

Supplementary Information

Synthesis and anticancer activity of benzoselenophene and heteroaromatic derivatives of 1,2,9,9a-tetrahydrocyclopropa[c]benzo[e]indol-4-one (CBI).

Content

Spectral Characterization of Compound 1a–h	S3
General synthetic procedure and spectral characterization of compound 2a–h	S4
General procedure for Synthesis of scaffold 12	S5
General synthetic procedure and spectral characterization of compound 15–16	S5
¹ H NMR and ¹³ C NMR spectra of compounds 1a–h	S6–13
¹ H NMR and ¹³ C NMR spectra of compounds 2a–h	S14–21
¹ H NMR and ¹³ C NMR spectra of compounds 3	S22
¹ H NMR and ¹³ C NMR spectra of intermediate of Compound 4	S23
¹ H NMR and ¹³ C NMR spectra of Compound 4	S24
¹ H NMR and ¹³ C NMR spectra of intermediate of Compound 5	S25
¹ H NMR and ¹³ C NMR spectra of Compound 5	S26
¹ H NMR and ¹³ C NMR spectra of intermediate of Compound 6	S27
¹ H NMR and ¹³ C NMR spectra of Compound 6	S28
¹ H NMR and ¹³ C NMR spectra of intermediate of Compound 7	S29
¹ H NMR and ¹³ C NMR spectra of Compound 7	S30
¹ H NMR and ¹³ C NMR spectra of intermediate of Compound 8	S31
¹ H NMR and ¹³ C NMR spectra of Compound 8	S32
¹ H NMR and ¹³ C NMR spectra of intermediate of Compound 9	S33
¹ H NMR and ¹³ C NMR spectra of Compound 9	S34

^1H NMR and ^{13}C NMR spectra of intermediate of Compound 10	S35
^1H NMR and ^{13}C NMR spectra of Compound 10	S36
^1H NMR and ^{13}C NMR spectra of intermediate of Compound 14a–p	S37–52
^1H NMR and ^{13}C NMR spectra of intermediate of Compound 15–16	S53–54

The synthetic procedure for compounds 1a-h is described in our patent.³⁸

Spectral Characterization data of 1a–h:

Ethyl 5-nitrobenzo[b]selenophene-2-carboxylate (**1a**), 94%; ¹H NMR (500.1 MHz, CDCl₃) δ 8.74 (d, *J* = 1.8 Hz, 1H), 8.37 (s, 1H), 8.20 (dd, *J* = 2.0, 8.8 Hz, 1H), 8.04 (d, *J* = 8.8 Hz, 1H), 4.42 (q, *J* = 7.2 Hz, 2H), 1.42 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (125.7 MHz, CDCl₃) δ 164.1, 151.6, 147.3, 142.3, 141.9, 134.8, 127.8, 123.7, 121.7, 63.4, 15.4; LCMS (ESI) *m/z* calcd. for C₁₁H₉NO₄Se[M]⁺ 298.97, found 300.2 [M + H]⁺.

Ethyl 5-methoxybenzo[b]selenophene-2-carboxylate (**1b**), 68%; ¹H NMR (500.1 MHz, CDCl₃) δ 8.22 (s, 1H), 7.75 (d, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 2.5 Hz, 1H), 7.04 (dd, *J* = 2.6, 8.8 Hz, 1H), 4.38 (q, *J* = 7.2 Hz, 2H), 3.87 (s, 3H), 1.40 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (125.7 MHz, CDCl₃) δ 161.6, 155.7, 139.9, 135.3, 133.6, 131.7, 124.1, 115.0, 107.0, 59.3, 53.2, 12.0; LCMS (ESI) *m/z* calcd. for C₁₂H₁₂O₃Se [M]⁺ 284.00, found 285.2 [M+H]⁺.

Ethyl 6-methoxybenzo[b]selenophene-2-carboxylate (**1c**), 86%; ¹H NMR (500.1 MHz, CDCl₃) δ 8.19 (s, 1H), 7.74 (d, *J* = 8.8 Hz, 1H), 7.36 (d, *J* = 1.8 Hz, 1H), 6.99 (dd, *J* = 2.2, 8.8 Hz, 1H), 4.36 (q, *J* = 7.2 Hz, 2H), 3.87 (s, 3H), 1.39 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (125.7 MHz, CDCl₃) δ 162.2, 157.5, 144.2, 133.3, 132.3, 131.5, 126.3, 113.4, 106.5, 59.7, 53.8, 12.6; LCMS (ESI) *m/z* calcd. for C₁₂H₁₂O₃Se [M]⁺ 284.00, found 285.0 [M+H]⁺.

ethyl 7-methoxybenzo[b]selenophene-2-carboxylate (**1d**), 79%; ¹H NMR (500.1 MHz, CDCl₃) δ 8.29 (s, 1H), 7.51 (d, *J* = 7.9 Hz, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 6.82 (d, *J* = 7.8 Hz, 1H), 4.39 (q, *J* = 7.2 Hz, 2H), 3.99 (s, 3H), 1.41 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (125.7 MHz, CDCl₃) δ 164.3, 156.5, 142.9, 137.2, 134.8, 133.4, 126.9, 120.1, 106.4, 61.9, 56.0, 14.6; LCMS (ESI) *m/z* calcd. for C₁₂H₁₂O₃Se [M]⁺ 284.00, found 285.0 [M + H]⁺.

ethyl 5,6-dimethoxybenzo[b]selenophene-2-carboxylate (**1e**), 87%; ¹H NMR (500.1 MHz, CDCl₃) δ 8.17 (s, 1H), 7.32 (s, 1H), 7.28 (s, 1H), 4.37 (q, *J* = 7.2 Hz, 2H), 3.97 (s, 3H), 3.94 (s, 3H), 1.39 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (125.7 MHz, CDCl₃) δ 162.1, 148.3, 146.7, 135.4, 132.7, 132.2 (2C), 106.1, 105.0, 59.5, 54.2, 54.1, 12.5; LCMS (ESI) *m/z* calcd. for C₁₃H₁₄O₄Se [M]⁺ 314.01, found 337.8 [M + Na]⁺.

ethyl selenopheno[2,3-b]pyridine-2-carboxylate (**1f**), 97%; ¹H NMR (500.1 MHz, CDCl₃) δ 8.58 (dd, *J* = 1.7, 4.7 Hz, 1H), 8.16 (s, 1H), 8.09 (dd, *J* = 1.7, 8.1 Hz, 1H), 7.33 (dd, *J* = 4.6, 8.0 Hz, 1H), 4.38 (q, *J* = 7.2 Hz, 2H), 1.39 (t, *J* = 7.1 Hz, 3H); ¹³C NMR (125.7 MHz, CDCl₃) δ 167.0, 163.6, 148.6, 137.5, 135.6, 134.4, 131.3, 120.3, 62.0, 14.3; LCMS (ESI) *m/z* calcd. for C₁₀H₉NO₂Se [M]⁺ 254.98, found 256.0 [M + H]⁺.

ethyl selenopheno[3,2-b]thiophene-5-carboxylate (**1g**), 95%; ¹H NMR (500.1 MHz, CDCl₃) δ 8.24 (s, 1H), 7.56 (d, *J* = 5.2 Hz, 1H), 7.33 (d, *J* = 5.3 Hz, 1H), 4.37 (q, *J* = 7.2 Hz, 2H), 1.39 (t, *J* = 7.1 Hz, 3H); ¹³C NMR (125.7 MHz, CDCl₃) δ 162.0, 142.1, 138.5, 136.8, 129.4, 126.6, 121.3, 59.8, 12.8; LCMS (ESI) *m/z* calcd. for C₉H₈O₂SSe [M]⁺ 259.94, found 261.0 [M + H]⁺.

ethyl selenopheno[3,2-b]furan-5-carboxylate (**1h**), 73%; ¹H NMR (500.1 MHz, CDCl₃) δ 8.06 (s, 1H), 7.63 (d, *J* = 1.2 Hz, 1H), 6.81 (s, 1H), 4.35 (q, *J* = 7.1 Hz, 2H), 1.38 (t, *J* = 7.1 Hz, 3H); ¹³C NMR (125.7 MHz, CDCl₃) δ 162.8, 156.4, 146.8, 135.0, 127.6, 118.4, 108.4, 60.4, 13.4; LCMS (ESI) *m/z* calcd. for C₉H₈O₃Se [M]⁺ 243.96, found 245.0 [M + H]⁺.

General procedure for Preparation of 2a–h: The benzoselenophene ester compound (0.1g) was dissolved in 2 mL MeOH-H₂O (9:1) mixture, then added NaOH (5 eq.) and stirred at room temperature for 24 h. After complete hydrolysis, the reaction mixture was concentrated under reduced pressure and added 5 mL water. The reaction solution was acidified with 20% HCl solution, the precipitated solid was filtered and further purified by silica column chromatography (eluent, 10% MeOH: CH₂Cl₂). The compounds 2a-h was obtained with excellent yields (> 80%).

Spectral Characterization data of 2a–h:

5-Nitrobenzo[b]selenophene-2-carboxylic acid (**2a**), 94%; ¹H NMR (500.1 MHz, MeOH-d₄) δ 8.86 (d, *J* = 1.7 Hz, 1H), 8.42 (s, 1H), 8.23-8.22 (m, 2H); ¹³C NMR (125.7 MHz, DMSO-d₆) δ 164.3, 150.3, 145.7, 142.3, 141.3, 133.8, 127.7, 122.7, 120.1.

5-Methoxybenzo[b]selenophene-2-carboxylic acid (**2b**), 89%; ¹H NMR (500.1 MHz, MeOH-d₄) δ 8.19 (s, 1H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.45 (d, *J* = 2.3 Hz, 1H), 7.03 (dd, *J* = 2.3, 8.8 Hz, 1H), 3.85 (s, 3H); ¹³C NMR (125.7 MHz, MeOH-d₄) δ 166.4, 158.8, 143.1, 139.1, 136.3, 134.3, 126.7, 117.4, 109.4, 55.1.

6-Methoxybenzo[b]selenophene-2-carboxylic acid (**2c**), 96%; ¹H NMR (500.1 MHz, MeOH-d₄) δ 8.16 (s, 1H), 7.86 (d, *J* = 8.8 Hz, 1H), 7.68 (d, *J* = 1.8 Hz, 1H), 7.03 (dd, *J* = 2.2, 8.7 Hz, 1H), 3.82 (s, 3H); ¹³C NMR (125.7 MHz, MeOH-d₄) δ 165.2, 158.6, 145.1, 136.3, 135.1, 132.8, 128.0, 114.8, 108.9, 55.5.

7-Methoxybenzo[b]selenophene-2-carboxylic acid (**2d**), 91%; ¹H NMR (500.1 MHz, MeOH-d₄) δ 8.28 (s, 1H), 7.60 (d, *J* = 7.7 Hz, 1H), 7.42 (t, *J* = 7.8 Hz, 1H), 7.01 (d, *J* = 7.7 Hz, 1H), 3.95 (s, 3H); ¹³C NMR (125.7 MHz, MeOH-d₄) δ 164.8, 155.7, 142.6, 139.1, 133.7, 131.4, 126.9, 119.9, 106.8, 55.8.

5,6-Dimethoxybenzo[b]selenophene-2-carboxylic acid (**2e**), 84%; ¹H NMR (500.1 MHz, MeOH-d₄) δ 8.10 (s, 1H), 7.48 (s, 1H), 7.40 (s, 1H), 3.90 (s, 3H), 3.87 (s, 3H); ¹³C NMR (125.7 MHz, MeOH-d₄) δ 168.5, 151.6, 150.1, 138.8, 136.5, 134.9, 134.8, 109.5, 108.6, 56.6, 56.5.

Selenopheno[2,3-b]pyridine-2-carboxylic acid (**2f**), 98%; ¹H NMR (500.1 MHz, MeOH-d₄) δ 8.75 (d, *J* = 4.9 Hz, 1H), 8.60 (d, *J* = 8.1 Hz, 1H), 8.34 (s, 1H), 7.71 (dd, *J* = 5.9, 7.7 Hz, 1H); ¹³C NMR (125.7 MHz, MeOH-d₄) δ 170.9, 166.9, 148.3, 148.2, 138.9, 135.8, 129.1, 121.4.

Selenopheno[3,2-b]thiophene-5-carboxylic acid (**2g**), 89%; ¹H NMR (500.1 MHz, MeOH-d₄) δ 8.24 (s, 1H), 7.71 (d, *J* = 5.1 Hz, 1H), 7.41 (d, *J* = 5.1 Hz, 1H); ¹³C NMR (125.7 MHz, MeOH-d₄) δ 167.0, 145.4, 141.5, 140.3, 132.4, 129.6, 124.3.

Selenopheno[3,2-b]furan-5-carboxylic acid (**2h**), 93%; ¹H NMR (500.1 MHz, MeOH-d₄) δ 7.94 (s, 1H), 7.71 (s, 1H), 6.85 (s, 1H); ¹³C NMR (125.7 MHz, MeOH-d₄) δ 168.3, 158.8, 149.3, 140.0, 129.8, 119.8, 110.5.

Synthetic procedure of scaffold 12 (One-pot synthesis): A solution of compound **11** (3.5 g, 10.02 mmol) in anhydrous THF (150 mL) was cooled to -78 °C then treated with catalytic amount H₂SO₄ (60 µL) in THF (5 mL). After 15 min stirring, a solution of NIS (2.7 g, 12.02 mmol) in THF (15 mL) was added and the reaction mixture was stirred at same temperature for 2 h, and then at room temperature for 30 min. The progress of the reaction was monitored by TLC. After complete conversion of starting compound, NaH (60% dispersion in mineral oil, 3.26 g, 80.16 mmol) was added in portion under N₂ atmosphere at 0 °C and then stirred the reaction mixture at room temperature for 30 min. Glycidyl nosylate (3.12 g, 12.02 mmol) was added under N₂ atmosphere and the mixture was stirred for 3 to 5 h at room temperature. On complete conversion of intermediate, 3 M solution of EtMgBr in diethyl ether (10 mL, 30.06 mmol) was added slowly and stirred continuously for 2 h. The reaction mixture was quenched with saturated NH₄Cl at 0 °C, and then extracted with ethyl acetate (3 x 150 mL). The combine organic layer was washed with aqueous NaCl and dried over Na₂SO₄, filtered and concentrated under vacuum to get crude residue. which was purified by flash column chromatography on silica gel using 40% ethyl acetate in hexane as an eluent to provide **12** (3.08 g, 76%). Spectral characterization of scaffold **12** described in the ref.³⁴

General procedures for the synthesis of spirocyclized CBI derivatives (15–16):

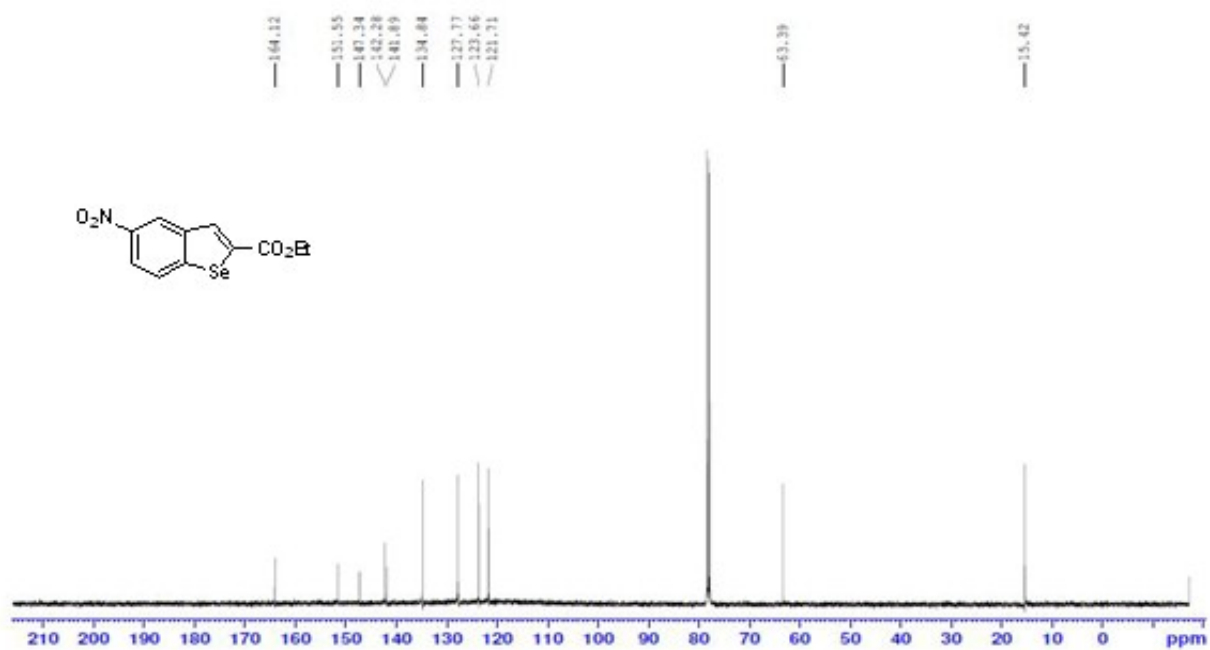
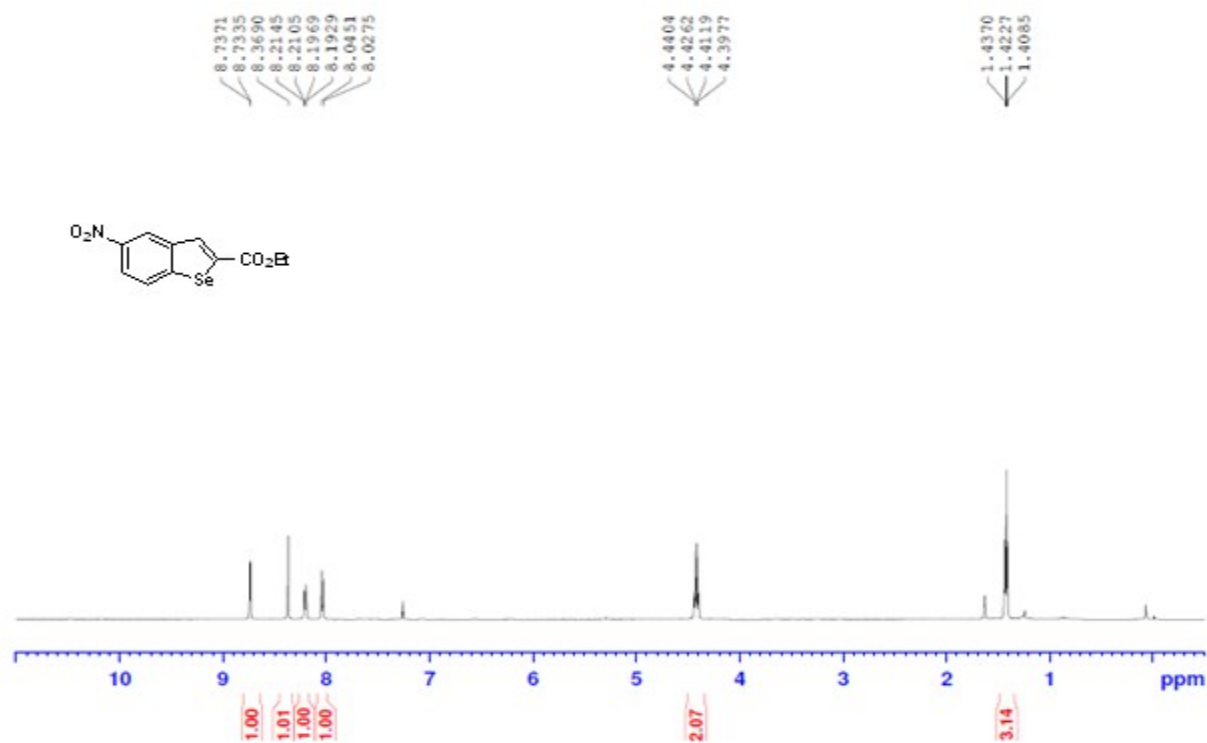
To the solution of compound **14d** or **14g** (20 mg, 1 eq.) in DMF (0.2 mL), 15% aqueous solution of NaHCO₃ (0.2 mL) was slowly added at 0 °C and stirred continuously at room temperature for 3 h. After complete the reaction, the mixture was diluted with water and then product was extracted with ethyl acetate (3 x 2 mL). The organic layers was combined, washed with brine, dried over Mg₂SO₄, filtered and concentrate under vacuum to get crude residue. The residue purified by column chromatography using 60 % ethyl acetate in hexane as an eluent to get the pure desired product.

Spectral Characterization data for 15–16:

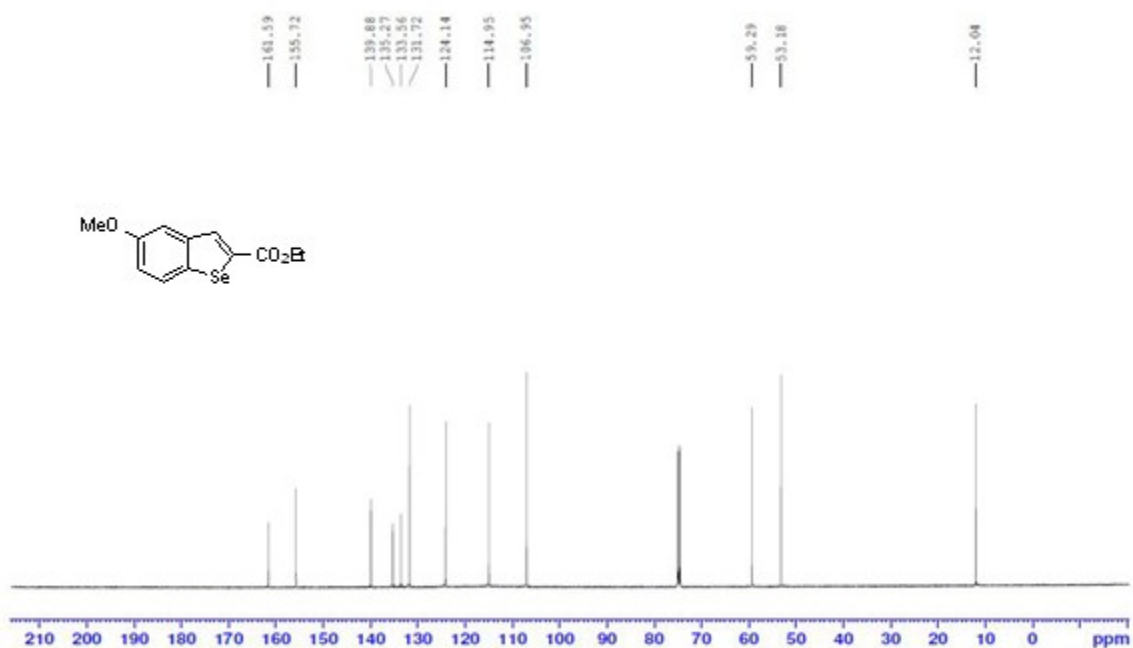
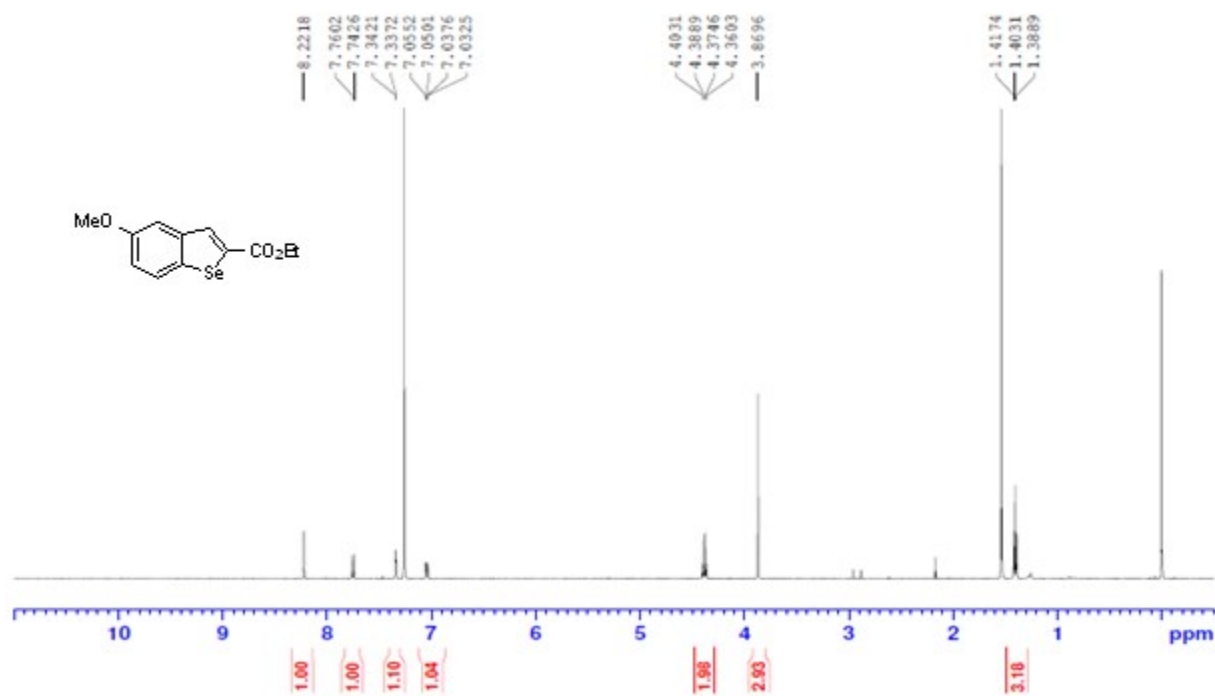
(8bR,9aS)-2-(5,6-dimethoxybenzo[b]selenophene-2-carbonyl)-9,9a-dihydro-1H-benzo[e]cyclopropa[c]indol-4(2H)-one (**15**), yellow solid, 94%; ¹H NMR (500.1 MHz, Acetone-d₆) δ 8.21 (s, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 7.67 (s, 1H), 7.59 (t, *J* = 7.1 Hz, 1H), 7.50 (s, 1H), 7.43 (t, *J* = 7.0 Hz, 1H), 7.21 (d, *J* = 7.9 Hz, 1H), 6.80 (s, 1H), 4.61-4.59 (m, 1H), 4.45 (d, *J* = 10.2 Hz, 1H), 3.92 (s, 3H), 3.87 (s, 3H), 3.22-3.18 (m, 1H), 1.83-1.80 (m, 2H); ¹³C NMR (125.7 MHz, Acetone-d₆) δ 185.5, 165.1, 161.7, 151.8, 150.2, 141.7, 139.9, 137.4, 136.3, 133.8, 132.9, 132.8, 127.3, 127.0, 123.2, 112.0, 109.7, 108.3, 56.5, 56.3, 55.6, 33.6, 28.8, 25.3; HRMS Calcd for (C₂₄H₁₉NO₄Se) 466.0558 [M+H]⁺, found 466.0560.

N-(2-((8bR,9aS)-4-oxo-2,4,9,9a-tetrahydro-1H-benzo[e]cyclopropa[c]indole-2-carbonyl)benzo[b]selenophen-5-yl)butyramide (**16**), yellow solid, 92%; IR (KBr cm⁻¹) 2952, 2928, 2859, 1736, 1660, 1606, 1579, 1518, 1448, 1380, 1226, 1153, 1046, 808, 759; ¹H NMR (500.1 MHz, Acetone-d₆) δ 9.27 (s, 1H), 8.54 (s, 1H), 8.24 (s, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.99 (d, *J* = 8.7 Hz, 1H), 7.60-7.52 (m, 2H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 6.84 (s, 1H), 4.64 (dd, *J* = 10.4, 4.9 Hz, 1H), 4.48 (d, *J* = 10.4 Hz, 1H), 3.18 (dd, *J* = 11.6, 5.5 Hz, 1H), 2.36 (t, *J* = 7.4 Hz, 2H), 1.81 (d, *J* = 6.4 Hz, 2H), 1.71 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H); ¹³C NMR (125.7 MHz, Acetone-d₆) δ 185.4, 172.0, 165.0, 161.3, 143.3, 143.0, 141.6, 138.4, 133.7, 132.8, 132.4, 127.2, 126.9, 126.6, 123.1, 122.9, 120.4, 118.1, 112.2, 55.6, 39.6, 33.5, 28.7, 25.2, 19.6, 14.0; HRMS Calcd for (C₂₆H₂₂N₂O₃Se) 491.0874 [M+H]⁺, found 491.0877.

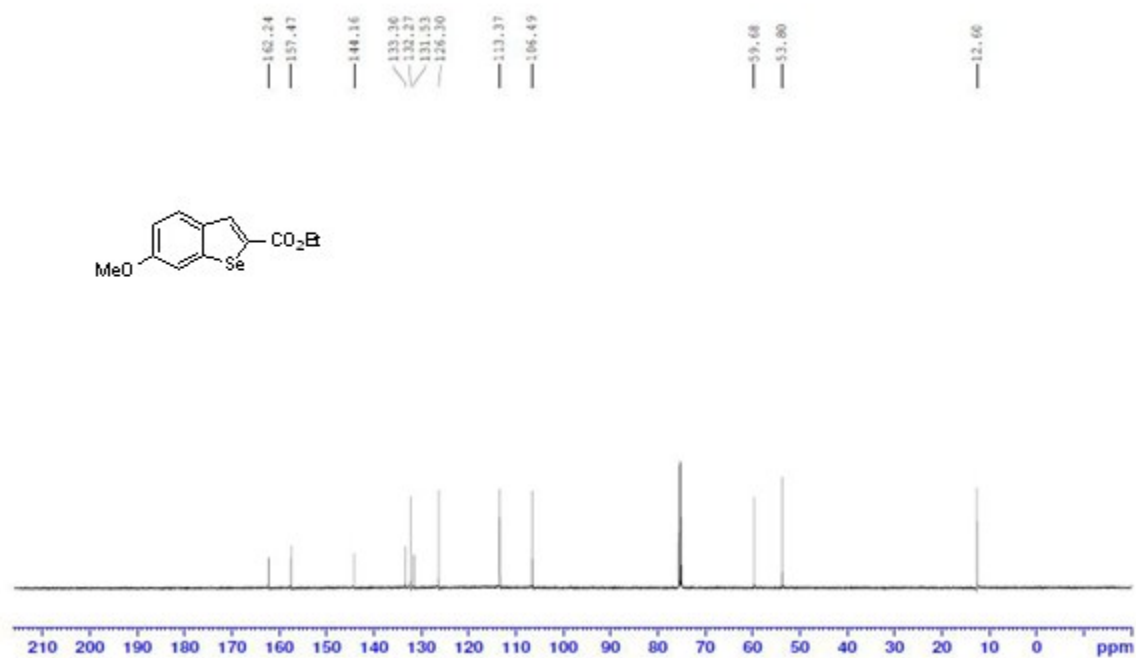
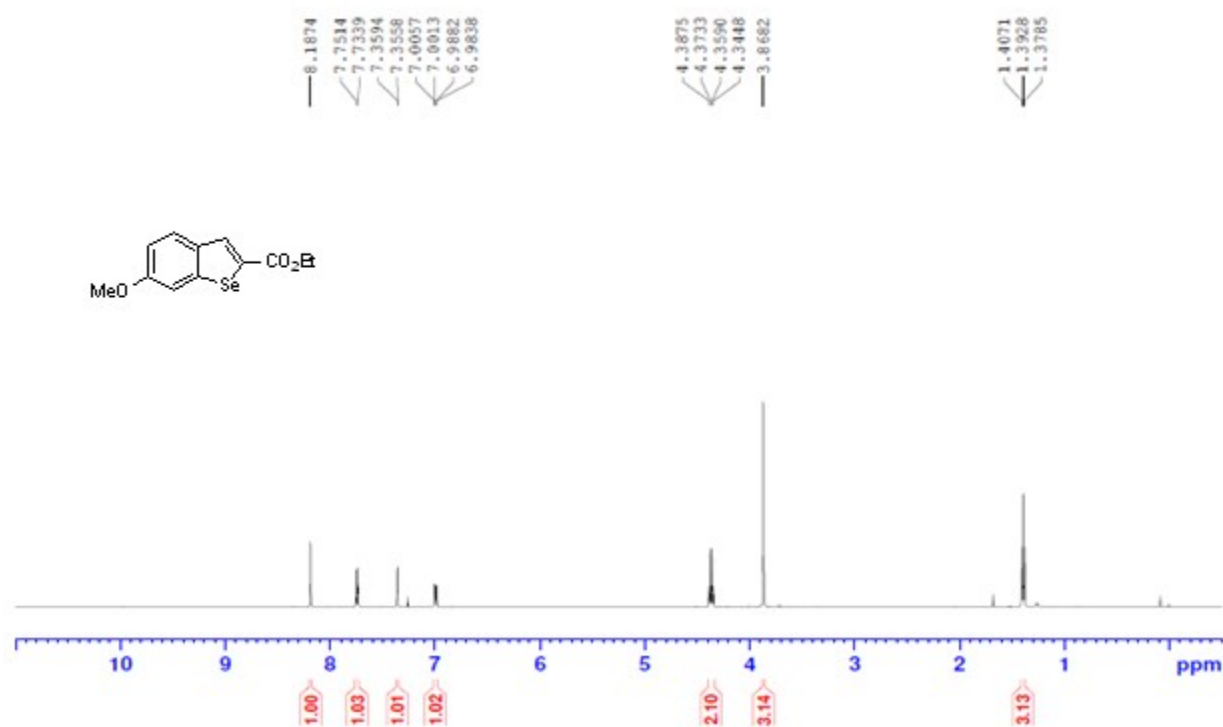
Compound **1a**



