Hypocretenolides: Collective Total Syntheses and Activities toward Metastatic Colon Cancer

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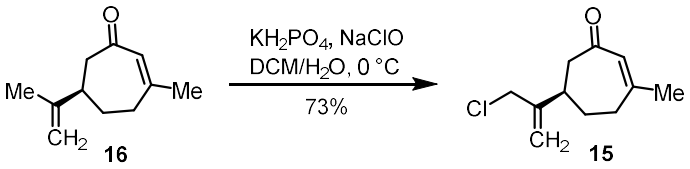
Part I. General Information

All air and moisture sensitive reactions were performed under an atmosphere of argon. Reagents purchased from Acros, Aldrich, J&K, and Aladdin were used without further purification. THF was dried by distillation over Na/benzophenone or purchased directly as ultra dry solvents. Dichloromethane (DCM) was dried by distillation over CaH_2 or purchased directly as ultra dry solvent. Petroleum ether (PE) and ethyl acetate (EA) etc. brought from Titan and Tianjin Bohua chemical reagents Co. etc were used directly unless otherwise indicated. TLC inspections were on silica gel GF254 plates. Column chromatography was performed on silica gel (200–300 mesh).

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE AV400 (400 MHz and 100 MHz). Signal positions were recorded in ppm with the abbreviations s, d, t, m, and bs denoting singlet, doublet, triplet, multiplet, and broad singlet, respectively. All NMR chemical shifts were referenced to residual solvent peaks or to $Si(CH_3)_4$ as an internal standard ($CDCl_3$ ^{1}H NMR = 7.26 ppm, ^{13}C NMR = 77.16 ppm; CD_3OD ^{1}H NMR = 3.31 ppm, ^{13}C NMR = 49.00 ppm). All coupling constants *J* are quoted in Hz. High resolution mass spectra (HRMS) were obtained on an IonSpec QFT mass spectrometer with ESI ionization. FTIR spectra were obtained with a Bruker Tensor 27 instrument. All IR samples were prepared as thin film and reported in wave numbers (cm^{-1}). Optical rotations were recorded on a Perkin–Elmer 341 polarimeter (using the sodium D line; 589 nm). The X-ray single-crystal determination was realized on a Rigaku XtalAB Pro MM007 DW diffractometer with graphite monochromated Cu Kα radiation.

Part II. Experimental Procedures and Spectroscopic Data

1. Organic Synthesis



To a stirred solution of **16**[1] (49.6 g, 302 mmol, 1.0 equiv.) in DCM/H_2O (690/690 mL) at 0 °C was added KH_2PO_4 (82.3 g, 604 mmol, 2.0 equiv.), 8–10% wt. NaClO (499 g, 604 mmol, 2.0 equiv.) in sequence. The mixture was stirred for 2 h before it was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (400 mL). The mixture was extracted with DCM (3 × 500 mL). The combined organic phases were washed with brine (400 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (PE : EA = 30 : 1 → 6 : 1) to provide **15** (43.7 g, 221 mmol, 73%) as a yellow liquid.

Data for 15:

R_f = 0.29 (PE : EA = 6 : 1);

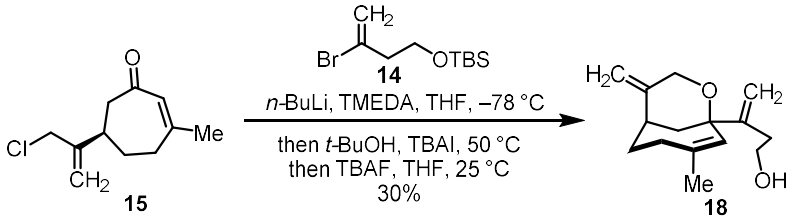
$[\alpha]_D^{25}$ = +5.24 (*c* = 1.0, $CHCl_3$);

IR (thin film) 2934, 2361, 1650, 1558, 1263, 753 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.96 – 5.89 (m, 1H), 5.20 (d, *J* = 1.0 Hz, 1H), 5.07 (d, *J* = 1.0 Hz, 1H), 4.08 (s, 2H), 2.85 – 2.77 (m, 1H), 2.77 – 2.65 (m, 2H), 2.51 (ddd, *J* = 17.6, 8.3, 3.4 Hz, 1H), 2.39 (ddd, *J* = 17.6, 8.5, 3.4 Hz, 1H), 2.08 (m, 1H), 1.96 (d, *J* = 1.3 Hz, 3H), 1.79 (m, 1H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ 201.6, 159.1, 148.3, 129.9, 114.8, 47.9, 47.6, 36.1, 33.7, 31.8, 27.6 ppm;

HRMS(ESI-TOF): Calcd for $C_{11}H_{16}ClO^+$ $[M+H]^+$ 199.0884, 201.0855 found: 199.0886, 201.0855.



To a stirred solution of **14**[2] (116 g, 440 mmol, 2.0 equiv.) in THF (1 L) under argon at −78 °C was added *n*-BuLi (183 mL, 2.4 M in hexane, 440 mmol, 2.0 equiv.) dropwise over 10 min. After stirring for 30 min, TMEDA (76.7 g, 660 mmol, 3.0 equiv.) was added into the mixture dropwise via syringe. The resulting mixture was stirred for 10 min followed by addition of a solution of **15**

(43.5 g, 220 mmol, 1.0 equiv.) in THF (100 mL) dropwise via syringe. The mixture was stirred for 5 h at 0 °C before it was quenched with *t*-BuOH (14.7 g, 198 mmol, 0.9 equiv.) and TBAI (16.3 g, 44.0 mmol, 0.2 equiv.). The resulting mixture was stirred for 5 h at 50 °C before it was quenched with a saturated aqueous solution of $NaHCO_3$ (600 mL). The mixture was extracted with Et_2O (3 × 600 mL). The combined organic phases were washed with brine (600 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to provide a crude product. To a stirred solution of the crude product in THF (1 L) was added TBAF (440 mL, 1 M in THF, 440 mmol, 2.0 equiv.). The mixture was stirred for 5 h before it was quenched with H_2O (500 mL). The resulting mixture was extracted with Et_2O (3 × 500 mL). The combined organic phases were washed with brine (500 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (PE : EA = 50 : 1 → 10 : 1 → 5 : 1) to provide **18** (15.5 g, 66.2 mmol, 30%) as a yellow oil.

Data for 18:

R_f = 0.45 (PE : EA = 2 : 1);

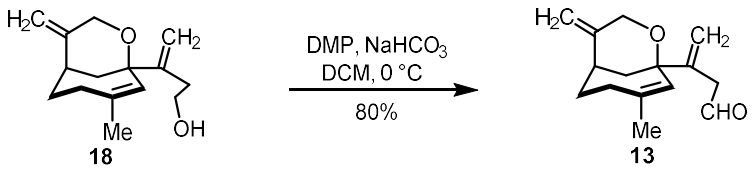
$[\alpha]_D^{25}$ = −14.9 (*c* = 1.0, $CHCl_3$);

IR (thin film) 2924, 1443, 1052, 898, 771 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.14 (d, *J* = 1.2 Hz, 1H), 5.11 (h, *J* = 1.6 Hz, 1H), 4.90 (t, *J* = 1.2 Hz, 1H), 4.76 – 4.71 (m, 2H), 4.37 – 4.30 (m, 1H), 3.98 (d, *J* = 12.9 Hz, 1H), 3.72 (q, *J* = 6.7 Hz, 2H), 2.75 (m, 1H), 2.51 (t, *J* = 4.4 Hz, 1H), 2.47 – 2.30 (m, 3H), 2.16 (m, 1H), 2.12 – 1.97 (m, 2H), 1.85 (d, *J* = 0.7 Hz, 3H), 1.82 (ddd, *J* = 13.9, 5.1, 2.0 Hz, 1H), 1.57 – 1.49 (m, 1H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ 152.4, 148.4, 143.0, 126.1, 111.2, 108.5, 78.9, 64.2, 62.9, 36.3, 35.8, 34.6, 32.0, 30.2, 27.8 ppm;

HRMS(ESI-TOF): Calcd for $C_{15}H_{23}O_2^+$ $[M+H]^+$ 235.1693, found: 235.1687.



To a stirred solution of **18** (18.0 g, 77.0 mmol, 1.0 equiv.) and $NaHCO_3$ (19.4 g, 231 mmol, 3.0 equiv.) in DCM (385 mL) was added Dess–Martin periodinane (49.0 g, 116 mmol, 1.5 equiv.) at 0 °C. The mixture was stirred at the same temperature for 2 h before it was quenched with a saturated

aqueous solution of $Na_2S_2O_3$ (80.0 mL). The resulting mixture was extracted with DCM (3 × 100 mL). The combined organic phases were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (PE : EA = 200 : 1 → 50 : 1) to provide **13** (14.3 g, 61.6 mmol, 80%) as a yellow oil.

Data for 13:

R_f = 0.35 (PE : EA = 20 : 1);

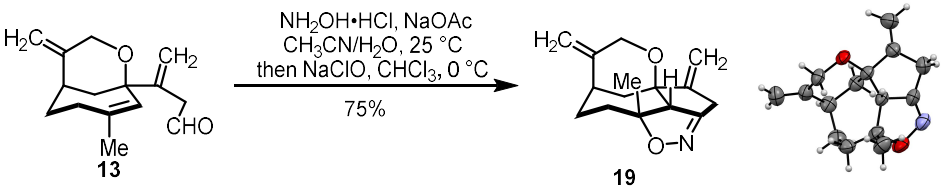
$[\alpha]_D^{25}$ = −17.14 (*c* = 1.0, $CHCl_3$);

IR (thin film) 2925, 2851, 1724, 1665, 1447, 1055, 901 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.56 (s, 1H), 5.28 (s, 1H), 5.06 (s, 1H), 4.96 (s, 1H), 4.73 (d, *J* = 7.2 Hz, 2H), 4.29 (d, *J* = 13.0 Hz, 1H), 3.95 (d, *J* = 13.0 Hz, 1H), 3.19 – 3.02 (m, 2H), 2.76 (d, *J* = 8.4 Hz, 1H), 2.37 (t, *J* = 13.4 Hz, 1H), 2.16 – 2.09 (m, 1H), 2.04 (m, 1H), 1.85 (s, 3H), 1.76 (dd, *J* = 13.9, 5.1 Hz, 1H), 1.52 (m, 1H), 1.25 (s, 1H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ 200.7, 148.5, 146.9, 144.1, 125.7, 114.5, 108.6, 78.5, 64.3, 47.3, 36.0, 35.8, 32.2, 30.3, 27.9 ppm;

HRMS(ESI-TOF): Calcd for $C_{15}H_{21}O_2^+$ $[M+H]^+$ 233.1536, found: 233.1534.



To a stirred solution of **13** (14.0 g, 60.3 mmol, 1.0 equiv.) and NaOAc (9.89 g, 121 mmol, 2.0 equiv.) in CH_3CN/H_2O (225 mL/75 mL) was added $NH_2OH·HCl$ (6.29 g, 90.5 mmol, 1.5 equiv.) at 25 °C. The mixture was stirred at the same temperature for 40 min before it was extracted with Et_2O (3 × 100 mL). The combined organic phases were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to provide a crude product. To a stirred solution of the crude product in $CHCl_3$ (300 mL) was added 8–10% wt. NaClO (124 g, 151 mmol, 2.5 equiv.) at 0 °C. The mixture was stirred at the same temperature for 30 min before it was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (100 mL). The resulting mixture was extracted with DCM (3 × 100 mL). The combined organic phases were washed with brine (200 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was

purified by column chromatography (PE : EA = 50 : 1 → 10 : 1 → 5 : 1) to provide **19** (11.1 g, 45.3 mmol, 75%) as a white solid.

Data for 19:

R_f = 0.36 (PE : EA = 4 : 1);

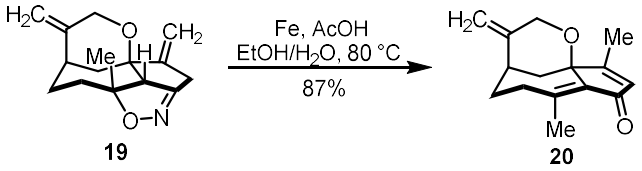
$[\alpha]_D^{25}$ = −17.04 (*c* = 1.0, $CHCl_3$);

IR (thin film) 3838, 3748, 3673, 3649, 3615, 1687, 1650, 1540, 1521, 770 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.31 (t, *J* = 2.6 Hz, 1H), 5.05 (t, *J* = 2.3 Hz, 1H), 4.97 (s, 1H), 4.87 (s, 1H), 4.40 (d, *J* = 13.9 Hz, 1H), 4.16 (m, 1H), 3.40 (s, 1H), 3.29 (m, 2H), 2.74 – 2.66 (m, 1H), 2.14 – 1.96 (m, 2H), 1.90 (m, 1H), 1.80 (m, 1H), 1.70 – 1.63 (m, 2H), 1.43 (s, 3H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ 161.6, 152.8, 144.7, 111.8, 110.2, 88.1, 82.1, 71.7, 70.1, 38.9, 37.0, 34.8, 32.4, 30.6, 28.2 ppm;

HRMS(ESI-TOF): Calcd for $C_{15}H_{20}NO_2^+$ $[M+H]^+$ 246.1489, found: 246.1491.



19 20

To a stirred solution of **19** (14.0 g, 57.1 mmol, 1.0 equiv.) and AcOH (34.3 g, 571 mmol, 10.0 equiv.) in EtOH/H_2O (300 mL/300 mL) was added iron powder (32.0 g, 571 mmol, 10.0 equiv.) at 25 °C. The mixture was stirred at 80 °C for 1 h before it was cooled to 25 °C. The mixture was extracted with Et_2O (3 × 200 mL). The combined organic phases were washed with brine (200 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (PE : EA = 10 : 1 → 5 : 1) to provide **20** (11.4 g, 49.5 mmol, 87%) as a white solid.

Data for 20:

R_f = 0.30 (PE : EA = 4 : 1);

$[\alpha]_D^{25}$ = +3.20 (*c* = 1.0, $CHCl_3$);

IR (thin film) 3747, 3546, 2928, 2360, 1688, 1648, 1540,1521,1269, 1059, 764 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.97 (q, *J* = 1.4 Hz, 1H), 5.03 (s, 1H), 4.95 – 4.89 (m, 1H), 4.48 (m, 1H), 4.30 (d, *J* = 14.4 Hz, 1H), 3.10 – 3.01 (m, 2H), 2.39 – 2.31 (m, 4H), 2.11 (d, *J* = 1.4 Hz, 3H), 1.96 – 1.83 (m, 2H), 1.80 – 1.72 (m, 1H), 1.54 (dd, *J* = 13.9, 2.4 Hz, 1H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ 194.8, 170.5, 153.1, 147.0, 138.1, 133.8, 109.9, 81.3, 67.6, 35.6, 34.8, 32.5, 31.1, 21.6, 13.4 ppm;

HRMS(ESI-TOF): Calcd for $C_{15}H_{19}O_2^+$ $[M+H]^+$ 231.1380, found: 231.1384.

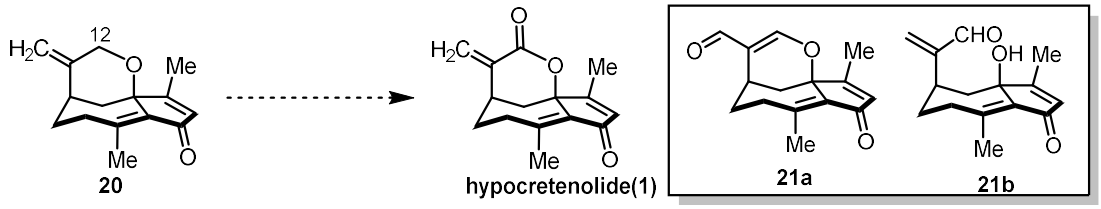
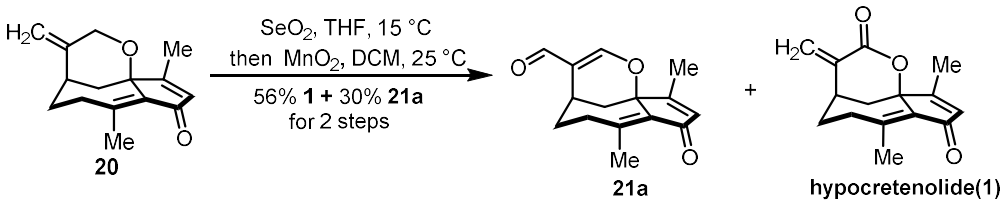


Table S1. Screening of the conditions for the oxidation of C12.

Entry	Conditions	Results
1	CrO_3, 3,5-DMP, DCM, 0 °C	60% **21a**
2	CrO_3, Py, DCM, 0 °C	45% **21a**
3	PCC, NaOAc, Celite, CCl_4, 0 °C	No reaction
4	PCC, NaOAc, Celite, PhH, 0 °C	No reaction
5	PCC, Ac_2O, DCM, 0 °C	40% **21a**
6	PDC, DMF, 0 °C	No reaction
7	PDC, *t*BuOOH, PhH, 0 °C	Decomposed
8	$Mn(OAc)_3$, *t*BuOOH, 3Å MS, EA, rt	No reaction
9	$RuCl_3$, $NaIO_4$, CH_3CN/CCl_4, H_2O, 0 °C	No reaction
10	SeO_2, 1,4-dioxane, 15 °C	**21b**
11	SeO_2, THF, 15 °C	**21b**
12	SeO_2, *t*BuOOH, DCM, 0 °C	Unidentified compound

21b is unstable and readily decomposed on silica gel.



To a stirred solution of **20** (11.2 g, 48.7 mmol, 1.0 equiv.) in THF (2.44 L) was added SeO_2 (10.8 g, 97.4 mmol, 2.0 equiv.) at 15 °C. The mixture was stirred at the same temperature for 4 h before it was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (200 mL). The resulting

mixture was extracted with Et_2O (3 × 800 mL). The combined organic phases were washed with brine (800 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to provide a crude containing **21b**. To a stirred solution of the crude product in DCM (487 mL) was added MnO_2 (112 g, 1000% w.t.) at 25 °C. After stirring for 24 h at 25 ºC, the reaction mixture was filtered through a pad of celite and concentrated under reduced pressure. The residue was purified by column chromatography (DCM : MeOH = 500 : 1 → 100 : 1) to provide **21a** (3.6 g, 14.7 mmol, 30%) as a white solid and **hypocretenolide (1)** (6.7 g, 27.4 mmol, 56%) as a white solid.

Data for 21a:

$\mathbf{R}_f$ = 0.28 (PE : EA = 2 : 1);

$[\alpha]_D^{25}$ = −41.57 (*c* = 1.0, $CHCl_3$);

IR (thin film) 3747, 3648, 3613, 3587, 2360, 1696, 1557, 1521, 1473, 1217, 768 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.29 (s, 1H), 7.62 (s, 1H), 6.16 (s, 1H), 3.31 – 3.22 (m, 1H), 2.61 (t, *J* = 14.7 Hz, 1H), 2.36 (s, 3H), 2.14 (dd, *J* = 15.3, 3.3 Hz, 1H), 1.96 (s, 3H), 1.94 – 1.80 (m, 3H), 1.67 (t, *J* = 14.3 Hz, 1H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ 194.0, 189.0, 166.3, 165.9, 155.9, 135.5, 134.5, 120.4, 87.1, 33.9, 32.3, 26.9, 26.3 22.2, 12.7 ppm;

HRMS(ESI-TOF): Calcd for $C_{15}H_{17}O_3^+$ $[M+H]^+$ 245.1172, found: 245.1176.

Data for hypocretenolide (1):

$\mathbf{R}_f$ = 0.28 (PE : EA = 2 : 1);

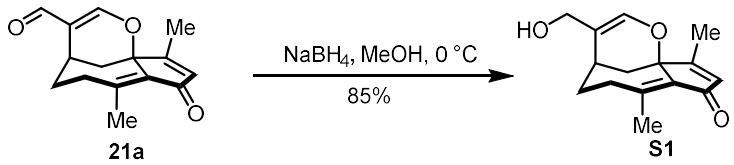
$[\alpha]_D^{25}$ = −2.80 (*c* = 0.5, $CHCl_3$);

IR (thin film) 3747, 3502, 2361, 1696, 1649, 1540, 1521, 768 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.60 (d, *J* = 1.4 Hz, 1H), 6.15 (q, *J* = 1.4 Hz, 1H), 5.70 (t, *J* = 1.1 Hz, 1H), 3.25 (m, 1H), 2.73 (m, 1H), 2.40 – 2.34 (m, 4H), 2.10 – 2.04 (m, 1H), 2.03 (d, *J* = 1.5 Hz, 3H), 1.96 – 1.82 (m, 3H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ 193.4, 166.8, 164.9, 154.2, 136.8, 135.3, 134.9, 129.7, 89.3, 37.4, 33.5, 32.5, 32.2, 21.8, 12.8 ppm;

HRMS(ESI-TOF): Calcd for $C_{15}H_{17}O_3^+$ $[M+H]^+$ 245.1172, found: 245.1176.



