

Supporting Information

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A New Labdane Diterpene from the Aerial Parts of *Chloranthus serratus*

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S1: Bioactivity Assays

Cell Culture: The RAW 264.7 mouse macrophage cell line was purchased from the Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China), and was cultured in Dulbecco's modified Eagle medium (DMEM, Gibco Invitrogen Corp., Carlsbad, CA, USA) which was supplemented with 10% FBS, 100 Units/mL penicillin and 100 µg/mL streptomycin. The cells were placed at 37 °C in a humidified incubator containing 5% CO₂. The inhibitory activity of the isolated compounds toward NO production was determined using the Griess reagent system (Beyotime, Beijing, China). Cells were cultured in each well of 96-well plates in the density of 5×10^4 cells/well with 100 µL DMEM for 24 h. Then test samples which dissolved in DMSO and diluted with DMEM were added as well as LPS 2 µg/mL as stimuli. The final drug concentration was 50 (25 or 12.5) µM with 1% DMSO. Wells treated with only LPS were served as model control and wells treated with neither LPS nor test samples were served as blank control (all contained 1% DMSO). After 24 h incubation, half of the medium (50 µL) in each well was harvested.

MTT Assay: The cytotoxicity of the isolated compounds toward RAW 264.7 cells was determined by MTT assay. RAW264.7 cells were planted in 96-well plates (5×10^3 /well) for 24 h. Then they were treated with test samples which dissolved in DMSO and diluted in 100 µL DMEM making the final drug concentration 50 (25 or 12.5) µM and 1% DMSO. 1% DMSO served as solvent control. Wells without cells contain only 100 µL DMEM were served as blank control. 24 h later, 20 µL solution MTT was added in each well. After incubation for 4 h, the medium was removed and 100 µL DMSO was added in each well, then the absorbance (A) was detected at 490 nm using a microplate reader. The inhibition of cell growth was calculated according to the following formula: % Inhibition = $[1 - (A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{solvent}} - A_{\text{blank}})] \times 100$.

Inhibitory activity toward NO: Nitric oxide release was assessed by a colorimetric assay based on a diazotization reaction using the Griess reagent system. After 50 µL Griess reagent I and 50 µL Griess reagent II were added in each well, the absorbance (A) was measured at 540 nm using a microplate reader. The inhibition of NO release was calculated according to the following formula: % Inhibition = $[1 - (A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{model}} - A_{\text{blank}})] \times 100$

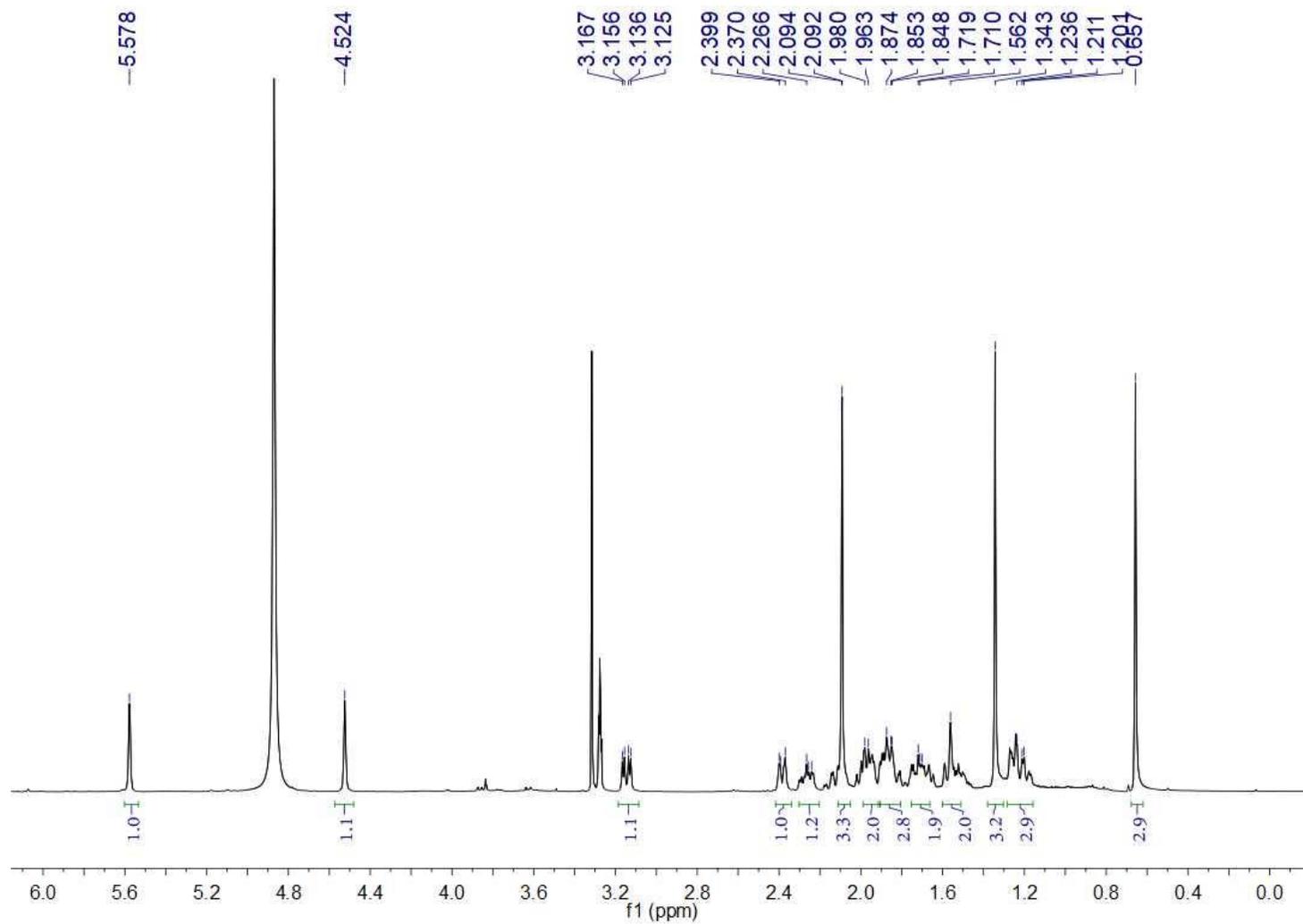


Figure S1: ^1H NMR Spectrum of **1** in CD_3OD (400 MHz)

