SUPPORTING INFORMATION

Design and Synthesis of Paracaseolide A Analogues as Selective Protein Tyrosine Phosphatase 1B Inhibitors

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Part A: Biology Experimental Procedures

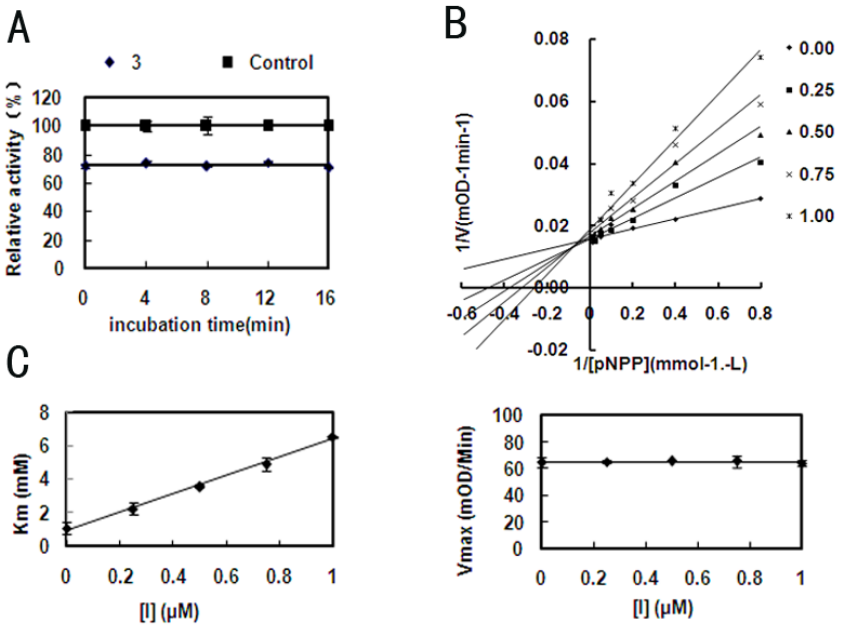
1. PTP1B and related PTPs biological assay

A colorimetric assay to measure inhibition against PTP1B was performed in 96-well plates. Briefly, the tested compounds were solublized in DMSO and serially diluted into concentrations for the inhibitory test. The assays were carried out in a final volume of 100 μL containing 50 mmol/L MOPS, pH 6.5, 2 mmol/L pNPP, 30 nmol/L GST-TCPTP, and 2% DMSO, and the catalysis of pNPP was continuously monitored on a SpectraMax 340 microplate reader at 405 nm for 3 min at 30 °C. The IC_{50} value was calculated from the nonlinear curve fitting of the percent inhibition [inhibition (%)] vs the inhibitor concentration using the following equation: %inhibition = 100/□1+IC_{50}/[*I*]*k*□, where *k* is the Hill coefficient. To study the inhibition on the other PTPase family members, SHP1, SHP2, and LAR were prepared and assays were performed according to procedures described previously.

2. Characterization of the PTP1B inhibitor

In the time-independent inhibition experiment, PTP1B were preincubated with compounds (2% DMSO) on the ice for different times, and then add 10 μL mixture of enzyme and compounds to 90 μL assay system. To characterize the inhibitor of PTP1B, the assay was carried out in a 100 μL system containing 50 mmol/L MOPS, pH 6.5, 14 nmol/L PTP1B, pNPP in 2-fold dilution from 80 mmol/L, and different concentrations of the inhibitor. In the presence of the competitive inhibitor, the Michaelis-Menten equation is described as $1/v = (K_m/[V_{max}[S]])(1+[I]/K_i)+1/V_{max}$, where K_m is the Michaelis constant, v is the initial rate, V_{max} is the maximum rate, and [*S*] is the substrate concentration. The K_i value was obtained by the linear replot of apparent K_m/V_{max} (slope) from the primary reciprocal plot versus the inhibitor concentration [*I*] according to the equation $K_m/V_{max} = 1+ [I]/K_i$.

Figure 1. Characterization of **9** to PTP1B



(A) Time-independent inhibition of PTP1B by **9**.

(B) Typical competitive inhibition of **9** shown by Lineweaver-Burk plot.

(C) At various fixed concentrations of **9** the initial velocity was determined with various concentrations of pNPP.

3. Effect of PTP1B inhibitors on phosphorylation level of CHO / hIR cell line

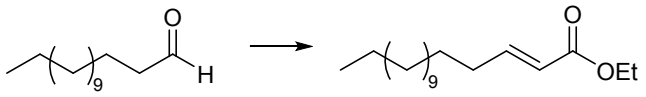
CHO/hIR cell were cultured in F12 medium supplemented with 10% (*V*/*V*) FBS, 100 units/ml Penicillin and 100 μg/ml streptomycin with 5% CO_2 at 37 °C.[1] Cell were serum free starve for 2 hour, then incubated with compounds for 1 hour, followed with Indulin (10 nM, Lilly) for 10min before harvested. Then Cells were washed twice with precooled 1X PBS and lysed with loading buffer. Cell lysates were subjected to 8% SDS-polyarylamide gel and transferred to NC (nitrocellulose) membranes were blocked for 2 hour with 5% BSA (*W*/*V*). The primary antibodies incubated overnight at 4 °C and secondary antibodies for 1 hour at room temperature. The primary antibody p-Tyr (PY20) and IRβ were from Santa cruz and β-action from Sigma, secondary antibody was from Jackson Immuno Research. The chemiluminescence were detected by Bio-Rad.

Part B: Computational Method

Molecular docking was performed using the LibDock docking package of Discovery Studio 2.1 to explore the interaction between the receptor and molecular. The crystal structures of the PTP1B were downloaded from the RCSB protein data bank (PDB code: 1NNY). The proteins' ligands and the water have been removed and the pockets which were used to combine with molecules were exposed before docking. The binding site sphere for LibDock calculation had a radius of 14 Å. ''Number of Hotspots'' was ''5000''. ''Conformation Method'' was ''BEST''. Other parameters were the default values.

Part C: Chemistry Experimental Procedures

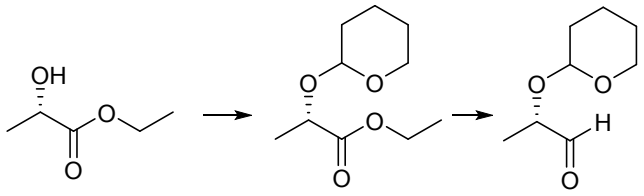
Preparation of α, β-unsaturated ester 2



1 **2**

To a solution of the aldehyde **1** (3.82 g, 18.0 mmol) in dry CH_2Cl_2 (50 ml) at room temperature, Wittig reagent were added. The reaction mixture was stirred for 10 hours at room temperature and then the solution was concentrated in vacuo and purified by column chromatography (silica gel, petroleum ether:EtOAc = 30:1) to afford the α,β-unsaturated ester **2** (4.40 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (t, J=8.0Hz, 3H), 1.25-1.32 (m, 25H), 1.43-1.46 (m, 2H), 2.16-2.22 (m, 2H), 4.18 (q, 8.0Hz, 2H), 5.81 (dt, J_1=16.0Hz, J_2=4.0Hz, 1H), 6.93-7.00 (m, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.11, 14.27, 22.68, 28.00, 29.14, 29.35, 29.38, 29.51 (2C), 29.64 (3C), 31.91, 32.19, 60.11, 121.16, 149.54, 166.81 ppm. HRMS (TOF ESI): calcd for $C_{18}H_{34}NaO_2$: 305.2457 $[M + Na]^+$; found: 305.2458.

(s)-2-Tetrahydropyranyloxypropanal 4

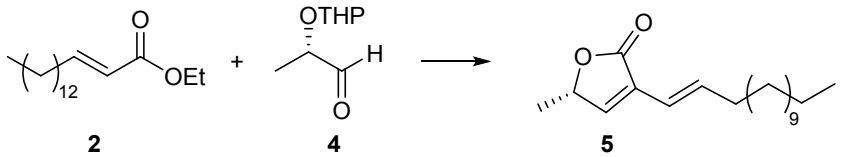


3 **S1** **4**

To a solution of (s)-Ethyl-lactate **3** (2.0 g, 16.95 mmol) in CH_2Cl_2 (30 ml) at room temperature, Pyridinium p-toluenesulfonate (424 mg, 1.69 mmol), 3, 4-2H-dihydropyran (2.14 g, 25.42 mmol) were added. After the reaction solution had been stirred for 10 hours at this temperature, Et_2O (70 mL) and water (70 ml) were added, The layers were separated and the organic layer was washed again with water (2×), dried with $MgSO_4$ and concentrated in vacuo, The crude product **S1** was used for the next step without further purification. The product **S1** consisted of an almost equal amount of the two diastereomers due to its tetrahydropyranyl group. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.27 (two t, J=6.0Hz, 3H), 1.38-1.46 (two d, J=6.0Hz, 3H), 1.54-1.57 (m, 2H), 1.65-1.73 (m, 2H), 1.84-1.87 (m, 2H), 3.48-3.53 (m, 1H), 3.81-3.92 (m, 1H), 4.16-4.23 (m, 2H) 4.41 (q, J=6.0Hz, 1H), 4.70-4.73 (m, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.12, 14.18, 18.02, 18.74, 19.08, 19.13, 25.25, 25.33, 30.33, 30.40, 60.69, 60.77, 62.25, 62.41, 69.93, 72.45, 97.53, 98.21, 173.24, 173.35 ppm. HRMS (TOF ESI): calcd for $C_{10}H_{18}NaO_4$: 225.1103 $[M + Na]^+$; found: 225.1186.

The crude product ester **S1** (3.42 g, 16.95 mmol) was dissolved in Et_2O (50 mL) and cooled to -78 °C. And then Diisobutylaluminium hydride (25.4 ml, 25.4 mmol) was added dropwise. After being stirred for 40 minutes at -78 °C, MeOH (1 ml), H_2O (2.5 ml) were added. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. Then the saturated Potassium sodium tartrate tetrahydrate was added and srirred until the mixture become clear, the layers were separated and the aqueous phase was extracted with Et_2O (2×). The combined organic phases were dried ($MgSO_4$) and concentrated. Purification by chromatography (silica gel, petroleum ether:Et_2O = 15:1) afforded the aldehyde **4** (2.62 g, 16.58 mmol, 98%, 2 steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.27 (d, J=6.0Hz, 3H), 1.54-1.56 (m, 4H), 1.75-1.85 (m, 2H), 3.46-3.50 (m, 1H), 3.88-3.97 (m, 1H), 3.97-4.00 (m, 1H), 4.63 (t, J=3.0Hz, 1H), 9.65 (d, J=3.0Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 15.17, 15.70, 19.33, 19.96, 25.14, 25.24, 30.53, 30.65, 62.73, 63.53, 76.51, 78.56, 98.28, 99.35, 203.12, 203.44 ppm. HRMS (TOF ESI): calcd for $C_8H_{14}NaO_3$: 181.0841 $[M + Na]^+$; found: 181.0832.

Preparation of diene 5



2 **4** **5**

To a solution of hexamethylphosphoramide (8.26 g, 47.47 mmol) in tetrahydrofuran (150 mL) at -78 °C, Lithium diisopropylamide (23.8 ml, 47.47 mmol) was added. After stirring for 10min at -78 °C, α, β-unsaturated ester **2** (12.2 g, 43 mmol) in THF (10 mL) was added dropwise. The reaction mixture was stirred for 30 min at this temperature, aldehyde **4** (7.50 g, 47.47 mmol) in tetrahydrofuran was added dropwise. The reaction mixture was stirred for 1 h at -78 °C. H_2O was added, the phases were separated and the aqueous layer was extracted with Et_2O(3×). The combined organic phases were washed

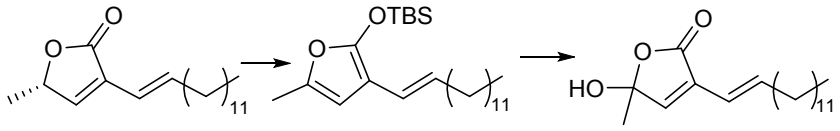
with water (3×) and dried ($MgSO_4$), concentrated in vacuo. The crude product β-hydroxy ester was used for the next step without further purification.

The crude product (18.92 g, 43 mmol) was dissolved in MeOH (200 ml), and *p*-Toluenesulfonic acid (1.72 g, 10 mmol) was added at room temperature. The reaction mixture was stirred overnight. H_2O was added, the phases were separated and the aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution and water. And then dried ($MgSO_4$) and concentrated in vacuo. The crude productβ-hydroxy lactone was used for the next step without further purification.

To a solution of crude product alcohol in CH_2Cl_2 (200 ml) at 0 °C was added Methanesulfonyl chloride (6.87 g, 60.0 mmol) followed by NEt_3 (16.8 ml, 120.0 mmol). The reaction mixture was stirred for 2 h at 0 °C and subsequently diluted with H_2O. The layers were separated and the aqueous phases were extracted with CH_2Cl_2 (3×). The combined organic phases were washed with water and dried over ($MgSO_4$), concentrated in vacuo. Purification by chromatography (silica gel, petroleum ether:EtOAc = 30:1) to afford the diene *Z*- and *E*-**5** (7.2g, 57.3%, 3 steps, *Z*:*E*=8:1). *E*-**5**: $[\alpha]^{25}_D$ +32.4 (c 1.41, CHCl3); E-**5**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.87 (t, J=6.0Hz, 3H), 1.25 (brs, 20H), 1.41 (d, J=6.0Hz, 3H), 2.14 (q, J=6.0Hz, 2H), 5.02 (q, J=6.0Hz, 2H), 6.08 (d, J=15.0Hz, 1H), 6.73-6.83 (m, 1H), 7.03 (d, J=3.0Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.08, 19.15, 22.65, 28.74, 29.21, 29.32, 29.44, 29.54(C), 29.62(3C), 31.88, 33.39, 76.87, 118.25, 129.38, 138.82, 146.80, 172.02 ppm. HRMS (TOF ESI): calcd for $C_{19}H_{32}NaO_2$: 315.2300 $[M + Na]^+$; found: 315.2324.

To a solution of the mixture of *Z* and *E*-**5** isomers (7.1 g, 24.3 mmol) in CH_2Cl_2 (300 ml), I_2 (122 mg, 0.48 mmol) was added. The reaction mixture was stirred for 8 h under direct sunlight. And then diluted with saturated aqueous $Na_2S_2O_3$ solution, the layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3×). The combined organic phases were dried ($MgSO_4$) and concentrated. Purification by chromatography (silica gel, petroleum ether:EtOAc = 30:1) to afford the pure *E*-**5** (6.03g, 20.6 mmol, 85%).

Preparation of 4-hydroxybutenolide 12



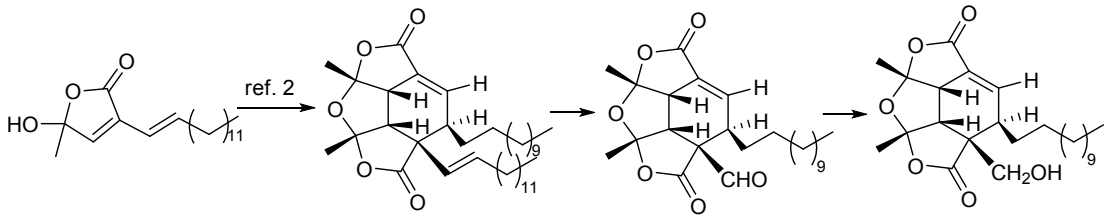
5 **11** **12**

To a stirred solution of diene *E*-**5** (117 mg, 0.4 mmol) in anhydrous CH_2Cl_2 (10 mL) at room temperature was added triethylamine (166 μL, 1.2 mmol), Subsequently, TBSOTf (183 μL, 0.8 mmol) was added dropwise. The reaction mixture was was stirred for 8 hours at room temperture and subsequently diluted the addition of saturated aqueous NH_4Cl solution, The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (2×). The combined organic layers were dried ($MgSO_4$) and the solvents were removed under reduced pressure. Purification by chromatography (silica gel, petroleum ether:EtOAc = 40:1) to afford the TBS-protected furanol **11** (148 mg, 91%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.23 (s, 6H), 0.88 (t, J=6.0Hz, 3H), 0.98 (s, 9H), 1.26 (brs, 20H), 2.08-2.11 (m, 2H), 2.13 (s, 3H), 5.61 (m, 1H), 5.91 (s, 1H), 6.00 (d, J=15.0Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = -4.48(2C), -2.94, 13.52, 14.13, 18.06, 22.71, 25.53(2C), 25.70, 29.22, 29.38, 29.59, 29.68(2C), 29.71, 29.80, 31.95, 33.10, 98.53, 104.11, 119.15, 126.09, 141.50, 151.06 ppm.