To a stirred solution of **21a** (3.5 g, 14.3 mmol, 1.0 equiv.) in MeOH (143 mL) was added $NaBH_4$ (649 mg, 17.2 mmol, 1.2 equiv.) at 0 °C. The mixture was stirred at 0 °C for 1 h before it was quenched with H_2O (10 mL). The resulting mixture was extracted with EA (3 × 100 mL). The combined organic phases were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced. The residue was purified by column chromatography (PE : EA = 4 : 1 → 1 : 1) to provide **S1** (3.0 g, 12.2 mmol, 85%) as colourless oil.

Data for S1:

R_f = 0.50 (PE : EA = 1 : 1);

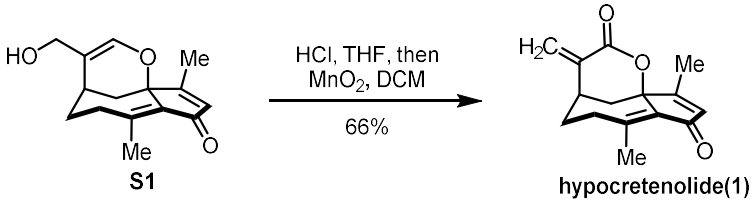
$[\alpha]_D^{25}$ = −83.40 (*c* = 1.0, $CHCl_3$);

IR (thin film) 2923, 1691, 1433, 1368, 1311, 1274, 1162, 1134, 1049, 995, 880, 794, 756 cm^{-1};

^{1}H NMR (400 MHz, MeOD) δ 6.77 (s, 1H), 6.06 (s, 1H), 4.06 – 3.90 (m, 2H), 2.92 (t, *J* = 14.4 Hz, 1H), 2.80 (m, 1H), 2.33 (s, 3H), 2.26 (m, 1H), 1.98 (s, 3H), 1.97 – 1.79 (m, 3H), 1.67 (t, *J* = 13.5 Hz, 1H) ppm;

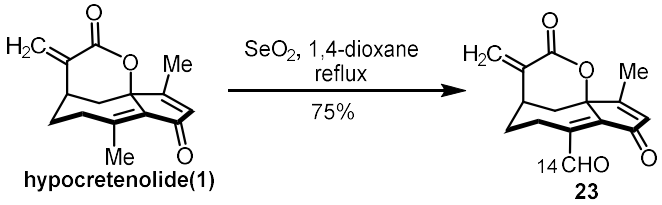
^{13}C NMR (100 MHz, MeOD) δ 197.2, 172.0, 156.4, 144.3, 137.5, 134.4, 114.7, 84.0, 62.6, 34.9, 34.4, 31.5, 27.9, 22.0, 12.6 ppm;

HRMS(ESI-TOF): Calcd for $C_{15}H_{19}O_3^+$ $[M+H]^+$ 247.1329, found: 247.1330.



To a stirred solution of **S1** (2.9 g, 11.8 mmol, 1.0 equiv.) in THF (59.0 mL) was added 1 M HCl (11.8 mL) at 25 °C. The mixture was stirred at the same temperature for 8 h before it was extracted with Et_2O (3 × 80 mL). The combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$ (80 mL) and brine (80 mL) in sequence, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to provide crude product. To a stirred solution of the crude product in DCM (59.0 mL) was added MnO_2 (29 g, 1000% w.t.) at 25 °C. After stirring for 24 h at 25 ºC, the reaction mixture was filtered through a pad of celite and concentrated under

reduced pressure. The residue was purified by column chromatography (DCM : MeOH = 500 : 1 → 100 : 1) to provide **hypocretenolide(1)** (1.9 g, 7.78 mmol, 66%) as a white solid.



To a stirred solution of **hypocretenolide(1)** (200 mg, 0.82 mmol, 1.0 equiv.) in 1, 4-dioxane (8.2 mL) was added SeO_2 (273.1 mg, 2.46 mmol, 3.0 equiv.). The mixture was stirred at 110 °C for 8 h before it was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (10 mL). The resulting mixture was extracted with Et_2O (3 × 10 mL). The combined organic phases were was washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced. The residue was purified by column chromatography (PE : EA = 10 : 1 → 2 : 1) to provide **23** (158.7 mg, 0.615 mmol, 75%) as a yellow solid.

Data for 23:

R_f = 0.40 (PE : EA = 1 : 1);

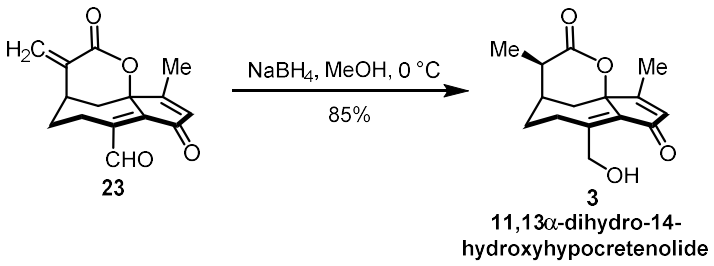
$[\alpha]_D^{25}$ = −3.21 (*c* = 0.5, $CHCl_3$);

IR (thin film) 3820, 3748, 3674, 3649, 3592, 2361, 1697, 1649, 1521, 1457, 770 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.86 (s, 1H), 6.62 (s, 1H), 6.28 (q, *J* = 1.4 Hz, 1H), 5.76 (d, *J* = 1.0 Hz, 1H), 3.29 (m, 1H), 3.12 (m, 1H), 2.45 (m, 1H), 2.22 – 2.15 (m, 1H), 2.12 (d, *J* = 1.4 Hz, 3H), 2.06 – 2.00 (m, 1H), 1.94 (dd, *J* = 14.2, 2.5 Hz, 1H), 1.73 (m, 1H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ 192.7, 192.2, 170.8, 164.0, 146.6, 146.5, 135.8, 135.2, 130.9, 88.0, 37.1, 33.1, 31.4, 18.3, 13.2 ppm;

HRMS(ESI-TOF): Calcd for $C_{15}H_{15}O_4^+$ $[M+H]^+$ 259.0965, found: 259.0966.



To a stirred solution of **23** (30.0 mg, 0.116 mmol, 1.0 equiv.) in MeOH (1.2 mL) was added $NaBH_4$ (9.1 mg, 0.24 mmol, 2.0 equiv.) at 0 °C. The mixture was stirred at 0 °C for 1 h before it

was quenched with H_2O (10 mL). The resulting mixture was extracted with EA (3 × 3.0 mL). The combined organic phases were washed with brine (5.0 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced. The residue was purified by column chromatography (PE : EA = 4 : 1 → 1 : 1) to provide **3** (**11,13α-dihydro-14-hydroxyhypocretenolide**) (25.8 mg, 0.0985 mmol, 85%) as colourless oil.

Data for 11,13α-dihydro-14-hydroxyhypocretenolide:

R_f = 0.31 (PE : EA = 1 : 1);

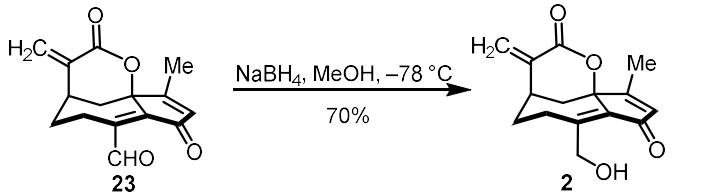
$[\alpha]_D^{25}$ = −0.92 (*c* = 0.5, $CHCl_3$);

IR (thin film) 3747, 3648, 3587, 2360, 1699, 1650, 1521, 1473, 771 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.24 – 6.16 (m, 1H), 4.66 (d, *J* = 15.2 Hz, 1H), 4.40 (d, *J* = 15.2 Hz, 1H), 2.76 (m, 1H), 2.56 – 2.45 (m, 2H), 2.42 (m, 1H), 2.35 (m, 1H), 2.13 – 2.18 (m, 1H), 2.11 (s, 3H), 1.91 (dd, *J* = 13.9, 3.1 Hz, 1H), 1.69 (m, 1H), 1.37 (d, *J* = 7.0 Hz, 3H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ 194.1, 173.7, 169.8, 156.3, 137.8, 134.7, 89.0, 65.0, 38.2, 34.8, 34.1, 27.5, 26.0, 14.0, 13.1 ppm;

HRMS(ESI-TOF): Calcd for $C_{15}H_{18}NaO_4^+$ $[M+Na]^+$ 285.1097, found: 285.1097.



To a stirred solution of **23** (300.0 mg, 1.16 mmol, 1.0 equiv.) in MeOH (11.6 mL) was added $NaBH_4$ (35.0 mg, 0.93 mmol, 0.8 equiv.) at −78 °C. The mixture was stirred at −78 °C for 0.5 h before it was quenched with H_2O (10.0 mL). The resulting mixture was extracted with EA (3 × 15.0 mL). The combined organic phases were washed with brine (10.0 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced. The residue was purified by column chromatography (PE : EA = 4 : 1 → 1 : 1) to provide **2** (**14-hydroxyhypocretenolide**) (210.0 mg, 0.808 mmol, 70%) as colourless oil.

Data for 14-hydroxyhypocretenolide:

R_f = 0.31 (PE : EA = 1 : 1);

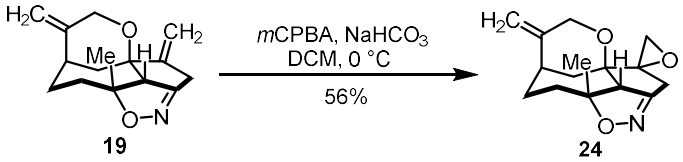
$[\alpha]_D^{25}$ = −2.13 (*c* = 0.5, $CHCl_3$);

IR (thin film) 3748, 3673, 3648, 3614, 3588, 3565, 3546, 2360, 1698, 1650, 1540, 1521 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.63 (d, *J* = 1.0 Hz, 1H), 6.21 (s, 1H), 5.73 (s, 1H), 4.69 (d, *J* = 14.9 Hz, 1H), 4.43 (d, *J* = 14.9 Hz, 1H), 4.03 (s, 1H), 3.35 – 3.22 (m, 1H), 2.71 – 2.63 (m, 1H), 2.49 – 2.36 (m, 2H), 2.09 (d, *J* = 1.4 Hz, 3H), 2.06 – 1.97 (m, 1H), 1.97 – 1.86 (m, 2H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ 194.2, 169.8, 164.6, 157.0, 136.7, 136.4, 134.7, 130.3, 88.8, 65.1, 37.3, 33.9, 32.2, 27.8, 13.1 ppm;

HRMS(ESI-TOF): Calcd for $C_{15}H_{17}O_4^+$ $[M+H]^+$ 261.1121, found: 261.1121.



To a stirred solution of **19** (100.0 mg, 0.41 mmol, 1.0 equiv.) and $NaHCO_3$ (68.9 mg, 0.82 mmol, 2.0 equiv.) in DCM (4.1 mL) was added *m*CPBA (83.2 mg, 0.41 mmol, 1.0 equiv.) at 0 °C. The mixture was stirred at the same temperature for 12 h before it was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (2.0 mL). The resulting mixture was extracted with DCM (3 × 5.0 mL). The combined organic phases were washed with brine (8.0 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (PE : EA = 10 : 1 → 2 : 1) to provide **24** (59.9 mg, 0.23 mmol, 56%) as a white solid.

Data for 24:

R_f = 0.28 (PE : EA = 2 : 1);

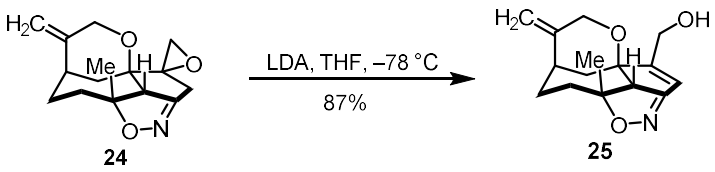
$[\alpha]_D^{25}$ = −34.40 (*c* = 1.0, $CHCl_3$);

IR (thin film) 2923, 2853, 1736, 1646, 1448, 1370, 1099, 901, 802, 774 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.94 (s, 1H), 4.87 (s, 1H), 4.32 (d, *J* = 13.9 Hz, 1H), 4.12 (d, *J* = 14.0 Hz, 1H), 3.68 (d, *J* = 1.8 Hz, 1H), 3.10 (m, 1H), 2.87 (d, *J* = 4.6 Hz, 1H), 2.83 (d, *J* = 4.6 Hz, 1H), 2.74 (q, *J* = 4.0 Hz, 1H), 2.61 (m, 1H), 2.08 (m, 1H), 2.02 – 1.89 (m, 2H), 1.79 (d, *J* = 13.7 Hz, 1H), 1.74 – 1.63 (m, 2H), 1.49 (s, 3H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ 144.2, 112.1, 78.0, 70.5, 69.8, 68.9, 47.2, 36.2, 35.1, 30.7, 29.8, 29.1 ppm;

HRMS(ESI-TOF): Calcd for $C_{15}H_{20}NO_3^+$ $[M+H]^+$ 262.1438, found: 262.1438.



To a stirred solution of **24** (50.0 mg, 0.19 mmol, 1.0 equiv.) in THF (1.9 mL) under argon at −78 °C was added LDA (0.29 mL, 1 M in THF, 0.29 mmol, 1.5 equiv.). The mixture was stirred at the same temperature for 1 h before it was quenched with a saturated aqueous solution of $NaHCO_3$ (1.0 mL). The resulting mixture was extracted with EA (3 × 3.0 mL). The combined organic phases were washed with brine (5.0 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (PE : EA = 5 : 1 → 1 : 1) to provide **25** (43.2 mg, 0.165 mmol, 87%) as a white solid.

Data for 25:

R_f = 0.35 (PE : EA = 1 : 1);

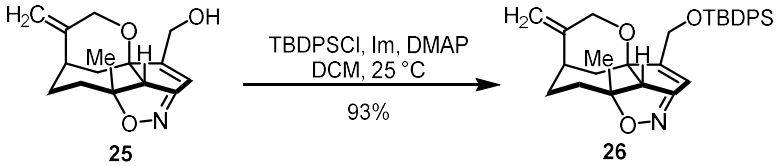
$[\alpha]_D^{25}$ = −3.73 (*c* = 1.0, $CHCl_3$);

IR (thin film) 3649, 2926, 1654, 1219, 1058, 772 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.32 (s, 1H), 4.82 (d, *J* = 6.2 Hz, 2H), 4.44 (dd, *J* = 15.9, 1.7 Hz, 1H), 4.27 (d, *J* = 16.2 Hz, 1H), 4.13 (d, *J* = 12.6 Hz, 1H), 3.98 (d, *J* = 12.2 Hz, 1H), 3.42 (s, 1H), 2.80 – 2.61 (m, 2H), 2.16 m, 1H), 1.97 (m, 2H), 1.78 (dd, *J* = 14.2, 2.2 Hz, 1H), 1.73 – 1.66 (m, 1H), 1.63 (s, 3H), 1.32 – 1.26 (m, 1H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 165.8, 146.2, 116.0, 110.9, 89.5, 85.3, 69.6, 66.5, 59.1, 33.5, 32.3, 32.1, 26.2, 25.4 ppm;

HRMS(ESI-TOF): Calcd for $C_{15}H_{20}NO_3^+$ $[M+H]^+$ 262.1438, found: 262.1439.



To a stirred solution of **25** (40.0 mg, 0.15 mmol, 1.0 equiv.) in DCM (1.5 mL) under argon at 25 °C was added imidazole (82.5 mg, 0.30 mmol, 2.0 equiv.), DMAP (3.7 mg, 0.031 mmol, 0.2 equiv.) and TBDPSCl (126.1 mg, 0.46 mmol, 3.0 equiv.). The mixture was stirred at the same temperature for 20 min before it was quenched with H_2O (1.0 mL). The resulting mixture was extracted with DCM (3 × 2.0 mL). The combined organic phases were washed with brine (3.0 mL),

dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (PE : EA = 50 : 1 → 10 : 1) to provide **26** (71.2 mg, 0.143 mmol, 93%) as a white solid.

Data for 26:

R_f = 0.60 (PE : EA = 4 : 1);

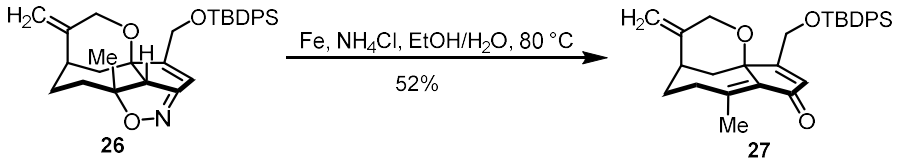
$[\alpha]_D^{25}$ = +1.72 (*c* = 1.0, $CHCl_3$);

IR (thin film) 3649, 3291, 3182, 2931, 1698, 1473, 1384, 772 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (m, 4H), 7.46 – 7.37 (m, 6H), 6.57 (t, *J* = 2.0 Hz, 1H), 4.75 (d, *J* = 5.1 Hz, 2H), 4.52 (dd, *J* = 17.8, 2. Hz, 1H), 4.33 (dd, *J* = 17.8, 2.1 Hz, 1H), 4.02 (d, *J* = 13.3 Hz, 1H), 3.92 (d, *J* = 13.4 Hz, 1H), 3.41 (s, 1H), 2.70 – 2.60 (m, 1H), 2.14 (m, 1H), 2.02 (m, 1H), 1.71 (t, *J* = 5.9 Hz, 3H), 1.64 (s, 3H), 1.28 – 1.24 (m, 1H), 1.07 (s, 9H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9, 166.1, 146.5, 135.6, 133.10, 133.05, 130.0, 127.94, 127.91, 114.9, 110.6, 89.2, 84.4, 69.8, 66.3, 60.2, 33.6, 32.4, 32.2, 26.9, 26.4, 25.4, 19.4 ppm;

HRMS(ESI-TOF): Calcd for $C_{31}H_{38}NO_3Si^+$ $[M+H]^+$ 500.2615, found: 500.2610.



26 27

To a stirred solution of **26** (60.0 mg, 0.12 mmol, 1.0 equiv.) and NH_4Cl (64.8 mg, 1.20 mmol, 10.0 equiv.) in EtOH-H_2O (1.2 mL-1.2 mL) was added iron powder (67.2 mg, 1.20 mmol, 10.0 equiv.) at 25 °C. The mixture was stirred at 80 °C for 2 h before it was cooled to 25 °C. The mixture was extracted with Et_2O (3 × 2.0 mL). The combined solvent was washed with brine (2.0 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (PE : EA = 50 : 1 → 10 : 1) to provide **27** (30.2 mg, 0.062 mmol, 52%) as a white solid.

Data for 27:

R_f = 0.74 (PE : EA = 4 : 1);

$[\alpha]_D^{25}$ = +1.88 (*c* = 1.0, $CHCl_3$);

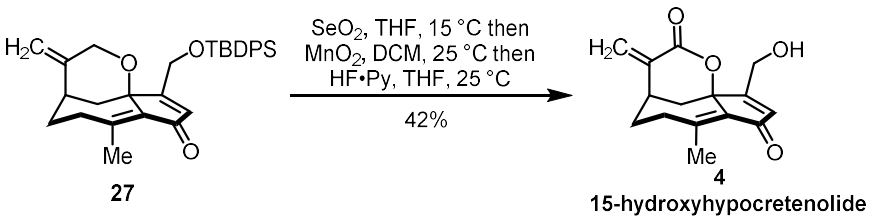
IR (thin film) 3649, 3291, 2856, 1654, 1541, 1220, 1108, 873 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 – 7.65 (m, 4H), 7.48 – 7.36 (m, 6H), 6.50 (t, *J* = 1.9 Hz, 1H),

4.95 (s, 1H), 4.86 (s, 1H), 4.72 – 4.54 (m, 2H), 4.17 – 4.03 (m, 2H), 3.09 – 2.98 (m, 1H), 2.91 (s, 1H), 2.37 (s, 3H), 2.13 (dd, J = 13.8, 4.9 Hz, 1H), 1.91 – 1.82 (m, 2H), 1.73 (m, 1H), 1.57 (s, 1H), 1.53 (dd, J = 14.0, 2.3 Hz, 1H), 1.07 (s, 9H) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ 194.4, 173.3, 153.8, 146.7, 138.6, 135.6, 133.1, 132.8, 131.9, 130.1, 128.1, 128.0, 110.1, 80.8, 67.3, 59.7, 35.7, 34.8, 32.7, 31.9, 26.8, 21.7, 19.4 ppm;

HRMS(ESI-TOF): Calcd for $C_{31}H_{37}O_3Si^+$ $[M+H]^+$ 485.2506, found: 485.2502.



To a stirred solution of **27** (25 mg, 0.052 mmol, 1.0 equiv.) in THF (1.0 mL) was added SeO_2 (11.5 mg, 0.10 mmol, 2.0 equiv.) at 15 °C. The mixture was stirred at the same temperature for 6 h before it was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (1.0 mL). The resulting mixture was extracted with Et_2O (3 × 2.0 mL). The combined organic phases were washed with brine (3.0 mL), dried over anhydrous Na_2SO_4, filtered through a pad of celite, and concentrated under reduced pressure to provide a crude product. To a stirred solution of the crude product in DCM (1.0 mL) was added MnO_2 (250 mg, 1000% w.t.) at 25 °C. After stirring for 12 h at 25 ºC, the reaction mixture was filtered through a pad of celite and concentrated under reduced pressure to provide a crude product. To a stirred solution of the crude product in THF (1.0 mL) was added 65−80% wt. HF·Py (0.1 mL) at 25 °C. The mixture was stirred at the same temperature for 2 h before it was quenched with a saturated aqueous solution of $NaHCO_3$ (0.5 mL). The resulting mixture was extracted with EA (3 × 1.0 mL). The combined organic phases were washed with brine (1.0 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (PE : EA = 4 : 1 → 1 : 2) to provide **4** (**15-hydroxyhypocretenolide**) (5.7 mg, 0.022 mmol, 42%) as a white solid.

