Supporting Information

Asymmetric synthesis and biological evaluation of ottensinin and its analogues

Jun-Ting Liang,‡ Zichen Xu,‡ Yong-Bin Xie, Wen-Tao Chen, Yu-Tao He,* and Ya-Jian Hu*

State Key Laboratory of Bioactive Molecules and Druggability Assessment, Institute for Advanced and Applied Chemical Synthesis, College of Pharmacy, Jinan University, Guangzhou 510632, China

‡ These authors contributed equally.

* Corresponding authors.

Emails: heyutao7@jnu.edu.cn (Y.-T. He); yajianhu@jnu.edu.cn (Y.-J. Hu)

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1. General Information

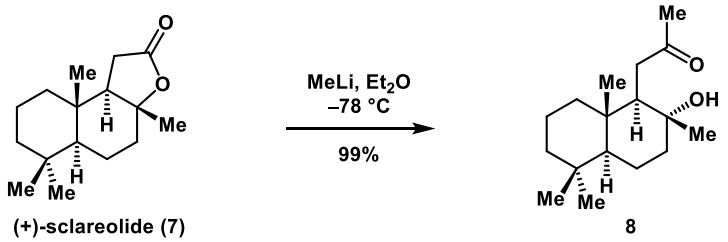
Unless otherwise mentioned, all reactions were carried out under argon atmosphere under anhydrous conditions and all reagents were purchased from commercial suppliers without further purification. Anhydrous solvents were distilled prior to use (toluene, THF, and Et_2O from Na/benzophenone; MeCN, DCM, and pyridine from CaH_2). Yields refer to chromatographically and spectroscopically (^1H NMR) homogeneous material, unless otherwise stated. Lower temperatures were maintained using dry ice/acetone (−78 °C), dry ice/MeCN (−40 °C), ice/water (0 °C) baths, and low temperature reactor.

Reactions were monitored by thin layer chromatography on plates (GF254) supplied by Yantai Chemicals (China), using UV light as the visualizing agent and/or ethanolic phosphomolybdic acid, acidic ethanolic anisaldehyde, or basic aqueous $KMnO_4$ and heat as developing agents. If not specially noted, flash column chromatography was performed on silica gel (200–300 mesh) supplied by Tsingtao Haiyang Chemicals (China) and preparative thin layer chromatography (PTLC) separations were carried out 0.50 mm Yantai (China) silica gel plates.

NMR spectra were recorded on 300, 500, and 600 MHz Bruker Avance spectrometers and calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain NMR peak multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High-resolution mass spectra (HRMS) were recorded on a Bruker Apex IV FTMS mass spectrometer using electrospray ionization (ESI). Infrared spectra were recorded on a Shimadzu IR Prestige 21, using thin films of the sample on KBr plates. Optical rotations were measured with a Rudolph autopol I automatic polarimeter using 10 cm glass cells with a sodium 589 nm filter. Data were reported as follow: optical rotation (*c* (g/100 mL), solvent). Melting points were obtained on an MP450-01 micro-melting point apparatus (Hanon Instrument, Shandong, China) without correction.

2. Synthetic Procedures and Characterization Data

Synthesis of compound 8



To a stirred solution of (+)-sclareolide (**7**, 10.0 g, 40.0 mmol, 1 equiv) in Et_2O (80 mL) was added MeLi (1.6 M in Et_2O, 40.0 mL, 64.0 mmol, 1.6 equiv) dropwise at –78 ºC. After 1 h, the mixture was quenched with sat. aq. NH_4Cl (20 mL) and extracted with EtOAc (3 × 150 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (gradient from 10:1 to 5:1, v/v) as an eluent to afford **8** (10.5 g, 99% yield) as a white solid.

TLC: R_f = 0.2 (petroleum ether/EtOAc = 4:1);

$[\alpha]_D^{22}$ = –5.7 (*c* = 1.0, $CHCl_3$);

m.p. = 63 – 65 ℃;

IR (film): ν_{max} = 3446, 2925, 1703, 1470, 1387, 1168, 1069, 750, 665 cm^{-1};

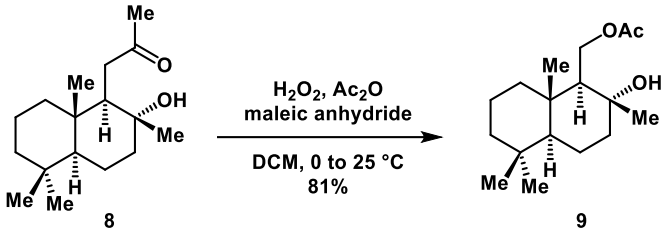
^{1}H NMR (300 MHz, $CDCl_3$) δ2.52 – 2.31 (m, 2H), 2.13 (s, 3H), 1.92 – 1.84 (m, 3H), 1.67 – 1.59 (m, 1H), 1.50 (s, 1H), 1.41 – 1.32 (m, 3H), 1.29 (d, *J* = 2.9 Hz, 1H), 1.25 – 1.19 (m, 1H), 1.14 (d, *J* = 3.9 Hz, 1H), 1.06 (s, 3H), 0.97 (dd, *J* = 2.3, 12.1 Hz, 1H), 0.83 (s, 3H), 0.76 – 0.73 (m, 6H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 210.2, 72.9, 55.8, 55.7, 44.3, 41.7, 39.5, 39.3, 38.2, 33.3, 33.1, 30.2, 23.1, 21.3, 20.5, 18.3, 15.6;

HRMS (ESI): *m/z* calcd for $C_{17}H_{30}NaO_2^+$ $[M+Na]^+$ 289.2138, found 289.2134.

The analytical data matched those previously reported.[1]

Synthesis of compound 9



To a mixture of acetic anhydride (Ac_2O, 42.3 mL, 451 mmol, 12 equiv) in DCM (200 mL) was added 30% hydrogen peroxide solution (49.0 mL, 488 mmol, 13 equiv) at 0 ºC. After 1 h, maleic anhydride (29.5 g, 301 mmol, 8 equiv) was added, and stirring was continued for another 2 h. A solution of keto-alcohol **8** (10.0 g, 37.6 mmol, 1 equiv) in DCM (40 mL) was added dropwise and stirring was continued for another 16 h at 25 ºC. The mixture was quenched with sat. aq. $Na_2S_2O_3$ (50 mL) and $NaHCO_3$ (50 mL) carefully at 0 ºC and extracted with DCM (3 × 200 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (gradient from 10:1 to 6:1, v/v) as an eluent to afford **9** (8.59 g, 81% yield) as a colorless oil.

TLC: R_f = 0.3 (petroleum ether/EtOAc = 4:1);

[α]$_D^{22}$ = –7.25 (c = 0.91, $CHCl_3$);

m.p. = 74 – 76 ºC;

IR (film): ν_{max} = 3446, 2923, 1723, 1464, 1387, 1239 1071, 752 cm^{-1};

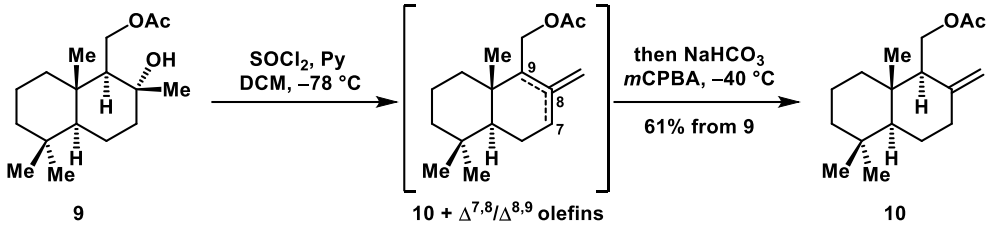
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.37 – 4.21 (m, 2H), 2.28 – 2.24 (m, 1H), 2.03 (s, 3H), 1.91 – 1.84 (m, 1H), 1.68 – 1.61 (m, 2H), 1.52 (d, J = 4.7 Hz, 1H), 1.50 – 1.42 (m, 2H), 1.40 – 1.35 (m, 1H), 1.33 – 1.20 (m, 2H), 1.16 (d, J = 1.0 Hz, 3H), 1.05 (dd, J = 3.5, 12.9 Hz, 1H), 0.94 (dd, J = 2.1, 12.1 Hz, 1H), 0.86 (d, J = 6.1 Hz, 6H), 0.79 (s, 3H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 210.2, 72.9, 55.8, 55.7, 44.3, 41.7, 39.5, 39.3, 38.2, 33.3, 33.1, 30.2, 23.1, 21.3, 20.5, 18.3, 15.6;

HRMS (ESI): *m/z* calcd for $C_{17}H_{30}NaO_3^+$ $[M+Na]^+$ 305.2087, found 305.2082.

The analytical data matched those previously reported.[1]

Synthesis of compound 10



To a stirred solution of **9** (9.10 g, 32.3 mmol, 1 equiv) in DCM (100 mL) cooled to –78 ºC was added sequentially pyridine (2.59 mL, 323 mmol, 10 equiv) and thionyl chloride (1.17 mL, 162 mmol, 5 equiv). After 2 h, the reaction mixture was concentrated in vacuum to give crude *exo* olefin **10** (contaminated with small amount of $\Delta^{7,8}$ and $\Delta^{8,9}$ *endo* olefins. The above crude mixture was azeotropically dried with toluene (50 mL) before it was dissolved in DCM (100 mL) and cooled to –40 ºC. $NaHCO_3$ (13.5 g, 162 mmol, 5 equiv) and *meta*-chloroperoxybenzoic acid (*m*CPBA, 1.95 g, 11.3 mmol, 0.35 equiv) were added sequentially. After 2 h, the reaction mixture was quenched with sat. aq. $NaHCO_3$ (20 mL) and extracted with DCM (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (gradient from 200:1 to 150:1, v/v) as an eluent to afford pure **10** (5.20 g, 61% yield) as a colorless oil.

TLC: R_f = 0.8 (petroleum ether/EtOAc = 4:1);

$[\alpha]_D^{22}$ = +22.0 (c = 0.5, $CHCl_3$);

IR (film): ν_{max} = 2925, 2866, 1737, 1459, 1365, 1231, 1027, 888 cm^{-1};

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.84 (d, *J* = 1.6 Hz, 1H), 4.50 (d, *J* = 1.6 Hz, 1H), 4.35 – 4.29 (m, 1H), 4.21 – 4.13 (m, 1H), 2.43 – 2.36 (m, 1H), 2.03 (d, *J* = 3.8 Hz, 2H), 2.01

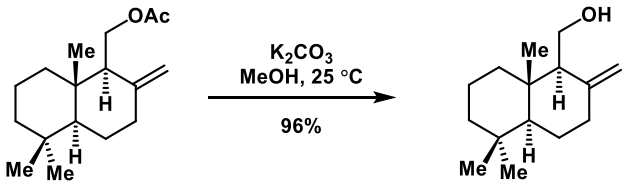
(s, 3H), 1.70 (d, J = 2.8 Hz, 1H), 1.64 (d, J = 3.7 Hz, 1H), 1.50 (d, J = 1.4 Hz, 1H), 1.45 – 1.41 (m, 1H), 1.39 – 1.36 (m, 1H), 1.33 (s, 1H), 1.27 – 1.23 (m, 1H), 1.17 (d, J = 2.2 Hz, 1H), 1.14 (d, J = 2.4 Hz, 1H), 0.87 (s, 3H), 0.80 (s, 3H), 0.74 (s, 3H);

^{13}C NMR (75 MHz, $CDCl_3$) δ171.5, 146.9, 107.3, 61.6, 55.2, 54.8, 42.0, 39.1, 39.1, 37.7, 33.8, 33.6, 24.0, 21.9, 21.3, 19.3, 15.2;

HRMS (ESI): *m/z* calcd for $C_{17}H_{28}NaO_2^+$ $[M+Na]^+$ 287.1982, found 287.1980.

The analytical data matched those previously reported.[2]

Synthesis of compound 11



To a stirred solution of compound **10** (5.20 g, 19.7 mmol, 1 equiv) in MeOH (50 mL) was added potassium carbonate (K_2CO_3, 13.6 g, 98.5 mmol, 5 equiv) at 25 ºC. The mixture was stirred for 4 h before it was quenched with sat. aq. NH_4Cl (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (gradient from 30:1 to 15:1, v/v) as an eluent to afford **11** (4.20 g, 96% yield) as a colorless solid.

TLC: R_f = 0.8 (petroleum ether/EtOAc = 10:1);

$[\alpha]_D^{22}$ = +13.8 (c = 0.5, $CHCl_3$);

m.p. = 62 – 65 °C;

IR (film): ν_{max} = 3358, 2923, 2866, 2844, 1458, 1440, 1020, 754 cm^{-1};

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.94 (s, 1H), 4.64 (s, 1H), 3.88 – 3.74 (m, 2H), 2.47 –

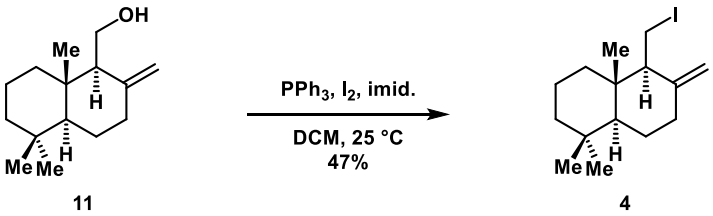
2.38 (m, 1H), 2.04 (s, 1H), 1.99 (s, 1H), 1.71 (s, 1H), 1.68 – 1.62 (m, 1H), 1.50 (s, 3H), 1.37 (d, J = 4.2 Hz, 1H), 1.25 (s, 1H), 1.20 (s, 1H), 1.10 (s, 1H), 0.87 (s, 3H), 0.80 (s, 3H), 0.71 (s, 3H);

^{13}C NMR (75 MHz, $CDCl_3$) δ148.0, 106.4, 59.3, 58.9, 55.3, 42.1, 39.1, 39.1, 38.0, 33.76, 33.6, 24.3, 21.9, 19.3, 15.4;

HRMS (ESI): *m/z* calcd for $C_{15}H_{26}NaO^+$ $[M+Na]^+$ 245.1876, found 245.1875.

The analytical data matched those previously reported.[2]

Synthesis of compound 4



To a stirred solution of **11** (100 mg, 0.450 mmol, 1 equiv) in DCM (5 mL) was sequentially added imidazole (61.2 mg, 0.899 mmol, 2 equiv) and PPh_3 (177 mg, 0.675 mmol, 1.5 equiv) and I_2 (171 mg, 0.675 mmol, 1.5 equiv) at 0 °C. The reaction mixture was warmed to 25 °C and stirred for 4 h before it was quenched with sat. aq. Na_2SO_3 (10 mL). Then the aqueous phase was extracted with DCM (3 × 25 mL) and the combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography with petroleum ether as an eluent to afford homoallylic iodide **4** (70.0 mg, 47% yield) as a colorless oil.

TLC: R_f = 0.95 (petroleum ether);

$[\alpha]_D^{25}$ = +109.7 (c = 1.0, $CHCl_3$);

IR (film): ν_{max} = 2922, 2846, 1647, 1459, 1387, 1206, 886 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ5.00 (s, 1H), 4.64 (s, 1H), 3.66 (dd, J = 2.3, 10.0 Hz,

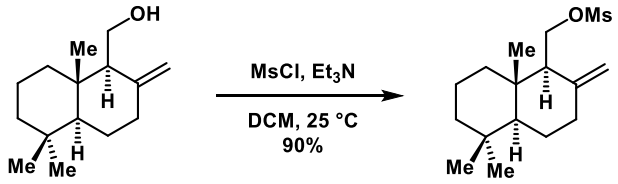
1H), 3.04 (dd, J = 10.0, 11.3 Hz, 1H), 2.50 – 2.35 (m, 1H), 2.17 (dd, J = 2.2, 11.3 Hz, 1H), 2.11 – 2.05 (m, 1H), 1.78 – 1.71 (m, 2H), 1.58 – 1.55 (m, 1H), 1.54 – 1.49 (m, 1H), 1.44 – 1.38 (m, 1H), 1.37 – 1.28 (m, 1H), 1.27 – 1.17 (m, 2H), 1.11 (dd, J = 2.8, 12.6 Hz, 1H), 0.88 (s, 3H), 0.80 (s, 3H), 0.70 (s, 3H);

^{13}C NMR (126 MHz, $CDCl_3$) δ 146.1, 108.2, 60.5, 55.3, 42.0, 41.8, 39.3, 37.8, 33.8, 33.7, 24.4, 21.84, 19.4, 13.9, 2.6;

HRMS (ESI): *m/z* calcd for $C_{15}H_{26}I^+$ $[M+H]^+$ 333.1074, found 333.1079.

The analytical data matched those previously reported.[3]

Synthesis of compound 12



11 **12**

To a stirred solution of **11** (241 mg, 1.09 mmol, 1 equiv) in DCM (10 mL) was sequentially added Et_3N (452 μL, 3.27 mmol, 3 equiv) and MsCl (169 μL, 2.18 mmol, 2 equiv) at 0 °C. The reaction mixture was warmed to 25 °C for 4 h before being quenched with sat. aq. NH_4Cl (10 mL). Then, the resulting mixture was extracted with DCM (3 × 10 mL), and the combined organic phases were washed with brine (10 mL) and dried over anhydrous Na_2SO_4 filtered and concentrated. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (gradient from 30:1 to 15:1, v/v) as an eluent to afford **12** (294 mg, 90% yield) as a colorless oil.

TLC: R_f = 0.5 (petroleum ether/EtOAc = 5:1);

$[\alpha]_D^{25}$ = +18.9 (c = 0.5, $CHCl_3$);

IR (film): ν_{max} = 2926, 2846, 1647, 1460, 1387, 1355, 1173, 945, 888 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.91 (s, 1H), 4.62 (s, 1H), 4.49 (dd, J = 3.8, 10.0 Hz,

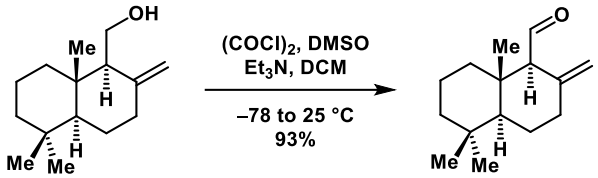
1H), 4.35 (t, J = 9.5 Hz, 1H), 2.98 (s, 3H), 2.44 – 2.40 (m, 1H), 2.16 – 2.13 (m, 1H), 2.07 – 2.00 (m, 1H), 1.76 – 1.68 (m, 2H), 1.62 – 1.48 (m, 2H), 1.43 – 1.39 (m, 1H), 1.36 – 1.32 (m, 1H), 1.28 – 1.20 (m, 2H), 1.14 (dd, J = 2.8, 12.6 Hz, 1H), 0.88 (s, 3H), 0.81 (s, 3H), 0.76 (s, 3H);

^{13}C NMR (126 MHz, $CDCl_3$) δ 145.8, 107.8, 66.8, 55.2, 55.1, 41.9, 39.3, 39.3, 37.7, 37.6, 33.7, 33.7, 23.9, 21.9, 19.2, 15.4;

HRMS (ESI): *m/z* calcd for $C_{15}H_{28}NaOS^+$ $[M+Na]^+$ 323.1651, found 323.1653.

