

## **Supporting Information for**

### **Clerodane Diterpenoids Isolated from the Leaves of *Casearia graveolens***

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## 1. Extraction and Isolation

The leaves of *C. graveolens* (14.0 kg) were extracted with MeOH ( $3 \times 140$  L) under reflux. The organic solvent was evaporated to afford a crude methanol extract (2.4 kg), which was suspended in H<sub>2</sub>O (2.4 L), and then partitioned successively with petroleum ether and EtOAc to give the resulting portions.

The ethyl acetate-soluble portion (280 g) was subjected to silica gel column chromatography (silica gel, 1.0 kg; column,  $9 \times 70$  cm), using a gradient solvent system of petroleum ether-acetone (100: 0, 100: 2, 100: 4, 100: 8, 100: 12, 100: 17, 100: 25, 100: 38, 21 L for each gradient elution), to afford eight fractions (EF<sub>1</sub>–EF<sub>8</sub>) based on TLC analysis. Fraction EF<sub>4</sub> was further purified by MPLC over ODS eluting with a step gradient of 65–92% MeOH in H<sub>2</sub>O to give eight subfractions, EF<sub>4-1</sub>–EF<sub>4-8</sub>. Using preparative HPLC (YMC-pack ODS-AM column,  $20 \times 250$  mm) for the purification of these subfractions, compounds **1** ( $t_R$  34 min, 13.4 mg) and **3** ( $t_R$  38 min, 7.8 mg) were obtained from EF<sub>4-4</sub> (85% MeOH in H<sub>2</sub>O) and EF<sub>4-3</sub> (81% MeOH in H<sub>2</sub>O), respectively. EF<sub>4-6</sub> (87% MeOH in H<sub>2</sub>O), when purified by the same HPLC procedure, afforded compounds **2** ( $t_R$  45 min, 7.1 mg) and **4** ( $t_R$  35 min, 4.8 mg).

The petroleum-ether soluble portion (450 g) was subjected to a passage silica gel column with the same gradient system as that used for the ethyl acetate portion, to give eight fractions, PF<sub>1</sub>–PF<sub>8</sub>, after TLC detection. Fraction PF<sub>6</sub> was subjected to the above-mentioned MPLC separation, eluting with 62–92% MeOH in H<sub>2</sub>O, to give subfractions PF<sub>6-1</sub>–PF<sub>6-10</sub>. Subfraction PF<sub>6-5</sub> was purified with the above-mentioned preparative HPLC system, to yield compound **5** ( $t_R$  37 min, 13.2 mg). Using the same protocols for the above fractions, fractions PF<sub>3</sub>, PF<sub>4</sub>, and PF<sub>5</sub> produced subfractions PF<sub>3-1</sub>–PF<sub>3-7</sub>, PF<sub>4-1</sub>–PF<sub>4-7</sub>, and PF<sub>5-1</sub>–PF<sub>5-10</sub>, respectively. The following purification of PF<sub>5-3</sub> (82% MeOH in H<sub>2</sub>O), PF<sub>4-4</sub> (86% MeOH in H<sub>2</sub>O), PF<sub>4-3</sub> (82% MeOH in H<sub>2</sub>O), and PF<sub>3-2</sub> (82% MeOH in H<sub>2</sub>O) with the above-mentioned HPLC system, yielded compounds **6** ( $t_R$  40 min, 19.0 mg), **7** ( $t_R$  64 min, 18.5 mg), **8** ( $t_R$  27 min, 20.5 mg), and **9** ( $t_R$  55 min, 14.9 mg), respectively.

## 2. Cytotoxicity Evaluation

The cytotoxic activities were evaluated using a MTT assay. Briefly, cells were seeded in 96-well plates ( $1 \times 10^4$  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 a continuous incubation for 48 h, 20  $\mu$ L MTT solution (5 mg/mL, Solarbio, Beijing, People's Republic of China) were added in each well for 4 h. Then, the medium was removed and 150  $\mu$ L DMSO were 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<sub>50</sub> 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, A549 cells were treated with various concentrations (1, 2, and 4  $\mu$ M) of the test compound. After an 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 adding 5  $\mu$ L Annexin V-FITC and 10  $\mu$ L 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. A549 cells ( $2 \times 10^5$  cells/well) in exponential growth phase were treated with different concentrations of the selected compounds (1, 1.5, and 2  $\mu$ M). 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 BDLSR Fortessa flow cytometry. Data were processed using ModFit LT Software.

