Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Splitting of β-carotene in the sexual interaction of *Phycomyces*

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Experimental Data

Strains and Culture Conditions

Strains of *Phycomyces blakesleeanus* Bgff: Strain NRRL1555, sexually *(–)*, is the standard wild-type; Strain NRRL1554, *(+)* is a different natural isolate. Both were obtained originally from the Northern Regional Research Laboratory (now National Center for Agricultural Utilization Research, Peoria, IL). Strain A56 is a *(+)* wild type derived from ten successive backcrosses into NRRL1555, and therefore largely consanguineous with this strain.[1] Strain C5 is a white mutant, isolated from a spore of NRRL1555 that had been exposed to N-methyl-N'-nitro-N-nitrosoguanidine.[2] Strain S342, genotype

[1]M. I. Alvarez and A. P. Eslava, *Genetics,* 1983, **105**, 873-879.

[2]E. Cerdá-Olmedo, *Meth. Enzymol.,* 1985, **110**, 220-243.

carB10 nicA101 (+), derived from crosses involving C5 and other strains, is white and auxotrophic for nicotinic acid.

Standard culture media and handling were used.[3] Plates containing 25 ml minimal agar were inoculated with 10^4 heat-activated spores each and incubated in the dark at 22 ºC for five days. Growth on the same agar enriched with yeast extract (1 g/L) did not sensibly modify the results. Mated cultures were incubated with 5 x 10^3 spores of each sex. Chemicals to be tested for biological effects were either added to the molten agar or placed in a well at the center of the plate.[4]

Extraction and fractionation of apocarotenoids

The initial extracts for apocarotenoid analyses were obtained by freezing (–20 ºC for at least 2 h) and thawing (22 ºC for 1 h) the media and centrifuging the liquid (4000 x *g*, 15 min). Neutral extracts were obtained by adjusting the initial extracts to pH 8.0 with KOH and extracting thrice with EtOAc. Acid extracts were obtained by adjusting the remaining aqueous phase to pH 2.0 with HCl and extracting with EtOAc. Water was removed by mixing with anhydrous Na_2SO_4 and filtering; the organic solvent was removed by evaporation under low pressure. For the sake of chemical stability, all procedures were carried out under dim light. Mated cultures (A56 and NRRL1555) yielded in the average 212 mg dry acid extract per L of medium; single cultures, 54 mg/L.

Chemical procedures.

NMR spectra (^{1}H, ^{13}C and 1D TOCSY) were recorded with Varian Direct-Drive 400 (^{1}H 400 MHz/^{13}C 100 MHz) and 500 (^{1}H 500 MHz/^{13}C 125 MHz) spectrometers. For high-resolution MS we used an Autospec-Q VG-Analytical (Fisons) mass spectrometer. GC/MS analyses were carried out in a Hewlett Packard 6890 chromatograph connected to a Hewlett-Packard 5988A mass spectrometer using

[3]E. Cerdá-Olmedo, in *Phycomyces*; ed. E. Cerdá-Olmedo, and E. D. Lipson, Cold Spring Harbor Laboratory: Cold Spring Harbor, New York, USA, 1987, pp 337-339.

[4]E. Cerdá-Olmedo and A. Hüttermann, *Angew. Botan.,* 1986, **60**, 59-70

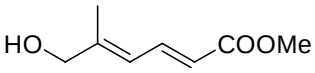
an ionization voltage of 70 eV. The GC conditions were: HP-1 methyl silicone capillary column (25 m x 0.2 mm); He 1.9 mL/min; the injection and detector heater temperature were 250 ºC and 280 ºC, respectively; the temperature was increased from 60 º to 300 ºC at 10 ºC/min. For semi-preparative normal-phase HPLC the neutral and methylated acid extracts were dissolved in *t*-BuOMe (at 20 g dry extract/L). Aliquots (0.5 mL) were injected into a column (10 by 250 mm; 5 µm silica particles; Agilent) with a 15 mm refillable guard pre-column filled with the same material in a Series 1100 liquid chromatograph (Agilent). The column was eluted at room temperature at a flow rate of 2 mL/min for 25 min with *t*-BuOMe and monitored with a refractometer. For the methylated acid extracts the eluent was *n*-hexane/*t*-BuOMe (1:4, v/v). Air- and water-sensitive reactions were performed under an argon atmosphere in flasks that had been flame-dried under an argon flow. The solvents were re-purified and stored under argon.

Isolation of 1 and 2 as methyl esters, 1m and 2m

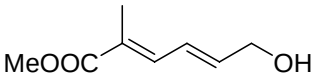
$TMSCHN_2$ 2M in Et_2O (0.3 mL) was added under stirring to a solution of the acid fraction (97 mg) in C_6H_6:MeOH (4:1 v/v) (2.8 mL) at 0 ºC. The solution was left for 5 min at room temperature and the solvent was evaporated at low pressure. The residue (104.3 mg) was fractionated by semi-preparative HPLC. The fraction with RT = 13.4 - 15.9 min contained a 2:1 mixture of **1m** and **2m** (19 mg).

*Methyl (2E,4E)-6-hydroxy-5-methylhexa-2,4-dienoate (**1m**) and methyl (2E,4E)-6-hydroxy-2-methylhexa-2,4-dienoate (**1b**)*: Colorless syrup. UV: λ_{max} = 252 nm (MeOH); HRFABMS *m/z* calcd for $C_8H_{12}O_3Na$ $[M+Na]^+$ 179.0681, found 179.0681.

*Methyl ester **1a***: EIMS (probe) 70 eV, m/z (rel. int.): 156 $[M]^+$ (25); δ_H (500 MHz, $CDCl_3$): 1.87 (3H, s, H-7), 3.74 (3H, s, OMe), 4.14 (2H, s, H-6), 5.87 (1H, d, J = 15.2 Hz, H-2), 6.25 (1H, d, J = 11.7 Hz, H-4), 7.59 (1H, dd, J = 11.7 and 15.2 Hz, H-3); δ_C (125 MHz, $CDCl_3$): 14.6 (CH_3, C-7), 51.6 (CH_3, OMe), 67.5 (CH_2, C-6), 120.4 (CH, C-2), 121.6 (CH, C-4), 140.3 (CH, C-3), 147.5 (C, C-5), 167.9 (C, C-1).



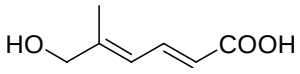
Methyl ester ***2m***: EIMS (probe) 70 eV, m/z (rel. int.): 156 $[M]^+$ (27); δ_H (500 MHz, $CDCl_3$): 1.95 (3H, s, H-7), 3.75 (3H, s, OMe), 4.29 (2H, d, *J* = 5.1 Hz, H-6), 6.17 (1H, dt, *J* = 5.1 and 15.1 Hz, H-5), 6.58 (1H, ddt, *J* = 1.5, 11.2 and 15.1 Hz, H-4), 7.18 (1H, d, *J* = 11.2 Hz, H-3); δ_C (125 MHz, $CDCl_3$): 12.7 (CH_3, C-7), 51.9 (CH_3, OMe), 63.1 (CH_2, C-6), 125.6 (CH, C-5), 127.3 (C, C-2), 137.6 (CH, C-4), 139.7 (CH, C-3), 169.0 (C, C-1).