TBS-protected furanol **11** (148 mg, 0.36 mmol) was dissolved in CH_2Cl_2 (10 ml). *m*-CPBA (68 mg, 0.4 mmol) was then added at -78 °C. The reaction mixture was stried for 30 minutes at this temperature, and then diluted with saturated aqueous $Na_2S_2O_3$ solution, the phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3×). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution(2×),

dried ($MgSO_4$) and the solvents were evaporated at reduced pressure. Purification by chromatography (silica gel, petroleum ether:EtOAc = 4:1) to afford 4-hydroxybutenolide **12** (103 mg, 0.33 mmol, 92%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, J=9.0Hz, 3H), 1.25 (brs, 20H), 1.43 (t, 6.0Hz, 2H), 1.70 (s, 3H), 2.12-2.19 (m, 2H), 3.46 (brs, 1H), 6.05 (dd, J1=3.0Hz, J2=15.0Hz , 1H), 6.76-6.85 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.10, 22.67, 24.81, 28.63, 29.22, 29.34, 29.44, 29.56, 29.64 (3H), 31.90, 33.50, 103.75, 117.70, 130.85, 141.16, 143.67, 169.70 ppm. HRMS (TOF ESI): calcd for $C_{19}H_{32}NaO_3$: 331.2244 $[M + Na]^+$; found: 331.2243.

Preparation of derivation 15



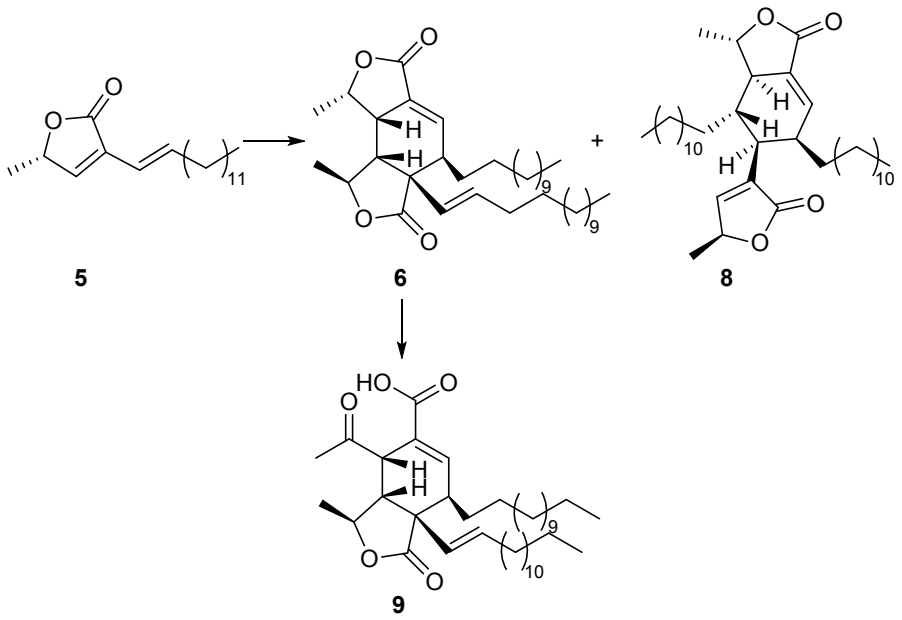
12 **13** Paracaseolide A **14** **15**

13: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, J=5.0Hz, 6H), 1.25 (brs, 42H), 1.38 (m, 3H), 1.62 (s, 3H), 1.76 (s, 3H), 2.11 (q, J=5.0Hz, 2H), 3.04 (m, 1H), 3.30 (d, J=10.0Hz, 1H), 3.37 (dd, J_1=10.0Hz, J_2=3.0Hz), 5.49 (d, J=15Hz, 1H), 5.83 (m, 1H), 7.25 (dd, J1=5.0Hz, J2=10.0Hz, 1H) ppm; ^{13}C NMR (100 MHz, CDCl3): δ = 14.16 (2C), 22.72 (2C), 25.72, 26.59, 28.22, 29.03, 29.14, 29.39, 29.44 (2C), 29.57, 29.66 (2C), 29.68 (8C), 31.94 (2C), 32.72, 45.06, 46.69, 50.22, 58.34, 113.83, 115.40, 126.25, 129.31, 135.12, 144.74, 166.22, 175.10 ppm. HRMS (TOF ESI): calcd for $C_{38}H_{64}NaO_5$: 621.4495 $[M + Na]^+$; found: 621.4487.

Ozonic was bubbled gently through the solution of nature product **13** (15 mg, 0.0250 mmol) in CH_2Cl_2 (30 ml) at -78 °C for 20 seconds. Subsequently, Argon was bubbled through it, and the $S(CH_3)_2$ (0.5 ml) was added. The mixture was warmed to room temperature overnight. Then concentrated in vacuo. Purification by chromatography (silica gel, petroleum ether:EtOAc = 5:1) to afford the aldehyde **14** (10.5 mg, 0.0243 mmol, 97.2%). ^{1}H NMR (300 MHz, CDCl3): δ = 0.88 (t, J=6.0Hz, 3H), 1.25 (brs, 22H), 1.58 (s, 3H), 1.79 (s, 3H), 3.36 (dd, J_1=3.0Hz, J_2=9.0Hz, 1H), 3.69-3.71 (m, 1H), 3.93 (d, J=9.0Hz, 1H), 9.60 (s, 1H) ppm; HRMS (TOF ESI): calcd for $C_{25}H_{36}NaO_6$: 455.2410 $[M + Na]^+$; found: 455.2414.

To a solution of aldehyde **14** (6.0 mg, 0.0138 mmol) in tetrahydrofuran /AcOH (10:1 1.0 ml) at 0 °C was added $NaBH_3CN$ (1.2 mg, 0.0179 mmol, 1.3 eq). Stired at 0 °C for 30 minues, and then warmed to room temperature for 20 minues. Subsequently, H_2O and $CHCl_3$ were added. The layers were separated and the aqueous phase was extracted with $CHCl_3$ (3×). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution(2×), dried ($MgSO_4$) and the solvents were evaporated at reduced pressure. Purification by chromatography (silica gel, petroleum ether:EtOAc = 4:1) to afford alcohol **15** (5.0mg, 0.0114mmol, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, J=6.0Hz, 3H), 1.25 (brs, 22H), 1.57 (brs, 4H), 1.75 (s, 6H), 3.17-3.20 (m, 2H), 3.36 (dd, J_1=3.0Hz, J_2=9.0Hz, 1H), 3.92 (d2, J_1=3.0Hz J_2=15.0Hz, 1H), 7.24 (dd, J_1=3.3Hz, J_2=7.5Hz, 1H) ppm. ^{13}C NMR (100 MHz, CDCl3): δ = 14.16, 22.72, 24.98, 26.82, 27.91, 28.29, 29.37, 29.41, 29.54, 29.65 (4C), 31.93, 40.30, 46.73, 49.74, 57.86, 65.46, 113.64, 115.66, 130.03, 143.54, 166.36, 176.82 ppm. HRMS (TOF ESI): calcd for $C_{25}H_{38}NaO_6$: 457.2566 $[M + Na]^+$; found: 457.2568.

Preparation of acid 9



5 **6** **8**

9

To solution of diene *E*-**5** (200 mg, 0.685 mmol) in toluene / water=2:1 (2 ml) was added BHT (5 mg) under argon, and heated in a sealed tube at 170 ℃ for 36 hours. The reaction was left to cool to room temperature. The mixture was purified by flash column chromatography (silica gel, petroleum Ether:Chloroform:EtOAc = 40:10:3) to afford dimer product **6** (86 mg, 43%) and **8** (20 mg, 10%). (74 mg, 37% of *E*-**5** recovered)

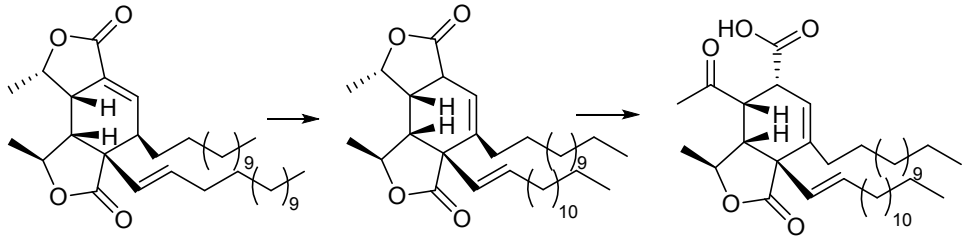
To solution of *E*-**5** (30 mg, 0.103 mmol) in toluene (0.3ml) was added BHT (3 mg) under argon, and heated in a sealed tube at 210℃ for 20 hours. The reaction was left to cool to room temperature. The mixture was purified by flash column chromatography (silica gel, petroleum ether:Chloroform:EtOAc = 40:10:3) to afford dimer product **8** (12 mg, 40%) and **2** (3 mg, 10%). (6 mg, 20% of *E*-**5** recovered). **6**: $[\alpha]^{25}_{D}$ -34.8 (c 1.61, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.87 (t, J=8.0Hz, 6H), 1.25 (brs, 42H), 1.48 (d, J=4.0Hz, 3H), 1.56 (d, J=4.0Hz, 3H), 1.95-2.00 (m, 2H), 2.18 (q, J=4.0Hz, 2H), 2.33 (q, J=4.0Hz, 1H), 4.22-4.28 (m, 2H), 5.38-5.39 (m, 2H), 6.75 (t, J=4.0Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.15 (2C), 19.60, 20.38, 22.71 (2C), 29.00, 29.11, 29.39 (4C), 29.45, 29.58 (2C), 29.68 (8C), 31.94 (2C), 32.78, 40.52, 45.40, 53.08, 53.70, 77.75, 79.95, 127.01, 131.67, 134.49, 138.55, 168.05, 176.12 ppm. HRMS (TOF ESI): calcd for $C_{38}H_{64}NaO_4$: 607.4702 $[M + Na]^+$; found: 607.4696. **8**: $[\alpha]^{25}_{D}$ -77.7 (c 1.02, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.87 (t, J=6.0Hz, 6H), 1.24 (brs, 44H), 1.41 (d, J=6.0Hz, 3H), 1.52 (d, J=6.0Hz, 3H), 1.83-1.85 (m, 1H), 2.45 (brs, 2H), 2.92 (t, J=6.0Hz, 1H), 4.17-4.24 (m, 1H), 5.04 (q, J=6.0Hz, 1H), 6.84 (s, 1H), 6.95 (s, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.08 (2C), 19.49, 21.56, 22.90 (2C), 24.46, 27.66, 29.55 (2C), 29.59 (2C), 29.78, 29.80(8C), 29.84, 31.50, 31.74, 32.12 (2C), 38.08, 38.24, 38.84, 47.81, 77.00, 81.03, 131.28, 133.60, 138.37, 151.48, 169.19, 173.31 ppm. HRMS (TOF ESI): calcd for $C_{38}H_{64}NaO_4$: 607.4702 $[M + Na]^+$; found: 607.4697.

Compound **6** (49 mg, 0.0838 mmol) was dissolved in t-BuOH / 1M KOH aq. solution =1:1 (5 mL). The resulting suspension was stirred for 3 hours at room temperature. Then the reaction mixture was added 1M aqueous HCl solution to PH=3, and $CHCl_3$. The layers were separated and the aqueous phase was extracted with $CHCl_3$ (3×). The combined organic phases were washed with water, dried $NaSO_4$ and the solvents were evaporated at reduced pressure. The crude product was used for the next step without further purification.

To solution of crude product acid (49 mg, 0.0838 mmol) in CH_2Cl_2 (5 ml) was added Dess-Martin periodinane (71 mg, 0.167 mmol). The reaction mixture was stirred at room temperature for 6 hours. Subsequently, saturated aqueous $Na_2S_2O_3$ solution and $CHCl_3$ were added. The layers were separated and the aqueous phase was extracted with $CHCl_3$ (3×). The combined organic phases were evaporated at reduced pressure. Purification by

chromatography (silica gel, petroleum ether:EtOAc:MeOH = 40:10:3) to afford acid **9** (34mg, 0.0566mmol, 68% , 2 steps). $[\alpha]^{25}_{D}$ -16.1 (c 0.70, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, J=6.0Hz, 6H), 1.25 (brs, 42H), 1.53 (d, J=6.0Hz, 3H), 1.75 (brs, 1H), 1.94-1.99 (m, 2H), 2.22 (s, 3H), 2.38 (brs, 1H), 2.49 (d, J=9.0Hz, 1H), 3.42 (brs, 1H), 4.14-4.19 (m, 1H), 5.24 (d, J=15.0Hz, 1H), 5.73-5.83 (m, 1H), 7.34 (brs, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.12 (2C), 19.06, 22.69 (2C), 28.18, 28.93, 29.17, 29.36 (2C), 29.48 (3C), 29.58, 29.67 (6C), 29.70 (4C), 31.93 (2C), 32.76, 38.98, 46.56, 49.83, 51.01, 125.73, 126.62, 134.39, 145.47, 170.34, 176.43, 204.81 ppm. HRMS (TOF ESI): calcd for $C_{38}H_{64}NaO_5$: 623.4651 $[M + Na]^+$; found: 623.4646.

Preparation of acid 10



6 **7** **10**

To a solution of compound **6** (70 mg, 0.120 mmol) in MeOH (12 ml) was added NaOMe (33 mg, 0.60 mmol) at 50 °C overnight. Then the water was added. The layers were separated and the aqueous phase was extracted with $CHCl_3$ (3×). The combined organic phases were washed with water , dried $NaSO_4$ and the solvents were evaporated at reduced pressure. The mixture was purified by flash column chromatography (silica gel, petroleum ether:Chloroform:EtOAc = 40:10:3) to afford **7** (60 mg, 0.10 mmol, 83%). $[\alpha]^{25}_{D}$ +55.1 (c 0.91, CHCl3); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (t, J=4.0Hz, 6H), 1.25 (brs, 40H), 1.38 (d, J=4.0Hz, 3H), 1.44 (d, J=4.0Hz, 3H), 1.62 (m, 2H), 2.06 (q, 8.0Hz, 2H), 2.12-2.17 (m, 2H), 2.21 (d, J=8.0Hz,1H), 2.30 (t, 8.0Hz, 1H),3.33 (d, J=8.0Hz, 1H), 4.12-4.19 (m, 1H), 4.64-4,71 (m, 1H), 5.42-5.55 (m, 2H), 5.80 (d, J=4.0Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.11 (2C), 18.59, 19.17, 22.68 (2C), 29.34 (2C), 29.44 (3C), 29.55, 29.63 (8C), 29.66 (4C), 31.90 (2C), 39.89, 40.55, 48.47, 52.56, 76.28, 77.00, 118.45, 129.60, 135.58, 136.52, 175.02, 175.15 ppm. HRMS (TOF ESI): calcd for $C_{38}H_{64}NaO_4$: 607.4702 $[M + Na]^+$; found: 607.4698.

10 was synthesized in the same process as **9** (65%)

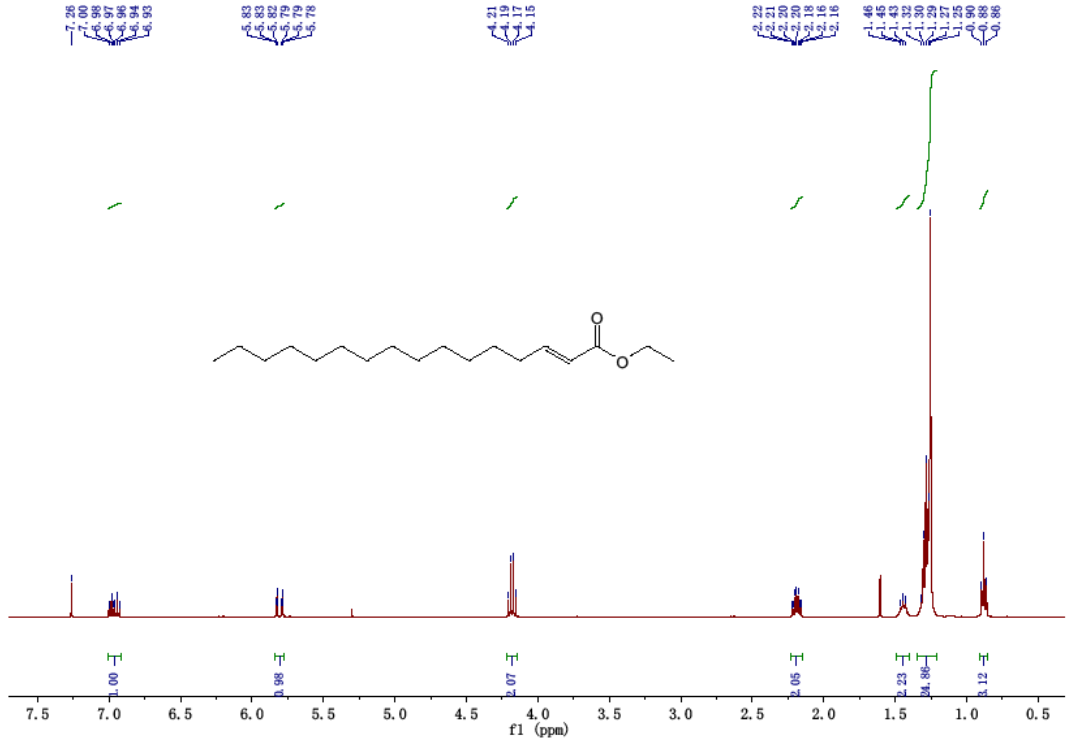
10: $[\alpha]^{25}_{D}$ +5.0 (c 0.71, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (t, J=4.0Hz, 6H), 1.24 (brs, 40H), 1.41 (m, 2H), 1.50 (d, J=8.0Hz,3H), 1.95-2.09 (m, 4H), 2.21(s, 3H), 2.63 (dd, J_1=4.0Hz, J_2=8.0Hz, 1H), 3.25 (t, J=4.0Hz, 1H), 4.07 (q, J=4.0Hz, 1H), 4.19-4.23 (m, 1H), 5.23 (d, J=16Hz, 1H), 5.34-5.41 (m, 1H), 5.98 (d, J=4.0Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.11 (2C), 18.47, 22.68 (2C), 28.20, 28.32, 28.68, 29.29, 29.35 (2C), 29.45 (2C), 29.50, 29.60 (2C), 29.65 (4C), 29.68 (2C), 31.22, 31.91 (2C), 32.45, 37.99, 45.34, 50.33, 53.09, 77.00, 120.15, 126.78, 134.20, 136.66, 175.31, 177.33, 205.41 ppm. HRMS (TOF ESI): calcd for $C_{38}H_{64}NaO_5$: 623.4651 $[M + Na]^+$; found: 623.4646.

