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メタデータ	言語: English 出版者: 公開日: 2020-07-28 キーワード (Ja): キーワード (En): 作成者: Takeuchi, Jun, Nagamiya, Hikaru, Moroi, Sayaka, Ohnishi, Toshiyuki, Todoroki, Yasushi メールアドレス: 所属:
URL	http://hdl.handle.net/10297/00027562

Design of potent ABA receptor antagonists based on a conformational restriction approach

Jun Takeuchi, Hikaru Nagamiya, Sayaka Moroi, Toshiyuki Ohnishi & Yasushi Todoroki

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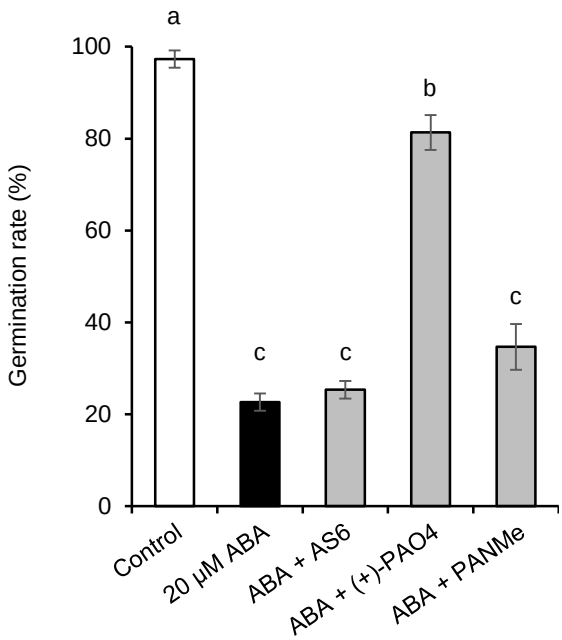
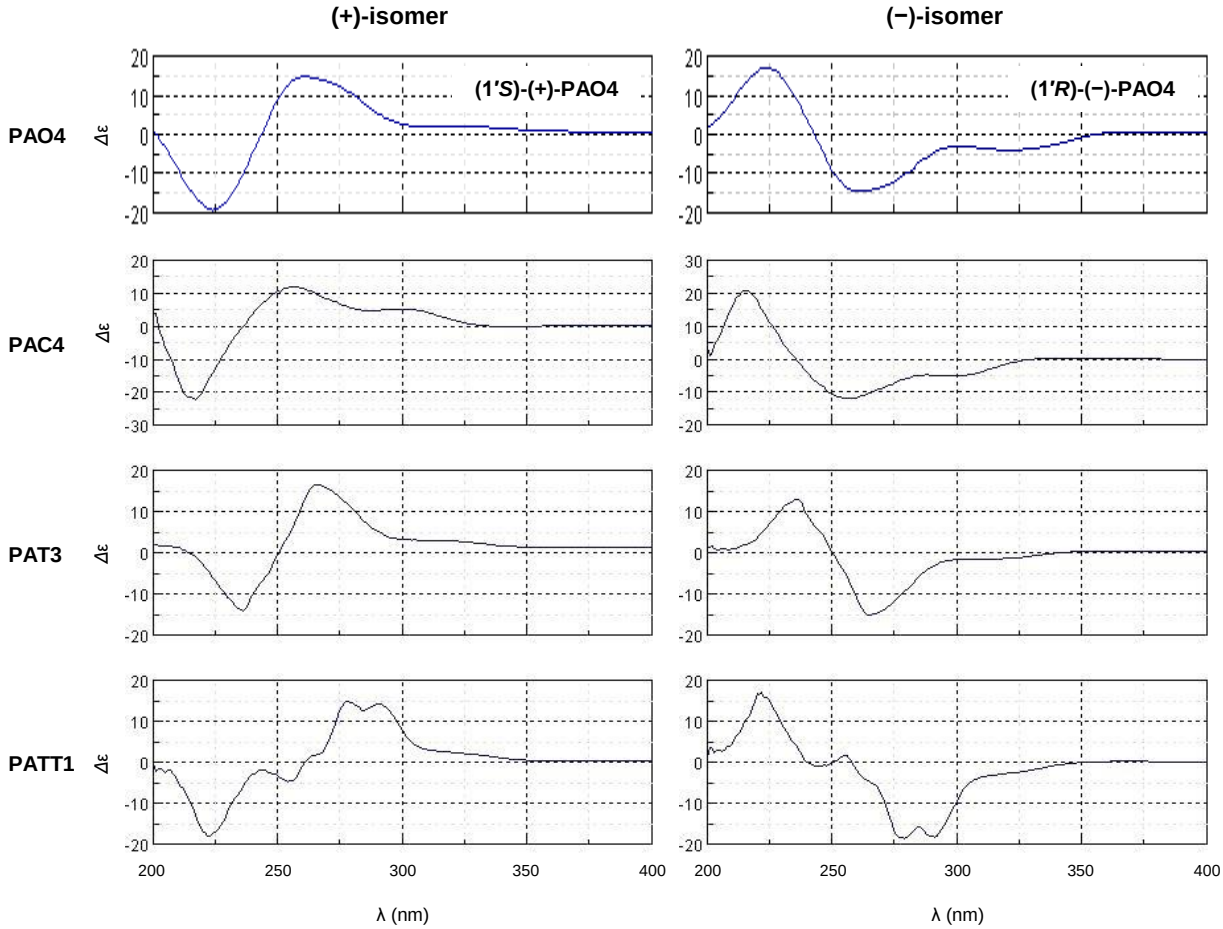
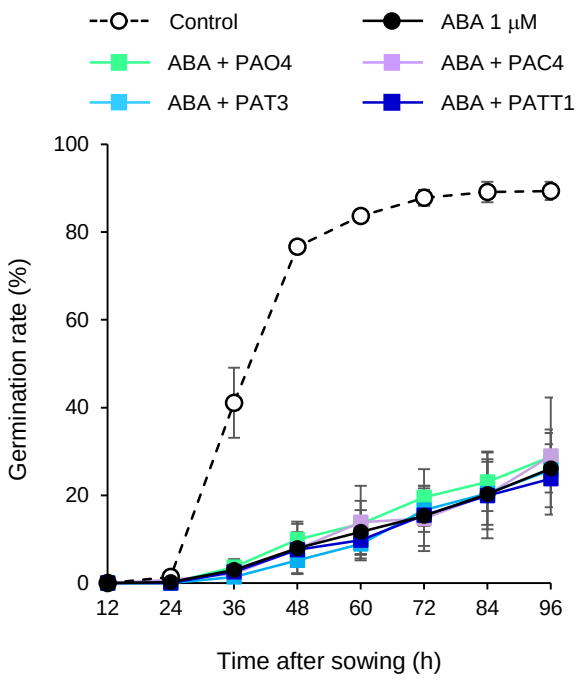


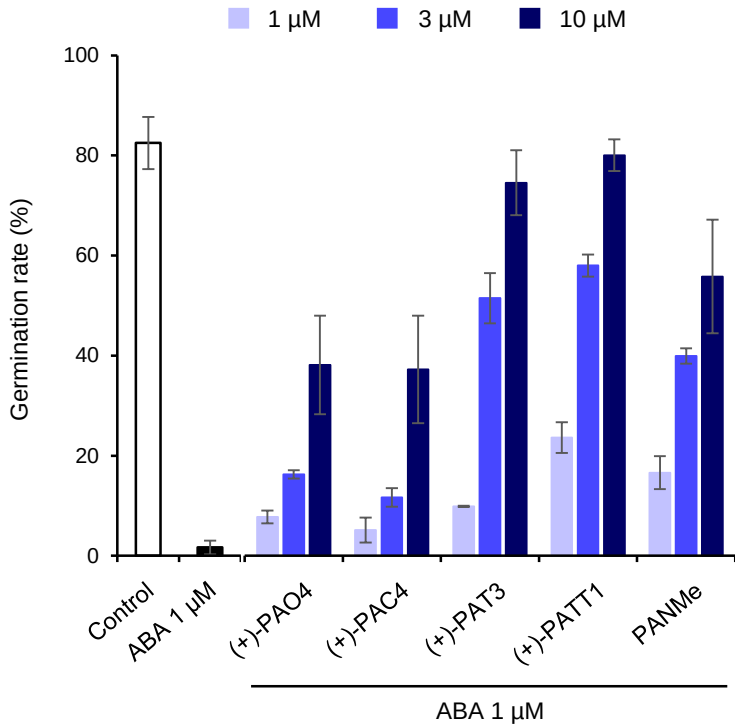
Supplementary Figure 1 Effects of AS6, (+)-PAO4 and PANMe on rice seed germination. Seed germination rate in the presence of 20 μM ABA and 30 μM AS6, (+)-PAO4 or PANMe at 60 h after sowing. Values marked with different letters were statistically significantly different between the treatments (*P*-value < 0.05, Tukey's test).



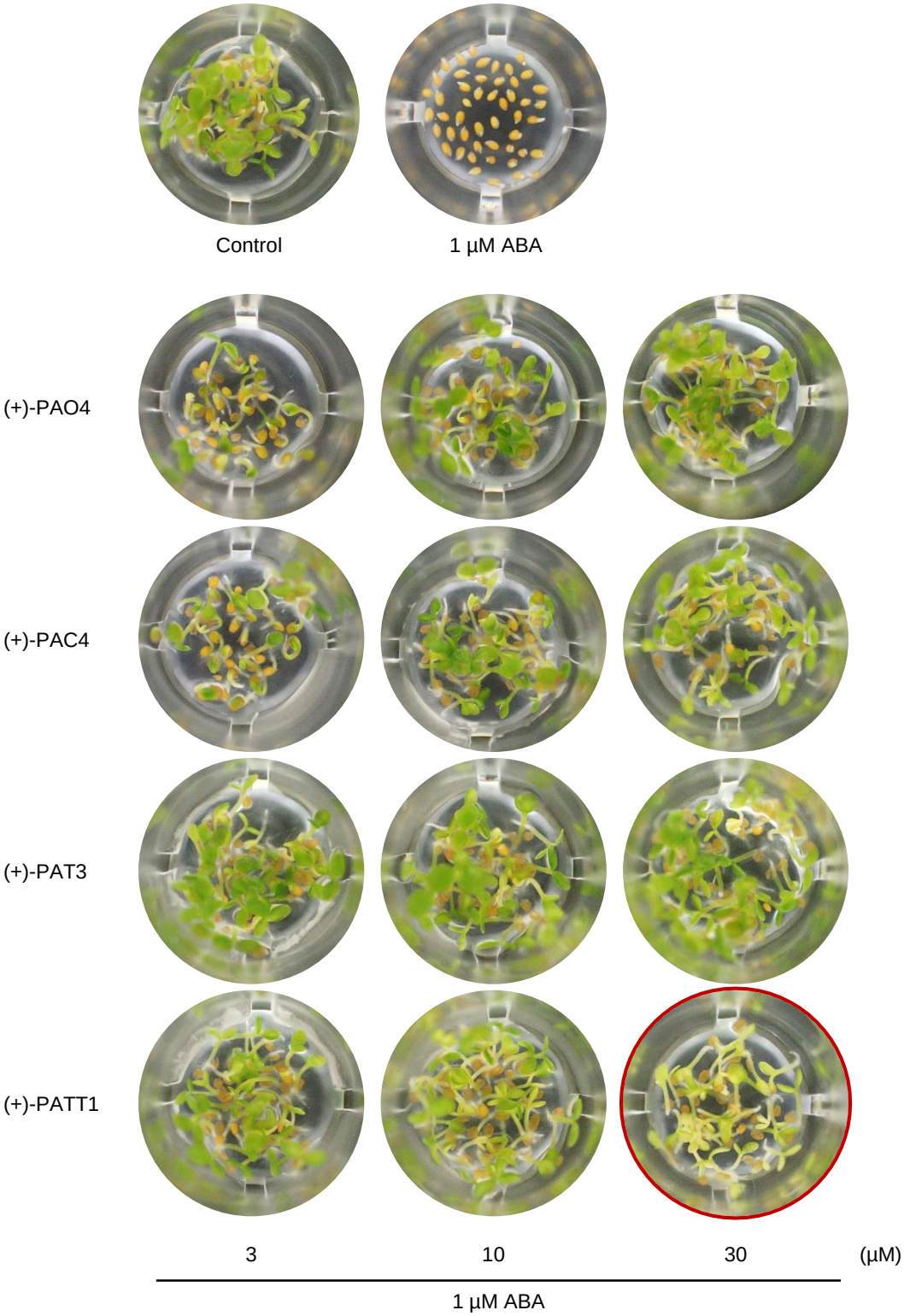
Supplementary Figure 2 Experimental CD spectra of enantiomers of PAO4, PAC4, PAT3 and PATT1.



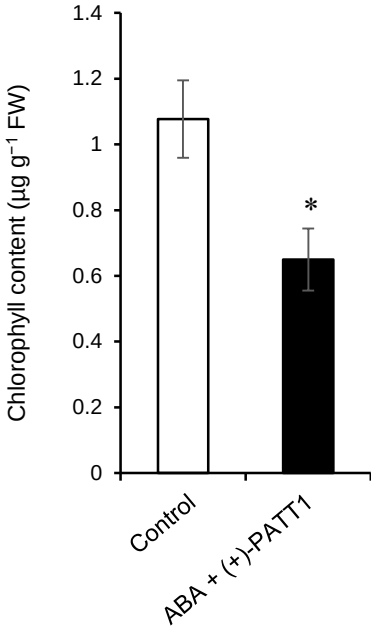
Supplementary Figure 3 Effects of (−)-PAC4, (−)-PAT3 and (−)-PATT1 on *Arabidopsis* seed germination. Seed germination rate in the presence of 1 μM ABA and 10 μM (−)-PAO4 analogs.



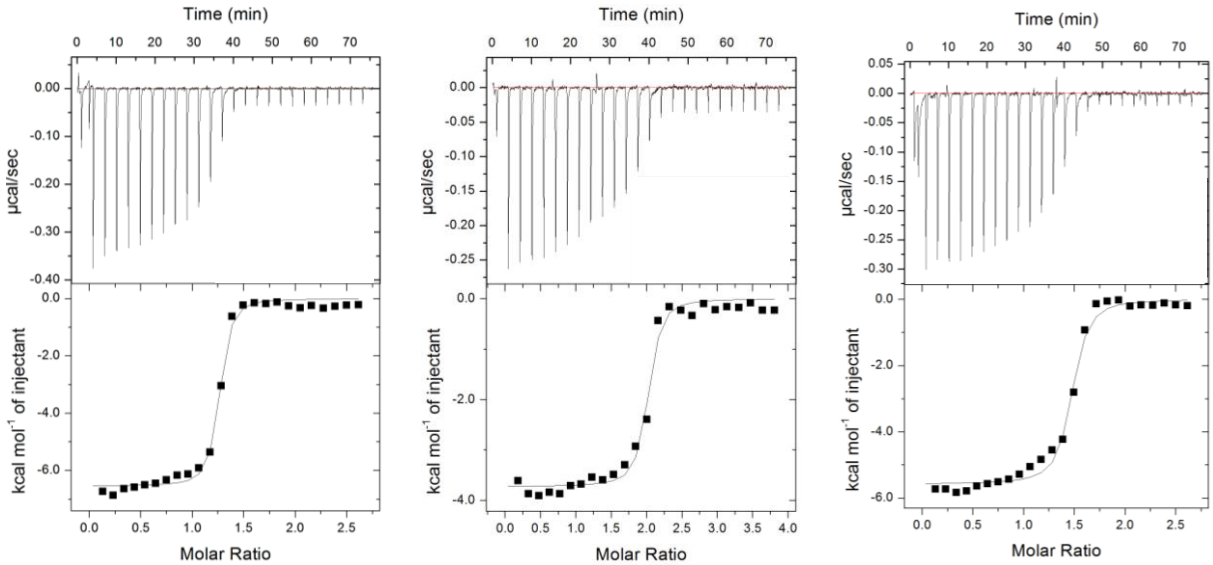
Supplementary Figure 4 Effects of (+)-PAO4 analogs on *Arabidopsis* seed germination compared with that of PANMe. Seed germination rate in the presence of 1 μM ABA and (+)-PAO4 analogs or PANMe at 36 h after stratification (n = 3, error bars represent SEs).



Supplementary Figure 5 Effects of (+)-PAO4 analogs on early seedling growth of *Arabidopsis*. Seedlings grown on test media agar containing 1 μM ABA and indicated concentrations of (+)-PAO4 analogs for 84 h. Similar results obtained from three independent experiments using different seed batches.



Supplementary Figure 6 Effect of (+)-PATT1 on total chlorophyll content. *Arabidopsis* seedlings grown on test media agar containing 1 µM ABA and 30 µM (+)-PATT1 for 84 h (n =3, error bars represent SDs).*P < 0.05, significant difference between the 2 values with Student's t test.

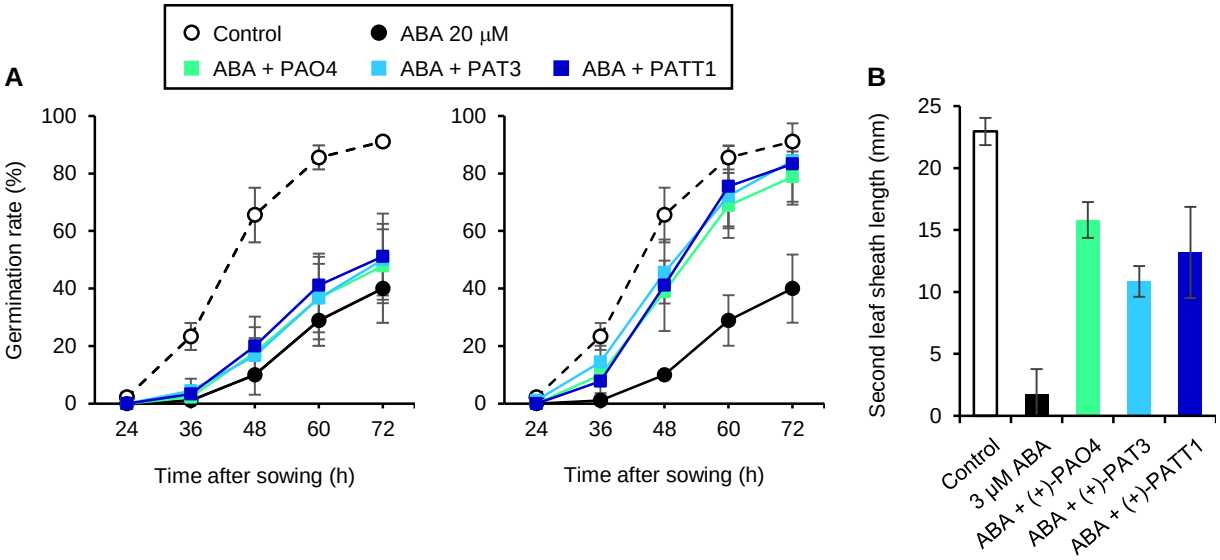


[(+)-PAC4/PYL5]
K_d = 68 ± 18 nM

[(+)-PAT3/PYL5]
K_d = 93 ± 37 nM

[(+)-PATT1/PYL5]
K_d = 129 ± 33 nM

Supplementary Figure 7 Isothermal titration calorimetry profiles and thermodynamic data for (+)-PAC4-, (+)-PAT3- and (+)-PATT1-PYL5 binding experiments. The data were corrected by subtraction the mixing enthalpies of (+)-PAC4, (+)-PAT3 and (+)-PATT1 solution into protein-free solution and fitted by Origin for ITC with 1:1 binding model.



Supplementary Figure 8 Effects of (+)-PAT3 and (+)-PATT1 on rice compared with that of (+)-PAO4. (A) Seed germination rate in the presence of 20 μM ABA and 3 μM (left) or 10 μM (right) of (+)-PAO4 analogs (*n* = 3, error bars represent SDs). (B) Seedlings were grown on test media containing 3 μM ABA and 30 μM (+)-PAO4 analogs for 7 days (*n* =3, error bars represent SDs).

Experimental

General procedures

ABA was a gift from Dr. Y. Kamuro and Toray Industries Inc., Tokyo, Japan. ^{1}H NMR spectra were recorded with tetramethylsilane as the internal standard using JEOLJNM-EX270 (270 MHz) NMR spectrometers (JEOL Ltd., Tokyo, Japan). All peak assignments refer to the numbering in structure **(+)-PAO4** (Fig. 1). High resolution mass spectra were obtained with a JEOL JMS-T100LC AccuTOF mass spectrometer (ESI-TOF, positive mode; JEOL Ltd.). Optical rotations were recorded with a Jasco DIP-1000 digital polarimeter. Circular dichroism spectra were recorded with a Jasco J-820 spectrophotometer. Column chromatography was performed using silica gel (Wakosil C-200, Wako P22ure Chemical Industries Ltd.).

