Supporting Information

Chemical Constituents of the *Morinda tomentosa* Leaves and their α-Glucosidase Inhibitory Activity

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1. General procedures

Optical rotations were determined on a Jasco DIP-370 automatic polarimeter. Preparative HPLC was carried out using a Waters HPLC system (600 pump, 600 controller, and a 996 photodiode array detector). The NMR spectra were recorded using a Bruker DRX 500 spectrometer (^{1}H, 500 MHz; ^{13}C, 125 MHz) and Jeol ECS 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz). The ESI-MS were obtained from an Agilent 1200 Series LC-MSD Trap spectrometer. The HR-ESI-MS were obtained from an Agilent 6530 Accurate-Mass Q-TOF LC/MS system. GC spectra were recorded on a Shimadzu-2010 spectrometer. Column chromatography was performed using a silica gel 60 (70-230 mesh or 230-400 mesh, Merck, Germany) or YMC RP-18 resins (30-50 μm, Fujisilisa Chemical Ltd., Japan), and thin layer chromatography (TLC) using a pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254}S plates (0.25 mm, Merck).

2. Extraction and Isolation

The dried leaves of *M. tomentosa* (3.0 kg) were extracted with MeOH three times at room temperature to yield 226 g of a dark solid extract, which was then suspended in water and successively partitioned with n-hexane and EtOAc to obtain *n*-hexane (MT1, 70.0 g), EtOAc (MT2, 37.0 g), and water (MT3, 115.0 g) extracts after removal solvent *in vacuo*. MT1 (70.0 g) was chromatographed on a silica gel column and eluted with gradient of *n*-hexane – acetone (40:1 → 1:1, v/v) to obtain five sub-fractions, MT1A (15.0 g), MT1B (12.0 g), MT1C (10.0 g), MT1D (13.0 g), and MT1E (15.0 g). Sub-fraction MT1B was chromatographed on a silica gel column eluting with *n*-hexane – EtOAc (10:1, v/v) to yield **9** (8.0 mg). Sub-fraction MT1C was chromatographed on a silica gel column eluting with $CHCl_3$ – MeOH (20:1, v/v) to give three smaller fractions, MT1C1 (3.0 g), MT1C2 (4.0 g), and MT1C3 (2.0 g). Fraction MT1C1 was chromatographed on an YMC RP-18 column eluting with acetone – water (4:1, v/v) to yield **10** (12.0 mg), **11** (9.0 mg), and **12** (8.5 mg). The fraction MT1C2 was chromatographed on a silica gel column and eluted with $CHCl_3$ – methanol (10:1, v/v) to yield **8** (10.0 mg). The water soluble fraction (MT3, 115.0 g) was chromatographed on a Diaion HP-20P column eluting with water containing increasing concentrations of MeOH (0%, 25%, 50%, 75%, and 100%) to obtain five sub-fractions MT3A (40.0 g), MT3B (15.0 g), MT3C (32.0 g), MT3D (12.0 g), and MT3E (15.0 g). The fraction MT3C (32.0 g) was chromatographed on a silica gel column eluting with $CHCl_3$ – MeOH – water (5:1:0.1, v/v/v) to give three smaller fractions, MT3C1 (10 g), MT3C2 (8.0 g), and MT3C3 (11.0 g). Sub-fraction MT3C1 was chromatographed on a silica gel column eluting with $CHCl_3$ – acetone – water (1:2:0.1, v/v/v) to yield **4** (7.0 mg). The fraction MT3C3 was chromatographed on an YMC RP-18 column eluting with acetone – water (1:2, v/v) to yield **1** (6.0 mg), **2** (8.0 mg), and **3** (15.0 mg). The fraction MT3E was chromatographed on a silica column eluting with $CHCl_3$ – MeOH – water (5:1:0.1, v/v/v) to give three smaller fractions, MT3E1 (5.0 g), MT3E2 (4.0 g), and MT3E3 (4.0 g). The fraction MT3E3 was chromatographed on an YMC RP-18 column eluting with acetone – water (1:2, v/v) to yield **5** (18.0 mg) and **6** (4.0 mg).

