Supporting Information

Development of a Hydrolysis-Based Small-Molecule Hydrogen Selenide (H_2Se) Donor

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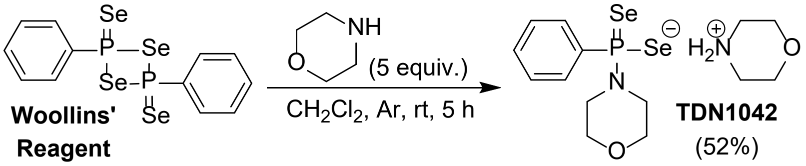
Materials and Methods

Reagents were purchased from Sigma-Aldrich, Alfa Aesar, and TCI Chemicals and were and used directly as received. Deuterated solvents were purchased from Cambridge Isotope Laboratories and used directly as received. ^{1}H, ^{13}C{^{1}H}, ^{31}P, and ^{77}Se NMR spectra were recorded on Bruker 500 and 600 MHz instruments. Chemical shifts are reported relative to residual protic solvent resonances for ^{1}H and ^{13}C{^{1}H} spectra. All air-free manipulations were performed in an inert atmosphere using standard Schlenk techniques or an Innovative Atmospheres N_2-filled glove box.

Synthesis of TDN1042



Scheme S1. Synthesis of TDN1042 from Woollins' Reagent

Morpholinium morpholinophenylphosphinodiselenoate (TDN1042). Woollins' reagent (0.54 g, 1.0 mmol) was added to anhydrous CH_2Cl_2 (10 mL) in a flame-dried round bottom flask under argon. Morpholine (0.44 mL, 5.1 mmol) was added using an air-tight syringe, and the resultant reaction mixture was allowed to stir for 5 h at room temperature. The reaction mixture was then filtered, leaving behind a black precipitate. The resultant golden-yellow filtrate was concentrated under reduced pressure to approximately 10% of the initial volume and cooled to 0 °C to promote crystallization. The resulting precipitate was isolated via filtration, washed with CH_2Cl_2 (3.0 mL), and dried overnight under reduced pressure to afford a white, microcrystalline solid (0.46 g, 52% yield). The identity of the product was verified by ^{1}H, ^{13}C{^{1}H}, ^{31}P, and ^{77}Se-NMR. Crystals suitable for structural determination were obtained by slow layering of hexane into a dilute solution of the product and CH_2Cl_2. ^{1}H NMR (600 MHz, DMSO-d^{6}) δ: 8.67 (s, 2H), 8.08 (dt, 1H, $^{3}J_{P\text{-}H}$ = 2 Hz), 8.07(dt, 1H, $^{3}J_{P\text{-}H}$ = 1 Hz), 7.30 (m, 3H), 3.76 (m, 4H), 3.50 (t, 4H), 3.11 (m, 4H), 2.75 (m, 4H). ^{13}C{^{1}H} NMR (151 MHz, DMSO-d^{6}) δ: 142.97, 141.95, 130.78, 128.68, 126.57, 66.21, 63.34, 45.45, 42.93. ^{31}P NMR (241 MHz, DMSO-d^{6}) δ: 62.10 (s, $^{1}J_{P\text{-}Se}$ = 671 Hz). ^{77}Se NMR (115 MHz, DMSO-d^{6}) δ: 8.22 (d, ^{1}J = 671 Hz). TOF MS (ES^{-}) (*m*/*z*): [M + H]$^{+}$ calc'd for $C_{10}H_{13}NOPSe_2$ 353.9065; found 353.9064.

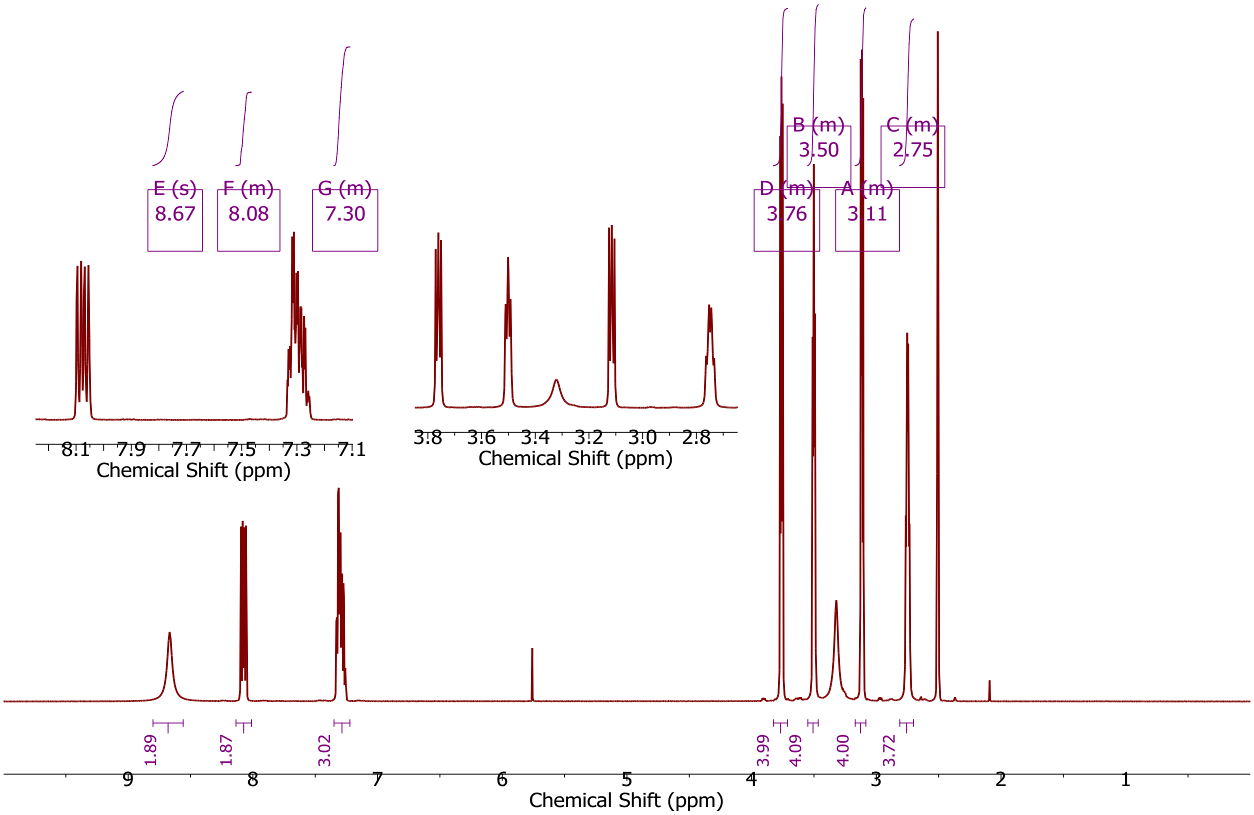


Figure S1. ^{1}H NMR (600 MHz, DMSO-d^6) spectrum of TDN1042.

