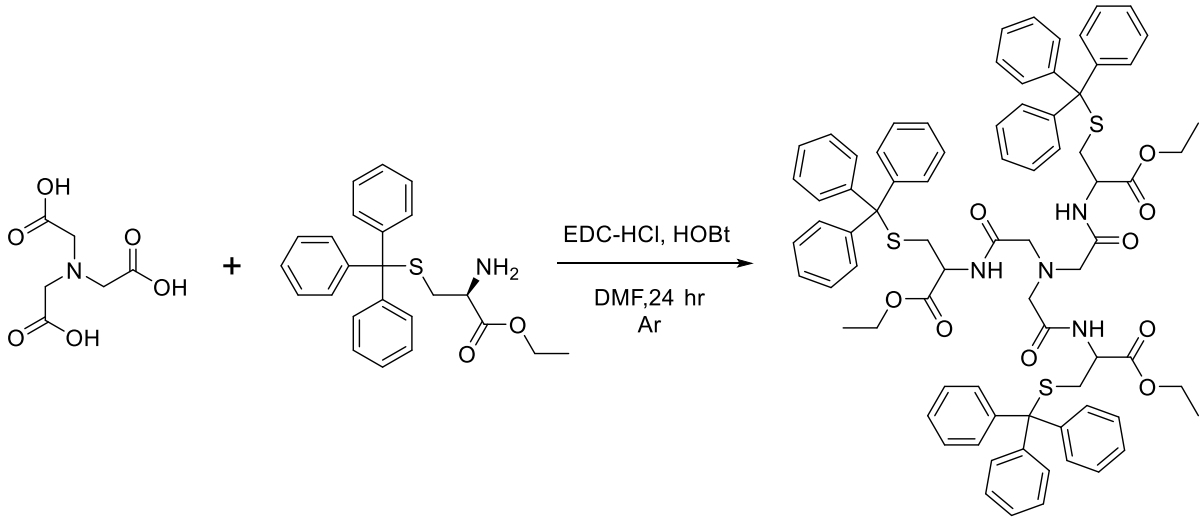
**$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 162.82, 156.13, 155.00, 150.53, 124.40, 108.63, 106.35, 105.37, 97.75, 60.92, 44.72, 12.44.




**Synthesis of compound 7**: In a 100 ml round bottom flask **6** (700 mg, 2.83 mmol, 1eq) was added and dissolved in 20 ml DCM, under ice-cold condition mesityl chloride (742 mg, 3.40 mmol, 1.2 eq) and triethyl amine (0.8 ml, 5.66 mmol, 2 eq) were added. After 1 hour reaction was stopped and work-up was done with DCM/Water. The solvent was evaporated and the solids were redissolved in 10 ml THF, lithium bromide (983 mg, 11.32 mmol, 4 mmol) was added to the reaction. After 2 hours THF was evaporated, work-up was done again in DCM/water. The resulting solid (310 mg, 37% yield) was used without further purification.

**$^{1}$H NMR** (500 MHz, $CDCl_3$) δ = 7.50 (d, *J*=9.0, 1H), 6.64 (dd, *J*=9.0, 2.6, 1H), 6.52 (d, *J*=2.6, 1H), 6.15 (s, 1H), 4.41 (s, 2H), 3.43 (q, *J*=7.1, 4H), 1.23 (t, *J*=7.1, 6H).

**$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 161.75, 156.67, 150.87, 150.38, 125.38, 109.19, 109.16, 108.66, 106.14, 97.83, 97.79, 77.34, 77.09, 76.84, 44.79, 27.15, 12.46, 12.43.



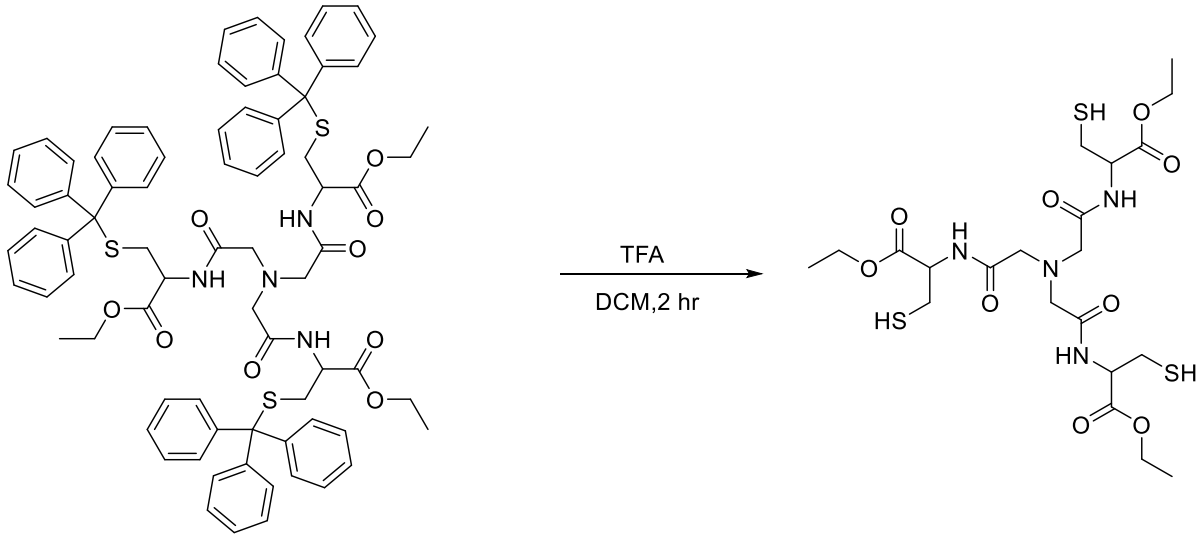

**9**

**Synthesis of compound 9**: In a 100 ml round bottom flask nitrilotriacetic acid (239 mg, 0.52 mmol,1 eq) and trityl protected L-cysteine ethyl ester (1.97 g, 5.03 mmol, 4 eq) (**8**) were taken, under argon atmosphere DMF was added. Under ice-cold condition EDC-HCl and HOBt were added. The reaction mixture was stirred at room temperature under argon atmosphere for 24 hours. Work-up was done with ethyl acetate and water and the compound was purified by silica gel column chromatography using DCM/Ethyl acetate as mobile phase. A white solid was obtained (600 mg, 37% yield) after drying with high vacuum.

**$^{1}$H NMR** (500 MHz, $CDCl_3$) δ = 7.69 (d, *J*=8.6, 3H), 7.39 (d, *J*=7.8, 18H), 7.23 (dt, *J*=29.9, 7.4, 28H), 4.46 (td, *J*=8.3, 4.1, 3H), 4.07 (dt, *J*=10.4, 7.1, 3H), 4.01 – 3.91 (m, 3H), 3.41 (d, *J*=15.2, 3H), 3.29 (d, *J*=15.2, 3H), 2.80 (dd, *J*=12.7, 8.1, 3H), 2.50 (dd, *J*=12.7, 4.2, 3H), 1.17 (t, *J*=7.1, 9H).

**$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 171.30, 170.43, 144.36, 129.56, 127.99, 126.83, 67.12, 61.89, 60.42, 57.64, 51.79, 33.33, 14.04.

**HRMS(M+H):** Theoretical: 1311.5009 found: 1311.4980

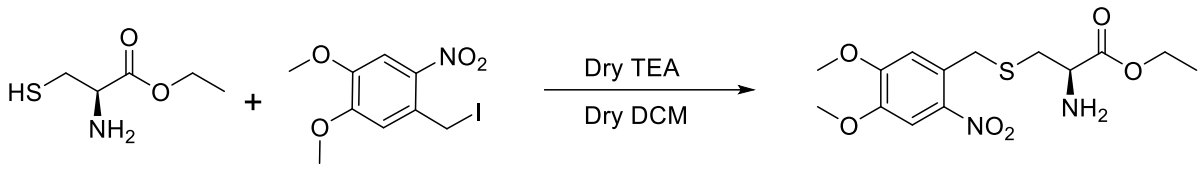



**Synthesis of NTASH**: In a 50 ml R.B. **9 (**490 mg, 0.37 mmol, 1 eq) was taken and dissolved in DCM. Under ice-cold condition TFA (1.5 ml, 25 eq) was added dropwise. The reaction was continued for two hours in RT. After two hours TFA and DCM were evaporated and work-up was done with ethyl acetate/water. The compound was purified with chromatography using DCM/methanol (9:1) as mobile phase. The compound was obtained as white solid (200 mg, 92% yield).

**$^{1}$H NMR** (500 MHz, $CDCl_3$) δ = 7.94 (d, *J*=8.3, 2H), 4.81 (dt, *J*=8.4, 5.2, 3H), 4.25 – 4.10 (m, 6H), 3.57 (d, *J*=15.7, 3H), 3.44 (d, *J*=15.8, 2H), 2.96 (dt, *J*=9.3, 4.8, 6H), 1.69 (t, *J*=8.9, 3H), 1.24 (t, *J*=7.2, 9H).

**$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 170.36, 62.14, 58.43, 54.14, 26.33, 14.09.

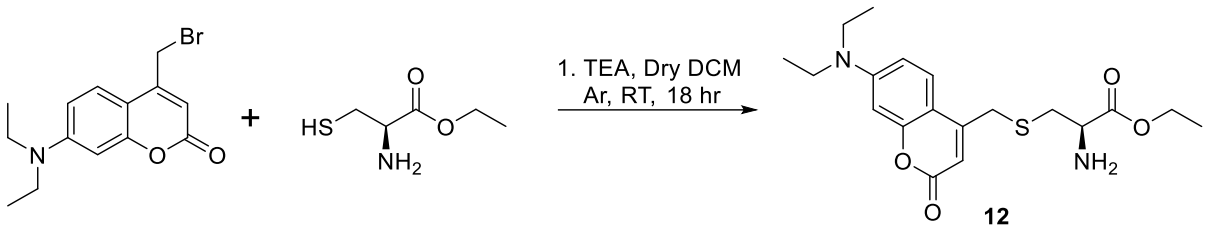
**HRMS(M+H):** Theoretical: 585.1723 Obtained: 585.1714




**Synthesis of compound 11**: In a 100 ml round bottom flask L-cysteine ethyl ester (300 mg, 1.62 mmol, 1 eq) was taken and dissolved in 10 ml dry DCM and triethylamine (0.7 ml, 4.8 mmol, 3 eq) was added and stirred for 30 mins. In another round bottom flask **5** (630mg, 1.94 mmol, 1.2 eq) was taken and dissolved in 5 ml dry DCM under argon atmosphere. In a syringe the stirring solution was taken and added to the second flask dropwise. The reaction was stirred at room temperature overnight. Work-up was done with DCM/Water, purified by column chromatography using DCM/MeOH (90:10) as mobile phase. The final compound was obtained as yellowish solid (252 mg, 45% yield).

**$^{1}$H NMR** (500 MHz, $CDCl_3$) δ = 7.65 (s, 1H), 6.96 (s, 1H), 4.20 (dd, *J*=8.4, 5.8, 2H), 4.19 – 4.11 (m, 2H), 4.00 (s, 3H), 3.95 (s, 3H), 3.66 (dd, *J*=7.5, 4.6, 1H), 2.93 (dd, *J*=13.6, 4.6, 1H), 2.76 (dd, *J*=13.6, 7.5, 1H), 1.28 (t, *J*=7.1, 3H).
**$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 173.70, 152.97, 147.92, 140.63, 128.64, 113.38, 113.32, 108.64, 61.31, 56.55, 56.45, 56.41, 56.29, 54.32, 54.26, 37.26, 37.17, 37.09, 34.40, 34.30, 34.19, 14.18, 14.12.

**HRMS(M+H):** Theoretical: 345.1120 Obtained: 345.1136

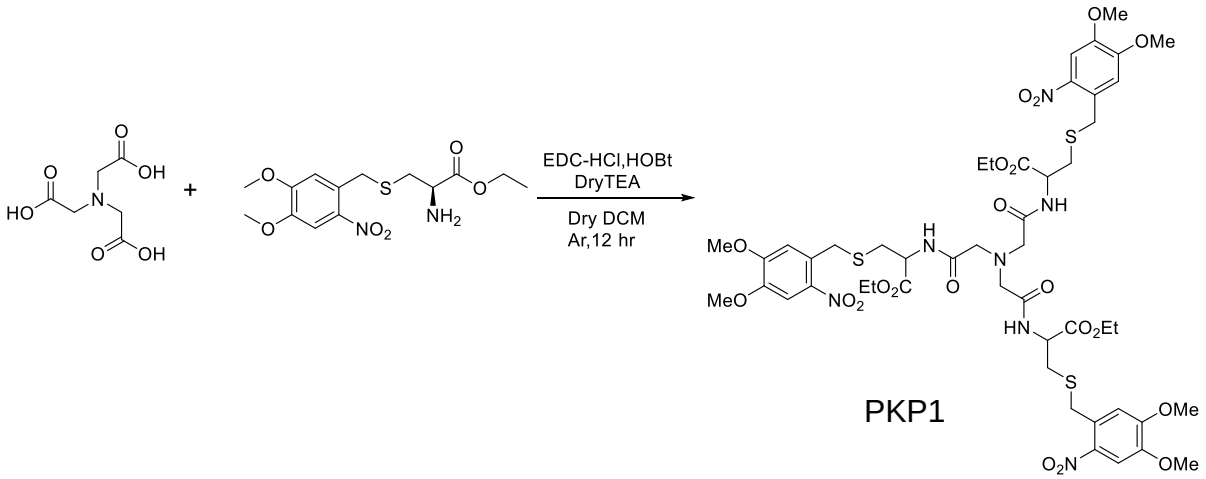



**Synthesis of compound 12**: In a 100 ml round bottom flask. L-cysteine ethyl ester (240 mg, 1.3 mmol, 1eq) was taken and dissolved in dry DCM, dry TEA was added. Compound **7** (500 mg, 1.61 mmol, 1.2 eq) was dissolved in DCM and added to the R.B. under argon atmosphere with a syringe. After overnight stirring the reaction was stopped and work-up was done with DCM/Water. The compound was purified by flash column chromatography using DCM/MeOH (Gradient 100:0 to 90:10). Yellowish oily solid (452 mg, 91% yield) was obtained as product.

**$^1$H NMR** (500 MHz, $CDCl_3$) δ = 7.43 (d, *J*=9.0, 1H), 6.54 (dd, *J*=9.0, 2.6, 1H), 6.44 (d, *J*=2.6, 1H), 5.99 (s, 1H), 4.14 (q, *J*=9.2, 8.1, 2H), 3.72 (s, 2H), 3.63 (dd, *J*=7.3, 4.7, 1H), 3.36 (q, *J*=7.1, 4H), 2.88 (dd, *J*=13.6, 4.6, 1H), 2.74 (dd, *J*=13.6, 7.2, 1H), 1.22 (t, *J*=7.3, 3H), 1.16 (t, *J*=7.1, 6H).

**$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 173.67, 161.71, 156.61, 151.44, 150.66, 125.65, 108.65, 108.54, 108.51, 106.85, 97.71, 97.67, 61.37, 54.25, 54.21, 44.69, 36.90, 32.74, 14.15, 14.12, 12.44, 12.41.

**HRMS(M+H):** Theoretical: 379.1692 Obtained: 379.1707

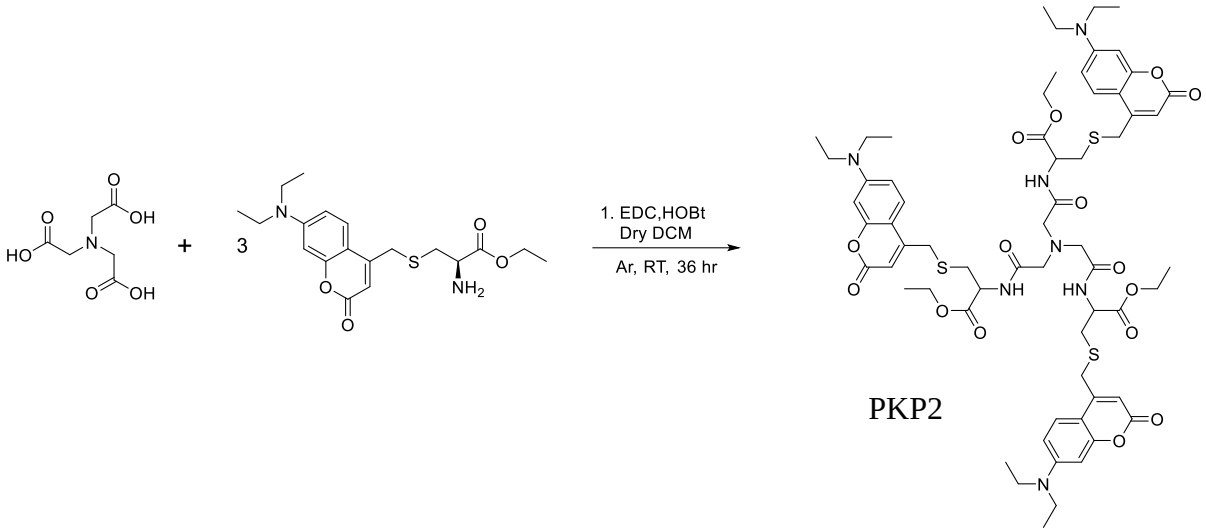



**Synthesis of PKP1**: In a 100 ml R.B. nitrilotriacetic acid (38 mg,0.2 mmol,1 eq) and **11** (252 mg, 0.73 mmol, 3.5 eq) was taken and vacuumed, under argon atmosphere in 10 ml dry DCM and dry TEA were added. Under ice-cold condition, EDC-HCl and HOBt were added to the stirring solution. Reaction was continued for 36 hours under argon atmosphere. Work-up was done with DCM/Water, compound was purified by flash column chromatography using DCM/MeOH (Gradient 100/0 to 85/15 for 30 mins). Product was obtained as pale yellow puffy solid (124 mg, 53% yield).

**$^1$H NMR** (500 MHz, $CDCl_3$) δ = 7.73 (d, *J*=8.5, 2H), 7.64 (s, 3H), 6.90 (s, 3H), 4.82 (dt, *J*=8.1, 4.0, 3H), 4.23 – 4.13 (m, 9H), 4.07 (d, *J*=13.3, 3H), 3.97 (d, *J*=28.6, 17H), 3.54 – 3.34 (m, 6H), 3.07 – 2.84 (m, 6H), 1.25 (t, 9H).

**$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 171.02, 170.35, 153.01, 147.96, 140.41, 128.43, 113.58, 113.55, 108.77, 62.08, 58.55, 56.60, 56.53, 56.38, 56.31, 51.62, 34.16, 33.83, 14.07, 14.04.

**HRLCMS(M+H):** Theoretical:1170.3317 found: 1170.3286

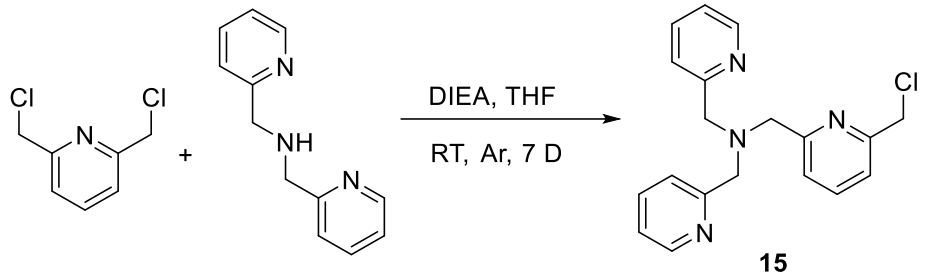



**Synthesis of PKP2**: In a 100 ml R.B. nitrilotriacetic acid (49 mg,0.25 mmol, 1eq) and **12** (340 mg, 0.89, 3.5 eq) were taken, vacuumed and under argon atmosphere dry DCM was added. Under ice-cold condition EDC-HCl and HOBt were added. The reaction was continued at RT for 36 hours under argon. Work-up was done with DCM/Water. The compound was purified by flash chromatography with DCM/MeOH (Gradient 100:0 to 85:15). The product was obtained as a yellow solid (264 mg, 81% yield).

**$^1$H NMR** (500 MHz, $CDCl_3$) δ = 7.94 (d, *J*=9.1, 2H), 7.44 (d, *J*=9.1, 3H), 6.62 – 6.54 (m, 3H), 6.46 (d, *J*=2.4, 3H), 6.06 (d, *J*=3.3, 3H), 4.85 (dd, *J*=9.1, 4.5, 3H), 4.15 (t, *J*=6.8, 6H), 3.73 (s, 6H), 3.55 – 3.44 (m, 6H), 3.40 (q, *J*=7.2, 12H), 3.07 (dd, *J*=14.1, 4.6, 3H), 2.94 – 2.82 (m, 3H), 1.25 (t, *J*=7.3, 9H), 1.19 (t, *J*=7.0, 18H).

**$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 171.11, 170.60, 161.93, 156.61, 151.24, 150.71, 125.68, 108.63, 106.84, 97.68, 97.66, 62.27, 58.52, 51.30, 51.18, 44.73, 33.74, 32.04, 14.06, 12.46, 12.44.

**HRMS(M+H):** Theoretical: 1272.5031 Obtained:1272.5004

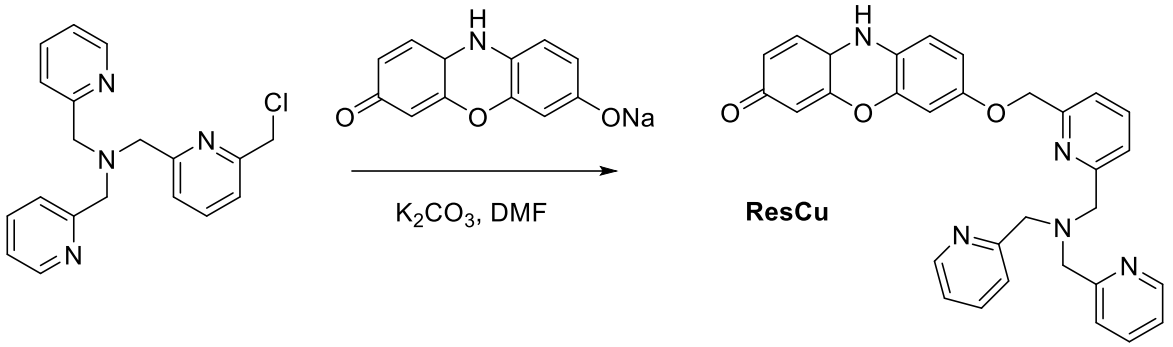



**15**

**Synthesis of 15:** 2,6-bis(chloromethyl)pyridine (270 mg, 1.55 mmol, 1 eq) and bis(pyridin-2-ylmethyl)amine (298 mg, 1.5 mmol, 1eq) was taken in a R.B. and under Ar atmosphere dissolved in dry THF. DIEA (0.8 ml, 8.25 mmol, 5.5 eq) was added dropwise, and reaction was stirred under Ar for 7 days. After completion of the reaction THF was removed and the compound was purified by column chromatography with DCM/Methanol (80:20). Yellowish white solid (242 mg, 46% yield) was obtained as product.

**$^1$H NMR** (500 MHz, $CDCl_3$) δ = 8.57 – 8.52 (m, 2H), 7.72 – 7.63 (m, 3H), 7.56 (dd, *J*=20.1, 7.8, 3H), 7.34 (d, *J*=7.6, 1H), 7.15 (ddd, *J*=7.7, 4.9, 1.3, 2H), 4.66 (s, 2H), 3.90 (d, *J*=3.5, 6H).

**$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 159.31, 159.24, 155.85, 149.06, 137.41, 136.44, 123.03, 122.21, 122.02, 121.00, 60.21, 59.97, 46.77.




**Synthesis of 16:** Compound **15** (100 mg) and $K_2CO_3$ (88 mg) was taken in a round bottom flask dissolved in 10 ml dry DMF, resorufin sodium salt (160 mg) was added to it. The reaction mixture was stirred at 90˚C with a condenser overnight. After cooling down to room temperature, DMF was evaporated. The residue was dissolved in DCM, washed with water, brine, and dried over anhydrous $Na_2SO_4$. Column chromatography was performed with DCM/Methanol (95:5) to purify the compound. Red crystalline solid (105 mg, 68% yield) was obtained as the product.

**$^1$H NMR** (500 MHz, $CDCl_3$) δ = 8.54 (d, *J*=4.9, 2H), 7.76 – 7.63 (m, 4H), 7.57 (q, *J*=6.0, 3H), 7.41 (d, *J*=9.8, 1H), 7.35 (d, *J*=7.8, 1H), 7.16 (dd, *J*=7.5, 4.9, 2H), 7.04 (dd, *J*=9.0, 2.7, 1H), 6.91 (d, *J*=2.6, 1H), 6.83 (dd, *J*=9.9, 2.0, 1H), 6.29 (s, 1H), 5.29 (s, 2H), 3.92 (d, *J*=12.4, 6H).

**$^{13}$C NMR** (126 MHz, $CDCl_3$) δ 186.29, 162.36, 159.35, 159.18, 154.87, 149.75, 149.11, 145.76, 145.55, 137.45, 136.50, 134.69, 134.25, 131.64, 128.57, 123.01, 122.47, 122.11, 119.78, 114.14, 106.77, 101.34, 71.51, 60.13, 60.01, 29.69.

