Supplementary Materials

Constituents from the Fruiting Bodies of *Trametes Cubensis* and *Trametes Suaveolens* in Vietnam and Their Anti-inflammatory Bioactivity

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Contents

S1. Anti-inflammatory bioactivity examination.

1. Human neutrophil preparation

Neutrophils were isolated using a standard dextran sedimentation method prior to centrifugation on a Ficoll Hypaque gradient and hypotonic lysis of erythrocytes. Blood was drawn from healthy human donors (20 to 30 years old) by venipuncture into heparin-coated vacutainer tubes using a protocol approved by the institutional review board at Chang Gung Memorial Hospital. Blood samples were mixed gently with an equal volume of a 3 % dextran solution. Leukocyte-rich plasma was collected after sedimentation of the red cells for 30 min at room temperature. The leukocyte-rich plasma was then transferred onto 20 mL of Ficoll solution (1.077 g/mL) and centrifuged at 400 g for 40 min at 20 °C. The granulocyte/erythrocyte pellets were resuspended in ice-cold 0.2 % NaCl and lysed. After 30 sec, the same 1.6 % NaCl solution volume was added to reconstitute the isotonic condition. Purified neutrophils were pelleted and then resuspended in calcium (Ca^{2+})-free Hank's balanced salt solution (HBSS) buffer at pH 7.4 and were maintained at 4 °C before use.

2. Superoxide anion generation measurement

The assay for measuring superoxide anion generation was based on the SOD-inhibitable reduction of ferricytochrome *c*. Briefly, after supplementation with 0.5 mg/mL ferricytochrome *c* and 1 mM Ca^{2+}, neutrophils (6 x 10^5 cells/mL) were equilibrated at 37 °C for 2 min and incubated with 100 nM fMLF during preincubation with 1 μg/mL cytochalasin B (fMLF/CB) for 3 min. Changes in the 550 nm absorbance reflecting a reduction in ferricytochrome *c* were continuously monitored using a double-beam, six-cell positioner spectrophotometer with constant stirring (Hitachi U03010, Tokyo, Japan). Calculations were based on differences in the reactions with and without SOD (100 U/mL) divided by the extinction coefficient for the reduction of ferricytochrome c (ε = 21.1/mM/10 mm).

3. Elastase release assay

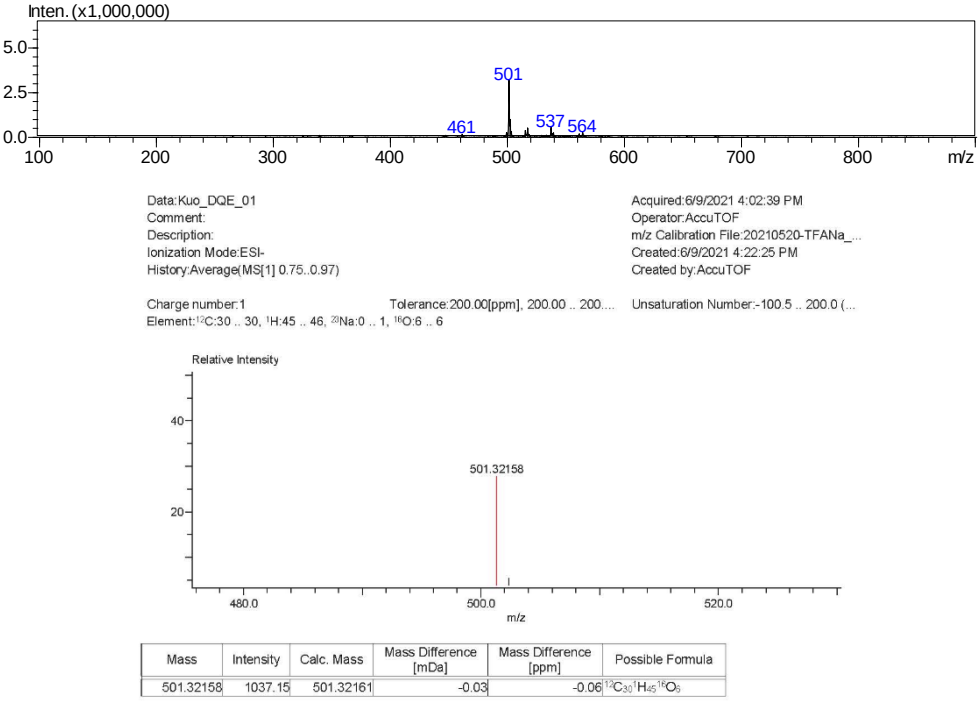
Degranulation of azurophilic granules was determined by measuring the release of elastase as previously described. Experiments were performed using MeO-Suc-Ala-Ala-Pro-Val-p-nitroanilide as the elastase substrate. Briefly, after supplementation with MeO-Suc-Ala-Ala-Pro-Val-p-nitroanilide (100 μM), neutrophils (6 x 10^5/mL) were equilibrated at 37 °C for 2 min and incubated with drugs or an equal volume of vehicle (0.1 % DMSO, negative control) for 5

min. Cells were activated using 100 nM fMLF and 0.5 μg/mL cytochalasin B, and changes in the 405 nm absorbance were continuously monitored to assay elastase release. The results are expressed as the percent of elastase release in the fMLF/CB-activated, drug-free control system.

