Supporting Information

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Steroidal Components from the Roots and Rhizomes of *Smilacina henryi* and Their Cytotoxic Activities

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Table of Contents	page

S1: Experimental section

S.1.General experimental procedures

Optical rotation was measured using a Rudolph Autopol VI polarimeter (Rudolph, USA); IR spectra were recorded on a Nicolet iS10 instrument (Thermo Fisher Scientific, USA); 1D and 2D NMR spectra were recorded on a Bruker-Avance 400 instrument (Bruker Corp. Karlsruhe, Germany); Semipreparative HPLC was performed on Agilent infinity II system equipped with a UV detector and a YMC-Pack-ODS-A (10 mm × 250 mm, 5μm particles) column. The HR-ESI-MS spectra were taken on an Agilent Technologies 6650 Q-TOF (Agilent Technologies). Sephadex LH-20 gel and ODS C_{18} (5 μm) silica gel was purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Silica gel was purchased from Qingdao Haiyang Chemical Group Corporation (Qingdao, China).

S.1.2.Extraction and Isolation

The air-dried roots and rhizomes of *S. henryi* (6.6 kg) were extracted with 80% EtOH under reflux for three times (2h, 2h, 1h, successfully). The concentrated residue was partitioned with petroleum ether (PE) and *n*-BuOH successively. The *n*-BuOH extract (130.2 g) was subjected to column chromatography (CC) on silica gel (1 kg), eluting with gradient solvent system (CH_2Cl_2-MeOH-H_2O, 100:0:0 − 60:40:10) to give six fractions (Fr.1 – Fr.6). Fr.2 (8.5 g) was subjected to column chromatography (CC) on silica gel (100 g), eluting with (PE-EtOAC, 20:1−1:1) to give eight subfractions (Fr.2-1−Fr.2-8). Fr.2-4 (0.6 g) was purified by HPLC (YMC-Pack-ODS-A, 10 mm × 250 mm, 5 μm particles, flow rate: 2 mL/min) with MeCN-H_2O (82:18) as mobile phase to afford compound 8 (15.2 mg; t_R = 35 min) and compound 9 (6.1 mg; t_R = 26 min). Fr.2-6 (0.3 g) was purified by HPLC (YMC-Pack-ODS-A, 10 mm × 250 mm, 5 μm particles, flow rate: 2 mL/min) with MeCN-H_2O (78:82) as mobile phase to afford compound 6 (7.2 mg; t_R = 31 min) and compound 7 (8.0 mg; t_R = 28 min). Fr.4 (19.1 g) was subjected to CC on silica gel (200 g), eluting with (CH_2Cl_2-MeOH-H_2O, 100:10:0−80:20:5) to give six subfractions (Fr.4-1−Fr.4-6). Fr.4-2 (1.4 g) was subjected to CC on Sephadex LH-20 gel (100 g) eluting with (CH_2Cl_2-MeOH 100:100) to give six subfractions (Fr.4-2-1−Fr.4-2-6). Fr.4-2-6 (74.5 mg) was purified by HPLC (YMC-Pack-ODS-A, 10 mm × 250 mm, 5 μm particles, flow rate: 2.0 mL/min) with MeCN-H_2O (68:32) as mobile phase to afford compound 1 (15.7 mg; t_R = 38 min); Fr.4-4 (4.6 g) was subjected to CC on Sephadex LH-20 gel (100 g) eluting with (CH2Cl2-MeOH 100:100) to give eleven subfractions (Fr.4-4-1−Fr.4-4-11). Fr.4-4-2 (142.6 mg) was purified by HPLC (YMC-Pack-ODS-A, 10 mm × 250 mm, 5 μm particles, flow rate: 1.5 mL/min) with MeCN-H_2O (74:26) as mobile phase to afford compound 2 (7.6 mg; t_R = 41 min) and 3 (5.8 mg; t_R = 34 min); Fr.4-4-3 (213.7 mg) was purified by HPLC (YMC-Pack-ODS-A, 10 mm × 250 mm, 5 μm particles, flow rate: 1.5 mL/min) with MeCN-H_2O (75:25) as mobile phase to afford compound 4 (10.6 mg; t_R = 32 min) and 5 (16.9 mg; t_R = 27 min).

S.1.3. Cytotoxic activity assay

The cytotoxic activities assays toward the human HepG2 and SW620 cell lines were measured by the MTT method. Briefly, 1×10^4 ml^{-1} cells were seeded into 96-well plates and allowed to adhere for 24 h. Compounds **1—9** were dissolved in DMSO and diluted with complete medium to 6 degrees of concentration for inhibition rate determination. After incubation at 37.8℃ for 4 h, the supernatant was removed before adding DMSO (100 μL) to each well.

S.1.4. Acid Hydrolysis

Solution of **1** (6 mg) was hydrolyzed in 2 M hydrochloric acid (10 mL) at 80 ℃ for 2 h. After cooling, the solution was concentrated under vacuum, dissolved with water, and extracted twice with dichloromethane (CH_2Cl_2). The aqueous part was subjected to CC on ODS C_{18} silica gel (10 g), eluting with (MeCN-H_2O, 5:95) to give one product. The D configuration of the glucose moiety in **1** was confirmed through its optical rotation data (Glc: $[\alpha]_D^{20}$ +40.5, MeOH) and R_f values (BuOH-AcOH-H_2O, 4:1:5 upper layer Glc: 0.36) with the authentic sugar sample.

