

SUPPLEMENTARY MATERIAL

In vitro* study on α -amylase inhibitory and α -glucosidase of a new stigmastane-type steroid saponin from the leaves of *Vernonia amygdalina

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Abstract

Using various chromatographic separations, four compounds, including one new steroid saponin named vernoamyoside E (**1**), were isolated from the leaves of the Vietnamese medicinal plant *Vernonia amygdalina* Delile (Asteraceae). Their structures were established by spectroscopic methods such as 1D- and 2D-NMR, HR-ESI-MS, and HPLC analysis. The inhibitory activities against α -glucosidase and α -amylase of the isolated compounds from *V. amygdalina* were reported for the first time. The results indicated that compound **1** significantly inhibited both against α -amylase and α -glucosidase activity.

Keywords: *Vernonia amygdalina*, Asteraceae, vernoamyoside E, α -amylase inhibitory, α -glucosidase inhibitor

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Acid hydrolysis and sugar identification

Compounds **1** (2.3 mg) were dissolved in 3 mL of 10% HCl 1M-dioxan (1:1, v/v) and then heated at 60 °C for 2 h. After the solvent was removed *in vacuo*, the residue was partitioned successively with CH₂Cl₂ and H₂O to give the aglycon and the sugar, respectively. The aqueous phase was concentrated to provide a residue. After drying, the residue was dissolved in anhydrous pyridine (100 µL) and then mixed with a pyridine solution of 0.1 M L-cysteine methyl ester hydrochloride (100 µL). After warming at 60°C for 1 h, phenyl isothiocyanate solution was added, and the reaction solution was maintained at 60°C for 1 h. The reaction mixture was evaporated *in vacuo* to give a dried product. Using column (Phenomenex: 4.6 mm×250 mm, 5µm) with a mobile phase MeCN/water (30:70, flow rate of 1 ml/min), detected at the UV 250 nm, analysis of the standard D-glucose and L-glucose was performed under the same experimental conditions. The retention time of derivatized sugar was found to be 27.7 min compared with the retention time of standard persilylated D-glucose of 27.7 min, and L-glucose of 28.2.

Assay for α-amylase inhibition

The porcine pancreas α-amylase (A3176, Sigma-Aldrich) enzyme inhibitory activity was carried out according to the standard method with minor modification by Luyen et al. with slight modifications (Luyen et al. 2019). The substrate was prepared by boiling 100 mg potato starch in 5 mL phosphate buffer (pH 7.0) for 5 min, then cooling to room temperature. The sample (2 mL dissolved in DMSO) and substrate (50 mL) were mixed in 30 mL of 0.1 M phosphate buffer (pH 7.0). After 5 min pre-incubation, 5 mg/mL α-amylase solution (20 mL) was added and the solution was incubated at 37 °C for 15 min. The reaction was stopped by adding 50 mL 1 M HCl, and then 50 mL iodine solution was added. The absorbance was measured at 650 nm by a microplate reader. Acarbose was used as a positive control.

The inhibitory activity was calculated by the following equation: α-amylase inhibitory activity (%) = $(1 - A/A_0) \times 100$, where A is the absorbance of the sample and A₀ is the absorbance of the blank, respectively. IC₅₀ value was calculated by GraphPad Prism.

Assay for α-glucosidase inhibition

The yeast α-glucosidase (G0660, Sigma-Aldrich) inhibition assay was performed using the substrate *p*-nitrophenyl-α-D-glucopyranoside (Luyen et al. 2019). Briefly, samples and acarbose were prepared by dissolving at 2 mg/mL (with extracts) or 0.8 mM (with pure compounds) in DMSO and 0.5 U/mL α-glucosidase (40 mL) were mixed in 120 mL of 0.1 M phosphate buffer (pH 6.8). After 5 min pre-incubation, 5 mM *p*-nitrophenyl-α-D-

glucopyranoside solution (40 mL) was added and the solution was incubated at 37 °C for 30 min. Pipette the following reagents into 96 wells plate. Each concentration of samples was carried out in triplicate. The absorbance of released 4-nitrophenol was measured at 405 nm by using a microplate reader (Xmark, Biorad, USA). Acarbose was used as the positive control. The inhibitory activity was calculated by the following equation: α -glucosidase inhibitory activity (%) = $(1 - A/A_0) \times 100$, where A is the absorbance of the sample and A₀ is the absorbance of the blank, respectively. IC₅₀ value was calculated by GraphPad Prism.

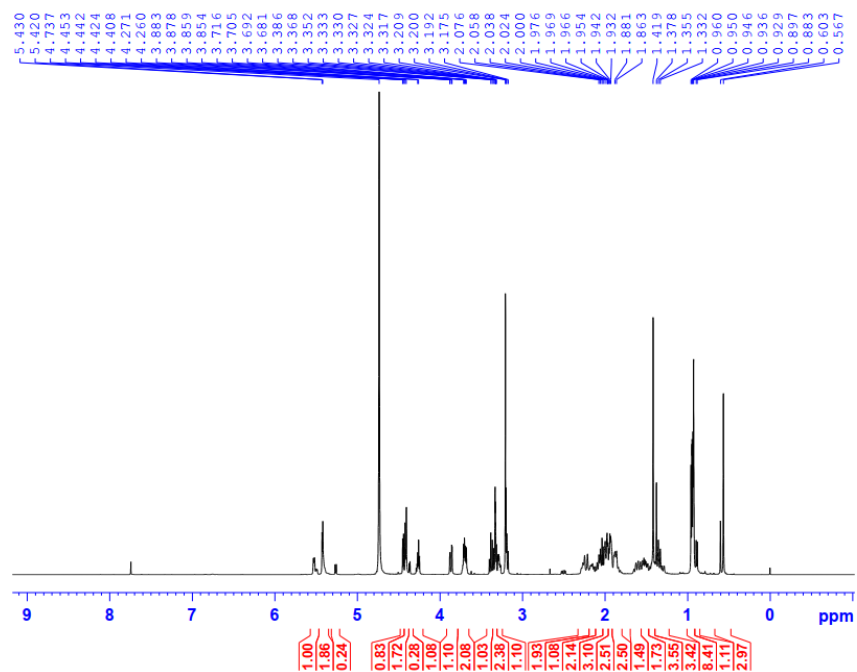


Figure S1. ^1H NMR spectrum (MeOD- d_4 , 500 MHz) of compound (1)

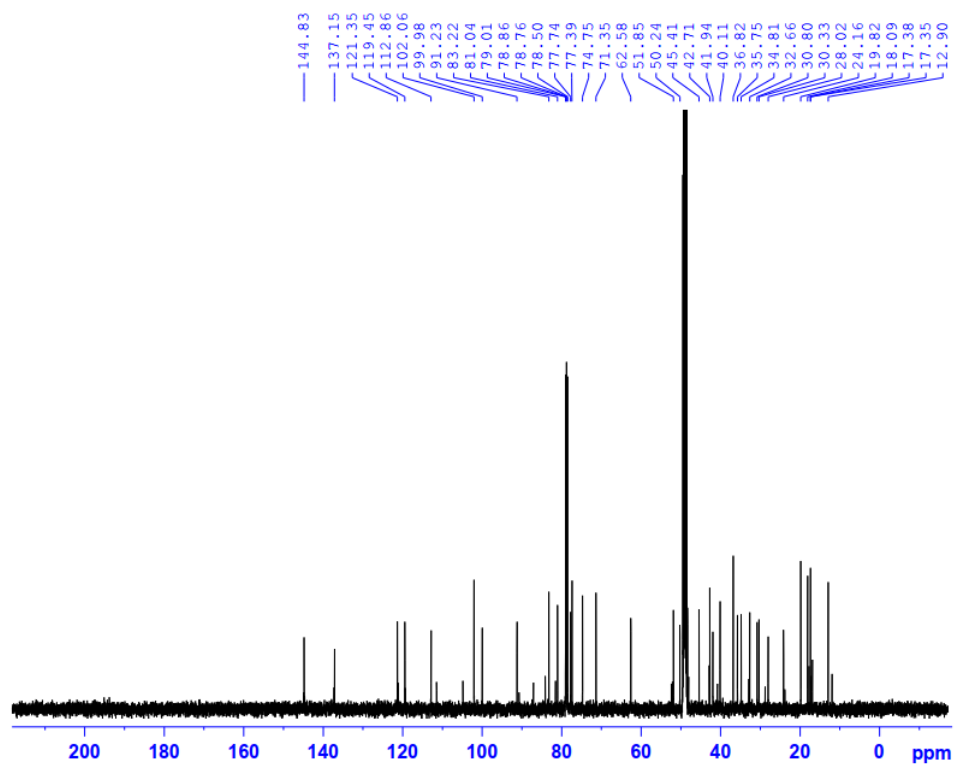


Figure S2. ^{13}C NMR spectrum (MeOD- d_4 , 125 MHz) of compound (1)

