New Journal of Chemistry

Electronic Supplementary Information

Aromatic selenocyanates as a unique class of non-mutagenic antimicrobial selenium compounds with pronounced activity against multidrug resistant ESKAPE bacteria

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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General procedure for the synthesis of selenocyanates

Selenocyantes were synthesized using the general protocol described by Wheeler and Merriam with some modifications.[1] According to the procedure, alkyl halides (10-20 mmol) were treated with KSeCN (12-25 mmols) in the presence of ethanol (10-20 mL). The reaction mixture was refluxed for 6 h and the progress of the reaction was monitored periodically by Thin Layer Chromatography (TLC). After the completion of the reaction, the inorganic salt was separated by filtration and the filtrate was heated with charcoal. The reaction mixture was filtered hot and the filtrate was left for cooling. On cooling, the solution yielded crystals which were separated by filtration. TLC was performed to evaluate the purity of the compound. Once purified, the samples were analysed using Mass Spectroscopy (MS) and Nuclear Magnetic Resonance (NMR) for structural confirmations as well as purity. Synthesis and chemical characteristics of compounds **1**, **3**-**7** and **10**-**12** have been described before and our values are in agreement with the reported values [2-4].

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

Synthesis of Benzyl selenocyanate (1)

Benzyl bromide (1.71 g, 10 mmol), KSeCN (1.73 g. 12 mmol) and ethanol (10 mL) were employed. The compound (**1**) was obtained as light crystals after purification by recrystallization with ethanol. Yield 72.5 % (1.43 g, 7.25 mmol). m.p.= 71-72 °C, TLC R*f* (DCM, 100 %): 0.64, ^{1}H NMR (DMSO-d_6, ppm): δ 7.36(m, 3H, 3C-H), 7.35 (m, 2H, 2 C-H), 4.30 (t, *J*=9.15 Hz, 2H, CH_2). ^{13}C NMR (DMSO-d_6, ppm): δ 138.79, 129.32 (2C), 129.04 (2C), 128.26,105.36(Se-CN), 33.08. LC–MS: purity 100 %, t_R = 5.52, (ESI) m/z: calculated for C_8H_7NSe $[M+H]^+$. 91.05, found: 91.00.

Synthesis of 3-Methylbenzyl selenocyanate (3)

3-Methylbenzyl chloride (2.812 g , 20 mmol) , KSeCN (3.6 g. 25 mmol) and ethanol (20 mL) were employed. The compound (**3**) was obtained as light crystals after purification by recrystallization with ethanol. Yield 83.5 % (3.51 g, 16.7 mmol). m.p.= 55.5-56.5 °C, TLC R*f* (DCM, 100 %): 0.65, ^{1}H NMR (DMSO-d_6, ppm): δ 7.23(m, 1H, CH), 7.16 (m, 2H, 2CH), 7.11 (m, 1H, CH), 4.26 (t, *J*=9.10 Hz, 2H, CH_2), 3.24 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, ppm): δ 138.60, 138.15, 129.82 (2C), 128.93 (2C), 126.42, 105.34 (Se-CN), 33.09, 21.42. LC–MS: purity 98.57 %, t_R = 6.24, (ESI) m/z: calculated for C_9H_9NSe $[M+H]^+$: 105.07, found: 105.02.

Synthesis of 4-trifluoromethylbenzyl selenocyanate (4)

4-Trifluoromethylbenzyl bromide (4.73 g, 20 mmol), KSeCN (3.6 g. 25 mmol) and ethanol (20 mL) were employed. The compound (**4**) was obtained as light crystals after purification by recrystallization with ethanol. Yield 81.75% (4.32 g, 16.35 mmol). m.p.= 54-55 °C, TLC R*f* (DCM, 100%): 0.94, ^{1}H NMR (DMSO-d_6, ppm): δ 7.64 (d, *J*=8.21 Hz, 2H, 2 C-H), 7.42(d, *J*=8.21 Hz, 2H, 2 C-H), 4.02 (t, *J*=8.21 Hz, 2H, CH_2). ^{13}C NMR (DMSO-d_6, ppm): δ 144.67, 130.06, 128.07, 127.64, 125.56, 110.00, 30.87. LC–MS: purity 95.66 %, t_R = 9.82, (ESI) m/z: calculated for $C_9H_6F_3NSe$ $[M+H]^+$: 159.04, found: 159.04.

Synthesis of 4-fluorobenzyl selenocyanate (5)

4-Fluorobenzyl chloride (2.89 g, 20 mmol), KSeCN (3.6 g. 25 mmol) and ethanol (20 mL) were employed. The compound (**5**) was obtained as light crystals after purification by recrystallization with ethanol. Yield 83.45% (3.57 3g, 16.69 mmol). m.p.= 64-65 °C, TLC R*f* (PE:EA; 4:1) : 0.60, ^{1}H NMR (DMSO-d_6, ppm): δ 7.25 (m, 2H, 2 CH), 7.12 (m, 2H, 2 C-H), 3.92 (t, *J*=7.62 Hz, 2H, CH_2). ^{13}C NMR (DMSO-d_6, ppm): δ 163.30, 160.07, 135.94, 131.29, 115.43, 30.93. LC–MS: purity 96.30 %, t_R = 9.06, (ESI) m/z: calculated for C_8H_6FNSe $[M+H]^+$: 109.05, found: 109.00.

Synthesis of 2-fluorobenzyl selenocyanate (6)

2-Fluorobenzyl chloride (2.892g, 20 mmol), KSeCN (3.6g. 25 mmol) and ethanol (20 mL) were employed. The compound (**6**) was obtained as light crystals after purification by recrystallization. Yield 75.45% (3.231g, 15.09 mmol). m.p.= 48-50 °C, TLC R*f* (DCM, 100%): 0.53, ^{1}H NMR (DMSO-d_6, ppm): δ 7.44 (m, 1H, 1 CH), 7.36 (m, 1H, 1 CH), 7.21(m, 2H, 2 CH), 4.33 (t, *J*=9.14 Hz, 2H, CH_2). ^{13}C NMR (DMSO-d_6, ppm): δ 161.13, 159.16, 131.56, 130.16, 124.51, 115.59, 104.39 (Se-CN), 25.37. LC–MS: purity 96.30 %, t_R = 9.06, (ESI) m/z: calculated for C_8H_6FNSe $[M+H]^+$: 109.05, found: 109.00.

Synthesis of 4-chlorobenzyl selenocyanate (7)

4-Chlorobenzyl bromide (2.673g, 16.6 mmol), KSeCN (2.8 g, 19.4 mmol) and ethanol (20 mL) were employed. The compound (**7**) was obtained as light crystals after purification by recrystallization with ethanol. Yield 85.24% (3.263g, 14.15 mmol). m.p.= 58-59 °C, TLC R*f* (DCM, 100%): 0.75, ^{1}H NMR (DMSO-d_6, ppm): δ 7.34 (d, *J*=8.79 Hz, 2H, 2 CH), 7.25 (d, *J*=8.21 Hz, 2H, 2 CH), 3.93 (t def., 2H, CH_2). ^{13}C NMR (DMSO-d_6, ppm): δ 138.78, 131.96, 131.15, 128.72, 30.87. LC–MS: purity 99.35 %, t_R = 9.99, (ESI) m/z: calculated for C_8H_6ClNSe $[M+H]^+$: 125.02, found: 125.02.

Synthesis of 4-bromobenzyl selenocyanate (10)

4-Bromobenzyl bromide (5 g, 20 mmol), KSeCN (3.6 g. 25 mmol) and ethanol (20 mL) were employed. The compound (**10**) was obtained as light crystals after purification by recrystallization with ethanol. Yield 62.15% (3.417 g, 12.43 mmol). m.p.= 102-103 °C, TLC R*f* (DCM, 100%): 0.86, ^{1}H NMR (DMSO-d_6, ppm): δ 7.47 (d, *J*=8.21 Hz, 2H, CH), 7.18 (d, *J*=8.21 Hz, 2H, CH), 3.91 (t, *J*=7.62 Hz , 2H, CH_2). ^{13}C NMR (DMSO-d_6, ppm): δ 139.18, 131.65, 120.46, 30.93. LC–MS: purity 100 %, t_R = 10.23, (ESI) m/z: calculated for C_8H_6BrNSe $[M+H]^+$: 168.97, found: 168.94.

