Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

3’-(Phenyl alkynyl) Analogs of Abscisic Acid: Synthesis and Biological Activity of Potent ABA Antagonists

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Supporting Information

1. Chemistry

a) General methods

Anhydrous solvents were distilled under argon atmosphere as follows: tetrahydrofuran (THF) from benzophenone sodium ketyl and stored over 4 Å molecular sieves. All experiments involving air and/or moisture-sensitive compounds were conducted in an oven dried round-bottom flask capped with a rubber septum and attached via a needle and connecting tubing to argon atmosphere. Concentration refers to removal of volatiles at water aspirator pressure on a rotary evaporator. Evacuation at ca. 0.1 torr with a vacuum pump generally followed rotary evaporation.

Preparative TLC was carried out on glass plates (20x20 cm) pre-coated (0.25 mm) with silica gel 60 F254. Materials were detected by visualization under an ultraviolet lamp (254 nm) and/or by treating a 1 cm vertical strip removed from the plate with a solution of phosphomolybdic acid (PMA) solution [PMA (40 g), $Ce(SO_4)_2$ (10 g) and H_2SO_4 (50 mL) and diluted to 1 L with water] followed by charring on a hot plate. Flash column chromatography (FCC) was performed according to Still et al.[1] with Merck Silica Gel 60 (40-63 mm). All mixed solvent eluents are reported as v/v solutions. Unless otherwise noted, all reported compounds were homogeneous by thin layer chromatography (TLC) and by ^{1}H NMR spectroscopy.

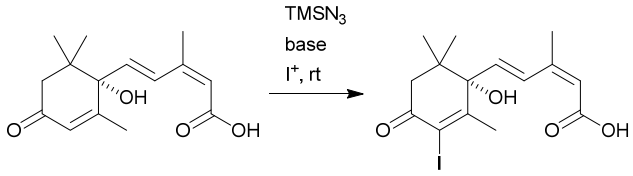
High resolution mass spectra (HRMS) were obtained on a JEOL AccuTOF 4G GCv Mass spectrometer using field desorption (FD) ionization or a QSTAR XL MS/MS Mass spectrometer using electrospray ionization (ESI) method; only partial data are reported. Alternatively, HRMS was obtained on a LC-MS/MS time-of-flight high resolution spectrometer with electrospray ionization (ESI) from methanol solution. Infrared spectra were recorded on a Bio-Rad FTS-40 Fourier transform interferometer using a diffuse reflectance cell (DRIFT); only diagnostic and/or intense peaks are reported.

The NMR solvent $CDCl_3$ was passed through small plug of basic alumina prior to use. Unless otherwise noted, NMR spectra were measured in $CDCl_3$ or CD_3OD solution at 500 or 600 MHz (Bruker Avance) for 1H and 125 MHz for 13C. Signals due to the solvent (13C NMR) or residual protonated solvent (1H NMR) served as the internal standard: $CDCl_3$ (7.26 δH, 77.23 δC); CD_3OD (3.31 δH, 49.00 δC). The 1H NMR chemical shifts and coupling constants were determined assuming first-order behavior. Multiplicity is indicated by one or more of the following: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad), ap (apparent); the list of couplings constants (*J*) corresponds to the order of the multiplicity assignment. Coupling constants are reported to the nearest 0.5 Hz (consistent with the digital resolution of ca. 0.2 Hz/pt). The NMR assignments were made on the basis of chemical shifts, coupling constants (*J*) and multiplicity and were confirmed, where necessary, by homonuclear decoupling and/or two-dimensional correlation experiments (gCOSY, gHSQC, gHMBC).[2] The multiplicity of 13C NMR signals refers to the number of H's attached (i.e., s = C, d = CH, t = CH_2, q = CH_3) and was determined by gHSQC.

Et_3N was distilled from CaH_2 under argon and stored in a Schlenk RBF over KOH. $TMSN_3$ was distilled under argon and stored neat in a Schlenk RBF. 4-ethynylphenol was prepared according to the published procedure.[3] All other reagents were commercially available and, unless otherwise noted, were used as received.

b) Experimental procedures and spectral data for compounds

Optimization of transformation of (*S*)-ABA to 3'-I ABA



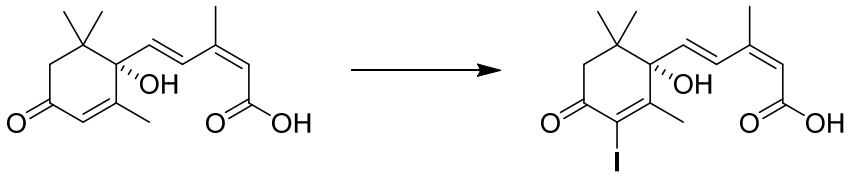
(*S*)-ABA, **1** 3'-I ABA, **6**

Entry	$TMSN_3$ (equiv.)	Pyridine (equiv.)	I^+ (equiv.)	Solvent	[rxn](M)	Time (days)	% Yield[b]	
							3'-I ABA	Recovered ABA
1	2	6	I_2 (2)	CCl4	0.2	1	43	53
2	2	6	I_2 (2)	toluene	0.2	1	40	58
3	2	6	I_2 (2)	CH_2Cl_2	0.2	1	43	54
4	2	6	IBr (2)	CH_2Cl_2	0.2	1	42	42
5	2	6	ICl (2)	CH_2Cl_2	0.2	1	NA	NA
6	12	6	I_2 (2)	CH_2Cl_2	0.2	1	39	54
7	2	2	I_2 (2)	CH_2Cl_2	0.2	1	23	75
8	2	6	I_2 (1)	CH_2Cl_2	0.2	1	19	79
9	2	6	I_2 (4)	CH_2Cl_2	0.2	1	35	35
10	2	6	I_2 (2)	CH_2Cl_2	0.4	1	29	52
11[a]	2	6	I_2 (2)	toluene	0.2	1	<10	90
12	2	6	I_2 (2)	CH_2Cl_2	0.2	2	27	42
13	2	6	I_2 (2)	CH_2Cl_2	0.2	4	20	54

Table 1: Optimization of one-step transformation of ABA to 3'-I ABA. All reactions in the table were based on 1 mmol scale. [a]Reaction performed at 80 °C. [b]Yield by ^{1}H NMR.

Adapting a reported α-iodination method,[4] we started optimizing the reaction using ABA with different solvents. The results showed negligible difference between CCl_4, CH_2Cl_2, and toluene (Table 1, entries 1-3). Using more electrophilic iodine equivalents (e.g., IBr and ICl) did not afford better results than using elemental iodine (Table 1, entries 4-5). Varying the quantity of $TMSN_3$, pyridine, and iodine did not improve the reaction (Table 1, entries 6-9). Increasing the reaction concentration or temperature or longer reaction time drastically reduced the yields (Table 1, entries 10-13). Among several amine bases screened (e.g., pyridine, piperidine, 2,6-lutidine, and DBU), pyridine was found to be most effective for this reaction. Using acidic and basic additives (e.g., TMSCl, TMSOTf, and DMAP) showed either negative effects or no influence on the reaction. Although re-subjection of the crude of entry 3 to the standard reaction condition gave slightly higher conversion, the recovery of starting material was poor.

(2*Z*,4*E*)-5-((*S*)-1-hydroxy-3-iodo-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid (6)



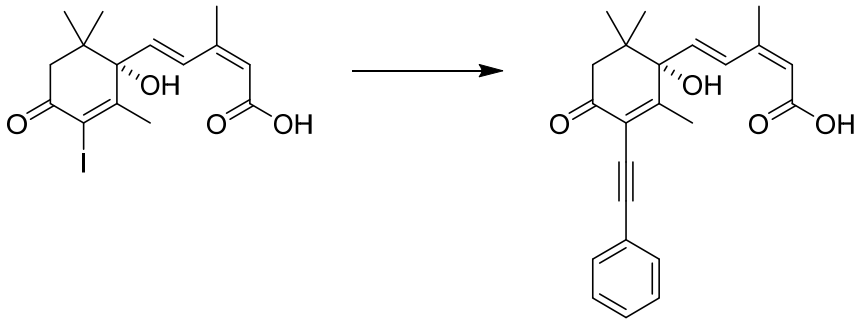
Adapting the known procedure,[4] under argon, ABA (2.6 g, 9.9 mmol), dichloromethane (50 mL), pyridine (5.0 mL), and $TMSN_3$ (2.6 mL, 20. mmol) were sequentially added to a flame-dried RBF at room temperature. To this mixture, iodine (5.04 g, 19.8 mmol), butylated hydroxytoluene (219 mg, 0.99 mmol) were added. After stirring for 24 hours in absence of light, the reaction was quenched by addition of 20% aq. $Na_2S_2O_3$ solution and extracted (3x) with EtOAc. The combined organic layers were washed twice with 10% aq. $CuSO_4$ solution. The combined $CuSO_4$ layers were back extracted once with EtOAc, and the combined organic layers were sequentially washed with water, brine, dried over Na_2SO_4, and concentrated to give a crude material. The crude was fractionated by FCC (30-50% ethyl acetate in hexanes with 0.1% of acetic acid) to give the title compound (1.521 g, 40 % or 80% brSM) and recovered SM (1.310 g, 50%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (1H, d, *J* = 16.0 Hz, HC-4), 6.11 (1H, d, *J* = 16.0 Hz, HC-5), 5.79 (1H, s, HC-2), 2.65 (1H, d, *J* = 17.0 Hz, HC-5'), 2.61 (1H, d, *J* = 17.0 Hz, HC-5'), 2.25 (3H, s, H_3C-7'), 2.04 (3H, d, *J* = 1.0 Hz, H_3C-6), 1.10 (3H, s), 1.04 (3H, s).

^{13}C NMR (125 MHz, $CDCl_3$) δ 191.0 (s, C-4'), 170.9 (s, C-1), 166.3 (s, C-2'), 151.6 (s, C-3), 135.9 (d, C-5), 129.1 (d, C-4), 118.6 (d, C-2), 109.0 (s, C-3'), 81.9 (s, C-1'), 48.2 (t, C-5'), 41.5 (s, C-6'), 27.1 (q, C-7'), 24.2 (q), 23.3 (q), 21.6 (q, C-6).

HRMS *m/z* calcd. for $C_{15}H_{19}IO_4$+Na^+ 413.0220, found 413.0230 (ESI).

(2*Z*,4*E*)-5-((*S*)-1-Hydroxy-2,6,6-trimethyl-4-oxo-3-(phenylethynyl)cyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid (7)



Under argon, 3'-iodo-(*S*)-ABA (2.00 g, 5.13 mmol), tetrakis(triphenylphosphine)palladium (0) (594 mg, 0.514 mmol) and copper (I) iodide (193 mg, 1.01 mmol) were transferred into a round bottom flask and THF (50 mL), triethylamine (2.55 mL) and ethynylbenzene (1.1 mL, 10. mmol) at room temperature were added sequentially. The flask was lowered into an oil bath set to 90 °C. After stirring for 30 minutes, the reaction was allowed to cool to ambient temperature and diluted with ethyl acetate. The organic phase was washed with 1.2 M HCl twice, brine once, dried over Na_2SO_4 and concentrated. The crude was fractionated by FCC (20% to 40% of ethyl acetate in hexanes with 0.1% of acetic acid) to give the title compound (1.50 g, 88%). $[\alpha_D]$ +304 (*c* 1.0, MeOH); UV (CH_3OH) λ_{max}, nm (log ε) 248 (4.6), 293 (sh, 4.4); MP range 144-146 °C.

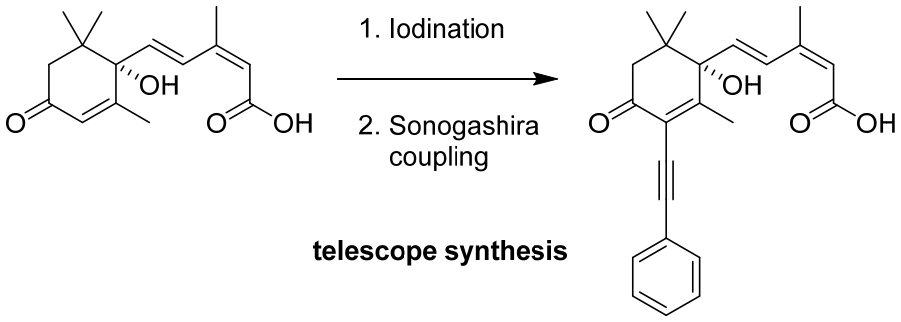
IR (DRIFT) ν_{max} 3452, 2963, 1678. 1632, 1599, 1248, 757, 736 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (1H, d, *J* = 16.0 Hz, HC-4), 7.50-7.54 (2H, m, HC-13' x2), 7.29-7.34 (3H, m, HC-14' x2, HC-15'), 6.17 (1H, d, *J* = 16.0 Hz, HC-5), 5.74 (1H, s, HC-2), 2.58 (1H, d, *J* = 17.0 Hz, HC-5'), 2.45 (1H, d, *J* = 17.0 Hz, HC-5'), 2.20 (3H, s, H_3C-7'), 2.03 (3H, d, *J* = 1.0 Hz, H_3C-6), 1.13 (3H, s), 1.03 (3H, s).

^{13}C NMR (125 MHz, $CDCl_3$) δ 194.3 (s, C-4'), 171.0 (s, C-1), 165.6 (s, C-2'), 151.7 (s, C-3), 136.4 (d, C-5), 131.9 (d, C-13' x2), 128.9 (d, C-4), 128.7 (d, C-15'), 128.4 (d, C-14' x2), 123.1 (s, C-12'), 122.4 (s, C-3'), 118.3 (d, C-2), 97.5 (s, C-11'), 83.0 (s, C-10'), 80.2 (s, C-1'), 49.5 (t, C-5'), 41.1 (s, C-6'), 24.5 (q), 23.3 (q), 21.6 (q, C-6), 18.4 (q, C-7').

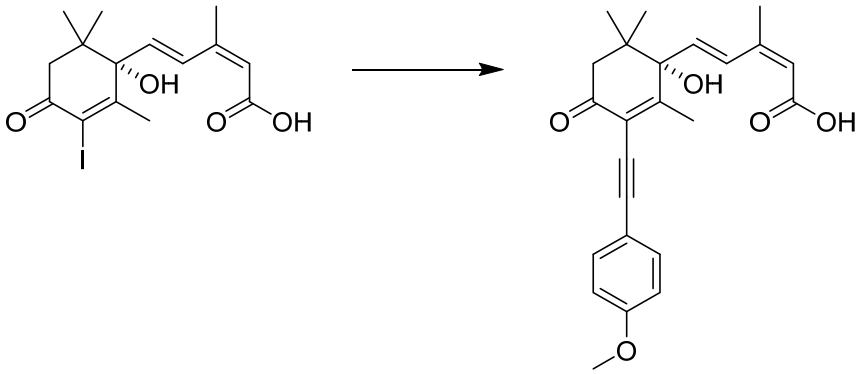
HRMS *m/z* calcd. for $C_{23}H_{24}O_4$+Na^+ 387.1567, found 387.1586 (ESI).

(2*Z*,4*E*)-5-((*S*)-1-Hydroxy-2,6,6-trimethyl-4-oxo-3-(phenylethynyl)cyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid (7)



Under argon, ABA (1.31 g, 5.0 mmol), dichloromethane (25 mL), pyridine (2.5 mL), and $TMSN_3$ (1.3 mL, 10.0 mmol) were sequentially added to a flame-dried RBF at room temperature. To this mixture, iodine (2.55 g, 10.0 mmol), butylated hydroxytoluene (108 mg, 0.5 mmol) were added. After stirring for 24 hours in absence of light, the reaction was quenched by addition of 20% aq. $Na_2S_2O_3$ solution, and extracted (3x) with EtOAc. The combined organic layers were washed twice with 10% aq. $CuSO_4$ solution. The combined $CuSO_4$ layers were back extracted once with EtOAc, and the combined organic layers were sequentially washed with water, brine, dried over Na_2SO_4, and concentrated to give a crude material. To the crude ^1H NMR indicated the presence of a 1.5:1 mixture of ABA and 3'-I ABA. To the crude of 3'-I ABA (~1.95 mmol), THF (30 mL), CuI (75 mg, 0.39 mmol), Et_3N (3 mL), phenylacetylene (0.42 mL, 3.9 mmol) were sequentially added and the mixture was degassed under argon for 10 minutes. $Pd(PPh_3)_4$ (336 mg, 0.29 mmol) was added to the stirring mixture, the flask was immersed into a preheated oil bath (90 °C), and refluxed for 45 minutes. The reaction was cooled to room temperature, diluted with EtOAc, and washed sequentially with 1.2 M HCl, water, and brine. Organic layer was dried over Na_2SO_4 and concentrated to give a crude material. The crude was fractionated by FCC (30-50% ethyl acetate in hexanes with 0.1% of acetic acid) to give the title compound (521 mg, 29% or 74% brSM) and recovered SM (795 mg, 61%).

(2*Z*,4*E*)-5-((*S*)-1-Hydroxy-3-((4-methoxyphenyl)ethynyl)-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid (8)



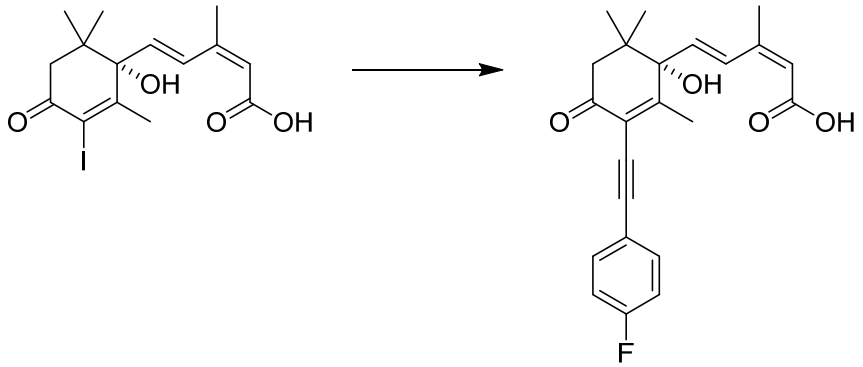
Under argon, 3'-iodo-(*S*)-ABA (108 mg, 0.277 mmol), tetrakis(triphenylphosphine)palladium (0) (160 mg, 0.138 mmol) and copper (I) iodide (27 mg, 0.14 mmol) were transferred to a RBF and sequentially was added THF (2.7 mL), triethylamine (2.7 mL) and 1-ethynyl-4-methoxybenzene (54 mg, 0.41 mmol) at room temperature. The suspension was placed in an oil bath at 100 °C. After stirring for 2 hours, the reaction was allowed to cool to ambient temperature and diluted with ethyl acetate. The organic phase was washed with 1.2 M HCl twice, brine once, dried over Na_2SO_4 and concentrated. The crude was fractionated by FCC (20% to 40% of acetone in hexanes with 0.1% of acetic acid) to give the title compound (67 mg, 61%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (1H, d, *J* = 16.0 Hz, HC-4), 7.46 (2H, ap d, *J* = 9.0 Hz, HC-13' x2), 6.84 (2H, ap d, *J* = 9.0 Hz, HC-14' x2), 6.18 (1H, d, *J* = 16.0 Hz, HC-5), 5.77 (1H, s, HC-2), 3.81 (3H, s, H_3CO), 2.57 (1H, d, *J* = 17.0 Hz, HC-5'), 2.46 (1H, d, *J* = 17.0 Hz, HC-5'), 2.20 (3H, s, H_3C-7'), 2.04 (3H, d, *J* = 1.0 Hz, H_3C-6), 1.13 (3H, s), 1.04 (3H, s).

^{13}C NMR (125 MHz, $CDCl_3$) δ 194.1 (s, C-4'), 170.1 (s, C-1), 164.4 (s, C-2'), 160.0, (s, C-15'), 151.7 (s, C-3), 136.4 (d, C-5), 133.5 (d, C-13' x2), 128.7 (d, C-4), 122.7 (s, C-3'), 118.1 (d, C-2), 115.3 (s, C-12'), 114.1 (d, C-14' x2), 97.7 (s, C-11'), 81.8 (s, C-10'), 80.2 (s, C-1'), 55.5 (q, C-16'), 49.6 (t, C-5'), 41.1 (s, C-6'), 24.5 (q), 23.3 (q), 21.6 (q, C-6), 18.3 (q, C-7').

HRMS *m/z* calcd. for $C_{24}H_{26}O_5$+Na^+ 417.1672, found 417.1686 (ESI).

(2*Z*,4*E*)-5-((*S*)-3-((4-Fluorophenyl)ethynyl)-1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid (9)



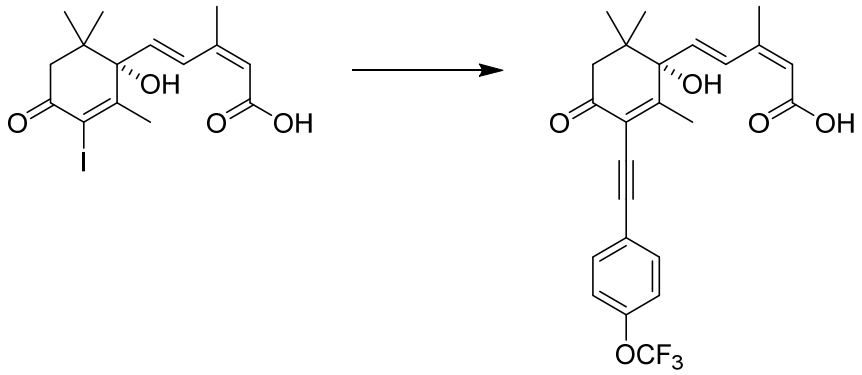
Under argon, 3'-iodo-(*S*)-ABA (97 mg, 0.25 mmol), tetrakis(triphenylphosphine)palladium (0) (144 mg, 0.124 mmol) and copper (I) iodide (24 mg, 0.13 mmol) were transferred to a RBF and sequentially were added THF (2.5 mL), triethylamine (2.5 mL) and 1-ethynyl-4-fluorobenzene (44 mg, 0.37 mmol) at rt. The suspension was placed in an oil bath at 95 °C. After stirring for 1 hour, the reaction was allowed to cool to ambient temperature and diluted with ethyl acetate. The organic phase was washed with 1.2 M HCl twice, brine once, dried over Na_2SO_4 and concentrated. The crude was fractionated by FCC (40% of diethyl ether in toluene with 0.1% of acetic acid) to give the title compound (46 mg, 48%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (1H, d, *J* = 16.0 Hz, HC-4), 7.47-7.53 (2H, m, HC-13' x2), 6.97-7.05 (2H, m, HC-14' x2), 6.17 (1H, d, *J* = 16.0 Hz, HC-5), 5.76 (1H, s, HC-2), 2.57 (1H, d, *J* = 17.0 Hz, HC-5'), 2.45 (1H, d, *J* = 17.0 Hz, HC-5'), 2.19 (3H, s, H_3C-7'), 2.04 (3H, d, *J* = 1.0 Hz, H_3C-6), 1.14 (3H, s), 1.04 (3H, s).

