Supplementary information

Design and Synthesis of Novel Phthalocyanines as Potential Antioxidant and Antitumor Agents Starting with New Synthesized Phthalonitrile derivatives

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Abstract

Formation of new phthalonitrile derivatives from reaction of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) considered as the key intermediate for the synthesis of new phthalocyanines. Moreover, new phthalonitrile derivatives **2, 5, 9, 10, 15** and **16** were reacted with 1,4-diazabicyclo[2.2.2]octane (DBO) or hydroquinone to afford the corresponding new phthalocyanine dyes **3, 6, 11, 12, 17** and **18**, respectively. In addition, cyclotetramerization of phthalic anhydride derivative **20** afforded new phthalocyanine dye **22** . The correct structures of the newly synthesized phthalocyanines were confirmed by spectral and elemental analyses. The antioxidant and cytotoxicity of the new compounds were studied and showed that compounds **17** and **18** have a very strong activity against all cell lines and act as good antitumor and antioxidant agents.

Keywords: Phthalonitrile, cyclotetramerization, phthalocyanines, disilazane, antioxidant activity and antitumor activity

Experimental Section

General remarks

Melting points were measured using an Electro thermal IA 9100 apparatus with open capillary tube and are uncorrected. All experiments were carried out using drying solvents. Products were purified by recrystallization. All reaction was carried out under microwave (Discover^{TM} by CEM, 2450 MHz, 20 bar, 300 W, 180 ^{o}C). The UV spectra were recorded on Bye-Unicam SP-1800 spectrometer. The IR spectrum (KBr discs) was recorded on a Pye Unicam Sp-^{3}-300 or a Shimadzu FTIR 8101 PC infrared spectrophotometer. The ^{1}H NMR 400 MHz and ^{13}CNMR 100 MHz spectrum were measured on a JEOL-JNM-LA spectrometer using DMSO as a solvent. All chemical shifts were expressed on the δ (ppm) scale using TMS as an internal standard reference. The coupling constant (*J)* values are given in Hz. Analytical data were obtained from the Micro analytical Center, Faculty of pharmacy, Cairo University, Cairo, Egypt. The mass spectra were recorded on a MS-S988 instrument operating at 70 eV. [23].

3.3. Biochemical assays (Antioxidant and antitumor properties)

3.3.1. Antioxidant properties

3.3.1.1. DPPH radical scavenging capacity

The evaluation of the antioxidant activity of new Phthalocyanines was done by (DPPH) radical scavenging activity. CuONPs, PdNPs and L-ascorbic corrosive were estimated as far as hydrogen giving or revolutionary searching capacity utilizing the steady extremist DPPH. About 0.1 mM t of DPPH in ethanol was ready and was added to 3.0 ml of new Phthalocyanines (20-100 µg/ml). After thirty minutes, the absorbance was estimated at 517 nm. The IC_{50} esteem was characterized as the focus (in µg/ml) of concentrates that restrains the development of DPPH revolutionaries by 50 %. The aftereffects of hostile to

oxidant action of Phthalocyanines, utilizing DPPH free revolutionary searching technique is arranged in table 2.

3.3.1.2. Hydroxyl-radical scavenging assay:

The degradation of 2-deoxy-Dribose by OH radicals generated in situ in Fenton's reaction [24] was used to determine the studied compounds' hydroxyl-radical scavenging capacity (HO• RSC). These radicals breakdown the sugar 2-deoxy-D-ribose into a set of fragments, some or all of which react with 2-TBA at low pH to create a pink chromogen that can be measured.by spectrophotometric method at 532 nm. Different aliquots (0.005–0.5 mL) of sample solution in methanol were added to test tubes (final concentration ranged between 0.01 and 8 m*M*), each containing 0.1 mL of 5 m*M* H_2O_2, 0.1 mL of 10 m*M* $FeSO_4$ and 0.1 mL of 0.05 *M* 2-deoxy-D-ribose and 0.067 M KH2PO4-K2HPO4 buffer pH 7.4 to a final volume of 3 mL. Another reaction mixture under the same condition and without sample was used as the control. After an incubation period of 1 h at 37 °C, 2 mL of TBA reagent (10.4 mL of 60% (v/v) $HClO_4$, 3 g TBA and 120 g of trichloroacetic acid, and0.2 mL of 0.1 M EDTA were added to the reaction mixture, and the tubes were heated at 100°C for 20 min. After cooling, absorbance of the reaction mixtures and control was recorded at 532 nm. Percentage of HO• RSC was calculated using the following equation:

$$RSC\ (\%) = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Three replicates were recorded for each sample; BHT and BHA were used as reference compounds. See table 2.

3.3.2. Cytotocity assays

The cytotoxic activity of the newly synthesised Phthalocyanines a dyes was tested against two cell lines, human heptacellular liver carcinoma cell line (HepG2) and human breast adenocarcinoma cell line (MCF-7) with human lung fibroblast cell line (WI-38) and adult African green monkey kidney cell line (VERO) as controls. After a 7-day bath culture, the cells were planted in 96-well plates at 37 °C for 24 h. Under 5% CO_2. The

cells were cultured either alone (negative control) or with various concentrations of sample (1000, 500, 200, 50 mg/mL). In 96-well flat bottom microplates, cells were suspended in RPMI-1640 media with 1% antibiotic-antimycotic mixture (104 m/mL potassium penicillin, 104 mg/mL streptomycin sulphate, and 25 mg/mL Amphotericin B) and 1% L-glutamin at 37 °C under 5 % CO2. After 96 h of incubation, the medium was again aspirated, trays were inverted onto a pad of paper towels, the remaining cells rinsed carefully with medium, and fixed with 3.7% (v/v) formaldehyde in saline for at least 30 min. The % viability of cells was examined visually as described previously. [25, 26]. See table 3.

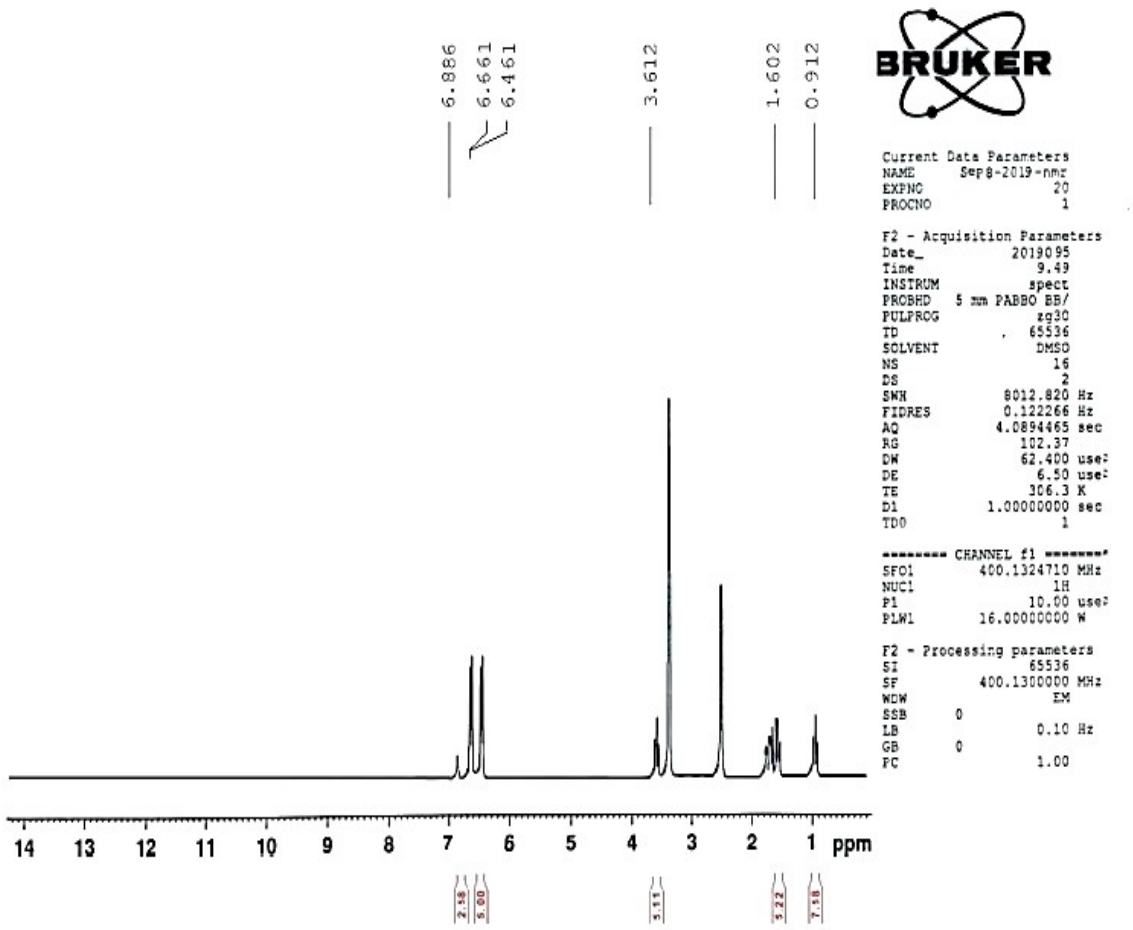


Fig. (S1): ^{1}H NMR Spectrum of Compound (1)

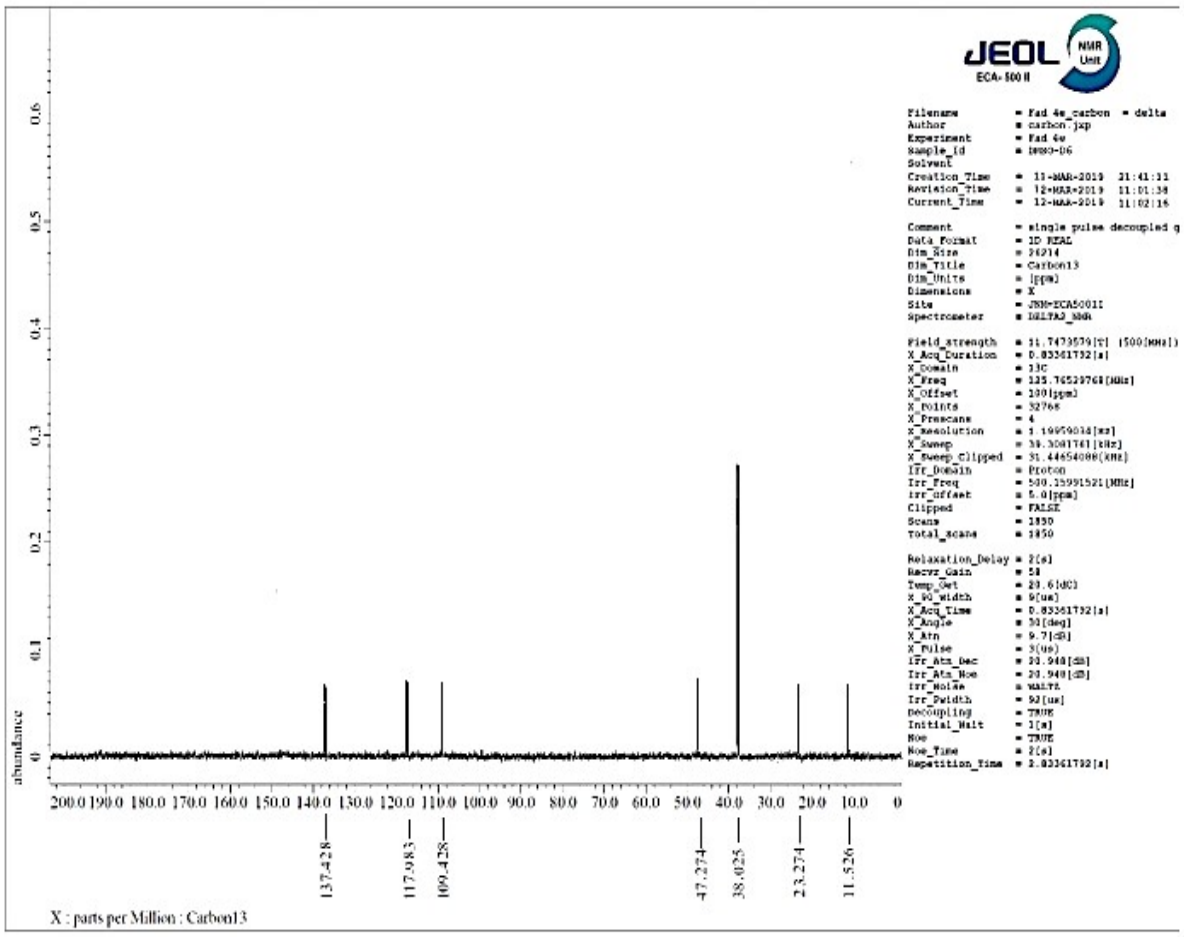


Fig. (S2): ^{13}C NMR Spectrum of Compound (1)

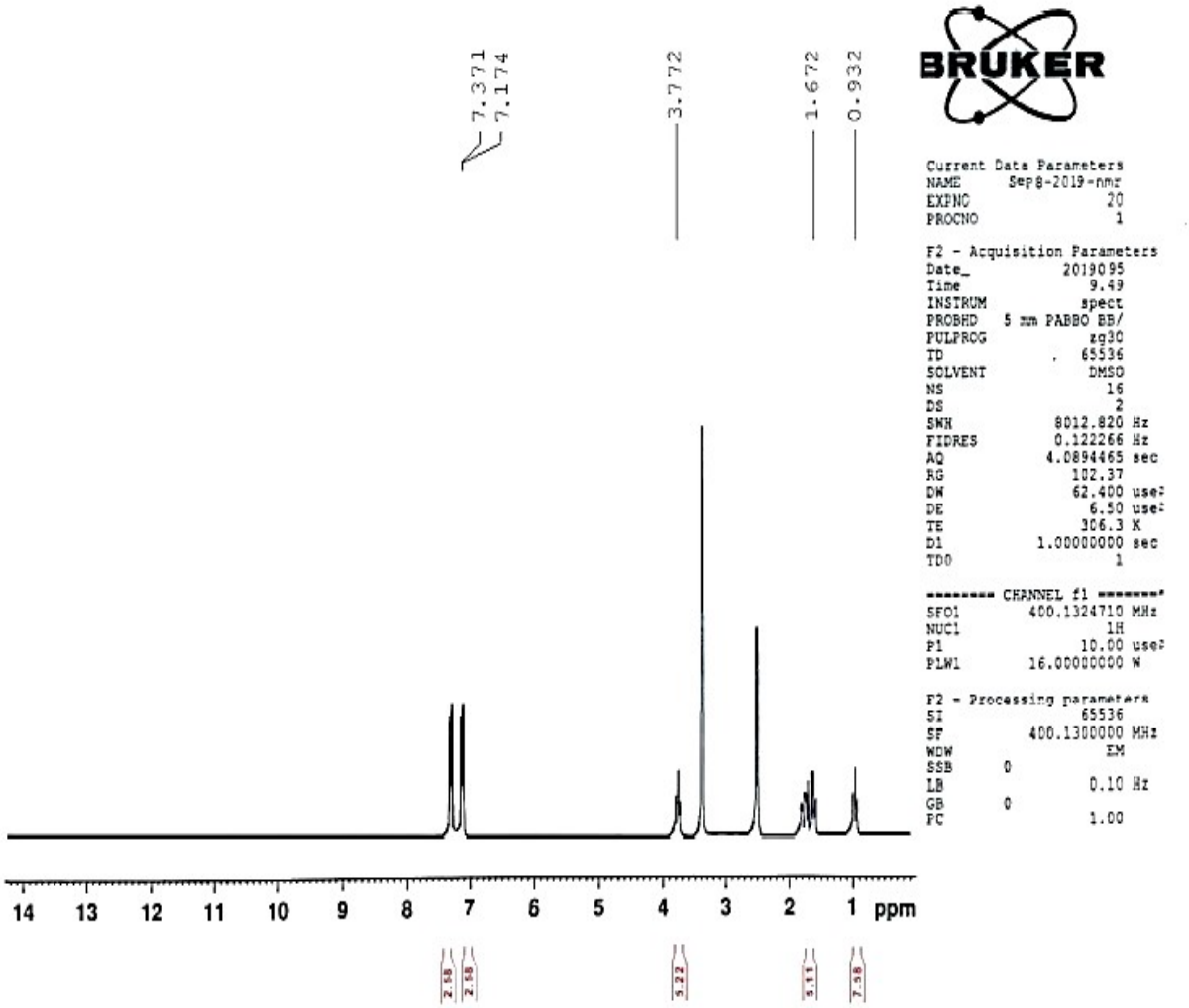


Fig. (S3): ^{1}H NMR Spectrum of Compound (2)

