Electronic Supplementary Information (ESI)

for

Phenacylselenoesters Allow Facile Selenium Transfer and Hydrogen Selenide Generation

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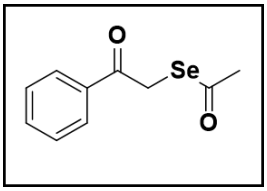
1. General methods

Unless otherwise specified, all reagents, starting materials, and dry solvents were purchased from commercial suppliers and used as received without further purification. All reactions were conducted under a nitrogen atmosphere. The moisture-sensitive reactions were conducted using oven-dried glassware, degassed solvents, and Schlenk lines. Thin-layer Chromatography (TLC) analyses were performed using pre-coated TLC sheets from MERCK® SIL G/UV254. Visualization was accomplished with UV light (λ_{max} = 254 nm). Column chromatography was performed on Rankem silica gel (100-200 mesh). The following abbreviations are used to signify the chemicals utilized in various assays: ES (Porcine liver esterase), wt 3-MST (wildtype 3-Mercaptopyruvate sulfurtransferase), PMSF (Phenylmethylsulfonyl fluoride), BSA (Bovine serum albumin), DTT (Dithiothreitol), GSH (Glutathione), GSSG (glutathionedisulfide), NAC (*N*-Acetyl Cysteine), BME (*β*-mercaptoethanol) and HPE-IAM (*β*-hydroxyphenyl-ethyl-iodoacetamide). ^{1}H and ^{13}C NMR spectra were recorded either on BRUKER 400 MHz or JEOL 400 MHz (or 100 MHz for ^{13}C) spectrometers using tetramethylsilane (δ_H = 0.00, δ_C = 0.00) as an internal standard or residual solvent [$CHCl_3$ δH, (7.26 ppm, δ_C 77.2 ppm) signals as reference. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (*J*) in Hz. The following notations are used to indicate the multiplicity of the signals: s (singlet), d (doublet), t (triplet), dd (doublet of doublet), q (quartet), m (multiplet). High-Resolution Mass Spectra (HRMS) were obtained from HRMS-ESI-Q-Time of Flight LC/MS. Infrared (IR) spectra were recorded using BRUKER- ALPHA FT-IR spectrometer. High-Performance Liquid Chromatography (HPLC) was performed on an Agilent Technologies 1260 infinity with Eclipse plus C-18 reversed-phase column (250 mm × 4.6 mm, 5μm). The purity of donors **1a**, **1b**, and **1c** was determined by HPLC analysis. Fluorescence and other spectrophotometric measurements were performed using EnSight Multimode Plate Reader under

the Perkin Elmer-IISER Pune, Centre of Excellence facility.

2. Synthesis and characterization

2.1. Se-(2-oxo-2-phenylethyl) ethaneselenoate (1a).

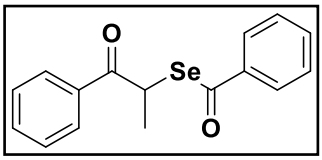


Compound **1a** was synthesized from 2- bromo-1-phenylethan-1-one using a reported protocol with a few modifications.[1] To a two-necked round bottom flask, under nitrogen atmosphere, selenium powder (0.395 g, 5 mmol) and degassed ethanol (20 mL) were added, followed by the addition of $NaBH_4$ (0.378 g, 10 mmol). The mixture was stirred until the solution became colorless (5-10 min). Subsequently, acetyl chloride (5 mmol) was added to the reaction medium resulting in an immediate change in color to milky-yellow. After 30 min, 2- bromo-1-phenylethan-1-one (0.85 g, 4.26 mmol) was added. The reaction was monitored by TLC until complete consumption of 2-bromo-1-phenylethan-1-one. After 2 h, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography using 100-200 silica mesh. The desired product was obtained with 100% hexane as the eluent and compound **1a** was isolated as a yellow liquid (0.34 g, 33%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99, 7.97 (dd, J = 8.1 Hz, 2H), 7.59 (t, J = 7.4 Hz, 1H), 7.45 (dd, J = 5.0 Hz, 2H), 4.35 (s, 2H), 2.45 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.7, 194.9, 135.1, 133.4, 128.5, 33.8, 30.9; ^{77}Se NMR (76.3 MHz, $CDCl_3$): δ 563.2-562.9; HRMS (ESI-TOF) for $C_{10}H_{10}O_2Se$ $[M+H]^+$: Calcd. 242.9926, Found 242.9924; FT-IR (ν_{max}, cm^{-1}): 2923, 2852, 1714,1676.

2.2. General protocol for the synthesis of ethanethioate derivatives (1b and 1c).

Compounds **1b** and **1c** were synthesized using a reported protocol with a few additional modifications.[2] To a two-necked round bottom flask, under nitrogen atmosphere, selenium powder

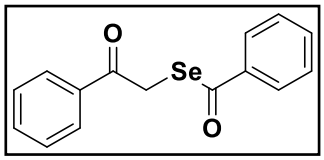
(0.281 g, 3.56 mmol) and degassed water (20 mL) were added followed by the addition of $NaBH_4$ (0.283 g, 7.47 mmol). The mixture stirred until the solution became colorless within 5-10 min. Benzoyl chloride (0.50 g, 3.56 mmol) was then added and the reaction mixture was stirred for 90 min at 50 -70 °C.



Subsequently, the crude reaction mixture was filtered to eliminate formed boron salts. The appropriate electrophile (phenacyl bromide or 2-bromopropiophenone) was added to the filtrate, and the reaction was stirred at 50 ºC for 1 h, followed by room temperature for an additional hour. The reaction mixture was extracted thrice with ethyl acetate/water, the organic phase was dried over Na_2SO_4 and concentrated by evaporating the solvent *in vacuo*. The crude product was isolated by column chromatography using 100-200 silica mesh as the stationary phase and ethyl acetate/hexane as the mobile phase.

2.2.1. Se-(2-oxo-2-phenylethyl) benzoselenoate (1b).

Following the above general synthetic protocol, 2-bromo-1-phenylethan-1-one (0.708 g, 3.56 mmol) was utilised as the electrophile in the last step of the reaction. A gradient starting from 1% ethyl acetate/hexane was used as the mobile phase and the desired product was obtained with 1.5% ethyl acetate/hexane as eluent. Compound **1b** was obtained as a light pink solid (0.629 g, 58%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (dd, *J* = 8.2, 1.1 Hz, 2H), 7.85 (dd, *J* = 8.2, 1.0 Hz, 2H), 7.60 (dd, *J* = 14.9, 7.4 Hz, 2H), 7.47 (dd, *J* = 14.1, 7.4 Hz, 4H), 4.54 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.4, 193.0, 138.2, 135.6, 134.2, 133.7, 129.1, 128.9, 127.5, 31.2; ^{77}Se NMR (76.3 MHz, $CDCl_3$): δ 533.8; HRMS (ESI-TOF) for $C_{15}H_{12}O_2Se$ $[M+Na]^+$: Calcd. 326.9900, Found, 326.9899; FT-IR (ν_{max}, cm^{-1}): 1672



2.2.2. Se-(1-oxo-1-phenylpropan-2-yl) benzoselenoate (1c). Following the above general synthetic protocol, the last step used 2-bromo-1-phenylpropan-1-one (0.644 g, 3.02 mmol) as the

electrophile. For the purification, a solvent gradient starting from 100% hexane was used as the mobile phase, and the desired product was purified with 1% ethyl acetate/hexane as eluent. Compound **1c** was obtained as a yellow liquid (0.263 g, 28%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (dd, *J* = 7.3 Hz, 2H), 7.87 (dd, *J* = 7.3 Hz, 2H), 7.58 (dt, *J* = 7.4 Hz, 2H), 7.47-7.42 (m, 4H), 5.44 (q, *J* = 6.8 Hz, 1H), 1.83 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.2, 193.2, 138.2, 135.1, 134.3, 133.5, 129.1, 128.8, 128.6, 127.5, 39.8, 18.6; ^{77}Se NMR (76.3 MHz, $CDCl_3$): δ 670.3; HRMS (ESI-TOF) for $C_{16}H_{14}O_2Se$ $[M+Na]^+$: Calcd. 341.0056, Found 341.0053; FT-IR (ν_{max}, cm^{-1}): 2927, 1666.

2.3. Preparation of single crystal of 1b

Crystals of the compound were grown by slow evaporation from a solution of $CHCl_3$. A single crystal **1b** was mounted on a loop with a small amount of the paraffin oil. (Refer to **Figure 2**). Tables S1-S4 contain data pertinent to the crystal structure determination.

3. Experimental Protocols

3.1.1. Lead acetate assay to detect H_2Se

General protocol: The assay was conducted in the presence of $Zn(OAc)_2$ as a trapping agent following which as previously reported, [3,4] the aliquot was treated with lead acetate in acidic medium. Briefly, 10 mM DMSO stock solutions of the selenoesters **1a-1c** were freshly prepared. Stocks of DTT, GSH, NAC and/or β-ME (10 mM) and $Zn(OAc)_2 \cdot 2H_2O$ (40 mM) were prepared in deionized water while ES (100 U/mL) was prepared in phosphate buffered saline (10 mM, pH 7.4). The stock solution of lead acetate ($Pb(OAc)_2 \cdot 3H_2O$, 5 mM) was prepared in 4.2 N HCl.

The reaction samples were prepared by sequentially adding 400 μM of $Zn(OAc)_2$ (10 μL, 40 mM stock), 10 μL of DMSO, 100 μM of the phenacylselenoester **1** (10 μL, 10 mM stock), 0.1 U/mL ES (10 μL, 10 U/mL stock) and 200 μM DTT (20 μL, 10 mM stock). The final concentration of DMSO was maintained at 2% in each reaction. The final volume was adjusted to 1.0 mL using 50 mM HEPES-NaOH pH 7.4 buffer and placed in a shaker incubator maintained at 37 ºC and 300 rpm.

Reaction of Na_2SeO_3 with GSH. A 10 mM stock solution of sodium selenite (Na_2SeO_3).[5] Sequential additions were made as follows: 400 μM $Zn(OAc)_2 \cdot 2H_2O$ (10 μL, 40 mM stock), 20 μL DMSO, 100 μM Na_2SeO_3 (10 μL, 10 mM stock), 400 μM GSH (10 μL, 40 mM stock), and 200 μM DTT (20 μL, 10 mM stock). The final concentration of DMSO was maintained at 2% in each reaction. The final volume was adjusted to 1.0 mL using 50 mM HEPES- NaOH buffer at pH 7.4. (**Figure S1**).

For the control reactions, 400 μM of $Zn(OAc)_2$ (10 μL, 40 mM), 10 μL of DMSO, 100 μM of **1** (10 μL, 10 mM) along with either 0.1 U/mL ES (10 μL, 10 U/mL) or 200 μM DTT (20 μL, 10 mM) were added sequentially. The final volume was adjusted to 1.0 mL using 50 mM HEPES- NaOH pH 7.4 buffer.

Reaction of **1b** in the presence of lysine and other amino acids. The reactivity of **1b** in the presence of lysine and other nucleophilic amino acids was assessed in the absence and presence of ES respectively. Herein, the reaction samples were prepared by adding 400 μM of $Zn(OAc)_2$ (10 μL, 40 mM stock), 10 μL of DMSO, 100 μM of **1b** (10 μL, 10 mM stock), 1 mM of amino acids (100 μL, 10 mM stock) with or without 0.1 U/mL ES (10 μL, 10 U/mL stock). The effective concentration of DMSO was maintained at 2% in each reaction. The final volume was adjusted to 1.0 mL using 50 mM HEPES-NaOH pH 7.4 buffer and then placed in a shaker incubator maintained at 37 ºC and 300 rpm for 60 min. No other thiols were added in this case. **(Figures S8 and S9)**

Reaction of **1b** in the presence of bovine serum albumin (BSA). Under the standard reaction conditions, this assay could not be carried out since coagulation was seen in the presence of $Zn(OAc)_2$ and BSA **(Figure S10).** Instead, we carried out this assay in a 96-well plate without zinc acetate and in the presence of 5% BSA (*w/v*) **(Figure S11)**.

