Supplementary data for

Diterpenoids from the leaves of *Casearia kurzii* showing cytotoxic activities

Contents

1. Experimental Section

General experimental procedures. Optical rotations were recorded on an InsMark IP120 automatic polarimeter (InsMark Instrument Co., Ltd., Shanghai, People's Republic of China). ECD spectra were obtained on a JASCO J-715 CD spectrometer (JASCO Corporation, Tokyo, Japan). Infrared (IR) spectra (KBr disks) were recorded on a Bruker Tensor 27 FT-IR spectrometer (Bruker Optics, Ettlingen, Germany). 1D and 2D NMR experiments were performed on a Bruker AV 400 instrument (Bruker, Switzerland, 100 MHz for ^{13}C and 400 MHz for ^{1}H) with TMS as an internal reference at room temperature. HRESIMS were recorded by an IonSpec 7.0 T FTICR MS (IonSpec Co., Ltd., Lake Forest, CA, USA). HPLC separations were conducted on a CXTH system, equipped with a Shodex RI-102 detector (Showa Denko Co., Ltd., Tokyo, Japan) and a YMC-pack ODS-AM (20 × 250 mm) column (YMC Co. Ltd., Kyoto, Japan). Medium pressure liquid chromatography (MPLC) was run on a P0100 pump with an ultraviolet (UV) detector (Huideyi Co., Beijing, People's Republic of China) and a column (40 × 400 nm) filled by octadecylsilyl (ODS, 50 μM, YMC Co., Ltd.). Silica gel (200-300 mesh) used for column chromatography was purchased from Qingdao Haiyang Chemical Group Co., Ltd. (Qingdao, People's Republic of China). Chemical reagents (analytical grade) and biological reagents were provided by Tianjin Chemical Reagent Co. (Tianjin, People's Republic of China) and Sigma Co., respectively. A549, K562, HeLa and HepG2 cell lines were from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, People's Republic of China).

Computational analysis. According to the conformation of every compound deduced from the NOESY spectrum and Chem3D modeling, systematic conformational searches were performed firstly using MOE software and appropriate conformers were selected for geometry optimizations. Geometry optimizations and re-optimizations on the B3LYP/6-31+G(d,p) level were performed by the Gaussian 09 package [1]. The TDDFT ECD calculations for the optimized conformers were carried out at the CAM-B3LYP/SVP level with a CPCM solvent model in acetonitrile, and the

calculated ECD spectra of different conformers were simulated with a half bandwidth of ~0.4 eV. The ECD curves were extracted by SpecDis 1.62 software [2]. The overall ECD curves of all the compounds were weighted by Boltzmann distribution after UV correction.

Cytotoxic activity assay. The cytotoxic activities were evaluated using MTT assay [3,4]. Cells were cultured in RPMI-1640 (A549, K562, and HeLa cells) or DMEM (HepG2 cells) medium supplemented with 10% (v/v) fetal bovine serum and 100 U/mL penicillin/streptomycin under a water-saturated atmosphere of 95% air and 5% CO_2. After reaching approximate 80% confluence, the cells were harvested and seeded in 96-well plates (1×10^4 cells/well) and allowed to adhere for 24 h at 37°C. Then, the cells were treated with the test samples dissolved in DMSO at different concentrations, including the positive and the negative controls. Etoposide was used as a positive control. After a continuous incubation for 48 h, 20 μL MTT solution (5 mg/mL, Solarbio, Beijing, People's Republic of China) were added in each well for 4 hours. Then, the medium was replaced with 150 μL DMSO and the absorbance was measured at 492 nm using microplate reader (Thermo Fisher Scientific Inc. America). The experiments were performed in triplicate, and the IC_{50} value was defined as the concentration of the compounds that inhibited cell proliferation by 50%.

Apoptosis analysis by flow cytometry. The apoptosis analysis of HepG2 cells induced by the tested compound was accomplished by flow cytometry using Annexin V-FITC Apoptosis Detection Kit (Beyotime, Shanghai, People's Republic of China) according to the manufacturer's instruction [5,6]. Briefly, HepG2 cells were treated with various concentrations (3, 9, and 27 μM) of selected compound. After an incubation of 48 h, the cells were washed twice with PBS and resuspended in the binding buffer (Beyotime, Shanghai, People's Republic of China). This suspension was incubated for 20 min at room temperature in the dark after adding 10 μL Annexin V-FITC and 5 μL PI. Then, cell apoptosis was examined by BD LSRFortessa flow cytometry (BD Biosciences). The cell apoptosis data were obtained with FLOWJO flow cytometry analysis software (FLOWJO LLC, USA).

Cell cycle analysis. The distribution of cell cycle of HepG2 cells affected by the tested compound was performed using Flow cytometric analysis [7,8]. HepG2 cells in exponential growth phase were seeded in 12 well plate and treated with different concentrations of selected compounds (6, 9, and 12 μM). After an exposure to the test sample for 48 h, the cells were harvested, washed with PBS twice, and fixed in 70% ice-cold ethanol at 4°C overnight. Then, the cells were washed with PBS twice and treated with propidium iodide staining buffer containing RNase (Beyotime, Shanghai, People's Republic of China) for 30 minutes at 37 °C in the dark, followed immediately by cellar DNA analysis using BDLSR Fortessa flow cytometry. Data were processed using ModFit LT Software.

References

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2. NMR Spectra of Compounds 1–6