Data for 15-hydroxyhypocretenolide:

R_f = 0.22 (PE : EA = 1 : 2);

$[\alpha]_D^{25}$ = −0.69 (c = 0.5, $CHCl_3$);

IR (thin film) 3434, 2923, 1687, 1634, 1260, 1023, 780 cm^{-1};

^{1}H NMR (400 MHz, MeOD) δ 6.56 (d, *J* = 1.5 Hz, 1H), 6.39 (t, *J* = 1.9 Hz, 1H), 5.84 – 5.80 (m, 1H), 4.51 (dd, *J* = 18.1, 2.0 Hz, 1H), 4.41 (dd, *J* = 18.1, 2.0 Hz, 1H), 2.66 (m, 1H), 2.49 (m, 1H), 2.39 (s, 3H), 2.18 – 2.11 (m, 1H), 2.01 (dd, *J* = 14.1, 2.6 Hz, 1H), 1.98 – 1.92 (m, 2H) ppm;

^{13}C NMR (100 MHz, MeOD) δ 194.9, 173.3, 166.8, 156.9, 138.5, 136.6, 133.4, 130.6, 90.2, 58.1, 38.6, 34.2, 33.8, 33.1, 21.7 ppm;

HRMS(ESI-TOF): Calcd for $C_{15}H_{17}O_4^+$ $[M+H]^+$ 261.1121, found: 261.1118.

2. CCK-8 Assay

The colon cancer cells including MC-38, CT-26, HCT116 and HT-29 were all cultured with RPMI-1640 supplemented with 10% FBS in a cell incubator at 37 °C with 5% CO_2. Colon cancer cells in logarithmic growth phase were seeded into 96 well plates. After cell adhesion, hypocretenolide, 5-FU and ACT001 were added with different concentrations respectively. After incubation for 72 h, CCK-8 was added into each well and incubated. Then the absorbance at 450 nm was measured. The IC_{50} was calculated by GraphPad Prism.

3. Wound Healing Assay

CT-26 colon cancer cells were seeded into 6 well plates. After reaching 80% confluency, cells were scratched with pipette tip, washed with PBS buffer and treated with hypocretenolide, 5-FU and ACT001 at different concentrations. The width of scratches was recorded at 0 h, 24 h and 48 h respectively. The wound healing rates were calculated.

4. Transwell Assay

CT-26 cells were collected, counted and resuspended with serum-free medium. Then the cells were seeded into the upper chamber of transwell chambers filters and the lower chambers containing 700 μL DMEM with 20% FBS as a chemoattractant. After 48 h, the medium in the upper chamber was discarded and 4% paraformaldehyde was used to fix cells. Then the cells were stained with crystal viol at 37 °C for 30 min. After removing the cells in the upper chamber, the cells migrated to the lower surface were observed and counted.

5. Western Blot Assay

Colon cancer cells were first treated with hypocretenolide, 5-FU and ACT001 and then collected and lysed with RIPA buffer respectively. The protein concentration was detected and equivalent amount of protein were separated with SDS polyacrylamide gel and then transferred to PVDF membranes. After that, the membranes were blocked with 5% skim milk at room temperature for at least 1 h. The membranes were incubated with the primary antibodies specific for E-cadherin, vimentin and GAPDH at 4 °C overnight. Then the membranes were washed with PBST for 5 times and incubated with respective second antibodies. After washed with PBST for 5 times the membranes were developed with the ECL.

5. Liver Metastasis Mouse Assay

CT-26-luci cells were collected, counted and injected into BAL B/c mice spleen. After 3 days, the bioluminescence intensity was detected after D-luciferin potassium salt was administrated by intraperitoneal injection. Then the mice were grouped according to the bioluminescence intensity. Then hypocretenolide was administrated by intraperitoneal injection every three days, ACT001 was administrated orally every other day and 5-FU was administrated twice a week by intravenous injection. After administration for 12 days, the bioluminescence intensity was detected.

Animal studies were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals (Tianjin, revised in June 2018) and approved by the Institutional Animal Care and Use Committee (IACUC) of Nankai University (Tianjin, China) (Approval number: 2022-SYDWLL-000511).

6. Pulmonary Metastasis Mouse Assay

CT-26-luci cells were collected, counted and injected into BAL B/c mice through the tail vein. After 7 days, the bioluminescence intensity was detected and the mice was grouped. Then hypocretenolide was administrated by intraperitoneal injection every three days, ACT001 was administrated orally every other day and 5-FU was administrated twice a week by intravenous injection. The survival time was recorded and the survival rates were calculated.

Animal studies were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals (Tianjin, revised in June 2018) and approved by the Institutional Animal Care and Use Committee (IACUC) of Nankai University (Tianjin, China) (Approval number: 2023-SYDWLL-000518).

Part III. Comparison of the Spectra of Isolated and Synthetic Compounds

Table S2. ^{1}H NMR comparison of natural **hypocretenolide**[3] (**1**) ($CDCl_3$, 400 M) and synthetic **hypocretenolide** (**1**) ($CDCl_3$, 400 M).

No	Natural **hypocretenolide** (**1**) δ ^{1}H [ppm, mult, *J* (Hz)]	Synthetic **hypocretenolide** (**1**) δ ^{1}H[ppm, mult, *J* (Hz)]
1		
2		
3	6.19 q	6.15 q (1.4)
4		
5		
6	1.88 dd; 2.36 dd	1.89 – 1.87 m; 2.40 – 2.35 m
7	3.20 s br	3.27 – 3.23 m
8	1.66 m; 1.93 ddd	1.85 – 1.83 m; 1.95 – 1.91 m
9	2.08 ddd; 2.75 ddd br	2.10 – 2.04 m; 2.77 – 2.69 m
10		
11		
12		
13	6.62 d; 5.71 s br	6.60 d (1.4); 5.70 t (1.1)
14	2.40 s	2.38 s
15	2.06 d	2.03 d (1.5)

The separation literature only provided ^{1}H NMR.

Table S3. ^{1}H NMR comparison of natural **11,13α-dihydro-14-hydroxyhypocretenolide**[4] (**3**) ($CDCl_3$, 400 M) and synthetic **11,13α-dihydro-14-hydroxyhypocretenolide** ($CDCl_3$, 400 M).

No	Natural **11,13α-dihydro-14-hydroxyhypocretenolide** (**3**) δ ^{1}H [ppm, mult, *J* (Hz)]	Synthetic **11,13α-dihydro-14-hydroxyhypocretenolide** (**3**) δ ^{1}H [ppm, mult, *J* (Hz)]
1		
2		
3	6.21 q	6.24 – 6.16 m
4		
5		
6	1.93 dd; 2.36 ddd	1.91 dd (13.9, 3.1); 2.37 – 2.33 m
7	2.48 s br	2.48 – 2.45 m
8	2.41 d br; 1.71 dddd	2.45 – 2.39 m; 1.73 – 1.65 m
9	2.17 d br; 2.54 ddd	2.18 – 2.13 m; 2.55 – 2.48 m
10		
11	2.77 dq	2.76 m
12		
13	1.38 d	1.37 d (7.0)
14	4.66 d; 4.39 d	4.66 d (15.2); 4.40 d (15.2)
15	2.12 d	2.11 s

The separation literature only provided ^{1}H NMR.

Table S4. ^{1}H NMR comparison of natural **14-hydroxyhypocretenolide**[4] (**2**) ($CDCl_3$, 400 M) and synthetic **14-hydroxyhypocretenolide** (**2**) ($CDCl_3$, 400 M).

No	Natural **14-hydroxyhypocretenolide** (**2**) δ ^{1}H [ppm, mult, *J* (Hz)]	Synthetic **14-hydroxyhypocretenolide** (**2**) δ ^{1}H [ppm, mult, *J* (Hz)]
1		
2		
3	6.22 q	6.21 s
4		
5		
6	1.92 dd; 2.41 dd br	1.88 – 1.92 m; 2.41 – 2.37 m
7	3.29 s br	3.35 – 3.22 m
8	2.41 d br; 1.96 dd br	2.45 – 2.41 m; 1.92 – 1.95 m
9	2.03 d br; 2.75 ddd	2.04 – 2.00 m; 2.71 – 2.63 m
10		
11		
12		
13	6.64 d; 5.74 br s	6.63 d (1.0); 5.73 s
14	4.68 d; 4.43 d	4.69 d (14.9); 4.43 d (14.9)
15	2.10 d	2.09 d (1.4)
OH		4.03 s

The separation literature only provided ^{1}H NMR.

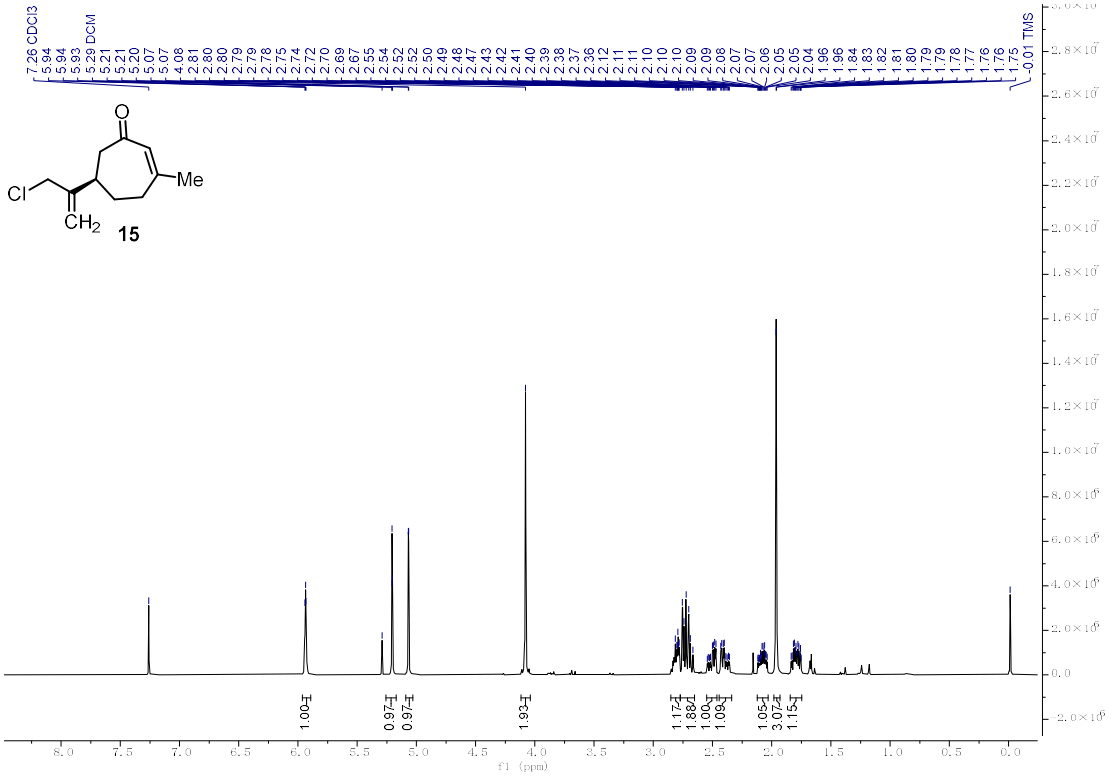
Table S5. ^{1}H NMR comparison of natural **15-hydroxyhypocretenolide**[5] (**4**) (CD_3OD, 400 M) and synthetic **15-hydroxyhypocretenolide** (CD_3OD, 400 M).

No	Natural **15-hydroxyhypocretenolide** (**4**) δ ^{1}H [ppm, mult, *J* (Hz)]	Synthetic **15-hydroxyhypocretenolide** (**4**) δ ^{1}H [ppm, mult, *J* (Hz)]
1		
2		
3	6.38 t (2.0)	6.39 t (1.9)
4		
5		
6	2.51 m; 1.98 m	1.93 – 1.97 m
7	3.30 m	--
8	2.50 m; 2.01 m	2.49 m; 2.01 m
9	2.62 m; 2.17 m	2.66 m; 2.15m
10		
11		
12		
13	6.55 d (1.5); 5.81 dd (1.5, 1.0)	6.56 d (1.5); 5.84 – 5.80 m
14	2.39 s	2.39 s
15	4.58 dd (18.0, 2.0); 4.45 dd (18.0, 2.0)	4.51 dd (18.1, 2.0); 4.41 dd (18.1, 2.0)

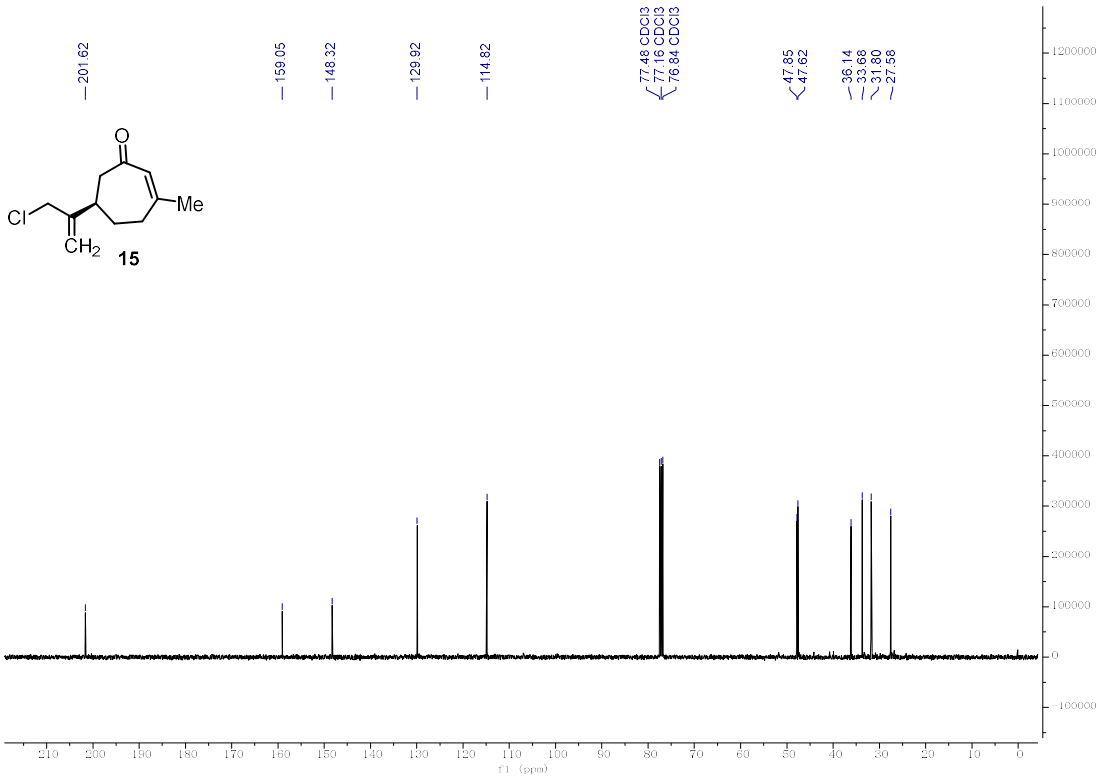
Table S6. ^{13}C NMR comparison of natural **15-hydroxyhypocretenolide**[5] (**4**) (CD_3OD, 100 M) and synthetic **15-hydroxyhypocretenolide** (**4**) (CD_3OD, 100 M).

No	Natural **15-hydroxyhypocretenolide** (**4**) δ ^{13}C (ppm)	Synthetic **15-hydroxyhypocretenolide** (**4**) δ ^{13}C (ppm)	Difference value
1	136.5	136.6	0.1
2	194.7	194.9	0.2
3	133.0	133.4	0.4
4	173.2	173.3	0.1
5	90.3	90.2	−0.1
6	35.1	34.2	−0.9
7	38.0	38.6	0.6
8	33.5	33.8	0.3
9	32.9	33.1	0.2
10	156.9	156.9	0.0
11	138.3	138.5	0.2
12	166.5	166.8	0.3
13	130.4	130.6	0.2
14	21.5	21.7	0.2
15	57.9	58.1	0.2

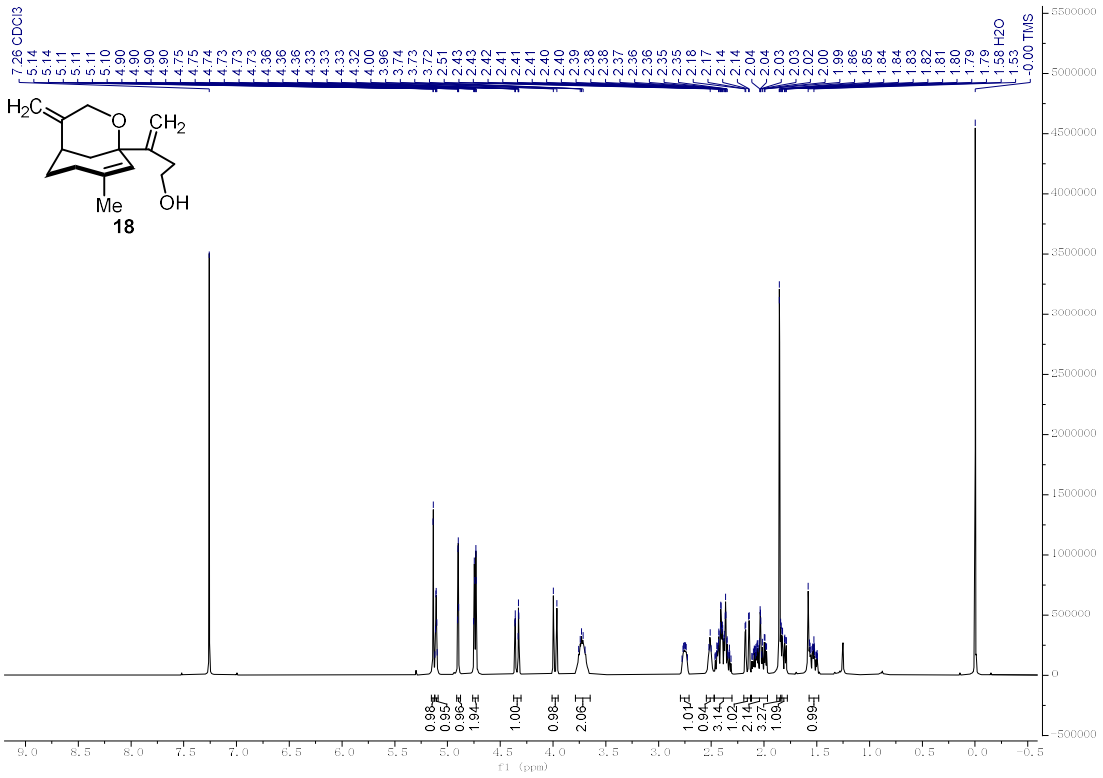
Part IV. NMR Spectra for the Synthetic Compounds



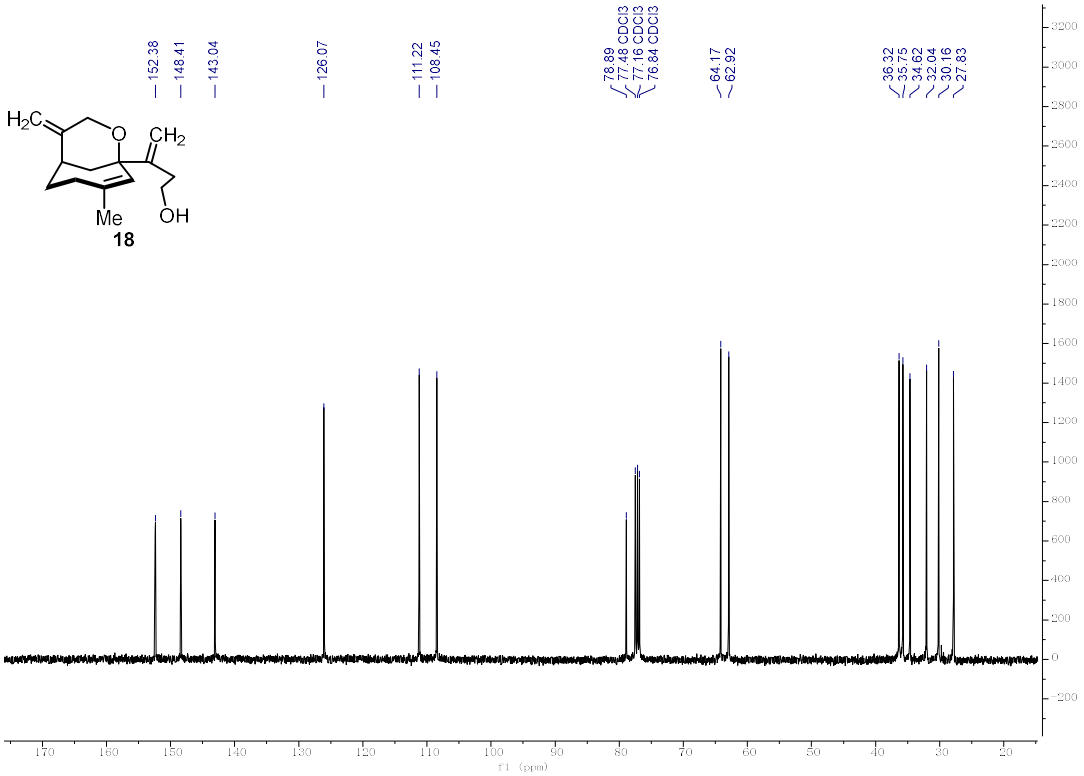
^{1}H NMR spectrum of **15** (400 MHz, $CDCl_3$)