4. Statistical analysis

The results are expressed as mean ± SEM. Computation of 50 % inhibitory concentrations (IC_{50}) were performed using PHARM/PCS v.4.2 software. Statistical comparisons were made between groups using Student's *t*-test. Values of $p < 0.05$ were considered to be statistically significant.

Figure S1. HRMS & MS spectra of **1**.



Mass	Intensity	Calc. Mass	Mass Difference [mDa]	Mass Difference [ppm]	Possible Formula
501.32158	1037.15	501.32161	-0.03	-0.06	$^{12}C_{30}{}^{1}H_{45}{}^{16}O_{6}$

Figure S2. ^{1}H NMR spectrum of **1** (CD_3OD, 400 MHz).

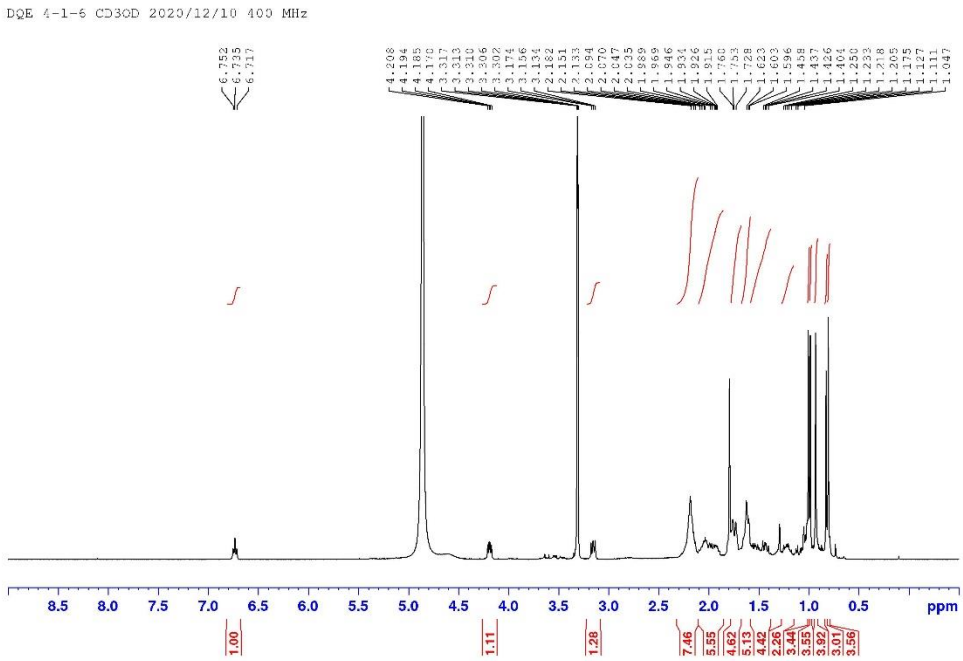


Figure S3. ^{13}C and DEPT NMR spectrum of **1** (CD_3OD, 100 MHz).