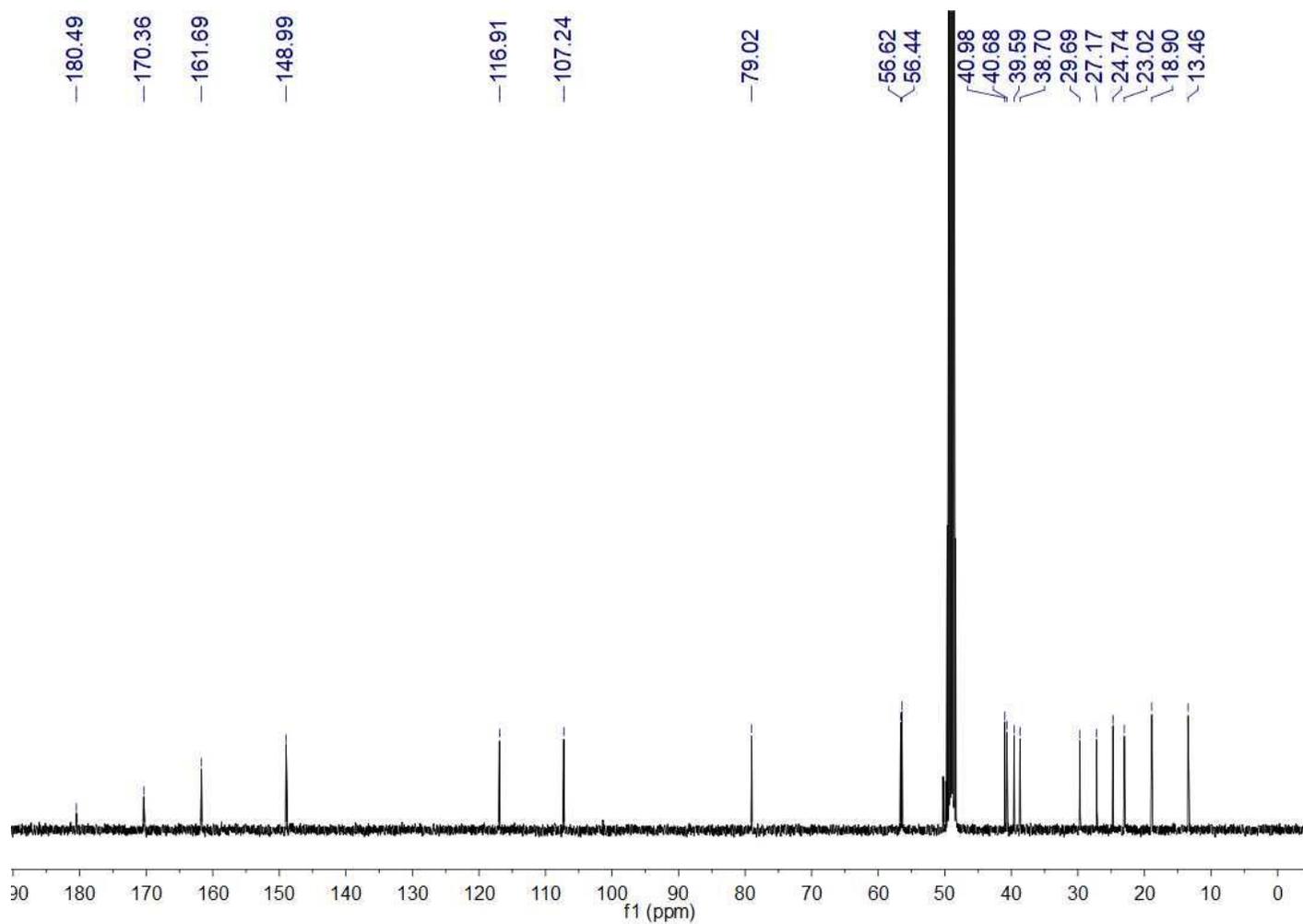


Figure S2: ^{13}C NMR Spectrum of **1** in CD_3OD (100 MHz)

