*SUPPLEMENTARY MATERIALS*

# Design of fungal co-cultivation based on comparative metabolomics and bioactivity for discovery of marine fungal agrochemicals

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**List of Tables** **Page No.**



**List of Figures**

**Table S1.** Identification of 8 sediment-derived fungal strains isolated from Windebyer Noor (Nov., 2015) according to the two new BLAST searches (13-08-2019). Self-hits are excluded from the similarity table. BLAST-ALL: shows results from nucleotide BLAST against highly similar sequences. BLAST-TYPE: shows results from nucleotide BLAST against highly similar sequences only from type material. Acc. No. is accession number.

| Acc. No. of strains used | BLAST-ALL | | | BLAST-TYPE | | | Taxonomic assignment |
|---|---|---|---|---|---|---|---|
| | Acc. No. | Name | % similarity | Acc. No. | Name | % similarity | |
| MH791233 | JF740228.1 | *Plenodomus influorescens* strain CBS 143.84 | 100 | NR_111619.1 | *Plenodomus enteroleucus* CBS 142.84 TYPE | 96.23 | *Plenodomus influorescens* |
| | MK495988.1 | *Plenodomus lindquistii* voucher MF-Ha16-005 | 97.74 | NR_119957.1 | *Plenodomus visci* TYPE | 95.88 | |
| | MK495987.1 | *Plenodomus lindquistii* voucher MF-Ha16-004 | 97.74 | MH858430.1 | *Plenodomus congestus* strain CBS 244.64 TYPE | 95.49 | |
| | MK495986.1 | *Plenodomus lindquistii* voucher MF-Ha16-001 | 97.74 | NR_111068.1 | | | |
| | | | | NR_153896.1 | *Comoclathris spartii MFLUCC 13-0214 TYPE* | 95.09 | |
| MH791253 | MN105533.1 | *Penicillium* sp. isolate SL71_37c_D | 100 | MH854996.1 | *Penicillium bialowiezense* strain CBS 227.28 TYPE | 100 | *Penicillium bialowiezense* |
| | | | | NR_111323.1 | | | |
| | MN105477.1 | *Penicillium* sp. isolate SL16_77a_G2 | 100 | KC411734.1 | *Penicillium brunneostoloniferum* strain CBS 317.59 | 98.02 | |
| | MN105342.1 | *Penicillium* sp. isolate SL63_46_G | 100 | NR_121299.1 | *Penicillium brevicompactum* NRRL 2011 | 98.02 | |
| | MN105326.1 | *Penicillium* sp. isolate SL61_6 | 100 | KF465776.1 | *Penicillium brevicompactum* strain CBS 257.29 | 98.02 | |
| MH791254 | MK907754.1 | *Sarocladium strictum isolate Z210A* | 99.33 | GQ376096.2 | *Sarocladium strictum isolate CBS 346.70 TYPE* | 99.33 | *Sarocladium strictum* |
| | | | | NR_111145.1 | | | |
| | LC433837.1 | *Sarocladium sp. MAFF307188* | 99.33 | AY138845.1 | | | |
| | | | | AY566998.1 | | | |
| | MK299148.1 | *Acremonium sp. (in: Hypocreales) isolate LWU_50* | 99.33 | FN691453.1 | *Acremonium strictum nucleomorph CBS:346.70* | 99.33 | |
| | | | | MH859409.1 | *Sarocladium bactrocephalum strain CBS 749.69* | 97.99 | |
| | MH268081.1 | *Sarocladium sp. strain AHB01_8B* | 99.33 | NR_145044.1 | | | |
| | | | | NR_145046.1 | *Sarocladium pseudostrictum UTHSC 02-1892* | 97.09 | |
