Supporting Information for

Cytotoxic and Antiangiogenetic Xanthones Inhibiting Tumor Proliferation and Metastasis from *Garcinia xipshuanbannaensis*

Contents

1. Extraction and Isolation

The leaves of *G. xipshuanbannaensis* (5.0 kg) were extracted with MeOH (3 × 50 L) under reflux. The organic solvent was evaporated to afford a crude methanol extract (1.4 kg), which was suspended in H_2O (1.0 L), and then partitioned with petroleum ether and EtOAc successively to give the resulting portions.

The ethyl acetate-soluble portion (350.0 g) was subjected to silica gel column chromatography (silica gel, 1.5 kg; column, 9 × 70 cm), using a gradient solvent system of petroleum ether-acetone (100: 0, 100: 2, 100: 4, 100: 6, 100: 9, 100: 12, 100: 17, 100: 25, and 100: 32; 21 L for each gradient elution), to afford ten fractions (A–J) based on TLC analysis. Fraction D was further separated by MPLC over ODS eluting with a step gradient of 66−94% MeOH in H_2O to give subfractions D1−D13. Using preparative HPLC (YMC-pack ODS-AM column, 20 × 250 mm) for the purification, compounds **1** (t_R = 55 min, 9.7 mg), **3** (t_R = 38 min, 37.9 mg), and **4** (t_R = 62 min, 10.8 mg) were obtained from D9 (93% MeOH in H_2O). Fraction F (63−93% MeOH in H_2O) was fractionated by the above MPLC to give subfractions F1−F17, and the purification of subfraction F13 (94% MeOH in H_2O) resulted in the isolation of compound **2** (t_R = 37 min, 10.3 mg) using the above HPLC. Fraction I was subjected to the same MPLC with the eluent of 62–92% MeOH in H_2O to provide subfractions I1–I13. The subsequent purification of subfraction I4 (84% MeOH in H_2O) was conducted with the same HPLC to produce compound **5** (t_R = 23 min, 12.0 mg). Fractionation of fraction C (69–95% MeOH in H_2O) yielded subfractions C1–C10 using the above MPLC system, and the purification of C3 (91% MeOH in H_2O) and C5 (93% MeOH in H_2O) yielded compounds **6** (t_R = 26 min, 13.7 mg) and **7** (t_R = 30 min, 10.4 mg), respectively, with the same HPLC system. With the same procedure as used for fraction C, 12 subfractions G1–G12 were obtained from fraction G, and compound **8** (t_R = 27 min, 11.0 mg) was acquired by preparative HPLC (88% MeOH in H_2O).

2. Cytotoxicity Evaluation

The cytotoxic activities were evaluated using MTT assay.[1,2] Briefly, cells were 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 each test sample dissolved in DMSO at different concentrations including the positive control. After continuous incubation for 48 h, 20 μL MTT solution (5 mg/mL, Solarbio, Beijing, People's Republic of China) was added in each well for 4 h. Then, the medium was removed and 150 μL DMSO was added. After vibrating for 7 min, the absorbance was measured at 492 nm using microplate reader (Thermo Fisher Scientific Inc.). 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%.

3. Cell Apoptosis Analysis

Cell apoptosis was analyzed by flow cytometry using Annexin V-FITC Apoptosis Detection Kit (Beyotime, Shanghai, People's Republic of China) according to the manufacturer's instructions. Briefly, HeLa cells were treated with various concentrations (6, 12, and 24 μM) of the test compound. After incubation of 48 h, the cells were washed twice with PBS and resuspended in the binding buffer (Beyotime). This suspension was incubated for 20 min at room temperature in the dark after adding 5 μL Annexin V-FITC and 10 μL propidium iodide (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, Ashland, OR, USA).

4. Cell Cycle Analysis

Flow cytometric analysis was performed to evaluate the distribution of the cell cycle.[3] HeLa cells (2×10^5 cells/well) in exponential growth phase were treated with different concentrations of the selected compound (6, 12, and 24 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) for 30 min at 37 °C, followed immediately by cellular DNA analysis using BD LSR Fortessa flow cytometry. Data were processed using ModFit LT Software.

5. Reactive Oxygen Species (ROS) Detection

The levels of intracellular ROS were measured by using the oxidation-sensitive dye 2′,7′-dichlorofluorescein diacetate (DCFH-DA).[4] HeLa cells were seeded into 12-well plates at a density of 2×10^5 cells/well and treated with various concentrations of the selected compound (7.5, 15, and 30 μM) for 48 h. After washed three times with serum-free medium and incubated with 10 μM DCFH-DA for 30 min at 37 °C in the dark, cells were washed again, harvested and resuspended in serum-free medium. The samples were then detected by flow cytometry using BD LSR Fortessa flow cytometry (BD Biosciences). The data were obtained with FLOWJO flow cytometry analysis software (FLOWJO LLC, Ashland, OR, USA).

6. Western Blotting Analysis

The STAT3 and p-STAT3 (Tyr705) protein levels affected by bioactive compounds were detected using Western blotting.[5,6] HeLa cells were seeded in 6-well plate at the density of 1×10^6 cells/well for 24 h. Then, the cells were pretreated with various concentrations of the tested compound (6, 12, and 24 μM). After continuous incubation for 12 h, the cells were washed with cold PBS twice and collected. The cells were lysed by lysis buffer with freshly added protease inhibitor cocktail and phenymethyl sulfonylfluoride. Then, the lysates were centrifuged at 10,000 rpm for 10 min and the supernatants were collected to acquire the total proteins. The protein concentration was determined by the BCA protein assay kit (Beyotime, Shanghai, People's Republic of China). Equal amounts of proteins (25 μg) were separated by SDS-PAGE gel electrophoresis and transferred to polyvinylidene difluoride membranes. The membrane was blocked with 5% skim milk for 2 h at room temperature and then incubated with primary antibody overnight at 4 ℃. After being washed with TBST for 30 min, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (diluted 1:5000 in 5% skim milk) for 1 h at room temperature and then washed with TBST for another 30 min. Lastly, the protein blots were visualized using an ECL detection kit (Beyotime, Shanghai, People's Republic of China). β-Actin protein was used as internal reference. Each band was quantified by Image J software.

References

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(6) Cao, H. H.; Zhang, W. Q.; Liu, D. Y.; Hou, M. T.; Liu, S. Y.; He, W. K.; Lin, J. W.; Shao, M. *Bioorg. Chem.* **2020**, *96*, 103655.