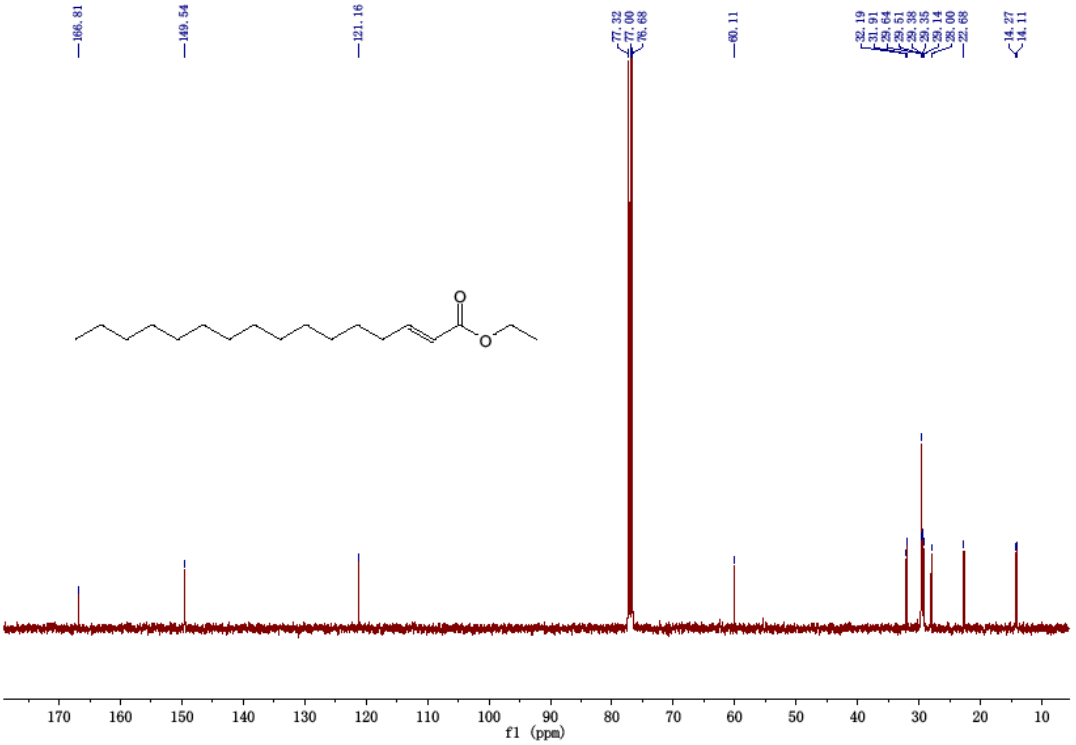
Reference:

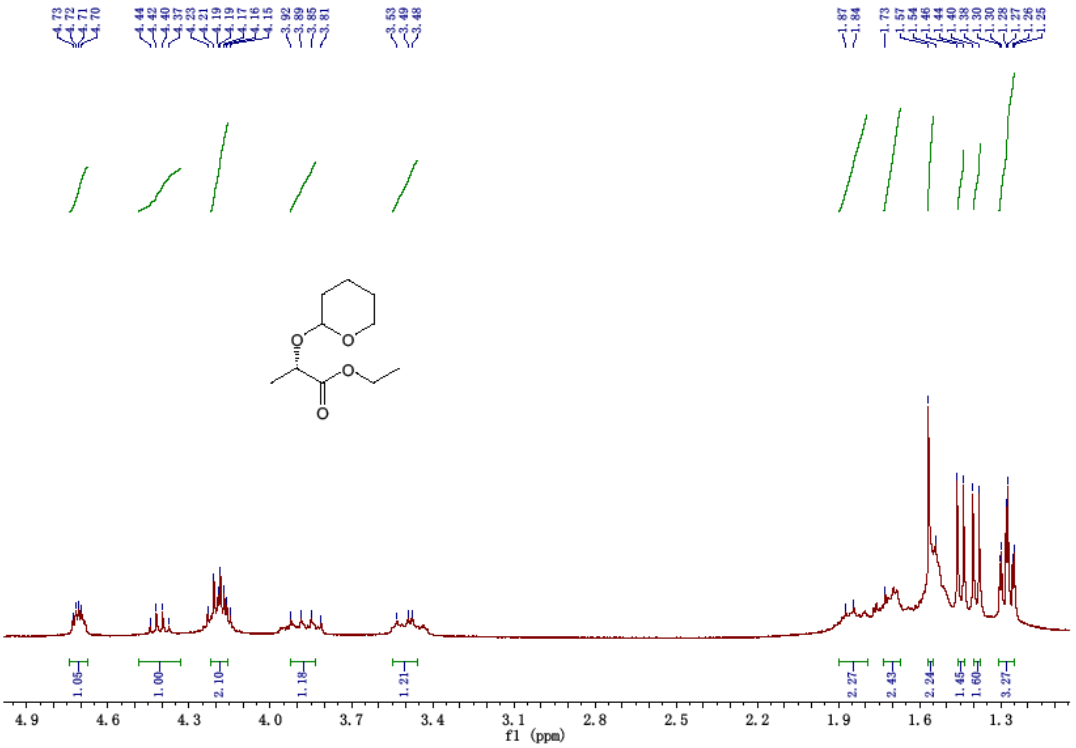
1 Yi-Nan Zhang, Wei Zhang, Di Hong, Lei Shi, Qiang Shen, Jing-Ya Li, Jia Li, Li-Hong Hu, *Bioorganic & medicinal chemistry*, 2008, **16**, 8697-8705.

2 Noutsias, D.; Vassilikogiannakis, G. *Org. Lett.* 2012, **14**, 3565-3567.

Part D: Copies of ^{1}H, ^{13}C and 2D NMR spectra



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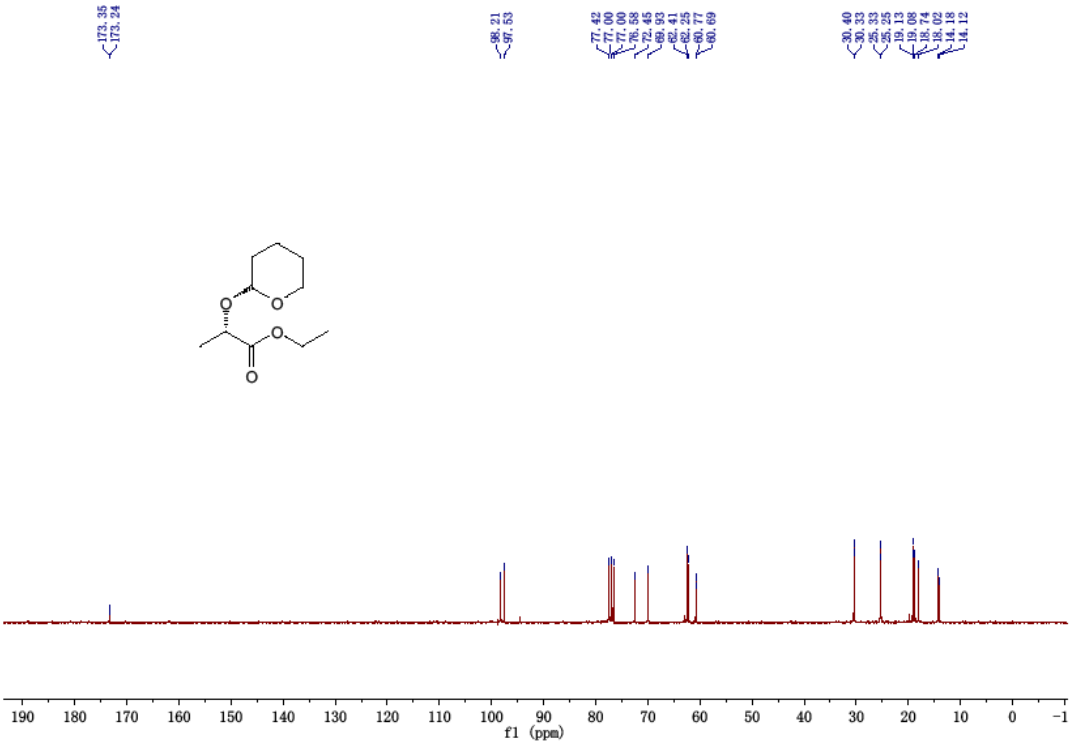
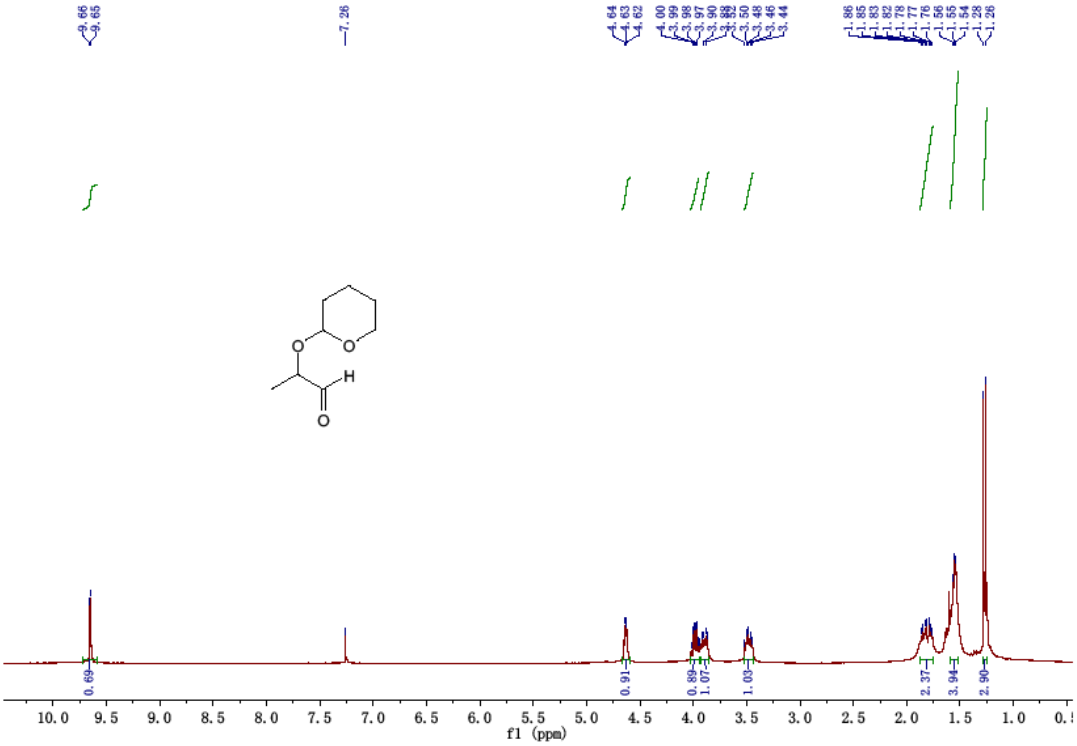
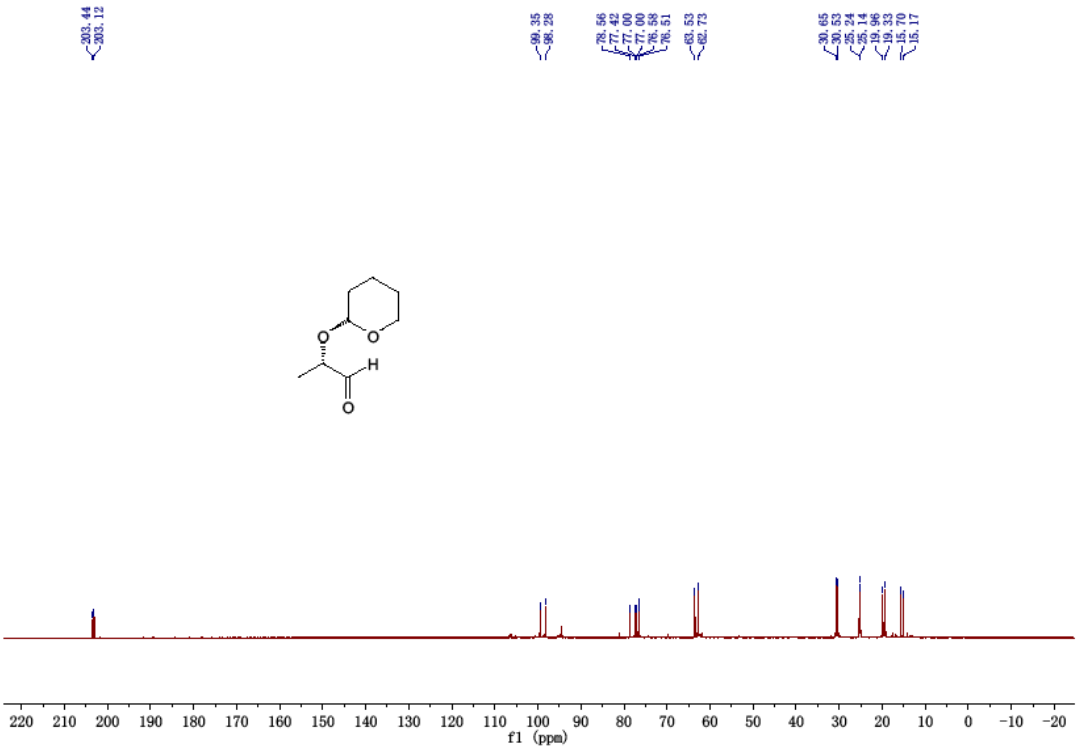
4.72
4.71
4.70
4.44
4.42
4.40
4.37
4.23
4.21
4.19
4.17
4.16
4.15
3.92
3.89
3.85
3.81
3.53
3.49
3.48
1.87
1.84
1.73
1.57
1.54
1.46
1.44
1.40
1.38
1.30
1.30
1.29
1.27
1.26
1.25
1.05
1.00
2.10
1.18
1.21
2.27
2.43
2.24
1.45
1.60
3.27
4.9
4.6
4.3
4.0
3.7
3.4
3.1
2.8
2.5
2.2
1.9
1.6
1.3
f1 (ppm)


173.35
173.24
98.21
97.53
77.42
77.00
77.00
76.58
72.45
69.93
62.41
62.25
60.77
60.69
30.40
30.33
25.33
25.25
19.13
19.08
18.74
18.02
14.18
14.12
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)


