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Synthesis and Biological Evaluation of Pharbinilic Acid and Derivatives as Potent Inhibitors of NF-κB

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Supporting Information

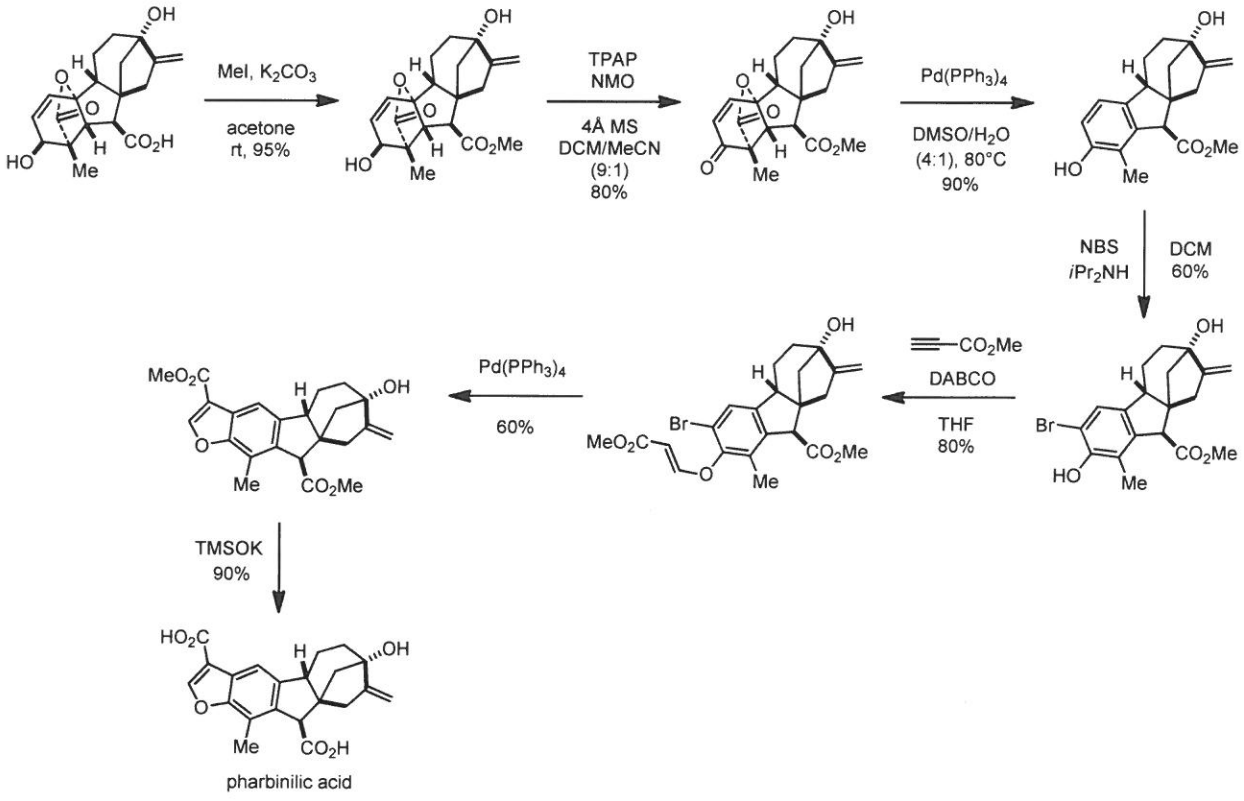
1. General Information

General Laboratory Procedures. All moisture-sensitive reactions were performed under an atmosphere of nitrogen in flame-dried round bottom flasks or glass vials fitted with rubber septa and/or septa equipped screw caps. For reactions run at low temperatures the caps were wrapped with Teflon® tape and parafilm to minimize the introduction of adventitious water. Stainless steel syringes were used to transfer air or moisture-sensitive liquids. Flash chromatography was performed using silica gel Silia Flash® 40-63 micron (230-400 mesh) from Silicycle.

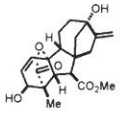
Materials and Instrumentation. All chemicals were purchased from Sigma-Aldrich, VWR or Acros and were used as received unless otherwise stated. Solvents were dried by passing through columns of activated alumina. Triethylamine and N,N-diisopropylethylamine were distilled from CaH_2 at 760 Torr. Proton Nuclear Magnetic Resonance NMR (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Varian Unity Plus 400, Varian MR400, Varian vnmrs 500, Varian Inova 500, Varian Mercury 500, and Varian vnmrs 700 spectrometers. Chemical shifts for protons are reported in parts per million and are references to the NMR solvent peak ($CDCl_3$: δ7.27). Chemical shifts for carbons are reported in parts per million and are referenced to the carbon resonances of the NMR solvent ($CDCl_3$: d77.0). Data are represented as follows: chemical shift, integration, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet), and coupling constants in Hertz (Hz). Mass spectroscopic (MS) data was recorded at the Mass Spectrometry Facility at the Department of Chemistry of the University of Michigan in Ann Arbor, MI on a Agilent Q-TOF HPLC-MS with ESI high resolution mass spectrometer. Infrared (IR) spectra were obtained using either an Avatar 360 FT-IR or Perkin Elmer Spectrum BX FT-IR spectrometer. Data are represented as follows: frequency of absorption (cm-1), intensity of absorption (s = strong, m = medium, w = weak) and b = broad. Luciferase assay signal was collected using a Molecular Devices LMax luminometer. β-Gal assay signal was collected using a Molecular Devices VersaMax Microplate Reader. RT-qPCR was performed using the Applied Biosystems StepOnePlus system.

Abbreviations used: Et_3N = triethylamine, EtOAc = ethyl acetate, AcOH = acetic acid, DCM = dichloromethane, HCl = hydrogen chloride, $NaHCO_3$ = sodium bicarbonate, NaOAc = sodium acetate, NaOMe = sodium methoxide, MeOH = methanol, DIPEA = *N,N*-diisopropylethylamine, TFA – trifluoroacetic acid, THF = tetrahydrofuran.

2. Synthesis of Pharbinilic Acid

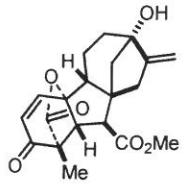


gibberellic acid methyl ester (2).



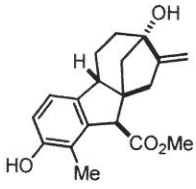
To a stirred solution of Gibberellic Acid **(1)** (5.00g, 14.4 mmol) in acetone (500mL) at room temperature was added solid K_2CO_3 (3.00g, 21.7 mmol) and then CH_3I (2.50 g, 17.6 mmol). Stirring at room temperature, under nitrogen was continued overnight. The mixture was then separated in 250mL water and 250mL ethyl acetate. The aqueous layer was extracted twice with 200mL ethyl acetate. The combined organic layer was washed with water, dried using $MgSO_4$, and concentrated to give gibberellic acid methyl ester **(2)** (4.98 g, 95.4% yield) as a white powder, the spectral characteristics of which matched that previously reported in the literature.[1] **^{1}H NMR** (400 MHz, acetone-d6) δ 6.30 (d, J = 9.3 Hz, 1H), 5.89 (dd, J = 9.3, 3.7 Hz, 1H), 5.19 (s, 1H), 4.86 (s, 1H), 4.66 (s, 1H), 4.15 (d, J = 3.7 Hz, 1H), 3.73 (s, 3H), 3.20 (d, J = 10.7 Hz, 1H), 2.78 (d, J = 10.7 Hz, 1H), 2.23 – 1.62 (m, 10H), 1.23 (s, 3H); **^{13}C NMR** (100 MHz, cdcl$_3$) δ 178.55, 172.66, 156.31, 132.89, 132.30, 107.94, 90.54, 78.51, 77.19, 69.79, 53.44, 52.76, 52.27, 50.93, 50.52, 44.63, 42.94, 38.06, 16.95, 14.35; **HRMS** calculated for $C_{20}H_{24}O_6$: 360.1573; found: 360.1460.

keto-gibberellic acid methyl ester (3).



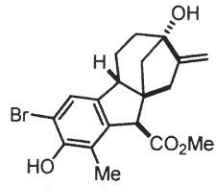
To a solution of gibberellic acid methyl ester **(2)** (2.00 g, 5.55 mmol) over activated 4Å molecular sieves (3.0 g) in dry 9:1 methylene chloride: acetonitrile (90 mL) was added N-methylmorpholine N-oxide (1.30 g, 11.1 mmol) and TPAP (195.0 mg, 0.555 mmol) slowly at room temperature. Stirring at room temperature was continued for 4 hours under nitrogen. The reaction was quenched with 50 mL of saturated ammonium chloride. The aqueous phase was extracted once with methylene chloride (25 mL) and the combined organic phases were washed with brine, dried using $MgSO_4$ filtered and concentrated onto silica. The crude material was purified using column chromatography and hexane/EtOAc (1:1) as eluent to yield keto-gibberellic acid methyl ester **(3)** (1.6 g, 80.5% yield) as a white solid, the spectral characteristics of which match those previously reported in the literature.[2] **^{1}H NMR** (400 MHz, cdcl$_3$) δ 7.25 (d, J= 9.4 Hz, 1H), 6.04 (d, *J* = 9.4 Hz, 1H), 5.29 (m, 1H), 4.98 (s, 1H), 3.73 (s, 3H), 3.51 (d, *J* = 10.5 Hz, 1H), 2.88 (d, *J* = 10.5 Hz, 1H), 2.27 – 1.68 (m, 10H), 1.26 (s, 3H); **^{13}C NMR** (100 MHz, cdcl$_3$) δ 191.51, 172.97, 171.56, 156.17, 147.13, 129.19, 108.04, 89.34, 77.98, 77.18, 64.91, 62.07, 52.39, 51.34, 49.95, 44.94, 42.92, 37.94, 16.99, 11.42; **IR** (thin film, cm^{-1}) 3500.7, 2955.0 , 1778.4, 1731.0, 1695.4, 1437.2, 1380.2, 1319.1, 1259.3, 1197.0, 1148.9, 1092.0, 1020.2, 991.6, 939.9, 896.5, 749.5; **HRMS** calculated for $C_{20}H_{23}O_6$: 358.1416; found: 358.1309.

1-hydroxy-allogibberellic acid methyl ester (4).



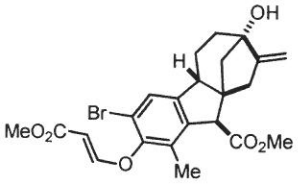
To vacuum dried keto-gibberellic acid methyl ester **(3)** (1.26 g, 3.52 mmol) and vacuum dried anthraquinone 2-sulfonic acid sodium salt (167.3 mg, 0.540 mmol) palladium tetrakis (213 mg, 0.184 mmol) was added atmosphere free under nitrogen and the reaction vessel was sealed. Under nitrogen, to the sealed vessel was added 4:1 water: DMSO (6.75 mL) and the reaction was brought to 80°C. Stirring of the reaction mixture was continued for 24 hours at 80°C. Upon completion, the reaction was added to a slurry 15mL of saturated ammonium chloride and 15mL ethyl acetate. The aqueous phase was then extracted twice with ethyl acetate (15 mL) and the combined organic phases were washed with brine, dried using $MgSO_4$, filtered and concentrated onto silica. The crude reaction mixture was purified using column chromatography with hexanes/EtOAc (1:2) as eluent to yield the desired product as a white solid (966 mg, 89.5% yield. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.84 (d, *J* = 7.9 Hz, 1H), 6.67 (d, *J* = 7.9 Hz, 1H), 5.22 (m, 1H), 5.06 (s, 1H), 3.68 (s, 3H), 3.65 (s, 1H), 3.37 (d, *J* = 7.5 Hz, 1H), 2.77 (dt, *J* = 16.3, 2.9 Hz, 1H), 2.50 (dd, *J* = 16.3, 2.2 Hz, 1H), 2.14 (s, 3H), 2.02 (m, 2H), 1.84 – 1.71 (m, 2H), 1.60 (ddd, *J* = 12.4, 10.7, 2.2 Hz, 2H); **^{13}C NMR** (126 MHz, cdcl$_3$) δ 172.54, 155.19, 152.48, 141.08, 138.56, 121.41, 120.19, 114.15, 106.77, 78.69, 56.44, 53.97, 51.77, 48.69, 47.46, 38.49, 38.24, 21.10, 12.08; **IR** (thin film, cm^{-1}) 3397.5, 3010.5, 2934.8, 2863.8, 1716.0, 1603.8, 1495.7, 1435.3, 1330.4, 1248.0, 1215.4, 1163.1, 1118.0, 1091.0, 1051.4, 1015.6, 997.2, 893.7, 823.4, 747.3, 666.0, 632.9, 620.4, 613.9; **HRMS** calculated for $C_{19}H_{22}O_4$: 314.1518; found: 314.1589.