The analytical data matched those previously reported.[4]

Synthesis of compound 13



11 13

To a stirred solution of oxalyl chloride (2.23 mL, 26.3 mmol, 1.5 equiv) in DCM (50 mL) was dropwise added DMSO (2.49 mL, 35.1 mmol, 2 equiv) at –78 ºC. After 10 min, a solution of **11** (3.90 g, 17.5 mmol, 1 equiv) in DCM (10 mL) was added, and stirring was continued for another 20 min. Then Et_3N (12.2 mL, 87.7 mmol, 5 equiv) was added, and the mixture was allowed to warm to room temperature and stirred for another 1 h before it was quenched with sat. aq. NH_4Cl (50 mL). The resulting mixture was extracted with DCM (3 × 50 mL) and the combined organic phases were washed with brine (50 mL) and dried over anhydrous Na_2SO_4 filtered and concentrated. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (gradient from 200:1 to 150:1, v/v) as an eluent to afford aldehyde **13** (3.60 g, 93% yield) as a colorless oil.

TLC: R_f = 0.8 (petroleum ether/EtOAc = 10:1);

$[\alpha]_D^{22}$ = –70.6 (c = 0.435, $CHCl_3$);

IR (film): ν_{max} = 2926, 2845, 1714, 1458, 1388, 1109, 893, 753 cm^{-1};

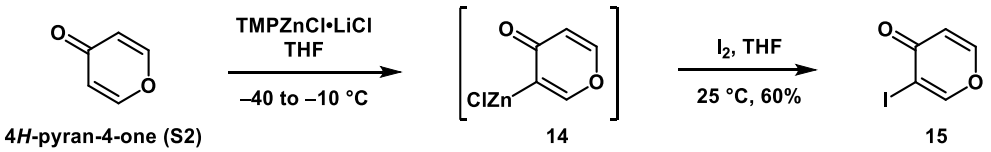
^{1}H NMR (300 MHz, $CDCl_3$) δ 9.85 (d, *J* = 4.9 Hz, 1H), 4.90 (s, 1H), 4.48 (s, 1H), 2.42 (d, *J* = 4.6 Hz, 2H), 2.10 – 1.99 (m, 1H), 1.74 – 1.66 (m, 1H), 1.60 – 1.52 (m, 2H), 1.51 – 1.32 (m, 4H), 1.25 – 1.20 (m, 1H), 1.19 – 1.16 (m, 1H), 1.13 (s, 3H), 0.86 (s, 3H), 0.84 (s, 3H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 205.8, 145.1, 109.3, 68.0, 54.1, 42.0, 40.0, 39.1, 36.8, 33.6, 33.5, 23.2, 22.0, 18.8, 16.1;

HRMS (ESI): *m/z* calcd for $C_{15}H_{25}O^+$ $[M+H]^+$ 221.1900, found 221.1897.

The analytical data matched those previously reported.[2]

Synthesis of compound 15



4*H*-pyran-4-one (S2) 14 15

Preparation of TMPZnCl•LiCl:[5] To a stirred solution of 2,2,6,6-tetramethylpiperidine (TMP, freshly distilled from CaH_2, 10.0 g, 7.08 mmol) in THF (68 mL) was added *n*-BuLi (2.5 M in hexane, 31.2 mL, 7.81 mmol) dropwise at –40 °C. After the addition, the reaction mixture is slowly warmed to –10 °C. After 1 h, $ZnCl_2$ (1.0 M in THF, 78.1 mL, 78.1 mmol) is added dropwise. The reaction mixture was slowed warmed to 25 °C and stirred for additional 1 h to give a THF solution of TMPZnCl•LiCl (0.4 M, theoretical), which was used in the next step quickly.

To a stirred solution of TMPZnCl•LiCl (0.4 M in THF, 78 mL, 31.2 mmol, 3 equiv) was added 4*H*-pyran-4-one (**S2**, 1.00 g, 10.4 mmol, 1 equiv) at 0 ºC. After 1 h, iodine (7.92 g, 31.2 mmol, 3 equiv) was added. The mixture was warmed to room temperature and stirred overnight before it was quenched with aq. $Na_2S_2O_3$ (4.0 M, 30 mL) and sat.

aq. NH_4Cl (30 mL). The resulting mixture was extracted with EtOAc (3 × 50 mL), and the combined organic phases were washed with brine (20 mL) and dried over anhydrous Na_2SO_4 filtered and concentrated. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (gradient from 5:1 to 2:1, v/v) as an eluent to afford **15** (1.39 g, 60% yield) as a yellow solid.

TLC: R_f = 0.5 (petroleum ether/EtOAc = 1:1);

m.p. = 137 – 139 ºC;

IR (film): ν_{max} = 3435, 3073, 3002, 1629, 1309, 1025, 943, 831, 749 cm^{-1};

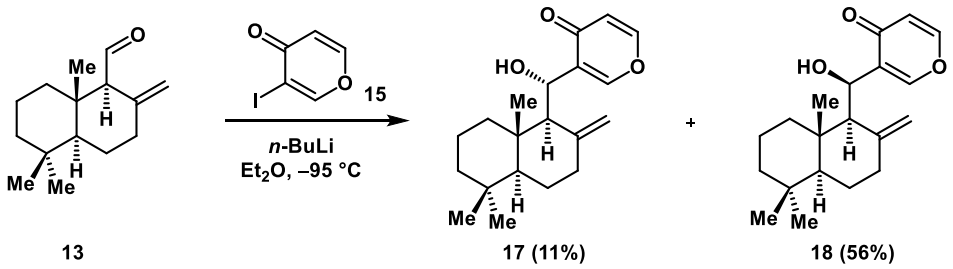
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, J = 1.0 Hz, 1H), 7.75 (dd, J = 1.0, 5.8 Hz, 1H), 6.33 (d, J = 5.7 Hz, 1H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 158.1, 155.4, 114.06, 93.7;

HRMS (ESI): *m/z* calcd for $C_5H_3INaO_2^+$ $[M+Na]^+$ 244.9070, found 244.9068.

The analytical data matched those previously reported.[5]

Synthesis of compounds 17 and 18



To a stirred solution of 3-iodo-4*H*-pyran-4-one (**15**, 753 mg, 3.39 mmol, 1.5 equiv) in Et_2O (3 mL) was added of *n*-BuLi (2.5 M solution in hexane 1.54 mL, 3.84 mmol, 1.7 equiv) dropwise at –95 ºC. After 1 h, a solution of **13** (500 mg, 2.26 mmol, 1 equiv) in Et_2O (3 mL) was added dropwise, and stirring was continued for another 1 h before it was quenched with sat. aq. NH_4Cl (5 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic phases were

washed with brine (5 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (gradient from 10:1 to 4:1, v/v) as an eluent to afford **17** (75 mg, 11% yield) as a colorless oil and **18** (407 mg, 56% yield) as a colorless oil.

Data for compound **17**:

TLC: R_f = 0.4 (petroleum ether/EtOAc = 2:1);

$[\alpha]_D^{22}$ = –68.6 (c = 1.0, $CHCl_3$);

IR (film): ν_{max} = 3387, 2925, 1644, 1596, 1436, 1322, 837, 734 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (dd, *J* = 1.0, 5.7 Hz, 1H), 7.67 (s, 1H), 6.33 (d, *J* = 5.8 Hz, 1H), 4.89 (d, *J* = 8.4 Hz, 1H), 4.78 (s, 1H), 4.32 (s, 1H), 2.53 (d, *J* = 8.4 Hz, 1H), 2.27 – 2.18 (m, 2H), 2.03 – 1.97 (m, 1H), 1.76 – 1.69 (m, 1H), 1.62 – 1.51 (m, 1H), 1.45 – 1.39 (m, 1H), 1.39 – 1.33 (m, 2H), 1.33 – 1.07 (m, 4H), 1.03 (s, 3H), 0.84 (s, 3H), 0.83 (s, 3H);

^{13}C NMR (126 MHz, $CDCl_3$) δ 178.9, 155.1, 153.4, 149.1, 132.19, 117.5, 109.8, 69.6, 58.3, 54.2, 42.2, 41.8, 37.06, 34.0, 33.8, 24.5, 22.1, 19.5, 15.9;

HRMS (ESI): *m/z* calcd for $C_{20}H_{28}NaO_3^+$ $[M+Na]^+$ 339.1931, found 339.1928.

Data for compound **18**:

TLC: R_f = 0.5 (petroleum ether/EtOAc = 2:1);

$[\alpha]_D^{22}$ = –5.4 (c = 0.343, $CHCl_3$);

IR (film): ν_{max} = 2925, 2852, 1651, 1461, 1264, 1081, 896, 704 cm^{-1};

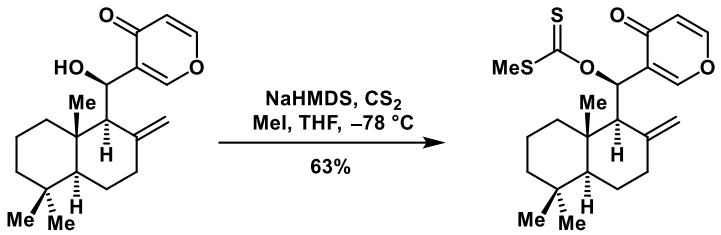
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.73 (d, *J* = 5.4 Hz, 1H), 5.41 – 5.34 (m, 1H), 5.09 (d, *J* = 2.0 Hz, 1H), 4.94 (d, *J* = 2.1 Hz, 1H), 2.36 – 2.29 (m, 2H), 2.16 (s, 1H), 2.05 – 1.97 (m, 1H), 1.93 (d, *J* = 12.5 Hz, 2H), 1.75 – 1.69 (m, 1H), 1.43 – 1.38 (m, 2H), 1.35 – 1.32 (m, 1H), 1.30 – 1.27 (m, 1H), 1.22 – 1.19 (m, 1H), 1.13 (s, 3H),

0.86 (d, J = 4.7 Hz, 6H);

^{13}C NMR (126 MHz, $CDCl_3$) δ 179.2, 155.2, 154.1, 145.6, 131.7, 117.2, 111.4, 66.3, 56.9, 55.9, 42.1, 40.5, 39.3, 39.0, 33.9, 29.8, 24.5, 21.9, 19.4, 17.0;

HRMS (ESI): *m/z* calcd for $C_{20}H_{28}NaO_3^+$ $[M+Na]^+$ 339.1931, found 339.1928.

Synthesis of compound 19



18 19

To a stirred solution of **18** (100 mg, 0.316 mmol, 1 equiv) in THF (2 mL) were added sodium bis(trimethylsilyl)amide (NaHMDS, 1.0 M in THF, 0.475 mL, 0.475 mmol, 1.5 equiv) and carbon disulfide (CS_2, 380 μL, 6.33 mmol, 20 equiv) sequentially at –78 ºC. After 1 h, iodomethane (390 μL, 6.33 mmol, 20 equiv) was added and stirring was continued for another 1 h. The reaction mixture was quenched with sat. aq. NH_4Cl (5 mL) and extracted with EtOA (3 × 5 mL). The combined organic phases were washed with brine (5 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (gradient from 15:1 to 8:1, v/v) as an eluent to afford **19** (80 mg, 63% yield) as a yellow solid.

TLC: R_f = 0.8 (petroleum ether/EtOAc = 2:1);

$[\alpha]_D^{22}$ = –175.6 (c = 0.475, $CHCl_3$);

m.p. = 100 – 102 ºC;

IR (film): ν_{max} = 3243, 2921, 2848, 1652, 1610, 1420, 1323, 1138, 838, 809 cm^{-1};

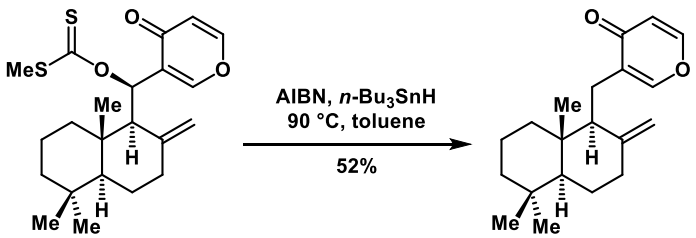
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (d, J = 1.0, 5.8 Hz, 1H), 7.55 (s, 1H), 7.05 (s, 1H),

6.31 (d, J = 5.7 Hz, 1H), 4.95 (s, 2H), 2.59 (s, 3H), 2.32 – 2.23 (m, 1H), 1.99 – 1.91 (m, 1H), 1.85 – 1.77 (m, 1H), 1.72 – 1.67 (m, 1H), 1.66 – 1.63 (m, 1H), 1.60 – 1.54 (m, 3H), 1.43 – 1.35 (m, 3H), 1.22 – 1.15 (m, 1H), 0.93 (s, 3H), 0.84 (s, 3H), 0.82 (s, 3H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 214.0, 176.7, 155.0, 153.5, 145.7, 128.3, 117.3, 110.2, 56.9, 55.6, 41.9, 40.5, 39.2, 39.0, 33. 8, 33.7, 24.7, 21.9, 19.5, 19.4, 16.5, 1.2;

HRMS (ESI): *m/z* calcd for $C_{22}H_{30}NaO_3S_2^+$ $[M+Na]^+$ 429.1529, found 429.1525.

Synthesis of ottensinin (2)



19 ottensinin (2)

To a stirred solution of **19** (80 mg, 0.192 mmol, 1 equiv) in toluene (2 mL) was added AIBN (6.3 mg, 38.3 μmol, 0.2 equiv) and tri-*n*-butyltin hydride (*n*-Bu_3SnH, 100 μL, 0.383 mmol, 2 equiv). The reaction mixture was degassed by freeze-pump-thaw for three cycles before it was heated to 90 ºC and stirred for 4 h. After being cooled to room temperature, the mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (gradient from 10:1 to 4:1, v/v) as an eluent to afford ottensinin (**2**, 30 mg, 52% yield) as a colorless oil, which solidified upon standing.

TLC: R_f = 0.5 (petroleum ether/EtOAc = 1:1);

$[\alpha]_D^{20}$ = +8.2 (c = 0.61, $CHCl_3$);

m.p. = 118 – 120 ºC;

IR (film): ν_{max} = 2925, 2849, 1695, 1649, 1617, 1388, 1325, 1259, 1215, 836 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, J = 5.7 Hz, 1H), 7.52 (s, 1H), 6.31 (d, J = 5.8

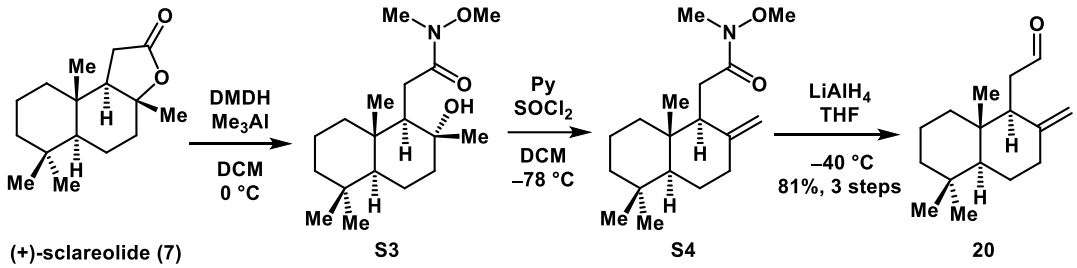
Hz, 1H), 4.81 (s, 1H), 4.46 (s, 1H), 2.69 (d, J = 16.2 Hz, 1H), 2.44 (dd, J = 16.2, 11.3 Hz, 1H), 2.40 – 2.35 (m, 1H), 2.02 (d, J = 11.0 Hz, 1H), 1.97 (dd, J = 12.9, 5.3 Hz, 1H), 1.89 – 1.85 (m, 1H), 1.77 – 1.73 (m, 1H), 1.62 – 1.56 (m, 1H), 1.54 – 1.50 (m, 1H), 1.42 – 1.39 (m, 1H), 1.36 (dd, J = 12.9, 4.3 Hz, 1H), 1.24 – 1.21 (m, 1H), 1.20 – 1.18 (m, 1H), 1.18 – 1.14 (m, 1H), 0.88 (s, 3H), 0.82 (s, 3H), 0.78 (s, 3H);

^{13}C NMR (126 MHz, $CDCl_3$) δ 178.8, 154.7, 153.1, 147.9, 130.0, 116.5, 107.9, 55.7, 54.1, 42.2, 40.1, 39.2, 38.2, 33.8 (×2), 24.5, 21.9, 19.7, 19.5, 14.5;

HRMS (ESI): *m/z* calcd for $C_{20}H_{28}NaO_2^+$ $[M+Na]^+$ 323.1982, found 323.1980.

The analytical data matched those previously reported.[6]

Synthesis of compound 20



Aldehyde **20** was prepared following the reported procedure.[7]

To a stirred solution of *N,O*-dimethylhydroxylamine hydrochloride (DMDH, 3.9 g, 40.0 mmol, 2 equiv) in DCM (40 mL) was added Me_3Al (2.0 M in hexane, 22 mL, 44.0 mmol, 2.2 equiv) dropwise at 0 ºC. After 2 h, a solution of (+)-sclareolide (**7**, 5.00 g, 20.0 mmol, 1 equiv) in DCM (40 mL) was added and stirring was continued for another 2 h. The mixture was quenched with 2 M H_2SO_4 (20 mL) and extracted with DCM (3 × 200 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated to give crude **S3** (R_f = 0.3, petroleum ether/EtOAc = 1:1), which was azeotropically dried with PhMe (2 × 20 mL) and used in the next step without further purification.

To a stirred solution of above crude **S3** in DCM (40 mL) was added pyridine (16.1

mL, 200 mmol, 10 equiv) and thionyl chloride (7.24 mL, 100 mmol, 5 equiv) sequentially at –78 ºC. After 2 h, the mixture was quenched with sat. aq. $NaHCO_3$ (40 mL) and extracted with DCM (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (gradient from 10:1 to 5:1, v/v) as an eluent to afford crude **S4** (R_f = 0.5, petroleum ether/EtOAc = 5:1), which was azeotropically dried with PhMe (2 × 20 mL) and used in the next step without further purification.

To a stirred solution of the crude **S4** in THF (30 mL) was added lithium aluminum hydride ($LiAlH_4$, 760 mg, 20.0 mmol, 1 equiv) at –40 ºC portionwise over 10 min. After 2 h, the reaction mixture was quenched with sat. aq. potassium sodium tartrate (100 mL) carefully, stirred vigorously at 25 ºC for 3 h, and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (gradient from 100:1 to 50:1, v/v) as an eluent to afford aldehyde **20** (3.79 g, 81% yield over 3 steps) as a yellow oil.

