Supporting Information for

A Selenolesterase Enzyme Activity of Carbonic Anhydrases

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Abstract: Carbonic Anhydrases (CAs, E.C. 4.2.1.1) are metalloenzymes expressed on a variety of cell types. Their overexpression leads to serious pathologies, including cancer. The discovery of a series of selenolesters with high structural diversity as novel CA inhibitors is reported here. These compounds showed a remarkable *in vitro* inhibition against a panel of human CA isoforms such as hCA I, II, IX and XII. We observed that they undergo a CA mediated hydrolisis, releasing different active selenol fragments, which act as CA inhibitors. Notably, to the best of our knowledge, this is the first example of an enzyme with selenolesterase activity. In addition, X-ray crystallographic data support the proposed mechanism, proving selenolesters as novel pro-drug inhibitors with potential pharmacologic applications.

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Experimental Procedures

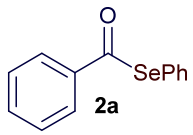
General information

All commercial materials were purchased from Merck - Sigma-Aldrich and used as received, without further purification. Solvents were dried using a solvent purification system (Pure-Solv™). Flash column chromatography purifications were performed with Silica gel 60 (230-400 mesh). Thin layer chromatography was performed with TLC plates Silica gel 60 F_{254}, which was visualised under UV light, or by staining with an ethanolic acid solution of *p*-anisaldehyde followed by heating. High resolution mass spectra (HRMS) were recorded by Electrospray Ionization (ESI). ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using Varian Mercury 400 and Bruker 400 Ultrashield spectrometers operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. ^{77}Se NMR spectra were recorded using a Bruker 400 Ultrashield spectrometer, operating at 76 MHz. NMR signals were referenced to nondeuterated residual solvent signals (7.26 ppm for ^{1}H, 77.0 ppm for ^{13}C). Diphenyl diselenide $(PhSe)_2$ was used as an external reference for ^{77}Se NMR (δ = 461 ppm). Chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are given in Hertz (Hz), rounded to the nearest 0.1 Hz. ^{1}H NMR data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, ap d = apparent doublet, m = multiplet, dd = doublet of doublet, bs = broad singlet, bd = broad doublet, ecc.), coupling constant (J) or line separation (ls), and assignment. Aliphatic[1] and aromatic[2] selenols were synthesised following reported procedures.

General procedure for the synthesis of selenolesters 2a-t

A solution of selenol **1a-h** (0.5 mmol, 1.0 eq.) in dry CH_2Cl_2 (2 mL) was cooled at 0 °C under inert atmosphere (N_2) and treated with Et_3N (0.6 mmol, 1.2 eq.). The mixture was stirred for 5 minutes and then a solution of acyl chloride (0.6 mmol, 1.2 eq.) in dry CH_2Cl_2 (1 mL) was slowly added. The reaction was allowed to warm to room temperature and stirred for additional 2h. Afterwards, a saturated solution of *aq*. NH_4Cl was added and the organic phase was extracted with Et_2O (3x10 mL), washed with brine (2x5 mL) and dried over Na_2SO_4. The solvent was removed under vacuum and the crude material was purified by flash column chromatography (silica gel) to afford pure selenolesters **2a-t**.

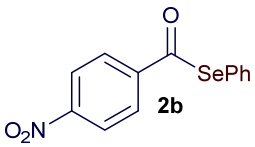
Synthesis of Se-phenyl benzoselenoate **2a**



2a

Following the general procedure, benzeneselenol **1a** (39 mg, 0.25 mmol) and benzoyl chloride (42 mg, 0.30 mmol) gave, after purification by flash column chromatography (petroleum ether : Et_2O 20:1), **2a** as a white solid (59 mg, 91%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 7.43-7.48 (3H, m), 7.50 (2H, ap t, J = 7.4 Hz), 7.61-7.65 (3H, m), 7.95-7.98 (2H, m). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 125.7, 127.3, 128.9, 129.0, 129.3, 133.8, 136.3, 138.5, 193.3. **^{77}Se NMR** ($CDCl_3$, 76 MHz) δ (ppm): 639.4. **MS** (ESI positive) *m/z*: 263 $[M+H]^+$. Spectroscopic data matched those previously reported in the literature.[3,4]

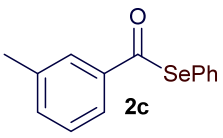
Synthesis of Se-phenyl 4-nitrobenzoselenoate **2b**



2b

Following the general procedure, benzeneselenol **1a** (39 mg, 0.25 mmol) and 4-nitrobenzoyl chloride (56 mg, 0.30 mmol) gave, after purification by flash column chromatography (petroleum ether : Et_2O 10:1), **2b** as a yellow solid (66 mg, 86%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 7.43-7.48 (3H, m), 7.58-7.61 (2H, m), 8.08 (2H, d, J = 8.7 Hz), 8.34 (2H, d, J = 8.7 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm) 124.2, 125.0, 128.1, 129.56, 129.62, 136.1, 143.0, 150.7, 192.5. **^{77}Se NMR** ($CDCl_3$, 76 MHz) δ (ppm): 656.8. **MS** (ESI positive) *m/z*: 330 $[M+Na]^+$. Spectroscopic data matched those previously reported in the literature.[4]

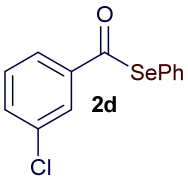
Synthesis of Se-phenyl 3-methylbenzoselenoate **2c**



2c

Following the general procedure, benzeneselenol **1a** (39 mg, 0.25 mmol) and 3-methylbenzoyl chloride (47 mg, 0.30 mmol) gave, after purification by flash column chromatography (petroleum ether : Et_2O 20:1), **2c** as a colourless glassy solid (61 mg, 88%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 2.45 (3H, s), 7.38 (1H, t, J = 7.5 Hz), 7.43-7.46 (4H, m), 7.61-7.64 (2H, m), 7.76-7.78 (2H, m). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm) 21.3, 124.5, 125.9, 127.7, 128.8, 128.9, 129.3, 134.6, 136.3, 138.5, 138.8, 193.3. **^{77}Se NMR** ($CDCl_3$, 76 MHz) δ (ppm): 638.7. **MS** (ESI positive) *m/z*: 277 $[M+H]^+$. Spectroscopic data matched those previously reported in the literature.[5]

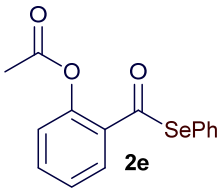
Synthesis of Se-phenyl 3-chlorobenzoselenoate **2d**



2d

Following the general procedure, benzeneselenol **1a** (39 mg, 0.25 mmol) and 3-chlorobenzoyl chloride (53 mg, 0.30 mmol) gave, after purification by flash column chromatography (petroleum ether : Et_2O 25:1), **2d** as a colourless glassy solid (56 mg, 76%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 7.42-7.45 (4H, m), 7.58-7.60 (3H, m), 7.82 (1H, ap d, J = 7.8 Hz), 7.90 (bs, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm) 125.38, 125.42, 127.1, 129.3, 129.5, 130.2, 133.7, 135.2, 136.2, 140.1, 192.3. **^{77}Se NMR** ($CDCl_3$, 76 MHz) δ (ppm): 645.2. **MS** (ESI positive) *m/z*: 319 $[M+Na]^+$. Spectroscopic data matched those previously reported in the literature.[5]

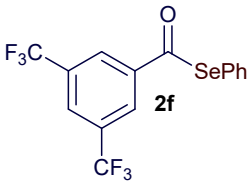
Synthesis of 2-((phenylselanyl)carbonyl)phenyl acetate **2e**[6]



2e

Following the general procedure, benzeneselenol **1a** (39 mg, 0.25 mmol) and 2-(chlorocarbonyl)phenyl acetate (60 mg, 0.30 mmol) gave, after purification by flash column chromatography (petroleum ether : Et_2O 5:1), **2e** as a white solid (67 mg, 84%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 2.34 (3H, s), 7.16 (1H, d, J = 8.1 Hz), 7.37 (1H, t, J = 7.8 Hz), 7.40-7.44 (3H, m), 7.56-7.61 (3H, m), 7.97 (1H, d, J = 7.8 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm) 21.3, 124.1, 126.3, 129.1, 129.4, 129.7, 131.4, 134.0, 136.2, 147.4, 169.2, 190.7. **^{77}Se NMR** ($CDCl_3$, 76 MHz) δ (ppm): 676.8. **HRMS** (ESI) calc. $C_{15}H_{12}NaO_3Se$ $[M+Na]^+$ 342.9849, found 342.9865.

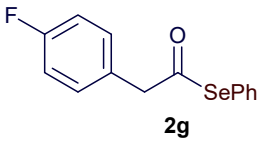
Synthesis of Se-phenyl 3,5-bis(trifluoromethyl)benzoselenoate **2f**



2f

Following the general procedure, benzeneselenol **1a** (39 mg, 0.25 mmol) and 3,5-bis(trifluoromethyl)benzoyl chloride (83 mg, 0.30 mmol) gave, after purification by flash column chromatography (petroleum ether : Et_2O 50:1, then petroleum ether : Et_2O 1:1), **2f** as a yellowish oil (80 mg, 81%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 7.44-7.50 (3H, m), 7.59-7.61 (2H, m), 8.13 (1H, s), 8.35 (2H, s). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm) 122.7 (q, $^1J_{C\text{-}F}$ = 273.2 Hz), 124.6, 126.9 (hept, $^3J_{C\text{-}F}$ = 3.6 Hz), 127.1 (q, $^3J_{C\text{-}F}$ = 3.3 Hz), 132.7 (q, $^2J_{C\text{-}F}$ = 34.2 Hz), 136.1, 140.2, 191.6. **^{19}F NMR** ($CDCl_3$, 376 MHz) δ (ppm): -63.0. **^{77}Se NMR** ($CDCl_3$, 76 MHz) δ (ppm): 651.6. **HRMS** (ESI) calc. $C_{15}H_8F_6NaOSe$ $[M+Na]^+$ 420.9542, found 420.9563.

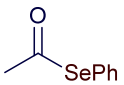
Synthesis of Se-phenyl 2-(4-fluorophenyl)ethaneselenoate **2g**



2g

Following the general procedure, benzeneselenol **1a** (39 mg, 0.25 mmol) and 2-(4-fluorophenyl)acetyl chloride (52 mg, 0.30 mmol) gave, after purification by flash column chromatography (petroleum ether : Et_2O 30:1), **2g** as a white glassy solid (56 mg, 76%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 3.90 (2H. s), 7.06 (2H, ap t, J = 8.7 Hz), 7.29 (2H, ap dd, J = 5.3, 8.3 Hz), 7.35-7.39 (3H, m), 7.46-7.48 (2H, m). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 52.7, 115.7 (d, $^2J_{C\text{-}F}$ = 21.6 Hz), 126.4, 128.4 (d, $^4J_{C\text{-}F}$ = 3.3 Hz), 129.0, 129.3, 131.6 (d, $^3J_{C\text{-}F}$ = 8.2 Hz), 135.7, 162.5 (d, $^1J_{C\text{-}F}$ = 246.8 Hz), 198.6. **^{19}F NMR** ($CDCl_3$, 376 MHz) δ (ppm): -114.3. **^{77}Se NMR** ($CDCl_3$, 76 MHz) δ (ppm): 666.6. **HRMS** (ESI) calc. $C_{14}H_{11}FNaOSe$ $[M+Na]^+$ 316.9857, found 316.9848.

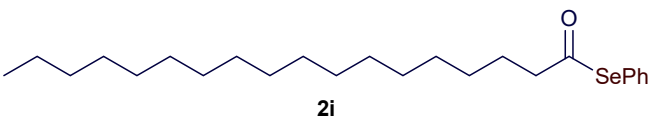
Synthesis of Se-phenyl ethaneselenoate **2h**



2h

Following the general procedure, benzeneselenol **1a** (39 mg, 0.25 mmol) and acetyl chloride (23 mg, 0.30 mmol) gave, after purification by flash column chromatography (petroleum ether : Et_2O 20:1), **2h** as a yellowish oil (36 mg, 72%). **^{1}H NMR** ($CDCl_3$, 200 MHz) δ (ppm): 2.47 (3H, s), 7.37-7.42 (3H, m), 7.50-7.55 (2H, m). **^{13}C NMR** ($CDCl_3$, 50 MHz) δ (ppm): 34.0, 126.8, 128.9, 129.3, 135.7, 196.6. **MS** (ESI positive) *m/z*: 201 $[M+H]^+$. Spectroscopic data matched those previously reported in the literature.[3,4]

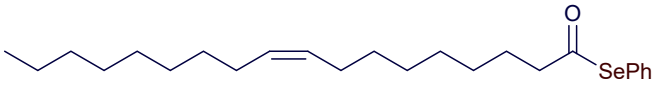
Synthesis of Se-phenyl octadecaneselenoate **2i**[7]



2i

Following the general procedure, benzeneselenol **1a** (39 mg, 0.25 mmol) and stearoyl chloride (91 mg, 0.30 mmol) gave, after purification by flash column chromatography (petroleum ether, then petroleum ether : Et_2O 1:1), **2i** as a white glassy solid (90 mg, 85%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 0.88 (3H, t, J = 6.8 Hz), 1.26-1.30 (28 H, m), 1.64-1.73 (2H, m), 2.70 (2H, t, J = 7.5 Hz), 7.37-7-39 (3H, m), 7.49-7.52 (2H, m). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 14.1, 22.7, 25.4, 28.8, 29.2, 29.36, 29.38, 29.56, 29.62, 29.66, 29.7, 31.9, 47.6, 126.6, 128.8, 129.3, 135.8, 200.4. **HRMS** (ESI) calc. $C_{24}H_{41}OSe$ $[M+H]^+$ 425.2323, found 425.2335.

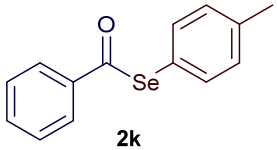
Synthesis of Se-phenyl (Z)-octadec-9-eneselenoate **2j**



2j

Following the general procedure, benzeneselenol **1a** (39 mg, 0.25 mmol) and oleoyl chloride (90 mg, 0.30 mmol) gave, after purification by flash column chromatography (petroleum ether, then petroleum ether : Et_2O 3:1), **2j** as a colourless oil (82 mg, 78%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 0.89 (3H, t, J = 6.4 Hz), 1.27-1.35 (20H, m), 1.64-1.74 (2H, m), 2.00-2.04 (4H, m), 2.70 (2H, t, J = 7.5 Hz), 5.31-5.40 (2H, m), 7.35-7.40 (3H, m), 7.49-7.53 (2H, m). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 14.1, 22.7, 25.4, 27.1, 27.2, 28.8, 29.0, 29.1, 29.3, 29.5, 29.6, 29.8, 31.9, 47.5, 126.5, 128.8, 129.3, 129.7, 130.0, 135.8, 200.4. **^{77}Se NMR** ($CDCl_3$, 76 MHz) δ (ppm): 656.5. **HRMS** (ESI) calc. $C_{24}H_{38}NaOSe$ [M+Na]$^+$ 445.1986, found 445.2003.

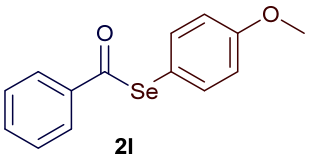
Synthesis of Se-(p-tolyl) benzoselenoate **2k**



2k

Following the general procedure, 4-methylbenzeneselenol **1b** (34 mg, 0.20 mmol) and benzoyl chloride (34 mg, 0.24 mmol) gave, after purification by flash column chromatography (petroleum ether : Et_2O 25:1), **2k** as a yellowish glassy solid (46 mg, 83%). Spectroscopic data matched those previously reported in the literature.[3]

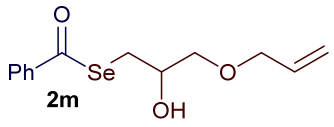
Synthesis of Se-(4-methoxyphenyl) benzoselenoate **2l**



2l

Following the general procedure, 4-methoxybenzeneselenol **1c** (37 mg, 0.20 mmol) and benzoyl chloride (34 mg, 0.24 mmol) gave, after purification by flash column chromatography (petroleum ether : Et_2O 3:1), **2l** as a yellowish glassy solid (34 mg, 75%). Spectroscopic data matched those previously reported in the literature.[8]

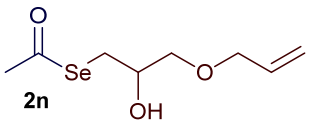
Synthesis of Se-(3-(allyloxy)-2-hydroxypropyl) benzoselenoate **2m**



2m

Following the general procedure, 1-(allyloxy)-3-hydroselenopropan-2-ol **1d** (39 mg, 0.20 mmol) and benzoyl chloride (22 mg, 0.16 mmol, 0.8 eq.) gave, after purification by flash column chromatography (petroleum ether : Et_2O 6:1), **2m** as a yellowish oil (31 mg, 65%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 2.81 (1H, bs, OH), 3.22 (1H, dd, J = 6.9, 13.0 Hz, C**H$_a$**H$_b$Se), 3.33 (1H, dd, J = 4.9, 13.0 Hz, CH$_a$**H$_b$**Se), 3.48 (1H, dd, J = 6.7, 9.6 Hz, C**H$_a$**H$_b$O), 3.59 (1H, dd, J = 3.9, 9.6 Hz, CH$_a$**H$_b$**O), 3.97-4.11 (3H, m, C**H**OH overlapped with C**H$_2$**CH=CH$_2$), 5.19-5.32 (2H, m), 5.87-5.96 (1H, m), 7.44-7.50 (3H, m), 7.89-7.95 (2H, m). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 28.8, 70.0, 72.3, 73.3, 117.4, 127.3, 128.8, 130.2, 133.8, 134.3, 192.8. **HRMS** (ESI) calc. $C_{13}H_{16}NaO_3Se$ [M+Na]$^+$ 323.0162, found 323.0156.

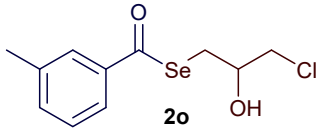
Synthesis of Se-(3-(allyloxy)-2-hydroxypropyl) ethaneselenoate **2n**



2n

Following the general procedure, 1-(allyloxy)-3-hydroselenopropan-2-ol **1d** (59 mg, 0.30 mmol) and benzoyl chloride (34 mg, 0.24 mmol, 0.8 eq.) gave, after purification by flash column chromatography (petroleum ether : Et_2O 2:1), **2n** as a yellowish oil (39 mg, 68%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 2.42 (3H, s), 3.01 (1H, dd, J = 6.8, 13.1 Hz, C**H$_a$**H$_b$Se), 3.13 (1H, dd, J = 5.1, 13.1 Hz, CH$_a$**H$_b$**Se), 3.40 (1H, dd, J = 6.7, 9.6 Hz, C**H$_a$**H$_b$O), 3.52 (1H, dd, J = 3.9, 9.6 Hz, CH$_a$**H$_b$**O), 3.91-3.98 (1H, C**H**OH), 3.97-4.05 (2H, m, C**H$_2$**CH=CH$_2$), 5.16-5.32 (2H, m), 5.80-5.99 (1H, m). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 29.1, 34.6, 69.9, 72.3, 73.3, 117.4, 134.3, 198.0. **^{77}Se NMR** ($CDCl_3$, 76 MHz) δ (ppm): 520.3. **HRMS** (ESI) calc. $C_8H_{14}NaO_3Se$ [M+Na]$^+$ 261.0006, found 260.9995.

Synthesis of Se-(3-chloro-2-hydroxypropyl) 3-methylbenzoselenoate **2o**

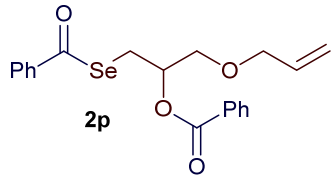


2o

Following the general procedure, 1-chloro-3-hydroselenopropan-2-ol **1e** (43 mg, 0.25 mmol) and 3-methylbenzoyl chloride (31 mg, 0.20 mmol, 0.8 eq.) gave, after purification by flash column chromatography (petroleum ether : Et_2O 3:1), **2o** as a yellowish oil (42 mg, 73%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 2.43 (3H, s), 2.74 (1H, bs, OH), 3.28 (1H, dd, J = 6.8, 13.2 Hz, C**H$_a$**H$_b$Se), 3.37 (1H, dd, J = 4.8, 13.2 Hz, CH$_a$**H$_b$**Se), 3.65 (1H, dd, J = 6.2, 11.2 Hz, C**H$_a$**H$_b$Cl), 3.72 (1H, dd, J = 4.5, 11.2 Hz, CH$_a$**H$_b$**Cl), 4.06-

4.15 (1H, m, CHOH), 7.35 (1H, ap t, *J* = 7.9 Hz), 7.43 (1H, ap d, *J* = 7.9 Hz), 7.69-7.74 (2H, m). **^{13}C NMR** ($CDCl_3$, 100 MHz) *δ* (ppm): 21.3, 29.2, 48.8, 71.0, 124.7, 127.8, 128.8, 134.9, 138.4, 138.9, 195.0. **HRMS** (ESI) calc. $C_{11}H_{13}ClNaO_2Se$ $[M+Na]^+$ 314.9667, found 314.9671.

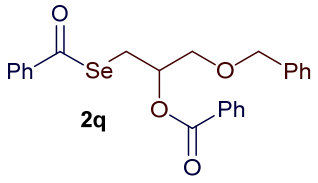
Synthesis of 1-(allyloxy)-3-(benzoylselanyl)propan-2-yl benzoate **2p**



2p

Following the general procedure, 1-(allyloxy)-3-hydroselenopropan-2-ol **1d** (39 mg, 0.20 mmol) and benzoyl chloride (70 mg, 0.50 mmol, 2.5 eq.) gave, after purification by flash column chromatography (petroleum ether : Et_2O 8:1), **2p** as a pale yellowish oil (45 mg, 56%). **^{1}H NMR** ($CDCl_3$, 400 MHz) *δ* (ppm): 3.49 (1H, dd, *J* = 6.7, 13.0 Hz, C**H$_a$**H$_b$Se), 3.57 (1H, dd, *J* = 5.5, 13.0 Hz, CH$_a$**H$_b$**Se), 3.73 (1H, dd, *J* = 4.6, 10.7 Hz, C**H$_a$**H$_b$O), 3.80 (1H, dd, *J* = 5.3, 10.7 Hz, CH$_a$**H$_b$**O), 4.01-4.10 (2H, m, C**H$_2$**CH=CH$_2$), 5.17-5.31 (2H, m), 5.44-5.50 (1H, m, C**H**O), 5.85-5.94 (1H, m), 7.41-7.46 (4H, m), 7.53-7.61 (2H, m), 7.87-7.89 (2H, m), 8.03-8.05 (2H, m). **^{13}C NMR** ($CDCl_3$, 100 MHz) *δ* (ppm): 26.5, 71.2, 72.9, 73.0, 117.9, 127.9, 128.9, 129.4, 130.4, 130.7, 133.6, 134.4, 135.0, 139.3, 166.4, 194.1. **HRMS** (ESI) calc. $C_{20}H_{24}NO_4Se$ $[M+NH_4]^+$ 422.0871, found 422.0854.