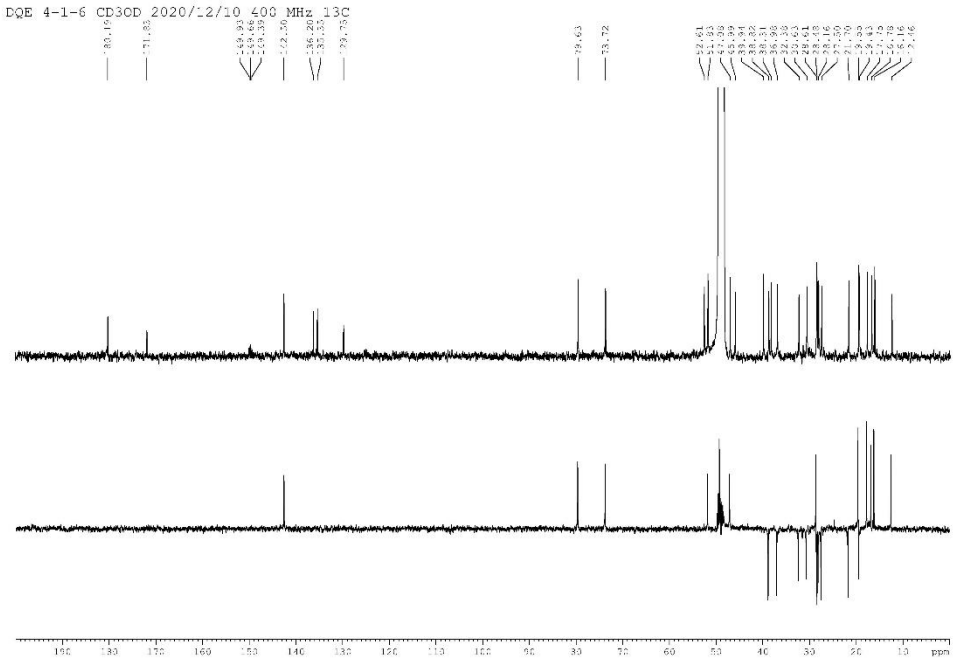


Figure S4. HSQC spectrum of **1** (CD_3OD, 400 MHz).

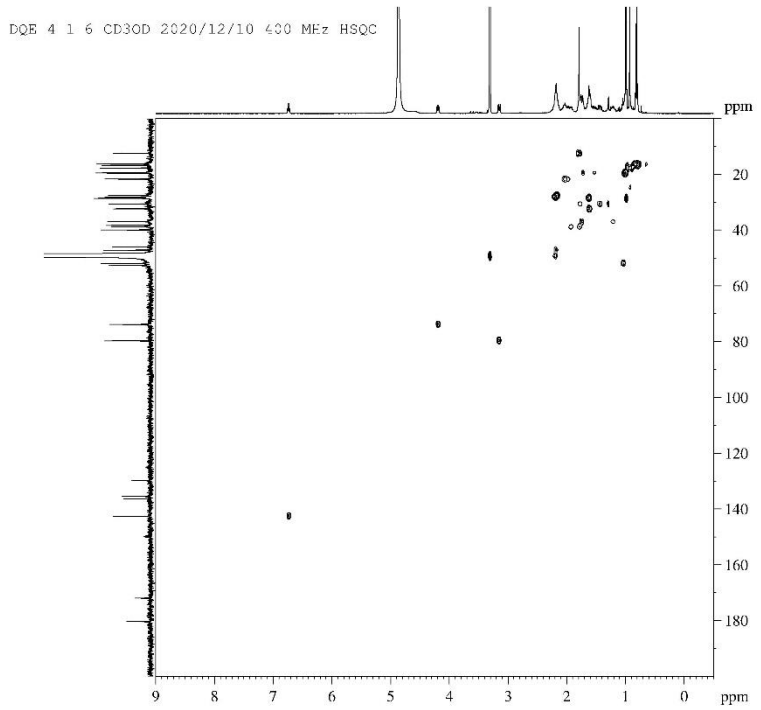


Figure S5. HMBC spectrum of **1** (CD_3OD, 400 MHz).

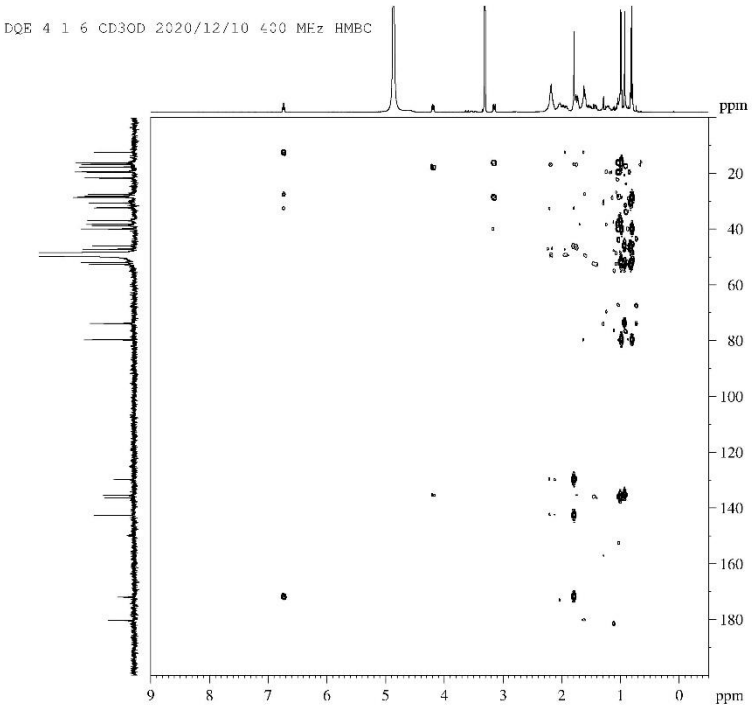


Figure S6. NOESY spectrum of **1** (CD_3OD, 400 MHz).