9.66
9.65
7.26
4.64
4.63
4.62
4.00
3.99
3.98
3.97
3.90
3.50
3.48
3.46
3.44
1.86
1.85
1.83
1.82
1.78
1.77
1.76
1.56
1.55
1.54
1.28
1.26
O
O
H
O
0.69
0.91
0.89
1.07
1.03
2.37
3.94
2.90
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f1 (ppm)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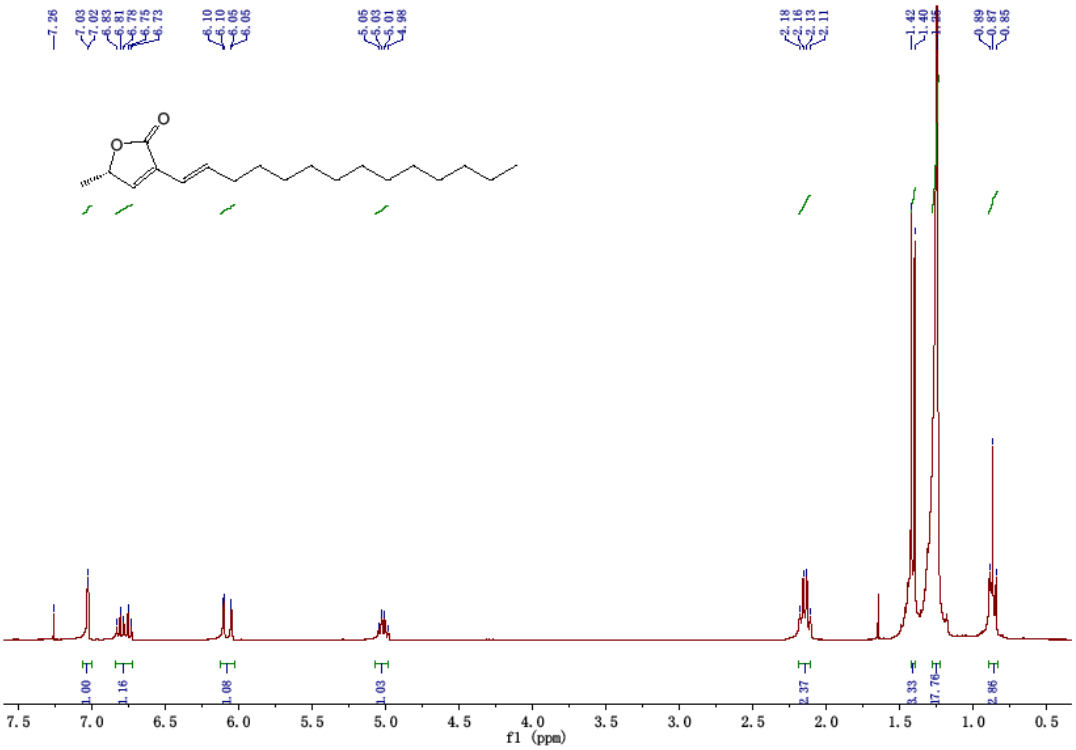
203.12
99.35
96.28
78.56
77.42
77.00
76.58
76.51
63.53
62.73
30.65
30.53
25.24
25.14
19.96
19.33
15.70
15.17
O
O
H
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)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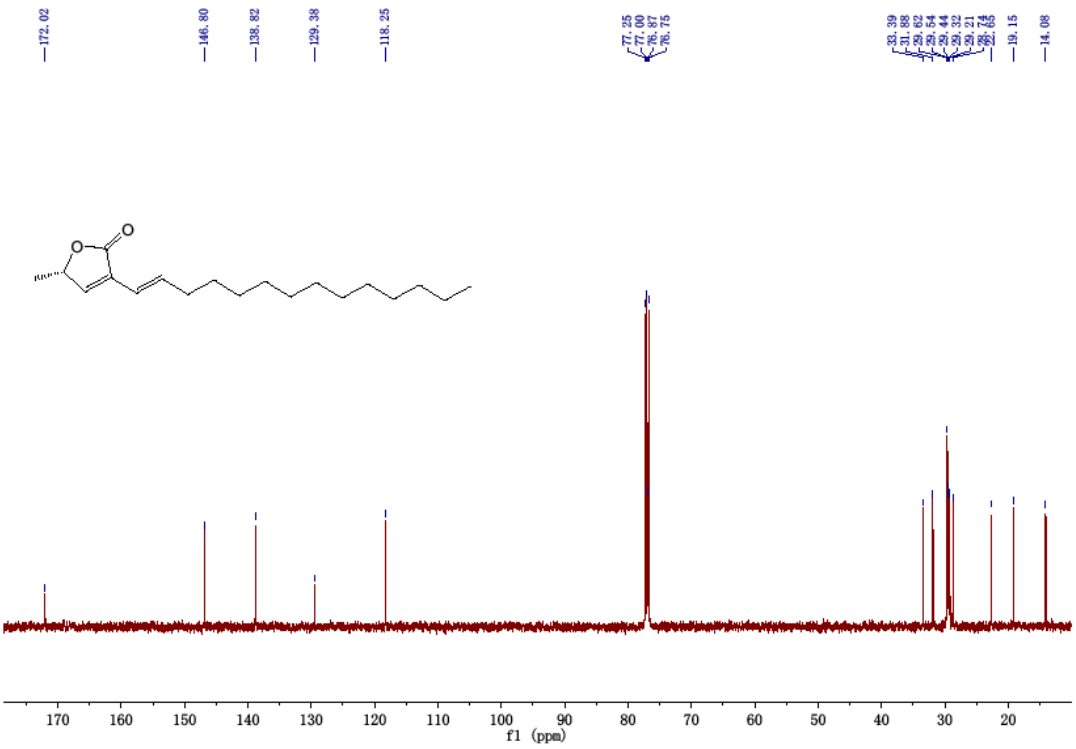
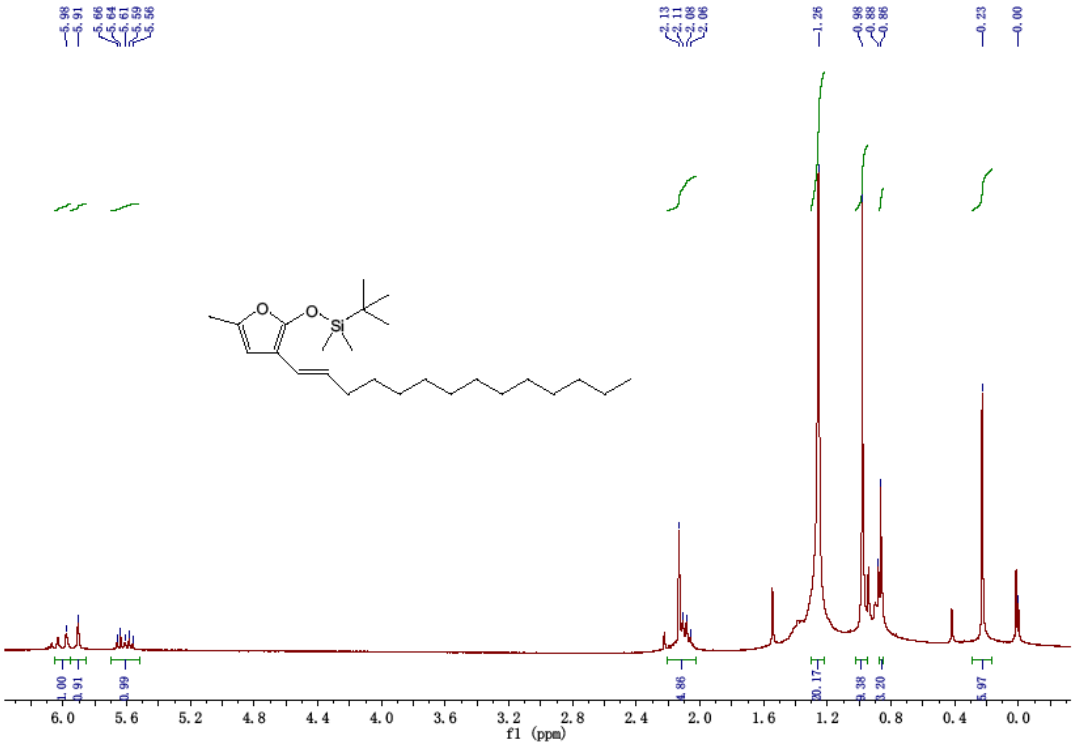
7.03
7.02
6.83
6.81
6.78
6.75
6.73
6.10
6.10
6.05
6.05
5.05
5.03
5.01
4.98
2.18
2.16
2.13
2.11
1.42
1.40
0.89
0.87
0.85
1.00
1.16
1.08
1.03
2.37
3.33
17.76
2.86
f1 (ppm)


172.02
146.80
138.82
129.38
118.25
77.25
77.00
76.87
76.75
33.39
31.88
29.62
29.54
29.44
29.32
29.21
28.74
28.65
22.65
19.15
14.08
f1 (ppm)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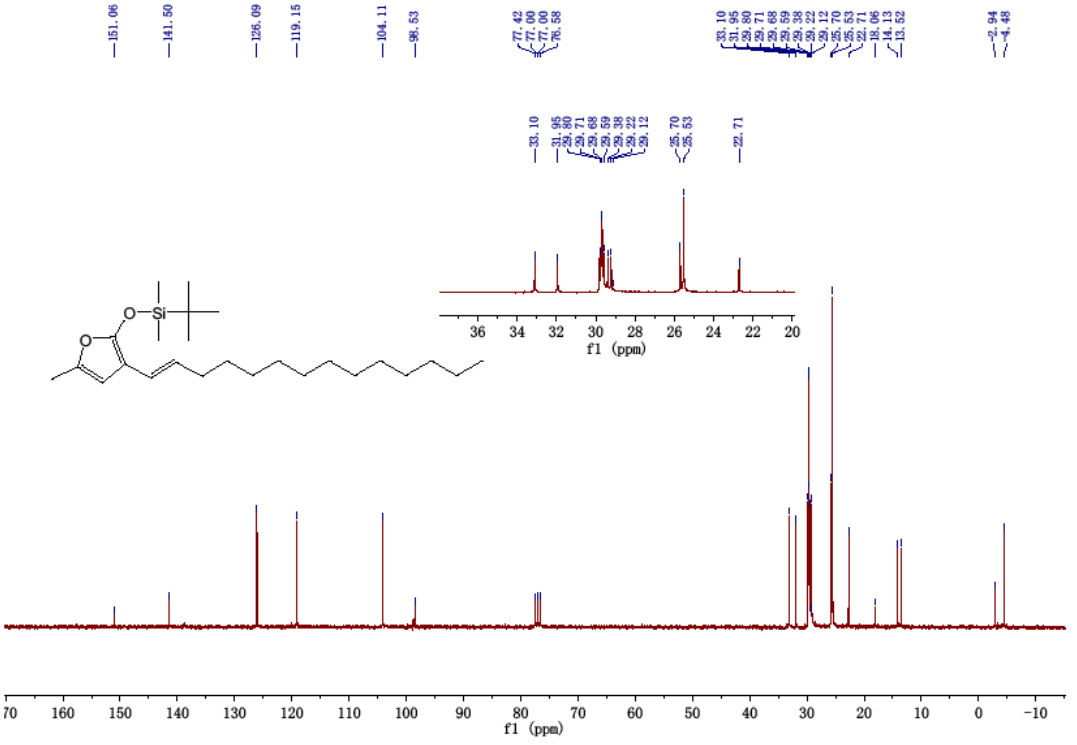
5.91
5.66
5.64
5.61
5.59
5.56
2.13
2.11
2.08
2.06
1.26
0.98
0.88
0.86
0.23
0.00
O
O
Si
1.00
0.91
1.99
4.86
20.17
4.38
3.20
5.97
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
f1 (ppm)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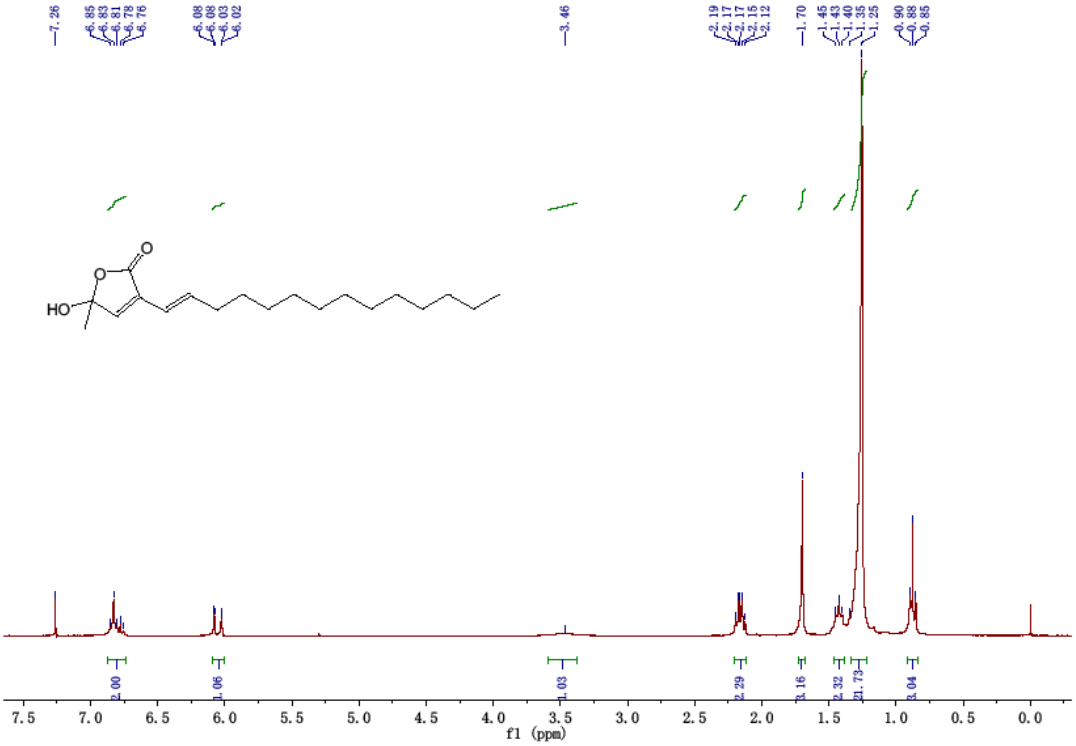
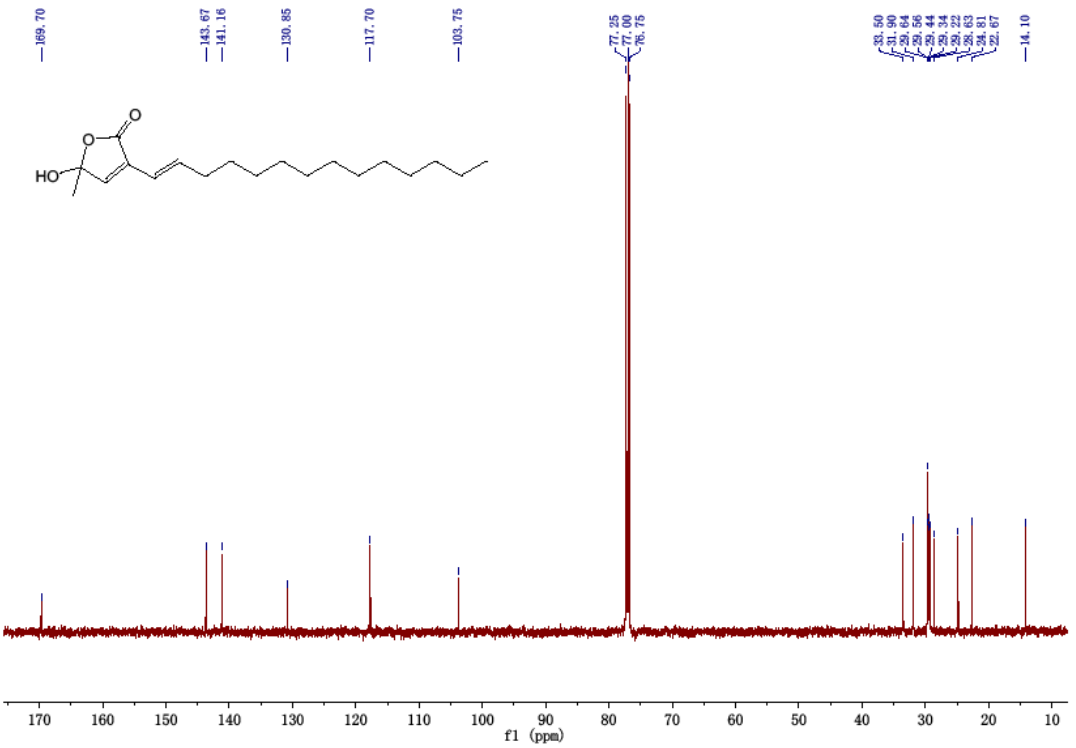
126.09
119.15
104.11
98.53
77.42
77.00
76.58
33.10
31.95
29.80
29.71
29.68
29.59
29.38
29.22
29.12
25.70
25.53
22.71
18.06
14.13
13.52
-2.94
-4.48
O
Si
O
36
34
32
30
28
26
24
22
20
f1 (ppm)
70
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