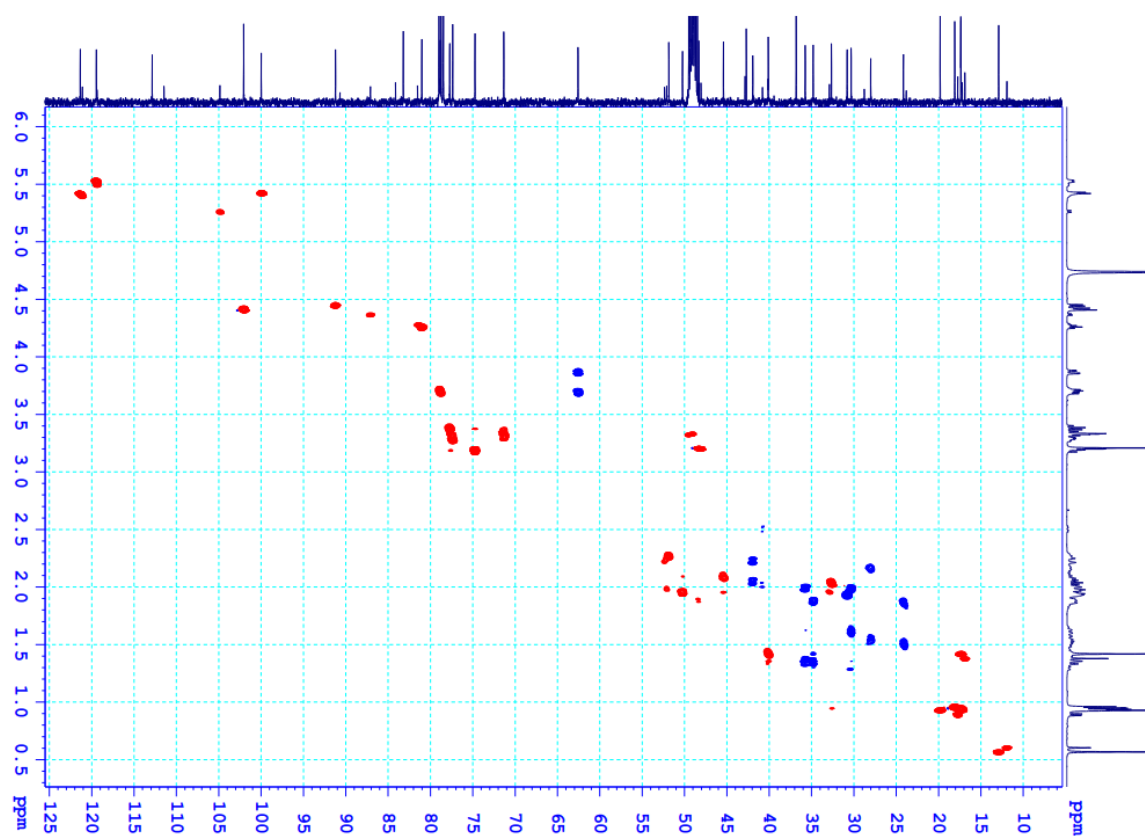


Figure S3. HSQC spectrum (MeOD-*d*₄, 500 MHz) of compound (**1**)

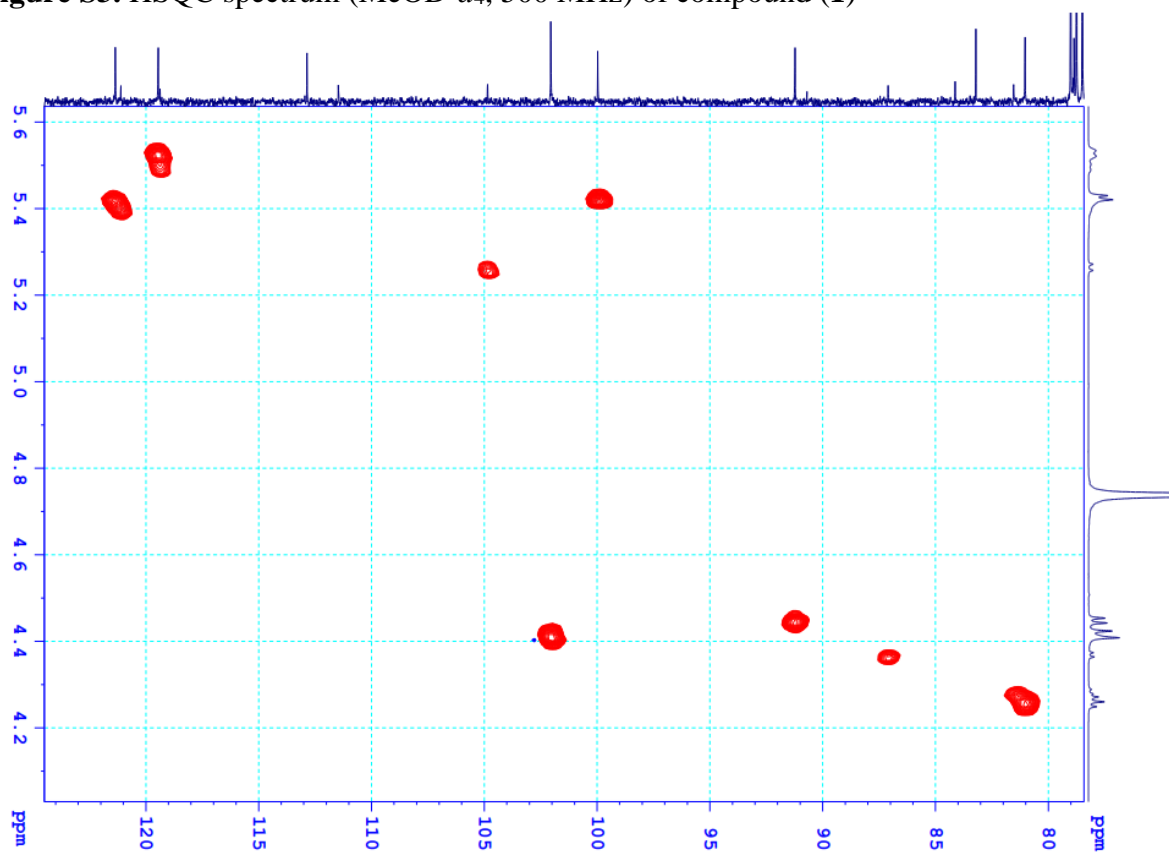


Figure S4. HSQC spectrum ex-1 (MeOD-*d*₄, 500 MHz) of compound (**1**)