^{13}C NMR (125 MHz, $CDCl_3$) δ 194.1 (s, C-4'), 170.3 (s, C-1), 165.3 (s, C-2'), 162.9 (s, C-15', $^1J_{CF}$ = 249.8 Hz), 151.7 (s, C-3), 136.3 (d, C-5), 133.9 (d, C-13' x2, $^3J_{CF}$ = 8.4 Hz), 128.9 (d, C-4), 122.4 (s, C-3'), 119.3 (s, C-12', $^4J_{CF}$ = 3.5 Hz), 118.2 (d , C-2), 115.8 (d, C-14' x2, $^2J_{CF}$ = 22.1 Hz), 97.5 (s, C-11'), 82.7 (s, C-10'), 80.2 (s, C-1'), 49.6 (t, C-5'), 41.2 (s, C-6'), 24.5 (q), 23.3 (q), 21.6 (q, C-6), 18.4 (q, C-7').

HRMS *m/z* calcd. for $C_{23}H_{23}FO_4$+Na^+ 405.1473, found 405.1456 (ESI).

(2*Z*,4*E*)-5-((*S*)-1-Hydroxy-2,6,6-trimethyl-4-oxo-3-((4-(trifluoromethoxy)phenyl)ethynyl)cyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid (10)



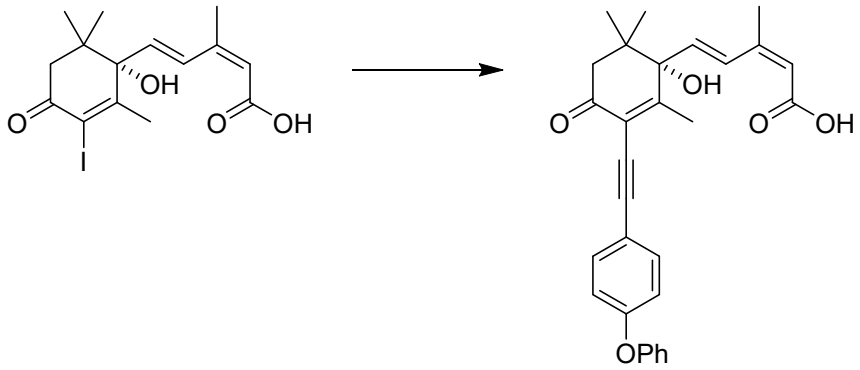
Under argon, 3'-iodo-(*S*)-ABA (100 mg, 0.256 mmol), tetrakis(triphenylphosphine)palladium (0) (149 mg, 0.129 mmol) and copper (I) iodide (25 mg, 0.13 mmol) were transferred to a RBF and sequentially was added THF (2.6 mL), triethylamine (2.6 mL) and 1-ethynyl-4-(trifluoromethoxy)benzene (0.07 mL, 0.5 mmol) at room temperature. The suspension was placed in an oil bath at 95 °C. After stirring for 1 hour, the reaction was allowed to cool to ambient temperature and diluted with ethyl acetate. The organic phase was washed with 1.2 M HCl twice, brine once, dried over Na_2SO_4 and concentrated. The crude was fractionated by FCC (40% of diethyl ether in toluene with 0.1% of acetic acid) to give the title compound (71 mg, 61%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (1H, d, *J* = 16.0 Hz, HC-4), 7.54 (2H, ap d, *J* = 8.5 Hz, HC-13' x2), 7.16 (2H, ap d, *J* = 8.5 Hz, HC-14' x2), 6.17 (1H, d, *J* = 16.0 Hz, HC-5), 5.76 (1H, s, HC-2), 2.57 (1H, d, *J* = 17.0 Hz, HC-5'), 2.46 (1H, d, *J* = 17.0 Hz, HC-5'), 2.19 (3H, s, H_3C-7'), 2.04 (3H, d, *J* = 1.0 Hz, H_3C-6), 1.14 (3H, s), 1.04 (3H, s).

^{13}C NMR (125 MHz, $CDCl_3$) δ 194.0 (s, C-4'), 170.3 (s, C-1), 165.9 (s, C-2'), 151.7 (s, C-3), 149.3 (s, C-15', $^3J_{CF}$ = 1.9 Hz), 136.3 (d, C-5), 133.5 (d, C-13' x2), 129.0 (d, C-4), 122.3 (s, C-3'), 121.9 (s, C-12'), 120.9 (d, C-14' x2, $^4J_{CF}$ = 1.1 Hz), 120.6 (s, C-16', $^1J_{CF}$ = 257.9 Hz), 118.2 (d, C-2), 96.0 (s, C-11'), 83.8 (s, C-10'), 80.2 (s, C-1'), 49.5 (t, C-5'), 41.2 (s, C-6'), 24.5 (q), 23.3 (q), 21.6 (q, C-6), 18.5 (q, C-7').

HRMS *m/z* calcd. for $C_{24}H_{23}F_3O_4$+Na^+ 471.1395, found 471.1409 (ESI).

(2*Z*,4*E*)-5-((*S*)-1-Hydroxy-2,6,6-trimethyl-4-oxo-3-((4-phenoxyphenyl)ethynyl)cyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid (11)



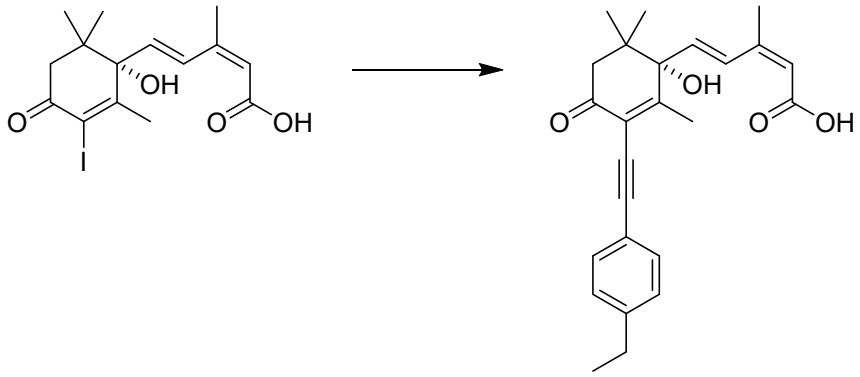
Under argon, 3'-iodo-(*S*)-ABA (100 mg, 0.256 mmol), tetrakis(triphenylphosphine)palladium (0) (148 mg, 0.128 mmol) and copper (I) iodide (26 mg, 0.14 mmol) were transferred to a RBF and sequentially were added THF (2.6 mL), triethylamine (2.6 mL) and 1-ethynyl-4-phenoxybenzene (0.07 mL, 0.4 mmol) at rt. The suspension was placed in an oil bath at 95 °C. After stirring for 1 hour, the reaction was allowed to cool to ambient temperature and diluted with ethyl acetate. The organic phase was washed with 1.2 M HCl twice, brine once, dried over Na_2SO_4 and concentrated. The crude was fractionated by FCC (30% of acetone in hexanes with 0.1% of acetic acid) to give the title compound (77 mg, 65%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 (1H, bd, *J* = 16.0 Hz, HC-4), 7.48 (2H, d, *J* = 8.5 Hz, HC-13' x2), 7.35 (2H, dd, *J* = 7.5, 8.5 Hz, HC-18' x2), 7.14 (1H, t, *J* = 7.5 Hz, HC-19'), 7.02 (2H, d, *J* = 8.5 Hz, HC-17' x2), 6.92 (2H, d, *J* = 8.5 Hz, HC-14' x2), 6.17 (1H, d, *J* = 16.0 Hz, HC-5), 5.78 (1H, bs, HC-2), 2.57 (1H, d, *J* = 17.0 Hz, HC-5'), 2.46 (1H, d, *J* = 17.0 Hz, HC-5'), 2.20 (3H, s, H_3C-7'), 2.04 (3H, s, H_3C-6), 1.14 (3H, s), 1.04 (3H, s).

^{13}C NMR (125 MHz, $CDCl_3$) δ 194.2 (s, C-4'), 170.1 (s, C-1), 165.0 (s, C-2'), 158.0 (s, C-15'), 156.5 (s, C-16'), 151.7 (s, C-3), 136.4 (d, C-5), 133.6 (d, C-13' x2), 130.1 (d, C-18' x2), 128.8 (d, C-4), 124.1, (d, C-19'), 122.6 (s, C-3'), 119.7 (d, C-17' x2), 118.4 (d, C-14' x2), 118.2 (d, C-2), 117.6 (s, C-12'), 97.2 (s, C-11'), 82.4 (s, C-10'), 80.2 (s, C-1'), 49.6 (t, C-5'), 41.1 (s, C-6'), 24.5 (q), 23.3 (q), 21.6 (q, C-6), 18.4 (q, C-7').

HRMS *m/z* calcd. for $C_{29}H_{28}O_5$+Na^+ 479.1829, found 479.1849 (ESI).

(2*Z*,4*E*)-5-((*S*)-3-((4-Ethylphenyl)ethynyl)-1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid (12)



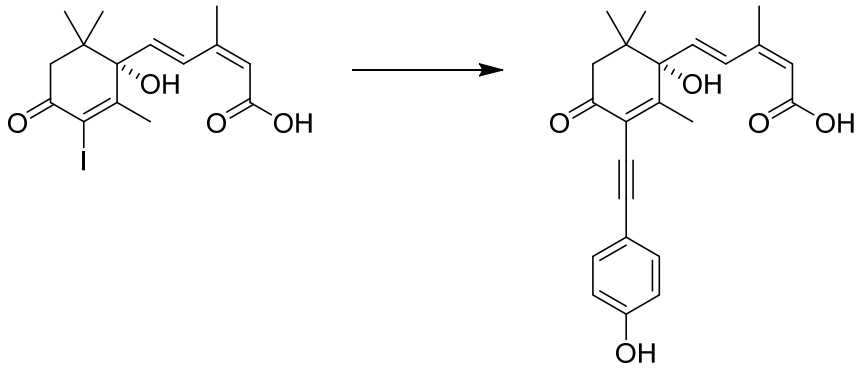
Under argon, 3'-iodo-(*S*)-ABA (101 mg, 0.259 mmol), tetrakis(triphenylphosphine)palladium (0) (149 mg, 0.129 mmol) and copper (I) iodide (25 mg, 0.13 mmol) were transferred to a RBF and sequentially were added THF (2.6 mL), triethylamine (2.6 mL) and 1-ethyl-4-ethynylbenzene (0.06 mL, 0.4 mmol) at rt. The flask was placed in an oil bath at 95 °C. After stirring for 1 hour, the reaction was allowed to cool to ambient temperature and diluted with ethyl acetate. The organic phase was washed with 1.2 M HCl twice, brine once, dried over Na_2SO_4 and concentrated. The crude was fractionated by FCC (30% of diethyl ether in toluene with 0.1% of acetic acid) to give the title compound (61 mg, 59%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (1H, d, *J* = 16.0 Hz, HC-4), 7.43 (2H, d, *J* = 8.0 Hz, HC-13' x2), 7.14 (2H, d, *J* = 8.0 Hz, HC-14' x2), 6.17 (1H, d, *J* = 16.0 Hz, HC-5), 5.77 (1H, s, HC-2), 2.64 (2H, q, *J* = 7.5 Hz, H_2C-16'), 2.57 (1H, d, *J* = 17.0 Hz, HC-5'), 2.46 (1H, d, *J* = 17.0 Hz, HC-5'), 2.19 (3H, s, H_3C-7'), 2.04 (3H, d, *J* = 1.0 Hz, H_3C-6), 1.22 (3H, t, *J* = 7.5 Hz, H_3C-17'), 1.13 (3H, s), 1.03 (3H, s).

^{13}C NMR (125 MHz, $CDCl_3$) δ 194.2 (s, C-4'), 170.3 (s, C-1), 165.0 (s, C-2'), 151.7 (s, C-3), 145.2 (s, C-15'), 136.4 (d, C-5), 132.0 (d, C-13' x2), 128.8 (d, C-4), 128.0 (d, C-14' x2), 122.6 (s, C-3'), 120.3 (s, C-12'), 118.2 (d, C-2), 97.8 (s, C-11'), 82.3 (s, C-10'), 80.2 (s, C-1'), 49.6 (t, C-5'), 41.1 (s, C-6'), 29.0 (t, C-16'), 24.5 (q), 23.3 (q), 21.6 (q, C-6), 18.3 (q, C-7'), 15.5 (q, C-17').

HRMS *m/z* calcd. for $C_{25}H_{28}O_4$+Na^+ 415.1885, found 415.1881 (ESI).

(2*Z*,4*E*)-5-((*S*)-1-hydroxy-3-((4-hydroxyphenyl)ethynyl)-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid (13).



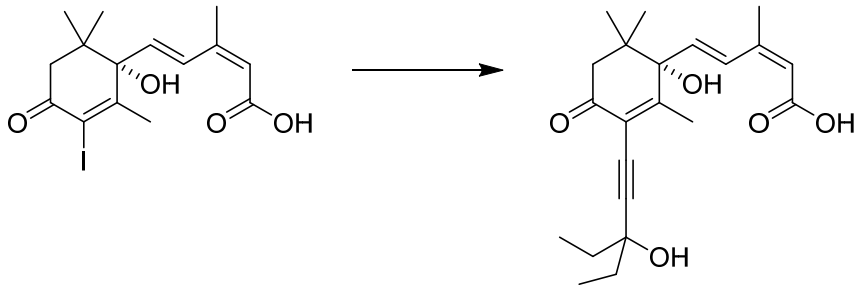
Under argon, 3'-iodo-(*S*)-ABA (157 mg, 0.40 mmol), 4-ethynylphenol[3] (94 mg, 0.80 mmol), copper (I) iodide (39 mg, 0.20 mmol), triethylamine (0.8 mL) and THF (4.0 mL) were transferred to a RBF and the mixture was degassed with argon for 10 minutes. Tetrakis(triphenylphosphine)palladium (0) (233 mg, 0.20 mmol) was added to the reaction mixture and the flask was placed in an oil bath at 90 °C. After stirring for 1.5 hours, the reaction was allowed to cool to ambient temperature, cooled to 0 °C and quenched with 1 N HCl. The mixture was diluted with ethyl acetate, separated the layers and the organic phase was washed with brine once, dried over Na_2SO_4 and concentrated. The crude was fractionated by FCC (30% to 100% of ethyl acetate in hexanes with 0.2% of acetic acid) to give a semi pure compound that was further purified through PTLC (15% of isopropanol in hexanes with 0.2% of acetic acid) to give the title compound (21 mg, 14%).

^{1}H NMR (500 MHz, CD_3OD) δ 7.81 (1H, d, *J* = 16.1 Hz, HC-4), 7.35 (2H, d, *J* = 8.7 Hz, HC-13'), 6.76 (2H, d, *J* = 8.7 Hz, HC-14'), 6.27 (1H, d, *J* = 16.1 Hz, HC-5) 5.75 (1H, s, HC-2), 2.64 (1H, d, *J* = 16.9 Hz, HC-5'), 2.34 (1H, d, *J* = 16.9 Hz, HC-5'), 2.20 (3H, s, H_3C-7'), 2.05 (3H, d, *J* = 1.0 Hz, H_3C-6), 1.07 (3H, s, H_3C-8' or H_3C-9'), 1.04 (3H, s, H_3C-8' or H_3C-9').

^{13}C NMR (125 MHz, CD_3OD) δ 197.2 (s, C-4'), 169.5 (s, C-1), 167.3 (s, C-2'), 159.3 (s, C-15'), 151.0 (s, C-3), 137.5 (s, C-5), 134.2 (d, C-13'), 129.8 (d, C-4), 123.4 (s, C-3'), 119.7 (d, C-2), 116.4 (d, C-14'), 115.1 (s, C-12'), 98.7 (s, C-11'), 81.8 (s, C-10'), 80.7 (s, C-1'), 50.4 (t, C-5'), 42.2 (s, C-6'), 24.7 (q, C-8' or C-9'), 23.6 (q, C-8' or C-9'), 21.3 (q, C-6), 18.8 (q, C-7').

HRMS *m/z* calcd for $C_{23}H_{25}O_5Na^+$ 403.1515, found 403.1530 (ESI).

(2*Z*,4*E*)-5-((*S*)-3-(3-ethyl-3-hydroxypent-1-yn-1-yl)-1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid (14).



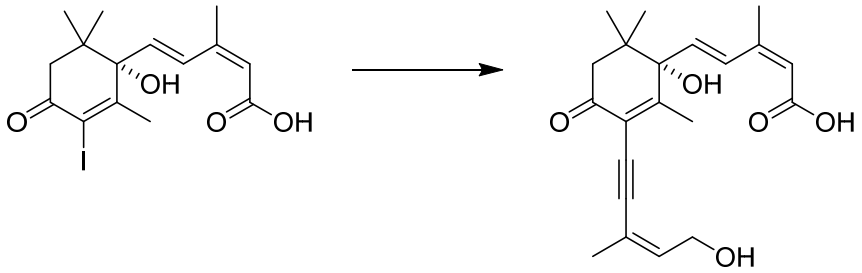
Under argon, 3'-iodo-(*S*)-ABA (105 mg, 0.27 mmol), tetrakis(triphenylphosphine)palladium (0) (94 mg, 0.081 mmol) and copper (I) iodide (16 mg, 0.084 mmol) were transferred to a RBF and sequentially were added THF (2.7 mL), triethylamine (0.54 mL) and 3-ethyl-1-pentyn-3-ol (0.05 mL, 0.4 mmol) at rt. The flask was placed in an oil bath at 95 °C. After stirring for 1 hour, the reaction was allowed to cool to ambient temperature and diluted with ethyl acetate. The organic phase was washed with 1.2 M HCl twice, brine once, dried over Na_2SO_4 and concentrated. The crude was fractionated by FCC (30% of acetone in hexanes with 0.1% of acetic acid) to give the title compound (56 mg, 55%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (1H, d, *J* = 16.0 Hz, HC-4), 6.13 (1H, d, *J* = 16.0 Hz, HC-5), 5.77 (1H, bs, HC-2), 2.50 (1H, d, *J* = 17.0 Hz, HC-5'), 2.37 (1H, d, *J* = 17.0 Hz, HC-5'), 2.13 (3H, s, H_3C-7'), 2.04 (3H, s, H_3C-6), 1.67-1.81 (4H, m, H_2C-13' x2), 1.10 (3H, s), 1.08 (6H, dt, *J* = 1.5, 7.5 Hz, H_3C-14' x2), 1.03 (3H, s).

^{13}C NMR (125 MHz, $CDCl_3$) δ 194.5 (s, C-4'), 170.5 (s, C-1), 165.3 (s, C-2'), 151.5 (s, C-3), 136.3 (d, C-5), 129.1 (d, C-4), 122.3 (s, C-3'), 118.6 (d, C-2), 100.5 (s, C-11'), 80.4 (s, C-10'), 77.8 (s, C-1'), 72.8 (s, C-12'), 49.5 (t, C-5'), 41.2 (s, C-6'), 34.50 (t, C-13'), 34.45 (t, C-13'), 24.4 (q), 23.3 (q), 21.6 (q, C-6), 18.6 (q, C-7'), 8.93 (q, C-14'), 8.91 (q, C-14').

HRMS *m/z* calcd. for $C_{22}H_{30}O_5$+Na^+ 397.1985, found 397.1973 (ESI).

(2*Z*,4*E*)-5-((*S*)-1-hydroxy-3-((*Z*)-5-hydroxy-3-methylpent-3-en-1-yn-1-yl)-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid (15).



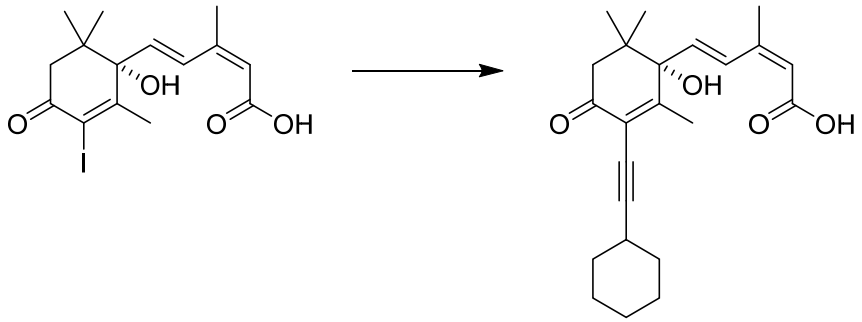
Under argon, 3'-iodo-(*S*)-ABA (205 mg, 0.526 mmol), tetrakis(triphenylphosphine)palladium (0) (184 mg, 0.16 mmol) and copper (I) iodide 29.5 mg, 0.15 mmol) were weighted into a RBF and sequentially added THF (5.4 mL), triethylamine (1.1 mL) and (*Z*)-3-methylpent-2-en-4-yn-1-ol (81.5 mg, 0.85 mmol) at rt. The suspension was submerged to a pre-heated 95 °C oil bath. After stirring for 1 hour, the reaction was allowed to cool to ambient temperature and diluted with ethyl acetate. The organic phase was washed with 1.2 M HCl twice, brine once, dried over Na_2SO_4 and concentrated. The crude was fractionated by FCC (40% of acetone in hexanes with 0.1% of acetic acid) to give the title compound (95.5 mg, 51%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (1H, d, *J* = 16.0 Hz, HC-4), 6.13 (1H, d, *J* = 16.0 Hz, HC-5), 5.99 (1H, dt, *J* = 1.0, 6.5 Hz, HC-13'), 5.76 (1H, s, HC-2), 4.35 (2H, dd, *J* = 6.5, 6.5 Hz, H_2C-14'), 2.52 (1H, d, *J* = 17.0 Hz, HC-5'), 2.40 (1H, d, *J* = 17.0 Hz, HC-5'), 2.15 (3H, s, H_3C-7'), 2.03 (3H, d, *J* = 1.0 Hz, H_3C-6), 1.94 (3H, d, *J* = 1.0 Hz, H_3C-15'), 1.10 (3H, s), 1.04 (3H, s).

^{13}C NMR (125 MHz, $CDCl_3$) δ 194.7 (s, C-4'), 169.7 (s, C-1), 165.3 (s, C-2'), 151.0 (s, C-3), 136.8 (d, C-13'), 136.1 (d, C-5), 129.1 (d, C-4), 122.5 (s, C-3'), 121.7 (s, C-12'), 118.2 (d, C-2), 96.1 (s, C-11'), 87.8 (s, C-10'), 80.3 (s, C-1'), 60.9 (t, C-14'), 49.5 (t, C-5'), 41.2 (s, C-6'), 24.4 (q), 23.3 (q), 23.0 (q, C-15'), 21.5 (q, C-6), 18.7 (q, C-7').

HRMS *m/z* calcd. for $C_{21}H_{26}O_5$+Na^+ 381.1672, found 381.1681 (ESI).

(2*Z*,4*E*)-5-((*S*)-3-(cyclohexylethynyl)-1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid (16).