3. Acid hydrolysis

Each compound (**1** and **6**, 2.0 mg) was dissolved in 1.0 N HCl (dioxane/H_2O, 1:1, v/v, 1.0 mL) and then heated to 80 °C in a water bath for 3 h. The acidic solution was neutralized with silver carbonate and the solvent thoroughly driven out under N_2 gas overnight. After extraction with $CHCl_3$, the aqueous layer was concentrated to dryness using N_2 gas. The residue was dissolved in 0.1 mL of dry pyridine, and then L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 mL) was added to the solution. The reaction mixture was heated at 60 °C for 2 h, and 0.1 mL of

trimethylsilylimidazole solution was added, followed by heating at 60 °C for 1.5 h. The dried product was partitioned with *n*-hexane and H_2O (0.1 mL, each), and the organic layer was analyzed by gas liquid chromatography (GC): Column: column SPB-1 (0.25 mm × 30 m); detector FID, column temperature 210 °C, injector temperature 270 °C, detector temperature 300 °C, carrier gas He (2.0 mL/min). The retention time of persilylated glucose was found to be 14.11 min. When standard solution was prepared by the same reaction from the standard glucose, the retention times of persilylated standard D-glucose and L-glucose were 14.11 and 14.26 min, respectively. From the comparison of retention times, glucose was found as D-glucose.

4. Inhibition Assay for α-Glucosidase Activity

The α-glucosidase inhibition assay was performed according to (Li *et al.*, 2009) with some modification. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Briefly, a mixture of 50 μL samples and 50 μL of 0.1 M phosphate buffer (pH 7.0) containing α-glucosidase solution (0.3 U/mL) and 50 μL distilled water was incubated in 96 well plates at 37 °C for 15 min. After pre-incubation, 100 μL of 3 mM *p*-NPG solution in 0.1 M phosphate buffer (pH 7.0) was added to each well at timed intervals. The reaction mixtures were incubated at 37 °C for 10 min and stopped by adding 750 μL of 0.1 M Na_2CO_3. The absorbance was recorded at 405 nm by FLUOstar Optima (BMG Labtech, Offenburg, Germany). The results were expressed as a percent of α-glucosidase inhibition and calculated according to the following equation:

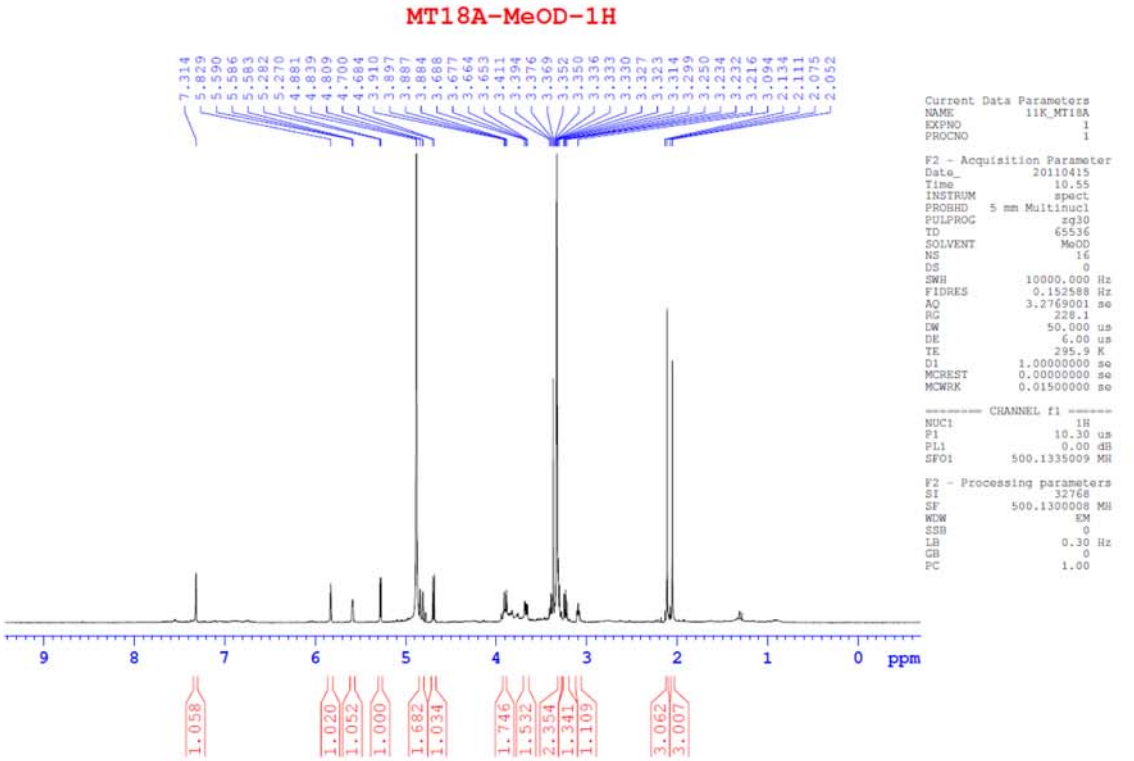
$$\% \text{ inhibition} = \frac{A^{control} - A^{compound}}{A^{control}} \times 100$$