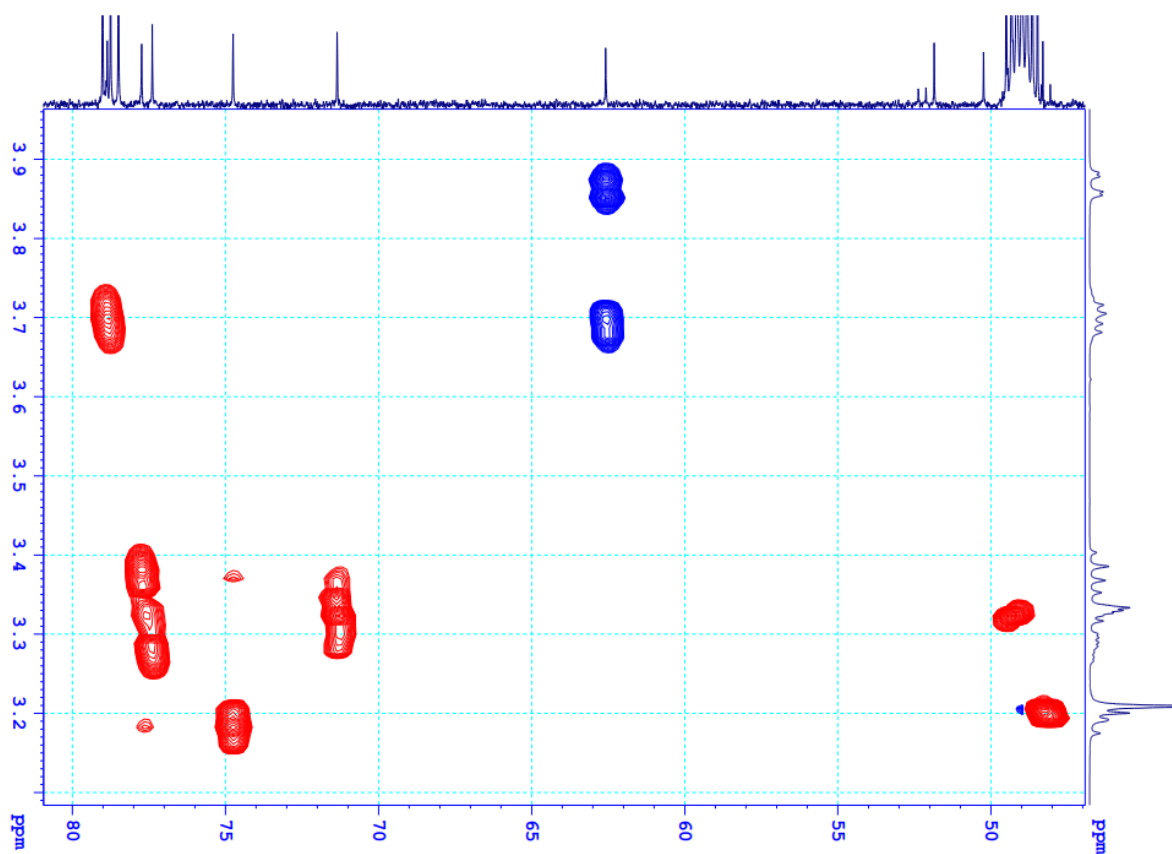


Figure S5. HSQC spectrum ex-2 (MeOD- d_4 , 500 MHz) of compound (**1**)

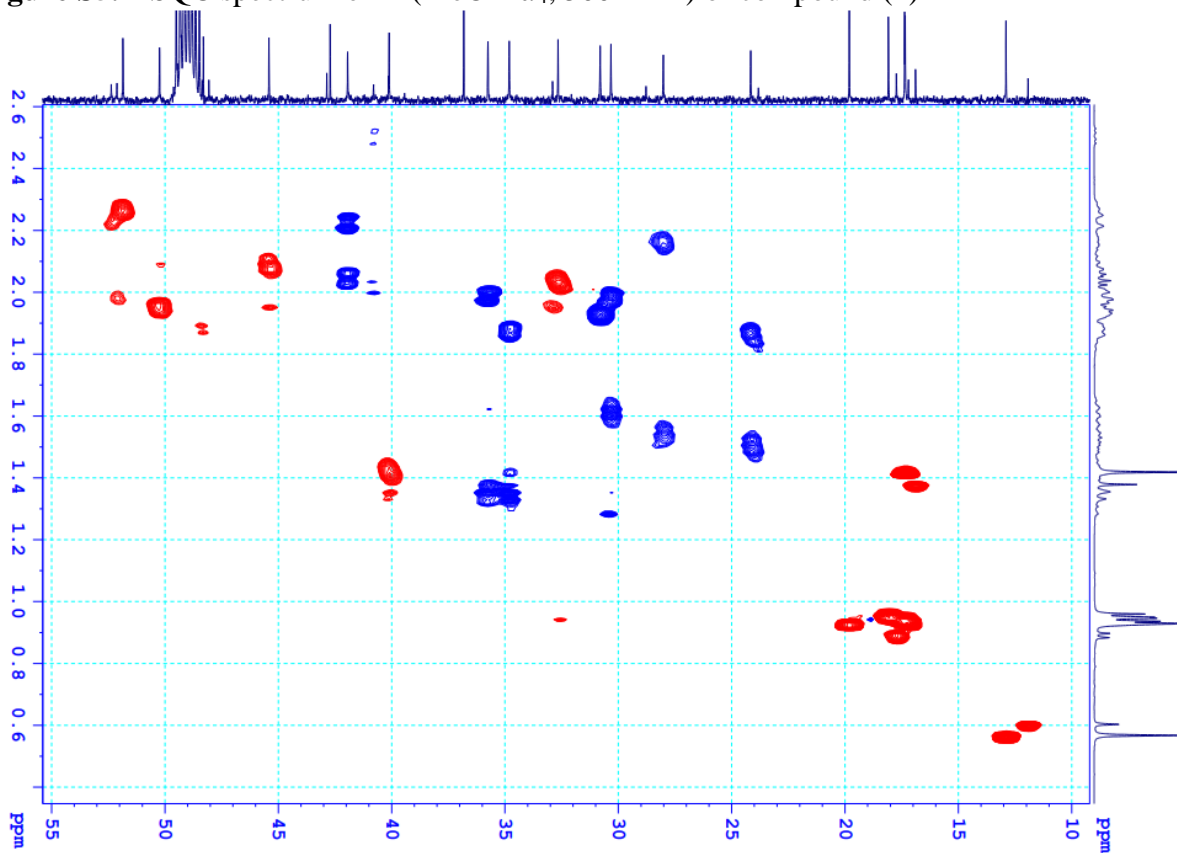


Figure S6. HSQC spectrum ex-3 (MeOD- d_4 , 500 MHz) of compound (**1**)