**HRMS(M):** Theoretical: 517.2114 Obtained: 517.2120

## HPLC Study with Light Irradiation

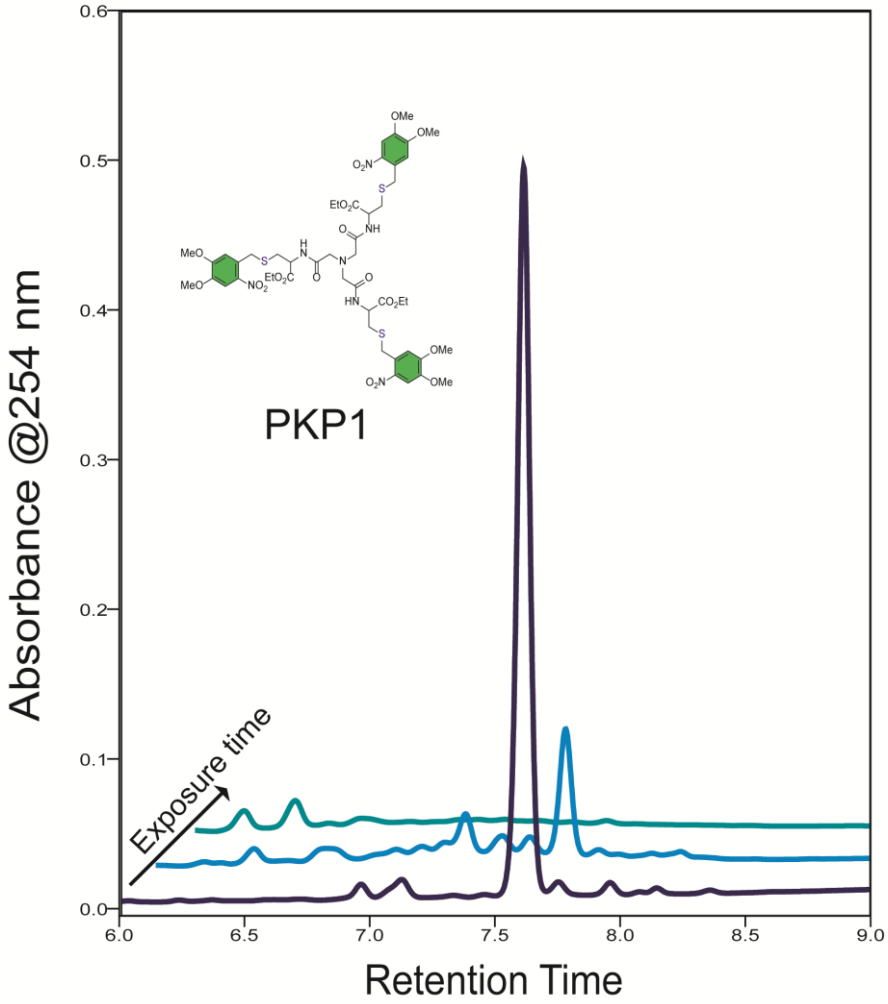



**Figure S1:** HPLC Chromatogram of PKP1 with increasing exposure time to 365 nm light

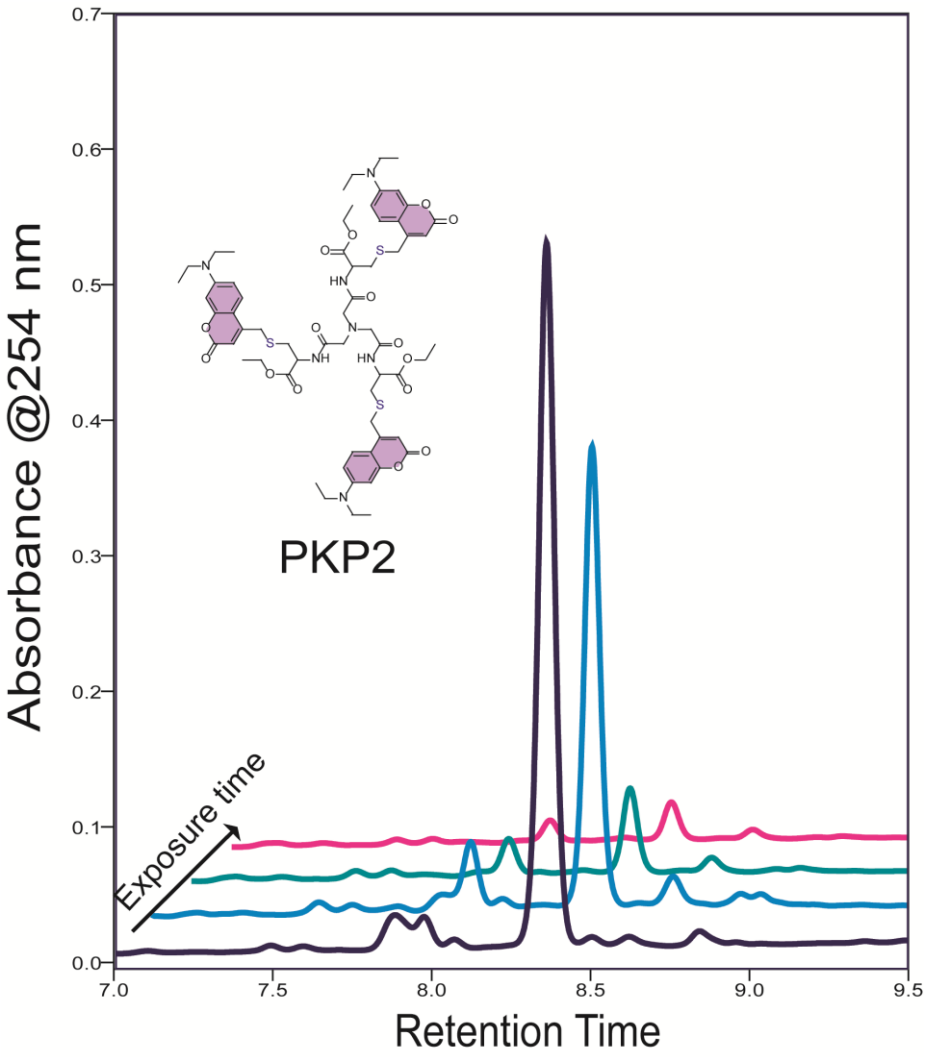



**Figure S2:** HPLC Chromatogram of PKP2 with increasing exposure time to 365 nm light

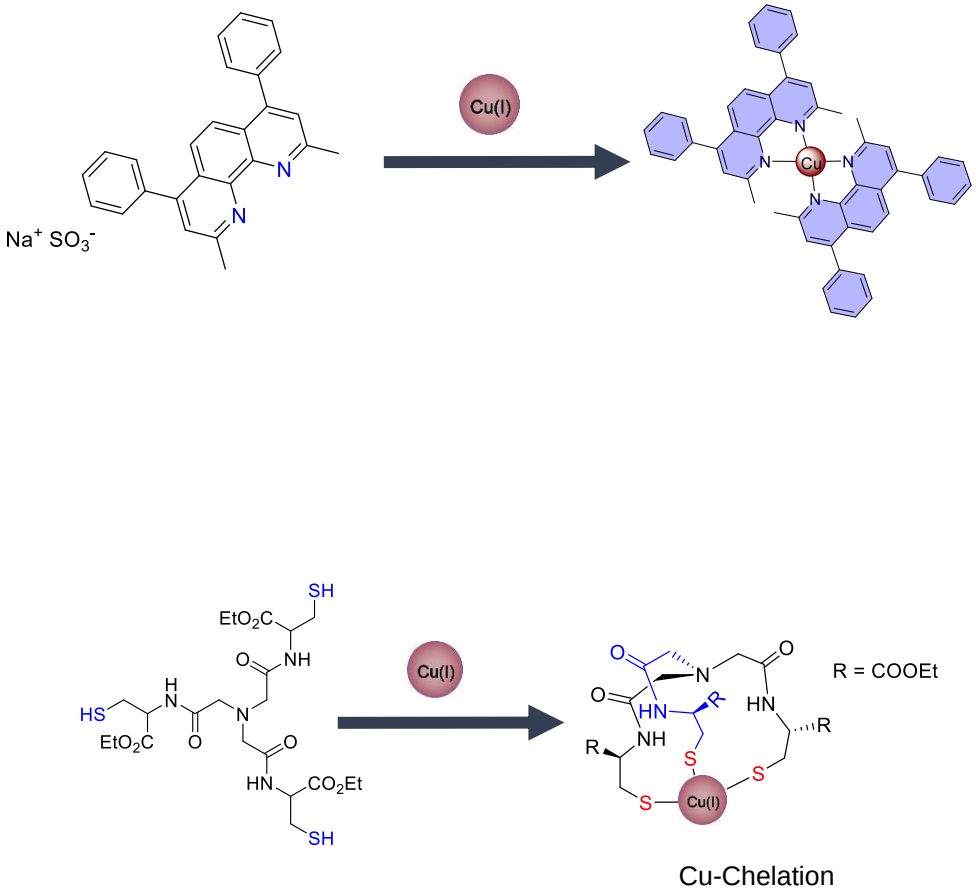



**Figure S3:** Schematic representation of BCS salt forming complex with Cu(I) and shows MLCT band**.** (top) Copper Chelation by tripodal NTASH chelator. (bottom)

## UV Titration of Copper Chelation with NTASH Chelator

BCS Salt (100 µM) and Cu(I) (50 µM) were added to 50 mM phosphate buffer and kept for 2 hours for formation of the complex. Then NTASH was added, mixed and absorbance was measured, the decrease of the absorbance maxima was observed with increasing NTASH concentration. The colour of BCS-Cu complex changed from orange to colourless with increasing concentration.

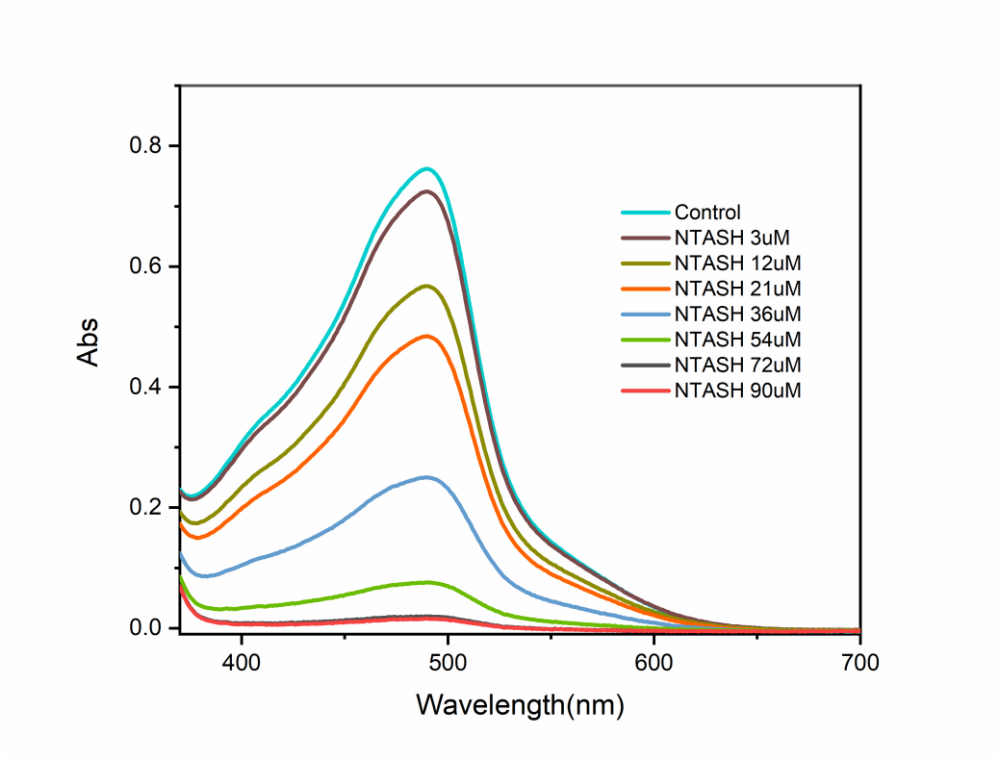



**Figure S4:** UV-Vis Absorbance study of copper chelation with NTASH chelator by varying concentration in presence of BCS Salt.

## Absorbance studies with PKP1 and Irradiation

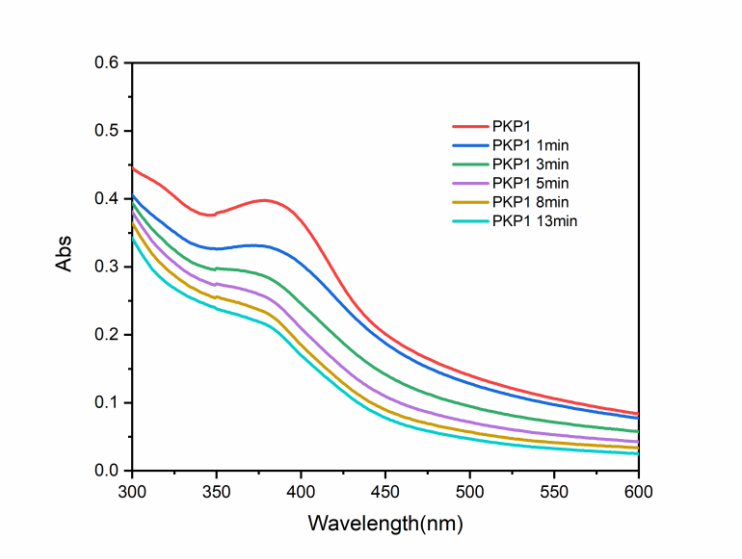



**Figure S5:** The decrease in the absorbance with irradiation time confirming the uncaging of PKP1. PKP1 (25 μM) taken in phosphate buffer (50 mM, 7.2 pH). The solution was irradiated with 365 nm light and absorbance was measured at different time interval.

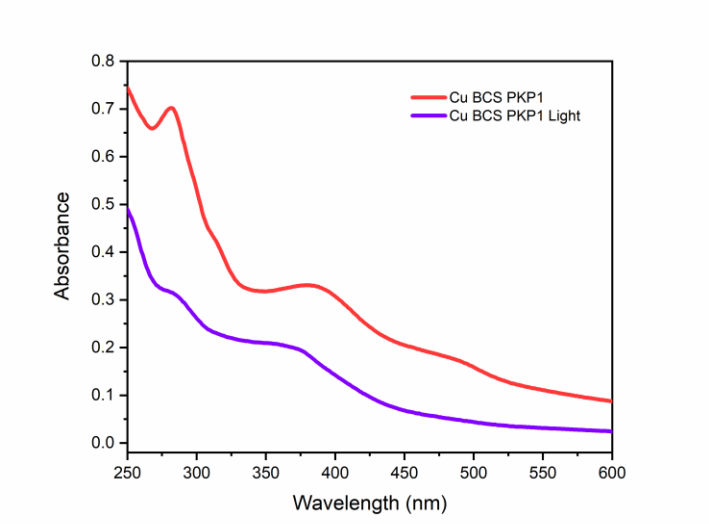



**Figure S6:** Copper (3 μM) and BCS (10 μM) was taken in phosphate buffer. After 1-hour PKP1 (15 μM) was added and then irradiated with 365 nm light for 10 minutes. The copper-BCS PKP1 absorbance maxima decreased after irradiation.

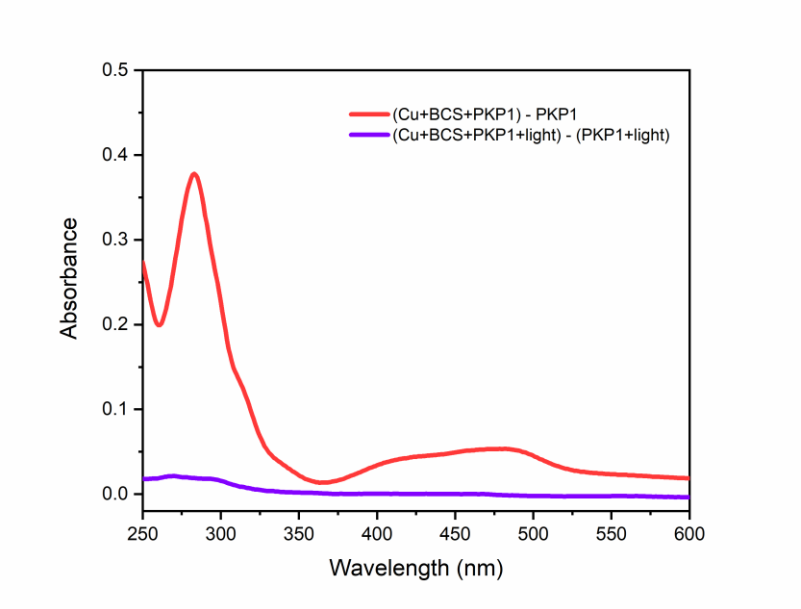



**Figure S7:** We subtracted the PKP1 absorbance to clearly show the MLCT band at 481 nm. In the UV spectra of [(Cu+BCS+PKP1)- (PKP1)], $Cu(BCS)_2$ is clearly visible. Upon light irradiation, the intensity of $Cu(BCS)_2$ peak almost disappeared in the UV spectra of [ (Cu+BCS+PKP1+light) – (PKP1+light)]

## Fluorescence studies

In 3 ml HEPES buffer solution (50 mM, 7.2 pH) in presence of 2 mM GSH, 6 µM Cu(I) salt was added (10 mM in ACN Stock). 2 µM ResCu was added, and spectra was measured. In another cuvette with same buffer, NTASH chelator (25 µM) was added and incubated for 30 mins. Similarly, in the identical condition just the fluorophore (2 µM) was added and spectra was recorded. The spectral intensity for copper and chelator was reduced than copper without chelator. (Excitation at 540 nm, slit width = 2, integration time =0.6 s, corrections were used for both S1 and R1)

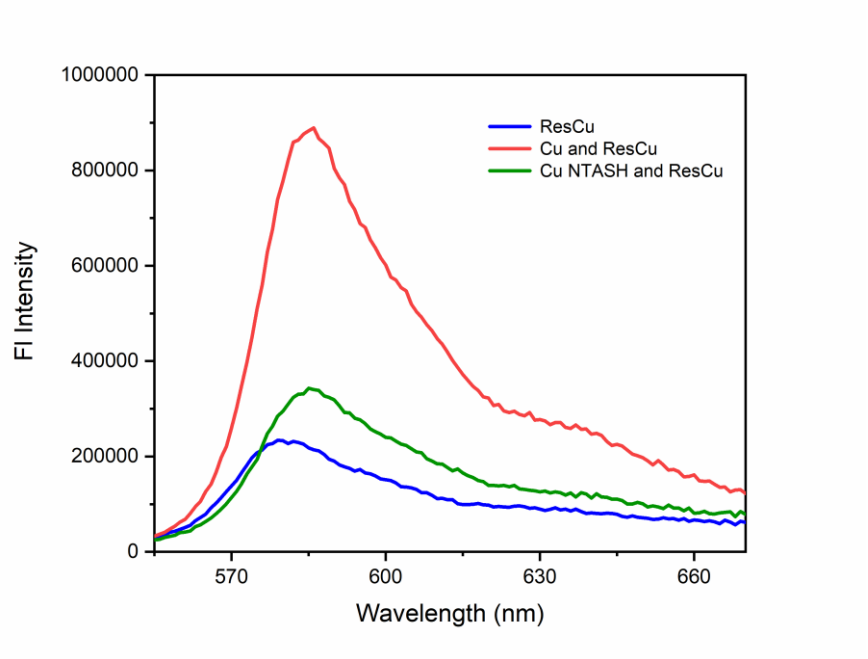
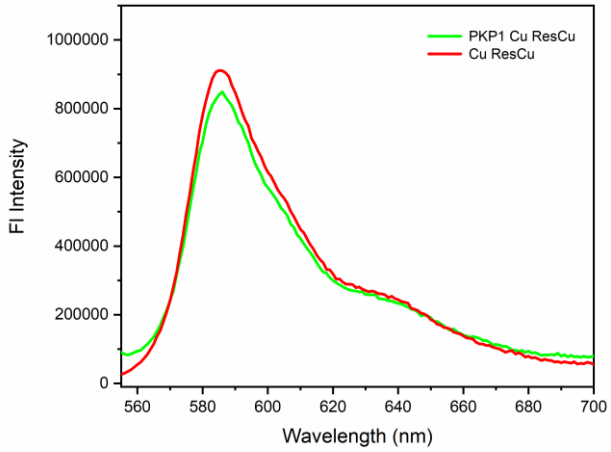



**Figure S8:** Emission Spectra of fluorophore(blue); copper and fluorophore(red); copper fluorophore and NTASH chelator(green).

## Fluorescence studies with photocaged probes without light

Fluorescence spectra was measured for two photocaged compounds with copper and fluorophore to confirm that photocaged PKP1 and PKP2 does not chelate Cu(I). 6 µM Cu(I) salt was added in HEPES buffer (50 mM, 7.2 pH, 2 mM GSH) followed by 25 PKP1 or PKP2. Then 2 µM ResCu probe was added and spectra was recorded. Almost similar pattern of intensity was observed in case of Cu(I)+ResCu and with both the photocaged probes (PKP1, PKP2).



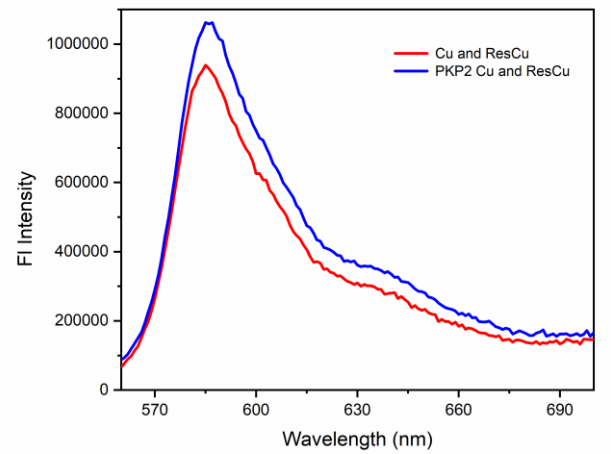




**Figure S9:** Emission Spectra of copper, ResCu, and Photo-caged compounds (PKP1, PKP2) without irradiation.

## Fluorescence Studies with Photocaged probes with Light

The **ResCu** probe (2 μM) was incubated in HEPES buffer with 6 μM Cu(I) in presence of **PKP1** under light irradiation. The fluorescent intensity decreased with exposure of 365 nm light which confirms chelation of Cu(I) only after uncaging of PKP1.

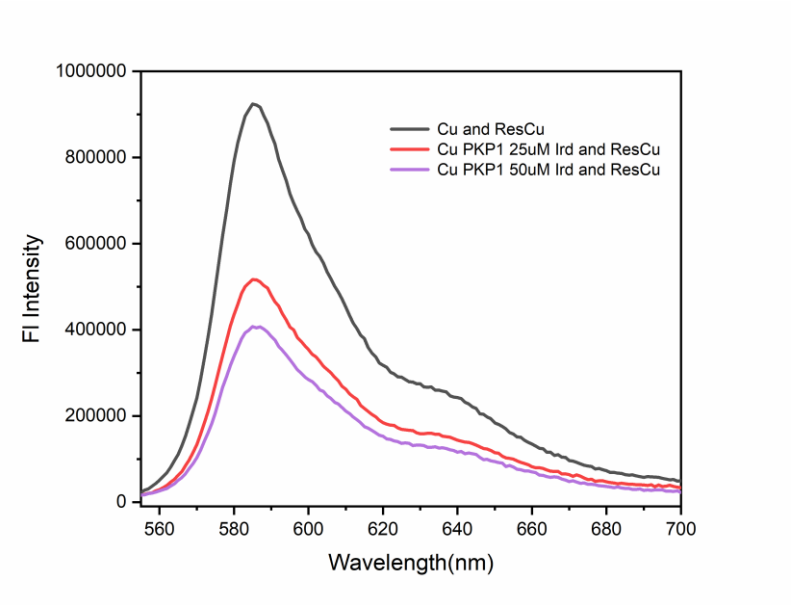



**Figure S10:** Emission spectra of ResCu+Cu(I) in presence of PKP1 with irradiation. Decrease of fluorescence showing chelation of copper

Due to increase in fluorescence after uncaging with PKP2 probe, the fluorescence studies were not conclusive. The excitation of coumarin group is 480 nm, the spectral overlap is causing the increase in Fl intensity after excitation at 540 nm.

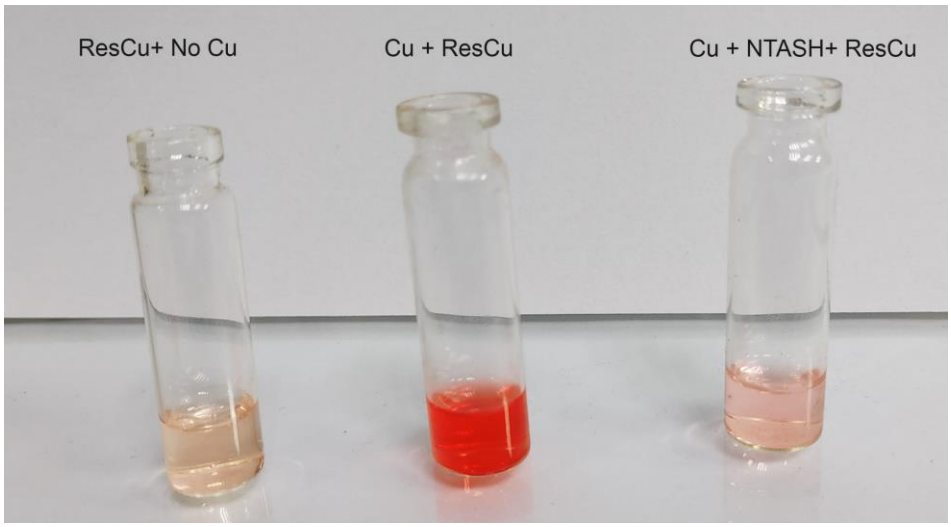



**Figure S11:** Visible color change of the Cu(I) and ResCu probe under different conditions

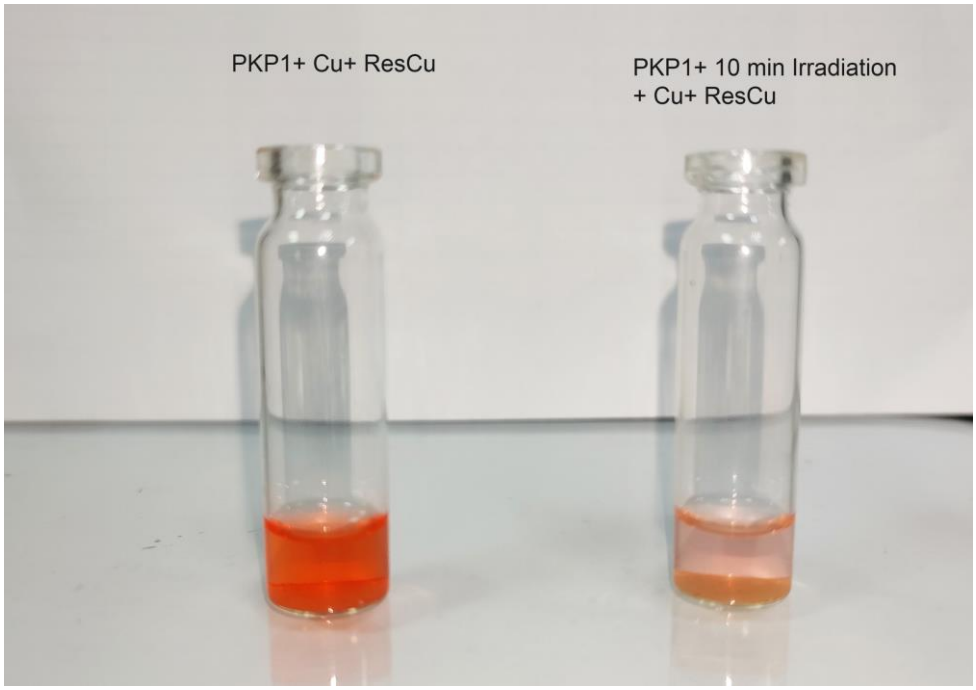



**Figure S12:** Visible color change of the Cu(I) and ResCu probe under different conditions with PKP1, with and without irradiation.