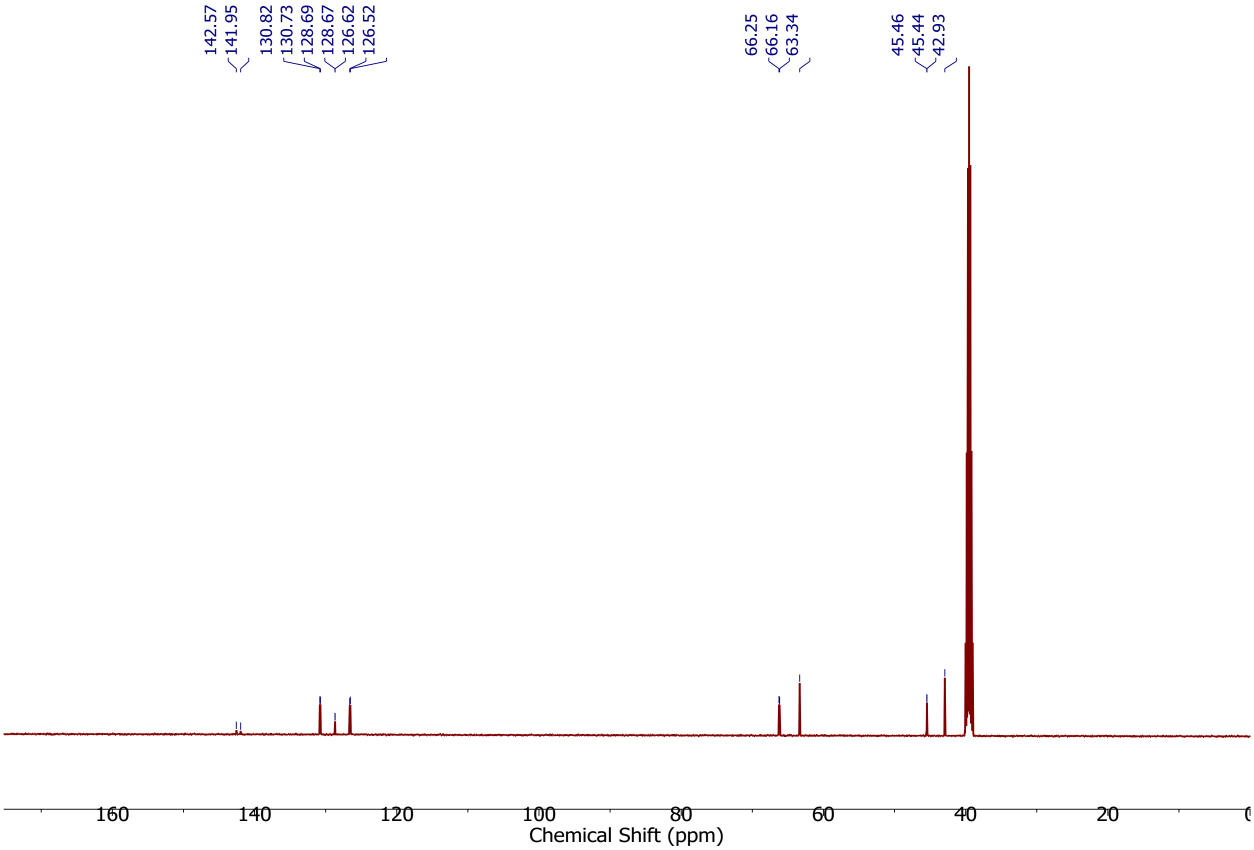


Figure S2. $^{13}C\{^1H\}$ NMR (151 MHz, DMSO-d^6) spectrum of TDN1042.

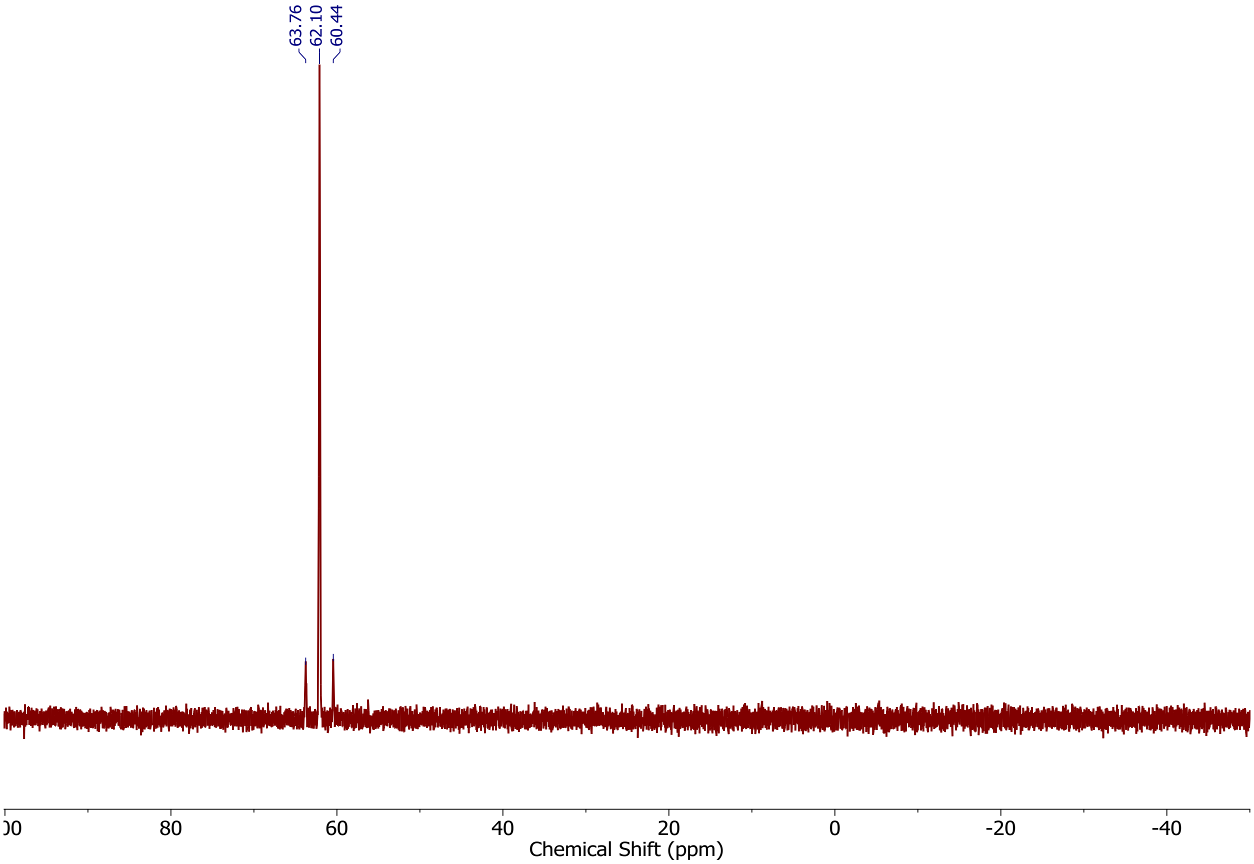


Figure S3. ^{31}P NMR (241 MHz, DMSO-d^6) spectrum of TDN1042.

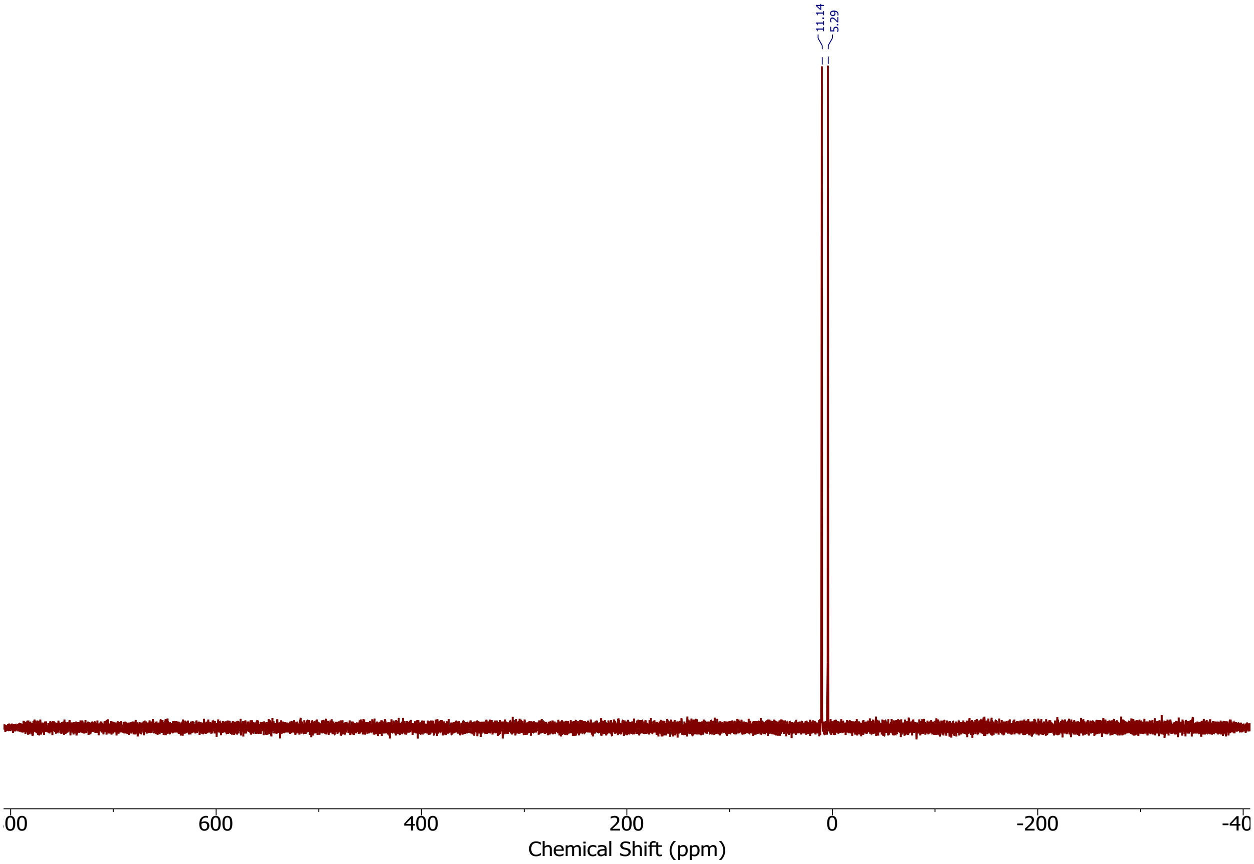


Figure S4. ^{77}Se NMR (115 MHz, DMSO-d^6) spectrum of TDN1042.

Hydrolysis Studies

Stock solutions of TDN1042 (70 mM) and triethylphosphate (35 mM) were prepared in DMSO-d^6 in GC vials in a glovebox. Aliquots of 0.10 mL of each stock solution were added to 0.50 mL citrate or HEPES buffers (pH 3.0-7.4, 50 mM) in NMR tubes. These NMR tubes were removed from the glovebox and flame sealed under vacuum.

