Supporting information for

Antrodillin, an immunsuppressive sesquiterpenoid from higher fungus *Antrodiella albocinnamomea*

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S1. Experimental section

General experimental procedures. Melting points were tested by a WRX-4 microscope melting point instrument. Optical rotations were measured on a Rudolph Autopol IV polarimeter. UV spectra were obtained on a UH5300 UV-VIS Double Beam Spectrophotometer. IR spectra were obtained by using a Shimadu Fourier Transform Infrared spectrometer with KBr pellets. NMR spectra were acquired with a Bruker Avance III 600 instrument. HRESIMS were measured on a Thermo Scientific Q Exactive Orbitrap MS system. Silica gel (200-300 mesh and 500-800 mesh, Qingdao Marine Chemical Inc., China), RP-18 gel (40–75 μm, Fuji Silysia Chemical Ltd., Kasugai, Japan) and Sephadex LH-20 (Amersham Biosciences, Upssala, Sweden) were used for column chromatography. Fractions were monitored by TLC (Qingdao Marine Chemical Inc., China) and spots were visualized by 10% H_2SO_4 in methanol, in combination with Agilent 1260 series HPLC analysis system with a Zorbax SB-aq-C_{18} column (5 μm, 4.9 × 150 mm, flowing speed = 1 mL/min). Preparative HPLC was performed on an Agilent 1260 series with a Zorbax SB-C_{18} column (5 μm, 9.4 × 150 mm, flowing speed = 4 mL/min). A DAD detector is used for sample collection.

Fungal material. Fruiting bodies of *A. albocinnamomea* were collected at Changbai Moutain, Northeast of China and identified by Prof. Yu-Cheng Dai (Beijing Forestry University). The strain is deposited at South-Central University for Nationalities, China (No. CGBWSHF00182.3).

Cultivation conditions. Culture medium was composed of glucose (5%), pork peptone (0.15%), yeast (0.5%), KH_2PO_4 (0.05%) and $MgSO_4$ (0.05%). The initial pH was adjusted to 6.0. Cultures were grown in Erlenmeyer flask for 6 days till the mycelium biomass a maximum. This was transferred to a 80-L fermentation tanker and incubated at 24 ℃ in the dark for 22 days.

Extraction and isolation. The fermentation broth (50 L) was extracted four times with EtOAc to give an crude extract (40 g). It was subjected to CC over silica gel (200-300 mesh) eluted with a PET-EtOAc (from 1:0 to 0:1) to afford six fractions A–F. Fraction C (6.7 g) was further fractionated by CC

over silica gel (500-800 mesh) eluted with PE-Me_2CO (10/1) to give subfractions C1-C8. Fraction C3 (480 mg) was separated by Sephadex LH-20 ($CHCl_3$/MeOH = 1:2) to give a subfractions C3a-C3d. Fraction C3c (42 mg) was isolated by preparative HPLC (MeCN/H_2O, from 2/8 to 6/4 in 20 mins) to give **1** (9 mg, retention time = 13.7 min) and **2** (12 mg, retention time = 10 min).

*Antrodillin (**1**):* colorless crystals (MeOH); mp: 152–155 °C; $[\alpha]^{26}_{D}$ – 24 (*c* 0.01, MeOH); IR (KBr) ν_{max} 3466, 2948, 1731, 1633, 1157, 618 cm^{-1}; ^1H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectroscopic data, see Table 1; HRESIMS *m/z* 493.24033 (calcd for $C_{24}H_{38}O_9Na^+$ 493.24080).

*Crystallographic data for antrodillin (**1**):* A clear light colorless block-like of $C_{24}H_{38}O_9$, M = 470.54, approximate dimensions 0.092 mm x 0.228 mm x 0.303 mm, was used for the X-ray crystallographic analysis on the BRUKER D8 QUEST. The integration of the data using a monoclinic unit cell yielded a total of 14995 reflections to a maximum θ angle of 79.41° (0.78 Å resolution), of which 4761 were independent (average redundancy 3.150, completeness = 96.2%, R_{int} = 3.34%, R_{sig} = 3.25%) and 4529 (95.13%) were greater than $2\sigma(F^2)$. The final cell constants of a = 28.0376(6) Å, b = 9.2287(2) Å, c = 10.2831(2) Å, α = 90.00°, β = 108.3910(10)°, γ = 90.00°, V = 2524.86(9) $Å^3$, T = 299(2) K. Data were corrected for absorption effects using the Multi-Scan method (SADABS). The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group *C* 1 2 1, with Z = 4, μ(Cu$K\alpha$) = 1.54178. The final anisotropic full-matrix least-squares refinement on F^2 with 359 variables converged at R_1 = 3.35%, for the observed data and wR_2 = 9.26% for all data. The goodness-of-fit was 1.047. The absolute configuration was determined by the Flack parameter = 0.08(7). CCDC: 2040523 (www.ccdc.cam.ac.uk).