## Cell based experiments and microscopic analysis

**Cell Growth:** HeLa cells were obtained from NCCS (National Centre for Cell Science, Pune, India). Cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (Foetal Bovine Serum) and 1% Pen-Strep. Cells were incubated at 37˚C incubator with 5% $CO_2$. Cells were washed with pre-warmed PBS (Phosphate buffer Saline, pH 7.4) and supplemented with fresh medium every 48 hours.

**Copper chelation in Cells with NTASH chelator and fluorescence imaging:** Cells were seeded in a six well plate with coverslips after it reached 70% confluency. After 18 hours, cells were checked for perfect morphology. Cells were incubated with copper (50 μM) in serum free media for 4 hours. Cells were washed with PBS and excess copper was removed, in fresh serum free media NTASH chelator (50 μM) was added in one well and kept for incubation for 8 hours. Cells were washed again and in fresh serum free media, Fluorophore (5 μM) was added and kept in incubation for 2 hours. Then cells were washed again and fixed with 4% PFA (Paraformaldehyde), kept under airflow for 15 mins and washed thrice with PBS. Cells were stained with 0.1% Hoest (Invitrogen) dye for nucleus staining and kept under dark for 15 mins and washed with PBS. Cells were mounted in the coverslips with Mowiol (Sigma Aldrich) in a glass slide. A similar procedure was followed for another condition where no chelator was added. Experiments were performed more than three times to reconfirm the findings.

The cells were viewed under fluorescence microscope, DAPI channel is showing the blue fluorescence coming from the nucleus of the cells, TRITC channel is showing the red fluorescence coming from the cells due to release of the active fluorophore. The distinct difference in red fluorescence between chelator and without chelator is visible. In presence of NTASH chelator the copper is getting chelated, thus very minimal amount of fluorescence is visible.

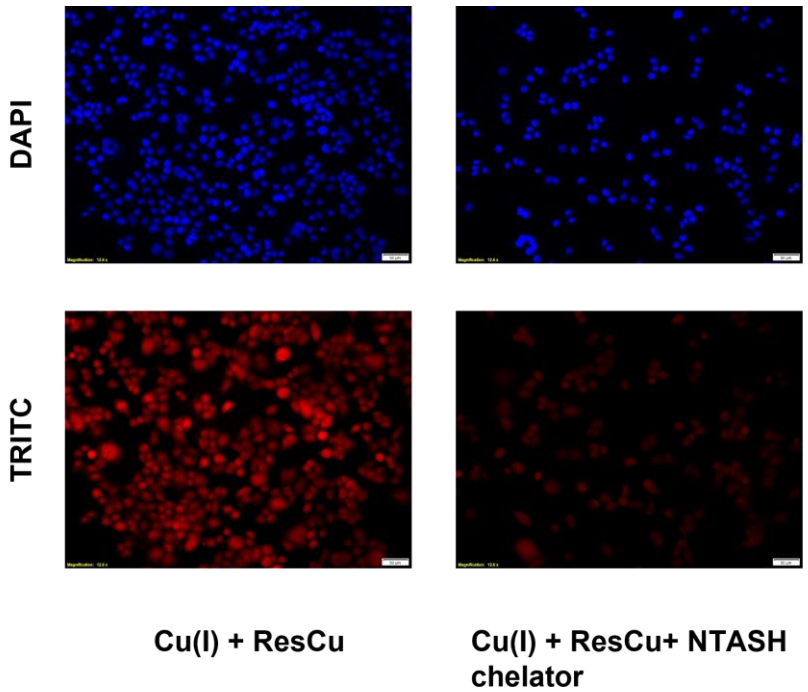



**Figure S13:** HeLa cells incubated with NTASH chelator showing decrease in fluorescence intensity compared to cells without NTASH chelator.

## Light controlled Cu(I) chelation inside cells

Cells were seeded after reaching 70% confluency in a six well plate on with coverslips. Cells were incubated with copper for 4 hours in serum free media. Cells were washed with PBS, PKP1 was added after diluting it in serum free media and incubated for 6-8 hours. After a PBS wash, the ResCu probe (5 μM) was added and incubated for 2 hours. In all the below three cases the conditions were kept the same with an additional step of light irradiation (5 & 10 mins respectively) for the last two wells.

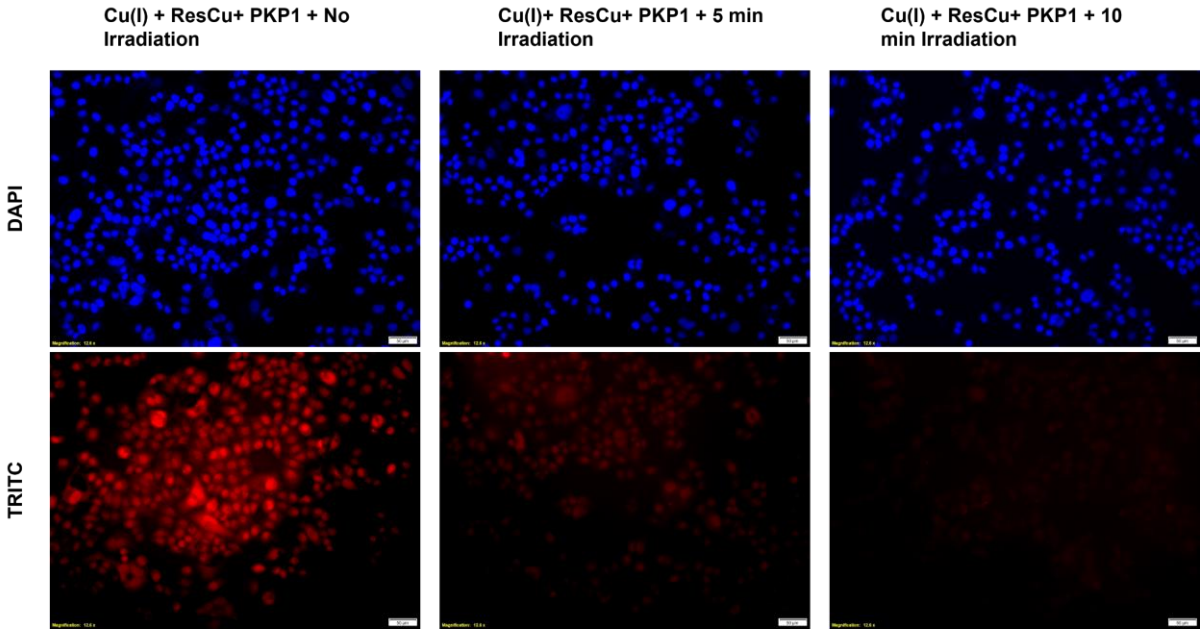



**Figure S14:** HeLa cells were incubated with copper, fluorophore and PKP1. Increasing irradiation time showing decrease in fluorescence proving copper chelation.

In fluorescence microscopy, cells incubated with PKP1, and no irradiation shows highest fluorescence intensity. The intensity decreases gradually with increase in irradiation time, the intensity of red fluorescence was lesser in cells irradiated for 10 mins with PKP1 compared to 5 min irradiation. With more irradiation, the uncaging of the photocages will be enhanced, thus more copper chelation.

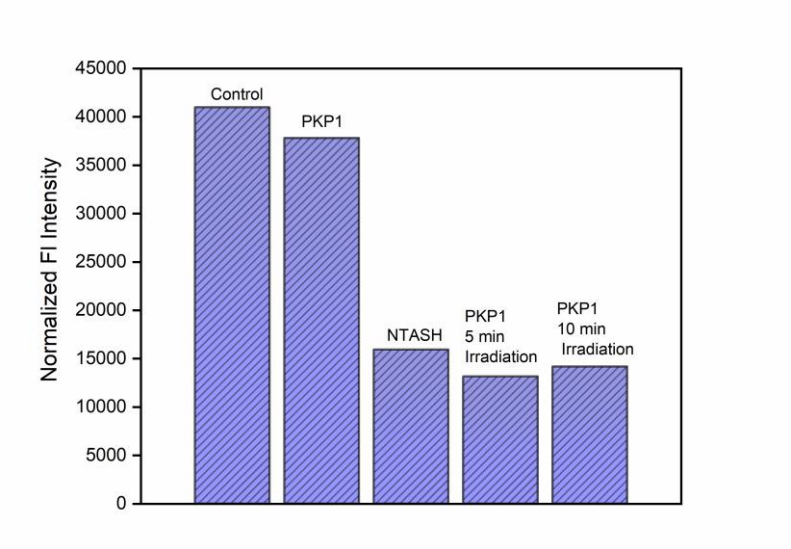



**Figure S15:** Average fluorescence intensity from HeLa under different conditions

## Cell Viability assay with MTT dye:

HEK293 and Hela cells were seeded at a concentration of $10^5$ cells/ml in 96-well plates in DMEM supplemented with 10% FBS and 1% antibiotic solution (penicillin & streptomycin) for the MTT assay. After overnight incubation at 37°C in humidified chamber under 5% $CO_2$, Scavenger and the photocaged scavenger were added in a diluted form in media at a final concentration of 25 µM, 50 µM, 100 µM (In each well amount of DMSO was 2 µL). Cells were incubated with the respective compounds for 24 hours at 37°C followed by changing the compound containing media with 100uL of fresh media. After that 10uL of MTT dye (12 mM) was added the plate was incubated in the dark at 37°C. After 2.5 hours of incubation with MTT dye the media was carefully removed from the top (without disturbing the crystals) and 50 µL of DMSO was added in each well and kept on a shaker for half an hour. The absorbance of each well was measured at 540 nm using a plate reader. The assay was performed in replicates and the average absorbance value was calculated with the error bar for each.

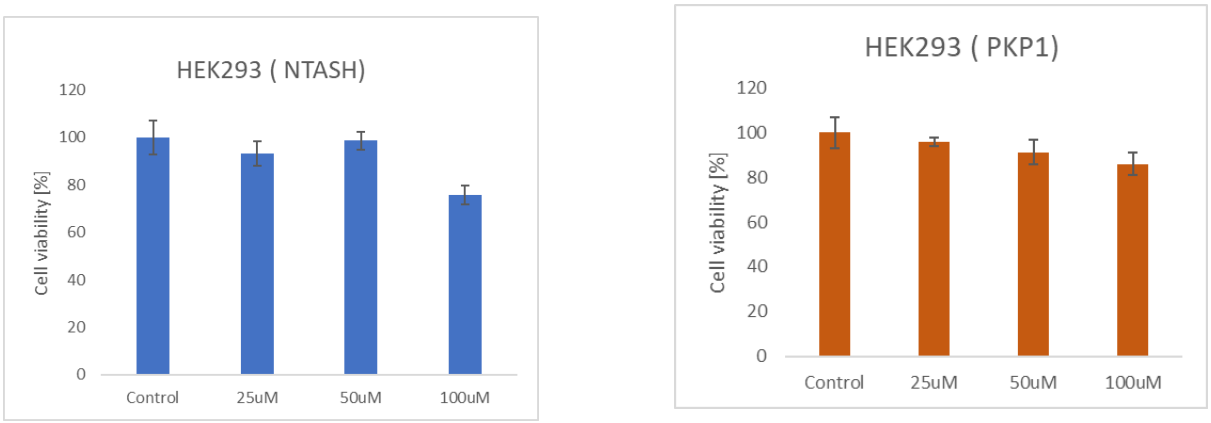



**Figure S16**. Cell viability assay of NTASH and PKP1 in HEK293 cell line

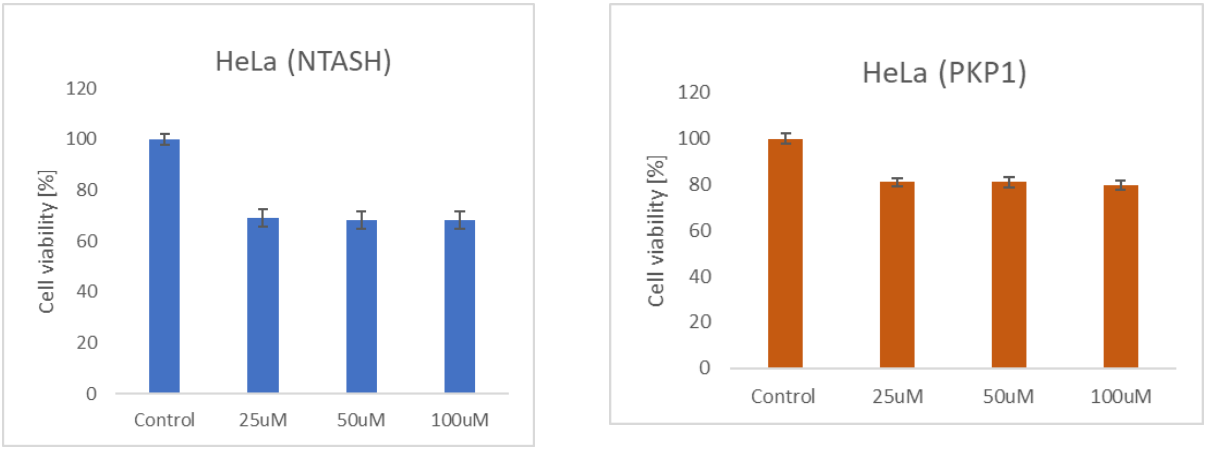



**Figure S17**. Cell viability assay of NTASH and PKP1 in HeLa cell line

## Effect of EDTA wash: Copper chelation in Cells by PKP1 and NTASH

The experiments were repeated as reported for S13 with one additional step. After giving the treatment with the Cu(I) the cells were washed two times with 50 μM EDTA in PBS and followed by another PBS wash for complete removal of Cu(I) from outside the cells.

Cells were seeded after reaching 70% confluency in a six well plate on coverslips. Cells were incubated with Fluorophore for 2 hours in serum free media. In one well after giving the treatment with the Fluorophore the cells were irradiated with the light for 10 minutes. All that remaining steps like fixing, nucleus staining, and mounting were done for the sake of simplicity in proper imaging.

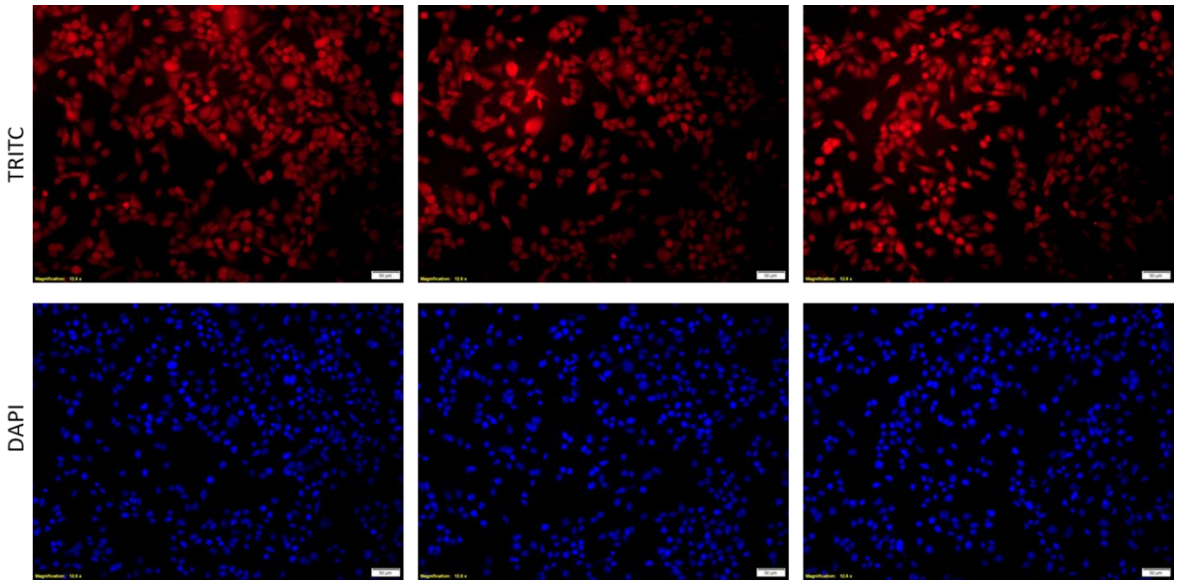



**Figure S18:** Fluorescence microscopy images of HeLa cells where cells were treated with 50 μM Cu(I) and washed twice with 50 μM EDTA in PBS followed by PBS wash.

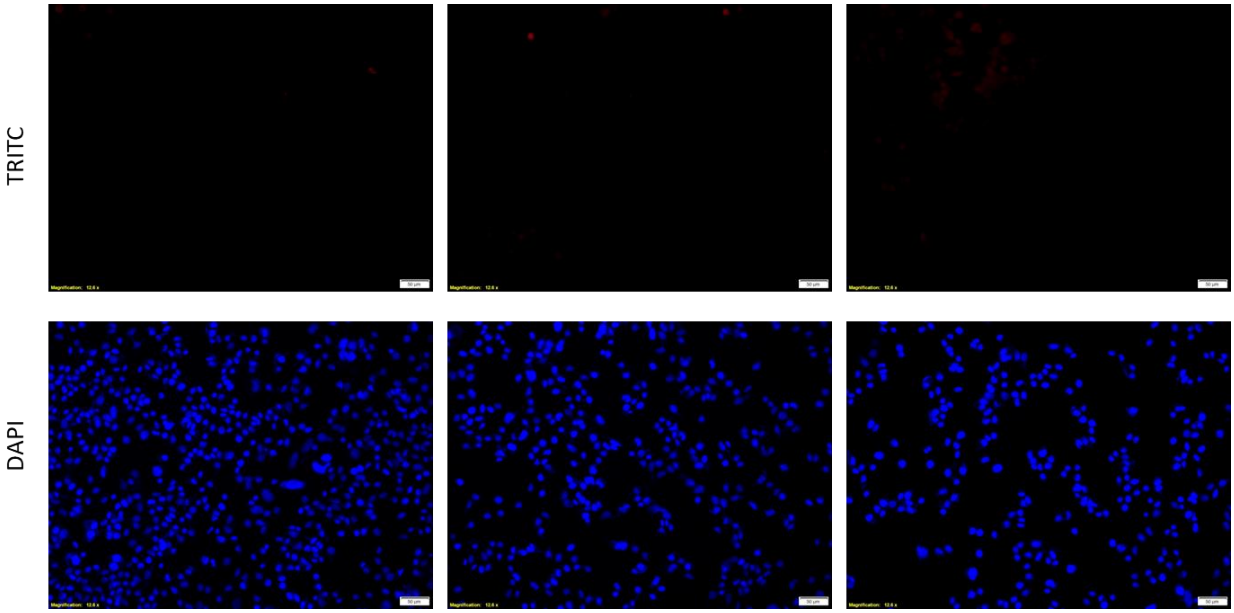



**Figure S19:** Fluorescence microscopy images of HeLa cells in presence of active chelator NTASH. Cells were washed with EDTA as described in Figure S18.

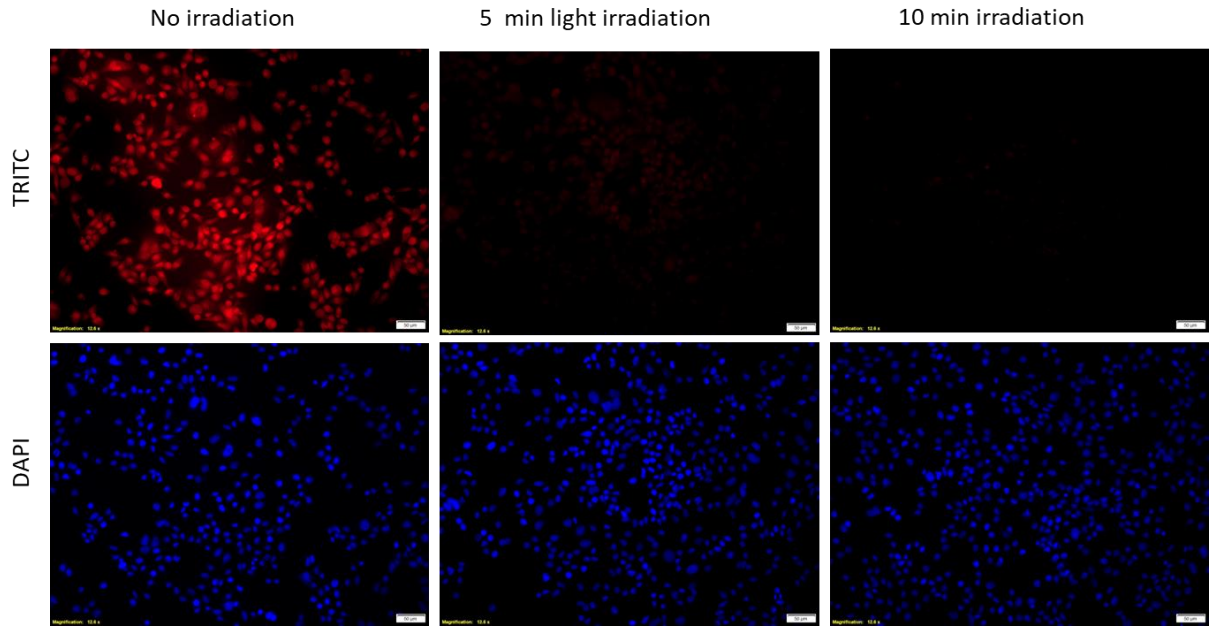



**Figure S20:** Fluorescence microscopy images of HeLa cells in presence of photocaged chelator PKP1 and 365 nm light source with different exposure time. Cells were washed with EDTA as described in Figure S18.

.

## ATP7A Translocation experiment: Probing copper chelation in Cells by PKP1 upon irradiation

HeLa cells were cultured in Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with 10% FBS (Himedia) and 1% Penicillin-Streptomycin antibiotic solution (Gibco). The cells were grown at 37 °C temperature under an atmosphere of humidified air containing 5% $CO_2$. Cells were seeded and grown on coverslips in a 6-well plate. The growth medium was then replaced with fresh DMEM containing 50 µM Cu(I) (Tetrakis(acetonitrile)copper(I) hexafluorophosphateand) and incubated at 37°C, 5% CO2 atmosphere for 4 hours. After the incubation period, the copper supplemented media was removed, and the cells were thoroughly washed with PBS. The cells were further incubated either with 50 µM NTASH or 50 µM PKP1 (according to plate map designed for the experiment for 8 hours at 37°C and 5% CO2 atmosphere. The well containing PKP1 was irradiated with 365 nm light for 10 min (1 second turn-on and one second turn-off pulse). The medium was then removed, and the cells were washed with PBS and fixed with 4% paraformaldehyde at room temperature for 20 min. The fixative was aspirated off and the cells were washed three times with PBS and permeabilized with 0.5% Triton X-100 for 10 min. After washing three-times with PBS, the cells were incubated with 5% BSA in PBS-T (0.05% Tween-20 in PBS) for 1 hour at room temperature. The cells were then incubated with mouse monoclonal anti-ATP7A primary antibody (Santa Cruz Biotechnology) at a dilution of 1:100 overnight at 4°C, followed by Goat anti-Mouse IgH (H+L) Cross-Absorbed secondary antibody, Texas Red-X (Invitrogen) at a dilution of 1:800 for 1 hour at room temperature. The cells were then washed thoroughly with PBS and counterstained with Hoechst 33258 (5 µM in PBS), mounted on glass slides using Mowiol (Sigma-Aldrich), and analyzed using an Olympus confocal Laser Scanning microscope.

In HeLa cells we have tried to confirm the chelation effects of PKP1 after light irradiation by checking the trafficking effects of ATP7A. Under normal conditions the ATPase is localized to Trans Golgi network (TGN) close to nucleus (Fig S21 A). In presence of 50 μM Cu(I) salt, trafficking of ATPase from Golgi to cytoplasm is observed (Fig S21B). In presence of the active Cu(I) chelator 50 μM NTASH re-localized back to golgi (Fig S21C). After supplementation with 50 μM PKP1 without irradiation with 50 μM Cu(I) salt it shows similar trafficking to cytoplasm (Figure S21D). After irradiation to PKP1 by 365 nm light, ATPase re-localized to trans golgi network confirming the Cu(I) chelation (Figure S21E).

