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Electronic Supplementary Information

Design and synthesis of simple, yet potent and selective non-ring-A pyripyropene A-based inhibitors of acyl-coenzyme A: cholesterol acyltransferase 2 (ACAT2)

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General Materials and methods

Cholesterol was purchased from Sigma. ACAT2-specific inhibitor Pyripyropene A was from ALEXIS Biochemicals. ACAT1-specific inhibitor K604 and NBD22-sterol were synthesized in our lab. Pyripyropene A, K604 and synthesized compounds were dissolved in DMSO. NBD22-sterol was dissolved in ethanol. All reactions sensitive to air or moisture were carried out under argon or nitrogen atmosphere in dry and freshly distilled solvents under anhydrous conditions, unless otherwise noted. Anhydrous THF and toluene were distilled over sodium benzophenone ketyl under Ar. Anhydrous CH_2Cl_2 was distilled over calcium hydride under Ar. All other solvents and reagents were used as obtained from commercial sources without further purification, unless otherwise stated. Optical rotations were measured on a polarimeter using a 10 cm cell at approximately 20 °C. NMR spectra were recorded at 300 and 75 MHz for ^{1}H and ^{13}C nuclei, or at 400 and 100 MHz for for ^{1}H and ^{13}C nuclei, respectively. Chemical shifts are reported in parts per million (ppm) relative to the tetramethylsilane peak recorded as δ0.00 ppm in $CDCl_3$/ TMS solvent, or the residual chloroform (δ7.26 ppm) or methanol (δ3.31 ppm) peaks. The ^{13}C NMR values were referenced to the residual chloroform (δ77.0 ppm), or methanol (δ49.0 ppm) peaks. ^{13}C NMR values are reported as chemical shift δ, multiplicity and assignment. ^{1}H NMR shift values are reported as chemical shift δ, relative integral, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constant (*J* in Hz) and assignment. High resolution mass spectroscopy (HRMS) was performed on a TOF instrument with ESI in positive ionization mode.

1. Biological assay protocol:

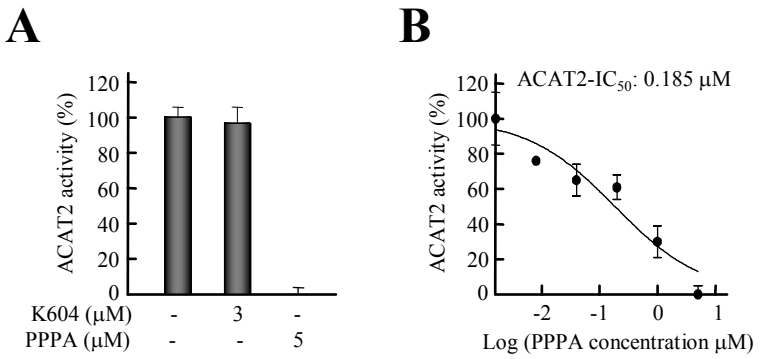
Cell lines and culture

Human hepatoma cell line HepG2 which expresses both ACAT2 and ACAT1 was obtained from American Type Culture Collection. Human normal liver cell line L02

was obtained from Shanghai Cell Bank of Chinese Academy of Sciences. Cells were maintained in DEME medium supplemented with 100 units/ml penicillin, 100 mg/ml streptomycin sulfate, plus 10% (v/v) FBS at 37°C in 5% CO2.

a. Determining IC_{50} of a synthesized compound by fluorescence assay for the ACAT2-catalyzed NBD22-steryl esters in the secreted lipoproteins

Because the ACAT1-specific inhibitor K604 doesn't reduce fluorescence intensity of NBD22-steryl esters in the secreted lipoproteins although HepG2 cells contain both ACAT1 and ACAT2 (Figure S1A) and moreover the determined ACAT2-IC_{50} of the inhibitor PPPA (0.185 μM) shown in Figure S1B is very similar to that reported in literature (0.190 μM) [29], it means that the fluorescence assay is specifically for the ACAT2-catalyzed NBD22-steryl esters in the secreted lipoproteins.



Supplementary Figure S1. Determination of the ACAT2 activity and ACAT2-IC_{50} of the inhibitor PPPA by fluorescence assay

A, ACAT2 activity (%) was determined after the 9 h incubation with 3 μM of K604 and 5 μM of PPPA.

B, The ACAT2-IC_{50} was obtained by using the different concentrations of the inhibitor PPPA (0.008 to 5 μM).

HepG2 cells were cultured overnight, and then incubated with a sterol mixture contain 0.5 μg/ml NBD22-sterols and the synthesized compound with the different concentrations ranging from 0.008 to 5 μM. After the 9 h incubation, the media fluorescence intensity (FI) of the secreted lipoproteins containing NBD22-steryl esters which are specifically catalyzed by ACAT2 was measured using the Envision Multilabel Reader by setting the excitation and emission wavelength to 488 nm and 535 nm, respectively. The FI of medium without cell used as the blank control was minus from the FI of medium with cells. The synthesized compound was replaced by

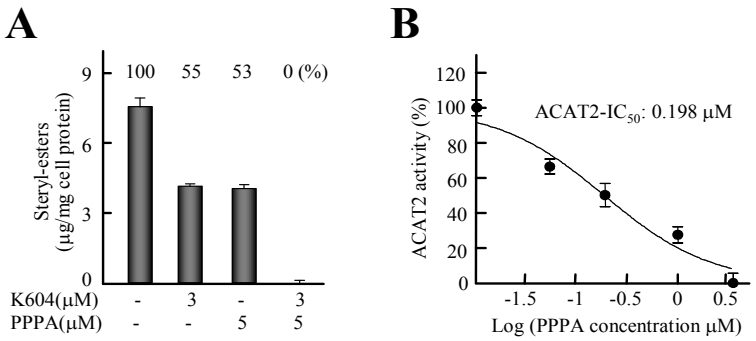
DMSO and 5 μM of ACAT2-specific inhibitor PPPA as the controls of no inhibition (NI) and positive inhibition (PI), respectively. Then, the ACAT2 activity (%) was calculated by the following formula:

$$\text{ACAT2 activity \%} = 100\% - (FI_{NI} - FI_{SC})/(FI_{NI} - FI_{PI}) \times 100\%$$

And the ACAT2-IC_{50} of a synthesized compound was obtained through non-linear fitting of the concentration-dependent curve by using Graphpad Prism 5 as Figure S1B.

b. Determining IC_{50} of a synthesized compound by cholesterol oxidase assay for the cellular steryl-esters catalyzed by ACAT.

Because ACAT1 and ACAT2 in HepG2 cells contribute separately about 50% of the cellular steryl-esters shown by inhibiting with PPPA, K604 and PPPA plus K604 (Figure S2A) under the condition of delivering cholesterols and moreover the determined ACAT2-IC_{50} of the inhibitor PPPA (0.198 μM) shown in Figure S2B is very similar to that reported in literature (0.190 μM) [29], it means that the cholesterol oxidase assay for the cellular steryl-esters can also be used to identify the different inhibition of a synthesized compound to ACAT1 and ACAT2, respectively.



Supplementary Figure S2. Determination of the cellular steryl-esters and ACAT2-IC_{50} of the inhibitor PPPA by cholesterol oxidase assay

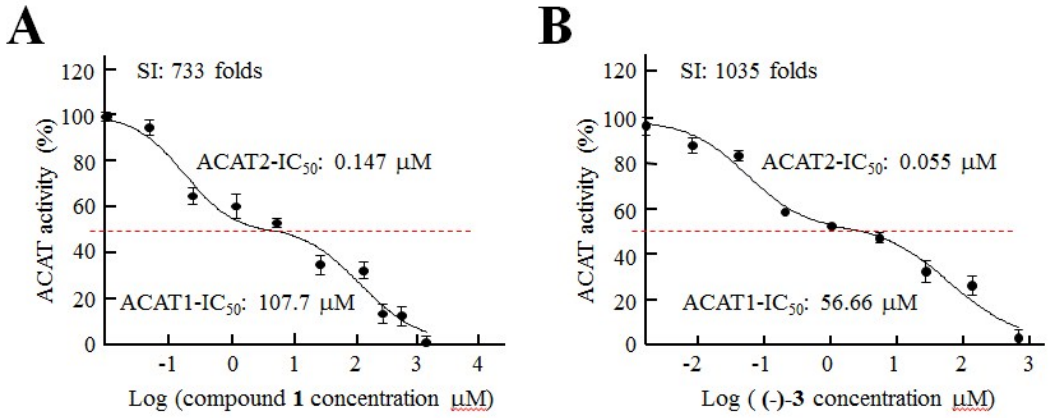
A, The cellular steryl-esters were determined after the 9 h incubation with 3 μM of K604 and 5 μM of PPPA.

B, The ACAT2-IC_{50} was obtained by using the different concentrations of inhibitor PPPA (0.01 to 5 μM).

HepG2 cells were cultured overnight, and then incubated with the different concentrations ranging more extensively from 0.008 to 625 μM of a synthesized compound. After the 9 h incubation, the cellular lipids were extracted by using the Folch method. The cellular steryl-esters (SE) and proteins of each sample were determined with Amplex Red Cholesterol Assay kit (Invitrogen, Carlsbad, USA) and BCA Protein Assay kit according to the manufacturer's instructions, respectively. The determined SE was normalized by the cellular proteins. The synthesized compound (SC) was replaced by DMSO and 3 μM of ACAT1 inhibitor K604 plus 5 μM of ACAT2 inhibitor PPPA as the controls of no inhibition (NI) and total inhibiton (TI), respectively. Then, the ACAT activity (%) was calculated as following formula:

$$\text{ACAT activity \%} = 100\% - (SE_{NI} - SE_{SC})/(SE_{NI} - SE_{TI}) \times 100\%$$

And the different ACAT2- and ACAT1-IC_{50} were obtained through hyperbolic non-linear fitting of the concentration-dependent curves respectively by using Graphpad Prism 5 as shown in Figure S3.



Supplementary Figure S3. Determination of the ACAT2- and ACAT1-IC_{50} of a synthesized compound by cholesterol oxidase assay

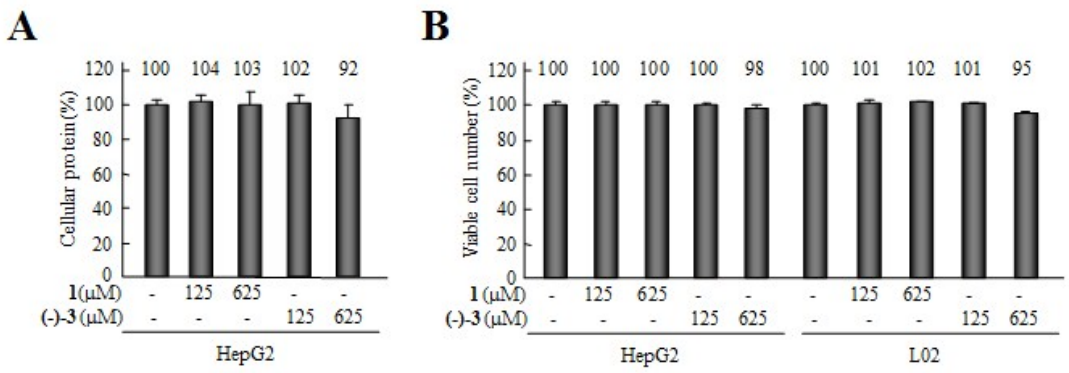
A and B, The ACAT2- and ACAT1-IC_{50} were obtained by using the extensive concentrations of the synthesized compounds **1** (0.008 to 1250 μM) and **(-)-3** (0.0016 to 625 μM), respectively.

c. Determining the effect of a synthesized compound on the growth of HepG2 and

L02 cells

HepG2 and L02 cells were cultured overnight, and then incubated with the synthesized compounds **1** and **(-)-3**, respectively. After the 9 h incubation, the effect of cells growth were evaluated by determining cellular proteins as above and measuring the viable cell number with Cell Counting Kit CCK8 according to the manufacturer's instructions.

Because the high concentrations of **1** and **(-)-3** do not reduce the cellular proteins in the hepatocellular carcinoma cell line HepG2 as shown in Figure S4A and the viable cell numbers of both HepG2 and L02 (a normal liver cell line) shown in Figure S4B, it indicates that these compounds are non-toxic both in hepatocellular carcinoma and non-hepatocellular carcinoma cell lines.

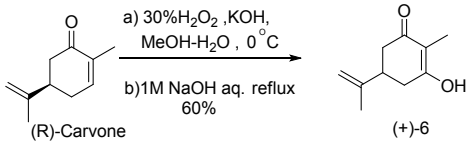


Supplementary Figure S4. Determination of the cellular proteins and the viable cell numbers of HepG2 and L02 cells after treating with high concentrations of the synthesized compounds

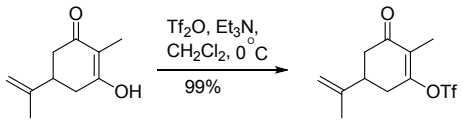
The cellular proteins of HepG2 (A) and the viable cell numbers of HepG2 and L02 (B) were determined after treating with high concentrations of **1** and **(-)-3**.

2. Experimental Procedures

Preparation of (±)-1:

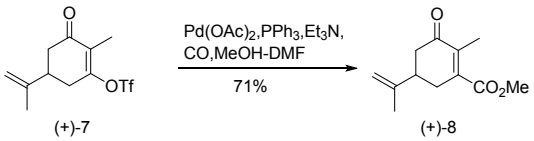


To a soln of 40.0g (0.266mol) (R)-carvone m 40ml MeOH , previously cooled to 0 ℃. was added with stirring a soln of 32.0g (0.57mol) KOH in 40ml water and 120ml MeOH. To the resulting mixture at -5℃ was added in one portion, 30m1 30% H_2O_2, previously cooled to - 13℃. The temperature rose to 15℃ after 10 min. Another 35 ml portion of 30% H_2O_2 was added after 25min , by which time the temperature had fallen to - 3℃. The mixture was stirred at or slightly below 0 ℃ for 2. 5 hrs .The reaction was then diluted with ice water, extracted with EtOAc (2 × 500 mL), and the combined organic layer dried (Na_2SO_4) and concentrated to give pale yellow epoxide, which was sufficiently pure for further use; To 1L of I N NaOH aq.at room temperature was added crude epoxide , and the heterogeneous mixture was heated at reflux for approximately 1 hr and then cooled to room temperature.The soln was acidified with 20% HCl aq., filtered and the crude wet residue was dried through vacuum drying to obtain hydroxycarvone (±)-**6** (racemic mixture 29.8g, 0.180mol, 67%).



(+)-6 (+)-7

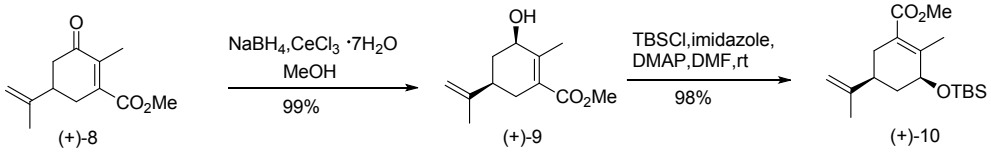
A solution of diketones (±)-6 (380mg, 2.29mmol, 1.00equiv) and triethylamine (302mg, 2.98mmol, 1.30equiv) in CH_2Cl_2 (15ml) was stirred at 0℃for 5 min and treated with trifluoromethanesulfonic anhydride (0.50ml, 2.98mmol, 1.30equiv). The mixture was stirred at 0℃for 1hr with TLC monitoring (hexane/EtOAc = 10/1). After the reaction is complete judged by TLC, to this suspension was added petroleum ether/ether = 1/1 mixture and purified by flash chromatography on silica gel (hexane/EtOAc = 10/1) providing (±)-7 (726mg, 2.44mmol, quantitative).



(+)-7 (+)-8

A stream of CO was passed through a solution of enol triflate (±)-7 (100mg, 0.34mmol, 1.00equiv), $Pd(OAc)_2$ (8.0mg, 0.04mmol, 0.10equiv), PPh_3 (11.0mg, 0.04mmol, 0.10equiv) and Et_3N (0.15ml, 1.02mmol, 3.0 equiv) in MeOH (2.0ml) and DMF(3.0ml) at room temperature for 30 min. A CO-filled balloon was then fitted to the apparatus and the reaction mixture was heated at reflux for overnight, cooled to

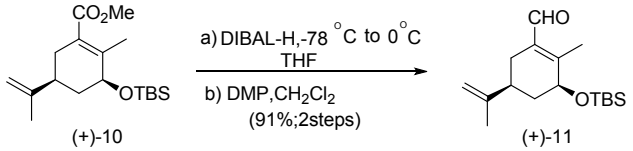
room temperature, filtered and concentrated. The residue was dissolved in EtOAc(30ml) and washed with brine, and the organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 25:1) to give (±)-8 (50mg, 0.24mmol, 71%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.81 (s, 1H), 4.75 (s, 1H), 3.80 (s, 3H), 2.72-2.30 (m, 5H), 1.92 (s, 3H), 1.79 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.3, 168.6, 145.9, 143.3, 137.1, 110.9, 52.1, 42.6, 41.1, 32.3, 20.4, 12.5.



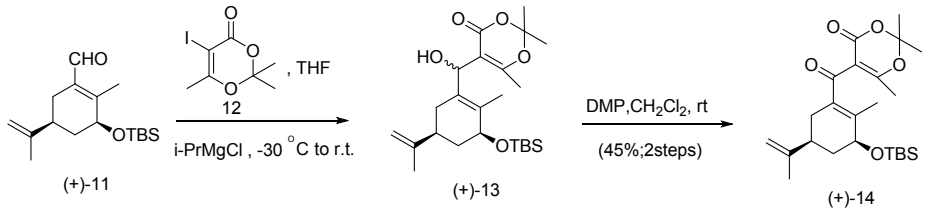
A solution of ketone (±)-8 (45mg, 0.22mmol, 1.00equiv) and cerium chloride heptahydrate (121mg, 0.33mmol, 1.50equiv) in methanol(10ml) was cooled to 0℃ and treated with sodium borohydride(13mg, 0.33mmol, 1.50equiv). The mixture was stirred at 0℃ for 30 min and then concentrated.The residue was dissolved in CH_2Cl_2(20ml) and the resultant solution was washed with H_2O, dried over Na_2SO_4, filtered and concentrated to give compound (±)-9 (45mg, 100%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.75 (dd, *J* = 1.2, 5.7 Hz, 2H), 3.75 (brs, 1H), 3.71 (s, 3H), 2.45 (brd, *J* =14.4 Hz, 1H), 2.30-2.11 (m, 3H), 2.03 (s, 3H), 1.68 (s, 3H), 1.52 (td, *J* = 12, 9.9 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.4, 148.1, 145.3, 126.1, 109.7, 71.8, 51.5, 39.3, 37.1, 32.3, 20.5, 16.5. HRMS (TOF ESI) calcd for $C_{12}H_{18}NaO_3$ 233.1148 $[M + Na]^+$, found 233.1145.

To a suspension of alcohol (±)-9 (2.35 g, 11.17mmol, 1.00 equiv) in DMF (50ml) at room temperature was added imidazole (1.60 g, 22.34mmol, 2.00 equiv). Then TBSCl (3.40 g, 22.34mmol, 2.00 equiv) was added. The mixture was stirred at room temperature for 12 h, then quenched by the addition of H_2O, and extracted with EtOAc (100 mL). The organic solution was washed with brine, dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane:EtOAc = 50:1) providing (±)-10 (3.55g, 10.94mmol, 98%) as a clear, colorless oil that was a 1:1mixture of enantiomers at the position of two chiral center: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.74 (s, 2H), 4.24 (brs, 1H), 3.72 (s, 3H), 2.41 (brd, *J* = 14.1 Hz, 1H), 2.23-2.02 (m, 3H), 1.98 (s, 3H), 1.68 (s, 3H), 1.52 (td, *J* = 12.3, 10.2 Hz, 1H), 0.90 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.4,

148.3, 146.1, 125.8, 109.6, 72.7, 51.4, 39.7, 37.7, 32.4, 25.8 (3C), 20.3, 18.1, 16.9, -4.0, -4.9. HRMS (TOF ESI) calcd for $C_{18}H_{32}NaO_3Si$ 347.2013 $[M + Na]^+$, found 347.2014.

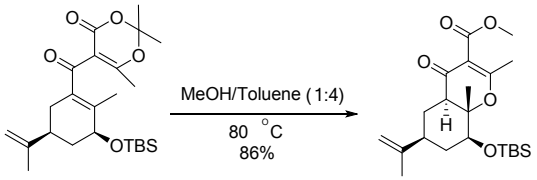


Neat DIBAL-H (24.10 ml, 24.10mmol, 2.2equiv) was added dropwise to a cooled (-78 °C) stirred solution of (±)-**10** (3.55 g, 10.94mmol, 1.0equiv) in THF (50 ml) and the reaction mixture was allowed to stir and warm to r.t. over 2 h. The mixture was then cooled (-15 °C) and saturated $NaHCO_3$ solution (30 ml) was added and the mixture was allowed to warm to r.t. and then filtered through celite (washing through with Et_2O). The filtrate was collected and washed with brine. The ethereal layers combined, dried (Na_2SO_4), filtered and the solvent removed in vacuo. The residue was dissolved in CH_2Cl_2 (50ml). DMP (5.60g, 13.20 mmol,1.2equiv) was added at 0 °C, after addition the resulting mixture was warmed up to rt. and stirred for 12 h. Saturated aqueous $NaHCO_3/Na_2S_2O_3$=1/1 (100ml) was added and the reaction mixture was stirred for additional 30 min. The organic layer was collected and washed with brine, dried over Na_2SO_4 and purified by flash column chromatography (hexane:EtOAc = 50:1) to afford (±)-**11** as a light yellow oil (2.928g, 91%, two steps): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.18 (s, 1H), 4.75 (s, 2H), 4.34 (brs, 1H), 2.52 (brd, *J* = 15.3 Hz), 2.16 (s, 3H), 2.12-1.83 (m, 3H), 1.75 (s, 3H), 1.52 (td, *J* = 12.6, 10.2 Hz, 1H), 0.93 (s, 9H), 0.14 (s, 3H), 0.12 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 191.2, 155.8, 147.8, 133.4, 109.5, 72.9, 38.8, 37.5, 28.2, 25.7 (3C), 20.3, 17.9, 13.3, -4.1, -5.1. HRMS (TOF ESI) calcd for $C_{17}H_{30}NaO_2Si$ 317.1907 $[M + Na]^+$, found 317.1905.

