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

Muhammad Jawad Nasim [1], Karolina Witek [2,3], Annamária Kincses [4], Muhammad Sarfraz [1], Ewa Żesławska [5], Małgorzata Anna Marć [2], Mário Gajdács [4], Gabriella Spengler [4], Wojciech Nitek [6], Gniewomir Latacz [2], Elżbieta Karczewska [3], Katarzyna Kieć-Kononowicz [2], Claus Jacob [1,*], Jadwiga Handzlik [2,*]

Supplementary

1. 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 (10-20mmol) were treated with KSeCN (12-25 mmols) in the presence of ethanol (10-20 ml). The reaction mixture was refluxed for 6 hours 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].

1.1. Synthesis of Benzyl selenocyanate ***(1)***

Benzyl bromide (1.71g, 10 mmol), KSeCN (1.73 g. 12 mmol) and ethanol (10 ml) were used. The compound (**1**) was obtained as light crystals after purification by recrystallization with ethanol. Yield 72.5% (1.43 g, 7.25 mmol). TLC R*f* (DCM, 100%): 0.64, ^{1}H NMR (DMSO-d6, 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-d6, 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.

1.2. Synthesis of 3-Methylbenzyl selenocyanate ***(3)***

3-Methylbenzyl chloride (2.81g, 20 mmol), KSeCN (3.6g, 25 mmol) and ethanol (20 ml) were used. The compound (**3**) was obtained as light crystals after purification by recrystallization with ethanol. Yield 83.5% (3.51g, 16.7mmol). TLC R*f* (DCM, 100%): 0.65, ^{1}H NMR (DMSO-d6, 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-d6, ppm): δ 138.60, 138.15,129.82, 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.

1.3. Synthesis of 4-trifluoromethylbenzyl selenocyanate ***(4)***

4-Trifluoromethylbenzyl bromide (4.73g, 20 mmol), KSeCN (3.6g, 25 mmol) and ethanol (20 ml) were used. The compound (**4**) was obtained as light crystals after purification by recrystallization with ethanol. Yield 81.75% (4.32g, 16.35mmol). TLC R*f* (DCM, 100%): 0.94, ^{1}H NMR (DMSO-d6, 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-d6, 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.

1.4. Synthesis of 4-fluorobenzyl selenocyanate ***(5)***

4-Fluorobenzyl chloride (2.89g, 20 mmol), KSeCN (3.6g, 25 mmol) and ethanol (20 ml) were used. The compound (**5**) was obtained as light crystals after purification by recrystallization with ethanol. Yield 83.45% (3.57g, 16.69 mmol). TLC R*f* (PE:EA; 4:1): 0.60, ^{1}H NMR (DMSO-d6, 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-d6, 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.

1.5. Synthesis of 2-fluorobenzyl selenocyanate ***(6)***

2-Fluorobenzyl chloride (2.89g, 20 mmol), KSeCN (3.6g, 25 mmol) and ethanol (20 ml) were used. The compound (**6**) was obtained as light crystals after purification by recrystallization. Yield 75.45% (3.23g, 15.09mmol). TLC R*f* (DCM, 100%): 0.53, ^{1}H NMR (DMSO-d6, 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-d6, 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.

1.6. Synthesis of 4-chlorobenzyl selenocyanate ***(7)***

4-Chlorobenzyl bromide (2.67g, 16.6 mmol), KSeCN (2.8 g, 19.4 mmol) and ethanol (20 ml) were used. The compound (**7**) was obtained as light crystals after purification by recrystallization with ethanol. Yield 85.24% (3.26 g, 14.15 mmol). TLC R*f* (DCM, 100%): 0.75, ^{1}H NMR (DMSO-d6, 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-d6, 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.

1.7. Synthesis of 4-bromobenzyl selenocyanate ***(10)***

4-Bromobenzyl bromide (5g, 20 mmol), KSeCN (3.6g, 25 mmol) and ethanol (20 ml) were used. The compound (**10**) was obtained as light crystals after purification by recrystallization with ethanol. Yield 62.15% (3.42g, 12.43mmol). TLC R*f* (DCM, 100%): 0.86, ^{1}H NMR (DMSO-d6, 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-d6, 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.