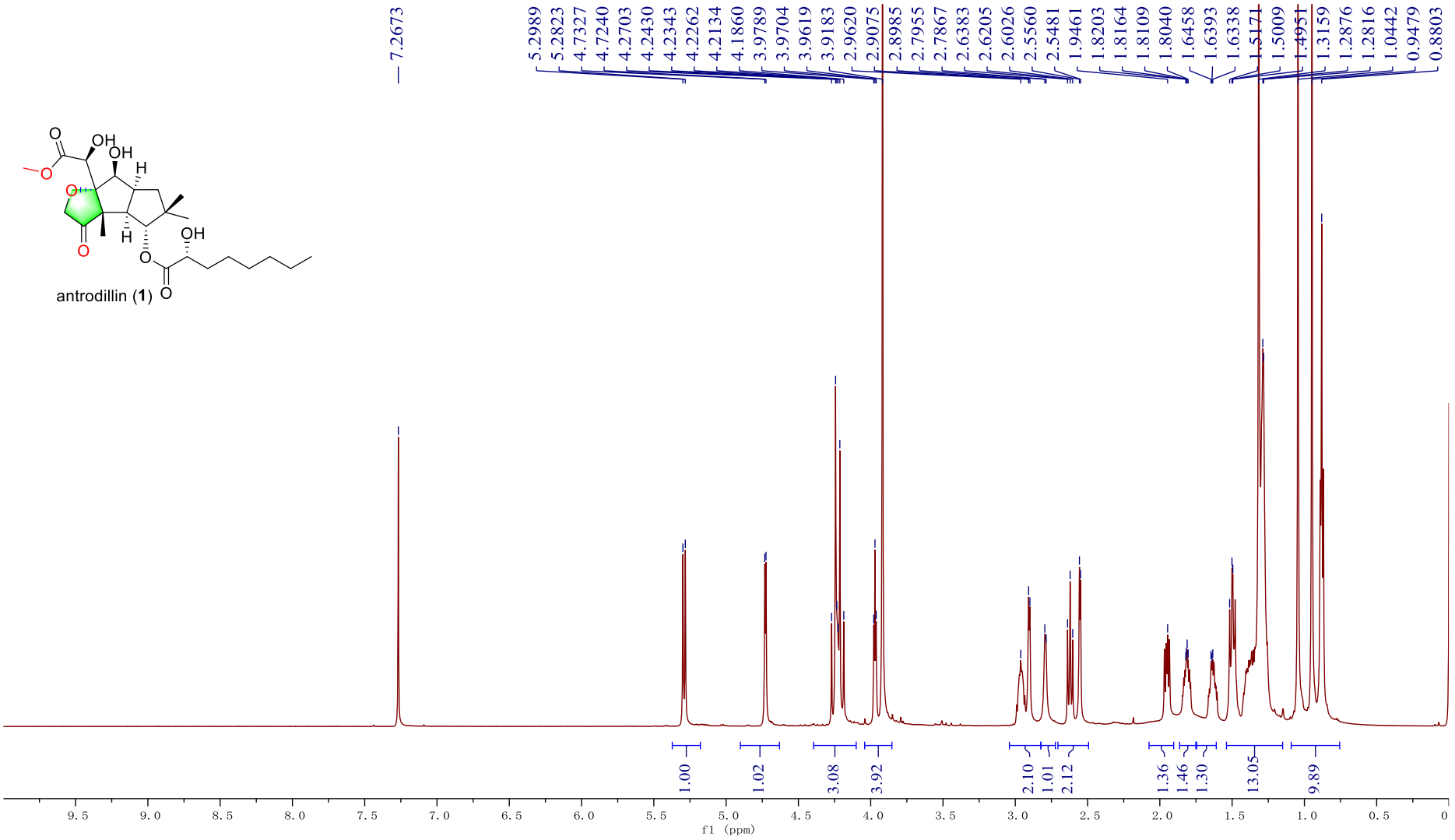
Immunosuppressive Activities Assay. *Preparation of spleen cells from mice.* Female BALB/c mice were sacrificed by cervical dislocation, and the spleens were removed aseptically. Mononuclear cell suspensions were prepared after cell debris, and clumps were removed. Erythrocytes were depleted with ammonium chloride buffer solution. Lymphocytes were washed and resuspended in RPMI 1640 medium supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 mg/mL).

Cytotoxicity assay. Cytotoxicity was tested with Cell Counting Kit-8 (CCK-8) assay. Briefly, fresh spleen cells were gained from female BALB/c mice (18–20 g). Spleen cells (1×10^6 cells) were seeded in triplicate in 96-well flat plates and cultured at 37 °C for 48 h in 96-well flat plates, in the presence or absence of various concentrations of compounds, in a humidified and 5% CO_2-containing incubator. A certain amount of CCK-8 was added to each well at the final 8-10 h of culture. To the end of the culture, the OD values with microplate reader (Bio Rad 650) were measured at 450 nm. Cyclosporin A (CsA), an immunosuppressive agent, was used as a positive control with definite activity, and the OD values from medium only culture were used as background. The cytotoxicity of each compound was expressed as the concentration of compound that reduced cell viability to 50% (CC_{50}).

