**SUPPLEMENTARY MATERIAL**

# Antioxidant activity-guided isolation of constituents from *Euphorbia gaditana* Coss. and their antioxidant and tyrosinase inhibitory activities

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**ABSTRACT**

Two previously unreported compounds, 4-*O*-(2″-*O*-galloyl-*β*-D-glucopyranosyl)-3,5-dihydroxyacetophenone (**1**) and 5-isopropyl-2-oxo-3,6-dihydropyran-4-carboxylic acid (**2**), along with twenty-nine known compounds (**3-31**) were isolated from the aerial parts of *Euphorbia gaditana* Coss. Their structures were elucidated based on extensive spectroscopic analysis 1D and 2D-NMR, mass spectrometry HR-ESI-MS, optical rotation $[\alpha]_D$, acid hydrolysis and the comparison of NMR data with those described in literature. The antioxidant activity-guided study was conducted using DPPH and CUPRAC methods started from the extracts to bioactive isolated molecules. Most of the isolates (**1-31**) showed a good to excellent antioxidant activity compared to the standards BHT and ascorbic acid. Furthermore, **1** and **2** exhibited moderate tyrosinase inhibitory activity ($IC_{50}$ 89.78 ± 0.93 and 52.39 ± 0.69 μg/mL, respectively) compared to the standard kojic acid ($IC_{50}$ 25.23 ± 0.78 μg/mL).

**Keywords:** Euphorbiaceae; *Euphorbia gaditana* Coss.; 4-*O*-(2″-*O*-galloyl-*β*-D-glucopyranosyl)-3,5-dihydroxyacetophenone; 5-isopropyl-2-oxo-3,6-dihydropyran-4-carboxylic acid; antioxidant activity; tyrosinase inhibitory activity

## General methods

### *1. Total bioactive contents*

The Folin-Ciocalteu method (Muller et al., 2010) was used for the measurement of total phenolic content (TPC) for the obtained extracts (PE, $CHCl_3$, EtOAc and *n*-BuOH). For the calibration, the gallic acid solution at various concentrations was used and the result was expressed as gallic acid equivalents (mg GAE/g extract).

To measure the total flavonoids content (TFC) the trichloroaluminum method (Topçu et al., 2007) was applied. The TFC of extracts was given as quercetin equivalents (mg QE/g extract), with quercetin for calibration.

### *2. Biological activities*

#### *2.1. DPPH free radical scavenging assay*

Following Blois method with a slight modification (Blois 1958), the free radical scavenging assay was evaluated by the DPPH. Briefly, 160 µL of DPPH solution (0.4 mM) was added to 40 µL of samples dissolved in MeOH at different concentrations; after 30 min in darkness the absorbance was measured at 517 nm. A solution containing 40 µL of MeOH with 160 µL of DPPH solution was used as control.

#### *2.2. Cupric reducing antioxidant capacity (CUPRAC) assay*

The Apak method (Apak et al., 2004) was applied in order to measure cupric-reducing antioxidant capacity (CUPRAC). To each well, in a 96-well plate, 40 µL of the sample (dissolved in MeOH) at different concentrations as well as 60 µL ammonium acetate buffer (1 M, pH 7.0), 50 µL 7.5 mM neocuproine and 50 µL 10 mM copper (II) chloride solutions were added. As control, the 40 µL of the sample was substituted by 40 µL of MeOH. After 60 min, the absorbance was measured at 450 nm.

### *2.3. Tyrosinase assay*

The tyrosinase enzyme inhibitory activity was measured using Deveci method (Deveci et al., 2018). 150 µL of sodium phosphate buffer (pH 6.8), 10 µL of sample at different concentrations and 20 µL of tyrosinase enzyme solution were added in 96-well micro-plate and incubated for 10 min at 37°C. After that, 20 µL of L-DOPA was added and incubated again for 10 min at 37°C. The absorbance was read at 475 nm.

## References


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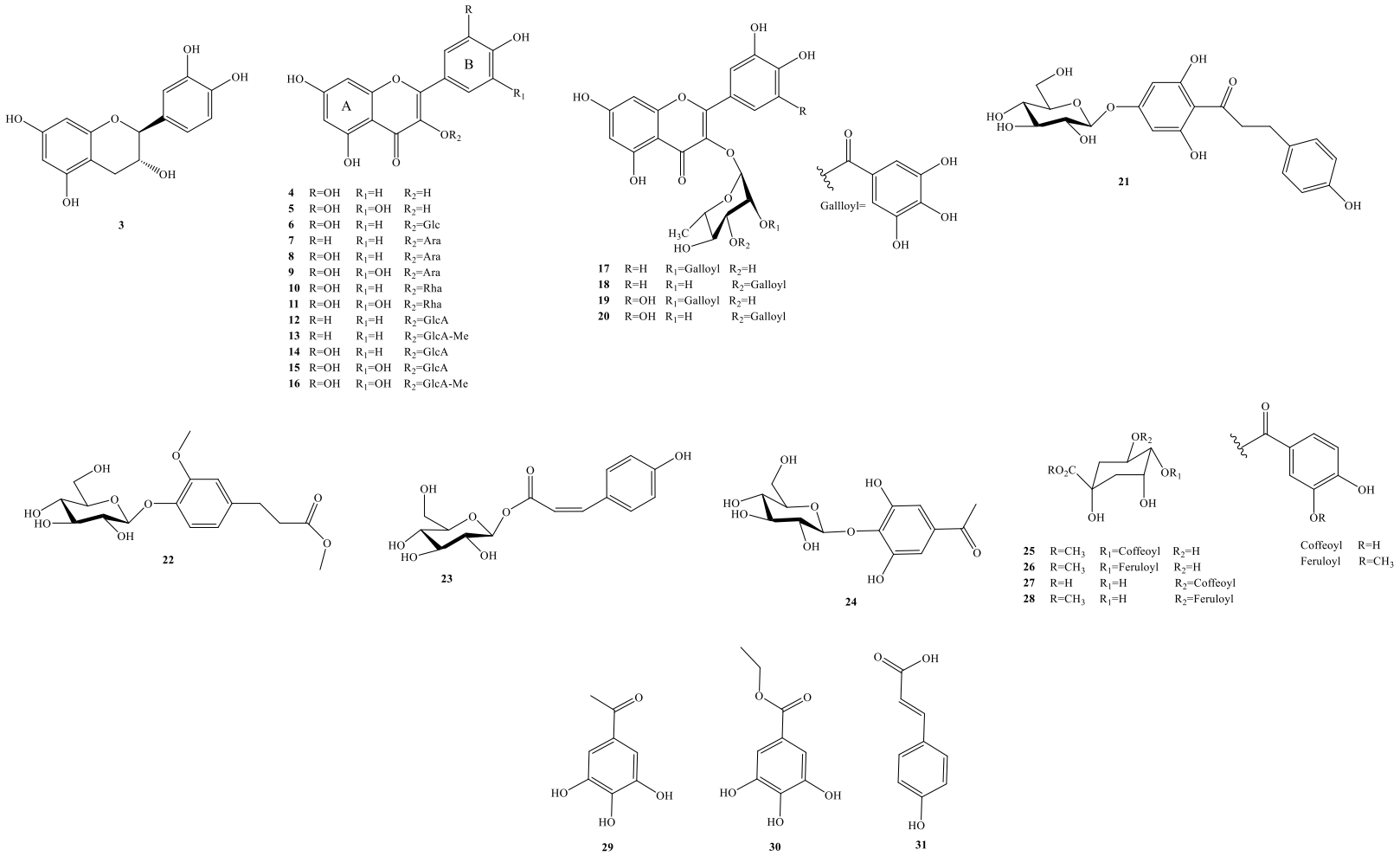