Reaction of **1b** in the presence of 3-MST. H_2Se formation was assessed with **1b** in the presence of *E. coli* wildtype 3-MST and C238A mutant. Herein, the reaction samples were prepared by adding 400 μM of $Zn(OAc)_2$ (10 μL, 40 mM stock), 10 μL of DMSO, 100 μM of **1b** (10 μL, 10 mM stock), 0.1 U/mL ES (10 μL, 10 U/mL stock) along with 1 μM of either wildtype 3-MST or C238A mutant enzyme. The effective concentration of DMSO was maintained at 2% in each reaction. The final volume was adjusted to 1.0 mL using 50 mM HEPES-NaOH pH 7.4 buffer and then placed in a shaker incubator maintained at 37 ºC and 300 rpm for 30 min. Afterwards, 200 μM DTT (20 μL, 10 mM stock) was added to each of the reaction mixtures and those were incubated at 37 ºC and 300 rpm shaking. (**Figure S19**). At predetermined time points, equal volumes (100 μL) of $Pb(OAc)_2$ and aliquots from the above reaction samples were mixed and incubated at 37 ºC; 150

μL was transferred to 96-well plate and the absorbance at 400 nm was recorded using a multimode microplate reader (Perkin-Elmer Ensight).

3.1.2. FESEM data of colloidal PbSe particles

A fresh stock solution of **1b** (15 mM) was freshly prepared in DMSO. Stocks of DTT (50 mM) and $Zn(OAc)_2 \cdot 2H_2O$ (40 mM) were prepared in deionized water while ES (100 U/mL) was prepared in phosphate buffered saline (10 mM, pH 7.4). The stock solution of lead acetate ($Pb(OAc)_2 \cdot 3H_2O$, 5 mM) was prepared in 4.2 N HCl. The reaction sample was prepared by sequentially adding 400 μM of $Zn(OAc)_2$ (10 μL, 40 mM), 300 μM of **1b** (20 μL, 15 mM), 1 U/mL ES (100 μL, 10 U/mL) and 5 mM DTT (500 μL, 50 mM). The final concentration of DMSO was maintained at 2% in the reaction. The final volume was adjusted to 1.0 mL using 50 mM HEPES-NaOH pH 7.4 buffer and placed in a shaker incubator maintained at 37 ºC and 300 rpm up to 60 min. After 1 h, equal volumes (100 μL) of $Pb(OAc)_2 \cdot 3H_2O$ and aliquots from the above reaction sample were mixed and incubated at 37 ºC produce brown PbSe particles. The precipitated particles were collected by centrifugation at 10,000 rpm for 10 min, washed with 10 mM PBS, and dried to remove residual water. The resulting precipitate was then visualized under Field-Emission Scanning Electron Microscopy (FESEM) imaging, wherein, Energy-Dispersive X-Ray Spectroscopy (EDXS) was carried out, revealing 42.10 wt% of Pb and 43.86 wt% of Se. In addition, 14.04 wt% presence of S was also detected, likely due to PbS. The uniform distribution of all the respective elements was confirmed from elemental mapping. (**Figure S3**).

3.2. Stability and Cleavage Studies by HPLC Analysis

Fresh stock solutions of the selenoesters, **1a**, **1b** and **1c** (10 mM) were prepared in DMSO while the stocks of $Zn(OAc)_2$ (40 mM), DTT (10 mM) and ES (100 U/mL) were prepared in deionized water. The reaction mixtures were prepared in 50 mM HEPES-NaOH buffer (pH 7.4). For the reactions, 100 μM of the substrate, 400 μM $Zn(OAc)_2$, 200 μM of DTT, 10 μL DMSO and 0.1 U/mL ES were added and placed in a shaker incubator maintained at 300 rpm and 37 ºC. Aliquots (100 μL) were treated with ACN (100 μL), filtered through 0.22 μm syringe filters and flash-frozen using liquid nitrogen and thawed prior to HPLC analysis (250 nm). A Phenomenex Luna C-18 reverse phase column, 100 Å particle size and 5 μm pore size (250 x 4.6 mm) was used as the stationary phase. The mobile phase used was water: ACN with a multistep gradient of 40:60→ 0 min, 40:60 to 30:70→ 0-5 min, 30:70 to 20:80→ 5-10 min, 20:80 to 10:90→ 10-13 min, 10:90 to 20:80→ 13-16 min, 20:80 to 30:70→ 16-20 min, 30:70 to 40:60→ 20-25 min at a flow-rate of 0.5 mL/min. (Refer to **Figure 4B** and **Figure 5A**)

Stability experiments in buffer. A 10 mM stock solution of **1b** was prepared in DMSO. The reaction mixture contained 100 μM of **1b** (10 μL, 10 mM stock), 10 μL of DMSO and 400 μM of $Zn(OAc)_2$ (10 μL, 40 mM). The remaining volume was adjusted to 1.0 mL with ACN and 50 mM HEPES-NaOH pH 7.4 buffer. The reactions were placed in a shaker incubator maintained at 37 ºC and 300 rpm. Aliquots were quenched with ACN, filtered through 0.22 μm syringe filters and analysed by HPLC. (**Figure S14**). A similar protocol was followed to test the stability of **1b** in pH 5.0 (**Figure S15**) and pH 9.0 (**Figure S16**) buffer.

Stability of **1a-1c** in the presence of H_2O_2[6]: The susceptibility of the phenacylselenoesters, **1a-1c**, was tested by performing a TLC experiment in the presence of H_2O_2. The reaction mixture contained 1 mM **1b** (10 μL, 100 mM stock), 2 mM H_2O_2 (20 μL, 100 mM stock) and 10 μL of

DMSO. The effective concentration of DMSO was maintained at 2% in the reaction. The final volume of the reaction was adjusted to 1.0 mL using ACN and then placed in a shaker incubator maintained at 37 ºC and 300 rpm for 120 min. The TLC of the reaction was monitored at different time intervals using 20% EtOAc-Hexane as the solvent system. (**Figure S17**)

3.4. General protocols for protein expression and purification for wildtype *E. coli* 3-MST[7]

The pET28a constructs of 3-MST were transformed into *E. coli* BL21 (DE3) cells for overexpression of the respective proteins. Overnight primary cultures of *E. coli* BL21 (DE3) cells containing the respective 3-MST pET28a plasmids were grown in Luria-Bertani (LB) medium with kanamycin (25 μg/mL) at 37 ºC, 180 rpm. These cultures were transferred into large secondary cultures of LB-Kan medium at 1% *v/v* inoculum and were grown at 37 ºC, 180 rpm to an OD_{600} of 0.6. Protein overexpression was achieved with 1 mM of isopropyl β-D-1-thiogalactopyranoside (IPTG), following which the cultures were incubated at 18 ºC for 18 h at 180 rpm. The cells were harvested from the large cultures by centrifugation at 6500 rpm, 4 ºC and the pellets were flash-frozen in liquid nitrogen and stored at -80 ºC till further use. The cell pellets obtained were resuspended in lysis buffer (50 mM Tris-HCl, pH 8.0) containing 300 mM NaCl, 10 mM $MgCl_2$, 0.05 mM phenylmethylsulfonyl fluoride and 0.025% β-mercaptoethanol (BME) and were lysed by sonication. The cell lysate was loaded onto a Ni-NTA column pre-incubated with 50 mM Tris-HCl, pH 8.0, containing 300 mM NaCl, 0.025% BME and 10 mM imidazole. The column was then washed with 50 mM Tris-HCl, pH 8.0, containing 300 mM NaCl, 0.025% BME and 50 mM imidazole, and the proteins were eluted in 50 mM Tris-HCl, pH 8.0, containing 300 mM NaCl, 0.025% BME and 250 mM imidazole. To check purity, the eluted protein fractions were run in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The purified proteins were buffer exchanged in 50 mM Tris-HCl, pH 8.0, containing 100 mM NaCl and 0.025%

BME using Econo-Pac 10 DG prepacked desalting columns (Bio-Rad), and were stored with 15% v/v glycerol in -80 ºC. (**Figure S18**).

3.5. Docking methods for *h*3-MST with phenacylselenols derived from 1a, 1b and 1c

Autodock Vina (V. 1.1.2) software was employed to conduct docking studies within the active site of ***h*3-MST** (Protein data bank (PDB) file: **4JGT**). Conversion of molecules from SMILES to PDB format as required for docking was executed using an online chemoinformatics tool developed by the National Cancer Institute (NCI) and the National Institute of Health (NIH).[8] Docking was carried out with an exhaustiveness parameter of 8, while all other parameters were maintained to the vina default. The parameters defining the docking sites are:

Box Coordinates: Centre: (144.968, -50.5523, -7.08),

Box Dimensions (Å): 18 × 17.5 × 19 (**Table S8**).

3.6. MALDI-TOF analysis

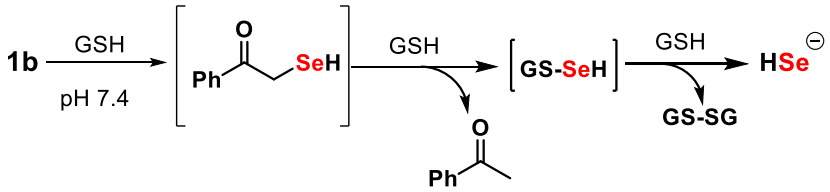
3.6.1. Trapping Experiment with *β*-hydroxyphenyl-ethyl-Iodoacetamide (HPE-IAM)

The stock solutions of **1b** (10 mM) and HPE-IAM (10 mM) were freshly prepared in DMSO. Next, a fresh stock solution of porcine liver esterase (100 U/mL; Sigma Aldrich, E3019) was prepared in deionized water. The reaction mixture consisted of **1b** (10 µL, 10 mM) along with HPE-IAM (200 µL, 10 mM) and esterase (10 µL, 10 U/mL). The volume was adjusted to 1 mL using 50 mM HEPES-NaOH pH 7.4 buffer and the reaction mixture was incubated at 37 ºC and 300 rpm for 30 min. 200 µL aliquot of the reaction mixture were taken and extracted by adding DCM. The resultant organic layer was analyzed using MALDI-TOF, and the following mass fragments were observed: m/z $[M+K]^+$ 344.2138 (**HPE-IAM**) and 416.4073 (**1b-Se-HPE-AM**) (**Scheme S2** and **Figure S13**).

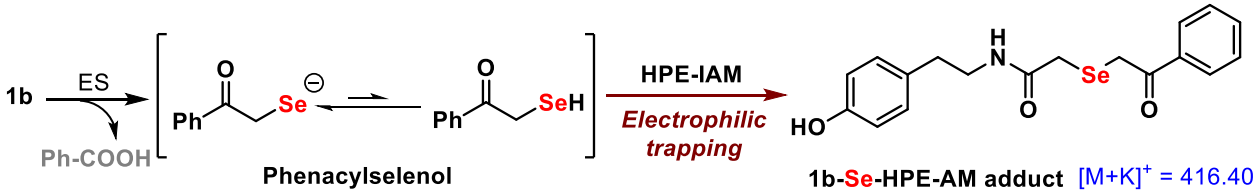
3.6.2. Detection of Selenodiglutathione (GSSeSG)[9]

The reaction mixture was prepared by adding **1b** (10 µL, 10 mM), esterase (10 µL, 10 U/mL) along with GSH (20 µL, 10 mM stock) and GSSG (20 µL, 1 mM in deionized water) and the final volume was adjusted to 1 mL by adding 50 mM HEPES-NaOH pH 7.4 buffer. The reaction mixture was incubated at 37 ºC and 300 rpm and after 30 min, a 250 µL aliquot was taken and extracted with 100 µL of DCM. The resulting organic layer was analyzed using mass spectrometry and the following adducts were detected: m/z $[M+K]^+$ 437.32 (**phenacyl diselenide**), 731.36 (**selenodiglutathione**) and 543.43 (**selenol-SG adduct**). (**Scheme S3** and **Figure S20**).

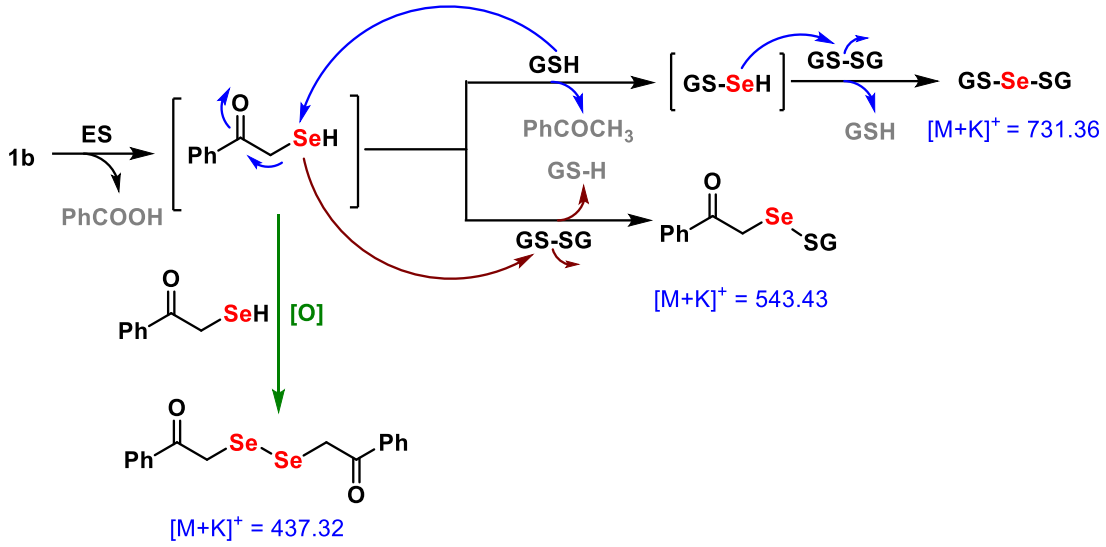
4. Supplementary Schemes



Scheme S1: Reaction of **1b** in the presence of glutathione



Scheme S2: Detection of the intermediate, phenacylselenol, during the reaction of **1b** under the standard reaction conditions in the presence of HPE-IAM (2 mM). (Refer to **Figure S13** for MS trace).