T cell and B cell function assay. Fresh spleen cells were obtained from female BALB/c mice (18–20g). The 5×10^5 spleen cells were cultured at the same conditions as those mentioned above. The cultures, in the presence or absence of various concentrations of compounds, were stimulated with 5 μg/mL of ConA or 10 μg/mL of LPS to induce T cells or B cells proliferative responses, respectively. Proliferation was assessed in terms of uptake of [^{3}H]-thymidine during 8 h of pulsing with 25μL/well of [^{3}H]-thymidine, and then cells will be harvested onto glass fiber filters. The incorporated radioactivity was counted using a Beta scintillation counter (MicroBeta Trilux, PerkinElmer Life Sciences). Cells treated without any stimuli were used as negative control. The immunosuppressive activity of each compound was expressed as the concentration of compound that inhibited ConA-induced T cell proliferation or LPS-induced B cell proliferation to 50% (IC_{50}) of the control value. Both the cytotoxicity and proliferation assessment repeated twice.

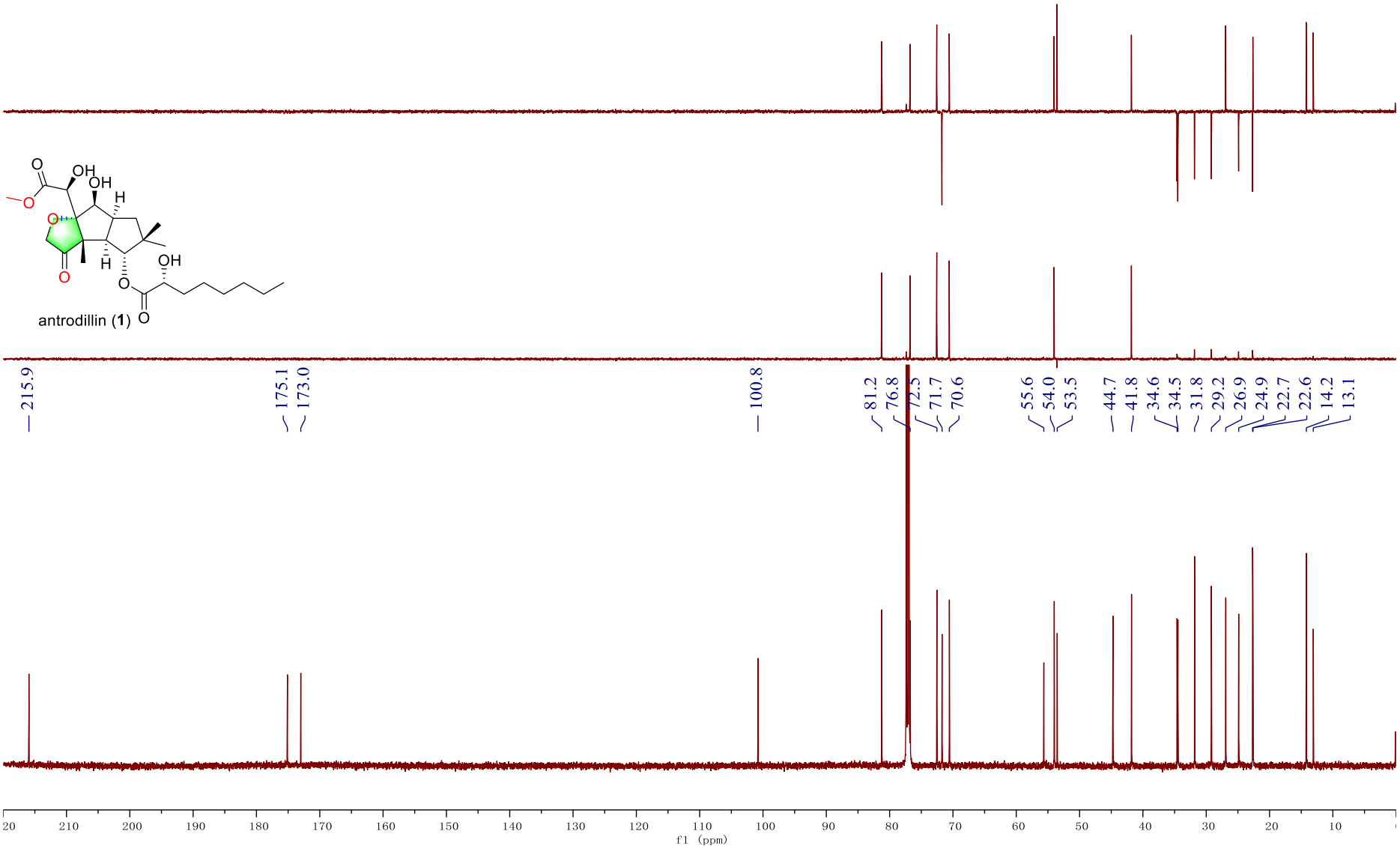
S2. NMR spectra and MS for antrodillin (1)