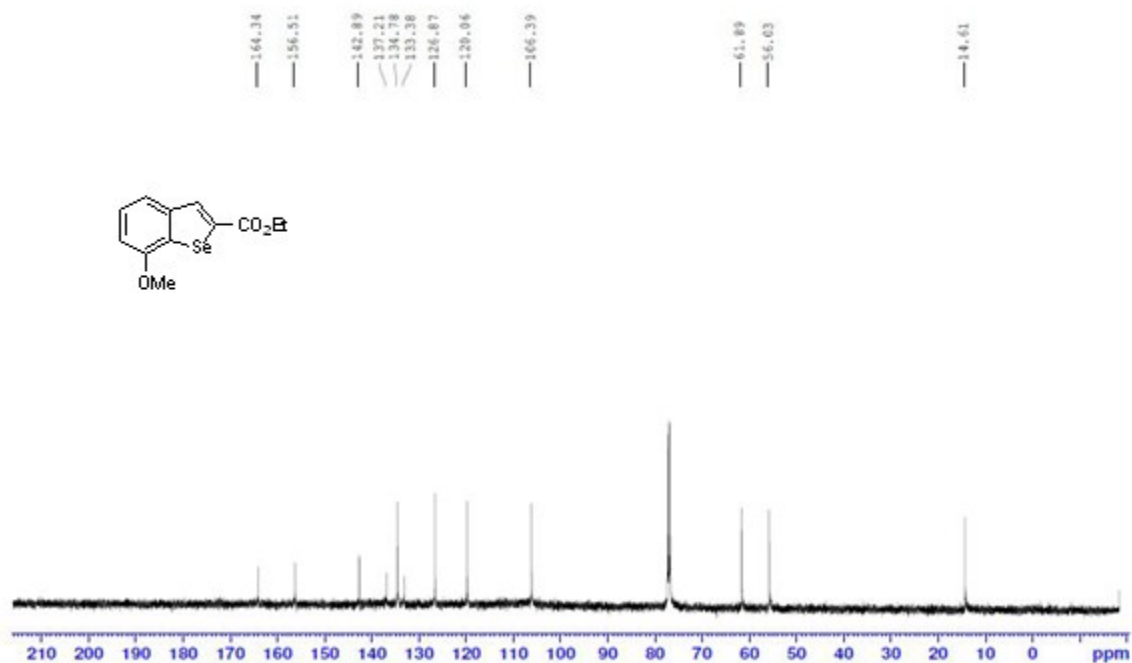
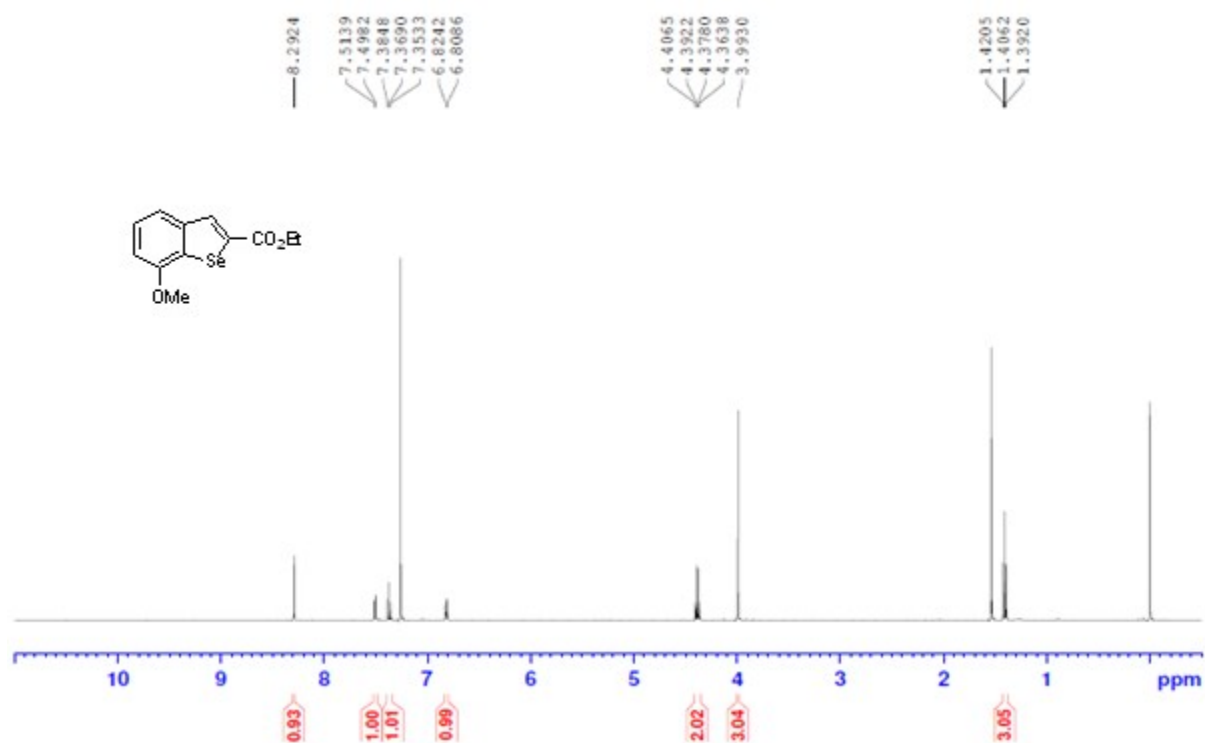
Compound **1b**



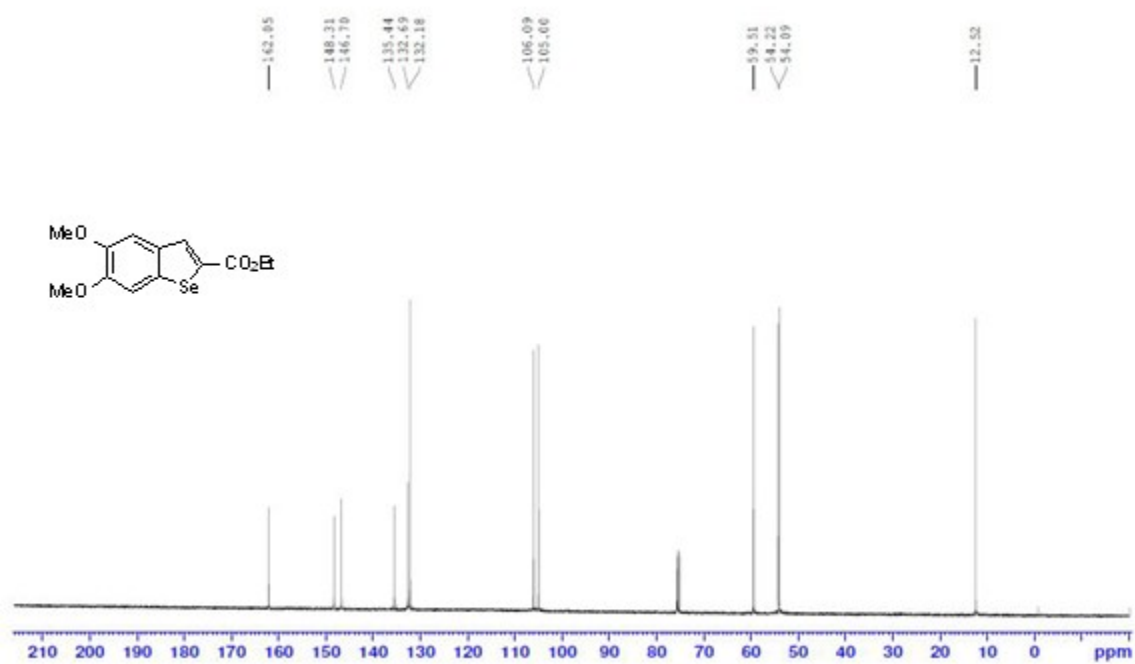
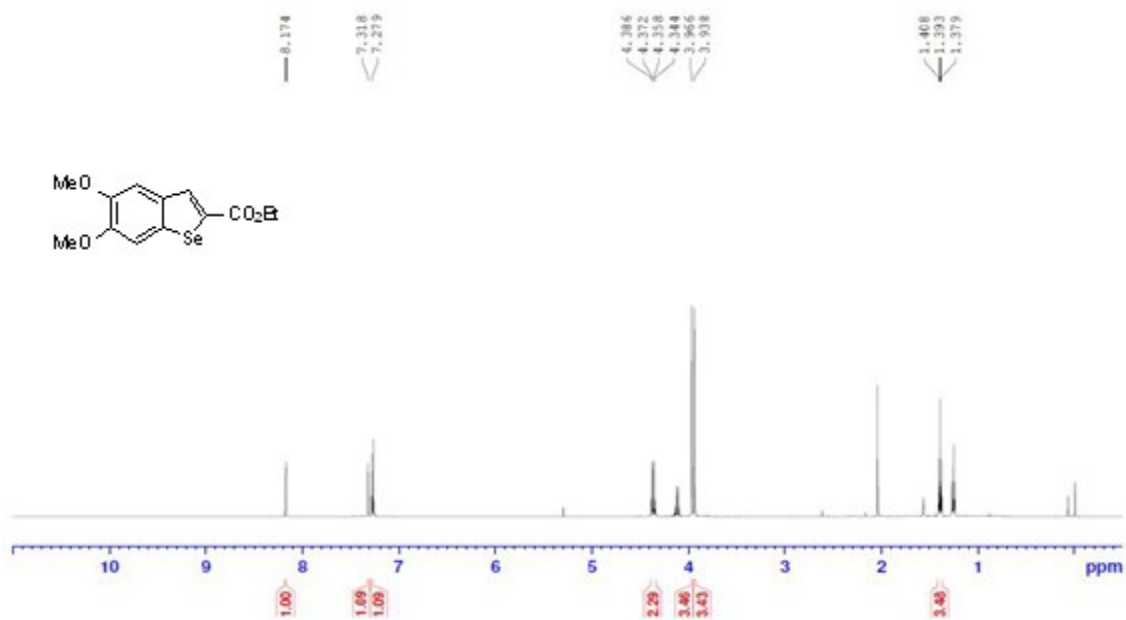
Compound **1c**



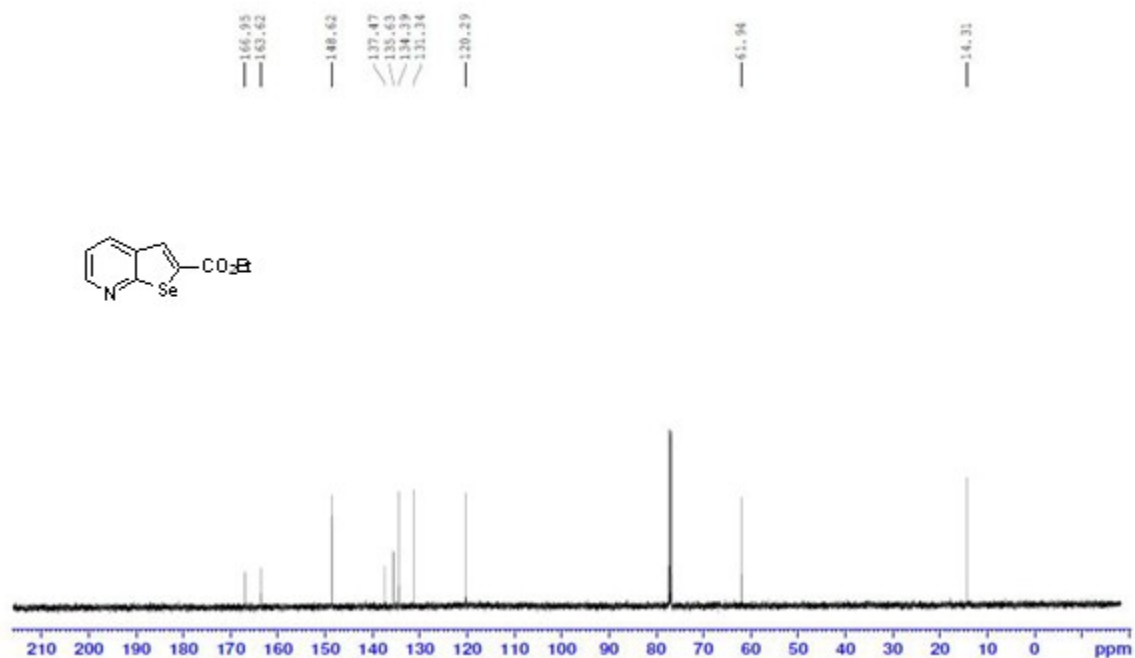
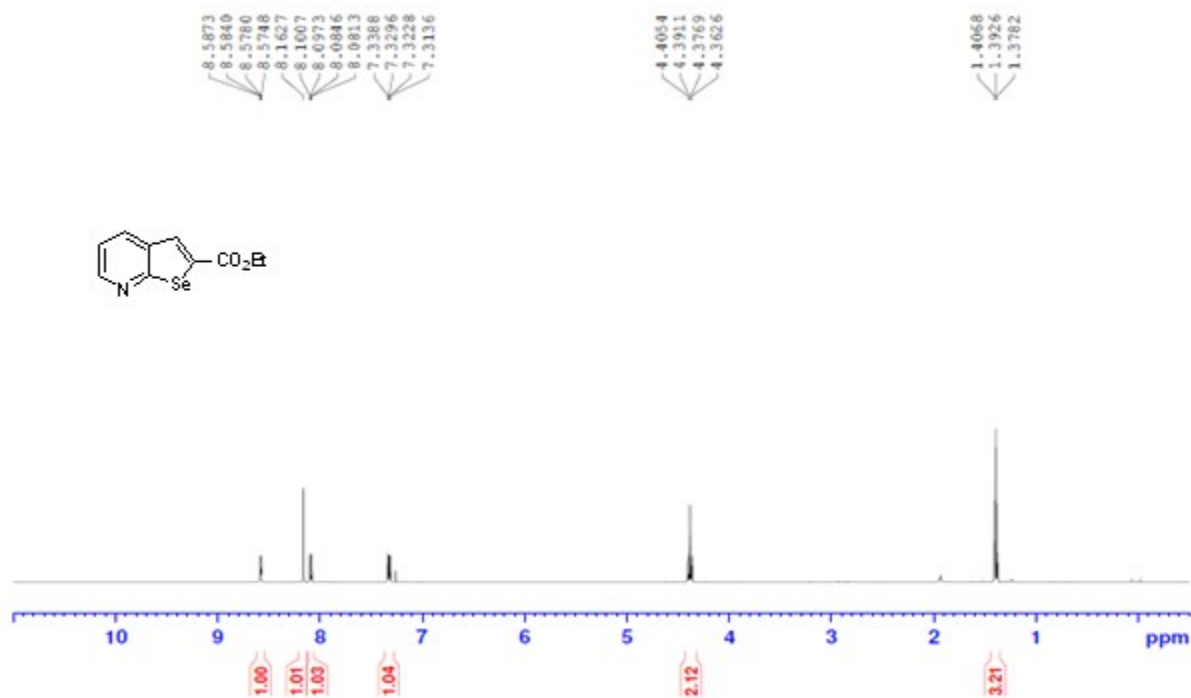
Compound **1d**



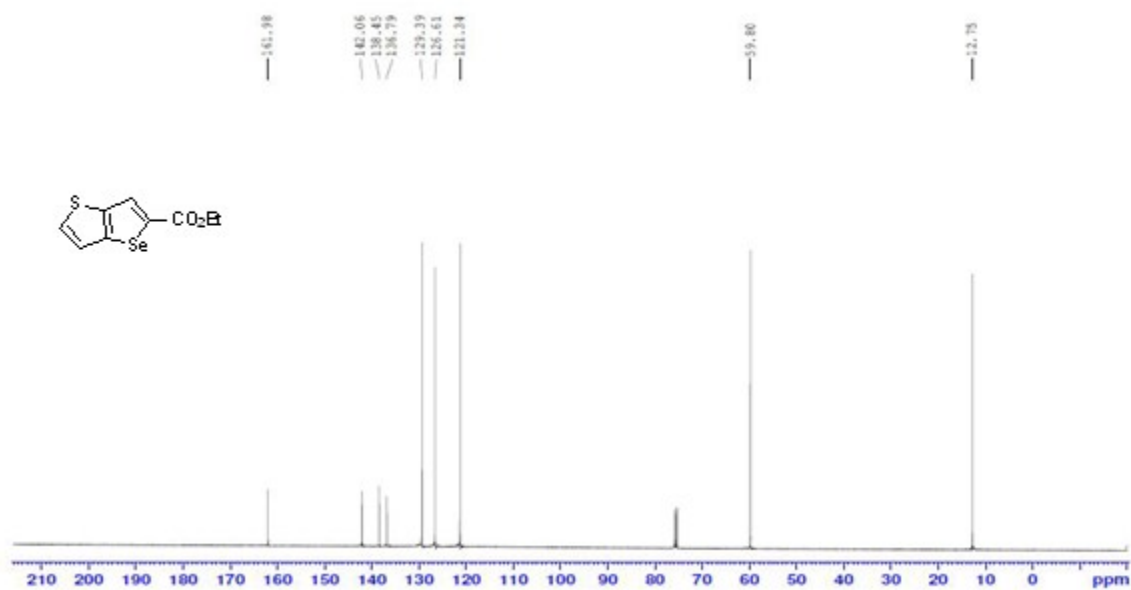
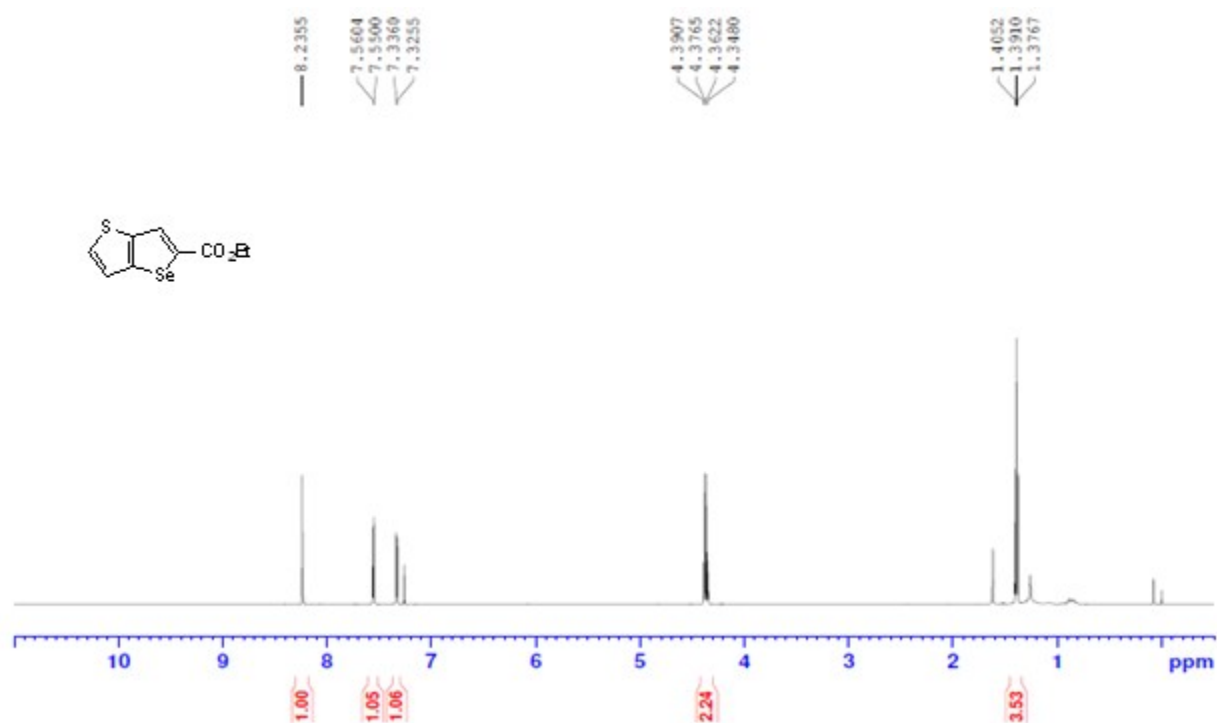
Compound **1e**



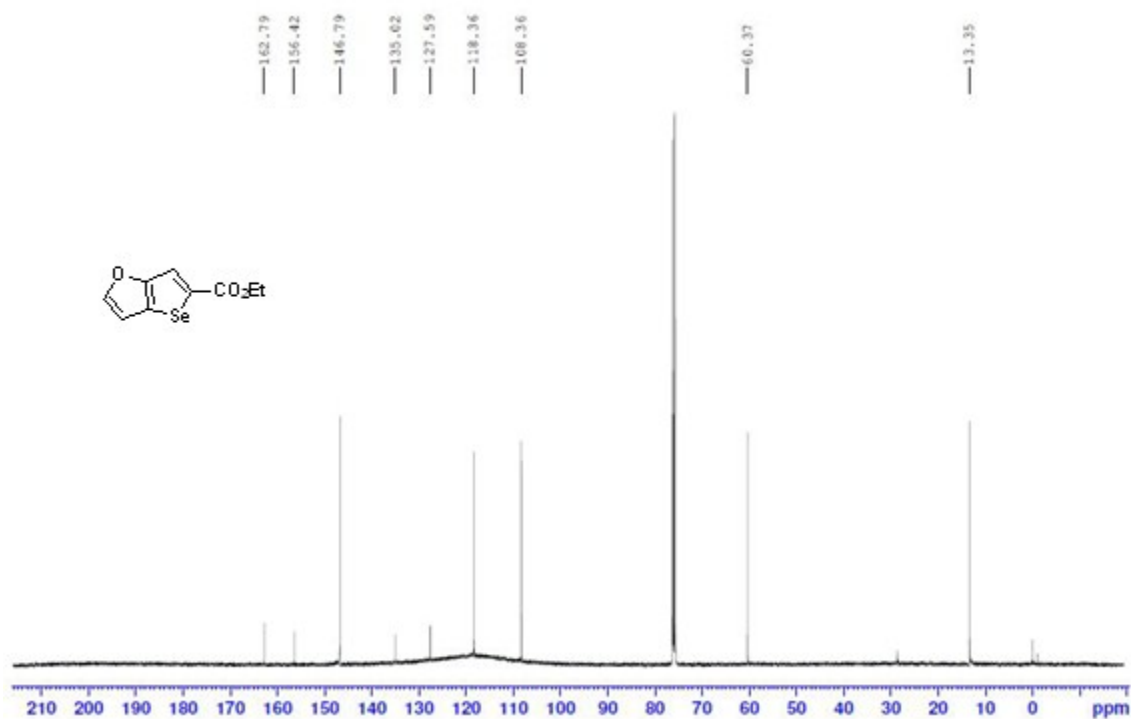
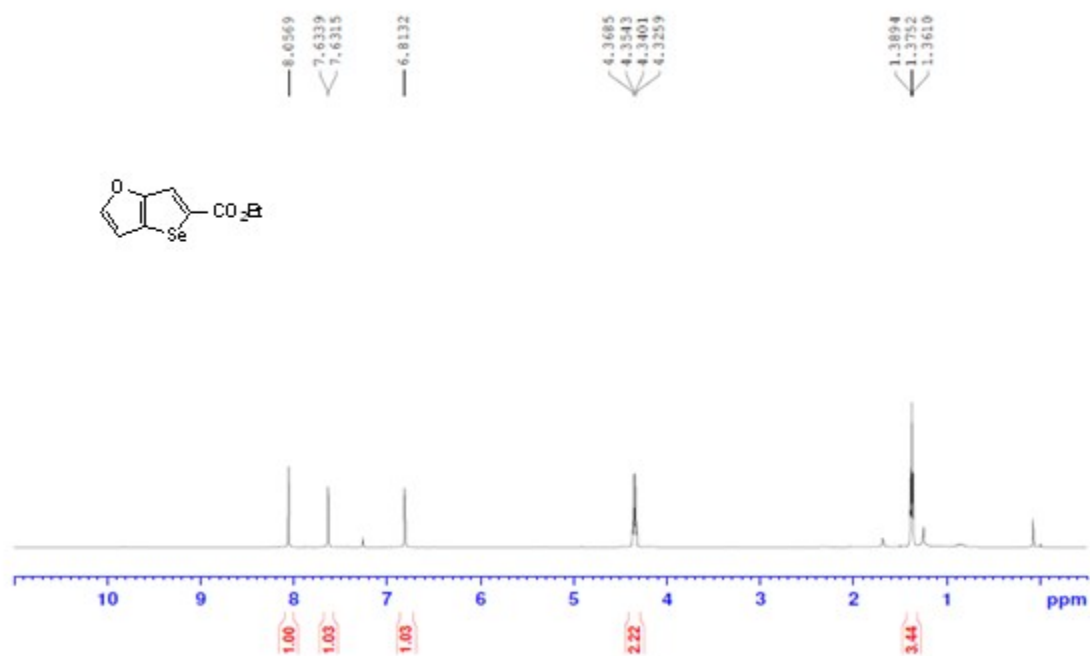
Compound **1f**



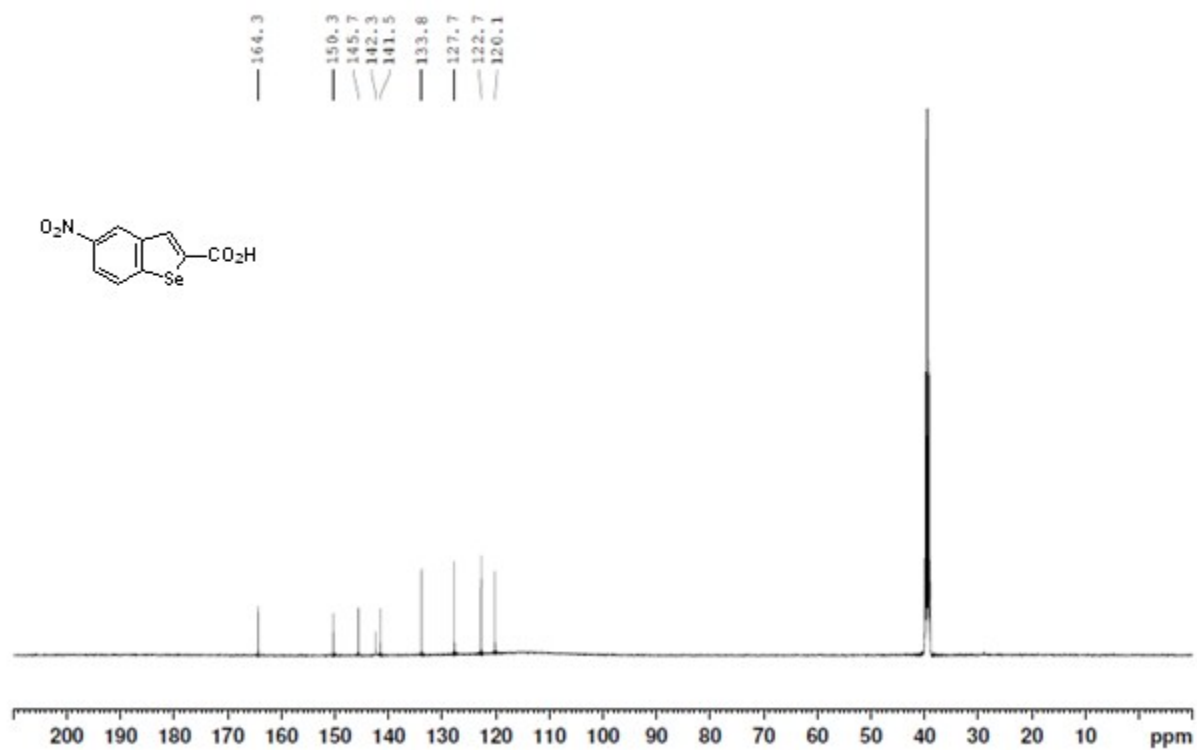
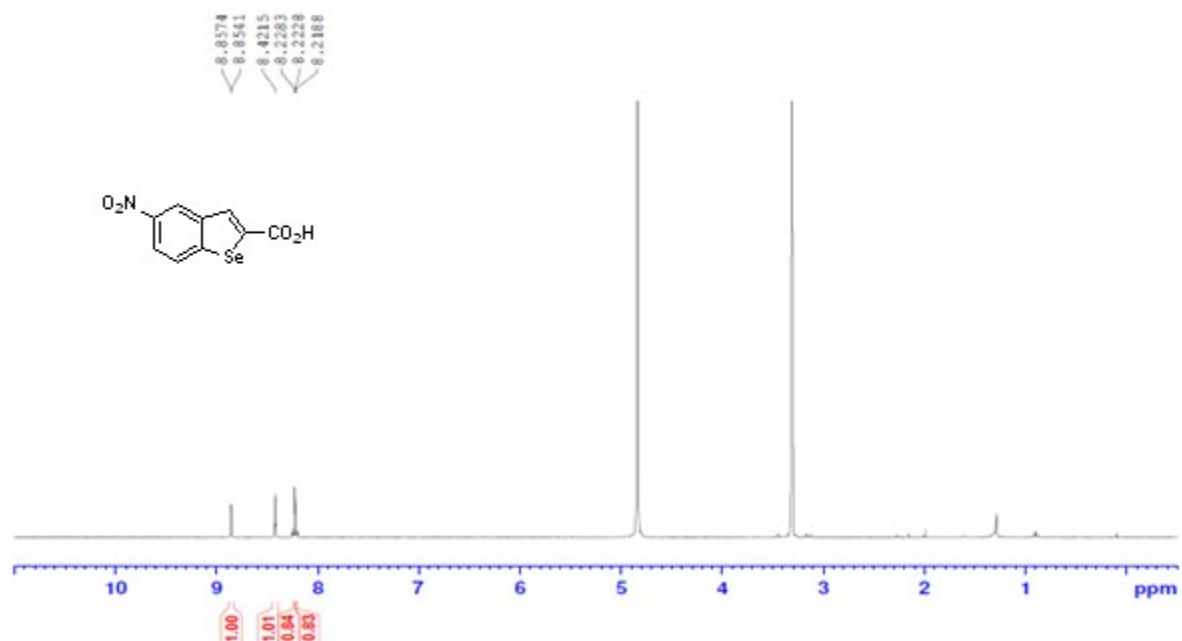
Compound **1g**



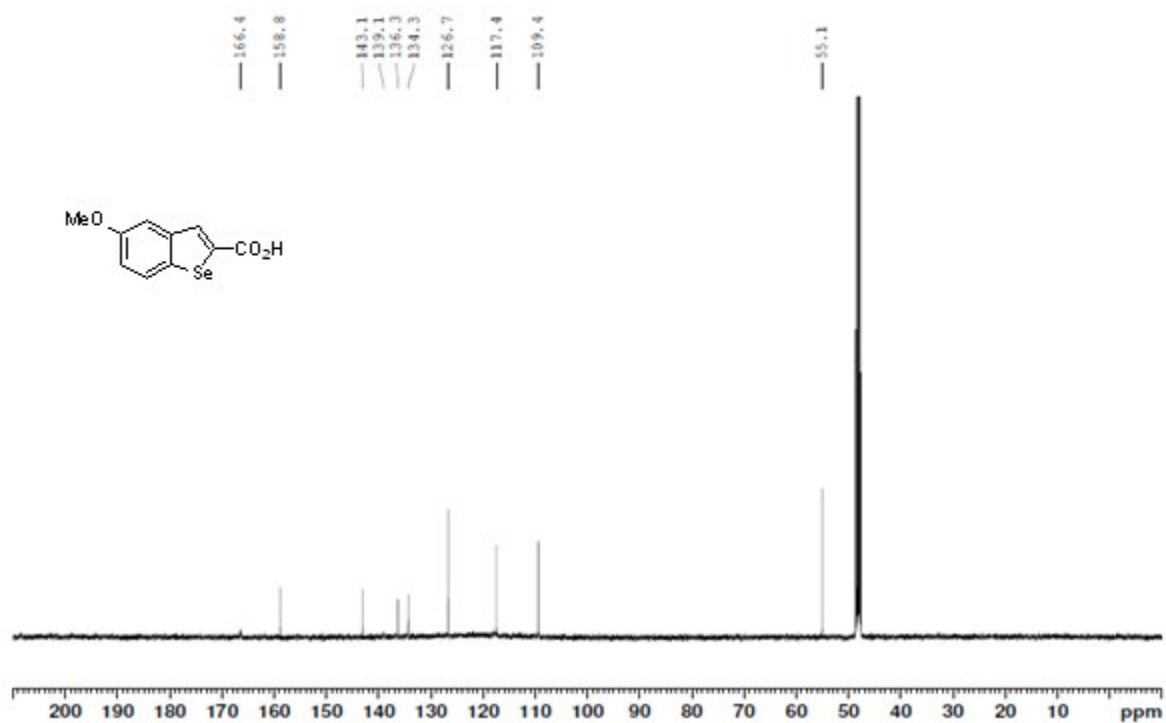
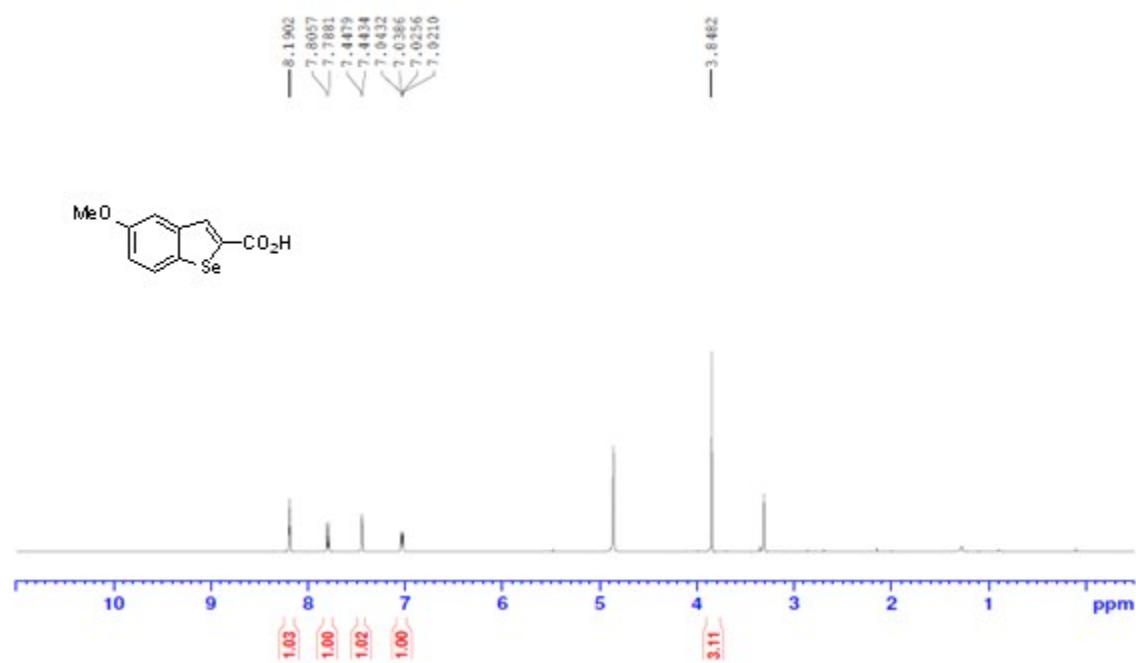
Compound **1h**