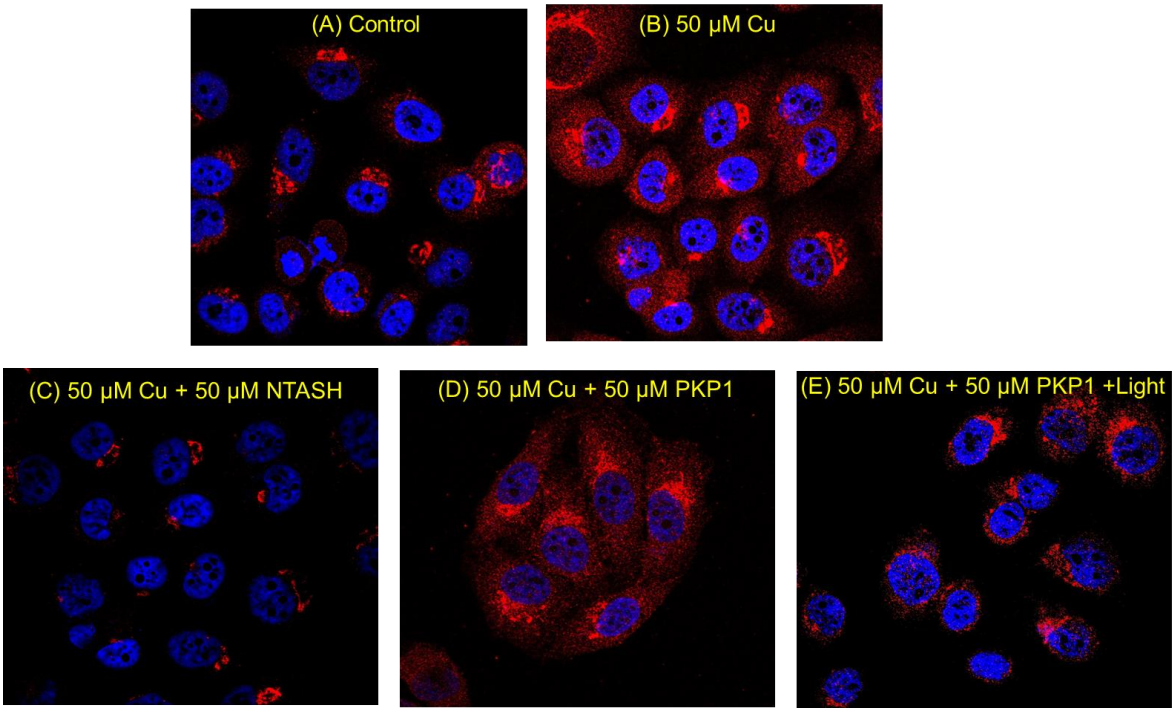



**Figure S21:** Cu(I) dependent translocation of ATP7A in HeLa cells upon treatment of PKP1 and light irradiation. Relocation of ATP7A was visualized by immunohistochemistry under various conditions (A) absence of any Cu(I) supplement (B) after treating the cells with 50 μM Cu(I) (C) Cu(I) treatment followed by treatment with active chelator NTASH (D) Cu(I) supplement followed by treatment with PKP1 and no light irradiation (E) supplement followed by treatment with PKP1 and 10 min light irradiation

## Laccase Activity Study with NTASH Chelator:

Laccase from *Trametes versicolor* [**1.10.3.2** (**BRENDA**, **IUBMB**)] was purchased from Sigma Aldrich. Laccase activity is normally measured by checking $OD_{510}$ of the adduct formation by 4-Aminoanitipyrine (AP) and 2,4 Dichlorophenol (DP) in presence of Laccase.[5] Aqueous solution was prepared with 1mg/ml concentration of both the compounds and Laccase. The reaction was done with MES buffer (30 mM in pH 6.8) in well-plates with continuous linear shaking at 25˚C. The O.D. values were measured with a multimode reader Kinetic measurement for 65 mins in 5 min intervals.

Control: 10 µL AP + 10 µL DP+ 10 µL Laccase + 70 µL MES buffer; in other wells NTASH was added from 20 µM to 400 µM.

NTASH were used in multiple concentrations, we have observed a gradual decrease in Laccase activity with increasing concentrations.

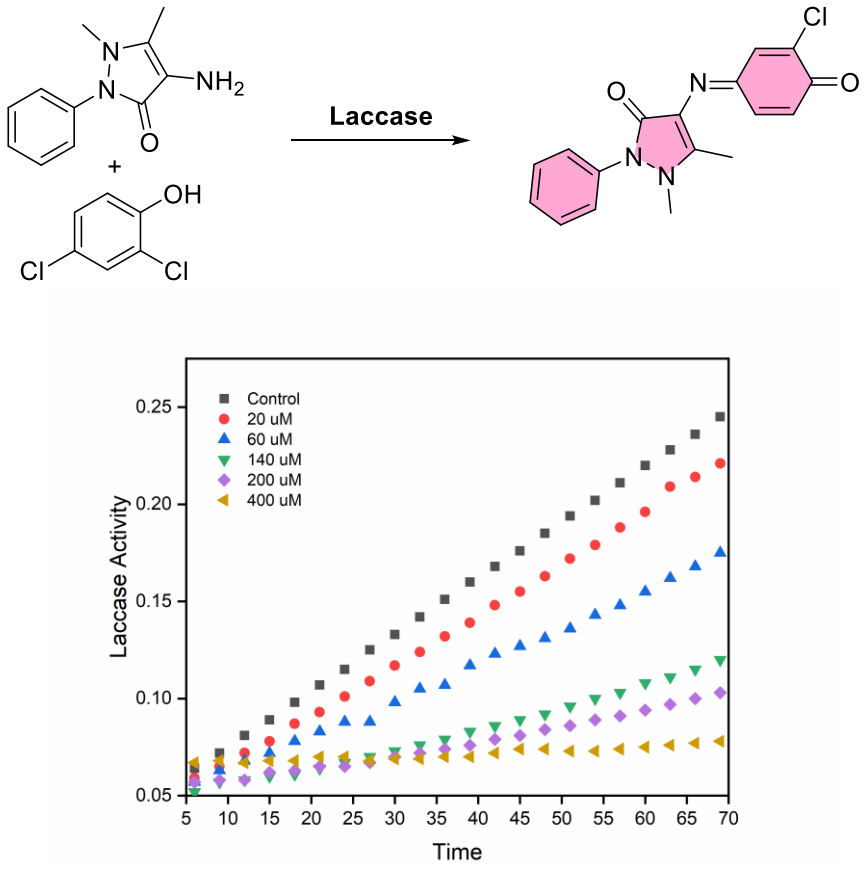



**Figure S22:** Scheme of Laccase Activity using 4-AP and 2,4 DP. (Top) Decrease of Laccase activity in presence of NTASH Chelator. (Bottom)

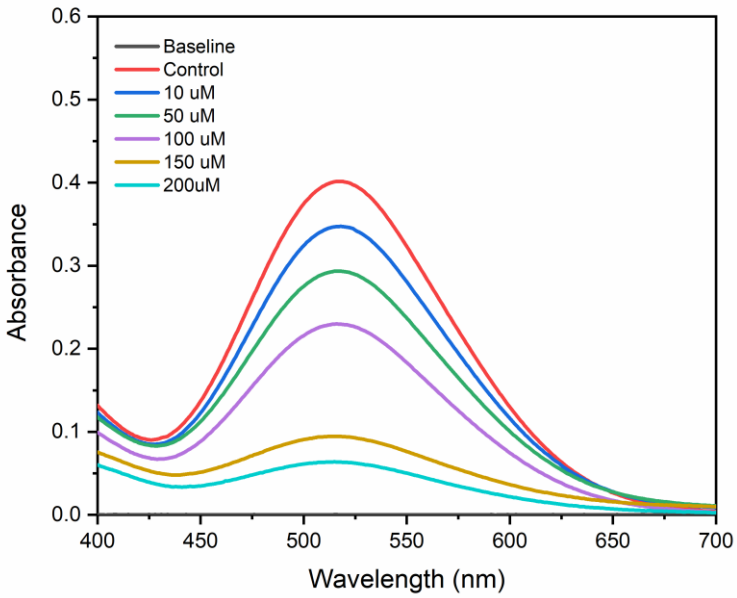



**Figure S23:** Gradual decrease of absorption maxima at 510 nm with increasing concentration of NTASH in presence of 4-AP and 2-DP in pH 6.8 MES Buffer confirming Laccase inhibition

## Laccase Activity Study with Photocaged Chelator PKP1:

Similar study was performed with Photocaged scavenger PKP1, where Laccase activity was checked with and without using light irradiation. In Control, no chelator was used, scavenger is NTASH and Photocaged scavenger is PKP1 without irradiation. Finally, the uncaged scavenger is PKP1 after irradiation of 10 mins.

Control: 5 µL AP + 5 µL DP+ 5 µL Laccase + 70 µL MES buffer; in another three wells NTASH was added 5 µL along with control, another well was incubated with PKP1 (200 µM), and no light was irradiated. In another well PKP1 (200 µM) was added to each well with the buffer and light was irradiated, then all the other components were added. Laccase was added to the wells at last quickly and measurement was started as soon as addition was over.