| MH791292 | MK796144.1 | *Neocucurbitaria cava* isolate cp75.3 | 100 | NR_160112.1 | *Neocucurbitaria cava* CBS 257.68 TYPE | 99.73 | *Neocucurbitaria sp.* |
| | MK460389.1 | *Neocucurbitaria* sp. strain EXF-12877 | 100 | JF740260.1 | | | |
| | MK460388.1 | *Neocucurbitaria* sp. strain EXF-12880 | 100 | NR_156358.1 | *Neocucurbitaria juglandicola* CBS 142390 TYPE | 99.73 | |
| | MK460387.1 | *Neocucurbitaria* sp. strain EXF-12446 | 100 | MF795773.1 | *Neocucurbitaria juglandicola* strain BW6 | 99.73 | |
| | | | | NR_156359.1 | *Neocucurbitaria populi* CBS 142393 TYPE | 99.47 | |
| MH791174 | MH791174.1 | *Pyrenochaeta nobilis* isolate SICB | 100 | NG_062727.1 | *Pyrenochaeta nobilis* CBS 407.76 TYPE | 98.38 | *Pyrenochaeta nobilis* |
| | NG_062727.1 | *Pyrenochaeta nobilis* CBS 407.76 TYPE | 98.38 | MF795792.1 | | | |
| | MF795792.1 | | | DQ898287.1 | | | |
| | DQ898287.1 | | | MF795794.1 | *Seltsamia ulmi* strain L150 | 98.24 | |
| | EU710832.1 | *Pyrenochaeta* sp. 14009 | 98.31 | NG_062728.1 | *Pseudopyrenochaeta lycopersici* CBS 306.65 TYPE | 98.11 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | U42481.1 | *Cucurbitaria berberidis* | 98.31 | DQ898289.1 | | | |
| | | | | NG_063079.1 | *Septoriella leuchtmannii CBS 459.84 TYPE* | 98.04 | |
| MH791258 * | KT923227.1 | Fungal sp. strain OTU53 | 99.79 | MH862690.1 | *Helicodendron microsporum* strain CBS 100149 TYPE | 91.50 | Helotiales sp. |
| | | | | NR_137974.1 | | | |
| | JX507714.1 | Helotiales sp. 203 OA-2013 | 98.90 | MH862609.1 | *Helicodendron websteri* strain CBS 745.96 | 91.50 | |
| | JX507688.1 | Helotiales sp. 104 OA-2013 | 98.89 | MH857844.1 | *Dimorphospora foliicola* strain CBS 221.59 TYPE | 91.24 | |
| | | | | NR_153969.1 | | | |
| | | | | DQ202518.1 | | | |
| | JX507683.1 | Helotiales sp. 96 OA-2013 | 98.89 | NR_160181.1 | *Hydrocina chaetocladia* CBS 249.90 | 87.41 | |
| | | | | MH862207.1 | | | |
| MH791244 ** | MH791287.1 | *Phoma* sp. isolate 41RWS2 | 99.71 | NR_154108.1 | *Lentithecium pseudoclioninum* HHUF 29055 TYPE | 93.64 | *Lentithecium* sp.** |
| | MH791275.1 | *Phoma* sp. isolate 8ES2 | 99.71 | AB809633.1 | *Lentithecium pseudoclioninum* strain: KT 1113 | 93.64 | |
| | MH791270.1 | *Phoma* sp. isolate 26XWS2 | 99.71 | NR_154137.1 | *Lentithecium clioninum* HHUF 28199 | 93.35 | |
| | MH791242.1 | *Phoma* sp. isolate 27XWS2 | 99.71 | LC014566.1 | *Lentithecium clioninum* KT1149A | 93.35 | |
| MH791275 ** | MH791287.1 | *Phoma* sp. isolate 41RWS2 | 100 | NR_158534.1 | *Lentithecium carbonneanum* CBS 144076 TYPE | 95.21 | *Lentithecium* sp.** |
| | MH791273.1 | *Phoma* sp. isolate 27XWS2 | 99.78 | MH062991.1 | *Lentithecium carbonneanum* | 95.21 | |
| | MH791270.1 | *Phoma* sp. isolate 26XWS2 | 99.57 | NR_154108.1 | *Lentithecium pseudoclioninum* HHUF 29055 TYPE | 92.75 | |
| | KU179250.1 | Fungal endophyte isolate 15 | 97.01 | AB809633.1 | *Lentithecium pseudoclioninum* KT 1113 | 92.75 | |