Synthesis of PAC4

6,6-dimethyl-5-oxo-5,6,7,8-tetrahydronaphthalen-2-yl trifluoromethanesulfonate (6)

To a stirred solution of **5** (1.00 g, 5.26 mmol) in dry CH_2Cl_2 was added 2,6-lutidine (0.92 mL, 7.88mmol) under an atmosphere of Ar. After stirring the mixture for 15 min at 0 °C, trifluoromethanesulfonic anhydride (1.29 mL, 7.88 mmol) was added dropsies to the mixture. The mixture was stirred for 1 h at 0 °C and then the ice bath was removed. The reaction mixture was stirred at room temperature for 1 h. After quenching with 1M HCl (10 mL), it was extracted with EtOAc (20 mL × 3). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residual oil was purified by silica gel chromatography (2% EtOAc/ hexane) to obtain **6** (1.73 g, quantitative yield) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 1.23 (6H, s, 2 × CH_3), 2.02 (2H, t, *J*=6.3 Hz, H_2-5′), 3.03 (2H, t, *J*=6.3 Hz, H_2-4′), 7.15 (1H, d, *J*=2.3Hz, H-12′), 7.22 (1H, dd, *J*=8.6 and 2.3 Hz, H-10′), 8.14 (1H, d, *J*=8.6 Hz, H-7′); ^{13}C NMR (68 MHz, $CDCl_3$): δ_C 24.1, 24.1, 25.8, 36.2, 41.6, 116.3, 119.6, 121.2, 130.8, 131.3, 146.0, 152.2, 201.1. The data were consistent with the previous data[1].

2,2-dimethyl-6-pentyl-3,4-dihydronaphthalen-1(2H)-one (7)

9-BBN (0.5 M solution in THF, 9.3 mL, 4.6 mmol) was added to 1-penten (0.54 mL, 4.6 mmol) at room temperature. The solution was stirred at room temperature overnight. After this time, K_3PO_4 (987 mg, 4.6 mmol), $Pd(PPh_3)_4$ (65 mg, 0.056 mmol), KBr (433 mg, 3.7 mmol) and degassed H_2O (0.061 mL, 3.4 mmol) were added. This was followed by a solution of **6** (1.00 g, 3.1 mmol) in dry THF (3.5 mL). The reaction mixture was stirred for 2 h at 68 °C. After cooling, the solution was acidified to pH 2 and extracted with EtOAc (20 mL × 3). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residual oil was purified by silica gel chromatography (1.5% EtOAc/ hexane) to obtain **7** (765 mg, quantitative yield) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 0.90 (3H, t, *J*=6.9 Hz, H_3-5″), 1.21 (6H, s, 2 × CH_3), 1.30–1.35 (4H, m, H_2-3″ and 4″), 1.62 (2H, m,

H_2-2″), 1.97 (2H, t, *J*=6.3 Hz, H_2-5′), 2.61 (2H, t, *J*=7.6 Hz, H_2-1″), 2.95 (2H, t, *J*=6.3 Hz, H_2-4′), 7.02 (1H, br s, H-12′), 7.11 (1H, dd, *J*=7.9 and 1.3 Hz, H-10′), 7.95 (1H, d, *J*=7.9 Hz, H-7′); ^{13}C NMR (68 MHz, $CDCl_3$): δ_C 14.0, 22.5, 24.4, 24.4, 25.7, 30.8, 31.5, 36.0, 36.7, 41.5, 127.0, 128.1, 128.4, 129.3, 143.3, 148.7, 202.7; HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{17}H_{24}ONa$, 267.1725; found, 267.1730.

(*Z*)-1-(5-hydroxy-3-methylpent-3-en-1-yn-1-yl)-2,2-dimethyl-6-pentyl-1,2,3,4-tetrahydronaphthalen-1-ol (8)

(*Z*)-3-Mehylpent-2-en-4-yn-1-ol (214 mg, 2.22 mmol) in dry THF (7 mL) was cooled to −80 °C under an atmosphere of Ar. *n*-Butyllithium (2.9 mL, 1.6 M) was then added slowly. After being stirred for 40 min at −80 °C, a solution of compound **7** (356 mg, 1.43 mmol) in dry THF (1.3 mL) was added dropwise to the stirred mixture. The reaction mixture was stirred for a further 10 min at −80 °C and then the ice bath was removed. The reaction mixture was stirred at room temperature for 30 min. After quenching with sat. NH_4Cl (8 mL), it was extracted with EtOAc (20 mL × 3). The organic layer was washed, dried and concentrated, as described above. The residual oil was purified by silica gel chromatography (0–25% EtOAc/ hexane) to obtain **8** (463 mg, 93%) as a yellow oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 0.90 (3H, t, *J*=6.9 Hz, H_3-5″), 1.08 (3H, s, H_3-8′ or 9′), 1.18 (3H, s, H_3-8′ or 9′), 1.30–1.36 (4H, m, H_2-3″ and 4″), 1.54–1.68 (3H, m, H-5′ and H_2-2″), 1.92 (3H, d, *J*=1.3 Hz, H_3-6), 2.04 (1H, m, H-5′), 2.55 (2H, t, *J*=7.9 Hz, H_2-1″), 2.82 (2H, m, H_2-4′), 4.32 (2H, d, *J*=6.3 Hz, H_2-1), 5.88 (1H, tq, *J*=6.3 and 1.3 Hz, H-2), 6.91 (1H, d, *J*=2.0 Hz, H-12′), 7.05 (1H, dd, *J*=7.9 and 2.0 Hz, H-10′), 7.69 (1H, d, *J*=7.9 Hz, H-7′); ^{13}C NMR (68 MHz, $CDCl_3$): δc 14.0, 22.5, 23.2, 23.8, 23.8, 25.8, 31.0, 31.2, 31.6, 35.5, 37.6, 61.4, 75.0, 84.2, 96.7, 120.8, 126.6, 128.2, 128.9, 134.7, 135.6, 136.1, 142.8; HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{23}H_{32}O_2Na$, 363.2300; found, 363.2297.

1-((1*E*,3*Z*)-5-hydroxy-3-methylpenta-1,3-dien-1-yl)-2,2-dimethyl-6-pentyl-1,2,3,4-tetrahydronaphthalen-1-ol (9)

To a stirred solution of **8** (463 mg, 1.36 mmol) in dry THF (12 mL) was cooled to 0 °C and added sodium bis (2-methoxyethoxy) aluminum hydride in toluene 65 % w/w (SMEAH) (2.3 mL, 8.16 mmol) under an atmosphere of Ar. The mixture was stirred for 50 min at room temperature. After quenching with sat. NH_4Cl aq. (5 mL), it was diluted with water (20 mL) and extracted with EtOAc (25 mL × 3). The organic layer was washed, dried, and concentrated as above. The residual oil was purified by silica gel chromatography (25 % EtOAc/ hexane) to obtain **9** (419 mg, 80%) as a pale-yellow oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 0.89 (3H, t, *J*=6.9 Hz, H_3-5″), 0.96 (3H, s, H_3-8′ or 9′), 0.99 (3H, s, H_3-8′ or 9′), 1.29–1.35 (4H, m, H_2-3″ and 4″), 1.53–1.63 (3H, m, H-5′ and H_2-2″), 1.69 (1H, s, -HO), 1.87 (3H, d, *J*=1.0 Hz, H_3-6), 1.90 (1H, m, H-5′), 2.54 (2H, t, *J*=7.6 Hz, H_2-1″), 2.83 (2H, t, *J*=6.6 Hz, H_2-4′), 4.31 (2H, m, H_2-1), 5.56 (1H, t, *J*=7.6 Hz, H-2), 5.98 (1H, d, *J*=15.5 Hz, H-5), 6.73 (1H, d, *J*=15.5 Hz, H-4), 6.92 (1H, d, *J*=1.6 Hz, H-12′), 6.98 (1H, dd, *J*=7.9 and 1.6 Hz, H-

10′), 7.25 (1H, d, *J*=7.9Hz, H-7′); ^{13}C NMR (68 MHz, $CDCl_3$): δ_C 14.0, 20.8, 22.5, 23.3, 23.9, 26.0, 31.1, 31.6, 33.0, 35.5, 37.1, 58.6, 77.9, 125.4, 126.5, 127.8, 128.1, 128.7, 134.9, 135.5, 135.6, 138.0, 142.0; HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{23}H_{34}O_2Na$, 365.2457; found, 365.2456.

methyl (2Z,4E)-5-(1-hydroxy-2,2-dimethyl-6-pentyl-1,2,3,4-tetrahydronaphthalen-1-yl)-3-methylpenta-2,4-dienoate (10)

To a stirred solution of **9** (200 mg, 0.58 mmol) in dry acetone (7.7 mL) was added MnO_2 (0.86 g, 9.9 mmol) at room temperature. After stirring at room temperature for 30 min, all the starting material had disappeared. The reaction mixture was then filtered through a pad of Celite® and concentrated *in vacuo*. The crude material (226 mg) was carried through to the next stage without further purification. The crude aldehyde (226 mg) was dissolved in MeOH (4.6 mL) and stirred with MnO_2 (0.86 g, 9.9 mmol), NaCN (86 mg, 1.8 mmol) and AcOH (34 μL, 0.58 mmol) at room temperature. After 50 min, the reaction mixture was filtered through a pad of Celite® and concentrated *in vacuo*. The residue was brought up in distilled water and extracted with EtOAc (20 mL × 3). The organic layer was washed, dried, and concentrated as above. The residual oil was purified by silica gel chromatography (8% EtOAc/ hexane) to obtain **10** (119 mg, 55%) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 0.89 (3H, t, *J*=6.9Hz, H_3-5″), 0.97 (3H, s, H_3-8′ or 9′), 1.01 (3H, s, H_3-8′ or 9′), 1.32 (4H, m, H_2-3″ and 4″), 1.54–1.71 (3H, m, H-5′ and H_2-2″), 1.83 (1H, s, -OH), 1.92 (1H, dt, *J*=13.5 and 6.6 Hz, H-5′), 1.99 (3H, d, *J*=1.3 Hz, H_3-6), 2.54 (2H, t, *J*=7.6 Hz, H_2-1″), 2.84 (2H, t, *J*=6.6 Hz, H_2-4′), 3.70 (3H, s, -OCH_3), 5.69 (1H, s, H-2), 6.33 (1H, d, *J*=16.2 Hz, H-5), 6.92 (1H, d, *J*=1.9 Hz, H-12′), 6.98 (1H, dd, *J*=7.9 and 1.9 Hz, H-10′), 7.26 (1H, d, *J*=7.9 Hz, H-7′), 7.85 (1H, d, *J*=16.2 Hz, H-4).^{13}C NMR (68 MHz, $CDCl_3$): δc 14.0, 21.3, 22.5, 23.2, 24.0, 26.0, 31.1, 31.6, 33.1, 35.5, 37.2, 51.0, 77.9, 116.8, 126.4, 126.6, 128.1, 128.7, 135.4, 137.6, 141.6, 142.0, 150.5, 166.7; HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{24}H_{34}O_3Na$, 393.2406; found, 393.2397.

(2Z,4E)-5-(1-hydroxy-2,2-dimethyl-4-oxo-6-pentyl-1,2,3,4-tetrahydronaphthalen-1-yl)-3-methylpenta-2,4-dienoic acid, (±)-PAC4 (2)

The methyl ester **10** (98.8 mg, 0.27 mmol) in dry benzene (2.9 mL) was added Celite® (0.6 g) and pyridinium dichromate (400 mg, 1.07 mmol). After being stirred for 10 min, 70% *tert*-butyl hydroperoxide (0.2 mL, 1.4 mmol) was added to the mixture. The reaction mixture was stirred for 2.5 h at room temperature, and then diluted with Et_2O (50 mL) and filtered over a bed of Celite®. Evaporation of solvent *in vacuo* and residual oil was purified by silica gel column chromatography (0–15% EtOAc/ hexane) to obtain methyl PAC4 (33 mg) as a colorless oil. A solution of 2 M NaOH (3.5 mL) was added to a solution of methyl PAC4 (33 mg) in MeOH (6 mL), and reaction mixture was stirred for 2.5 h at room temperature. The pH of the reaction mixture was adjusted to 2 using 1 M HCl and extracted with EtOAc (20 mL × 3). The organic layer was washed, dried, and concentrated as

above. The residual oil was purified by silica gel chromatography (35% EtOAc/ hexane containing 0.2% AcOH) to obtain **(±)-PAC4** (31.9 mg, 32%) as colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 0.89 (3H, t, *J*=6.9 Hz, H_3-5″), 1.05 (3H, s, H_3-8″ or 9″), 1.06 (3H, s, H_3-8″ or 9″), 1.62 (2H, tt, *J*=7.6 and 7.6 Hz, H_2-2″), 2.03 (3H, s, H_3-6), 2.56 (1H, d, *J*=17.1 Hz, H-5′), 2.63 (2H, t, *J*=7.6 Hz, H_2-1″), 2.82 (1H, d, *J*=17.1 Hz, H-5′), 5.74 (1H, s, H-2), 6.39 (1H, d, *J*=16.1 Hz, H-5), 7.37 (1H, dd, *J*=8.2 and 1.6 Hz, H-10′), 7.41 (1H, d, *J*=8.2 Hz, H-7′), 7.83 (1H, d, *J*=16.1 Hz, H-4), 7.86 (1H, d, *J*=1.6 Hz, H-12′). ^{13}C NMR (68MHz, $CDCl_3$): δ_C 14.0, 21.4, 22.5, 23.5, 24.4, 30.8, 31.5, 35.4, 41.1, 49.8, 78.2, 117.9, 126.3, 127.4, 128.0, 130.8, 134.7, 139.1, 143.0, 143.2, 151.3, 170.3, 197.7; UVλ_{max} (MeOH) nm (ε): 212.4 (23300), 255.8 (25400); HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{23}H_{30}O_4Na$, 393.2042; found, 393.2045.

A CHIRALART cellulose-SC HPLC column (250 × 10.0 mm i.d., YMC; solvent, 15% EtOAc in hexane containing 0.1% AcOH; flow rate, 4.7 mL/min; detection, 254 nm) was injected with (±)-PAC4. The material at t_R 13.5 and 16.8 min were collected to give (−)-PAC4 (3.2 mg) and the (+)-enantiomer (3.2 mg) with an optical purity of 100% and 99.9, respectively. (+)-PAC4: $[\alpha]_D^{30}$+ 185.0 (MeOH; *c* 0.21); CD λ_{ext} (MeOH) nm ($\Delta\varepsilon$): 258.0 (12.1), 217.0 (−22.2). (−)-PAC4: $[\alpha]_D^{30}$ − 198.4 (MeOH; *c* 0.21); CD λ_{ext} (MeOH) nm ($\Delta\varepsilon$): 258.0 (−11.9), 216.0 (20.9).

Synthesis of PAT3

6-iodo-2,2-dimethyl-3,4-dihydronaphthalen-1(2H)-one (12)

To a suspension of NaH (4.03 g, 101 mmol) in dry THF (30 mL) was added 6-iodo-1-tetralone **11** (2) (3.08 g, 11.3 mmol) dissolved in THF (10 mL). After stirring for 10 min at room temperature, methyl iodide (2.1 mL, 34 mmol) was added dropwise to the mixture. The mixture was stirred for 2 h at room temperature. After quenching with water (30 mL), it was then extracted with EtOAc (50 mL × 3). The organic layer was washed, dried, and concentrated as above. The residual oil was purified by silica gel chromatography (20% CH_2Cl_2/ hexane) to obtain **12** (2.37 g, 67%) as pale-yellow solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 1.20 (6H, s, 2 × CH_3), 1.96 (2H, t, *J*=6.4 Hz, H_2-5′), 2.93 (2H, t, *J*=6.4 Hz, H_2-4′), 7.64–7.74 (3H, m, H-7′, 10′ and 12′); ^{13}C NMR (68 MHz, $CDCl_3$): δ_C 24.2, 24.2, 25.3, 36.3, 41.5, 101.3, 129.5, 130.8, 136.0, 137.7, 144.9, 202.3.

(*Z*)-1-(5-hydroxy-3-methylpent-3-en-1-yn-1-yl)-6-iodo-2,2-dimethyl-1,2,3,4-tetrahydronaphthalen-1-ol (13)

(*Z*)-3-Mehylpent-2-en-4-yn-1-ol (1.21 g, 12.6 mmol) in dry THF (25 mL) was cooled to −80 °C under an atmosphere of Ar. *n*-Butyllithium (16.1 mL, 1.57 M) was then added slowly. After being stirred for 45 min at −80 °C, a solution of compound **12** (2.37 g, 7.90 mmol) in dry THF (16 mL) was added dropwise to the stirred mixture. The reaction mixture was stirred for a further 10 min at −80 °C and then the ice bath was removed. The reaction mixture was stirred at room temperature for 60 min. After

quenching with sat. NH_4Cl (40 mL), it was then diluted with water (10 mL) and extracted with EtOAc (200 mL × 3). The organic layer was washed, dried, and concentrated as above. The residual oil was purified by silica gel chromatography (25% EtOAc/ hexane) to obtain **13** (3.10 g, 99%) as a yellow oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 1.08 (3H, s, H_3-8′ or 9′), 1.14 (3H, s, H_3-8′ or 9′), 1.45 (1H, d, *J*=5.6 Hz, -OH), 1.68 (1H, dt, *J*=13.5 and 6.2 Hz, H-5′), 1.90 (3H, d, *J*=1.3 Hz, H_3-6), 1.97 (1H, dt, *J*=13.5 and 6.2 Hz, H-5′), 2.24 (1H, s, -HO), 2.80 (2H, t, *J*=6.2 Hz, H_2-4′), 4.30 (2H, t, *J*=6.5 Hz, H_2-1), 5.89 (1H, tq, *J*=6.5 and 1.3 Hz, H-2), 7.48–7.57 (3H, m, H-7′, 10′ and 12′); ^{13}C NMR (68 MHz, $CDCl_3$): δ_C 23.1, 23.2, 23.9, 25.4, 31.1, 37.5, 61.4, 74.8, 84.8, 93.9, 95.9, 120.4, 130.1, 135.5, 136.0, 137.5, 137.8, 138.7; HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{18}H_{21}O_2INa$, 419.0484; found, 419.0492.

(*Z*)-1-(5-hydroxy-3-methylpent-3-en-1-yn-1-yl)-2,2-dimethyl-6-(pent-1-yn-1-yl)-1,2,3,4-tetrahydronaphthalen-1-ol (14)

To a stirred solution of **16** (1.00 g, 2.52 mmol) in triethylamine (15 mL) was added CuI (40.5 mg, 0.21 mmol), bis(triphenylphosphine)palladium(II) dichloride (36 mg, 0.05 mmol) and 1-pentyne (0.35 mL, 3.63 mmol) under an atmosphere of Ar. The reaction mixture was stirred for 2 h at room temperature, and then it was filtered through silica gel (EtOAc). The filtrate was successively washed with 1 M HCl and brine, and then dried and concentrated as above. The same reaction was performed again. The total residual oil was purified by silica gel chromatography (30% EtOAc/ hexane) to obtain **14** (1.48 g, 87%) as a brown oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 1.04 (3H, t, *J*=7.3 Hz, H_3-5″), 1.08 (3H, s, H_3-8′ or 9′), 1.15 (3H, s, H_3-8′ or 9′), 1.62 (2H, tq, *J*=7.3 and 7.3 Hz, H_2-4″), 1.68 (1H, dt, *J*=13.5 and 6.3 Hz, H-5′), 1.90 (3H, dt, *J*=1.3 and 1.3 Hz, H_3-6), 1.98 (1H, m, H-5′), 2.26 (1H, s, -HO), 2.37 (2H, t, *J*=7.3 H, H_2-3″), 2.80 (2H, t, *J*=6.3 Hz, H_2-4′), 4.30 (2H, d, *J*=6.6 Hz, H_2-1), 5.87 (1H, tq, *J*=6.6 and 1.3 Hz, H-2), 7.16 (1H, d, *J*=1.6 Hz, H-12′), 7.25 (1H, dd, *J*=8.2 and 1.6 Hz, H-10′), 7.70 (1H, d, *J*=8.2 Hz, H-7′); ^{13}C NMR (68 MHz, $CDCl_3$): δ_C 13.5, 21.4, 22.2, 23.1, 23.3, 23.9, 25.5, 31.2, 37.5, 61.4, 74.9, 80.4, 84.7, 90.5, 96.2, 120.6, 123.6, 128.0, 129.5, 132.1, 134.9, 135.8, 138.2; HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{23}H_{28}O_2Na$, 359.1987; found, 359.1994.

