Supplementary Data

Caseahomopene A, a ring-expanded homotriterpenoid from *Casearia kurzii* showing anti-inflammatory activities *in vitro* and *in vivo*

Contents

1. Experimental Section

General experimental procedures. Optical rotations and infrared (IR) spectra were determined on an IP120 automatic polarimeter (InsMark) and a Tensor 27 FT-IR instrument (Bruker), respectively. ECD spectra were taken with a J-715 CD spectropolarimeter (JASCO). 1D and 2D NMR spectra were recorded on a AV 400 spectrometer (Bruker) using tetramethylsilane as internal references. HRESIMS data were recorded on an IonSpec 7.0 T FTICR MS (IonSpec Co., Ltd., Lake Forest, CA), while ESIMS data were acquired on a Thermo Finnigan LCQ-Advantage mass spectrometer. Preparative HPLC separations were conducted using a CXTH LC3000 system (Shodex RI-102 detector) equipped with a YMC-pack ODS-AM column (5 μm, 250 mm × 20 mm). Biological reagents and chemical reagents were purchased from Sigma Co. and Tianjin Chemical Reagent Co., respectively. Column chromatography was performed using silica gel (100–200 mesh) as stationary phase. The BV-2 cell line was provided by Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

Bioassay for NO production. The evaluation of NO inhibitory effect was tested by inhibiting NO release in LPS-induced murine microglial BV-2 cells [1]. The cells were cultured in DMEM with 10% (*v*/*v*) inactivated fetal bovine serum and 100 U/mL penicillin/streptomycin in a humidified incubator containing 95% air and 5% CO_2 at 37 °C. The cells were seeded into 96-well culture plate at a density of 5×10^4 cells/well and allowed to adhere for 24 h at 37 °C. The cells were incubated for 24 h with or without 0.2 μg/mL of LPS (Sigma Chemical Co., St. Louis, MO, U.S.A.) in the absence or presence of the test compounds. 2-Methyl-2-thiopseudourea, sulfate (SMT) was used as a positive control. As a parameter of NO synthesis, the nitrite concentration was measured by the Griess reaction using the supernatant of the BV-2 cells. Briefly, 50 μL of the cell culture supernatants were reacted with 50 μL of Griess reagent [1:1 mixture of 0.1% *N*-(1-naphtyl) ethylenediamine in H_2O and 1% sulfanilamide in 5% phosphoric acid] in a 96 well plate and the absorbance was read with a microplate reader (Thermo Fisher Scientific Inc. America) at 550 nm. The experiments were performed in parallel three times, and the results are presented as the mean ± SD.

Bioassay for PGE_2 production. The PGE_2 production in the culture supernatants was measured using ELISA kits (Cayman Chemical, USA) [2]. In brief, BV-2 cells were seeded in 24 well plate at a density of 5×10^5 cells per well. After 24 h, the cells were treated or untreated with various concentrations of the tested compounds for 1 h, and then treated with LPS (0.2 μg/mL) for another 24 h. 2-Methyl-2-thiopseudourea, sulfate (SMT) was used as a positive control. The cell supernatants were collected and stored at −20 °C before used. PGE_2 levels were tested with ELISA kit according to the manufacturer's instructions (Cayman Chemical, MI, USA), and the absorbance was read at 405 nm on a microplate spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) [3].

Western blotting analysis. The experiments were carried out as reported in the literature [4]. The BV-2 cells were seeded in 12-well plate for 24 h and pretreated with the compound **1** for 30 min, and then were stimulated with LPS. After continuous incubation for 20 h, cells were collected and lysed in lysis buffer, and centrifuged at 10,000 rpm for 10 min. The protein was obtained from supernatants and the concentration was quantified by BCA assay kit. Equal amounts of proteins (20 μg) were separated by SDS-PAGE gel electrophoresis and transferred to PVDF membrane. The membrane was blocked with 5% skim milk for 2 h and then incubated with primary antibody overnight against iNOS and COX-2. After washing with TBST thoroughly, horseradish peroxidase-conjugated secondary antibodies were applied, and the blots were developed using an ECL detection kit. The protein bands were quantified by Image J software (Version 1.51K, NIH, Bethesda, MD, USA).