TLC: R_f = 0.8 (petroleum ether/EtOAc = 5:1);

$[\alpha]_D^{23}$ = –27.0 (c = 1.0, $CHCl_3$);

IR (film): ν_{max} = 3344, 2924, 2867, 2846, 1708, 1644, 1459, 969 cm^{-1};

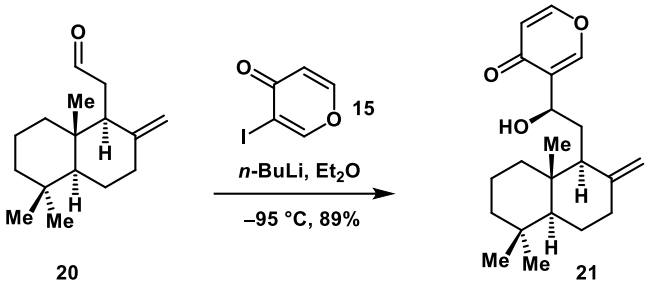
^{1}H NMR (500 MHz, $CDCl_3$) δ 9.62 (s, 1H), 4.80 (s, 1H), 4.38 (s, 1H), 2.50 – 2.41 (m, 2H), 2.41 – 2.37 (m, 1H), 2.36 – 2.33 (m, 1H), 2.11 – 2.04 (m, 1H), 1.77 – 1.73 (m, 1H), 1.59 – 1.39 (m, 4H), 1.39 – 1.29 (m, 1H), 1.20 (dd, J = 2.8, 12.7 Hz, 2H), 1.12 – 1.03 (m, 1H), 0.89 (s, 3H), 0.81 (s, 3H), 0.70 (s, 3H);

^{13}C NMR (126 MHz, $CDCl_3$) δ 203.6, 148.6, 108.2, 55.4, 51.1, 42.1, 39.9, 39.5, 39.0, 37.6, 33.7, 33.63, 24.0, 21.8, 19.4, 14.7;

HRMS (ESI): *m/z* calcd for $C_{16}H_{26}NaO^+$ $[M+Na]^+$ 257.1876, found 257.1871.

The analytical data matched those previously reported.[7]

Synthesis of compound 21



To a stirred solution of 3-iodo-4*H*-pyran-4-one (**15**, 142 mg, 0.64 mmol, 1.5 equiv) in Et_2O (3 mL) was added of *n*-BuLi (2.5 M in hexane, 0.29 mL, 0.726 mmol, 1.7 equiv) dropwise at –95 ºC. After 1 h, a solution of **20** (100 mg, 0.427 mmol, 1 equiv) in Et_2O (3 mL) was added dropwise, and stirring was continued for another 1 h. The resulting mixture was quenched with sat. aq. NH_4Cl (2 mL) and extracted with EtOAc (3 × 5 mL). The combined organic phases were washed with brine (2 mL) and dried over anhydrous Na_2SO_4 filtered and concentrated. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (gradient from 10:1 to 4:1, v/v) as an eluent to afford **21** (126 mg, 89% yield) as a white solid.

TLC: R_f = 0.5 (petroleum ether/EtOAc = 2:1);

$[\alpha]_D^{22}$ = +32.9 (*c* = 1.0, $CHCl_3$);

m.p. = 153 – 154 ºC;

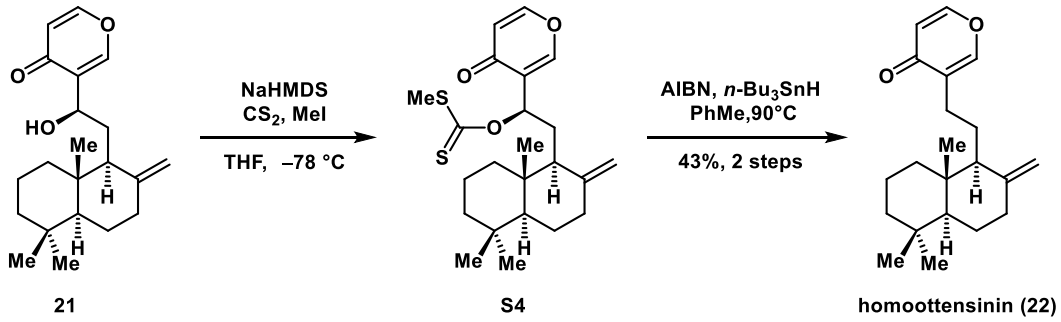
IR (film): ν_{max} = 2924, 2866, 2845, 1647, 1600, 1436, 1142, 837 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.74 (d, *J* = 5.8 Hz, 1H), 6.35 (d, *J* = 5.7 Hz, 1H), 4.87 (d, *J* = 1.6 Hz, 1H), 4.64 (s, 1H), 4.53 (dd, *J* = 2.3, 10.2 Hz, 1H), 2.44 – 2.37 (m, 1H), 2.11 (d, *J* = 11.2 Hz, 1H), 2.09 – 2.00 (m, 1H), 1.92 (dd, *J* = 10.2, 14.2 Hz, 1H), 1.77 – 1.71 (m, 3H), 1.56 – 1.46 (m, 2H), 1.41 – 1.30 (m, 2H), 1.24 – 1.16 (m, 2H), 1.13 – 1.06 (m, 1H), 0.87 (s, 3H), 0.79 (s, 3H), 0.66 (s, 3H);

^{13}C NMR (126 MHz, $CDCl_3$) δ 179.1, 155.5, 151.9, 148.9, 132.5, 117.5, 106.9, 67.8, 55.6, 52.3, 42.2, 39.5, 39.1, 38.4, 33.7, 30.8, 24.5, 21.1, 19.4, 14.8;

HRMS (ESI): *m/z* calcd for $C_{21}H_{30}NaO_3^+$ $[M+Na]^+$ 353.2087, found 353.2086.

Synthesis of compound 22



To a stirred solution of **21** (200 mg, 0.606 mmol, 1 equiv) in THF (3 mL) was added sodium bis(trimethylsilyl)amide (NaHMDS, 1.0 M in THF, 0.60 mL, 1.21 mmol, 2 equiv) and carbon disulfide (CS_2, 727 μL, 12.1 mmol, 20 equiv) sequentially at –78 ºC. After 1 h, iodomethane (755 μL, 12.1 mmol, 20 equiv) was added, and stirring was continued for another 1 h. The reaction mixture was quenched with sat. aq. NH_4Cl (2 mL) and extracted with EtOA (3 × 5 mL), and the combined organic phases were washed with brine (5 mL) and dried over anhydrous Na_2SO_4, filtered and concentrated to afford crude **S4**, which was directly used in the next step.

To a stirred solution of crude **S4** in toluene (2 mL) was added AIBN (19.8 mg, 0.121 mmol, 0.2 equiv) and tri-*n*-butyltin hydride (*n*-Bu_3SnH, 325 μL, 1.21 mmol, 2 equiv). The reaction mixture was degassed via three freeze-pump-thaw cycles under argon and then heated to 90 ºC. After 4 h, the reaction mixture was cooled to 25 ºC and concentrated under reduced pressure. The residue was purified by silica gel column chromatography with petroleum ether/EtOAc (gradient from 10:1 to 4:1, v/v) as an eluent to afford homoottensinin (**22**, 82 mg, 63% yield) as a colorless oil, which solidified upon standing.

TLC: R_f =0.8 (petroleum ether/EtOAc = 2:1);

[α]$_D^{23}$ = +27.1 (*c* = 1.0, $CHCl_3$);

m.p. = 115 – 116 ºC;

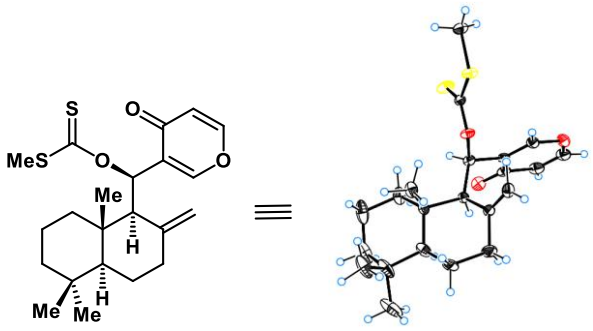
IR (film): ν_{max} = 3358, 2960, 2919, 2848, 1647, 1437, 1260, 796 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (d, *J* = 5.8 Hz, 1H), 7.59 (s, 1H), 6.30 (d, *J* = 5.8 Hz, 1H), 4.85 (d, *J* = 2.0 Hz, 1H), 4.64 (d, *J* = 2.0 Hz, 1H), 2.59 – 2.48 (m, 1H), 2.40 – 2.36 (m, 1H), 2.13 – 2.01 (m, 1H), 1.98 – 1.92 (m, 1H), 1.74 – 1.67 (m, 3H), 1.60 (d, *J* = 11.7 Hz, 1H), 1.55 – 1.41 (m, 2H), 1.37 – 1.24 (m, 2H), 1.15 (dd, *J* = 4.2, 13.5 Hz, 1H), 1.05 (dd, *J* = 2.8, 12.6 Hz, 1H), 0.99 – 0.88 (m, 2H), 0.84 (s, 3H), 0.77 (s, 3H), 0.64 (s, 3H);

^{13}C NMR (126 MHz, $CDCl_3$) δ 178.6, 155.1, 151.9, 148.2, 131.1, 116.8, 106.8, 56.6, 55.6, 42.2, 39.7, 39.1, 38.4, 33.7, 33.7, 25.1, 24.5, 22.0, 21.8, 19.4, 14.6;

HRMS (ESI): *m/z* calcd for $C_{21}H_{30}NaO_2^+$ $[M+Na]^+$ 337.2138, found 337.214.

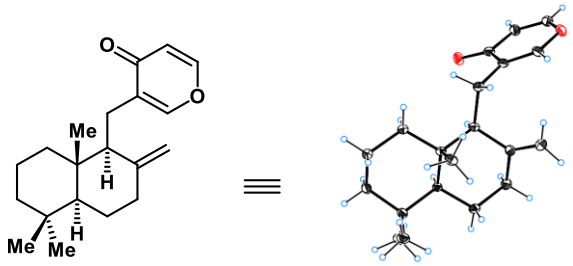
3. X-ray Crystallographic Data



19 CCDC 2464862

Crystal data and structure refinement for **19**

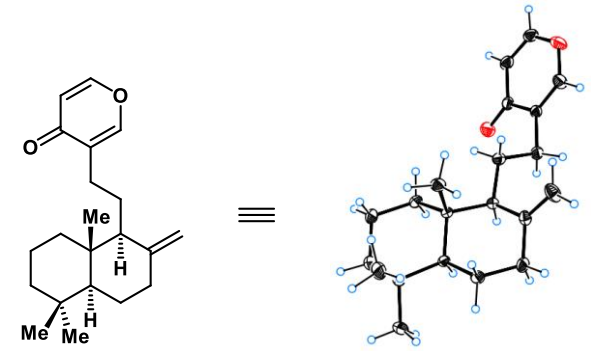
Empirical formula	$C_{22}H_{30}O_3S_2$
Formula weight	406.58
Temperature/K	169.99(10)
Crystal system	monoclinic
Space group	$P2_1$
a/Å	7.0253(2)
b/Å	7.5941(2)
c/Å	20.2221(7)
α/°	90
β/°	95.857(3)
γ/°	90
Volume/Å³	1073.23(6)
Z	2
ρ_{calc}g/cm³	1.258
μ/mm⁻¹	2.395
F(000)	436.0
Crystal size/mm³	0.16 × 0.12 × 0.11
Radiation	Cu Kα (λ = 1.54184)
2^{Θ} range for data collection/°	4.392 to 147.146
Index ranges	$-7 \leq h \leq 8$, $-9 \leq k \leq 9$, $-25 \leq l \leq 21$
Reflections collected	6701
Independent reflections	3975 [R_{int} = 0.0470, R_{sigma} = 0.0482]
Data/restraints/parameters	3975/1/256
Goodness-of-fit on F^2	1.068
Final R indexes [I>=2σ (I)]	R_1 = 0.0688, wR_2 = 0.1780
Final R indexes [all data]	R_1 = 0.0697, wR_2 = 0.1796
Largest diff. peak/hole / e Å⁻³	0.48/-0.64
Flack parameter	0.01(3)



ottensinin (2) ≡ CCDC 2464863

Crystal data and structure refinement for ottensinin (**2**)

Empirical formula	$C_{20}H_{28}O_2$
Formula weight	300.42
Temperature/K	293(2)
Crystal system	triclinic
Space group	P1
a/Å	6.3288(3)
b/Å	7.2917(4)
c/Å	20.5591(8)
α/°	91.255(4)
β/°	97.617(4)
γ/°	114.449(5)
Volume/Å^3	852.96(8)
Z	2
ρ_{calc}g/cm^3	1.170
μ/mm^{-1}	0.568
F(000)	328.0
Crystal size/mm^3	0.14 × 0.12 × 0.1
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	4.352 to 147.304
Index ranges	-7 ≤ h ≤ 7, -9 ≤ k ≤ 9, -25 ≤ l ≤ 24
Reflections collected	11232
Independent reflections	5791 [R_{int} = 0.0509, R_{sigma} = 0.0469]
Data/restraints/parameters	5791/3/403
Goodness-of-fit on F^2	1.054
Final R indexes [I>=2σ (I)]	R_1 = 0.0683, wR_2 = 0.1972
Final R indexes [all data]	R_1 = 0.0737, wR_2 = 0.2063
Largest diff. peak/hole / e Å^{-3}	0.58/-0.32
Flack parameter	-0.3(3)



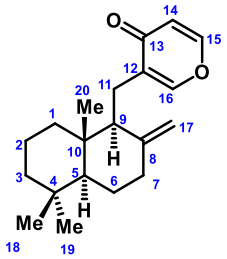
homoottensinin (22) CCDC 2464864

Crystal data and structure refinement for **22**

Empirical formula	$C_{21}H_{30}O_2$
Formula weight	314.45
Temperature/K	169.99(10)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	7.53830(10)
b/Å	10.13360(10)
c/Å	24.0107(4)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	1834.18(4)
Z	4
ρ_{calc}g/cm^3	1.139
μ/mm^{-1}	0.549
F(000)	688.0
Crystal size/mm^3	0.16 × 0.12 × 0.1
Radiation	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	7.364 to 148.132
Index ranges	$-6 \le h \le 9$, $-12 \le k \le 12$, $-29 \le l \le 28$
Reflections collected	11201
Independent reflections	3671 [R_{int} = 0.0338, R_{sigma} = 0.0310]
Data/restraints/parameters	3671/0/219
Goodness-of-fit on F^2	1.027
Final R indexes [I>=2σ (I)]	R_1 = 0.0437, wR_2 = 0.1157
Final R indexes [all data]	R_1 = 0.0467, wR_2 = 0.1184
Largest diff. peak/hole / e Å^{-3}	0.19/-0.24
Flack parameter	0.09(12)

4. Comparison of NMR Data of Ottensinin (2)

Table S1. Comparison of ^{1}H NMR data of natural and synthetic ottensinin (**2**) in $CDCl_3$



ottensinin (2)

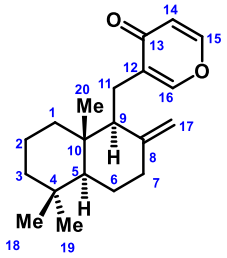
No.	Natural[8] (Kikuzaki)[a] (500 MHz)	Synthetic (us)[b] (500 M)	Δδ (*Nat-Syn*)
1	1.88, m	1.89 – 1.85, m	–
	1.18, br ddd (13, 13, 4)	1.20 – 1.18, m	–
2	1.60, ddddd (13×3, 3×2)	1.62 – 1.56, m	–
	1.53, m	1.54 – 1.50, m	–
3	1.41, dddd (13, 3×2, 2)	1.42 – 1.39, m	–
	1.20, br ddd (13×2, 4)	1.24 – 1.21, m	–
5	1.17, dd (13, 3)	1.18 – 1.14, m	–
6	1.36, dddd (13×3, 4)	1.36, dd (12.9, 4.3)	0
	1.76, dddd (13, 5, 3×2)	1.77 – 1.73, m	–
7	2.38, ddd, (13, 4, 2)	2.40 – 2.35, m	–
	1.99, br ddd (13×2, 5)	1.97, dd (12.9, 5.3)	+0.02
9	2.01, brd (12)	2.02, d (11.0)	–0.01
11	2.69, ddd (16, 2, 1)	2.69, d (16.2)	0
	2.45, ddd (16, 12, 1)	2.44, dd (16.2, 11.3)	+0.01
14	6.31, d (5.5)	6.31, d (5.8)	0
15	7.67, dd (6, 1)	7.66, d (5.7)	+0.01
16	7.52, ddd (1×3)	7.52 (s, 1H)	0
17	4.82, br d (1)	4.81 (s, 1H)	+0.01

	4.46, br d (1)	4.46 (s, 1H)	0
18	0.88, s	0.88 (s, 3H)	0
19	0.82, s	0.82 (s, 3H)	0
20	0.78, s	0.78 (s, 3H)	0

[a] Chemical shifts referenced to TMS peak.

[b] Chemical shifts referenced to residual undeuterated $CDCl_3$ at 7.26 ppm.

Table S2. Comparison of ^{13}C NMR data of natural and synthetic ottensinin (1) in $CDCl_3$



ottensinin (2)

No.	Natural[8] (Kikuzaki)[a] (125 MHz)	Synthetic (us)[b] (126 M)	Δδ (*Nat-Syn*)
1	39.0	39.2	–0.2
2	19.4	19.5	–0.1
3	42.0	42.2	–0.2
4	33.6	33.8	–0.2
5	55.5	55.7	–0.2
6	24.4	24.5	–0.1
7	38.1	38.2	–0.1
8	147.7	147.9	–0.2
9	53.9	54.1	–0.2
10	40.0	40.1	–0.1
11	19.5	19.7	–0.2
12	129.8	130.0	–0.2
13	178.8	178.8	0
14	116.4	116.5	–0.1
15	154.6	154.7	–0.1

16	153.0	153.1	–0.1
17	107.8	107.9	–0.1
18	33.6	33.8	–0.2
19	21.7	21.9	–0.2
20	14.4	14.5	–0.1

[a] Chemical shifts referenced to TMS peak.

[b] Chemical shifts referenced to $CDCl_3$ at 77.16 ppm.

5. Biological Study of Anti-Cancer Activity of Ottensinin and Its Analouges Against SMMC-7721 Celll Line

The biological bioassay was carried out following the reported procedure.[9,10] The Cell line used in this study is SMMC-7721 cells. This cell line was purchased from Cell Bank of Shanghai Institute of Biochemistry & Cell Biology, Chinese Academy of Sciences. Cells were cultured in 90% DMEM (GIBCO, Invitrogen Corporation, NY) supplemented with 10% fetal bovine serum (Sigma, USA), 100 U/ml benzyl penicillin and 100 U/ml streptomycin in a humidified environment with 5% CO_2 at 37 ℃.

