The Total Syntheses of Basiliolide C, epi-Basiliolide C, and Protecting-Group-Free Total Syntheses of Transtaganolides C and D **

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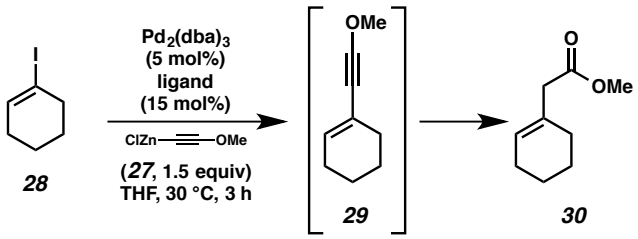
I. Materials and Methods

Unless otherwise stated, reactions were performed in flame-dried glassware under an argon or nitrogen atmosphere using dry deoxygenated solvents. Solvents were dried by passage through an activated alumina column under argon. $Pd(PPh_3)_4$ was prepared using known methods. Thin layer chromatography (TLC), both preparatory and analytical, was visualized by UV fluorescence quenching, *p*-anisaldehyde, I_2, or $KMnO_4$ staining. Analytical super critical fluid chromatography (SFC) was performed using an SFC and AD-H column. Preparatory SFC was performed with a SFC and a prep AD-H column (21 x 250 mm, 5mic part#19445). Silica gel (particle size 0.032-0.063 mm) was used for flash chromatography. ^1H NMR and ^{13}C NMR spectra were recorded at 300 MHz or 500 MHz. ^1H NMR spectra are reported relative to $CDCl_3$ (7.26 ppm). Data for ^1H NMR spectra are reported as follows: chemical shift (ppm), multiplicity, coupling constant (Hz), integration. Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, sept. = septet, m = multiplet, bs = broad singlet. ^{13}C NMR spectra are reported relative to $CDCl_3$ (77.16 ppm). FTIR spectra are reported in frequency of absorption (cm^{-1}). HRMS were acquired using a TOF with a multimode source in electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), or multimode-ESI/APCI.

II. Organozinc Cross-Coupling Optimization



Yields of methyl ester **30**[1] and consumption of vinyl iodide **28**[2] were quantified using the calibration curves (Figure SI-1A and B), which graph the area ratio of GC peaks as a function of the molar ratio of either the methyl ester **30** and the tridecane internal standard or substrate **28** and the tridecane internal standard. This data was collected from several individual GC runs depicted in Table SI-1.

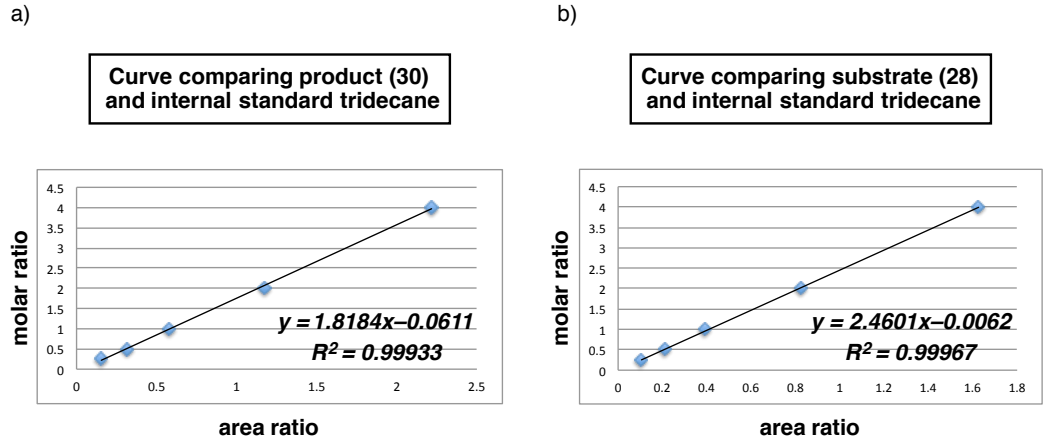
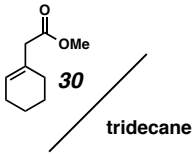


Figure SI-1. *(a) Calibration curve used to determine cross-coupling yields comparing methyl ester **30** and tridecane internal standard. (b) Calibration curve used to determine cross-coupling substrate consumption by comparing vinyl iodide **28** and tridecane internal standard.*

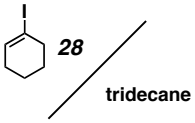
Table SI-1. *(a) The molar ratio of product **30** and tridecane with the associated peak area ratio obtained from GC analysis. (b) The molar ratio of substrate **28** and tridecane with the associated peak area ratio obtained from GC analysis.*

a)



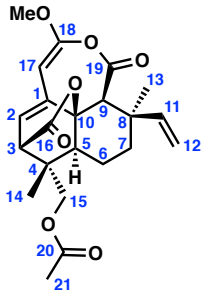
entry	molar ratio	area ratio
1	4	2.217
2	2	1.169
3	1	0.5758
4	0.5	0.3133
5	0.25	0.1547

b)



entry	molar ratio	area ratio
1	4	1.626
2	2	0.8269
3	1	0.3913
4	0.5	0.2134
5	0.25	0.1055

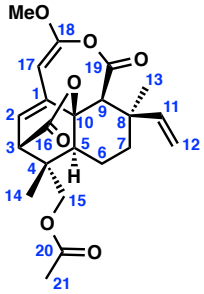
III. Comparison of Spectral Data for Synthetic and Reported Basiliolide C (8), Transtaganolide C (4), and Transtaganolide D (5)



basiliolide C (8)

Table SI-2. Comparison of ^{1}H NMR data for synthetic and reported natural[3] basiliolide C (**8**).

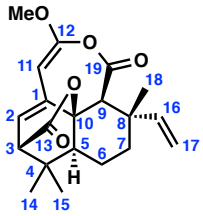
Assignment	Synthetic 8 (ppm)	Multiplicity, *J* (Hz)	Natural 8 (ppm)	Multiplicity, *J* (Hz)
C1	—	—	—	—
C2	6.05	dd, 6.5, 1.2	6.06	dd, 6.4, 1.1
C3	3.29	d, 6.5	3.29	d, 6.4
C4	—	—	—	—
C5	1.32–1.24	m	1.29	dd, 12.9, 4.6
C6	1.75	qd, 13.5, 3.0	1.76	dddd, 13.8, 13.0, 12.9, 2.8
	1.64–1.50	m	1.54	dddd, 13.8, 4.6, 3.9, 2.8
C7	1.93	dt, 13.5, 3.4	1.94	ddd, 13.7, 3.9, 2.8
	1.41	td, 13.5, 3.0	1.42	ddd, 13.7, 13.0, 2.8
C8	—	—	—	—
C9	3.14	s	3.15	s
C10	—	—	—	—
C11	7.00	dd, 17.7, 11.1	7.00	dd, 17.7, 11.1
C12	5.16	dd, 11.1, 1.4	5.17	dd, 11.0, 1.0
	5.06	dd, 17.7, 1.2	5.06	dd, 17.7, 1.0
C13	1.23	s	1.24	s
C14	1.12	s	1.12	s
C15	3.73	d, 10.8	3.74	d, 10.8
	3.69	d, 10.8	3.70	d, 10.8
C16	—	—	—	—
C17	5.00	d, 1.4	5.01	d, 1.1
C18	—	—	—	—
C19	—	—	—	—
C20	—	—	—	—
C21	2.08	s	2.09	s
OMe	3.73	s	3.74	s



basiliolide C (8)

Table SI-3. Comparison of ^{13}C NMR data for synthetic and reported natural[3] basiliolide C (**8**).

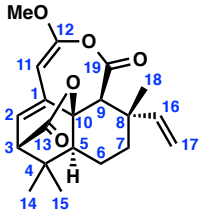
Assignment	Synthetic 8 (ppm)	Natural 8 (ppm)
C1	138.6	138.6
C2	123.0	122.8
C3	49.8	49.7
C4	37.1	37.0
C5	44.8	44.7
C6	21.2	21.1
C7	40.3	40.2
C8	38.6	38.4
C9	53.4	53.3
C10	87.0	86.9
C11	142.7	142.6
C12	112.5	112.3
C13	28.7	28.6
C14	19.8	19.6
C15	70.6	70.4
C16	170.7	170.7
C17	79.2	79.0
C18	157.0	156.9
C19	162.4	162.3
C20	171.0	170.8
C21	20.9	20.7
OMe	56.6	56.4



transtaganolide C (4)

Table SI-4. Comparison of ^{1}H NMR data for synthetic and reported natural[4] transtaganolide C (**4**).

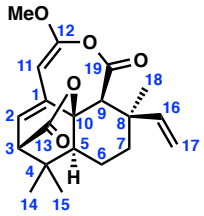
Assignment	Synthetic 4 (ppm)	Multiplicity, *J* (Hz)	Natural 4 (ppm)	Multiplicity, *J* (Hz)
C1	—	—	—	—
C2	6.07	dd, 6.5, 1.5	6.08	dd, 6.5, 1.1
C3	3.06	d, 6.5	3.07	d, 6.5
C4	—	—	—	—
C5	1.34–1.27	m	1.30	dd, 11.5, 6.5
C6	1.71–1.63	m	1.70–1.60	m
	1.71–1.63	m	1.70–1.60	m
C7	1.48–1.39	m	1.44	ddd, 13.0, 13.0, 3.3
	1.71–1.63	m	1.65	m
C8	—	—	—	—
C9	3.23	s	3.24	s
C10	—	—	—	—
C11	5.00	d, 1.5	5.01	d, 1.1
C12	—	—	—	—
C13	—	—	—	—
C14	0.97	s	0.98	s
C15	1.08	s	1.09	s
C16	5.80	dd, 17.5, 11.0	5.81	dd, 17.4, 10.8
C17	5.03	d, 11.0	5.05	br d, 10.7
	5.07	d, 17.5	5.08	br d, 17.4
C18	1.60	s	1.61	s
C19	—	—	—	—
OMe	3.71	s	3.72	s



transtaganolide C (4)

Table SI-5. Comparison of ^{13}C NMR data for synthetic and reported natural[4] transtaganolide C (**4**).

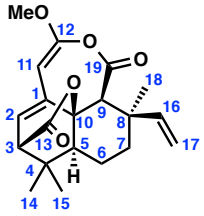
Assignment	Synthetic 4 (ppm)	Natural 4 (ppm)
C1	138.0	131.9
C2	123.6	123.5
C3	53.8	53.8
C4	33.3	33.2
C5	48.1	47.9
C6	19.9	19.8
C7	38.3	38.3
C8	38.4	38.4
C9	50.6	50.4
C10	87.3	87.4
C11	79.3	79.1
C12	156.7	156.7
C13	171.8	172.0
C14	29.9	29.6
C15	24.8	24.8
C16	146.5	146.5
C17	112.8	112.8
C18	19.2	19.1
C19	162.3	162.5
OMe	56.6	56.3



transtaganolide D (5)

Table SI-6. Comparison of ^{1}H NMR data for synthetic and reported natural[4] transtaganolide D (**5**).

Assignment	Synthetic 5 (ppm)	Multiplicity, *J* (Hz)	Natural 5 (ppm)	Multiplicity, *J* (Hz)
C1	—	—	—	—
C2	6.09	dd, 6.5, 1.0	6.08	dd, 6.5, 1.2
C3	3.06	d, 6.5	3.04	d, 6.6
C4	—	—	—	—
C5	1.33	dd, 13.5, 3.5	1.34	dd, 12.6, 5.3
C6	1.64	dquint, 13.5, 3.0	1.65–1.53	m
	1.59–1.56	m	1.65–1.53	m
C7	1.39	dt, 13.5, 3.5	1.39	ddd, 13.0, 13.0, 3.3
	1.91	dt, 13.5, 3.5	1.89	ddd, 13.0, 3.3, 3.3
C8	—	—	—	—
C9	3.13	s	3.14	s
C10	—	—	—	—
C11	5.02	d, 1.0	5.02	d, 1.1
C12	—	—	—	—
C13	—	—	—	—
C14	0.97	s	0.96	s
C15	1.04	s	1.02	s
C16	7.00	dd, 17.5, 11.0	6.98	dd, 17.8, 11.2
C17	5.15	dd, 11.0, 1.0	5.11	dd, 11.1, 1.1
	5.05	dd, 17.5, 1.0	5.03	dd, 17.8, 1.1
C18	1.22	s	1.21	s
C19	—	—	—	—
OMe	3.73	s	3.72	s



transtaganolide D (5)

Table SI-7. Comparison of ^{13}C NMR data for synthetic and reported natural[4] transtaganolide D (**5**).

Assignment	Synthetic 5 (ppm)	Natural 5 (ppm)
C1	137.7	137.5
C2	123.9	123.8
C3	54.0	53.9
C4	33.3	33.2
C5	48.4	48.2
C6	20.5	20.3
C7	40.5	40.3
C8	38.4	38.3
C9	53.3	53.1
C10	87.3	87.4
C11	79.4	79.2
C12	156.7	156.6
C13	171.7	171.9
C14	29.9	29.8
C15	24.8	24.7
C16	142.9	142.9
C17	112.1	111.9
C18	28.5	28.4
C19	162.6	162.7
OMe	56.3	56.3

IV. Bioactivity of Synthetic Transtaganolide C (4)

Appendino, Muñoz and coworkers assayed naturally isolated transtaganolide C (**4**) for its effect on calcium mobilization as well as cell viability following exposure to the natural product **4**.[5] To ensure the data obtained was not the result of trace thapsigargin (**1**) contamination in the isolation sample, we sent synthetic transtaganolide C (**4**) to be tested as a control. Both calcium mobilization (Figure SI-2)[6] and cell viability (Figure SI-3)[6] assays were consistent with those observed with naturally isolated transtaganolide C (**4**).

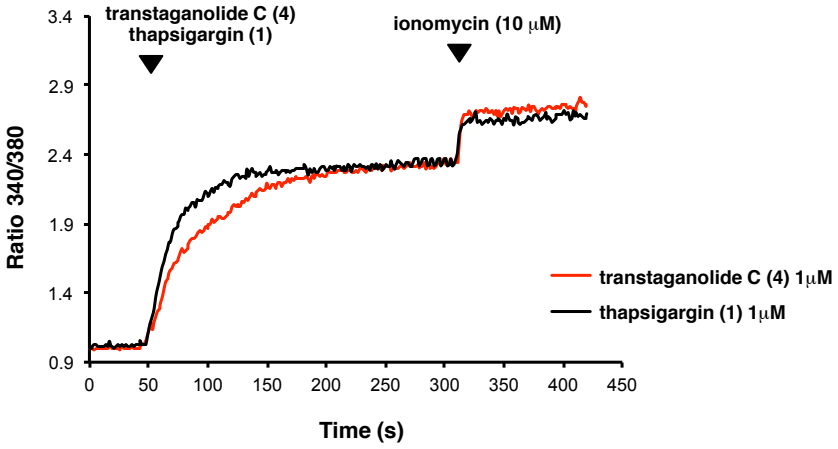


Figure SI-2. *The effect of synthetic transtaganolide C (**4**) or naturally isolated thapsigargin (**1**) on calcium mobilization as a function of time (arrows indicate the time at which a reagent was added).*[6]

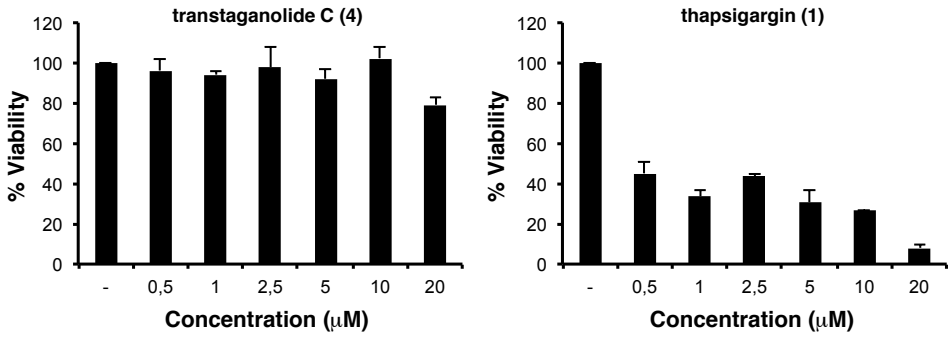


Figure SI-3. *Percentage of cells still viable after 24 hours of exposure to either synthetic transtaganolide C (**4**) or thapsigargin (**1**).*[6]

General procedure for calcium mobilization. Jurkat cells were incubated for 1 h at 37 ºC in Tyrode's salt solution (137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 0.4 mM NaH_2PO_4, 12.0 mM $NaHCO_3$, 5.6 mM D-glucose) containing 5 μM Indo1-AM (Invitrogen) for 30 minutes at 37 °C in the dark. Cells were then harvested, washed three times with buffer to remove extracellular Indo-1 dye, readjusted to 10^6 cells/mL in the appropriate buffer and analyzed in a spectrofluorimeter operated in the ratio mode (Hitachi F-2500 model, Hitachi Ltd.) under continuous stirring and at a constant temperature of 37 ºC using a water-

jacketed device. After five minutes for accommodation to equilibrate temperatures, samples were excited at 338 nm and emission was collected at 405 and 485 nm, corresponding to the fluorescence emitted by Ca^{2+}-bound and free Indo-1, respectively. $[Ca^{2+}]_i$ was calculated using the ratio values between bound- and free-Indo-1 fluorescence, and assuming an Indo-1 K_d for Ca^{2+} of 0.23 μM. Maximum and minimum ratio values for calculations were determined by the addition at the end of the measurements of 10 μM ionomycin. $[Ca^{2+}]_i$ changes are presented as changes in the ratio of bound-to-free calcium (340 nm/380 nm).[6]

General procedure for cell viability. For cytotoxicity analysis, Jurkat cells were seeded in 96-well plates in complete medium and treated with increasing doses of transtaganolide C (**4**) or thapsigargin (**1**) at the indicated concentrations (μM) for 24 hours. Samples were then diluted with 300 μL of PBS and incubated 1 minute at 23 °C in the presence of propidium iodine (10 μg/ml). After incubation, cells were immediately analyzed by flow cytometry. The results are represented as the percentage of viability considering 100% viability for the untreated cells.[6]

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[1] Methyl ester **30** was synthesized for GC studies using known methods: Kapferer, T.; Brückner, R. *Eur. J. Org. Chem.* **2006**, 2119–2133.