Scheme S3. Reaction of **1b** in the presence of esterase and GSH. Diselenide, $[M+K]^+$ = 437.32, can be formed by the oxidation of phenacylselenol. Phenacylselenol reacts with glutathione to generate GS-SeH, which could then react further with GS-SG and GS-Se-SG, $[M+K]^+$ = 731.36. The selenol-SG adduct, $[M+K]^+$ = 543.43, could be produced from the reaction of GS-SG with phenacylselenol (Refer to **Figure S20**).

5. Supplementary Figures

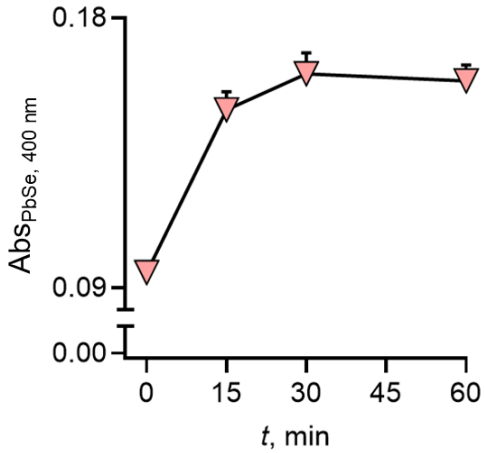


Figure S1: (A) Time course for PbSe formation from the reaction mixture containing Na_2SeO_3 (100 μM), GSH (400 μM), DTT (200 μM) and $Zn(OAc)_2$ (400 μM). Comparison of absorbance corresponding to the formation of PbSe during the incubation of SeO_3^{2-} and GSH with DTT. All data are expressed as mean ± SD (n = 3 per group). The error bars at each time point represent the

standard deviation. Student's two-tailed unpaired parametric *t*-test was carried out to determine statistical significance between $t = 0$ min and $t = 30$ min and the *p*-value was < 0.0001.

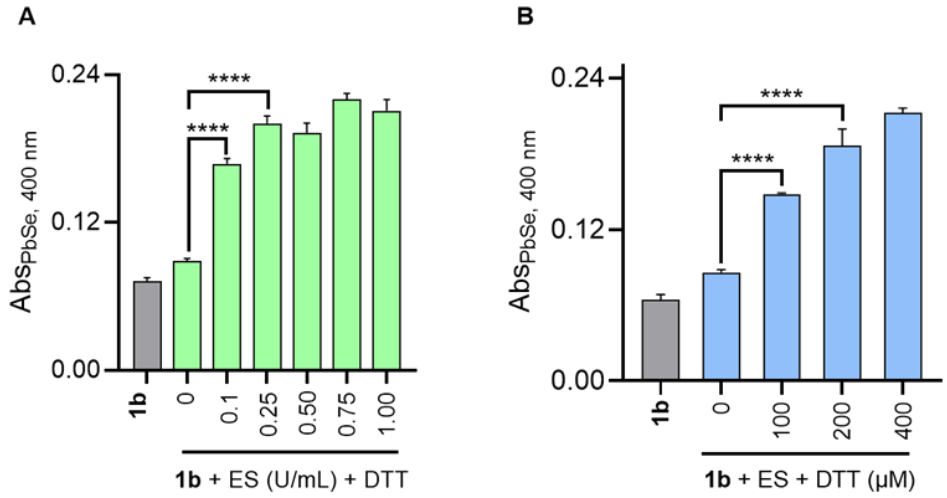


Figure S2: Data for the reaction of **1b** after 30 min under the standard reaction conditions. **(A)** Varying concentrations of ES (0-1.0 U/mL) **(B)** Varying concentrations of DTT (0-400 μM). Results are represented as mean ± SD (n = 3 per group). Student's two-tailed unpaired parametric *t*-test was carried out to determine the statistical significance (****$p < 0.0001$).

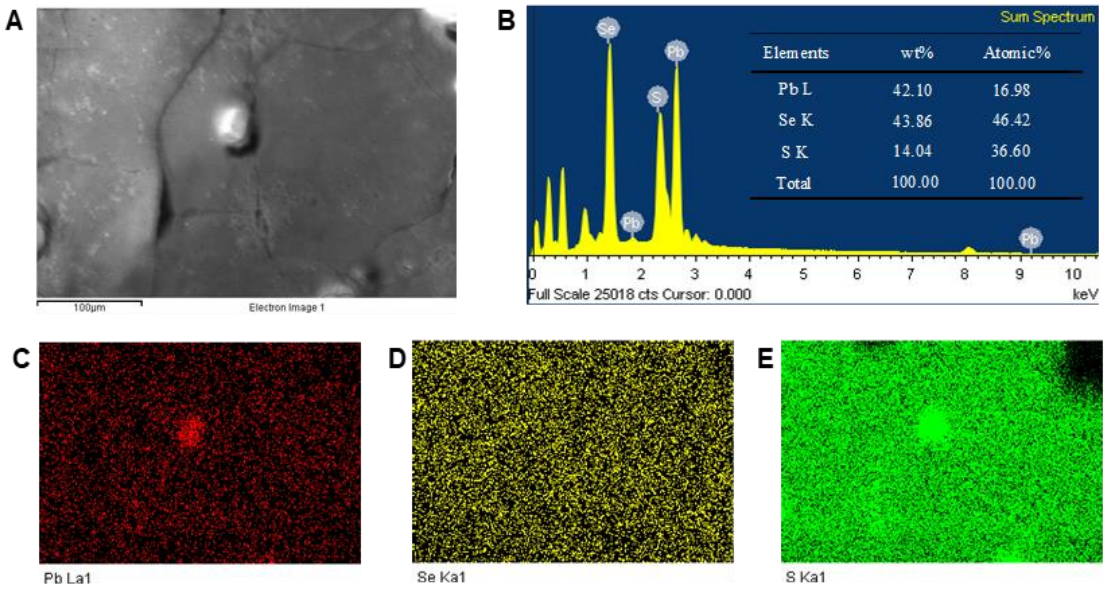


Figure S3. (A) FESEM imaging of the PbSe precipitate; **(B)** Energy-Dispersive X-ray

Spectroscopy (EDXS) analysis of the PbSe precipitate, revealing the presence of Pb and Se predominantly, along with the minimal signature of sulfur (S); **(C-E)** Elemental mapping of the PbSe precipitate, exhibiting the uniform distribution of all the respective element.

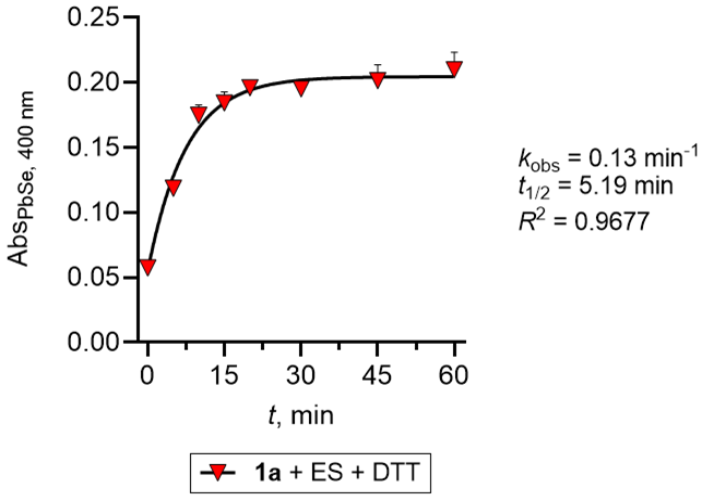


Figure S4: Kinetics of PbSe formation during the incubation of the compound **1a** under the standard reaction conditions. All the results are represented as mean ± SD (n = 3 per group). Curve fitting using the equation $y = y_0 + a\ (1\text{-}e^{-kx})$ gave the values listed beside the plot.

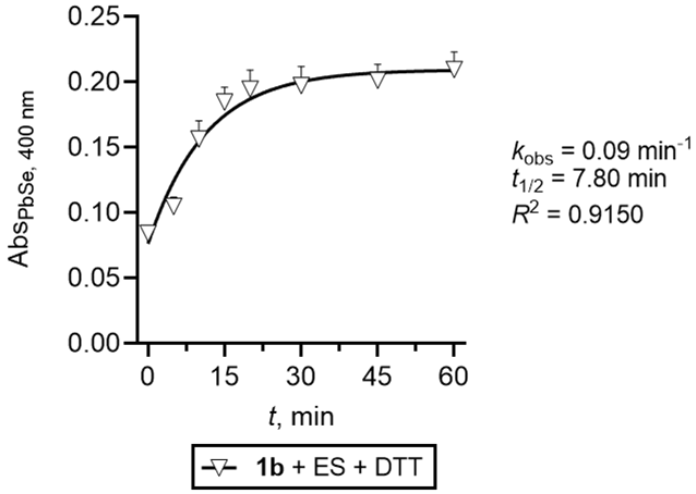


Figure S5: Kinetics of PbSe formation during the incubation of the compound **1b** under the standard reaction conditions. All the results are represented as mean ± SD (n = 3 per group). Curve fitting using the equation $y = y_0 + a\ (1\text{-}e^{-kx})$ gave the values listed beside the plot.

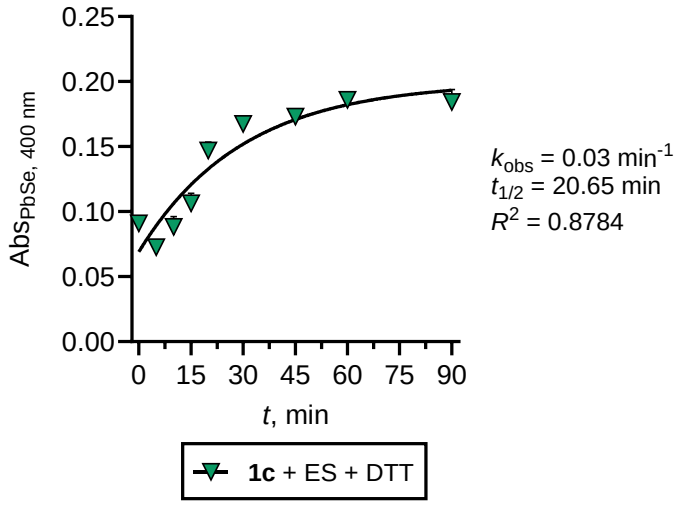


Figure S6: Kinetics of PbSe formation during the incubation of the compound **1c** under the standard reaction conditions. All the results are represented as mean ± SD (n = 3 per group). Curve fitting using the equation $y = y_0 + a(1-e^{-kx})$ gave the values listed beside the plot.

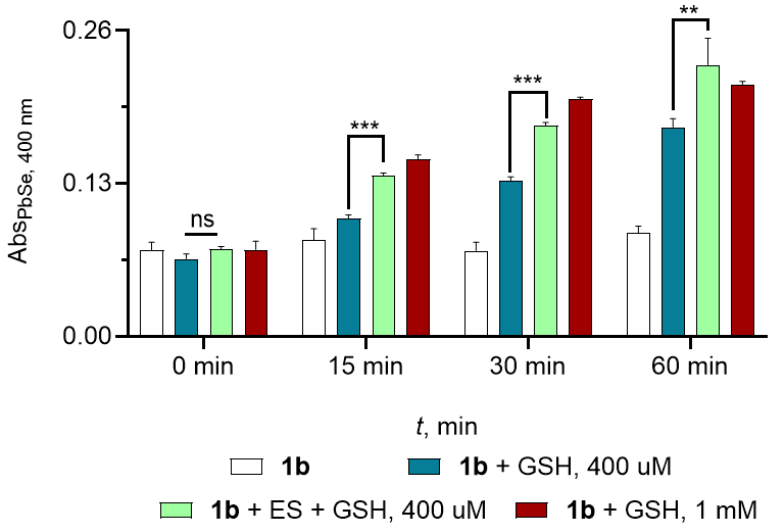


Figure S7: Data for the reaction of **1b** alone, with GSH (400 µM), with ES and GSH (400 µM) and with GSH (1 mM) in pH 7.4 HEPES-NaOH buffer. Results are represented as mean ± SD (n = 3 per group). Student's two-tailed unpaired parametric *t*-test was carried out to determine the statistical significance (ns indicates non-significant, $^{**}p < 0.01$ and $^{***}p < 0.001$).