The cytotoxicity of synthesized compounds against human SMMC-7721 cells was evaluated by MTT assays using 5-fluorouracil (5-FU) as positive controls. Initially, SMMC-7721 cells were distributed evenly across 96-well plates, ensuring a consistent density of 5×10^4 cells. Subsequently, the plates were incubated overnight in an incubator. Following this, the cells were exposed to various concentrations (120, 60, 30, 15, 7.5 μg/mL, n = 3) of compounds **2, 17, 18, 19, 21, 22** and 5-fluorouracil during a pre-treatment phase. Meanwhile, there was a blank control group (only culture medium added, without cell inoculation). The treated cells were then incubated for 48 hours in an incubator with a controlled atmosphere of 5% CO_2 at a temperature of 37 ℃. Next, 50 μL of MTT reagent (2.0 mg/mL), prepared in serum-free medium, was added carefully to each well under dark conditions, allowing the cells to incubate for an additional 2 hour. Finally, the absorbance of the contents in each well was measured at a wavelength of 450 nm using a precise microplate specifically, the SpectraMax 190 model manufactured by Molecular Devices in the United States. The operation procedure was followed by a colorimetric assay using MTT.

The calculation formula for cell viability is as follows:

Viability (%) = (OD value of drug-treated group − OD value of blank control group)/(OD value of untreated control group − OD value of blank control group) × 100%.

The calculation formula for cell growth inhibition rate is as follows:

Cell inhibition rate (%) = 1 – (absorbance of drug group/absorbance of control group) × 100%.

IC_{50} was taken as the concentration that caused 50% inhibition of cell viabilities and calculated by the Logit method.

The IC_{50} values of the tested compounds are presented in **Table S3**. The cell inhibition for the tested compounds is shown in **Fig S1**.

Table S3. IC_{50} values of compounds **2, 17, 18, 19, 21, 22** and 5-fluorouracil**.**

Compounds	IC_{50}±SD [μg/mL]
ottensinin (**2**)	28.69±3.24
17	26.69±2.27
18	18.68±1.57
19	28.15±3.12
21	33.42±1.71
22	21.22±1.68
5-fluorouracil	13.59±2.18

The statistical analysis was performed using GraphPad Prism 9.5.1 software. All data were presented as mean ± standard deviation (IC_{50} ± SD), and all experiments were repeated three times.

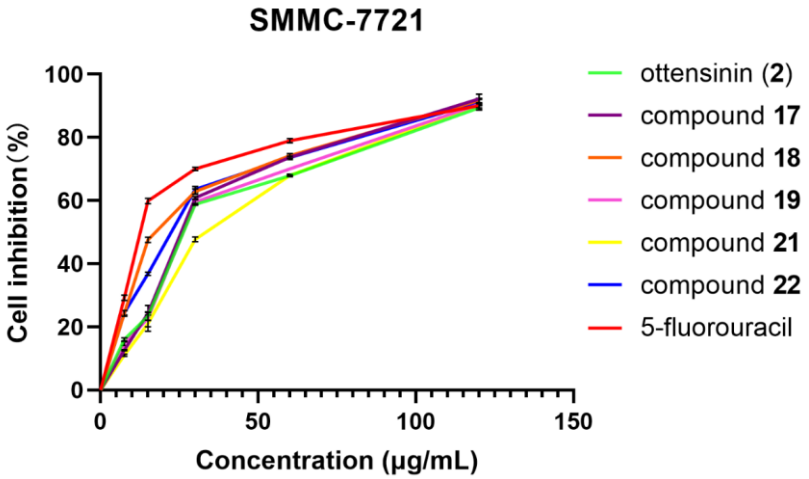


Fig. S1 Cell inhibition of compounds **2**, **17**, **18**, **19**, **21**, **22** and 5-fluorouracil.

6. Summary of Ottensinin (2) Syntheses

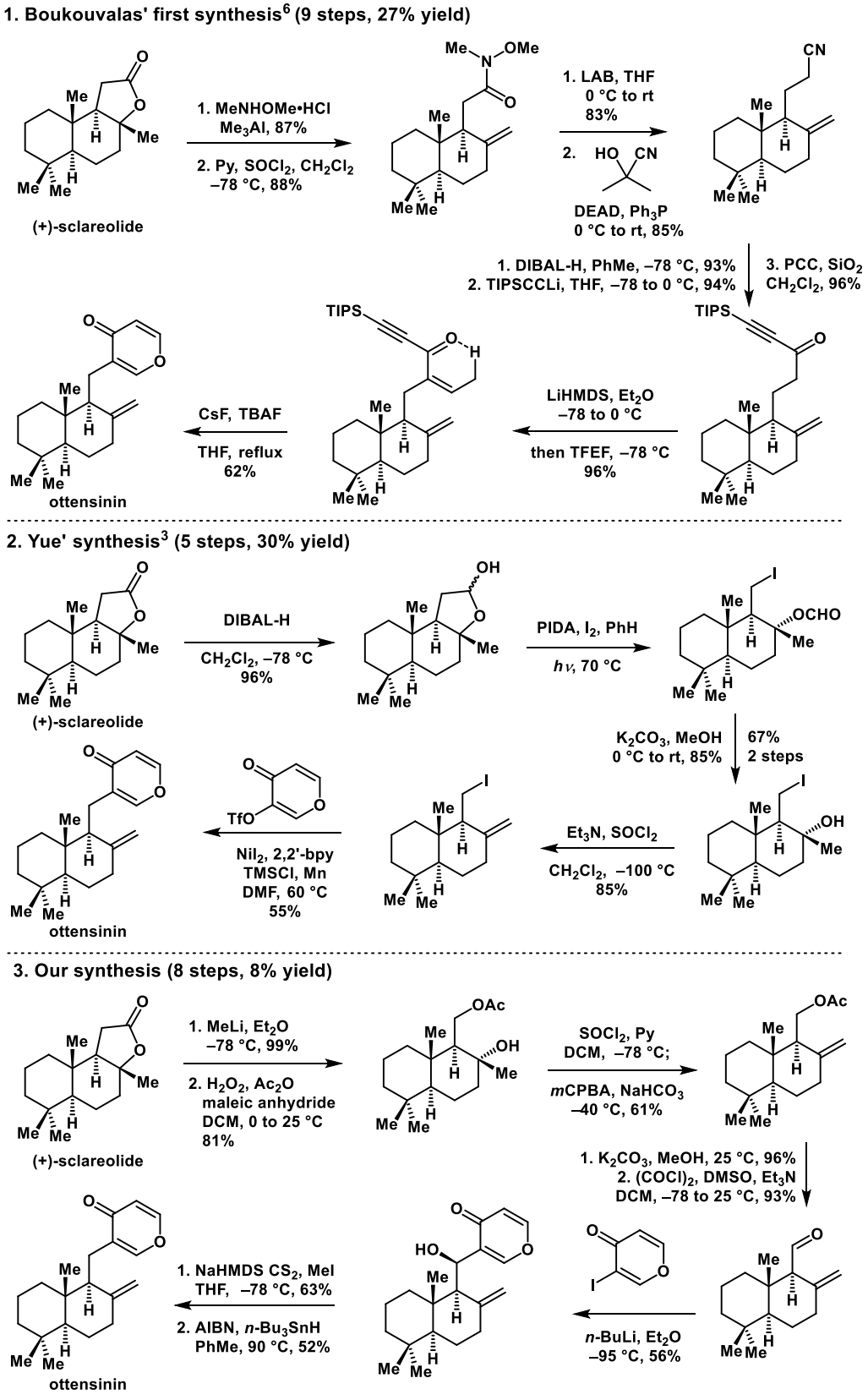
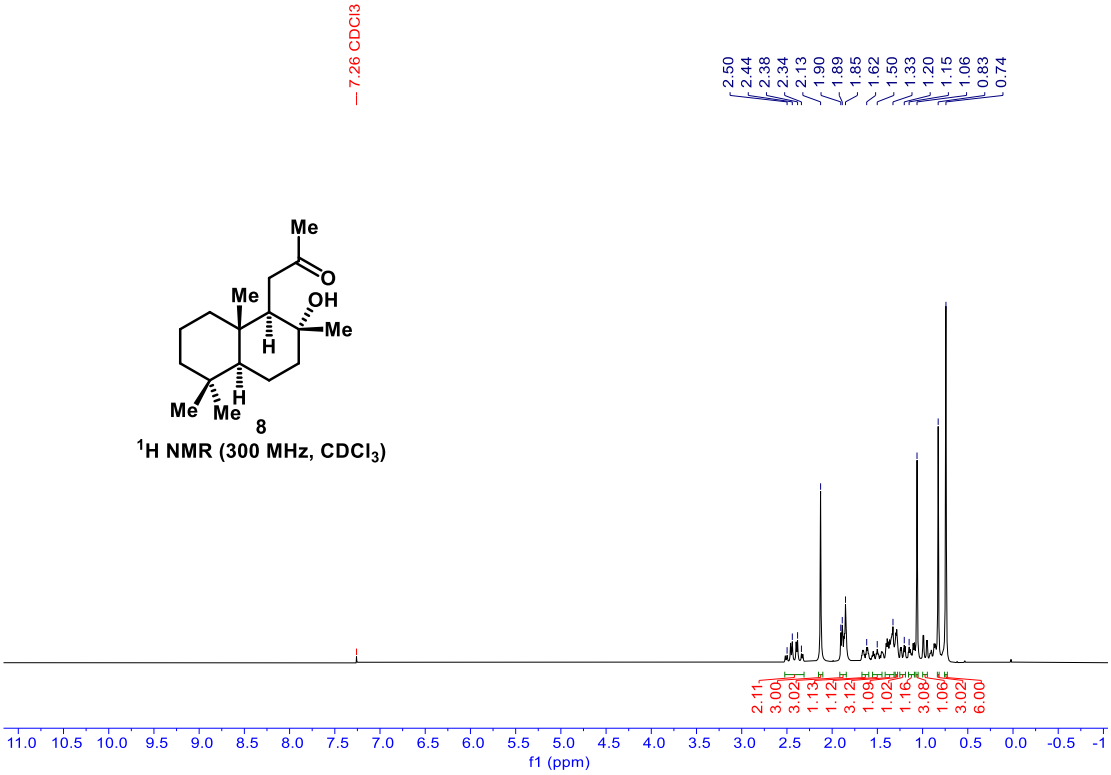


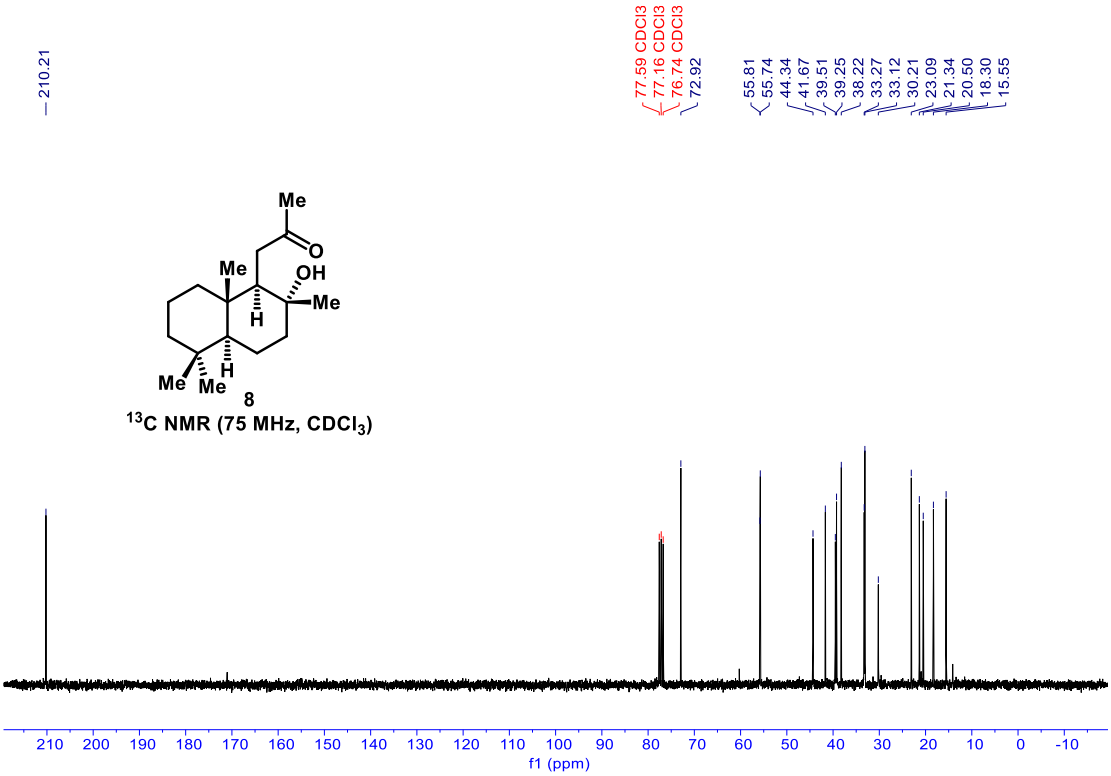
Fig. S2 Summary of ottensinin (**2**) syntheses.

7. NMR Spectra



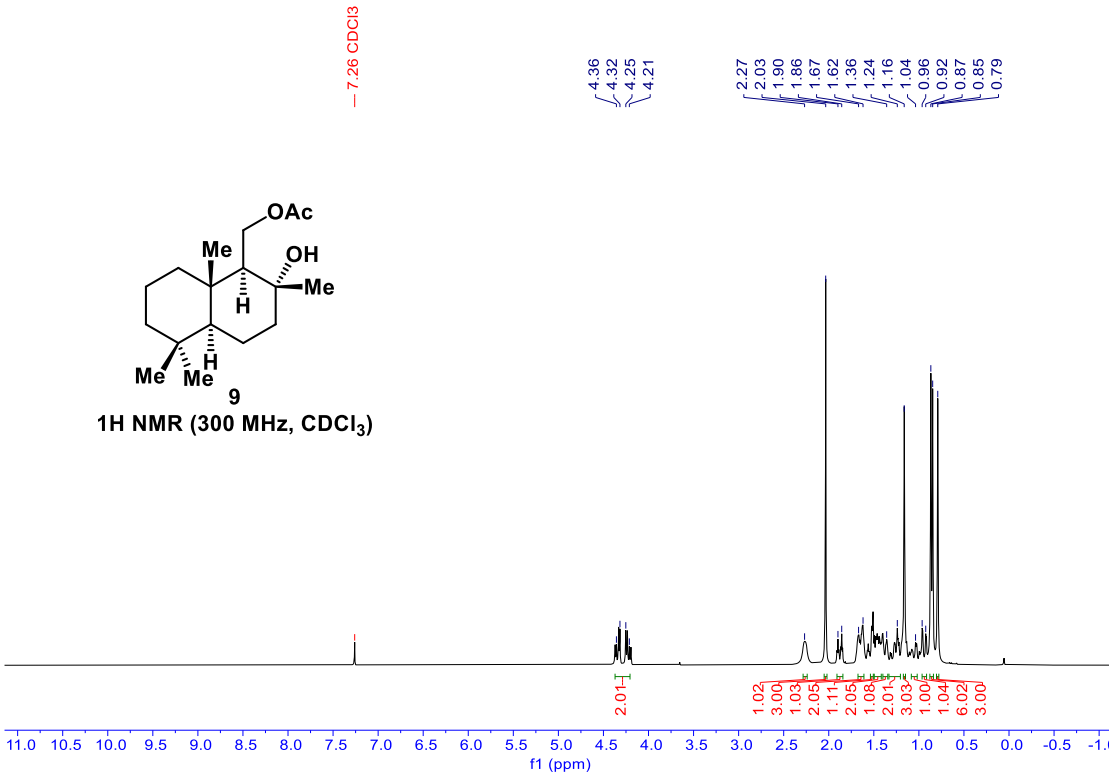
8

^{1}H NMR (300 MHz, $CDCl_3$)



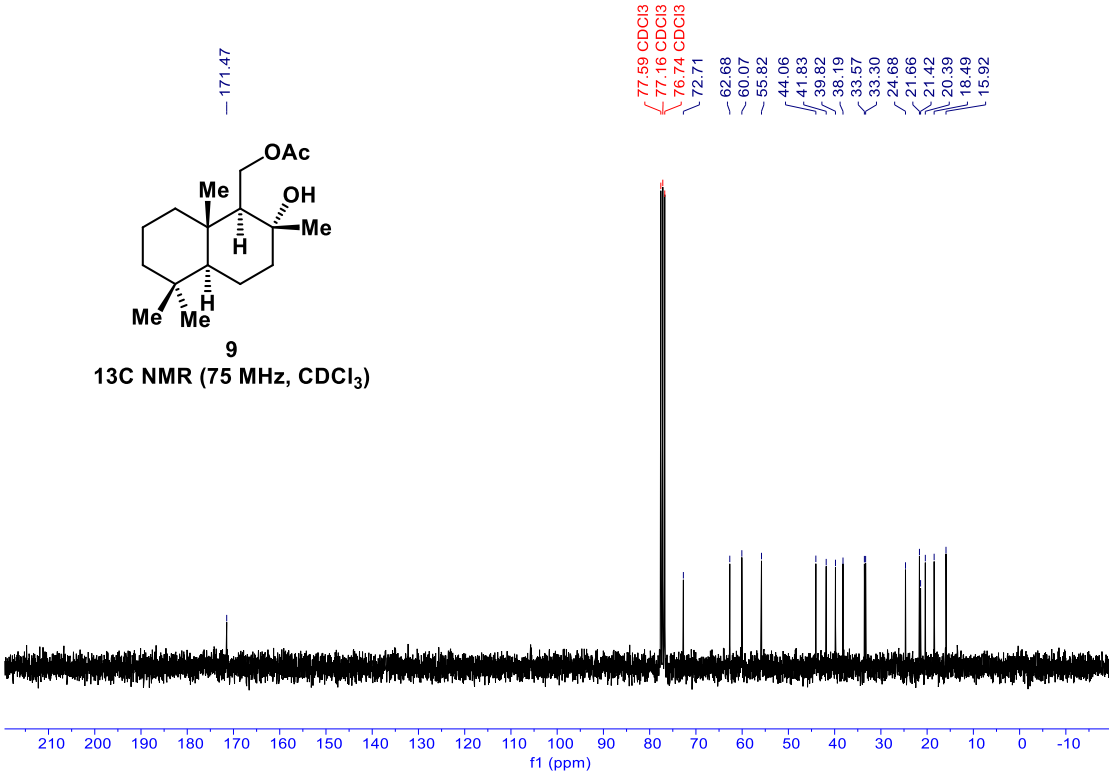
8

^{13}C NMR (75 MHz, $CDCl_3$)