Fig. S1 ^{1}H NMR spectrum for compound **1**

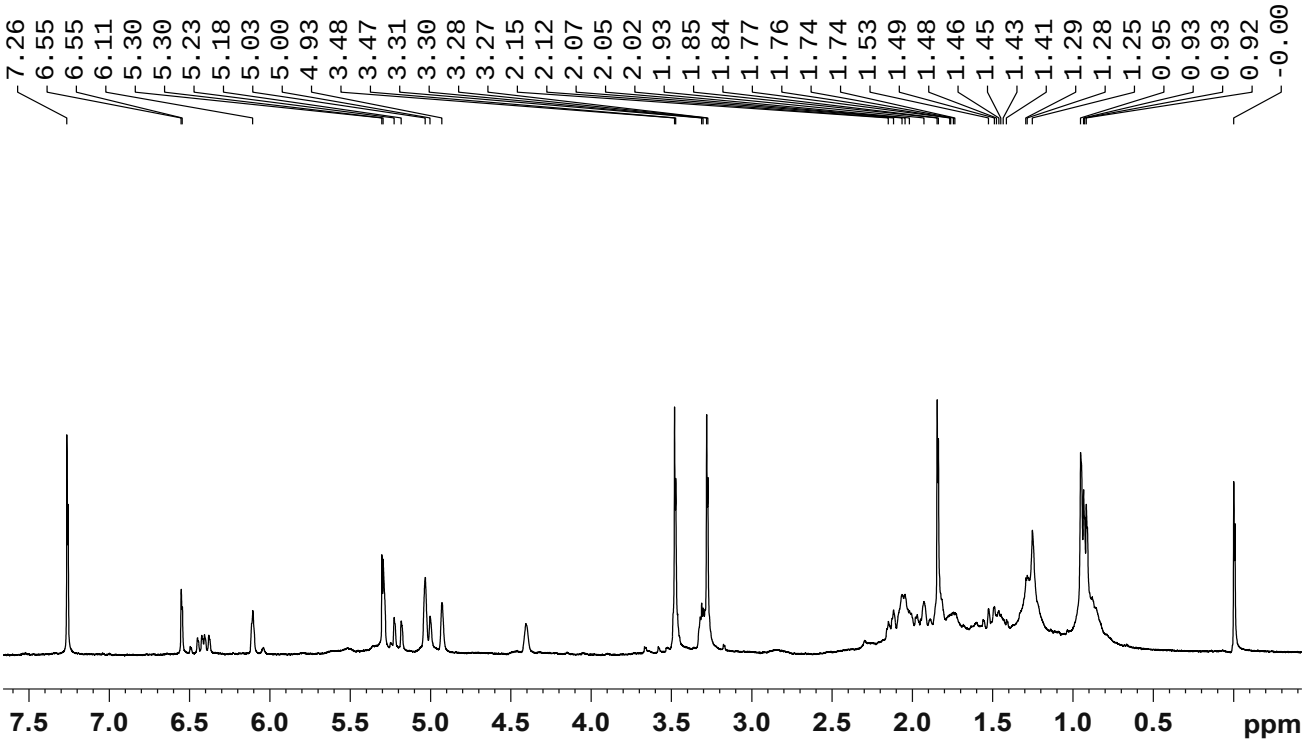

Fig. S2 ^{13}C NMR spectrum for compound **1**

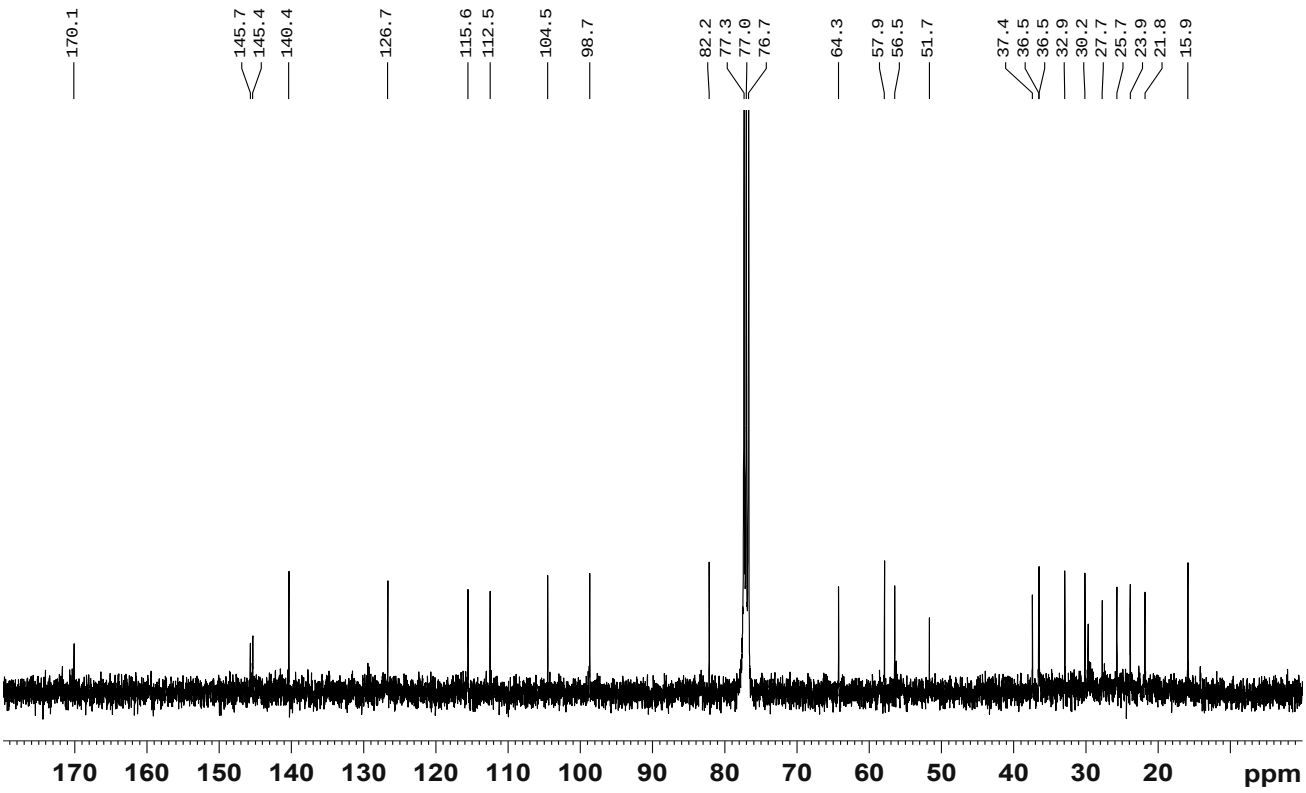

Fig. S3 DEPT (θ = 135°) NMR spectrum for compound **1**

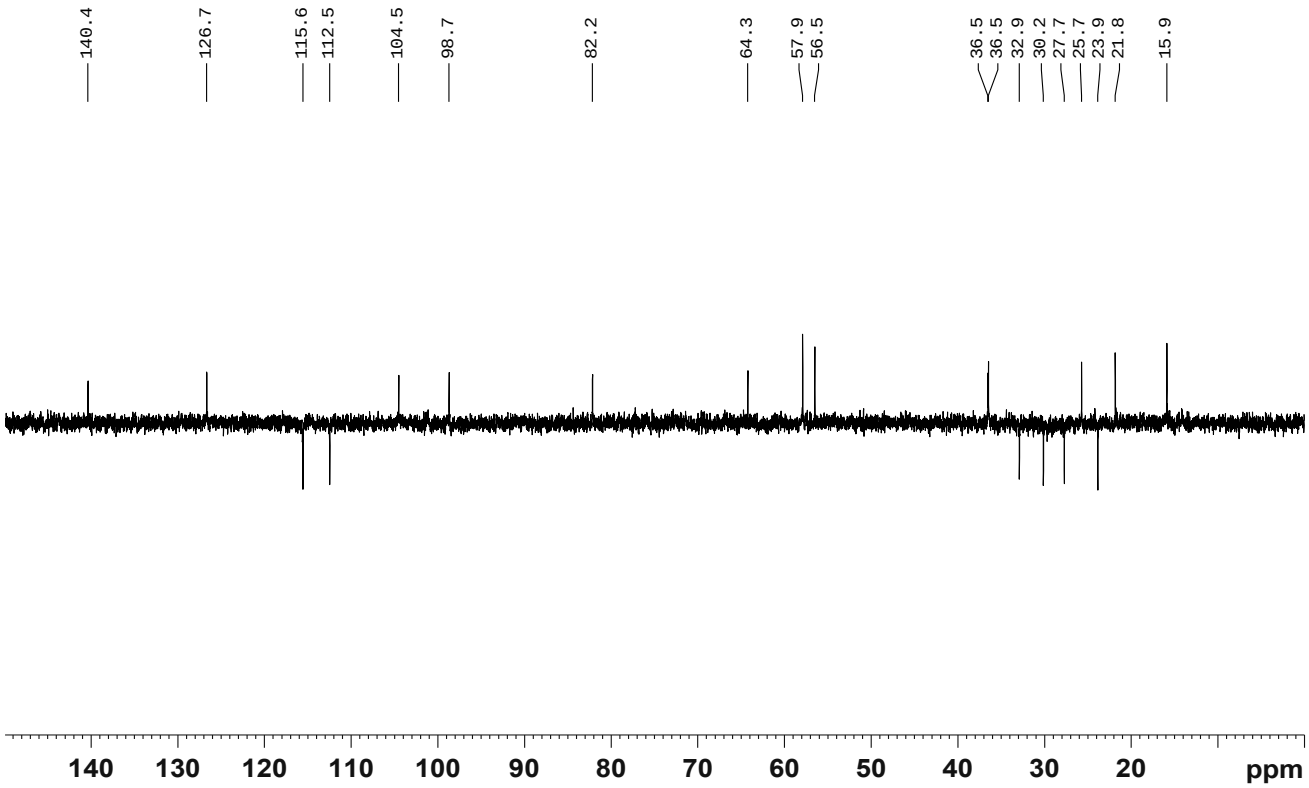

Fig. S4 HMQC spectrum for compound **1**

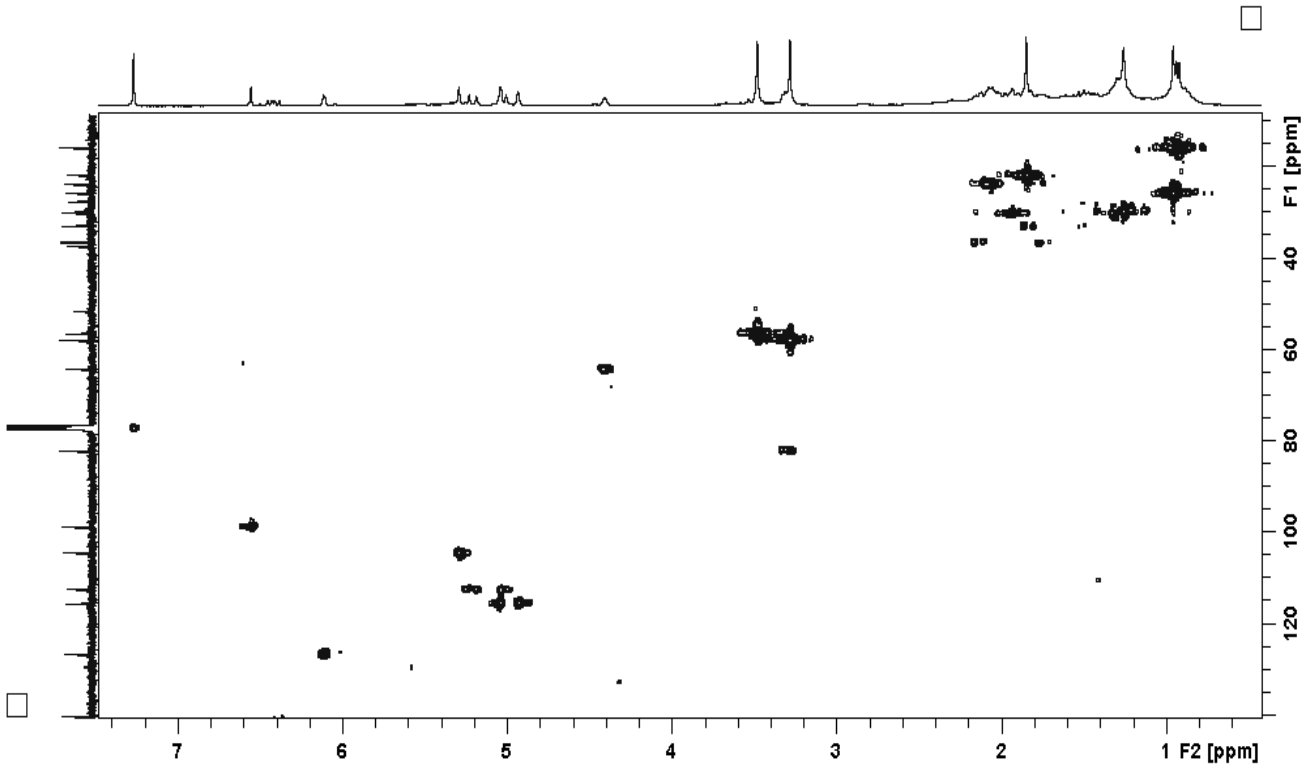

Fig. S5 HMBC spectrum for compound **1**