7.26
6.85
6.83
6.81
6.78
6.76
6.08
6.08
6.03
6.02
3.46
2.19
2.17
2.17
2.15
2.12
1.70
1.45
1.43
1.40
1.35
1.25
0.90
0.88
0.85
HO
O
O
2.00
1.06
1.03
2.29
3.16
2.32
21.73
3.04
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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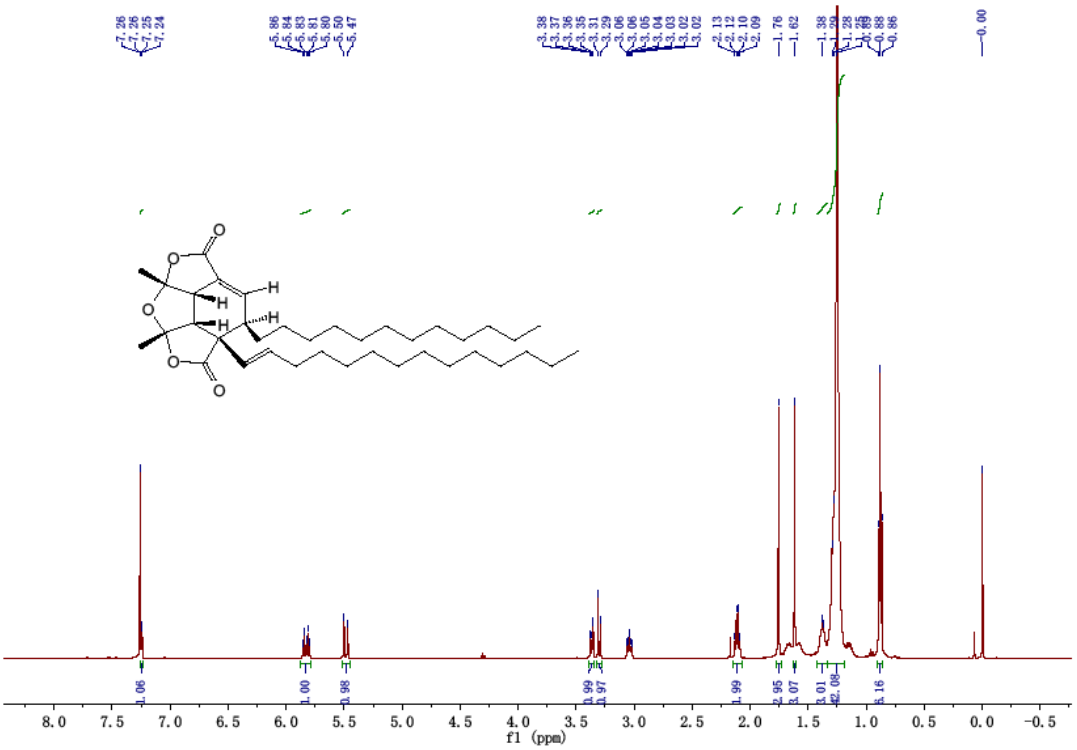
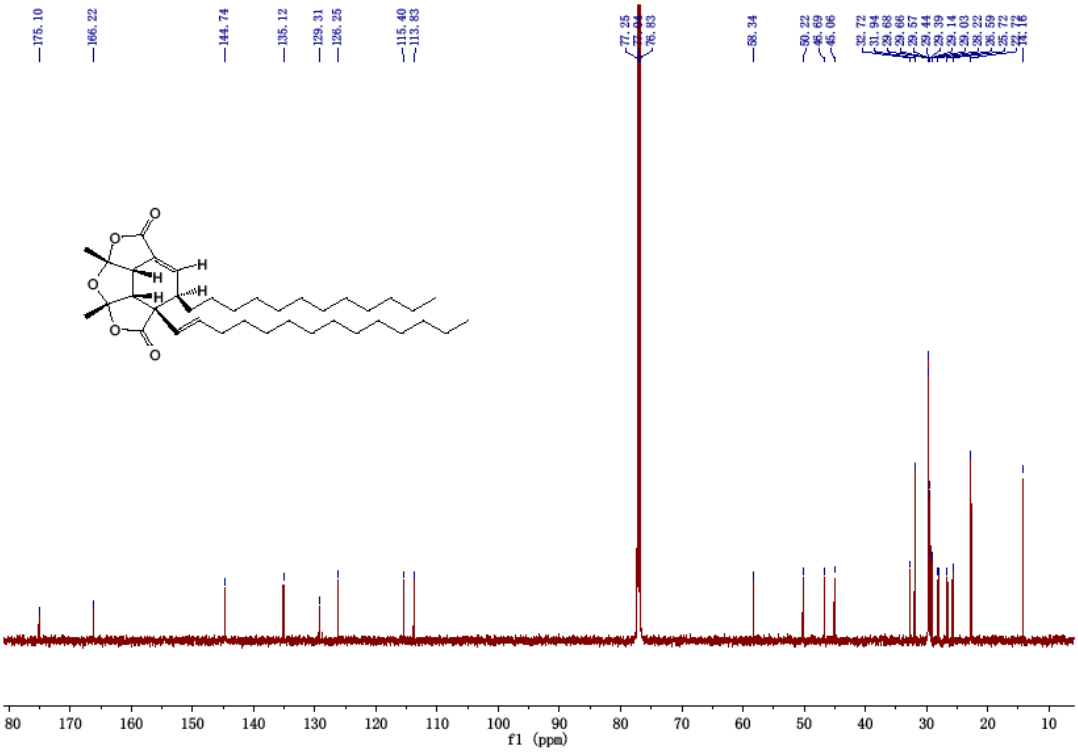
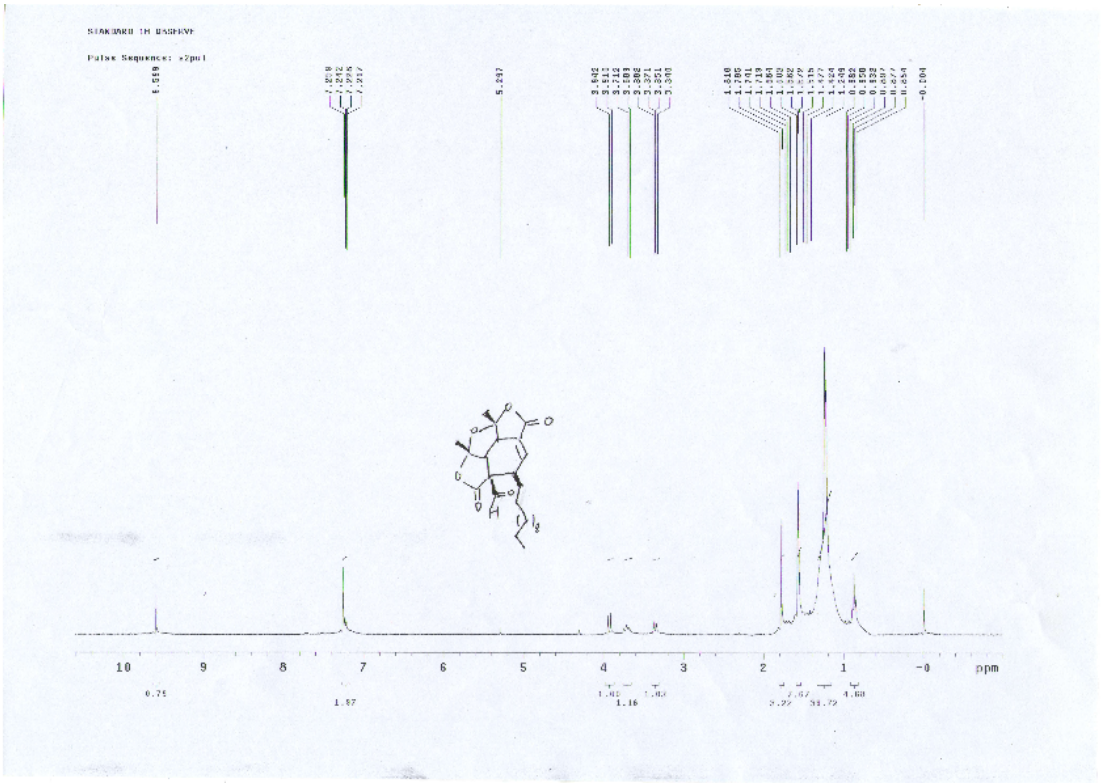
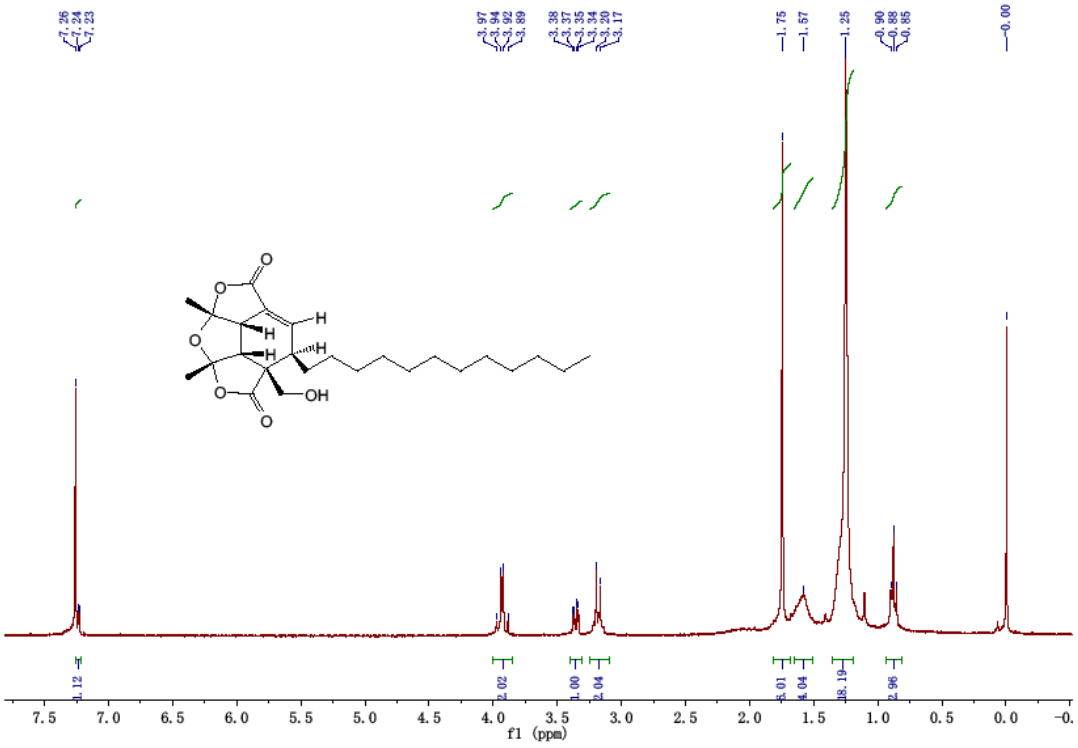
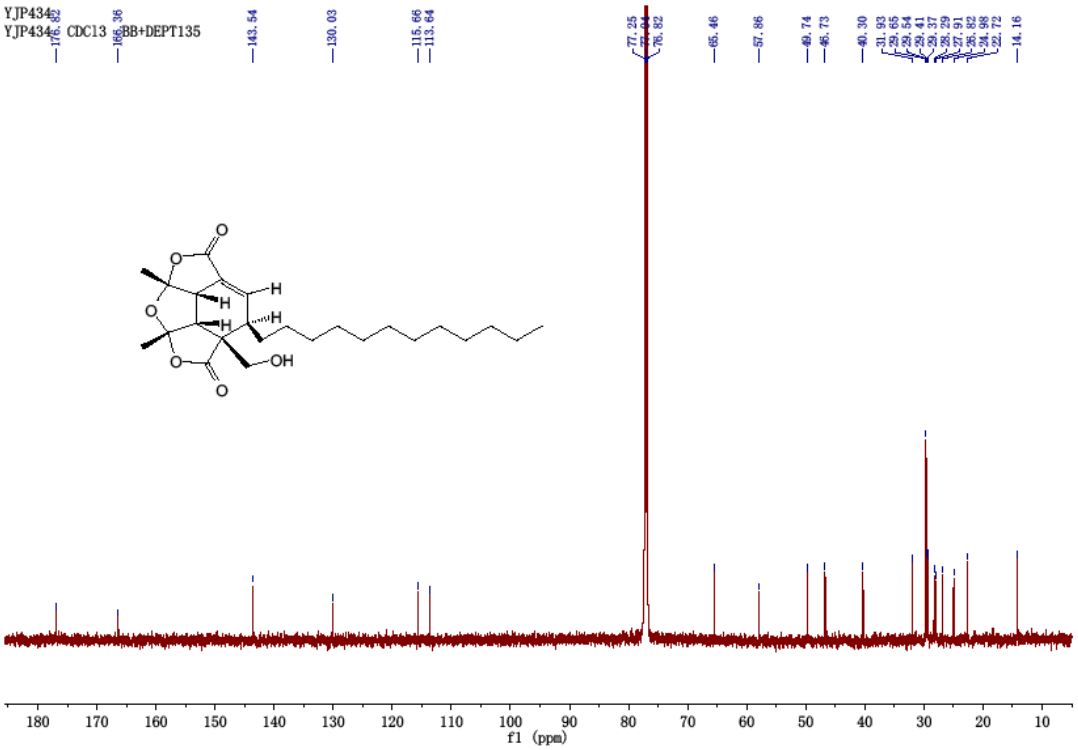
141.16
130.85
117.70
103.75
77.35
77.00
76.75
33.50
31.96
29.64
29.56
29.44
29.34
29.22
28.63
24.81
22.67
14.10
HO
O
O
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

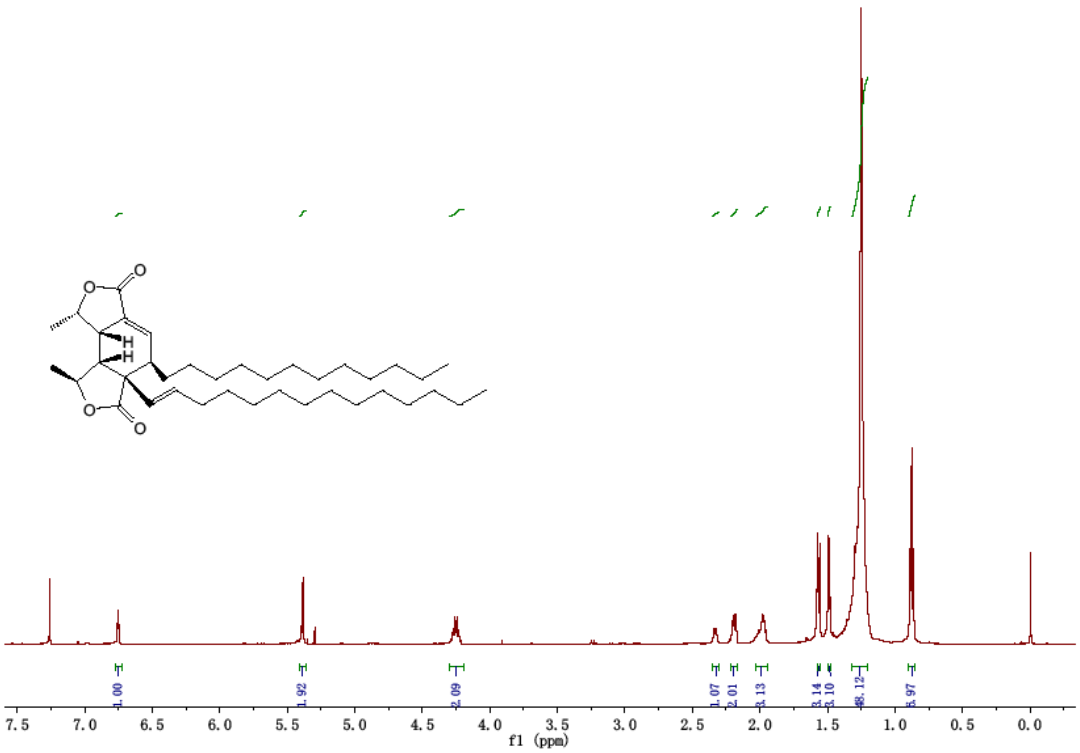

f1 (ppm)


f1 (ppm)