Recorded spectra:

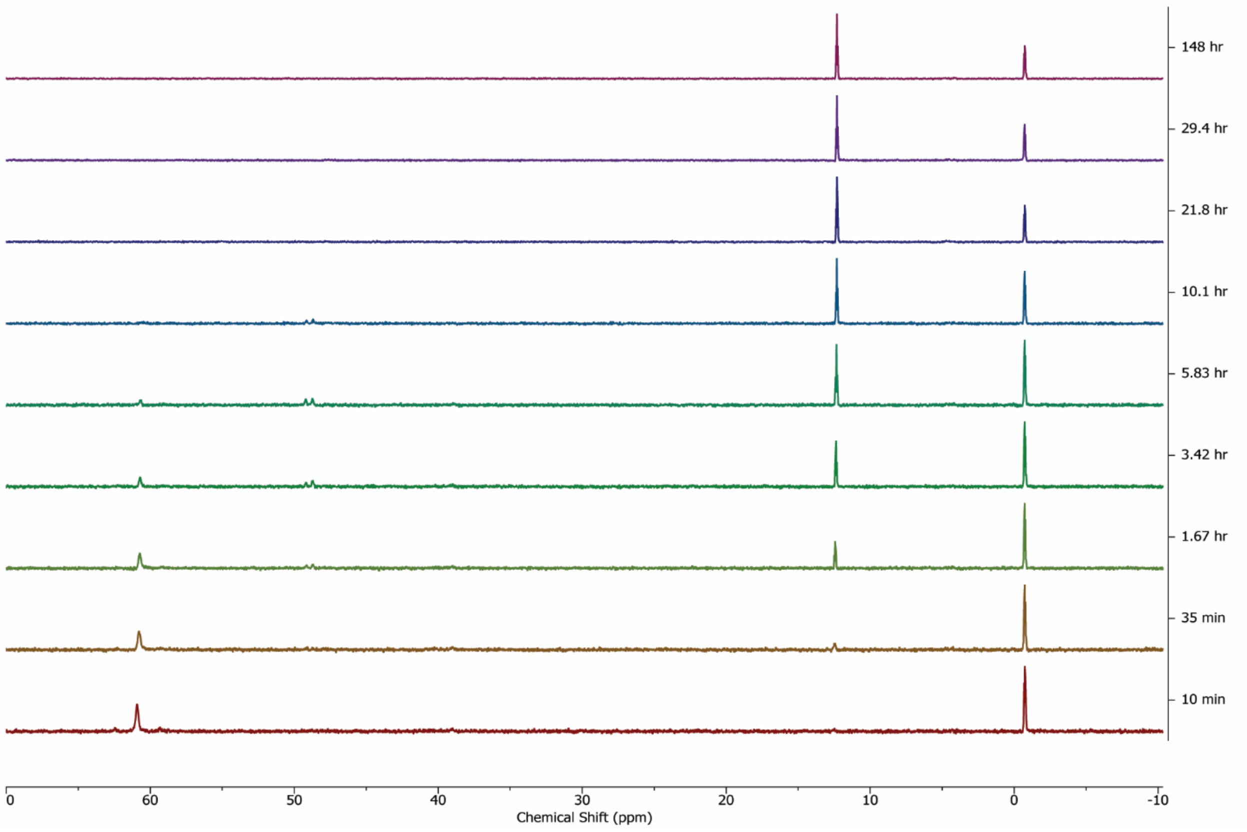


Figure S5. ^{31}P NMR (202 MHz, DMSO-d^6) spectra of TDN1042 hydrolysis at pH 3.0.

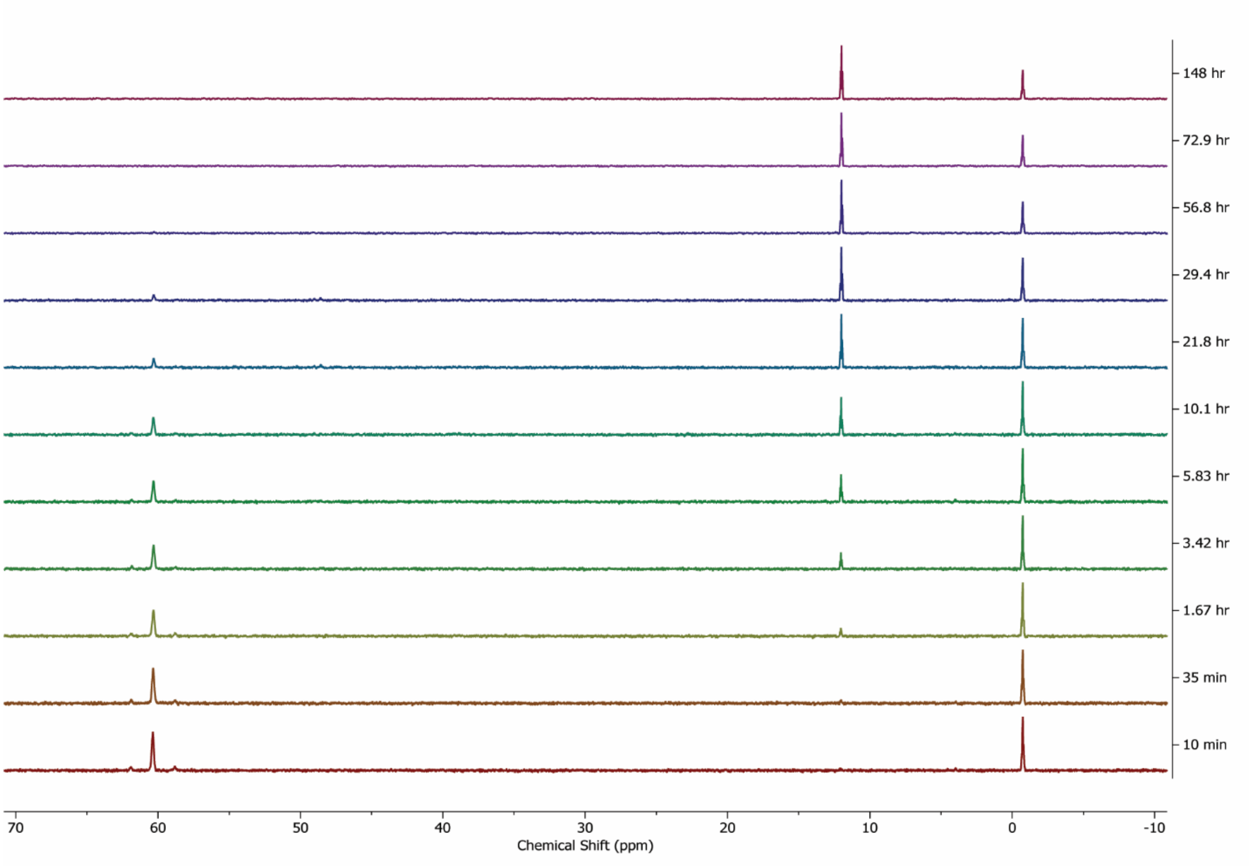


Figure S6. ^{31}P NMR (202 MHz, DMSO-d^6) spectra of TDN1042 hydrolysis at pH 3.6.