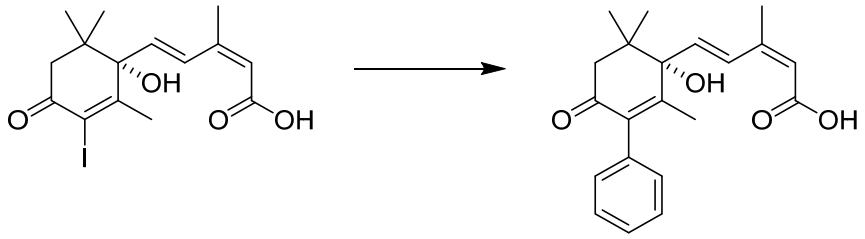
Under argon, 3'-iodo-(*S*)-ABA (157 mg, 0.40 mmol), ethynylcyclohexane (87 mg, 105 µL, 0.80 mmol), copper (I) iodide (39 mg, 0.20 mmol), triethylamine (0.8 mL) and THF (4.0 mL) were transferred to a RBF and the mixture was degassed with argon for 10 minutes. Tetrakis(triphenylphosphine)palladium (0) (137 mg, 0.12 mmol) was added to the reaction mixture and the flask was placed in an oil bath at 90 °C. After stirring for 1.5 hours, the reaction was allowed to cool to ambient temperature, cooled to 0 °C and quenched with 1 N HCl. The mixture was diluted with ethyl acetate, separated the layers and the organic phase was washed with brine once, dried over Na_2SO_4 and concentrated. The crude was fractionated by FCC (20% to 40% of ethyl acetate in hexanes with 0.2% of acetic acid) to give the title compound (71 mg, 47%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (1H, d, *J* = 15.9 Hz, HC-4), 6.16 (1H, d, *J* = 16.0 Hz, HC-5) 5.77 (1H, s, HC-2), 2.66-2.61(1H, m, HC-12'), 2.51 (1H, d, *J* = 17.2 Hz, HC-5'), 2.41 (1H, d, *J* = 17.2 Hz, HC-5'), 2.11 (3H, s, H_3C-7'), 2.04 (3H, d, *J* = 1.1 Hz, H_3C-6), 1.89-1.83 (2H, m, HC-13'), 1.76-1.69 (2H, m, HC-14'), 1.56-1.49 (3H, m, HC-13', HC-15'), 1.36-1.31 (3H, m, HC-14', HC-15'), 1.1 (3H, s, H_3C-8' or H_3C-9'), 1.0 (3H, s, H_3C-8' or H_3C-9').

^{13}C NMR (125 MHz, $CDCl_3$) δ 194.7 (s, C-4'), 170.9 (s, C-1), 164.1 (s, C-2'), 151.8 (s, C-3), 136.6 (s, C-5), 128.5 (d, C-4), 122.7 (s, C-3'), 118.1 (d, C-2), 103.2 (s, C-11'), 80.0 (s, C-1'), 74.0 (s, C-10'), 49.5 (t, C-5'), 41.0 (s, C-6'), 32.7 (t, C-13'), 30.0 (d, C-12'), 26.1 (t, C-15'), 25.0 (d, C-14'), 24.5 (q, C-8' or C-9'), 23.3 (q, C-8' or C-9'), 21.6 (q, C-6), 18.1 (q, C-7').

HRMS *m/z* calcd for $C_{23}H_{30}O_4Na^+$ 393.2036, found 393.2055 (ESI).

(2*Z*,4*E*)-5-((*S*)-3-hydroxy-2,4,4-trimethyl-6-oxo-3,4,5,6-tetrahydro-[1,1'-biphenyl]-3-yl)-3-methylpenta-2,4-dienoic acid (17).



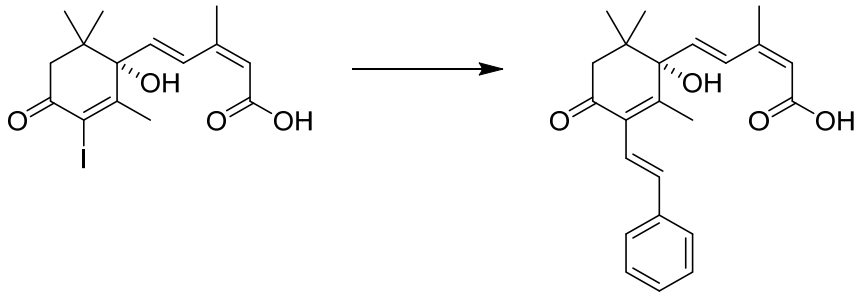
Under argon, 3'-iodo-(*S*)-ABA (45 mg, 0.11 mmol), tetrakis(triphenylphosphine)palladium (0) (13 mg, 0.011 mmol), phenylboronic acid (17 mg, 0.14 mmol) and potassium carbonate (77 mg, 0.55 mmol) were transferred to a RBF and were added a 9:1 mixture of THF, H_2O (2.0 mL). The flask was placed in an oil bath at 90 °C. After stirring for 21 hours, the reaction was allowed to cool to ambient temperature, cooled to 0 °C and quenched with 1 N HCl. The mixture was diluted with ethyl acetate, separated the layers and the organic phase was washed with brine once, dried over Na_2SO_4 and concentrated. The crude was fractionated by PTLC (10% of methanol in dichloromethane) to give the title compound (16 mg, 41%). The ^{1}H NMR data for **17** were consistent with those previously reported.[5]

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (1H, d, *J* = 16.2 Hz, HC-4), 7.36 (2H, t, *J* = 7.2 Hz, HC-12'), 7.29 (1H, t, *J* = 7.4 Hz, HC-13'), 7.07 (1H, d, *J* = 7.0 Hz, HC-11'), 6.26 (1H, d, *J* = 16.2 Hz, HC-5) 5.79 (1H, s, HC-2), 2.60 (1H, d, *J* = 17.0 Hz, HC-5'), 2.43 (1H, d, *J* = 17.0 Hz, HC-5'), 2.08 (3H, s, H_3C-6), 1.70 (3H, s, H_3C-7'), 1.21 (3H, s, H_3C-8' or H_3C-9'), 1.08 (3H, s, H_3C-8' or H_3C-9').

^{13}C NMR (125 MHz, $CDCl_3$) δ 196.7 (s, C-4'), 171.1 (s, C-1), 157.2 (s, C-2'), 151.5 (s, C-3), 138.8 (s, C-3'), 137.5 (d, C-5), 135.8 (s, C-10'), 129.9 (d, C-11'), 128.6 (d, C-4), 128.4 (d, C-12'), 127.6 (d, C-13'), 118.5 (d, C-2), 80.4 (s, C-1'), 49.8 (t, C-5'), 41.3 (s, C-6'), 24.6 (q, C-8' or C-9'), 23.3 (q, C-8' or C-9'), 21.6 (q, C-6), 17.4 (q, C-7').

HRMS *m/z* calcd for $C_{21}H_{23}O_4$ (M-1) 339.1601, found 339.1591 (ESI).

(2*Z*,4*E*)-5-((*S*)-1-hydroxy-2,6,6-trimethyl-4-oxo-3-((*E*)-styryl)cyclohex-2-en-1-yl)-3-methylpenta-2,4-dienoic acid (18).



Under argon, 3'-iodo-(*S*)-ABA (140 mg, 0.36 mmol), tetrakis(triphenylphosphine)palladium (0) (21 mg, 0.018 mmol), *trans*-2-phenylvinylboronic acid (108 mg, 0.72 mmol) and potassium carbonate (201 mg, 1.44 mmol) were transferred to a RBF and were added a 9:1 mixture of THF, H_2O (7.2 mL). The flask was placed in an oil bath at 90 °C. After stirring for 24 hours, the reaction was allowed to cool to ambient temperature, cooled to 0 °C and quenched with 1 N HCl. The mixture was diluted with ethyl acetate, separated the layers and the organic phase was washed with brine once, dried over Na_2SO_4 and concentrated. The crude was fractionated by FCC (40% of ethyl acetate in hexanes with 0.1% of acetic acid) to give the title compound (30 mg, 23%), [α_D] +319 (*c* 2.4, MeOH).

IR (DRIFT) ν_{max} 3450, 3027, 1669, 1598, 1449, 1374, 1248, 749, 694 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (1H, d, *J* = 16.0 Hz, HC-4), 7.46 (2H, d, *J* = 7.4 Hz, HC-13'), 7.32 (2H, t, *J* = 7.4 Hz, HC-14'), 7.25 (1H, t, *J* = 7.4 Hz, HC-15'), 7.0 (1H, d, *J* = 16.5 Hz, HC-11'), 6.84 (1H, d, *J* = 16.5 Hz, HC-10'), 6.21 (1H, d, *J* = 16.1 Hz, HC-5) 5.75 (1H, s, HC-2), 2.58 (1H, d, *J* = 16.9 Hz, HC-5'), 2.42 (1H, d, *J* = 16.9 Hz, HC-5'), 2.09 (1H, s, OH), 2.05 (6H, s, H_3C-6, H_3C-7'), 1.14 (3H, s, H_3C-8' or H_3C-9'), 1.04 (3H, s, H_3C-8' or H_3C-9').

^{13}C NMR (125 MHz, $CDCl_3$) δ 197.1 (s, C-4'), 171.1 (s, C-1), 156.3 (s, C-2'), 152.0 (s, C-3), 137.7 (s, C-12'), 137.3 (d, C-5), 135.9 (d, C-11'), 133.4 (s, C-3'), 128.7 (d, C-14'), 128.6 (d, C-4), 128.0 (d, C-15'), 126.8 (d, C-13'), 121.6 (d, C-10'), 118.0 (d, C-2), 80.7 (s, C-1'), 50.2 (t, C-5'), 40.8 (s, C-6'), 24.7 (q, C-8' or C-9'), 23.4 (q, C-8' or C-9'), 21.7 (q, C-6), 16.7 (q, C-7').

HRMS *m/z* calcd for $C_{23}H_{25}O_4$ (M-1) 365.1758, found 365.1744 (ESI).

c) References

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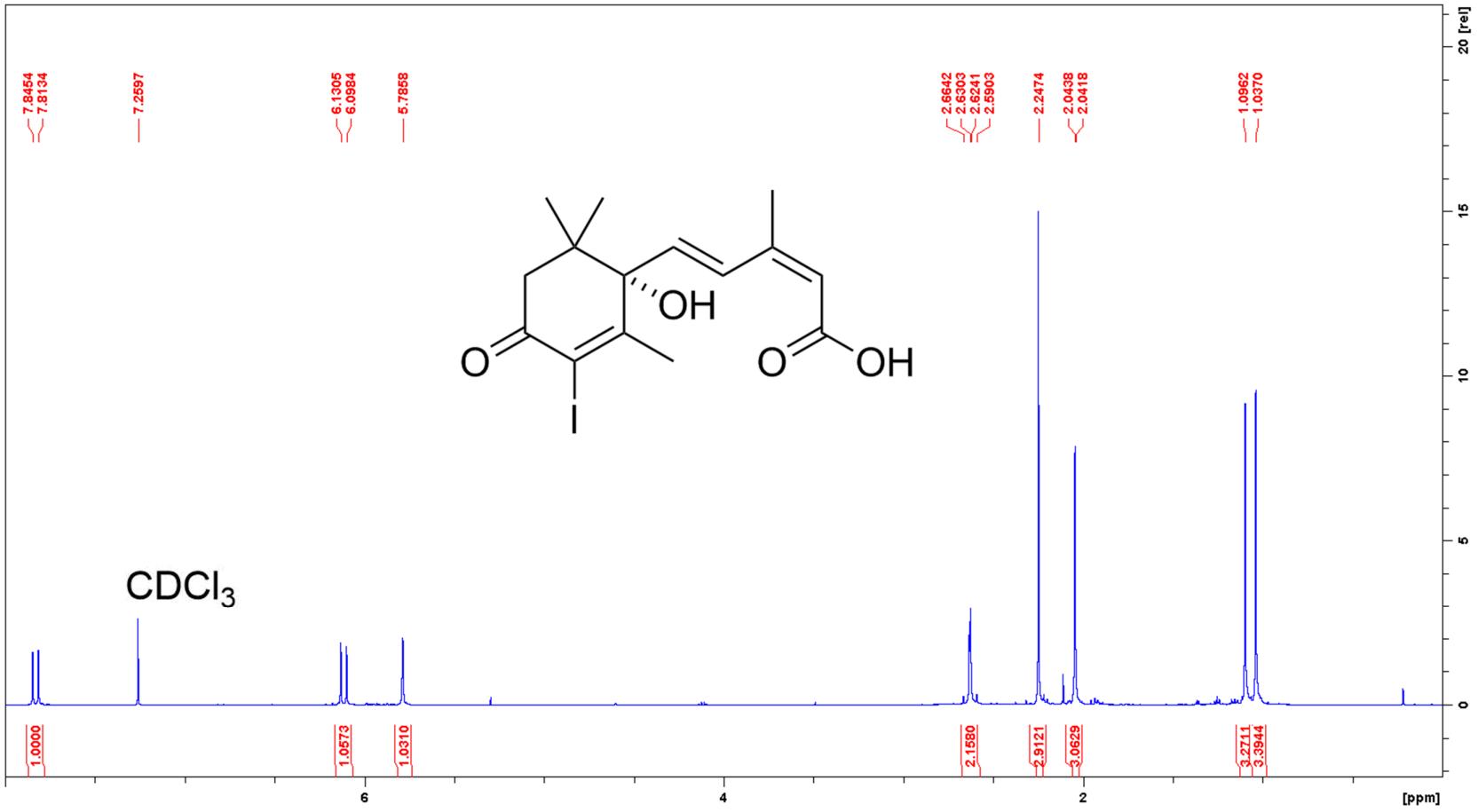
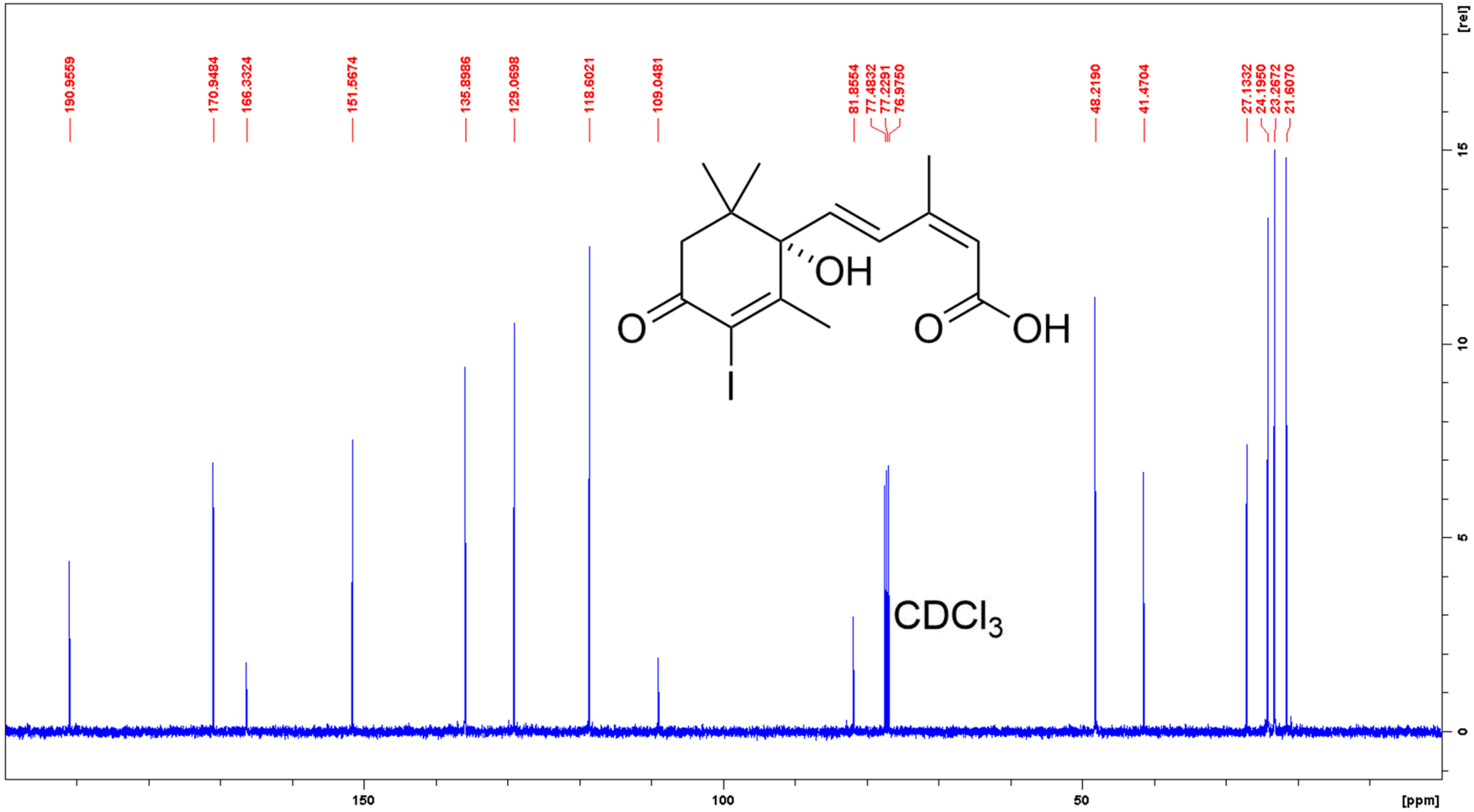
d) Spectra for compounds