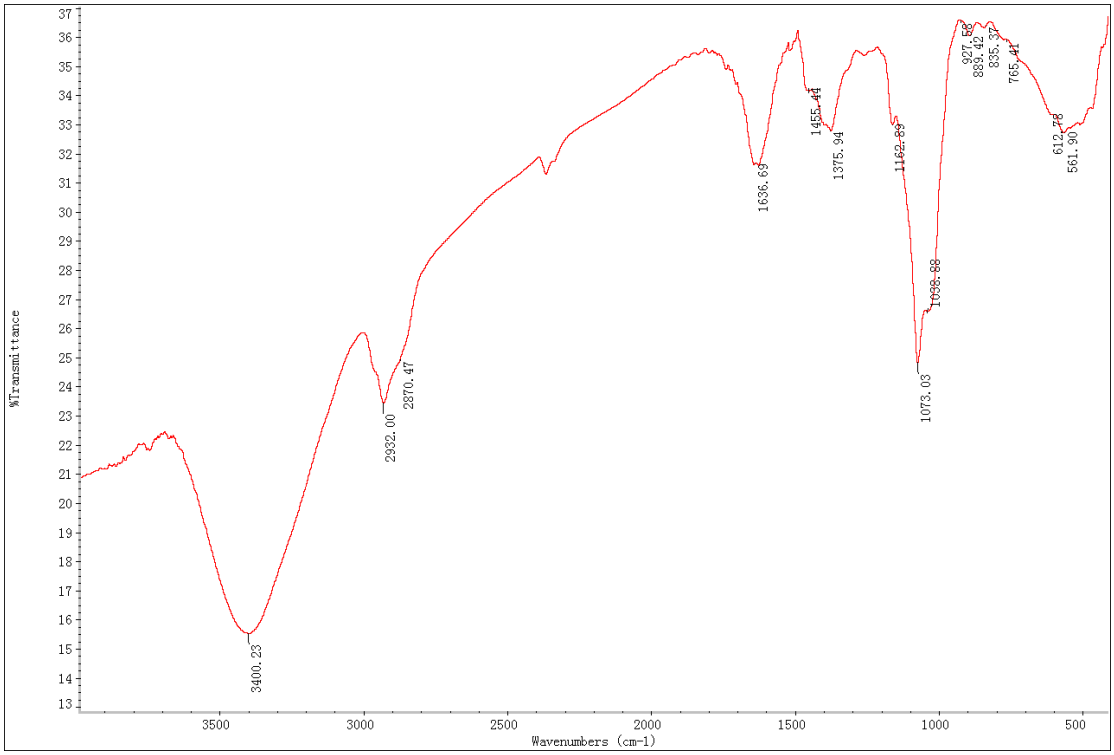


Figure S1: The IR spectrum of **1** (in KBr)

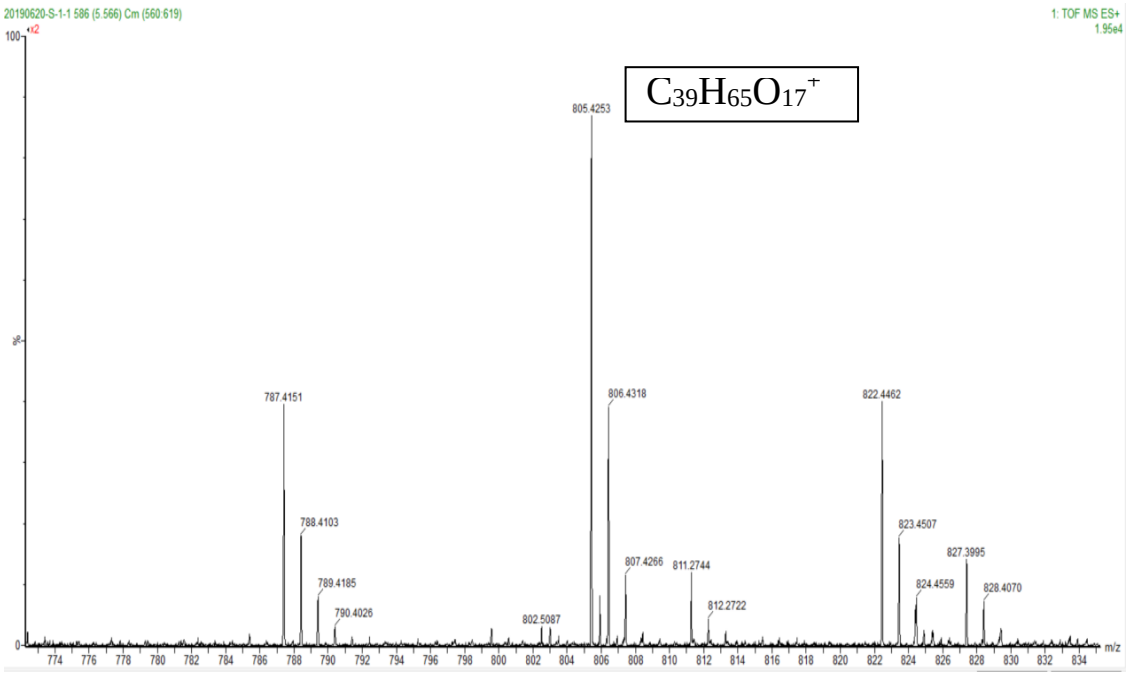
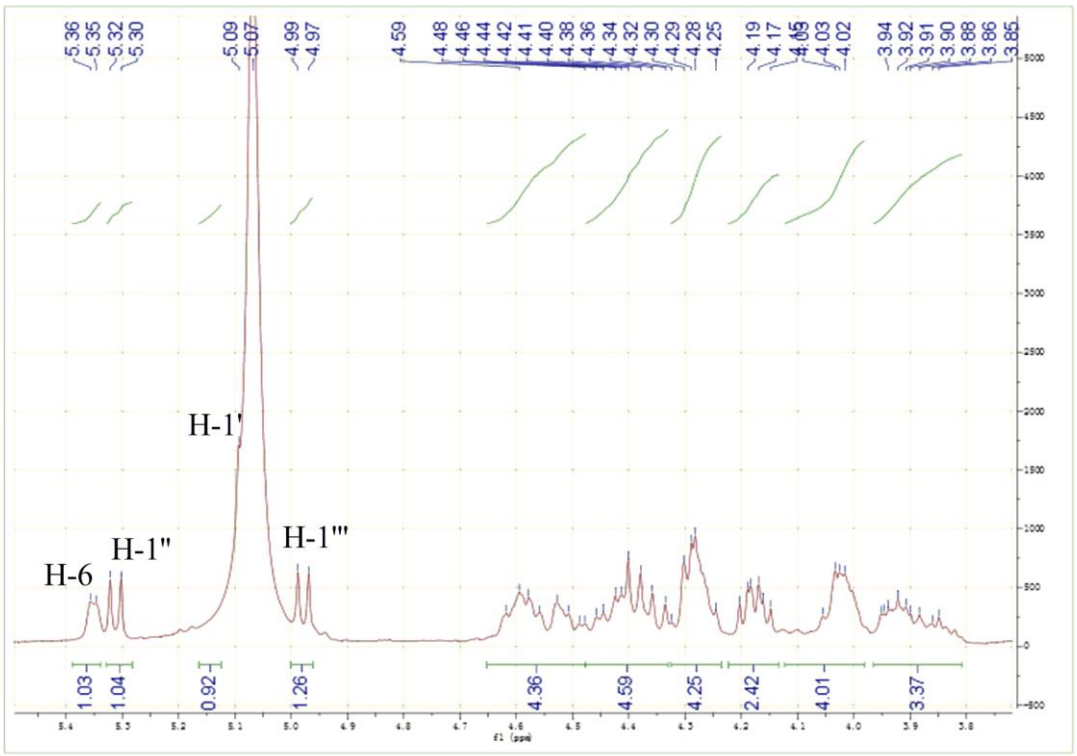