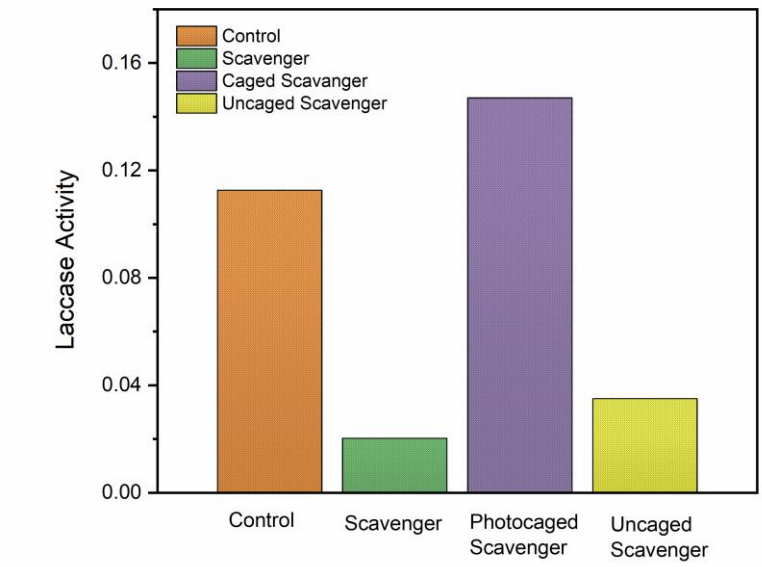



**Figure S24:** Decrease in Laccase activity before and after irradiation with PKP1

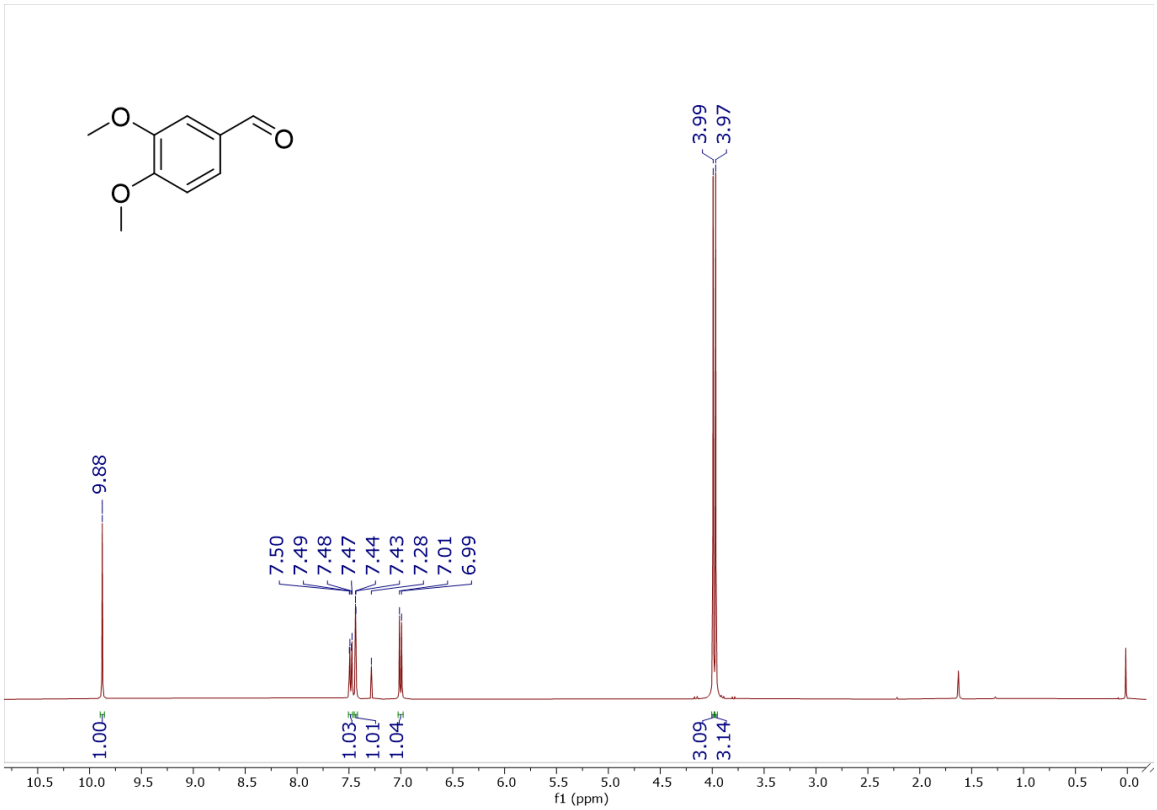



Figure S25: $^{1}H$ NMR of compound **1** in $CDCl_3$

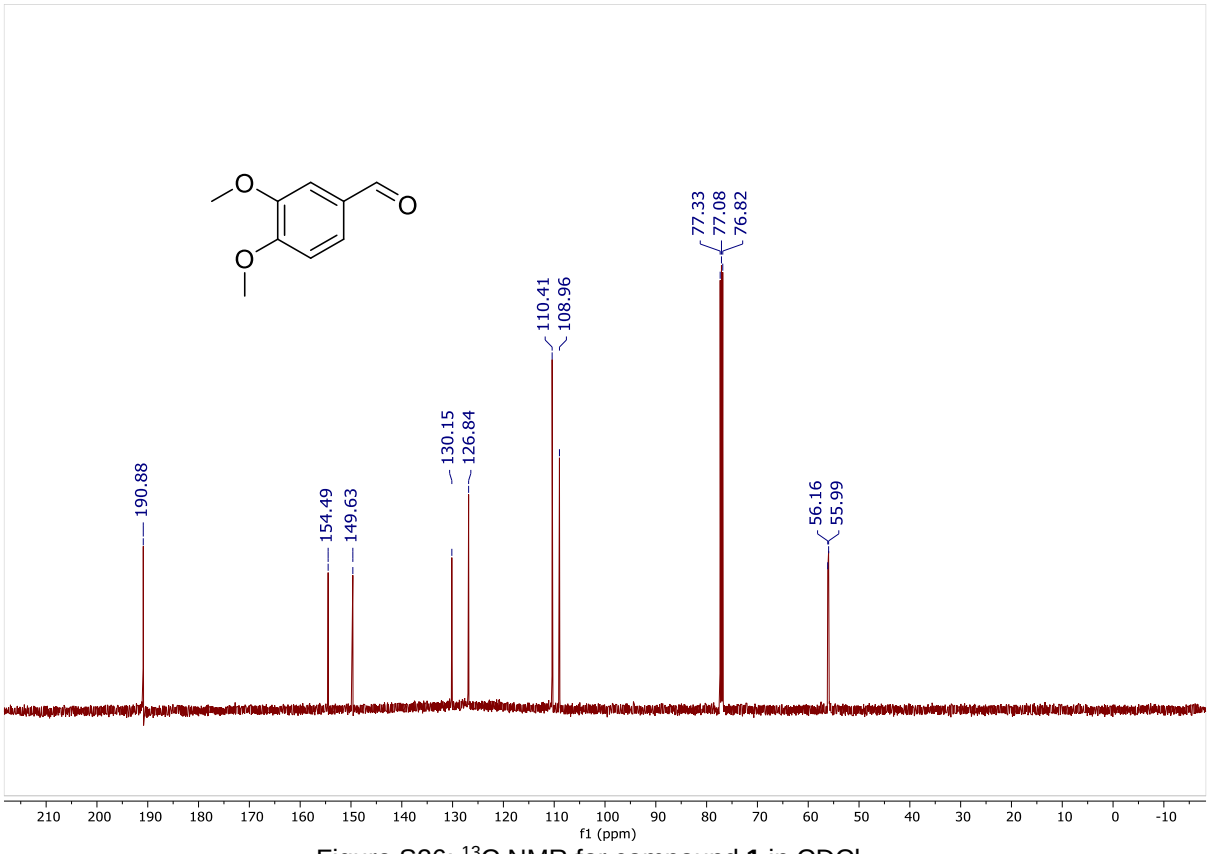



Figure S26: $^{13}C$ NMR for compound **1** in $CDCl_3$

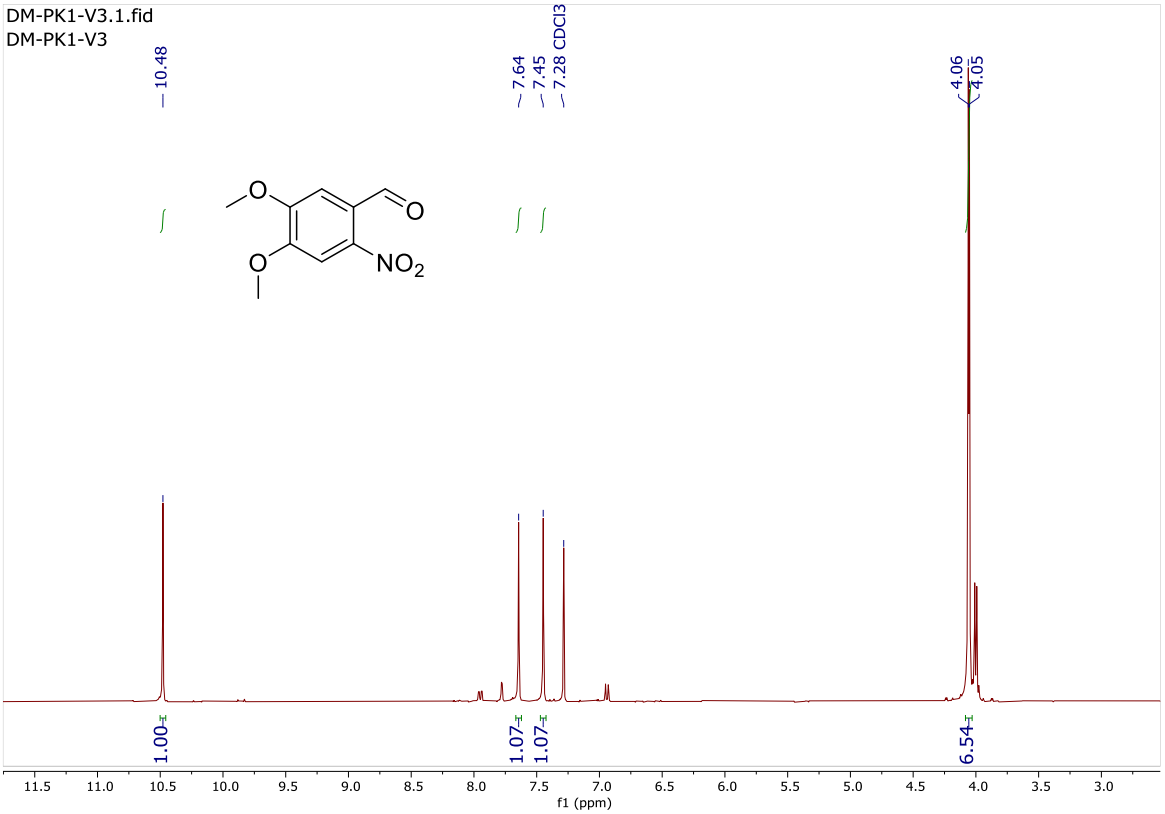



Figure S27: $^{1}H$ NMR of compound **2** in $CDCl_3$

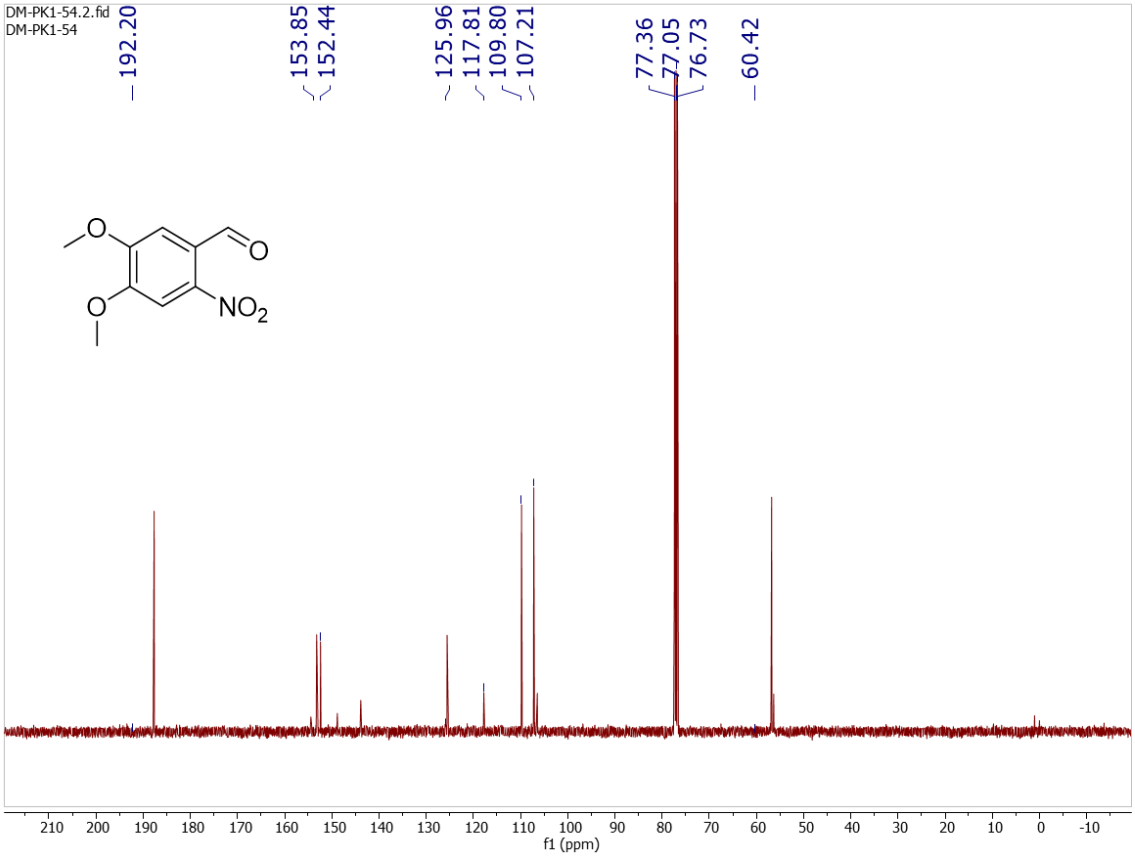



Figure S28: $^{13}C$ NMR of compound **2** in $CDCl_3$

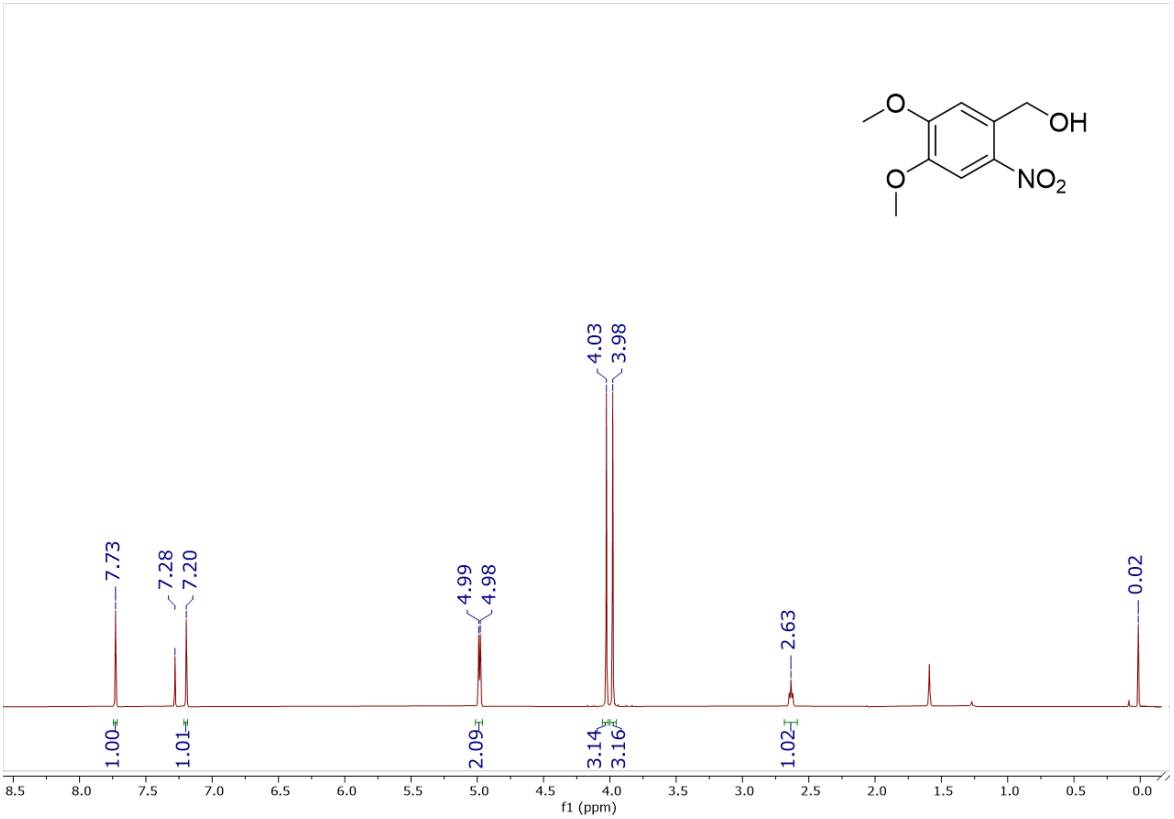



Figure S29: $^{1}H$ NMR of compound **3** in $CDCl_3$

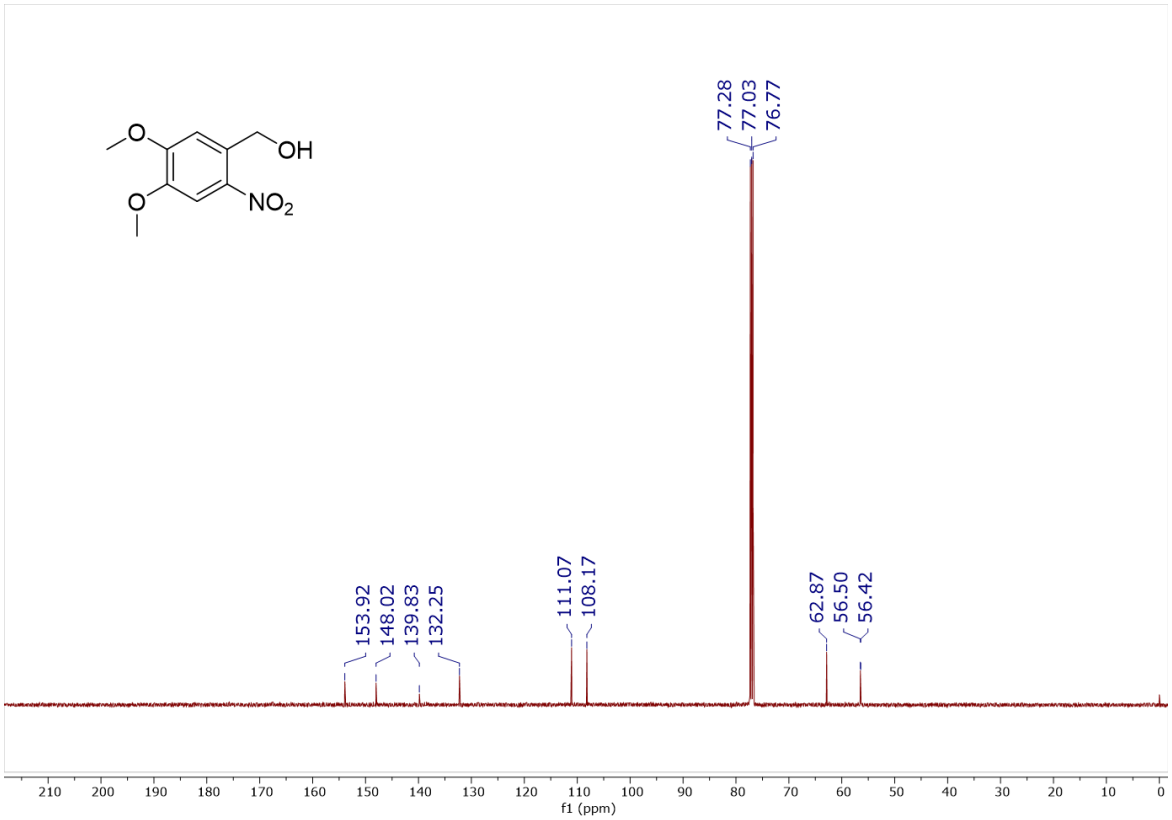



Figure S30: $^{13}C$ NMR of compound **3** in $CDCl_3$

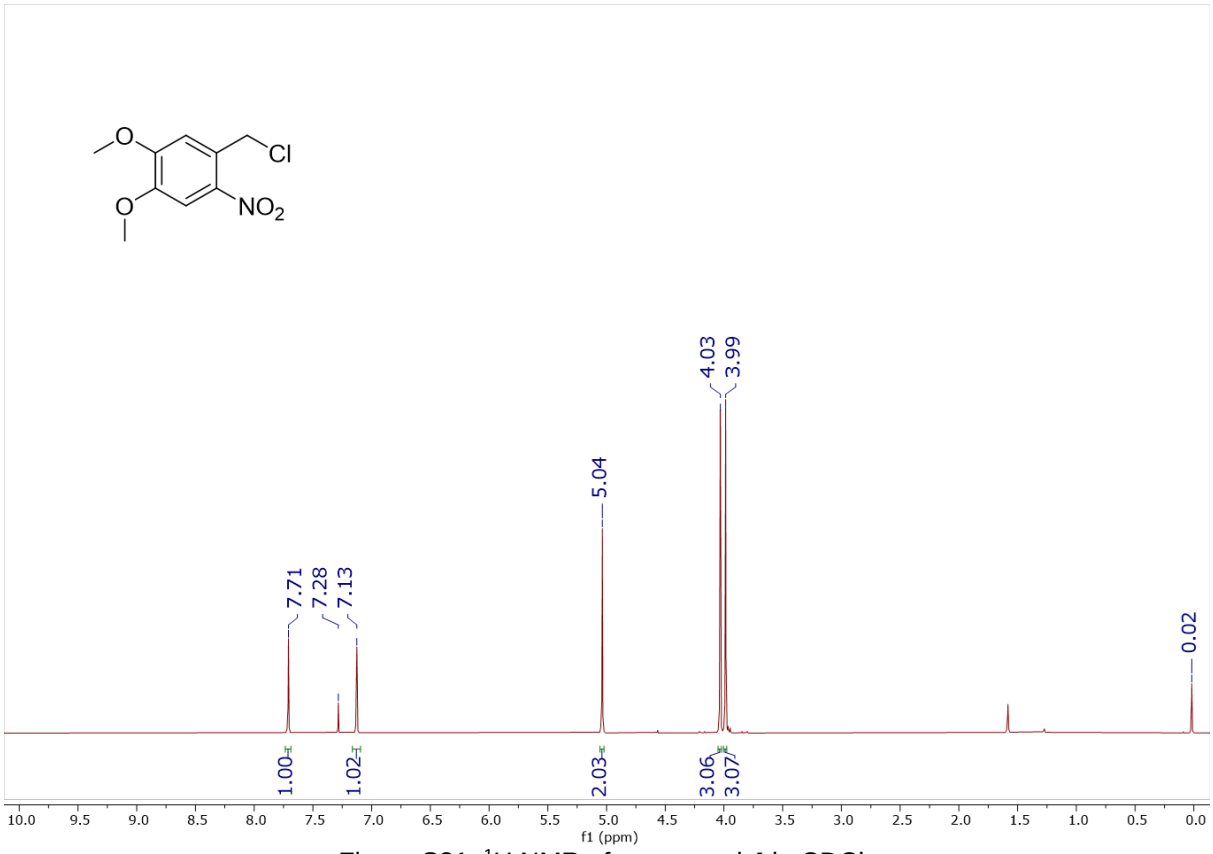



Figure S31: $^{1}$H NMR of compound **4** in $CDCl_3$

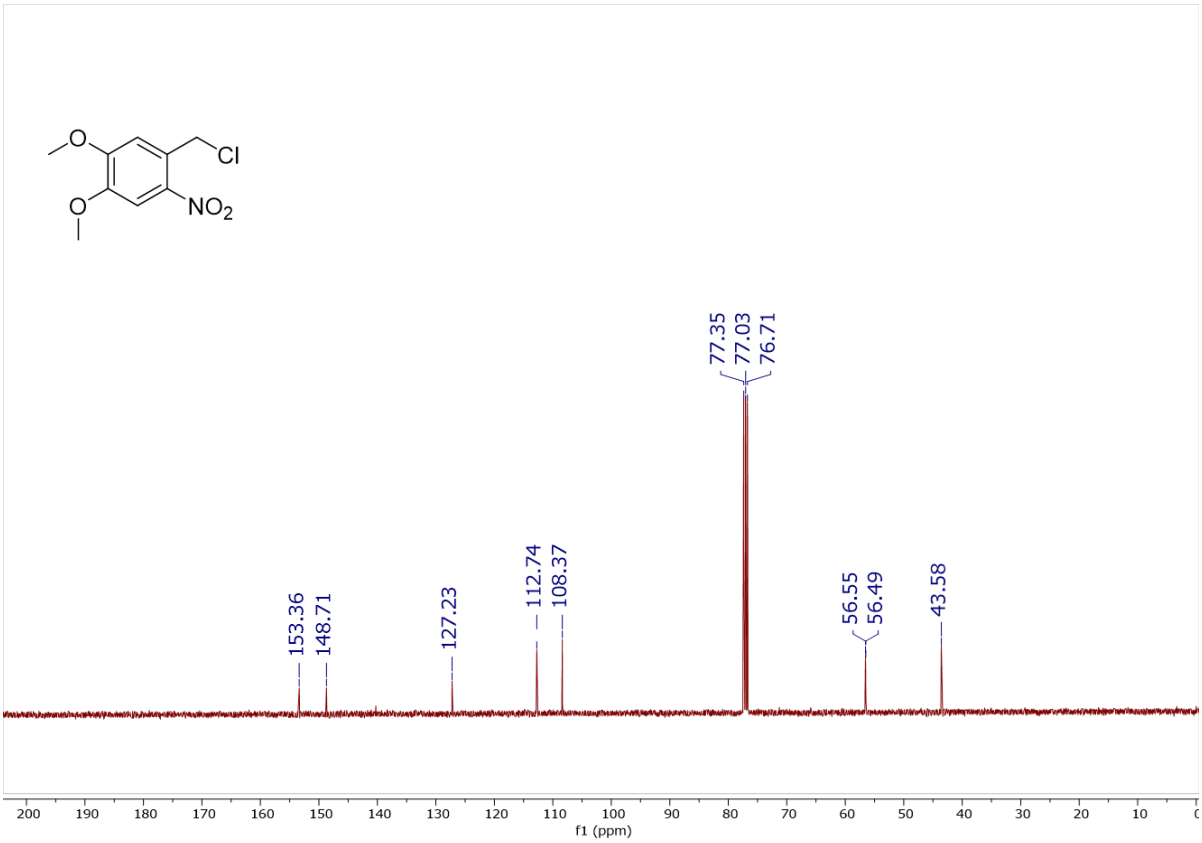



Figure S32: $^{1}$H NMR of compound **4** in $CDCl_3$

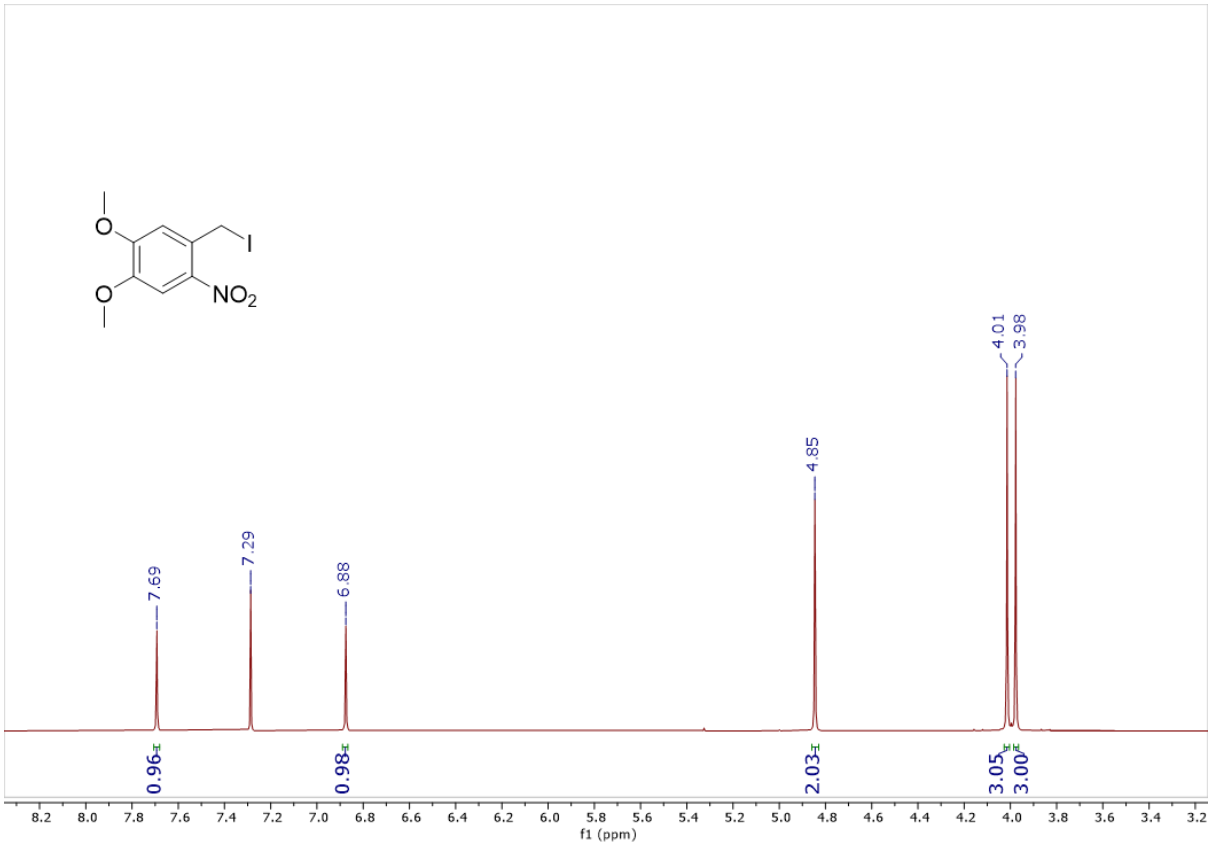



Figure S33: $^{1}$H NMR for compound **3** in $CDCl_3$

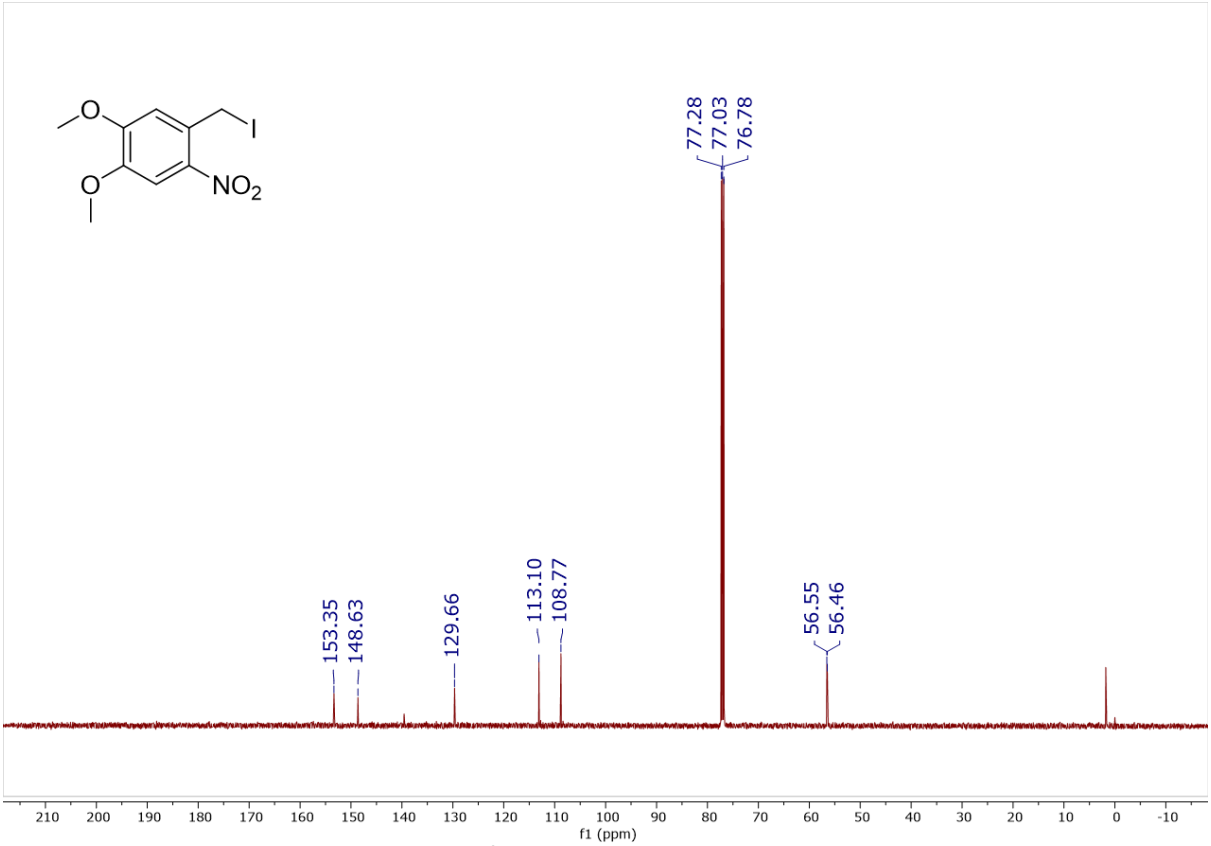



Figure S34: $^{13}$C NMR of compound **5** in $CDCl_3$