1-((1*E*,3*Z*)-5-hydroxy-3-methylpenta-1,3-dien-1-yl)-2,2-dimethyl-6-(pent-1-yn-1-yl)-1,2,3,4-tetrahydronaphthalen-1-ol (15)

To a stirred solution of **14** (1.48 g, 4.40 mmol) in dry THF (33 mL) was cooled to 0 °C and added SMEAH (4.2 mL, 15.1 mmol). The mixture was allowed to warm up slowly to room temperature and was stirred for 1 h. After quenching with sat. aq. Rochelle salt (30 mL), it was extracted with EtOAc (40 mL × 3). The organic layer was washed, dried, and concentrated as above. The residual oil was purified by silica gel chromatography (30% EtOAc/ hexane) to obtain **15** (1.42 g, 95%) as a yellow oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 0.96 (3H, s, H_3-8′ or 9′), 0.98 (3H, s, H_3-8′ or 9′), 1.04 (3H, t, *J*=7.3 Hz, H_3-5″), 1.62 (2H, tq, *J*=7.3 and 7.3 Hz, H_2-4″), 1.67 (1H, m, H-5′), 1.84 (1H, m, H-5′), 1.85

(3H, d, J=1.0 Hz, H_3-6), 2.37 (2H, t, J=7.3 H, H_2-3″), 2.81 (2H, t, J=6.6 Hz, H_2-4′), 4.29 (2H, d, J=6.9 Hz, H_2-1), 5.56 (1H, t, J=6.9 Hz, H-2), 5.94 (1H, d, J=16.5 Hz, H-5), 6.65 (1H, d, J=16.5 Hz, H-4), 7.17 (1H br s, H-12′), 7.18 (1H, dd, J=8.2 and 1.6 Hz, H-10′), 7.30 (1H, d, J=8.2 Hz, H-7′); ^{13}C NMR (68 MHz, $CDCl_3$): δ_C 13.5, 20.7, 21.4, 22.2, 22.9, 24.0, 25.7, 32.8, 37.0, 58.5, 78.0, 80.5, 90.2, 122.9, 125.7, 128.0, 128.1, 129.4, 131.9, 134.7, 135.2, 135.7, 140.2; HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{23}H_{30}O_2Na$, 361.2143; found, 361.2146.

Methyl (2*Z*,4*E*)-5-(1-hydroxy-2,2-dimethyl-6-(pent-1-yn-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yl)-3-methylpenta-2,4-dienoate (16)

To a stirred solution of **15** (1.41 g, 4.17 mmol) in dry acetone (45 mL) was added MnO_2 (7.24 g, 83.3 mmol) at room temperature. After stirring at room temperature for 30 min, all the starting material had disappeared. The reaction mixture was then filtered through a pad of Celite® and concentrated *in vacuo*. The crude aldehyde (1.36 g) was dissolved in MeOH (35 mL) and stirred with MnO_2 (7.24 g, 83.3 mmol), NaCN (612 mg, 12.5 mmol) and AcOH (0.24 mL, 4.17 mmol) at room temperature. After 60 min, the reaction mixture was filtered through a pad of Celite® and concentrated *in vacuo*. The residue was brought up in distilled water and extracted with EtOAc (30 mL × 3). The organic layer was washed, dried, and concentrated as above. The residual oil was purified by silica gel chromatography (12% EtOAc/ hexane) to obtain **16** (745 mg, 49%) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 0.96 (3H, s, H_3-8′ or 9′), 1.00 (3H, s, H_3-8′ or 9′), 1.04 (3H, t, J=7.3 Hz, H_3-5″), 1.55–1.74 (3H, m, H_2-4″ and H-5′), 1.89 (1H, m, H-5′), 1.98 (3H, s, H_3-6), 2.37 (2H, t, J=6.9 Hz, H_2-3″), 2.83 (2H, t, J=6.6 Hz, H_2-4′), 3.69 (3H, s, -OCH_3), 5.69 (1H, s, H-2), 6.28 (1H, d, J=15.8 Hz, H-5), 7.17 (1H, s, H-12′), 7.19 (1H, d, J=8.2 Hz, H-10′), 7.30 (1H, d, J=8.2 Hz, H-7′), 7.79 (1H, d, J=15.8 Hz, H-4); ^{13}C NMR (68 MHz, $CDCl_3$): δ_C 13.5, 21.2, 21.4, 22.2, 22.9, 24.1, 25.6, 32.9, 37.2, 51.0, 77.9, 80.5, 90.2, 117.1, 123.0, 126.7, 128.1, 129.5, 131.9, 135.6, 139.8, 141.0, 150.2, 166.6; HRMS (*m/z*) :$[M+Na]^+$ calcd. for $C_{24}H_{30}O_3Na$, 389.2092; found, 389.2096.

(2Z,4E)-5-(1-hydroxy-2,2-dimethyl-4-oxo-6-(pent-1-yn-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yl)-3-methylpenta-2,4-dienoic acid, (±)-PAT3 (3)

The methyl ester **19** (899 mg, 2.45 mmol) in dry benzene (20 mL) was added Celite® (4.5 g) and pyridinium dichromate (3.75 g, 10.0 mmol). After being stirred for 10 min, 70% *tert*-butyl hydroperoxide (1.65 mL, 12.9 mmol) was added to the mixture. The reaction mixture was stirred for 2.5 h at room temperature, and then filtered over a bed of Celite®. Evaporation of solvent *in vacuo* and residual oil was purified by silica gel column chromatography (0–20% EtOAc/ hexane) to obtain methyl PAT3 (378 mg) as a yellow oil. A solution of 1 M NaOH (10 mL) was added to a solution of methyl PAT3 (378 mg) in MeOH (20 mL), and reaction mixture was stirred for 7.5 h at room temperature. The pH of the reaction mixture was adjusted to 2 using 1 M HCl and extracted with

EtOAc (50 mL × 3). The organic layer was washed, dried, and concentrated as above. The residual oil was purified by silica gel chromatography (0–35% EtOAc/ hexane containing 0.2% AcOH) to obtain (±)-PAT3 (124 mg, 14%) as a pale-yellow oil, which was further purified for bioassays by HPLC (YMC ODS-AQ, 150 × 20.0 mm i.d.; solvent, 80% MeOH in water containing 0.05% AcOH; flow rate, 8 ml min^{-1}; detection, 254 nm) to obtain a colorless oil. ^{1}H NMR ($CDCl_3$, 270 MHz): δ_H 1.04 (3H, t, *J*=7.3 Hz, H_3-5″), 1.05 (3H, s, H_3-8′ or 9′), 1.07 (3H, s, H_3-8′ or 9′), 1.62 (2H, tq, *J*=7.3 and 7.3 Hz, H_2-4″), 2.02 (3H, s, H_3-6), 2.37 (1H, t, *J*=7.3 Hz, H_2-3″), 2.59 (1H, d, *J*=17.4 Hz, H-5′), 2.81 (1H, d, *J*=17.4 Hz, H-5′), 5.74 (1H, s, H-2), 6.36 (1H, d, *J*=15.8 Hz, H-5), 7.47 (1H, d, *J*=7.9 Hz, H-7′), 7.57 (1H, dd, *J*=7.9 and 1.6 Hz, H-10′), 7.80 (1H, d, *J*=15.8 Hz, H-4), 8.06 (1H, d, *J*=1.6 Hz, H-12′); ^{13}C NMR ($CDCl_3$, 68 MHz): δ_C 13.5, 21.4, 21.4, 22.1, 23.4, 24.3, 41.0, 49.7, 78.2, 79.6, 91.9, 117.9, 124.4, 127.3, 128.4, 129.8, 130.8, 137.1, 138.7, 144.6, 151.5, 170.7, 196.8; UVλ_{max} (MeOH) nm (ε) 236.7 (41,100), 255.1 (30,600), 316.6 (3,400); (HRMS (*m/z*): $[M+Na]^+$ calcd. for $C_{23}H_{26}O_4Na$, 389.1728; found, 389.1732.

A CHIRALART cellulose-SC HPLC column (250 × 10.0 mm i.d., YMC; solvent, 5% EtOAc in $CHCl_3$ containing 0.1% AcOH; flow rate, 4.7 mL/min; detection, 254 nm) was injected with (±)-PAT3. The material at t_R 8.8 and 10.7 min were collected to give (−)-PAT3 (7.2 mg) and the (+)-enantiomer (6.6 mg) with an optical purity of 99.8% and 98.9, respectively. (+)-PAT3: $[\alpha]_D^{25}$ +138.6 (MeOH; *c* 0.23); CD λ_{ext} (MeOH) nm ($\Delta\varepsilon$): 266.0 (16.4), 236.0 (−14.0). (−)-PAT3: $[\alpha]_D^{25}$ −132.4 (MeOH; *c* 0.27); CD λ_{ext} (MeOH) nm ($\Delta\varepsilon$): 265.0 (−15.0), 236.0 (12.9).

Synthesis of PATT1

(Z)-1-(5-hydroxy-3-methylpent-3-en-1-yn-1-yl)-2,2-dimethyl-6-((trimethylsilyl)ethynyl)-1,2,3,4-tetrahydronaphthalen-1-ol (17)

To a stirred solution of **13** (4.49 g, 11.33 mmol) in triethylamine (40 mL) was added CuI (151 mg, 0.80 mmol), bis(triphenylphosphine)palladium(II) dichloride (168 mg, 0.24 mmol) and trimethylsilylacetylene (2.35 mL, 17.0 mmol) under an atmosphere of Ar. The reaction mixture was stirred for 40 min at room temperature, and then it was filtered through silica gel (EtOAc). The filtrate was successively washed with 1 M HCl and brine, and then dried and concentrated as above. The residual oil was purified by silica gel chromatography (30% EtOAc/ hexane) to obtain **17** (3.75 g, 90%) as an orange oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 0.25 (9H, s, 3 × CH_3-Si), 1.08 (3H, s, H_3-8′or 9′), 1.13 (3H, s, H_3-8′ or 9′), 1.70 (1H, dt, *J*=13.6 and 6.3 Hz, H-5′),1.89 (3H, d, *J*=1.5 Hz ,H_3-6), 1.97 (1H, dt, *J*=13.6 and 6.3 Hz, H-5′), 2.80 (2H, t, *J*=6.3 Hz, H_2-4′), 4.28 (2H, d, *J*=6.4 Hz, H_2-1), 5.88 (1H, tq, *J*=6.4 and 1.5 Hz, H-2), 7.23 (1H, d, *J*=1.4 Hz, H-12′),7.32 (1H, dd, *J*=8.0 and 1.4 Hz, H-10′), 7.72 (1H, d, *J*=8.0 Hz, H-7′); ^{13}C NMR (68 MHz, $CDCl_3$): δ_C 0.41, 0.41, 0.41, 23.6, 23.6, 24.4, 25.9, 31.7, 38.0, 61.9, 75.3, 85.3, 94.8, 95.6, 105.3, 120.9, 123.0, 128.4, 130.3, 133.0, 135.4, 136.4, 139.8; HRMS (*m/z*) :$[M+Na]^+$ calcd. for $C_{23}H_{30}O_2Si_1Na$, 389.1913; found, 389.1910.

6-ethynyl-1-((1E,3Z)-5-hydroxy-3-methylpenta-1,3-dien-1-yl)-2,2-dimethyl-1,2,3,4-tetrahydronaphthalen-1-ol (18)

To a stirred solution of **17** (3.75 g, 10.23 mmol) in dry THF (50 mL) was cooled to 0 °C and added SMEAH (10.0 mL, 35.8 mmol). The mixture was allowed to warm up slowly to room temperature and was stirred for 1 h. After quenching with sat. aq. Rochelle salt (30 mL), it was extracted with EtOAc (60 mL × 3). The organic layer was washed, dried, and concentrated as above. The crude material (4.56 g) was carried through to the next stage without purification. The crude material was dissolved in MeOH (55 mL) and stirred with K_2CO_3 (2.68 g, 19.4 mmol) for 30 min at room temperature. After quenching with water (70 mL), it was concentrated *in vacuo* to remove MeOH. The resulting mixture was extracted with EtOAc (80 mL × 3), washed with brine, dried, and concentrated as above. The residual oil was purified by silica gel chromatography (40% EtOAc/ hexane) to obtain **18** (2.54 g, 84%) as an orange oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 0.97 (3H, s, H3-8′ or 9′), 0.98 (3H, s, H3-8′ or 9′), 1.69 (1H, dt, *J*=13.8 and 6.9 Hz, H-5′), 1.85 (1H, dt, *J*=13.8 and 6.9 Hz, H-5′), 1.85 (3H, d, *J*=1.0 Hz, H_3-6), 2.83 (2H, t, *J*=6.9 Hz, H_2-4′), 3.04 (1H, s, alkyne), 4.29 (2H, d, *J*=6.9 Hz, H_2-1), 5.57 (1H, t, *J*=6.9 Hz, H-2), 5.94 (1H, d, *J*=15.5 Hz, H-5), 6.65 (1H, dd, *J*=15.5 and 1.0 Hz, H-4), 7.26–7.37 (3H, m, H-7′, 10′ and 12′); ^{13}C NMR (68 MHz, $CDCl_3$): δ_C 20.7, 22.9, 24.0, 25.6, 32.8, 37.0, 58.5, 77.2, 78.0, 83.5, 120.9, 125.8, 128.2, 128.3, 130.0, 132.5, 134.6, 134.9, 135.9, 141.7; HRMS (*m/z*) :$[M+Na]^+$ calcd. for $C_{20}H_{24}O_2Na$, 319.1674; found, 319.1679.

methyl (2*Z*,4*E*)-5-(6-ethynyl-1-hydroxy-2,2-dimethyl-1,2,3,4-tetrahydronaphthalen-1-yl)-3-methylpenta-2,4-dienoate (19)

To a stirred solution of **18** (2.54 g, 8.57 mmol) in dry acetone (80 mL) was added MnO_2 (14.8 g, 171 mmol) at room temperature. After stirring at room temperature for 30 min, all the starting material had disappeared. The reaction mixture was then filtered through a pad of Celite® and concentrated *in vacuo*. The crude aldehyde was dissolved in MeOH (80 mL) and stirred with MnO_2 (14.8 g, 171 mmol), NaCN (1.26 g, 25.7 mmol) and AcOH (0.49 mL, 8.6 mmol) at room temperature. After 60 min, the reaction mixture was filtered through a pad of Celite® and concentrated *in vacuo*. The residue was brought up in distilled water and extracted with EtOAc (100 mL × 3). The organic layer was washed with brine, dried, and concentrated as above. The residual oil was purified by silica gel chromatography (10% EtOAc/ hexane) to obtain **19** (1.70 g, 61%) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 0.98 (3H, s, H_3-8′ or 9′), 1.00 (3H, s, H_3-8′ or 9′), 1.72 (1H, dt, *J*=13.8 and 6.9 Hz, H-5′), 1.87 (1H, dt, *J*=13.8 and 6.9 Hz, H-5′), 1.98 (3H, d, *J*=1.3 Hz, H_3-6), 2.85 (2H, t, *J*=6.9 Hz, H_2-4′), 3.03 (1H, s, alkyne), 3.69 (3H, s, -OCH_3), 5.70 (1H, br s, H-2), 6.28 (1H, d, *J*=15.8 Hz, H-5), 7.27–7.38 (3H, m, H-7′, 10′ and 12′), 7.78 (1H, d, *J*=15.8 Hz, H-4); ^{13}C NMR (68 MHz, $CDCl_3$): δ_C 21.2, 22.8, 24.1, 25.6, 32.8, 37.1, 51.0, 77.2, 78.0, 83.6, 117.3, 120.9, 126.8, 128.2, 130.0, 132.6, 135.8,

140.8, 141.3, 150.1, 166.6; HRMS (*m/z)* :[M+Na]$^+$ calcd. for $C_{21}H_{24}O_3Na$, 347.1623; found, 347.1631.

methyl (2*Z*,4*E*)-5-(6-(bromoethynyl)-1-hydroxy-2,2-dimethyl-1,2,3,4-tetrahydronaphthalen-1-yl)-3-methylpenta-2,4-dienoate (20)

To a stirred solution of **19** (1.69 g, 5.21 mmol) in acetone (30 mL) was added *N*-bromosuccinimide (1.12 g, 6.28 mmol) and silver nitrate (88 mg, 0.52 mmol) at room temperature. After being stirred for 60 min at room temperature, it was concentrated *in vacuo* to remove acetone. The residue was brought up in water and extracted with Et_2O (30 mL × 3). The organic layer was washed with brine, dried, and concentrated as above. The residual oil was purified by silica gel chromatography (10% EtOAc/ hexane) to obtain **20** (1.28 g, 61%) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 0.97 (3H, s, H_3-8′ or 9′), 1.00 (3H, s, H_3-8′ or 9′), 1.71 (1H, dt, *J*=13.8 and 6.9 Hz, H-5′), 1.86 (1H, *J*=13.8 and 6.9 Hz, H-5′), 1.98 (3H, d, *J*=1.0 Hz, H_3-6), 2.84 (2H, t, *J*=6.9 Hz, H_2-4′), 3.69 (3H, s, -OCH_3), 5.70 (1H, br s, H-2), 6.28 (1H, d, *J*=16.2 Hz, H-5), 7.22–7.37 (3H, m, H-7′, 10′ and 12′), 7.78 (1H, d, *J*=16.2 Hz, H-4); ^{13}C NMR (68 MHz, $CDCl_3$): δ_C 21.2, 22.8, 24.1, 25.6, 32.8, 37.1, 49.5, 51.1, 78.0, 79.9, 117.3, 121.5, 126.8, 128.3, 129.9, 132.4, 135.8, 140.7, 141.2, 150.1, 166.6; HRMS (*m/z*) :[M+Na]$^+$ calcd. for $C_{21}H_{24}O_3BrNa$, 425.0728; found, 425.0729.

methyl (2*Z*,4*E*)-5-(1-hydroxy-2,2-dimethyl-6-(penta-1,3-diyn-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yl)-3-methylpenta-2,4-dienoate (21)

To a stirred solution of **20** (0.73 g, 1.81 mmol) in THF (11 mL) was added propyne in THF 5% w/w (3.6 mL, 3.6 mmol), CuI (17 mg, 0.09 mmol), bis(triphenylphosphine)palladium(II) dichloride (32 mg, 0.05 mmol) and diisopropylamine (0.51 mL, 3.6 mmol) under an atmosphere of N_2. After stirring for 60 min at room temperature, it was quenched with 1 M HCl (15 mL) and extracted with EtOAc (30 mL × 3). The organic layer was washed, dried and concentrated, as described above. The residual oil was purified by silica gel chromatography (10% EtOAc/ hexane) to obtain **21** (295 mg, 45%) as a brown oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 0.97 (3H, s, H_3-8′ or 9′), 1.00 (3H, s, H_3-8′ or 9′), 1.71 (1H, dt, *J*=13.8 and 6.9 Hz, H-5′), 1.86 (1H, dt, *J*=13.8 and 6.9 Hz, H-5′), 1.88 (1H, s, -OH), 1.98 (3H, d, *J*=1.3 Hz, H_3-6), 2.01 (3H, s, -CCH_3), 2.83 (2H, t, *J*=6.9 Hz, H_2-4′), 3.69 (3H, s, -OCH_3), 5.70 (1H, br s, H-2), 6.27 (1H, d, *J*=16.1 Hz, H-5), 7.25 (1H, br s, H-12′), 7.26 (1H, dd, *J*=8.2 and 1.6 Hz, H-10′), 7.35 (1H, d, *J*=8.2 Hz, H-7′), 7.78 (1H, d, *J*=16.1 Hz, H-4); ^{13}C NMR (68 MHz, $CDCl_3$): δ_C 4.6, 21.2, 22.8, 24.1, 25.6, 32.8, 37.1, 51.1, 64.4, 74.1, 74.2, 78.0, 80.2, 117.3, 120.9, 126.8, 128.3, 130.4, 132.9, 135.8, 140.7, 141.4, 150.1, 166.6; HRMS (*m/z*) :[M+Na]$^+$ calcd. for $C_{24}H_{26}O_3Na_1$, 385.1779; found, 385.1778.

(2*Z*,4*E*)-5-(1-hydroxy-2,2-dimethyl-4-oxo-6-(penta-1,3-diyn-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yl)-3-methylpenta-2,4-dienoic acid, (±)-PATT1 (4)

To a stirred solution of **21** (348 mg, 0.96 mmol) in acetone (5 mL) was added $Co(acac)_2$ (25 mg, 0.096 mmol) and 70% *tert*-butyl hydroperoxide (0.15 mL, 1.15 mmol), and then the mixture stirred for 48 h at room temperature. After quenching with water (20 mL), the resulting mixture was extracted with EtOAc (20 mL × 3). The organic layer was washed, dried, and concentrated as above. The residual oil was purified by silica gel chromatography (0–25% EtOAc/ hexane) to obtain methyl PATT1 (85 mg) as a yellow oil. A solution of 2 M NaOH (4 mL) was added to a solution of methyl PATT1 (77 mg) in MeOH (5 mL), and reaction mixture was stirred for 3.5 h at room temperature. The pH of the reaction mixture was adjusted to 2 using 1 M HCl; it was diluted with water (10 mL) and extracted with EtOAc (15 mL × 3). The organic layer was washed, dried, and concentrated as above. The residual oil was purified by silica gel chromatography (0–35% EtOAc/ hexane containing 0.2% AcOH) to obtain (±)-PATT1 (58.7 mg, 17%) as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ_H 1.06 (3H, s, H_3-8′ or 9′), 1.10 (3H, s, H_3-8′ or 9′), 2.02 (3H, s, -CCH_3), 2.03 (3H, d, *J*=1.0 Hz, H_3-6), 2.63 (1H, d, *J*=17.1 Hz, H-5′), 2.78 (1H, d, *J*=17.1 Hz, H-5′), 5.75 (1H, br s, H-2), 6.37 (1H, d, *J*=15.8 Hz, H-5), 7.54 (1H, d, *J*=7.9 Hz, H-7′), 7.67 (1H, dd, *J*=7.9 and 1.6 Hz, H-10′), 7.74 (1H, d, *J*=15.8 Hz, H-4), 8.15 (1H, d, *J*=1.6 Hz, H-12′); ^{13}C NMR (68 MHz, CD_3OD): δ_C 3.9, 21.2, 23.8, 24.6, 42.2, 50.7, 64.6, 73.4, 76.2, 79.0, 81.9, 119.6, 123.0, 129.7, 130.0, 130.8, 132.6, 138.5, 140.1, 148.6, 151.0, 169.4, 198.5; UVλ_{max} (MeOH) nm (ε): 221.4 (40000), 251.2 (33300), 274.2 (29100), 289.6 (25200); HRMS (*m/z*) :$[M+Na]^+$ calcd. for $C_{23}H_{22}O_4Na$, 385.1416; found, 385.1417.