Synthesis of 4-nitrobenzyl selenocyanate (11)

4-Nitrobenzyl chloride (3.432 g, 20 mmol), KSeCN (3.6 g. 25 mmol) and ethanol (20 mL) were employed. The compound (**11**) was obtained as light crystals after purification by recrystallization with ethanol. Yield 72% (3.471g, 14.4 mmol). m.p.= 99-100 °C, TLC R*f* (DCM, 100%): 0.50, ^{1}H NMR (DMSO-d_6, ppm): δ 8.21 (m, 2H, CH), 7.63 (m, 2H, CH), 4.39 (t def, 2H, CH_2). ^{13}C NMR (DMSO-d_6, ppm): δ 146.97, 130.57, 124.26, 105.25, 31.61. LC–MS: purity 97.77%, t_R = 5.30, (ESI) m/z: calculated for $C_8H_6N_3O_2Se$[M+H]$^+$: 136.04, found: 135.98.

Synthesis of 2-(selenocyanatomethyl) naphthalene (12)

2- Chloromethyl naphthalene (2.51g, 14.2 mmol), KSeCN (2.6 g. 18 mmol) and ethanol (14 mL) were employed. The compound (**12**) was obtained as light crystals after purification by recrystallization wit ethanol. The compound was obtained in 81.27 % yield (2.85 g, 11.54 mmol). m.p.= 119-120 °C, TLC R*f* (DCM, 100%): 0.77, ^{1}H NMR (DMSO-d_6, ppm): δ 7.92 (m, 4H, CH), 7.53 (m, 3H, CH), 4.49 (t, *J*=9.14 Hz, 2H, CH_2). ^{13}C NMR (DMSO-d_6, ppm): δ 135.76, 132.65, 132.36, 128.40, 127.76, 127.60, 127.38, 126.89, 126.49, 126.33, 104.94 (SeCN), 33.12. LC–MS: purity 97.56%, t_R = 6.73, (ESI) m/z: calculated for $C_{12}H_9NSe$ $[M+H]^+$: 141.07, found: 141.03

Crystallographic Studies

The crystallographic studies have been performed to provide evidence about the stability of the arylmethylselenocyanates when exposed to different solvents and temperature, considering a potential reactivity of the selenocyanate moiety. The molecular structures and atomic-numbering schemes of **1** and **12** are presented and described in main article (Figure 2). Parameters of intermolecular C-H.···N interactions for **1** and **12** are shown in Table S1. The geometries of the methyleneselenocyanate groups in both compounds differ slightly. The responsible angles have values C1-Se1-C2 = 95.2° and 95.5°, Se1-C2-C3 = 113.7° and 114.3°, C1-Se1-C2-C3 = −62.4° and -66.5°, Se1-C2-C3-C4 = 98.6° and 98.8°, whereas the torsion angle N1-C1-Se1-C2 exhibits a significant difference of −164.4° and 67.3° for compound **1** and **12**, respectively. Methylselenocyanate fragments were also searched for in the Cambridge Structural Database (CSD, Version 5.37), which resulted in five crystal structures, in one case two independent molecules.[5] In these crystal structures, the values of torsion angles N1-C1-Se1-C2 were 66.8°, −170.9°, −139.5°, 29.4°, −131.7° and −173.1°.

Table S1. Parameters of intermolecular C-H.···N interactions for **1** and **12**.

Cpd	D-H.···A	H···A (Å)	D. ···A (Å)	D-H···A (°)	Symmetry Codes
1	C2-H2A···N1	2.59	3.559(3)	174	−x, −y, −z
	C2-H2B···N1	2.73	3.328(3)	119	x + 1, y, z
	C6-H6···N1	2.84	3.513(3)	128	−x, y − 1/2, −z + 1/2
12	C2-H2A···N1	2.58	3.487(2)	155	x + 1/2, −y + 1, −z
	C2-H2B···N1	2.76	3.333(1)	118	x, y − 1, z
	C8-H8···N1	2.75	3.520(2)	141	x − 1/2, y − 1/2, z − 1/2

Ames fluctuation assay

Compounds **1**, **2** and **13** were also non-mutagenic at the higher concentration of 10 μM which was employed in most of the assays. Only compound **4**, at the higher concentration (10 μM), exhibited an increased binomial B–value (B = 1.0), which may point towards a probable mutagenic potential. The mutagenicity of this derivative is rather ambiguous, as the value of the second parameter indicative of mutagenicity (MI = 1.75) was still below the threshold of 2.0, and substantially lower compared to the MI value for the mutagenic reference NQNO with a MI = 6.91 calculated at a concentration of 0.5 μM (Figure 1).

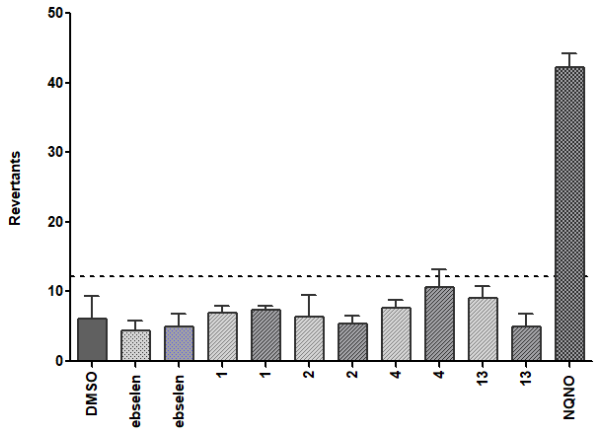

Figure 1. Results of the Ames liquid microtitre test indicative of mutagenic potential; DMSO (1 % in growth medium)-negative control, ebselen (reference compound) at the concentration 1 µM and 10 µM, NQNO (4-nitroquinoline-*N*-oxide, benchmark mutagenic agent) at concentration 0.5 µM; **1, 2**, **4** and **13** — selenocyanates at concentrations 1 µM and 10 µM, ----- baseline defining the mutagenicity threshold (significant mutagenicity above this line).

***In Vitro* PAMPA Permeability.**

The results calculated for compound **13** may be ambiguous due to the instability of the compound in phosphate buffered saline (pH 7.4), as around 50 % decomposition was determined by controlled LC-MS analysis (data not shown).

Table 2. ADMET properties of the most active compounds (**1**, **2**, **4**, **13**).

ADME–Tox Properties					
	Mutagenic Potential				PAMPA-Permeability
Compounds	MI (1 µM)	B	MI (10 µM)	B	Kp (cm s^{-1})
1	1.15	0.74	1.20	0.81	2.69×10^{-6}
2	1.04	0.56	0.87	0.28	3.17×10^{-6}
4	1.25	0.87	1.75	1.0	2.57×10^{-6}
13	1.47	0.96	0.82	0.14	2.25×10^{-6}
Ebselen	0.69	0.26	0.80	0.46	nd
Caffeine	nd	nd	Nd	nd	3.61×10^{-6}
Norfloxacin	nd	nd	Nd	nd	0.95×10^{-6}

Ebselen - selenium reference compound in Ames test. Reference compounds in PAMPA: high-permeable drug, caffeine; low-permeable drug, norfloxacin. MI - mutagenic index (the quotient of the number of revertant colonies induced in a test sample and the number of revertants in a negative control). B - Binomial B value, nd not determined; *K*p - permeability coefficient.

Notes and references

1 1. H. L. Wheeler and H. F. Merriam, *Journal of the American Chemical Society*, 1901, **23**, 283-299.