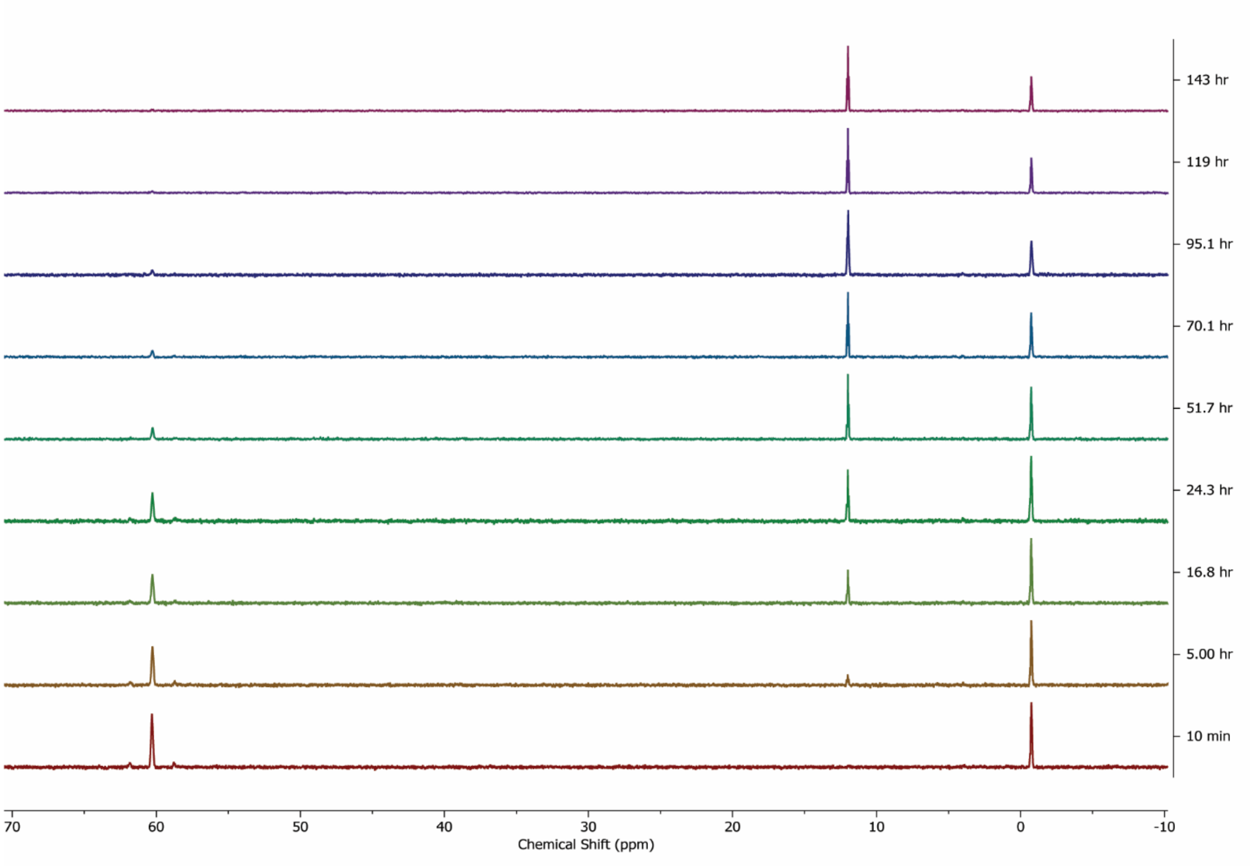


Figure S7. ^{31}P NMR (202 MHz, DMSO-d^{6}) spectra of TDN1042 hydrolysis at pH 4.0.

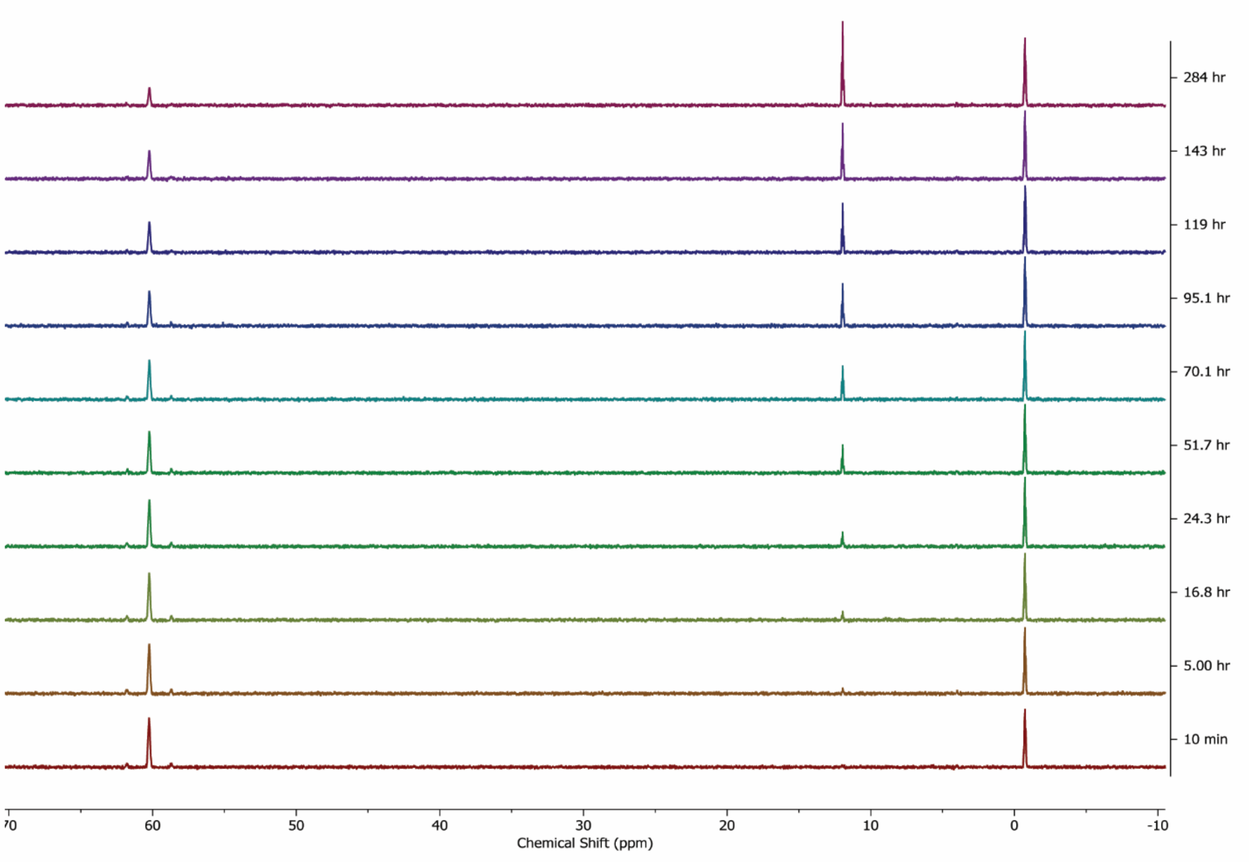


Figure S8. ^{31}P NMR (202 MHz, DMSO-d^{6}) spectra of TDN1042 hydrolysis at pH 5.0.

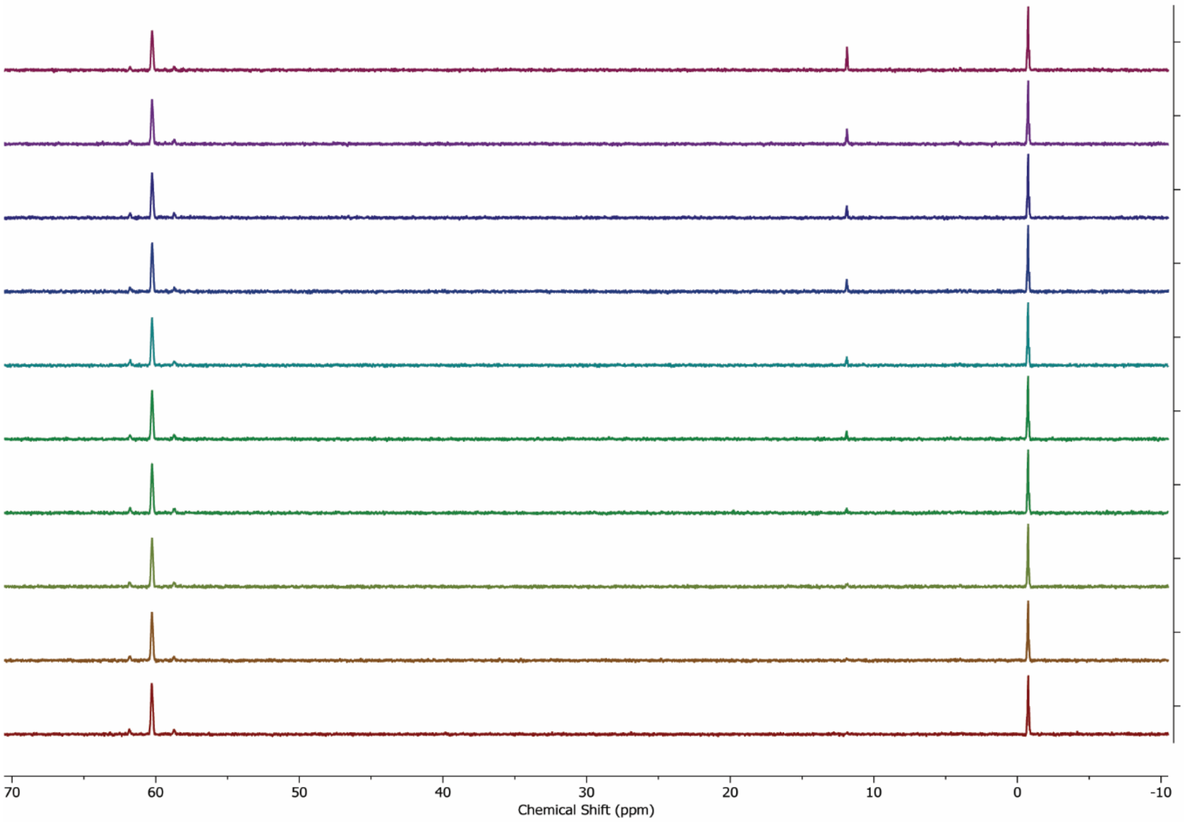
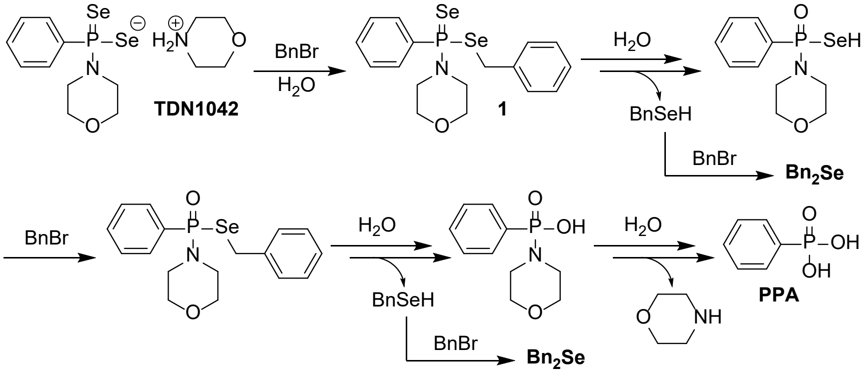


Figure S9. ^{31}P NMR (202 MHz, DMSO-d^{6}) spectra of TDN1042 hydrolysis at pH 6.0.