Figure S2: The HR-ESI-MS spectrum of **1**(in MeOH)

Figure S3: The ^{1}H-NMR spectrum of **1** (in pyridine-d_5)



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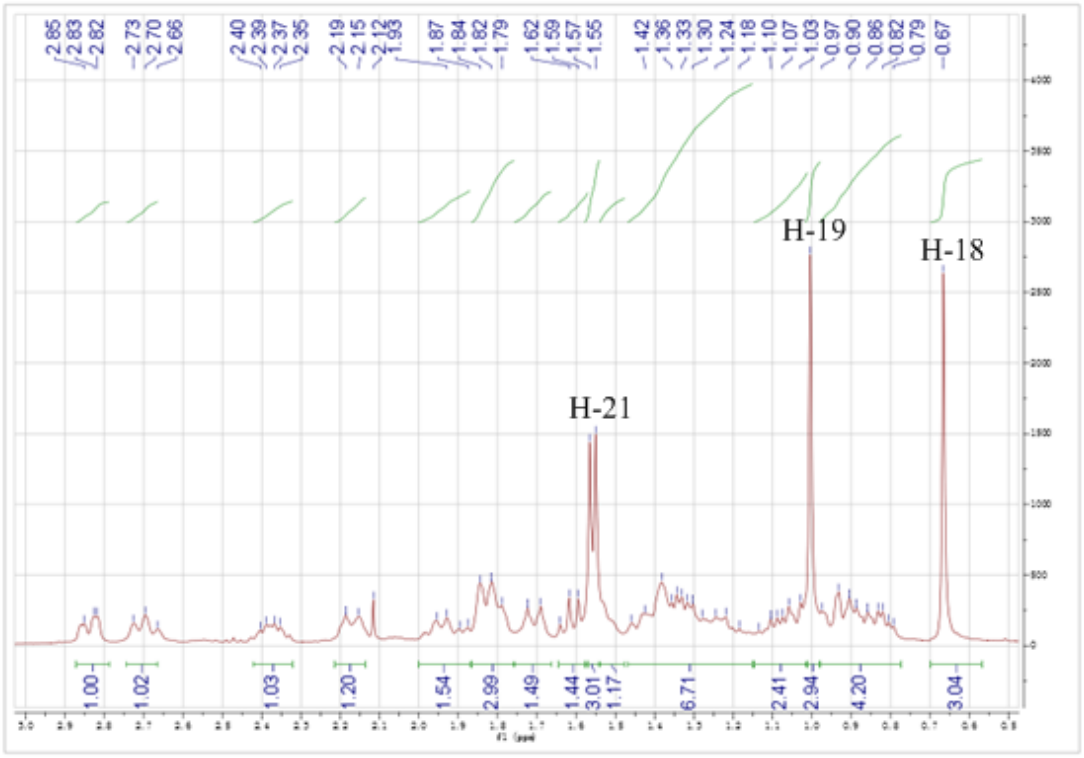