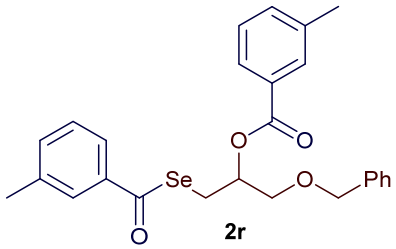
Synthesis of 1-(benzoylselanyl)-3-(benzyloxy)propan-2-yl benzoate **2q**



2q

Following the general procedure, 1-(benzyloxy)-3-hydroselenopropan-2-ol **1f** (37 mg, 0.15 mmol) and benzoyl chloride (53 mg, 0.38 mmol, 2.5 eq.) gave, after purification by flash column chromatography (petroleum ether : Et_2O 8:1), **2q** as a pale yellowish oil (39 mg, 57%). **^{1}H NMR** ($CDCl_3$, 400 MHz) *δ* (ppm): 3.53 (1H, dd, *J* = 6.7, 13.0 Hz, C**H$_a$**H$_b$Se), 3.60 (1H, dd, *J* = 5.5, 13.0 Hz, CH$_a$**H$_b$**Se), 3.79 (1H, dd, *J* = 4.6, 10.6 Hz, C**H$_a$**H$_b$O), 3.85 (1H, dd, *J* = 5.3, 10.6 Hz, CH$_a$**H$_b$**O), 4.60 (1H, d, *J* = 12.1 Hz, C**H$_a$**H$_b$Ph), 4.65 (1H, d, *J* = 12.1 Hz, CH$_a$**H$_b$**Ph), 5.51-5.56 (1H, m, C**H**O), 7.28-7.37 (5H, m), 7.45-7.49 (4H, m), 7.56-7.63 (2H, m), 7.89-7.91 (2H, m), 8.06-8.15 (2H, m). **^{13}C NMR** ($CDCl_3$, 100 MHz) *δ* (ppm): 25.9, 70.6, 72.4, 73.3, 127.3, 127.68, 127.70, 128.3, 128.4, 128.8, 129.8, 133.0, 133.8, 137.9, 138.6, 165.8, 193.5. **^{77}Se NMR** ($CDCl_3$, 76 MHz) *δ* (ppm): 489.9. **HRMS** (ESI) calc. $C_{24}H_{22}NaO_4Se$ $[M+Na]^+$ 477.0581, found 477.0605.

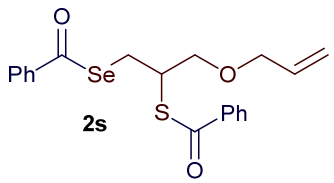
Synthesis of 1-(benzyloxy)-3-((3-methylbenzoyl)selanyl)propan-2-yl 3-methylbenzoate **2r**



2r

Following the general procedure, 1-(benzyloxy)-3-hydroselenopropan-2-ol **1f** (37 mg, 0.15 mmol) and 3-methylbenzoyl chloride (59 mg, 0.38 mmol, 2.5 eq.) gave, after purification by flash column chromatography (petroleum ether : Et_2O 8:1), **2r** as a pale yellowish oil (45 mg, 62%). **^{1}H NMR** ($CDCl_3$, 400 MHz) *δ* (ppm): 2.38 (3H, s), 2.40 (3H, s), 3.49 (1H, dd, *J* = 6.8, 13.0 Hz, C**H$_a$**H$_b$Se), 3.57 (1H, dd, *J* = 5.4, 13.0 Hz, CH$_a$**H$_b$**Se), 3.76 (1H, dd, *J* = 4.6, 10.7 Hz, C**H$_a$**H$_b$O), 3.82 (1H, dd, *J* = 5.3, 10.7 Hz, CH$_a$**H$_b$**O), 4.57 (1H, d, *J* = 12.0 Hz, C**H$_a$**H$_b$Ph), 4.62 (1H, d, *J* = 12.0 Hz, CH$_a$**H$_b$**Ph), 5.47-5.52 (1H, m, C**H**O), 7.27-7.40 (9H, m), 7.67-7.69 (2H, m), 7.83-7.84 (2H, m). **^{13}C NMR** ($CDCl_3$, 100 MHz) *δ* (ppm): 21.3, 21.9, 70.6, 72.3, 73.3, 124.5, 127.0, 127.7, 128.2, 128.4, 128.7, 130.0, 130.3, 133.8, 134.5, 137.9, 138.1, 138.66, 138.72, 166.0, 193.6. **HRMS** (ESI) calc. $C_{26}H_{26}NaO_4Se$ $[M+Na]^+$ 505.0894, found 505.0891.

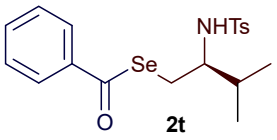
Synthesis of S-(1-(allyloxy)-3-(benzoylselanyl)propan-2-yl) benzothioate **2s**



2s

Following the general procedure, 1-(allyloxy)-3-hydroselenopropane-2-thiol **1g** (21 mg, 0.10 mmol) and benzoyl chloride (35 mg, 0.25 mmol, 2.5 eq.) gave, after purification by flash column chromatography (petroleum ether : Et_2O 5:1), **2s** as a pale yellowish oil (30 mg, 71%). **^{1}H NMR** ($CDCl_3$, 400 MHz) *δ* (ppm): 3.56 (1H, dd, *J* = 6.3, 12.6 Hz, C**H$_a$**H$_b$Se), 3.66 (1H, dd, *J* = 7.4, 12.6 Hz, CH$_a$**H$_b$**Se), 3.71 (1H, dd, *J* = 5.6, 10.1 Hz, C**H$_a$**H$_b$O), 3.86 (1H, dd, *J* = 4.7, 10.1 Hz, CH$_a$**H$_b$**O), 4.05-4.13 (2H, m, C**H$_2$**CH=CH$_2$), 4.25-4.31 (1H, m, C**H**S), 5.17-5.36 (2H, m), 5.88-6.00 (1H, m), 7.42-7.47 (3H, m), 7.51-7.59 (2H, m), 7.66-7.70 (1H, m), 7.92 (2H, ap d, *J* = 7.7 Hz), 7.97 (2H, ap d, *J* = 7.7 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz) *δ* (ppm): 27.9, 45.1, 72.1, 72.8, 117.9, 128.0, 129.2, 129.5, 131.2, 134.1, 135.1, 137.5, 139.4, 191.4, 194.2. **^{77}Se NMR** ($CDCl_3$, 76 MHz) *δ* (ppm): 513.7. **HRMS** (ESI) calc. $C_{20}H_{20}NaO_3SSe$ $[M+Na]^+$ 443.0196, found 443.0203.

Synthesis of (S)-Se-(3-methyl-2-((4-methylphenyl)sulfonamido)butyl) benzoselenoate **2t**



2t

Following the general procedure, (*S*)-*N*-(1-hydroseleno-3-methylbutan-2-yl)-4-methylbenzenesulfonamide **1h** (64 mg, 0.20 mmol) and benzoyl chloride (28 mg, 0.20 mmol) gave, after purification by flash column chromatography (petroleum ether : EtOAc 5:1), **2t** as a pale yellowish glassy solid (63 mg, 74%). **^{1}H NMR** ($CDCl_3$, 400 MHz) δ (ppm): 0.95 (6H, ap t, J = 6.9 Hz), 1.95-2.04 (1H, m), 2.28 (3H, s), 3.06 (2H, ap d, J = 6.4 Hz, CH_2Se), 3.34-3.41 (1H, m, C**H**NH), 4.81 (1H, d, J = 7.9 Hz, NH), 7.09 (2H, ap d, J = 8.0 Hz), 7.45 (2H, ap t, J = 7.4 Hz), 7.61 (1H, ap t, J = 7.4 Hz), 7.68 (2H, ap d, J = 8.0 Hz), 7.77 (2H, ap d, J = 7.4 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ (ppm): 18.0, 18.3, 21.4, 27.2, 33.0, 60.2, 127.1, 127.3, 128.8, 129.4, 133.9, 138.1, 138.4, 142.9, 194.8. **^{77}Se NMR** ($CDCl_3$, 76 MHz) δ (ppm): 499.9. **HRMS** (ESI) calc. $C_{19}H_{23}NNaO_3SSe$ [M+Na]$^+$ 448.0462, found 448.0468.

Carbonic anhydrase assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity.[9] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 6 hours at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng–Prusoff equation, as reported earlier,[10-12] and represent the mean from at least three different determinations. All CA isofoms were recombinant ones obtained in-house as reported earlier.[10-12]

Crystallization and X-ray data collection

Crystals of hCA II were obtained using the hanging drop vapor diffusion method using 24 well Linbro plate. 2 µl of 10 mg/ml solution of hCA II in Tris-HCl 20 mM pH 8.0 were mixed with 2 µl of a solution of 1.5 M sodium citrate, 0.1 M Tris pH 8.0 and were equilibrated against the same solution at 296 K. Crystals of the protein grew in one week. Afterwards hCAII crystals were soaked in 5mM inhibitor solution for 3 days. The crystals were flash-frozen at 100K using a solution obtained by adding 15% (v/v) glycerol to the mother liquor solution as cryoprotectant. Data on crystals of the complex were collected using synchrotron radiation at the ID-11.2C beamline at Elettra (Trieste, Italy) with a wavelength of 1.000 Å and a Pilatus3_6M Dectris CCD detector. Data were integrated and scaled using the program XDS.[13]

Structure determination

The crystal structure of hCA II (PDB accession code: 4FIK) without solvent molecules and other heteroatoms was used to obtain initial phases of the structures using Refmac5.[14] 5% of the unique reflections were selected randomly and excluded from the refinement data set for the purpose of Rfree calculations. The initial |Fo - Fc| difference electron density maps unambiguously showed the inhibitor molecules. Atomic models for inhibitors were calculated and energy minimized using the program JLigand 1.0.40.[15] Refinements proceeded using normal protocols of positional, isotropic atomic displacement parameters alternating with manual building of the models using COOT.[16] Solvent molecules were introduced automatically using the program ARP.[17] The quality of the final models were assessed with COOT and RAMPAGE.[18] Atomic coordinates were deposited in the Protein Data Bank (PDB accession code: 6XWZ). Graphical representations were generated with Chimera.[19]

Summary of Data Collection and Atomic Model Refinement Statistics.

	HCAII + **2a**
PDB ID	
Wavelength (Å)	1.000
Space Group	P21
Unit cell (a, b, c, α, β, γ) (Å,°)	42.32, 41.23, 72.34, 90.0, 104.22, 90.0
Limiting resolution (Å)	41.31-1.38 (1.40–1.38)
Unique reflections	50482 (2342)
Rmerge (%)	21.8 (112.7)
Rmeas (%)	22.0 (124.8)
Rpim (%)	3.3 (52.8)
Redundancy	31.0 (5.5)
Completeness overall (%)	99.8 (95.5)
<I/σ(I)>	11.1 (1.2)
CC (1/2)	89.0 (82.0)
Refinement statistics	
Resolution range (Å)	41.1-1.38
Unique reflections, working/free	50482/47967
Rfactor (%)	18.894
Rfree(%)	21.05
r.m.s.d. bonds(Å)	0.0128
r.m.s.d. angles (°)	1.8273
Ramachandran statistics (%)	
Most favored	97.6
additionally allowed	2.4
outlier regions	0.0
Average B factor (Å^2)	
All atoms	17.736
inhibitors	18.967
solvent	25.217

Control experiment. In order to verify that the hydrolysis of the selenolester moiety is CA-catalysed and does not occur under the experimental conditions, **2a** was stirred under the same conditions of temperature in the aqueous buffered solution used for kinetic assays up to 18 h with no enzyme. No traces of cleaved by-products (*ie*. PhSeSePh or PhCOOH) were detected by inspection of the ^{1}H-NMR spectra of raw material.

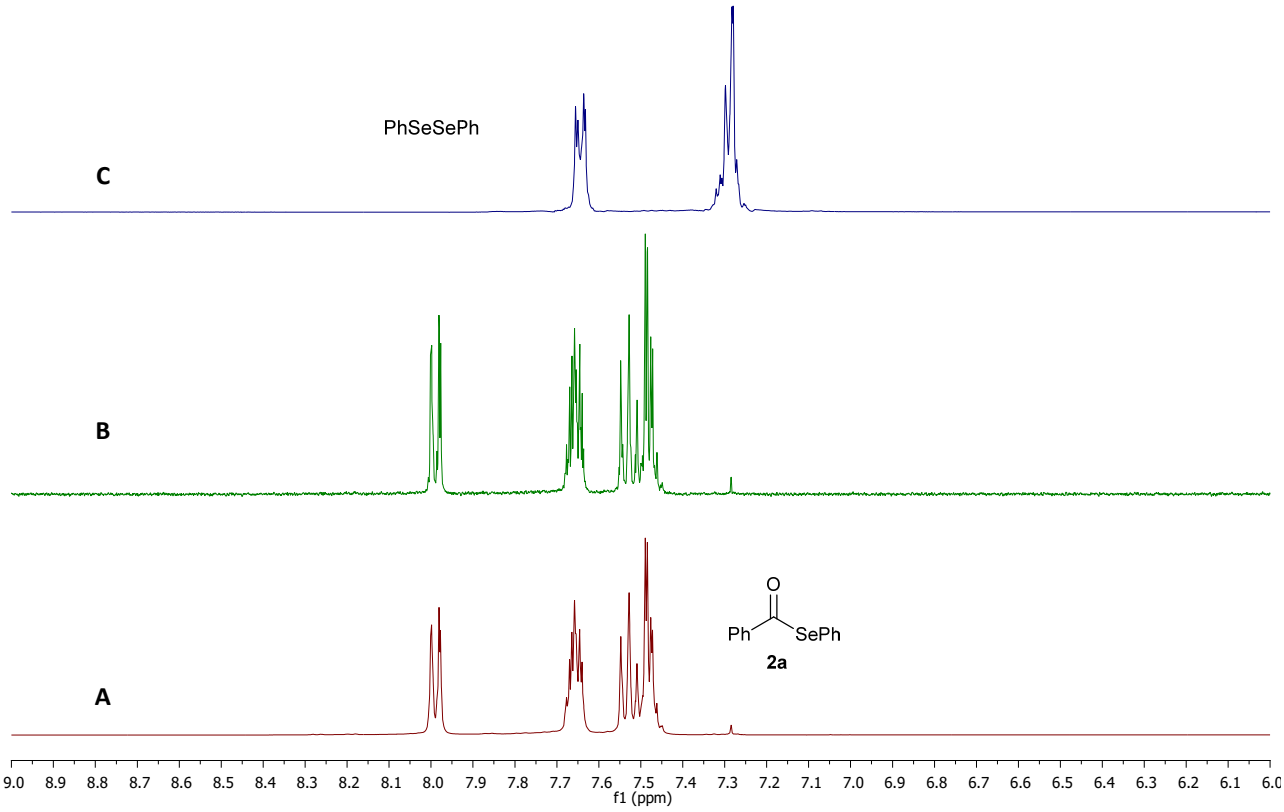
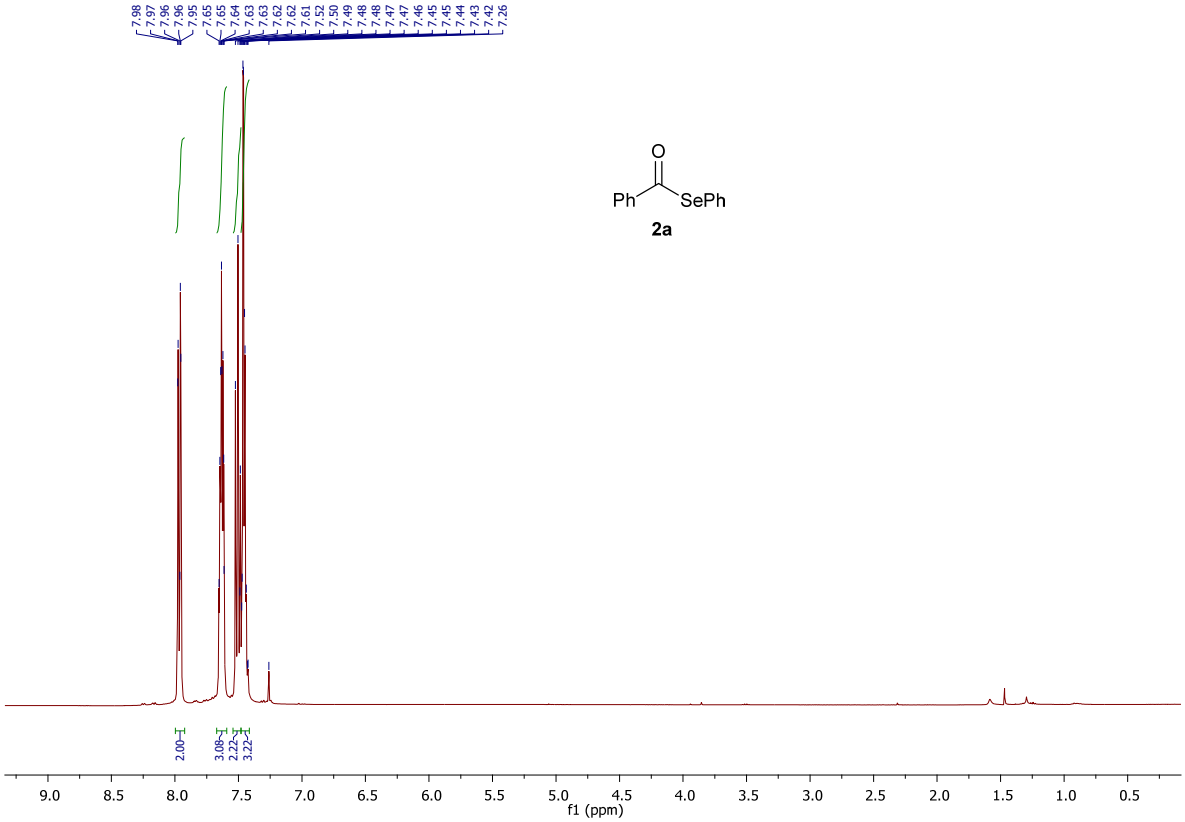


Figure S1. A) ^{1}H NMR spectrum of compound **2a**, *red curve*. B) ^{1}H NMR spectrum of the raw material recovered after stirring the selenolester **2a** as described above for the control experiment, *green curve*. C) ^{1}H NMR spectrum of diphenyl diselenide (PhSeSePh). Diphenyl diselenide represents the selenated product that would be formed through hydrolysis of **2a** under these conditions.

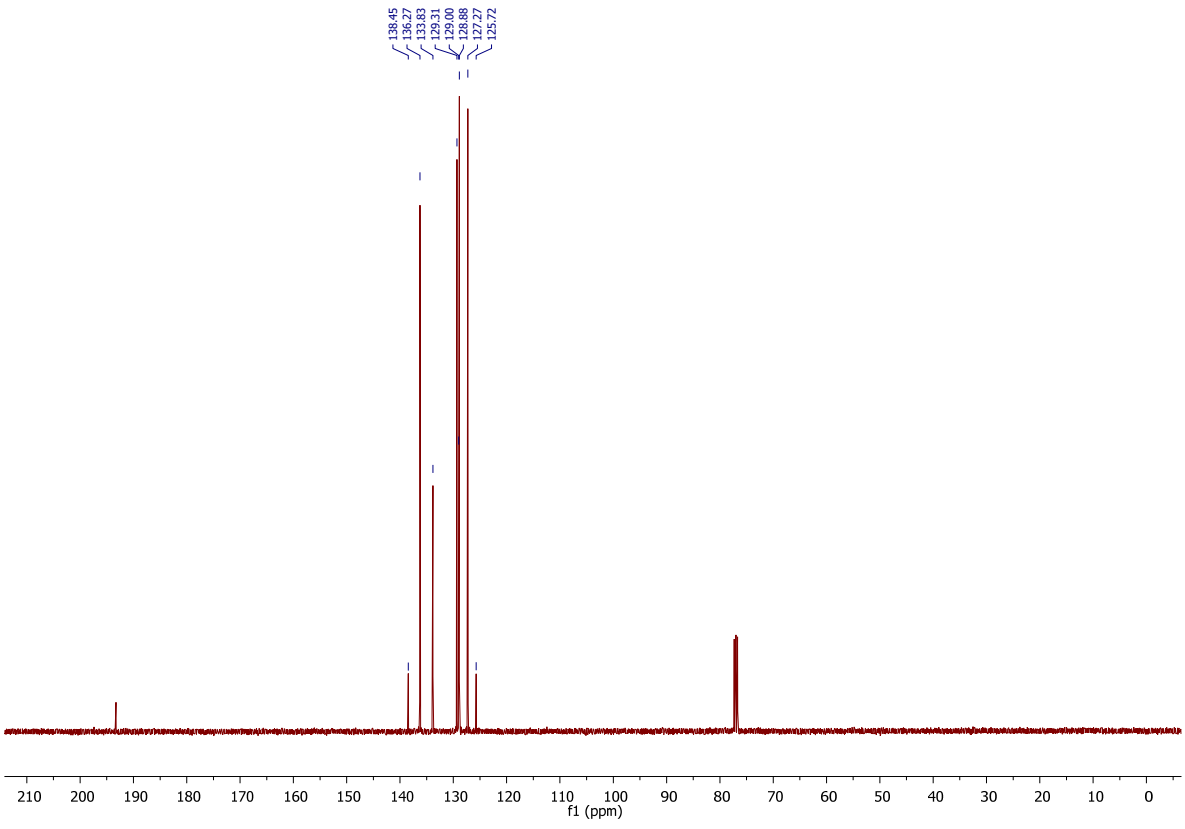
NMR spectra of new compounds

^{1}H NMR of compound **2a** ($CDCl_3$, 400 MHz)