## **5. NMR and HRESIMS Spectra of Compounds 1–9**

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

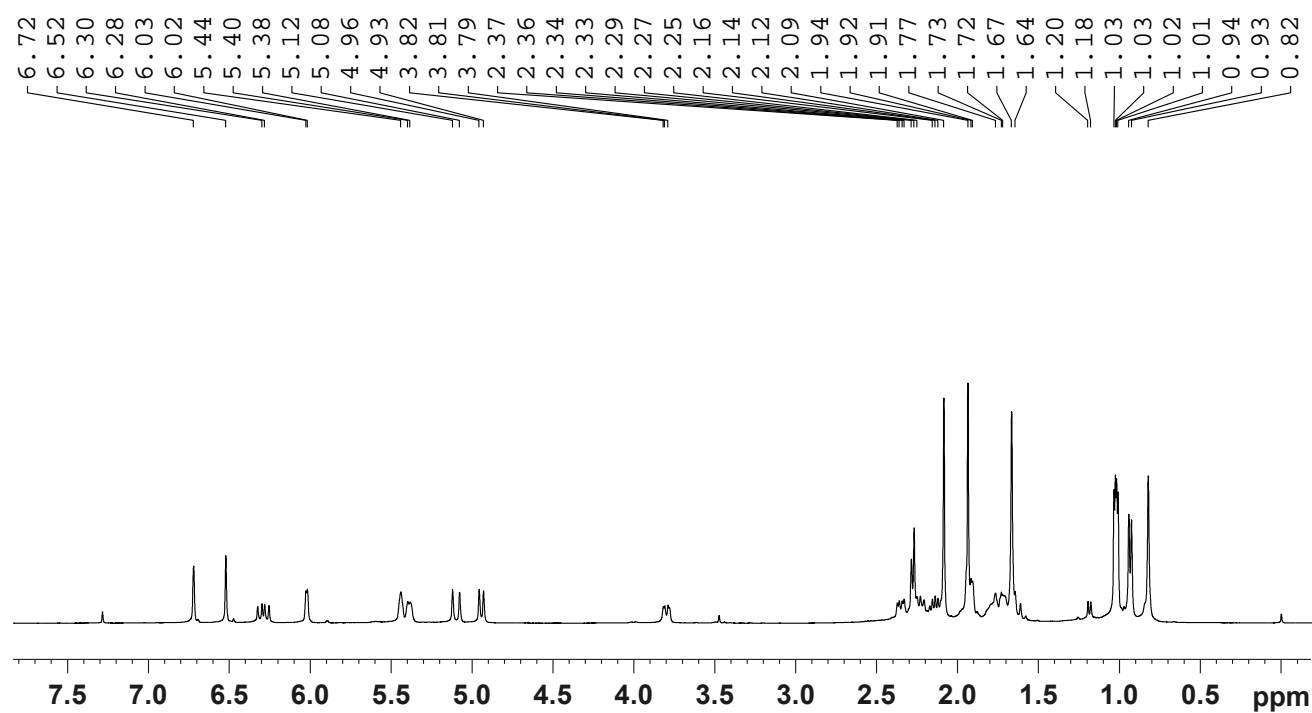


Fig. S2  $^{13}\text{C}$  NMR spectrum for compound **1**

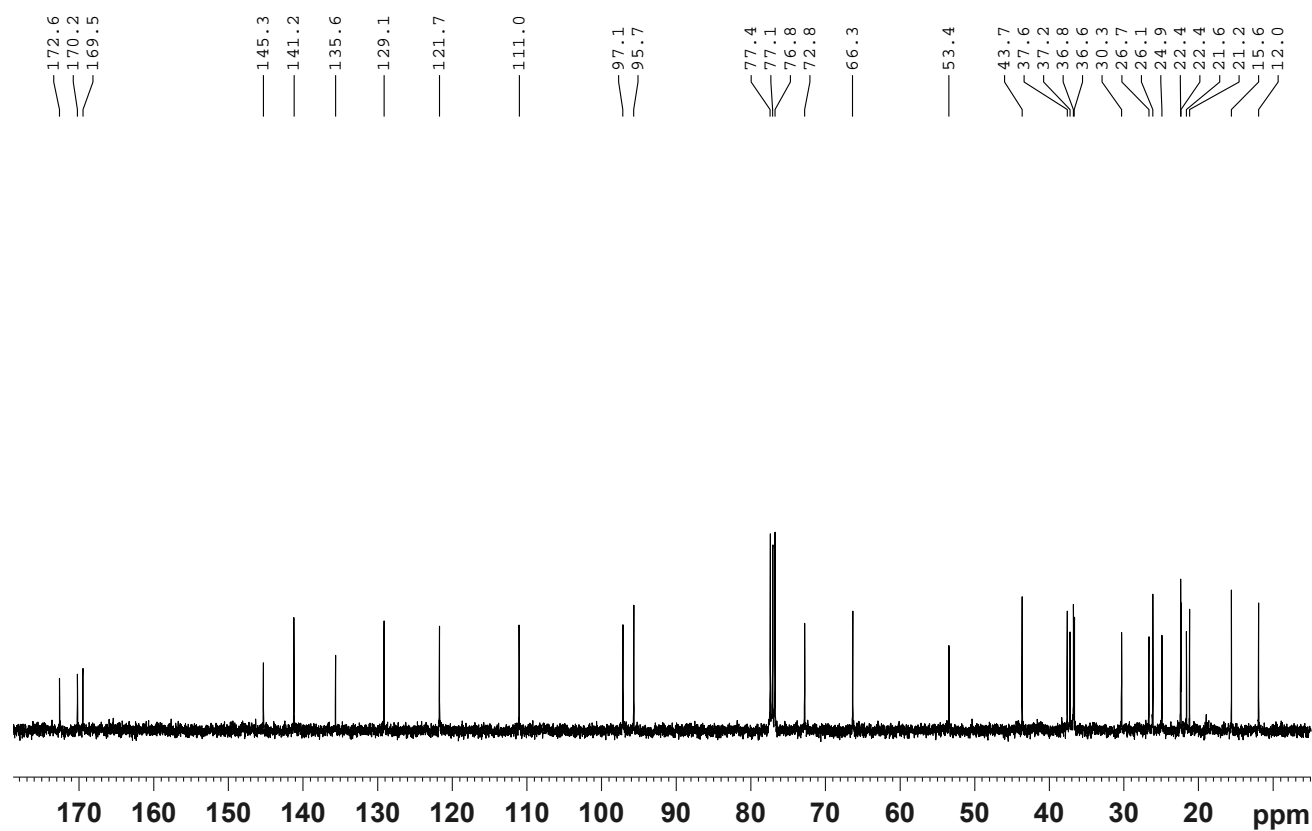




Fig. S3 DEPT ( $\theta = 135^\circ$ ) NMR spectrum for compound **1**

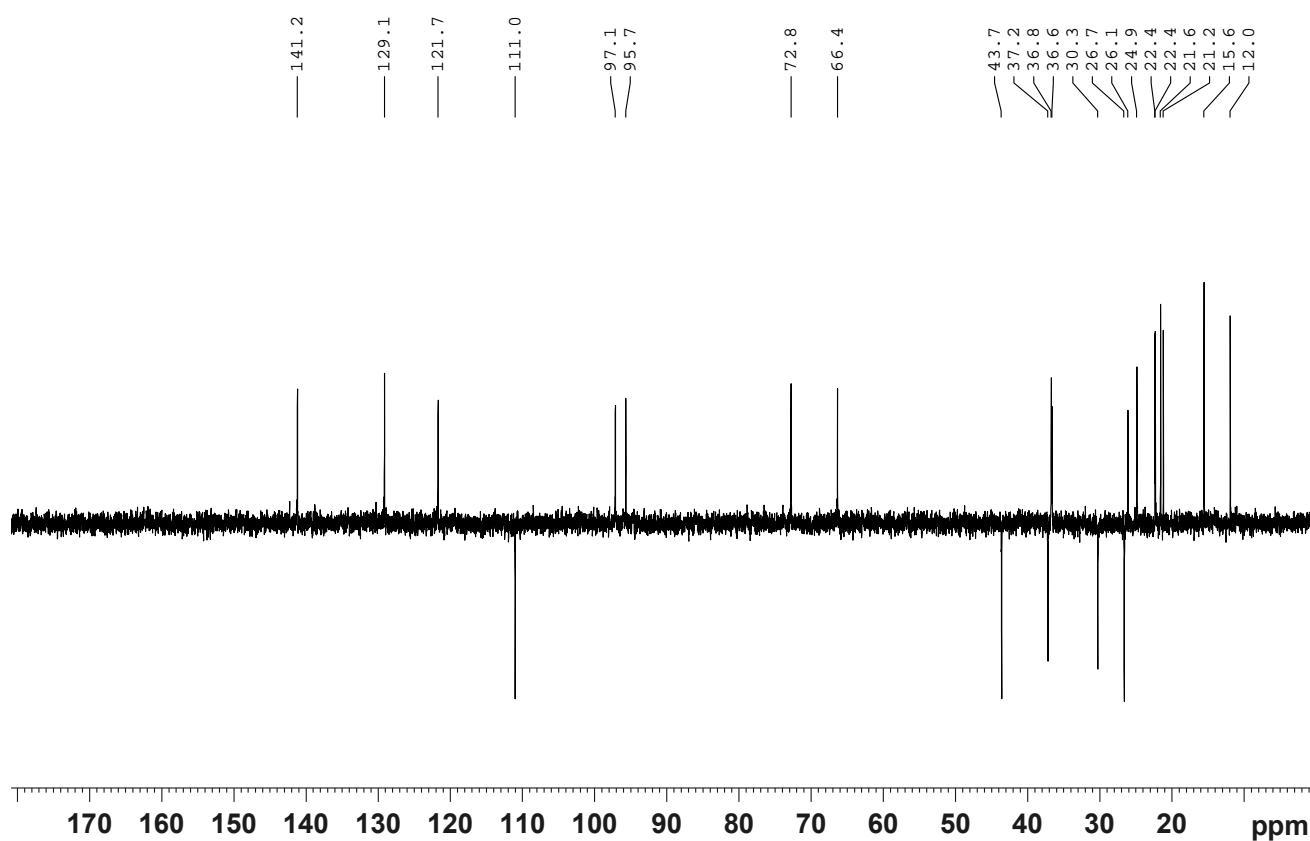


Fig. S4 HMQC spectrum for compound **1**

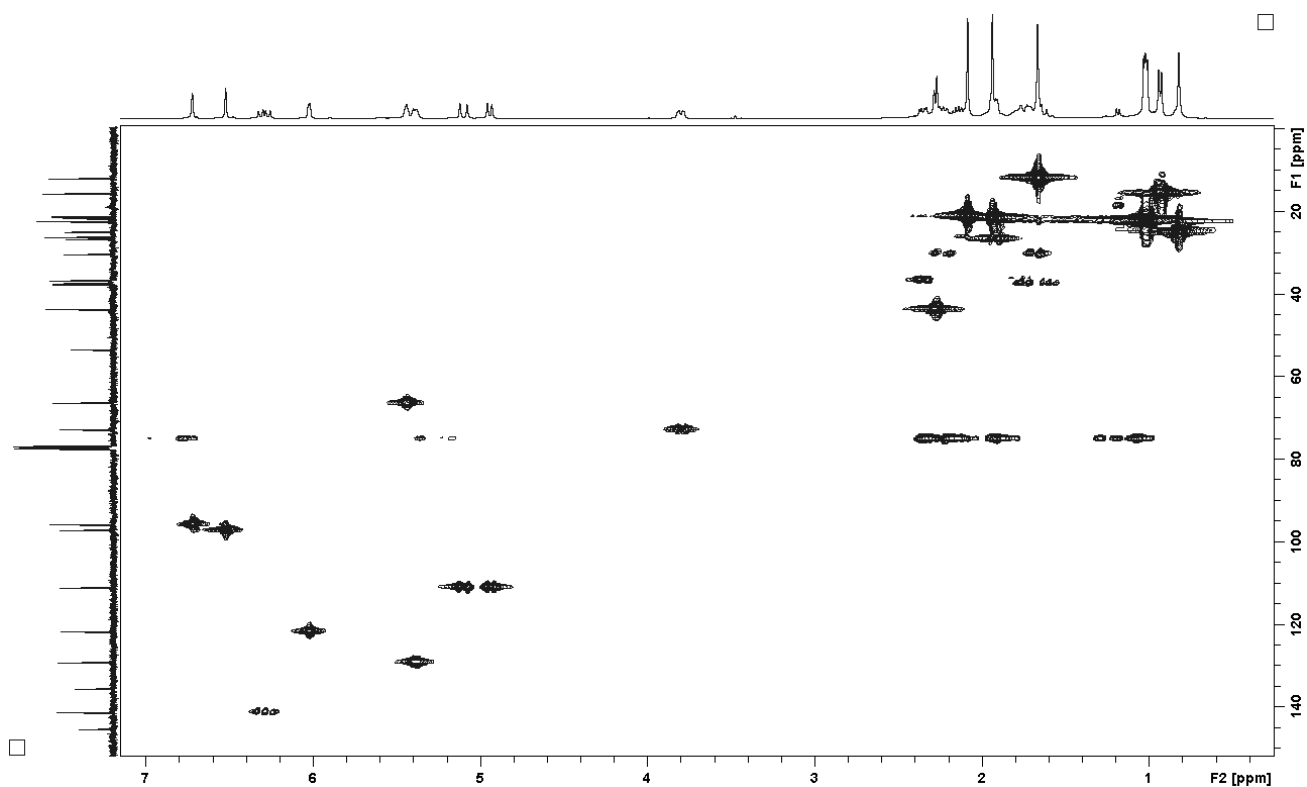


Fig. S5 HMBC spectrum for compound 1

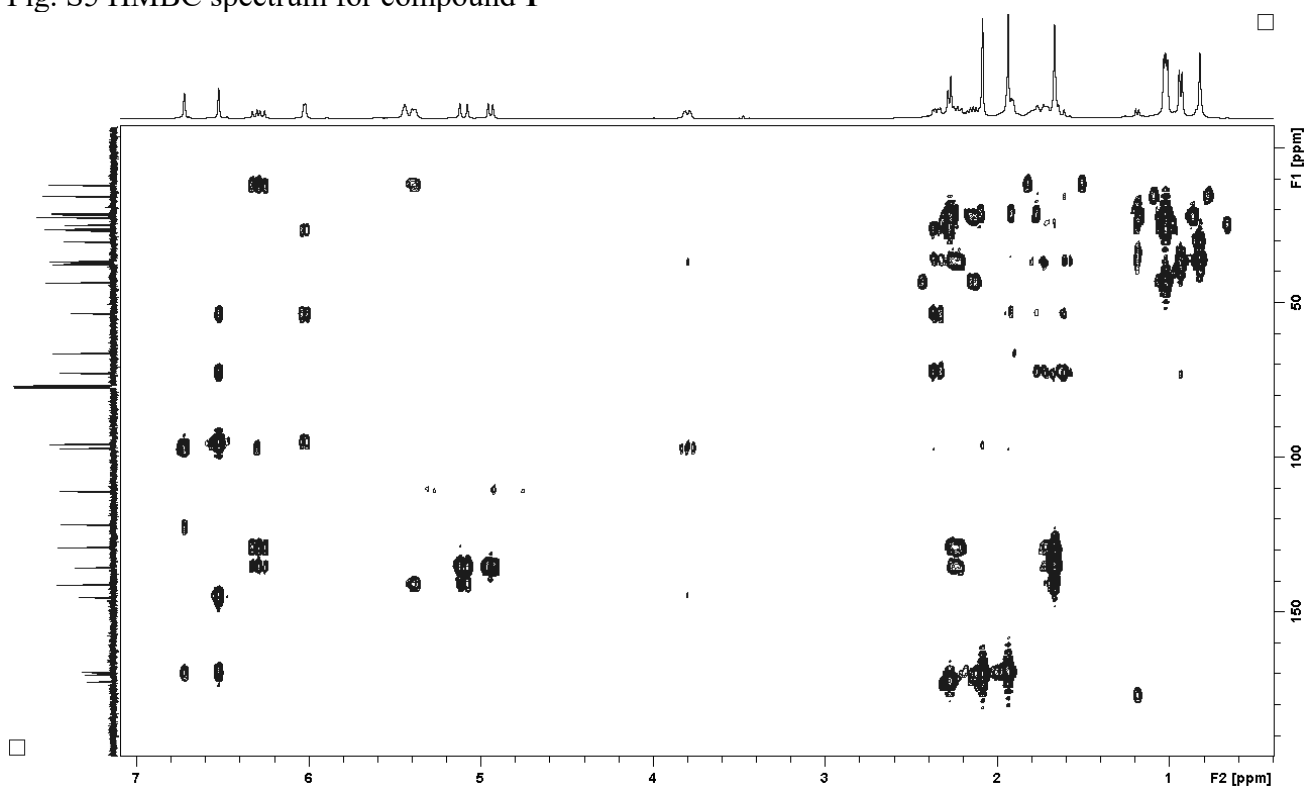


Fig. S6  $^1\text{H}$ - $^1\text{H}$  COSY spectrum for compound 1

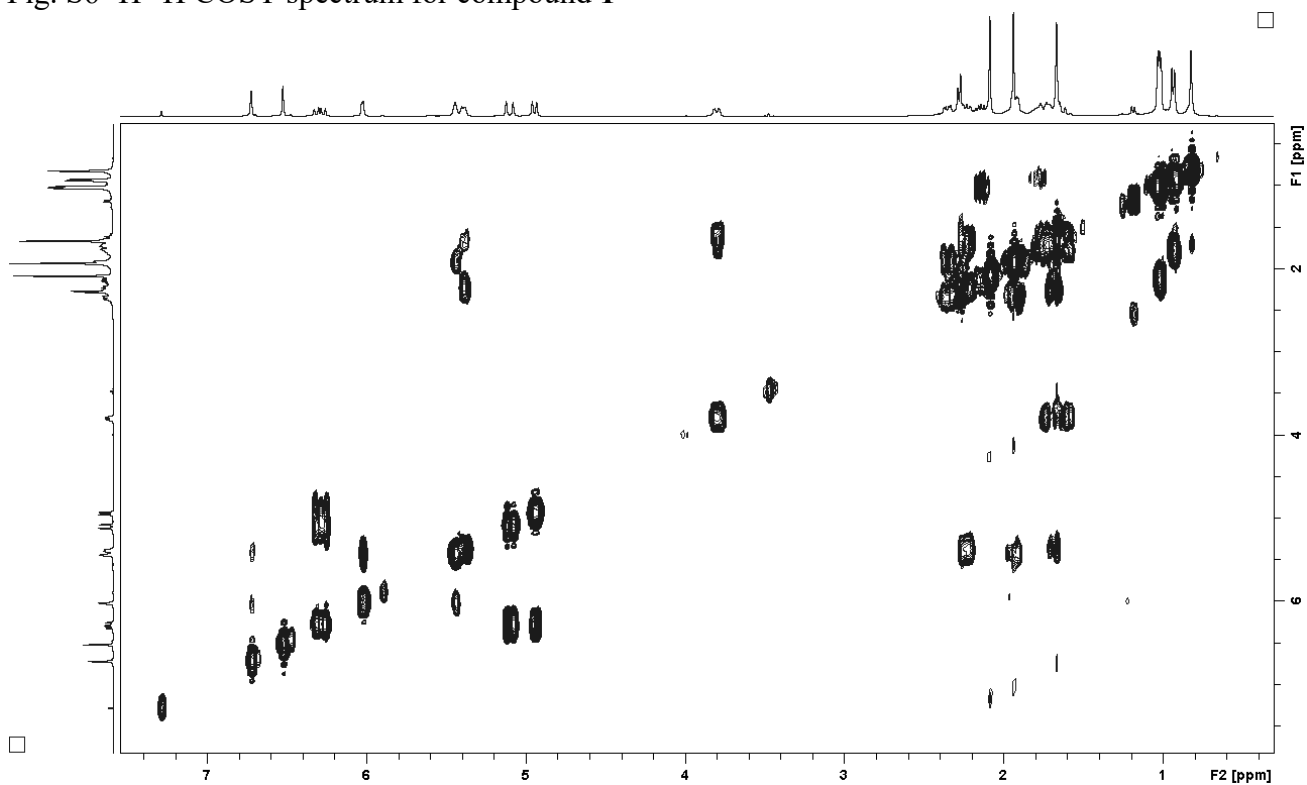


Fig. S7 NOESY spectrum for compound **1**

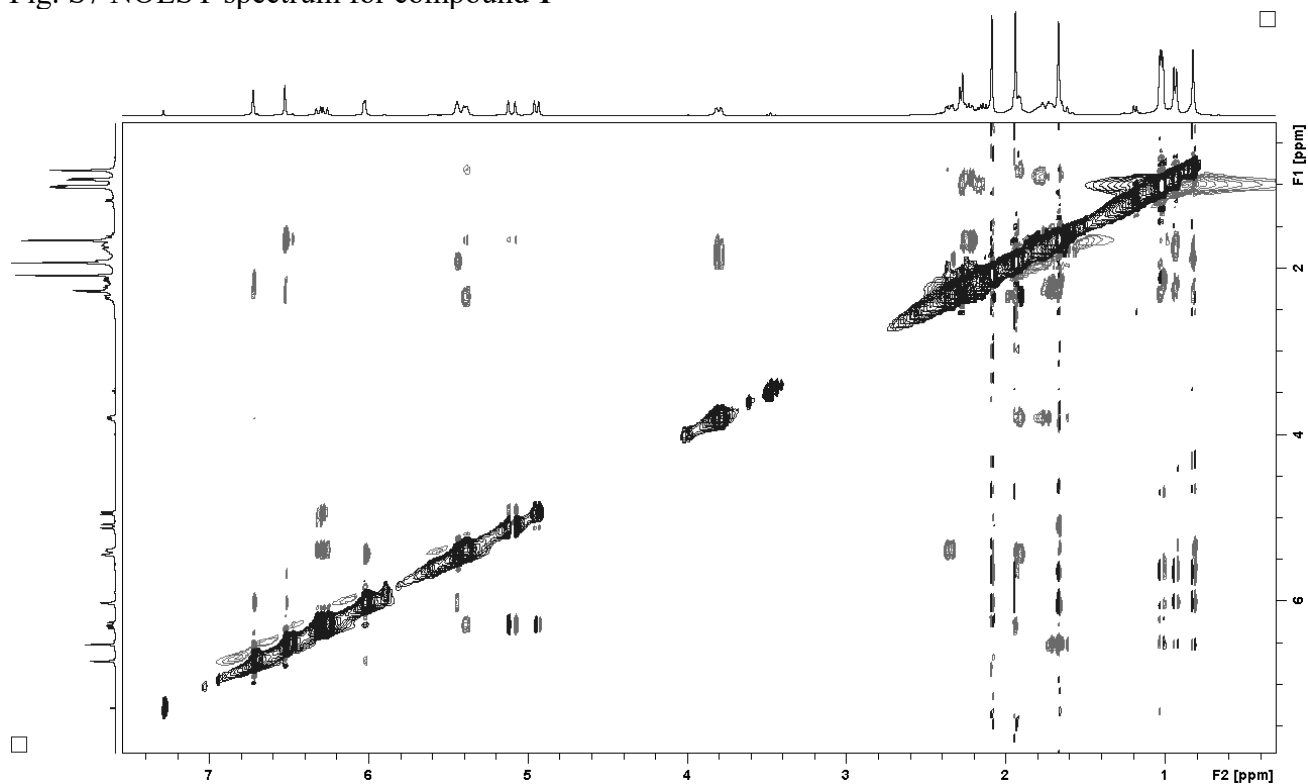


