Supplementary Information

for

Thioredoxin reductase-triggered fluorogenic donor of hydrogen sulfide: a model study with a symmetrical organopolysulfide probe with turn-on near-infrared fluorescent emission

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Abbreviations

Glu, L-Glutamic acid; Asp, L-Aspartic acid; Ser, L-Serine; Gly, L-Glycine; Met, L-Methionine; Arg, L-Arginine; Val, L-Valine; Ala, L-Alanine, NaCl, Sodium chloride; H_2O_2, Hydrogen peroxide; *t*BuOOH, *tert*-butyl hydroperoxide; BSA, Bovine serum albumin; GR, Glutathione disulfide reductase; GPx, Glutathione peroxidase; $Na_2S.9H_2O$, Sodium sulfide nonahydrate; DNCB, 2,4-Dinitrochlorobenzene, Ph_3PAuCl, Chlorotriphenyl phosphinegold(I); NADPH, β-Nicotinamide adenine dinucleotide phosphate reduced tetrasodium salt; PhSH, Thiophenol; 2-ME, 2-Mercaptoethanol; Cys, L-Cysteine; GSH; Glutathione reduced; DTT, Dithiothreitol; TCEP, Tris(2-carboxyl)phosphine.

Experimental section

Compound 2:[1] To a solution of 3, 5, 5-trimethyl-2-cyclohexen-1-one (0.20 g, 1.47 mmol) and malononitrile (0.11 g, 1.72 mmol) in absolute ethanol was added piperidine (13.00 mg, 0.15 mmol) and acetic acid (9.00 mg, 0.15 mmol) under argon atmosphere. The reaction mixture was refluxed at 90 °C for 12 h upon which the reaction mixture turned dark black in colour. The progress of the reaction was monitored by TLC analysis. After completion, the mixture was cooled to room temperature and the solvent was evaporated under reduced pressure and the residue obtained was dissolved in CH_2Cl_2. The organic layer was washed with water, brine and dried over anhy sodium sulfate. The solvent was evaporated to afford the crude product, which was subjected to silica gel column chromatography using pet ether and dichloromethane as eluents to afford the pure product **2** as a white solid. $R_f = 0.5$ (20% dichloromethane in pet ether). Yield: 0.20 g (74%). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 6.62 (s, 1H), 2.52 (s, 2H), 2.18 (s, 2H), 2.03 (s, 3H), 1.01 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 170.4, 159.9, 120.6, 113.2, 112.4, 78.2, 45.6, 42.6, 32.4, 27.8, 25.3.

Compound DCI-OH:[1] To a solution of 4-hydroxybenzaldehyde (0.20 g, 1.07 mmol) in acetonitrile (20 mL) was added compound **2** (0.14 g, 1.18 mmol) and piperidine (10 μl, 0.10 mmol). The mixture was heated to reflux at 90 °C for 5 h under inert atmosphere and the reaction mixture turned red in colour. Progress of the reaction was monitored by TLC analysis. Upon completion, the solvent was removed under reduced pressure. The resulting residue was dissolved in dichloromethane, washed with water and dried over anhy sodium sulfate. The crude product was purified by silica gel column chromatography using methanol and dichloromethane as eluents to afford the pure **DCI-OH** as an orange solid. $R_f = 0.5$ (30% ethyl acetate in pet ether). Yield: 0.20 g (41%). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.42 (d, J = 8.4 Hz, 2H), 7.01 (d, J

= 16.2 Hz, 1H), 6.87-6.84 (m, 3H), 6.80 (s, 1H), 2.59 (s, 1H), 2.46 (s, 1H), 1.08 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 169.4, 157.2, 154.4, 136.8, 129.4, 128.6, 127.0, 122.7, 116.1, 113.7, 112.9, 77.7, 43.0, 39.2, 32.0, 28.0. ESI-MS: *m/z* calcd. for $C_{19}H_{17}N_2O_2$ $[M-H]^-$: 289.1346; obs. $[M-H]^-$: 289.1448.

Compound 3:[2] To a solution of 2-mercaptoethanol (0.50 g, 6.40 mmol) in ethyl acetate (5.0 mL) was added a catalytic amount of NaI (5.00 mg, 0.03 mmol), followed by the dropwise addition of hydrogen peroxide (30% solution in water, 0.70 mL, 6.4 mmol), upon which the reaction mixture turned brown in colour. The reaction mixture was stirred at room temperature for additional 30 min and the colour of the solution turned brownish. The reaction was quenched by addition of saturated $Na_2S_2O_3$ solution (10.0 mL). Upon completion of the reaction, the organic layer was washed with saturated Na_2CO_3 solution (10.0 mL) followed by brine solution (10.0 mL). The organic layer was collected and dried over anhy. sodium sulfate. The solvent was evaporated under vacuum to afford compound **3** as colourless oil. R_f = 0.5 (60% ethyl acetate in pet ether). Yield: 0.20 g (48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.92 (t, *J* = 6.0 Hz, 4H), 2.89 (t, *J* = 5.6 Hz, 4H), 2.33 (d, *J* = 4.0 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 60.4, 41.3. ESI-MS: *m/z* calcd. for $C_4H_{10}NaO_2S_2$ $[M+Na]^+$: 177.0020; Obs. $[M+Na]^+$: 177.0036.

Compound 4:[3] To a solution of compound **3** (0.20 g, 1.30 mmol) in anhy dichloromethane (6.0 mL) was added pyridine (0.50 mL, 6.40 mmol) and the mixture was cooled to 0 °C. To the above mixture, a solution of 4-nitrophenyl chloroformate (0.60 g, 3.20 mmol) in anhy dichloromethane was added dropwise at 0 °C. The reaction mixture was then allowed to attain room temperature and stirred for 8 h. The progress of the reaction was monitored by TLC analysis. Upon completion, the mixture was diluted with dichloromethane and the mixture was sequentially washed with saturated $NaHCO_3$ (10.0 mL), water (10.0 mL), 10% citric acid (10.0 mL), water (10.0 mL) and brine (10.0 mL). The organic layer was dried over anhy sodium sulfate and the solvent was evaporated under reduced pressure to afford compound **4**. The crude residue was purified by silica gel column chromatography using ethyl acetate and pet ether as eluents to obtain pure compound **4** as white solid. R_f = 0.5 (30% ethyl acetate in pet ether) Yield: 0.40 g (62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.28 (d, *J* = 7.2 Hz, 4H), 7.39 (d, *J* = 9.2 Hz, 4H), 4.58 (t, *J* = 6.4 Hz, 4H), 3.09 (t, *J* = 6.4 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 155.3, 152.3, 145.5, 125.4, 121.8, 66.7 and 36.8. ESI-MS: *m/z* calcd. for $C_{14}H_{16}N_2O_{10}S_2$ $[M+K]^+$: 522.9883; obs. $[M+K]^+$: 522.9885.

Compound 6:[4, 5] To a solution of sodium sulfide nonahydrate (0.70 g, 3.10 mmol) in deionized water (12.0 mL) was added sulfur powder (0.20 g, 0.80 mmol) and the reaction mixture was

stirred for 2.5 h at room temperature during which the solution turned reddish brown indicating the formation of Na_2S_n (n = 2, 3, 4, 5) with Na_2S_3 as the major component. To the above solution, 2-chloroethanol (0.40 mL, 6.20 mmol) was added and the reaction mixture was stirred for 6 h at room temperature. Progress of the reaction was monitored by TLC analysis. Upon completion, the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with brine solution and dried over anhy sodium sulfate. The solvent was evaporated under reduced pressure to afford polysulfide **6** as faint yellow oil with almost quantitative yield. The crude compound was purified to remove the major amount of the disulfide from the mixture and the remaining inseparable polysulfide was used directly for the next step without any further purification. R_f = 0.5 (45% ethyl acetate in pet ether). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 4.01 – 3.97 (m, overlap of two triplets for –O$\underline{CH_2}$- group from trisulfide, tetrasulfide and pentasulfide, 4H), 3.18 (t, *J* = 6.0 Hz -S$\underline{CH_2}$- group from pentasulfide), 3.14 (t, *J* = 6.0 Hz, -S$\underline{CH_2}$- group from tetrasulfide) and 3.09 (t, *J* = 6.0 Hz, -S$\underline{CH_2}$- group from trisulfide). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 60.32, 59.97, 59.62, 42.44, 41.78, 41.67. ESI-MS: *m/z* calcd. for $C_4H_{10}O_2S_3$ (trisulfide) $[M+Na]^+$: 208.9741; Obs. $[M+Na]^+$: 208.9749, ESI-MS: *m/z* calcd. for $C_4H_{10}O_2S_4$ (tetrasulfide) $[M+Na]^+$: 240.9461; Obs. $[M+Na]^+$: 240.9468, ESI-MS: *m/z* calcd. for $C_4H_{10}O_2S_5$ (pentasulfide) $[M+Na]^+$: 272.9182; Obs. $[M+Na]^+$: 272.9196.

Preparation of polysulfide alcohol 6 with variation of the ratio of sulfur powder and $Na_2S.9H_2O$

General Procedure: To a solution of sodium sulfide nonahydrate (3-10 equiv) in deionized water (10 mL) was added sulfur powder (0.20 g, 0.78 mmol, 1 equiv) under argon atmosphere. The reaction was continued for 2.5 hours at room temperature and the reaction mixture turned yellow in colour that indicates the formation of polysulfide (Na_2S_n) species in the reaction mixture. After that 2-chloroethanol (0.50 g, 6.24 mmol) was added dropwise to the reaction mixture and the reaction was continued for 6 hours at room temperature. The progress of the reaction was monitored by thin layer chromatography. Upon completion of the reaction, the reaction mixture was extracted with ethyl acetate (3 x 25 mL). The organic layer was then washed with brine (30 mL) and dried over anhydrous sodium sulfate. The organic layer was evaporated under reduced pressure to afford the crude mixture as light yellow oil. The crude mixture was purified by silica gel column chromatography that mostly removed the disulfide component and afforded the polysulfide mixture as a colourless oil.

Synthesis of polysulfide alcohol 6 with 1:3 ratio of Sulfur powder to Sodium sulfide nonahydrate: Sulfur powder (0.20 g, 0.78 mmol) and sodium sulfide nonahydrate (0.56 g, 2.34

mmol). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 4.00-3.98 (m, overlap of three triplets for –O$\underline{CH_2}$- group from trisulfide, tetrasulfide and pentasulfide), 3.18 (t, *J* = 6.0 Hz -S$\underline{CH_2}$- group from pentasulfide), 3.14 (t, *J* = 6.0 Hz, -S$\underline{CH_2}$- group from tetrasulfide) and 3.09 (t, *J* = 6.0 Hz, -S$\underline{CH_2}$- group from trisulfide).

Synthesis of polysulfide alcohol 6 with 1:4 ratio of Sulfur powder to Sodium sulfide nonahydrate: Sulfur powder (0.20 g, 0.78 mmol) and sodium sulfide nonahydrate (0.75 g, 3.12 mmol). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 4.00-3.98 (m, overlap of three triplets for –O$\underline{CH_2}$- group from trisulfide, tetrasulfide and pentasulfide), 3.18 (t, *J* = 6.0 Hz -S$\underline{CH_2}$- group from pentasulfide), 3.14 (t, *J* = 6.0 Hz, -S$\underline{CH_2}$- group from tetrasulfide) and 3.09 (t, *J* = 6.0 Hz, -S$\underline{CH_2}$- group from trisulfide).

Synthesis of polysulfide alcohol 6 with 1:5 ratio of Sulfur powder to Sodium sulfide nonahydrate: Sulfur powder (0.20 g, 0.78 mmol) and sodium sulfide nonahydrate (0.94 g, 3.90 mmol). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 4.00-3.98 (m, overlap of two triplets for –O$\underline{CH_2}$- group from trisulfide and tetrasulfide), 3.14 (t, *J* = 6.0 Hz, -S$\underline{CH_2}$- group from tetrasulfide) and 3.09 (t, *J* = 6.0 Hz, -S$\underline{CH_2}$- group from trisulfide).

Synthesis of polysulfide alcohol 6 with 1:6 ratio of Sulfur powder to Sodium sulfide nonahydrate: Sulfur powder (0.20 g, 0.78 mmol) and sodium sulfide nonahydrate (1.12 g, 4.68 mmol). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 4.00-3.98 (m, overlap of two triplets for –O$\underline{CH_2}$- group from trisulfide, tetrasulfide and pentasulfide), 3.18 (t, *J* = 6.0 Hz -S$\underline{CH_2}$- group from pentasulfide), 3.14 (t, *J* = 6.0 Hz, -S$\underline{CH_2}$- group from tetrasulfide) and 3.09 (t, *J* = 6.0 Hz, -S$\underline{CH_2}$- group from trisulfide).

Synthesis of polysulfide alcohol 6 with 1:10 ratio of Sulfur powder to Sodium sulfide nonahydrate: Sulfur powder (0.20 g, 0.78 mmol) and sodium sulfide nonahydrate (1.87g, 7.80 mmol). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 4.00-3.98 (m, overlap of two triplets for –O$\underline{CH_2}$- group from trisulfide and tetrasulfide), 3.15 (t, *J* = 6.0 Hz, -S$\underline{CH_2}$- group from tetrasulfide) and 3.09 (t, *J* = 6.0 Hz, -S$\underline{CH_2}$- group from trisulfide).

Compound 7:[6] To a solution of polysulfide **6** (0.20 g, 1.0 mmol) in anhy. dichloromethane (6.0 mL) under inert atmosphere was added pyridine (0.45 mL, 5.30 mmol) and the mixture was cooled to 0 °C with external ice bath. A solution of 4-nitrophenyl chloroformate (0.54 g, 2.60 mmol) in anhy dichloromethane (6.0 mL) was added to the above reaction mixture in a dropwise manner at 0 °C. The resulting solution was allowed to attain room temperature and was stirred for another 8 h. The progress of the reaction was monitored by TLC analysis. Upon completion,

the mixture was diluted with dichloromethane and sequentially washed with saturated $NaHCO_3$ (15.0 mL), water (15.0 mL), 10% citric acid (15.0 mL) and brine solution (15.0 mL). The organic layer was dried over anhy. sodium sulfate and the solvent was evaporated under reduced pressure to afford compound **7** as white solid with almost quantitative yield, which was used for the next step without further purification. R_f = 0.5 (20% ethyl acetate in pet ether). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 8.29 (d, *J* = 9.6 Hz, 4H), 7.39 (d, *J* = 9.6 Hz, 4H), 4.64-4.62 (m, overlap of two triplets for –O$\underline{CH_2}$- group from trisulfide and tetrasulfide, 4H), [3.35-3.30 (m, overlap of two triplets for –O$\underline{CH_2}$- group from tetrasulfide and pentasulfide) and 3.26 (t, *J* = 6.4 Hz, –O$\underline{CH_2}$- group from trisulfide), 4H]. ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 155.3, 152.3, 145.5, 125.4, 121.7, 66.6, 66.4, 36.9, 36.4. ESI-MS: *m/z* calcd. for $C_{18}H_{16}N_2O_{10}S_3$ (trisulfide) $[M+Na]^+$: 538.9865; Obs. $[M+ Na]^+$: 538.9914, ESI-MS: *m/z* calcd. for $C_{18}H_{16}N_2O_{10}S_4$ (tetrasulfide) $[M+Na]^+$: 570.9585; Obs. $[M+Na]^+$: 570.9563, ESI-MS: *m/z* calcd. for $C_4H_{10}O_2S_5$ (pentasulfide) $[M+Na]^+$: 602.9306; Obs. $[M+Na]^+$: 602.9212.

Protein-ligand docking study

Protein Preparation: For the docking studies, the recently published crystal structure of TrxR1 (PDB: 1H6V) was extracted from RCSB (Protein Data Bank).[7] The protein structure was prepared using AutoDock 4.2 Tools (MGL Tools 1.5.6) and Pymol (Schrodinger LLC) software package.[8] Initially, the protein structure was assembled to the original form having all the important subunits depicted by the crystallographic study. During the protein preparation, addition of missing hydrogens and Gasteiger charges were applied.

Receptor grid generation: The protein TrxR1 was co-crystallized with FAD^+ and NADPH and the crystal structure contained Cysteine (Cys 498A) residue in its native form. A modified protein was prepared and used in the present study upon the point mutation at the cysteine residue (Cys 498A) with selenocysteine (Sec 498A) using Chimera 1.14 software package.[9, 10] In order to locate the binding site, the grid around the selenocysteine sites without altering the entire protein structure was calculated and the grid box was generated by performing the Auto grid. The grid size was set to 40 × 40 × 40 points with grid spacing of 1.000 Å and the grid was centred at Se atom (x = 0.938, y = 8.852, z = 156.484).