1.8.Synthesis of 4-nitrobenzyl selenocyanate ***(11)***

4-Nitrobenzyl chloride (3.43g, 20 mmol), KSeCN (3.6g, 25 mmol) and ethanol (20 ml) were used. The compound (**11**) was obtained as light crystals after purification by recrystallization with ethanol. Yield 72% (3.47g, 14.4mmol). TLC R*f* (DCM, 100%): 0.50, ^{1}H NMR (DMSO-d6, ppm): δ 8.21 (m, 2H, CH), 7.63 (m, 2H, CH), 4.39 (t def, 2H, CH_2). ^{13}C NMR (DMSO-d6, 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.

1.9.Synthesis of 2-(selenocyanatomethyl) naphthalene ***(12)***

2- Chloromethyl naphthalene (2.51g, 14.2 mmol), KSeCN (2.6g, 18 mmol) and ethanol (14 ml) were used. The compound (**12**) was obtained as light crystals after purification by recrystallization wit ethanol. The compound was obtained in 81.27 % yield (2.85g, 11.54mmol). TLC R*f* (DCM, 100%): 0.77, ^{1}H NMR (DMSO-d6, 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-d6, 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.

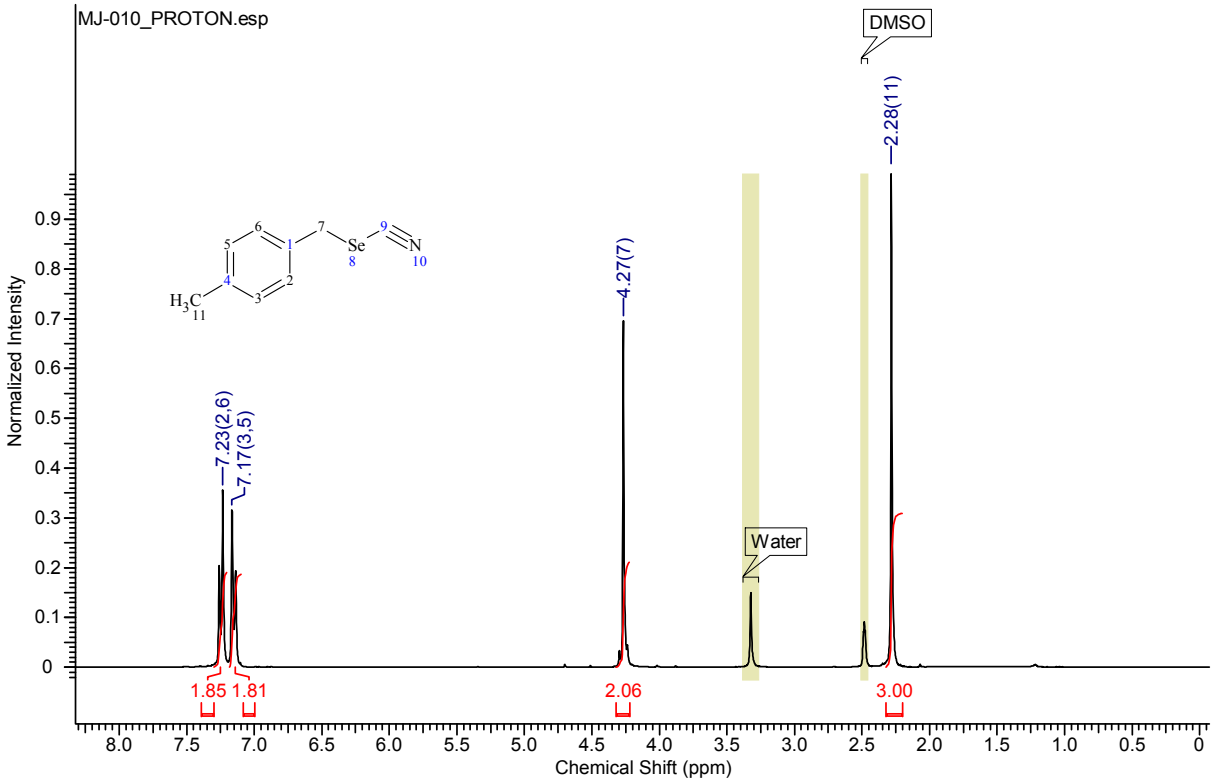
References

[1] Wheeler, H.L.; Merriam, H.F. On the action of alkylthiocyanates and alkyl isothiocyanates with thiol acids. *J. Am. Chem. Soc.* **1901**, *23*, 283-299.
[2] Plano, D.; Baquedano, Y.; Moreno-Mateos, D.; Font, M.; Jimenez-Ruiz, A.; Palop, J.A.; Sanmartin, C. Selenocyanates and diselenides: a new class of potent antileishmanial agents. *Eur. J. Med. Chem.* **2011**, *46*, 3315-3323.
[3] Suzuki, H.; Usuki, M.; Hanafusa, T. A photochemical route to some substituted benzyl isoselenocyanates. *Synthesis* **1979**, *1979*, 705-707.
[4] Jacob, L.A.; Matos, B.; Mostafa, C.; Rodriguez, J.; Tillotson, J.K. A facile synthesis of substituted benzyl selenocyanates *Molecules* **2004**, *9*, 622-626.