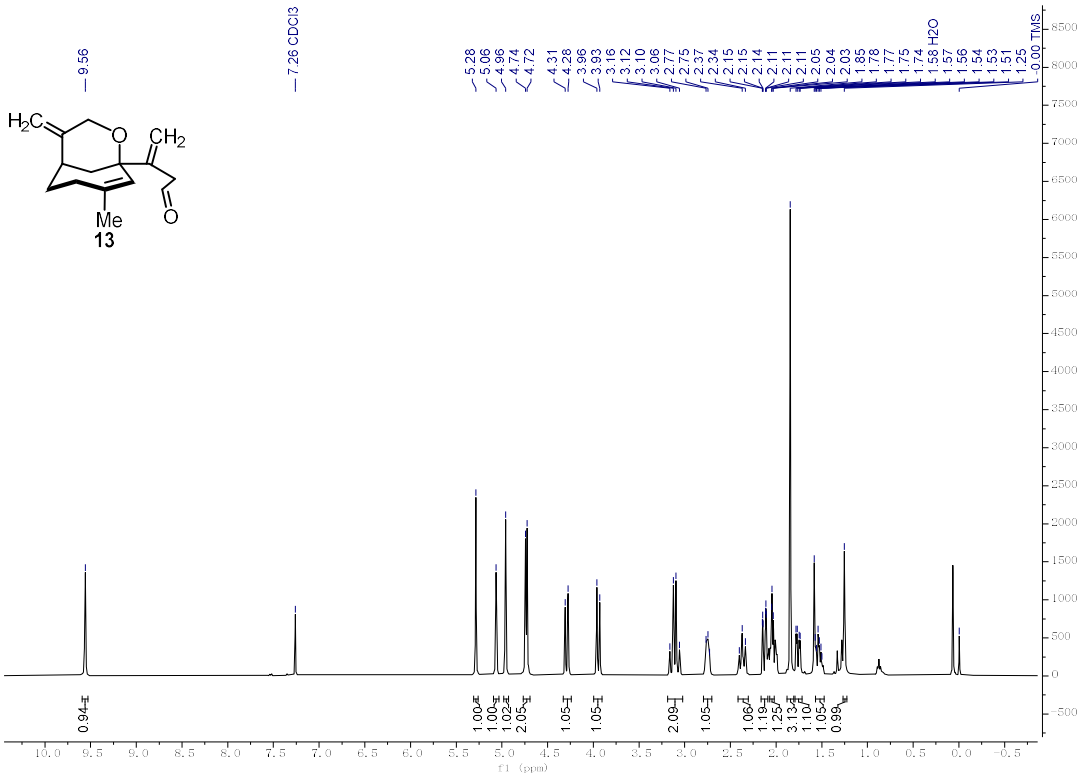
^{13}C NMR spectrum of **15** (100 MHz, $CDCl_3$)



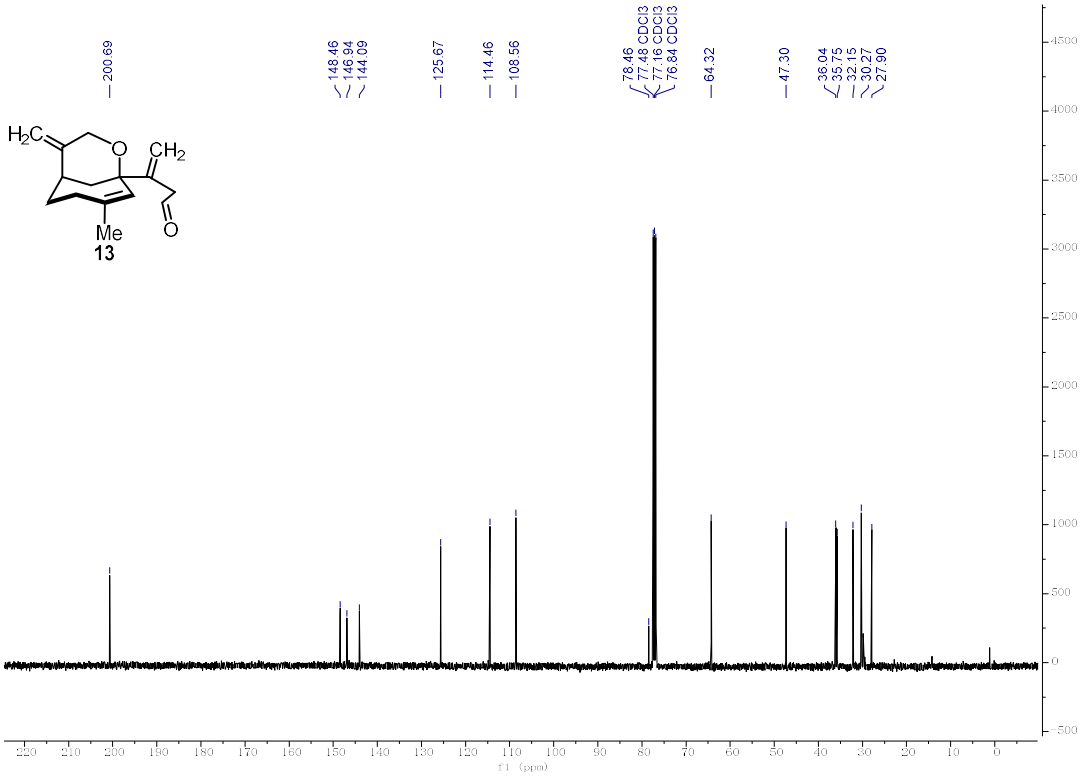
^{1}H NMR spectrum of **18** (400 MHz, $CDCl_3$)



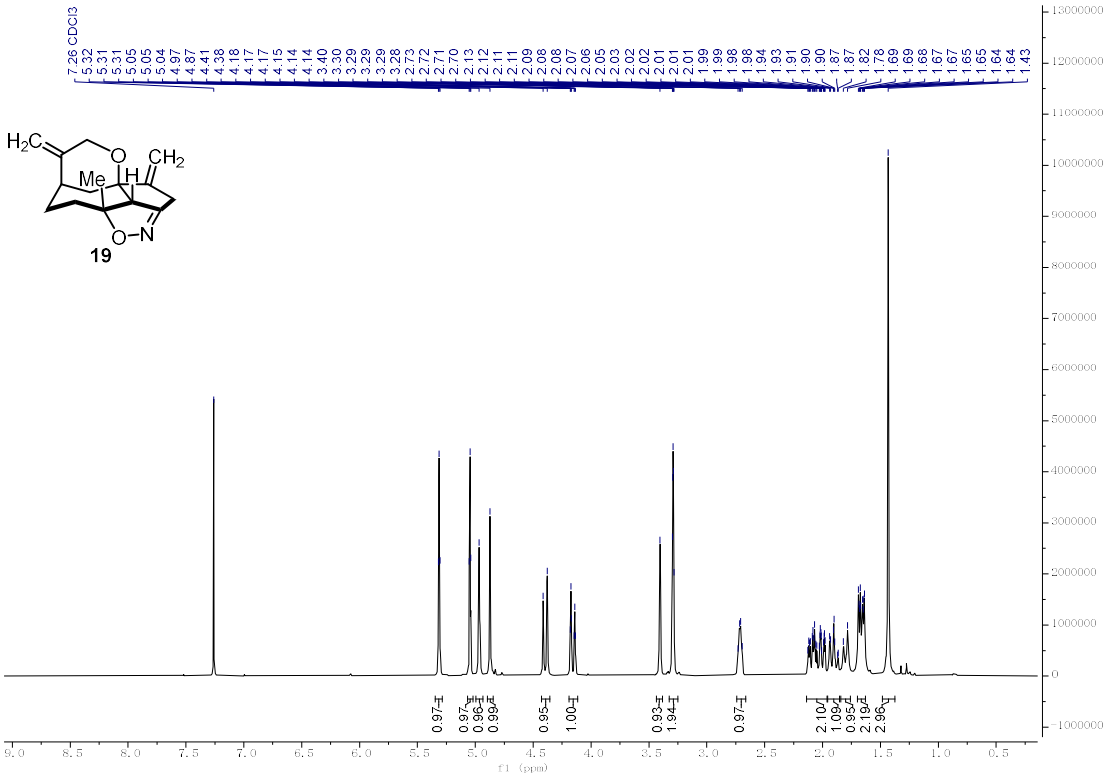
^{13}C NMR spectrum of **18** (100 MHz, $CDCl_3$)



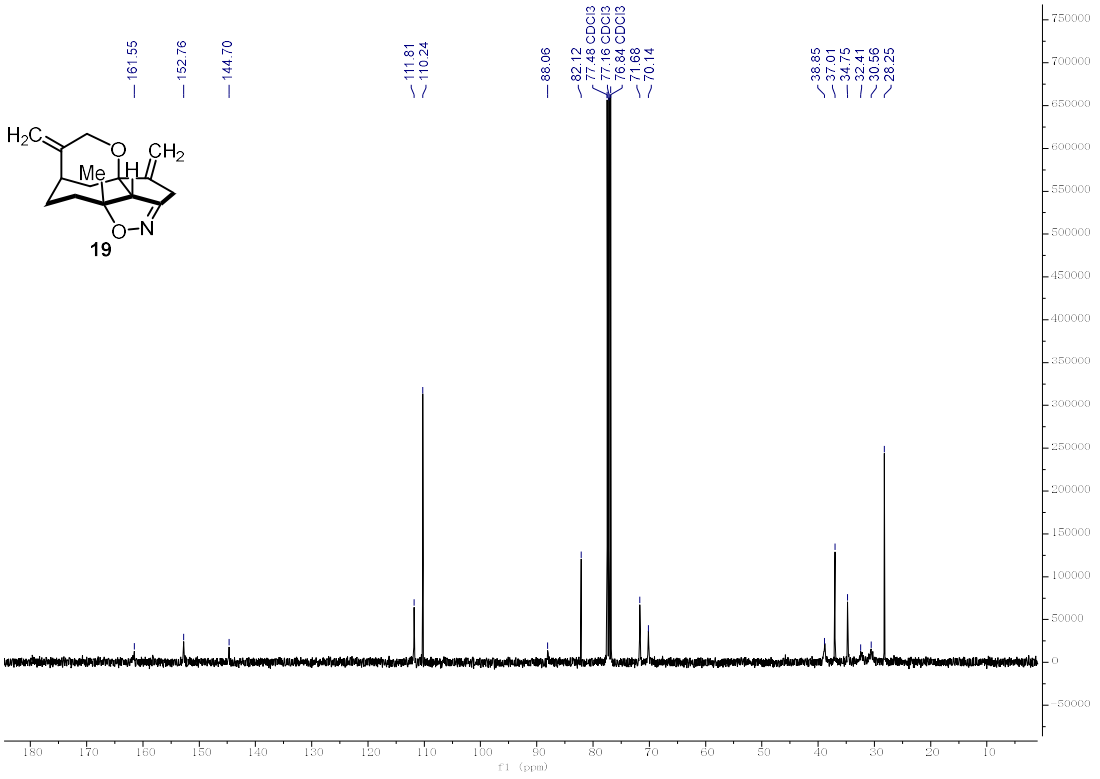
^{1}H NMR spectrum of **13** (400 MHz, $CDCl_3$)



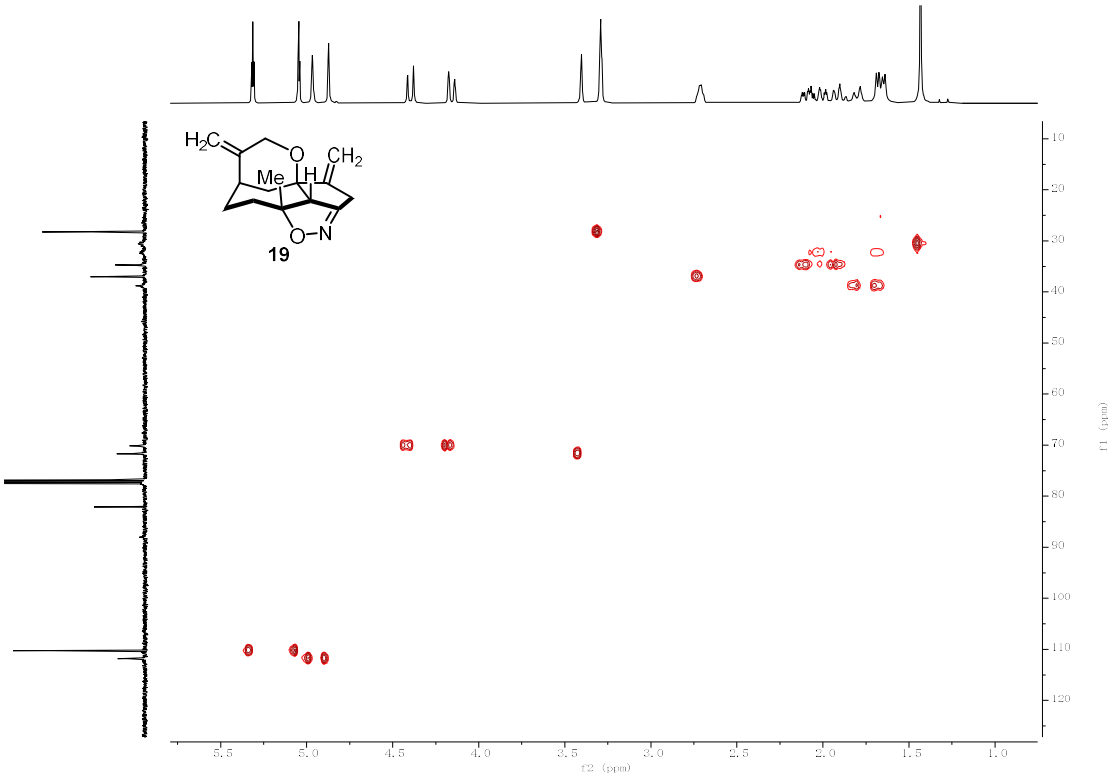
^{13}C NMR spectrum of **13** (100 MHz, $CDCl_3$)



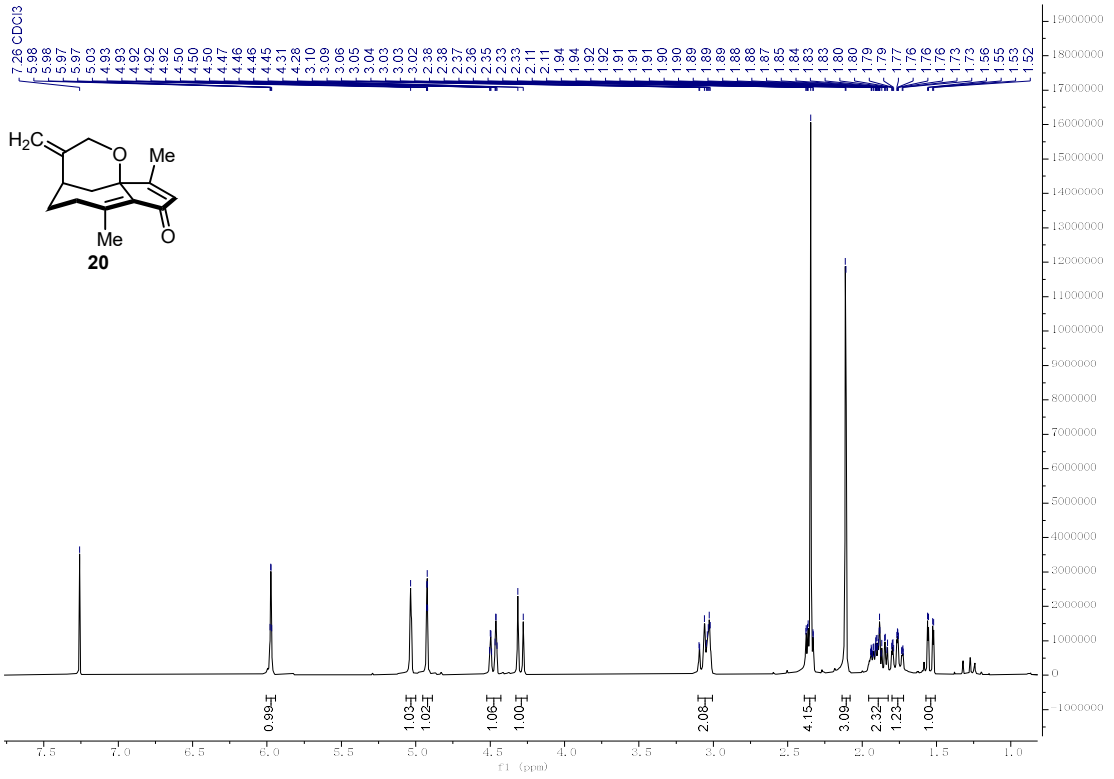
^{1}H NMR spectrum of **19** (400 MHz, $CDCl_3$)



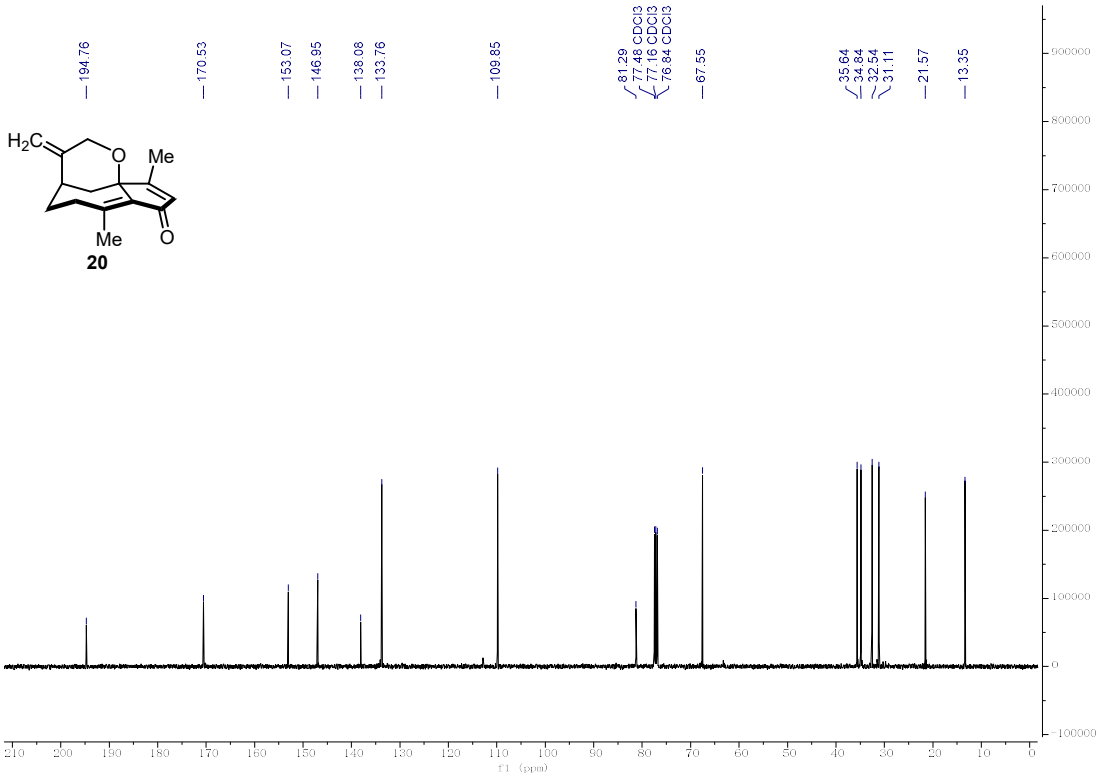
^{13}C NMR spectrum of **19** (100 MHz, $CDCl_3$)