**Fig. S1.** Chemical structures of known compounds **3-31** isolated from *E. gaditana*.

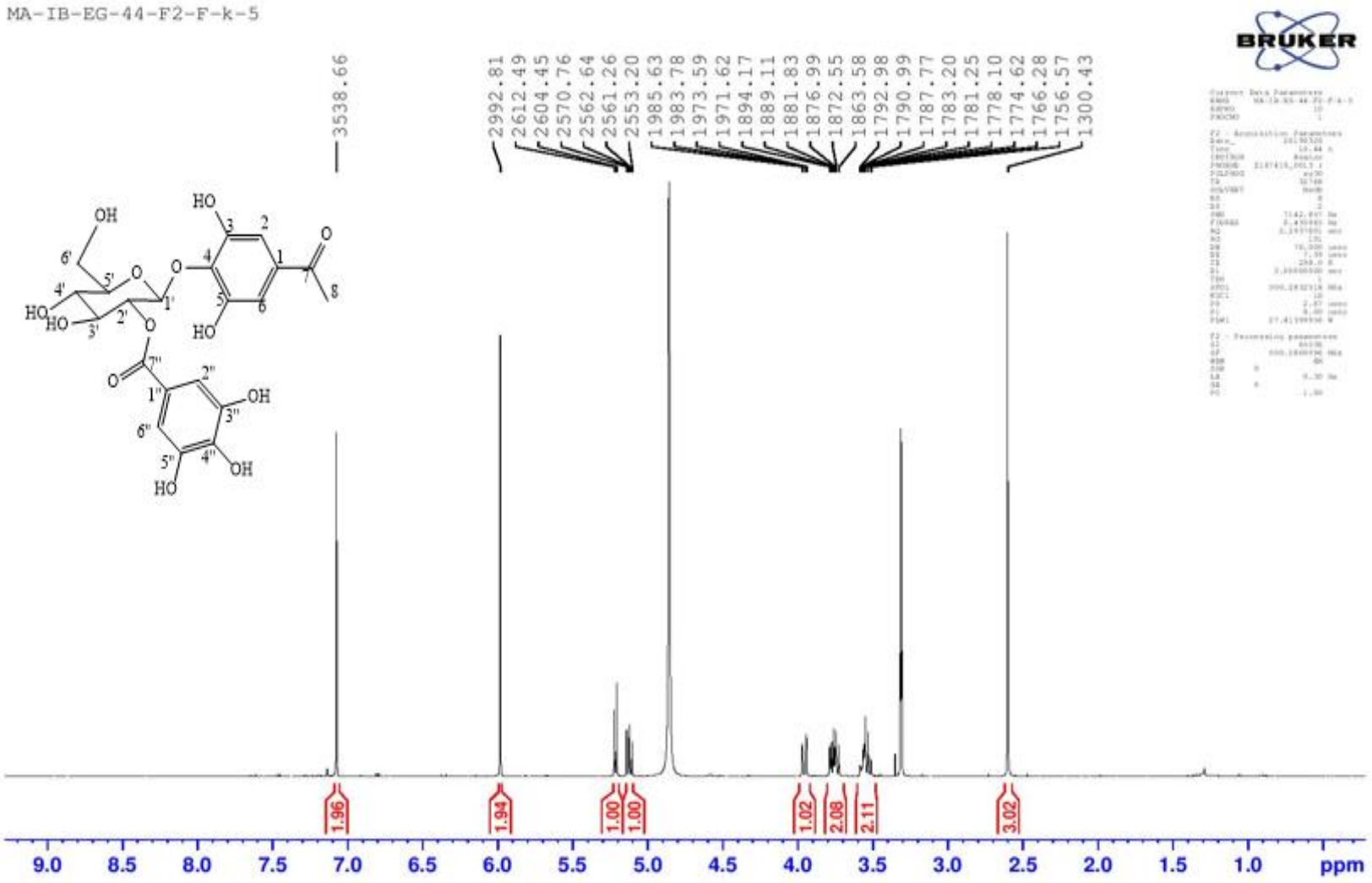


**Fig. S2:** $^{1}$H NMR spectrum of compound **1** (MeOH-$d_4$, 500 MHz)

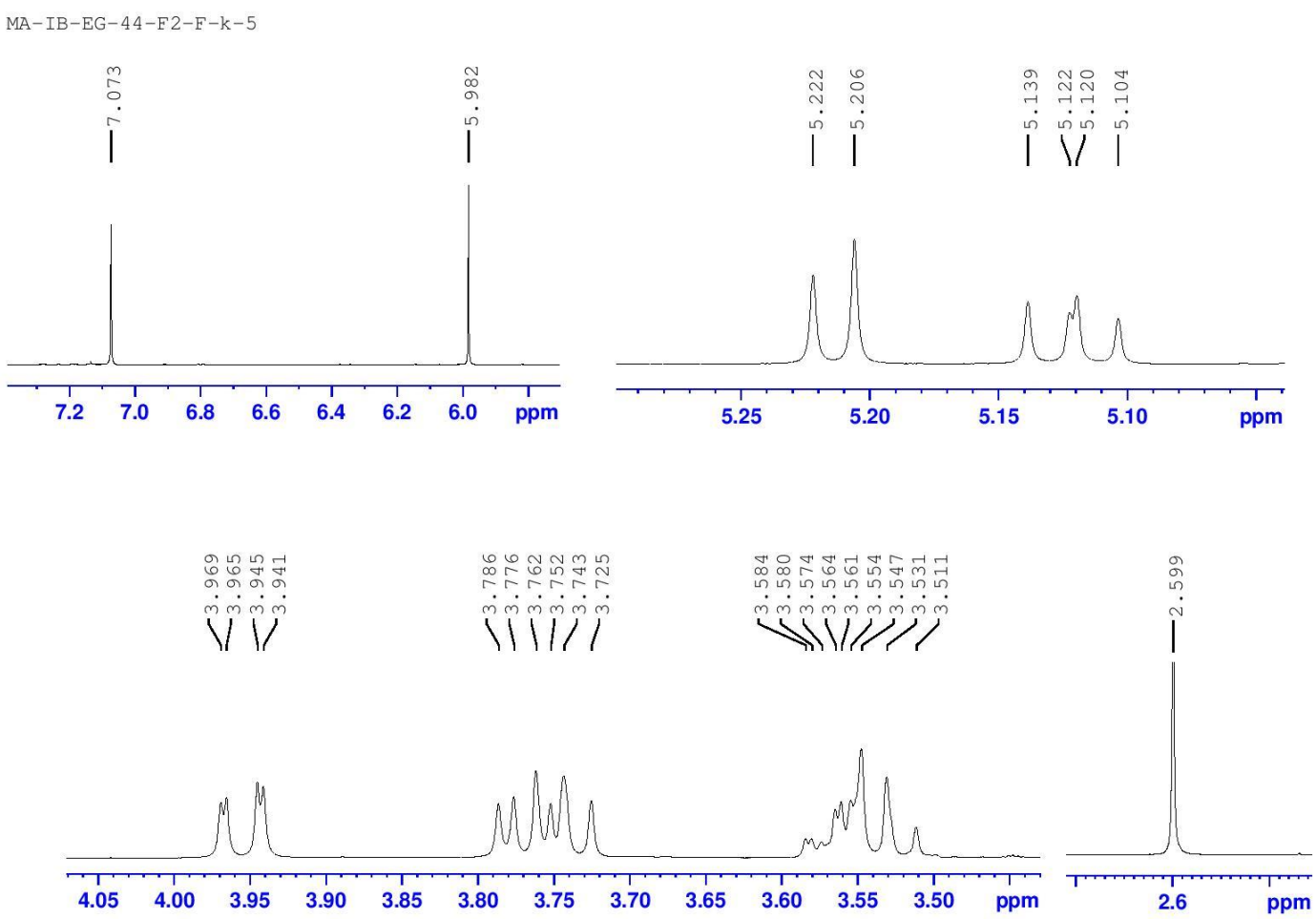


**Fig. S3:** $^{1}$H NMR staggering spectrum of compound **1** (MeOH-$d_4$, 500 MHz)

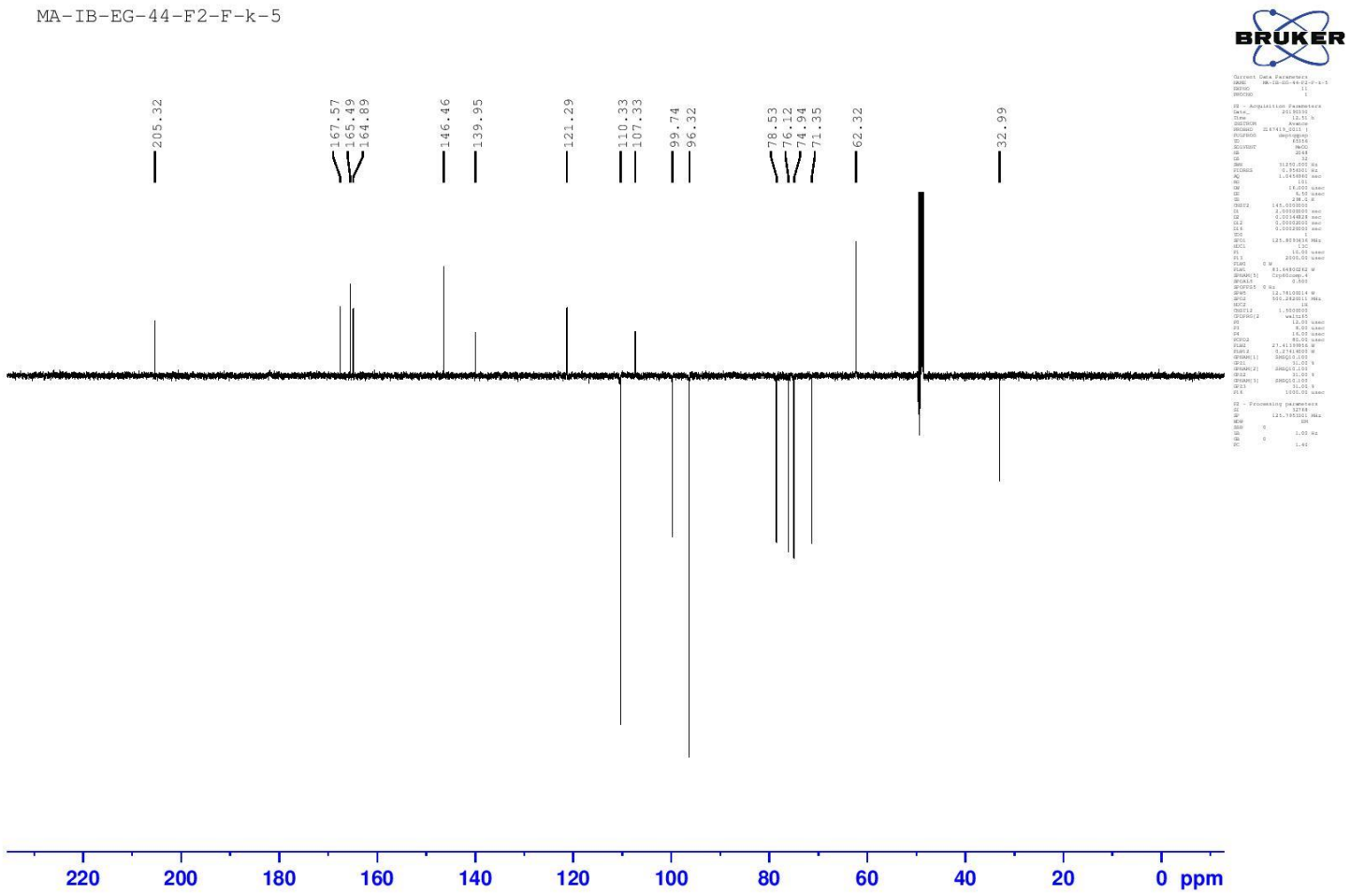