Fig. S8 HRESIMS spectrum for compound **1**

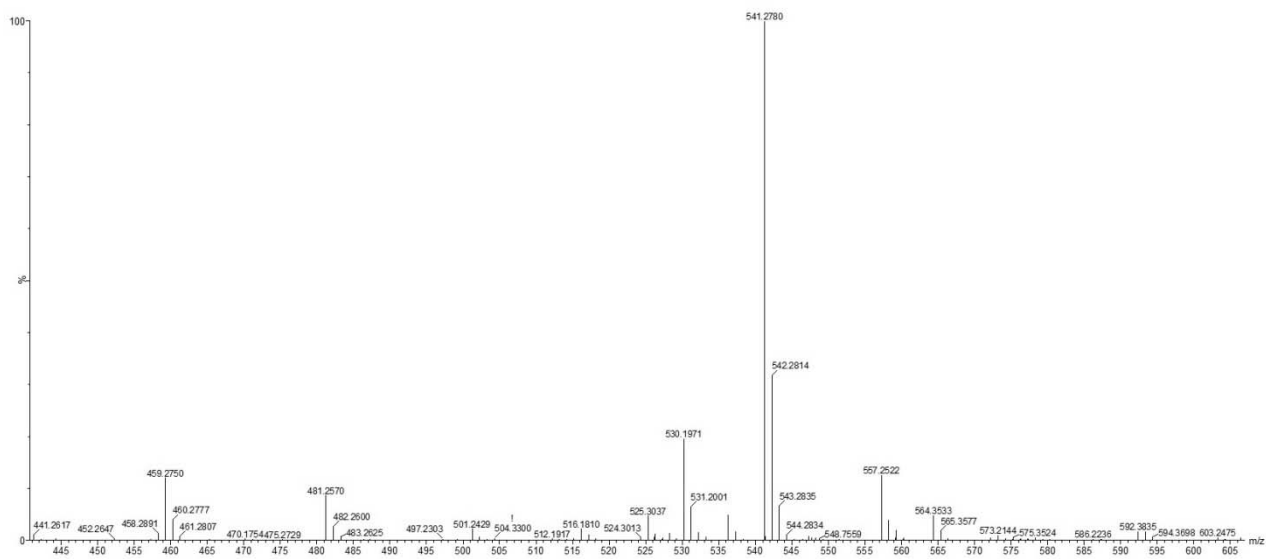


Fig. S9  $^1\text{H}$  NMR spectrum for compound **2**

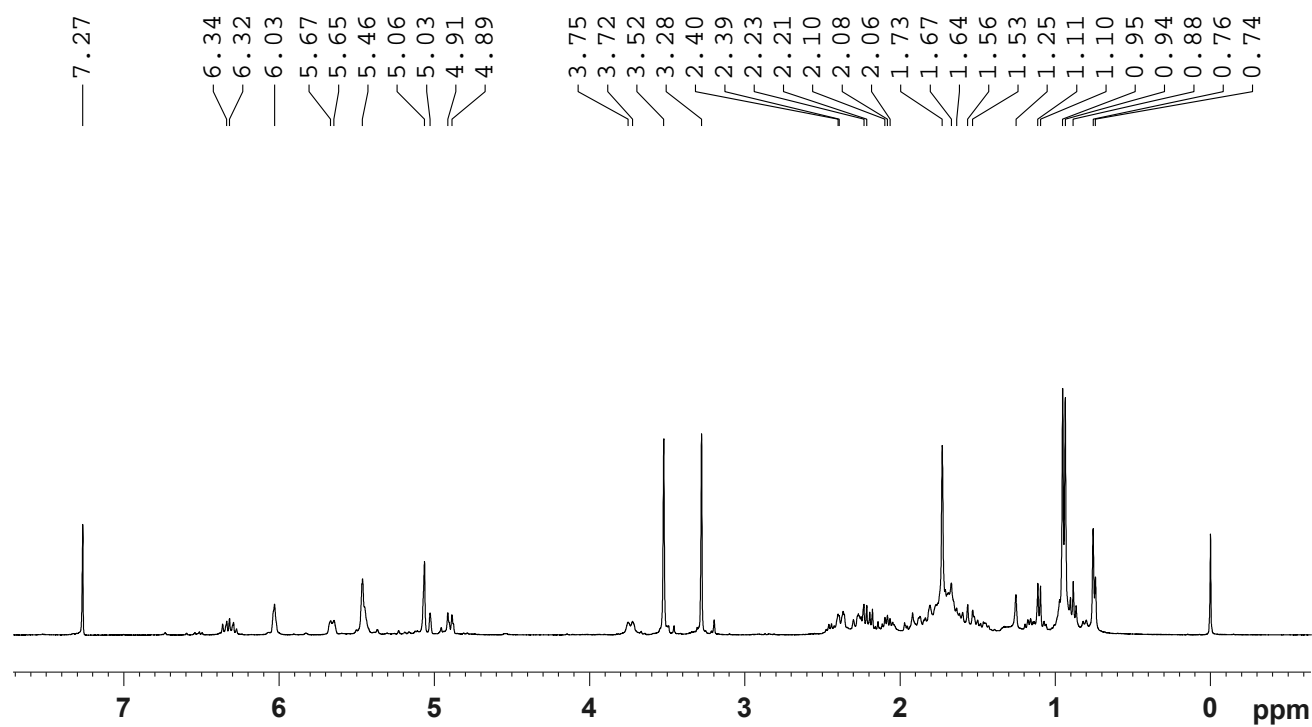


Fig. S10  $^{13}\text{C}$  NMR spectrum for compound **2**

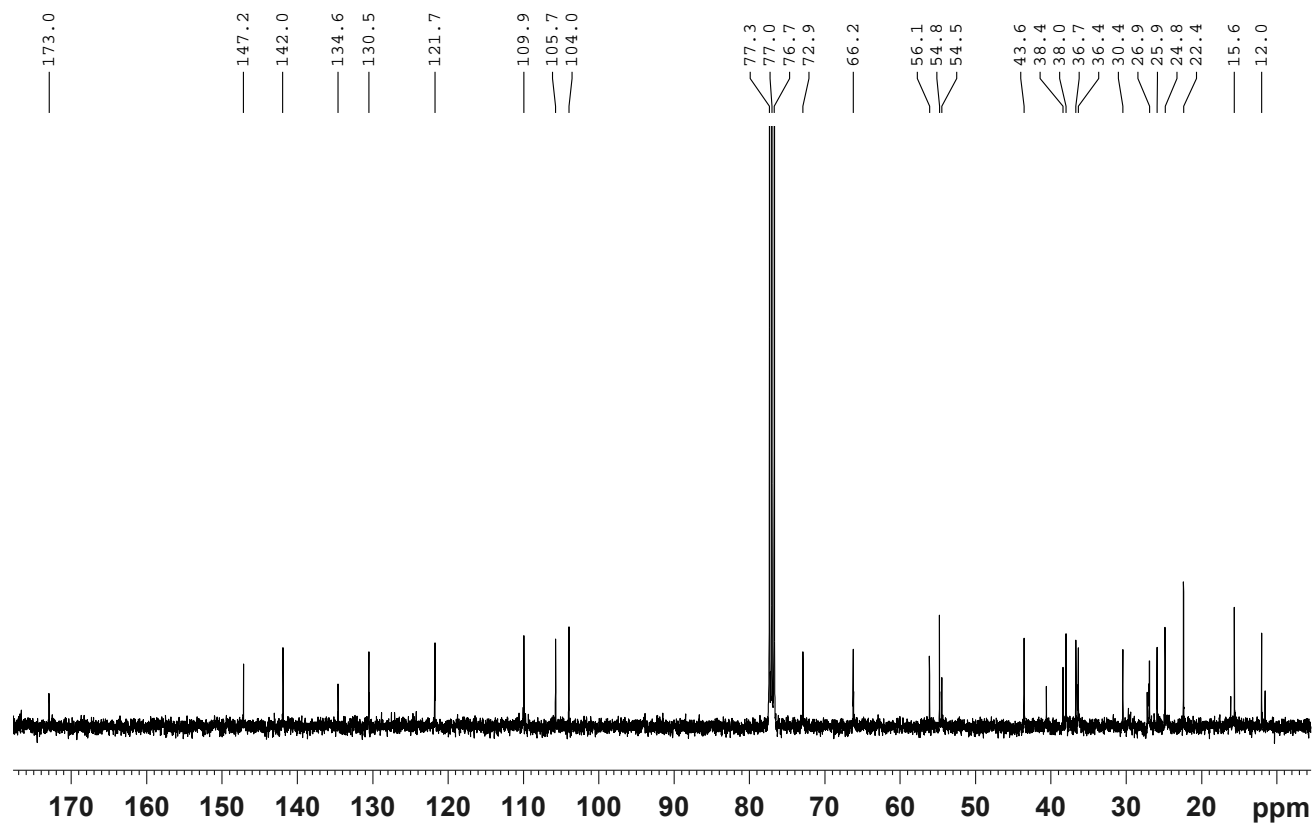


Fig. S11 HMQC spectrum for compound **2**

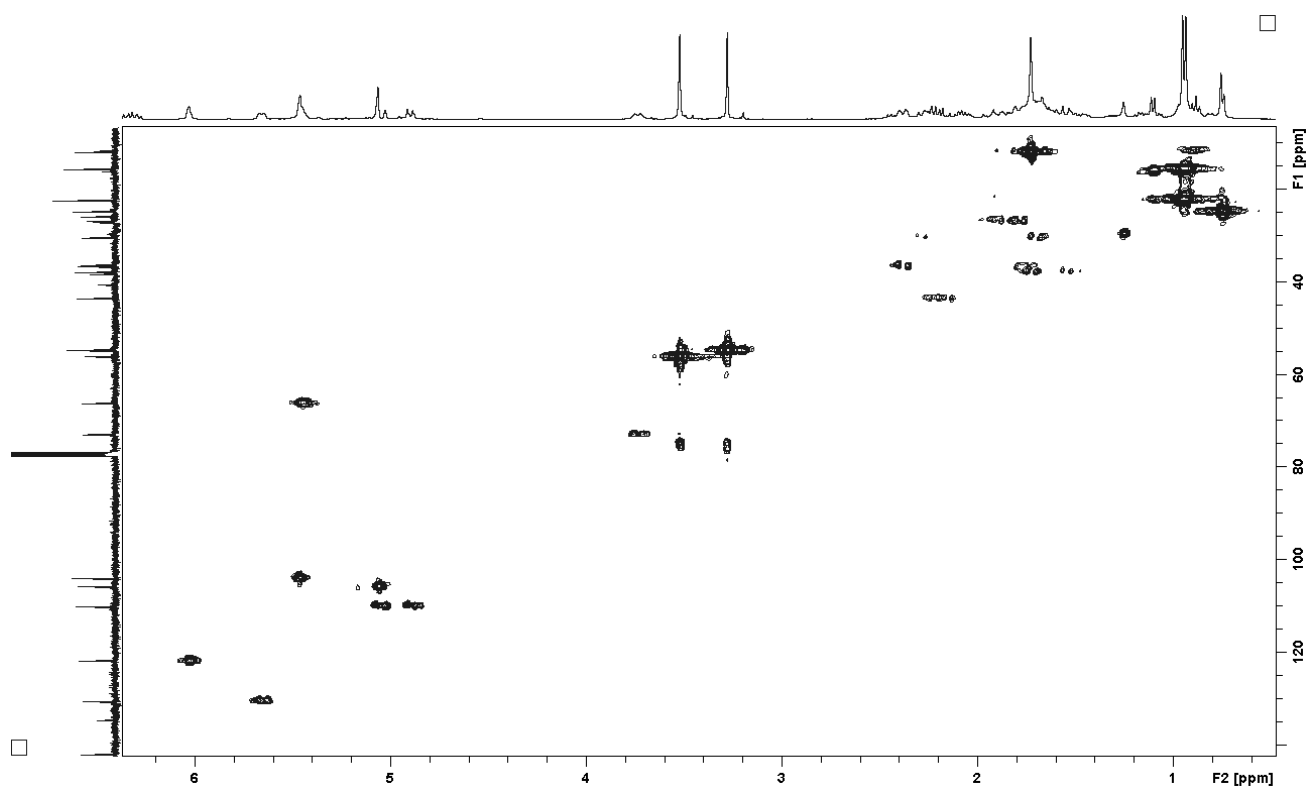


Fig. S12 HMBC spectrum for compound **2**

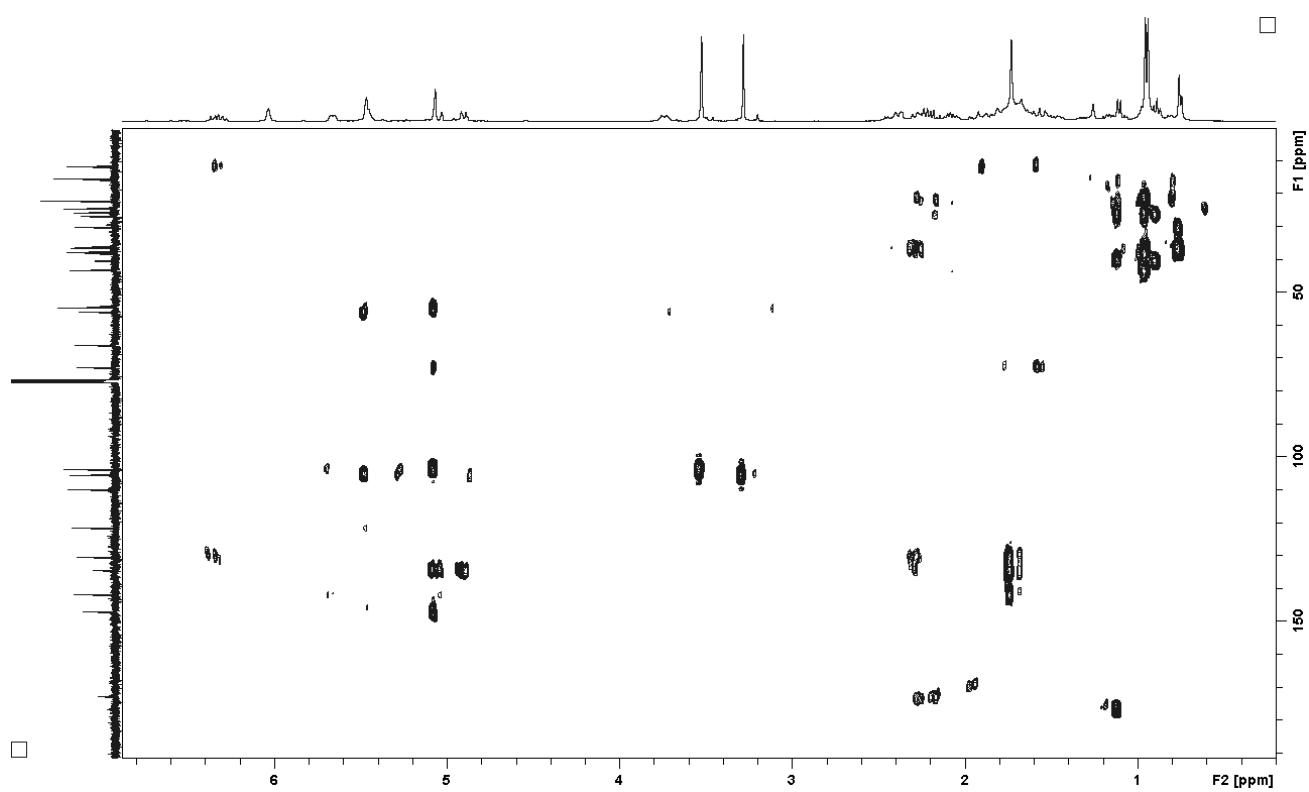


Fig. S13 NOESY spectrum for compound **2**

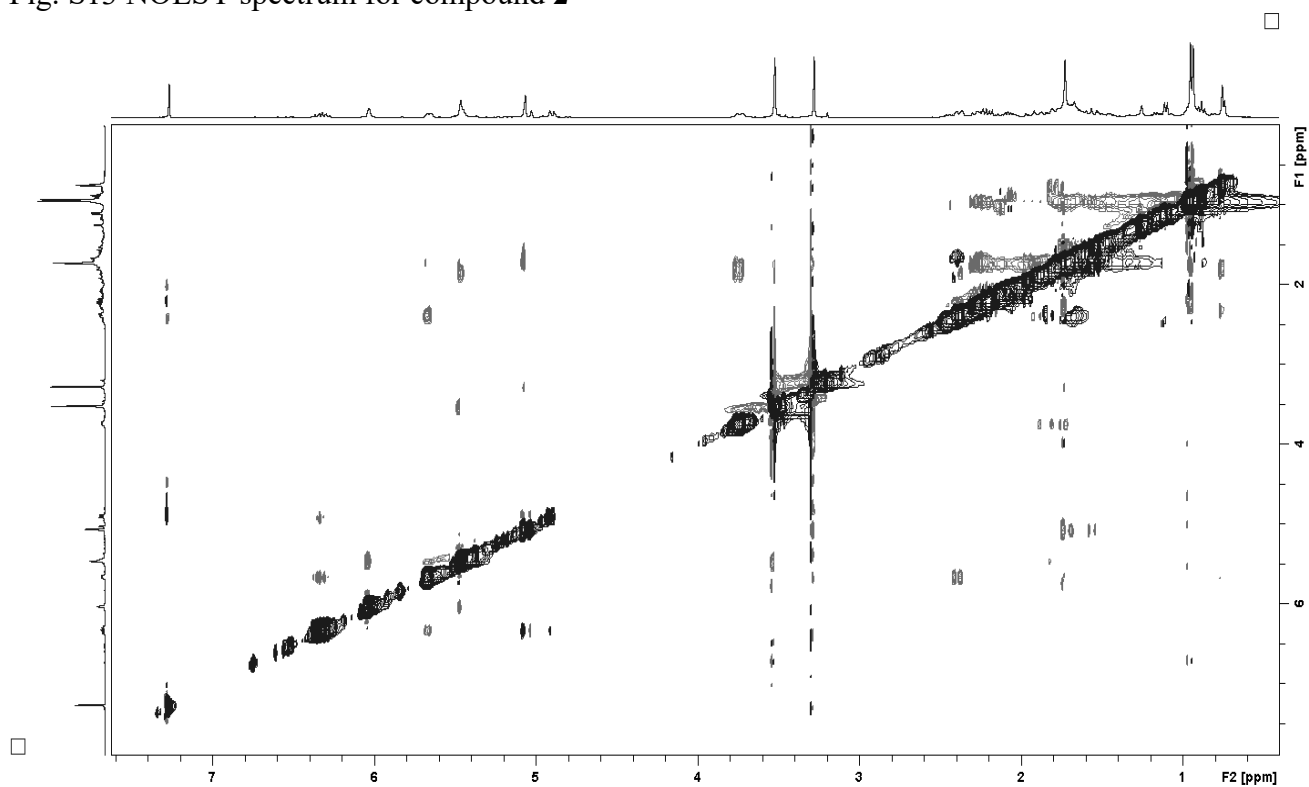


Fig. S14 HRESIMS spectrum for compound **2**

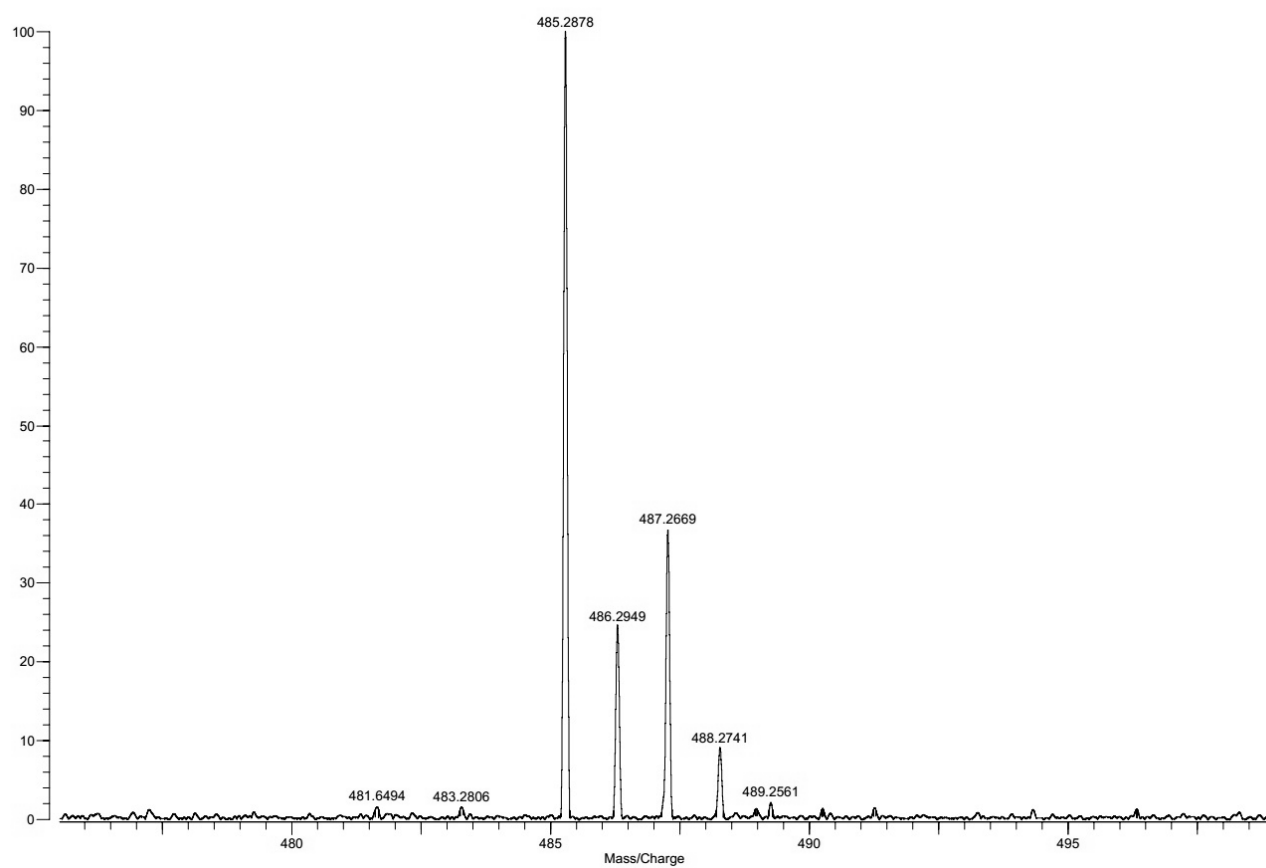


Fig. S15  $^1\text{H}$  NMR spectrum for compound **3**

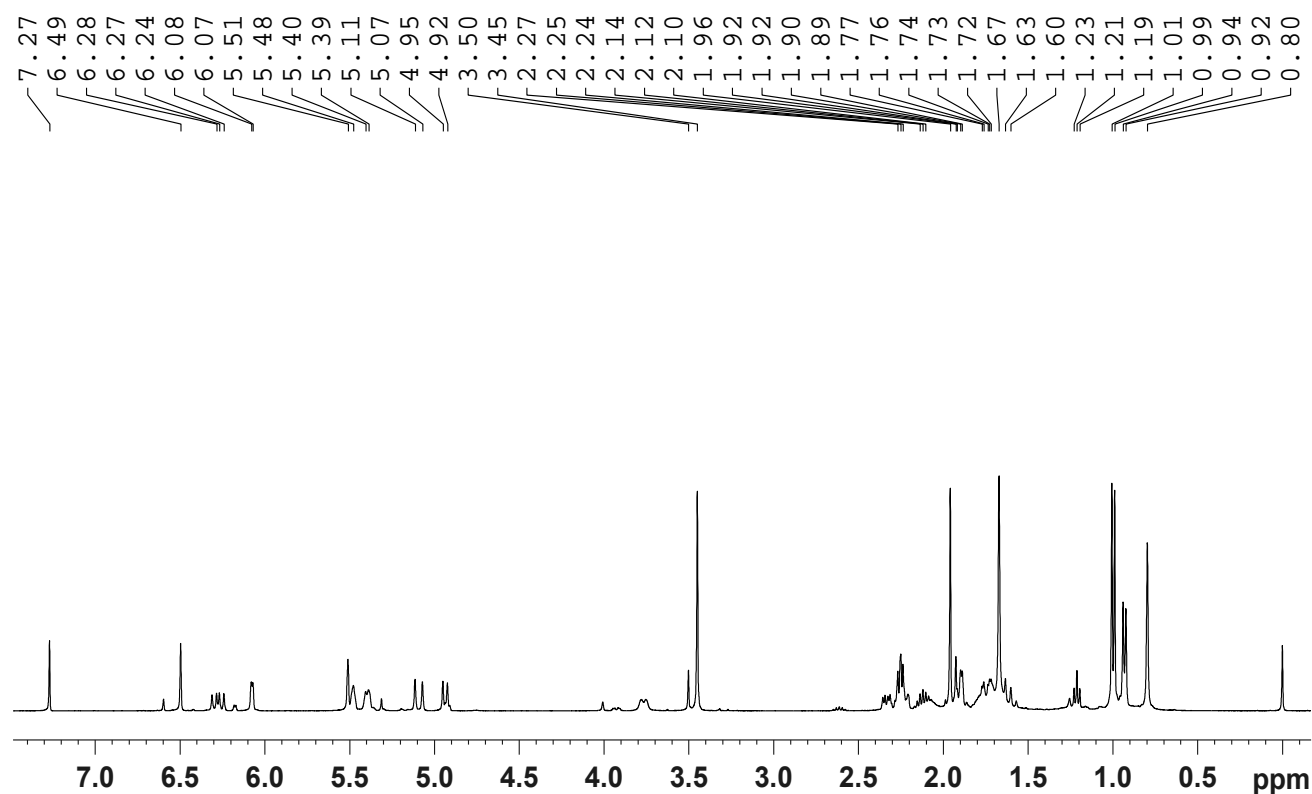