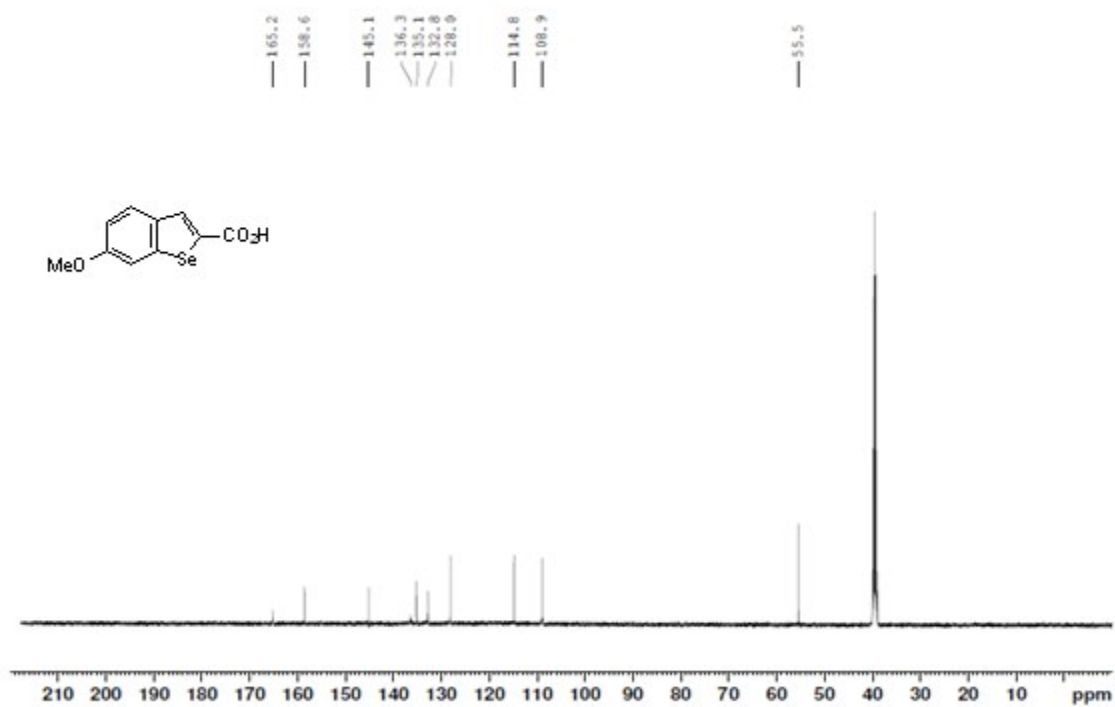
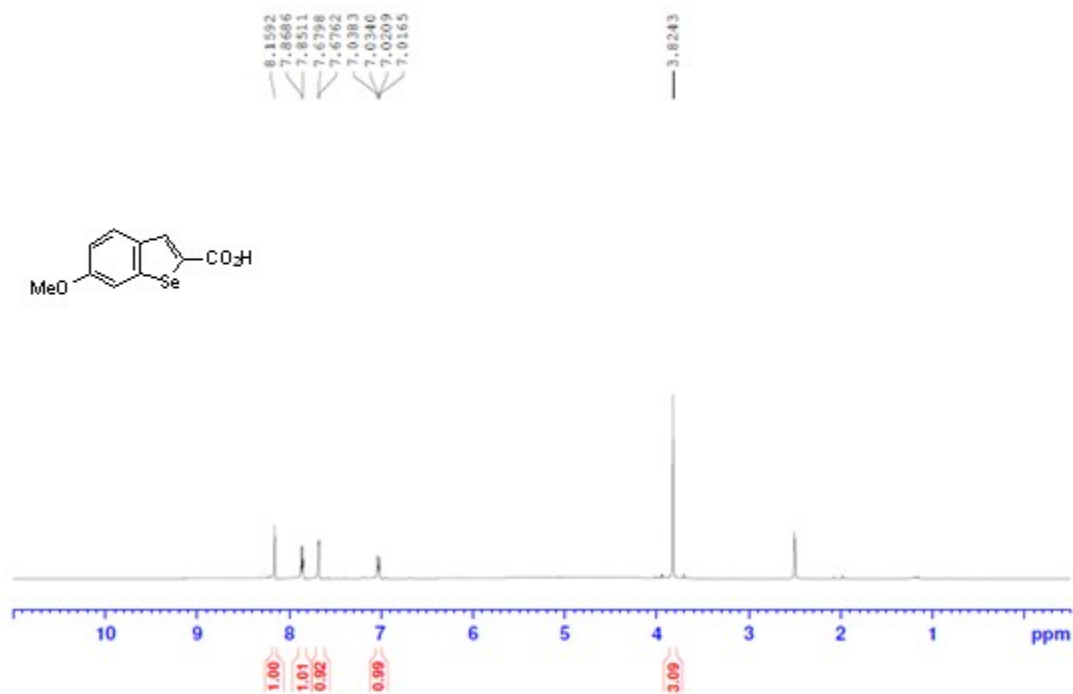
Compound **2a**



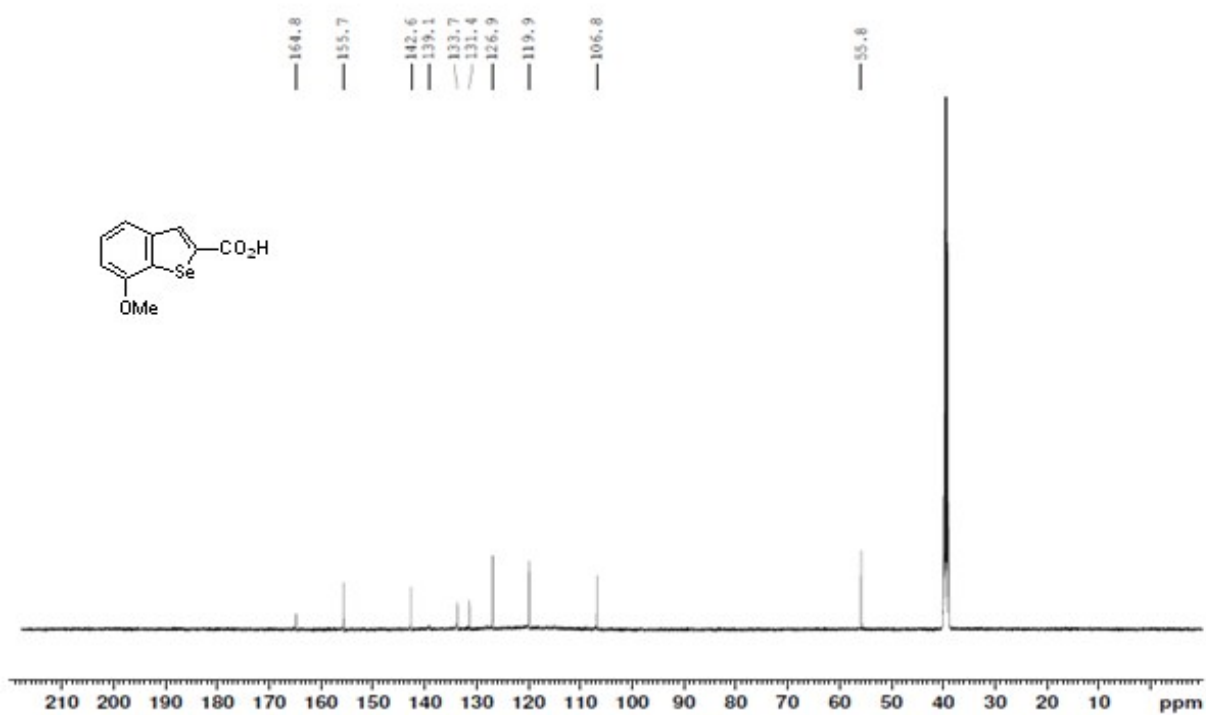
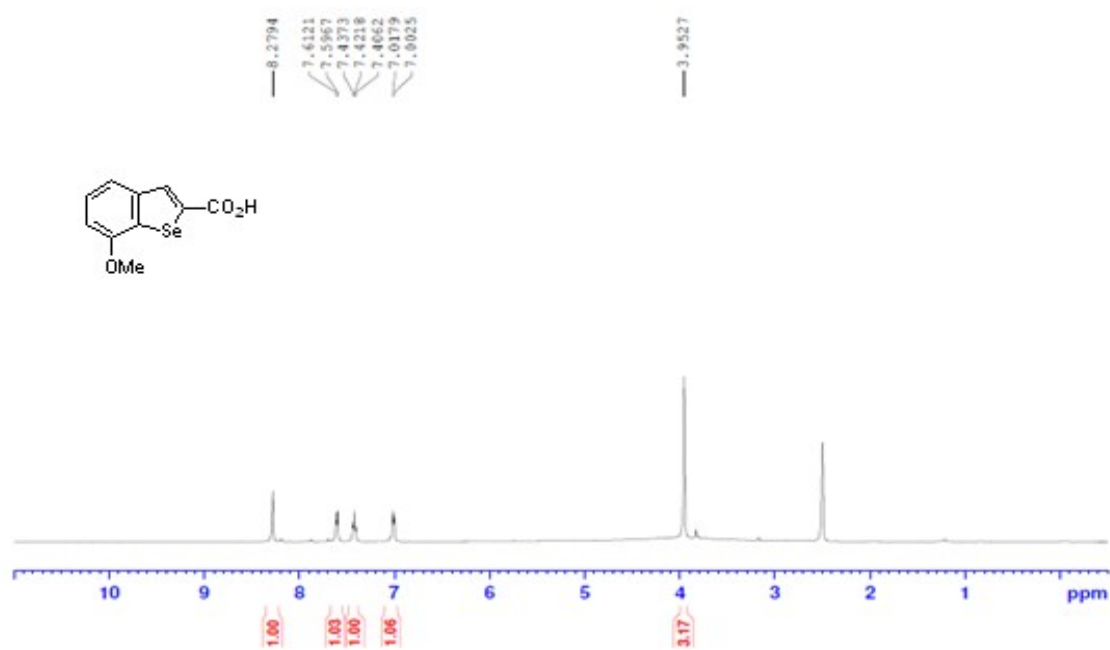
Compound **2b**



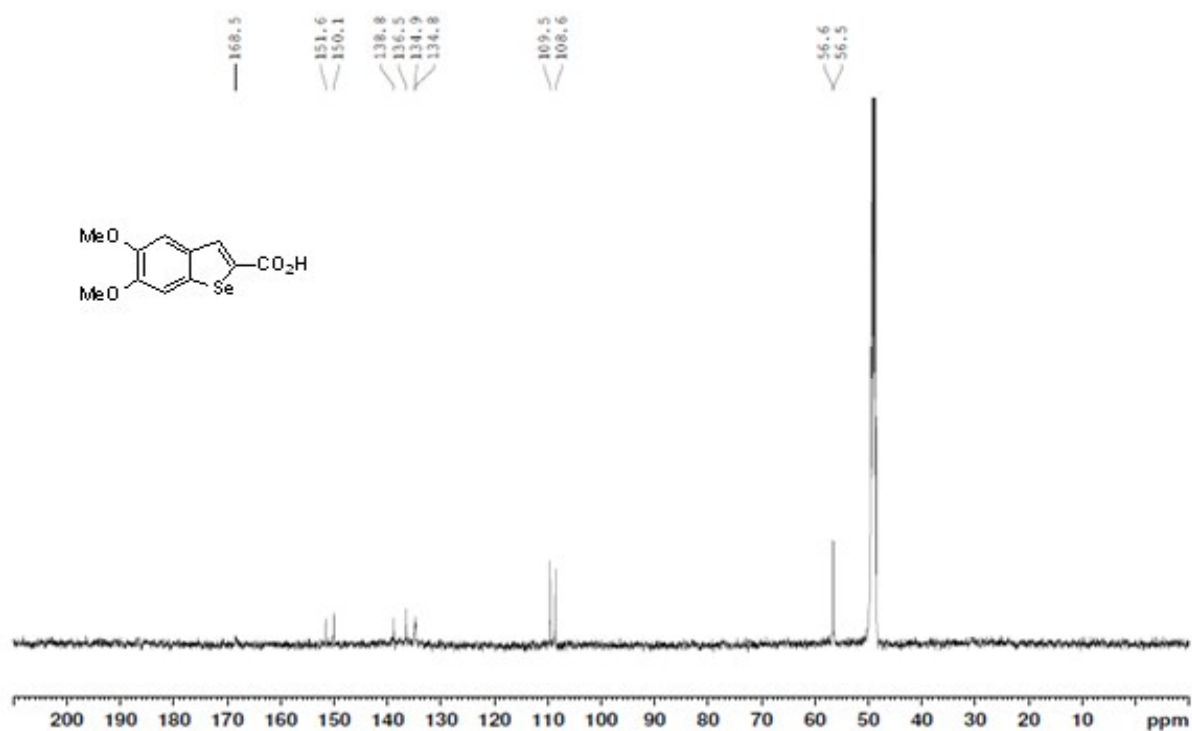
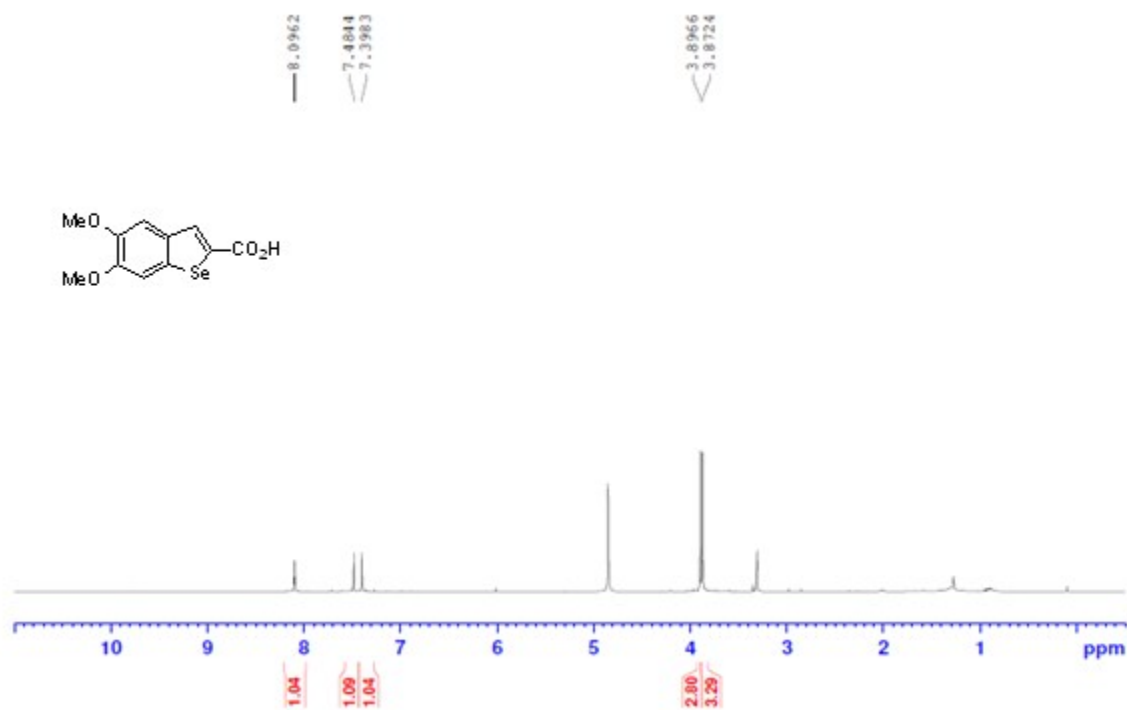
Compound **2c**



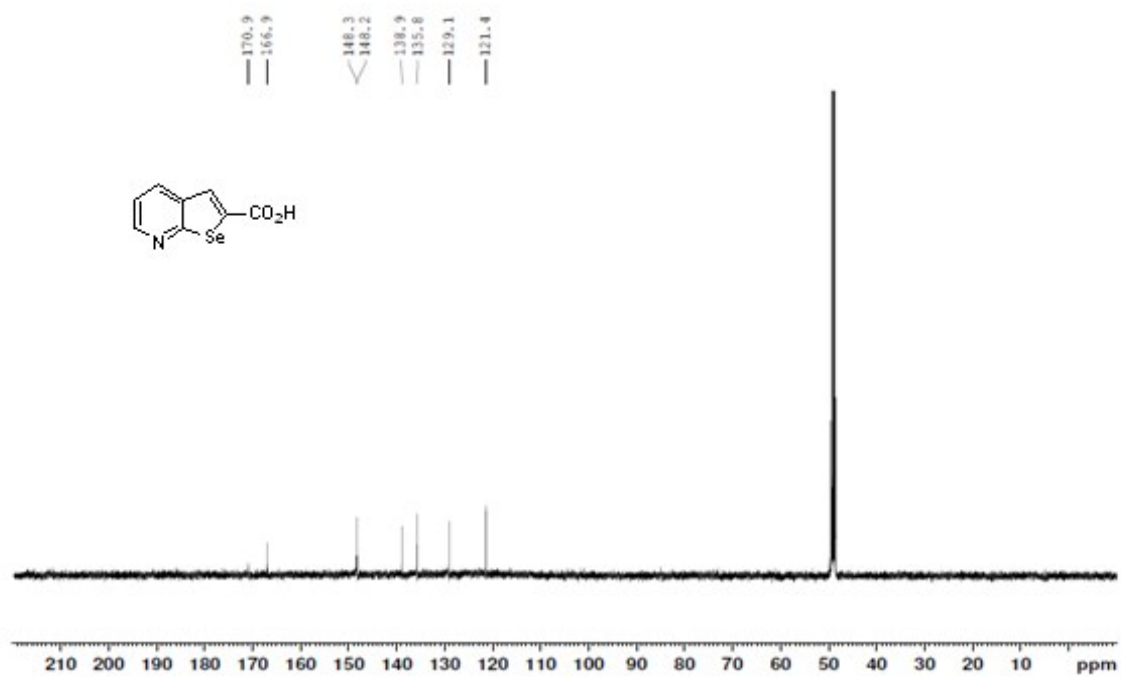
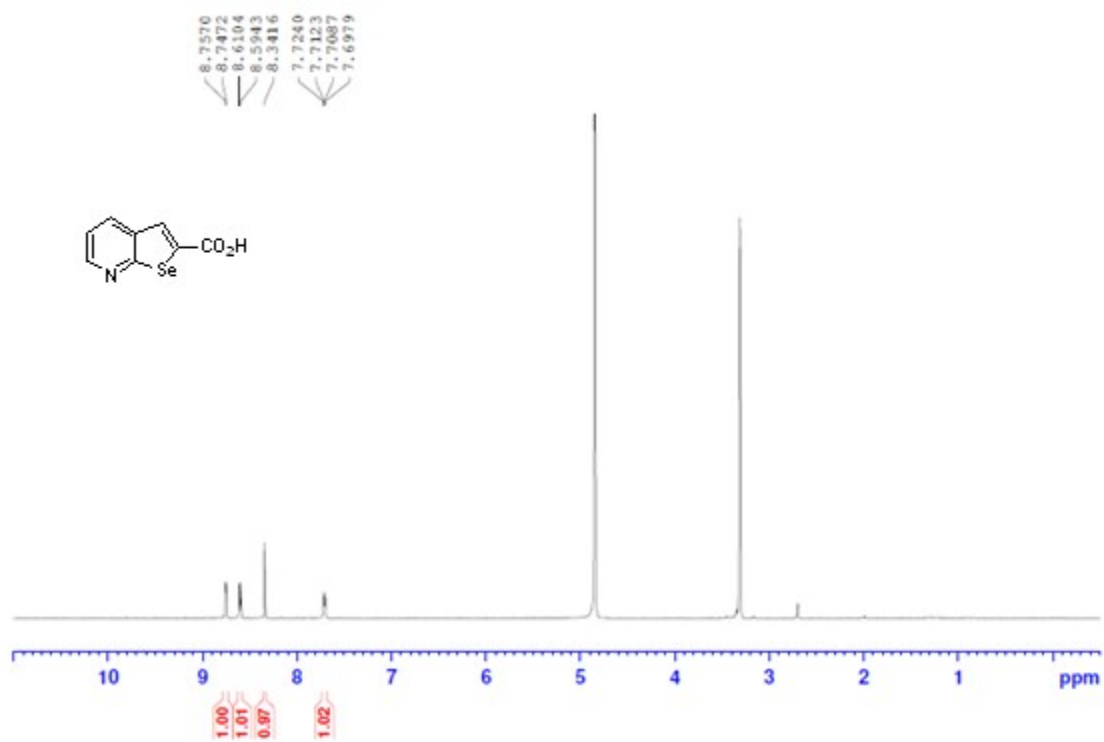
Compound **2d**



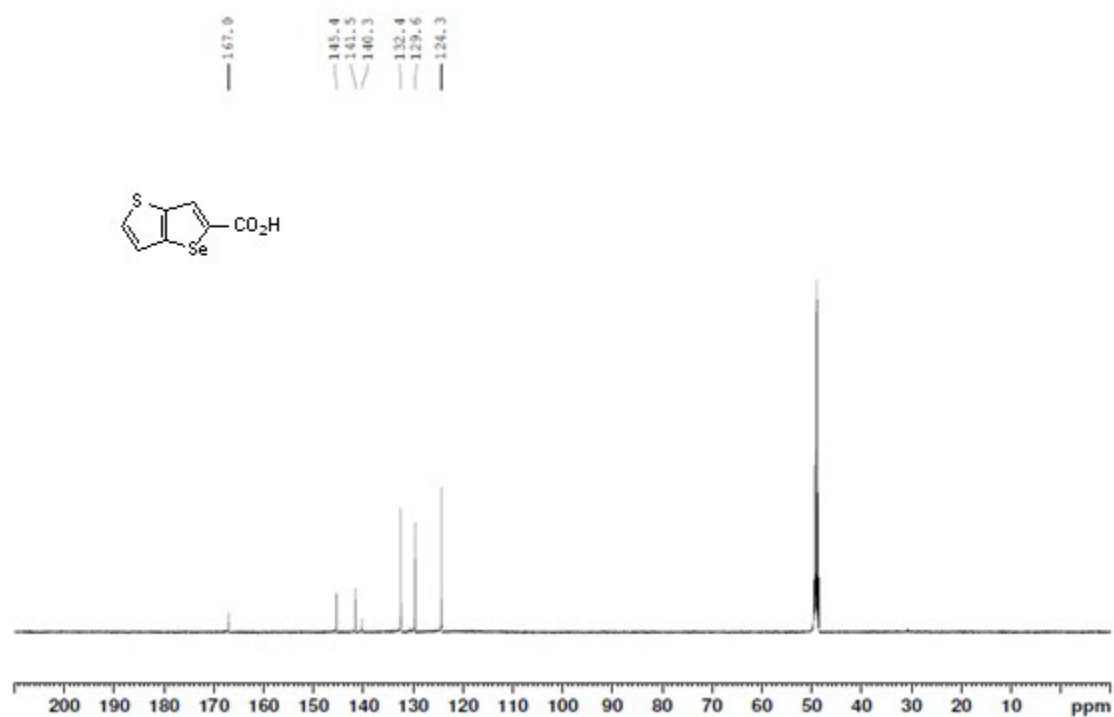
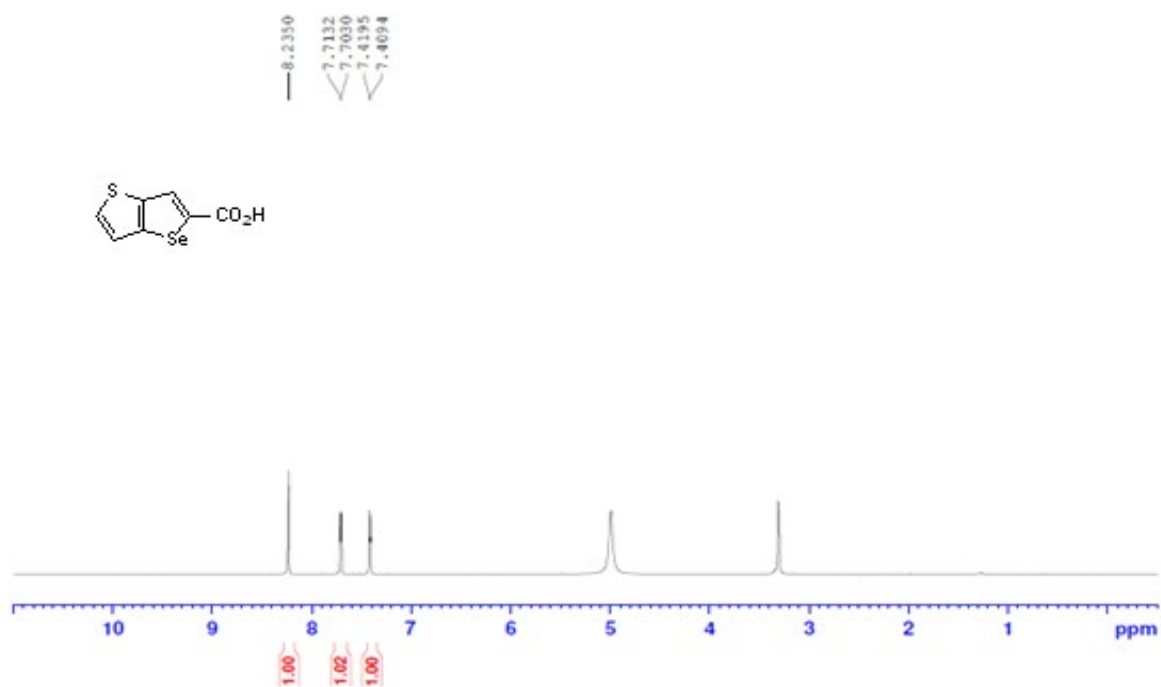
Compound **2e**



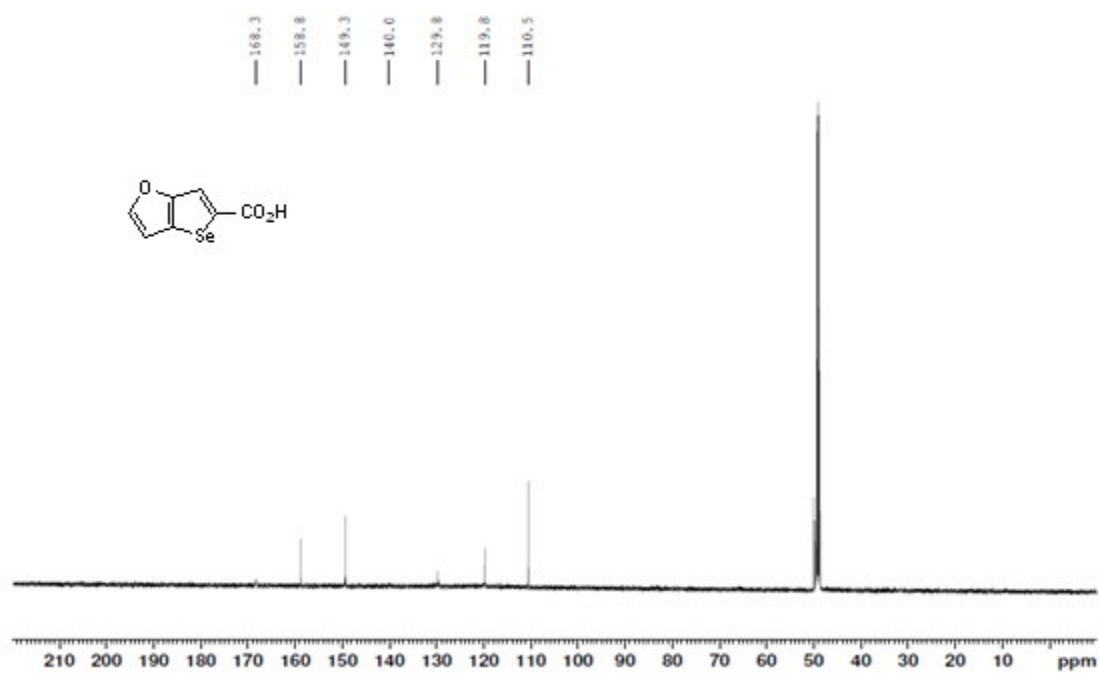
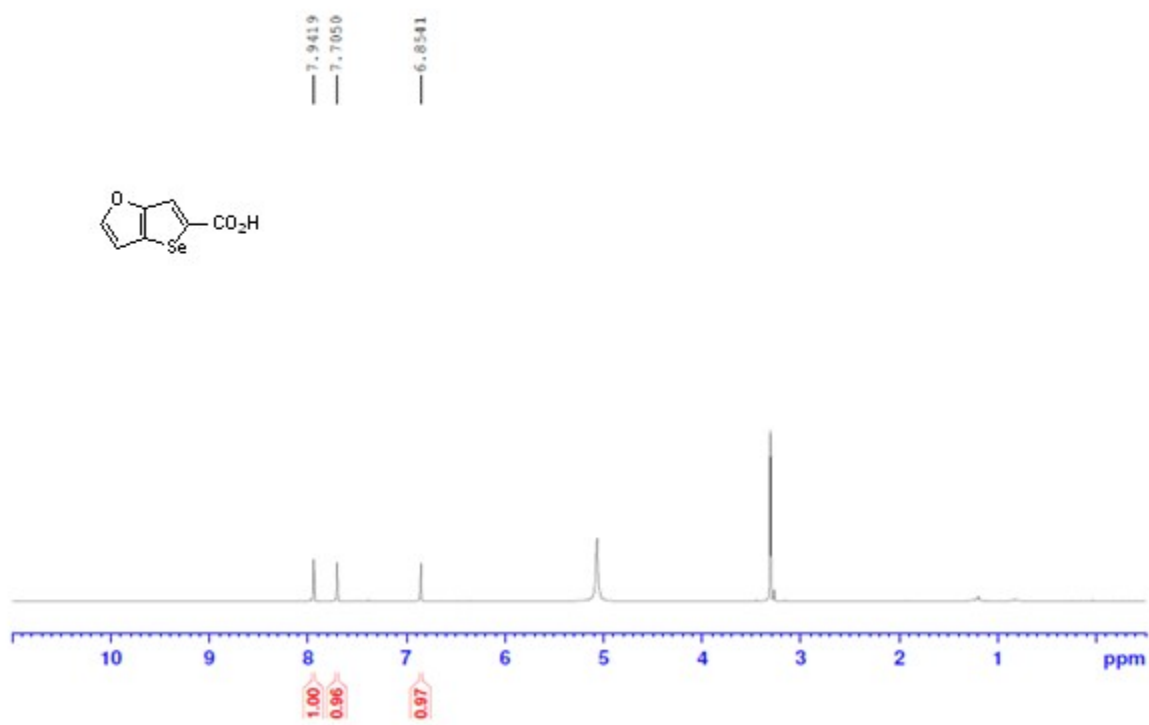
Compound **2f**