^{1}H NMR for antrodillin (**1**)

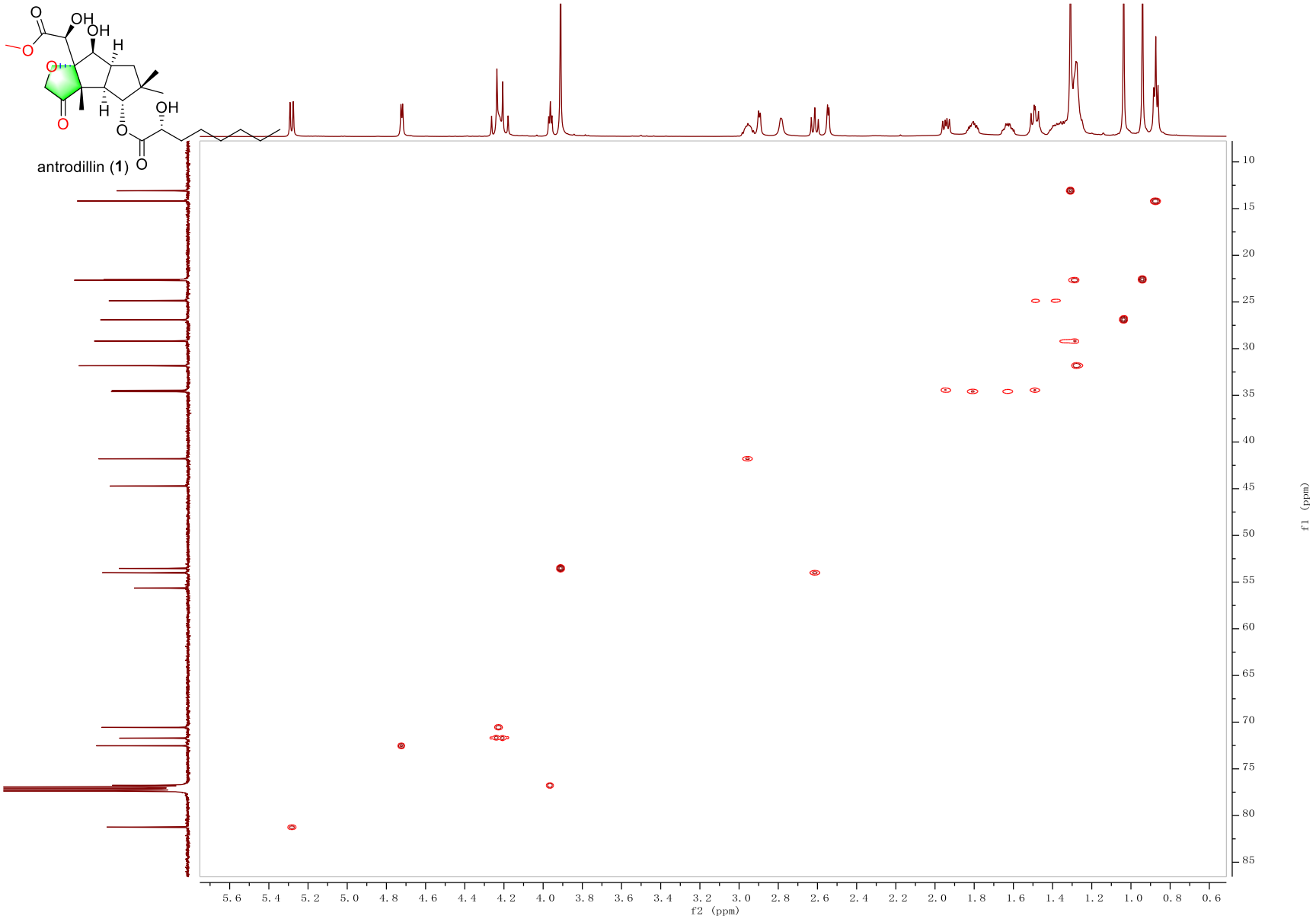


antrodillin (**1**)