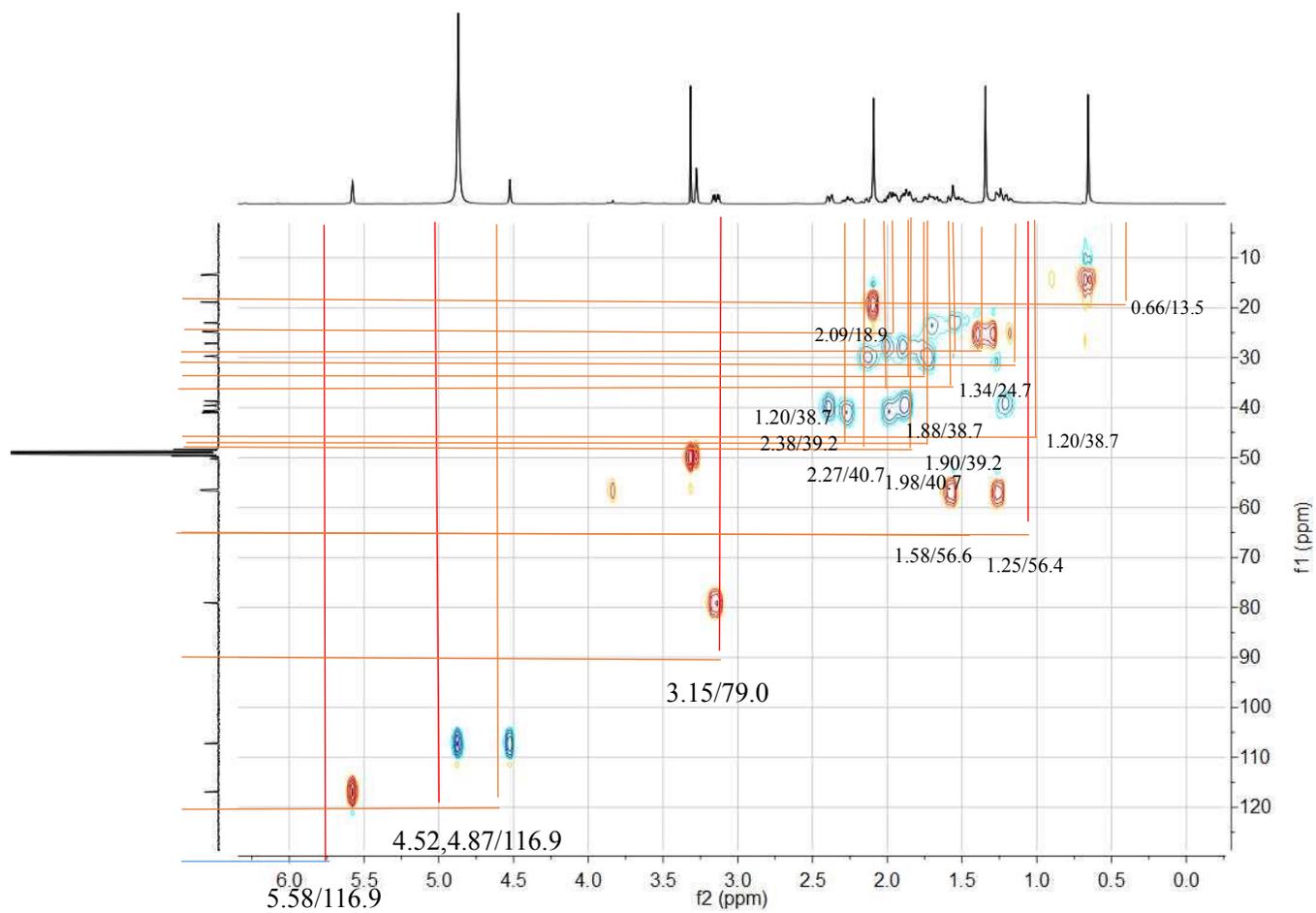


Figure S3: HSQC Spectrum of **1** in CD₃OD

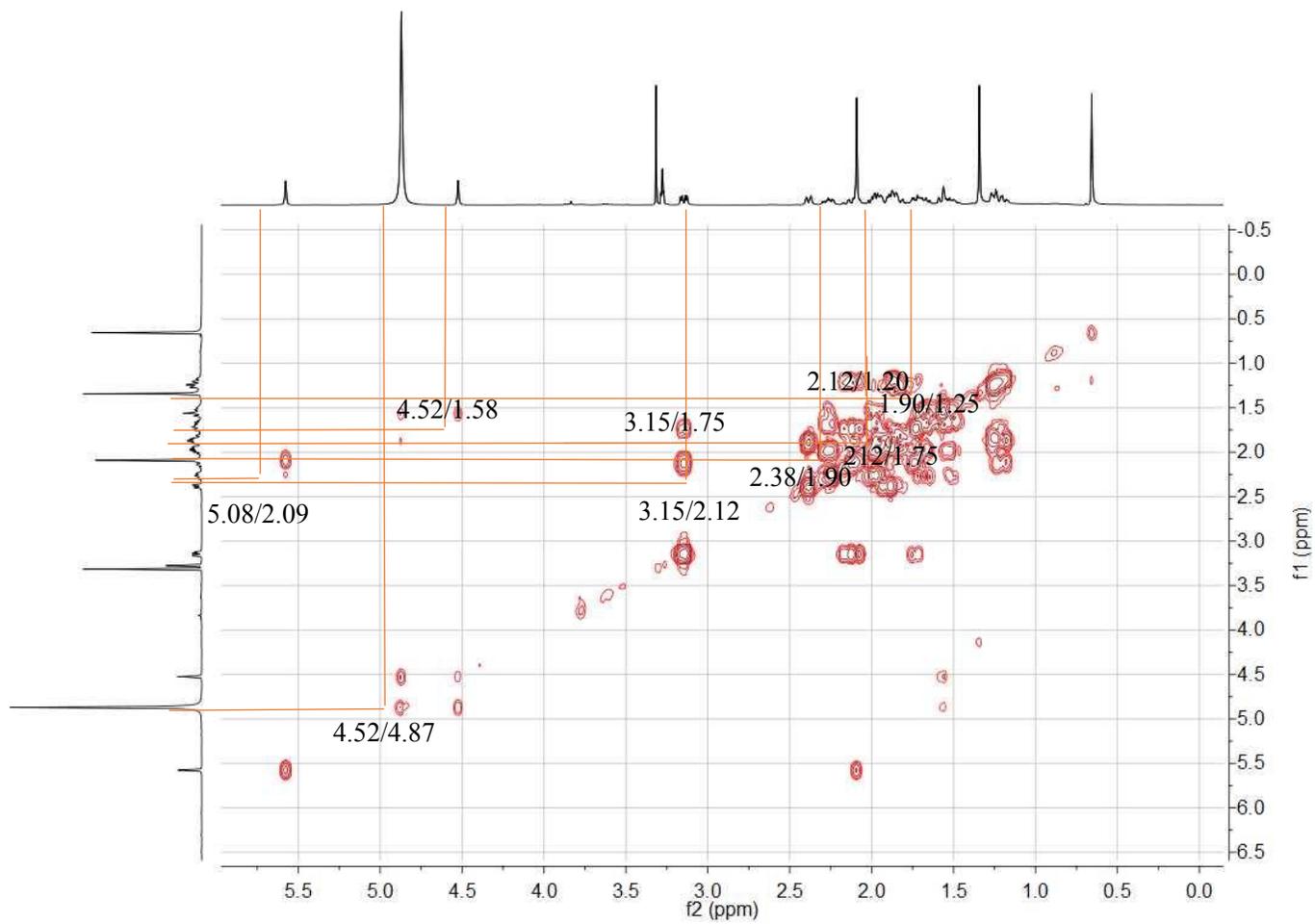


Figure S4: ^1H - ^1H COSY Spectrum of **1** in CD_3OD

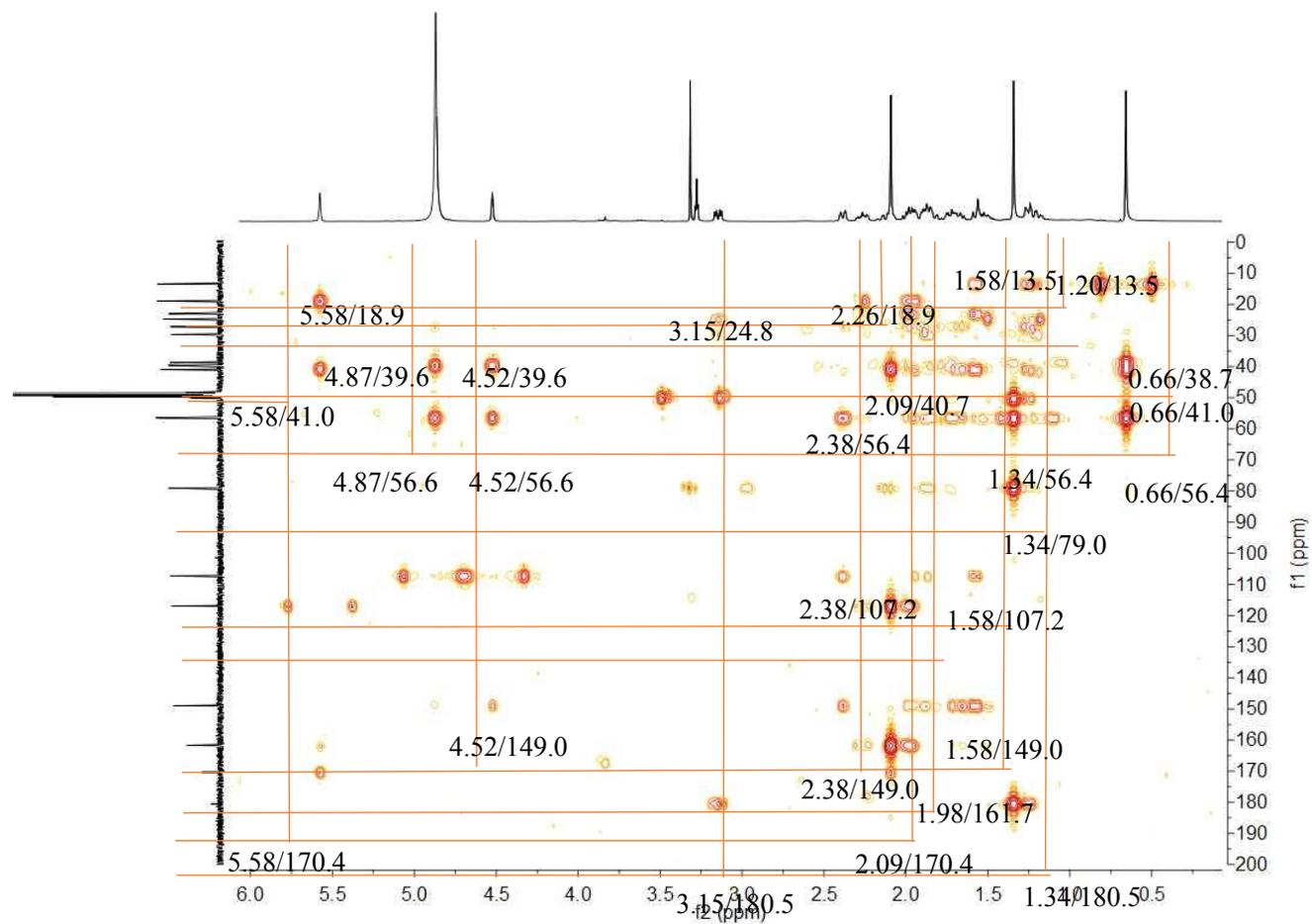


Figure S5: HMBC Spectrum of **1** in CD₃OD

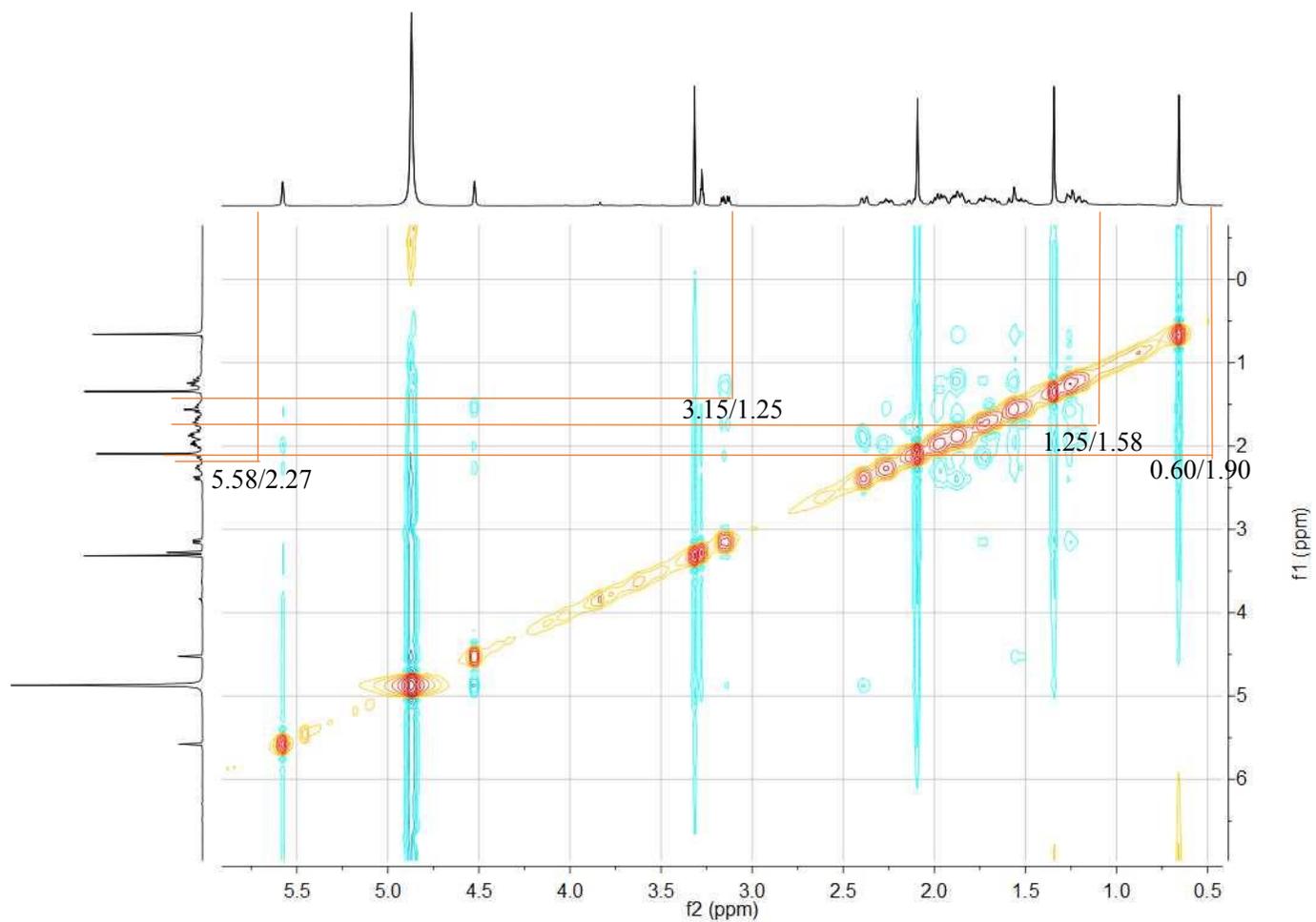


Figure S6: NOESY Spectrum of **1** in CD₃OD

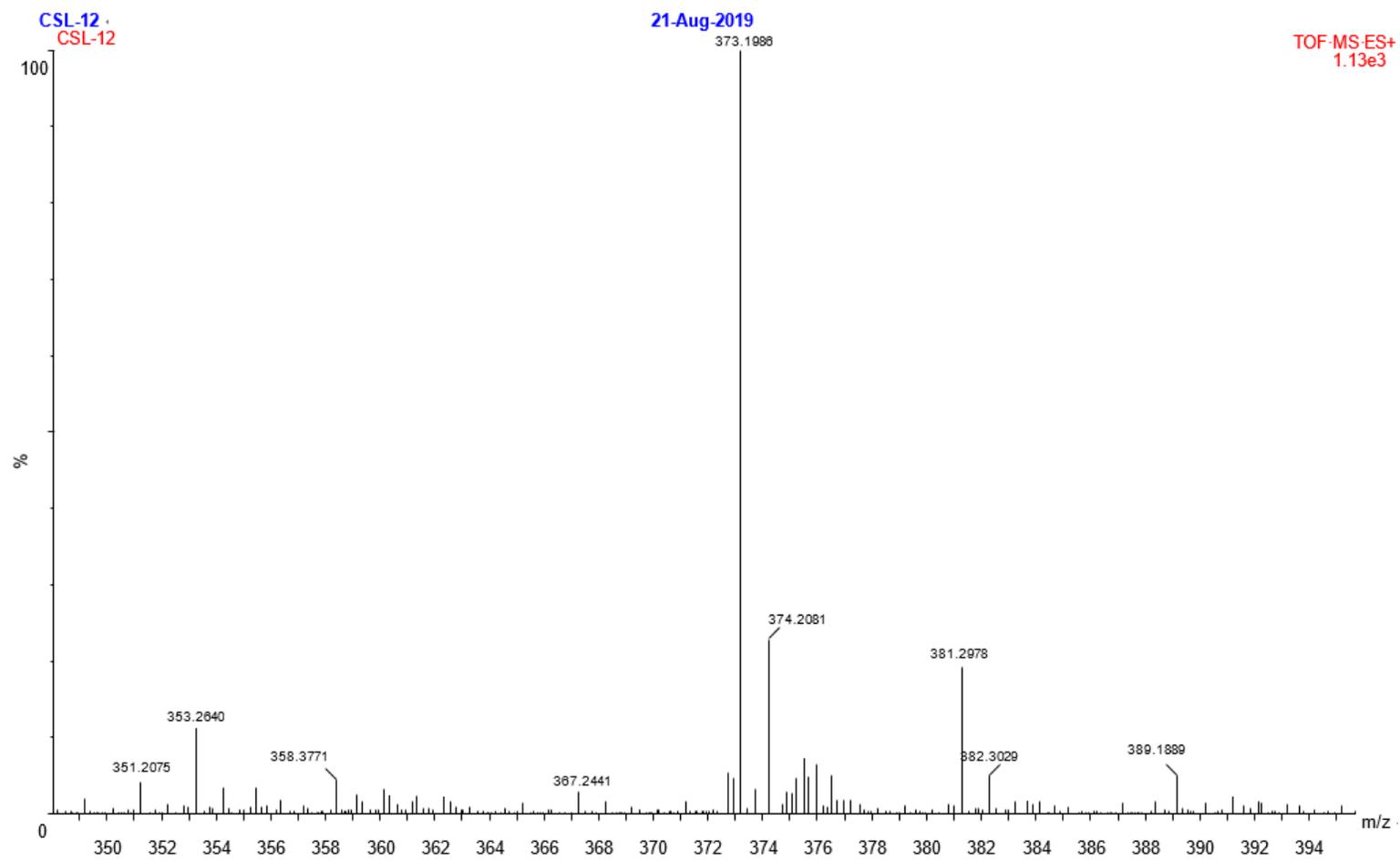


Figure S7: HRESIMS spectrum of **1**

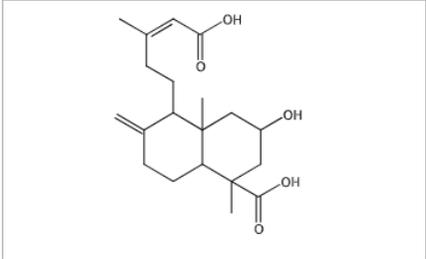
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0 of 8 Similarity Candidates Selected	
<input type="checkbox"/> ≥ 99 (most similar)	Substances: 0
<input type="checkbox"/> 95-98	2
<input type="checkbox"/> 90-94	22
<input type="checkbox"/> 85-89	93
<input type="checkbox"/> 80-84	261