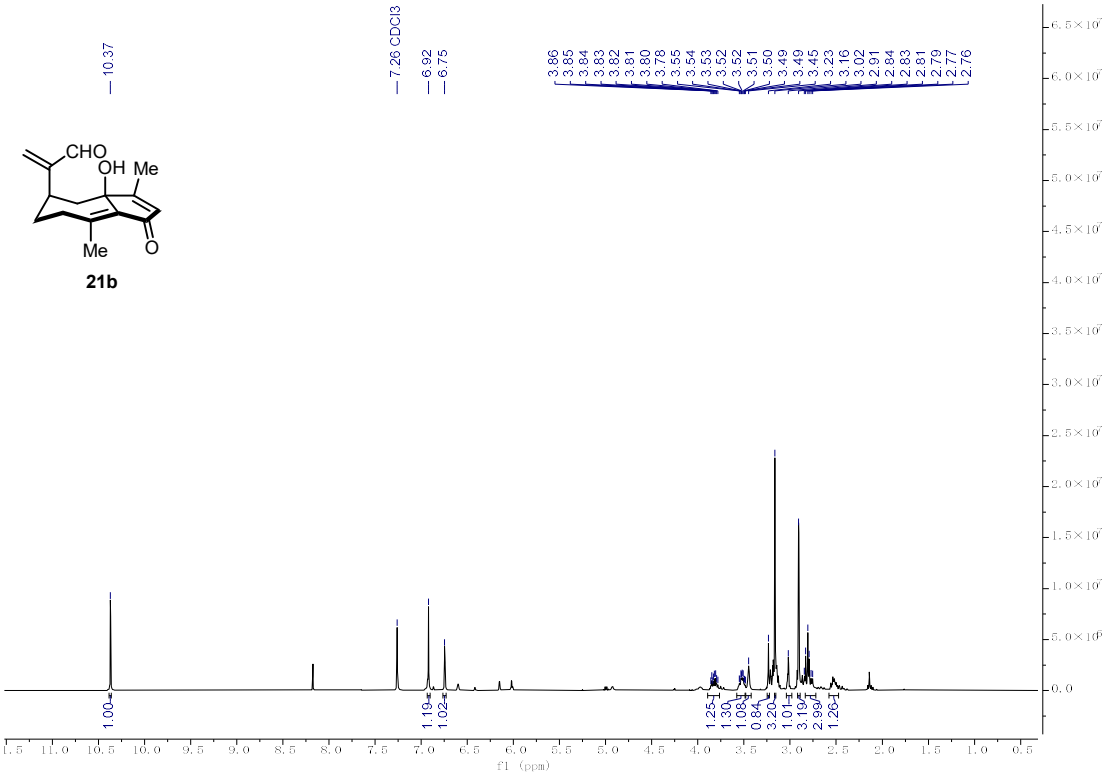
HSQC spectrum of **19** ($CDCl_3$)



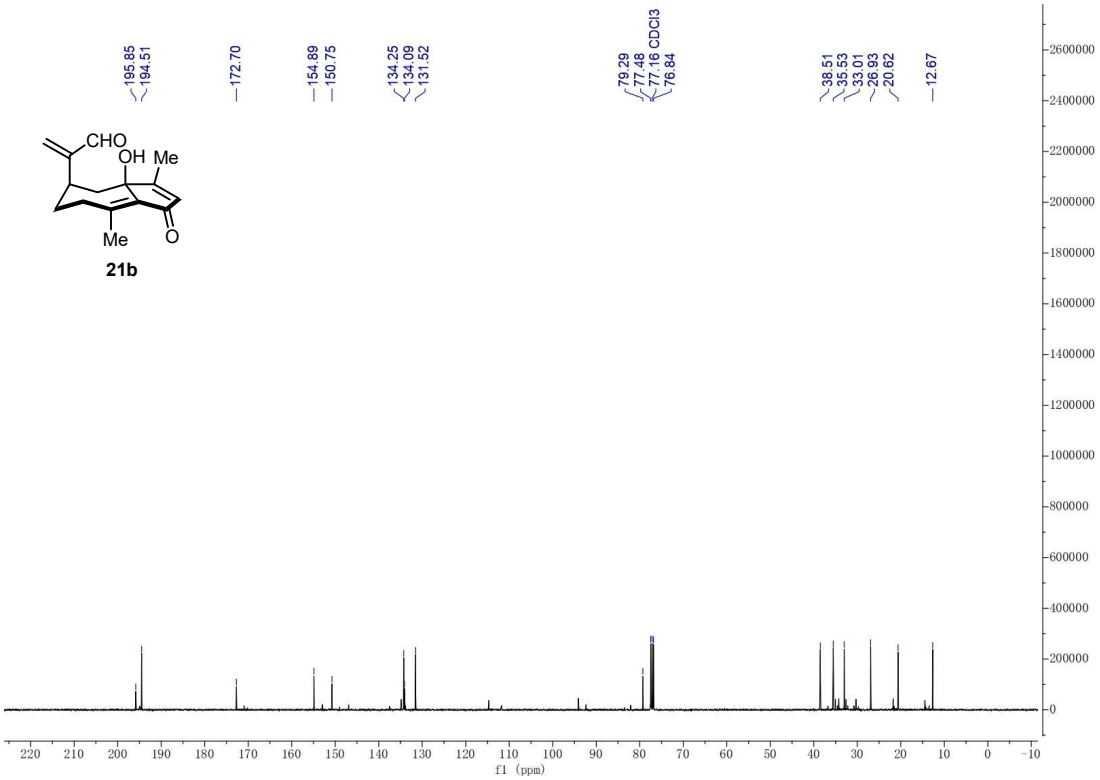
^{1}H NMR spectrum of **20** (400 MHz, $CDCl_3$)



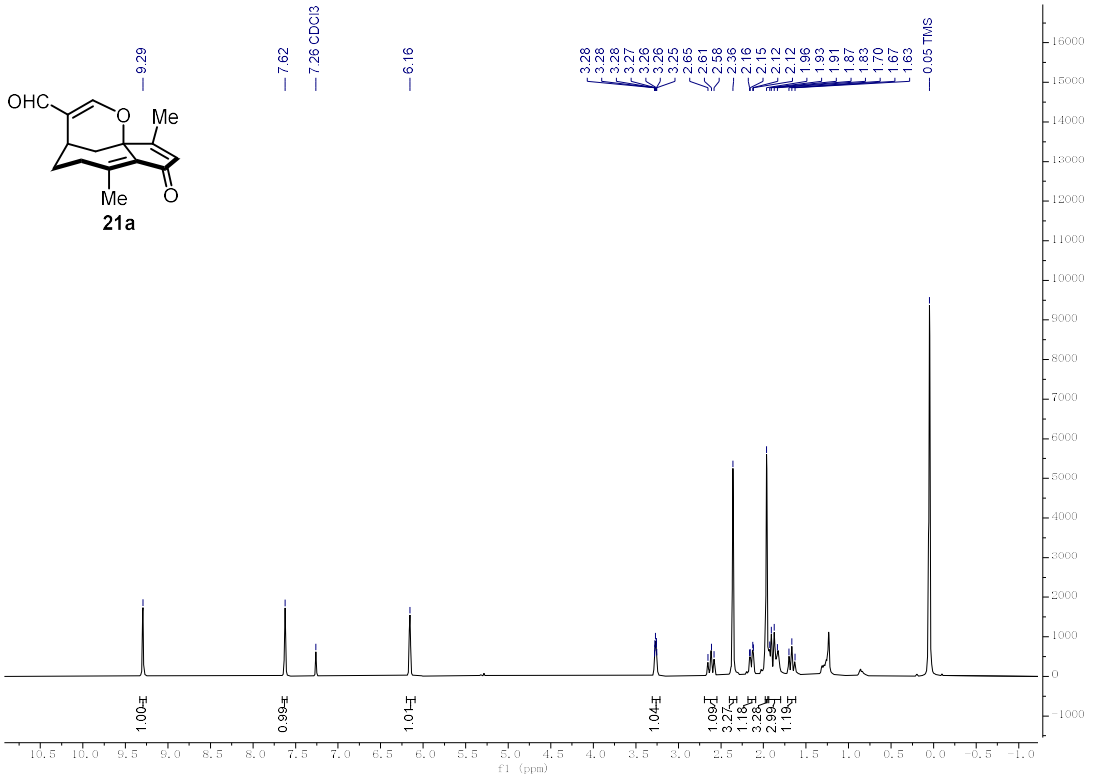
^{13}C NMR spectrum of **20** (100 MHz, $CDCl_3$)



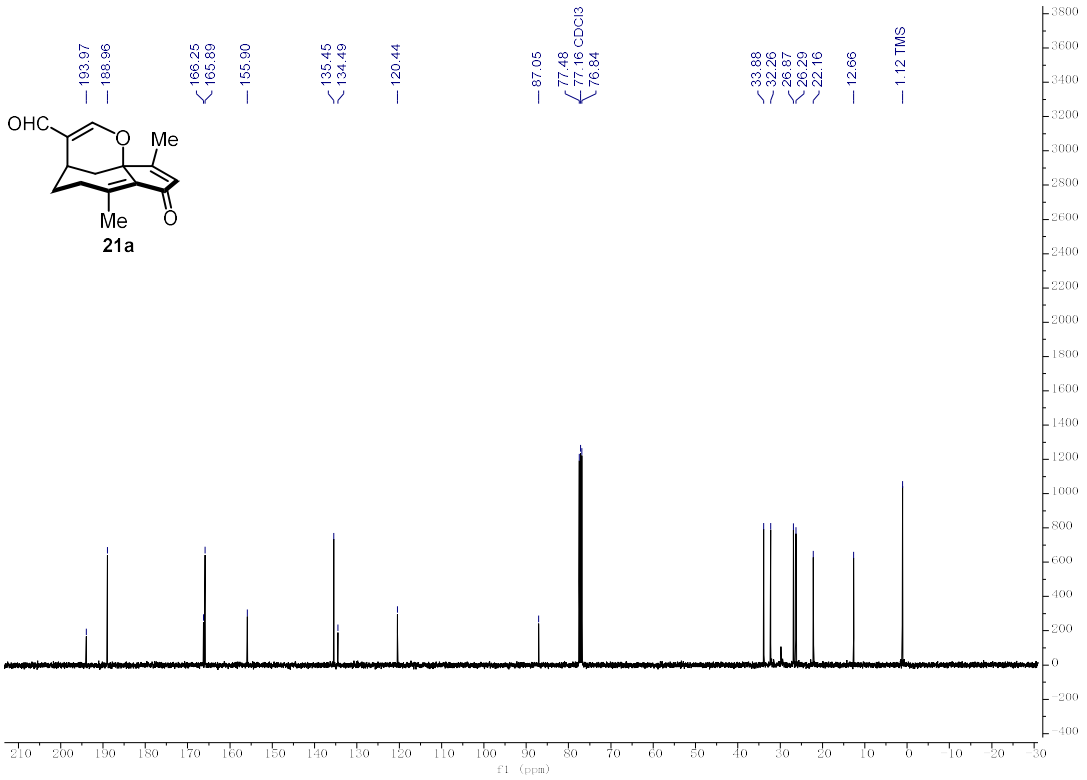
^{1}H NMR spectrum of the crude **21b** (400 MHz, $CDCl_3$)



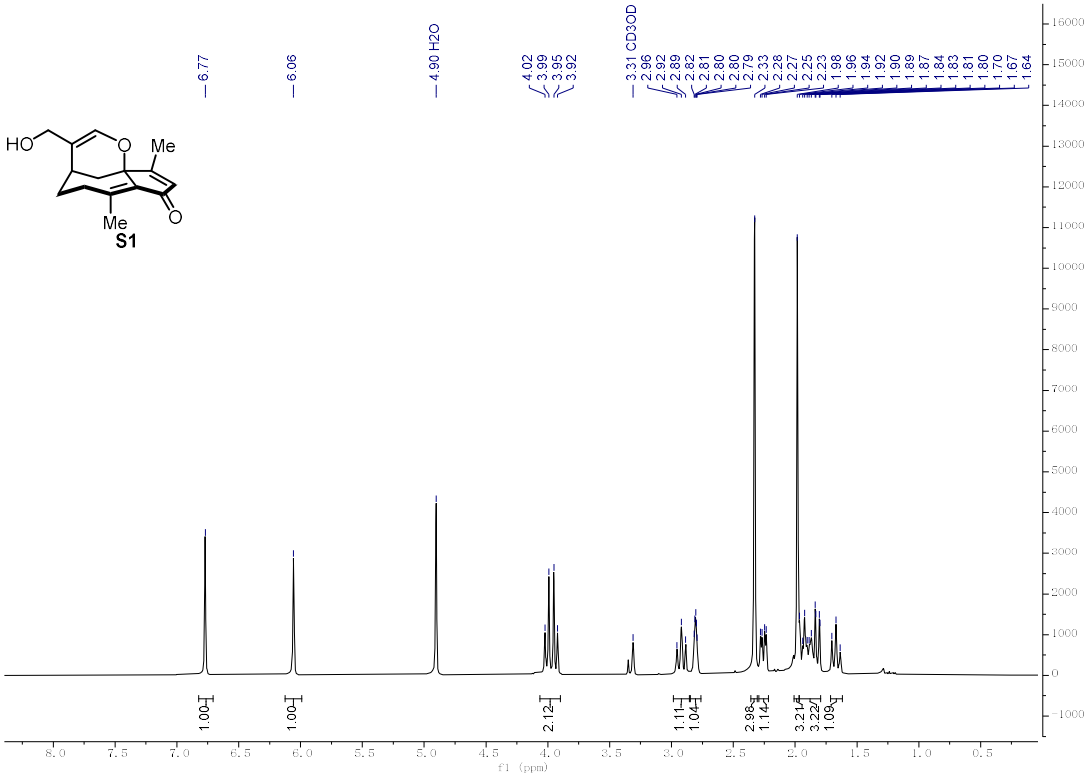
^{13}C NMR spectrum of the crude **21b** (100 MHz, $CDCl_3$)



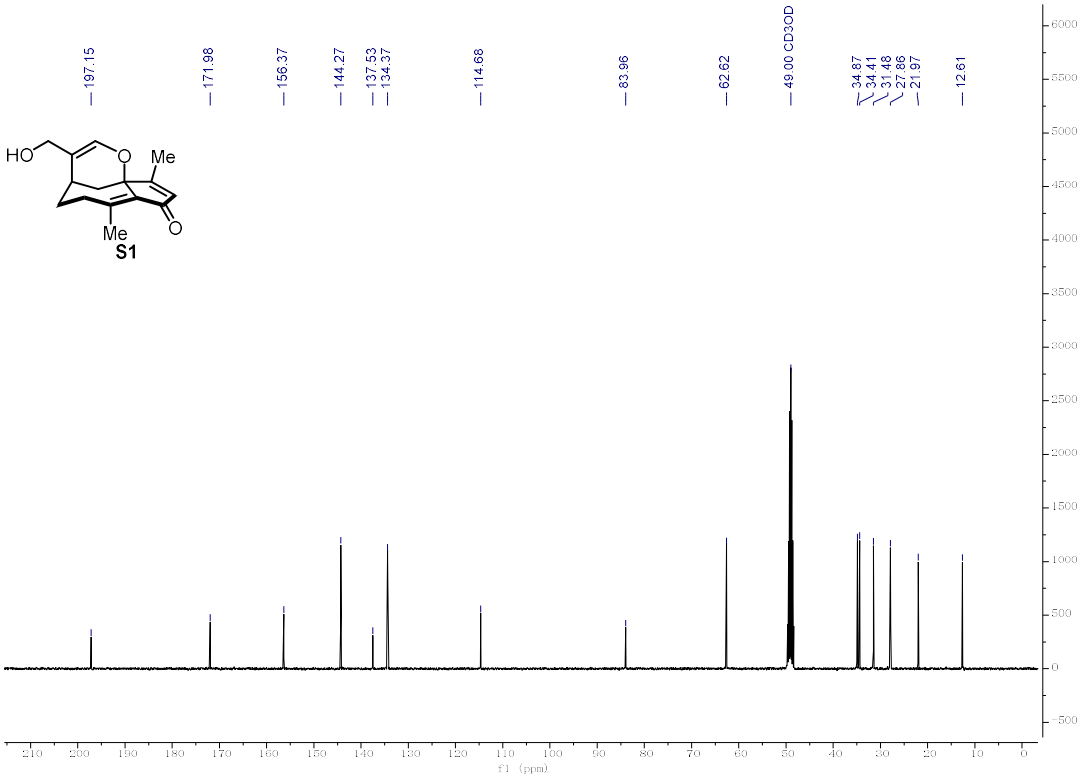
^{1}H NMR spectrum of **21a** (400 MHz, $CDCl_3$)



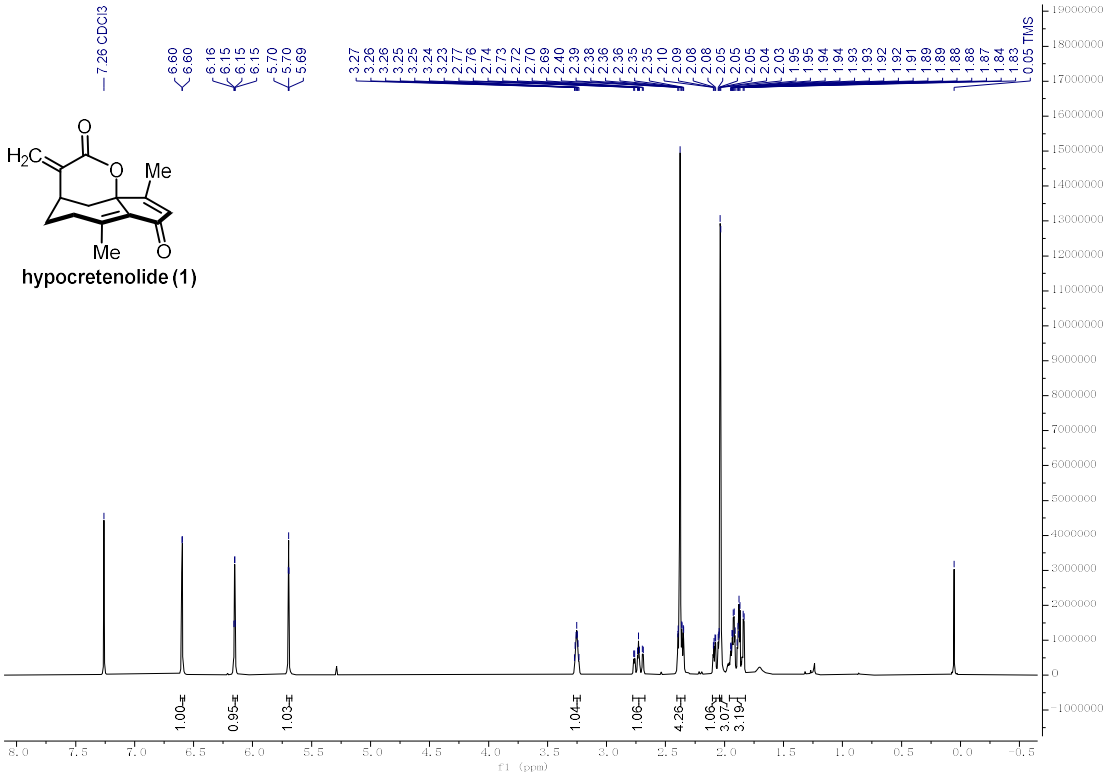
^{13}C NMR spectrum of **21a** (100 MHz, $CDCl_3$)



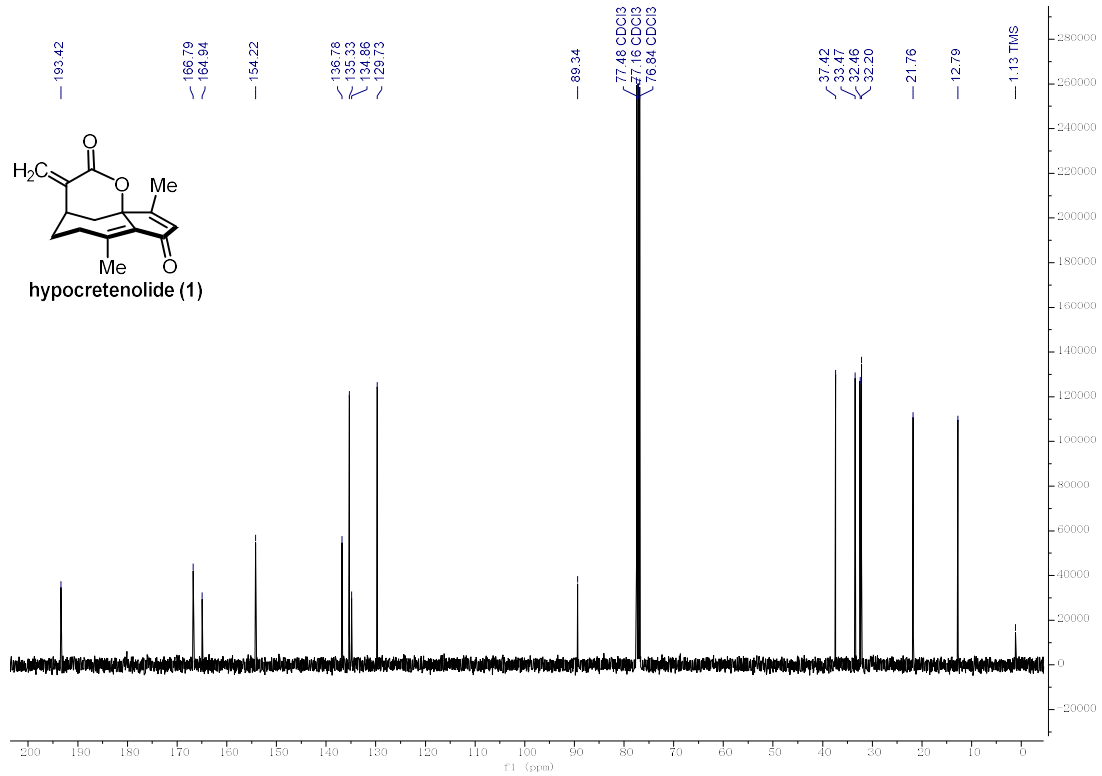
^{1}H NMR spectrum of **S1** (400 MHz, MeOD)



