Supporting Information

***Rec. Nat. Prod*. 17:6 (2023) 1024-1030**

A New Alkaloid from Marine-Derived Actinomycete *Actinoalloteichus cyanogriseus* G631

Do Thi Quynh[1,2], Trinh Thi Thanh Van[1], Nguyen Thuy Linh[1], Le Thi Hong Minh[1], Vu Thi Quyen[1], Brian T. Murphy[3], Doan Thi Mai Huong[1,2,*] and Pham Van Cuong[1,2*]

[1]*Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*

[2]*Graduate University of Science and Technology, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*

[3]*Department of Pharmaceutical Sciences, Center for Biomolecular Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, United States*

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S.1. General Experimental Procedures

Optical rotations were recorded on a Polax-2Lpolarimeter in $CHCl_3$. High resolution mass data were obtained from Korea Basic Science Institute (KBSI, Chuncheon Center). NMR spectra were performed on Bruker 600 MHz and 500 MHz spectrometers. TLC silica gel Merck 60 F254 was used as thin layer chromatography. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70 230 mesh and 230–400 mesh, Merck, Darmstadt, Germany), RP-18 resins (30–50 μm, Fuji Silysia Chemical Ltd., Japan). For thin-layer chromatography (TLC), pre-coated silica-gel 60 F254 (0.25 mm, Merck, Darmstadt, Germany) and RP-18 F254S (0.25 mm, Darmstadt, Merck, Germany) plates were used.

S.1.1. Fermentation

Actinoalloteichus cyanogriseus G631 was activated and inoculated into 1 L of A1 broth medium. After 7 days of incubation at 30 ºC while shaking at 150 rpm, the culture broth was used to spread on the agar surface of 50 flasks containing 1 L of rich-nutrient solid medium A1+ (g/L) [soluble starch (10.0 g), yeast extract (4.0 g), peptone (2.0 g), instant ocean salt (30.0 g)], KBr (5mL, 20 mg/mL), $FeSO_4$ (5 mL, 8 mg/mL), $CaCO_3$ (1.0 g), agar (15.0 g). The fermentation was incubated in an incubator at 30 ºC and harvested on the 20th day.

S.1.2. Actinobacteria Isolation

The sponge sample (0.5 g) was crushed by glass chopsticks in a falcon tube, 4.5 mL of sterile sea water was added, mixture was homogenized by vortexing for 1 minute, and the suspension was treated using a wet-heat technique (60 ºC for 6 minutes). Next, 0.5 ml of this suspension was transferred to another 4.5 ml sterile distilled water and this step was repeated to set up a ten-fold dilution series to 10^{-3}. At the final dilution step, aliquots of 50 µl of sample solution was spread on ISP1 medium pH 7.0 (g/l): (2.0 g yeast extract, 5.0 g casitone, 30.0 g instant ocean, 15.0 g agar) supplemented with 50 µg/mL polymycin B and cycloheximide to inhibit Gram - negative bacterial and fungal contaminations. After 10 days of aerobic incubation at 30 ºC, the colony of the G631 actinomycete strain was transferred onto a petri dish of A1 medium (g/l) (5.0 g soluble starch, 2.0 g yeast extract, 1.0 g peptone, 30.0 g instant ocean, 15.0 g agar) for purification (Figure S1). The crude extract of G631 fermentation exhibited antimicrobial activity against three Gram positive bacteria (*Enterococcus faecalis* ATCC 29212, *Stapphylococus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579), one Gram negative bacteria *Salmonella enterica* ATCC 13076 and one yeast strain *Candida albicans* ATCC 10231 with MIC values of 64, 2, 16, 256, 2 µg/ml, respectively.

S.1.3. Actinobacteria Identification

Genomic DNA of strain G631 was extracted by Gene JET Genomic DNA Purification Kit purchased from Thermo fisher (USA). The sequence of 16S rRNA was used for taxonomical identification of the actinomycete strain. Gene 16S rRNA amplification was performed in a 25.0 µl mixture containing: 10 µl of H_2O, 12.5 µl of PCR Master mix, 1.0 µl of 0.05 mM for both primers 9 F (5'-GAGTTTGATCCTGGCTCAG3') and 1541R (5'-AAGGAGGTGATCCAACC3') and 0.5 µl of genomic DNA. The reaction tube was then put into MJ Thermal cycler (Bio - Rad), with a pre-heating step at 94 ºC for 3 min, followed by 30 cycles of denaturation at 94 ºC for 1 min, annealing at 58 ºC for 30s and extension at 72 ºC for 45s before a final extension of 72 ºC for 8 min.

The estimated PCR product size was about 1500 bp (Figure 10S). PCR products were purified by DNA purification kit (Invitrogen) then sequenced by DNA Analyzer (ABI PRISM 3100, Applied Bioscience). Gene sequences were handled by BioEdit and compared with bacterial 16S rRNA sequences in GenBank database by NBCI Blast program. The results showed that strain G631 belonged to species *Actinoalloteichus cyanogriseus*. Strain G631 was registered with GenBank code: OM190414 (Figure S3).

S.2 Antimicrobial *Assays*

Antimicrobial assays were carried out using the reference microbes *Enterococcus faecalis* (ATCC29212), *Staphylococcus aureus* (ATCC25923), *Bacillus cereus* (ATCC14579), *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella enterica* (ATCC13076) and *Candida albicans* (ATCC10231). Stock solutions of samples were prepared in DMSO (10 mg/mL DMSO) and diluted to decreasing concentration range: 256, 128, 64, 32, and 16 µg/mL. The antimicrobial assays were carried out

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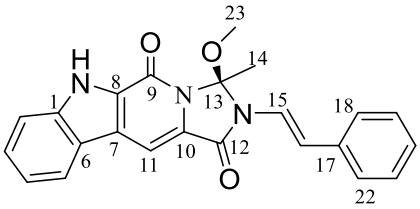
in 96-well microtiter plates against the microbial strains (2×10^5 CFU/mL) using the described protocol [1-3] (number of repeated experiments N=3). After incubation for 24 hours at 37°C, the UV absorption of each sample was measured at 610 nm and compared with the UV absorption of the media and the solvent DMSO as negative control. Streptomycin and cycloheximide were used as positive reference compounds for bacteria and yeast, respectively. MIC value was determined in wells with the lowest concentration of reagents that completely inhibits the growth of microorganisms after 24 h of incubation and was correctly identified based on data of cell turbidity measured by spectrophotometer Biotek and GraphPadPrism DaTa software.

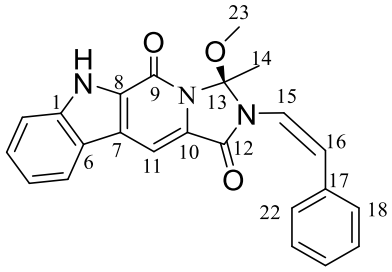
S3: Spectral data for Compounds

*Marinacarboline F (**3**)*



^{1}H-NMR (600 MHz, $CDCl_3$): δ_H (ppm): 2.41 (3H, s, CH_3-14); 3.13 (3H, s, OCH_3-23); 6.99 (1H, d, *J* = 15.0 Hz, H-15); 7.27 (1H, t, *J* = 7.2 Hz, H-20); 7.35 (1H, m, H-4); 7.37 (2H, m, H-19+H-21); 7.37 (1H, d, *J* = 15.0 Hz, H-16); 7.48 (2H, d, *J* = 7.2 Hz, H-18+22); 7.56 (1H, ddd, *J* = 7.2, 7.8, 1.2 Hz, H-3); 7.65 (1H, d, *J* = 8.4 Hz, H-2); 7.80 (1H, s, H-11); 8.26 (1H, d, *J* = 7.8 Hz, H-5); ^{13}C-NMR (150 MHz, $CDCl_3$): δ_C (ppm): 22.3 (CH_3, C-14); 50.0 (OCH_3, C-23); 100.9 (CH, C-11); 102.6 (C, C-13); 112.8 (CH, C-2); 117.5 (CH, C-16); 118.3 (CH, C-15);127.6 (CH, C-3); 121,5 (CH, C-4); 121.7 (CH, C-5); 122.9 (C, C-6); 124.2 (C, C-7); 126.1 (2xCH, C-18, C-22); 126.8 (C, C-10); 128.0 (CH, C-20); 128.8 (CH, C-19); 128.9 (CH, C-21); 130.7 (C, C-8); 135.9 (C, C-17); 139.7 (C, C-1); 152.4 (C=O, C-9); 157.2 (C=O, C-12).

*Marinacarboline H (**4**)*



^{1}H-NMR (600 MHz, $CDCl_3$): δ_H (ppm): 2.34 (3H, s, H-14); 3.05 (3H, s, OCH_3-23); 5.95 (1H, d, *J* = 9.6 Hz, H-15); 7.30 (1H, m, H-4); 6.99 (1H, d, *J* = 9.6 Hz, H-16); 7.23-7.35 (5H, m, H-18 →22); 7.54 (1H, ddd, *J* = 7.5, 7.6, 1.2 Hz, H-3); 7.64 (1H, d, *J* = 7.5 Hz, H-2); 7.72 (1H, s, H-11); 7.98 (1H, d, *J* = 7.8 Hz, H-5); ^{13}C-NMR (150 MHz, $CDCl_3$): δ_C (ppm): 23.3 (CH_3, C-14); 50.0 (OCH_3, C-23); 100.7 (CH, C-11); 102.1 (C, C-13); 112.8 (CH, C-2); 130.5 (CH, C-16); 115.7 (CH, C-15); 121.1 (C, C-6); 121.5 (CH, C-4); 121.6 (CH, C-5); 123.1 (C, C-7); 127.3 (CH, C-20); 127.4 (C, C-10); 127.9 (CH, C-3); 128.4 (2xCH, C-19, C-21); 128.5 (2xCH, C-18, C-22); 130.8 (C, C-8); 135.1 (C, C-17); 139.5 (C, C-1); 153.1 (C=O, C-9); 157.6 (C=O, C-12).