Statistical Analysis. All experiments were performed in triplicate. Data is presented as the means ± SD. The results were statistically analyzed by ANOVA and Duncan's multiple range tests. Statistical significance was accepted at a level of $p < 0.05$.

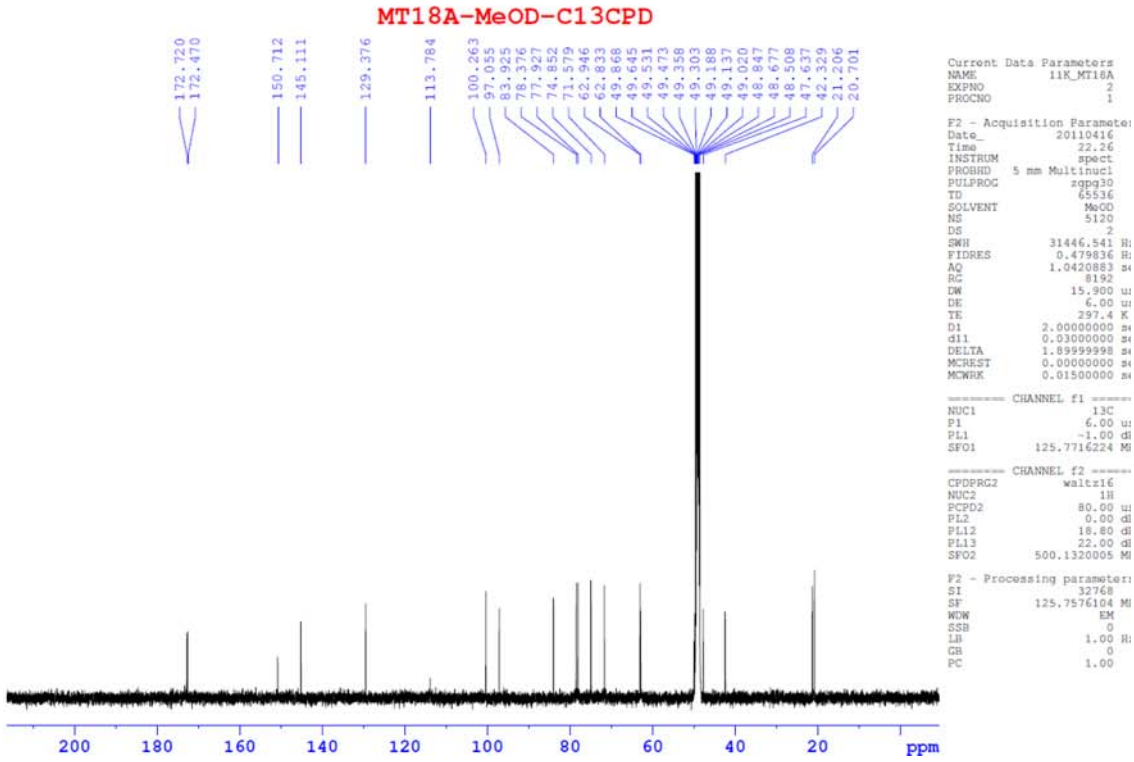
1. Li, H. L.; Song, F. R.; Xing, J. P.; Tsao, R.; Liu, Z. Q.; Liu, S. Y. Screening and structural characterization of α-glucosidase inhibitors from hawthorn leaf flavonoids extract by ultrafiltration LC-DAD-MSN and SORI-CID FTICR MS. *J. Am. Soc. Mass Spectrom.* **20**, 1496-1503 (2009).

