Electronic Supplementary Information

Transannular Selenocyclofunctionalization of 1,5-cyclooctadiene: The Antioxidant Properties of 9-selenabicyclo[3.3.1]nonane Derivatives and the Discovery of Increasing Both GPx and GR Activities

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Experimental

^{1}H (400.1 MHz), ^{13}C (100.6 MHz) NMR spectra were recorded on a Bruker DPX-400 spectrometer in 1–10% solution in D_2O, $CDCl_3$, DMSO-D_6, referenced to i-PrOH, HMDS (^{1}H and ^{13}C NMR, internal).

Crystal Structure Determination

Data were collected on a BRUKER D8 VENTURE PHOTON 100 CMOS diffractometer with MoKα radiation (α = 0.71073 Å) using the φ and ω scans technique. Using Olex2 [1], the structure was solved with the ShelXS [2] structure solution program using Direct Methods and refined with the XL [2] refinement package using Least Squares minimisation. Data were corrected for absorption effects using the multi-scan method (SADABS). All non-hydrogen atoms were refined anisotropically using SHELX [2]. The coordinates of the hydrogen atoms were calculated from geometrical positions.

Plant material

Studies were carried out in laboratory conditions on oilseed radish seeds (Raphanus sativus L. var. oleiferus Metzg.) of lines of Irkutsk State Agricultural Academy, with laboratory germinability of 80-98%, weighing 1,000 seeds 9.5g. Seeds were germinated on wet filter paper in Petri dishes at a constant temperature of 23 °C, in the dark, for 4 days, wetting them with the test solutions. The number of seeds in one cup was 30pcs. The experiment was repeated 3 times.

Evaluation of germinability and mass of seedlings

Germinability was analyzed according to the All-Union State Standard 10-14-86 “Oilseed Radish Seeds. Varietal and sowing qualities”. These indicators were determined in accordance with All-Union State Standard 12038-84 “Seeds of agricultural crops. Methods for determining germinability” [3]. The mass of shoots and roots was determined using the gravimetric analysis.

Determination of protein content

Protein contentwas determined by the degree of binding to the Coomassie blue dye (CBB G250 “Sigma”) according to the Bradford method [4].

Determination of glutation reductase activity

Glutathione reductase activity (EC 1.6.4.2) was measured according to the method described by Nigmatullina et al. [5]. The activity of glutathione reductase was determined by the change in absorption at 340 nm, caused by the oxidation of NADPH in 3.5 min with an interval of 1 s on the spectrophotometer. The enzyme activity was calculated using the extinction coefficient for $NADP^{+}$ at a wavelength of 340 nm, equal to 6.22 $mmol^{-1}cm^{-1}$.

Evaluation of diene conjugates

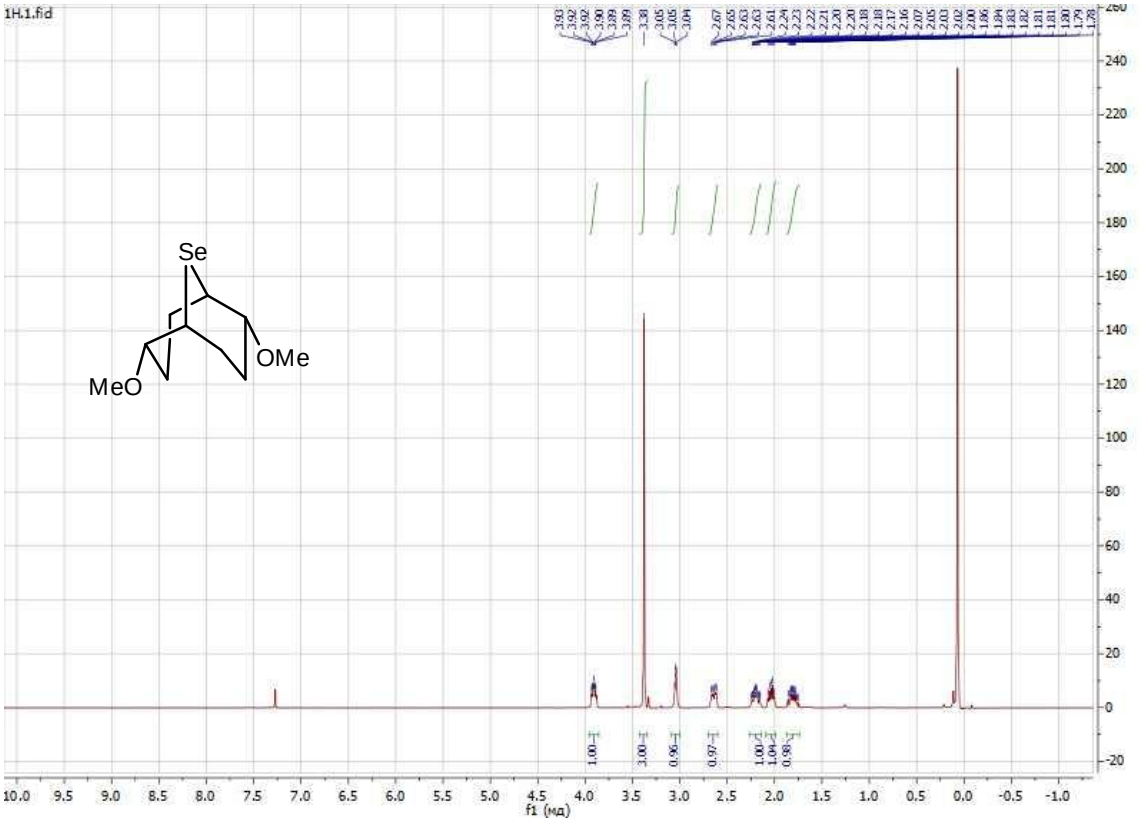
Analysis of the content of the primary products of lipid peroxidation – diene conjugates (DC) – was carried out according to the method in our modification [6]. The measurement was performed on a spectrophotometer at a wavelength of 203 nm. The obtained optical density (D) was used to calculate the concentration of diene conjugates (recalculated per 1 g wet mass) using an extinction coefficient equal to 2.2×105 $mol^{-1}cm^{-1}$. Salinisation was chosen as a stress, which was created with NaCl, a concentration of 200 mmol was taken from literature data [7]. This concentration causes stress, since it significantly increases the level of lipid peroxidation by almost two times compared with the control.