H_2Se Trapping Experiments

Trapping Experiments with BnBr.

In a glovebox, TDN1042 (20 mg, 0.045 mmol) was dissolved in DMSO-d^6 (0.50 mL) in an NMR tube, capped with a septum, and sealed with electrical tape. Benzyl bromide (BnBr, 44 µL) was dissolved in DMSO-d^6 (0.20 mL) in a septum-capped vial in a glovebox. Baseline ^{1}H, ^{13}C{^{1}H}, ^{31}P, and ^{77}Se NMR spectra of the TDN1042 solution were acquired prior to addition of the BnBr stock solution (0.10 mL, 0.18 mmol) and degassed Millipore water (25 µL, 1.4 mmol) were injected by syringe. This reaction was monitored by NMR spectroscopy over the course of two weeks. Analysis revealed the formation of an alkylated intermediate (**1**) (δ(^{31}P) = 69 ppm; δ(^{77}Se) = 354 ppm, -129 ppm) and the generation of both the Bn_2Se (δ(^{77}Se) = 330 ppm) and Bn_2Se_2 product (δ(^{77}Se) = 394 ppm), the latter of which is generated through auto-oxidation.



Scheme S3. Proposed pathway for the hydrolysis of TDN1042 in the presence of BnBr, leading to an alkylated intermediate (1) with further hydrolysis affording a trapped selenide product Bn_2Se (auto-oxidation pathway to Bn_2Se_2 is not included here).

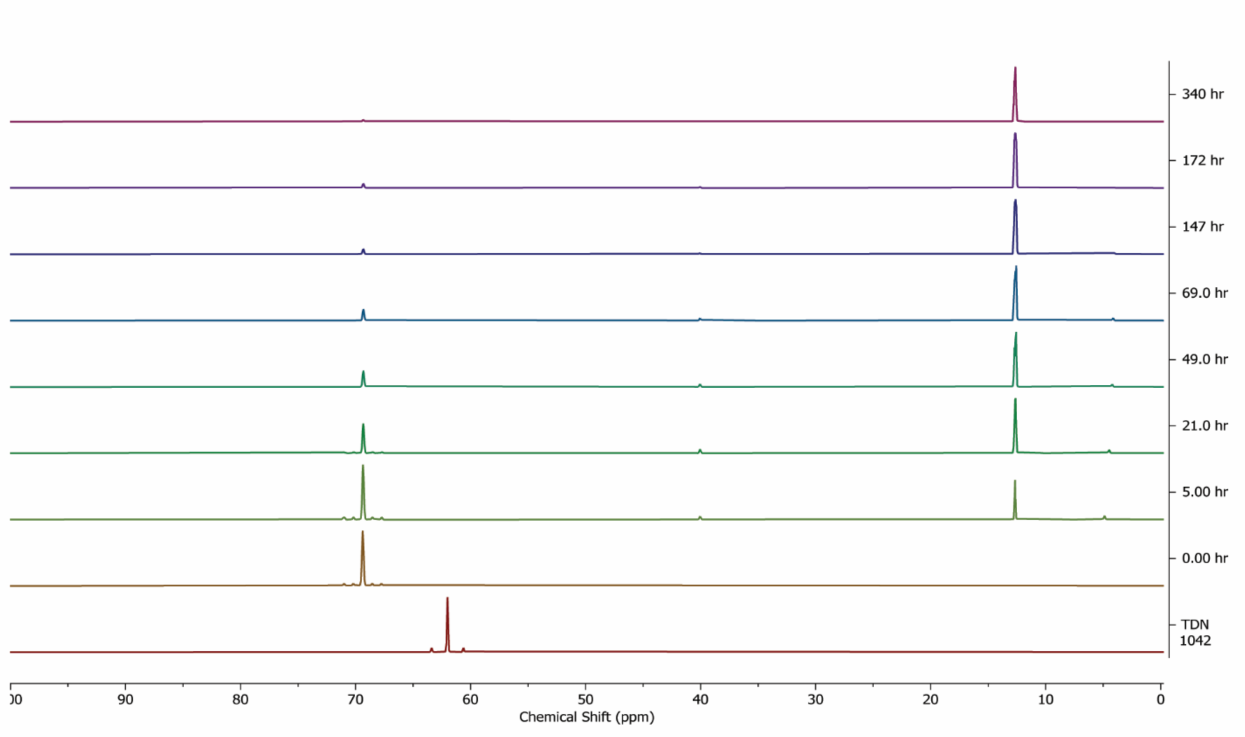


Figure S11. ^{31}P NMR (242 MHz, DMSO-d^6) spectra of TDN1042 hydrolysis in the presence of BnBr.

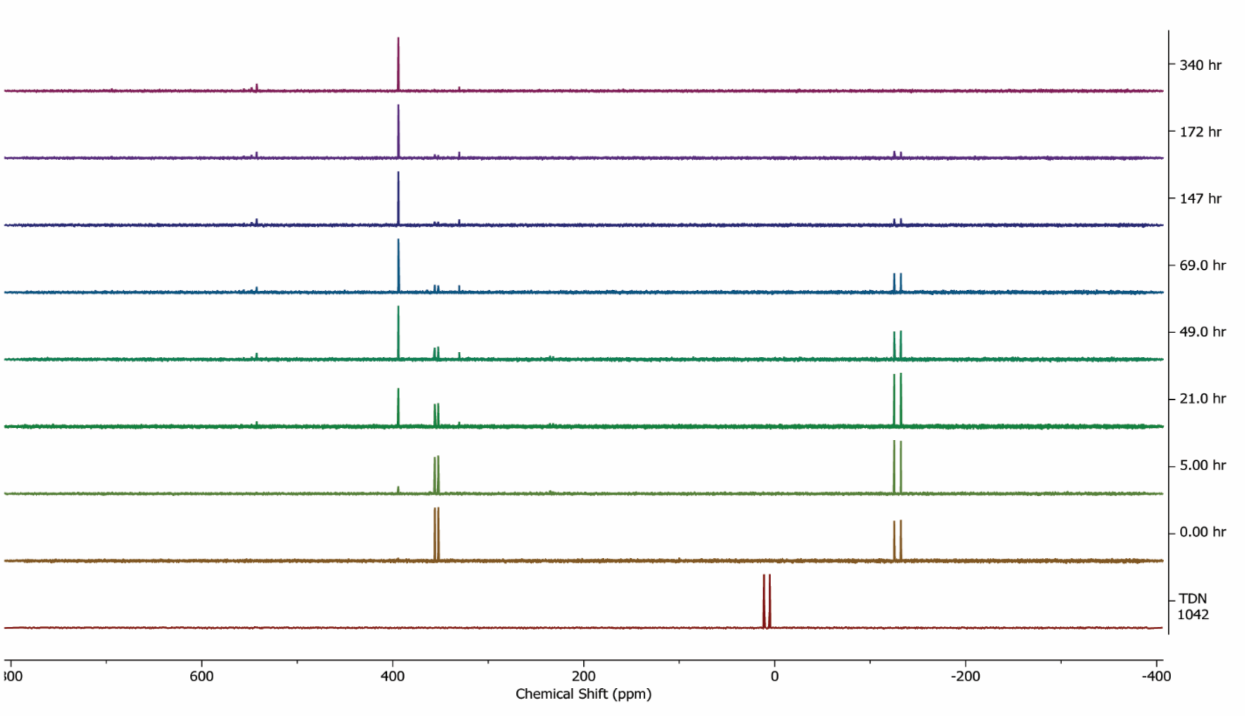


Figure S12. ^{77}Se NMR (115 MHz, DMSO-d^6) spectra of TDN1042 hydrolysis in the presence of BnBr.