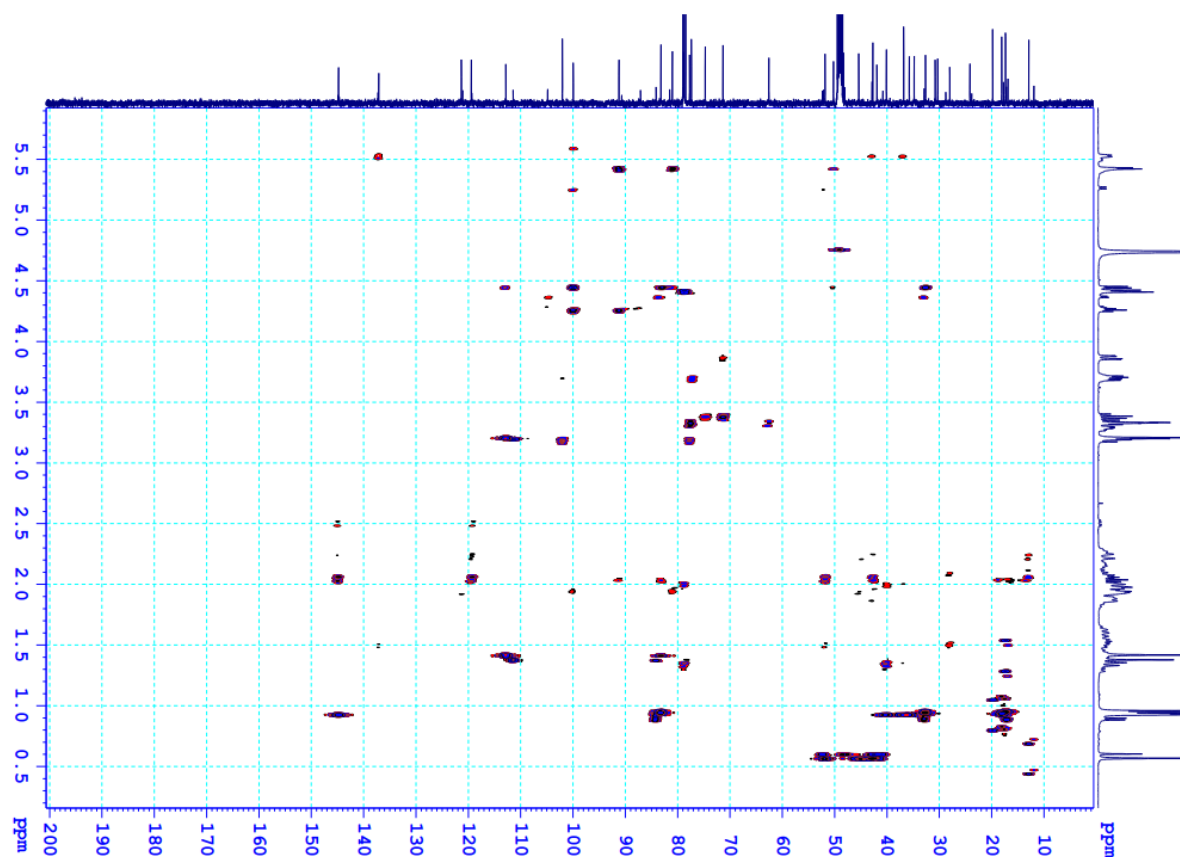


Figure S7. HMBC spectrum (MeOD- d_4 , 500 MHz) of compound (**1**)

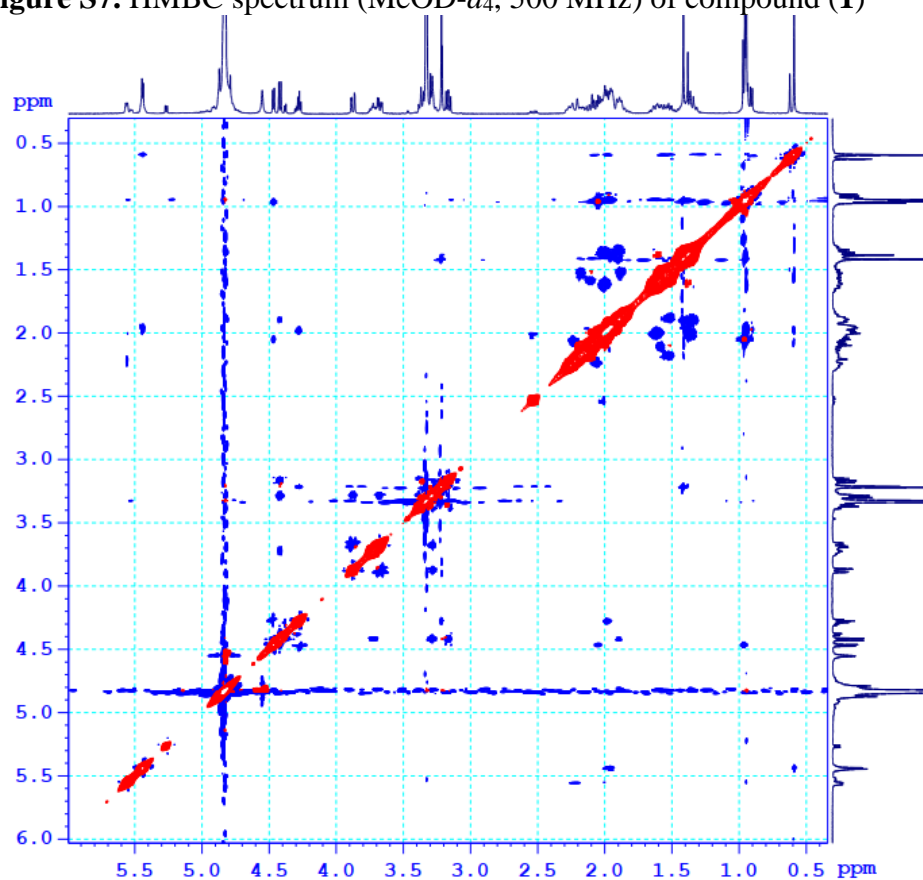


Figure S8. NOESY spectrum (MeOD- d_4 , 500 MHz) of compound (**1**)

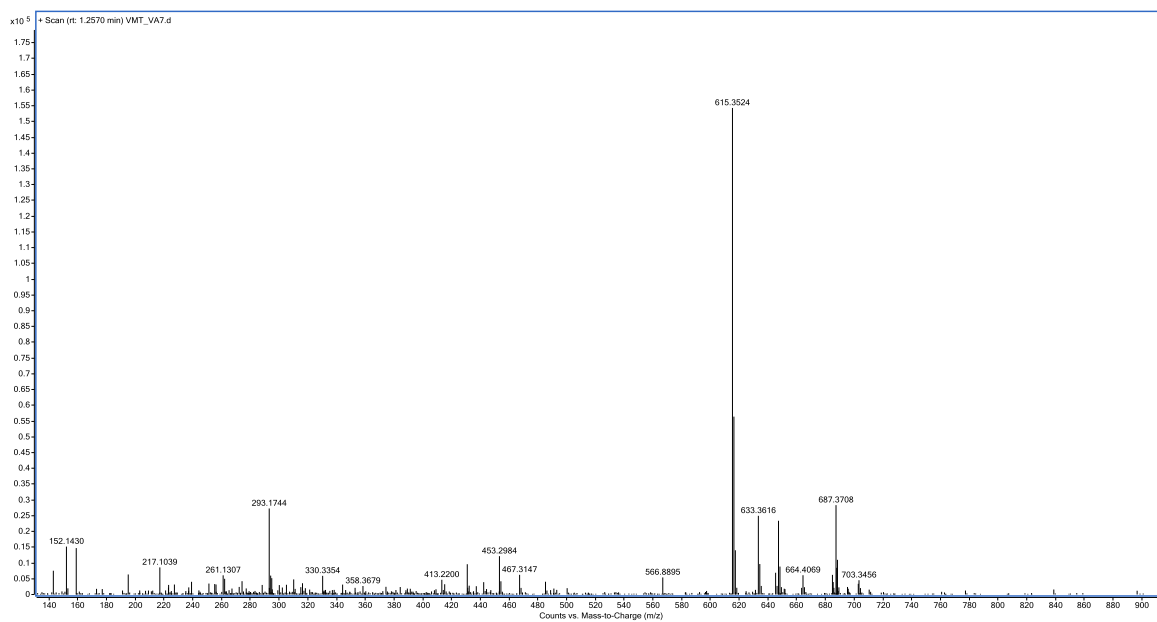


Figure S9. HR ESI MS of compound (1)