7. NMR Spectra of Compounds 1–4

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

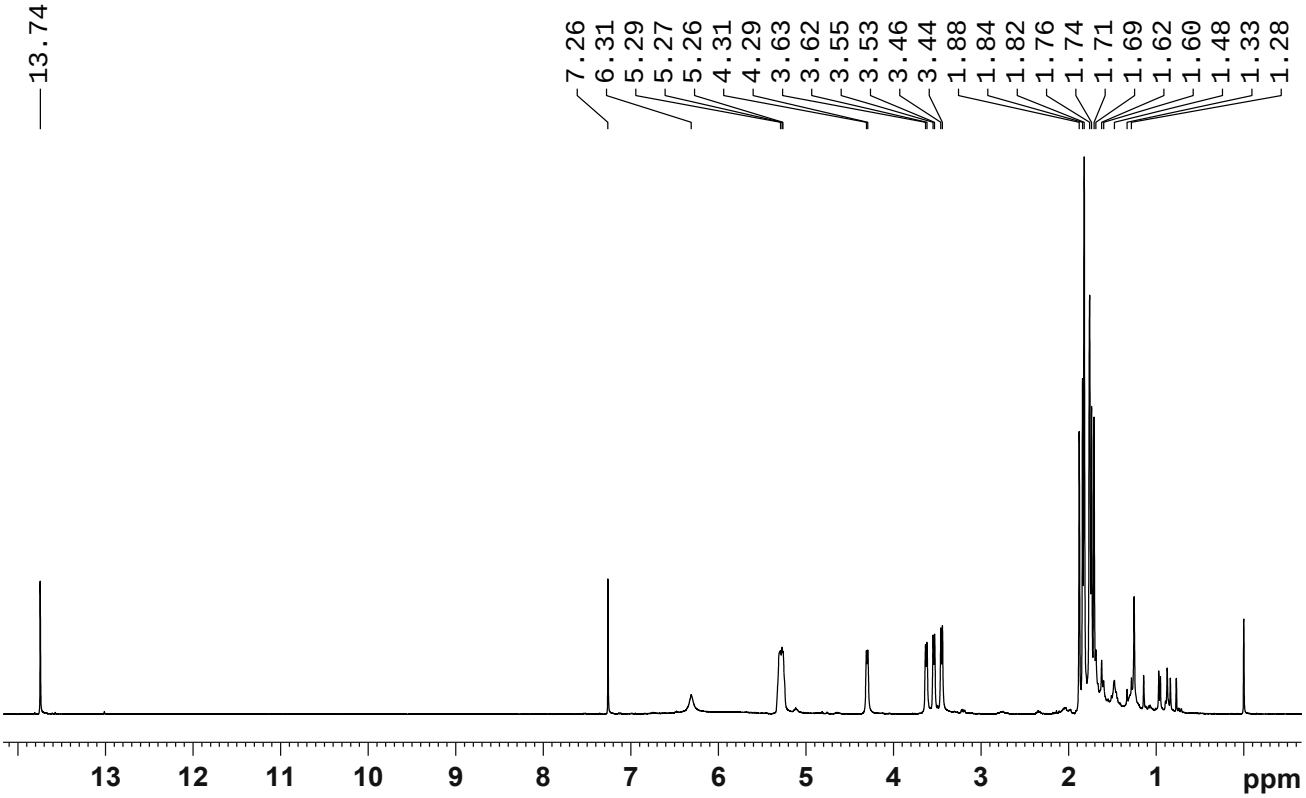

Fig. S2 ^{13}C NMR spectrum for compound **1**

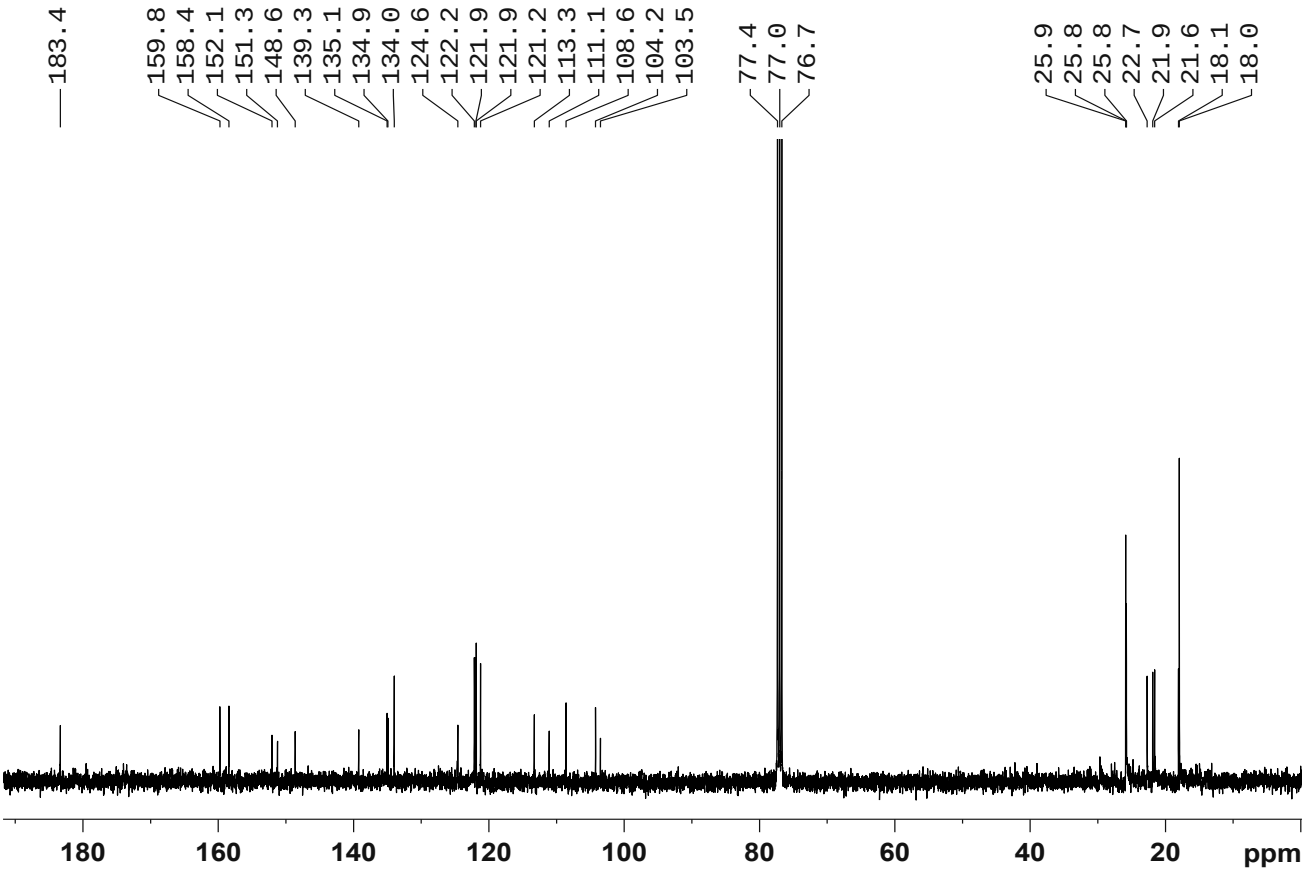

Fig. S3 DEPT (θ = 135°) NMR spectrum for compound **1**