[2] Iodo-cyclohexene (**28**) synthesized for GC studies using known methods: Kropp, P. J.; McNeely, S. A.; Davis, R. D. *J. Am. Chem. Soc.* **1983**, *105*, 6907–6915.

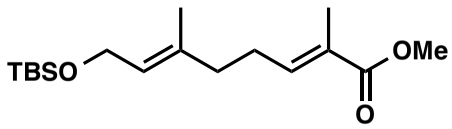
[3] Appendino, G.; Prosperini, S.; Valdivia, C.; Ballero, M.; Colombano, G.; Billington, R. A.; Genazzani, A. A.; Sterner, O. *J. Nat. Prod.* **2005**, *68*, 1213–1217.

[4] Saouf, A.; Guerra, F. M.; Rubal, J. J.; Moereno-Dorado, F. J.; Akssira, M.; Mellouki, F.; López, M.; Pujadas, A. J.; Jorge, Z. D.; Massanet, G. M. *Org Lett.* **2005**, *7*, 881–884.

[5] Navarrete, C.; Sancho, R.; Caballero, F. J.; Pollastro, F.; Fiebich, B. L.; Sterner, O.; Appendino, G.; Muñoz, E. *J. Pharmacol. Exp. Ther.* **2006**, *319*, 422–430.

[6] Muñoz, E. Departamento de Biología Celular, Fisiología e Inmunología, Facultad de Medicina, Universidad de Cordoba, Cordoba, Spain. Personal communication, July 2013.

V. Spectra



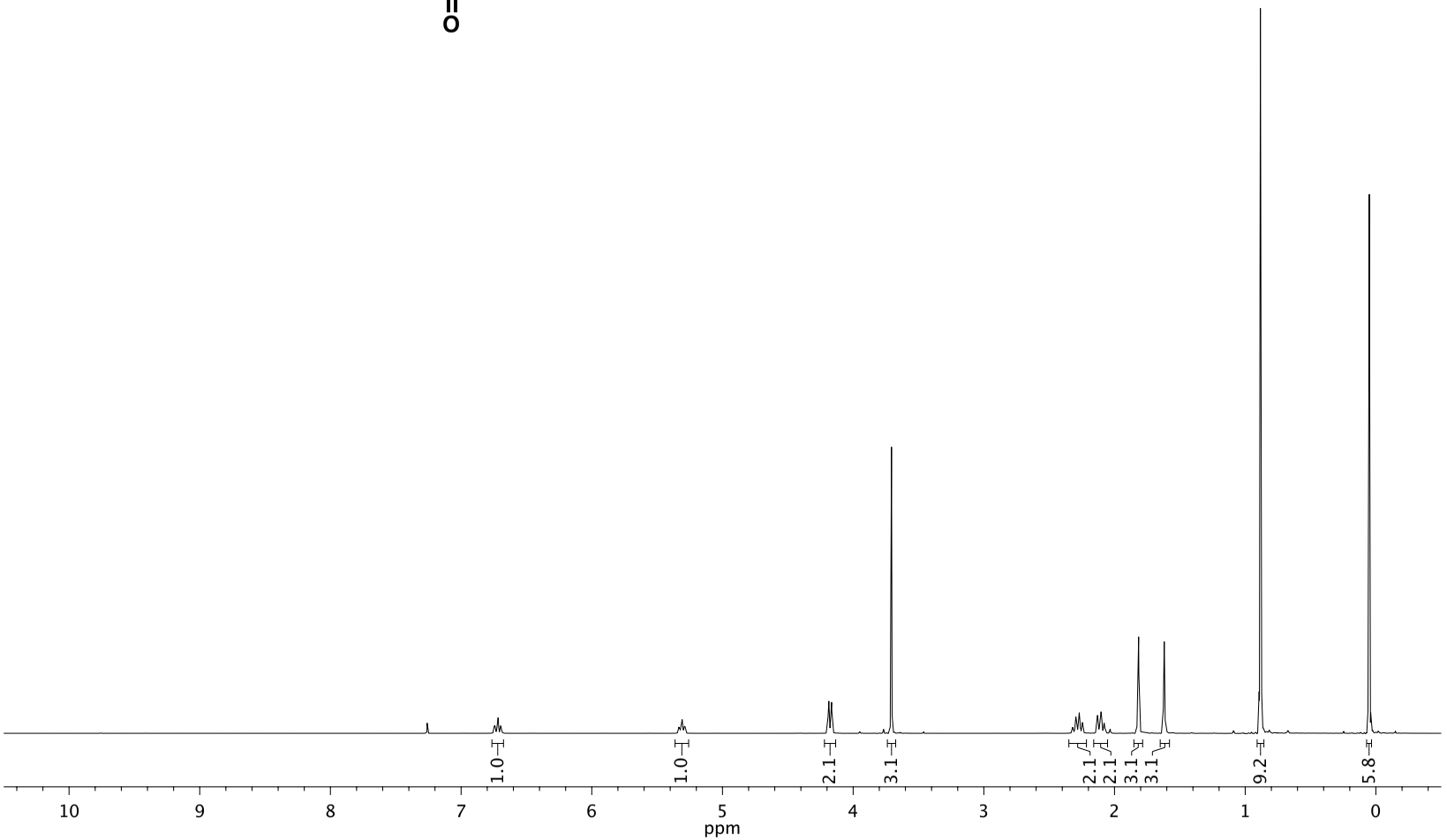


Figure SI-4A ^{1}H NMR 300 MHz, $CDCl_3$) of compound **17**.

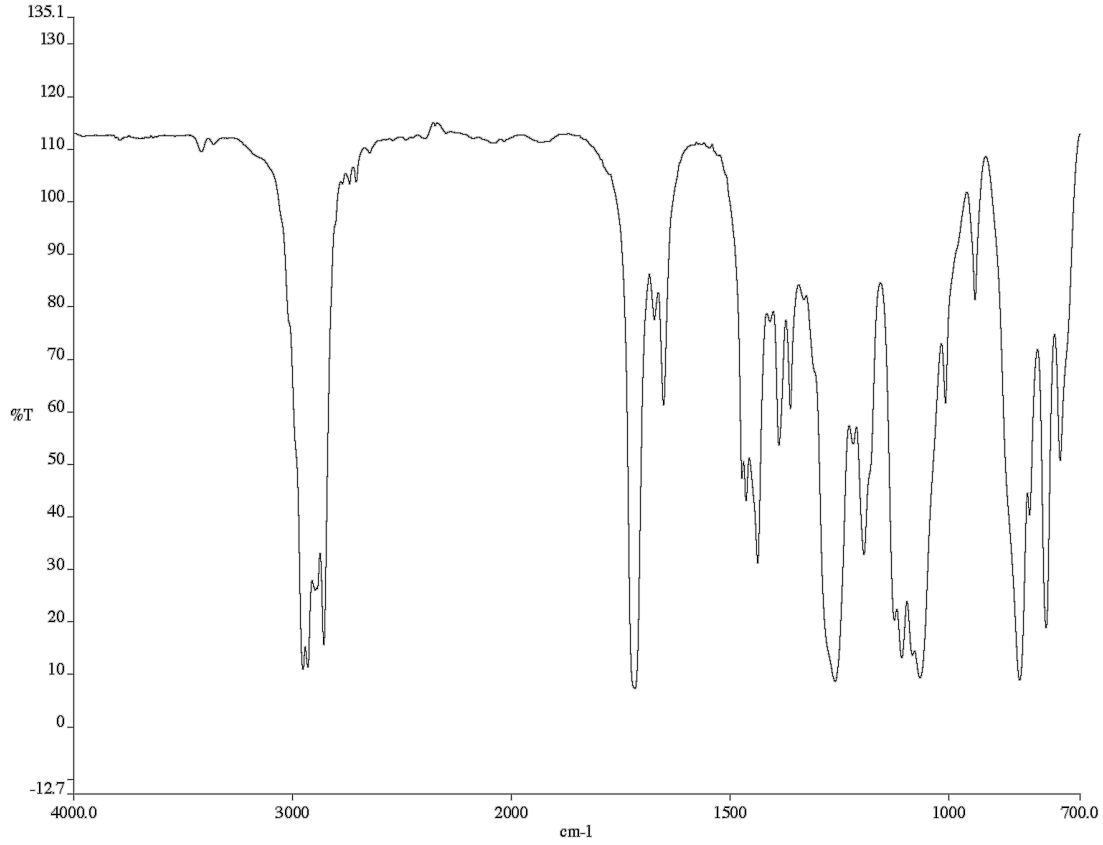


Figure SI-4B infrared spectrum (Thin Film, NaCl) of compound **17**.

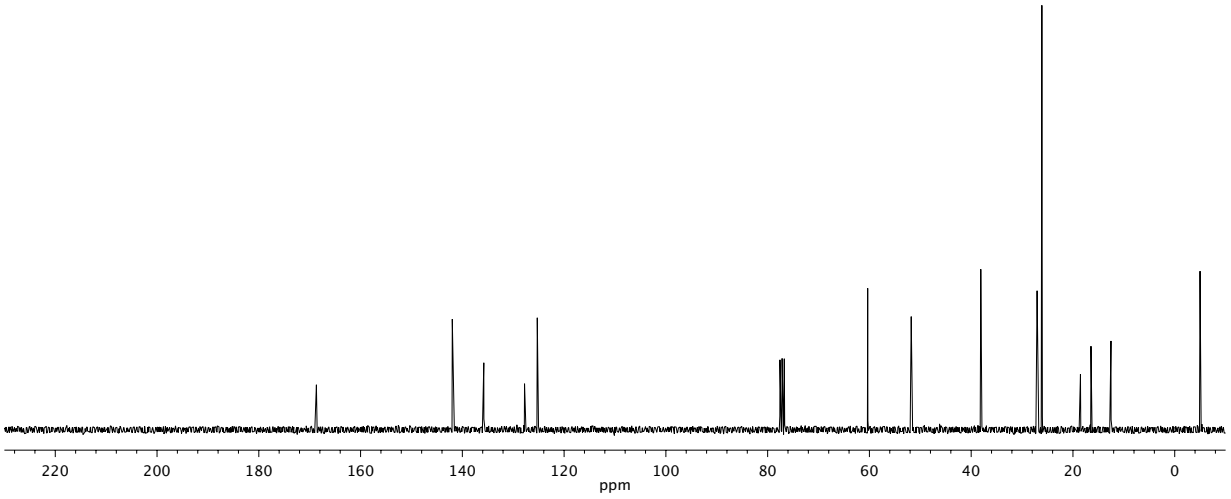


Figure SI-4C ^{13}C NMR (75 MHz, $CDCl_3$) of compound **17**.

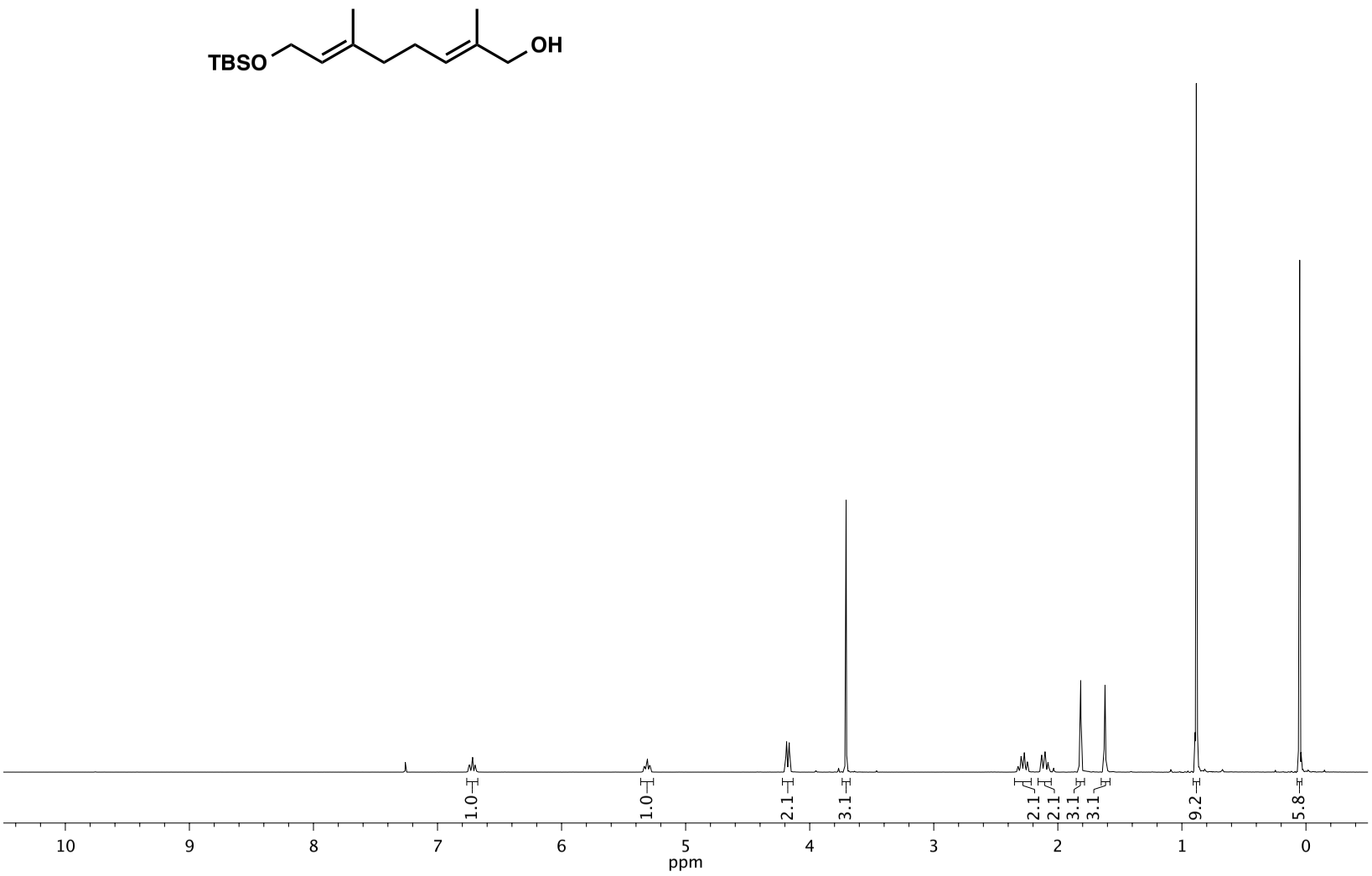


Figure SI-5A ^{1}H NMR 300 MHz, $CDCl_3$) of compound **18**.