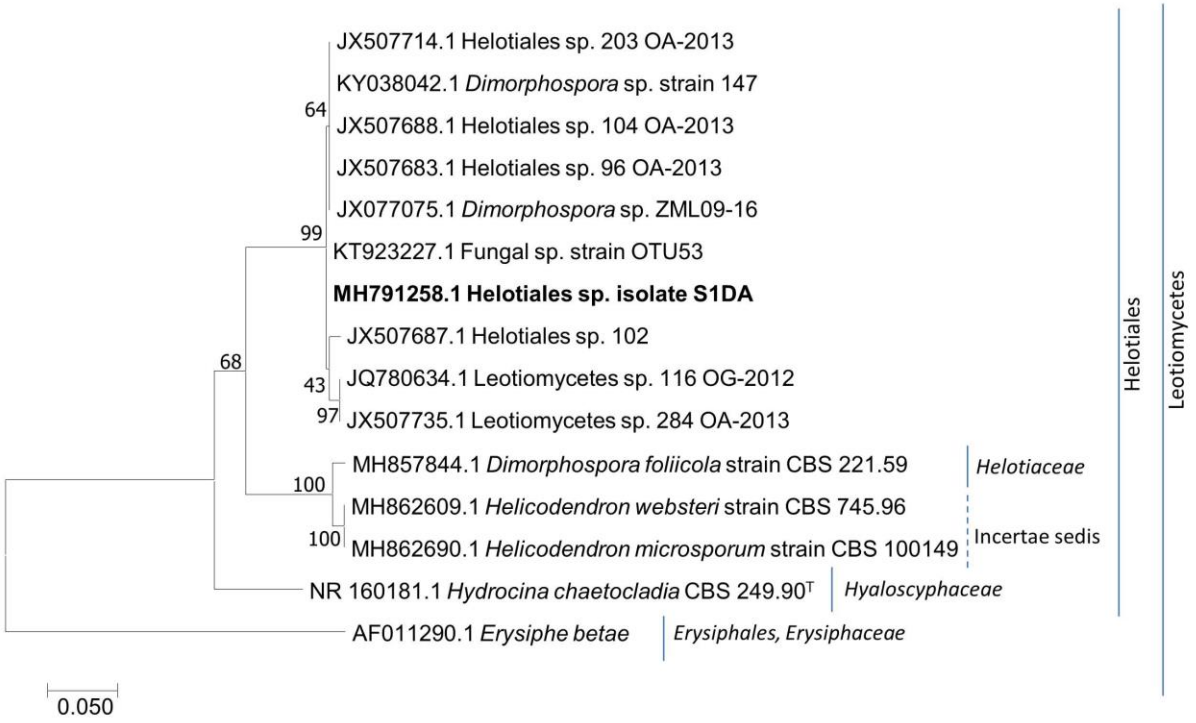
*: Taxonomic assignment was not possible to a higher rank than order level. Phylogenetic tree calculation (Fig. S27) suggests that this isolate may belong to a yet undescribed family within the order Helotiales