Fig. S16  $^{13}\text{C}$  NMR spectrum for compound **3**

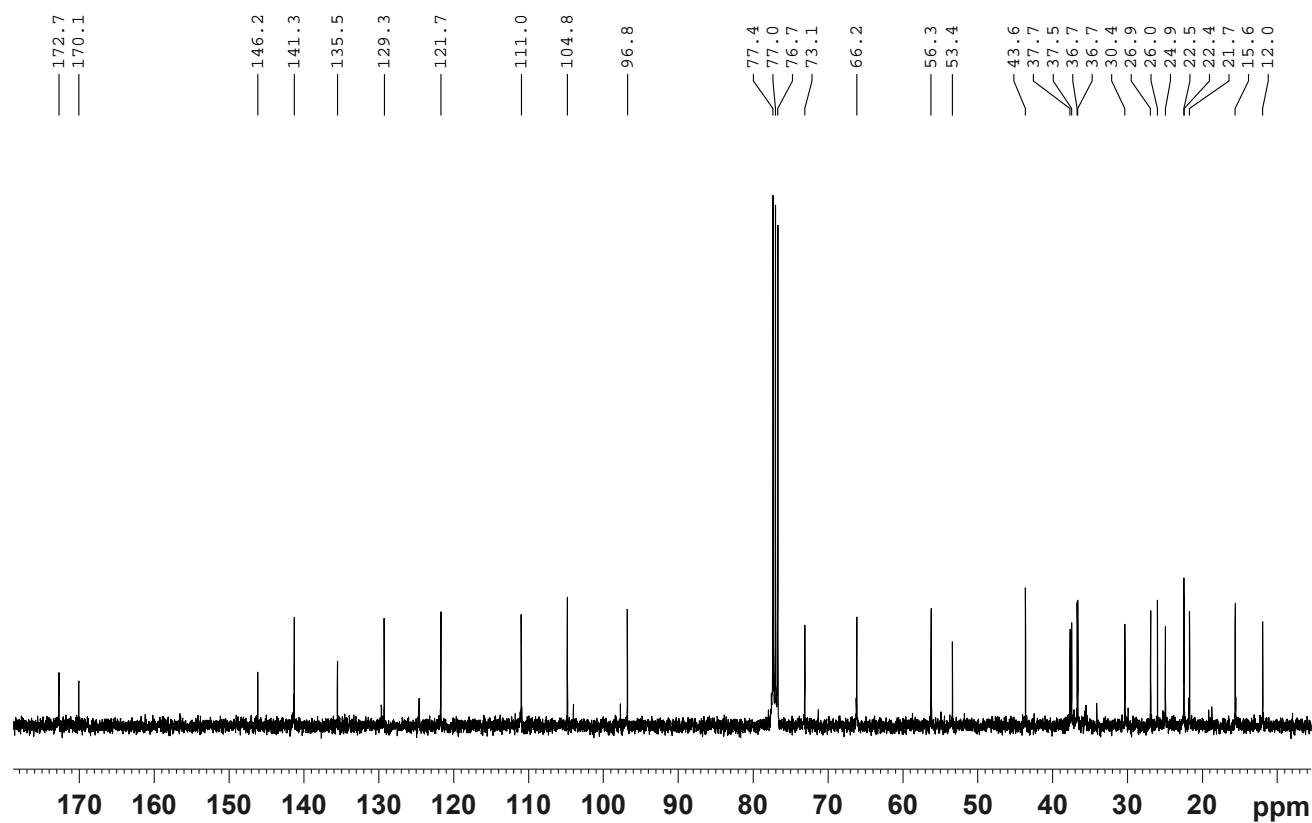


Fig. S17 HMQC spectrum for compound **3**

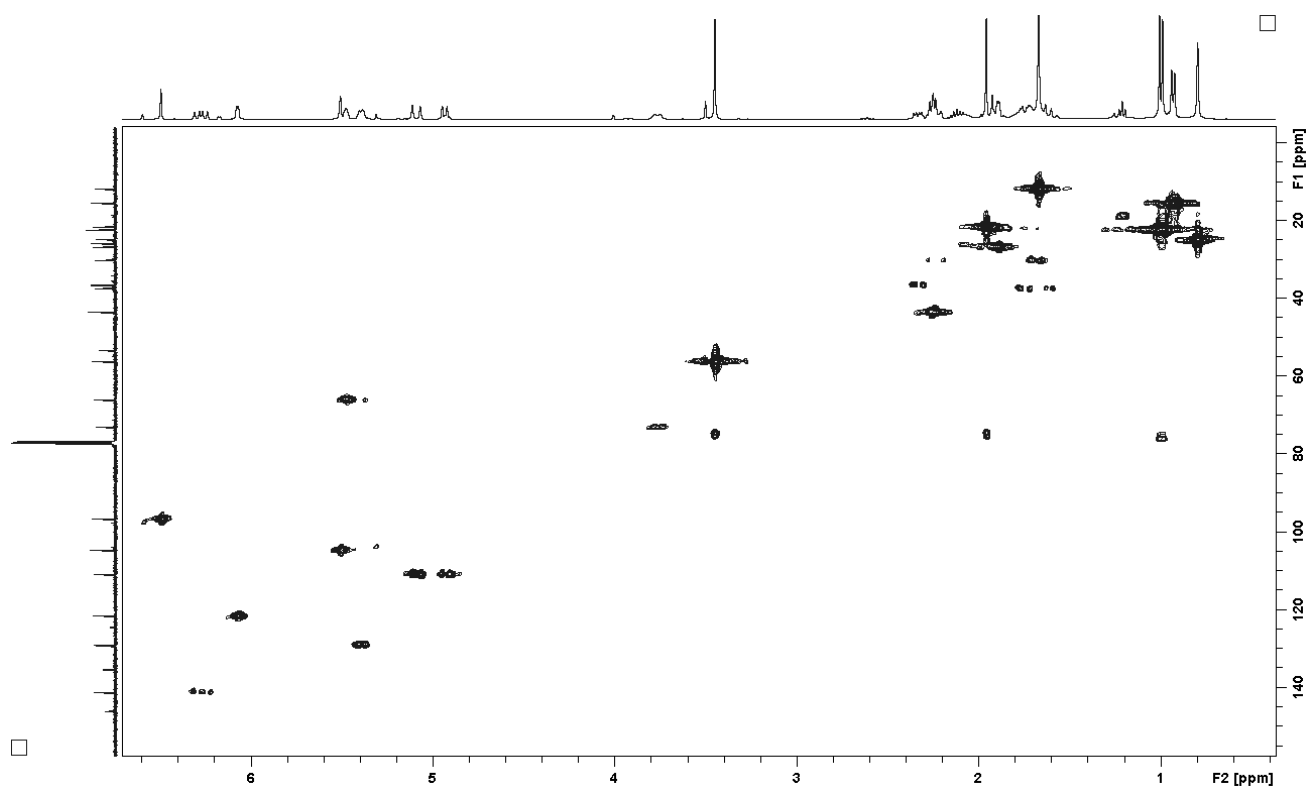


Fig. S18 HMBC spectrum for compound **3**

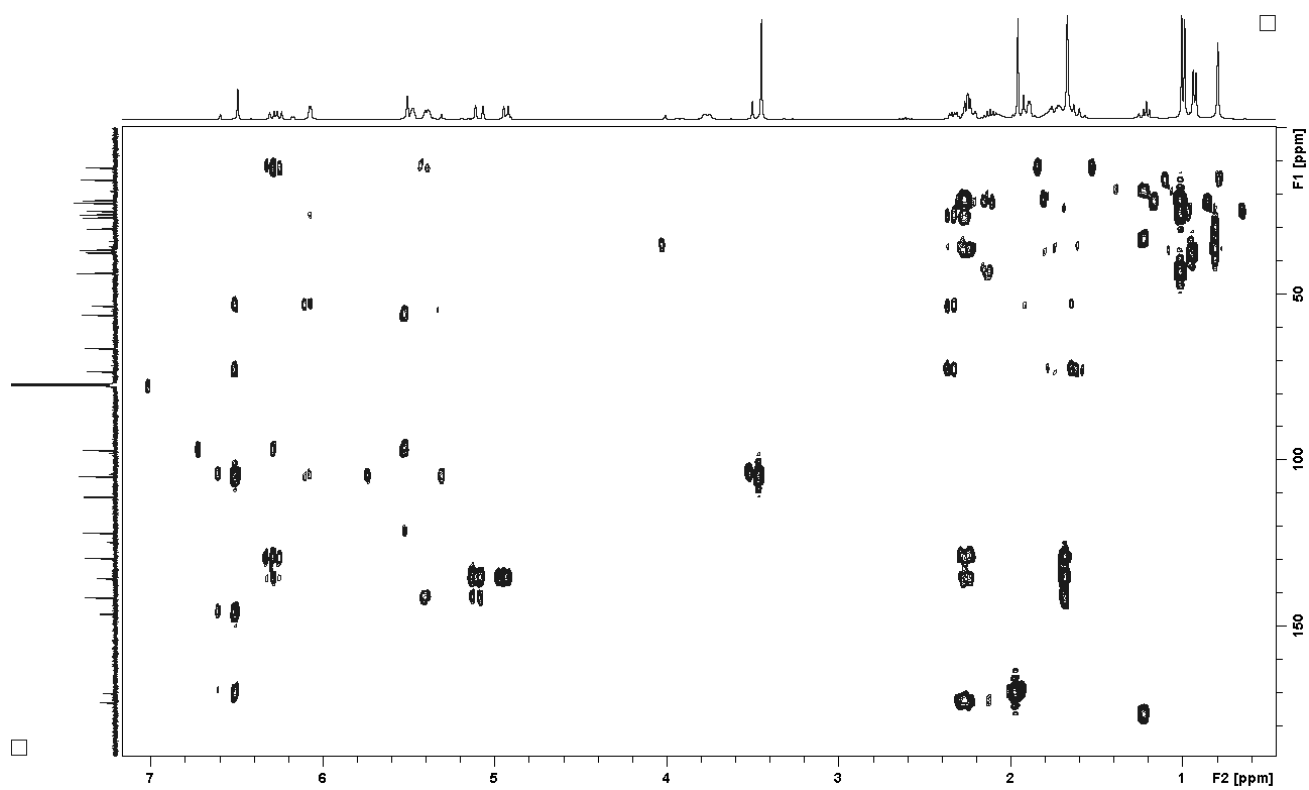




Fig. S19 NOESY spectrum for compound **3**

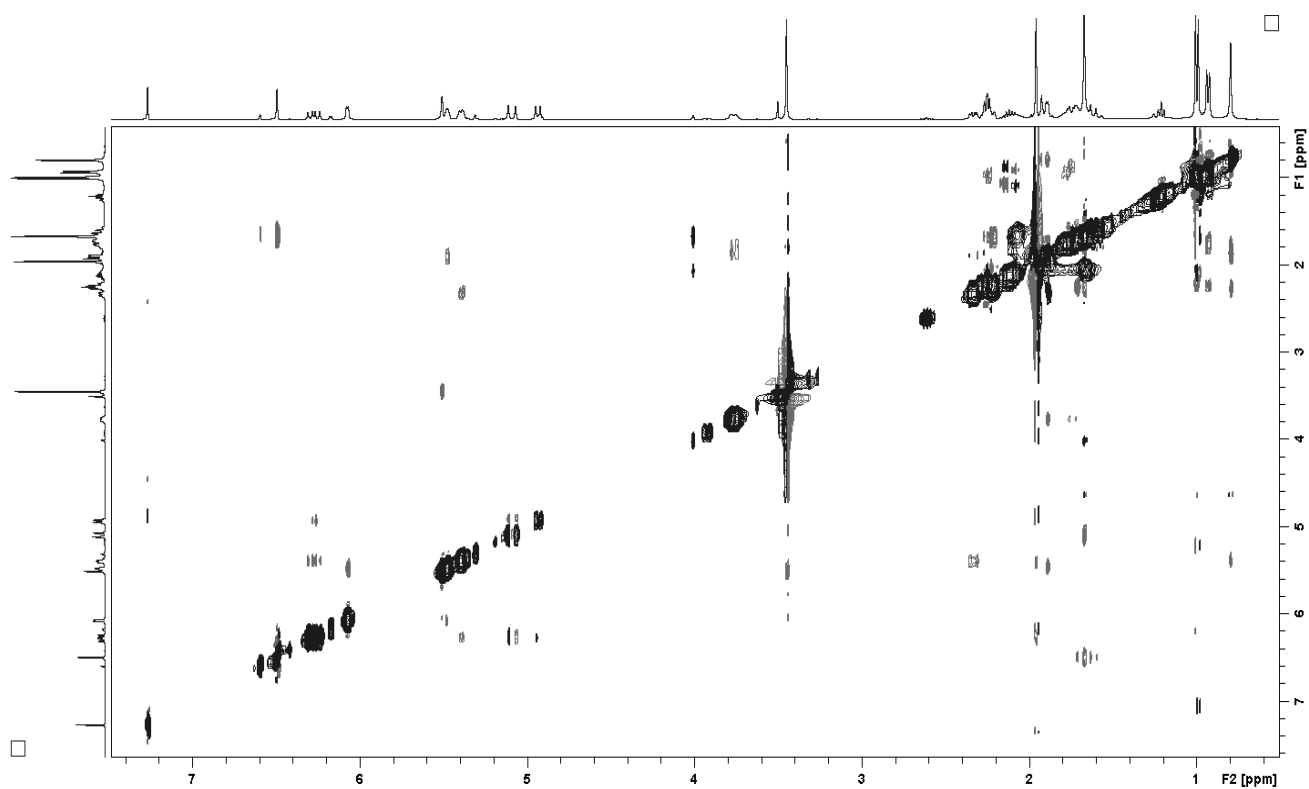


Fig. S20 HRESIMS spectrum for compound **3**

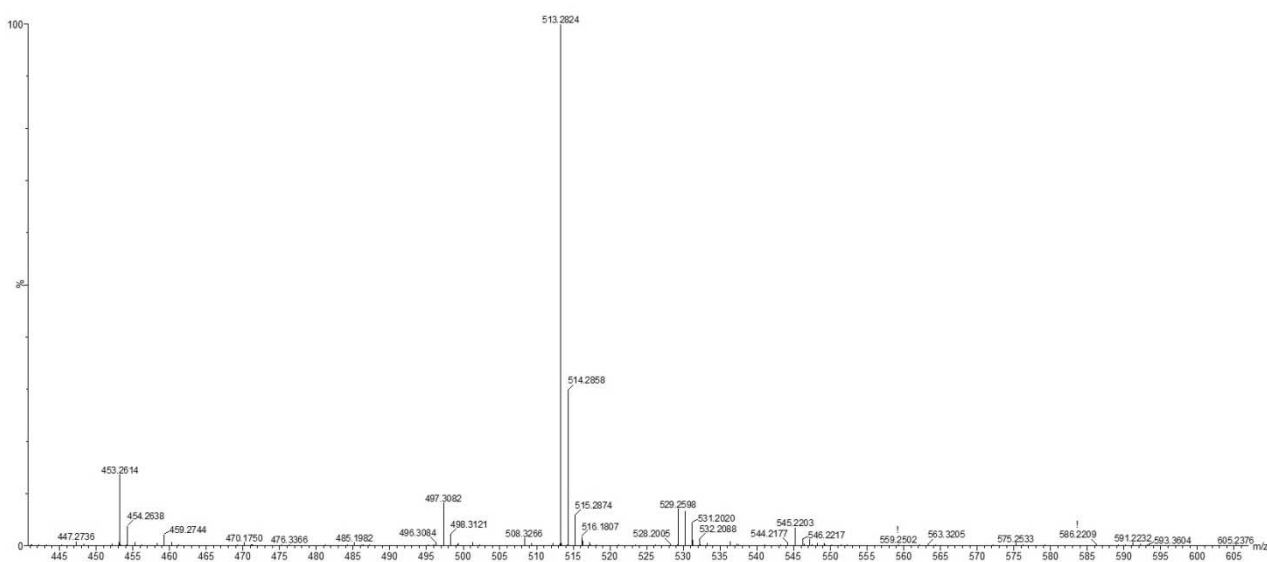


Fig. S21  $^1\text{H}$  NMR spectrum for compound **4**

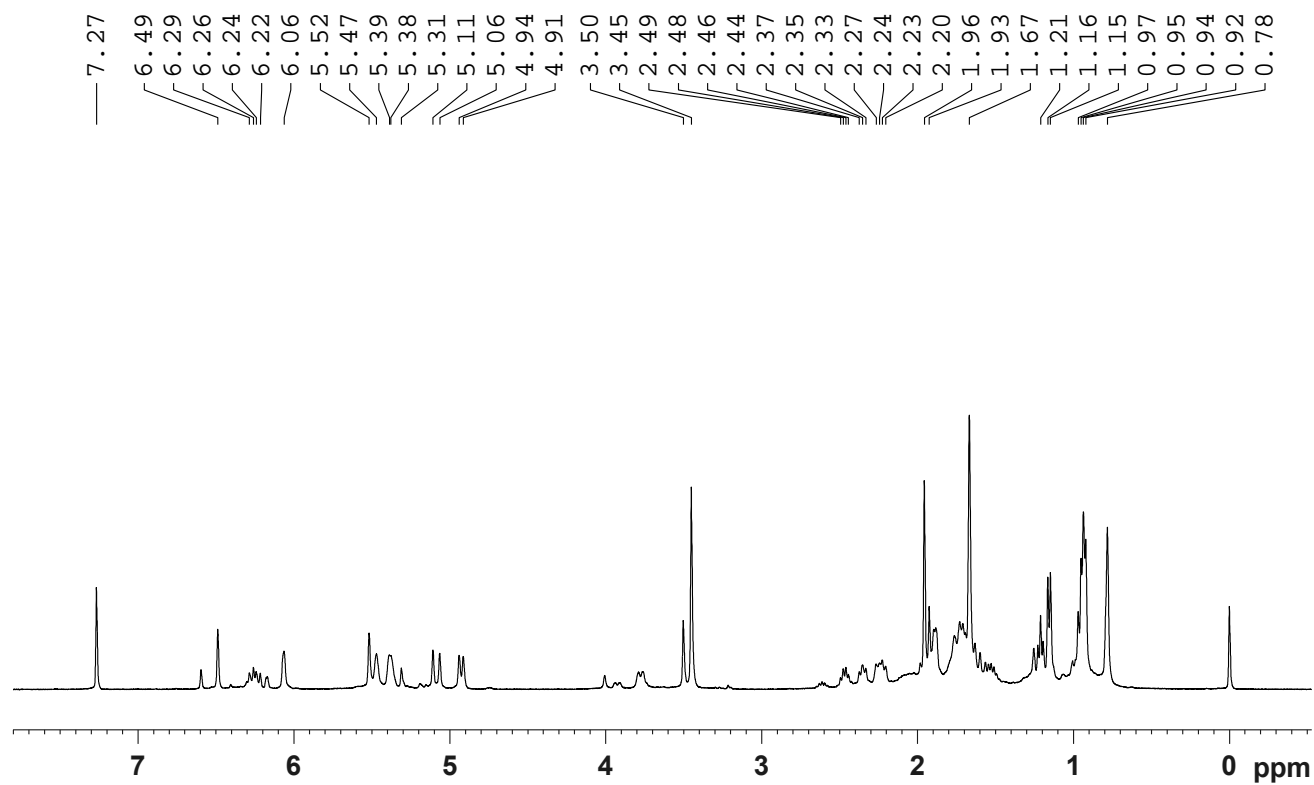


Fig. S22  $^{13}\text{C}$  NMR spectrum for compound **4**

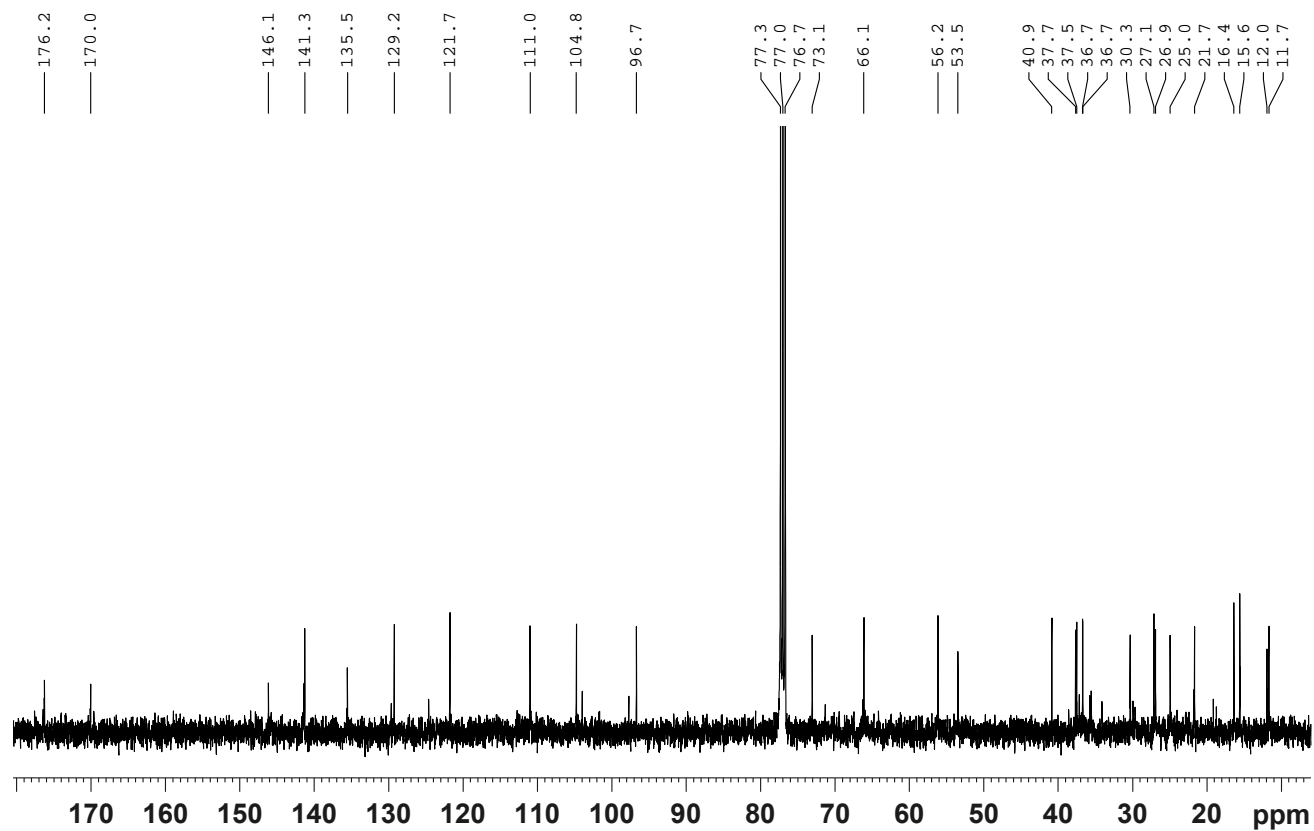


Fig. S23 HMQC spectrum for compound 4

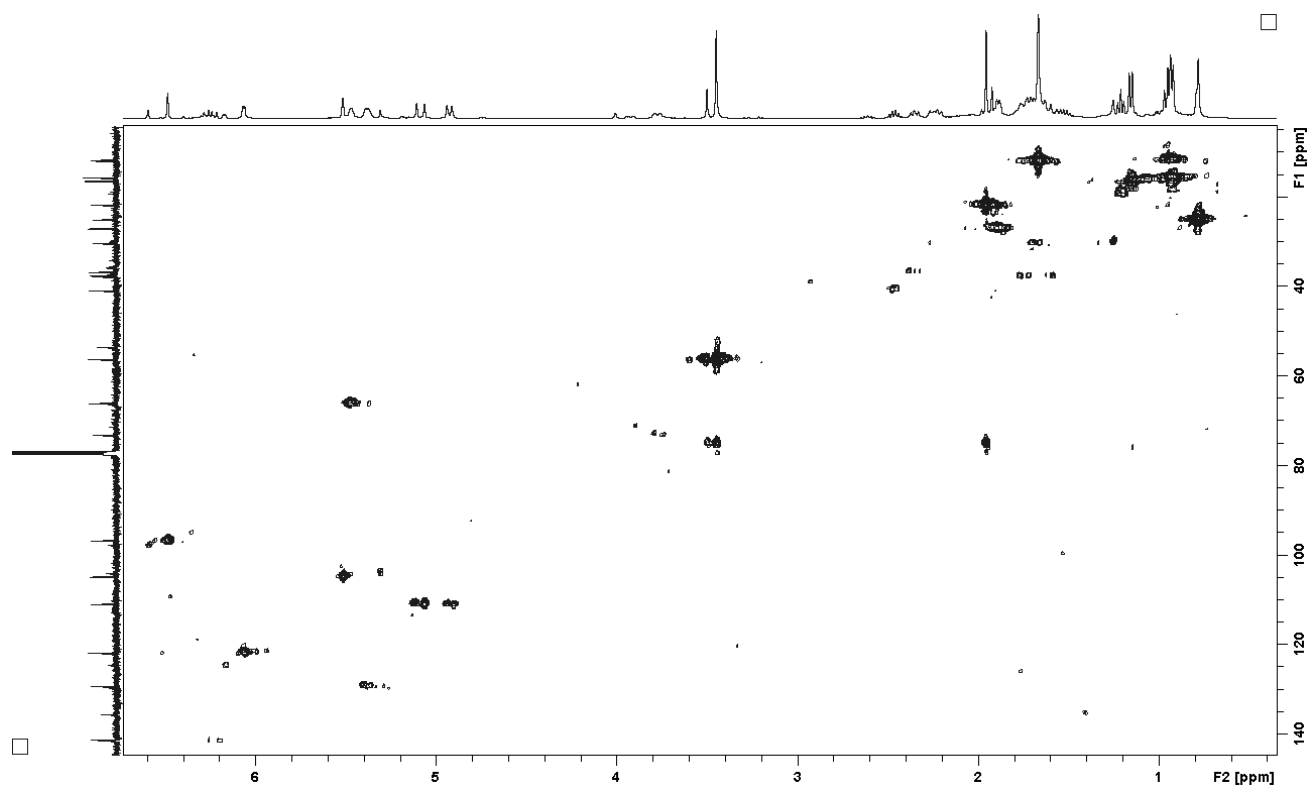