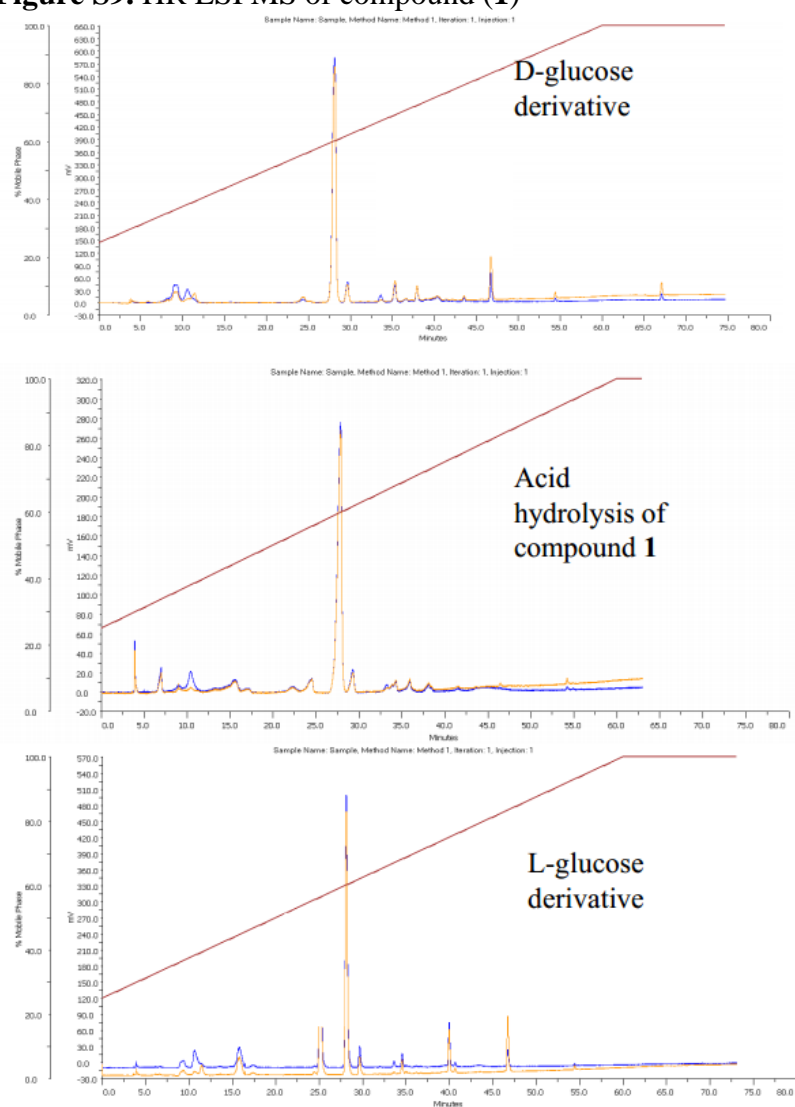


Figure S10. HPLC of compound (1) from acid hydrolysis

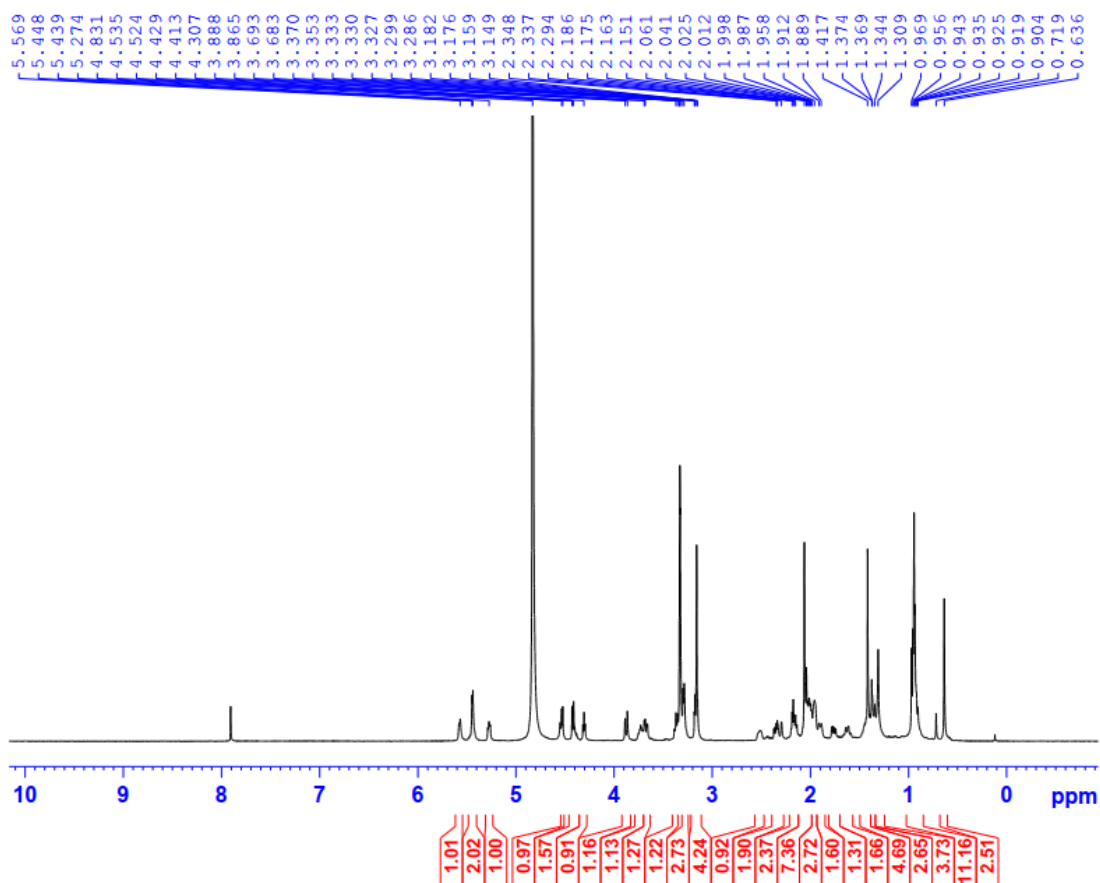


Figure S11. ^1H NMR spectrum (MeOD- d_4 , 500 MHz) of compound (2)

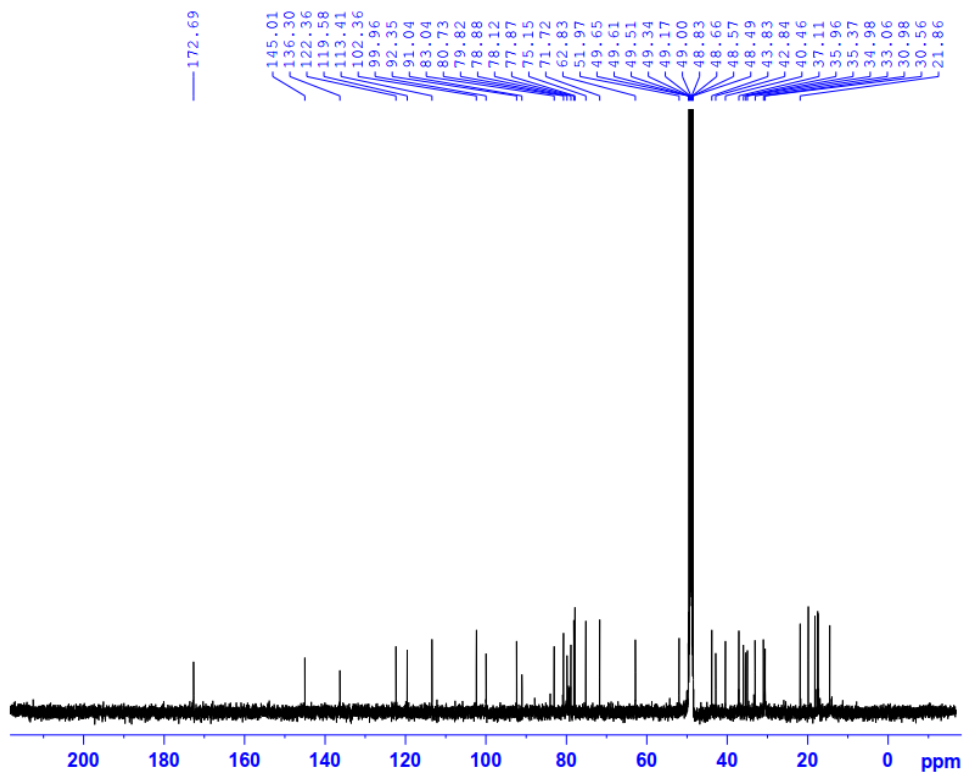


Figure S12. ^{13}C NMR spectrum (MeOD- d_4 , 125 MHz) of compound (2)