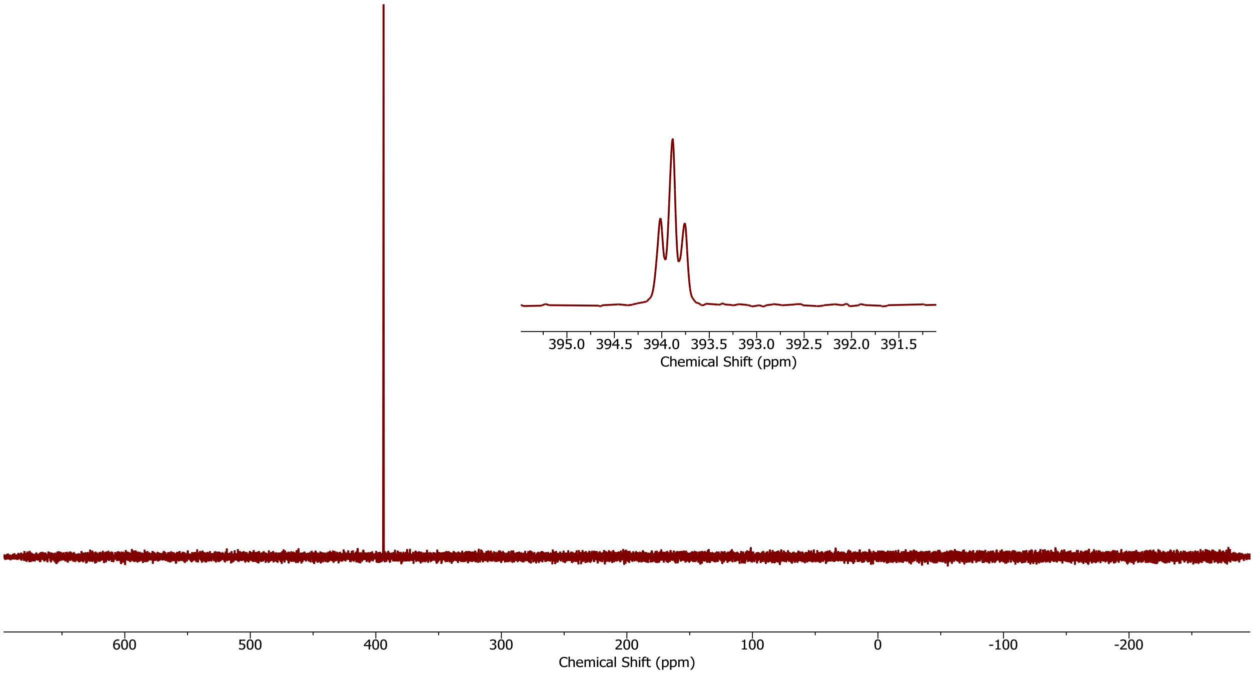
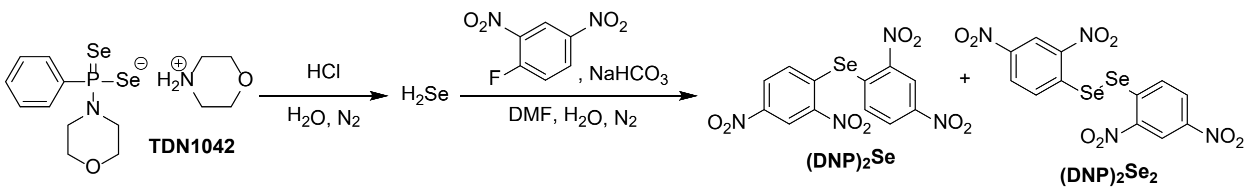


Figure S13. ^{77}Se NMR (115 MHz, DMSO-d^6) spectrum of an authentic sample of Bn_2Se_2.

Trapping Experiments with FDNB.

Volatilization and trapping apparatus can be seen in the main text (Figure 5a). The sample vial (centrifuge tube, 15 mL) contained H_2O (2.0 mL, 0.11 mol), TDN1042 (30 mg, 0.068 mmol), and *n*-octanol (2 drops) was connected with PE tubing to the trapping vial (15 mL glass tube). This trapping vial contained FDNB (15 µL, 0.12 mmol) and $NaHCO_3$ (15 mg, 0.18 mmol) in *N*,*N*-dimethylformamide (DMF, 1.6 mL) and H_2O (0.40 mL), and it was connected to the second trapping vial (15 mL glass tube) that contained a solution of $AgNO_3$ (3.0 mL, 0.10 M in H_2O, 0.30 mmol) meant for sequestering any excess H_2Se. Then, a N_2 line was connected to the Y-adapter, the Y-adapter's other side was sealed, and the Y-adapter was connected to the sample tube. The apparatus was purged with N_2 for 15 minutes, then concentrated HCl (3.0 mL, 36 mmol) was quickly injected through the sealed side of the Y-tube, and the reaction was allowed to proceed for 30 minutes. After this time, the FDNB-containing vial was extracted with benzene (3 x 10 mL), and this extract was dried over $MgSO_4$ and filtered before being concentrated *in vacuo*. The resulting oil was dissolved in DMSO and subjected to HPLC analysis.



Scheme S4. Pathway for the volatilization of H_2Se from TDN1042 and trapping with FDNB.

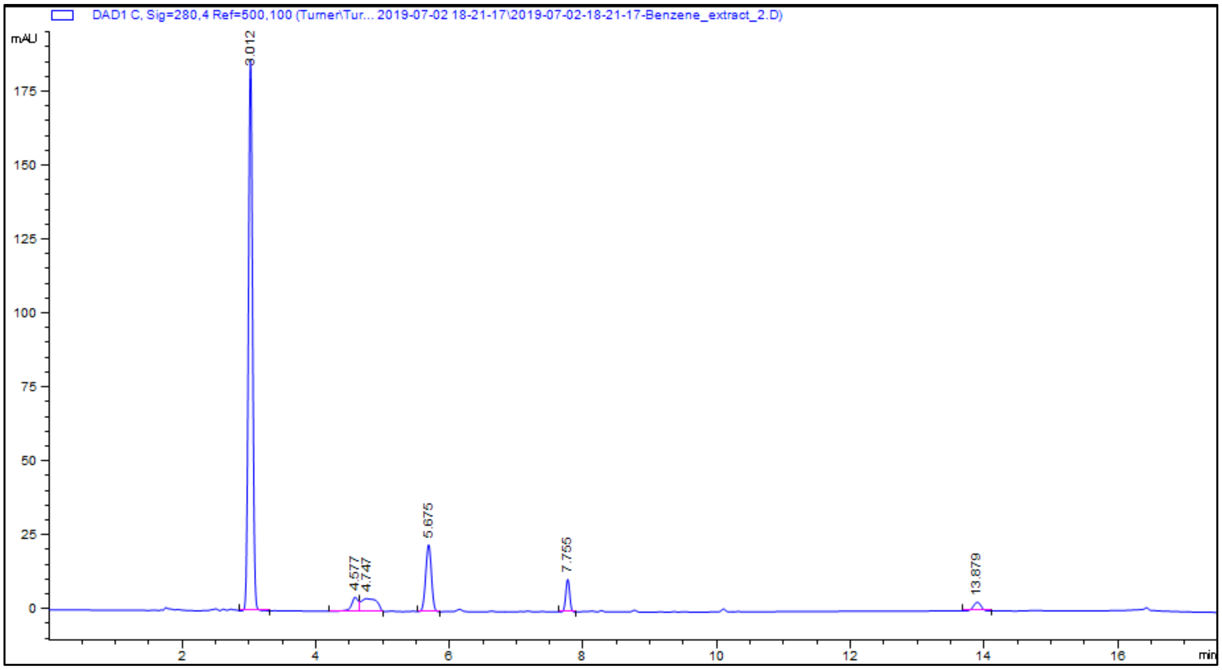
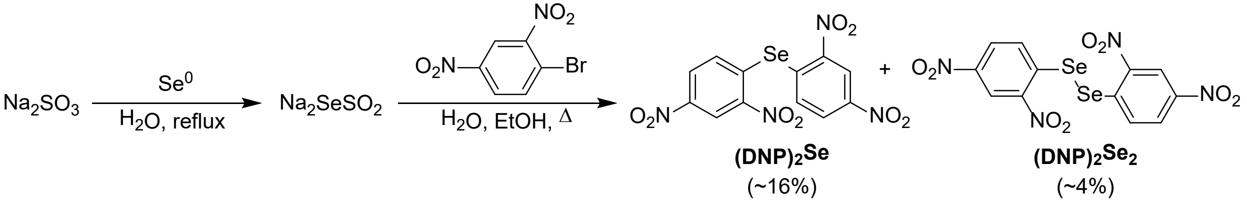


Figure S14. HPLC trace of volatilization-trapping reaction mixture (H_2O(5% MeOH):CH_3CN = 35:65, 280 nm) revealing the presence of FDNB (3.0 min), $(DNP)_2Se$ (5.7 min), and $(DNP)_2Se_2$ (7.8 min).

Authentic samples of $(DNP)_2Se$, $(DNP)_2Se_2$, and FDNB were used to verify retention times for HPLC experiments. $(DNP)_2Se$ and $(DNP)_2Se_2$ were prepared as described in the literature[1] and recrystallized from hot nitrobenzene layered with EtOH to afford yellow needles (152 mg, 20%). This preparation afforded a mixture of the monoselenide and diselenide, which is consistent with the reported reaction products.



Scheme S5. Synthesis of $(DNP)_2Se$ and $(DNP)_2Se_2$.