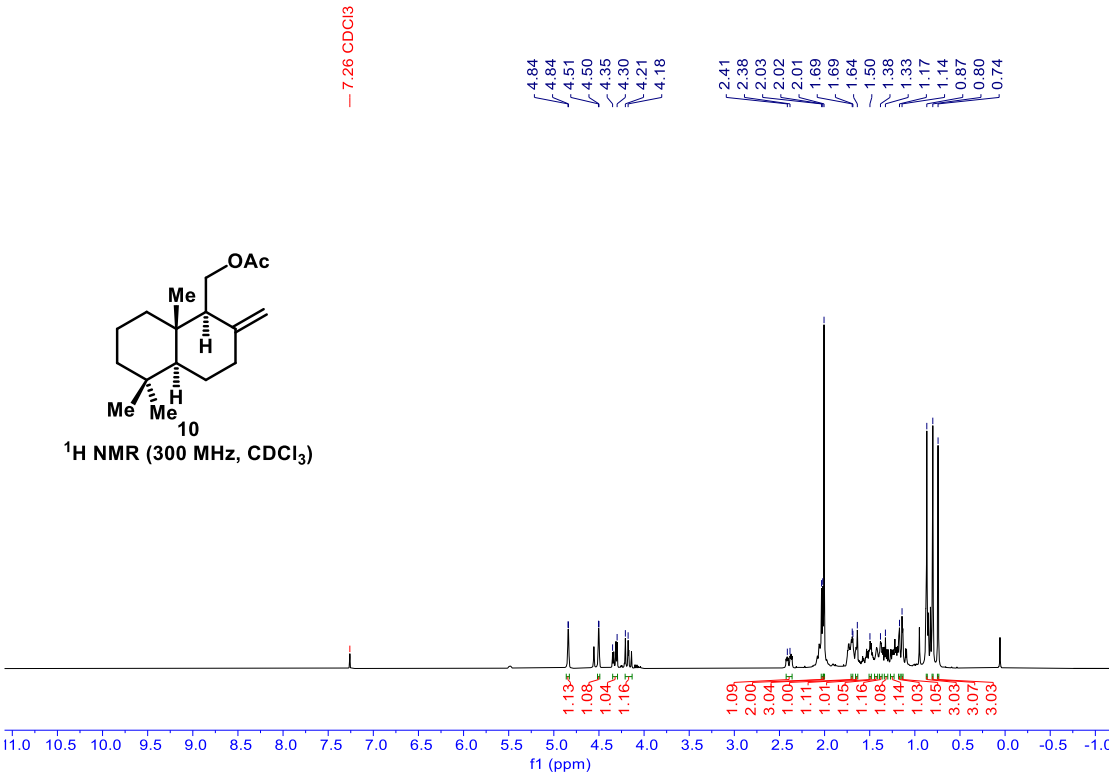
9

1H NMR (300 MHz, $CDCl_3$)

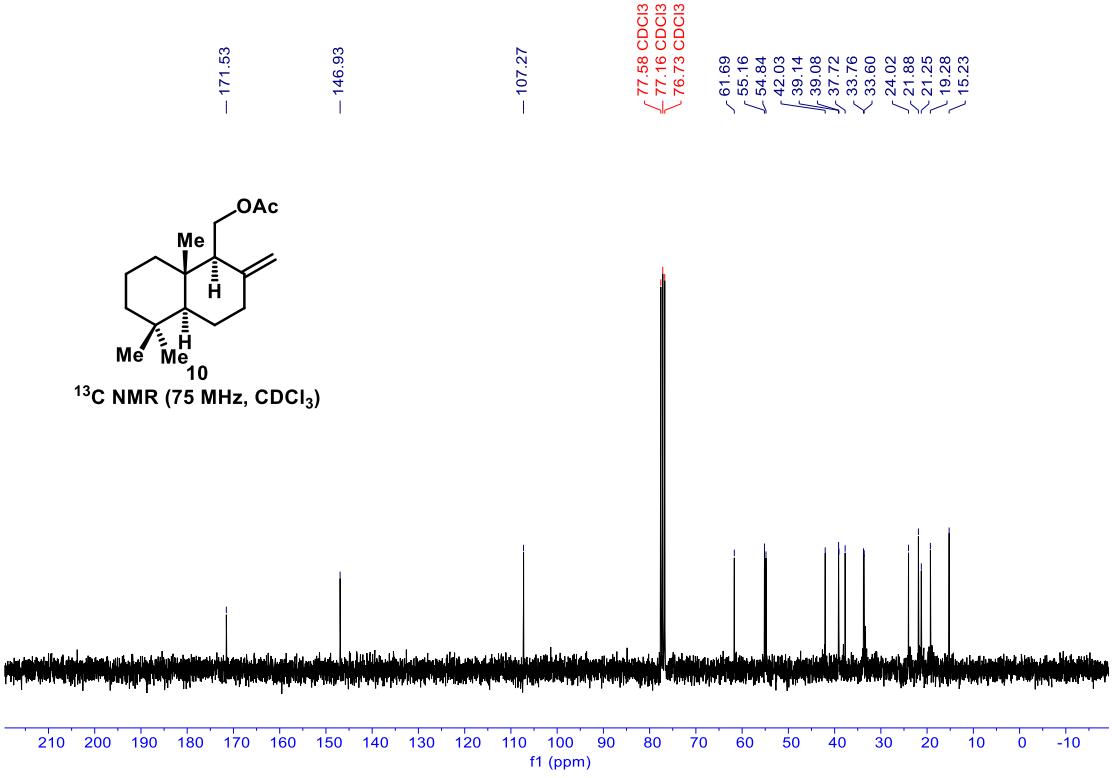


9

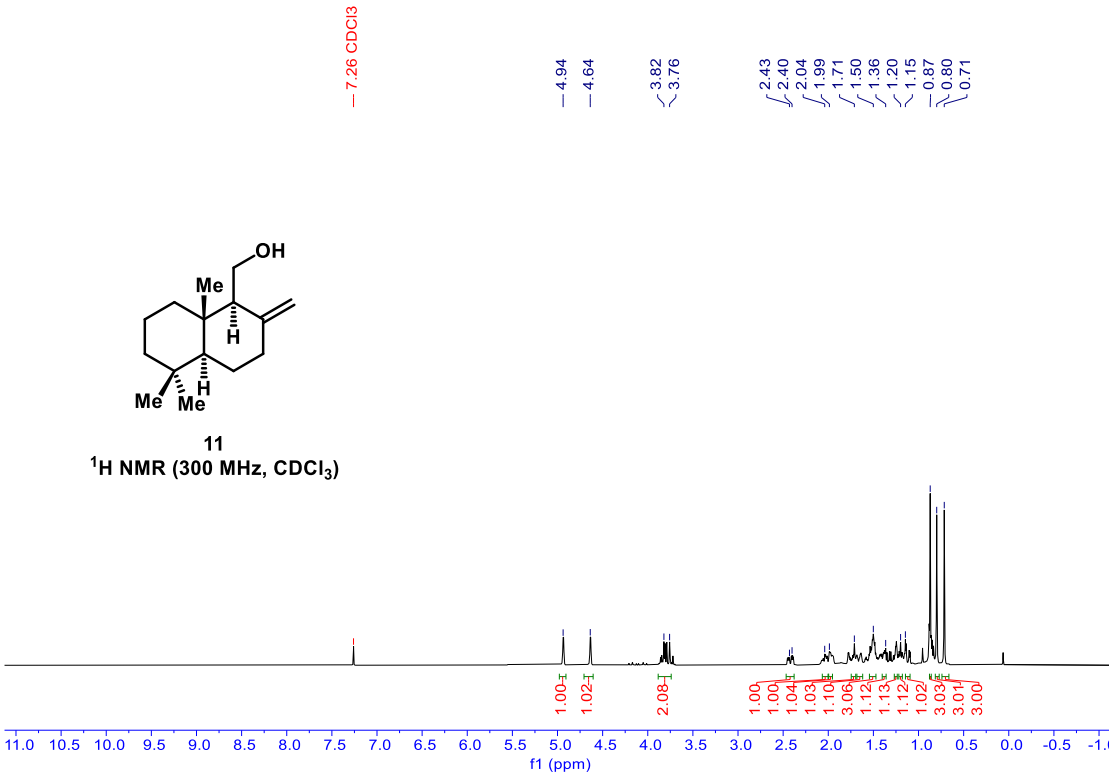
13C NMR (75 MHz, $CDCl_3$)



^{1}H NMR (300 MHz, $CDCl_3$)

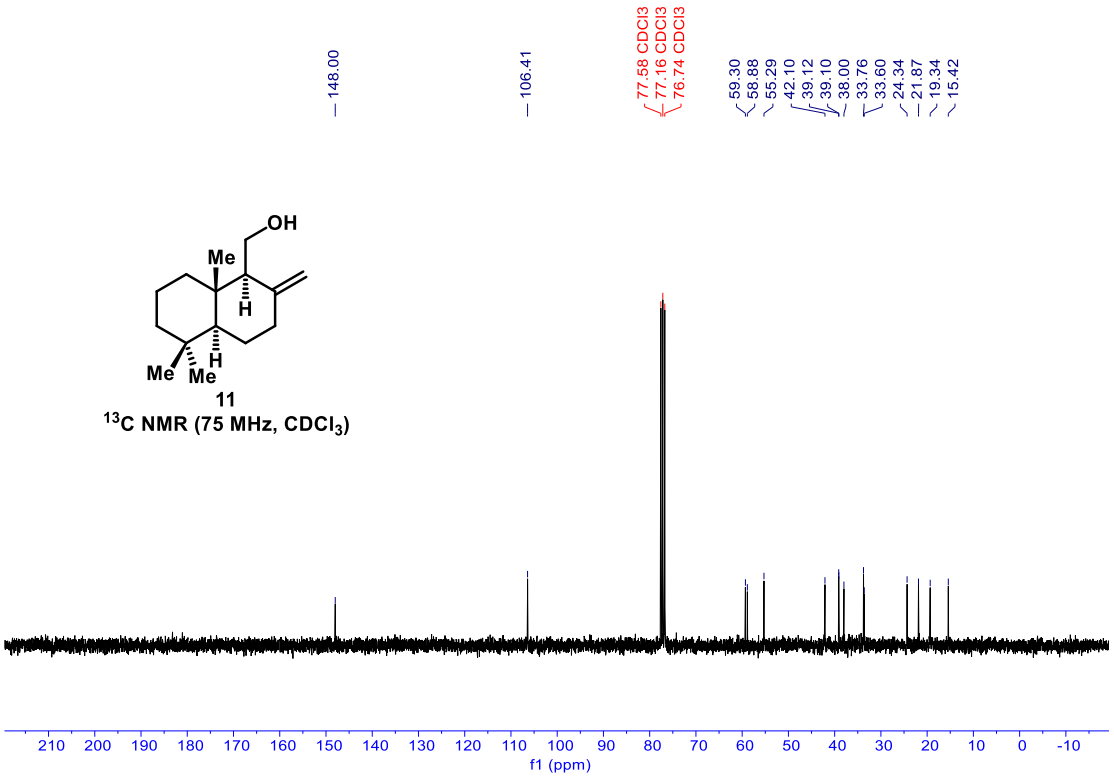


^{13}C NMR (75 MHz, $CDCl_3$)



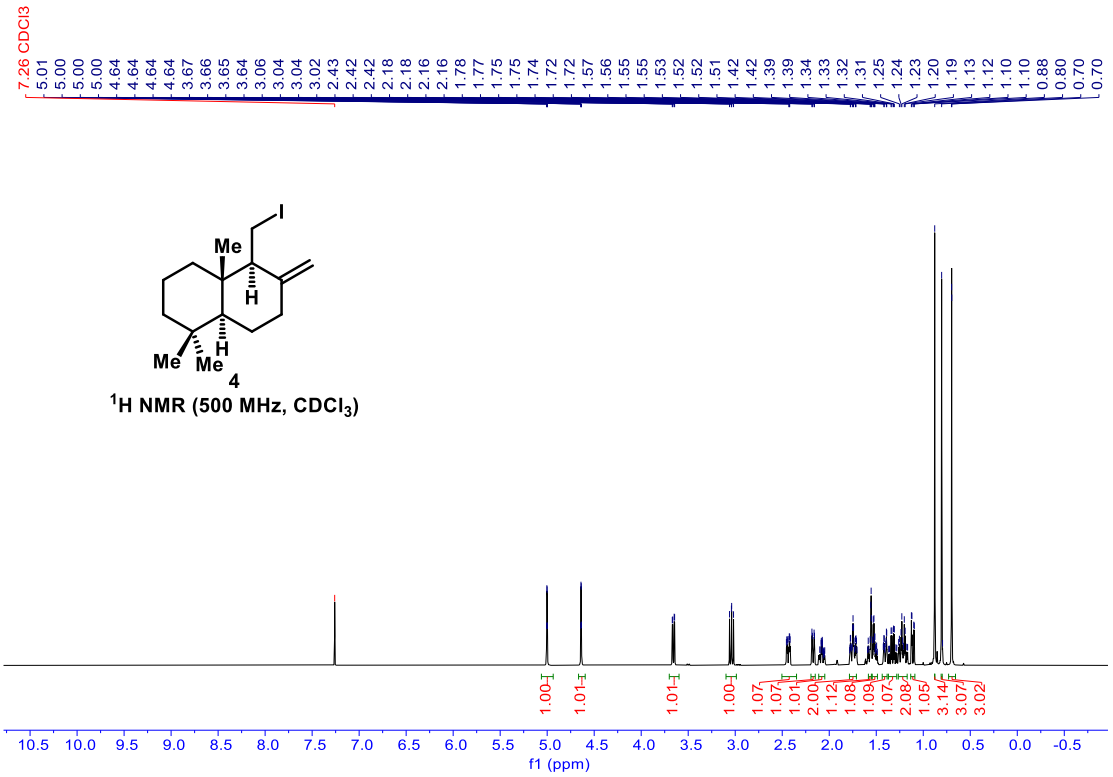
11

^{1}H NMR (300 MHz, $CDCl_3$)

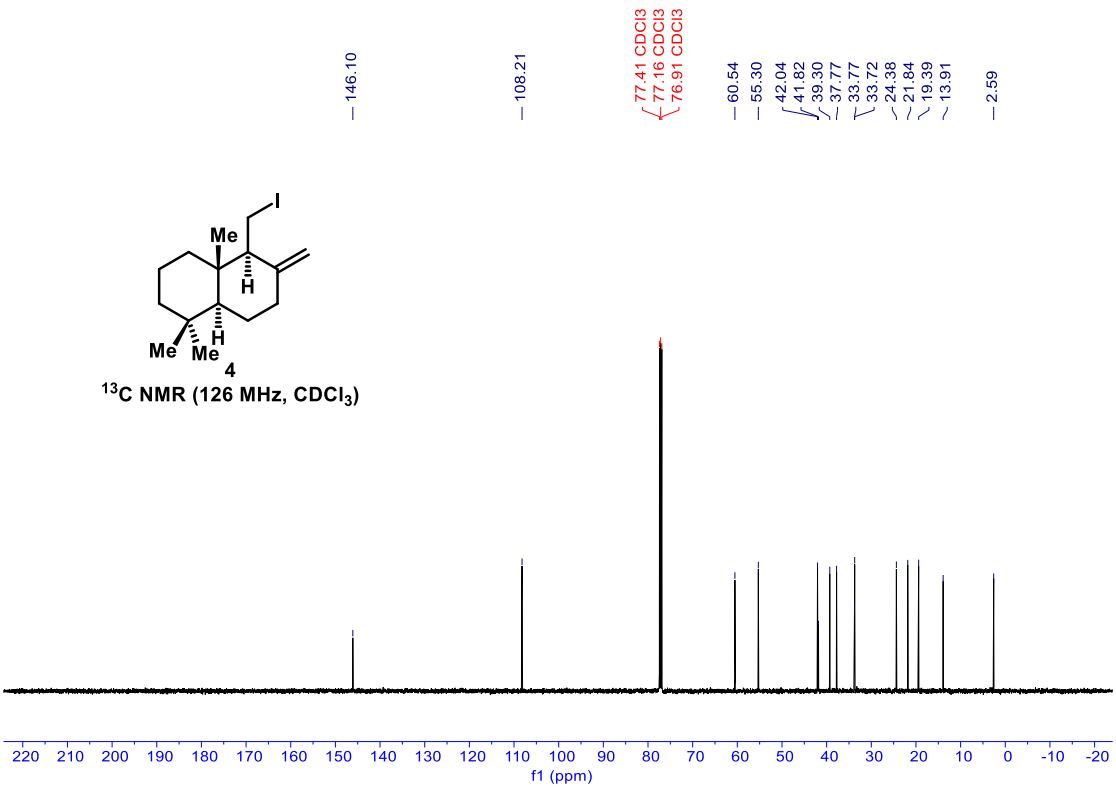


11

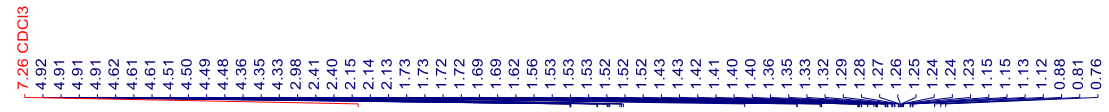
^{13}C NMR (75 MHz, $CDCl_3$)

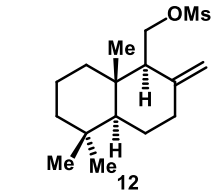


^{1}H NMR (500 MHz, $CDCl_3$)



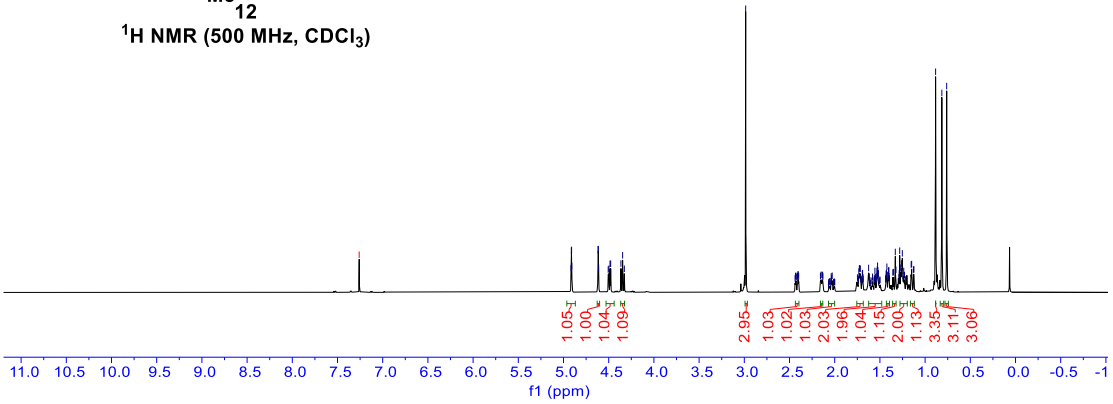
^{13}C NMR (126 MHz, $CDCl_3$)

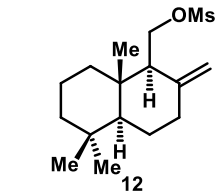




12

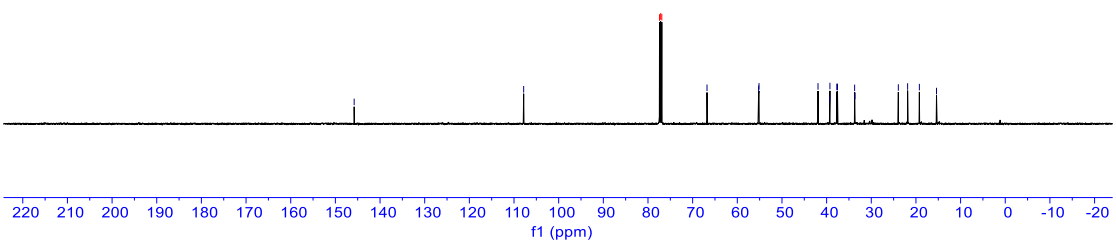
^{1}H NMR (500 MHz, $CDCl_3$)