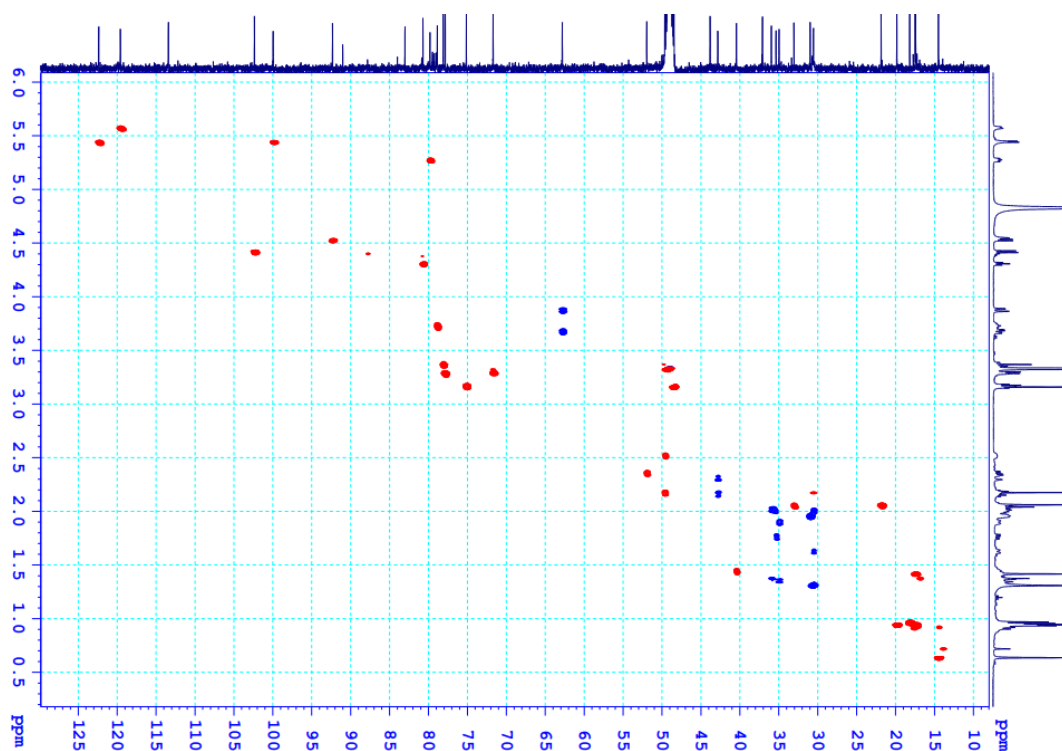


Figure S13. HSQC spectrum (MeOD-*d*₄, 500 MHz) of compound (2)

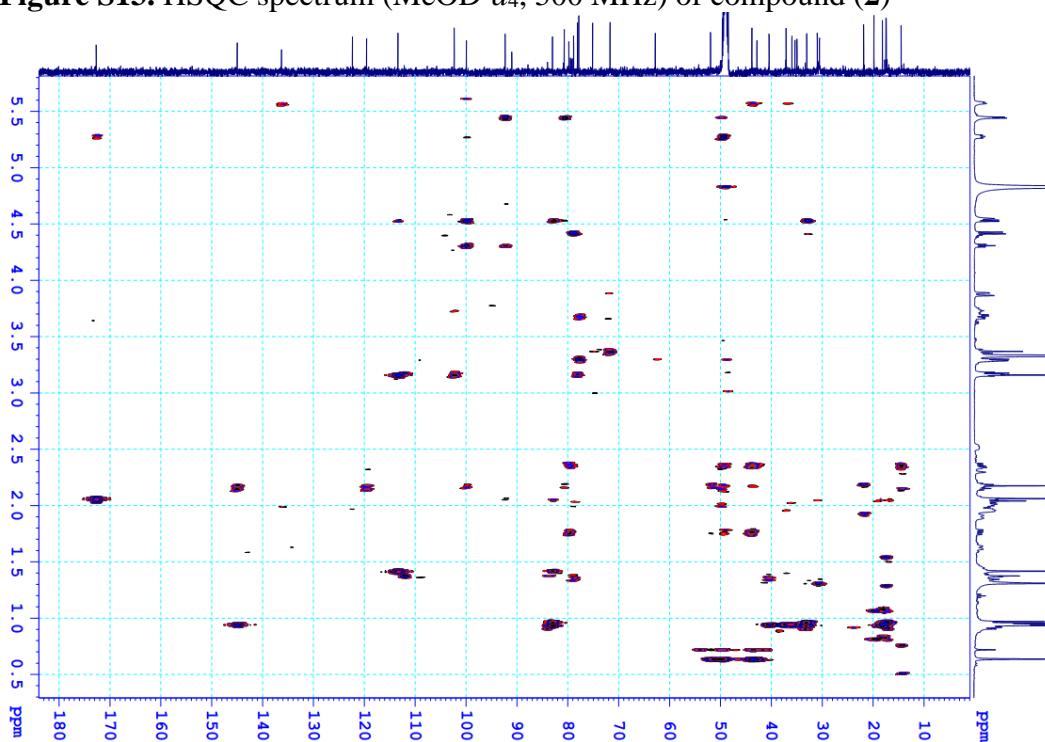


Figure S14. HMBC spectrum (MeOD-*d*₄, 500 MHz) of compound (2)

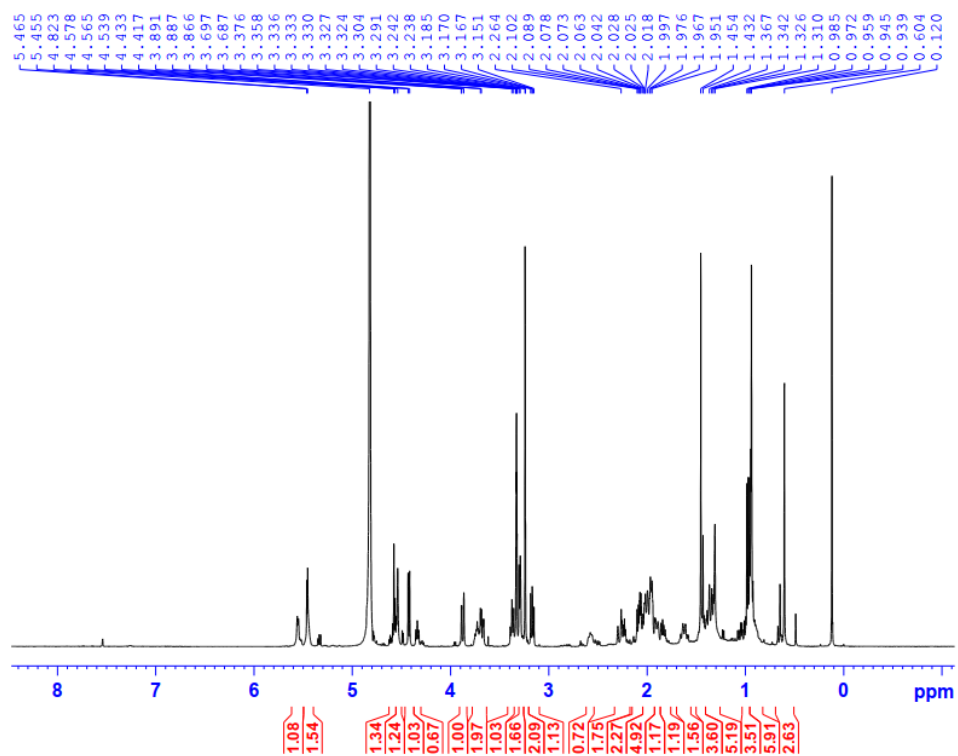


Figure S15. ^1H NMR spectrum ($\text{MeOD-}d_4$, 500 MHz) of compound (**3**)