**Fig. S4:** $^{13}$C NMR spectrum of compound **1** (MeOH-$d_4$, 500 MHz)

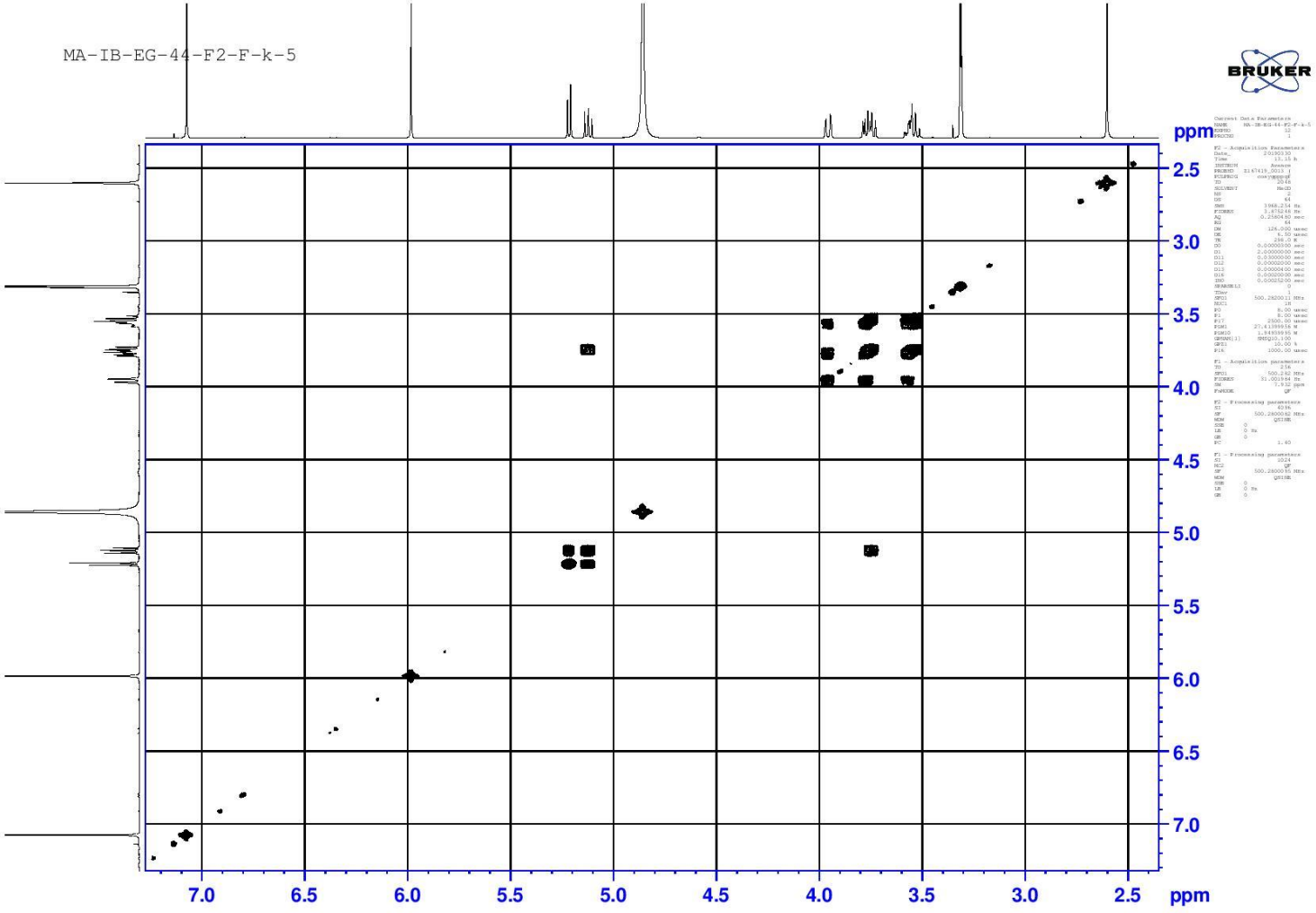


**Fig. S5:** $^{1}$H-$^{1}$H COSY spectrum of compound **1** (MeOH-$d_4$, 500 MHz)

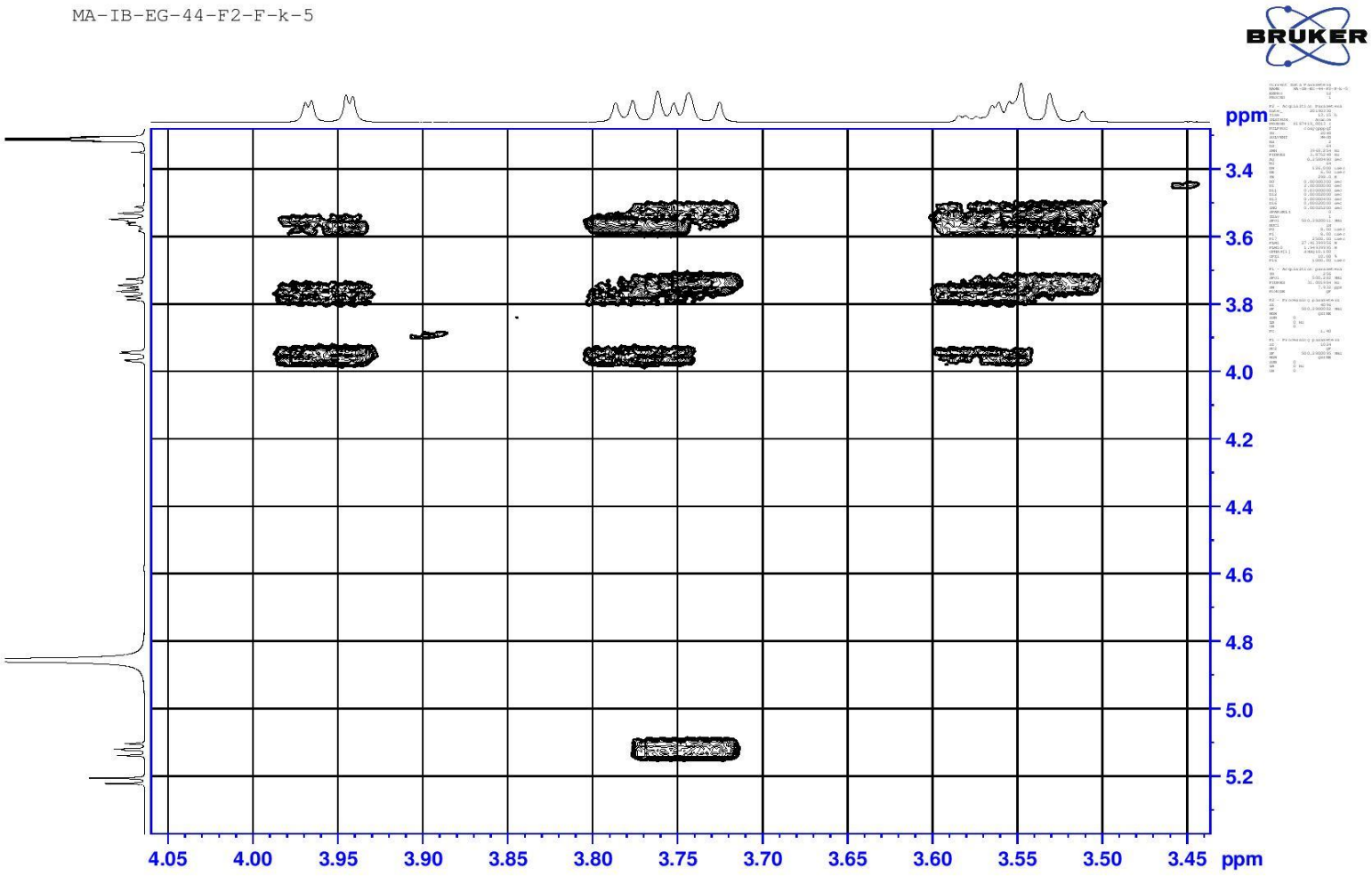


**Fig. S6:** $^{1}$H-$^{1}$H COSY staggering spectrum of compound **1** (MeOH-$d_4$, 500 MHz)

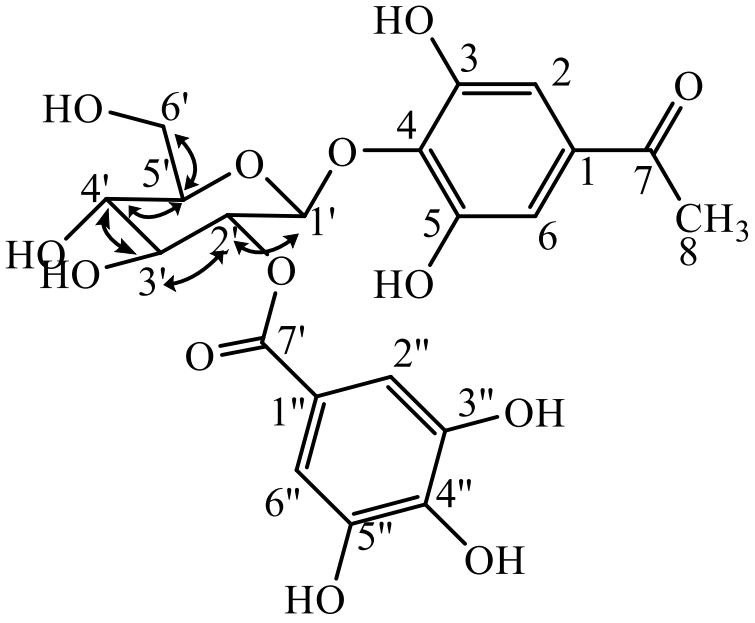


**Fig. S7:** Selected $^{1}$H-$^{1}$H COSY correlations of compound **1**

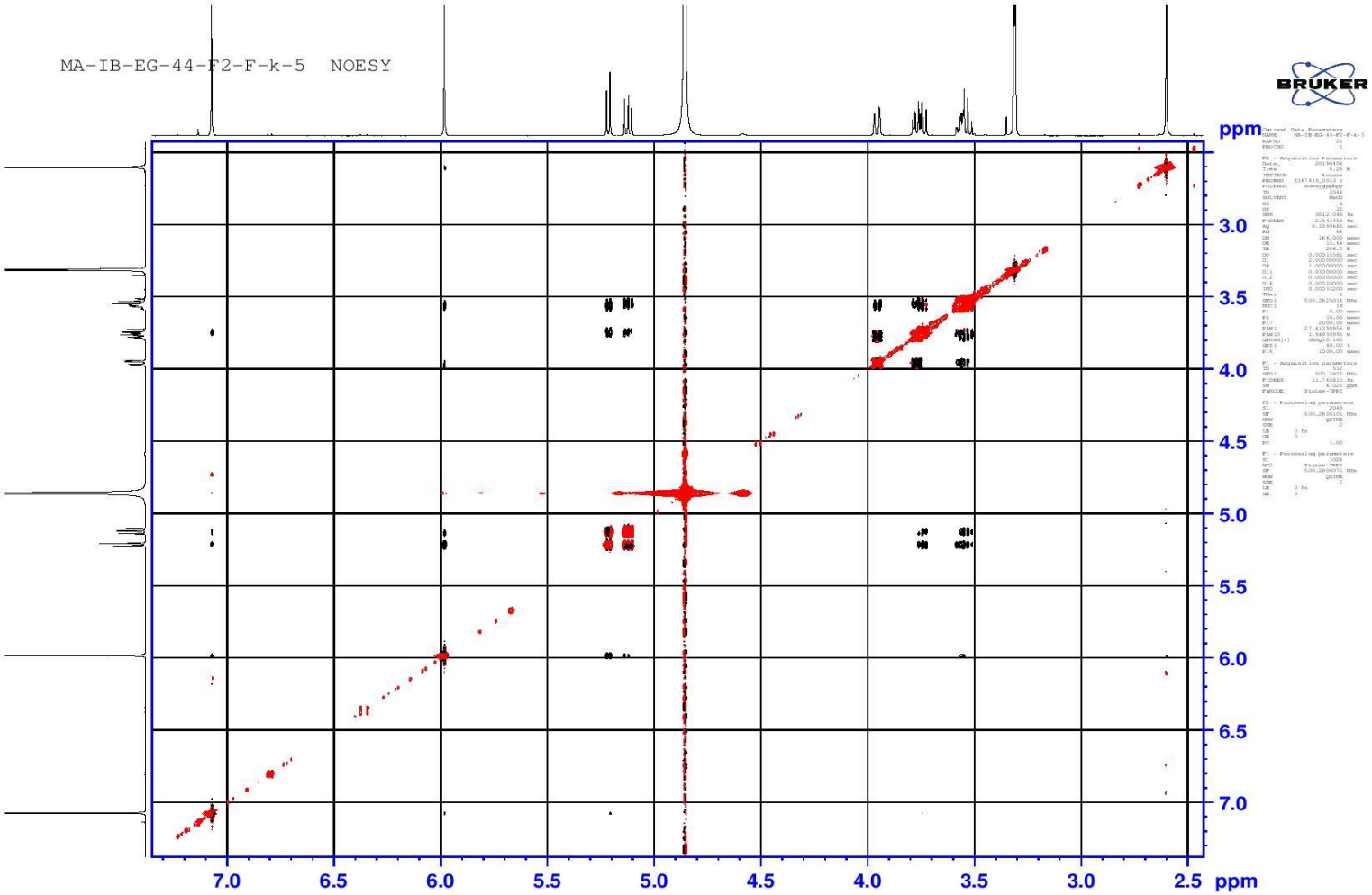