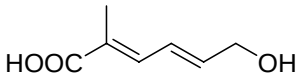
Saponification of 1m and 2m. Preparation of 1 and 2

The mixture (19 mg) of **1m** and **2m** was dissolved in EtOH (0.6 mL) and NaOH 1M (0.3 mL) was added dropwise at 0ºC. The mixture was then left at room temperature for 6.5 h. The solution was neutralized with HCl 1N (0.3 mL) and the solvent was evaporated under low pressure. The residue was extracted with EtOAc and the solvent was evaporated under low pressure to obtain a 2:1 mixture of **1** and **2** (15 mg, 87%).

*(2E,4E)-6-Hydroxy-5-methylhexa-2,4-dienoic acid (****1****)*: δ_H (500 MHz, $(CD_3)_2CO$): 1.86 (3H, s, H-7), 4.09 (2H, s, H-6), 5.86 (1H, d, *J* = 15.2 Hz, H-2), 6.34 (1H, br d, *J* = 11.7 Hz, H-4), 7.59 (1H, dd, *J* = 11.7 and 15.2 Hz, H-3); δ_C (125 MHz, $(CD_3)_2CO$): 13.6 (CH_3, C-7), 66.1 (CH_2, C-6), 119.9 (CH, C-2), 120.5 (CH, C-4), 140.3 (CH, C-3), 148.7 (C, C-5), 167.1 (C, C-1).



*(2E,4E)-6-Hydroxy-2-methylhexa-2,4-dienoic acid (****2****)*: δ_H (500 MHz, $(CD_3)_2CO$): 1.92 (3H, s, H-7), 4.24 (2H, d, *J* = 4.2 Hz, H-6), 6.25 (1H, dt, *J* = 4.2 and 15.1 Hz, H-5), 6.69 (1H, ddt, *J* = 1.8, 11.5 and 15.1 Hz, H-4), 7.23 (1H, d, *J* = 11.5 Hz, H-3); δ_C (125 MHz, $(CD_3)_2CO$): 11.7 (CH_3, C-7), 61.6 (CH_2, C-6), 125.2 (CH, C-5), 126.0 (C, C-2), 137.8 (CH, C-4), 141.7 (CH, C-3), 168.1 (C, C-1).

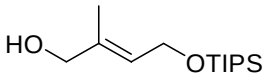


Preparation of (2*E*,4*E*)-6-hydroxy-5-methylhexa-2,4-dienoic acid (1)

*Preparation of the allylic alcohol **7**.* Imidazole (986 mg, 14.5 mmol) and TIPSCl (1335 mg, 6.83 mmol) were added to a solution of 3-methyl-2-buten-1-ol (**6**, 500 mg, 5.81 mmol) in dry DMF (1 mL) and stirring at room. After 50 min, the mixture was fractionated in *t*-BuOMe:H_2O (2:1). The organic phase was washed successively with HCl 2N and brine and dried over anhydrous Na_2SO_4. Once the solvent was evaporated under low pressure we obtained a crude product (1.32 g), which was dissolved in dry CH_2Cl_2 (19 mL) and chilled at 0ºC. After adding SeO_2 (0.35 g) and *t*-BuOOH 6M (1.1 mL) the solution was stirred at room temperature for 45 min, diluted in CH_2Cl_2 (30 mL), washed with brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated under low pressure to afford a crude product, which was chromatographed over silica gel column to obtain **7** (hexane/*t*- BuOMe, 1:1, 554 mg, 42%) and **8** (hexane/*t*- BuOMe, 93:7, 713 mg, 54%).

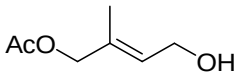
$NaBH_4$ (530 mg) was added to a solution of **8** (700 mg, 2.73 mmol) in dry MeOH (53 mL) at room temperature and stirring for 20 min. The solvent was evaporated under low pressure and the residue was suspended in water (50 mL) and extracted with *t*-BuOMe. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, and filtered. The residue obtained after evaporation of the solvent under low pressure was chromatographed in a silica gel column (hexane:*t*-BuOMe, 1:1) to obtain **7** (593 mg, 84%).

*(E)-4-Triisopropylsilyloxy-2-methylbut-2-en-1-ol (**7**)*: Colorless syrup. δ_H (500 MHz, $CDCl_3$): 1.04-1.18 (21H, m, TIPS), 1.66 (3H, s, H-5), 4.01 (2H, s, H-1), 4.30 (2H, d, J = 6.0 Hz, H-4), 5.59 (1H, tq, J = 1.2 and 6.0 Hz, H-3); δ_C (125 MHz, $CDCl_3$): 12.0 (3CH, Si(*CH*Me_2)$_3$), 13.8 (CH_3, *Me*C-2), (6CH_3, Si(CH*Me$_2$)$_3$*) 60.1 (CH_2, C-4), 68.3 (CH_2, C-1), 125.7 (CH, C-3), 135.6 (C, C-2); HRFABMS *m/z* calcd for $C_{14}H_{30}O_2SiNa$ $[M+Na]^+$ 281.1913, found 281.1912.



Preparation of the hydroxy-acetate ***9***. A mixture of **7** (231 mg, 0.89 mmol), dry pyridine (2 mL) and acetic anhydride (1.5 mL) was left at room temperature for 30 min and then was worked up as usual to give a crude product (258 mg), which was dissolved in dry THF (12 mL), mixed with 1M TBAF in THF (2.5 mL), stirred at room temperature for 90 min, diluted with Et_2O (15 mL), and washed with brine. The organic phase was dried over anhydrous Na_2SO_4 and filtered. The residue obtained after removing the solvent under low pressure was chromatographed in a silica gel column (hexane:*t*-BuOMe, 1:1) to obtain **7** (154 mg, 66.5%).

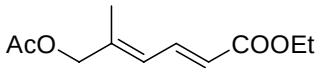
*(E)-4-Hydroxy-2-methylbut-2-enyl acetate (****9****)*: Colorless syrup. δ_H (500 MHz, $CDCl_3$): 1.70 (3H, s, H-5), 2.00 (3H, s, $COCH_3$), 4.22 (2H, d, J = 6.9 Hz, H-4), 4.48 (2H, s, H-1),5.67 (1H, tq, J = 1.1 and 6.9 Hz, H-3); δ_C (125 MHz, $CDCl_3$): 14.1 (CH_3, *Me*C-2), 21.0 (CH_3, CO*CH*$_3$) 59.1 (CH_2, C-4), 69.0 (CH_2, C-1), 127.1 (CH, C-3), 133.5 (C, C-2), 170.9 (C, *CO*CH_3); HRFABMS *m/z* calcd for $C_7H_{12}O_3Na$ $[M+Na]^+$ 167.1582, found 167.1583.