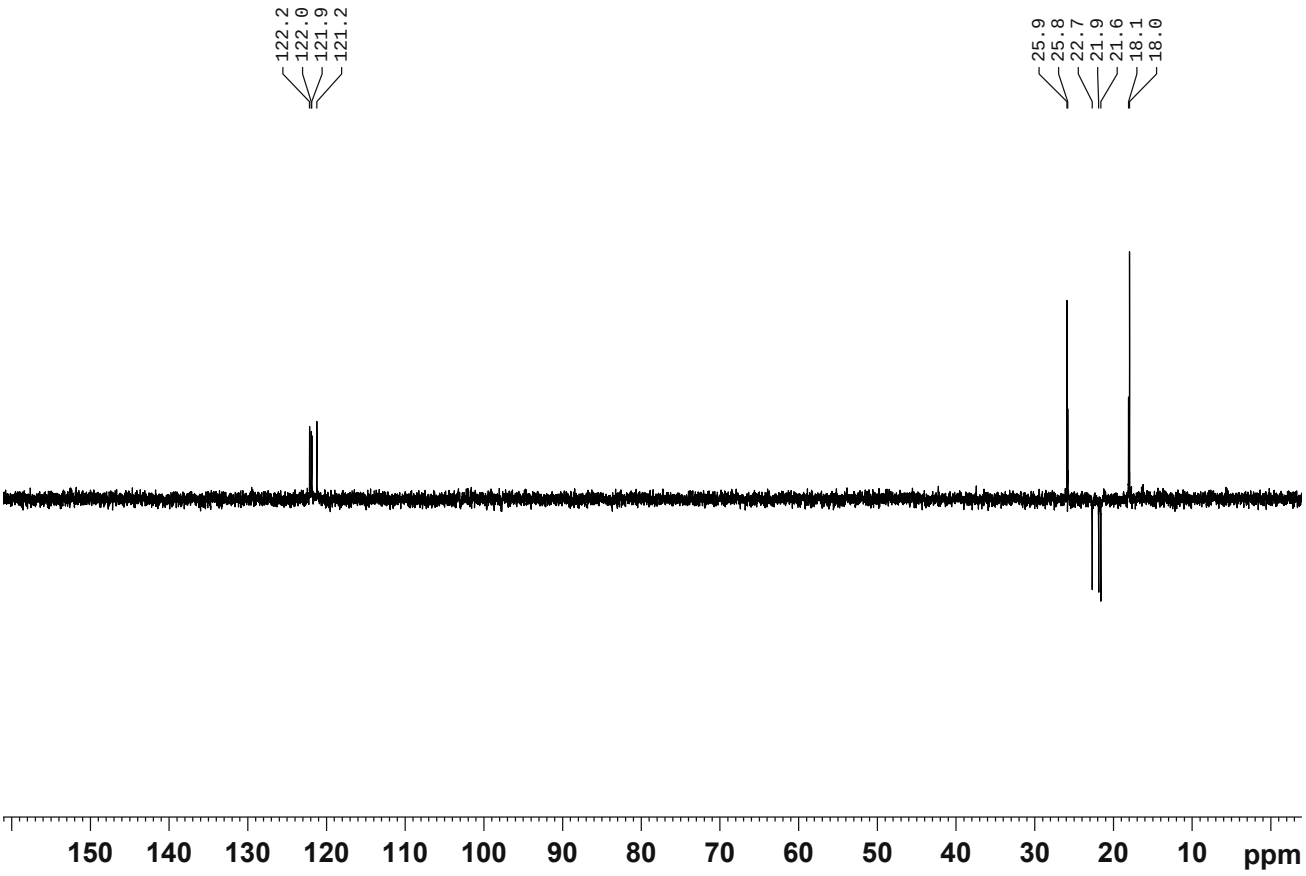

Fig. S4 HMQC spectrum for compound **1**

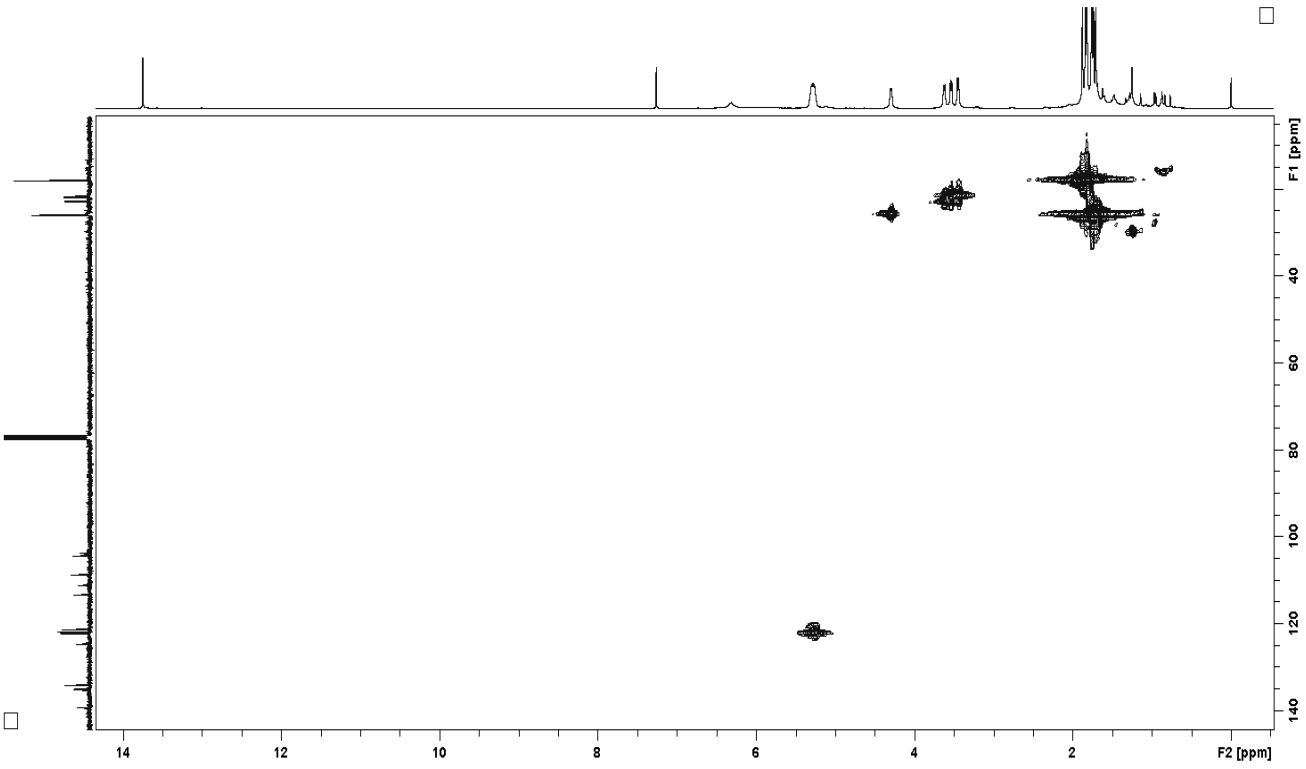

Fig. S5 HMBC spectrum for compound **1**

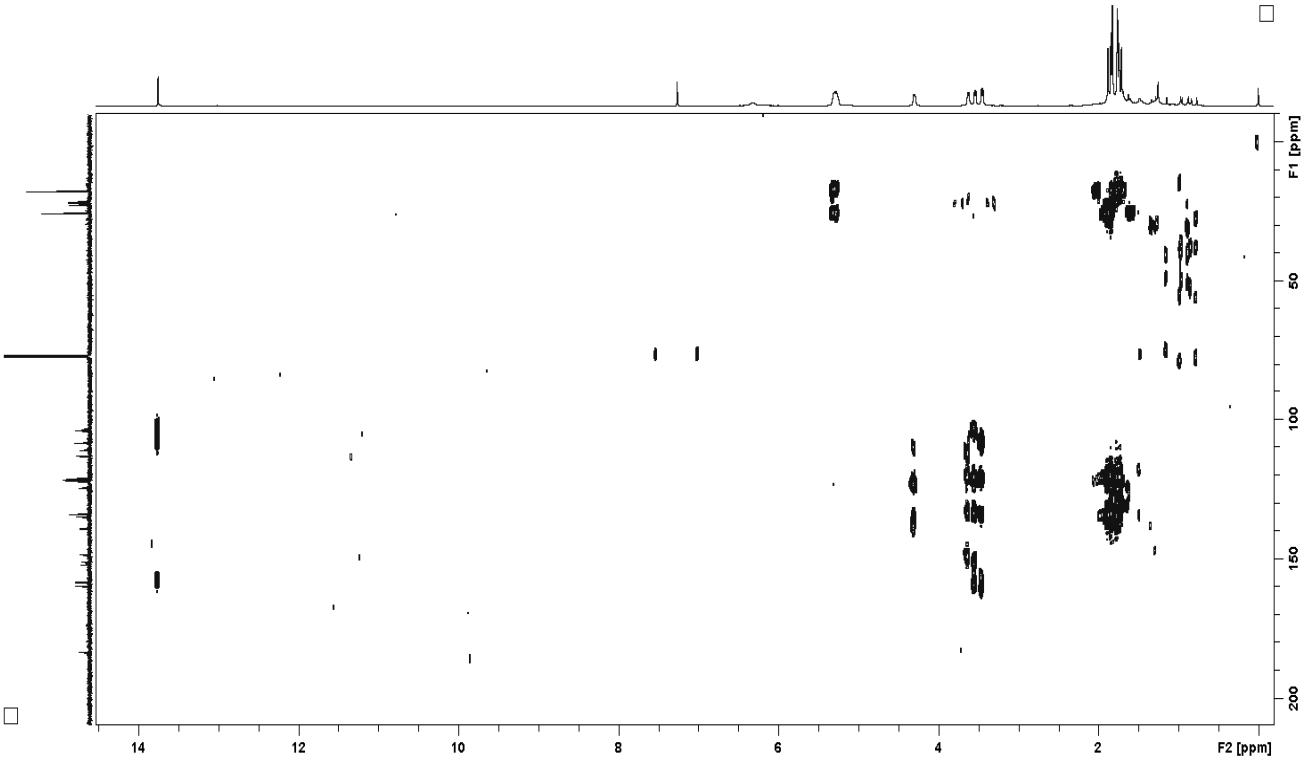

Fig. S6 ^{1}H-^{1}H COSY spectrum for compound **1**