**Fig. S8:** $^{1}$H-$^{1}$H NOESY spectrum of compound **1** (MeOH-$d_4$, 500 MHz)

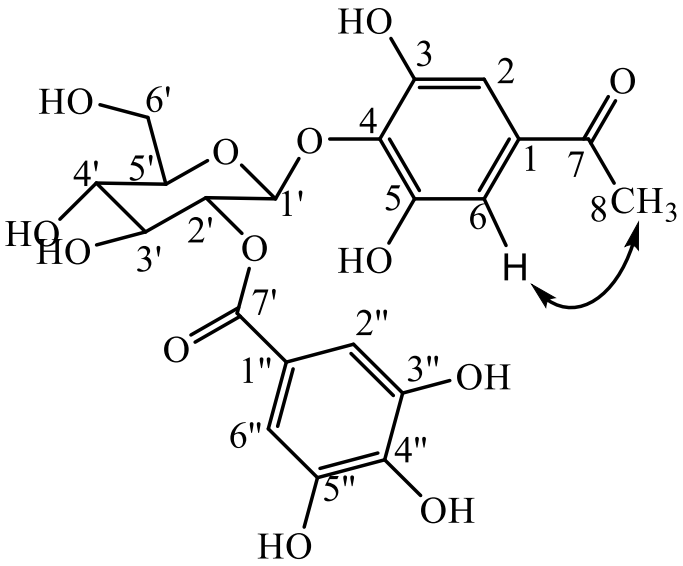


**Fig. S9:** Selected NOESY correlations of compound **1**

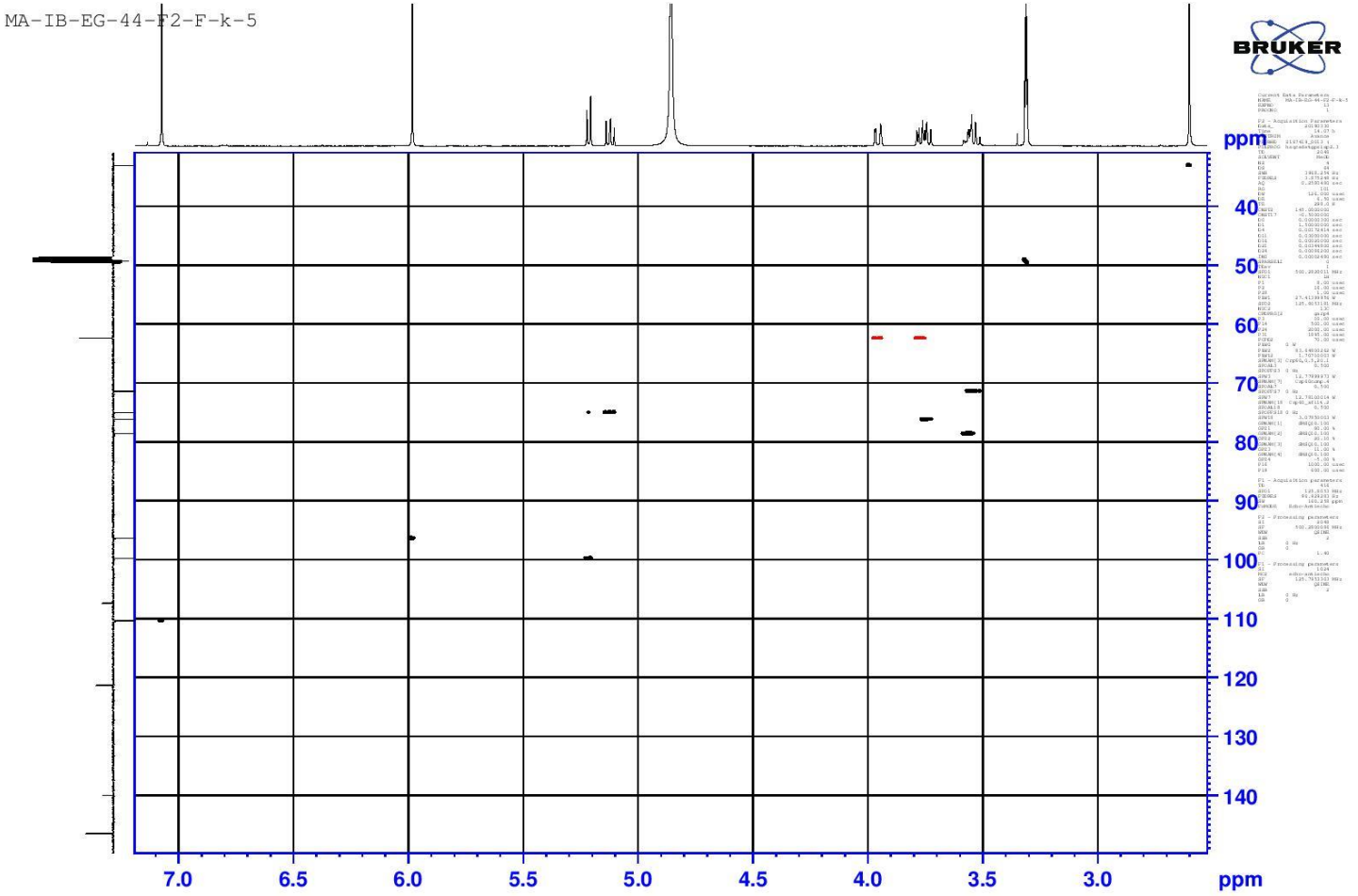


**Fig. S10:** HSQC spectrum of compound **1** (MeOH-$d_4$, 500 MHz)

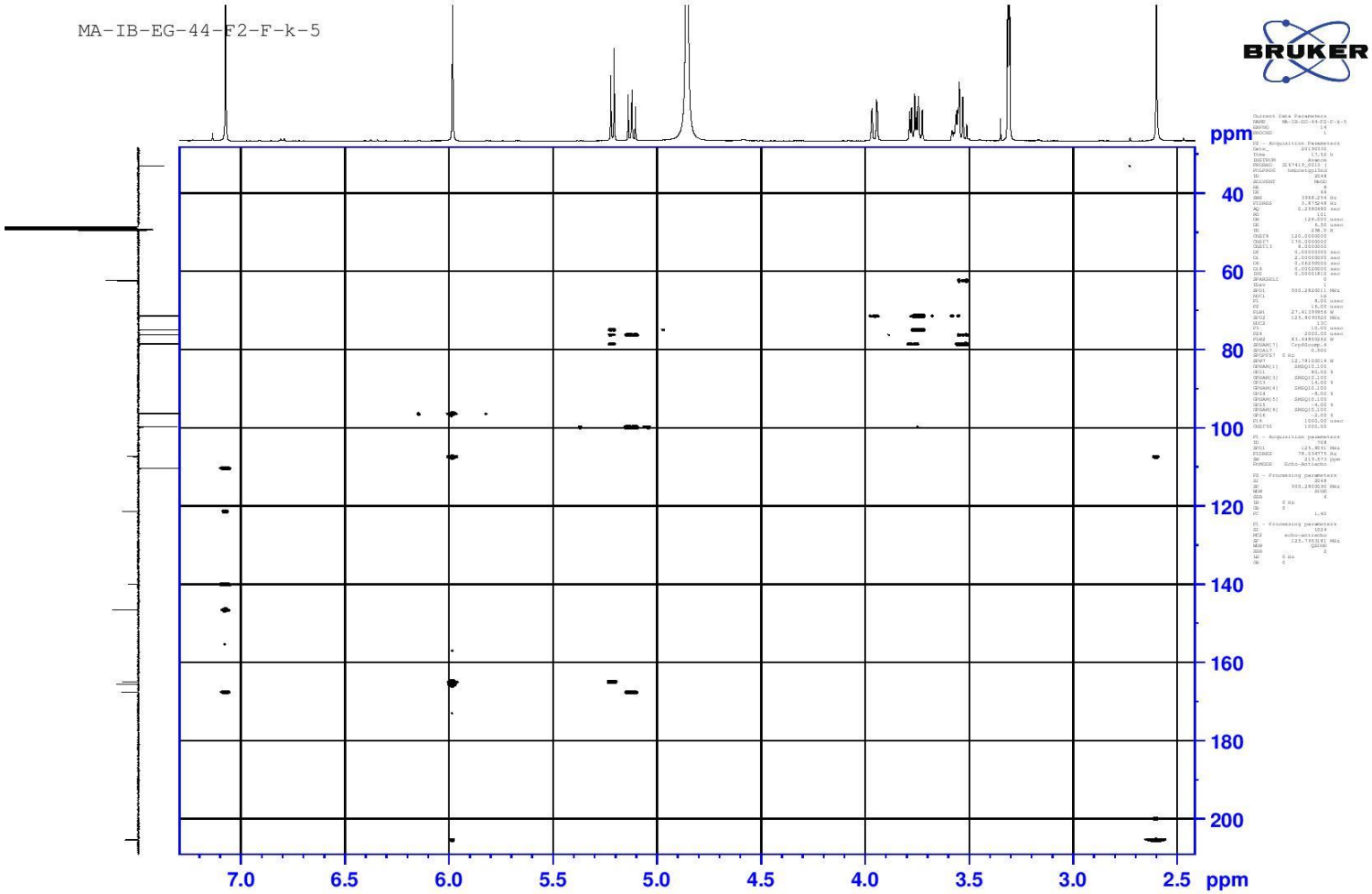