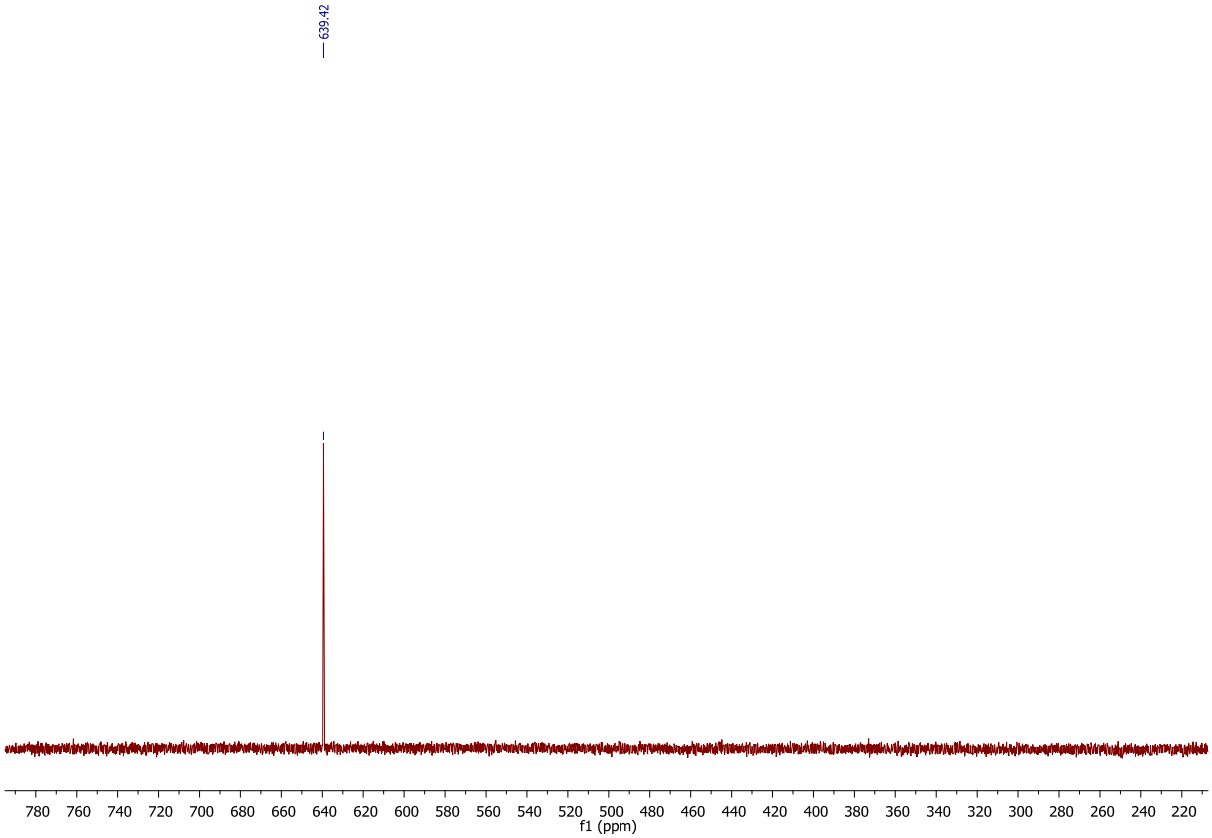


2a

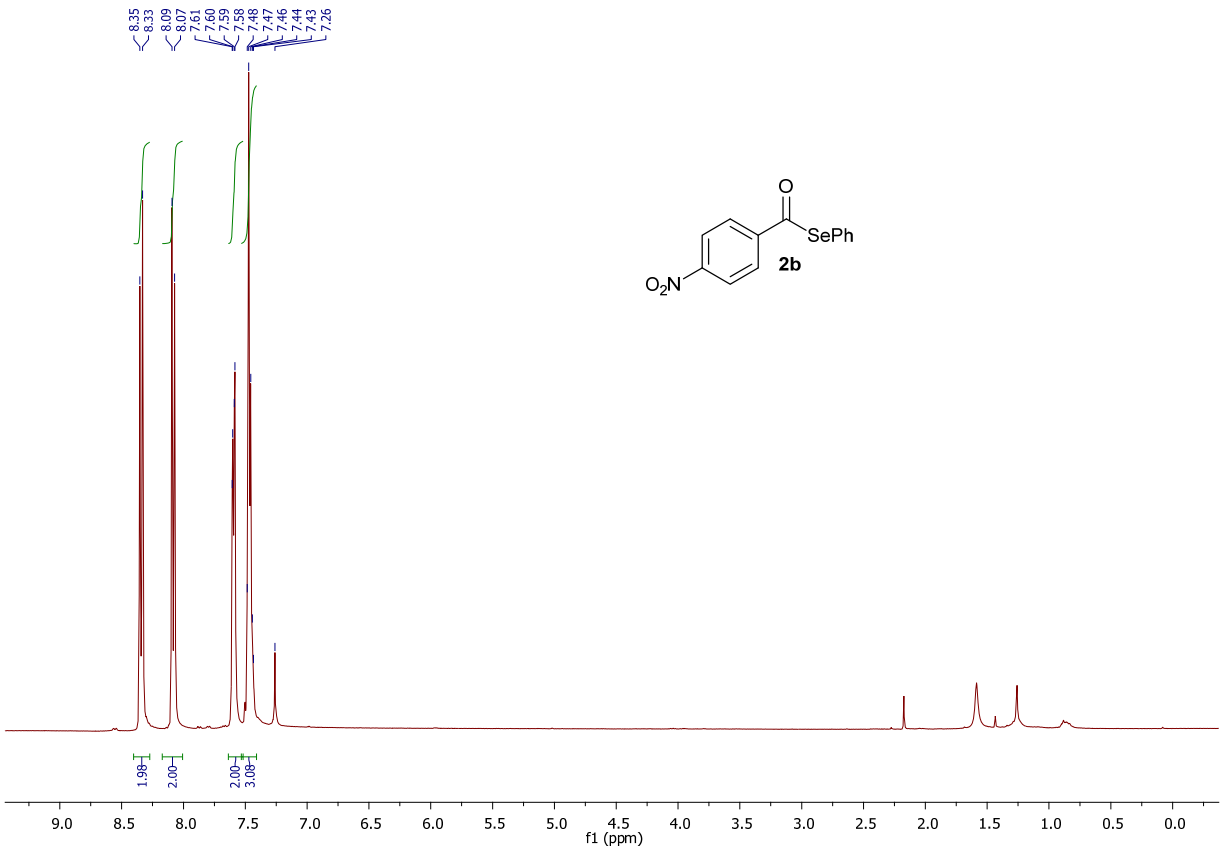
^{13}C NMR of compound **2a** ($CDCl_3$, 100 MHz)



^{77}Se NMR of compound **2a** ($CDCl_3$, 76 MHz)

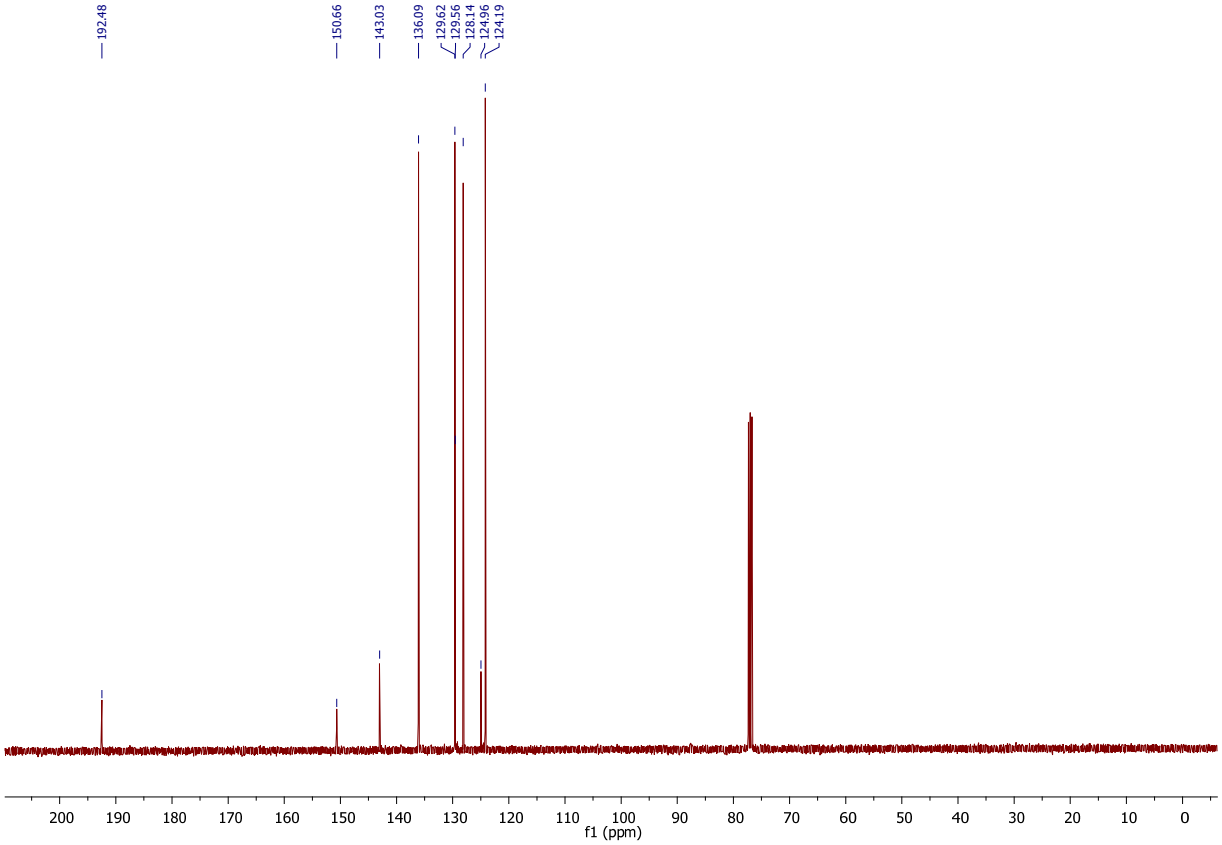


^{1}H NMR of compound **2b** ($CDCl_3$, 400 MHz)

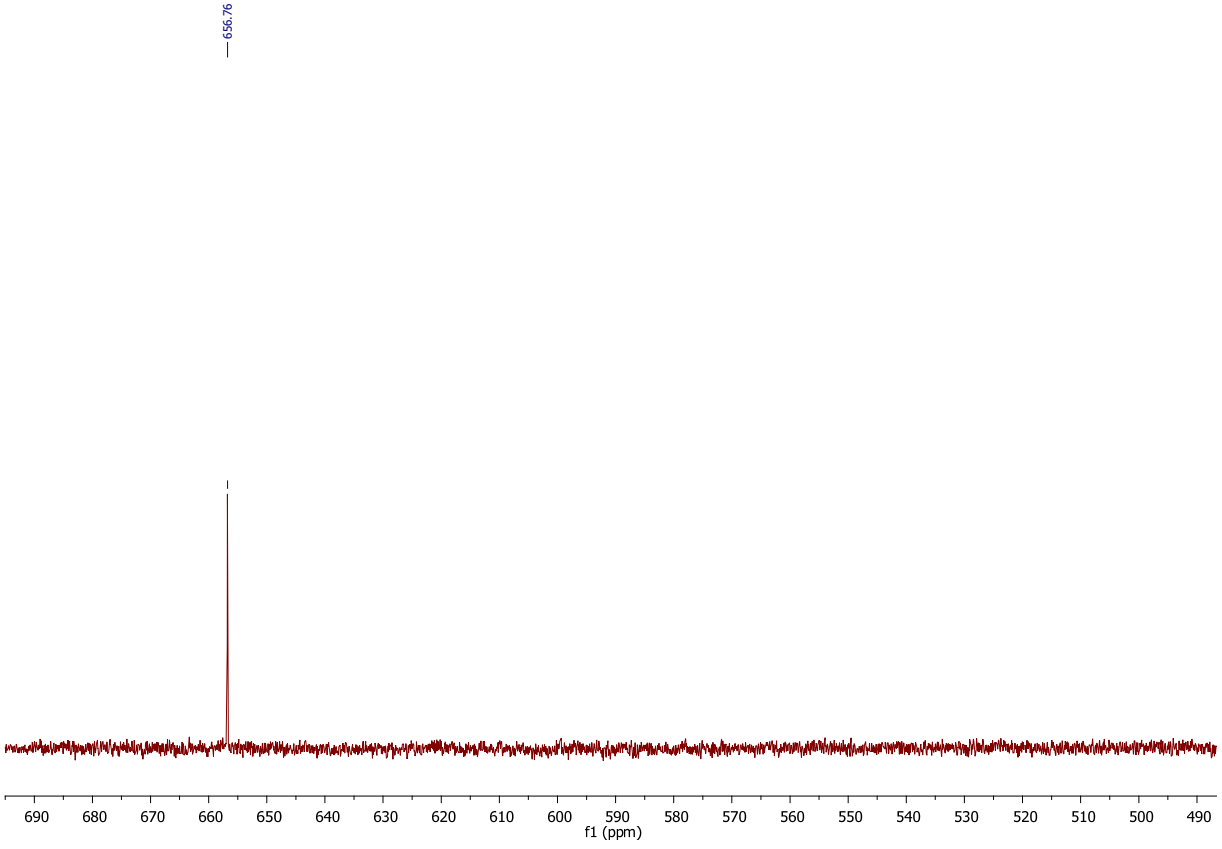


2b

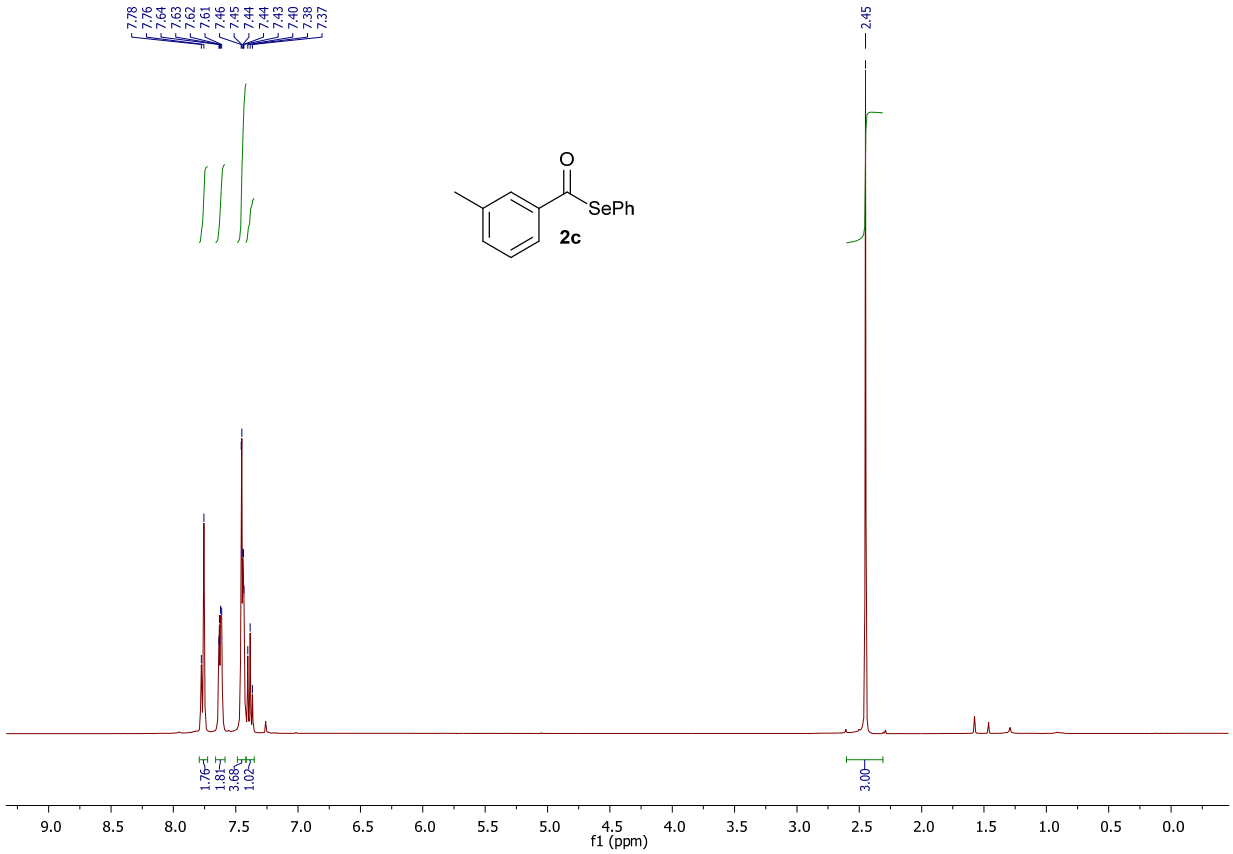
^{13}C NMR of compound **2b** ($CDCl_3$, 100 MHz)



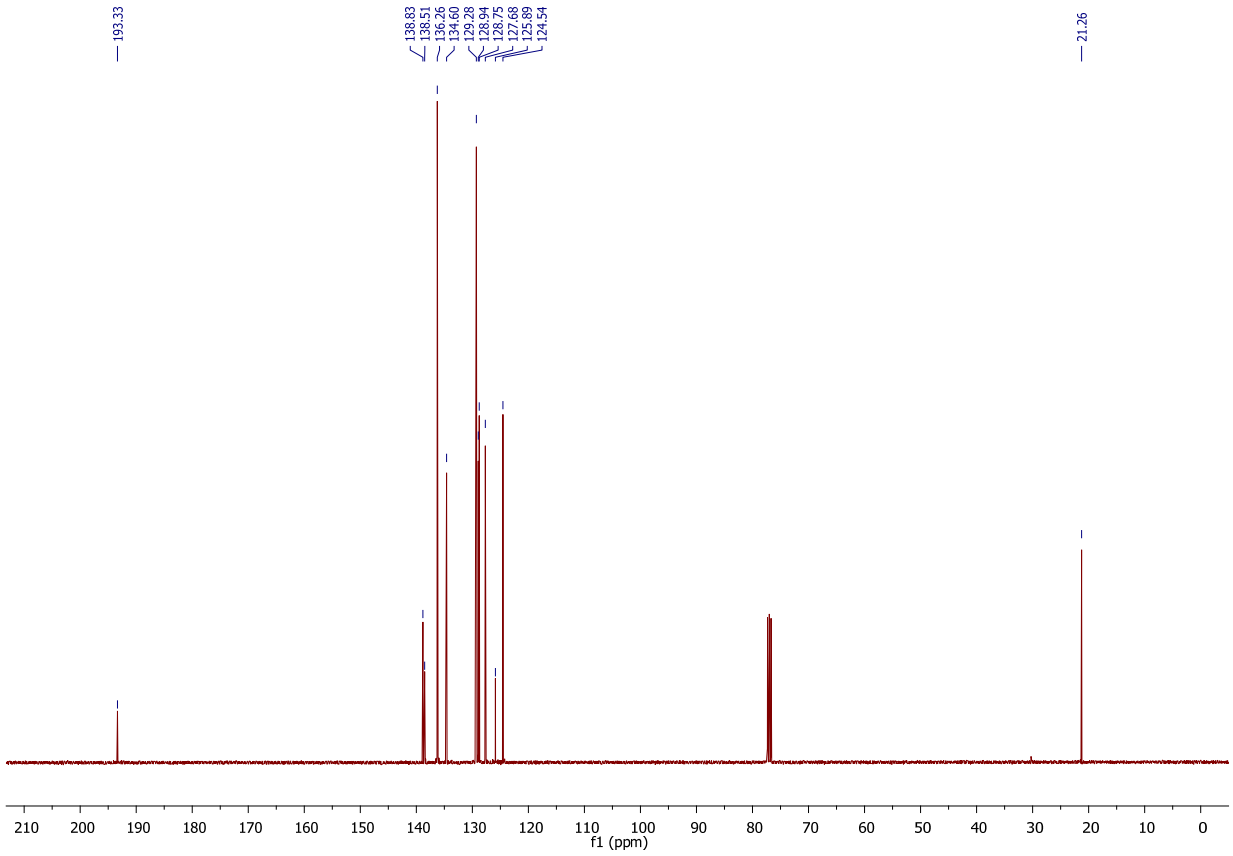
^{77}Se NMR of compound **2b** ($CDCl_3$, 76 MHz)



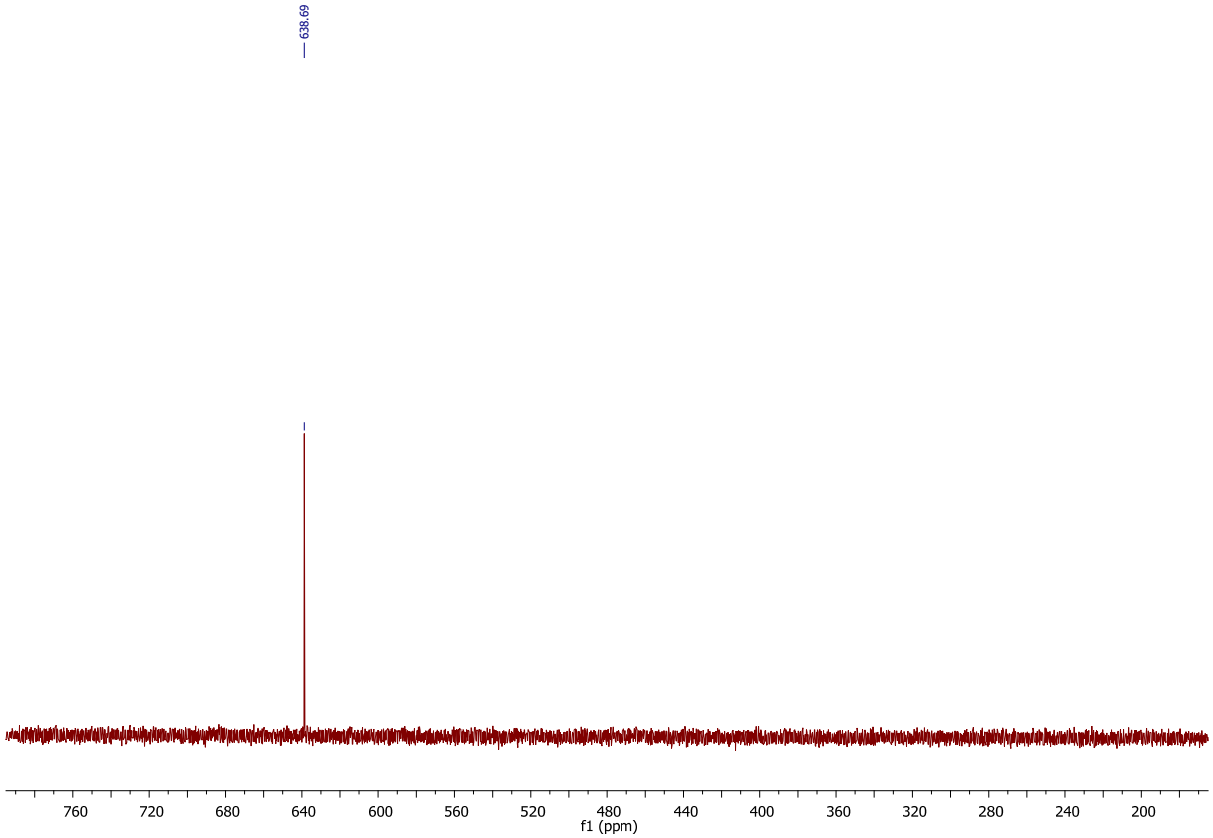
^{1}H NMR of compound **2c** ($CDCl_3$, 400 MHz)