<input type="checkbox"/> 75-79	653
<input type="checkbox"/> 70-74	1904
<input type="checkbox"/> 65-69	9221
<input type="checkbox"/> 0-64 (least similar)	28492
<input type="button" value="Get Substances"/>	

0 of 2 Substances Selected

Score: 96

1. **1217885-36-3** 🔍

~0 ~1

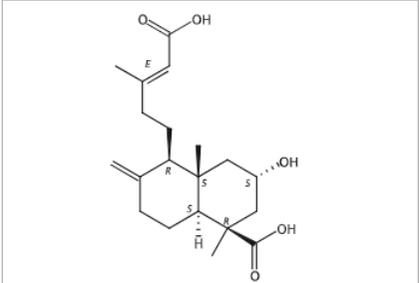


C₂₀H₃₀O₅
1-Naphthalenecarboxylic acid, 5-(4-carboxy-3-methyl-3-buten-1-yl)decahydro-3-hydroxy-1,4a-dimethyl-6-methylene-
▶ [Key Physical Properties](#)

Score: 96

2. **1788885-47-1** 🔍

~1



Double bond geometry as shown.,Relative stereochemistry.
C₂₀H₃₀O₅
1-Naphthalenecarboxylic acid, 5-[(3E)-4-carboxy-3-methyl-3-buten-1-yl]decahydro-3-hydroxy-1,4a-dimethyl-6-methylene-, (1R,3S,4aS,5R,8aS)-ref
▶ [Key Physical Properties](#)