Ligand preparation: The 2D structures of **DCI-DS** and **DCI-PS** were drawn carefully and converted to 3D structure using Chem3D Pro (Version: 19.0.1.28). The 3D structures of ligands were subjected to MM2 energy minimization calculations and the correct bond orders were assigned. Finally, for the docking studies, extended PDB format, termed PDBQT, was used for

coordinate files, which includes atomic partial charges and atom types. Torsion angles were calculated to assign the fixable and non-bonded rotation of molecules.[11]

Docking Study: Docking simulations were performed using AutoDock Vina software package by using Perl programming for handling multiple ligands at a time.[8] The number of runs is set by exhaustiveness parameter. Since the individual runs were executed in parallel, proper exhaustiveness parameter is essential. The exhaustiveness parameter of 8 was considered throughout the study.[10] Additionally, we have mentioned 20 binding modes and energy difference of 5 in the conformation file.

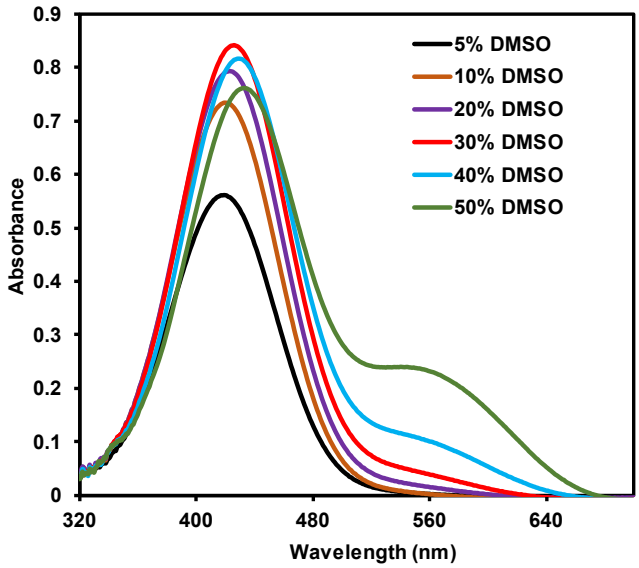

Figure **S1**. Absorption spectra of **DCI-OH** (5.0 μM) in phosphate buffered saline (20 mM) of pH 7.4 in the presence of varying percentage of DMSO (5% to 50%).

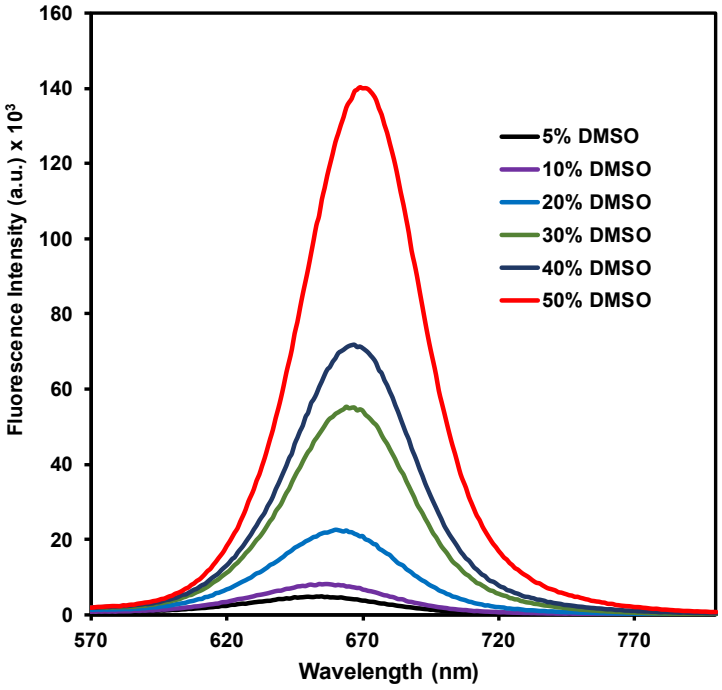

Figure **S2**. Emission spectra of **DCI-OH** (5.0 μM) in phosphate buffered saline (20 mM) of pH 7.4 in the presence of varying percentage of DMSO (5% to 50%).

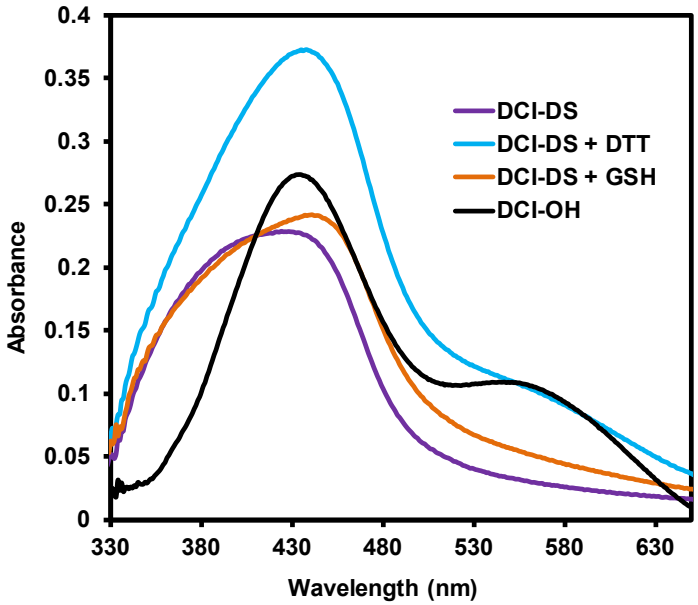

Figure **S3**. Absorption spectra of **DCI-DS** (5.0 μM) in the presence and absence of different thiols (200 μM) in phosphate buffered saline (20 mM) of pH 7.4 in the presence of 50% DMSO after incubating for 60 min. An absorption spectrum of **DCI-OH** was included for comparison.

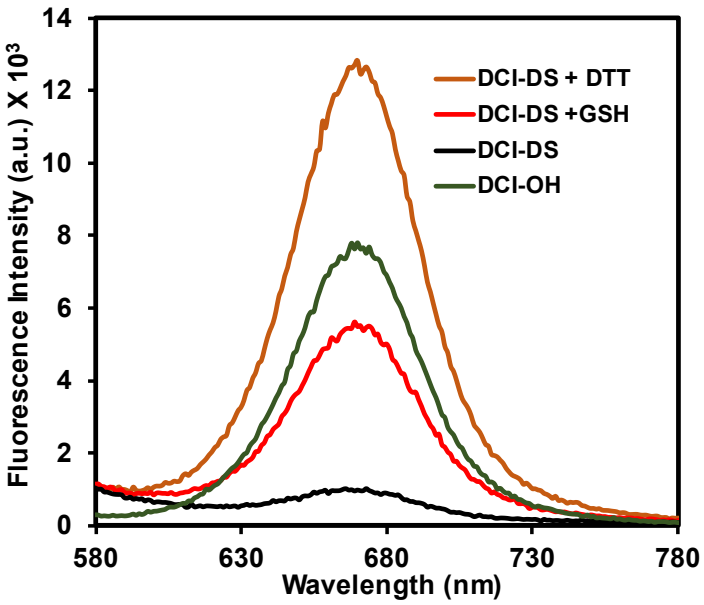

Figure **S4**. Emission spectra of **DCI-DS** (5.0 μM) in the presence and absence of different thiols (200 μM) in phosphate buffered saline (20 mM) of pH 7.4 in the presence of 50% DMSO (after incubating for 60 min) along with the emission spectrum of **DCI-OH.** λ_{ex} = 550 nm; λ_{em} = 570 - 800 nm; slit width 5/5 nm.

Table **S1**. Summary of the relative distribution of tetrasulfide and pentasulfide with respect to trisulfide as observed in ^{1}H NMR spectra (integration). The integration of the peak of –$(S)_n$-C**H$_2$**- group corresponding to the trisulfide was considered as 2.00.

S_8 : $Na_2S.9H_2O$	**Trisulfide : Tetrasulfide : Pentasulfide**
1 : 3	2.00 : 1.22 : 0.76
1 : 4	2.00 : 0.32 : 0.21
1 : 5	2.00 : 0.33
1 : 6	2.00 : 0.32 : 0.23
1 : 10	2.00 : 0.41

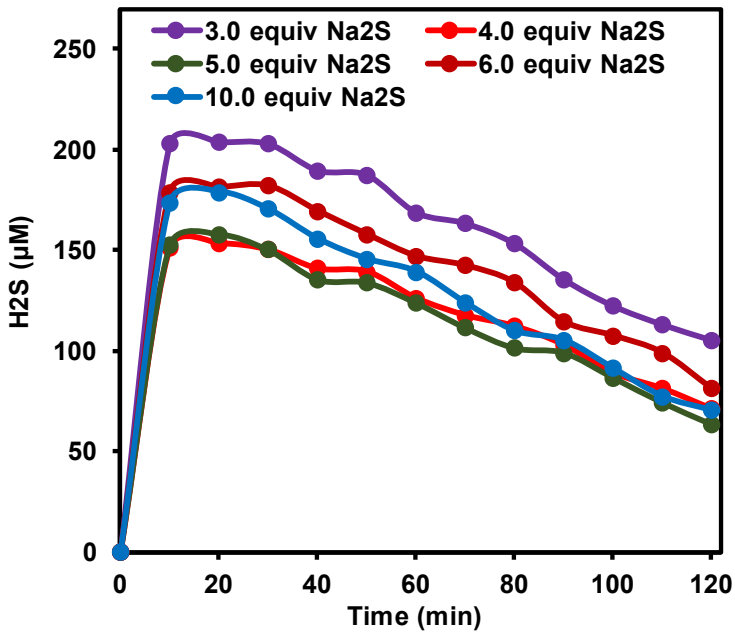

Figure **S5**. Comparative H_2S release profile of the different batches of polysulfide alcohols **6** (25 µg/mL) in the presence of DTT (1 mM) over 120 min. The batches were obtained upon the variation of the relative ratio of sulfur powder to $Na_2S.9H_2O$ during their synthesis.

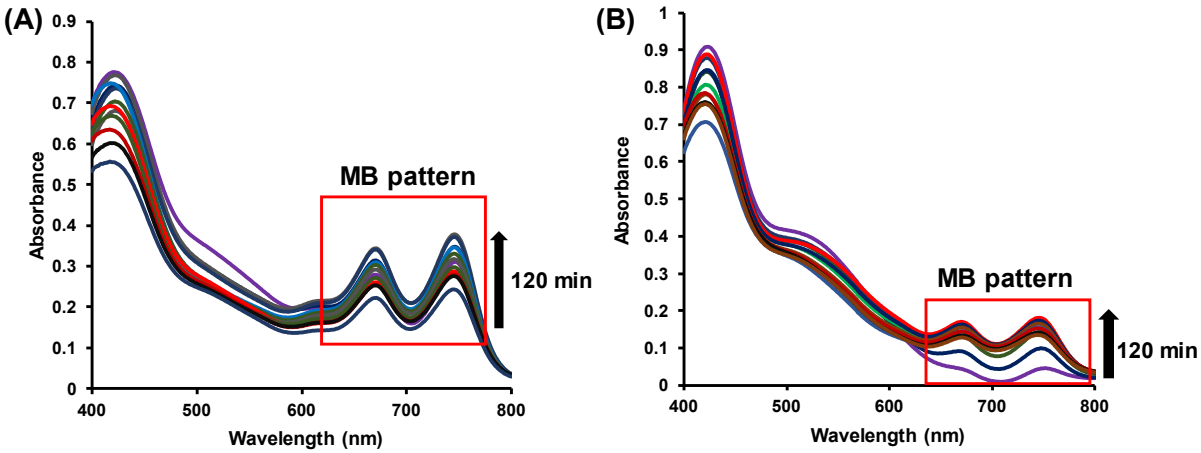

Figure **S6**. Time-dependent H_2S release profile of **DCI-PS** (25 µg/mL) in the presence of (A) DTT (1 mM) and (B) GSH (1 mM) over 120 min using MB assay. The representative methylene blue formation pattern was observed at 620-800 nm region.

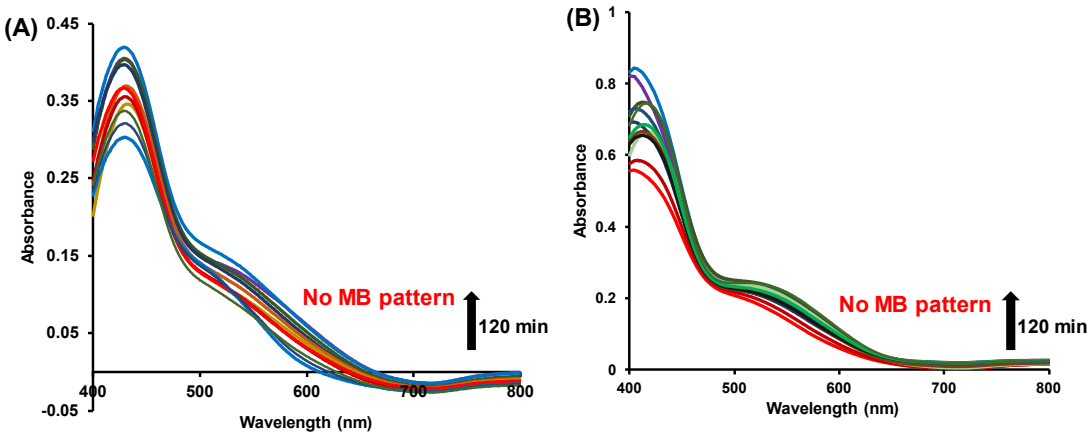

Figure **S7**. Time-dependent H_2S release profile of **DCI-DS** (25 μM) in the presence of (A) DTT (1 mM) and (B) GSH (1 mM) over 120 min using MB assay. The formation of methylene blue was not observed in the presence of both the thiols.

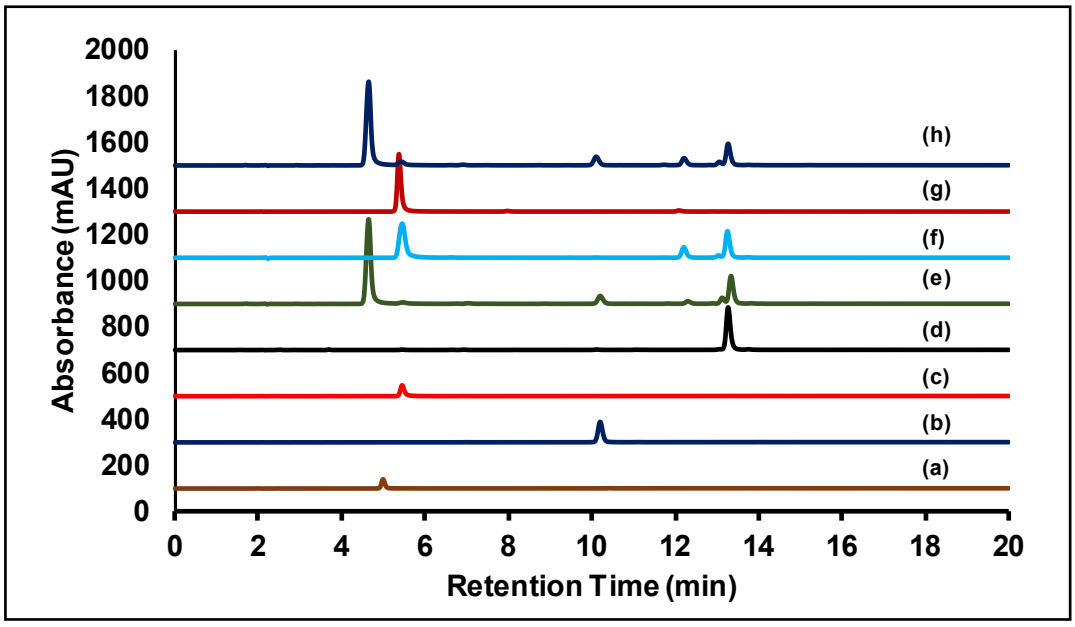

Figure **S8**. HPLC chromatogram (0 - 20 min) for the reaction of **DCI-DS** with PhSH. Lines: (a) PhSH; (b) PhSSPh; (c) **DCI-OH**; (d) **DCI-DS**; (e) **DCI-DS** + PhSH 1:1@254 nm; (f) **DCI-DS** + PhSH 1:1@433 nm; (g) **DCI-DS** + PhSH 1:5@ 433 nm; (h) **DCI-DS** + PhSH 1:5@ 254 nm. The chromatograms for the reaction mixtures were recorded after 60 min of incubation.