1-hydroxy-2-bromo-allogibberellic acid methyl ester (5).



To dry 1-hydroxy-allogibberellic acid methyl ester **(4)** (400 mg, 1.27 mmol) was added a solution of diisopropylamine (2.54 mL, 0.127 mmol, 50 mM in methylene chloride) and the solution was placed in a room temperature water bath. To the reaction mixture was added a solution of freshly recrystallized NBS (6.36 mL, 1.27 mmol, 200mM in methylene chloride) over 20 minutes under nitrogen. Stirring of the reaction mixture was continued for 5 minutes under nitrogen. The reaction mixture was acidified with 1 M HCl and diluted with one volume of water. The acidic aqueous layer was extracted twice with methylene chloride (10 mL). The combined organic phases were washed with brine, dried using $MgSO_4$, and concentrated onto silica. The crude reaction mixture was purified using column chromatography with hexanes/EtOAc (1:2) as eluent to yield the desired product as a white solid. (300 mg, 60% yield). **^{1}H NMR** (401 MHz, cdcl$_3$) δ 7.06 (s, 1H), 5.47 (s, 1H), 5.17 (s, 1H), 5.04 (s, 1H), 3.65 (s, 3H), 3.59 (s, 1H), 3.36 (d, J = 7.6 Hz, 1H), 2.75 (d, J = 16.4 Hz, 1H), 2.46 (d, J = 16.3 Hz, 1H), 2.17 (s, 3H), 2.09 – 1.85 (m, 2H), 1.84 – 1.50 (m, 6H); **^{13}C NMR** (101 MHz, cdcl$_3$) δ 171.95, 154.92, 148.91, 140.82, 139.35, 123.15, 122.80, 109.35, 106.91, 78.38, 56.15, 53.95, 51.83, 48.75, 47.31, 38.32, 38.16, 20.97, 13.12; **IR** (thin film, cm^{-1}) 3430.0, 2930.6,2858.1, 1716.4, 1434.1, 1327.5, 1285.6, 1213.7, 1159.2, 1118.4, 1090.3, 1053.1, 996.7, 893.7, 860.8, 750.6, 666.0, 649.9, 616.7; **HRMS** calculated for $C_{19}H_{21}BrO_4$: 392.0623; found: 392.0676.

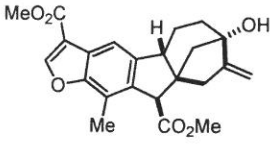
1-(propenoyl methyl ester vinyl ether)-2-bromo-allogibberellic acid (6).



To dry 1-hydroxy-2-bromo-allogibberellic acid **(5)** (222.7 mg, 0.566 mmol) a dry solution of DABCO (2.00 mL, 0.0566 mmol, 28 mM in methylene chloride) and the reaction mixture was brought to 0°C under nitrogen. To the 0°C reaction mixture was dropwise added a dry solution of methyl propynoate (2.00 mL, 0.566 mmol, 280 mM in methylene chloride). The reaction mixture was allowed to come to room temperature and stirring was continued for 90 minutes. Upon completion the reaction was quenched with 0.1 M HCl (10 mL) methylene chloride was added (10 mL) and the organic phase was separated. The organic phase was washed with saturated sodium bicarbonate, washed with brine, dried using $MgSO_4$, and concentrated to yield the crude product as a clear oil. The resulting crude product was purified using column chromatography with hexane/EtOAc (1:1) as eluent to form the desired product **(6)** (3.01g, 80% yield) as a clear oil. **^{1}H NMR** (500 MHz, cdcl$_3$) δ 7.68 (d, J = 12.4 Hz, 1H), 7.26 (s, 1H), 5.23 (dd, J = 2.9, 2.0 Hz, 1H), 5.16 (d, J = 12.4 Hz, 1H), 5.10 (s, 1H), 3.72 (s, 3H), 3.70 (s, 3H), 3.63 (s, 1H), 3.44 (d, J = 7.9 Hz, 1H), 2.81 (dt, J = 16.4, 2.9 Hz, 1H), 2.51 (dd, J = 16.4, 2.0 Hz, 1H), 2.14 (s, 3H), 2.12 – 2.08 (m, 1H), 2.04 – 1.96 (m, 1H), 1.85 – 1.73 (m, 2H), 1.65 – 1.57 (m, 2H); **^{13}C NMR** (126 MHz, cdcl$_3$) δ 171.42, 167.45, 160.17, 154.53, 148.31, 145.80, 141.11, 129.43, 125.06, 115.29, 107.16, 99.78, 78.25, 56.10, 54.07, 51.99, 51.32, 48.76, 47.65, 38.28, 38.14, 20.80, 13.34.; **IR** (thin film, cm^{-1}) 3414.3, 3010.2, 2948.6, 2858.2, 1708.9, 1628.9, 1435.5, 1415.5, 1319.3, 1287.6, 1241.8, 1197.4, 1158.7,

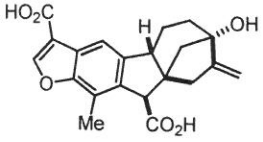
1119.9, 1046.5, 1016.7, 997.7, 958.1, 894.5, 869.6, 837.9, 747.4, 666.1, 623.7, 608.1; **HRMS** calculated for $C_{23}H_{25}BrO_6$: 476.0835; found: 476.0946.

Pharbinilic acid dimethyl ester (7).

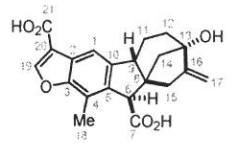


To vacuum dried 1-(propenoyl methyl ester vinyl ether)-2-bromo-allogibberellic acid **(6)** (74.4 mg, 0.156 mmol) under nitrogen was added palladium tetrakis (18 mg, 0.016 mmol) and the reaction vessel was sealed under nitrogen. To the sealed reaction vessel, under nitrogen was added dry acetonitrile (5 mL) and dry triethylamine (0.5 mL). The reaction mixture was brought to 80˚C and stirring of the reaction mixture was continued for 36 hours. The reaction mixture was quenched by adding 0.1 M HCl (10 mL) and the organic phase was separated in ethyl acetate (10 mL) The aqueous phase was extracted twice with ethyl acetate (10 mL) and the combined organic phases were washed with brine, dried using $MgSO_4$, filtered and concentrated. The resulting crude oil was purified using column chromatography with hexane/diethyl ether (4:1) as eluent to form the desired product **(7)** (36.9 mg, 59.7% yield) as a colorless foam. **^{1}H NMR** (400 MHz, cdcl$_3$) δ 8.21 (s, 1H), 7.63 (s, 1H), 5.18 (m, 1H), 5.07 (s, 1H), 3.92 (s, 3H), 3.72 (s, 1H), 3.66 (s, 3H), 3.53 (d, J = 8.0 Hz, 1H), 2.81 (dt, J = 16.4, 2.8 Hz, 1H), 2.61 – 2.48 (m, 1H), 2.41 (s, 3H), 2.31 (dd, J = 14.9, 6.4 Hz, 1H), 2.12 – 1.95 (m, 1H), 1.77 (m, 2H), 1.55 (m, 2H); **^{13}C NMR** (100 MHz, cdcl$_3$) δ 172.27, 164.08, 154.74, 153.94, 150.80, 142.84, 137.37, 123.87, 119.32, 114.54, 112.56, 106.94, 78.72, 55.52, 54.33, 51.81, 51.59, 48.85, 47.33, 38.36, 38.12, 20.87, 11.86; **IR** (thin film, cm^{-1}) 3382.4, 2932.1, 2860.2, 1715.1, 1563.2, 1486.6, 1435.3, 1373.7, 1329.4, 1286.8, 1244.4, 1194.3, 1160.8, 1139.3, 1111.6, 1074.0, 997.7, 917.9, 882.8, 840.6, 811.5, 751.6, 666.7, 636.5, 621.8; **HRMS** calculated for $C_{23}H_{24}O_6$: 396.1646; found: 396.1649.

Pharbinilic acid (8).

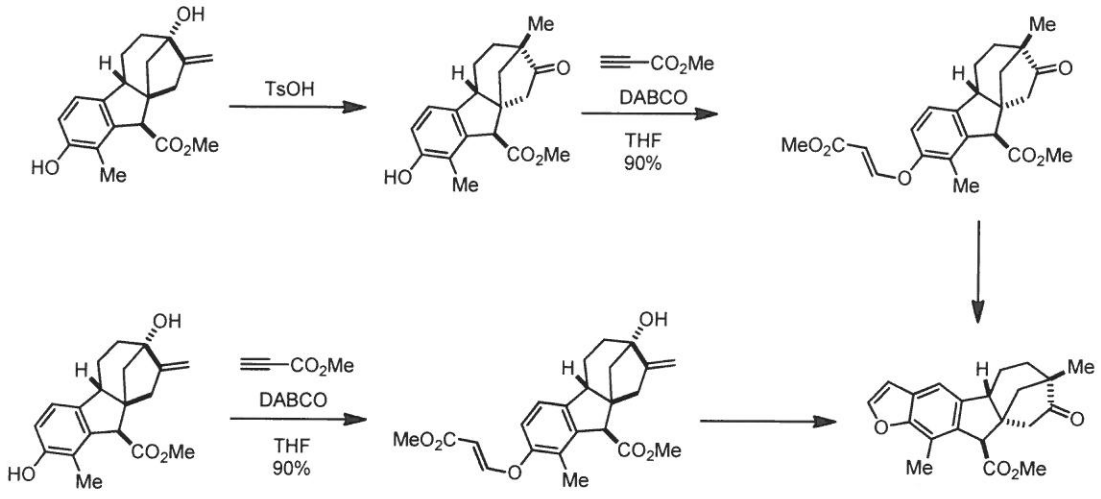


To a solution of pharbinilic acid dimethyl ester **(7)** (25.0 mg, 0.0631 mmol) in dry THF (3.00mL) was added TMSOK (40.5 mg, 0.316 mmol) and stirring of the reaction mixture was continued for 24 hours at room temperature. The solvent was subsequently removed in vacuum and the oil residue was dissolved in TFA acidified (0.1%) 2:1 Acetonitrile: water (300 uL). The crude product was purified under reversed phase conditions (Agilent 1100 system, Phenomenex LUNA 5u (C18) using acetonitrile/water/0.1% TFA as eluent) to form the desired product **(8)** (21.0 mg, 90.4% yield) as a white crystalline solid. $[\alpha]_D^{23}$ = -25.3˚ (c = 0.265, MeOH); **^{1}H NMR** (400 MHz, cd$_3$od) δ 8.36 (s, 1H), 7.63 (s, 1H), 5.15 (s, 1H), 5.06 (s, 1H), 3.69 (s, 1H), 3.50 (d, J = 8.0 Hz, 1H), 2.86 (dt, J = 16.4, 2.9 Hz, 1H), 2.57 (d, J = 16.4 Hz, 1H), 2.43 (s, 3H), 2.29 (dd, J = 14.7, 5.5 Hz, 1H), 2.09 – 1.96 (m, 1H), 1.78 – 1.59 (m, 3H), 1.41 (dd, J = 10.6, 2.5 Hz, 1H); **^{13}C NMR** (126 MHz, cd$_3$od) δ 174.01, 165.35, 154.77, 153.93, 151.20, 142.81, 138.07, 123.84, 118.92, 114.80, 111.91, 105.76, 77.58, 55.47, 53.95, 48.02, 47.85, 38.35, 37.99, 20.30, 10.39; **IR** (thin film, cm^{-1}) 3348, 2946, 2835, 2514, 2073, 1659, 1453, 1122, 1029; **HRMS** calculated for $C_{21}H_{20}O_6$: 369.1260; found: 368.1840.