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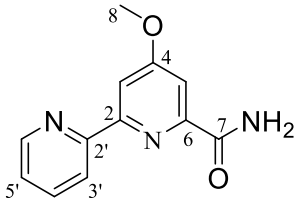
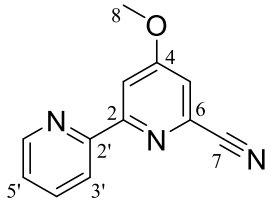
*Caerulomycinamide (**5**)*



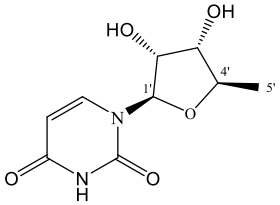
^{1}H-NMR (600 MHz, $CDCl_3$): δ_H (ppm): 4.00 (3H, s, OCH_3-8); 7.35 (1H, dd, *J* = 4.8, 7.8 Hz, H-5’); 7.78 (1H, d, *J* = 2.5 Hz, H-5); 7.83 (1H, t, *J* = 7.8 Hz, H-4’); 8.12 (1H, d, *J* = 2.4 Hz, H-3); 8.38 (1H, d, *J* = 7.8 Hz, H-3’); 8.69 (1H, d, *J* = 4.8 Hz, H-6’). ^{13}C-NMR (150 MHz, $CDCl_3$): δ_C (ppm) 55.8 (OCH_3, C-8); 108.9 (CH, C-5); 109.5 (CH, C-3); 121.1 (CH, C-3’); 124.2 (CH, C-5’); 136.9 (CH, C-4’); 149.3 (CH, C-6’); 150.8 (C, C-6); 155.1 (C, C-2’); 156.8 (C, C-2); 166.7 (C, C-4); 168.1 (C, C-7).

*Caerulomycinonitril (**6**)*



^{1}H-NMR (600 MHz, $CDCl_3$): δ_H (ppm): 4.00 (3H, s, OCH_3-8); 7.25 (1H, d, *J* = 2.5 Hz, H-5); 7.35 (1H, ddd, *J* = 1.5, 5.0, 7.5 Hz, H-5’); 7.85 (1H, td, *J* = 1.0, 8.0 Hz, H-4’); 8.20 (1H, d, *J* = 2.5 Hz, H-3); 8.45 (1H, d, *J* = 8.0 Hz, H-3’); 8.67 (1H, d, *J* = 5.0 Hz, H-6’). ^{13}C-NMR (150 MHz, $CDCl_3$): 56.0 (OCH_3, C-8); 108.6 (CH, C-3); 116.2 (CH, C-5); 117.4 (C, C-7); 121.8 (CH, C-3’); 124.8 (CH, C-5’); 134.1 (C, C-6); 137.2 (CH, C-4’); 149.1 (CH, C-6’); 154.0 (C, C-2’); 159.5 (C, C-2); 167.0 (C, C-4).

*5′-deoxyuridine (**7**)*



^{1}H-NMR (600 MHz, CD_3OD): δ_H (ppm): 1.40 (3H, d, *J* = 6.0 Hz, H-5′); 3.80 (1H, t, *J* = 5.4 Hz, H-4′); 4.02 (1H, t, *J* = 6.5 Hz, H-4′); 4.18 (1H, dd, *J* = 4.2, 5.4 Hz, H-2′); 5.74 (1H, d, *J* = 8.4 Hz, H-6); 5.79 (1H, d, *J* = 4.2 Hz, H-1′); 7.61 (1H, d, *J* = 8.4 Hz, H-5). ^{13}C-NMR (150 MHz, CD_3OD): 18.7 (CH_3, C-5′); 75.3 (CH, C-2′); 76.1 (CH, C-4′); 80.9 (CH, C-4′); 92.0 (CH, C-1′); 102.9 (CH, C-6); 142.5 (CH, C-5); 166.2 (C, C-4); 167.3 (C, C-2).

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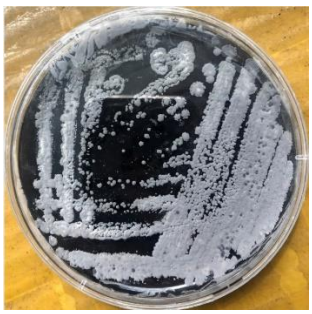

Figure S1: Morphological appearance of G631 strain's colonies on petri dish of A1 medium

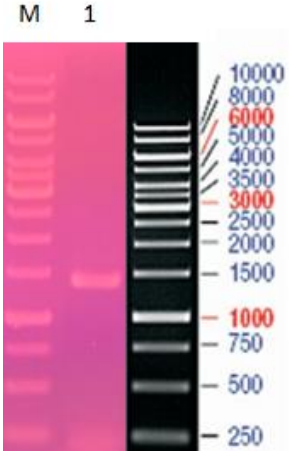

Figure S2: PCR product was run through the agarose gel electrophoresis

Lane 1: PCR product of G631 Lane M: 1 Kb Plus DNA ladder

Actinoalloteichus cyanogriseus strain G631 16S ribosomal RNA gene, partial sequence

GenBank: OM190414.1

FASTA Graphics

Go to:

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            Actinoalloteichus.
REFERENCE   1  (bases 1 to 1393)
  AUTHORS   Vu,Q.T., Pham,C.V., Nguyen,A.M., Doan,H.T., Vu,H.T., Phi,D.T. and
            Le,M.T.
  TITLE     Direct Submission
  JOURNAL   Submitted (10-JAN-2022) biotechnology, Institute of marine
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COMMENT     Sequences were screened for chimeras by the submitter using Bioedit
            7.2.5.

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Figure S3: The 16S rRNA gene sequence of strain G631 is displayed on GenBank

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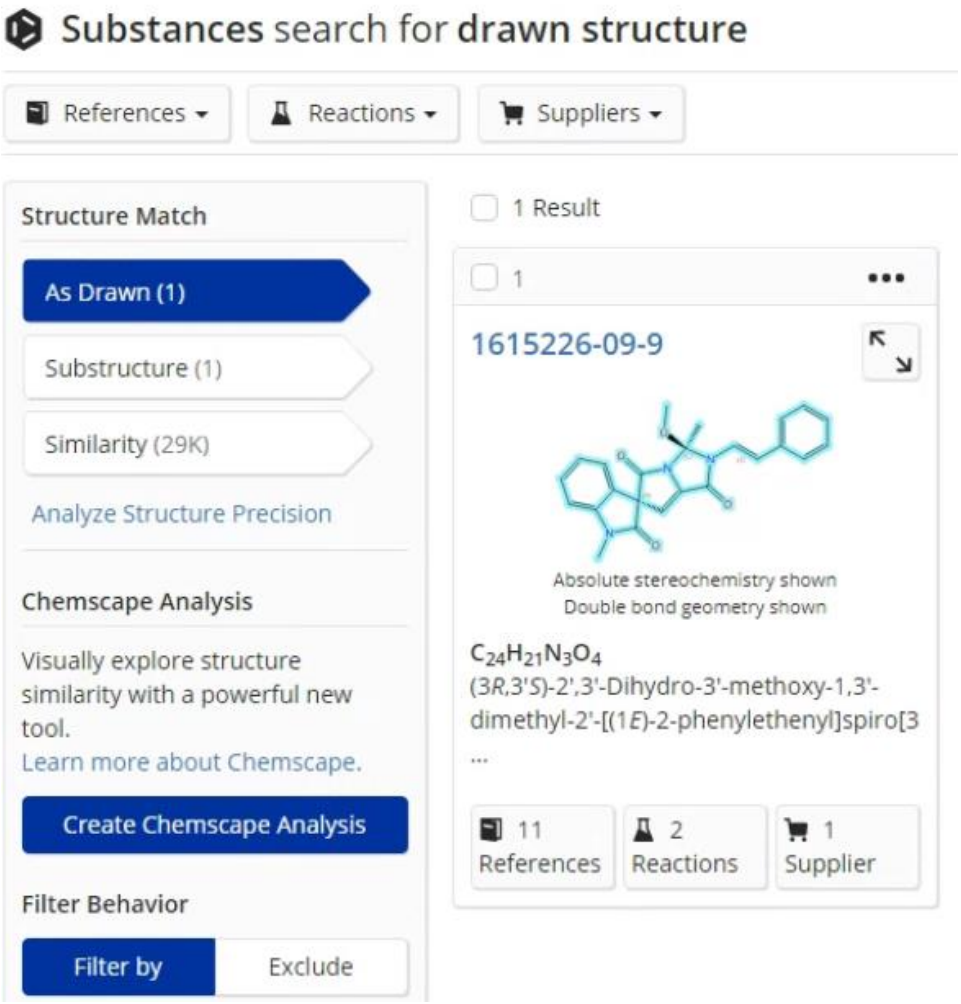