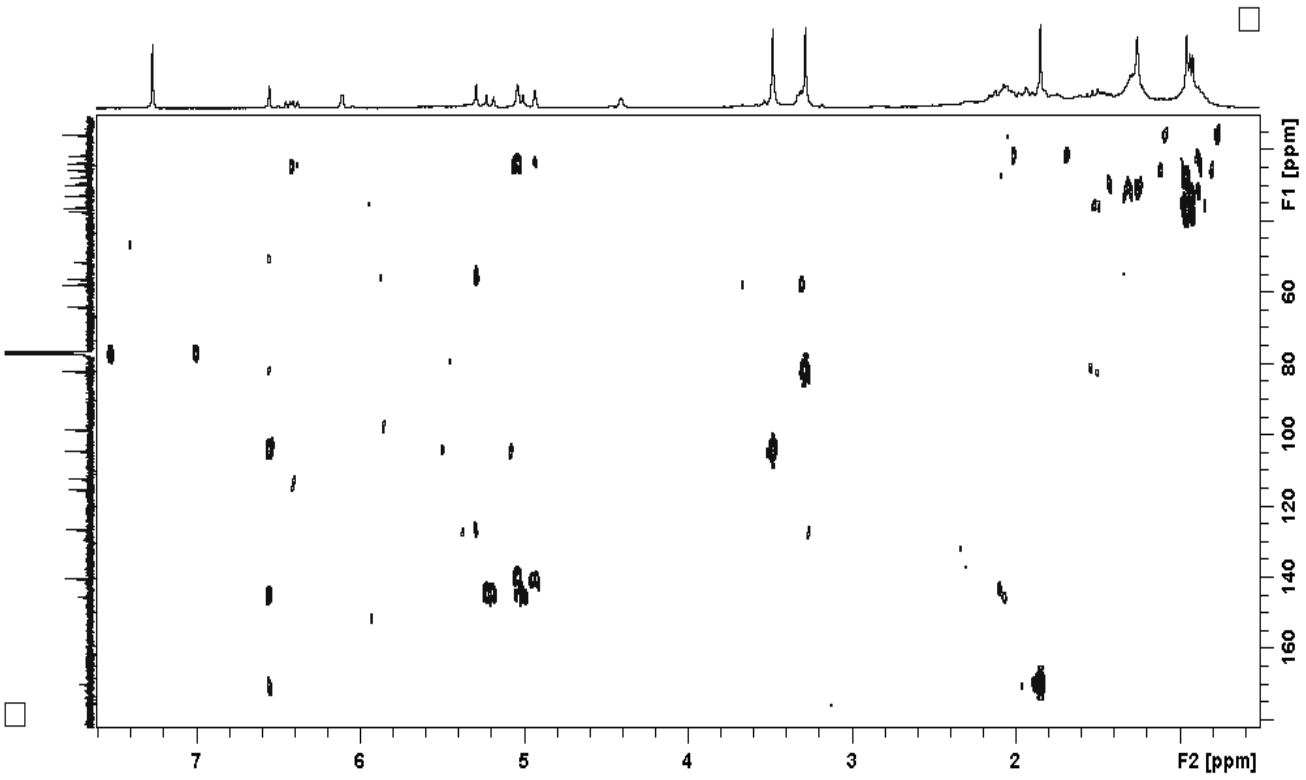

Fig. S6 ^{1}H-^{1}H COSY spectrum for compound **1**

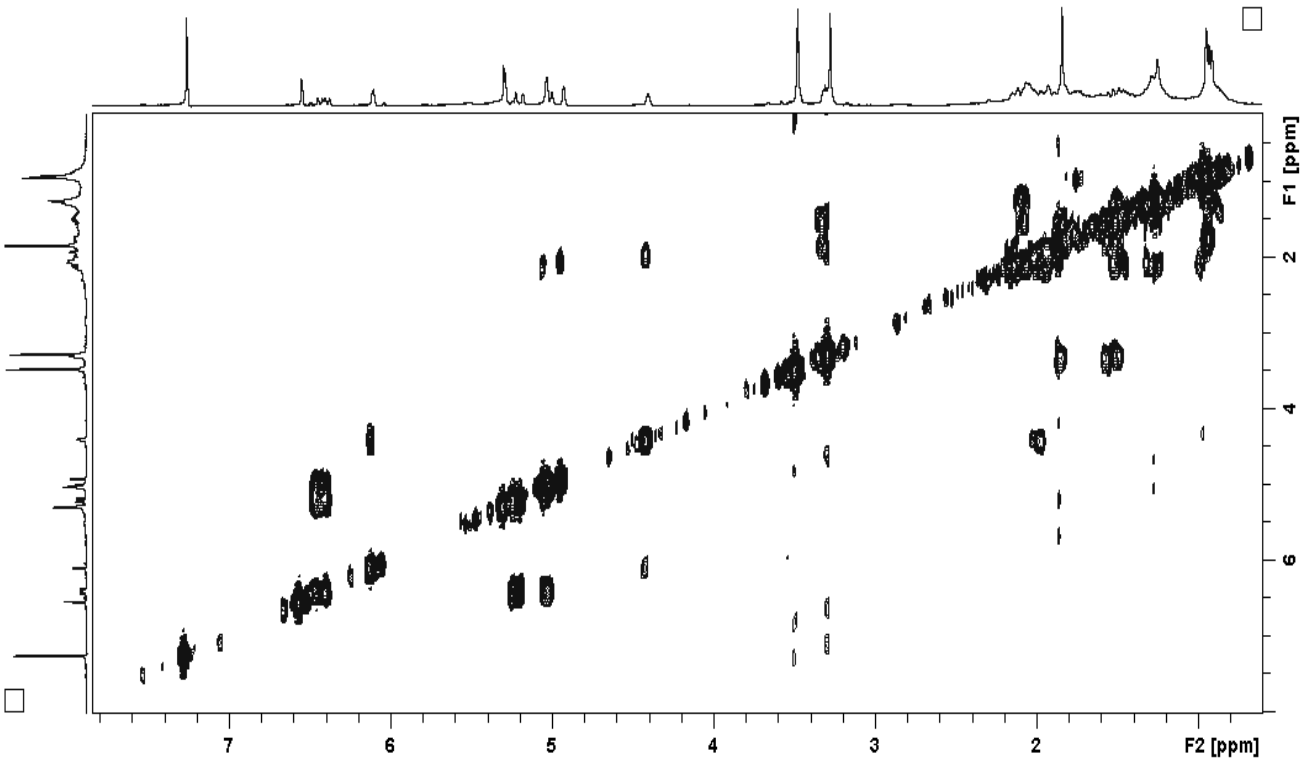

Fig. S7 NOESY spectrum for compound **1**

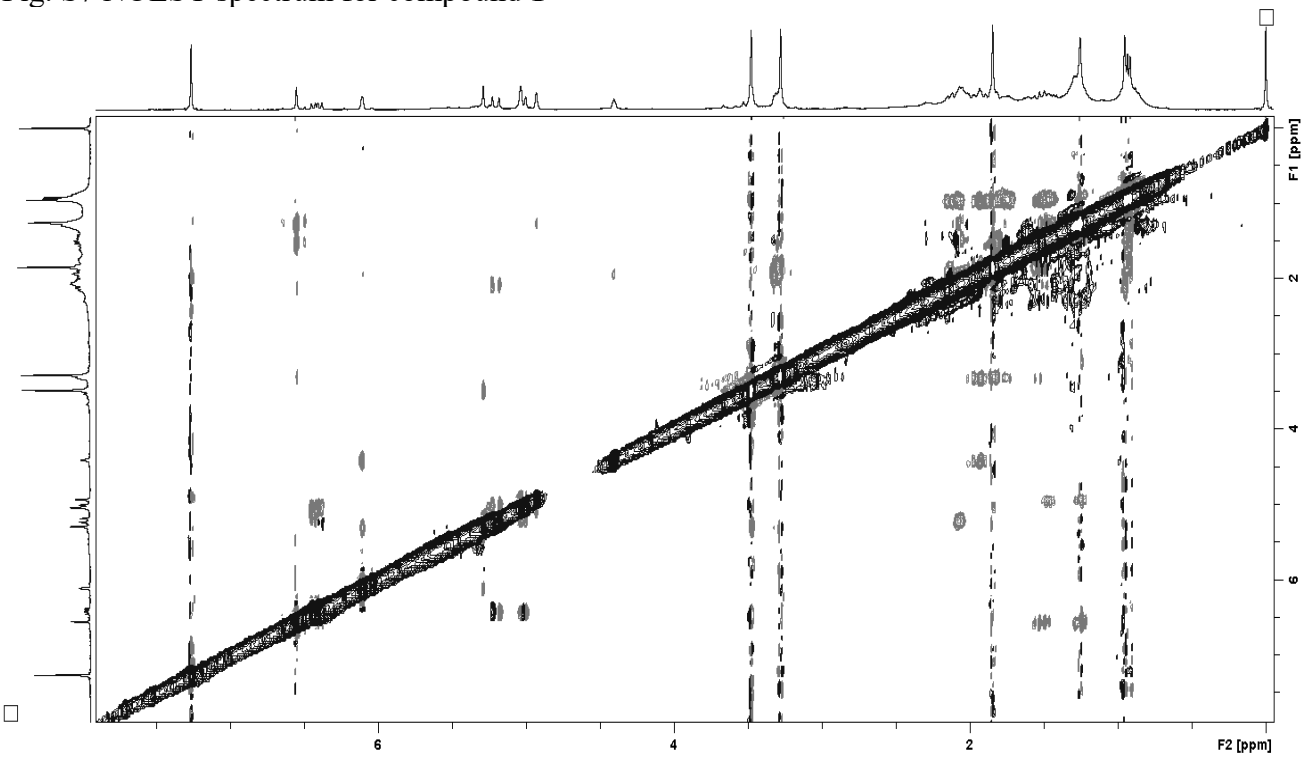

Fig. S8 ^{1}H NMR spectrum for compound **2**

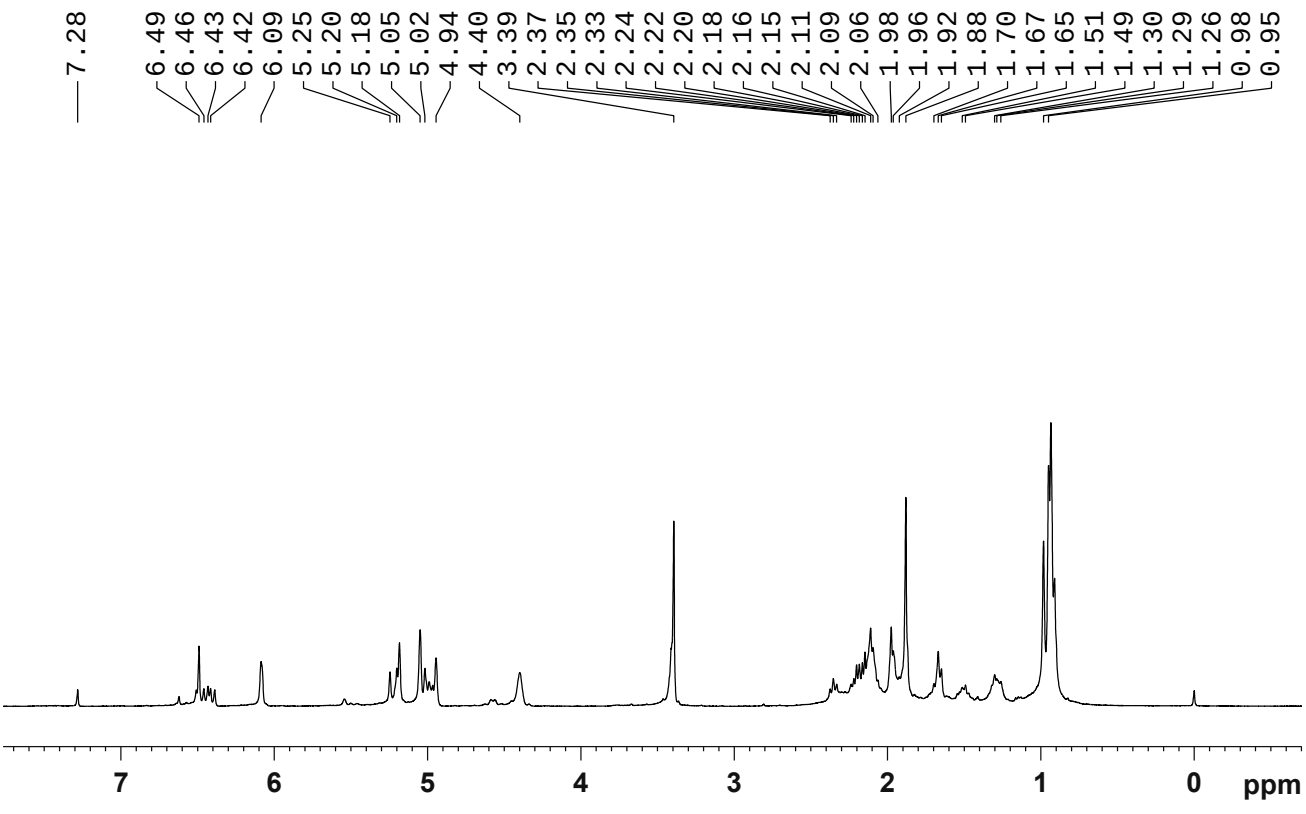

Fig. S9 ^{13}C NMR spectrum for compound **2**

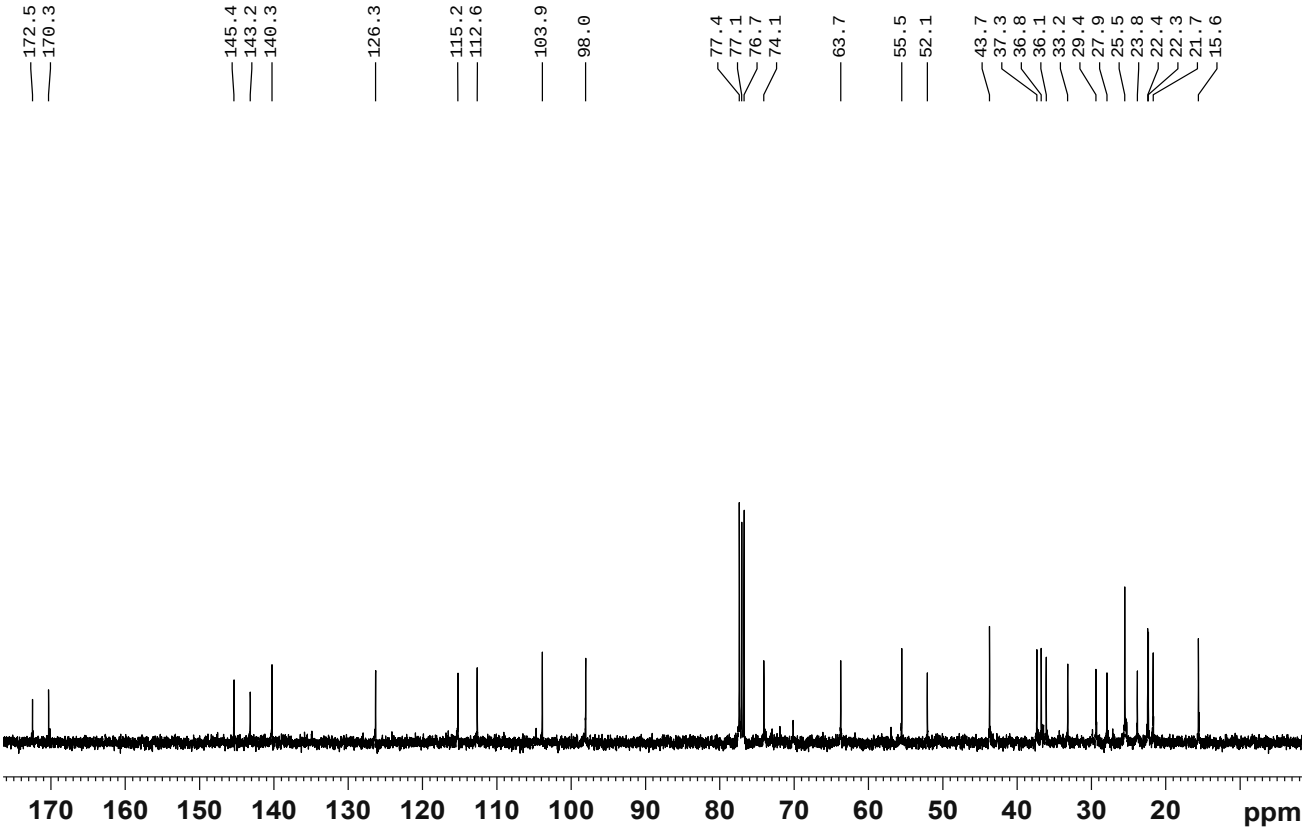