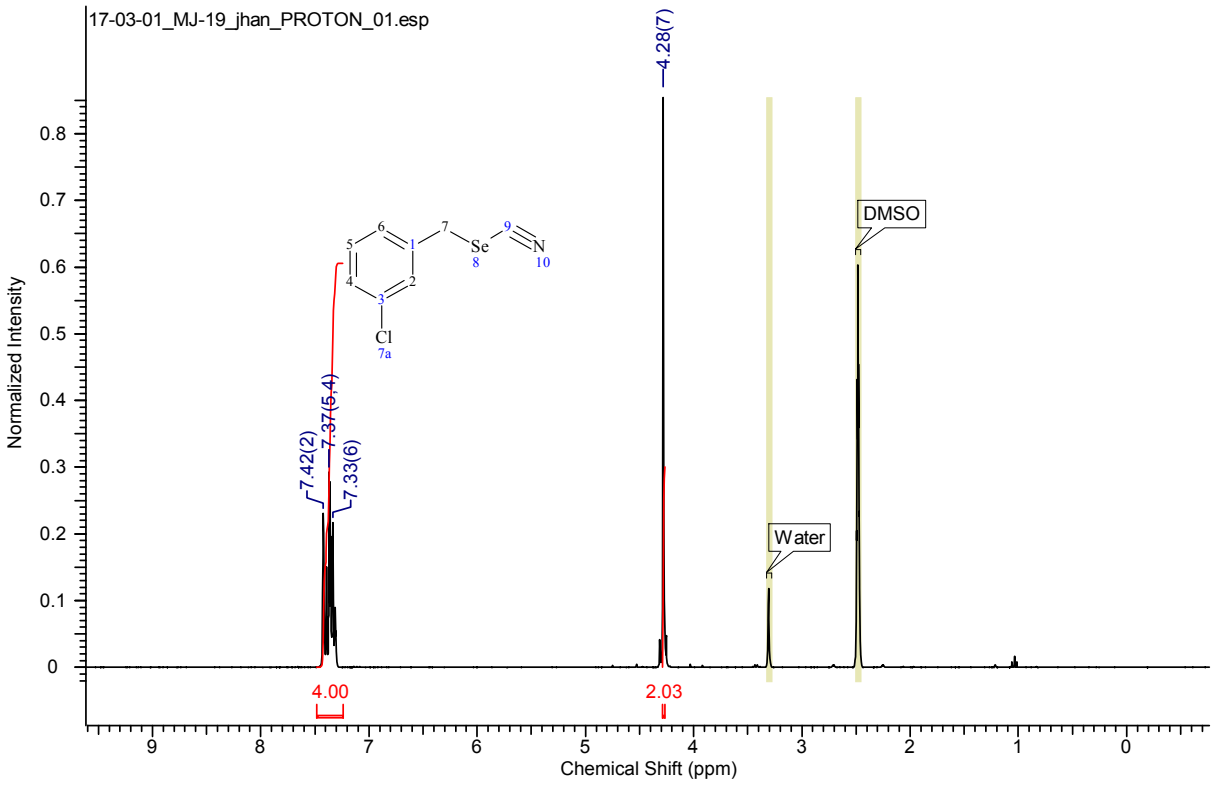
Spectral data for new compounds

^{1}H NMRs of Novel Compounds