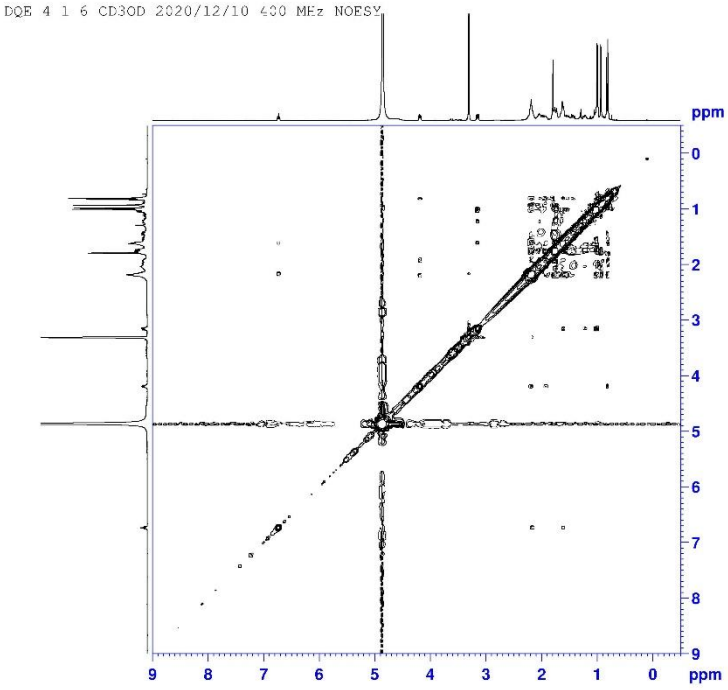


Figure S7. MS spectrum of **2**.

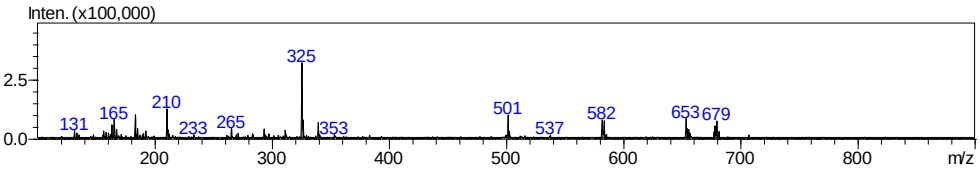


Figure S8. ^{1}H NMR spectrum of **2** (CD_3OD, 400 MHz).

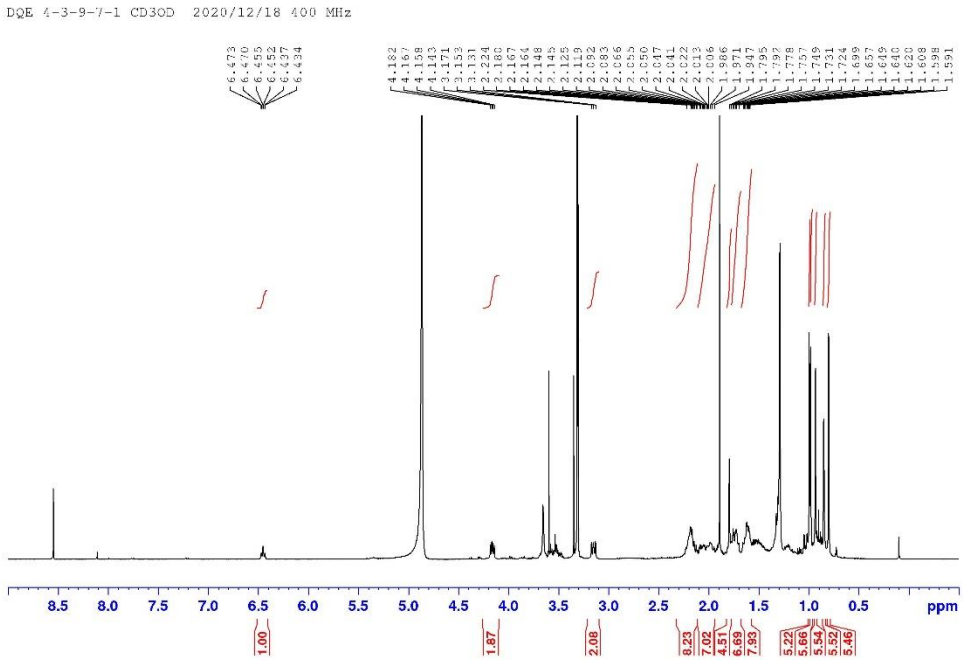


Figure S9. ^{13}C and DEPT NMR spectrum of **2** (CD_3OD, 100 MHz).