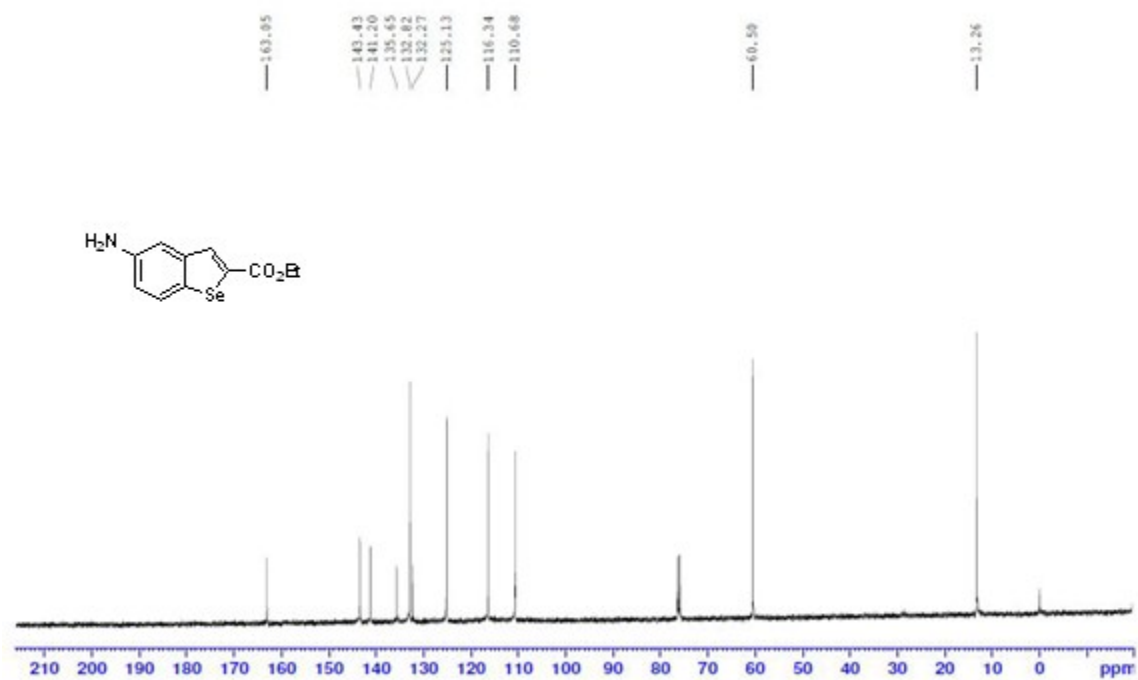
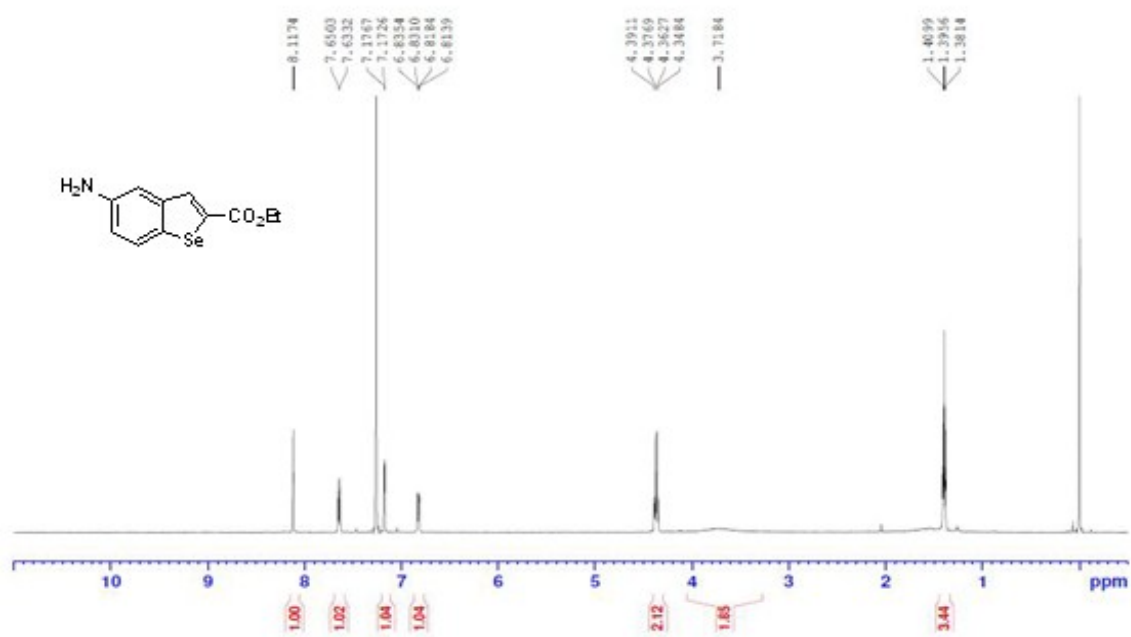
Compound **2g**



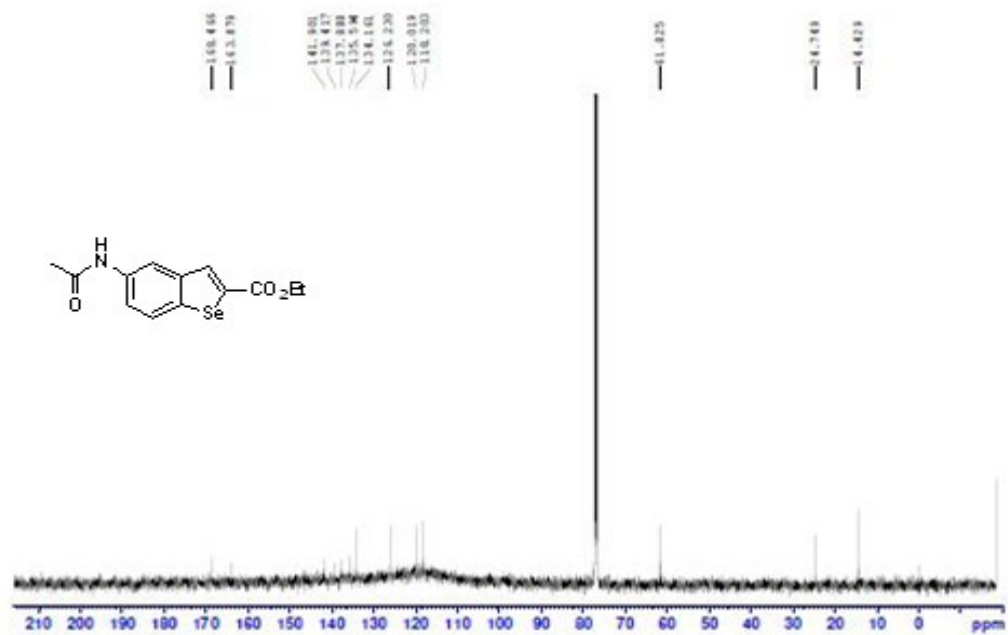
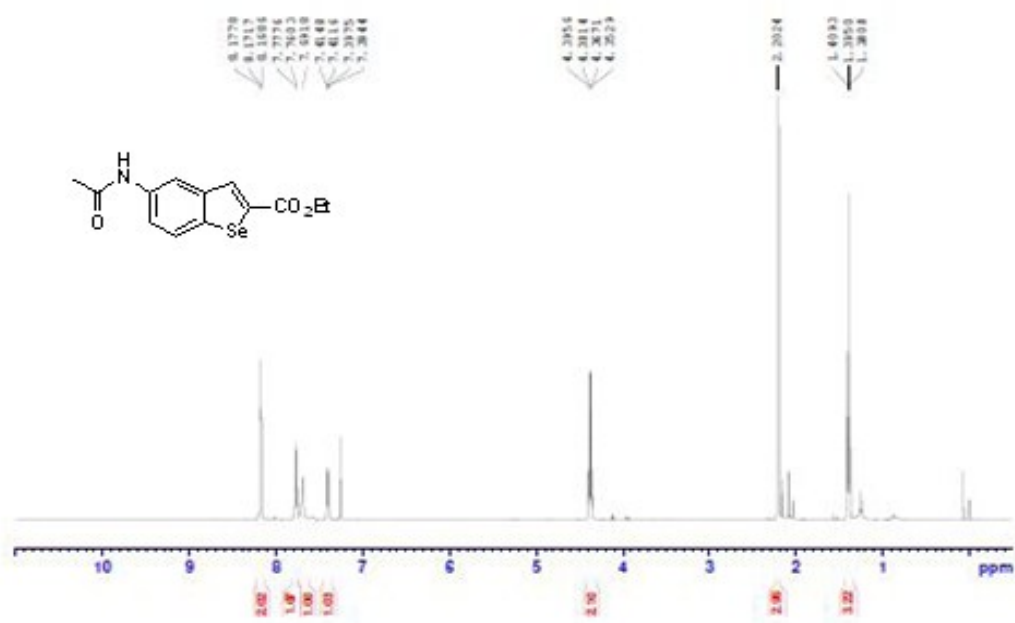
Compound **2h**



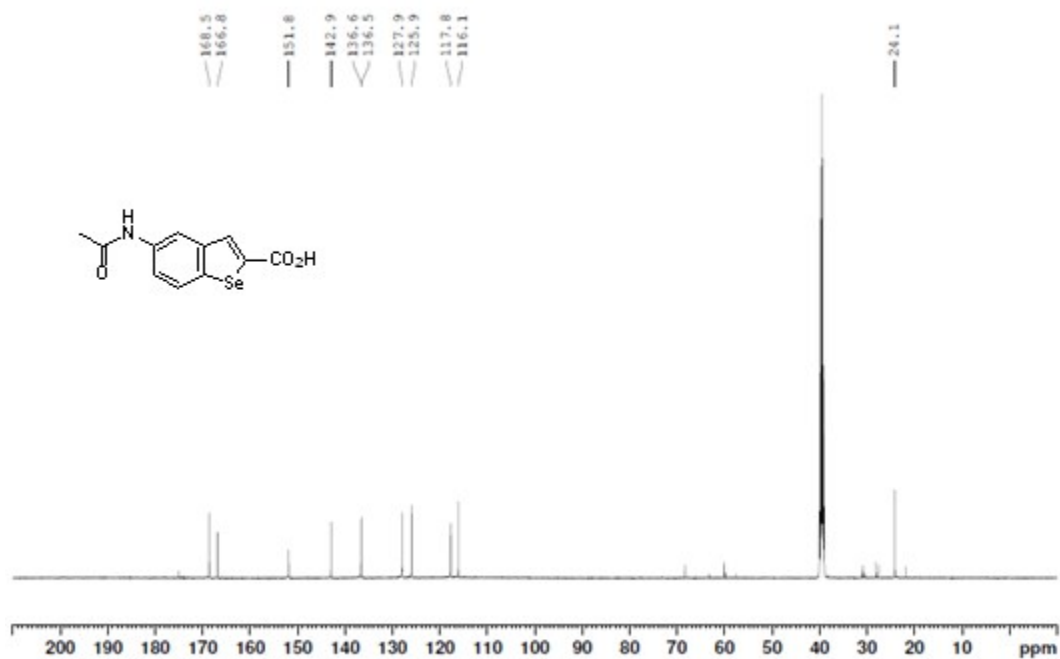
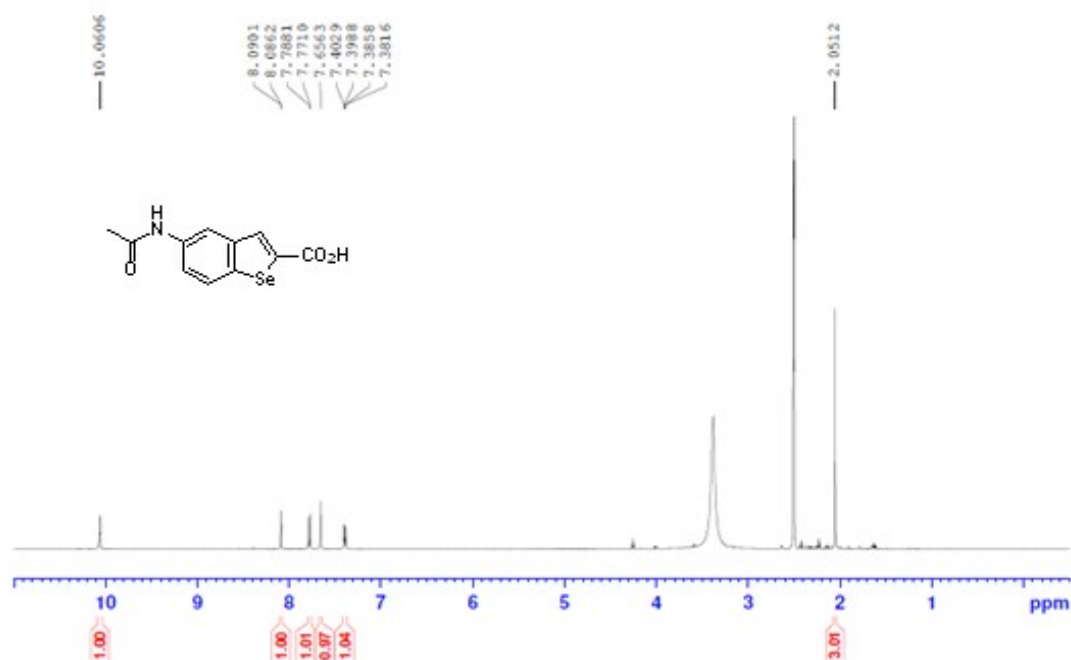
Compound **3**



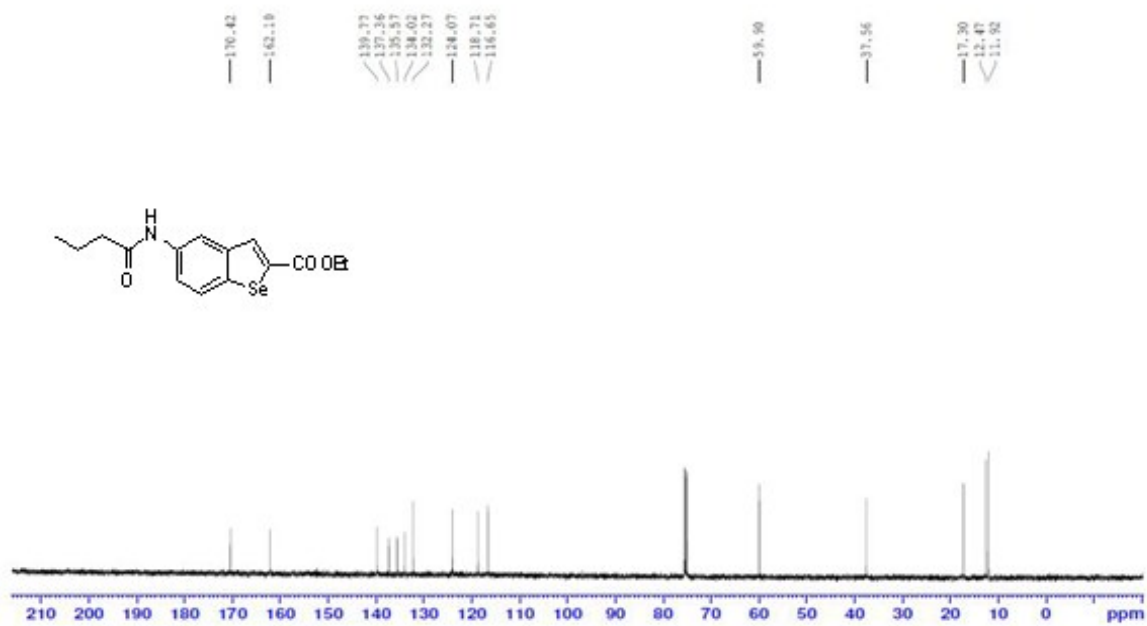
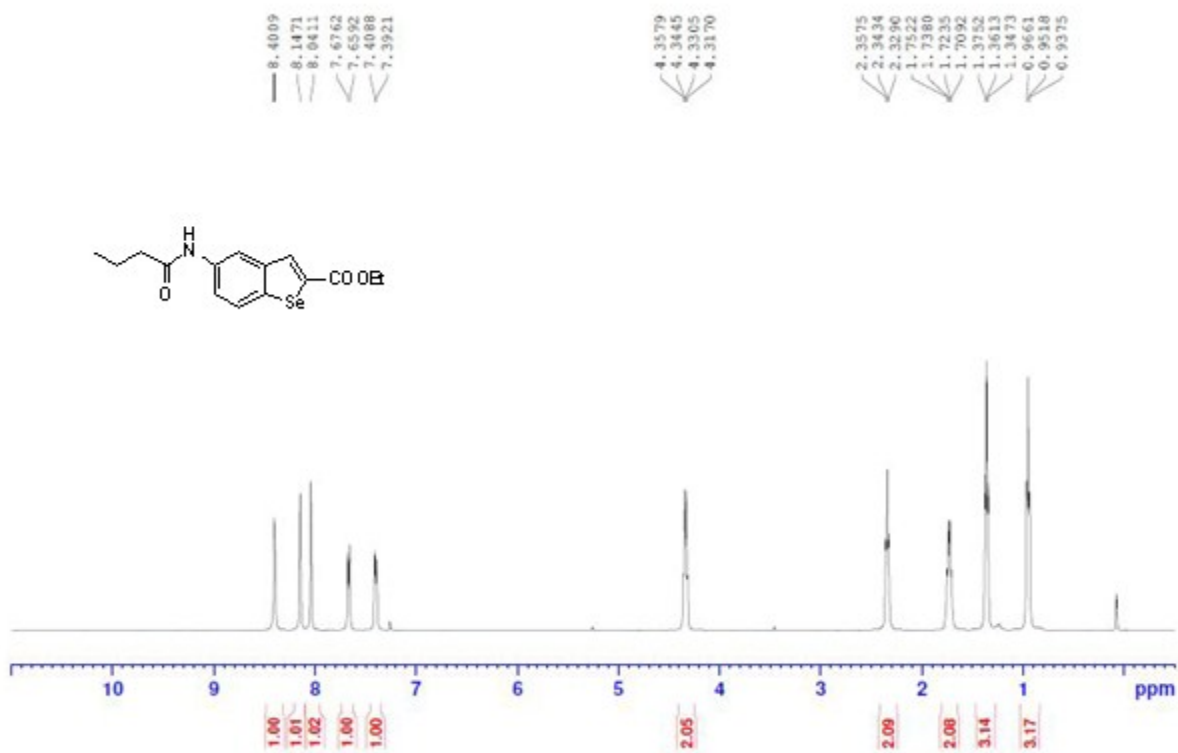
Intermediate of compound 4



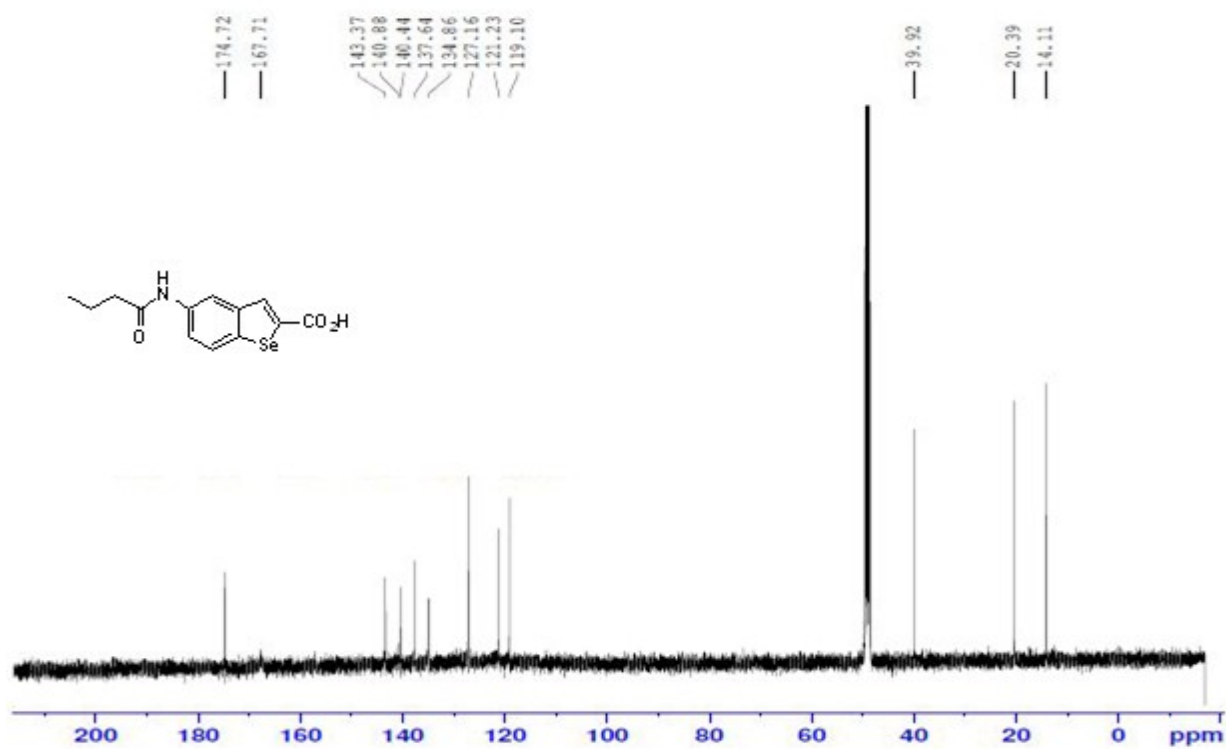
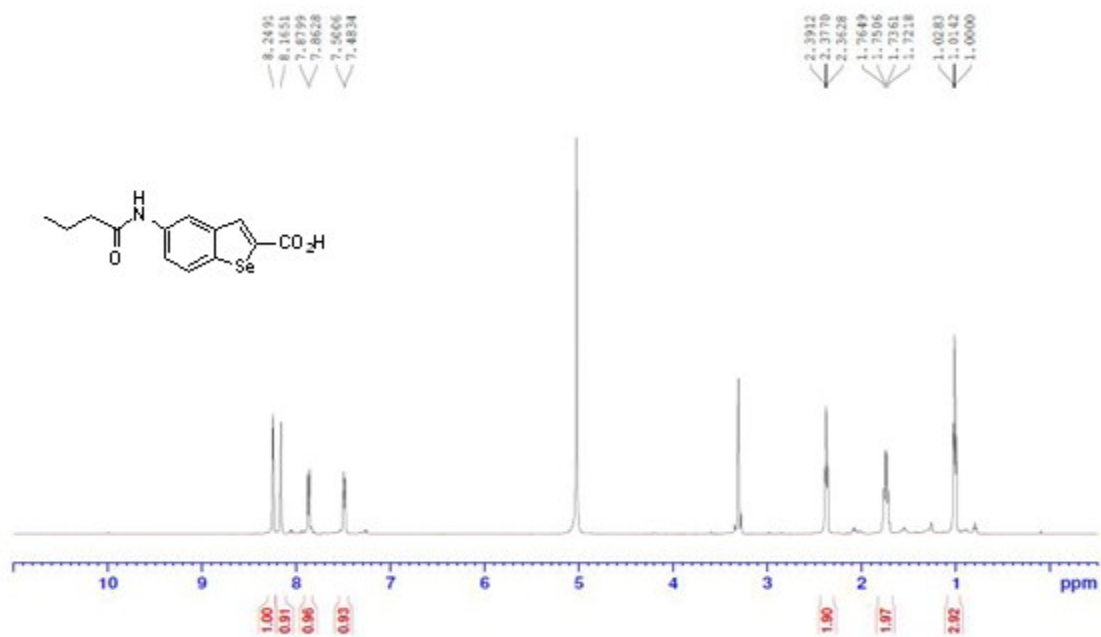
Compound 4



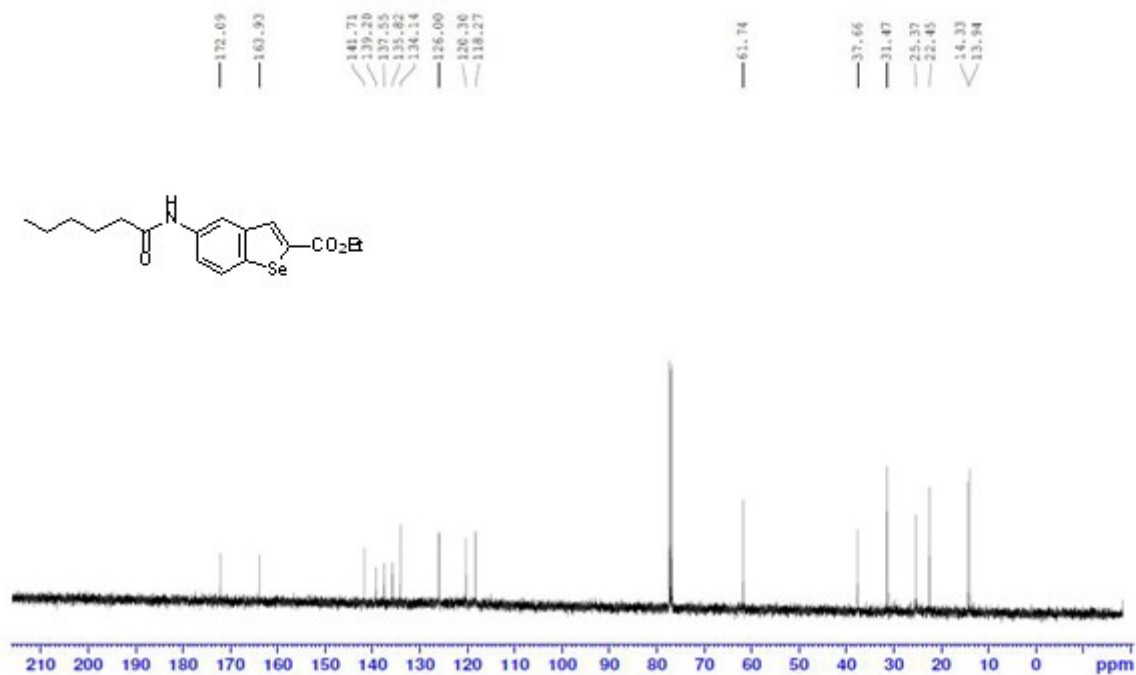
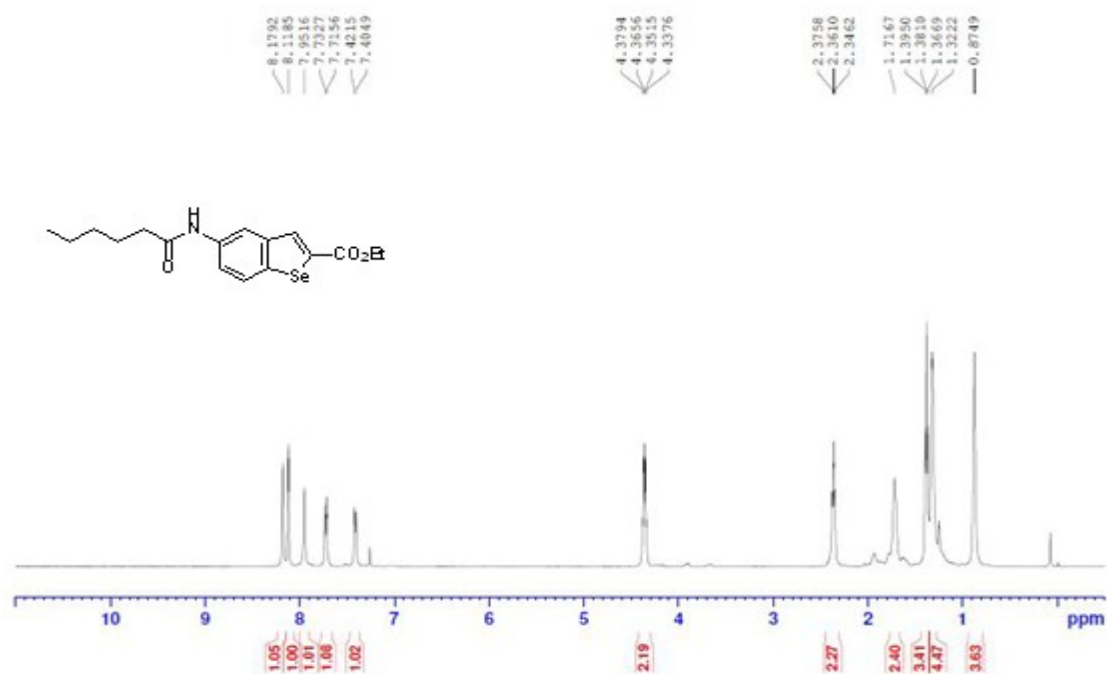
Intermediate of compound 5



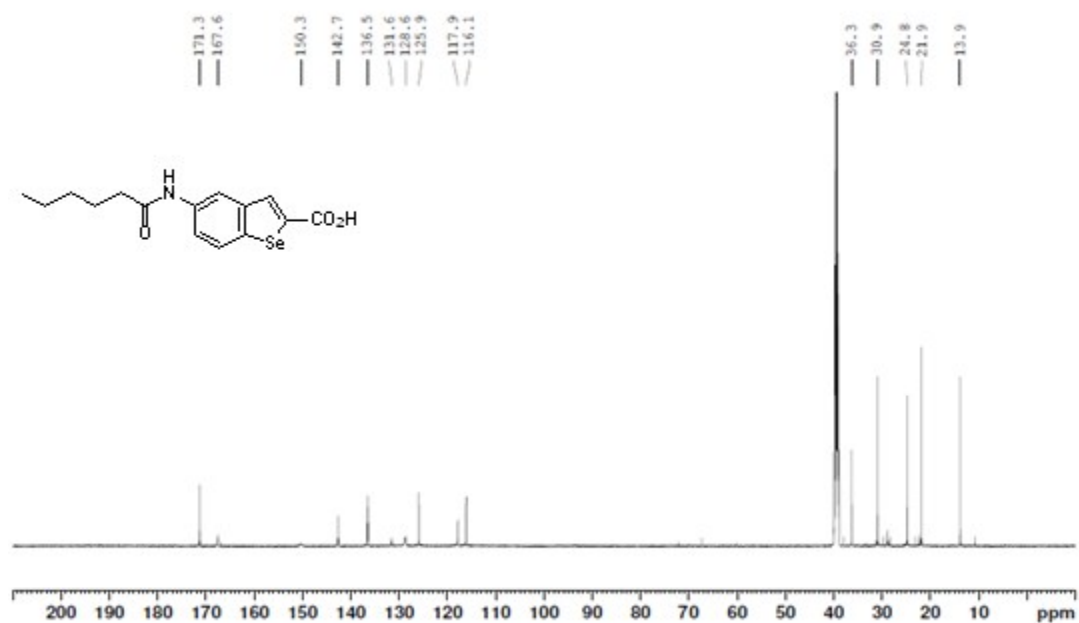
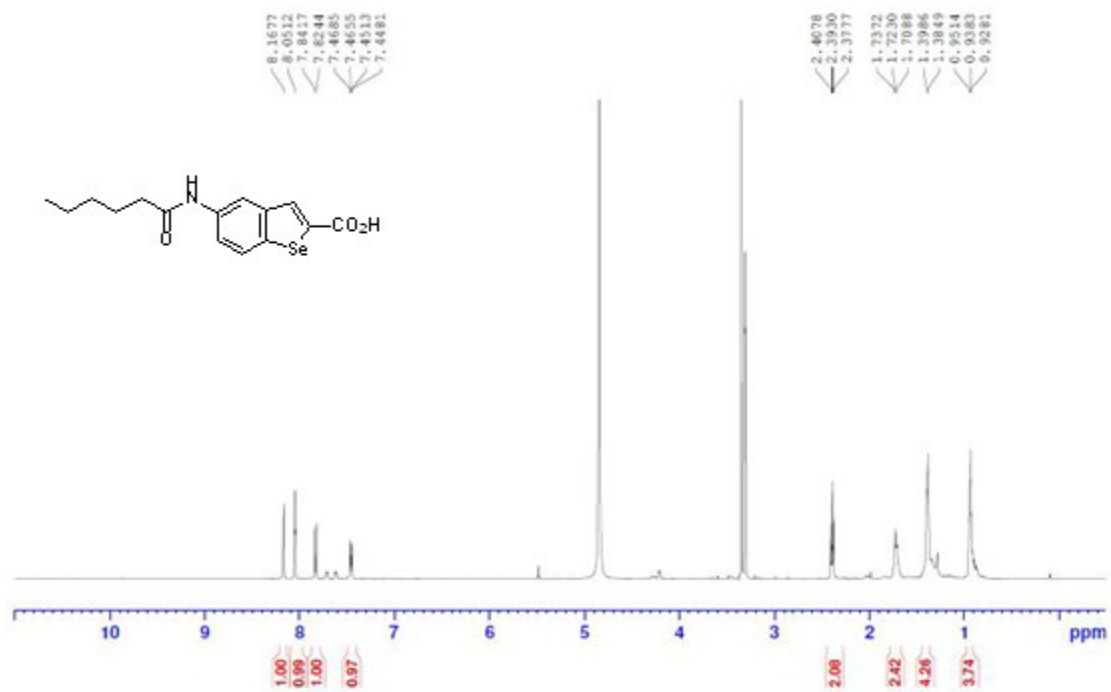
Compound 5