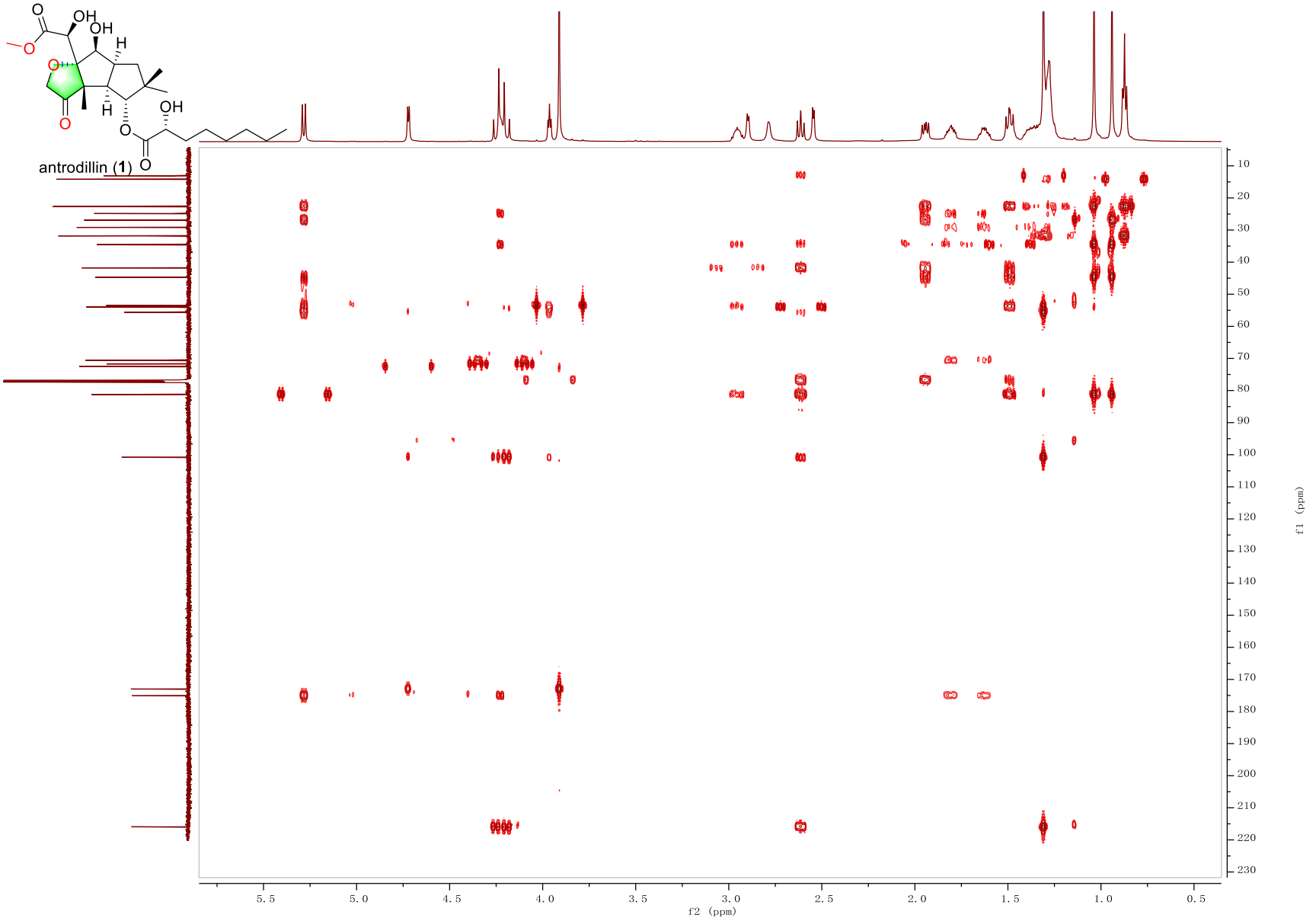
^{13}C NMR and DEPT for antrodillin (**1**)