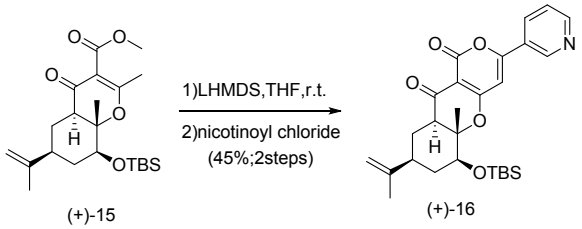


To a solution of 12 (656 mg, 2.45mmol, 3equiv) in THF (5.0 ml) at -30℃ was added dropwise i-PrMgCl (2.0 M in THF, 1.5ml, 2.45mmol, 3equiv). After being stirred for 0.5 h at -30℃, to the reaction mixture was added dropwise a solution of (±)-11 (240 mg, 0.82mmol, 1equiv) in THF (4.0 ml). The resulting mixture was warmed up to rt., stirred for 0.5 h, and quenched with a saturated aqueous NH_4Cl solution. The aqueous phase was extracted with EtOAc. The combined organic extracts were dried over

anhydrous Na_2SO_4 and concentrated in vacuo. This residue was employed in the next reaction without further purification. The crude (±)-13 was dissolved in CH_2Cl_2 (5.0 ml) and DMP (519 mg, 1.23mmol, 1.5equiv) was added. The resulting solution was stirred for 15 min at rt. and quenched with a saturated aqueous $Na_2S_2O_3$ solution and a saturated aqueous $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Flash column chromatography on silica gel (hexane:EtOAc = 25:1) afforded (±)-14 (157 mg, two steps 45%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.73 (s, 2H), 4.29 (s, 1H), 2.36 (dt, *J* = 7.8, 1.8 Hz, 1H), 2.30 (s, 3H), 2.26-1.97 (m, 3H), 1.72 (s, 6H), 1.68 (s, 6H), 1.58 (td, *J* = 12.6, 10.2 Hz, 1H), 0.88 (s, 9H), 0.08 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 195.6, 175.0, 157.9, 148.1, 137.2, 135.0, 108.9, 108.8, 105.8, 71.9, 39.5, 37.3, 32.1, 25.3 (3C), 24.9, 24.8, 19.9, 19.4, 17.6, 15.6, -4.5 (2C). HRMS (TOF ESI) calcd for $C_{24}H_{38}NaO_5Si$ 457.2381 [M + Na]$^+$, found 457.2377.

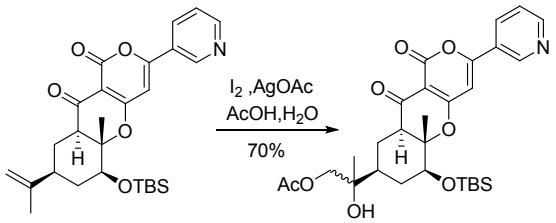


β-Ketoester (±)-14 (1.50g, 3.46mmol, 1equiv) was dissolved in toluene (60.0 ml) and MeOH (15.0 ml). The reaction mixture was stirred for 12 h at 80℃, cooled to rt, and concentrated in vacuo. Column chromatography on silica gel (hexane:EtOAc = 10:1→5:1) afforded (±)-15 (1.21g, 86%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.71 (s, 2H), 3.88 (dd, *J* = 6.0, 12.0 Hz, 1H), 3.75 (s, 3H), 2.52 (dd, *J* = 3.0, 12.0 Hz, 1H), 2.20 (s, 3H), 2.15-1.99 (m, 3H), 1.80-1.74 (m, 1H), 1.69 (s, 3H), 1.36 (td, *J* = 12.6, 10.2 Hz, 1H), 1.19 (s, 3H), 0.88 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 189.1, 175.5, 166.1, 147.2, 110.2, 109.9, 86.9, 75.7, 51.9, 49.7, 41.3, 37.1, 26.0, 25.7 (3C), 20.8, 20.7, 17.9, 10.1, -4.5 (2C). HRMS (TOF ESI) calcd for $C_{22}H_{36}NaO_5Si$ 431.2224 [M + Na]$^+$, found 431.2226.



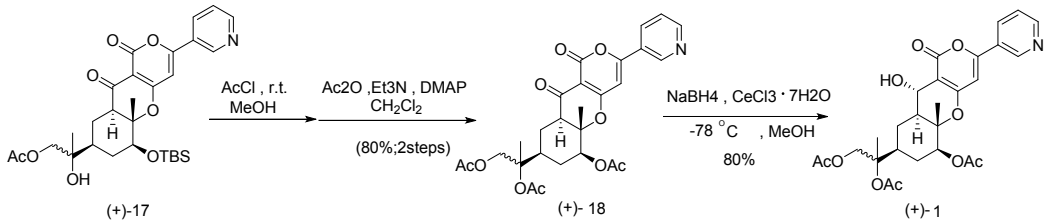
To a solution of LHMDS (1.0 M in THF, 2.00ml, 2.00mmol) in THF at 0 ℃ was

added dropwise a solution of (±)-15 (78.00 mg, 0.19mmol, 1equiv) in THF (2.00ml). The reaction mixture was warmed up to rt. and stirred for 4 h. To the mixture was added nicotinoyl chloride hydrochloride (107.00 mg, 0.57mmol) expeditiously. The resulting mixture was stirred for 2 h at rt., quenched with AcOH, and diluted with H_2O. The aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Column chromatography on silica gel (hexane:acetone=3:1) afforded (±)-16 (41.00 mg, 45%, two steps) as a white solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.06 (d, *J* = 1.2 Hz, 1H), 8.75 (d, *J* = 3.6 Hz, 1H), 8.19 (d, *J* = 8.4 Hz, 1H), 7.45 (dd, *J* = 4.8, 8.1 Hz, 1H), 6.50 (s, 1H), 4.89 (s, 2H), 4.03 (dd, *J* = 10.8, 4.8 Hz, 1H), 2.71 (dd, *J* = 12.3, 3.6 Hz, 1H), 2.28 (d, *J* = 13.5 Hz, 1H), 2.13 (t, *J* = 12.9 Hz, 1H), 1.88 (d, *J* = 13.8 Hz, 1H), 1.76 (s, 3H), 1.44 (q, *J* = 12.9 Hz, 1H), 1.34 (s, 3H), 1.31-1.23(m, 1H), 0.96 (s, 9H), 0.07 (s, 3H), 0.04 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 186.8, 172.7, 161.7, 156.2, 151.6, 146.6, 145.5, 133.8, 126.4, 123.6, 109.8, 99.7, 97.7, 88.9, 75.0, 50.8, 40.7, 36.7, 25.6, 25.3 (3C), 20.3, 17.7, 10.5, -4.9 (2C). HRMS (TOF ESI) calcd for $C_{27}H_{35}NaO_5SiN$ 504.2177 [M + Na]$^+$, found 504.2173.



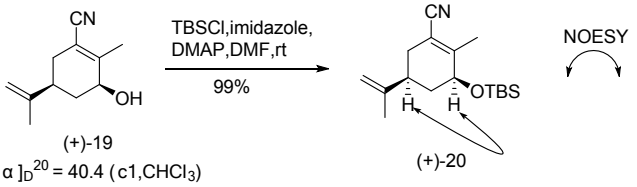
Pyridine pyrone (±)-16 (20.00mg, 0.04mmol, 1equiv) was dissolved in glacial HOAc (1.0ml) and H_2O (4.0 μl), followed by silver acetate (15.00mg, 0.08mmol, 2.0equiv) and powdered iodine(12.00mg, 0.05mmol, 1.1equiv).After stirring at rt for 12h, the mixture was diluted with 2:1 ether-CH_2Cl_2 and filtered through celite. The filtrate was partitioned with saturated $NaHCO_3$ and the organic layer was dried over Na_2SO_4 and concentrated in vacuo. Column chromatography on silica gel (CH_2Cl_2:MeOH = 25:1) afforded (±)-17 (17.00 mg, 70%) as a light yellow solid that was a 1:1 mixture of diastereomers at the position of hydroxyl: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.03 (d, *J* = 2.8Hz, 1H), 8.73 (d, *J* = 4.8 Hz, 1H), 8.16 (dd, *J* = 2.8, 10.4 Hz, 1H), 7.44 (dd, *J* = 6.8, 11.2 Hz, 1H), 6.44 (s, 1H), 4.08 (dd, *J* = 12.0, 32.0 Hz, 2H), 4.00 (dd, *J* = 4.0, 8.0 Hz,1H), 2.68 (dd, *J* = 4.0, 16.0 Hz, 1H), 2.36 (d, *J* = 16.0 Hz, 1H), 2.21 (d, *J* = 16.0 Hz, 1H), 2.11 (s, 3H), 2.01 (d, *J* = 12.0 Hz, 1H), 1.72 (t, *J* = 12.0 Hz, 1H), 1.37 (dd, *J* = 12.0, 24.0 Hz, 1H), 1.31 (s, 3H), 1.20 (s, 3H), 1.15 (dd, *J* = 8.0, 24.0 Hz, 1H), 0.95

(s, 9H), 0.19 (s, 3H), 0.14 (s, 3H). HRMS (TOF ESI) calcd for $C_{29}H_{39}NaO_8SiN$ 580.2337 $[M + Na]^+$, found 580.2338.



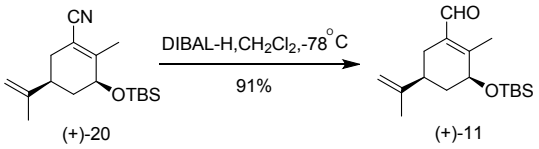
Acetyl chloride (0.17 mL, 1.58mmol) was added to MeOH (2.0 mL), and the mixture was stirred at rt for 5 min. A solution of (±)-17 (88.0 mg, 0.158mmol) in MeOH (5.0 mL) was added to the resulting MeOH solution, and the mixture was stirred at rt for 1h. The reaction mixture was concentrated in vacuo. A CH_2Cl_2 solution of crude triol (5.0mL) was treated with Ac_2O (0.11mL, 0.790mmol), Et_3N (0.24mL, 1.58mmol), and a catalytic amount of DMAP, and the mixture was stirred at rt for overnight. H_2O was added to the mixture and the aqueous layer was extracted with EtOAc. The organic layer was combined, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. A MeOH solution of crude triacetate (5.0mL) (±)-18 was treated with $CeCl_3 \cdot 7H_2O$ (410.1mg, 1.111mmol) and $NaBH_4$ (42.0mg, 1.111mmol), and the mixture was stirred at -78℃for 0.5 h. Acetone was added to the mixture and the resulting solution was diluted with EtOAc. The organic layer was washed with H_2O, brine, and dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (CH_2Cl_2/MeOH = 50:1) to afford (±)-1 (67.8mg, 80%): ^{1}HNMR ($CDCl_3$, 300 MHz) δ 9.01 (d, *J* = 4.0 Hz, 1H), 8.69 (dd, *J* = 4.0, 8.0 Hz, 1H), 8.09 (td, *J* = 4.0, 8.0 Hz, 1H), 7.41 (dd, *J* = 4.0, 8.0 Hz, 1H), 6.49 (s, 1H), 5.08 (dd, *J* = 4.0, 8.0 Hz, 1H), 4.56-4.34 (m, 3H), 2.34-1.99 (m, 2H), 2.17 (s, 3H), 2.07 (s, 3H), 1.99 (s, 3H), 1.89 (t, *J* = 12.0 Hz, 1H), 1.58-1.51 (m, 1H), 1.48 (s, 3H), 1.30 (s, 3H), 1.13 (dd, *J* = 12.0, 24.0 Hz, 1H). HRMS (TOF ESI) calcd for $C_{27}H_{31}NaO_{10}N$ 552.1840 $[M + Na]^+$, found 552.1840.

Preparation of single enantiomer (+)/(-)-2/3：



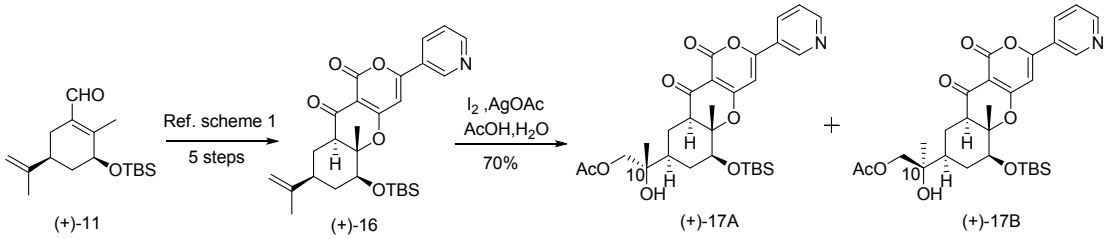
To a suspension of known allylic alcohol (+)-19 (131.0mg, 0.739mmol, 1.00 equiv) in DMF (50ml) at room temperature was added imidazole (100.8mg, 1.480mmol, 2.00 equiv) and a catalytic amount of DMAP. Then TBSCl (223.0mg, 1.480mmol, 2.00

equiv) was added. The mixture was stirred at room temperature for 12 h, then quenched by the addition of H_2O, and extracted with EtOAc (100 mL). The organic solution was washed with brine, dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography (hexane:EtOAc = 50:1) providing (+)-20 (213.0mg, 98%) as a clear, colorless oil: $[\alpha]_D^{20}$ = 27.80 (c1,$CHCl_3$), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.75 (s, 1H), 4.71 (s, 1H), 4.24 (brs, 1H), 2.30-2.01 (m, 4H), 1.99 (s, 3H), 1.69 (s, 3H), 1.49 (dd, *J* = 12.6, 22.8 Hz, 1H), 0.88 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.3, 146.9, 118.4, 110.4, 107.8, 71.2, 39.6, 37.3, 32.8, 25.8 (3C), 20.3, 18.9, 18.1, -4.1, -4.9. HRMS (TOF ESI) could not be obtained.



(+)-20 (+)-11

At -78 °C, DIBAL-H (11.20 ml, 11.17 mmol, 1.5equiv) was added dropwise to a cooled stirred solution of (+)-20 (2.17 g, 7.45 mmol, 1.0equiv) in CH_2Cl_2 (20 ml) and the reaction mixture was allowed to stir at this temperature over 2 h. The mixture was then added saturated Rochelle salt aqueous solution (30 ml) and the mixture was extracted with CH_2Cl_2. The organic solution was washed with brine, dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography (hexane:EtOAc = 50:1) providing (+)-11 (1.99g, 91%) as a yellow oil: $[\alpha]_D^{20}$ = 71.10 (c1,$CHCl_3$), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.15 (s, 1H), 4.71 (s, 2H), 4.34 (brs, 1H), 2.51 (d, *J* = 14.4 Hz, 1H), 2.16 (s, 3H), 2.11-1.84 (m, 3H), 1.74 (s, 3H), 1.49 (dd, *J* = 12.6, 22.8 Hz, 1H), 0.90 (s, 9H), 0.14 (s, 3H), 0.12 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 191.2, 155.8, 147.8, 133.4, 109.5, 72.9, 38.8, 37.5, 28.2, 25.7 (3C), 20.3, 17.9, 13.3, -4.1, -5.1. HRMS (TOF ESI) calcd for $C_{17}H_{30}NaO_2Si$ 317.1907 $[M + H]^+$, found 317.1906.

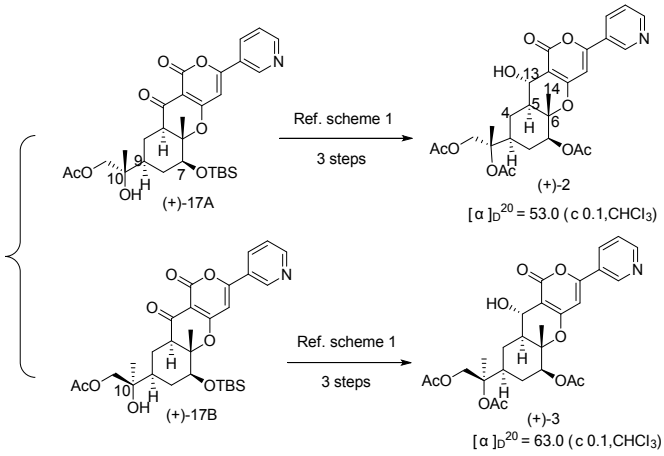


(+)-11 (+)-16 (+)-17A (+)-17B

The following synthetic procedure of (+)/(-)-2/3 is similar with that of racemic mixture (±)-1. Prevost dihydroxylation of (+)-16, furnished (+)-17A and (+)-17B as a 1:1 mixture of diastereomers at the newly formed stereocenter C_{10}, which can be isolated ((+)-17A and (+)-17B, separable by flash column chromatography), (+)-17A: $[\alpha]_D^{20}$ = 55.00 (c1,$CHCl_3$), ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.03 (d, *J* = 2.8Hz, 1H),

8.73 (d, *J* = 4.8 Hz, 1H), 8.16 (dd, *J* = 2.8, 10.4 Hz, 1H), 7.44 (dd, *J* = 6.8, 11.2 Hz, 1H), 6.44 (s, 1H), 4.08 (dd, *J* = 12.0, 32.0 Hz, 2H), 4.00 (dd, *J* = 4.0, 8.0 Hz,1H), 2.68 (dd, *J* = 4.0, 16.0 Hz, 1H), 2.21 (d, *J* = 16.0 Hz, 1H), 2.11 (s, 3H), 2.01 (d, *J* = 12.0 Hz, 1H), 1.72 (t, *J* = 12.0 Hz, 1H), 1.37 (dd, *J* = 12.0, 24.0 Hz, 1H), 1.31 (s, 3H), 1.20 (s, 3H), 1.15 (dd, *J* = 8.0, 24.0 Hz, 1H), 0.95 (s, 9H), 0.19 (s, 3H), 0.14 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 187.2, 173.1, 171.1, 162.5, 156.6, 152.6, 147.4, 133.8, 126.5, 123.8, 100.0, 97.8, 89.2, 75.5, 72.6, 69.9, 50.9, 40.6, 32.2, 25.7 (3C), 22.1, 21.1, 20.9, 18.2, 10.8, -4.5 (2C). HRMS (TOF ESI) calcd for $C_{29}H_{39}NaO_8SiN$ 580.2337 [M + Na]$^+$, found 580.2341.

(+)-17B: $[\alpha]_D^{20}$ - +44.00 (c0.1 in $CHCl_3$), ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.05 (s, 1H), 8.75 (d, *J* = 4.0 Hz, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 7.45 (dd, *J* = 4.0, 8.0 Hz, 1H), 6.43 (s, 1H), 4.07 (dd, *J* = 12.0, 32.0 Hz, 2H), 3.98 (dd, *J* = 4.0, 8.0 Hz,1H), 2.66 (dd, *J* = 4.0, 16.0 Hz, 1H), 2.36 (d, *J* = 16.0 Hz, 1H), 2.12 (s, 3H), 1.89 (d, *J* = 12.0 Hz, 1H), 1.70 (t, *J* = 12.0 Hz, 1H), 1.37-1.26 (m, 2H), 1.32 (s, 3H), 1.22 (s, 3H), 0.95(s, 9H), 0.19 (s, 3H), 0.14 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.1, 173.1, 171.0, 162.5, 156.6, 152.6, 147.5, 133.8, 126.6, 123.8, 100.0, 97.9, 89.1, 75.5, 72.6, 69.7, 51.0, 41.2, 33.1, 25.7 (3C), 21.8, 21.4, 20.9, 18.2, 10.8, -4.5 (2C). HRMS (TOF ESI) calcd for $C_{29}H_{39}NaO_8SiN$ 580.2337 [M + Na]$^+$, found 580.2338.



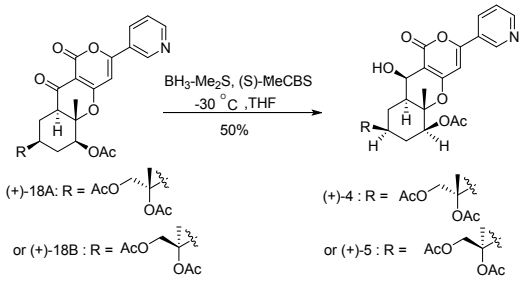
(+)-2: $[\alpha]_D^{20}$ - +53.0 (c0.1 in $CHCl_3$), ^{1}HNMR ($CDCl_3$, 300 MHz) δ 9.01 (s, 1H), 8.69 (d, J = 4.8 Hz, 1H), 8.09 (dd, *J* = 1.8, 8.1 Hz, 1H), 7.39 (dd, *J* = 4.8, 8.1 Hz, 1H), 6.49 (s, 1H), 5.08 (dd, *J* = 4.8, 12.0 Hz, 1H), 4.56-4.34 (m, 4H), 2.34-1.99 (m, 2H), 2.17 (s, 3H), 2.07 (s, 3H), 1.99 (s, 3H), 1.89 (t, *J* = 12.0 Hz, 1H), 1.58-1.51 (m, 1H), 1.48 (s, 3H), 1.30 (s, 3H), 1.13 (dd, *J* = 13.2, 26.1 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ170.3, 170.0, 169.6, 163.1, 162.6, 157.1, 151.3, 146.4, 132.5, 126.5, 123.2, 102.1, 98.9, 82.5, 82.3, 74.9, 64.7, 62.6, 43.9, 39.1, 28.4, 25.3, 21.7, 20.8, 20.4, 18.3, 11.5. HRMS (TOF ESI) calcd for $C_{27}H_{31}NaO_{10}N$ 552.1840 [M +Na]$^+$, found 552.1836.