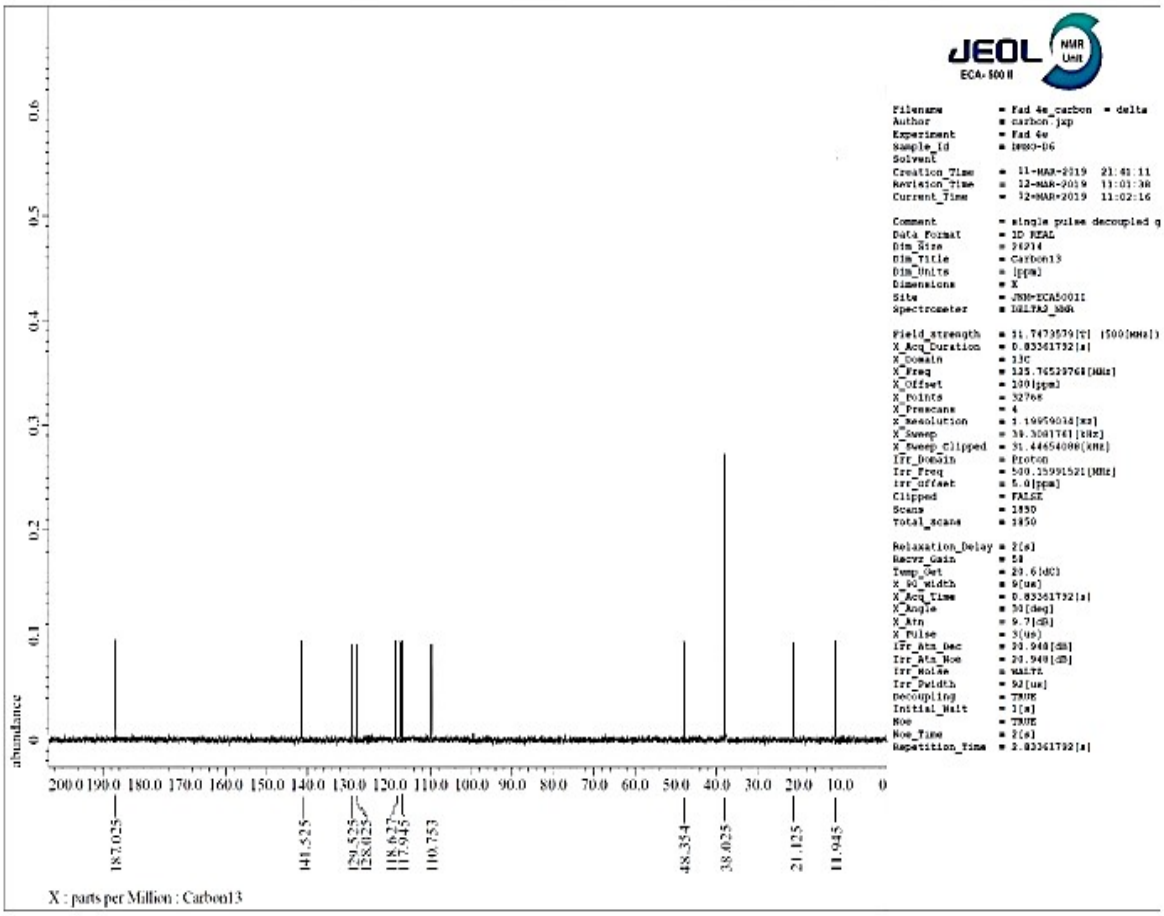


Fig. (S4): ^{13}C NMR Spectrum of Compound (2)

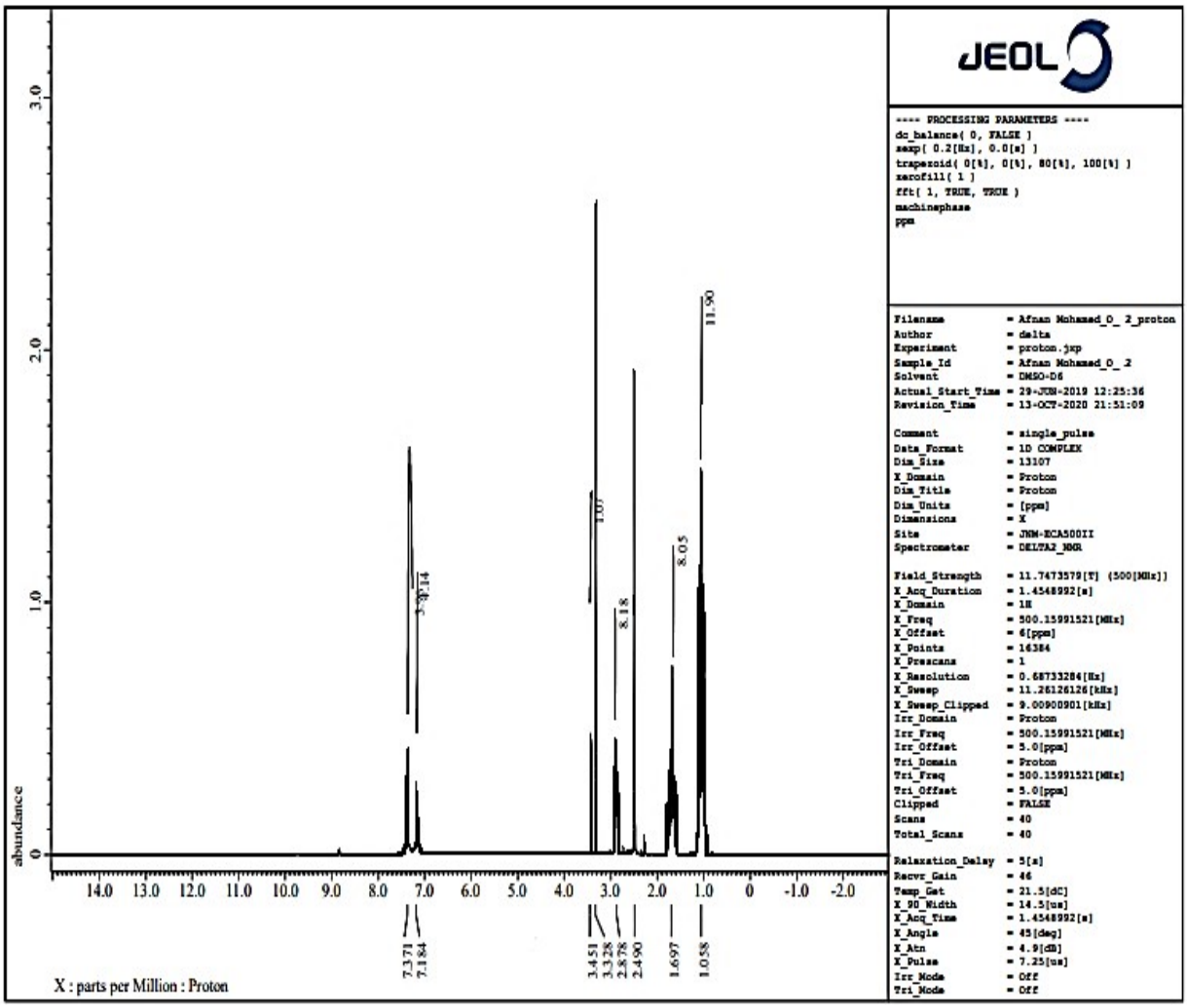


Fig. (S5): ^{1}H NMR Spectrum of Compound (3)

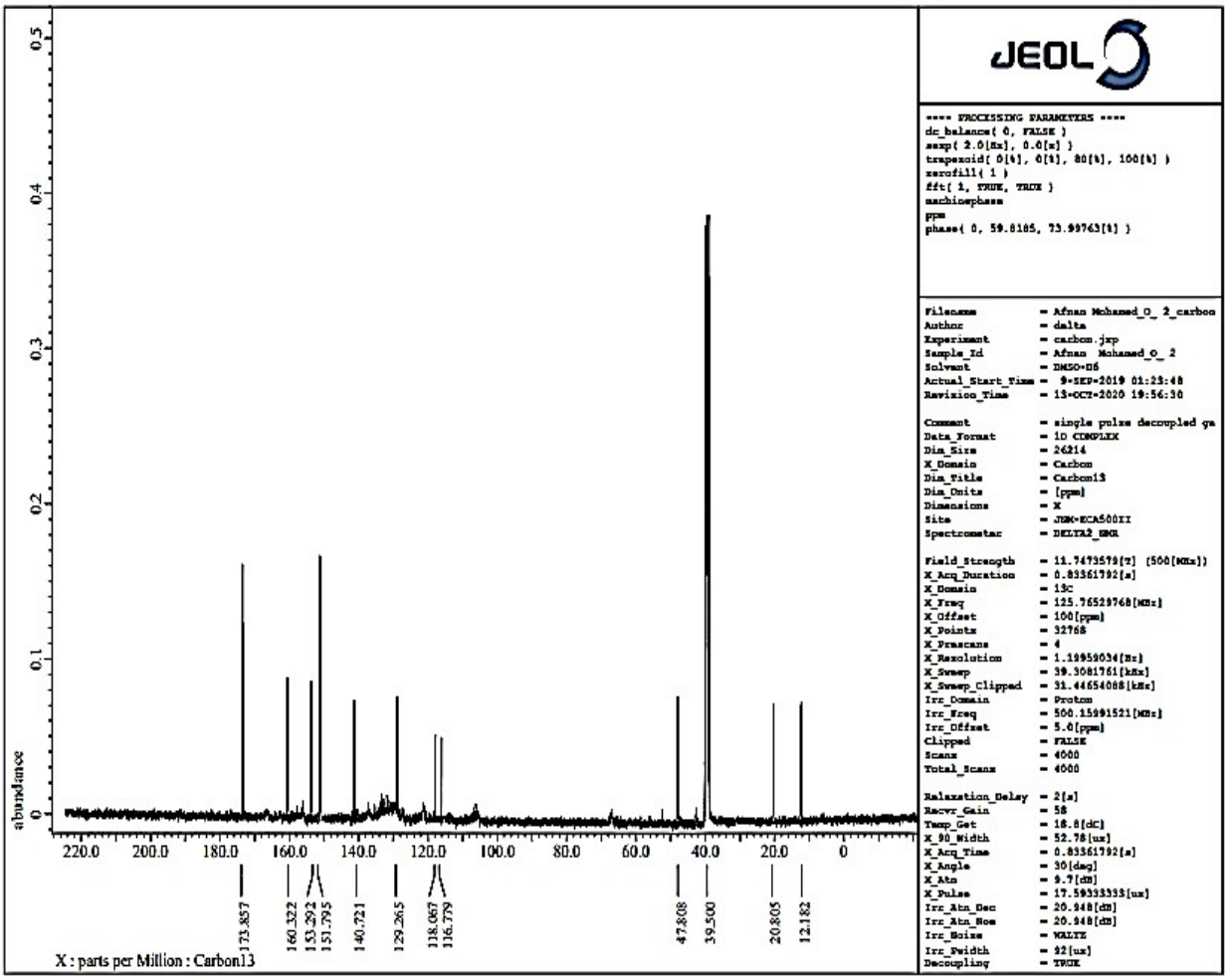


Fig. (S6): ^{13}C NMR Spectrum of Compound (3)

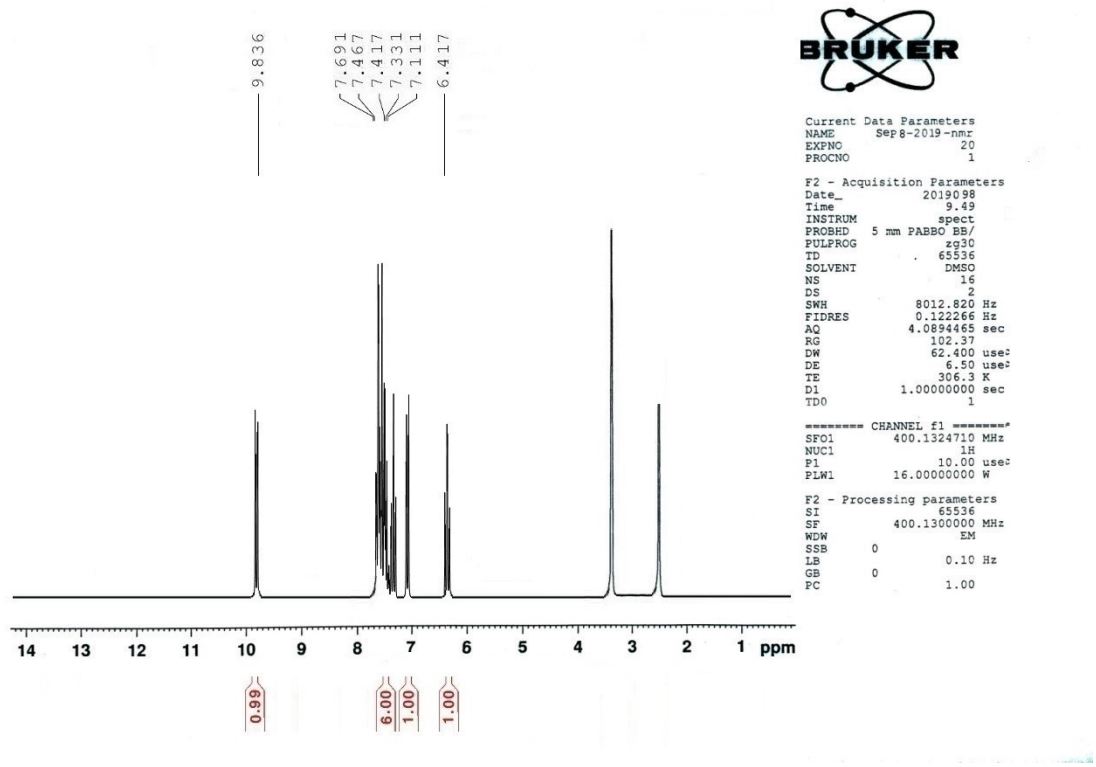


Fig. (S7): ^{1}H NMR Spectrum of Compound (5)