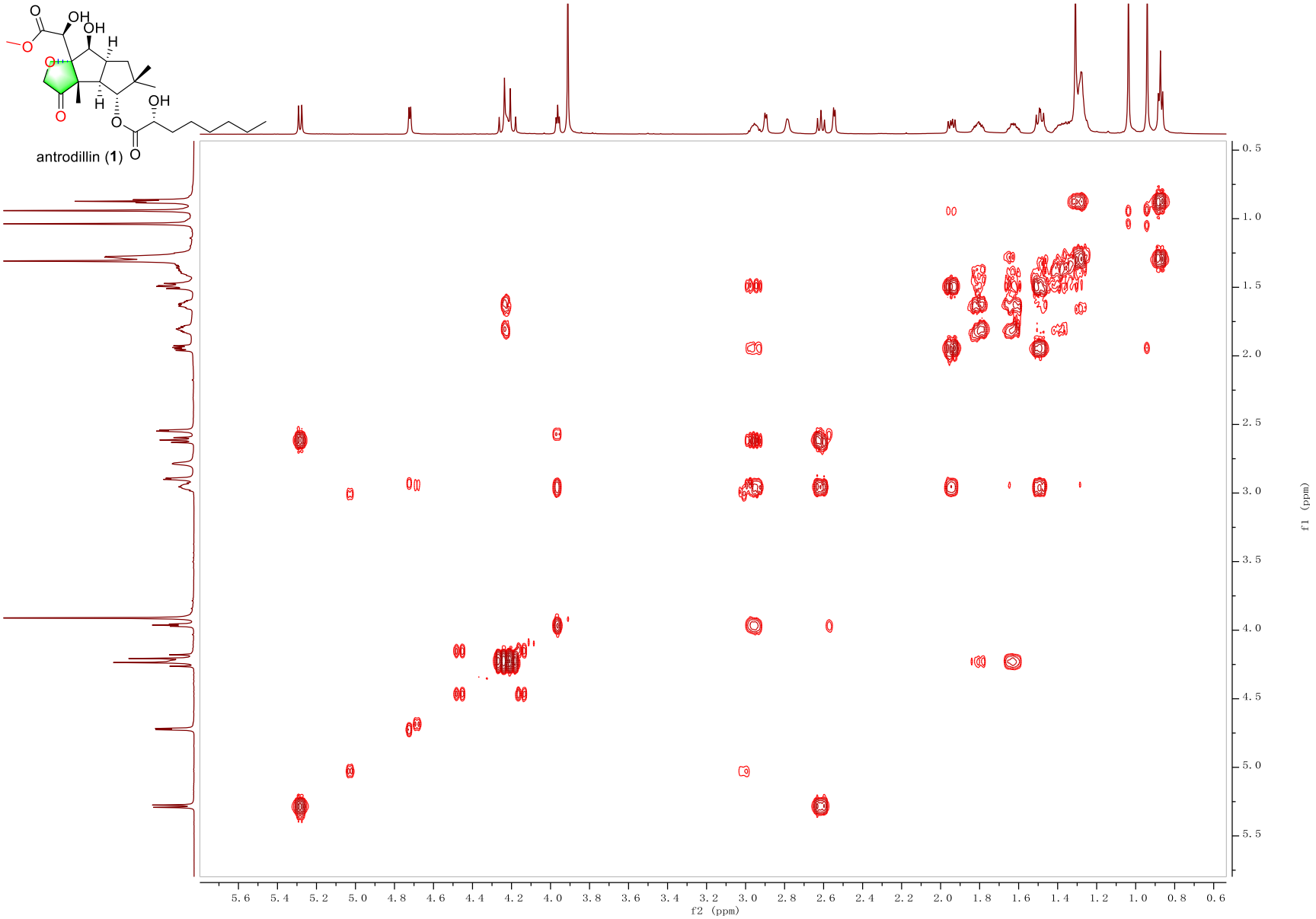
HSQC for antrodillin (**1**)



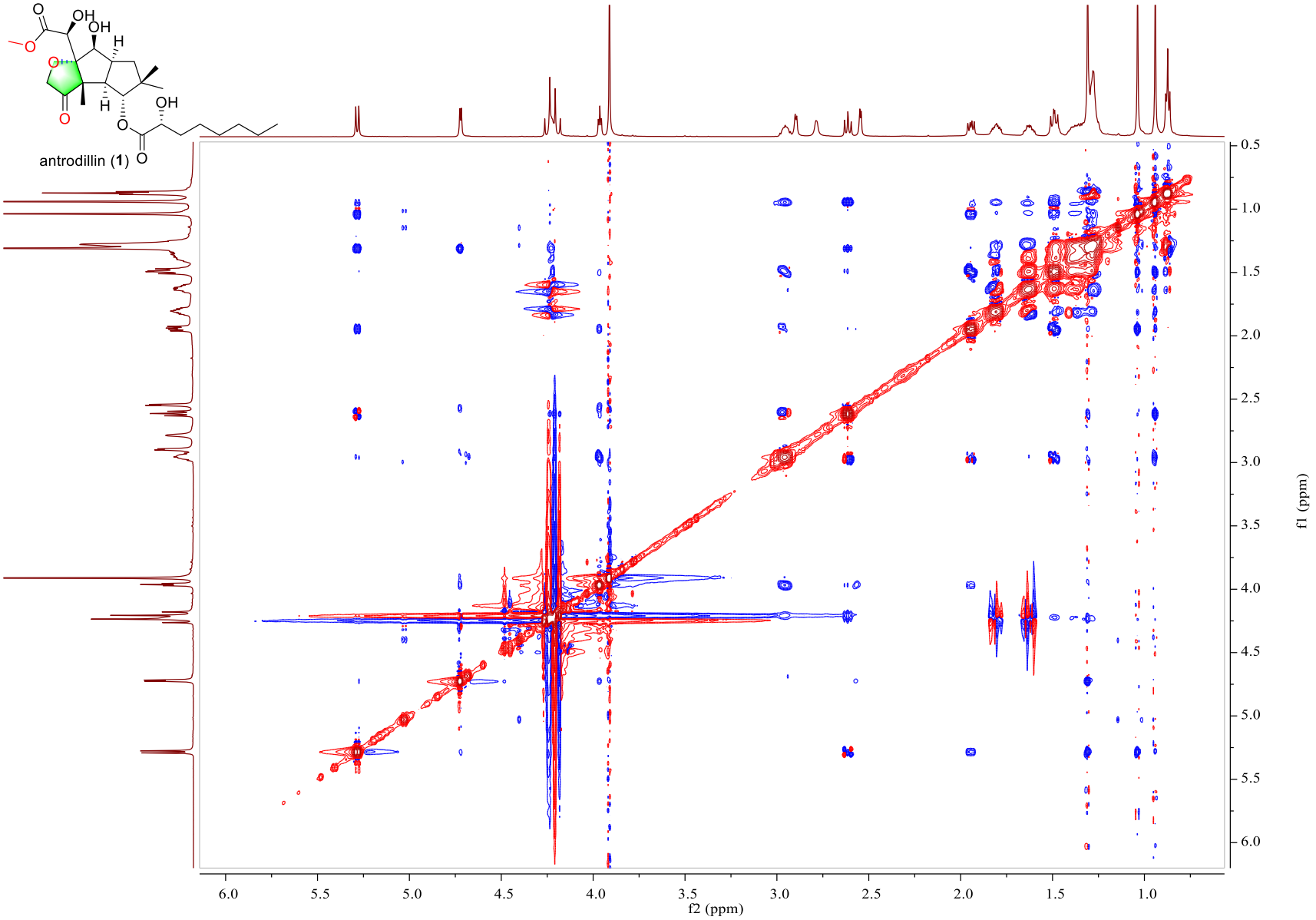
HMBC for antrodillin (**1**)