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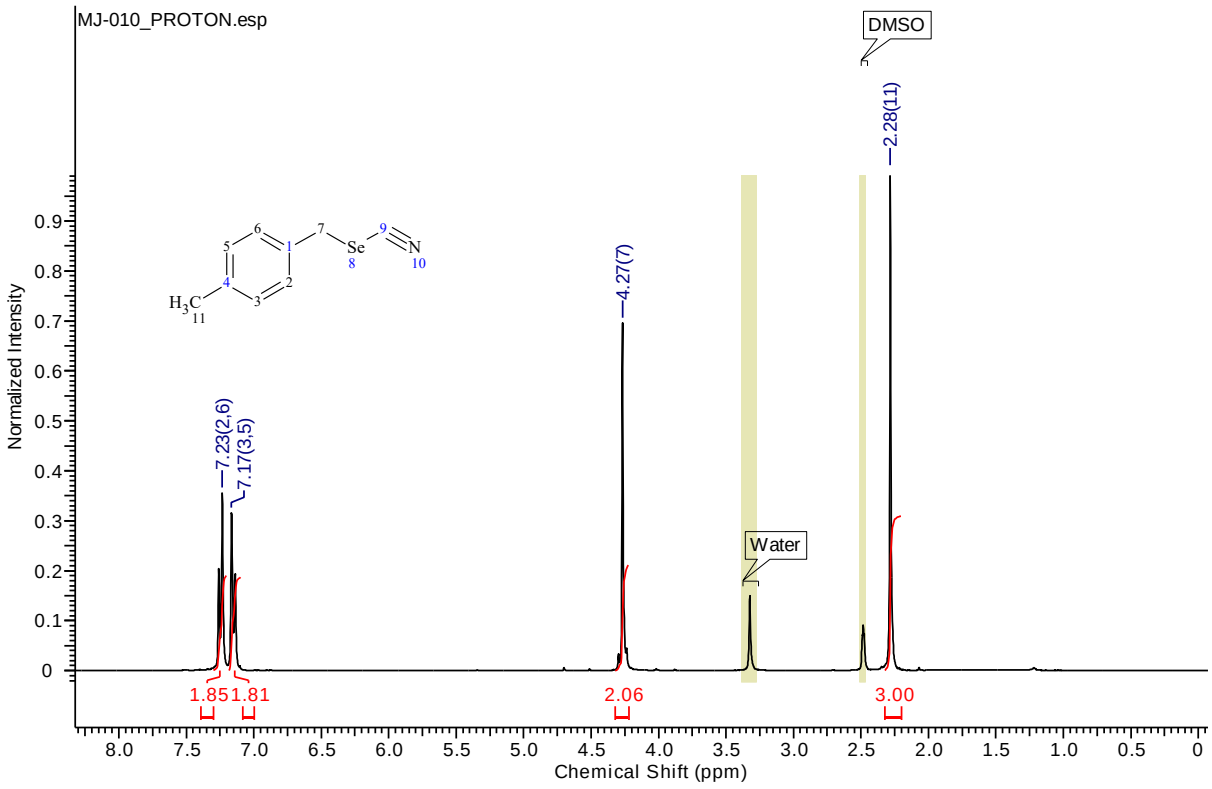
3 3. H. Suzuki, M. Usuki and T. Hanafusa, *Synthesis*, 1979, **1979**, 705-707.

4 4. L. A. Jacob, B. Matos, C. Mostafa, J. Rodriguez and J. K. Tillotson, *Molecules*, 2004, **9**, 622-626.

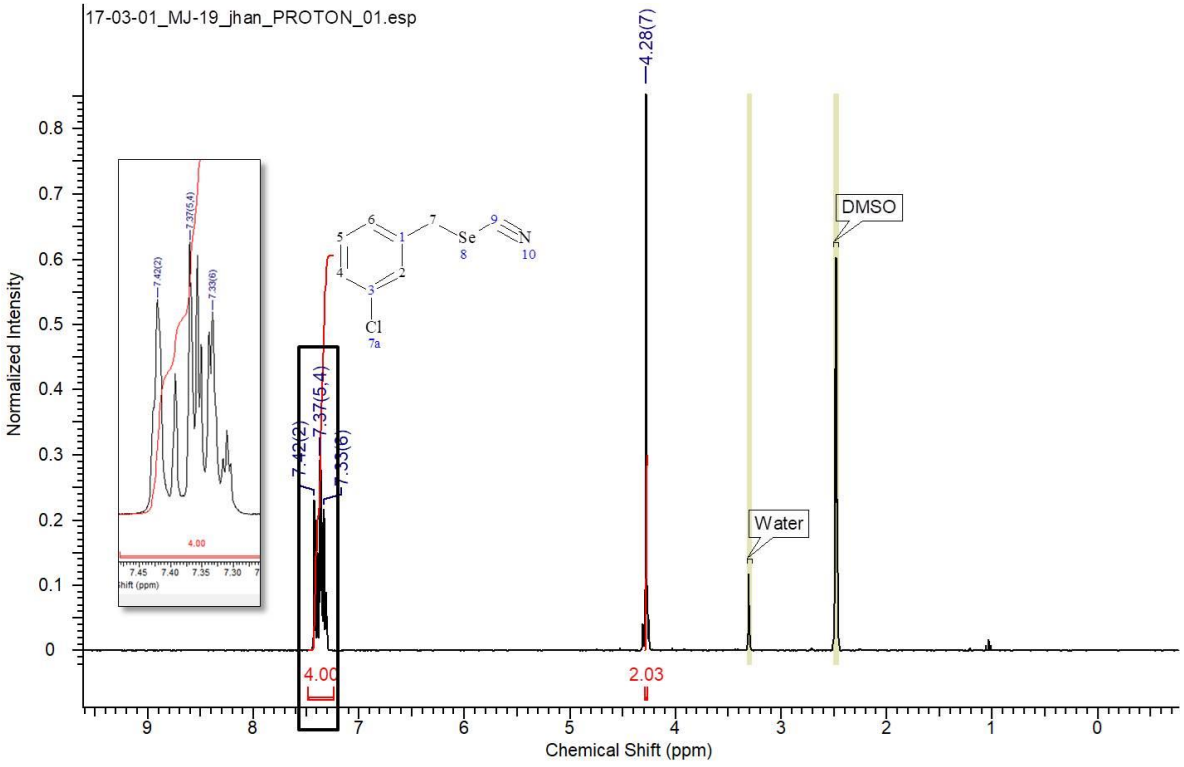
5 5. C. R. Groom, I. J. Bruno, M. P. Lightfoot and S. C. Ward, *Acta Crystallographica Section B-Structural Science Crystal Engineering and Materials*, 2016, **72**, 171-179.