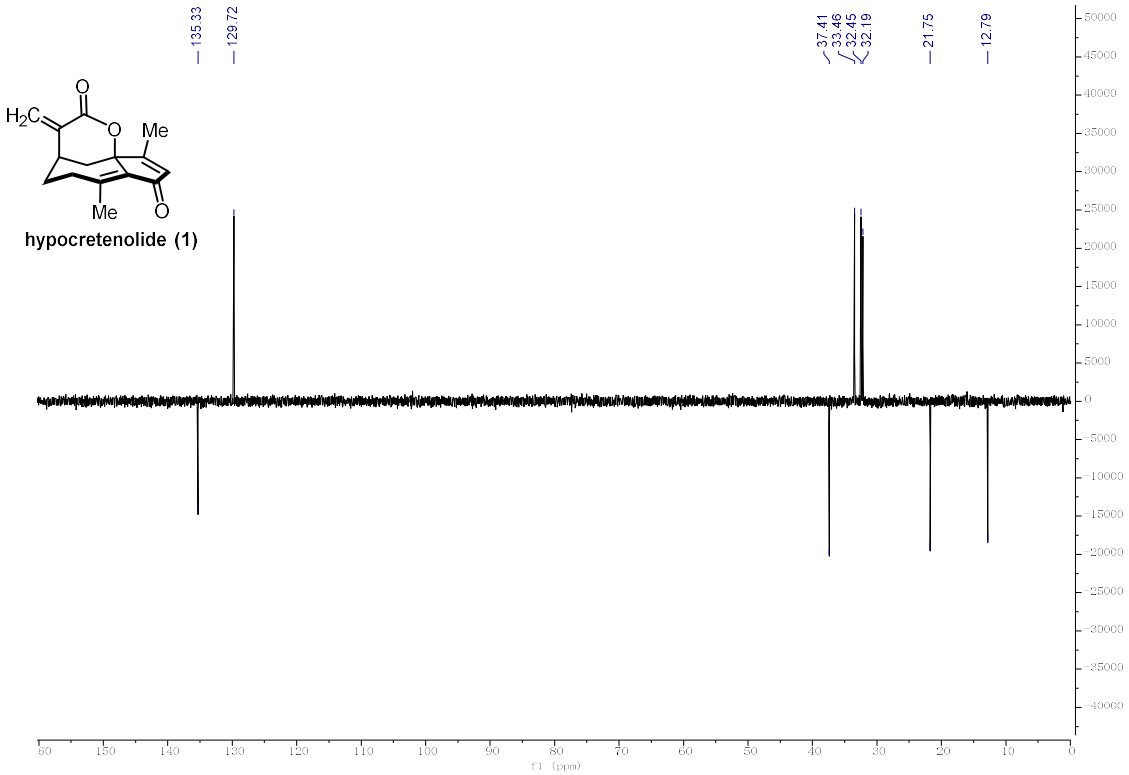
^{13}C NMR spectrum of **S1** (100 MHz, MeOD)



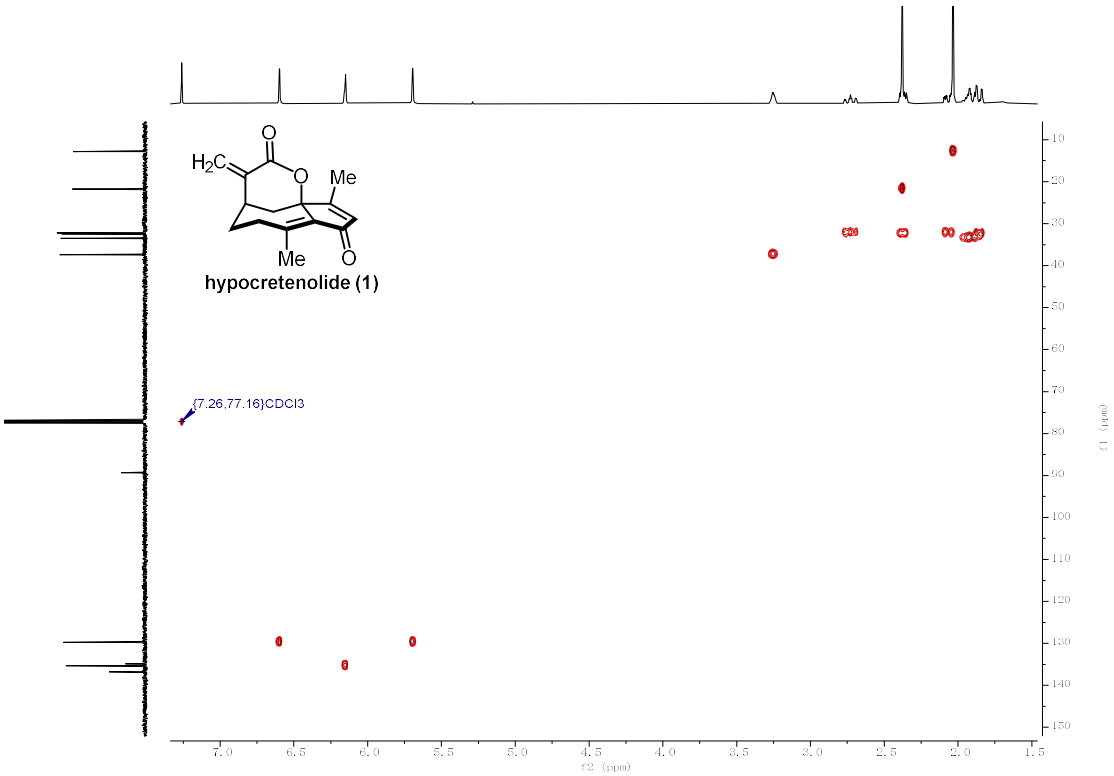
^{1}H NMR spectrum of **hypocretenolide (1)** (400 MHz, $CDCl_3$)



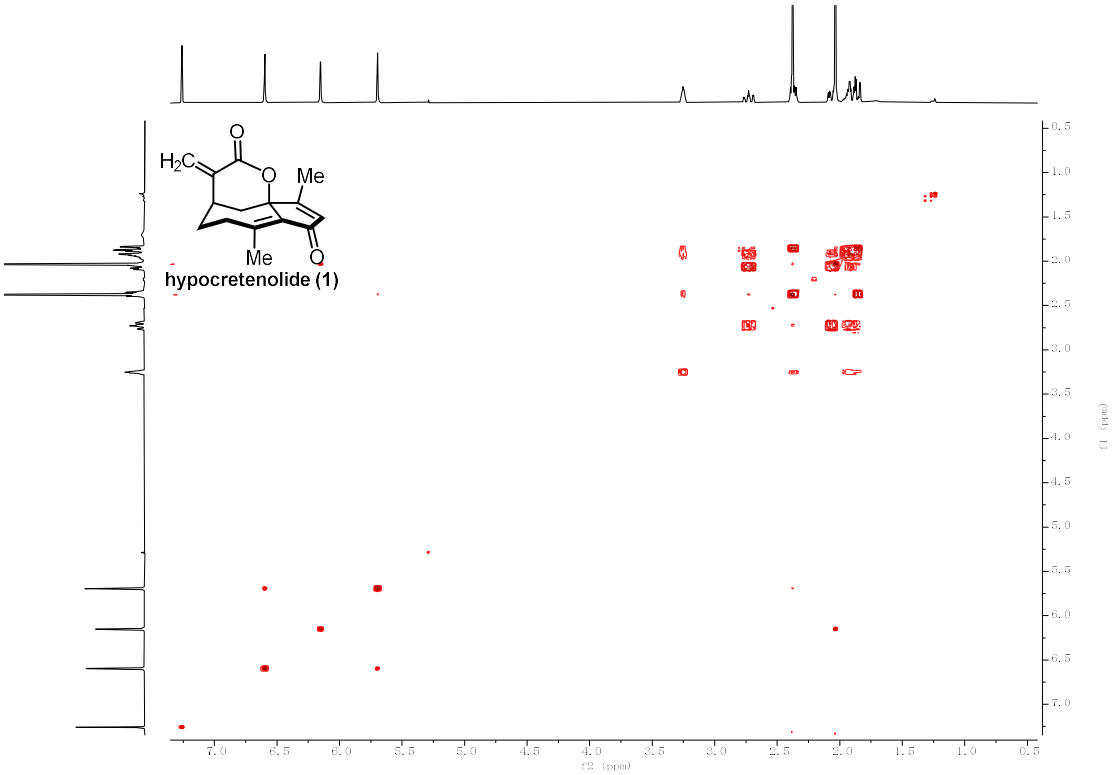
^{13}C NMR spectrum of **hypocretenolide (1)** (100 MHz, $CDCl_3$)



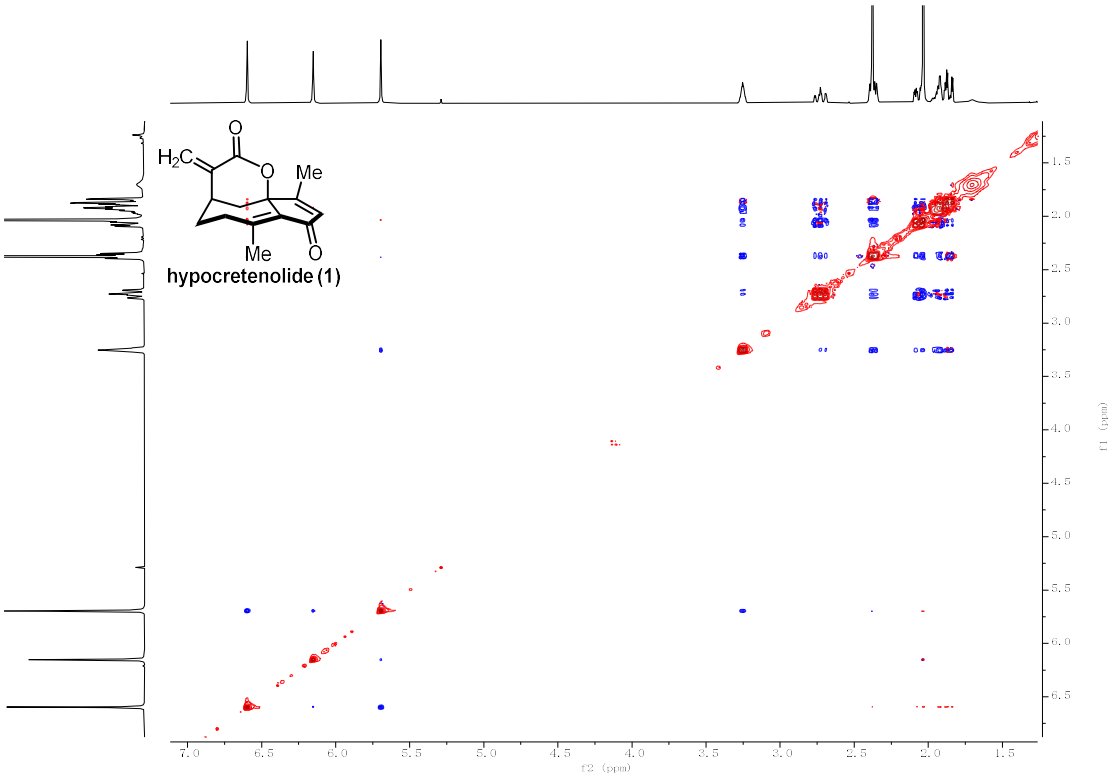
DEPT135 spectrum of **hypocretenolide (1)** ($CDCl_3$)



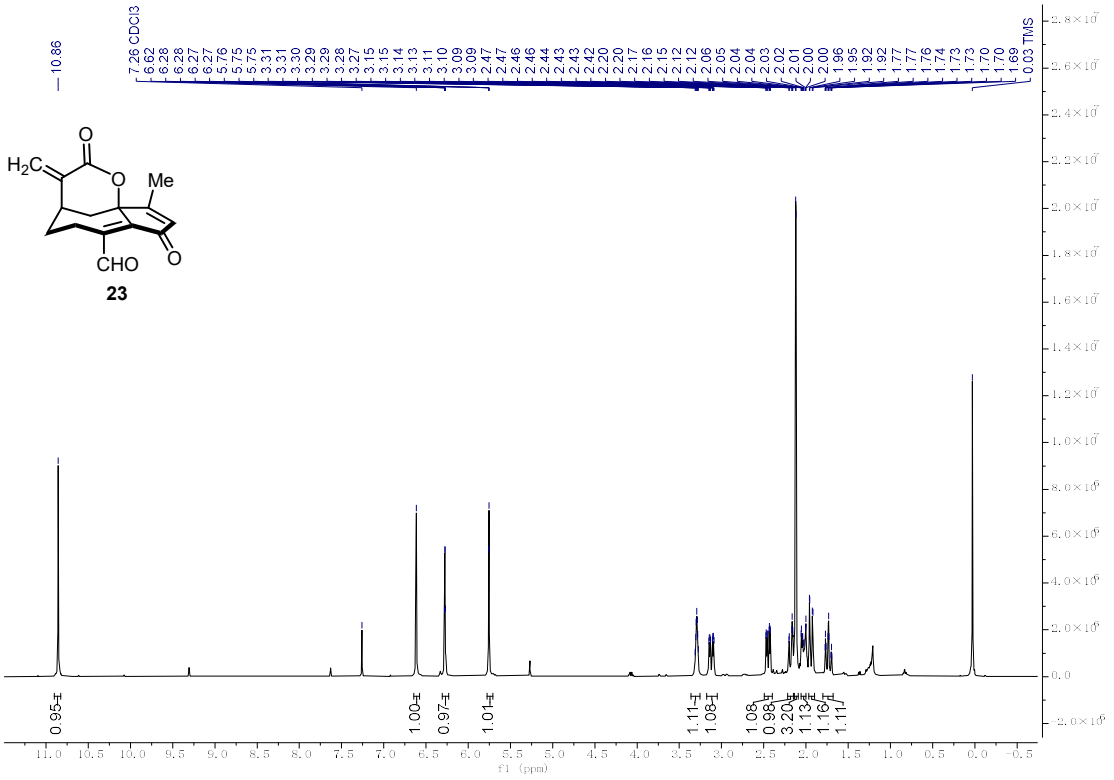
HSQC spectrum of **hypocretenolide (1)** ($CDCl_3$)



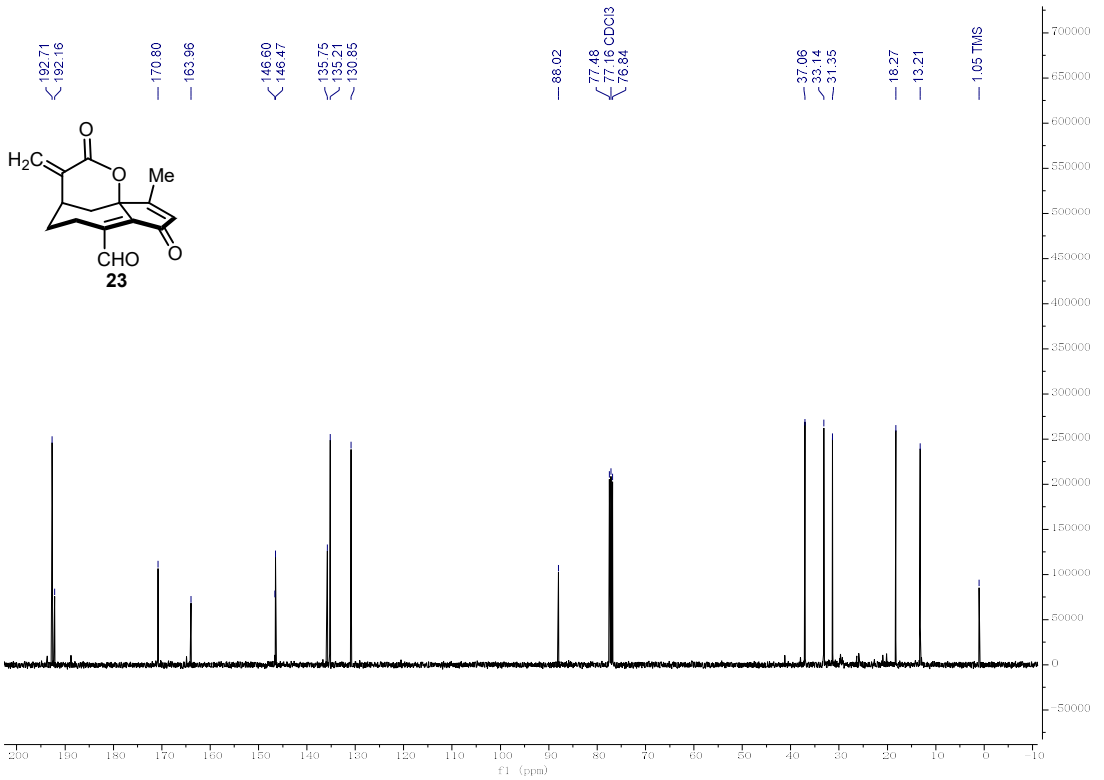
^{1}H-^{1}H COSY spectrum of **hypocretenolide (1)** ($CDCl_3$)



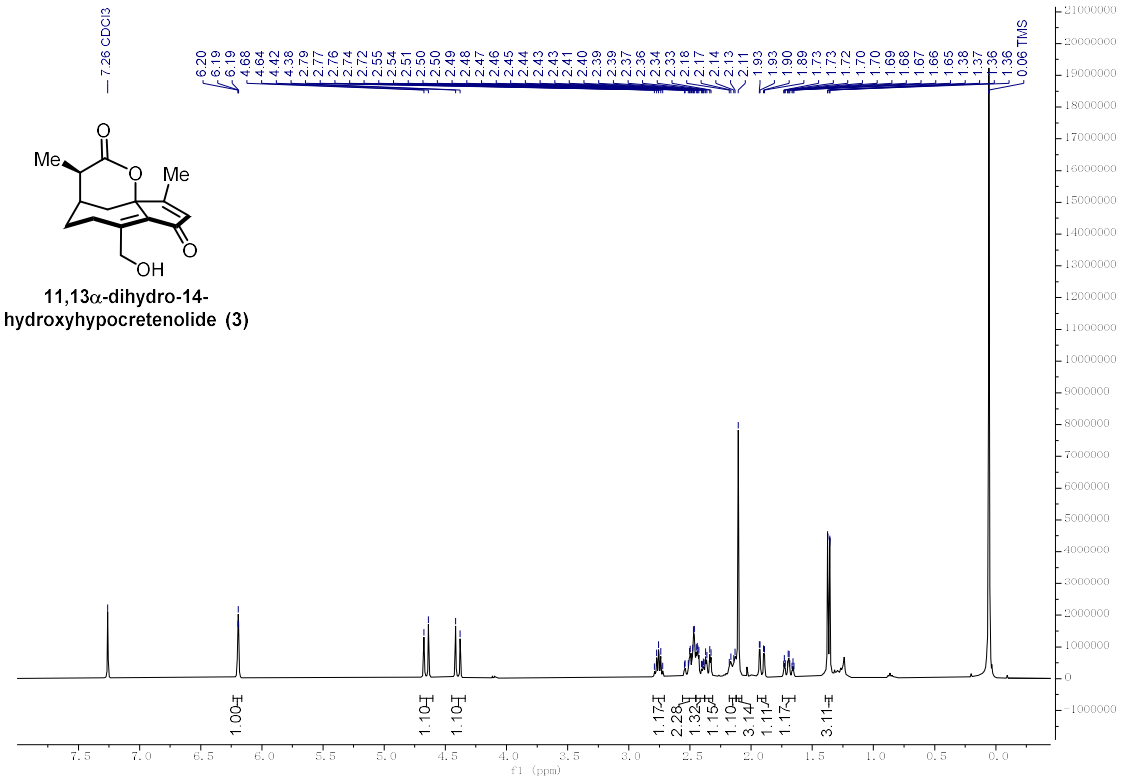
NOESY spectrum of **hypocretenolide (1)** ($CDCl_3$)