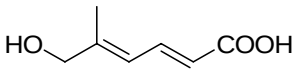
Preparation of the acetoxy-ester ***10***. The Dess-Martin reagent (152 mg) was added to a solution of **9** (150 mg, 1.04 mmol) in CH_2Cl_2 (5 mL) at room temperature. The mixture was left for 1 h under stirring. A saturated solution of $Na_2S_2O_3$ and $NaHCO_3$ was then added dropwise to the mixture and extracted with Et_2O. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, and filtered. The residue obtained after evaporating the solvent under low pressure was dissolved in petroleum ether (bp 30-40 ºC):Et_2O (3:1) and filtered through silica gel. The residue (119 mg) obtained after evaporating the solvent under low pressure was dissolved in dry THF (1.6 mL) and added to a suspension of NaH (45 mg) in dry THF (6.5 mL) to which had been added dropwise triethylphosphonoacetate (315 μL) at room temperature. The mixture was stirred for 5 min, diluted in Et_2O (15 mL). The organic phase was washed

successively with water and brine, dried over anhydrous Na_2SO_4 and filtered; the residue obtained after removing the solvent under low pressure was chromatographed in a silica gel column petroleum ether (bp 30-40 ºC):Et_2O (9:1) to obtain **10** (108 mg, 72%).

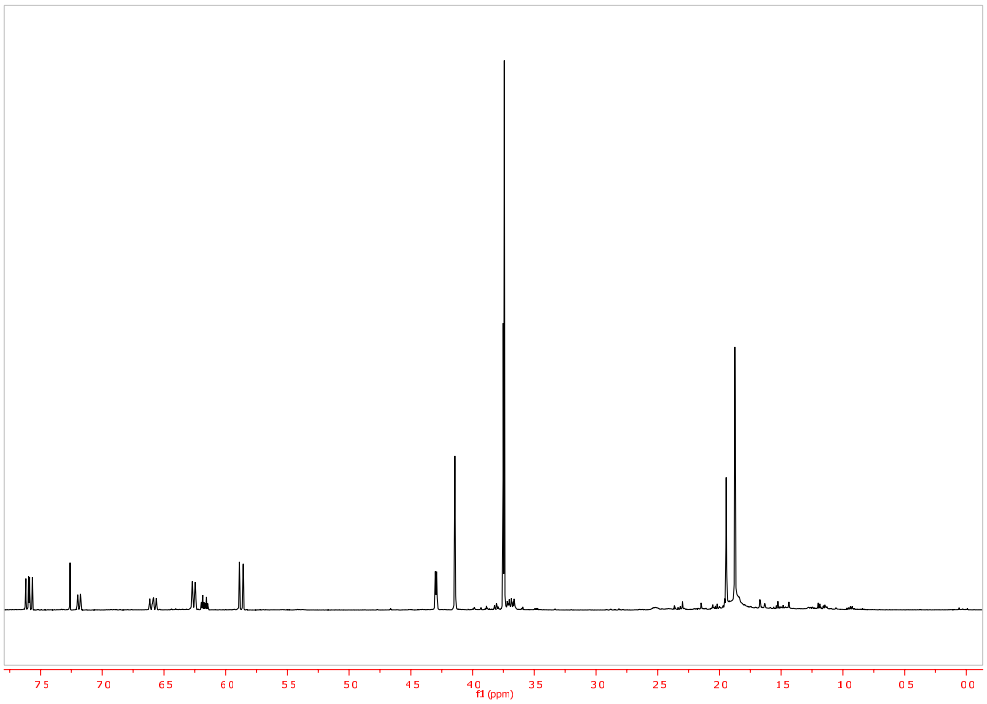
*Ethyl (2E,4E)-6-acetoxy-5-methylhexa-2,4-dienoate (**10**):* Colorless syrup. δ_H (400 MHz, $CDCl_3$): 1.30 (3H, t, *J* = 7.2 Hz, OCH_2CH_3), 1.90 (3H, s, H-7), 2.11 (3H, s, $COCH_3$), 4.21 (2H, q, *J* = 7.2 Hz, OCH_2CH_3), 4.57 (2H, s, H-6), 5.90 (1H, d, *J* = 15.2 Hz, H-2), 6.18 (1H, d, *J* = 11.6 Hz, H-4), 7.55 (1H, dd, *J* = 11.6 and 15.2 Hz, H-3); δ_C (100 MHz, $CDCl_3$): 14.4 (CH_3, C-7)[a], 15.0 (CH_3, OCH_2CH_3)[a], 20.9 (CH_3, $COCH_3$), 60.5 (CH_2, OCH_2CH_3), 68.5 (CH_2, C-6), 122.0 (CH, C-2), 124.6 (CH, C-4), 139.4 (CH, C-3), 141.7 (C, C-5), 167.2 (C, C-1), 170.7 (C, $COCH_3$), ([a]Signals with the same letter are exchangeable); HRFABMS *m/z* calcd for $C_{11}H_{16}O_4Na$ $[M+Na]^+$ 235.0947, found 235.0945.



*Saponification of **10***. The procedure to obtain the mixture of **1** and **2** was applied to a solution of **10** (90 mg, 0.42 mmol) in EtOH (4 mL) to obtain the hydroxyacid **1** (74 mg, 82%).



a)



b)

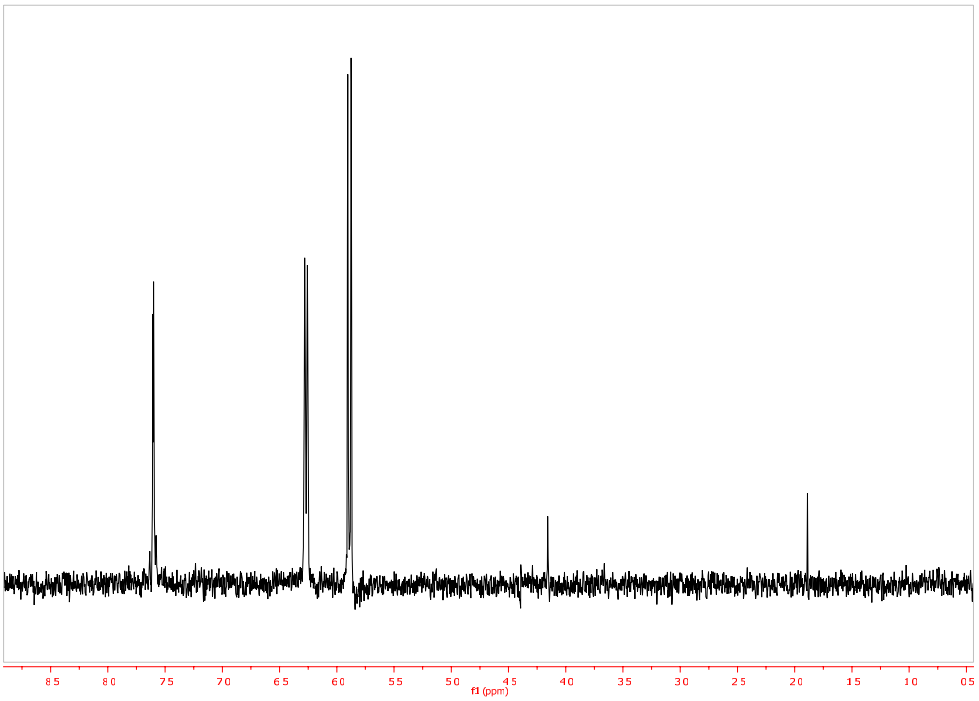


Figure S1. 500 MHz spectra of mixture of the methyl esters of **1** and **2** in CD_3Cl. a) Conventional ^1H NMR spectrum. b) 1D TOCSY experiment.

