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

Conformationally restricted 3′-modified ABA analogs for controlling ABA receptors

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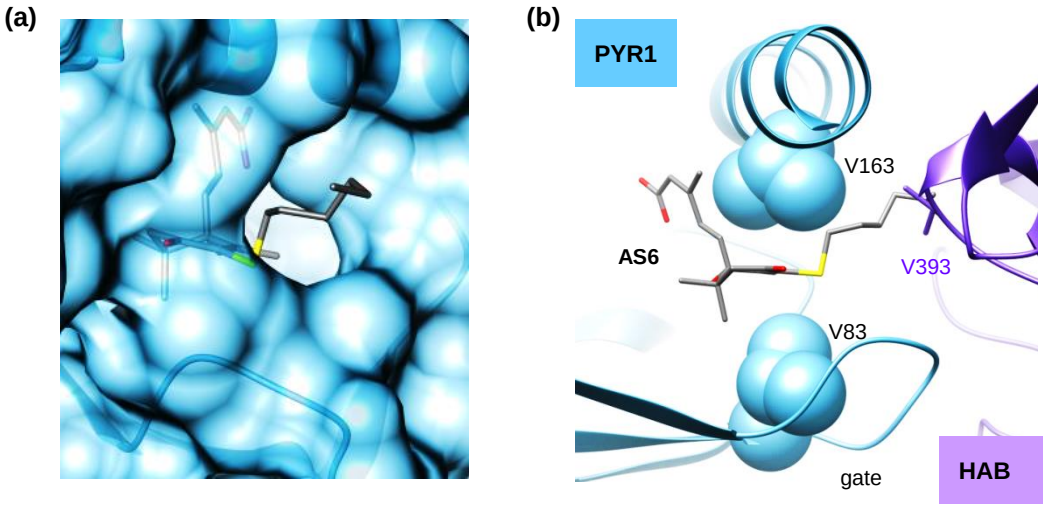
Supporting Information

1) Supplementary Figures

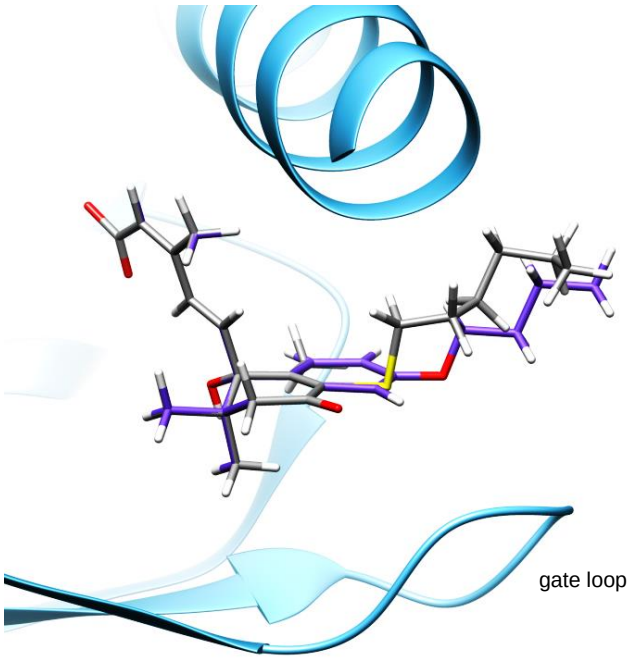
2) ^{1}H and ^{13}C NMR Spectrums of Synthesized Compounds

1) Supplementary Figures

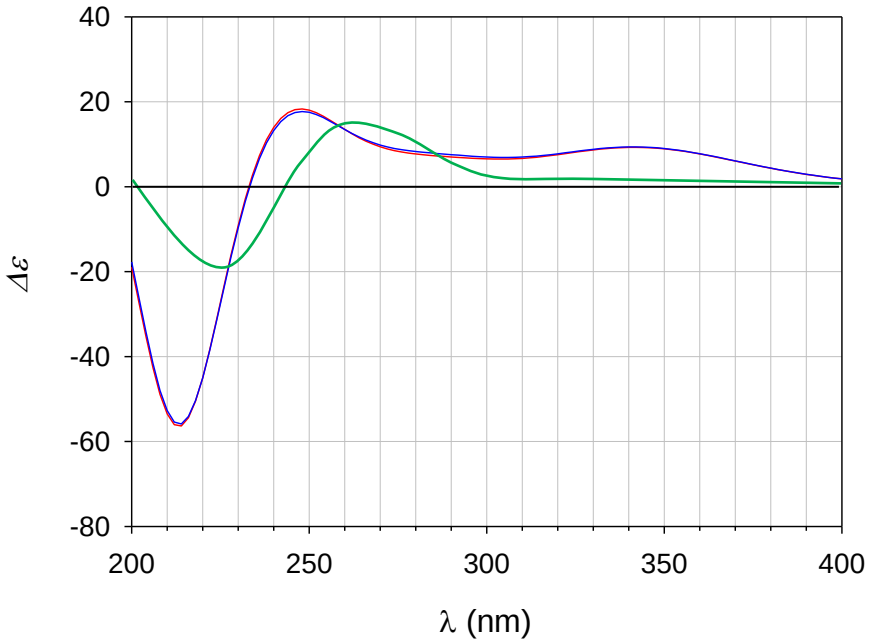


Supplementary Figure 1 The 3′-*S*-hexyl chain of AS6 protrudes through the narrow 3′-tunnel and blocks PP2C binding with no inhibiting the conformational change of PYL into the gate-closed form. (a) Crystal structure of PYR1-AS6 complex (PDB code 3WG8). The solvent-excluded surface (probe radius: 1.4 Å) prepared with Chimera software*. (b) Superposition of the PYR1-AS6 complex and the PYR1-ABA-HAB1 (purple, PDB code 3QN1) complex.

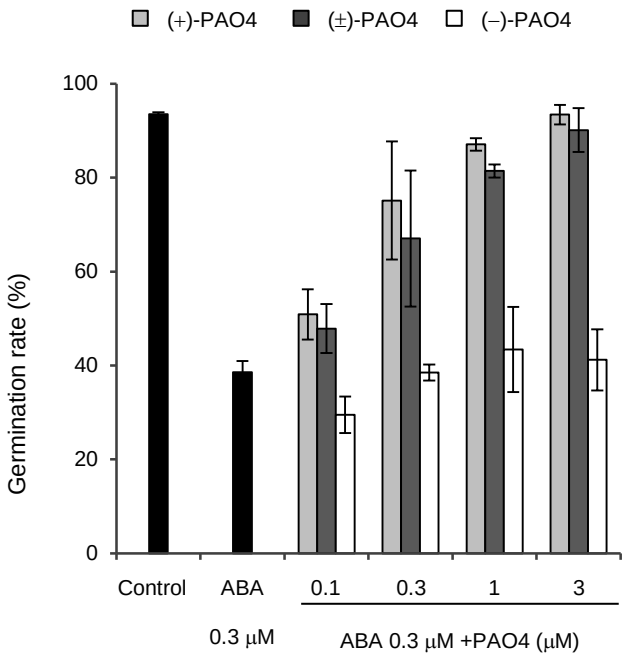
* E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng and T. E. Ferrin, *J. Comput. Chem.*, 2004, **25**, 1605–12.



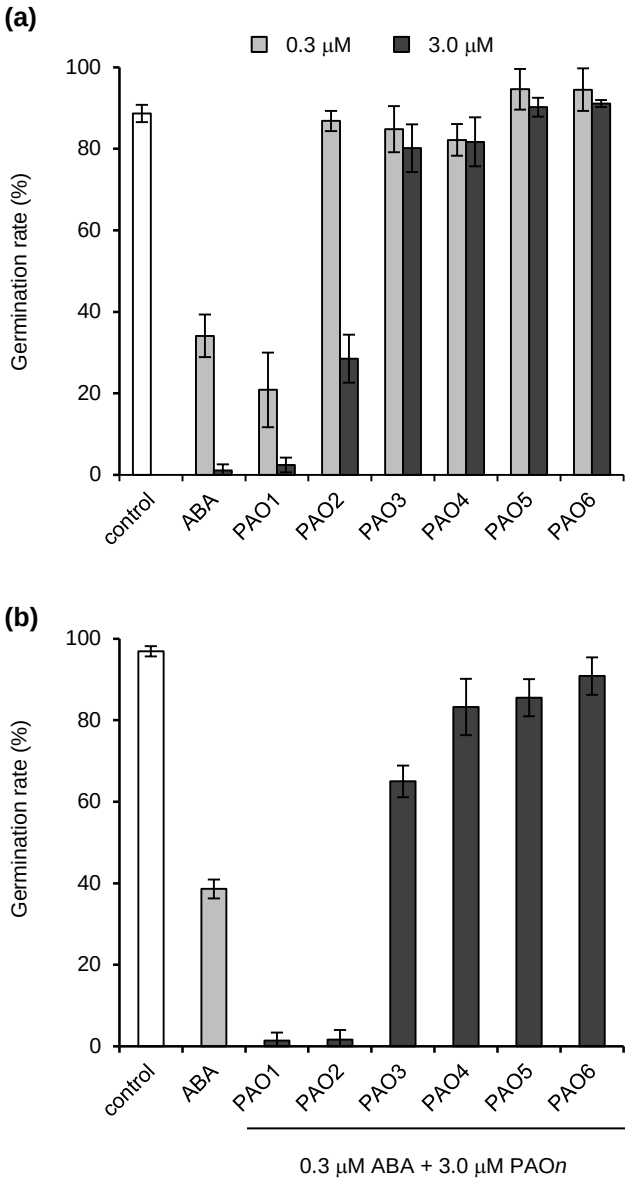
Supplementary Figure 2 Superposition of (+)-PAO4 and AS6 in the PYR1-AS6 complex (3WG8). AS6, gray sticks and (+)-PAO4, purple sticks.



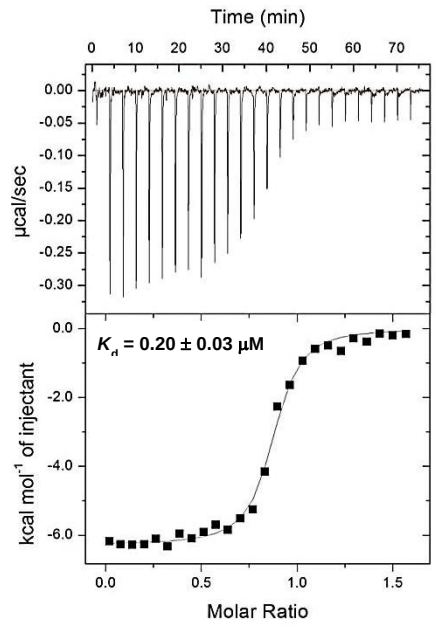
Supplementary Figure 3 Experimental CD spectrum of (+)-PAO4 in MeOH (green line) and theoretical CD curves of *S*-PAO4 calculated in the velocity (red line) and length (blue line) formalisms. The theoretical curves were obtained at the TDDFT/B3LYP/6-31G*, taking into account the 30 lowest energy transitions and assuming a Gaussian distribution with σi = 0.2 eV.



