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(B) ^{1}H expansions

(C) ^{13}C

(D) HMQC

(E) HMBC

(F) ^{1}H -^{1}H COSY

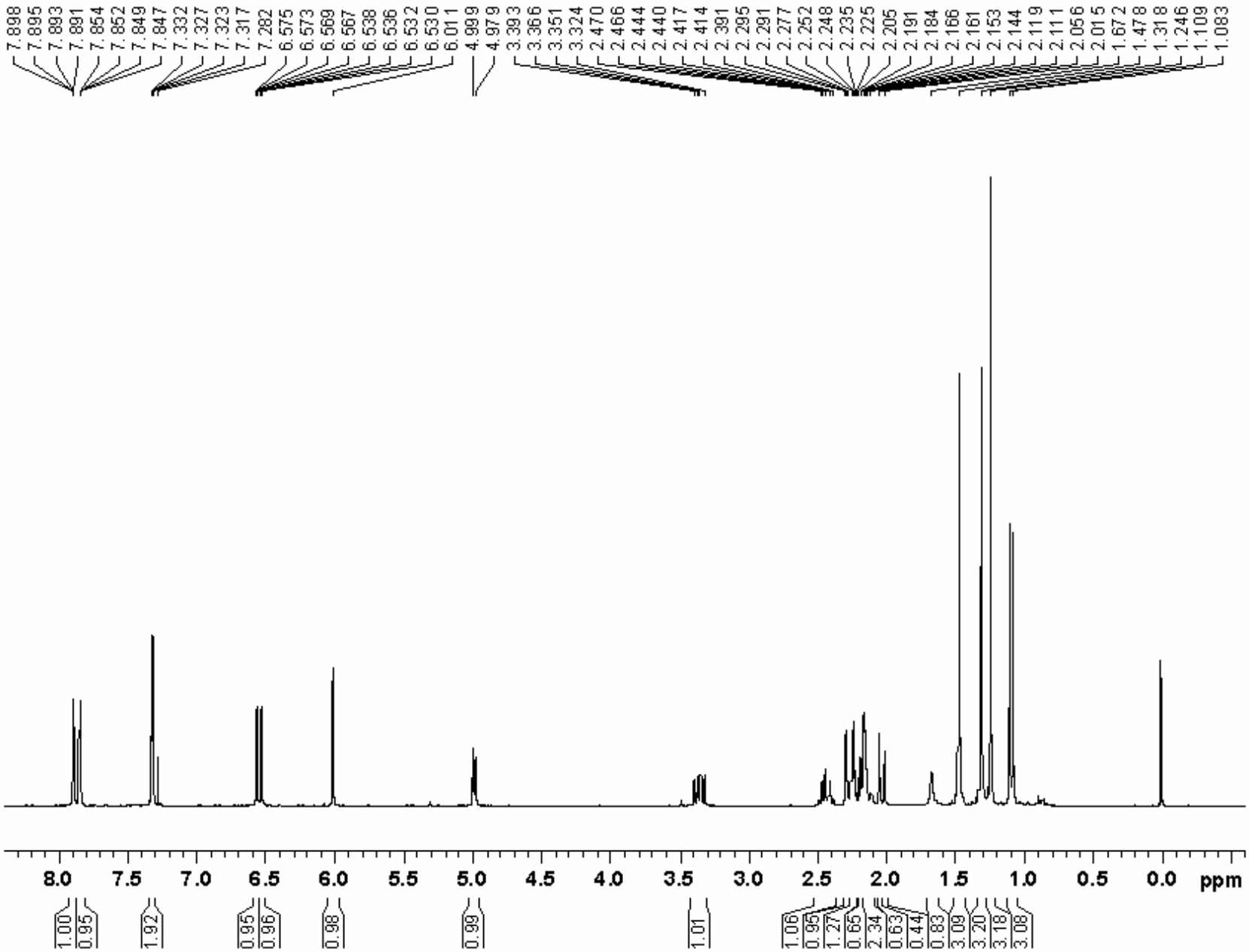
(G) HR EIMS

(H) FTIR-ATR

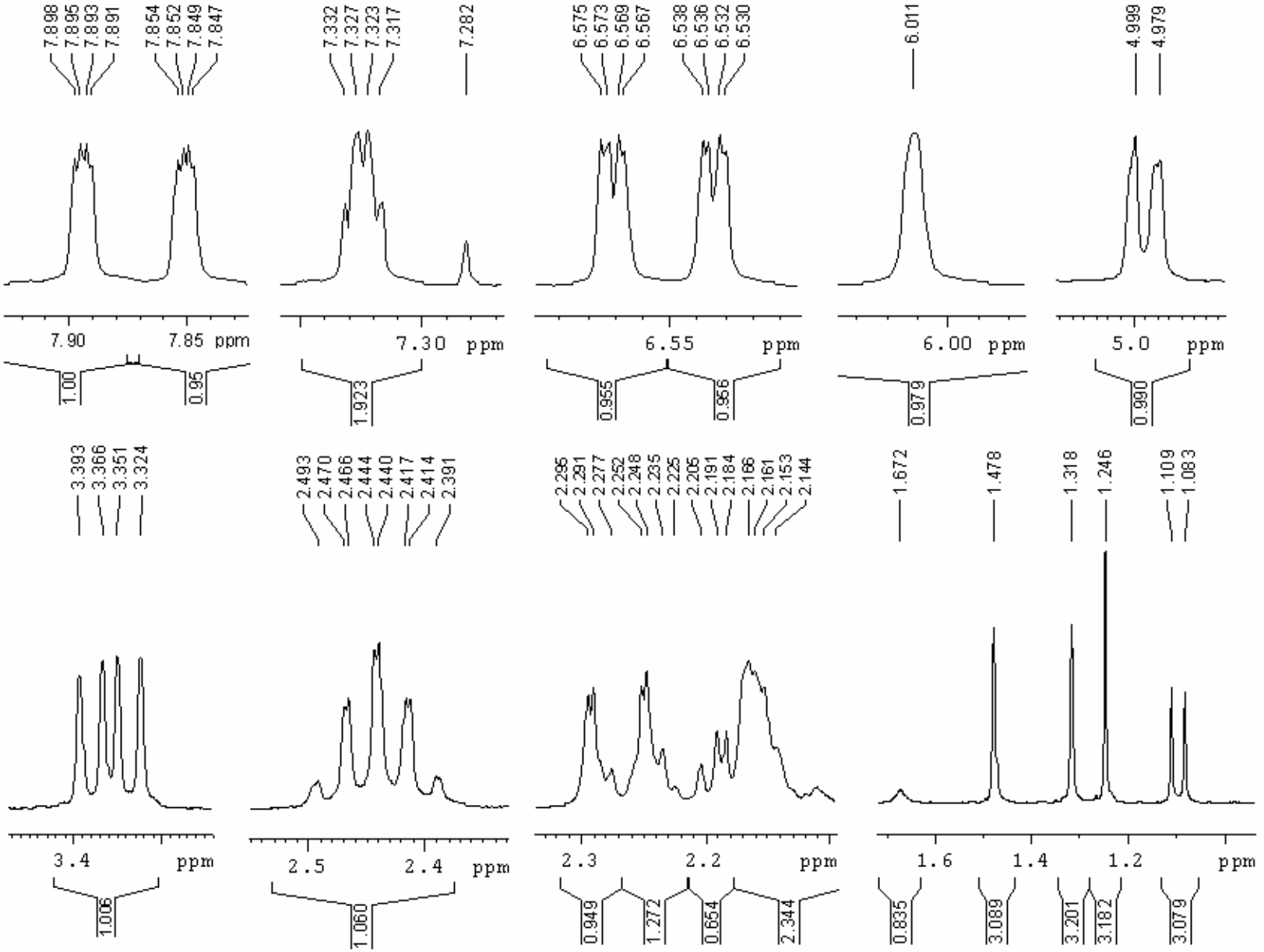
Pages 34-35: Procedure for Antimicrobial Evaluation by the Agar Overlay Method.

Pages 36-37: Unabridged Procedure for X-ray crystallography of Crystal 2

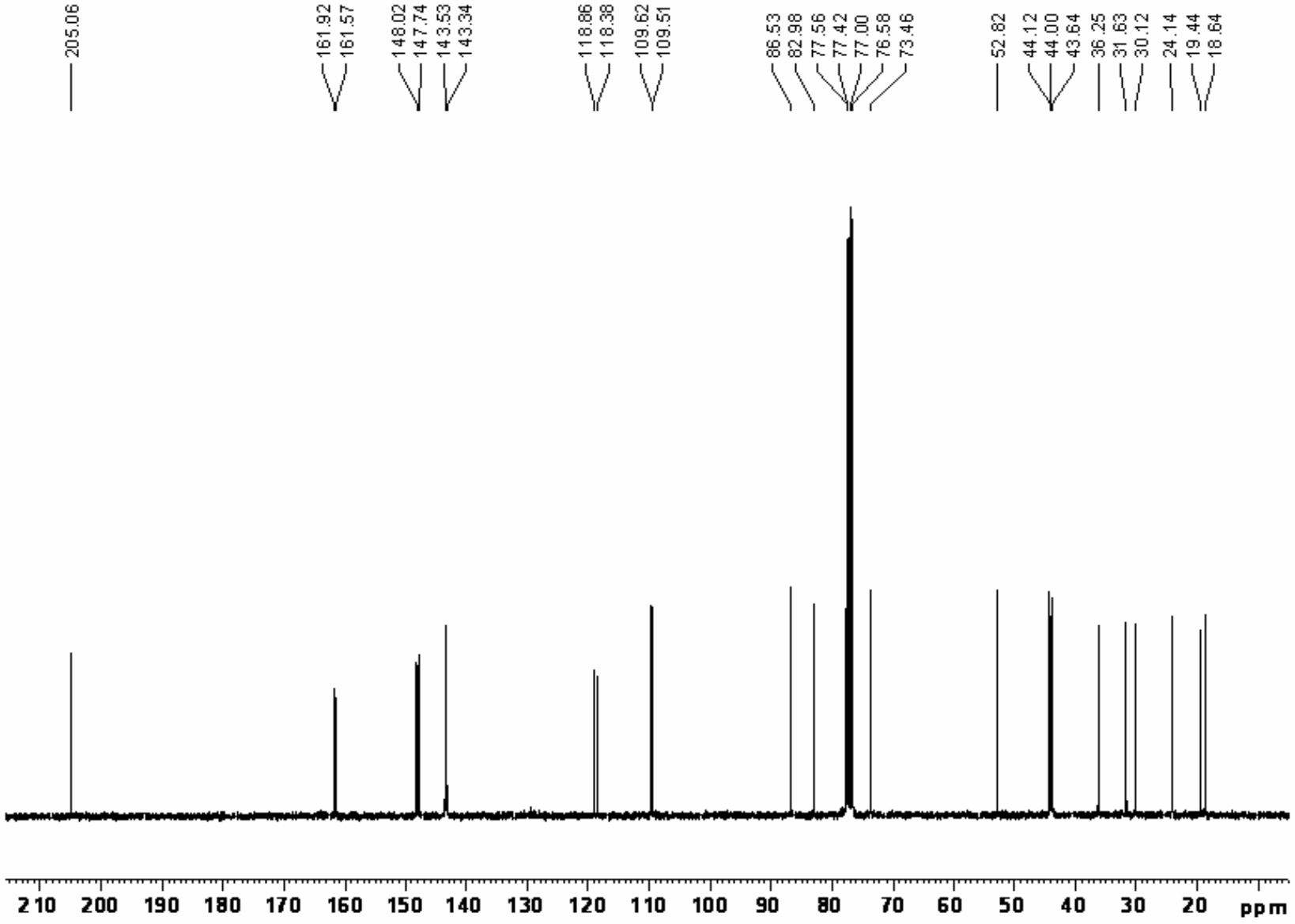
S1(A). ^{1}H NMR (300 MHz, $CDCl_3$) of Compound **1**



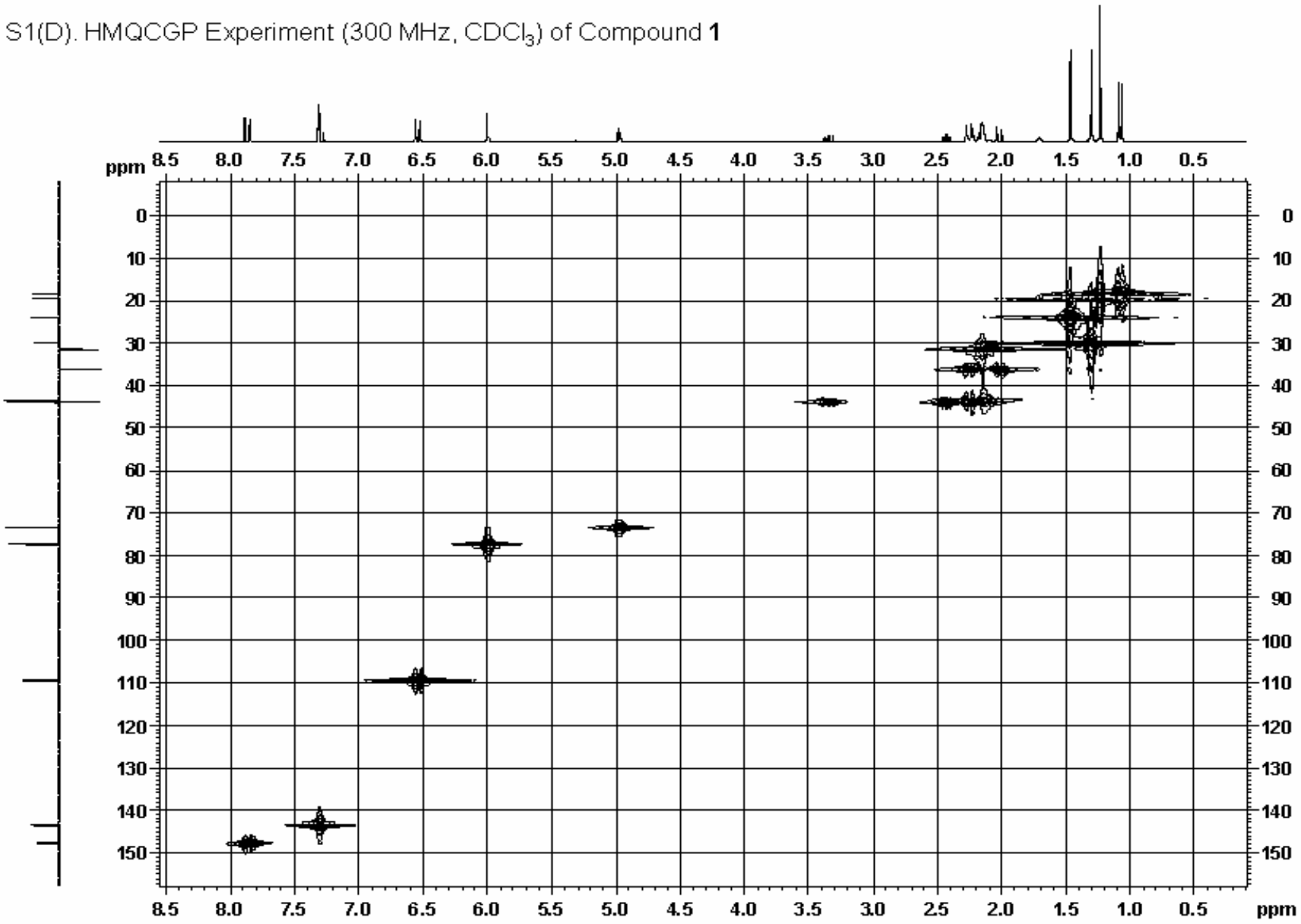
S1(B). Expansions of ^{1}H NMR (300 MHz, $CDCl_3$) of Compound **1**