5. NMR and MS spectra of compound 1

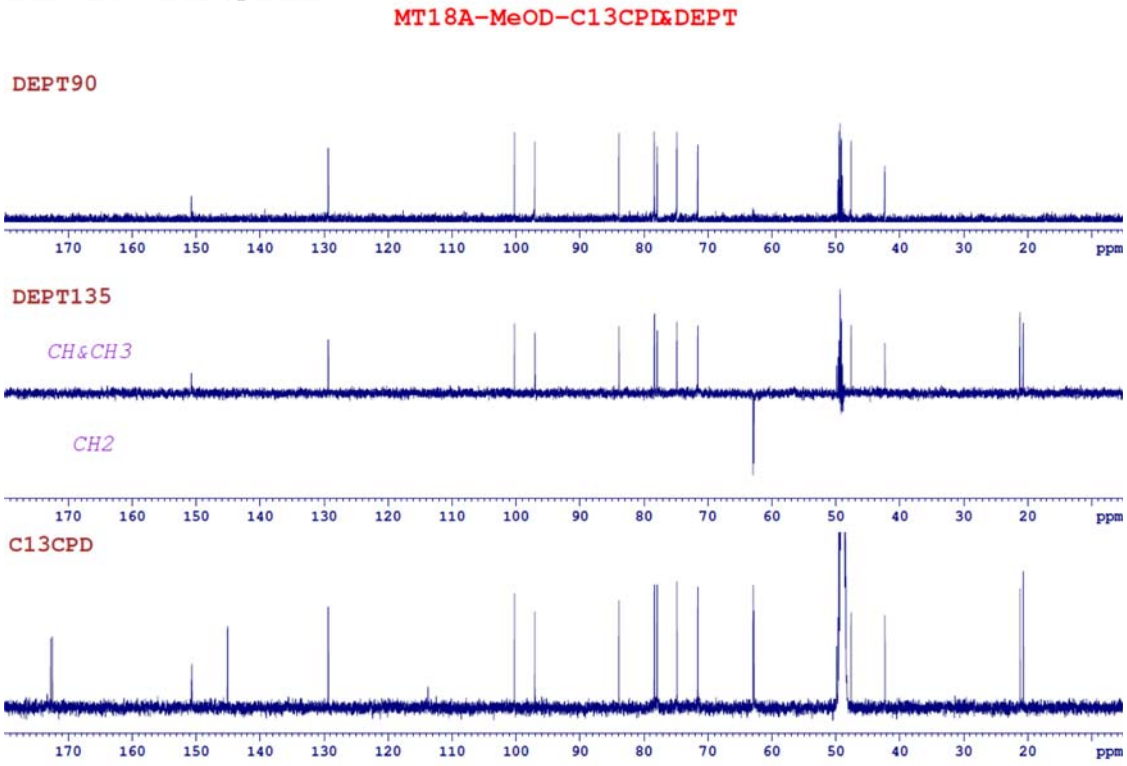
5.1. ^{1}H-NMR of compound **1**



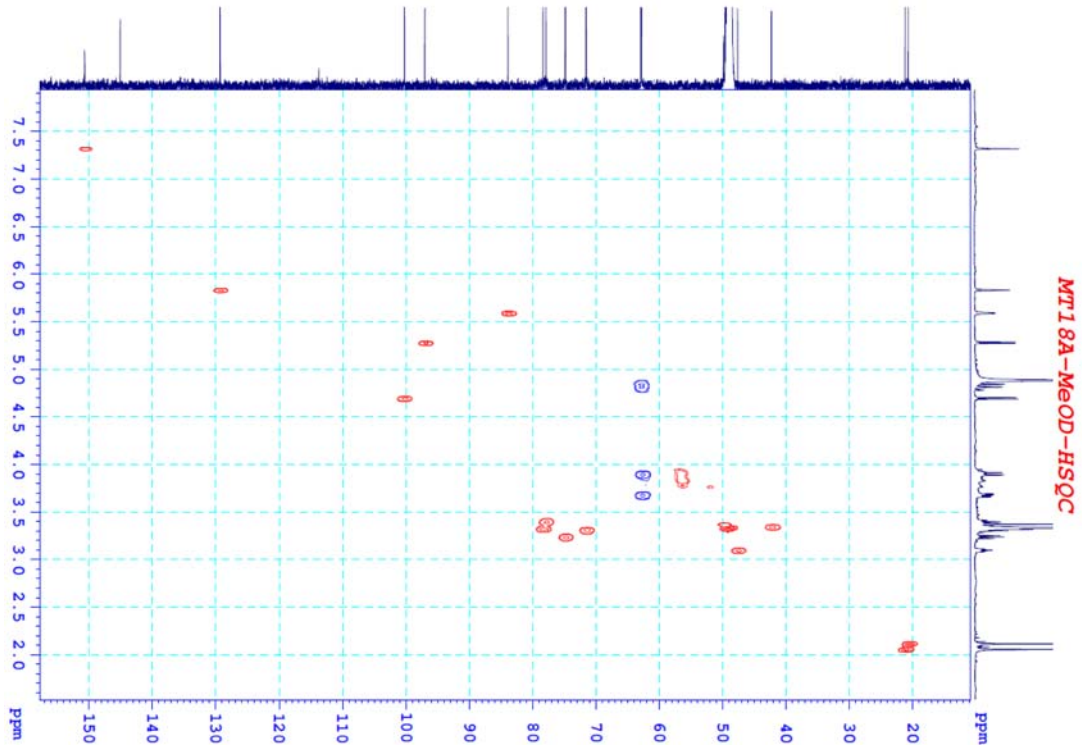