Figure S4: Illustrate for searching of new compound from SciFinder

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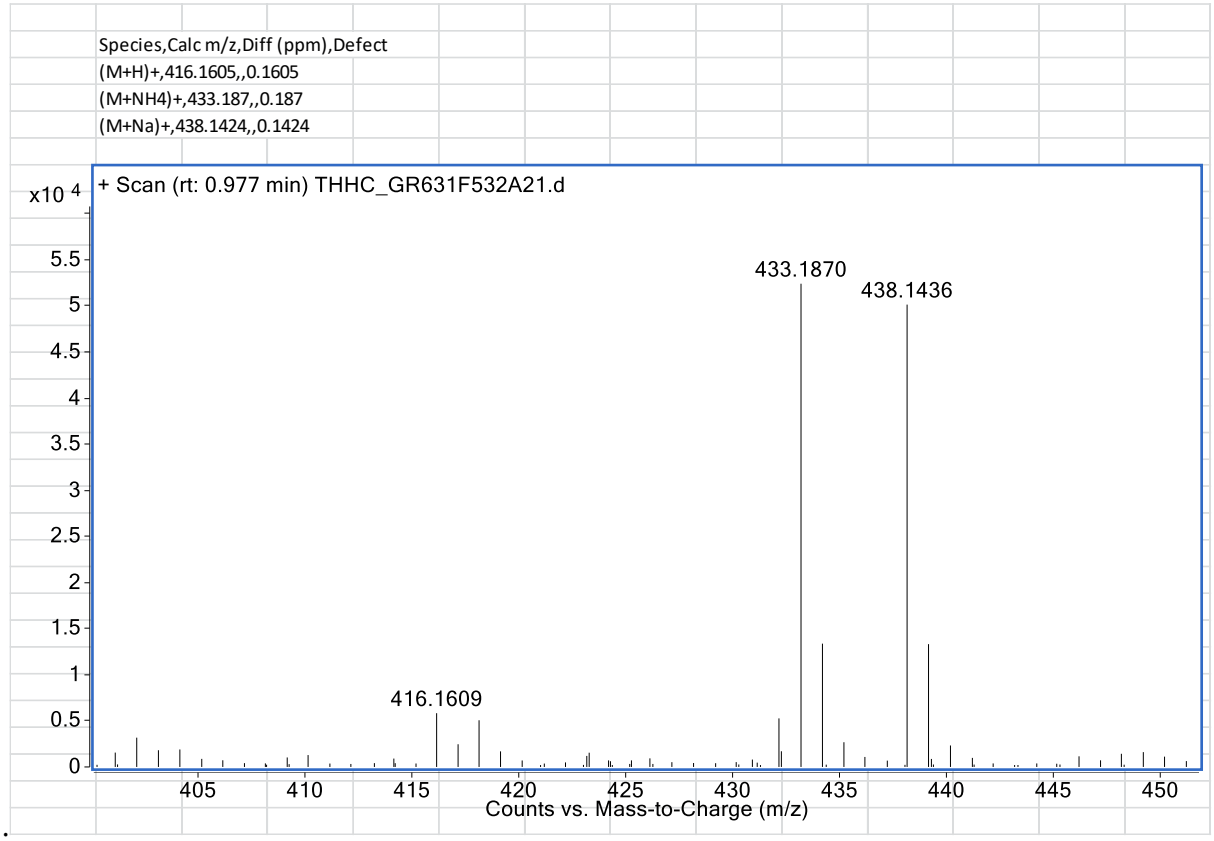

Figure S5: HR-ESI-MS spectrum of compound **1**

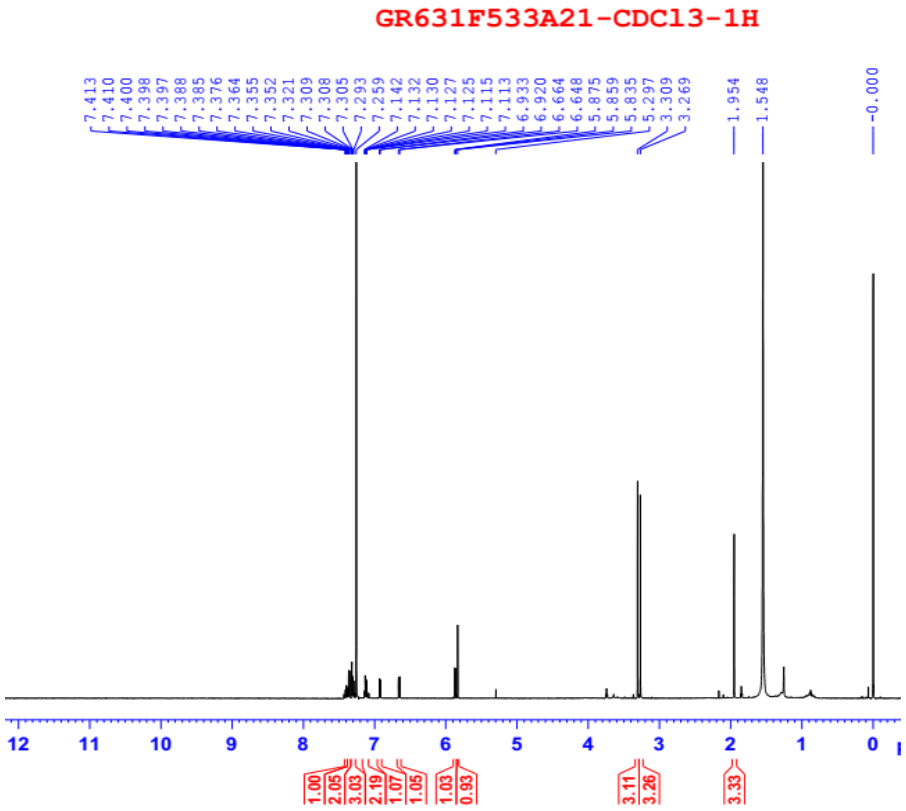

Figure S6: ^{1}H-NMR spectrum of compound **1**

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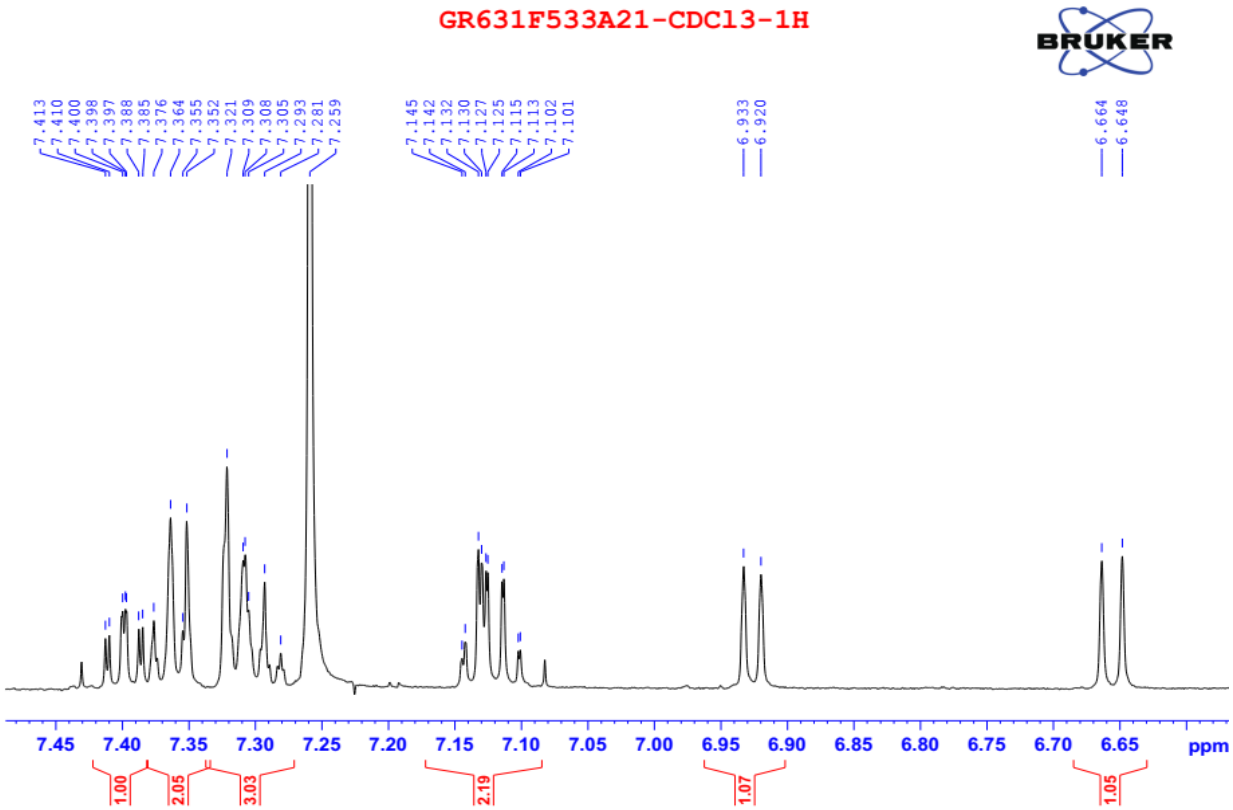

Figure S7: [1]H-NMR spectrum of compound **1** (Expanded)