A CHIRALART cellulose-SC HPLC column (250 × 10.0 mm i.d., YMC; solvent, 4% isopropanol in $CHCl_3$ containing 0.1% AcOH; flow rate, 4.7 mL/min; detection, 254 nm) was injected with (±)-PATT1. The material at t_R 7.7 and 9.5 min were collected to give (−)-PATT1 (8.7 mg) and the (+)-enantiomer (8.6 mg) with an optical purity of 100% and 99.9, respectively. (+)-PATT1: $[\alpha]_D^{25}$ +233 (MeOH; *c* 0.098); CD λ_{ext} (MeOH) nm ($\Delta\varepsilon$): 278.0 (14.8), 223.0 (−18.0). (−)-PATT1: $[\alpha]_D^{25}$ −239 (MeOH; *c* 0.098); CD λ_{ext} (MeOH) nm ($\Delta\varepsilon$): 279.0 (−18.5), 222.0 (17.0).

Seed germination assays

The classic definition of radical emergence was used for seed germination assays. All assays were performed at least three times. For *Arabidopsis*, 30–50 seeds (Columbia accession) were sterilized by soaking in 70% aqueous ethanol (EtOH, v/v) for 30 min and reagent-grade EtOH for 1 min. Seeds were then soaked in distilled water and incubated in the dark at 4°C for 3 days. The stratified seeds were then soaked in 0.1 mL of a test medium liquid agar containing 1/2 Murashige and Skoog (MS) in 96-well plates and allowed to germinate under continuous illumination at 22°C.

For rice, 30 seeds (*Oryza sativa* L. cv. Nipponbare) were sterilized with reagent-grade EtOH for 5 min and washed with running tap water. They were placed in a dish on two sheets of filter paper soaked in 4 mL of a test solution and allowed to germinate under continuous illumination at 30°C.

Rice seedling elongation assay

Seven seeds (*Oryza sativa* L. cv. Nipponbare) were sterilized with reagent-grade EtOH for 5 min and washed with running tap water. They were then soaked in distilled water and incubated under continuous illumination at 30°C for 2 days to germinate. The germinated seeds were then soaked in 2 mL of a test medium in a glass tube and grown under continuous illumination at 30°C. When the seedlings were 7 days old, the length of the second leaf sheath was measured. All assays were performed at least three times.

PP2C phosphatase assays

The PP2C phosphatase assays were performed as described previously[3] with some modification. Briefly, PYLs (AtPYLs and OsPYL2) and PP2Cs (HAB1 and OsPP2C06) were expressed in *E. coli* and purified by affinity column chromatography. Purified proteins were preincubated in 80 µL of a buffer containing 1.25 mM $MnCl_2$ and test compound at 22°C for 20 min. After adding 20 µL of substrate buffer (165 mM Tris-acetate, pH 7.9, 330 mM potassium acetate, 0.1% BSA, and 25 mM *p*NPP), reactions were immediately monitored for hydrolysis of *p*NPP at 405 nm using a microplate reader (Multiskan Sky, Thermo Fisher Scientific, USA). For AtPYL, reactions contained 600 nM HAB1 and 1200 nM AtPYL (PYR1, PYL1–6, and PYL8–9) proteins. For OsPYL, reactions contained 600 nM OsPP2C06 and 3000 nM OsPYL2.

Pull-down assay

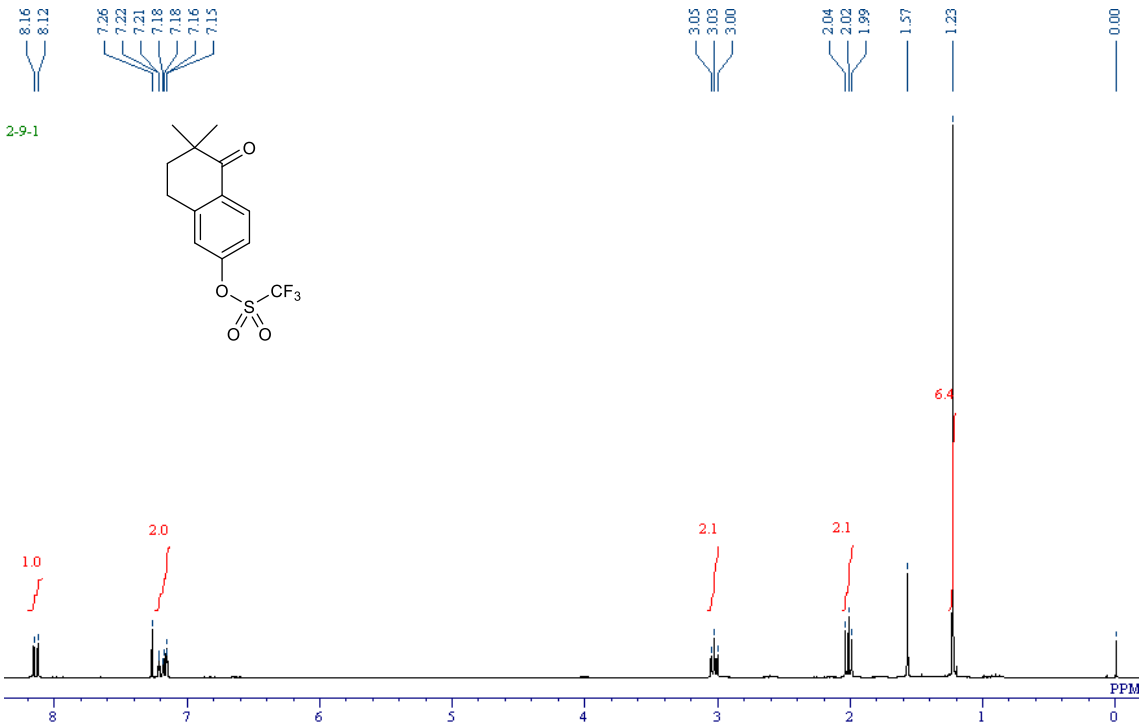
The protocol of the pull-down assay was described elsewhere[4]. Briefly, purified GST-HAB1 and 6xHis-tagged PYL2 were used 50 µg and 10 µg, respectively, and were incubated in 300 µL of Tris-buffered saline (TBS) containing 100 µg BAS, 0.025% 2-mercaptoethanol, 10 mM $MnCl_2$ and 20 µL Anti-His tag Beads (MBL, Co., Ltd.) in the presence or absence of test compounds with gentle shaking at 4 °C for 60 min. After washing the beads, bound proteins were eluted using a His-tagged protein purification kit (MBL, Co., Ltd.) according to the manufacturer's instructions. The eluted proteins were denatured with SDS-sample buffer at 95 °C for 5 min. Then, 5 µL of the denatured proteins were loaded on a 10% SDS-PAGE gel, and proteins were detected after development by EzStain AQua (ATTO, Co., Ltd.) staining.

Isothermal titration calorimetry

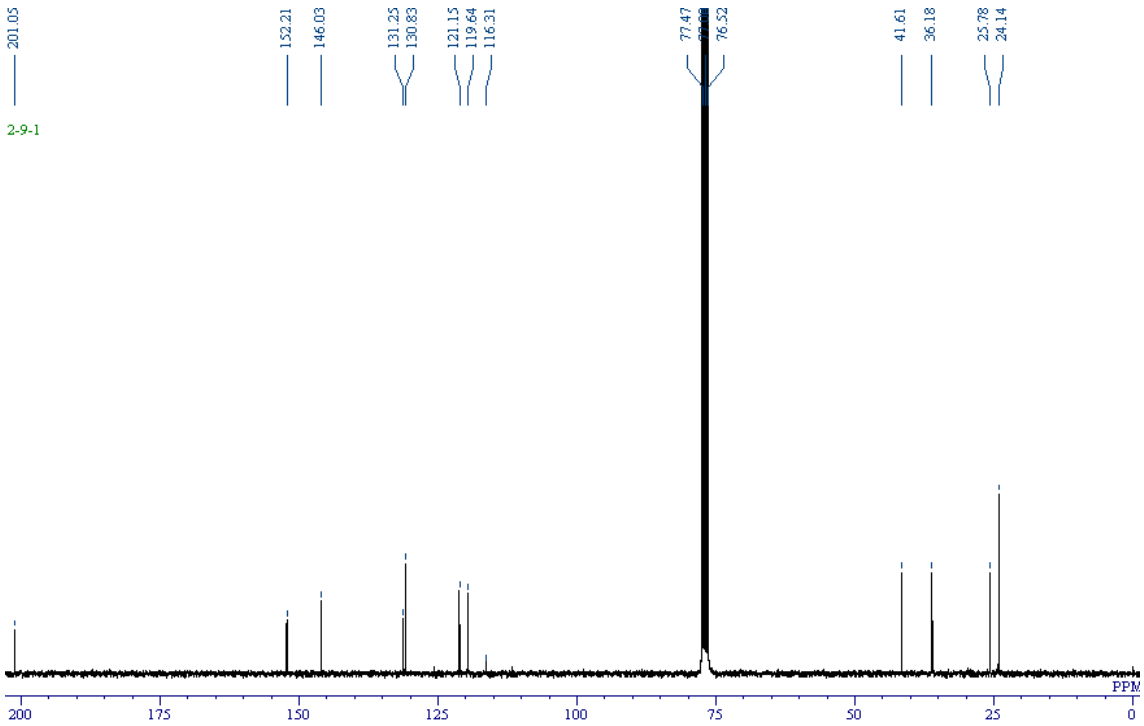
The ITC experiments were performed with an iTC_{200} calorimeter (Microcal, GE Healthcare Bio-Sciences AB) as described previously[3]. Briefly, His_6-tagged PYL5 was assayed at a concentration of 25 or 30 µM, with (+)-PAO4 analogs stock solutions in the injection syringe at a concentration of 500 µM. All titrations were carried out via a series of 25 injections of 1.25 µL or 1.5 µL each. The data were corrected by subtracting the mixing enthalpies for the (+)-PAO4 analogs solutions into protein-