(+)-3: $[\alpha]_D^{20}$ - +63.0 (c0.1 in $CHCl_3$), ^{1}HNMR ($CDCl_3$, 300 MHz) δ 9.00 (d, *J* = 2.1 Hz, 1H), 8.68 (dd, *J* = 0.9, 4.5 Hz, 1H), 8.09 (td, *J* = 1.5, 8.4 Hz, 1H), 7.40 (dd, *J* = 4.8, 8.1 Hz, 1H), 6.49 (s, 1H), 5.06 (dd, *J* = 5.1, 12.3 Hz, 1H), 4.52-4.32 (m, 4H), 2.39-2.23 (m, 2H), 2.16 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H), 2.02-1.85 (m, 2H), 1.54-

1.15 (m, 2H), 1.48 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 170.1, 170.0, 163.6, 163.0, 157.6, 151.7, 146.8, 133.0, 127.0, 123.7, 102.6, 99.4, 82.9, 82.7, 75.4, 65.2, 63.1, 44.3, 39.7, 29.2, 25.5, 22.1, 21.3, 20.9, 18.9, 11.9. HRMS (TOF ESI) calcd for $C_{27}H_{31}NaO_{10}N$ 552.1840 $[M+Na]^+$, found 552.1834.

The preparation and NMR spectra of (-)-2/3 is similar with that of (+)-2/3, but the minor difference is starting from commercial available material (S)-carvone. The final compound (-)-2: $[\alpha]_D^{20}$ - - 59.0 (c0.1 in $CHCl_3$); (-)-3: $[\alpha]_D^{20}$ - - 60.0 (c0.1 in $CHCl_3$).

Preparation of single enantiomer (+)/(-)-4/5:



A 10ml flask was charged with anhydrous THF, (S)-MeCBS catalyst (0.2mg, 0.001mmol, 0.05equiv) and BH_3-Me_2S (0.003ml, 0.023mmol, 1.5equiv) under nitrogen. The solution was cooled to -30℃ and enone (+)-18A (8.0mg, 0.015mmol, 1equiv) in anhydrous THF was added dropwise. After 2h, MeOH was added cautiously while maintaining the internal temperature below -5℃. The reaction mixture was warmed to room temperature, concentrated in vacuo, and purified via flash chromatography (CH_2Cl_2/MeOH = 50:1) to afford allylic alcohol (+)-4 (4.0mg, 50%): $[\alpha]_D^{20}$ - +53.0 (c0.1 in $CHCl_3$), ^{1}HNMR ($CDCl_3$, 300 MHz) δ 9.03 (s, 1H), 8.69 (dd, *J* = 4.8 Hz, 1H), 8.11 (d, *J* = 7.8 Hz, 1H), 7.40 (dd, *J* = 4.8, 7.8 Hz, 1H), 6.49 (s, 1H), 5.04 (dd, *J* = 3.9, 11.4 Hz, 1H), 4.65 (s, 1H), 4.48 (dd, *J* = 12.0, 65.4 Hz, 2H), 2.82 (s, 1H), 2.41-2.37 (m, 1H), 2.10-1.56 (m, 5H), 2.18 (s, 3H), 2.10 (s, 3H), 1.92 (s, 3H), 1.50 (s, 3H), 1.49 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 170.2, 170.1, 163.9, 162.9, 157.6, 151.7, 146.9, 133.0, 127.2, 123.7, 103.1, 99.4, 83.0, 82.1, 75.8, 65.1, 61.5, 43.0, 39.9, 28.9, 24.7, 22.2, 21.3, 20.9, 18.5, 12.7. HRMS (TOF ESI) calcd for $C_{27}H_{32}NO_{10}$ 530.2021 $[M+H]^+$, found 530.2015.

(+)-5: $[\alpha]_D^{20}$ = 64.0 (c0.1,$CHCl_3$), ^{1}HNMR ($CDCl_3$, 300 MHz) δ 9.02 (s, 1H), 8.69 (s, 1H), 8.10 (d, *J* = 7.8 Hz, 1H), 7.41 (dd, *J* = 4.8, 8.1 Hz, 1H), 6.48 (s, 1H), 5.03 (dd, *J* = 4.5, 11.7 Hz, 1H), 4.65 (d, *J* = 3.6 Hz, 1H), 4.45 (dd, *J* = 12.0, 47.7 Hz, 2H), 2.92 (s, 1H), 2.92-2.41 (m, 1H), 2.03-1.30 (m, 5H), 2.16 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H), 1.50 (s, 3H), 1.48 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 170.2, 170.1, 163.9, 162.9, 157.6, 151.7, 146.9, 133.0, 127.2, 123.7, 103.1, 99.4, 83.0, 82.1, 75.8, 65.2, 61.5, 42.9, 39.9, 29.3, 24.4, 22.2, 21.3, 20.8, 18.8, 12.7. HRMS (TOF ESI) calcd for $C_{27}H_{32}NO_{10}$ 530.2021 $[M+H]^+$, found 530.2018.

The preparation and NMR spectra of (-)-4/5 is similar with that of (+)-4/5, but the

minor difference is starting from commercial available material (S)-carvone. The final compound (-)-4: $[\alpha]_D^{20}$ - - 50.0 (c0.1 in $CHCl_3$); (-)-5: $[\alpha]_D^{20}$ - - 60.0 (c0.1 in $CHCl_3$).

Structure determination of (+)-(2)

Sample of (+)-2 was analyzed by NMR. Proton, carbon, proton-proton and proton-carbon correlation. NMR spectra of (+)-2 were consistent with the structure shown below (stereochemistry at C_5, C_6, C_7, C_9, C_{10}, and C_{13}).

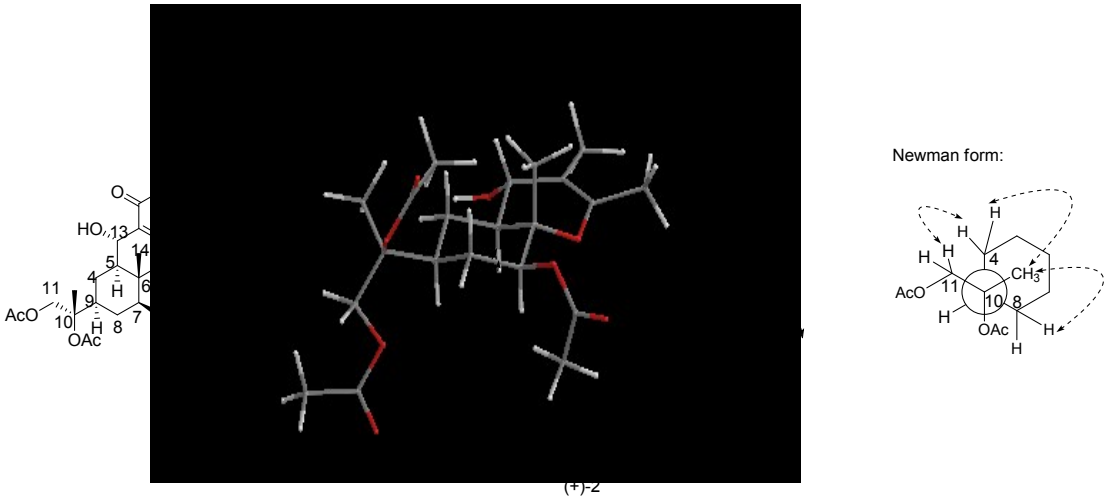


Figure S1. Key 2D NMR correlations for (+)-2

Proton and carbon resonances of (+)-2 were assigned by using COSY, NOESY, HSQC and HMBC experiments. Relative stereochemistry of C_5, C_6, C_7, C_9, C_{10}, and C_{13} centers was defined based on protonproton couplings and NOESY. Thus, C_{10} was determined in figure 1. NOE's between H_5, H_7 and H_9 protons indicated their cis stereochemistry and NOE between H_{13} and methyl-H_{14} protons, were consistent with the R,S,S,S,S,S-stereochemistry of C_5, C_6, C_7, C_9, C_{10}, and C_{13} centers (see NOE's and the conformation for that fragment of the molecule (+)-2 in the scheme above).