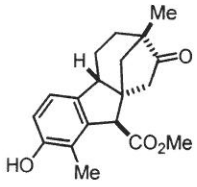


	natural pharbinilic acid		synthetic pharbinilic acid	
number	δ_H	δ_C	δ_H	δ_C
1	7.67 (s)	113.5	7.63 (s, 1H)	111.91
2		125.4		123.84
3		155.5		153.93
4		120.4		118.92
5		139.7		138.07
6	3.72 (s)	57.2	3.69 (s, 1H)	55.47
7		175.8		174.01
8		55.5		53.95
9	3.54 (br, d, J = 8.0 Hz)	48.9	3.50 (d, J = 8.0 Hz, 1H)	47.85
10		144.3		142.81
11	2.31 (m)	21.9	2.29 (dd, J = 14.7, 5.5 Hz, 1H)	20.30
	2.06 (m)		2.09 – 1.96 (m, 1H)	
12	1.75 (m)	39.5	1.78 – 1.59 (m, 3H)	37.99
	1.72 (m)			
13		79.1		77.58
14	1.67 (d, J = 11.0 Hz)	49.6	see 12	48.02
	1.45 (dd, J = 11.0, 2.5 Hz)		1.41 (dd, J = 10.6, 2.5 Hz, 1H)	
15	2.90 (d, J = 16.5 Hz)	39.9	2.86 (dt, J = 16.4, 2.9 Hz, 1H)	38.35
	2.60 (d, J = 16.5 Hz)		2.57 (d, J = 16.4 Hz, 1H)	
16		156.4		154.77
17	5.19 (s)	107.2	5.15 (s, 1H)	105.76
	5.08 (s)		5.06 (s, 1H)	
18	2.46 (s)	11.9	2.43 (s, 3H)	10.39
19	8.36 (s)	152.5	8.36 (s, 1H)	151.20
20		116.7		114.80
21		167.3		165.35

3. Synthesis of Gibberellin analogues

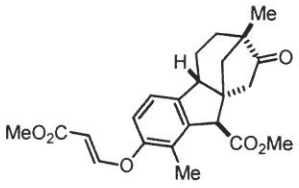


***Epi*-1-hydroxy-allogibberellic acid methyl ester (9).**



To a solution of 1-hydroxy-allogibberellic acid methyl ester **(4)** (700 mg, 2.23 mmol) in toluene (16 mL) was added pTsOH (1.23 g, 6.68 mmol) and stirring of the reaction mixture was continued for 20 minutes at reflux. The hot reaction mixture was added to a mixture of saturated sodium bicarbonate (50 mL) and ethyl acetate (50 mL). The organic phase was separated, the aqueous phase was extracted twice with ethyl acetate (20 mL), the combined organic phases were washed with brine, dried using $MgSO_4$, and concentrated onto silica. The resulting crude product was purified using column chromatography with hexane/ethyl acetate (1:2) as eluent to form the desired product **(9)** (699 mg, quantitative yield) as a white solid. **^{1}H NMR** (401 MHz, cdcl$_3$) δ 6.75 (d, J = 7.9 Hz, 1H), 6.60 (d, J = 7.9 Hz, 1H), 3.70 (s, 1H), 3.69 (s, 3H), 3.41 (dd, J = 12.5, 5.0 Hz, 1H), 2.22 (d, J = 3.6 Hz, 1H), 2.17 (d, J = 3.5 Hz, 1H), 2.10 (s, 3H), 2.07 – 1.89 (m, 4H), 1.71 – 1.55 (m, 2H), 1.37 (ddd, J = 26.0, 12.9, 5.8 Hz, 1H), 1.08 (s, 3H); **^{13}C NMR** (126 MHz, cdcl$_3$) δ 220.71, 172.18, 152.96, 140.32, 137.00, 121.41, 120.62, 114.46, 56.32, 52.09, 51.96, 50.85, 48.81, 48.57, 44.41, 36.67, 22.48, 19.66, 12.16; **IR** (thin film, cm^{-1}) 3772.7, 3696.6, 3660.0, 3407.7, 3016.3, 2926.8, 2868.7, 1725.2, 1641.0, 1602.2, 1548.0, 1499.0, 1453.6, 1433.9, 1400.7, 1338.9, 1266.5, 1226.3, 1196.8, 1161.8, 1085.7, 1056.3, 1004.3, 972.2, 940.8, 835.4, 816.1, 746.2, 665.9, 631.0, 619.4; **HRMS** calculated for $C_{19}H_{22}O_4$: 314.1518; found: 314.1410.

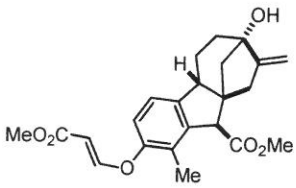
***Epi*-1-(propenoyl methyl ester vinyl ether)-allogibberellic acid (10).**



To a solution of *epi*-1-hydroxy-allogibberellic acid methyl ester **(9)** (570 mg, 1.81 mmol) in dry THF (10.0 mL) was added anhydrous DABCO (20.3 mg, 0.181 mmol) and the solution was cooled to 0°C. To the 0°C solution was added methyl propynoate (167.7 mg, 1.99 mmol). The reaction was allowed to come to room temperature. Stirring of the

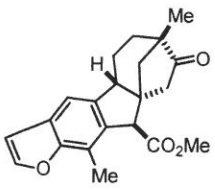
reaction mixture was continued for 40 minutes at room temperature. The reaction was quenched by addition of 10 mL saturated ammonium chloride. The aqueous layer was extracted twice with ethyl acetate (10 mL). The organic layers were combined, washed with brine, dried using $MgSO_4$, and concentrated onto silica. The resulting crude product was purified using column chromatography with hexane/EtOAc (2:1) as eluent to form the desired product **(10)** (648 mg, 89.7% yield) as a colorless foam. **^{1}H NMR** (500 MHz, cdcl$_3$) δ 7.75 (d, J = 12.3 Hz, 1H), 6.97 (d, J = 8.0 Hz, 1H), 6.91 (d, J = 8.0 Hz, 1H), 5.41 (d, J = 12.3 Hz, 1H), 3.76 (s, 1H), 3.74 (s, 3H), 3.73 (s, 3H), 3.50 (dd, J = 12.0, 5.7 Hz, 1H), 2.30 – 2.24 (m, 1H), 2.22 (dd, J = 18.6, 3.7 Hz, 1H), 2.13 (s, 3H), 2.10 – 1.94 (m, 3H), 1.63 (td, J = 12.8, 5.1 Hz, 1H), 1.51 – 1.41 (m, 1H), 1.12 (s, 3H); **^{13}C NMR** (101 MHz, cdcl$_3$) δ 218.46, 171.30, 167.68, 160.36, 152.88, 142.23, 141.19, 126.04, 121.16, 118.22, 100.69, 56.26, 52.11, 51.90, 51.22, 50.62, 49.02, 48.46, 44.48, 36.48, 22.31, 19.65, 12.36;
IR (thin film, cm^{-1}) 3023.5, 2952.0, 2927.9, 2873.2, 1731.9, 1712.4, 1644.2, 1628.2, 1599.1, 1471.4, 1434.7, 1402.1, 1376.6, 1319.6, 1287.0, 1226.1, 1201.7, 1160.0, 1122.0, 1044.1, 1004.3, 956.4, 836.6, 748.0, 666.2, 647.7, 625.9; **HRMS** calculated for $C_{23}H_{26}O_6$: 398.1729; found: 398.1810.

1-(propenoyl methyl ester vinyl ether)-allogibberellic acid (11).



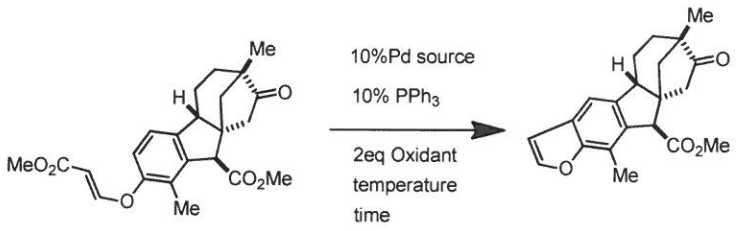
To a solution of *Epi*-1-(propenoyl methyl ester vinyl ether)-allogibberellic acid **(10)** (80.0 mg, 0.201 mmol) in dry THF (0.700 mL) was added a solution of anhydrous DABCO (0.500 mL, 0.0254 mmol, 50 mM in dry THF) and the reaction was brought to 0°C. To the cold reaction mixture was added a dry solution of methyl propynoate (0.100 mL, 0.254 mmol, 2.54 M in dry THF) and stirring was continued for 90 minutes at room temperature. The reaction was quenched with saturated ammonium chloride (10mL) and to the aqueous phase was added ethyl acetate (10 mL). The aqueous phase was then extracted twice with ethyl acetate (10 mL) and the combined organic phases were washed with brine, dried using $MgSO_4$, filtered and concentrated onto silica. The resulting crude product was purified using column chromatography with hexane/EtOAc (1:1) as eluent to form the desired product **(11)** (91 mg, 90% yield) as a colorless foam. **^{1}H NMR** (500 MHz, cdcl$_3$) δ 7.76 (d, J = 12.3 Hz, 1H), 7.01 (d, J = 8.1 Hz, 1H), 6.91 (d, J = 8.0 Hz, 1H), 5.40 (d, J = 12.3 Hz, 1H), 5.22 (dd, J = 2.9, 2.0 Hz, 1H), 5.08 (m, 1H), 3.73 (s, 3H), 3.69 (s, 3H), 3.67 (s, 1H), 3.43 (d, J = 7.6 Hz, 1H), 2.80 (dt, J = 16.4, 2.9 Hz, 1H), 2.52 (d, J = 16.4 Hz, 1H), 2.14 – 2.18 (m, 1H), 2.13 (s, 3H), 2.01 (ddt, J = 15.0, 10.7, 8.0 Hz, 1H), 1.84 – 1.72 (m, 2H), 1.60 (s, 2H); **^{13}C NMR** (101 MHz, cdcl$_3$) δ 171.91, 167.78, 160.56, 154.96, 152.58, 143.58, 141.77, 126.24, 120.64, 117.96, 106.86, 100.50, 78.41, 56.28, 54.03, 51.78, 51.22, 48.74, 47.63, 38.42, 38.23, 20.94, 12.32; **IR** (thin film, cm^{-1}) 3424.2, 2926.6, 2856.3, 1719.7, 1644.5, 1629.1, 1598.1, 1470.9, 1435.1, 1381.9, 1318.6, 1282.7, 1233.8, 1191.9, 1159.6, 1125.2, 1071.7, 1046.3, 1015.9, 997.0, 959.5, 890.6, 834.4, 743.2, 627.0, 619.8; **HRMS** calculated for $C_{23}H_{26}O_6$: 398.1729; found: 398.1800.

(4bR,7S,9aS,10R)-methyl,7,11-dimethyl-8-oxo-5,6,7,8,9,10-hexahydro-4bH-7,9a-methanocyclohepta[1,2]indeno[5,6-b]furan-10-carboxylate (12).