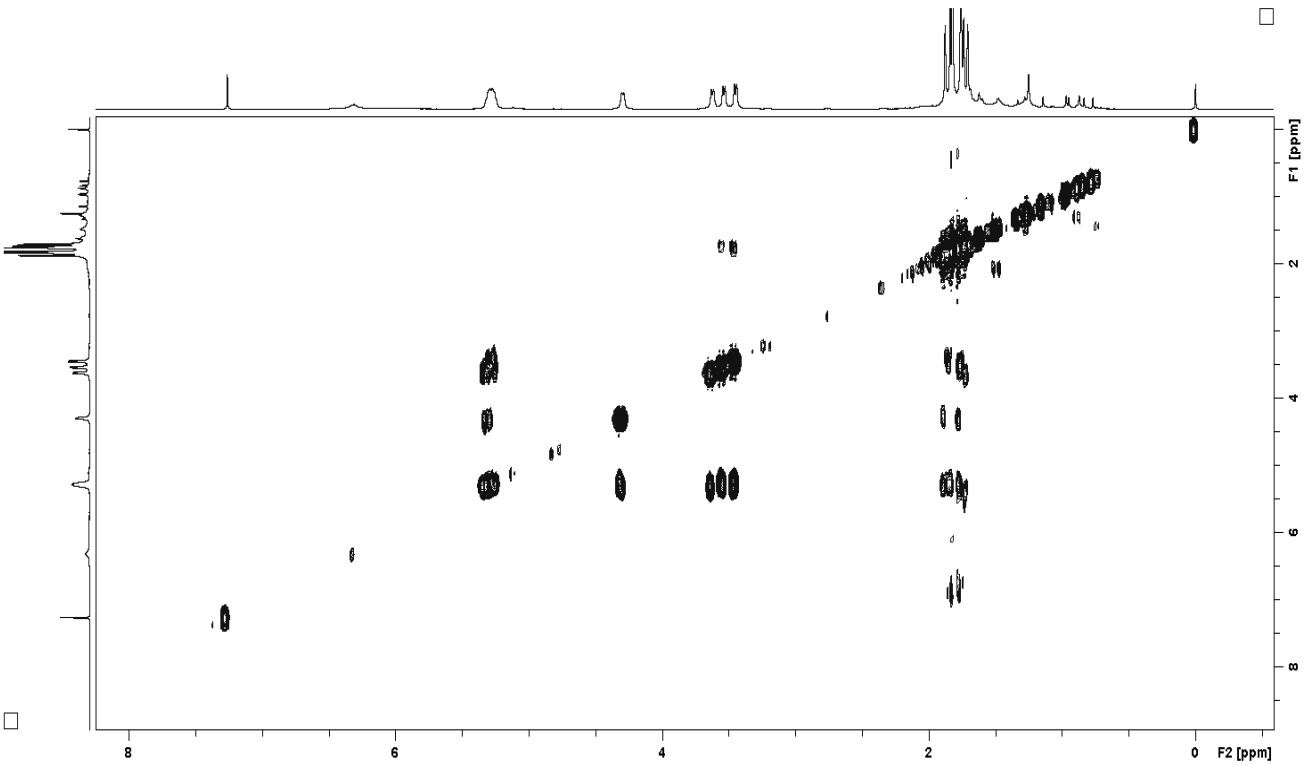

Fig. S7 HRESIMS spectrum for compound **1**

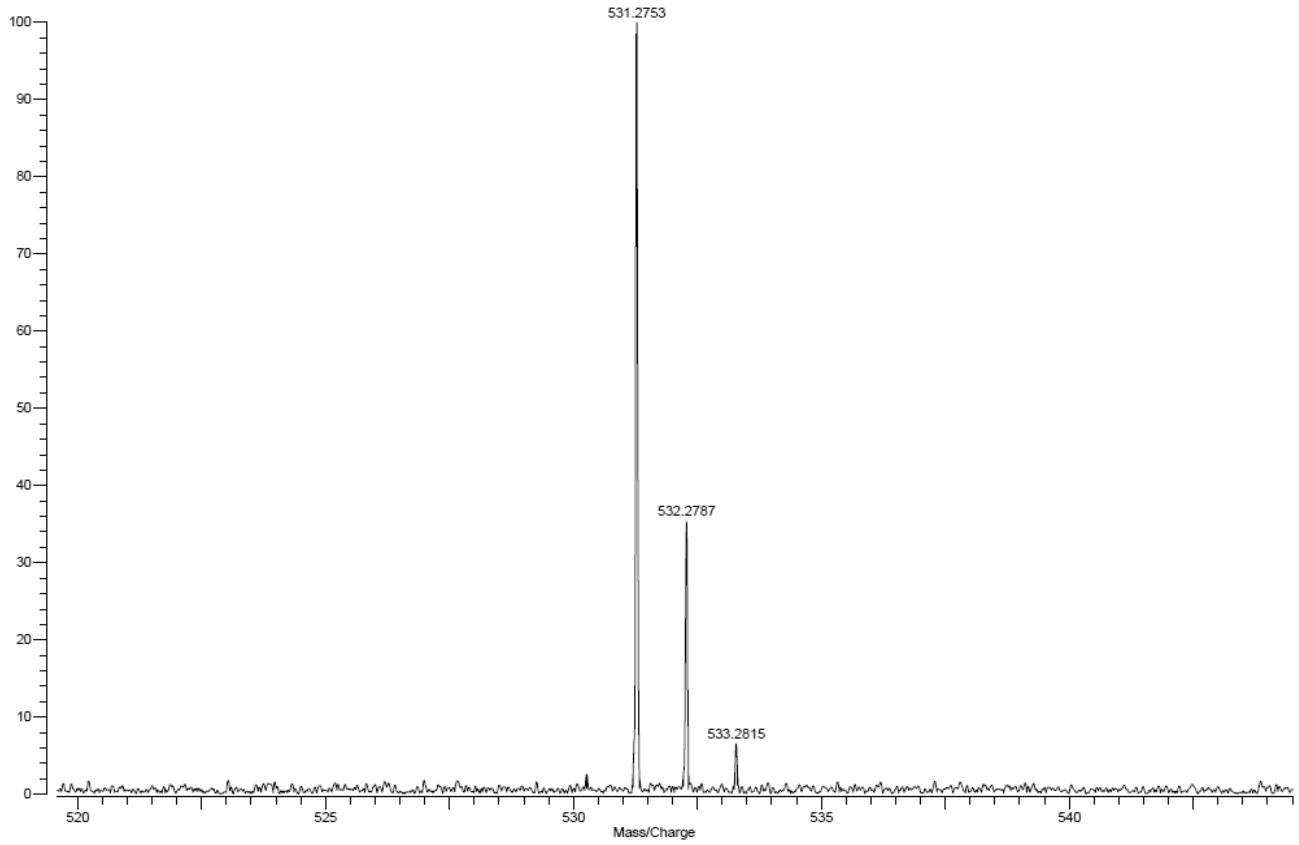

Fig. S8 ^{1}H NMR spectrum for compound **2**

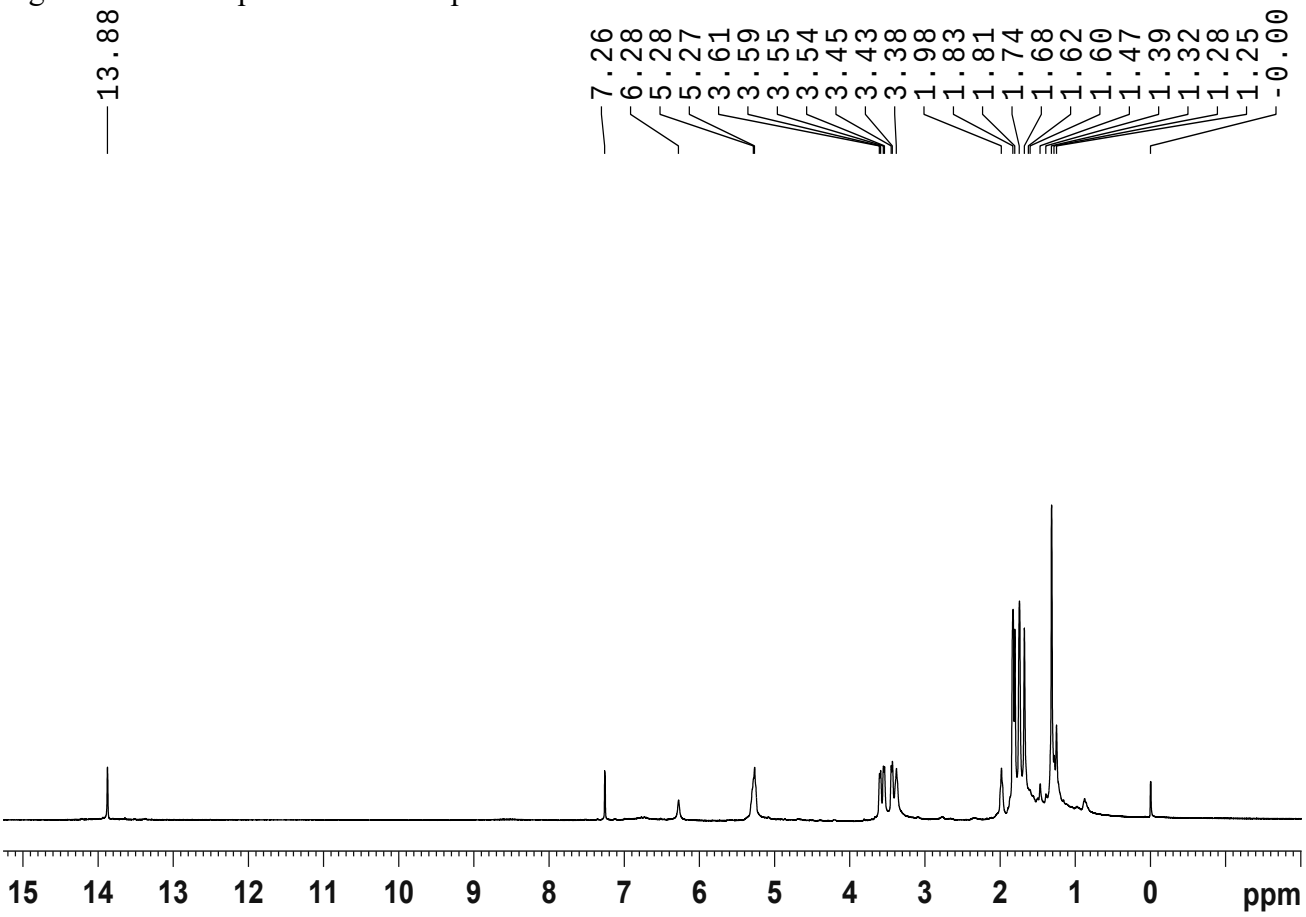

Fig. S9 ^{13}C NMR spectrum for compound **2**

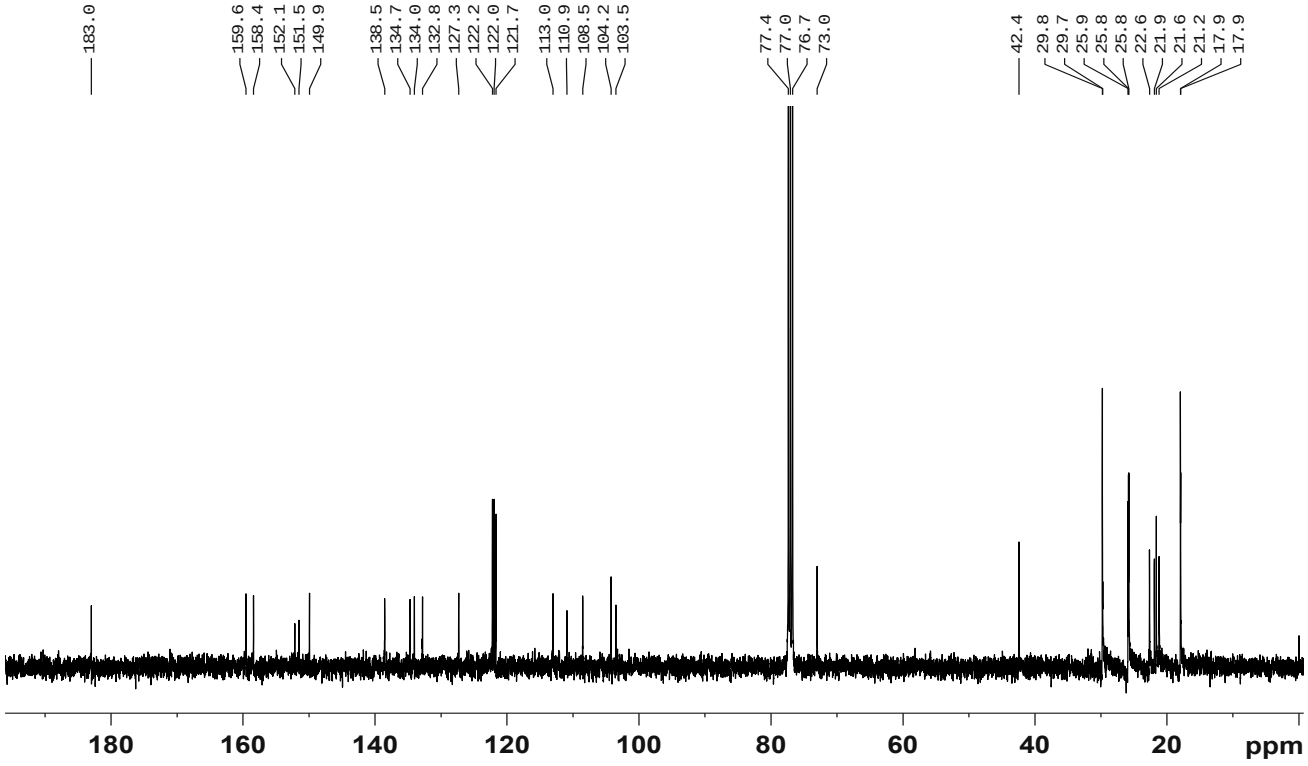