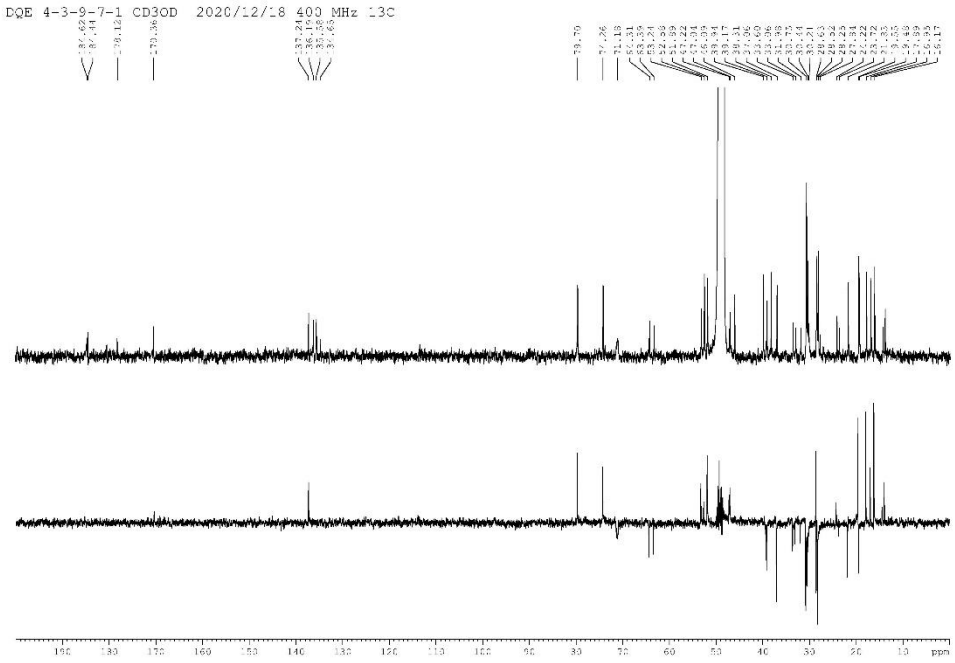


Figure S10. HSQC spectrum of **2** (CD_3OD, 400 MHz).

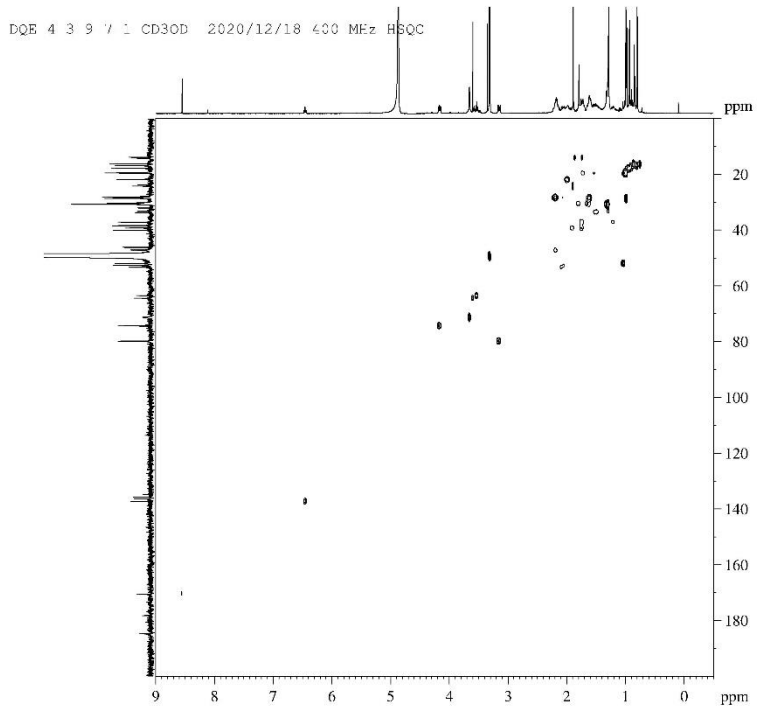


Figure S11. HMBC spectrum of **2** (CD_3OD, 400 MHz).