Statistics

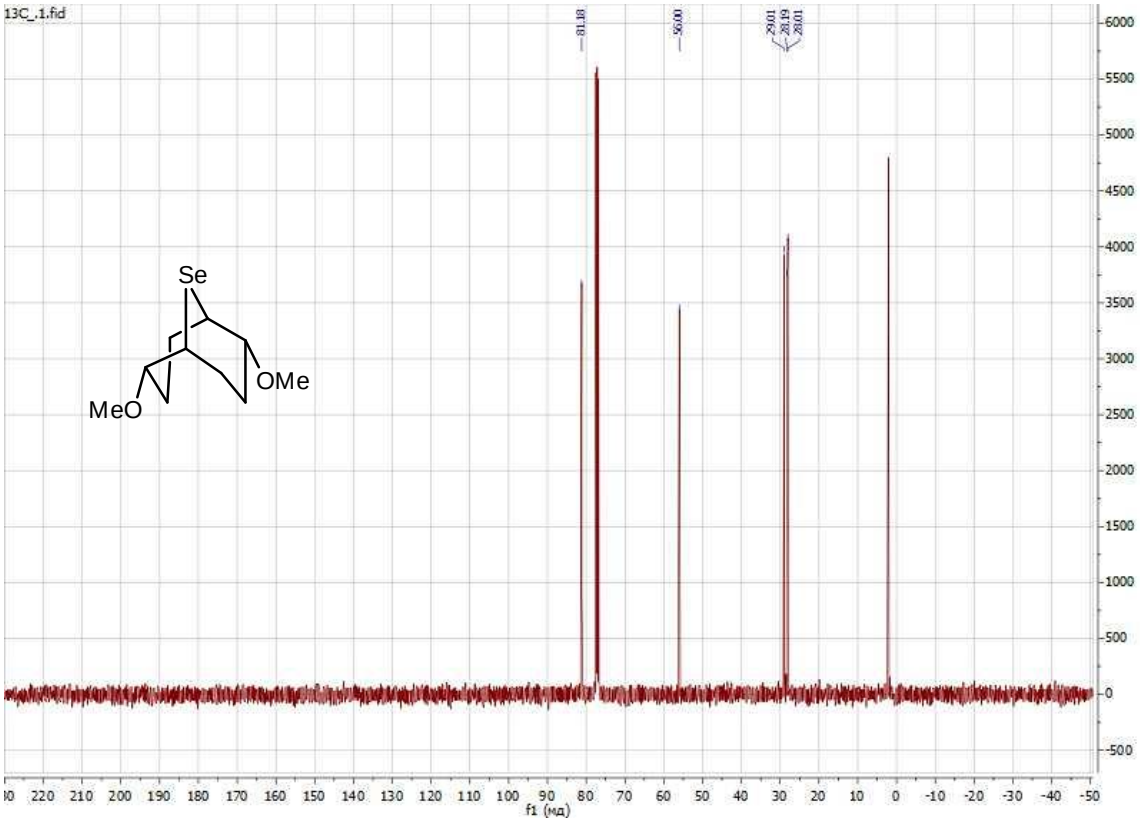
The data are presented as arithmetic mean values of quantities and their standard deviations, which were obtained in three independent experiments, calculated using Microsoft Excel.