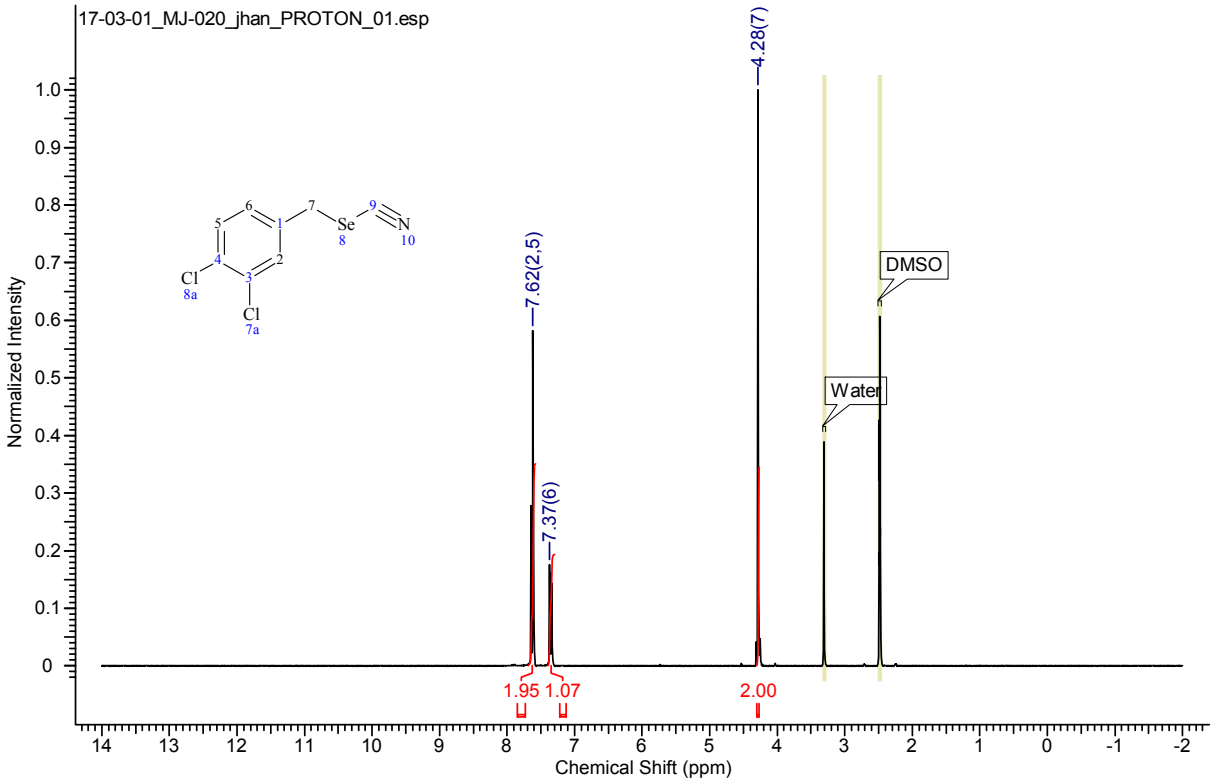
Compound 2



Compound 8



Compound 9



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