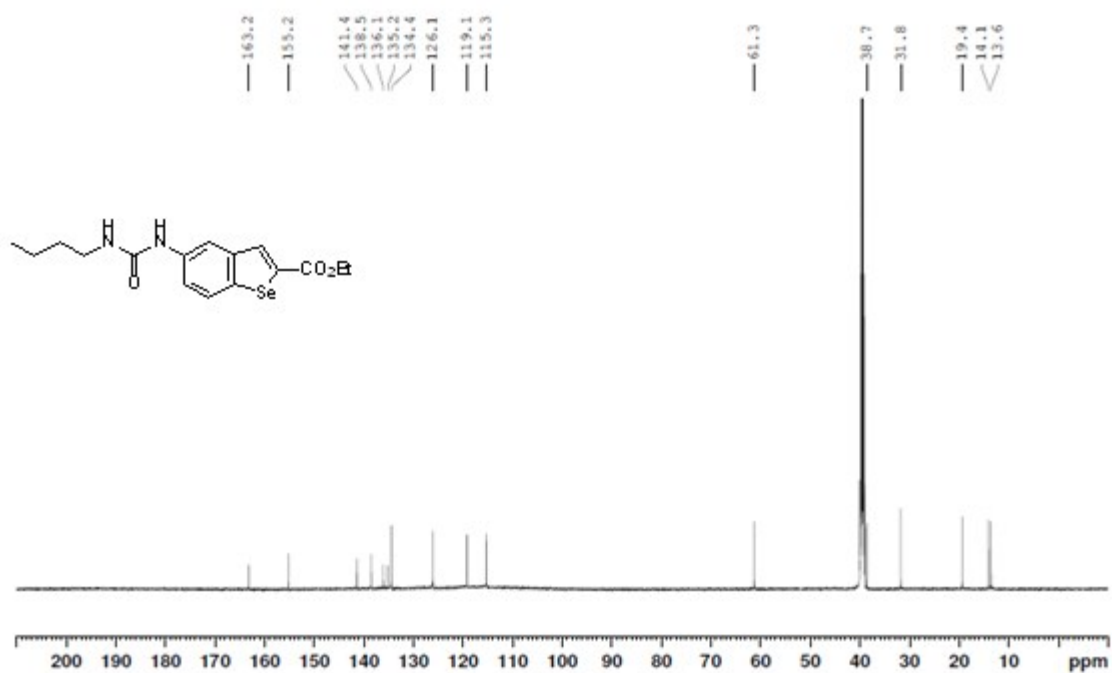
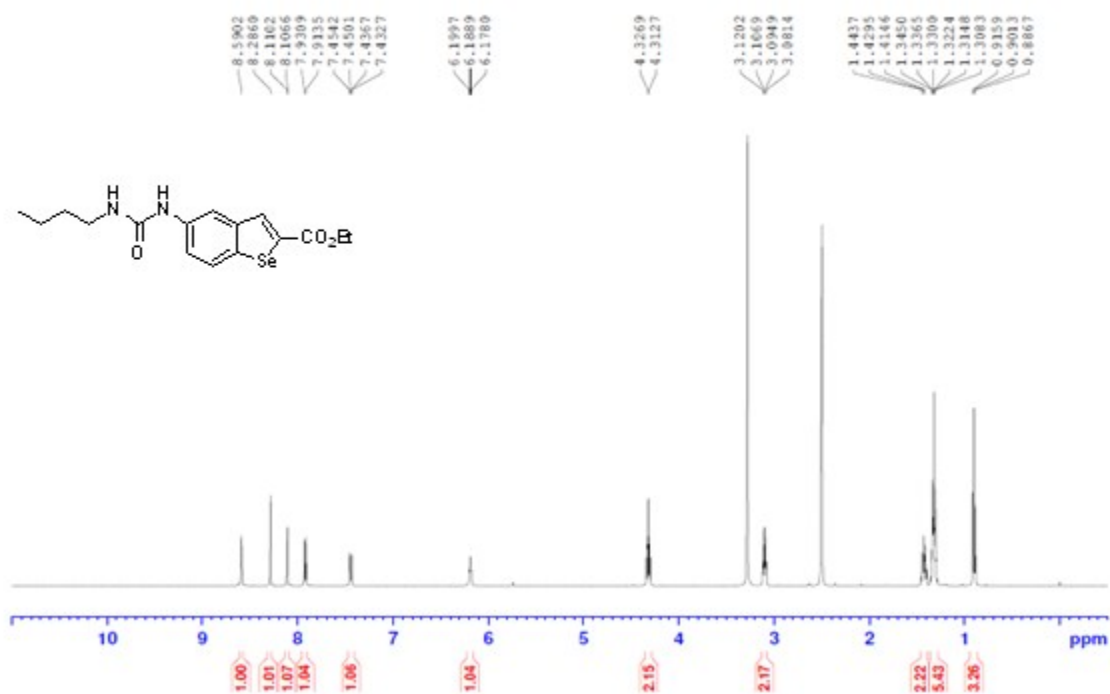
Intermediate of compound6



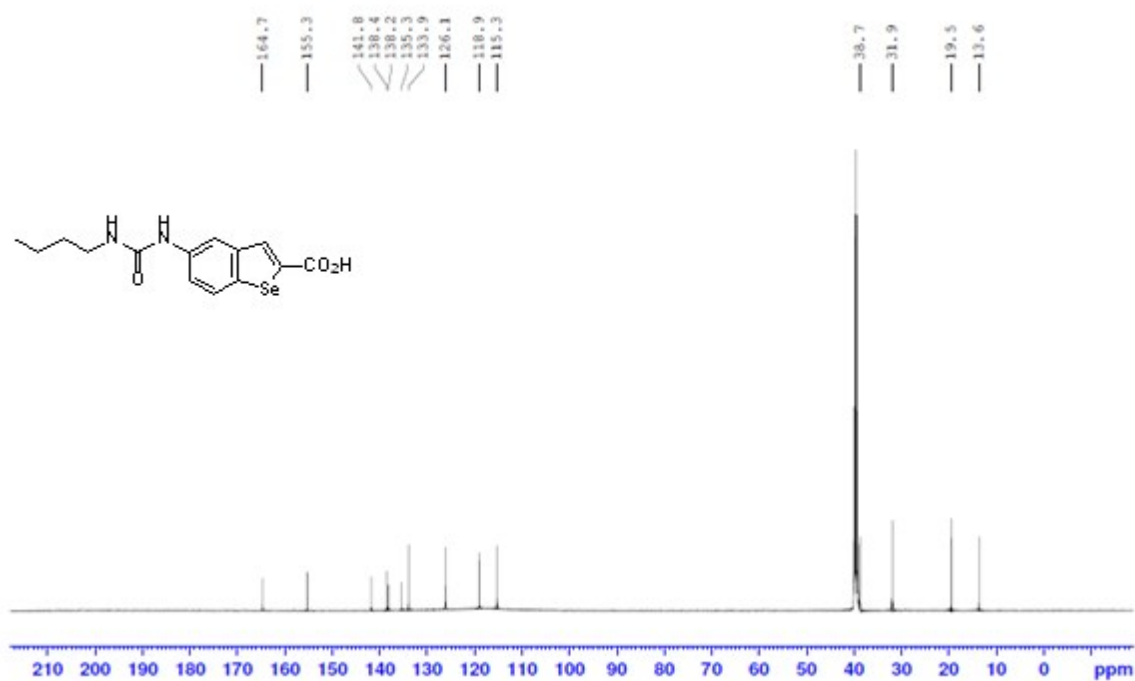
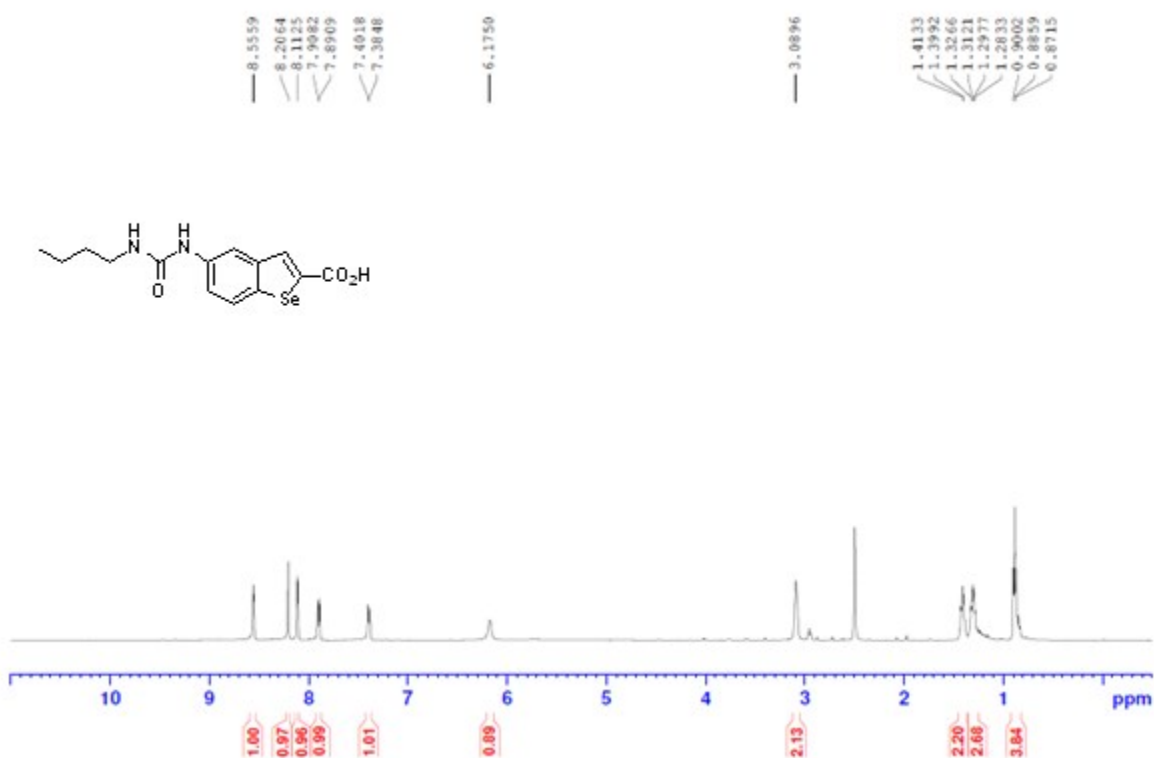
Compound 6



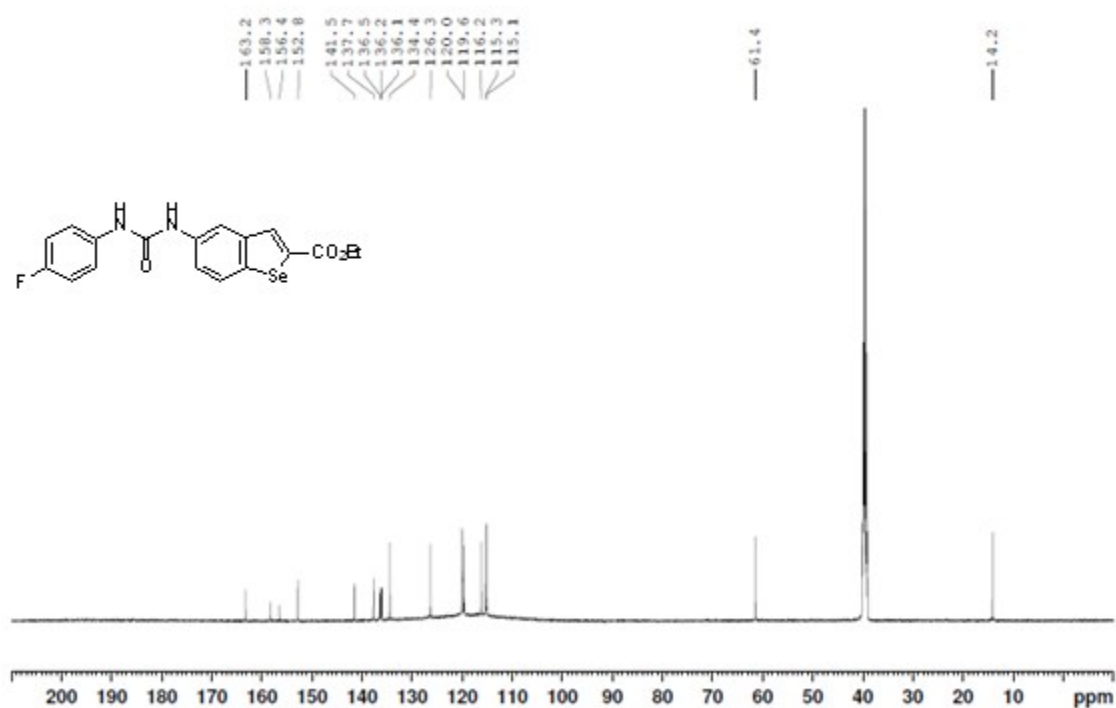
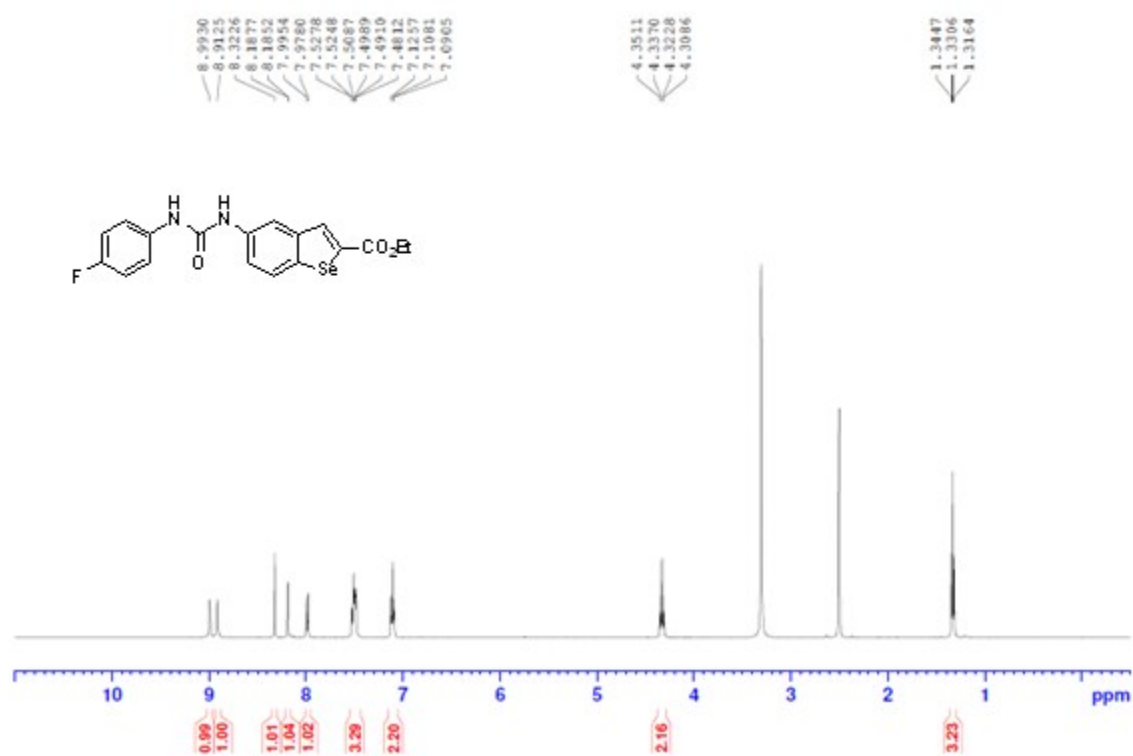
Intermediate of compound 7



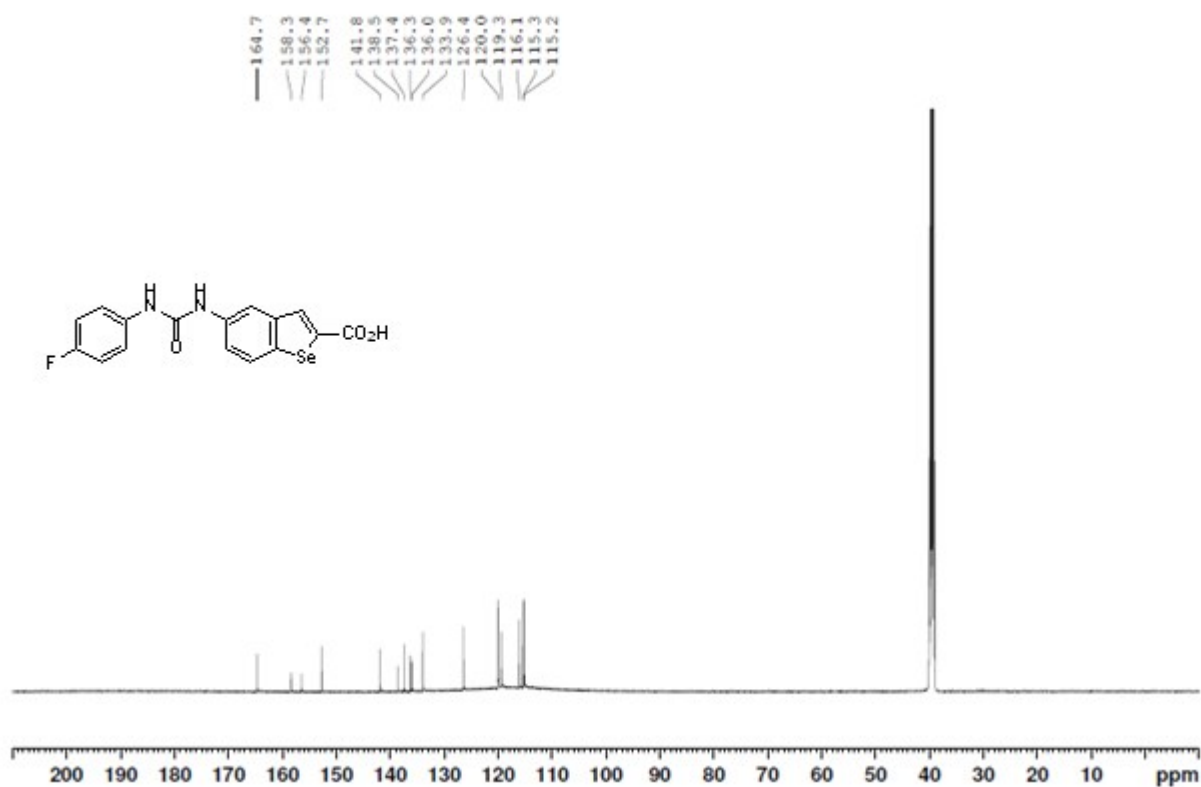
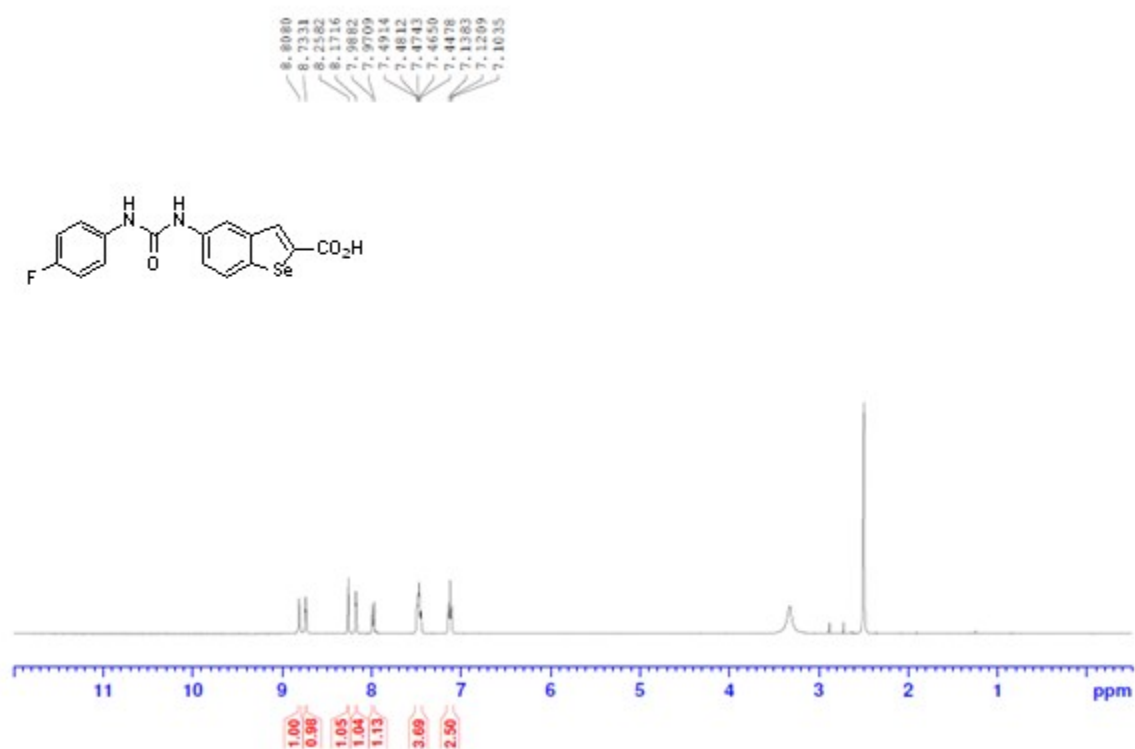
Compound 7



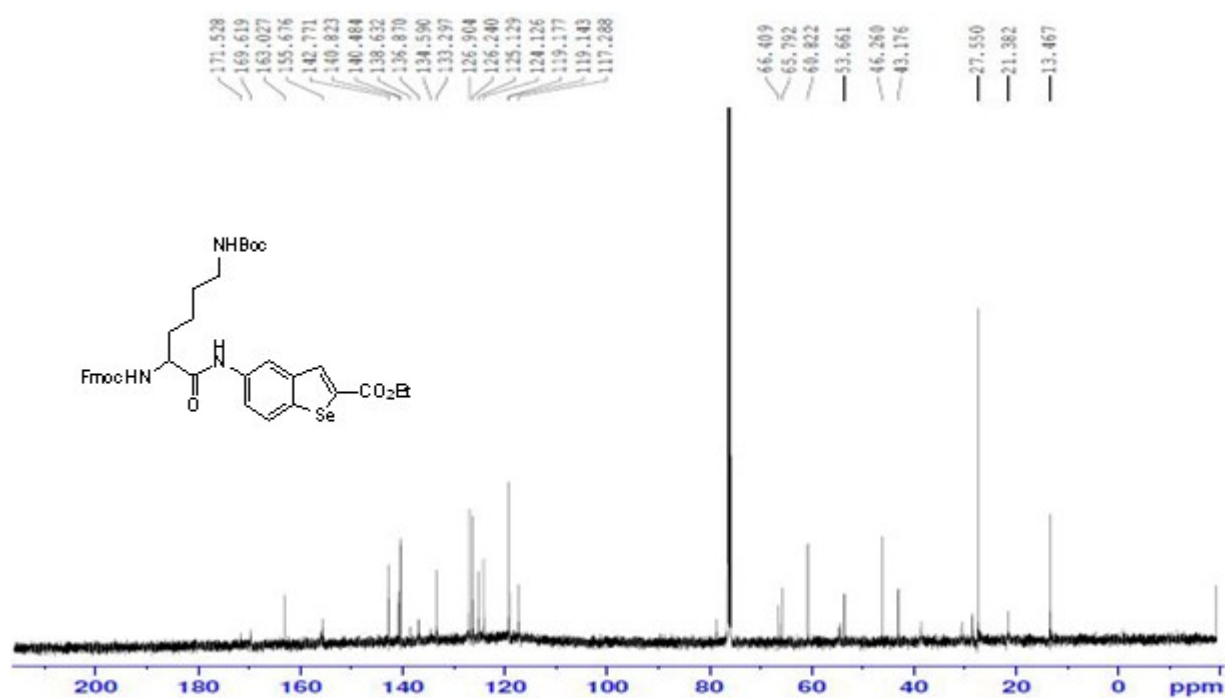
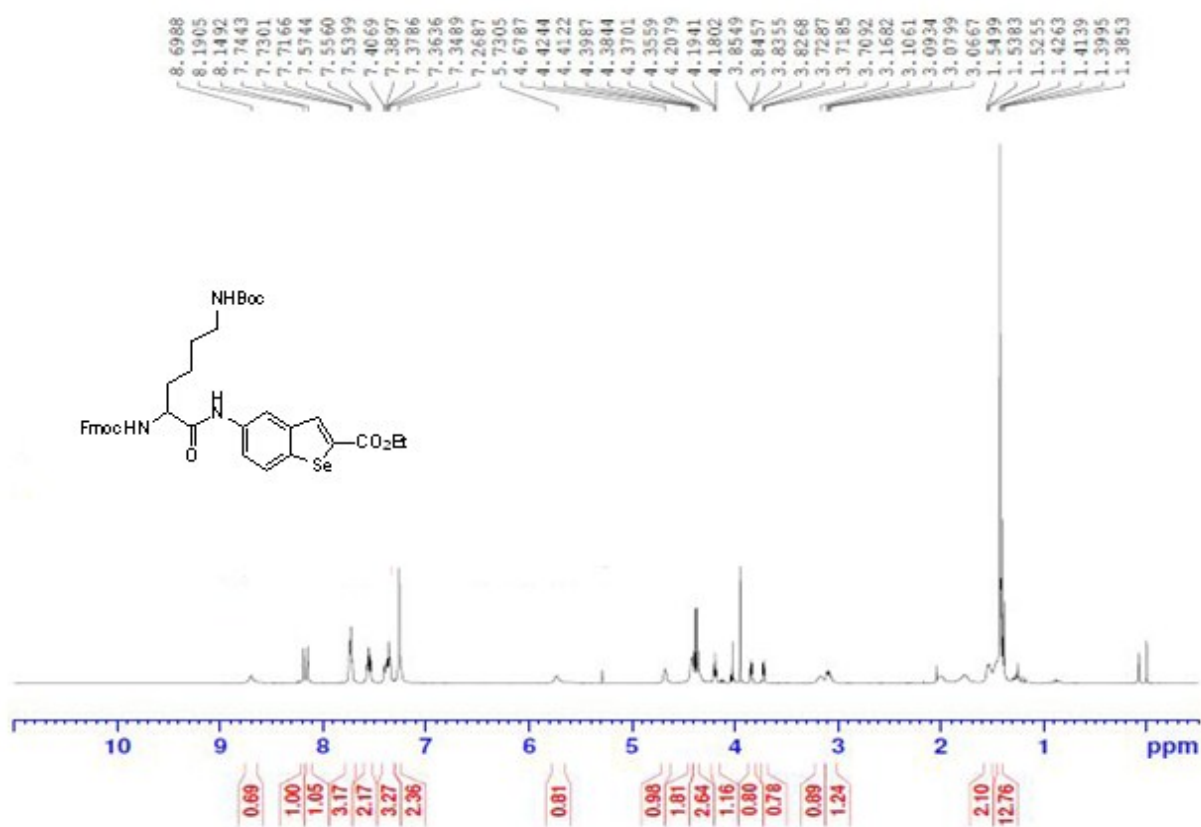
Intermediate of compound **8**



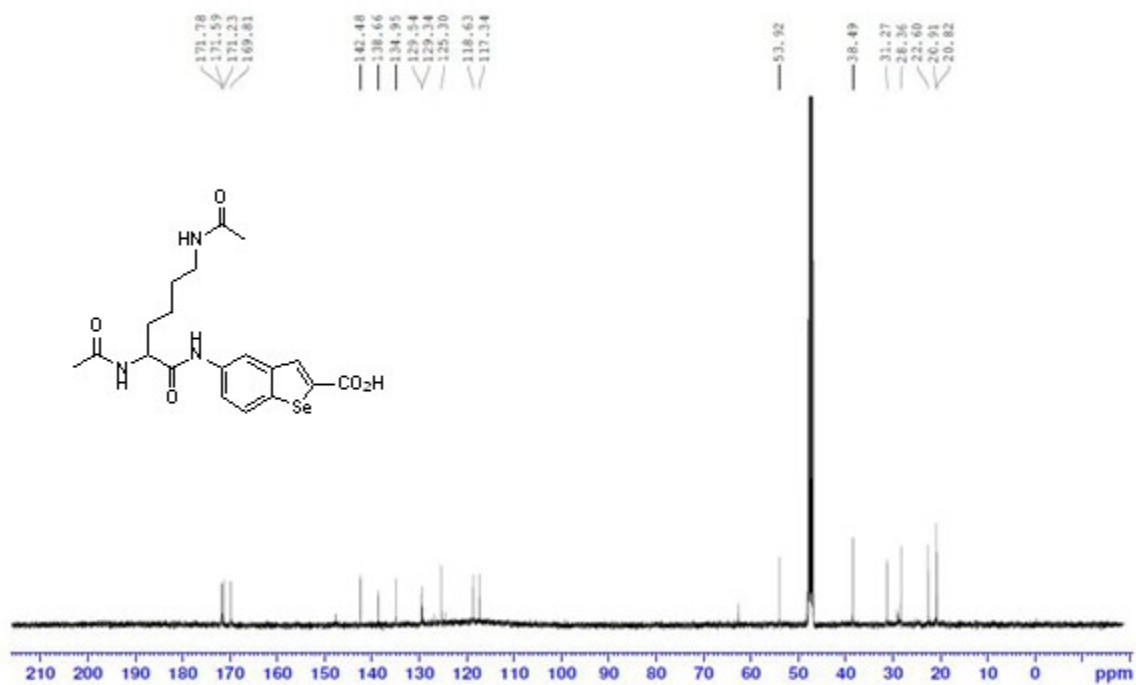
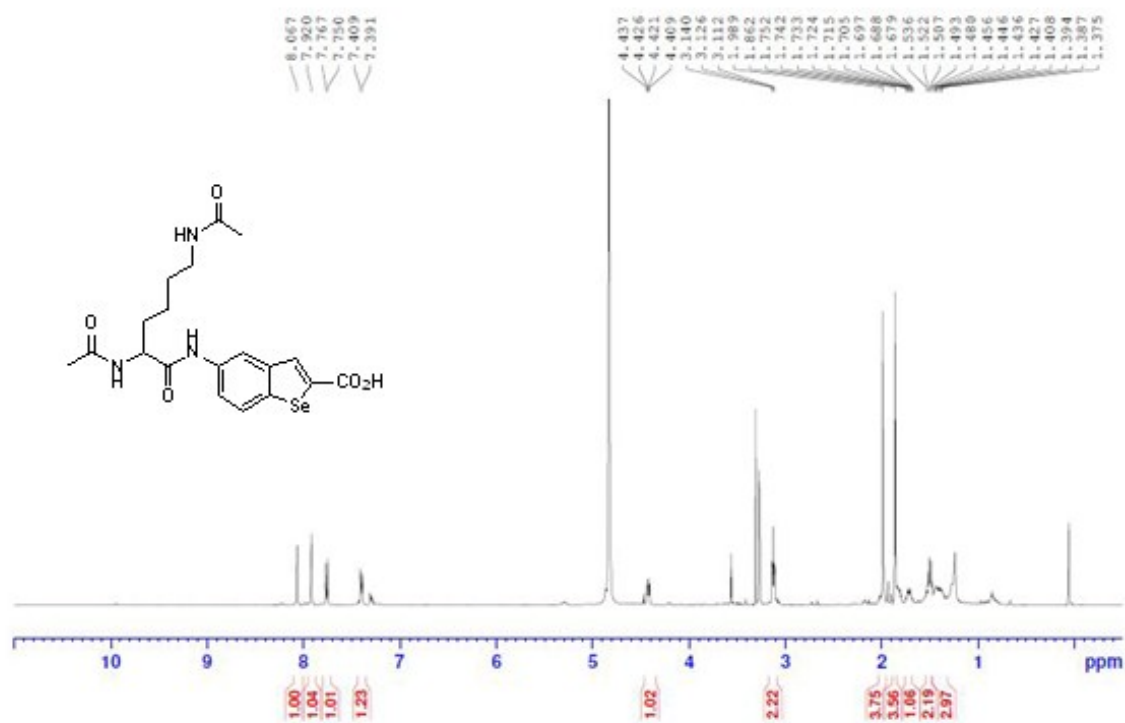
Compound **8**



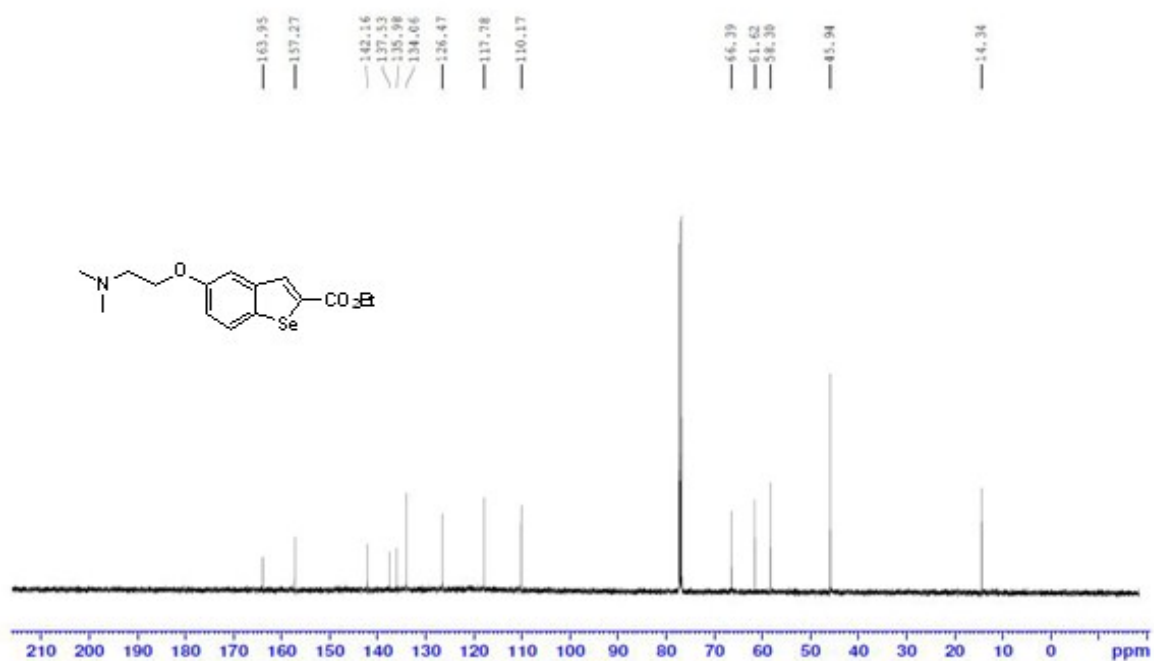
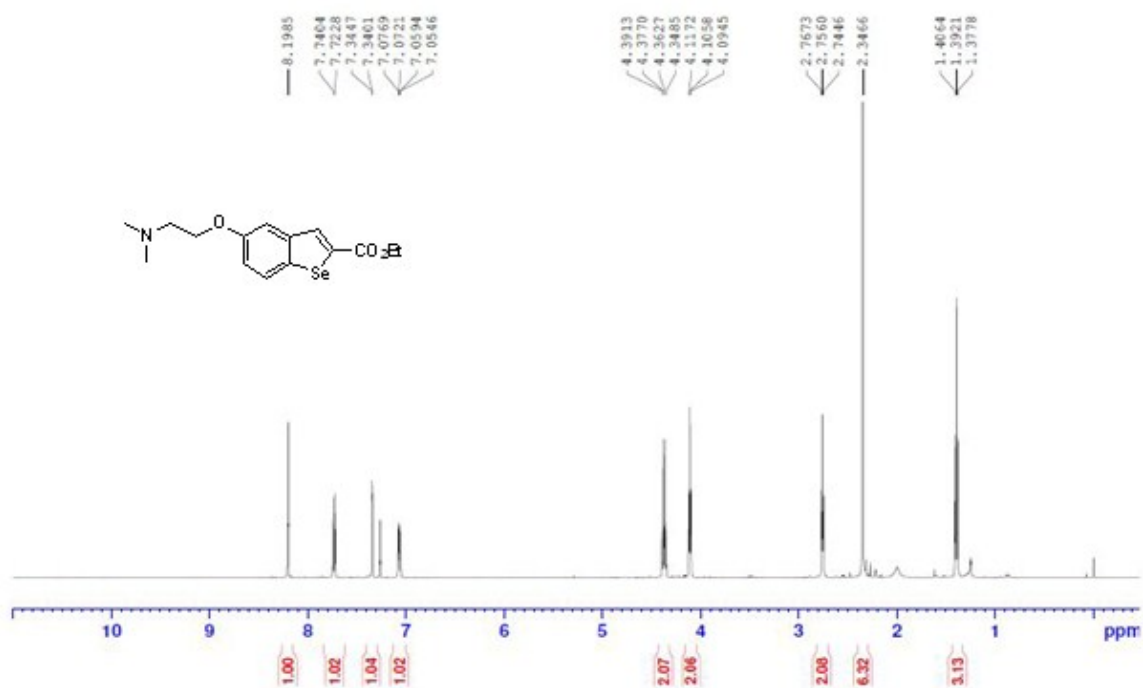
Intermediate of compound 9