3) ^{1}H and ^{13}C NMR Spectrums of Synthesized Compounds

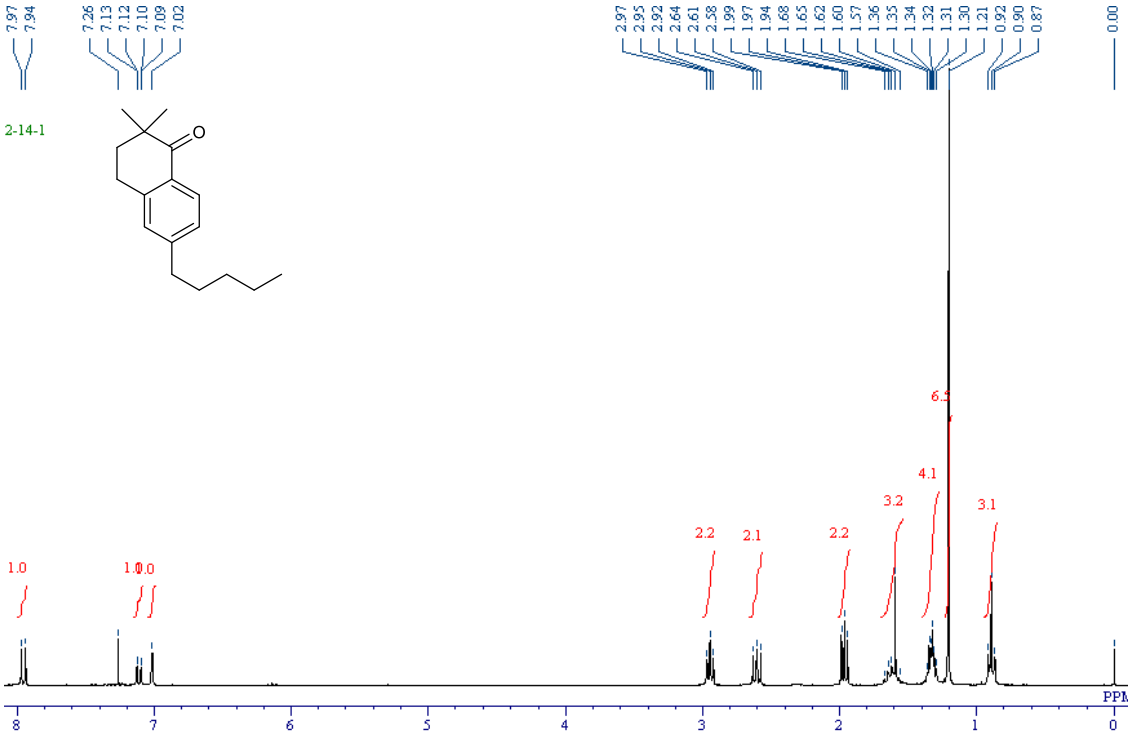
^{1}H NMR spectrum of Compound 6



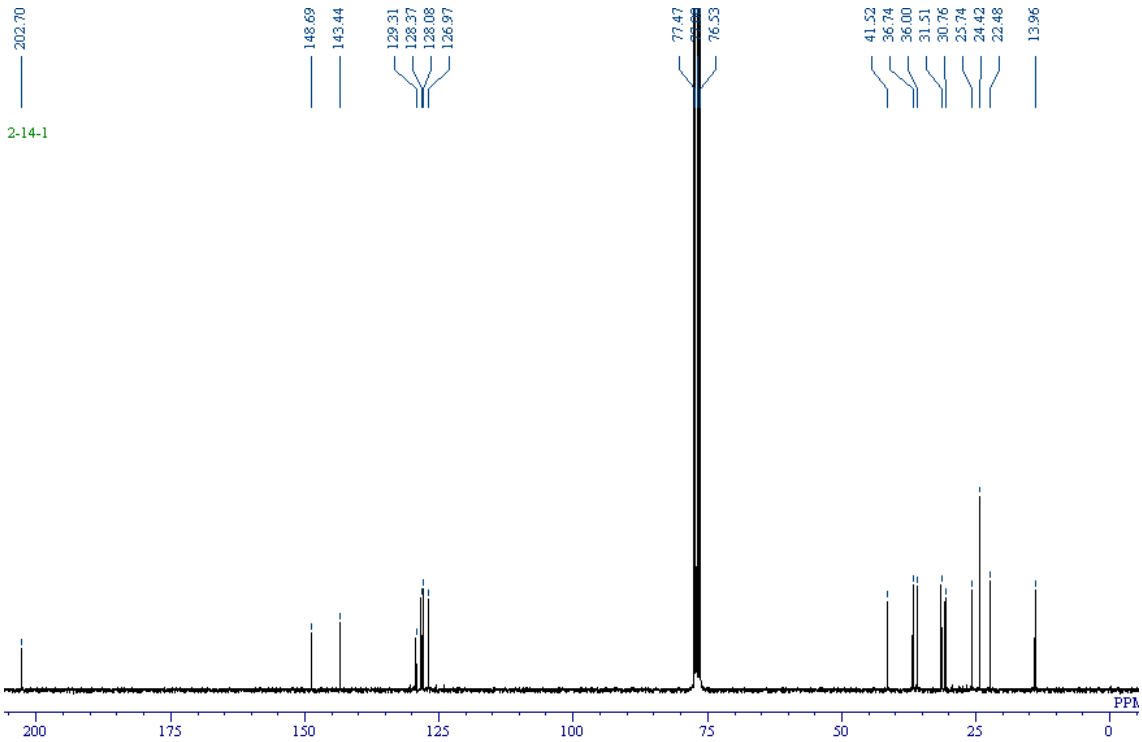
^{13}C NMR spectrum of Compound 6



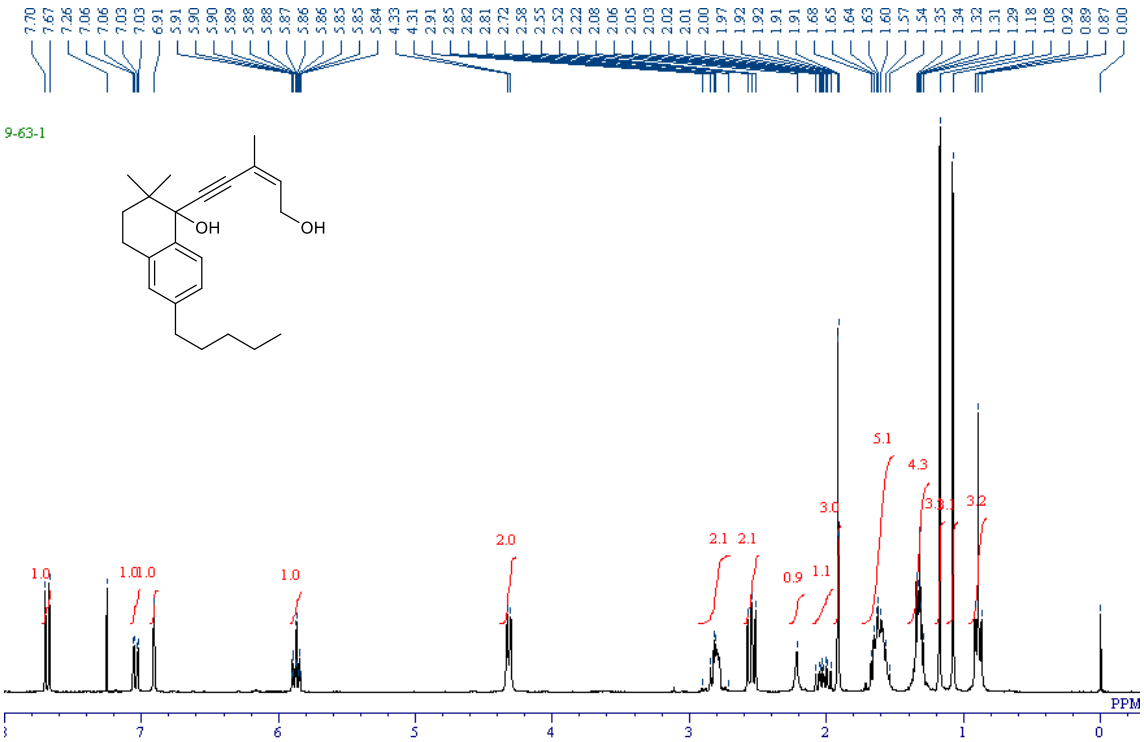
^{1}H NMR spectrum of Compound 7



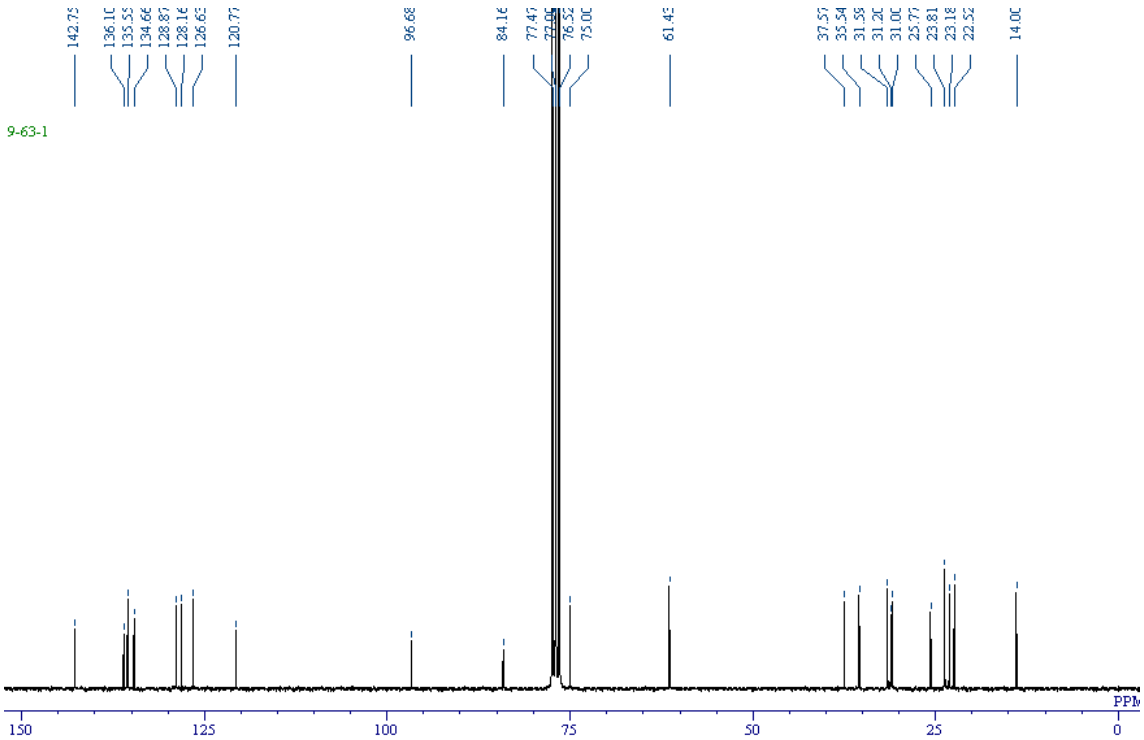
^{13}C NMR spectrum of Compound 7



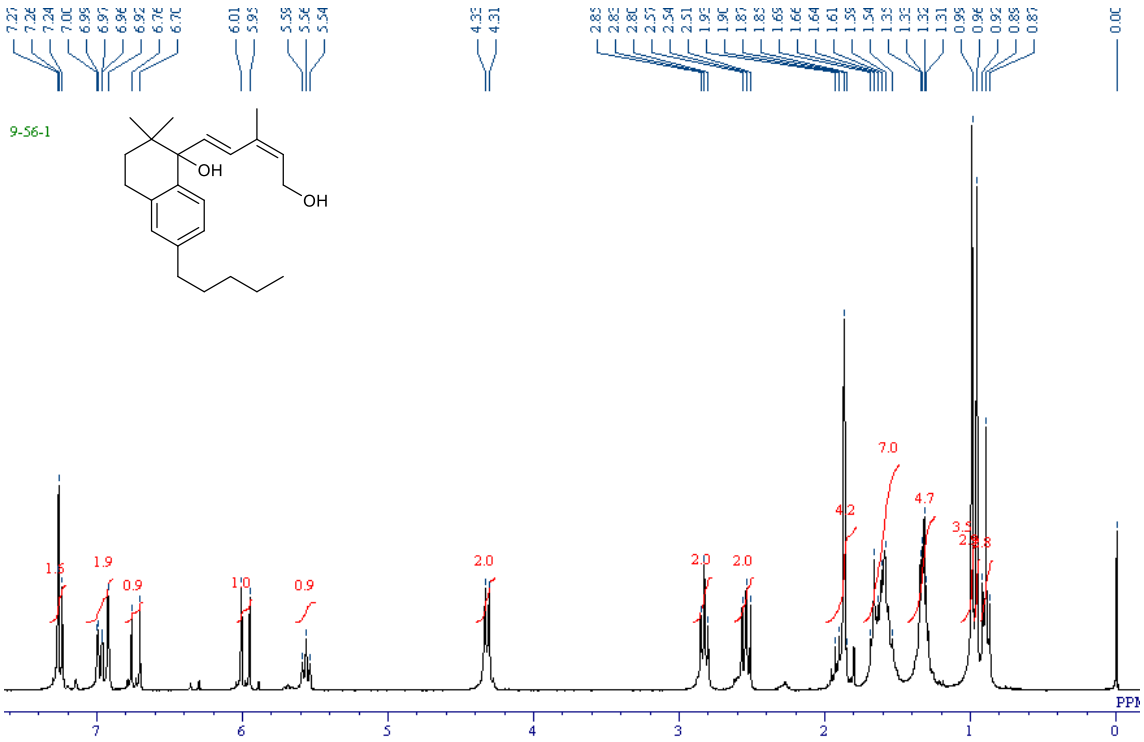
^{1}H NMR spectrum of Compound 8



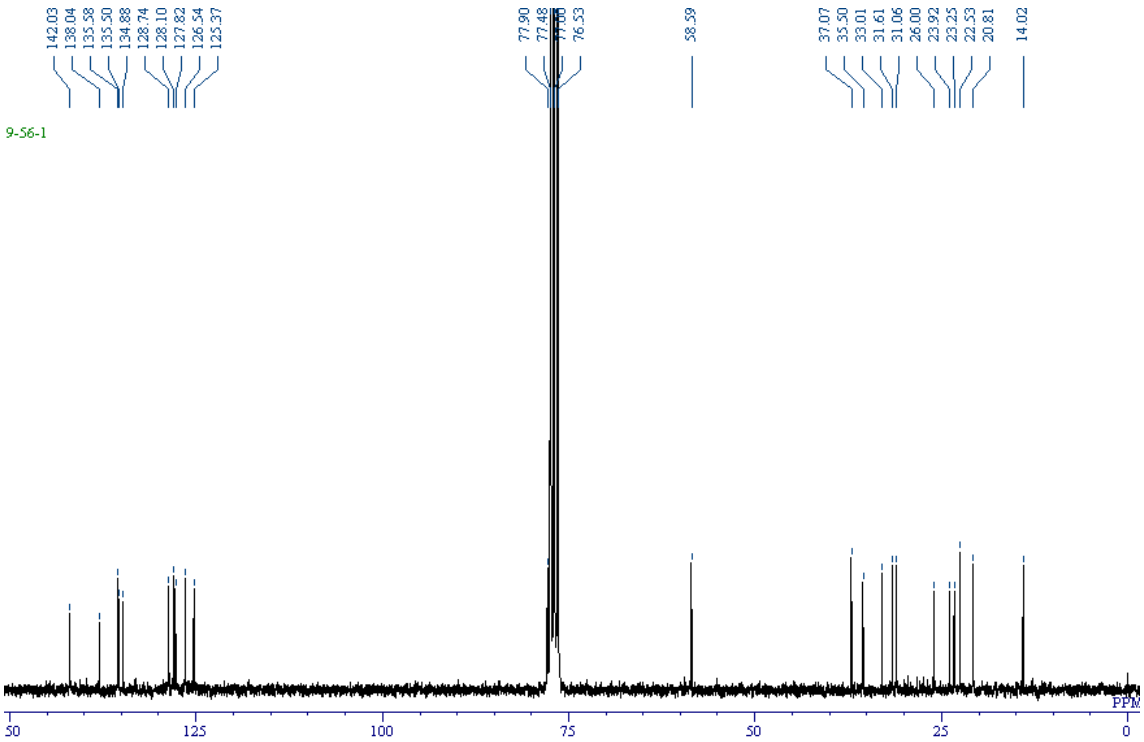
^{13}C NMR spectrum of Compound 8



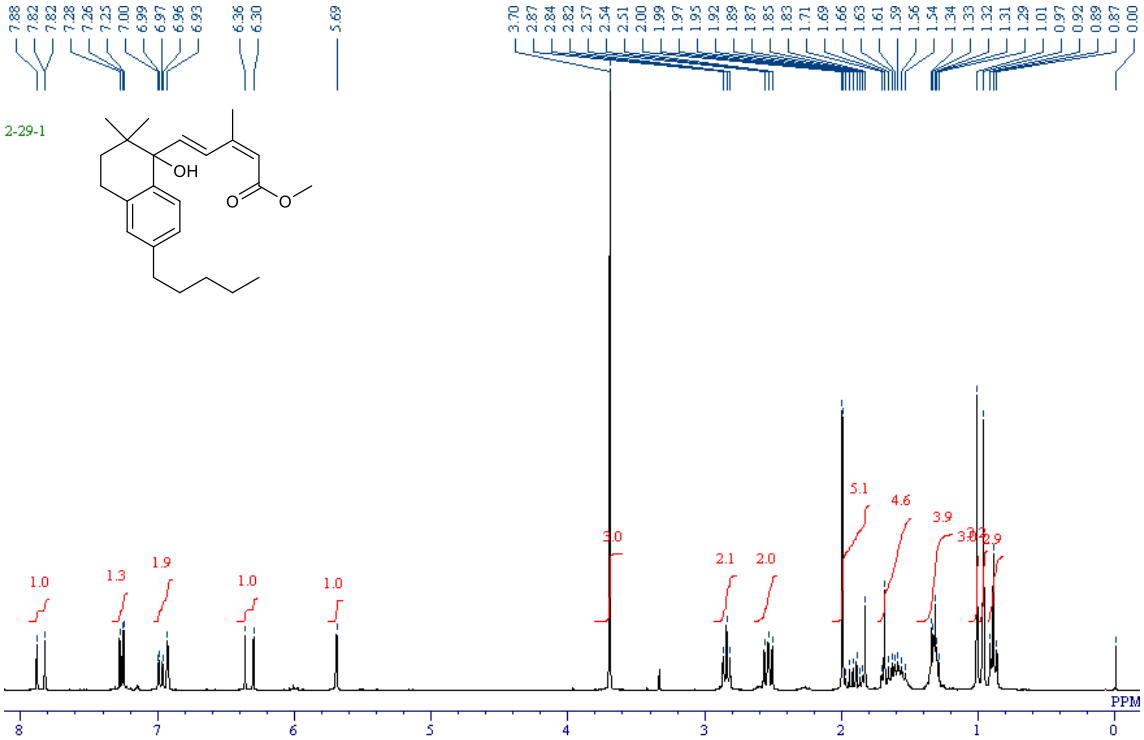
^{1}H NMR spectrum of Compound 9



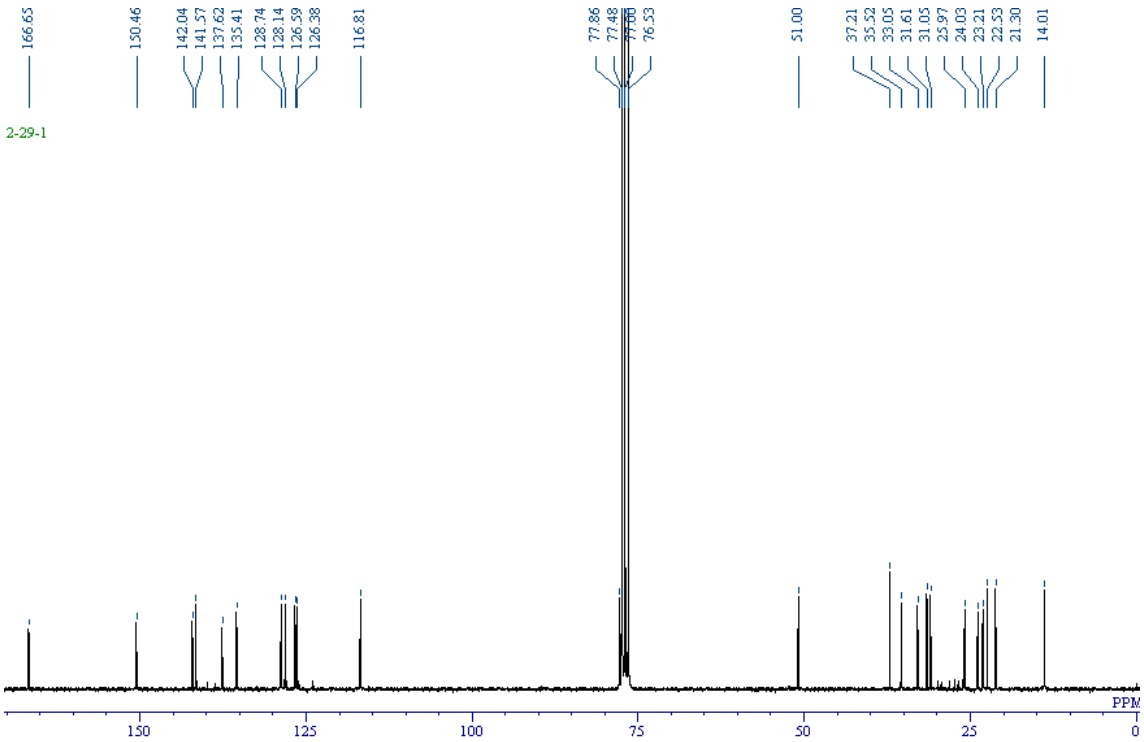
^{13}C NMR spectrum of Compound 9



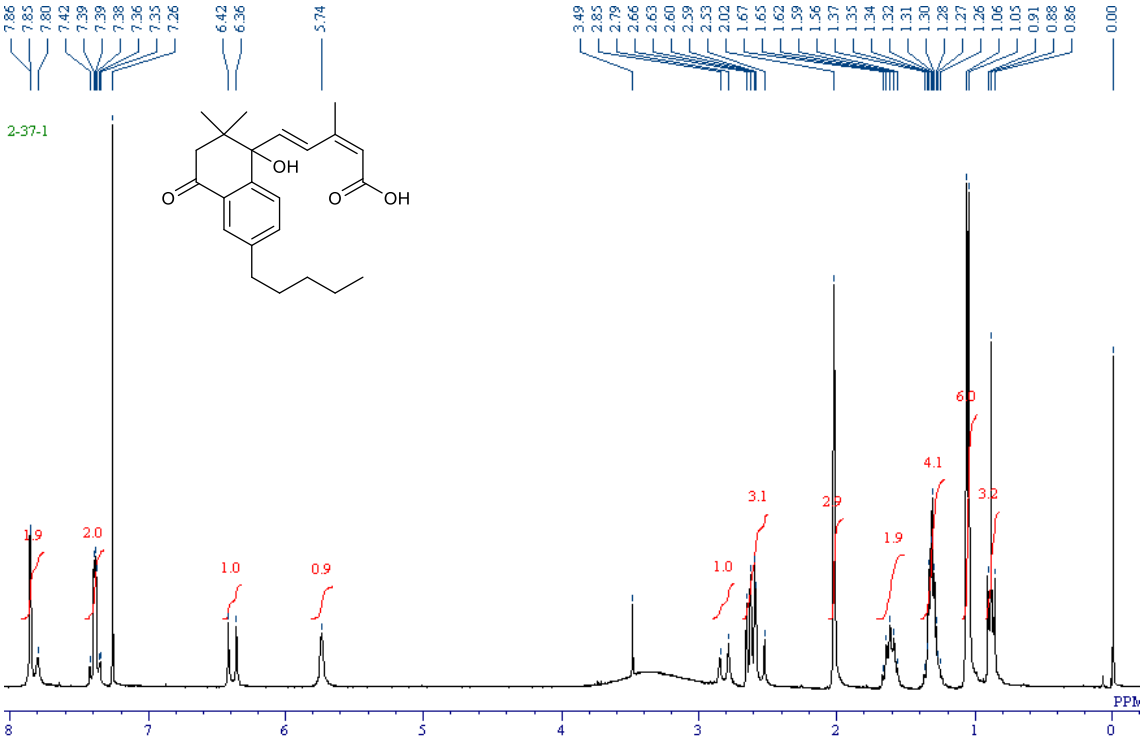
^{1}H NMR spectrum of Compound 10



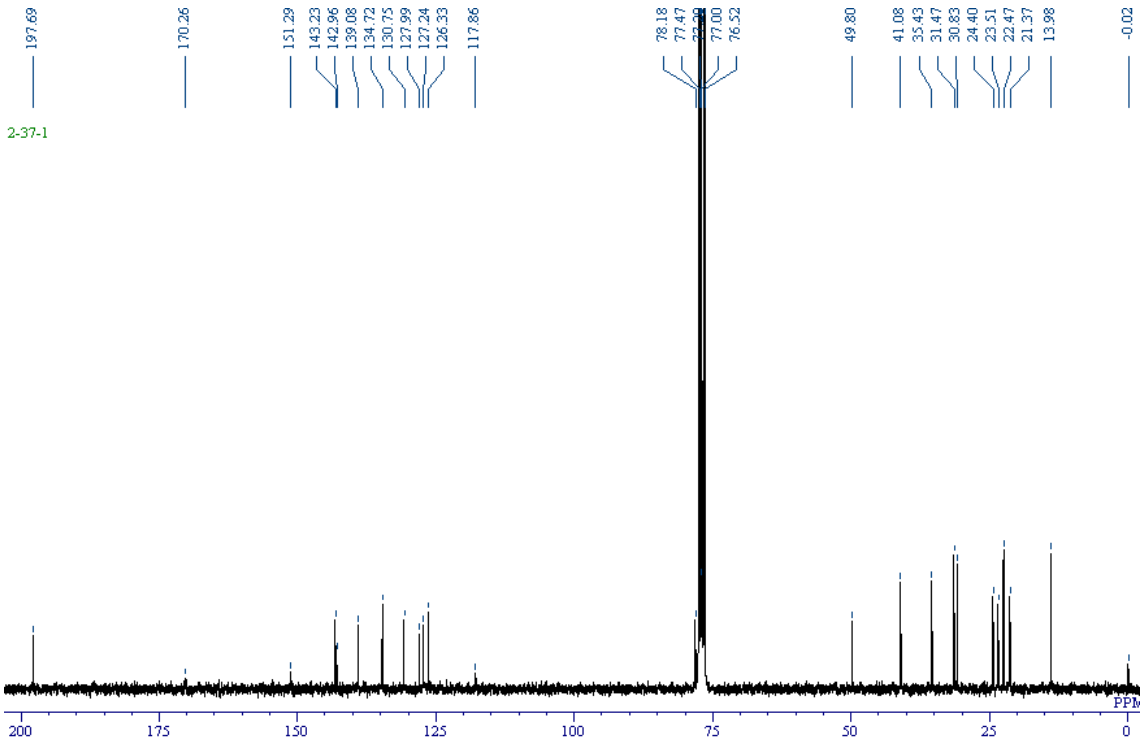
^{13}C NMR spectrum of Compound 10



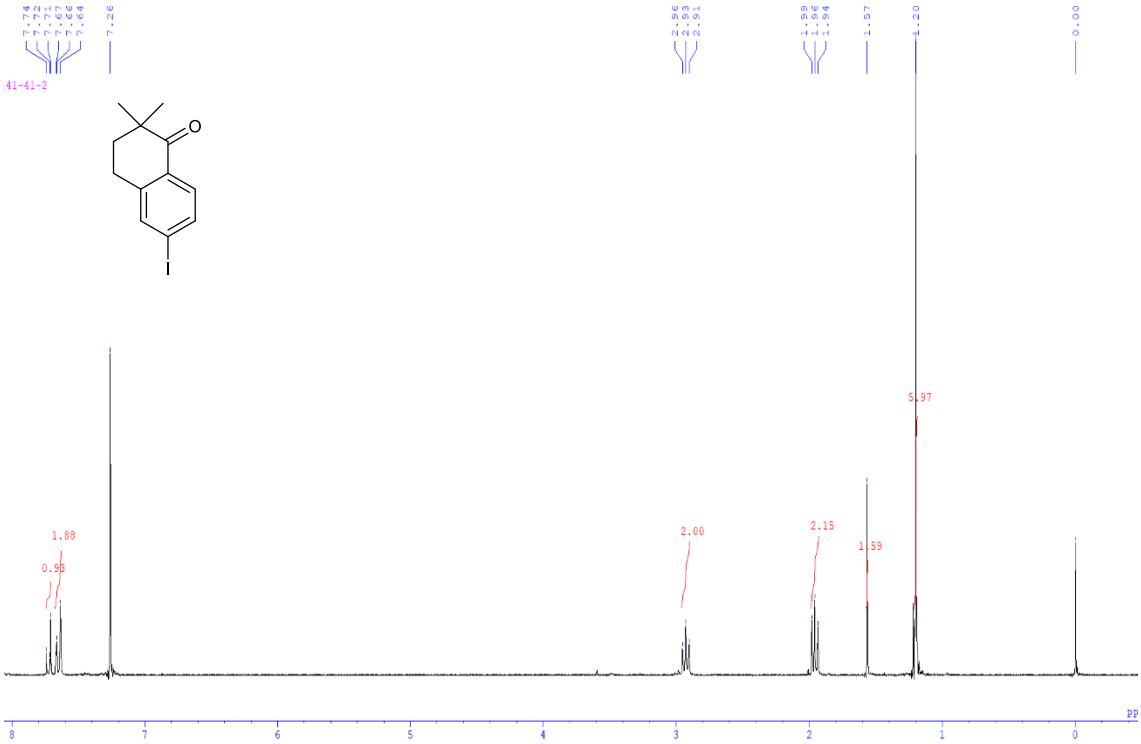
^{1}H NMR spectrum of Compound 2, (±)-PAC4