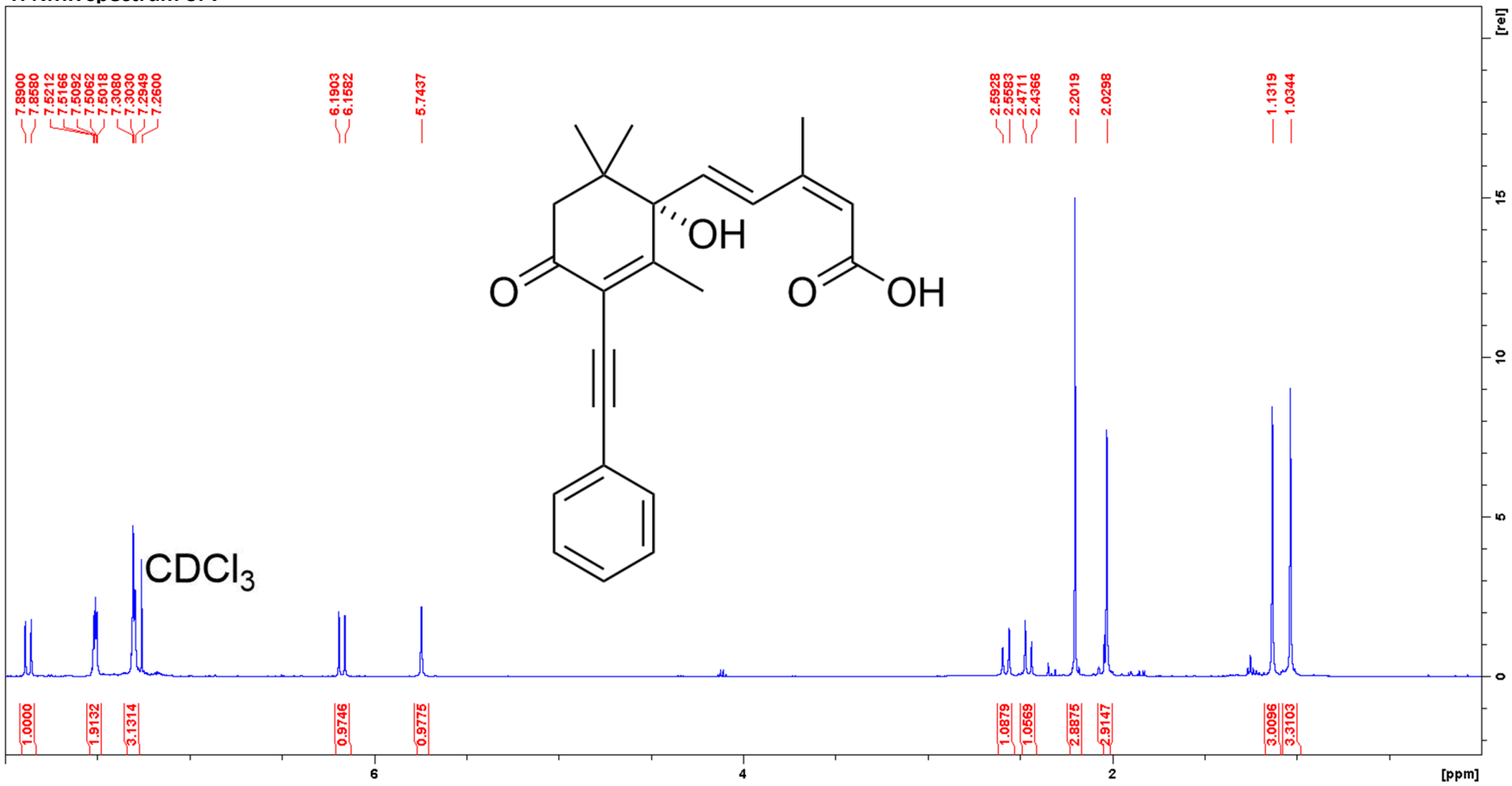
^{1}H NMR spectrum of 6



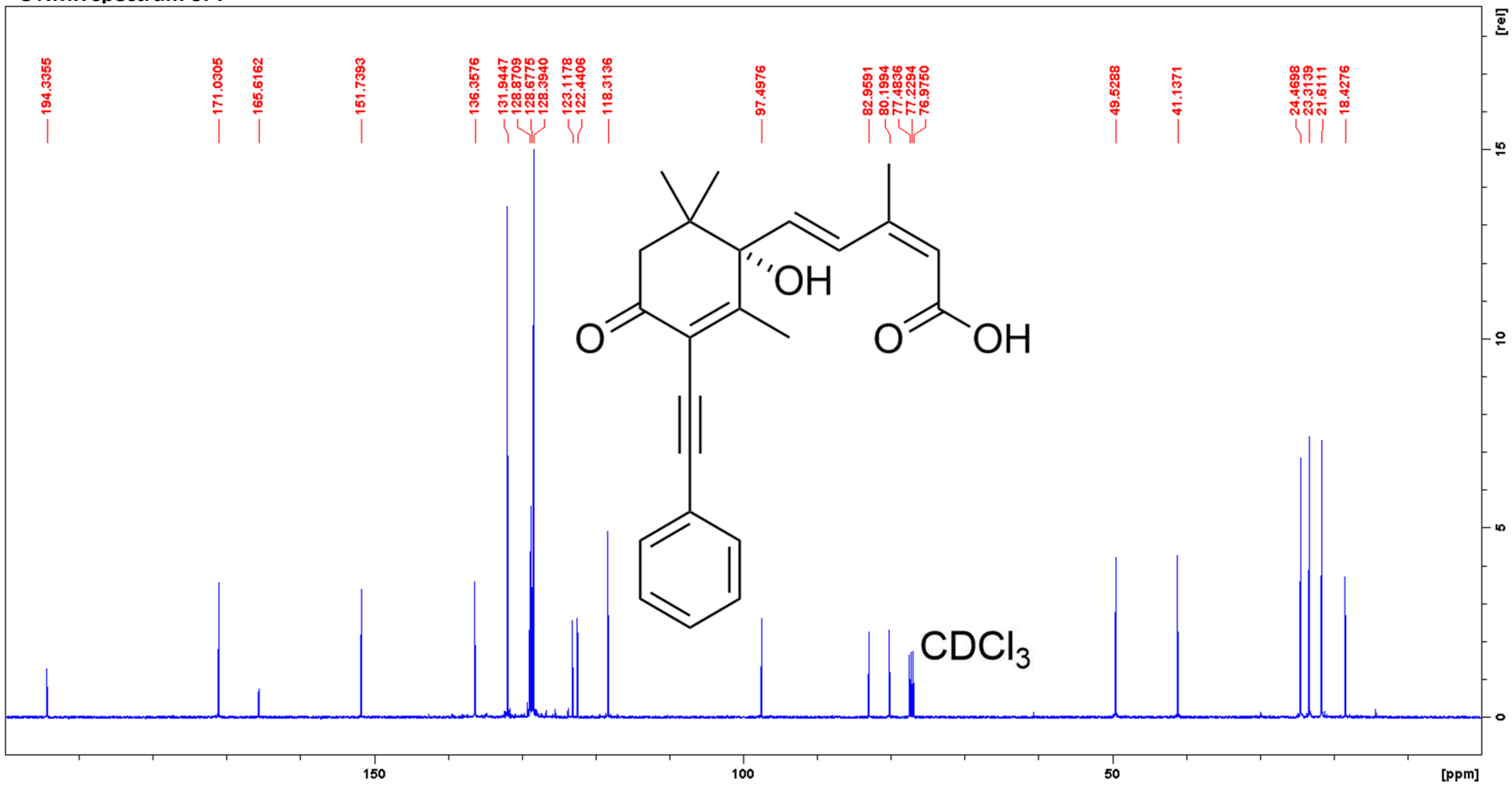
^{13}C NMR spectrum of 6



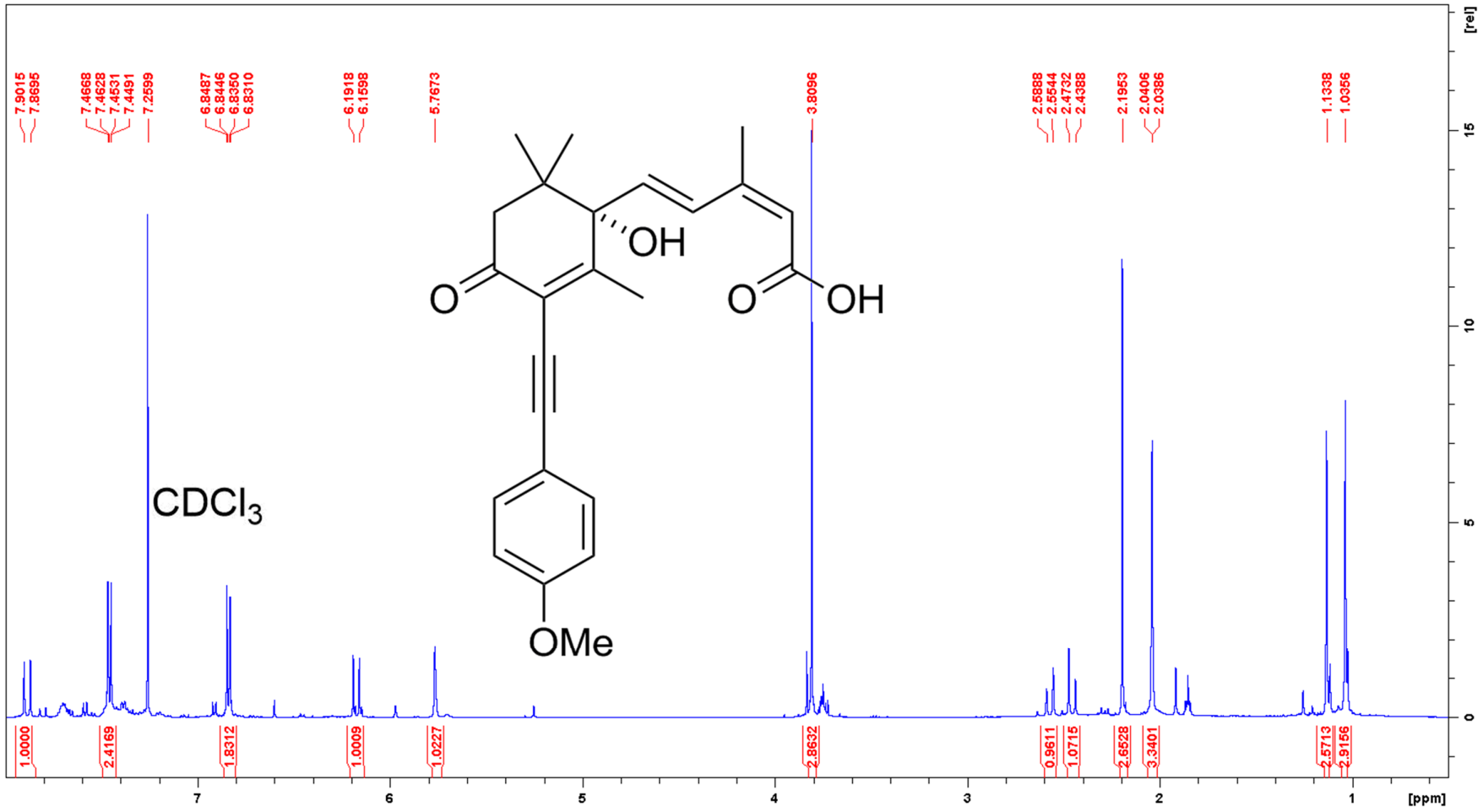
^{1}H NMR spectrum of 7



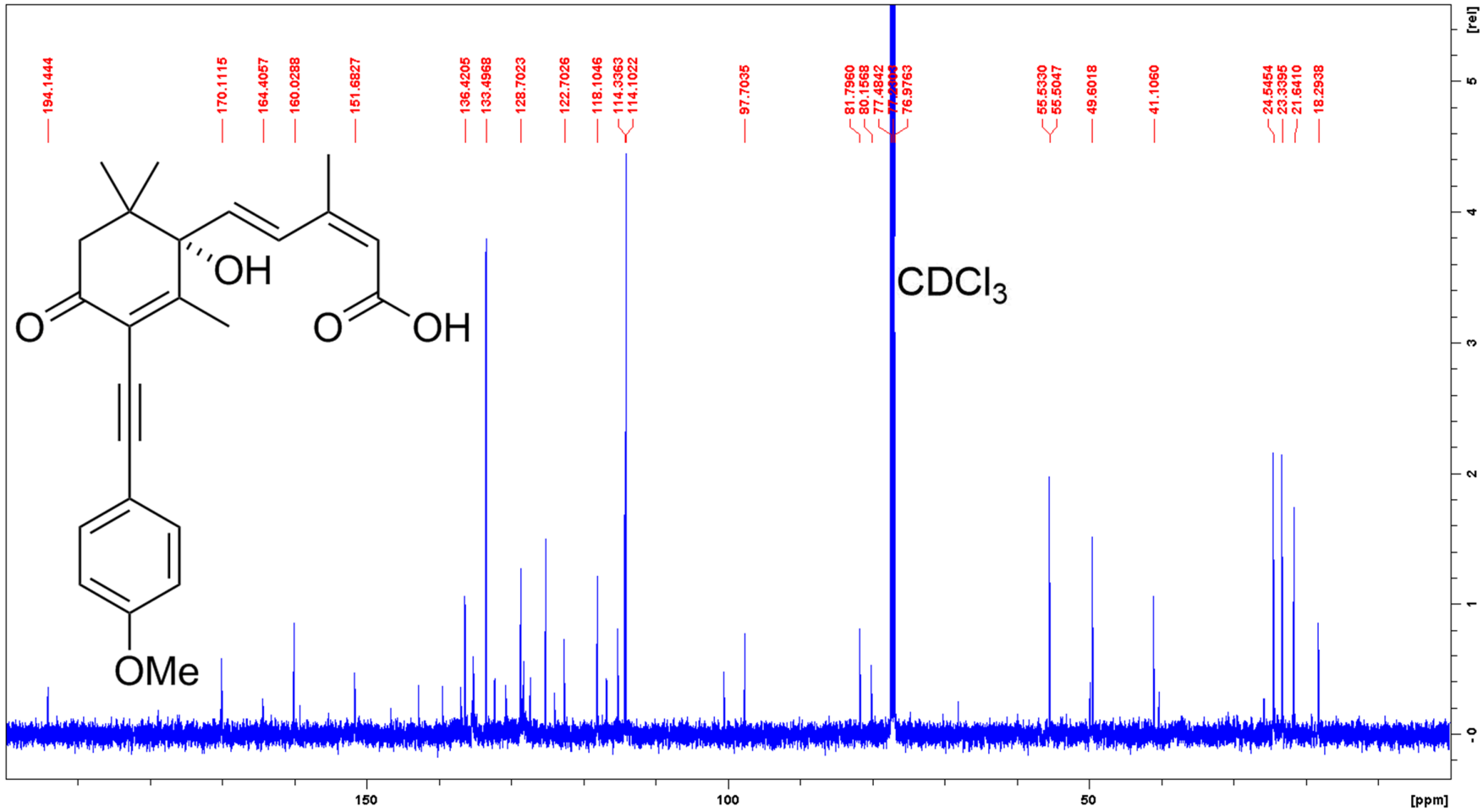
^{13}C NMR spectrum of 7



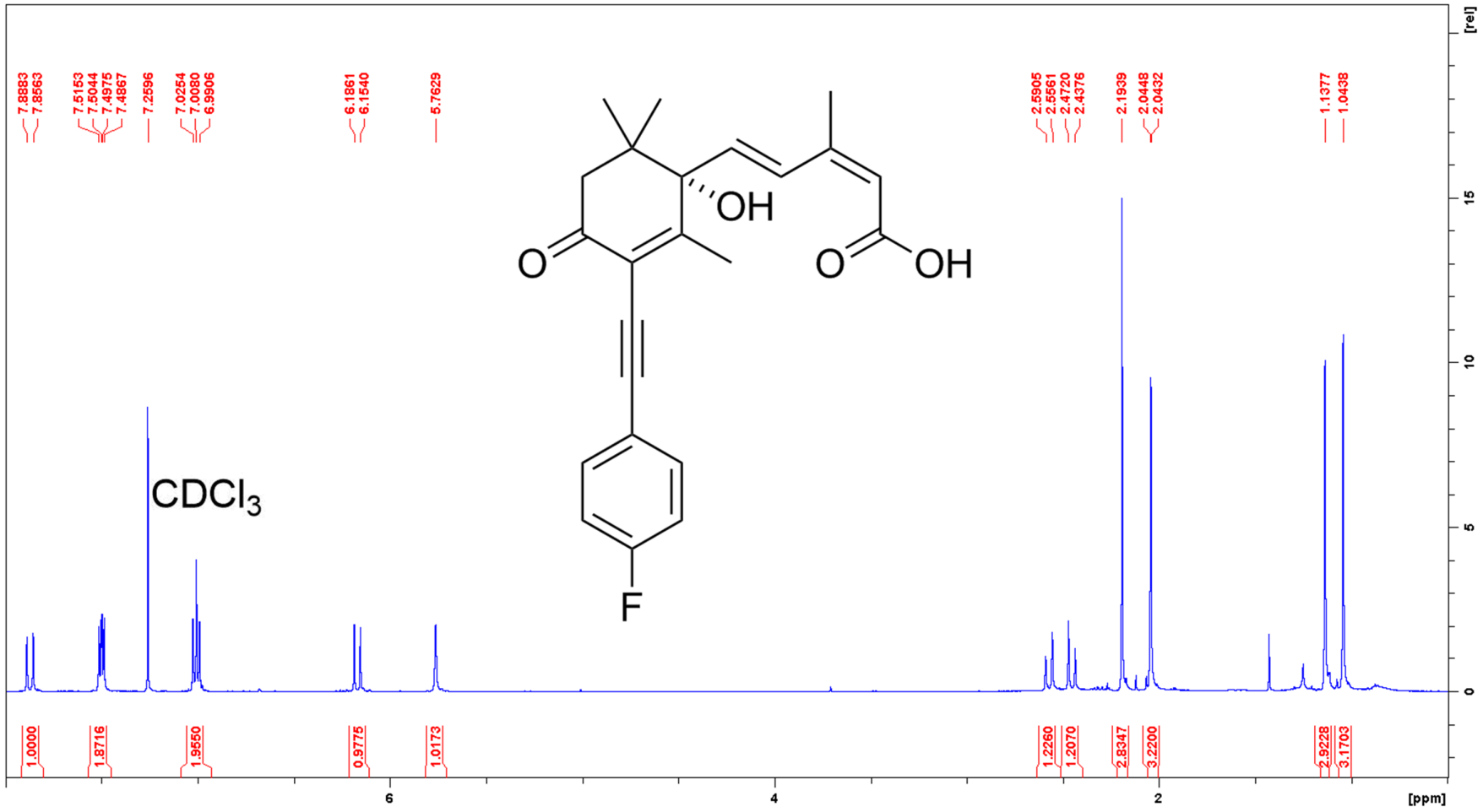
^{1}H NMR spectrum of 8



^{13}C NMR spectrum of 8



^{1}H NMR spectrum of 9



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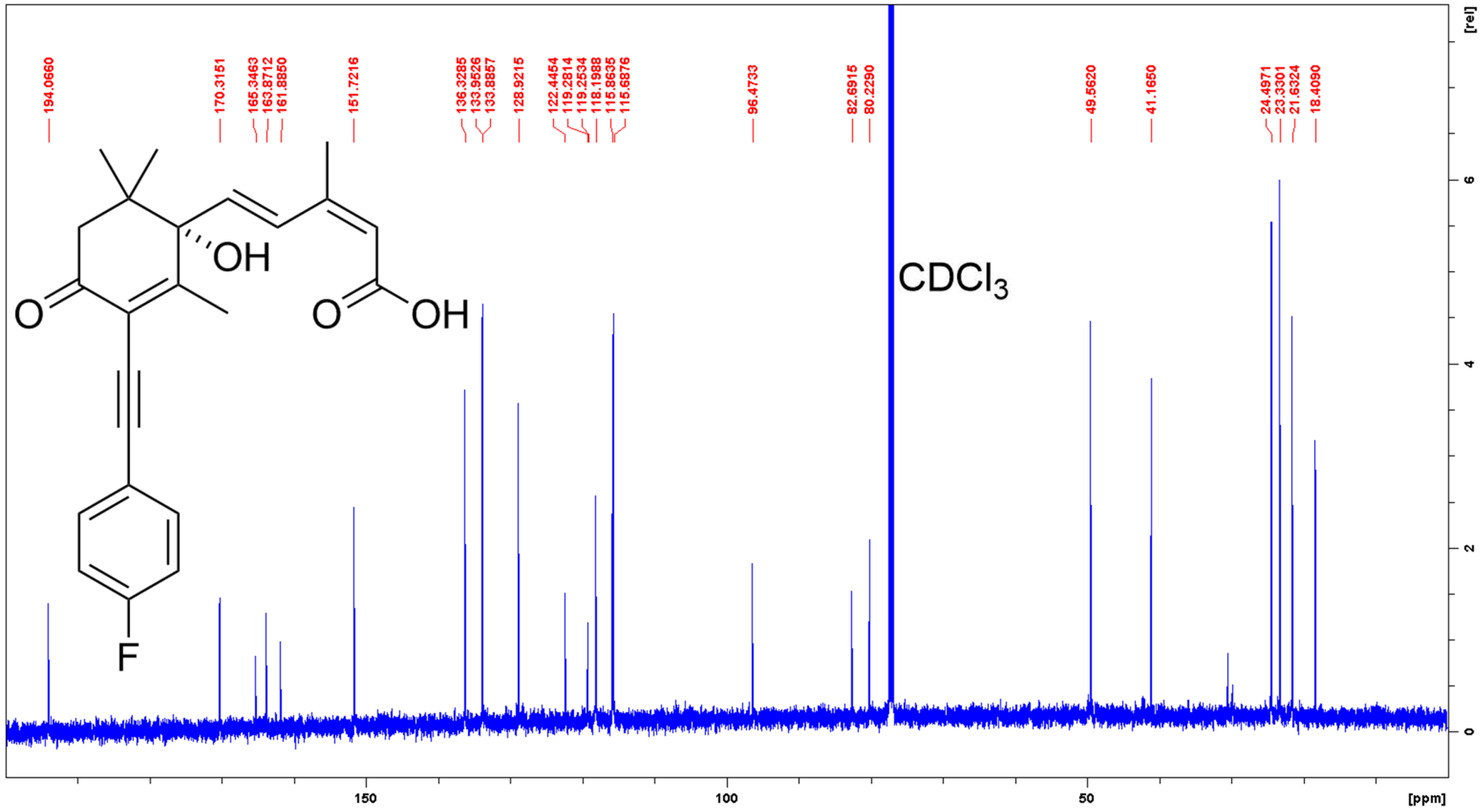
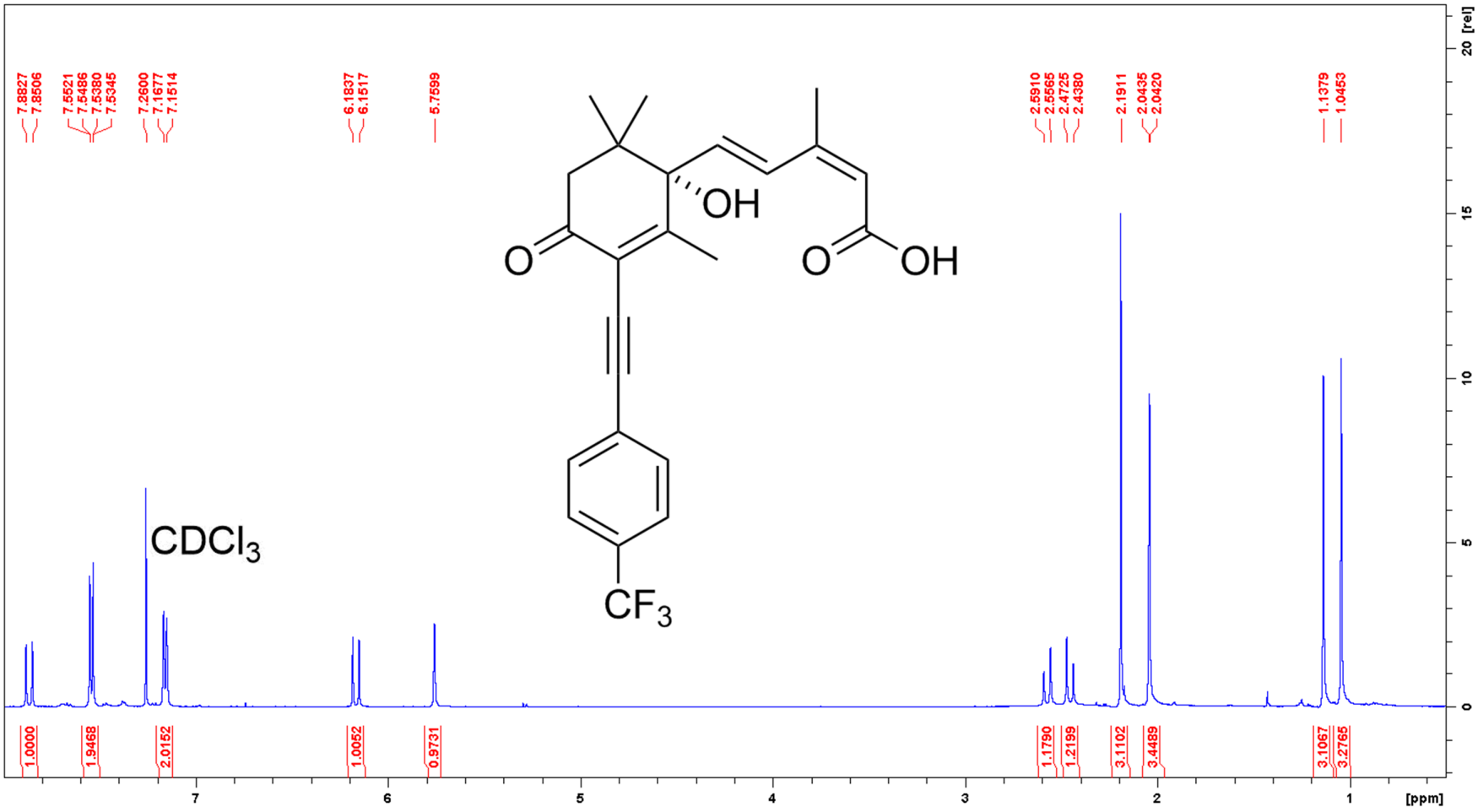
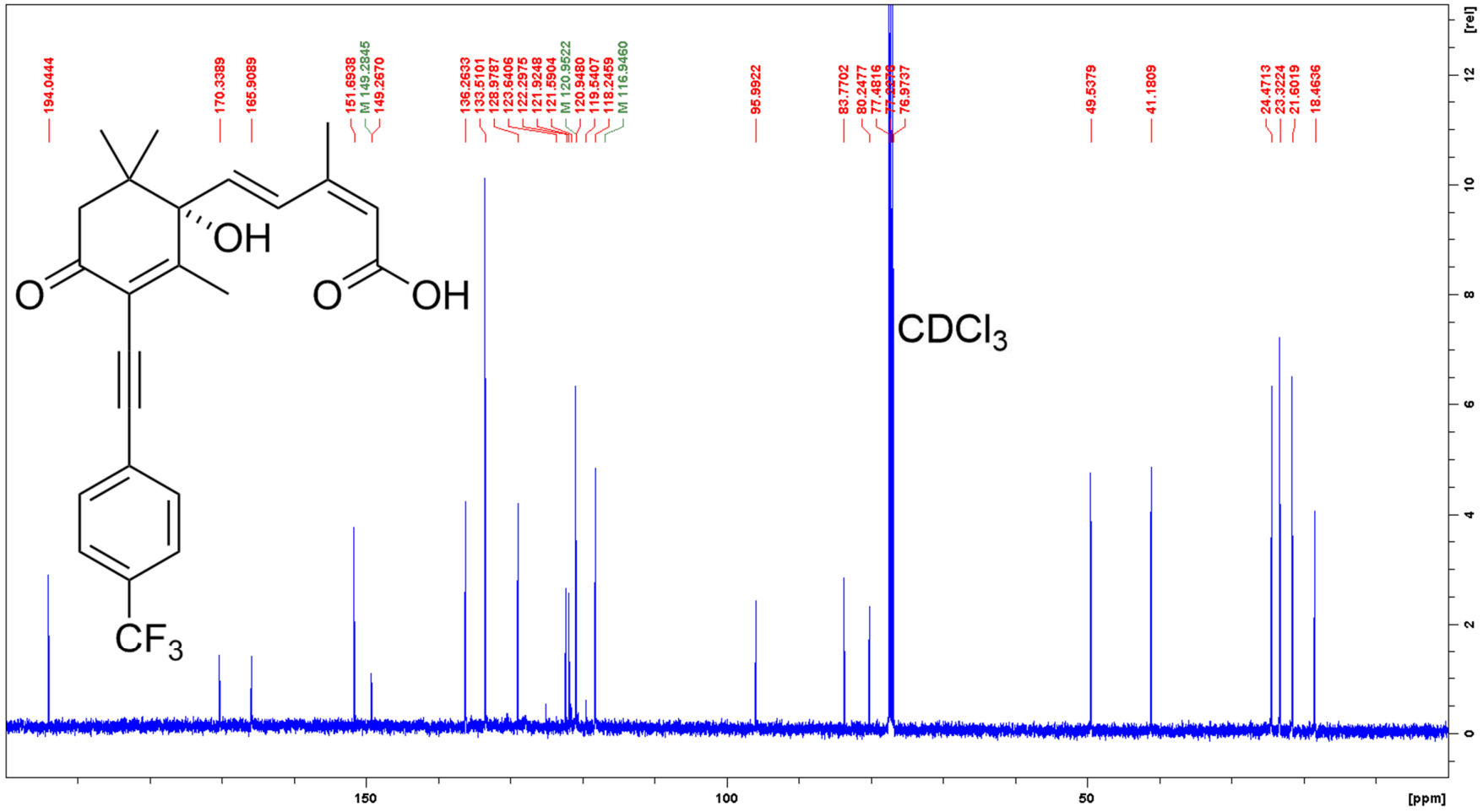