^{1}H and ^{13}C NMR spectra of the obtained compounds

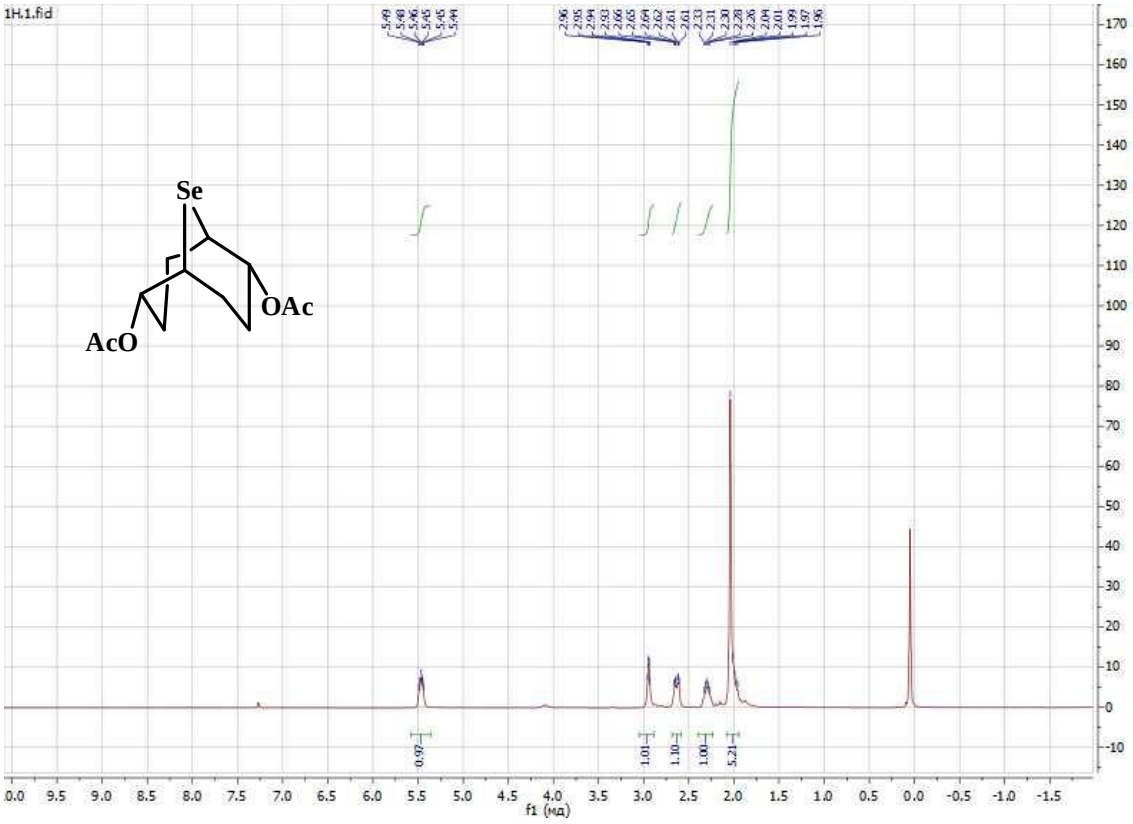
^{1}H NMR SPECTRUM OF COMPOUND **3**, **CDCl$_3$**, REFERENCED TO HMDS (INTERNAL)



^{13}C NMR SPECTRUM OF COMPOUND **3**, **CDCl$_3$** REFERENCED TO HMDS (INTERNAL)



^{1}H NMR SPECTRUM OF COMPOUND **5**, **$CDCl_3$**, REFERENCED TO HMDS (INTERNAL)