Fig. S10 HMQC spectrum for compound **2**

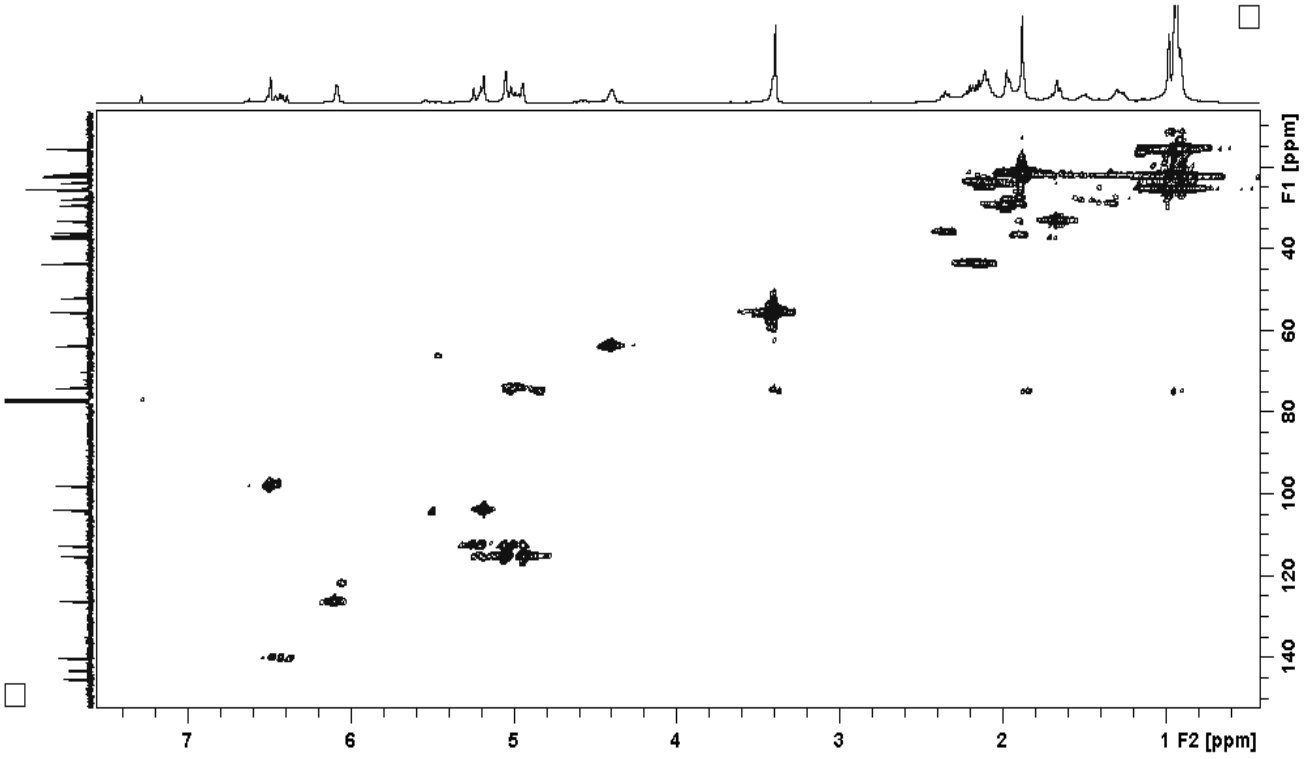

Fig. S11 HMBC spectrum for compound **2**

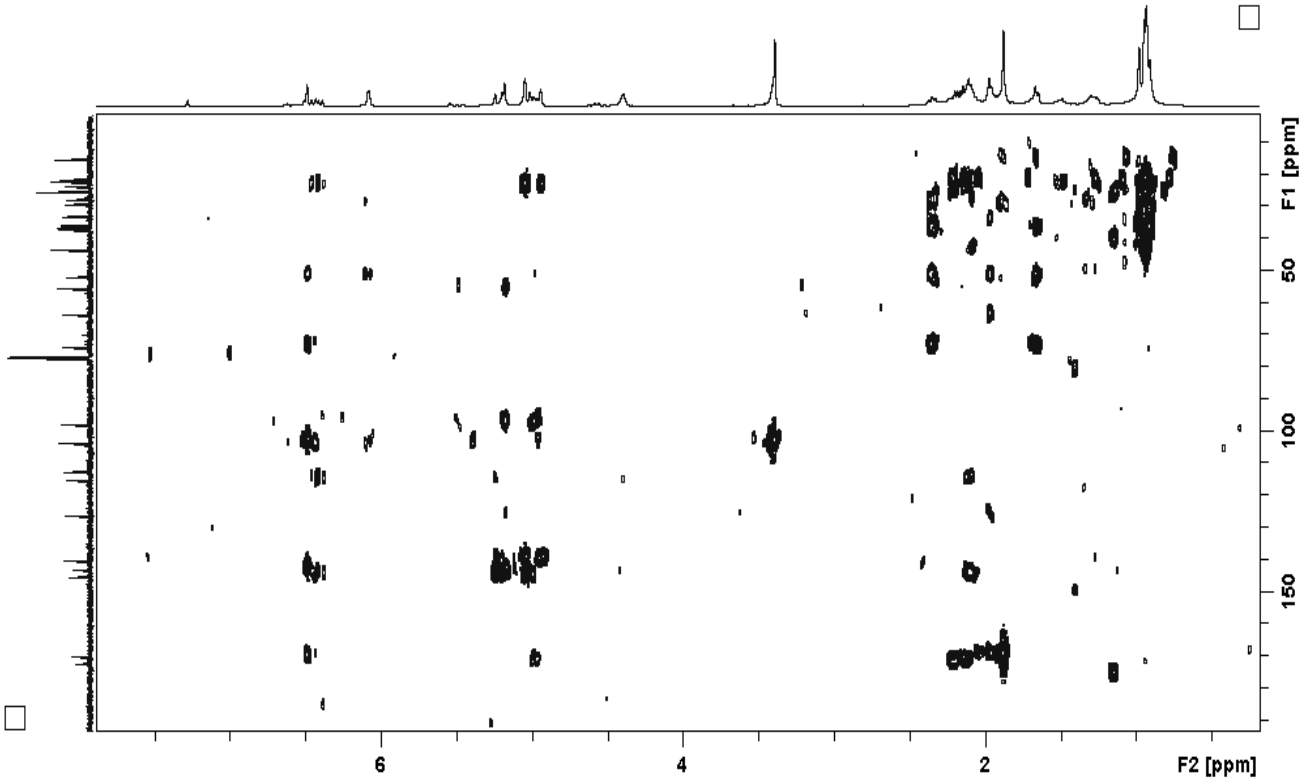

Fig. S12 NOESY spectrum for compound **2**

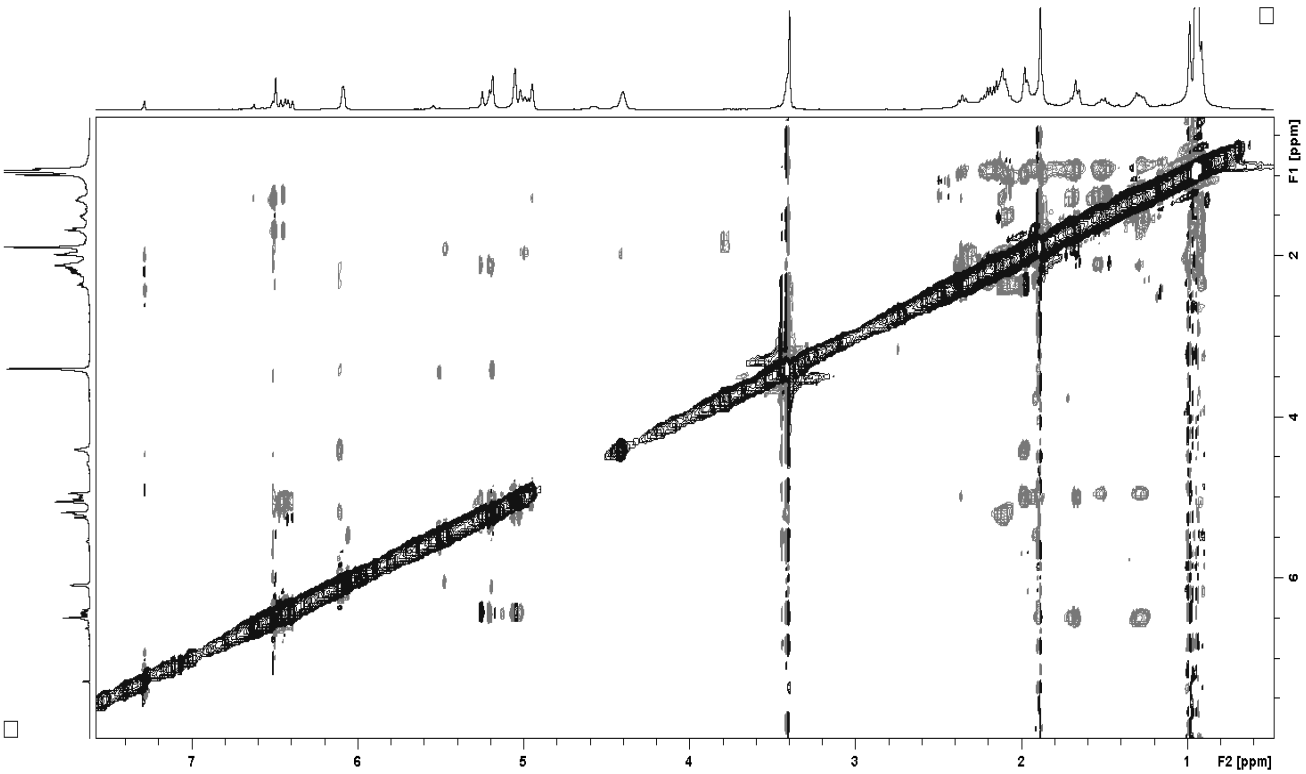

Fig. S13 ^{1}H NMR spectrum for compound **3**

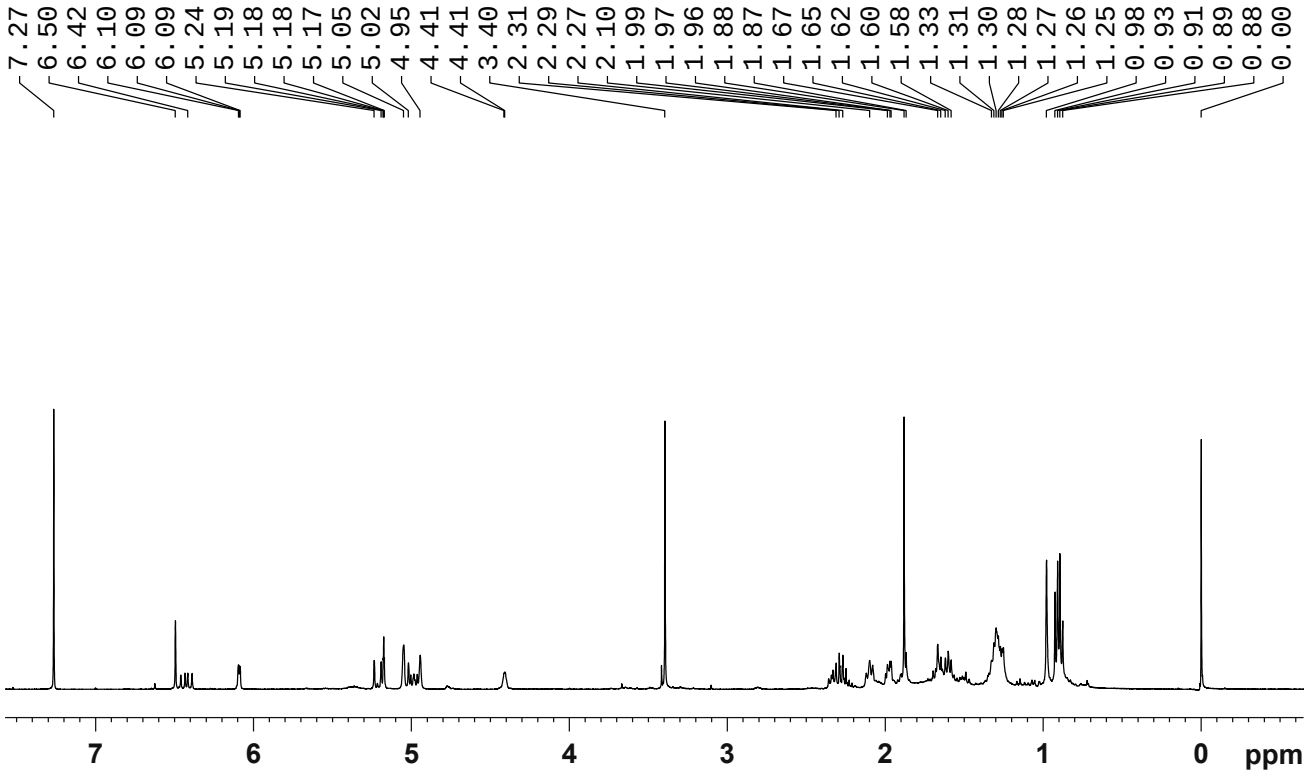

Fig. S14 ^{13}C NMR spectrum for compound **3**