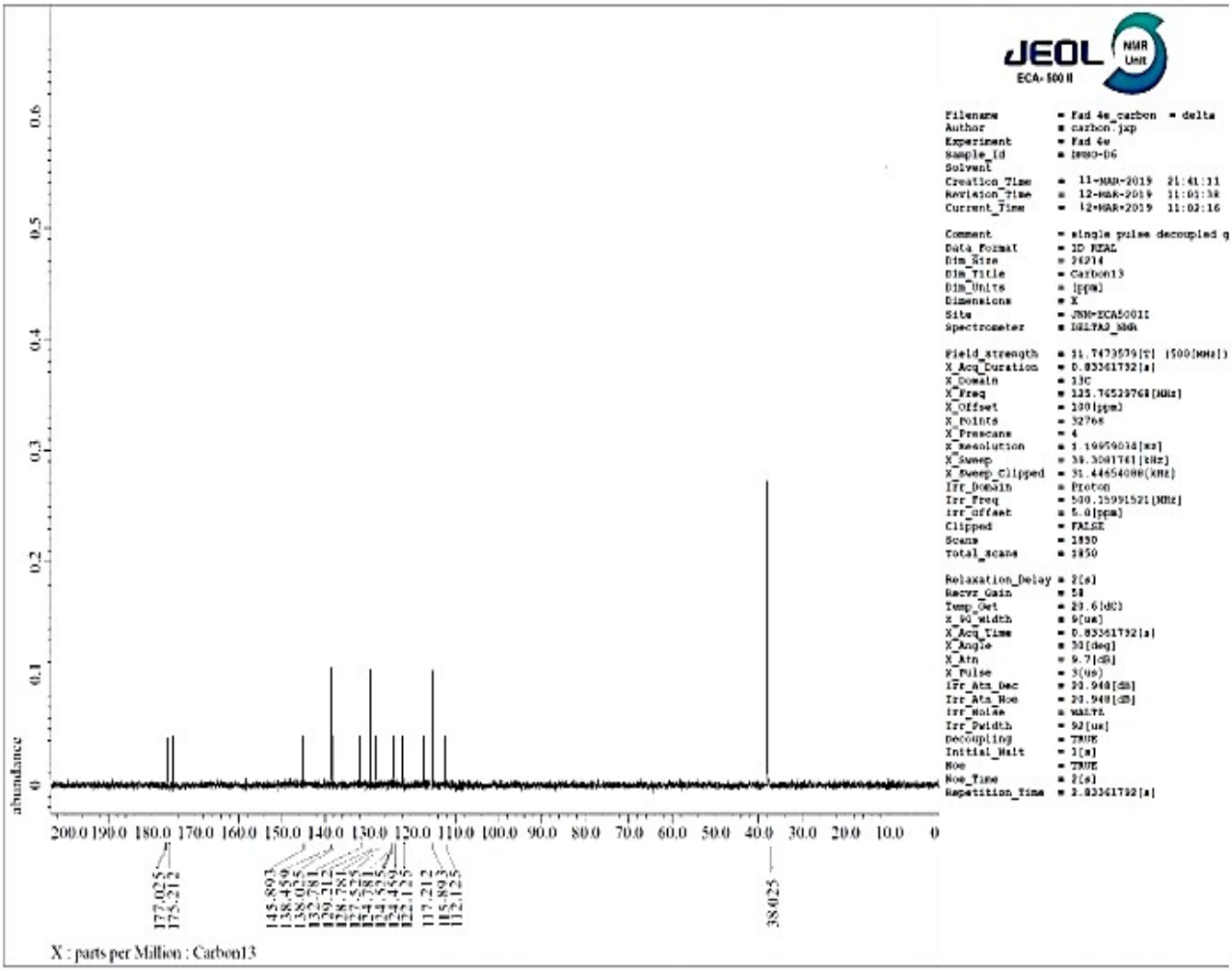


Fig. (S8): ^{13}C NMR Spectrum of Compound (5)

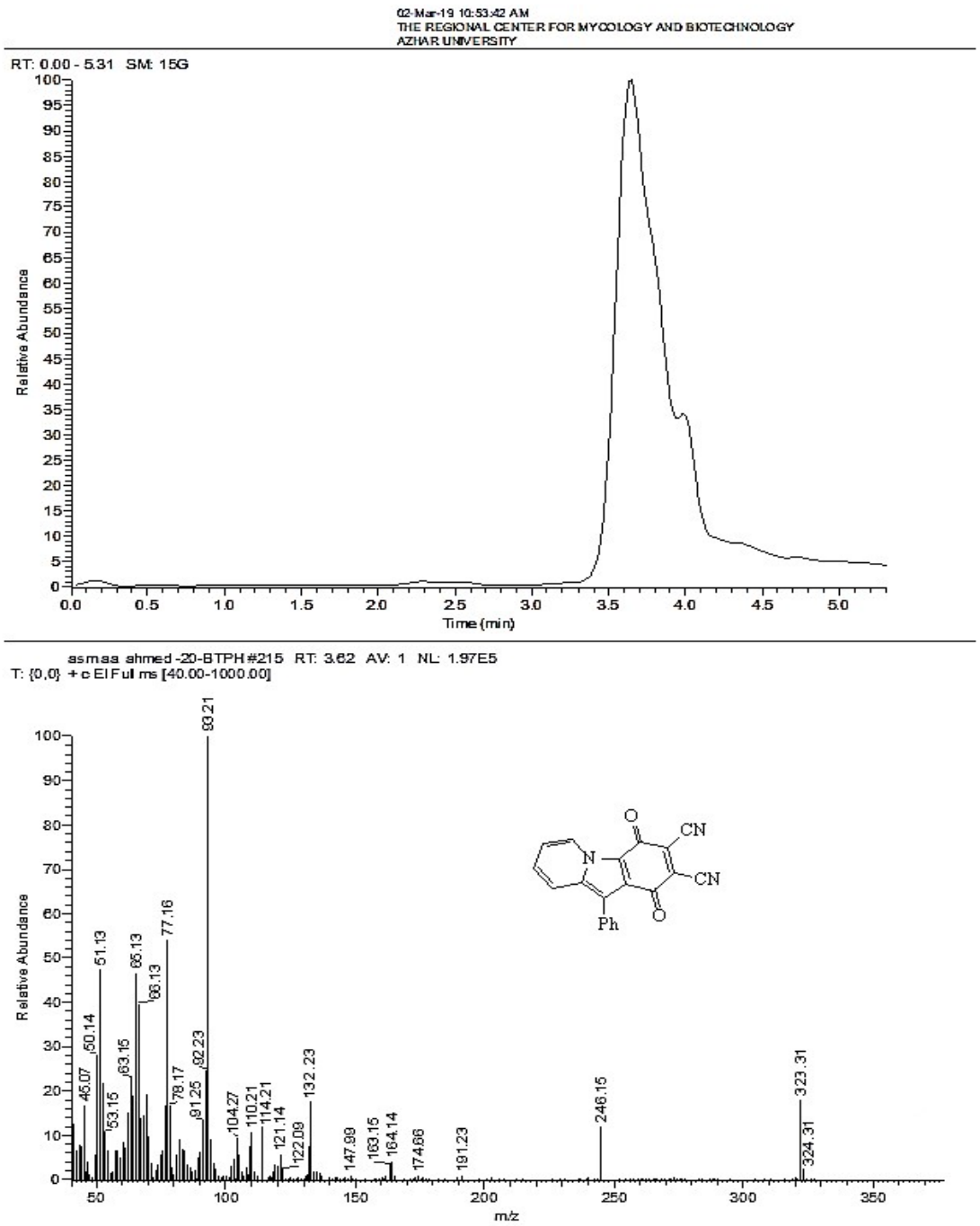


Fig. (S9): MS Spectrum of Compound (5)

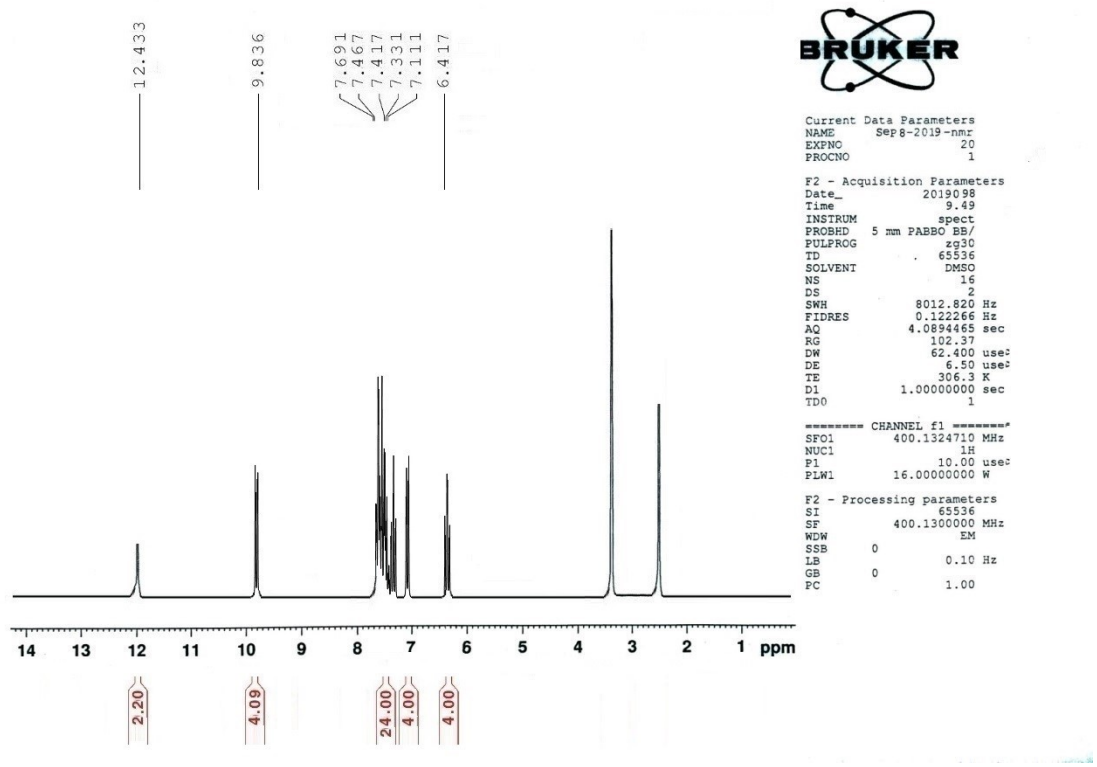


Fig. (S10): ^{1}H NMR Spectrum of Compound (6)

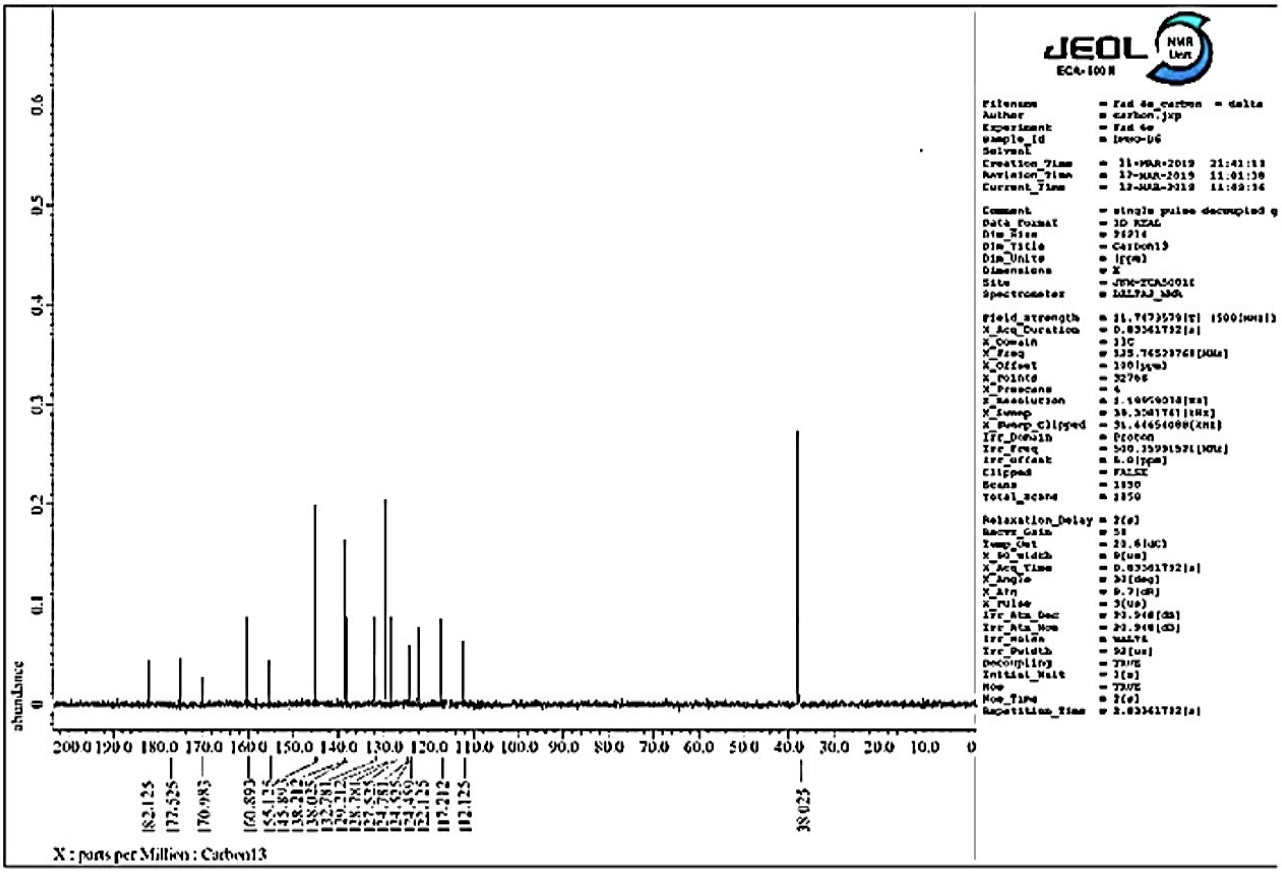


Fig. (S11): ^{13}C NMR Spectrum of Compound (6)