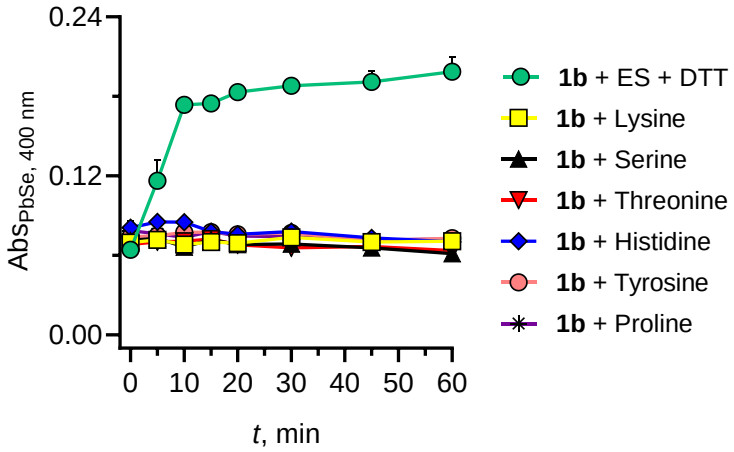


Figure S8: Time courses for the formation of PbSe during incubation of the compound **1b**, under standard reaction conditions, *i.e.*, **1b** + ES + DTT and compound with different nucleophilic amino acid residues: lysine, serine, threonine, histidine, tyrosine and proline in HEPES-NaOH pH 7.4 buffer up to 60 min. All data are represented here as mean ± SD. The error bars at each time point represent the SD.

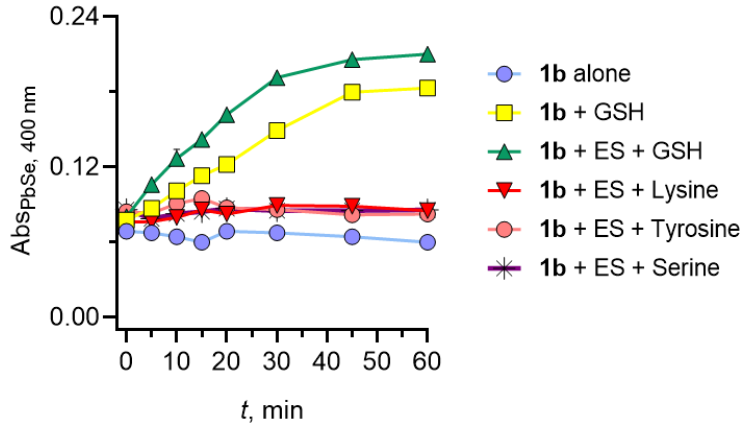
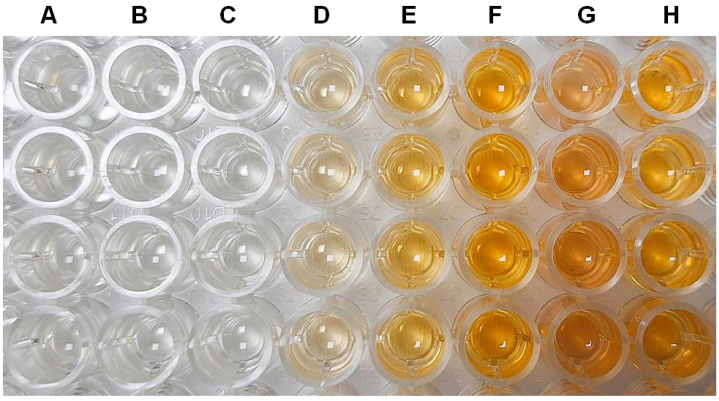


Figure S9: Time courses for the formation of PbSe during incubation of the compound, **1b** alone; with GSH (400 µM), with ES and GSH (400 µM) and with three different nucleophilic amino acids, *i.e.*, lysine, tyrosine and serine in the presence of ES under standard reaction conditions. All data are represented here as mean ± SD. The error bars at each time point represent the SD.

Figure S10: BSA protein is coagulated in the presence of $Zn(OAc)_2$ (400 µM)



5% BSA	+	+	+	+	+	+	+	+
Na_2SeO_3 (Varying)	-	1 mM	100 µM	250 µM	500 µM	1 mM	-	-
GSH (10 mM)	-	-	+	+	+	+	-	-
1b (1 mM)	+	-	-	-	-	-	+	+
Esterase (1 U/mL)	-	-	-	-	-	-	-	+

Figure S11: Formation of elemental selenium when Na_2SeO_3 and GSH are treated with BSA. Compound **1b** alone in BSA or in the presence of ES.

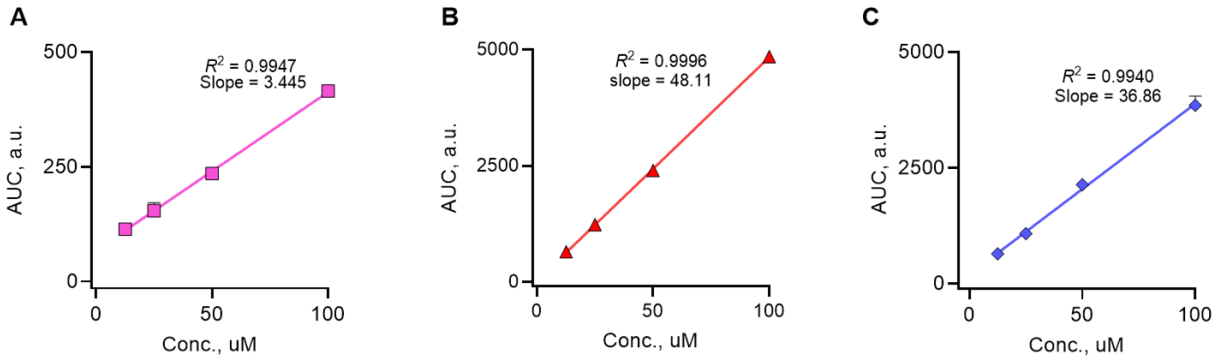


Figure S12: Calibration curves for **(A)** benzoic acid **(B)** Acetophenone, **2** and **(C)** Propiophenone, **3.**

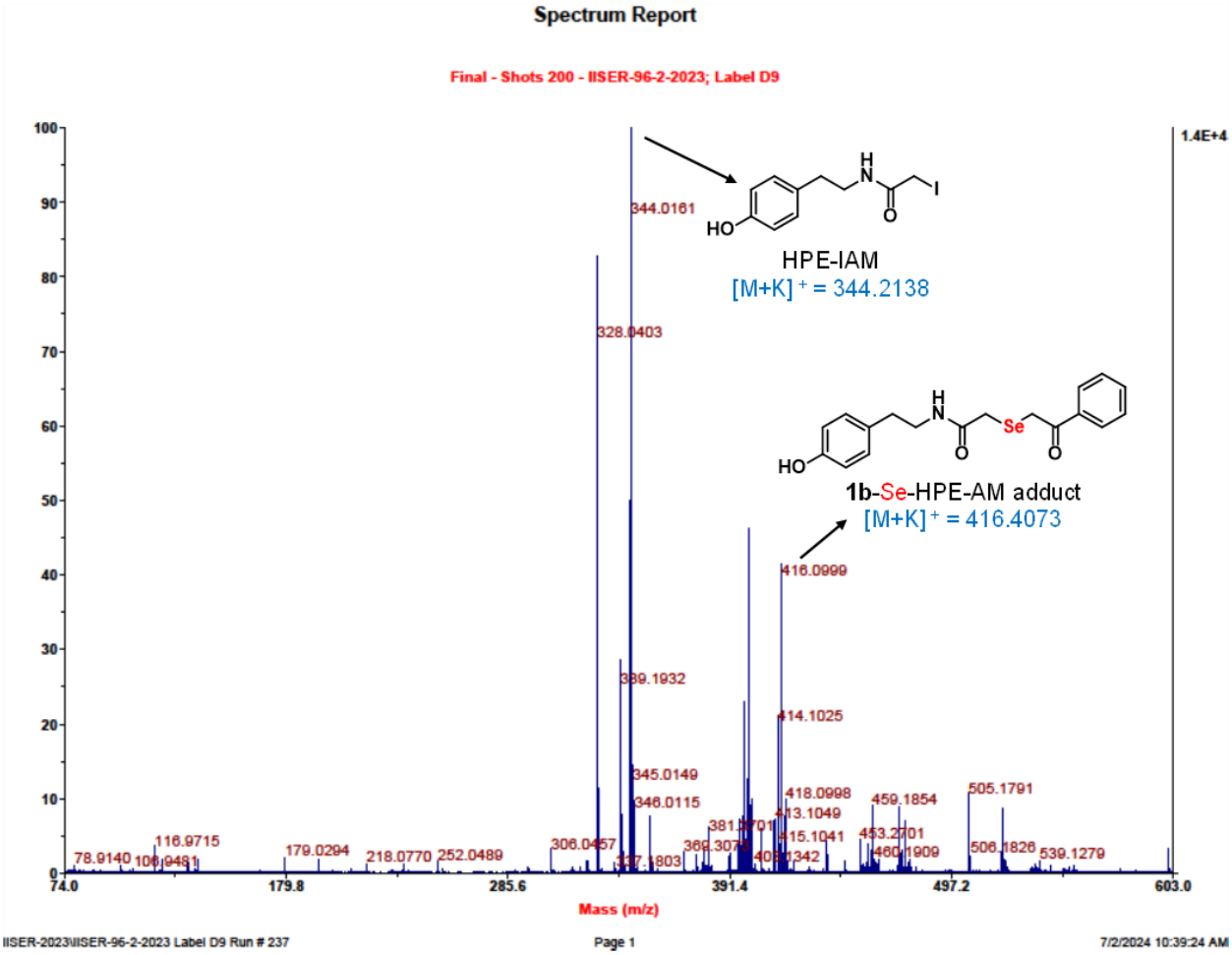


Figure S13. As per the **Scheme S2**, the reaction of **1b** in the presence of HPE-IAM analysed by mass spectrometry.

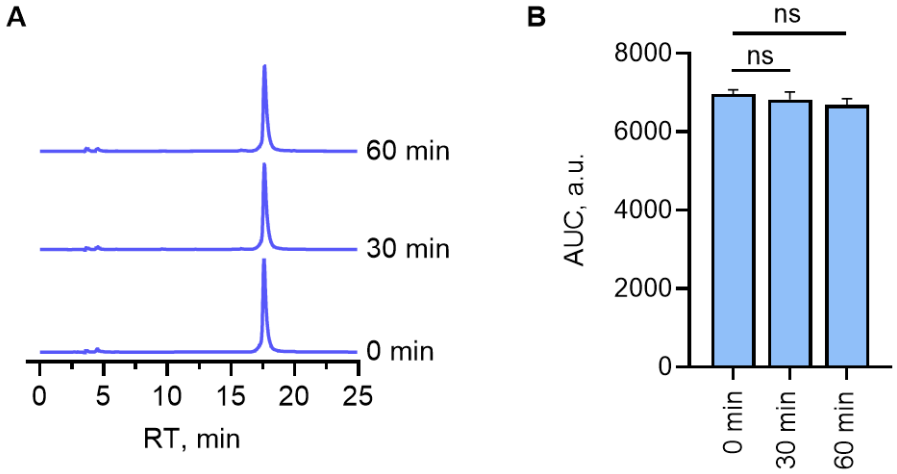


Figure S14: (A) HPLC traces of **1b** incubated in HEPES-NaOH pH 7.4 buffer with 50% MeCN

and 400 μM $Zn(OAc)_2$ up to 60 min (Abs = 250 nm). **(B)** The AUC values are plotted. All data are represented as mean ± SD (n = 3 per group). Student's two-tailed unpaired parametric *t*-test was carried out to determine significance (ns indicates not significant).