Dry 1-hydroxy-allogibberellic acid methyl ester **(11)** (50.0 mg, 0.125 mmol), dry palladium tetrakis (14.5 mg, 0.0125 mmol), dry triphenylphosphine (3.3 mg, 0.013 mmol), and dry silver trifluoroacetate (55.2 mg, 0.250 mmol) were combined in a dry flask which was purged with dry nitrogen. To the reaction vessel was added dry benzene (0.700 mL) and the reaction was brought to 80˚C and allowed to stir for 24 hr. The reaction was quenched with water (4mL) and to the aqueous phase was added ethyl acetate (4 mL). The aqueous phase was then extracted twice with ethyl acetate (4 mL) and the combined organic phases were washed with brine, dried using $MgSO_4$, filtered and concentrated onto silica. The resulting crude product was purified using column chromatography with hexane/EtOAc (1:1) as eluent to form the desired product **(12)** (22 mg, 56% yield) as a colorless foam.**^{1}H NMR** (401 MHz, $cdcl_3$) δ 7.69 (d, J = 9.5 Hz, 1H), 7.08 (s, 1H), 6.43 (d, J = 9.5 Hz, 1H), 3.87 (s, 1H), 3.76(s, 3H), 3.55 (dd, J = 12.2, 4.9 Hz, 1H), 2.38 (s, 3H), 2.32 (ddd, J = 10.8, 5.0, 3.7 Hz, 1H), 2.21 – 1.96 (m, 4H), 1.65 (dtd, J = 17.8, 12.8, 5.3 Hz, 2H), 1.52 – 1.39 (m, 1H), 1.10 (s, 3H); **^{13}C NMR** (126 MHz, $cdcl_3$) δ 218.13, 170.90, 161.05, 151.83, 143.84, 141.13, 123.49, 119.21, 118.38, 115.85, 56.31, 52.15, 51.94, 50.61, 48.73, 48.27, 44.43, 36.38, 22.39, 19.66, 12.09; **IR** (thin film, cm^{-1}) 2927.4, 2851.4, 1721.2, 1617.7, 1573.3, 1434.7, 1403.3, 1329.7, 1247.8, 1194.1, 1162.8, 1126.2, 1097.6, 1053.1, 997.3, 911.4, 877.9, 825.6, 726.7

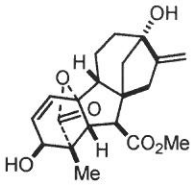
4. Approaches to Direct Palladium-Catalyzed Cross Coupling

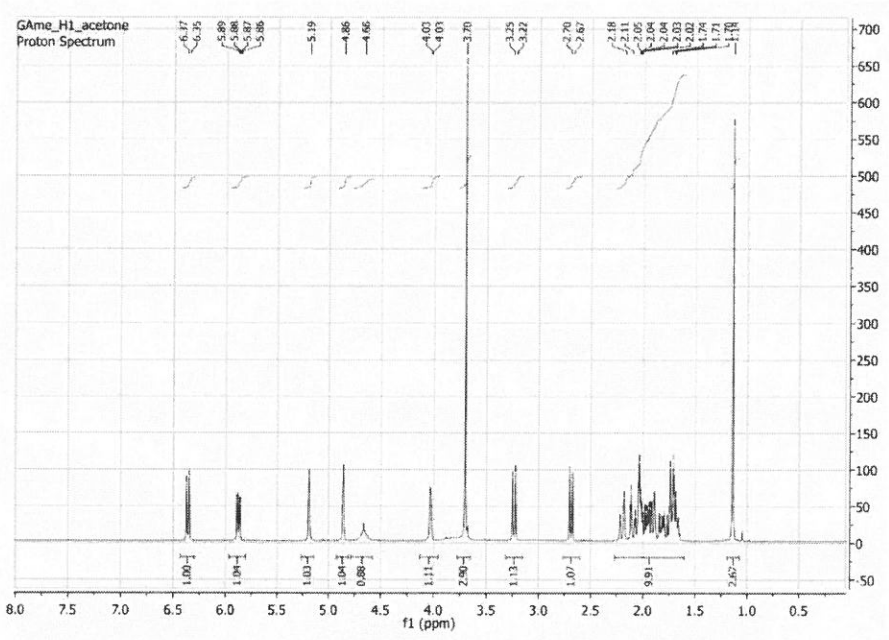


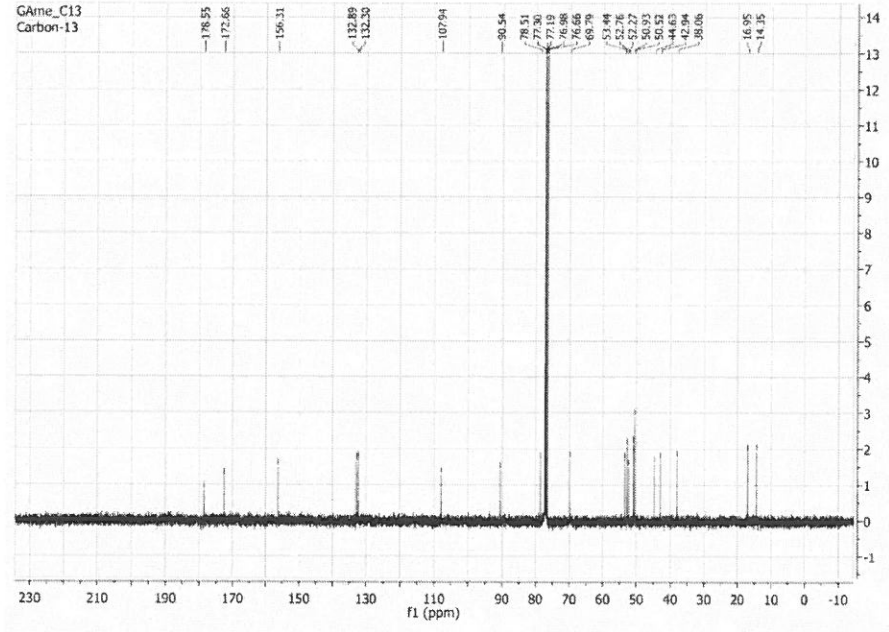
Catalyst	Oxidant	Solvent	temp	time	yield
$Pd(OAc)_2$	AgTFA	toluene	110	24	30
$Pd(OAc)_2$	AgTFA	toluene	80	24	32
$Pd(OAc)_2$	AgTFA	toluene	65	48	33
$Pd(OAc)_2$	AgTFA	toluene	55	48	28
$Pd(OAc)_2$	AgTFA	benzene	80	24	35
$Pd(OAc)_2$	AgOTf	toluene	110	24	31
$Pd(OAc)_2$	AgCl	toluene	110	24	complex mix
$Pd(OAc)_2$	O_2	toluene	110	24	no reaction
$Pd(OAc)_2$	DMP	toluene	110	24	no reaction
$Pd(OAc)_2$	DAIB	toluene	110	24	noreaction
$Pd(OAc)_2$	$NaIO_4$	toluene	110	24	no reaction
$Pd(OAc)_2$	stoichiometric	toluene	110	24	52
Pdtetrakis	AgTFA	toluene	110	24	56
Pdtetrakis	AgTFA	toluene	80	24	56
Pdtetrakis	AgTFA	toluene	65	48	50
Pdtetrakis	AgTFA	toluene	55	48	43
Pdtetrakis	AgTFA	benzene	80	24	56
Pdtetrakis	AgCl	toluene	110	24	complex mix
Pdtetrakis	O_2	toluene	110	24	no reaction
Pdtetrakis	DMP	toluene	110	24	no reaction
Pdtetrakis	DAIB	toluene	110	24	complex mix
Pdtetrakis	NaIO4	toluene	110	24	no reaction
Pdtetrakis	stoich	toluene	110	24	62
$PdCl_2$	AgTFA	toluene	80	24	complex mix
$PdCl_2$	DAIB	toluene	80	24	no reaction
$Pd(OTf)_2$	AgTFA	toluene	80	24	32
$Pd(OTf)_2$	DAIB	toluene	80	24	no reaction
none	AgTFA	toluene	110	24	no reaction

5. NMR Spectra

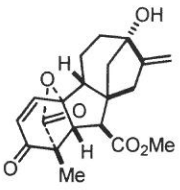
gibberellic acid methyl ester (2)

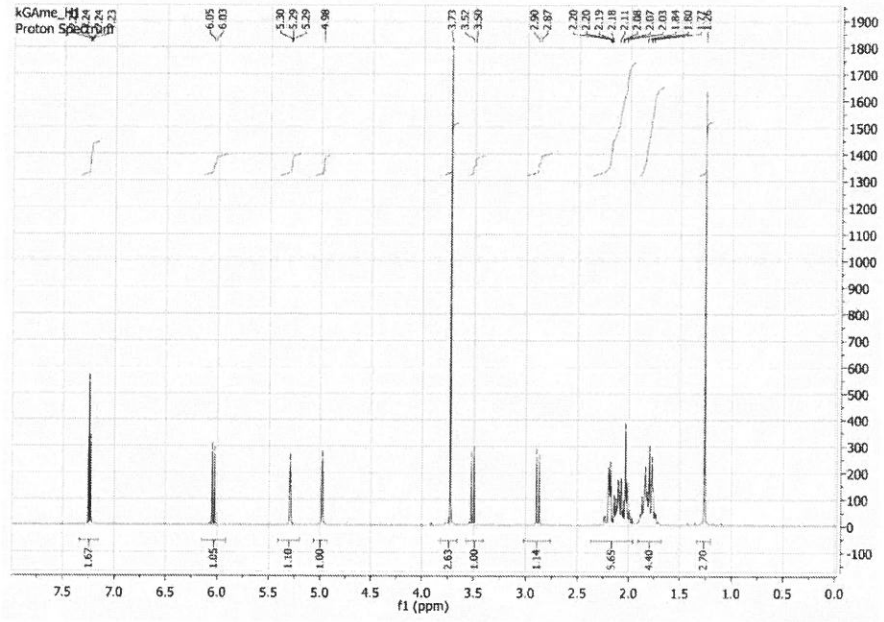


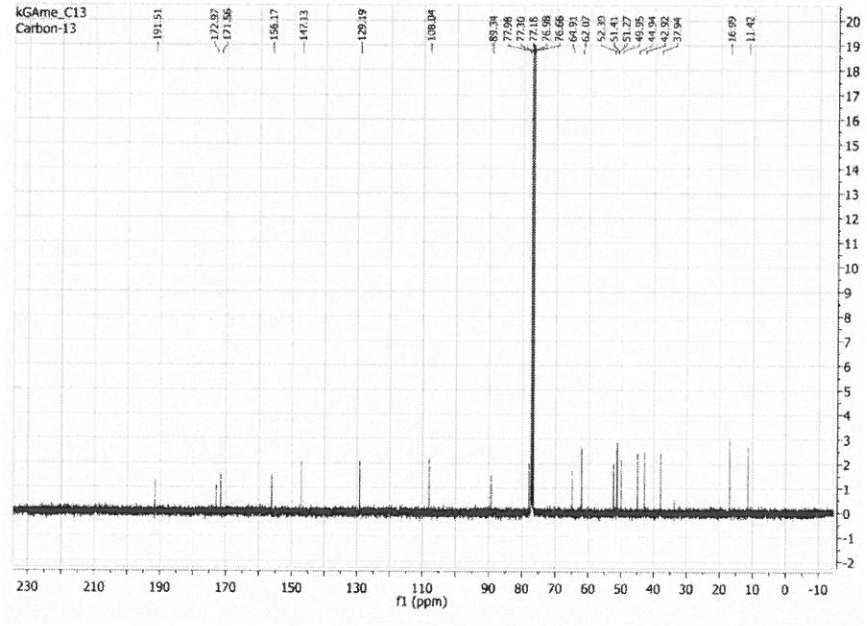




keto-gibberellic acid methyl ester (3)