Molecular docking studies. Molecular docking simulations were performed using the software AutoDock Vina along with AutoDock Tools (ADT 1.5.6) using the hybrid Lamarckian Genetic Algorithm (LGA). The three-dimensional (3D) crystal structures of iNOS (PDB code: 3E6T) and COX-2 (PDB code: 1PXX) were obtained from the RCSB Protein Data Bank, whose resolution was 2.5 Å and 2.9 Å, respectively. The standard 3D structures (PDB format) of selected compounds for molecular docking were constructed by chem3D Pro 14.0 software, whose configurations were determined by their NOESY spectra and Chem3D modeling. The cubic grid box of 20 Å size (x, y, z)

with a spacing of 1.000 Å and grid maps were built [5, 6]. All of the other parameters were used according to default settings of AutoDock Vina. Results differing by less than 2.0 Å in positional root mean-square deviation (RMSD) were clustered together, and the results of the most favorable free energy of binding were chosen as the resultant complex structures.

References

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2. NMR Spectra of Compound 1

Figure S1 ^{1}H NMR spectrum for compound **1**

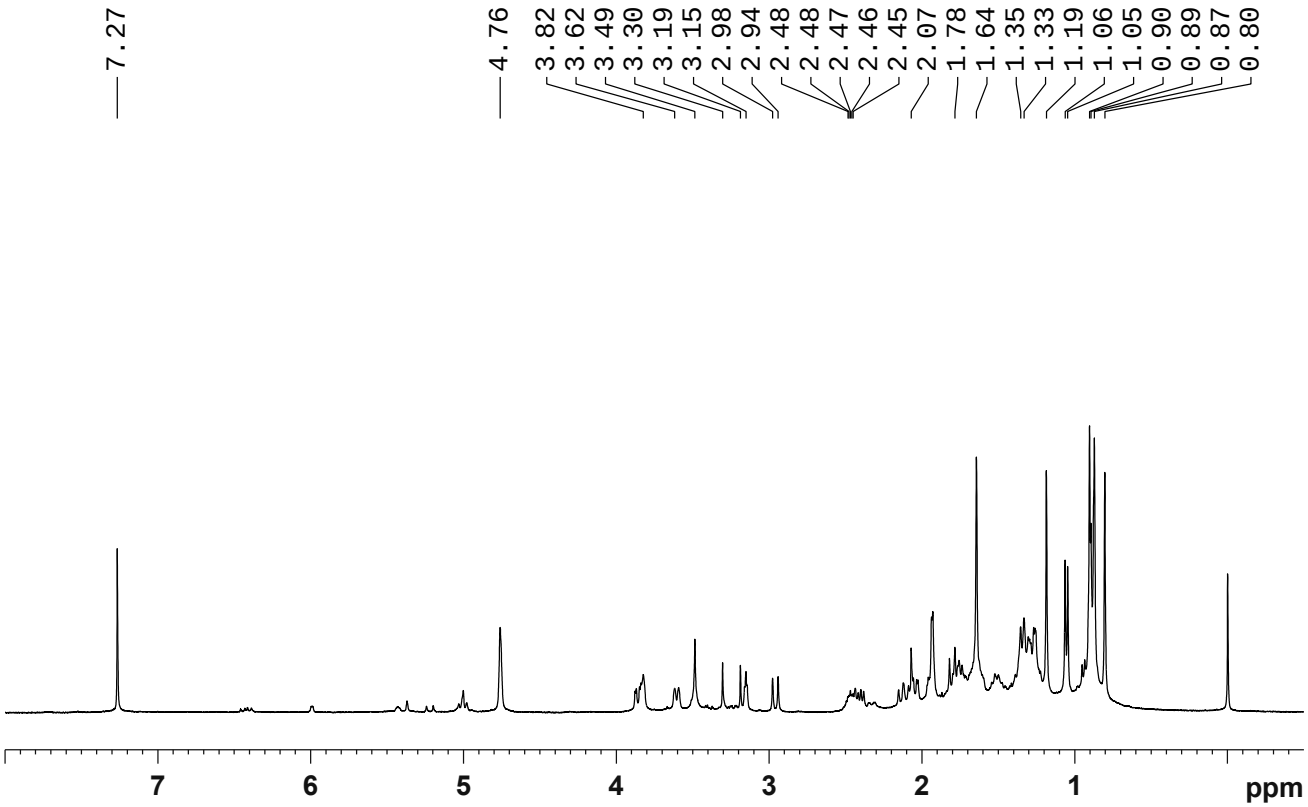

Figure S2 ^{13}C NMR spectrum for compound **1**

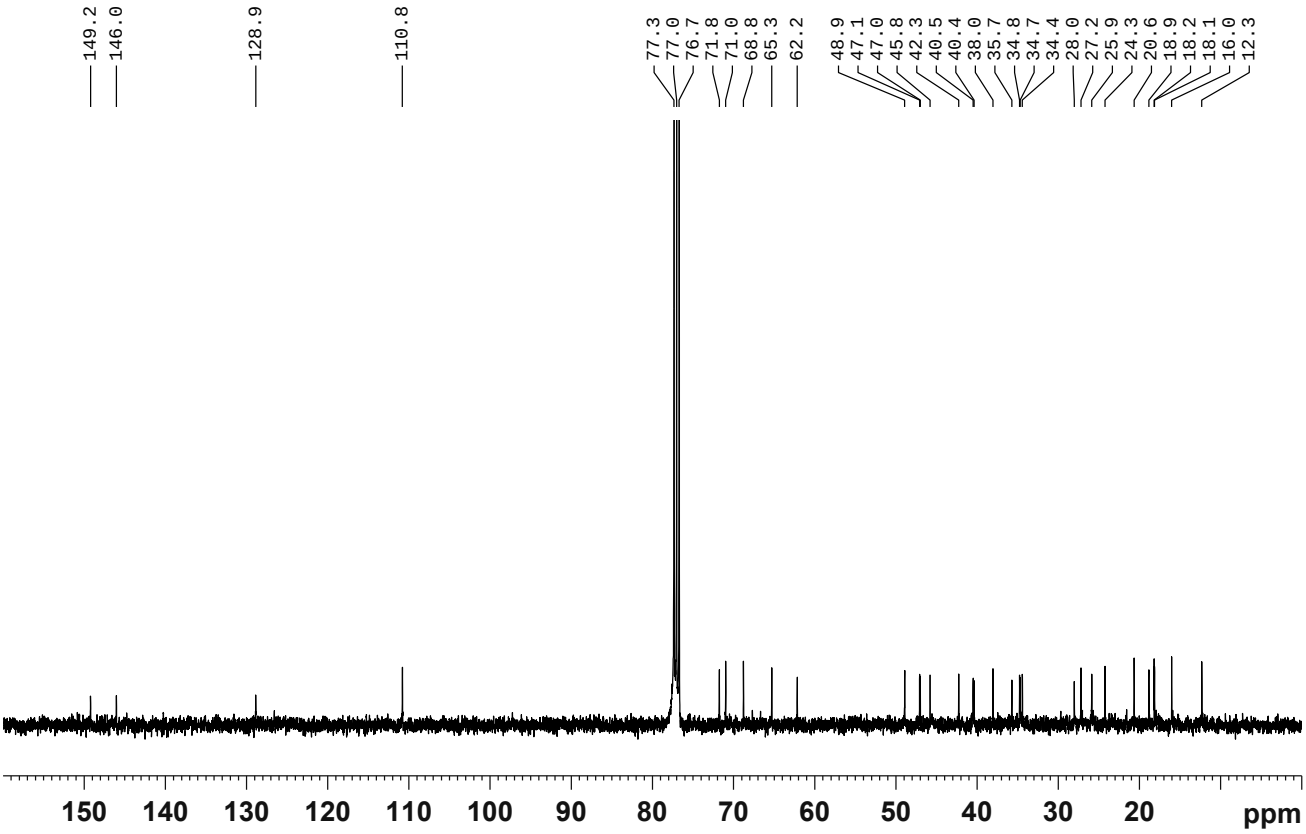

Figure S3 DEPT (θ = 135°) NMR spectrum for compound **1**