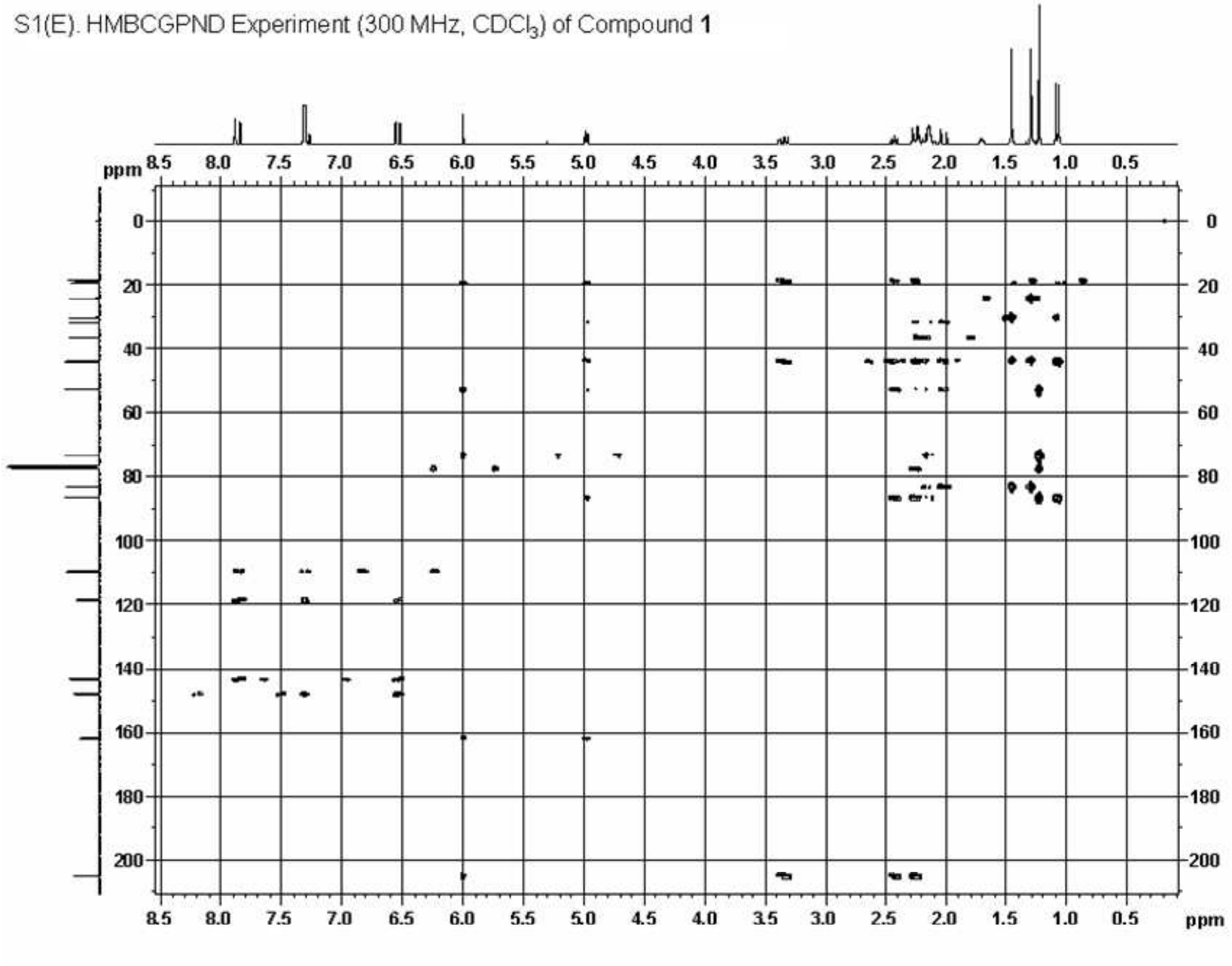
S1(C). ^{13}C NMR (75.5 MHz, $CDCl_3$) of Compound **1**



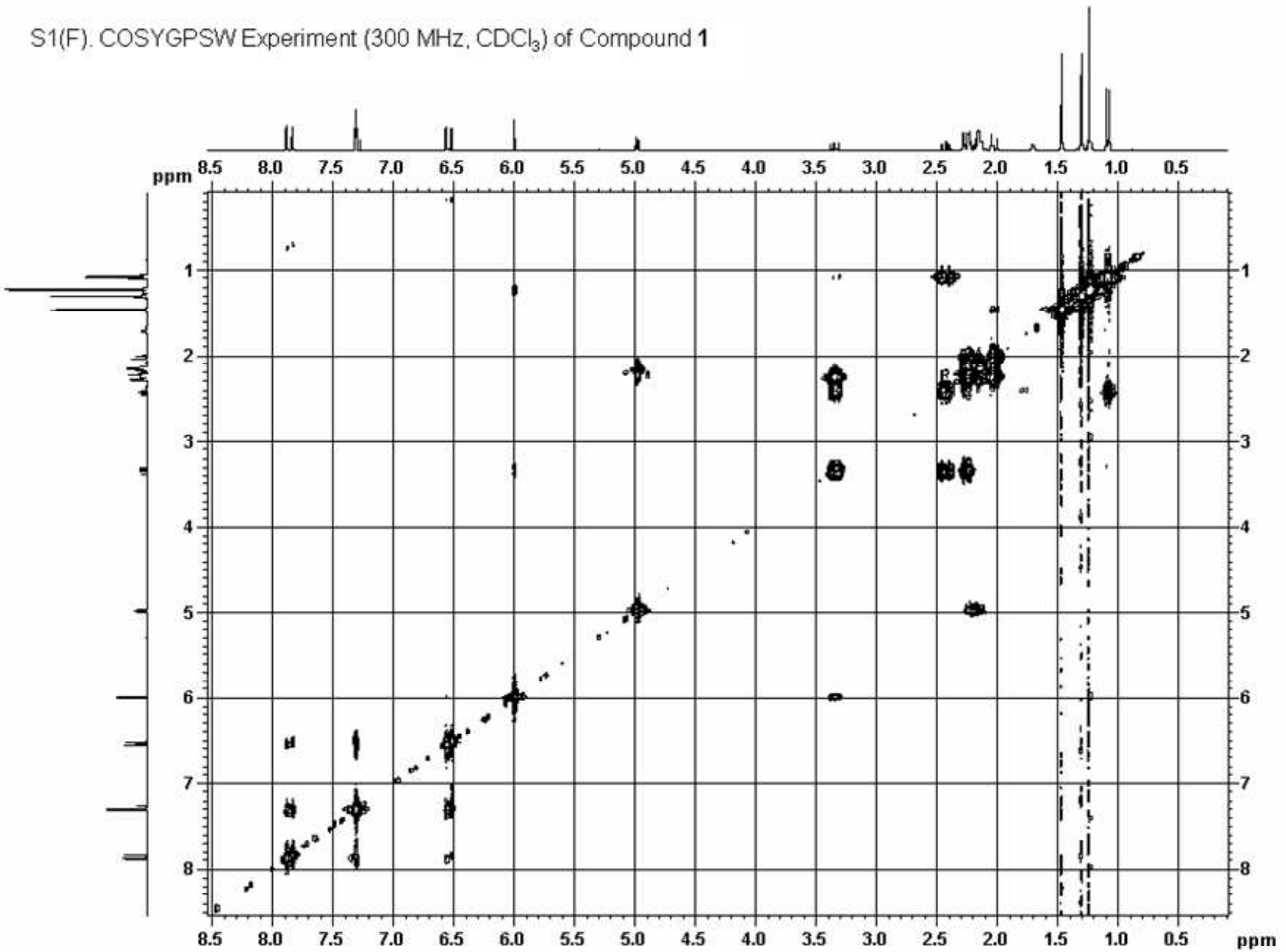
S1(D). HMQCGP Experiment (300 MHz, $CDCl_3$) of Compound **1**



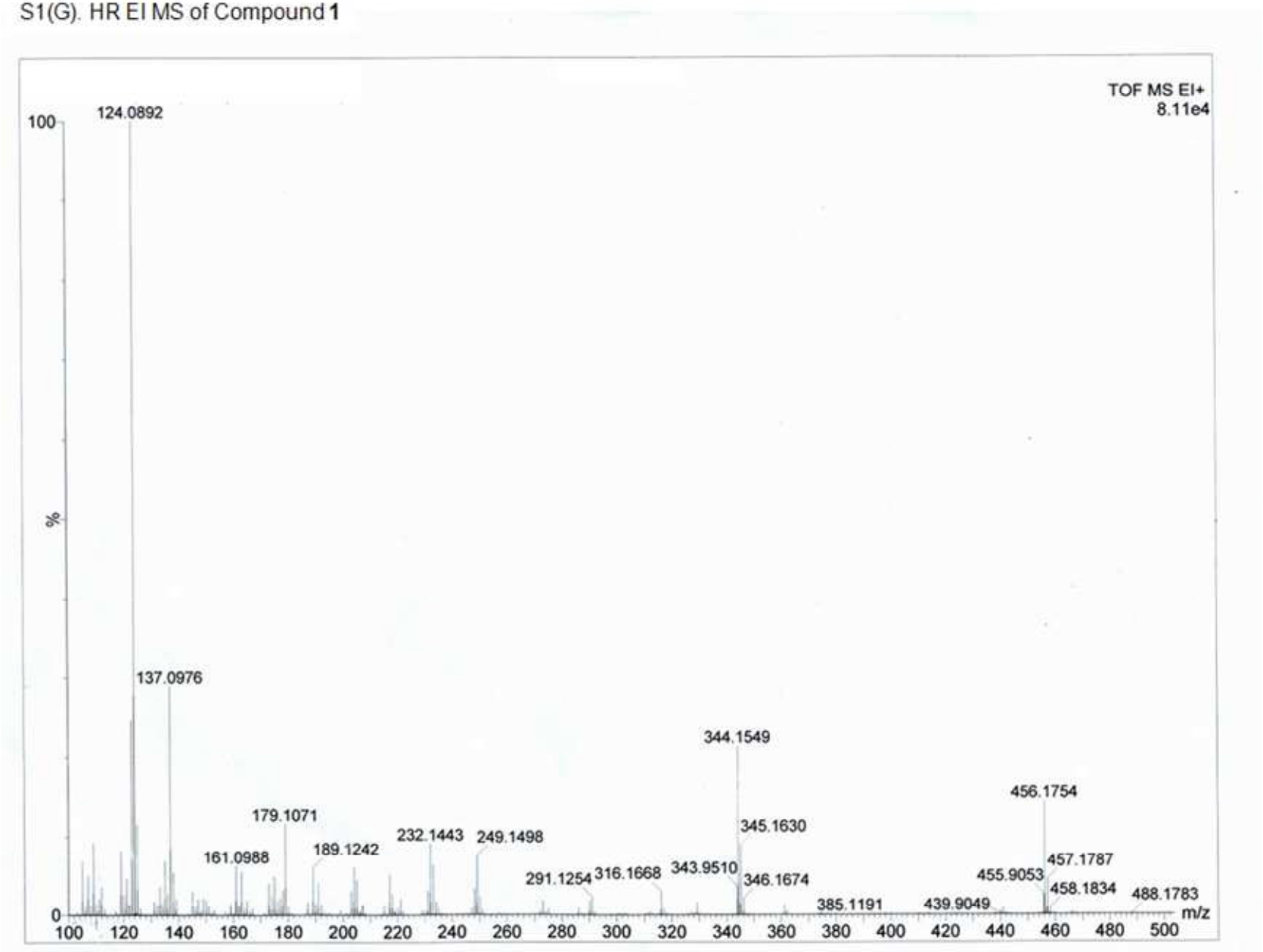
S1(E). HMBCGPND Experiment (300 MHz, $CDCl_3$) of Compound **1**



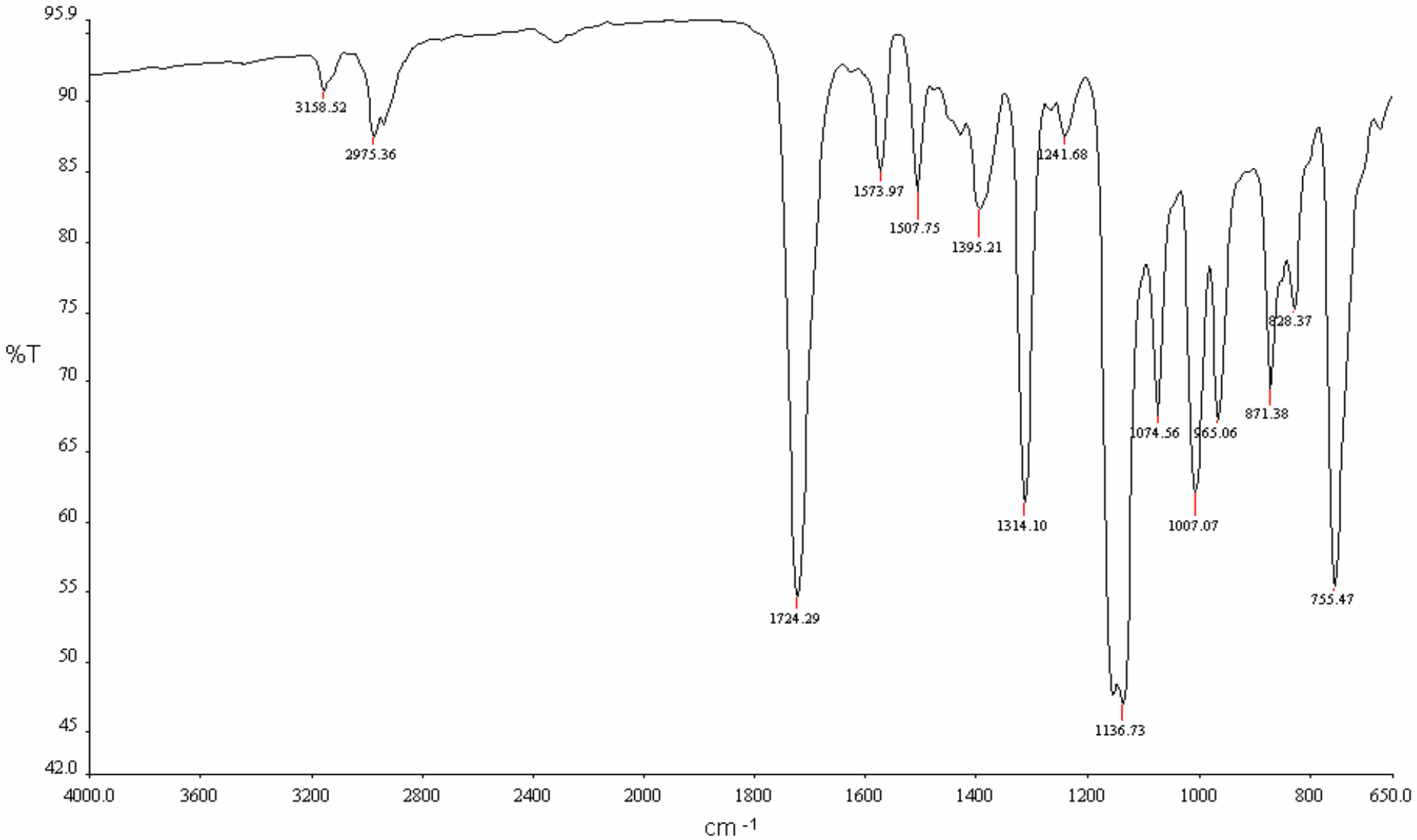
S1(F). COSYGPSW Experiment (300 MHz, $CDCl_3$) of Compound **1**