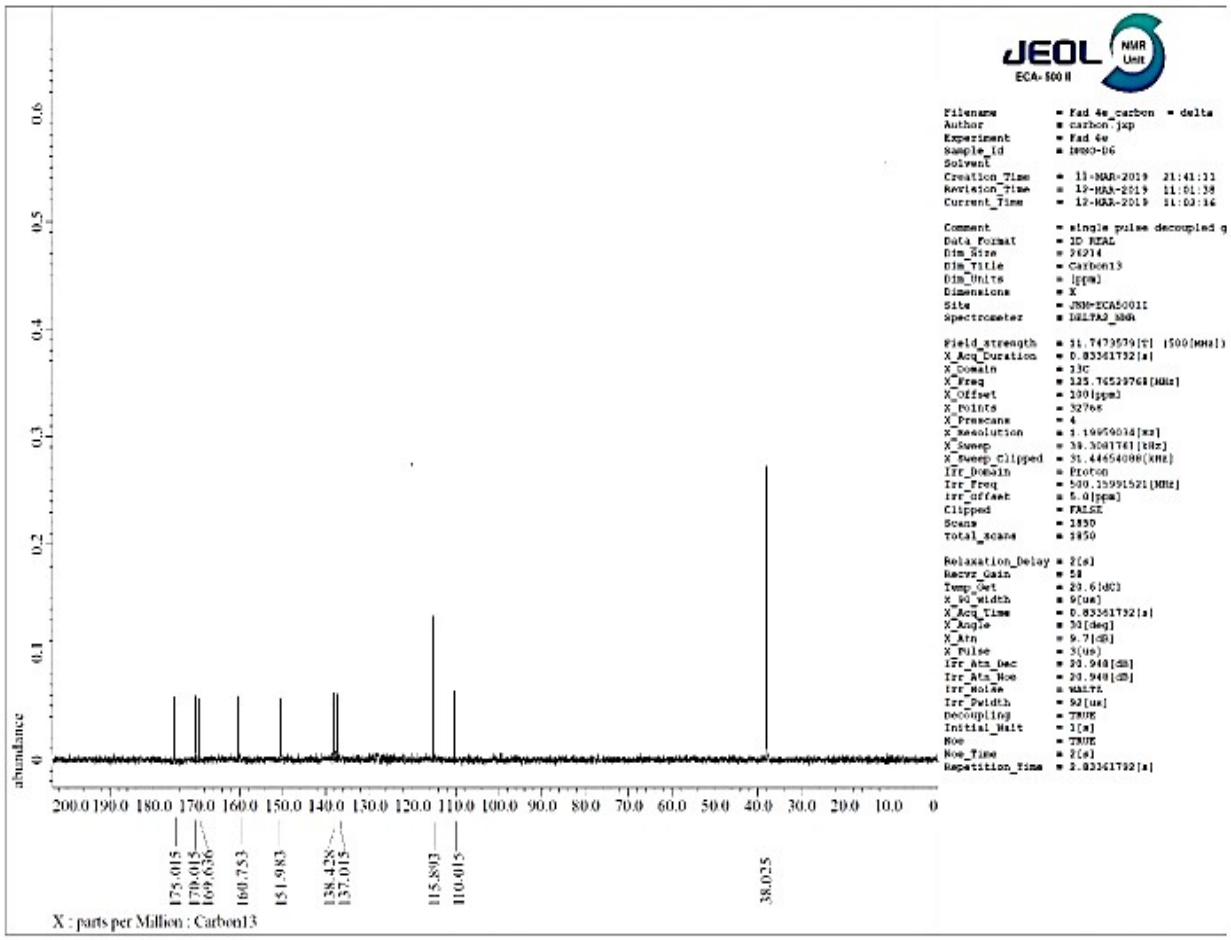
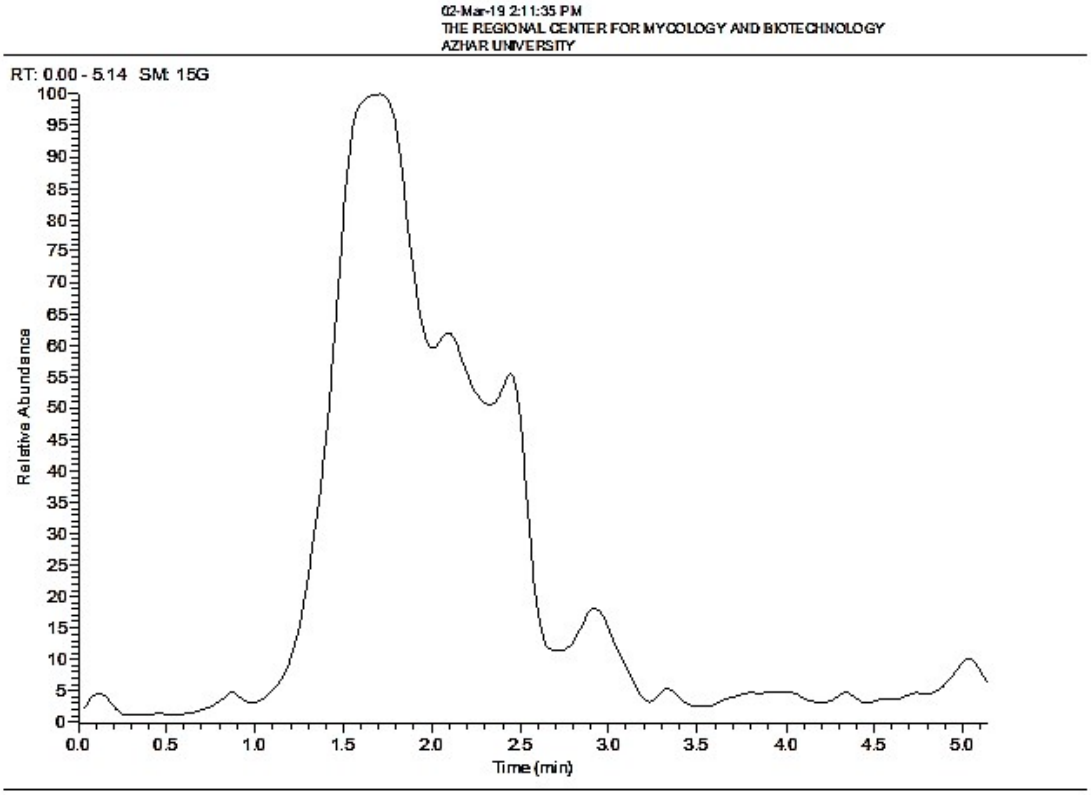


Fig. (S12): ^{13}C NMR Spectrum of Compound (9)



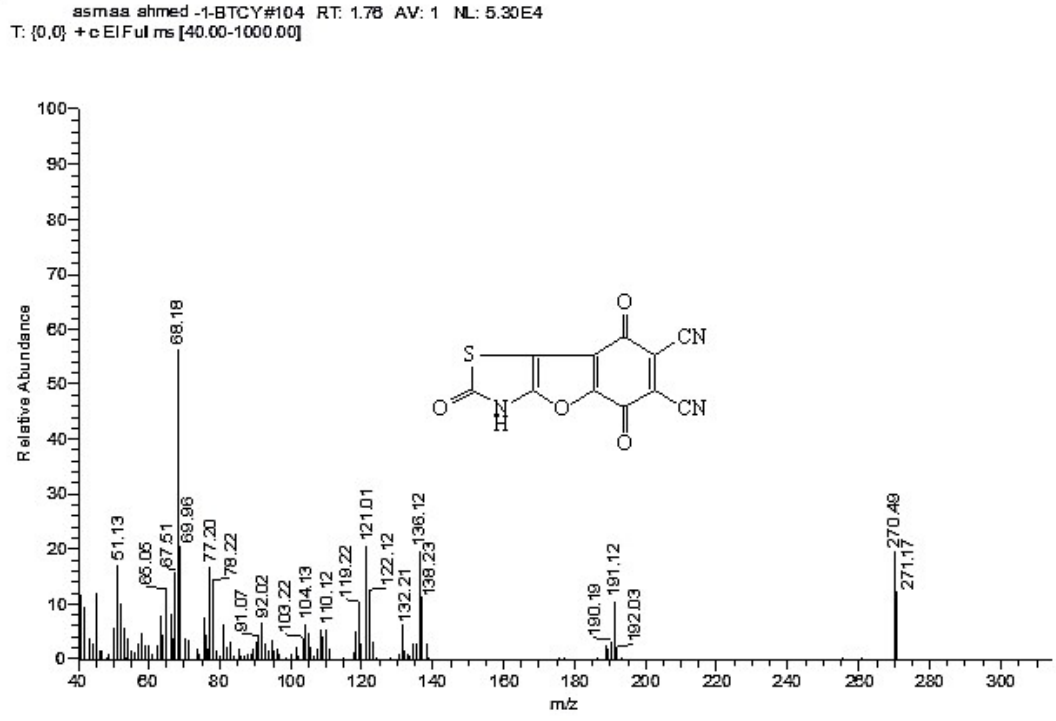


Fig. (S13): MS Spectrum of Compound (9)

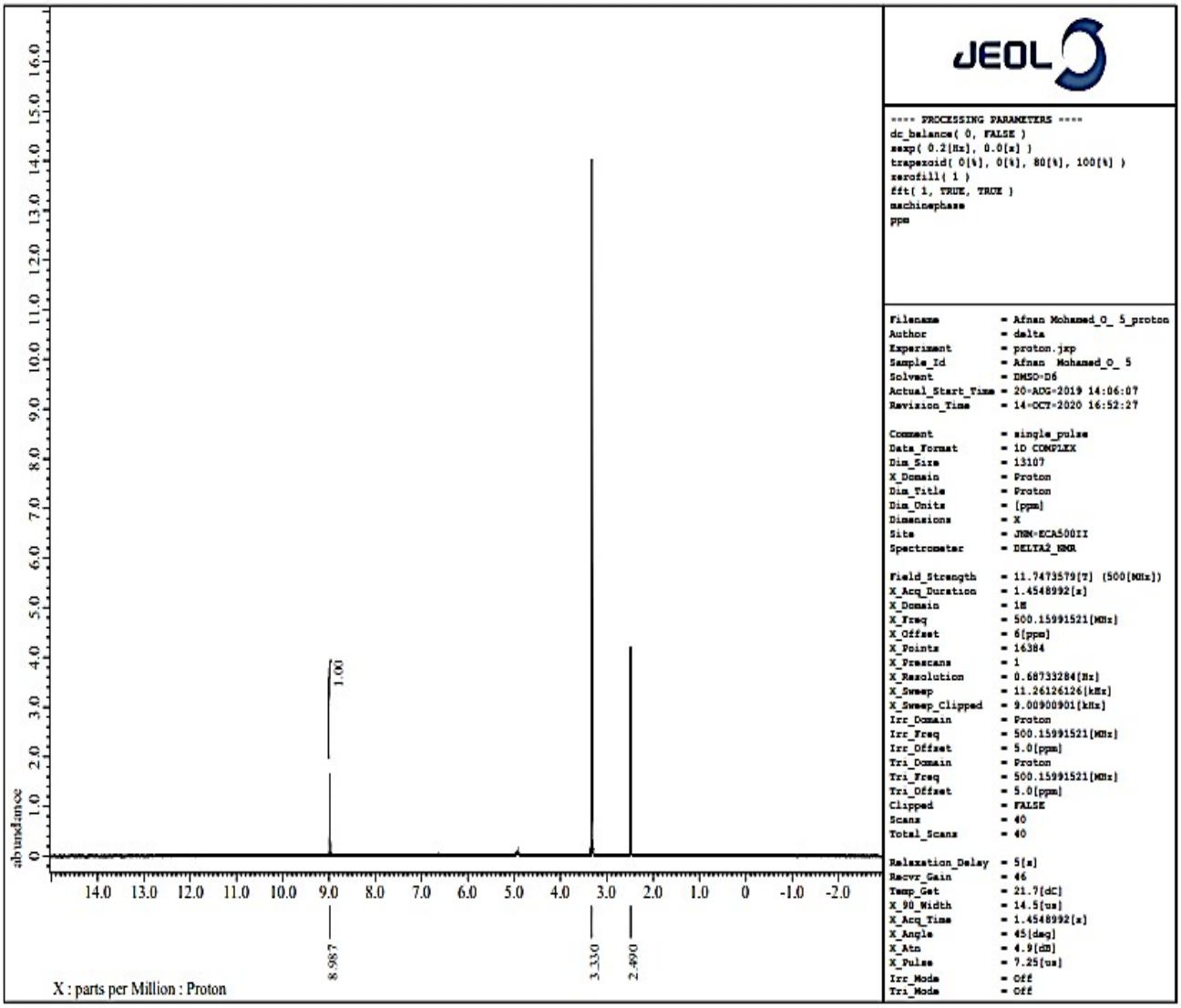


Fig. (S14): ^{1}H NMR Spectrum of Compound (10)

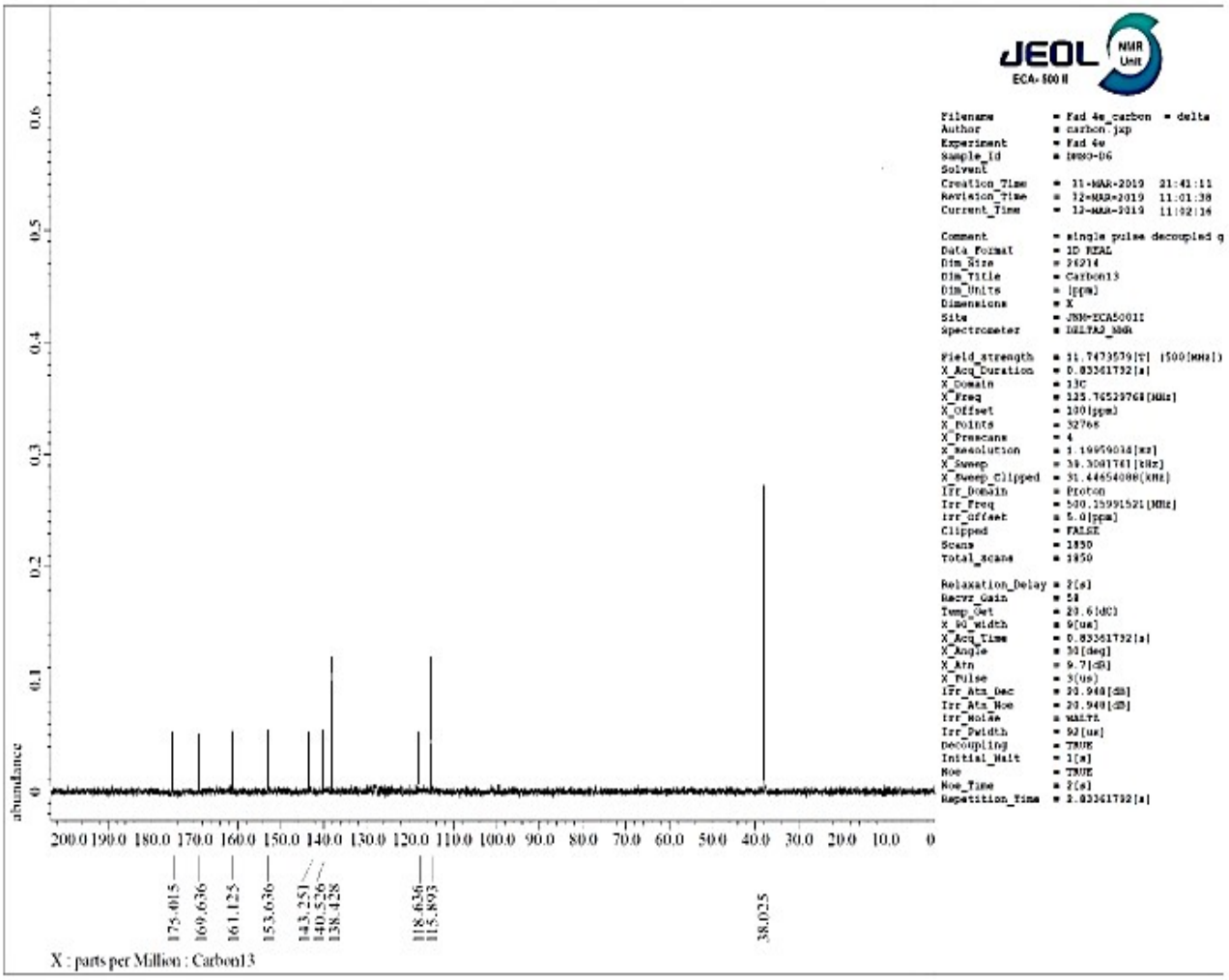


Fig. (S15): ^{13}C NMR Spectrum of Compound (10)

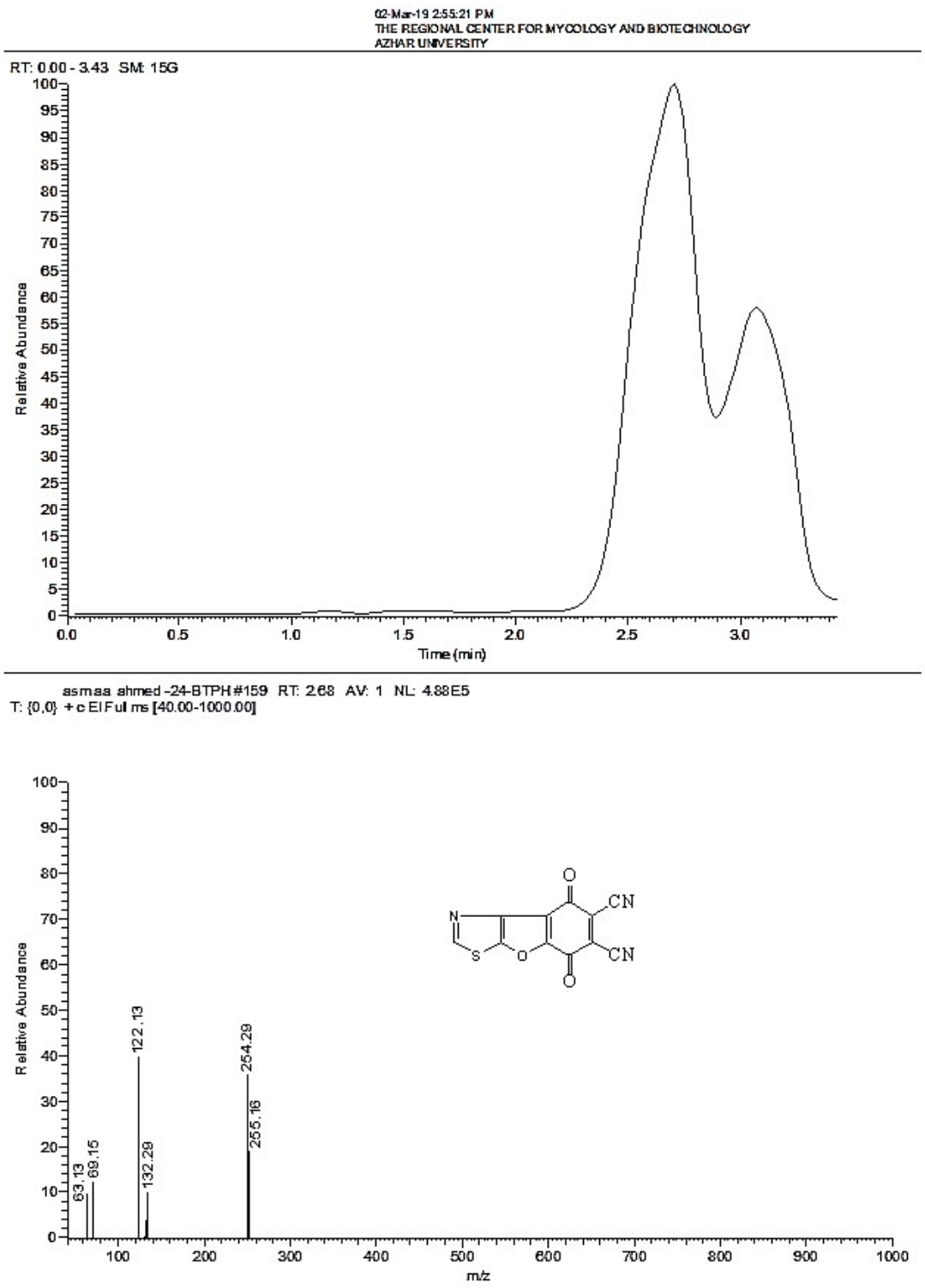


Fig. (S16): MS Spectrum of Compound (10)