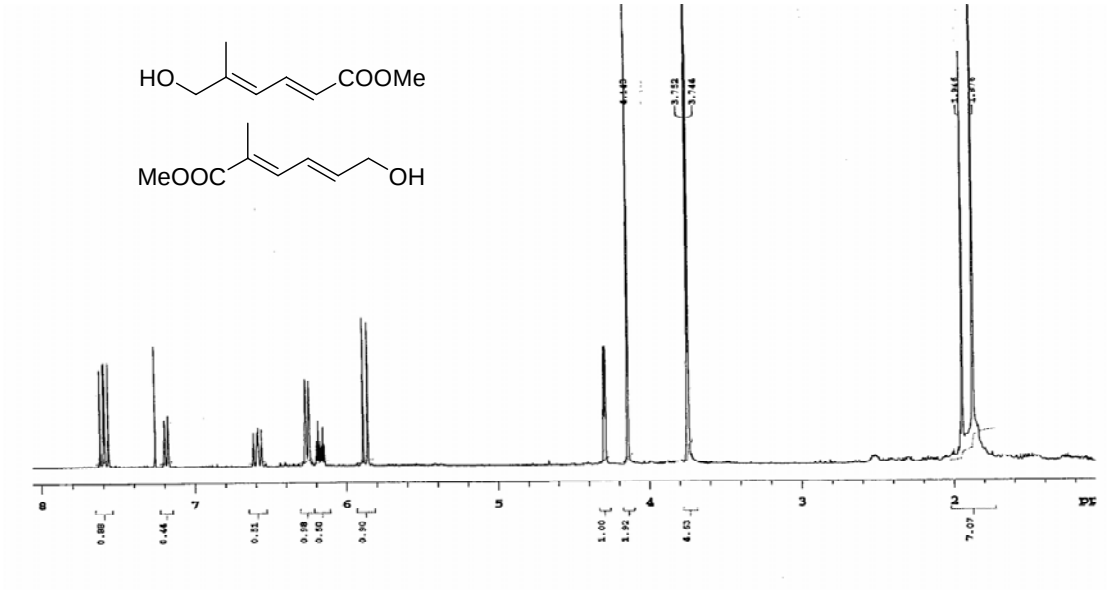


Figure S2. ^{1}H NMR spectrum of the **1m** and **2m** mixture.

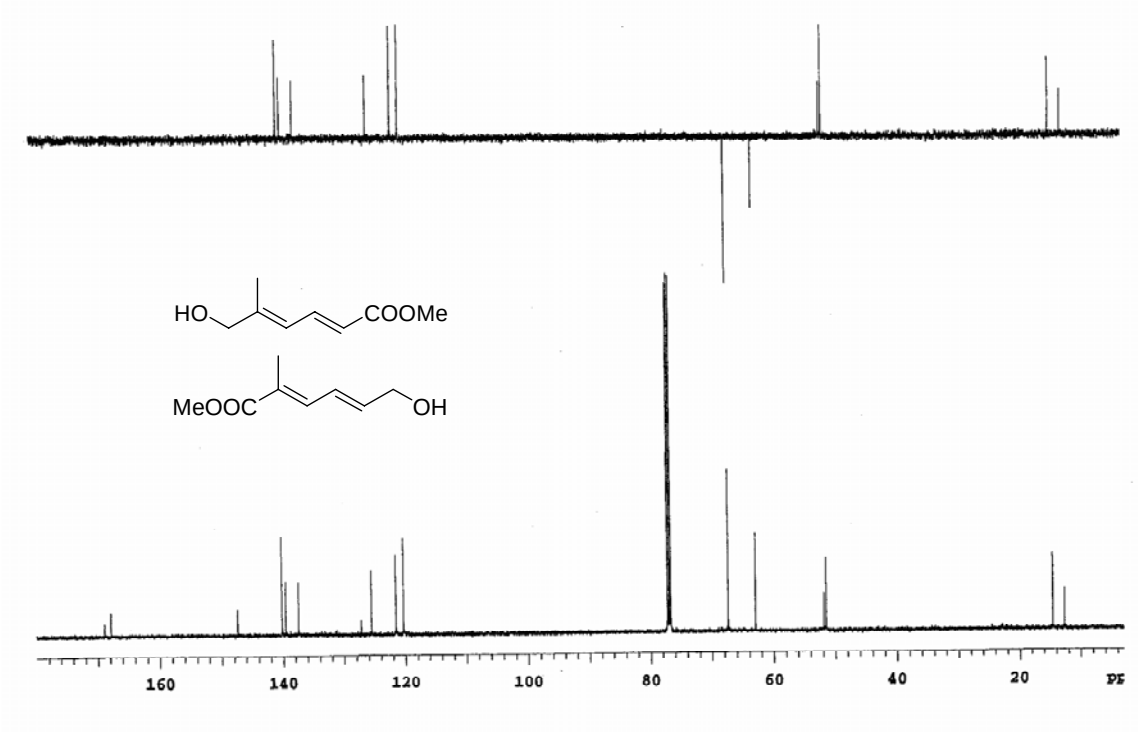


Figure S3. ^{13}C NMR spectrum of the **1m** and **2m** mixture.