5.2. ^{13}C-NMR of compound **1**



5.3. DEPT of compound **1**



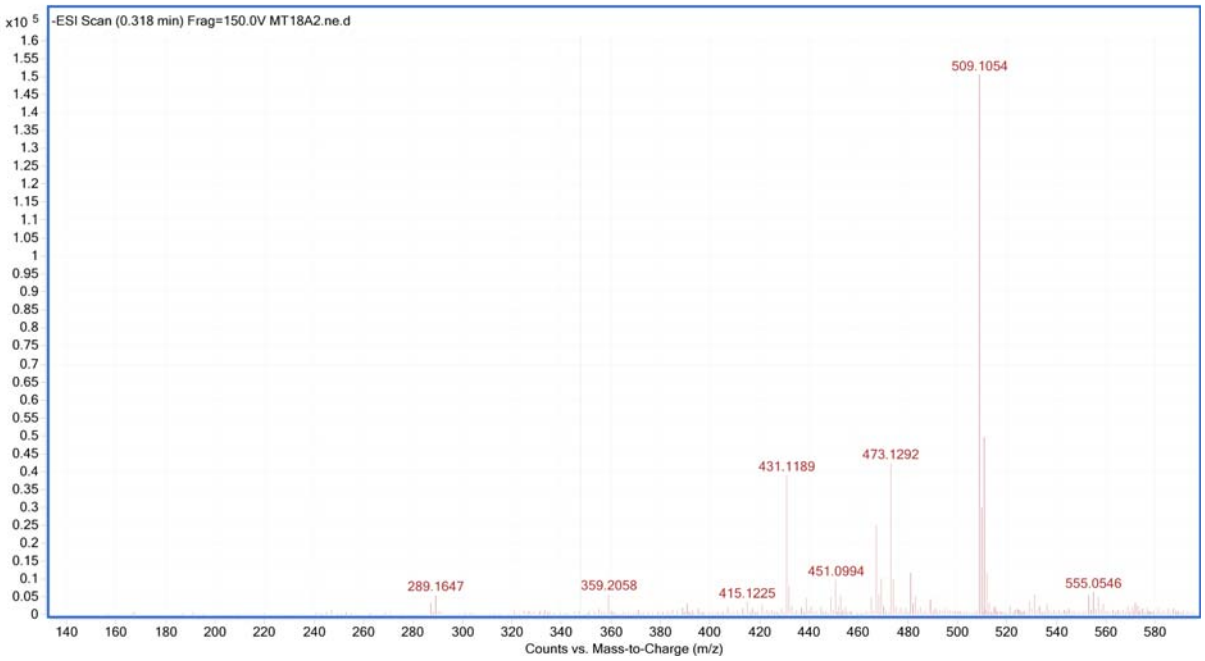
5.4. HSQC of compound **1**



5.5. HR-ESI-MS of compound **1**