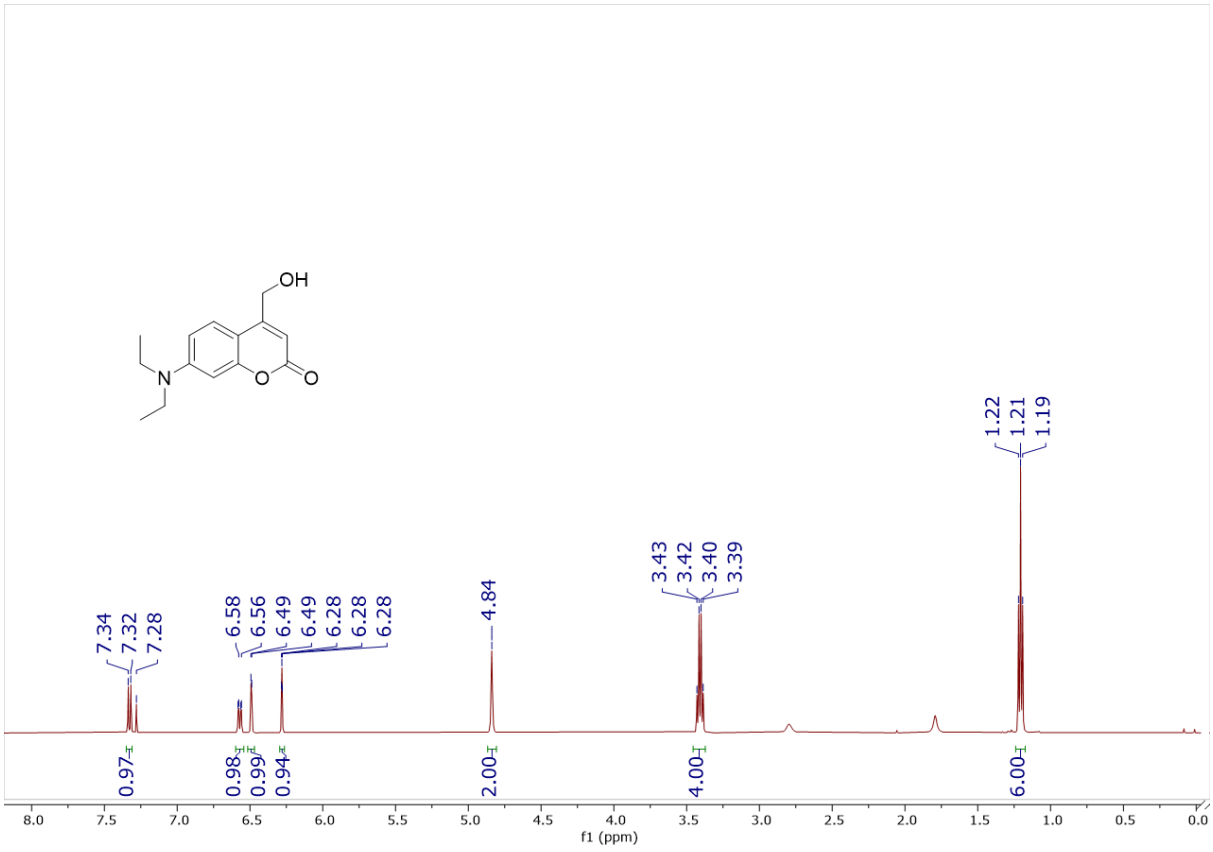



Figure S35: $^{1}H$ NMR of compound **6** in $CDCl_3$

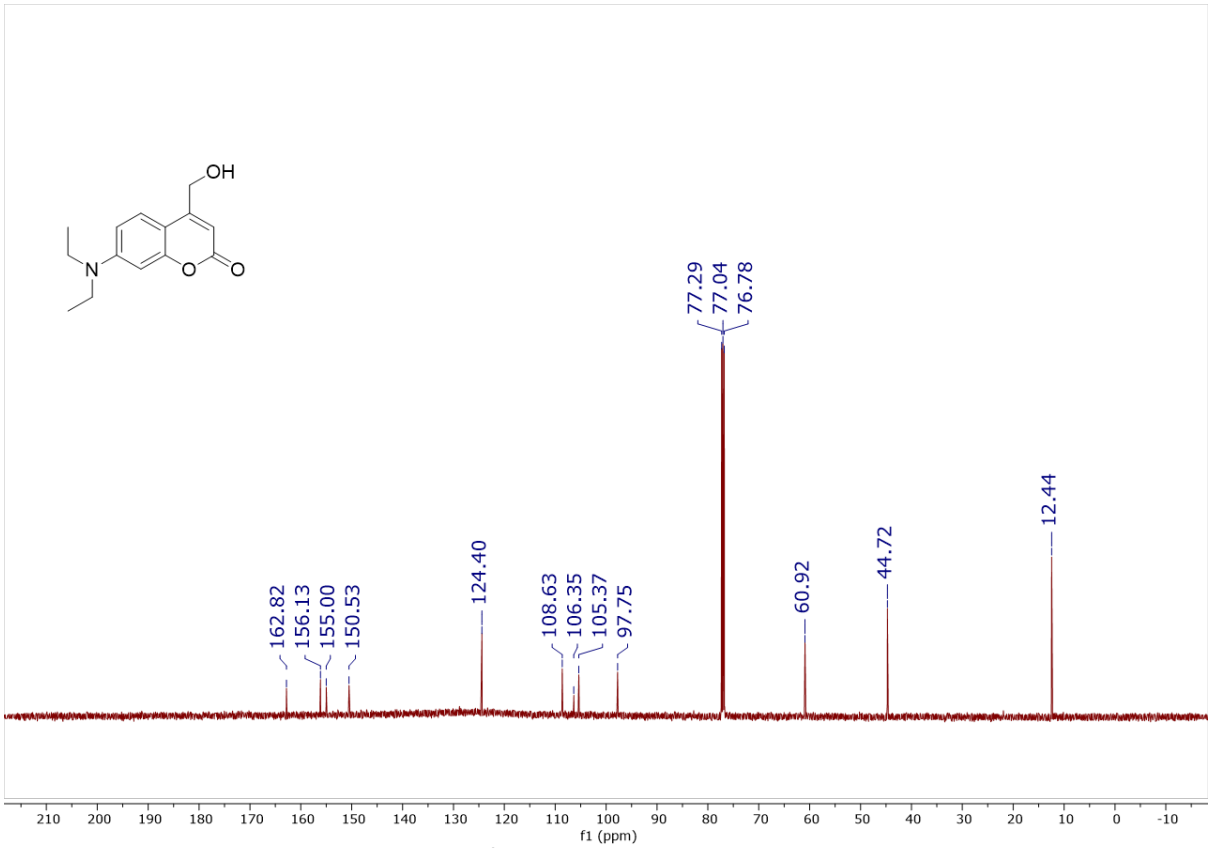



Figure S36: $^{13}C$ NMR of compound **6** in $CDCl_3$

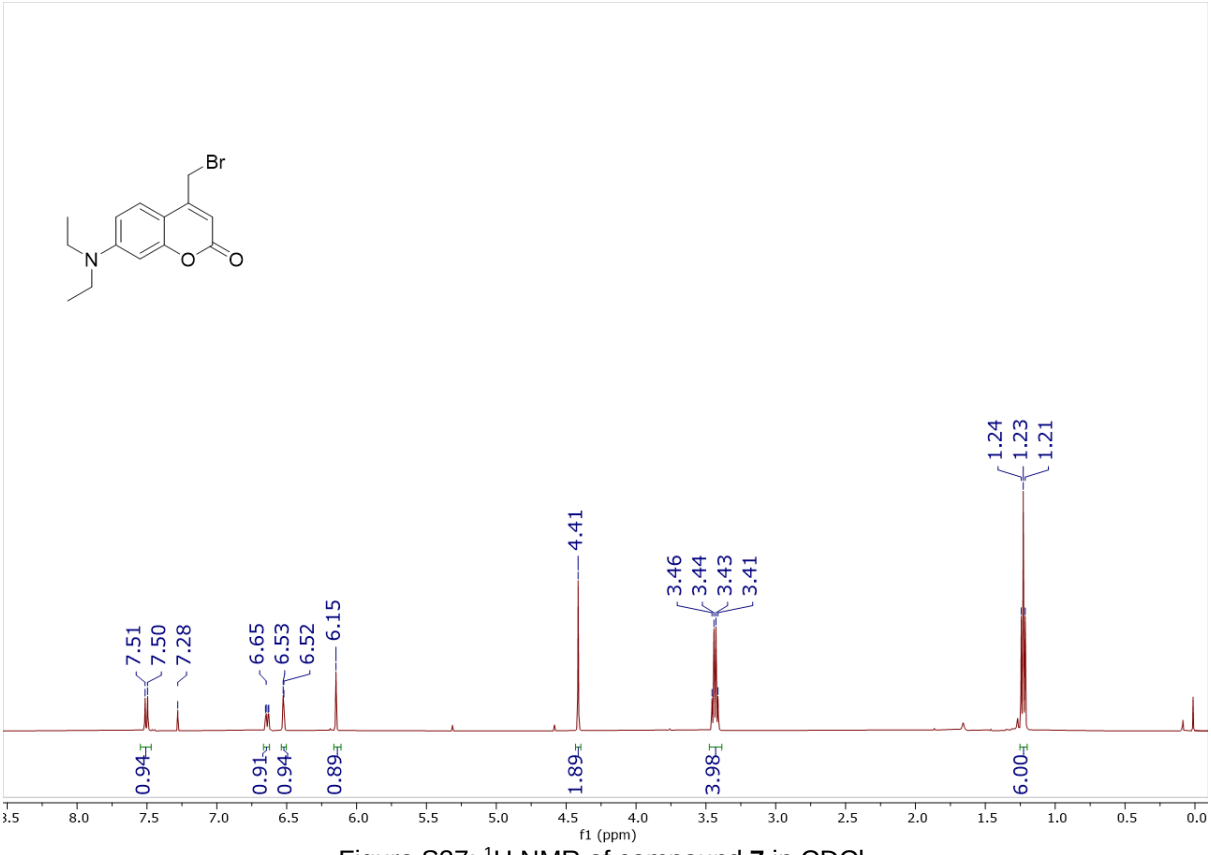



Figure S37: $^{1}H$ NMR of compound **7** in $CDCl_3$

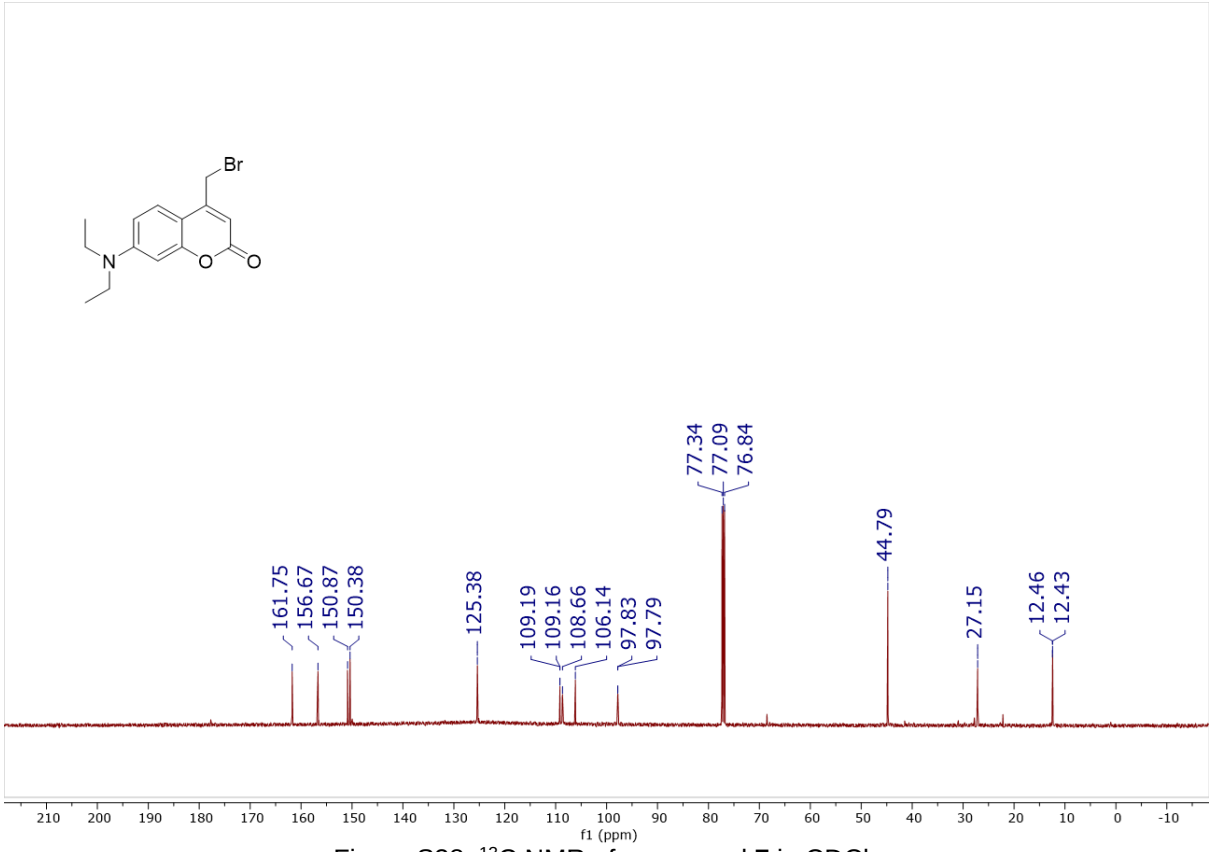



Figure S38: $^{13}C$ NMR of compound **7** in $CDCl_3$

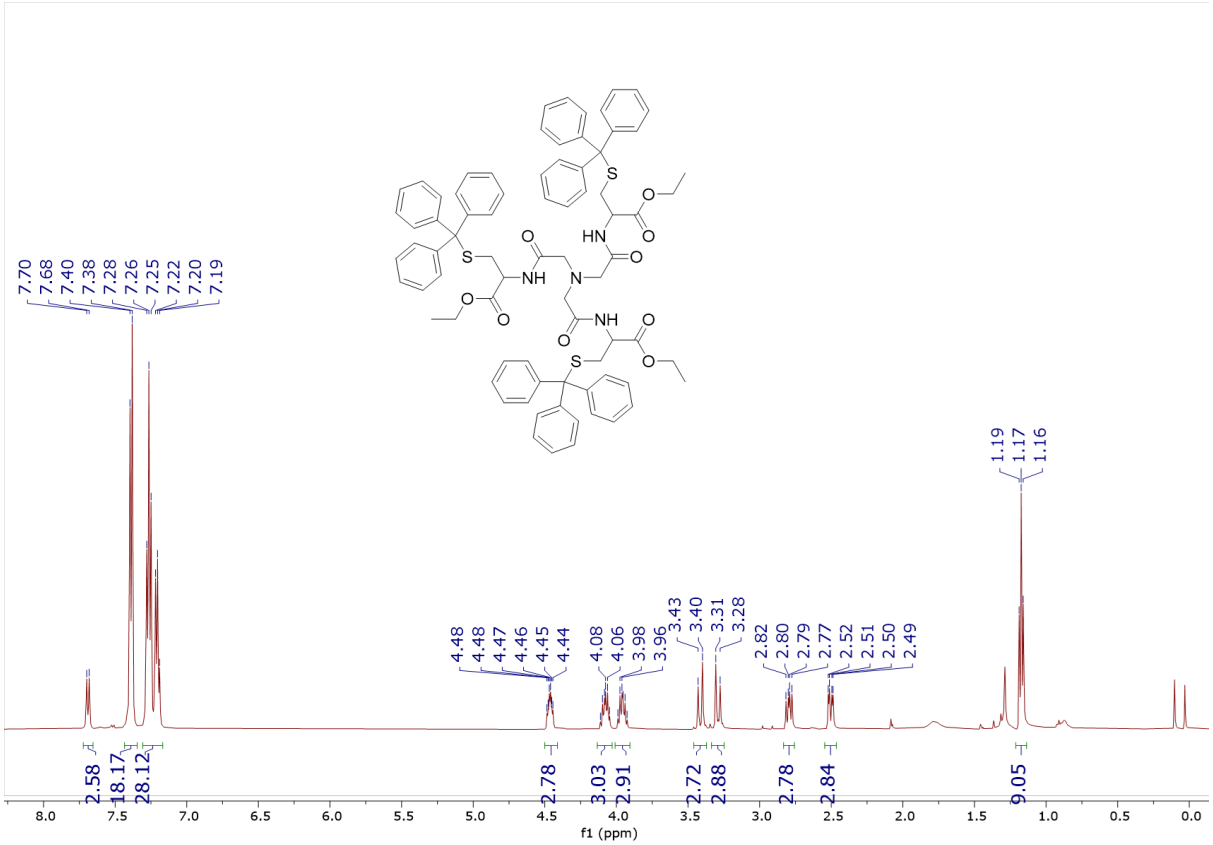



Figure S39: $^{1}$H NMR of compound **9** in $CDCl_3$

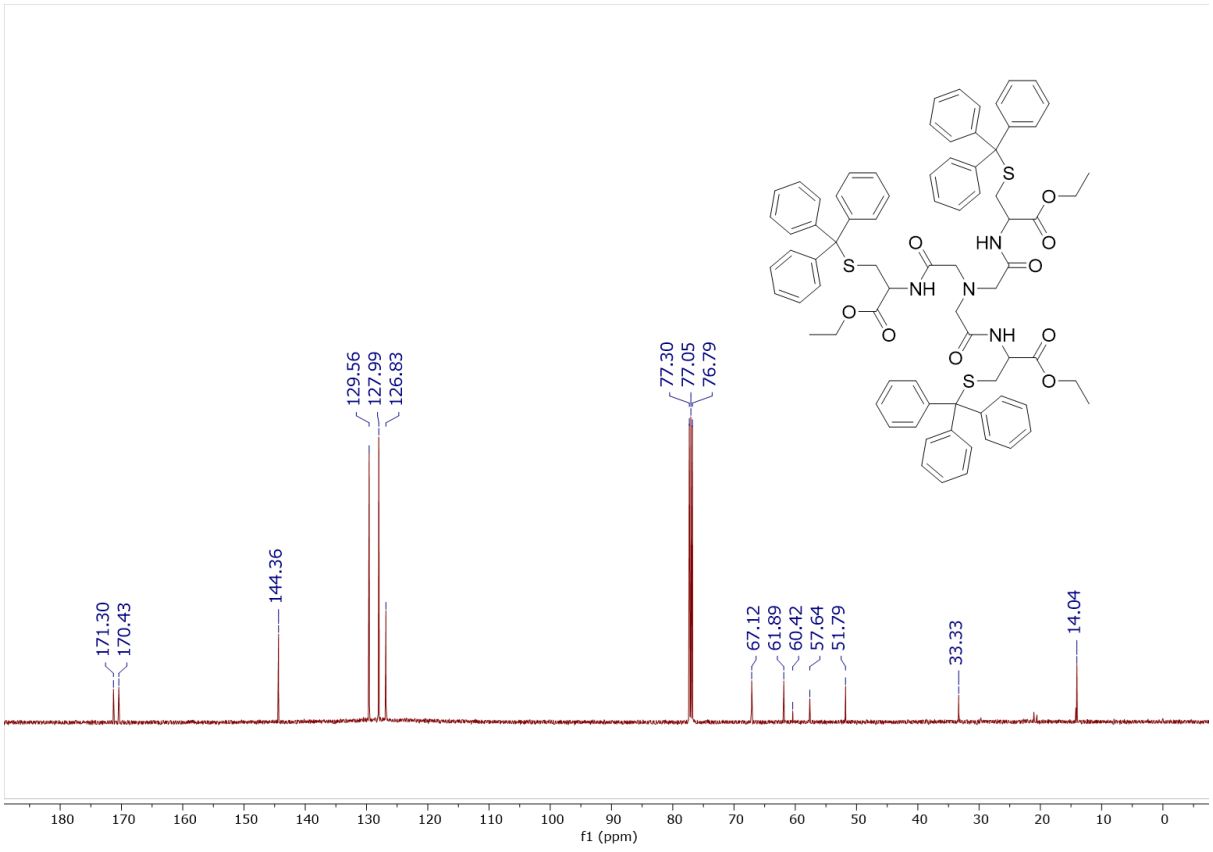



Figure S40: $^{13}$C NMR for compound **9** in $CDCl_3$

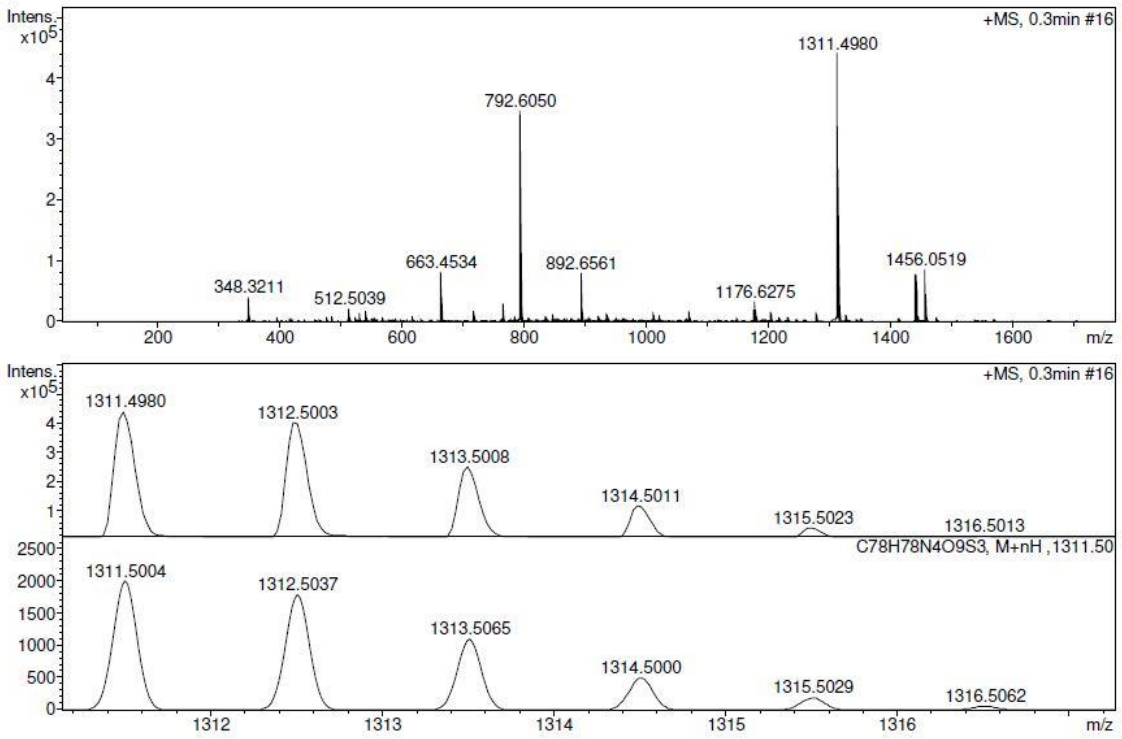



Figure S41: HRMS Spectra of Compound **9**

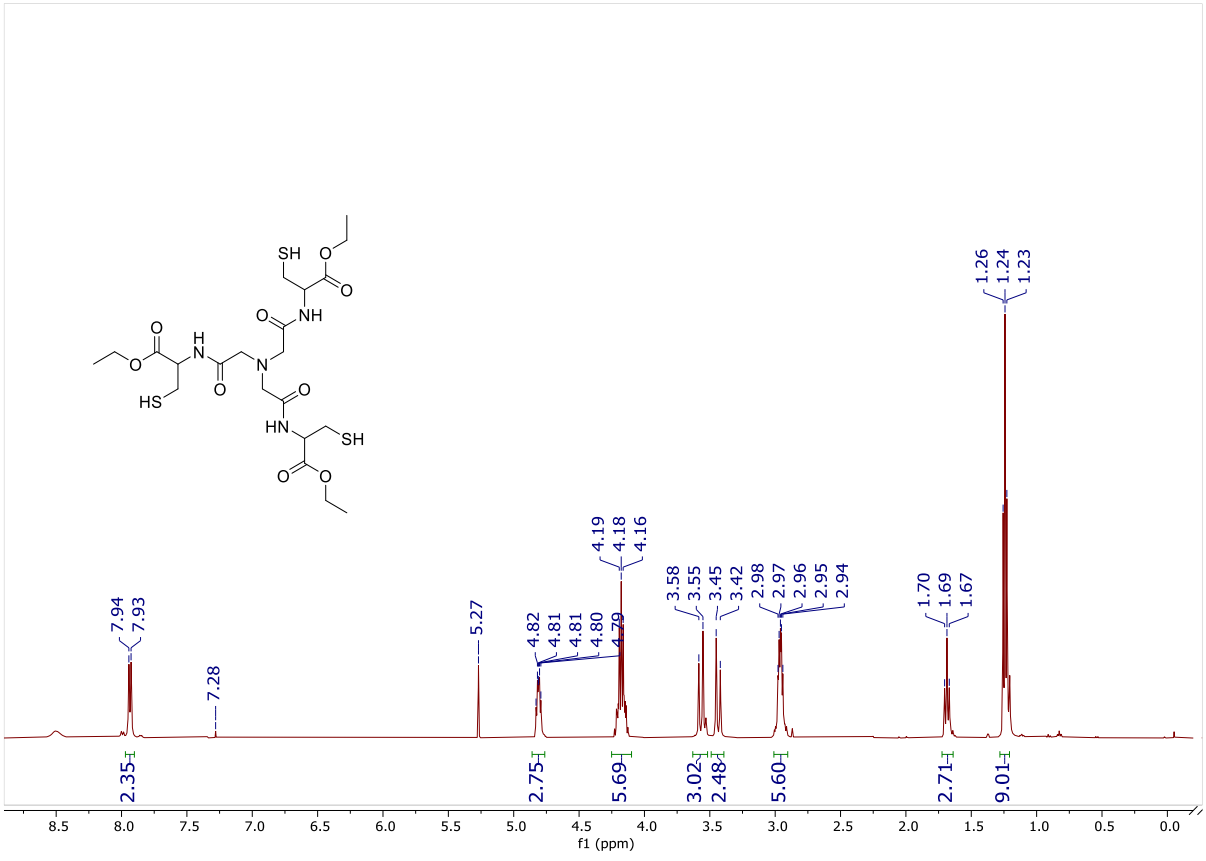



Figure S42: $^{1}H$ NMR of **NTASH** in $CDCl_3$

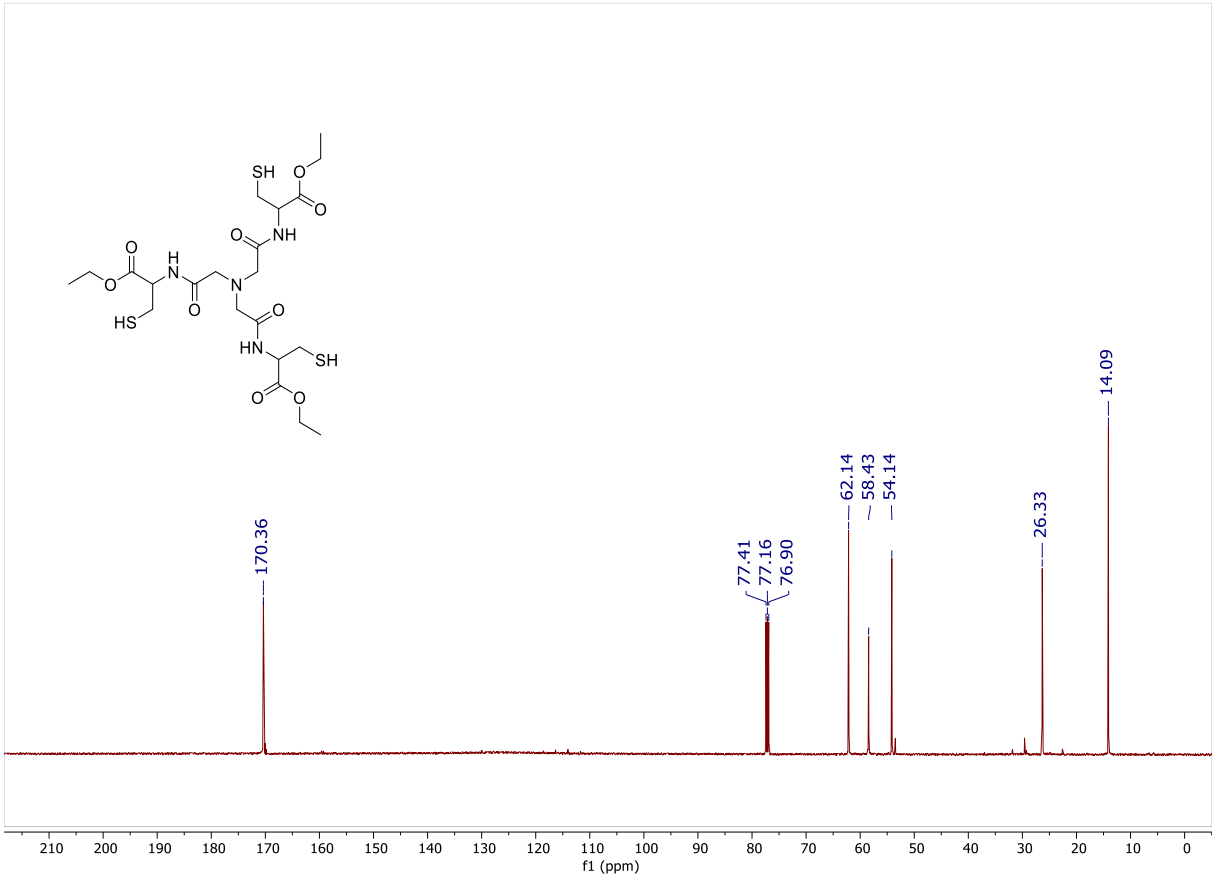



Figure S43: $^{13}C$ NMR for **NTASH** in $CDCl_3$

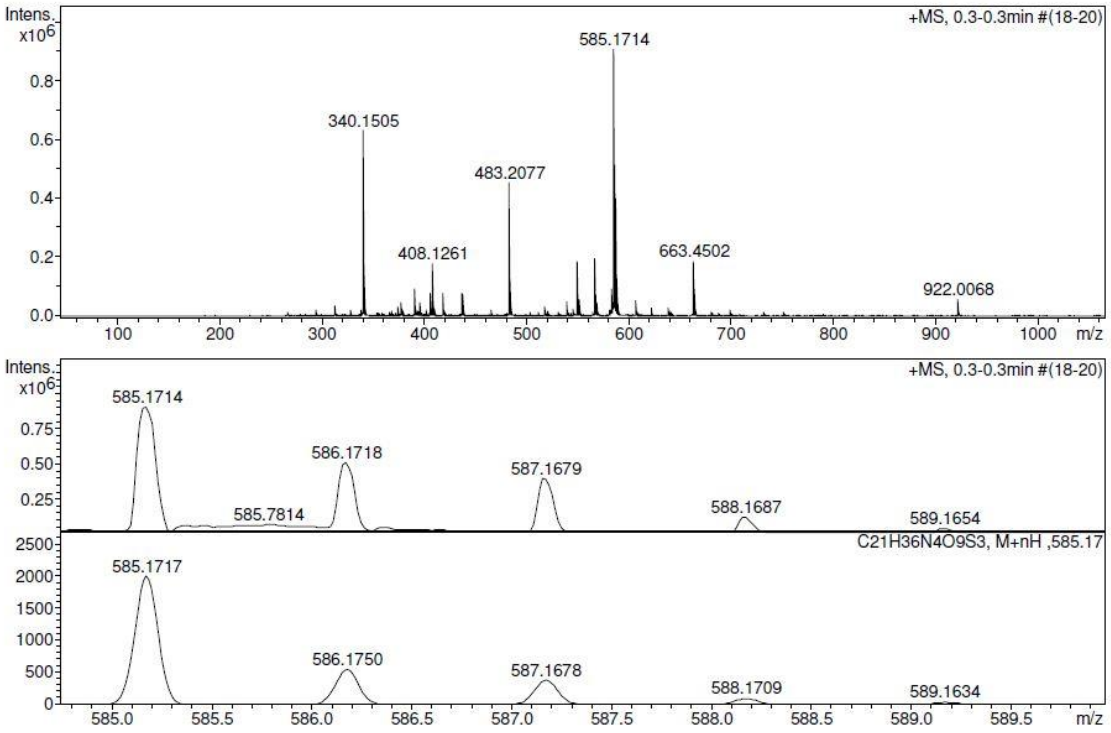



Figure S44: HRMS Spectra of **NTASH** compound

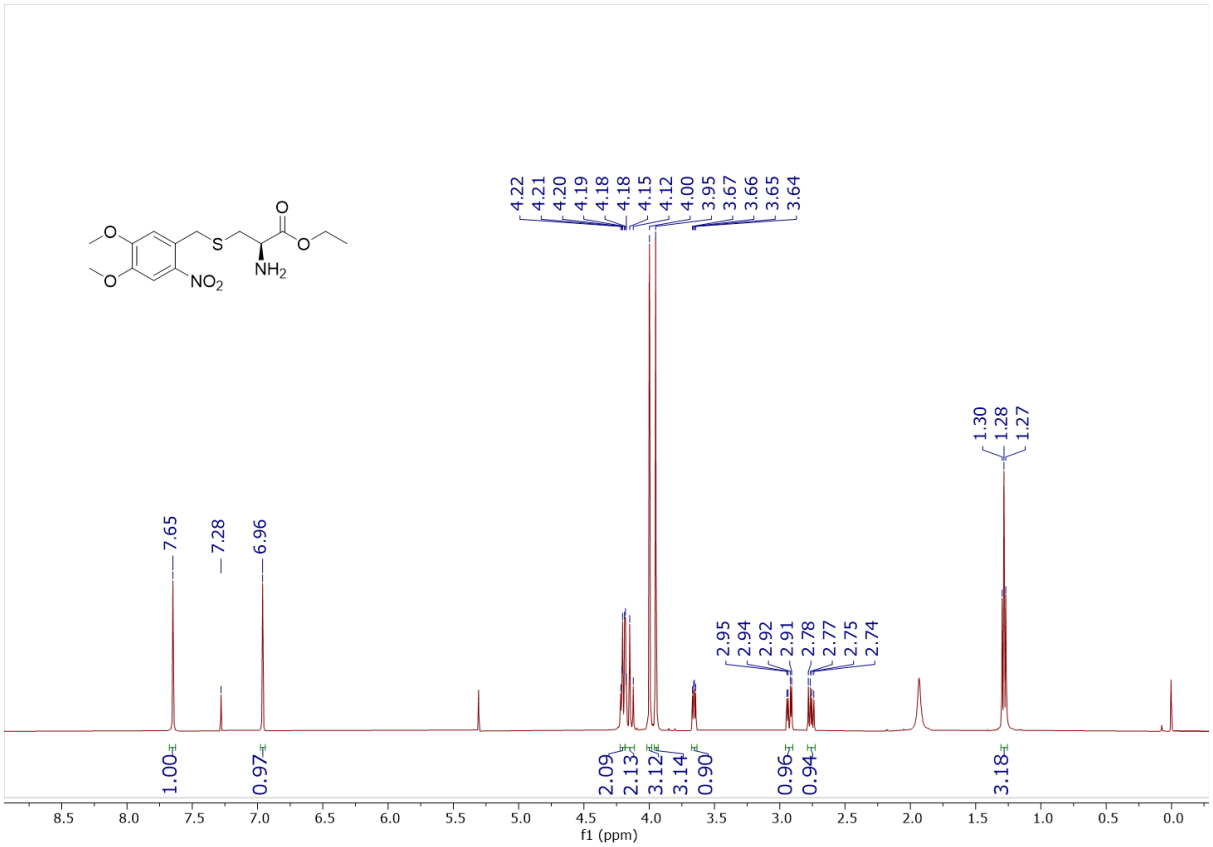



Figure S45: $^{1}$H NMR of compound **11** in $CDCl_3$