**Fig. S11:** HMBC spectrum of compound **1** (MeOH-$d_4$, 600 MHz)

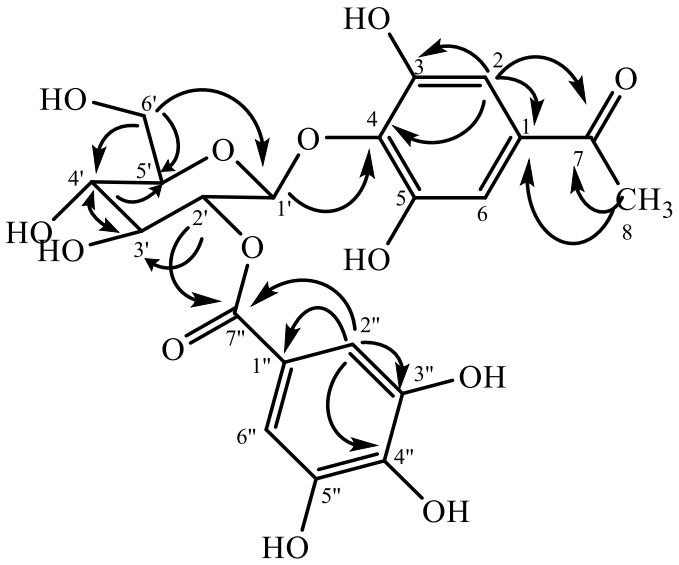


**Fig. S12:** Selected HMBC correlations of compound **1**

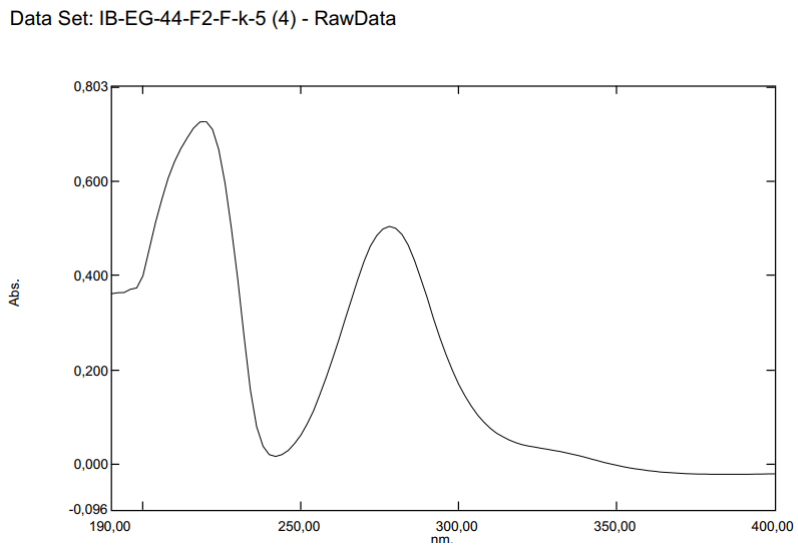


**Fig. S13:** UV spectrum of compound **1** (in MeOH)

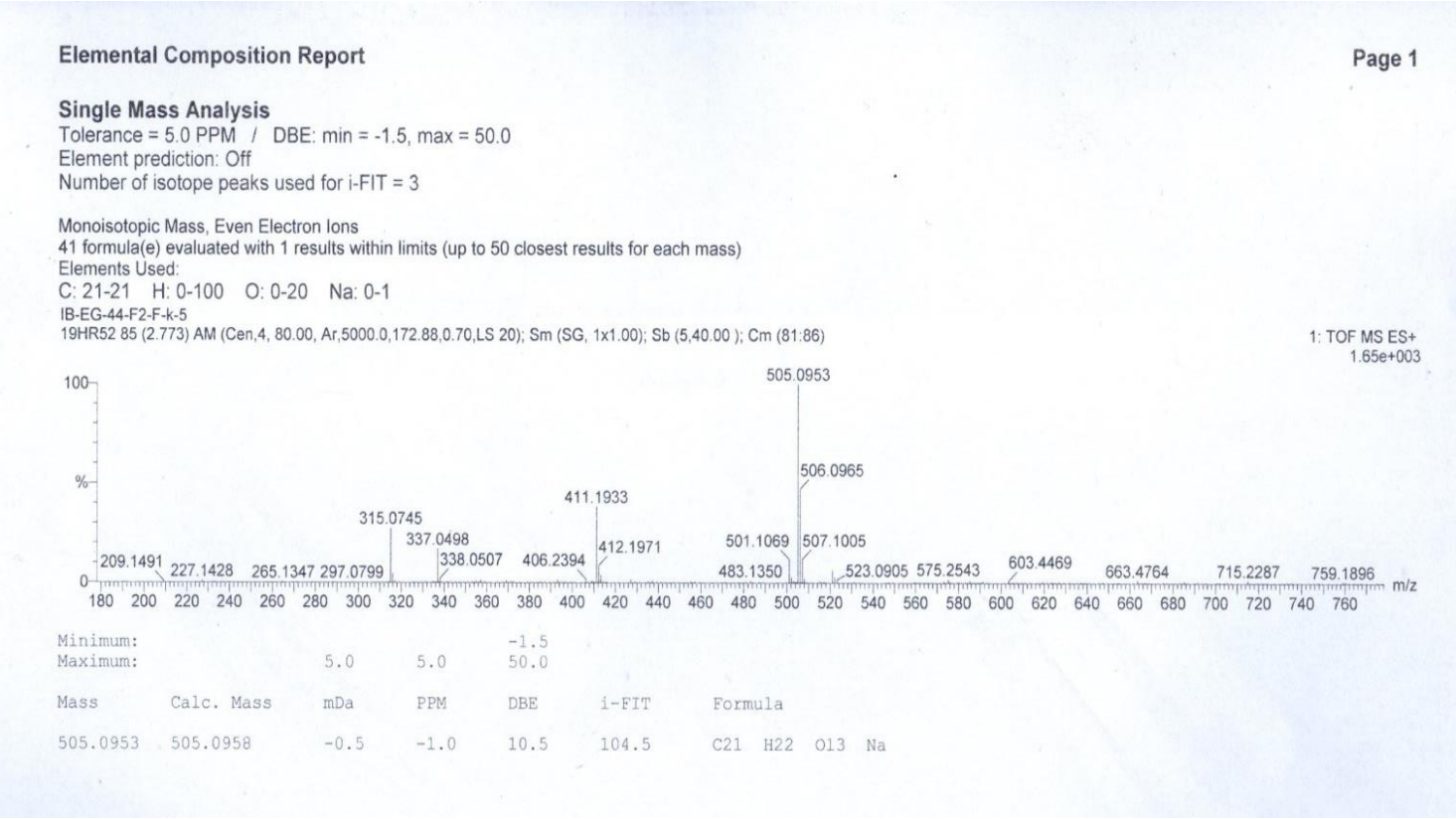


**Fig. S14:** HR-ESI-MS spectrum of compound **1**

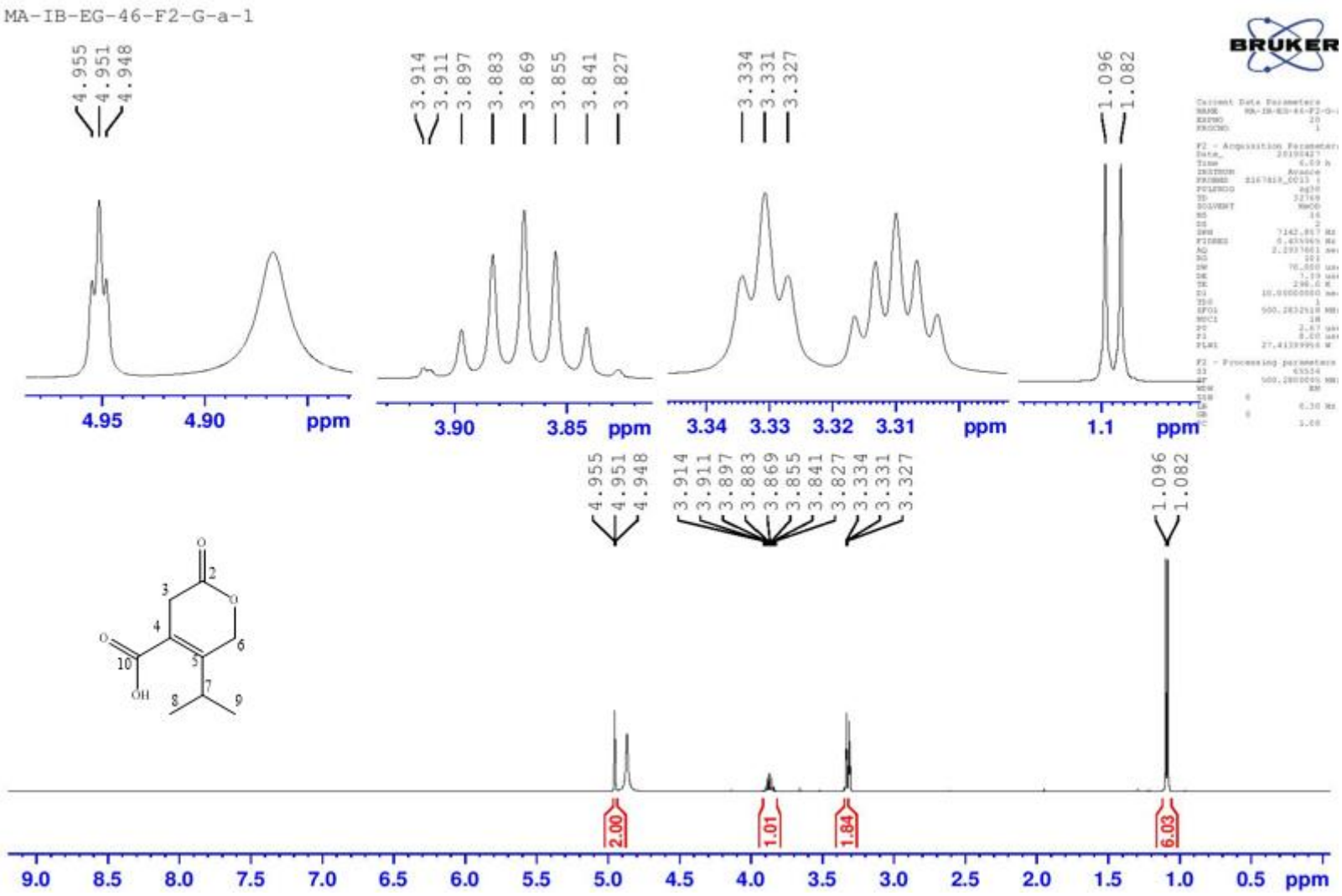