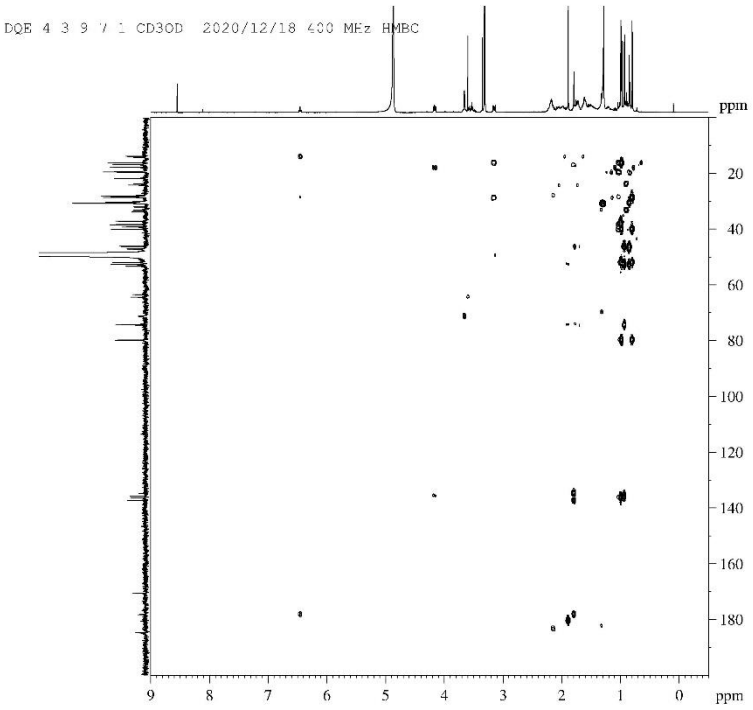


Figure S12. NOESY spectrum of **2** (CD_3OD, 400 MHz).

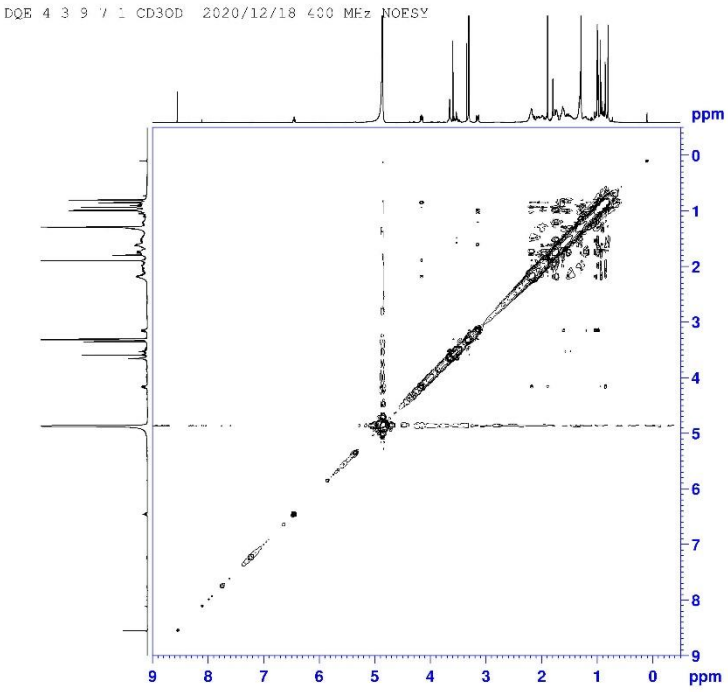
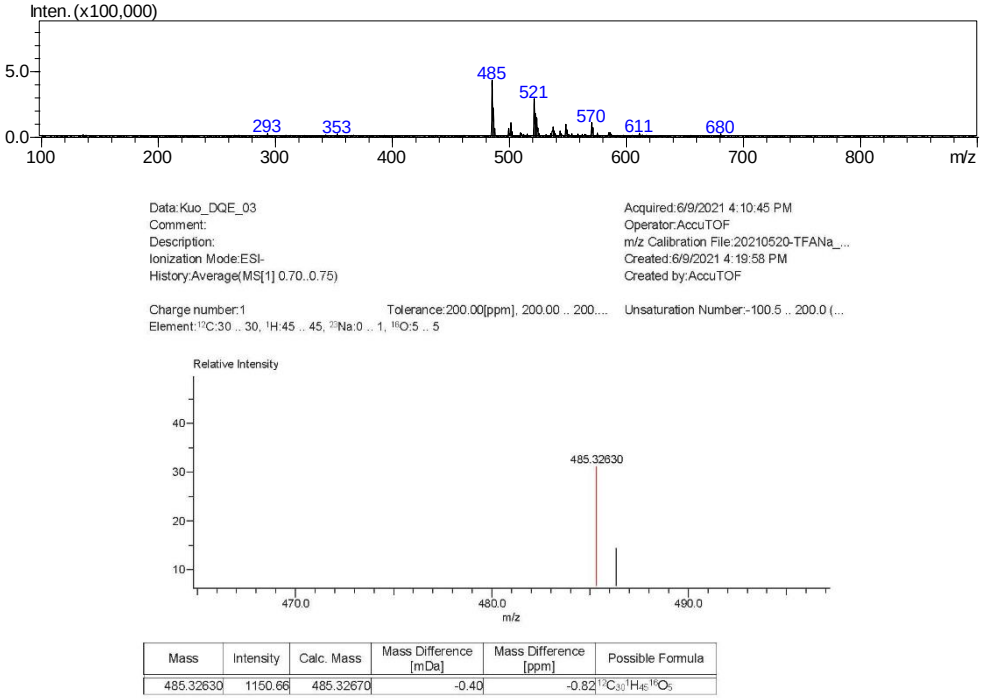