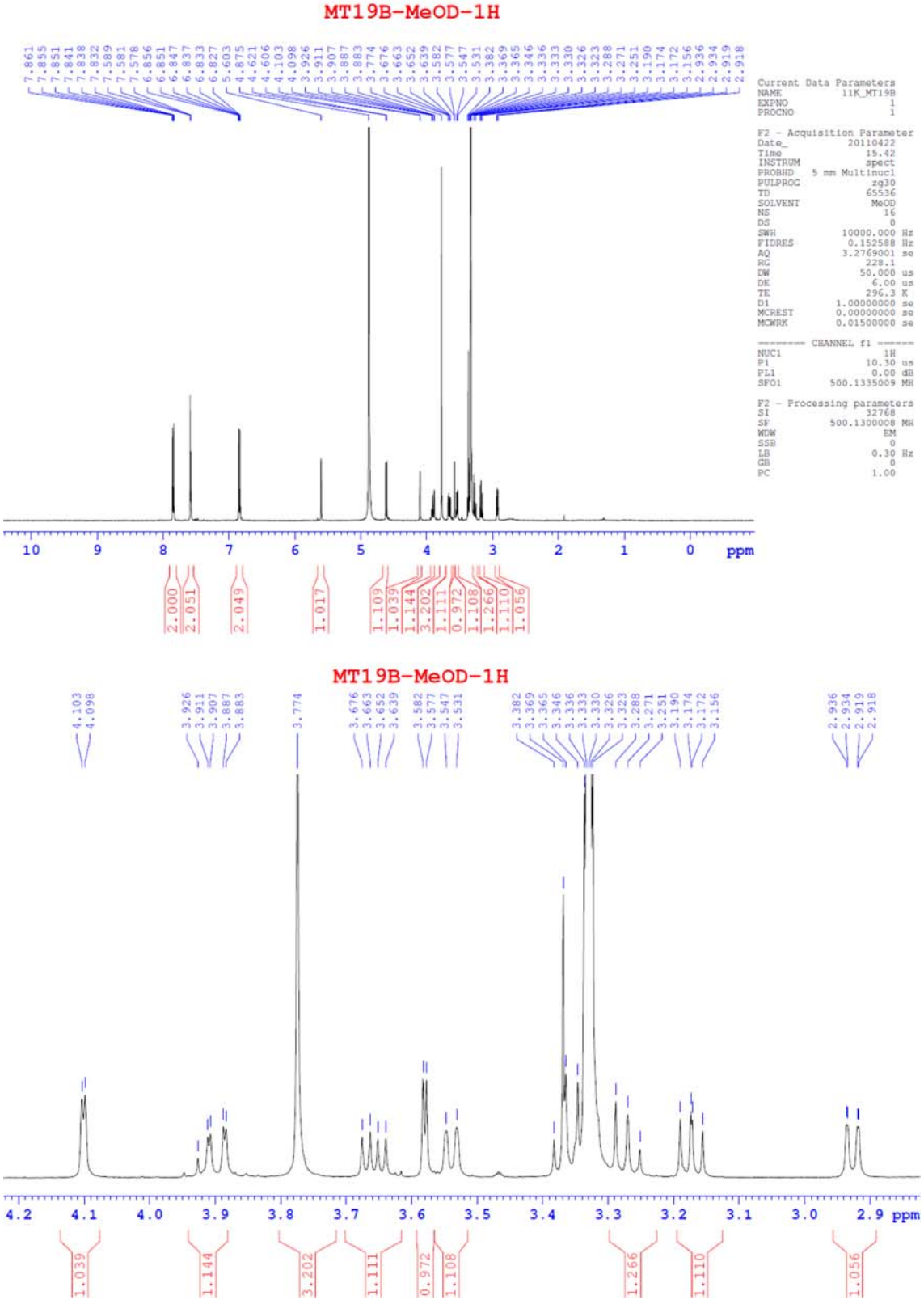
m/z 509.1054 $[M + Cl]^-$ (Calcd $C_{20}H_{26}O_{13}Cl$ for 509.1054)

m/z 473.1292 $[M - H]^-$ (Calcd $C_{20}H_{25}O_{13}$ for 473.1295)