YJP434
YJP434_CDC13_BB+DEPT135
176.82
166.36
143.54
130.03
115.66
113.64
77.25
76.82
65.46
57.86
49.74
46.73
40.30
31.93
29.65
29.54
29.41
29.37
28.29
27.91
25.82
24.98
22.72
14.16
OH
f1 (ppm)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3.13
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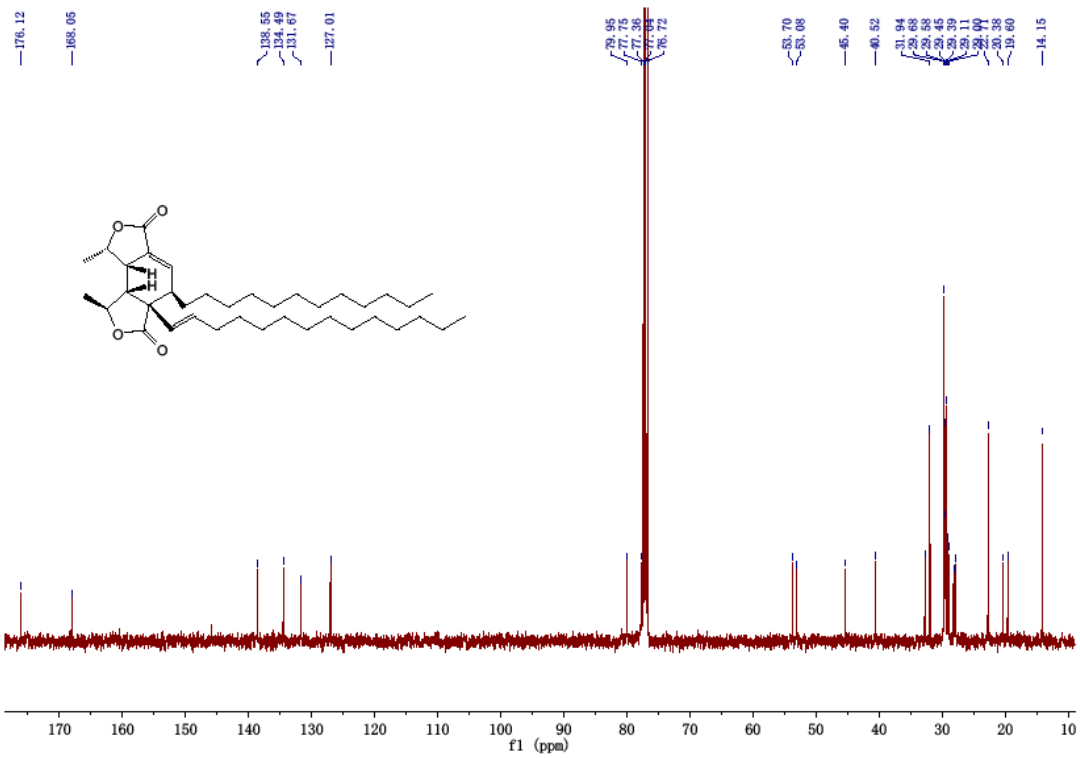
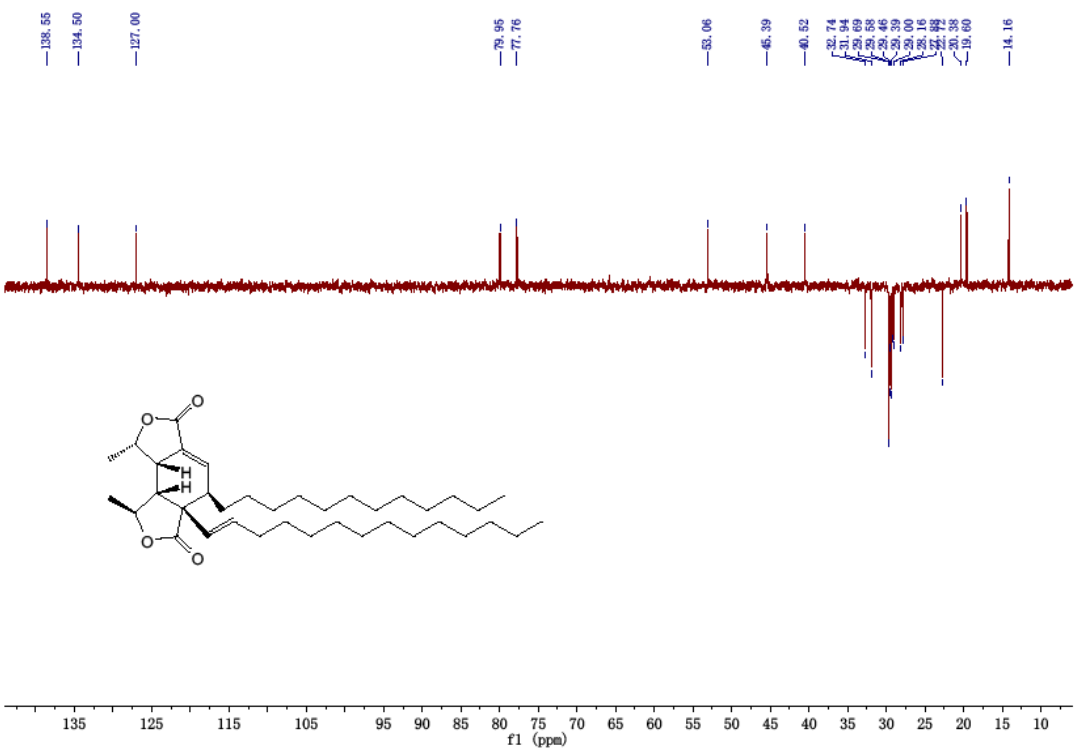
3.10
18.12
5.97
f1 (ppm)


176.12
168.05
138.55
134.49
131.67
127.01
79.95
77.75
77.36
76.72
53.70
53.08
45.40
40.52
31.94
29.68
29.58
29.45
29.39
29.11
20.38
19.60
14.15
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)


138.55
134.50
127.00
79.95
77.76
53.06
45.39
40.52
32.74
31.94
29.69
29.58
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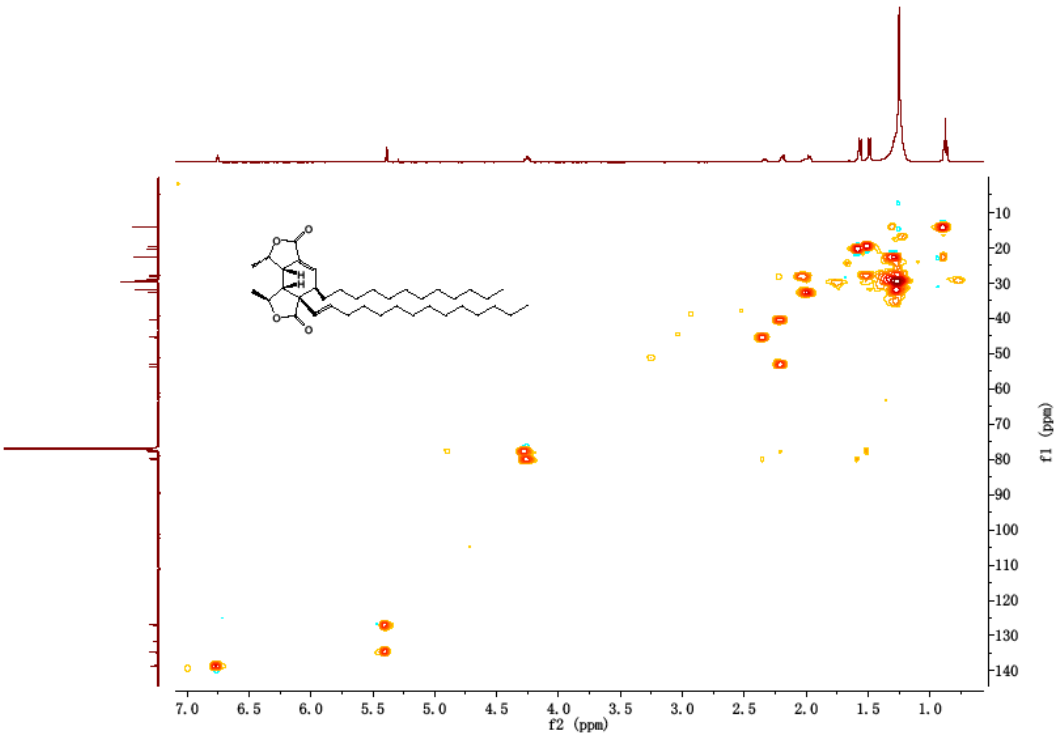
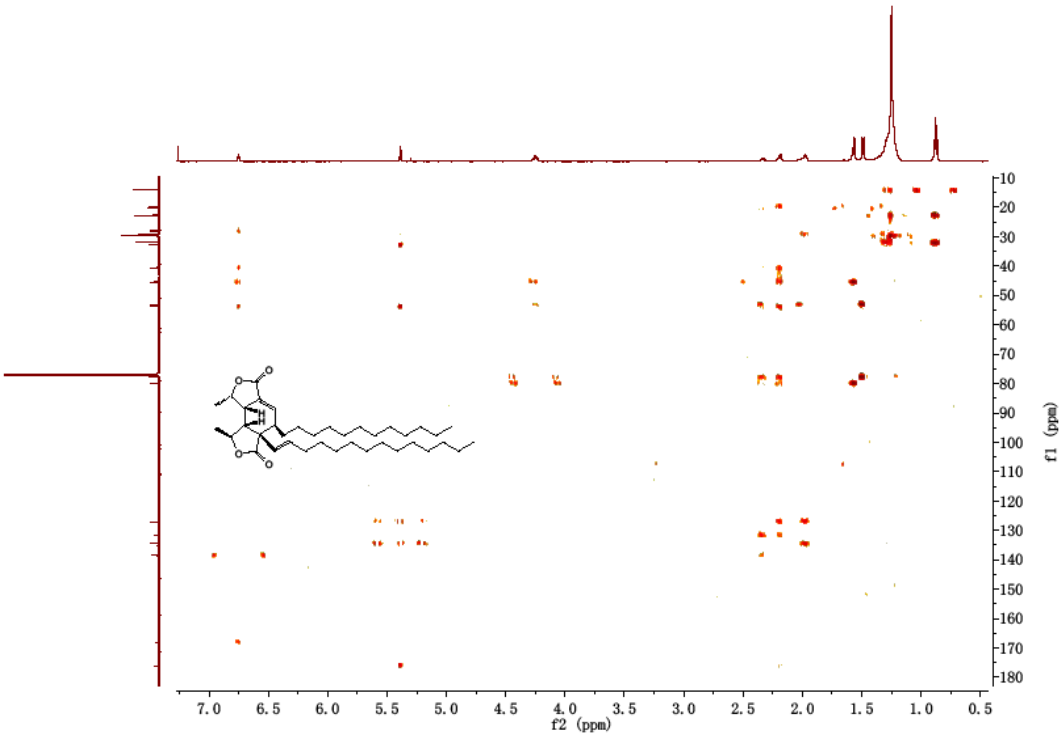
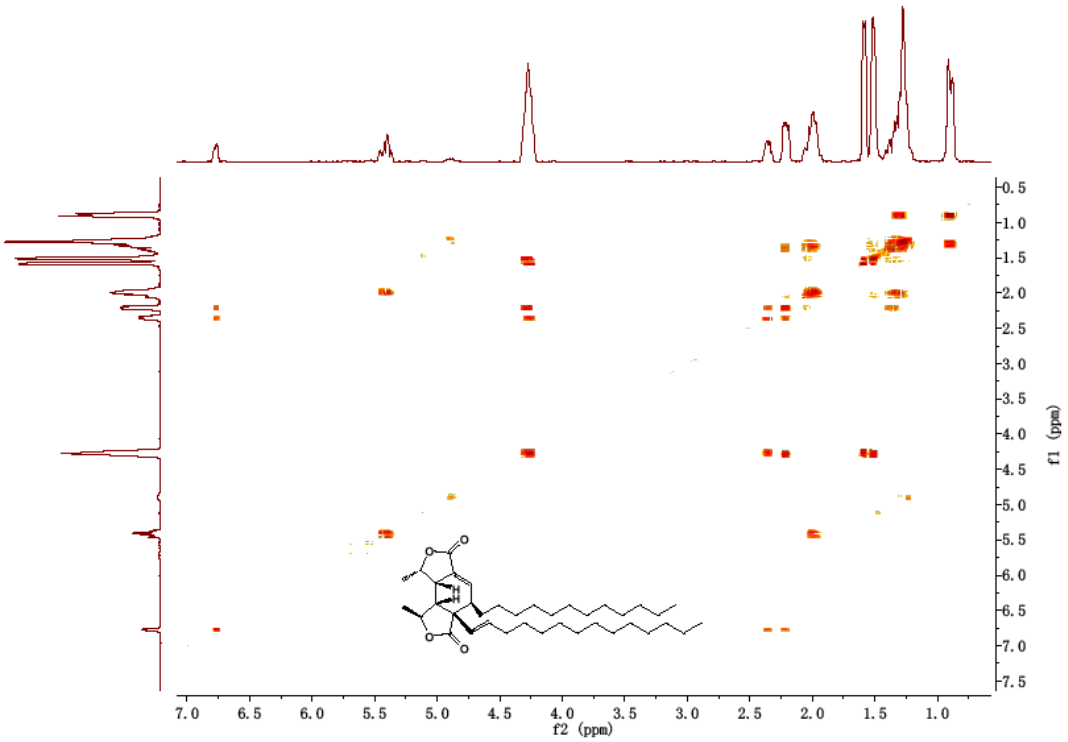
f1 (ppm)



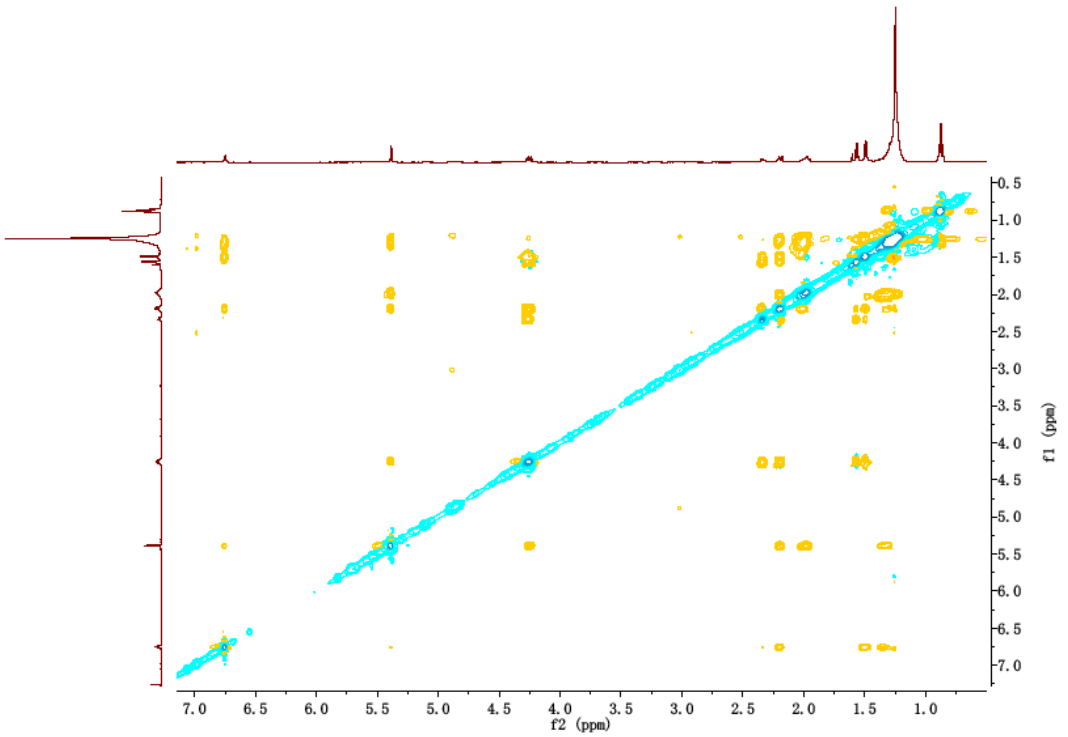
HSQC spectrum (400 MHz) of **6** in $CDCl_3$



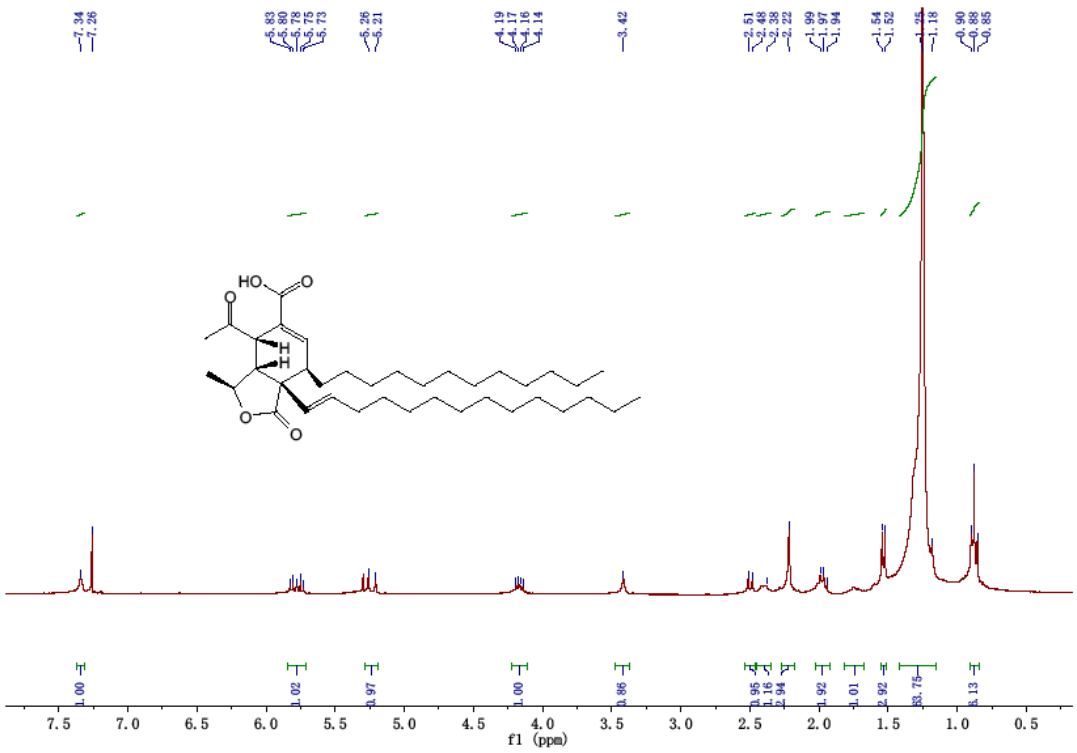
HMBC spectrum (400 MHz) of **6** in $CDCl_3$