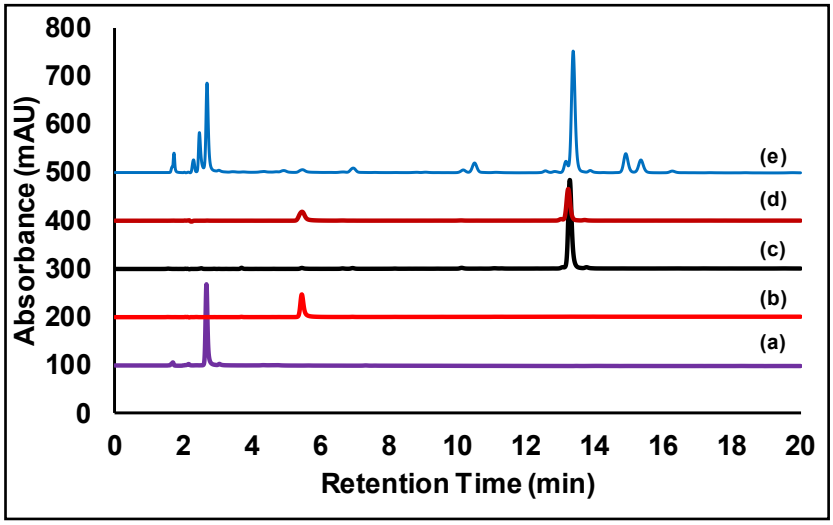

Figure **S9**. HPLC chromatogram (0-20 min) for the reaction of **DCI-DS** with DTT. Lines: (a) DTT @230 nm; (b) **DCI-OH**; (c) **DCI-DS**; (d) **DCI-DS** + DTT 1:1@ 433 nm; (e) **DCI-DS** + DTT 1:1@ 230 nm. The chromatograms for the reaction mixtures were recorded after 60 min of incubation.

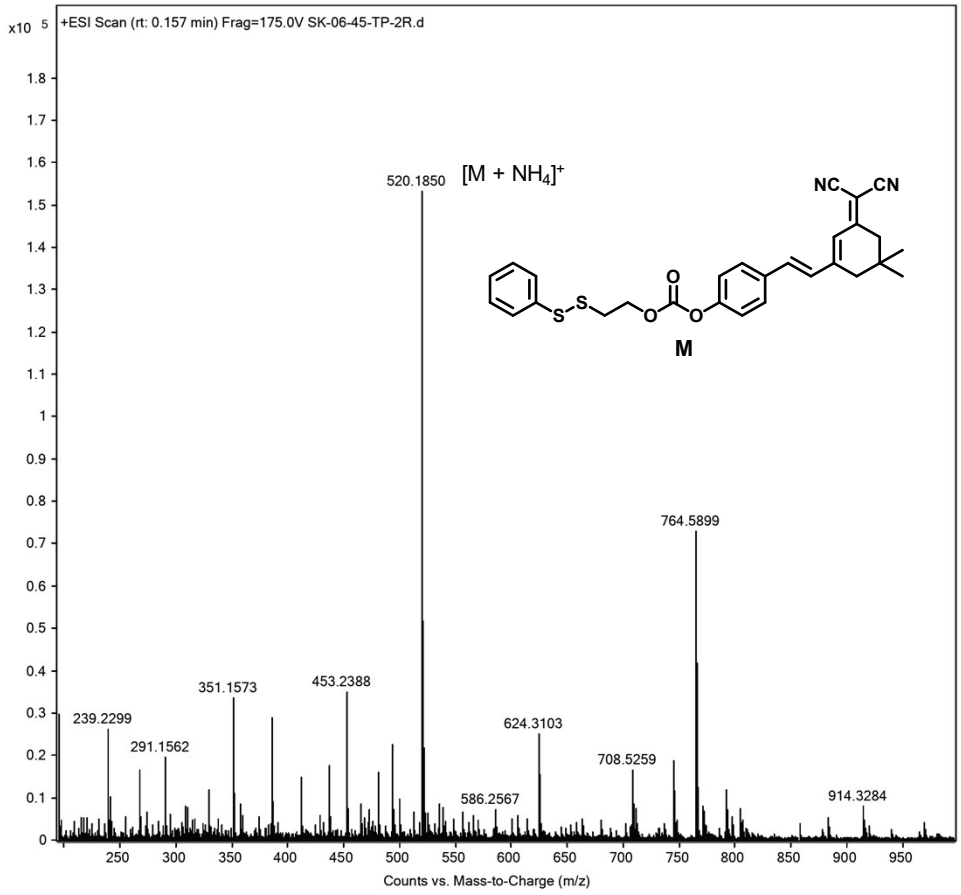

Figure **S10**. ESI-MS (+ve) spectrum of the reaction mixture of **DCI-DS** and PhSH (1:5) in acetonitrile, representing the formation of intermediate **8**. ESI-MS calcd. for $[M + NH_4]^+$ = 520.1729, observed $[M + NH_4]^+$ = 520.1850.

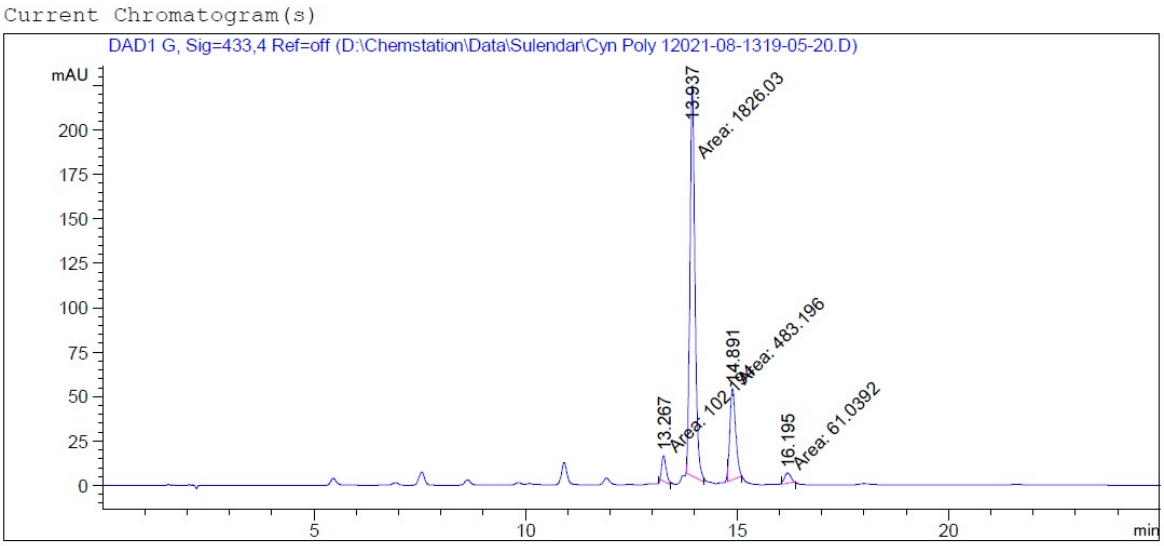

Figure **S11**. HPLC chromatogram of **DCI-PS** showing the presence of disulfide (4.1%), trisulfide (73.8%), tetrasulfide (19.5%) and pentasulfide (2.4%).

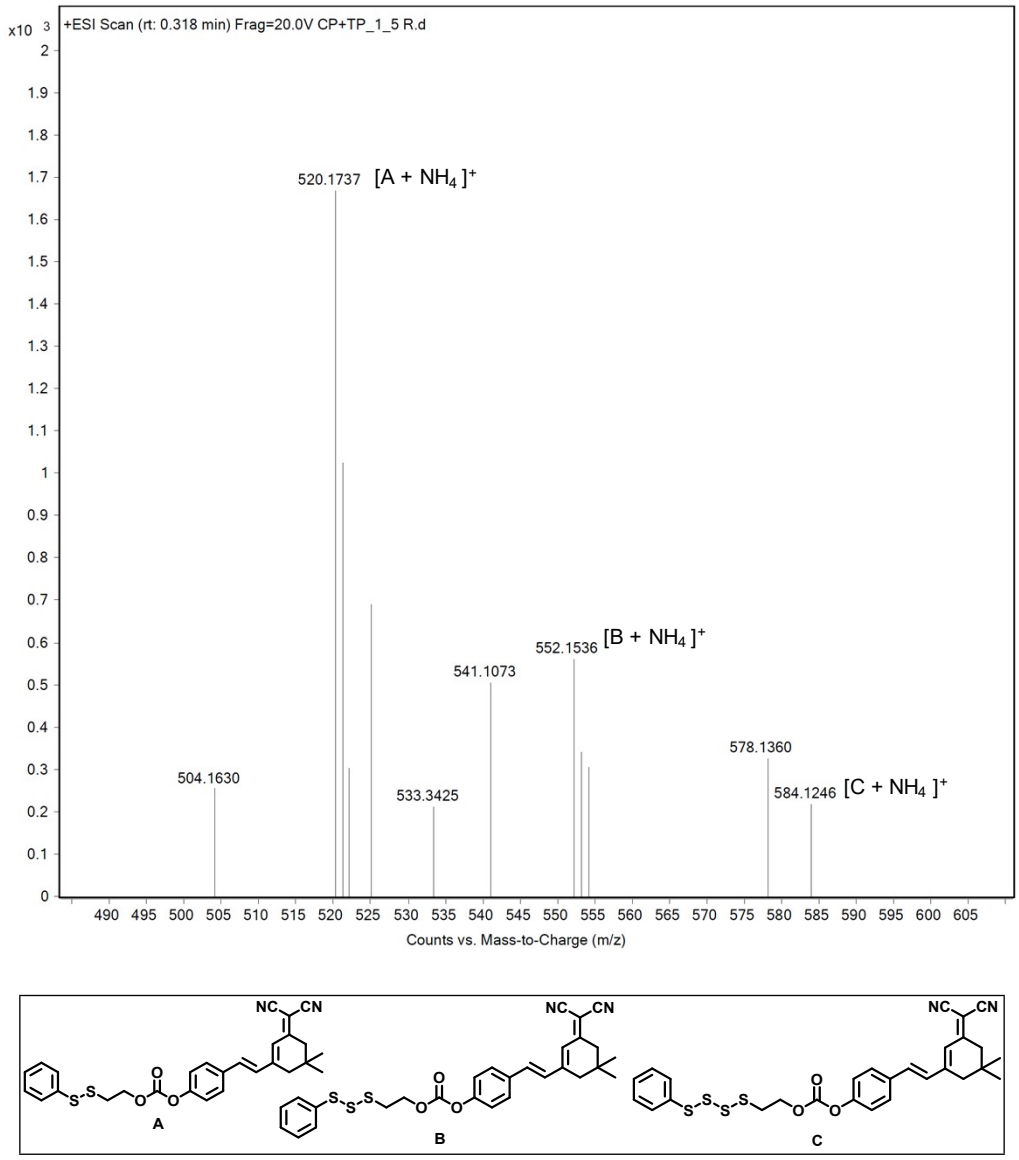

Figure **S12**. ESI-MS (+ve) spectrum of the reaction mixture of **DCI-PS** and **PhSH** (1:5) in acetonitrile. The formation of unsymmetrical polysulfide intermediates was detected in mass spectrometric condition. The structures and the detailed mass data of the intermediates A, B and C: ESI-MS calcd. for $C_{28}H_{26}N_2O_3S_2$ $[A + NH_4]^+$ = 520.1729, obs. $[A + NH_4]^+$ = 520.1737, ESI-MS calcd. for $C_{28}H_{26}N_2O_3S_3$ $[B + NH_4]^+$ = 552.1449, obs. $[B + NH_4]^+$ = 552.1536, calcd. for $C_{28}H_{26}N_2O_3S_4$ $[C + NH_4]^+$ = 584.1170, obs. $[C + NH_4]^+$ = 584.1246.

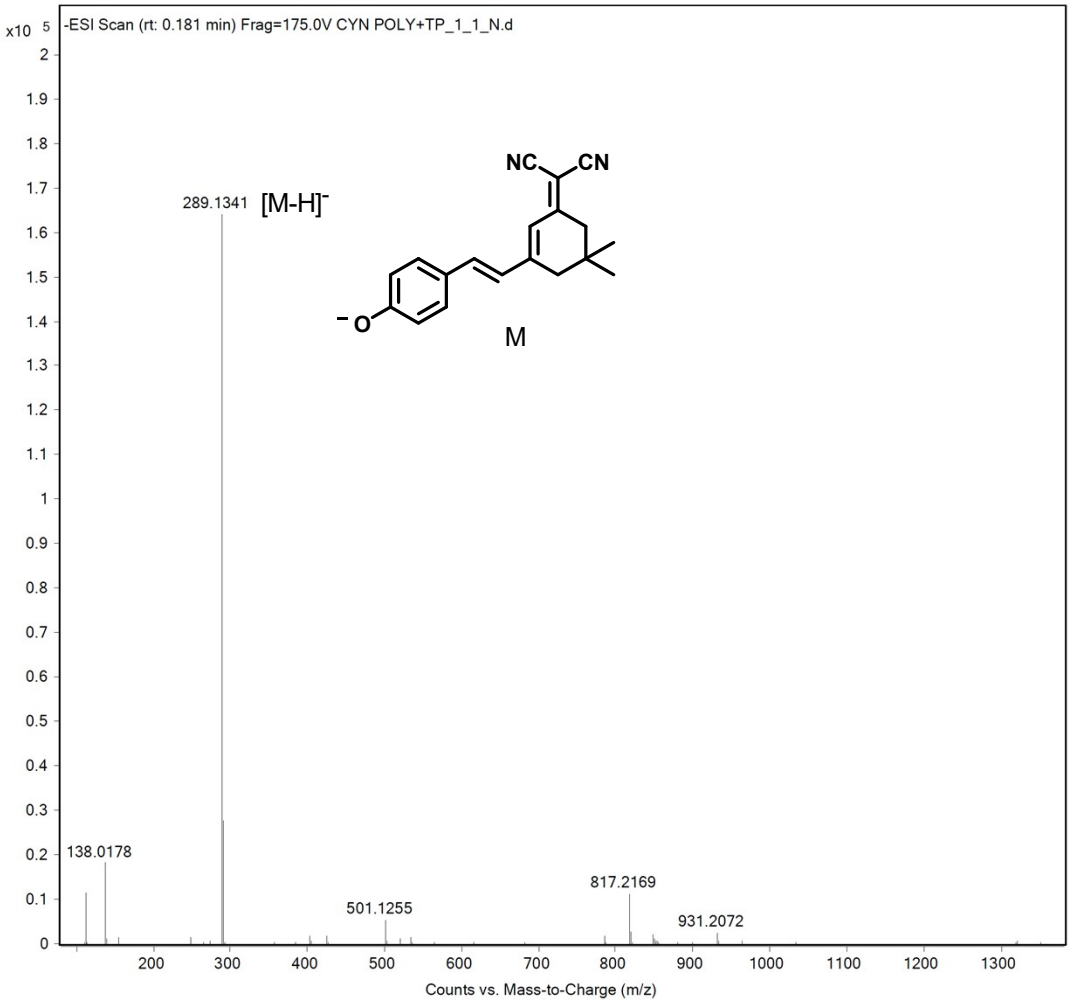

Figure **S13**. ESI-MS (-ve) spectrum of the reaction mixture of **DCI-PS** and **PhSH** (1:5) in acetonitrile. Formation of the released fluorophore **DCI-OH** was detected under the mass spectrometric condition. ESI-MS (-ve) calcd. for $C_{19}H_{18}N_2O$ $[M - H]^-$: 289.1346, obs. $[M - H]^-$: 289.1341.