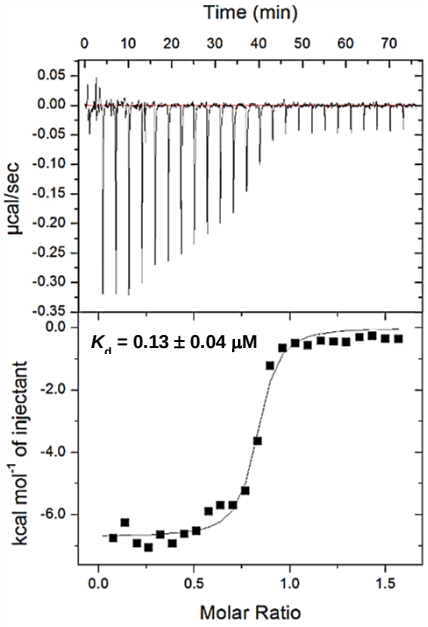
Supplementary Figure 4 Comparison of the activity of (+)-PAO4 and its racemate. *Arabidopsis* seed germination rate in response to 0.3 μM ABA and (+)-PAO4 or its racemate applied when the germination rate of ABA-treated plants was 40% (*n* = 3; error bars represent s.d.).



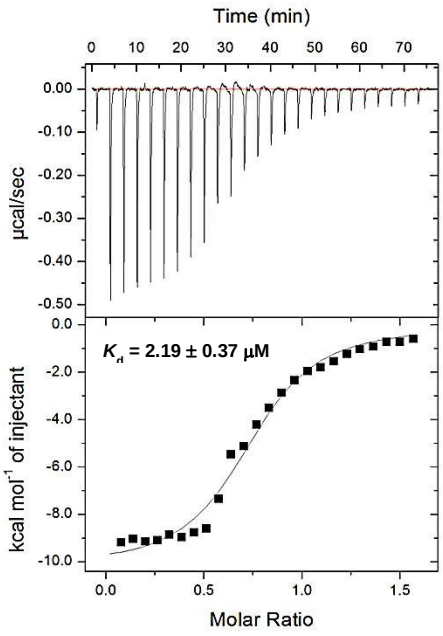
Supplementary Figure 5 Effect of (±)-PAO*n* compounds on *Arabidopsis* seed germination. (a) Seed germination rate in response to (±)-PAO*n* at 48 h after stratification (*n* = 3; error bars represent s.d.). (b) Seed germination rate in response to 0.3 μM ABA and 0.3 or 3.0 μM (±)-PAO*n* applied when the germination rate of ABA-treated plants was 40% (*n* = 3; error bars represent s.d.).



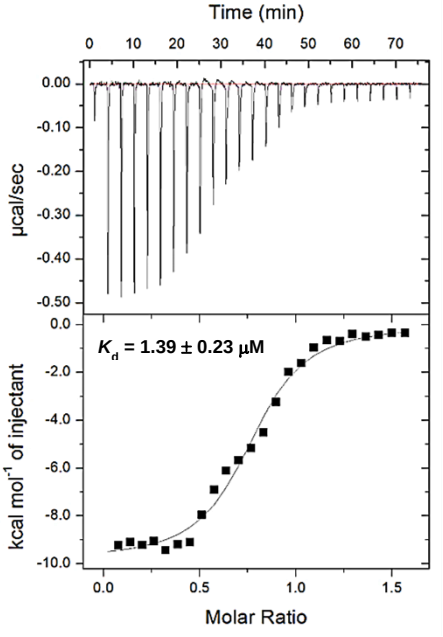
［(+)-PAO1/PYL5］



［(+)-PAO4/PYL5］



［(+)-PAO1/PYL10］



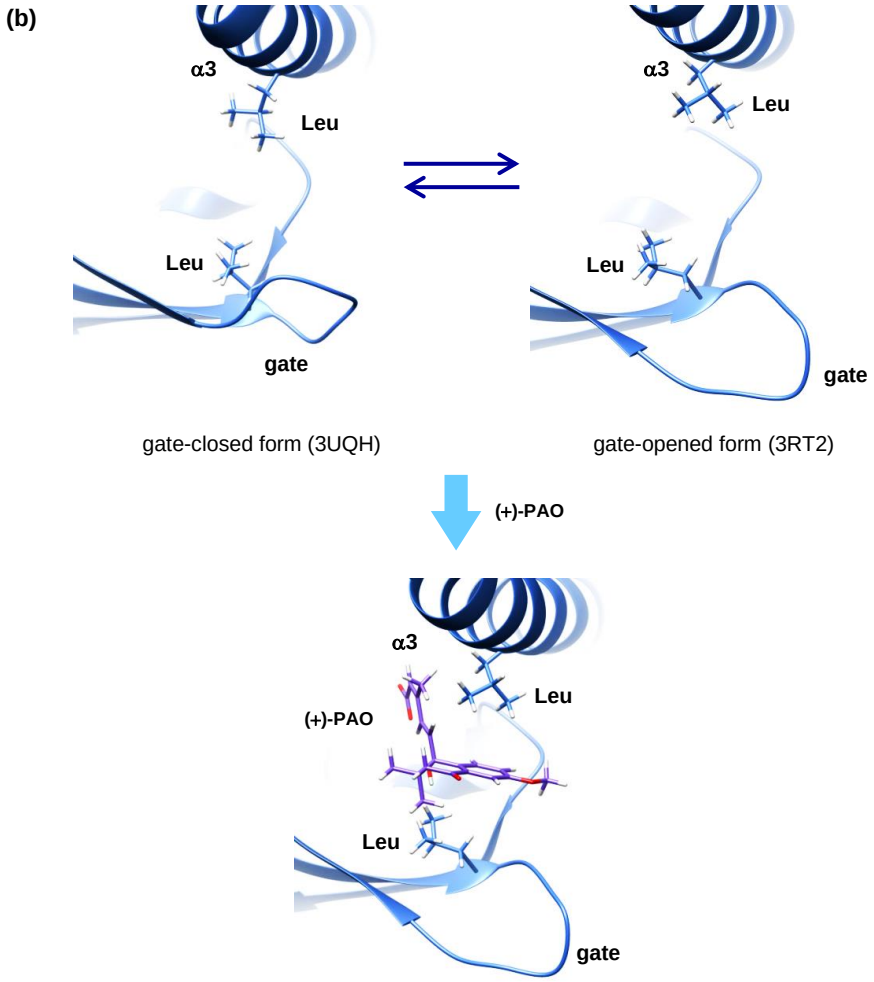
［(+)-PAO4/PYL10］

Supplementary Figure 6 Isothermal titration calorimetry profiles and thermodynamic data for (+)-PAO4-PYLs binding experiments. The data were corrected by subtraction the mixing enthalpies of (+)-PAO1 and (+)-PAO4 solution into protein-free solution and fitted by Origin for ITC with 1:1 binding model.

(a)

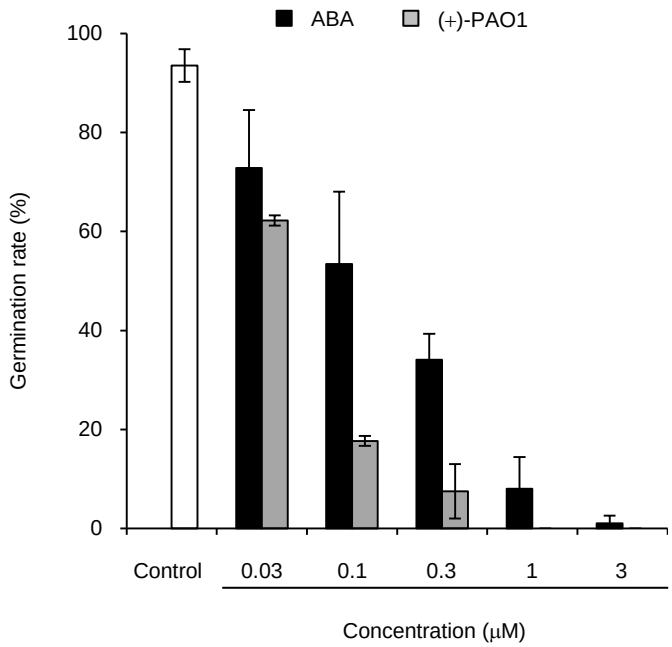
Amino acid (PYR1 #)	PYR1	PYL1	PYL2	PYL3	PYL4	PYL5	PYL6	PYL7	PYL8	PYL9	PYL10	PYL11	PYL12	PYL13
83[a]	V	V	V	V	V	V	V	V	V	V	**L**	V	V	L
163[b]	V	V	V	V	I	I	I	L	L	L	L	I	I	I

[a] The residue at the N-terminal of the gate loop region
[b] The residue at the α3 helix

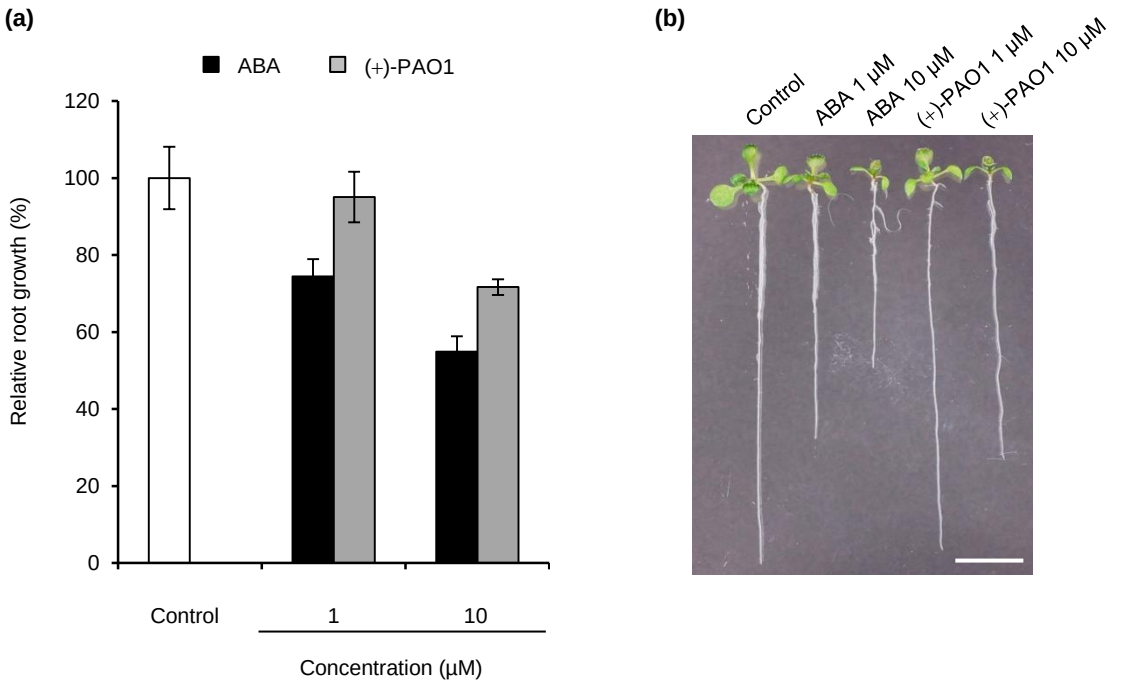


A model of PYL10-(+)-PAO1 complex.
(+)-PAO1 is inserted into PYL10 gate-opened form (3RT2).