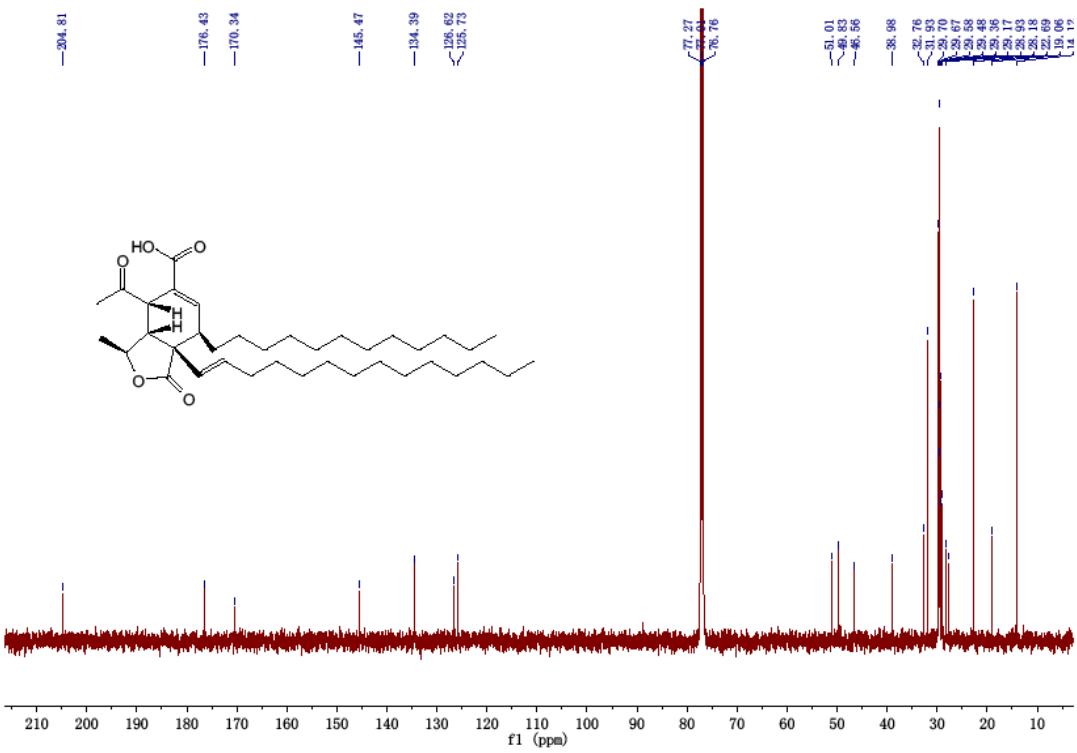


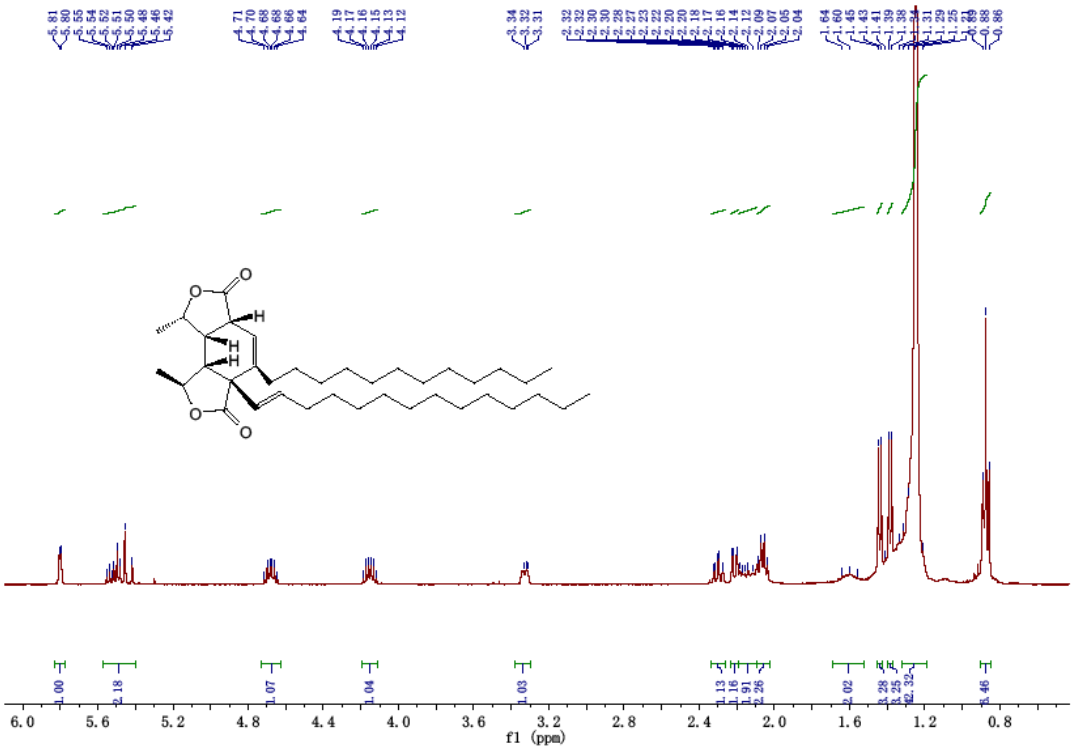
^{1}H-^{1}H COSY spectrum (400 MHz) of **6** in $CDCl_3$

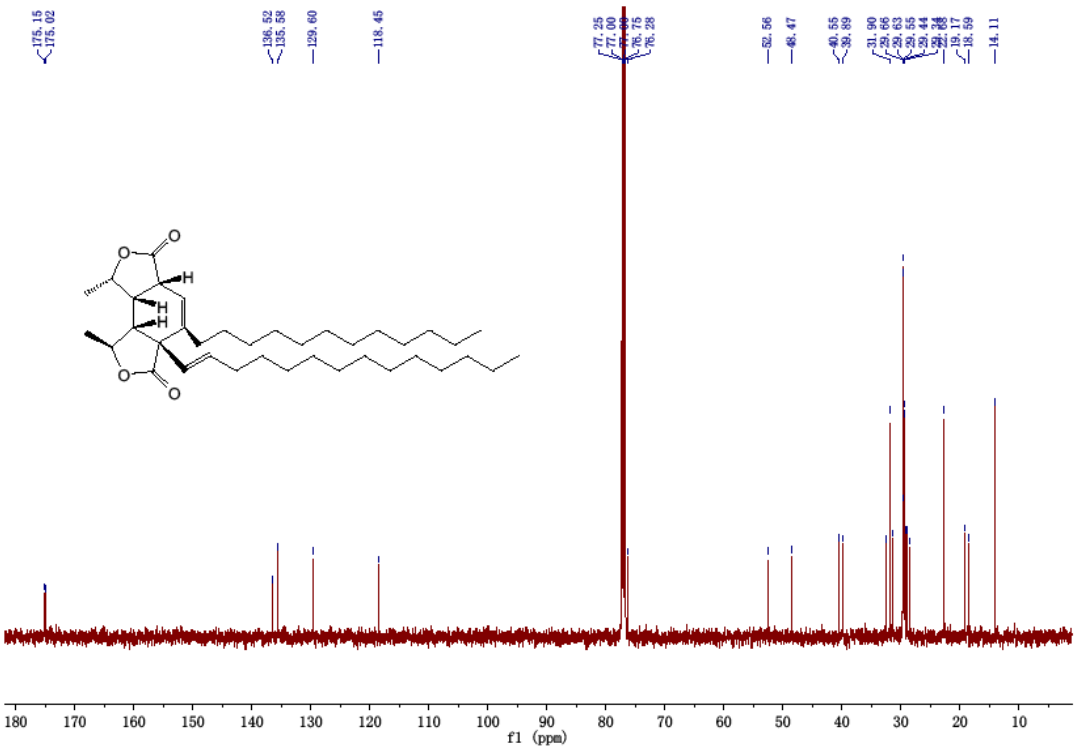


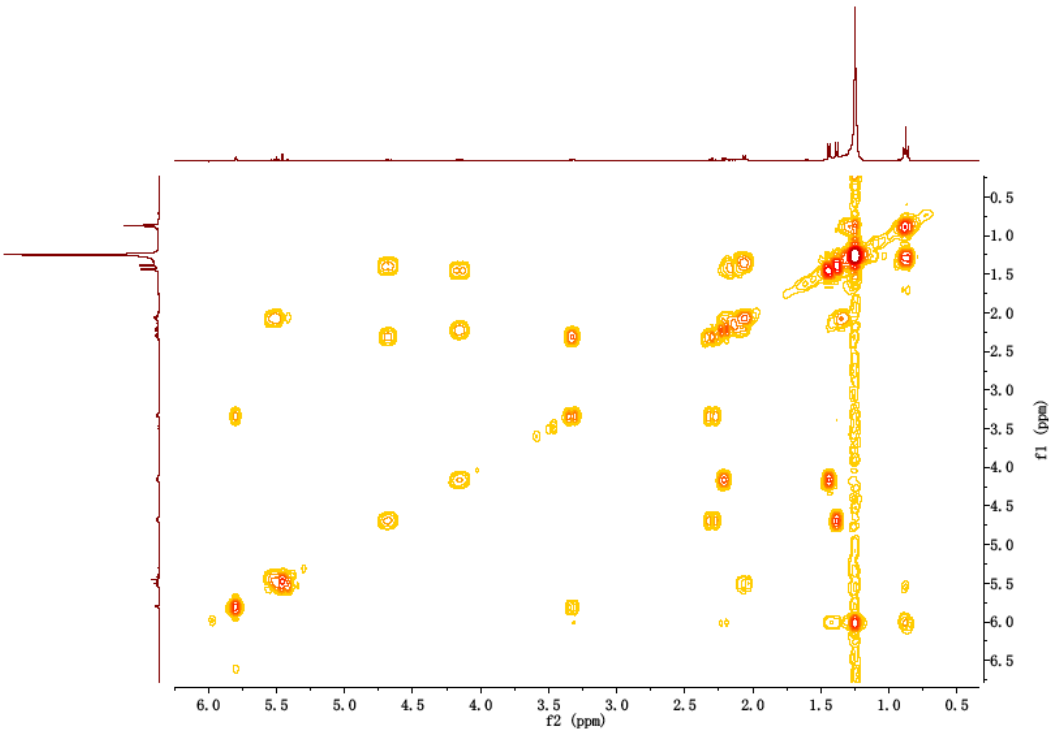
ROESY spectrum (400 MHz) of **6** in $CDCl_3$


7.34
7.26
5.83
5.80
5.78
5.75
5.73
5.26
5.21
4.19
4.17
4.16
4.14
3.42
2.51
2.48
2.38
2.22
1.99
1.97
1.94
1.54
1.52
1.25
1.18
0.90
0.88
0.85
HO
O
O
H
O
O
1.00
1.02
0.97
1.00
0.86
0.95
1.16
2.94
1.92
1.01
2.92
33.75
3.13
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

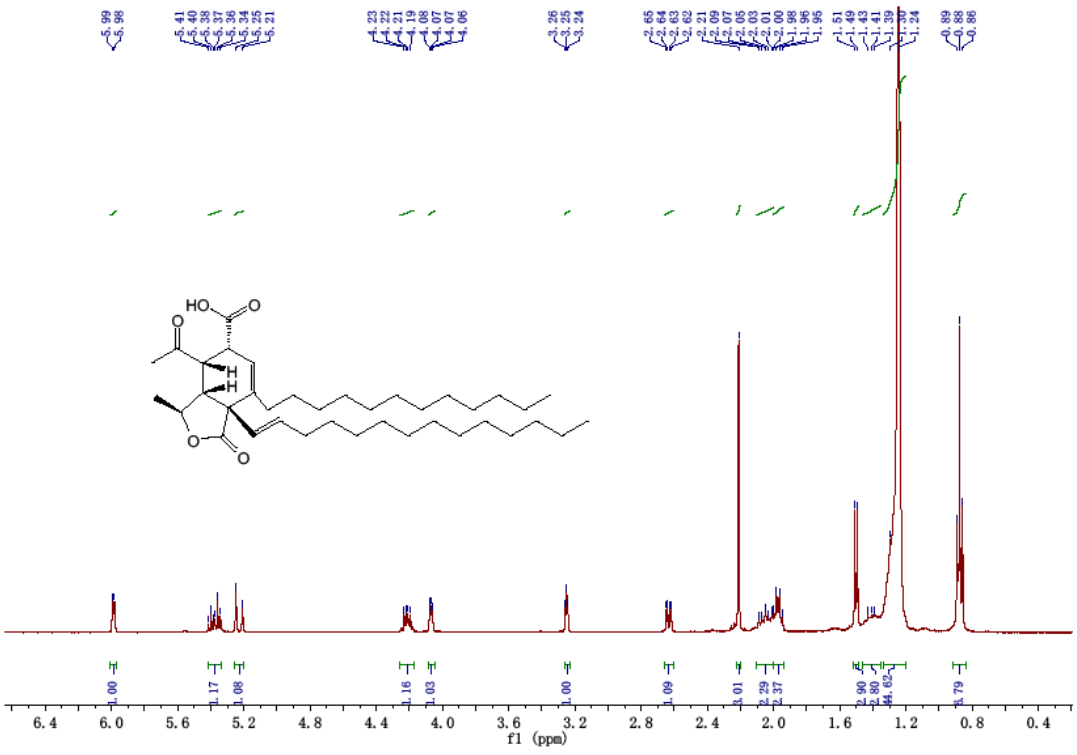

204.81
176.43
170.34
145.47
134.39
126.62
125.73
77.27
77.04
76.76
51.01
49.83
45.56
38.98
32.76
31.93
29.70
29.67
29.58
29.48
29.36
29.17
28.93
28.18
27.69
19.06
14.17
HO
O
O
H
O
O
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

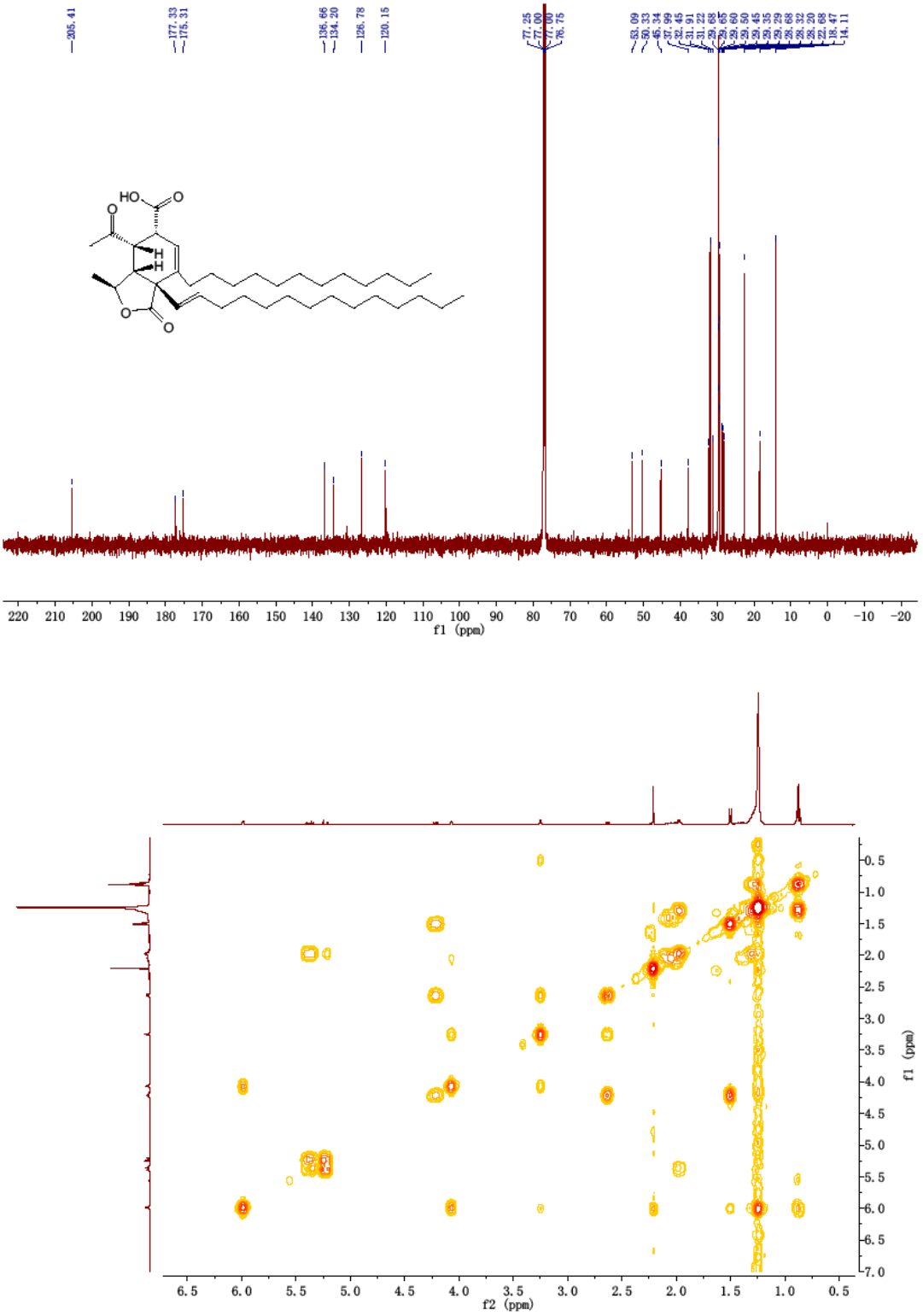

f1 (ppm)


f1 (ppm)