**Fig. S15:** $^{1}$H NMR spectrum of compound **2** (MeOH-$d_4$, 500 MHz)

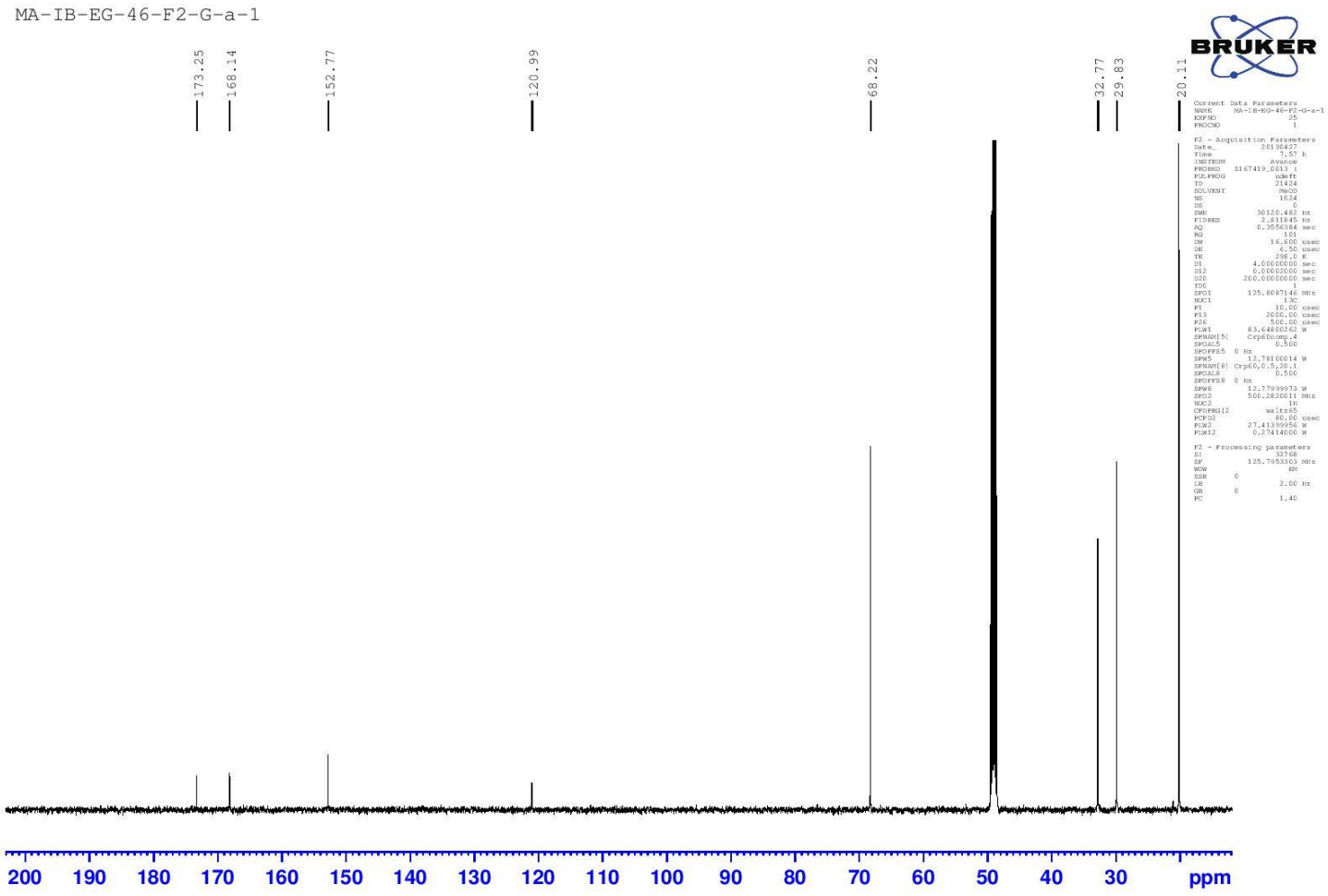


**Fig. S16:** $^{13}$C NMR spectrum of compound **2** (MeOH-$d_4$, 500 MHz)

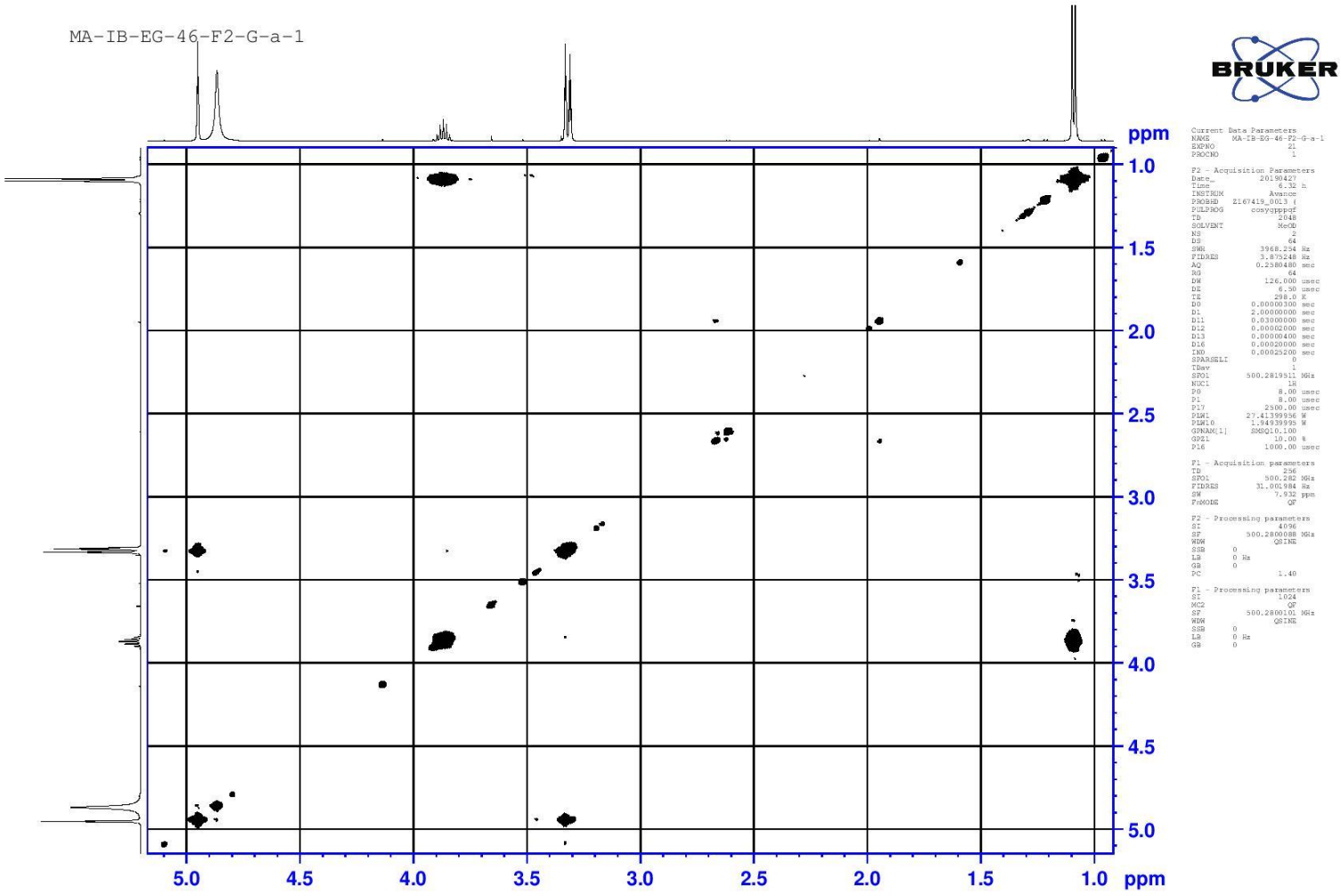


**Fig. S17:** $^{1}$H-$^{1}$H COSY spectrum of compound **2** (MeOH-$d_4$, 500 MHz)

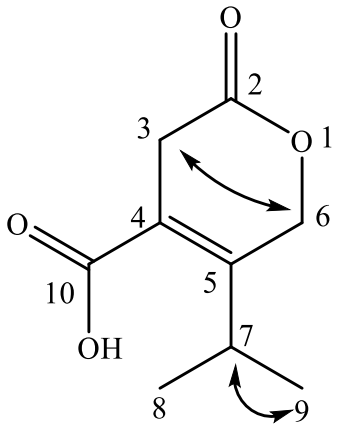


**Fig. S18:** Selected $^{1}$H-$^{1}$H COSY correlations of compound **2**

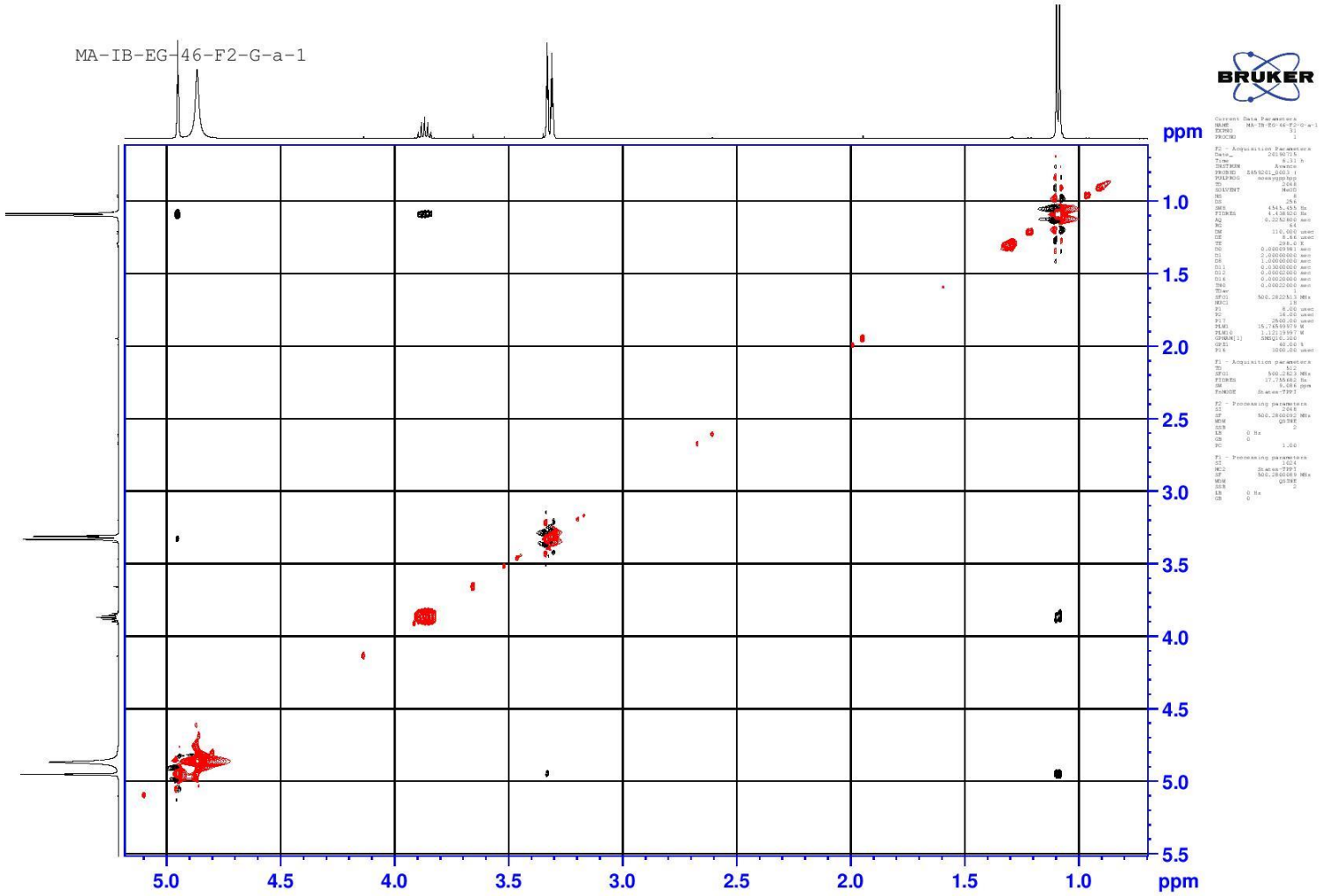