Figure S2. ^{1}H NMR spectrum of（±）-8 in $CDCl_3$

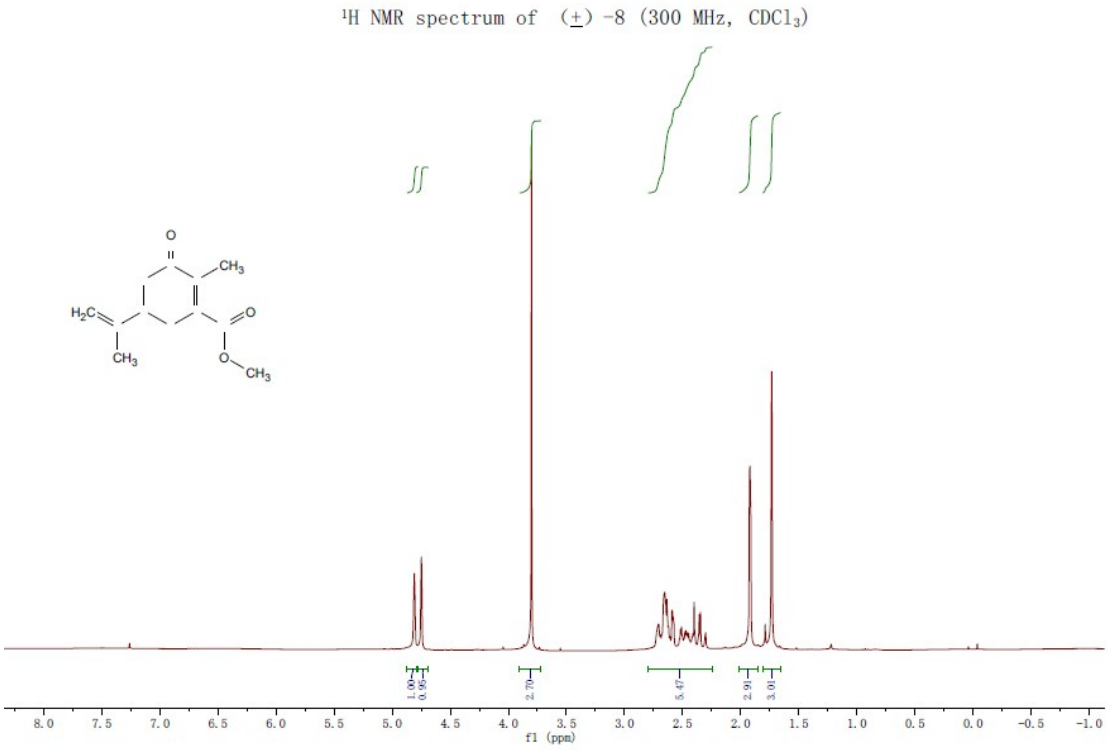


Figure S3. ^{13}C NMR spectrum of（±）-8 in $CDCl_3$

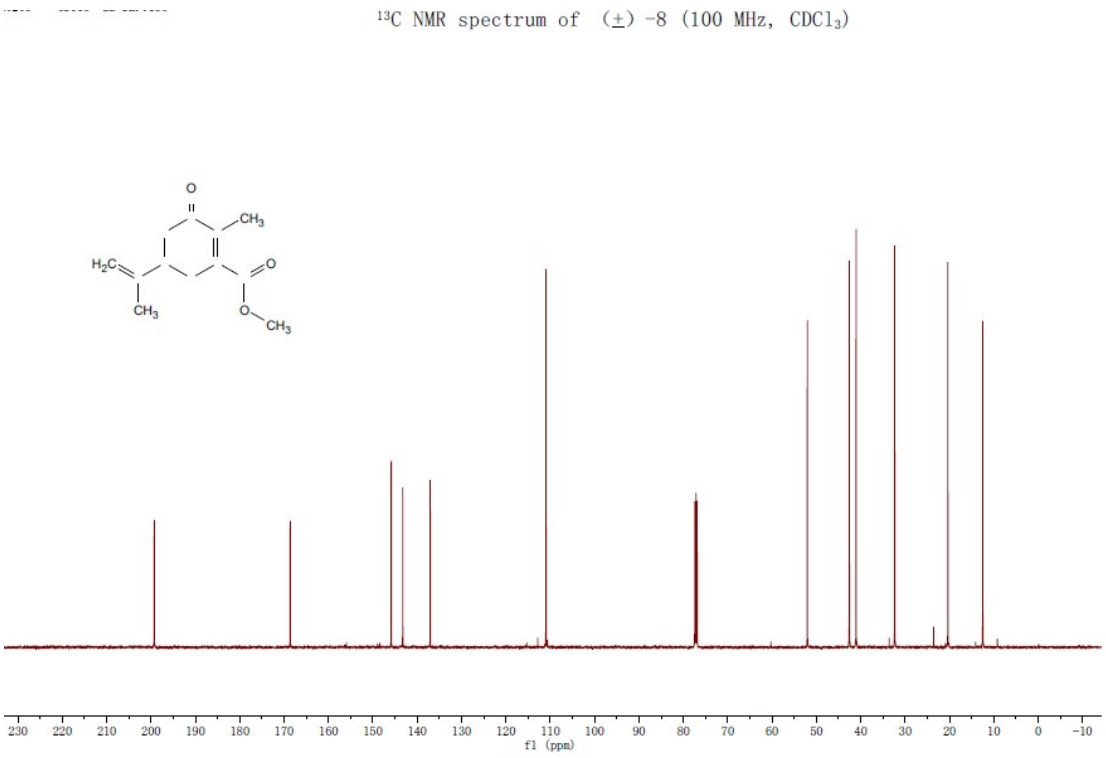


Figure S4. ^{1}H NMR spectrum of（±）-9 in $CDCl_3$

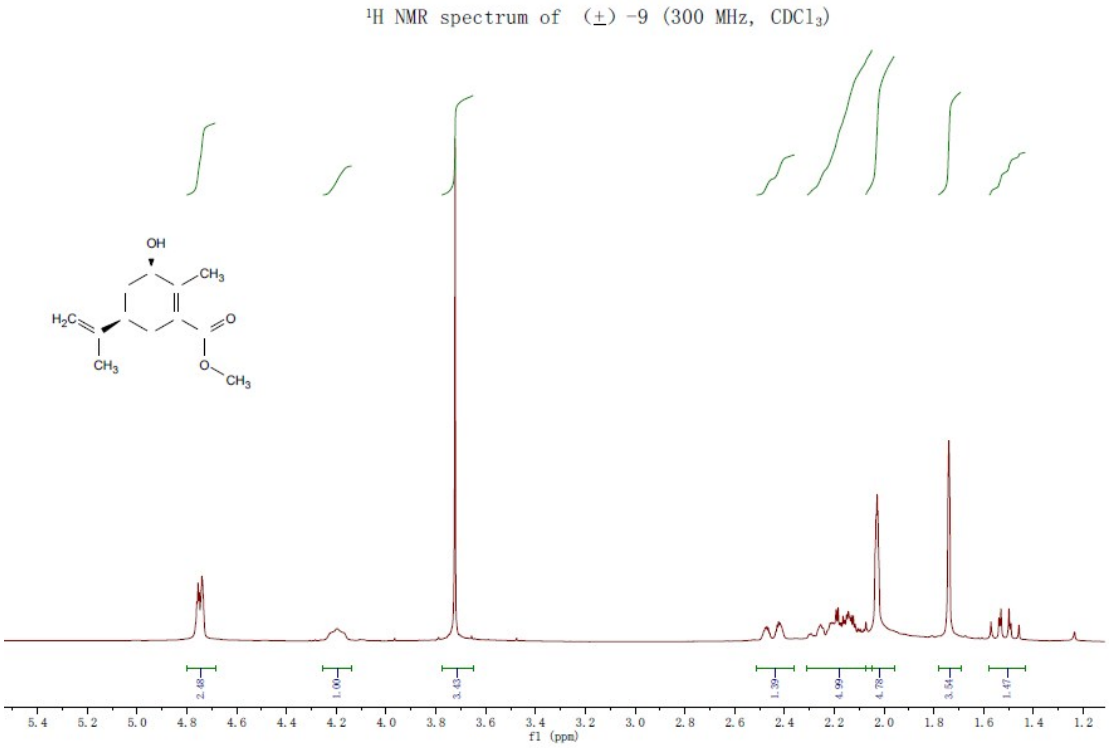


Figure S5. ^{13}C NMR spectrum of（±）-9 in $CDCl_3$

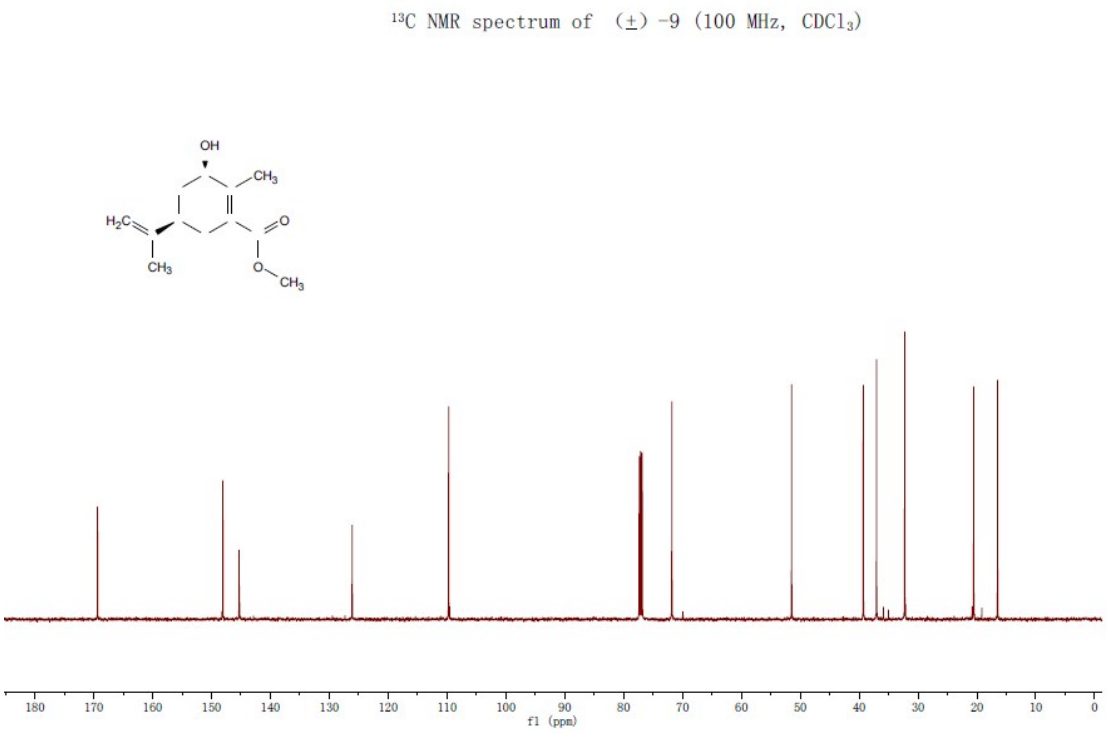


Figure S6. ^{1}H NMR spectrum of（±）-10 in $CDCl_3$

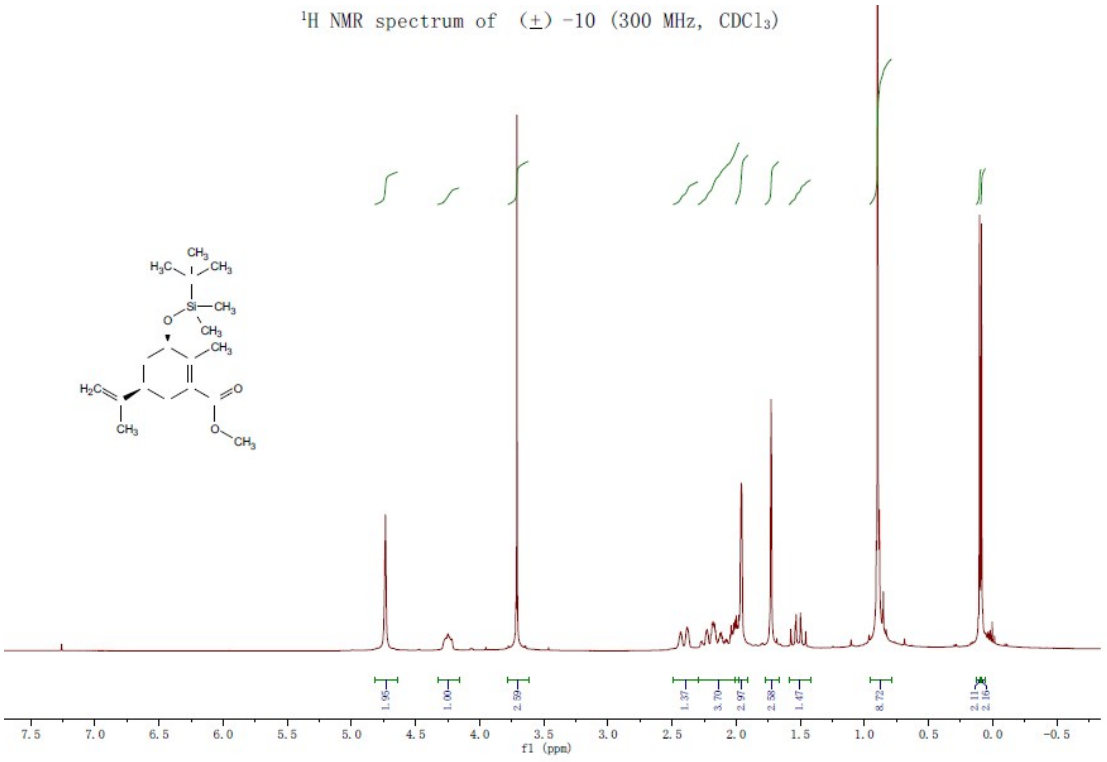


Figure S7. ^{13}C NMR spectrum of（±）-10 in $CDCl_3$

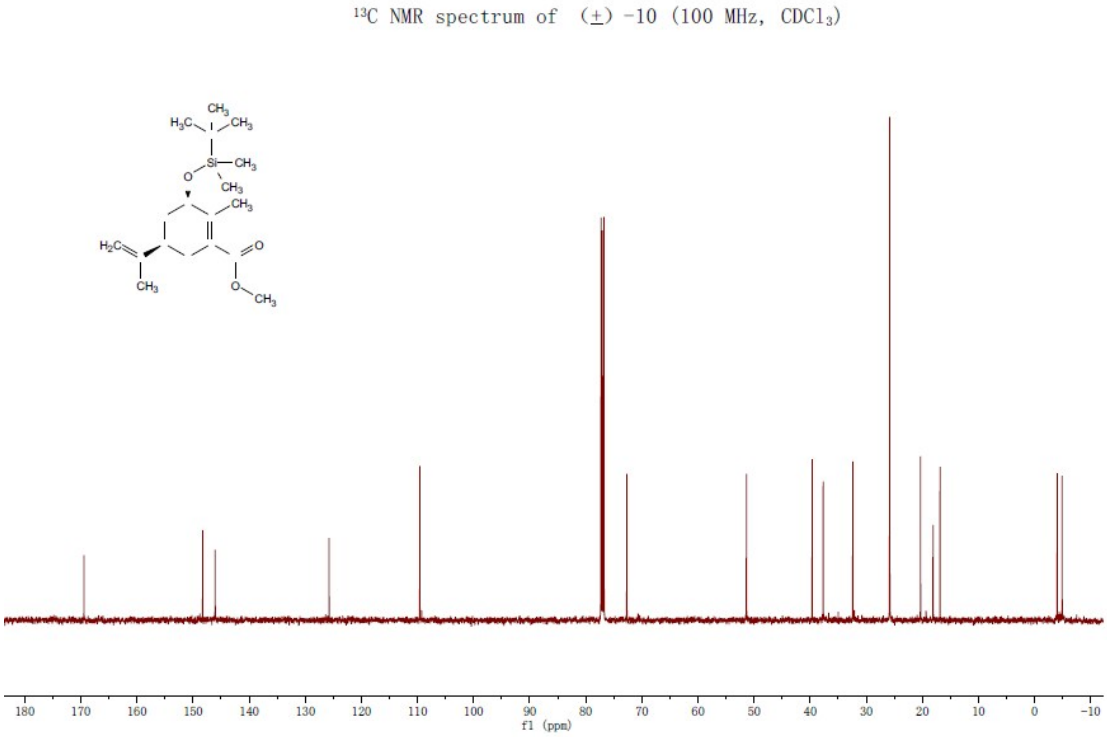


Figure S8. ^{1}H NMR spectrum of（+）-20 in $CDCl_3$

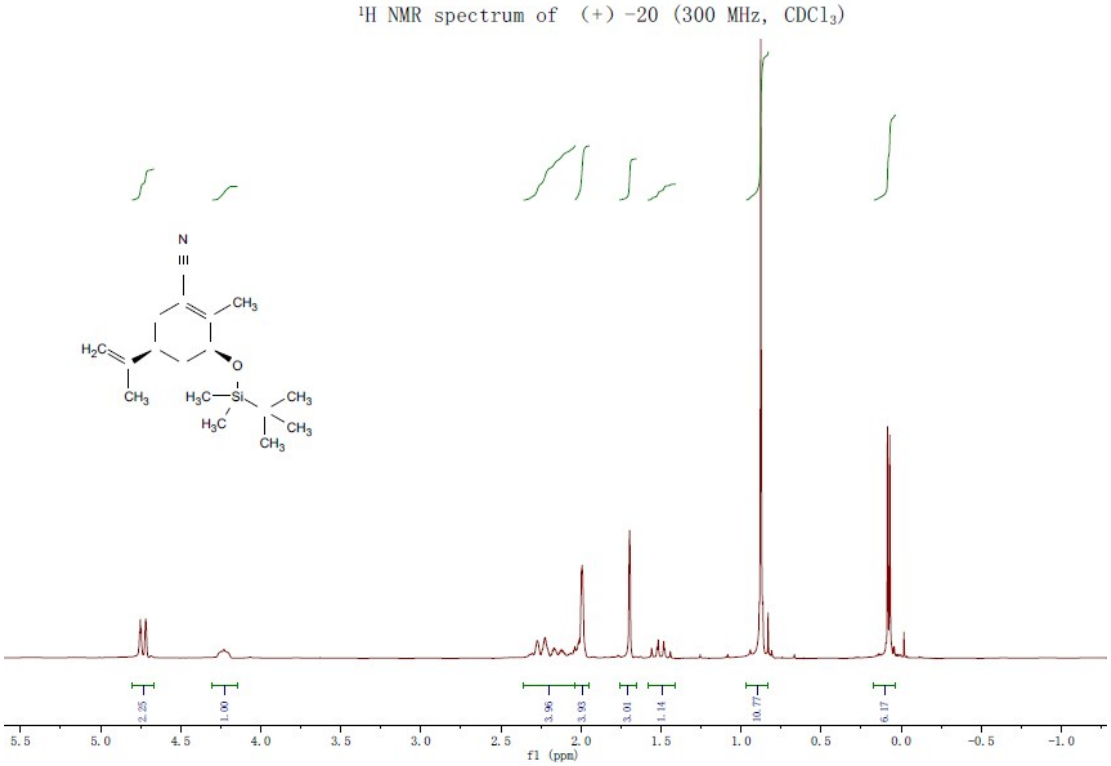


Figure S9. ^{13}C NMR spectrum of（+）-20 in $CDCl_3$

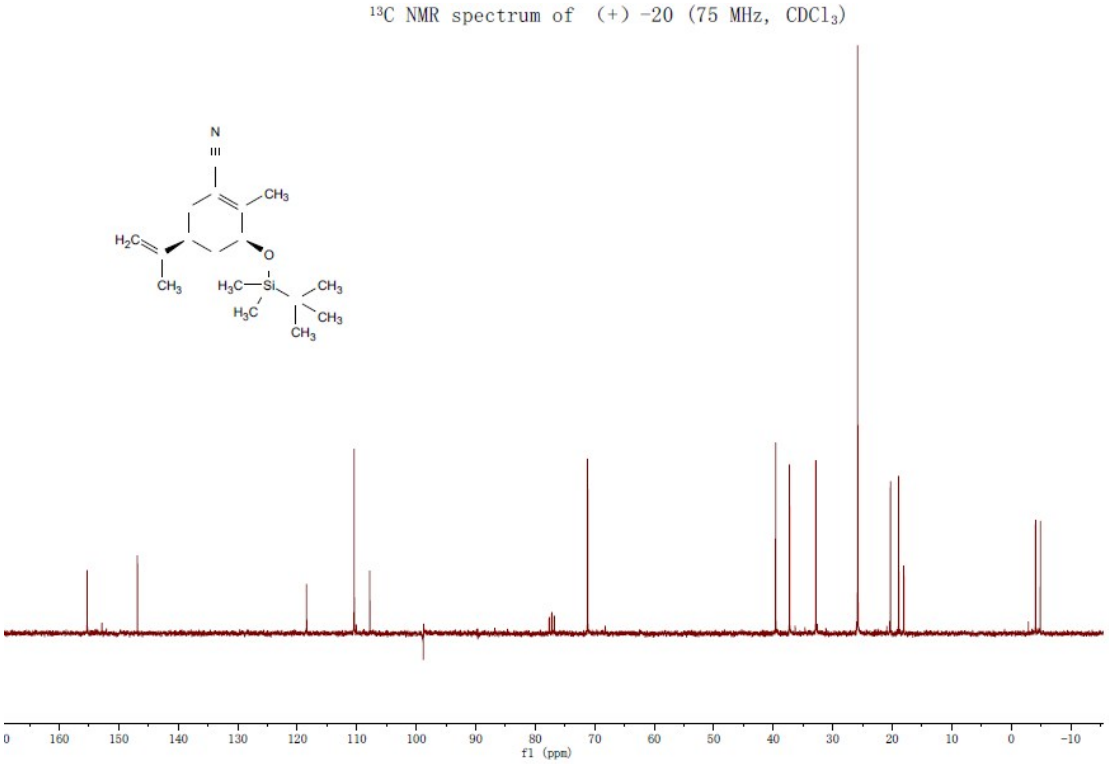


Figure S10. ^{1}H NMR spectrum of（+）-11 in $CDCl_3$

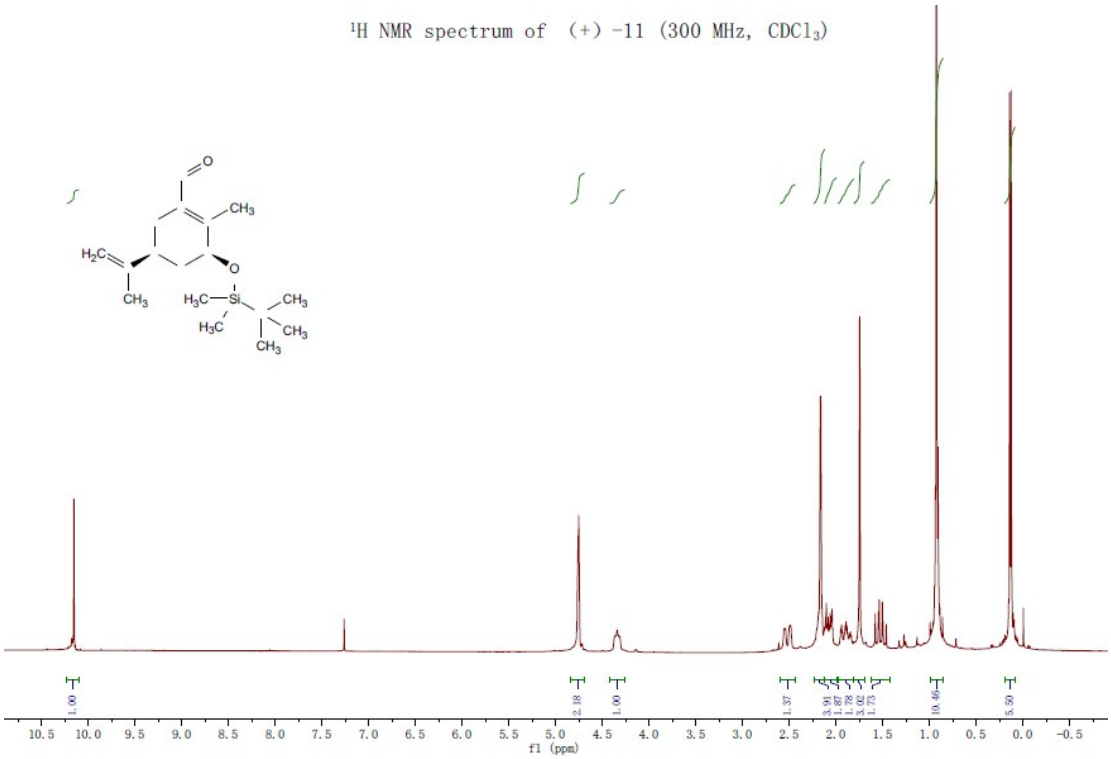