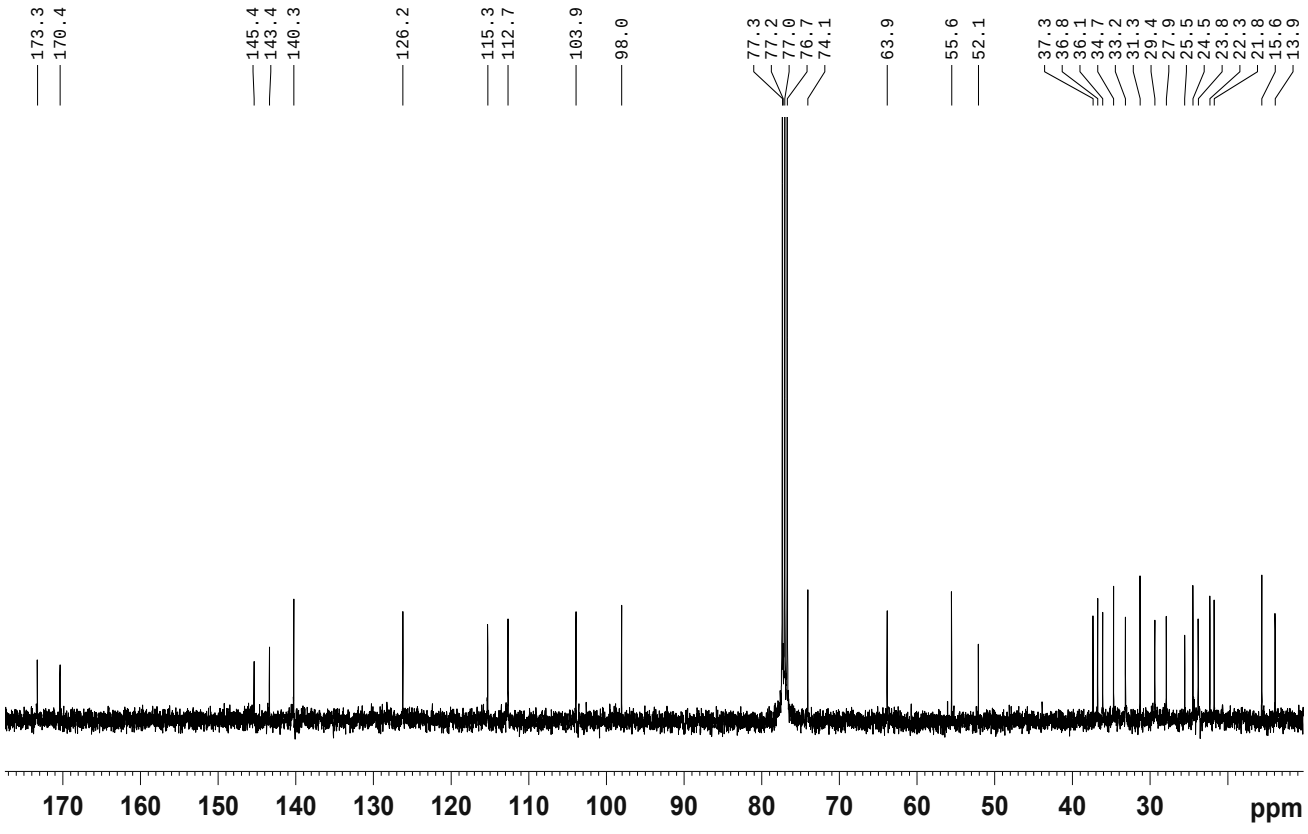

Fig. S15 HMQC spectrum for compound **3**

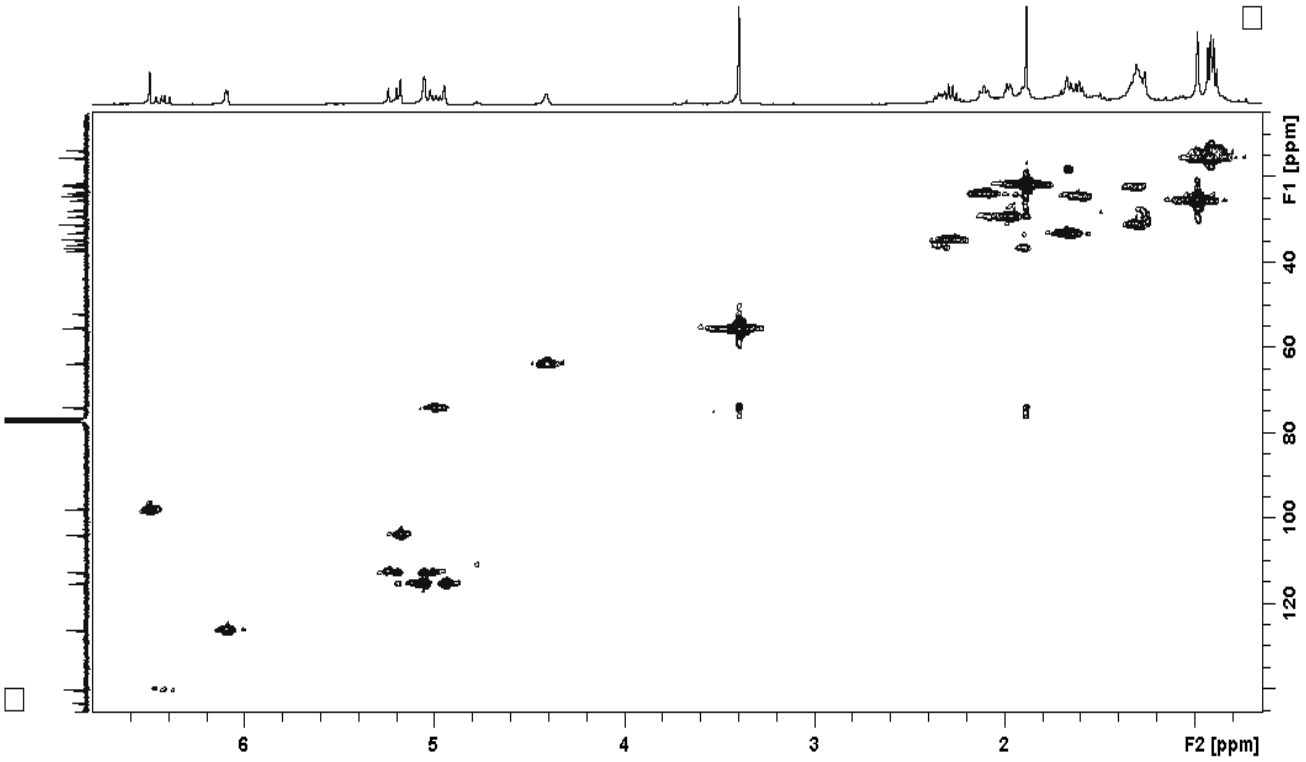

Fig. S16 HMBC spectrum for compound **3**

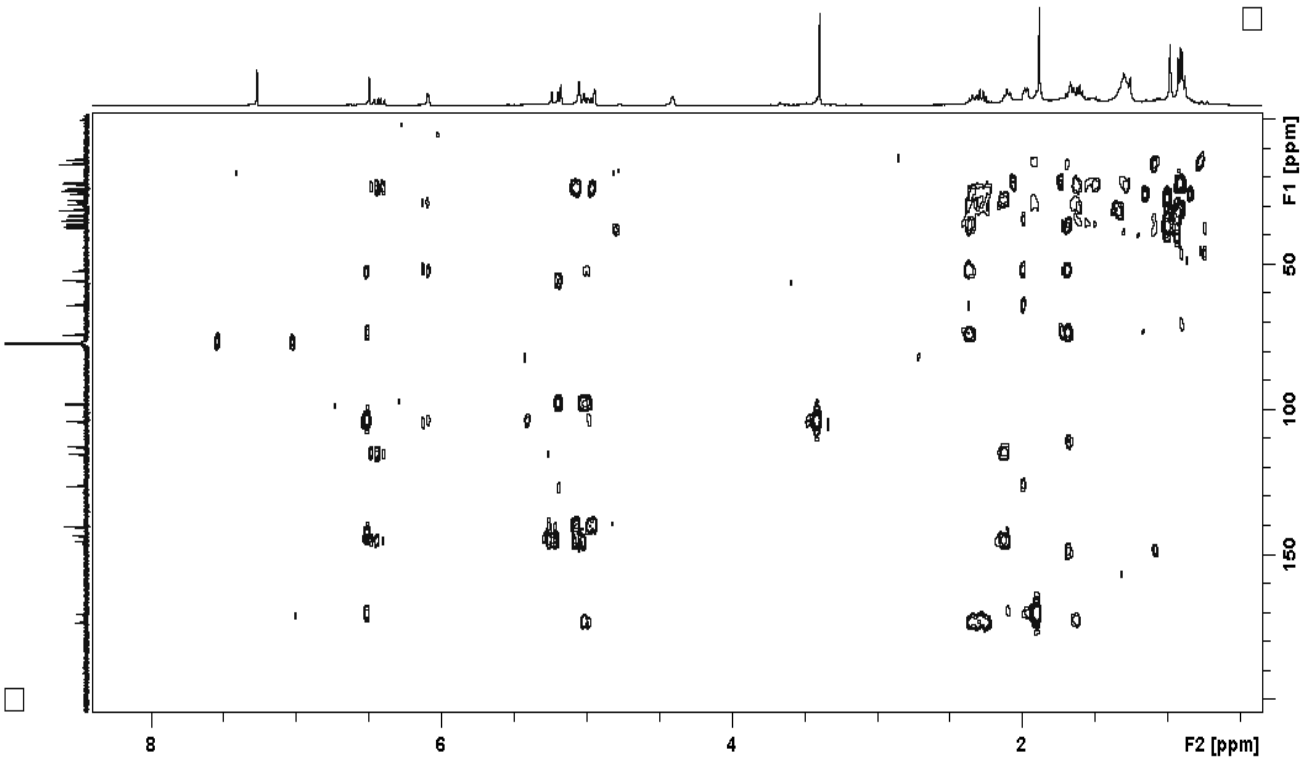

Fig. S17 NOESY spectrum for compound **3**

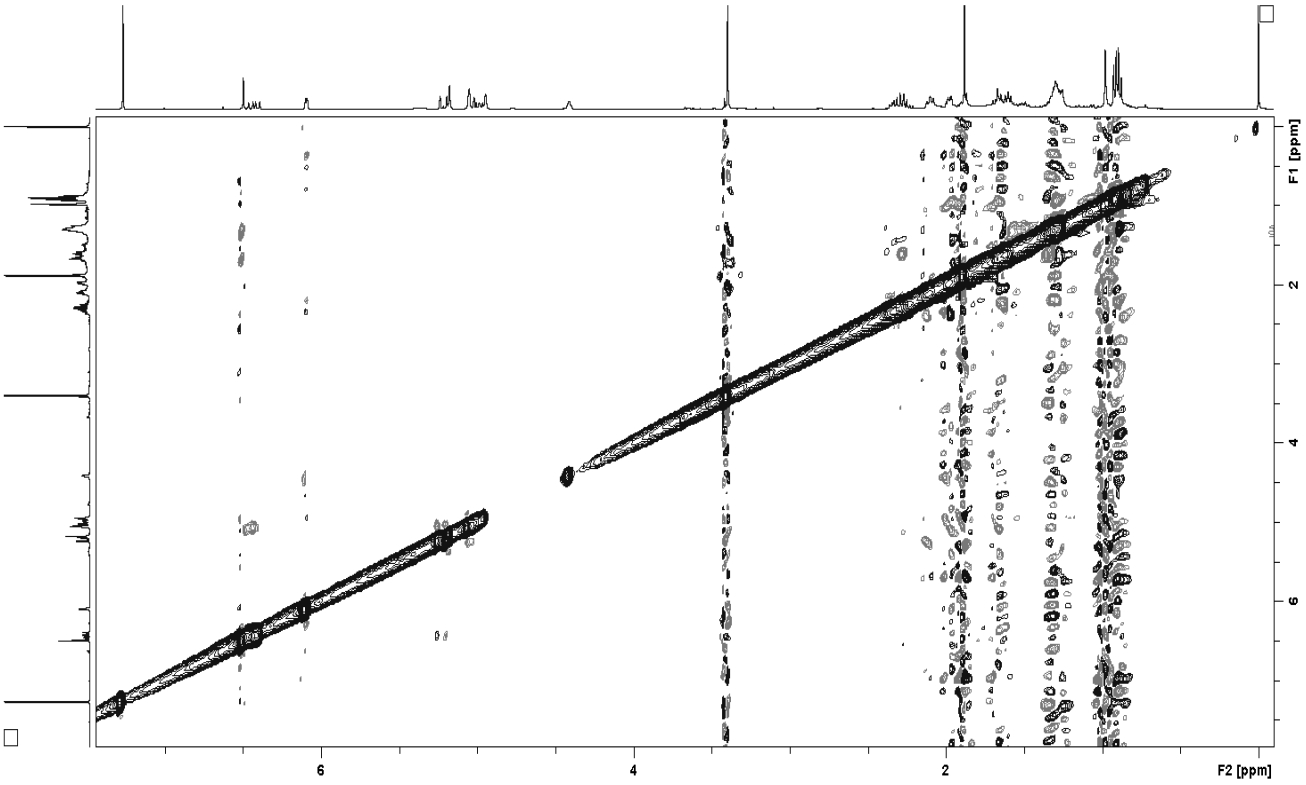

Fig. S18 ^{1}H NMR spectrum for compound **4**

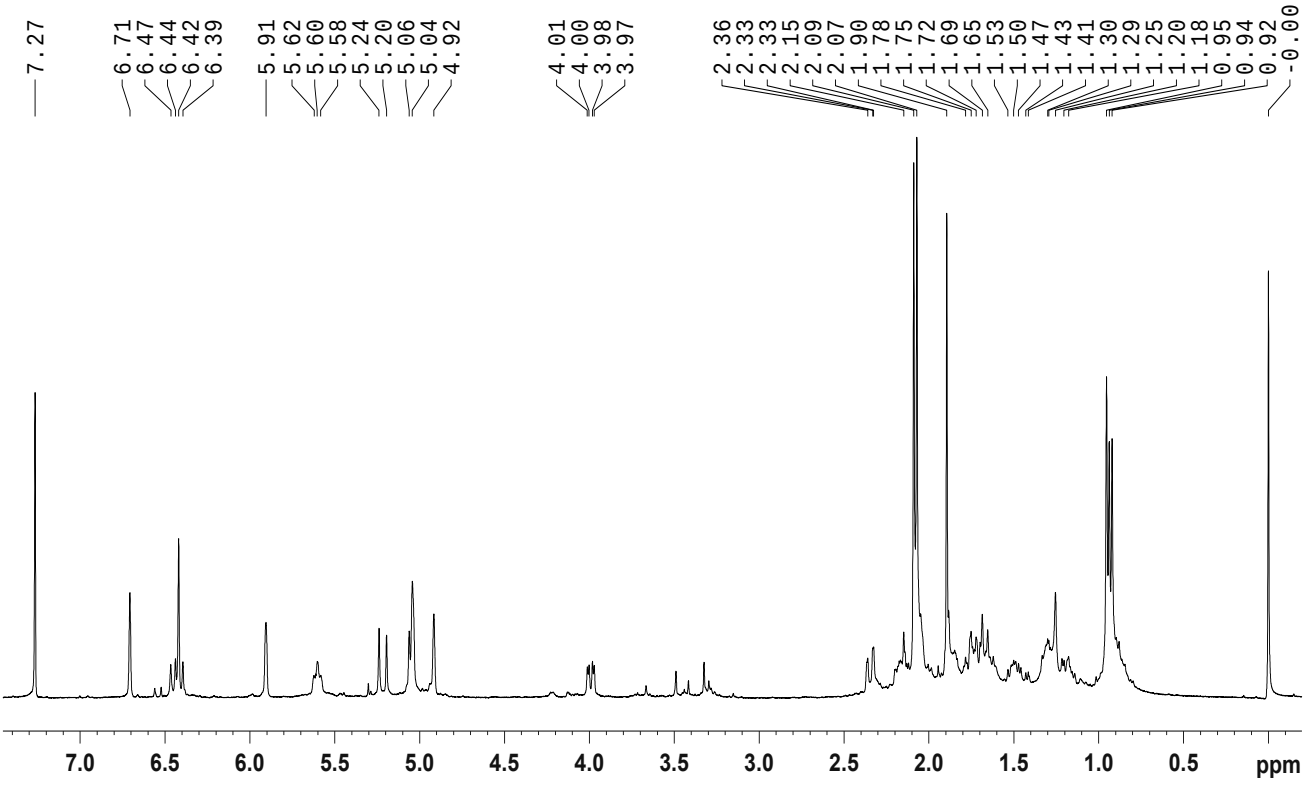