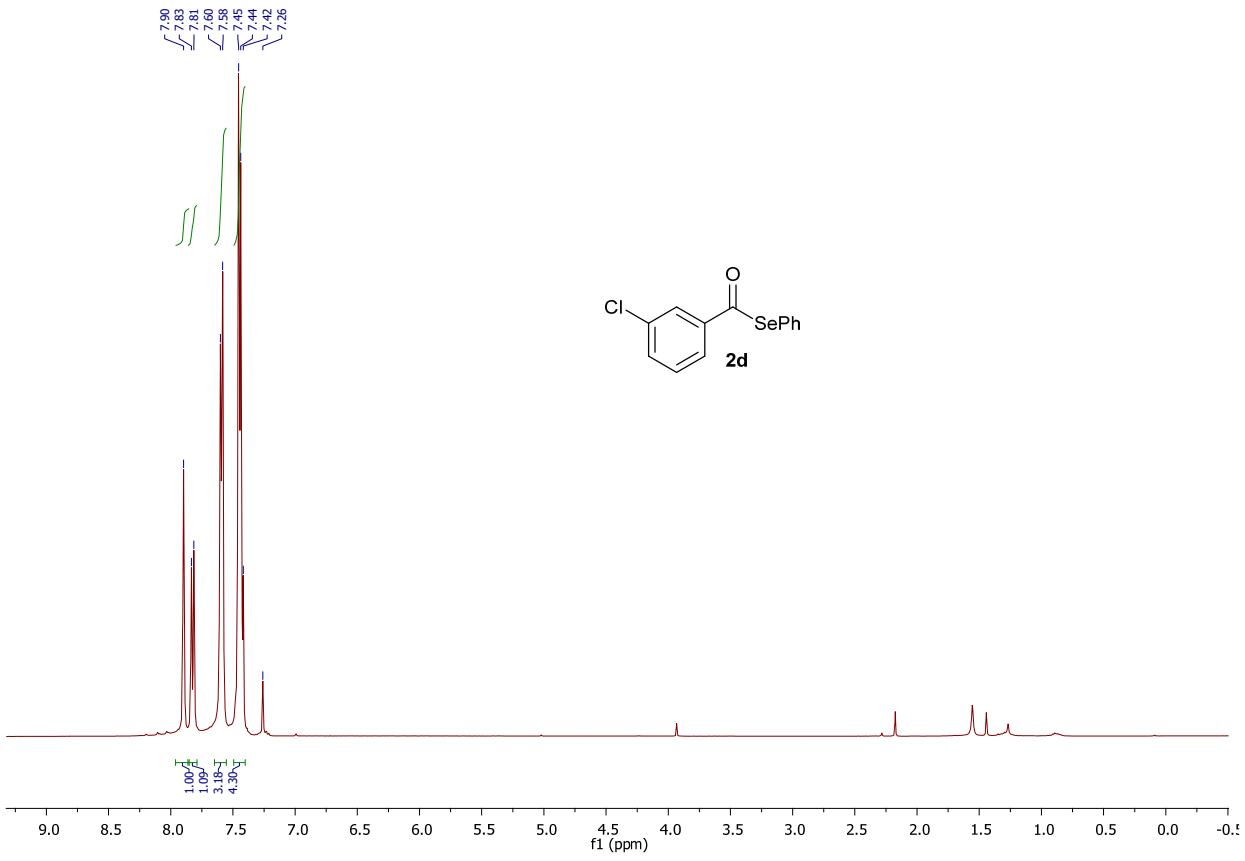
^{13}C NMR of compound **2c** ($CDCl_3$, 100 MHz)



^{77}Se NMR of compound **2c** ($CDCl_3$, 76 MHz)

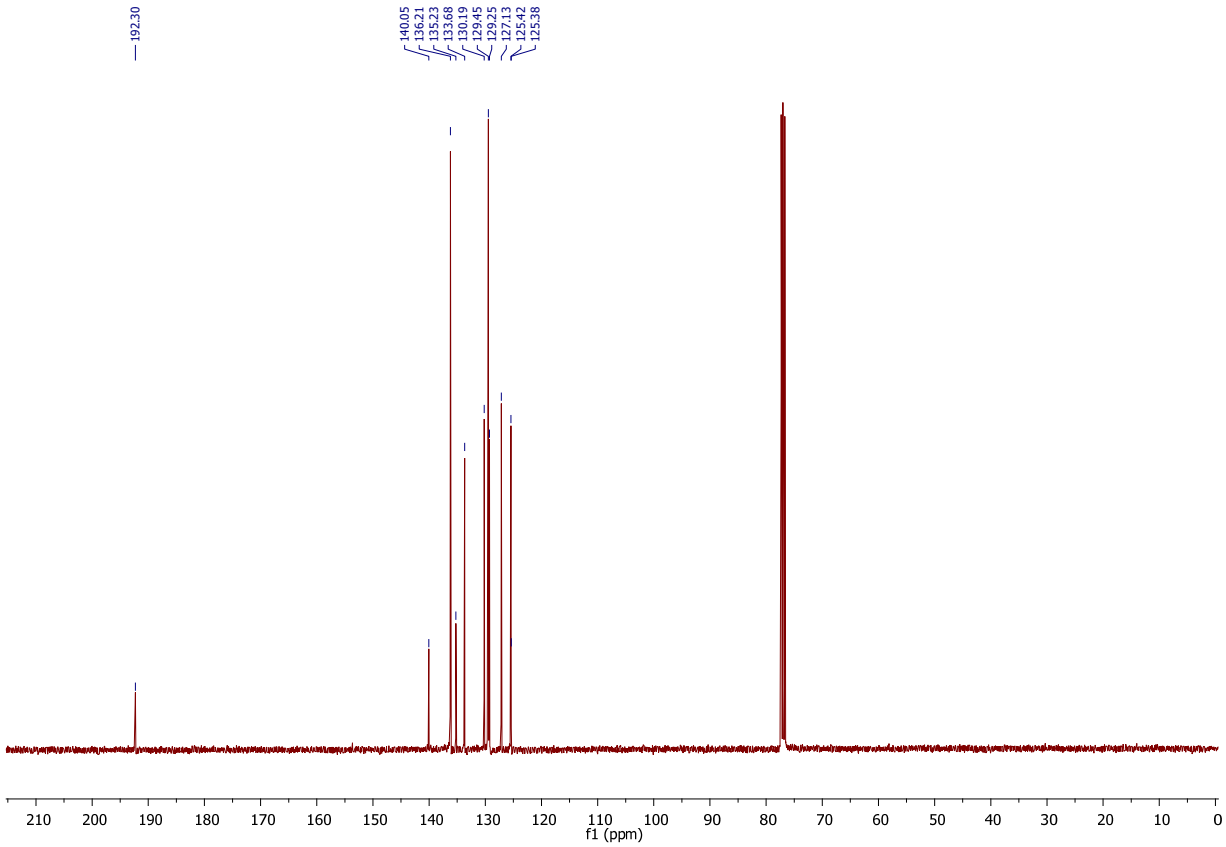


^{1}H NMR of compound **2d** ($CDCl_3$, 400 MHz)

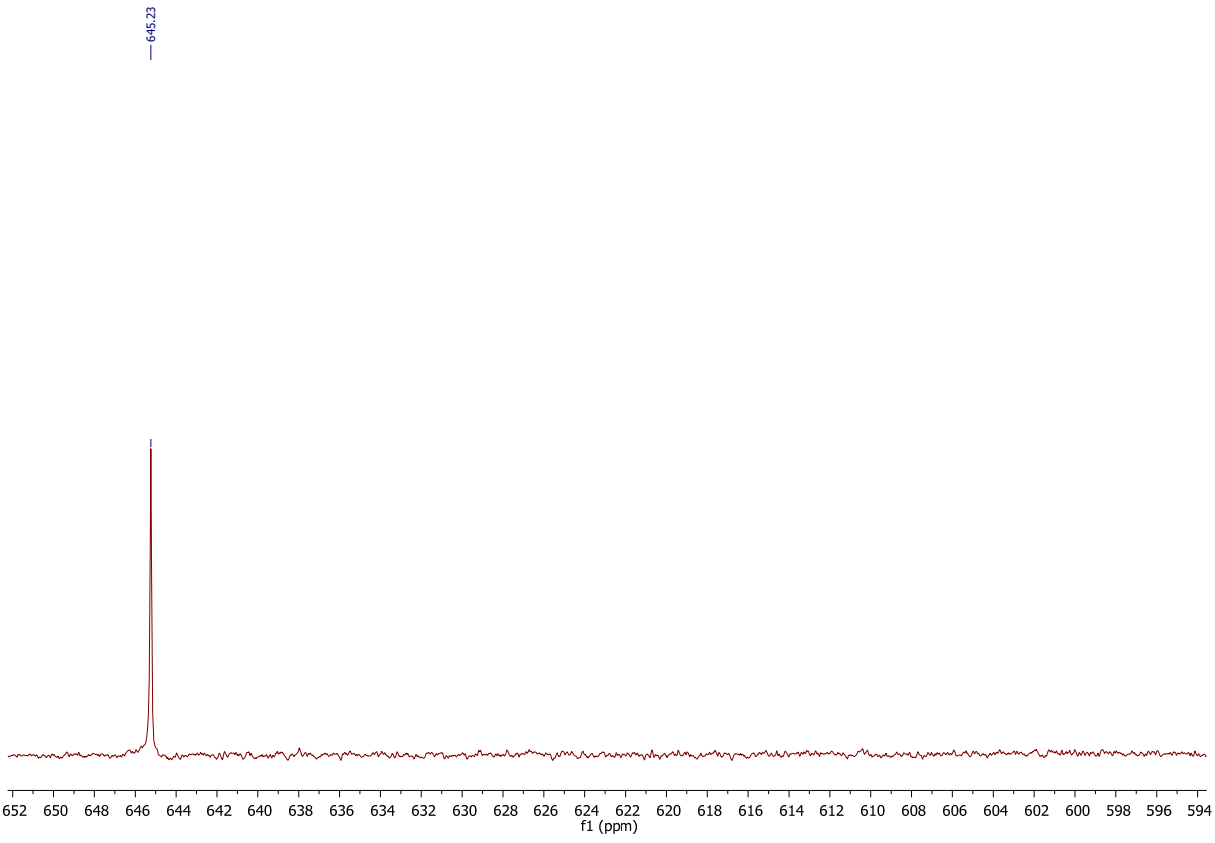


2d

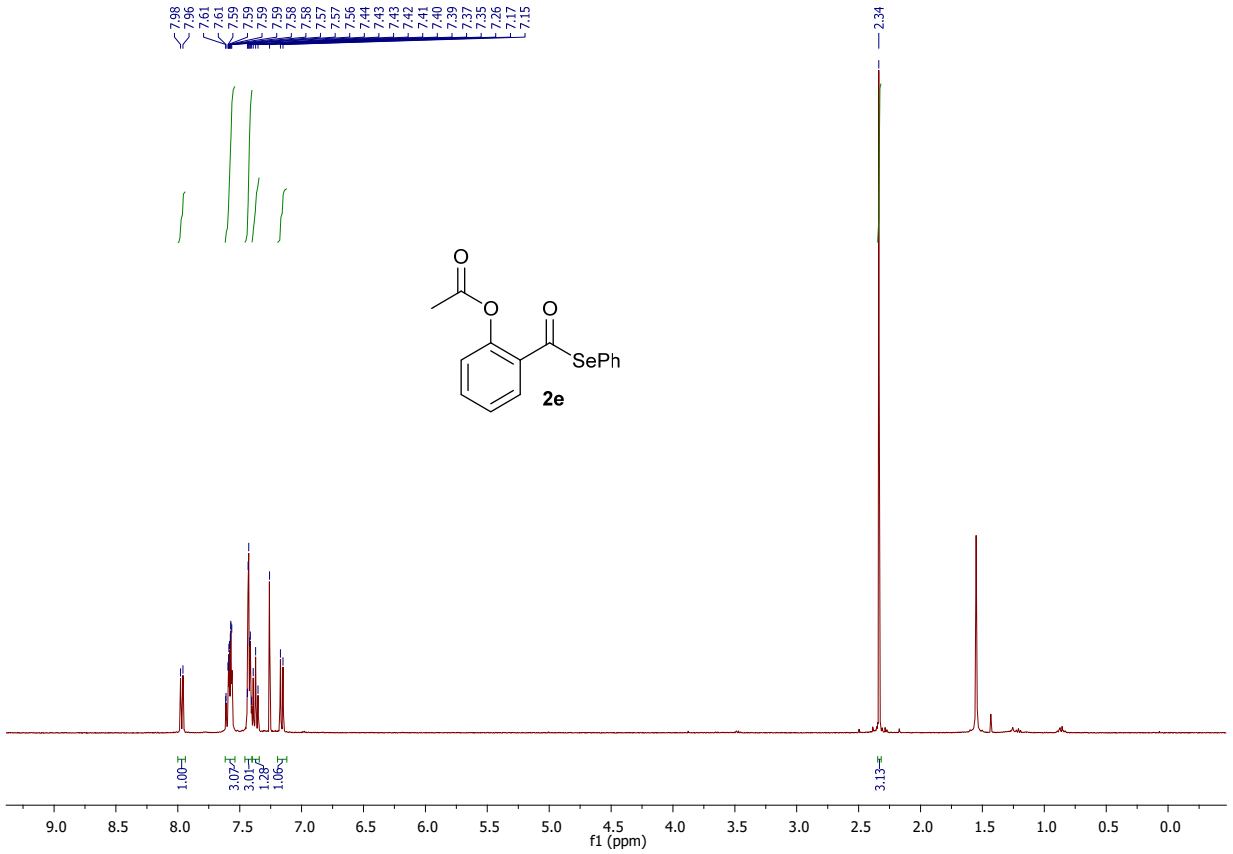
^{13}C NMR of compound **2d** ($CDCl_3$, 100 MHz)



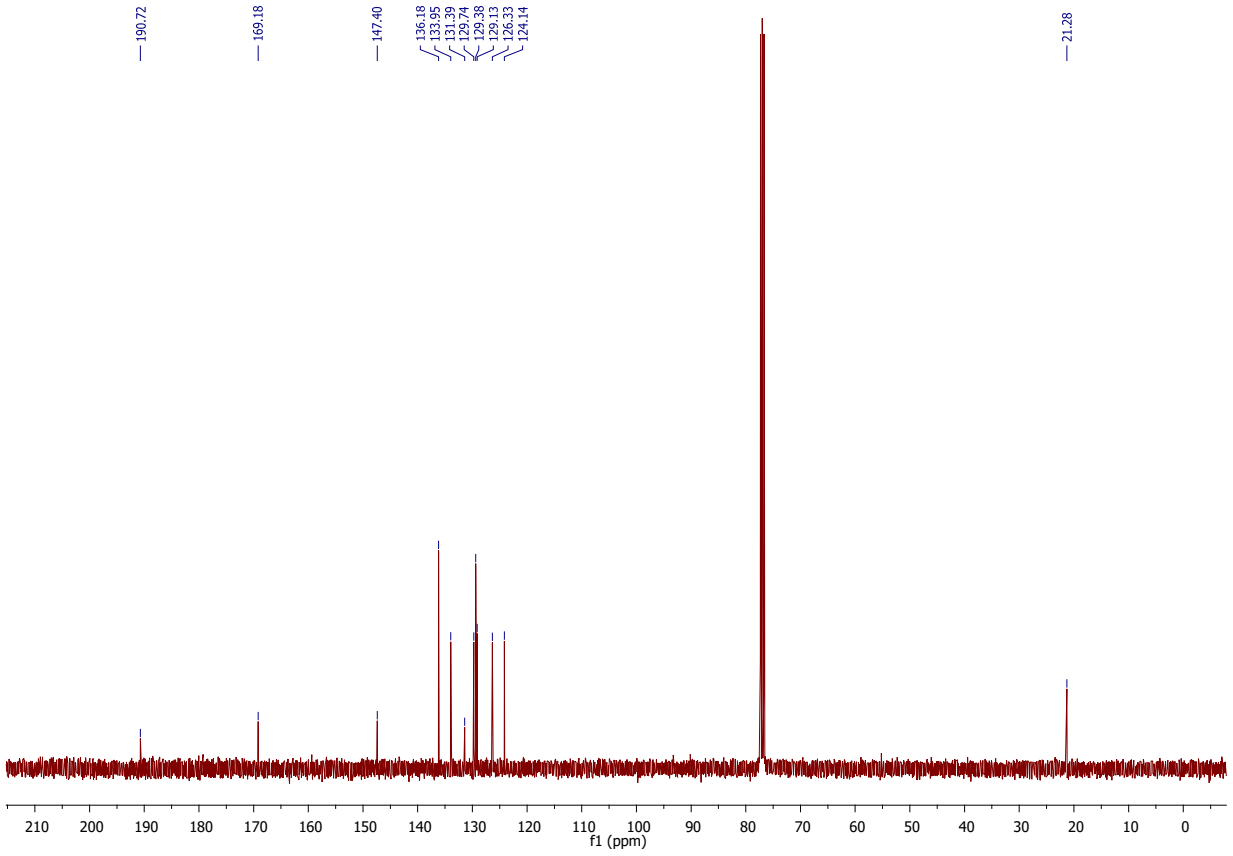
^{77}Se NMR of compound **2d** ($CDCl_3$, 76 MHz)



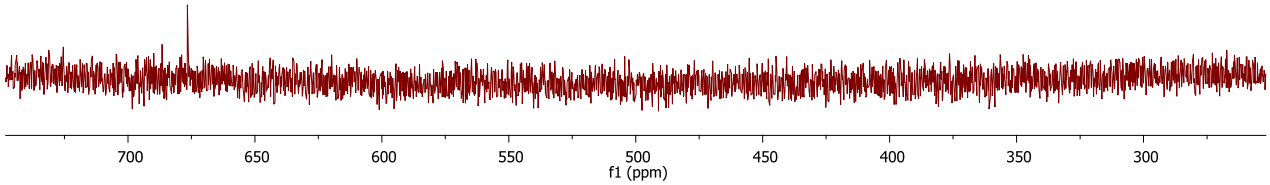
^{1}H NMR of compound **2e** ($CDCl_3$, 400 MHz)



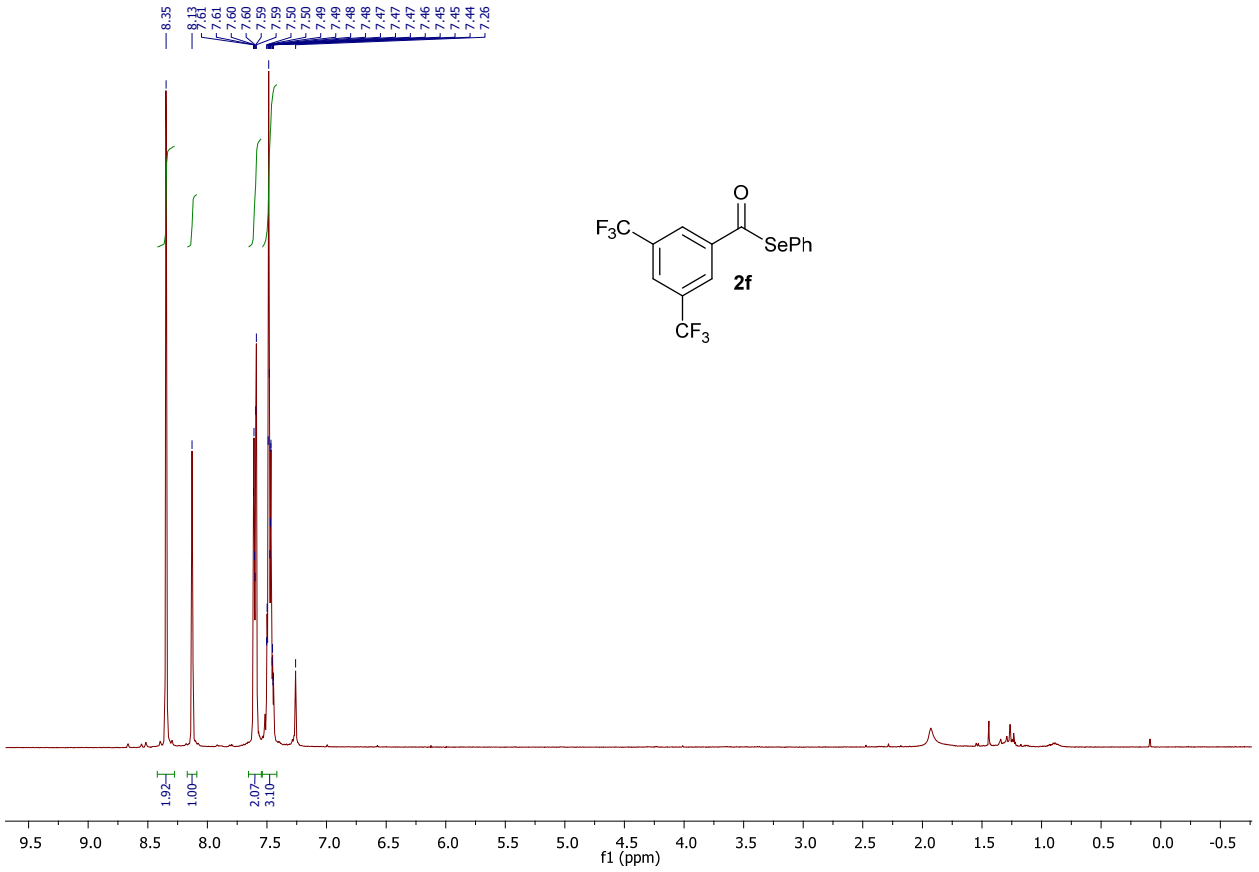
^{13}C NMR of compound **2e** ($CDCl_3$, 100 MHz)



^{77}Se NMR of compound **2e** ($CDCl_3$, 76 MHz)