Figure S11. ^{13}C NMR spectrum of（+）-11 in $CDCl_3$

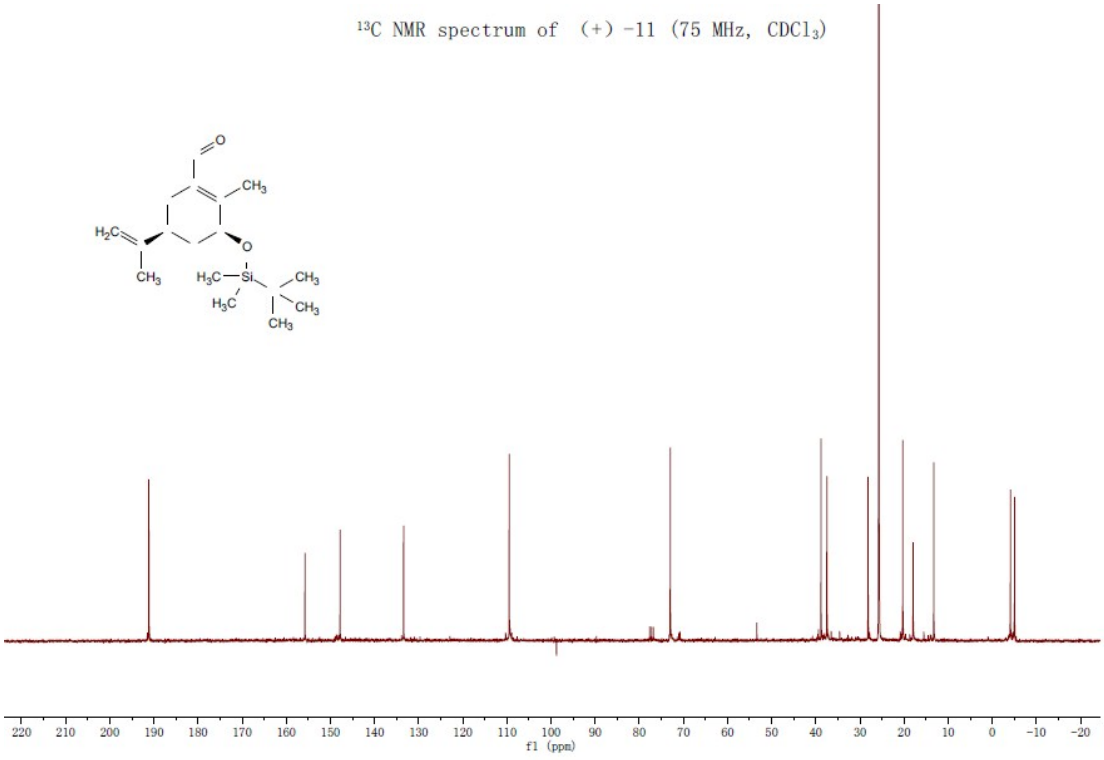


Figure S12. ^{1}H NMR spectrum of（+）-14 in $CDCl_3$

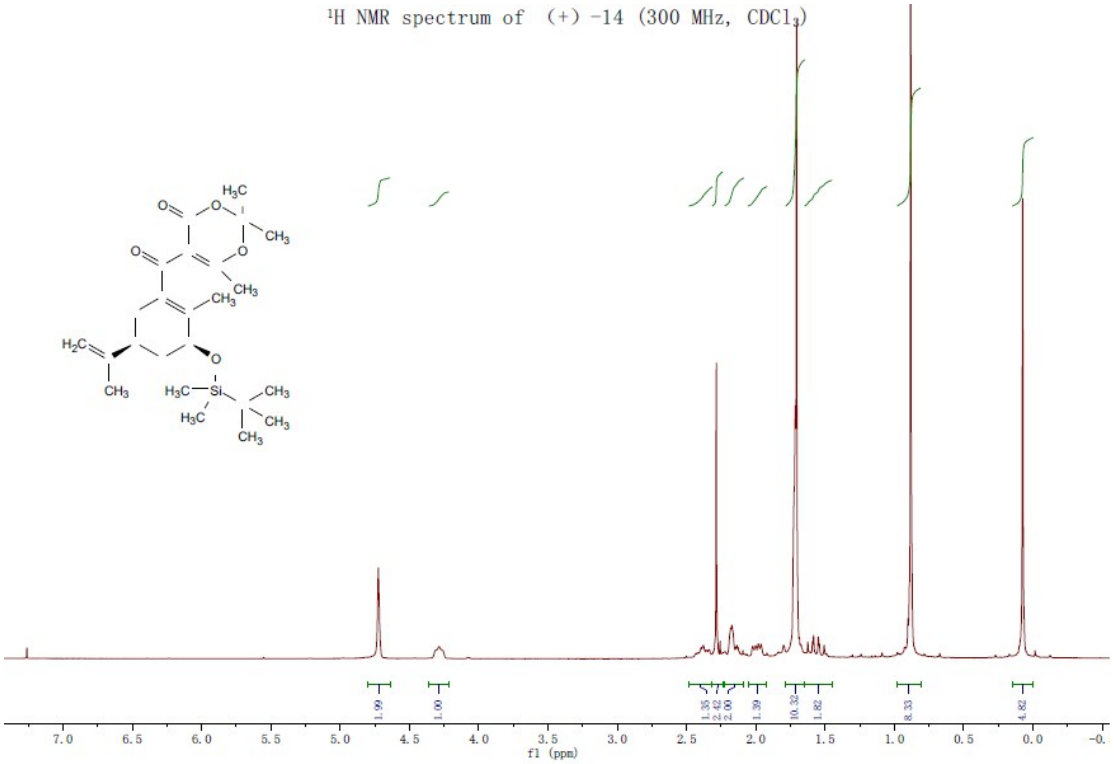


Figure S13. ^{13}C NMR spectrum of（+）-14 in $CDCl_3$

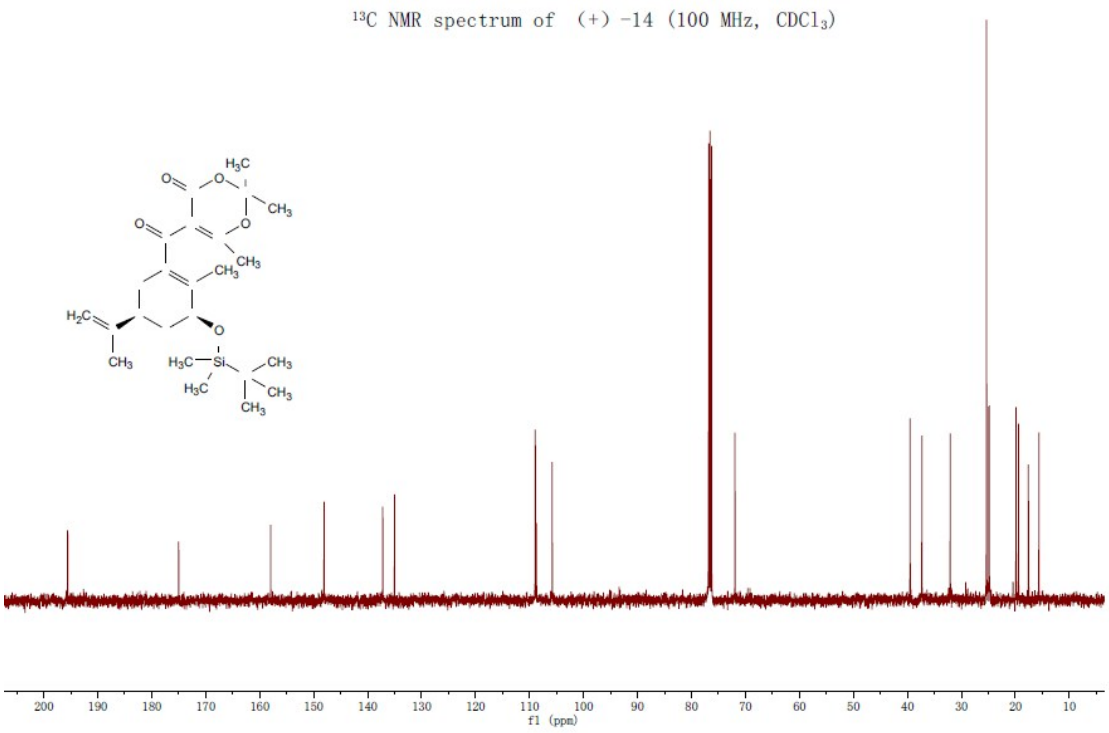


Figure S14. ^{1}H NMR spectrum of（+）-15 in $CDCl_3$

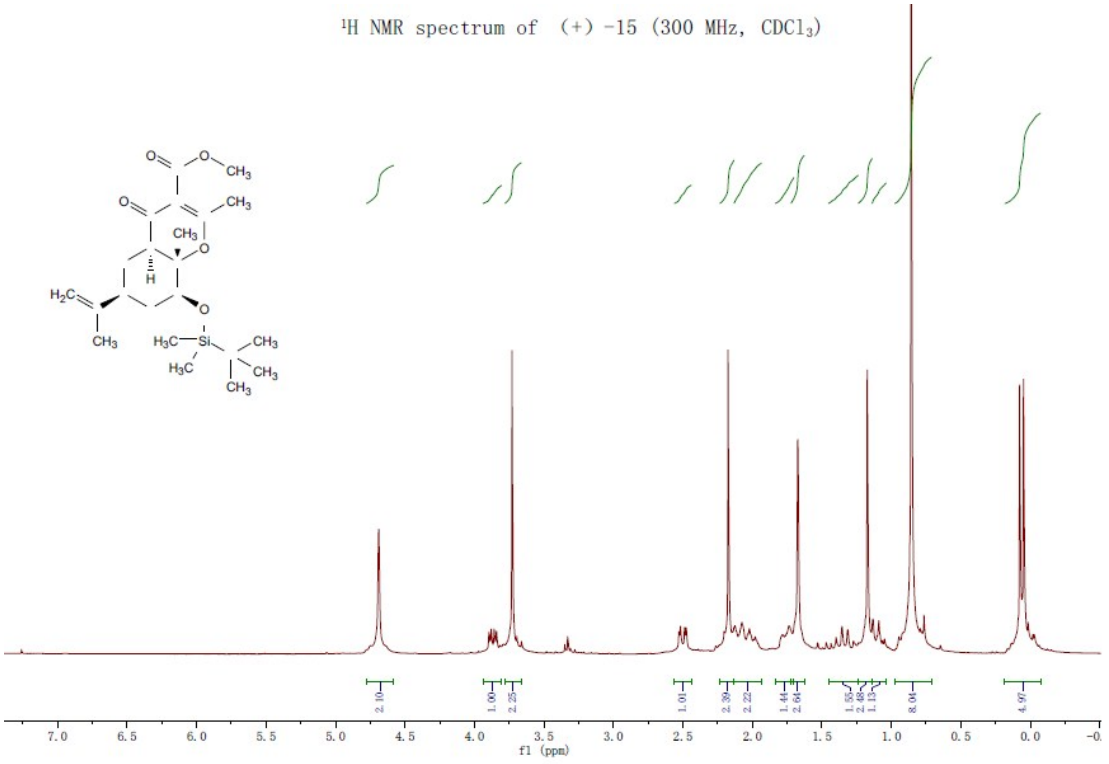


Figure S15. ^{1}H NMR spectrum of（+）-15 in $CDCl_3$

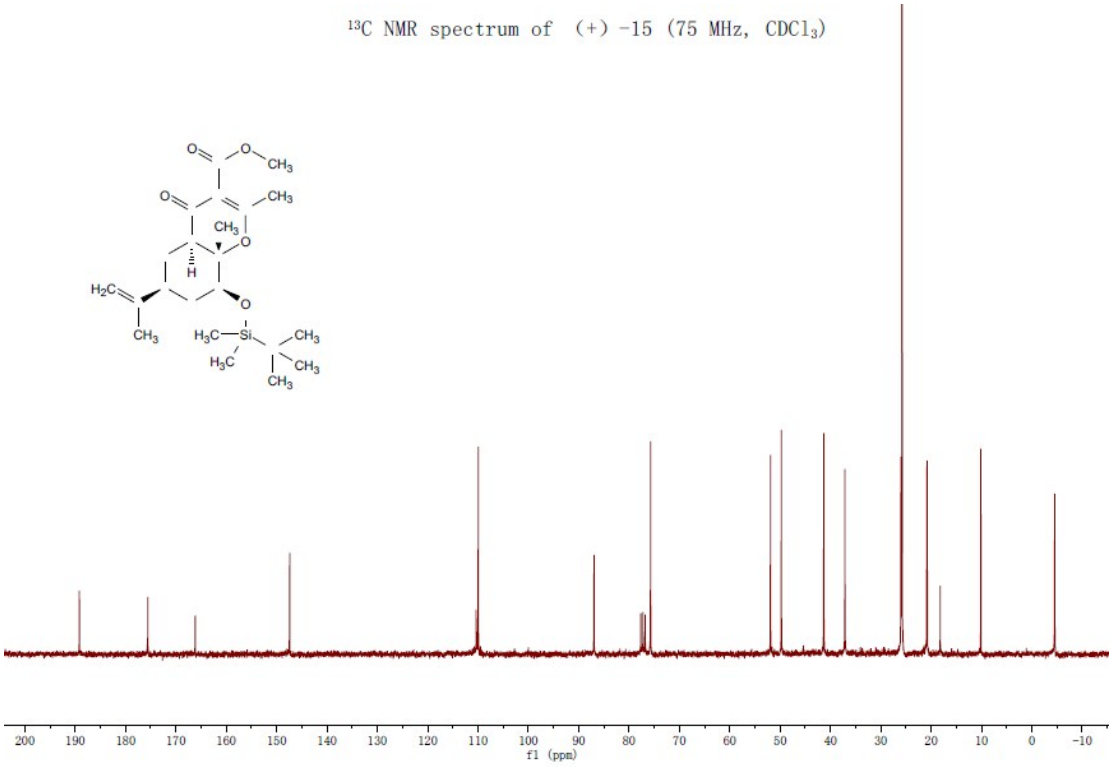


Figure S16. ^{1}H NMR spectrum of（+）-16 in $CDCl_3$

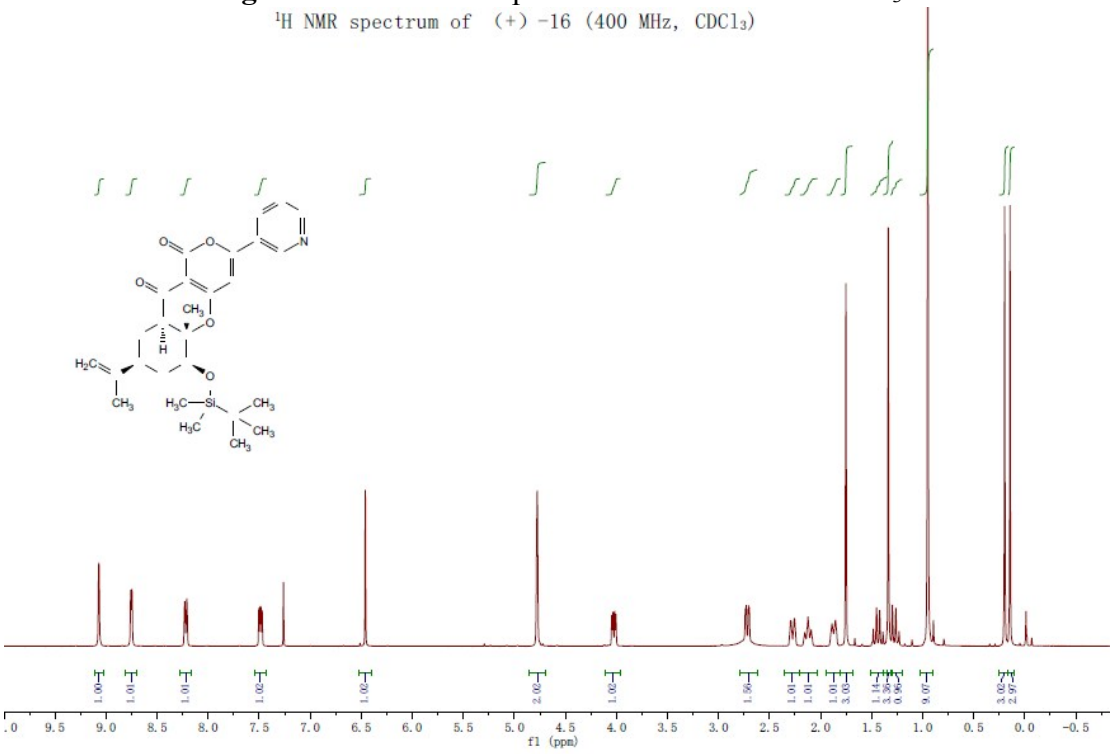


Figure S17. ^{13}C NMR spectrum of（+）-16 in $CDCl_3$

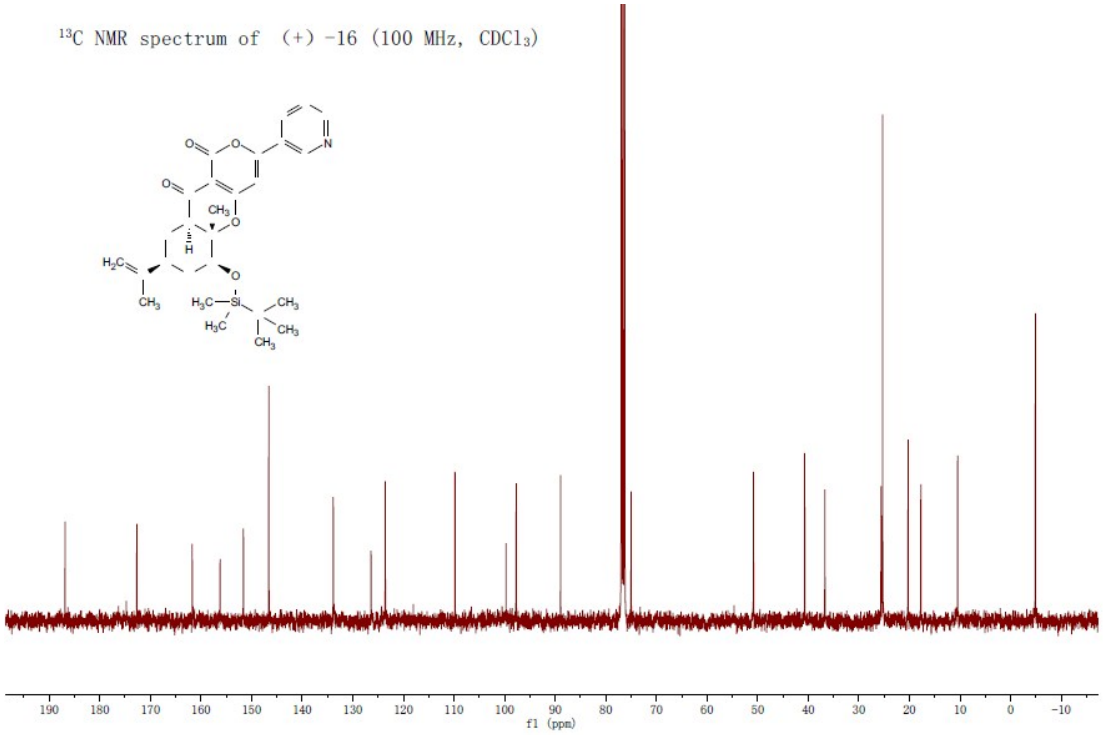


Figure S18. ^{1}H NMR spectrum of（+）-17A in $CDCl_3$

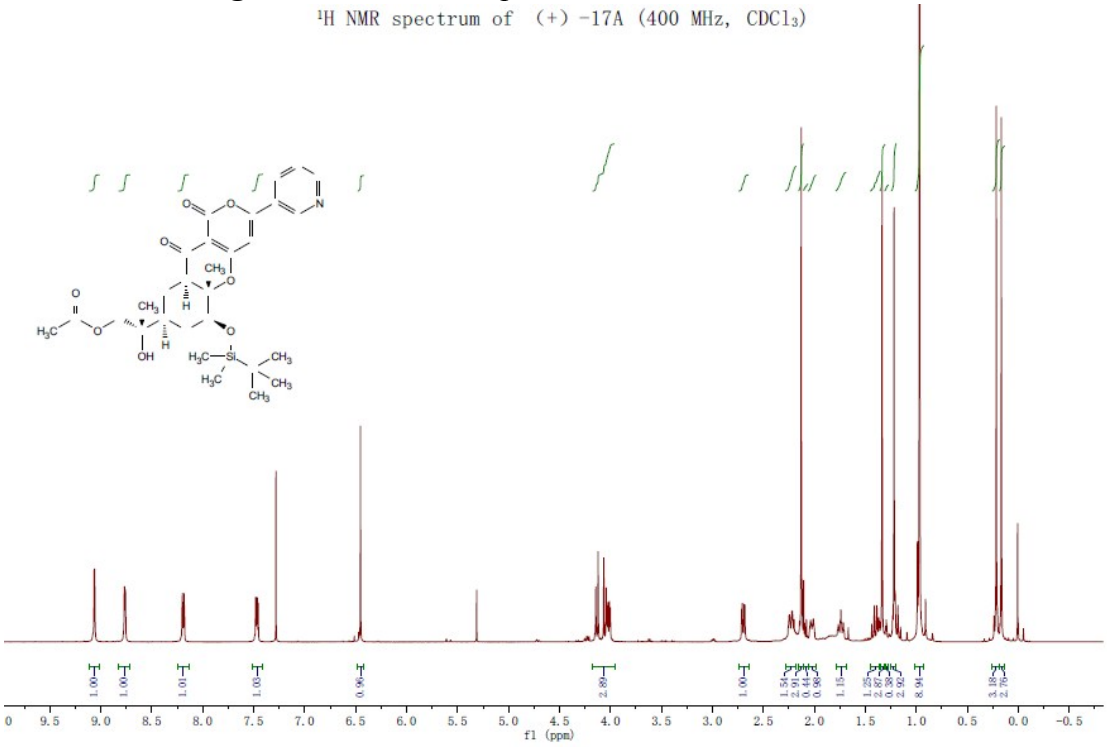


Figure S19. ^{13}C NMR spectrum of（+）-17A in $CDCl_3$