Fig. S19 ^{13}C NMR spectrum for compound **4**

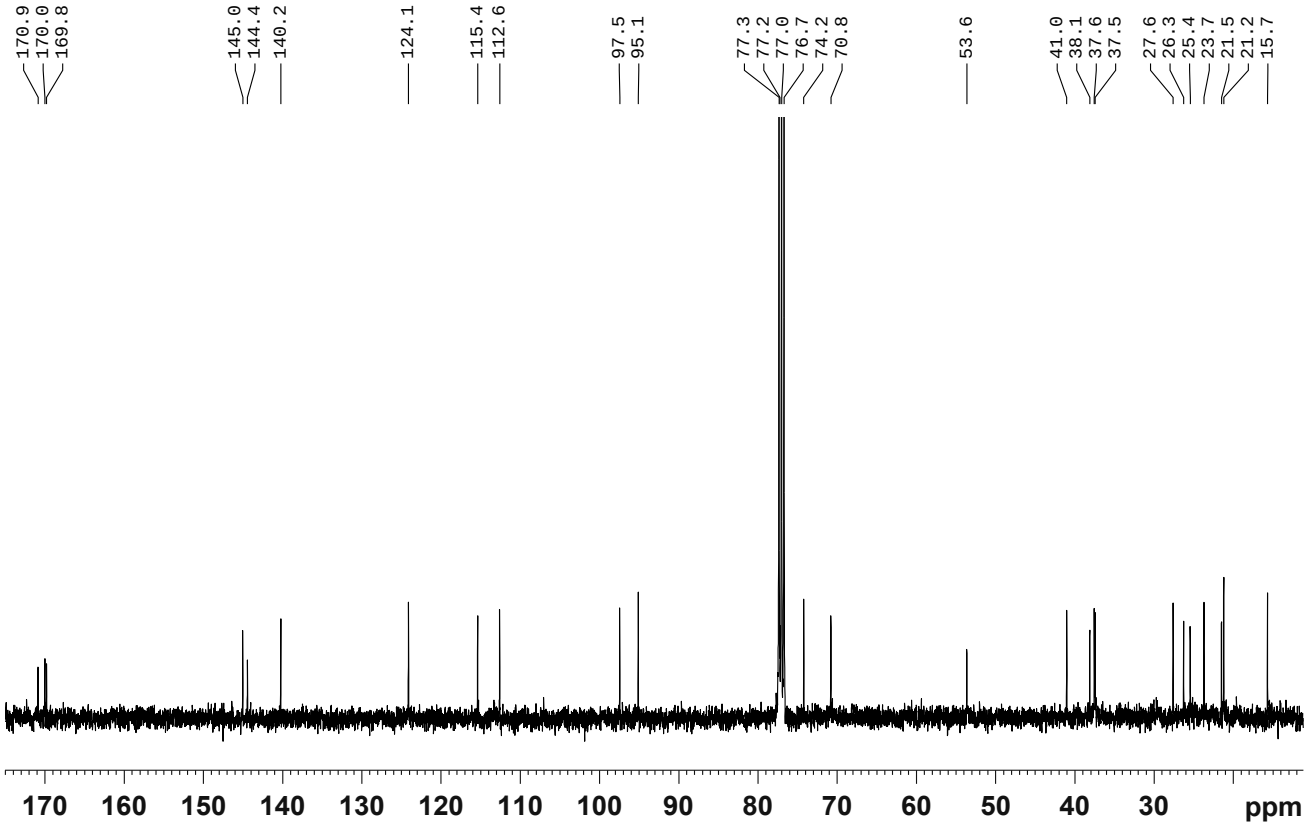

Fig. S20 HMQC spectrum for compound **4**

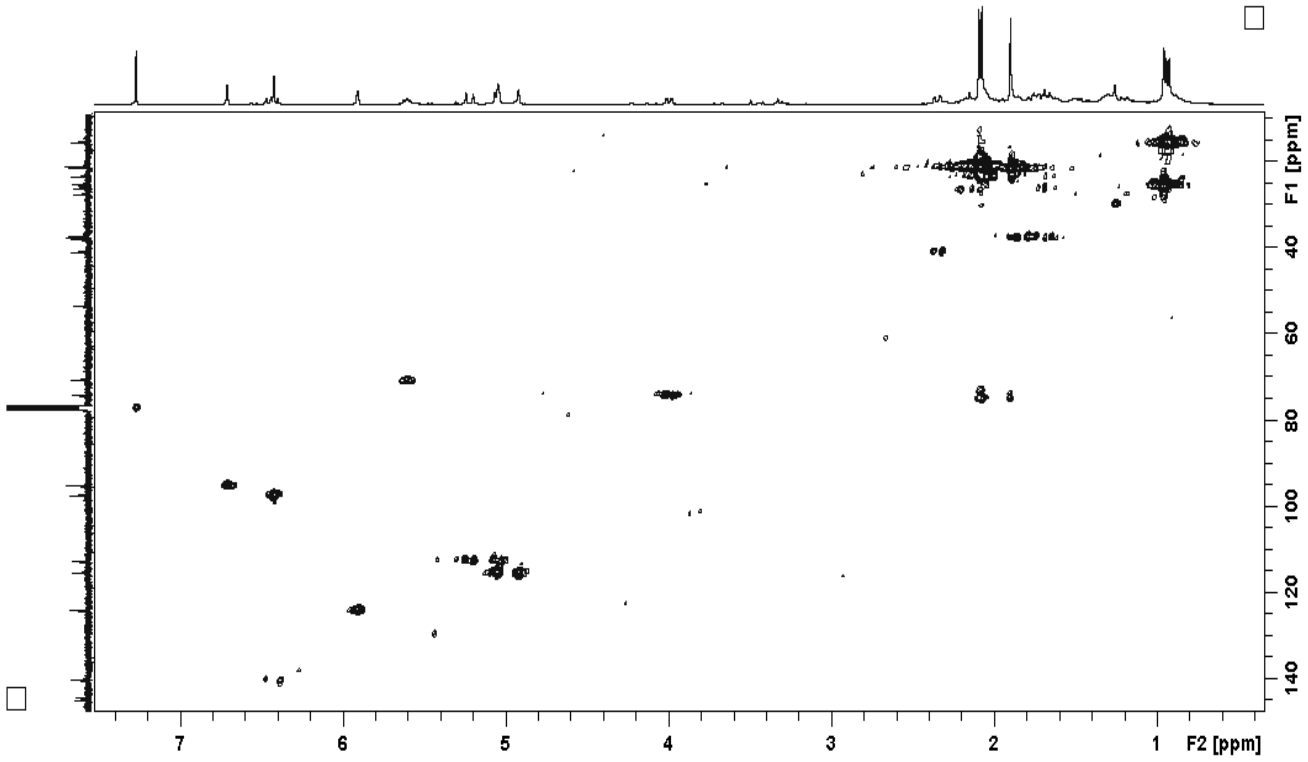

Fig. S21 HMBC spectrum for compound **4**

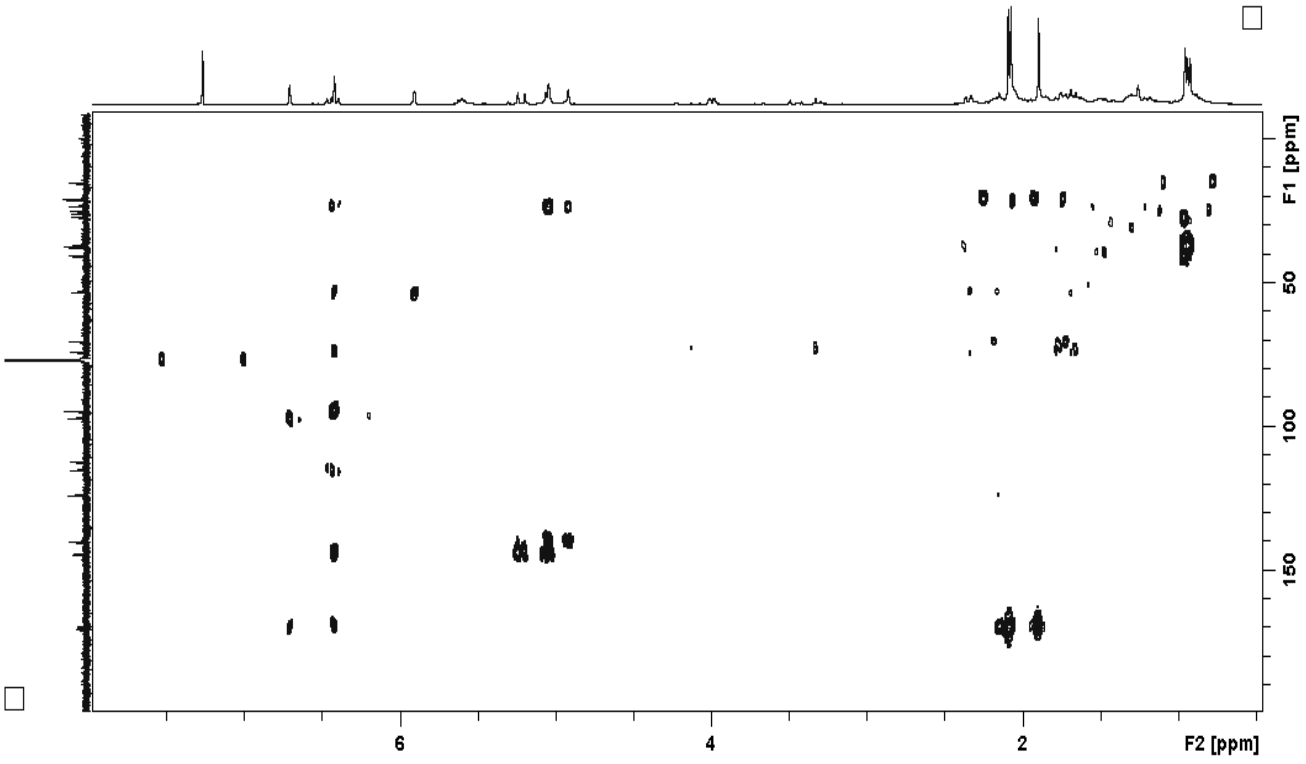

Fig. S22 NOESY spectrum for compound **4**

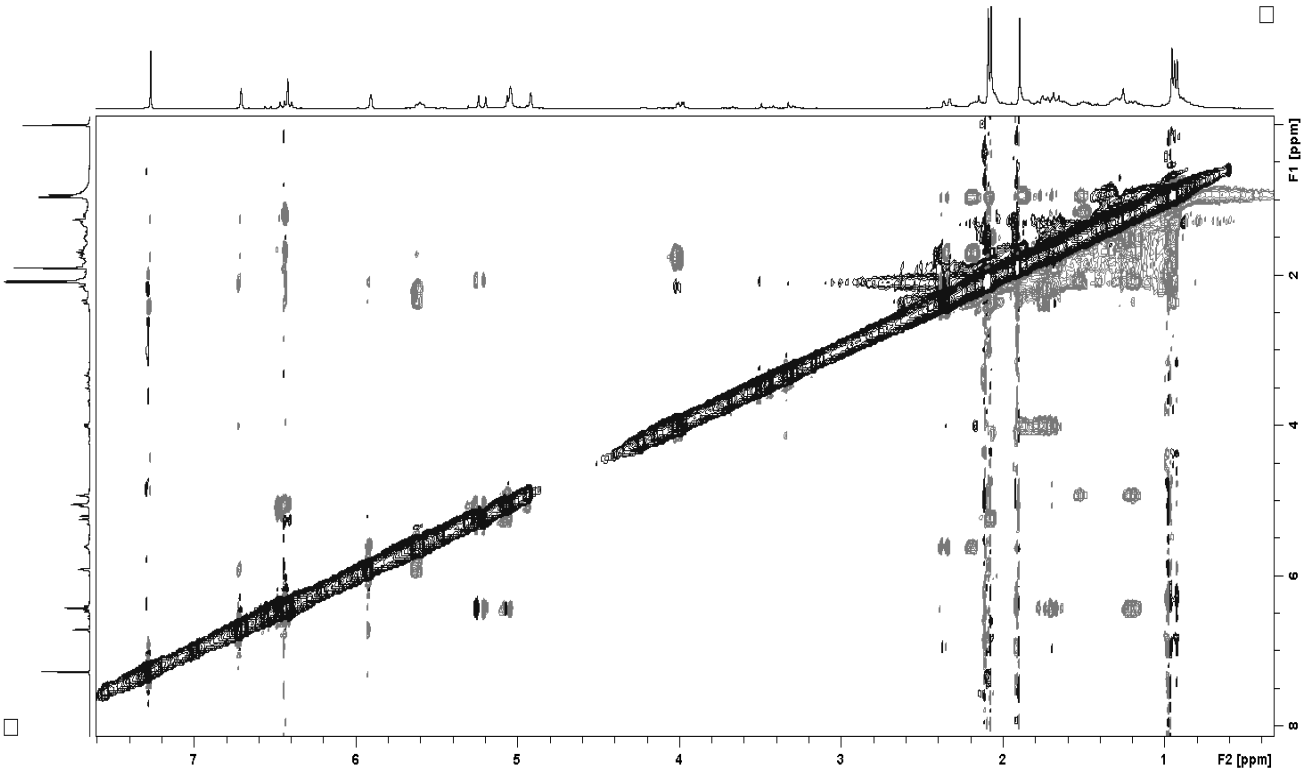

Fig. S23 ^{1}H NMR spectrum for compound **5**