Figure S13. HRMS & MS spectra of **3**.



Mass	Intensity	Calc. Mass	Mass Difference [mDa]	Mass Difference [ppm]	Possible Formula
485.32630	1150.66	485.32670	-0.40	-0.82	$^{12}C_{30}{}^{1}H_{45}{}^{16}O_5$

Figure S14. ^{1}H NMR spectrum of **3** (CD_3OD, 400 MHz).

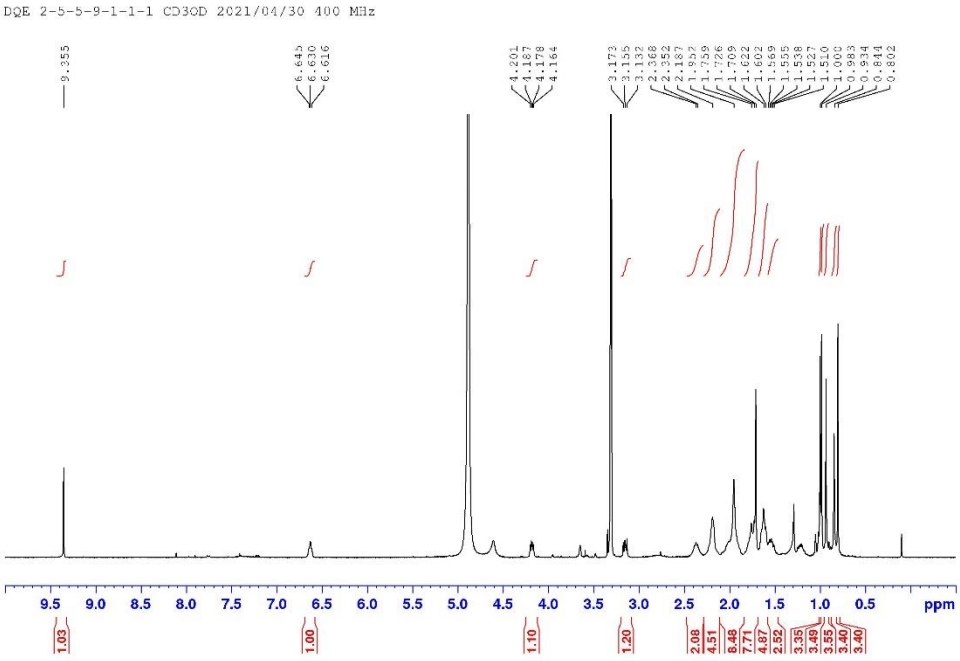


Figure S15. ^{13}C and DEPT NMR spectrum of **3** (CD_3OD, 100 MHz).