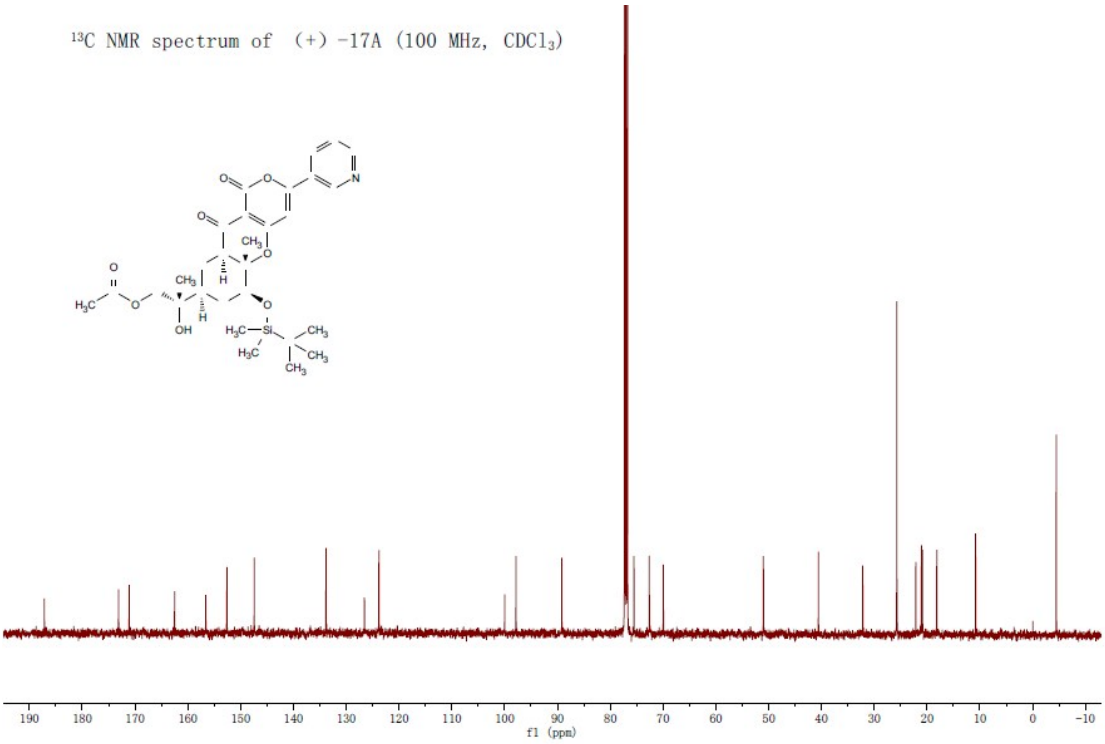


Figure S20. ^{1}H NMR spectrum of（+）-17B in $CDCl_3$

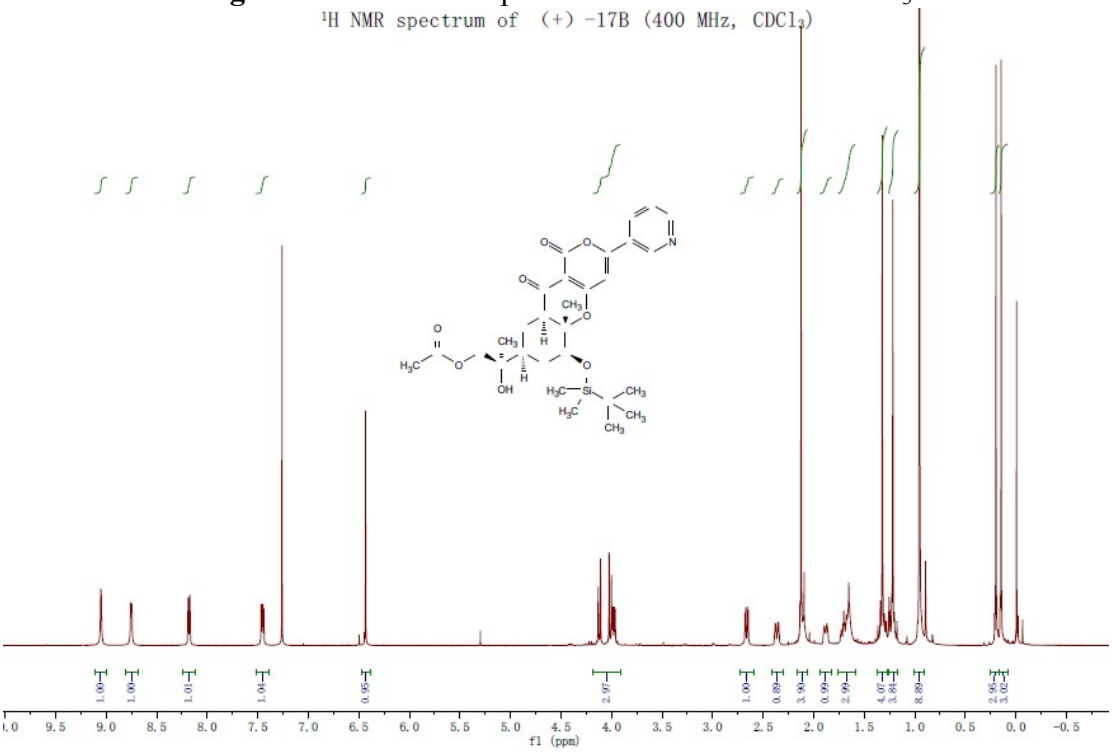


Figure S21. ^{13}C NMR spectrum of（+）-17B in $CDCl_3$

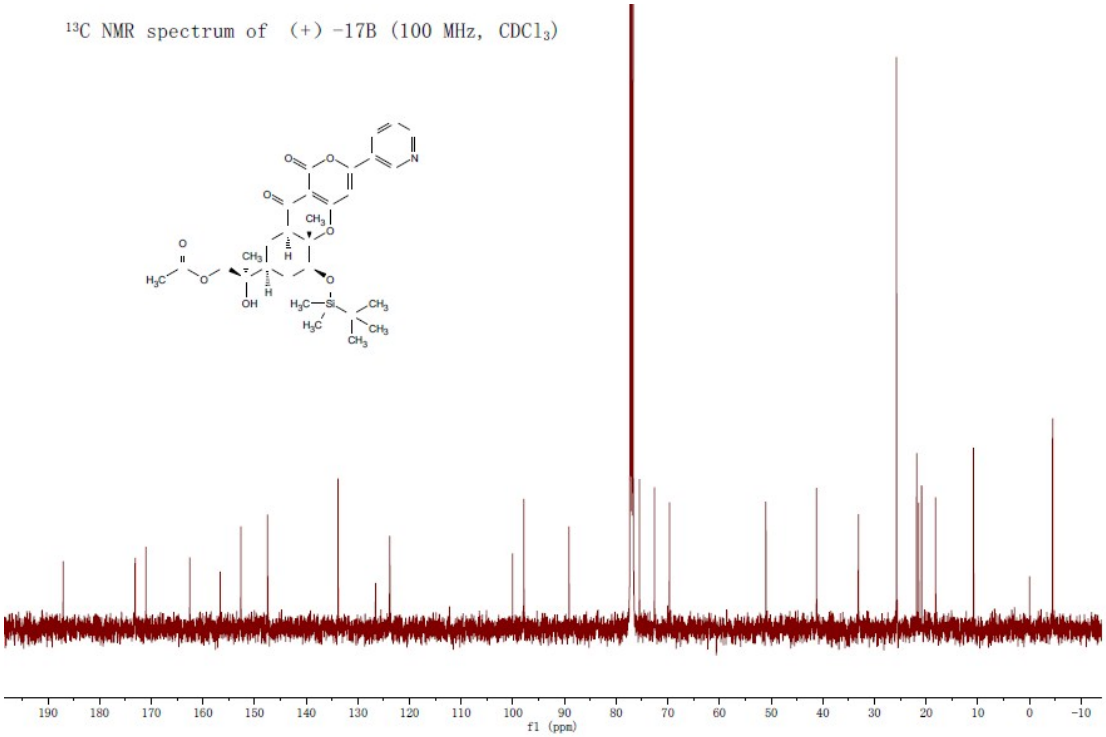


Figure S22. ^{1}H NMR spectrum of（+）-2 in $CDCl_3$

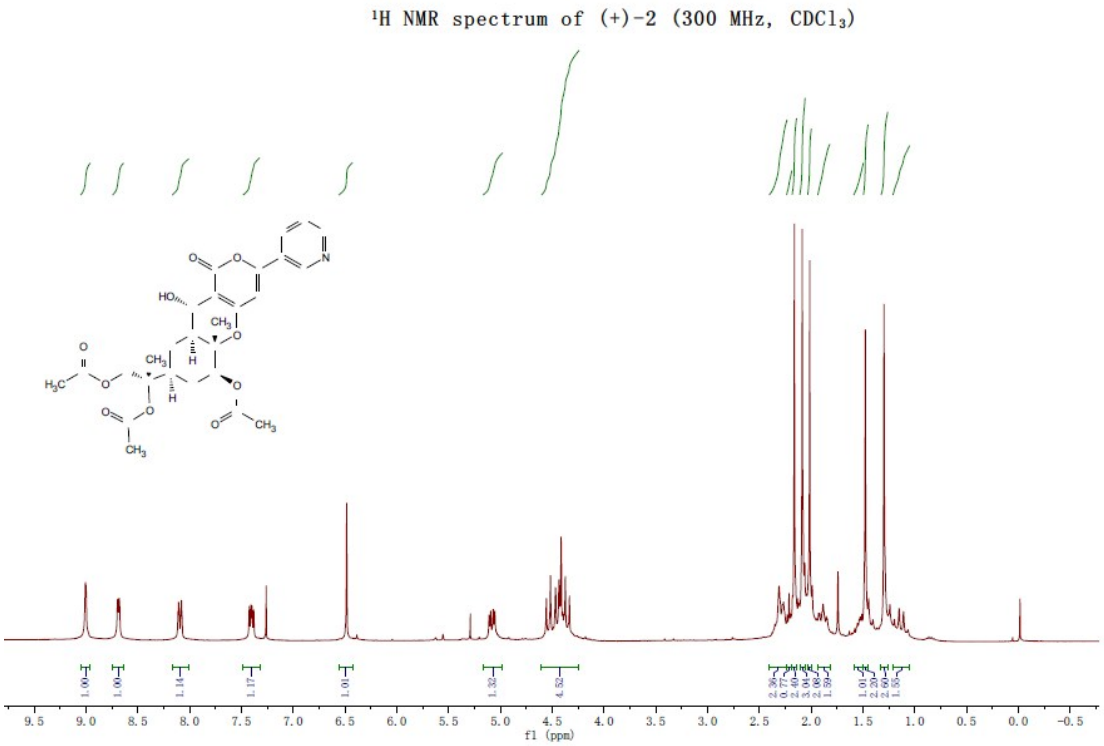


Figure S23. ^{13}C NMR spectrum of（+）-2 in $CDCl_3$

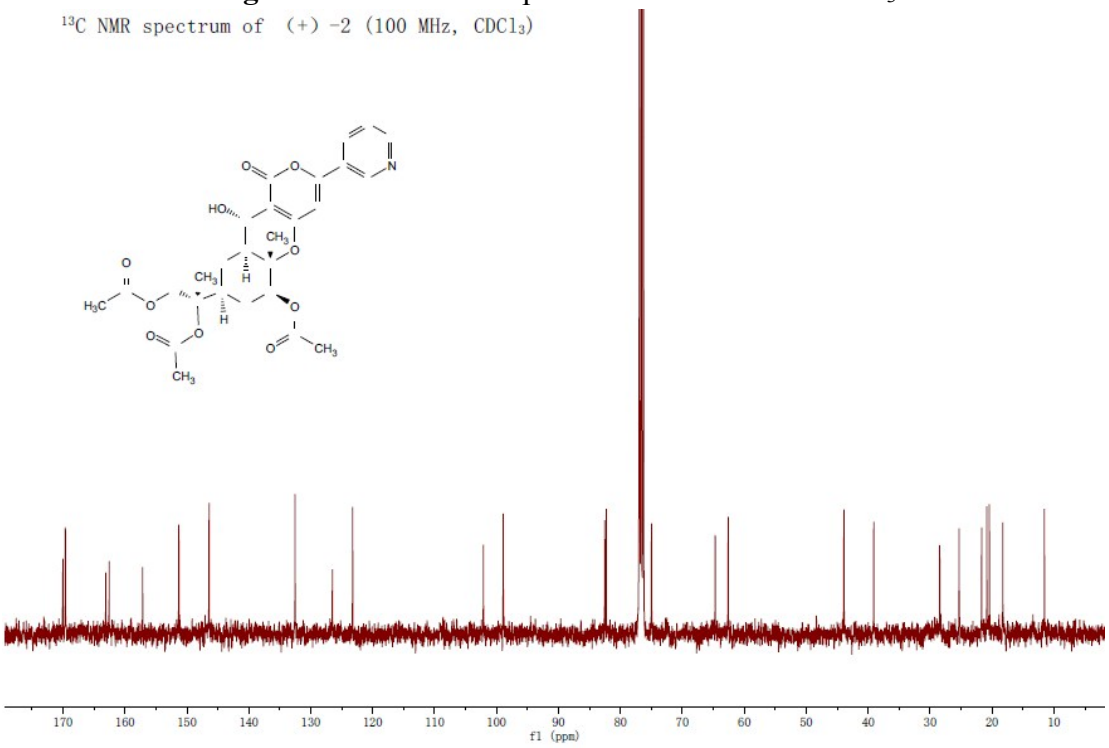


Figure S24. ^{1}H-^{1}H COSY spectrum of（+）-2 in $CDCl_3$

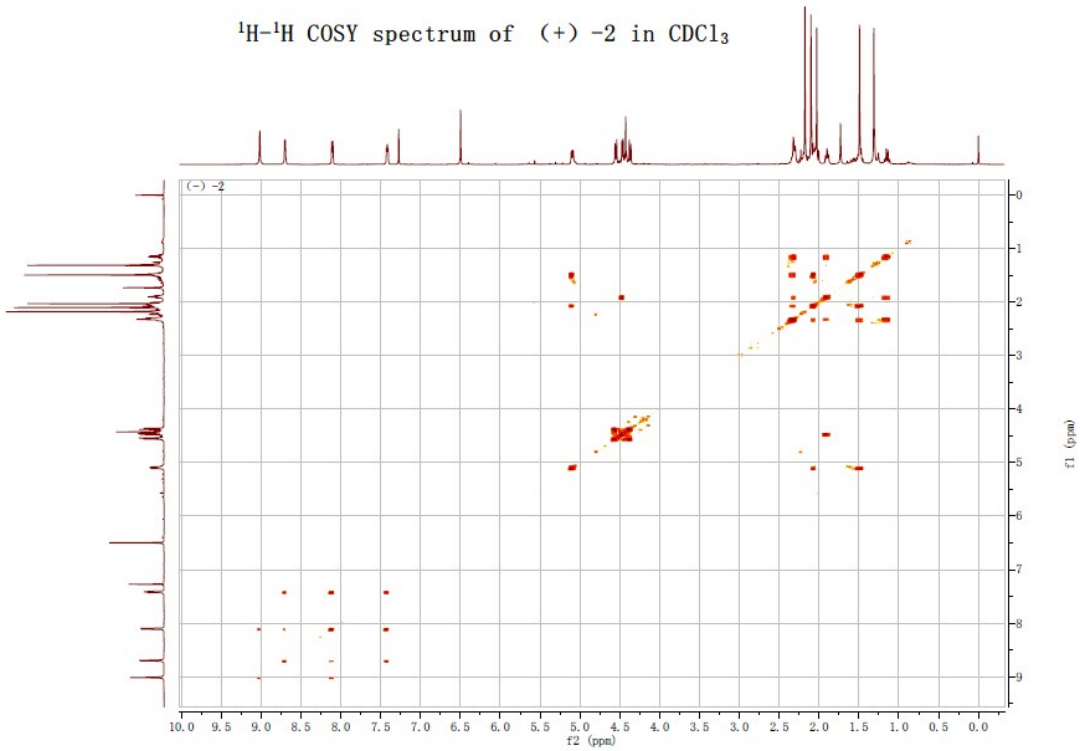


Figure S25. HMBC spectrum of（+）-2 in $CDCl_3$

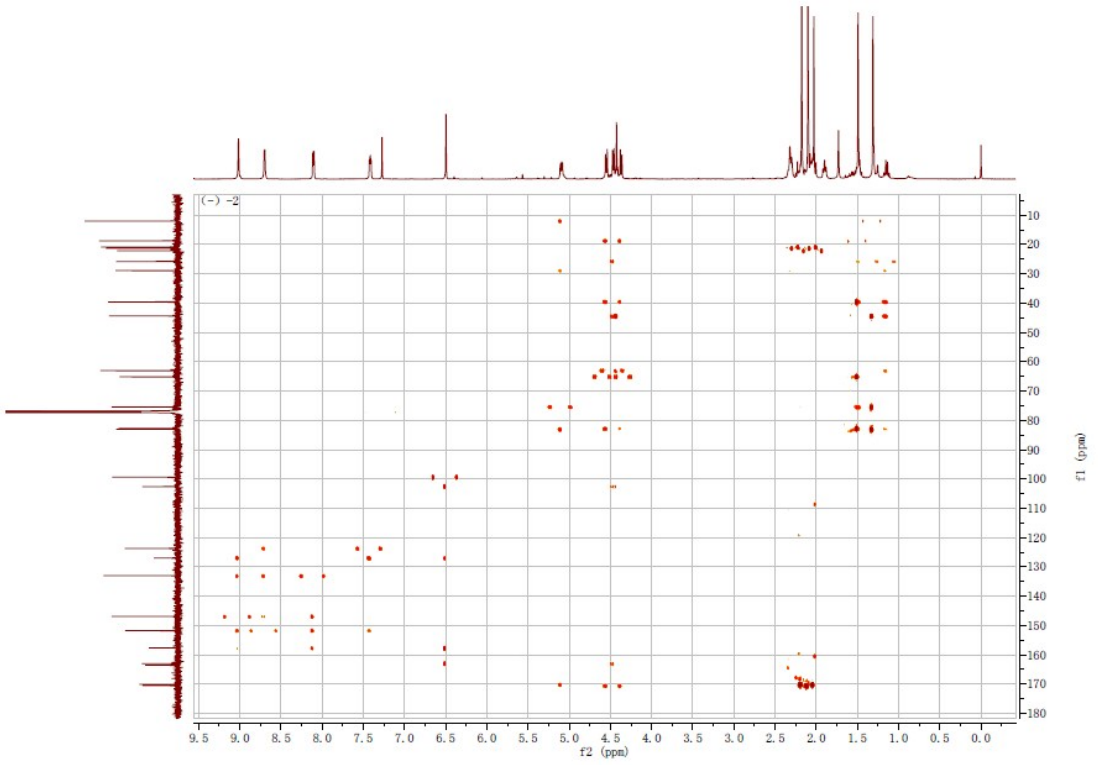


Figure S26. HSQC spectrum of（+）-2 in $CDCl_3$

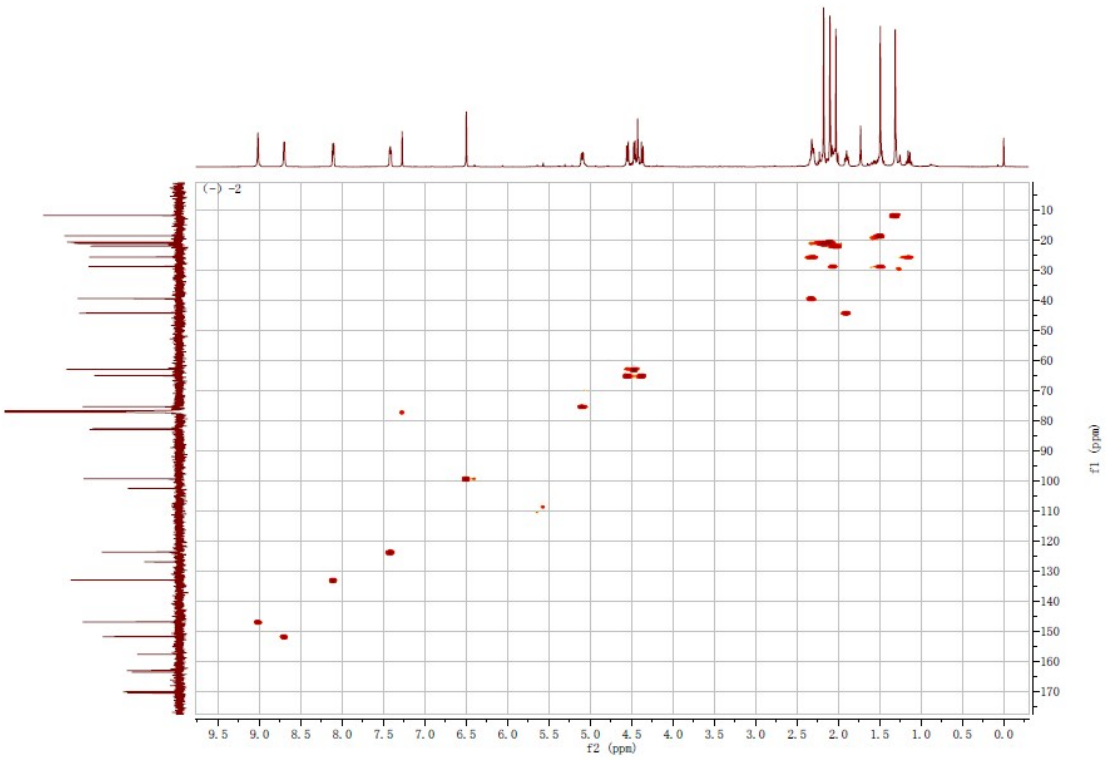


Figure S27. NOESY spectrum of（+）-2 in $CDCl_3$

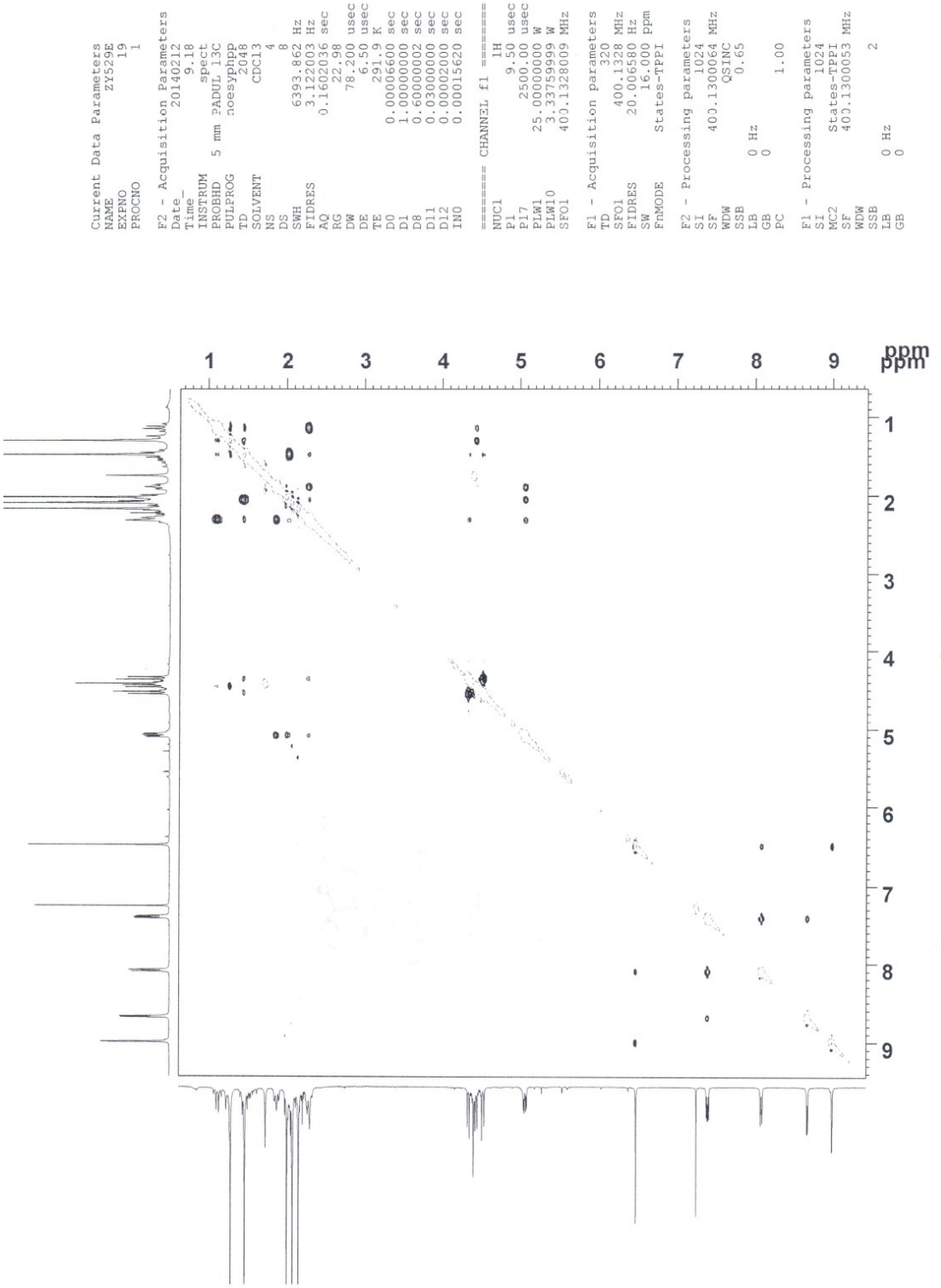


Figure S28. ^{1}H NMR spectrum of（+）-3 in $CDCl_3$