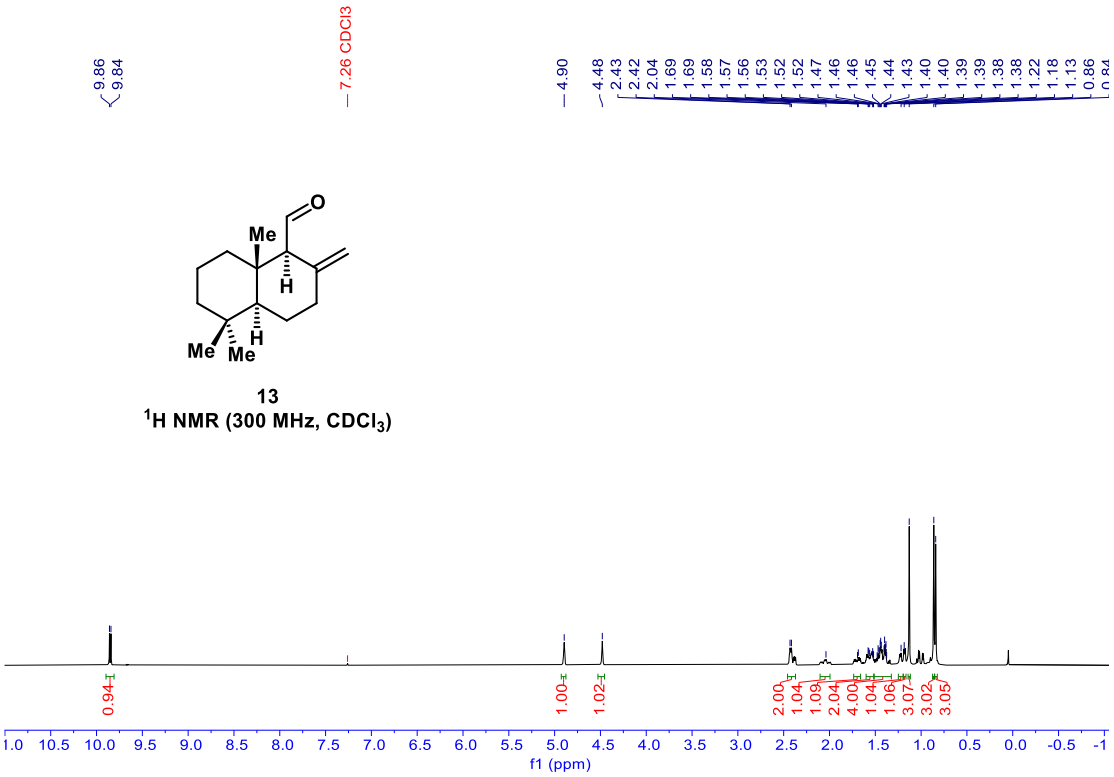




12

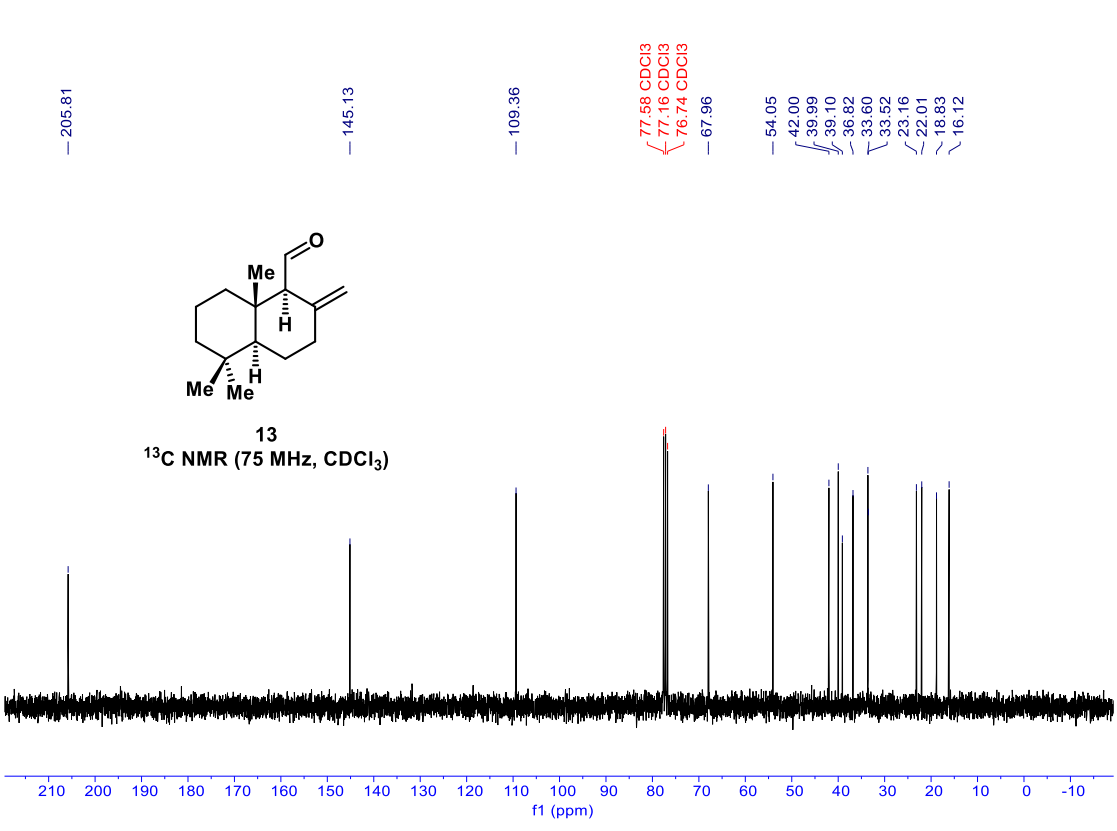
^{13}C NMR (126 MHz, $CDCl_3$)





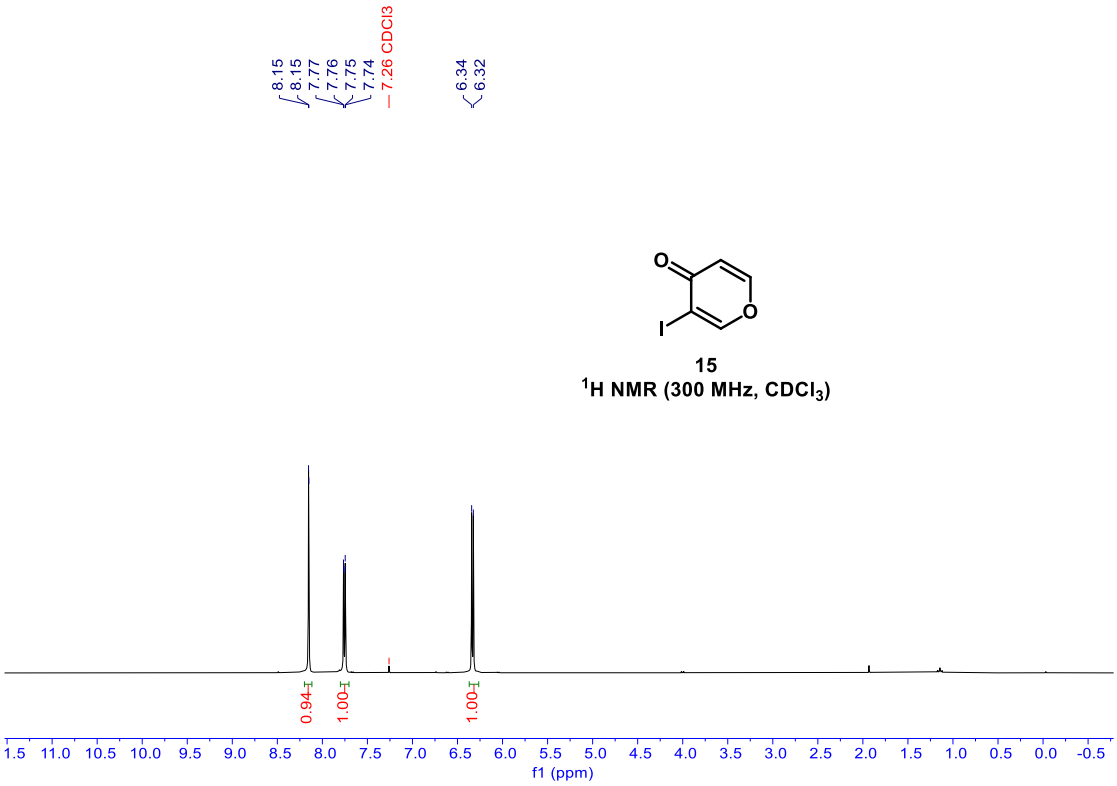
13

^{1}H NMR (300 MHz, $CDCl_3$)

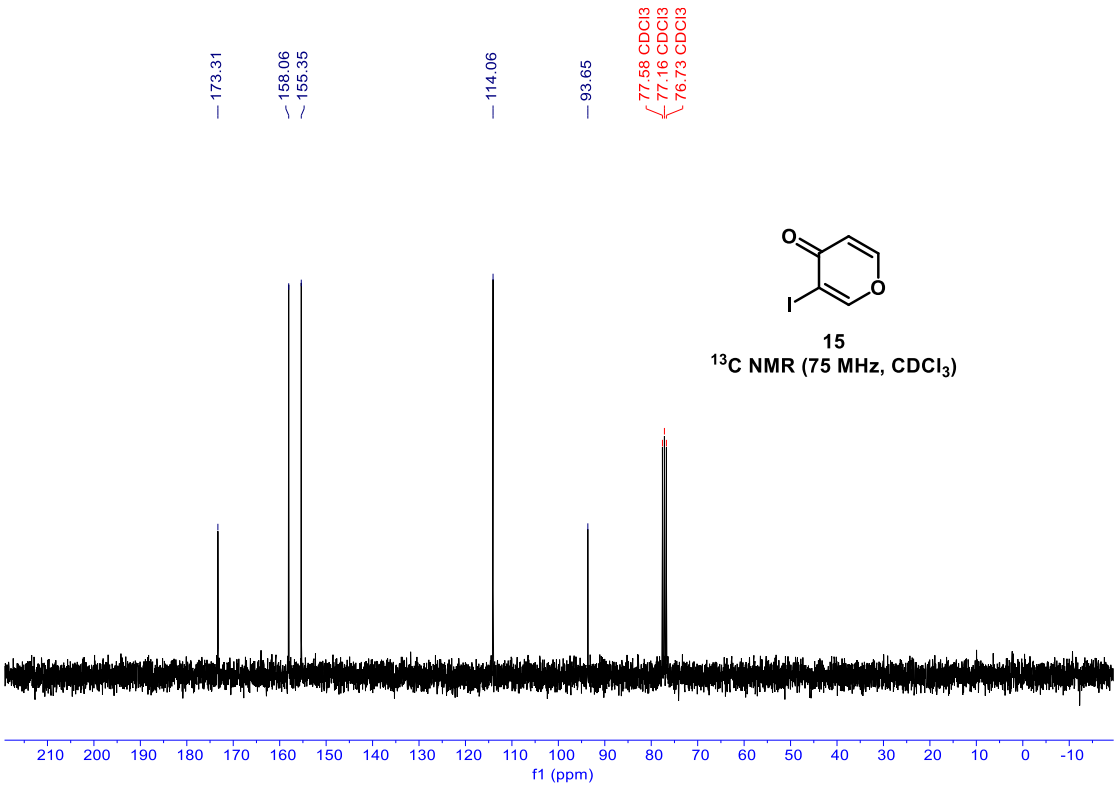


13

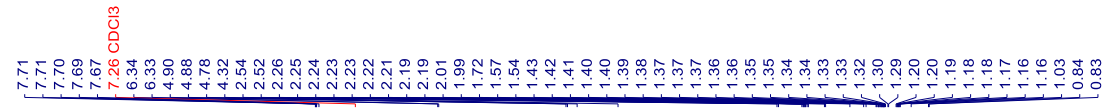
^{13}C NMR (75 MHz, $CDCl_3$)

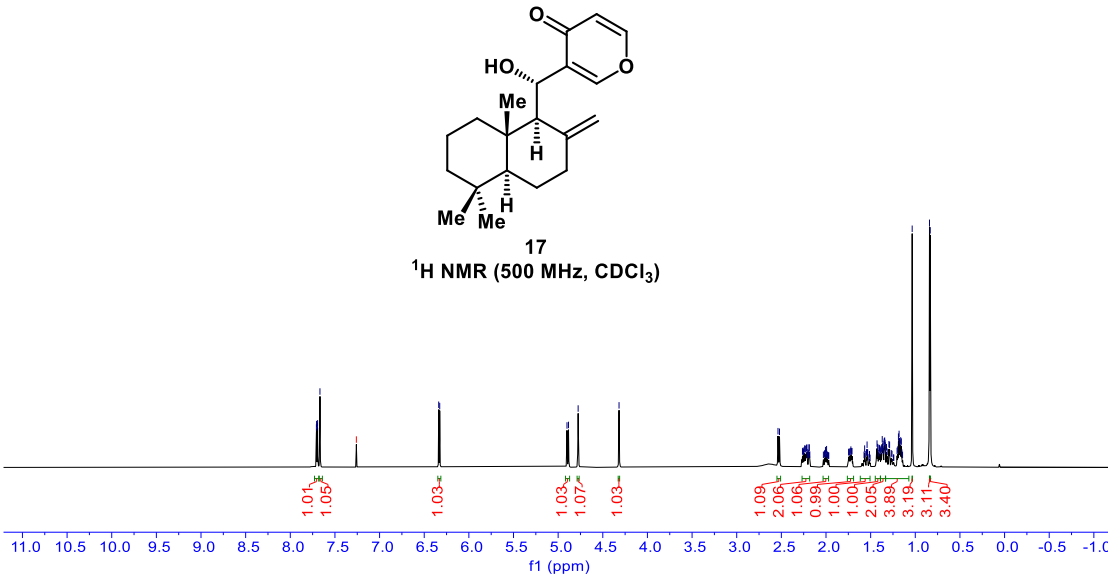


15
^{1}H NMR (300 MHz, $CDCl_3$)



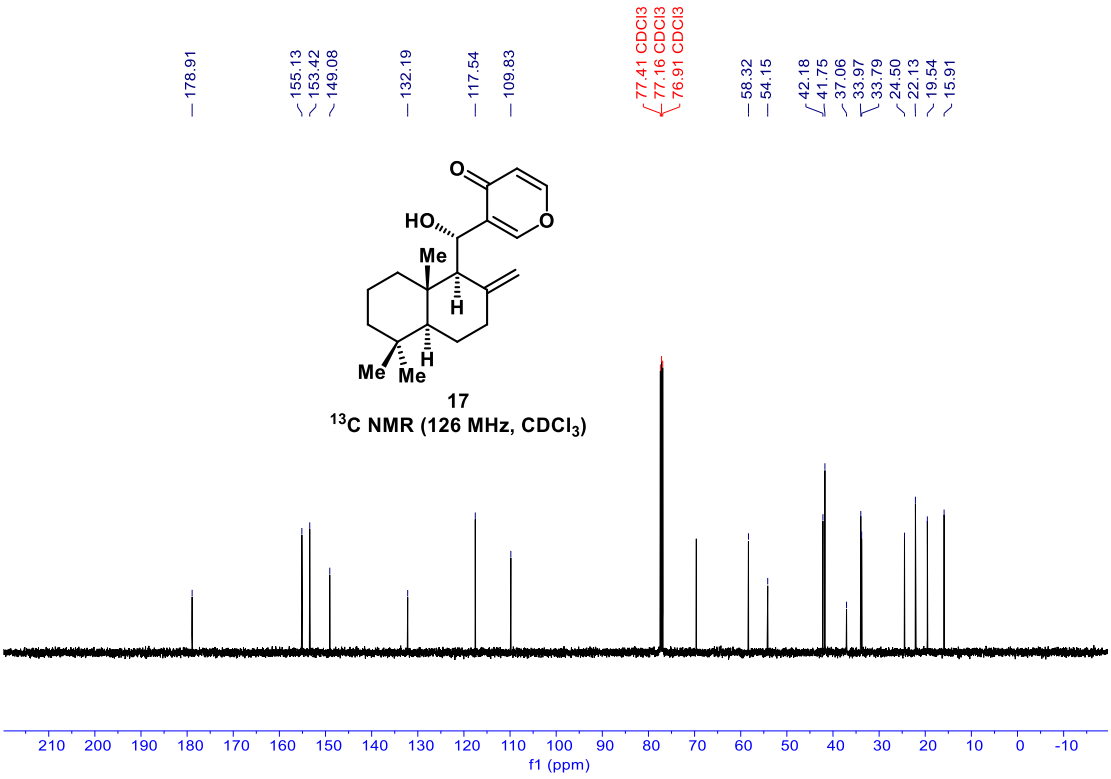
15
^{13}C NMR (75 MHz, $CDCl_3$)





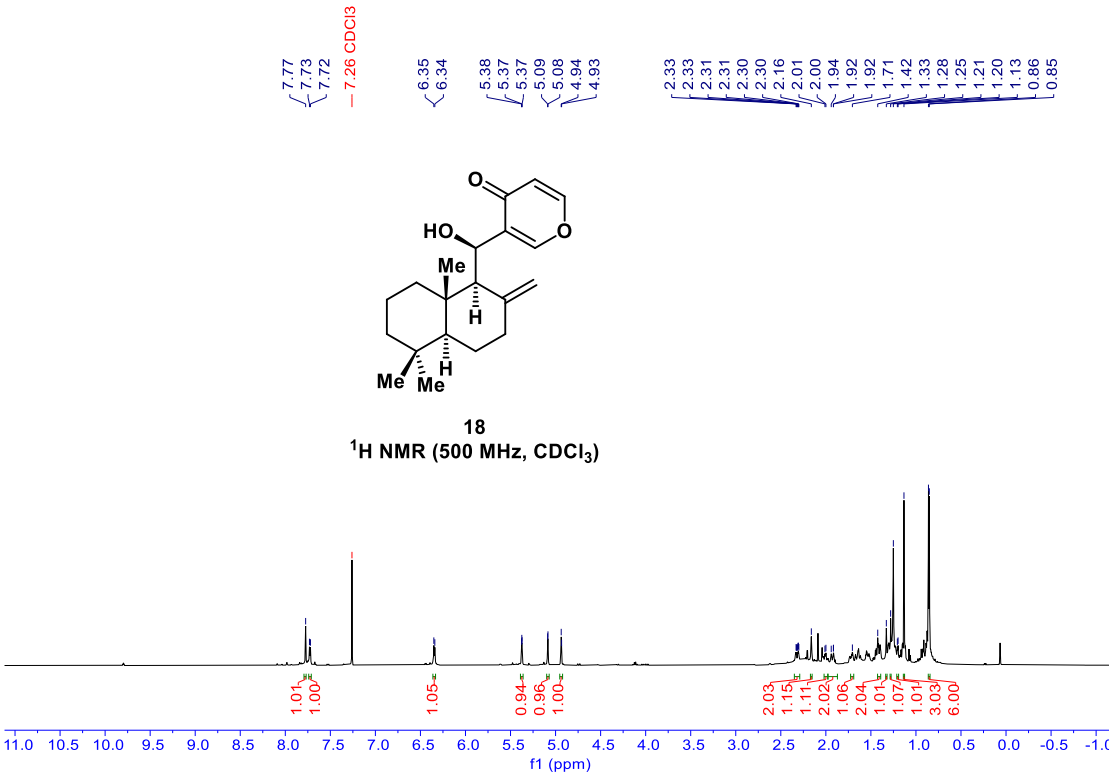
17

^{1}H NMR (500 MHz, $CDCl_3$)