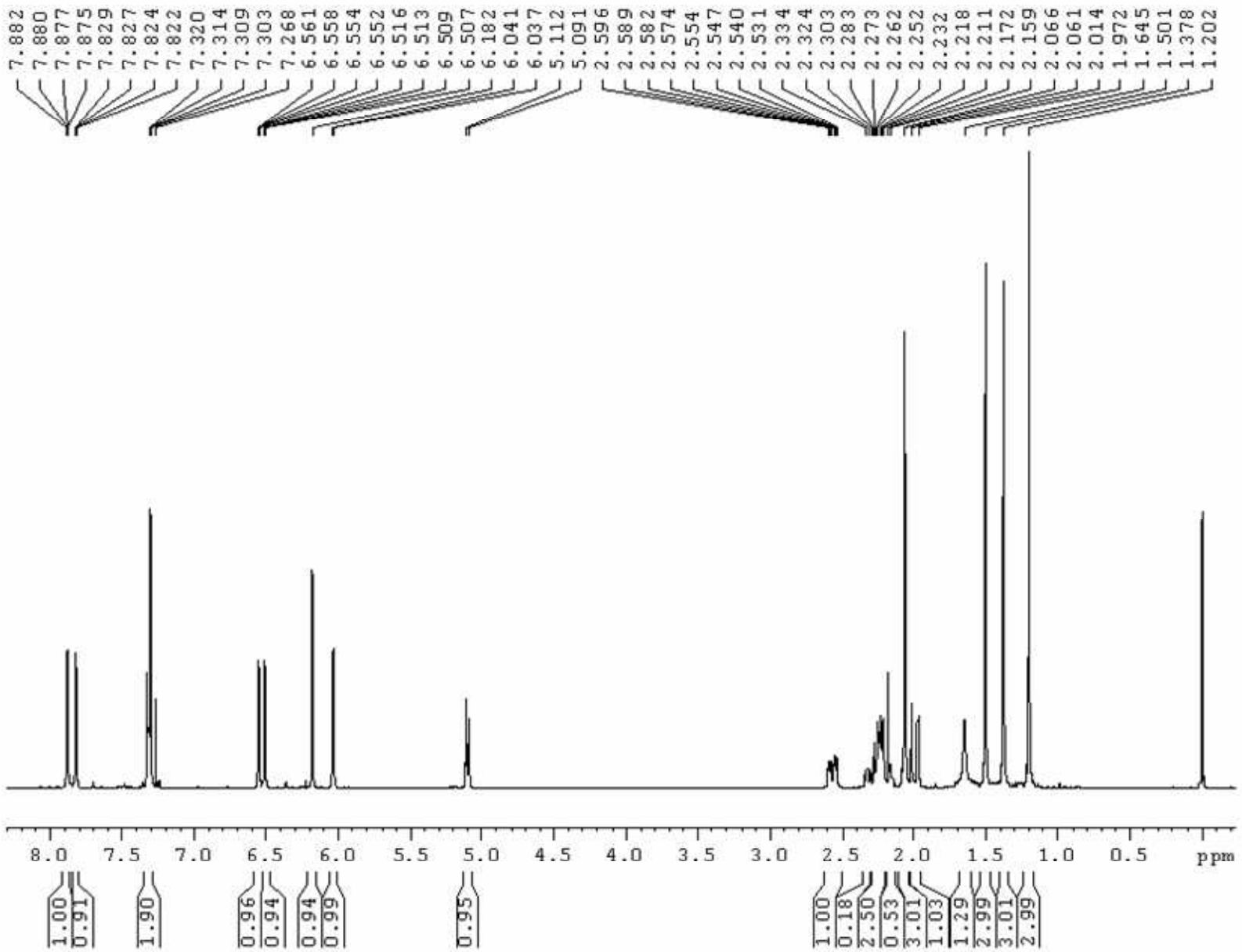
S1(G). HR EI MS of Compound **1**



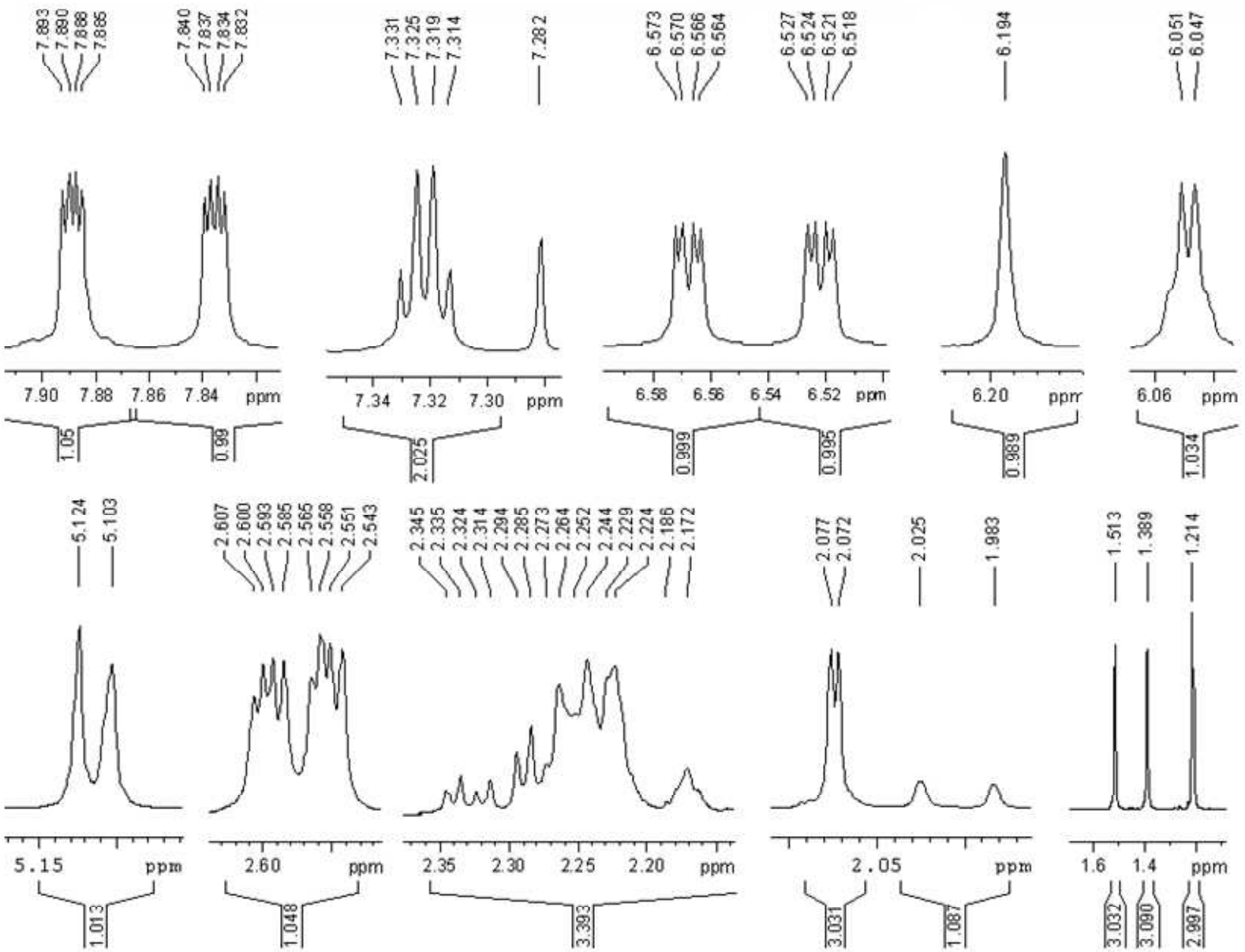
S1 (H). FTIR-ATR Spectrum of Compound **1**



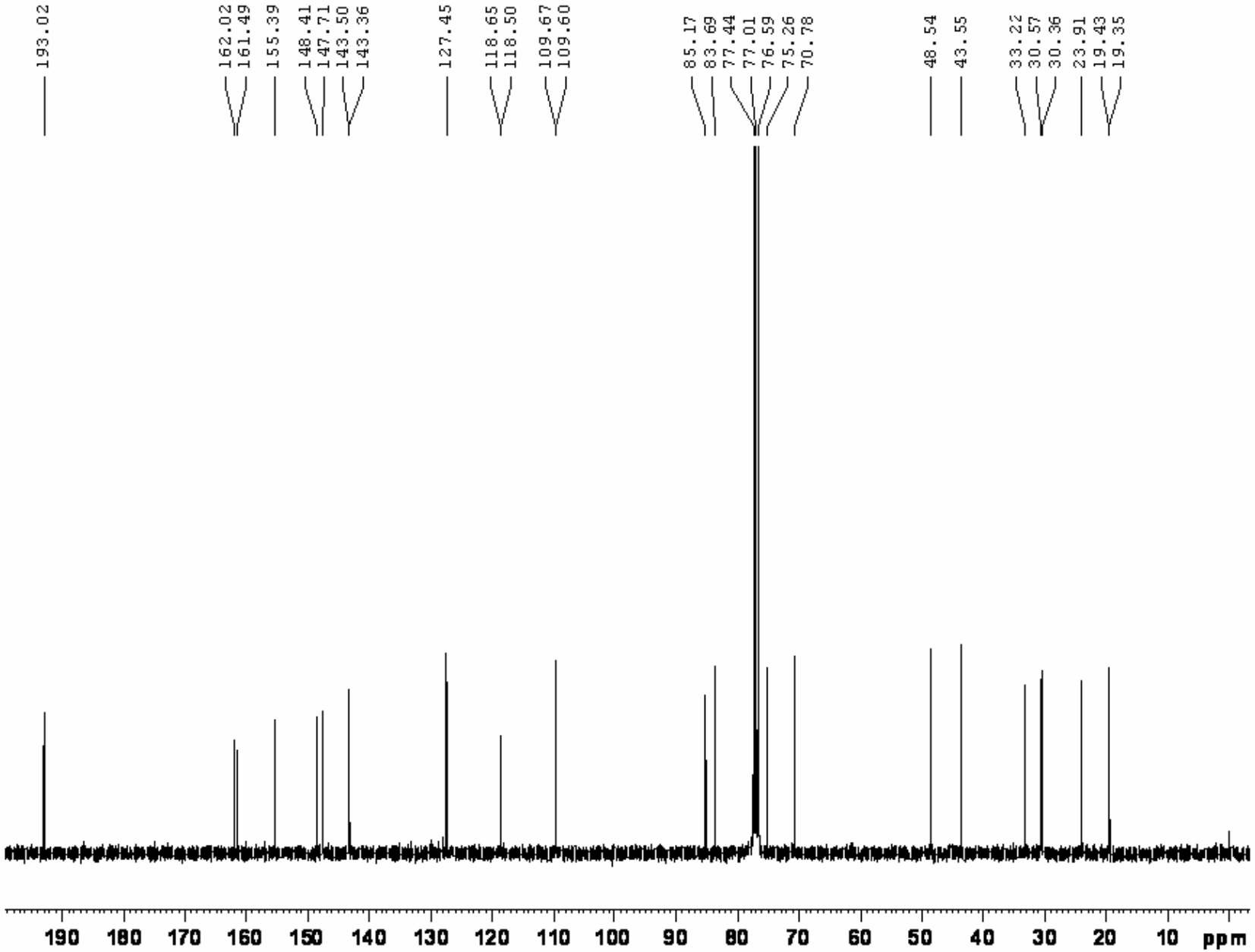
S2(A). ^{1}H NMR (300 MHz, $CDCl_3$) of Compound **2**



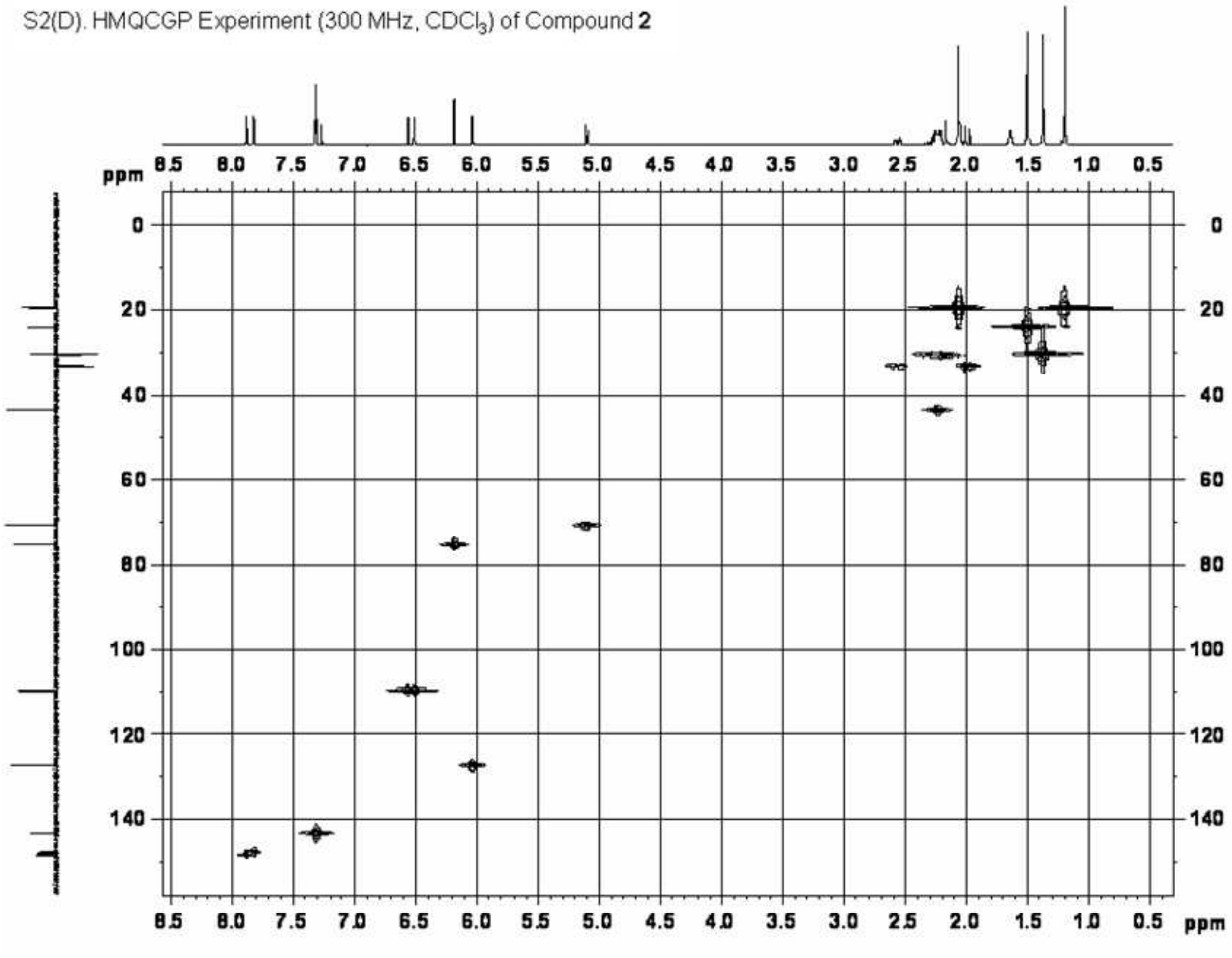
S2(B). Expansions of ^{1}H NMR (300 MHz, $CDCl_3$) of Compound **2**



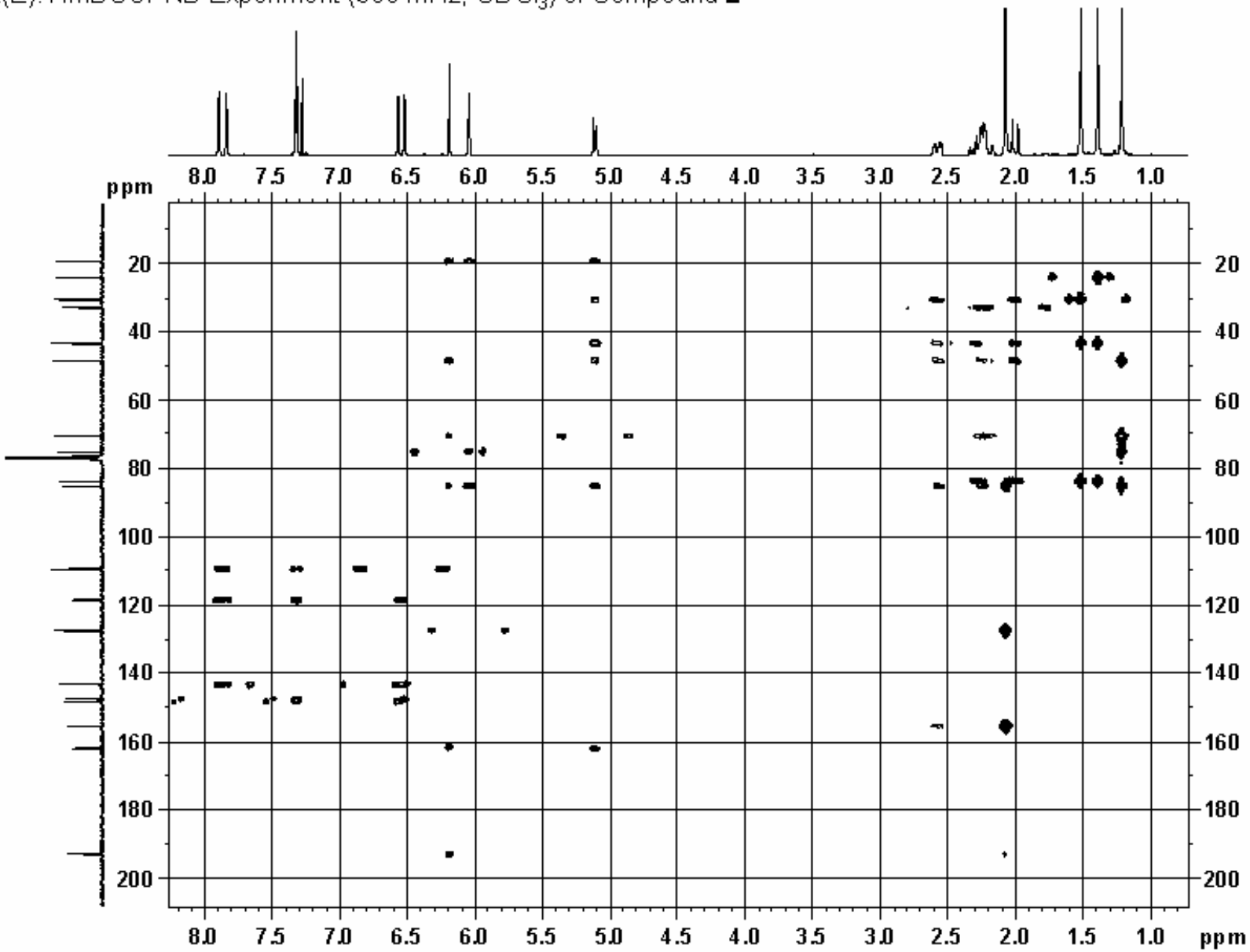
S2(C). ^{13}C NMR (75.5 MHz, $CDCl_3$) of Compound **2**