Fig. S24 HMBC spectrum for compound 4

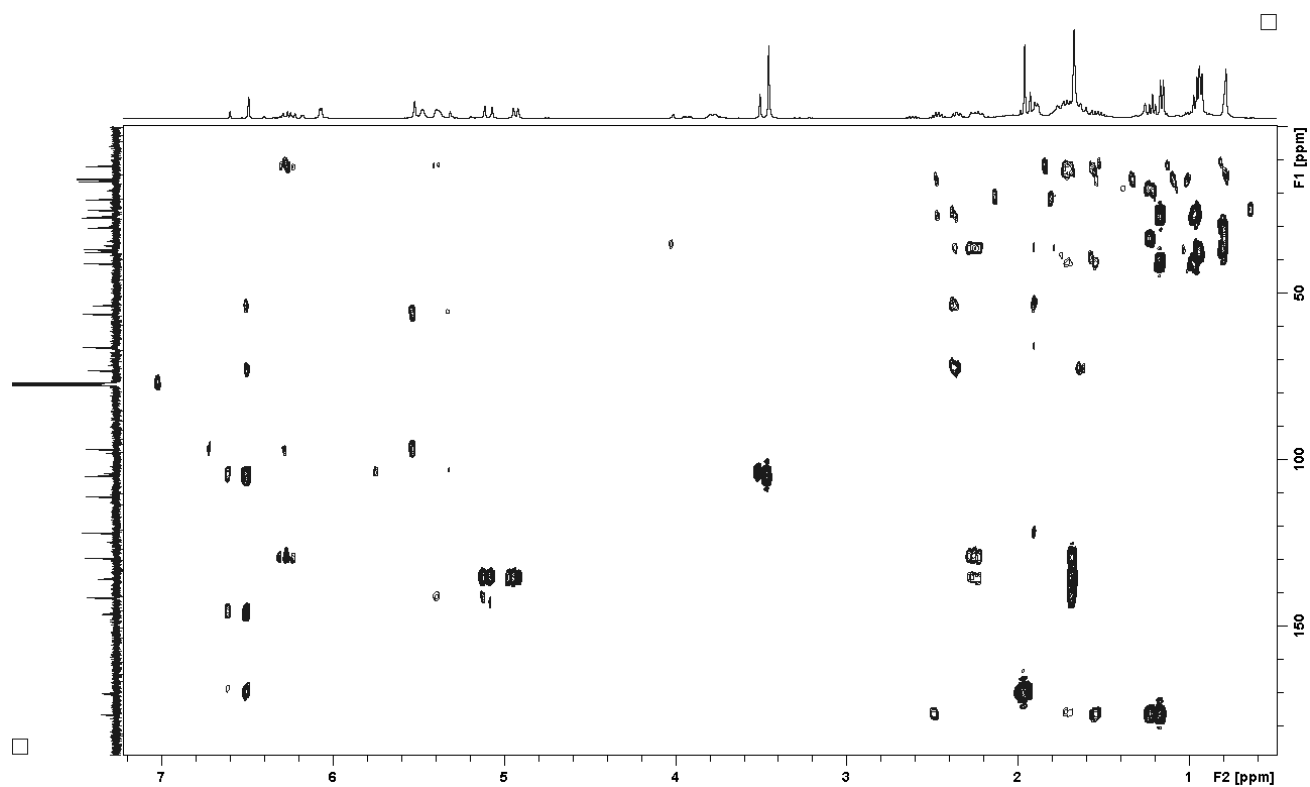


Fig. S25 NOESY spectrum for compound **4**

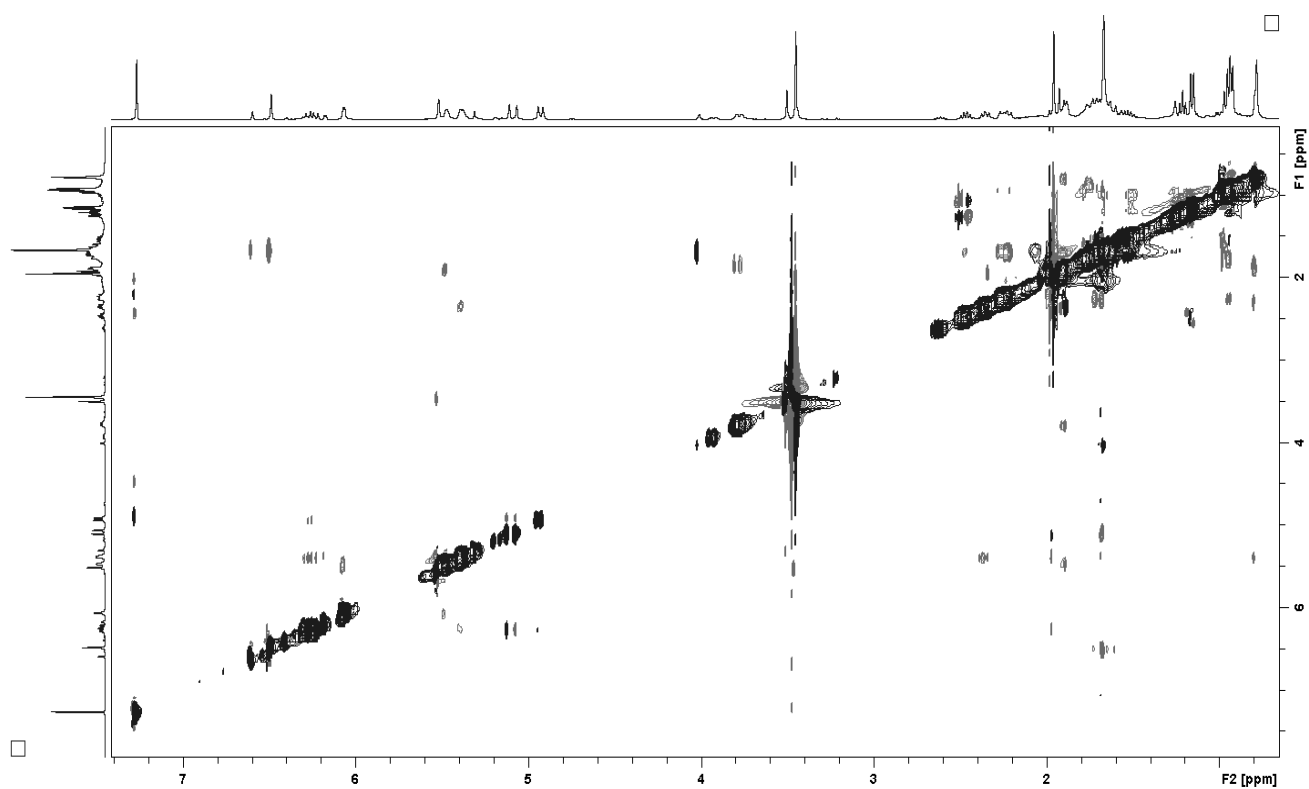


Fig. S26 HRESIMS spectrum for compound **4**

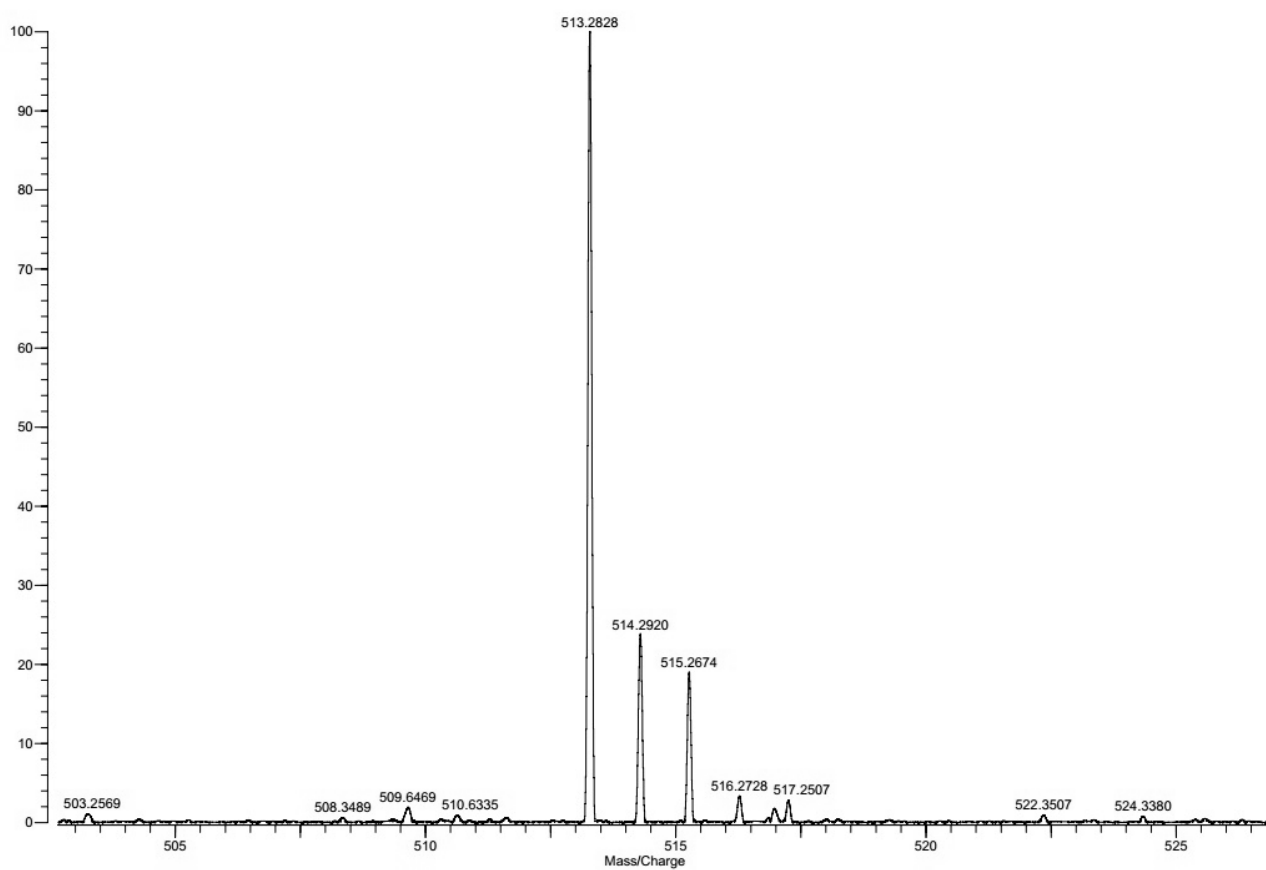


Fig. S27  $^1\text{H}$  NMR spectrum for compound **5**

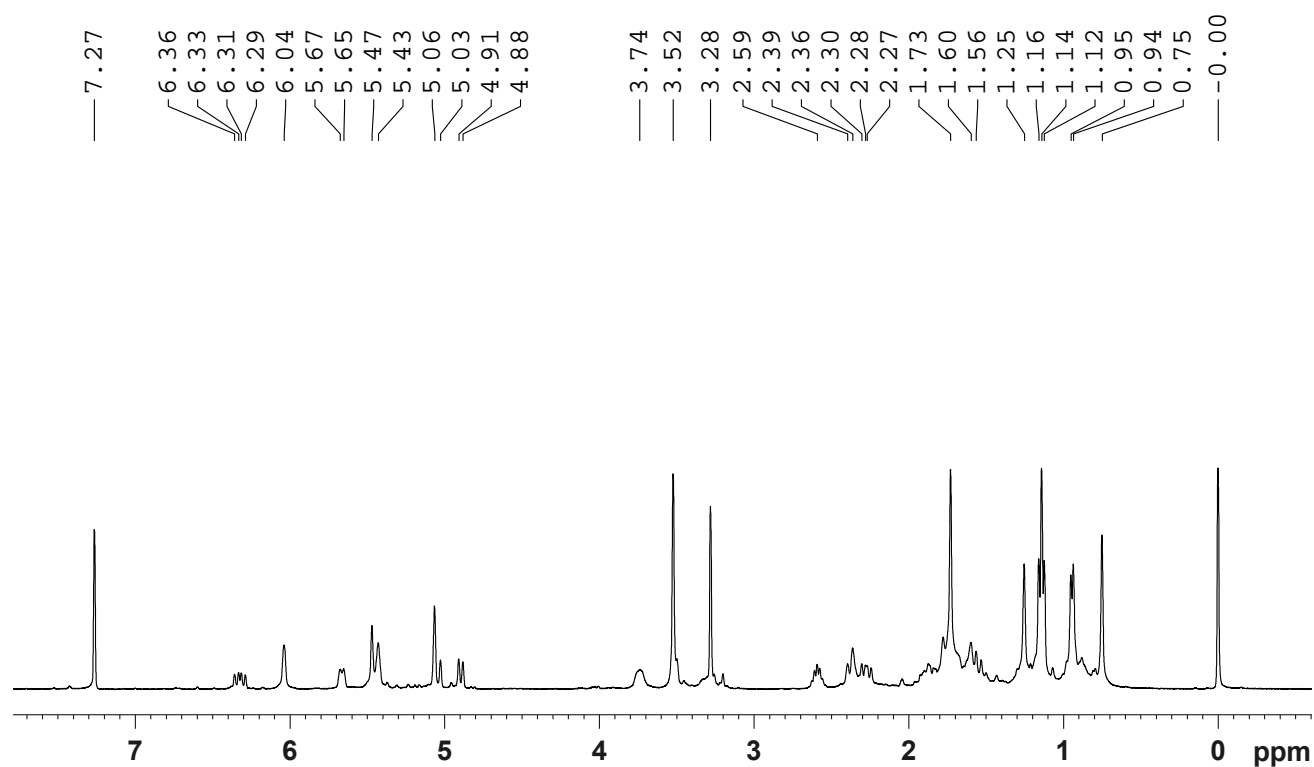


Fig. S28  $^{13}\text{C}$  NMR spectrum for compound **5**

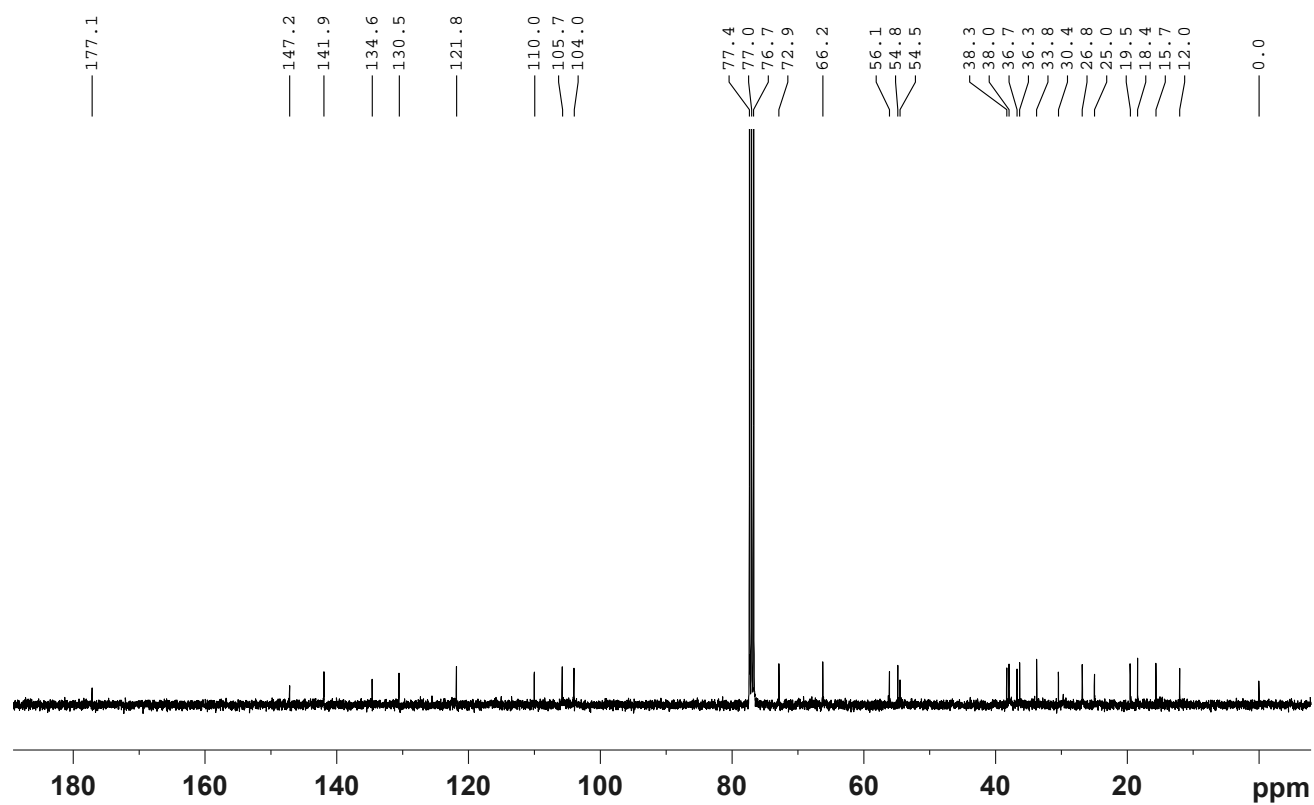


Fig. S29 HMQC spectrum for compound **5**

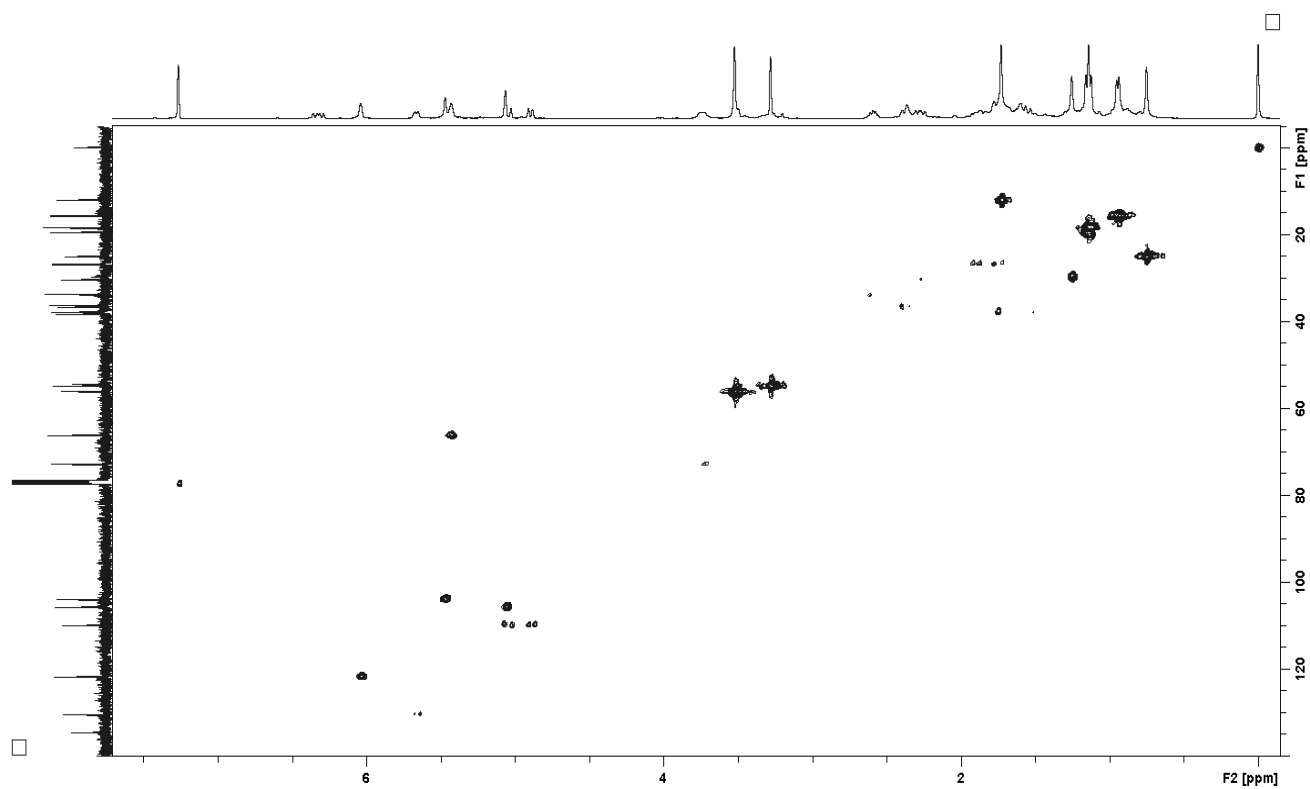


Fig. S30 HMBC spectrum for compound **5**

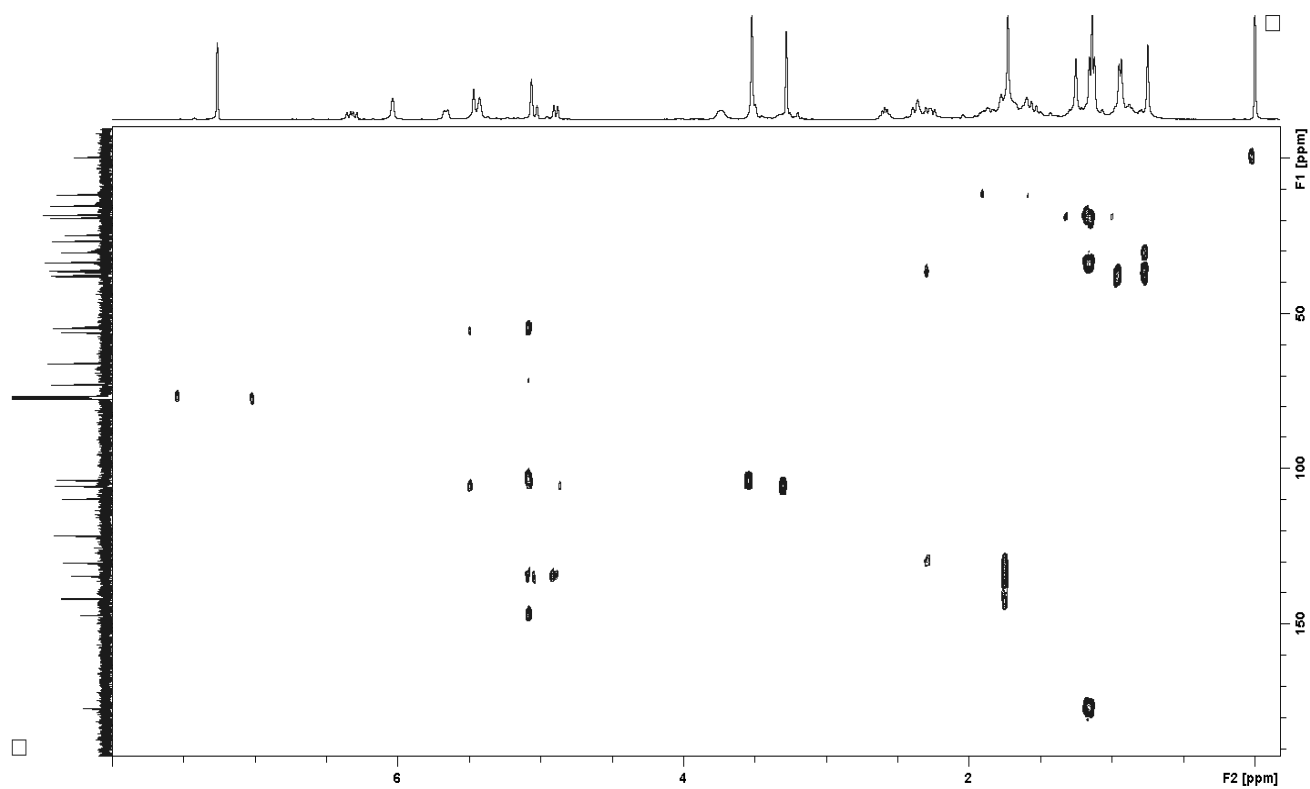


Fig. S31 NOESY spectrum for compound **5**

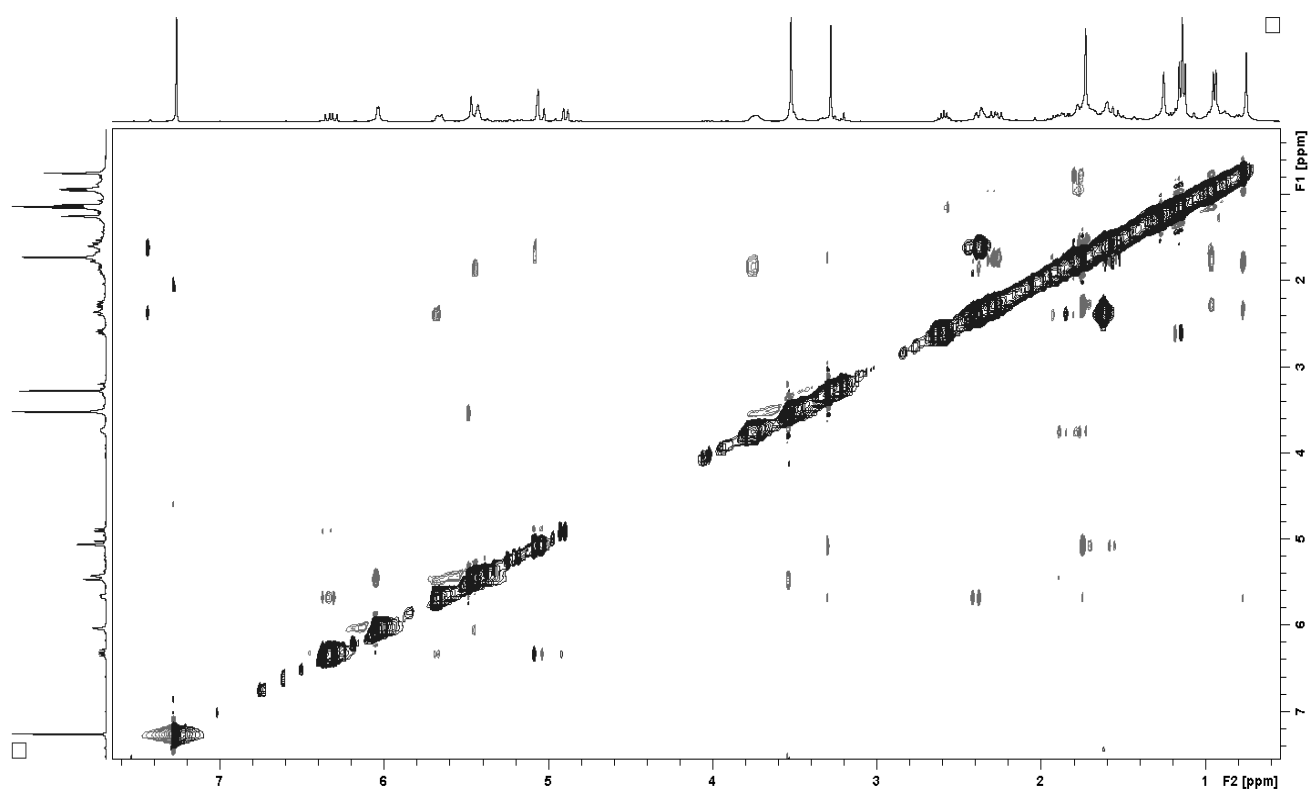