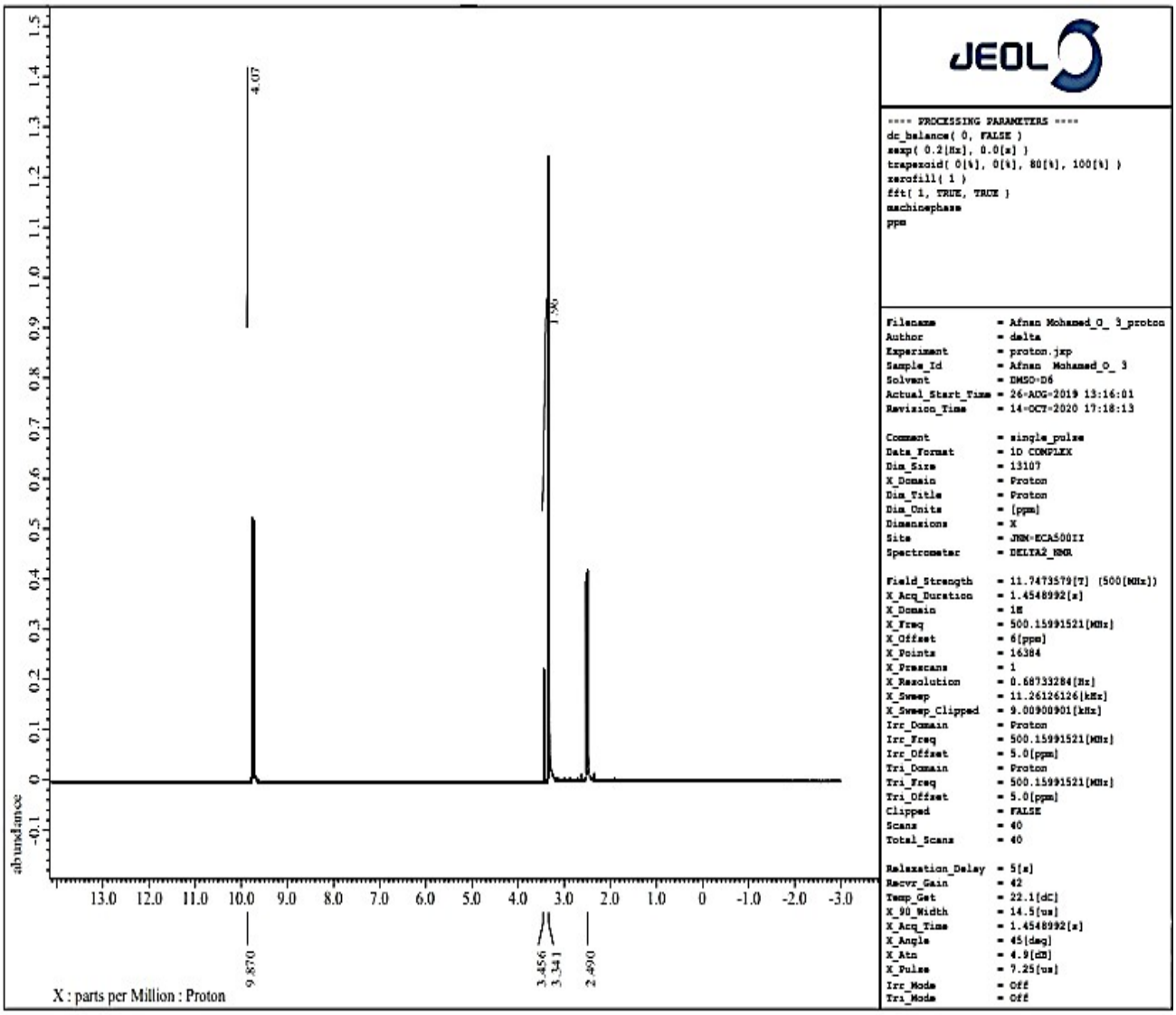


Fig. (S17): ^{1}H NMR Spectrum of Compound (11)

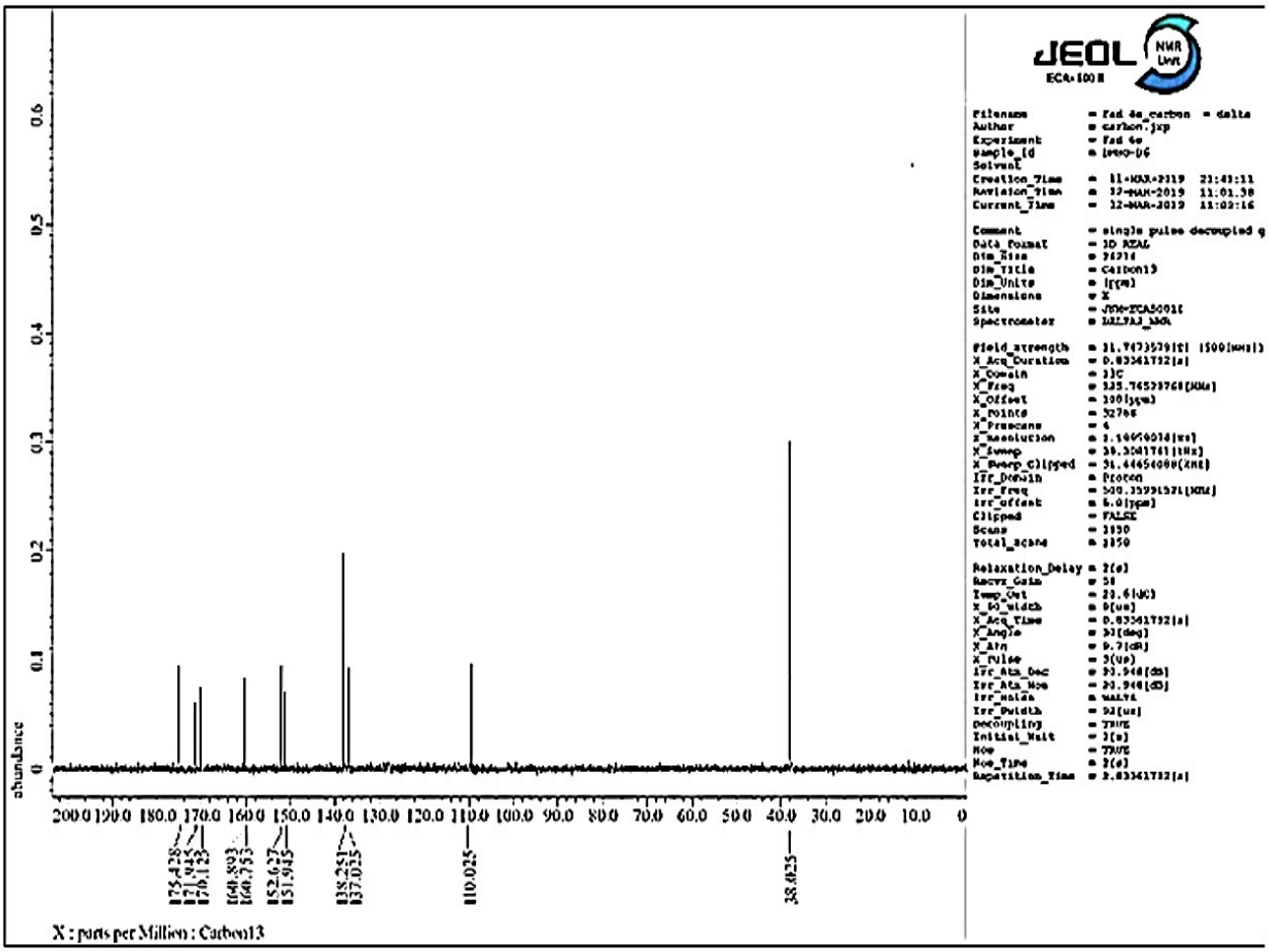


Fig. (S18): ^{13}C NMR Spectrum of Compound (11)

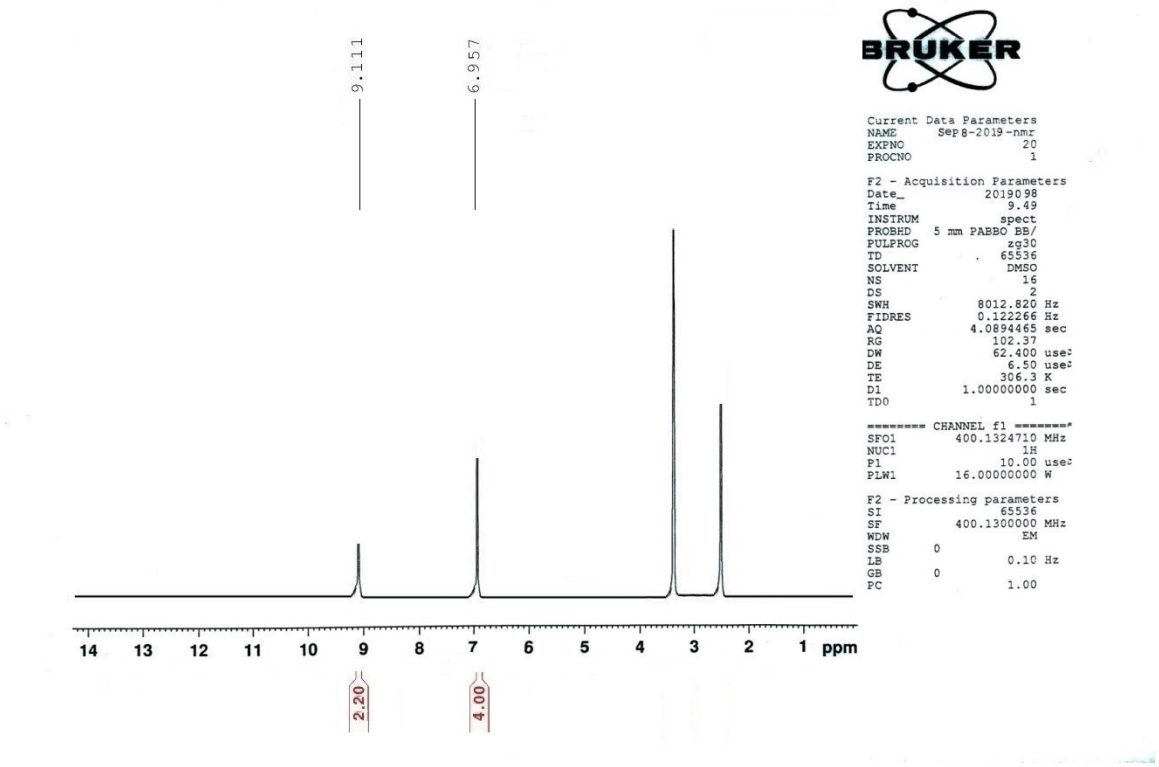


Fig. (S19): ^{1}H NMR Spectrum of Compound (12)

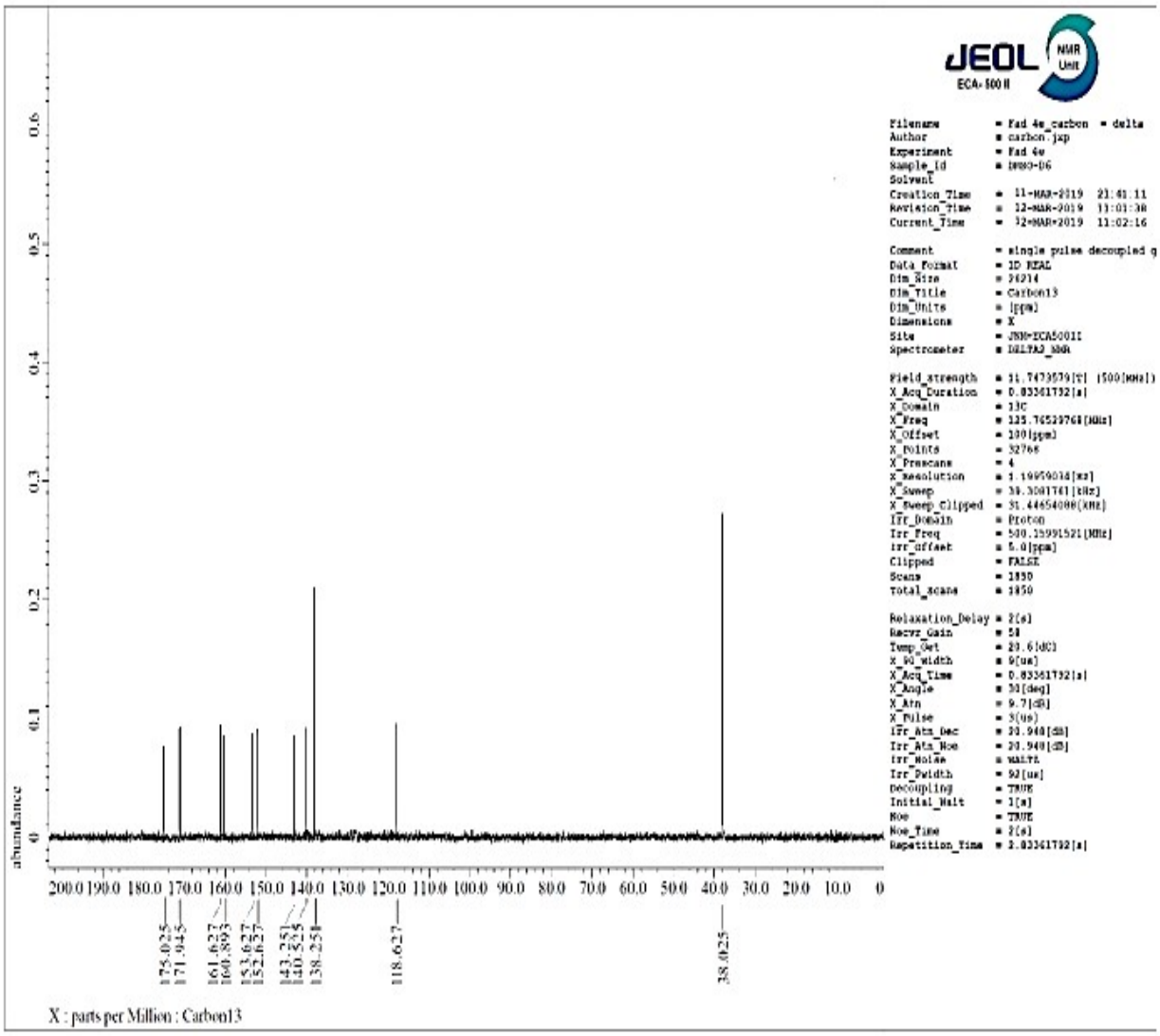


Fig. (S20): ^{13}C NMR Spectrum of Compound (12)