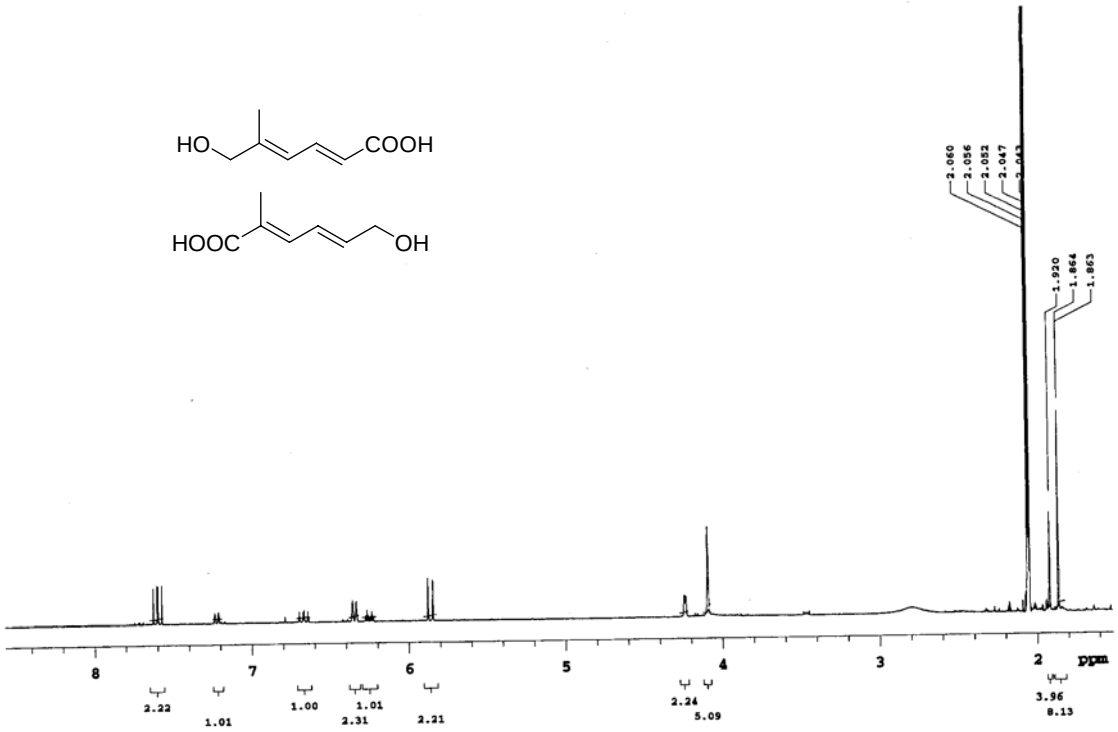


Figure S4. ^{1}H NMR spectrum of the **1** and **2** mixture.

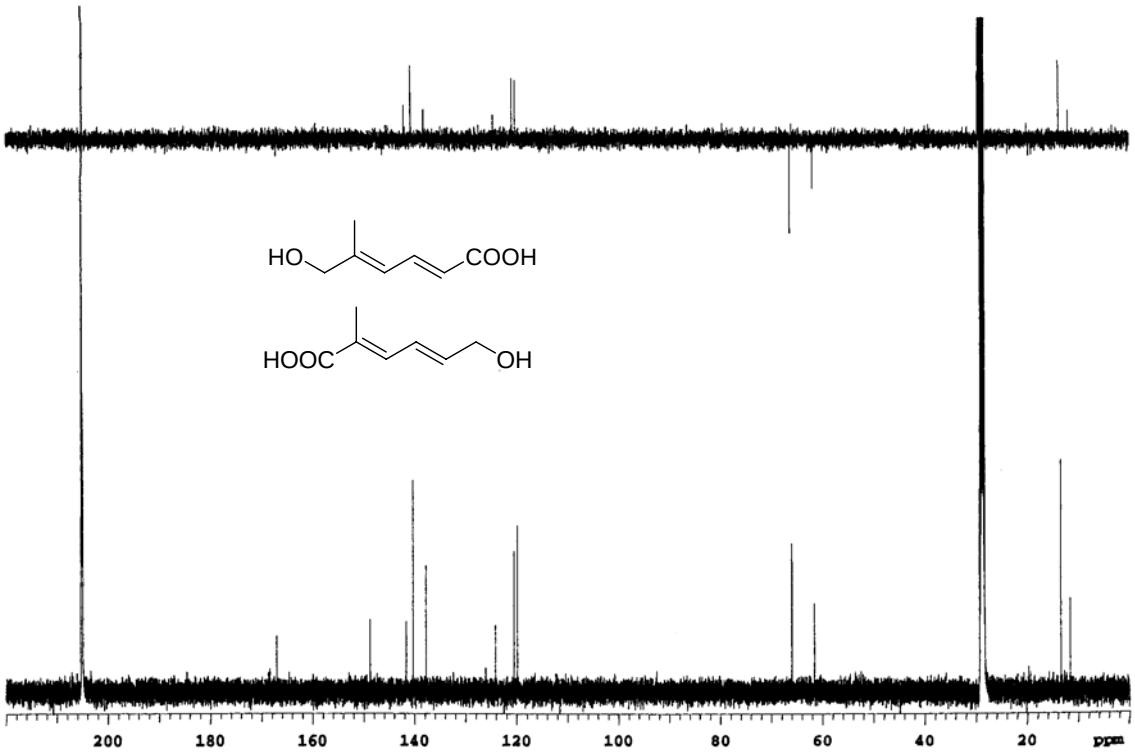


Figure S5. ^{13}C NMR spectrum of the **1** and **2** mixture.

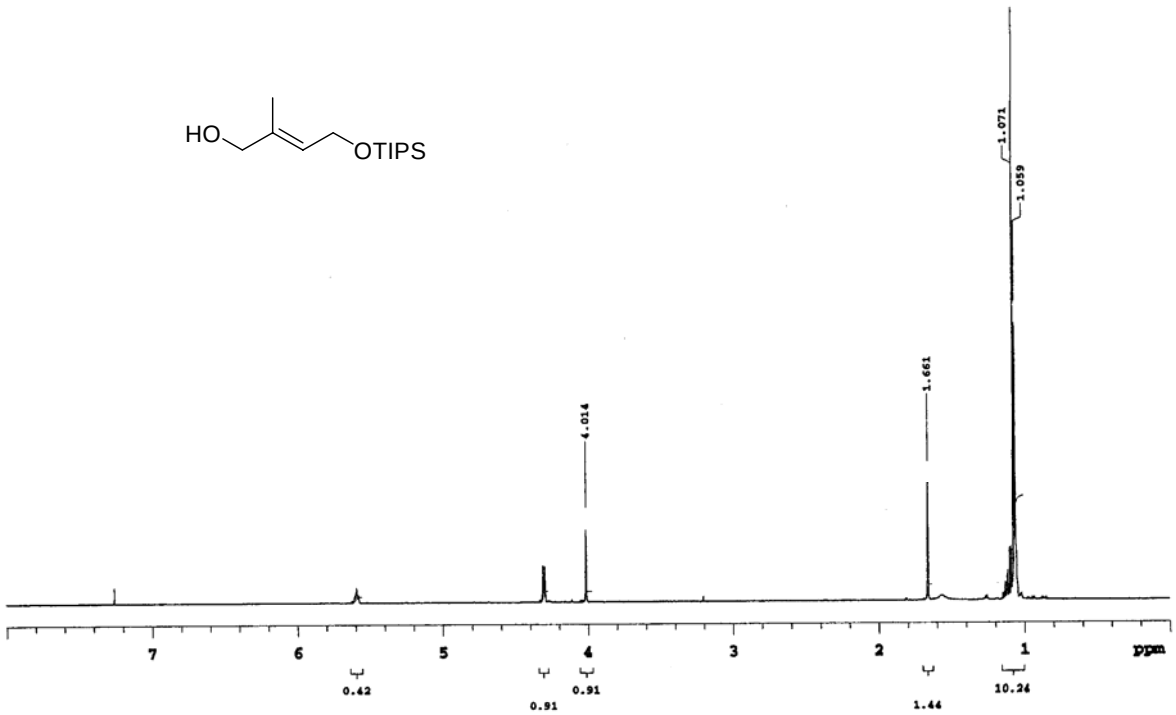


Figure S6. ^{1}H NMR spectrum of **7**.