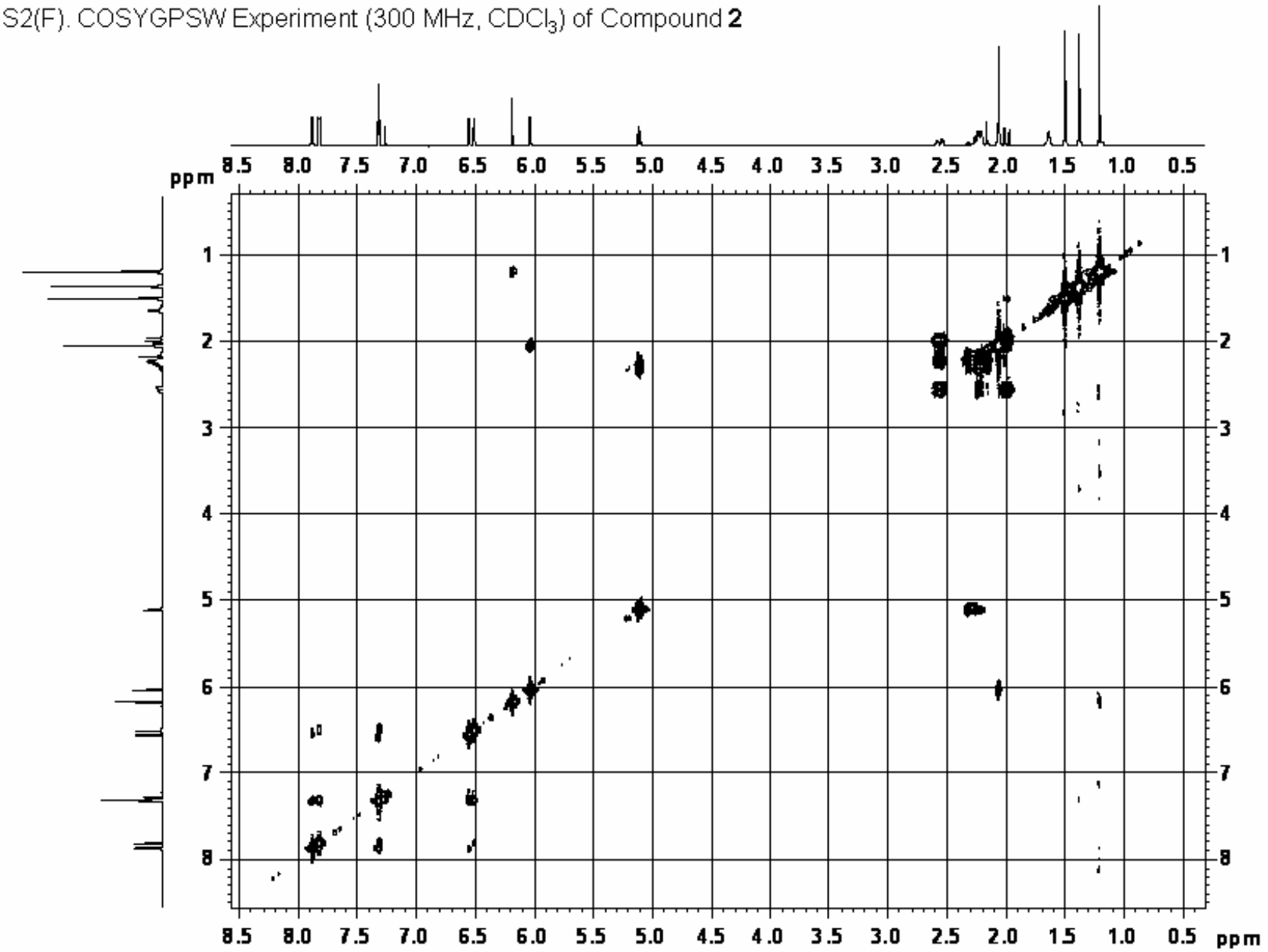
S2(D). HMQCGP Experiment (300 MHz, $CDCl_3$) of Compound **2**



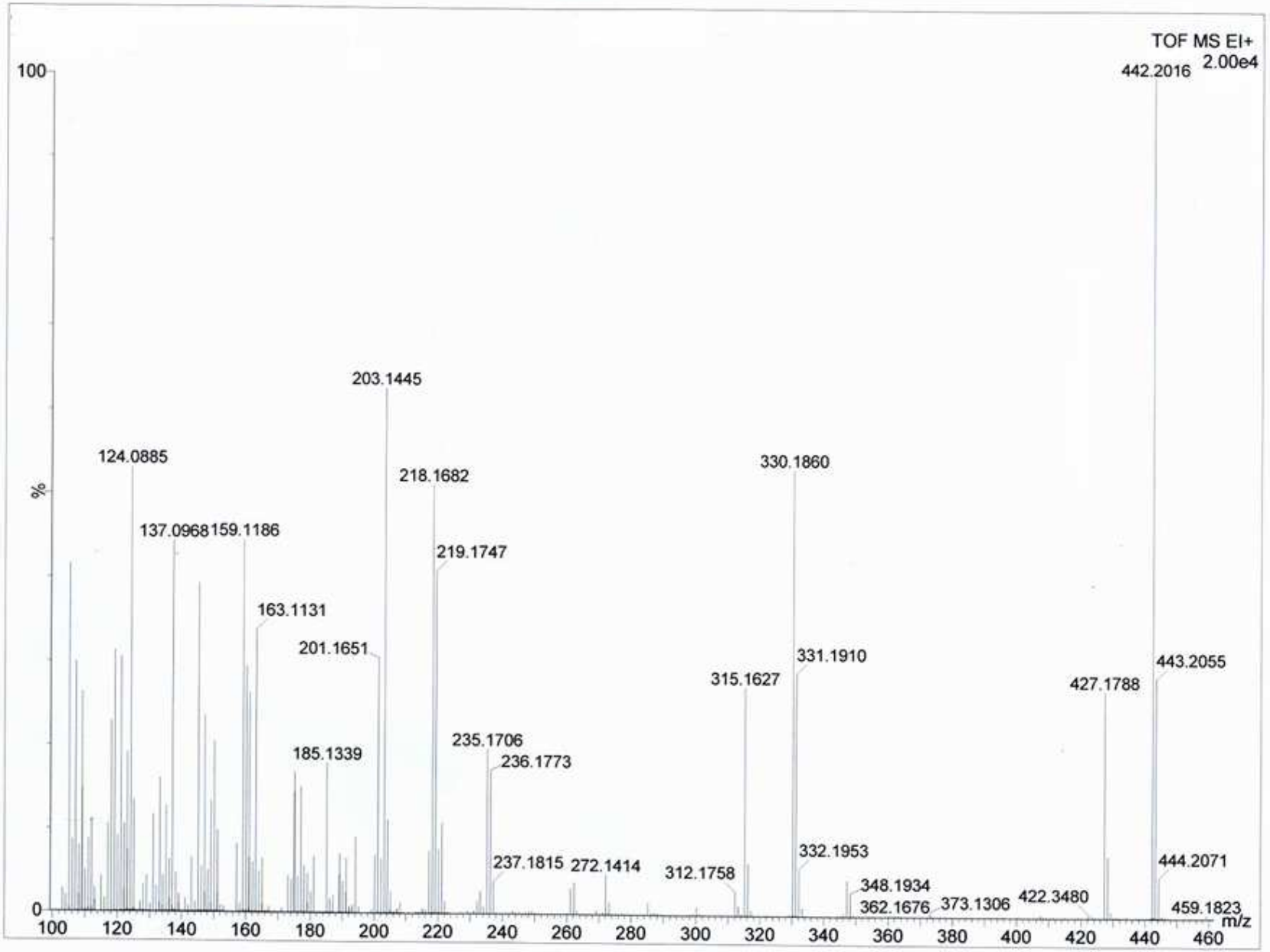
S2(E). HMBCGPND Experiment (300 MHz, $CDCl_3$) of Compound **2**



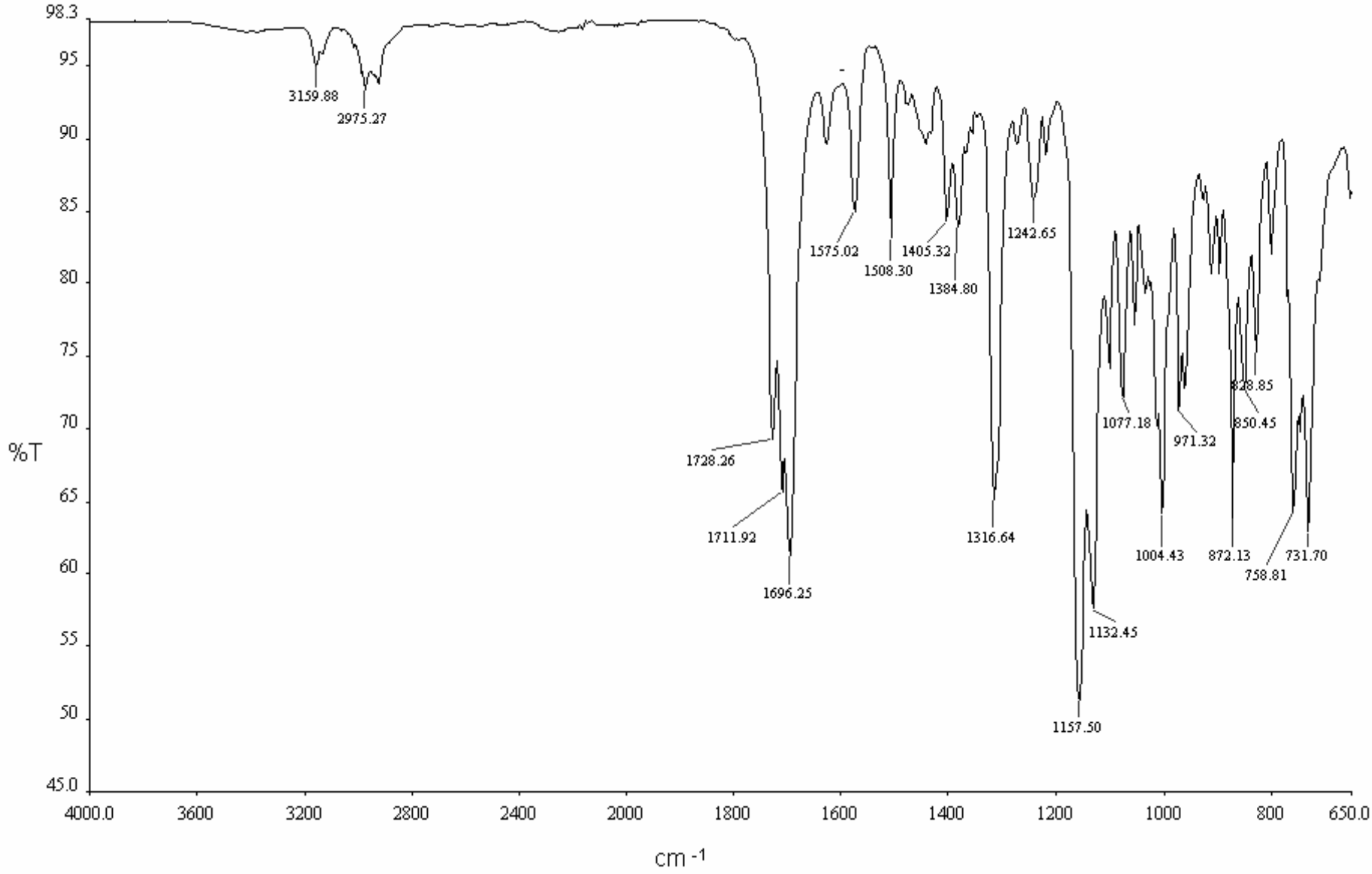
S2(F). COSYGPSW Experiment (300 MHz, $CDCl_3$) of Compound **2**