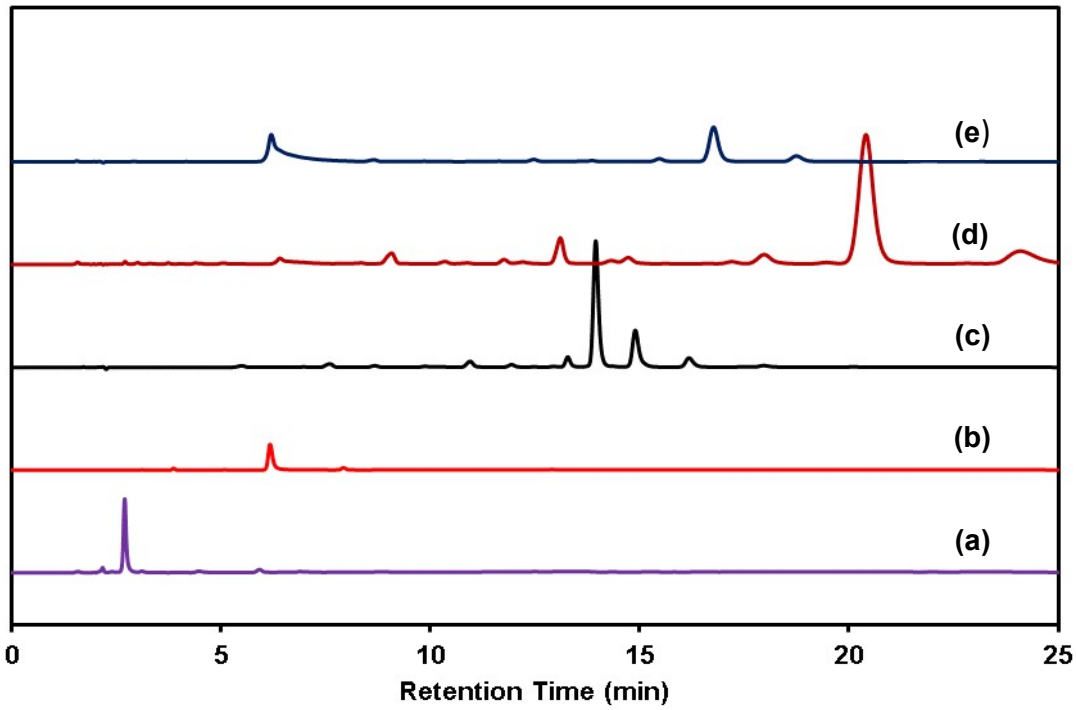

Figure **S14**. HPLC chromatogram (0-25 min) for the reaction of **DCI-PS** with DTT. Lines: (a) DTT @ 230 nm; (b) **DCI-OH**; (c) **DCI-PS**; (d) **DCI-PS** + DTT 1:1 @ 230 nm; (e) **DCI-PS** + DTT 1:1 @ 433 nm The chromatograms for the reaction mixtures were recorded after 60 min of incubation.

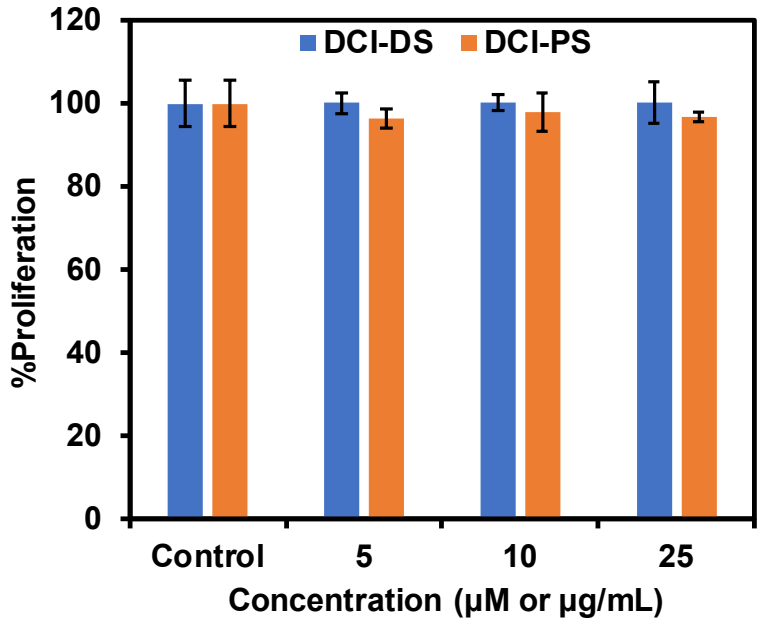

Figure **S15**. Dose-dependent anti-proliferative activities of **DCI-DS** (µM) and **DCI-PS** (µg/mL) in MDA-MB-231 cell line over an incubation over 48 h.

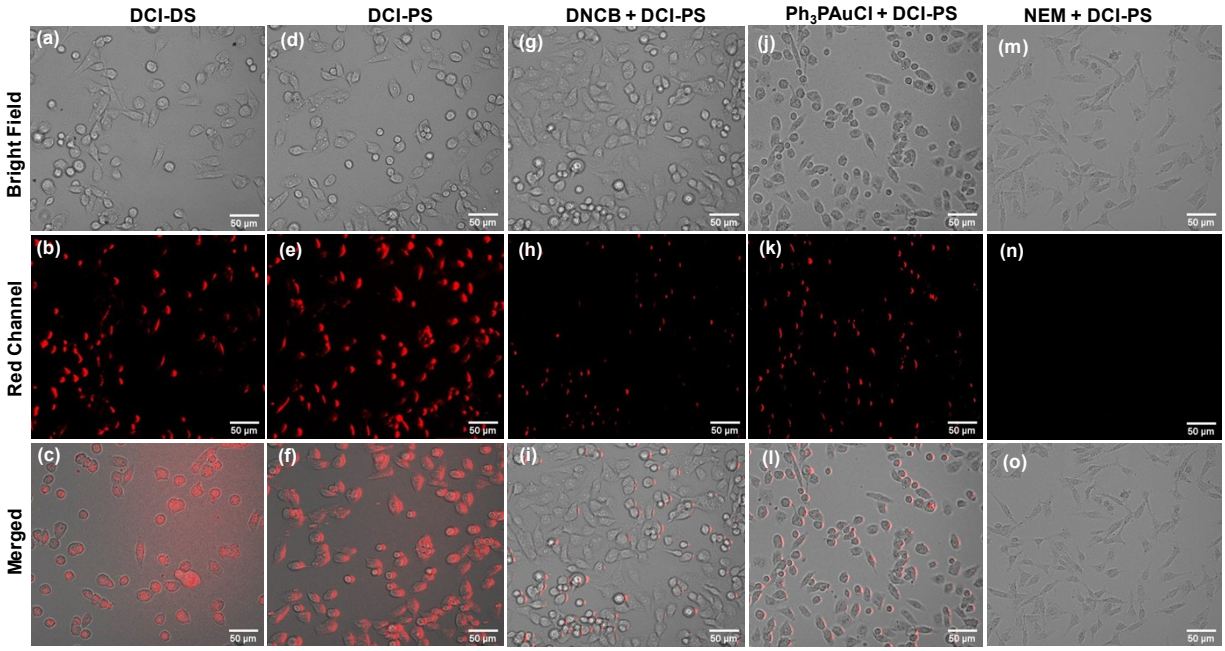

Figure **S16**. Fluorescence microscopic images (bright field, red channel and merged) of MDA-MB-231 cells for the bio-analytes-triggered release of **DCI-OH** from **DCI-DS** (a-c) and **DCI-PS** (d-f) and the inhibition of TrxR-triggered release of **DCI-OH** from **DCI-PS** using DNCB (10 μM, g-i) and PPh_3AuCl (2 μM, j-l). The endogenous thiol/selenol was quenched upon the pre-treatment of cells with NEM (2 mM, m-o). The scale bar represents 50 μm.

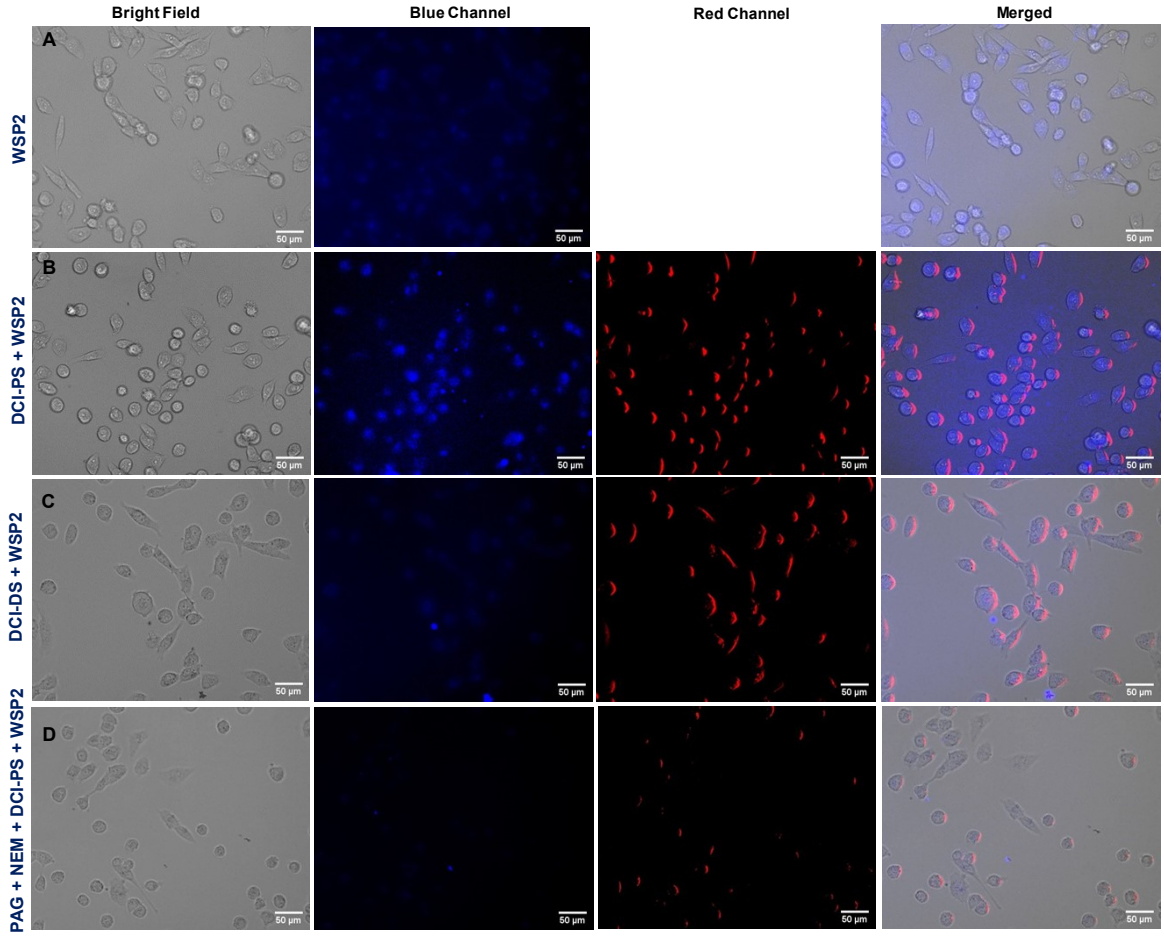

Figure **S17**. Fluorescence microscopic images (bright field, blue, red channels and merged) of MDA-MB-231 cells for the **WSP2**-mediated detection of (A) endogenous level of H_2S; (B) released H_2S from **DCI-PS** in the presence of bio-analytes; (C) released H_2S (if any) from **DCI-DS** in the presence of bio-analytes and (D) released H_2S from **DCI-PS** in the presence of bio-analytes upon the pre-incubation of cells with NEM (2 mM) and PAG (20 µM). The scale bar represents 50 µm.

Table **S2**. Summary of distances of various sulfur atoms of the ligands with the selenium center of TrxR1 at the active site and the list of proximal amino acid residues with which the ligands have non-bonding interactions.

Probes	**Binding Energy (ΔG_{bind}) (kcal/mol)**	**Distance of S atoms of ligands from the Se atom of Sec498 of TrxR (Å)**	**Amino acid residues involved in non-bonding interactions**
DCI-DS	-8.3	4.2, 4.5	K29, Y116, S404, W407, H472, G496
DCI-PS (n = 1, trisulfide)	-9.2	7.8, 8.1, 9.3	K33, K123, S404, F406, W407, L409, H472, V474, S483
DCI-PS (n =2, tetrasulfide)	-8.7	6.5, 7.6, 8.1, 8.1	K33, Y116, R351, W407, N419, H472, G499
DCI-PS (n = 3, pentasulfide)	-9.4	7.2, 7.4, 8.0, 8.4, 8.7	S22, K29, Y116, W407, L409, N418, V474, E477, Q494, G496

NMR (^{1}H, ^{13}C and Mass Spectrometric Data)

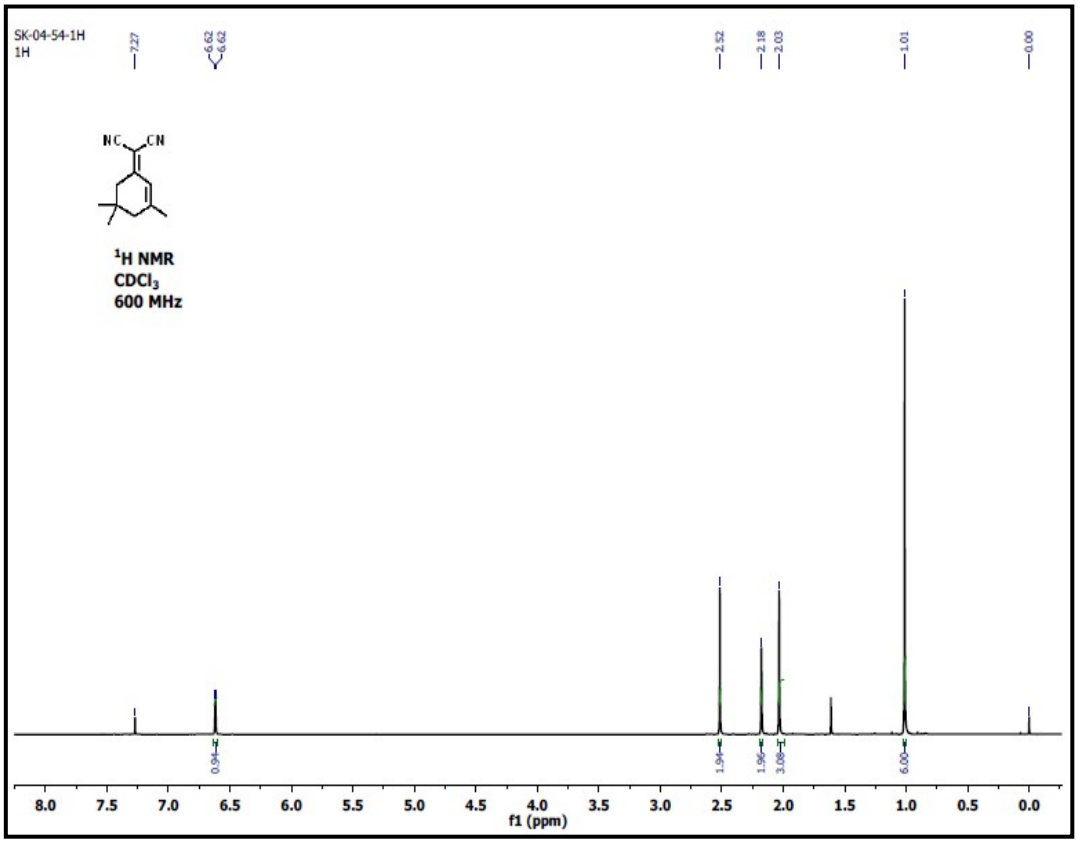

Figure **S18**. ^{1}H NMR (600 MHz) spectrum of compound **2** in $CDCl_3$.

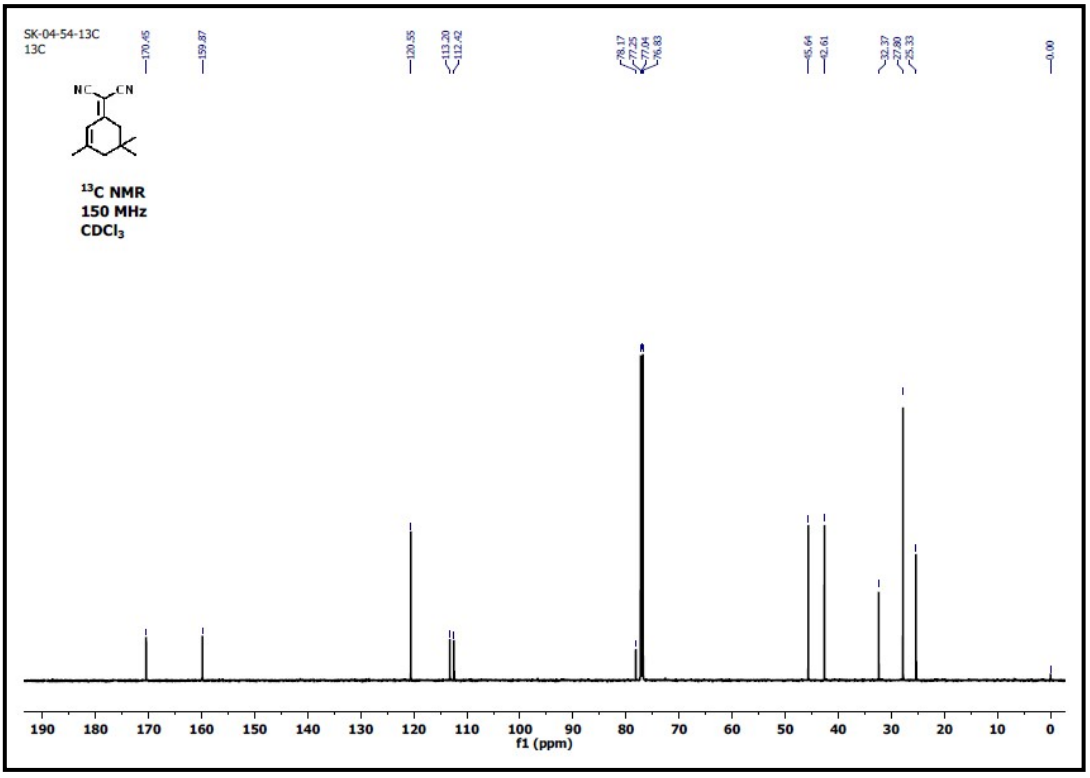

Figure **S19**. ^{13}C NMR (150 MHz) spectrum of compound **2** in $CDCl_3$.