^{1}H NMRs of Novel Compounds

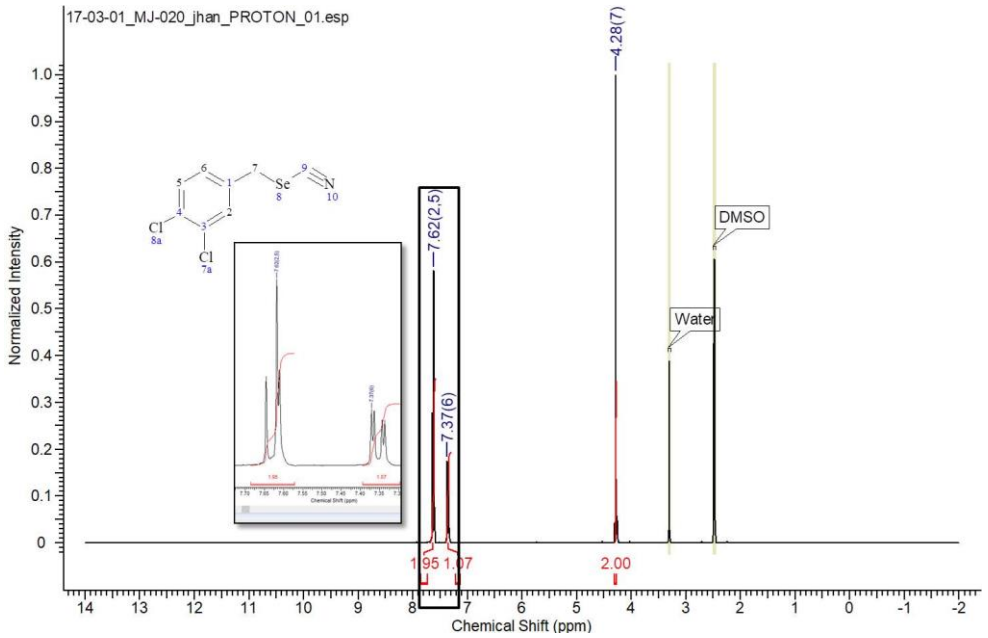
Compound 2



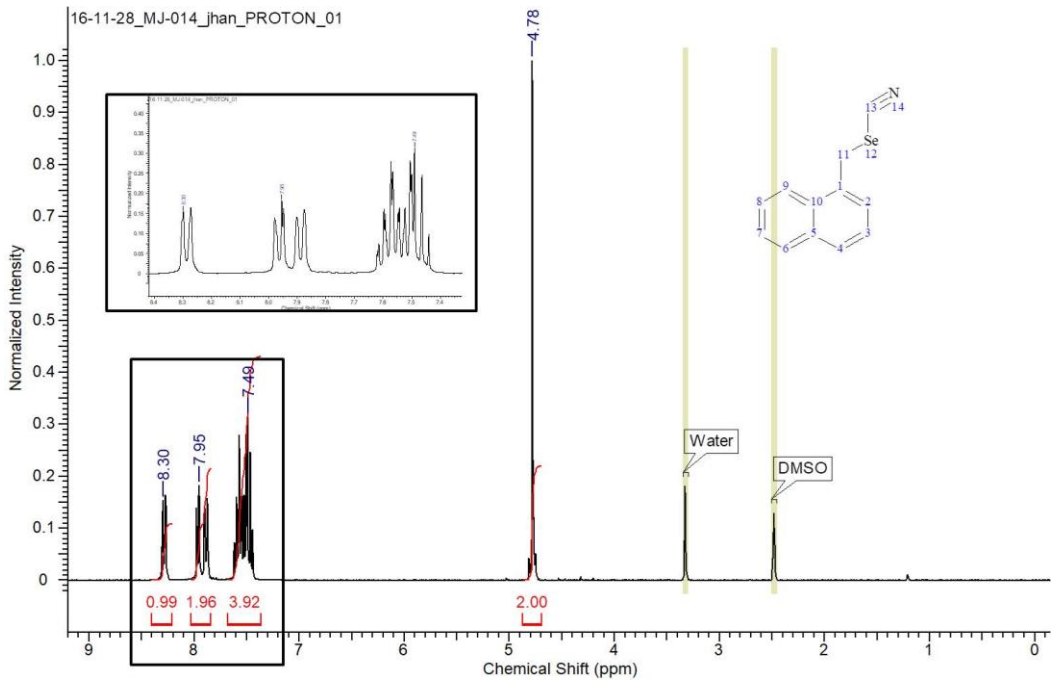
Compound 8



Compound 9

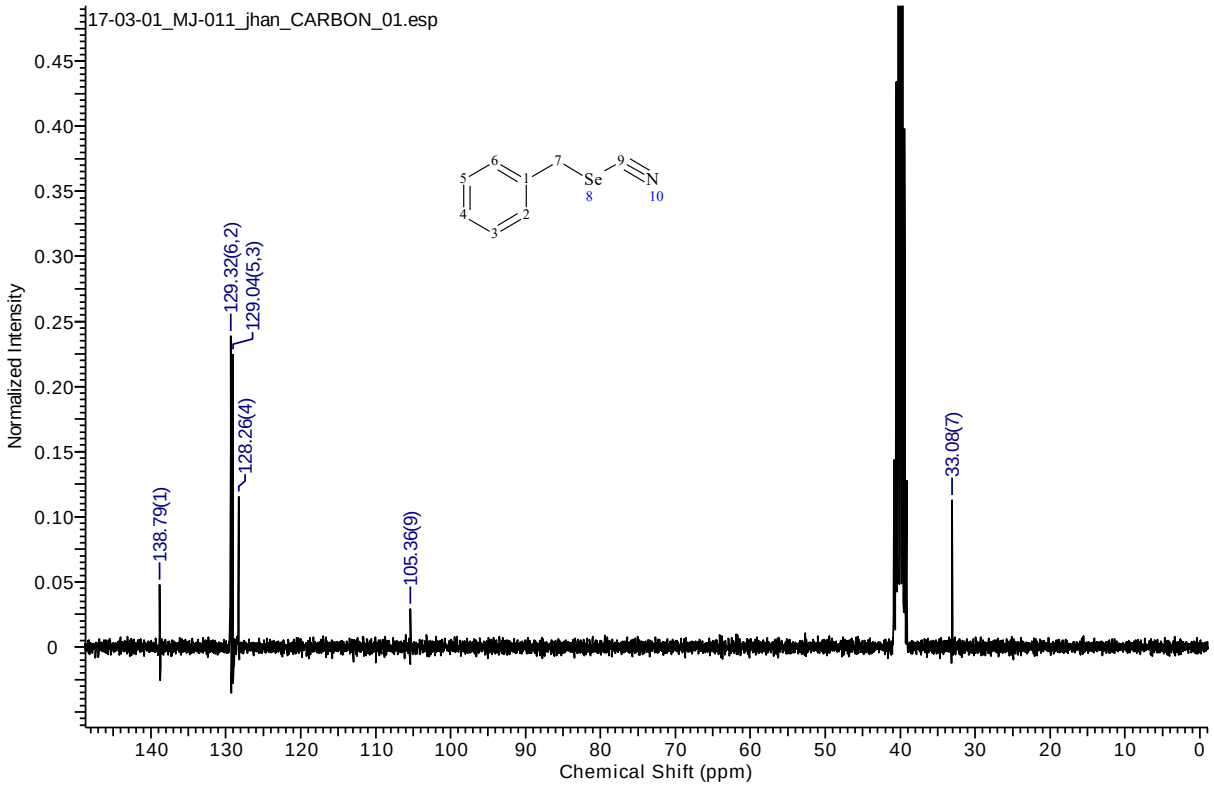