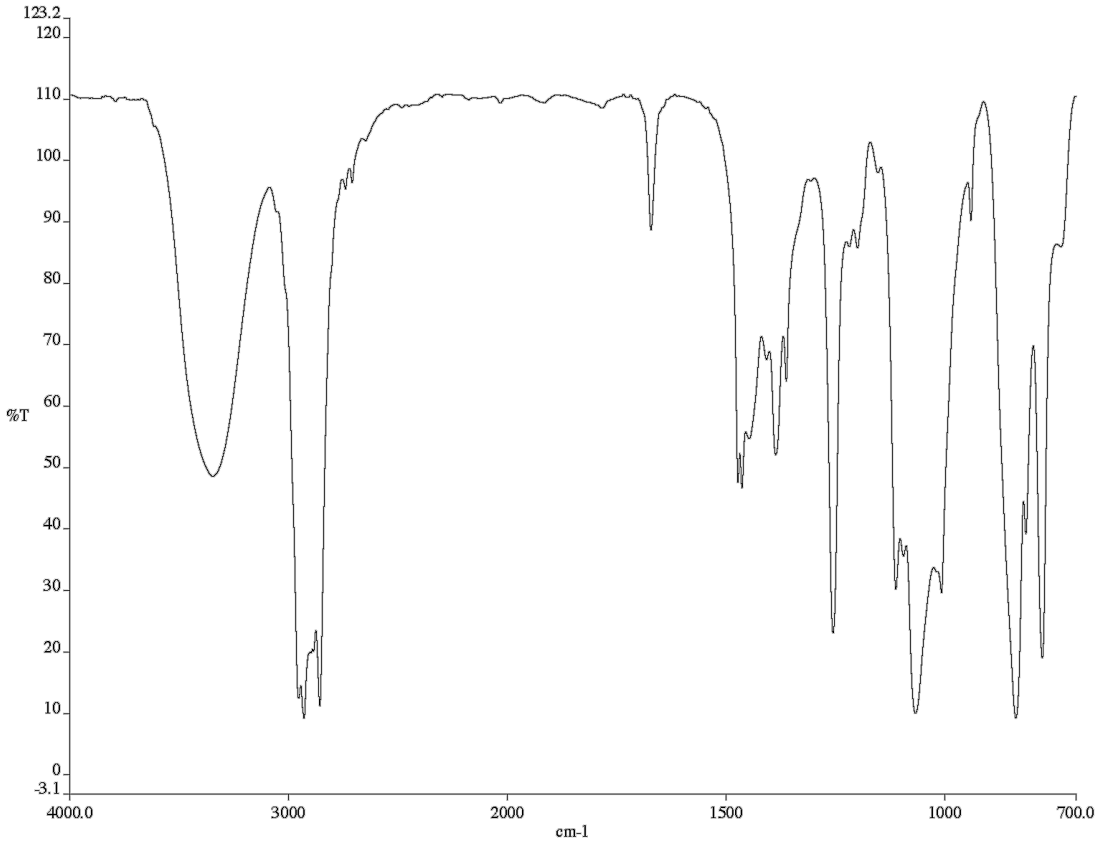


Figure SI-5B infrared spectrum (Thin Film, NaCl) of compound **18**.

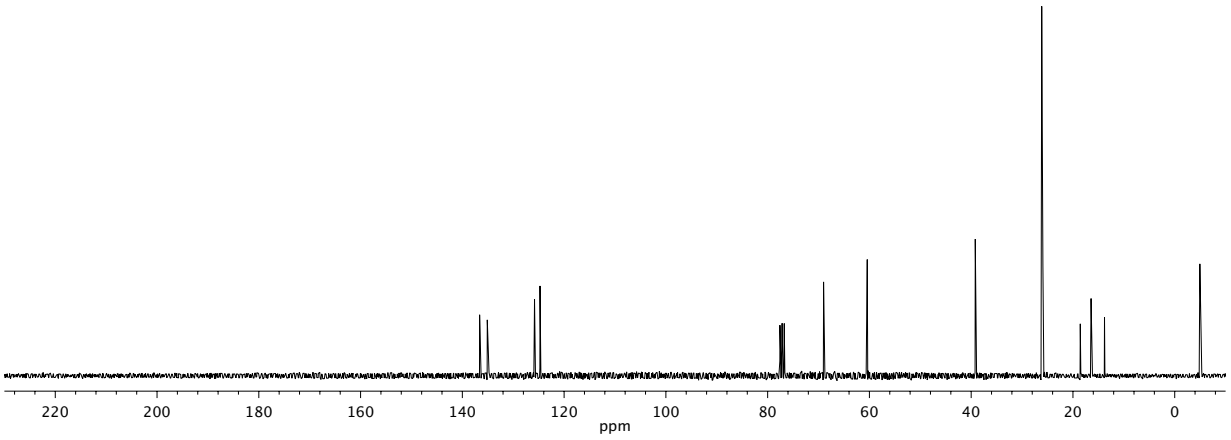


Figure SI-5C ^{13}C NMR (75 MHz, $CDCl_3$) of compound **18**.

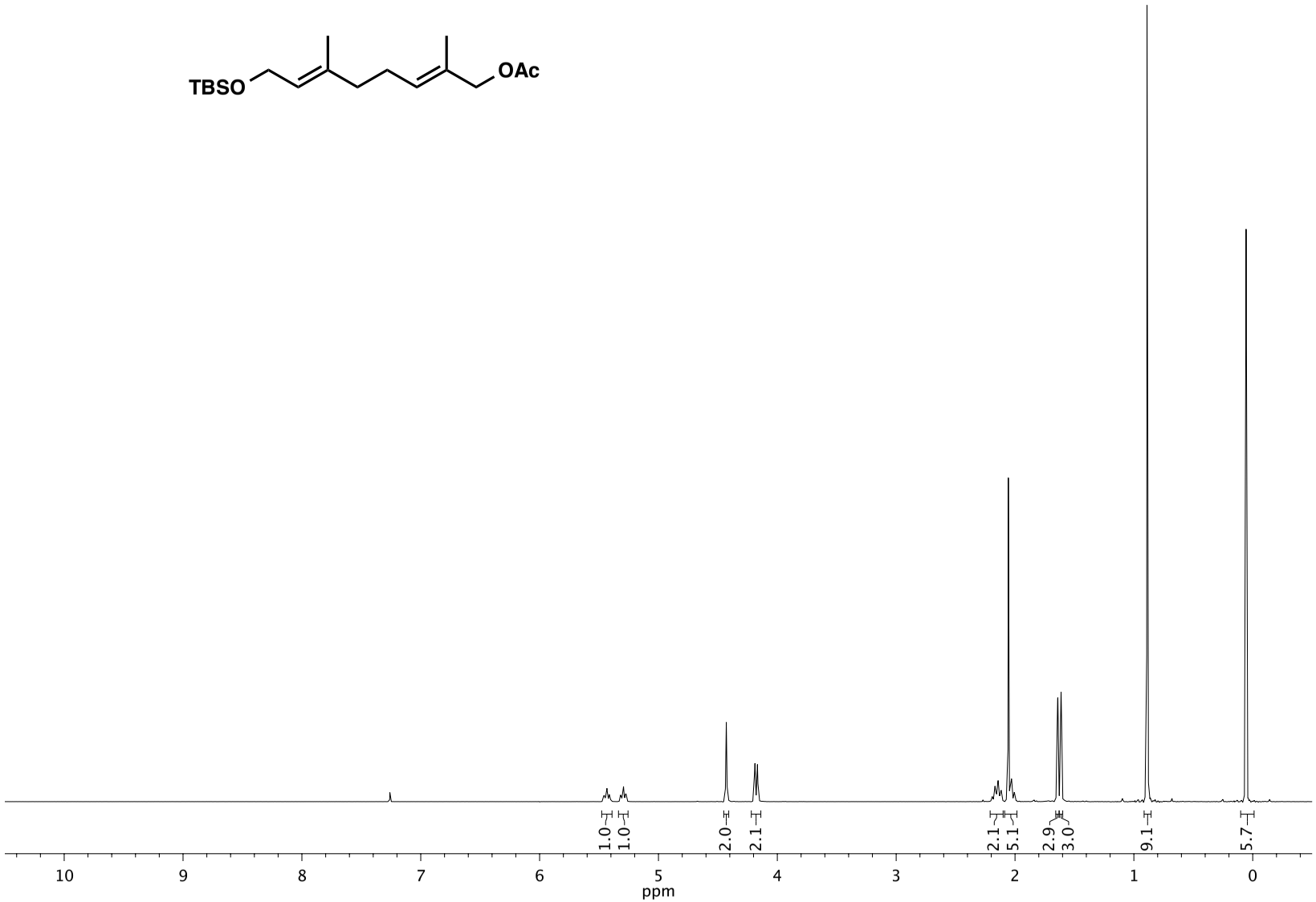


Figure SI-6A ^{1}H NMR 300 MHz, $CDCl_3$) of compound **SI-1**.

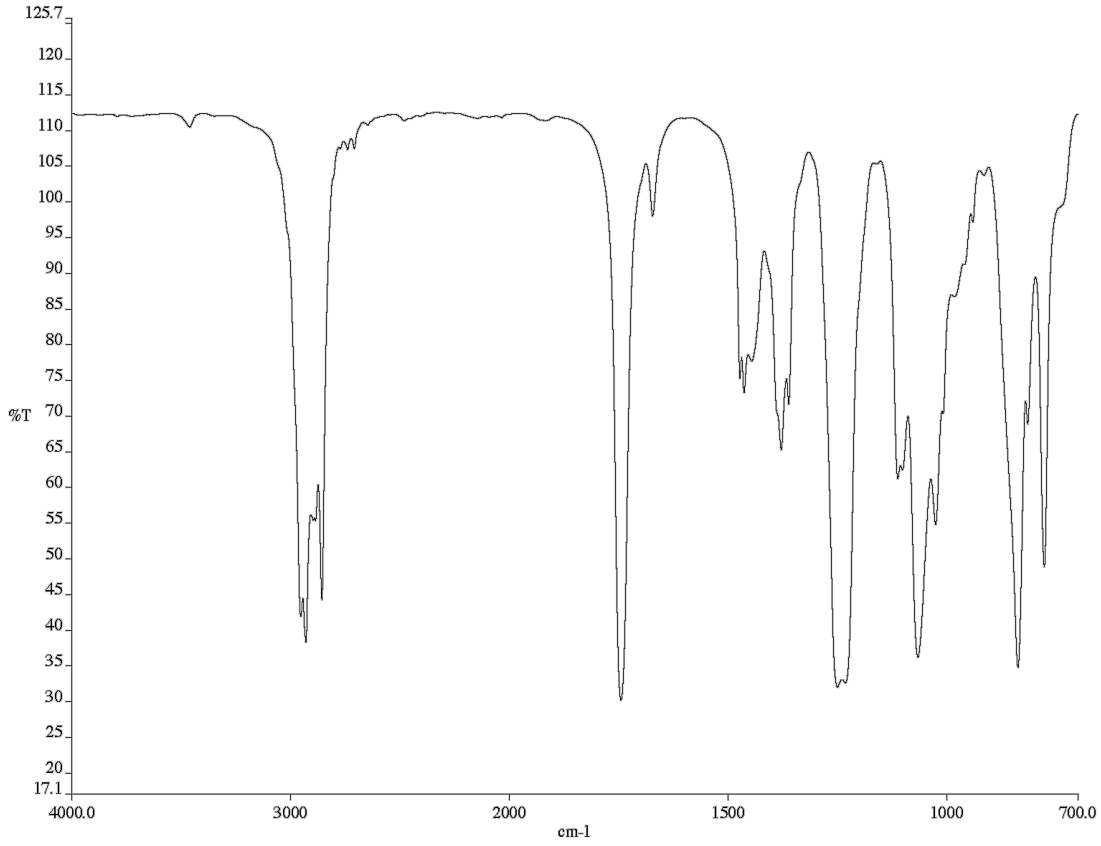


Figure SI-6B infrared spectrum (Thin Film, NaCl) of compound **SI-1**.

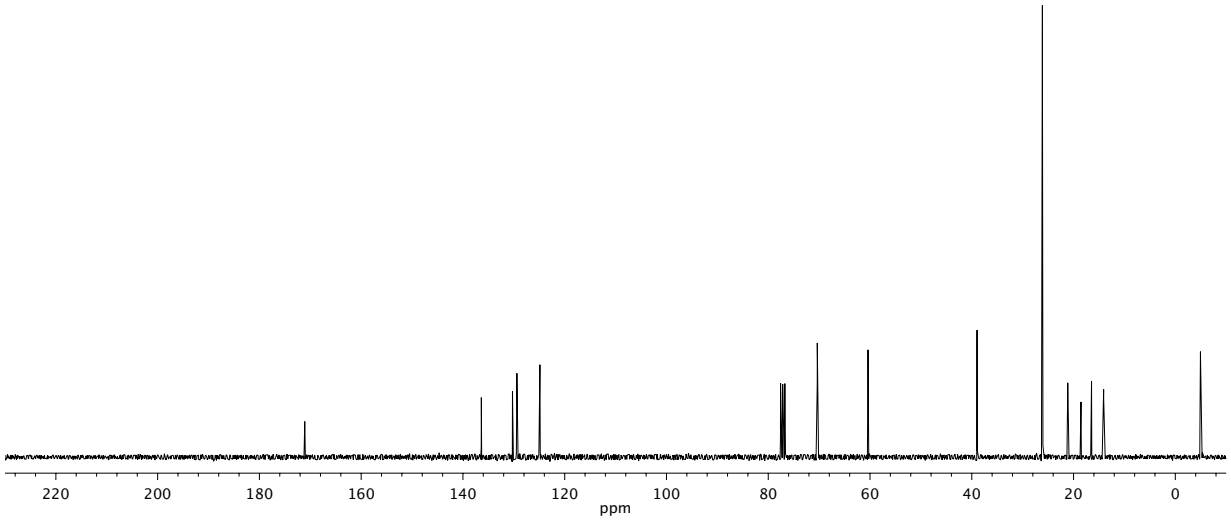
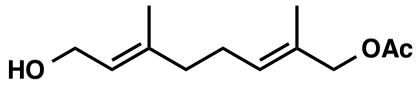


Figure SI-6C ^{13}C NMR (75 MHz, $CDCl_3$) of compound **SI-1**.



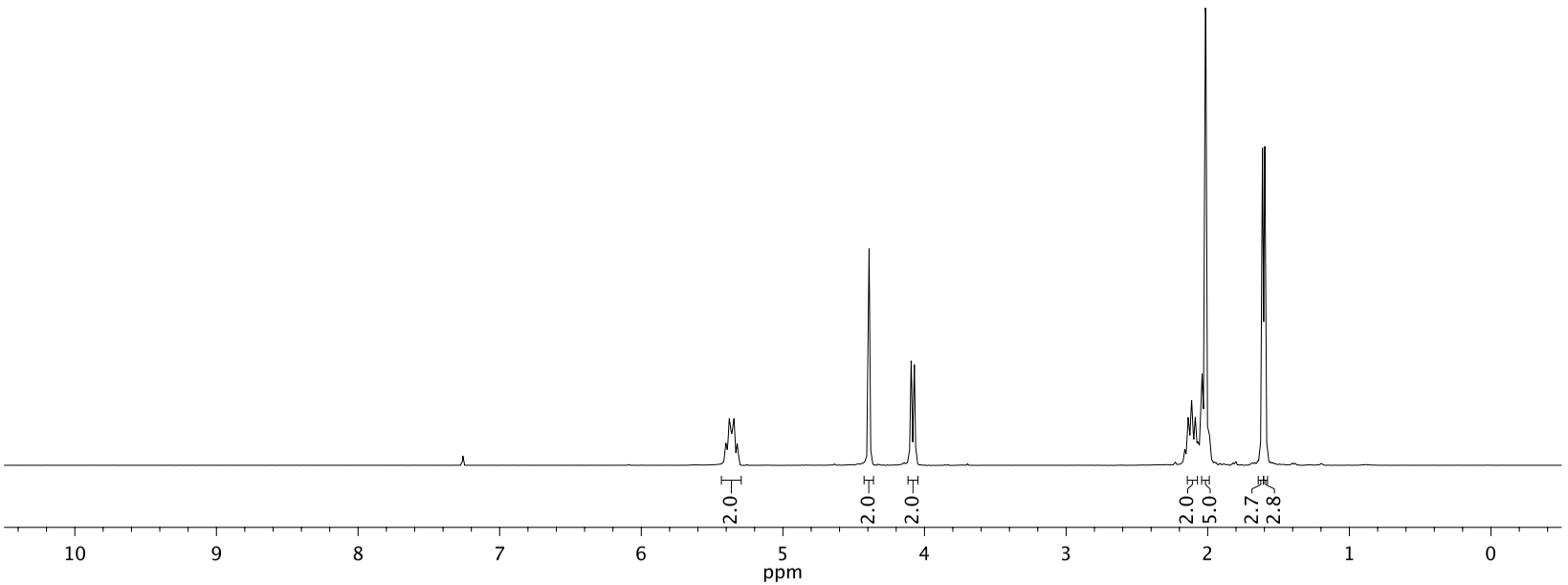


Figure SI-7A ^{1}H NMR 300 MHz, $CDCl_3$) of compound **19**.