Supplementary Figure 7 The complicated profile of (+)-PAO1 may be attributed to interactions between the 3'-substituent and the residues comprising the 3'-tunnel of PYLs. (a) Sequence alignment at the N-terminal of the gate loop and α3 helix of PYL subtypes. (b) (+)-PAO1 may stabilize the inactive gate-opened form of PYL10 by interactions with two Leu residues, that is, it may functions as an inverse agonist for PYL10.



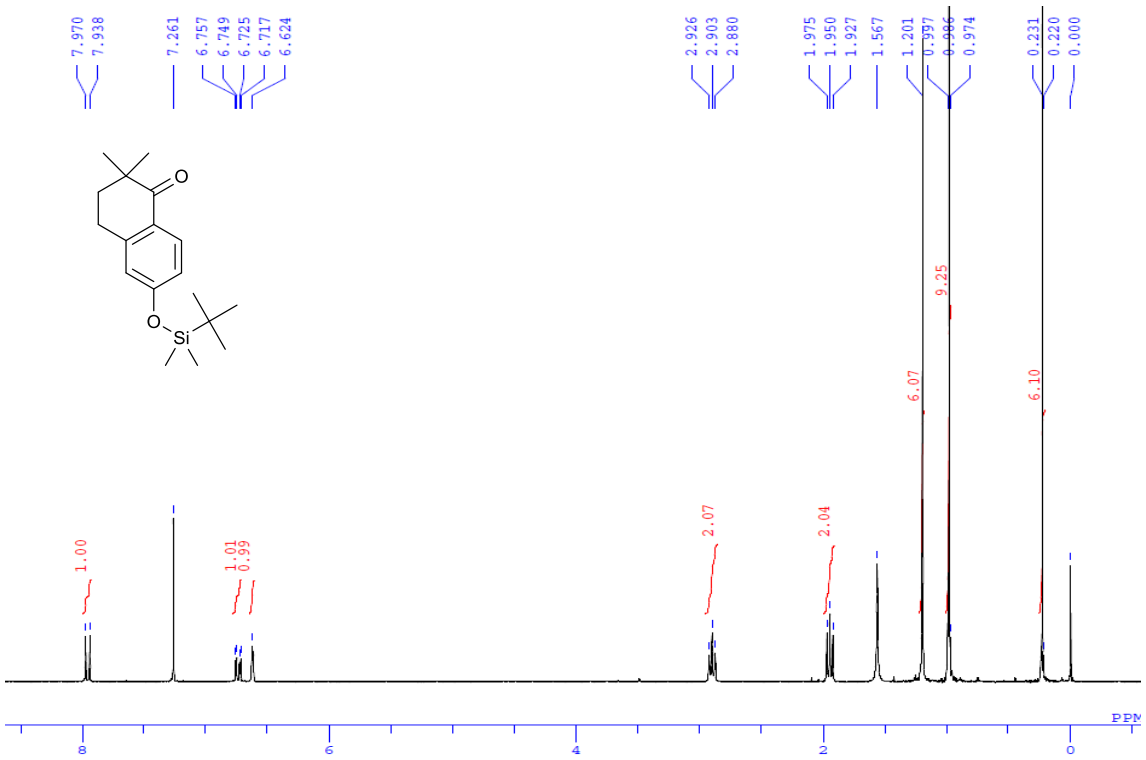
Supplementary Figure 8 Effect of (+)-PAO1 of *Arabidopsis* seed germination compared with that of ABA. *Arabidopsis* seed germination rate in response to (+)-PAO1 or ABA at 48 h after stratification (n = 3; error bars represent s.d.).



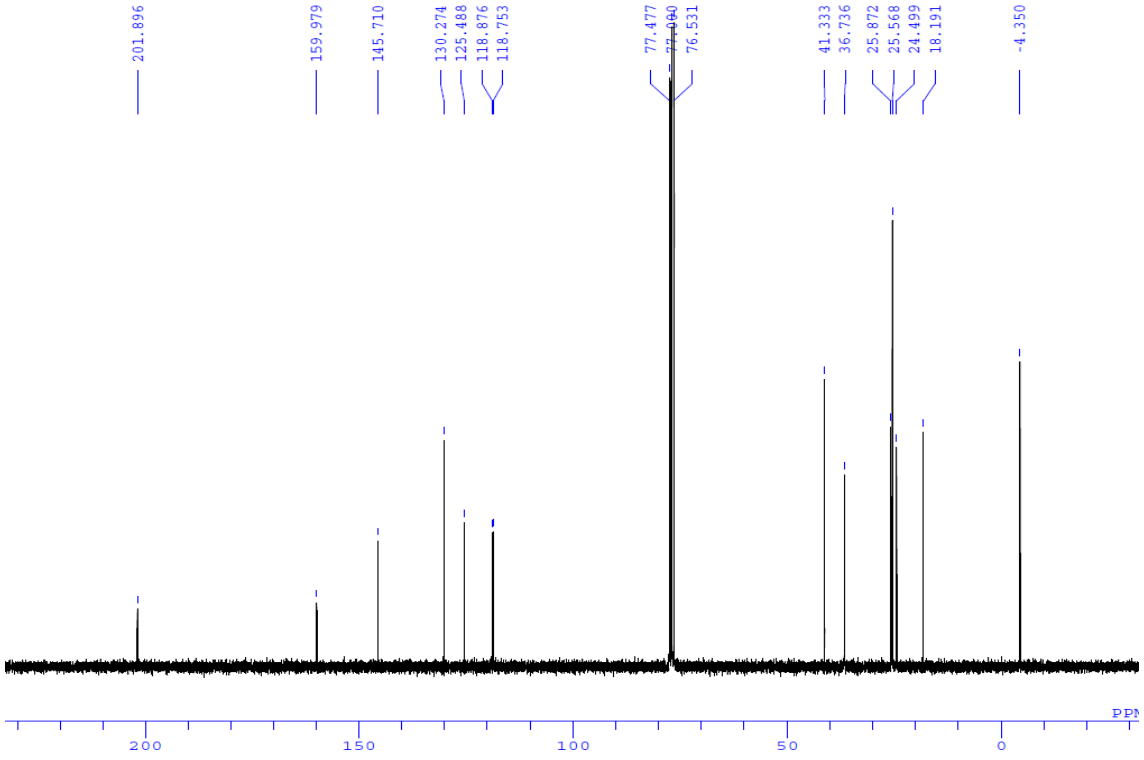
Supplementary Figure 9 Effect of (+)-PAO1 on *Arabidopsis* root growth compared with that of ABA. (a) Primary root lengths of 12 plants were measured after 7 d in medium lacking or supplemented with (+)-PAO1 or ABA. Data are averages from three independent experiments (n = 12 each; error bars represent s.d.). (b) The photograph shows representative seedlings 7 d after the transfer of 5-d-old seedlings from 1/2 MS medium to plates supplemented with (+)-PAO1 or ABA (scale bar represents 10 mm).