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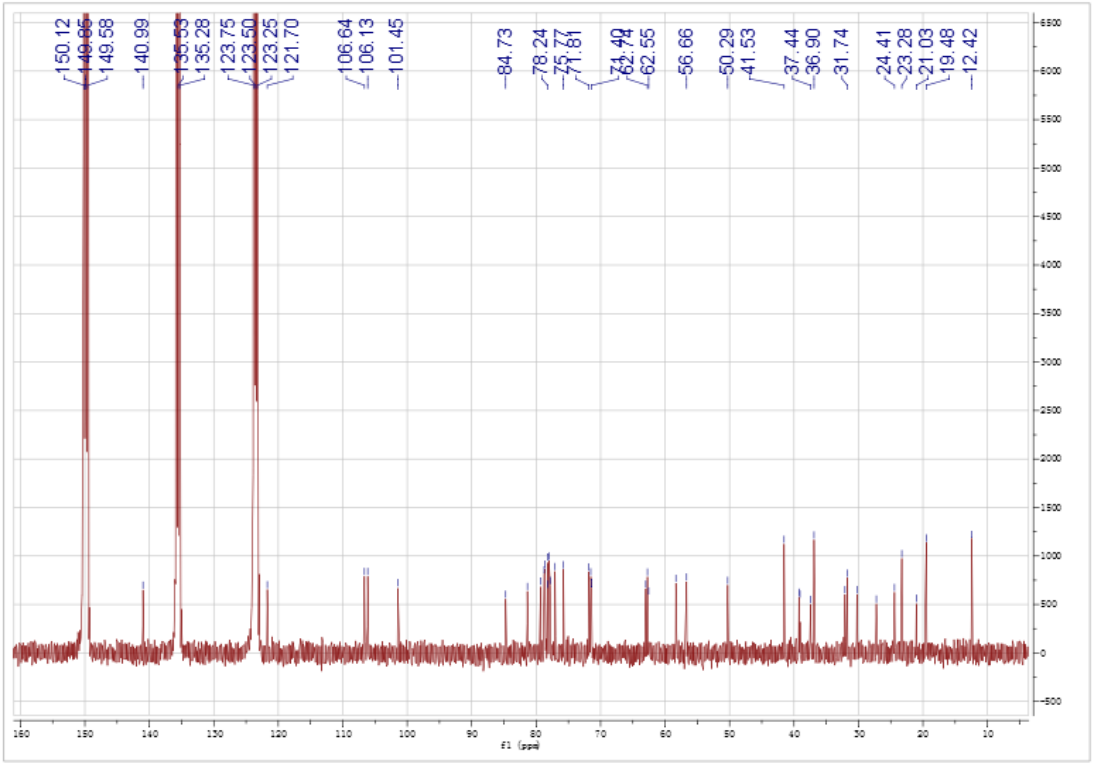

Figure S4: The ^{13}C-NMR spectrum of **1** (in pyridine-d_5)

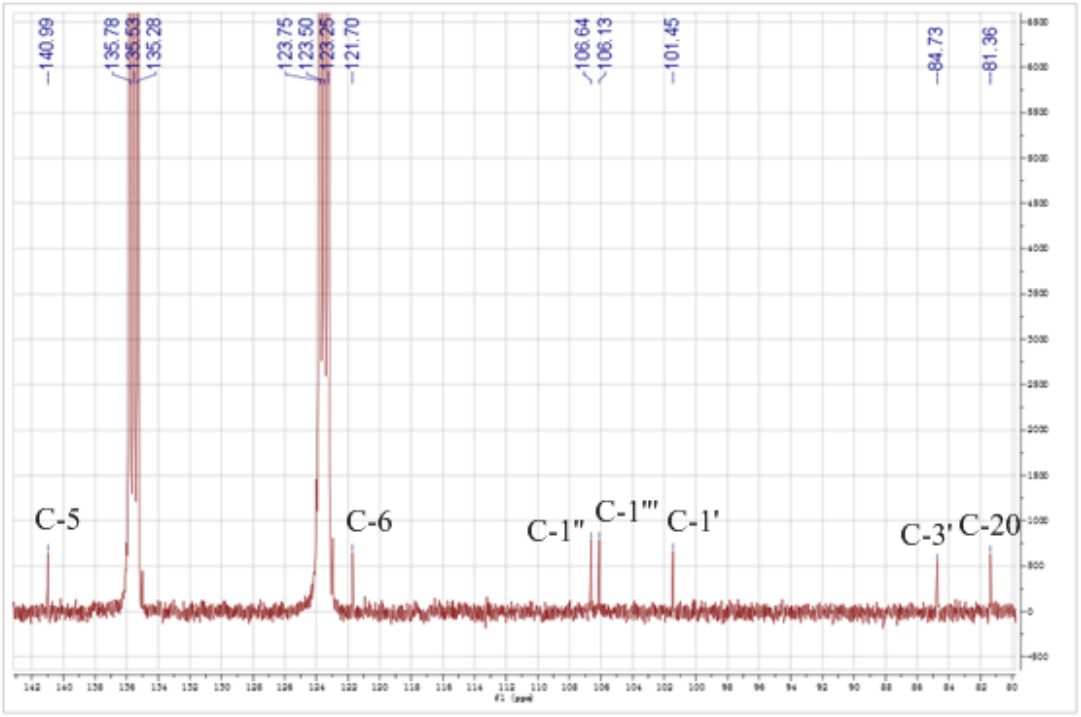

Figure S4: The ^{13}C-NMR spectrum of **1** (in pyridine-d_5)