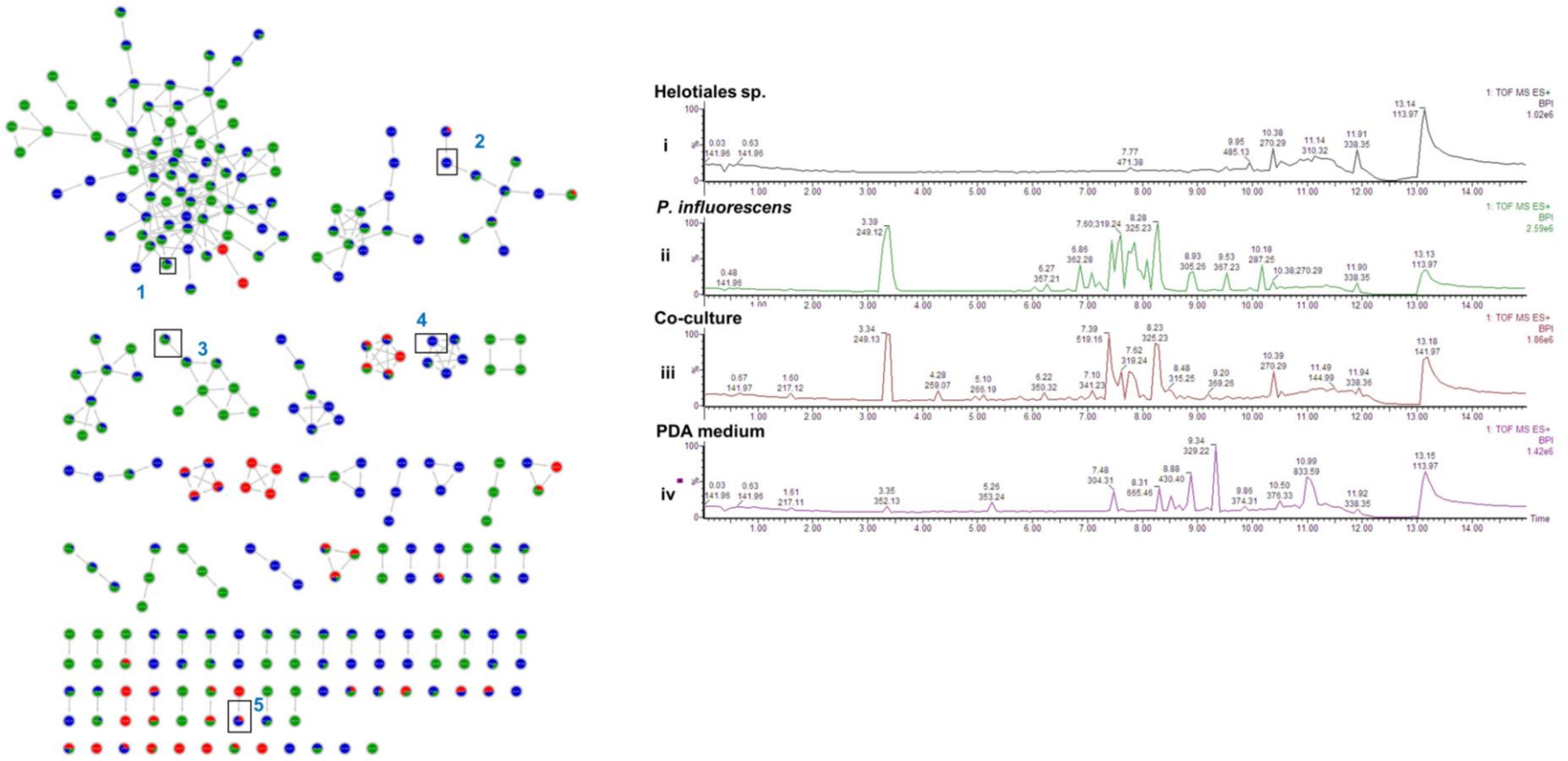
**: Re-sequencing the 28S rRNA gene of these isolates (9ES2- MH791244, 8ES2- MH791275) allowed classification as *Lentithecium* sp.

**Table S2**. In vitro antiphytopathogenic activity ($IC_{50}$ values in μg/mL) of the DCM subextract (D) and fractions obtained therefrom (D1–D11) by elution on a C18 SPE cartridge. Test phytopathogens include Pss, *P. syringae*; Xc, *X. campestris*, Ea, *E. amylovora*; Rs, *R. solanacearum*; Pi, *P. infestans*; Mo. *M. oryzae*. 0.5% DMSO was used as a solvent control. Positive controls for Xc, Ea and Pss: chloramphenicol, for Rs: tetracycline, for Mo: nystatin and for Pi: cycloheximide.

| Sample | **Pss** | **Xc** | **Ea** | **Rs** | **Pi** | **Mo** |
|---|---|---|---|---|---|---|
| D | > 100 | 0.9 | > 100 | > 100 | 2.3 | > 100 |
| D1/2 | > 100 | 34.1 | > 100 | > 100 | > 100 | > 100 |
| D 3 | > 100 | > 100 | > 100 | > 100 | > 100 | > 100 |
| D 4/5 | > 100 | 56.1 | > 100 | > 100 | > 100 | > 100 |
| D 6 | 83.8 | 8.5 | > 100 | > 100 | 15.6 | > 100 |
| D 7 | 18.8 | 3.1 | > 100 | > 100 | 4.3 | > 100 |
| D 8 | 17.2 | 2.1 | > 100 | > 100 | 3,1 | > 100 |
| D 9 | 32.0 | 3.9 | > 100 | > 100 | 3,1 | > 100 |
| D 10 | > 100 | 7.1 | > 100 | > 100 | > 100 | > 100 |
| D 11 | > 100 | 13.3 | > 100 | > 100 | > 100 | > 100 |
| Positive control | 0.7 | 0.5 | 0.7 | 1.0 | 0.3 | 0.4 |