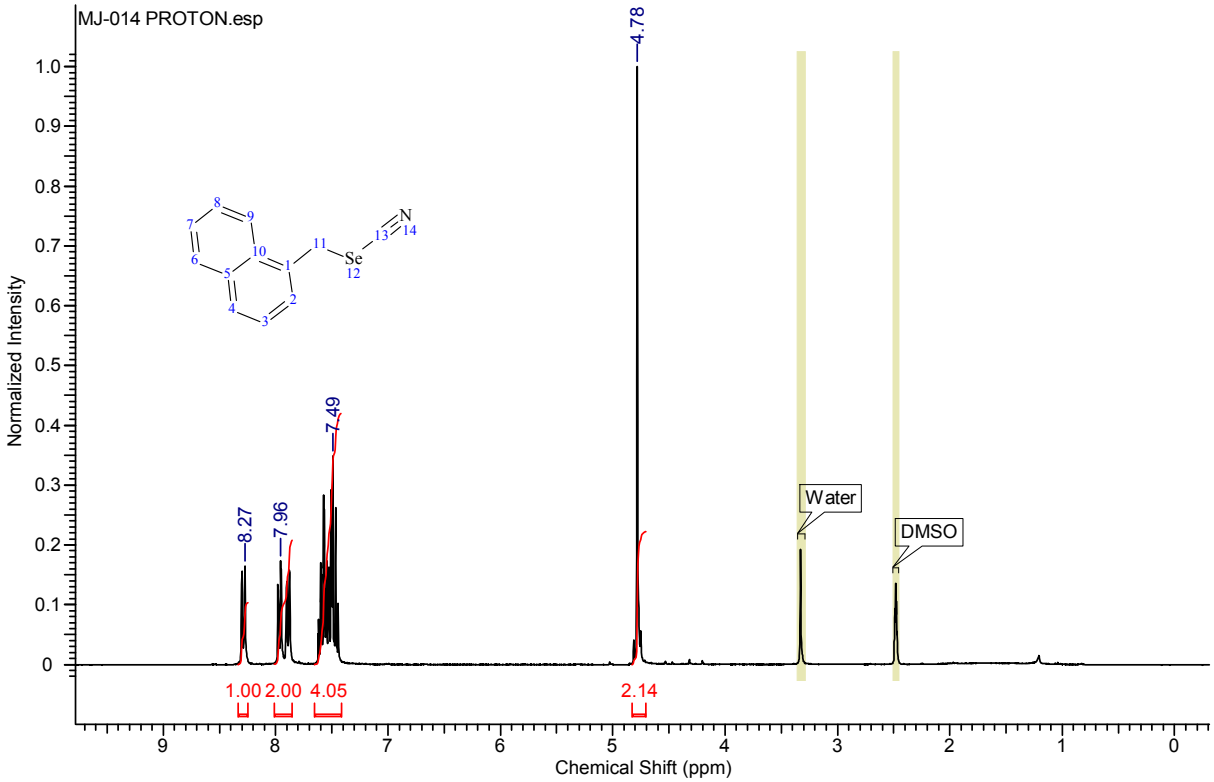
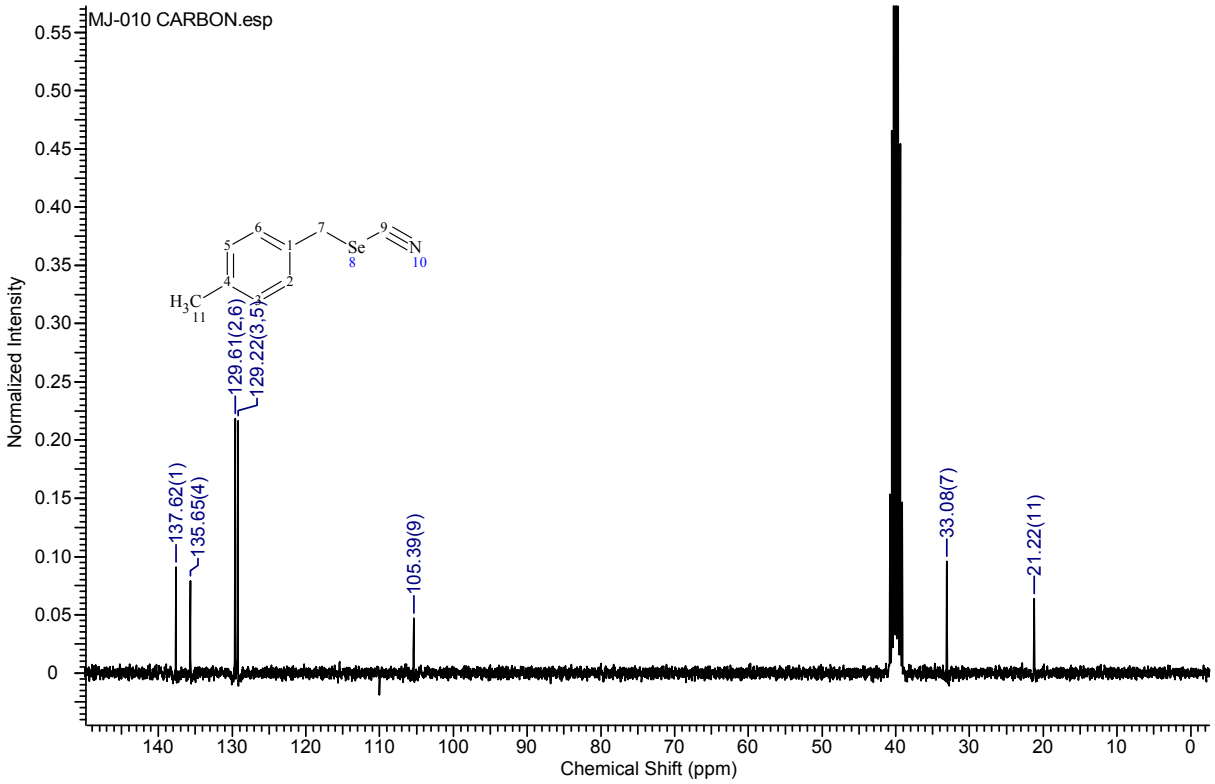