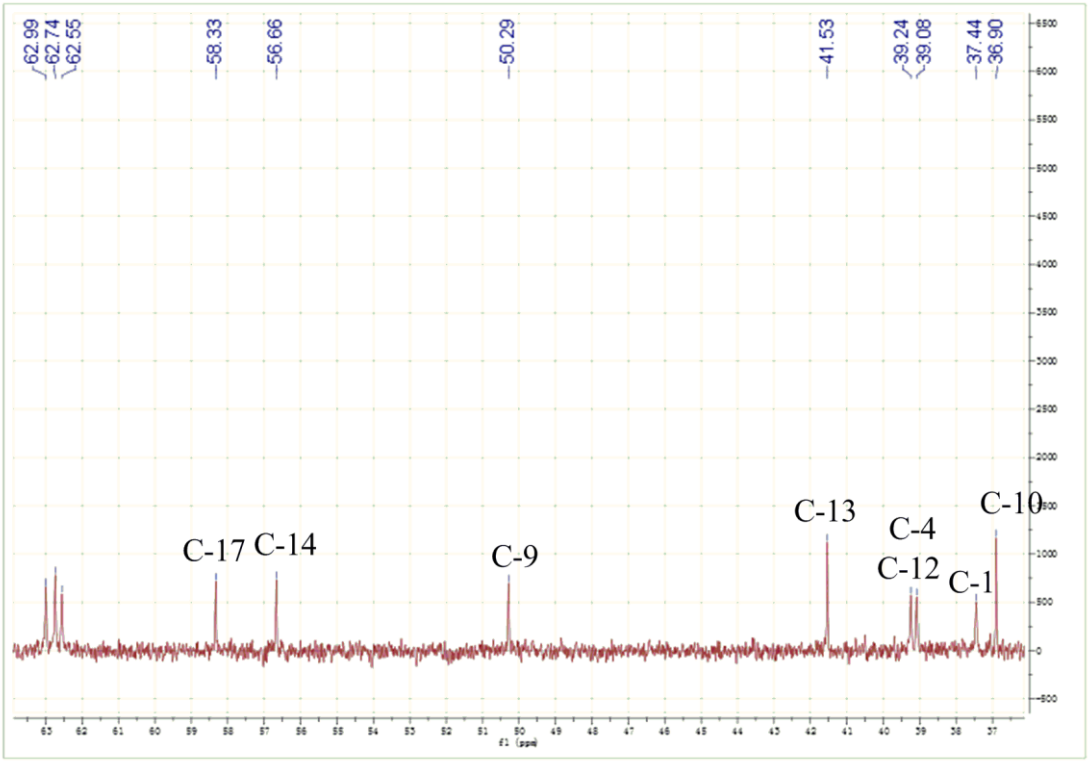

Figure S4: The ^{13}C-NMR spectrum of **1** (in pyridine-d_5)

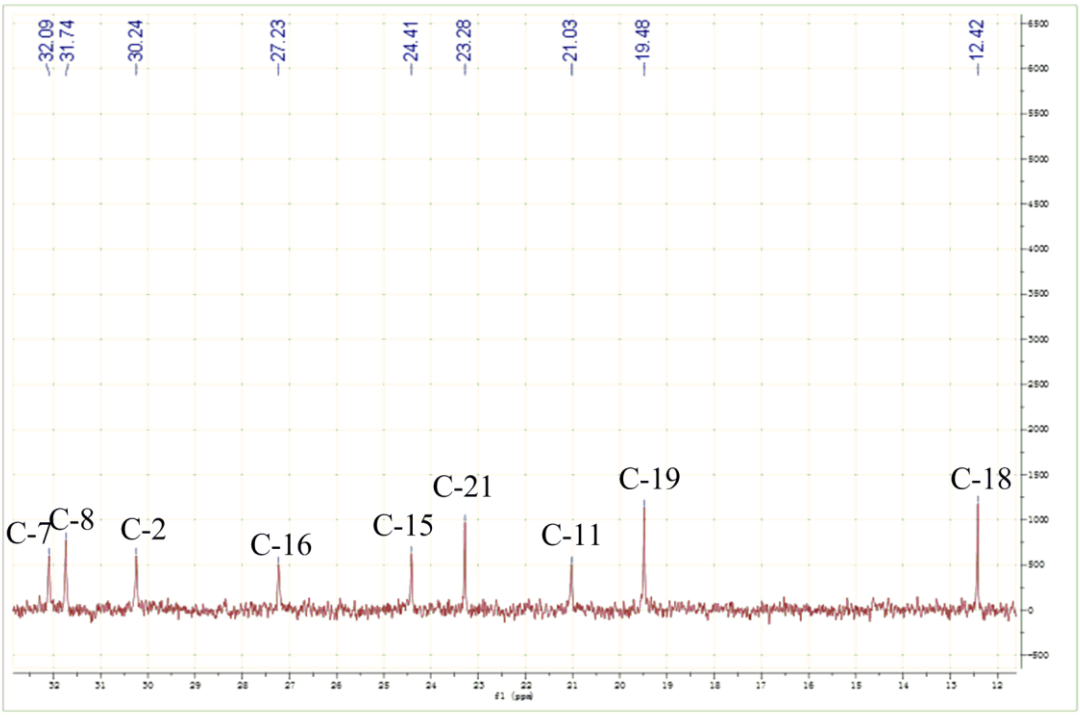

Figure S4: The ^{13}C-NMR spectrum of **1** (in pyridine-d_5)

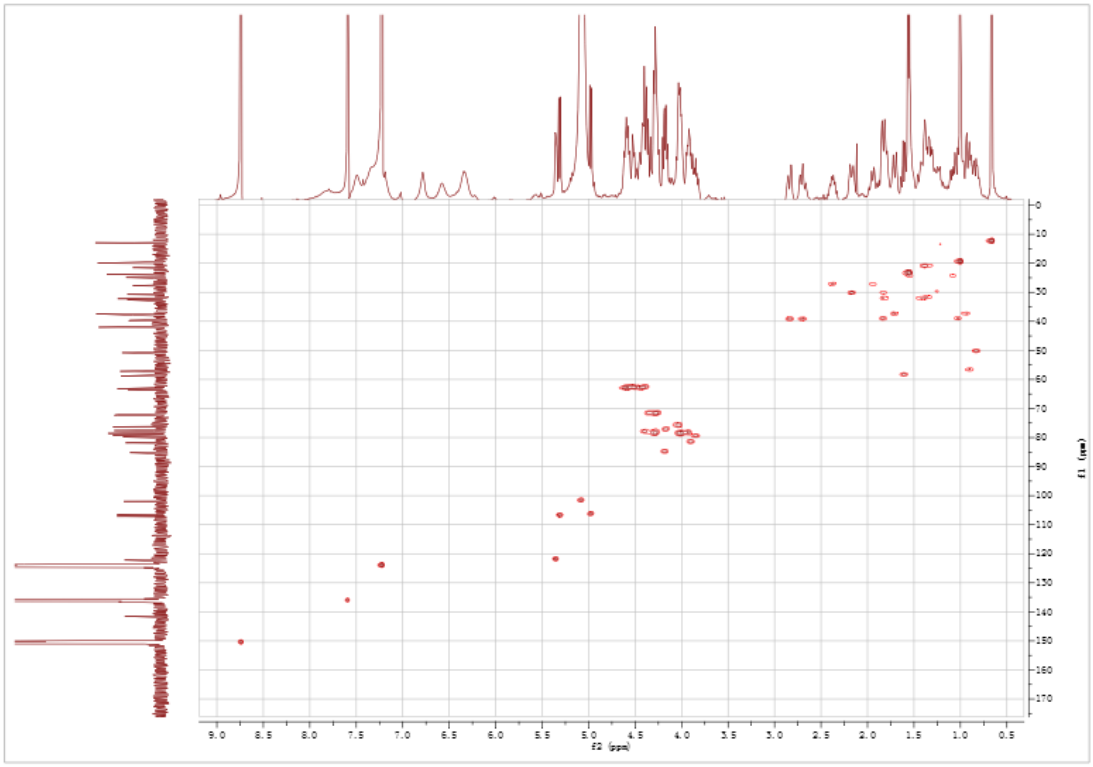

Figure S5: The HSQC spectrum of **1** (in pyridine-d_5)