2) ^{1}H and ^{13}C NMR Spectrums of Synthesized Compounds

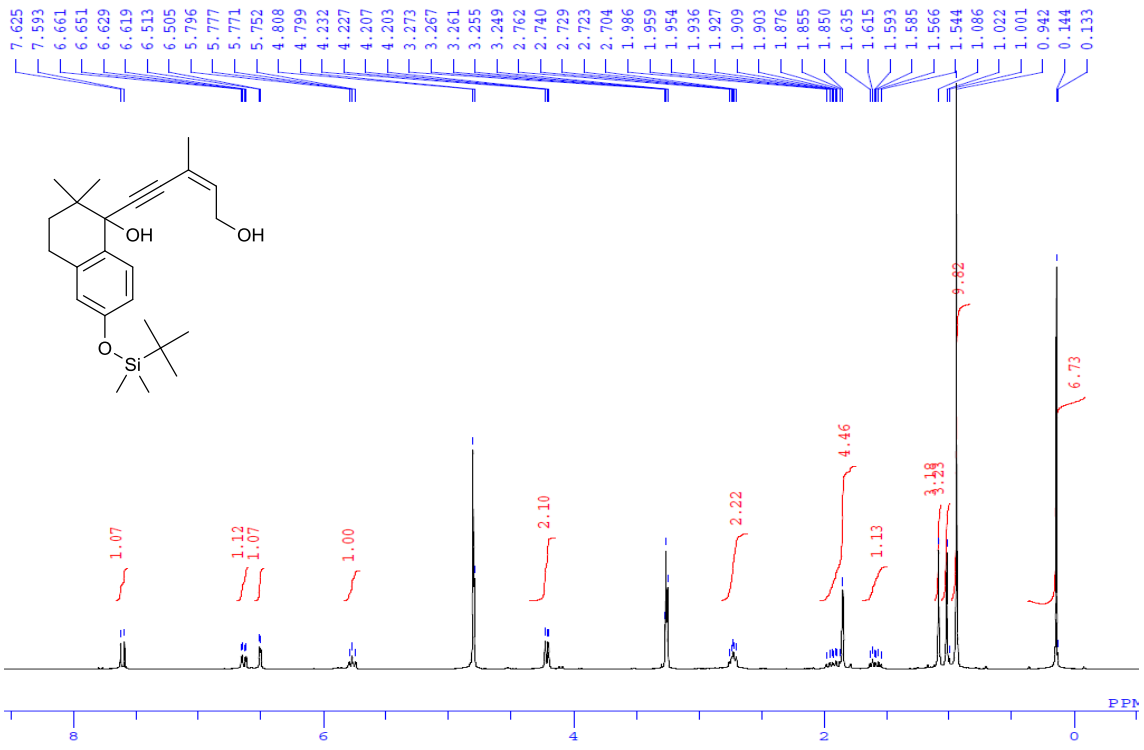
^{1}H NMR spectrum of Compound # **8**



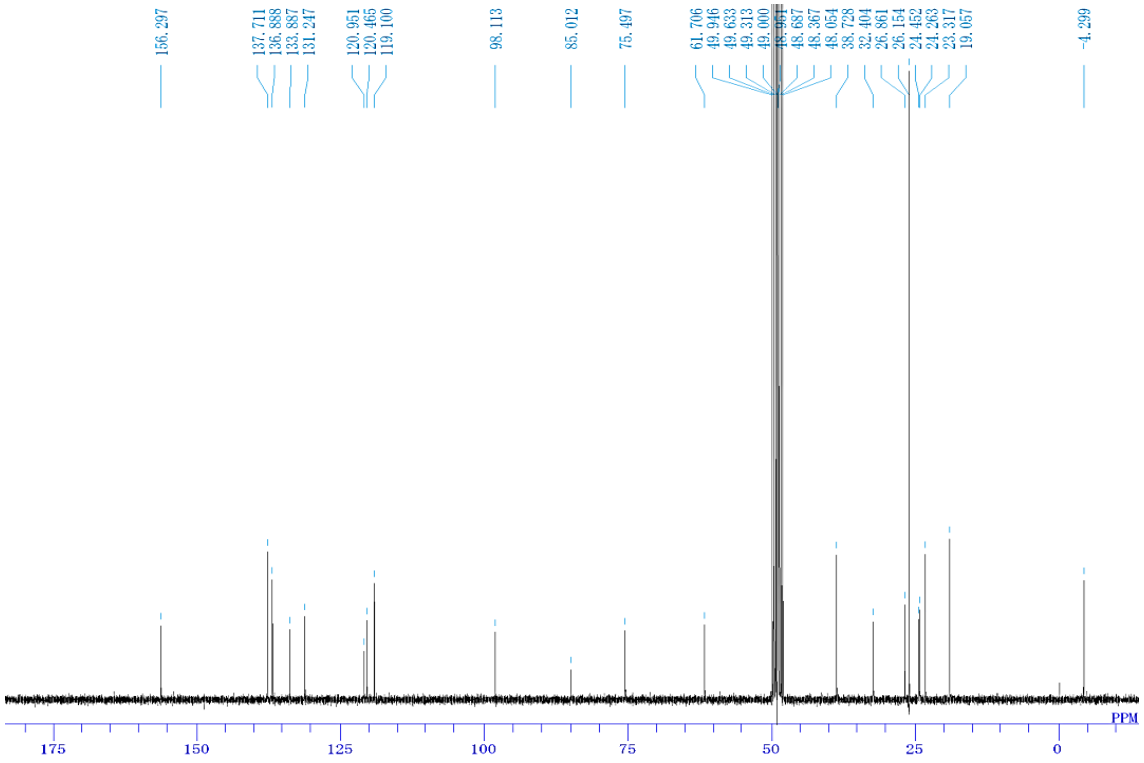
^{13}C NMR spectrum of Compound #**8**



^{1}H NMR spectrum of Compound #**9**



^{13}C NMR spectrum of Compound #**9**



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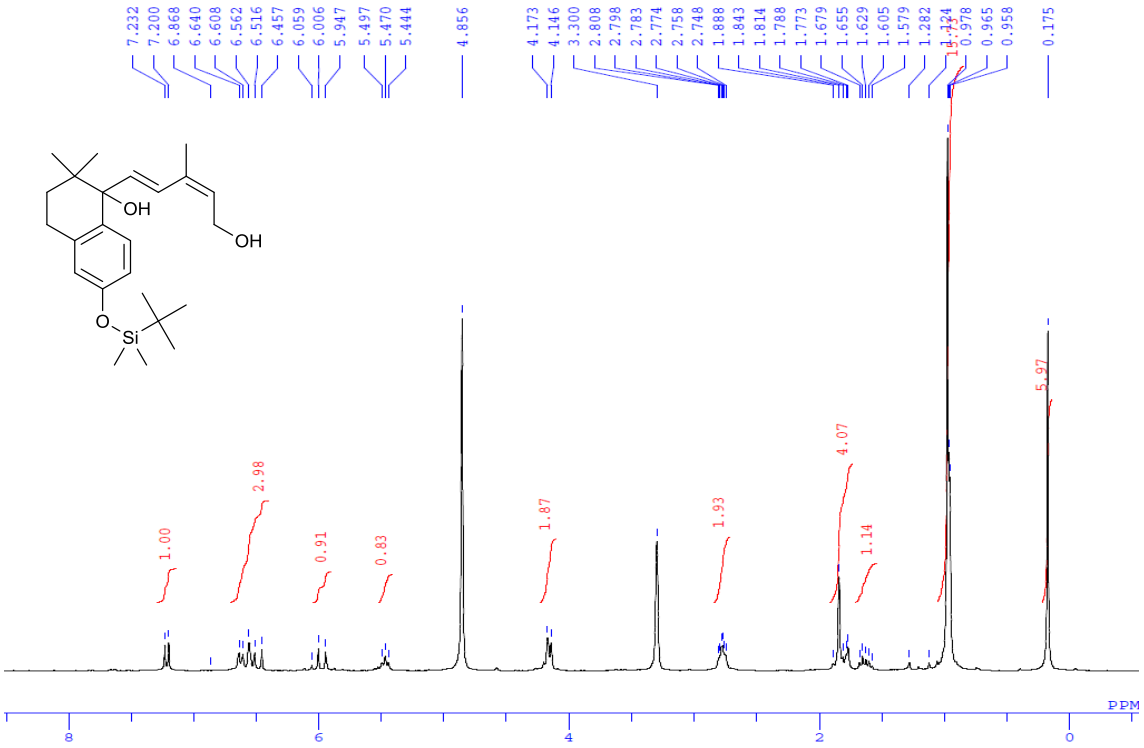
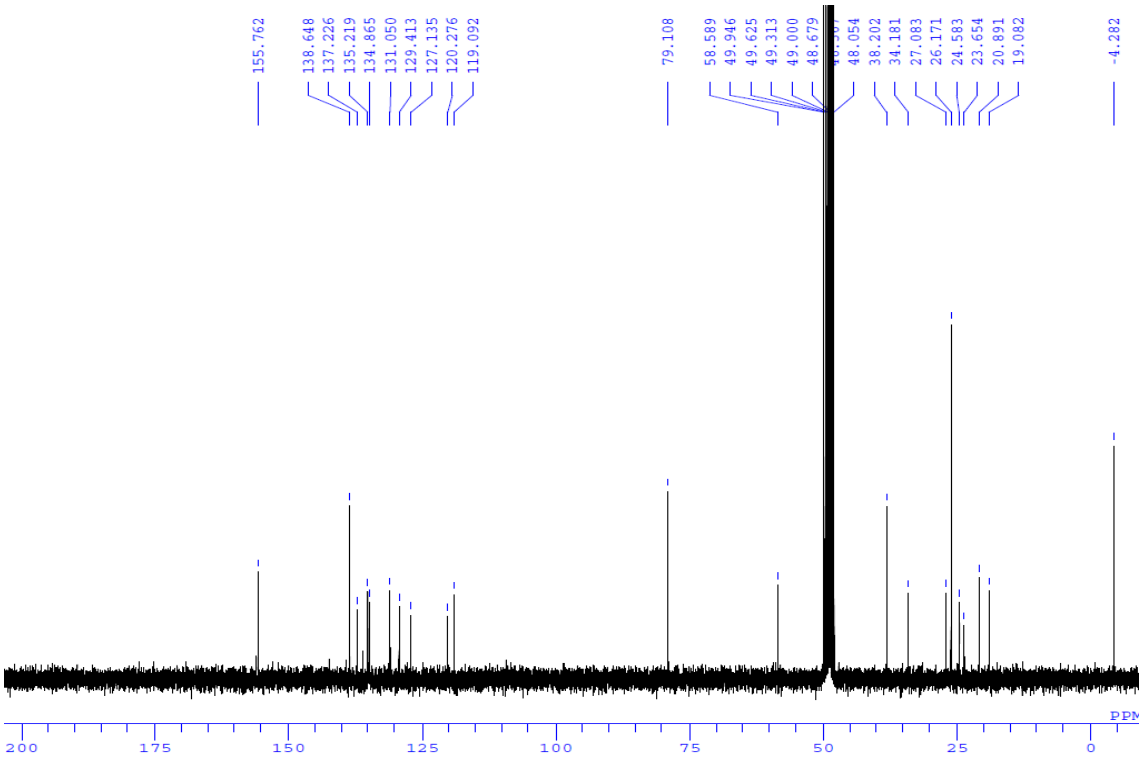


^{13}C NMR spectrum of Compound #**10**



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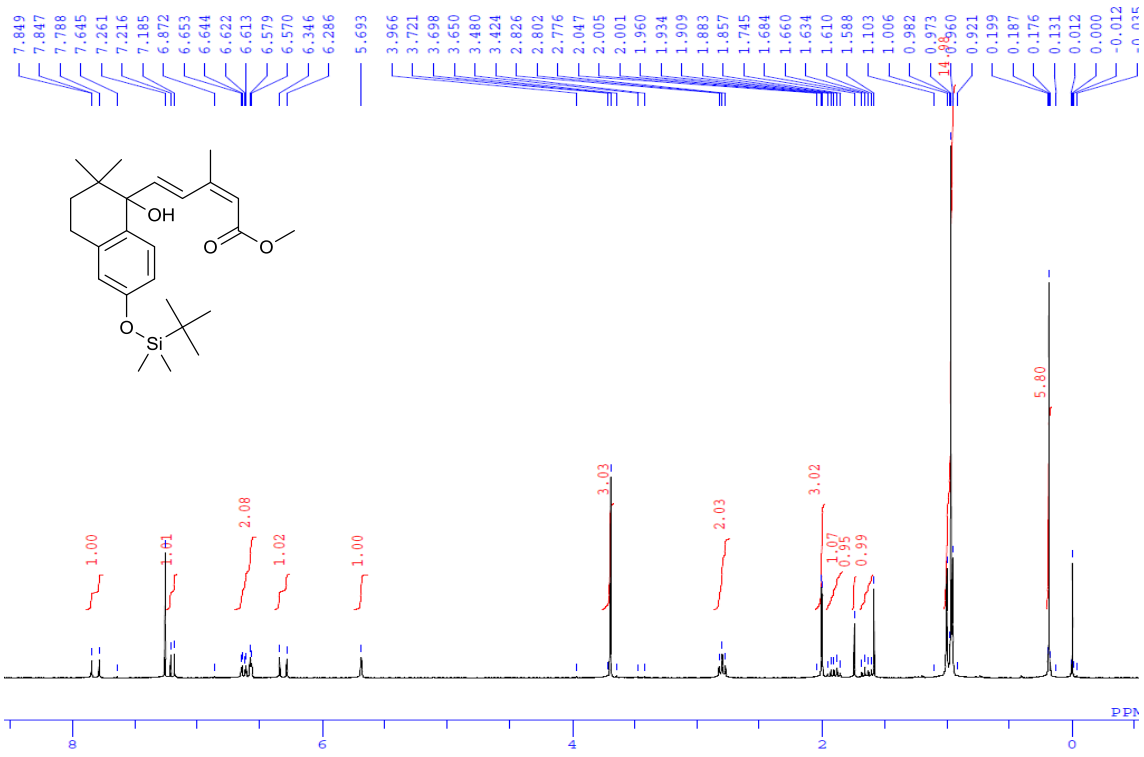
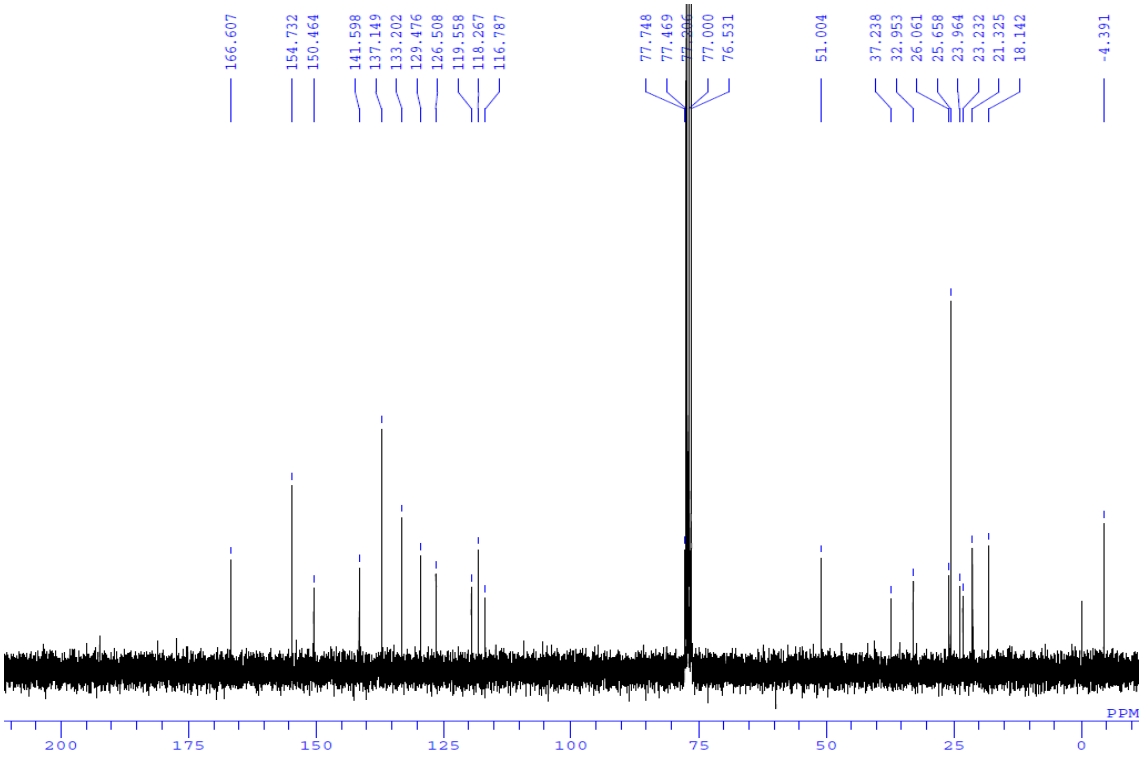