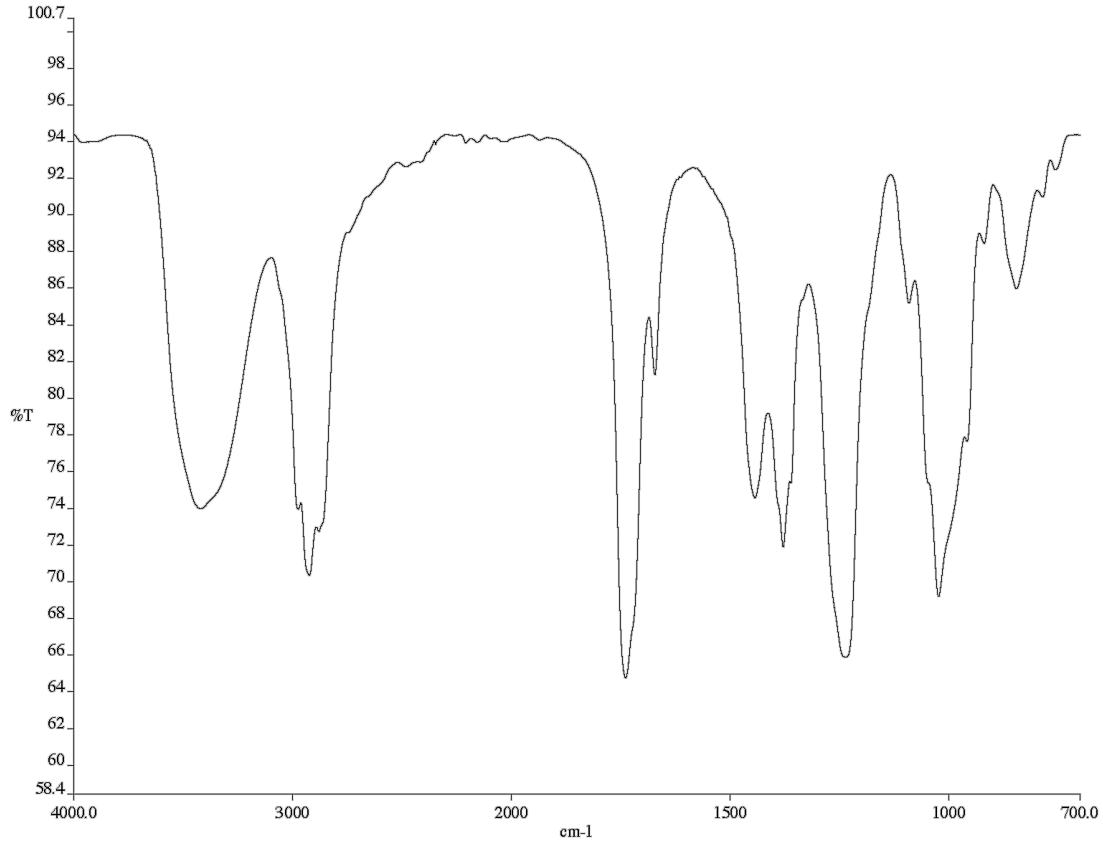


Figure SI-7B infrared spectrum (Thin Film, NaCl) of compound **19**.

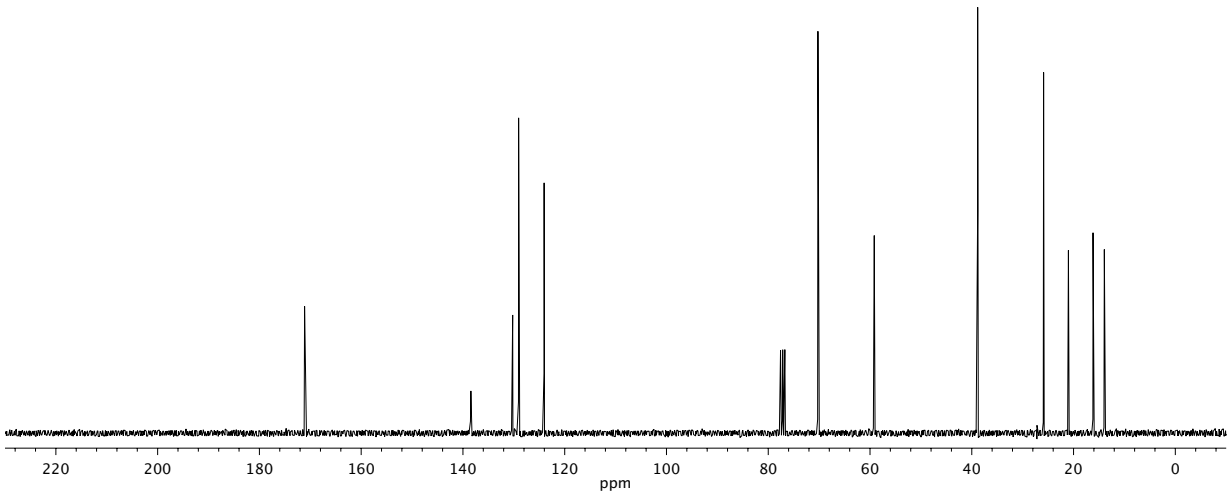


Figure SI-7C ^{13}C NMR (75 MHz, $CDCl_3$) of compound **19**.

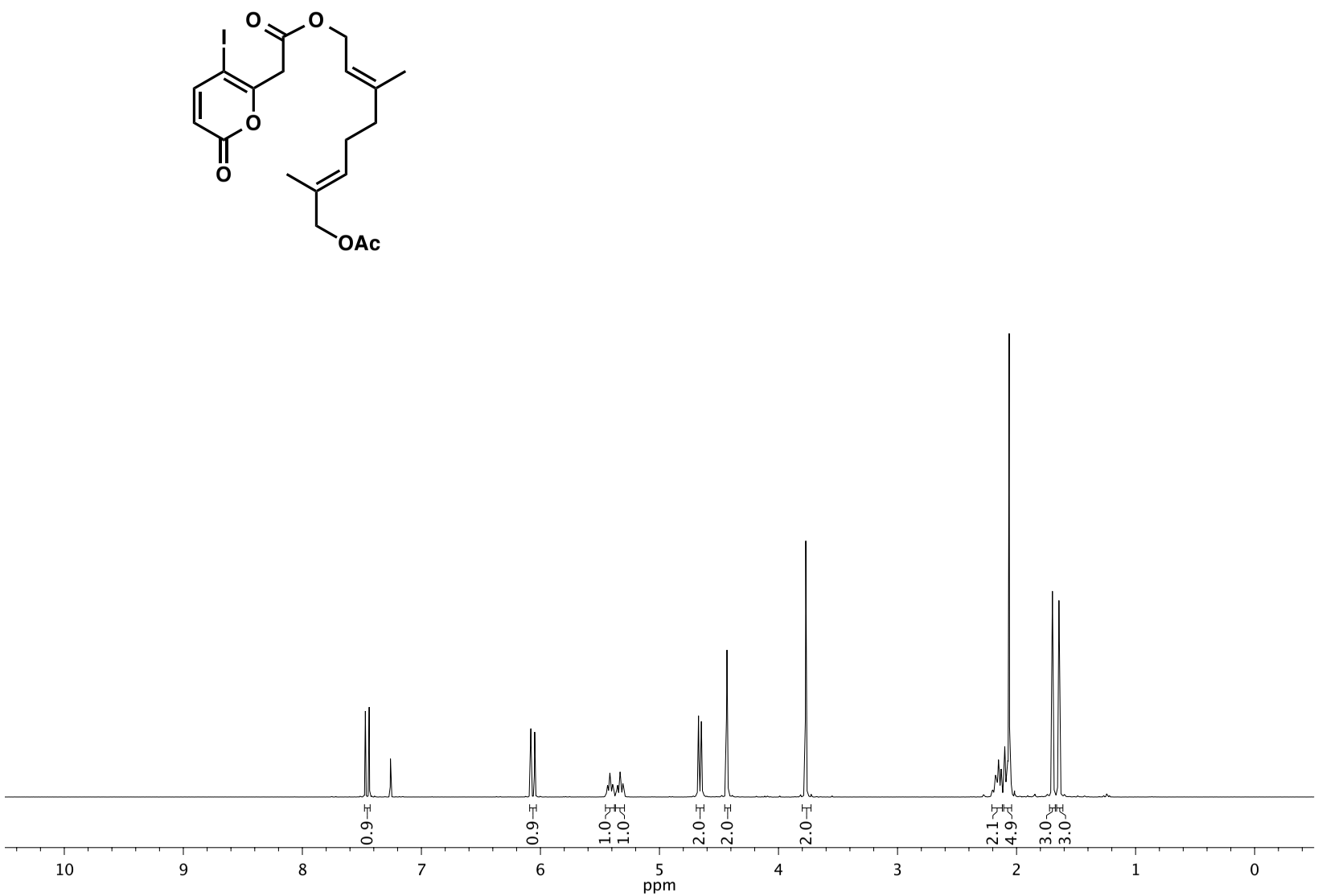


Figure SI-8A ^{1}H NMR 300 MHz, $CDCl_3$) of compound **14**.

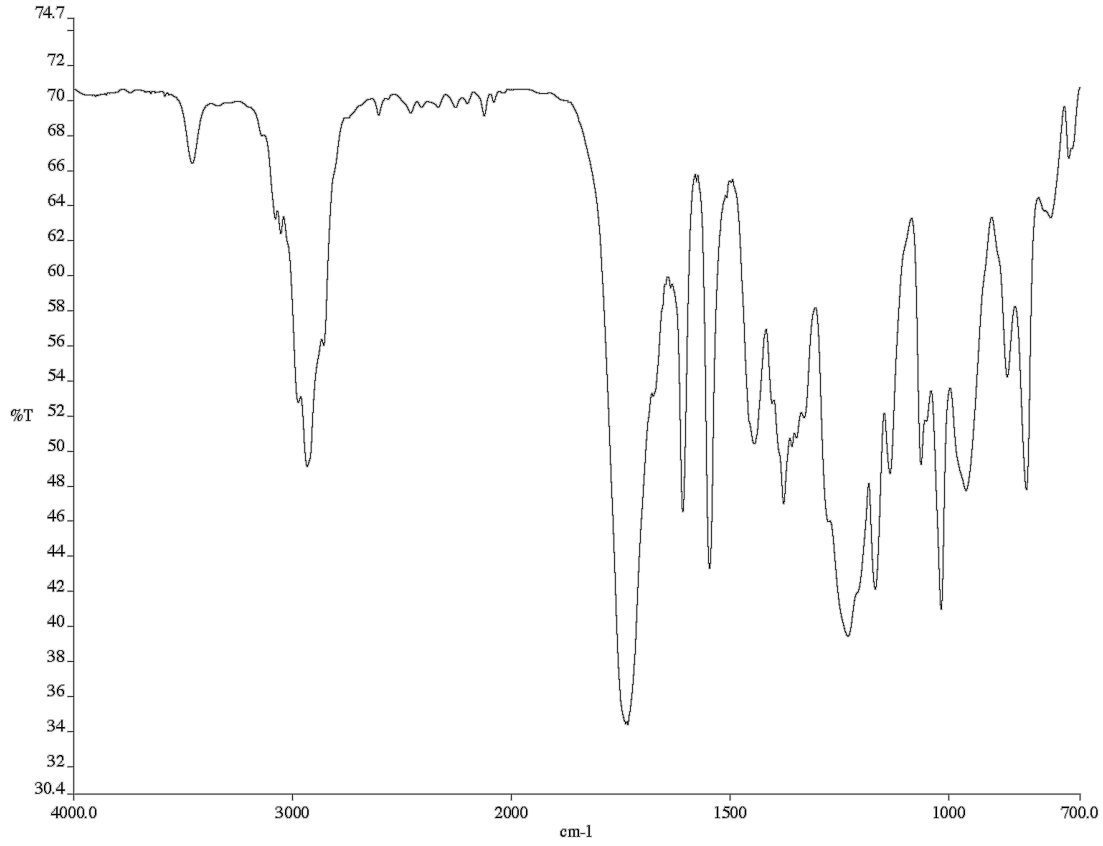


Figure SI-8B infrared spectrum (Thin Film, NaCl) of compound **14**.

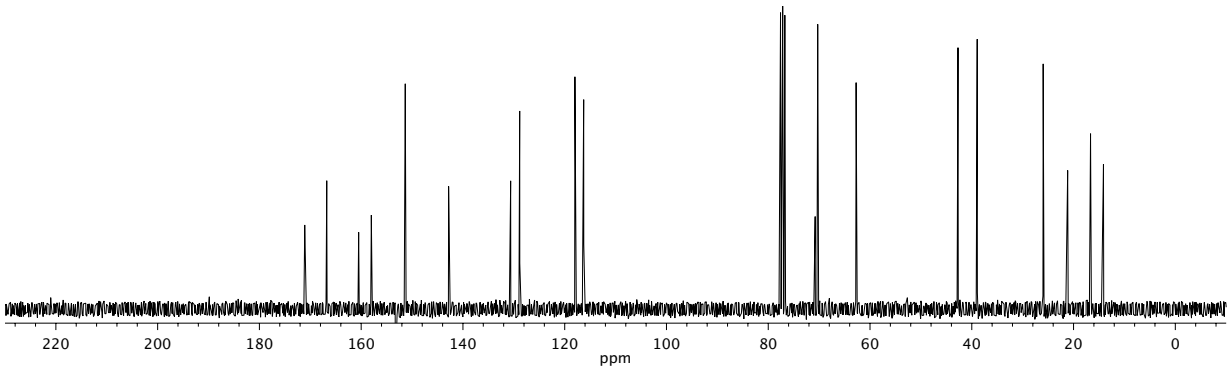
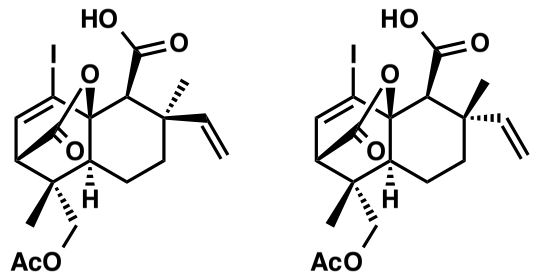


Figure SI-8C ^{13}C NMR (75 MHz, $CDCl_3$) of compound **14**.



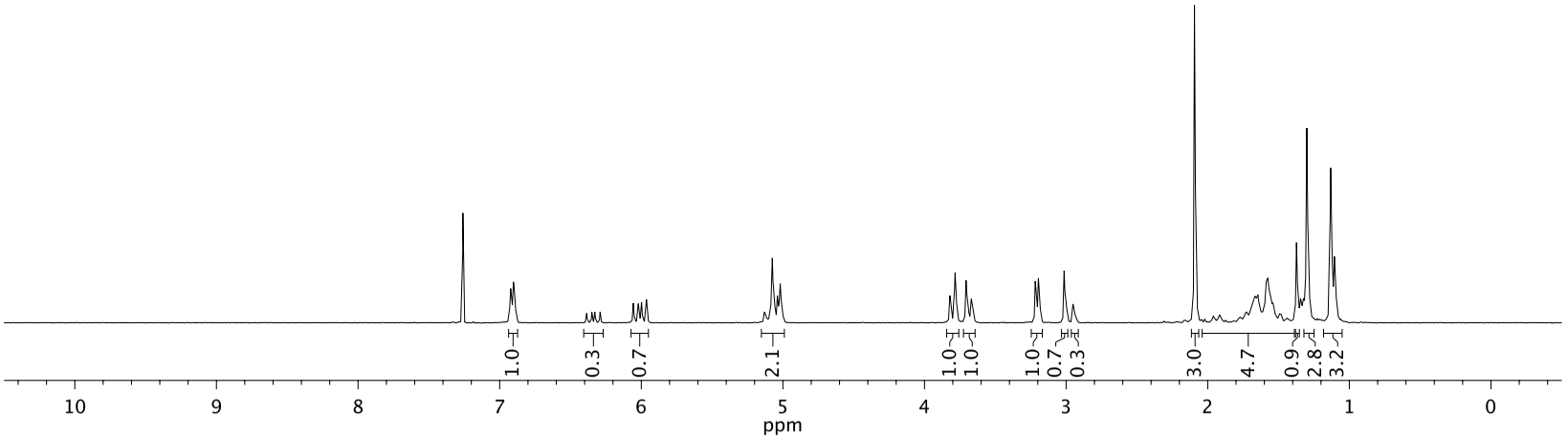


Figure SI-9A ^{1}H NMR 300 MHz, $CDCl_3$) of compounds **21** and **22**.