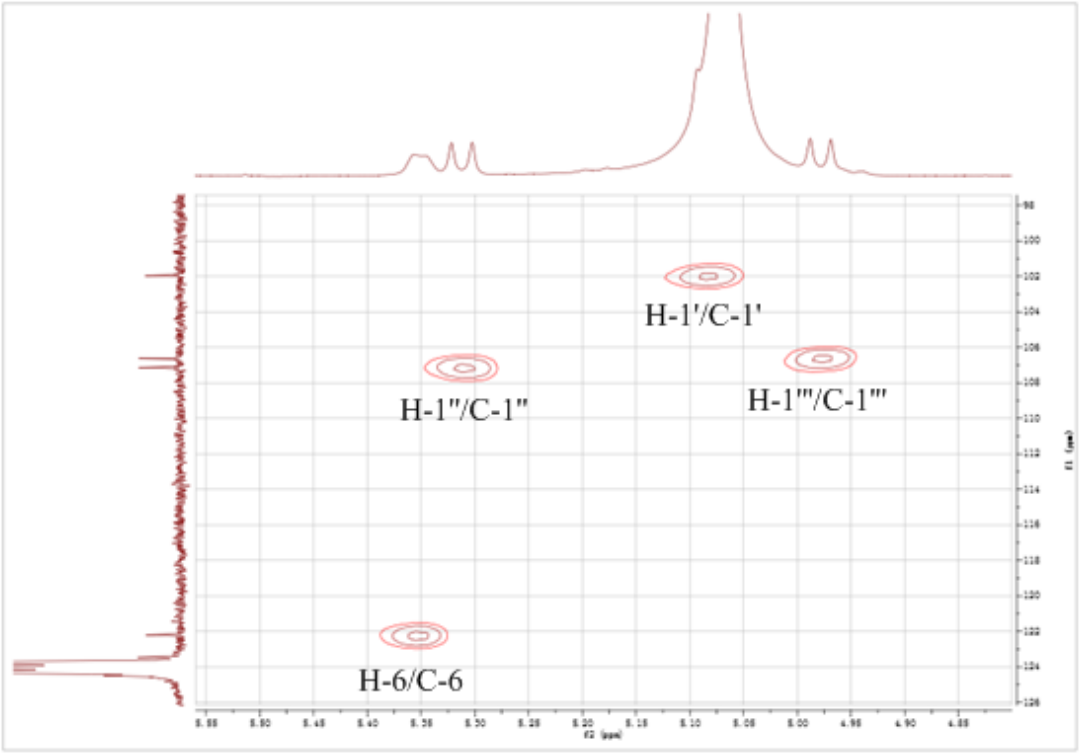

Figure S5: The HSQC spectrum of **1** (in pyridine-d_5)

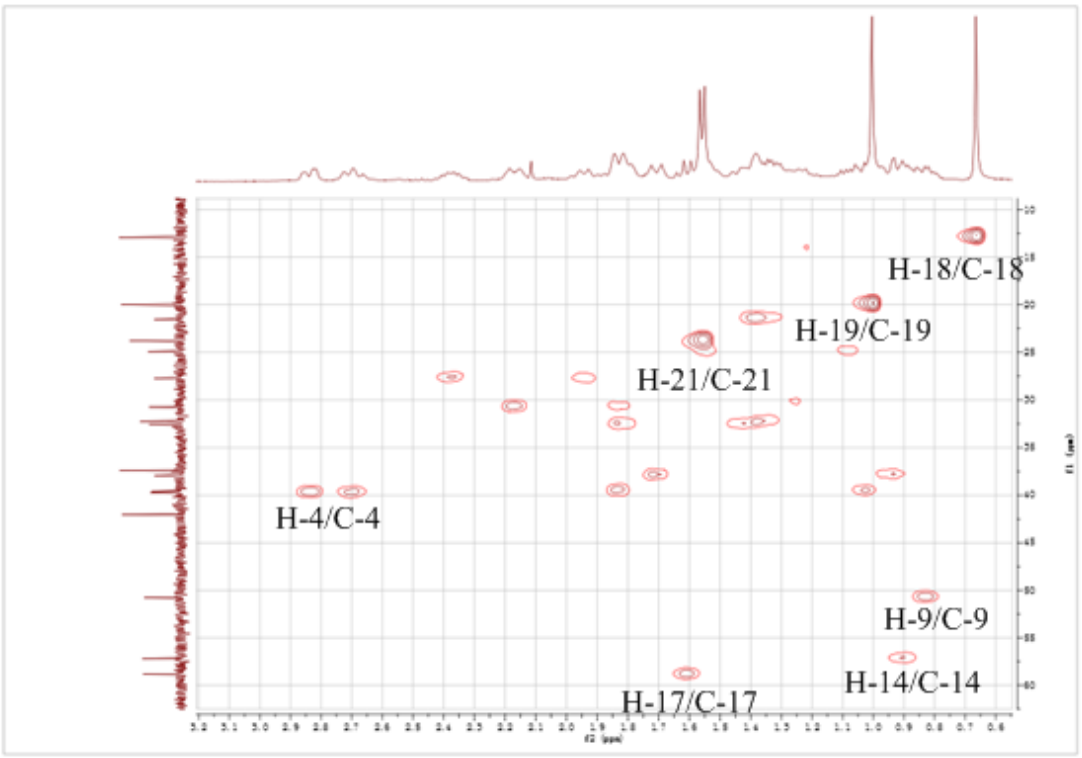

Figure S5: The HSQC spectrum of **1** (in pyridine-d_5)