Fig. S10 HMQC spectrum for compound **2**

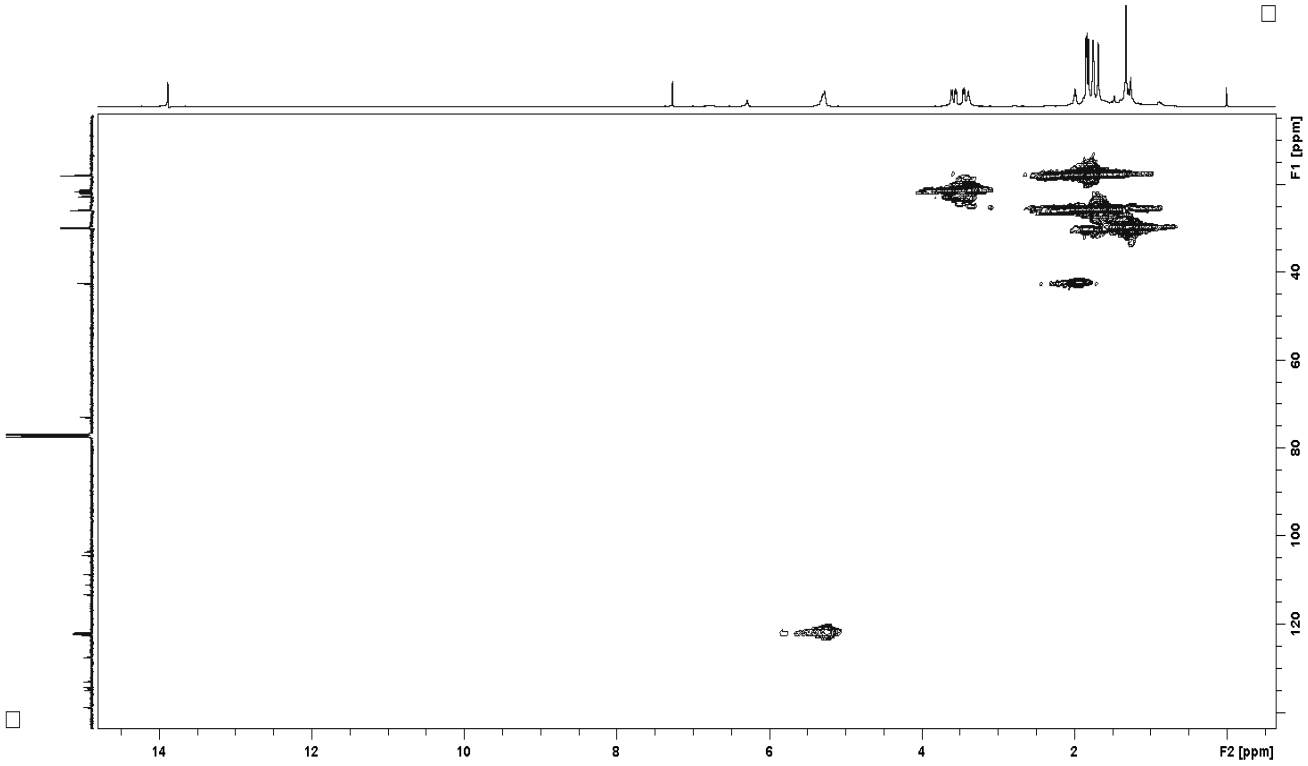

Fig. S11 HMBC spectrum for compound **2**

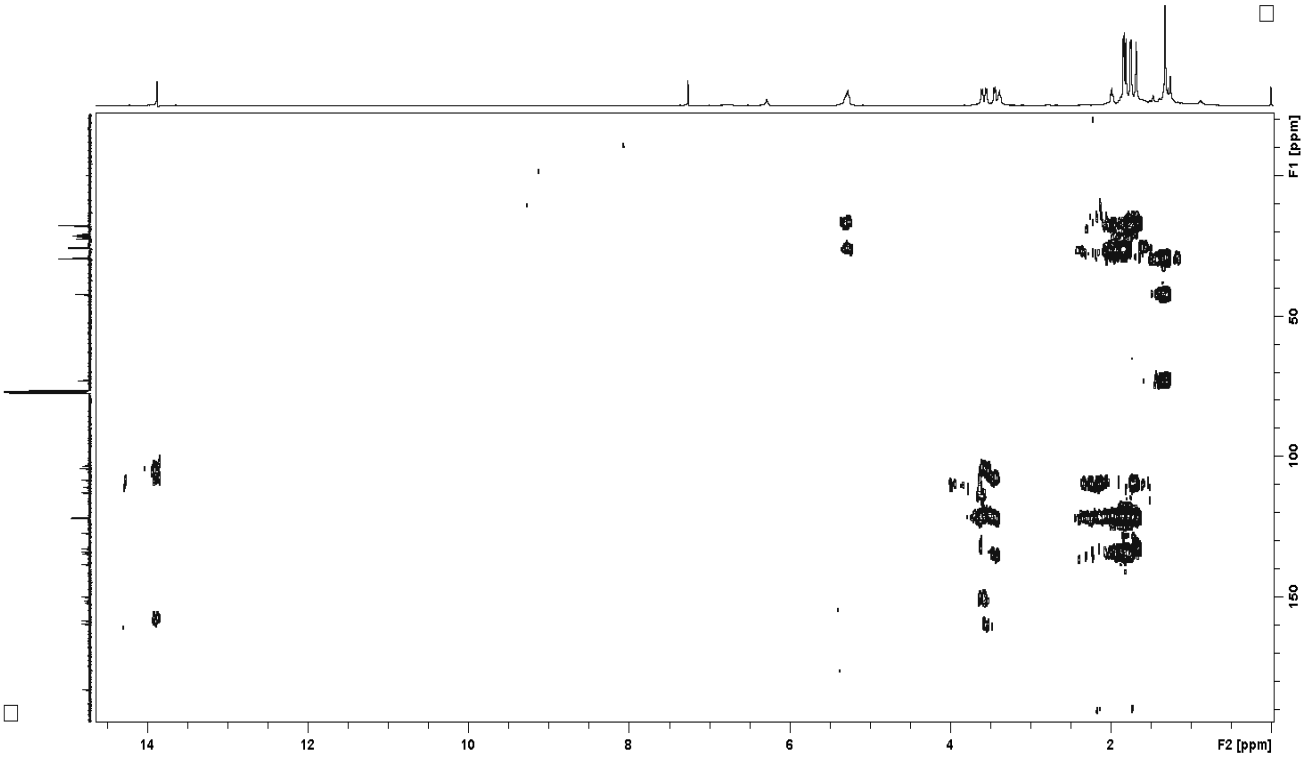

Fig. S12 HRESIMS spectrum for compound **2**

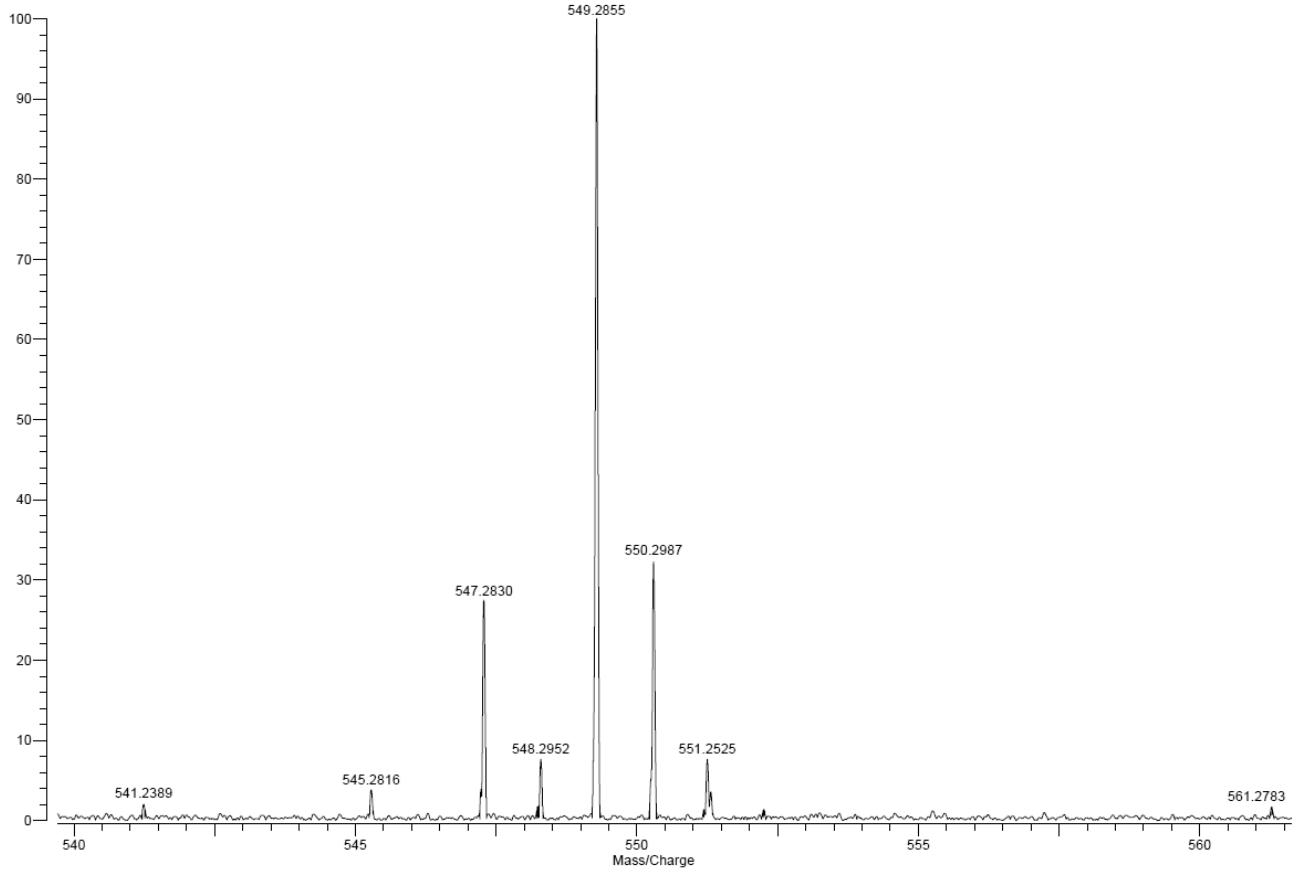