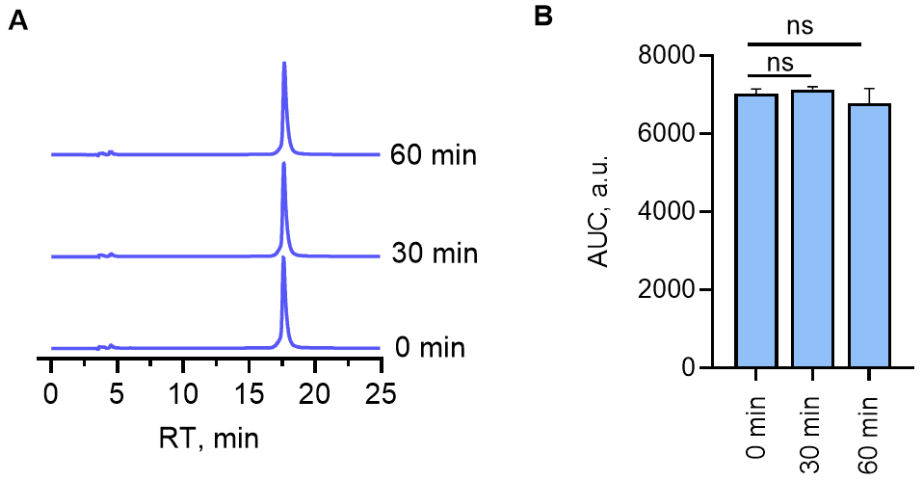


Figure S15: (A) HPLC traces of **1b** incubated in HEPES-NaOH pH 5.0 buffer with 50% MeCN and 400 μM $Zn(OAc)_2$ up to 60 min (Abs = 250 nm). **(B)** The AUC values are plotted. All data are represented as mean ± SD (n = 3 per group). Student's two-tailed unpaired parametric *t*-test was carried out to determine significance (ns indicates not significant).

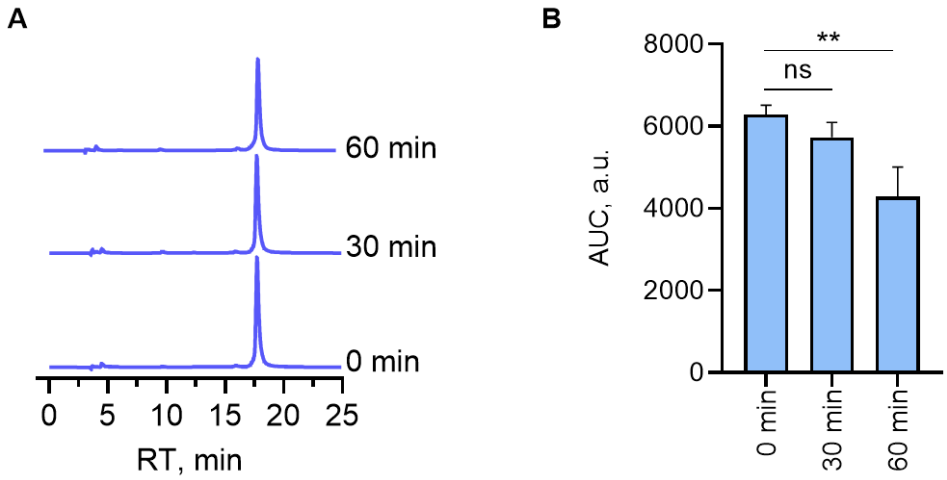


Figure S16: (A) HPLC traces of **1b** incubated in HEPES-NaOH pH 9.0 buffer with 50% MeCN and 400 μM $Zn(OAc)_2$ up to 60 min (Abs = 250 nm). **(B)** The AUC values are plotted. All data are

represented as mean ± SD (n = 3 per group). Student's two-tailed unpaired parametric *t*-test was carried out to determine significance (ns indicates not significant and $^{**}p < 0.01$).

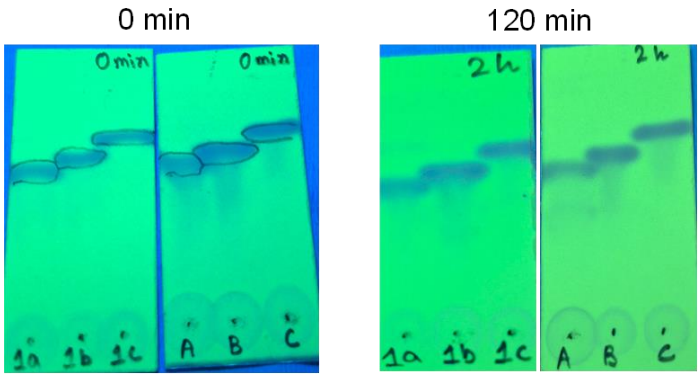


Figure S17: Stability of the compounds **1a**, **1b** and **1c** (1 mM) in ACN up to 2 h and **(A) 1a** + H_2O_2; **(B) 1b** + H_2O_2; **(C) 1c** + H_2O_2. Concentration of H_2O_2 was 2 mM. The solvent system used was 20% EtOAc-Hexane.

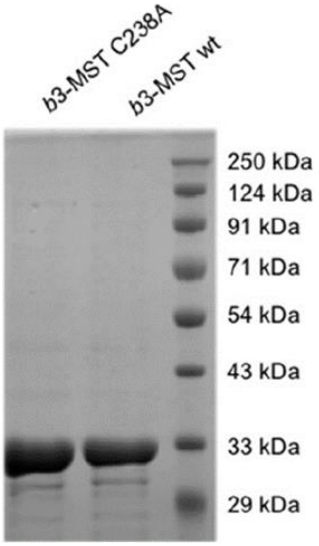


Figure S18: SDS-PAGE gel image for the purified proteins: wt *b*3-MST and *b*3-MST C238A mutant.

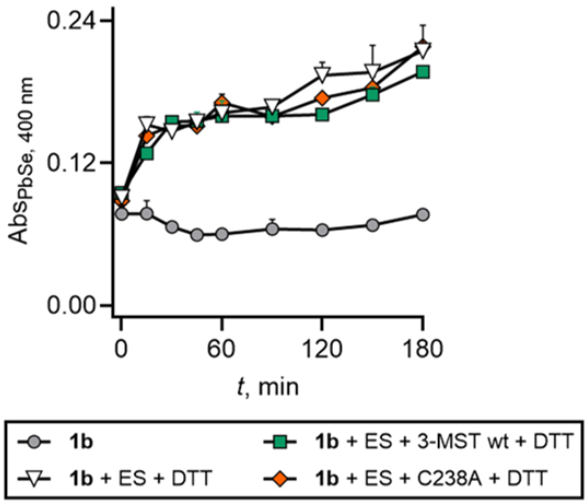


Figure S19: Time course for the formation of PbSe during incubation of **1b** (100 μM) with wt and mutant C238A 3-MST enzyme. All data are represented as mean ± SD (n = 3 per group).

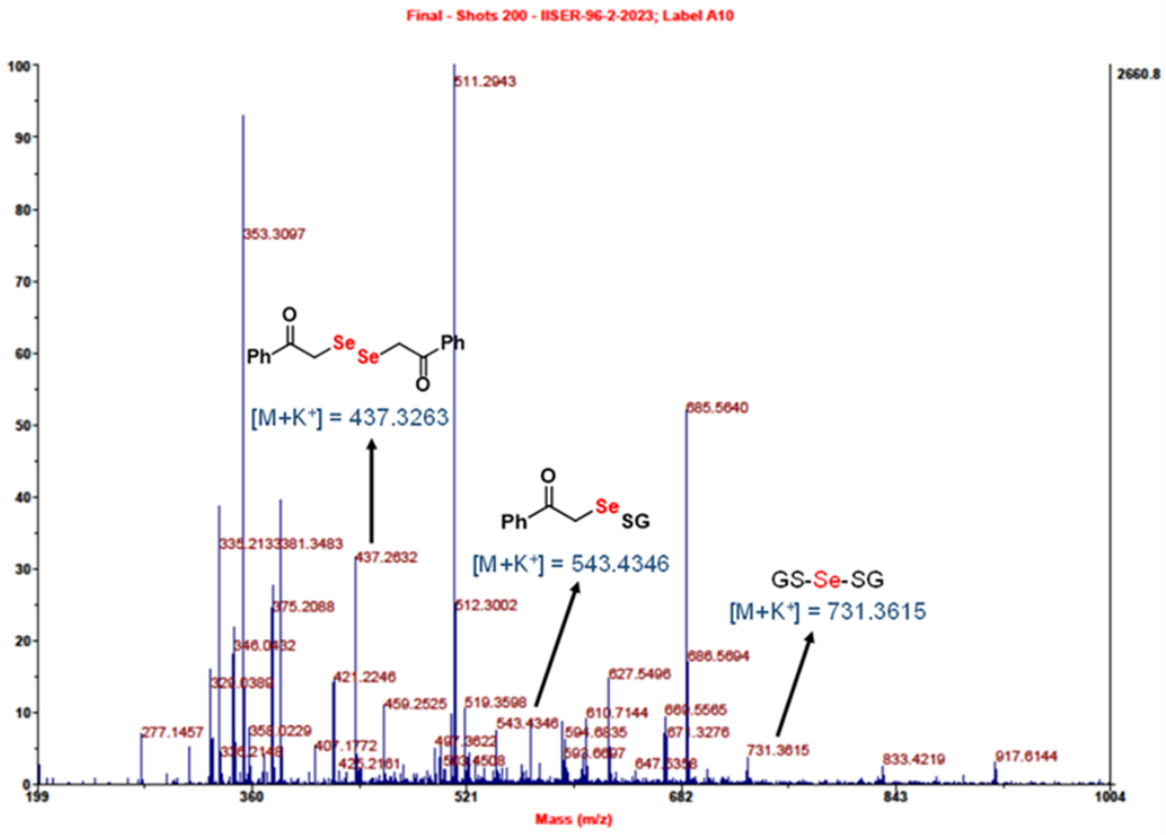


Figure S20. As per the **Scheme S3**, the reaction of **1b** in the presence of ES, GSH and GSSG analysed by mass spectrometry.

6. Supplementary Tables

Table S1. Crystal data and structure refinement for **1b**.

Empirical Formula	$C_{15}H_{12}O_2Se$
Formula Weight	303.21
Temperature/K	150.15
Crystal System	monoclinic
Space group	P 21/c
a/Å	13.0359(7)
b/Å	12.4928(7)
c/Å	7.6471(4)
α/°	90
β/°	97.074(2)
γ/°	90
Volume/Å^3	1235.89(12)
Z	4
Density (ρcalc)/g.cm^{-3}	1.630
Absorption coefficient (μ)/mm^{-1}	3.028
F(000)	608
Crystal size/mm^3	0.341 × 0.215 × 0.128
Radiation	MoKα (λ = 0.71073 Å)
2Θ range for data collection/°	4.532 to 50.138
Index ranges	$-15 \leq h \leq 15$, $-14 \leq k \leq 14$, $-8 \leq l \leq 9$
Reflections collected	34104
Independent reflections	2192 [Rint = 0.0460, Rsigma = 0.0164]
Data/restraints/parameters	2192/0/163
Goodness-of-fit on F^2	1.089
Final R indexes [I > 2σ(I)]	R1 = 0.0184, wR2 = 0.0445
Final R indexes [all data]	R1 = 0.0214, wR2 = 0.0459
Largest diff. peak and hole/ eÅ^{-3}	0.28 and -0.27

Table S2. Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for **1b.** U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*y*	*z*	U(eq)
Se1	3710.4(2)	7036.2(2)	3778.3(2)	18.42(7)
O1	3979.8(9)	4863.4(10)	3432.9(15)	16.9(3)
C1	4375.3(13)	5715.0(14)	3190(2)	13.3(3)
O2	1695.0(10)	6187.0(11)	1591.2(16)	21.9(3)
C2	5353.8(13)	5849.0(14)	2398(2)	13.2(3)
C3	5819.0(13)	4933.6(14)	1809(2)	15.9(4)
C6	6675.2(14)	6930.4(15)	1327(2)	19.9(4)
C5	7126.5(14)	6017.8(15)	728(2)	20.2(4)
C4	6700.9(14)	5018.3(15)	977(2)	18.6(4)
C7	5789.2(13)	6849.0(14)	2159(2)	16.3(4)
C8	2517.1(13)	6336.8(13)	4564(2)	12.8(3)
C9	1822.0(13)	5798.7(14)	3065(2)	15.2(4)
C10	1233.6(13)	4828.9(14)	3497(2)	14.4(4)
C11	1603.8(13)	4118.6(14)	4835(2)	15.6(4)
C12	1035.4(14)	3220.7(14)	5165(2)	18.7(4)
C13	90.7(14)	3022.9(15)	4171(3)	21.5(4)
C14	-288.2(14)	3730.0(16)	2847(2)	23.2(4)
C15	278.3(13)	4626.0(15)	2505(2)	19.1(4)