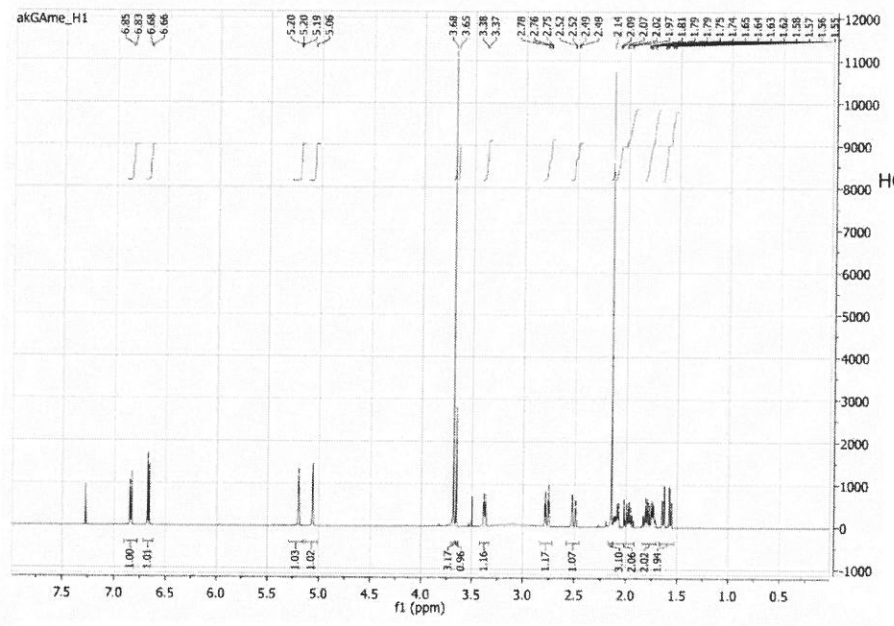


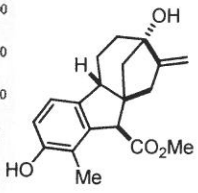


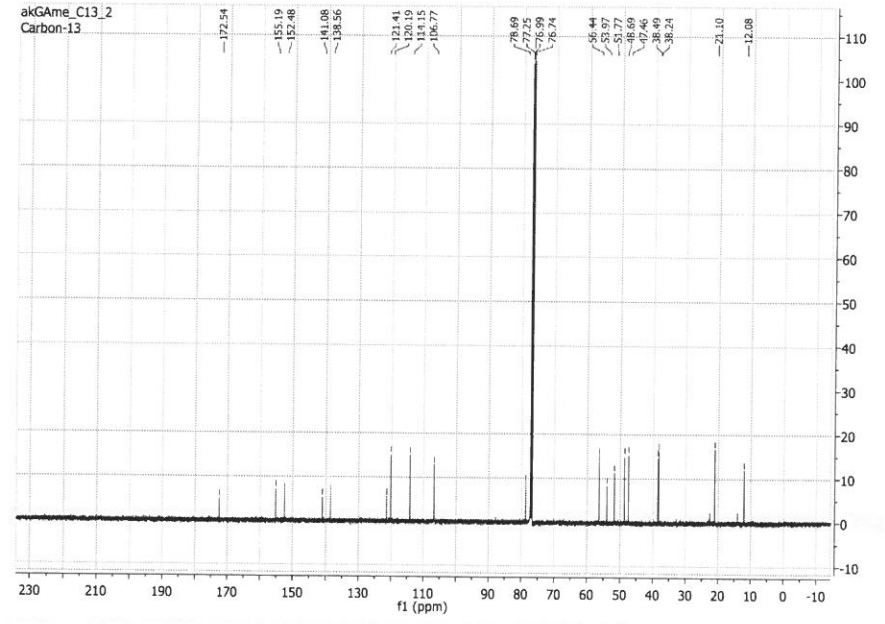


Solvent peak present under proton shift at 7.26 ppm in H^1-NMR

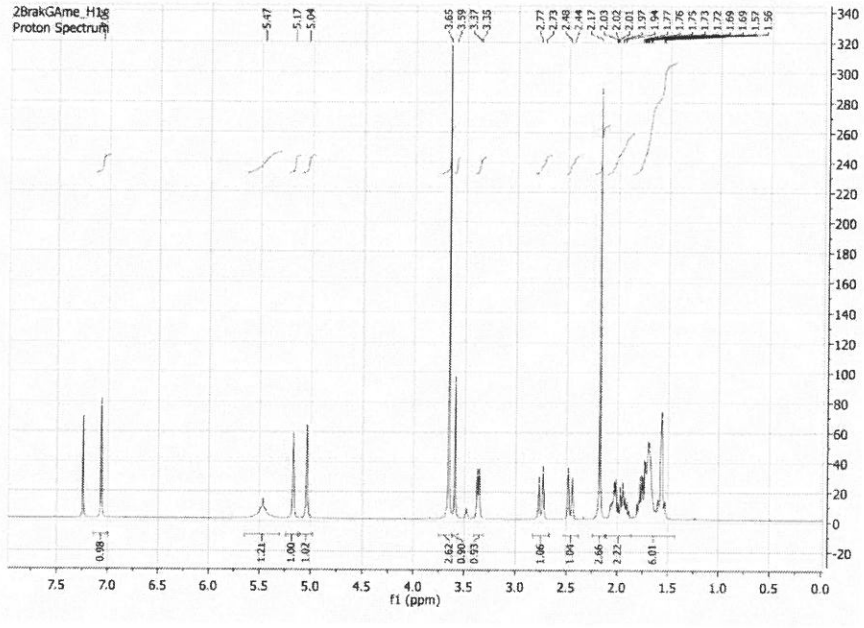
1-hydroxy-allogibberellic acid methyl ester (4)

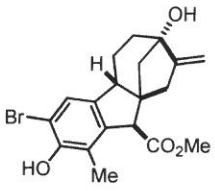


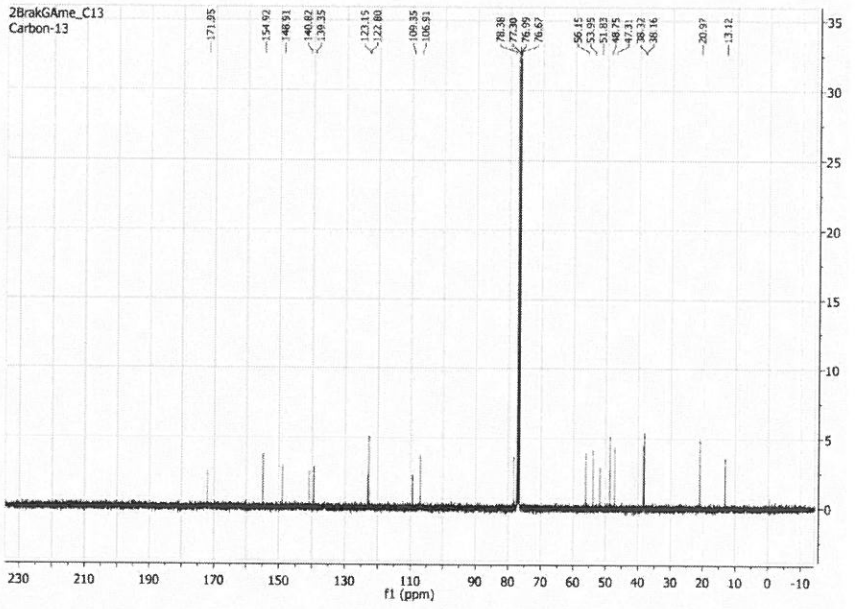




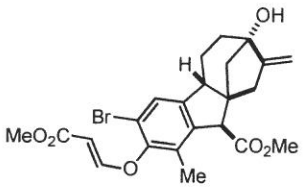
1-hydroxy-2-bromo-allogibberellic acid methyl ester (5)

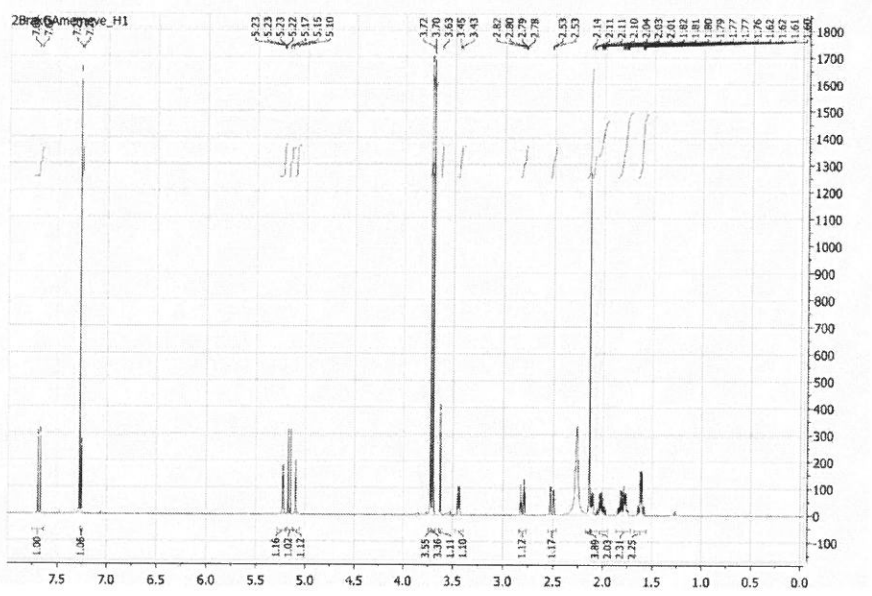


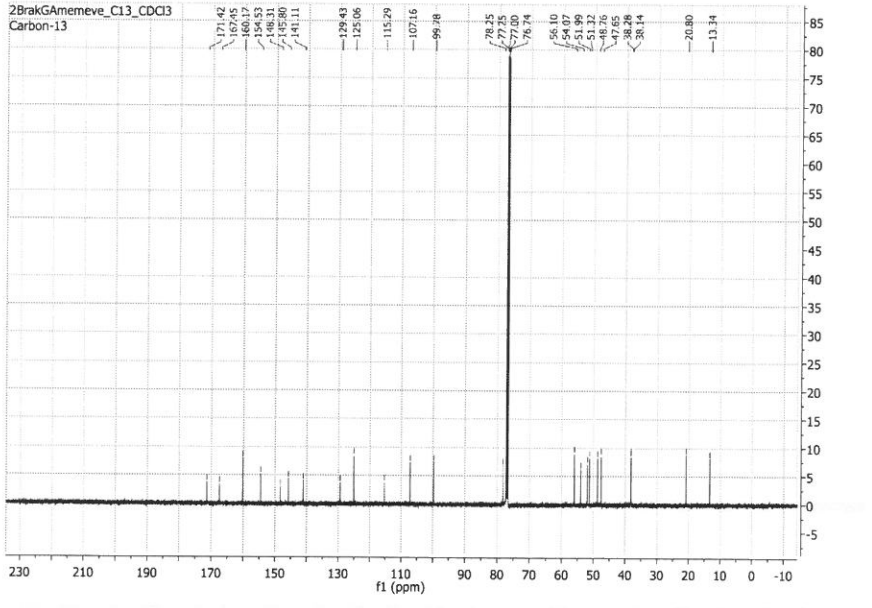




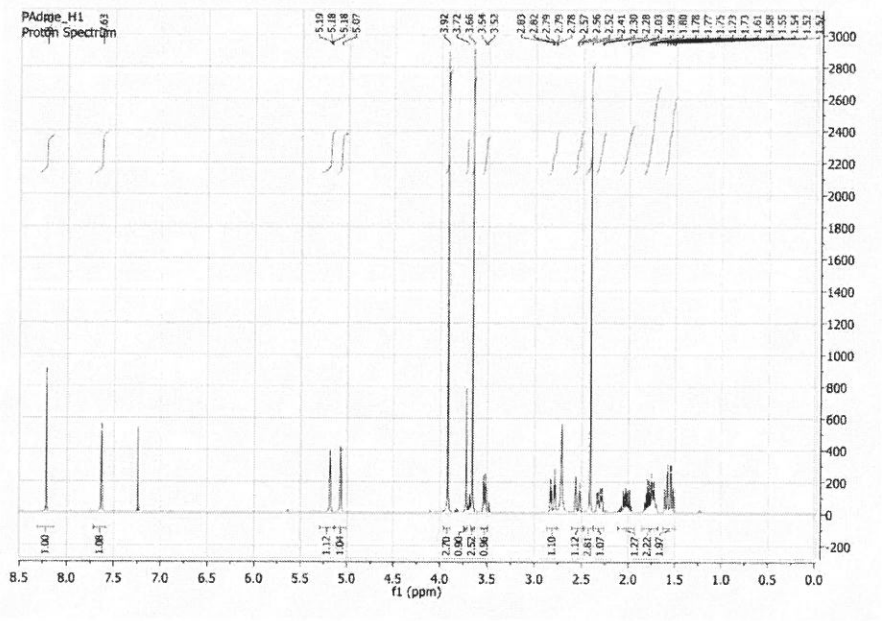
1-(propenoyl methyl ester vinyl ether)-2-bromo-allogibberellic acid (6)