Compound 13

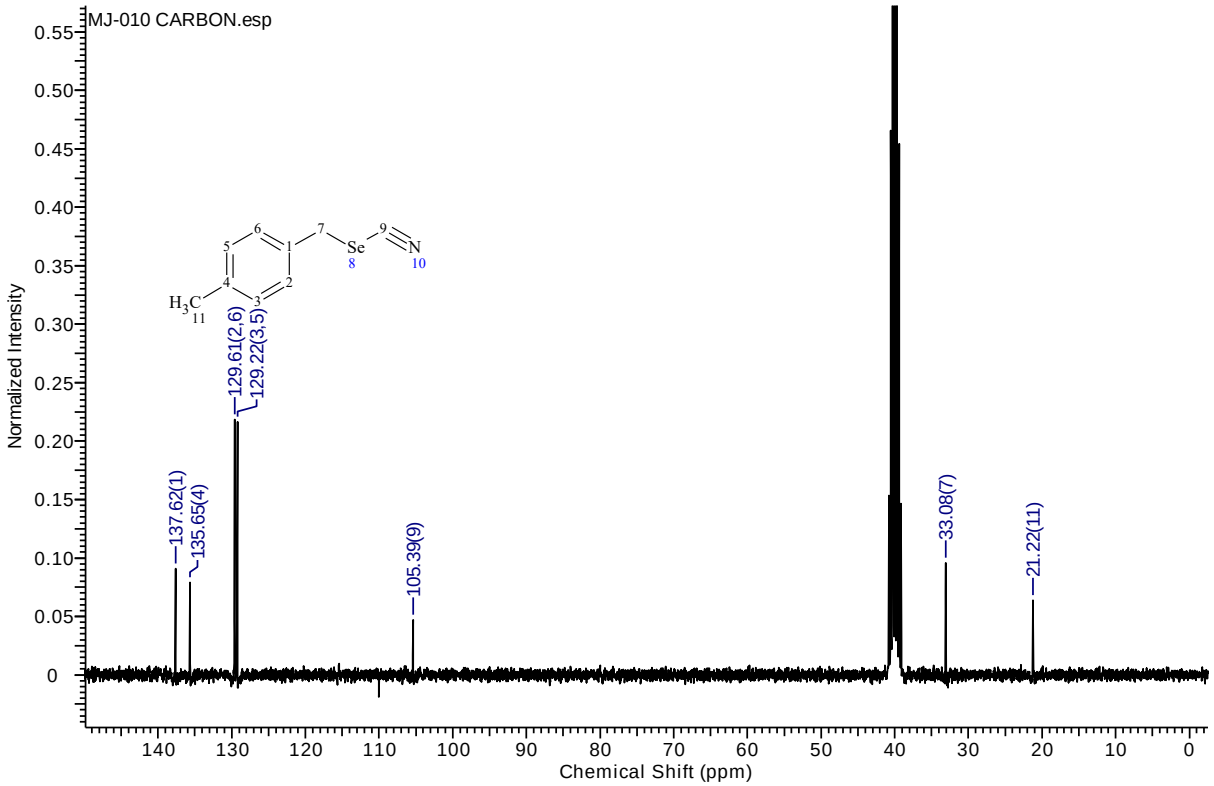


13 C NMRs of synthesized compounds

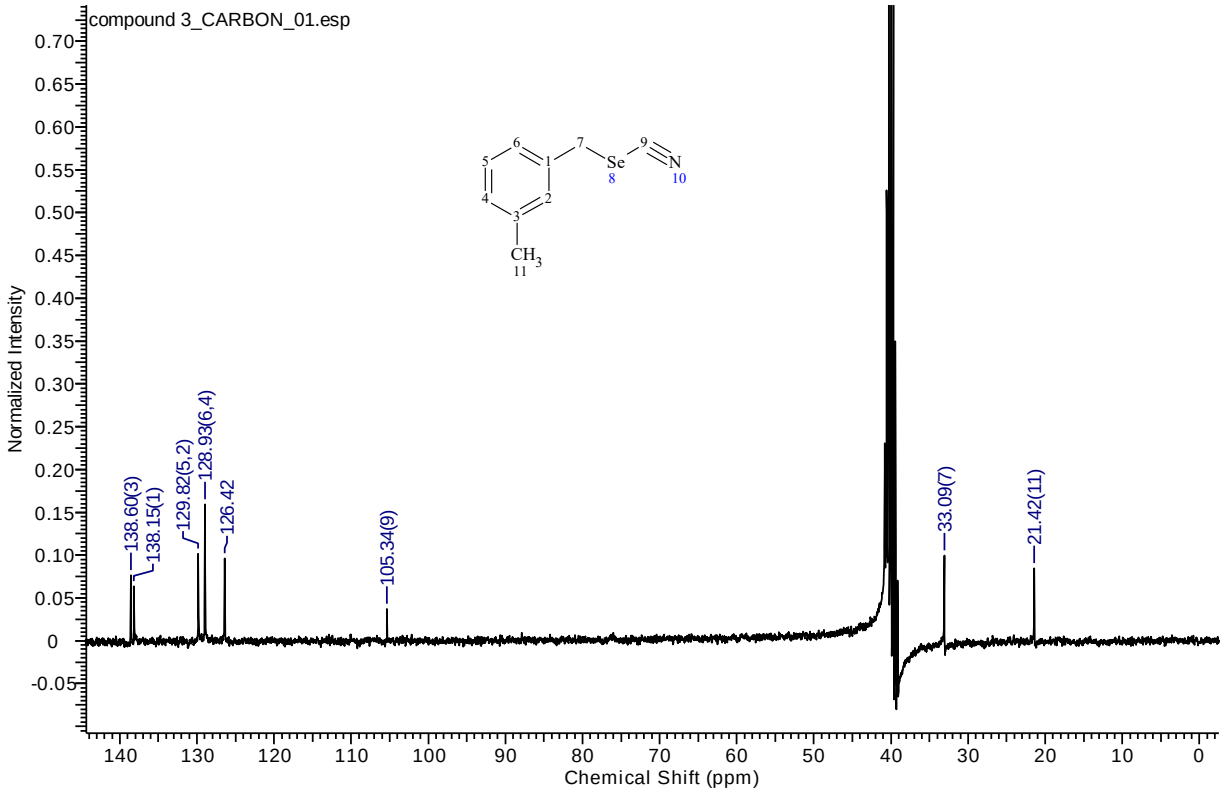
Compound 1