Table S3. Anisotropic Displacement Parameters ($Å^2 \times 10^3$) for **1b**. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+\ldots]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
Se1	18.21(11)	11.62(10)	27.11(11)	0.50(7)	9.53(7)	-0.30(7)
O1	19.5(6)	12.9(6)	18.6(6)	0.1(5)	4.3(5)	-2.5(5)
C1	15.0(9)	14.0(9)	10.3(8)	-0.5(7)	-0.8(6)	0.8(7)
O2	19.7(7)	28.6(7)	17.5(7)	7.9(6)	2.3(5)	5.9(6)
C2	13.7(8)	16.1(9)	9.1(8)	1.7(7)	-1.4(6)	0.2(7)
C3	18.3(9)	15.9(9)	13.0(8)	0.6(7)	-0.4(7)	-0.5(7)
C6	17.7(9)	20.4(10)	21.3(9)	6.8(7)	1.0(7)	-3.4(8)
C5	15.1(9)	30.5(11)	15.1(9)	3.7(8)	2.2(7)	0.2(8)
C4	18.2(9)	21.8(9)	15.6(9)	-1.2(7)	1.7(7)	4.2(8)
C7	17.0(9)	15.5(9)	15.7(9)	1.7(7)	-0.2(7)	1.1(7)
C8	11.4(8)	13.6(9)	14.0(8)	1.8(7)	4.3(7)	-0.7(7)
C9	12.2(8)	17.4(9)	16.6(9)	1.6(7)	4.3(7)	7.6(7)

C10	13.6(8)	17.7(9)	12.6(8)	-3.9(7)	4.1(7)	3.5(7)
C11	13.3(8)	16.5(9)	16.7(9)	-2.8(7)	0.5(7)	2.3(7)
C12	19.9(9)	16.6(9)	20.3(9)	-1.2(7)	5.5(7)	3.0(7)
C13	19.0(9)	19.2(10)	27.9(10)	-8.2(8)	9.1(8)	-3.7(8)
C14	13.4(9)	33.2(11)	22.7(10)	-11.0(8)	1.3(7)	-0.9(8)
C15	16.6(9)	26.2(10)	14.3(9)	-2.5(7)	1.4(7)	5.2(8)

Table S4. Bond lengths for **1b**.

Atom	**Atom**	**Length/Å**	**Atom**	**Atom**	**Length/Å**
Se1	C1	1.9427(17)	C5	C4	1.389(3)
Se1	C8	1.9429(16)	C8	C9	1.527(2)
O1	C1	1.206(2)	C9	C10	1.492(2)
C1	C2	1.487(2)	C10	C11	1.395(2)
O2	C9	1.220(2)	C10	C15	1.399(2)
C2	C3	1.395(2)	C11	C12	1.385(3)
C2	C7	1.393(2)	C12	C13	1.387(3)
C3	C4	1.385(3)	C13	C14	1.388(3)
C6	C5	1.386(3)	C14	C15	1.384(3)
C6	C7	1.389(3)			

Table S5. Bond Angles for **1b**.

Atom	**Atom**	**Atom**	**Angle/°**	**Atom**	**Atom**	**Atom**	**Angle/°**
C1	Se1	C8	94.96(7)	C9	C8	Se1	112.92(11)
O1	C1	Se1	120.15(13)	O2	C9	C8	121.29(16)
O1	C1	C2	124.47(15)	O2	C9	C10	120.82(16)
C2	C1	Se1	115.35(12)	C10	C9	C8	117.71(14)
C3	C2	C1	117.90(15)	C11	C10	C9	122.54(15)
C7	C2	C1	122.53(15)	C11	C10	C15	119.14(17)
C7	C2	C3	119.50(16)	C15	C10	C9	118.32(16)
C4	C3	C2	120.28(17)	C12	C11	C10	120.31(16)
C5	C6	C7	120.11(17)	C11	C12	C13	120.21(17)
C6	C5	C4	120.09(17)	C12	C13	C14	119.90(17)
C3	C4	C5	119.97(17)	C15	C14	C13	120.16(17)
C6	C7	C2	120.04(17)	C14	C15	C10	120.28(17)

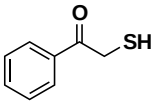
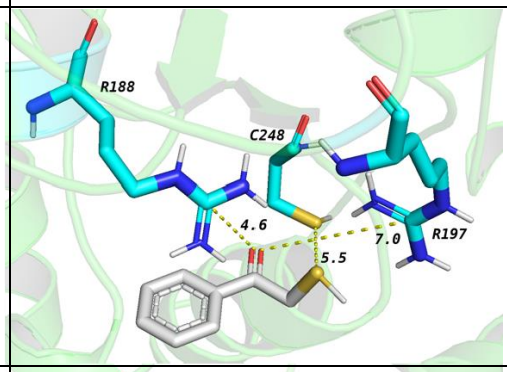
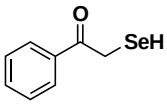
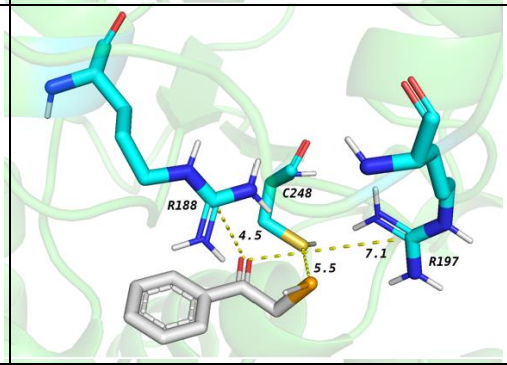
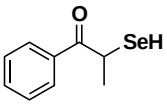
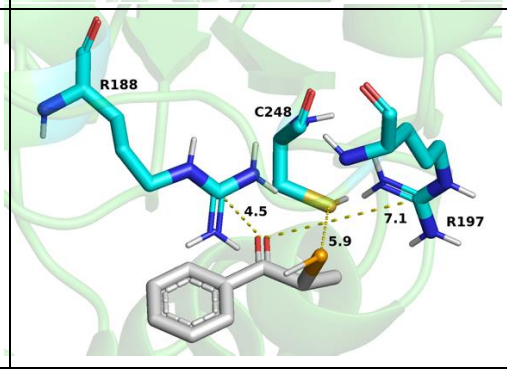
Table S6. Torsion Angles for **1b**.

A	B	C	D	Angle/°	A	B	C	D	Angle/°
Se1	C1	C2	C3	-174.58(12)	C5	C6	C7	C2	0.2(3)
Se1	C1	C2	C7	2.6(2)	C7	C2	C3	C4	-0.7(2)
Se1	C8	C9	O2	-35.0(2)	C7	C6	C5	C4	-0.8(3)
Se1	C8	C9	C10	149.87(12)	C8	C9	C10	C11	-30.9(2)
O1	C1	C2	C3	3.6(2)	C8	C9	C10	C15	149.65(15)
O1	C1	C2	C7	-179.28(16)	C9	C10	C11	C12	-178.98(15)
C1	C2	C3	C4	176.57(15)	C9	C10	C15	C14	179.30(15)
C1	C2	C7	C6	-176.58(15)	C10	C11	C12	C13	-0.3(3)
O2	C9	C10	C11	154.01(16)	C11	C10	C15	C14	-0.2(3)
O2	C9	C10	C15	-25.5(2)	C11	C12	C13	C14	-0.3(3)
C2	C3	C4	C5	0.1(3)	C12	C13	C14	C15	0.6(3)
C3	C2	C7	C6	0.5(2)	C13	C14	C15	C10	-0.3(3)
C6	C5	C4	C3	0.6(3)	C15	C10	C11	C12	0.5(2)

Table S7. Hydrogen Atom Coordinates (Å×10^4) and Isotropic Displacement Parameters (Å^2×10^3) for **1b.**

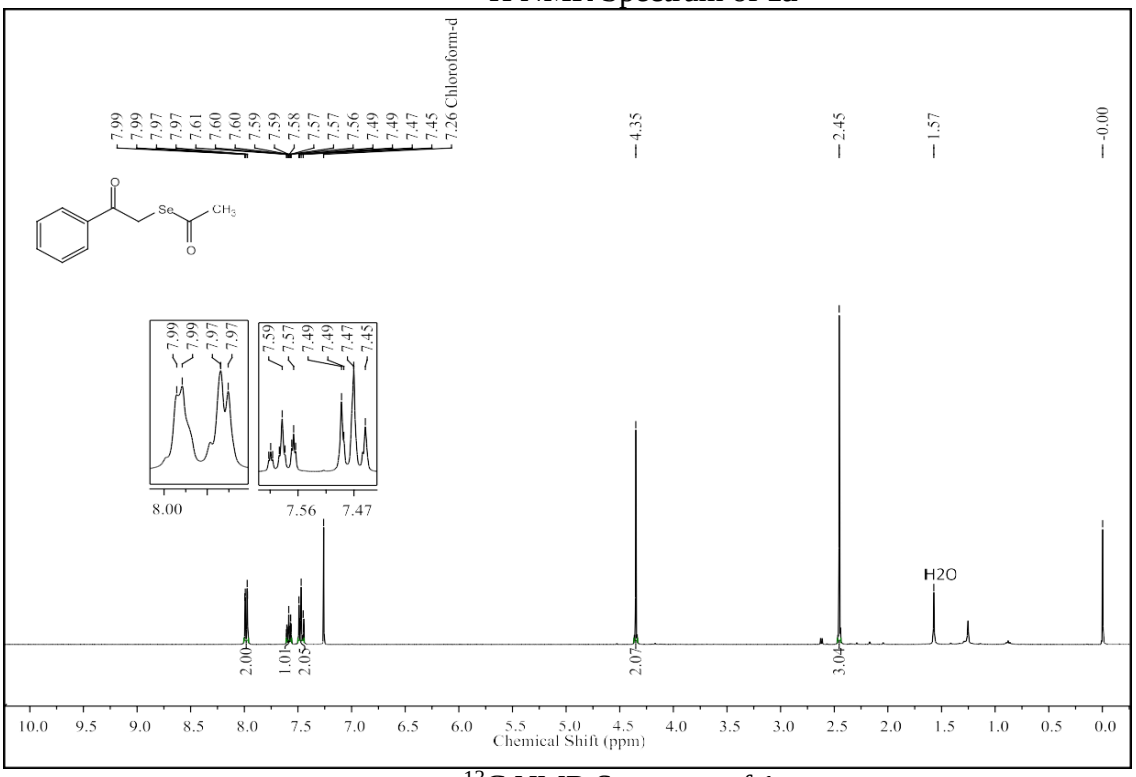
Atom	*x*	*y*	*z*	U(eq)
H3	5530	4249	1979	19
H6	6972	7613	1169	24
H5	7728	6076	147	24
H4	7015	4393	576	22
H7	5480	7475	2566	20
H8A	2758	5791	5461	15
H8B	2108	6874	5132	15
H11	2249	4252	5523	19
H12	1293	2739	6075	22
H13	-296	2405	4397	26
H14	-938	3598	2173	28
H15	18	5105	1592	23

Table S8: Artificial substrate **5** for 3-MST enzyme[6] (derived from **4**, the sulfur counterpart of **1a**) shows a favorable anchoring of the intermediate by the two arginine residues (R188 and R197). The C248 residue is at a distance of 5.5 Å from the Se atom. **(B-C)** Docking analysis of the α-selenoketone intermediate derived from **1b** and the methylated α-selenoketone intermediate derived from **1c** reveals a similar anchoring of the intermediate by the two arginine residues and the S-Se bond distance was found to be 5.9 Å.