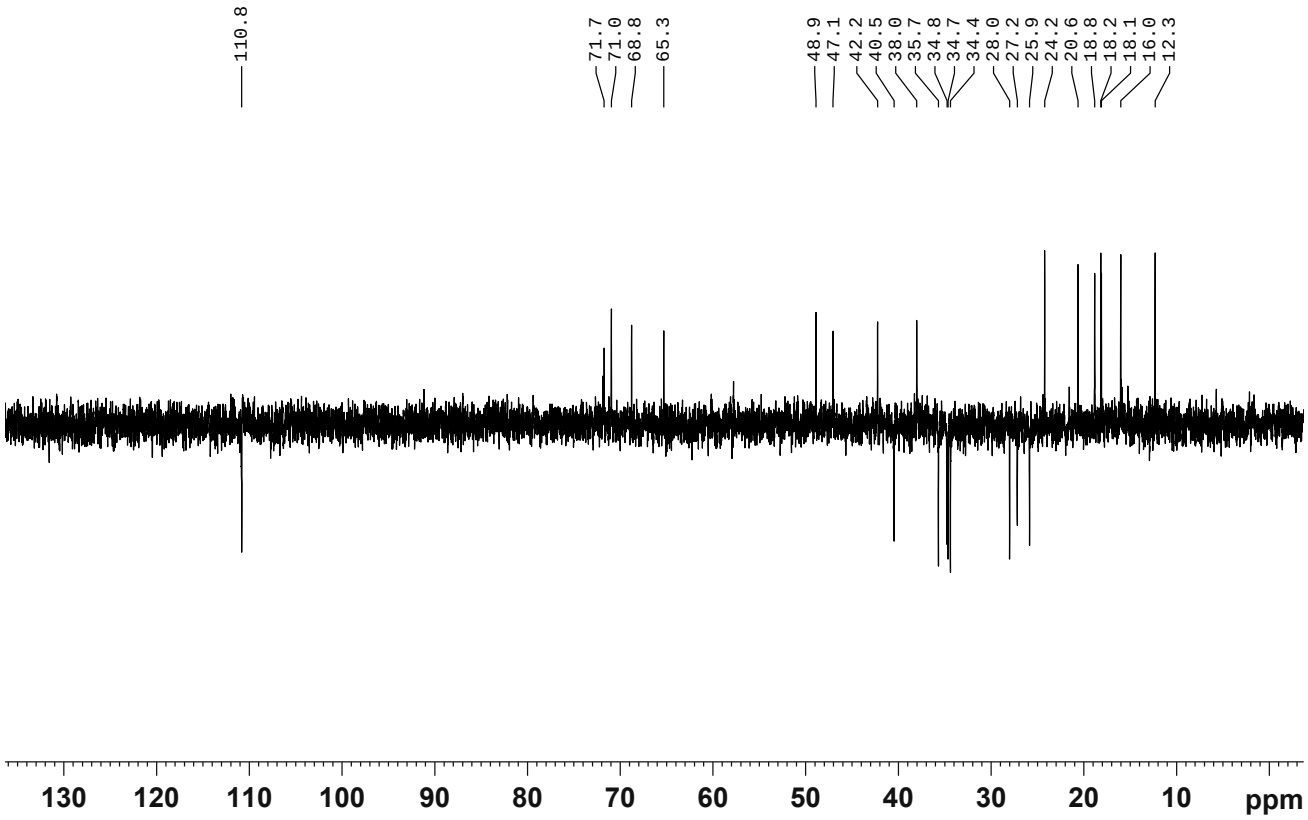

Figure S4 HMQC spectrum for compound **1**

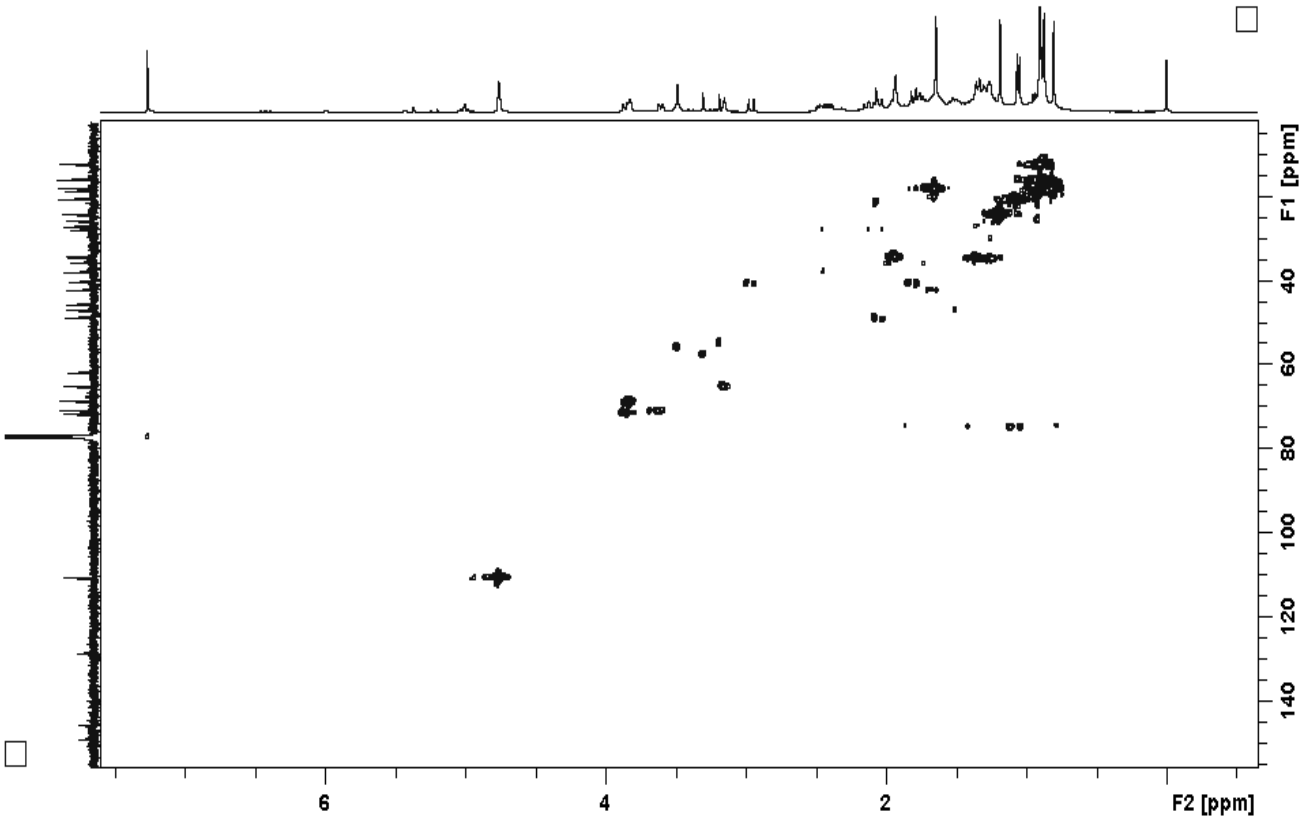

Figure S5 HMBC spectrum for compound **1**

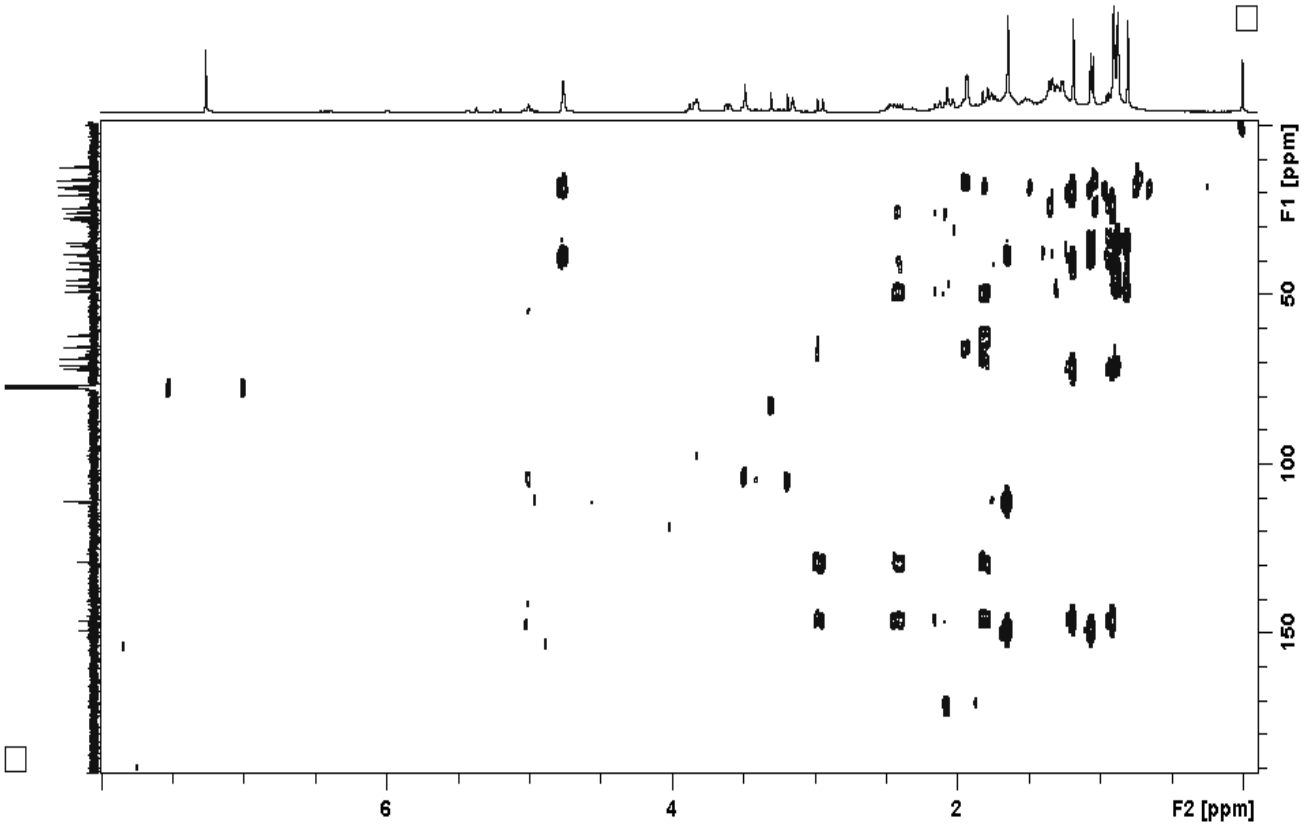

Figure S6 ^{1}H-^{1}H COSY spectrum for compound **1**

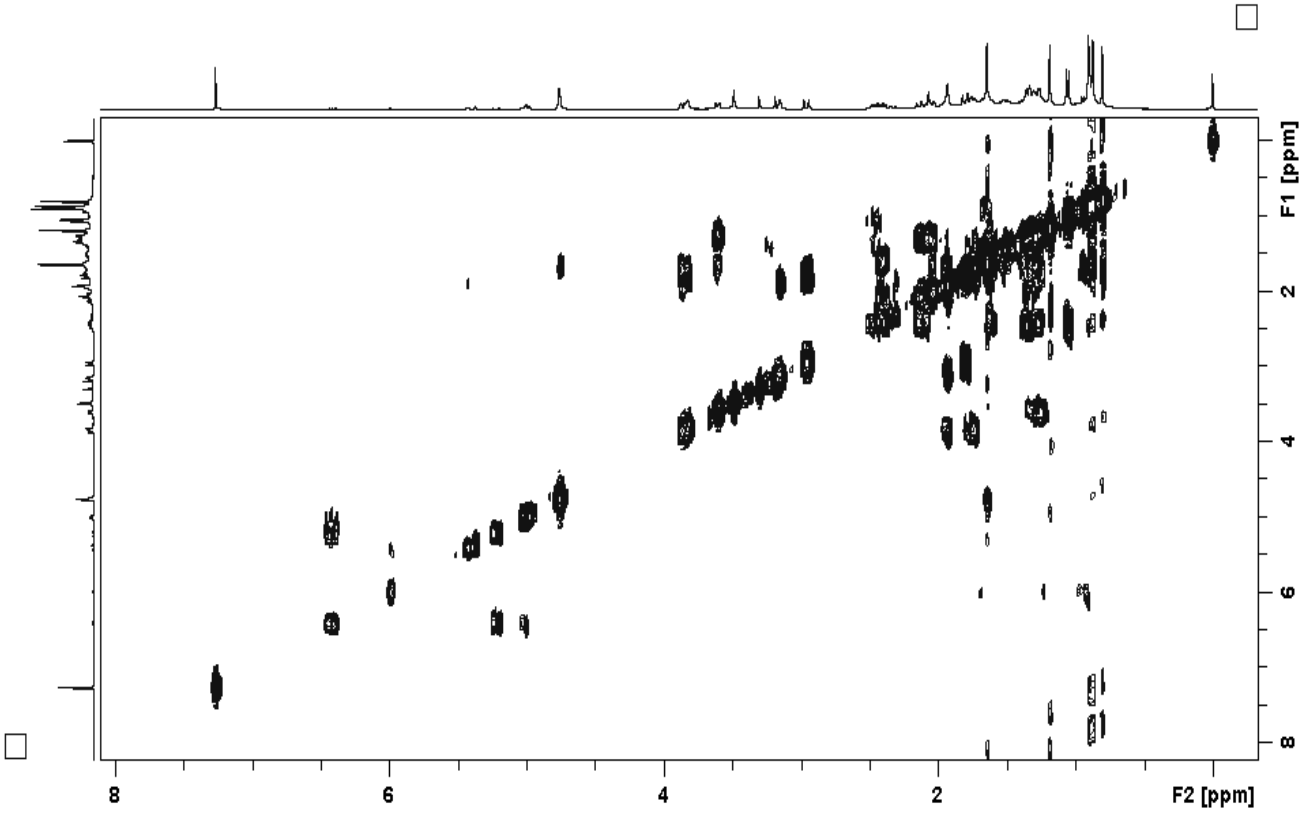

Figure S7 HRESIMS spectrum for compound **1**