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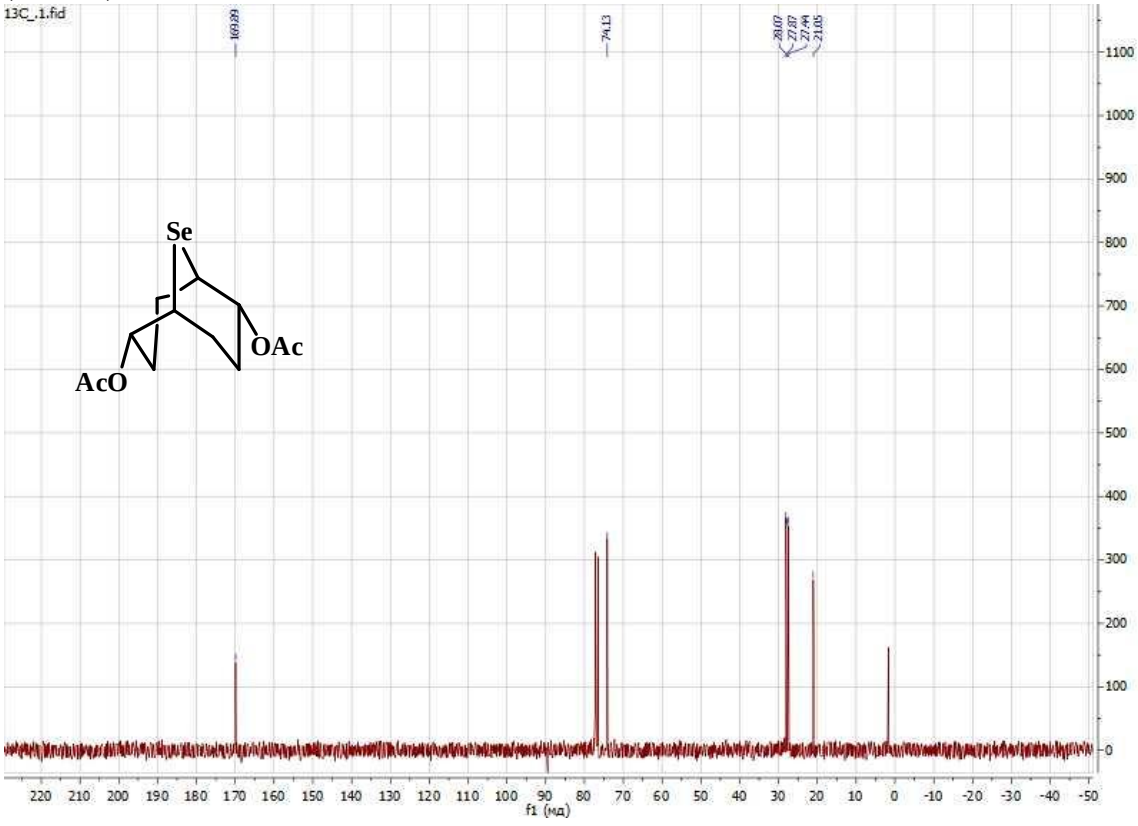
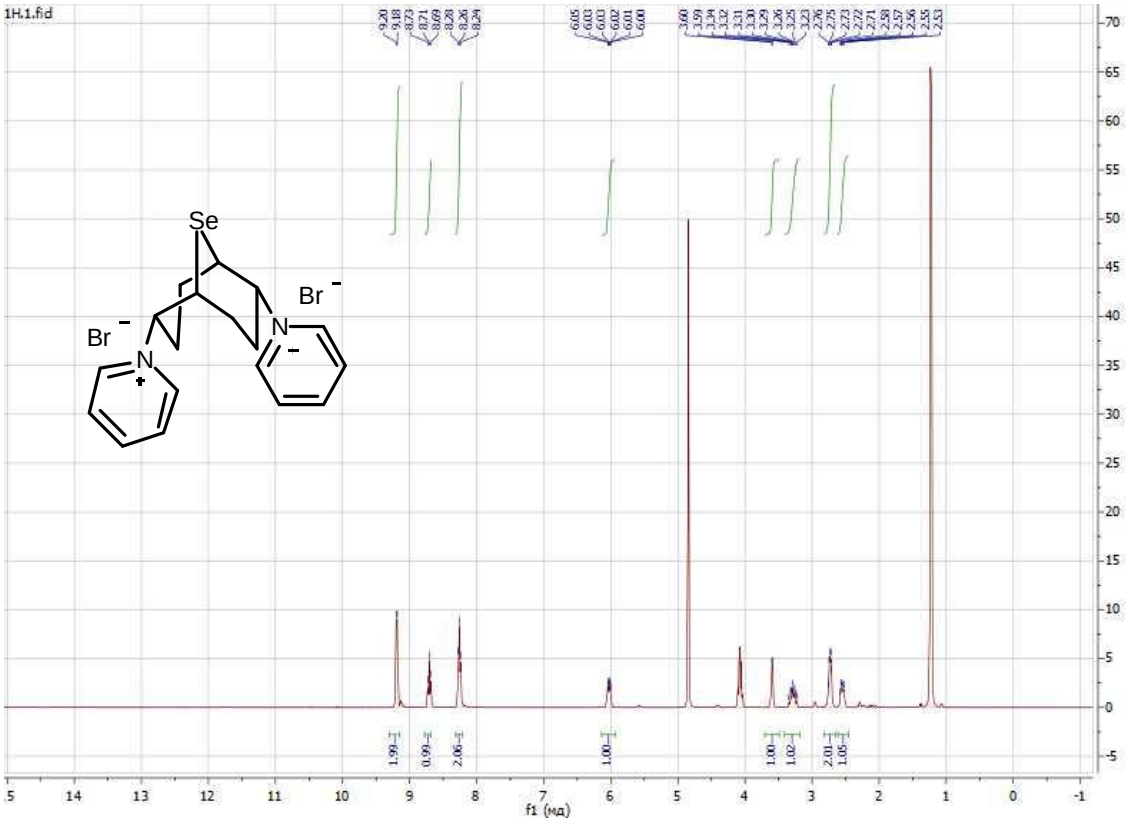
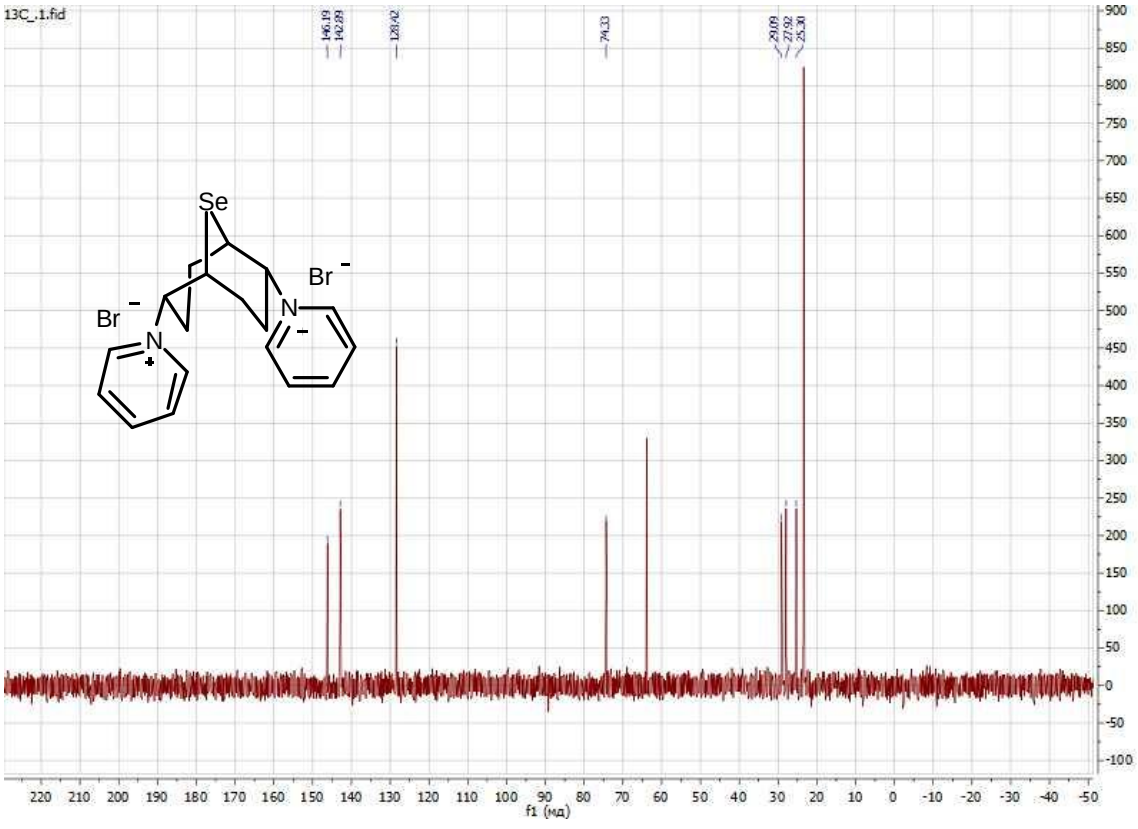