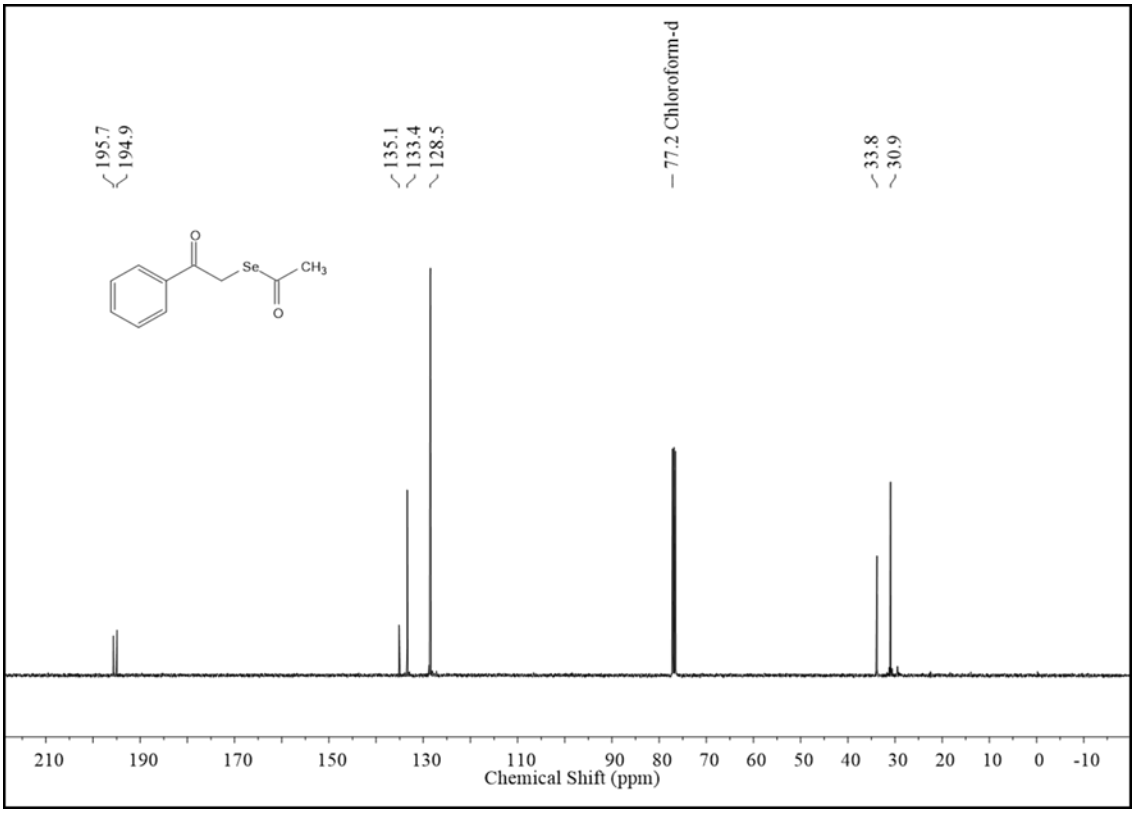
Comp.No.	Structure	Docking Score (kcal/mol)	S-Se or S-S Distance (Å)	Lowest Energy Conformation
Phenacylthiol		**-4.7**	**5.5**	
PhenacylSelenol		**-4.7**	**5.5**	
Methyl-PhenacylSelenol		**-5.2**	**5.9**	