Fig. S13 ^{1}H NMR spectrum for compound **3**

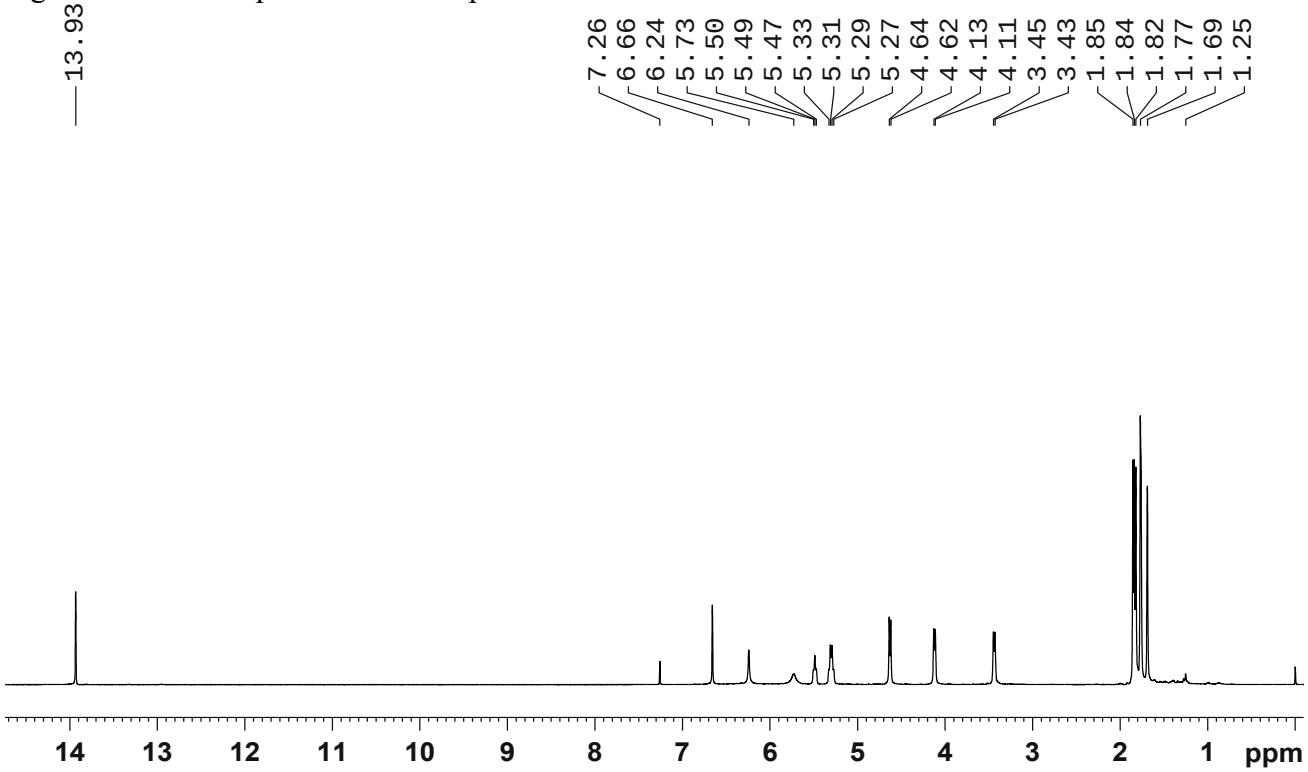

Fig. S14 ^{13}C NMR spectrum for compound **3**

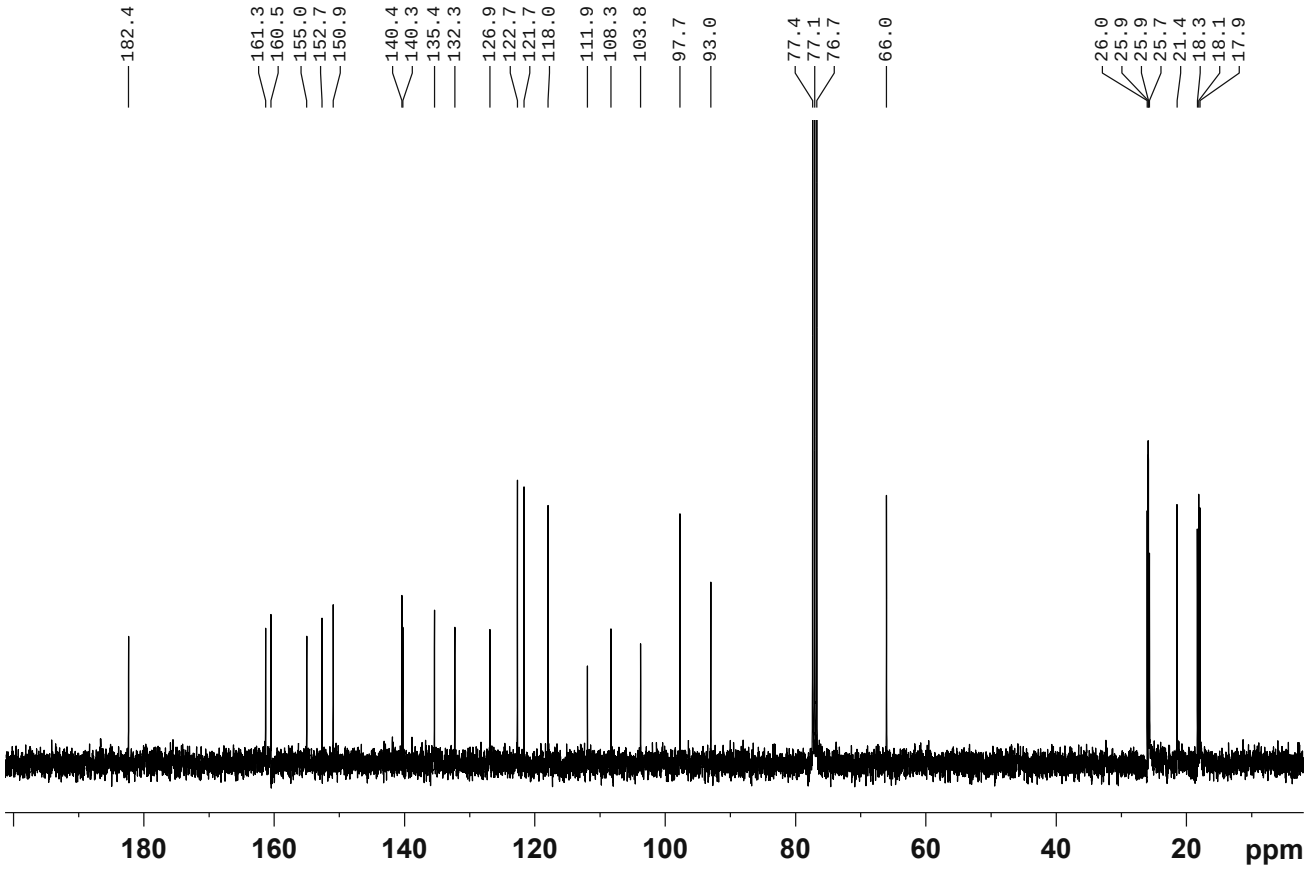

Fig. S15 HMQC spectrum for compound **3**

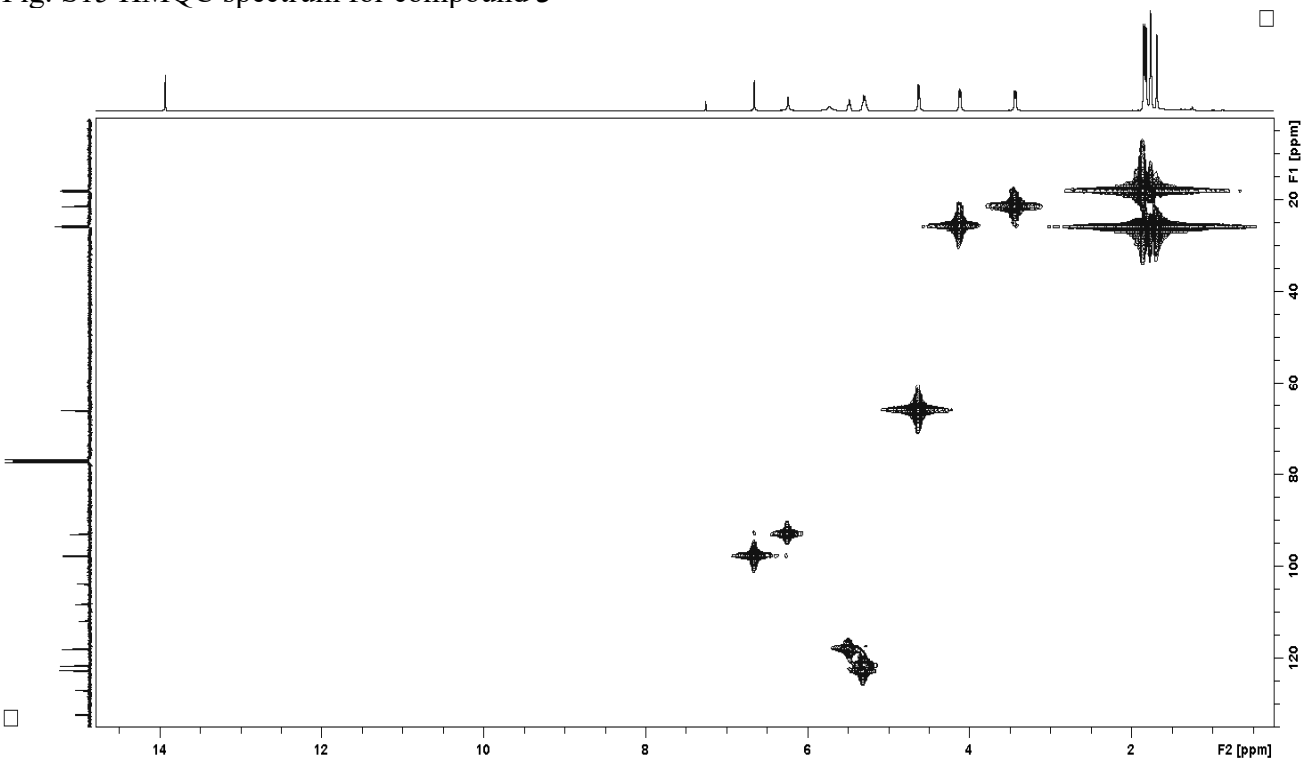