13 C NMRs of selected compounds

Compound 2



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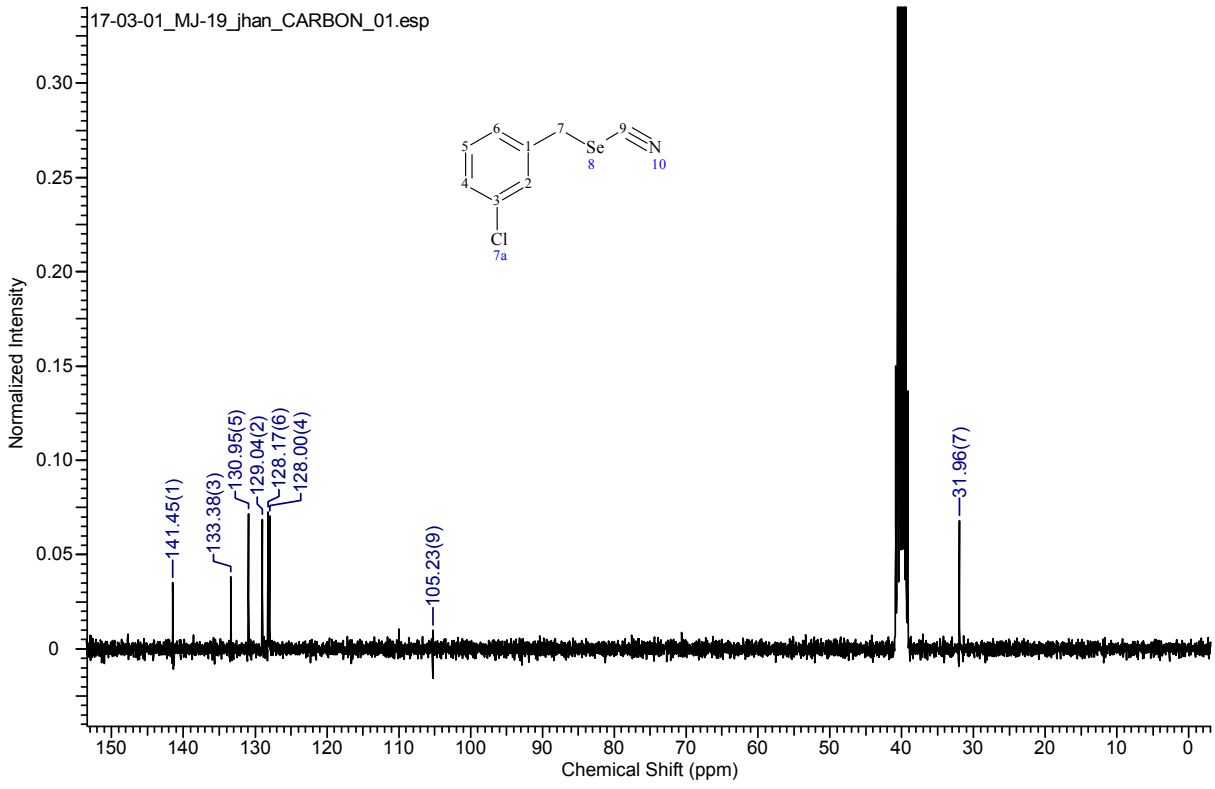

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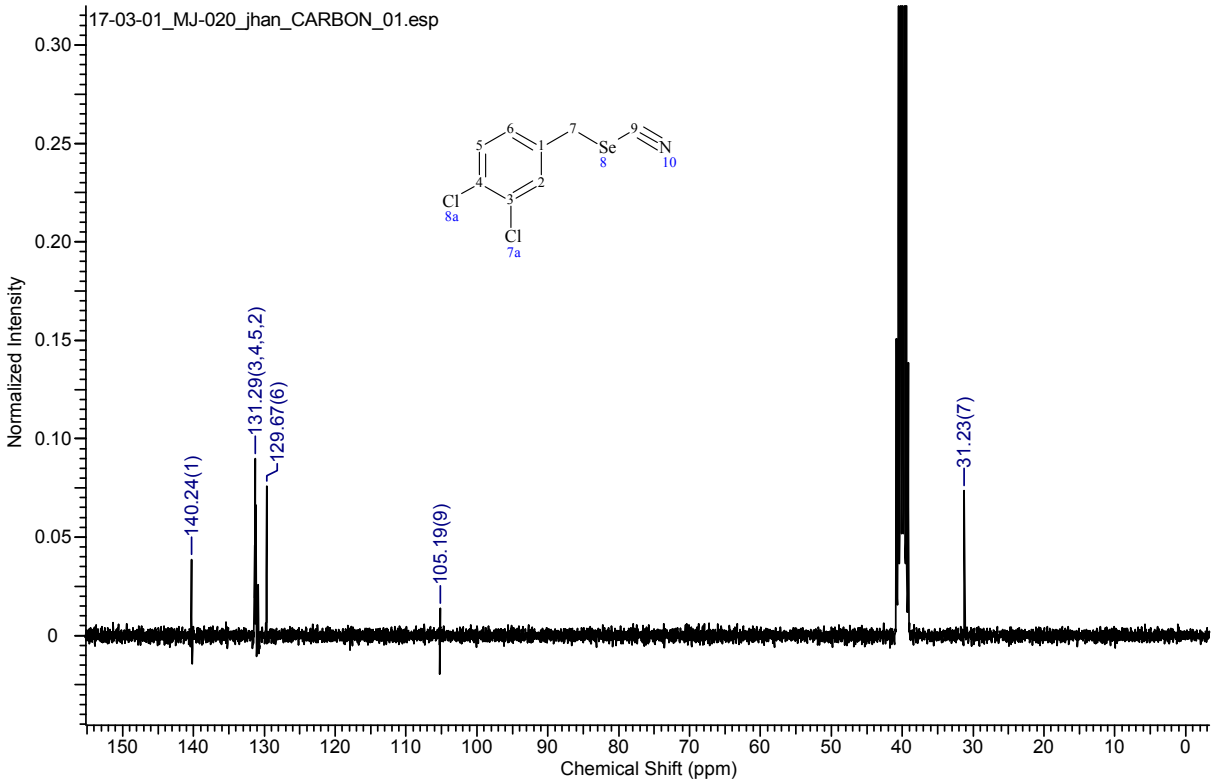