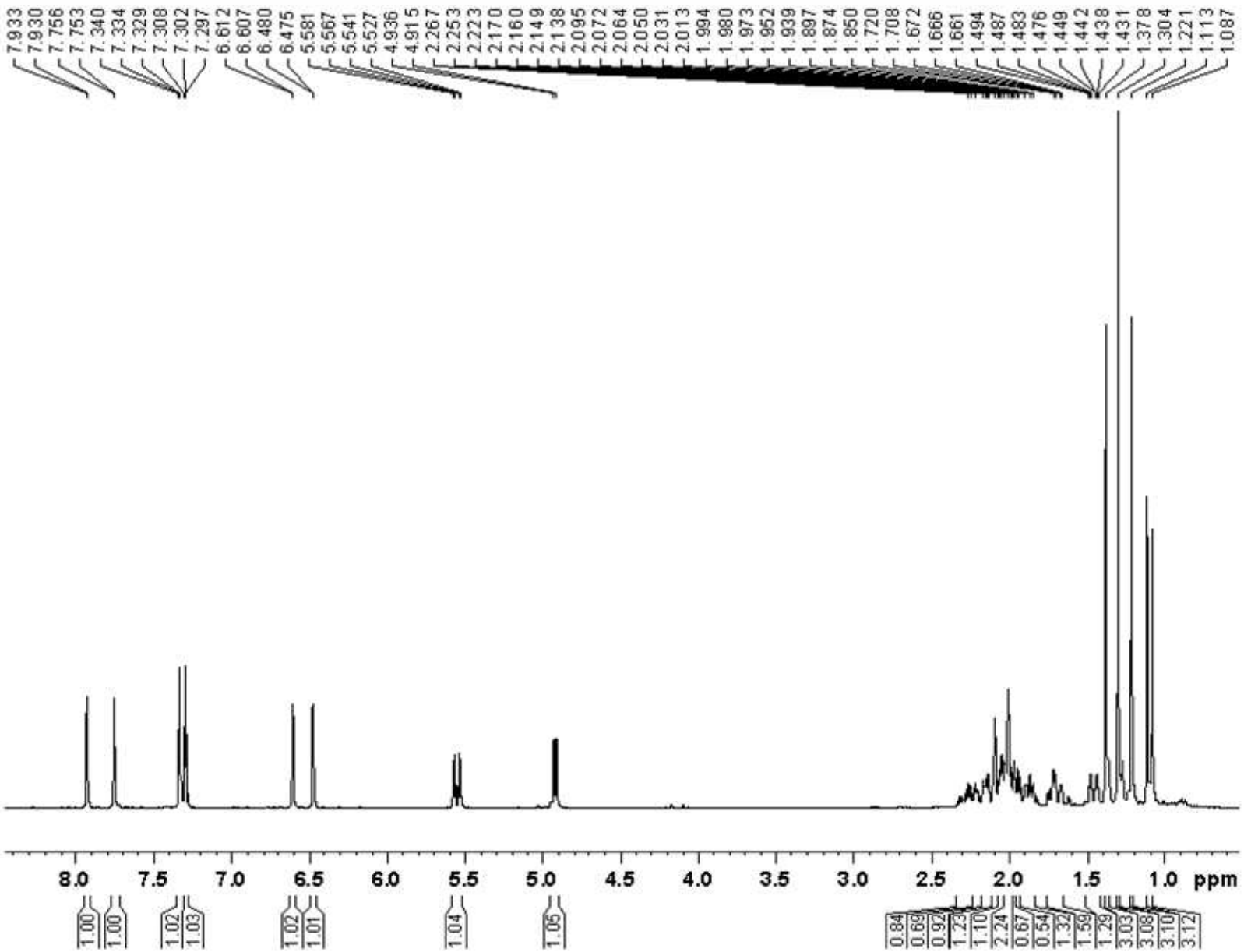
S3(G). HR EI MS of Compound **3**



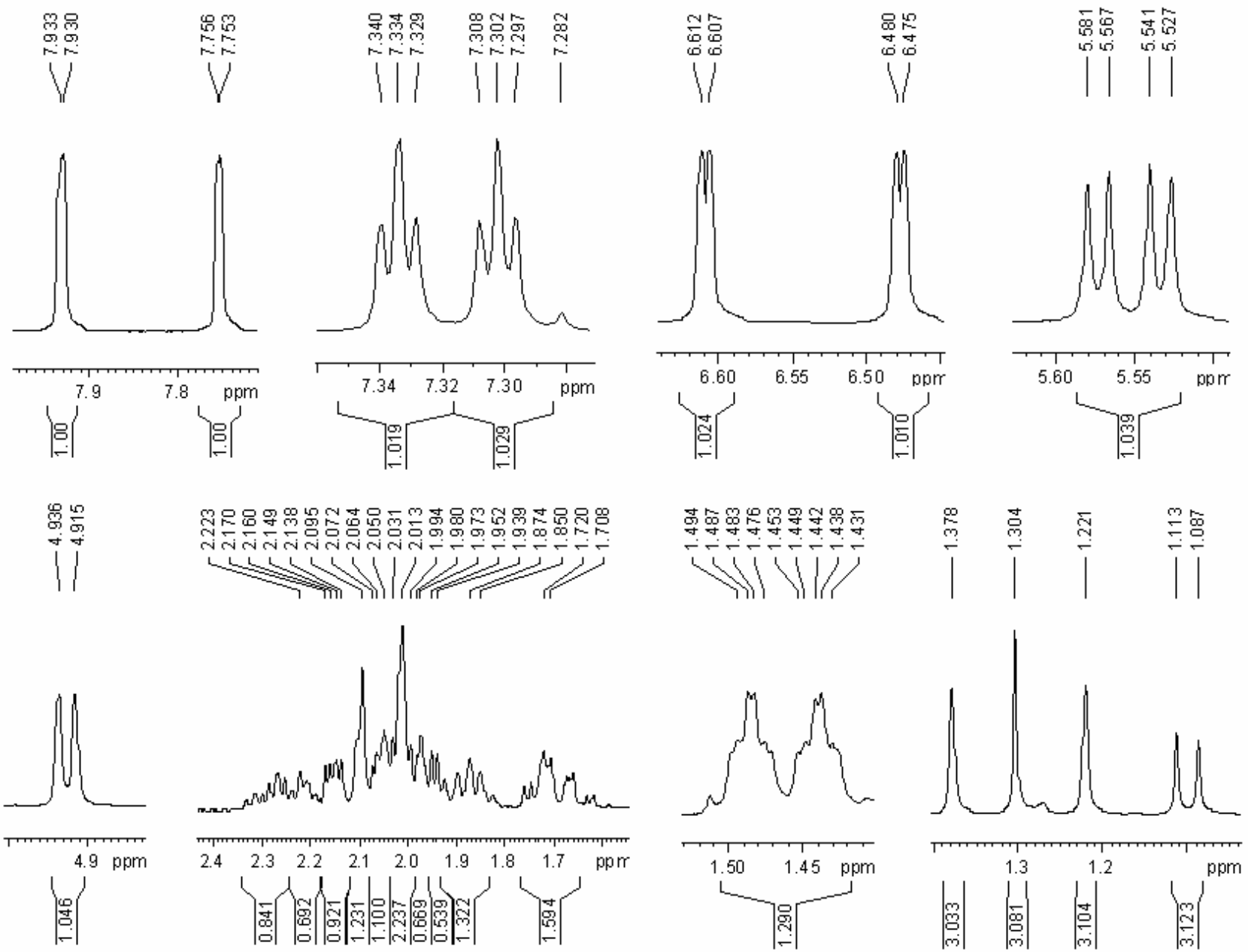
S2 (H). FTIR-ATR Spectrum of Compound **2**



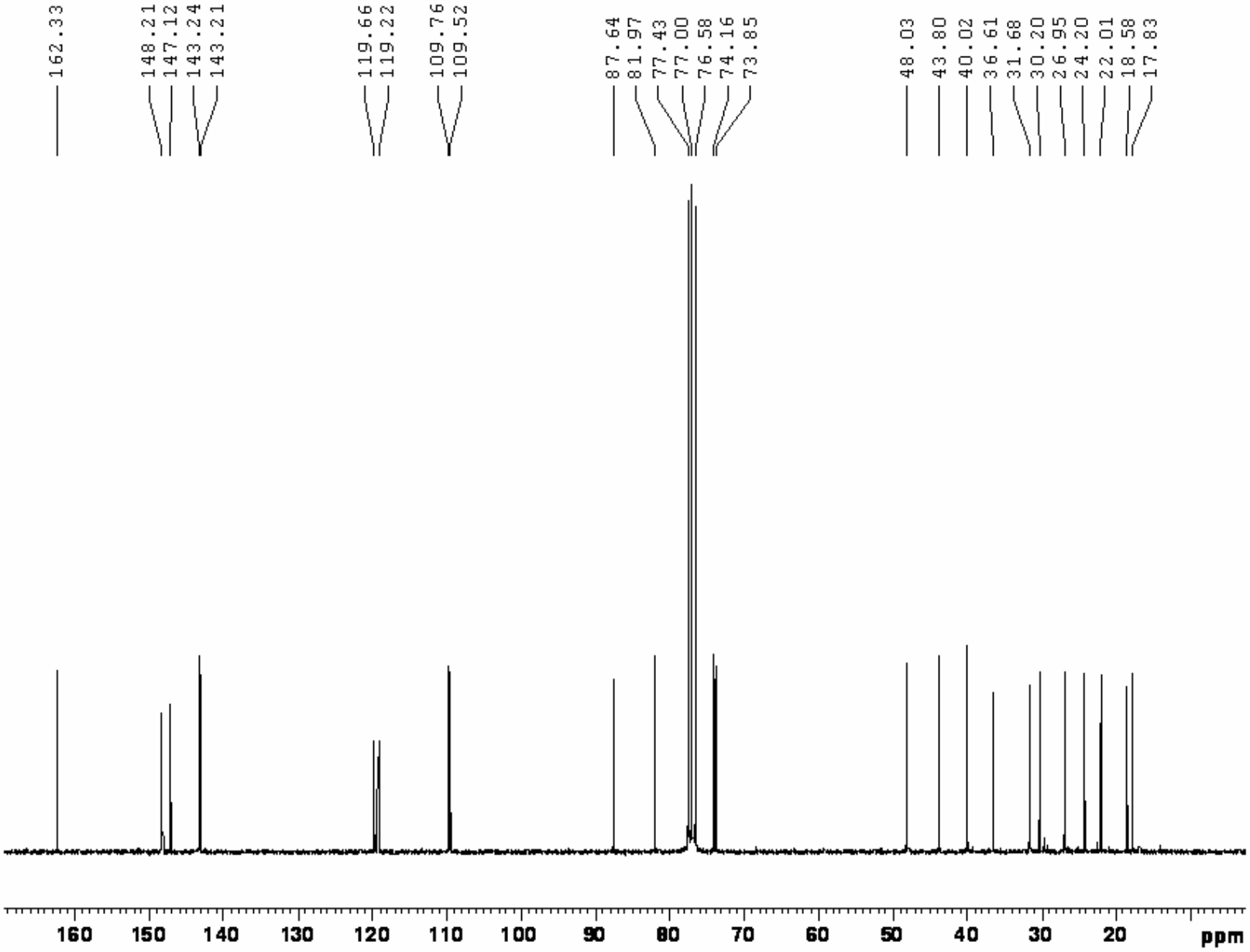
S3(A). ^{1}H NMR (300 MHz, $CDCl_3$) of Compound **3**



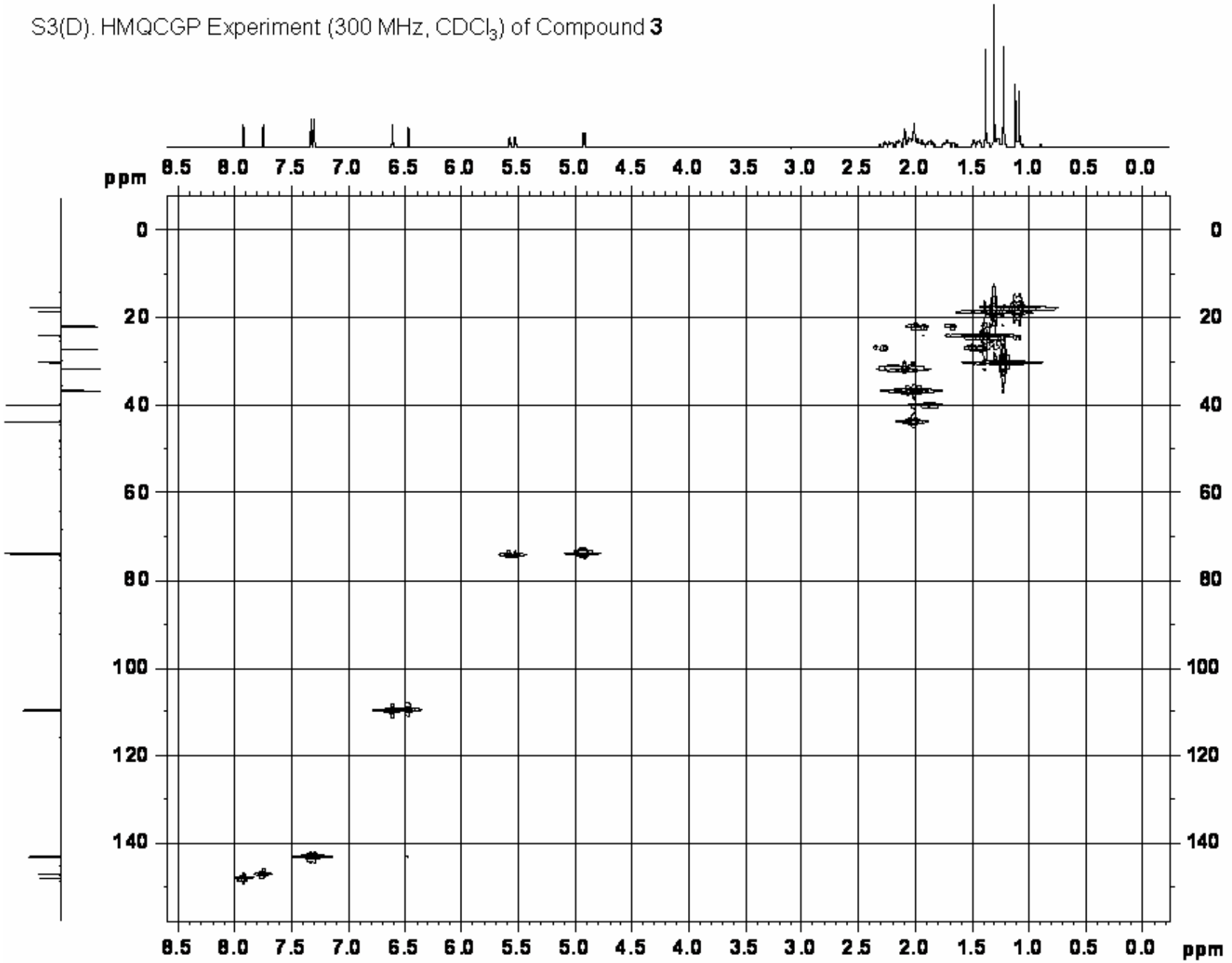
S3(B). Expansions of ^{1}H NMR (300 MHz, $CDCl_3$) of Compound **3**



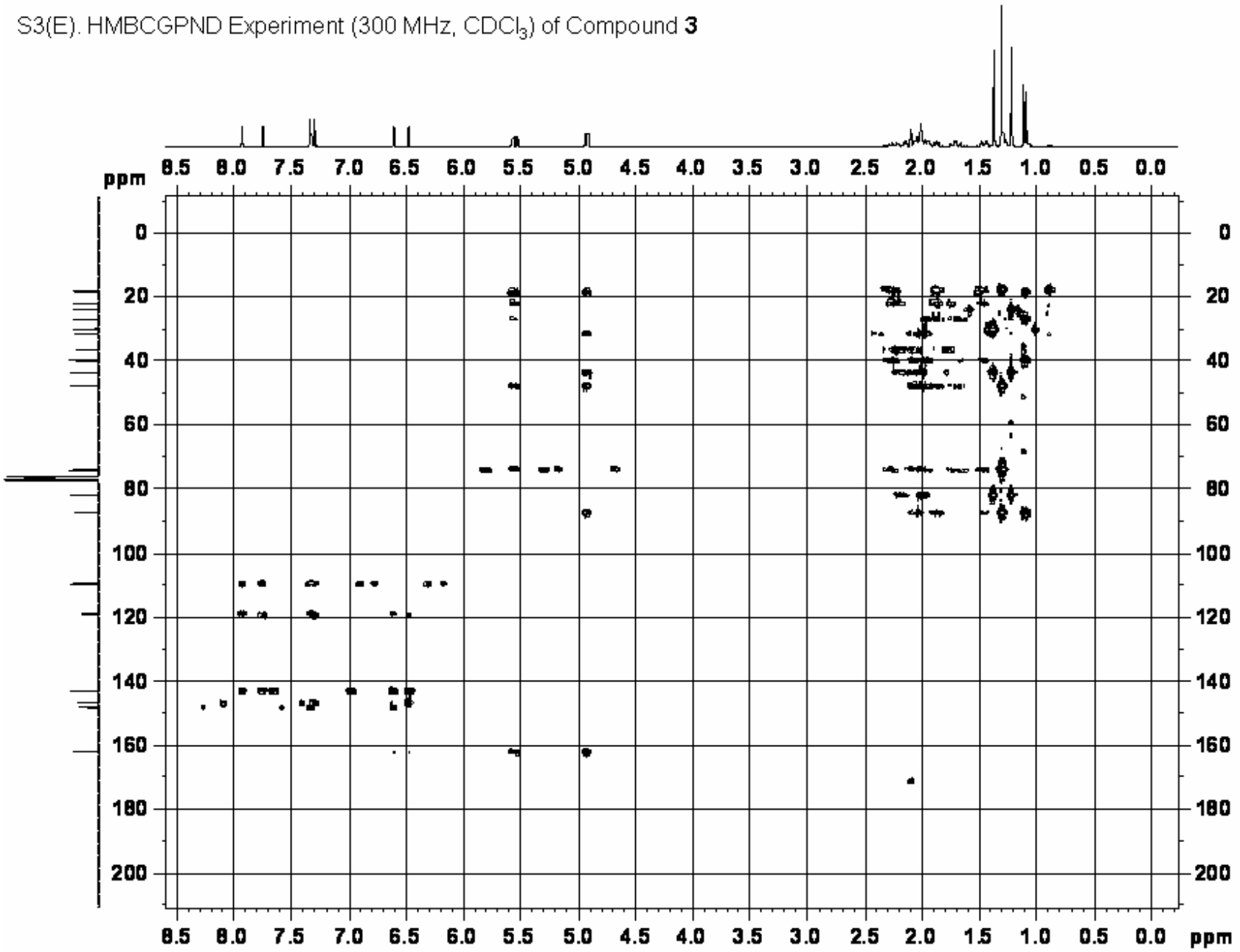
S3(C). ^{13}C NMR (75.5 MHz, $CDCl_3$) of Compound **3**



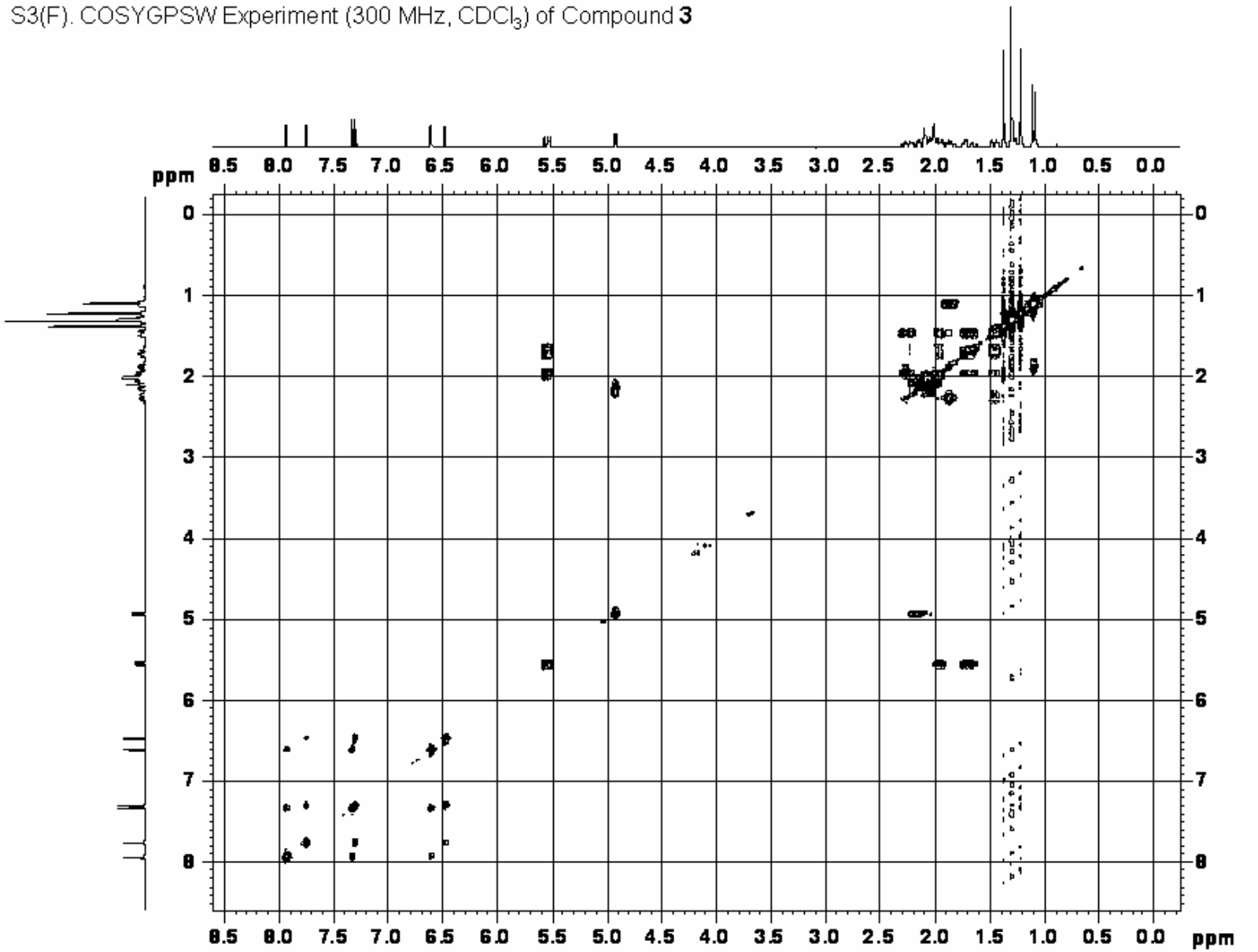
S3(D). HMQCGP Experiment (300 MHz, $CDCl_3$) of Compound **3**