Fig. S16 HMBC spectrum for compound **3**

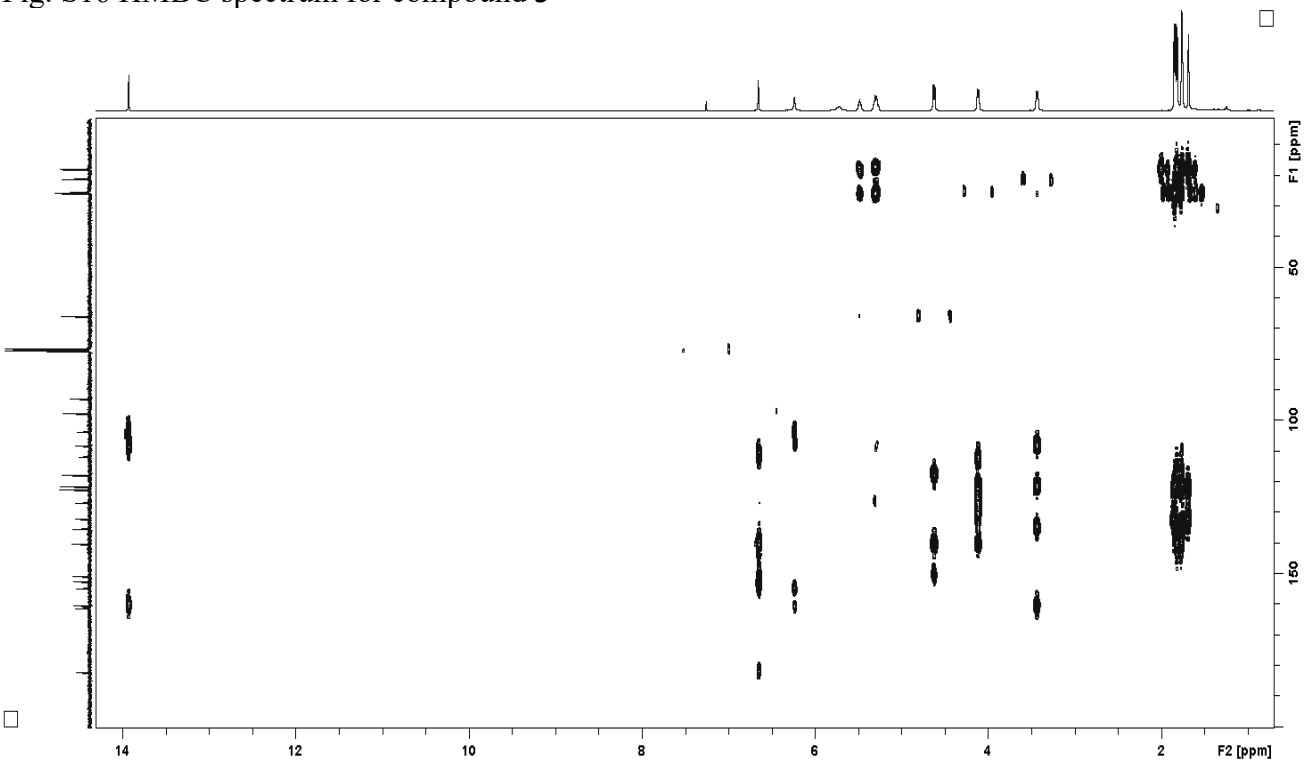

Fig. S17 HRESIMS spectrum for compound **3**

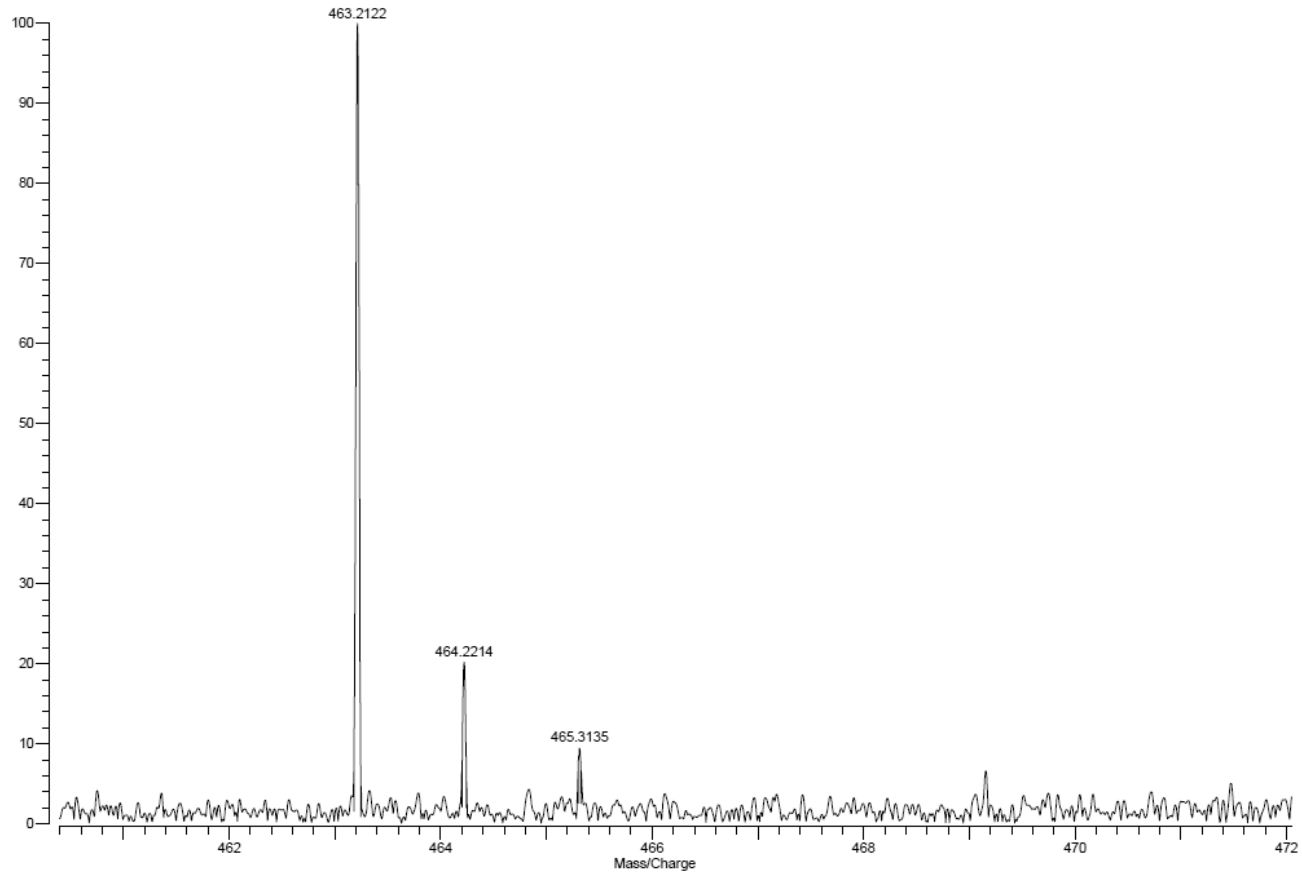

Fig. S18 ^{1}H NMR spectrum for compound **4**

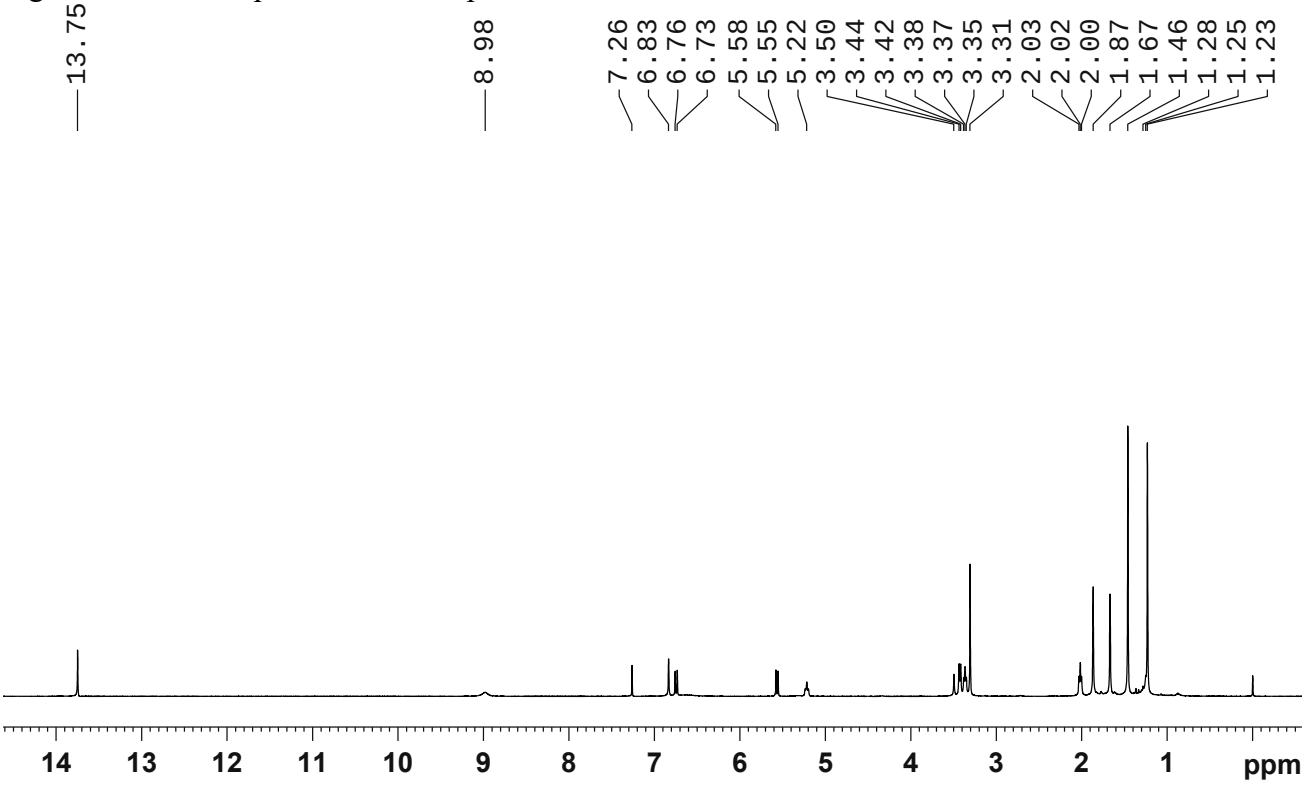

Fig. S19 ^{13}C NMR spectrum for compound **4**