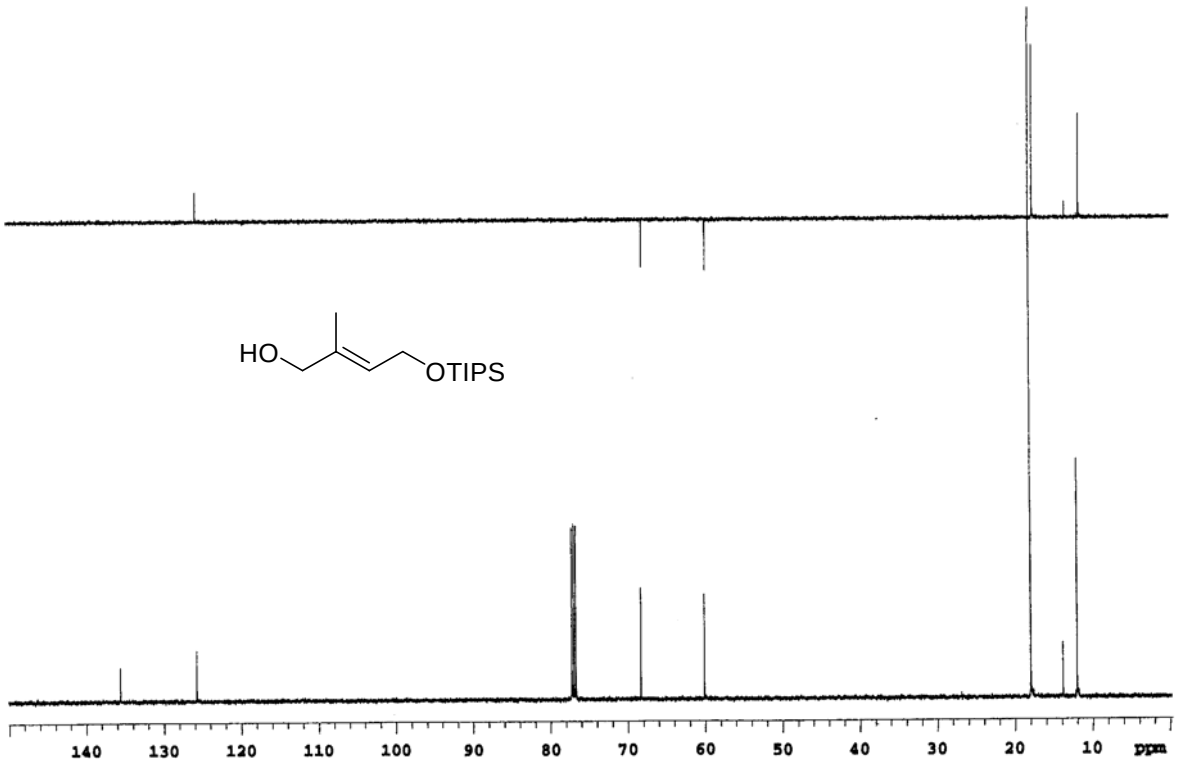


Figure S7. ^{13}C NMR spectrum of **7**.

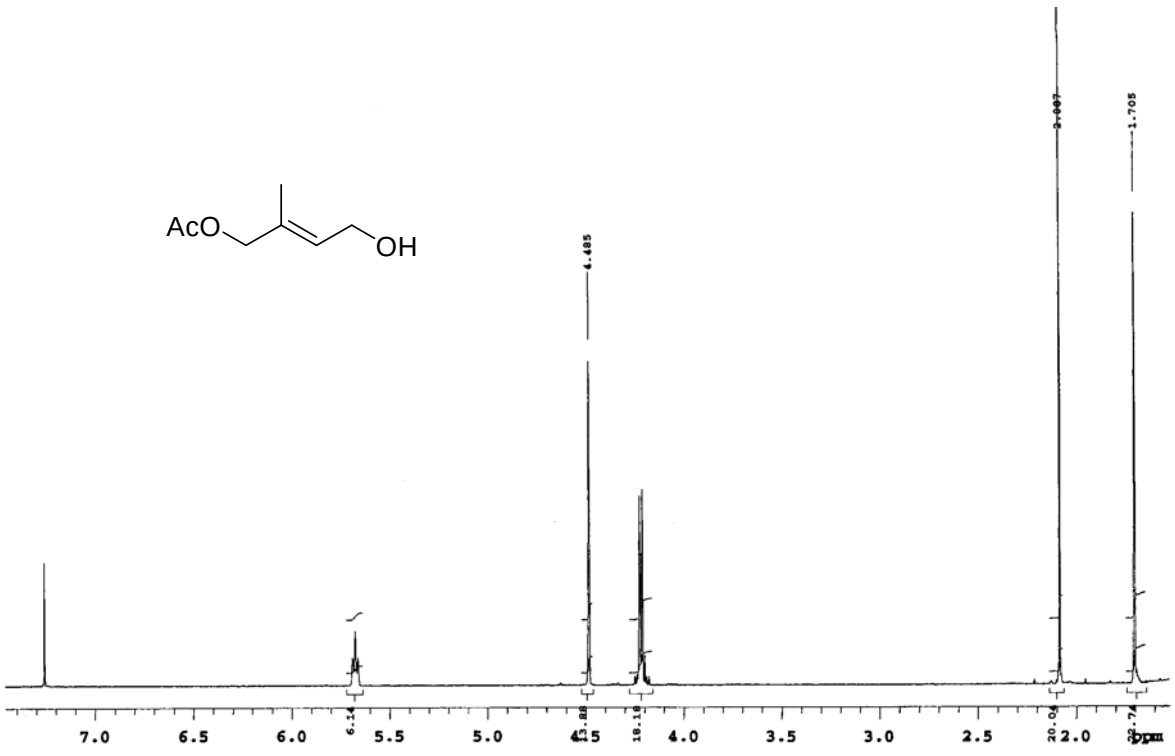


Figure S8. ^{1}H NMR spectrum of **9**.