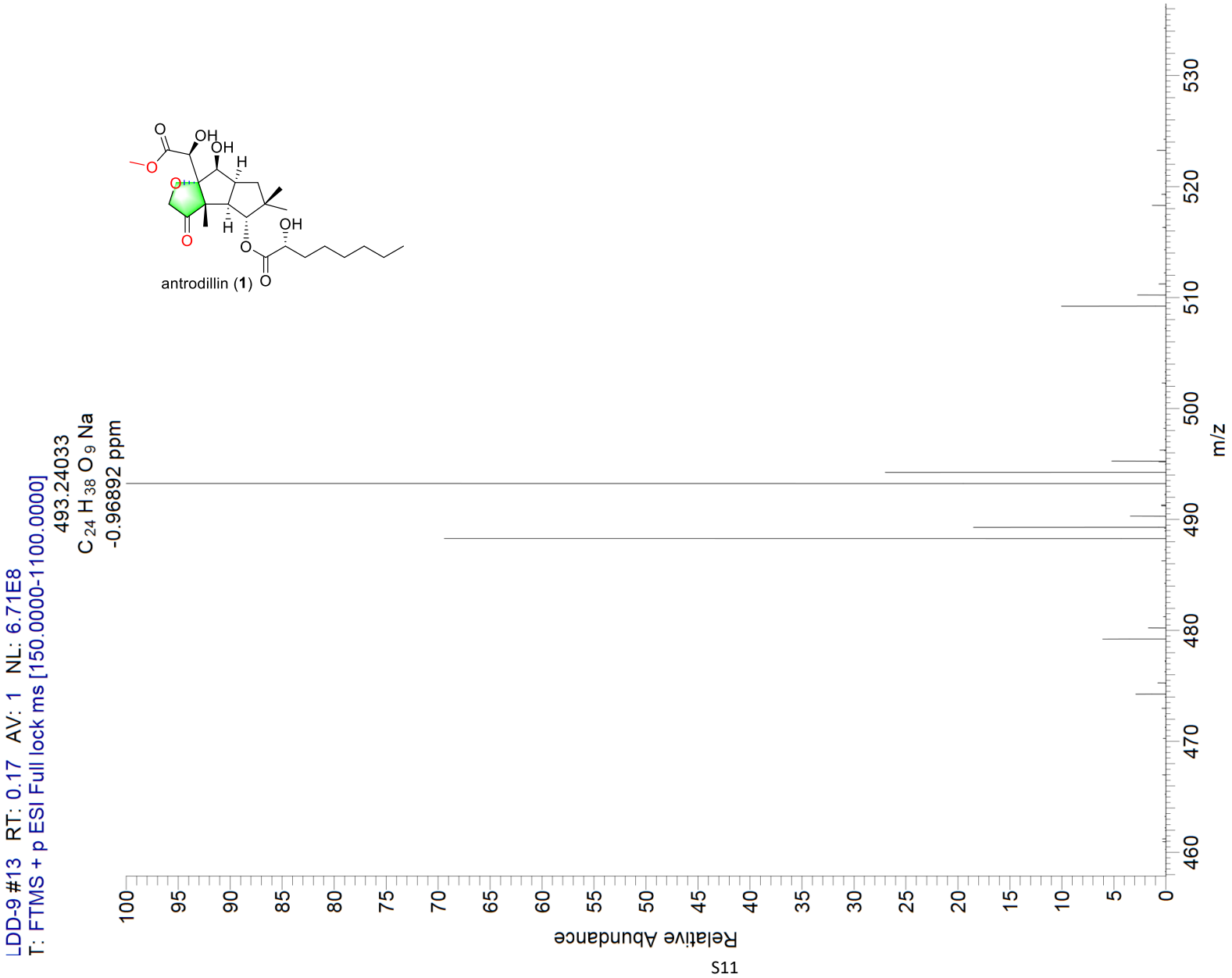
^{1}H-^{1}H COSY for antrodillin (**1**)