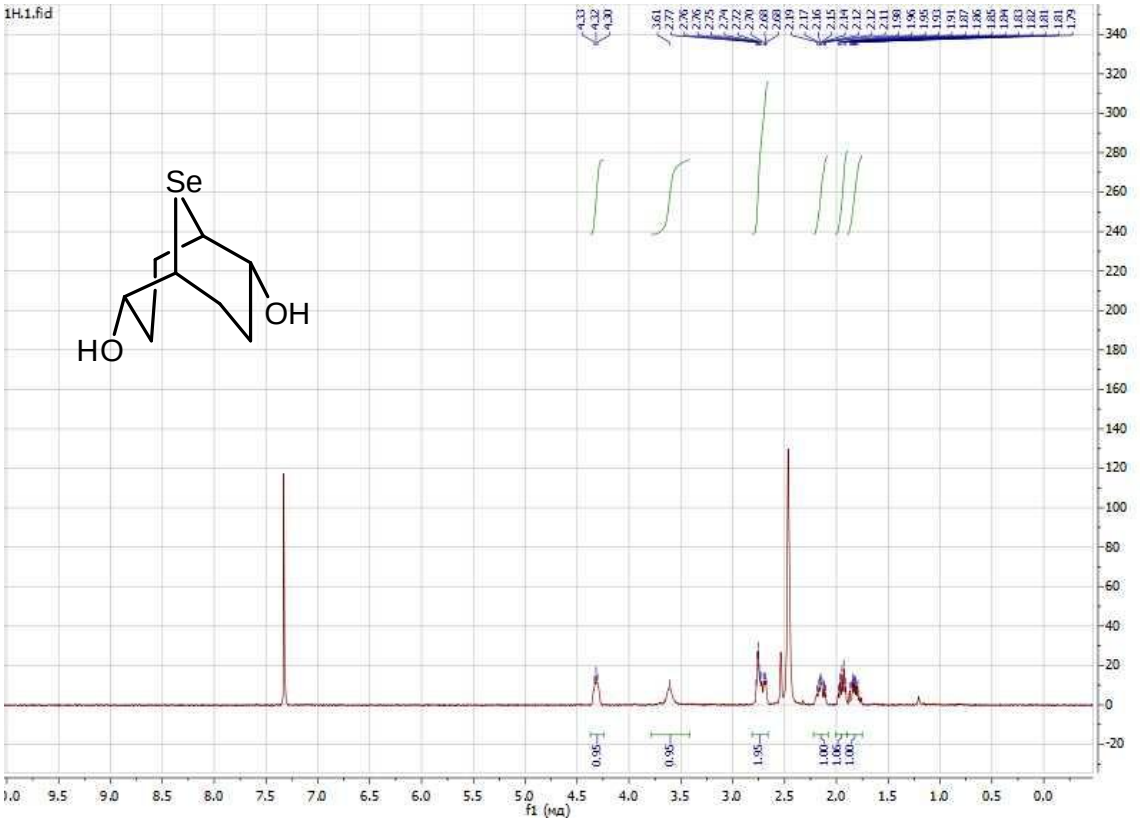
^{1}H NMR SPECTRUM OF COMPOUND **6, D_2O** REFERENCED TO i-PrOH (INTERNAL)



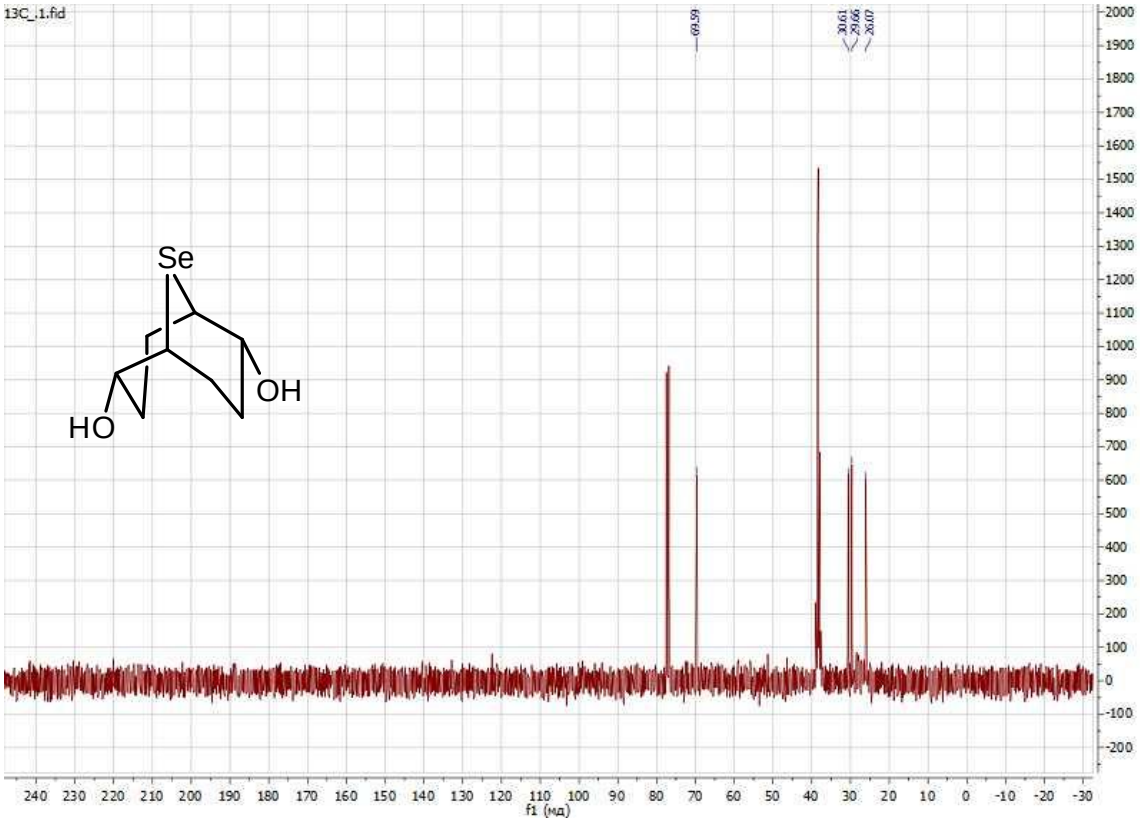
^{13}C NMR SPECTRUM OF COMPOUND **6, D_2O** REFERENCED TO i-PrOH (INTERNAL)