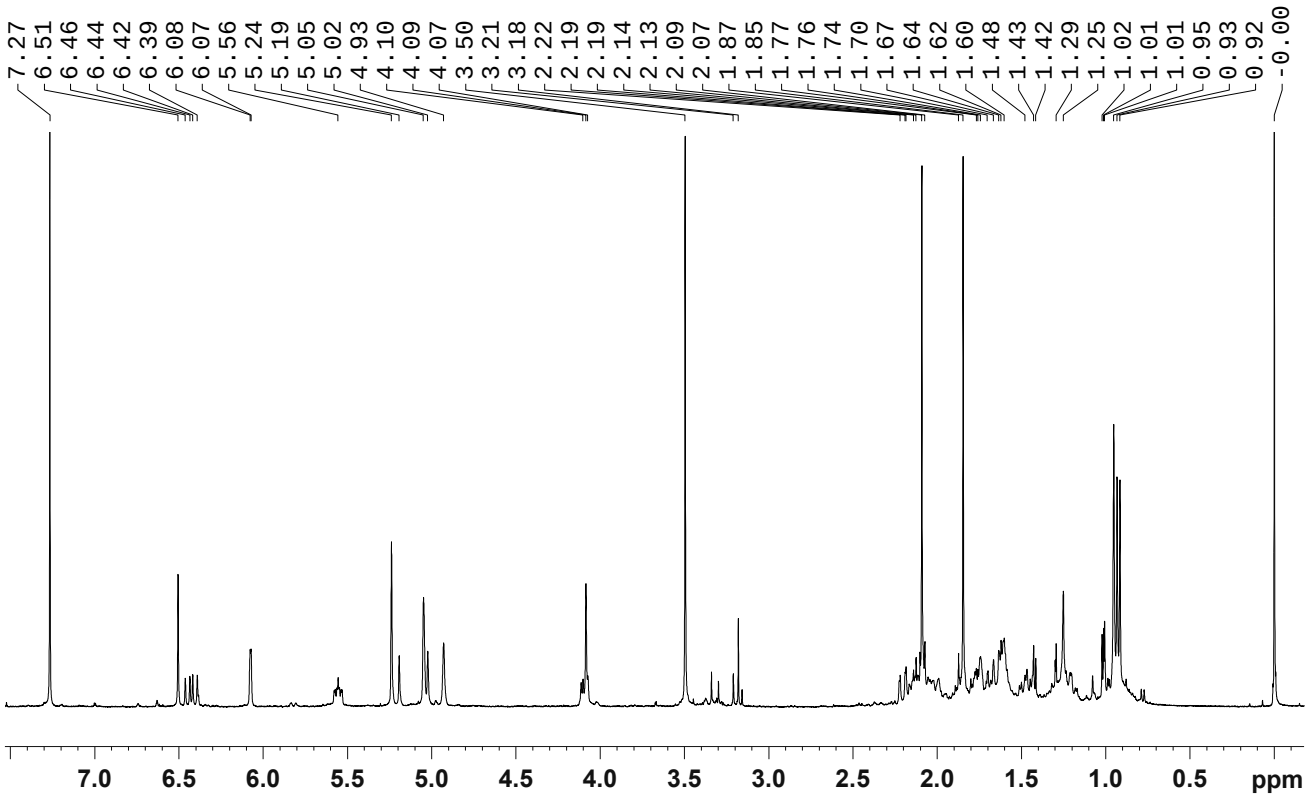

Fig. S24 ^{13}C NMR spectrum for compound **5**

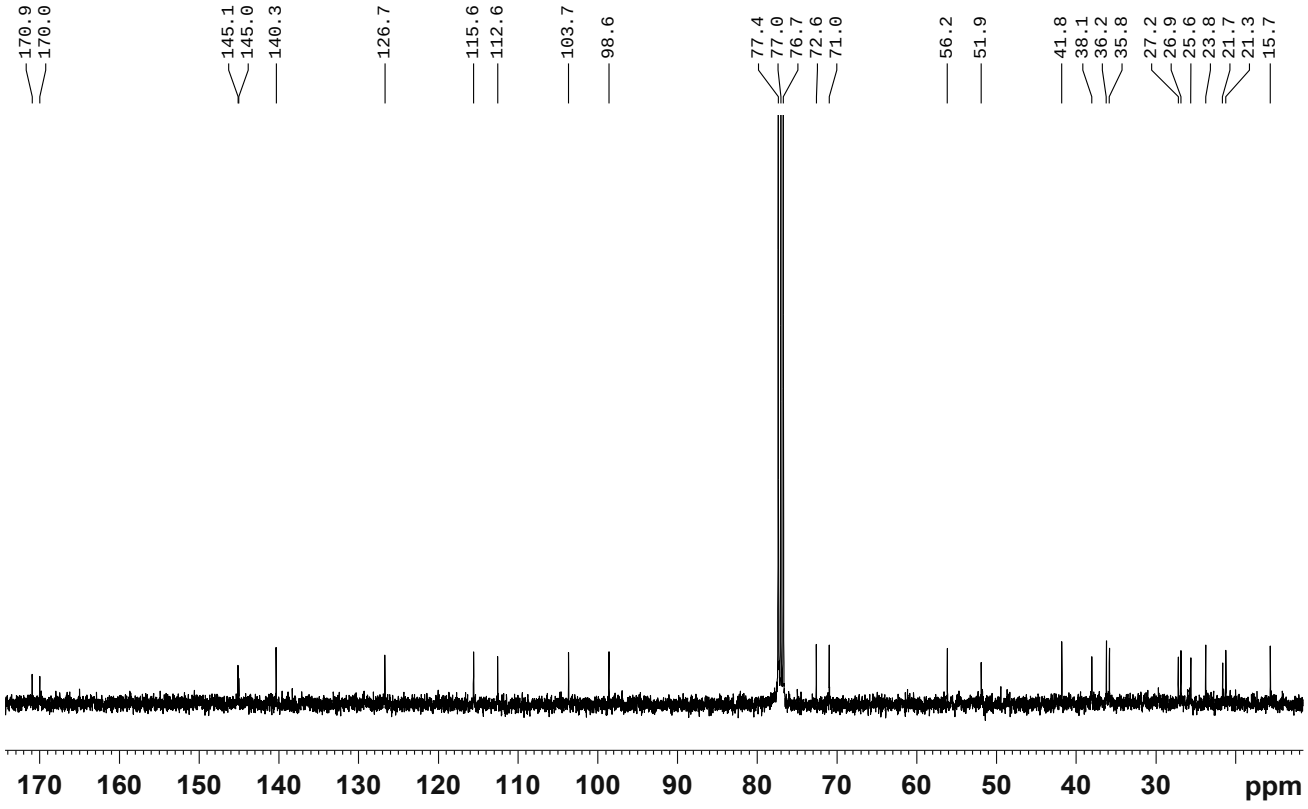

Fig. S25 HMQC spectrum for compound **5**

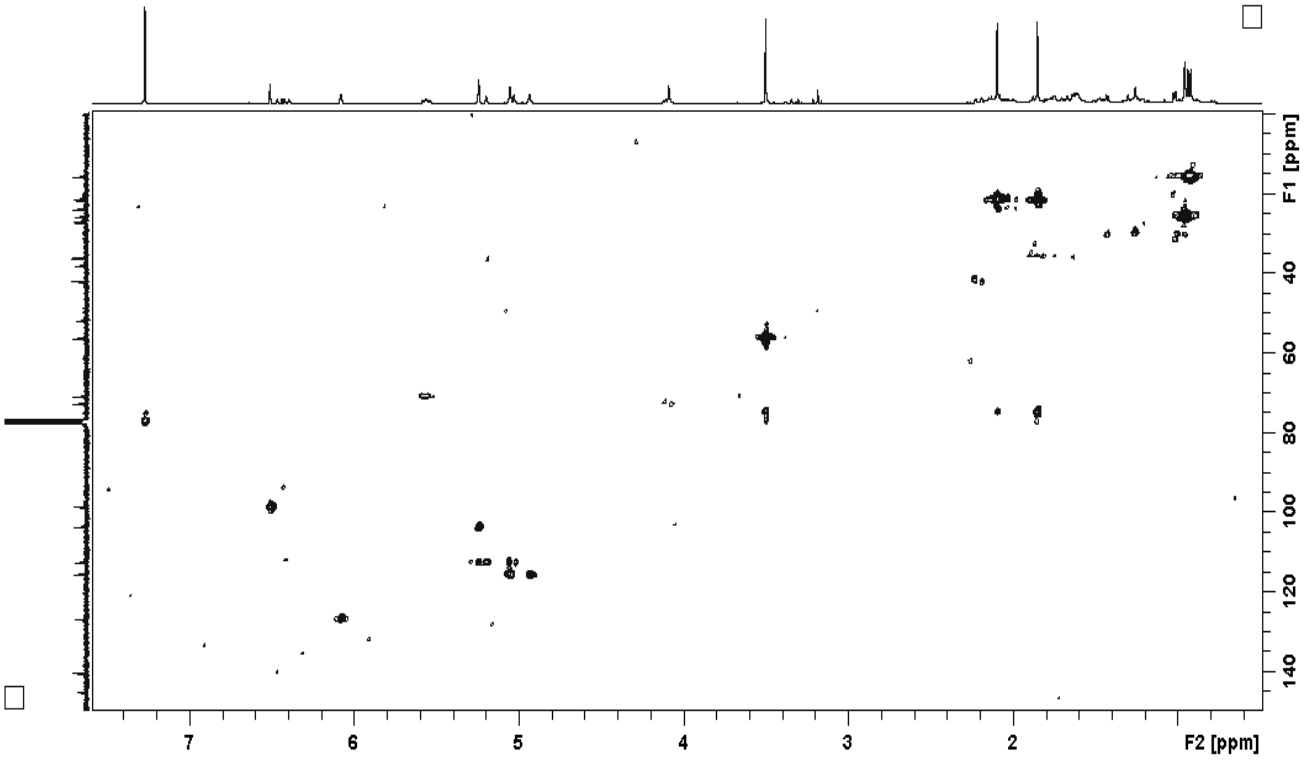

Fig. S26 HMBC spectrum for compound **5**

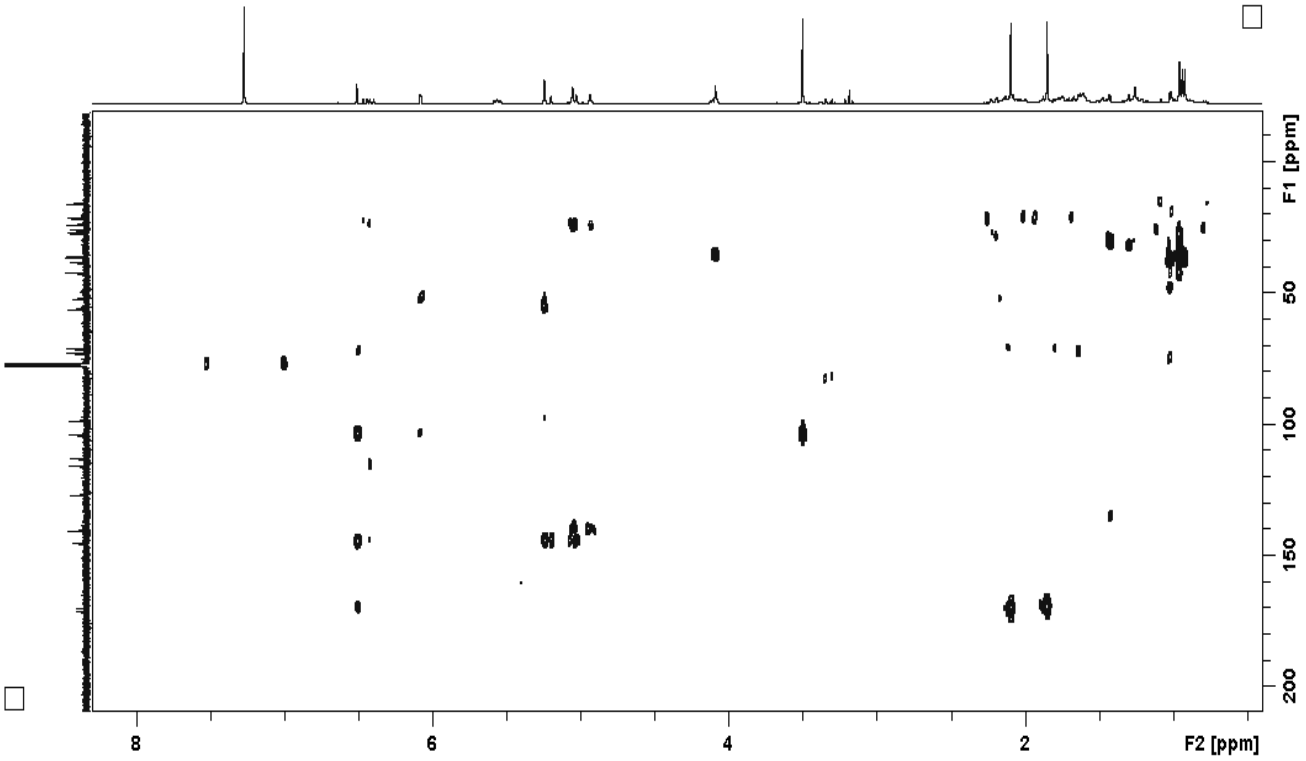

Fig. S27 NOESY spectrum for compound **5**

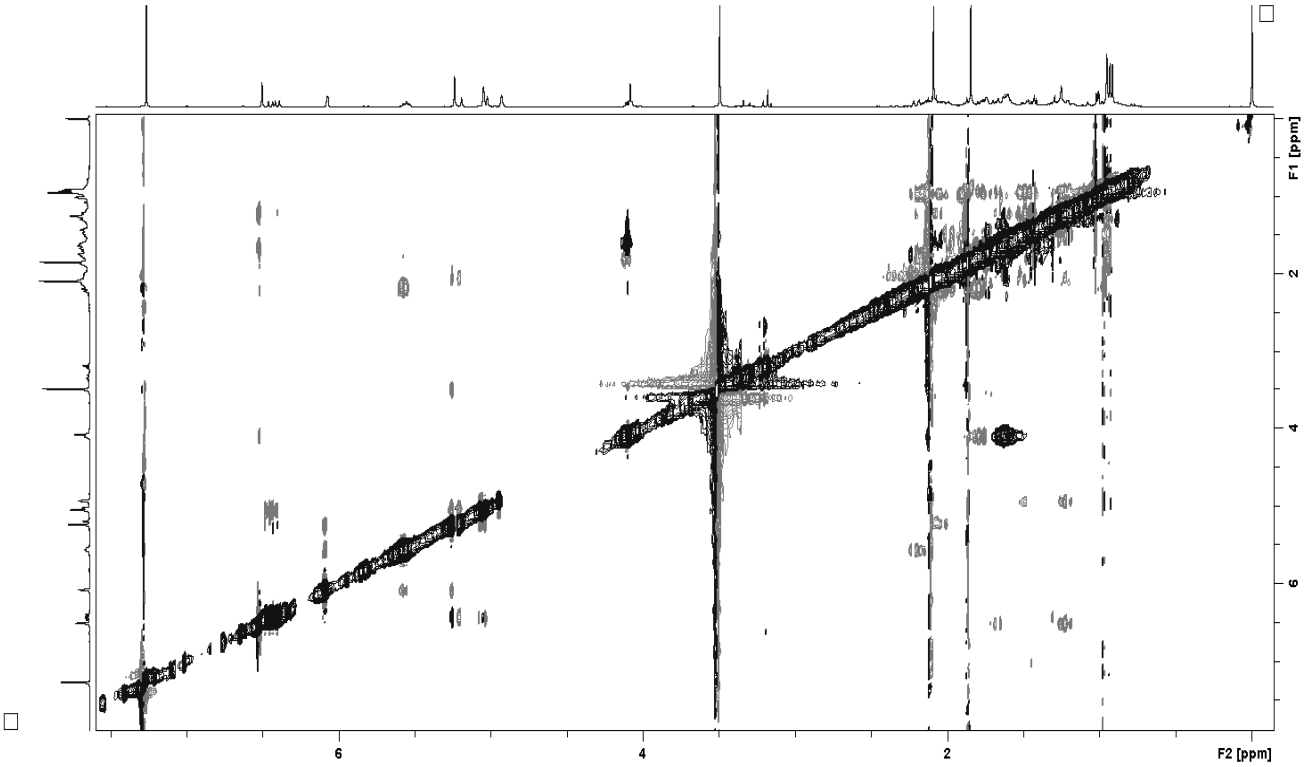