17

^{13}C NMR (126 MHz, $CDCl_3$)



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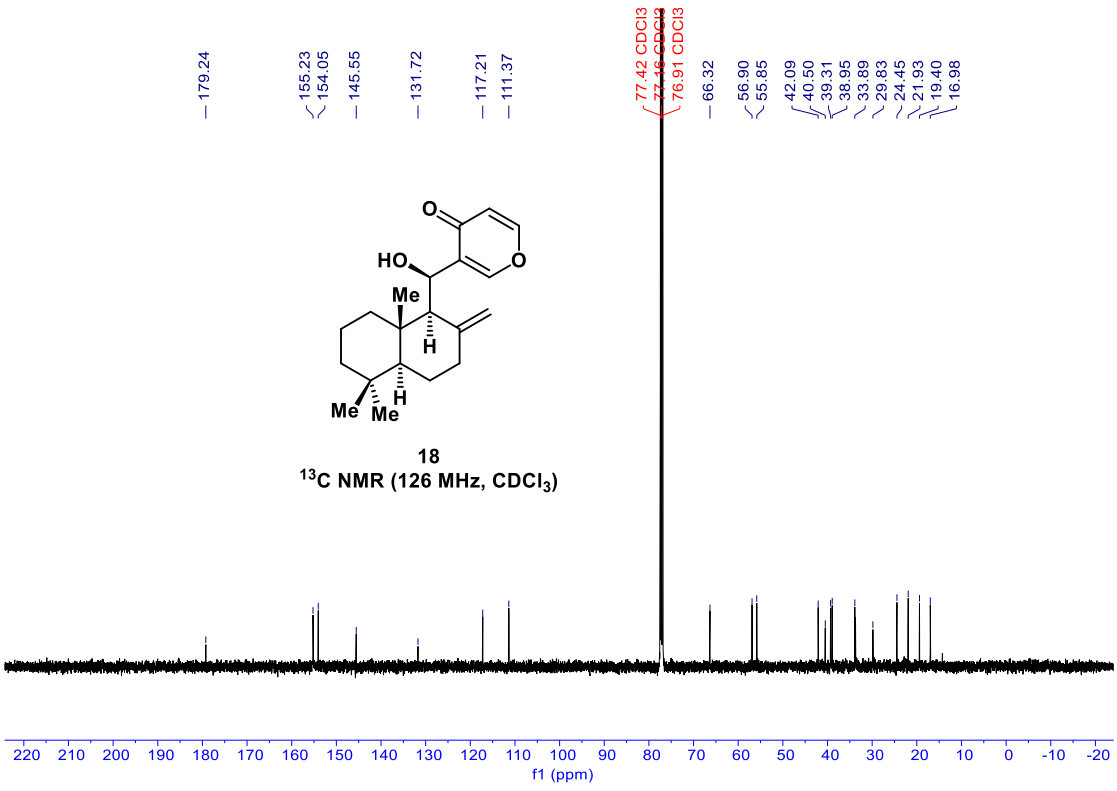
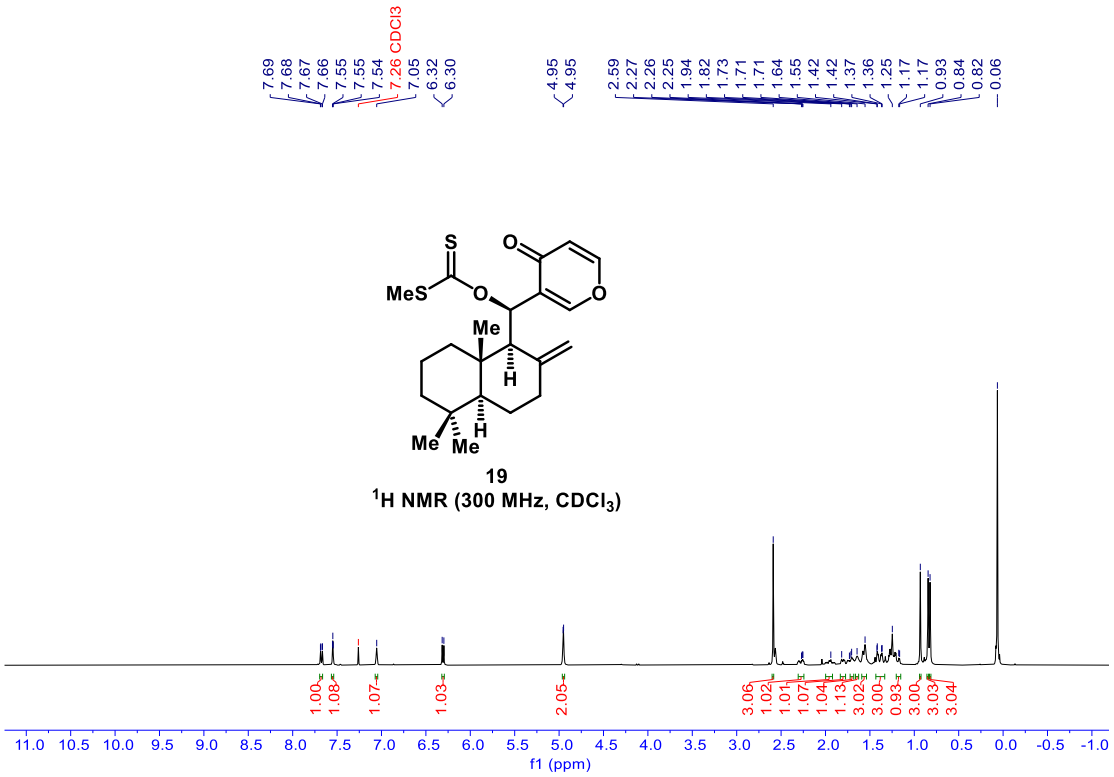
^{1}H NMR (500 MHz, $CDCl_3$)



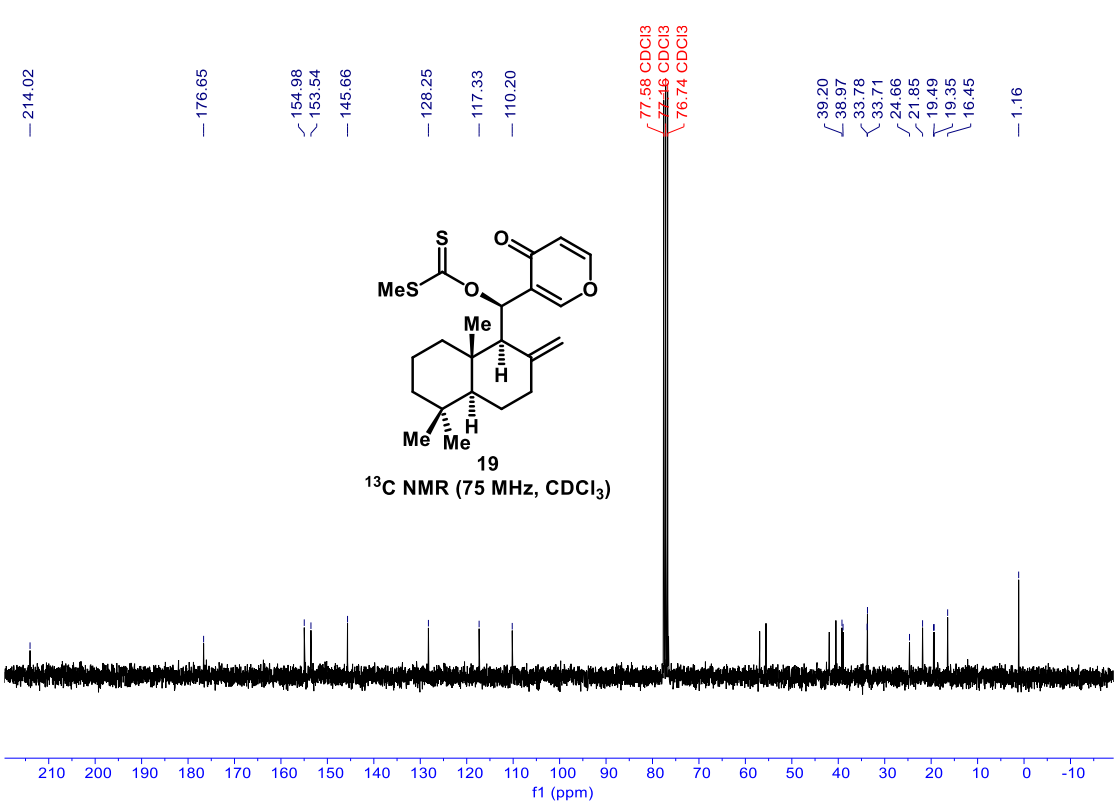
18

^{13}C NMR (126 MHz, $CDCl_3$)



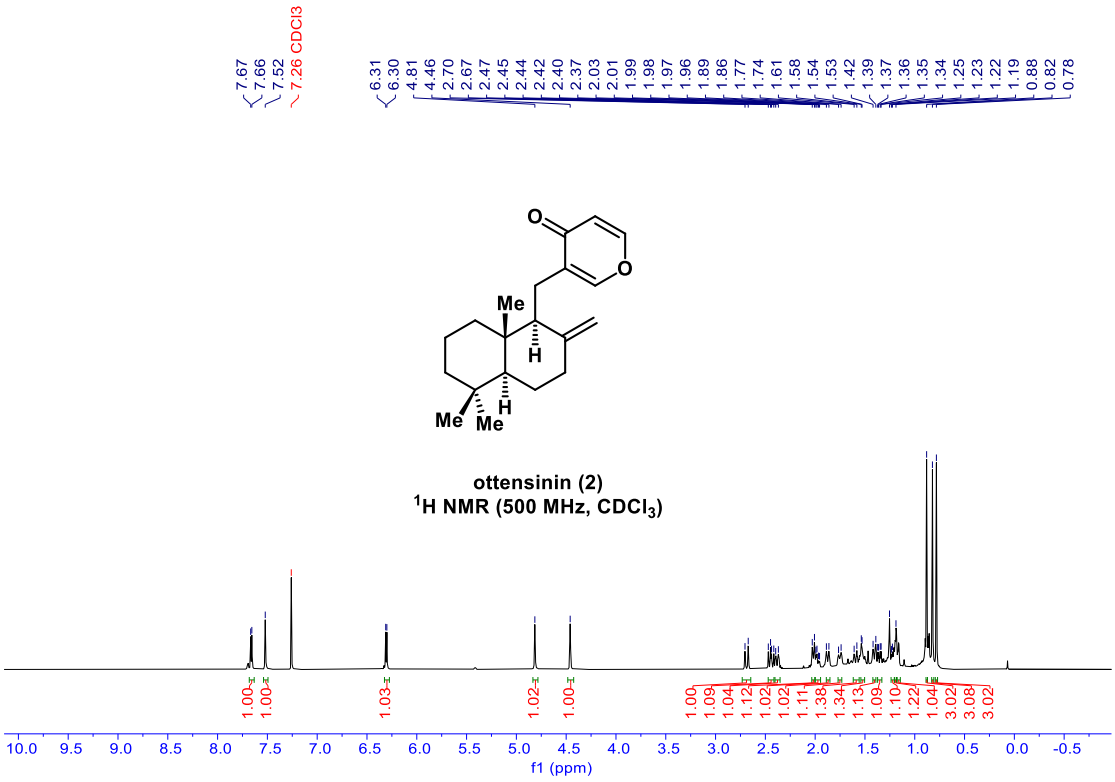
19

^{1}H NMR (300 MHz, $CDCl_3$)

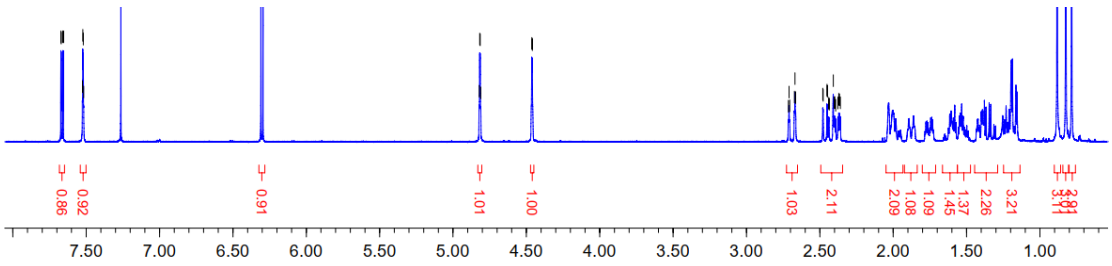


19

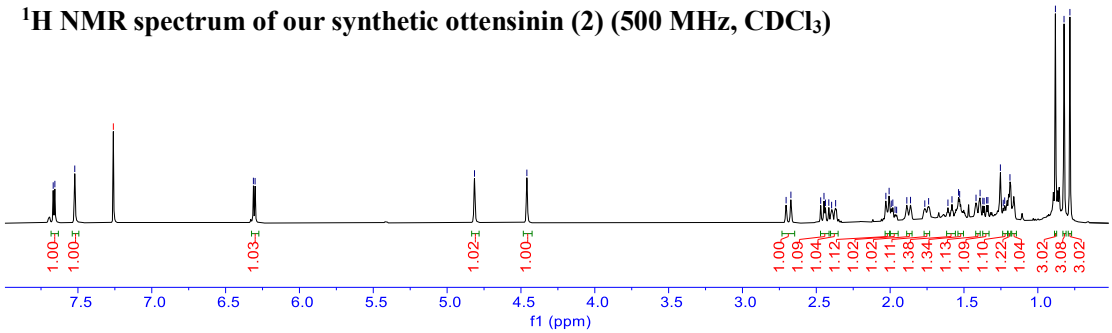
^{13}C NMR (75 MHz, $CDCl_3$)

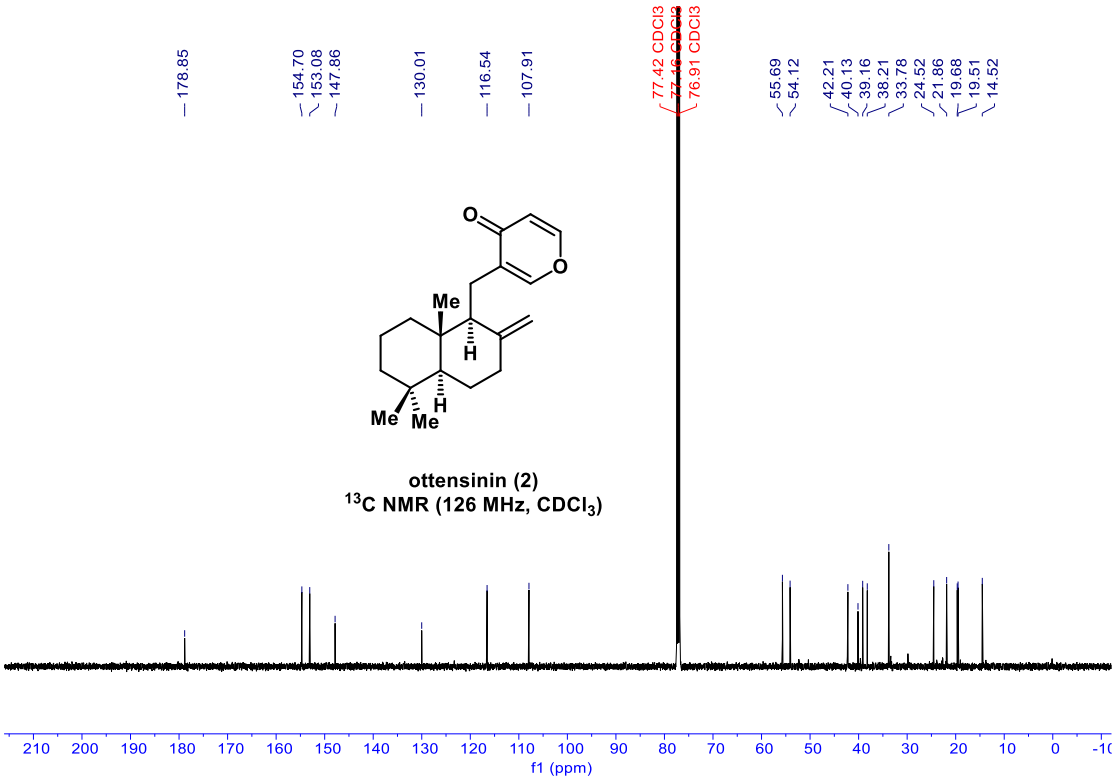


^{1}H NMR spectrum of Boukouvalas' synthetic ottensinin (2) (400 MHz, $CDCl_3$)

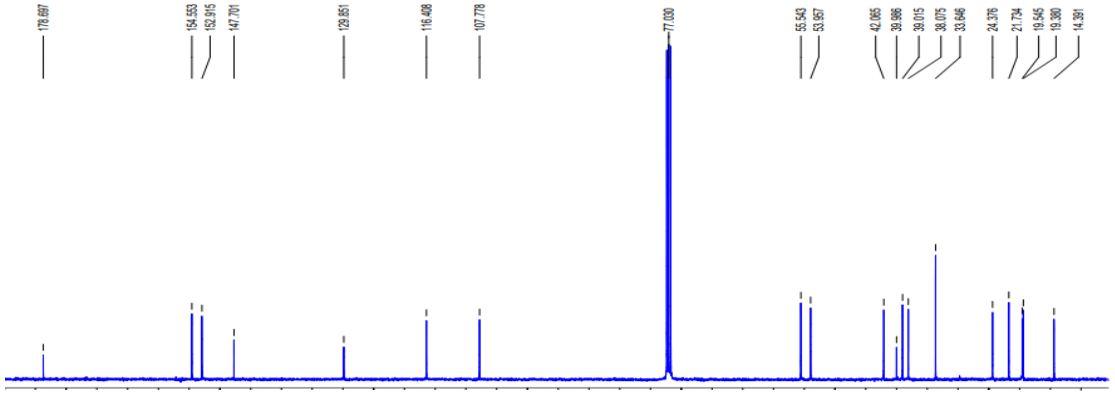


^{1}H NMR spectrum of our synthetic ottensinin (2) (500 MHz, $CDCl_3$)