Fig. S32 HRESIMS spectrum for compound **5**

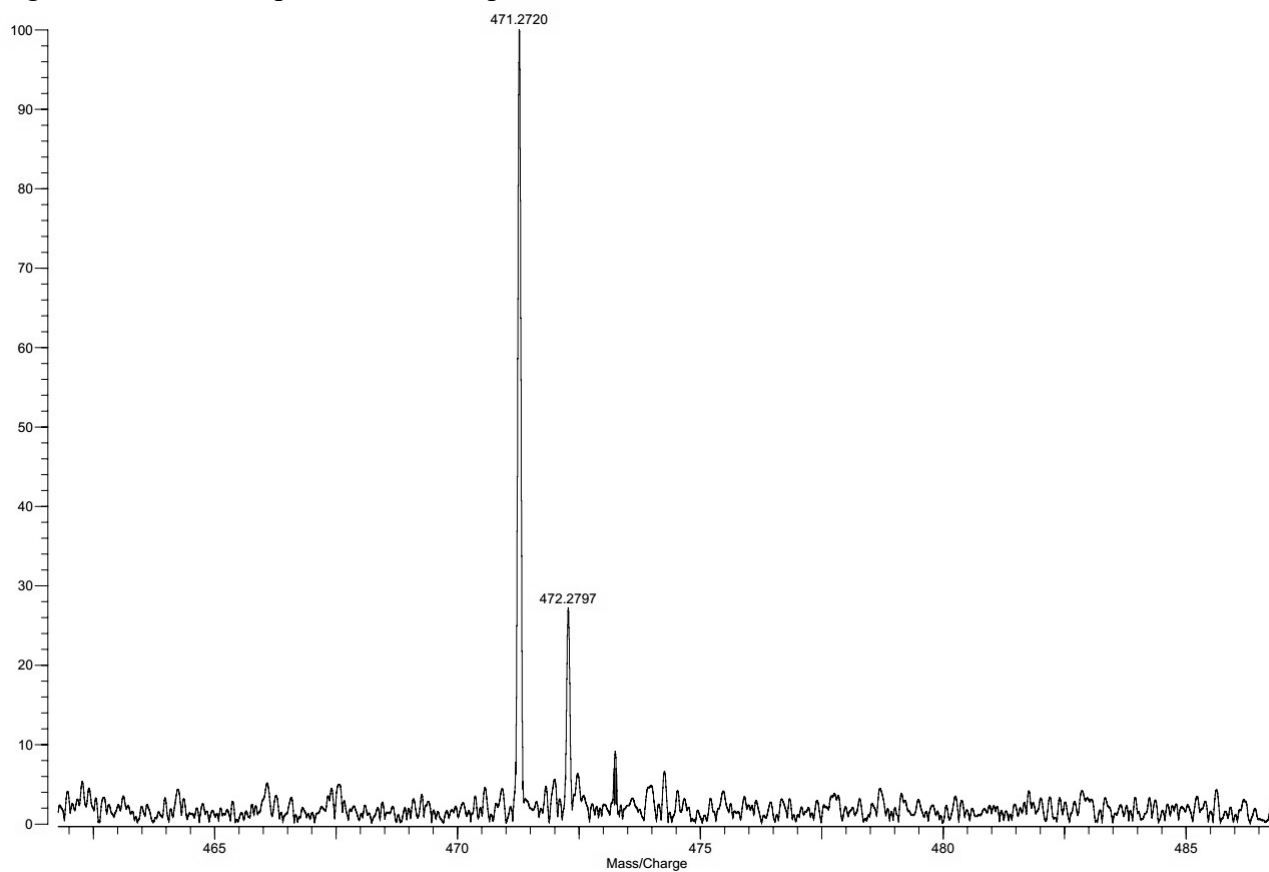


Fig. S33  $^1\text{H}$  NMR spectrum for compound **6**

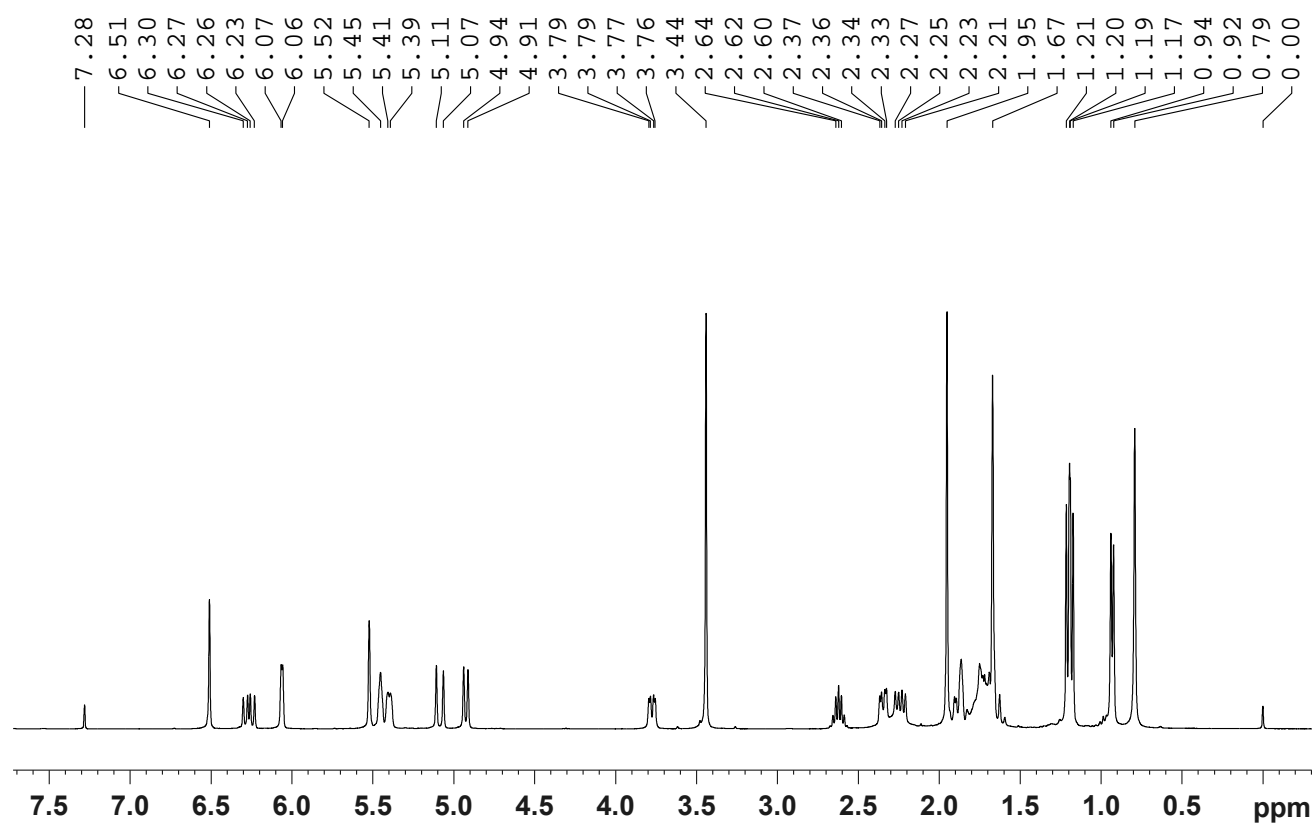


Fig. S34  $^{13}\text{C}$  NMR spectrum for compound **6**

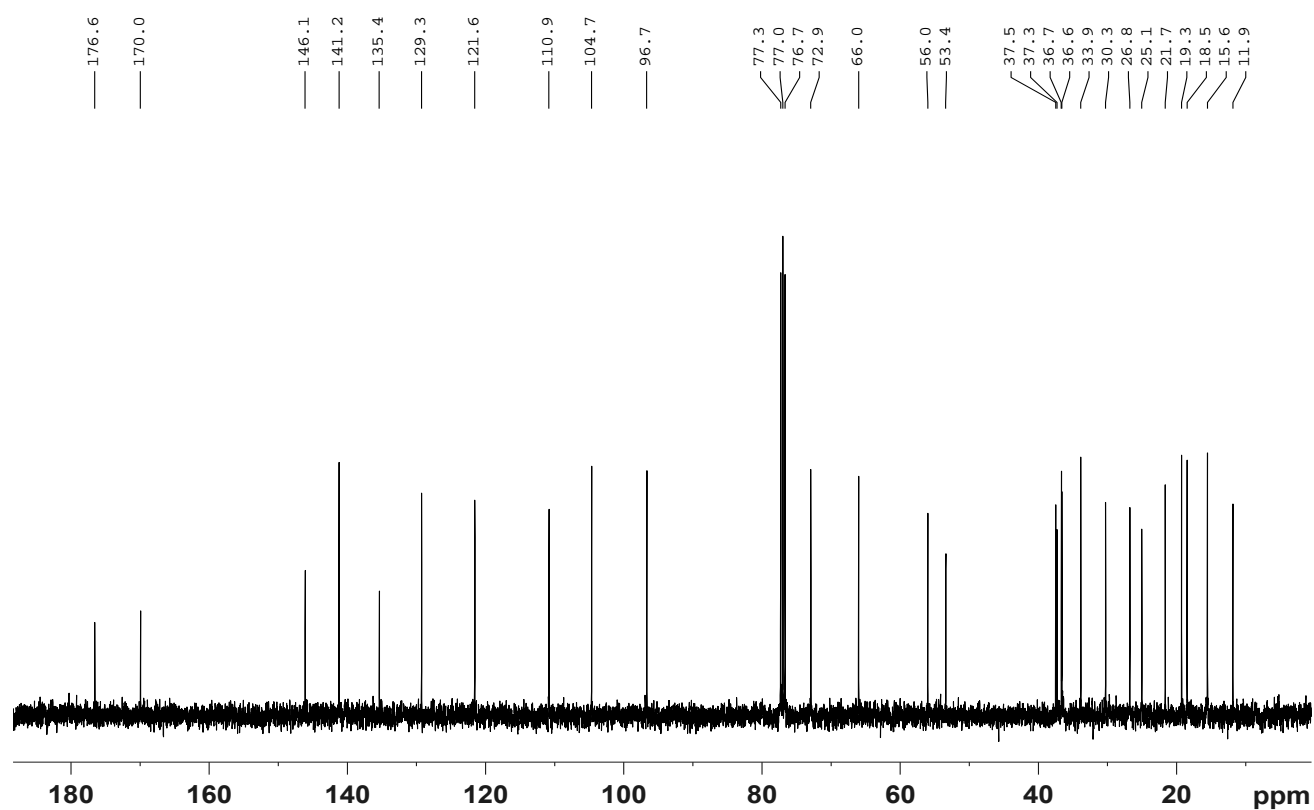




Fig. S35 HMQC spectrum for compound **6**

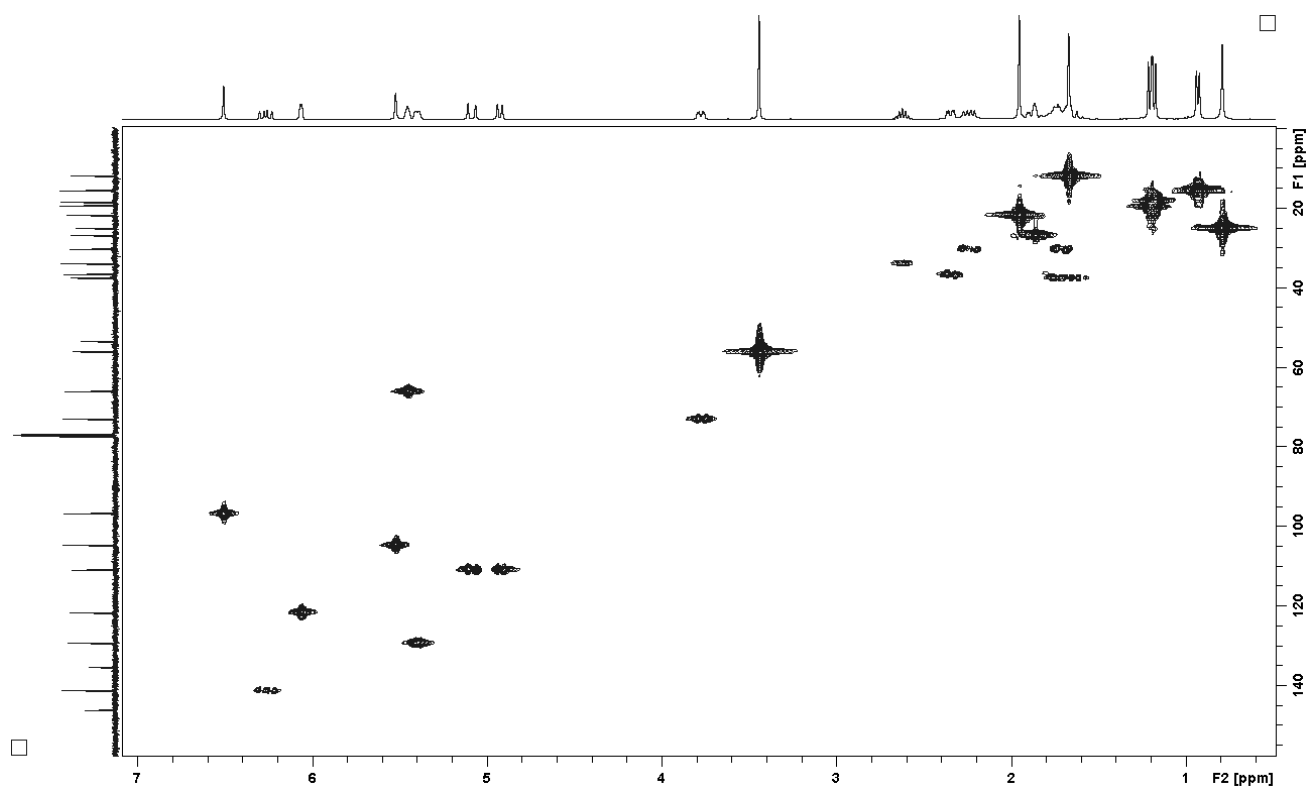


Fig. S36 HMBC spectrum for compound **6**

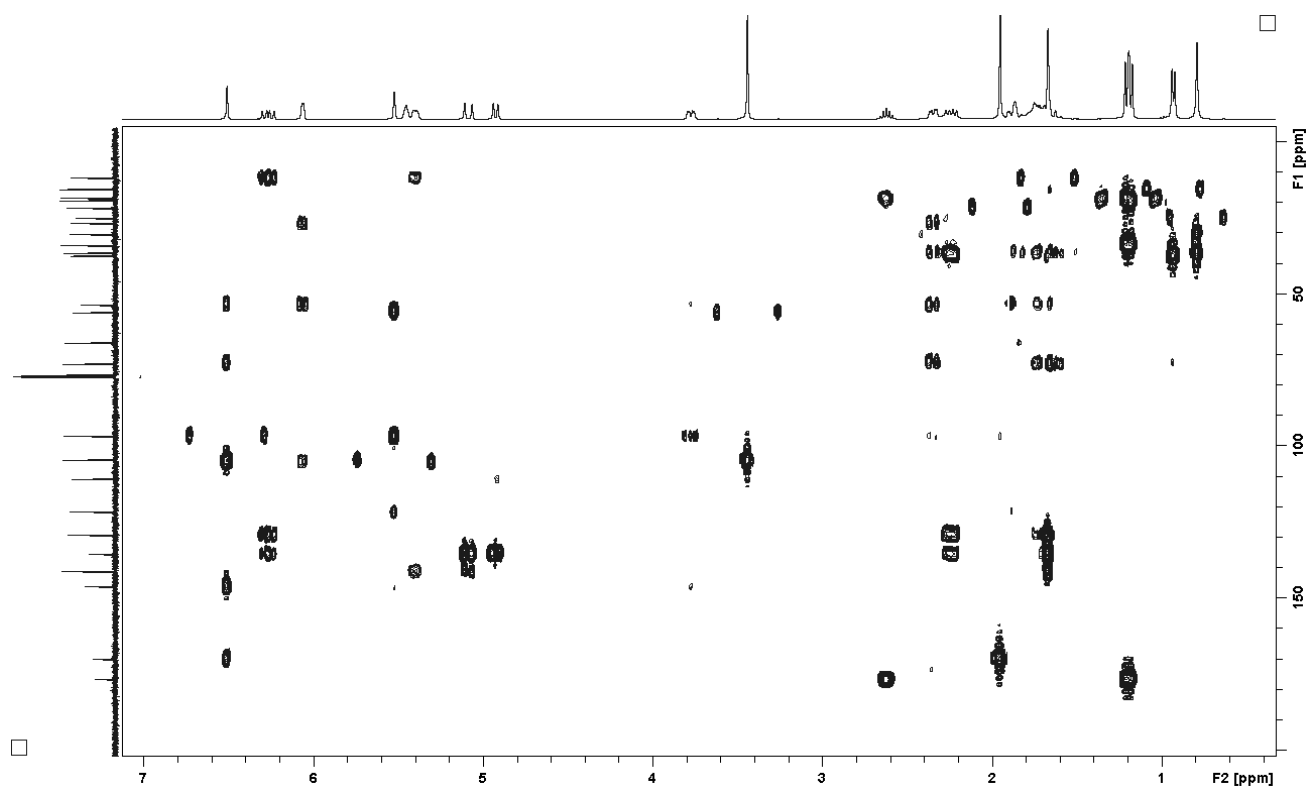


Fig. S37 NOESY spectrum for compound 6

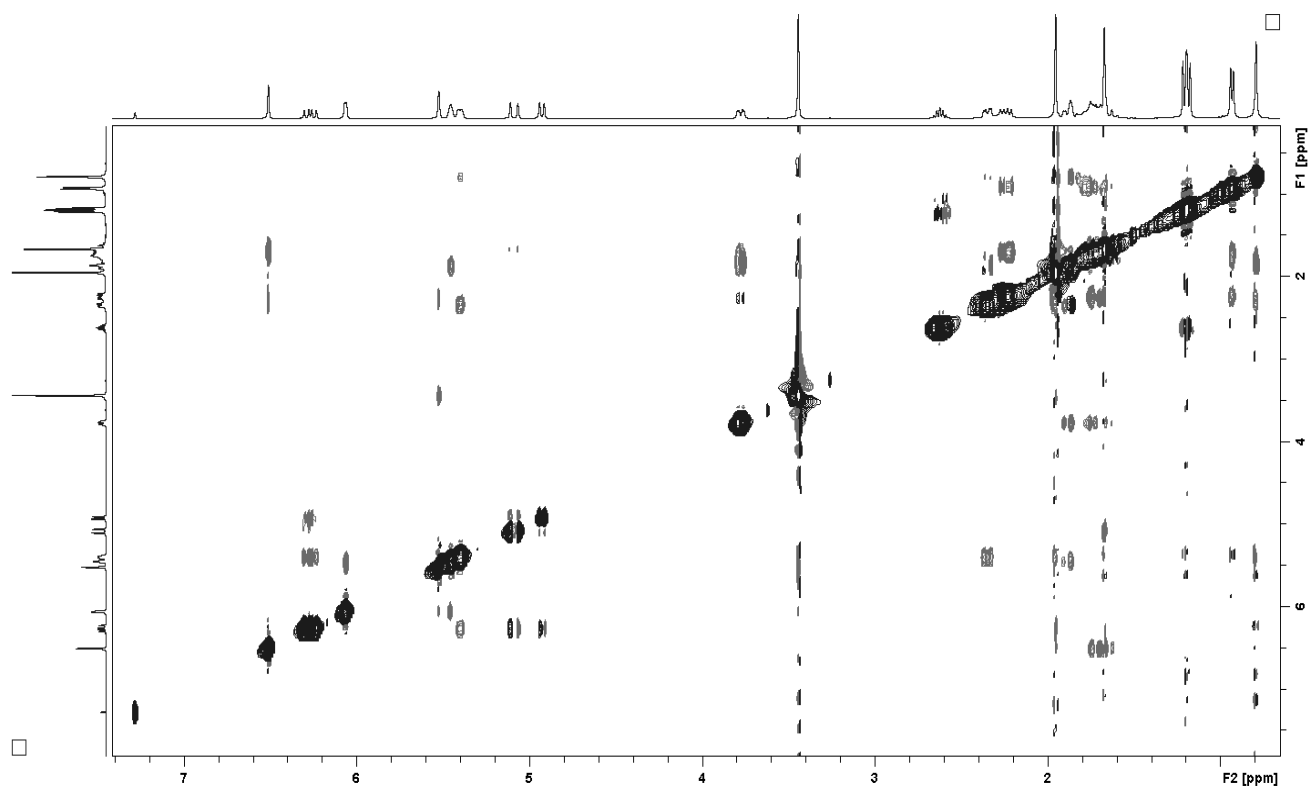


Fig. S38 HRESIMS spectrum for compound 6

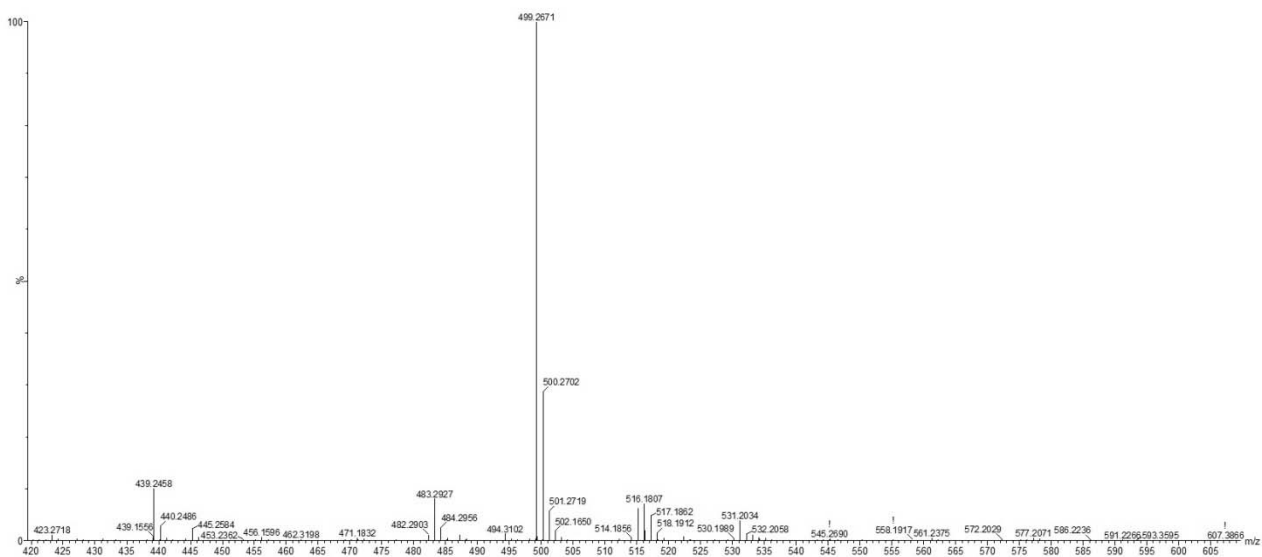


Fig. S39  $^1\text{H}$  NMR spectrum for compound **7**

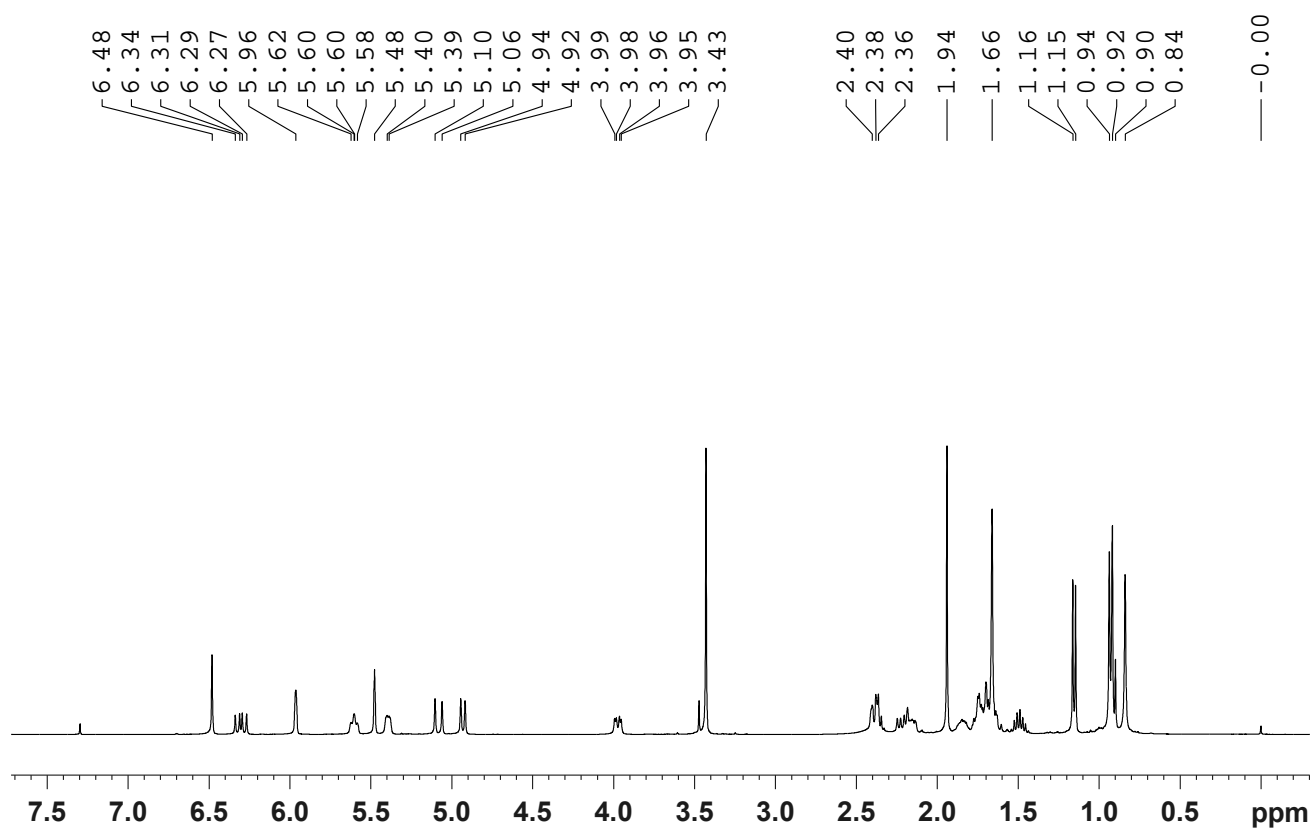