^{13}C NMR spectrum of Compound #**12**



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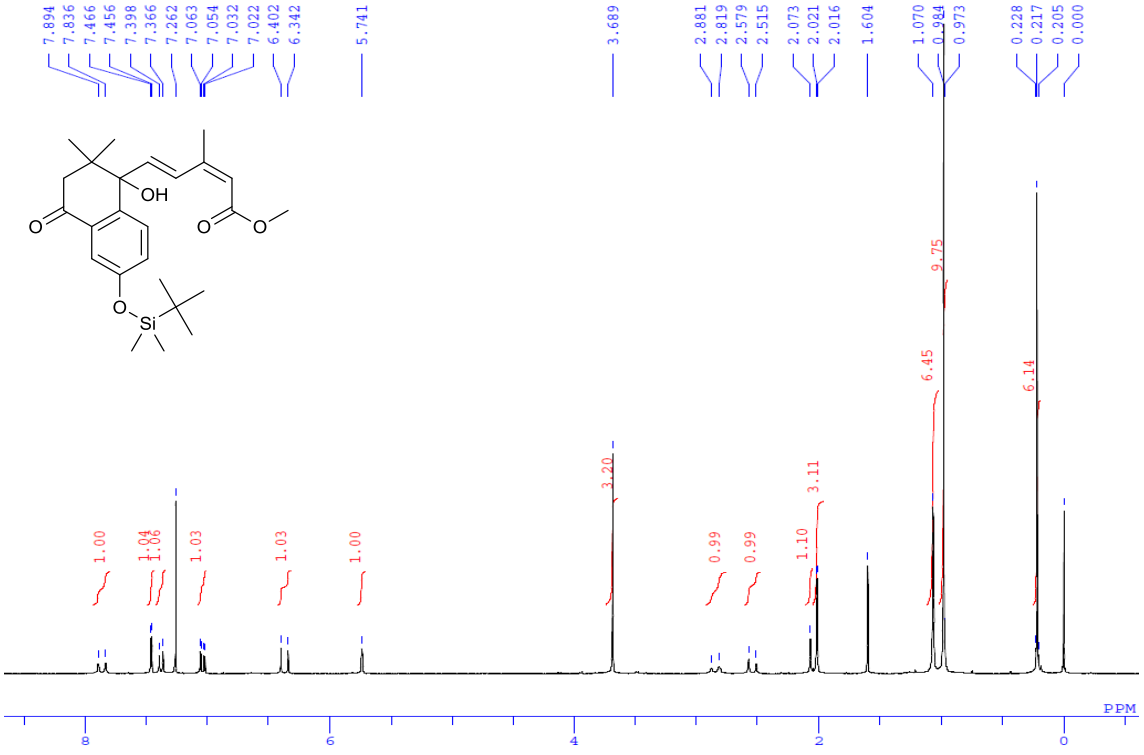
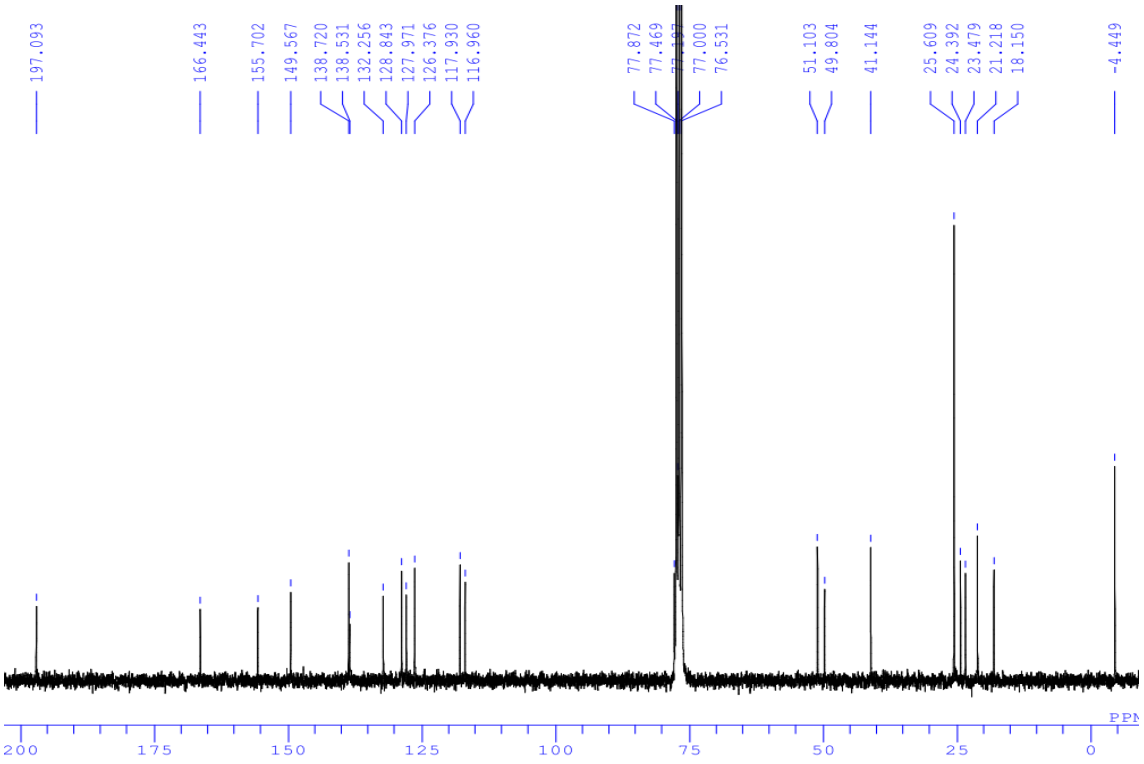