**Fig. S19:** $^{1}$H-$^{1}$H NOESY spectrum of compound **2** (MeOH-$d_4$, 500 MHz)

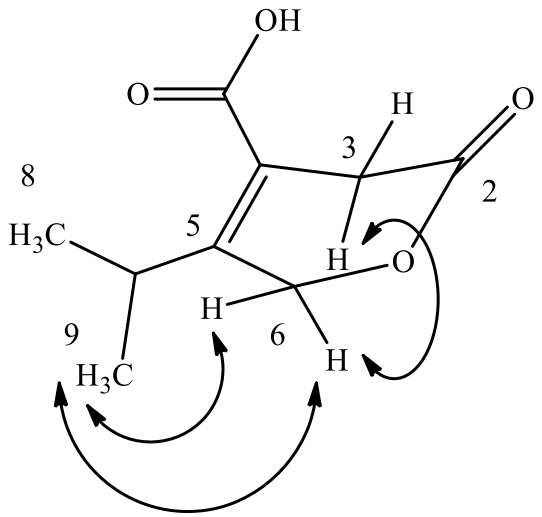


**Fig. S20:** Selected NOESY correlations of compound **2**

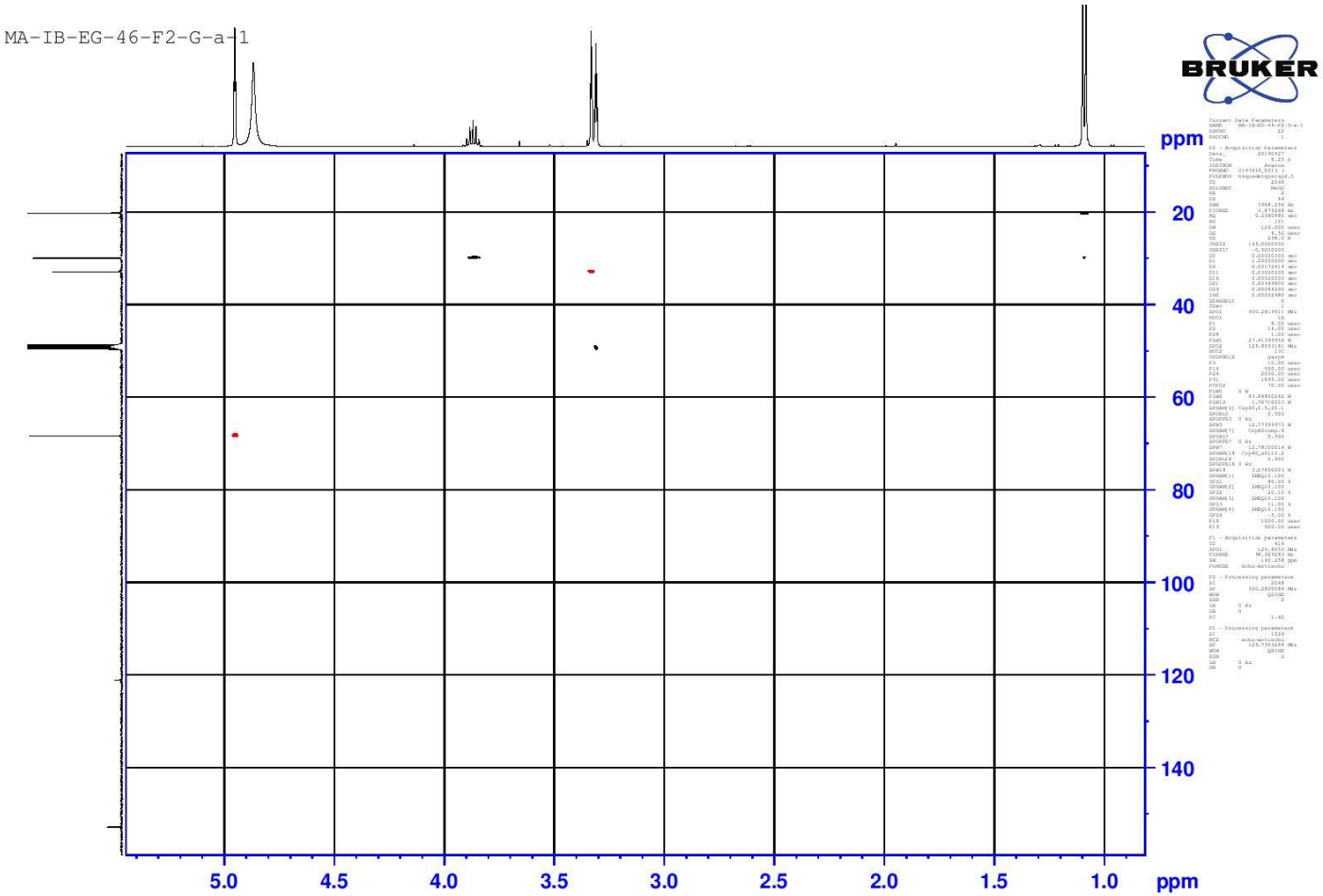


**Fig. S21:** HSQC spectrum of compound **2** (MeOH-$d_4$, 500 MHz)

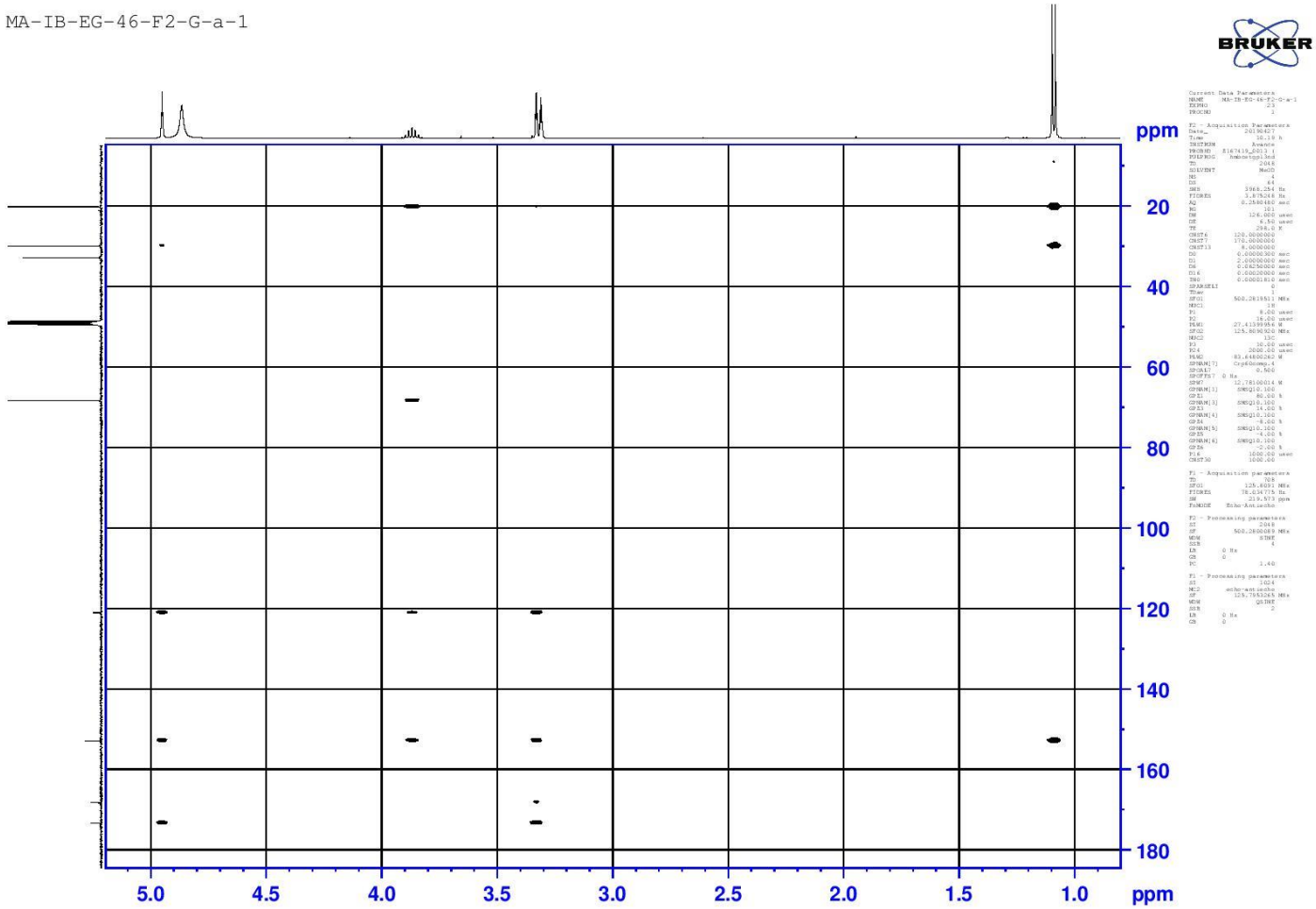