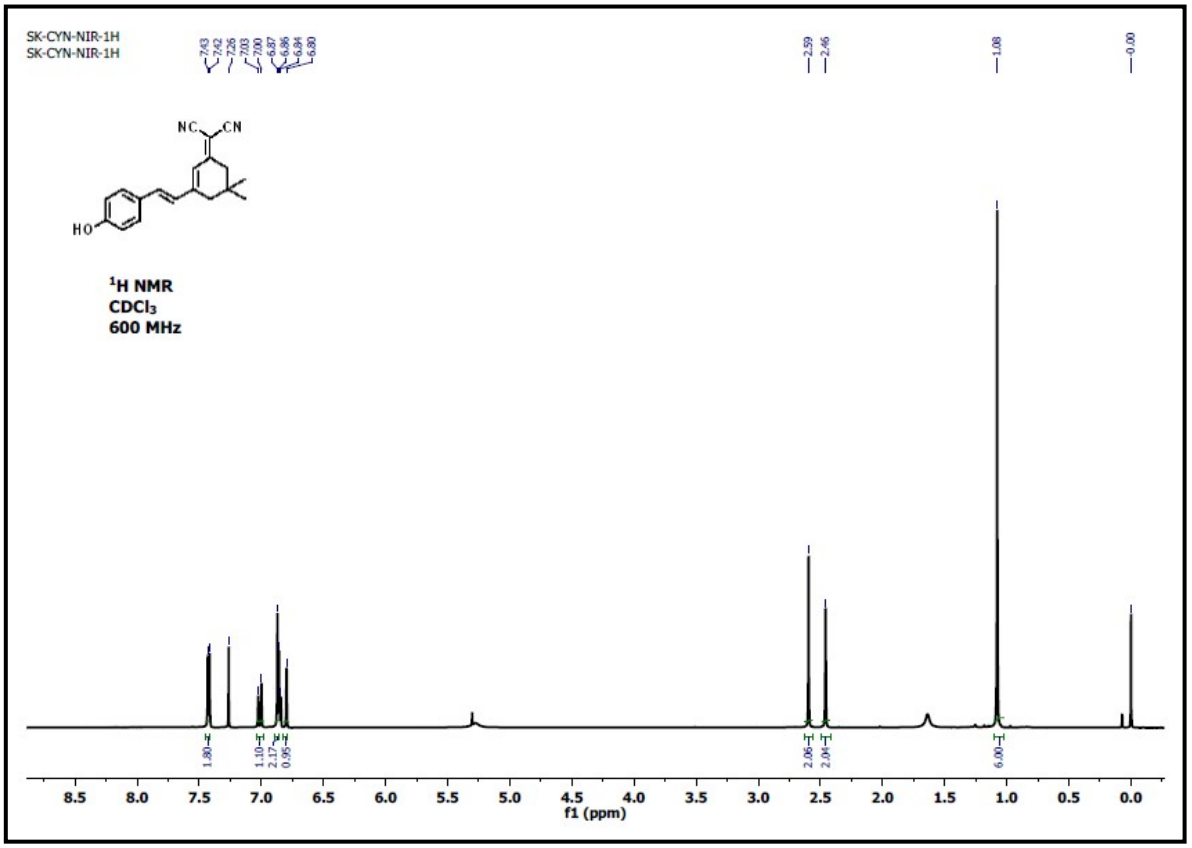

Figure **S20**. ^{1}H NMR (600 MHz) spectrum of **DCI-OH** in $CDCl_3$.

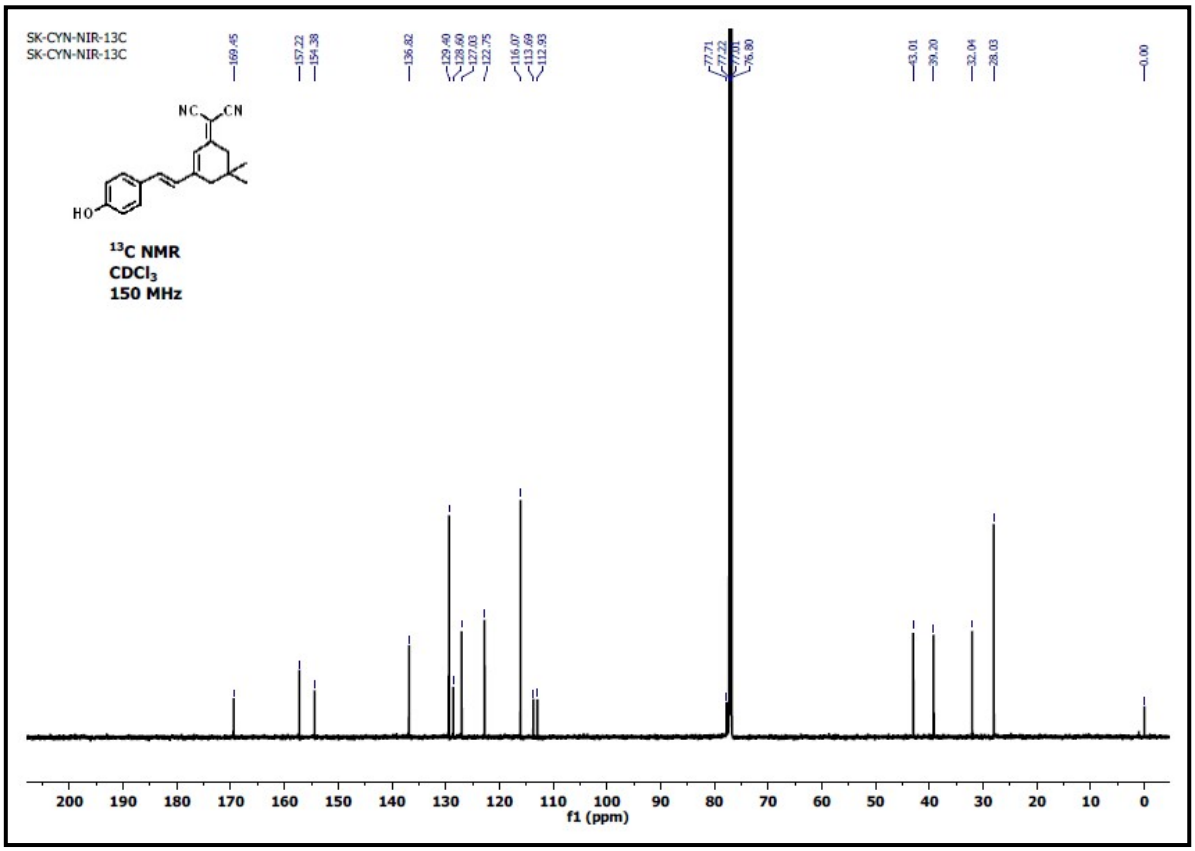

Figure **S21**. ^{13}C NMR (150 MHz) spectrum of **DCI-OH** in $CDCl_3$.

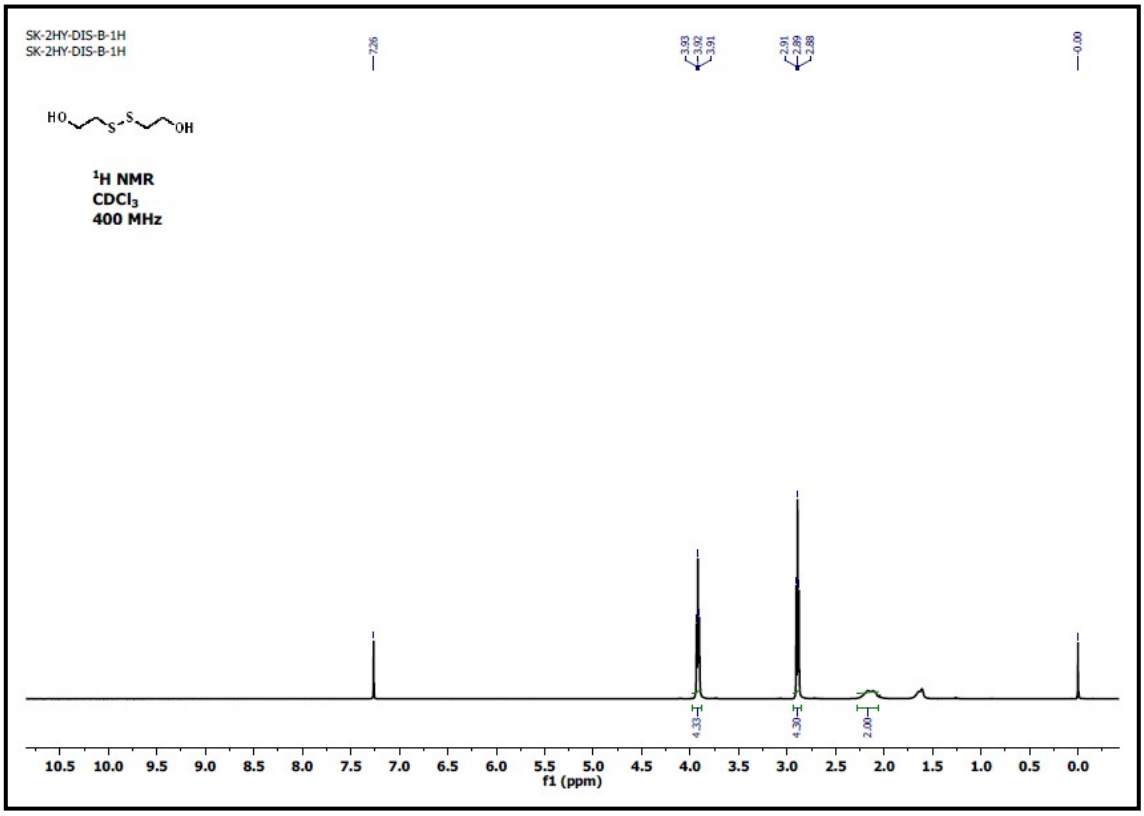

Figure **S22**. ^{1}H NMR (400 MHz) spectrum of compound **3** in $CDCl_3$.

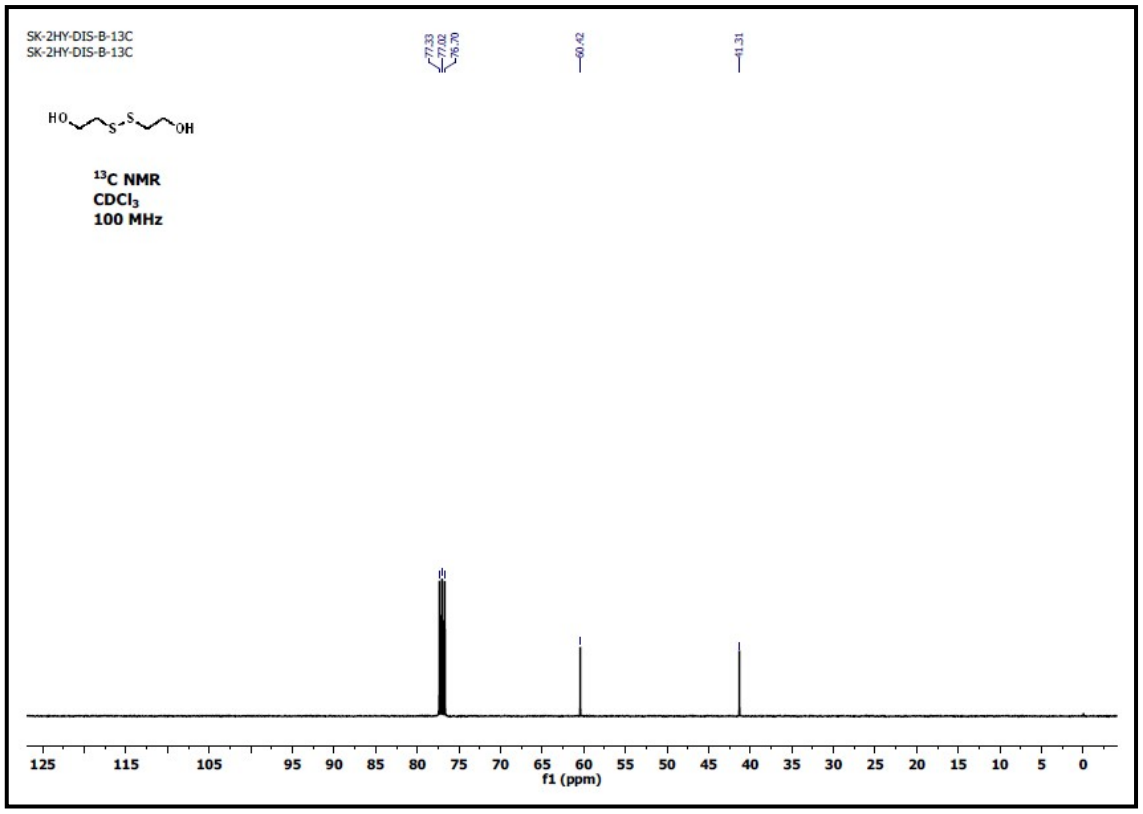

Figure **S23**. ^{13}C NMR (100 MHz) spectrum of compound **3** in $CDCl_3$.

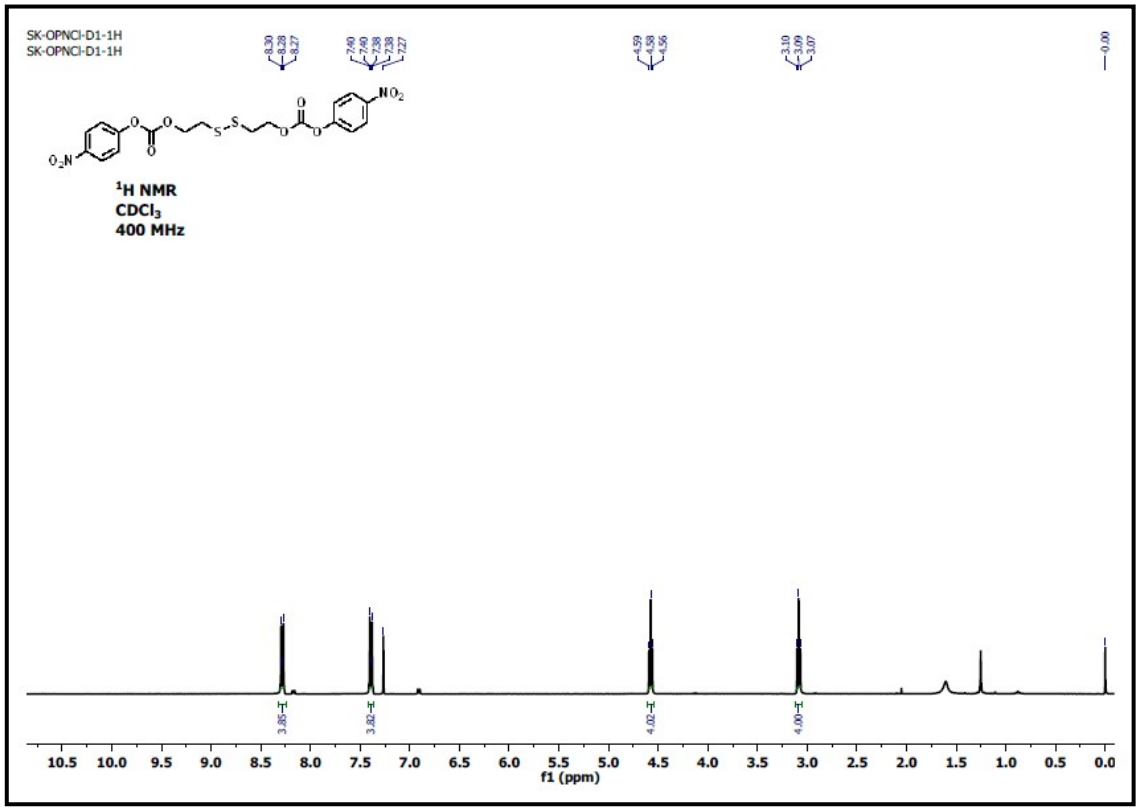

Figure **S24**. ^{1}H NMR (400 MHz) spectrum of compound **4** in $CDCl_3$.