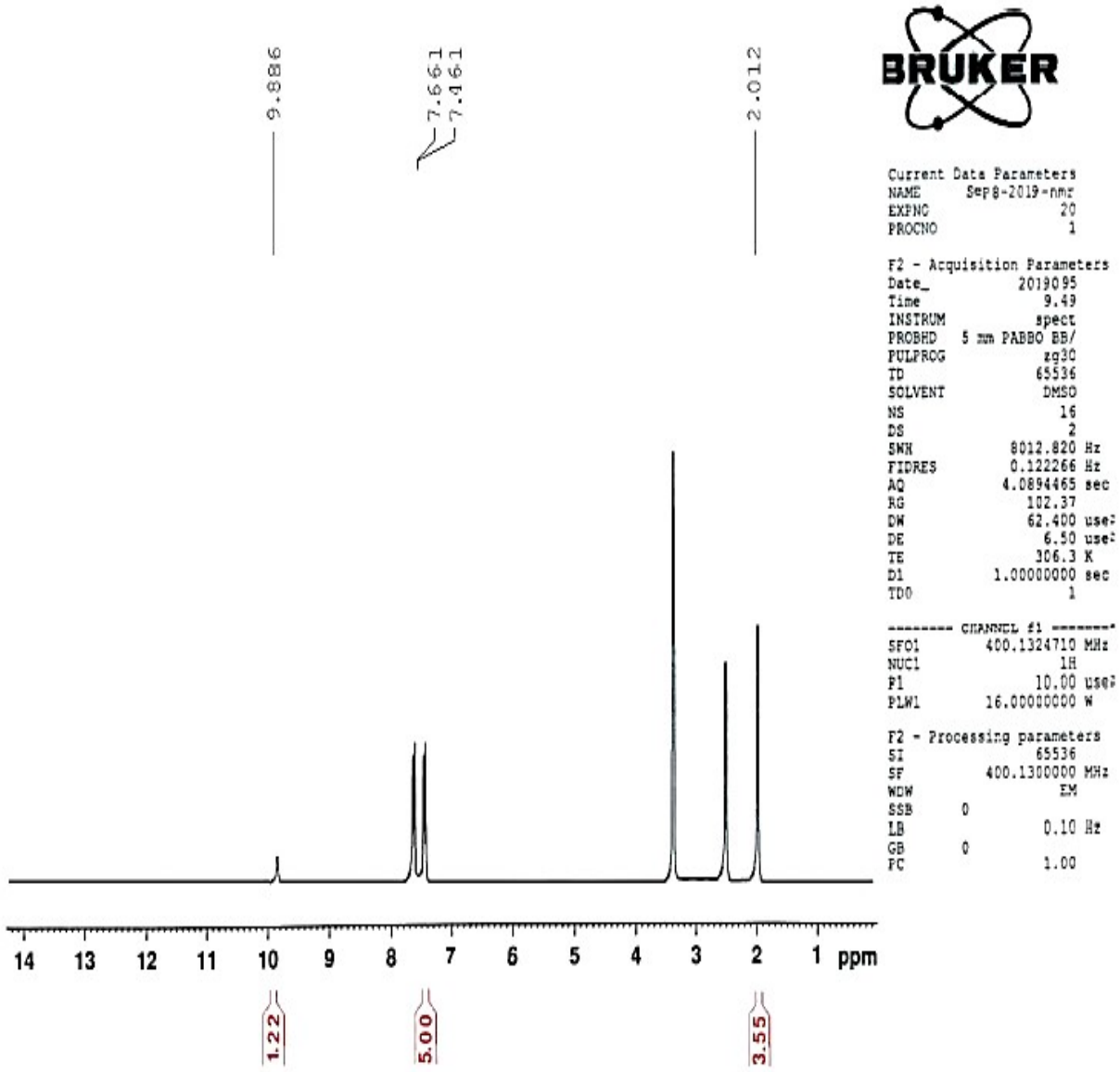


Fig. (S21): ^{1}H NMR Spectrum of Compound (15)

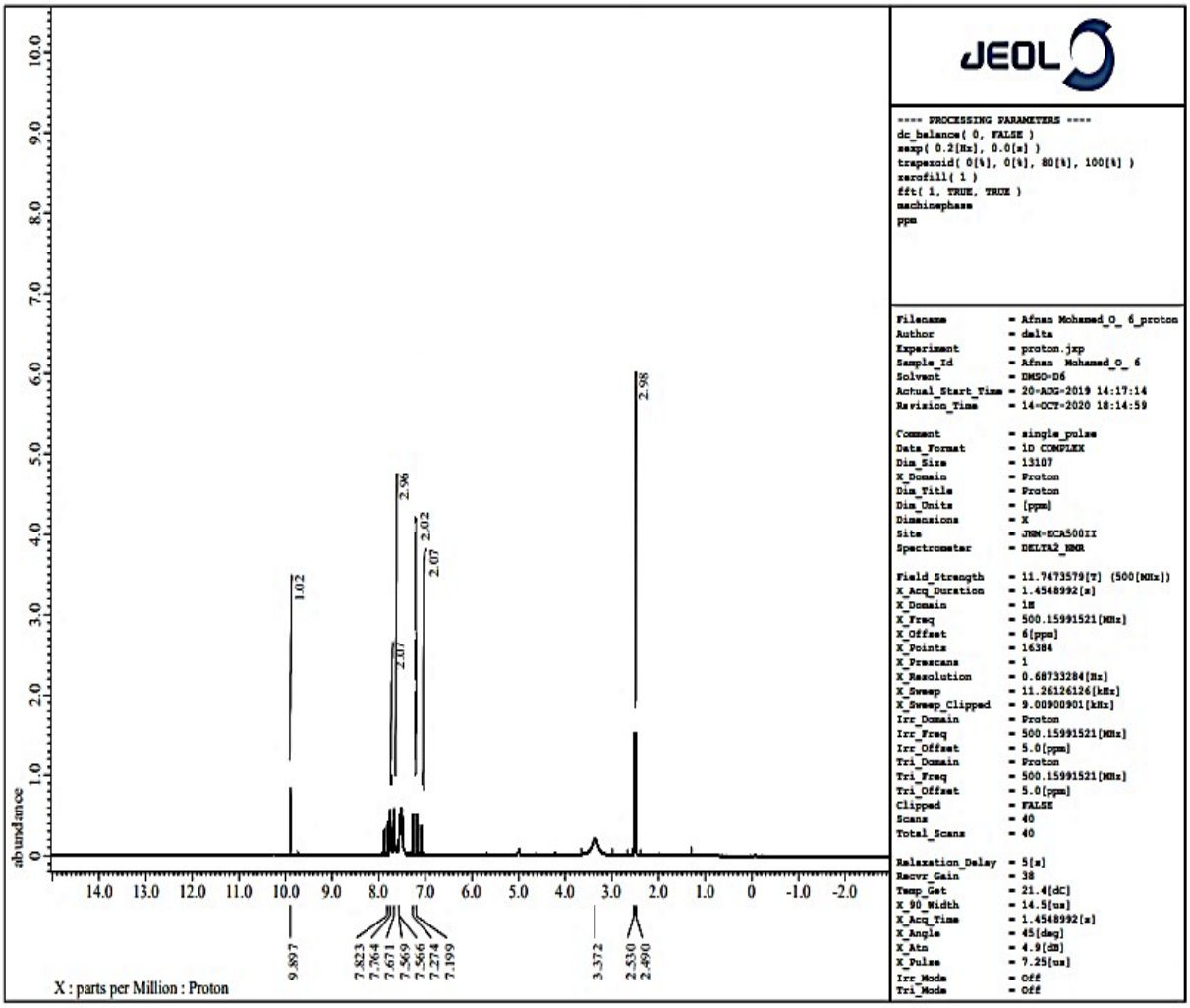


Fig. (S22): ^{1}H NMR Spectrum of Compound (16)

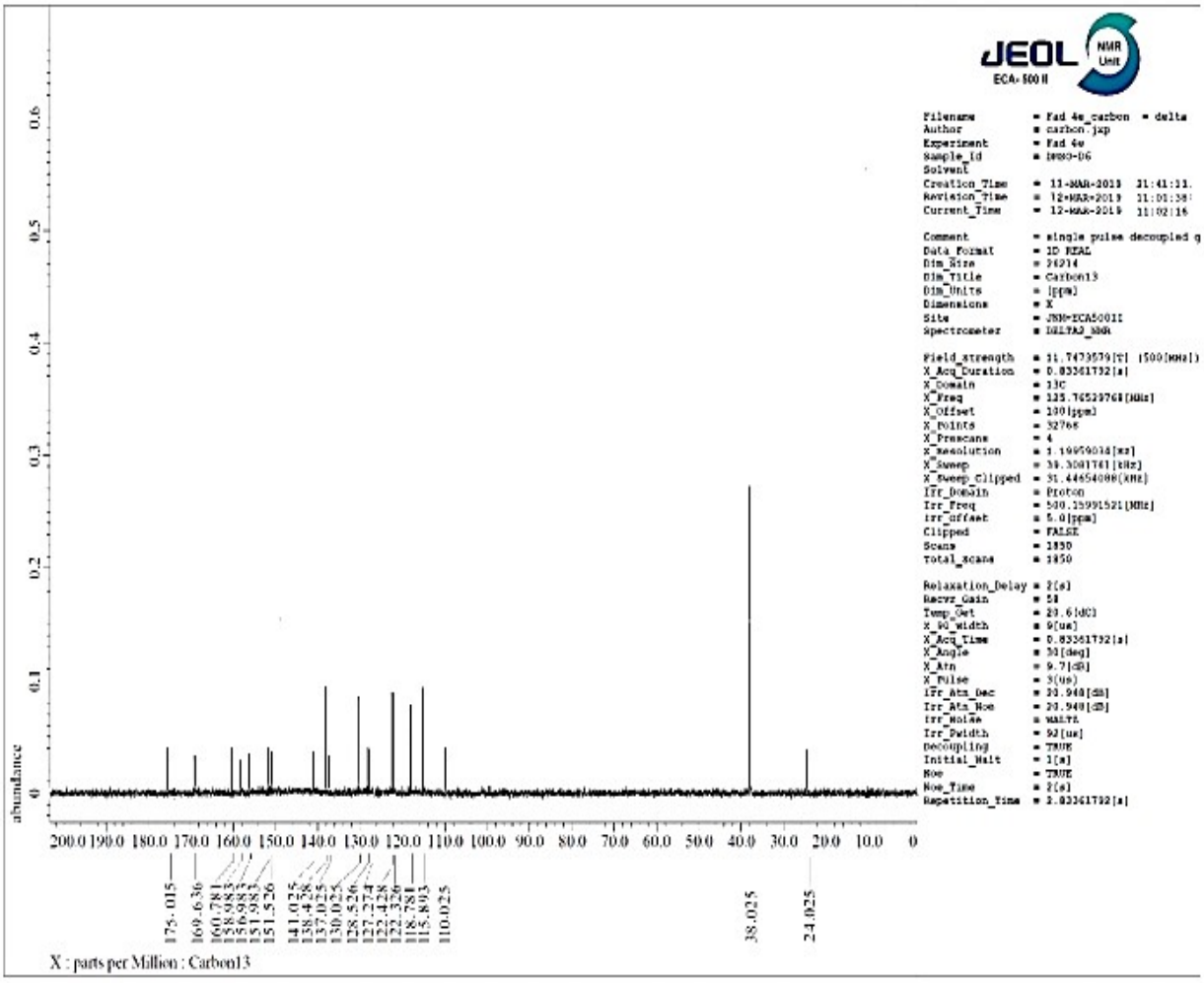


Fig. (S23): ^{13}C NMR Spectrum of Compound (16)

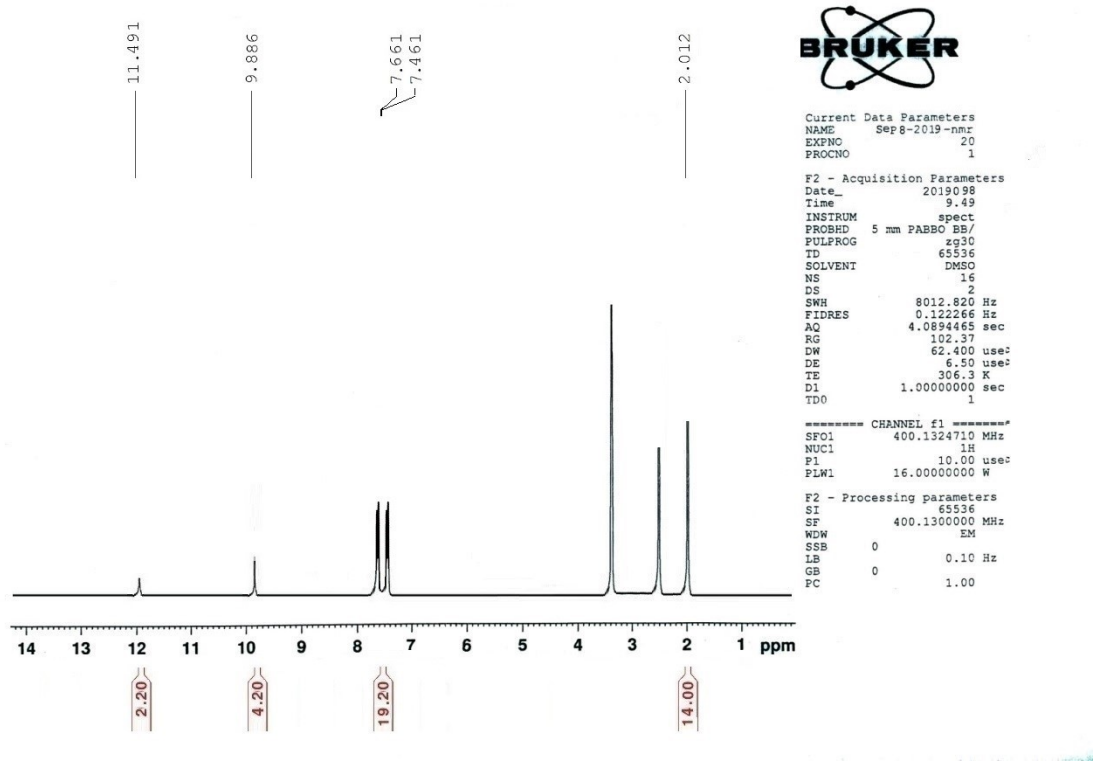


Fig. (S24): ^{1}H NMR Spectrum of Compound (17)