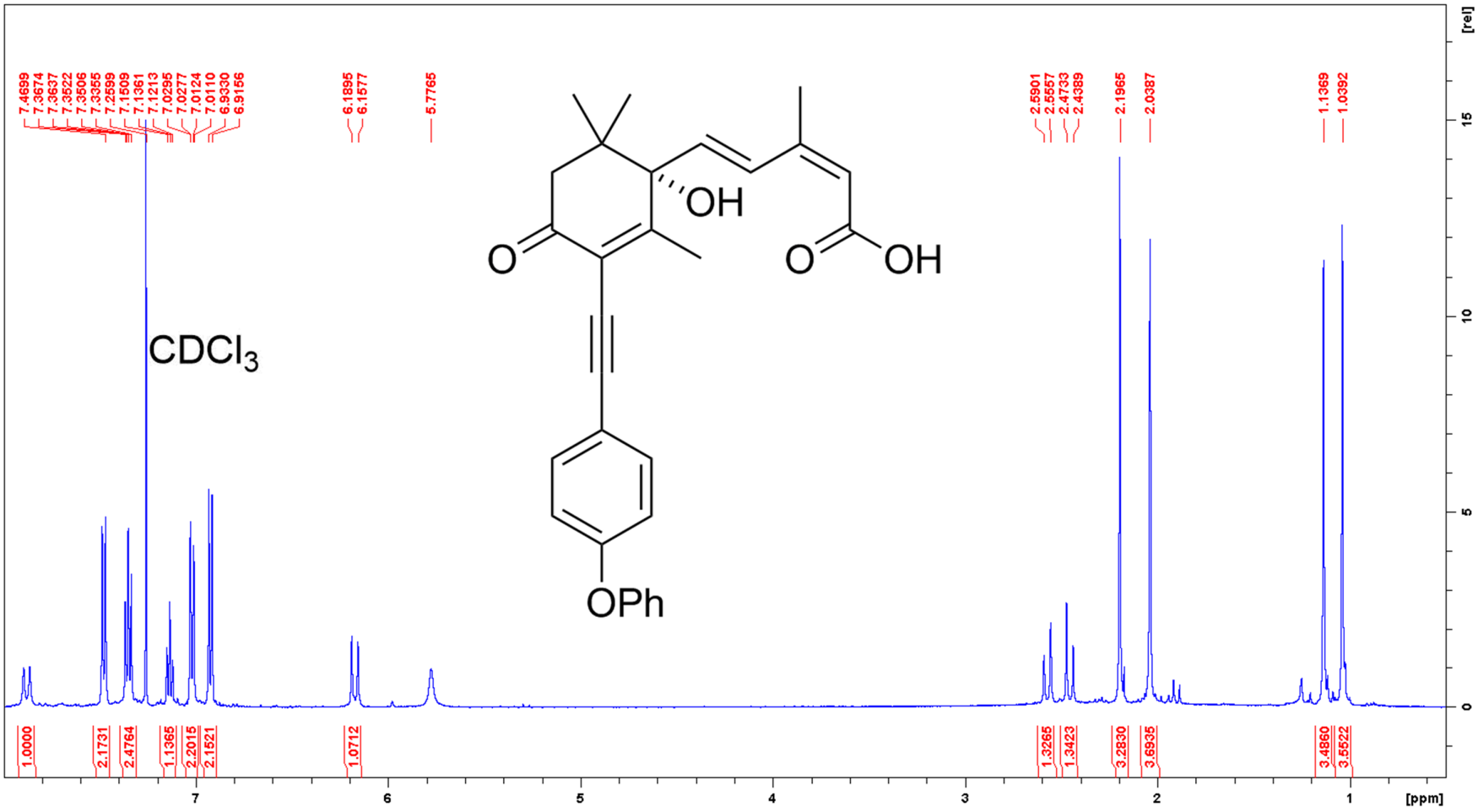
^{1}H NMR spectrum of 10



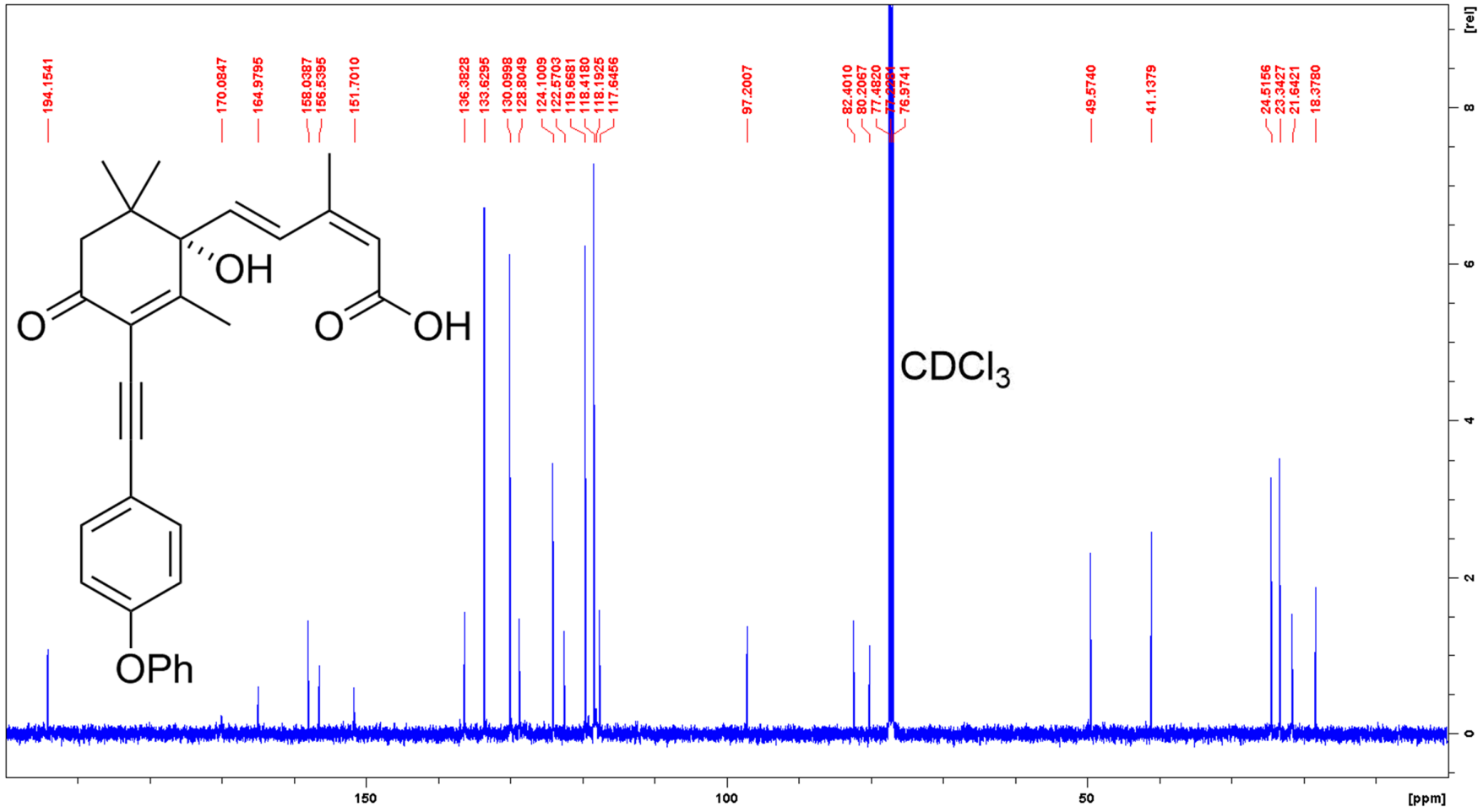
^{13}C NMR spectrum of 10



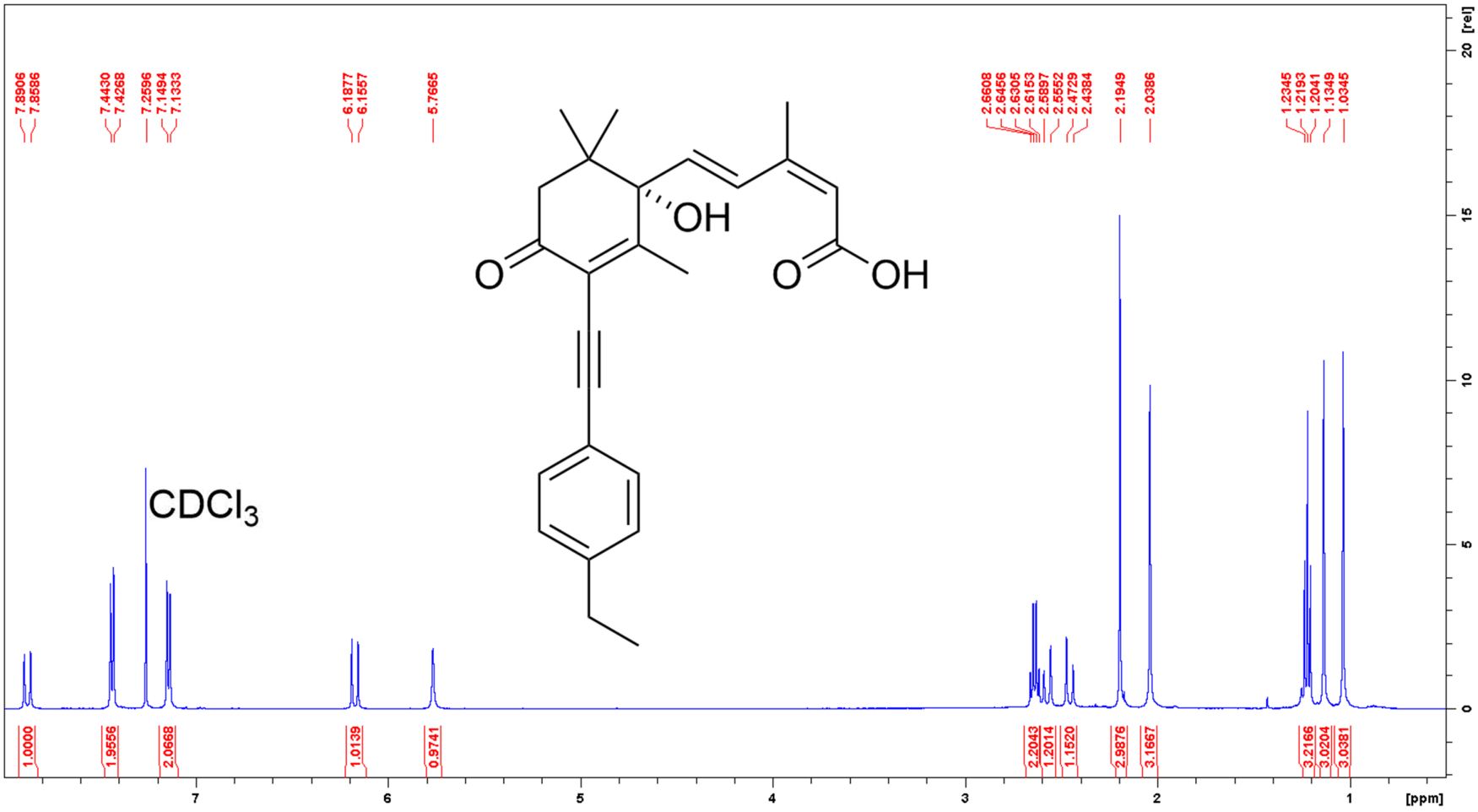
^{1}H NMR spectrum of 11



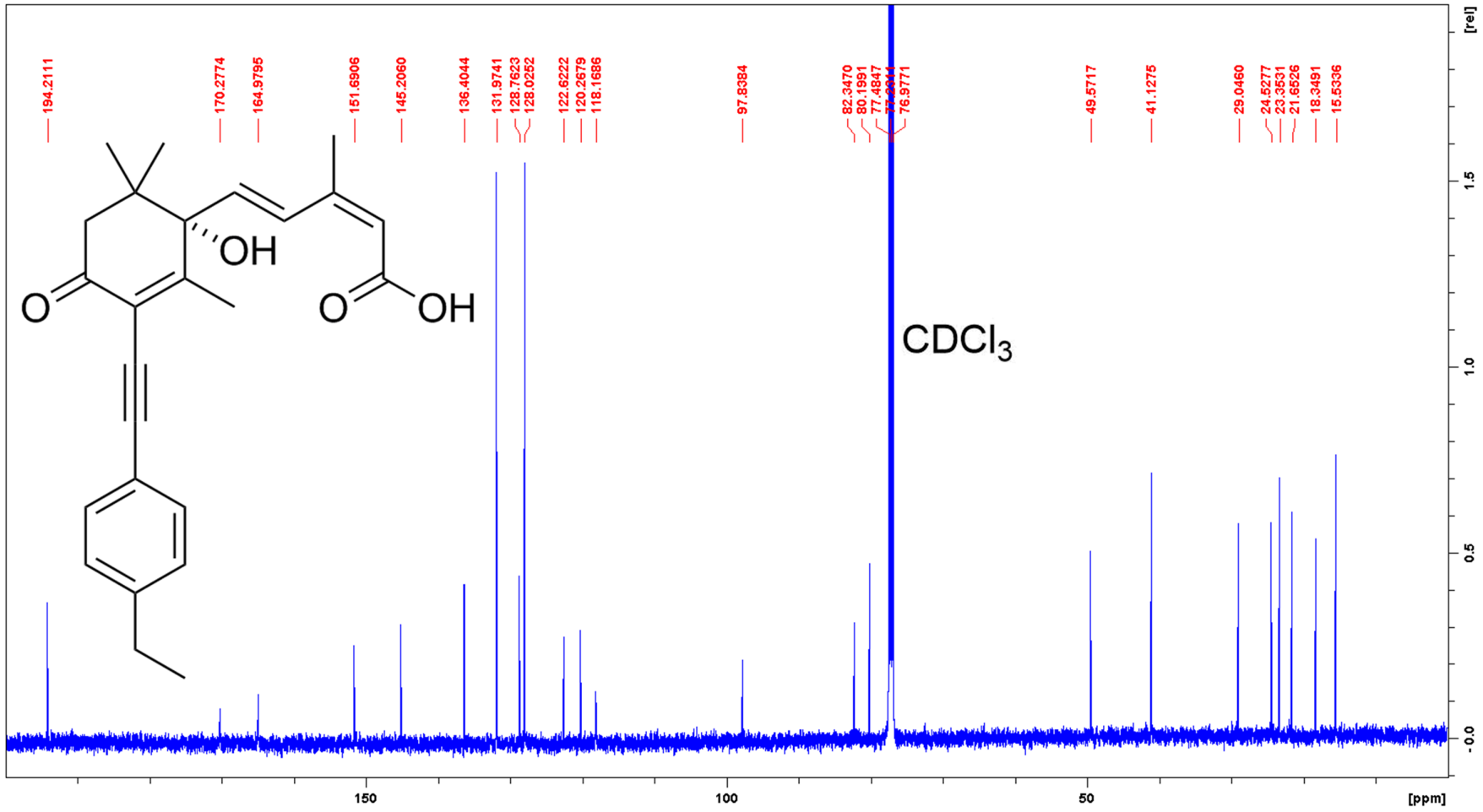
^{13}C NMR spectrum of 11



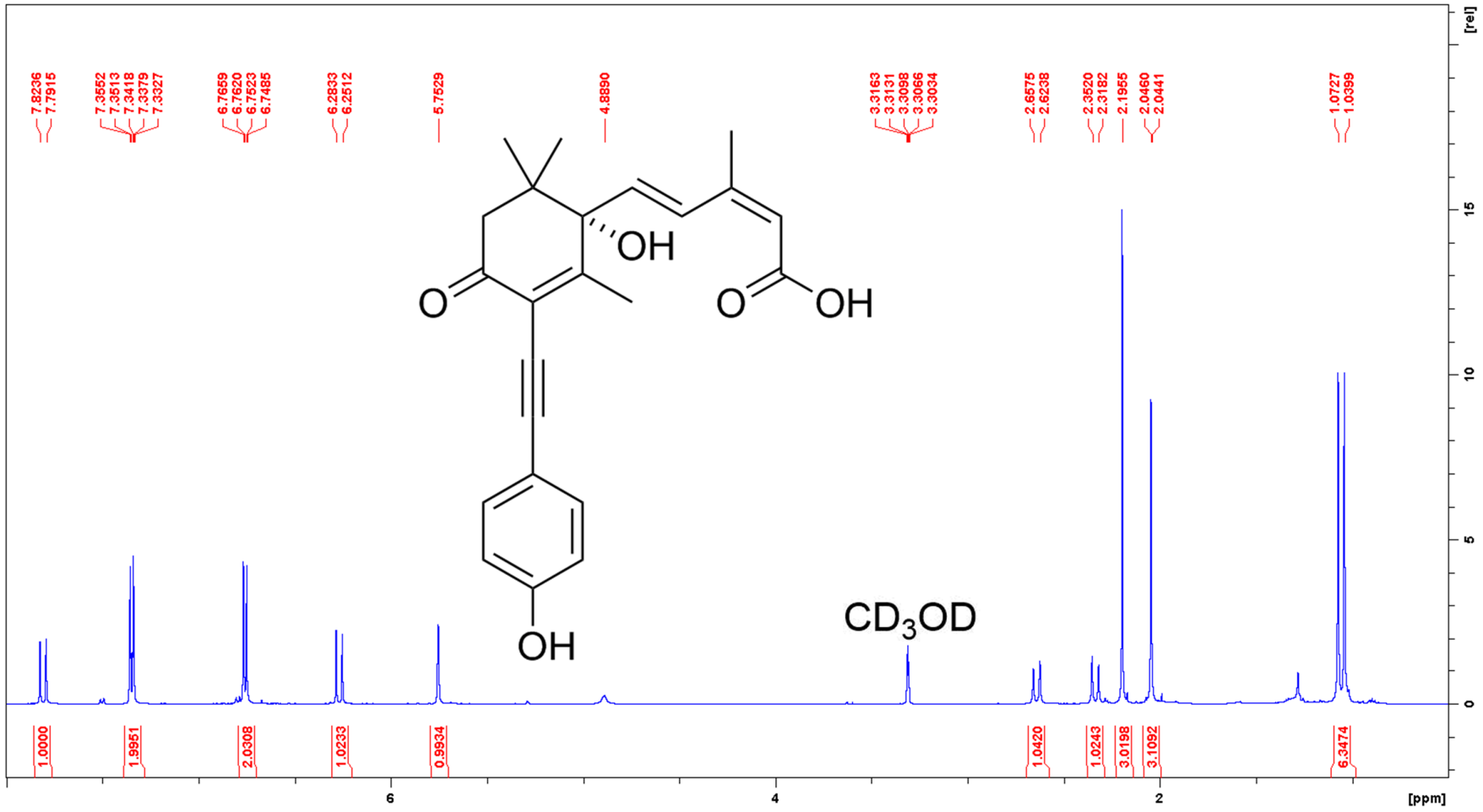
^{1}H NMR spectrum of 12



^{13}C NMR spectrum of 12



^{1}H NMR spectrum of 13



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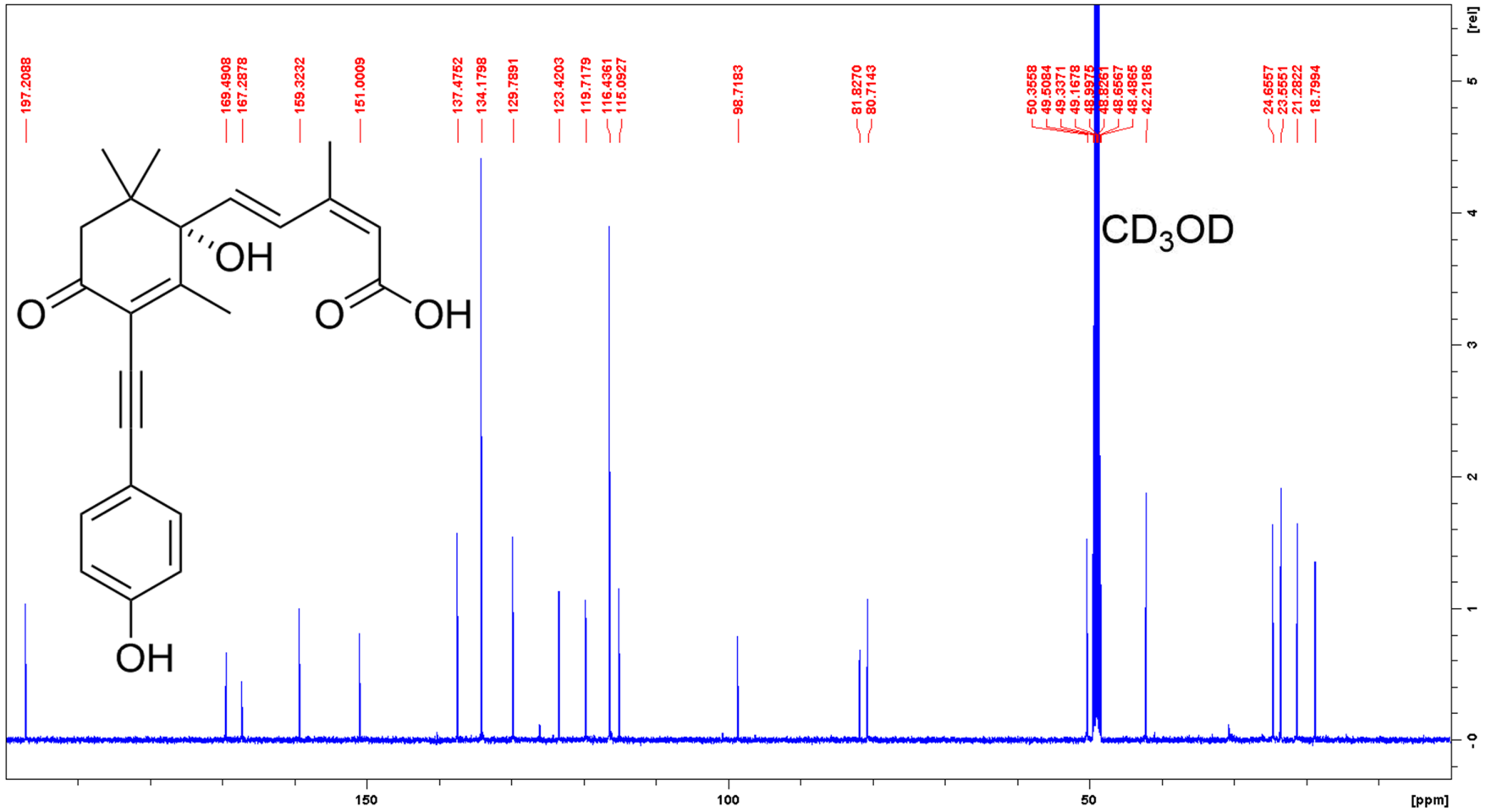
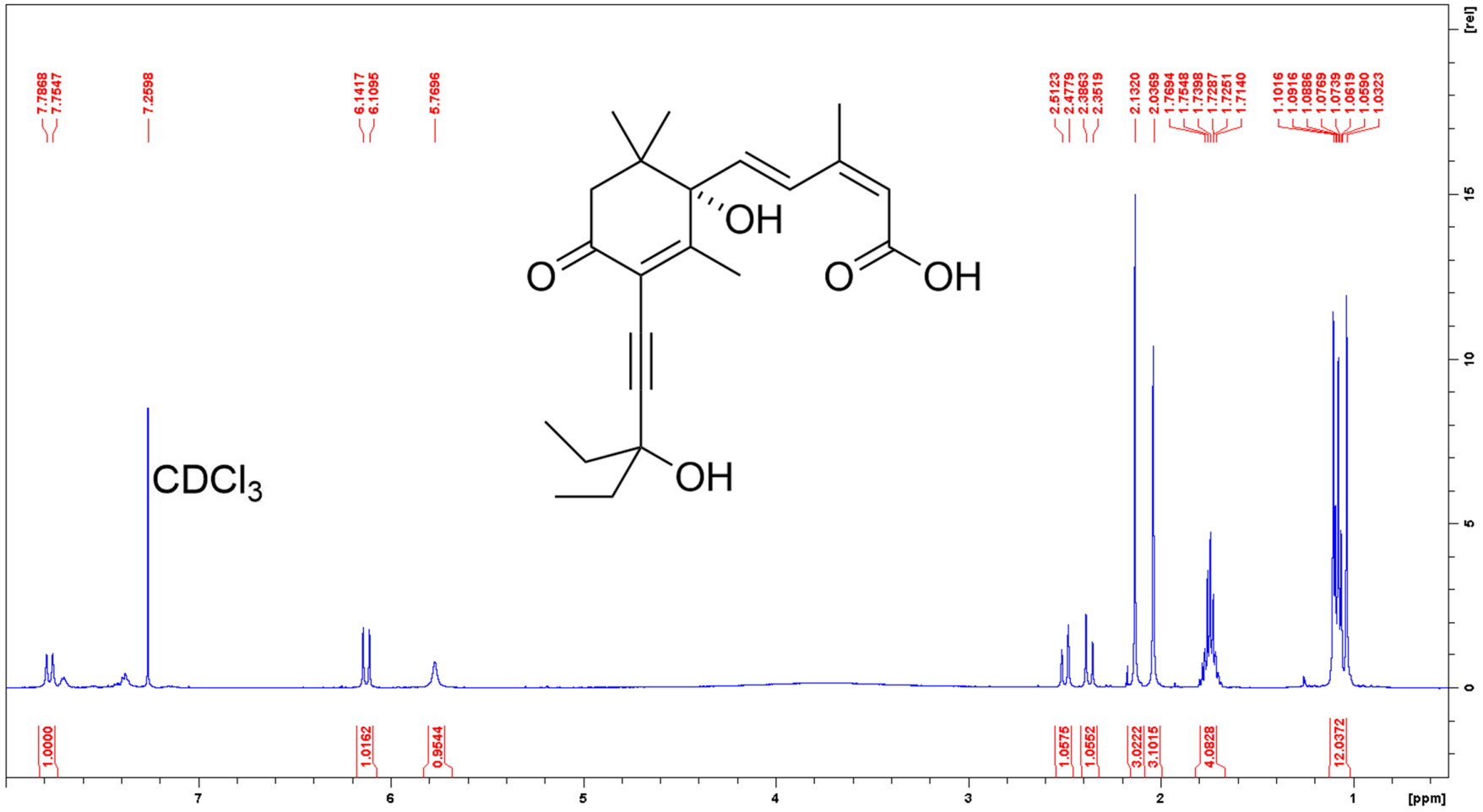
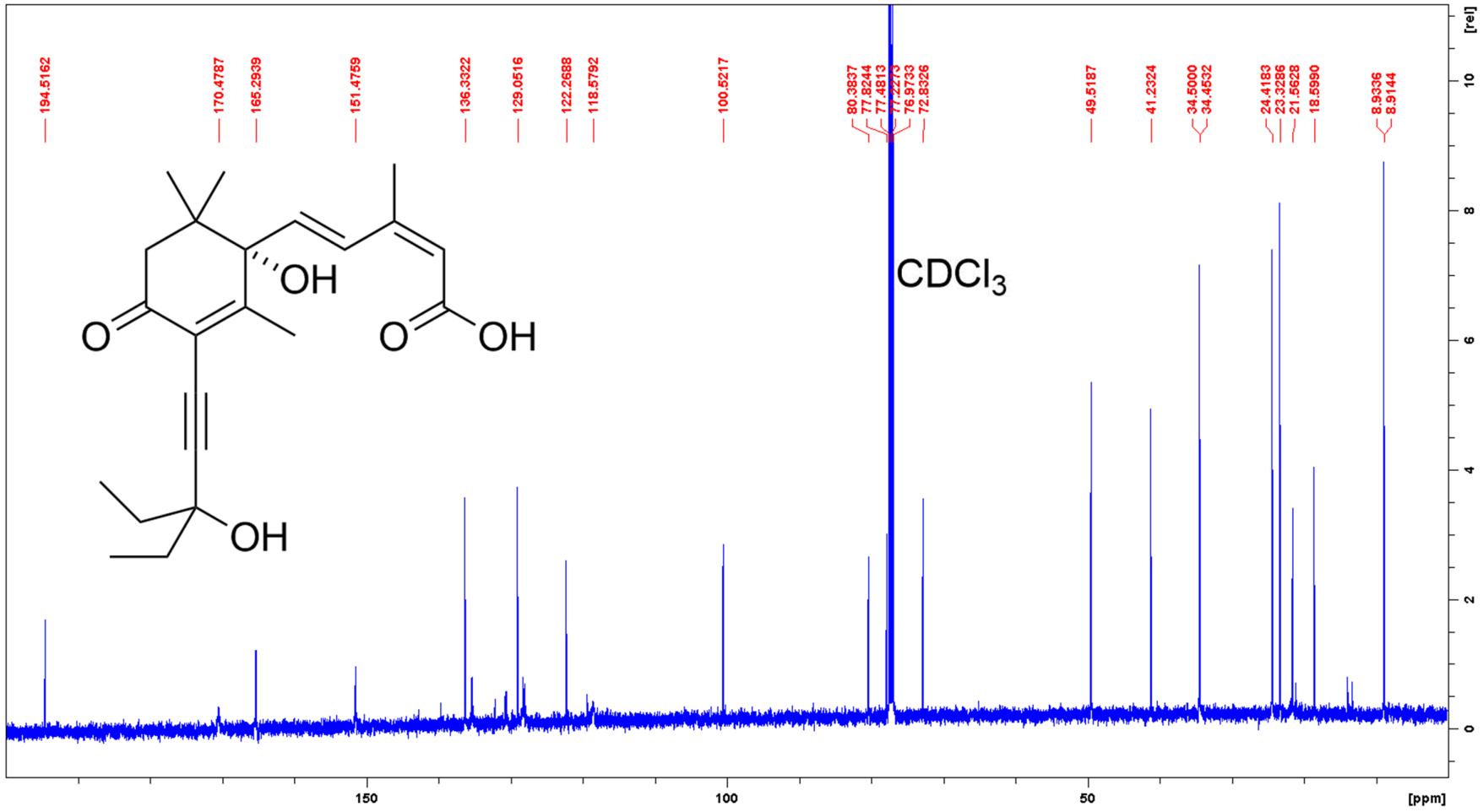