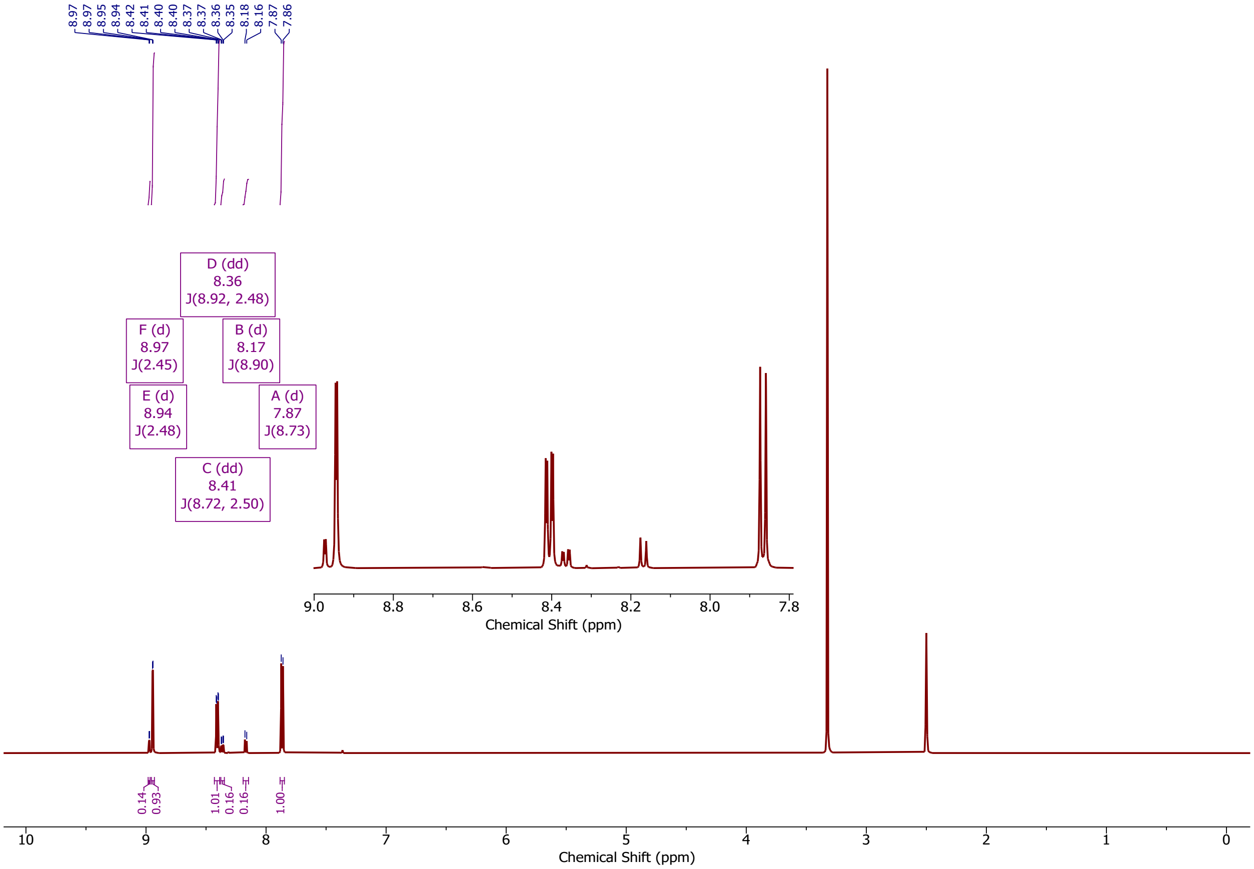


Figure S15. ^{1}H NMR (600 MHz, DMSO-d^6) spectrum of (DNP)$_2$Se and (DNP)$_2$Se$_2$ mixture.

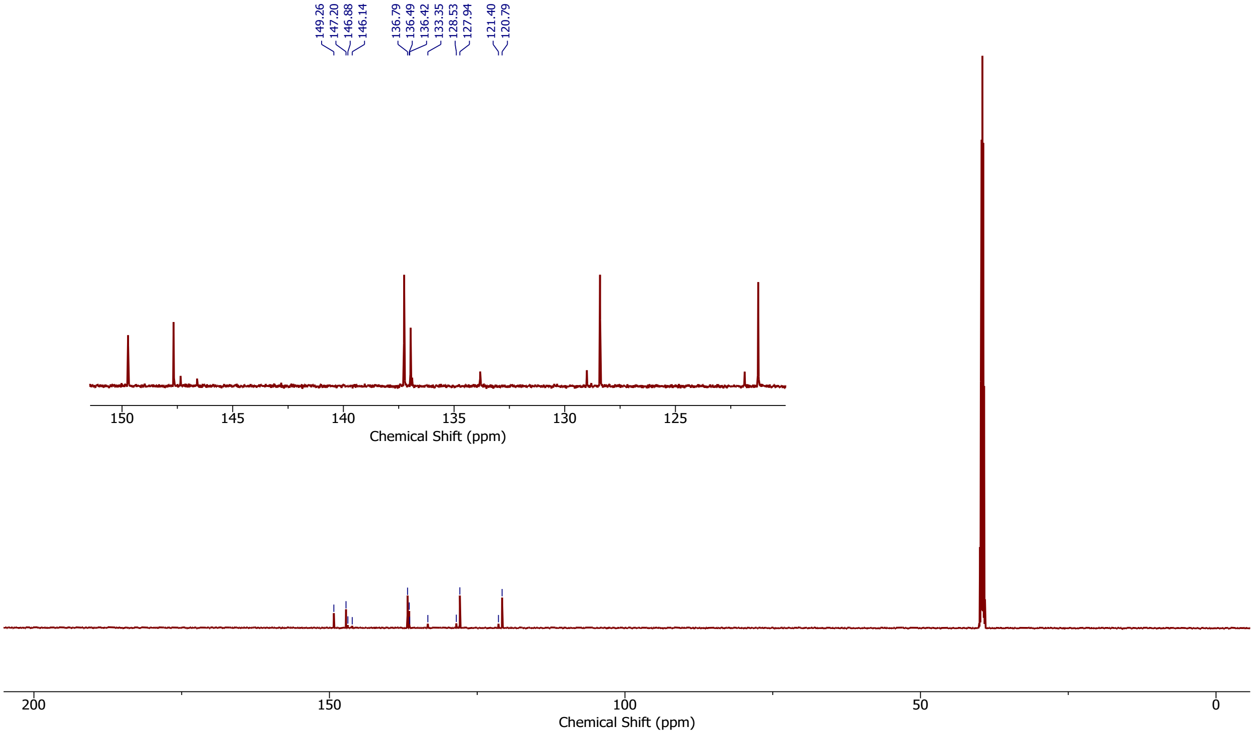


Figure S16. ^{13}C{^{1}H} NMR (151 MHz {600 MHz}, DMSO-d^6) spectrum of (DNP)$_2$Se and (DNP)$_2$Se$_2$ mixture.

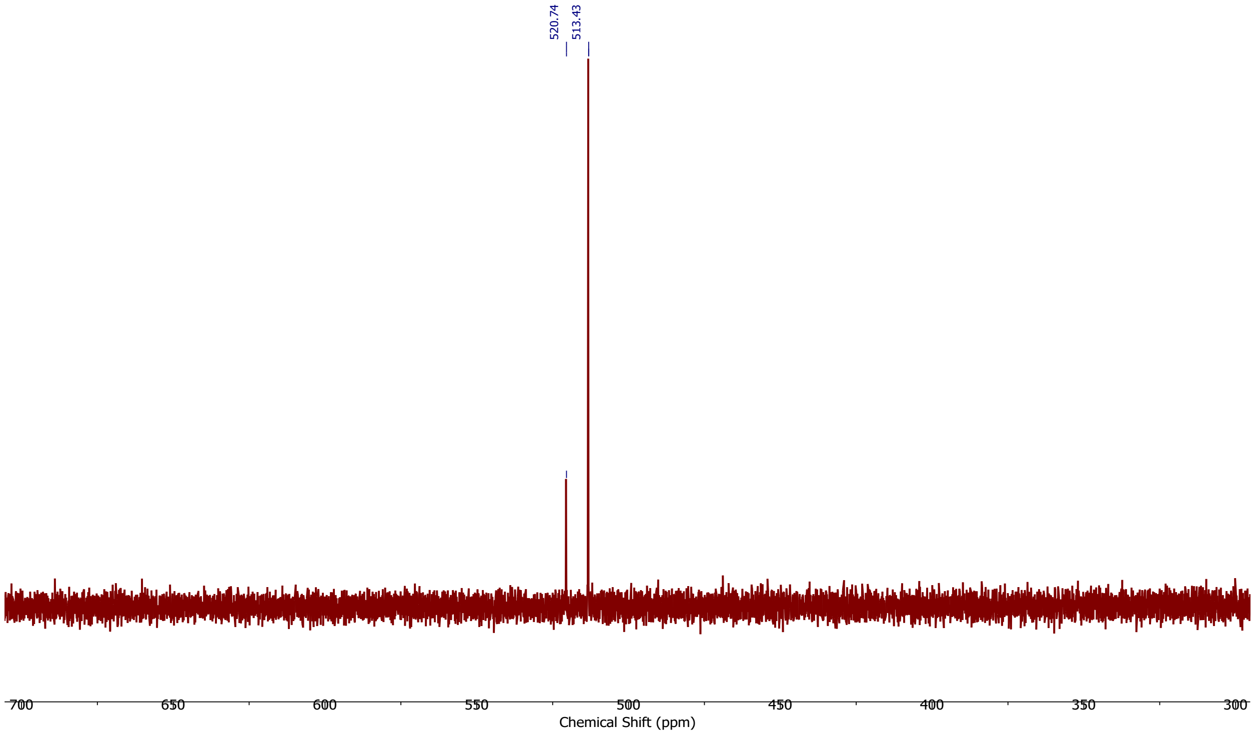


Figure S17. ^{77}Se NMR (115 MHz, DMSO-d^6) spectrum of (DNP)$_2$Se and (DNP)$_2$Se$_2$ mixture.