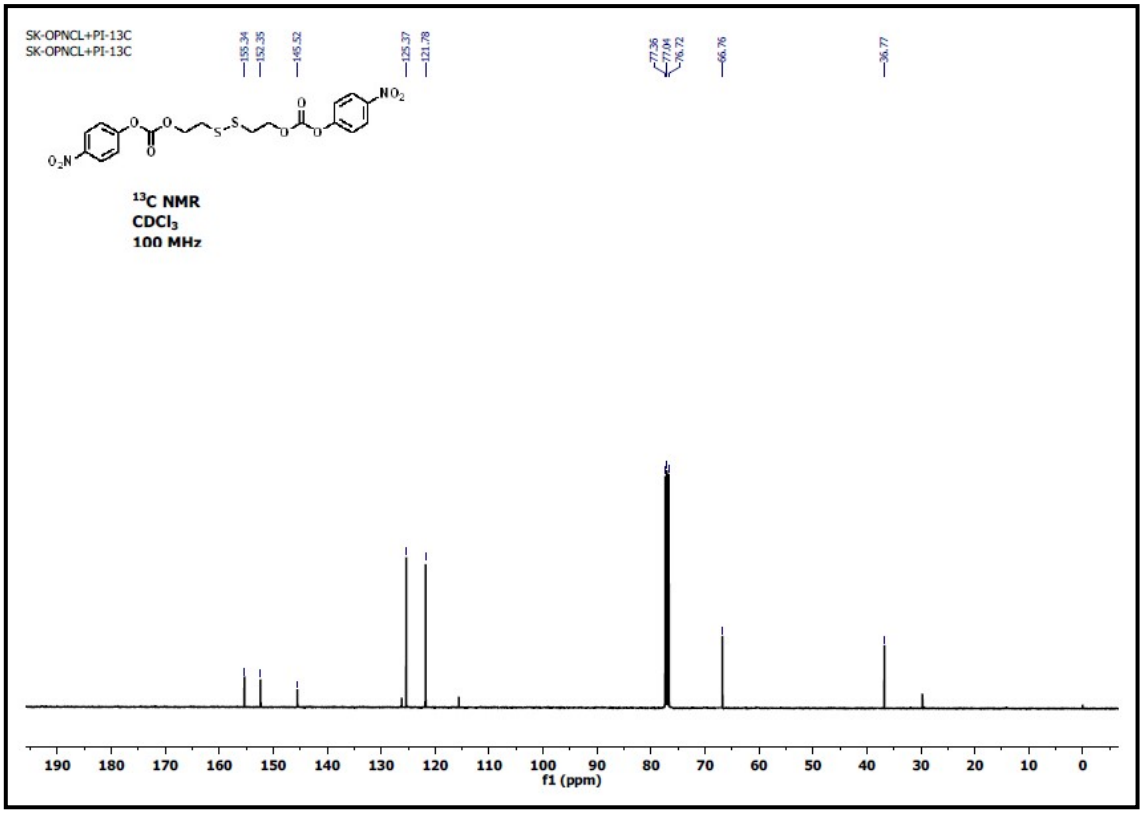

Figure **S25**. ^{13}C NMR (100 MHz) spectrum of compound **4** in $CDCl_3$.

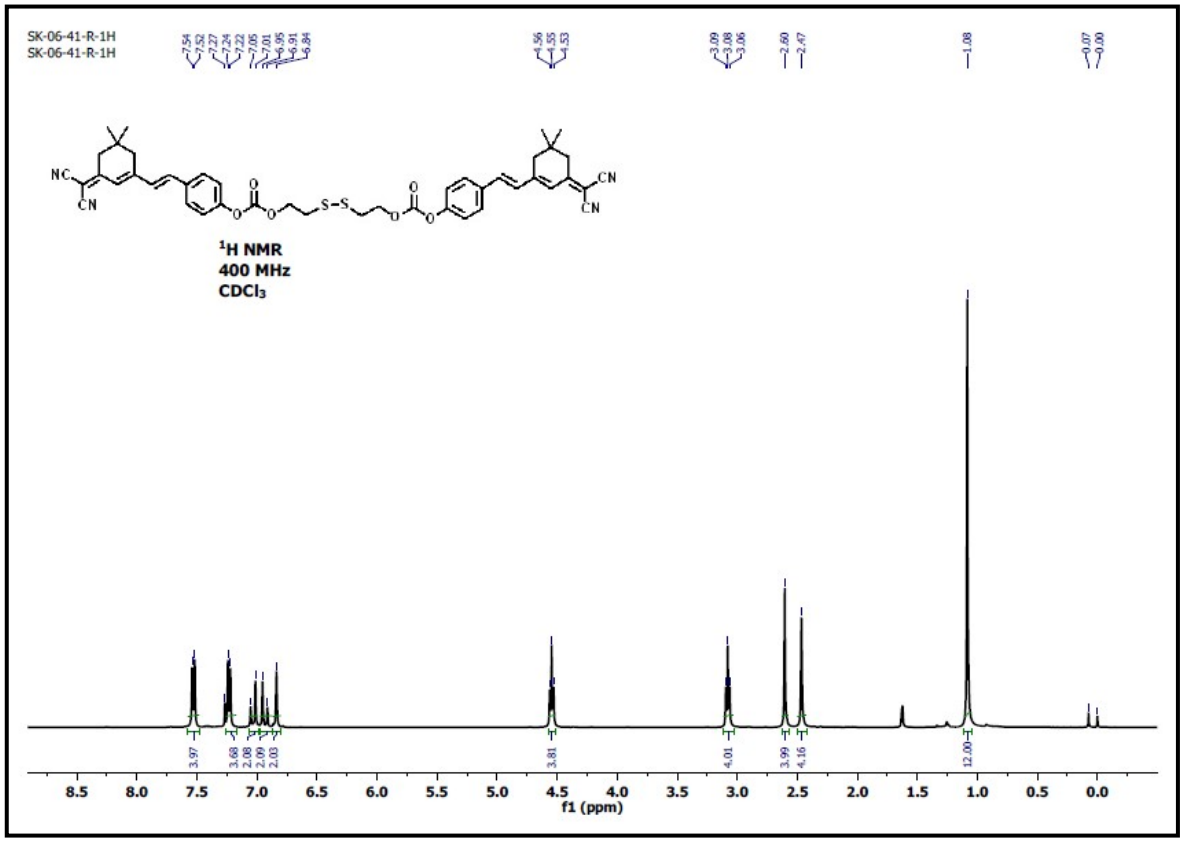

Figure **S26**. ^{1}H NMR (400 MHz) spectrum of **DCI-DS** in $CDCl_3$.

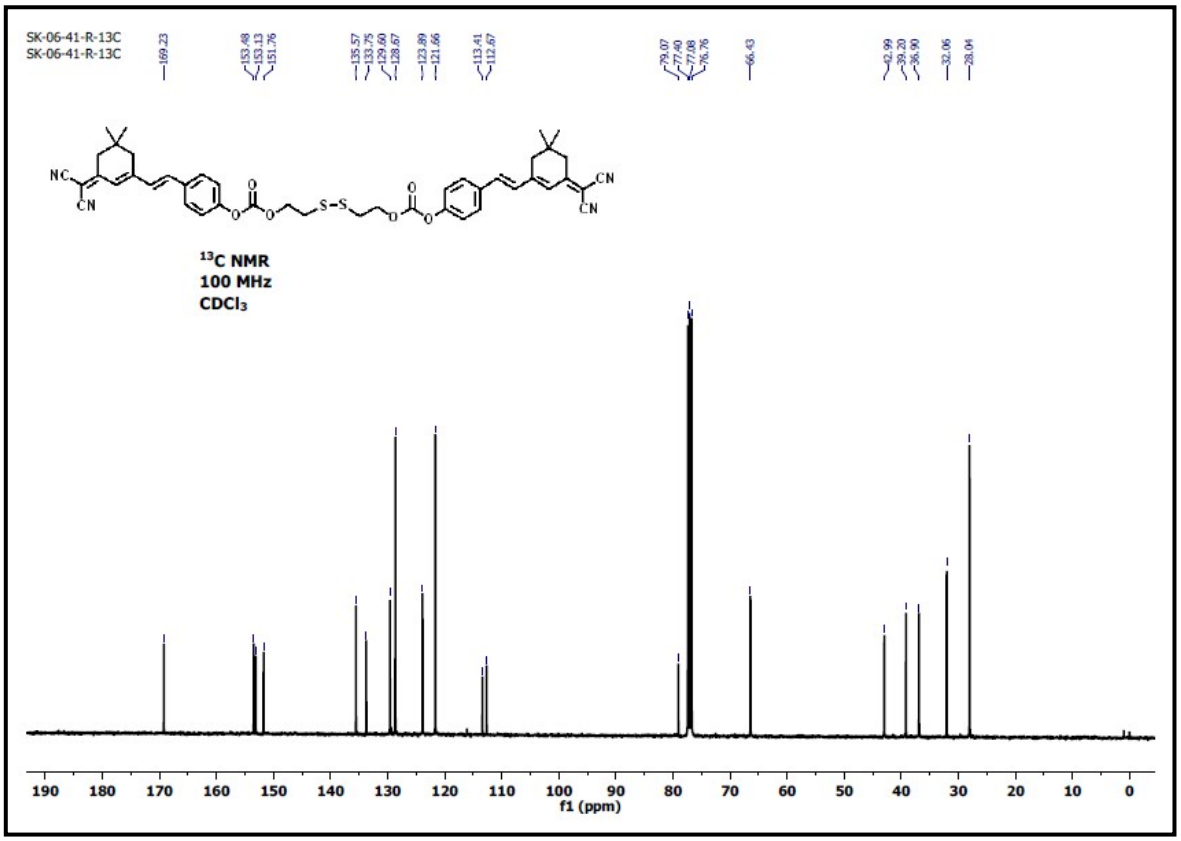

Figure **S27**. ^{13}C NMR (100 MHz) spectrum of **DCI-DS** in $CDCl_3$.

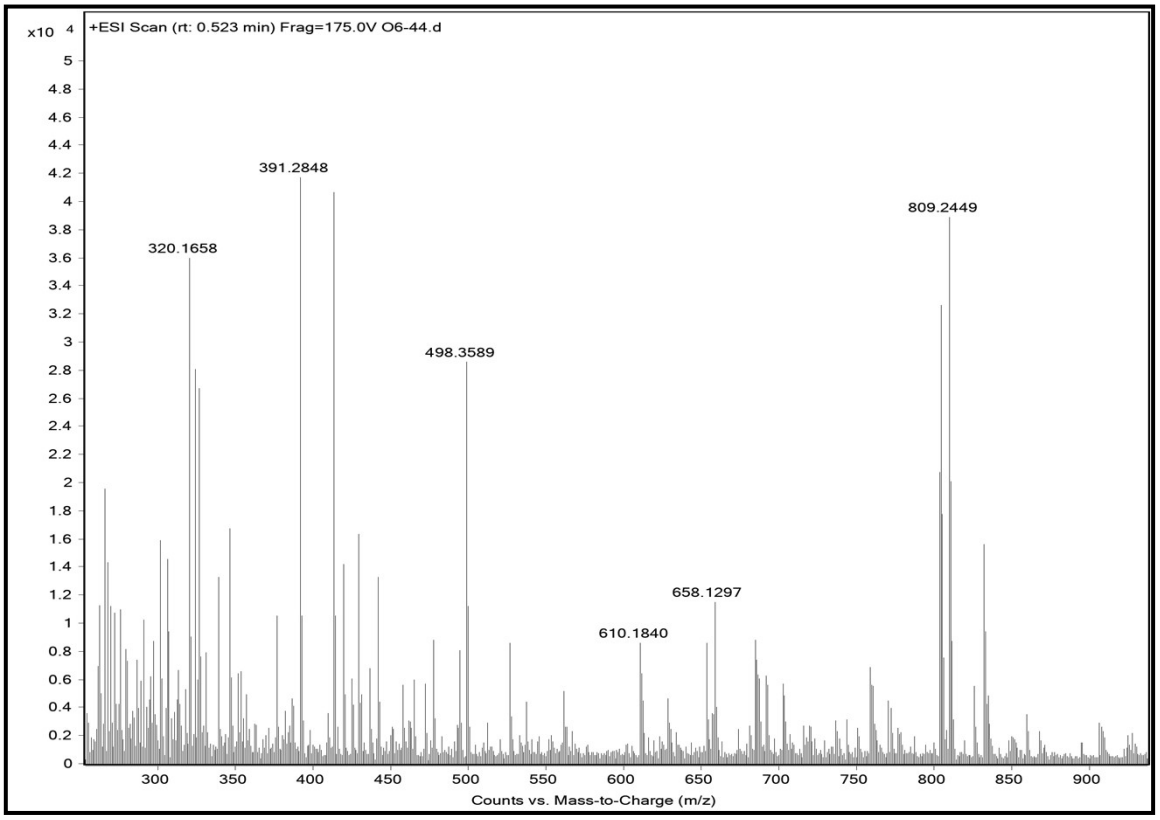

Figure **S28**. ESI-MS (+ ve) spectrum of **DCI-DS**. ESI-MS :m/z calcd. for $C_{44}H_{46}N_5O_6S_2$ $[M + Na]^+$ 809.2443 obs. $[M + Na]^+$:809.2449.

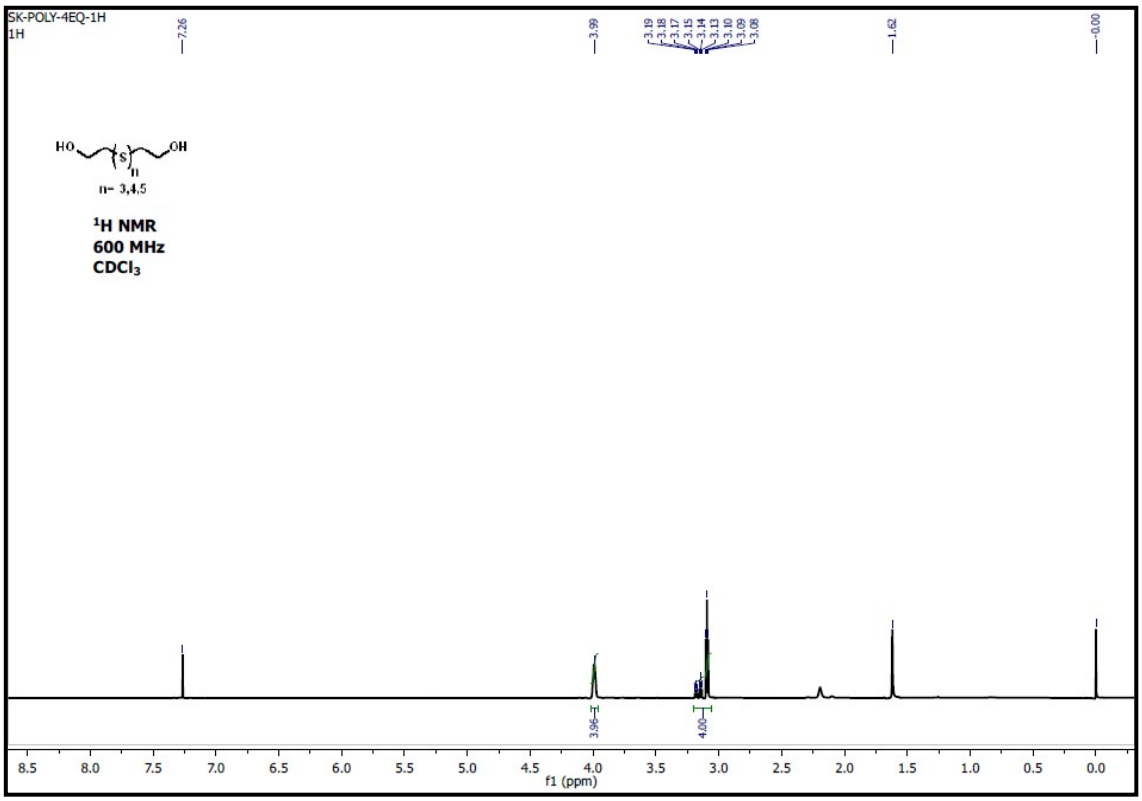

Figure **S29**. ^1H NMR (600 MHz) spectrum of compound **6** in $CDCl_3$.