Fig. S40  $^{13}\text{C}$  NMR spectrum for compound **7**

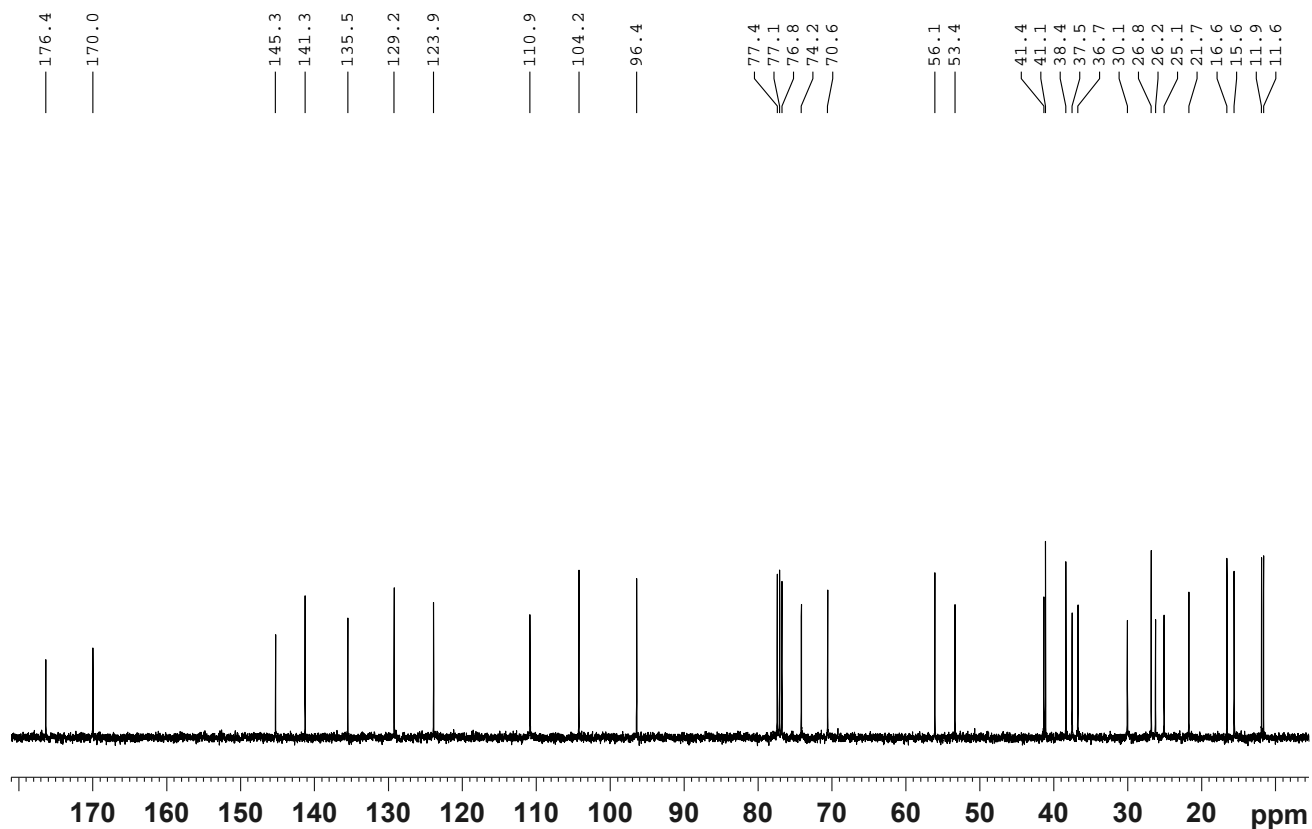


Fig. S41 HMQC spectrum for compound 7

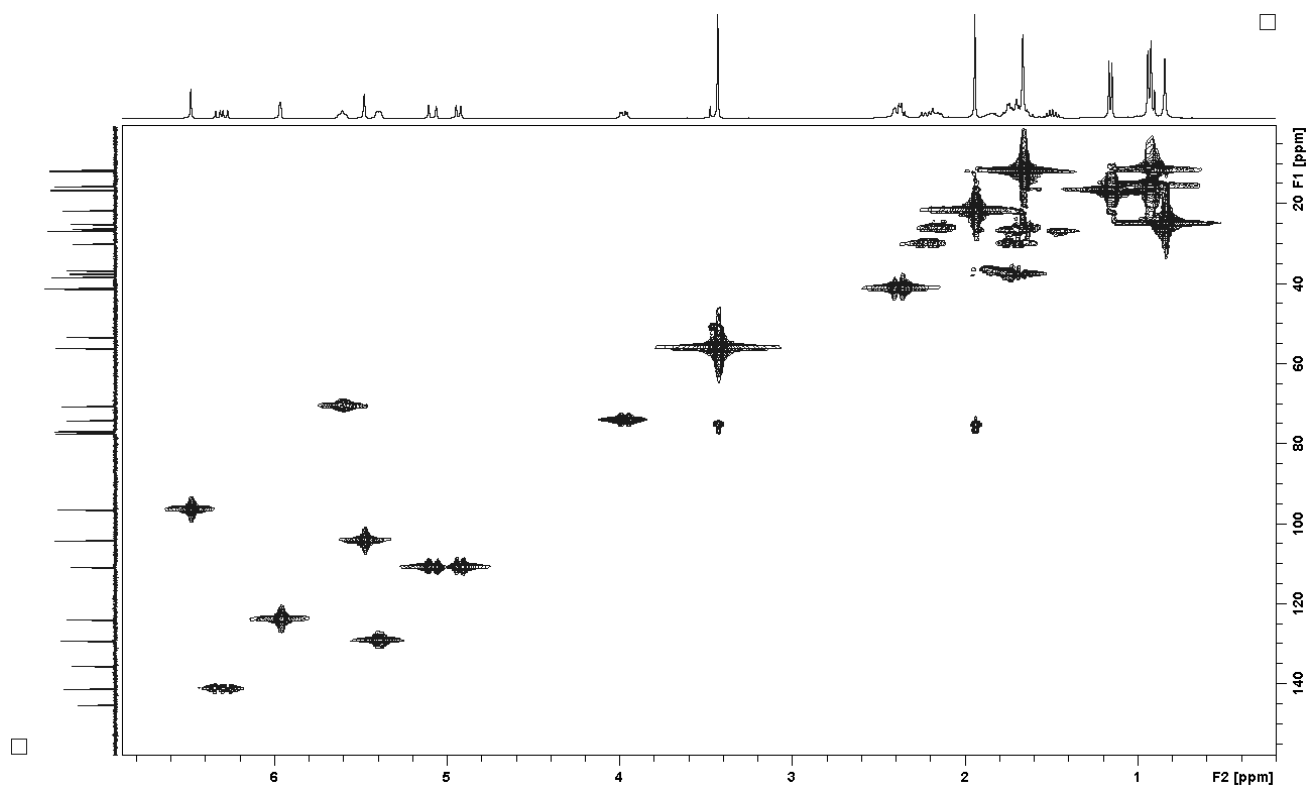


Fig. S42 HMBC spectrum for compound 7

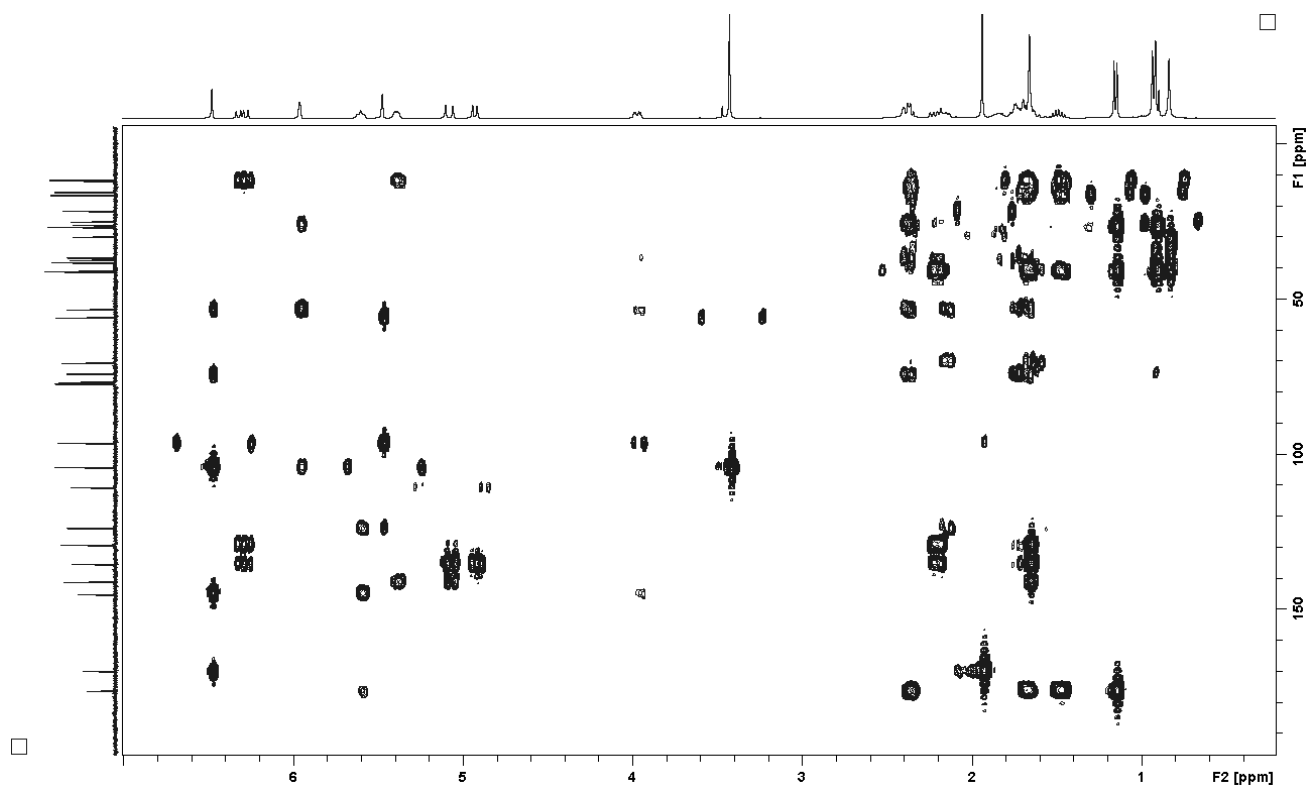


Fig. S43 NOESY spectrum for compound 7

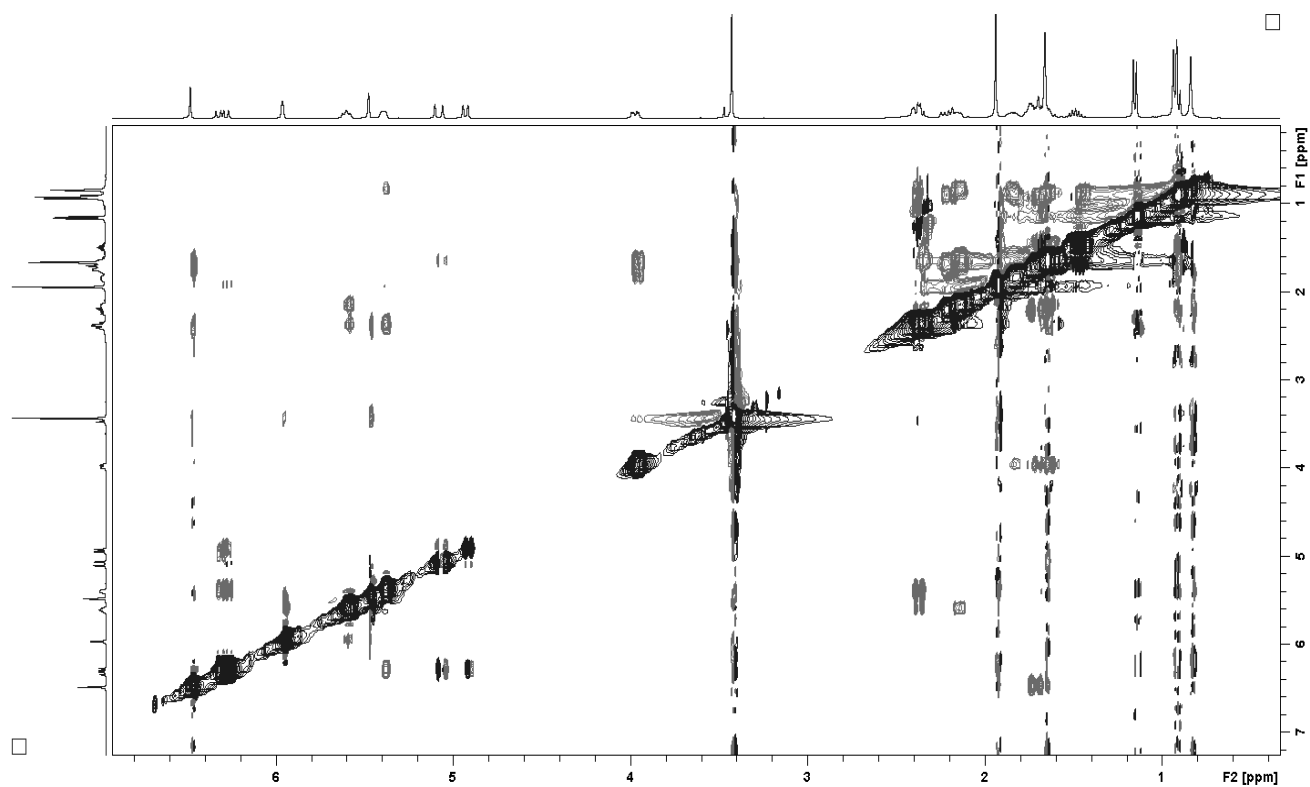


Fig. S44 HRESIMS spectrum for compound 7

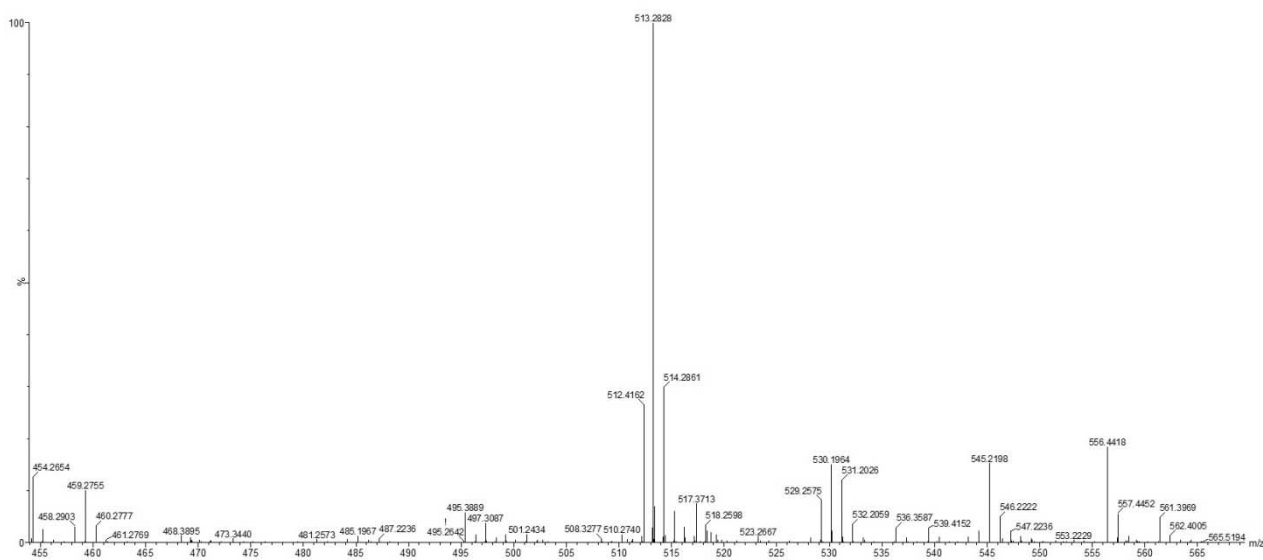


Fig. S45  $^1\text{H}$  NMR spectrum for compound **8**

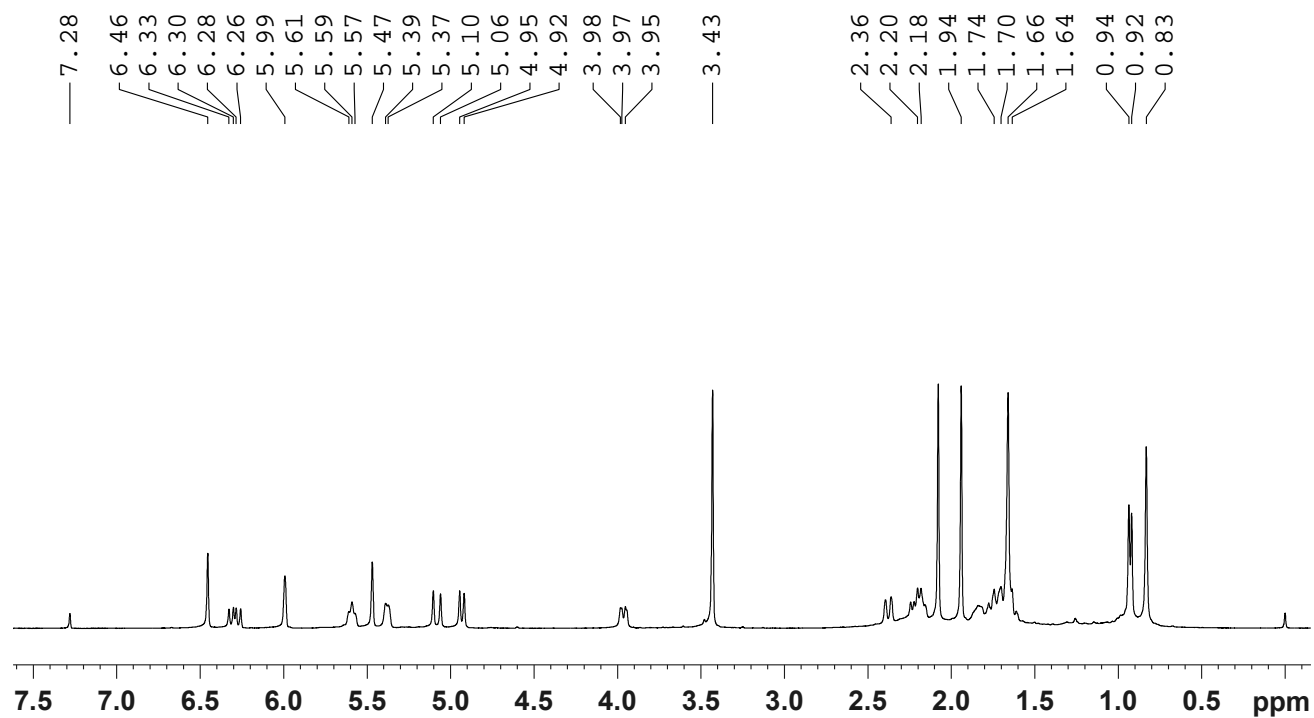


Fig. S46  $^{13}\text{C}$  NMR spectrum for compound **8**

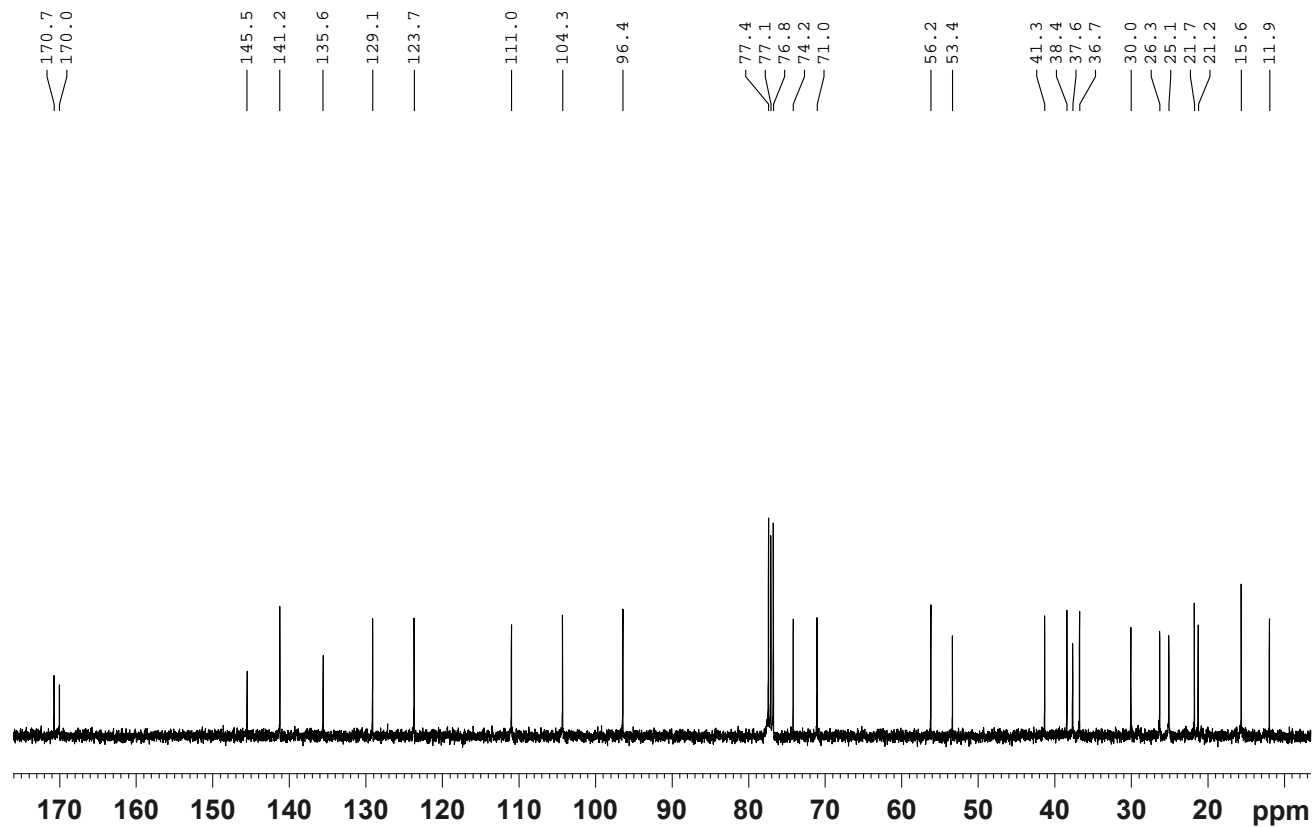


Fig. S47 HMQC spectrum for compound **8**

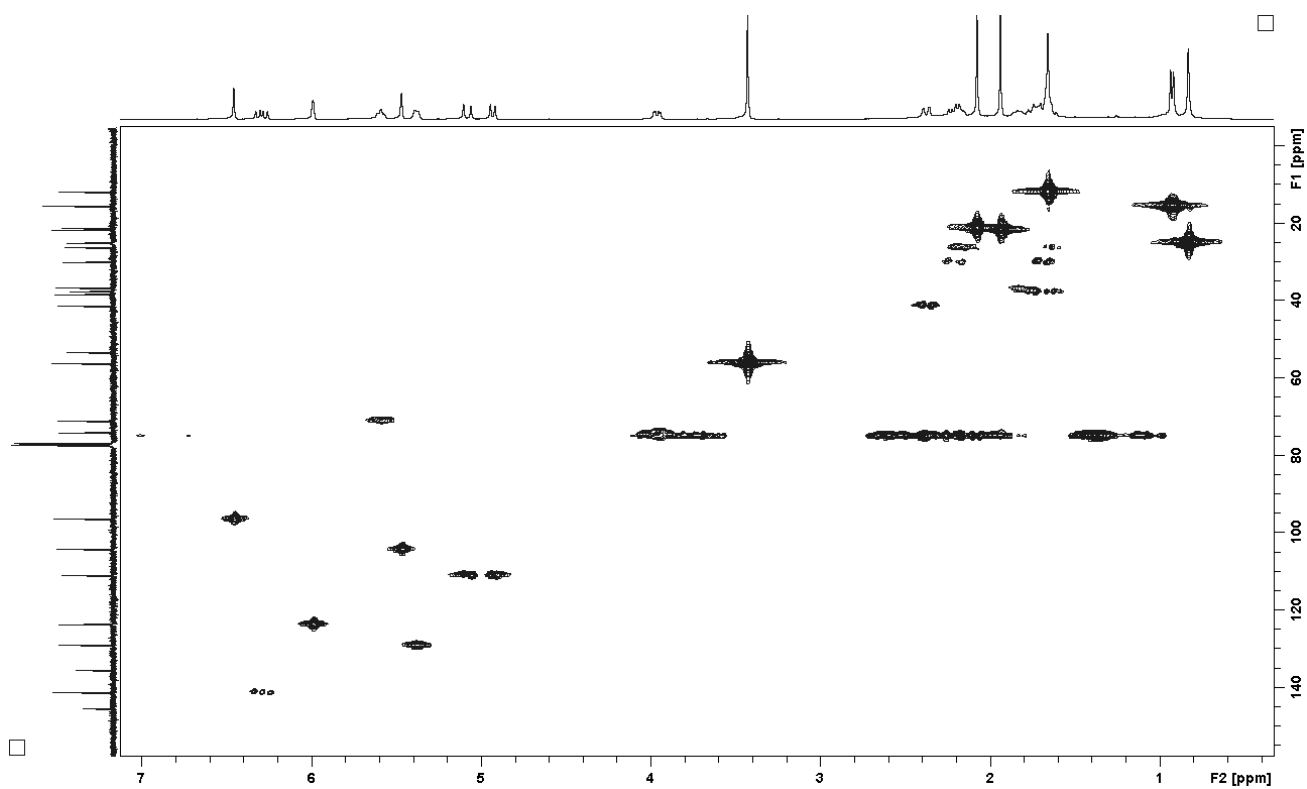


Fig. S48 HMBC spectrum for compound **8**