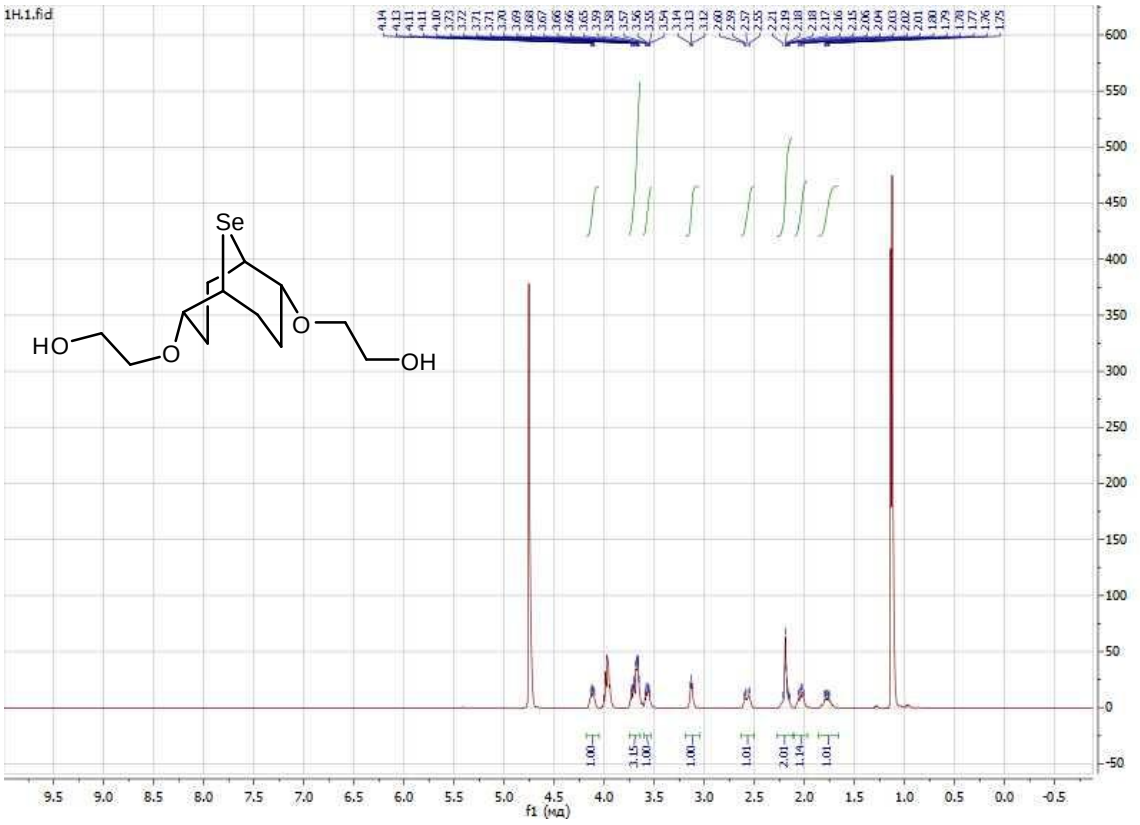
^{1}H NMR SPECTRUM OF COMPOUND **7, $CDCl_3$/DMSO-D6**, REFERENCED TO CHLOROFORM



^{13}C NMR SPECTRUM OF COMPOUND **7, $CDCl_3$/DMSO-D6,** REFERENCED TO CHLOROFORM



^{1}H NMR SPECTRUM OF COMPOUND **8, D_2O** REFERENCED TO i-PrOH (INTERNAL)