^{13}C NMR spectrum of Compound #**13**



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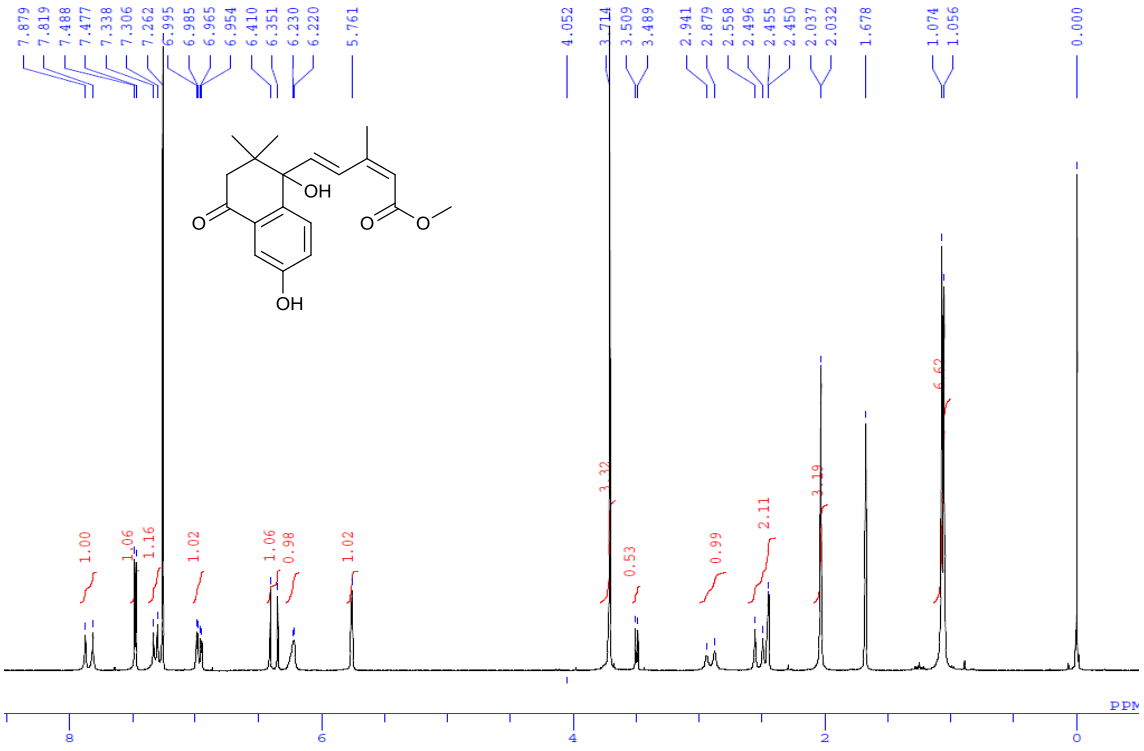
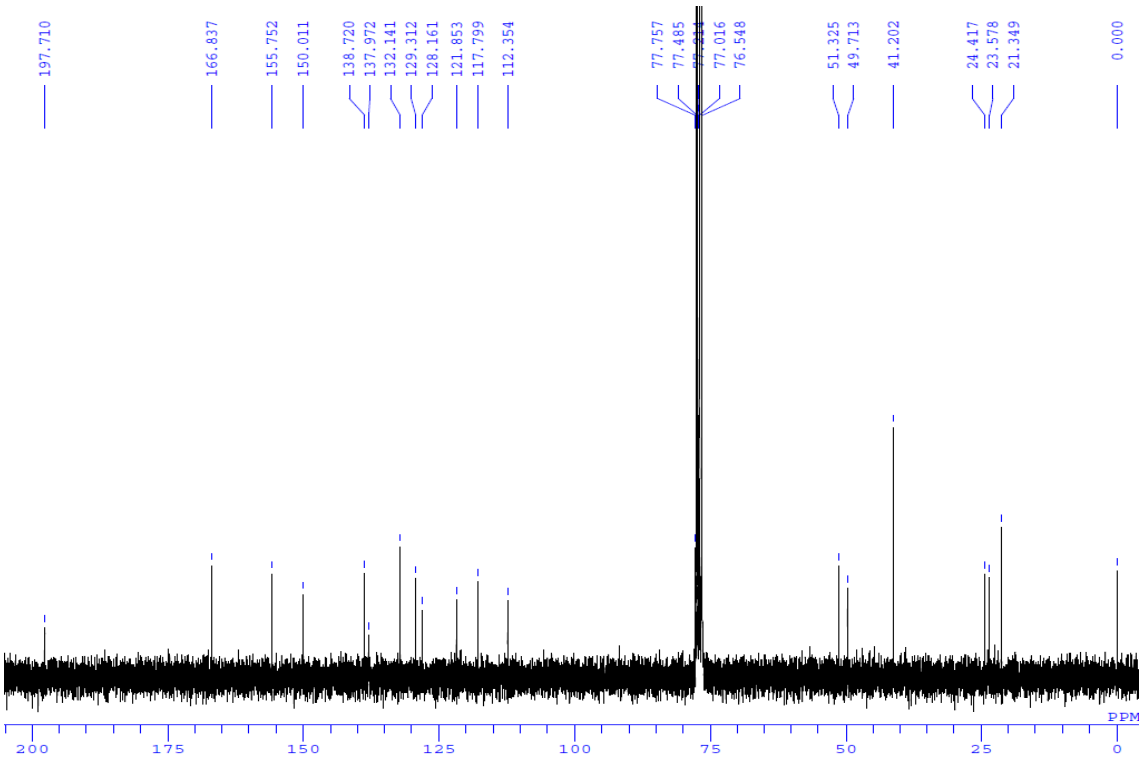
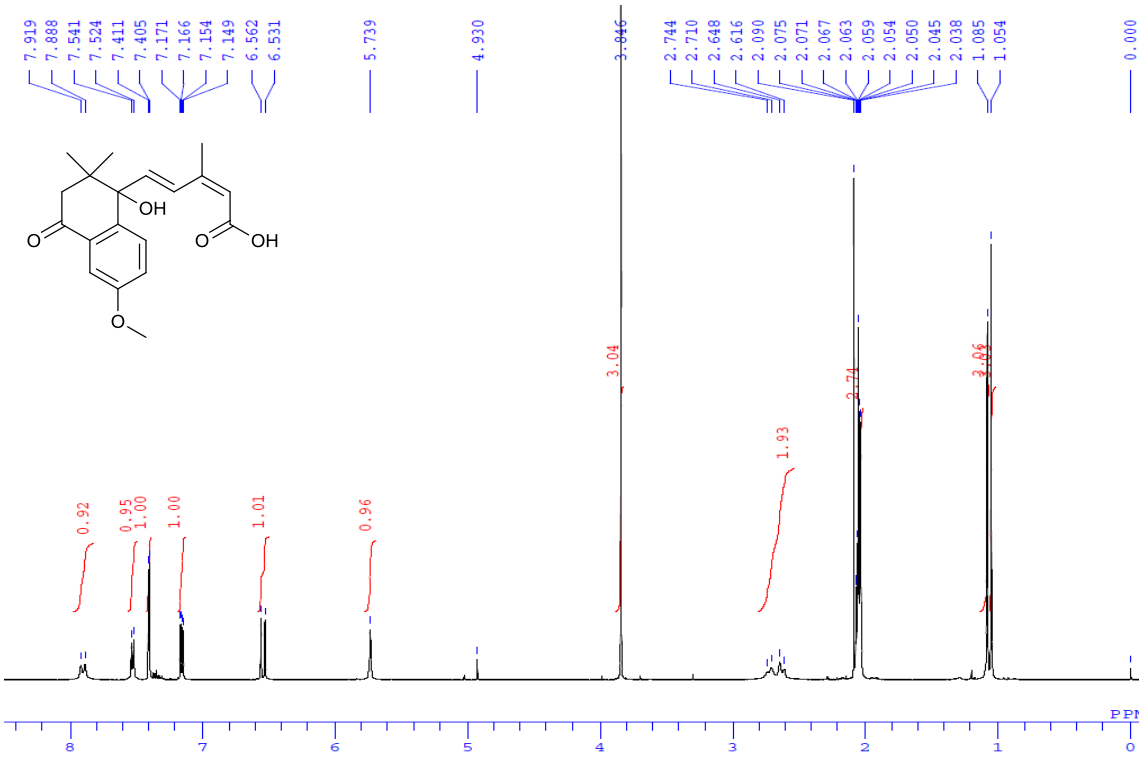


^{13}C NMR spectrum of Compound #**14**



^{1}H NMR spectrum of Compound #**15**



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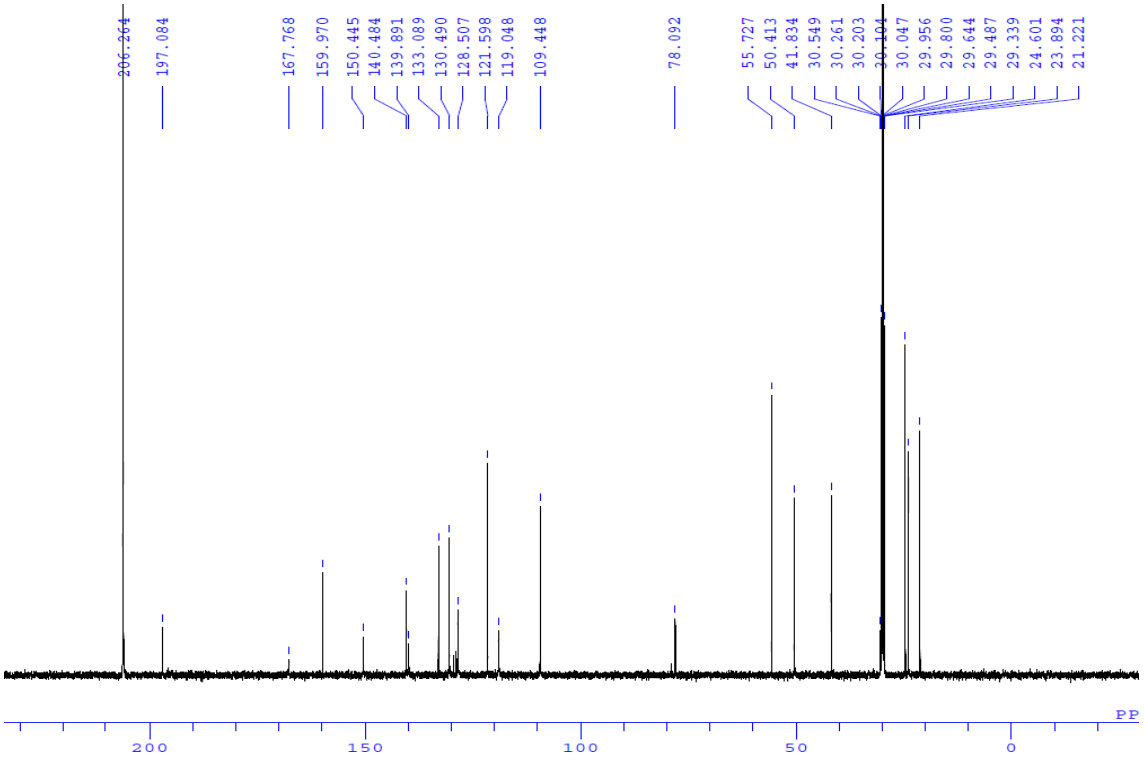
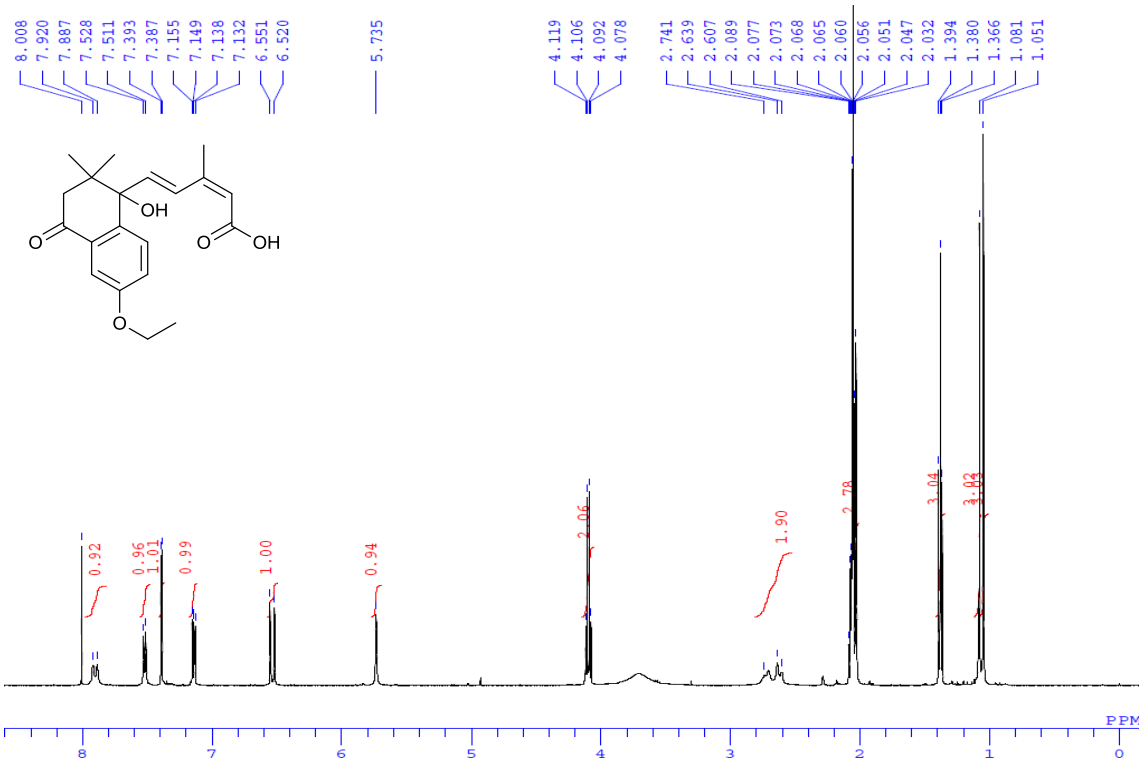
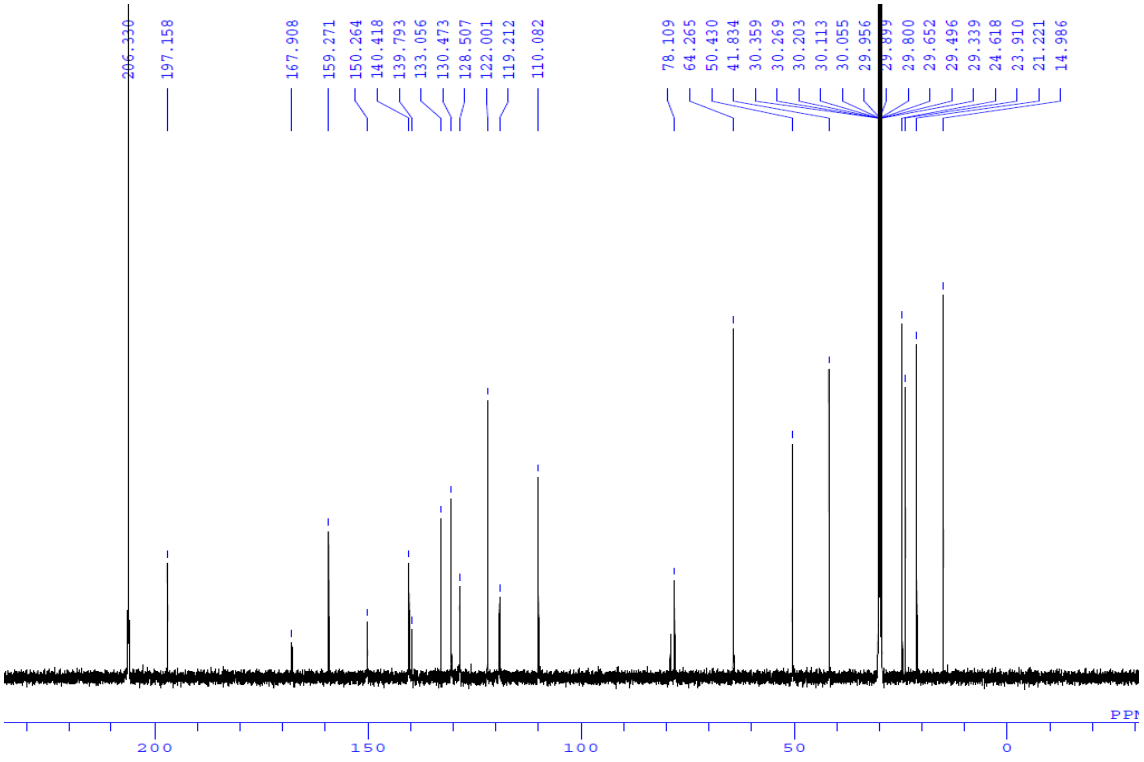