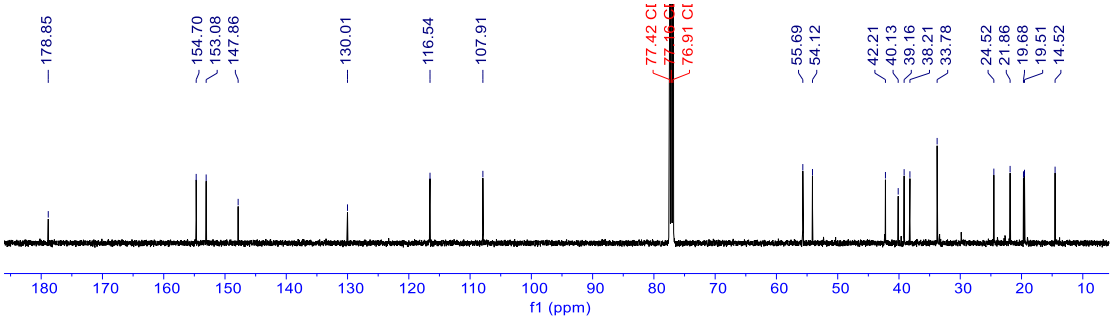


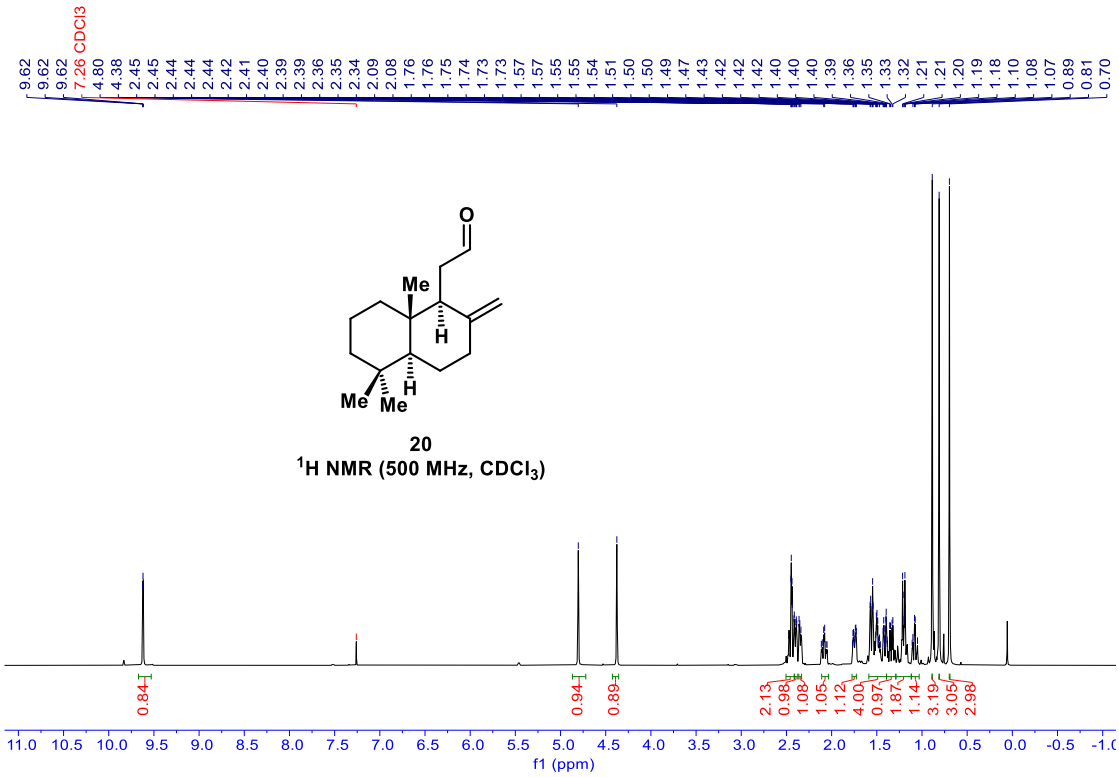


^{13}C NMR spectrum of Boukouvalas' synthetic ottensinin (2) (100 MHz, $CDCl_3$)



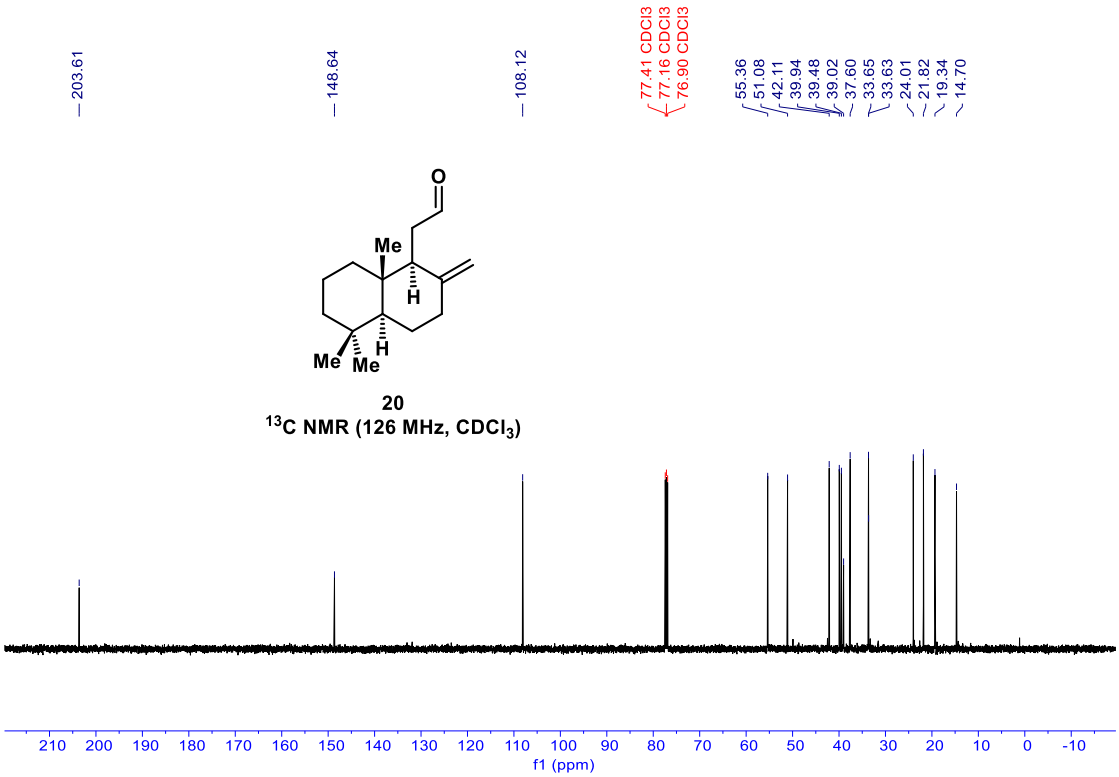
^{13}C NMR spectrum of our synthetic ottensinin (2) (126 MHz, $CDCl_3$)





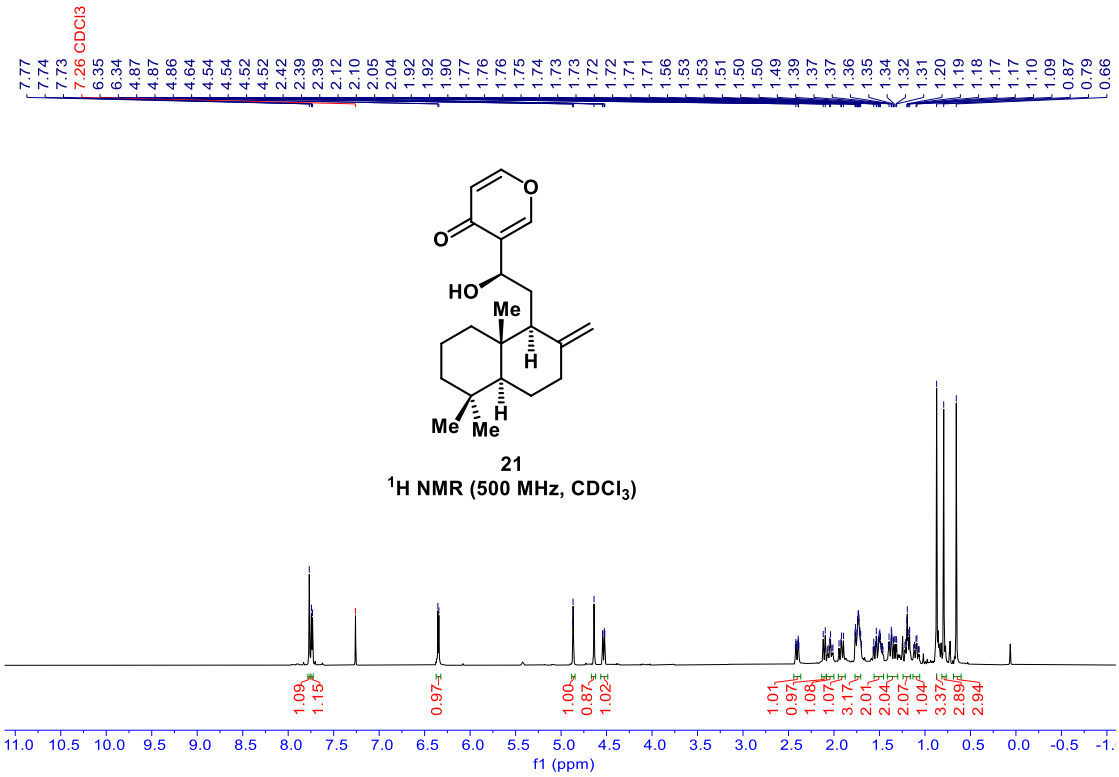
20

^{1}H NMR (500 MHz, $CDCl_3$)



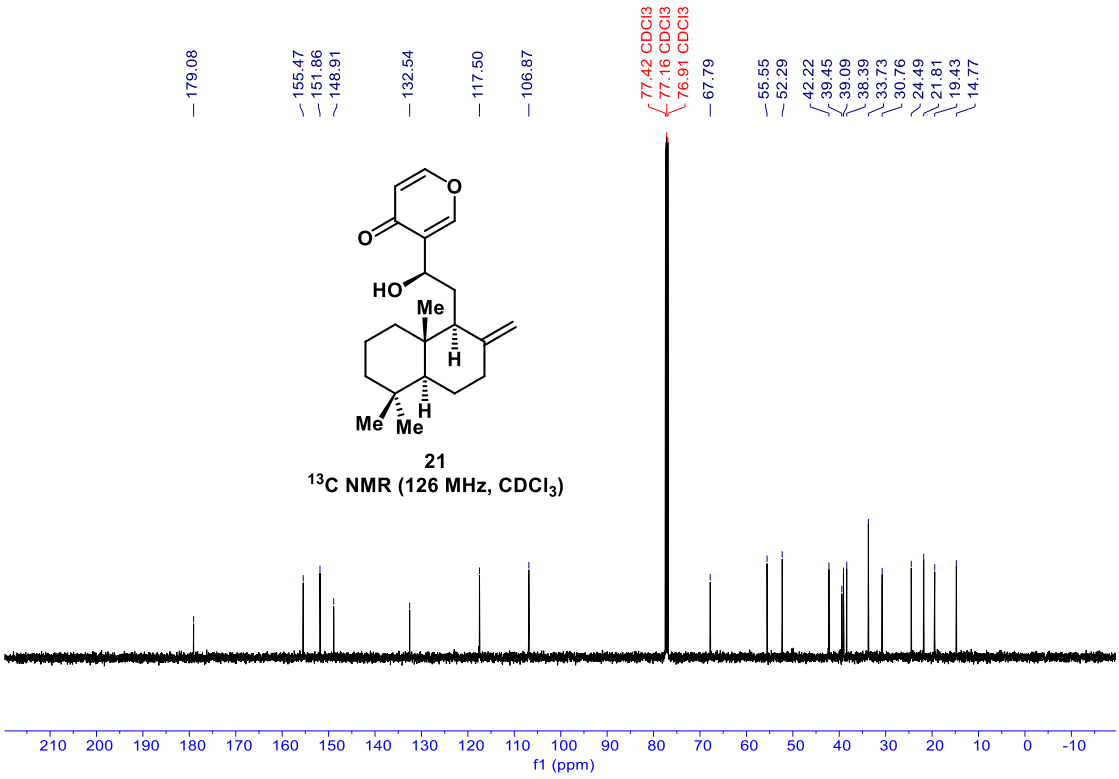
20

^{13}C NMR (126 MHz, $CDCl_3$)



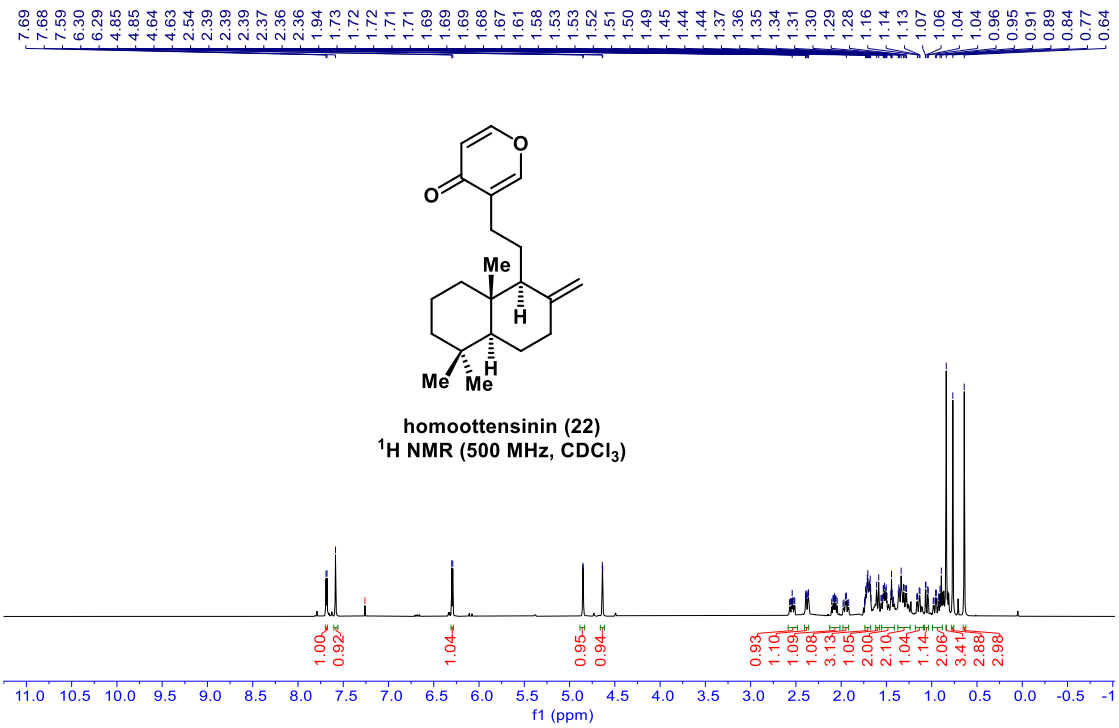
21

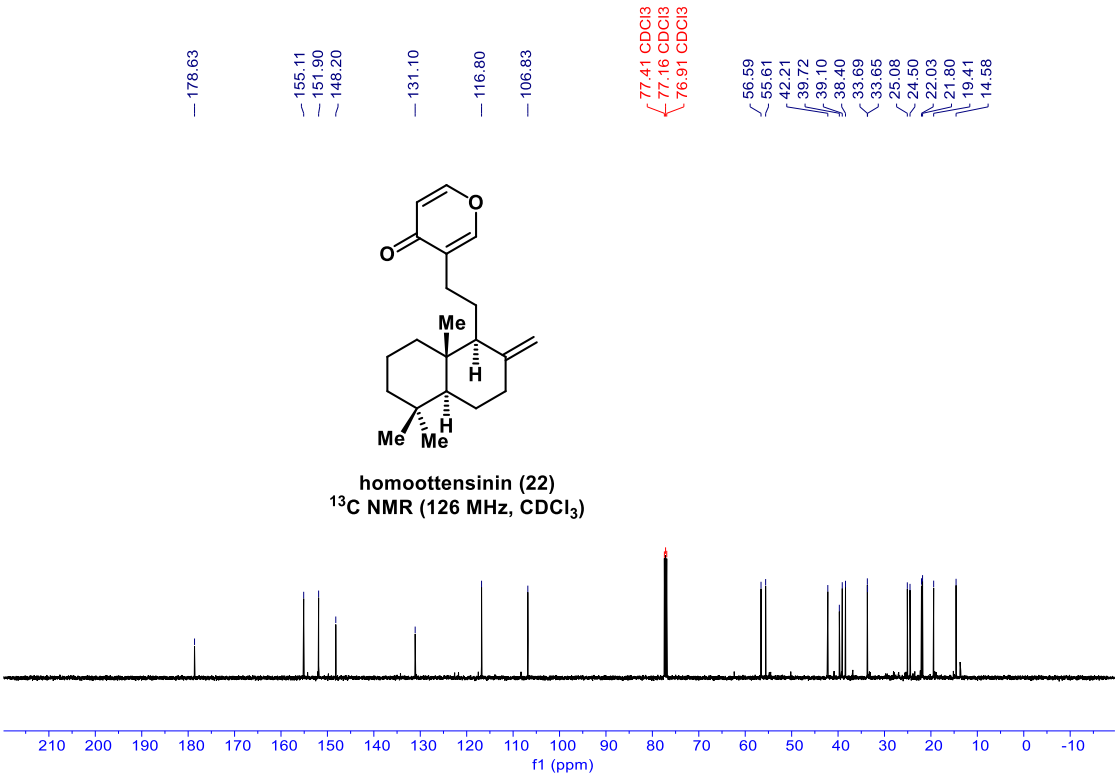
^{1}H NMR (500 MHz, $CDCl_3$)



21

^{13}C NMR (126 MHz, $CDCl_3$)


7.69 7.68 7.59 6.30 6.29 4.85 4.85 4.64 4.63 2.54 2.39 2.39 2.39 2.37 2.36 2.36 1.94 1.73 1.72 1.72 1.71 1.71 1.69 1.69 1.69 1.68 1.67 1.61 1.58 1.53 1.53 1.52 1.51 1.50 1.49 1.45 1.44 1.44 1.37 1.36 1.35 1.34 1.31 1.30 1.29 1.28 1.16 1.14 1.13 1.07 1.06 1.04 1.04 0.96 0.95 0.91 0.89 0.84 0.77 0.64
O
O
Me
H
Me
H
Me
homoottensinin (22)
1H NMR (500 MHz, CDCl3)
1.00 0.92 1.04 0.95 0.94 0.93 1.10 1.09 1.08 3.13 1.05 2.00 2.10 1.04 1.14 2.06 3.41 2.88 2.98
11.0 10.5 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 0.5 0.0 -0.5 -1
f1 (ppm)


178.63
155.11
151.90
148.20
131.10
116.80
106.83
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
56.59
55.61
42.21
39.72
39.10
38.40
33.69
33.65
25.08
24.50
22.03
21.80
19.41
14.58
O
O
Me
H
Me
H
Me
homoottensinin (22)
13C NMR (126 MHz, CDCl3)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

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