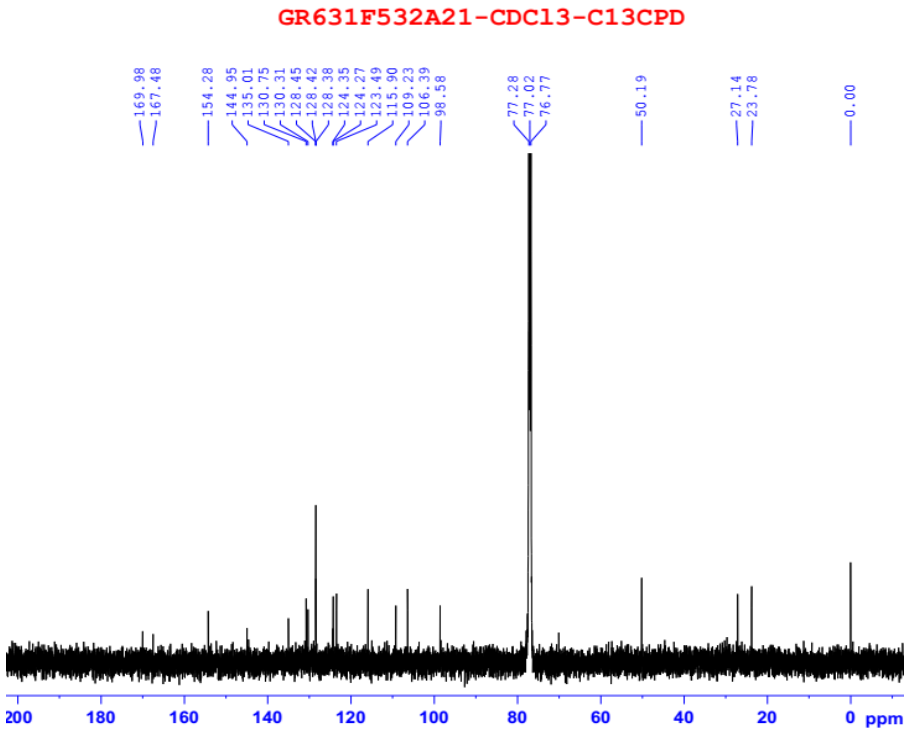

Figure S8: [13]C-NMR spectrum of compound **1**

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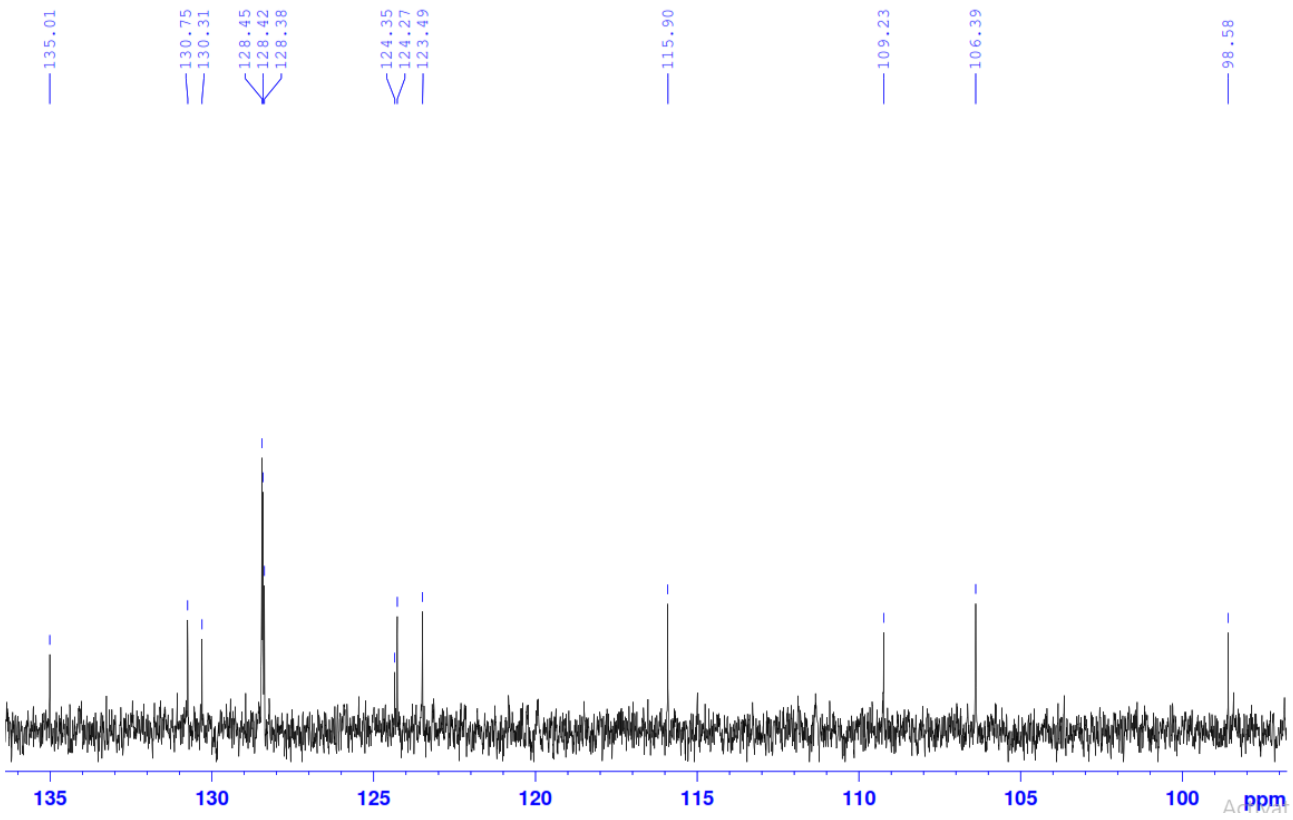

Figure S9: ^{13}C-NMR spectrum of compound **1** (Expanded)

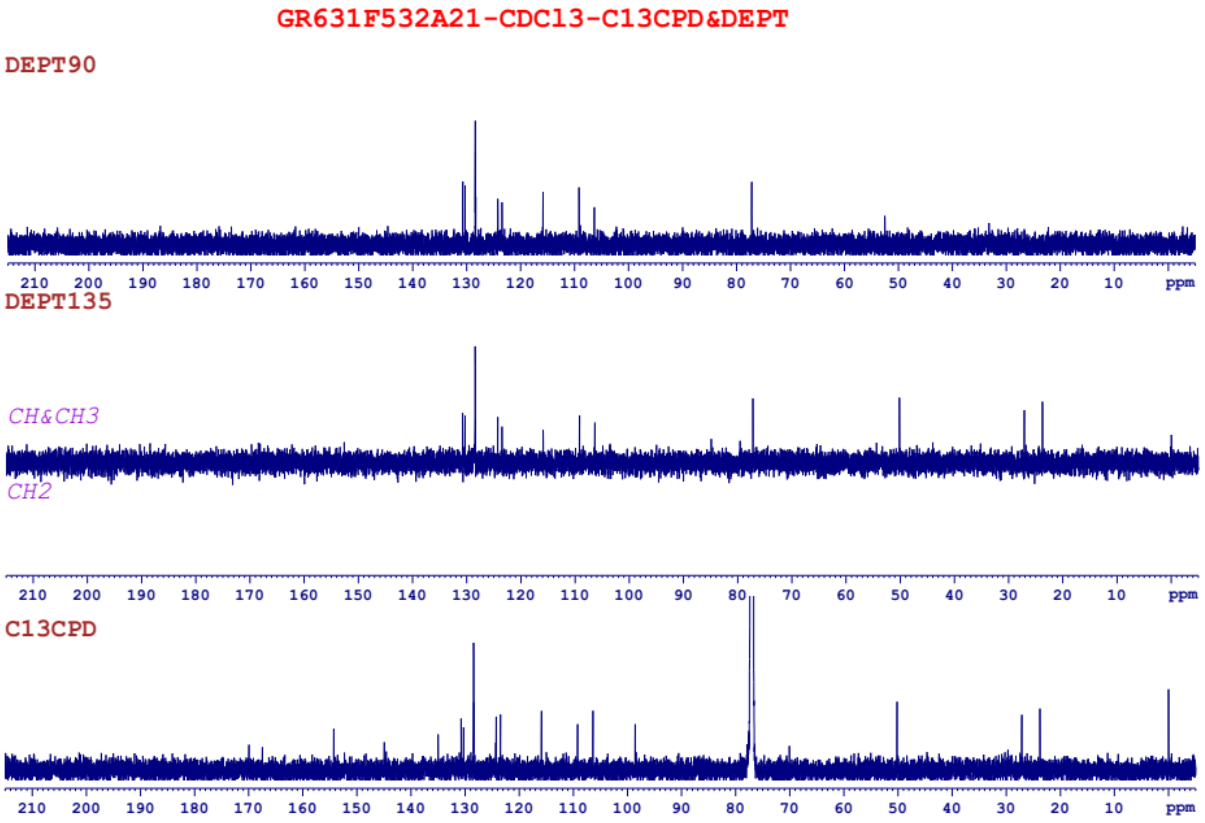

Figure S10: DEPT spectrum of compound **1**

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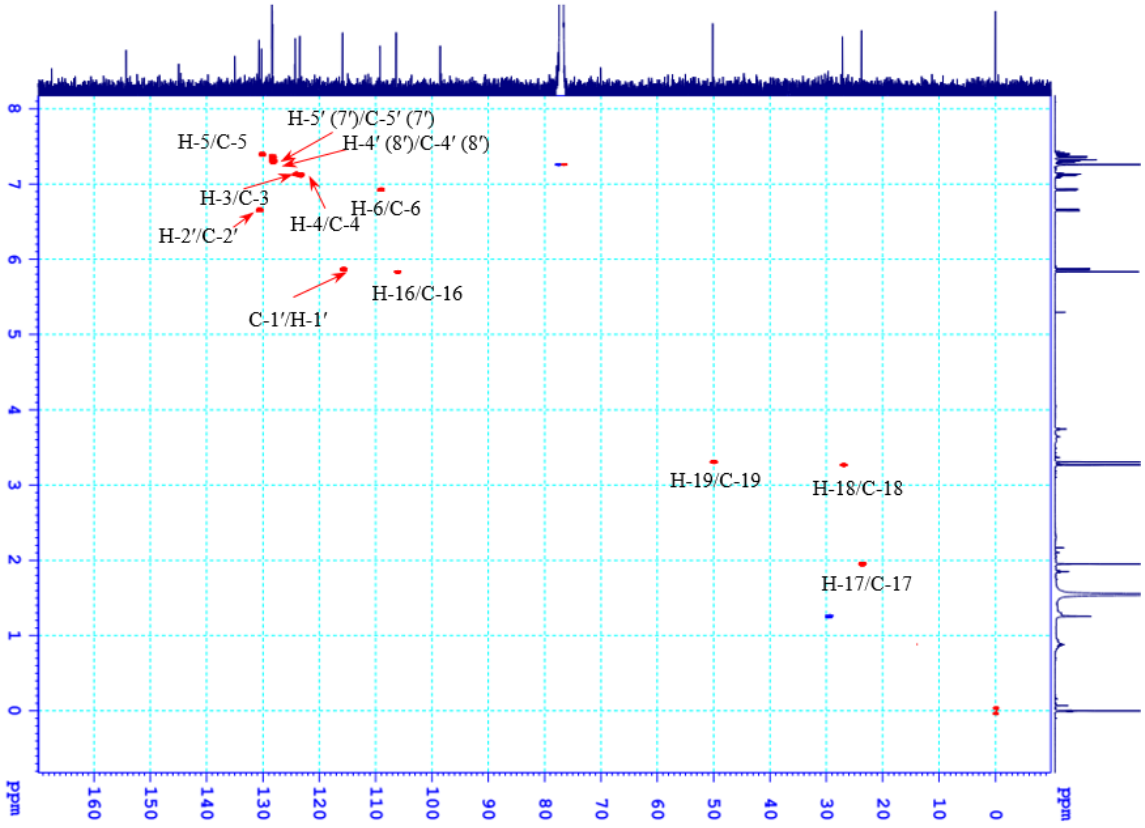