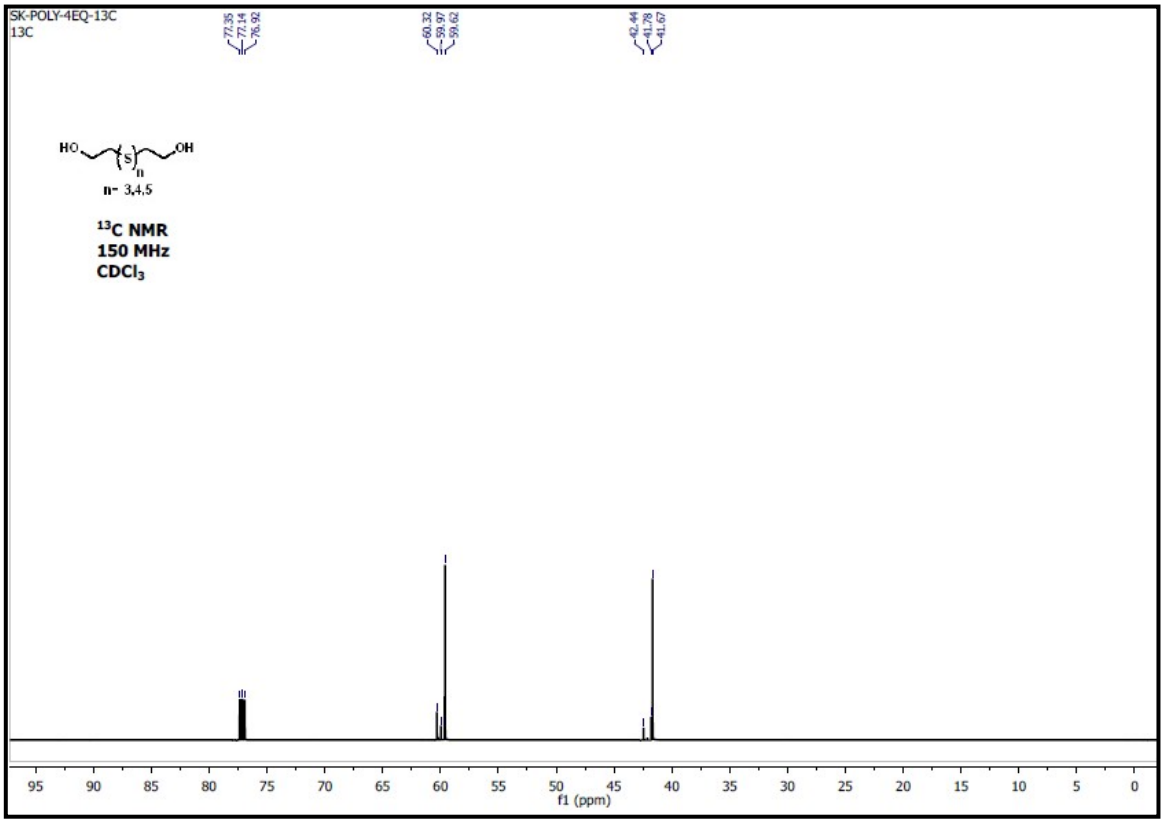

Figure **S30.** ^{13}C NMR (150 MHz) spectrum of compound **6** in $CDCl_3$

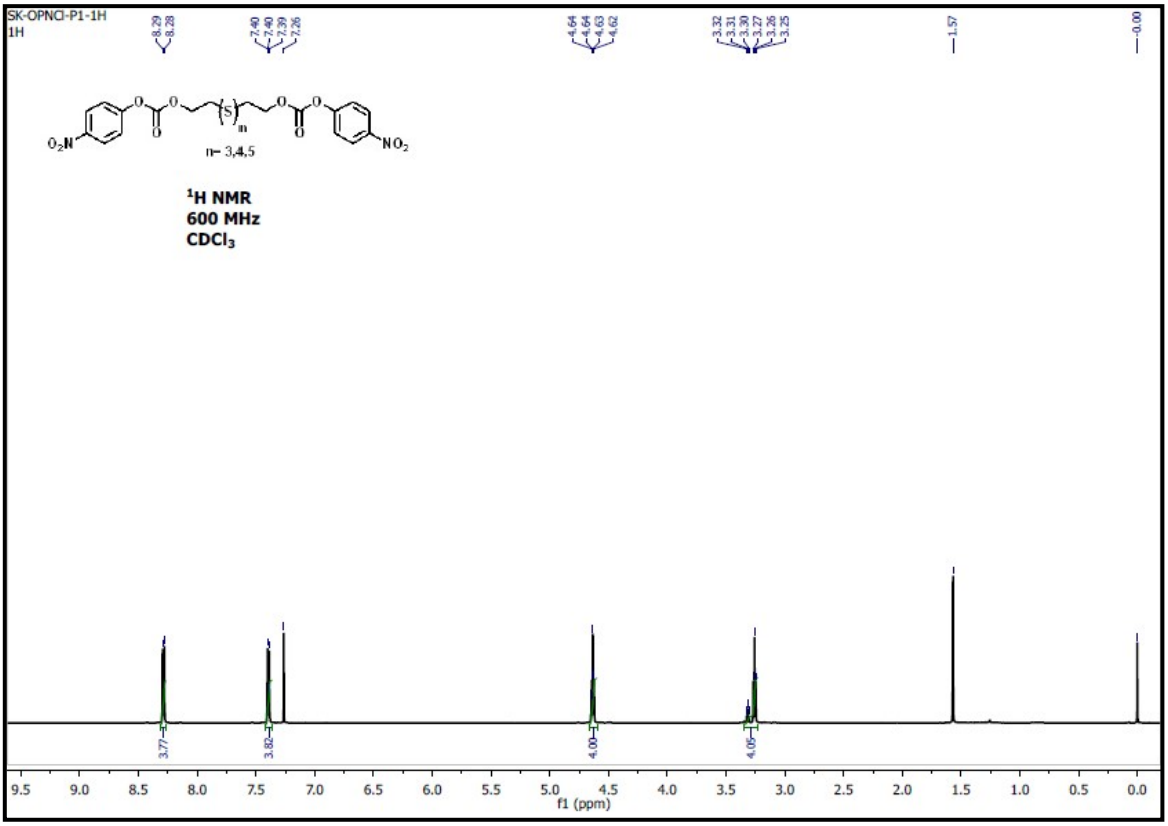

Figure **S31**. ^{1}H NMR (600 MHz) spectrum of compound **7** in $CDCl_3$.

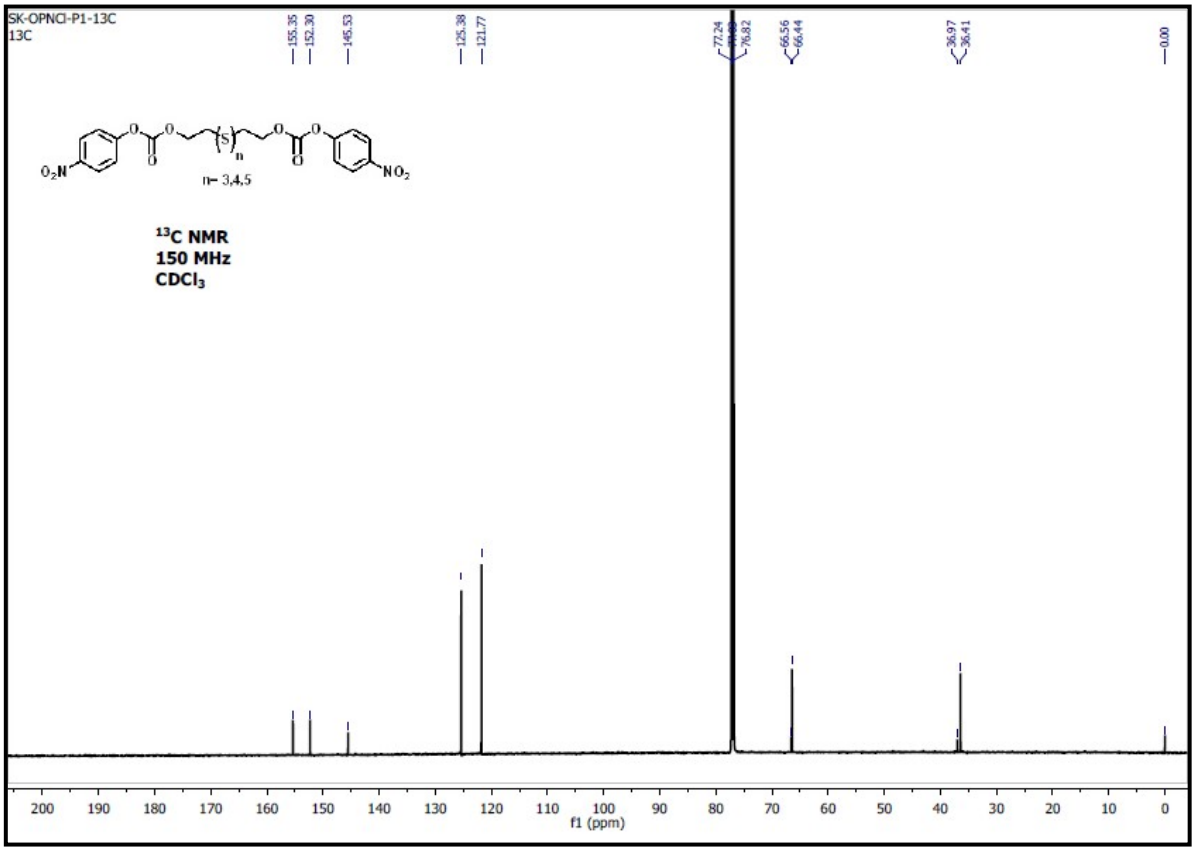

Figure **S32**. ^{13}C NMR (150 MHz) spectrum of compound **7** in $CDCl_3$.

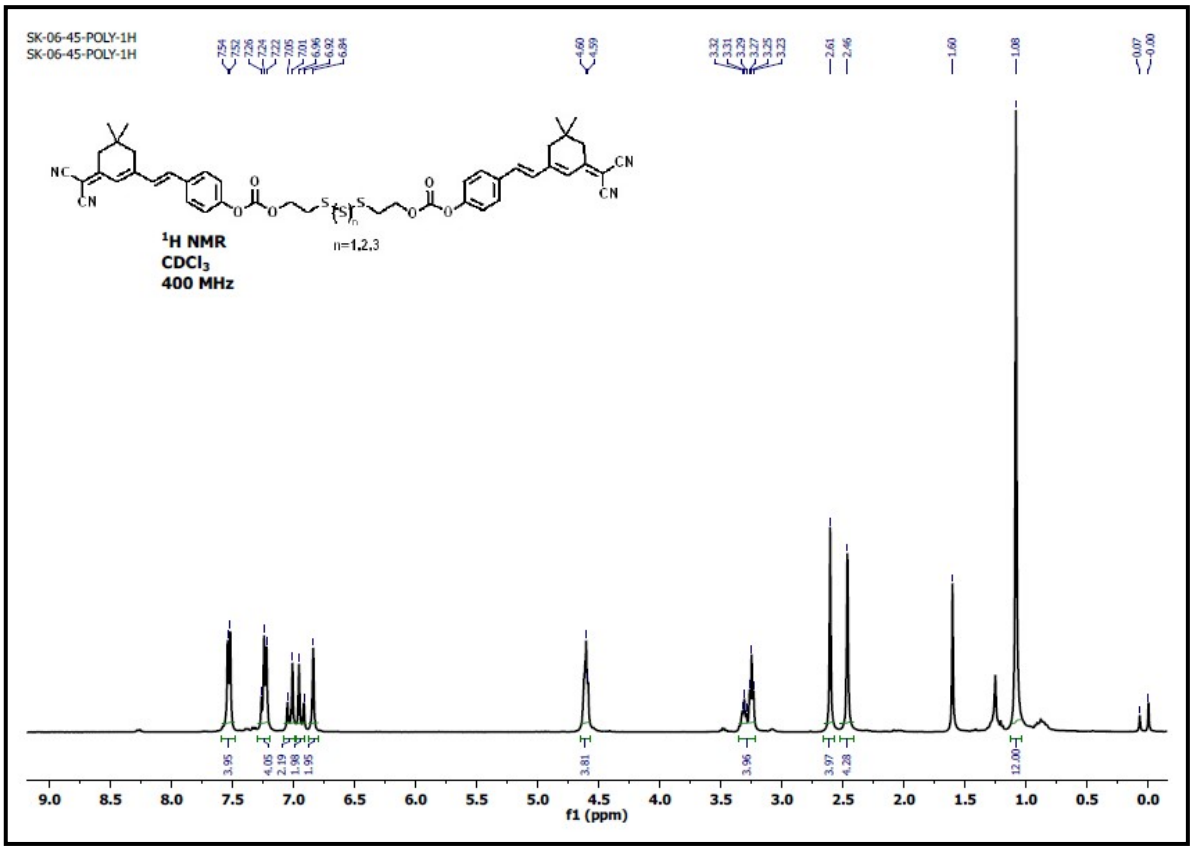

Figure **S33**. ^{1}H NMR (400 MHz) spectrum of **DCI-PS** in $CDCl_3$.

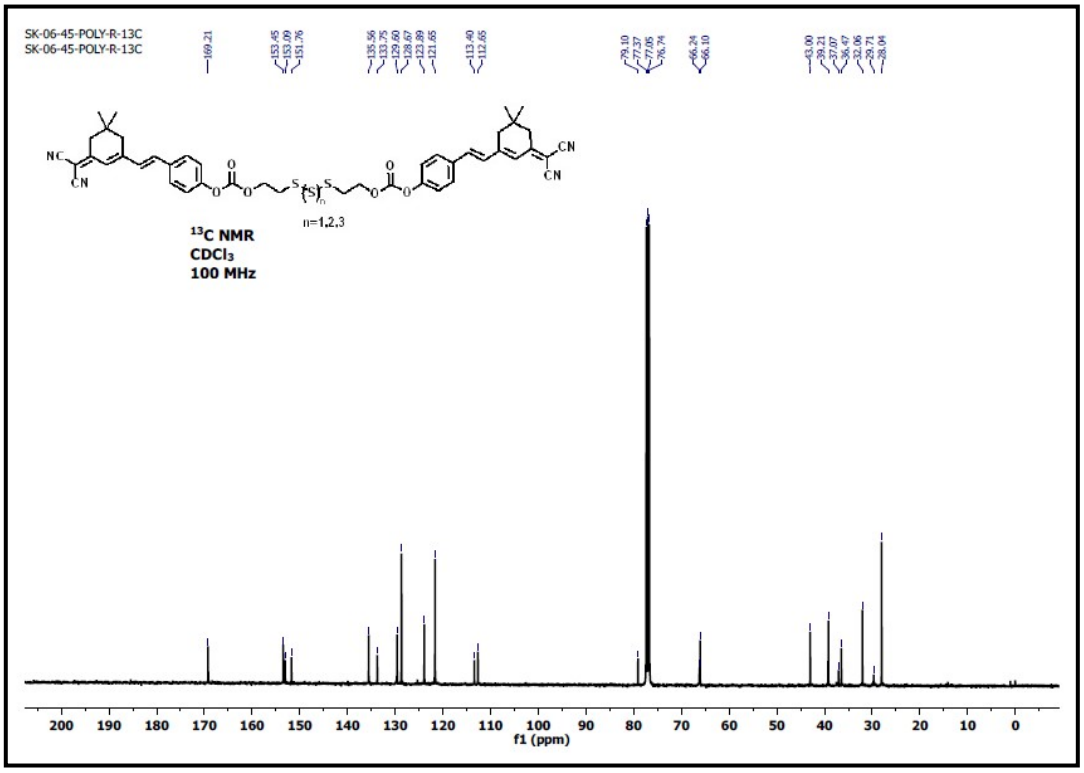

Figure **S34**. ^{13}C NMR (100 MHz) spectrum of **DCI-PS** in $CDCl_3$.