Figure S8: Scifinder report for 1

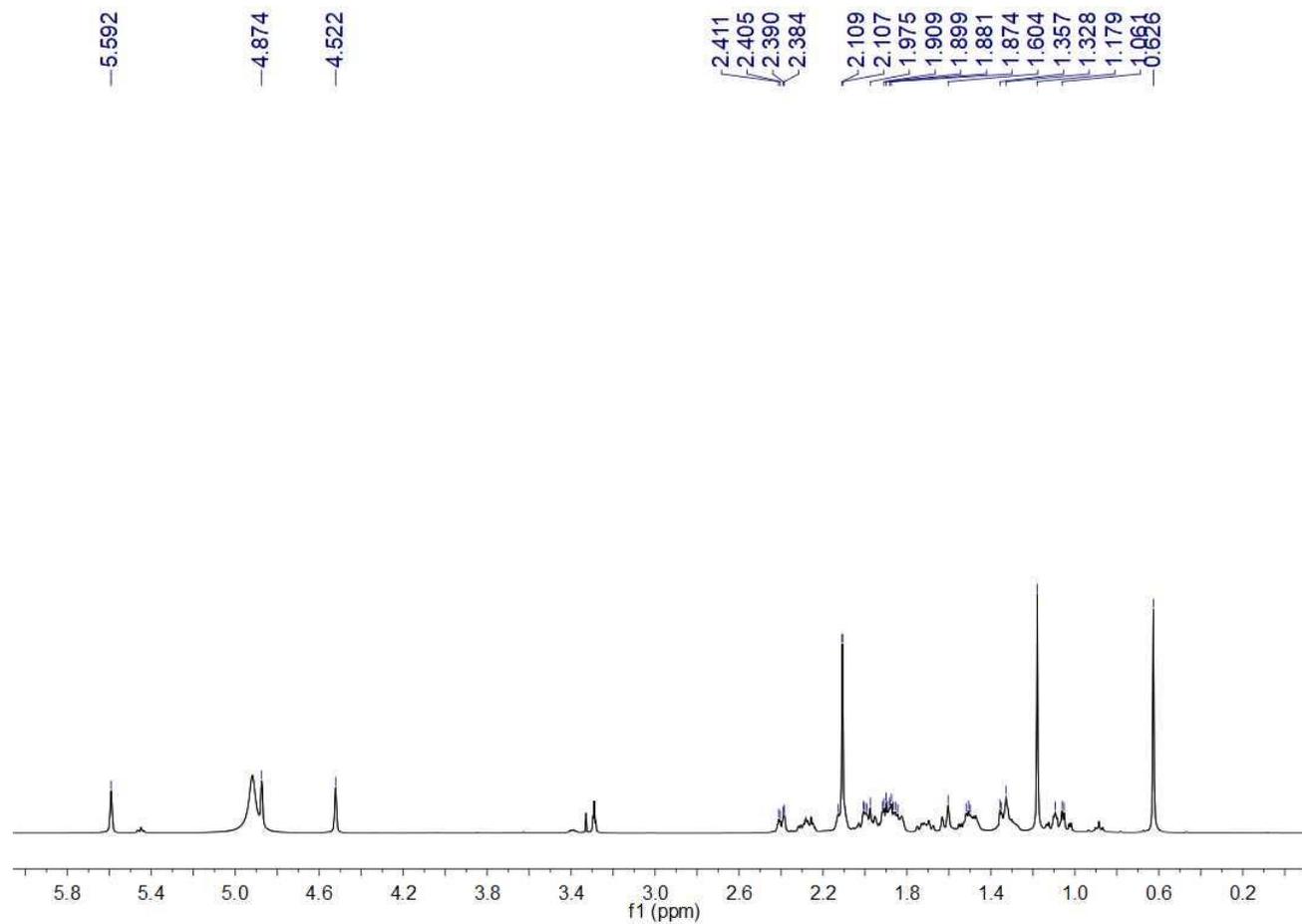


Figure S9: ^1H NMR Spectrum of **2** in CD_3OD (400 MHz)

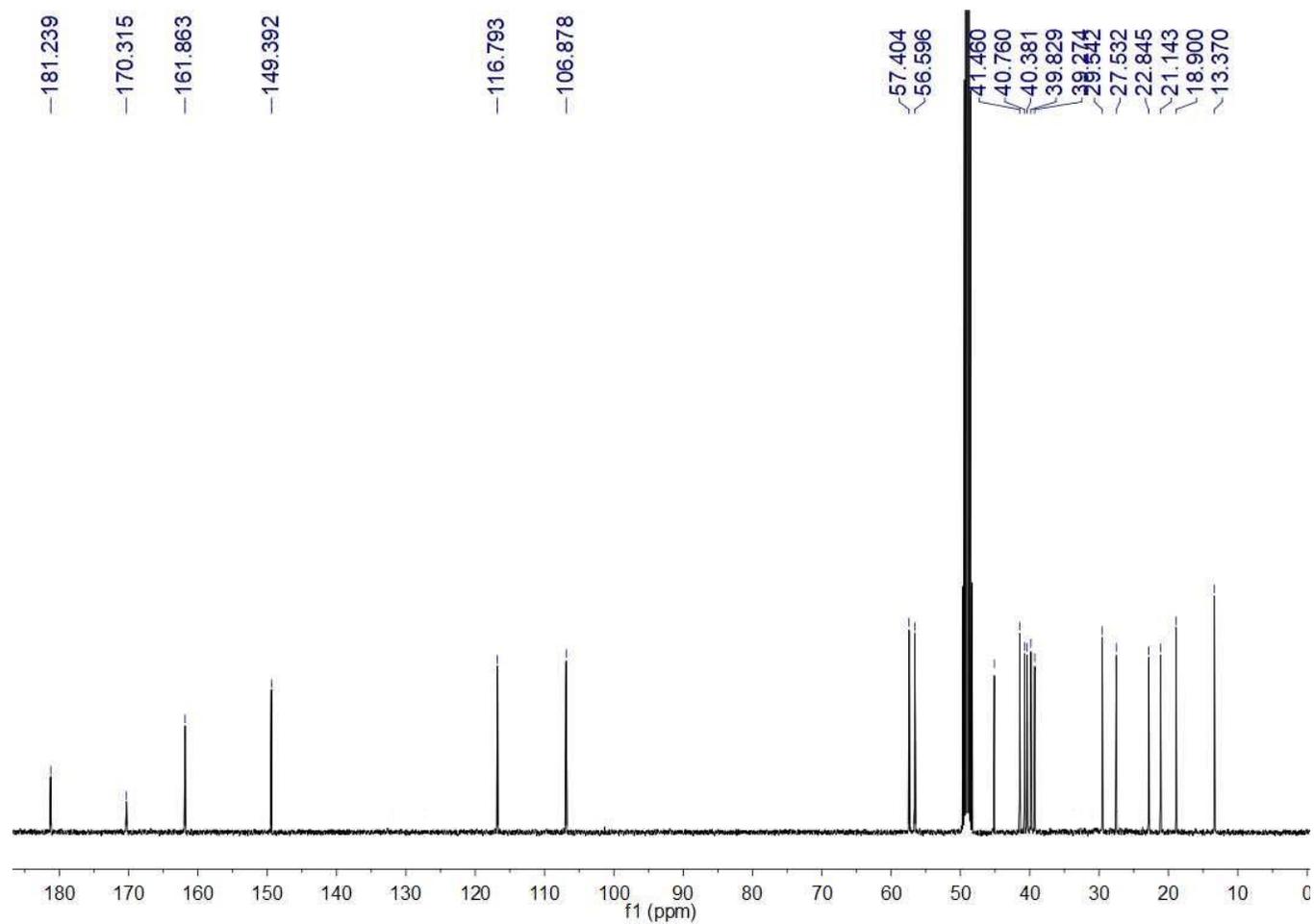


Figure S10: ^{13}C NMR Spectrum of **2** in CD_3OD (100 MHz)

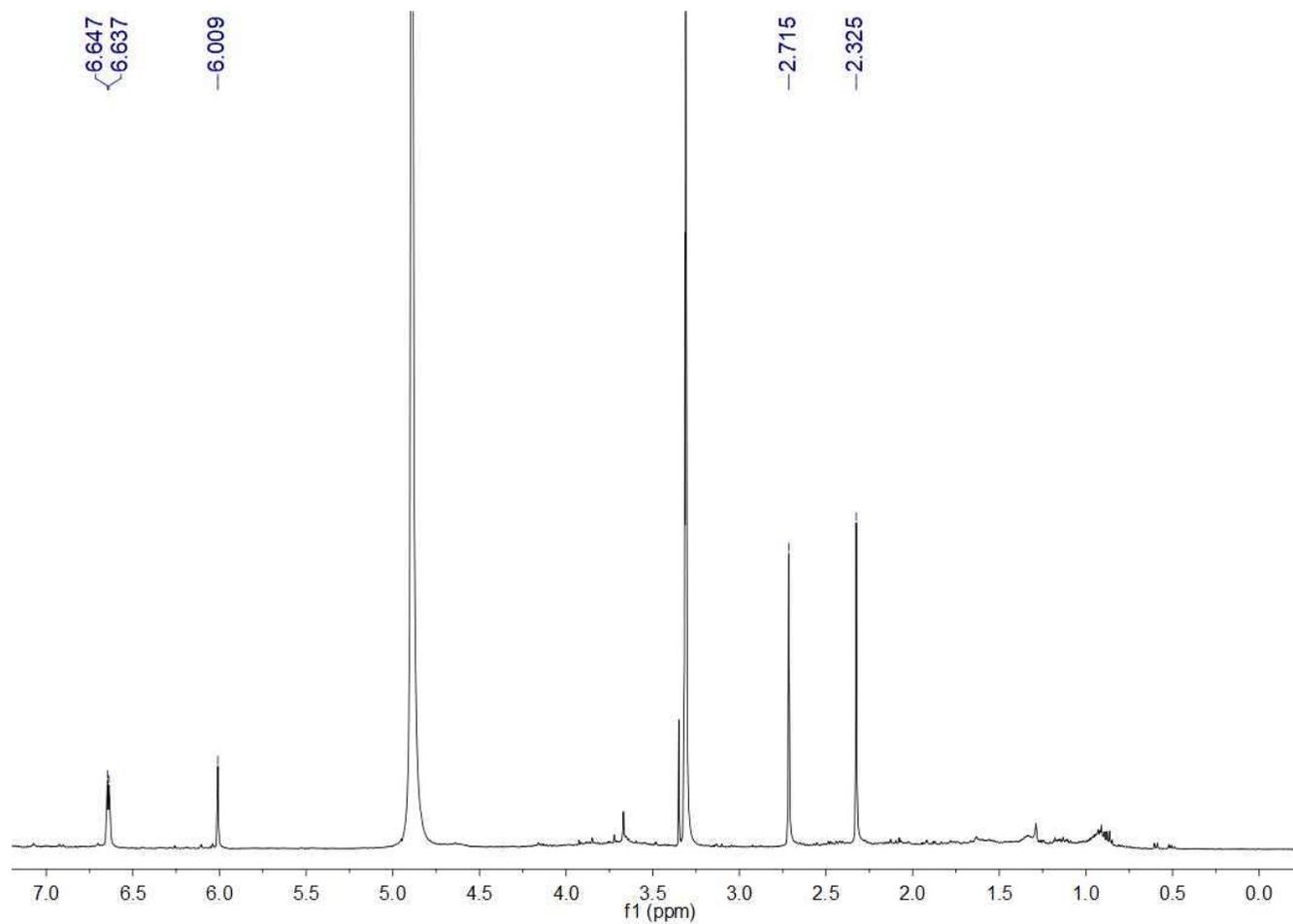


Figure S11: ¹H NMR Spectrum of **3** in CD₃OD (400 MHz)