Figure S11: HSQC spectrum of compound **1**

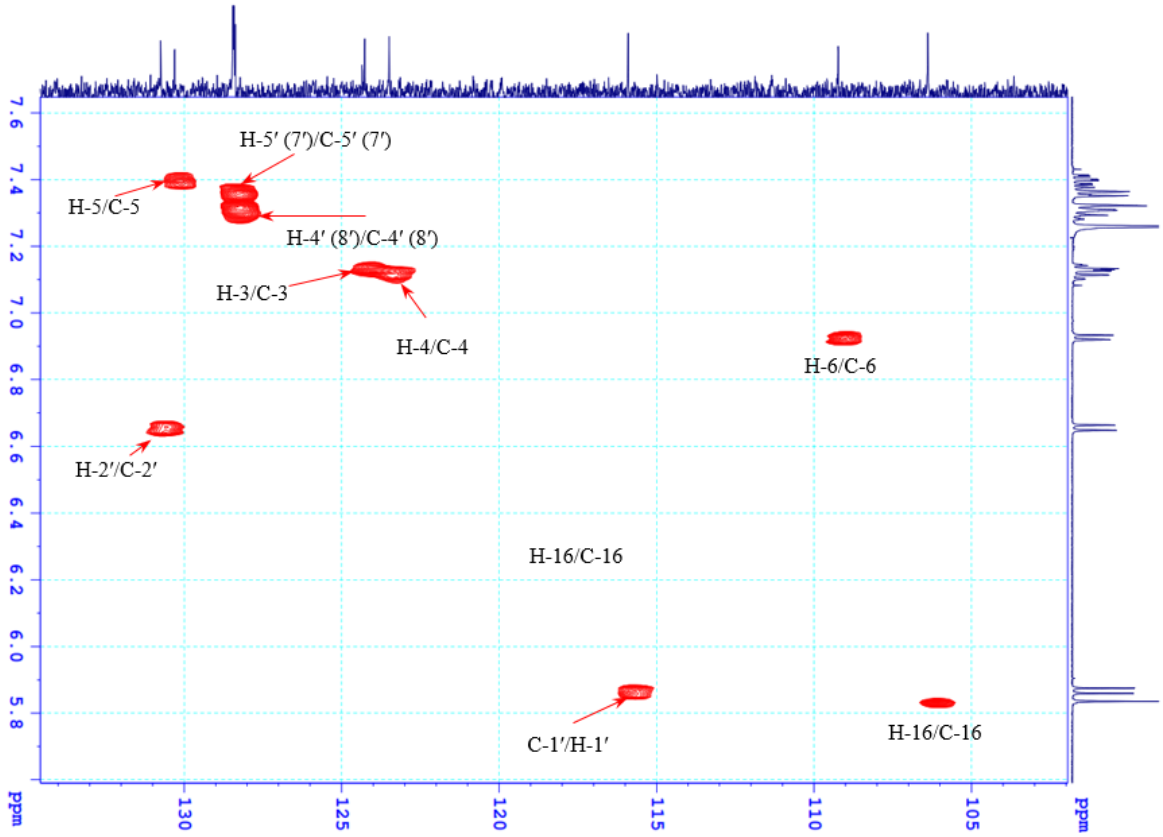

Figure S12: HSQC spectrum of compound **1** (Expanded)

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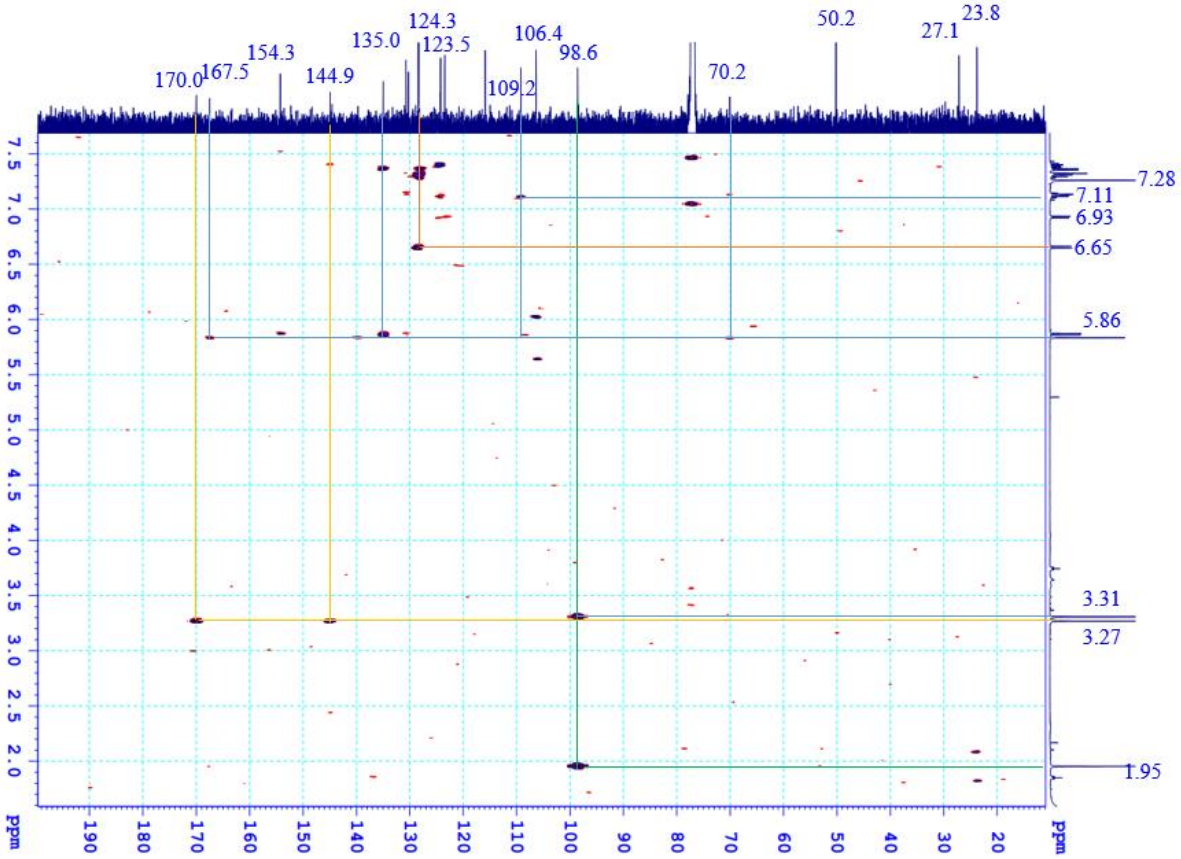

Figure S13: HMBC spectrum of compound **1**

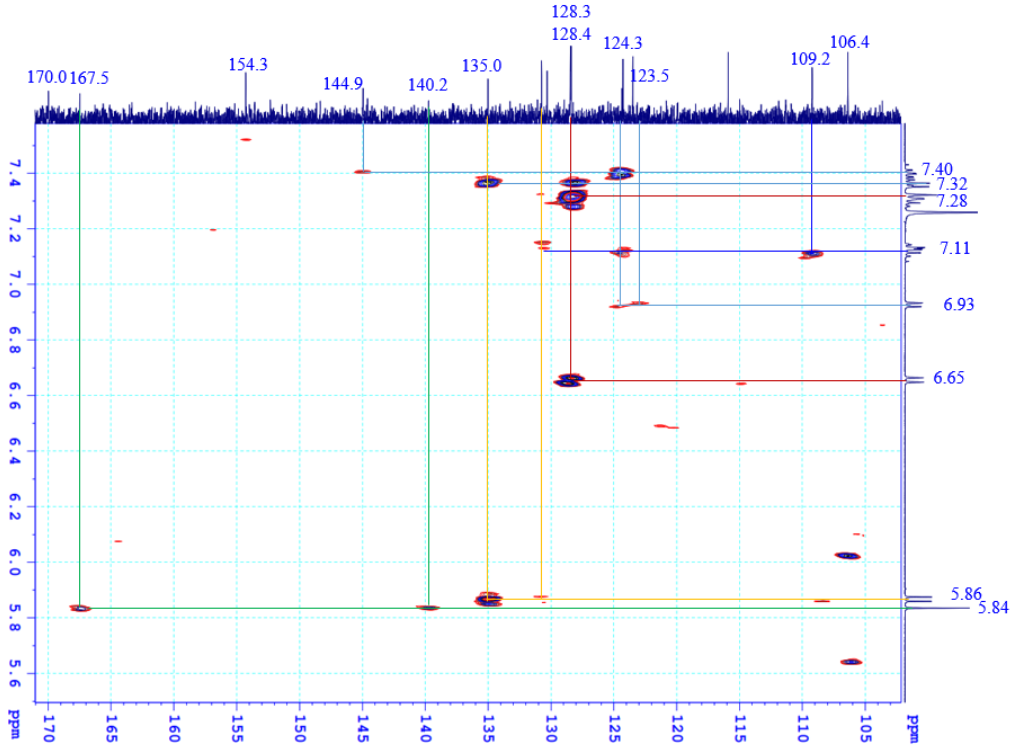

Figure S14: HMBC spectrum of compound **1** (Expanded)