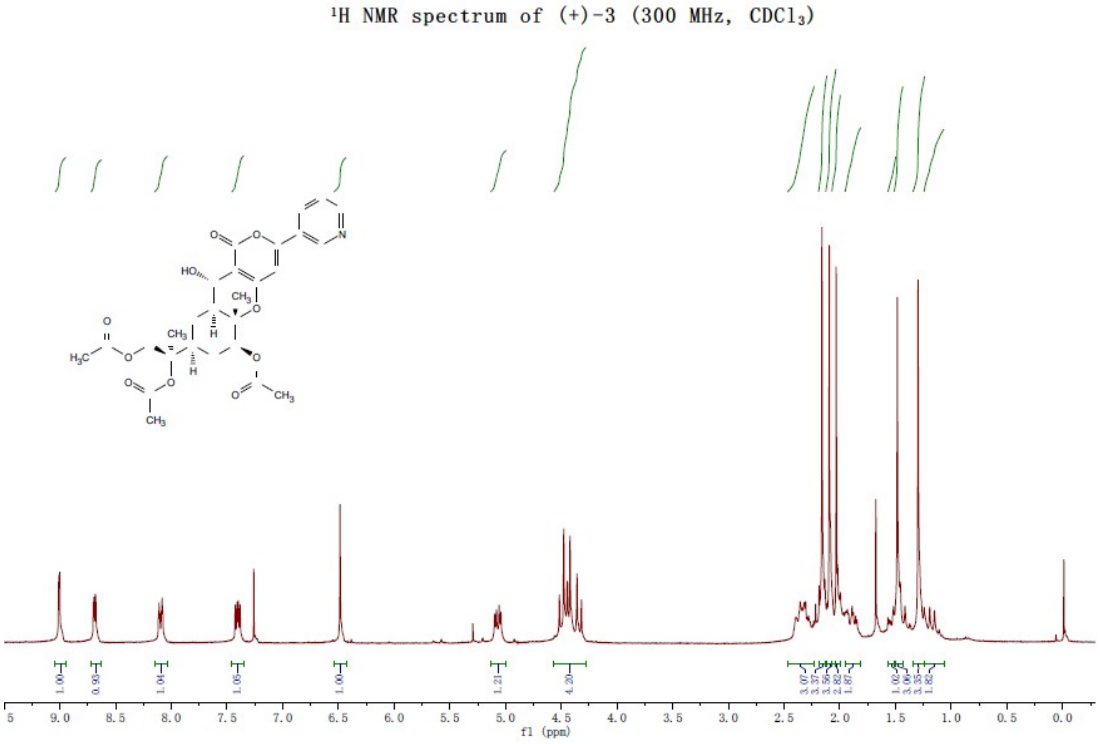


Figure S29. ^{13}C NMR spectrum of（+）-3 in $CDCl_3$

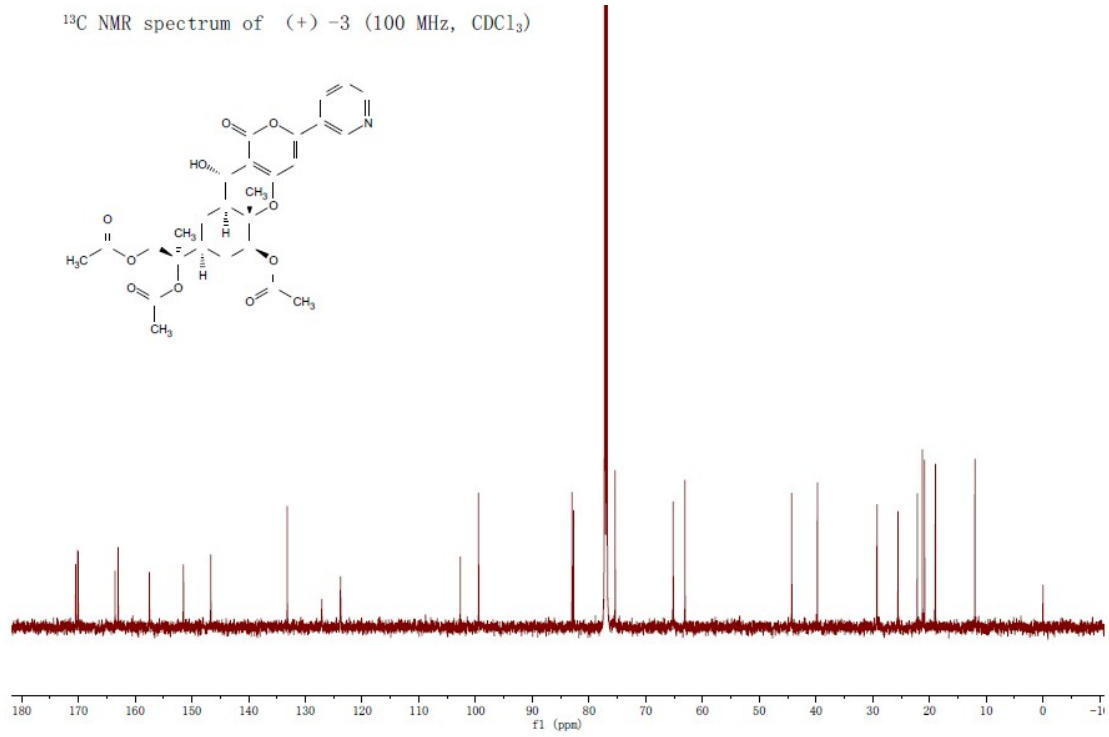


Figure S30. ^{1}H-^{1}H COSY spectrum of（+）-3 in $CDCl_3$

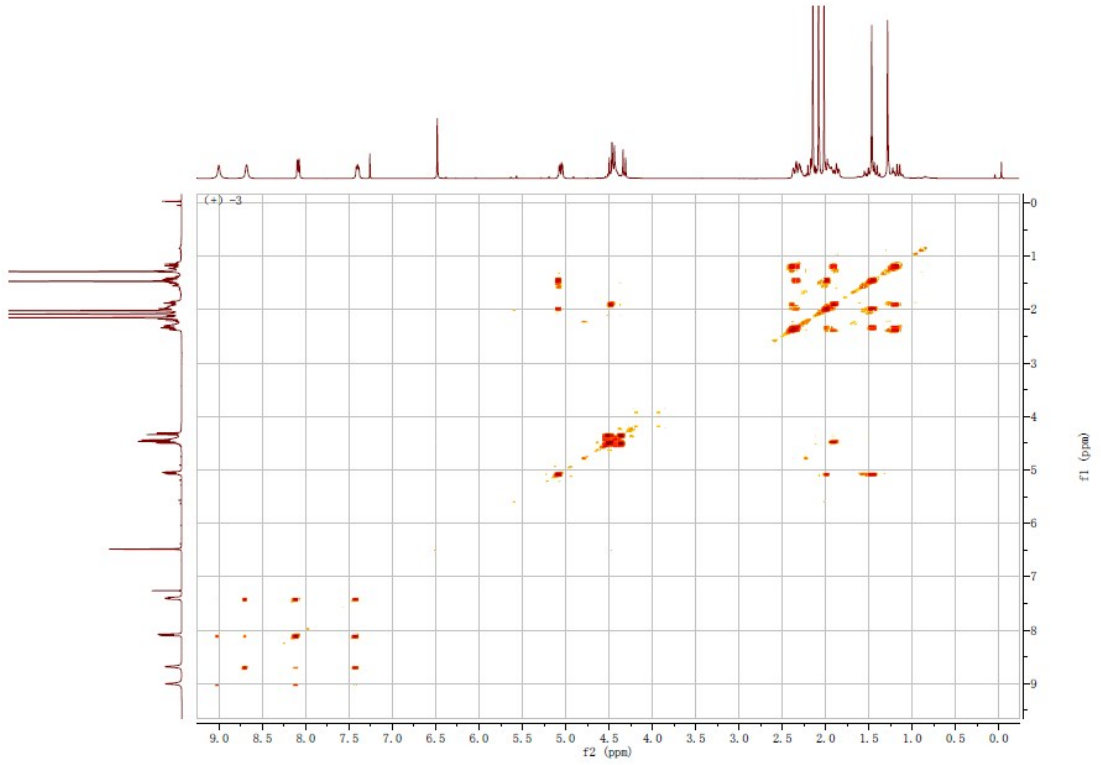


Figure S31. HMBC spectrum of（+）-3 in $CDCl_3$

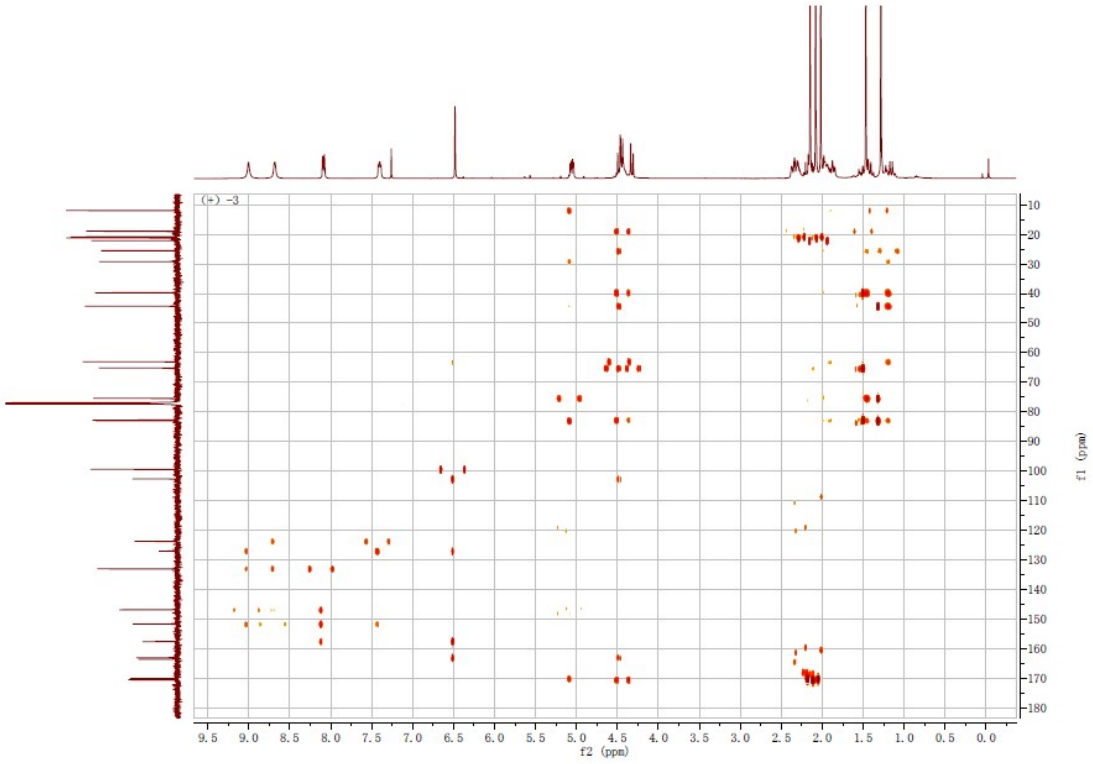


Figure S32. HSQC spectrum of（+）-3 in $CDCl_3$

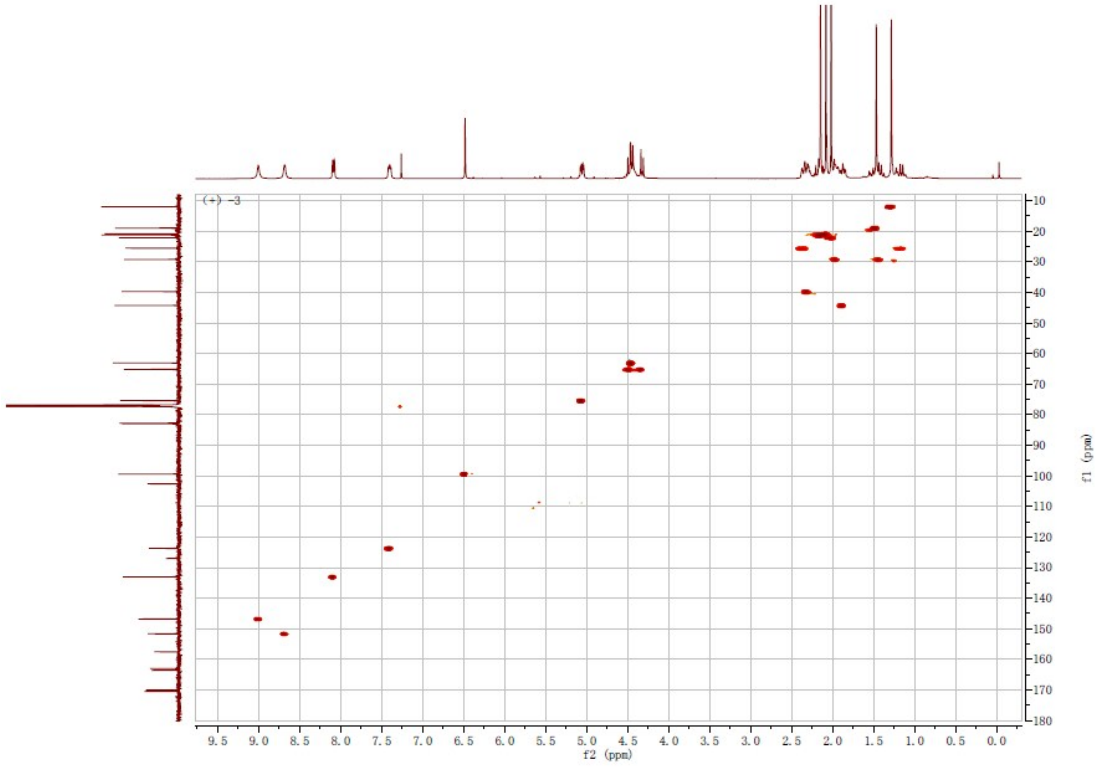


Figure S33. NOESY spectrum of（+）-3 in $CDCl_3$

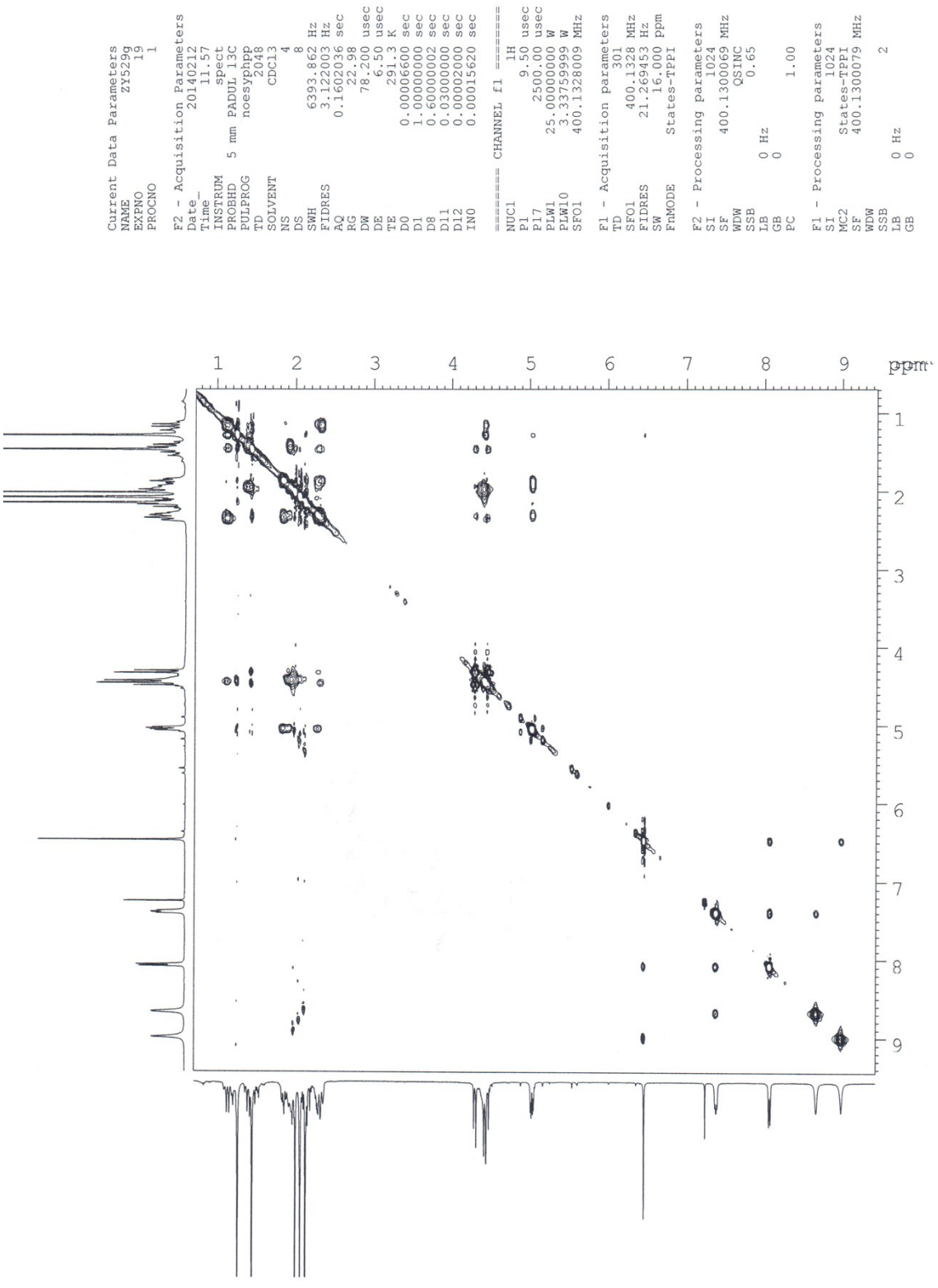


Figure S34. ^{1}H NMR spectrum of（+）-4 in $CDCl_3$

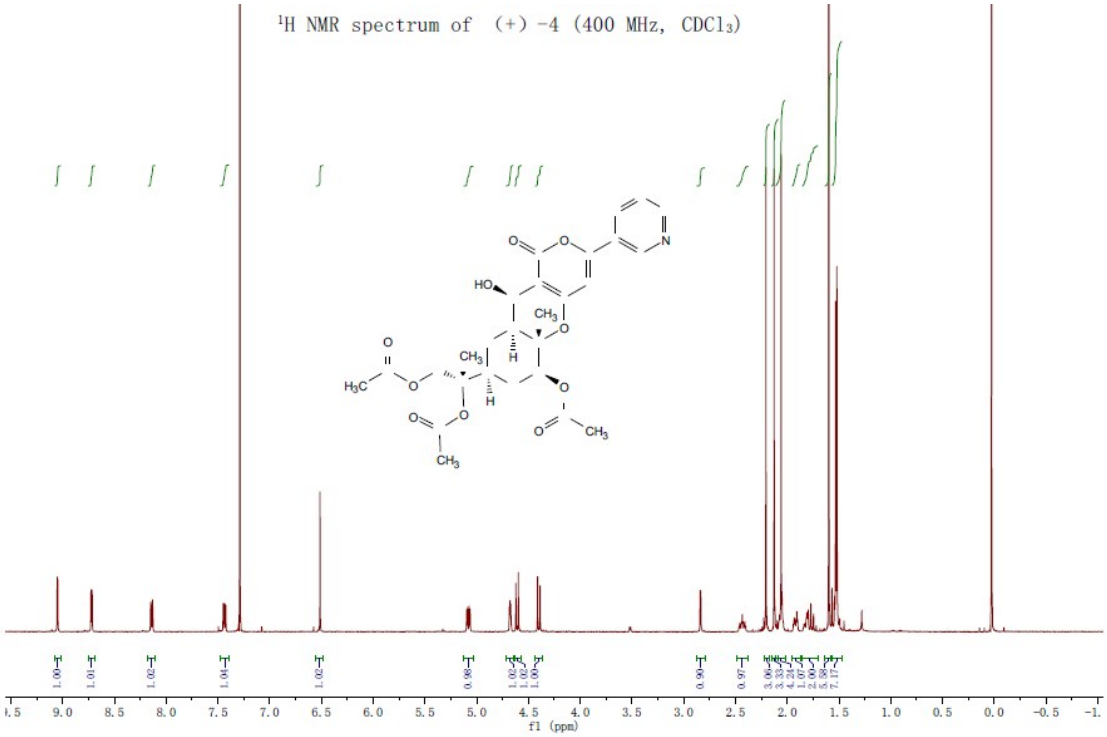


Figure S35. ^{13}C NMR spectrum of（+）-4 in $CDCl_3$

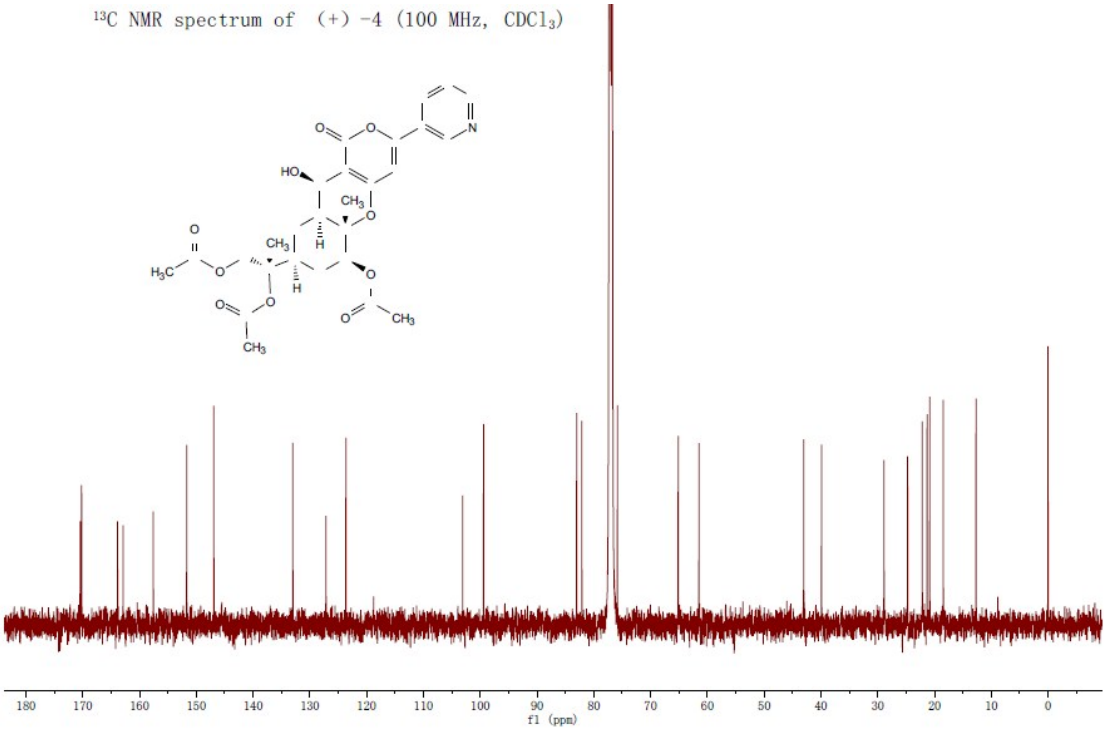


Figure S36. ^{1}H-^{1}H COSY spectrum of（+）-4 in $CDCl_3$

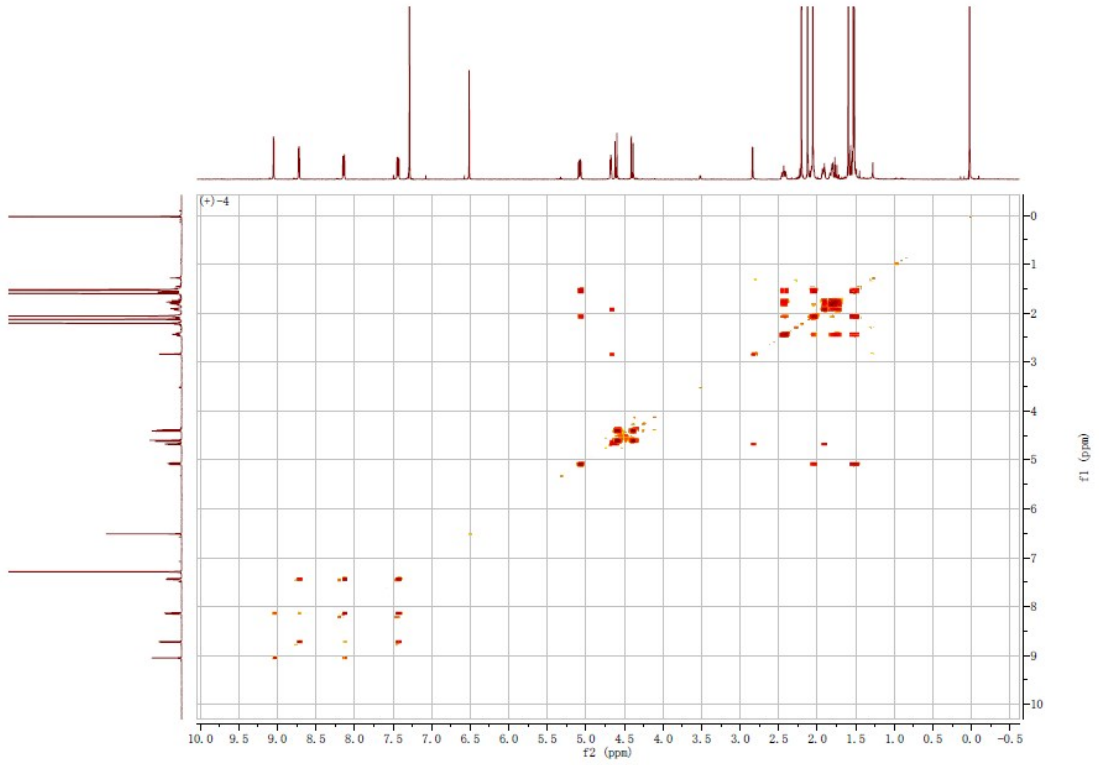


Figure S37. HMBC spectrum of（+）-4 in $CDCl_3$