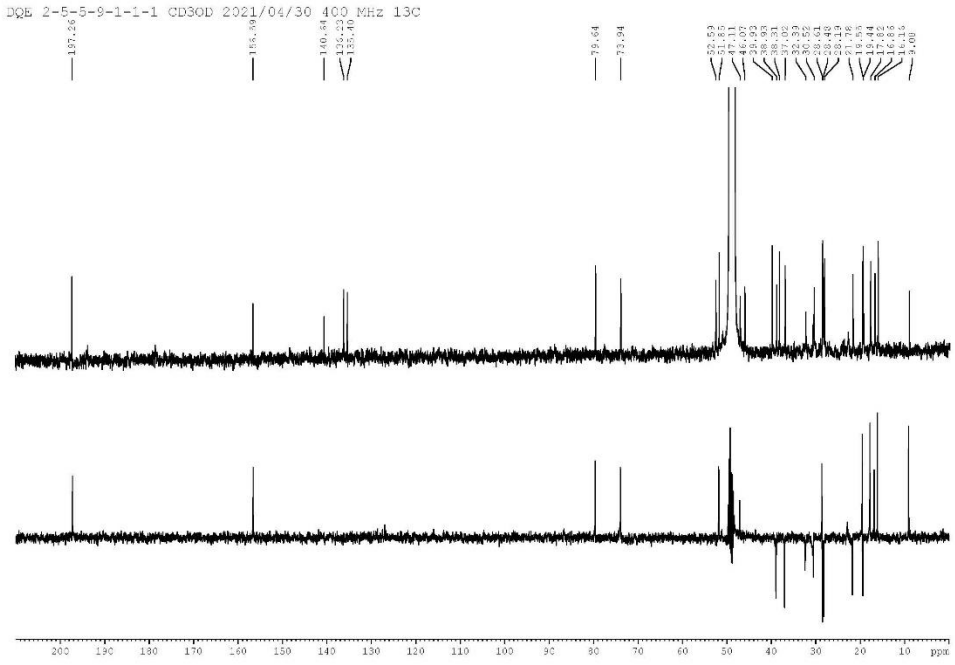


Figure S16. HSQC spectrum of **3** (CD_3OD, 400 MHz).

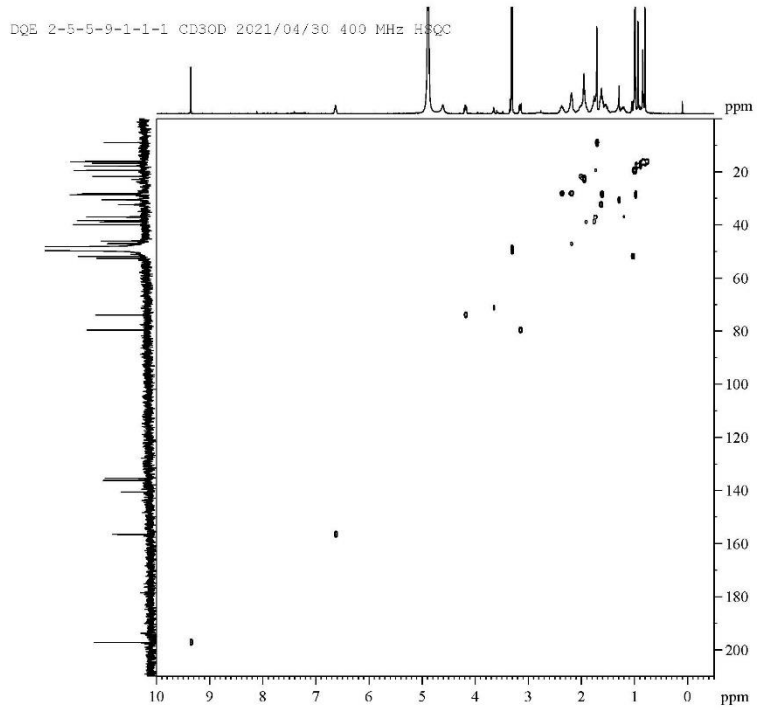


Figure S17. HMBC spectrum of **3** (CD_3OD, 400 MHz).

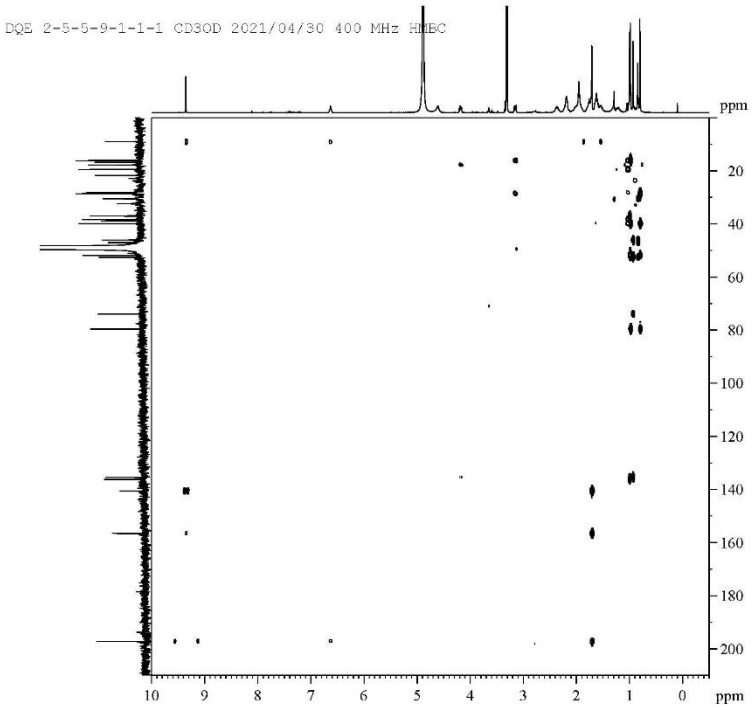


Figure S18. NOESY spectrum of **3** (CD_3OD, 400 MHz).