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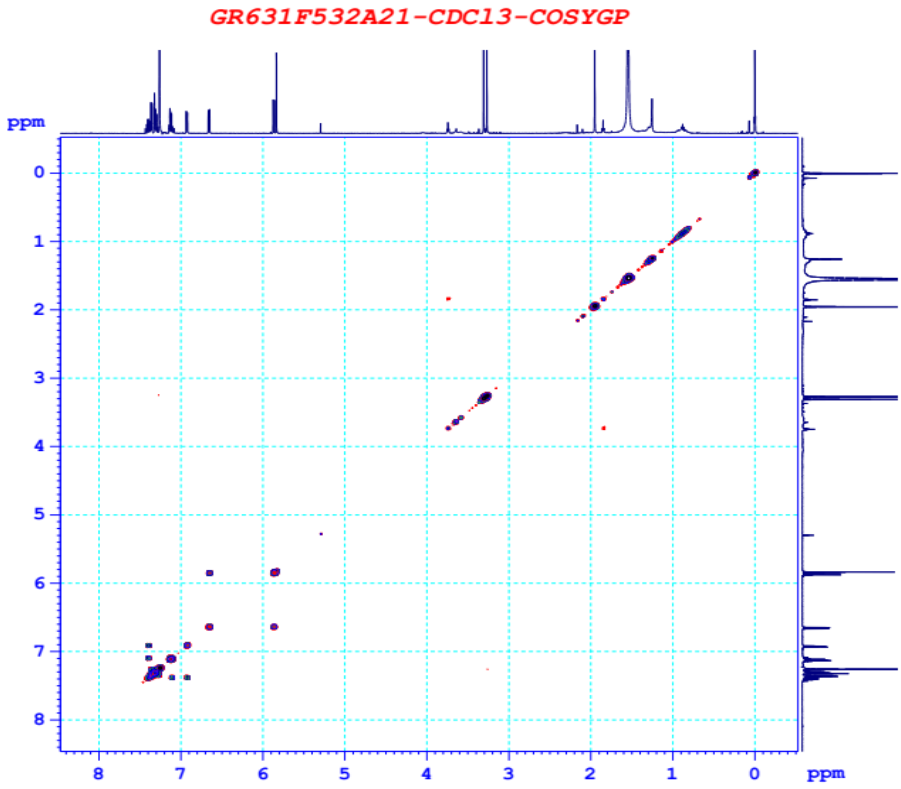

Figure S15: COSY spectrum of compound **1**

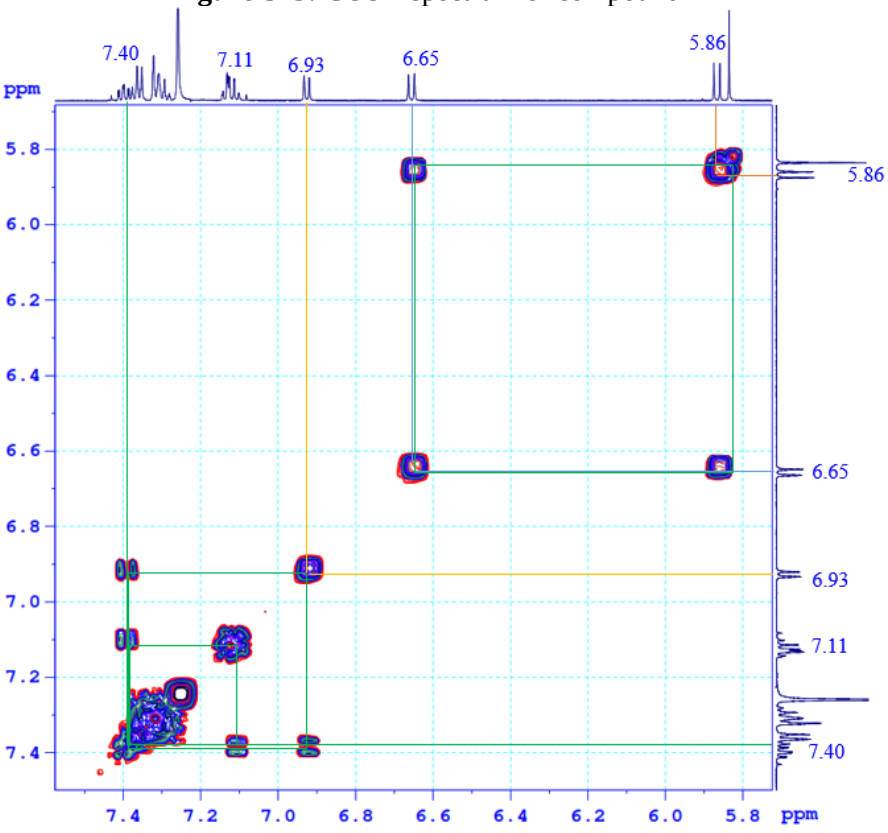

Figure S16: COSY spectrum of compound **1** (Expanded)

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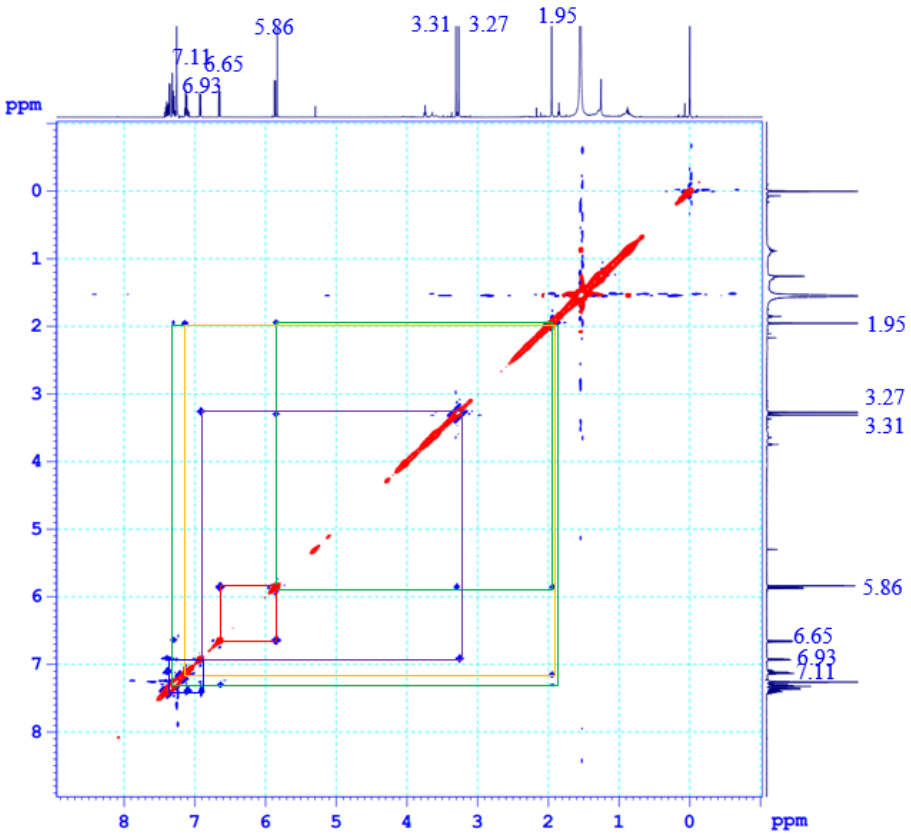

Figure S17: NOESY spectrum of compound **1**

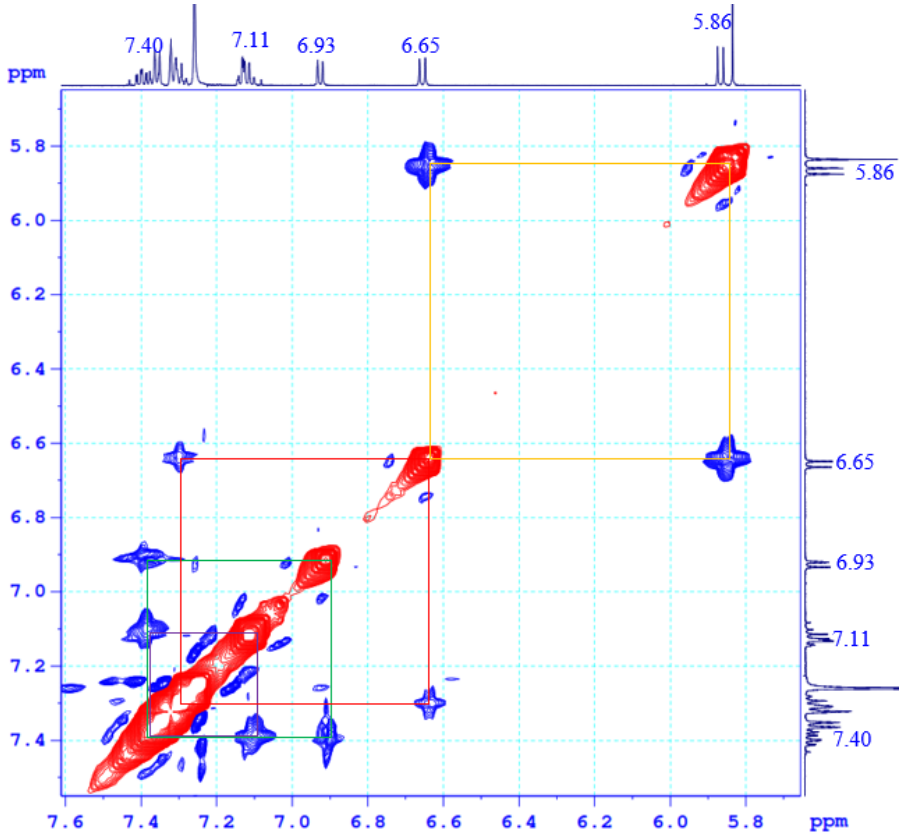

Figure S18: NOESY spectrum of compound **1** (Expanded)