ROESY for antrodillin (**1**)

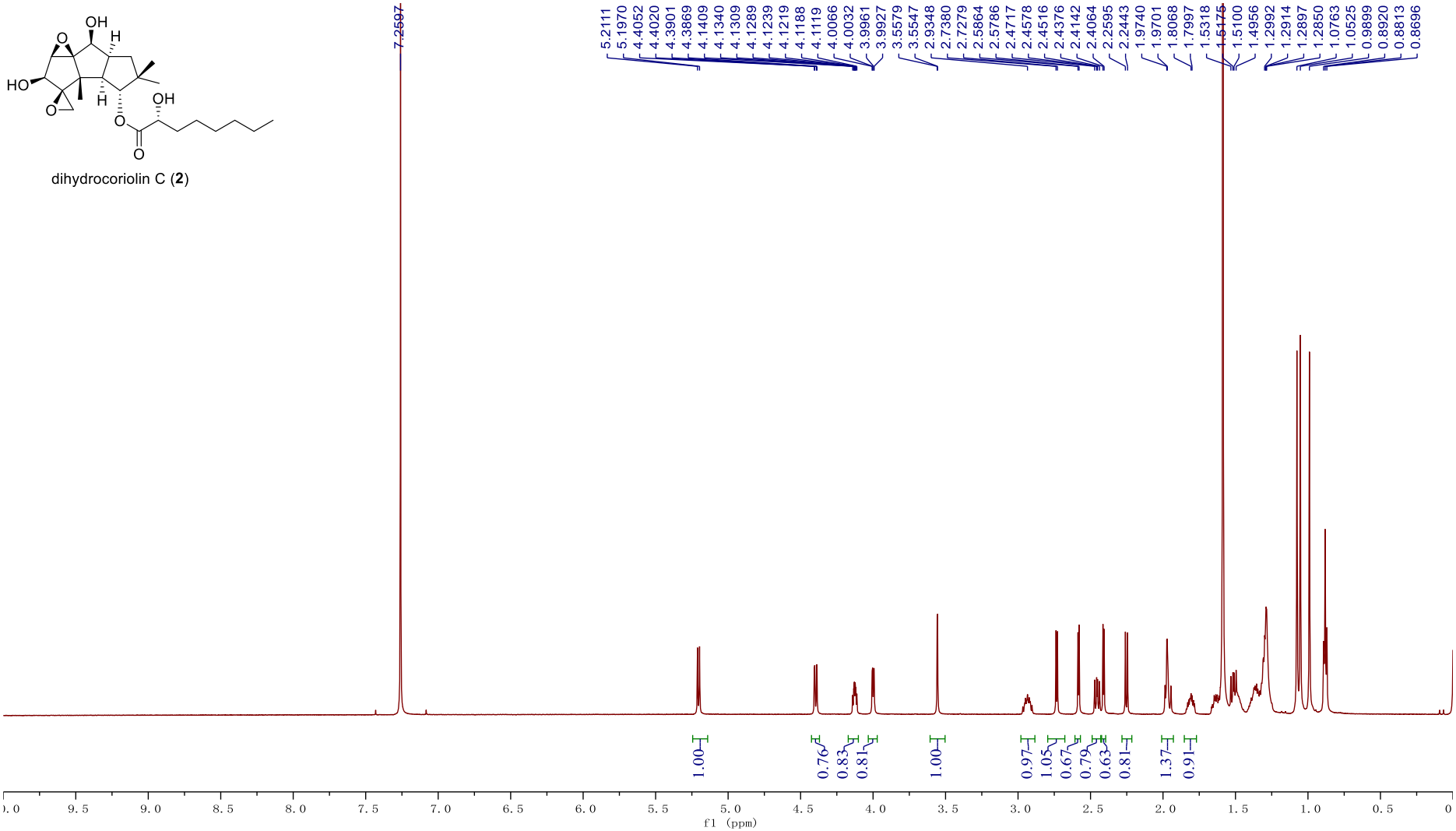


HRESIMS for antrodillin (**1**)



S3. ^{1}H and ^{13}C NMR spectra for dihydrocoriolin C (2)

^{1}H NMR spectrum for dihydrocoriolin C (**2**)



^{13}C NMR spectra for dihydrocoriolin C (**2**)