7. NMR Spectra

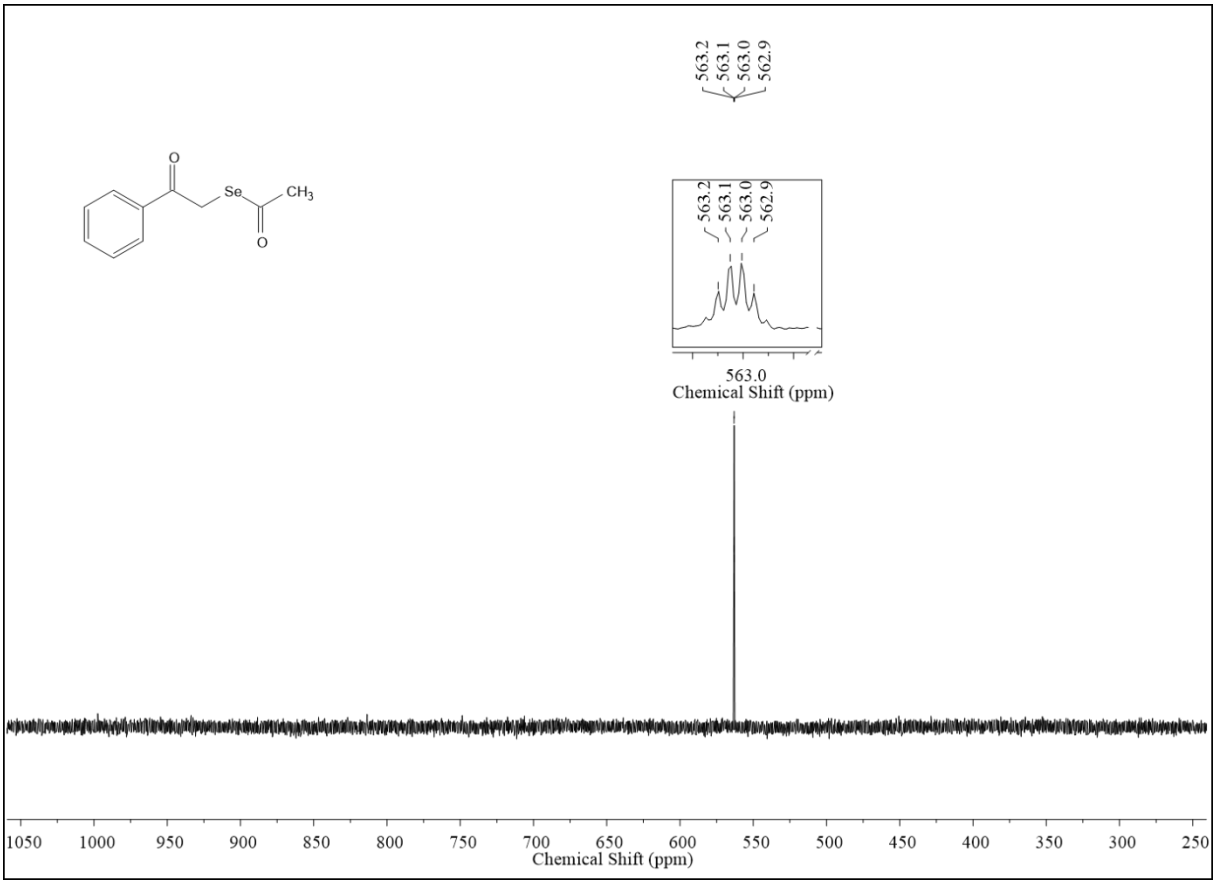
^{1}H NMR Spectrum of **1a**



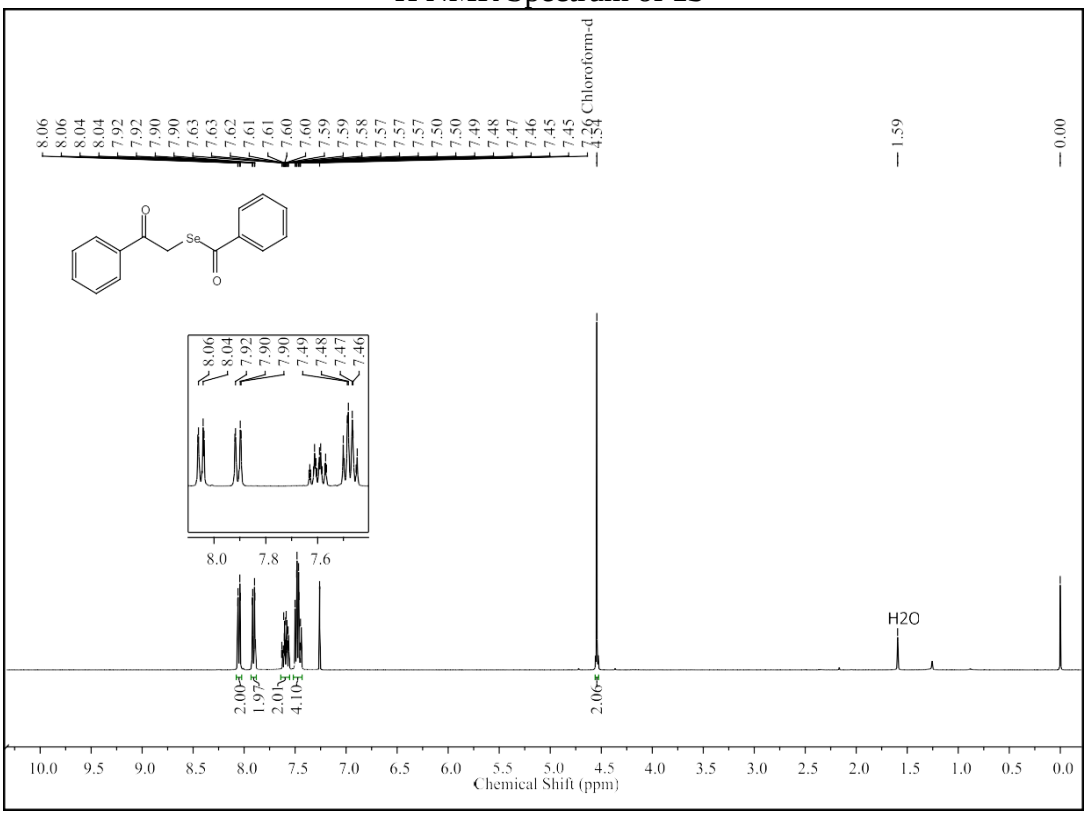
^{13}C NMR Spectrum of **1a**



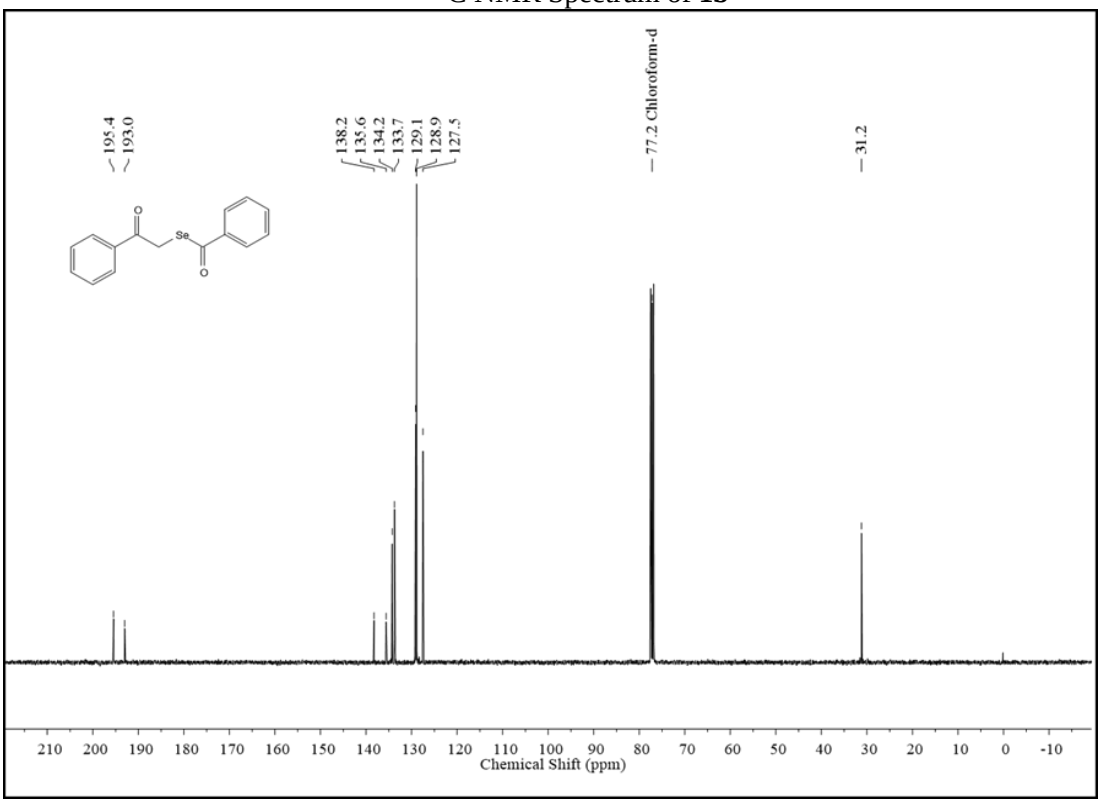
^{77}Se NMR Spectrum of **1a**



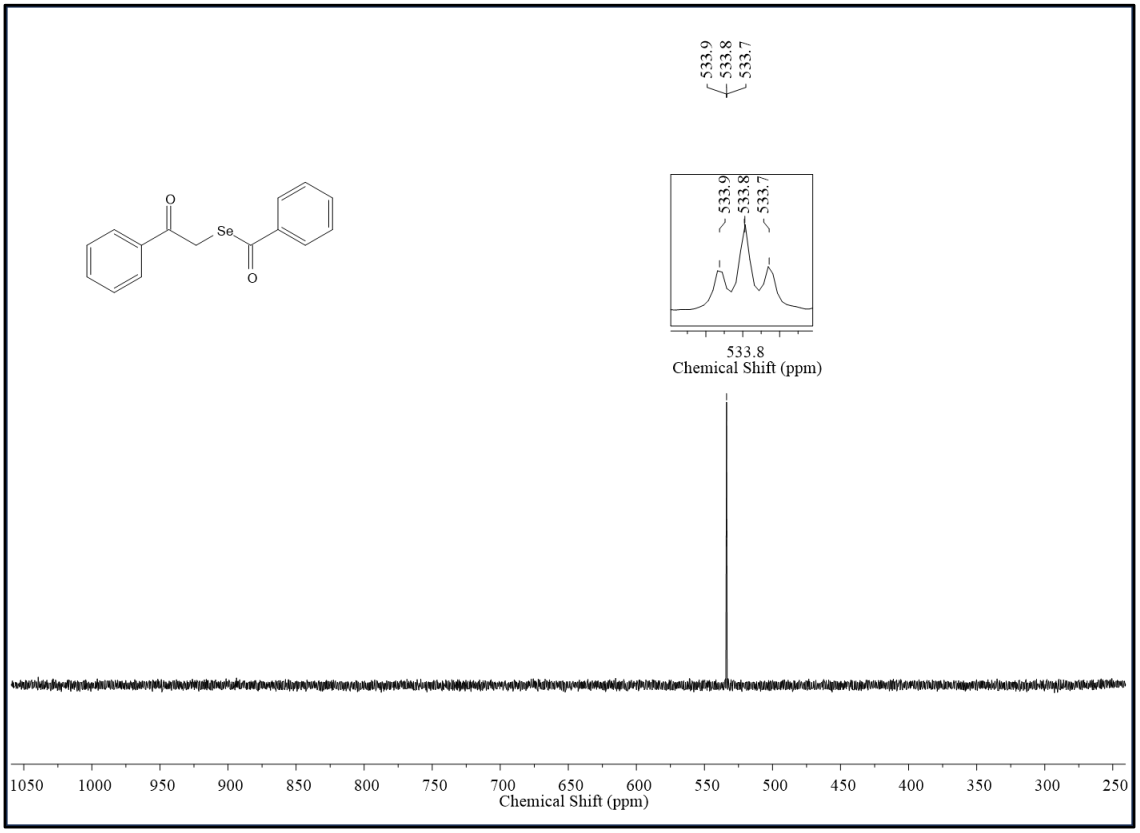
^{1}H NMR Spectrum of **1b**



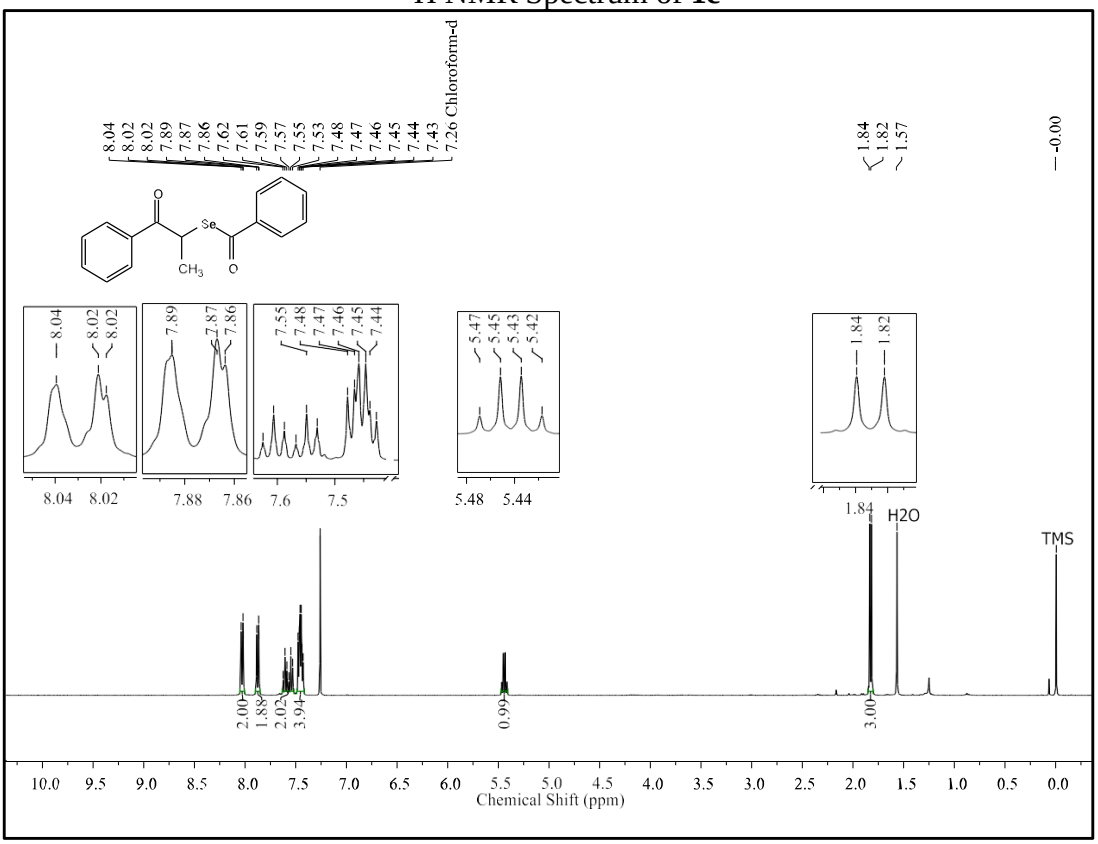
^{13}C NMR Spectrum of **1b**



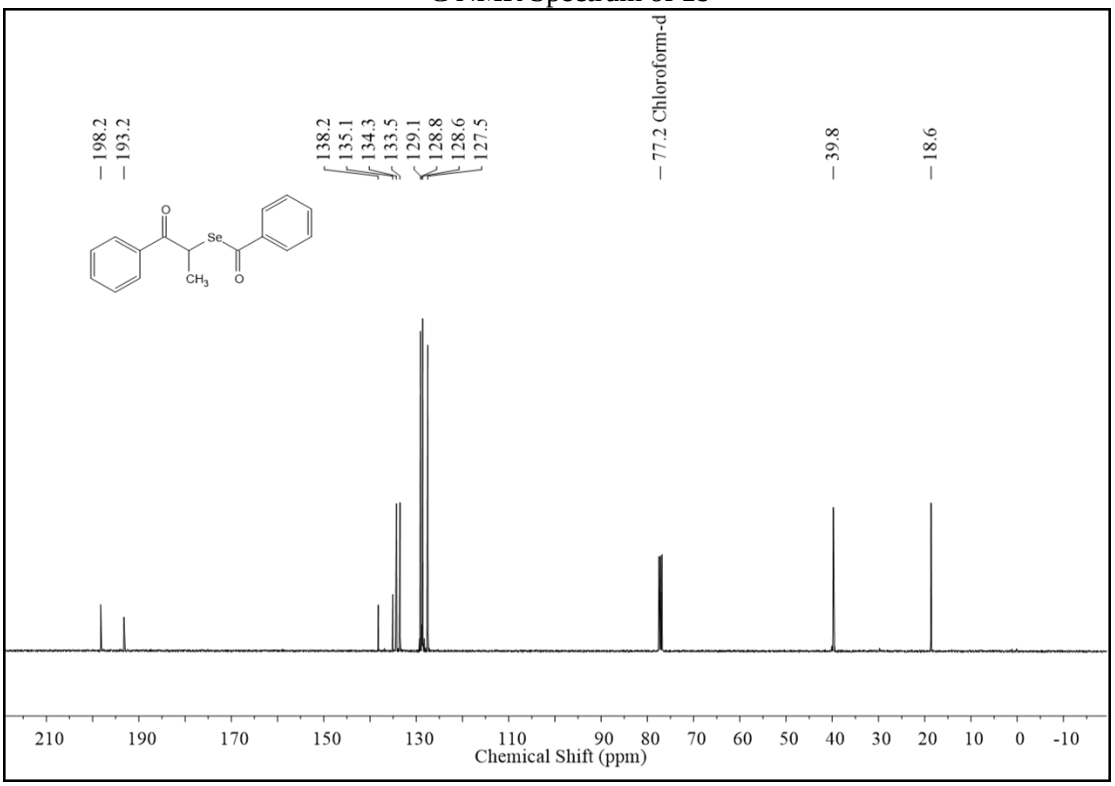
^{77}Se NMR Spectrum of **1b**



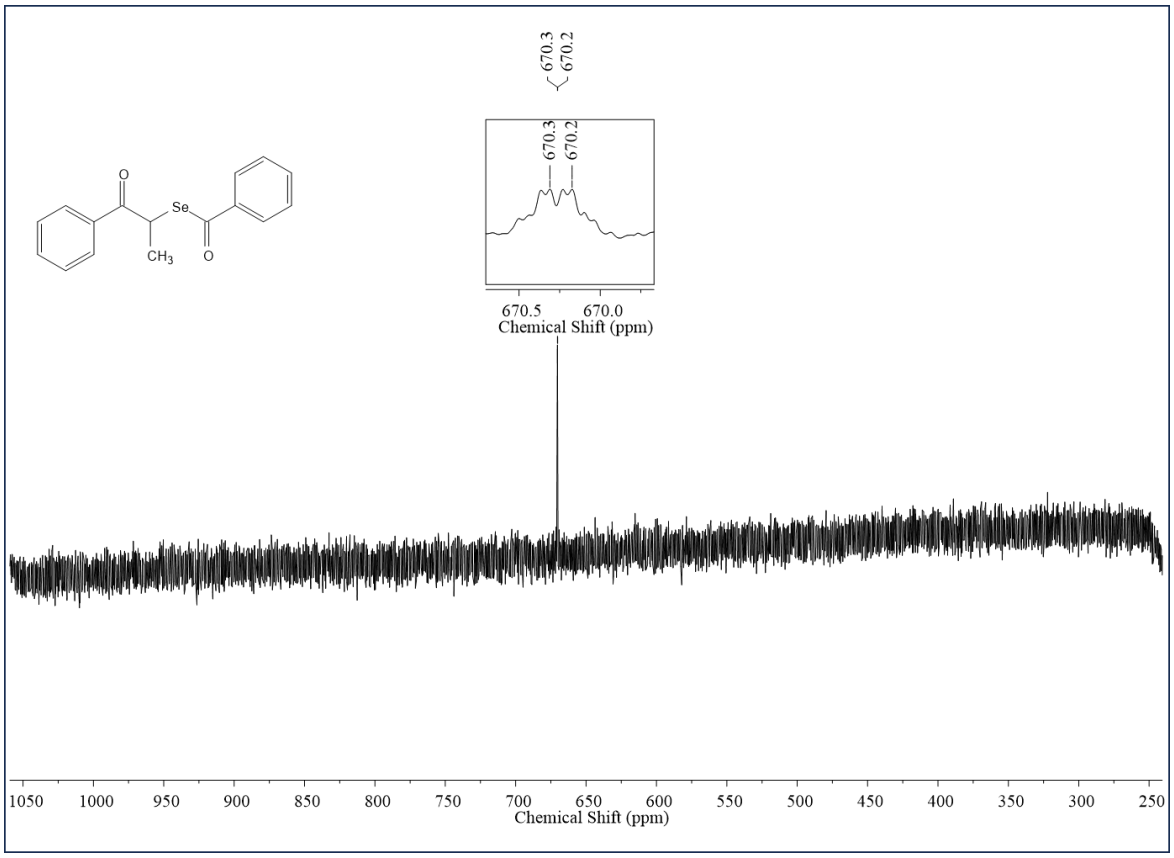
^{1}H NMR Spectrum of **1c**



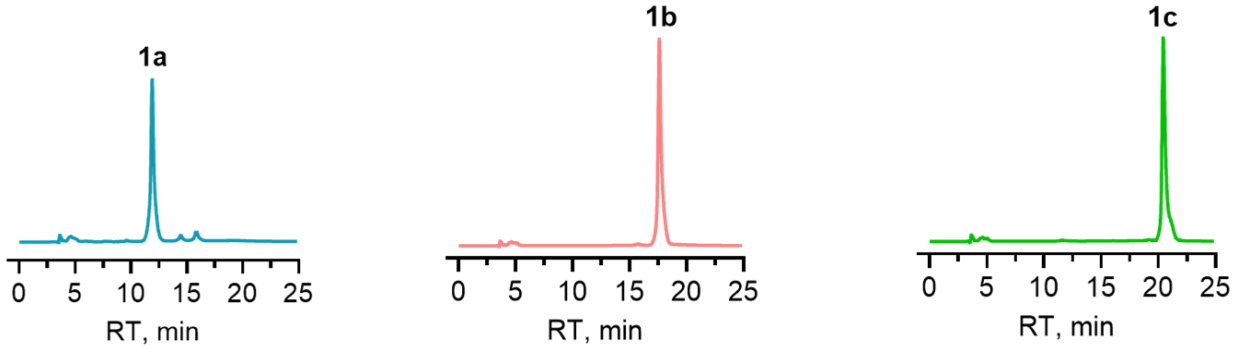
^{13}C NMR Spectrum of **1c**



^{77}Se NMR Spectrum of **1c**



8. HPLC traces for purity of the compounds



HPLC traces (250 nm) for **1a**, **1b** and **1c** with retention times (RT) 11.7 min, 17.3 min and 20.4 min respectively.

9. References

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