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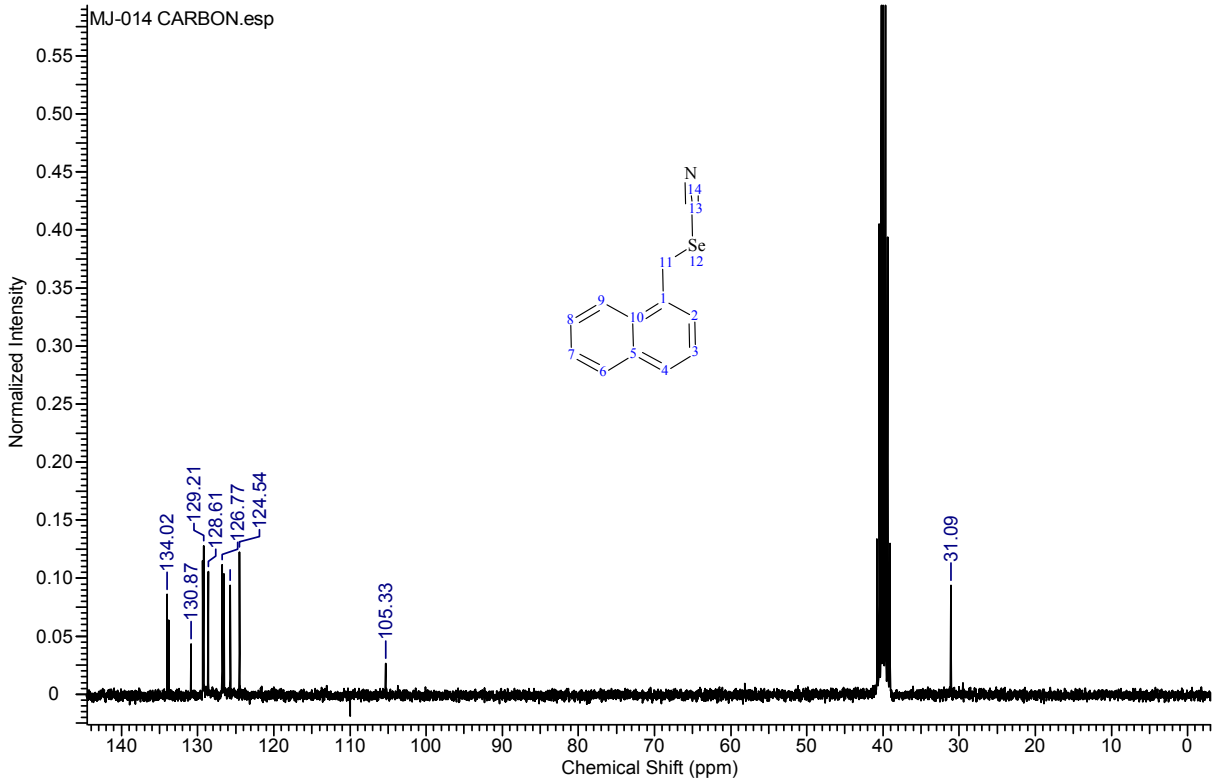

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