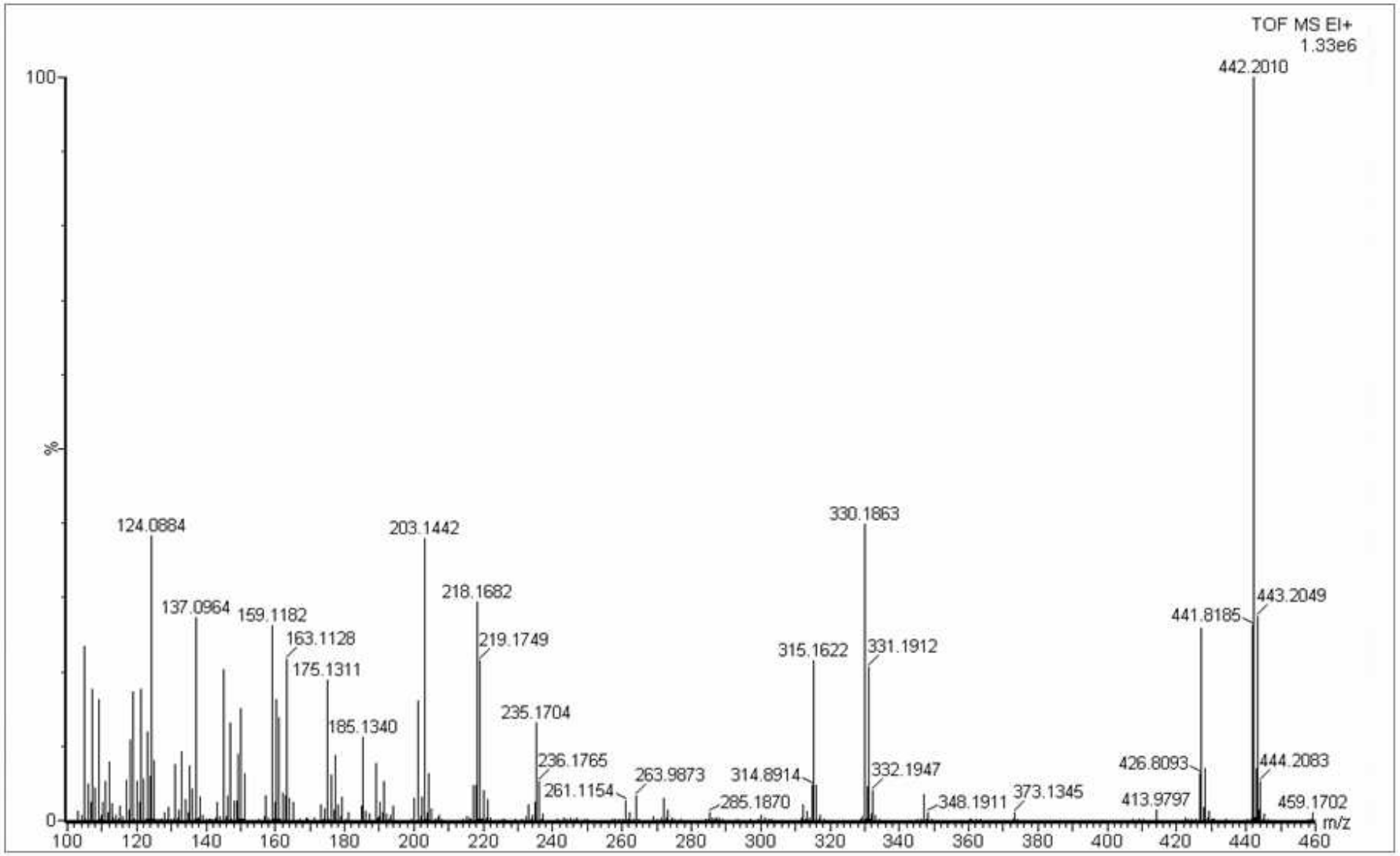
S3(E). HMBCGPND Experiment (300 MHz, $CDCl_3$) of Compound **3**



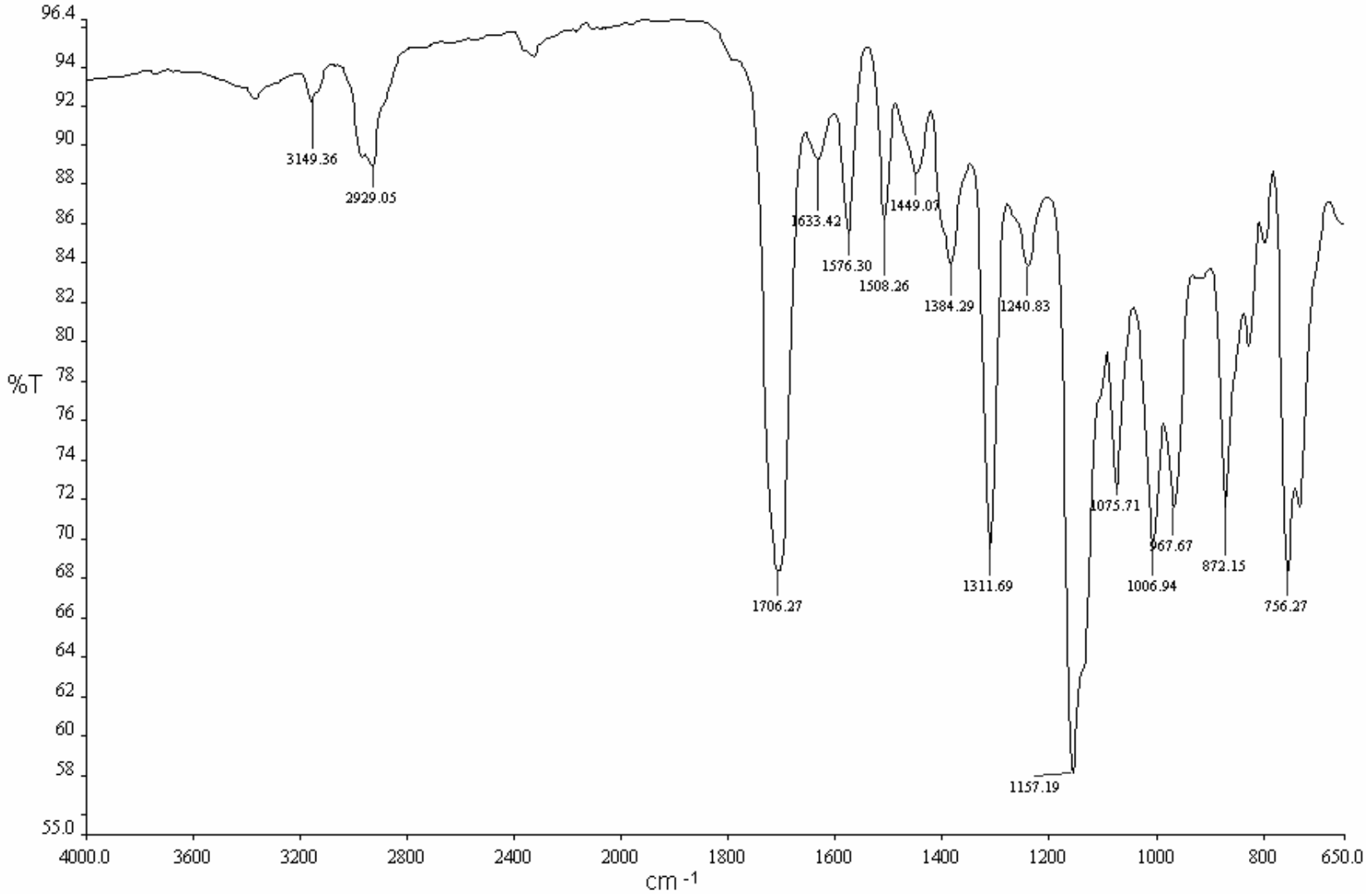
S3(F). COSYGPSW Experiment (300 MHz, $CDCl_3$) of Compound **3**



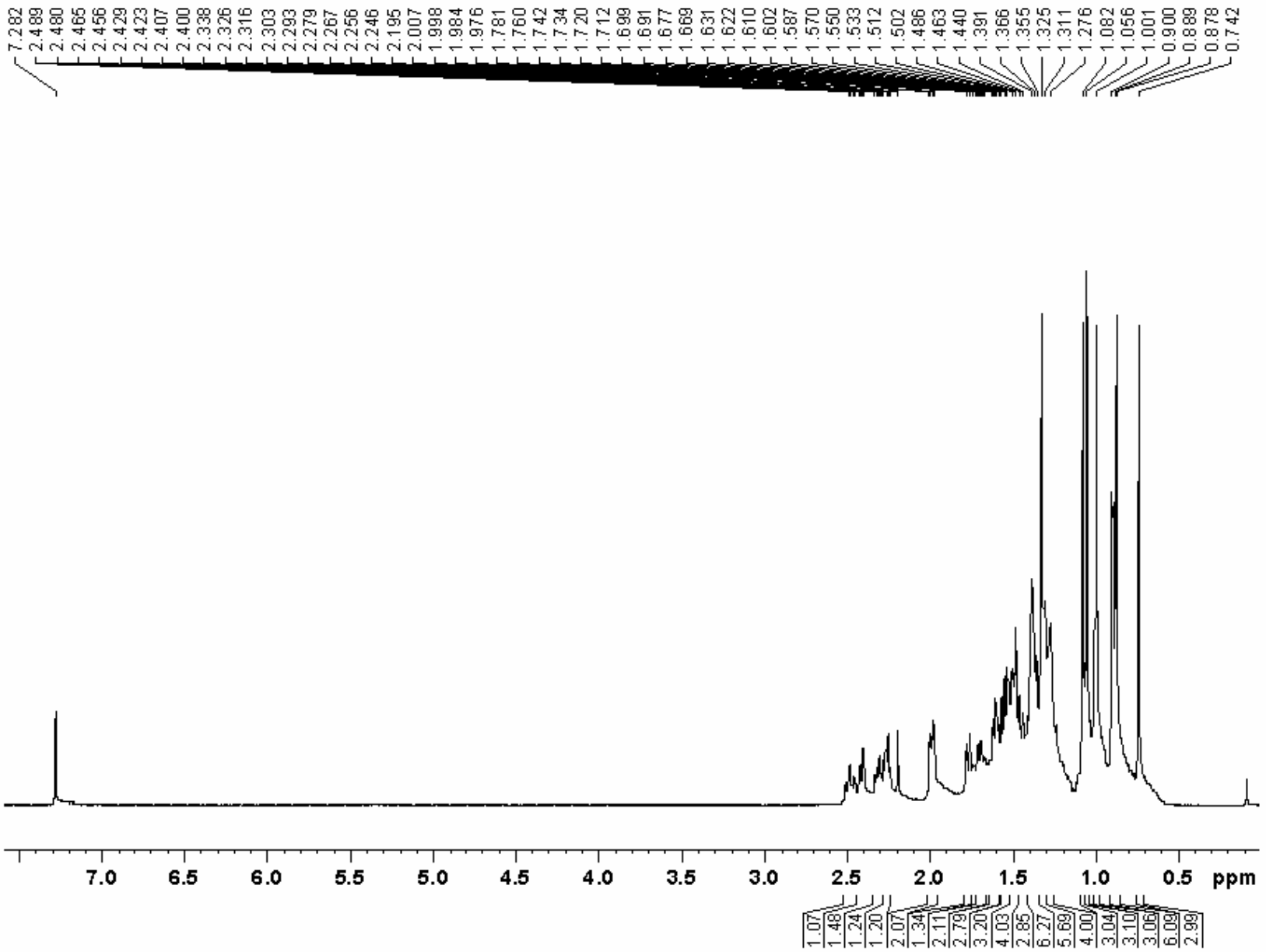
S3(G). HR EI MS of Compound **3**



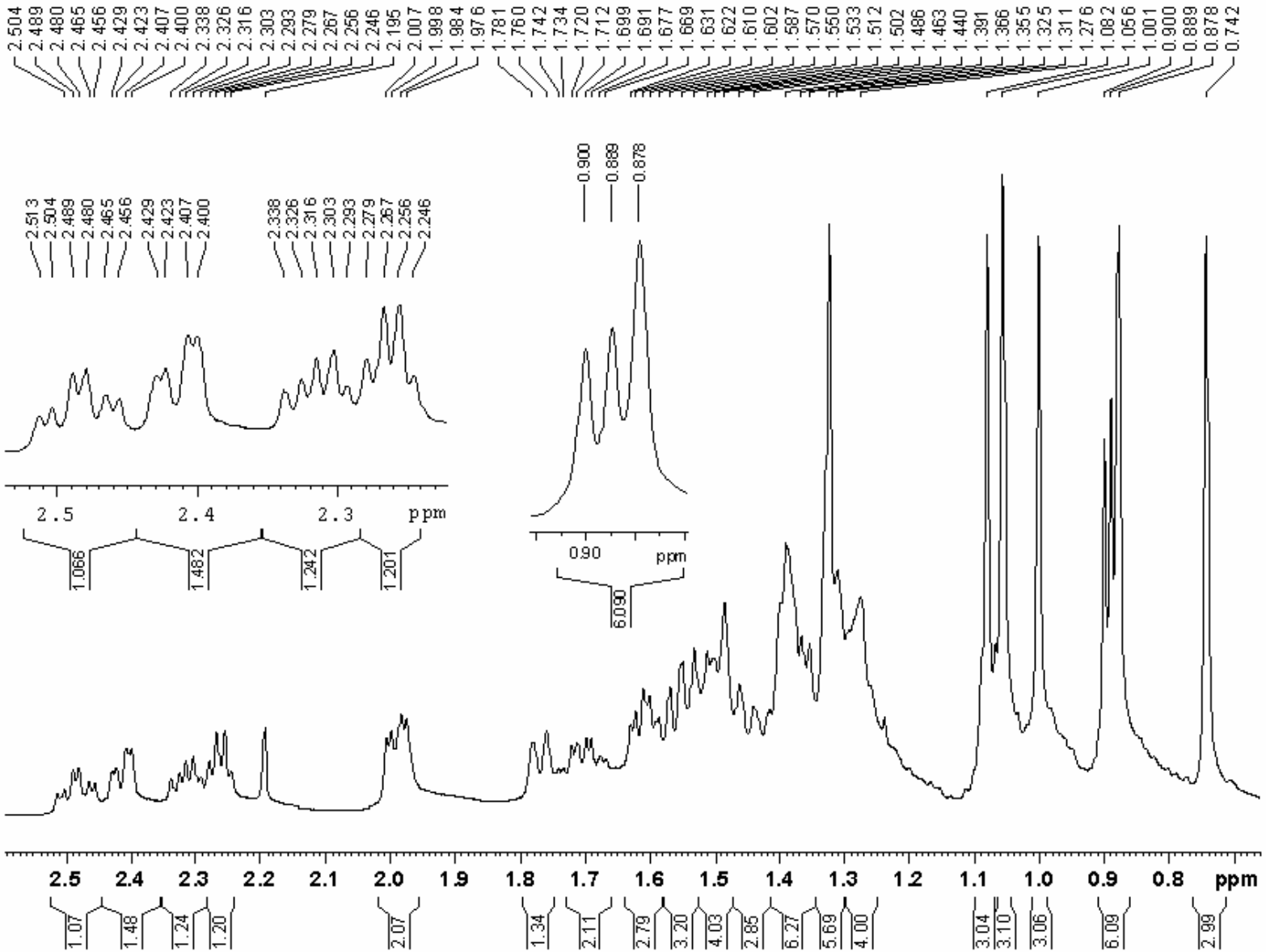
S3 (H). FTIR-ATR Spectrum of Compound **3**