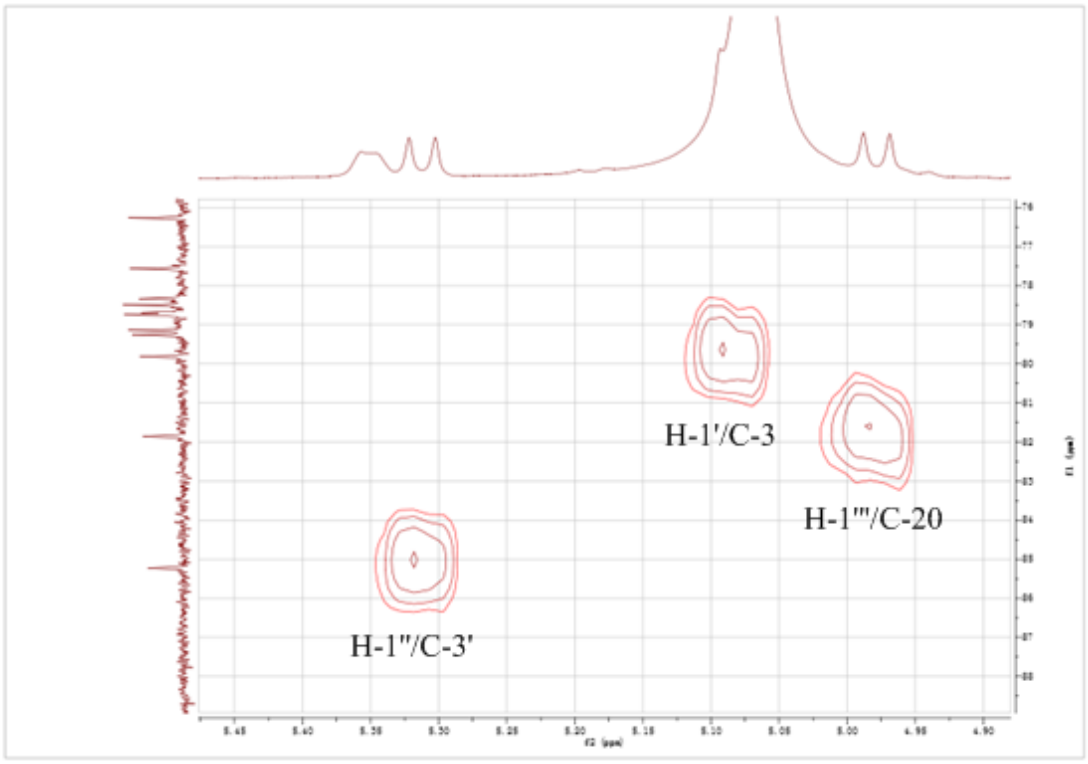

Figure S6: The HMBC spectrum of **1** (in pyridine-d_5)

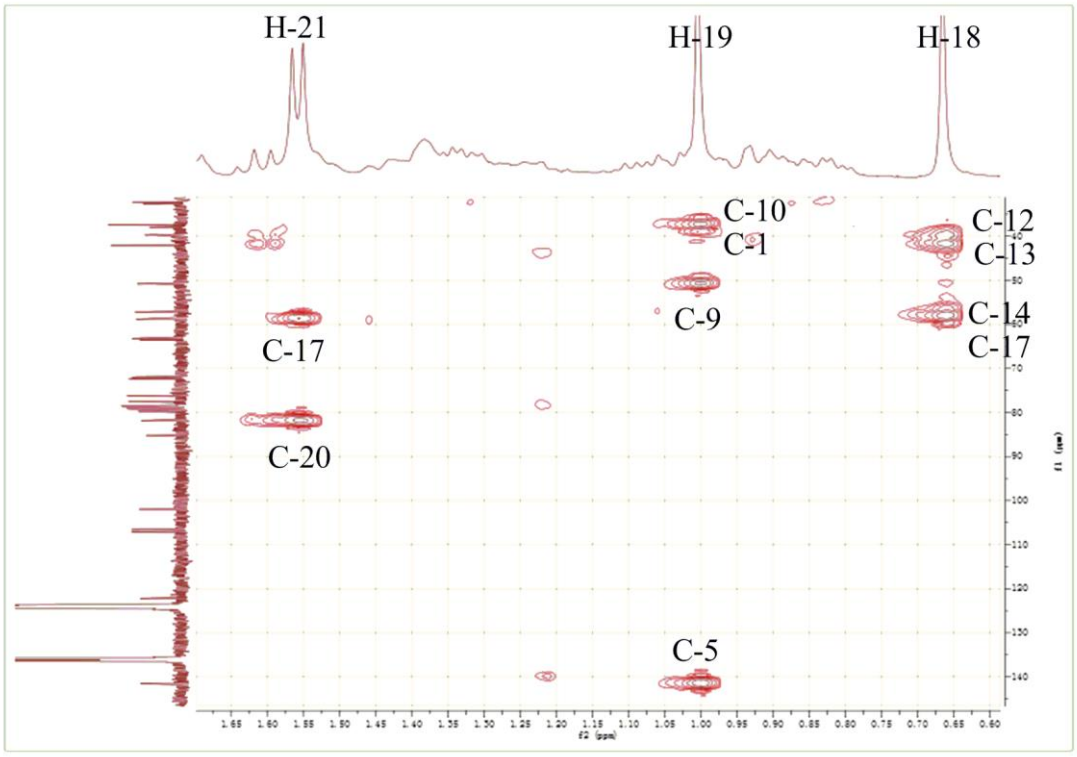

Figure S6: The HMBC spectrum of **1** (in pyridine-d_5)