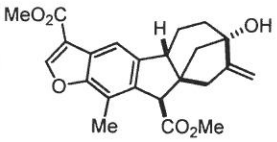


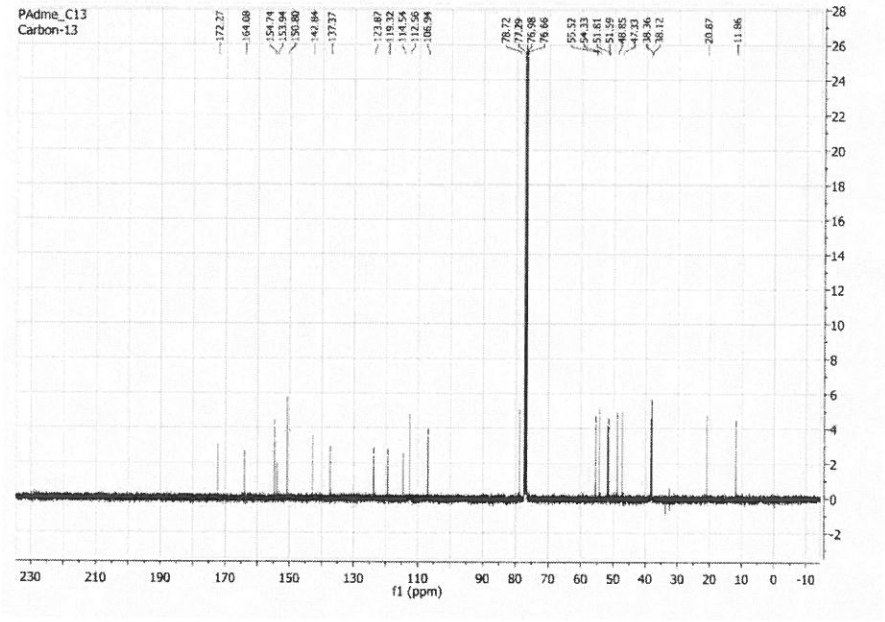




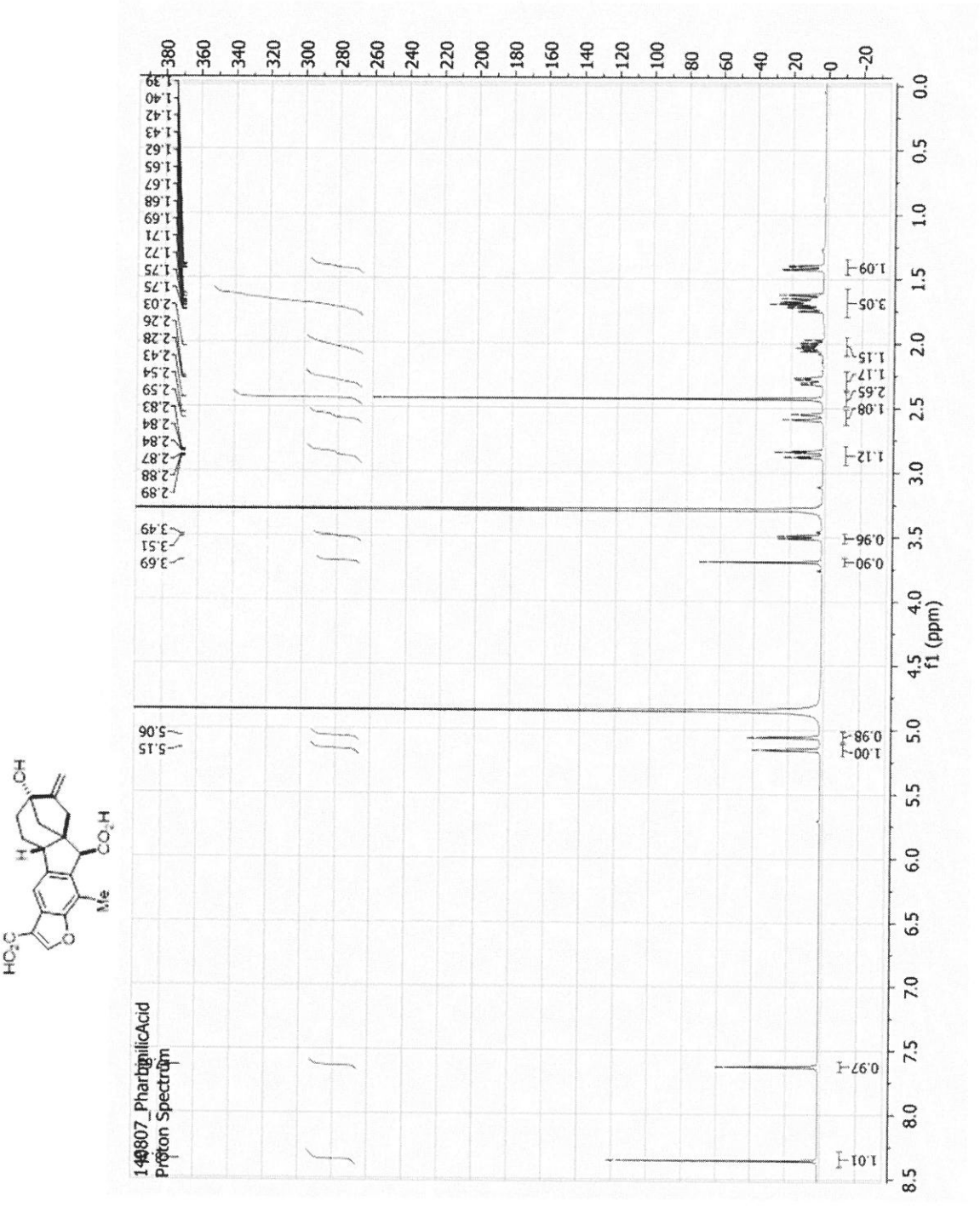
Pharbinilic acid dimethyl ester (7)

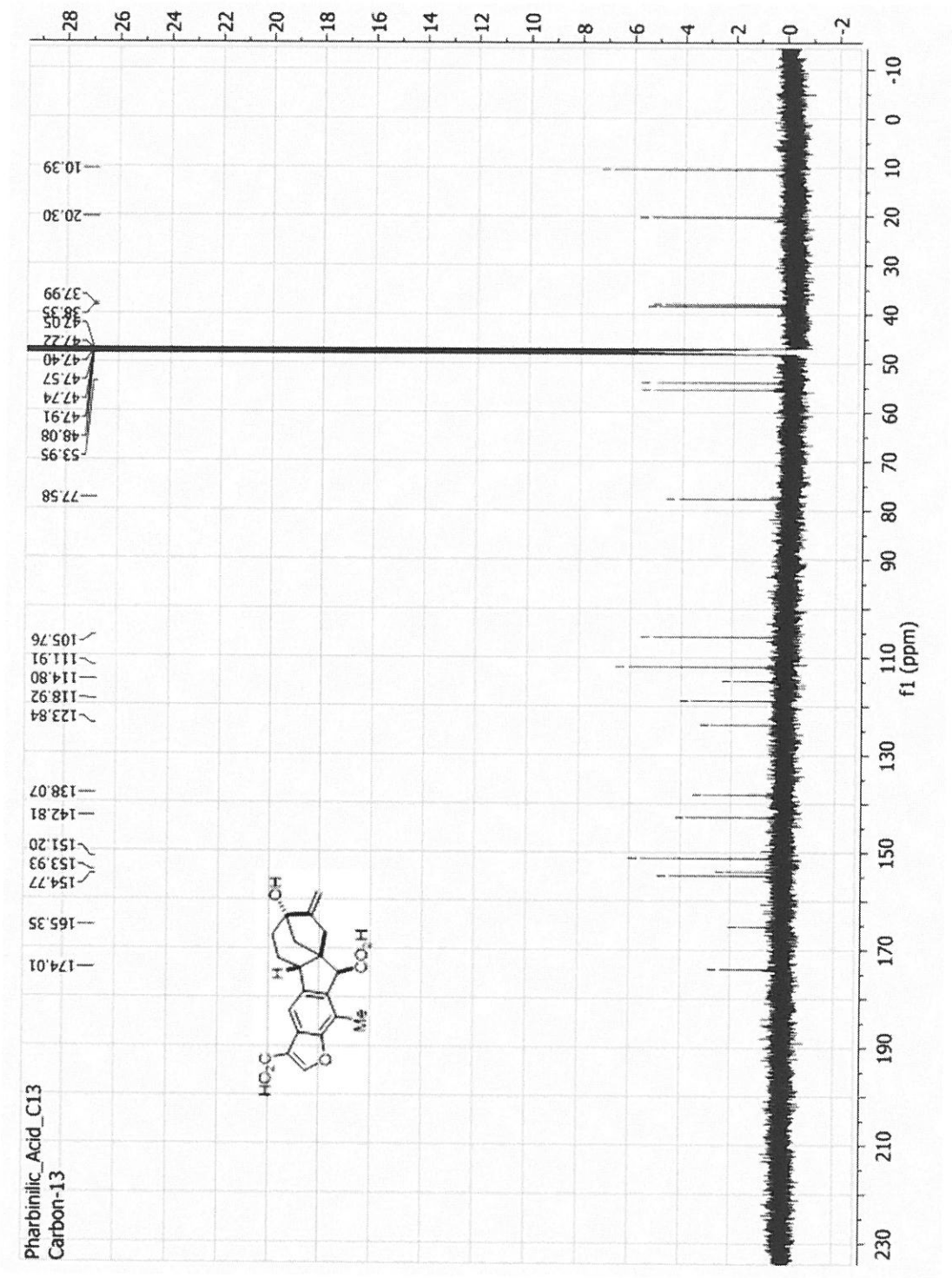




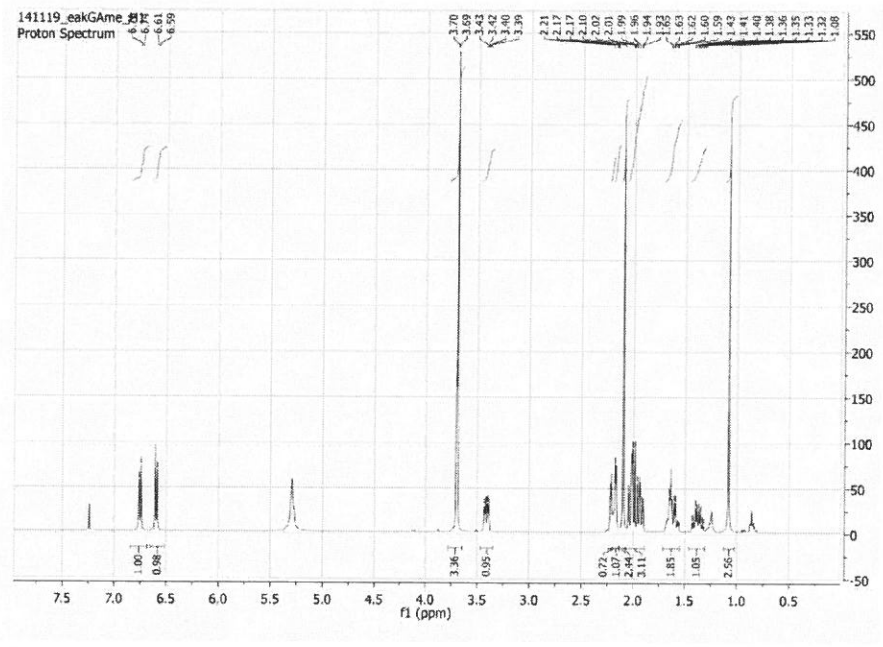


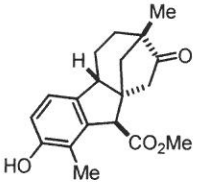
Pharbinilic acid (8)

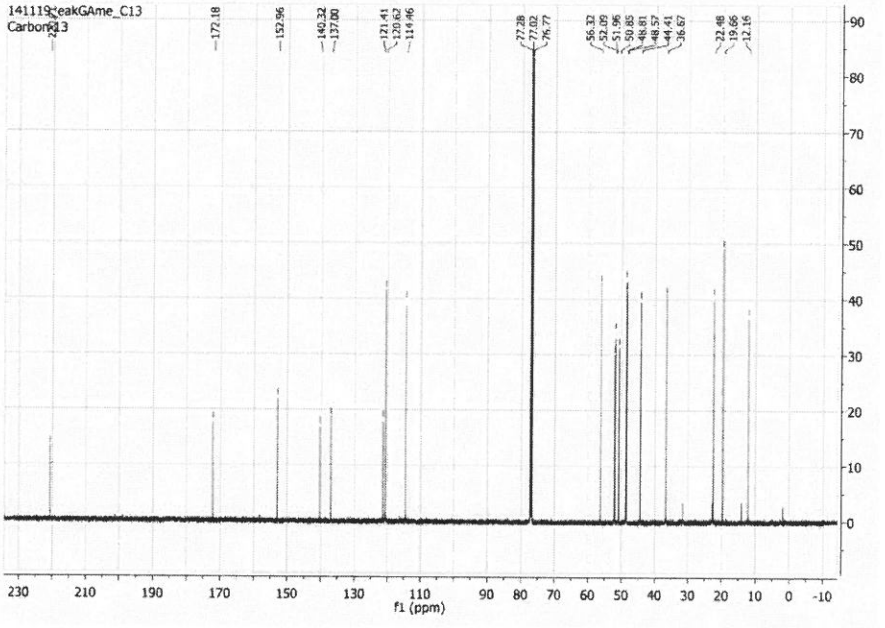



Pharbinilic_Acid_C13
Carbon-13
f1 (ppm)
10.39
20.30
37.99
38.35
47.05
47.22
47.40
47.57
47.74
47.91
48.08
53.95
77.58
105.76
111.91
114.80
118.92
123.84
138.07
142.81
151.20
153.93
154.77
165.35
174.01
OH
H
CO2H
Me
O
HO2C