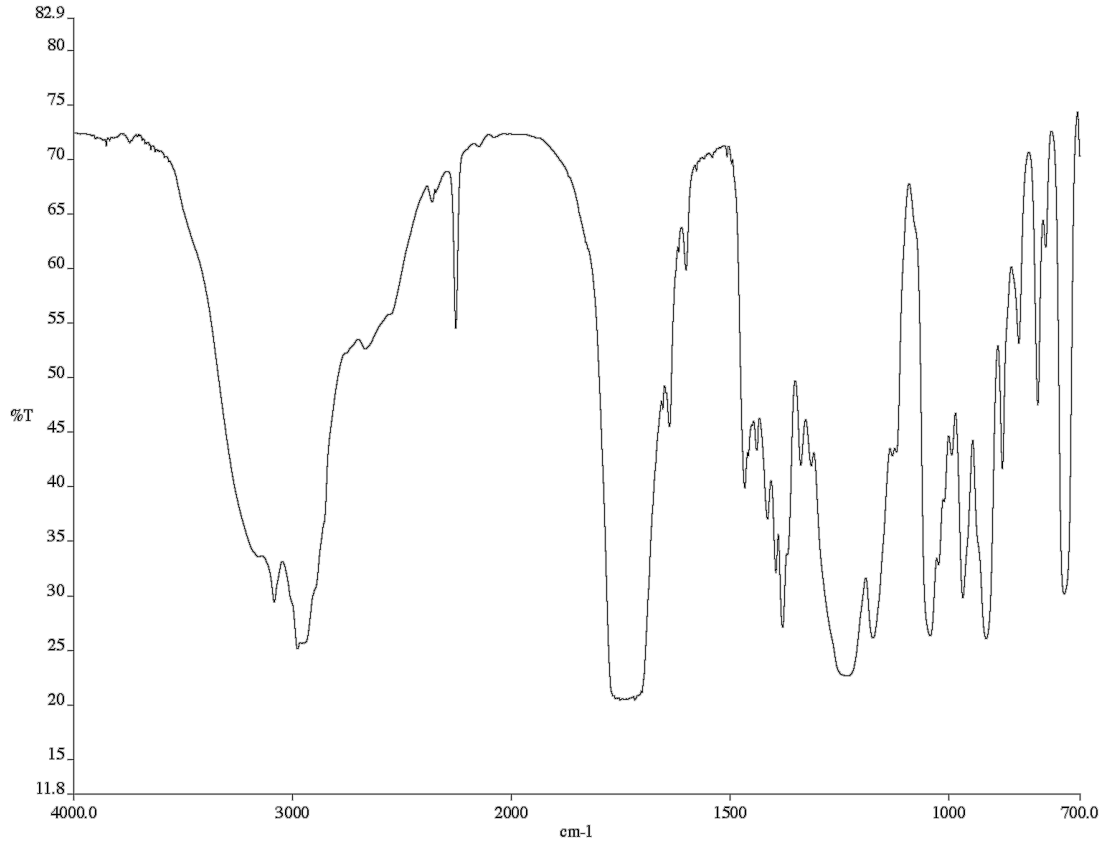


Figure SI-9B infrared spectrum (Thin Film, NaCl) of compounds **21** and **22**.

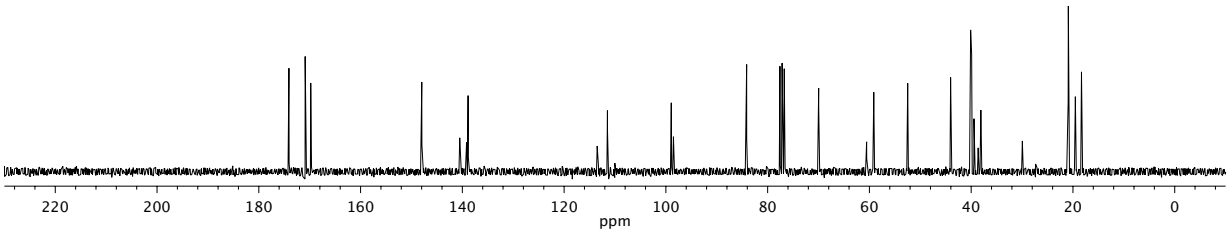


Figure SI-9C ^{13}C NMR (75 MHz, $CDCl_3$) of compounds **21** and **22**.

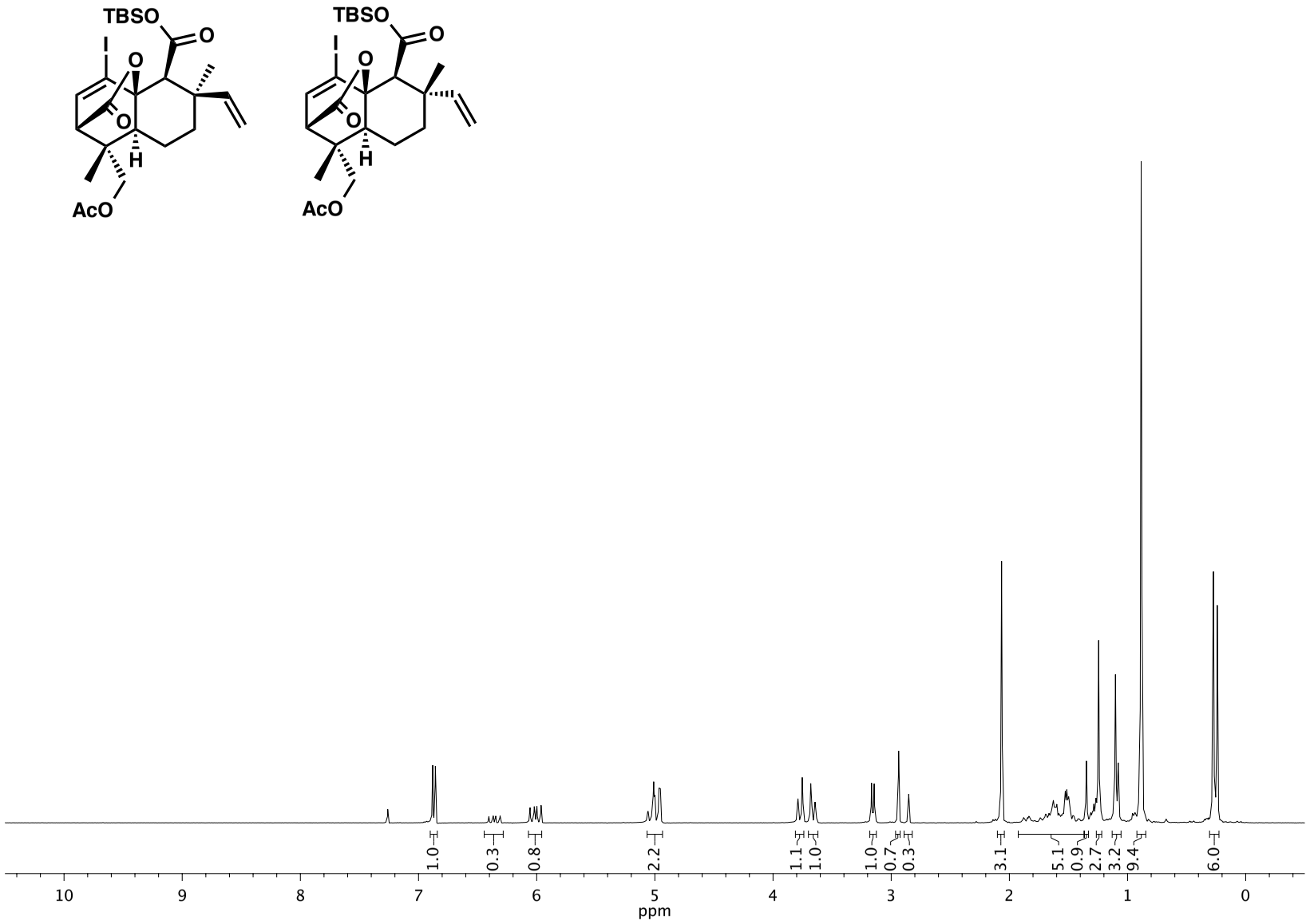


Figure SI-10A ^{1}H NMR 300 MHz, $CDCl_3$) of compounds **23** and **24**.

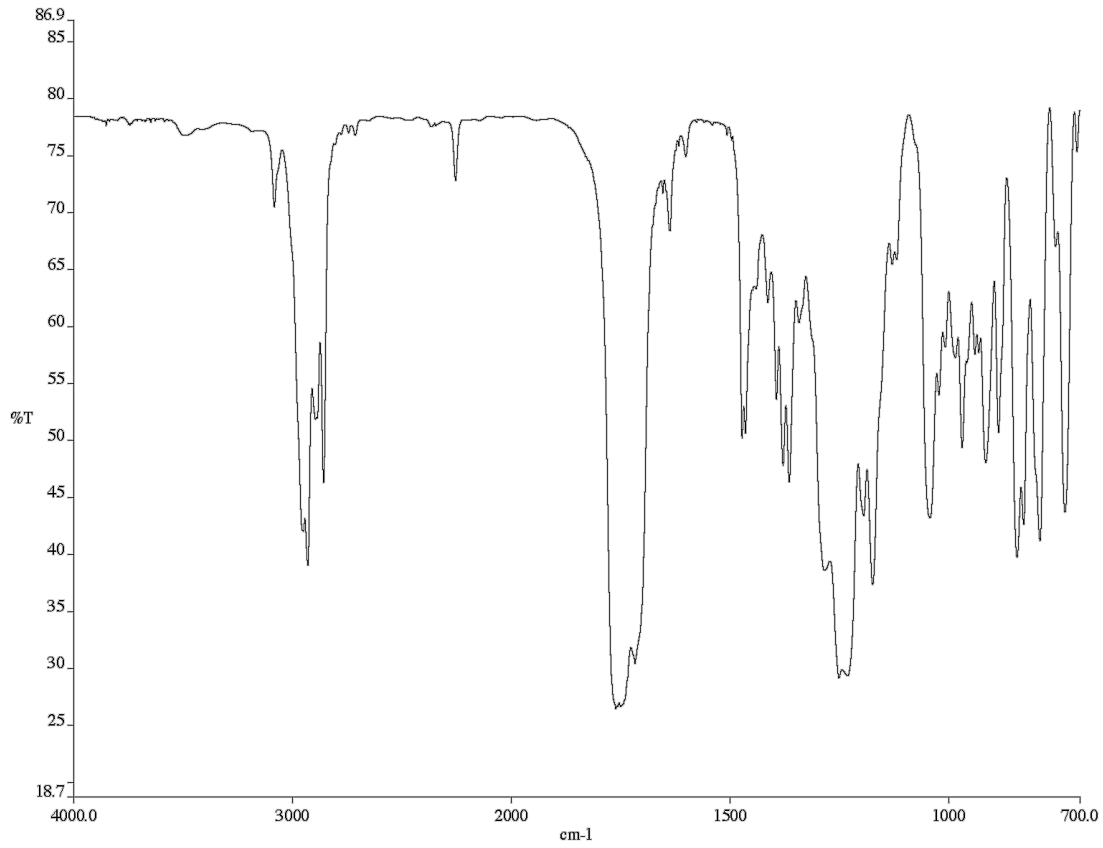


Figure SI-10B infrared spectrum (Thin Film, NaCl) of compounds **23** and **24**.

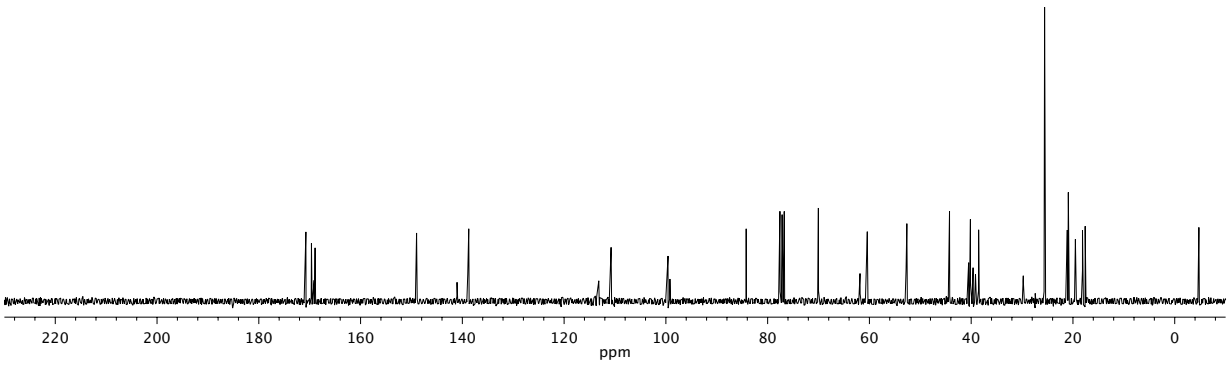
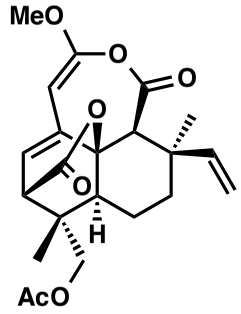


Figure SI-10C ^{13}C NMR (75 MHz, $CDCl_3$) of compounds **23** and **24**.



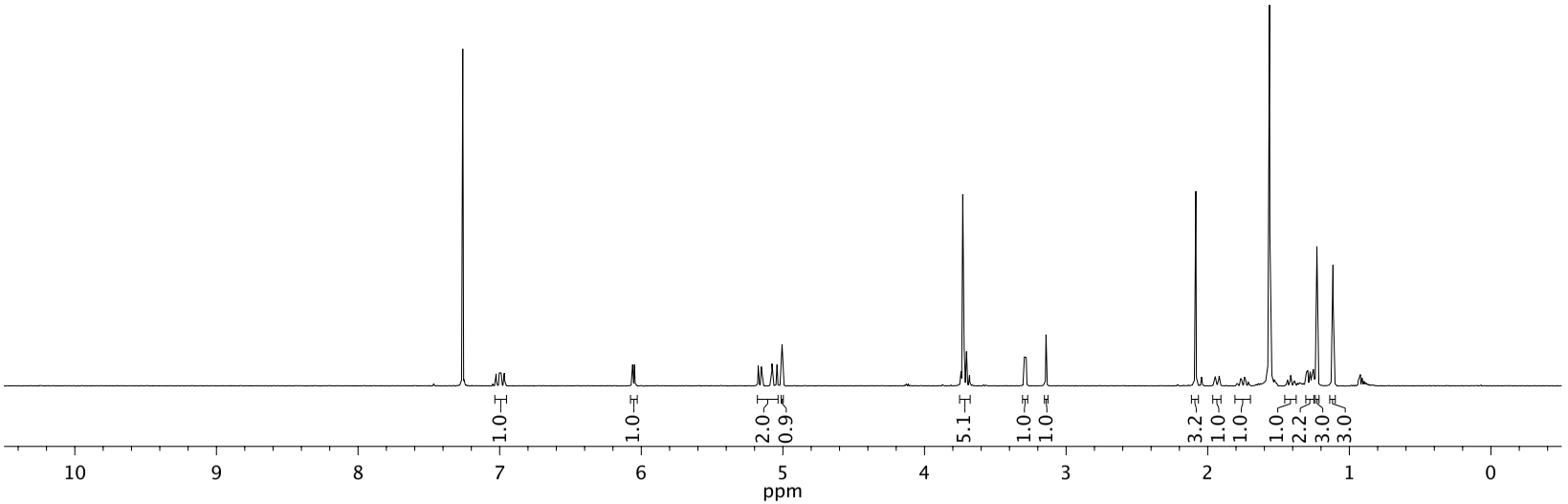


Figure SI-11A ^{1}H NMR (500 MHz, $CDCl_3$) of basiliolide C (**8**).