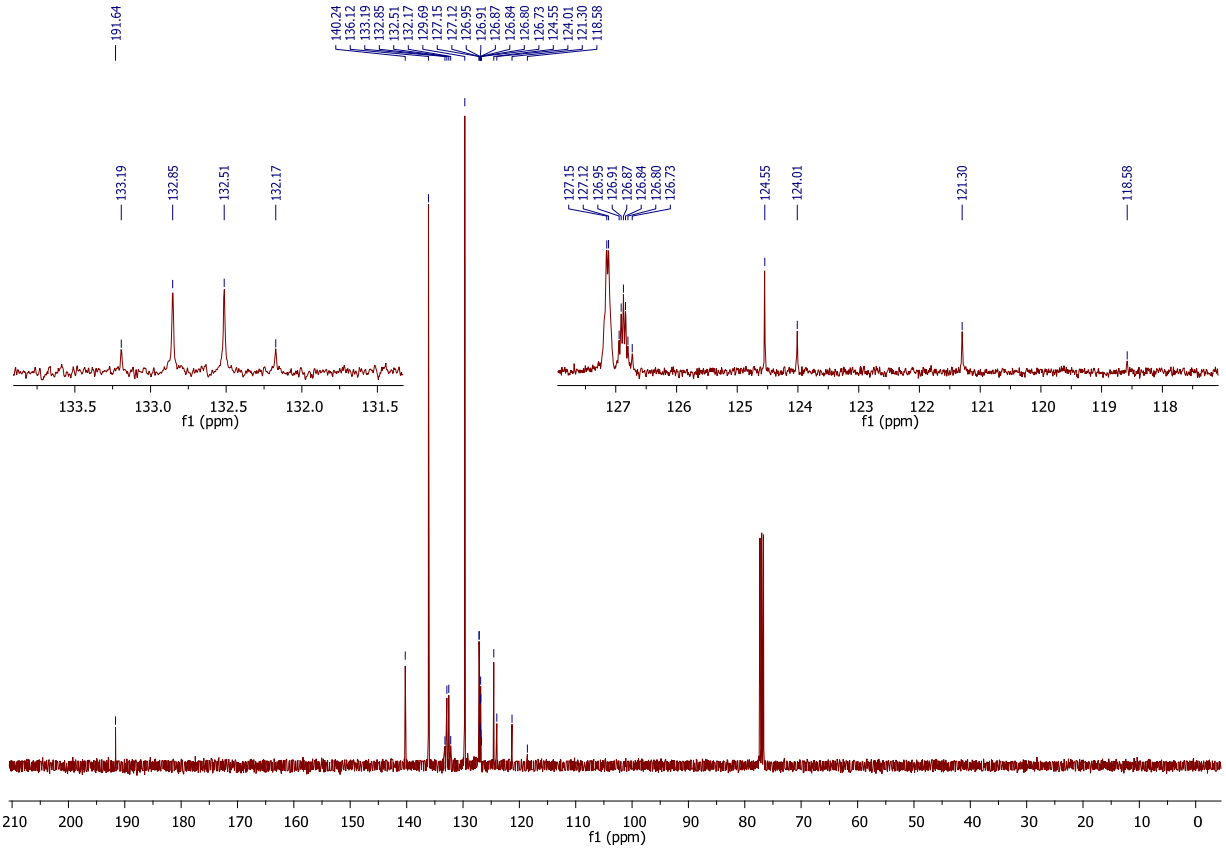


^{1}H NMR of compound **2f** ($CDCl_3$, 400 MHz)

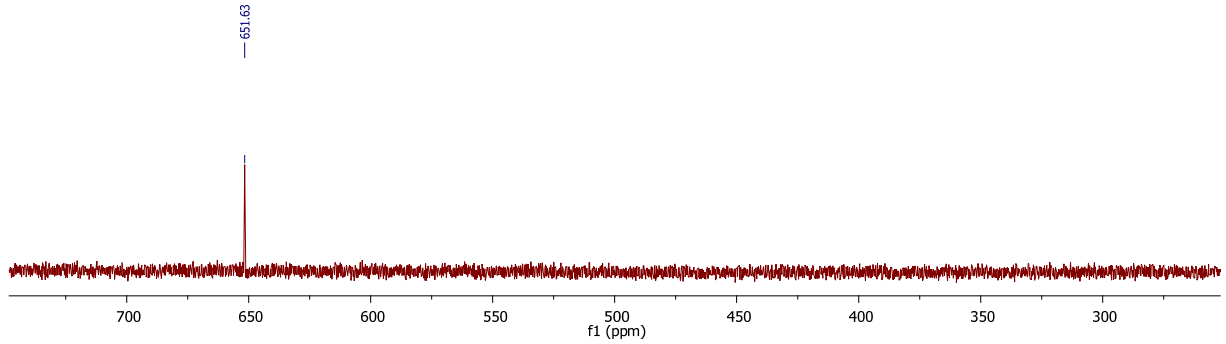


2f

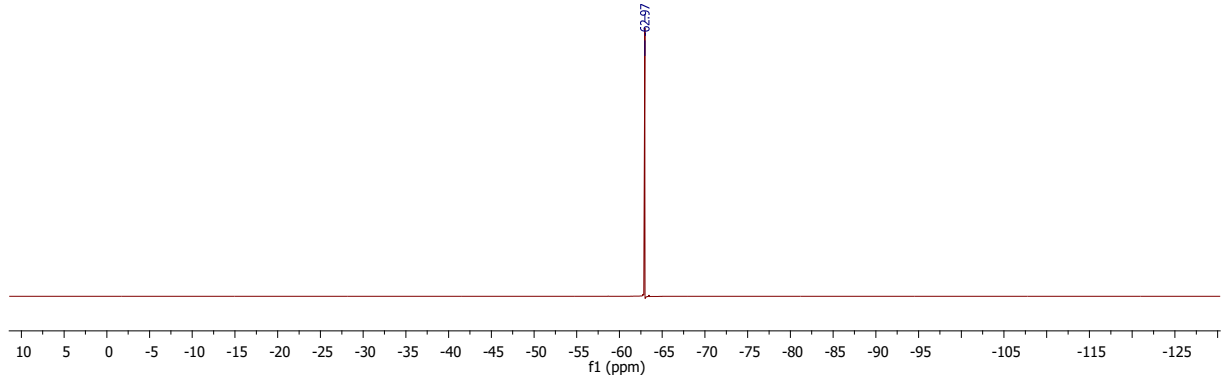
^{13}C NMR of compound **2f** ($CDCl_3$, 100 MHz)



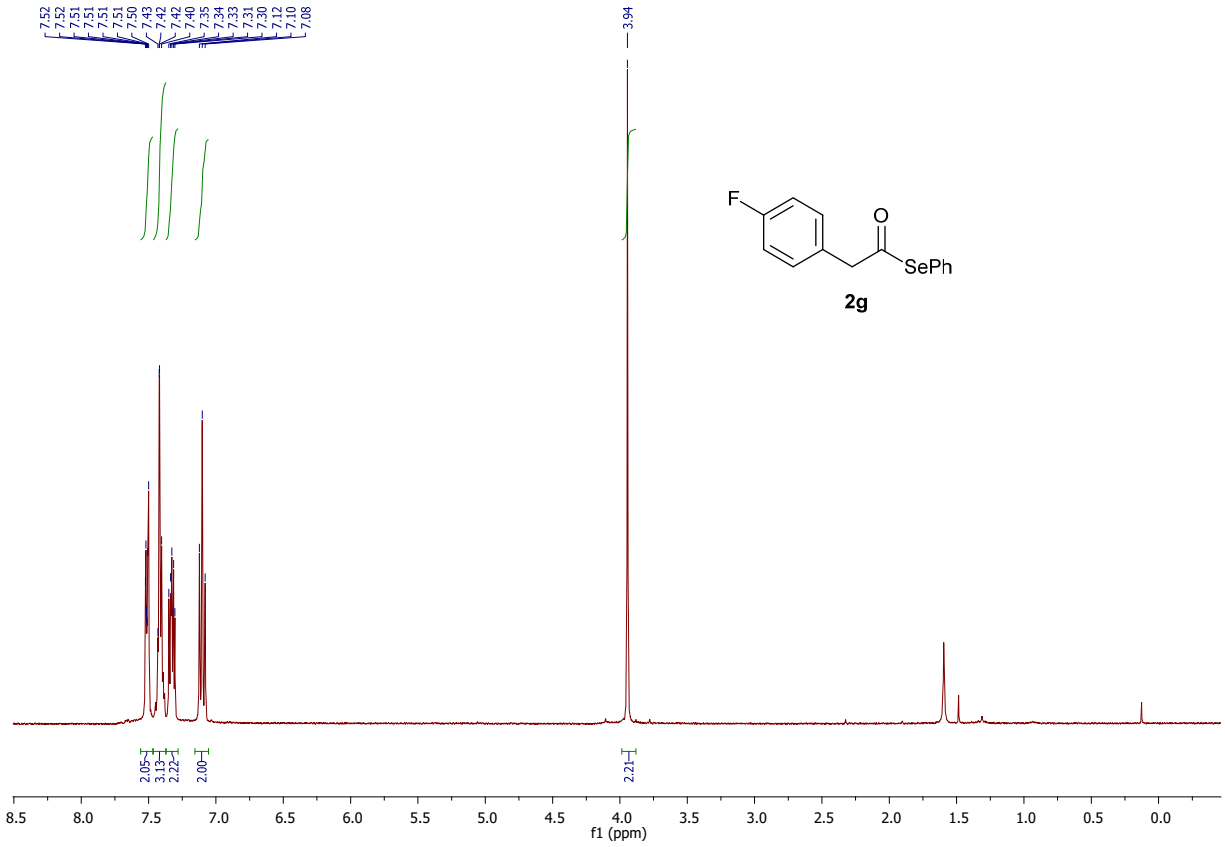
^{77}Se NMR of compound **2f** ($CDCl_3$, 76 MHz)



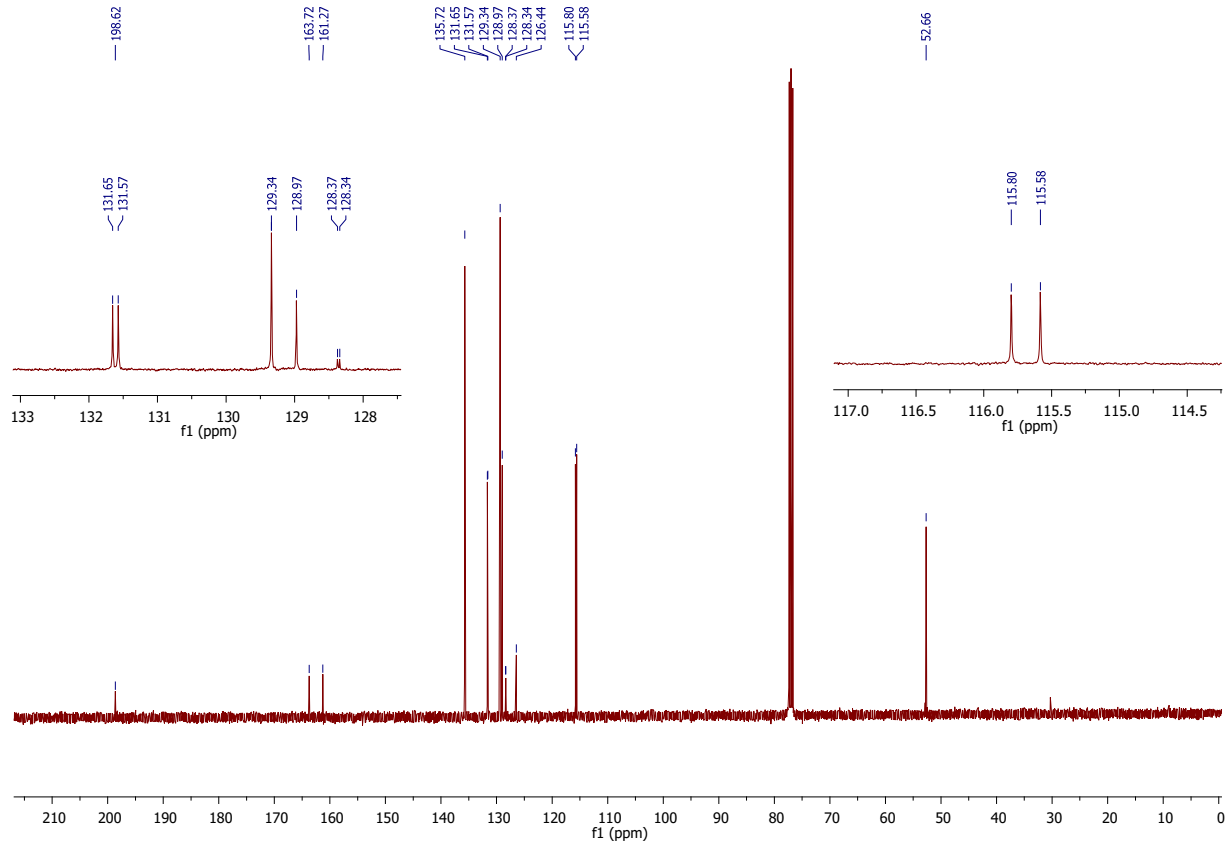
^{19}F NMR of compound **2f** ($CDCl_3$, 376 MHz)



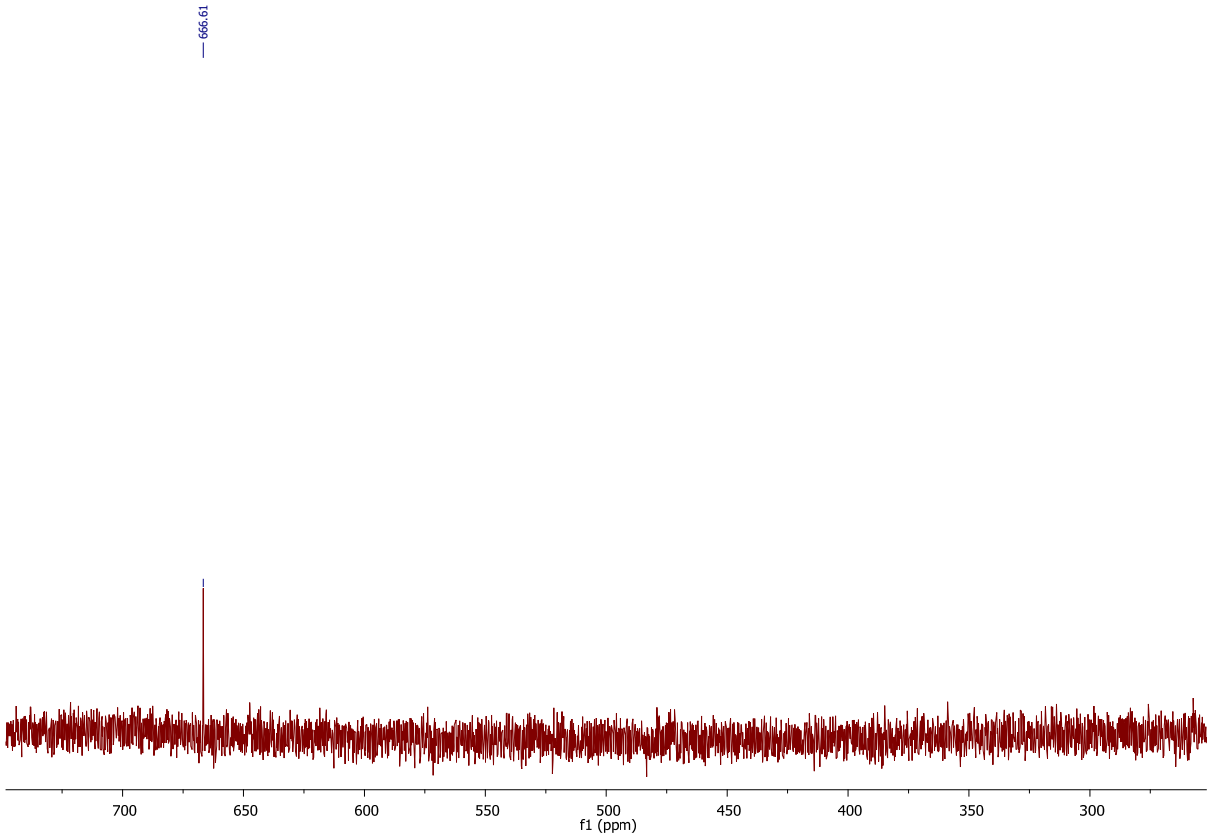
^{1}H NMR of compound **2g** ($CDCl_3$, 400 MHz)



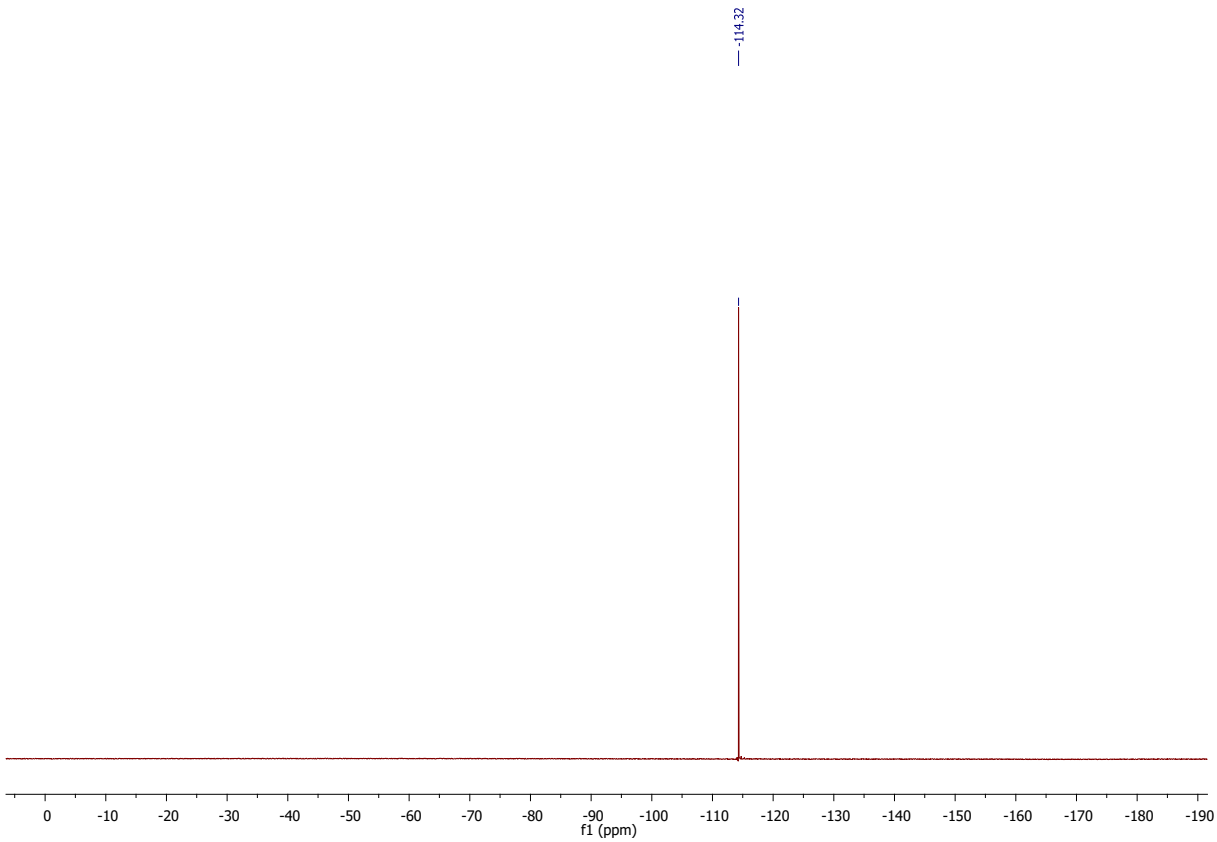
^{13}C NMR of compound **2g** ($CDCl_3$, 100 MHz)



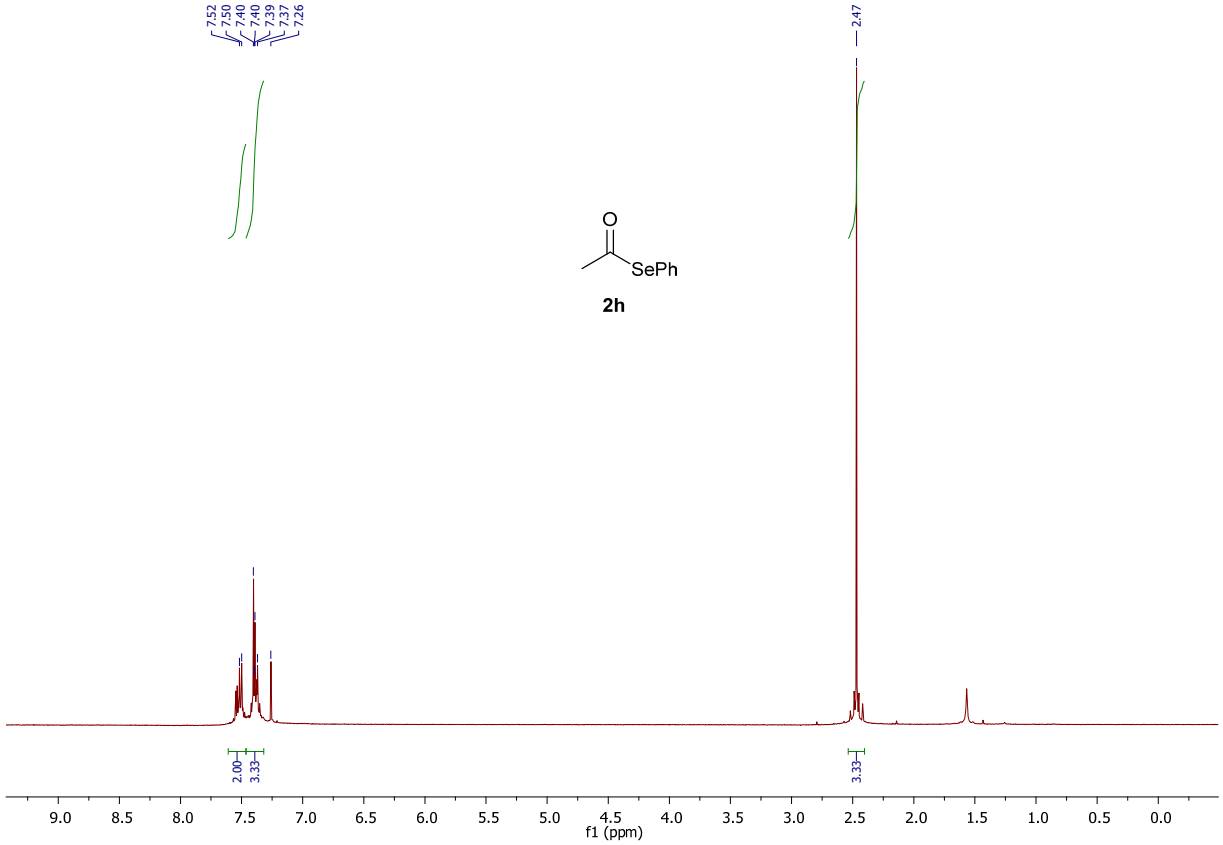
^{77}Se NMR of compound **2g** ($CDCl_3$, 76 MHz)