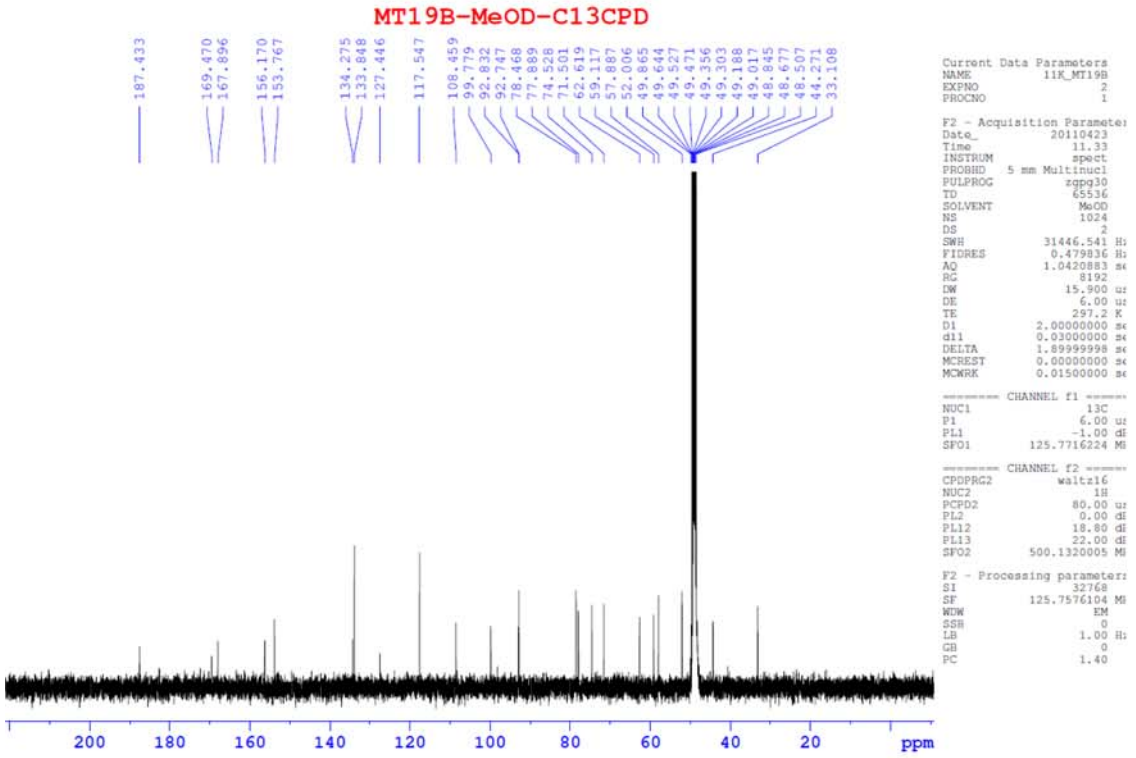


6. NMR and MS spectra of compound 5

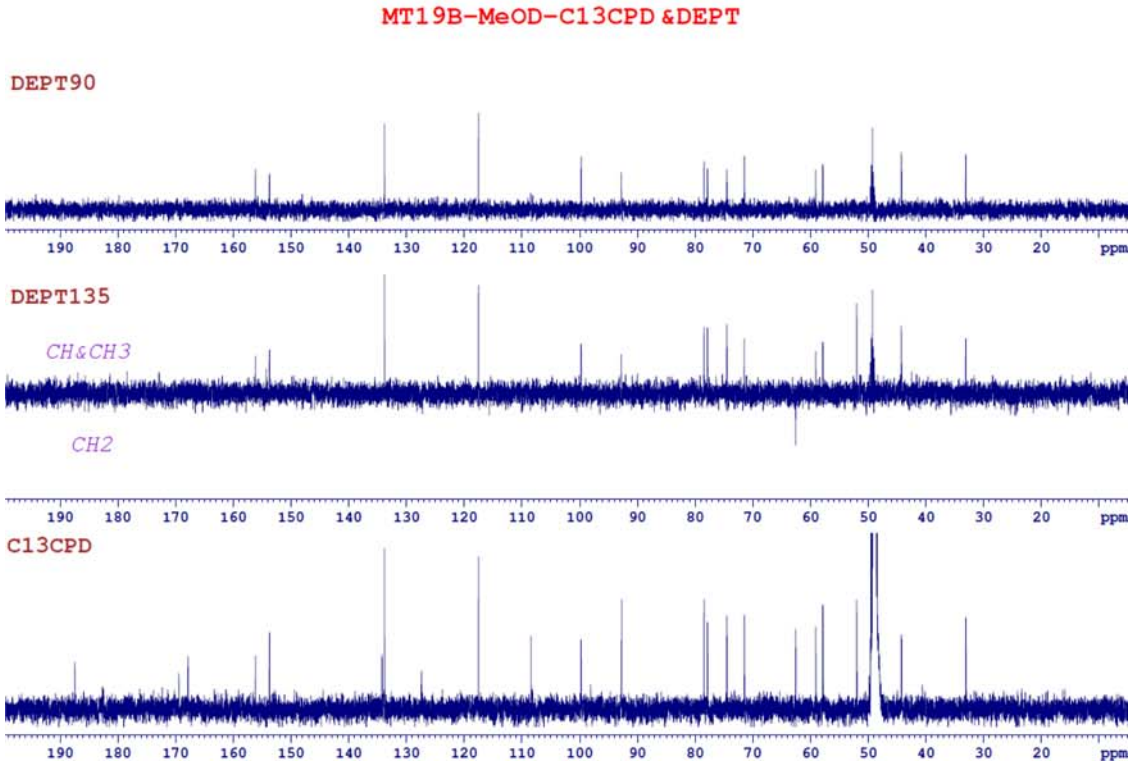
6.1. ^{1}H-NMR of compound **5**



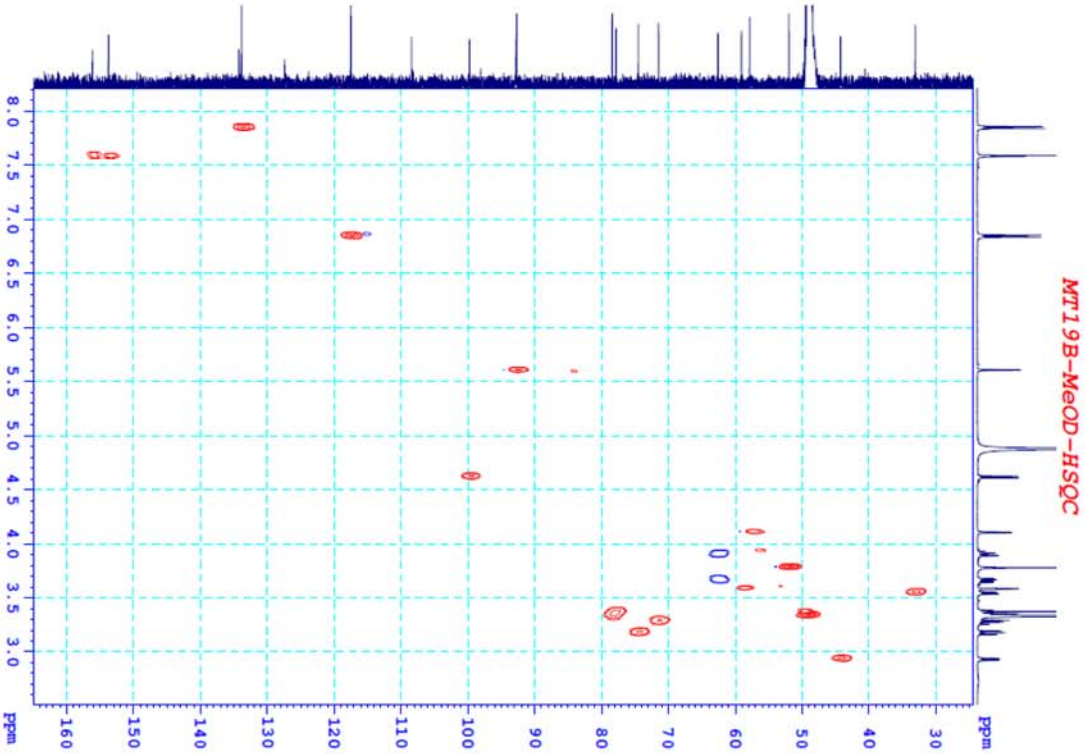
6.2. ^{13}C-NMR of compound **5**



6.3. DEPT of compound **5**



6.4. HMQC of compound **5**



6.5. ESI-MS of compound **5**

m/z 561.1247 $[M - H]^-$ (Calcd $C_{26}H_{25}O_{14}$ for 561.1244)

m/z 597.1008 $[M + Cl]^-$ (Calcd $C_{26}H_{26}O_{14}Cl$ for 597.1011)