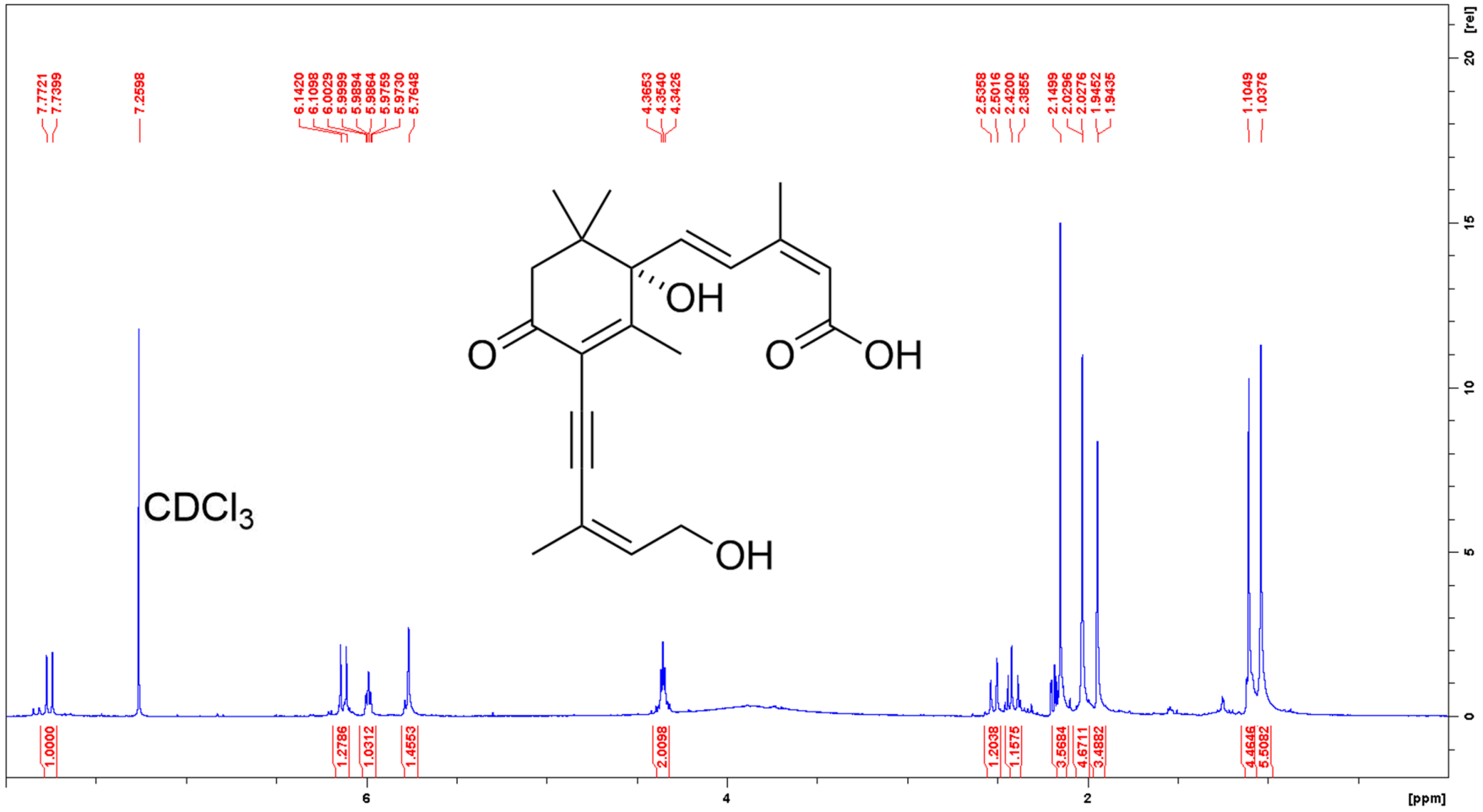
^{1}H NMR spectrum of 14



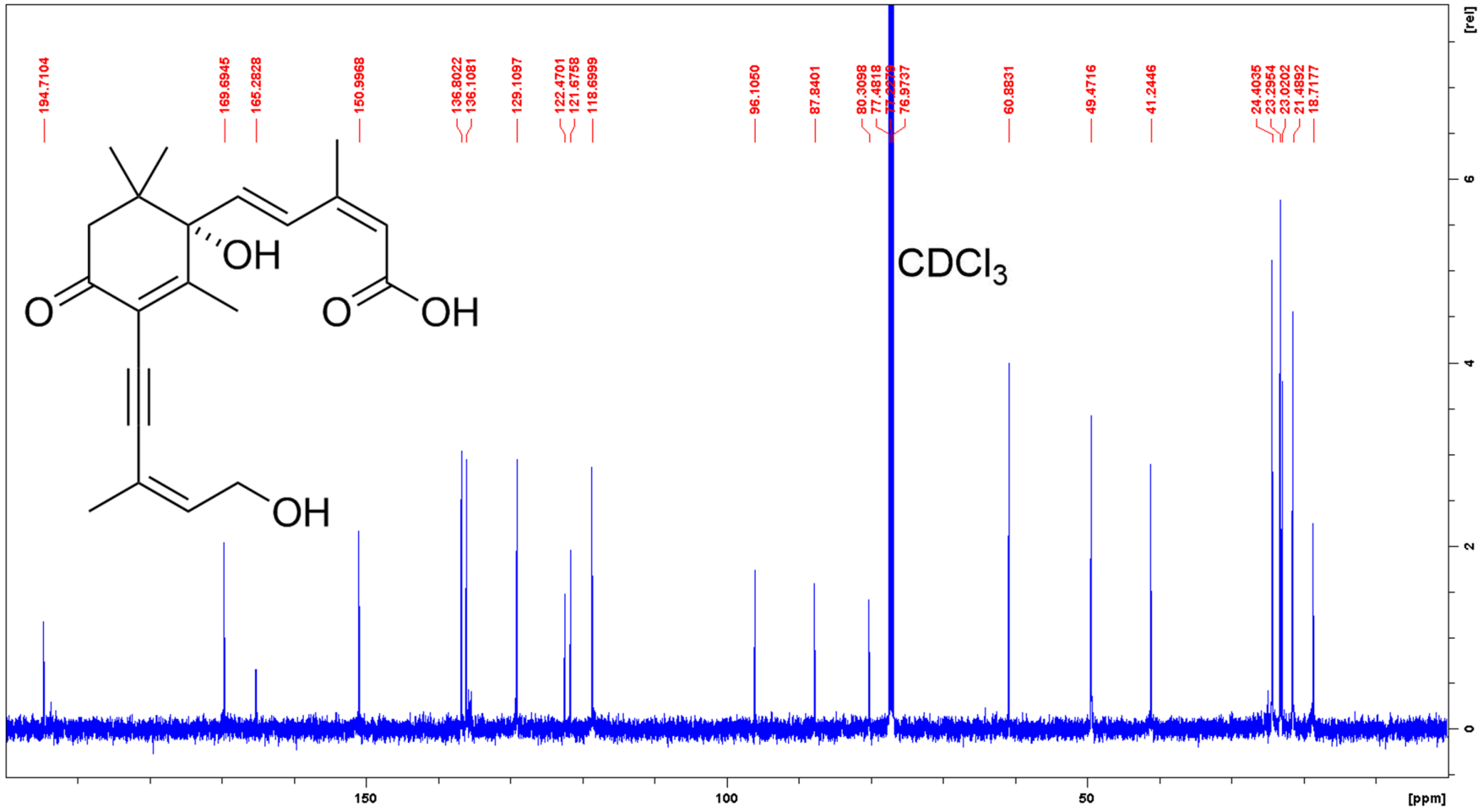
^{13}C NMR spectrum of 14



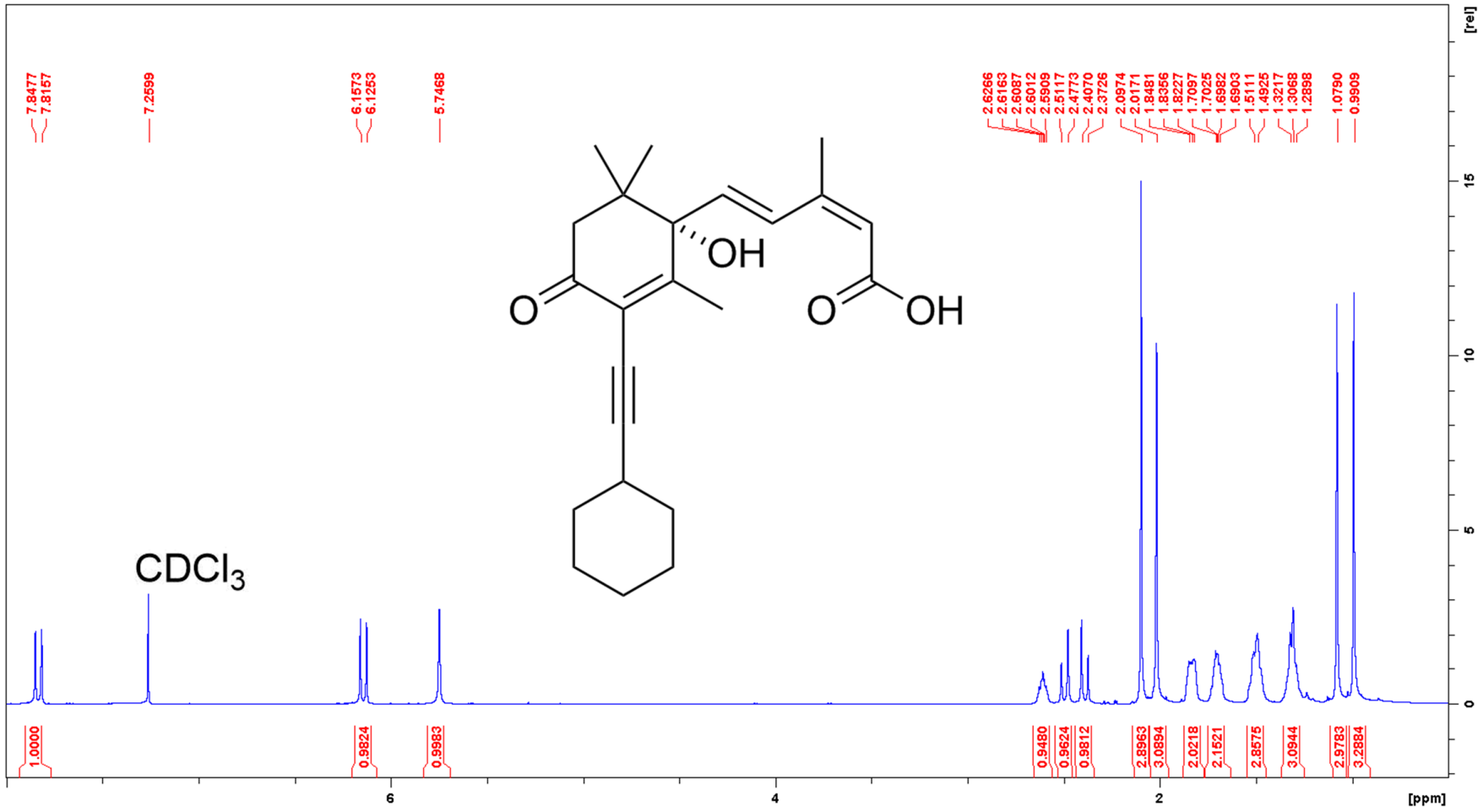
^{1}H NMR spectrum of 15



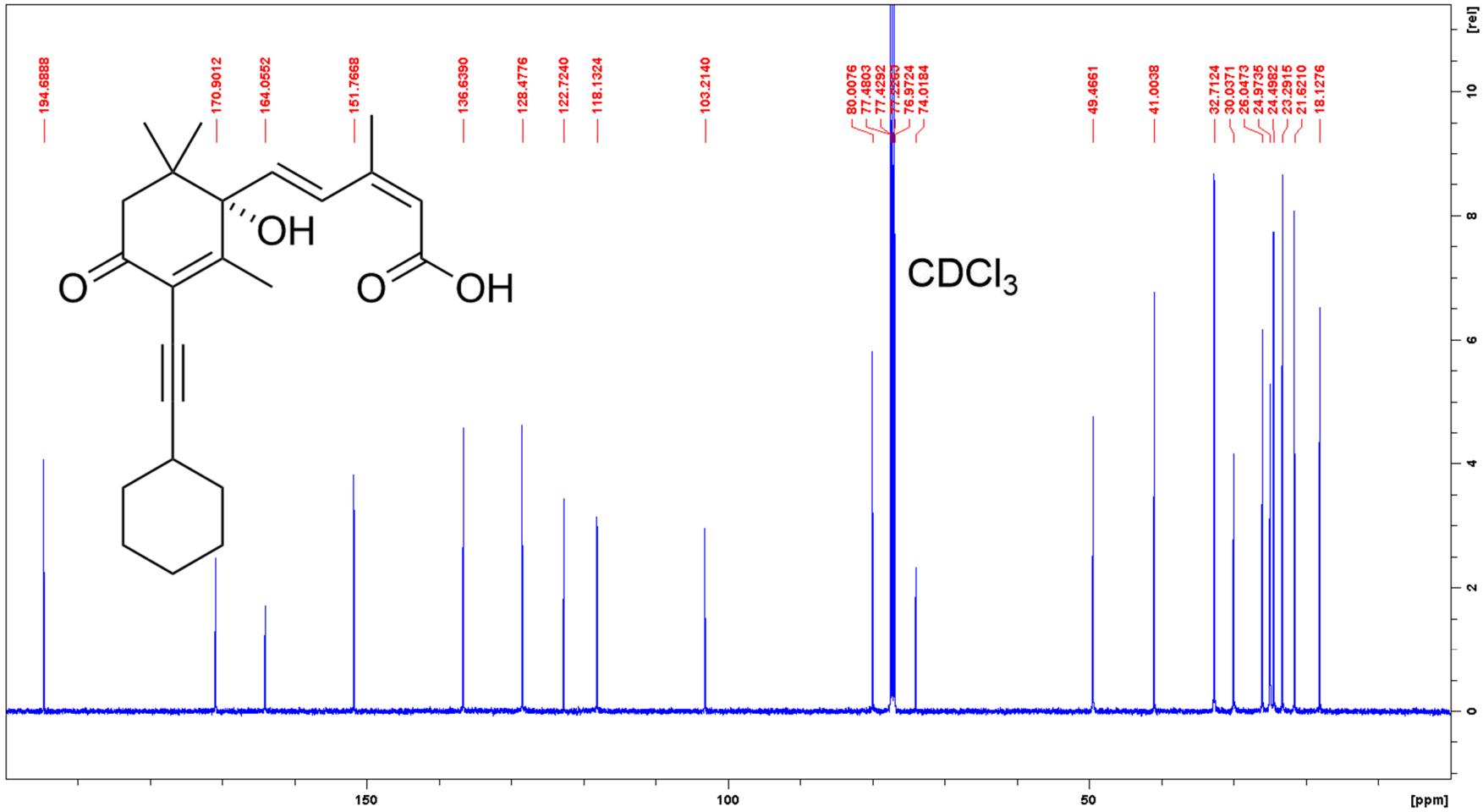
^{13}C NMR spectrum of 15



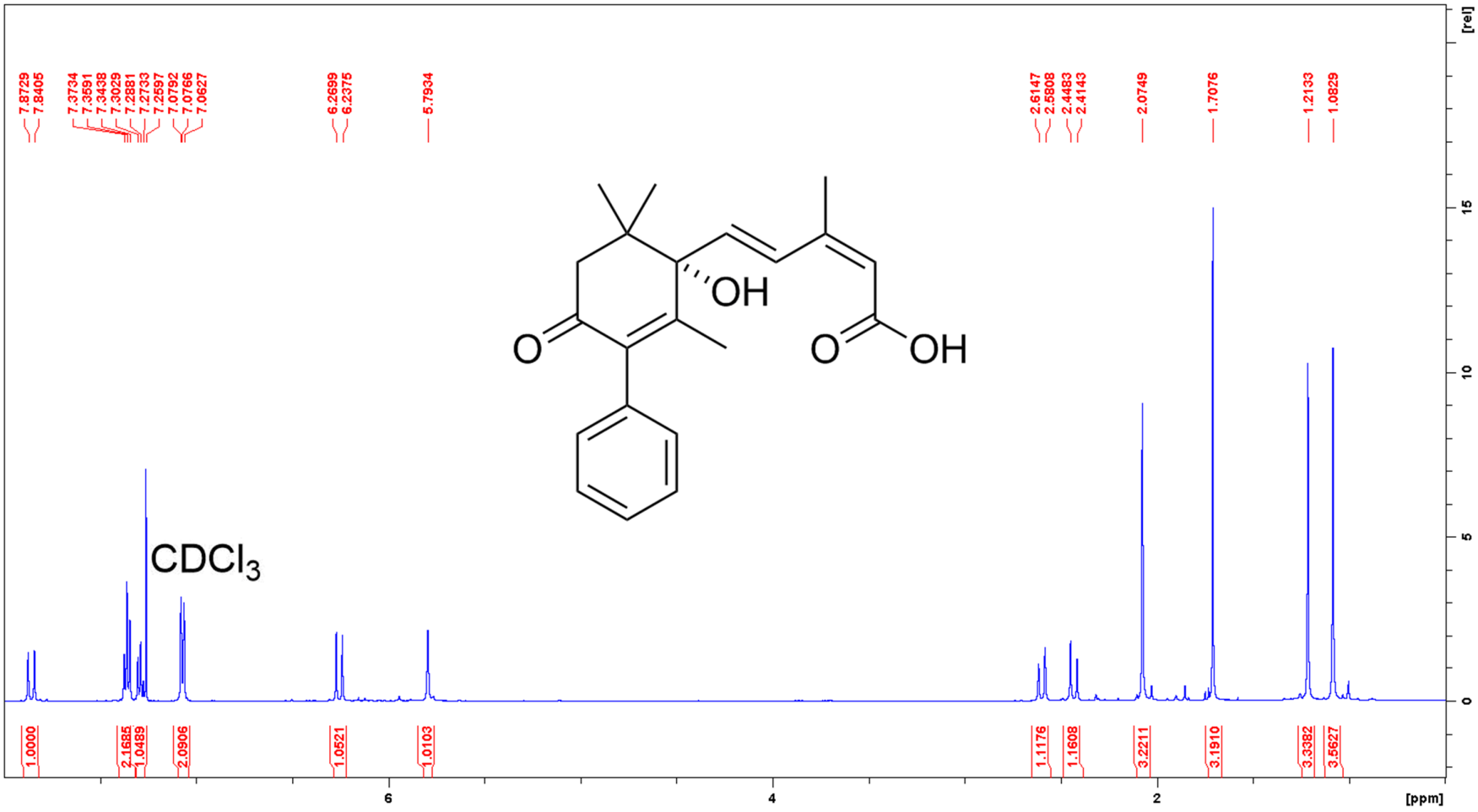
^{1}H NMR spectrum of 16



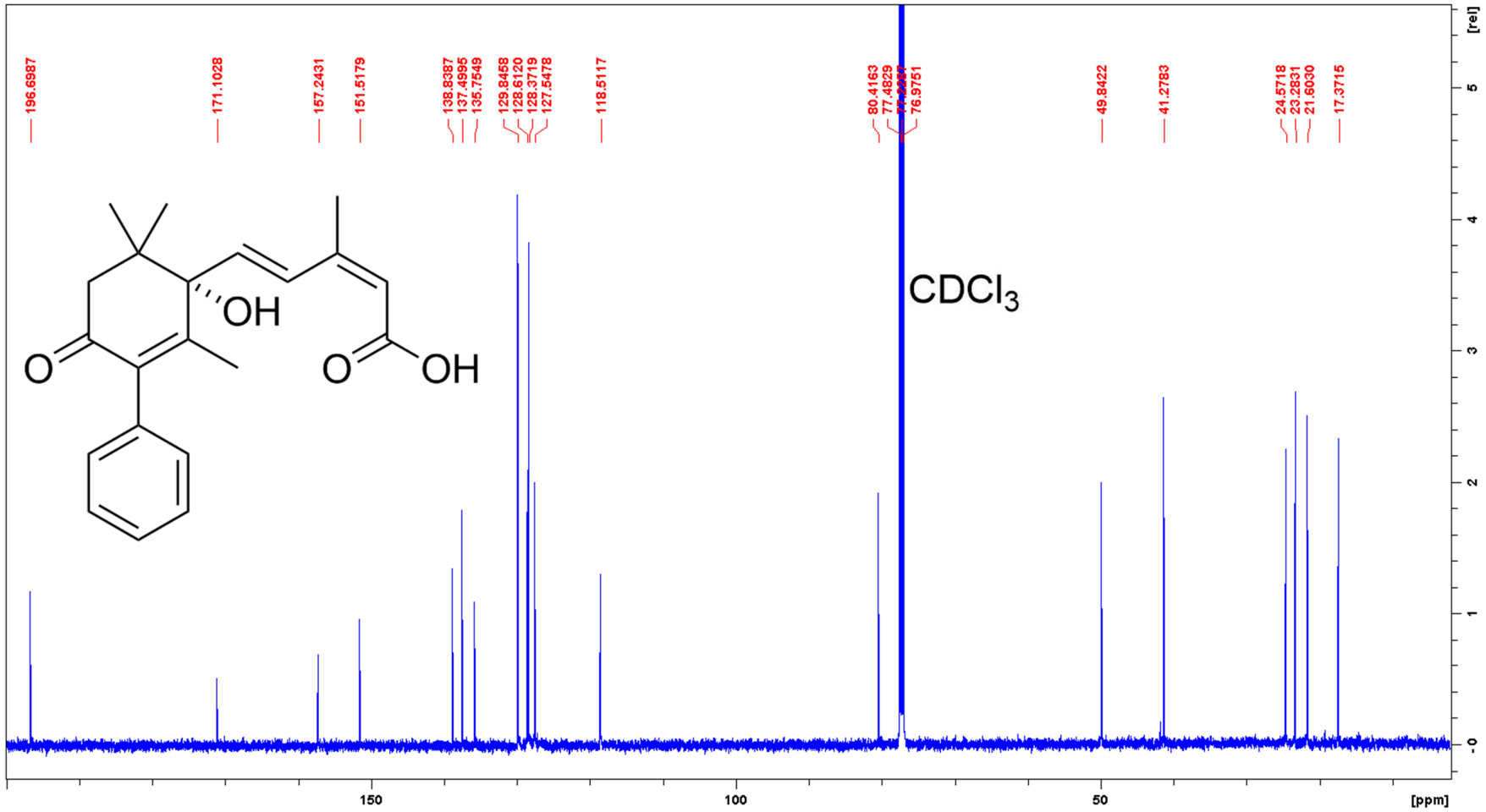
^{13}C NMR spectrum of 16



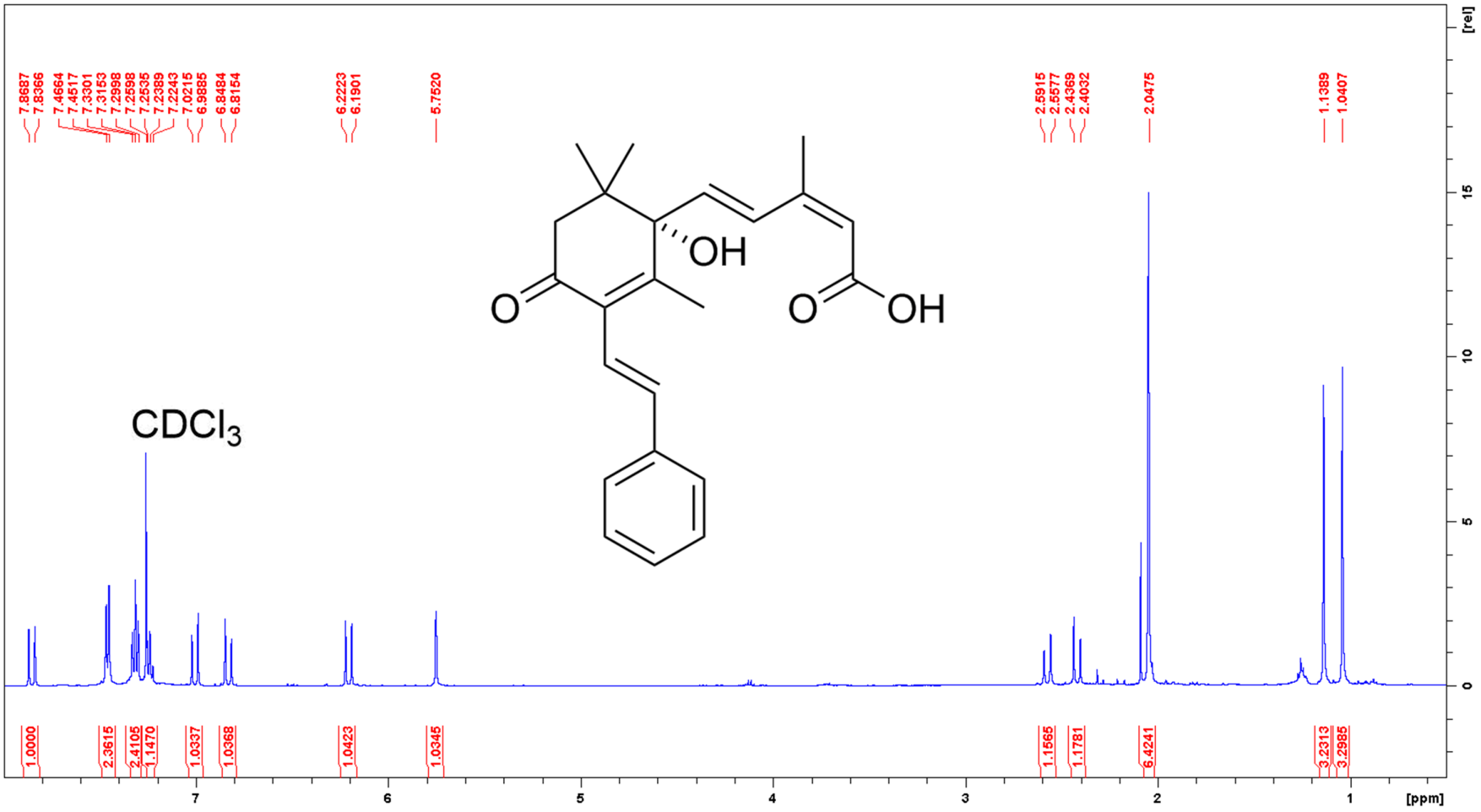
^{1}H NMR spectrum of 17



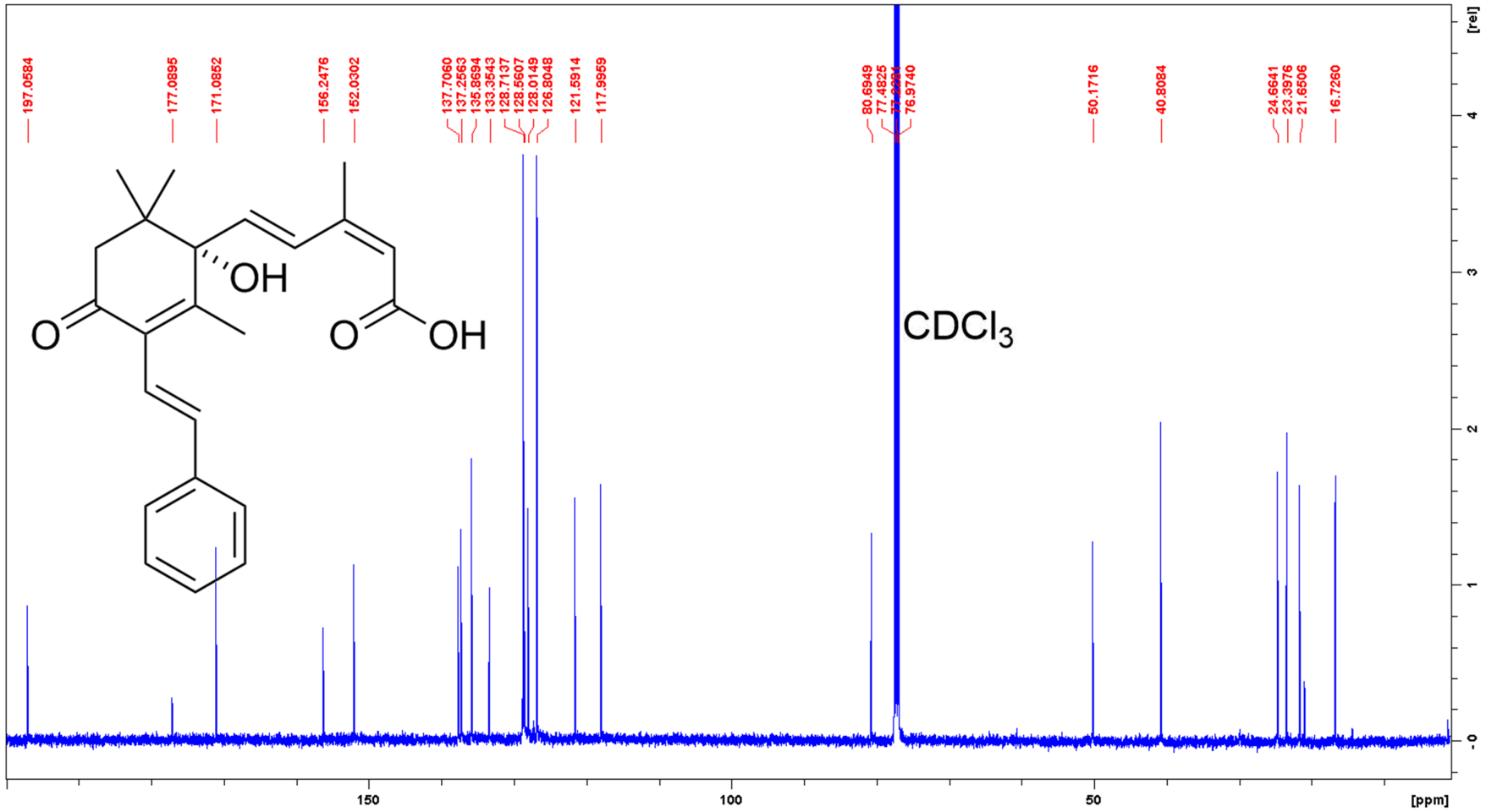
^{13}C NMR spectrum of 17



^{1}H NMR spectrum of 18



^{13}C NMR spectrum of 18



2. Biology

a) Effects of 7 in Arabidopsis

Arabidopsis seeds were soaked in 600 µl 10% bleach in 1.5 ml centrifuge tube for 10 min. Tube has been inverted regularly during 10 min. After spinning down the seeds and discarding bleach, seeds were washed with autoclaved dH2O for 10 times (30 sec each).

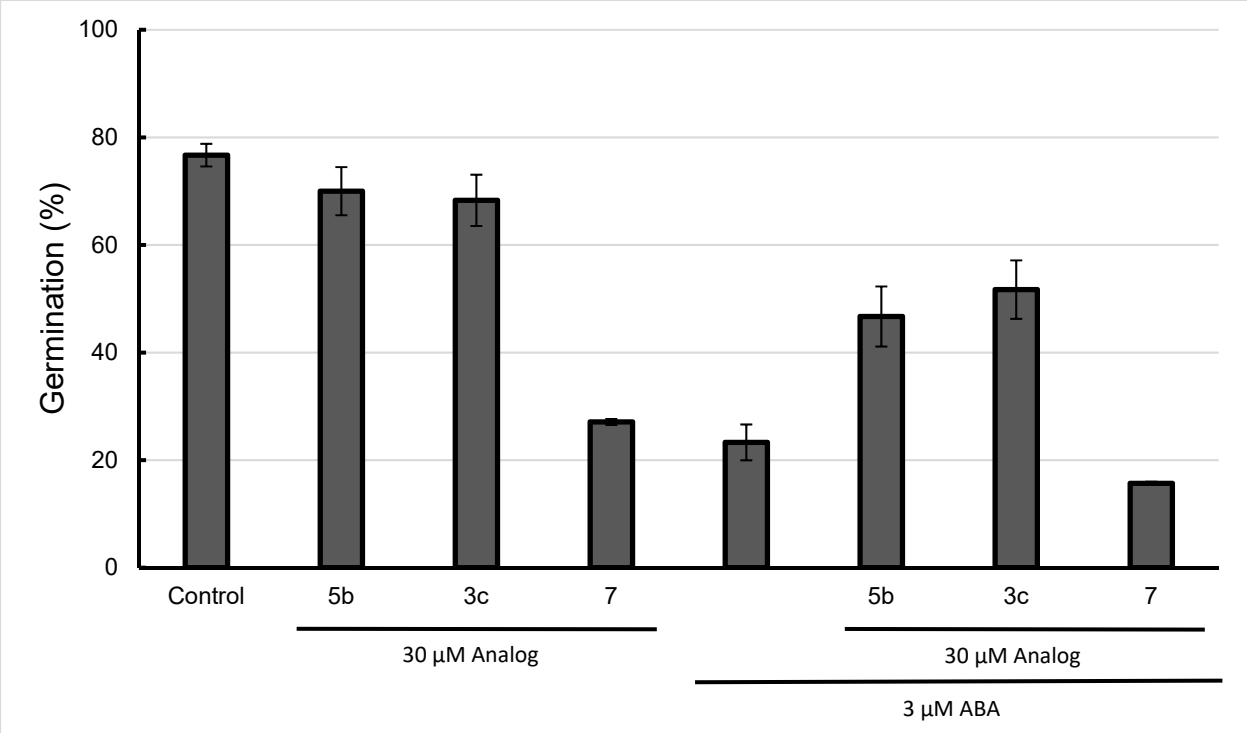