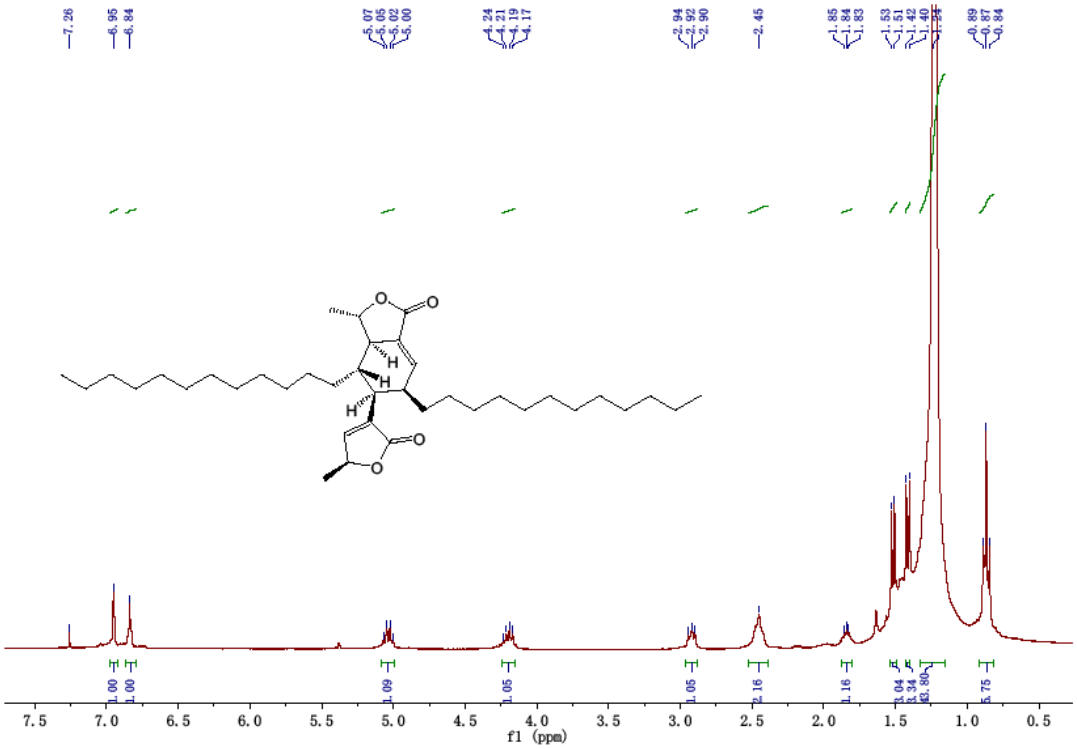


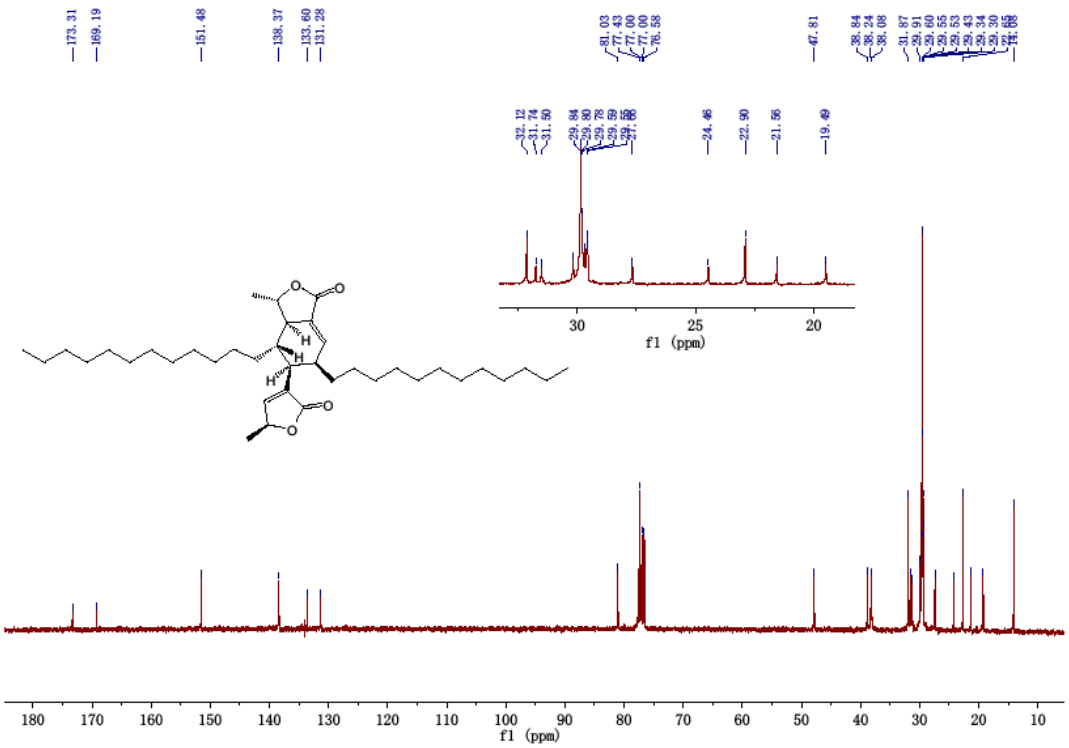
^{1}H-^{1}H COSY spectrum (400 MHz) of **7** in $CDCl_3$

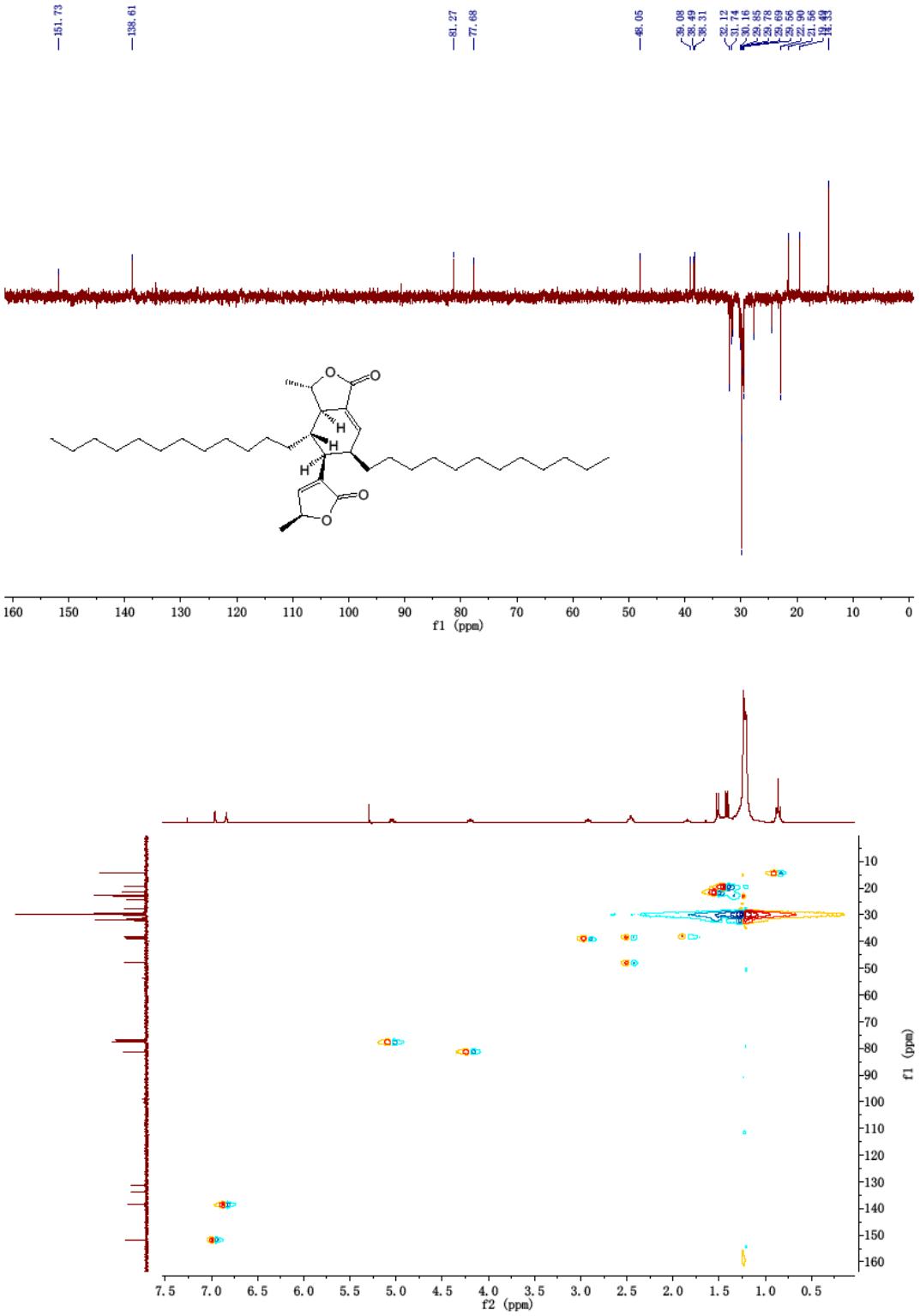




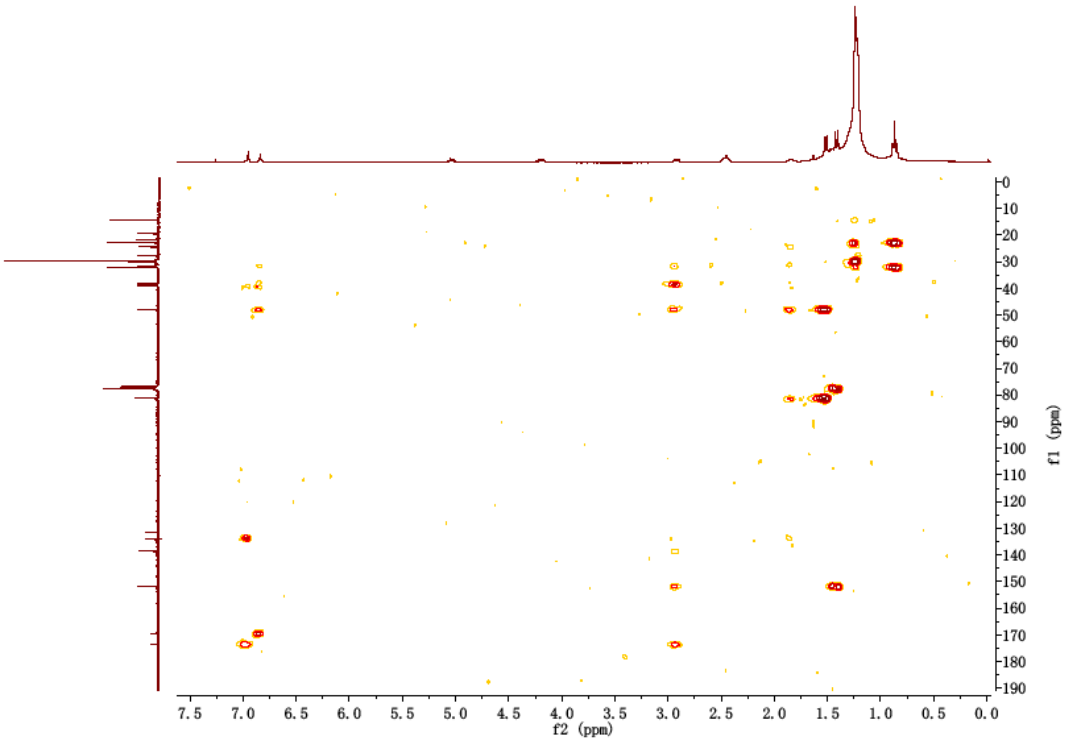
^{1}H-^{1}H COSY spectrum (400 MHz) of **10** in $CDCl_3$


f1 (ppm)

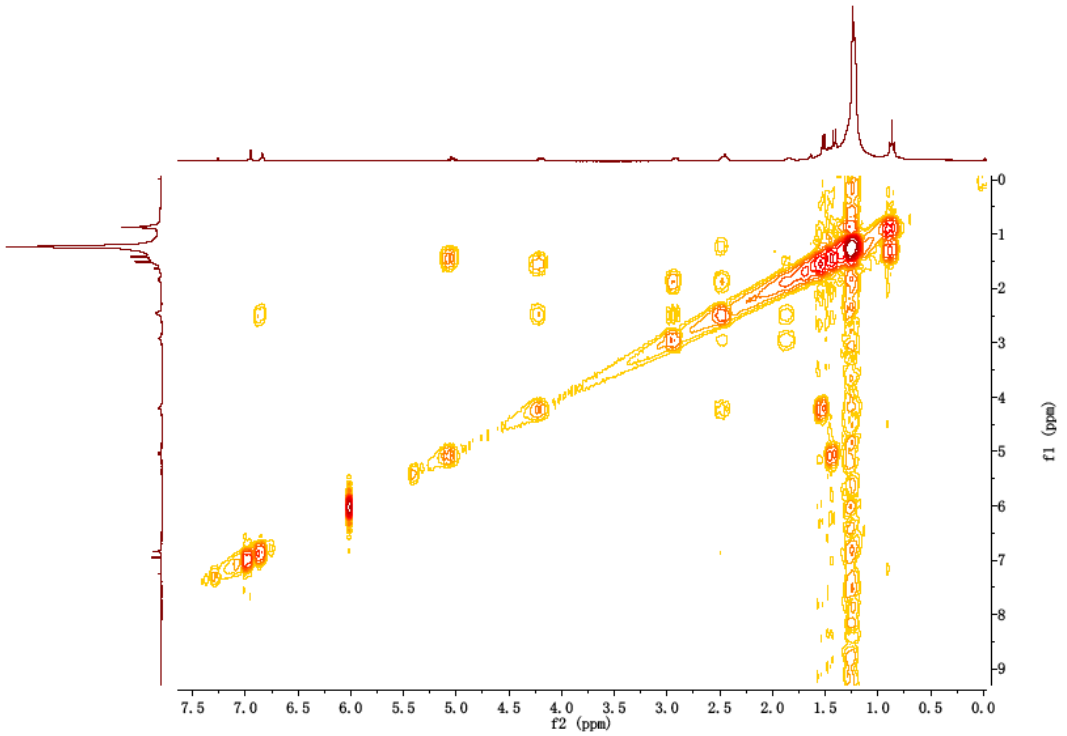

f1 (ppm)
f1 (ppm)



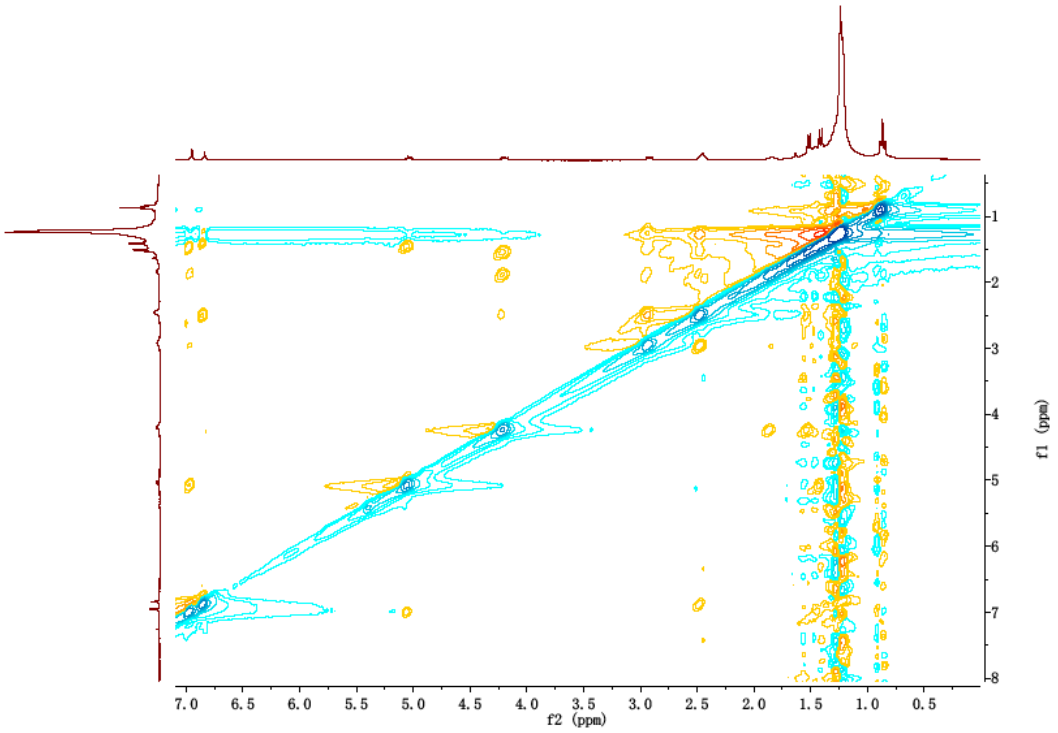
HSQC spectrum (300 MHz) of **8** in $CDCl_3$



HMBC spectrum (300 MHz) of **8** in $CDCl_3$



^1H-^1H COSY spectrum (300 MHz) of **8** in $CDCl_3$



ROESY spectrum (300 MHz) of **8** in $CDCl_3$