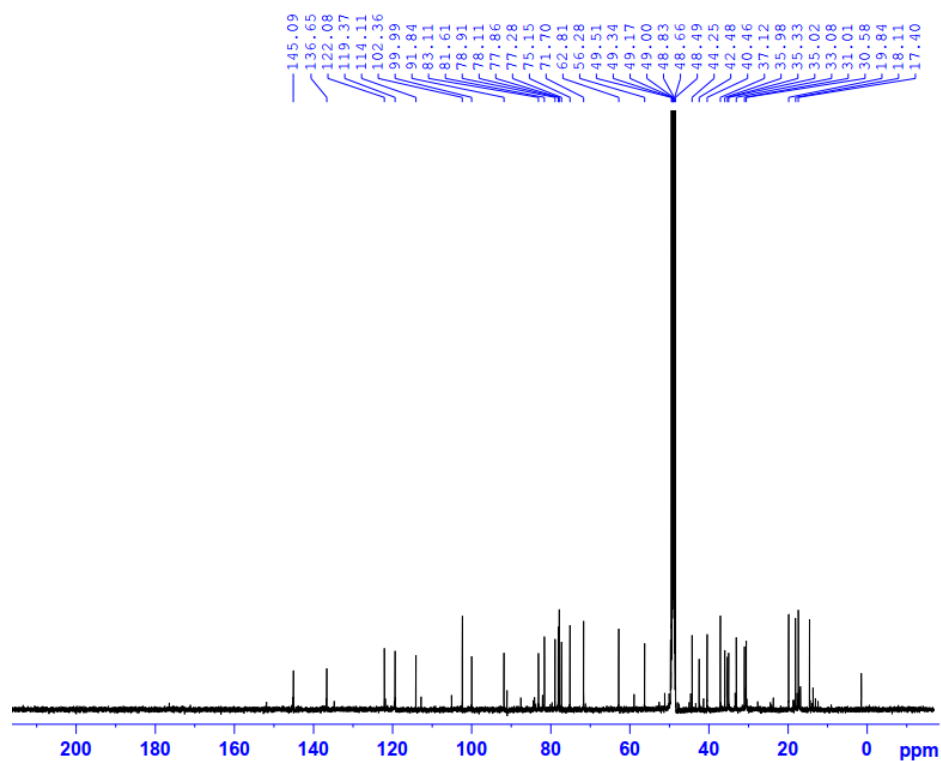


Figure S16. ^{13}C NMR spectrum ($\text{MeOD-}d_4$, 125 MHz) of compound (**3**)

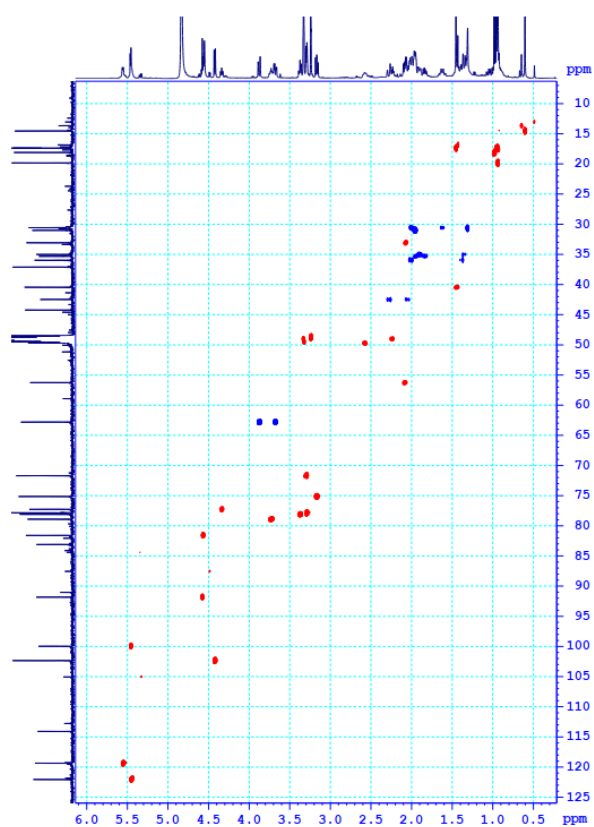


Figure S17. HSQC spectrum (MeOD-*d*₄, 500 MHz) of compound (**3**)

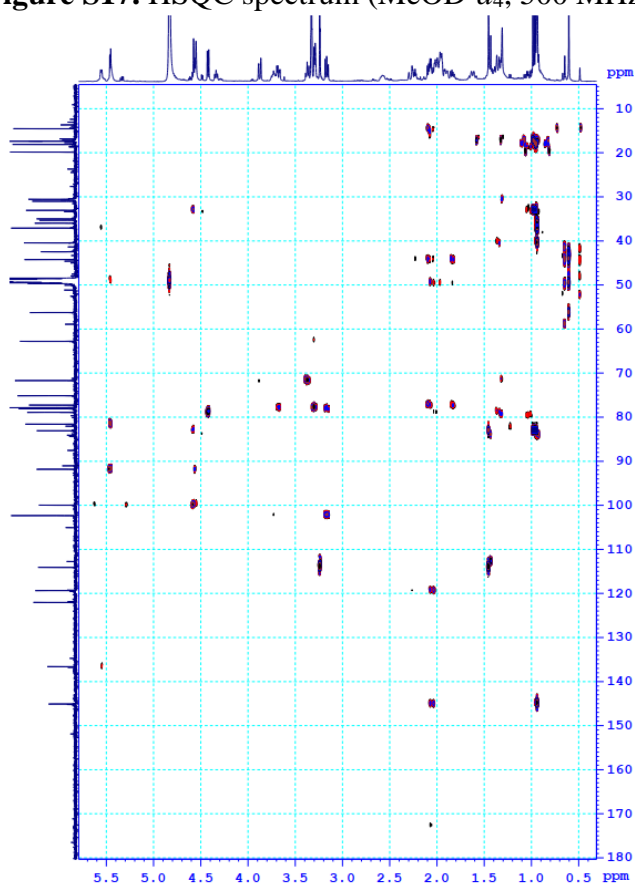


Figure S18. HMBC spectrum (MeOD-*d*₄, 500 MHz) of compound (**3**)

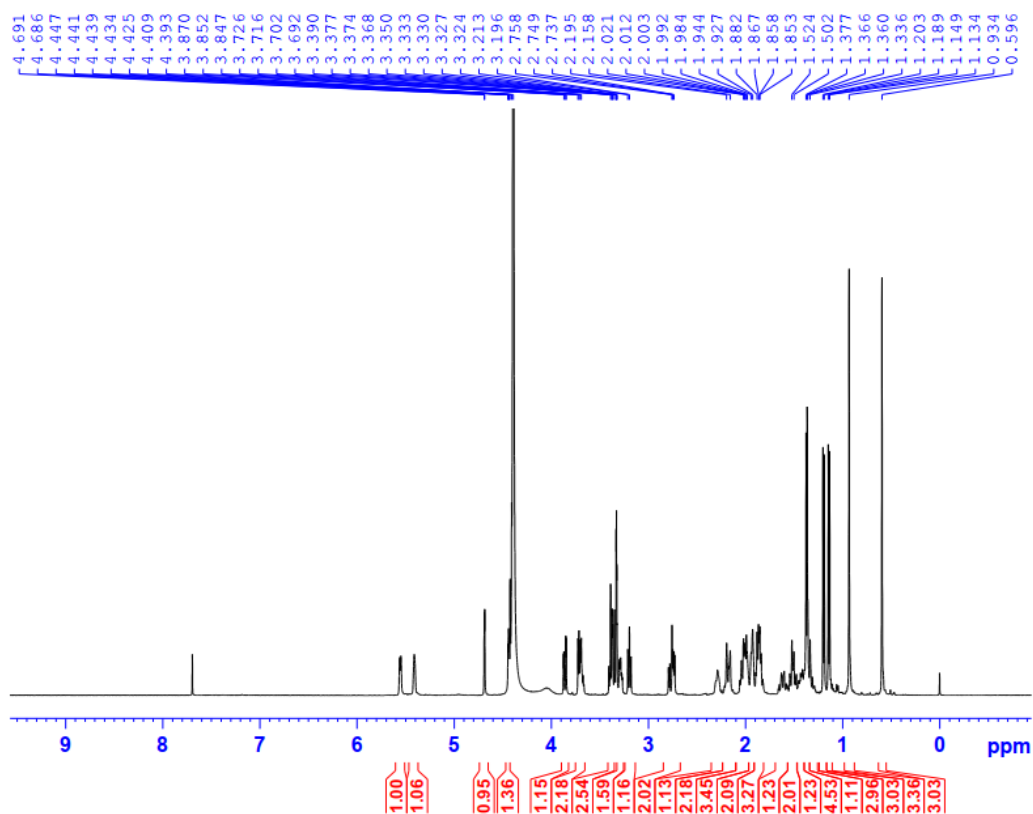


Figure S19. ^1H NMR spectrum ($\text{MeOD-}d_4$, 500 MHz) of compound (**4**)