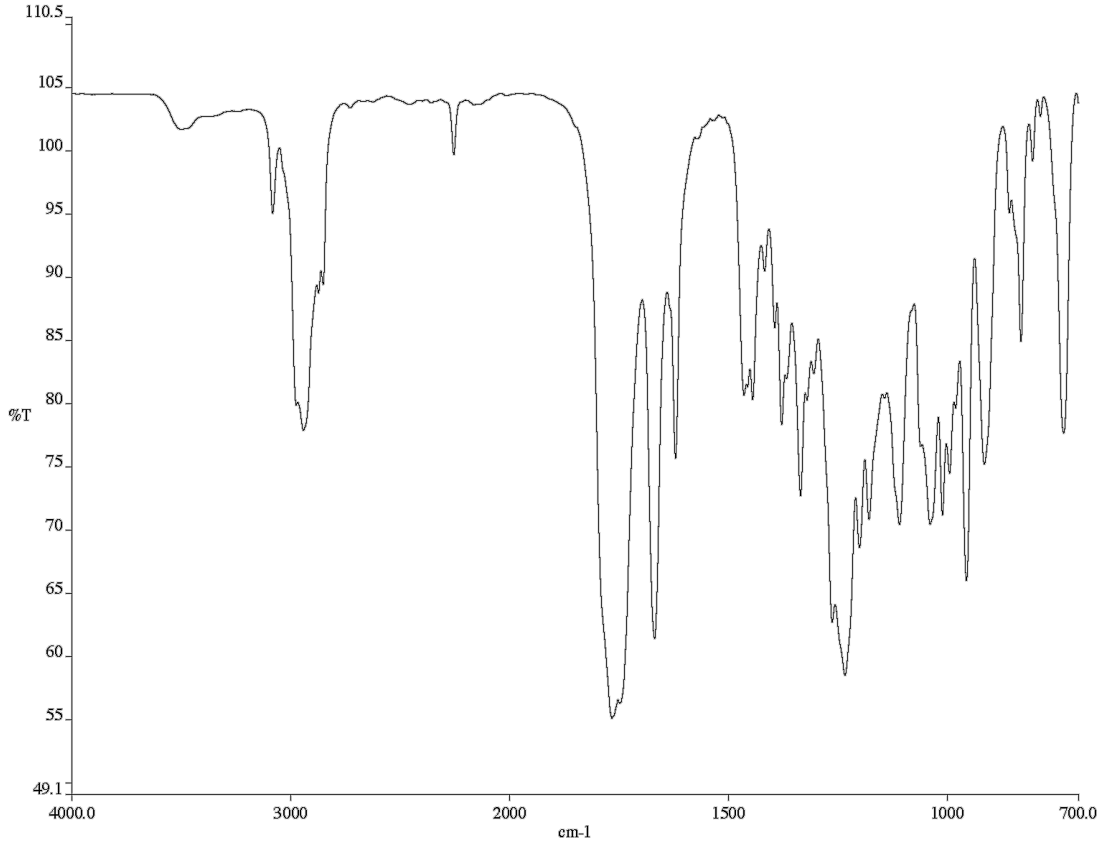


Figure SI-11B infrared spectrum (Thin Film, NaCl) of basiliolide C (**8**).

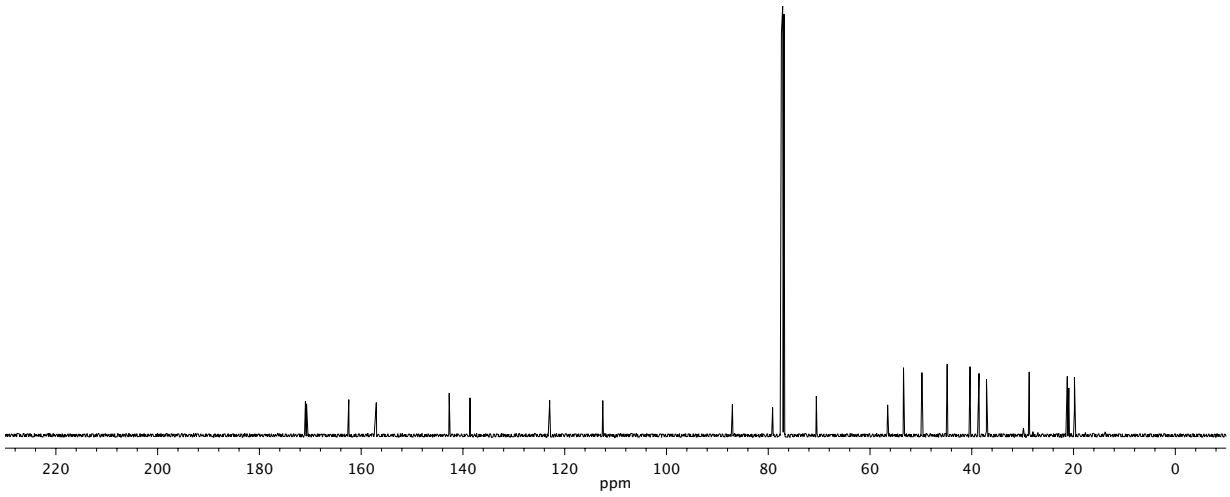
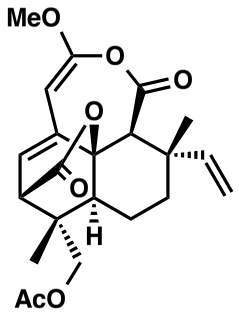


Figure SI-11C ^{13}C NMR (125 MHz, $CDCl_3$) of basiliolide C (**8**).



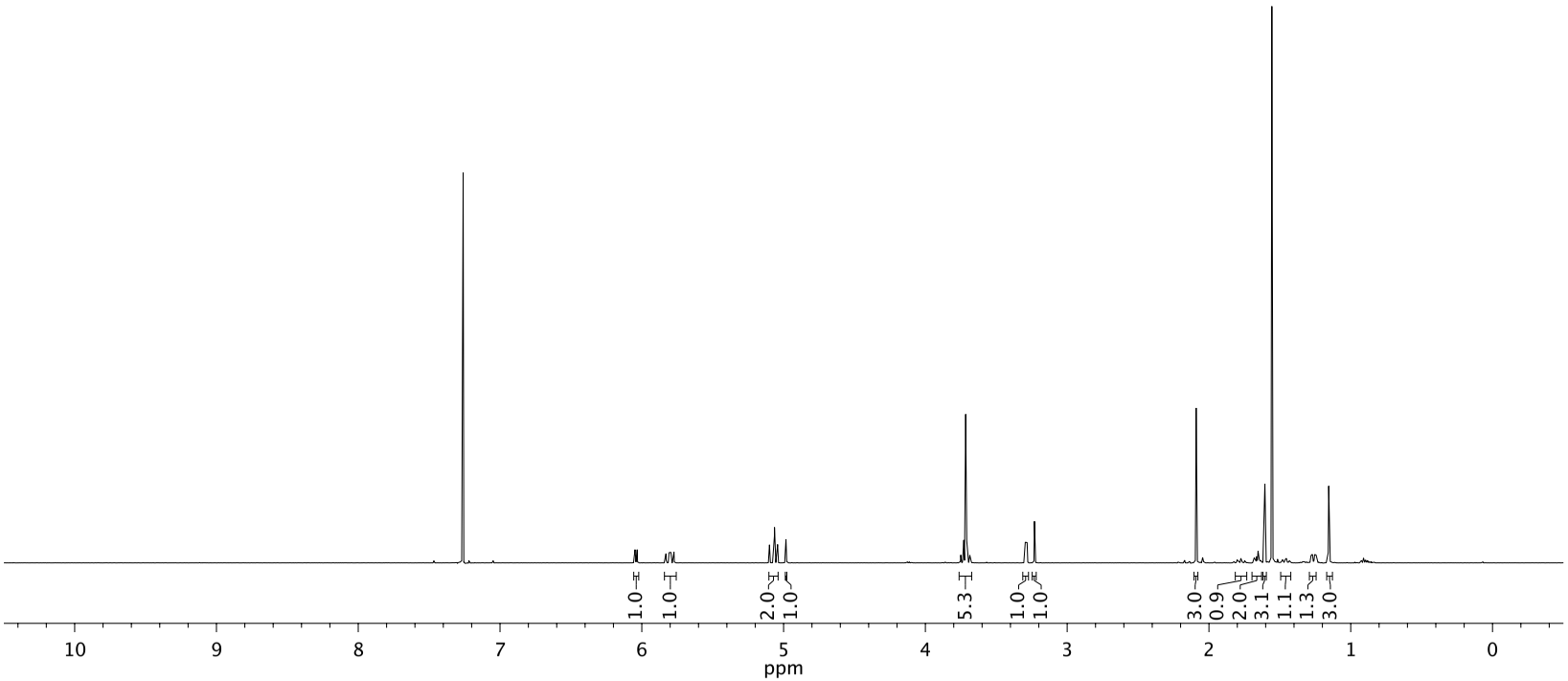


Figure SI-12A ^{1}H NMR (500 MHz, $CDCl_3$) of epi-basiliolide C (**9**).

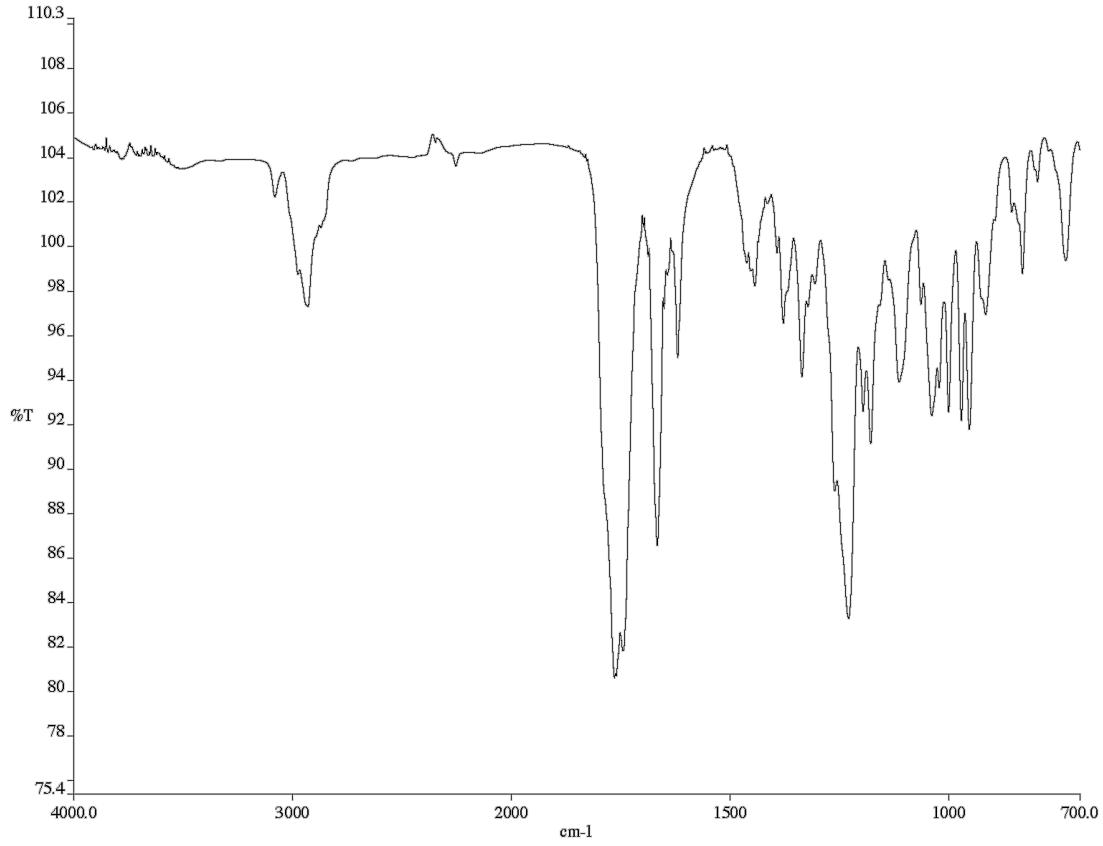


Figure SI-12B infrared spectrum (Thin Film, NaCl) of epi-basiliolide C (**9**).

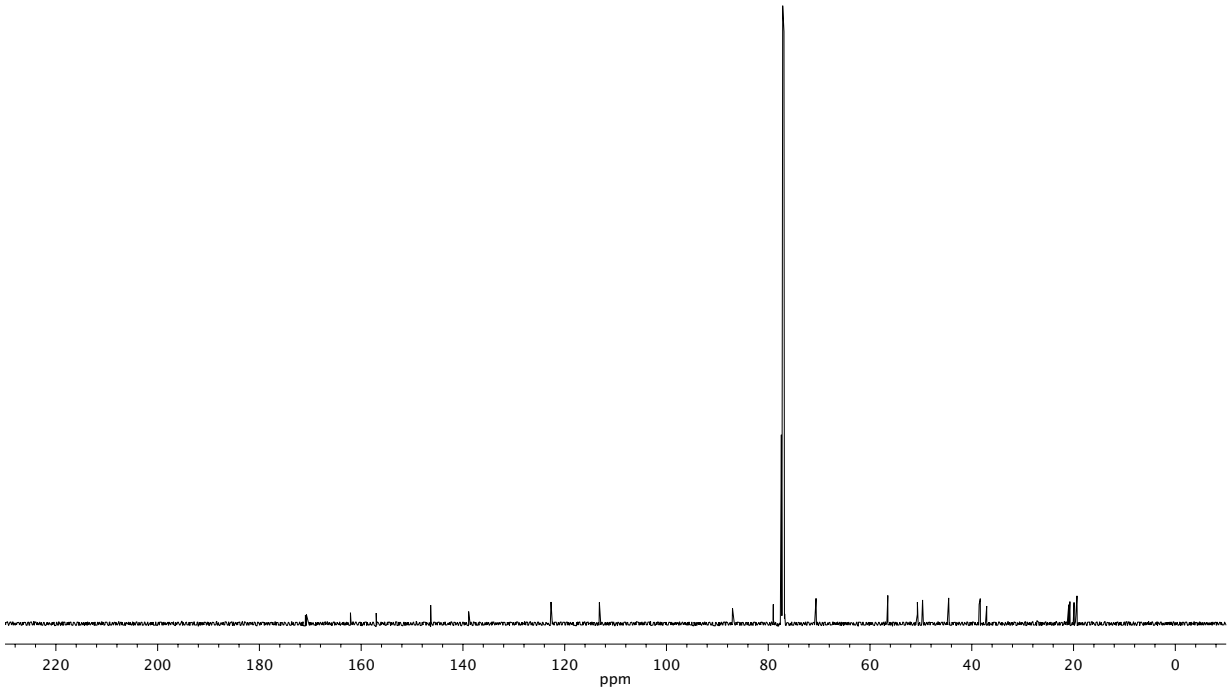


Figure SI-12C ^{13}C NMR (125 MHz, $CDCl_3$) of epi-basiliolide C (**9**).

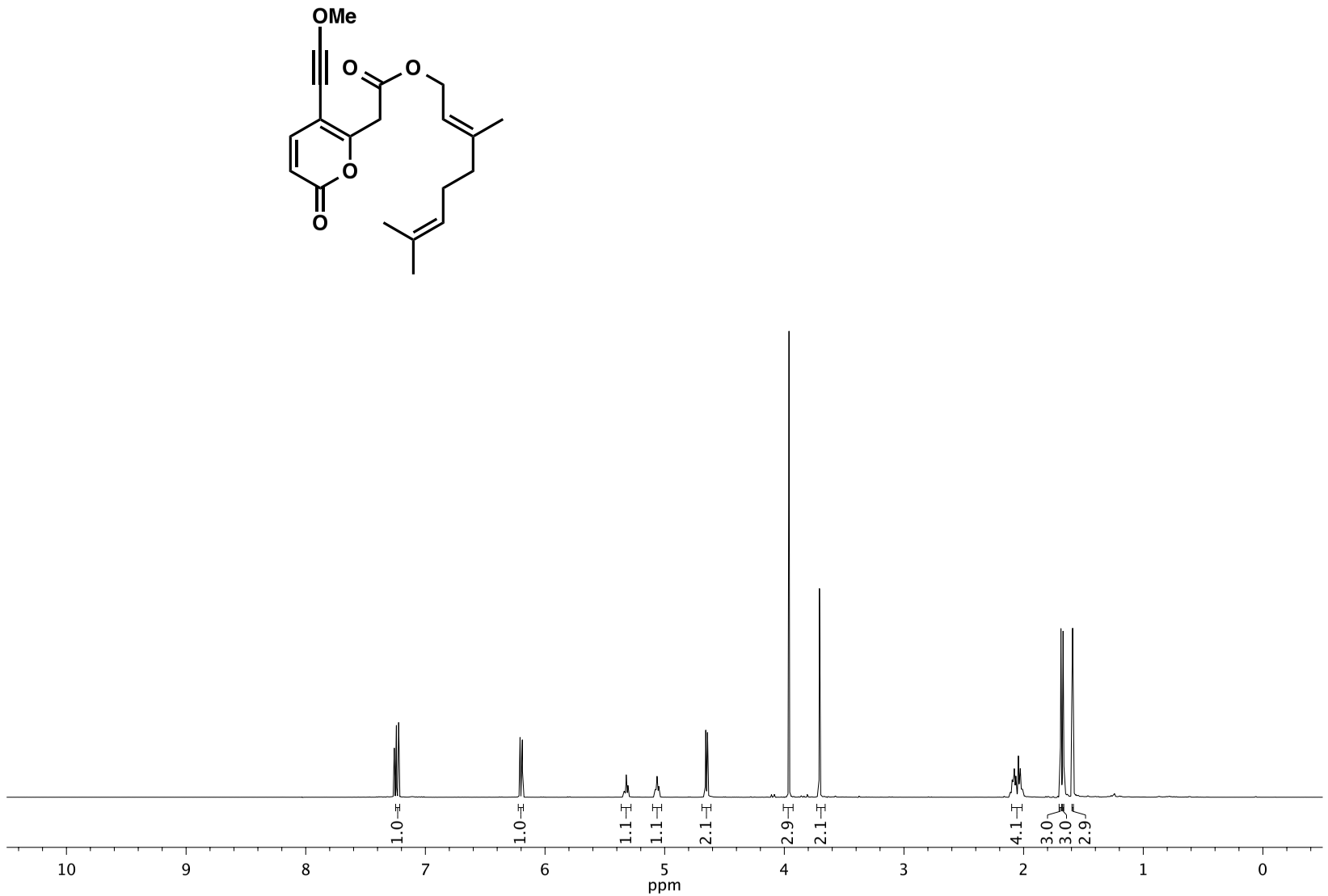


Figure SI-13A ^{1}H NMR 500 MHz, $CDCl_3$) of compound **11**.