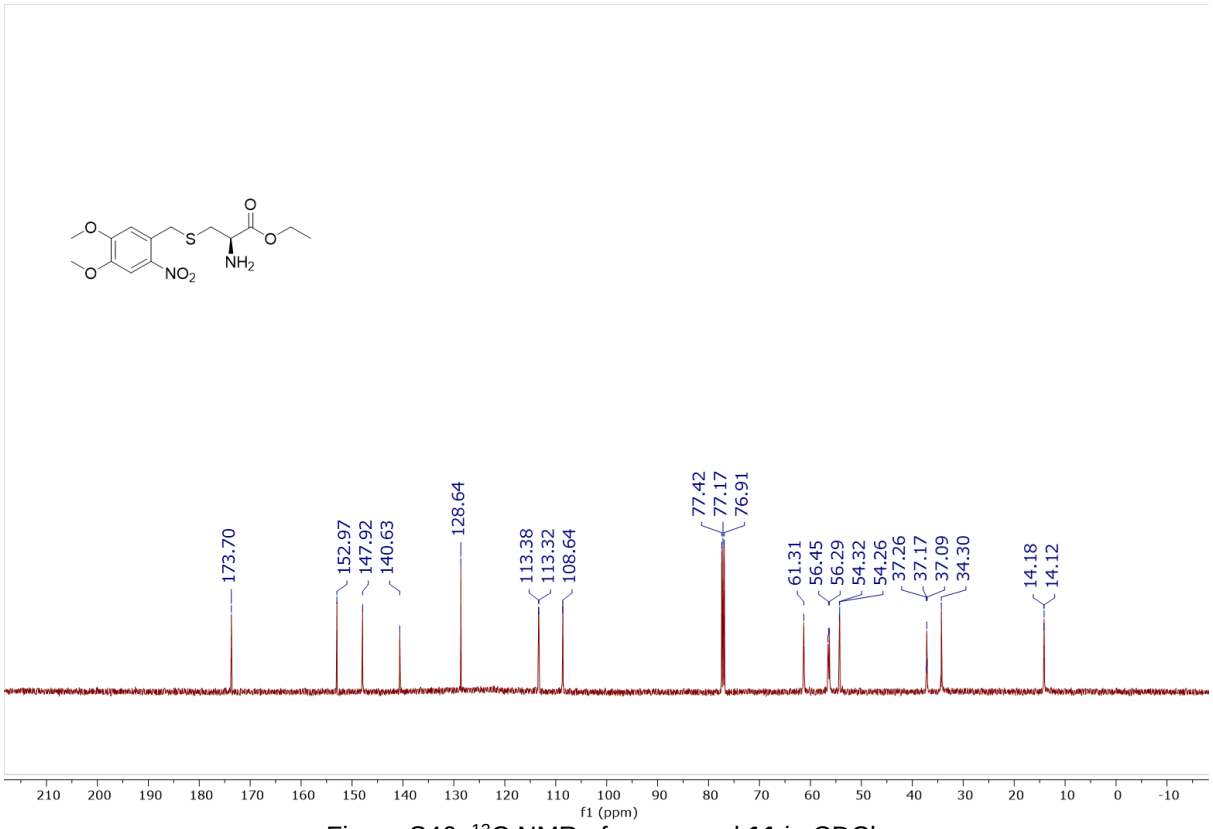



Figure S46: $^{13}$C NMR of compound **11** in $CDCl_3$

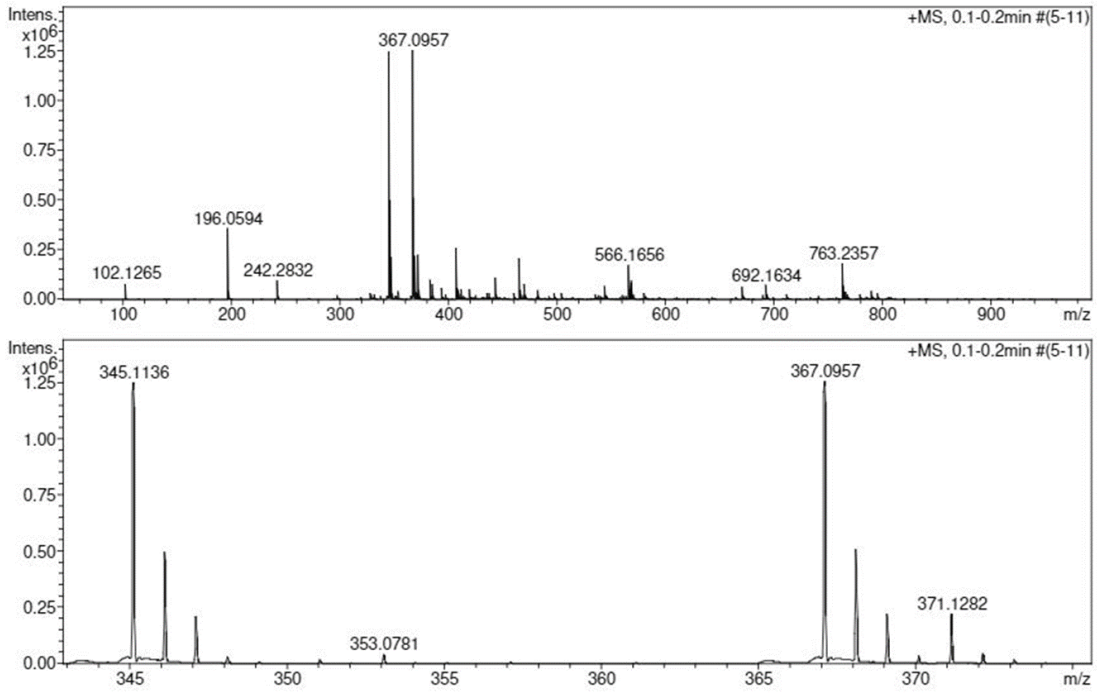



Figure S47: HRMS Spectra of compound **11**

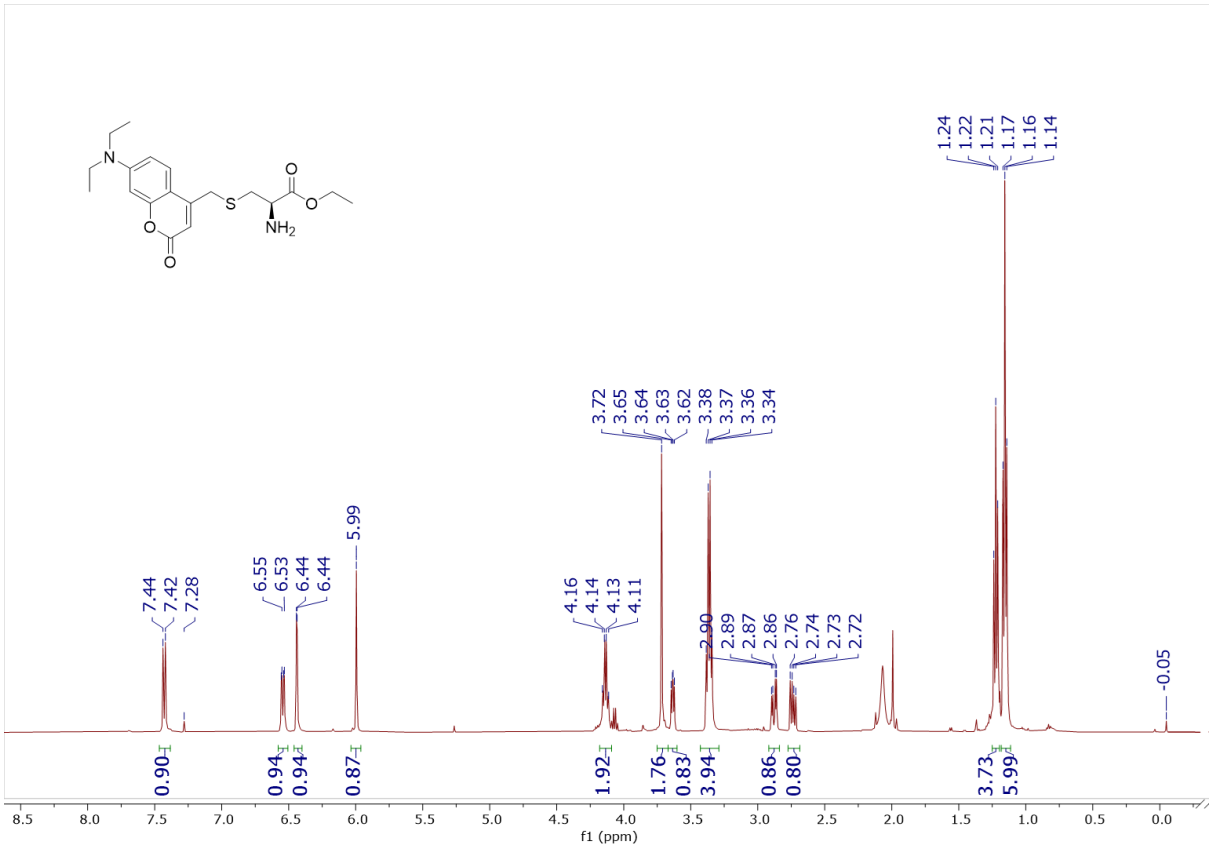



Figure S48: $^{1}H$ NMR of compound **12** in $CDCl_3$

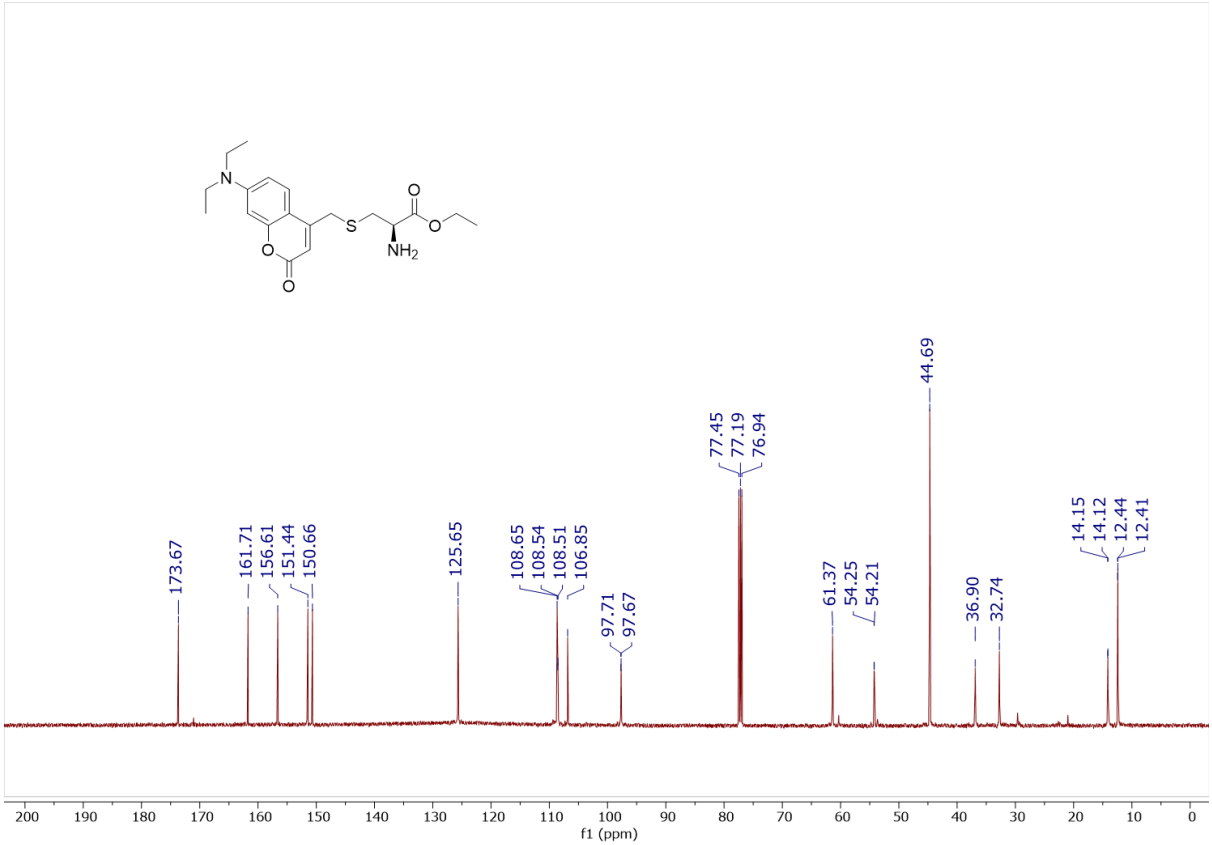



Figure S49: $^{13}C$ NMR of compound **12** in $CDCl_3$

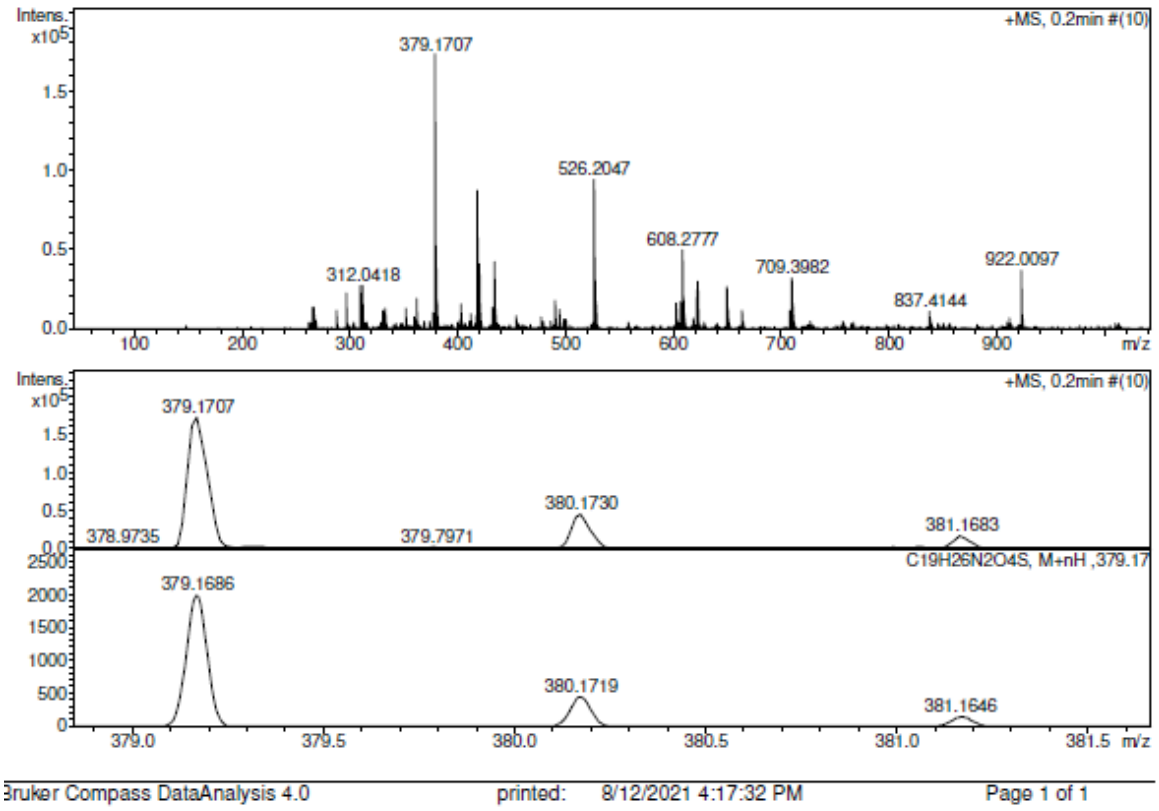



Figure S50: HRMS Spectra of compound **12**

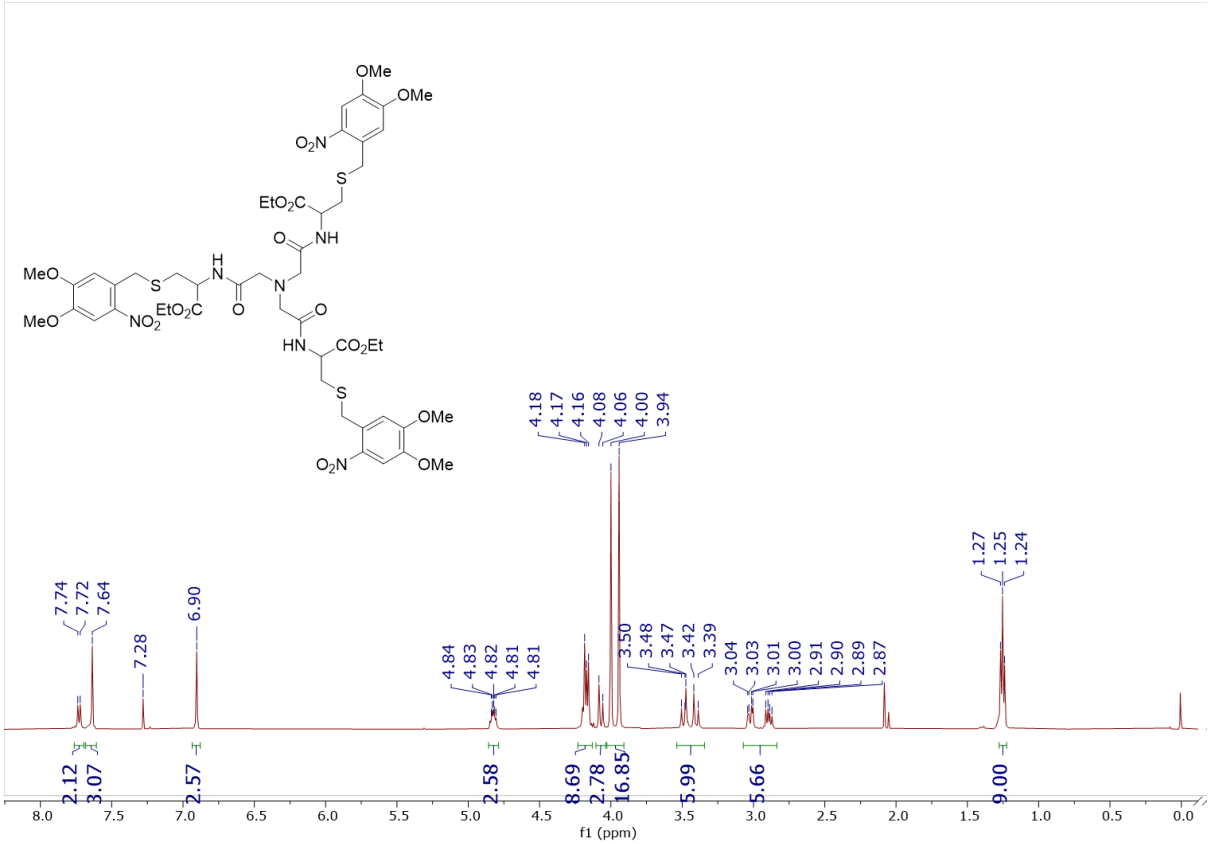



Figure S51: $^{1}$H NMR of compound **PKP1** in $CDCl_3$

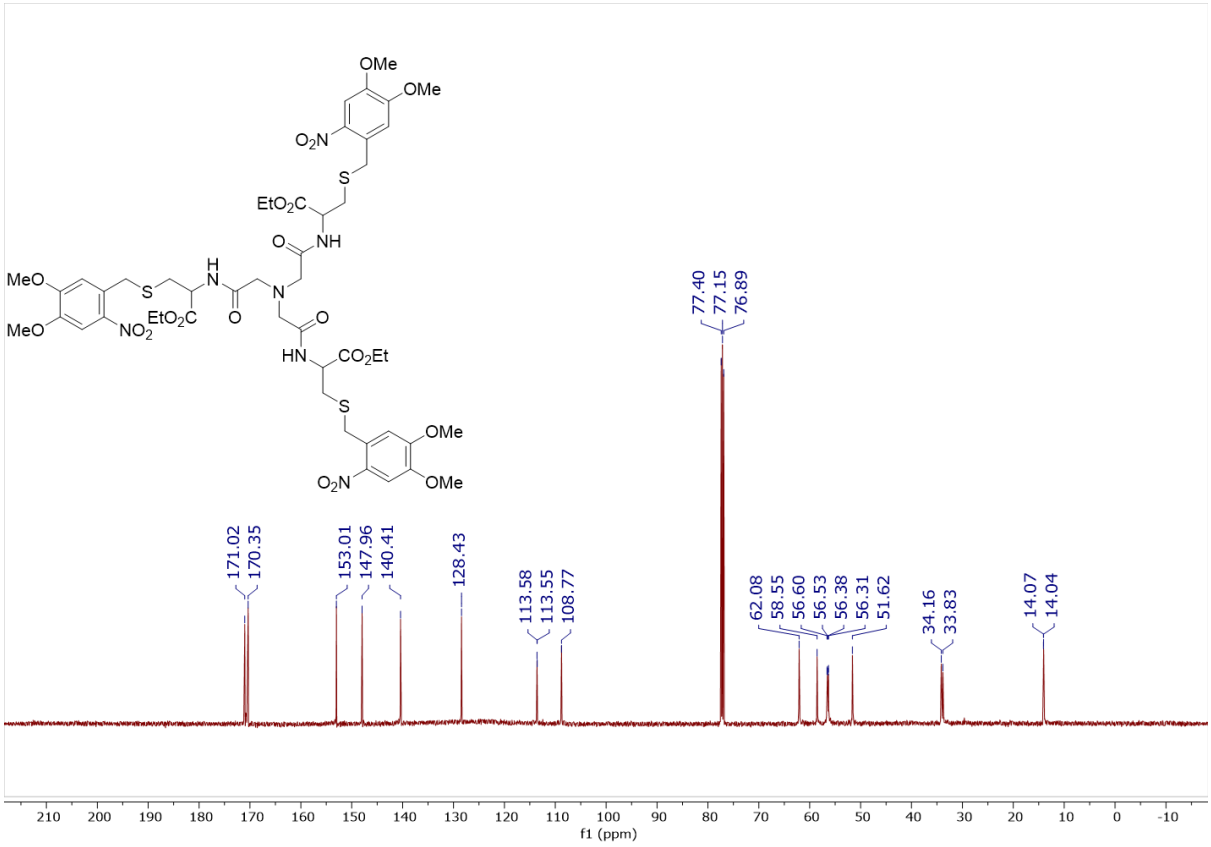



Figure S52: $^{13}$C NMR of compound **PKP1** in $CDCl_3$

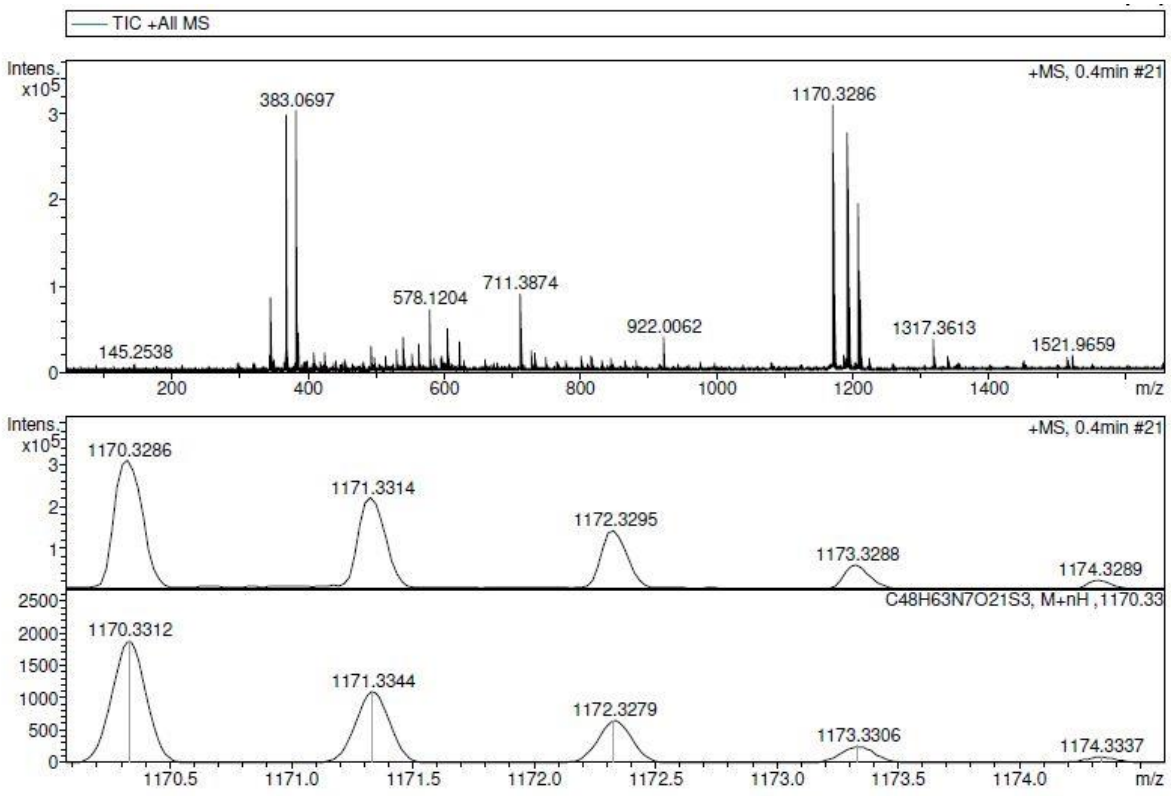



Figure S53: HRMS Spectra of compound **PKP1**

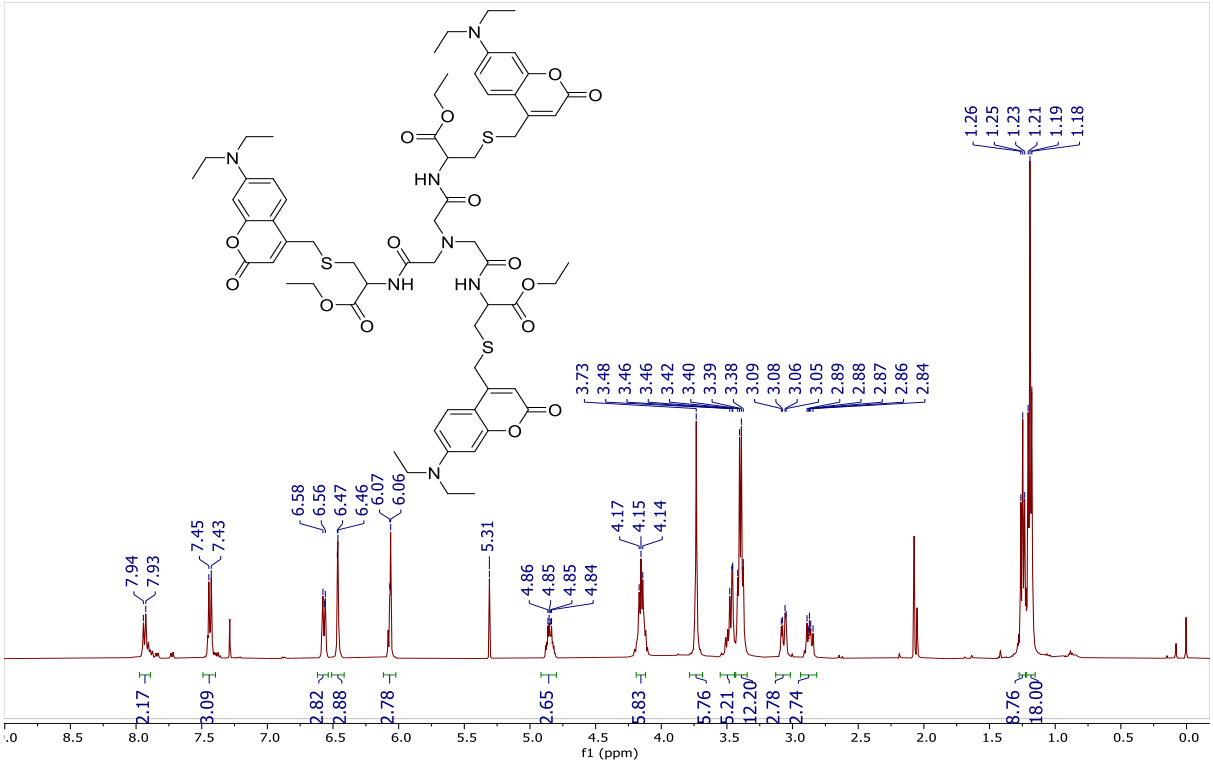



Figure S54: $^{1}H$ NMR of compound **PKP2** in $CDCl_3$

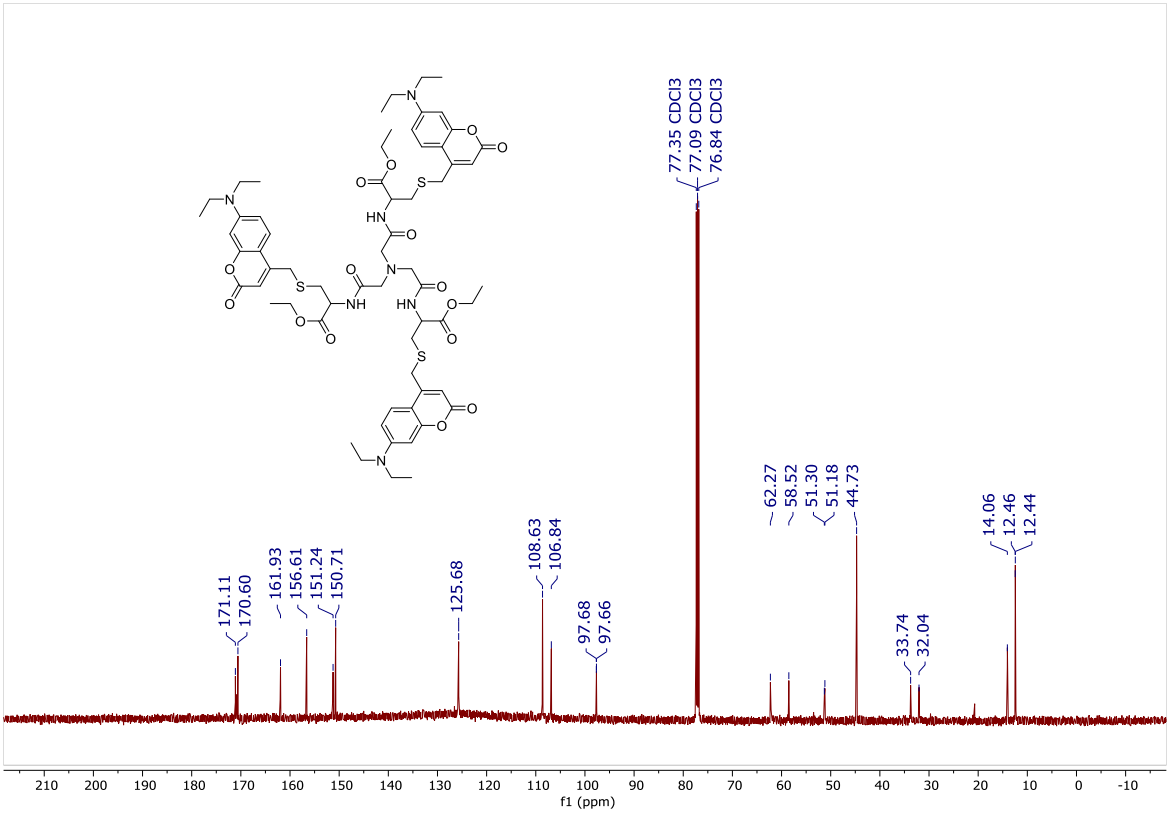



Figure S55: $^{13}C$ NMR of compound **PKP2** in $CDCl_3$

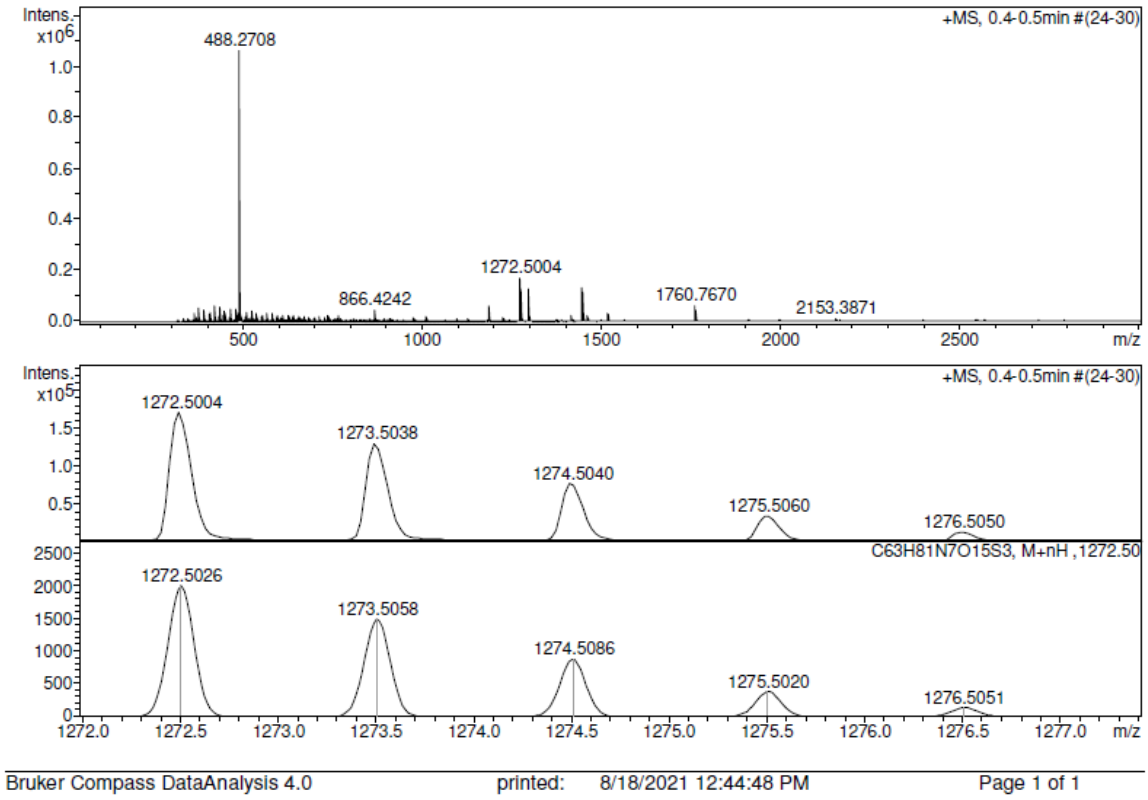



Figure 56: HRMS Spectra of compound **PKP2**

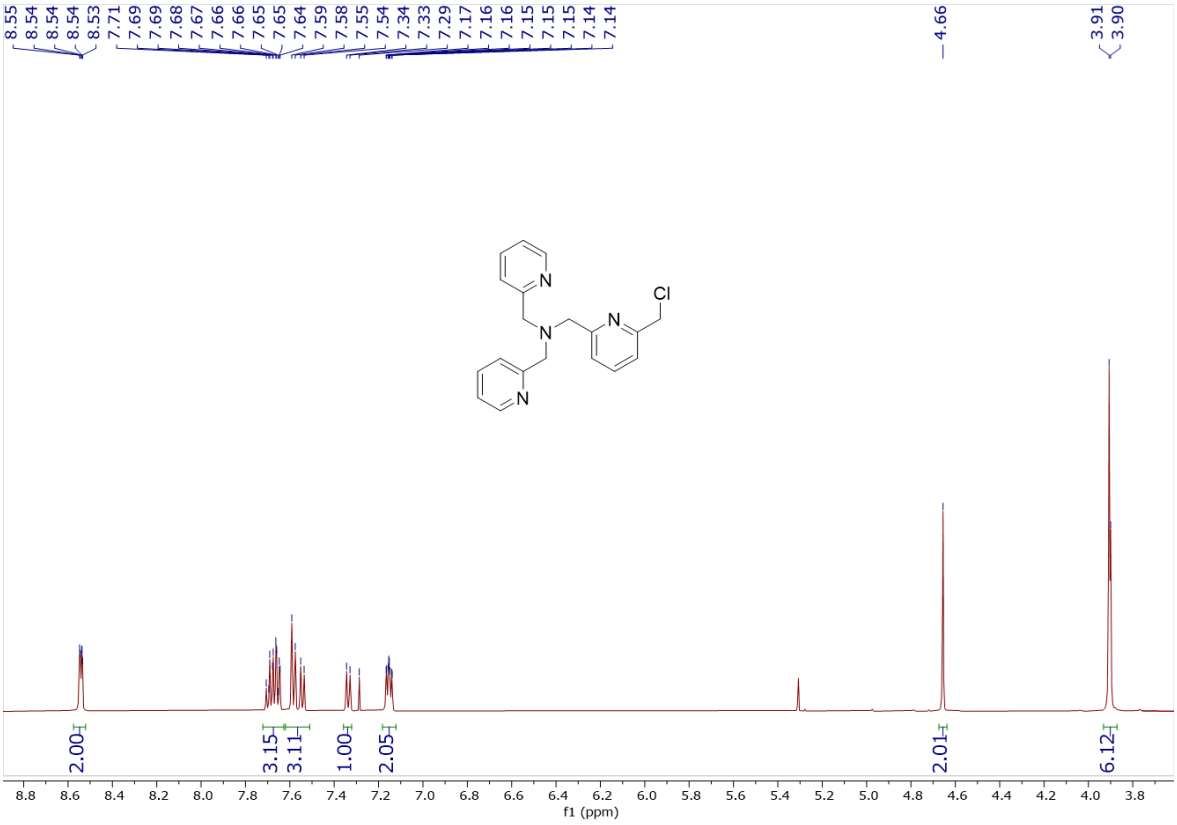