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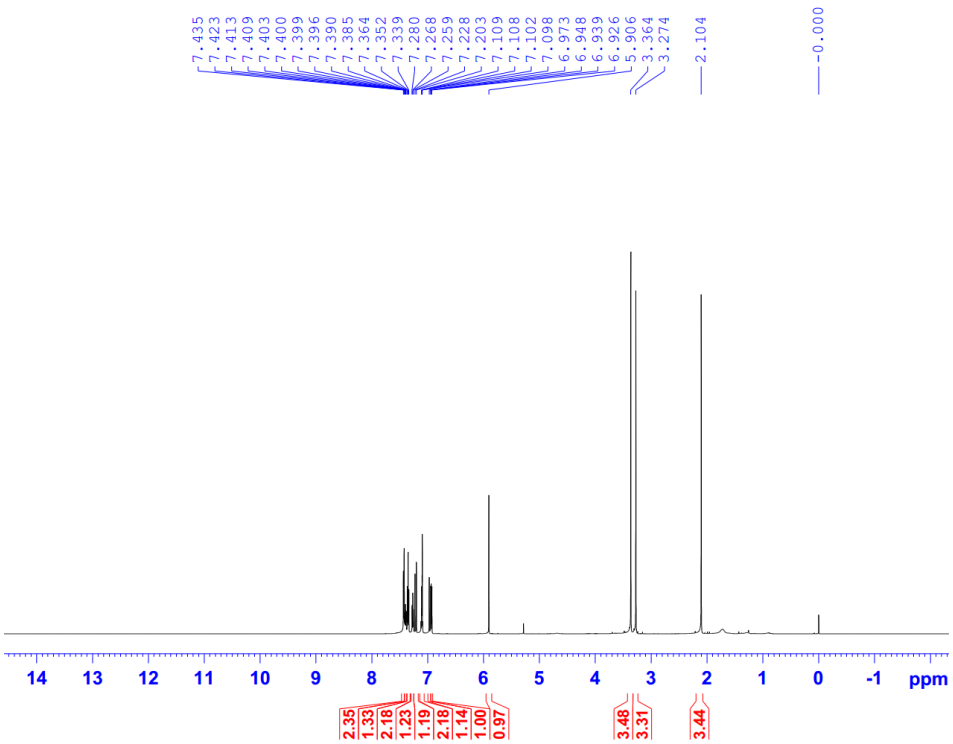

Figure S19: [1]H-NMR spectrum of compound **2**

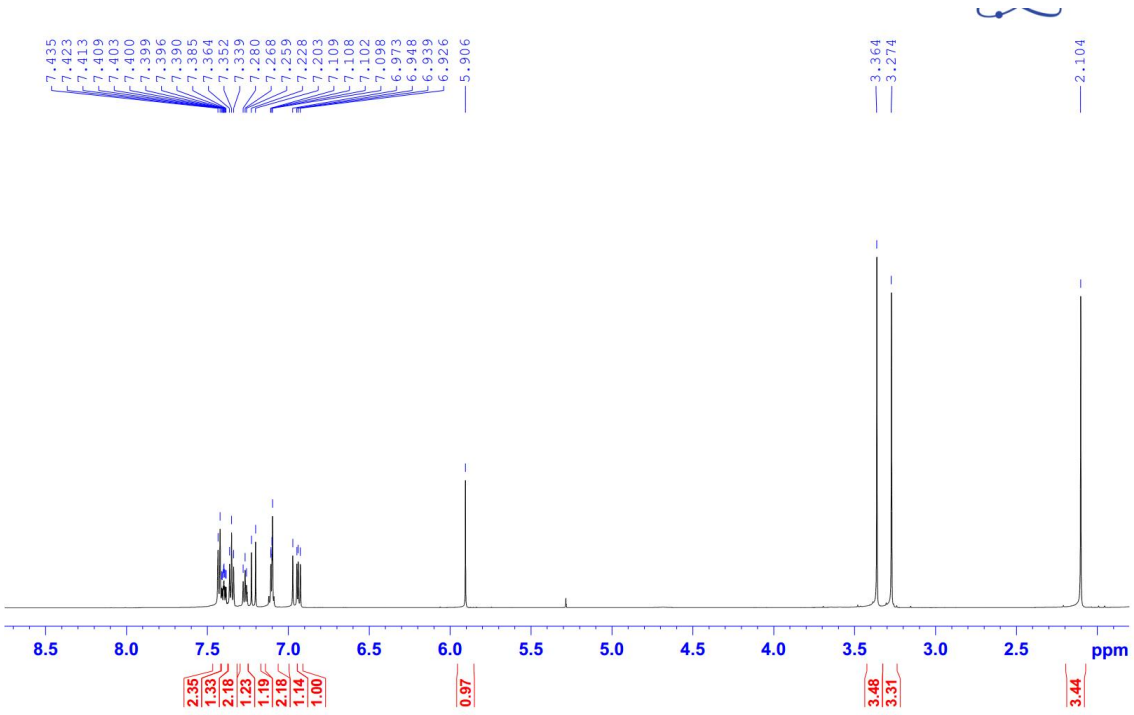

Figure S20: [1]H-NMR spectrum of compound **2** (Expanded)

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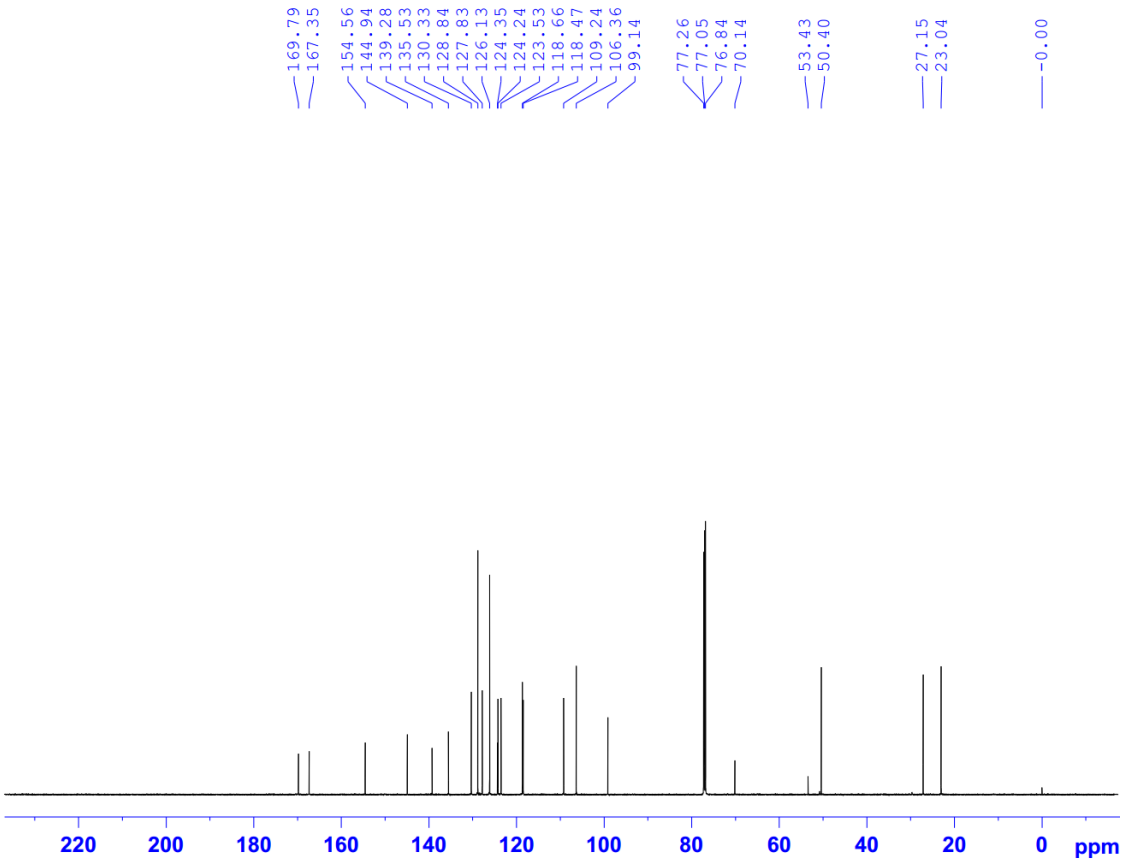