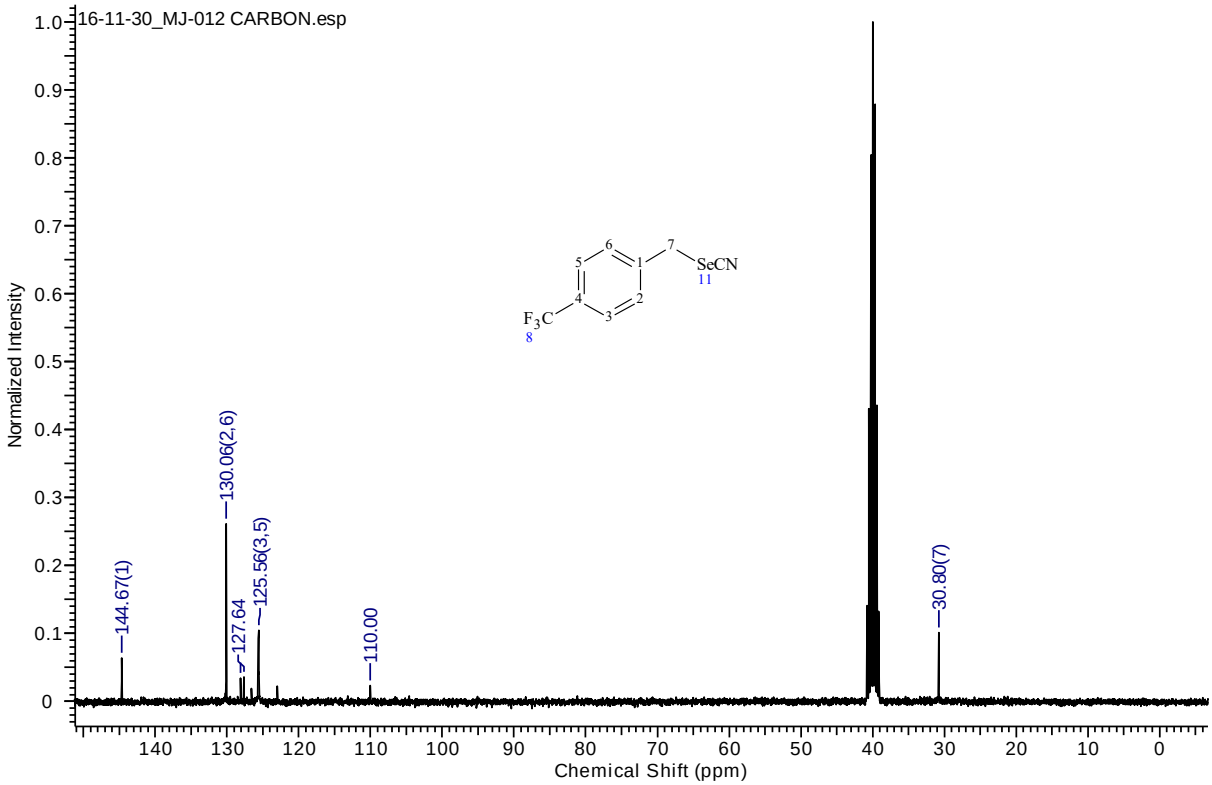
Compound 2



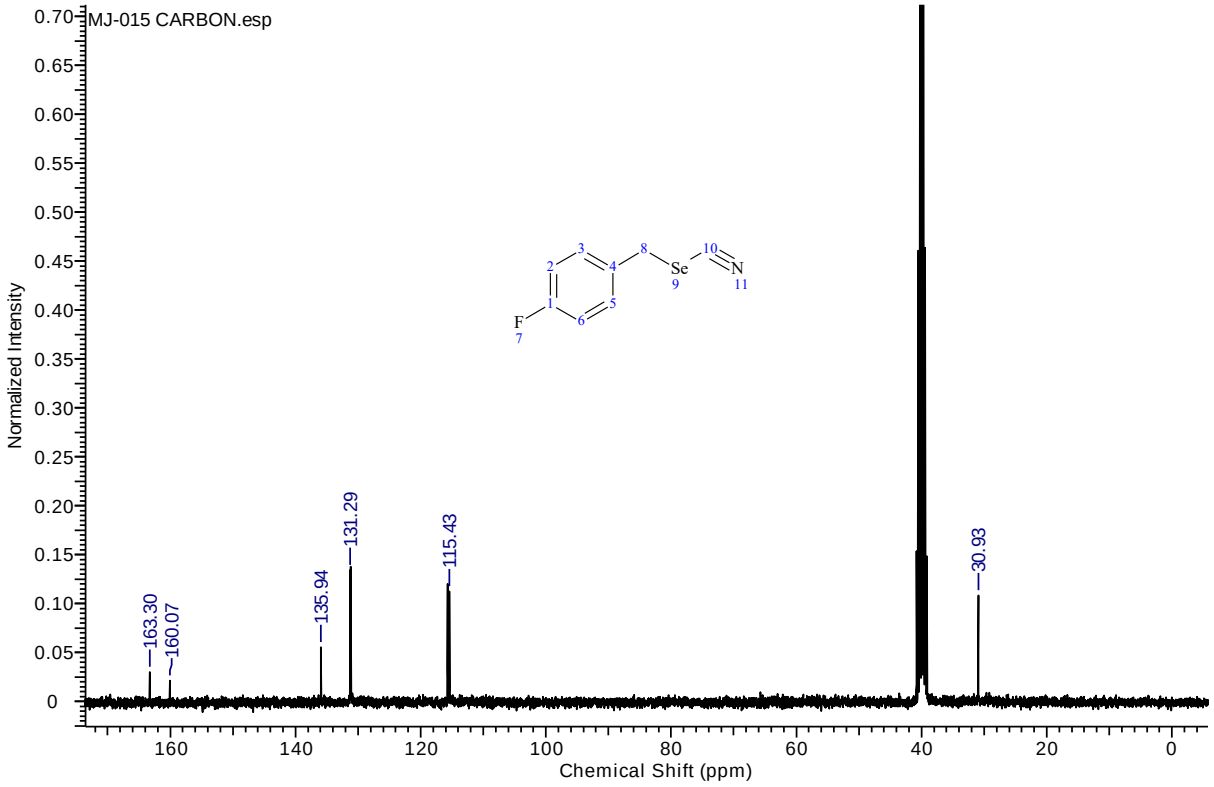
Compound 3



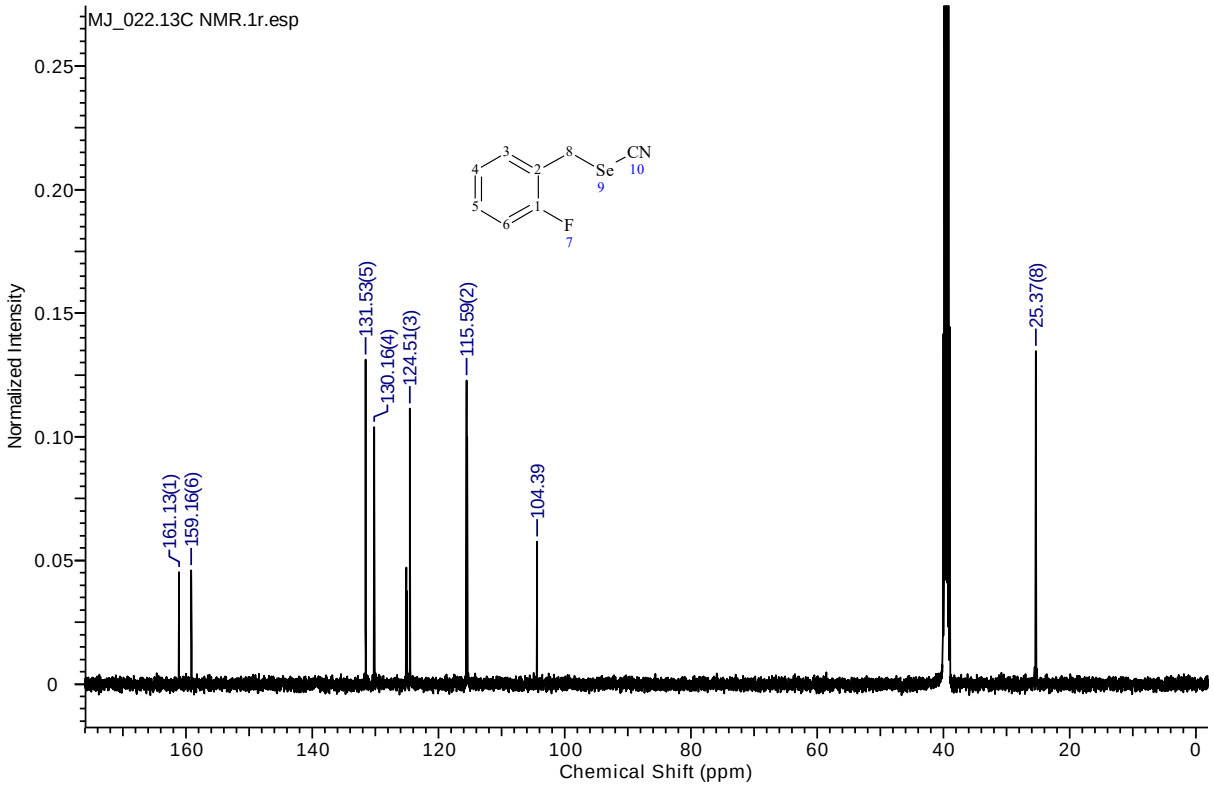
Compound 4