**Fig. S22:** HMBC spectrum of compound **2** (MeOH-$d_4$, 600 MHz)

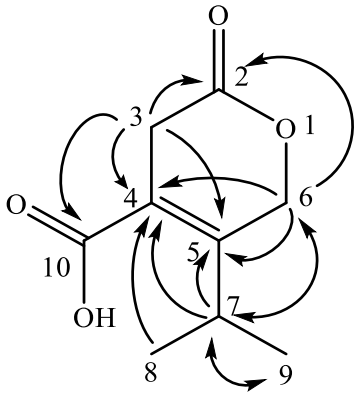


**Fig. S23:** Selected HMBC correlations of compound **2**

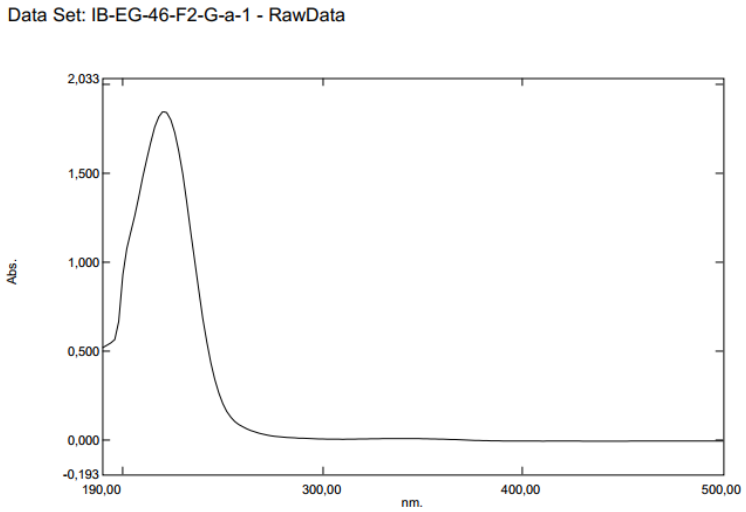


**Fig. S24:** UV spectrum of compound **2** (in MeOH)

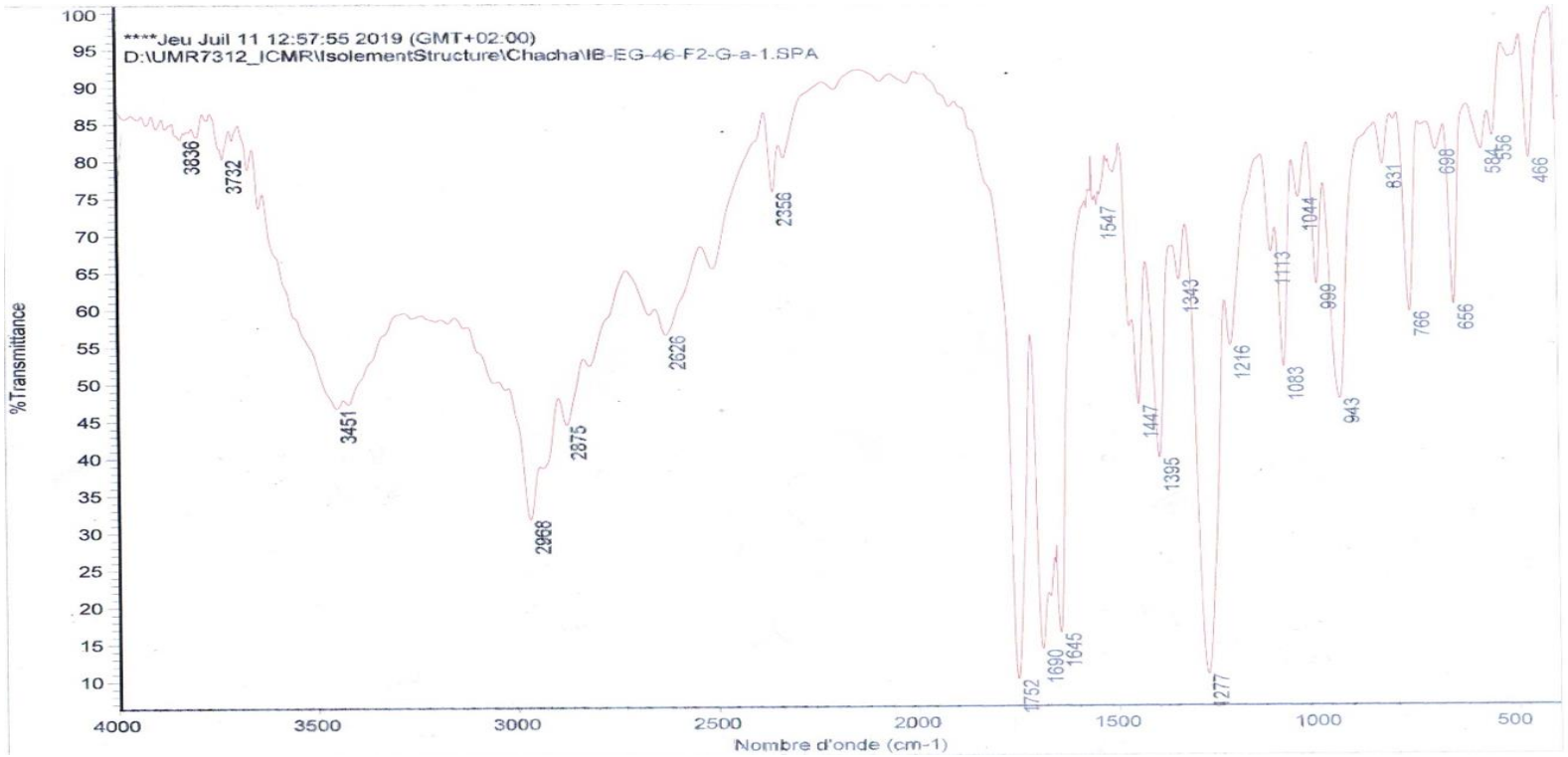


**Fig. S25:** IR spectrum of compound **2**

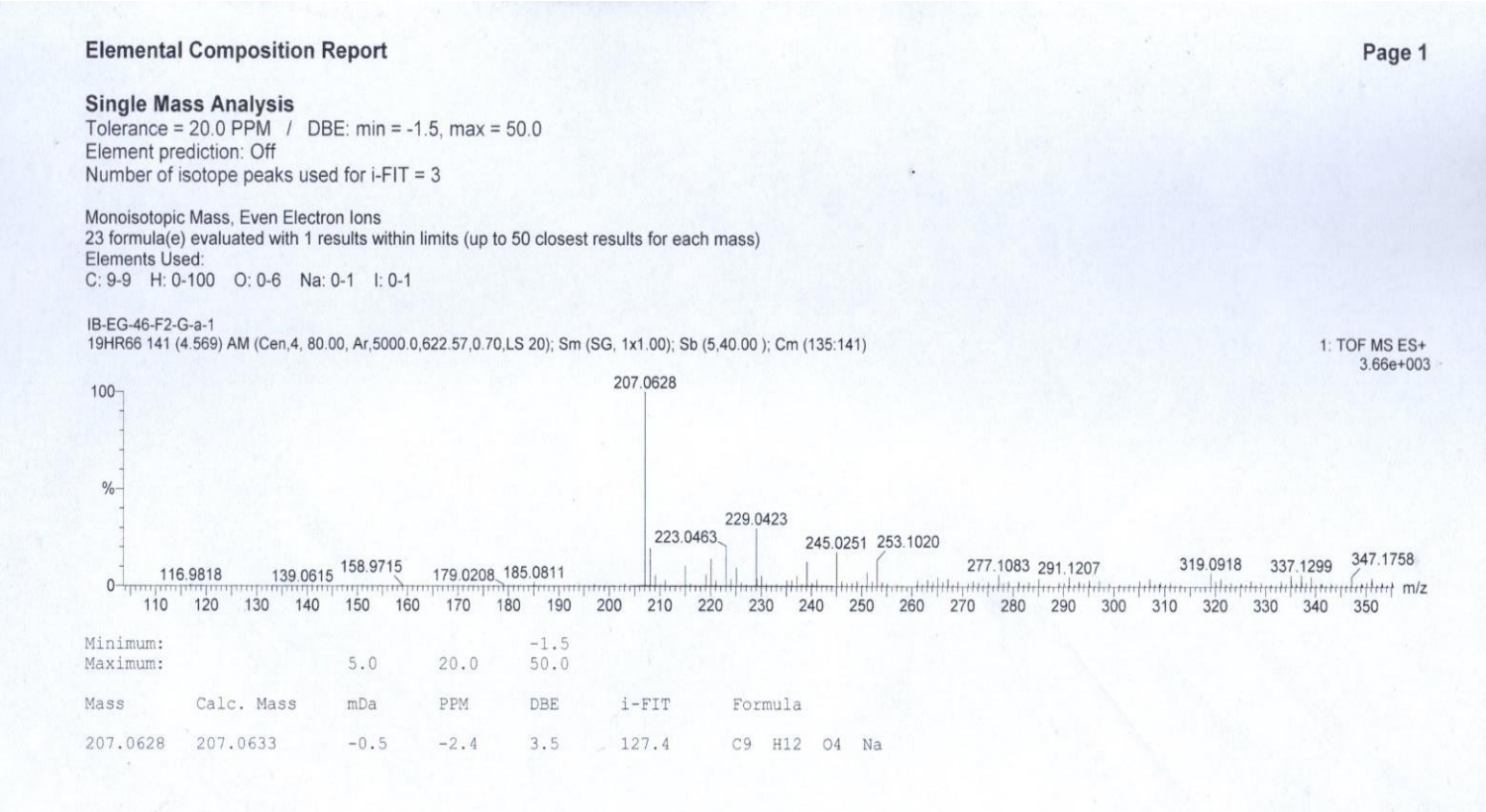


**Fig. S26:** HR-ESI-MS spectrum of compound **2**