^{1}H NMR spectrum of Compound #**16**



^{13}C NMR spectrum of Compound #**16**



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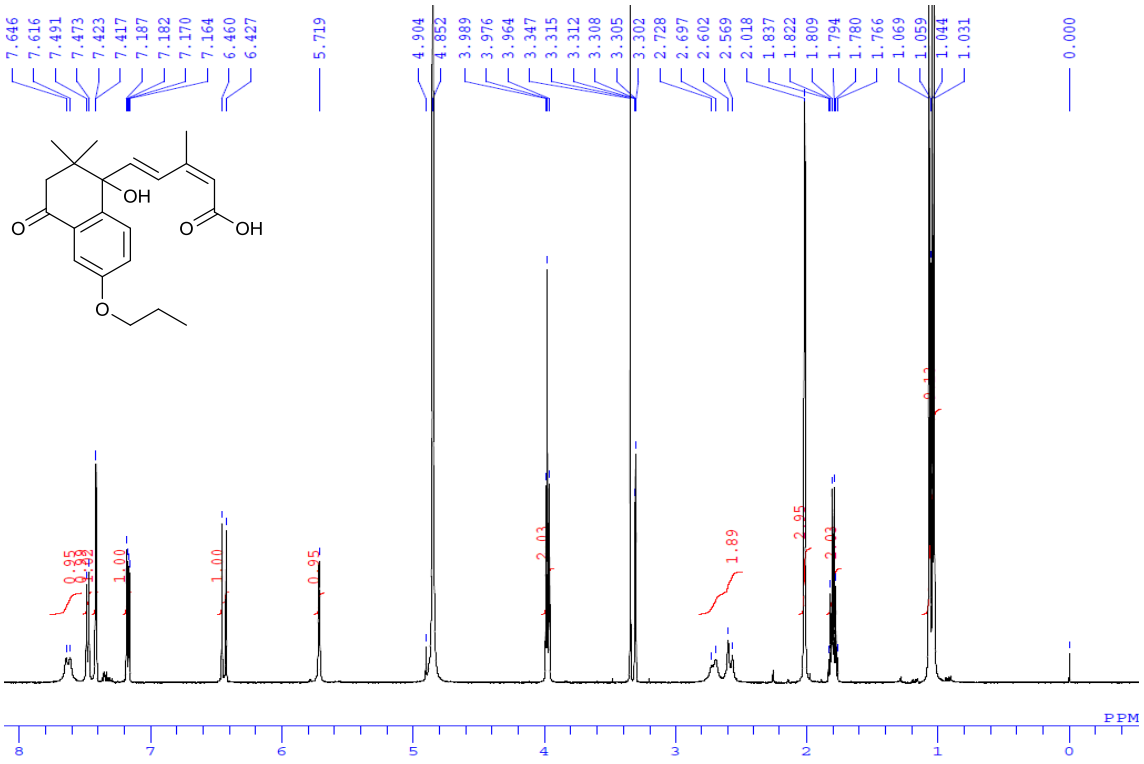

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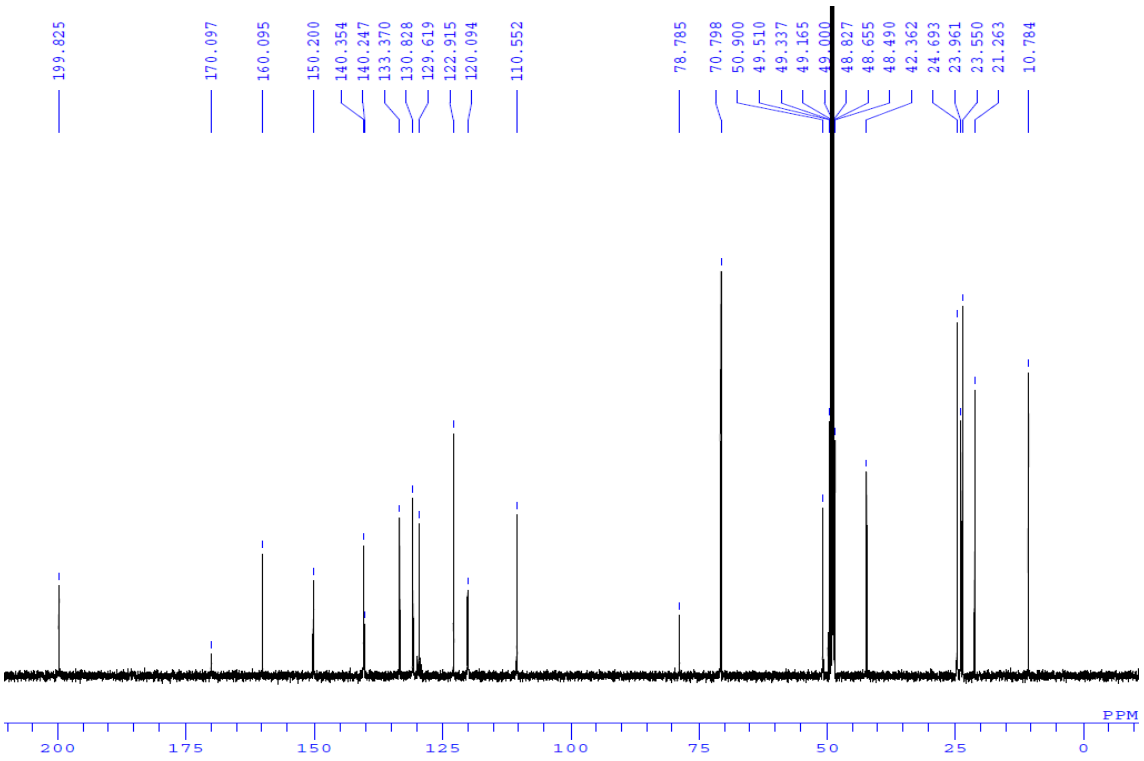

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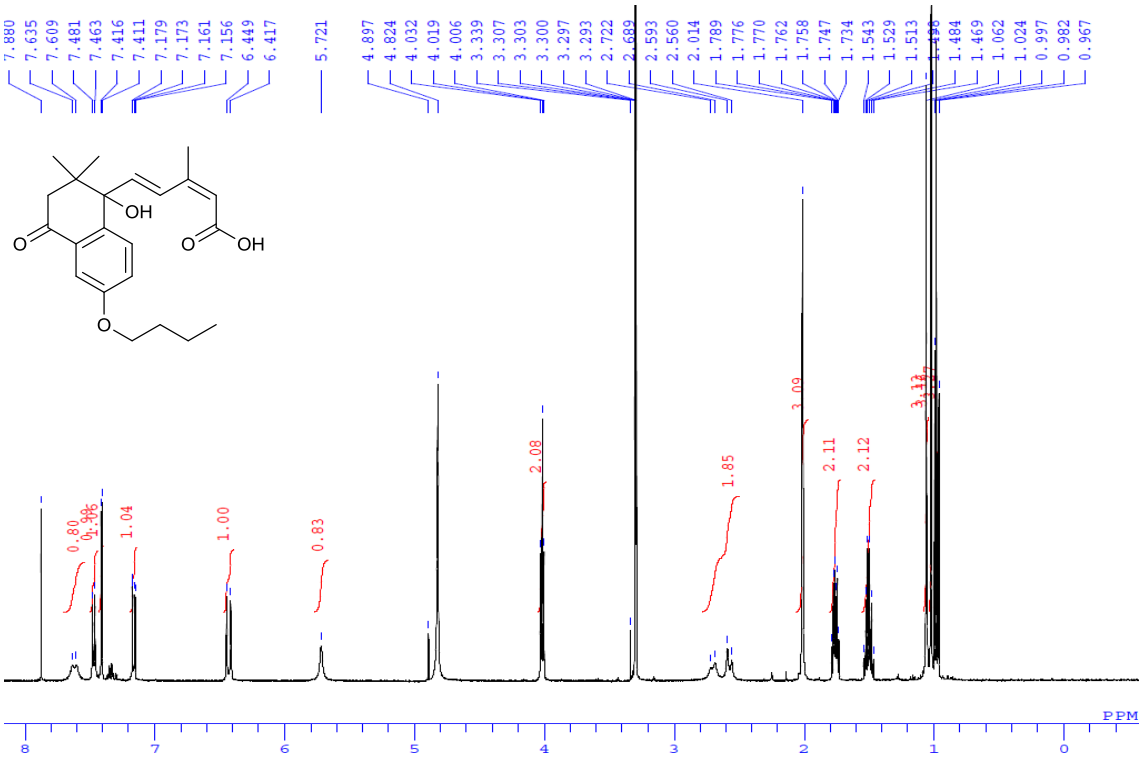