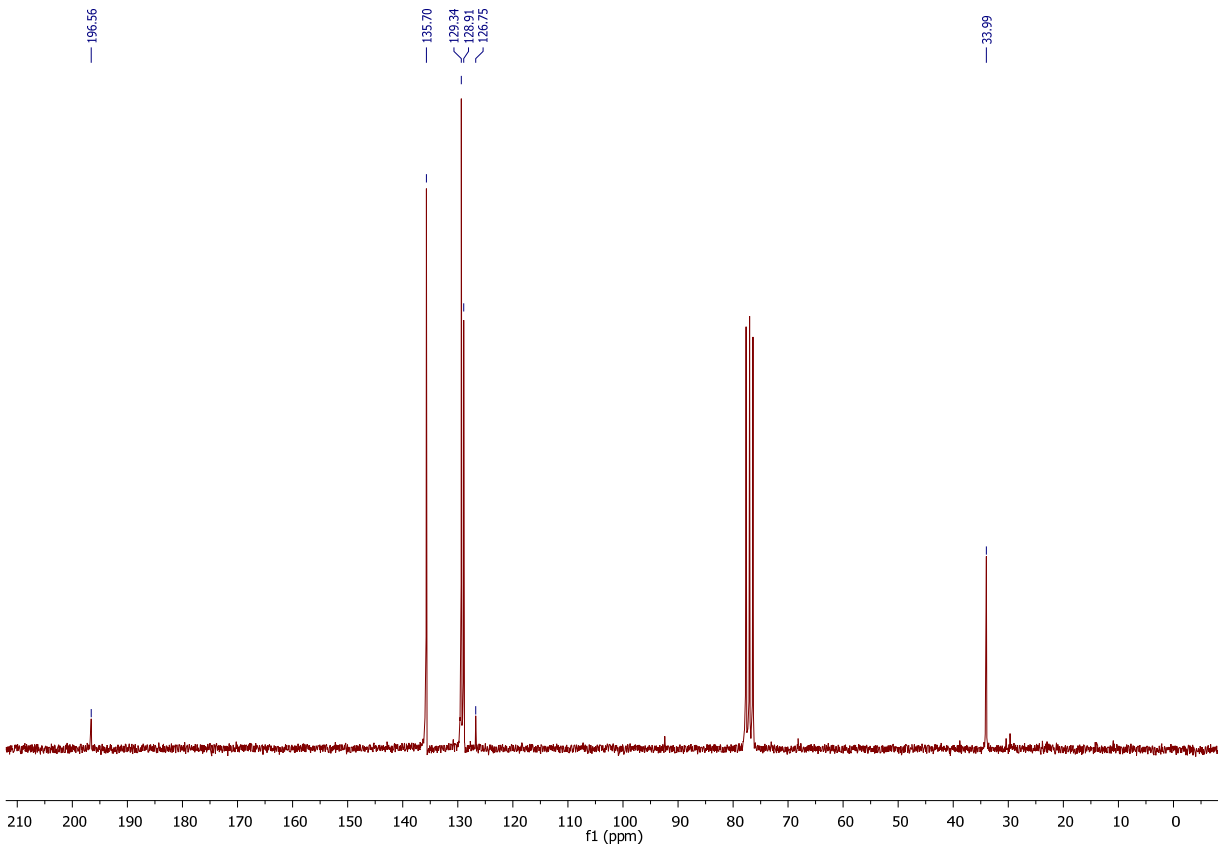
^{19}F NMR of compound **2g** ($CDCl_3$, 376 MHz)



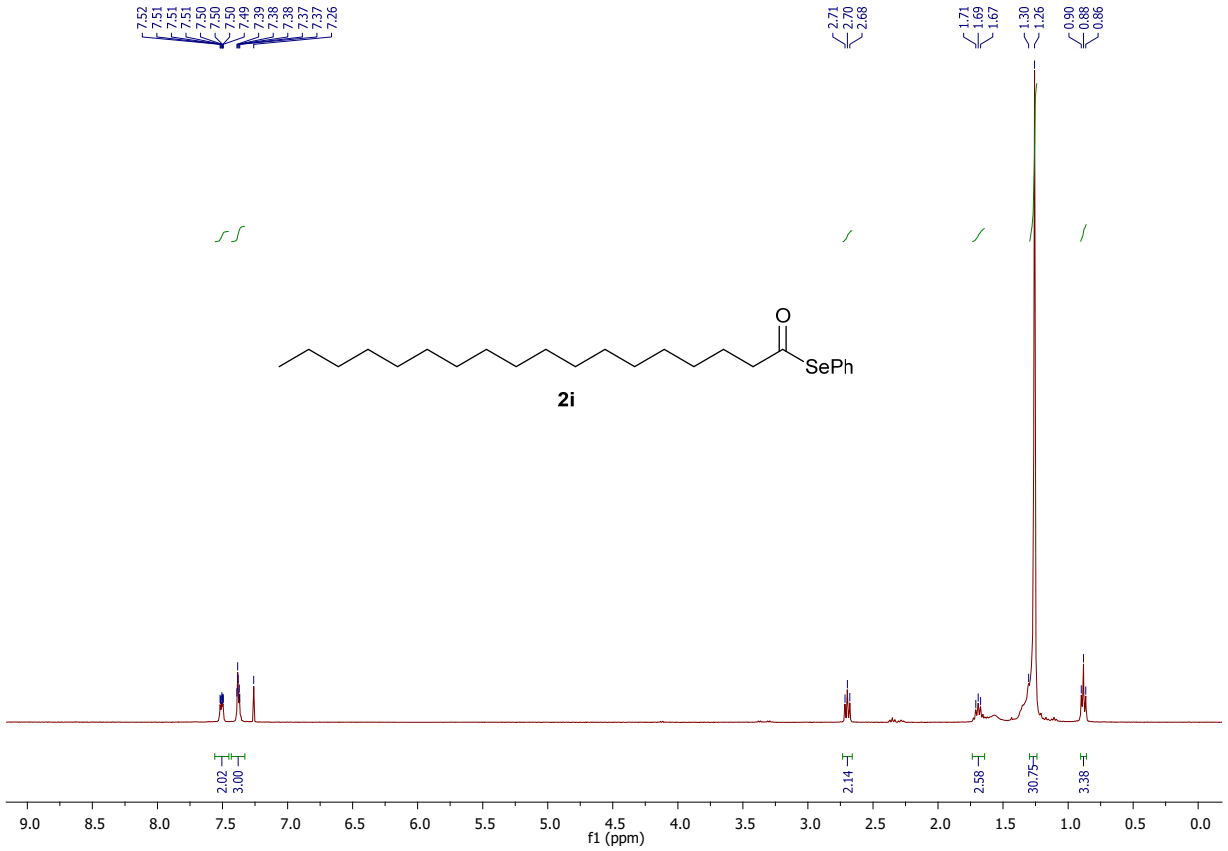
^{1}H NMR of compound **2h** ($CDCl_3$, 400 MHz)



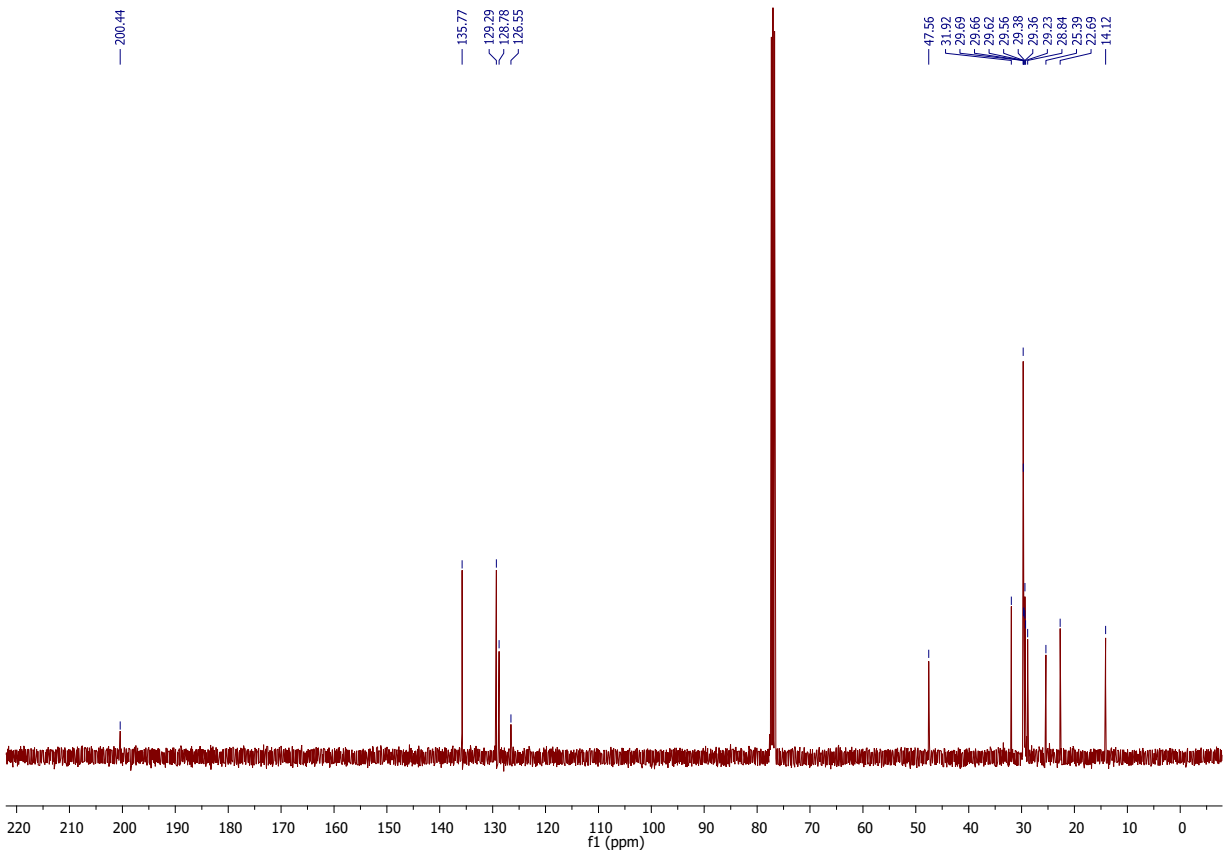
^{13}C NMR of compound **2h** ($CDCl_3$, 100 MHz)



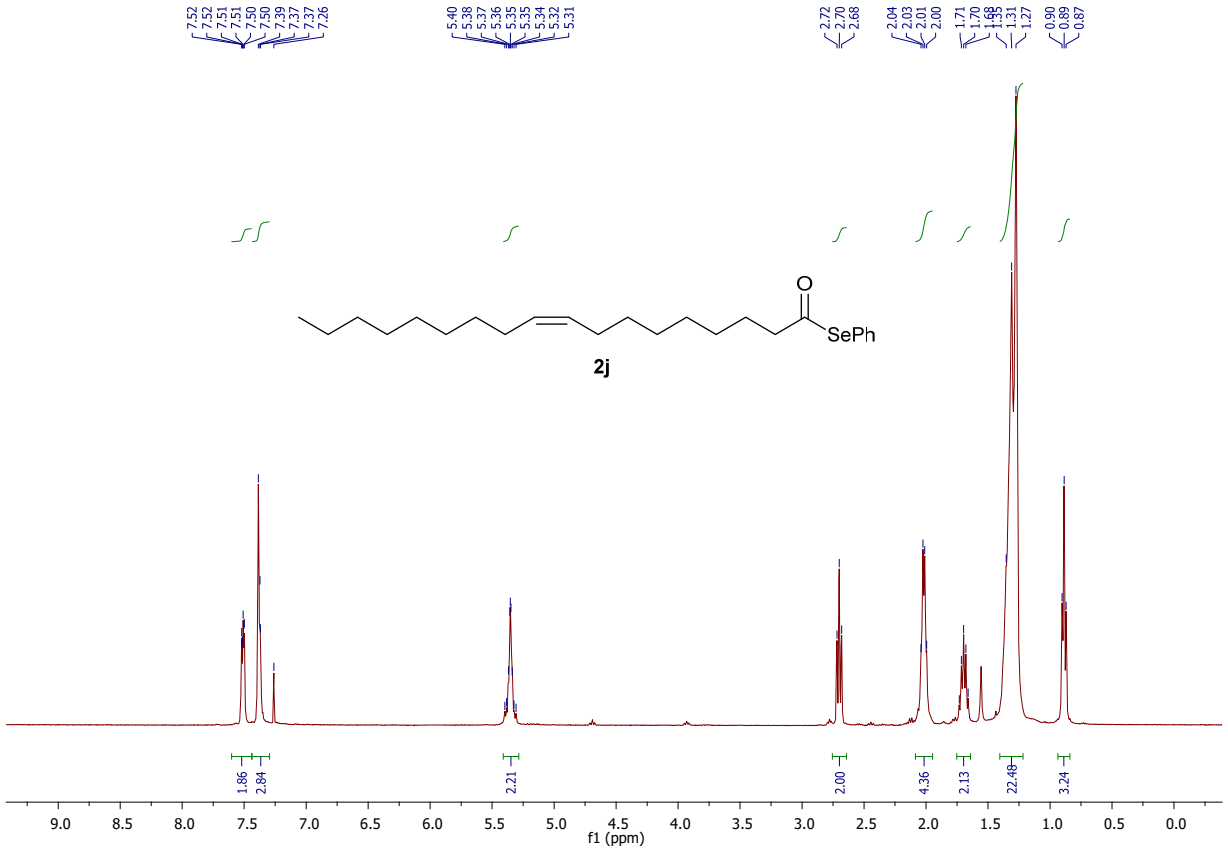
^{1}H NMR of compound **2i** ($CDCl_3$, 400 MHz)



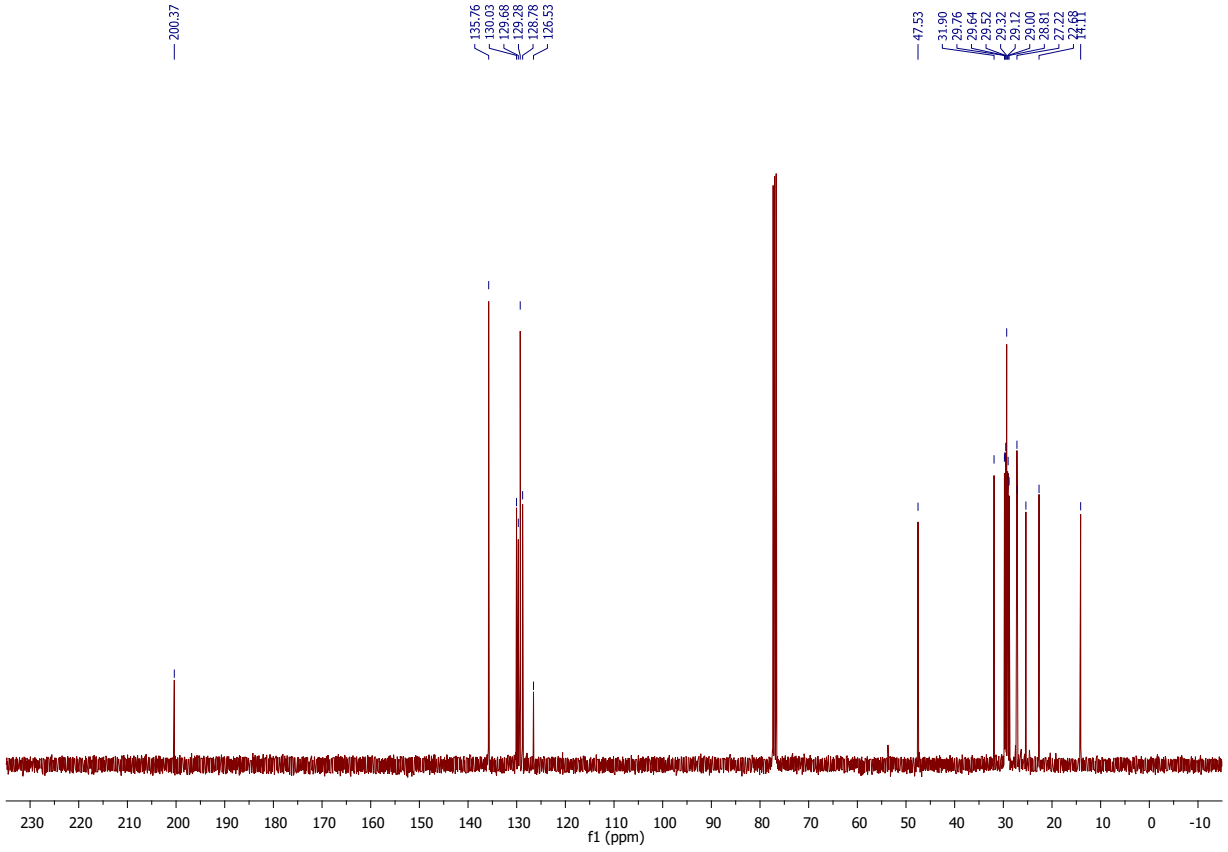
^{13}C NMR of compound **2i** ($CDCl_3$, 100 MHz)



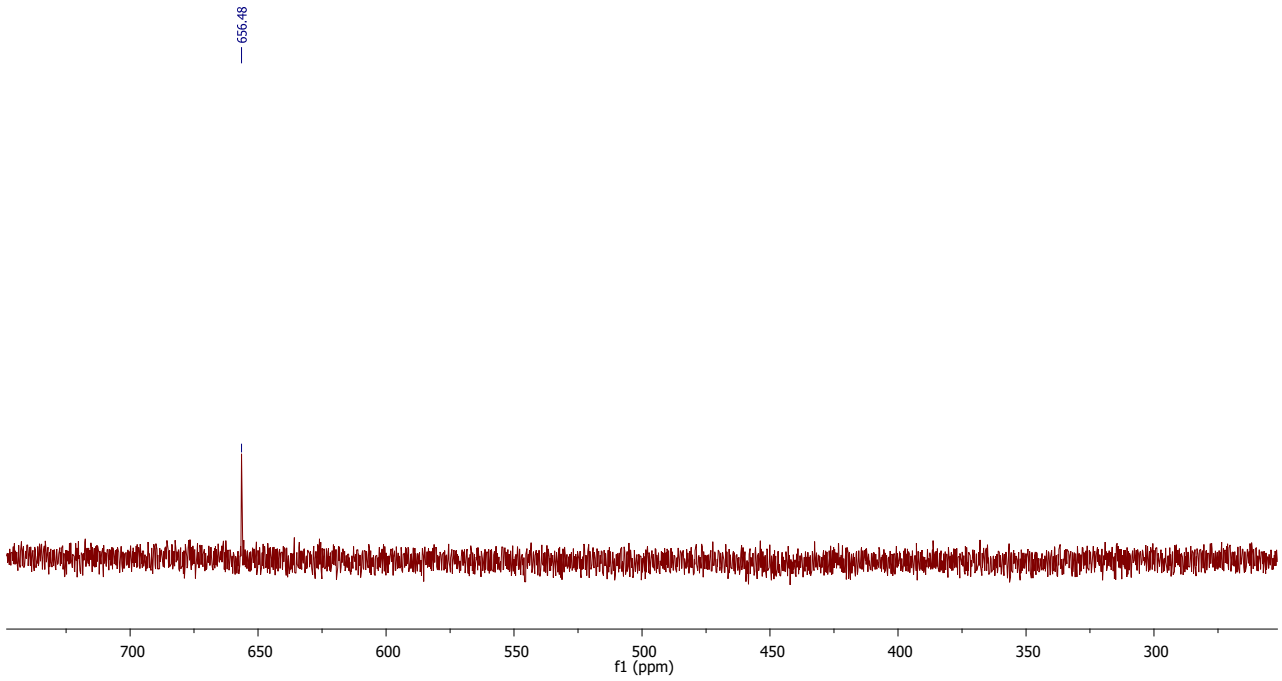
^{1}H NMR of compound **2j** ($CDCl_3$, 400 MHz)



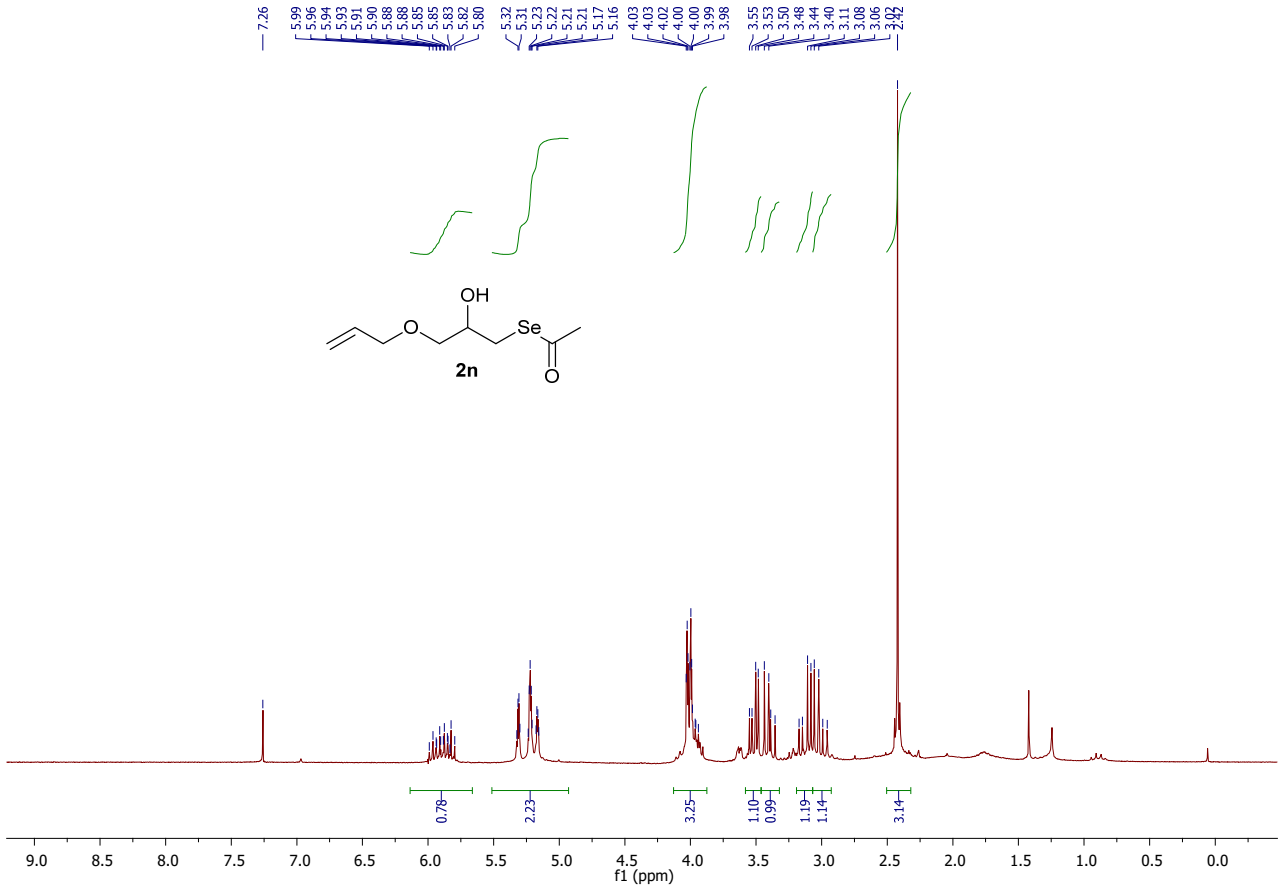
^{13}C NMR of compound **2j** ($CDCl_3$, 100 MHz)