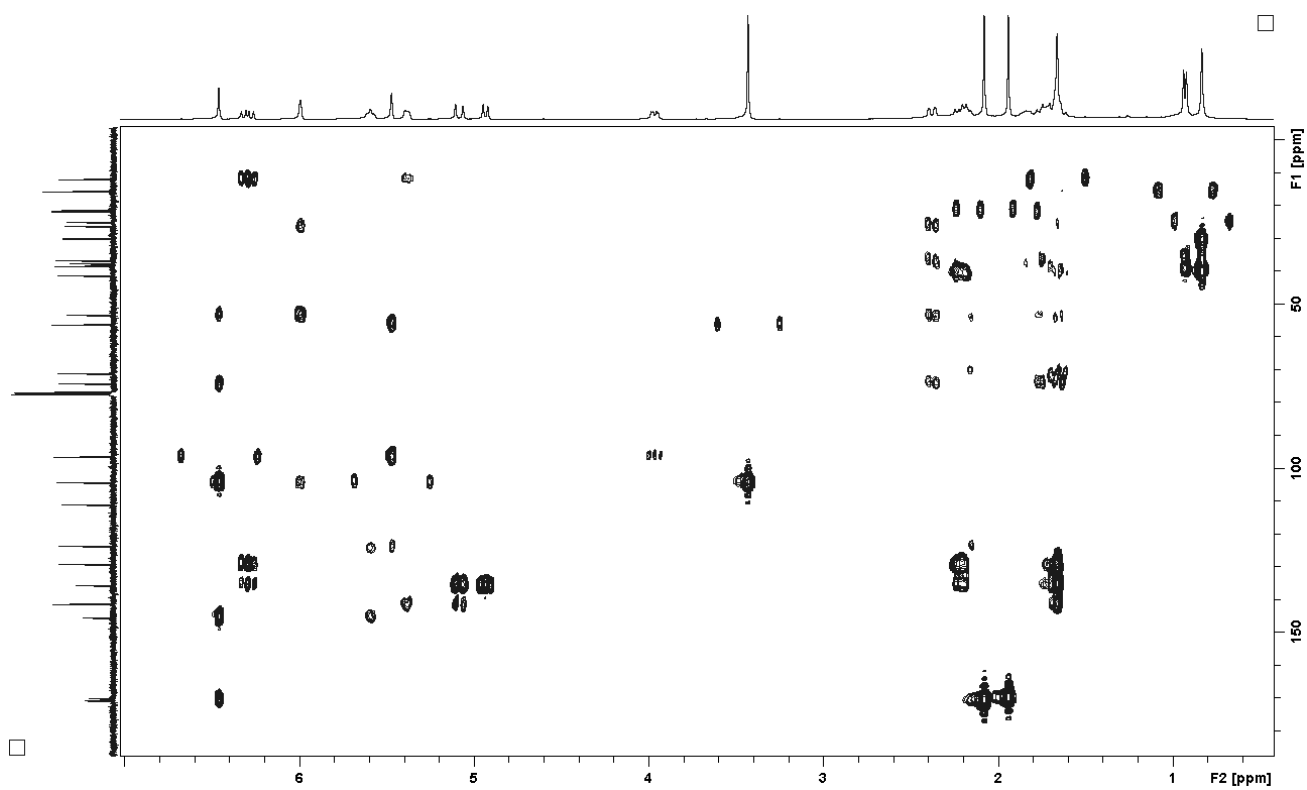


Fig. S49 NOESY spectrum for compound **8**

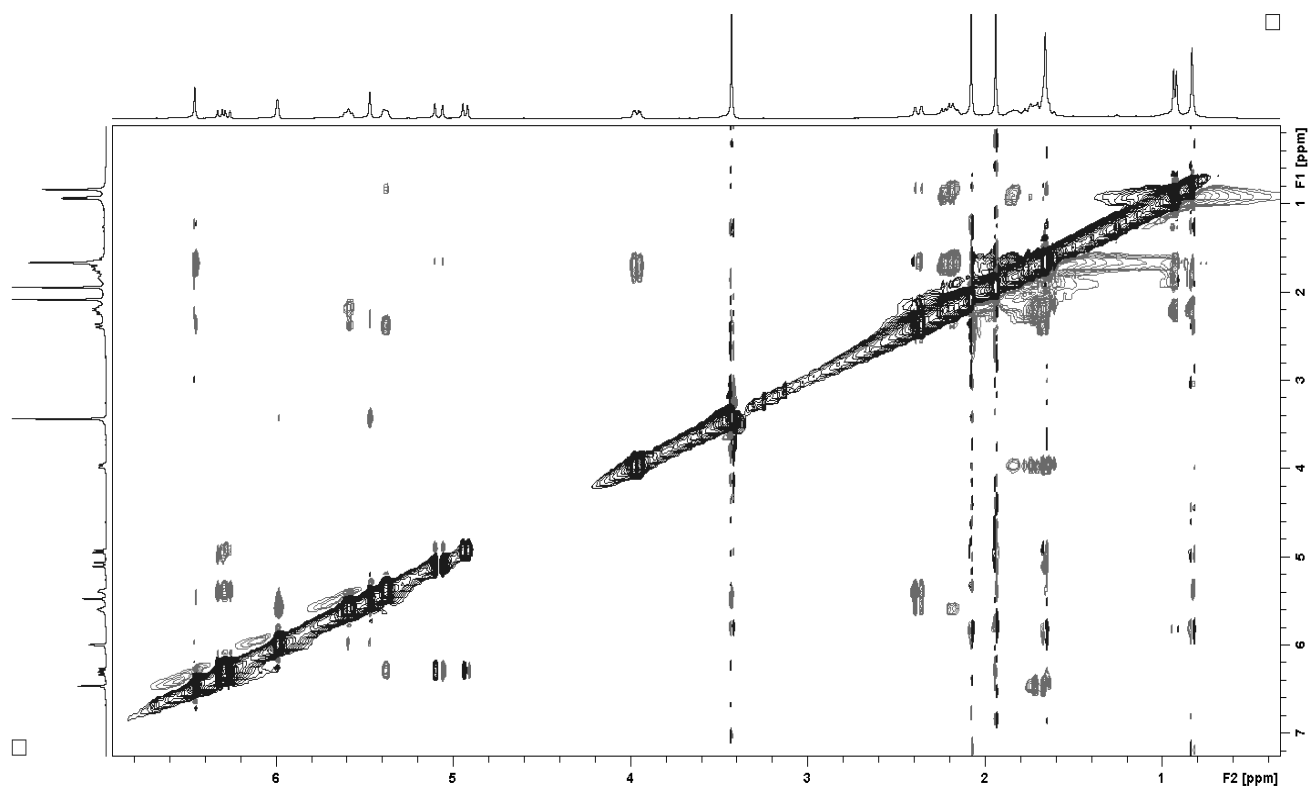


Fig. S50 HRESIMS spectrum for compound **8**

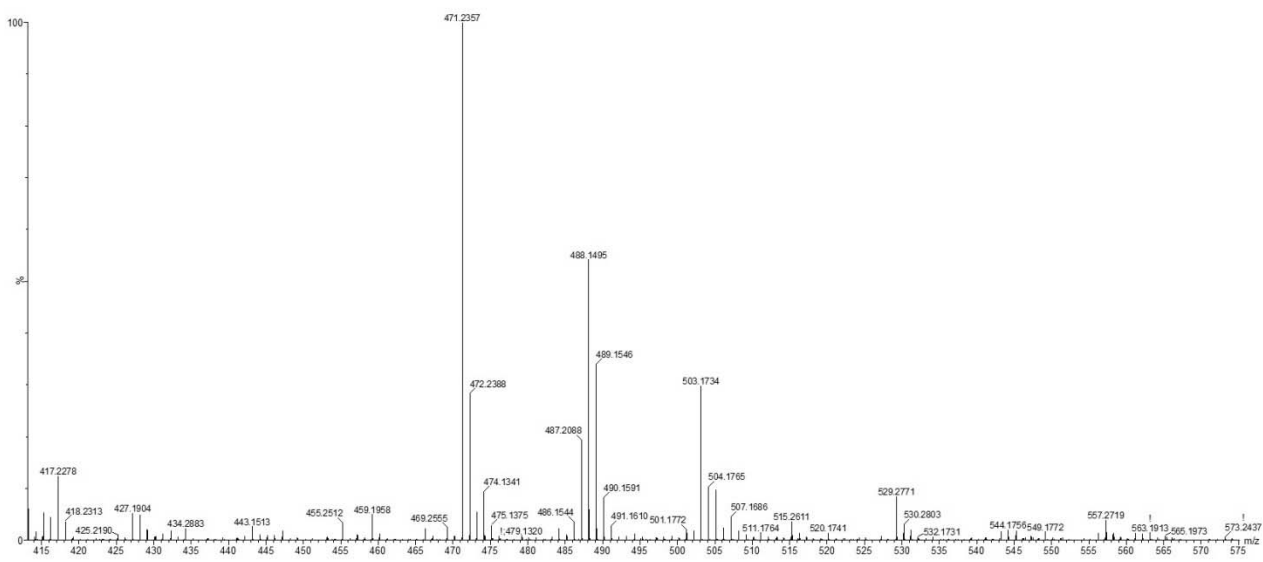




Fig. S51  $^1\text{H}$  NMR spectrum for compound **9**

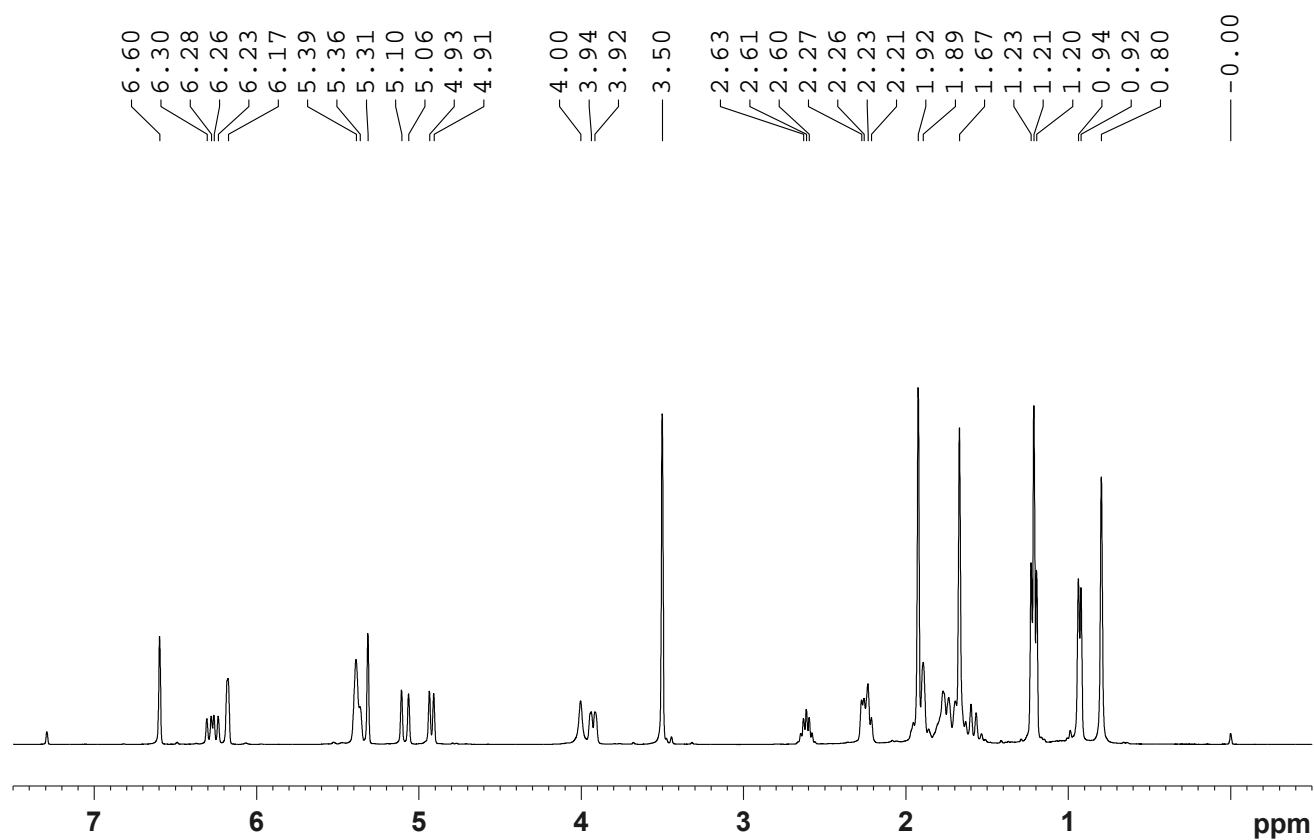


Fig. S52  $^{13}\text{C}$  NMR spectrum for compound **9**

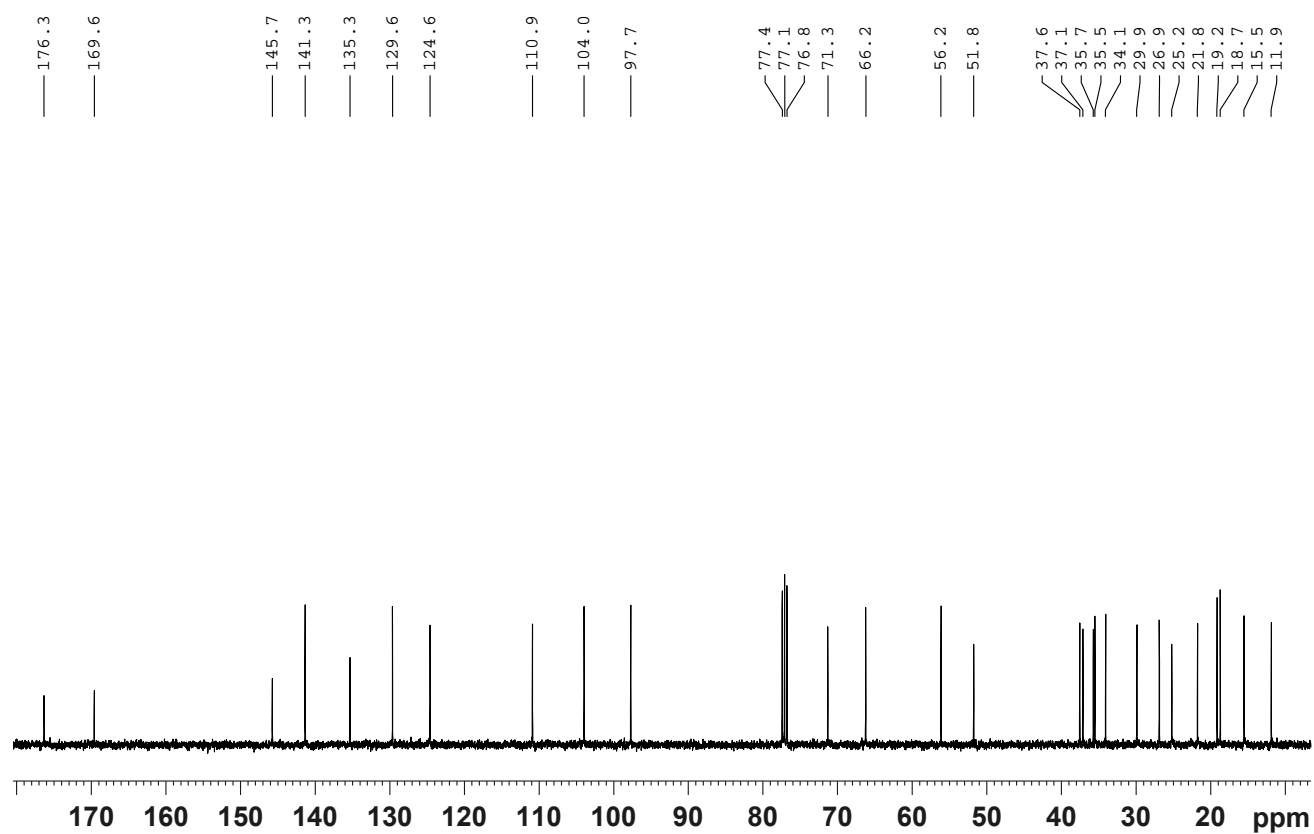


Fig. S53 HMQC spectrum for compound **9**

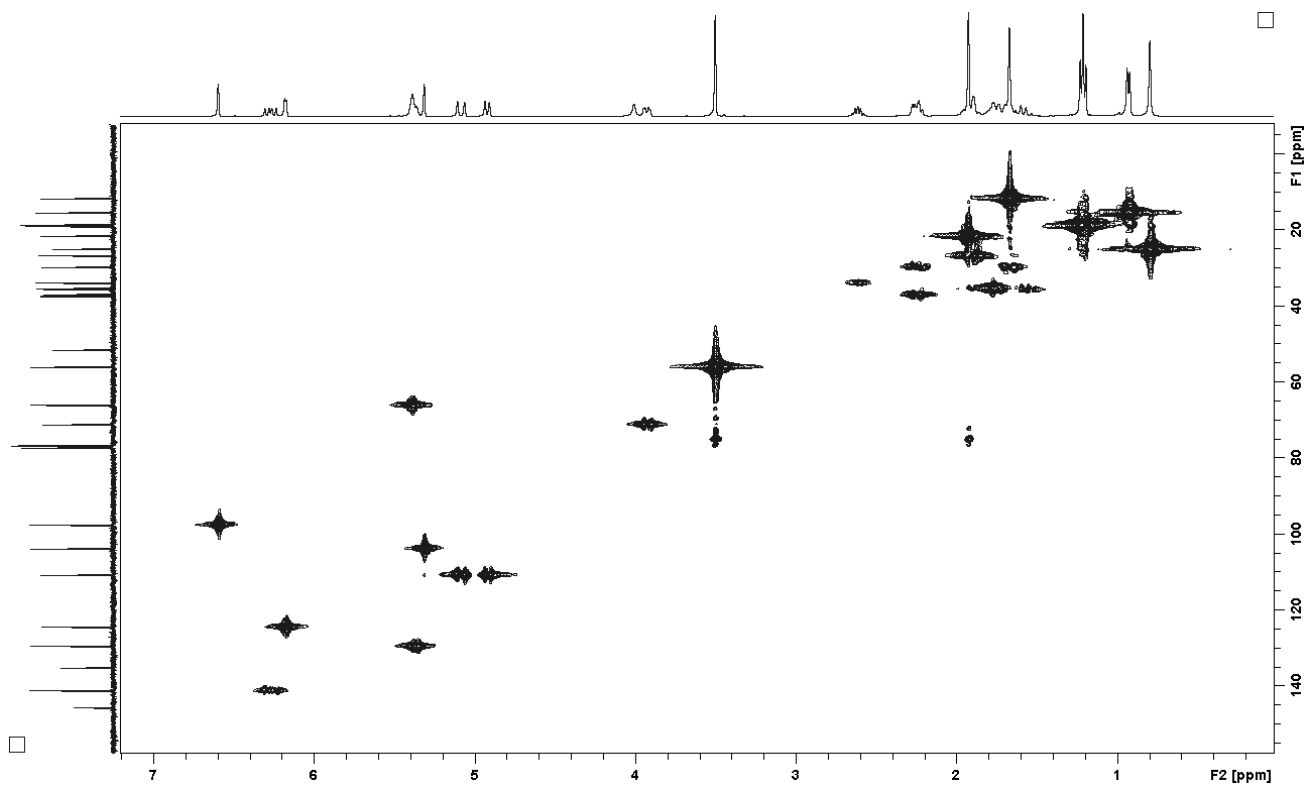


Fig. S54 HMBC spectrum for compound **9**

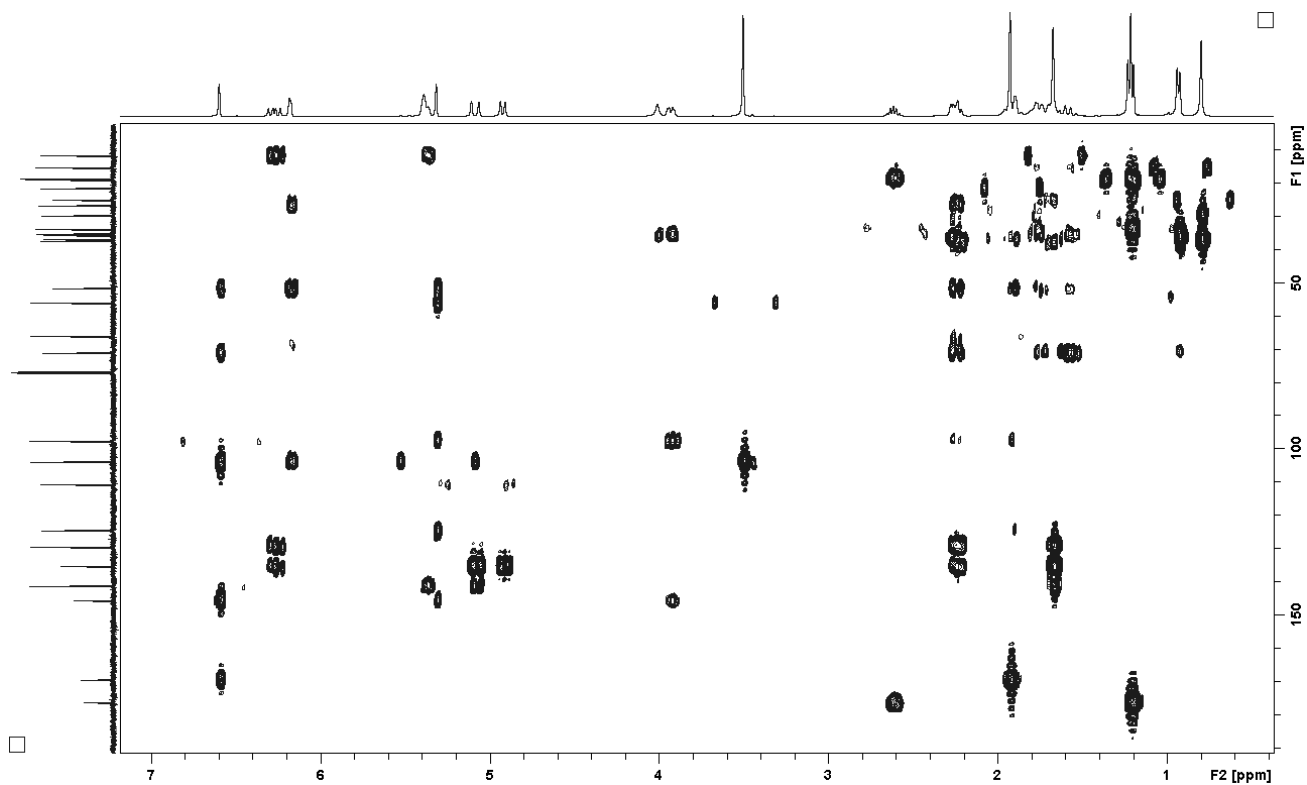


Fig. S55 NOESY spectrum for compound **9**

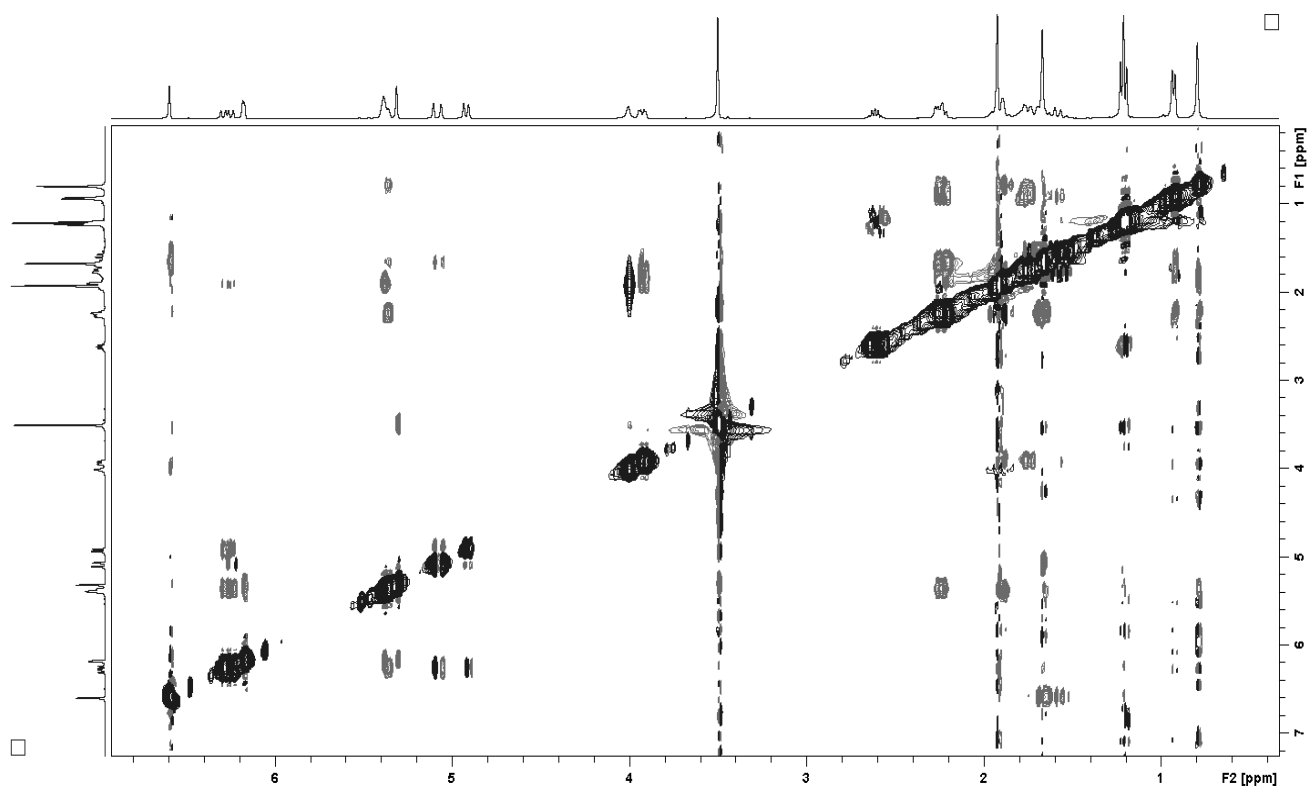


Fig. S56 HRESIMS spectrum for compound **9**