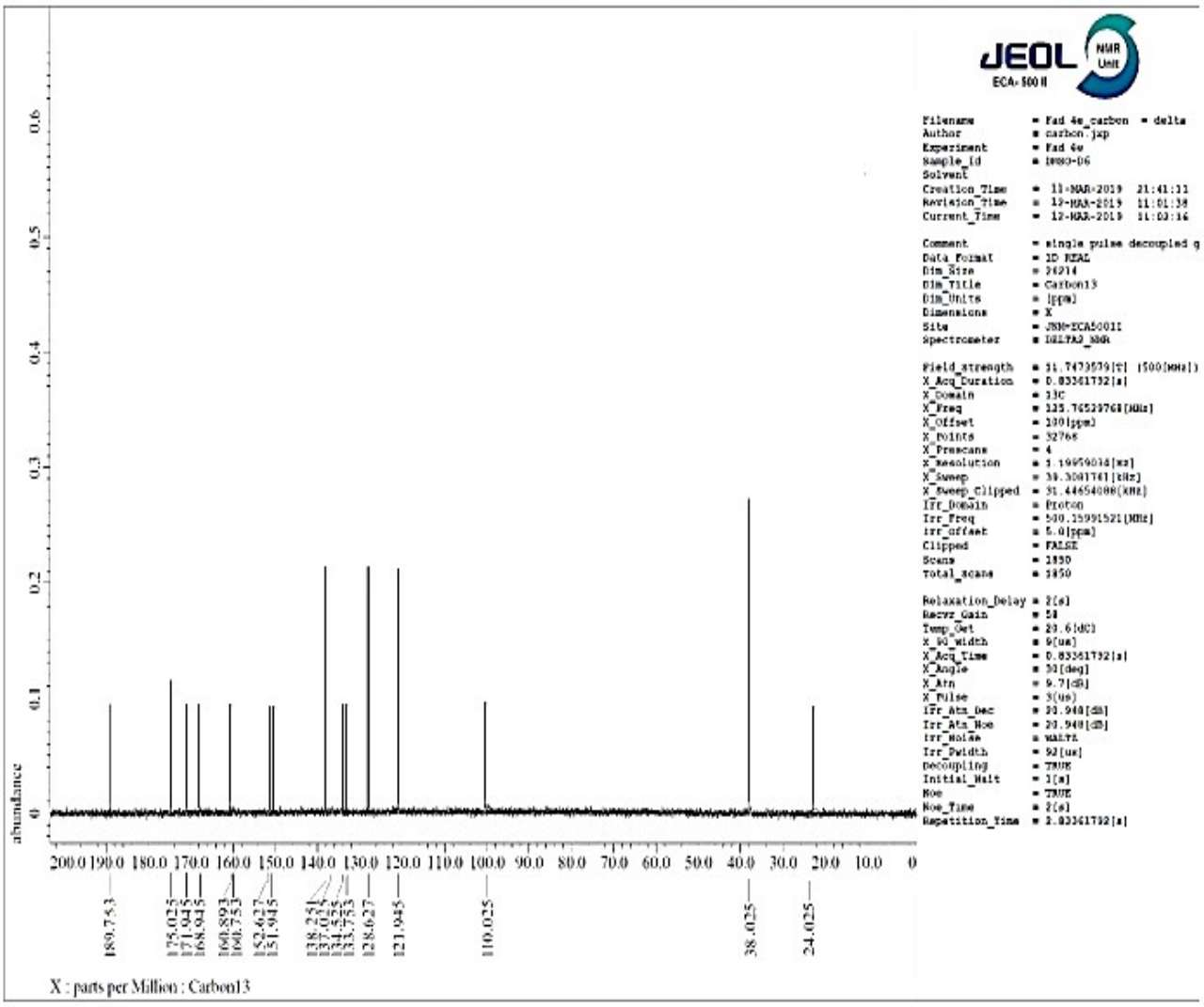


Fig. (S25): ^{13}C NMR Spectrum of Compound (17)

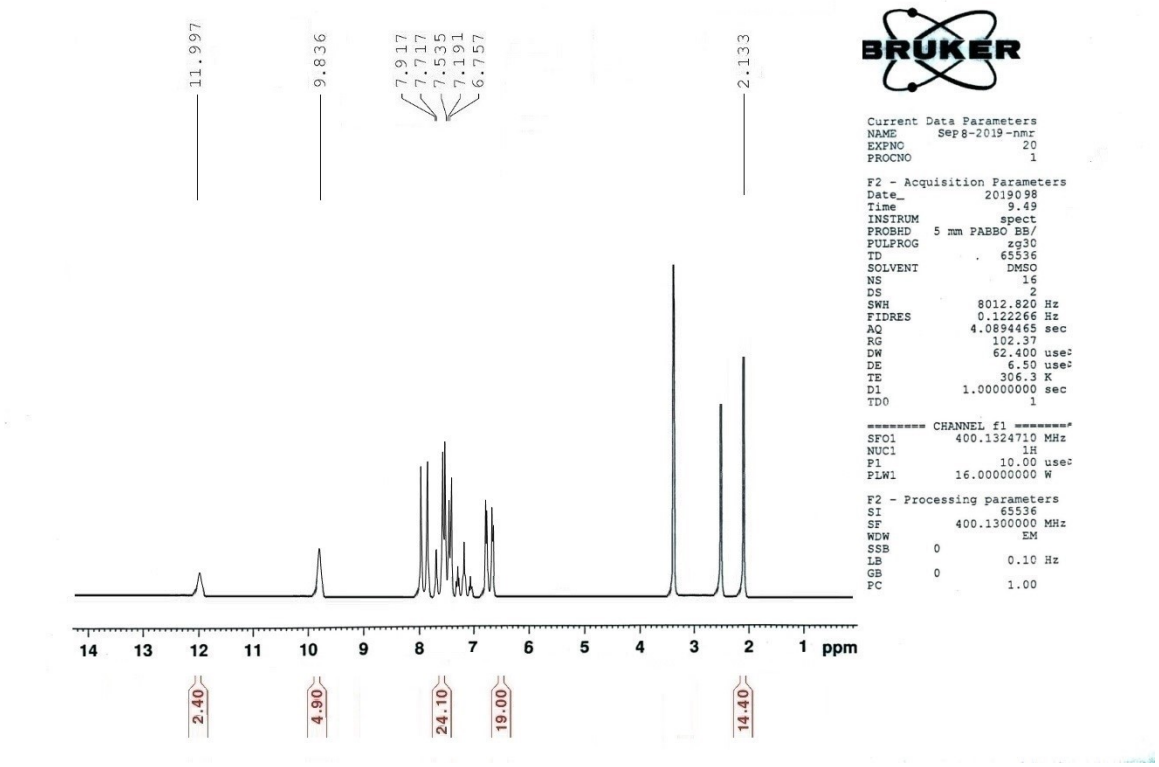


Fig. (S26): ^{1}H NMR Spectrum of Compound (18)

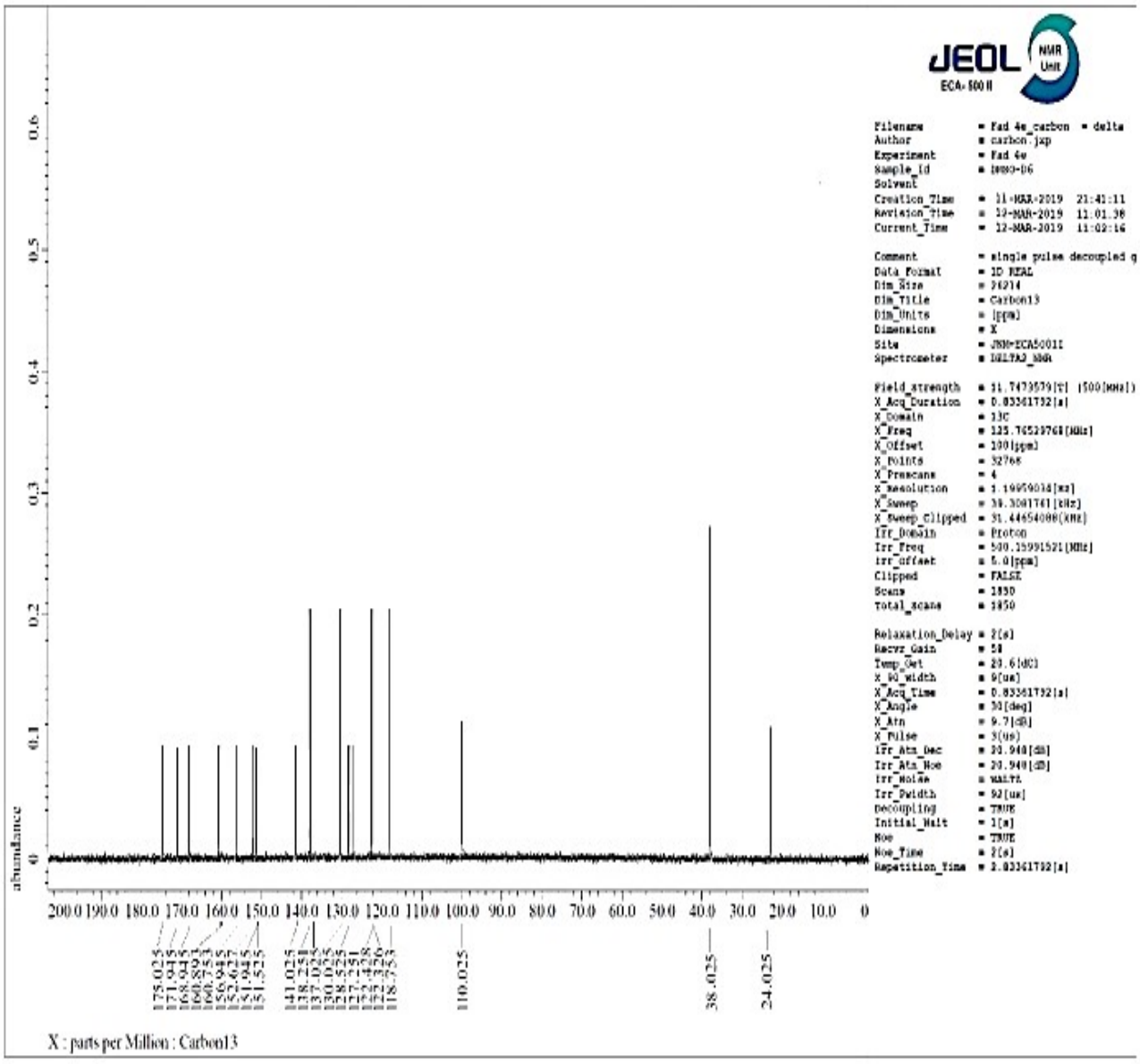


Fig. (S27): ^{13}C NMR Spectrum of Compound (18)

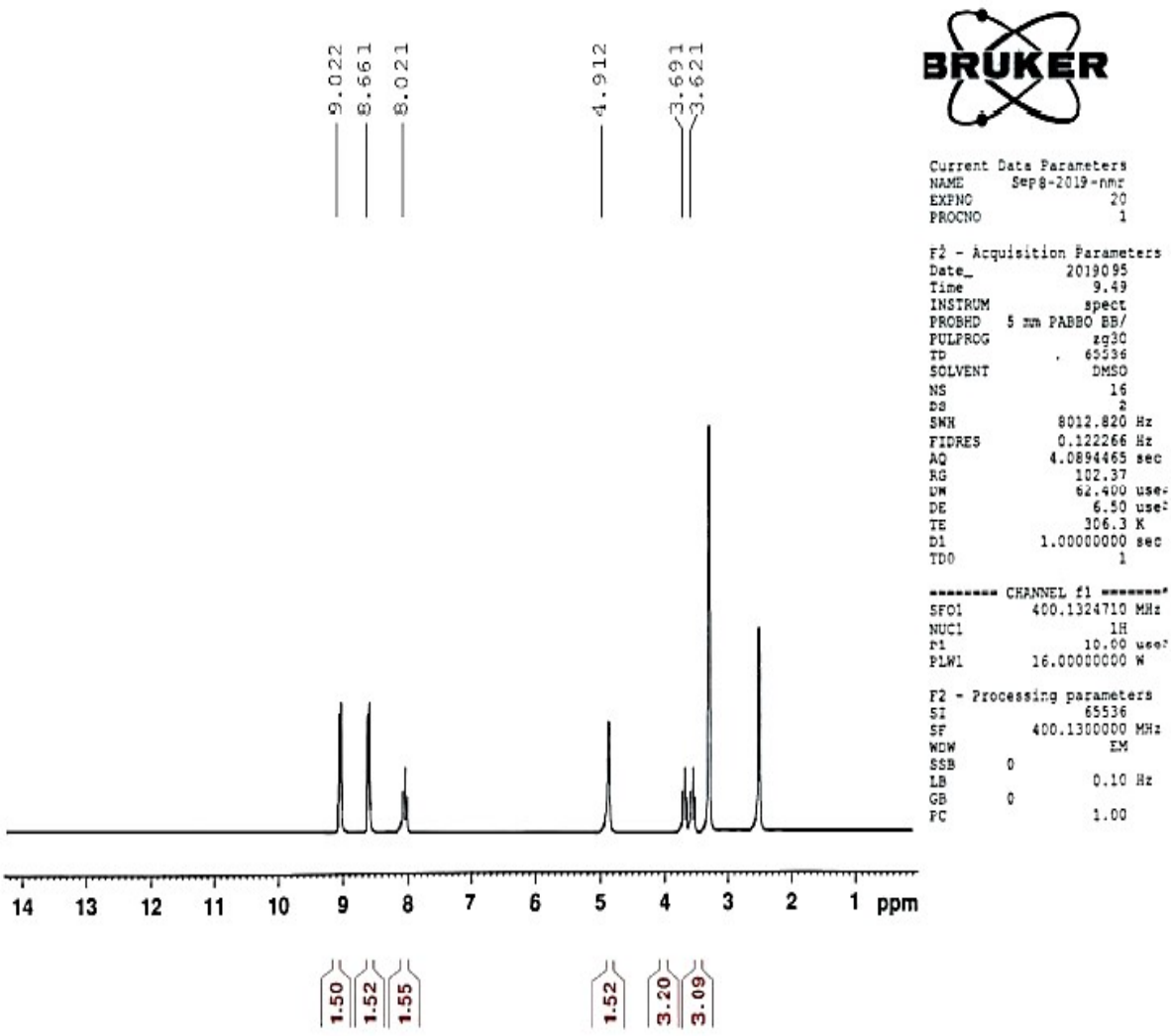


Fig. (S28): ^{1}H NMR Spectrum of Compound (19)

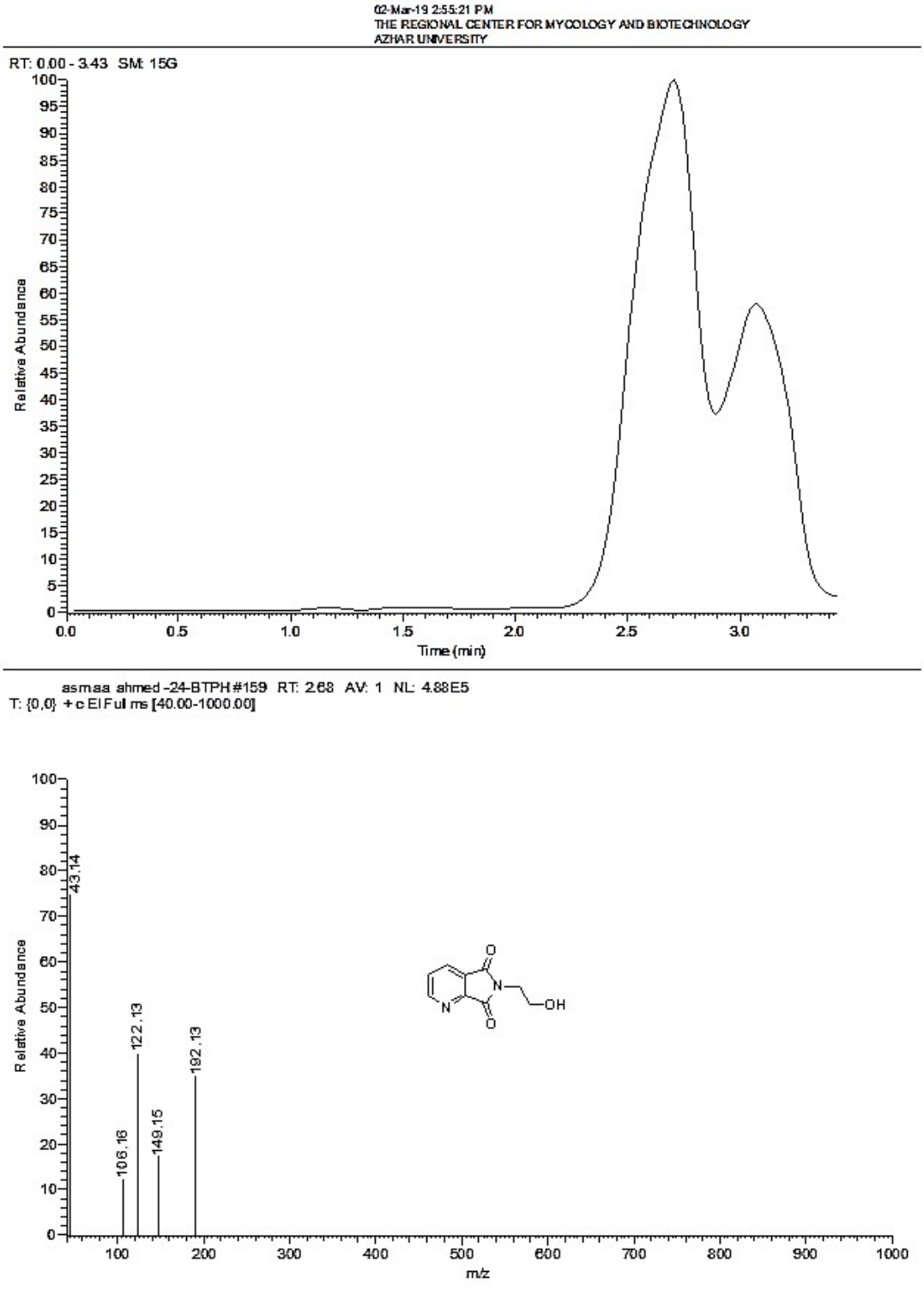


Fig. (S29): MS Spectrum of Compound (19)