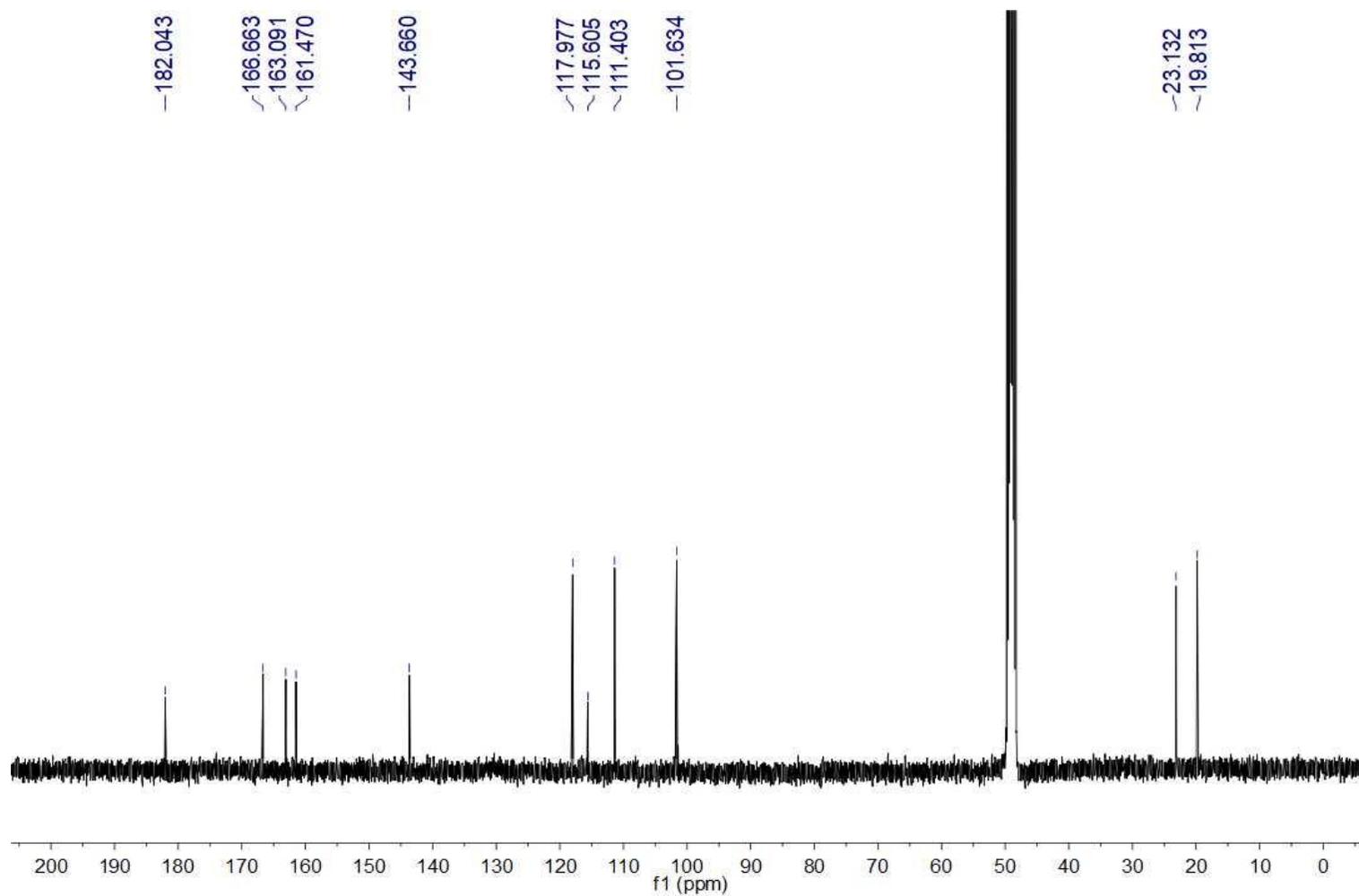


Figure S12: ^{13}C NMR Spectrum of **3** in CDCl_3 (100 MHz)

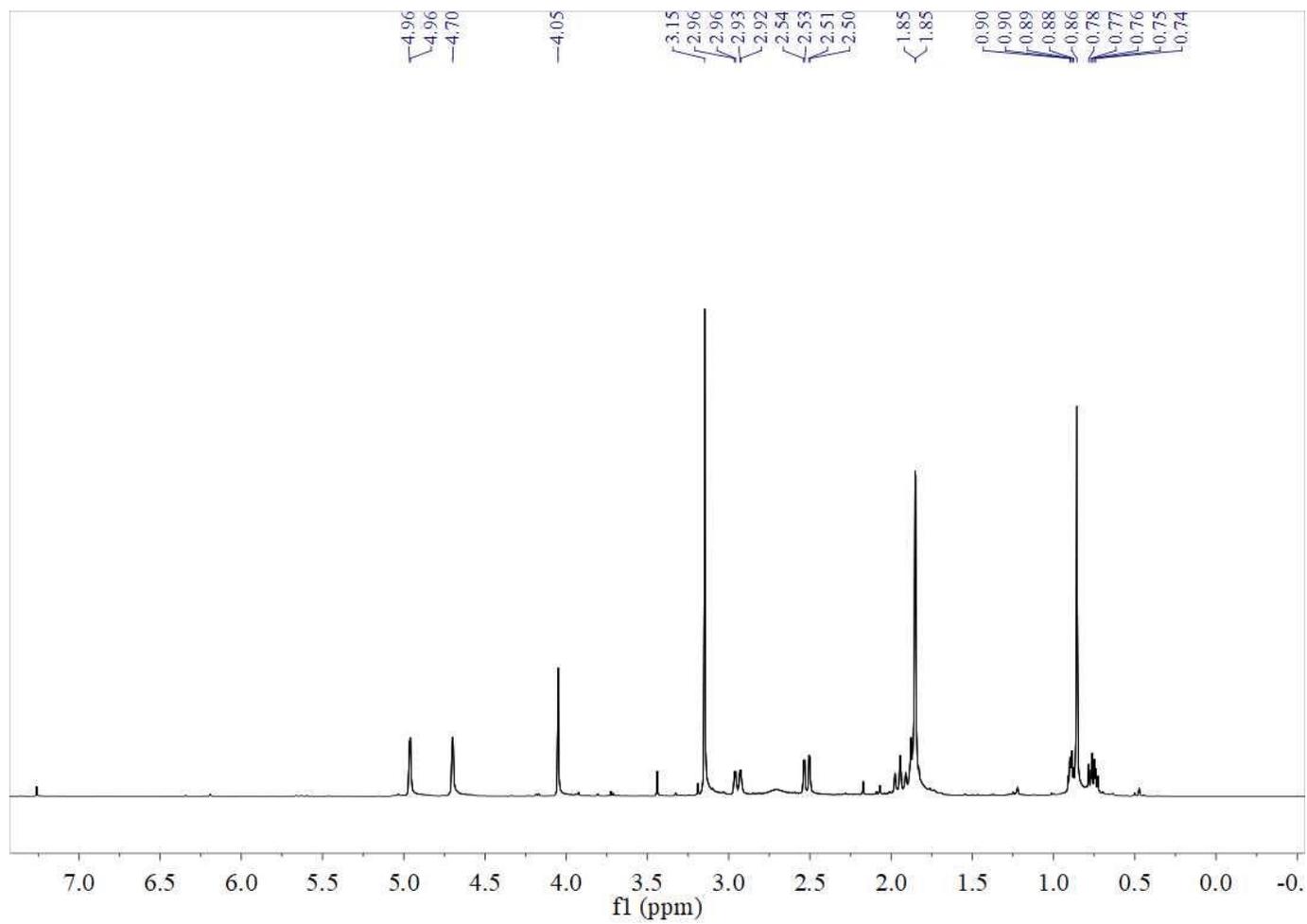


Figure S13: ¹H NMR Spectrum of **4** in CDCl₃ (400 MHz)