Figure S57: $^{1}$H NMR of compound **15** in $CDCl_3$

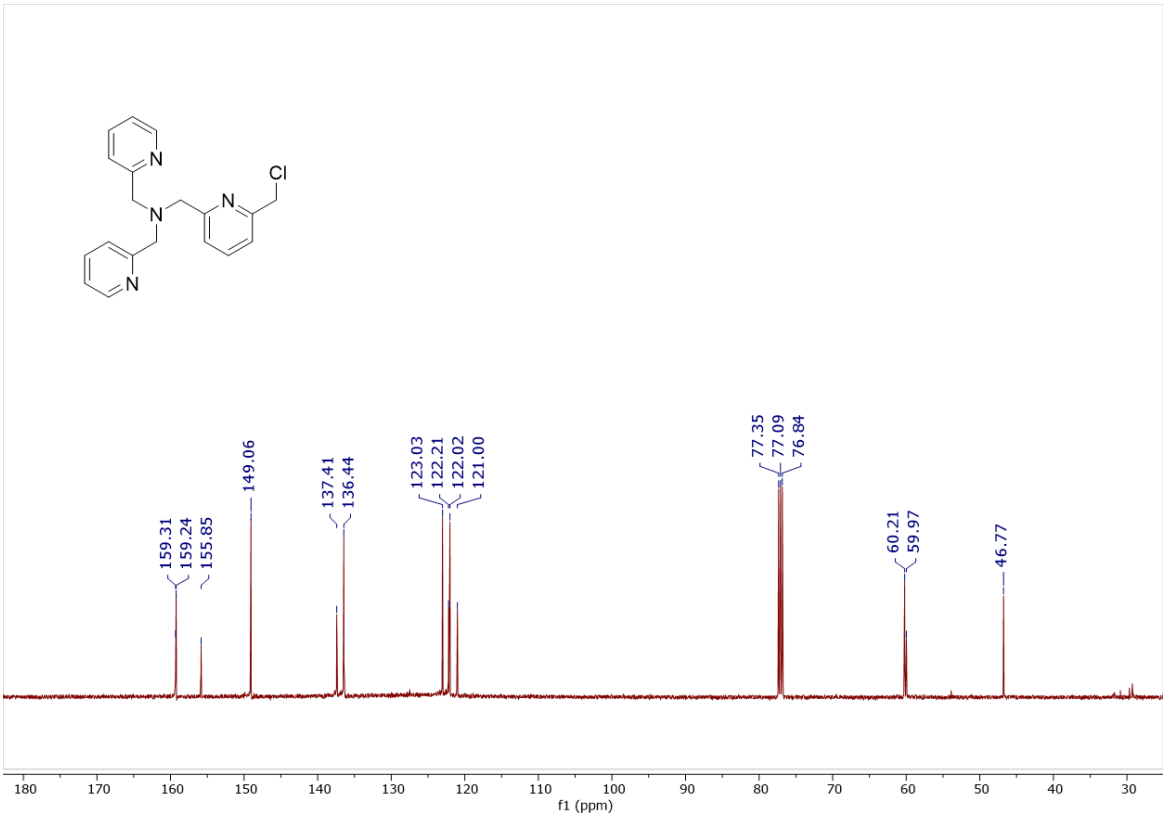



Figure S58: $^{13}$C NMR of compound **15** in $CDCl_3$

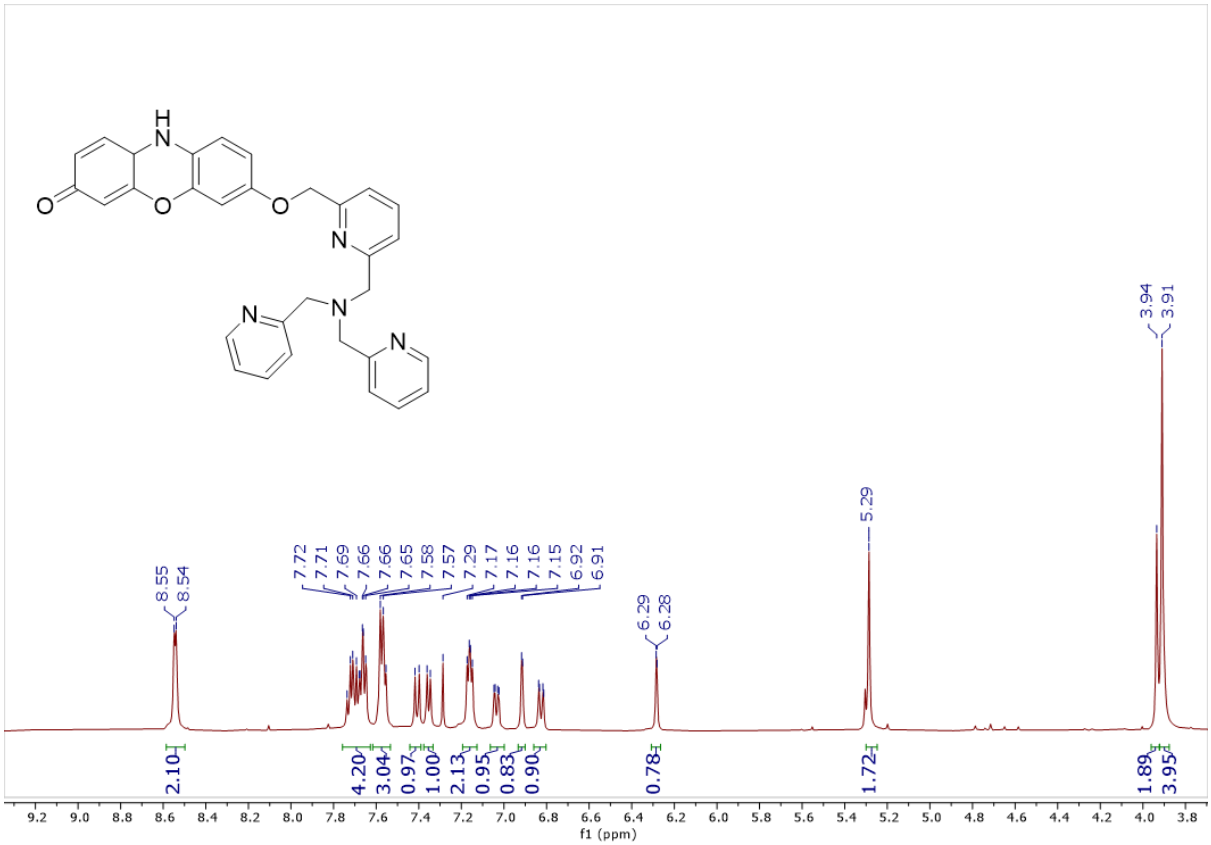



Figure S59: $^{1}H$ NMR of compound **16** in $CDCl_3$

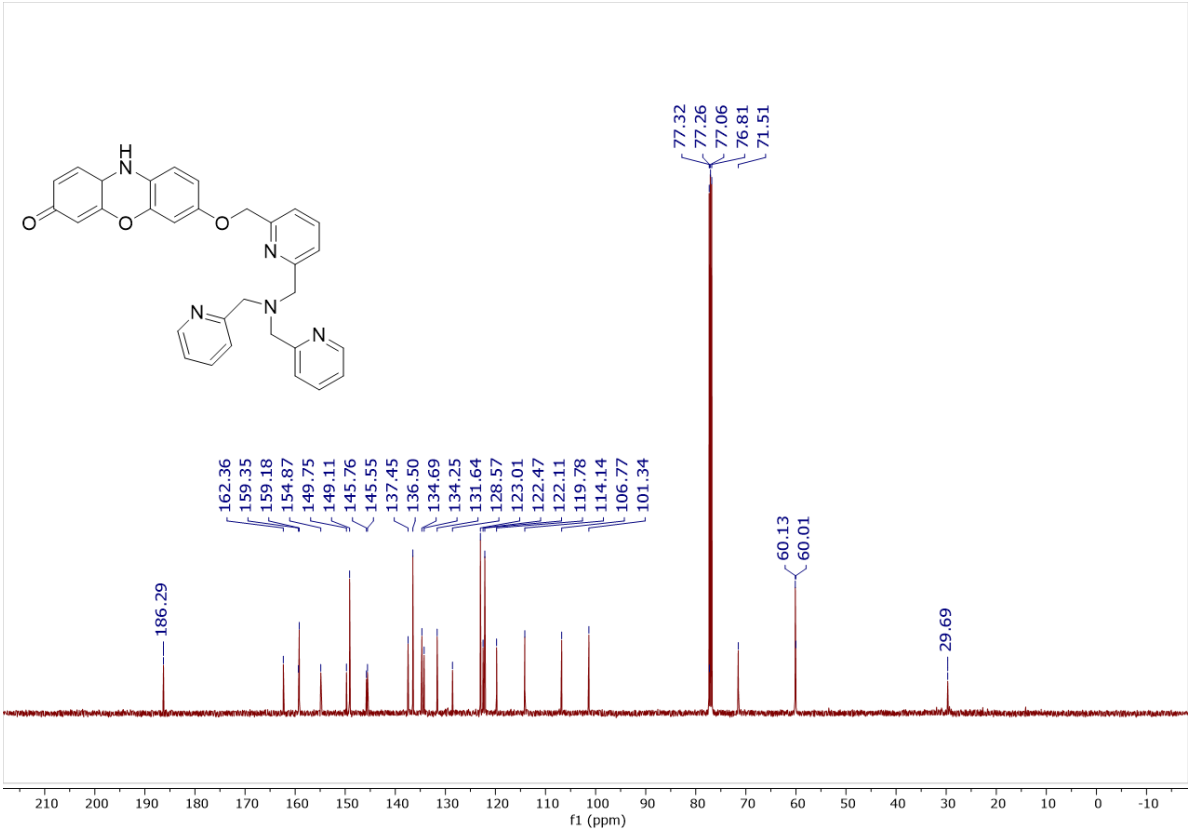



Figure S60: $^{13}C$ NMR of compound **16** in $CDCl_3$

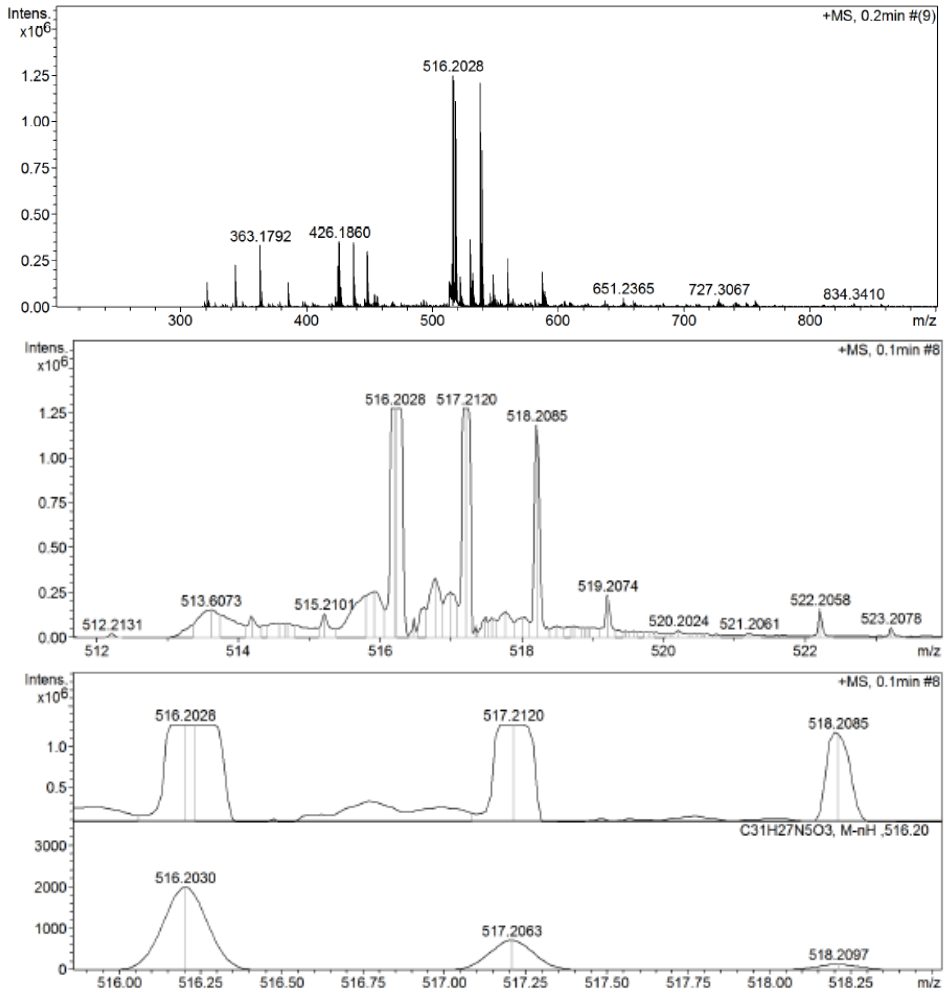



Figure S61: HRMS Spectra of compound **ResCu**

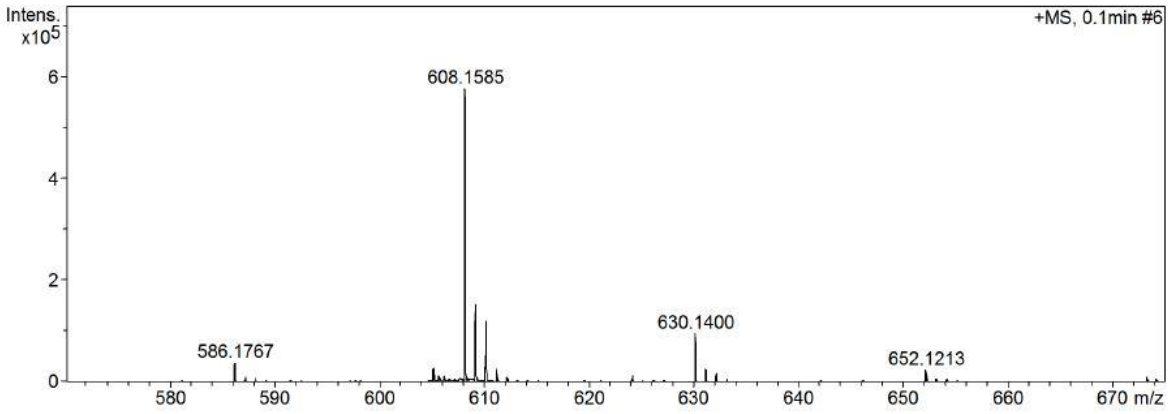



Figure S62: HRMS Spectra of PKP1 after uncaging (M+ Na)

1. A. M. Pujol, M. Cuillel, A. S. Jullien, C. Lebrun, D. Cassio, E. Mintz, C. Gateau and P. Delangle, *Angew Chem Int Ed Engl*, 2012, **51**, 7445-7448.
2. K. K. Behara, Y. Rajesh, Y. Venkatesh, B. R. Pinninti, M. Mandal and N. D. P. Singh, *Chem Commun*, 2017, **53**, 9470-9473.
3. L. Fournier, C. Gauron, L. Xu, I. Aujard, T. Le Saux, N. Gagey-Eilstein, S. Maurin, S. Dubruille, J. B. Baudin, D. Bensimon, M. Volovitch, S. Vriz and L. Jullien, *ACS Chem Biol*, 2013, **8**, 1528-1536.
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