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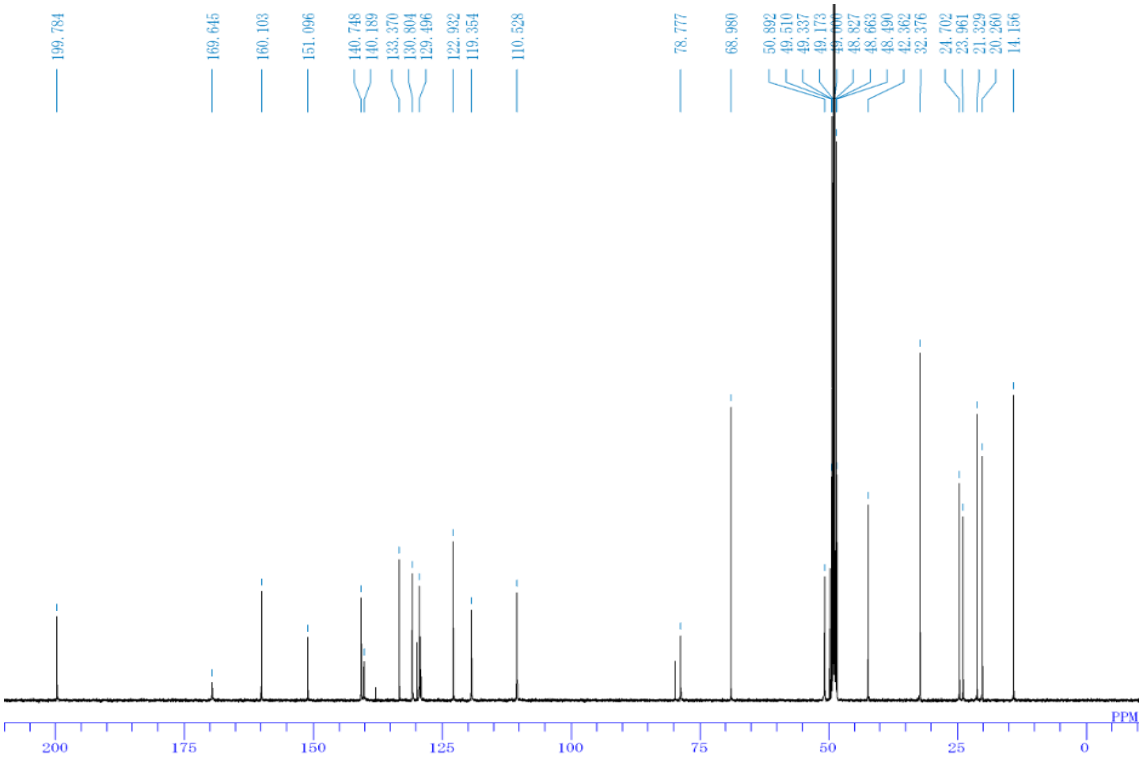

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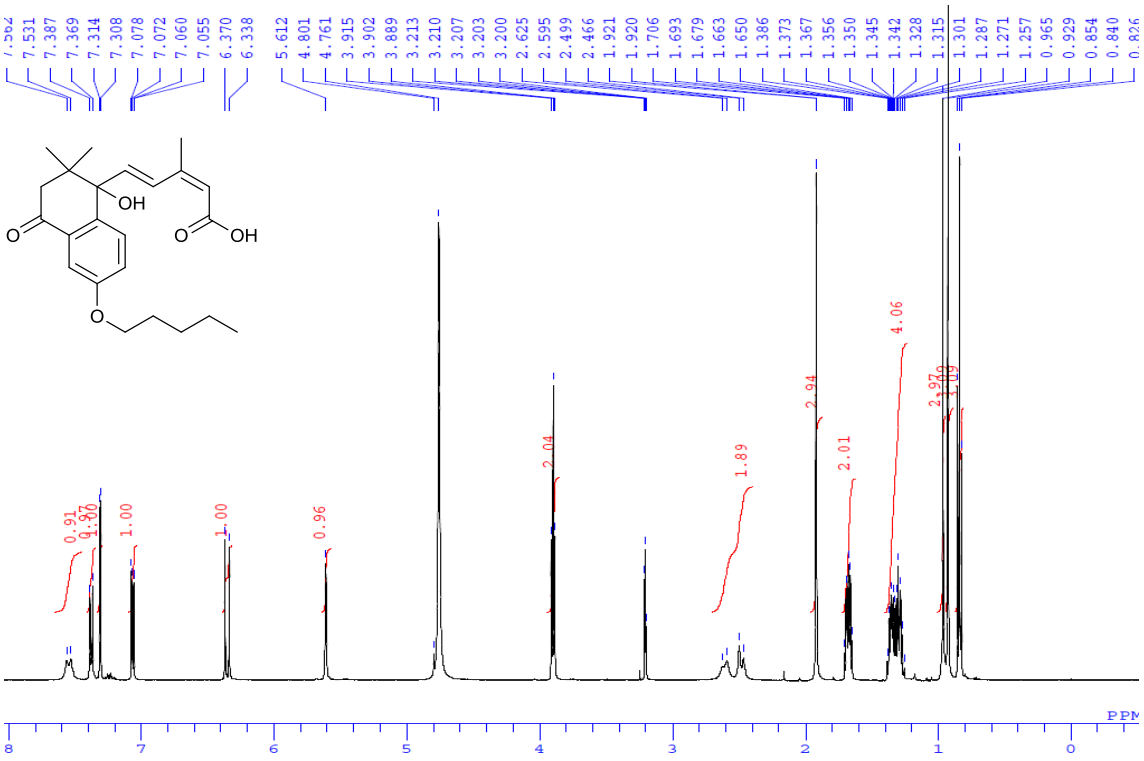
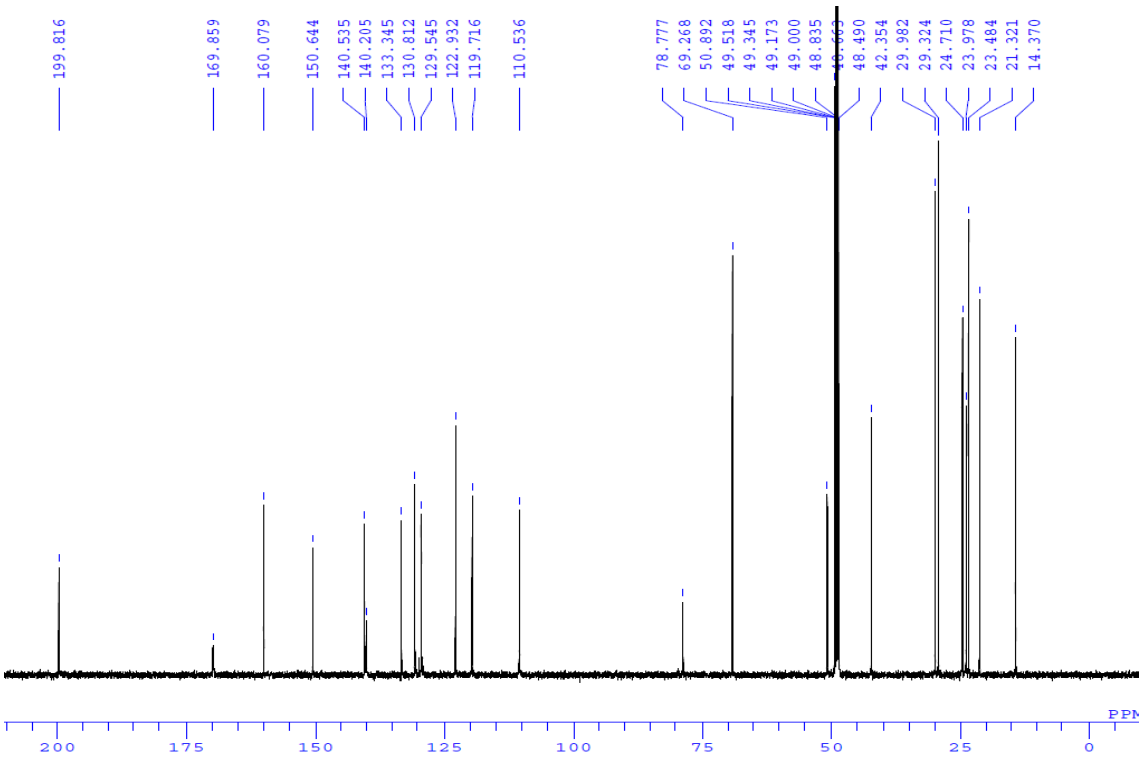


^{13}C NMR spectrum of Compound #**19**



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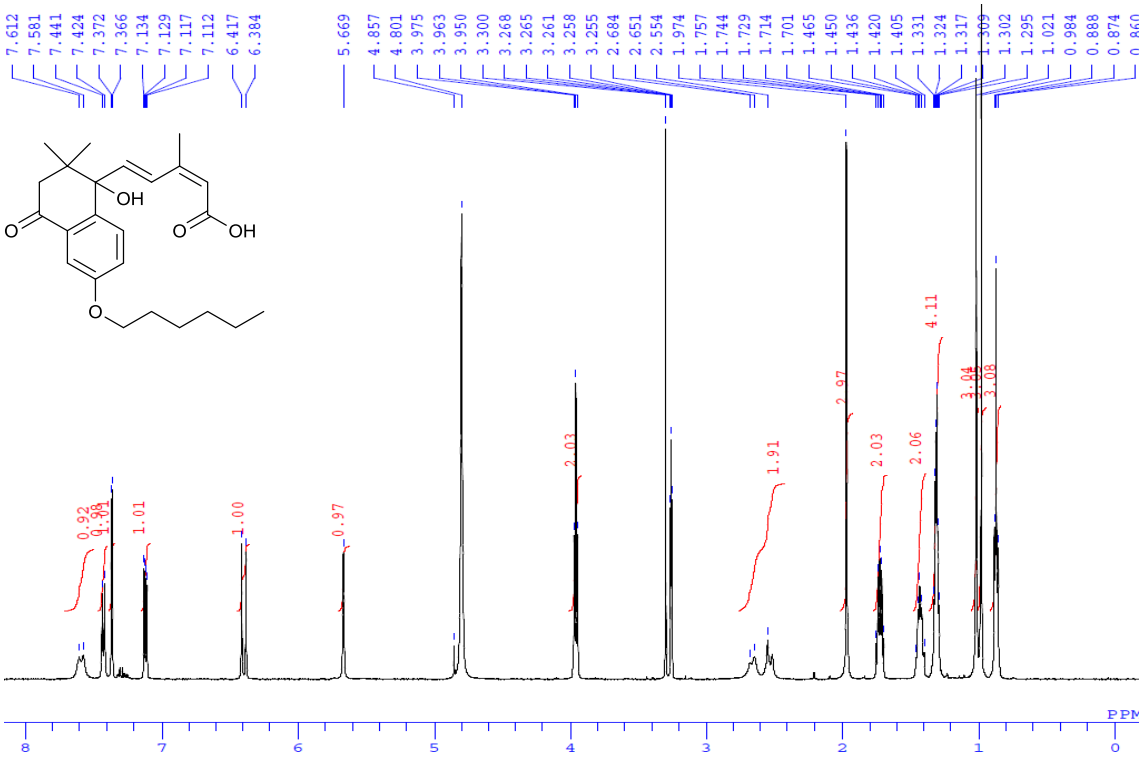

^{13}C NMR spectrum of Compound #**20**