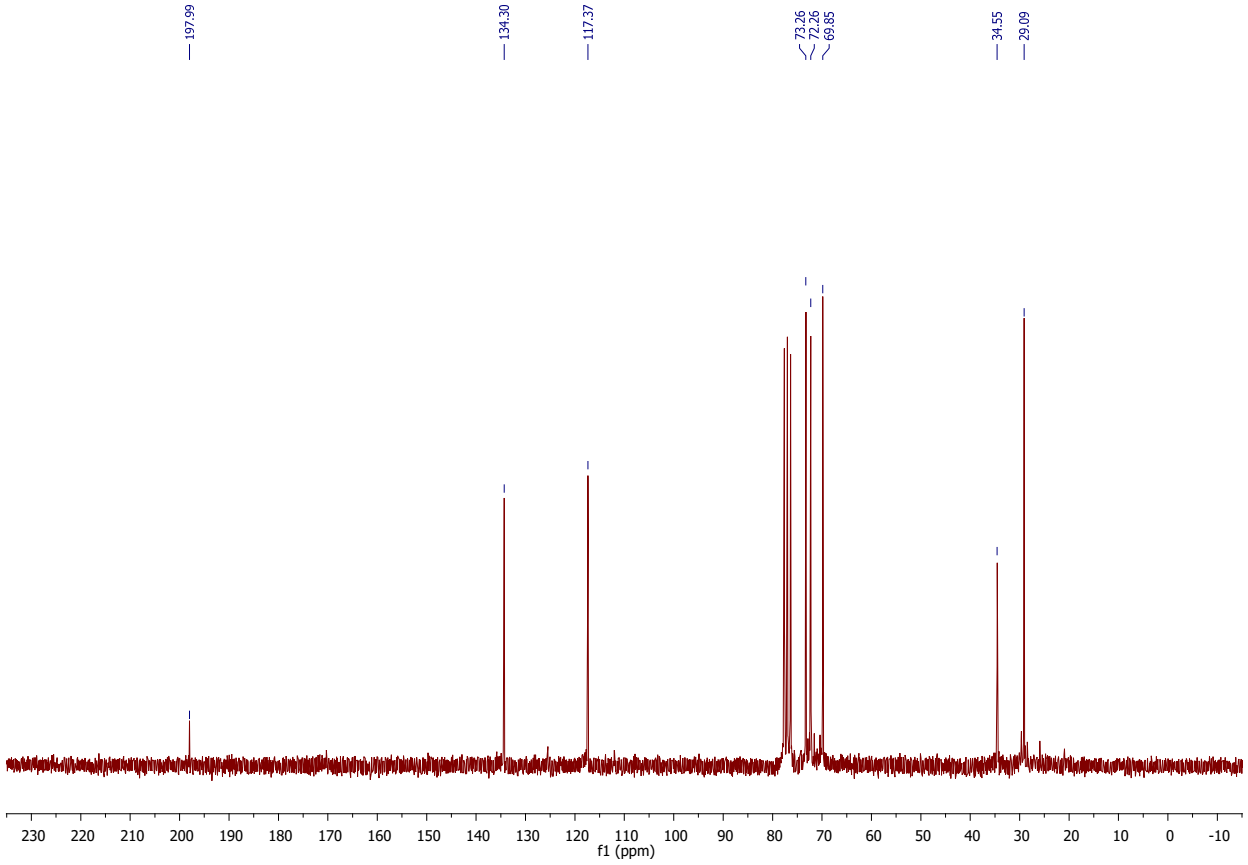
^{77}Se NMR of compound **2j** ($CDCl_3$, 76 MHz)



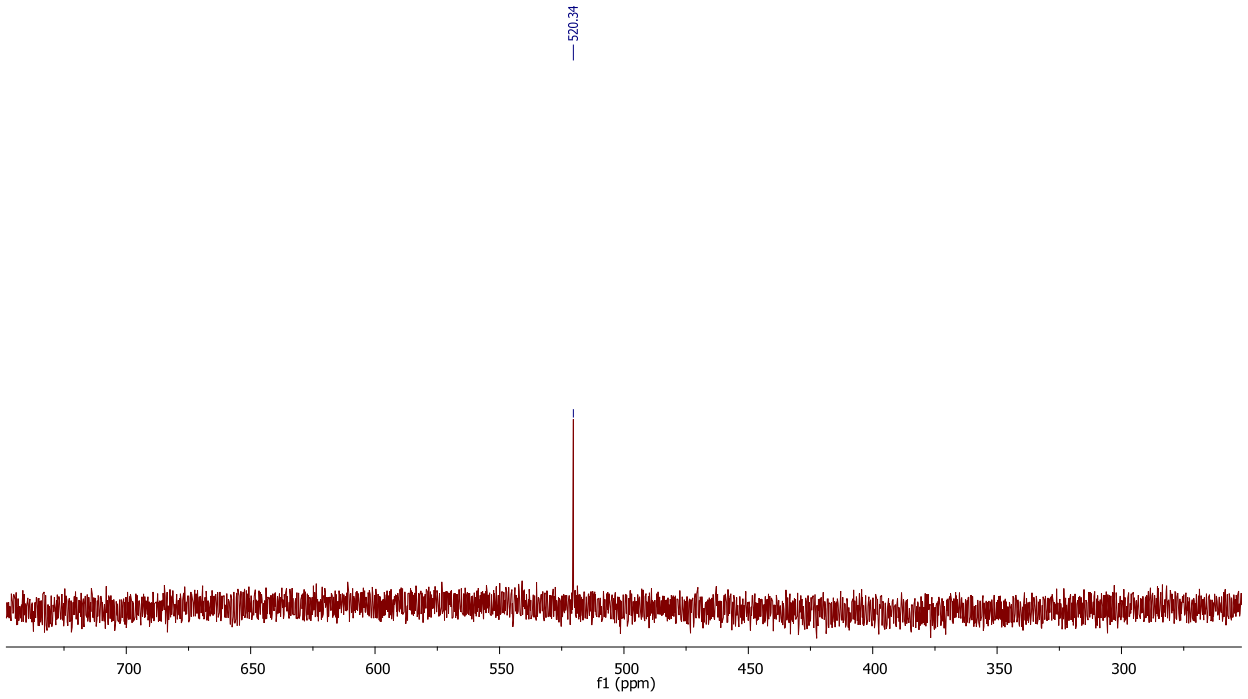
^{1}H NMR of compound **2n** ($CDCl_3$, 400 MHz)



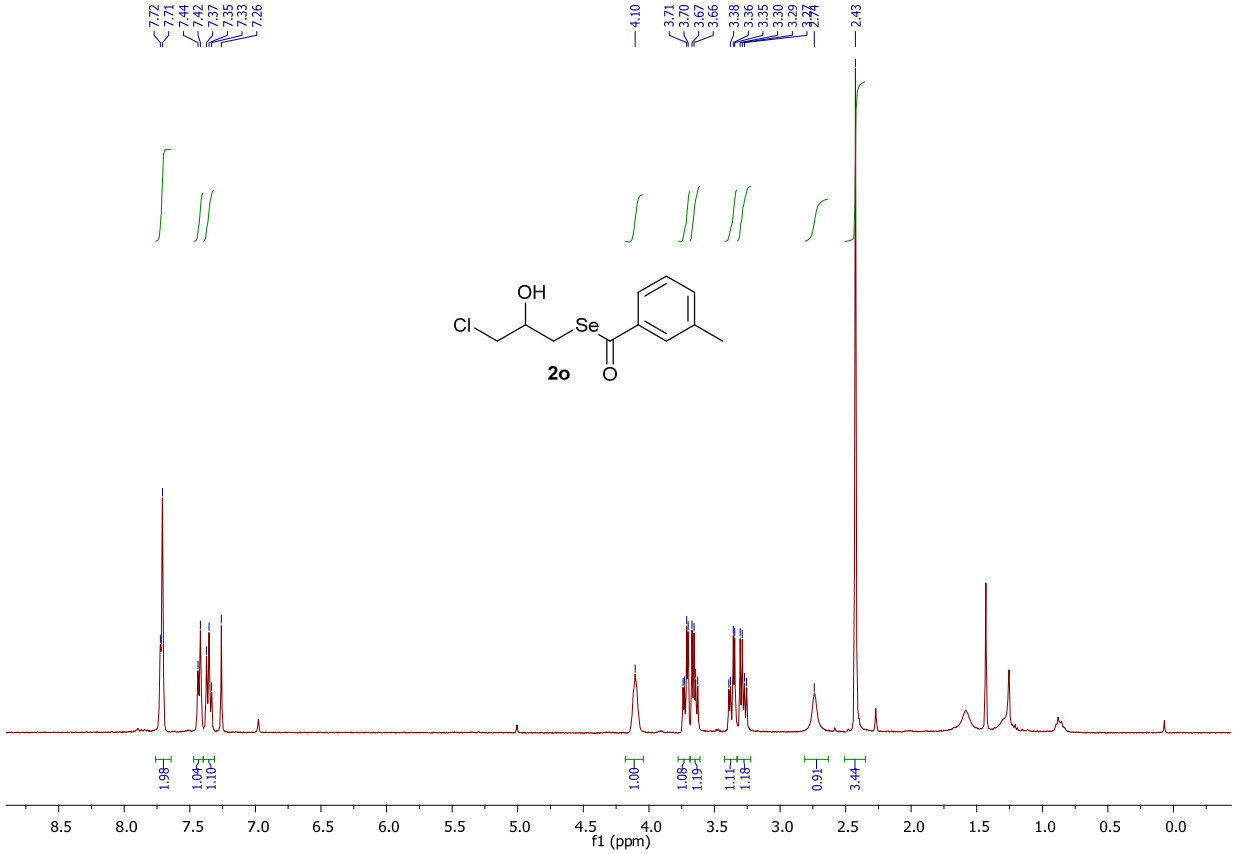
^{13}C NMR of compound **2n** ($CDCl_3$, 100 MHz)



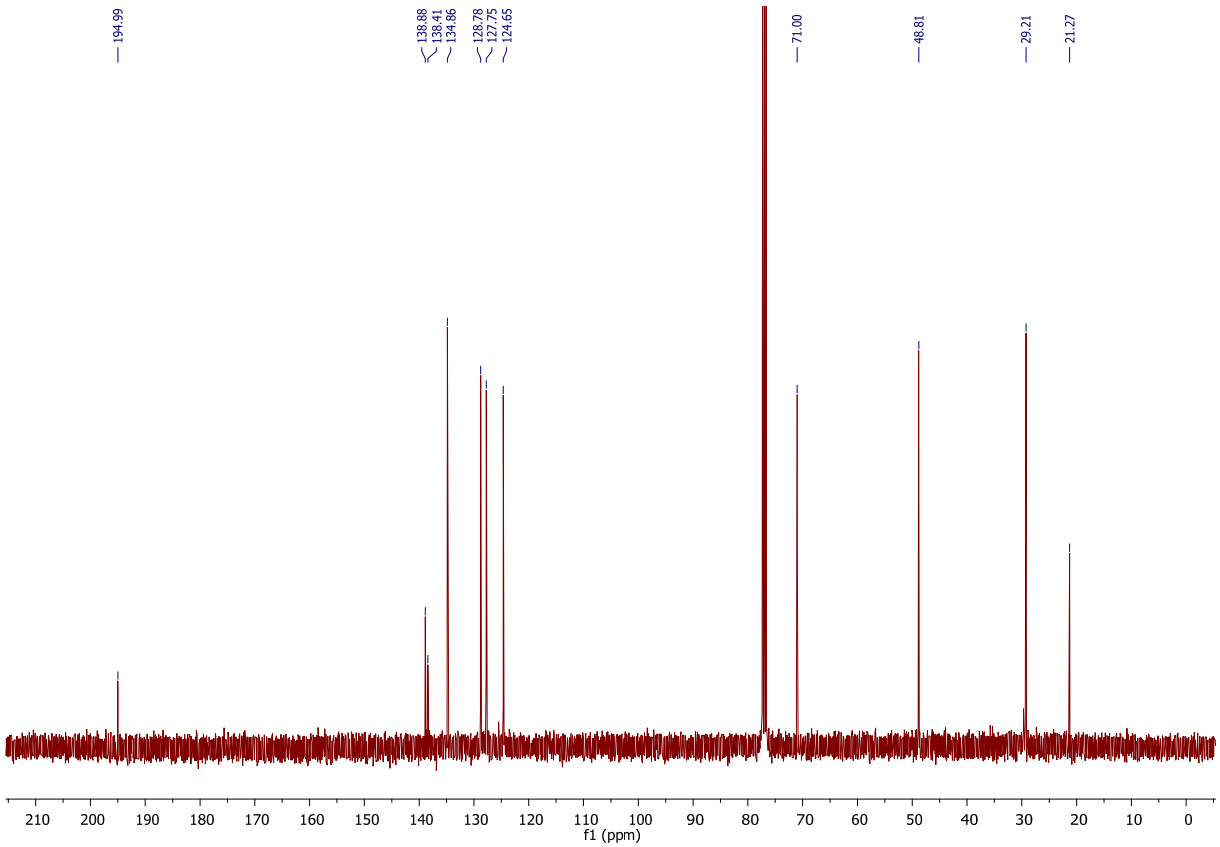
^{77}Se NMR of compound **2n** ($CDCl_3$, 76 MHz)



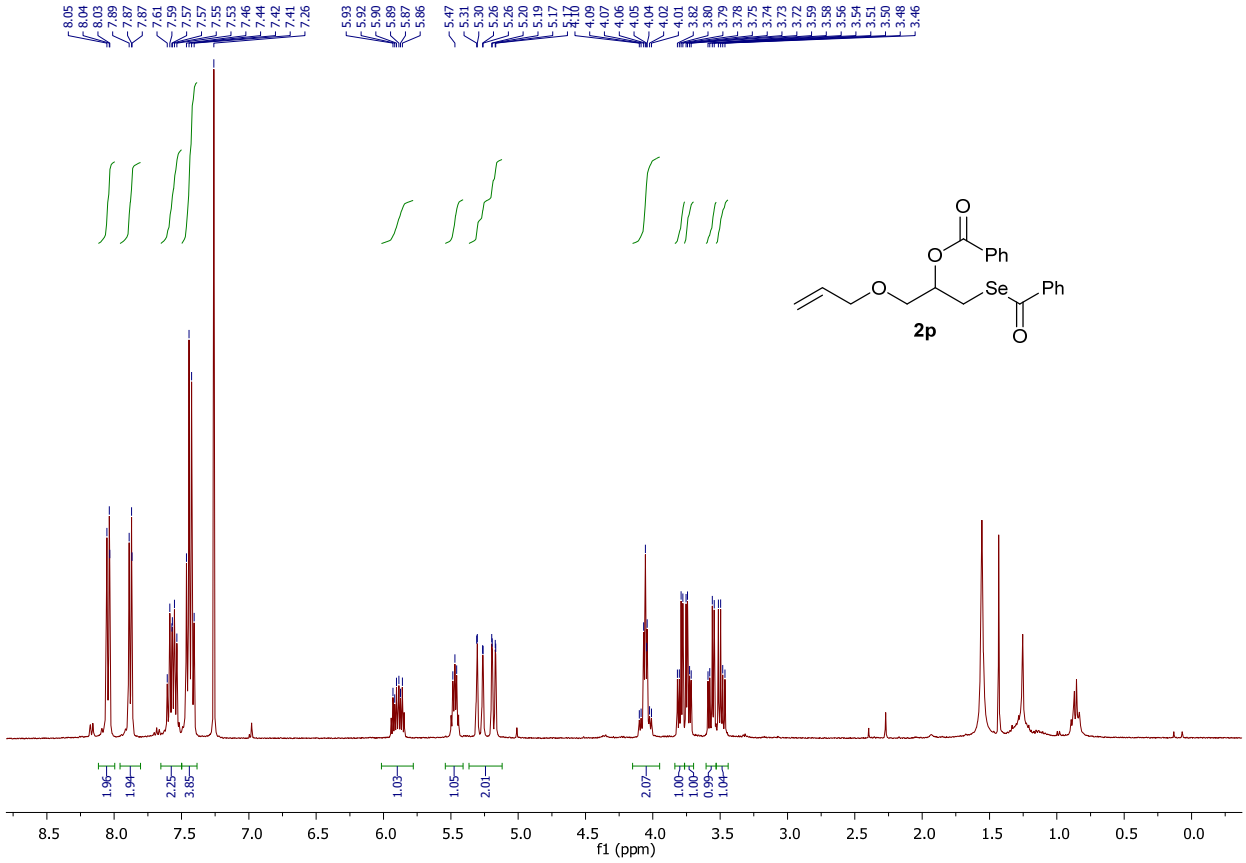
^{1}H NMR of compound **2o** ($CDCl_3$, 400 MHz)



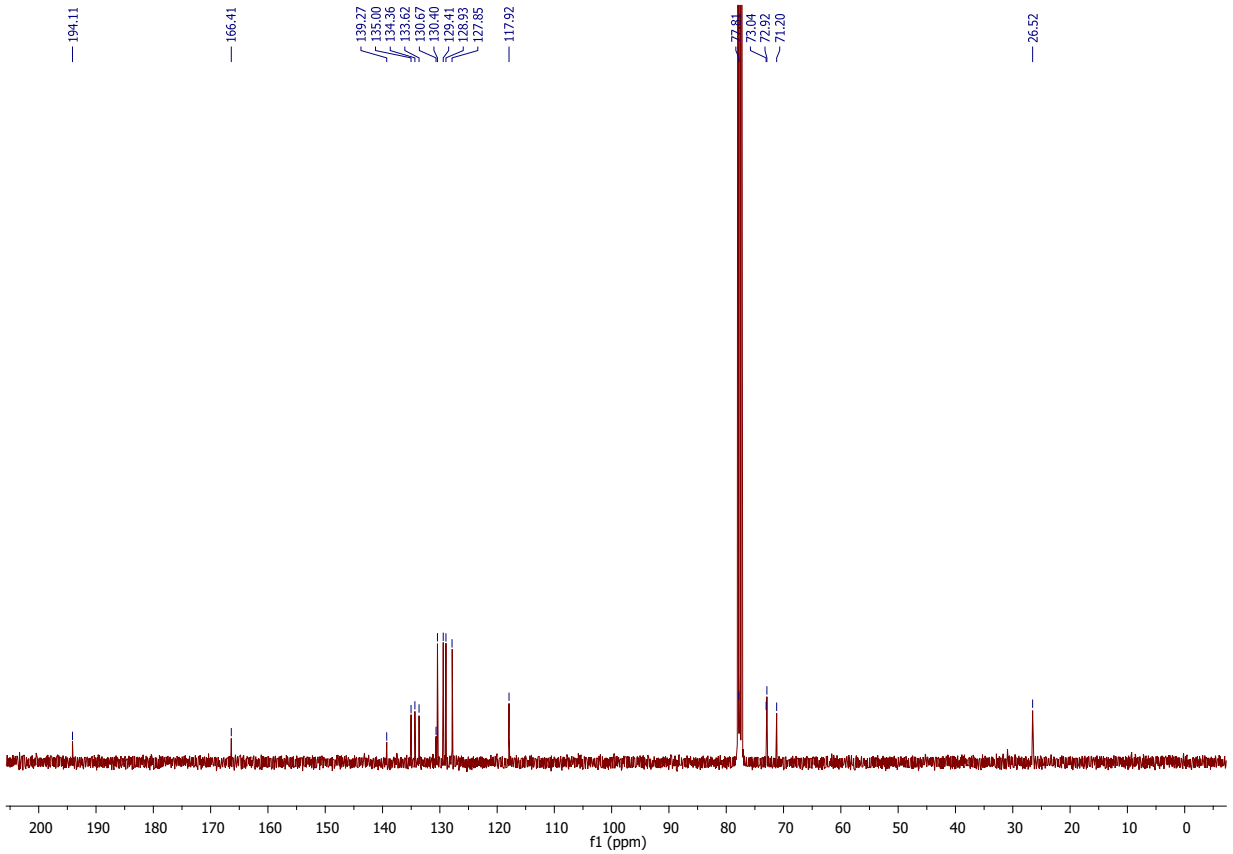
^{13}C NMR of compound **2o** ($CDCl_3$, 100 MHz)



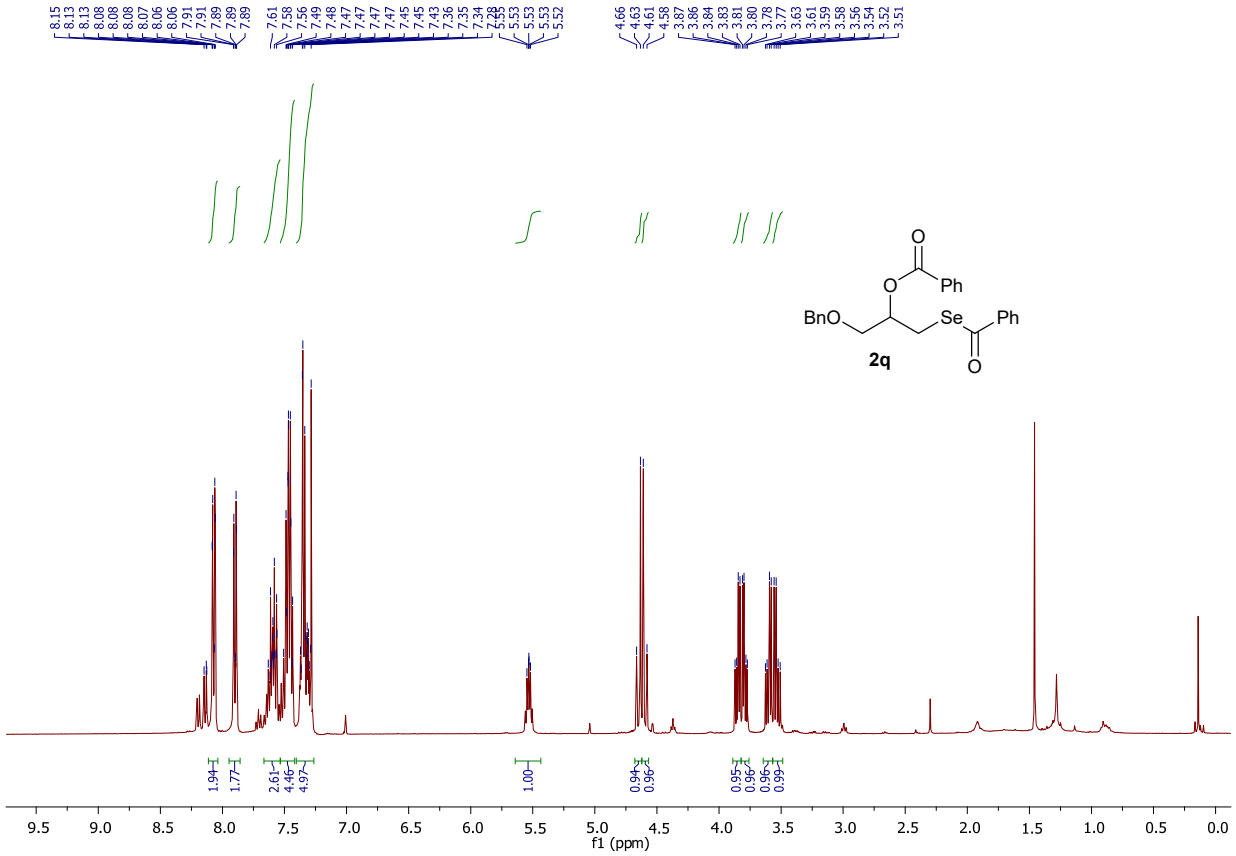
^{1}H NMR of compound **2p** ($CDCl_3$, 400 MHz)