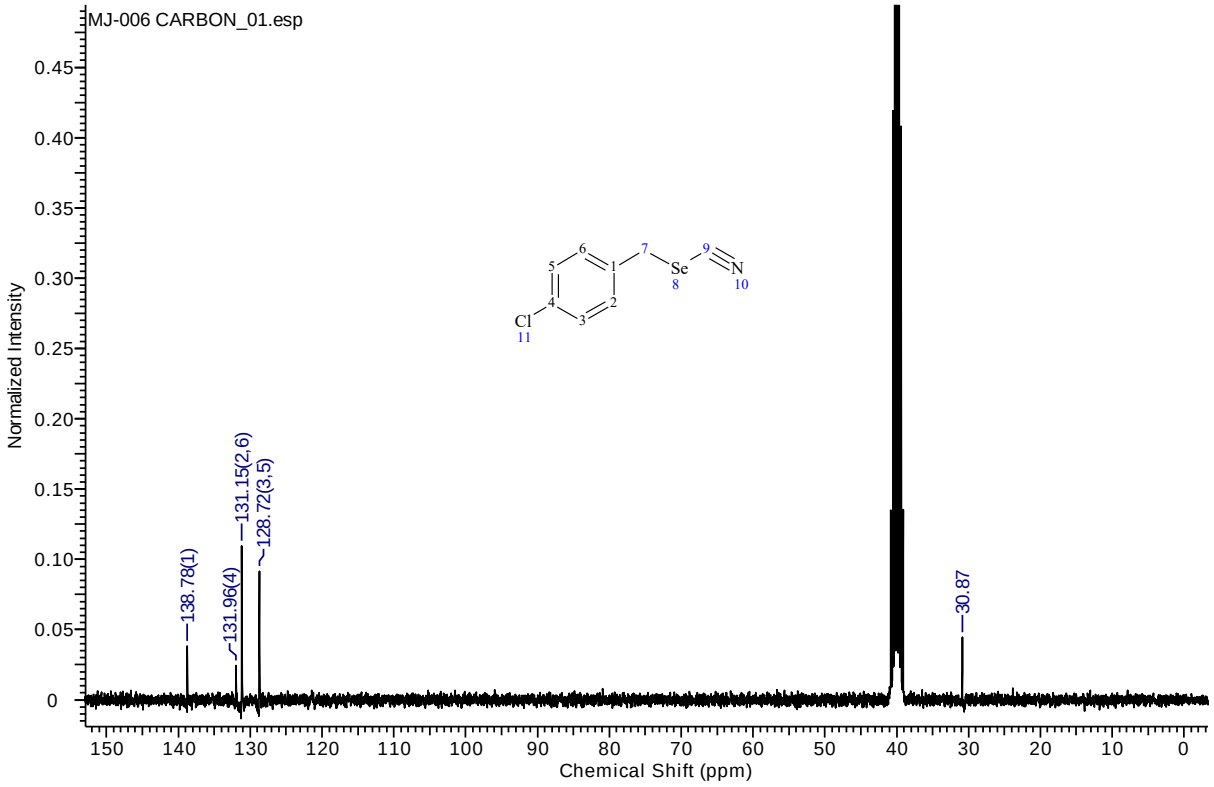
Compound 5



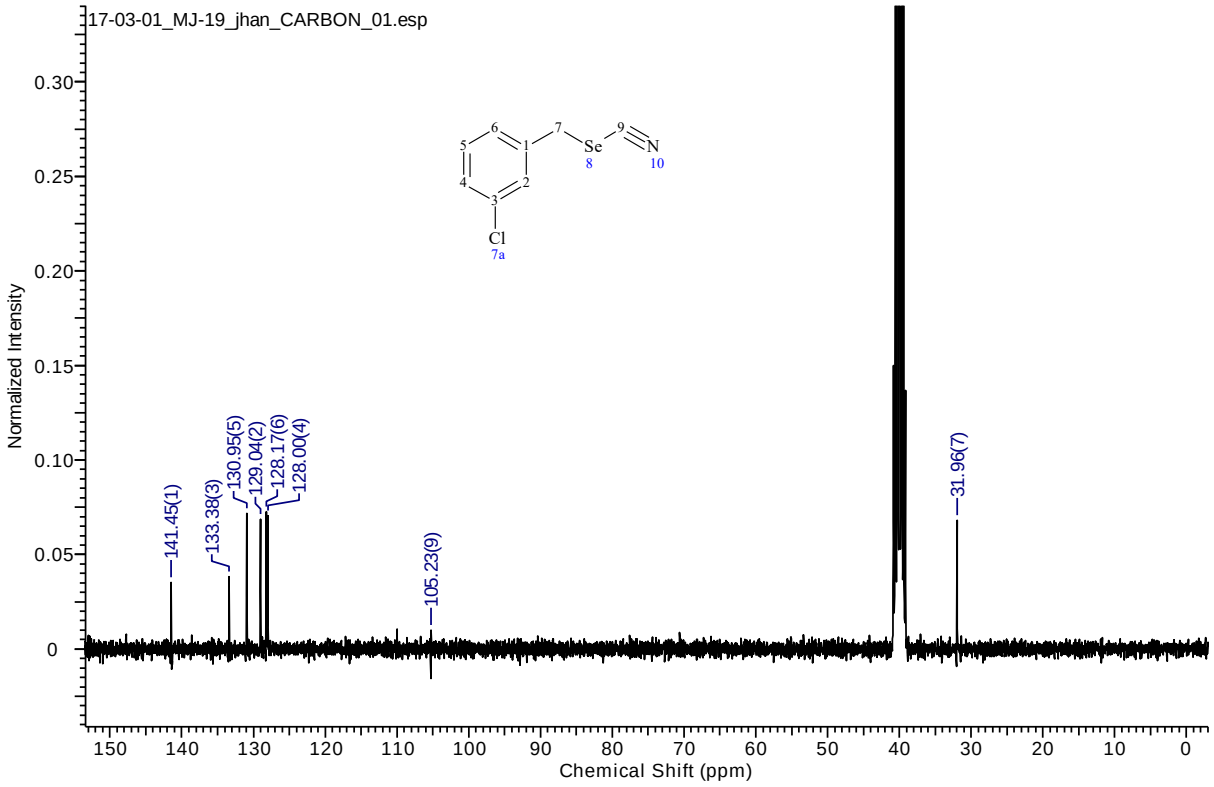
Compound 6



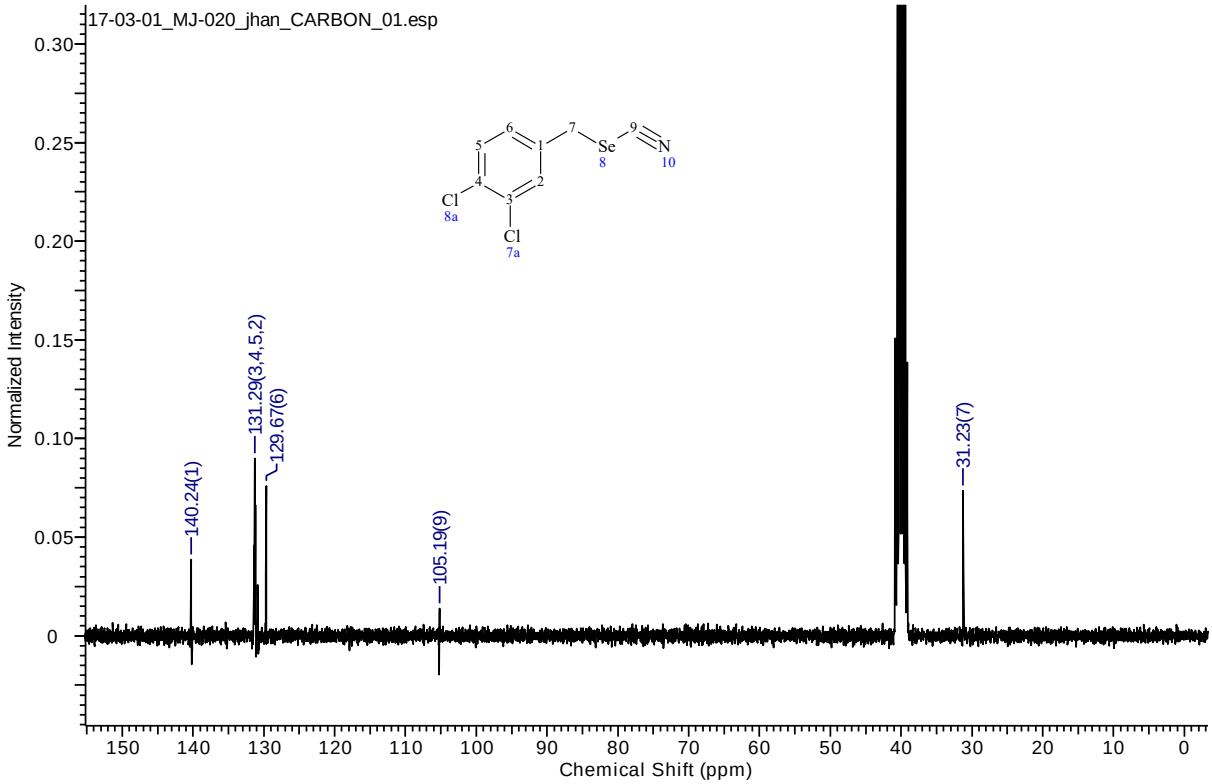
Compound 7



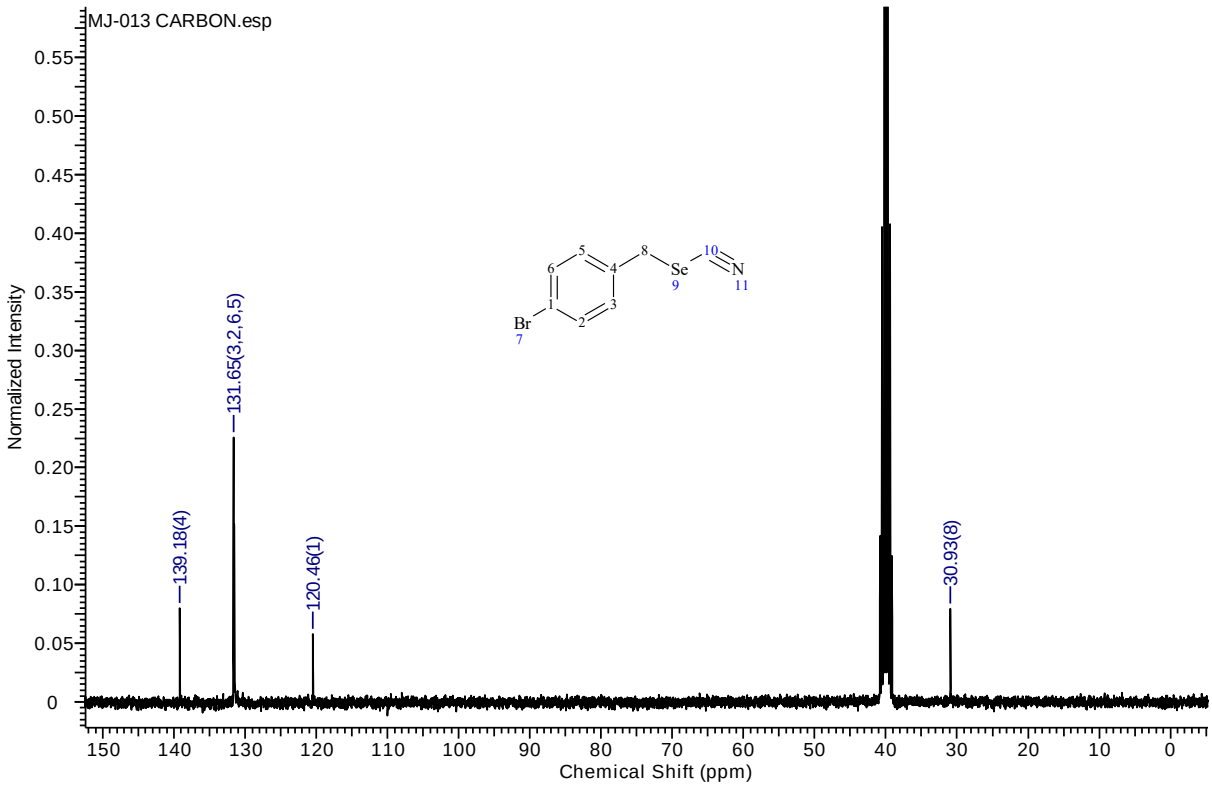