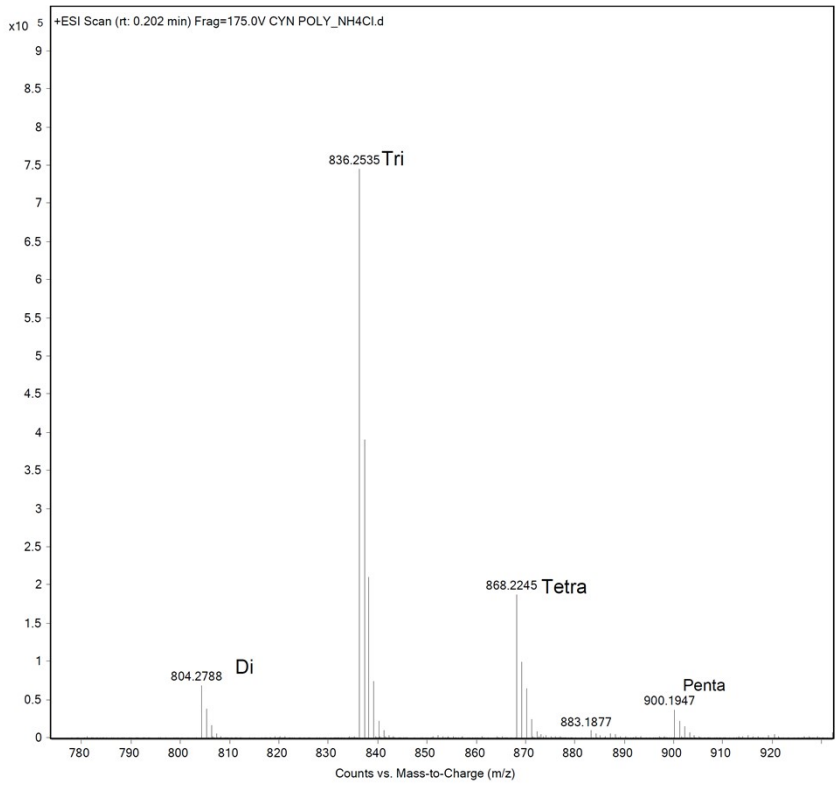

Figure **S35**. Zoomed ESI-MS (+ ve) of **DCI-PS** showing disulfide, trisulfide, tetrasulfide and pentasulfide forms of **DCI-PS**. ESI-MS *m/z* calcd. for $C_{44}H_{46}N_5O_6S_3$ $[M + NH_4]^+$ 836.2610 ,obs. 836.2535, *m/z* calcd. for $C_{44}H_{46}N_5O_6S_4$ $[M + NH_4]^+$: 868.2331, obs. 868.2245, *m/z* calcd. for $C_{44}H_{46}N_5O_6S_5$ $[M + NH_4]^+$: 900.2052 , obs. 900.1947.

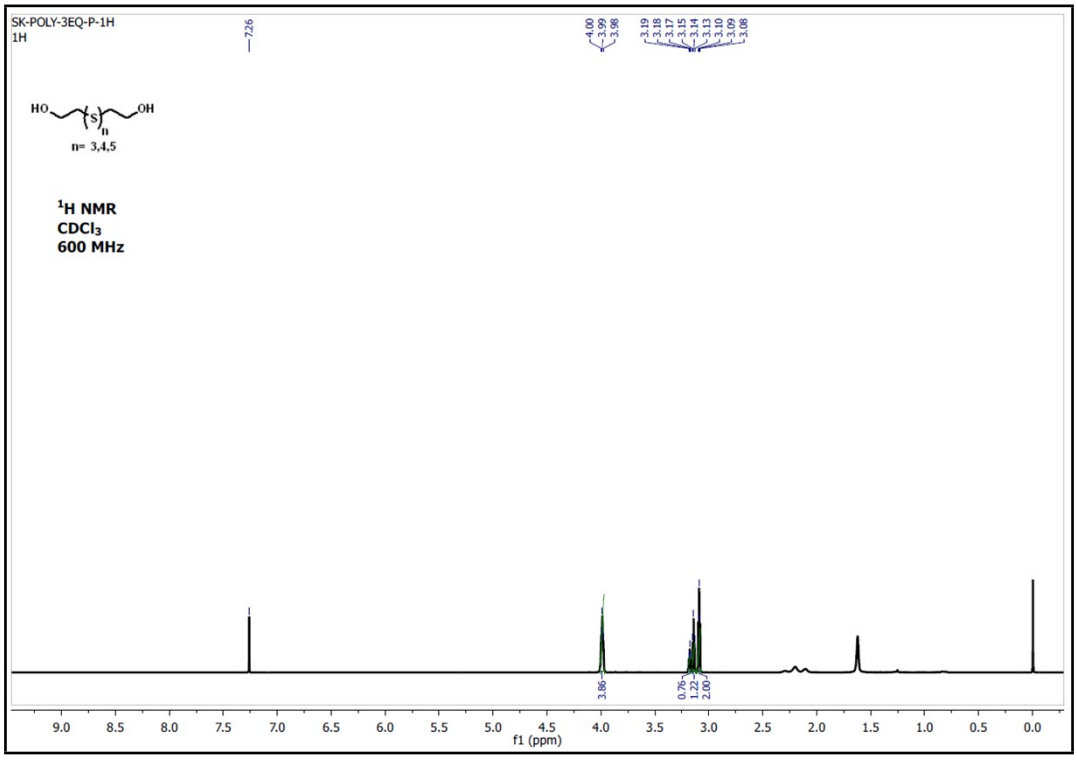

Figure **S36**. ^{1}H NMR (600 MHz) spectrum of compound **6** in $CDCl_3$.The compound was prepared using sulfur powder and $Na_2S.9H_2O$ in 1:3 ratio.

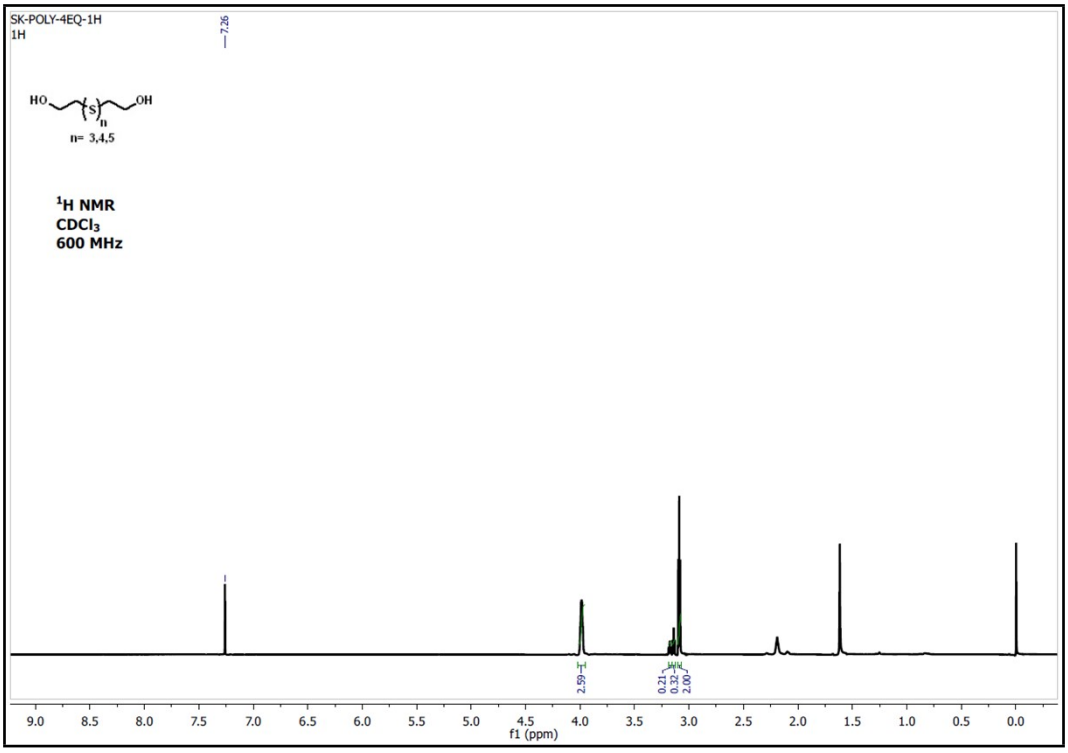

Figure **S37**. ^{1}H NMR (600 MHz) spectrum of compound **6** in $CDCl_3$.The compound was prepared using sulfur powder and $Na_2S.9H_2O$ in 1:4 ratio.

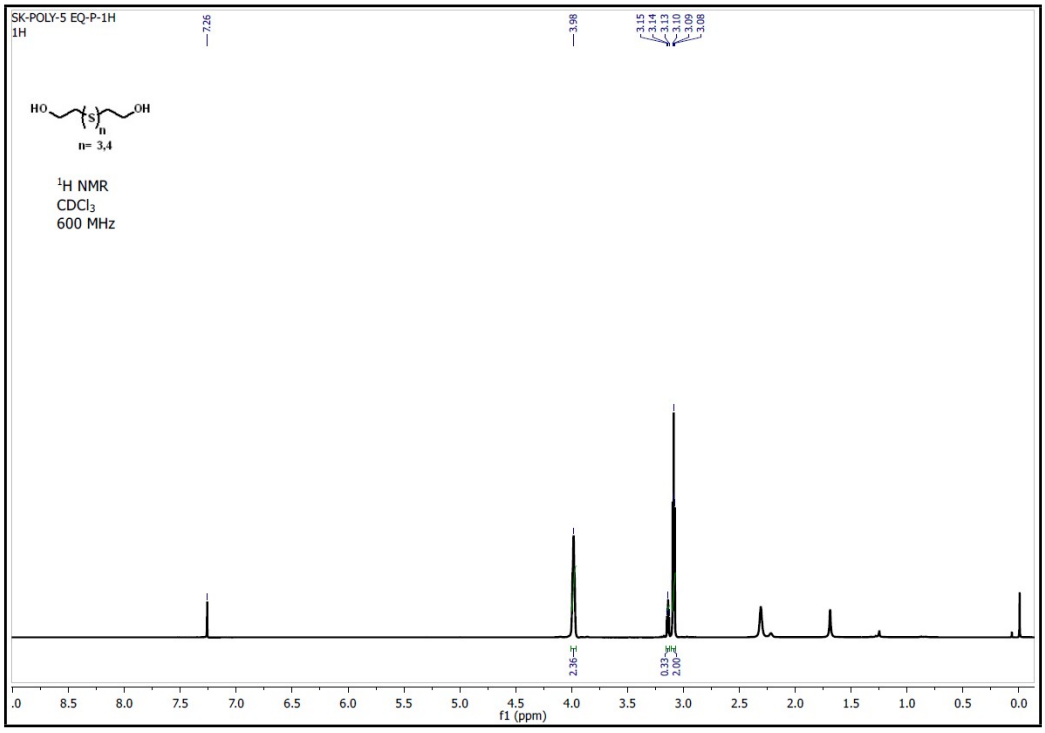

Figure **S38**. ^{1}H NMR (600 MHz) spectrum of compound **6** in $CDCl_3$.The compound was prepared using sulfur powder and $Na_2S.9H_2O$ in 1:5 ratio.

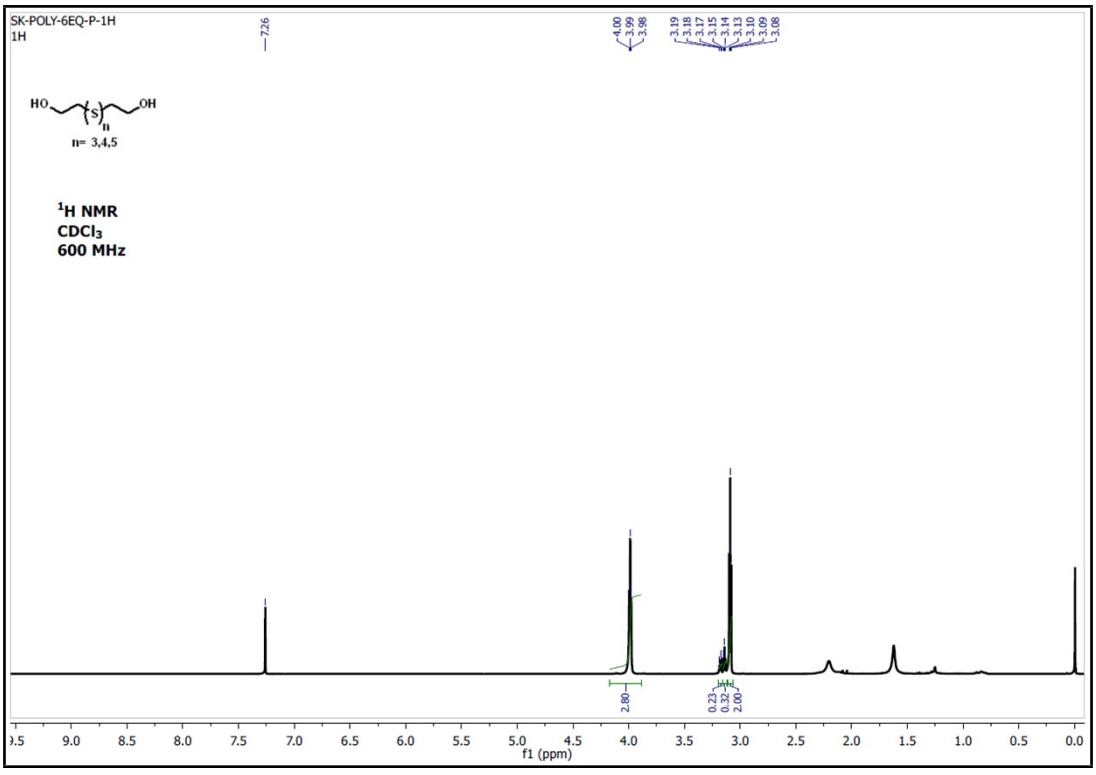

Figure **S39**. ^{1}H NMR (600 MHz) spectrum of compound **6** in $CDCl_3$.The compound was prepared using sulfur powder and $Na_2S.9H_2O$ in 1:6 ratio.

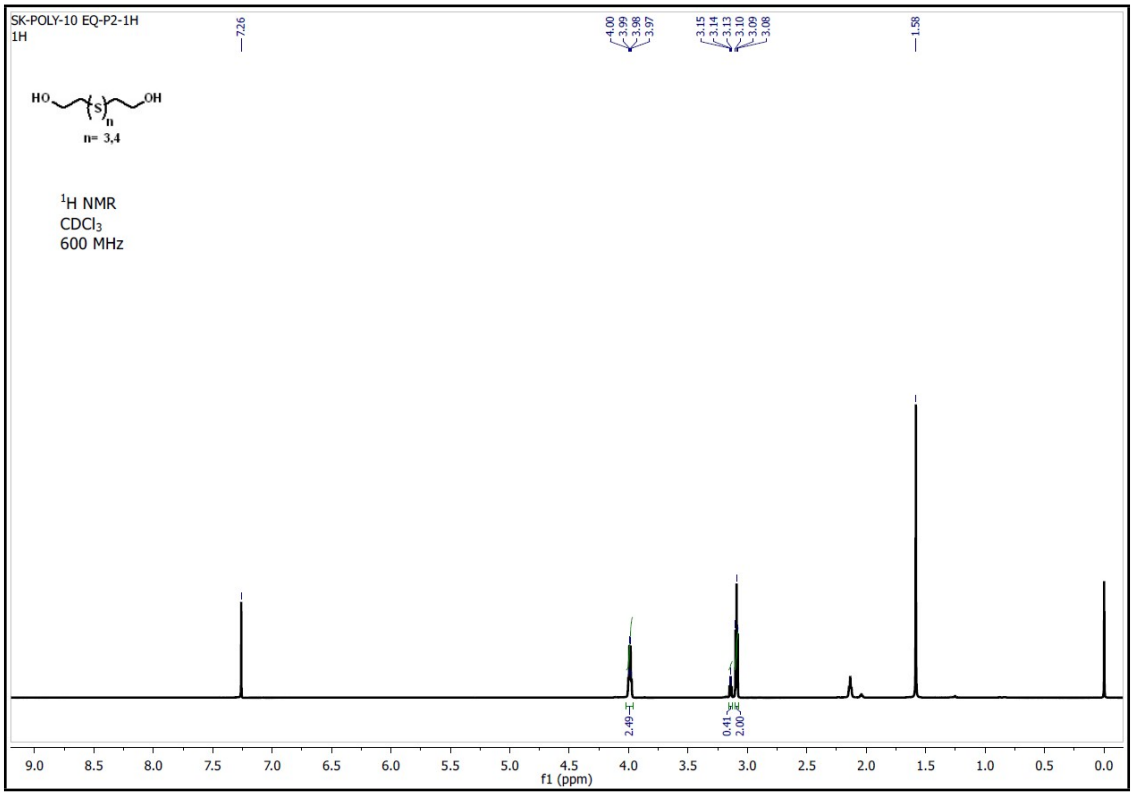

Figure **S40**. ^{1}H NMR (600 MHz) spectrum of compound **6** in $CDCl_3$.The compound was prepared using sulfur powder and $Na_2S.9H_2O$ in 1:10 ratio.

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