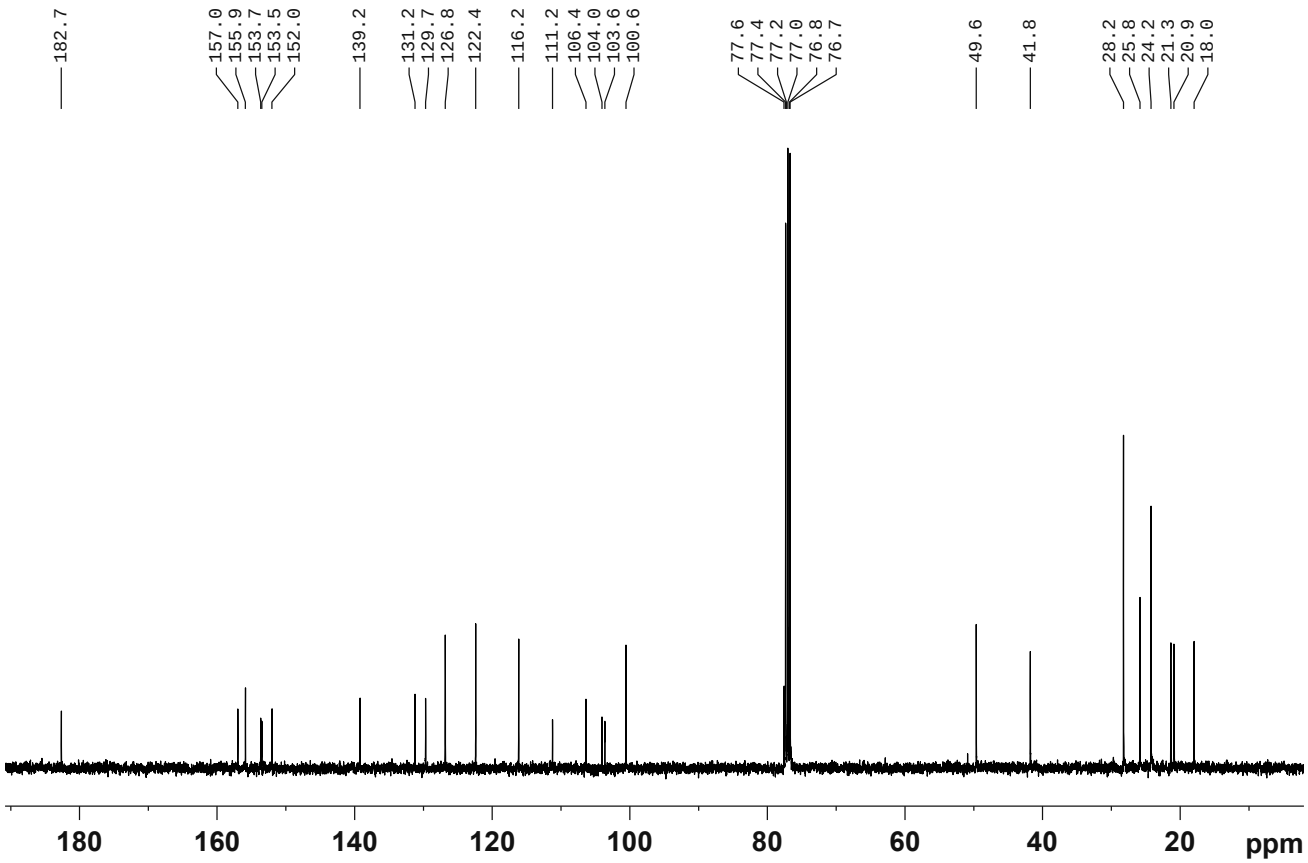

Fig. S20 HMQC spectrum for compound **4**

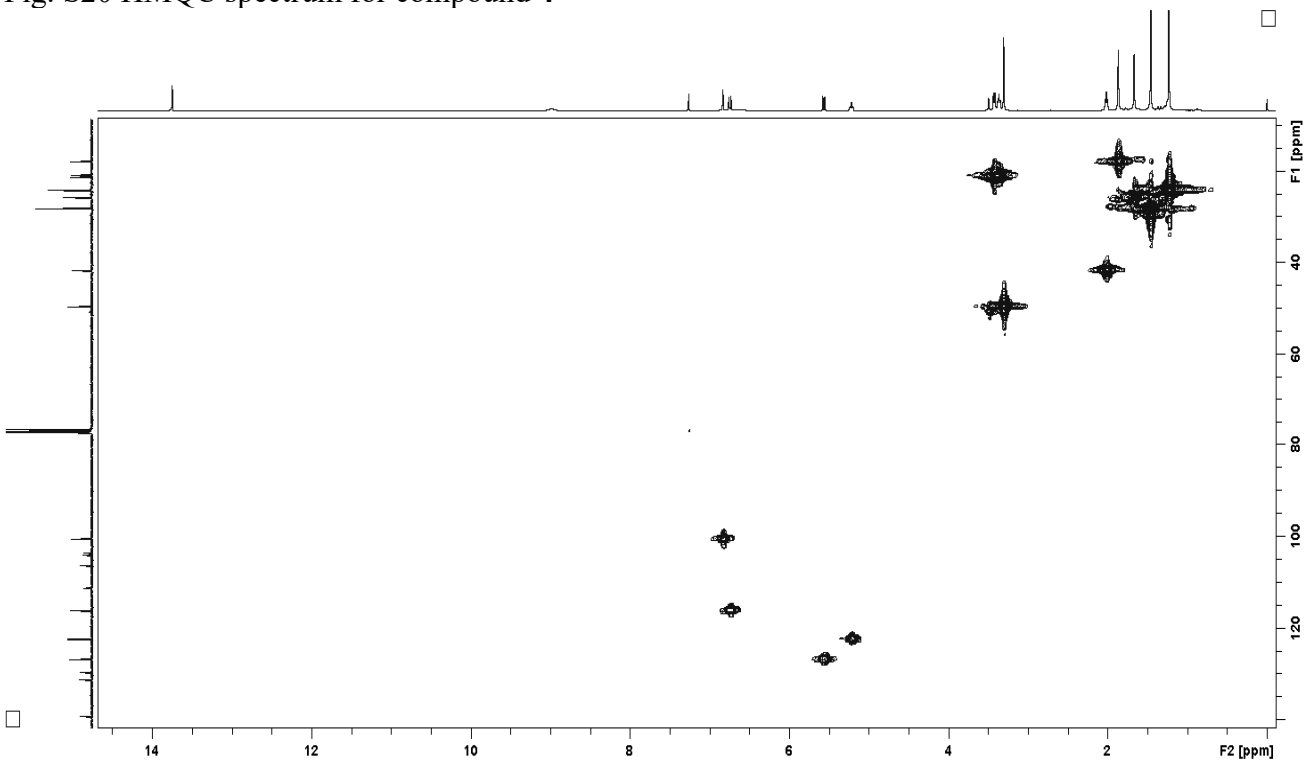

Fig. S21 HMBC spectrum for compound **4**

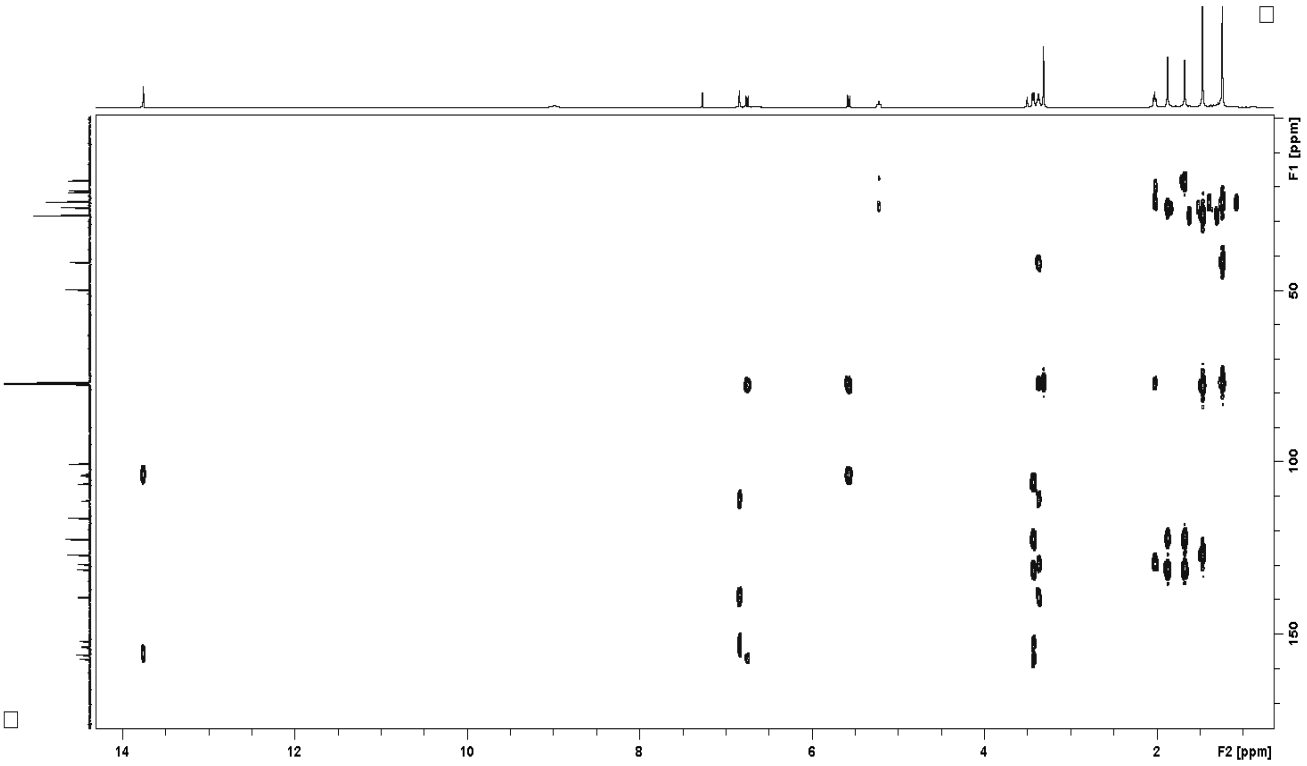

Fig. S22 HRESIMS spectrum for compound **4**