Figure 1a: Graph displaying the percent Arabidopsis (*thaliana*) germination observed three days after sowing in solutions of the treatments indicated. The bar height indicates the mean percent seed germination and the error bars display the standard error of the mean determined from six replicate estimations each containing 10 seeds.

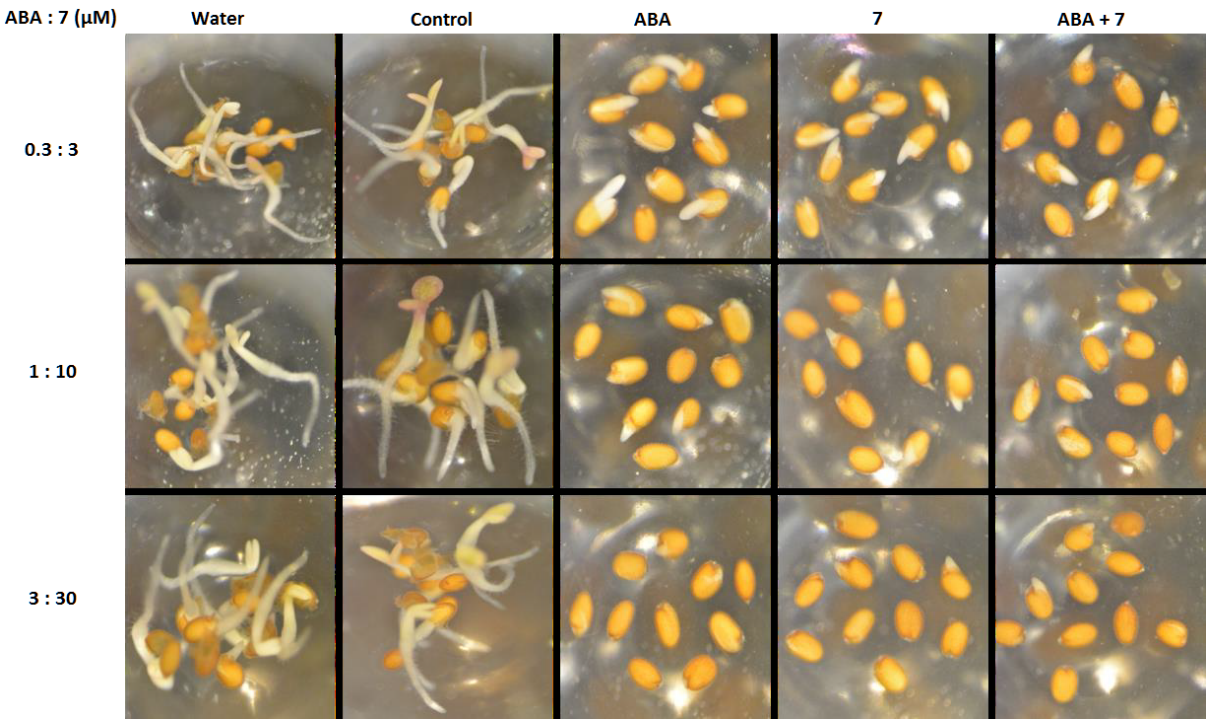


Figure 1b: Result at day 3. Ten seeds were counted and transferred into a fresh 1.5 ml centrifuge tubes. After spinning down the seeds and pouring off water, 100 µL of test solution were added into the tube. Then, seeds+100 µL of test solution were transferred into a well in 96-well Plates. Plate was sealed with parafilm and incubated in the fridge for three days. Then, transferred to the growth chamber (white growth chamber) and incubated there for five days. Number of germinated seeds were counted everyday using magnifying scope. Presence of radical was considered as an indicator of seed germination.