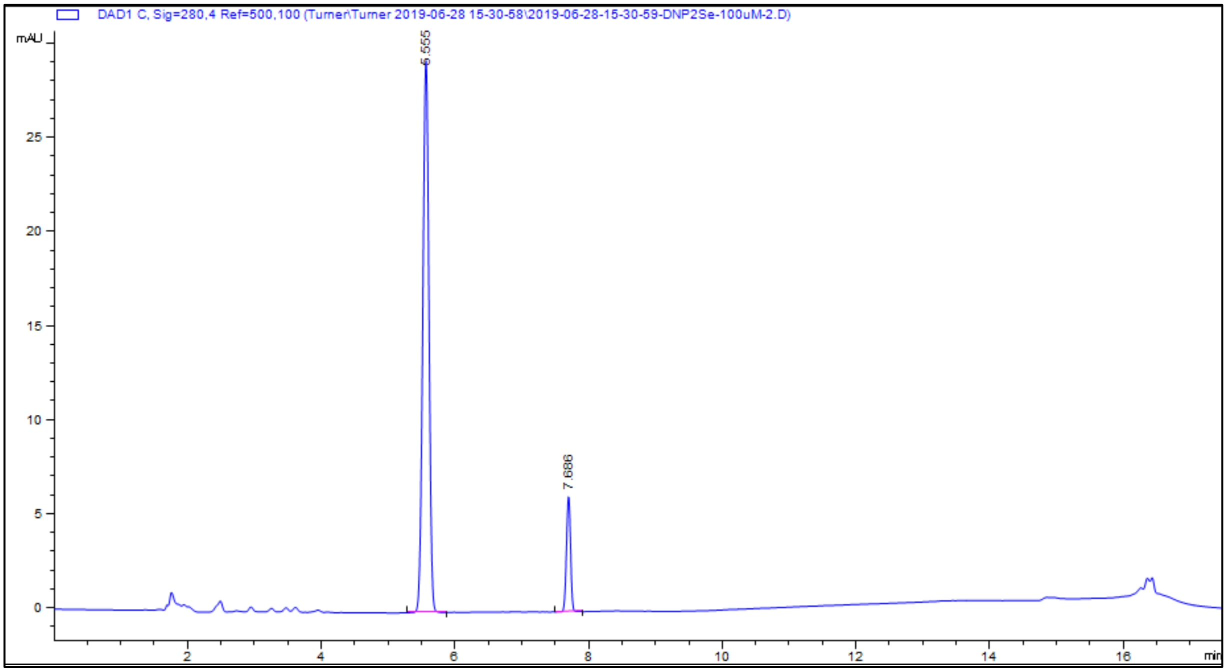


Figure S18. HPLC trace (H$_2$O(5% MeOH):CH$_3$CN = 35:65, 280 nm) of (DNP)$_2$Se (5.6 min) and (DNP)$_2$Se$_2$ (7.7 min) mixture.

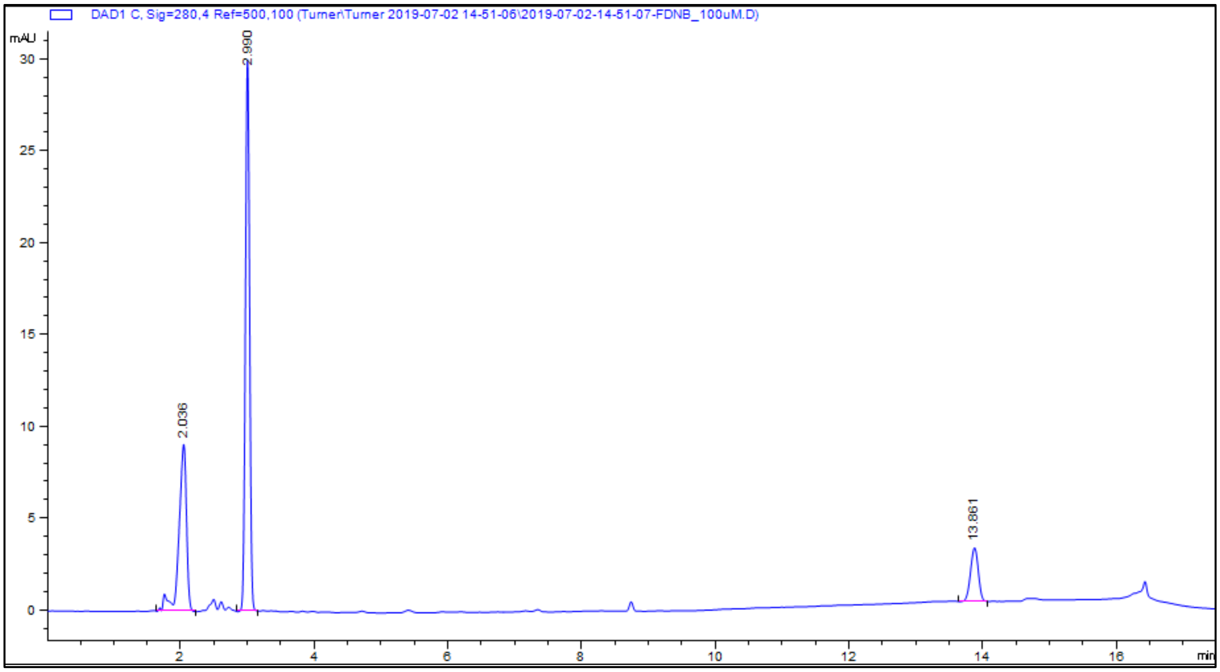


Figure S19. HPLC trace (H_2O(5% MeOH):CH_3CN = 35:65, 280 nm) of FDNB (3.0 min).

X-ray Crystallography

Low-temperature X-ray diffraction data for **Rtn2** were collected on a Rigaku XtaLAB Synergy diffractometer coupled to a Rigaku Hypix detector with Cu Kα radiation (λ = 1.54184 Å), from a PhotonJet micro-focus X-ray source at 200 K. The diffraction images were processed and scaled using the CrysAlisPro software.[2] The structures were solved through intrinsic phasing using SHELXT[3] and refined against F^2 on all data by full-matrix least squares with SHELXL[4] following established refinement strategies.[5] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms bound to carbon were included in the model at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the Ueq value of the atoms they are linked to (1.5 times for methyl groups). The solution was checked for missed symmetry using PLATON.[6] Data were treated as a racemic twin; the explicit refinement of the Flack parameter yielded a value of 0.428(16). Details of the data quality and a summary of the residual values of the refinements are listed in Table S1 .

Table S1. Crystal data and structure refinement for Rtn2.

Identification code	rtn2_abs	
Empirical formula	$C_{14} H_{23} N_2 O_2 P Se_2$	
Formula weight	440.23	
Temperature	199.99(10) K	
Wavelength	1.54184 Å	
Crystal system	Monoclinic	
Space group	P 1 c 1	
Unit cell dimensions	a = 12.50104(4) Å	α= 90°.
	b = 9.28018(3) Å	β= 90.6520(3)°.
	c = 30.05829(10) Å	γ = 90°.
Volume	3486.892(19) Å^3	
Z	8	
Density (calculated)	1.677 Mg/m^3	
Absorption coefficient	6.219 mm^{-1}	
F(000)	1760	
Crystal size	0.144 x 0.094 x 0.05 mm^3	
Theta range for data collection	2.940 to 78.077°.	
Index ranges	-15<=h<=15, -11<=k<=11, -38<=l<=38	
Reflections collected	156605	
Independent reflections	14852 [R(int) = 0.0484]	
Completeness to theta = 67.684°	100.0 %	
Absorption correction	Gaussian	
Max. and min. transmission	1.000 and 0.679	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	14852 / 10 / 782	
Goodness-of-fit on F^2	1.036	
Final R indices [I>2sigma(I)]	R1 = 0.0241, wR2 = 0.0630	
R indices (all data)	R1 = 0.0245, wR2 = 0.0632	
Absolute structure parameter	0.428(16)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.593 and -0.377 e.Å^{-3}	

References

1. Twiss, D. F., *J. Chem. Soc.* 1914, **105**, 1672-1678.
2. *CrysAlisPro*; Rigaku OD, The Woodlands, TX, 2015.
3. Sheldrick, G. M. *Acta Cryst.* 2015, **A71**, 3-8.
4. Sheldrick, G.M. *Acta Cryst.*, 2008, **A64**, 112-122.
5. Müller, P. *Crystallogr. Rev.* 2009, **15**, 57-83.
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