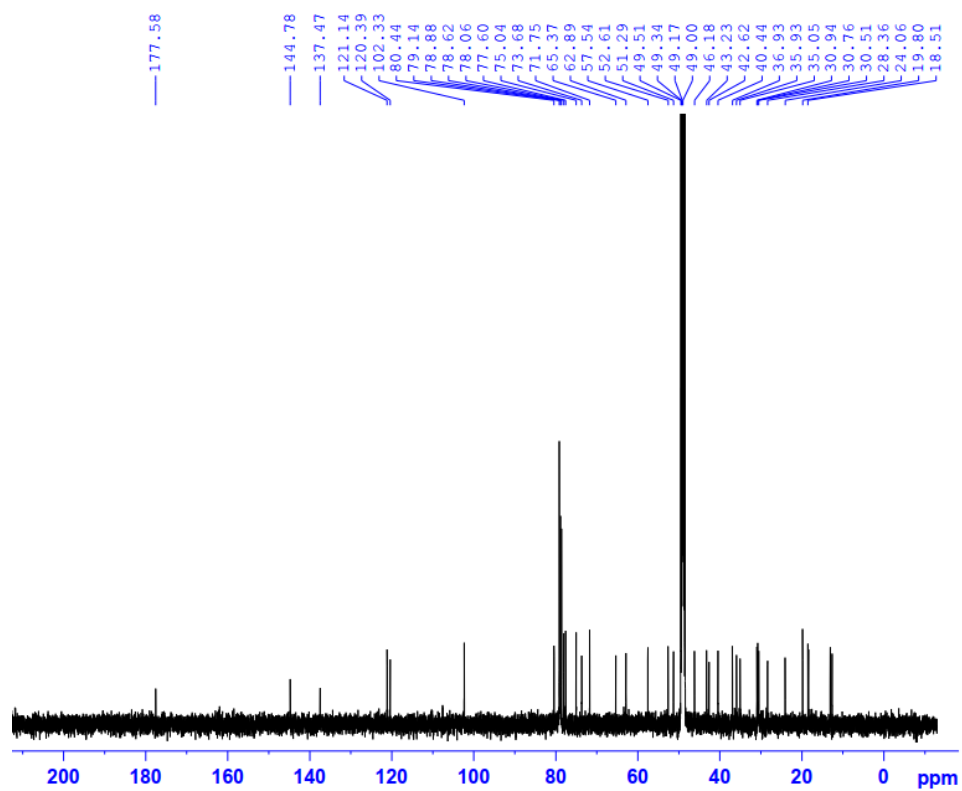


Figure S20. ^{13}C NMR spectrum ($\text{MeOD-}d_4$, 125 MHz) of compound (**4**)

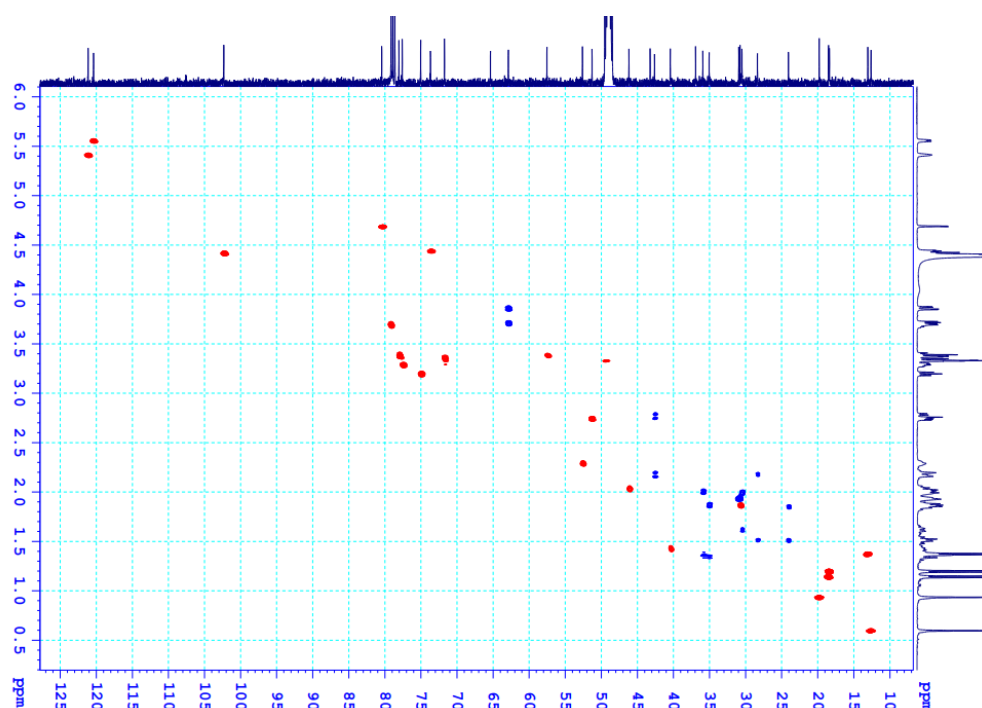


Figure S21. HSQC spectrum (MeOD-*d*₄, 500 MHz) of compound (**4**)

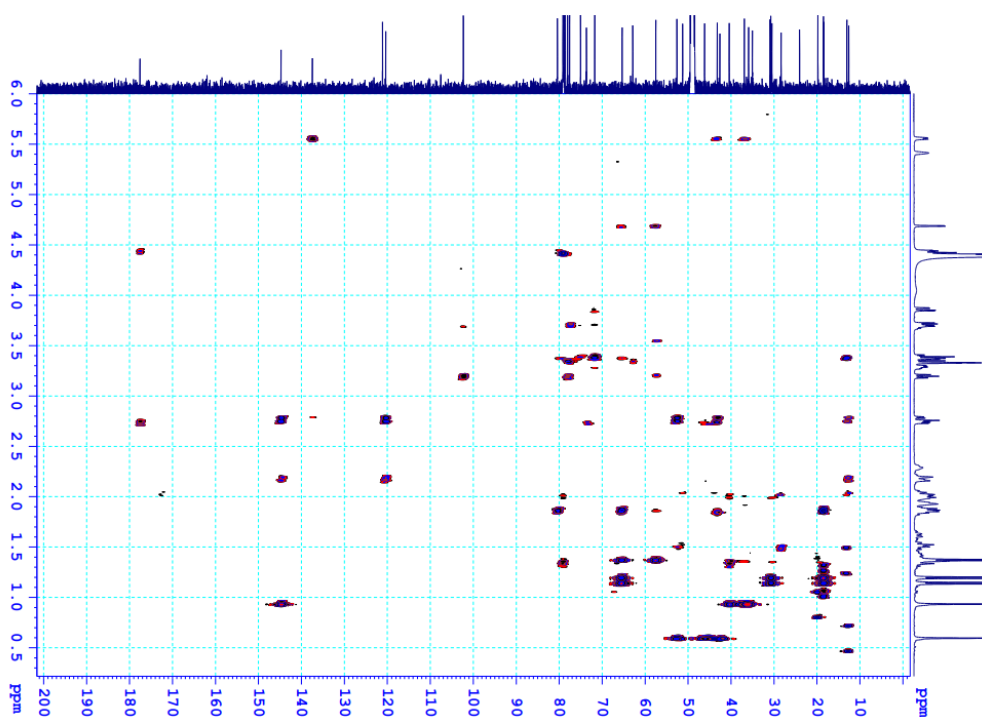


Figure S22. HMBC spectrum (MeOD-*d*₄, 500 MHz) of compound (**4**)

Luyen NT, Binh PT, Tham PT, Hung TM, Dang NH, Dat NT, Thao NP. 2019. Wedtrilosides A and B, two new diterpenoid glycosides from the leaves of *Wedelia trilobata* (L.) Hitchc. with α -amylase and α -glucosidase inhibitory activities. *Bioorg Chem.* 85:319-324.