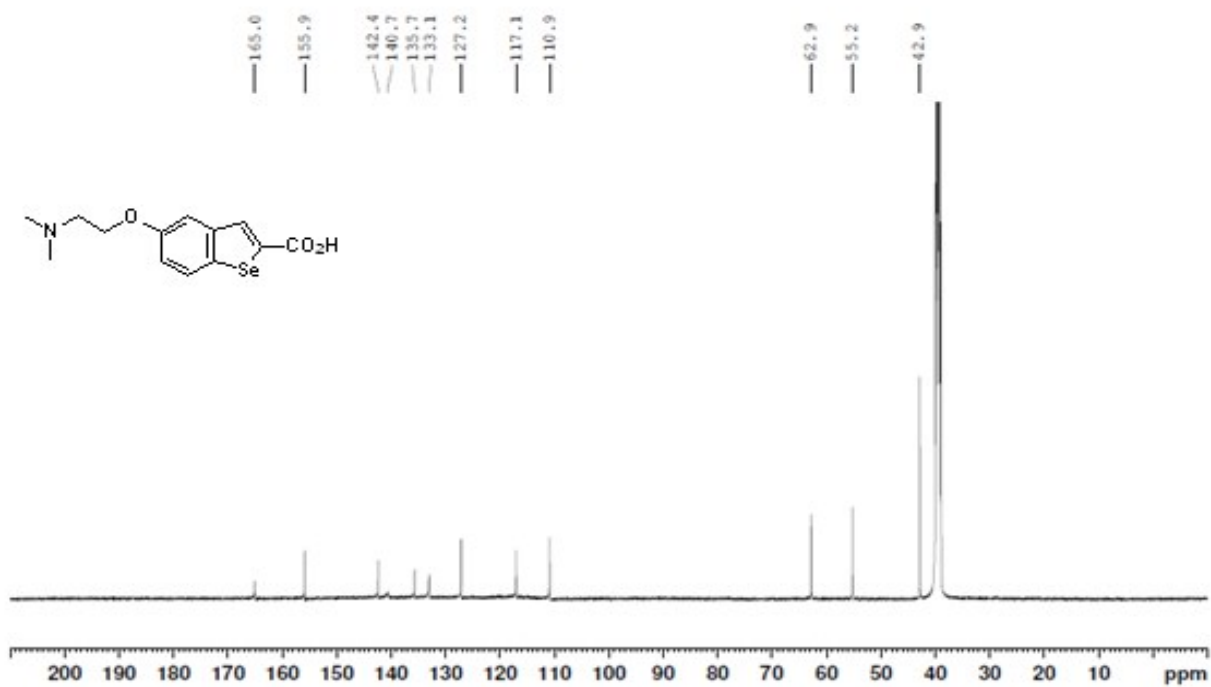
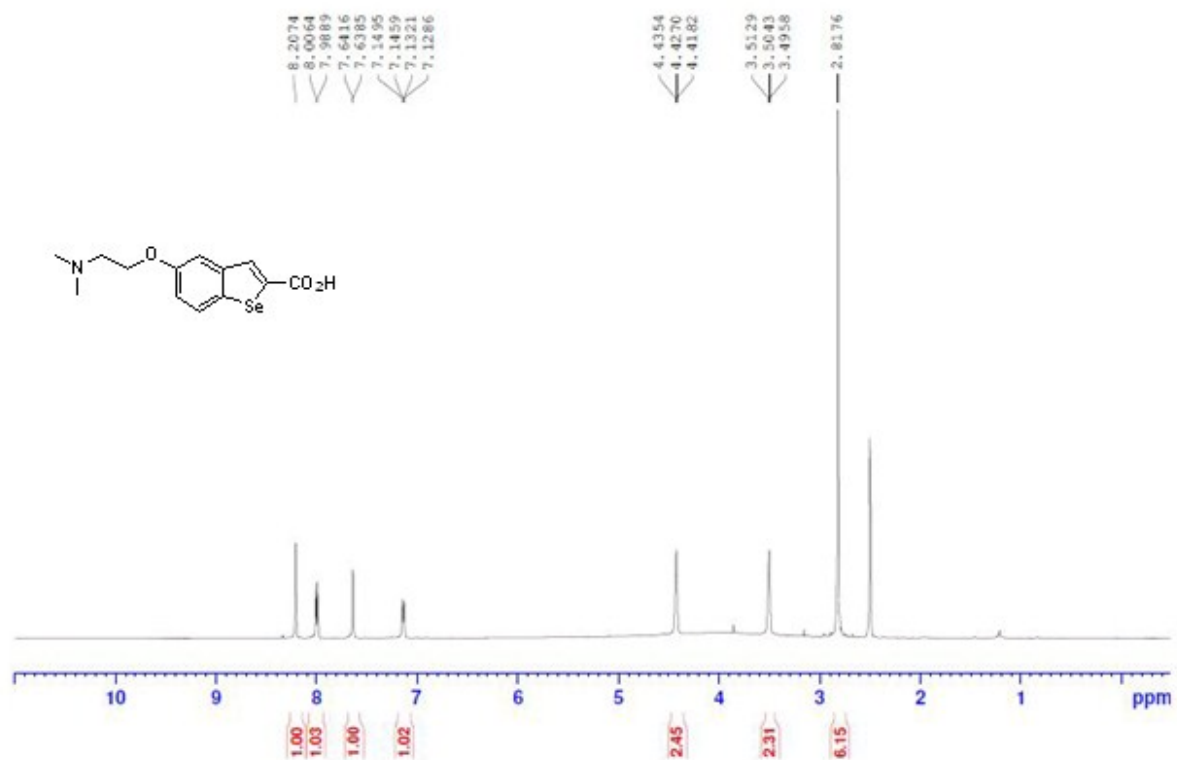
Compound 9



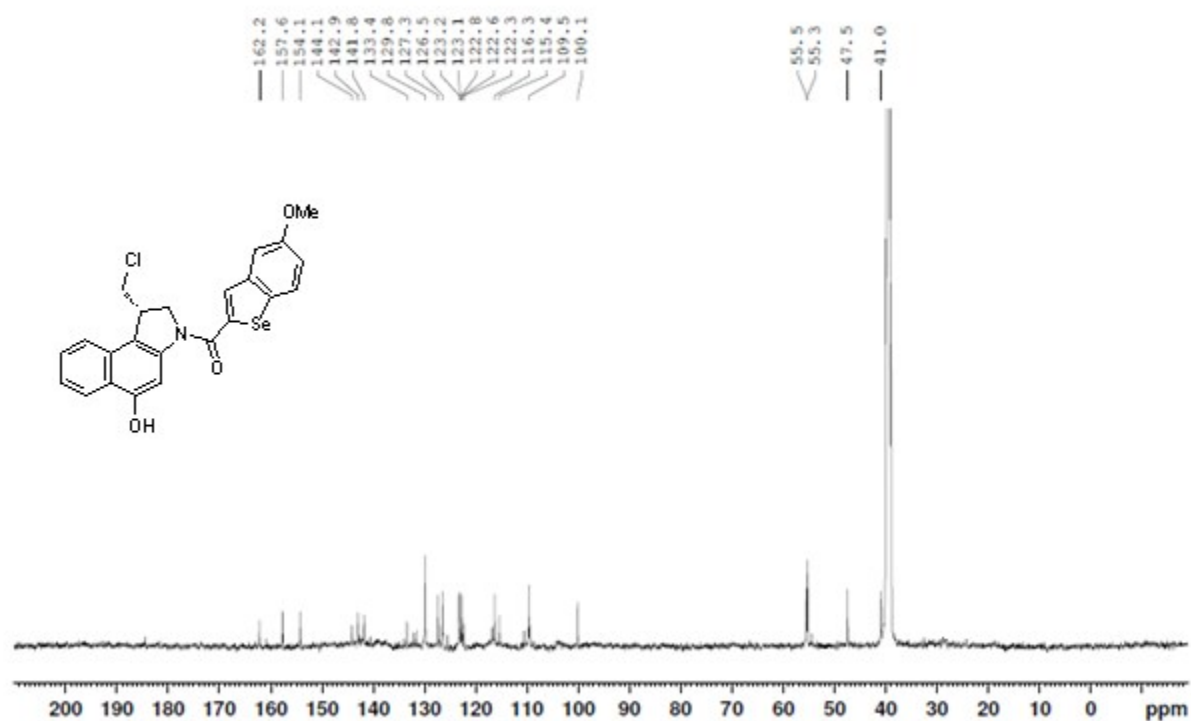
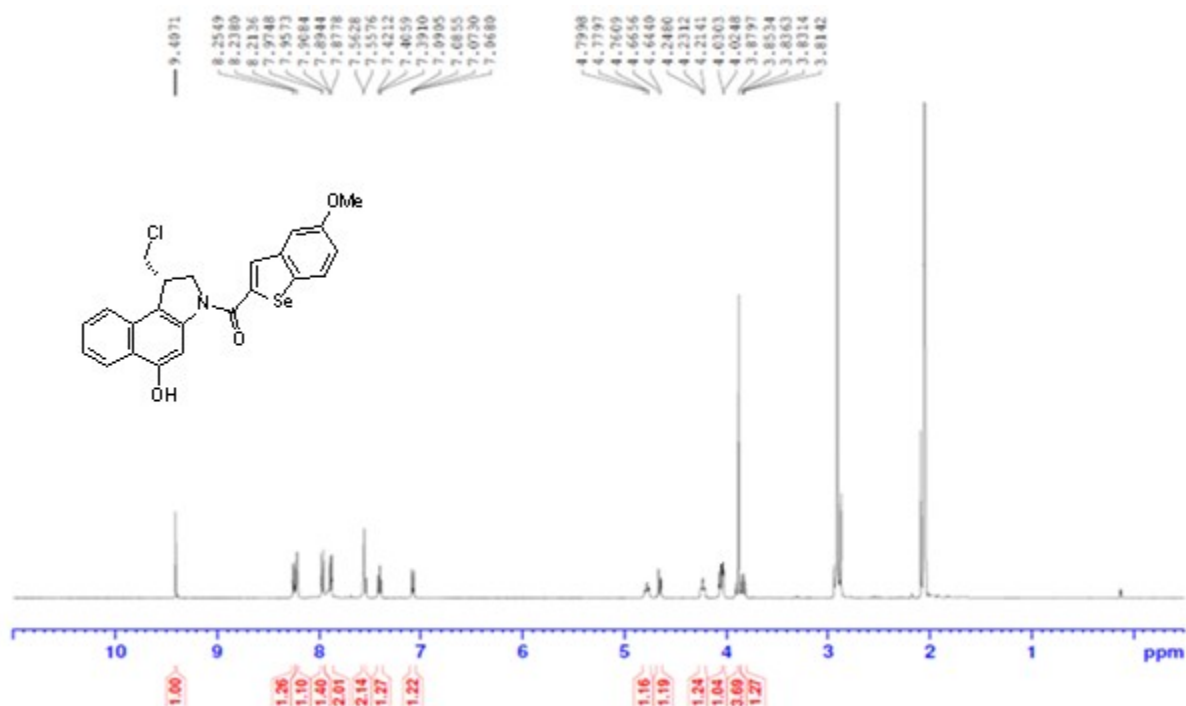
Intermediate of compound **10**



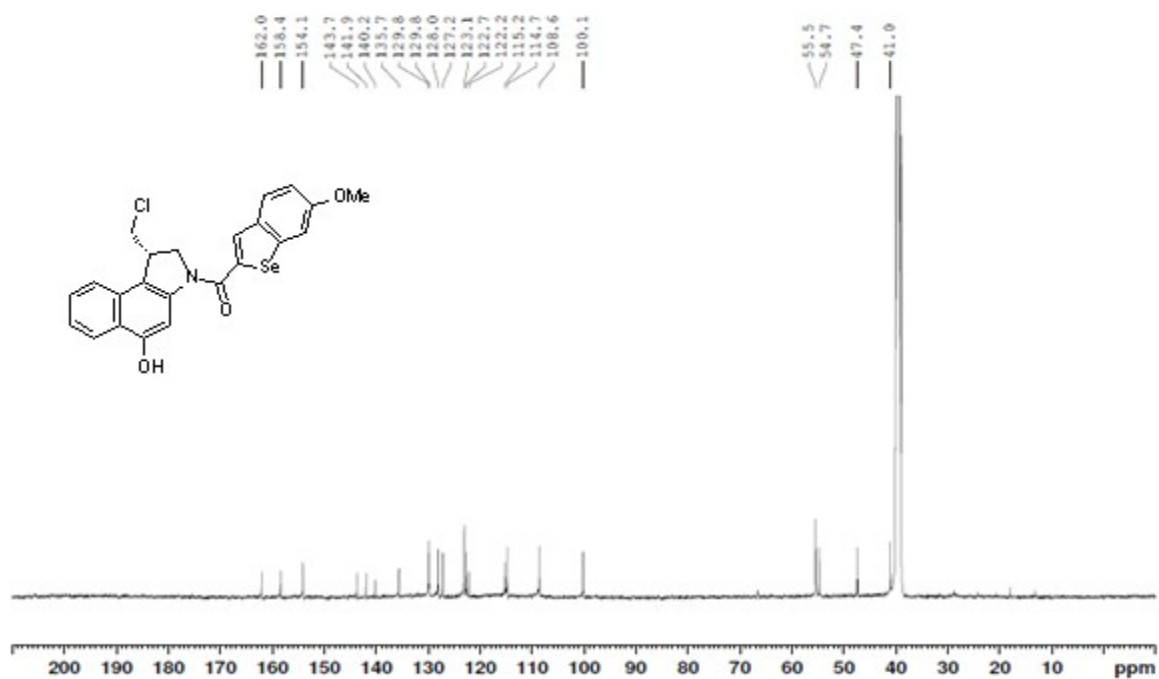
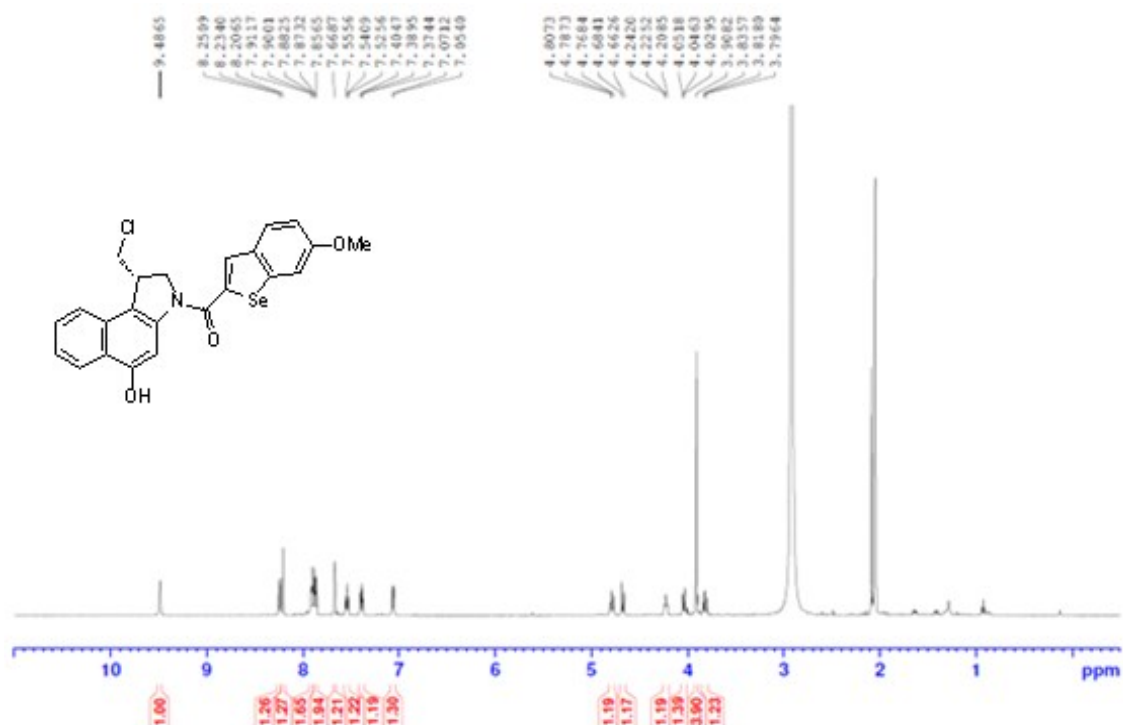
Compound **10**



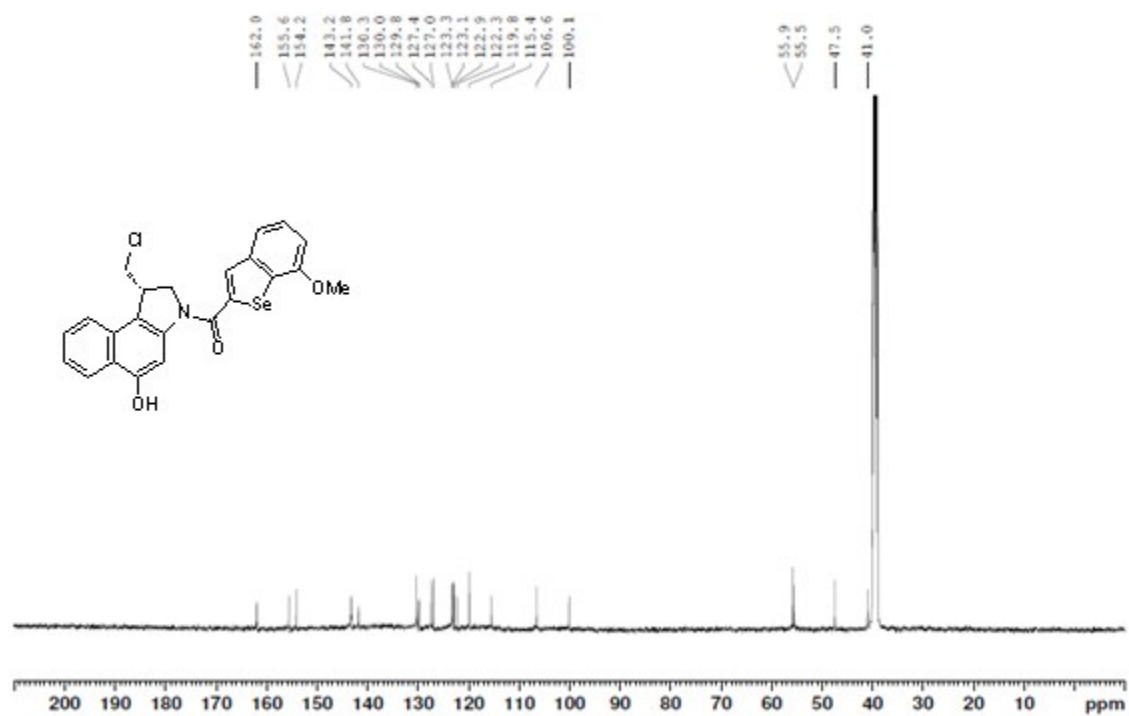
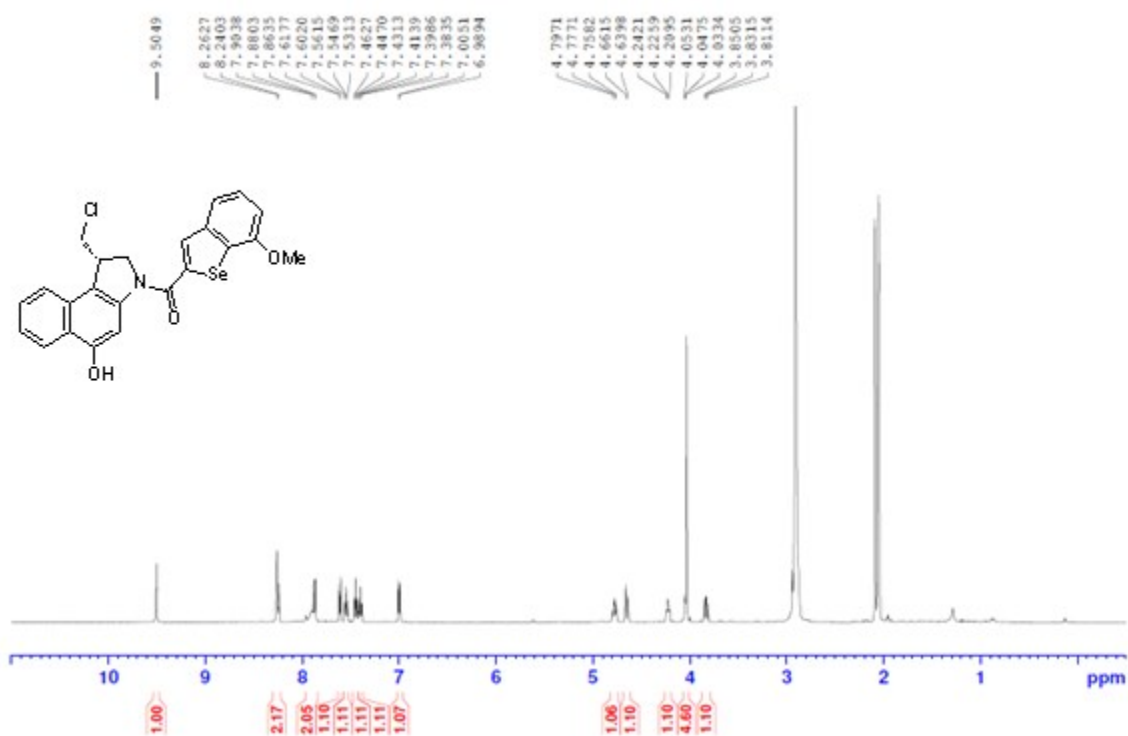
Compound **14a**



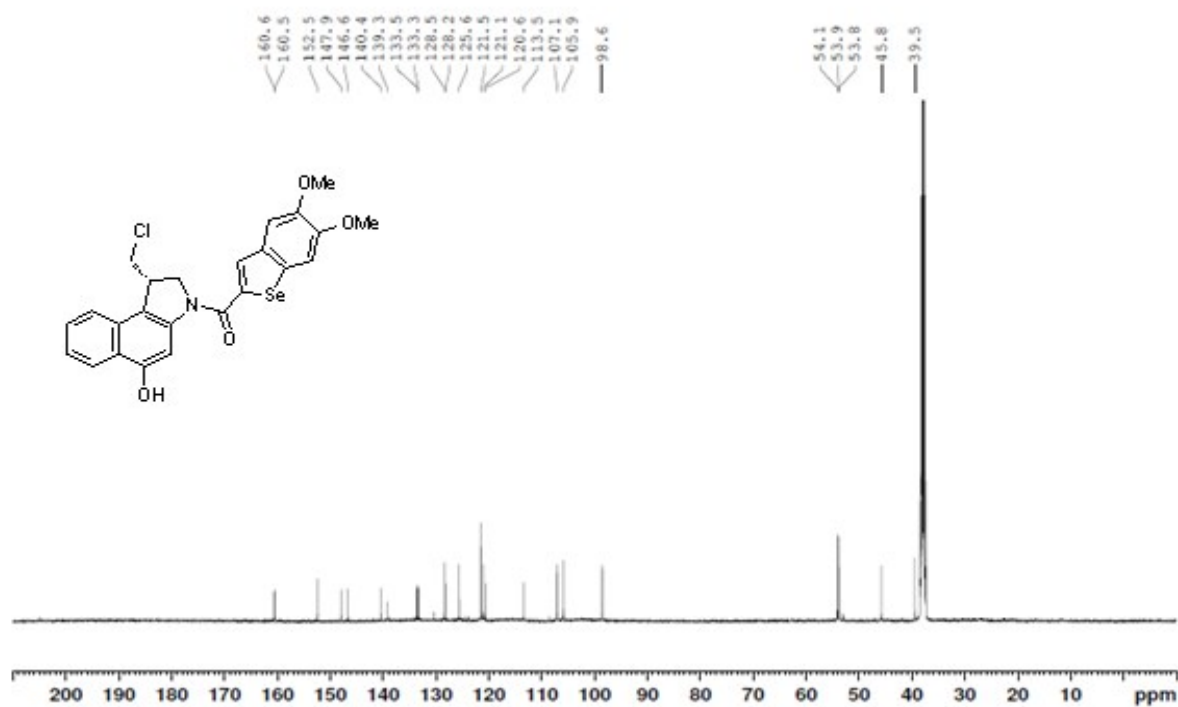
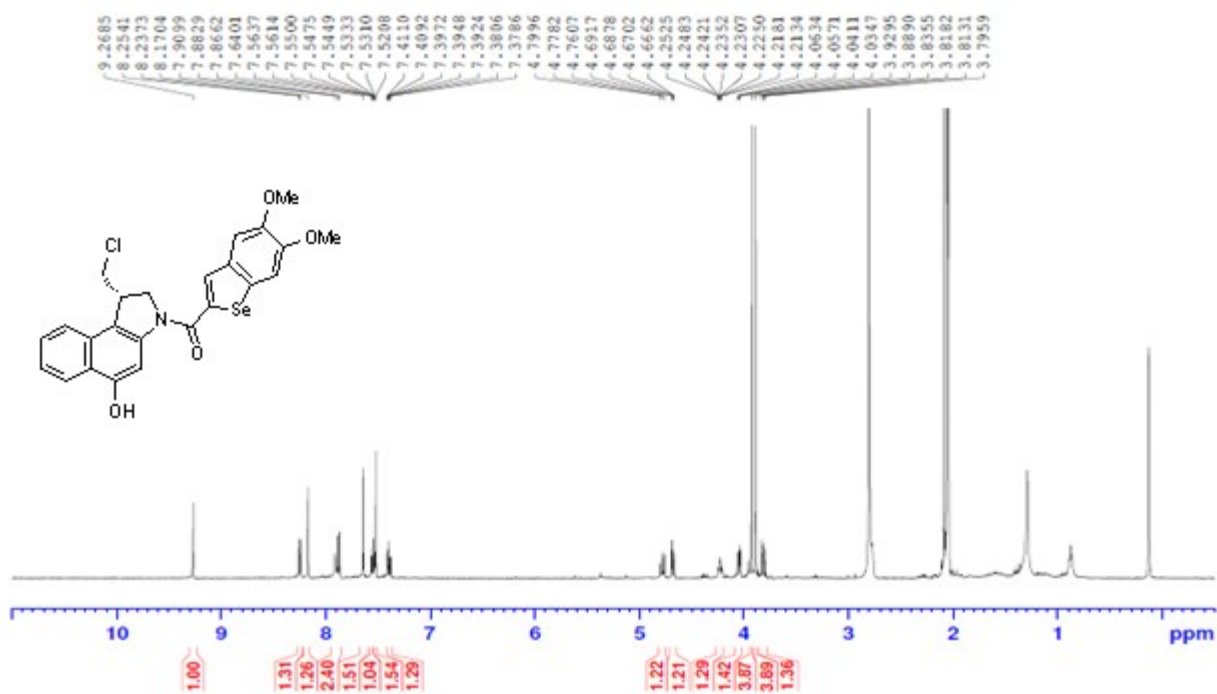
Compound **14b**



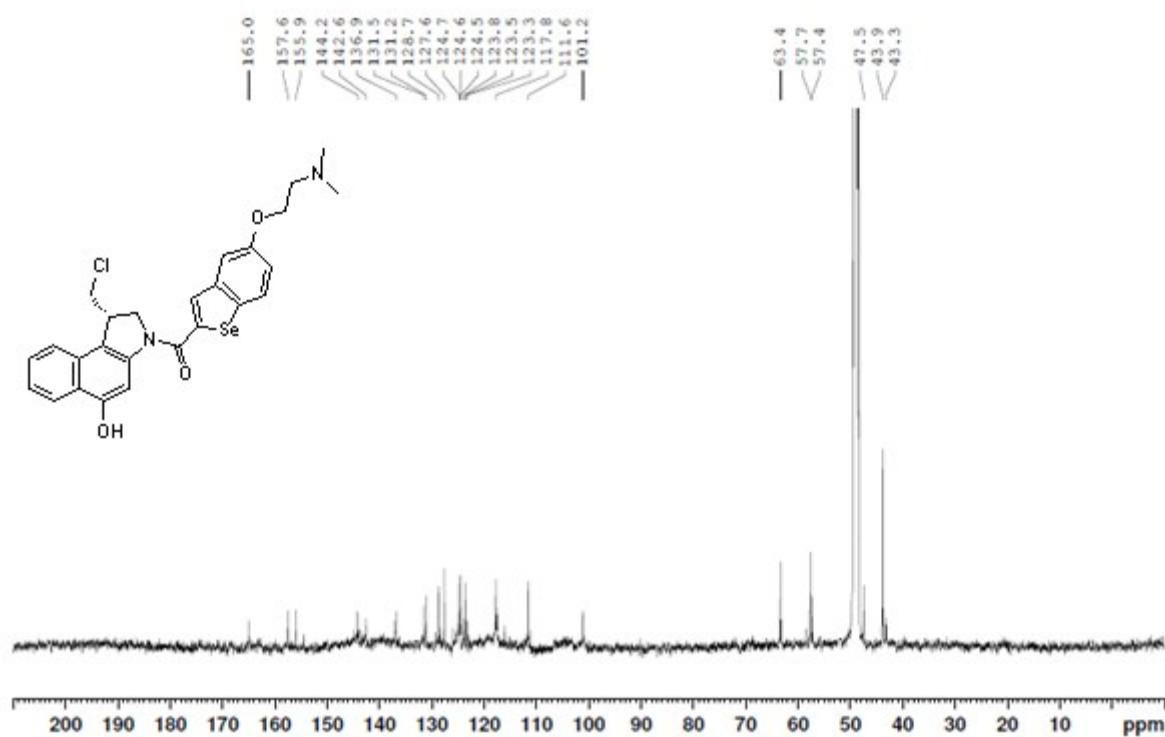
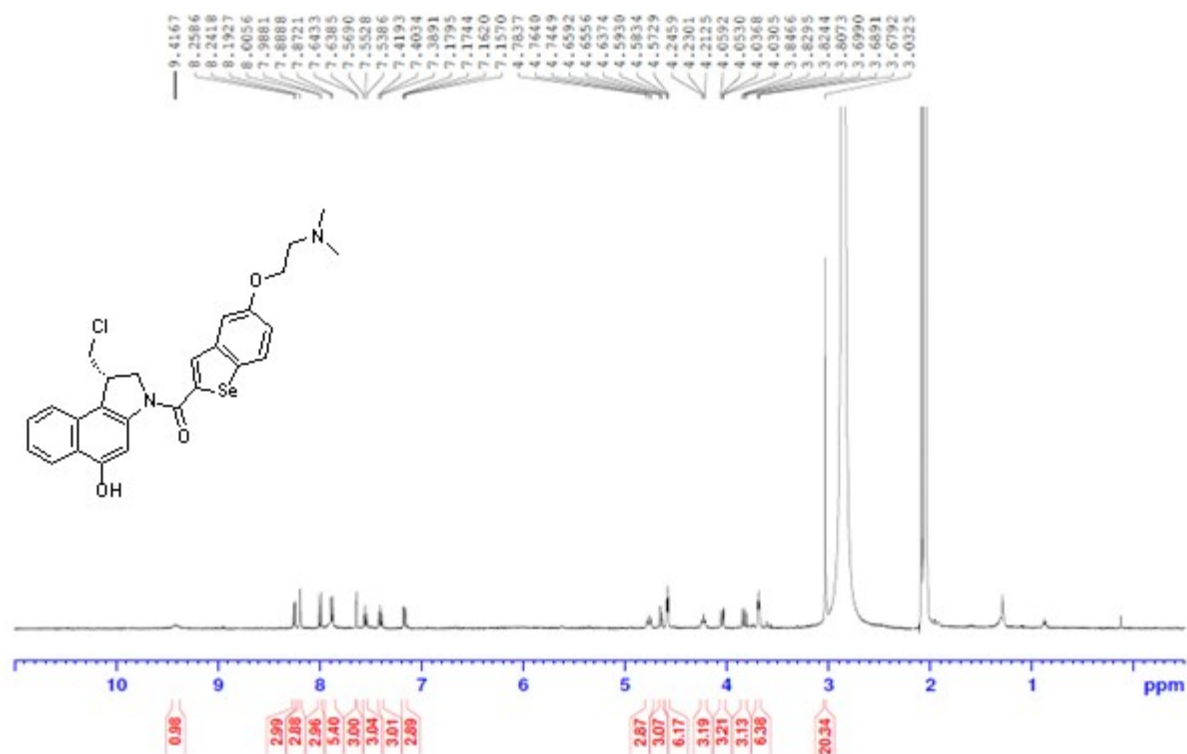
Compound **14c**