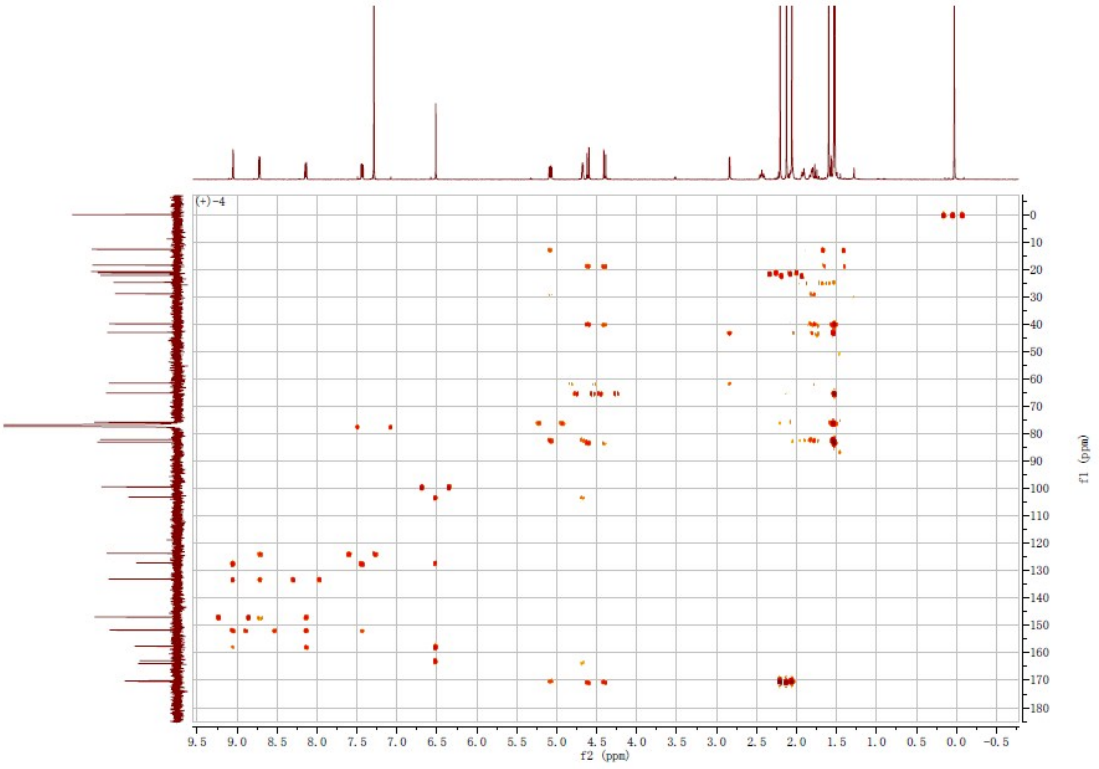


Figure S38. HSQC spectrum of（+）-4 in $CDCl_3$

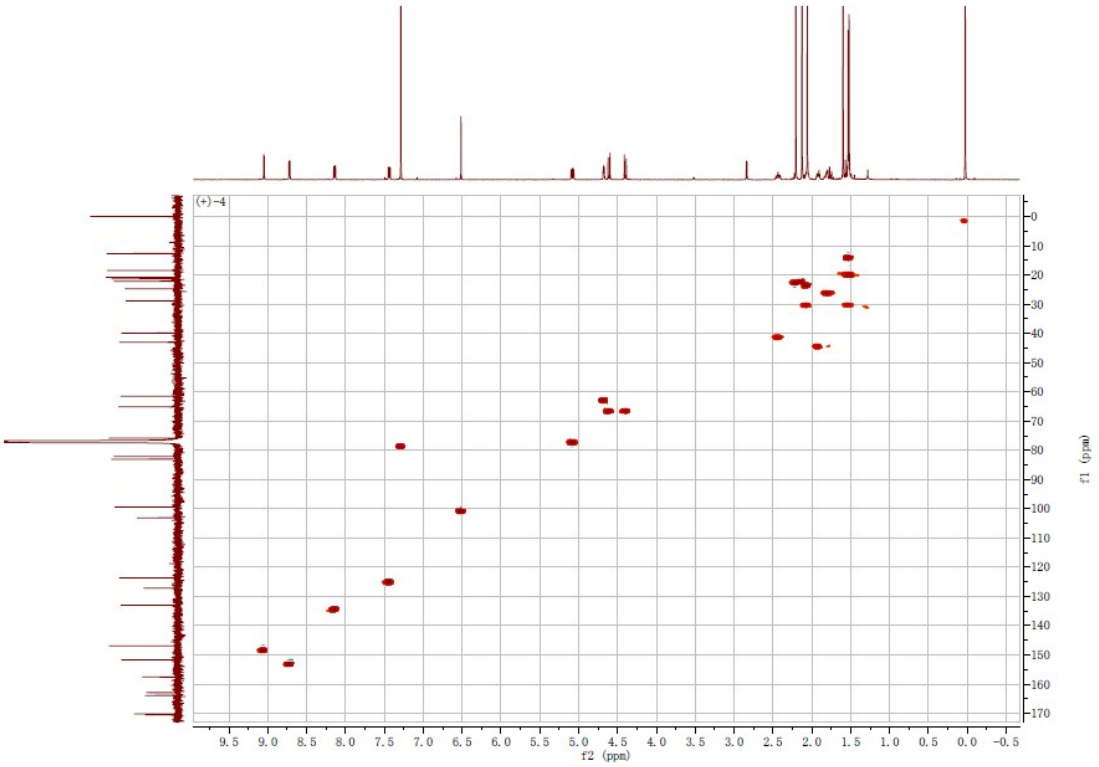


Figure S39. NOESY spectrum of（+）-4 in $CDCl_3$

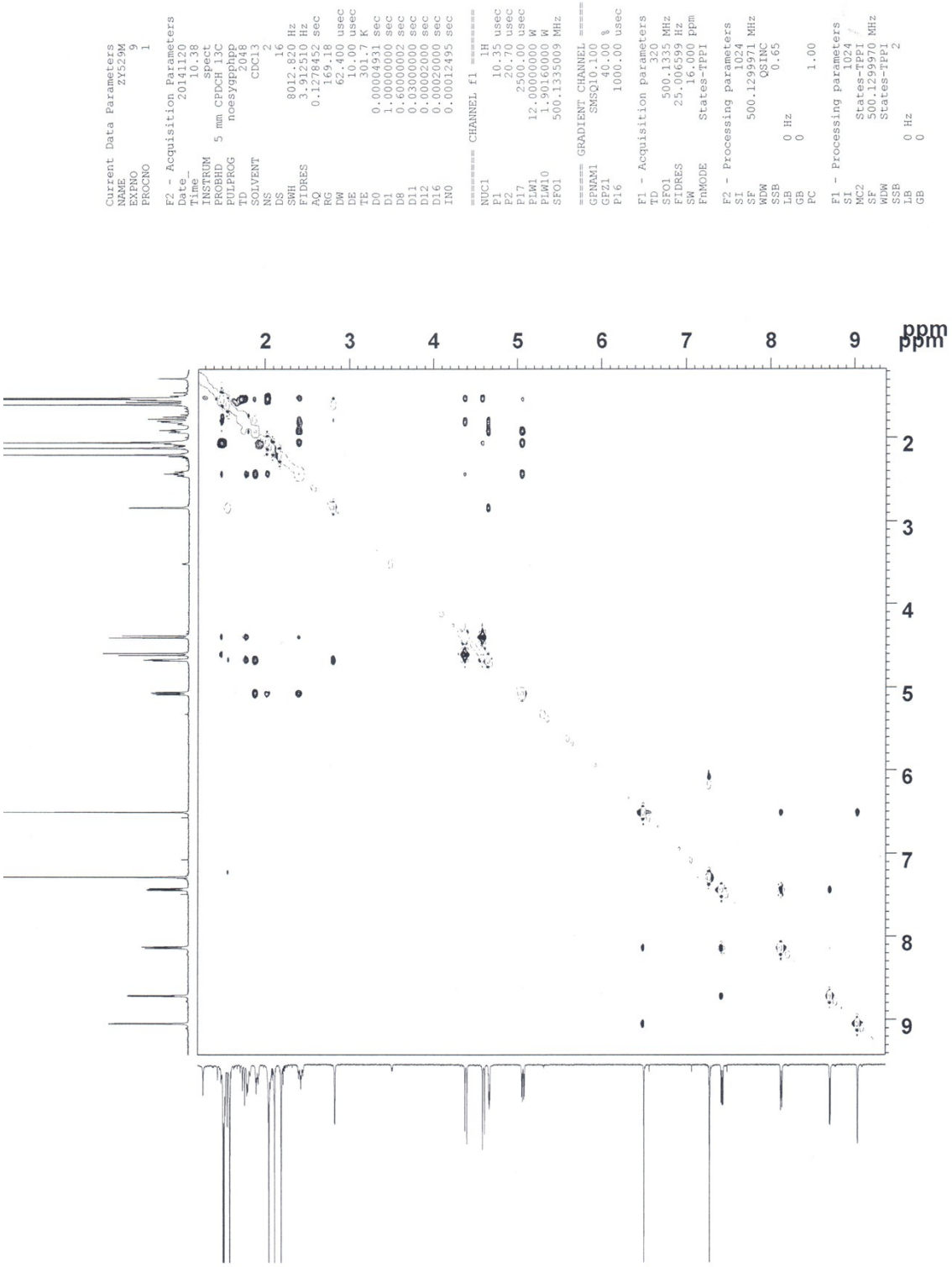


Figure S40. ^{1}H NMR spectrum of（+）-5 in $CDCl_3$

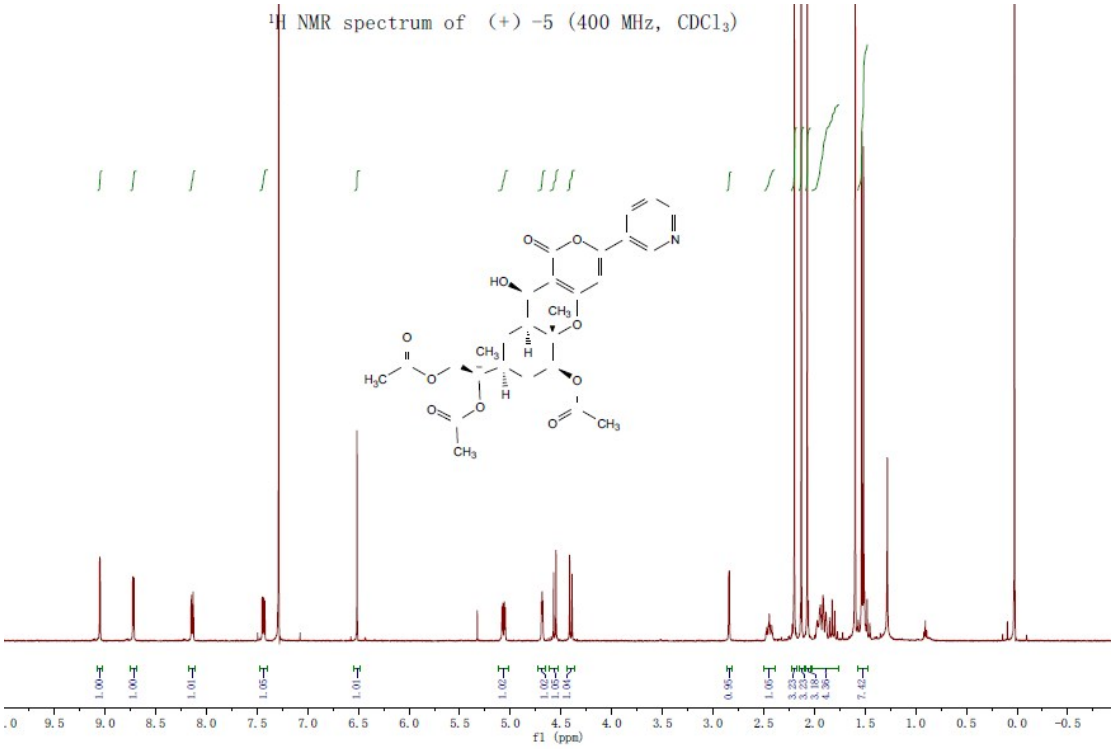


Figure S41. ^{13}C NMR spectrum of（+）-5 in $CDCl_3$

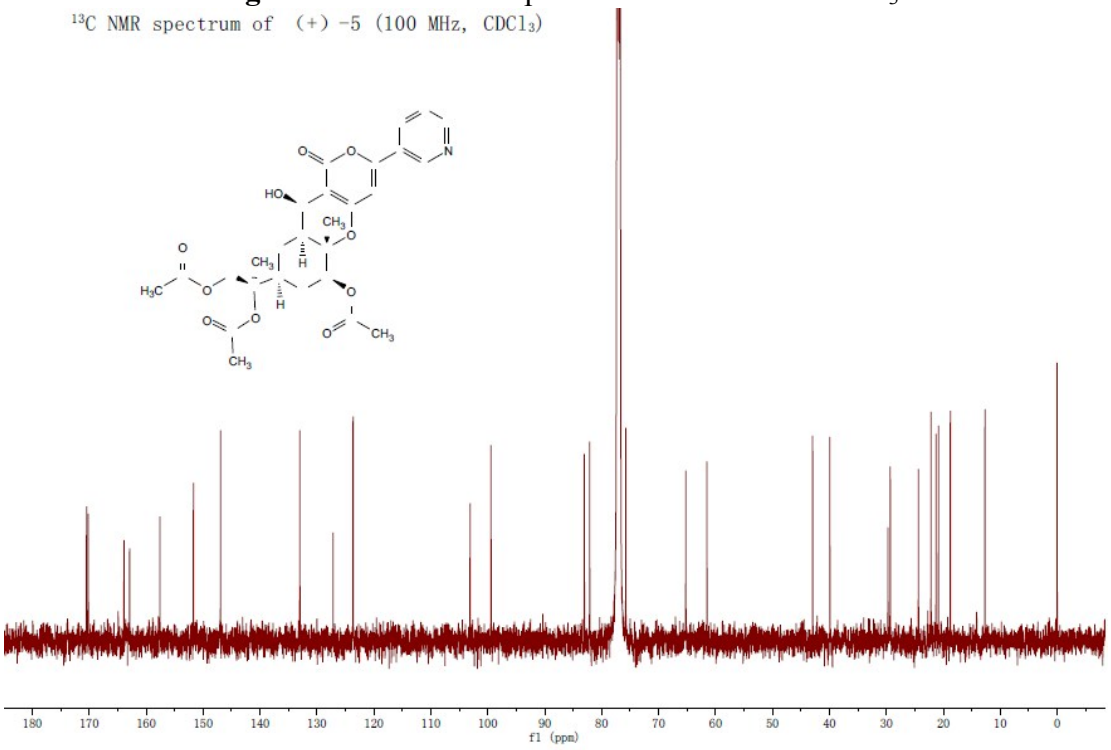


Figure S42. ^{1}H-^{1}H COSY spectrum of（+）-5 in $CDCl_3$

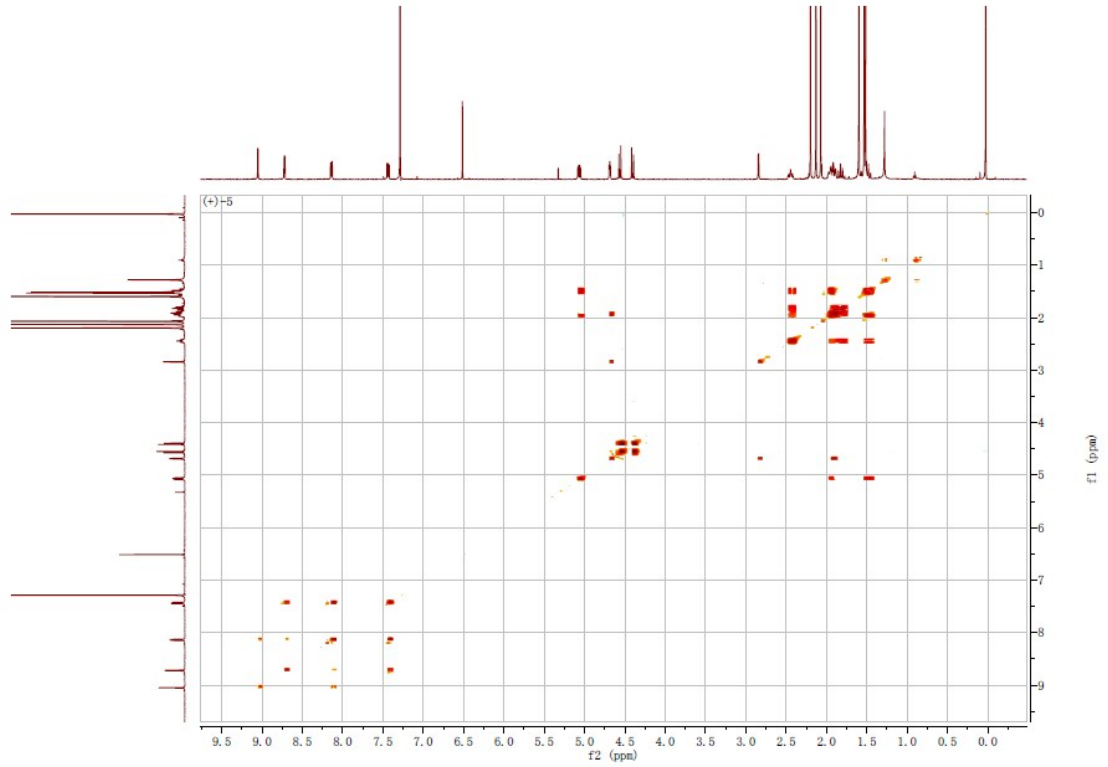


Figure S43. HMBC spectrum of（+）-5 in $CDCl_3$

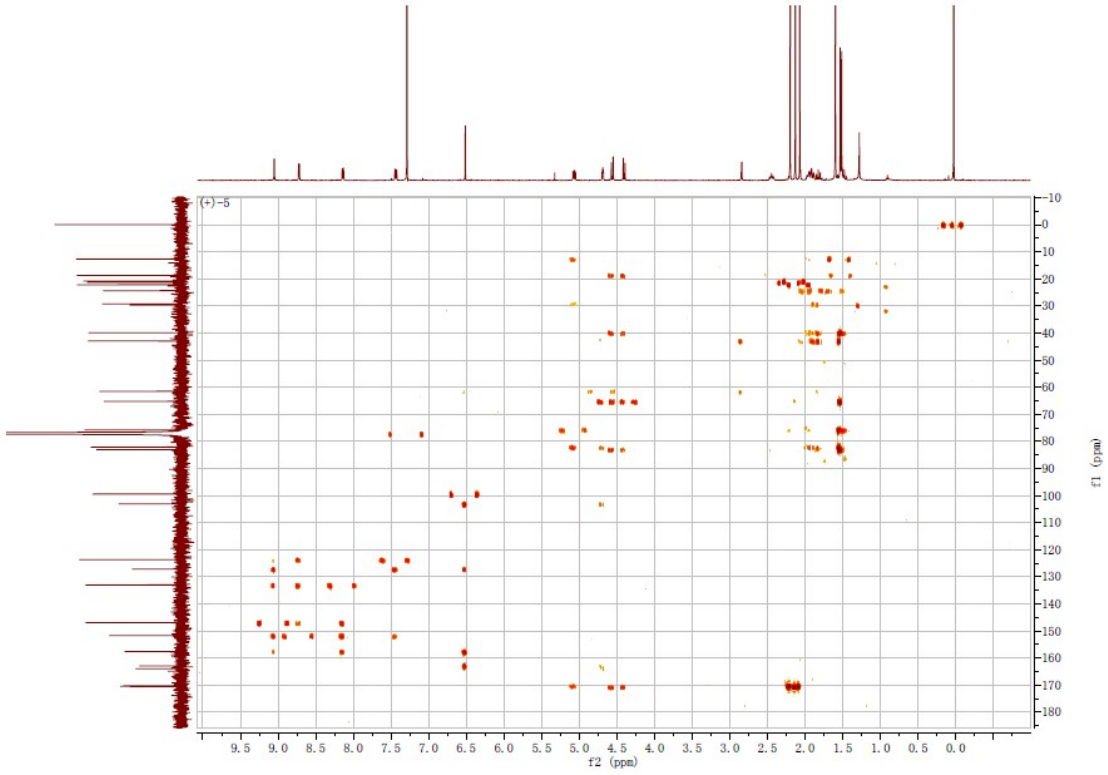


Figure S44. HSQC spectrum of（+）-5 in $CDCl_3$

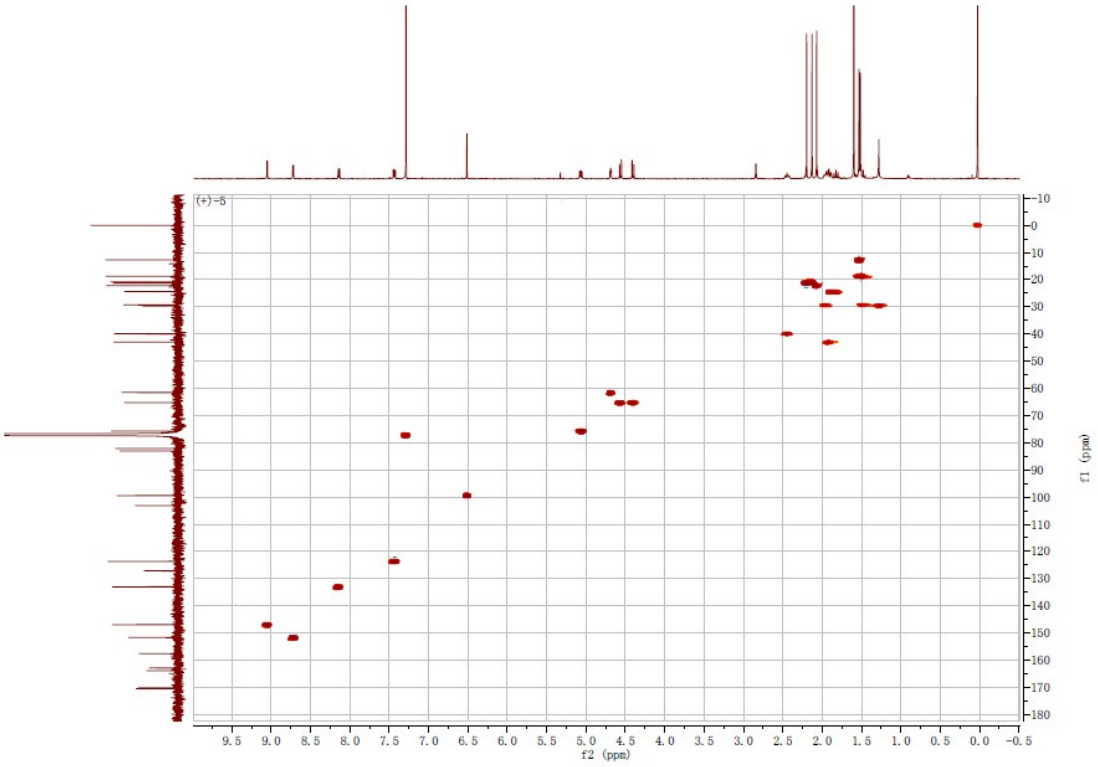
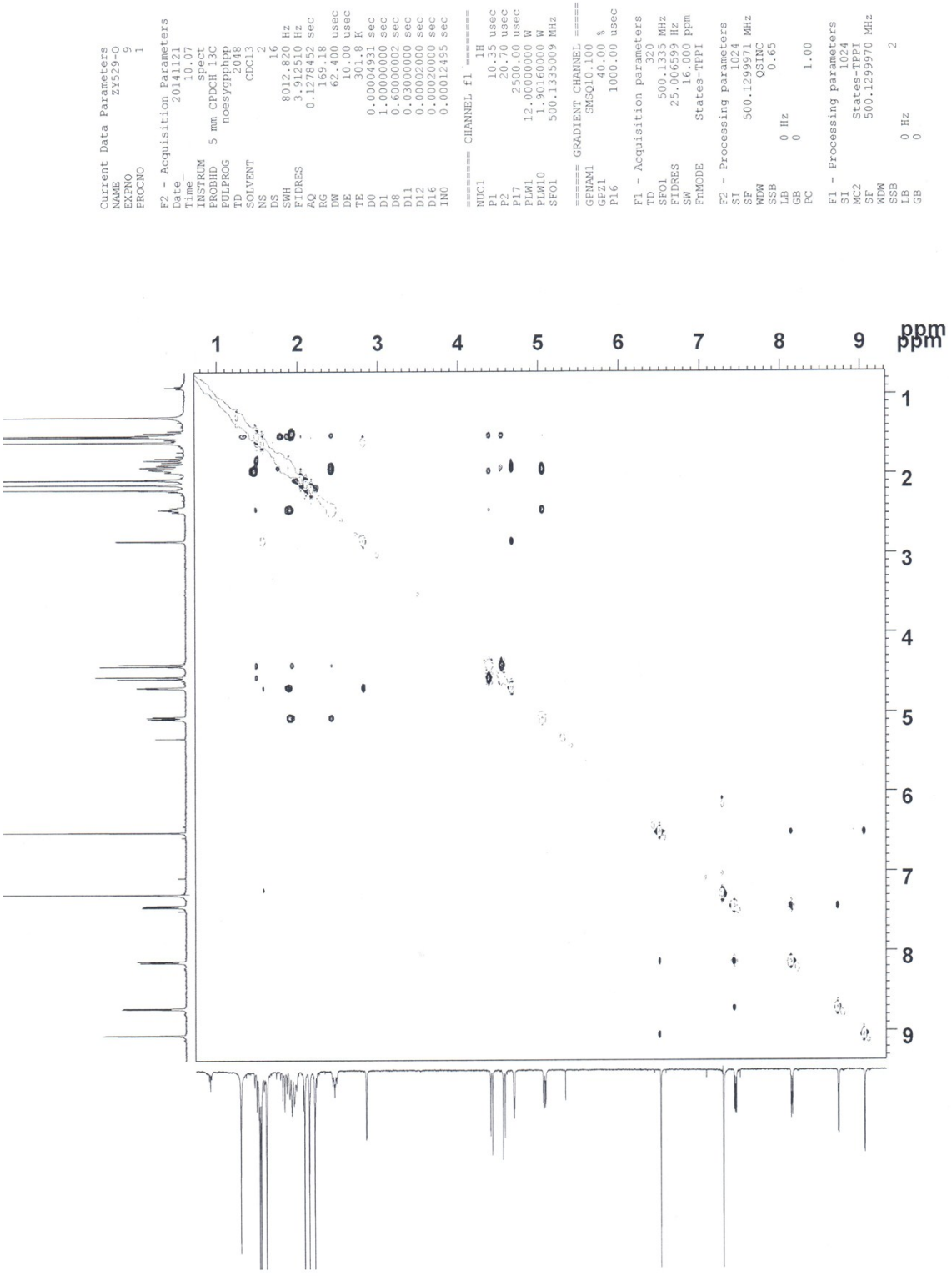


Figure S45. NOESY spectrum of（+）-5 in $CDCl_3$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