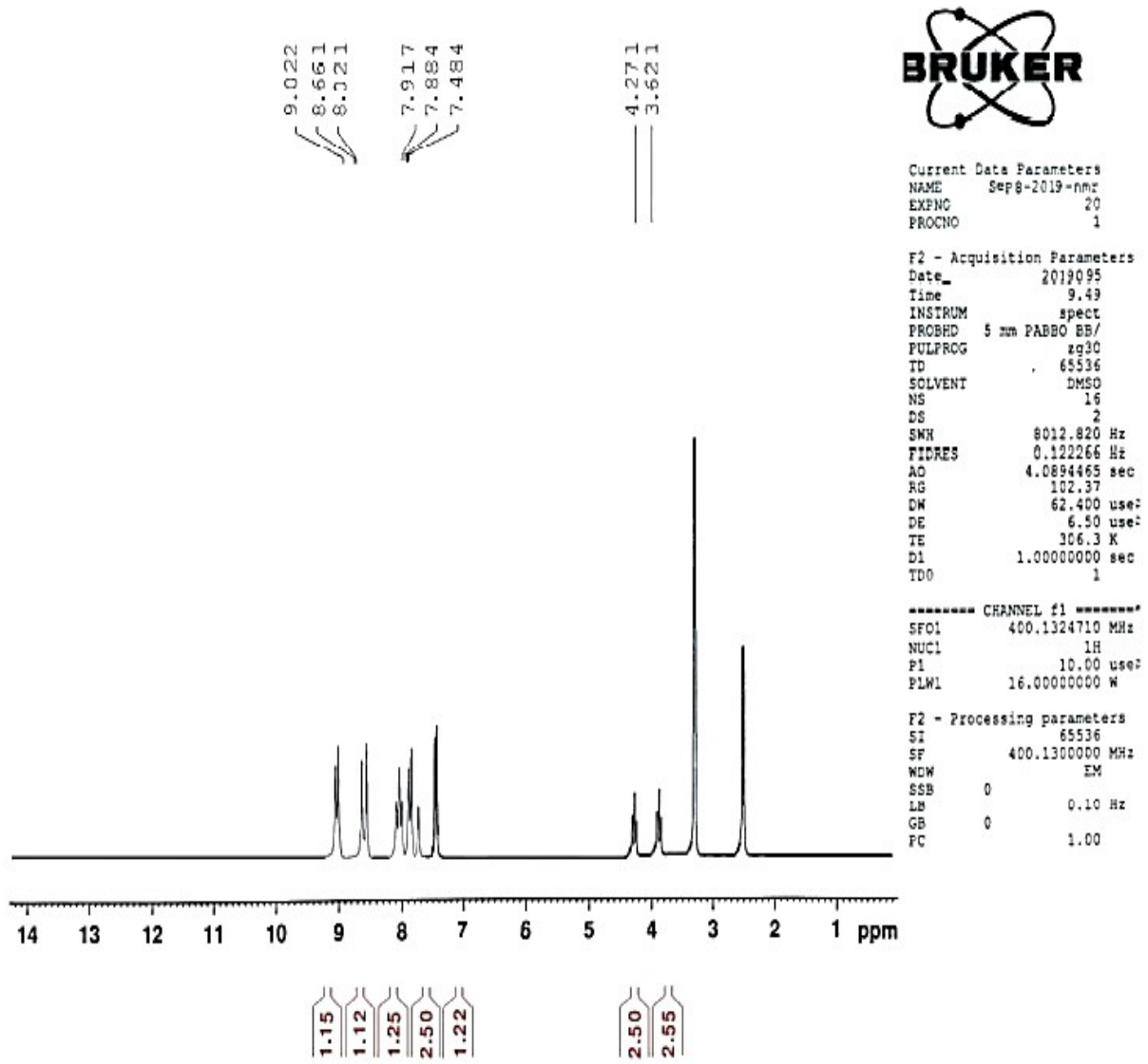


Fig. (S30): ^{1}H NMR Spectrum of Compound (20)

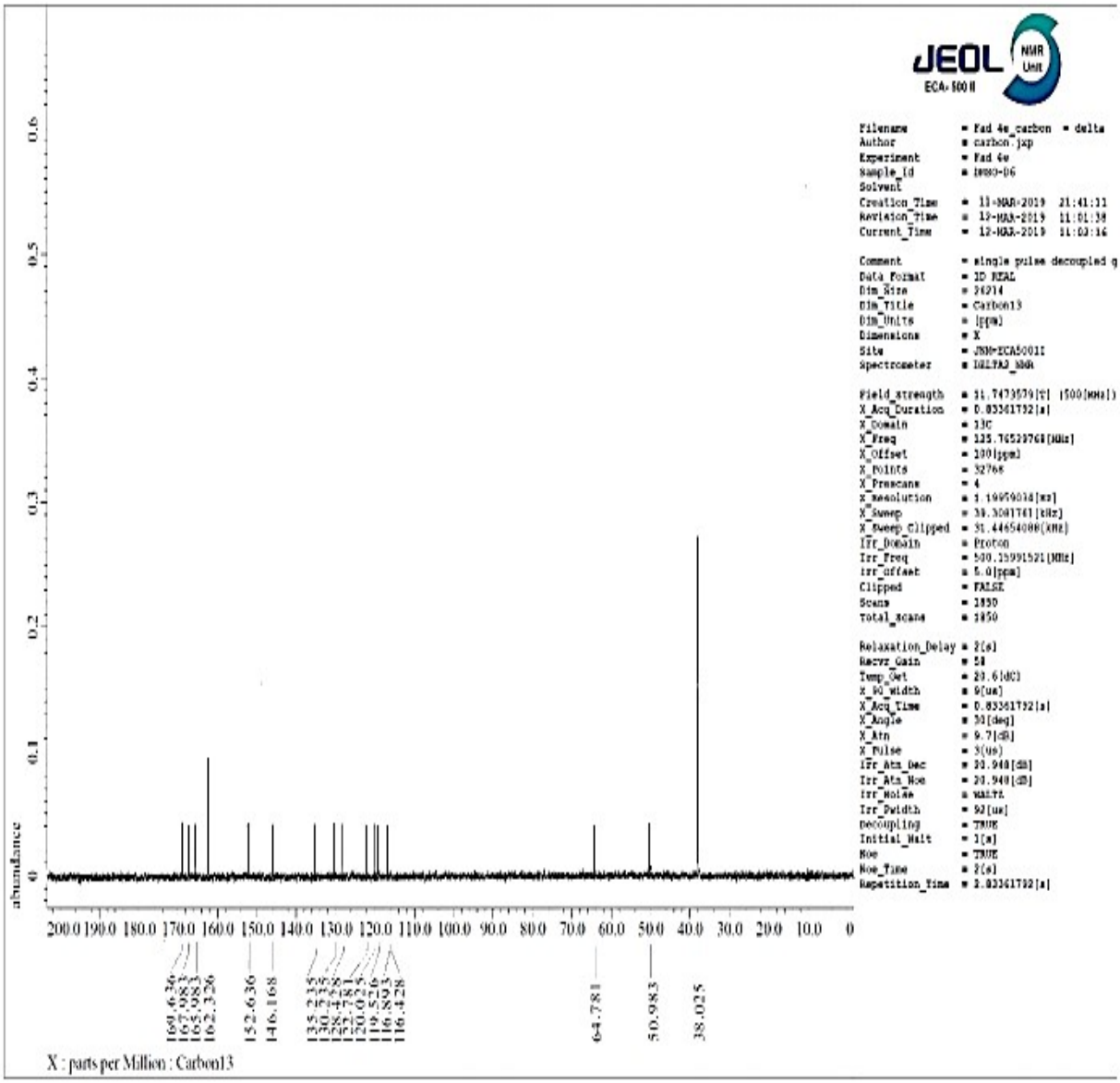


Fig. (S31): ^{13}C NMR Spectrum of Compound (20)

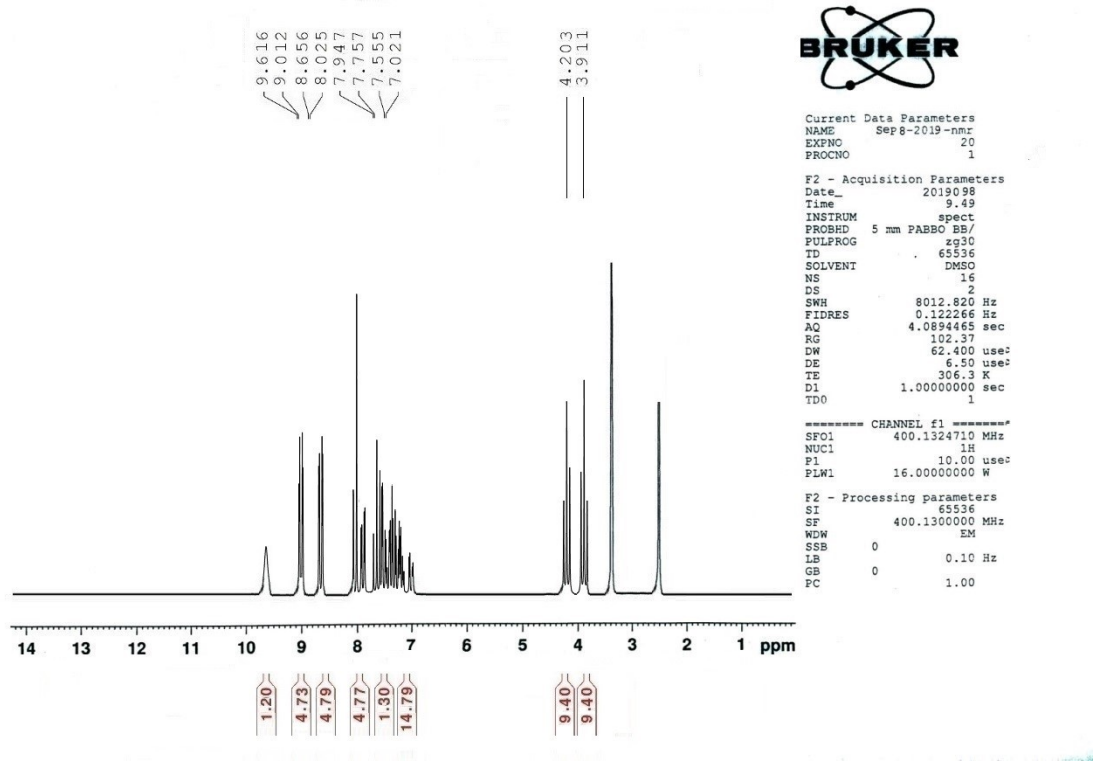


Fig. (S32): ^{1}H NMR Spectrum of Compound (22)

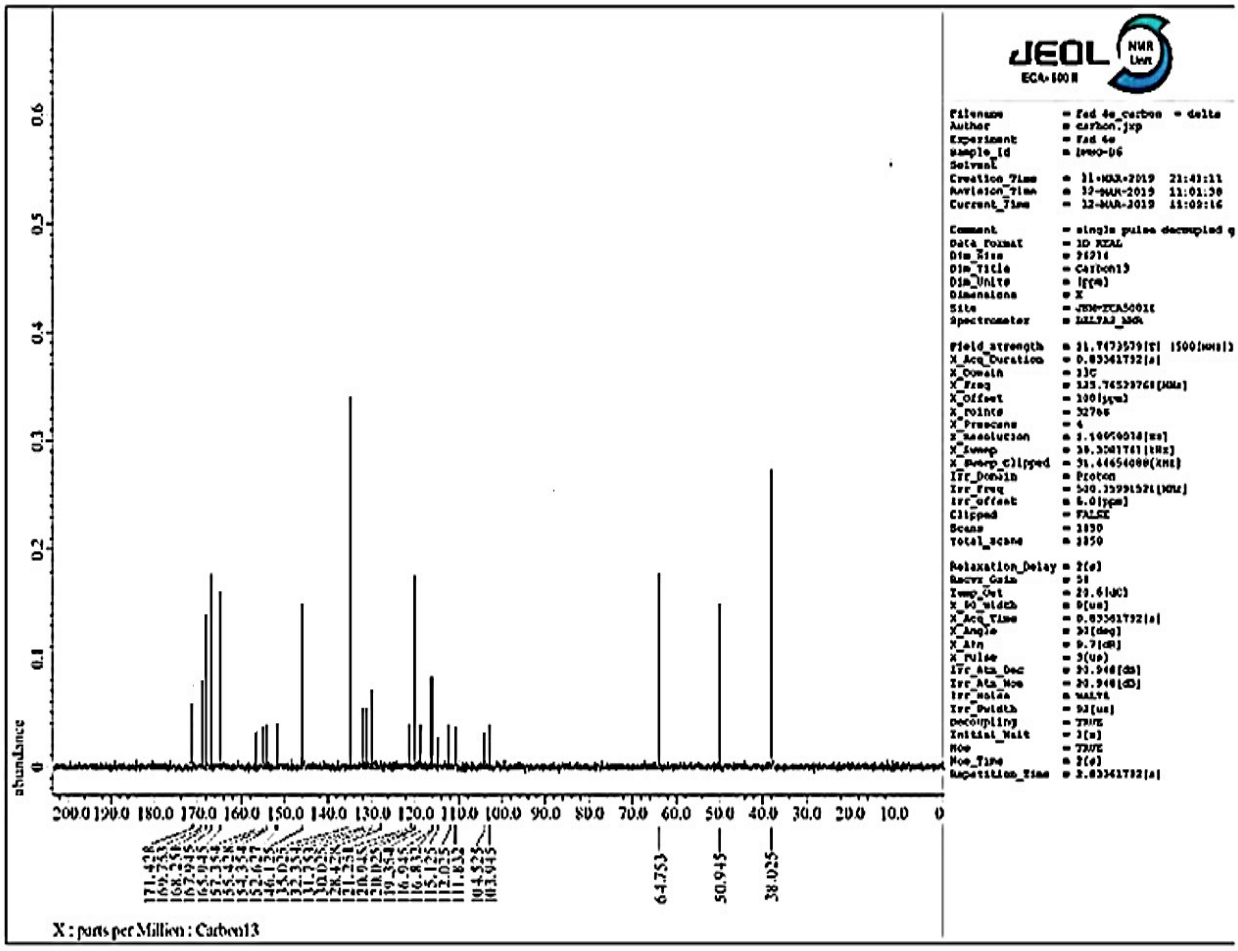


Fig. (S33): ^{13}C NMR Spectrum of Compound (22)