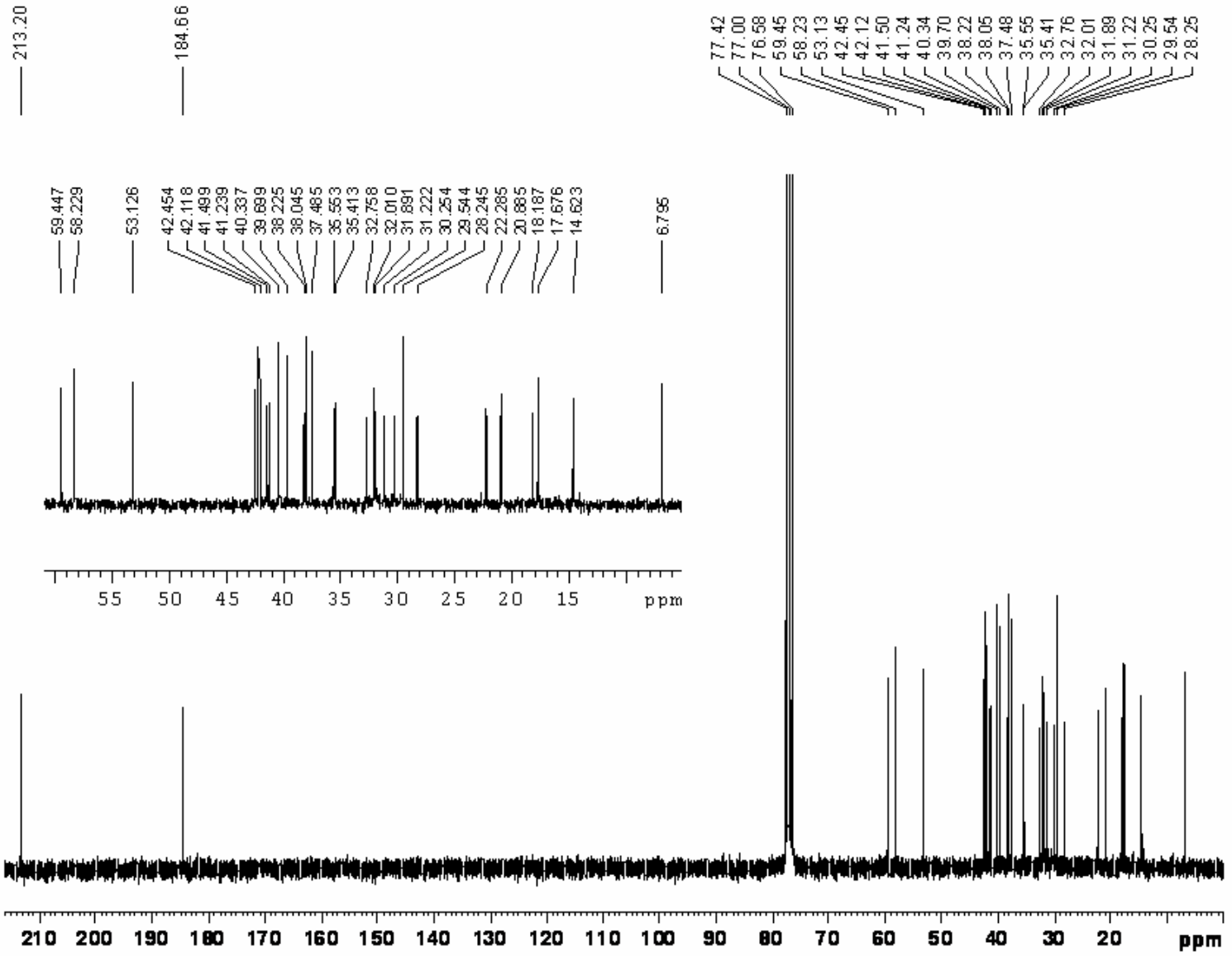
S4(A). ^{1}H NMR (600 MHz, $CDCl_3$) of Compound **4**



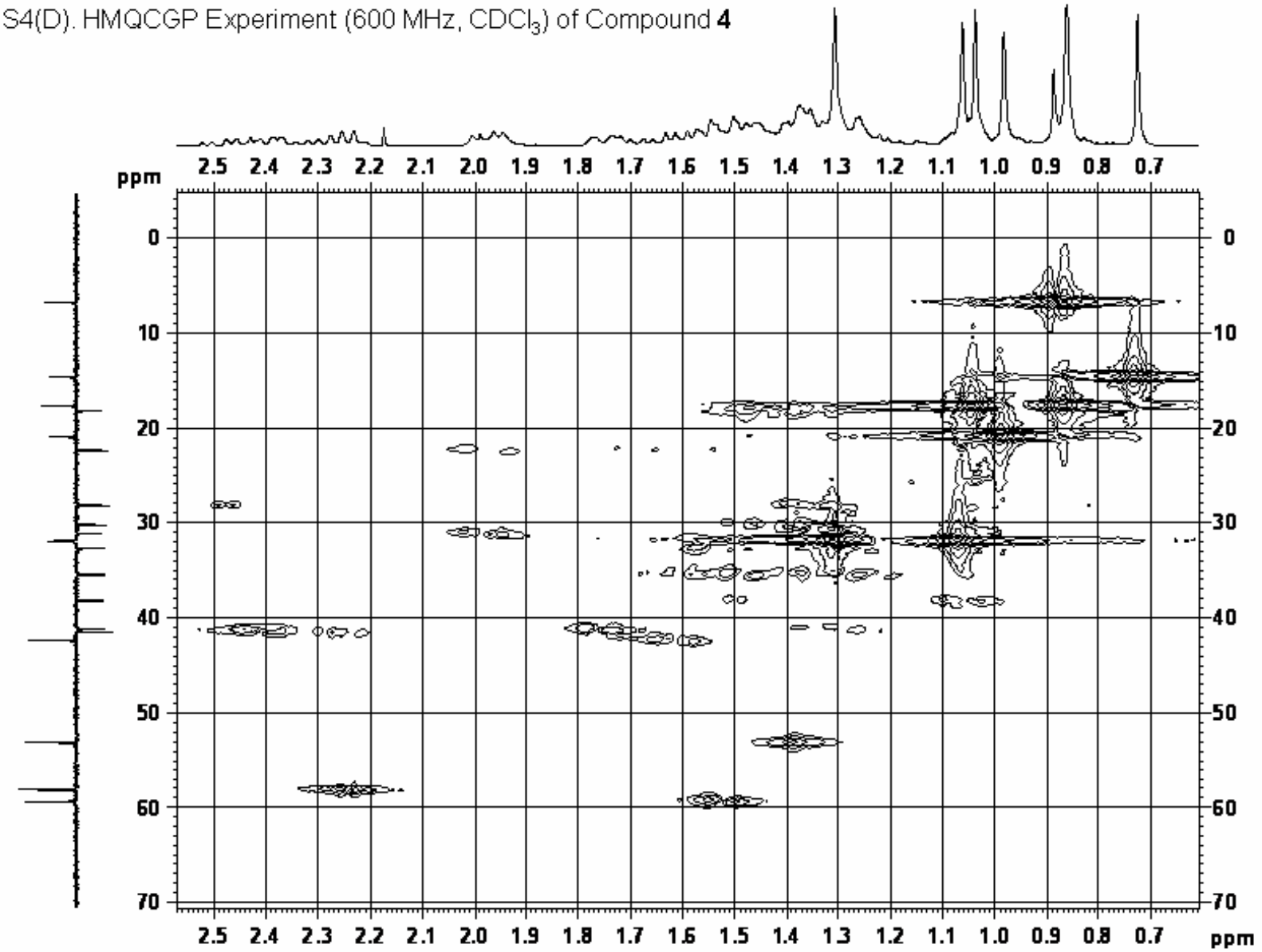
S4(B). Expansion of ^{1}H NMR (600 MHz, $CDCl_3$) of Compound **4**



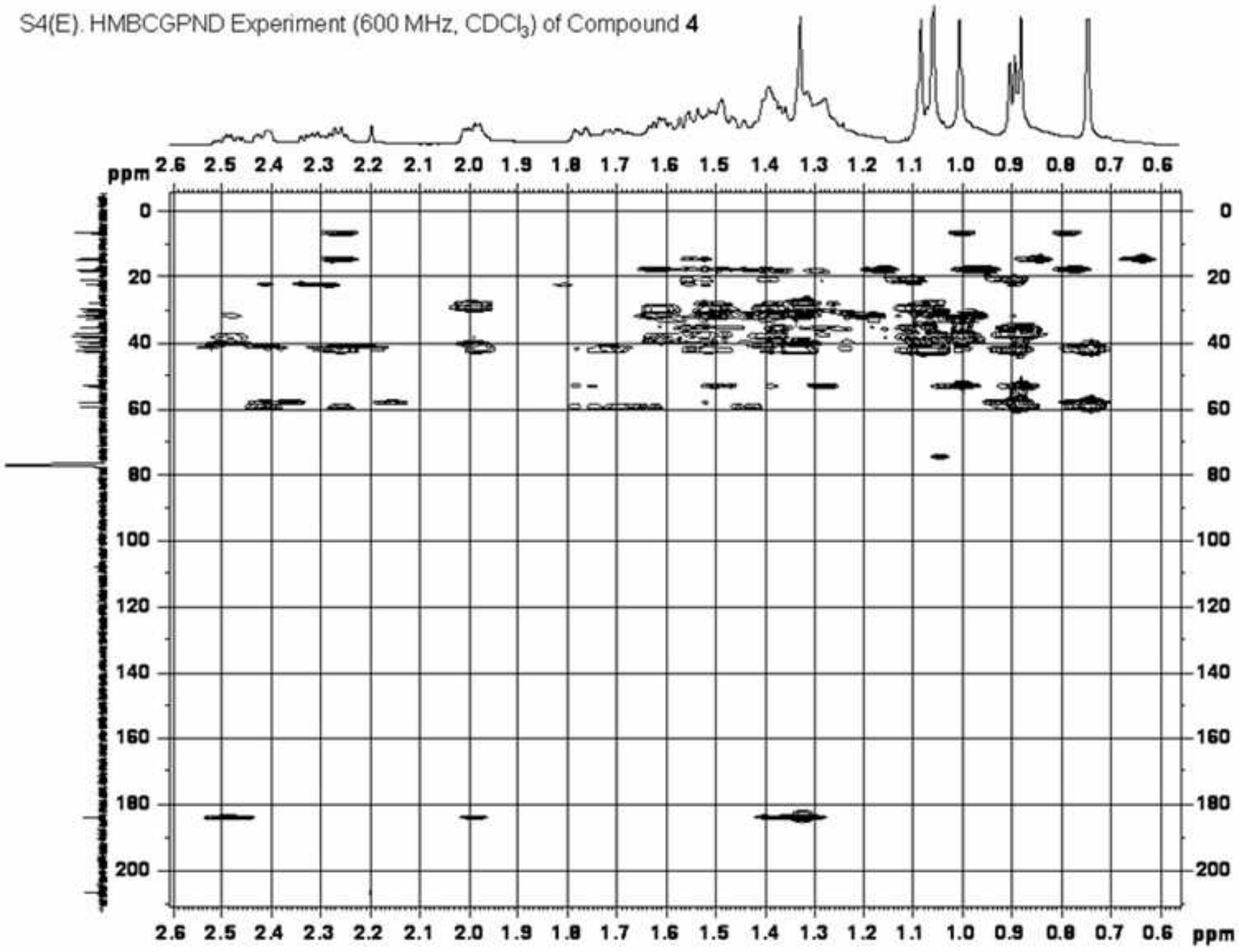
S4(C). ^{13}C NMR (150.9 MHz, $CDCl_3$) of Compound **4**



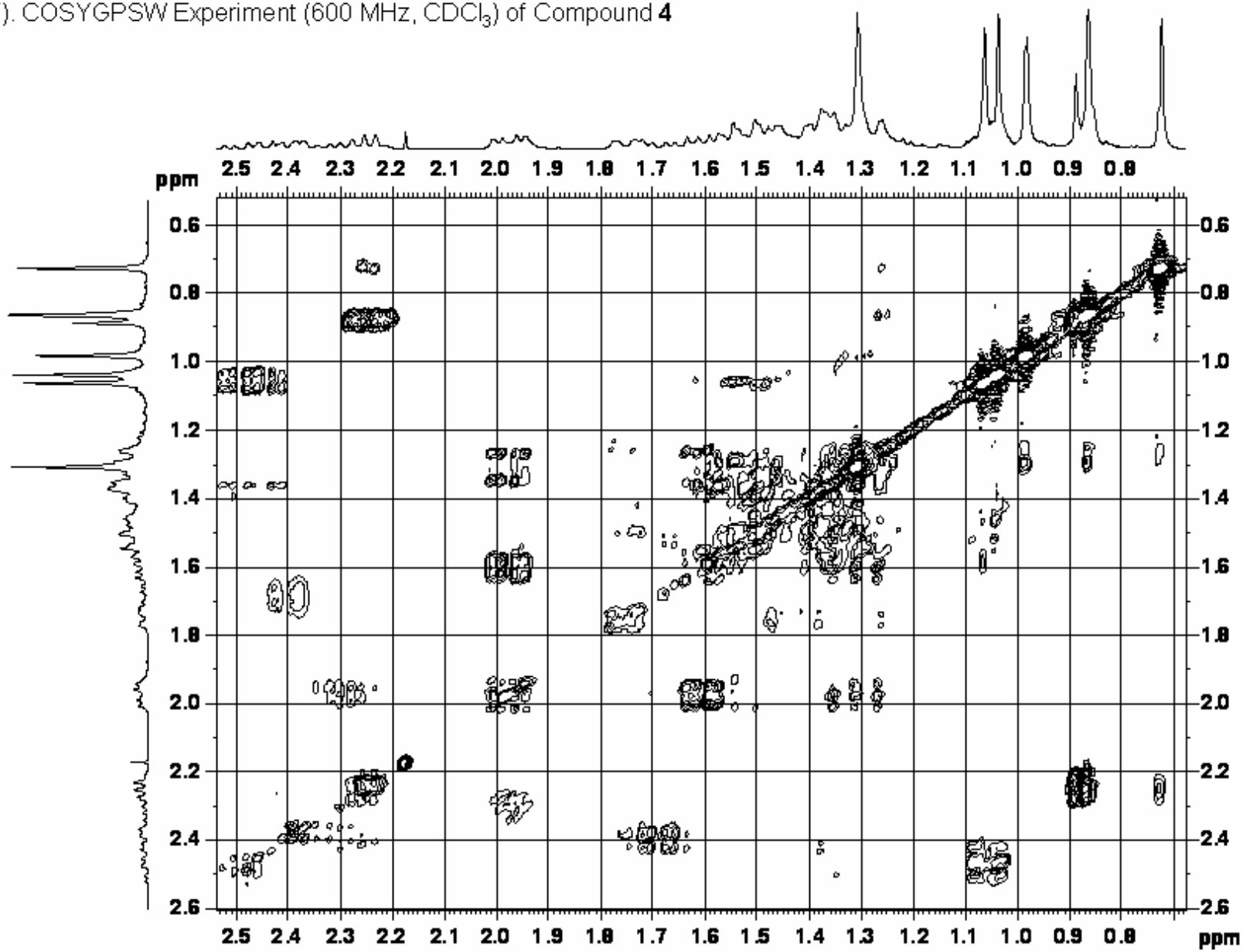
S4(D). HMQCGP Experiment (600 MHz, $CDCl_3$) of Compound **4**