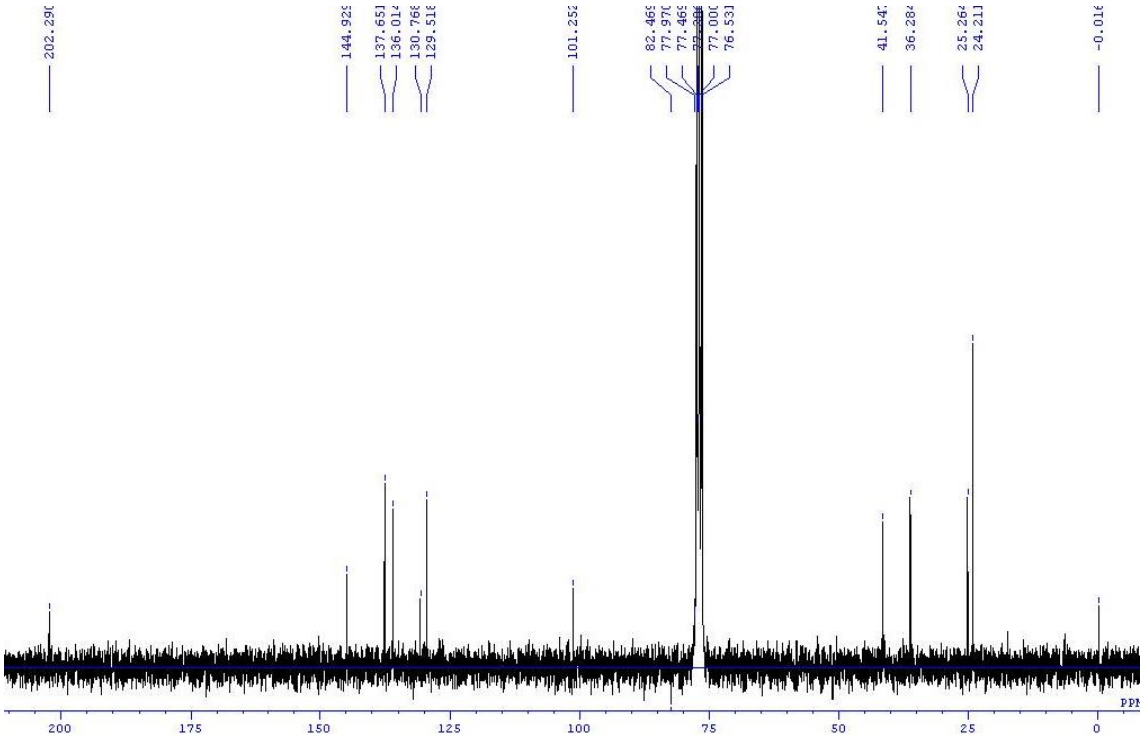
^{13}C NMR spectrum of Compound 2



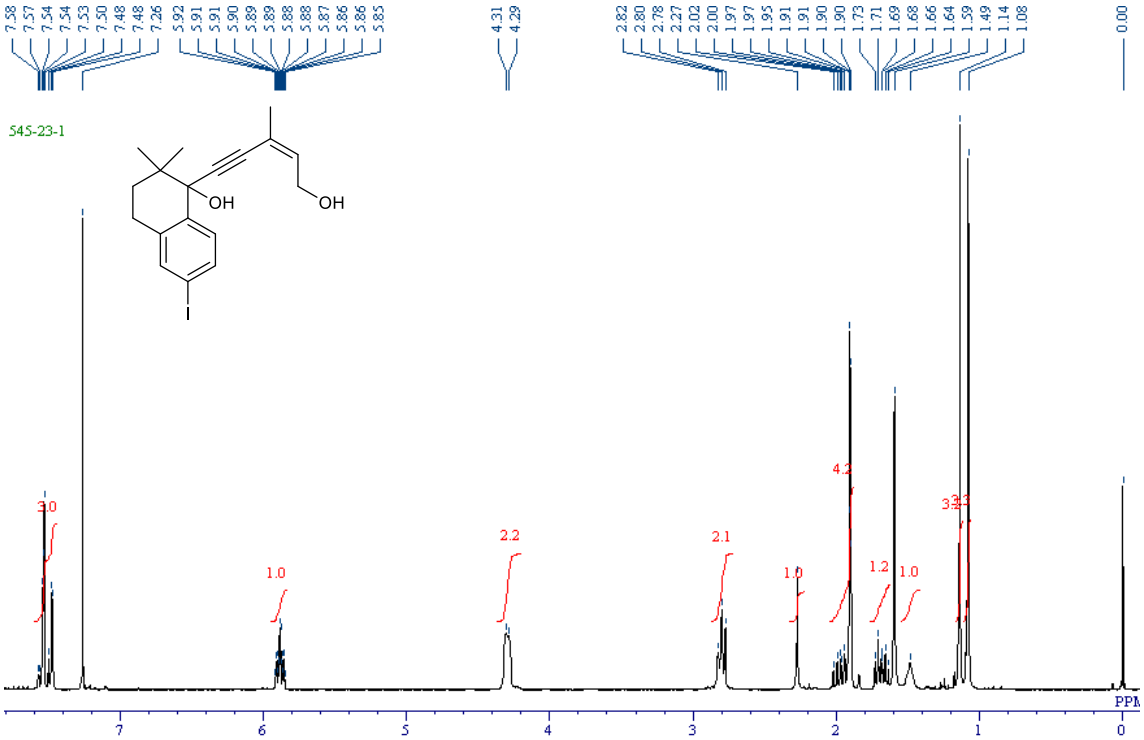
^{1}H NMR spectrum of Compound 12



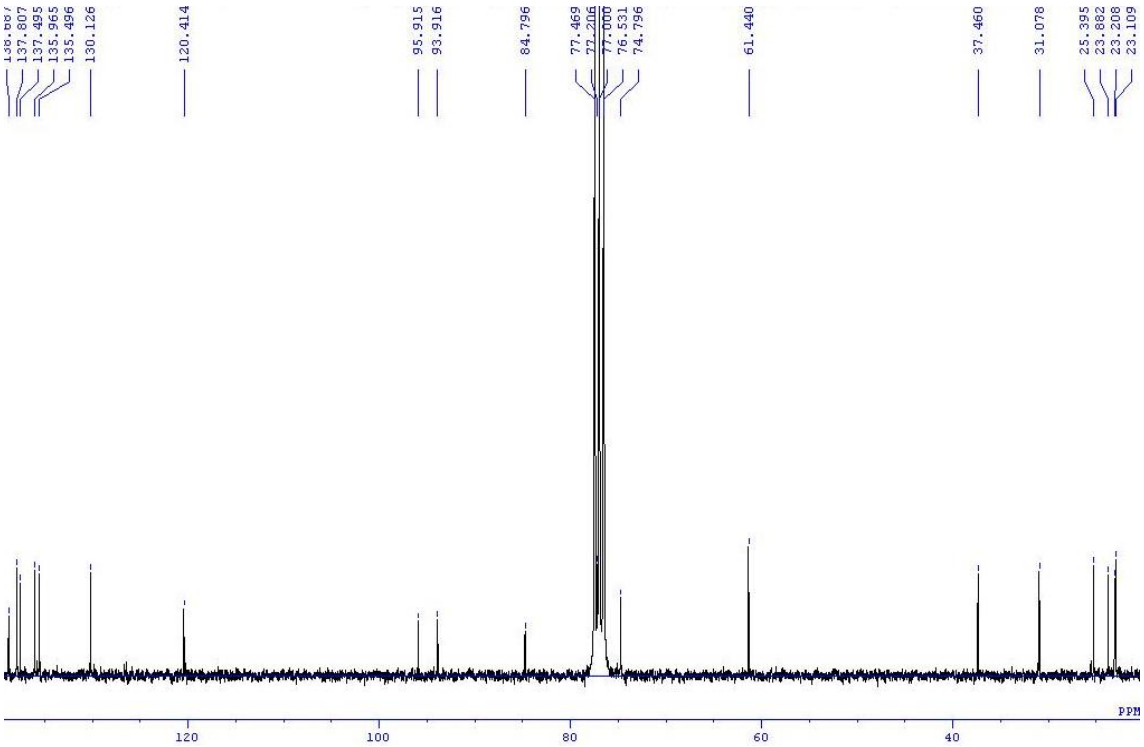
^{13}C NMR spectrum of Compound 12



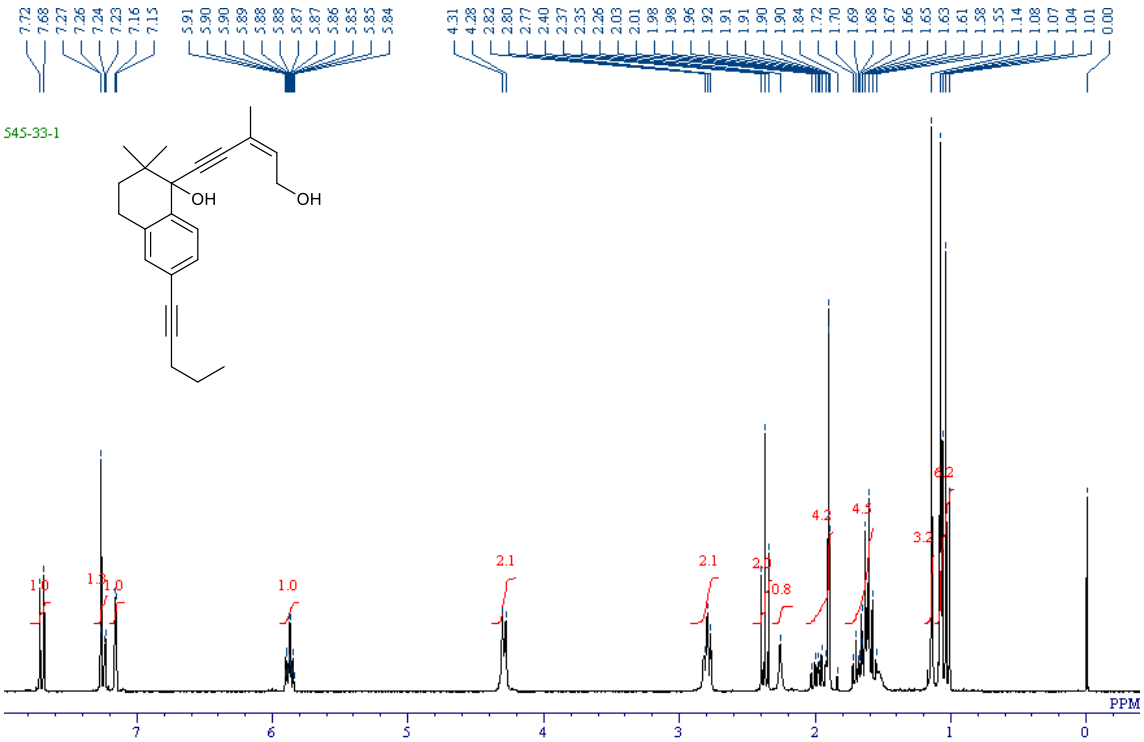
^{1}H NMR spectrum of Compound 13



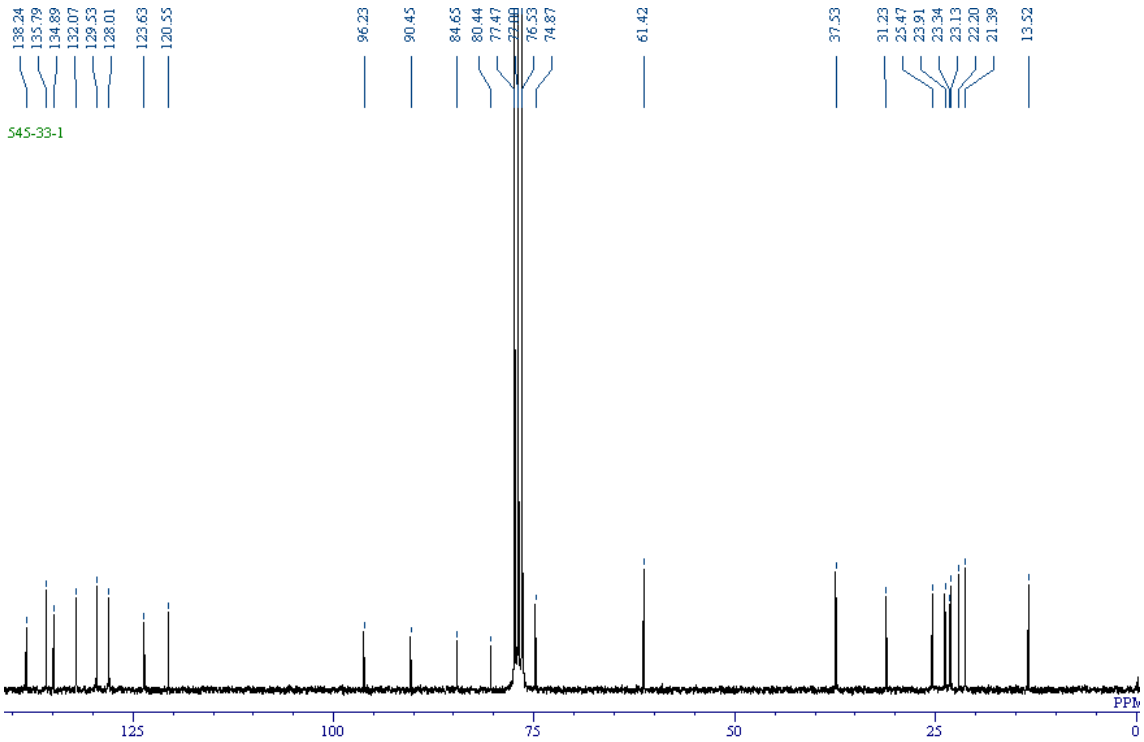
^{13}C NMR spectrum of Compound 13



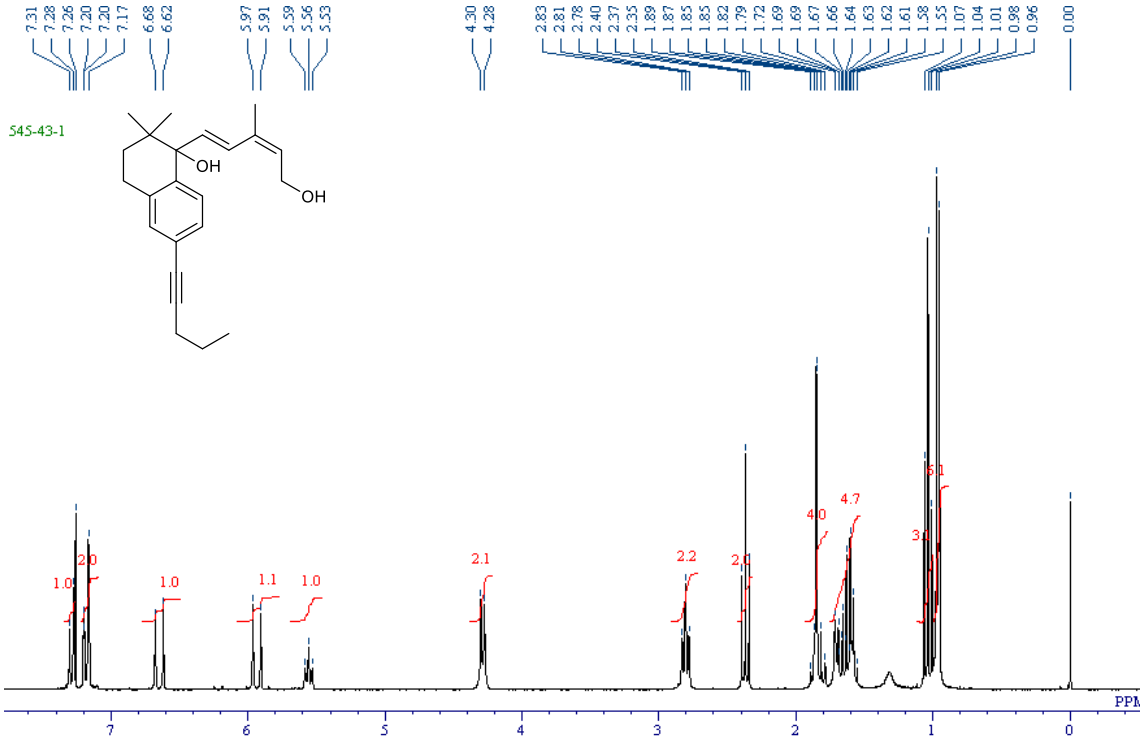
^{1}H NMR spectrum of Compound 14