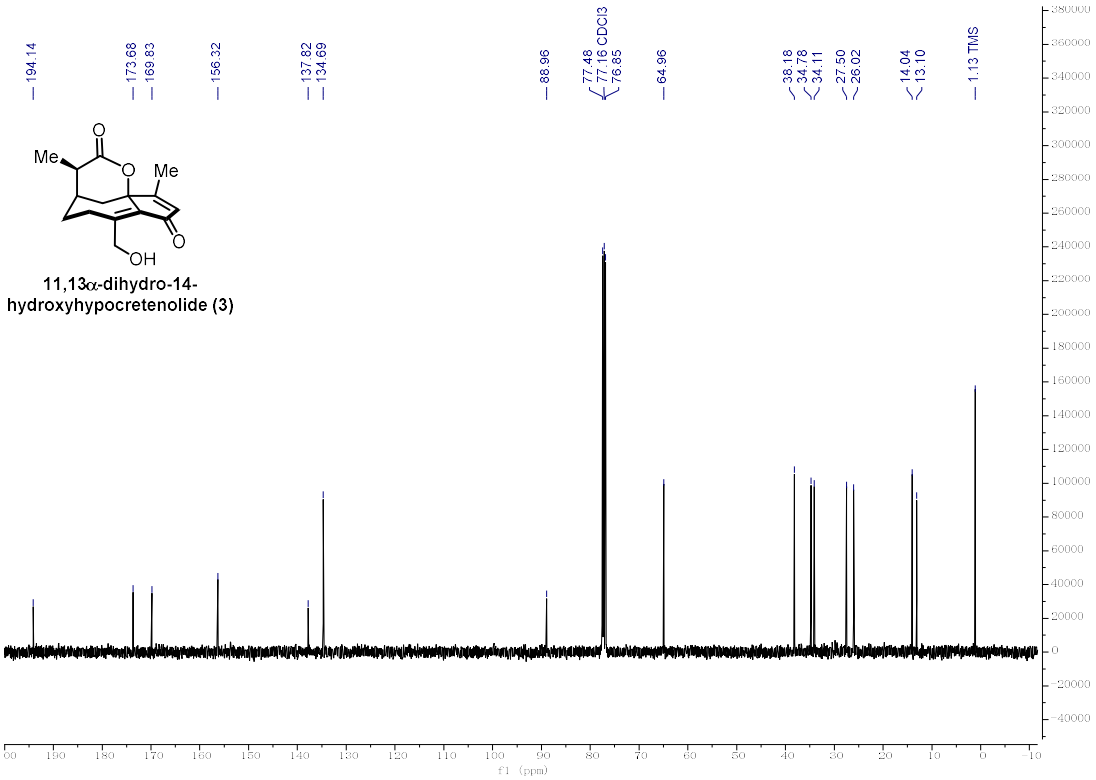
^{1}H NMR spectrum of **23** (400 MHz, $CDCl_3$)



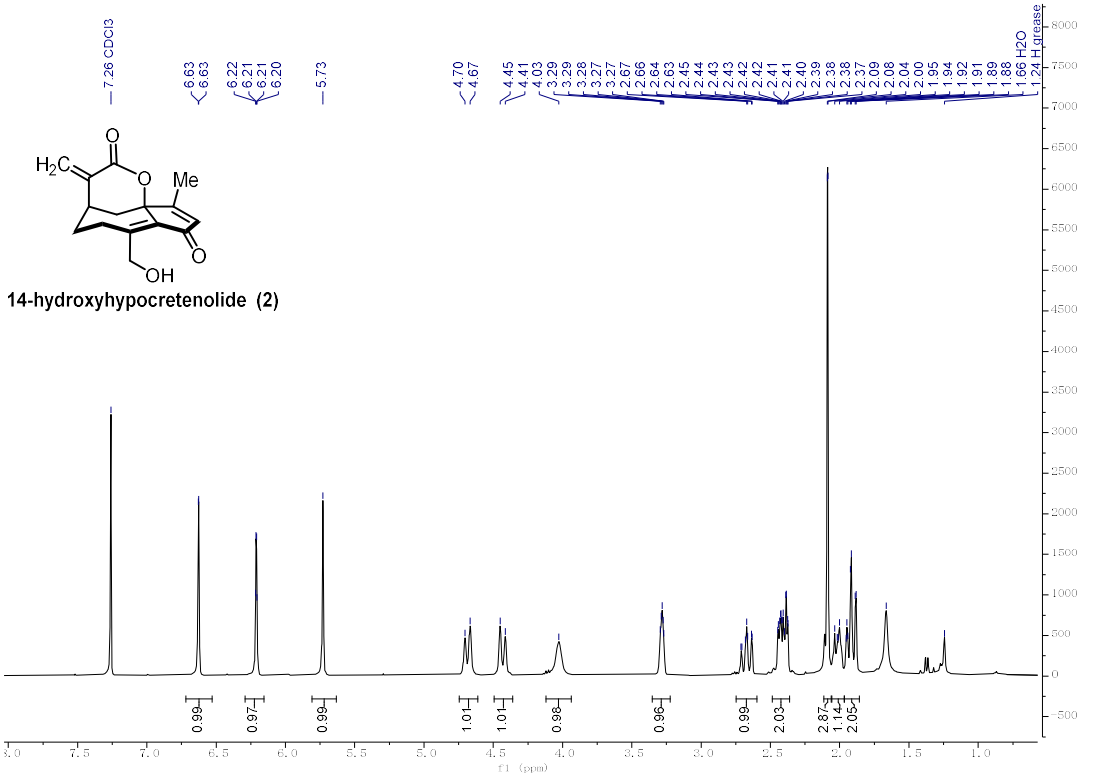
^{13}C NMR spectrum of **23** (100 MHz, $CDCl_3$)



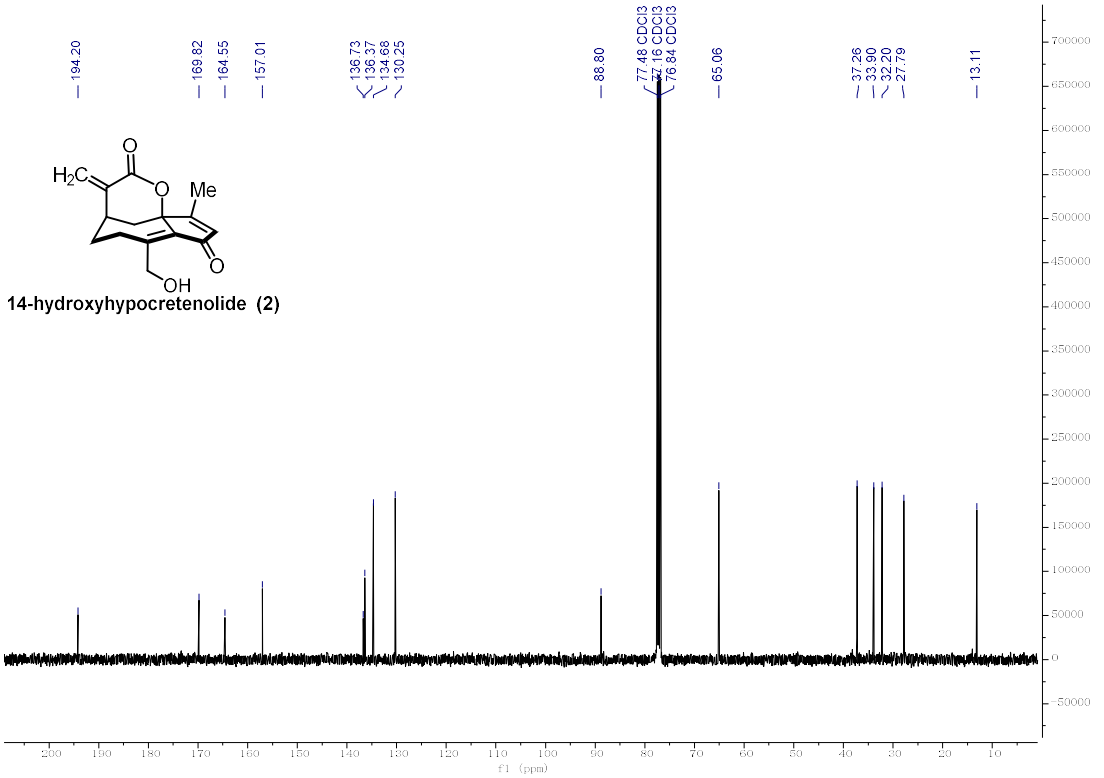
^{1}H NMR Spectrum of **11,13a-dihydro-14-hydroxyhypocretenolide (3)** (400 MHz, $CDCl_3$)



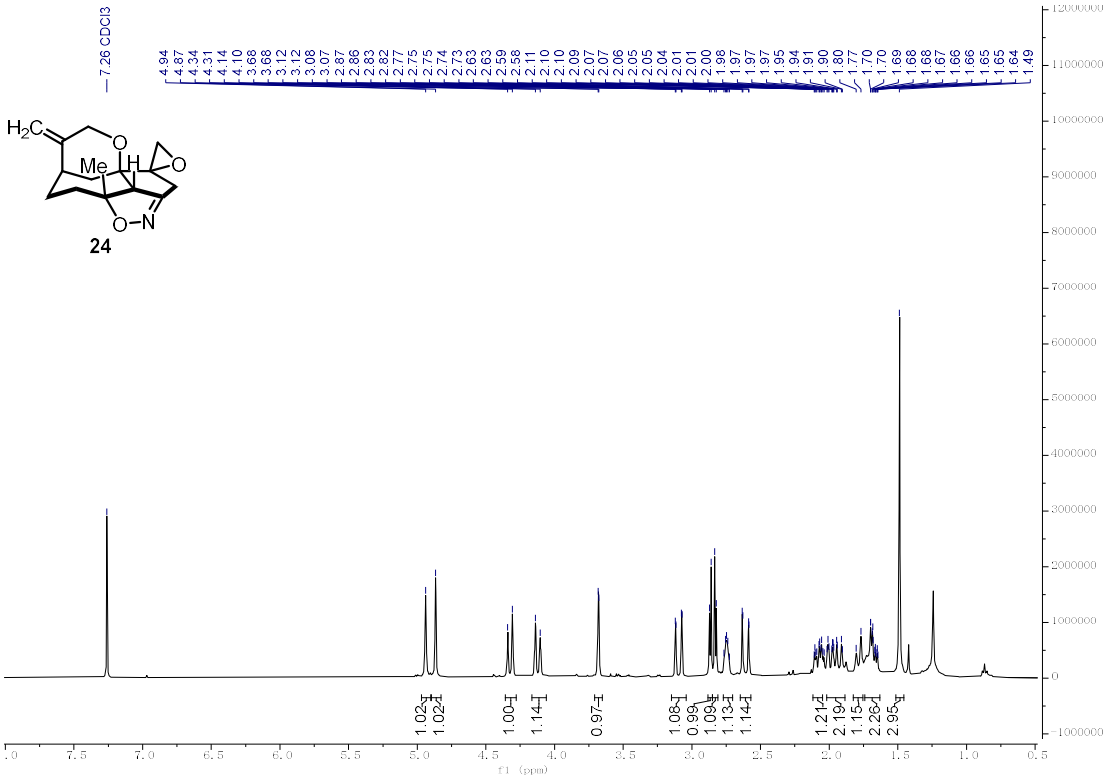
^{13}C NMR Spectrum of **11,13a-dihydro-14-hydroxyhypocretenolide (3)** (100 MHz, $CDCl_3$)



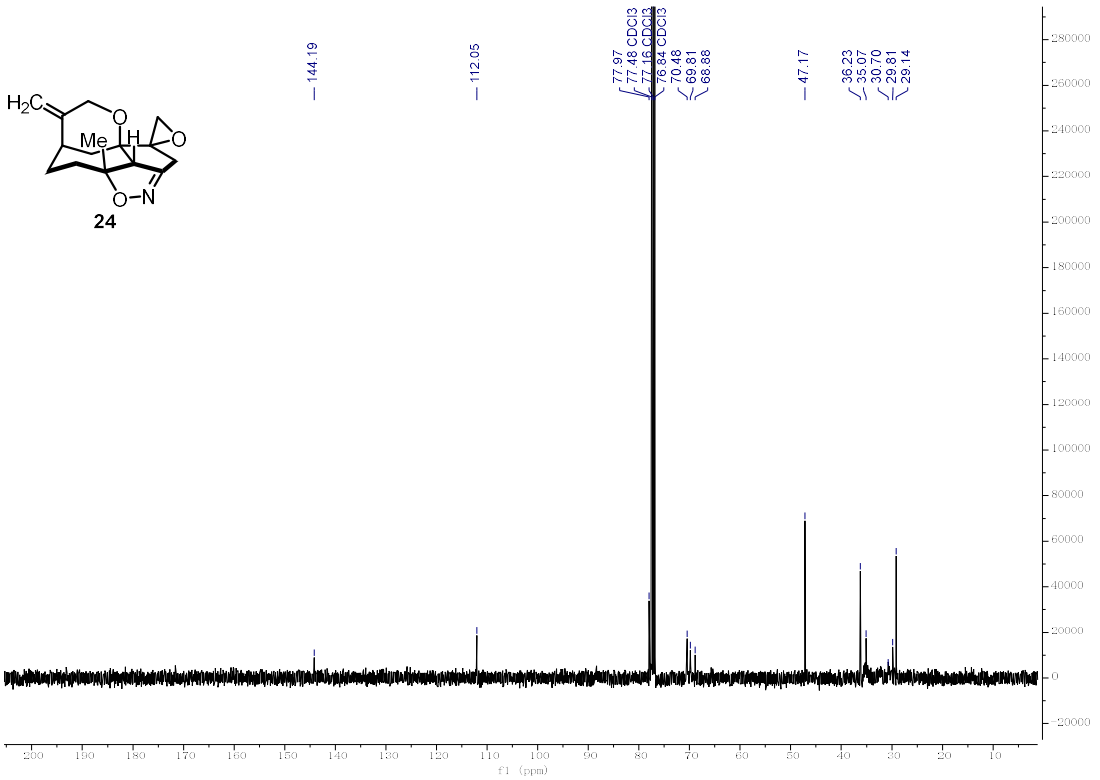
^{1}H NMR Spectrum of **14-hydroxyhypocretenolide (2)** (400 MHz, $CDCl_3$)



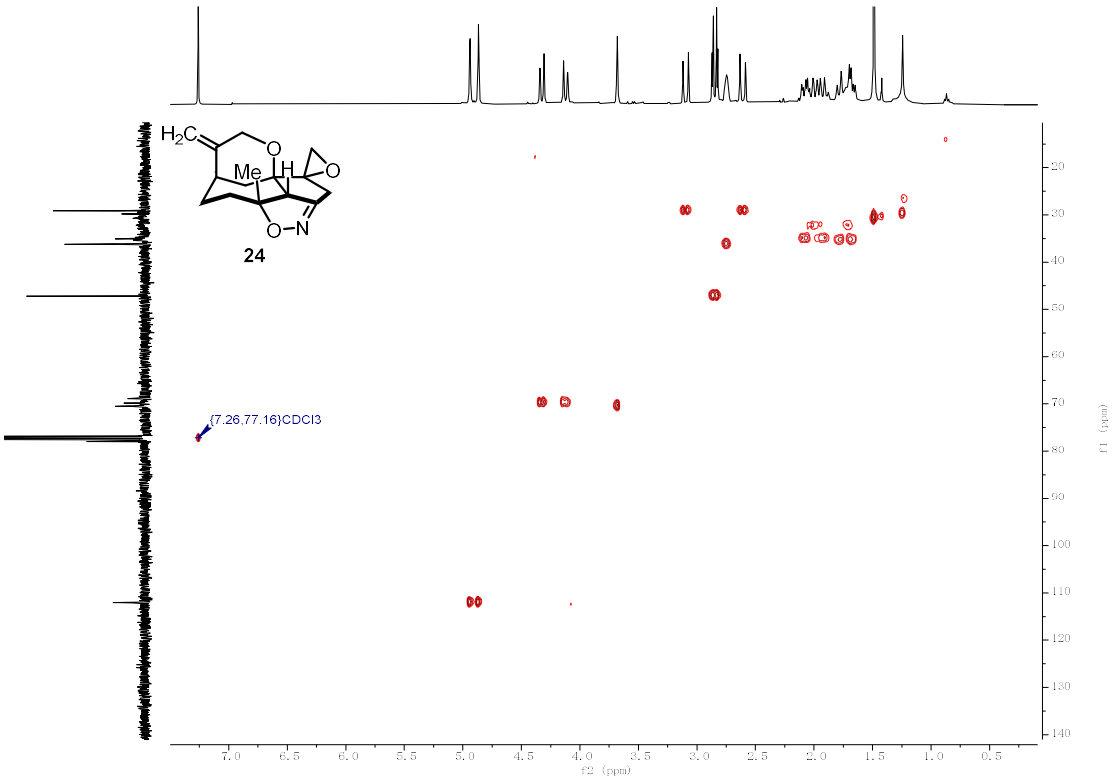
^{13}C NMR spectrum of **14-hydroxyhypocretenolide (2)** (100 MHz, $CDCl_3$)



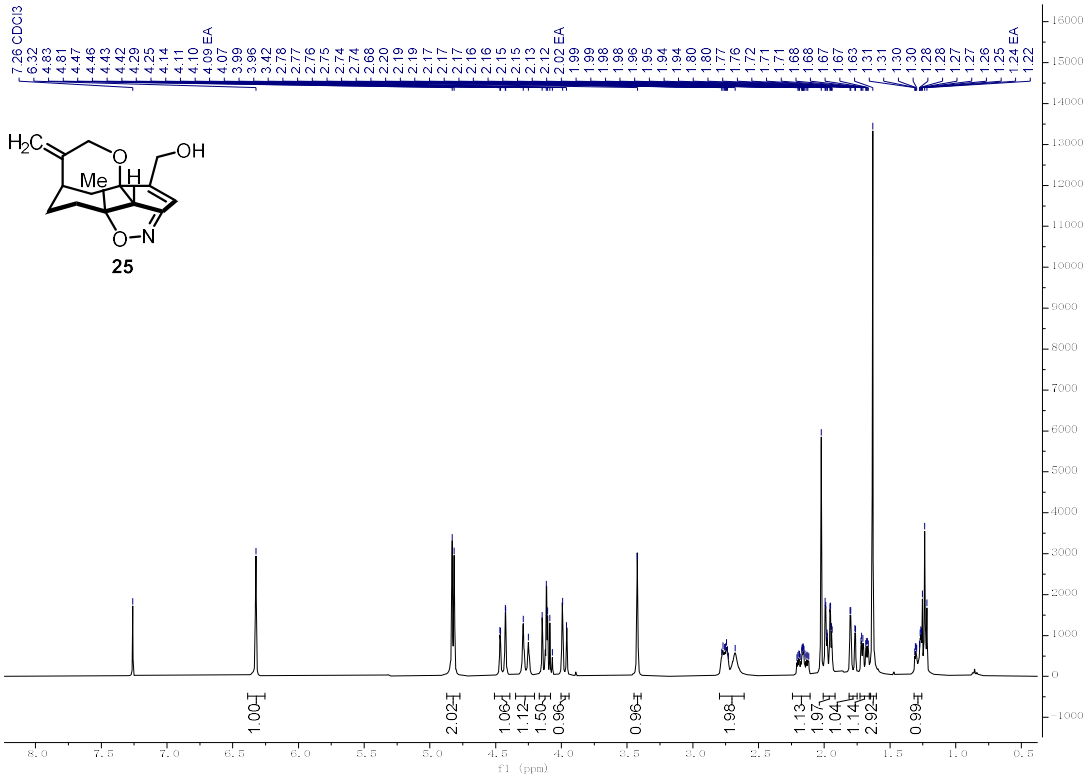
^{1}H NMR spectrum of **24** (400 MHz, $CDCl_3$)



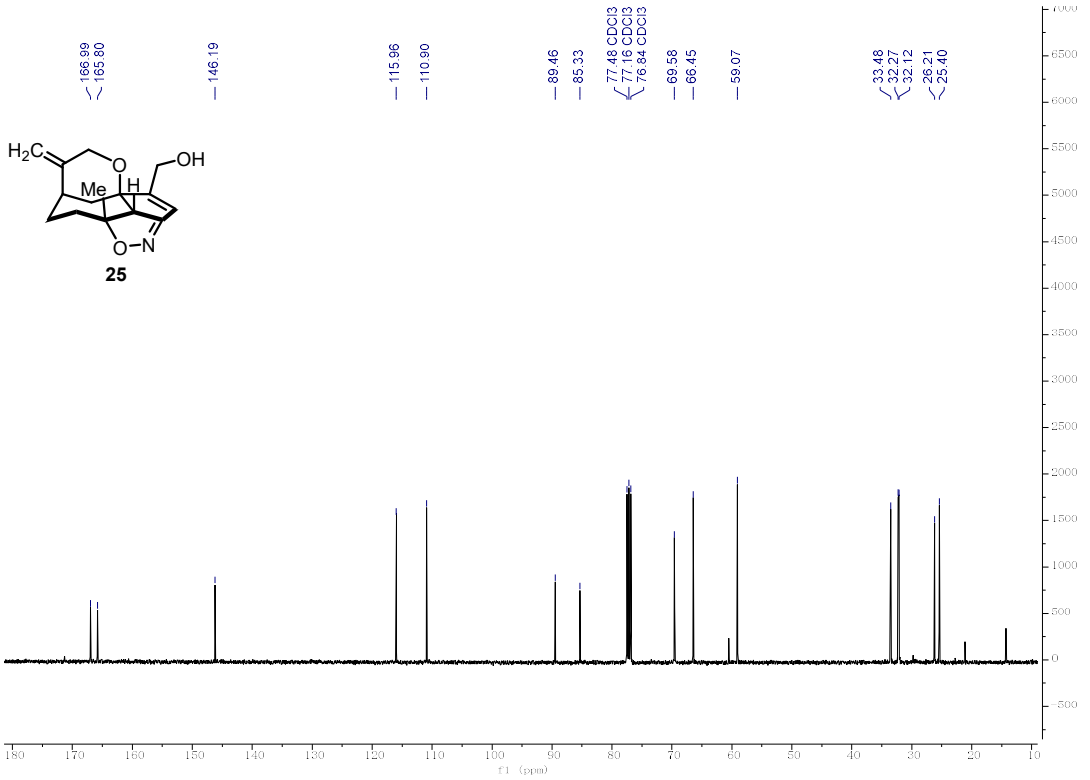
^{13}C NMR spectrum of **24** (100 MHz, $CDCl_3$)