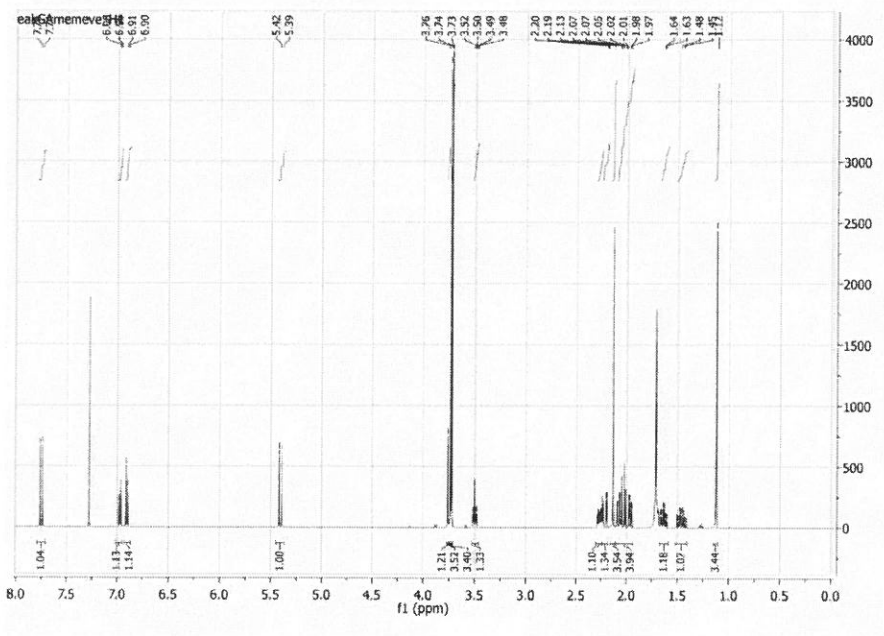
Epi-1-hydroxy-allogibberellic acid methyl ester (9)

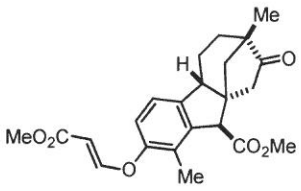


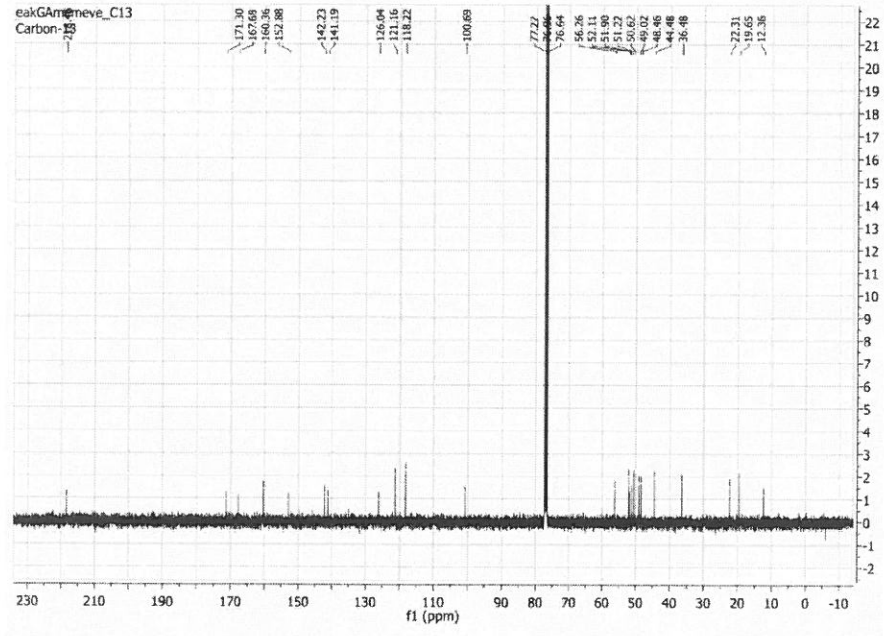




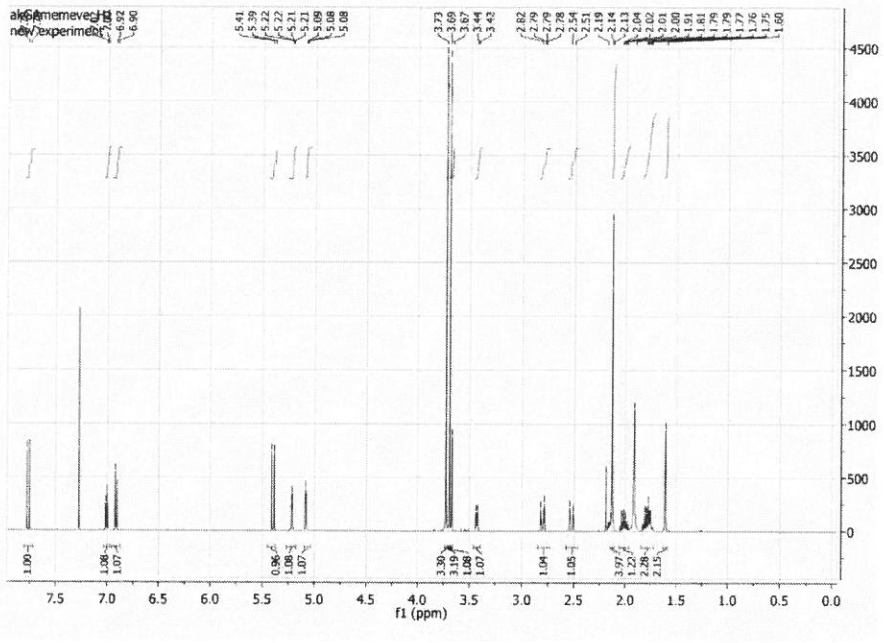
Epi-1-(propenoyl methyl ester vinyl ether)-allogibberellic acid (10)

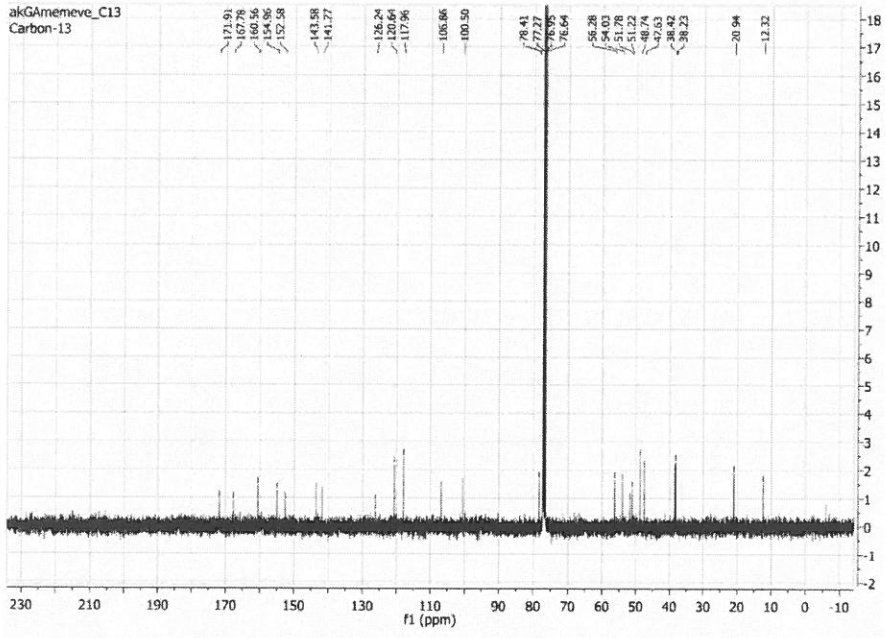




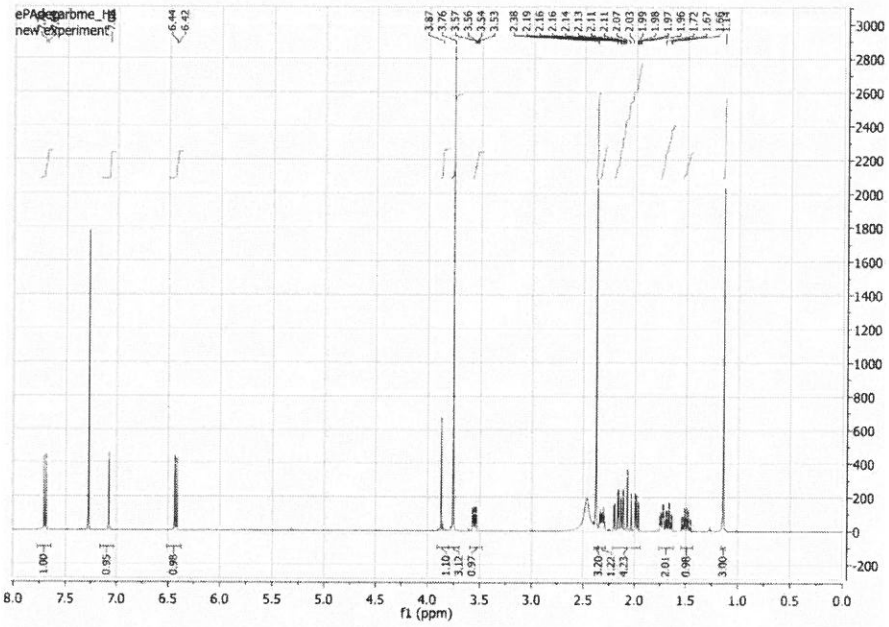


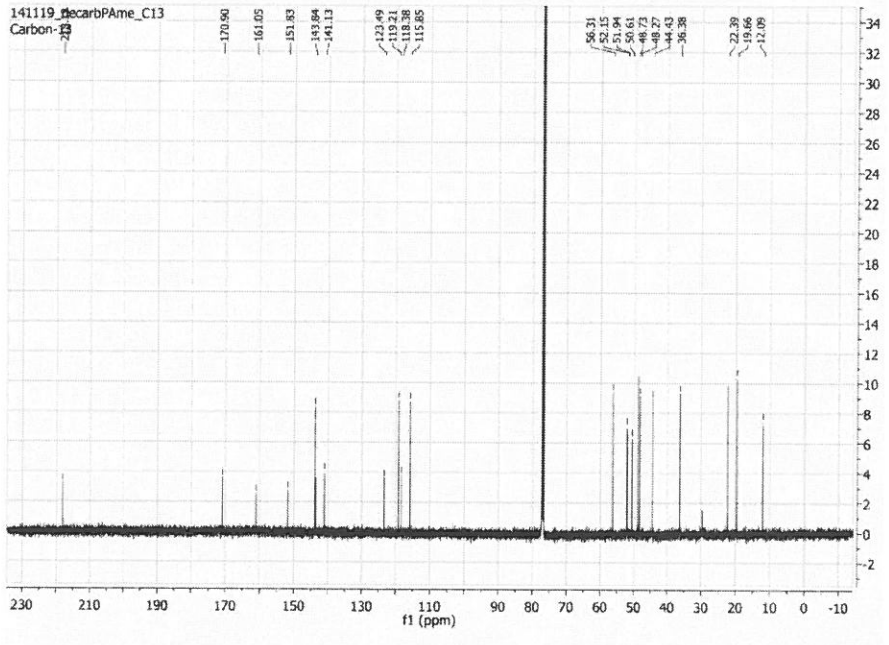
1-(propenoyl methyl ester vinyl ether)-allogibberellic acid (11)