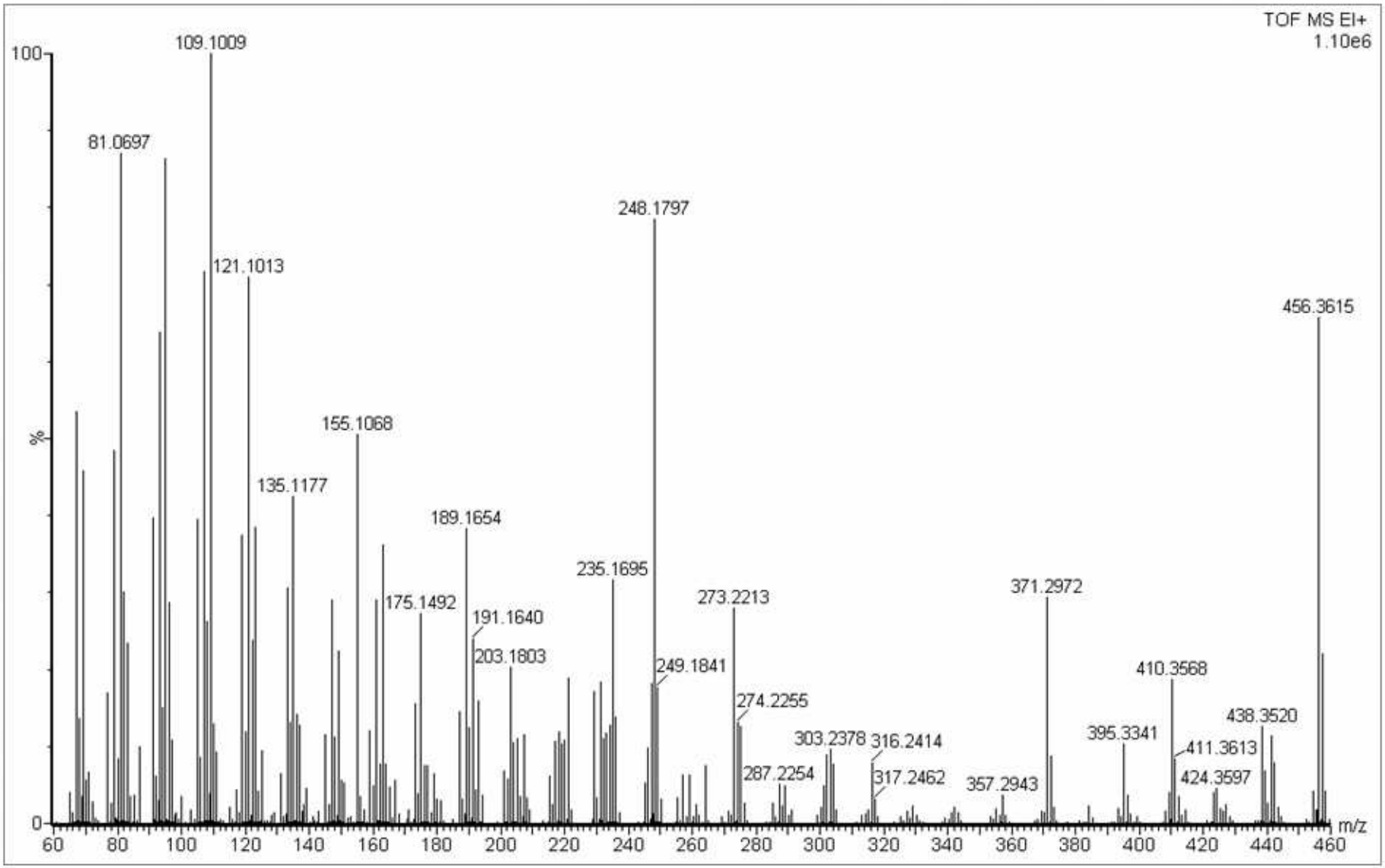
S4(E). HMBCGPND Experiment (600 MHz, $CDCl_3$) of Compound **4**



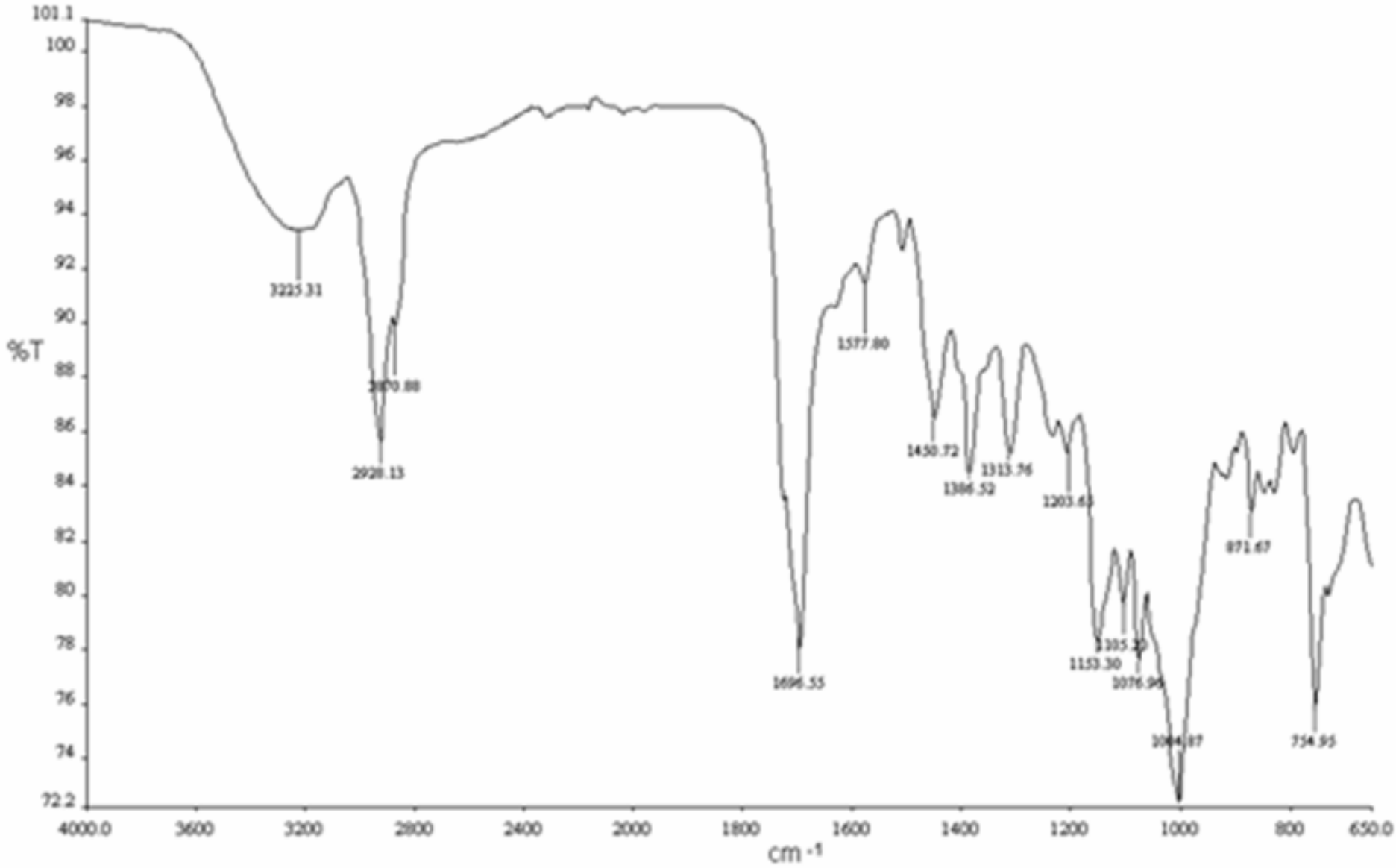
S4(F). COSYGPSW Experiment (600 MHz, $CDCl_3$) of Compound **4**



S4(G). HR EI MS of Compound **4**



S4 (H). FTIR-ATR Spectrum of Compound **4**



Experimental

Antimicrobial Activity by an Adaptation of the Agar Overlay Method

The antimicrobial activity of the compounds and the crude chloroform extract was evaluated using an adaptation of the agar overlay technique.[13] The microorganisms, two Gram-positive bacteria (*Bacillus subtilis* - ATCC 6633 and *Staphylococcus aureus* - ATCC 9144), two Gram-negative bacteria (*Pseudomonas aeruginosa* - NCTC 10332 and *Escherichia coli* - ATCC 11229) and one fungus (*Candida albicans* - ATCC 10231), were obtained from the Department of Biology, Microbiology section, of the University of Botswana. 10 µL aliquots containing different amounts (0.01 – 100 µg) of each compound were spotted onto faint pencil grids marked on aluminum backed TLC sheets (10 cm x 10 cm). Chloramphenicol (ChP) and miconazole (Mic), standards for the bacteria and for the fungus respectively, were spotted to a lower quantity of 0.0001µg/spot on separate sheets. The TLC sheets were spotted in duplicate for each microbe and were arranged in plastic bowls after spotting. Cultures of the microbes were prepared by aseptically transferring each bacteria from pure stock cultures in Petri dishes into 250 mL conical flasks containing 100 mL of sterilized nutrient broth (13 g/L) whilst the fungus was transferred into a 250 mL conical flask containing 100 mL of a mixture of 1 g of mycological peptone and 4 g of glucose. The cultures were incubated at 37 ^{0}C for 24 hours in a Lab-Line Orbit Auto Shaker (150 rpm). Growth of microorganisms was indicated by the mixtures turning cloudy. 10 mL aliquots of each of the bacteria suspensions were pipetted into 100 mL of sterilized nutrient agar (28 g/L) whilst 10 mL of fungus suspension was pipetted into 100 mL of sterilized Sabouraud dextrose agar (39 g/L). The sterilized agar solutions were maintained at 40^{o}C in a water bath before inoculation to prevent solidification. The inoculated agar mixtures were swirled to mix, quickly pipetted and uniformly spread onto the corresponding TLC sheets in the plastic bowls. The plastic bowls were covered and the agar was allowed to solidify on the TLC sheets which were then incubated for 24 hours at 25 ^{0}C (for *Bacillus subtilis, P. areuginosa* and *C. albicans*) and at 37 ^{0}C (for *Staphylococcus aureus* and *Escherichia coli*). After 24 hours, the TLC plates were sprayed with an aqueous solution (2.5 mg/mL) of thiazolyl blue, and incubated for a further 1 hour. The appearance of inhibition zones (white spots) against a blue-purple background was observed. The amount of sample (µg) that produced the least observable inhibition zone was recorded as the minimum inhibitory quantity (MIQ) (where the smaller the MIQ, the stronger the inhibitory potential of the sample). The strength of the inhibitory effect was further evaluated from the intensity and radius of the inhibition zones (the more intense and the larger the radius, the stronger the inhibitory effect of the compound at that amount). The whole experiment was repeated for the duplicate set of TLCs on a different day and the results compared. Strong activity was noted for **4** and **5** in the first trial thus in the second trial, lower quantities of **4** and **5** up to 0.0001 µg were spotted on additional TLC sheets.

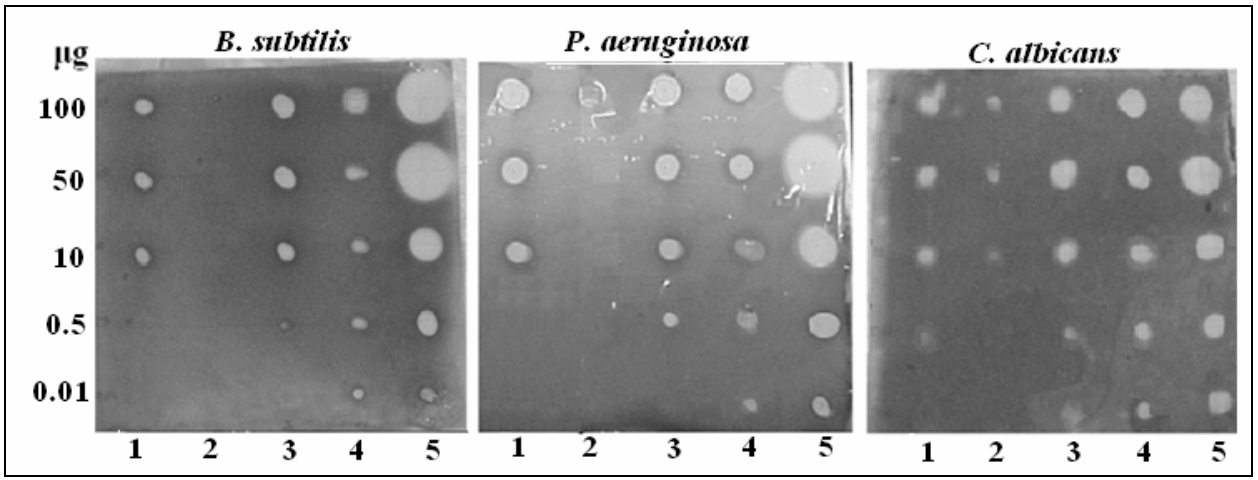


Figure 3 Sample photographs of TLCs showing inhibition zones for compounds **1**-**5** against *B. subtilis*, *P. aeruginosa* and *C. albicans*.

PROCEDURE FOR Crystal 2 (unabridged)

The X-ray intensity data were measured on a Siemens SMART 1K CCD area detector diffractometer system equipped with a graphite monochromator and Mo K_α fine-focus sealed tube (λ= 0.71073 Å) operated at 1.6 KW power (40 KV, 40 mA). The detector was placed at a distance of 4.00 cm from the crystal. Data was collected at room temperature (293 K). The initial unit cell and data collection was achieved by the SMART-NT software[i], utilizing COSMO[ii] for optimum collection of more than a hemisphere of reciprocal space. A total of 830 frames were collected with a 0.3° scan width in ω and an exposure time of 30 s.frame^{-1}. The first 50 frames were repeated at the end of collection to check for decay; none was found. The frames were integrated using a narrow-frame integration algorithm and reduced with the Bruker SAINT-Plus and XPREP[iii] software packages respectively. Data were corrected for absorption effects using the multi-scan technique SADABS[iv]. The structure was solved by the direct methods package SIR97[v] and refined using the WinGX software package[vi] incorporating SHELXL.[vii] The final anisotropic full-matrix least-squares refinement on F^2 with 302 variables converged at R1 = 0.0523 for the observed data and wR2 = 0.1397 for all data. The largest peak on the final difference electron density synthesis was 0.13 e.Å^{-3} at 1.04 Å from H13C and the deepest hole -0.17 e.Å^{-3} at 0.93 Å from C7 presented no physical meaning in the final refinements. The absolute conformation of the compound could not be determined from the refinement as no heavy scatterers are present in the coompound. The final value of the Flack parameter presented no physical meaning. The aromatic, methane, methylene and methyl H atoms were placed in geometrically idealized positions (C–H = 0.93 – 0.98 Å) and constrained to ride on their parent atoms with U_{iso}(H) = 1.2U_{eq}(C) for aromatic, methane, methylene and U_{iso}(H) = 1.5U_{eq}(C) for methyl. The methyl H's were located from a Fourier difference map and refined as a rigid rotor. Non-hydrogen atoms were refined with anisotropic displacement parameters. The molecular plot was drawn using the DIAMOND program[viii] with a 50% thermal envelope probability for non-hydrogen atoms. Hydrogen atoms were drawn as arbitrary sized spheres with radius of 0.135 Å.

The compound crystallised in the orthorhombic space group $P2_12_12_1$ (no. 19) with Z = 4, resulting in molecules lying on general positions in the unit cell. No classical hydrogen bonding is present in the crystal lattice, but several weaker non-classical C-H···O interactions can be detected in PLATON[ix]. The refinement of 1 showed large thermal vibrations on the C2' – C5' furoyl moiety indicating some freedom in the packing of the crystal lattice at this region. This was left untreated as it was not deemed necessary to execute a disorder refinement in the current scope of this research. Similar, but smaller vibrations are also noted in the low temperature collection of the compound.

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[i] Bruker (1998). SMART-NT. Version 5.050. Bruker AXS Inc., Madison, Wisconsin, USA.

[ii] COSMO. Version 1.53, Bruker AXS Inc., Madison, Wisconsin, USA, **2003**.

[iii] SAINT-Plus.Version 7.66A (including XPREP), Bruker AXS Inc., Madison, Wisconsin, USA, **2004**.

[iv] SADABS.Version 2004/1, Bruker AXS Inc., Madison, Wisconsin, USA, **1998**.

[v] Altomare, A.; Burla, M.C.; Camalli, M.; Cascarano, G.L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A.G.G.; Polidori, G.; Spagna, R. *J. Appl. Cryst.* **1999**, *32*, 115.

[vi] L.J. Farrugia, L.J. WinGX Version 1.70.01, *J. Appl. Cryst.* **1999**, *32*, 837.

[vii] Sheldrick, G.M. SHELXL97 Program for Crystal Structure Refinement, University of Göttingen, Germany, **1997**.

[viii] Brandenburg, K.; Putz, H., DIAMOND, Release 3.1a. Crystal Impact GbR, Bonn, Germany **2005**.

[ix] Spek, A. L. PLATON; J. Appl. Cryst. 36, 7-13. **2003**