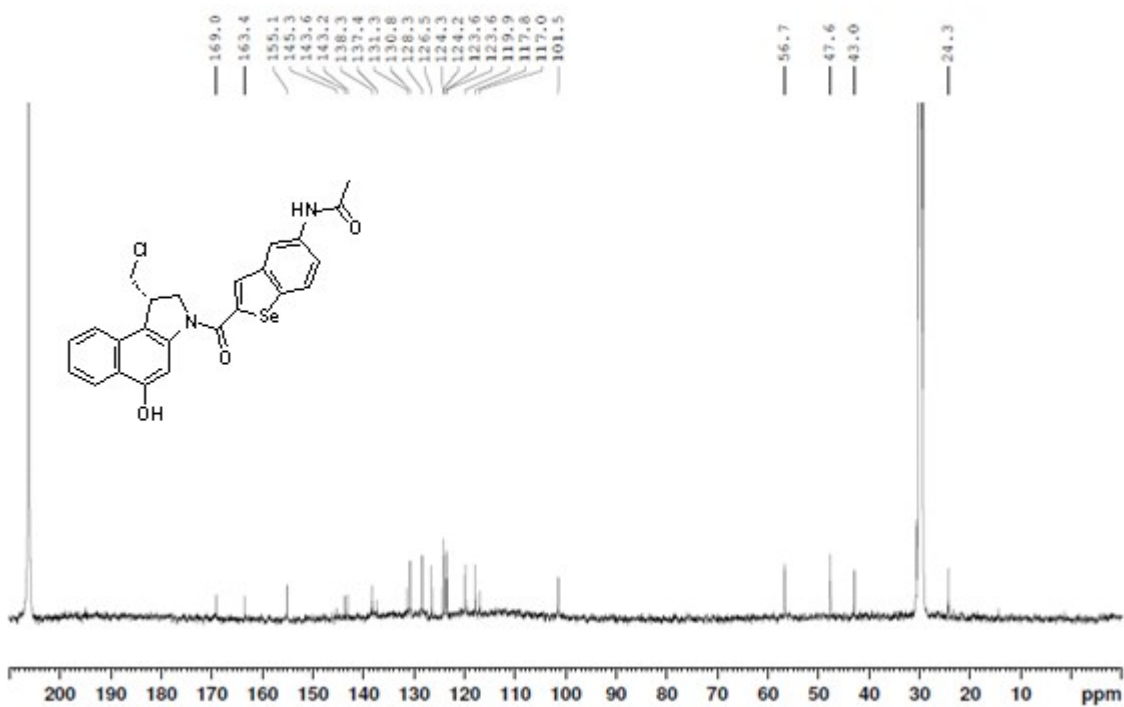
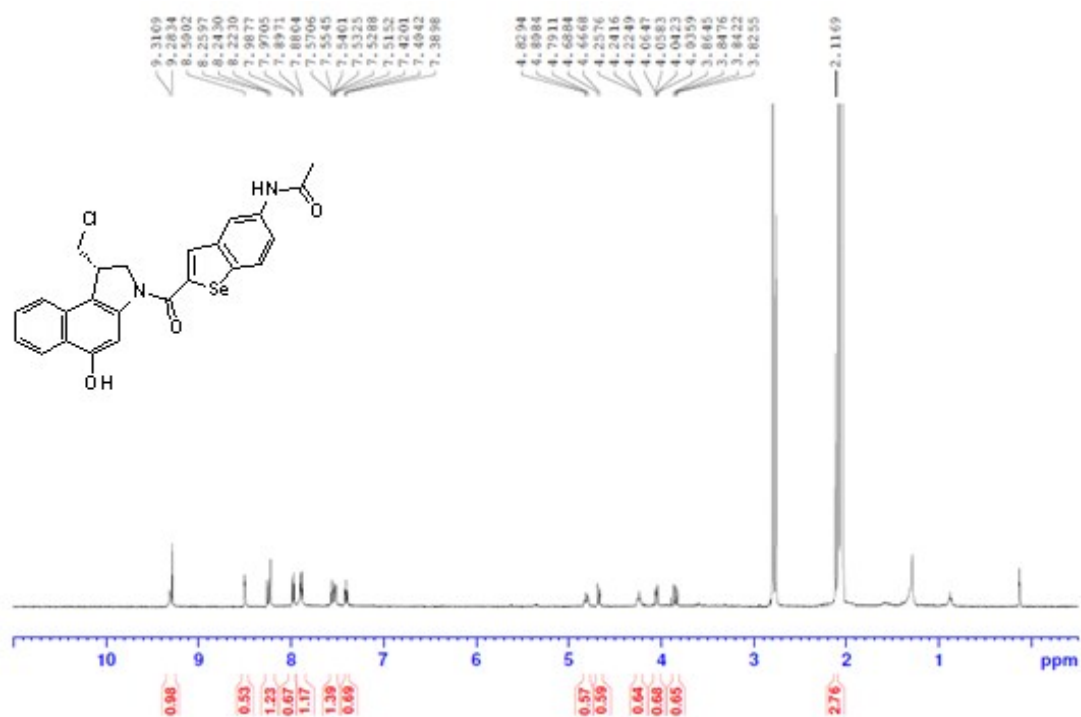
Compound **14d**



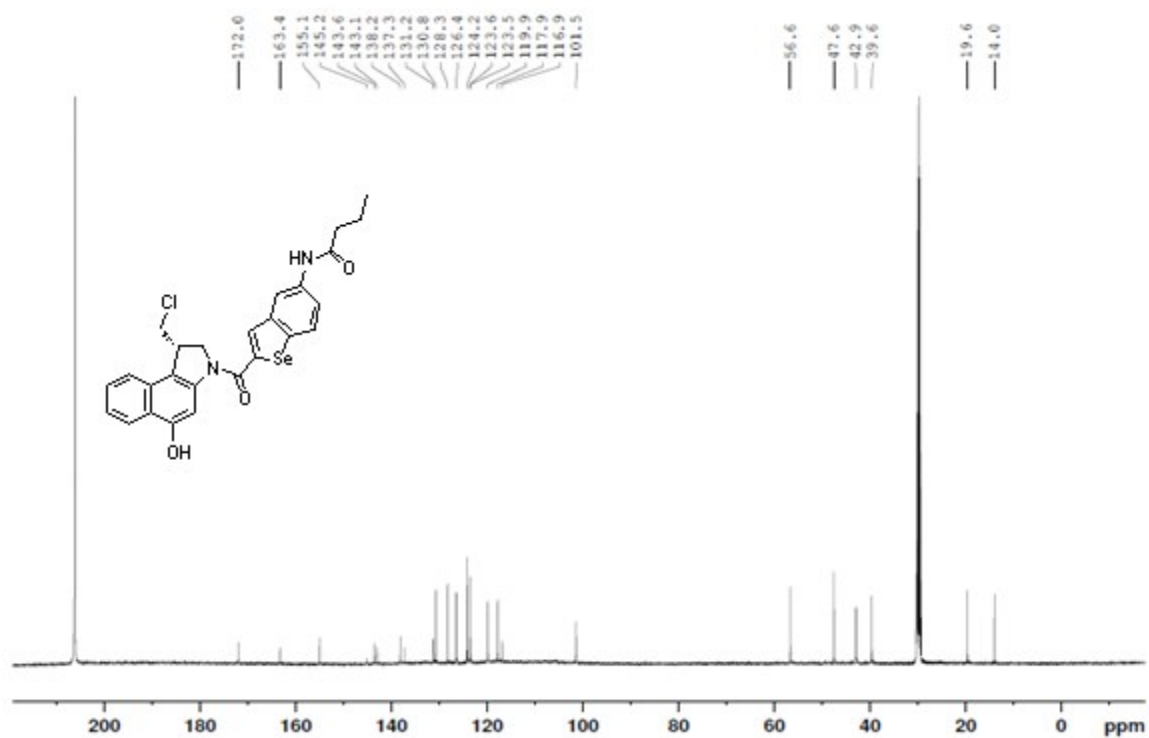
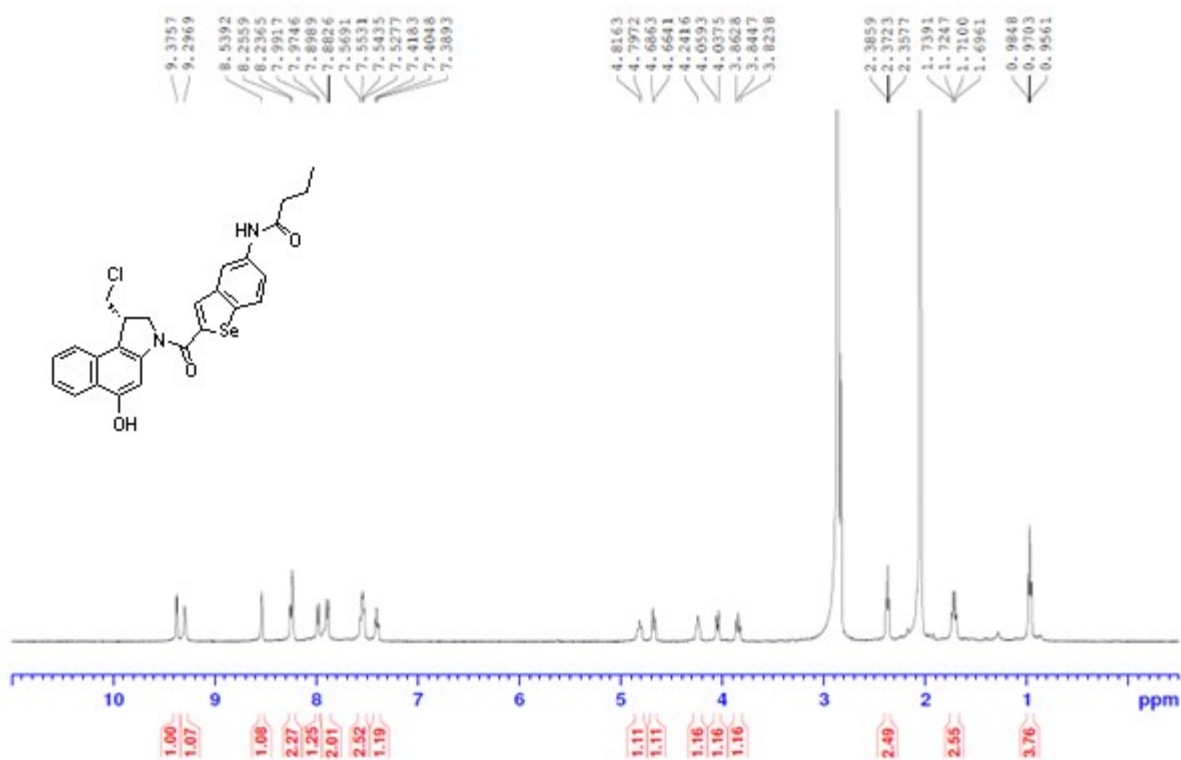
Compound **14e**



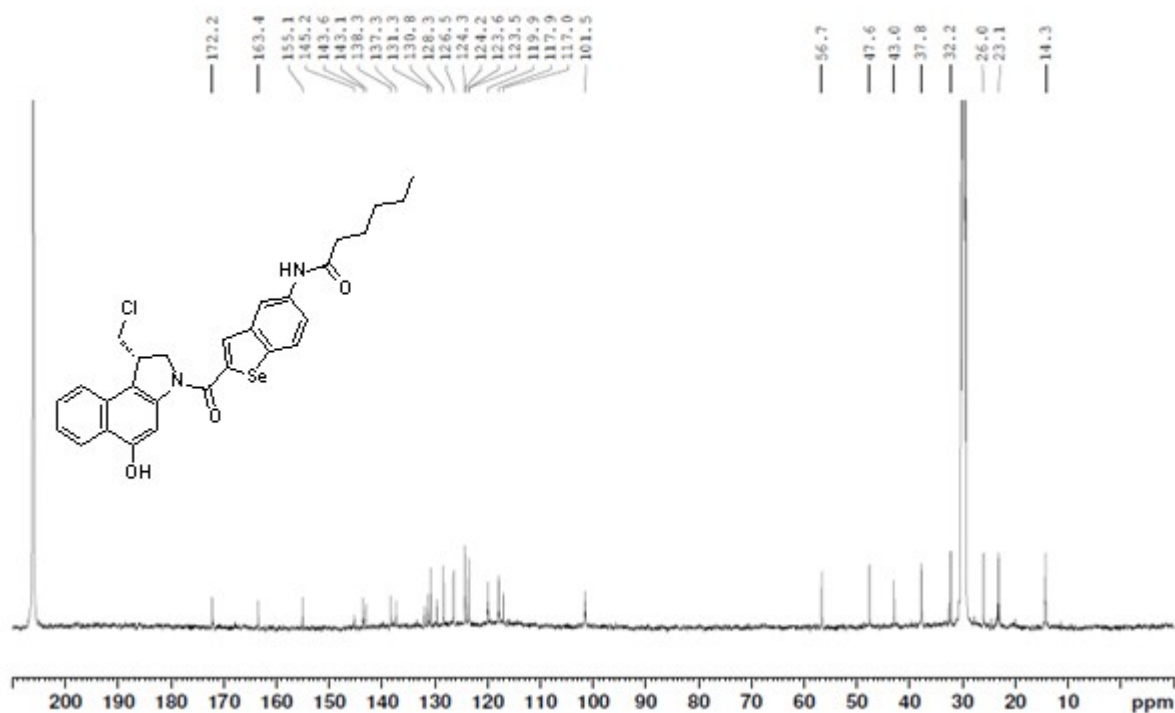
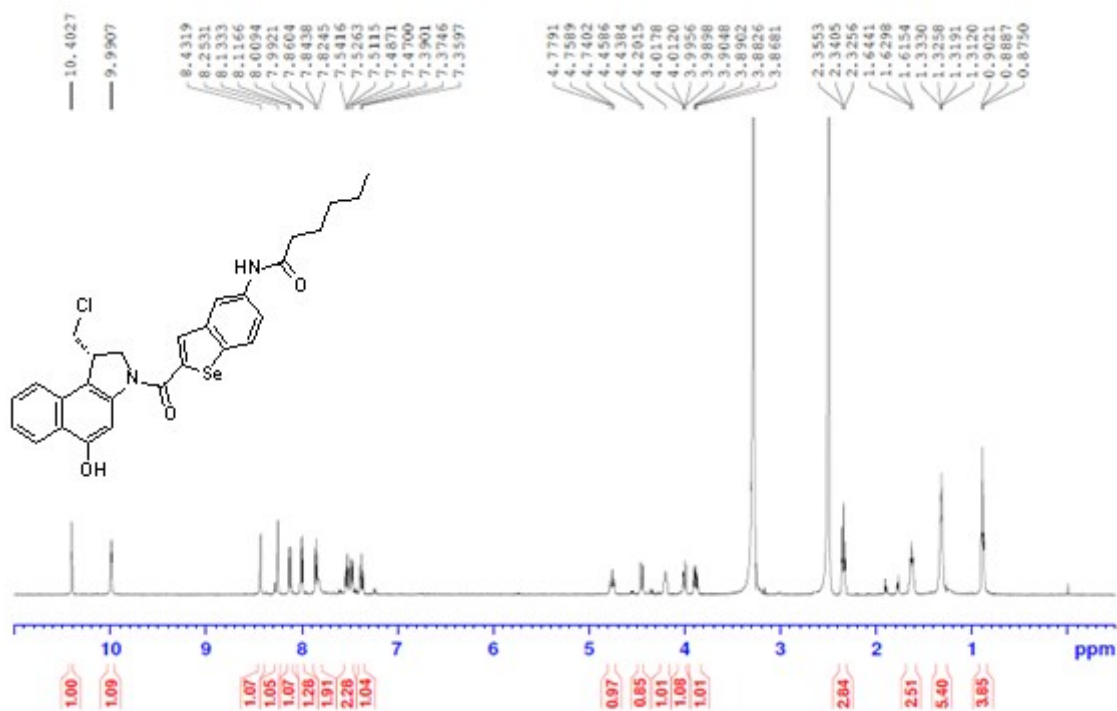
Compound **14f**



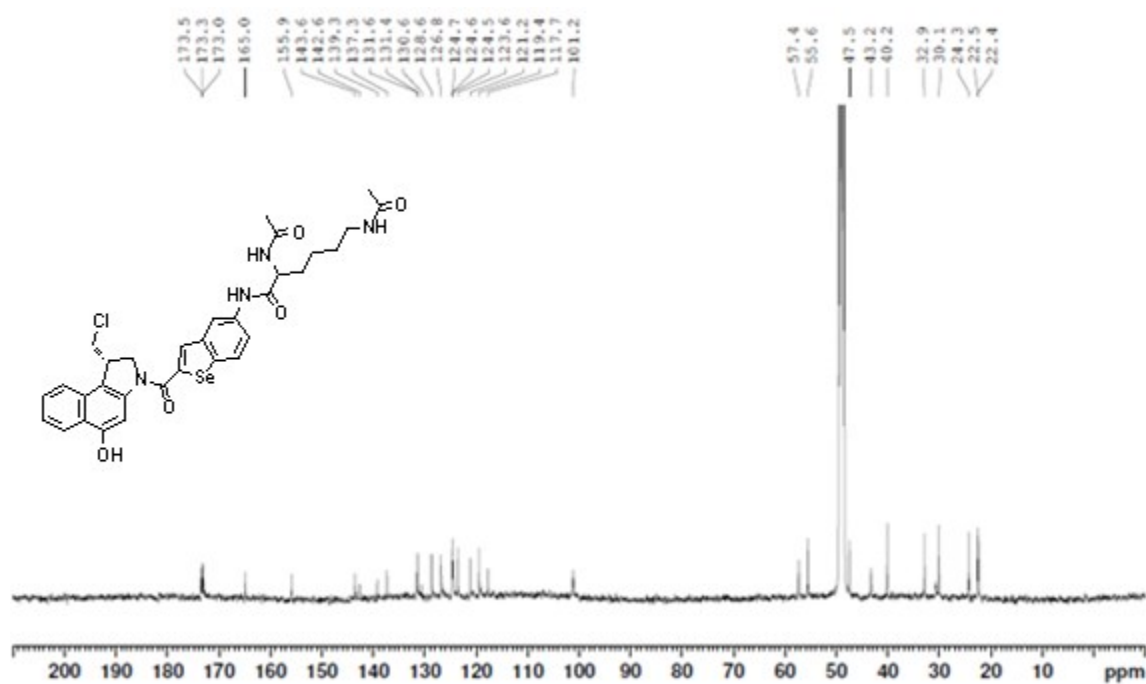
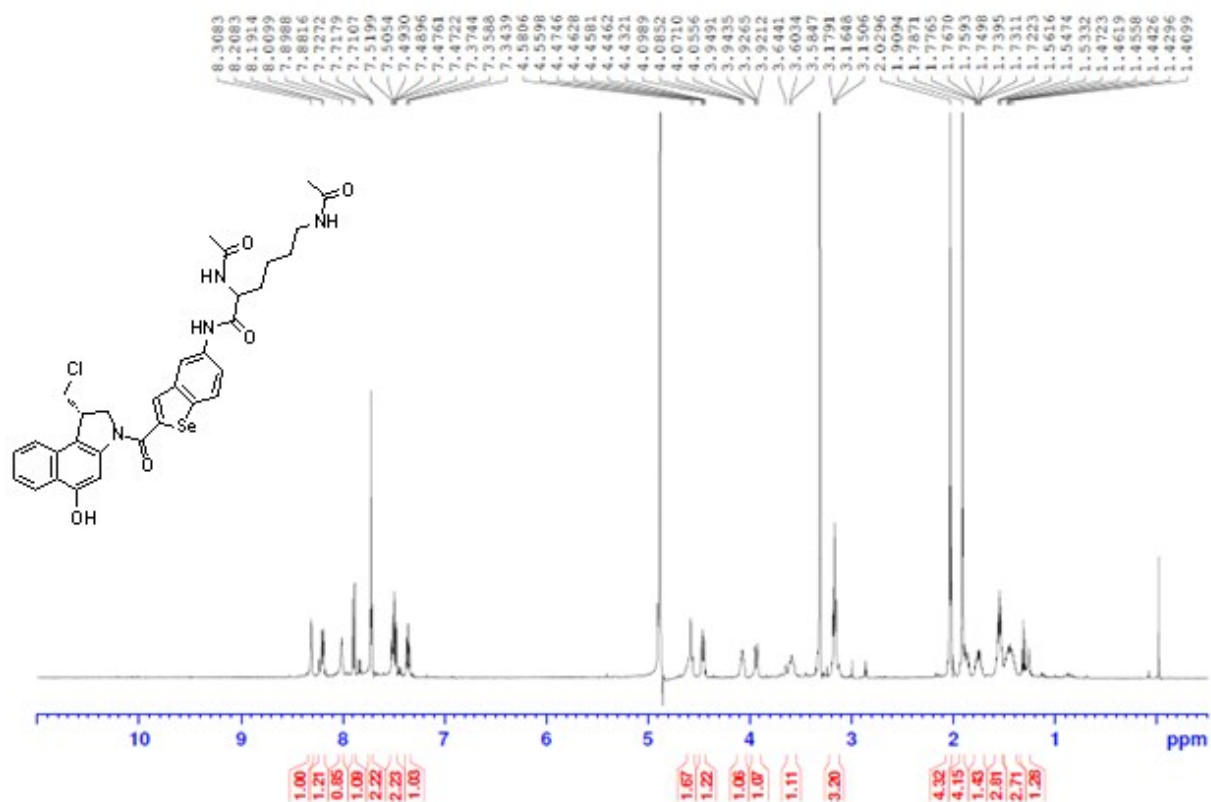
Compound **14g**



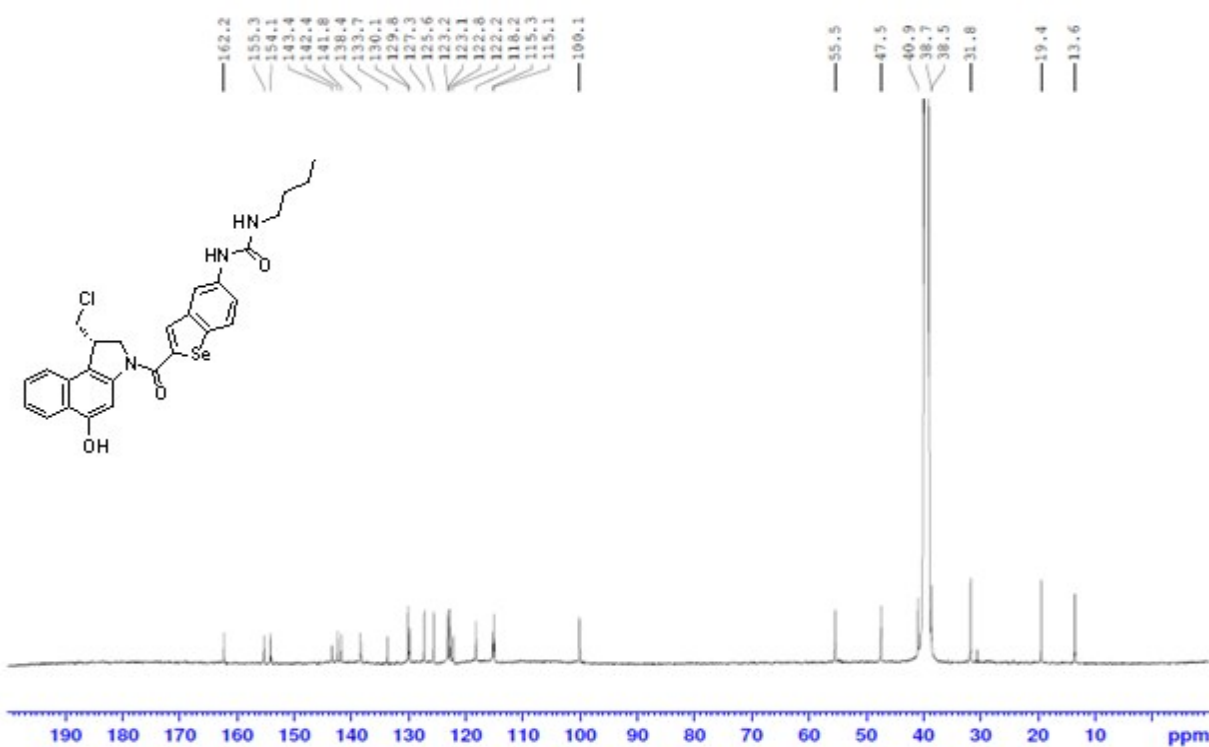
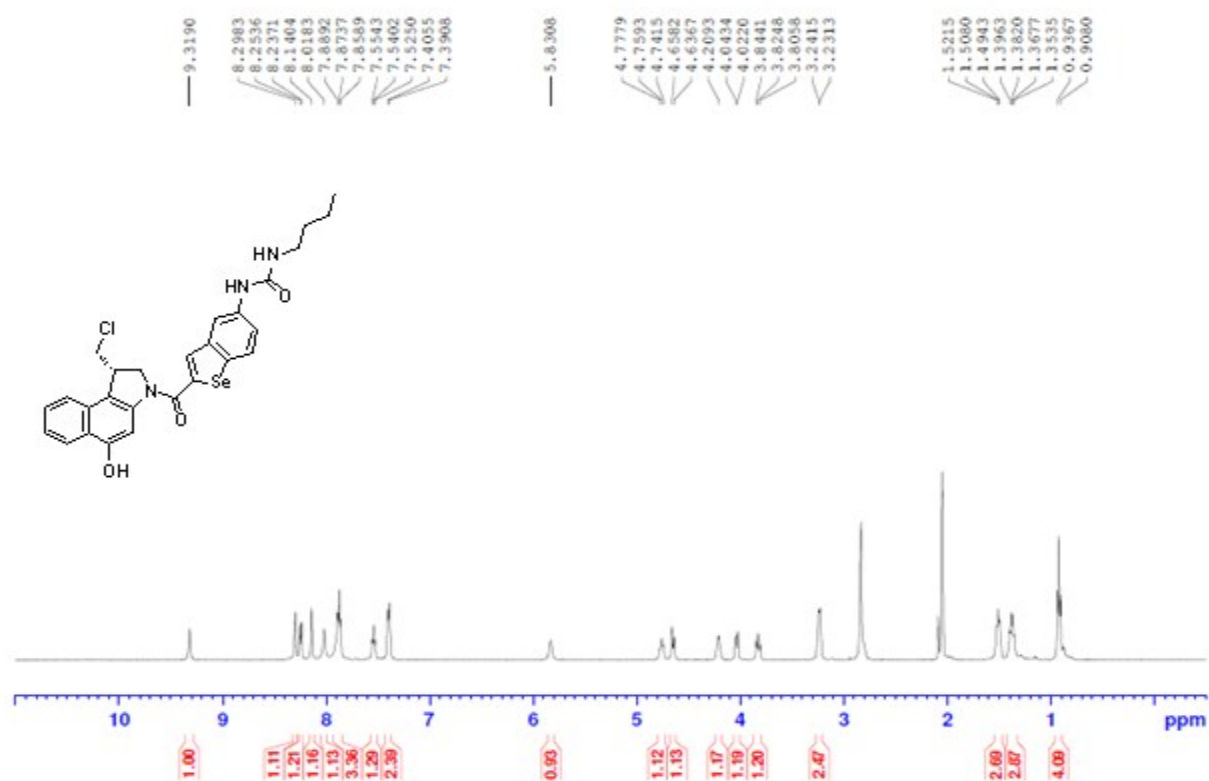
Compound **14h**



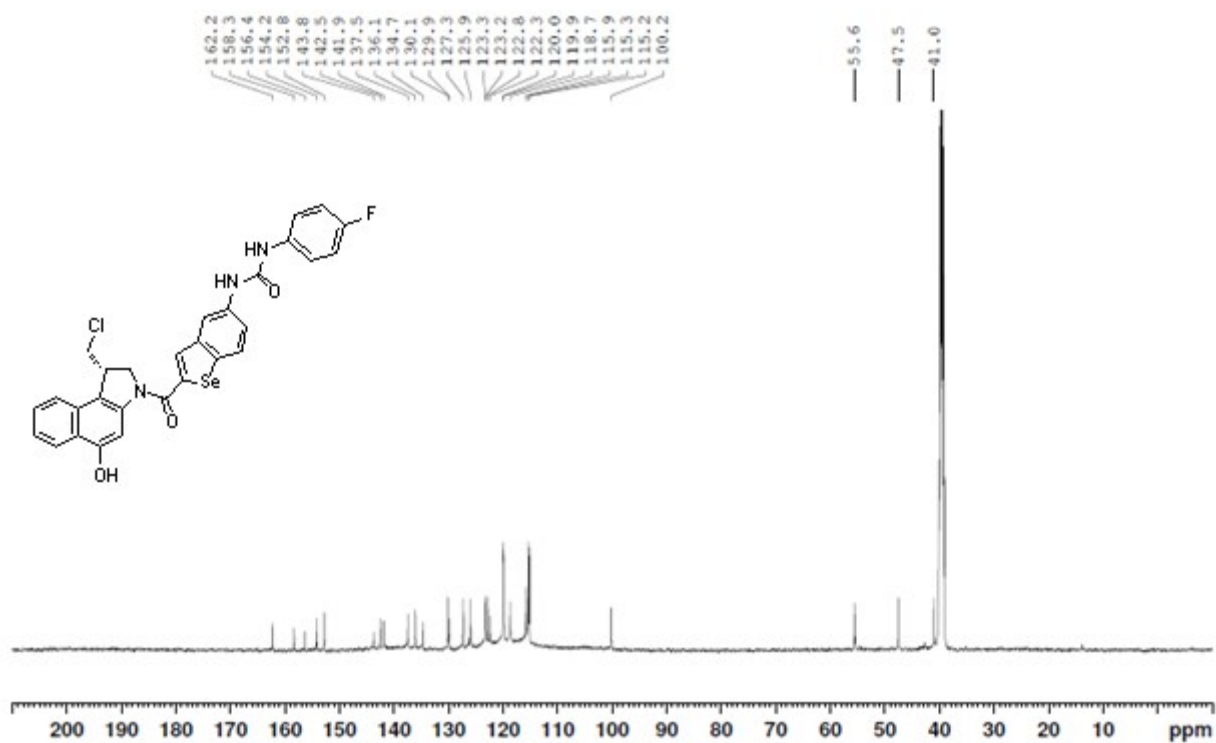
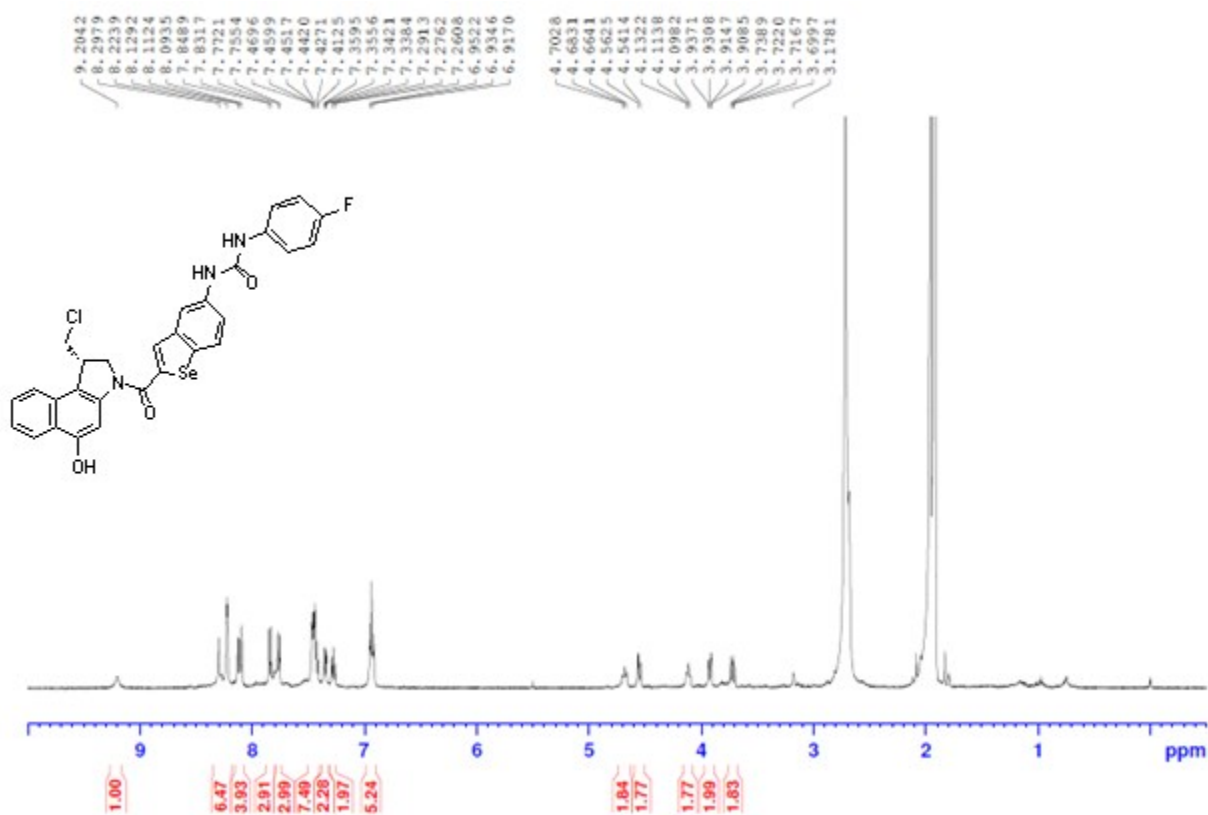
Compound **14i**