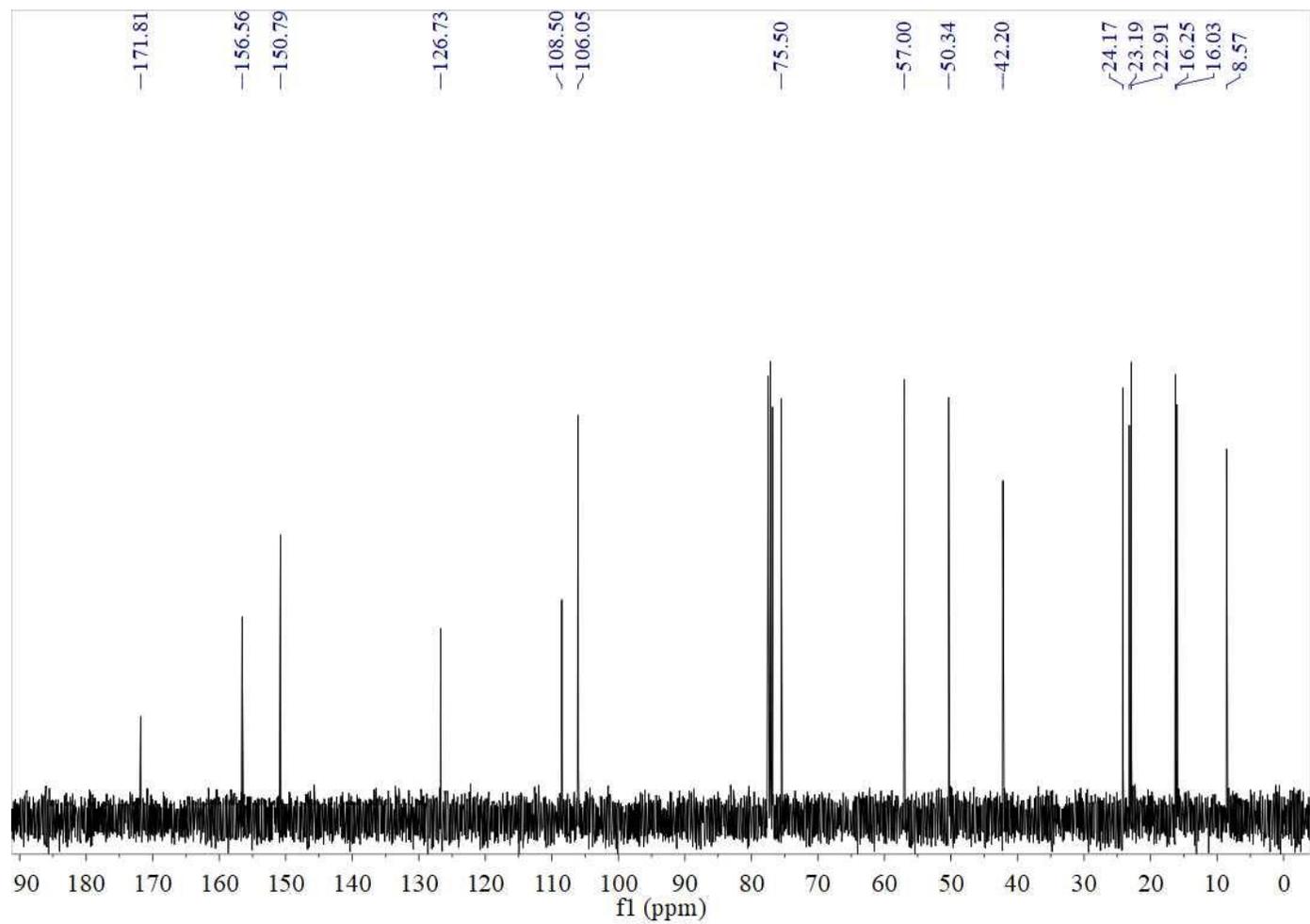


Figure S14: ^{13}C NMR Spectrum of **4** in CDCl_3 (100 MHz)

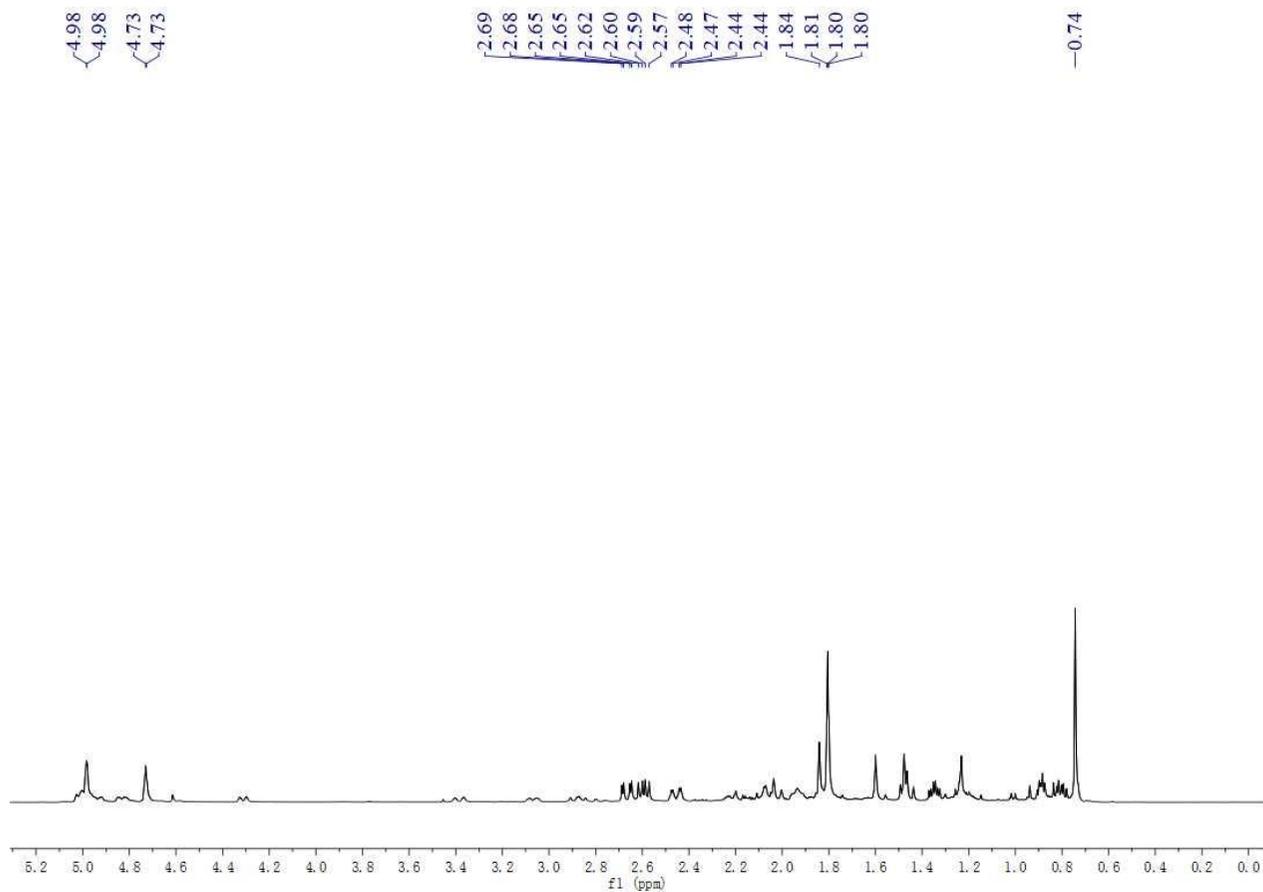


Figure S15: ^1H NMR Spectrum of **5** in CDCl_3 (400 MHz)

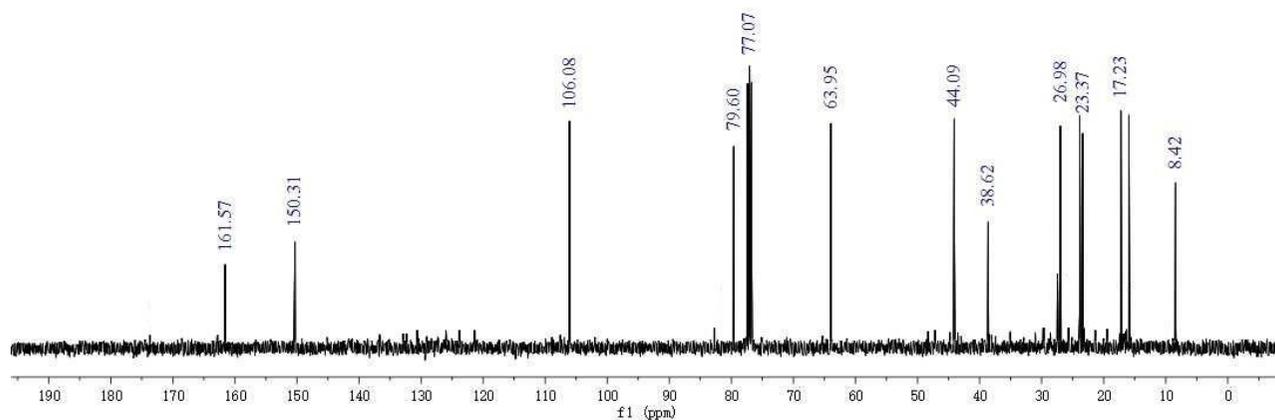


Figure S16: ^{13}C NMR Spectrum of **5** in CDCl_3 (100 MHz)