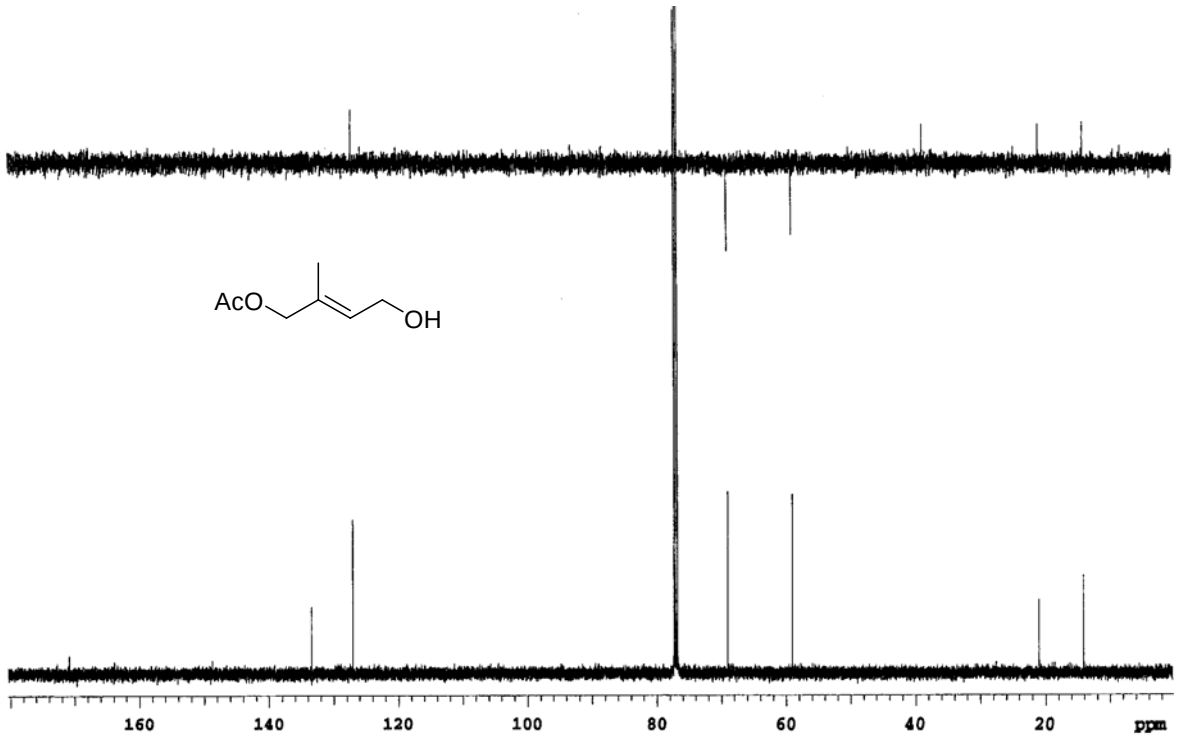


Figure S9. ^{13}C NMR spectrum of **9**.

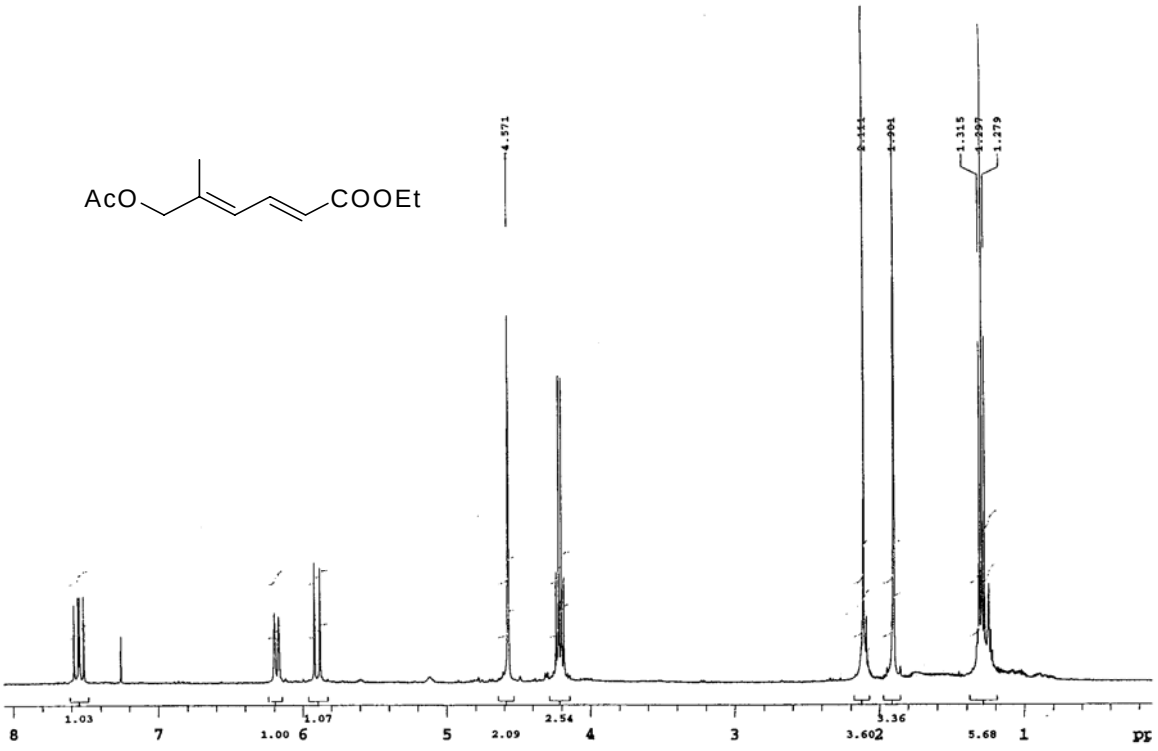


Figure S10. ^{1}H NMR spectrum of **10**.

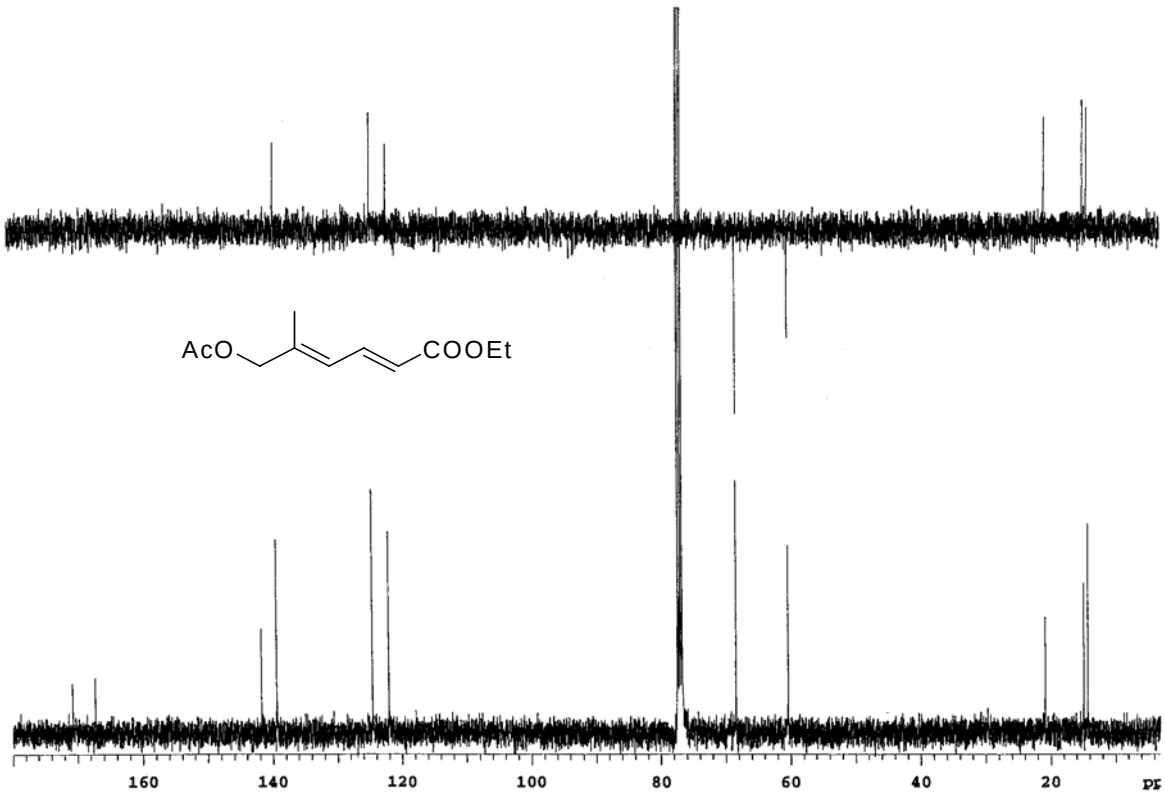


Figure S11. ^{13}C NMR spectrum of **10**.