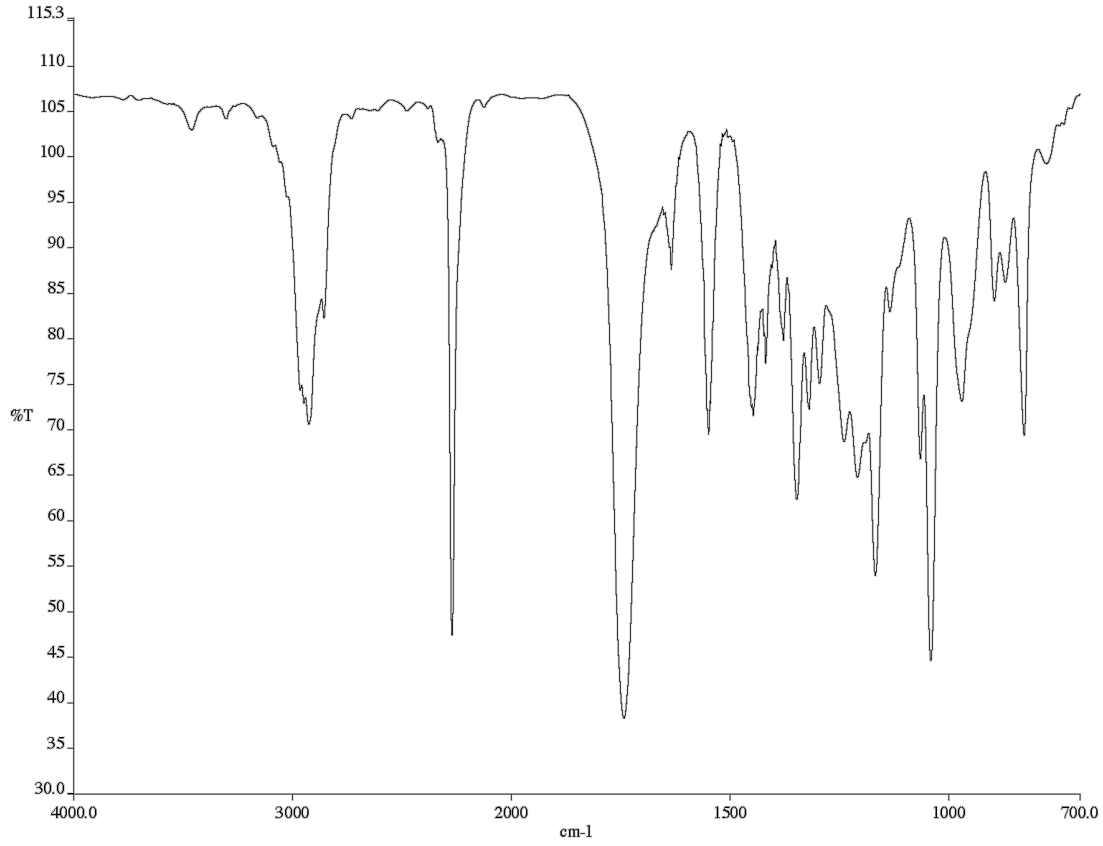


Figure SI-13B infrared spectrum (Thin Film, NaCl) of compound **11**.

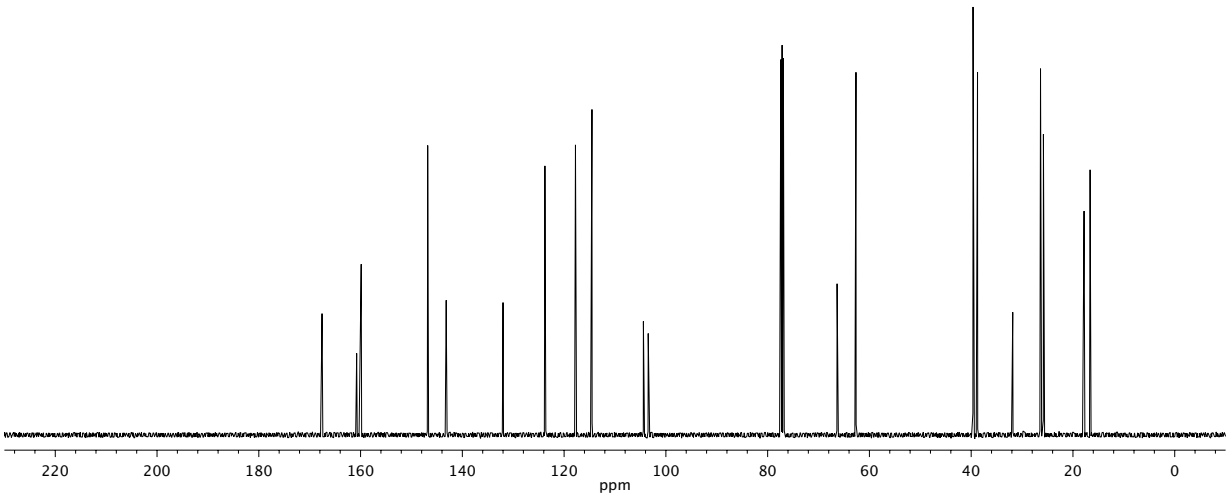
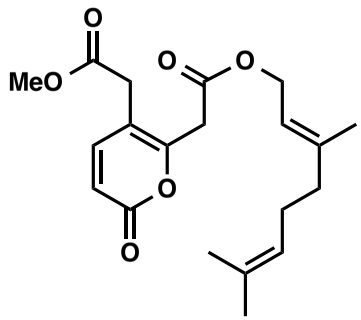


Figure SI-13C ^{13}C NMR (125 MHz, $CDCl_3$) of compound **11**.



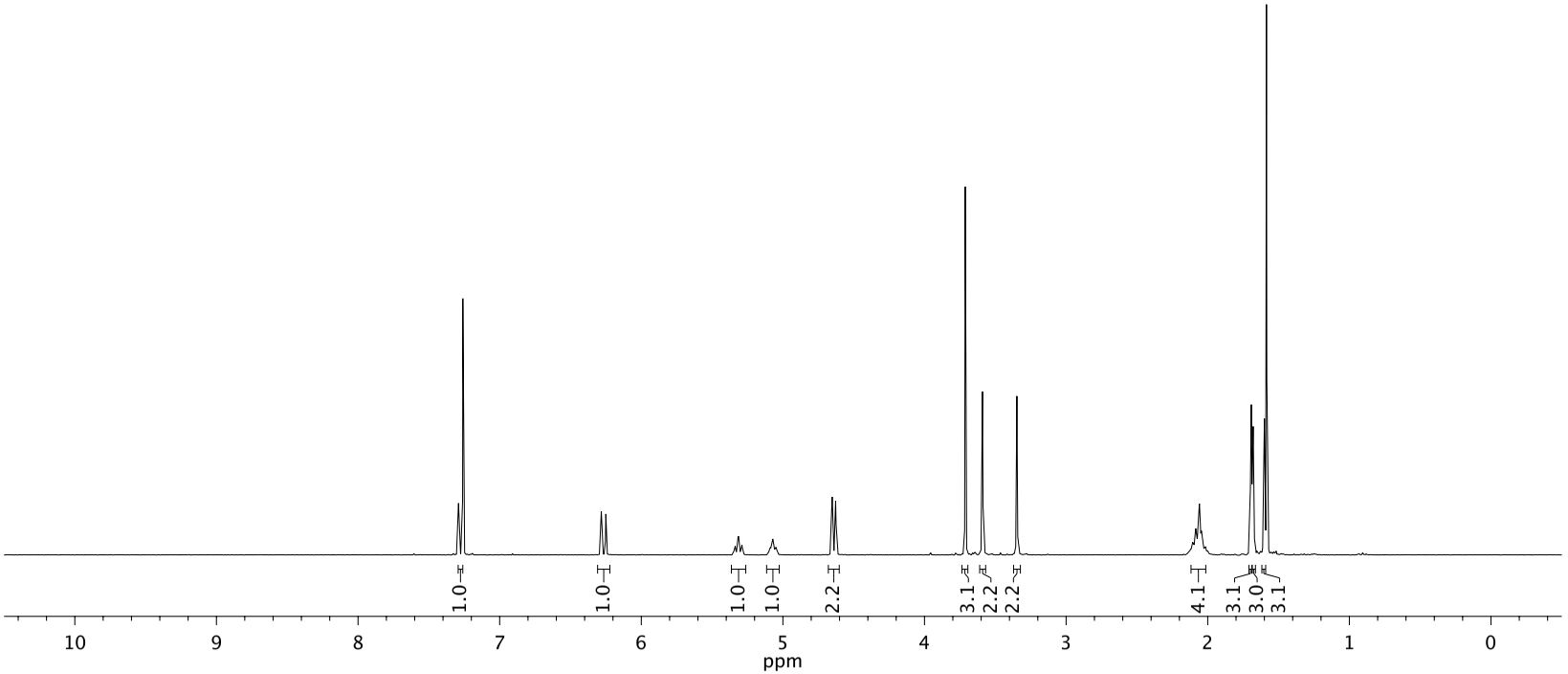


Figure SI-14A ^{1}H NMR 300 MHz, $CDCl_3$) of basiliopyrone (**10**).

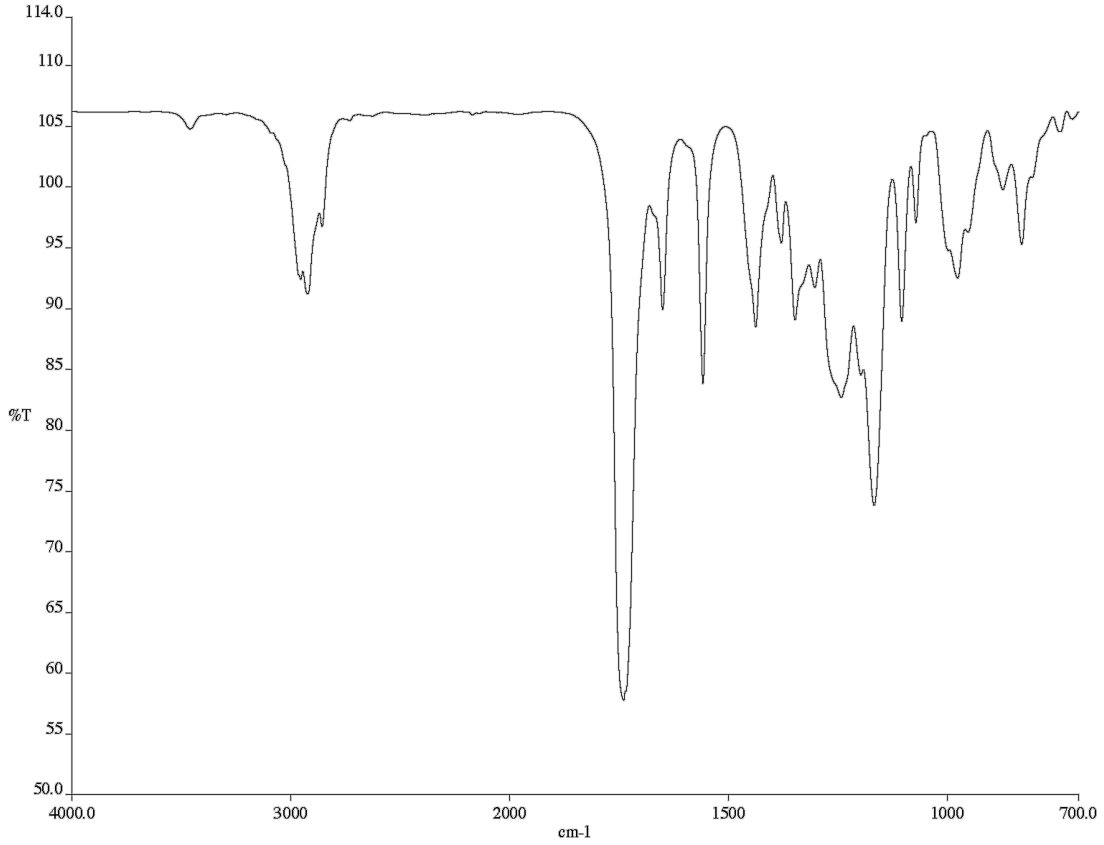


Figure SI-14B infrared spectrum (Thin Film, NaCl) of basiliopyrone (**10**).

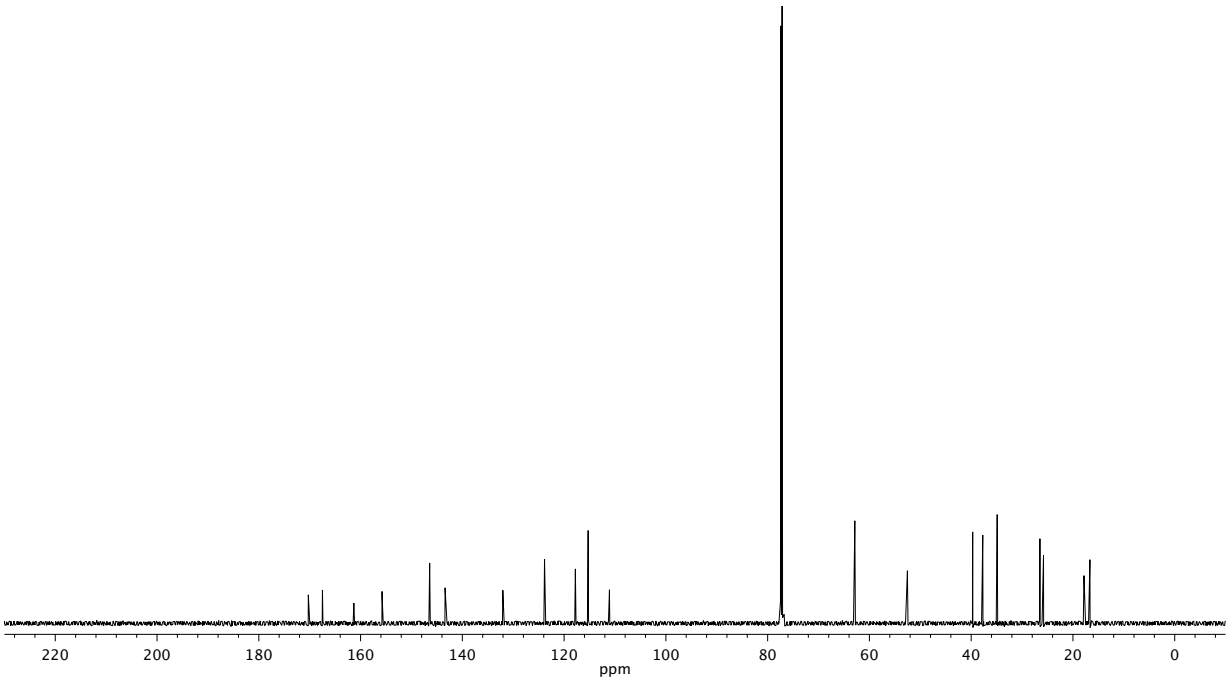
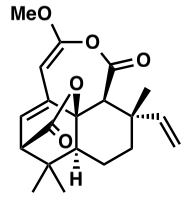


Figure SI-14C ^{13}C NMR (125 MHz, $CDCl_3$) of basiliopyrone (**10**).