Figure S21: ^{13}C-NMR spectrum of compound **2**

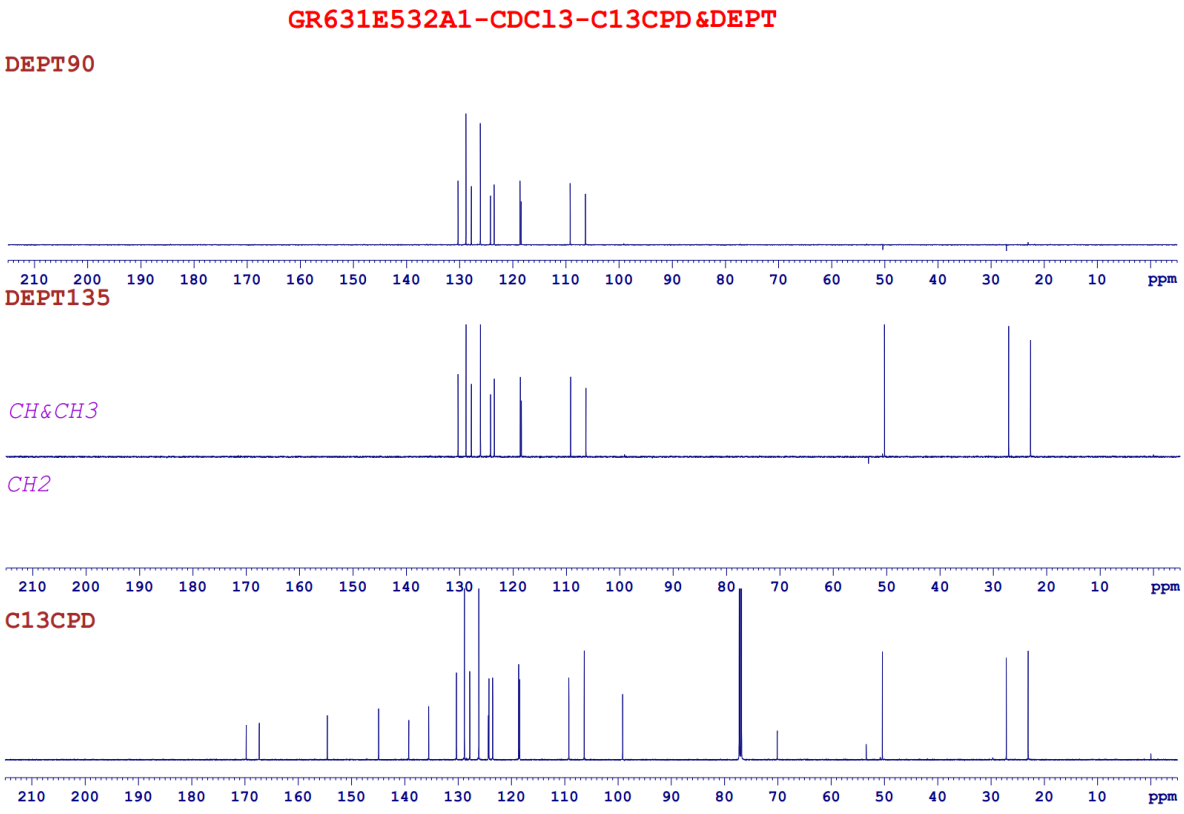

Figure S22: DEPT spectrum of compound **2**

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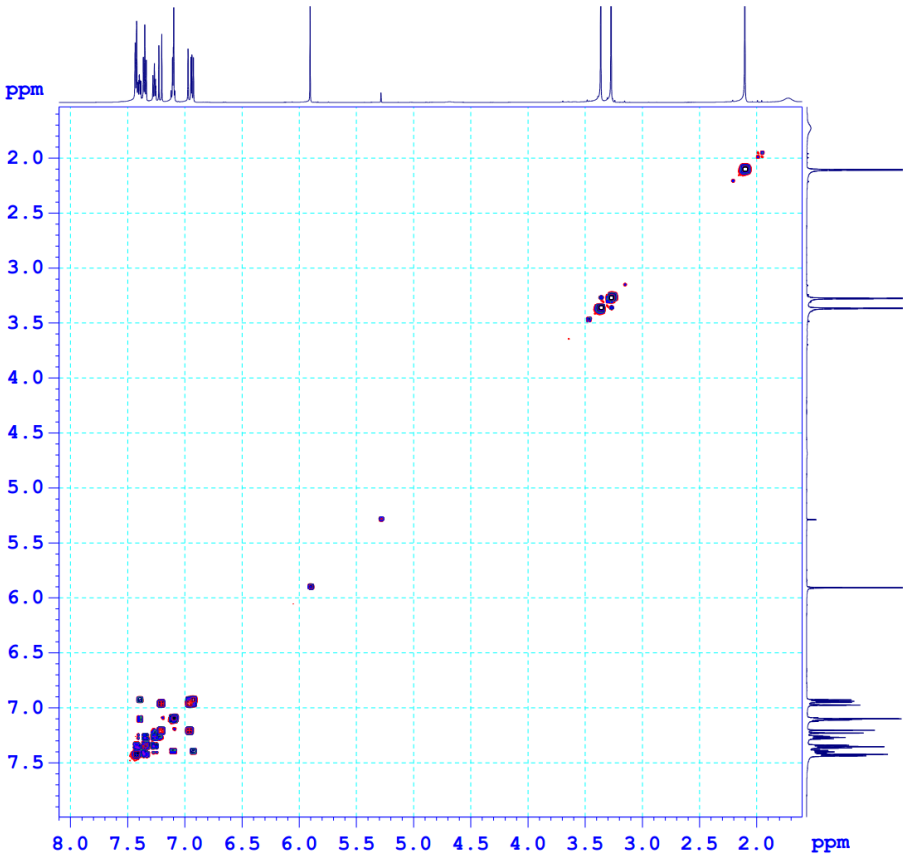

Figure S23: COSY spectrum of compound **2**

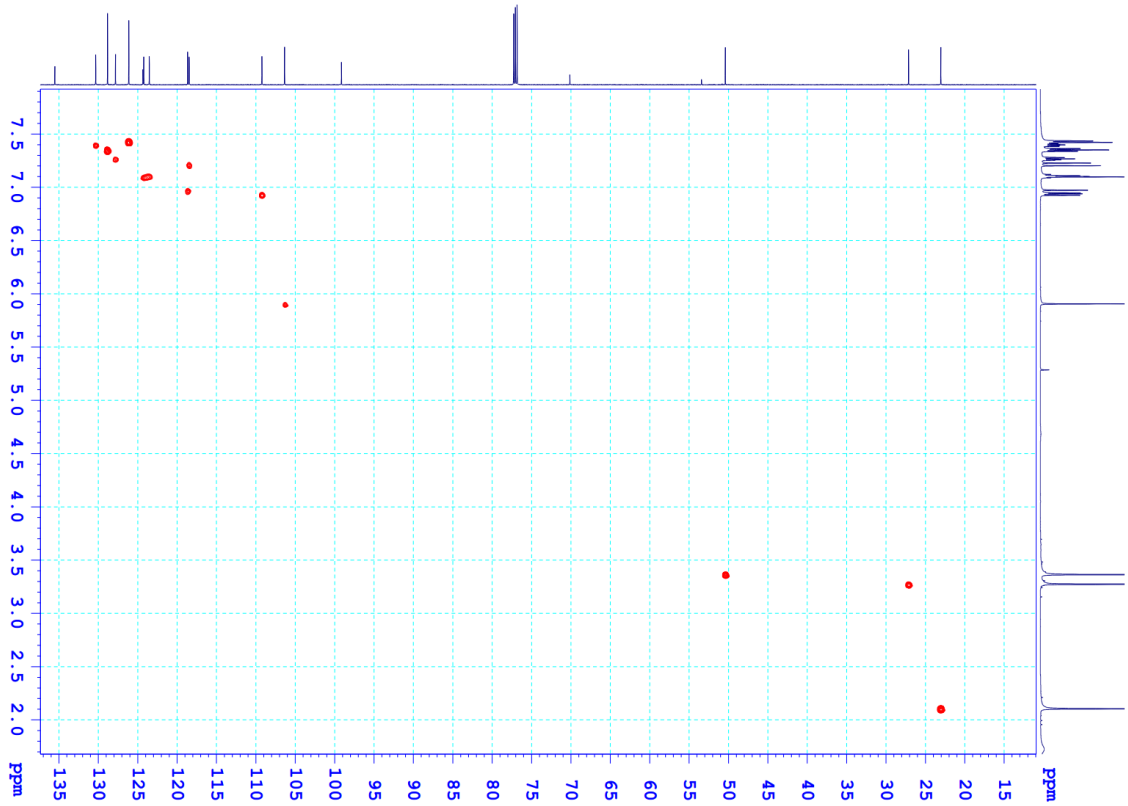

Figure S24: HSQC spectrum of compound **2**

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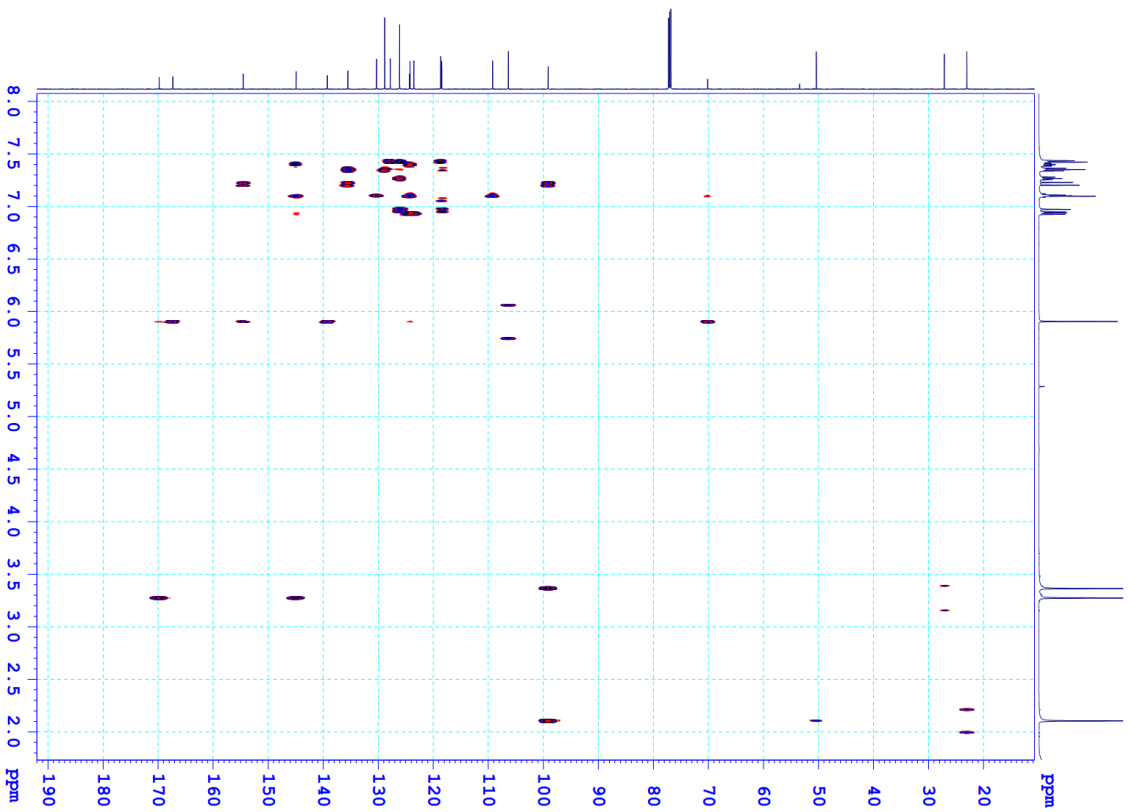

Figure S25: HMBC spectrum of compound **2**

References

[1] J. M. Andrews (2001). Determination of minimum inhibitory concentrations, *J Antimicrob Chemother* **48 Suppl 1**, 5-16.

[2] L.Yanping, S. Sheng J.Feng, Y. Wang, J. Guo, Y. Jiang and W. Wang (2022). New cyclic Peptides from the endophytic *Aspergillus versicolor* 0312 with their antimicrobial activity, *Rec. Nat. Prod*. **16**(**6**), 585-591.

[3] N. Peerakam, P. Phoowiang, S. Chansakaow, C. Thongpoon and S. Hadpech (2022). Chemical profiling revealed a dominant compound trans-anethole and biological evaluation of an edible plant clausena harmandiana containing essential oil, *Rec. Nat. Prod*. **16**(**2**), 118-127.

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