Fig. S27 ^{1}H NMR spectrum for compound **6**

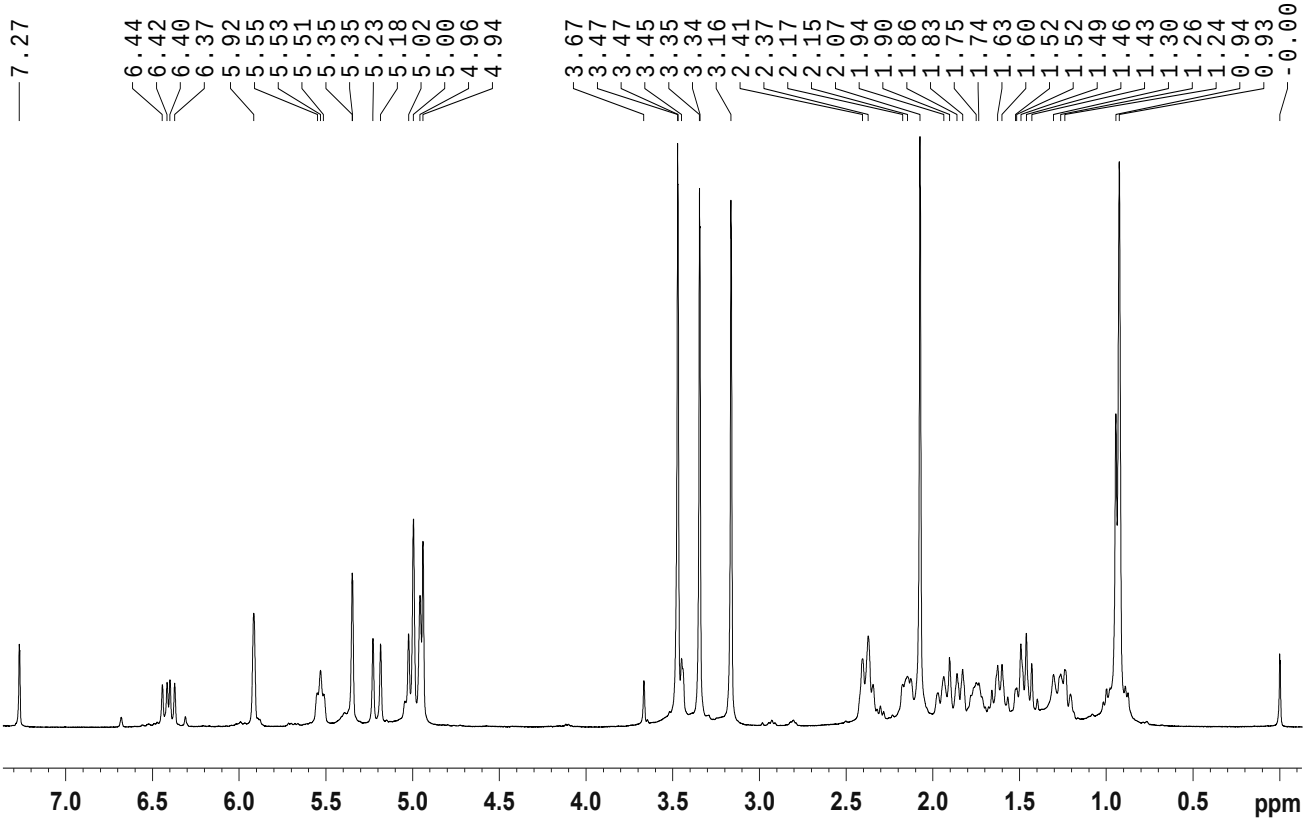

Fig. S28 ^{13}C NMR spectrum for compound **6**

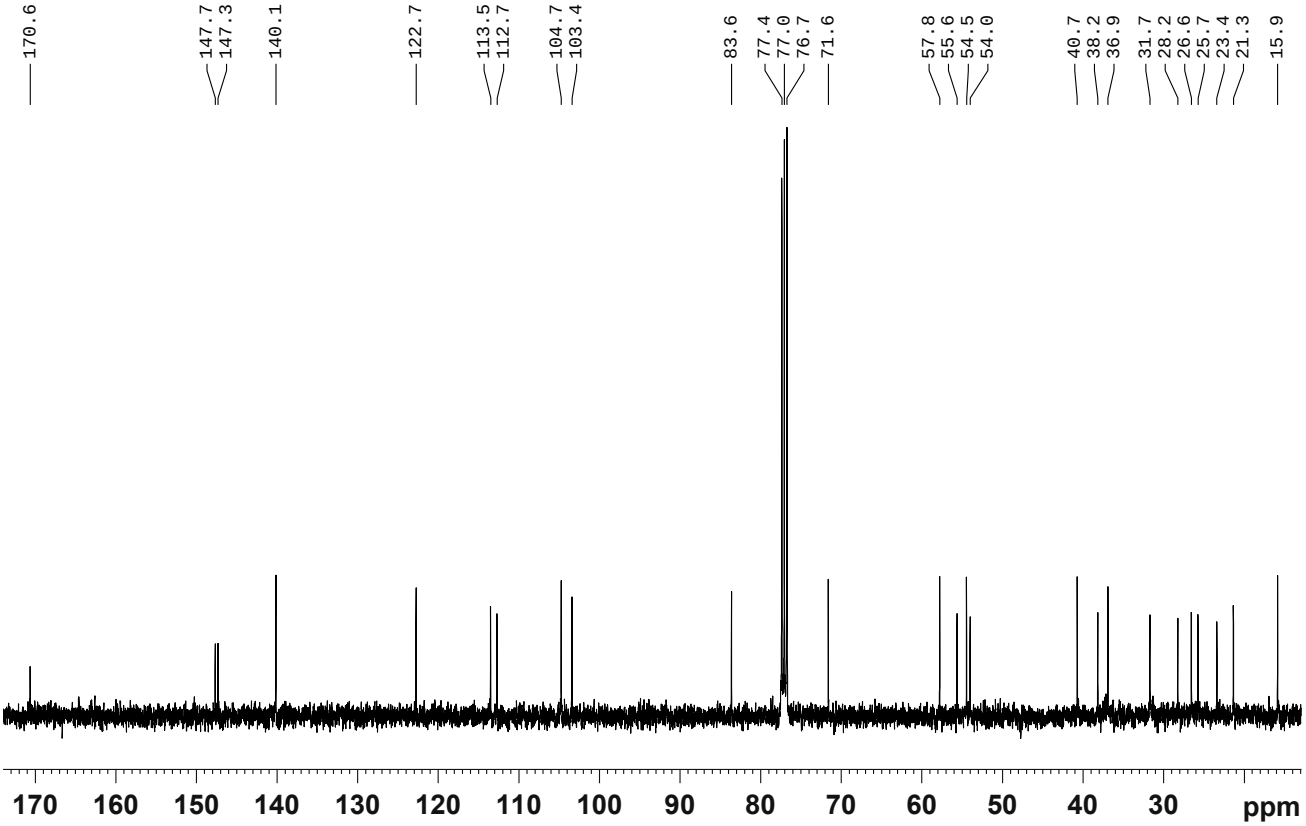

Fig. S29 HMQC spectrum for compound **6**

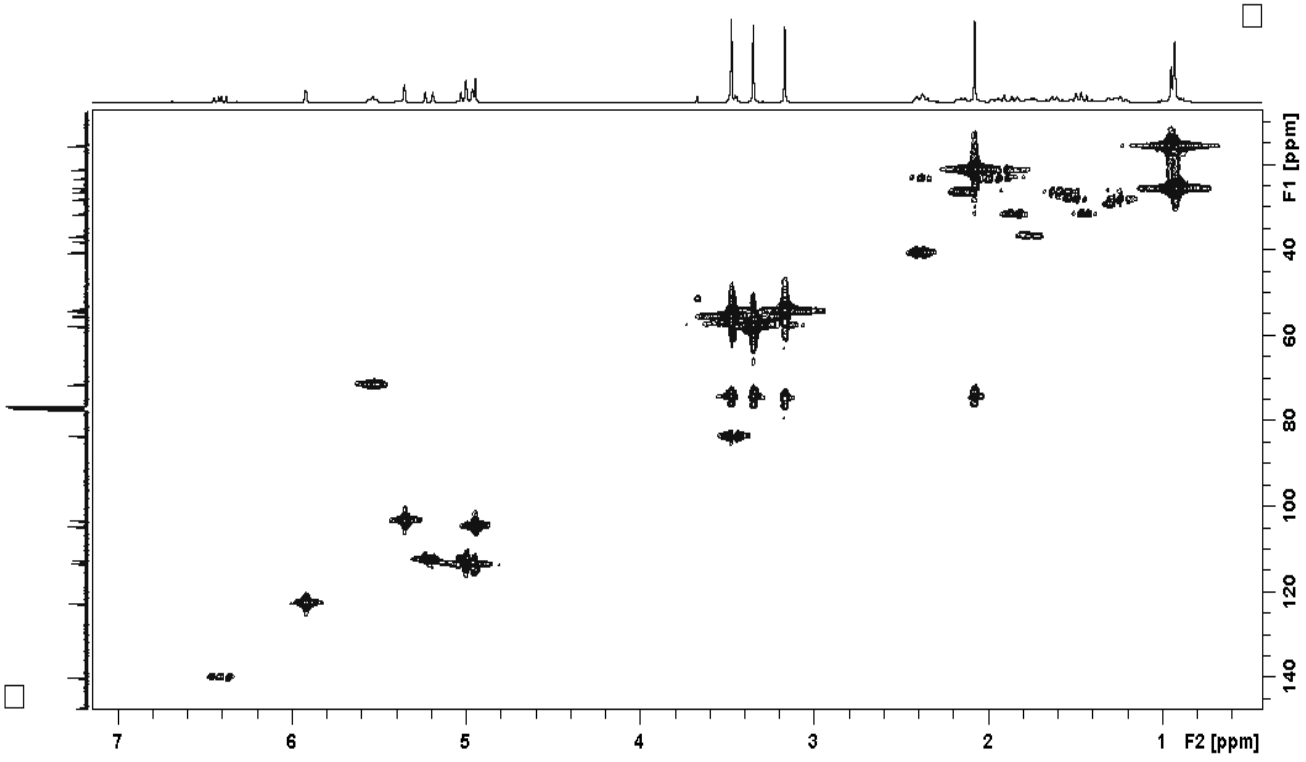

Fig. S30 HMBC spectrum for compound **6**

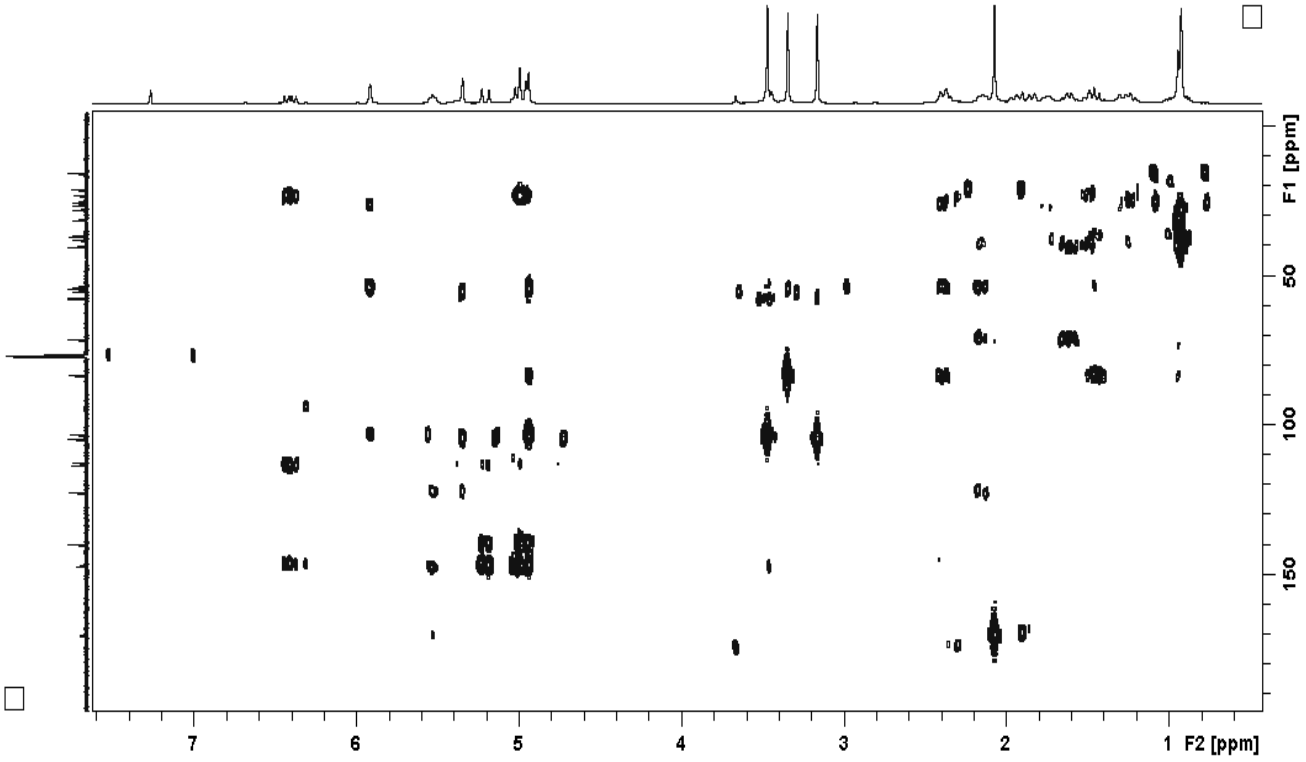

Fig. S32 NOESY spectrum for compound **6**