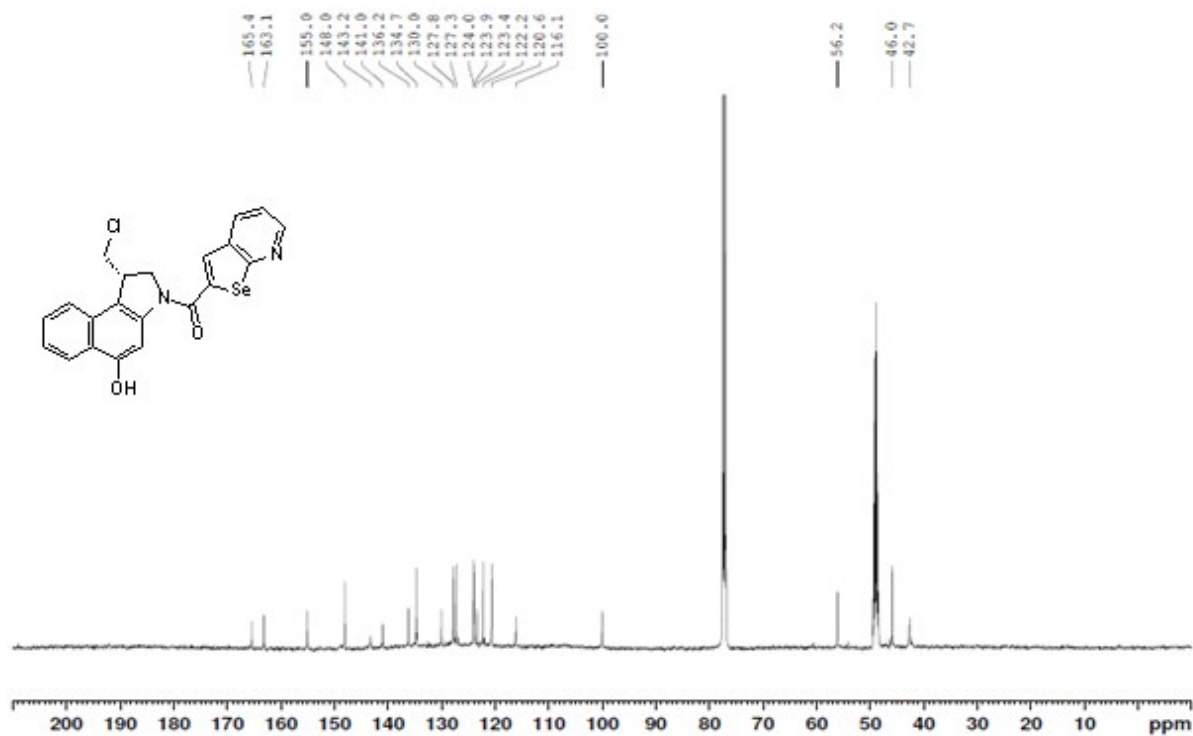
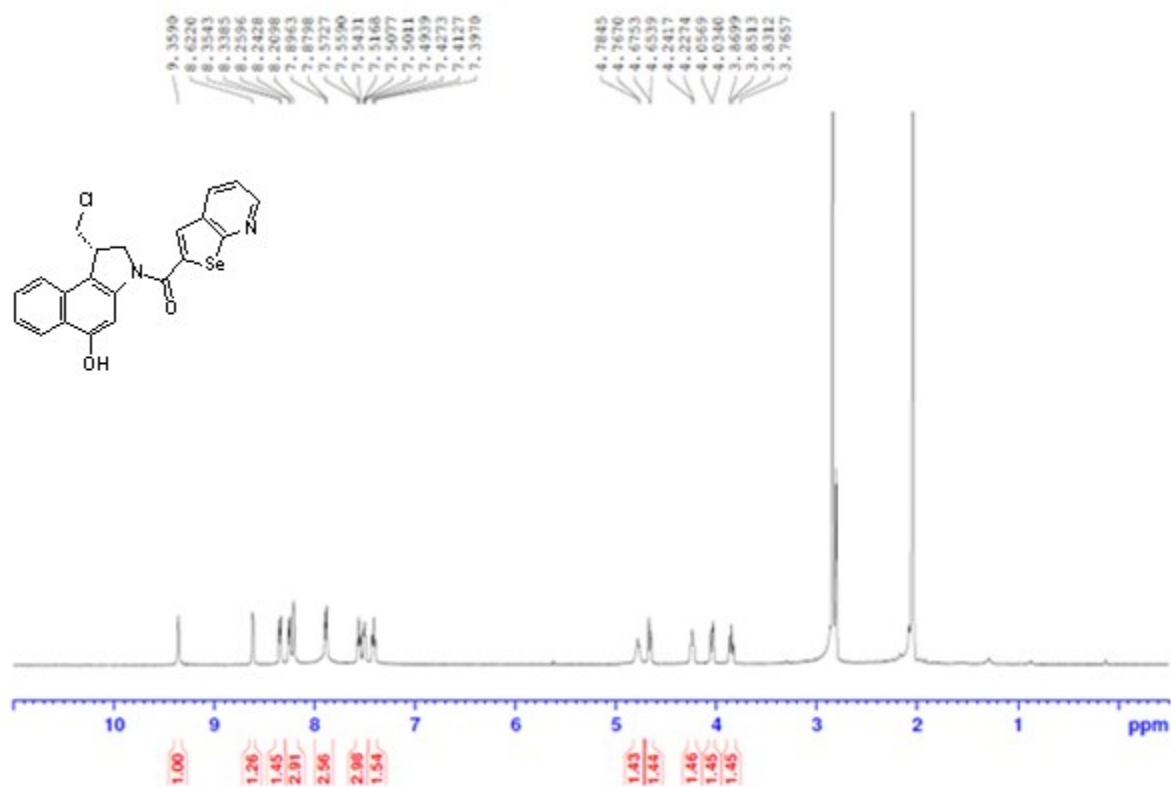
Compound **14j**



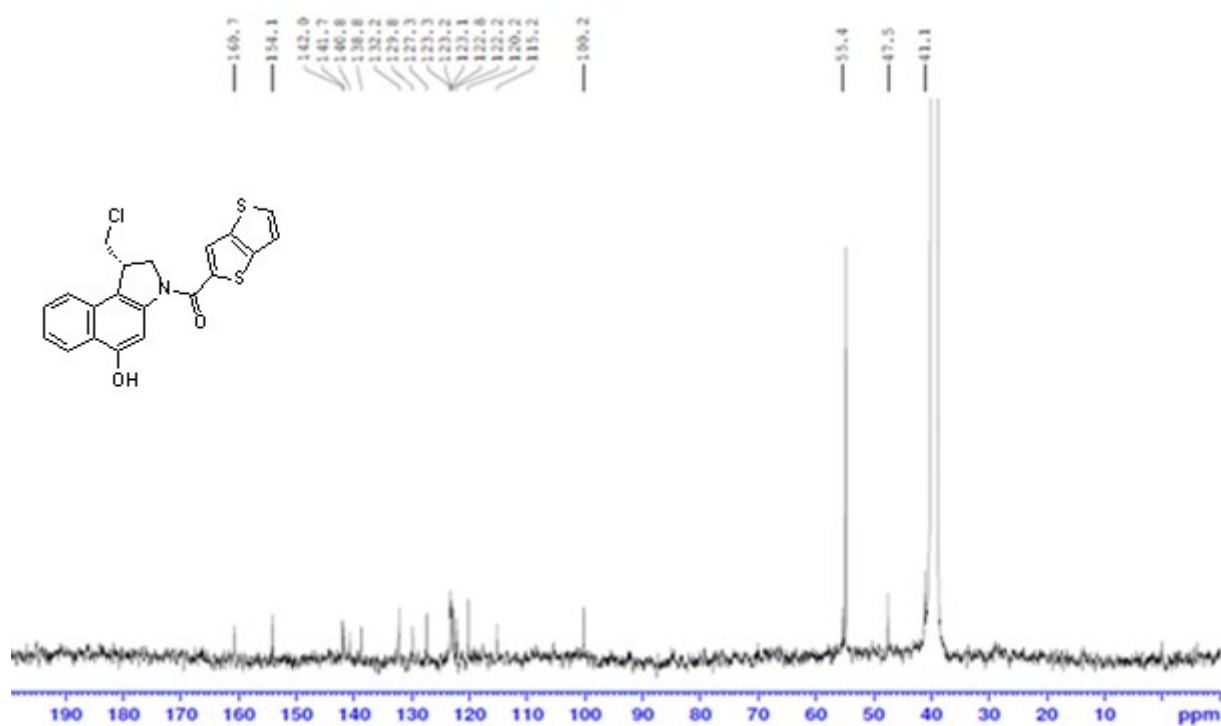
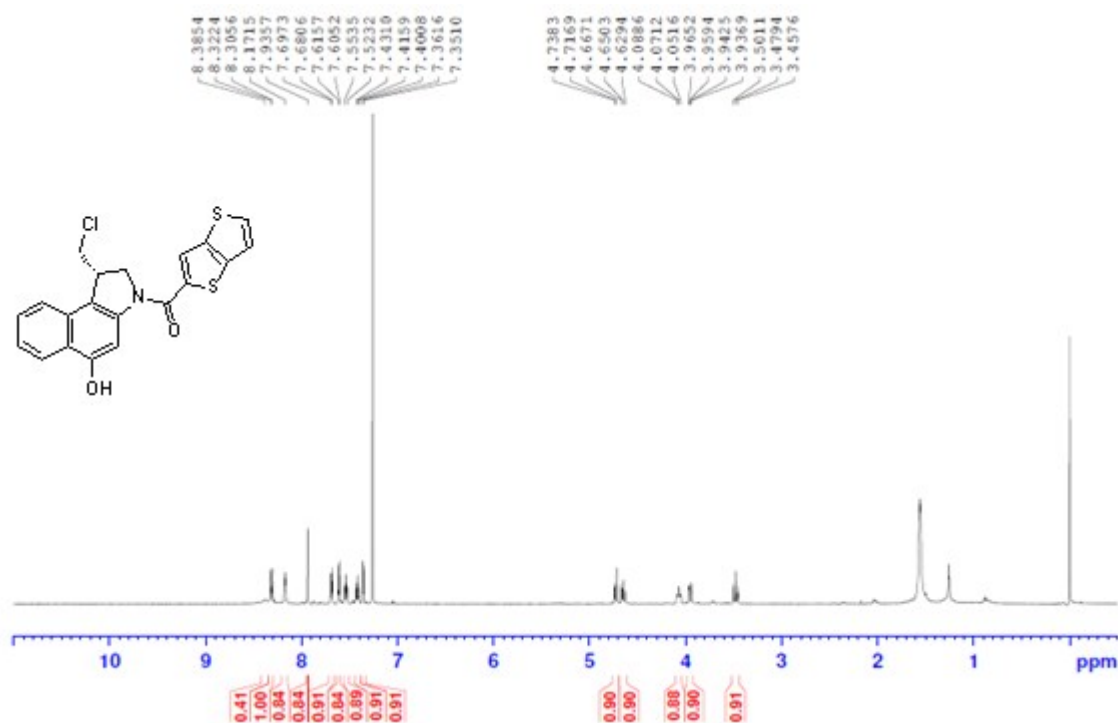
Compound **14k**



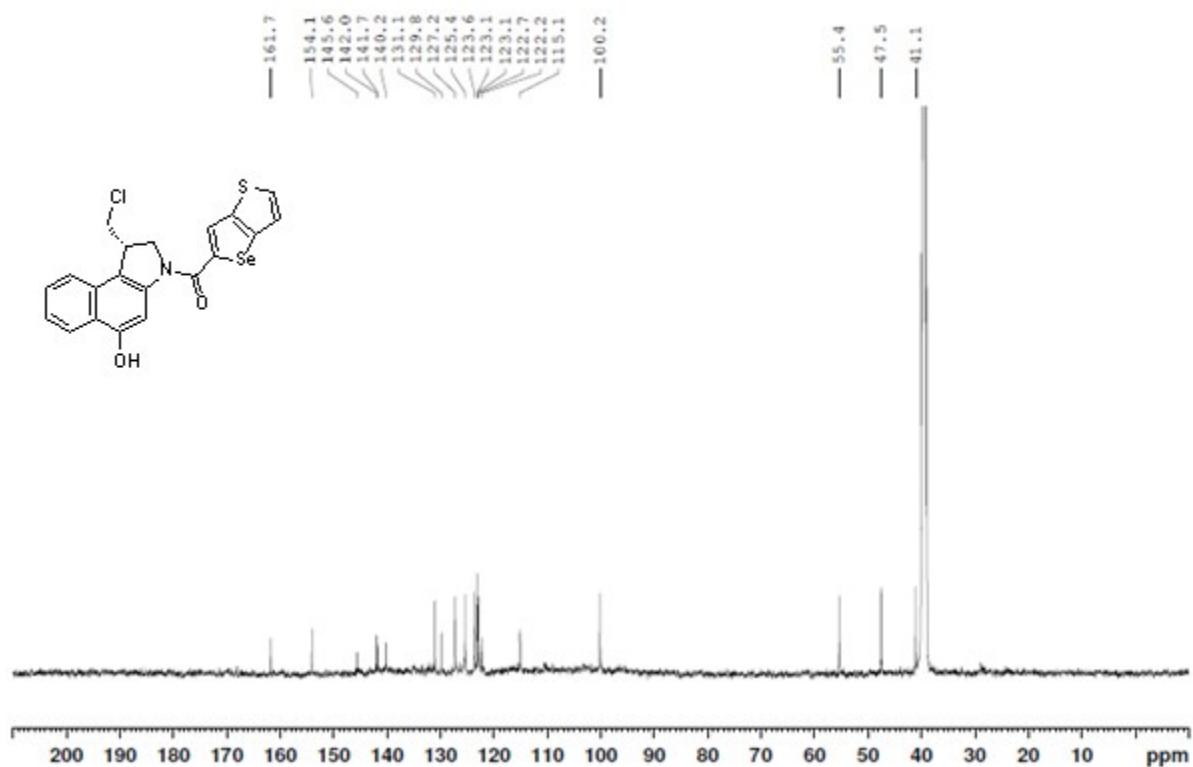
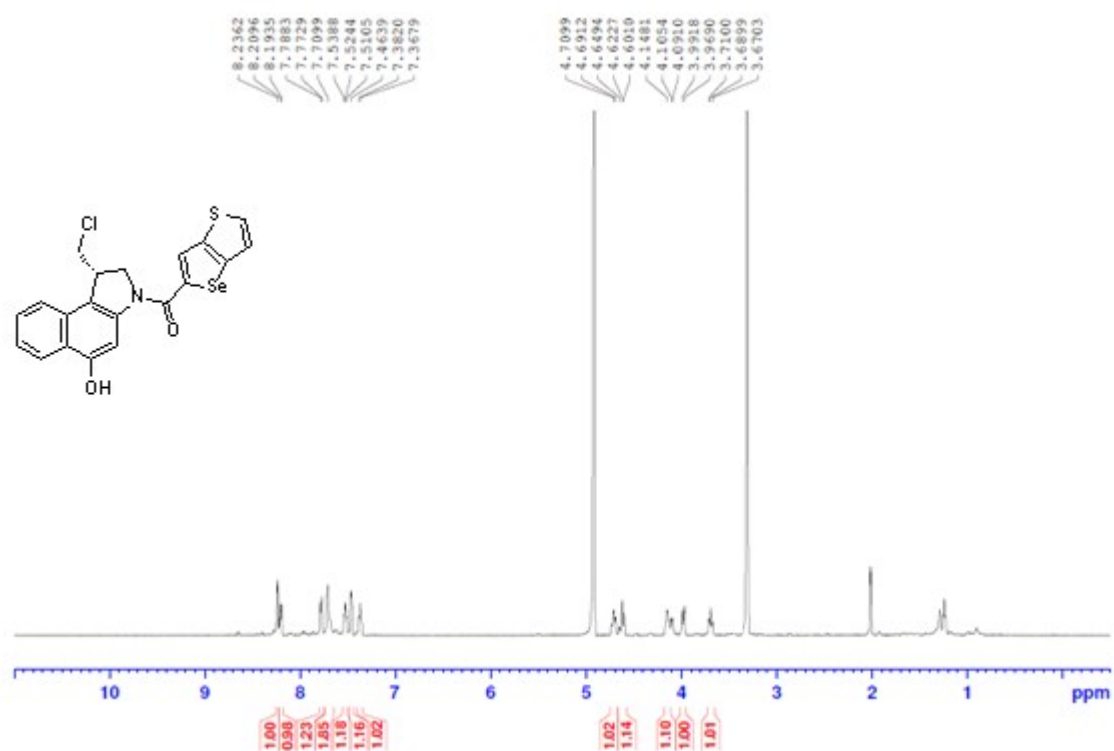
Compound **14l**



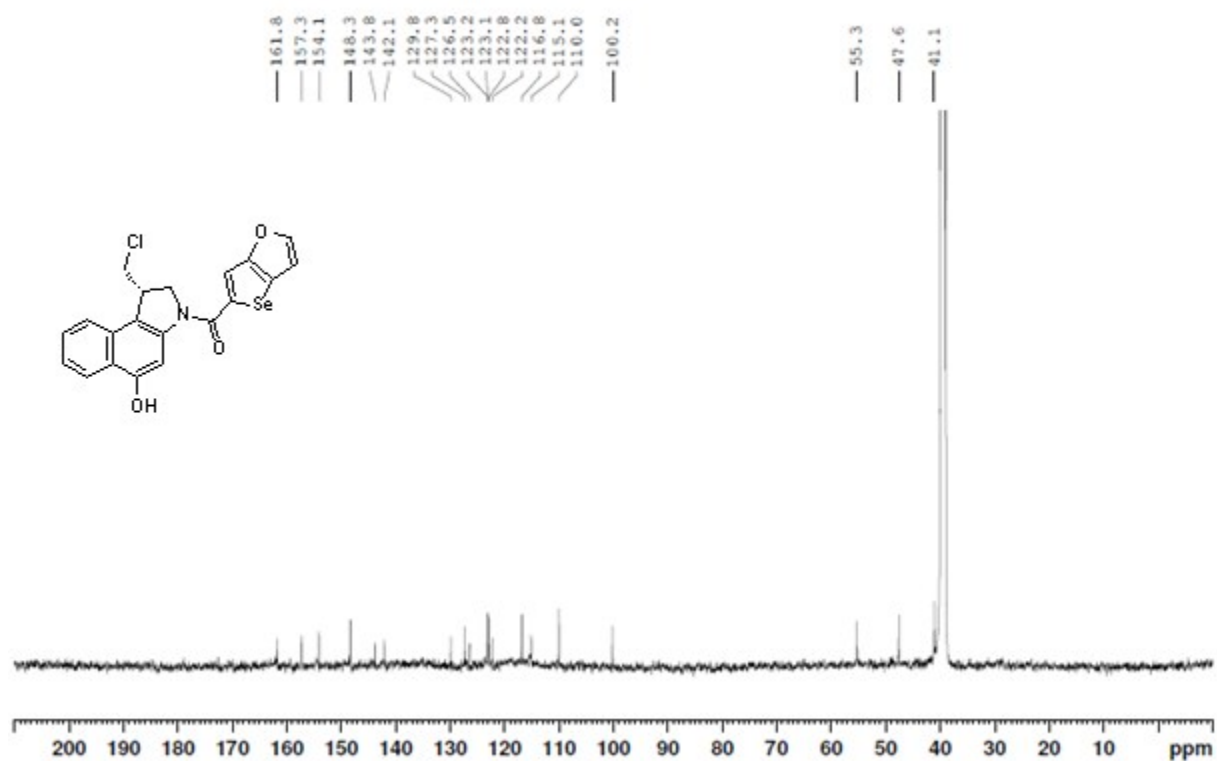
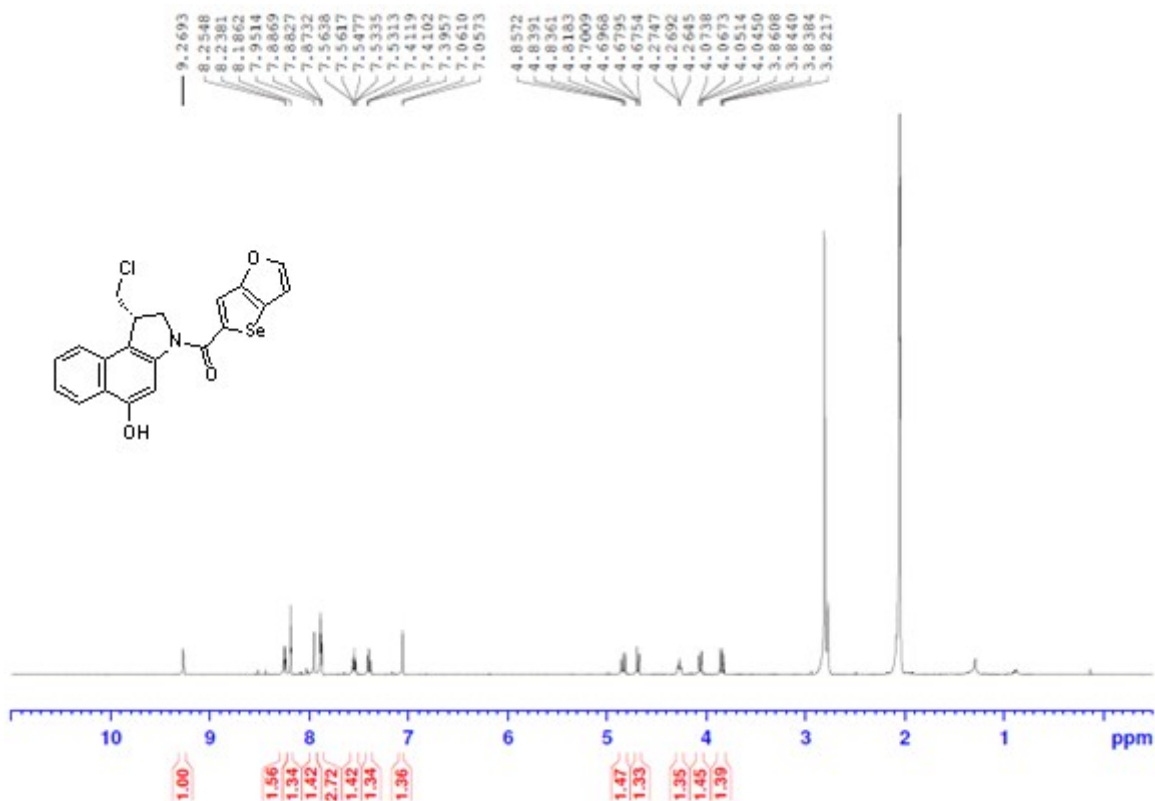
Compound **14m**



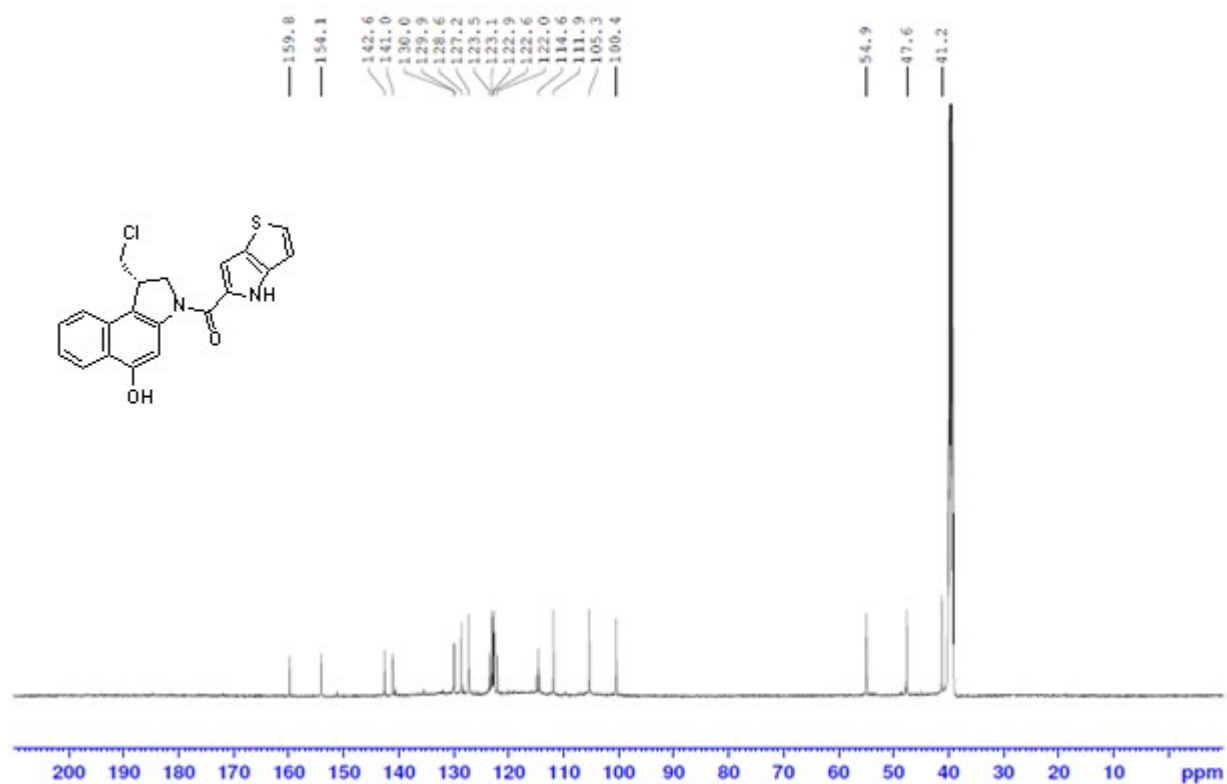
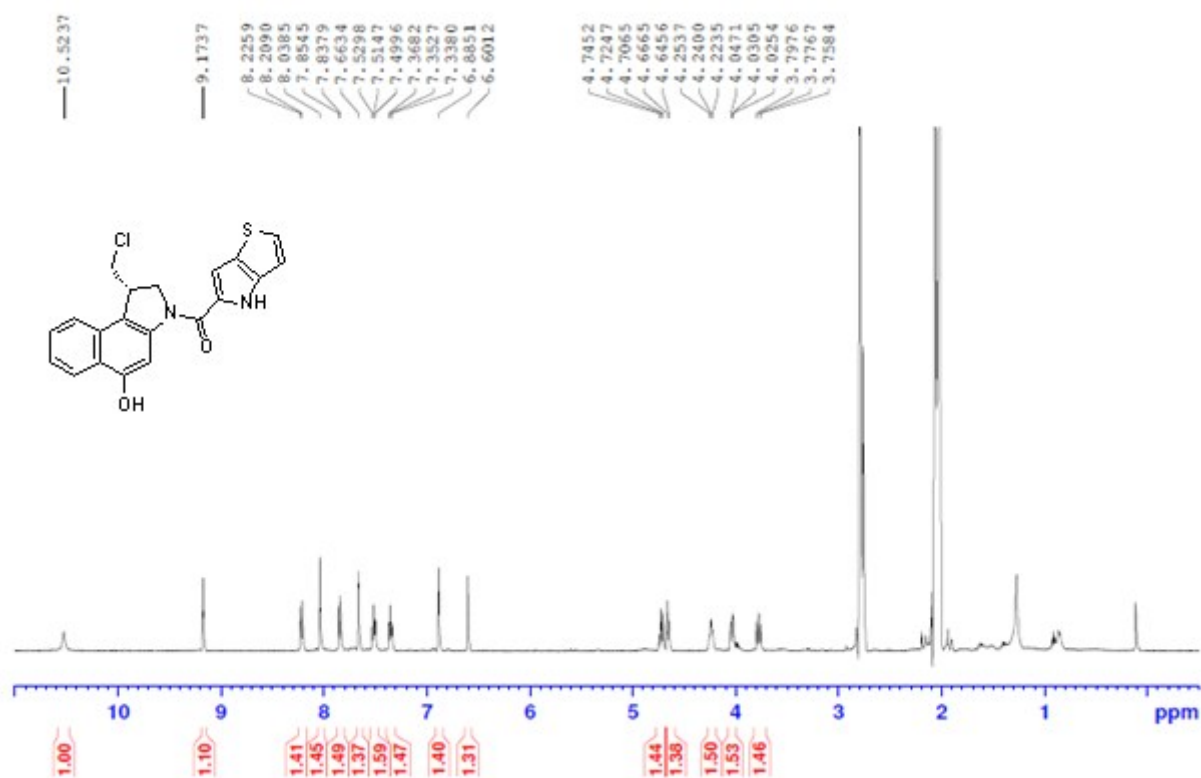
Compound **14n**



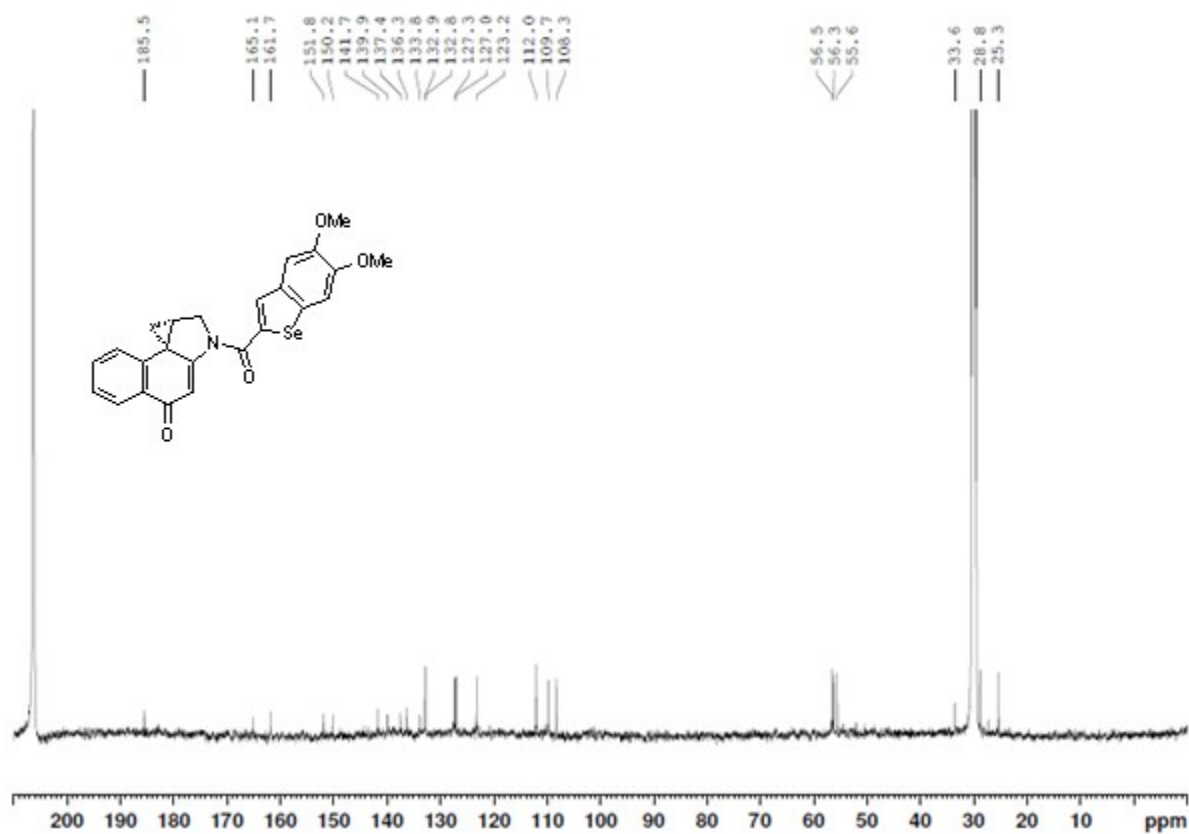
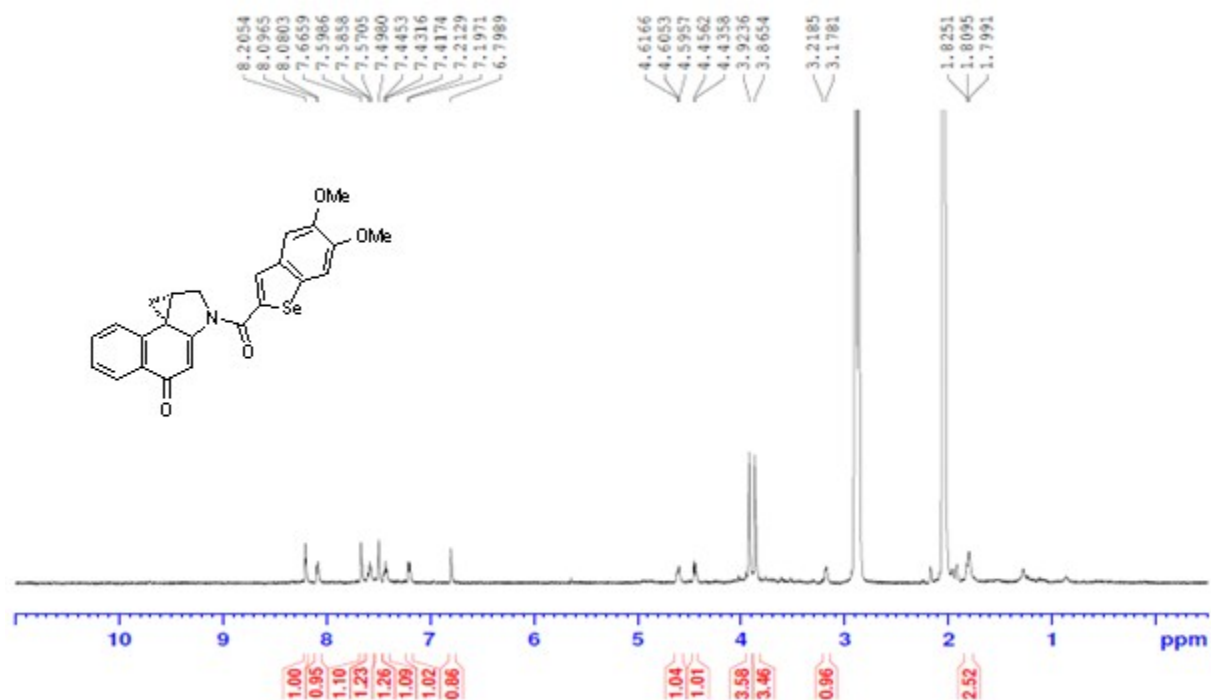
Compound **14o**



Compound **14p**



Compound 15



compound **16**