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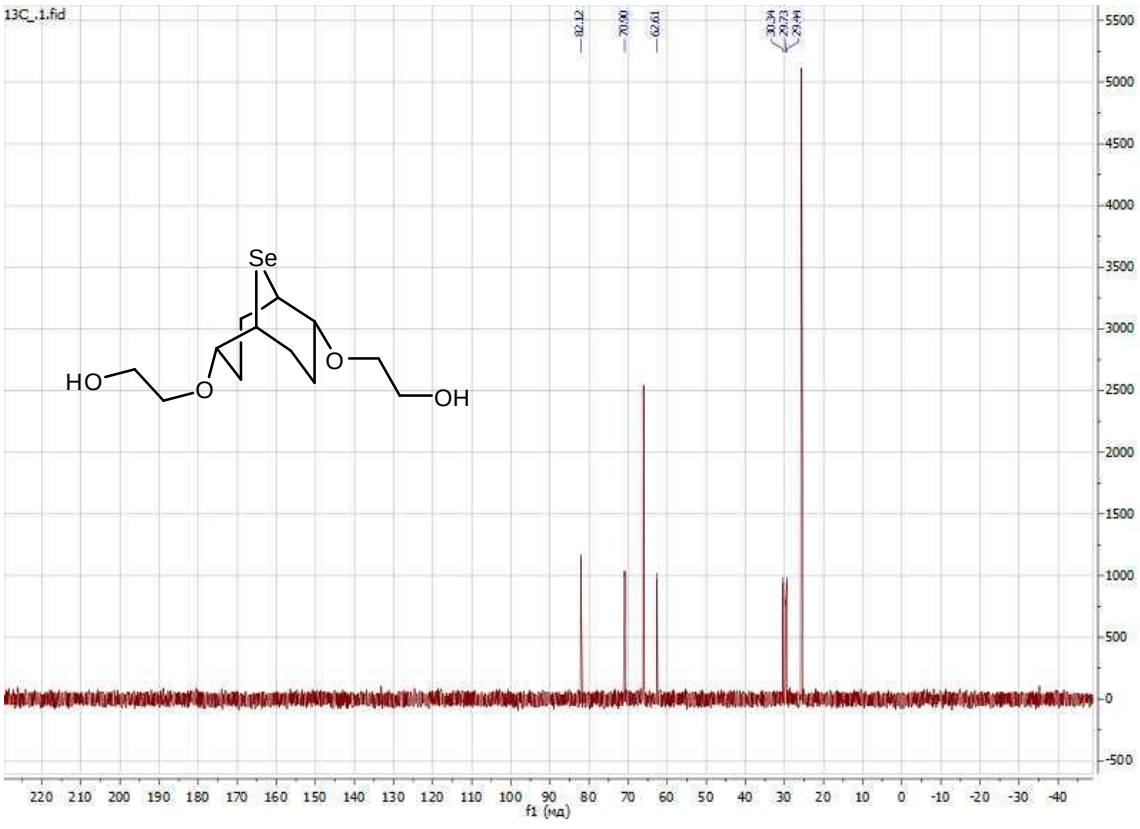

Table 1ESM. X-ray crystallographic data for compound **5**

Compound	**5**
CCDC number	**2277477**
Empirical formula	$C_{12}H_{18}O_4Se$
Formula weight / g·mol^{-1}	305.22
Crystal system	Monoclinic
Space group	*C 2/c*
a / Å	16.778(3)
b / Å	10.8062(15)
c / Å	7.4255(11)
α, β, γ / °	90.00, 105.777(5), 90.00
Volume / Å^3	1295.6 (3)
Z	4
Density (calculated) / g·cm^{-3}	1.565
Absorptions coefficient / mm^{-1}	2.899
Radiation (λ / Å)	MoKα (0.71073)
Temperature / K	100(2)
2Θ range / °	2.52 – 27.55
Crystal size / mm	0.16 × 0.15 × 0.11
Crystal habit	colourless, prism
F(000)	624
Index ranges	$-21 \leq h \leq 20$, $-14 \leq k \leq 14$, $-9 \leq l \leq 9$
Reflections collected	12339
Independent reflections	1486 [R_{int} = 0.0551, R_{sigma} = 0.0328]
Number of ref. parameters	79
R_1 / wR_2 [$I > 2\sigma(I)$]	0.0887 / 0.3091
R_1 / wR_2 (all data)	0.0953 / 0.3147
Goodness-of-fit on F^2	1.096
Completeness [%]	99.6
Largest diff. peak and hole / e·Å^{-3}	5.94/ -0.93
Weight scheme	$w=1/[\sigma^2(F_o^2)+(0.1916P)^2+56.1592P]$ where $P=(F_o^2+2F_c^2)/3$

Table S1ESM. Bond lengths, bond angles and torsion angles for compound **5**

Bond *l*, Å			Angle φ, °				Torsion angle θ, °				
Se1	C1	1.960(9)	C1	Se1	C1^1	90.3(5)	Se1	C1	C2	C3	-61.1(9)
Se1	C1^1	1.960(9)	C5	O1	C4	117.7(7)	Se1	C1	C4	O1	-177.1(5)
O1	C4	1.466(11)	C2	C1	Se^1	109.9(6)	Se1	C1	C4	C3^1	65.1(8)
O1	C5	1.344(10)	C2	C1	C4	117.2(7)	C1	C2	C3	C4^1	46.6(11)
O2	C5	1.213(11)	C4	C1	Se1	107.2(6)	C2	C1	C4	O1	58.9(9)
C1	C2	1.528(12)	C1	C2	C3	116.9(7)	C2	C1	C4	C3^1	-58.9(10)
C1	C4	1.541(11)	C4^1	C3	C2	115.0(7)	C4	O1	C5	O2	0.2(12)
C2	C3	1.533(13)	O1	C4	C1	108.6(6)	C4	O1	C5	C6	180.0(7)
C3	C4^1	1.525(12)	O1	C4	C3^1	104.3(7)	C4	C1	C2	C3	61.6(11)
C4	C3^1	1.525(12)	C3^1	C4	C1	117.4(7)	C5	O1	C4	C1	85.6(9)
C5	C6	1.488(13)	O1	C5	C6	111.7(8)	C5	O1	C4	C3^1	-148.6(7)
			O2	C5	O1	123.7(8)					
			O2	C5	C6	124.5(8)					