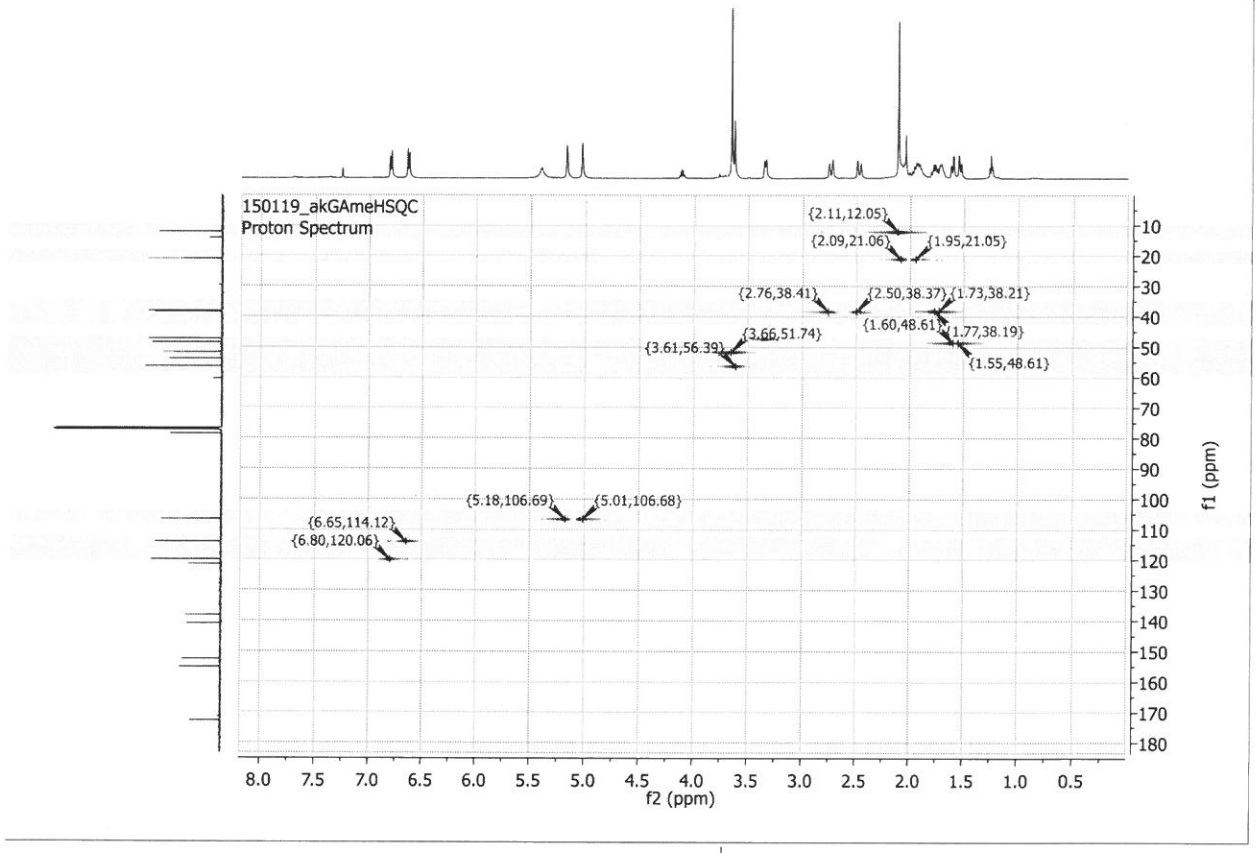


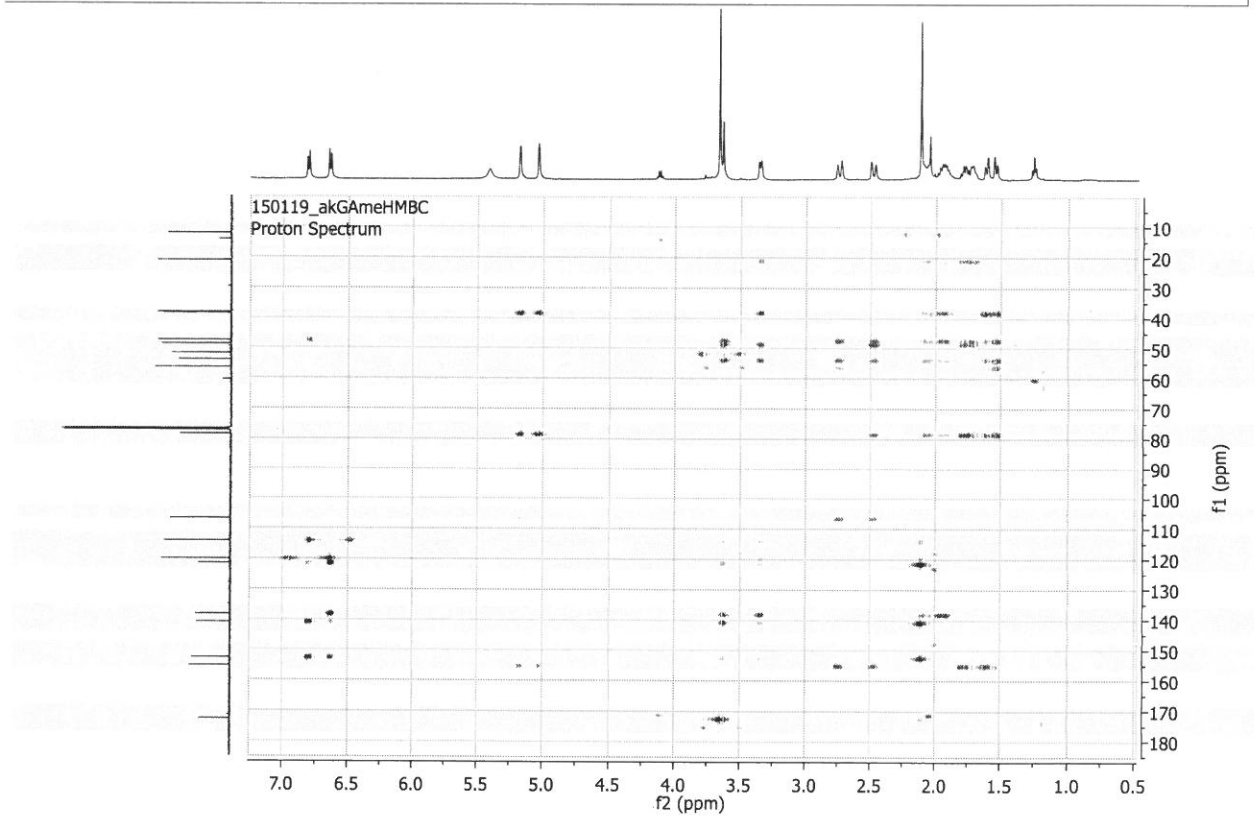
(4bR,7S,9aS,10R)-methyl,7,11-dimethyl-8-oxo-5,6,7,8,9,10-hexahydro-4bH-7,9a-methanocyclohepta[1,2]indeno[5,6-b]furan-10-carboxylate (12)

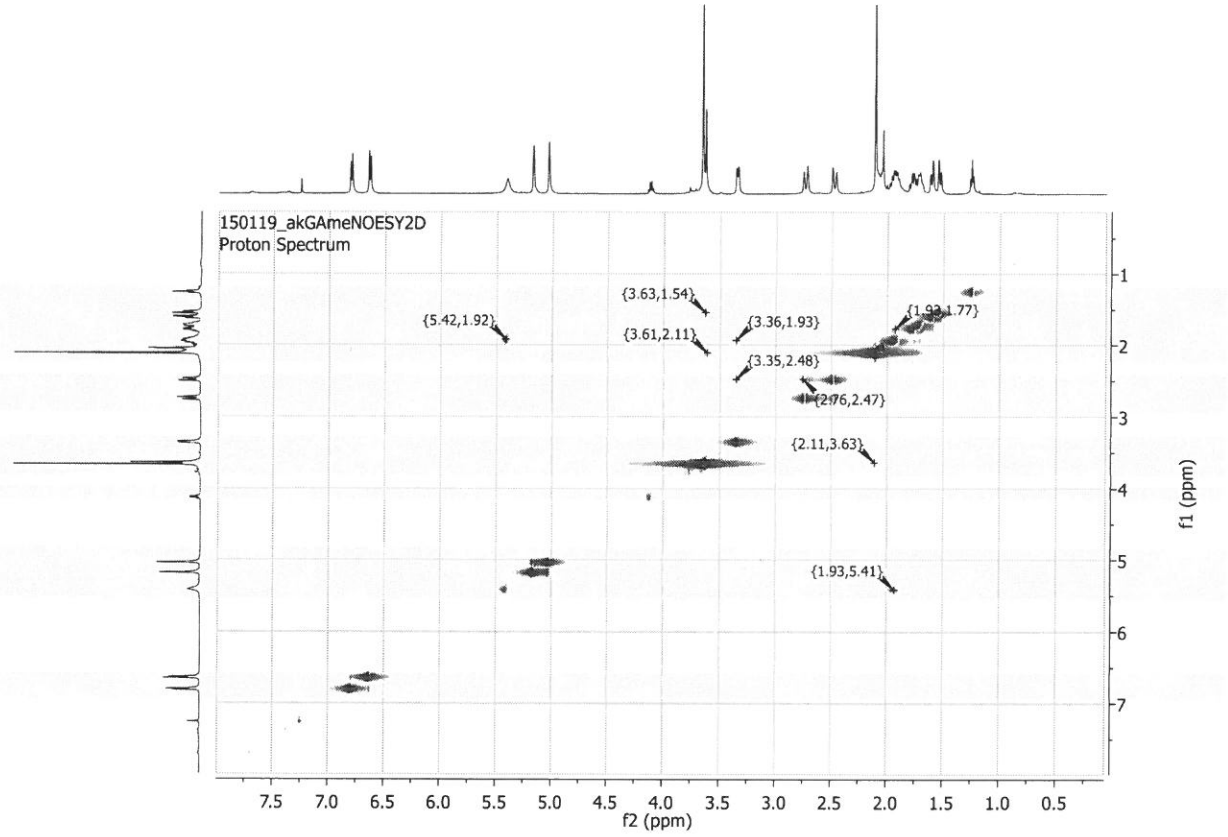


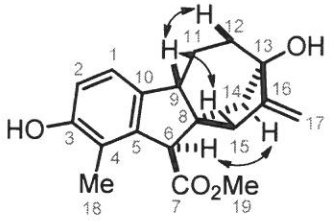


6. 2D NMR characterization of synthesized 1-hydroxy-allogibberellic acid methyl ester (4)









Arrows indicating key NOESY interactions required for assigning stereo centers. Interactions are observed between H9 and H12 and H15 as expected of the assigned stereo centers. H6 displays NOESY interactions with H14. Importantly no NOESY interaction is observed between H9 and H6.

7. Plasmids, Cell Culture and Transfections

The NF-κB luciferase reporter plasmid carrying 6 tandem κB-sites, NF-κB-luc, CMV-β-Gal, and pBSSK were generously provided by Dr. Jorge Iñigues-Lluhí (The University of Michigan Pharmacology Department).[3] All cells were maintained in 5% CO_2 at 37°C. HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% FBS. For luciferase assays, $4x10^5$ cells were seeded in a 6-well dish and allowed to adhere overnight. The media was removed and cells were transfected in Opti-Mem (Invitrogen) with 400 ng NF-κB-luc, 200 ng CMV-β-Gal, and 1,400 ng pBSSK using Lipofectamine 2000 (Life Technologies) according to manufacturer's instructions. After 4.5 h, transfection solution was removed and replaced with DMEM containing 10% FBS. At 24 h after transfection, cells were trypsinized and resuspended in DMEM supplemented with 10% FBS and seeded into a 96-well plate at a density of $8x10^3$ cells per well. After an additional 16 h, media was removed and replaced with Opti-Mem containing vehicle or molecules delivered in DMSO (0.1% v/v) at the indicated concentrations. After cells incubated with either vehicle or compound for 1 h, cells were treated with either PBS or IL-1β at a final concentration of 2 ng/mL. After an additional 3 h, media was removed and cells were lysed with 60 µL of passive lysis buffer. Luciferase and β-Galactosidase activities were determined as previously described.[4] NF-κB luciferase activity IC_{50}s were determined using 5 point dose curves. Response curve analysis was performed using GraphPad software. For endogenous gene expression analysis, $1x10^5$ cells were seeded into a 24-well plate and allowed to adhere overnight. Media was removed and replaced with Opti-Mem media containing vehicle or molecule delivered in DMSO (0.1% v/v) at the indicated concentrations. After incubating for 1 h, cells were treated with either PBS or IL-1β at a final concentration of 2 ng/mL. After 2 h, the media was removed and total RNA was isolated using RNeasy Plus RNA isolation kits (Qiagen) according to manufacturer's instructions. Each RNA sample was used to synthesize cDNA using iScript cDNA synthesis kits (Bio-Rad). Quantitative real-time PCR (qRT-PCR) reactions were carried out in triplicate in an Applied Biosystems StepPlusOne using SYBR green master mix and primers for human RPL19 (Forward, 5'-ATGTATCACAGCCTGTACCTG-3'; Reverse, 5'-TTCTTGGTCTCTCTTCCTCCTTG-3') and MIP3α[5] (Forward, 5'-CCTGGGGGAATATTCTGGTGGTGA-3'; Reverse, 5'-CATCGCTGCCTTGGGTGTTGTAT-3'). RT-qPCR analysis was carried out using the comparative C_T Method ($\Delta\Delta C_T$ Method) as previously described[6] to estimate MIP3α mRNA levels relative to the reference RPL19 mRNA levels.

8. References

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