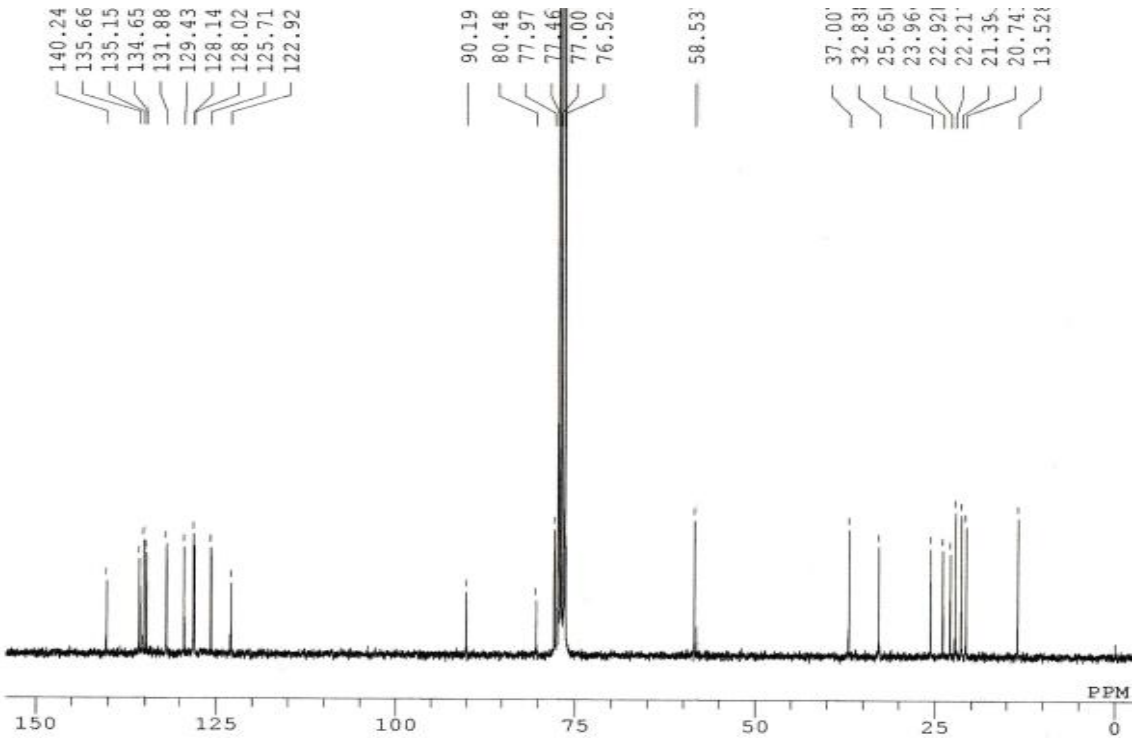
^{13}C NMR spectrum of Compound 14



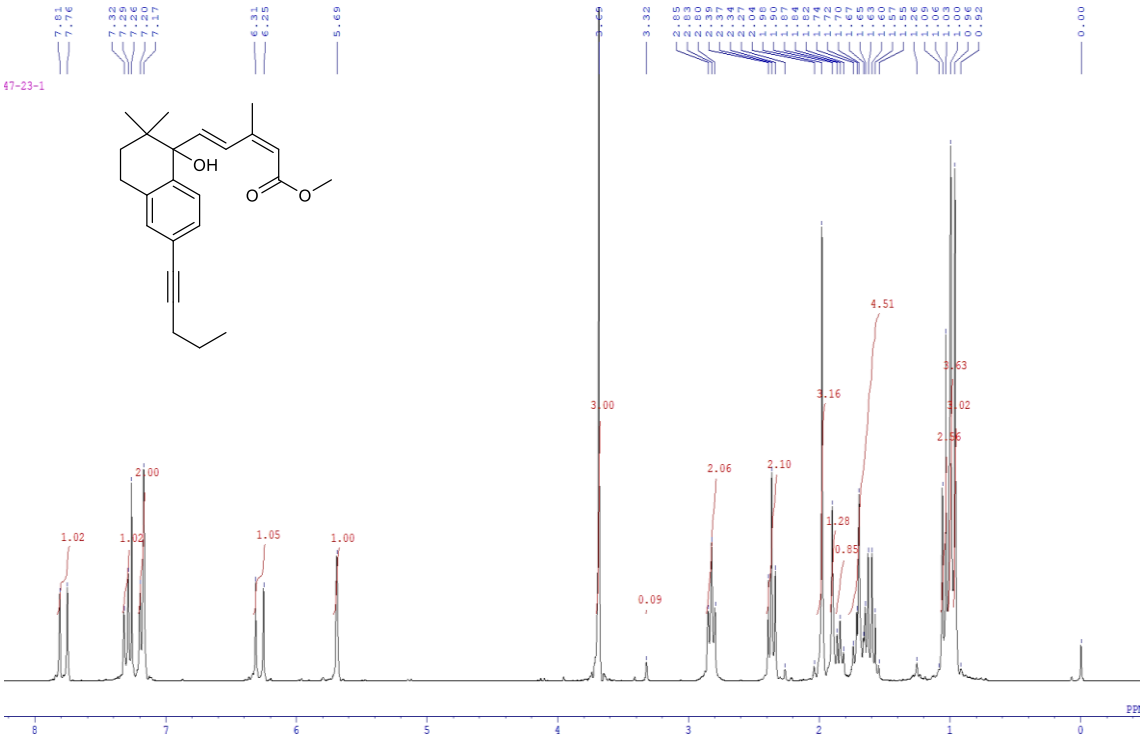
^{1}H NMR spectrum of Compound 15



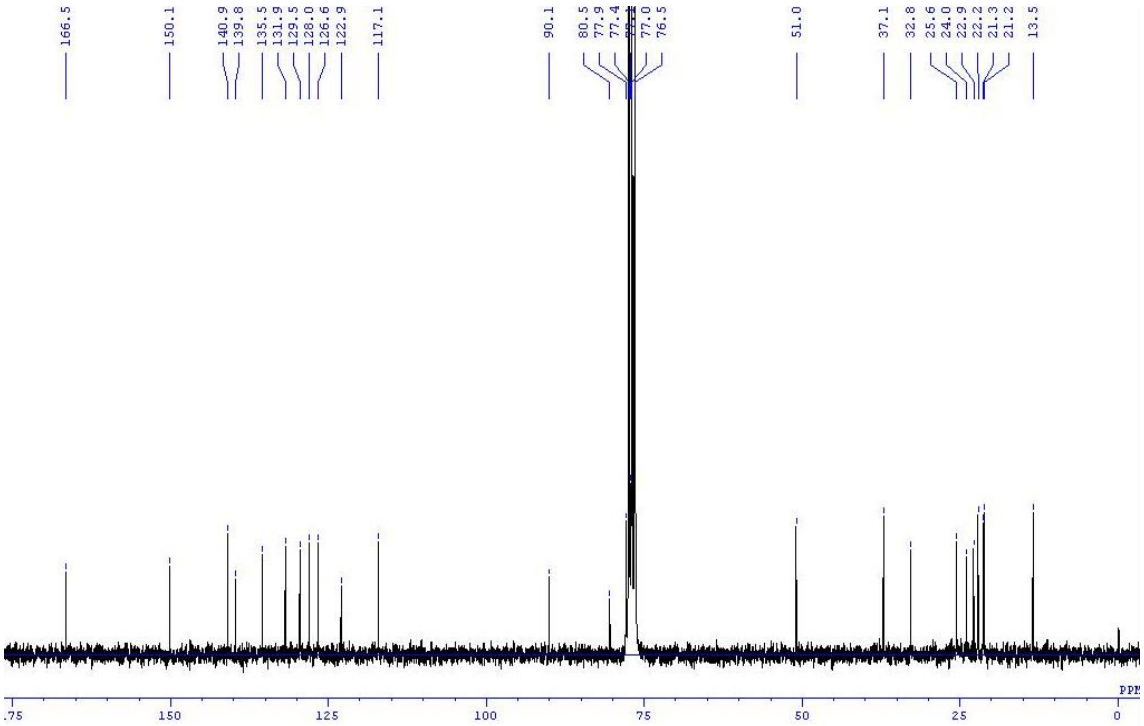
^{13}C NMR spectrum of Compound 15



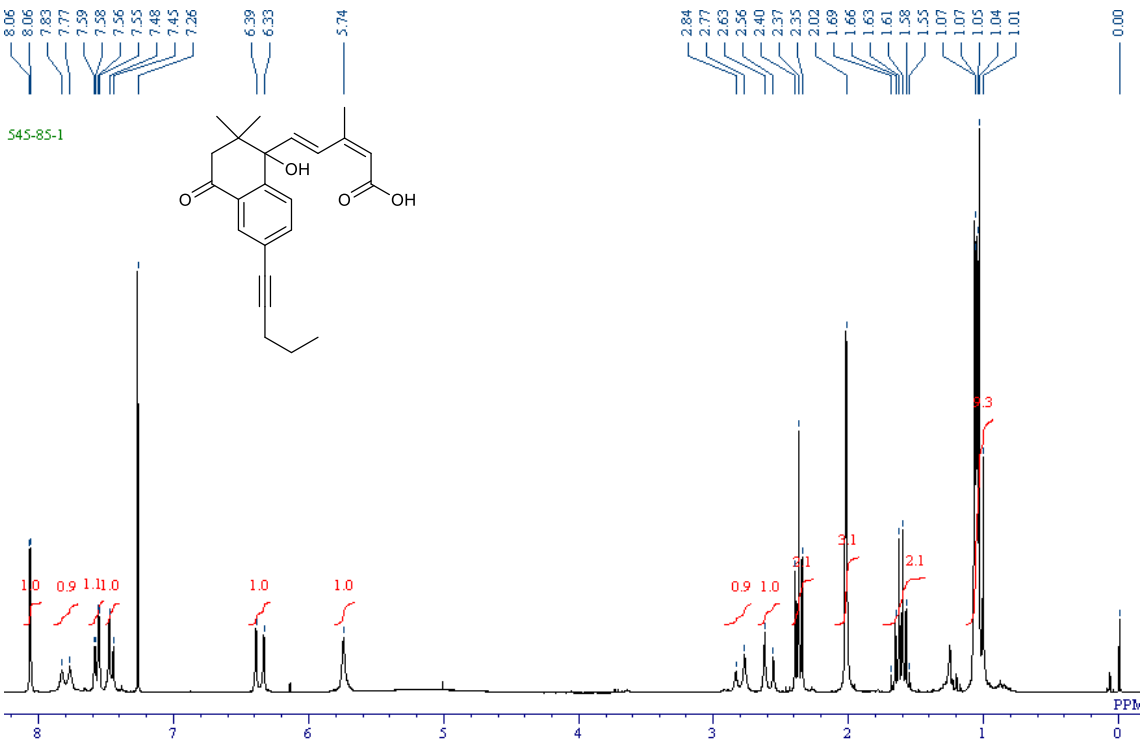
^{1}H NMR spectrum of Compound 16



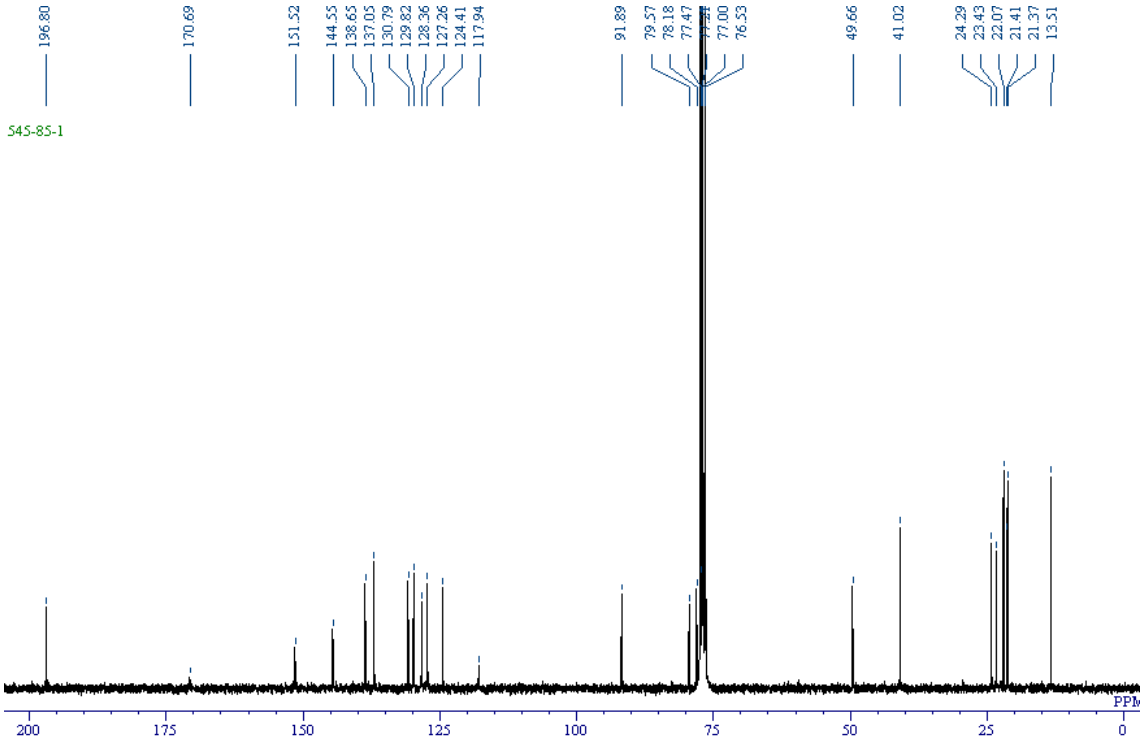
^{13}C NMR spectrum of Compound 16



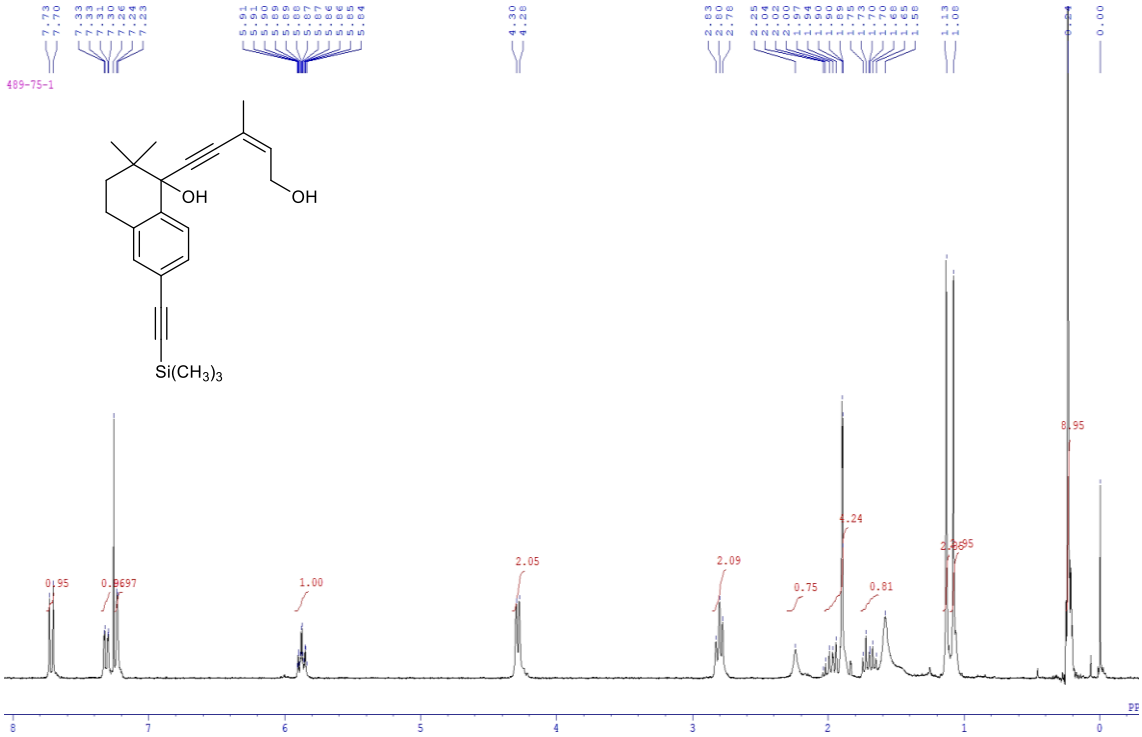
^{1}H NMR spectrum of Compound 3, (±)-PAT3