Evaluation of diene conjugates

Sample	nmol/1g wet mass			% to control		
	1000 µmol	100 µmol	10 µmol	1000 µmol	100 µmol	10 µmol
H_2O (control for Na_2SeO_3, comp. **6**, **7**, **8)**)	0,68±0,046			100		
1% Ethanol (control for Ph_2Se_2, comp. **1**, **3**, **5)**	1,28±0,018			100		
Ref.comp. Na_2SeO_3	2,40±0,074	0,92±0,016	0,60±0,010	352±10,9	135±2,3	88±1,5
comp. **6**	1,14±0,033	0,45±0,015	0,55±0,012	167±4,8	66±2,2	80±1,8
comp. **7**	0,42±0,01	0,44±0,02	0,54±0,02	62±1,5	64±2,9	79±2,9
comp. **8**	0,65±0,04	0,72±0,01	0,64±0,02	95±5,9	105±1,5	93±2,9
Ref.comp. (Ph_2Se_2)	2,95±0,103	1,36±0,033	1,01±0,010	230±8,1	106±2,5	78±0,8
comp. **1**	1,03±0,01	1,04±0,10	0,75±0,06	80±0,8	81±7,8	59±4,7
comp. **3**	1,05±0,005	1,40±0,032	0,66±0,068	82±0,4	109±2,5	52±5,4
comp. **5**	1,34±0,08	1,13±0,01	1,20±0,007	105±6,3	88±0,8	93±0,6

Glutathione reductase activity

Sample	Activity, mmol $min^{-1}mg^{-1}$			% to control		
	1000 µmol	100 µmol	10 µmol	1000 µmol	100 µmol	10 µmol
H_2O (control for Na_2SeO_3, comp. **6**, **7**, **8)**)	2,06±0,28			100		
1% Ethanol (control for Ph_2Se_2, comp. **1**, **3**, **5**	2,84±0,48			100		
Ref.comp. Na_2SeO_3	4,23±0,96	6,70±1,17	2,16±0,47	205±46,6	325±56,9	105±22,9
comp. **6**	8,39±1,41	3,61±1,10	1,58±0,40	407±68,0	175±53,4	77±19,5
comp. **7**	2,2±0,4	2,3±0,4	3,3±0,3	121±22,0	111±19,3	160±14,6
comp. **8**	3,1±0,6	2,5±0,1	4,4±0,2	151±29,3	121±4,8	213±9,6
Ref.comp. (Ph_2Se_2)	4,32±0,75	3,93±0,76	3,96±0,69	152±26,4	138±26,6	140±24,4
comp. **1**	3,0±0,4	0,6±0,4	0,5±0,3	104±13,8	22±14,3	18±1,1
comp. **3**	1,08±0,32	1,74±0,35	2,40±0,33	38±11,2	61±12,3	85±11,7
comp. **5**	1,2±0,4	3,0±1,3	2,2±0,3	43±14,3	105±45,5	77±10,5

Glutathion peroxidise activity

Sample	Activity, mmol $min^{-1}mg^{-1}$			% to control		
	1000 µmol	100 µmol	10 µmol	1000 µmol	100 µmol	10 µmol
H_2O (control for Na_2SeO_3, comp. **6**, **7**, **8)**)	8,59±0,34			100		
1% Ethanol (control for Ph_2Se_2, comp. **1**, **3**, **5**	8,61±0,68			100		
Ref.comp. Na_2SeO_3	6,84±0,33	6,30±0,31	5,90±0,04	80±3,8	73±56,9	67±0,5
comp. **6**	5,06±0,22	5,0±0,31	5,78±0,21	59±2,5	58±3,6	67±2,4
comp. **7**	10,1±0,86	13,7±1,17	12,2±0,11	118±10,0	160±13,6	143±1,3
comp. **8**	11,4±0,24	9,2±0,02	26,1±0,66	133±2,8	107±0,2	304±7,6
Ref.comp. (Ph_2Se_2)	8,25±0,28	9,31±0,67	9,41±0,11	96±3,3	108±7,8	109±1,3
comp. **1**	15,4±0,87	7,4±0,43	9,0±0,2	179±10,0	86±5,0	104±2,9
comp. **3**	10,08±0,39	9,08±0,89	9,3±0,15	117±4,6	105±10,3	108±1,7
comp. **5**	6,1±0,02	8,9±0,94	10,9±1,12	71±0,2	104±11,0	127±13,1

References

1. Dolomanov O.V., Bourhis L.J., Gildea R.J, Howard, J.A.K. & Puschmann, H. A Complete Structure Solution, Refinement and Analysis Program. ***J. Appl. Crystallography*** 2009, ***42***, 339. Doi: 10.1107/S0021889808042726
2. Sheldrick G.M. A short history of SHELX, ***Acta Crystallographica Section A. Foundations and Advances*** 2008, ***A64***, 112. Doi: 10.1107/S0108767307043930
3. Dorofeev, N.V.; Bojarkin, E.V.; Peshkova, A.A. Factors Defining Field Germination of Oilseed Radish Seeds. ***J. Stress Phys. Biochem***. 2013, ***9***, 159–168.
4. Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. ***Anal. Biochem.*** 1976, ***72***, 248–254.
5. Nigmatullina, L.R.; Rumyantseva, N.I.; Kostyukova, Y.A. The effect of D,L-buthionine-S,R-sulfoximine on the ratio of glutathione forms and the growth of Tatar buckwheat calli. ***Ontogenesis*** 2014, ***45***, 50–62..
6. Placer, Z. Lip peroxidation systeme im biologischen material. ***Nahrung*** 1968, ***12***, 679–684.
7. Ahmad, P.; Hashem, A.; Abd-Allah, E.F.; Alqarawi, A.A.; John, R.; Egamberdieva, D.; Gucel, S. Role of ***Trichoderma harzianum*** in mitigating NaCl stress in Indian mustard (***Brassica juncea*** L.) through antioxidative defense system. ***Front. Plant Sci.*** 2015, ***6***, 868–883.