Compound 8



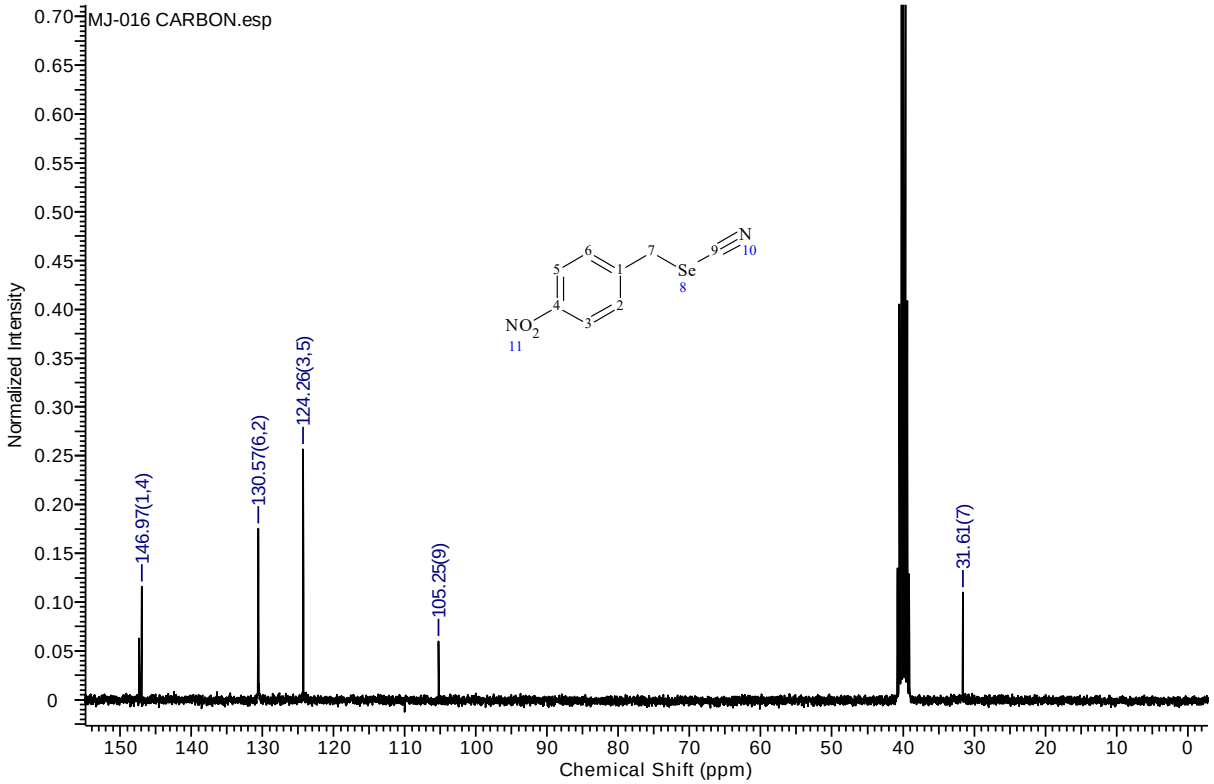
Compound 9



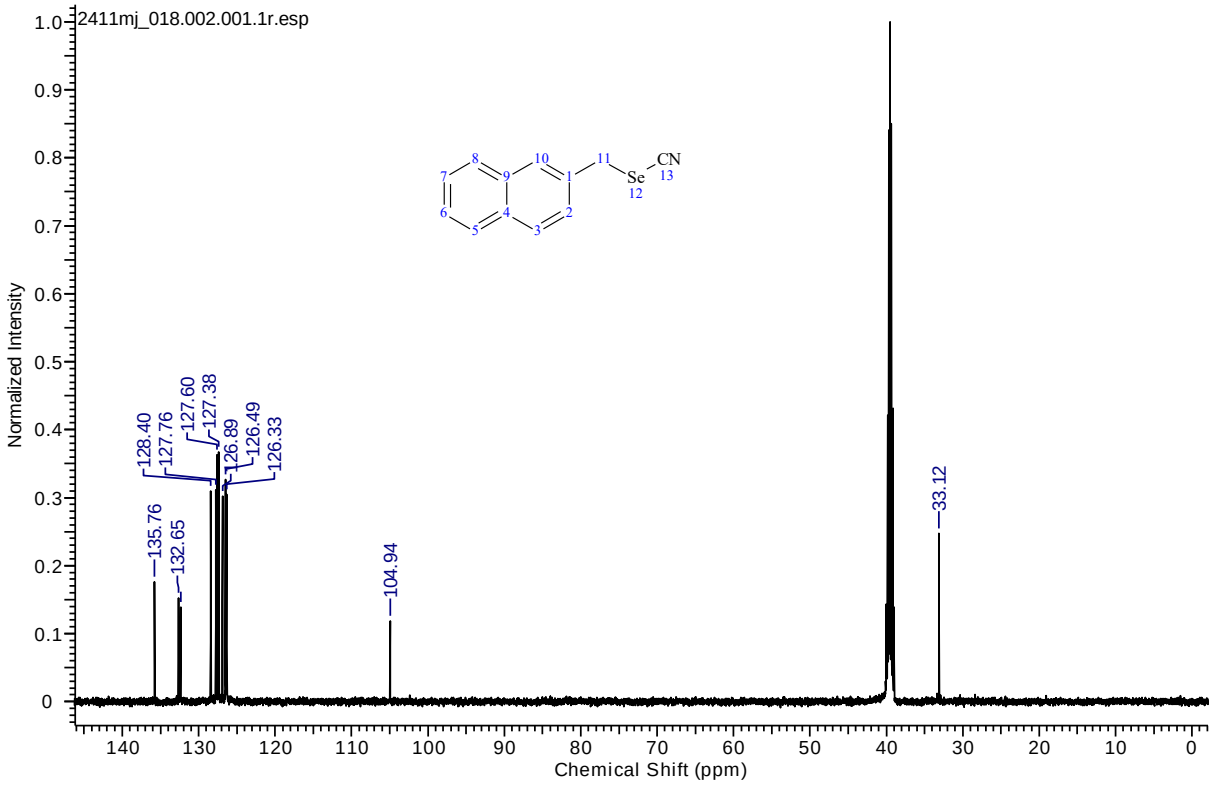
Compound 10



Compound 11



Compound 12



Compound 13