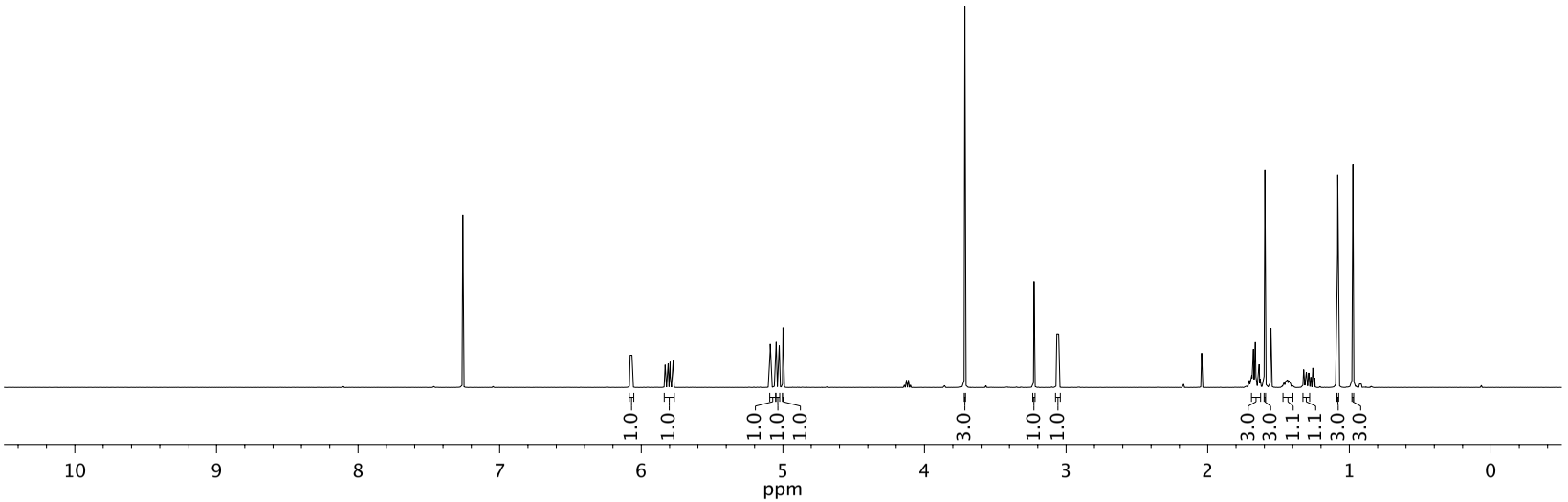


Figure SI-15A ^{1}H NMR 500 MHz, $CDCl_3$) of transtaganolide C (**4**).

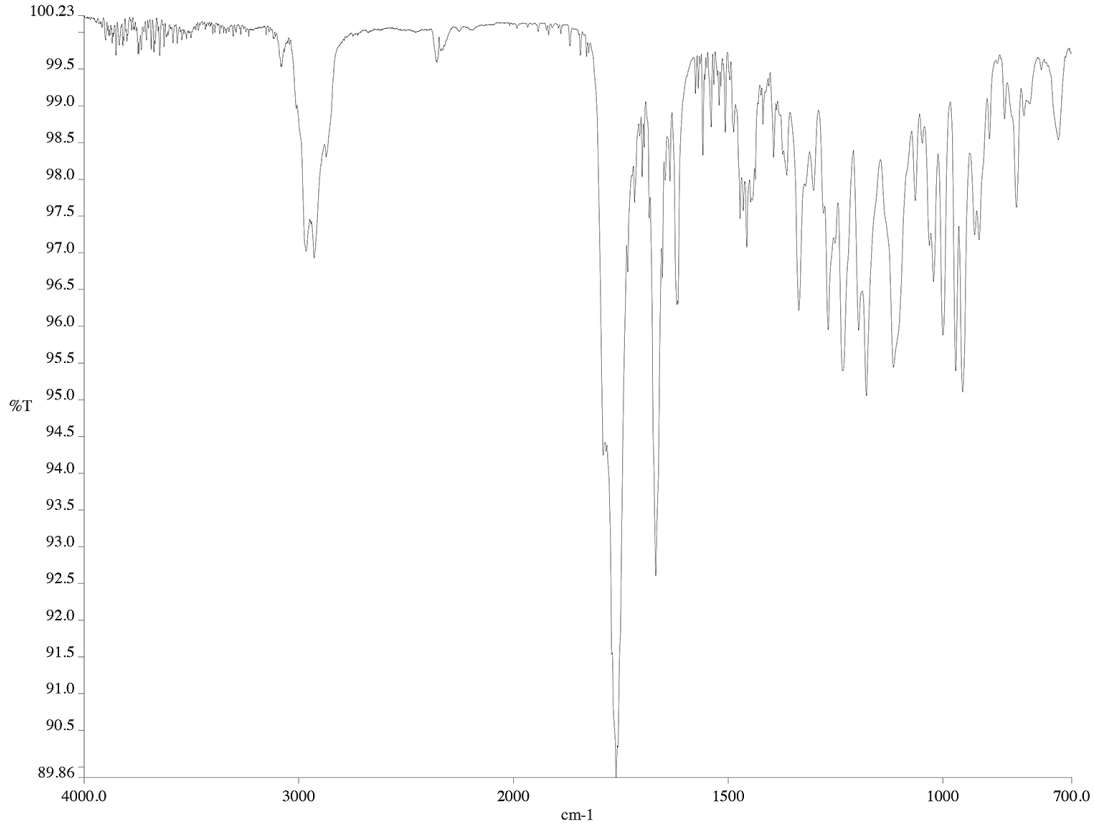


Figure SI-15B infrared spectrum (Thin Film, NaCl) of transtaganolide C (**4**).

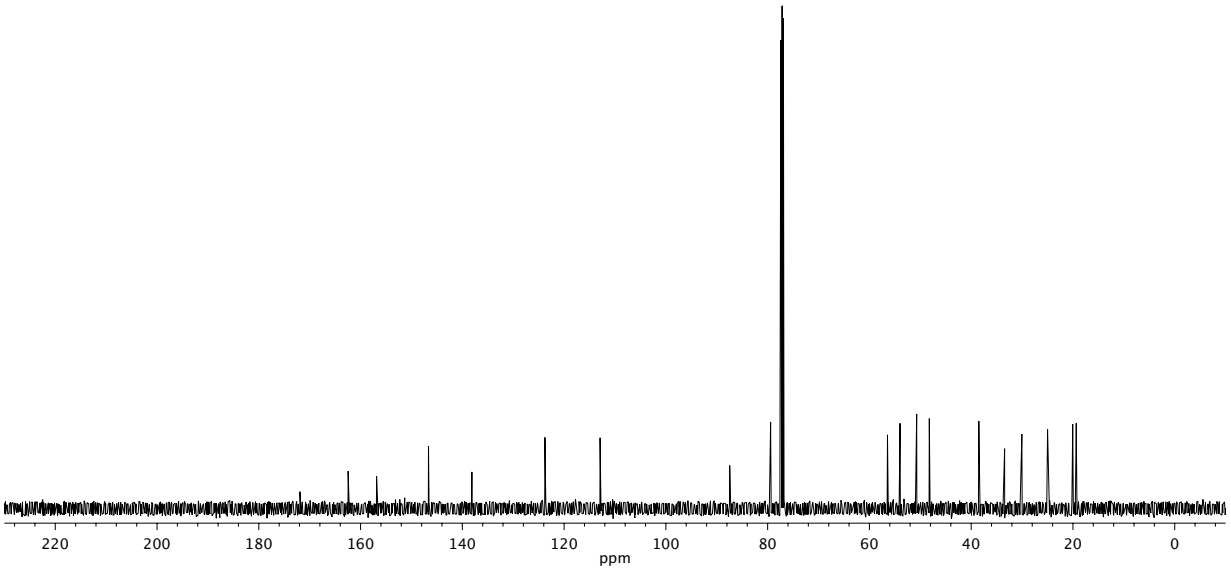
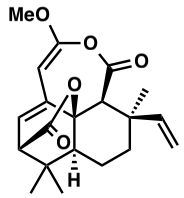


Figure SI-15C ^{13}C NMR (125 MHz, $CDCl_3$) of transtaganolide C (**4**).



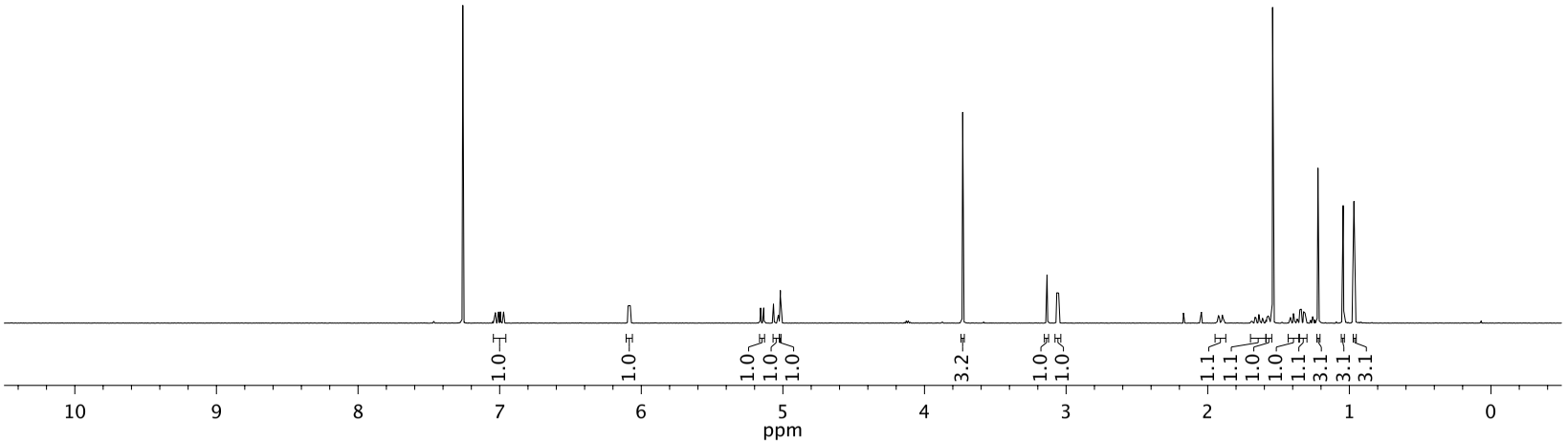


Figure SI-16A ^{1}H NMR 500 MHz, $CDCl_3$) of transtaganolide D (**5**).

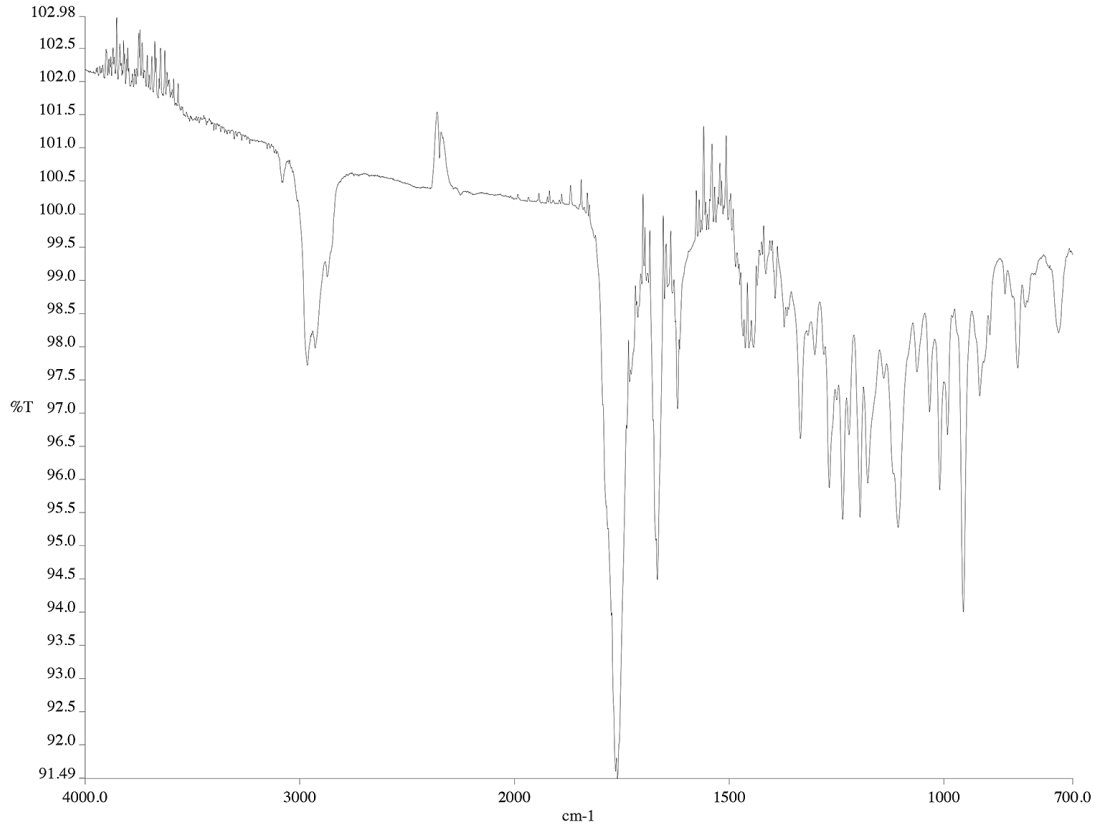


Figure SI-16B infrared spectrum (Thin Film, NaCl) of transtaganolide D (**5**).

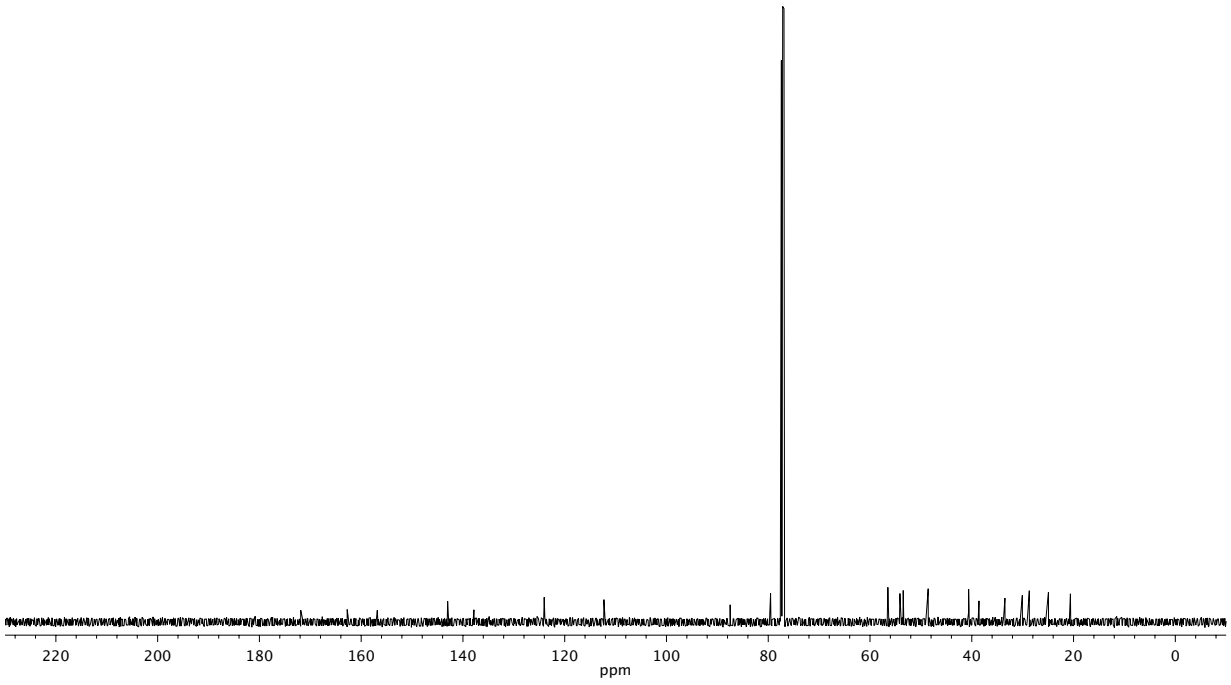


Figure SI-16C ^{13}C NMR (125 MHz, $CDCl_3$) of transtaganolide D (**5**).