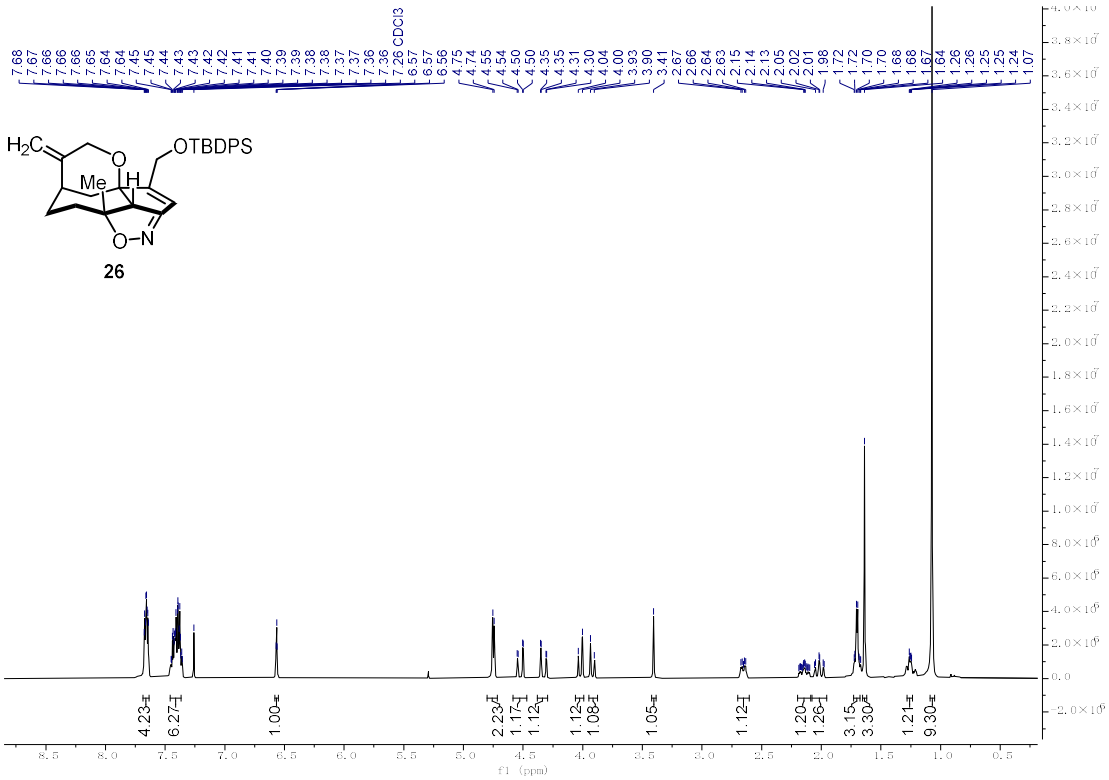
HSQC spectrum of **24** ($CDCl_3$)



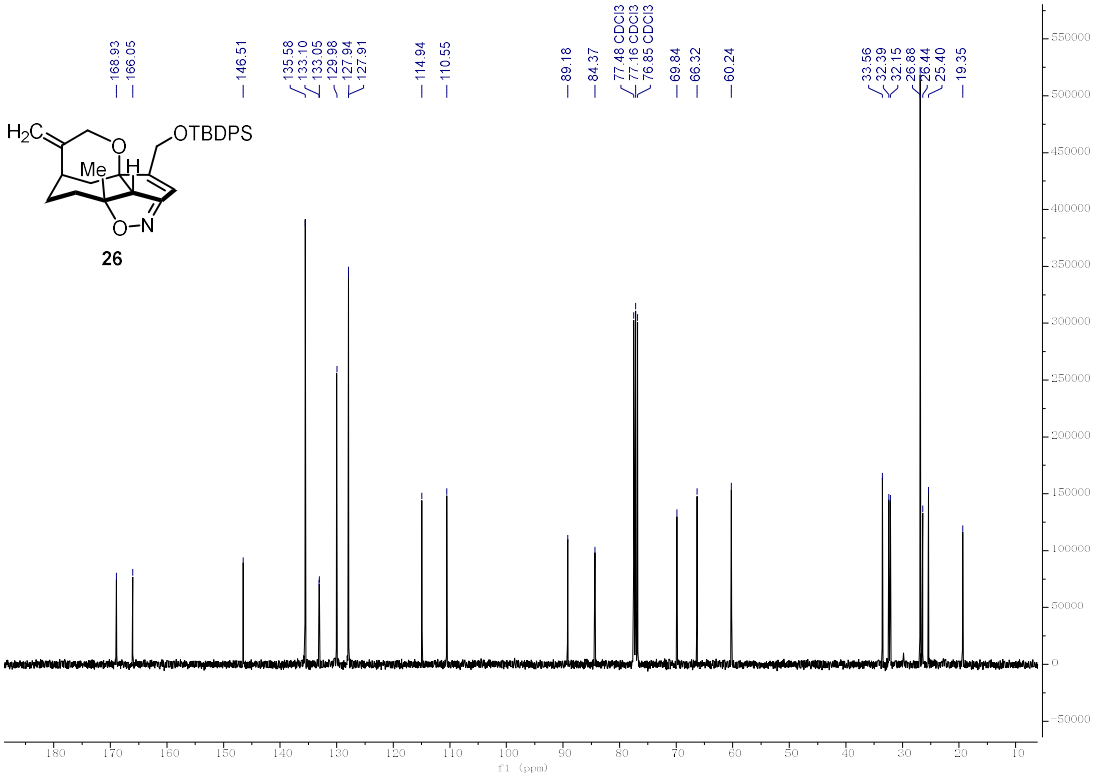
^{1}H NMR spectrum of **25** (400 MHz, $CDCl_3$)



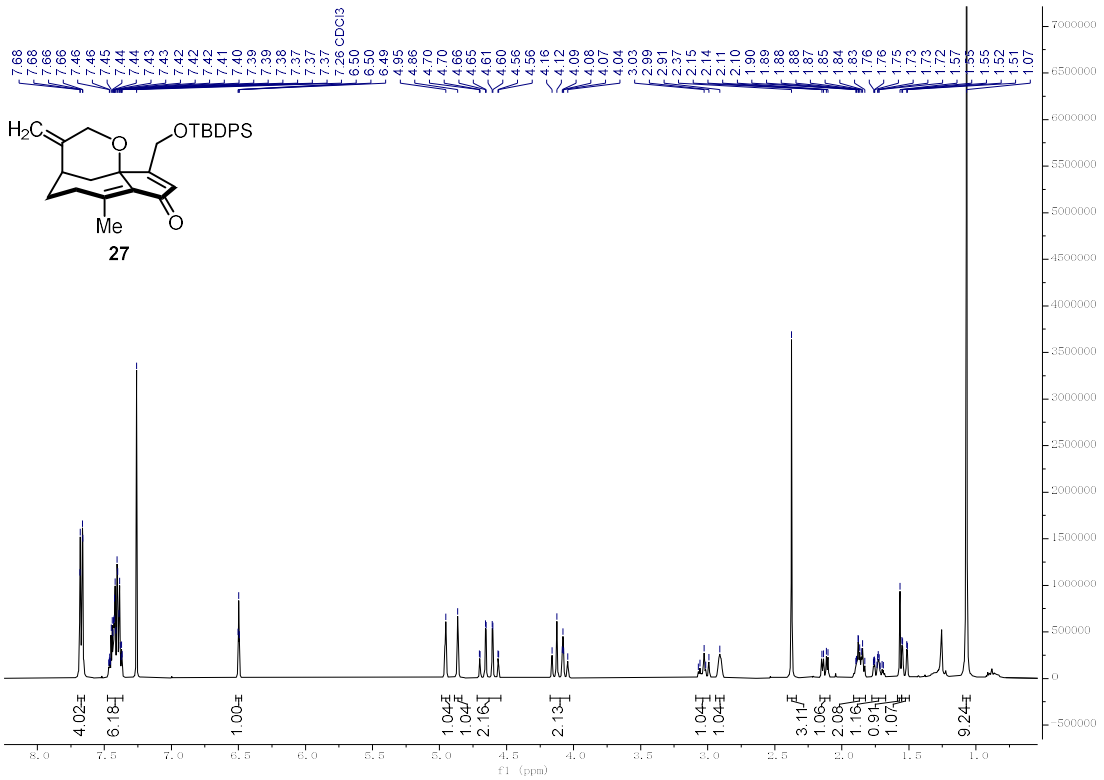
^{13}C NMR spectrum of **25** (100 MHz, $CDCl_3$)



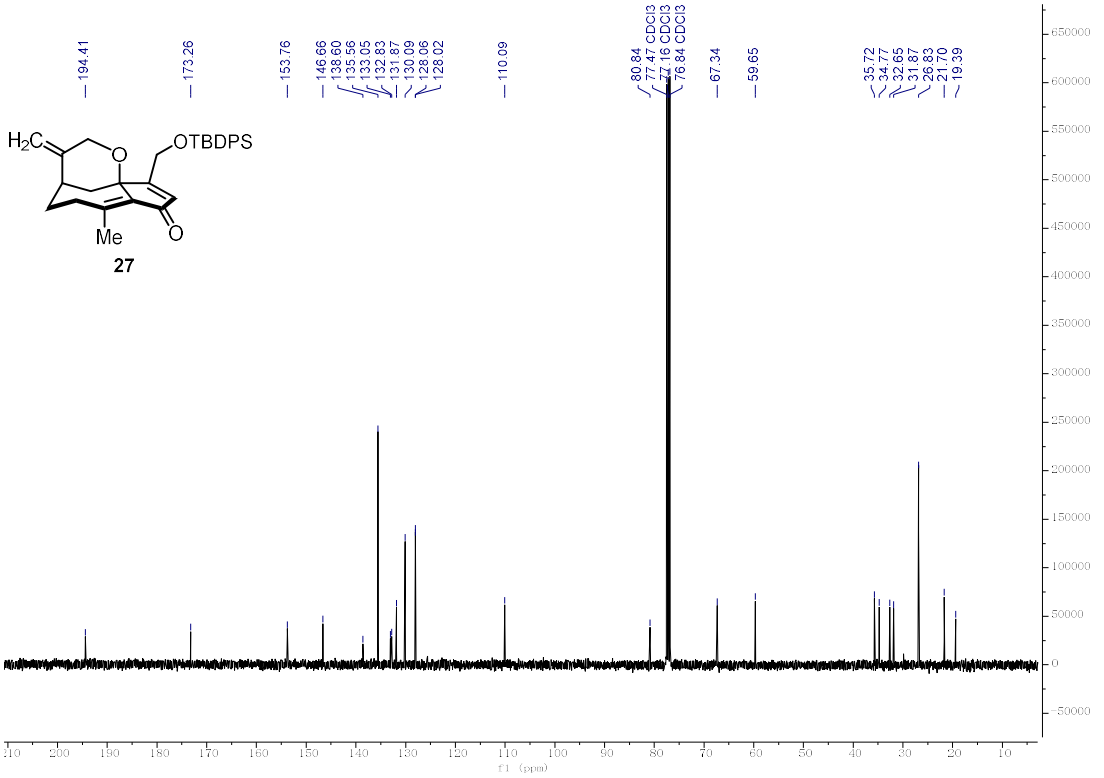
^{1}H NMR spectrum of **26** (400 MHz, $CDCl_3$)



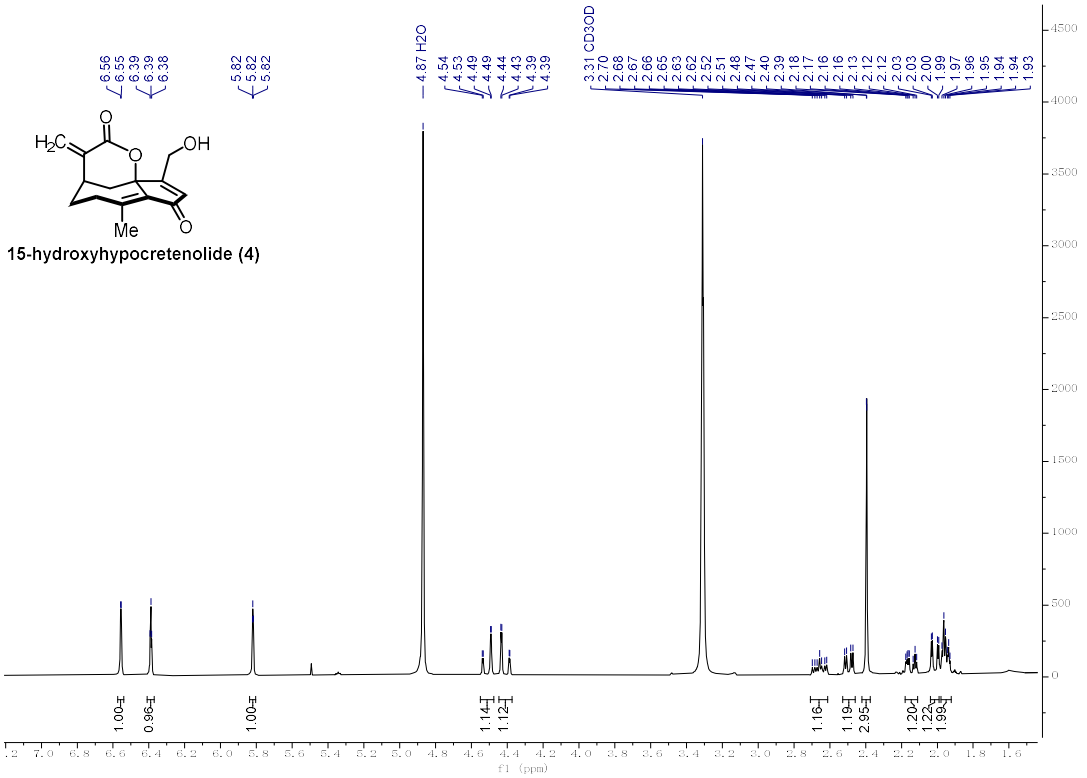
^{13}C NMR spectrum of **26** (100 MHz, $CDCl_3$)



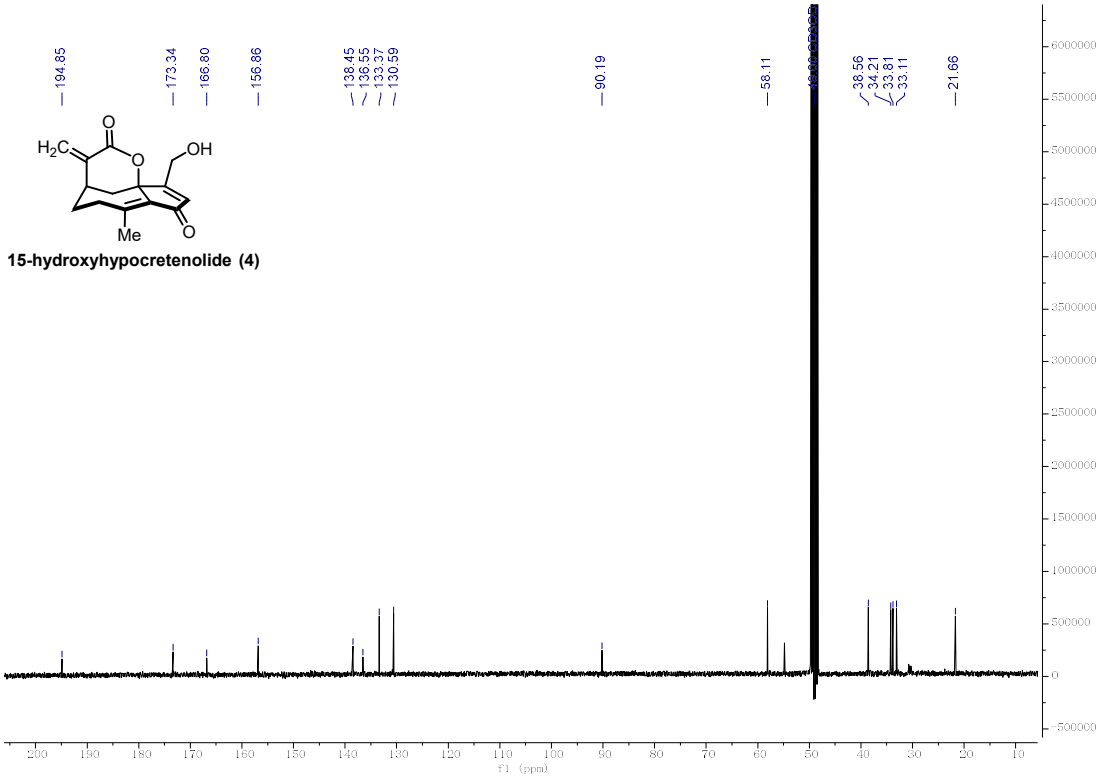
^{1}H NMR spectrum of **27** (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of **27**(100 MHz, $CDCl_3$)

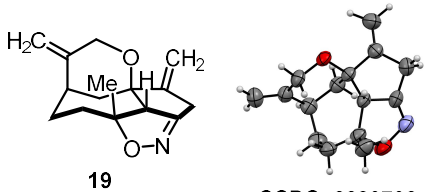


^{1}H NMR spectrum of **15-hydroxyhypocretenolide (4)** (400 MHz, MeOD)



^{13}C NMR spectrum of **15-hydroxyhypocretenolide (4)** (100 MHz, MeOD)

Part V. X-ray for the Synthetic Compounds



19

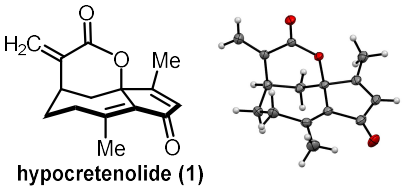
CCDC: 2280702

The ellipsoid contour at the 30% probability level.

Crystal Growing Method: About 20 mg of **19** was dissolved in around 0.1 mL of ethyl acetate and 0.8 mL of *n*-hexane in a vial, which was covered by the lid with a small hole. After slow evaporation in seven days, the colorless crystal was obtained and suitable for measurement.

Table S7. Crystal data and structure refinement for **19**

Identification code	**19**
Empirical formula	$C_{11}H_{10}NO$
Formula weight	172.20
Temperature/K	301.17(10)
Crystal system	triclinic
Space group	P-1
a/Å	8.5784(7)
b/Å	8.7025(4)
c/Å	9.1689(5)
α/°	101.163(4)
β/°	92.706(6)
γ/°	105.082(5)
Volume/Å^3	644.96(7)
Z	3
ρ_{calc}g/cm^3	1.330
μ/mm^{-1}	0.685
F(000)	273.0
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	9.886 to 151.398
Index ranges	$-10 \le h \le 9$, $-10 \le k \le 10$, $-11 \le l \le 10$
Reflections collected	5961
Independent reflections	2556 [R_{int} = 0.0394, R_{sigma} = 0.0466]
Data/restraints/parameters	2556/0/180
Goodness-of-fit on F^2	0.908
Final R indexes [I>=2σ (I)]	R_1 = 0.0517, wR_2 = 0.1588
Final R indexes [all data]	R_1 = 0.0615, wR_2 = 0.1668
Largest diff. peak/hole / e Å^{-3}	0.16/-0.18



hypocretenolide (1)

CCDC: 2280700

The ellipsoid contour at the 30% probability level.

Crystal Growing Method: About 20 mg of **hypocretenolide (1)** was dissolved in around 0.2 mL of ethyl acetate and 1.0 mL of *n*-hexane in a vial, which was covered by the lid with a small hole. After slow evaporation in seven days, the colorless crystal was obtained and suitable for measurement.

Table S8. Crystal data and structure refinement for **hypocretenolide (1)**.

Identification code	**Hypocretenolide (1)**
Empirical formula	$C_{30}H_{32}O_6$
Formula weight	488.55
Temperature/K	99.99(11)
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	22.3154(2)
b/Å	9.74840(10)
c/Å	11.29700(10)
α/°	90
β/°	92.6560(10)
γ/°	90
Volume/Å^3	2454.90(4)
Z	4
ρ_{calc}g/cm^3	1.322
μ/mm^{-1}	0.740
F(000)	1040.0
Crystal size/mm^3	0.27 × 0.16 × 0.16
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	9.904 to 152.788
Index ranges	-27 ≤ h ≤ 27, -12 ≤ k ≤ 12, -14 ≤ l ≤ 7
Reflections collected	19866
Independent reflections	5021 [R_{int} = 0.0255, R_{sigma} = 0.0214]
Data/restraints/parameters	5021/0/329
Goodness-of-fit on F^2	1.050
Final R indexes [I>=2σ (I)]	R_1 = 0.0355, wR_2 = 0.0902
Final R indexes [all data]	R_1 = 0.0379, wR_2 = 0.0918
Largest diff. peak/hole / e Å^{-3}	0.33/-0.21

Part VI. References

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