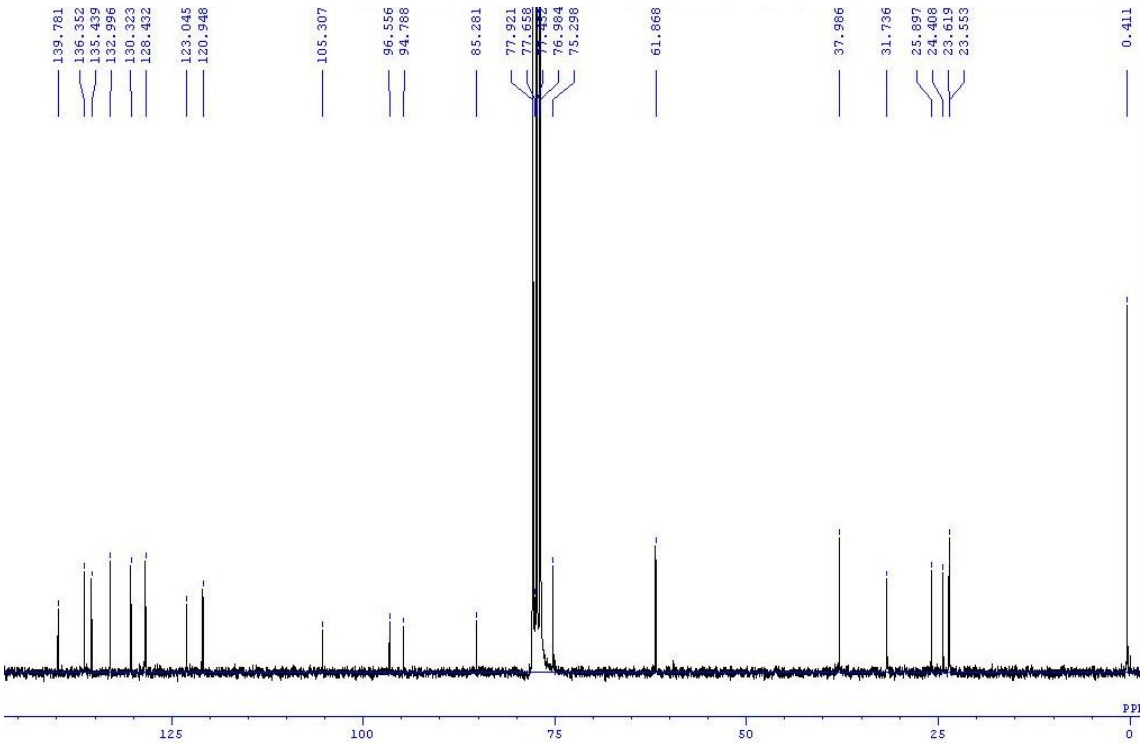
^{13}C NMR spectrum of Compound 3



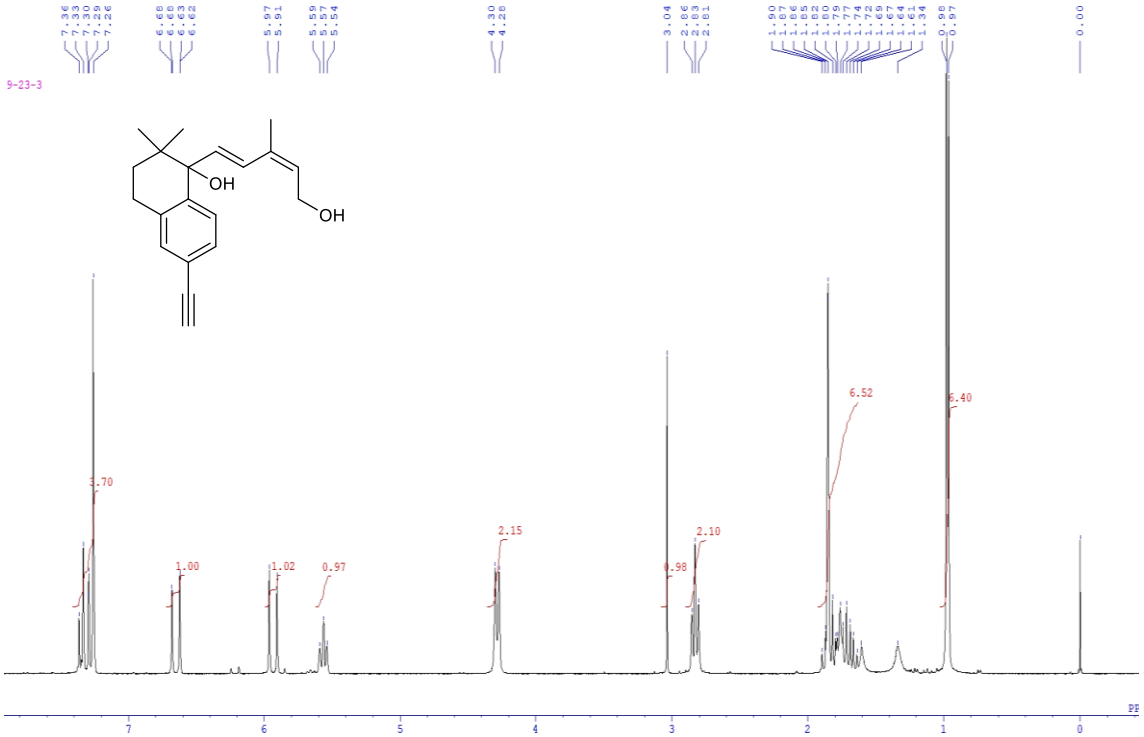
^{1}H NMR spectrum of Compound #17



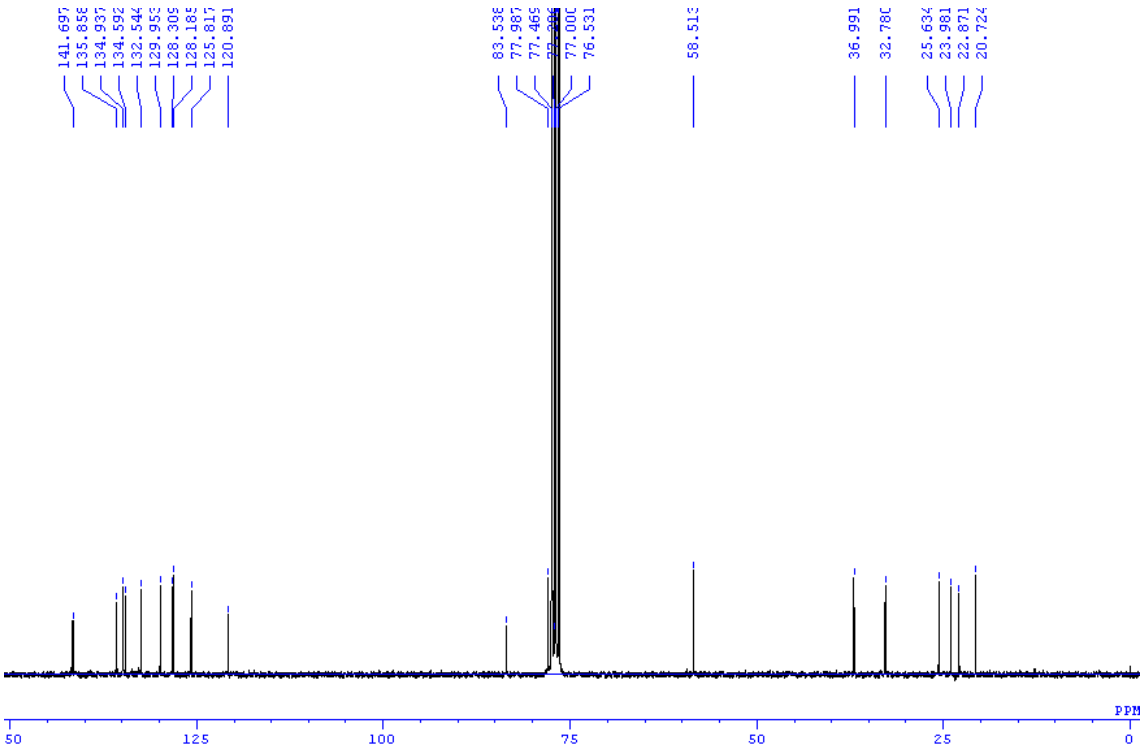
^{13}C NMR spectrum of Compound #17



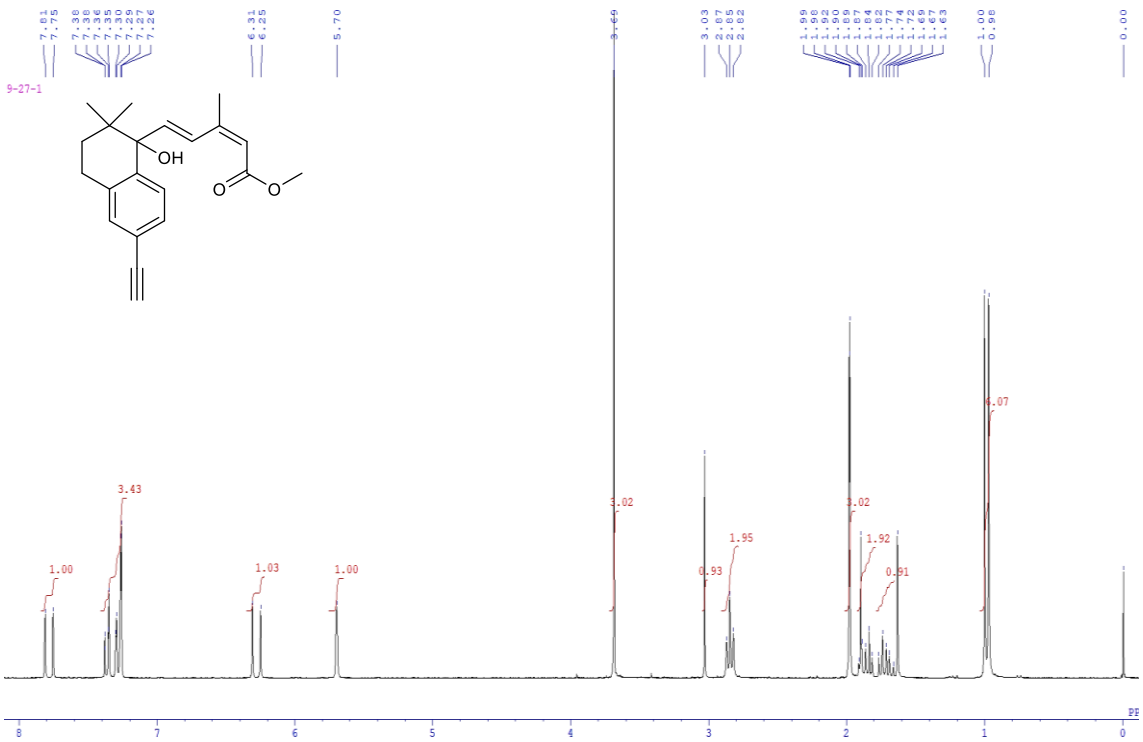
^{1}H NMR spectrum of Compound #18



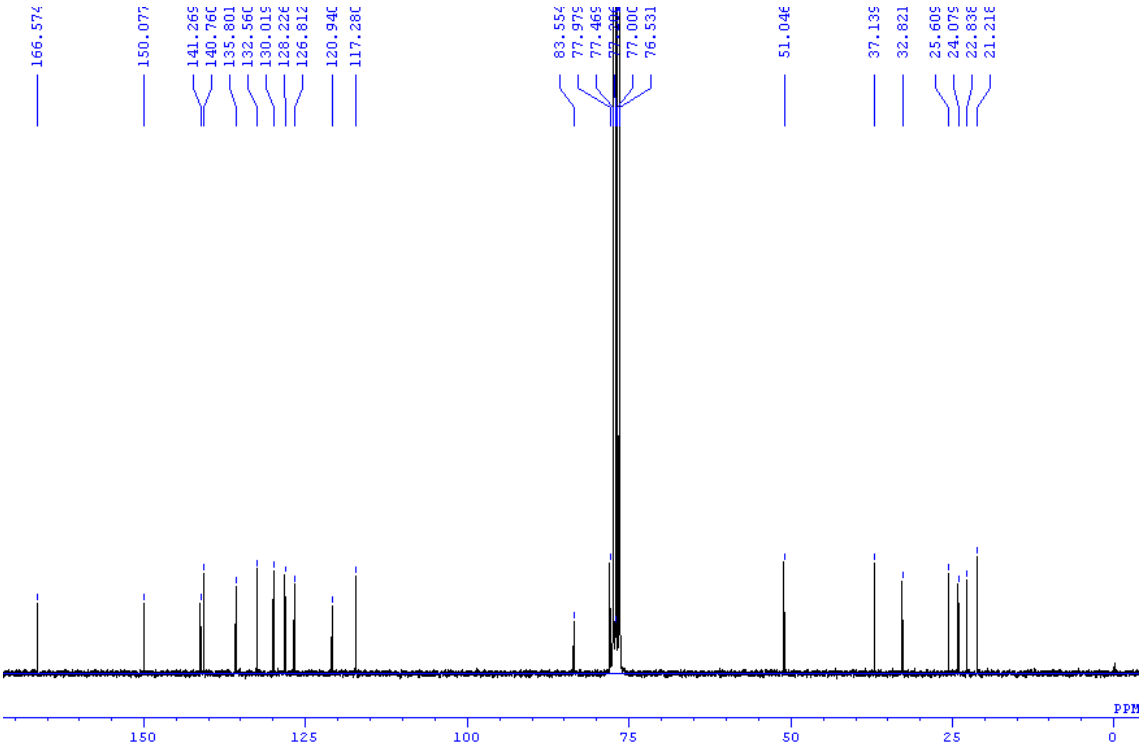
^{13}C NMR spectrum of Compound #18



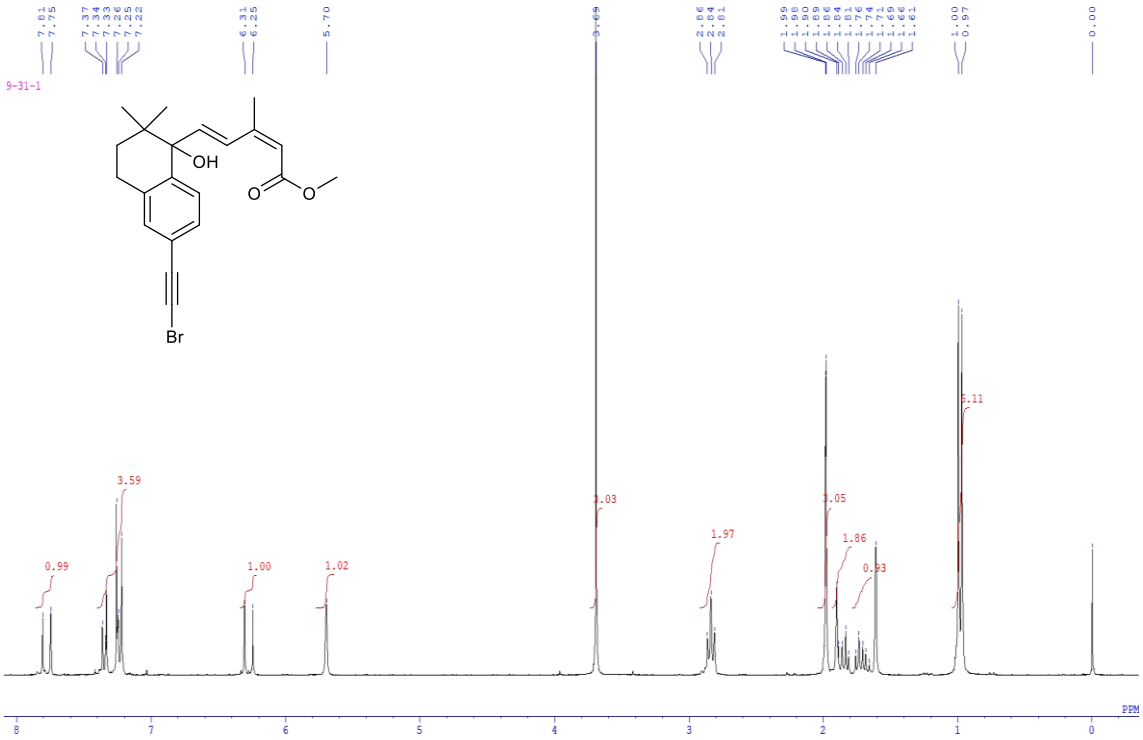
^{1}H NMR spectrum of Compound #19



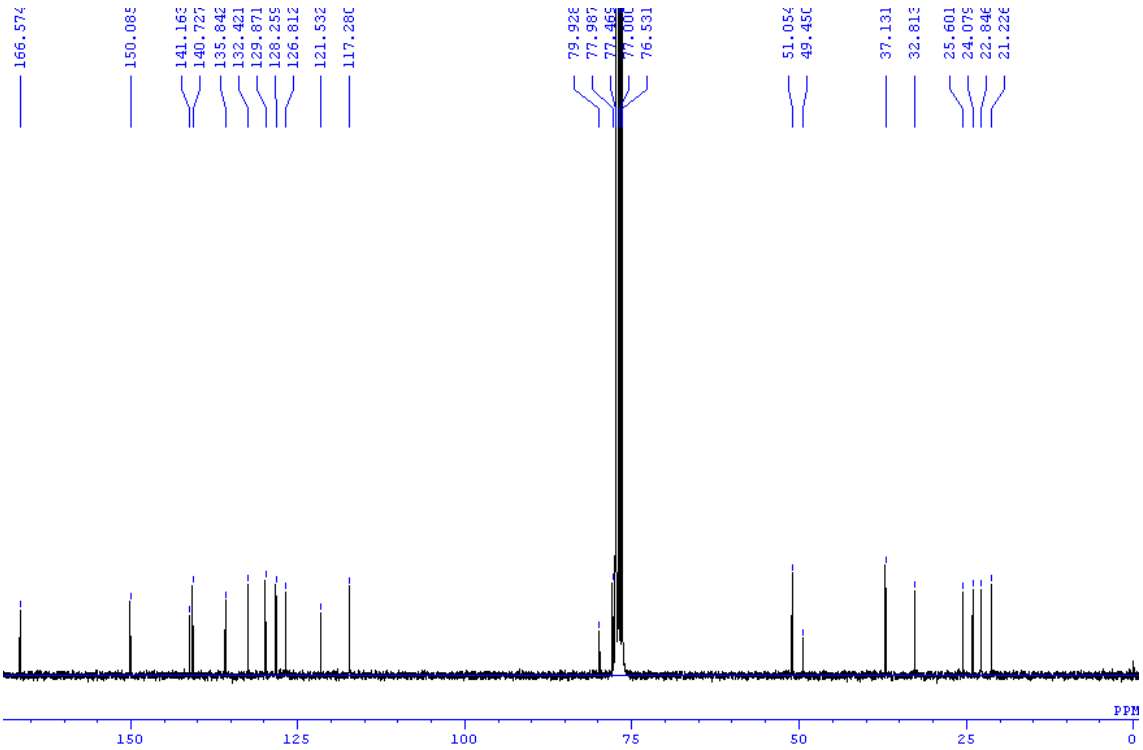
^{13}C NMR spectrum of Compound #19



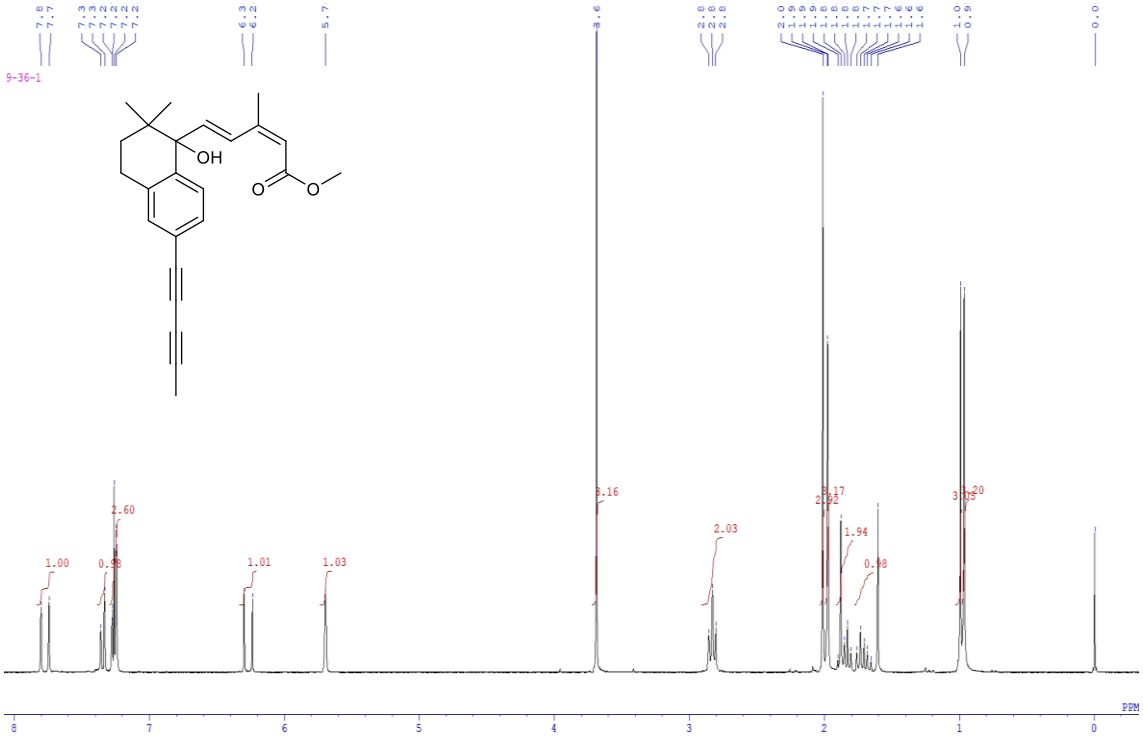
^{1}H NMR spectrum of Compound #20



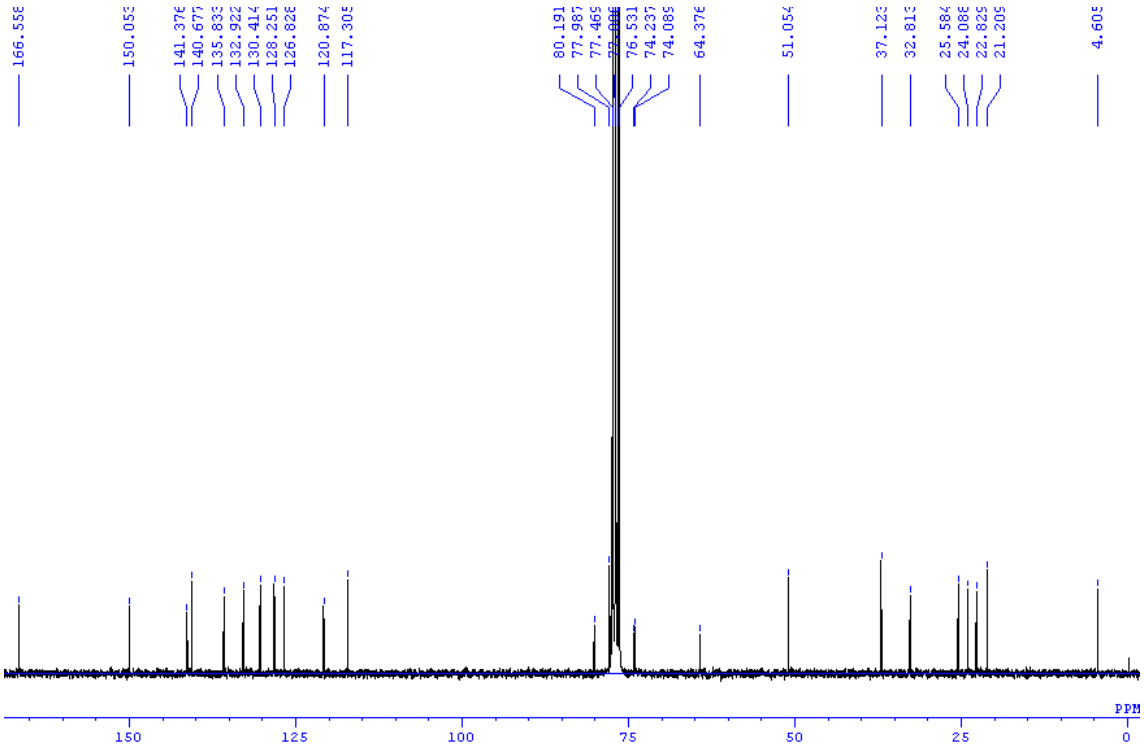
^{13}C NMR spectrum of Compound #20



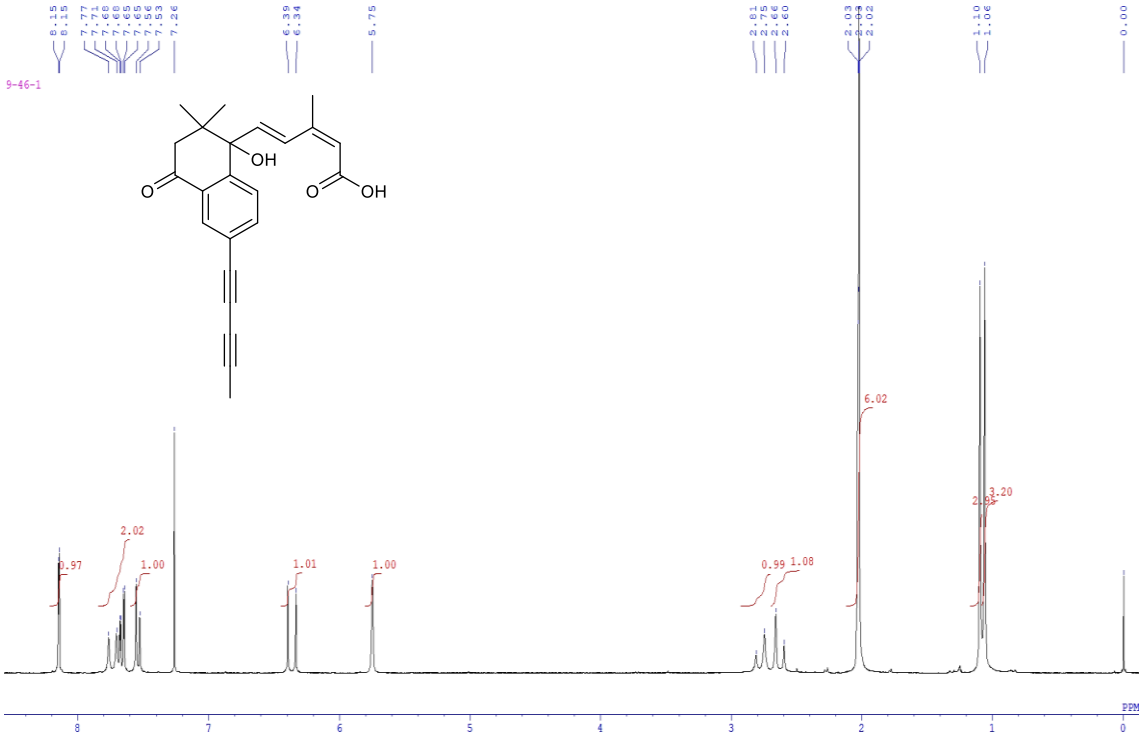
^{1}H NMR spectrum of Compound 21



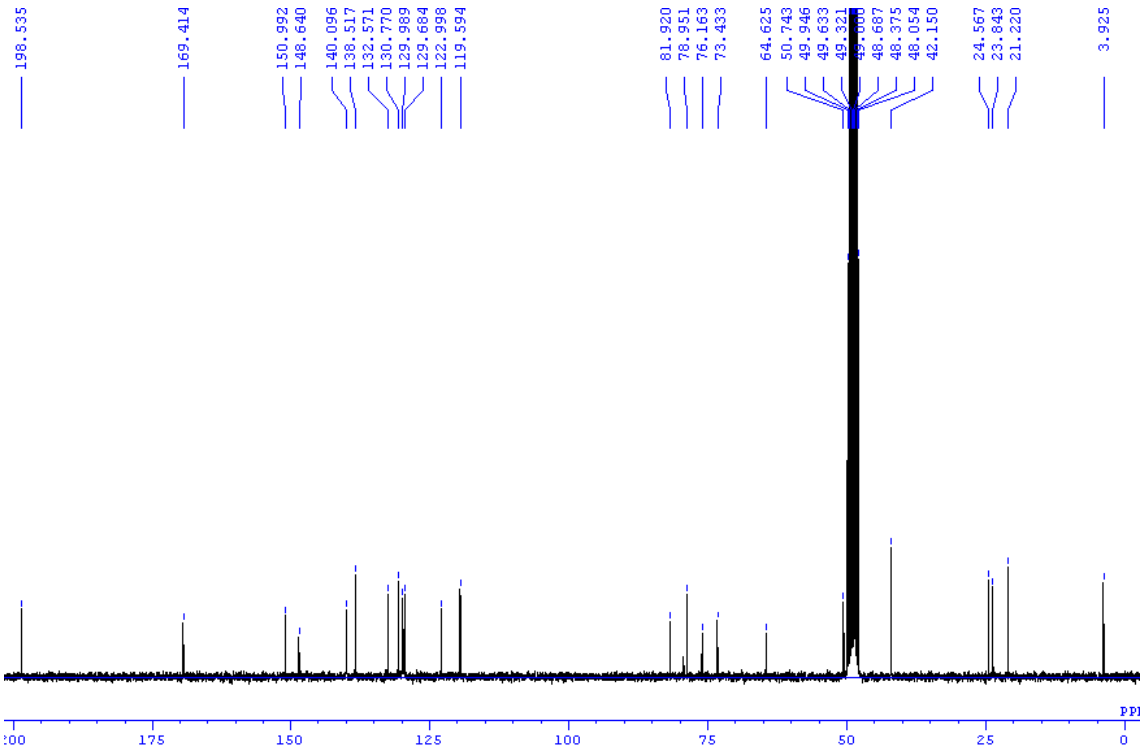
^{13}C NMR spectrum of Compound 21



^{1}H NMR spectrum of Compound 4, (±)-PATT1



^{13}C NMR spectrum of Compound 4



free solutions and fitted by Origin for ITC (GE Healthcare Bio-Sciences AB) with a 1/1 binding model.

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