b) Gene expression experiment in Arabidopsis

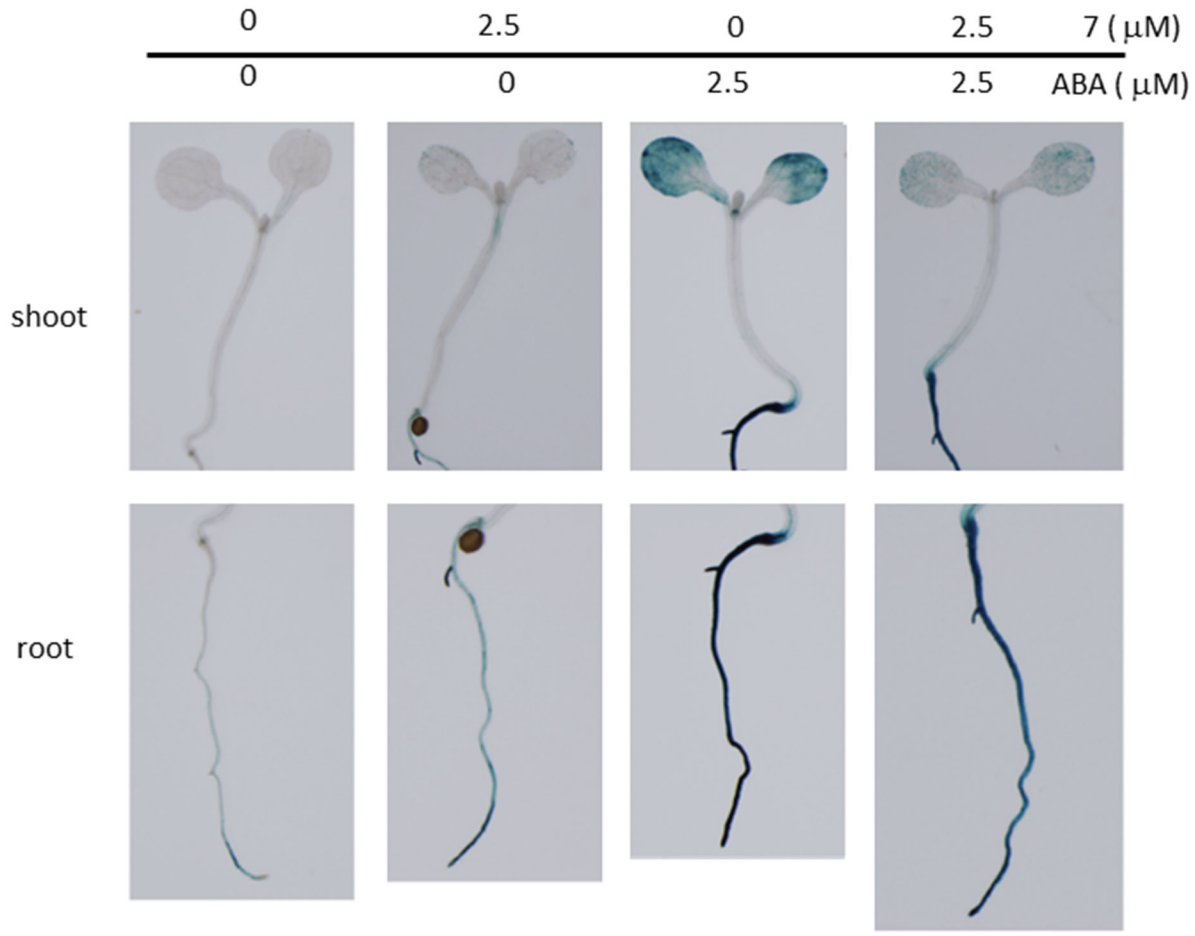


Figure 2: Effects of **7** on ABA-inducible gene expression MKKK18GUS plants harbor a β-glucuronidase (GUS) reporter gene fused to an ABA-inducible promoter of the MKKK18 gene (Okamoto et al., 2013). Six-day-old MKKK18GUS plants were treated with **7** alone or both **7** and ABA for 6 hours. Plants were then stained with 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid cyclohexyl ammonium salt (X-Glc) for 14 hours, and destained with 70% ethanol. **7** has a weak agonist activity when applied alone, while it showed an antagonist effect when co-applied with 2.5 µM ABA. Okamoto M, Petterson FC, Defries A, Park S-Y, Endo A, Nambara E, Volkman BF, Cutler SR. (2013) Activation of dimeric ABA receptors elicits guard cell closure, ABA-regulated gene expression, and drought tolerance. PNAS 110, 12132-12137.

b) Germination assays method

Seed germination assays were carried out at ambient temperature, in the dark. Unless noted, the experiments were performed in Petri dishes, with seeds imbibed on two layers of trimmed filter paper with 10 mL of treatment solutions. Germination of seed was determined by an appearance of visible radicle protruding through the seed coat. Seeds were observed every 24 h until one of the treatments reached 100% germination.

Rice, barley, and wheat seeds were surface sterilized before treatment using a 10% solution of sodium hypochlorite for 30 minutes. Four ml of test solutions were applied to 15 seeds per replicate, with three replicates per treatment. Root and shoot growth were measured 3 days post treatment for barley and wheat and 4.5 days post treatment for rice.

The presence of statistically significant differences among the germination rates was determined using Analysis of Variance and the significance of individual contrasts were determined using the least-squares means between factors in the linear model.
All statistical analyses were performed using the CAR package in the R language. All the graphs were prepared using R and the ggplot2 package. The germination frequency was estimated using at least four replicates for each treatment and the error bar represent the standard error of the mean, with the exception of the Cannabis data where only three replicates of each treatment were measured.

d) Additional supporting figures

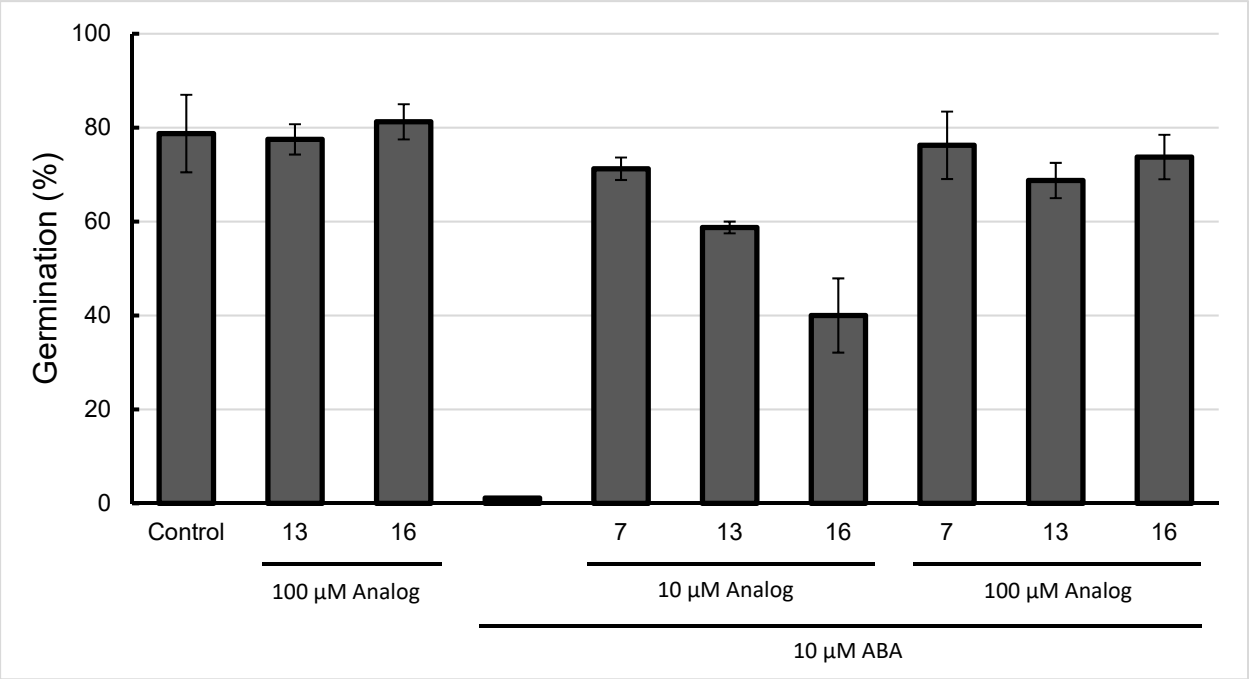


Figure 3: Graph displaying the percent lentil seed red cotyledon cultivar CDC Maxim (Seed Lot# Bt16 Lot A) germination observed two days after sowing in solutions of the treatments indicated. The bar height indicates the mean percent seed germination and the error bars display the standard error of the mean determined from four replicate estimations each containing 40 seeds.

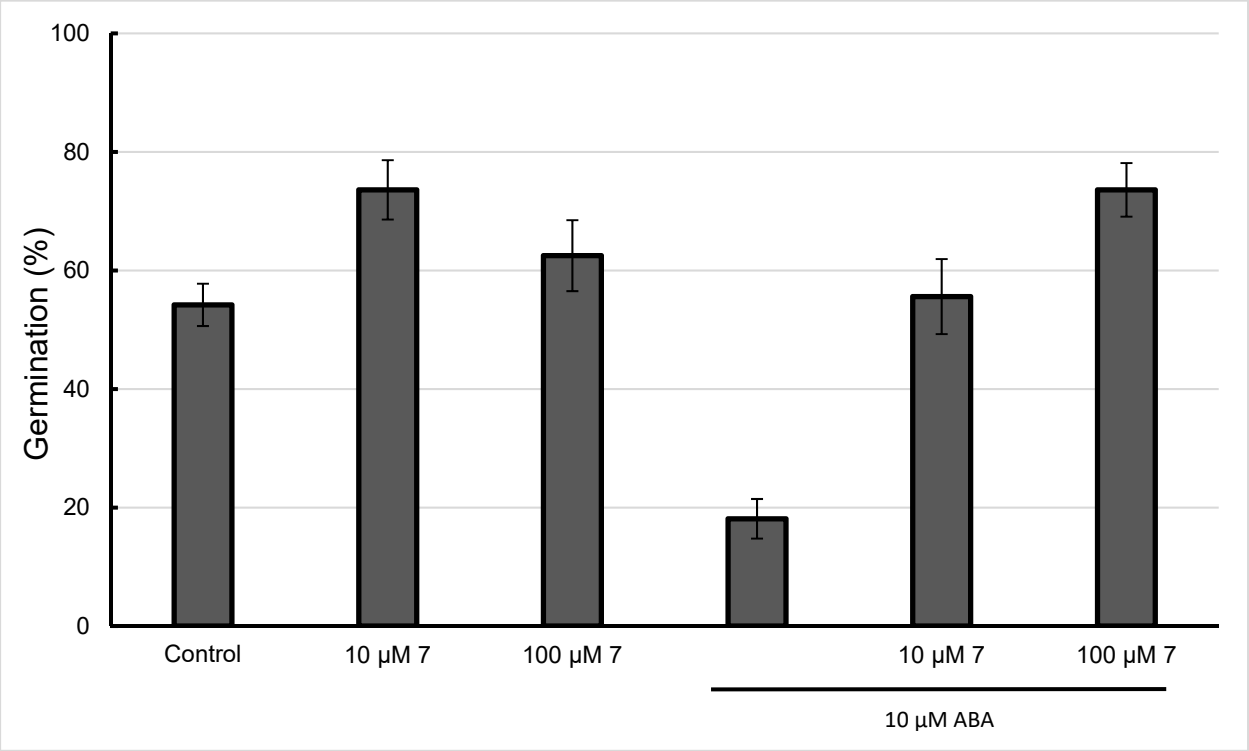


Figure 4: Graph displaying the percent soybean seed (AAC Edward; 2018; SBYT02 Investigation EN 4) germination observed two days after sowing in solutions of the treatments indicated. The bar height indicates the mean percent seed germination and the error bars display the standard error of the mean determined from six replicate estimations each containing 20 seeds.

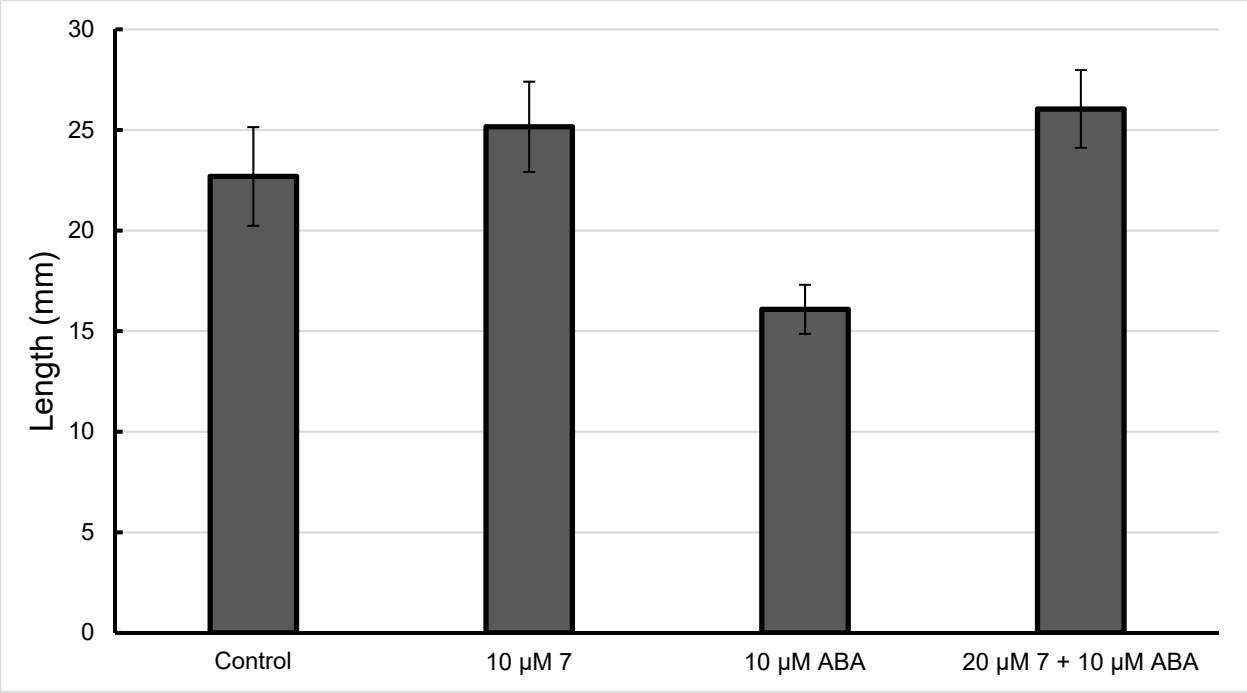


Figure 5: Graph displaying the length of barley (*Hordeum vulgare* cv. Morex) root measured four days after sowing in solutions of the treatments indicated. The bar height indicates the mean root length and the error bars display the standard error of the mean determined from forty-five replicate measurements.

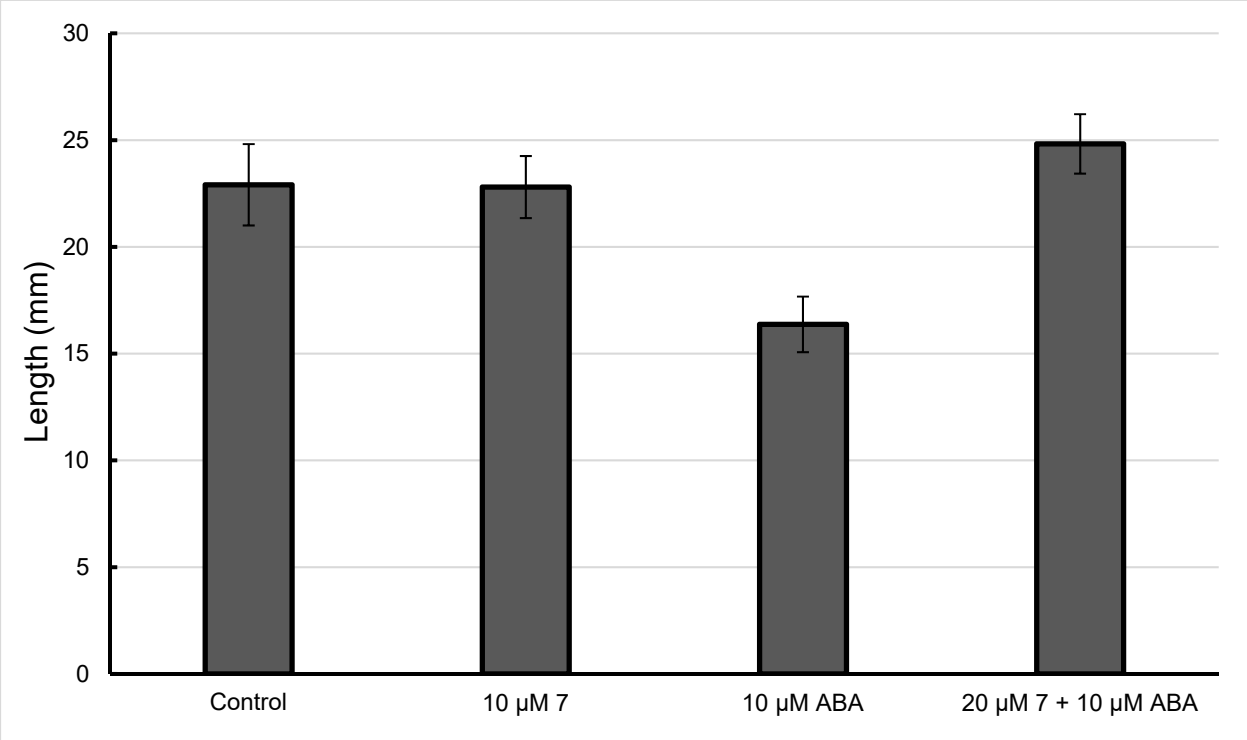


Figure 6: Graph displaying the length of wheat (*Triticum aestivum* L. cv. Byrd) root measured four days after sowing in solutions of the treatments indicated. The bar height indicates the mean root length and the error bars display the standard error of the mean determined from forty-four replicate measurements.

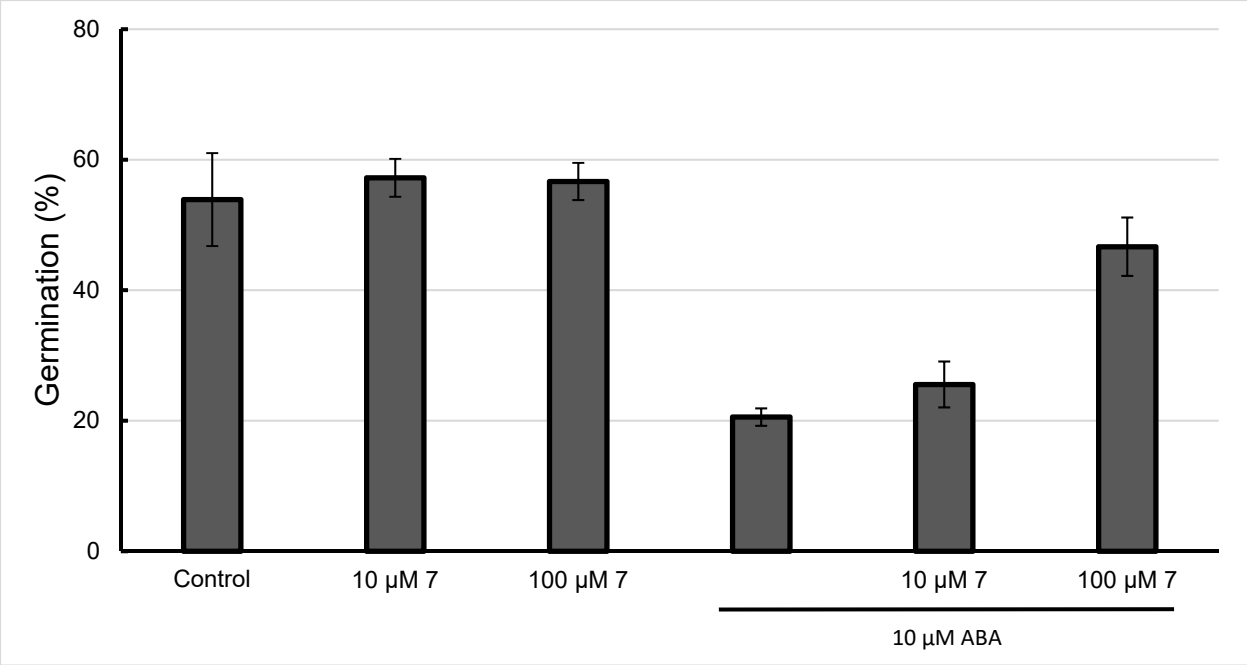


Figure 7: Graph displaying the percent canary seed (CDC Bastia; 2018; CANYT1 rep1; Kernen) germination observed three days after sowing in solutions of the treatments indicated. The bar height indicates the mean percent seed germination and the error bars display the standard error of the mean determined from six replicate estimations each containing 30 seeds.

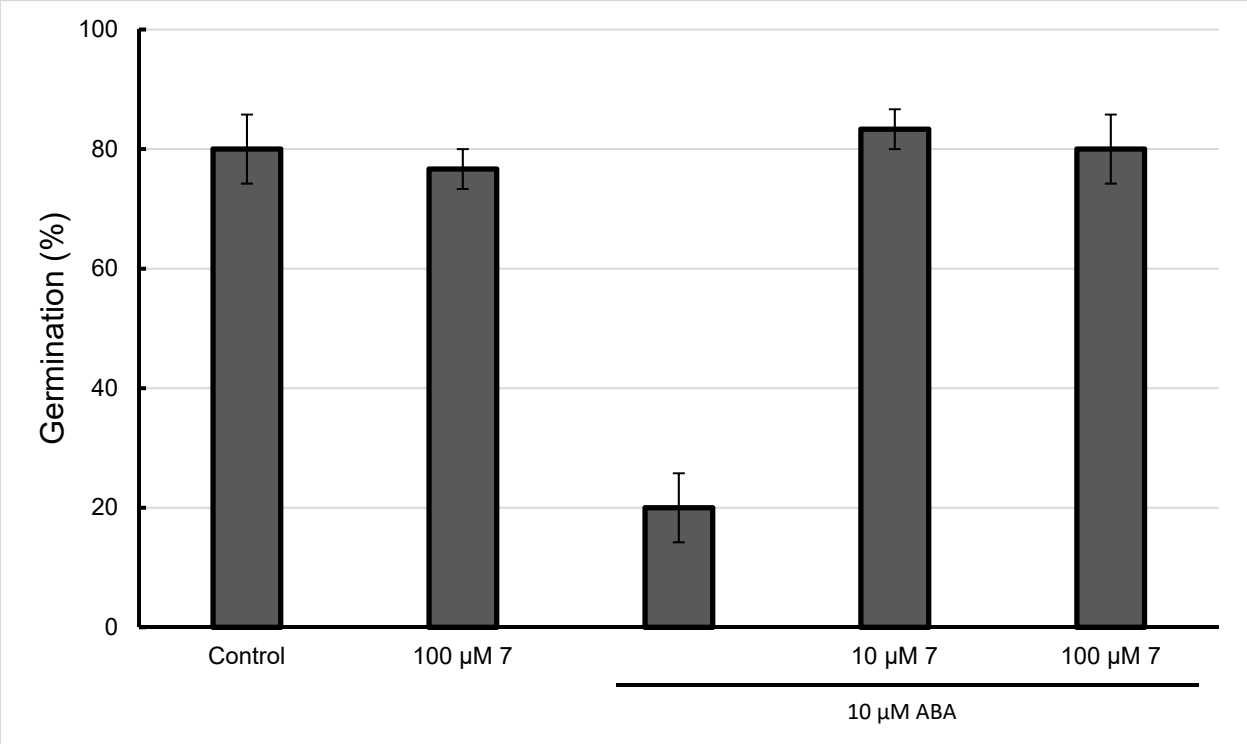


Figure 8: Graph displaying the percent cannabis seed (PAGE PK X Finola 2014) germination observed ten days after sowing in solutions of the treatments indicated. The bar height indicates the mean percent seed germination and the error bars display the standard error of the mean determined from three replicate estimations each containing 10 seeds.