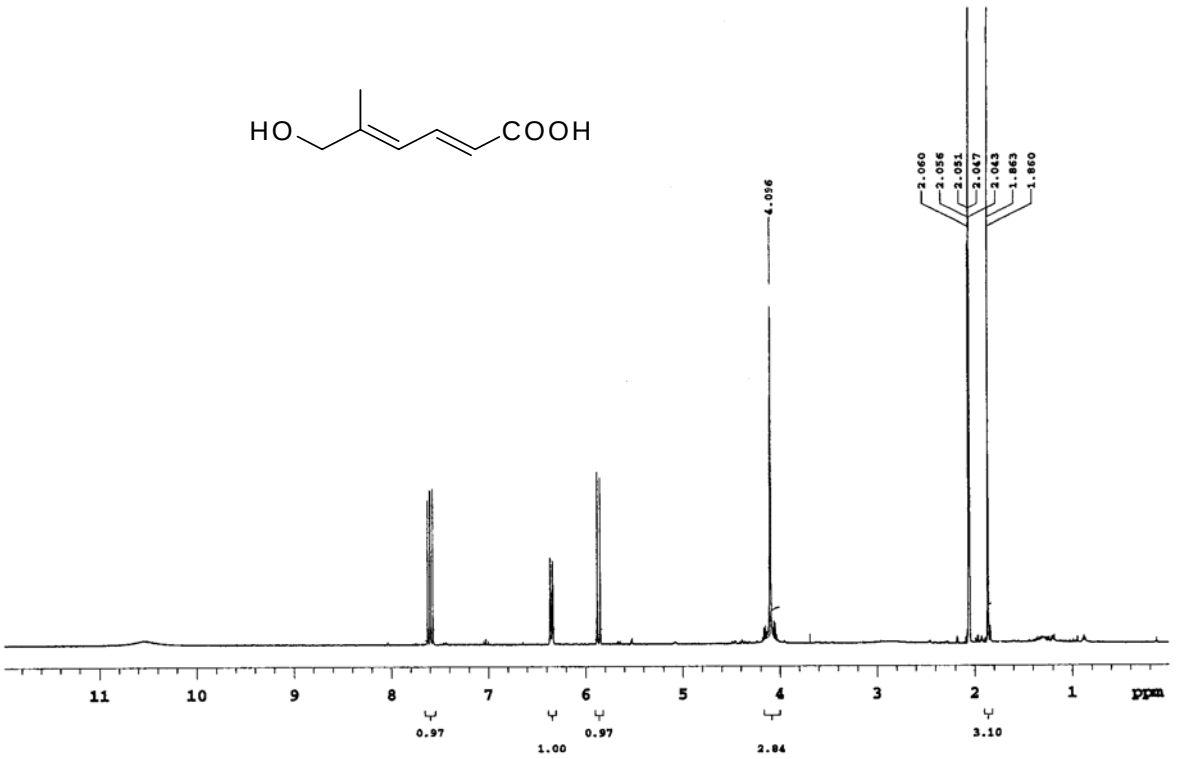


Figure S12. ^{1}H NMR spectrum of **1**.

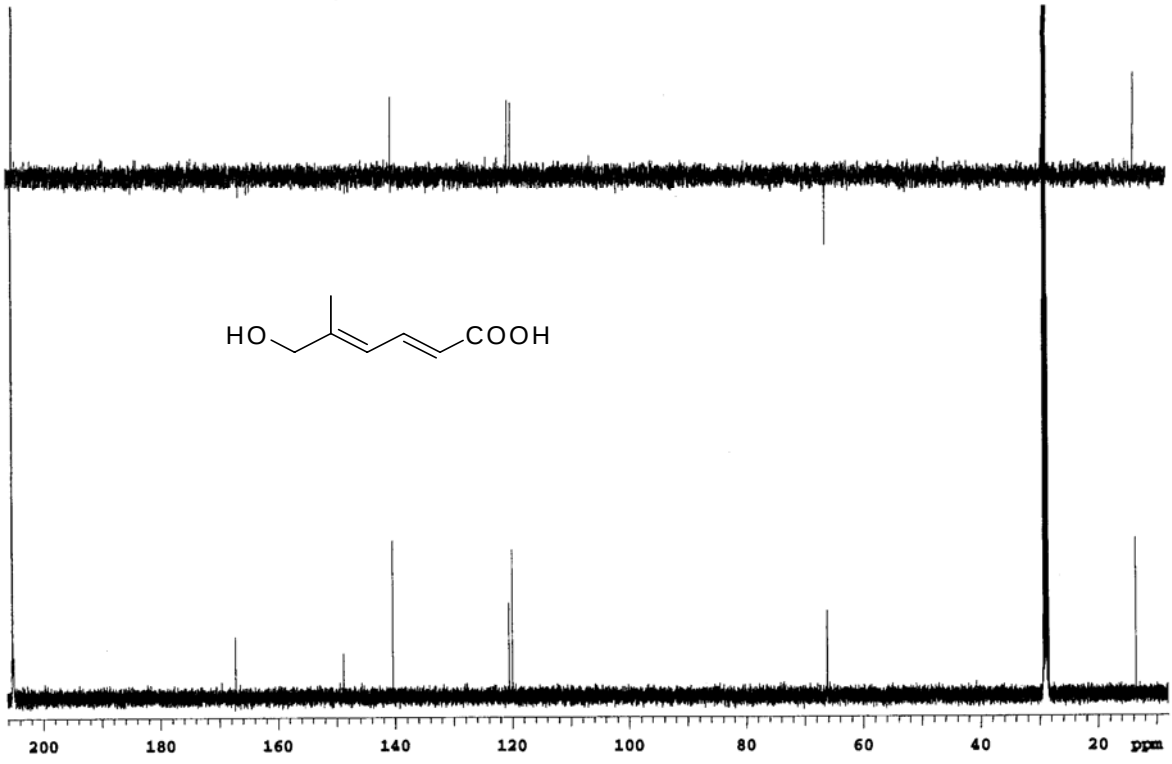


Figure S13. ^{13}C NMR spectrum of **1**.