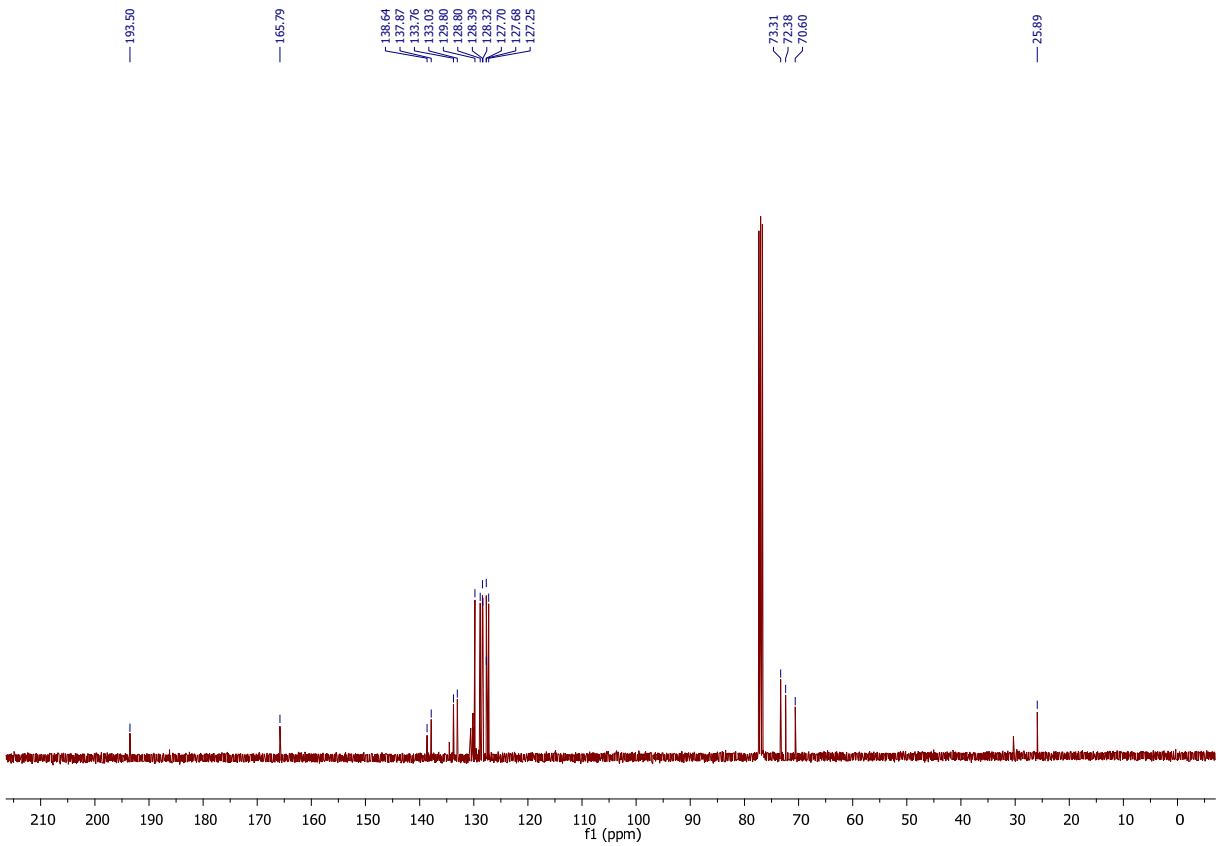
^{13}C NMR of compound **2p** ($CDCl_3$, 100 MHz)



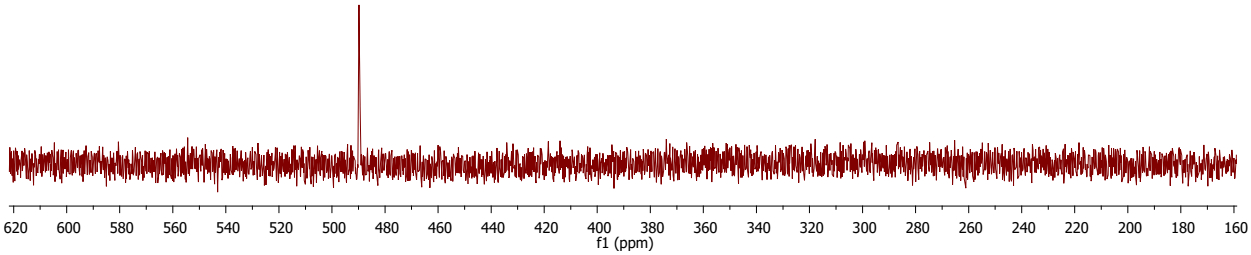
^{1}H NMR of compound **2q** ($CDCl_3$, 400 MHz)



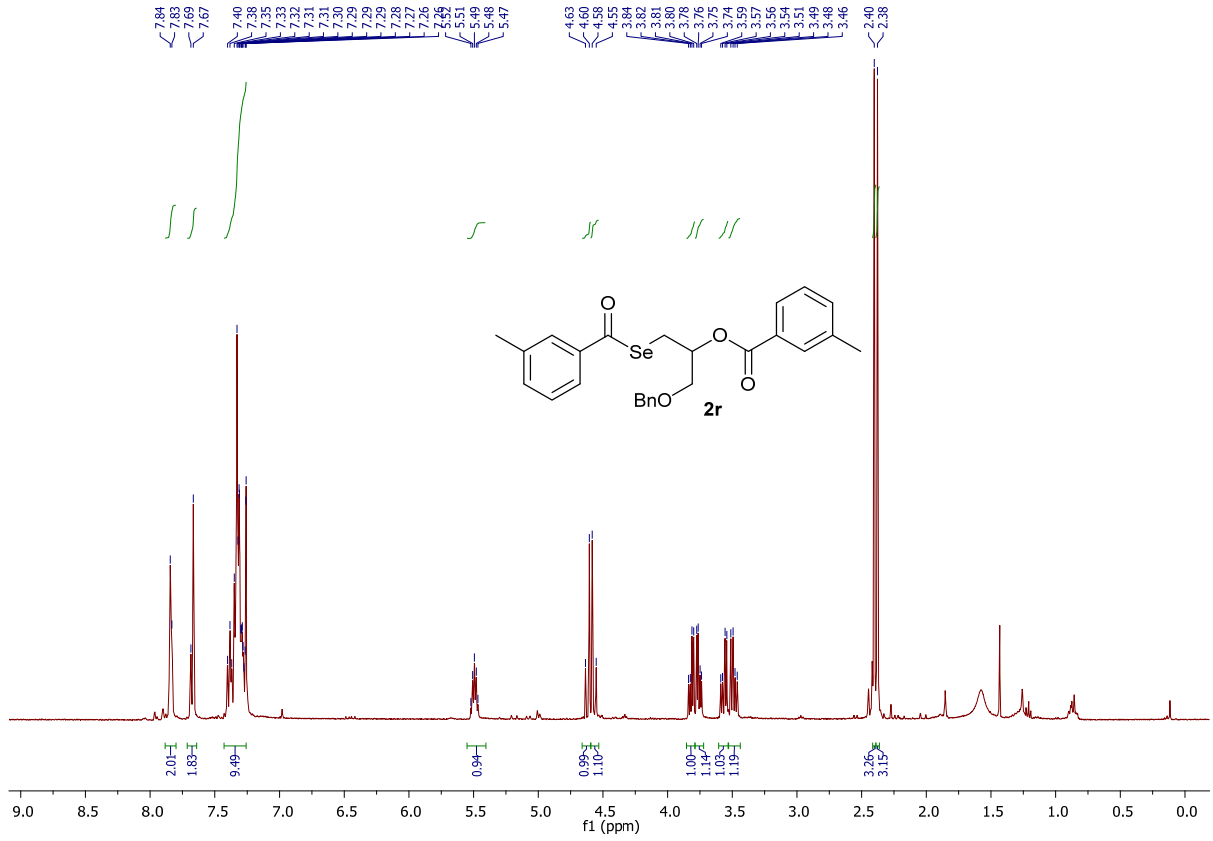
^{13}C NMR of compound **2q** ($CDCl_3$, 100 MHz)



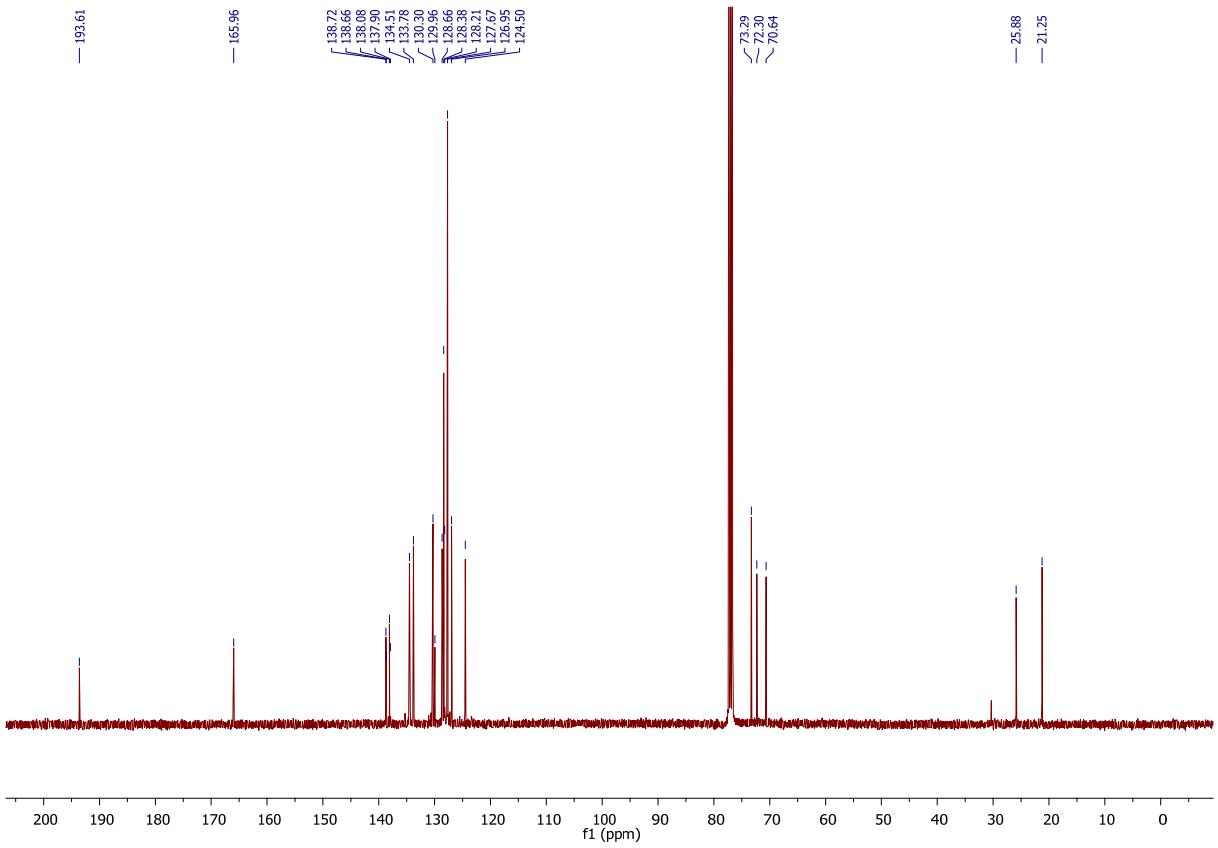
^{77}Se NMR of compound **2q** ($CDCl_3$, 76 MHz)



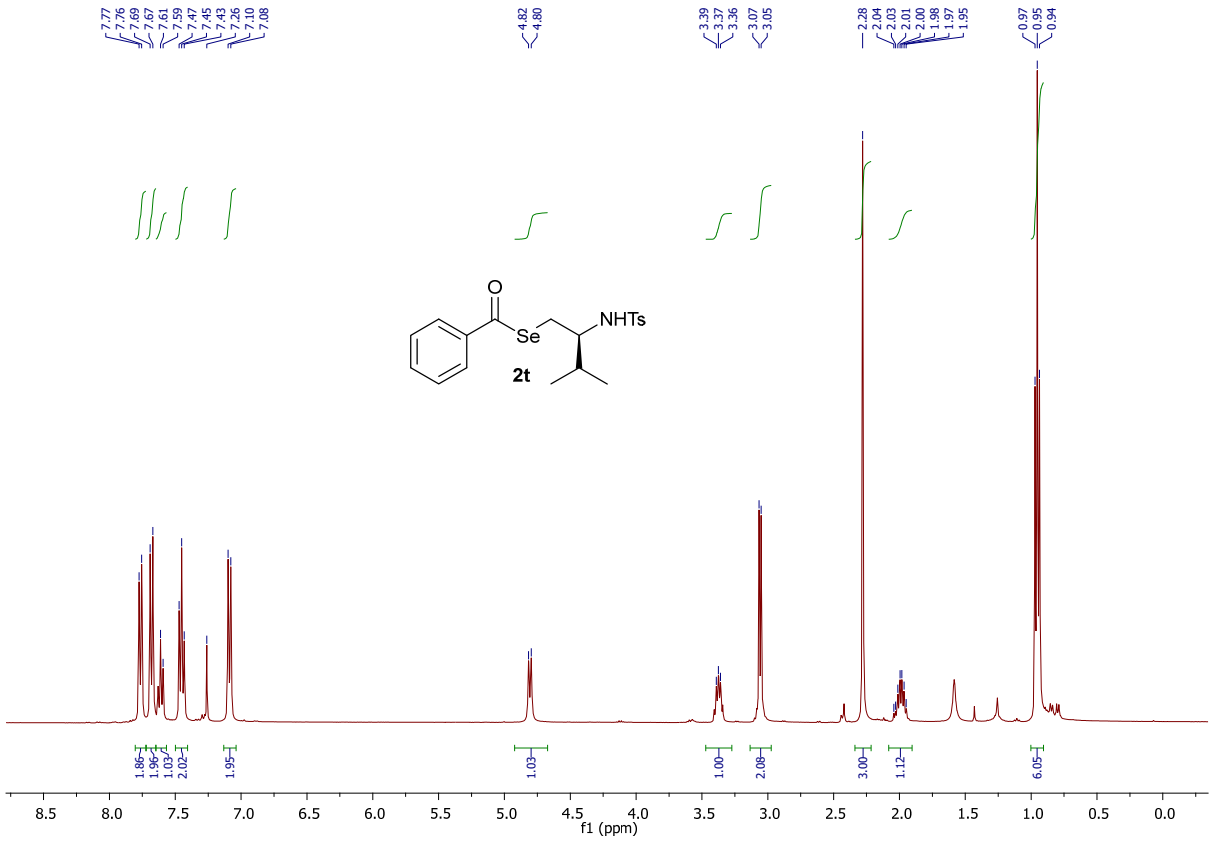
^{1}H NMR of compound **2r** ($CDCl_3$, 400 MHz)



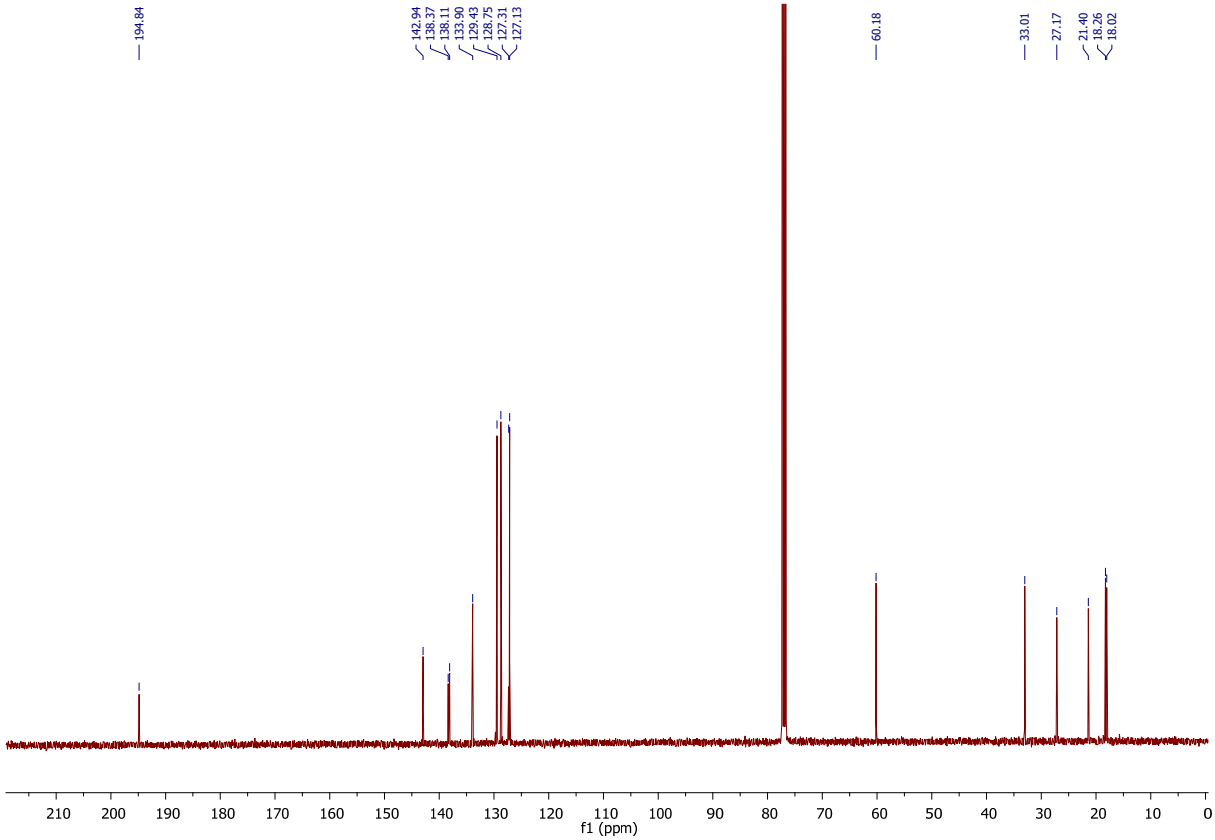
^{13}C NMR of compound **2r** ($CDCl_3$, 100 MHz)



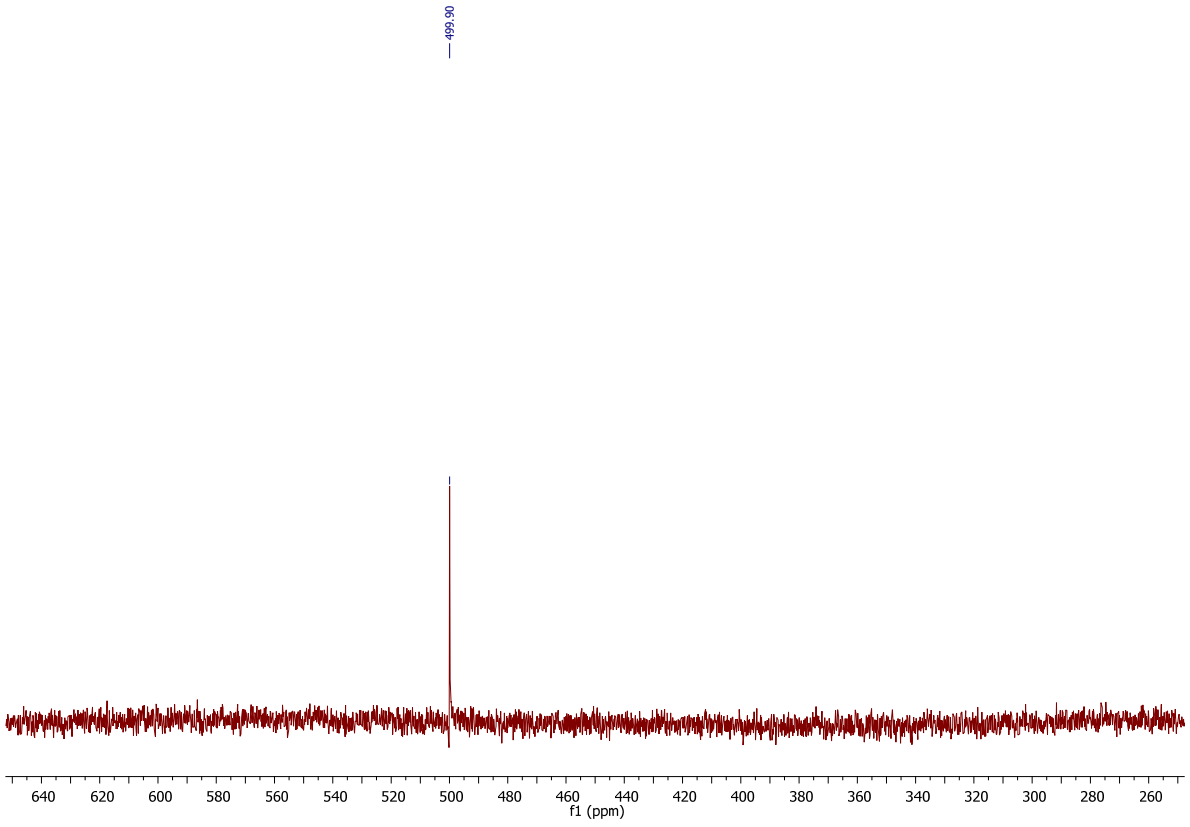
^{1}H NMR of compound **2t** ($CDCl_3$, 400 MHz)



^{13}C NMR of compound **2t** ($CDCl_3$, 100 MHz)



^{77}Se NMR of compound **2t** ($CDCl_3$, 76 MHz)



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