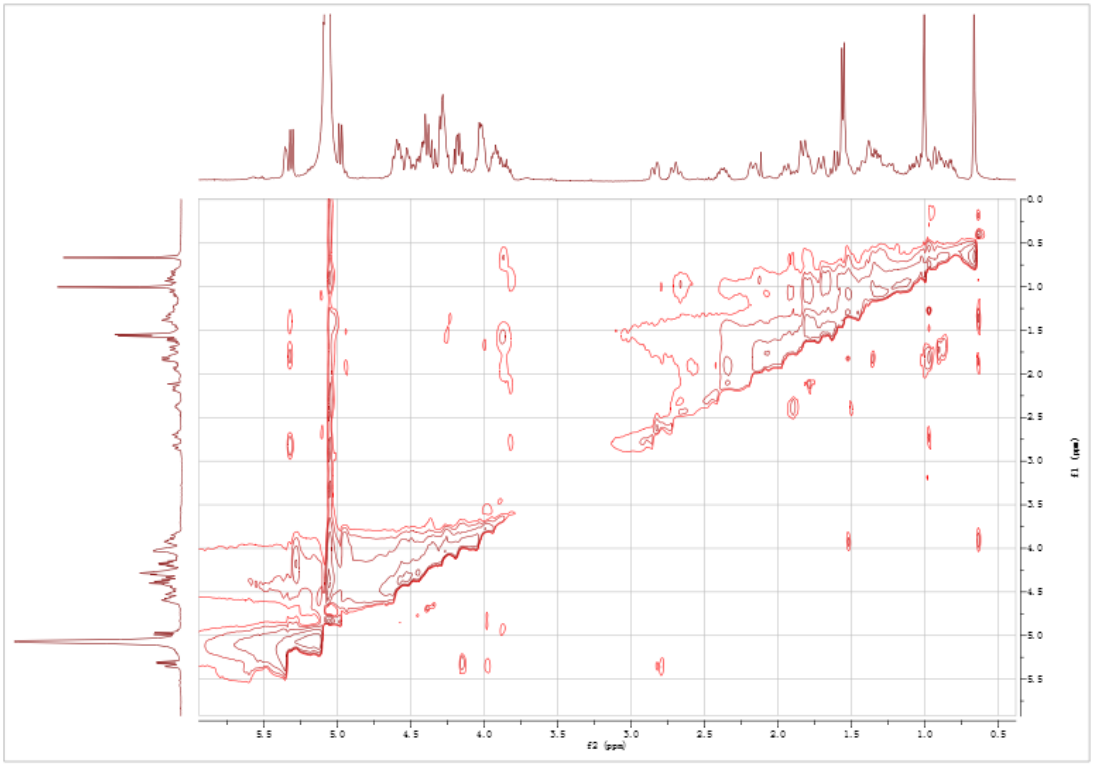

Figure S7: The NOESY spectrum of **1** (in pyridine-d_5)

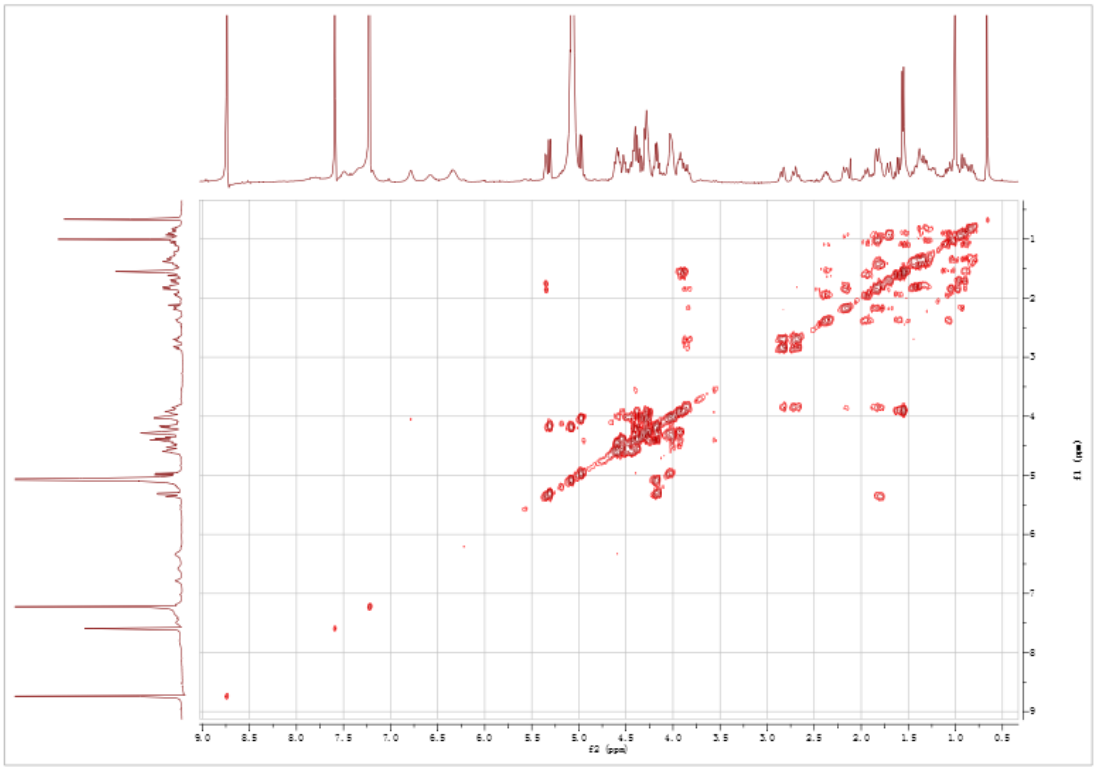

Figure S8: The ^{1}H-^{1}H COSY spectrum of **1** (in pyridine-d_5)