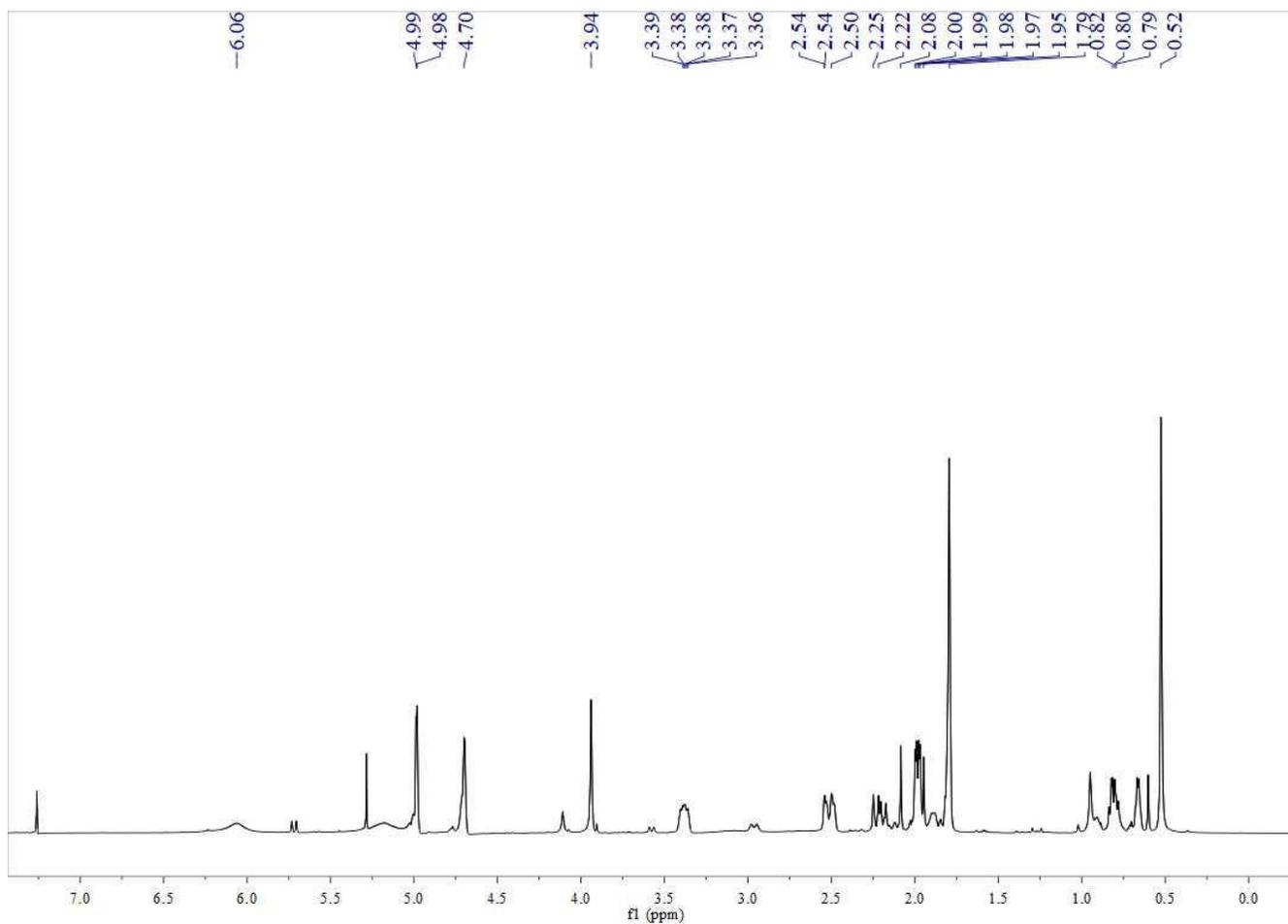


Figure S17: ^1H NMR Spectrum of **6** in CDCl_3 (400 MHz)

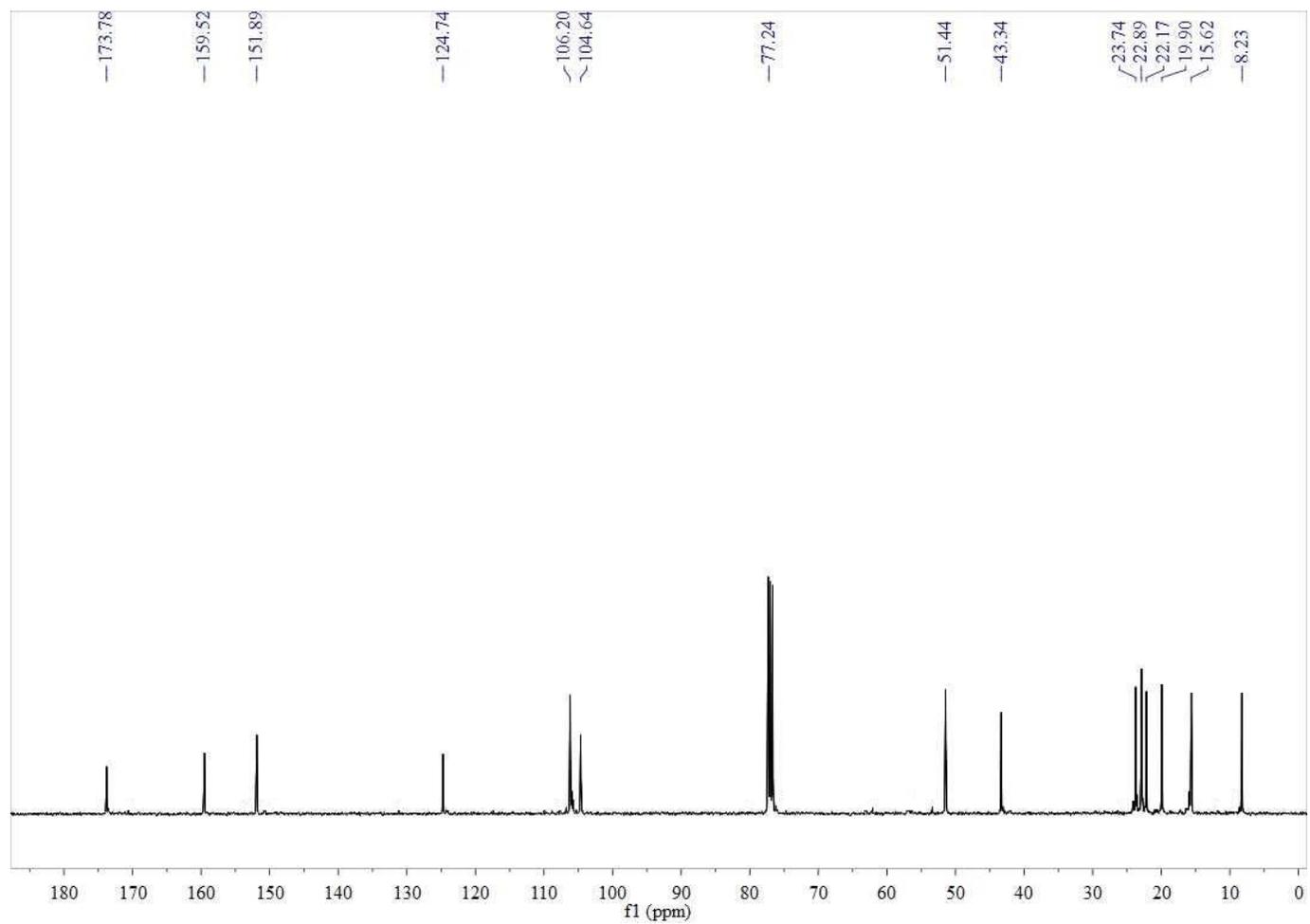


Figure S18: ^{13}C NMR Spectrum of **6** in CDCl_3 (100 MHz)