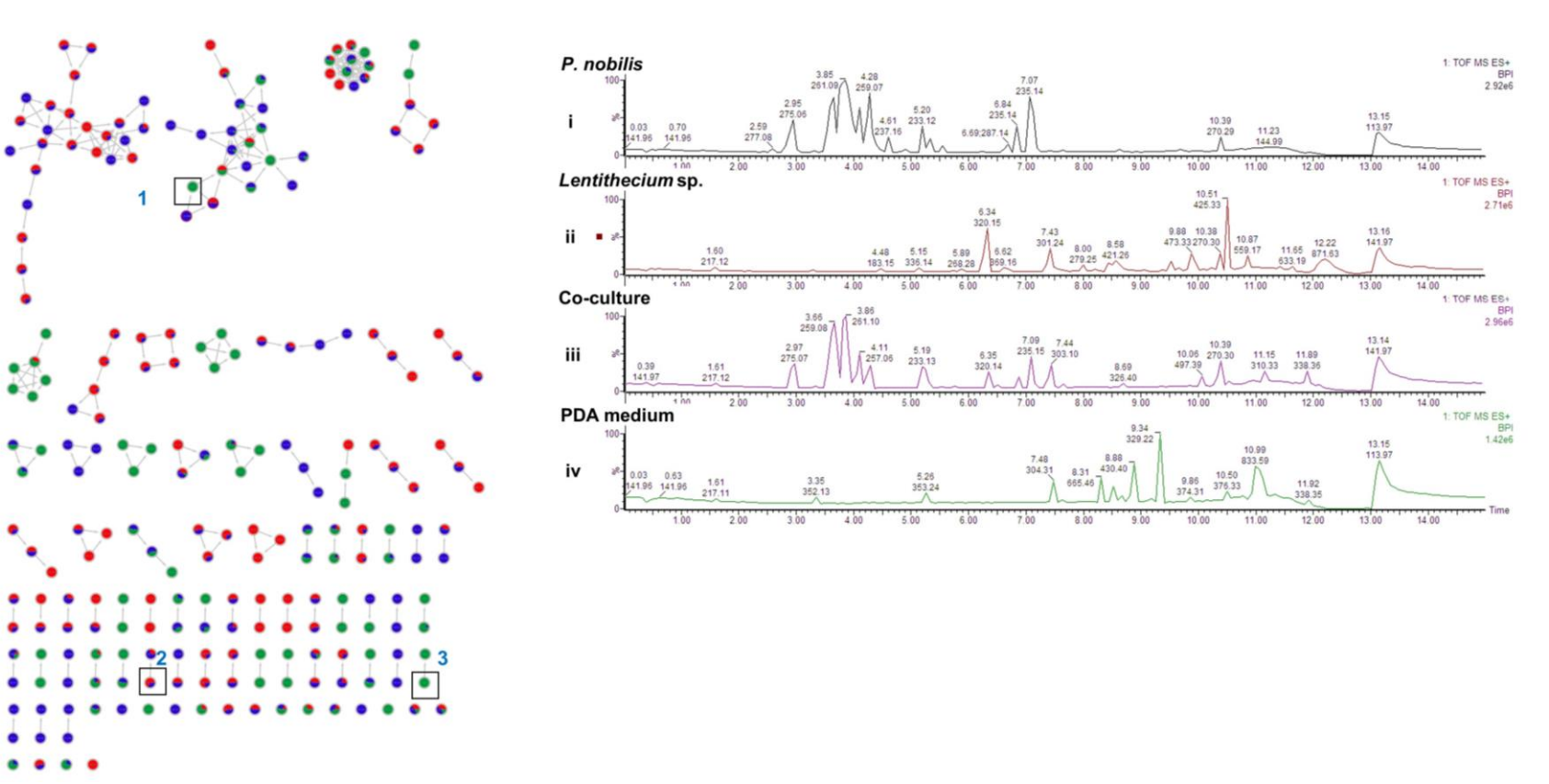
**Figure S1.** Phylogenetic tree for taxonomic assignment of isolate S1DA-Helotiales sp. based on sequencing of the ITS 1-5.8S rRNA gene-ITS2 fragment. Taxonomic affiliation is only possible to order level due to the low similarity (91.5%, Table S1) to related type strains. Closest relatives (98.8-99.8% similarity) of our isolate S1DA are other isolates only identified to order level.





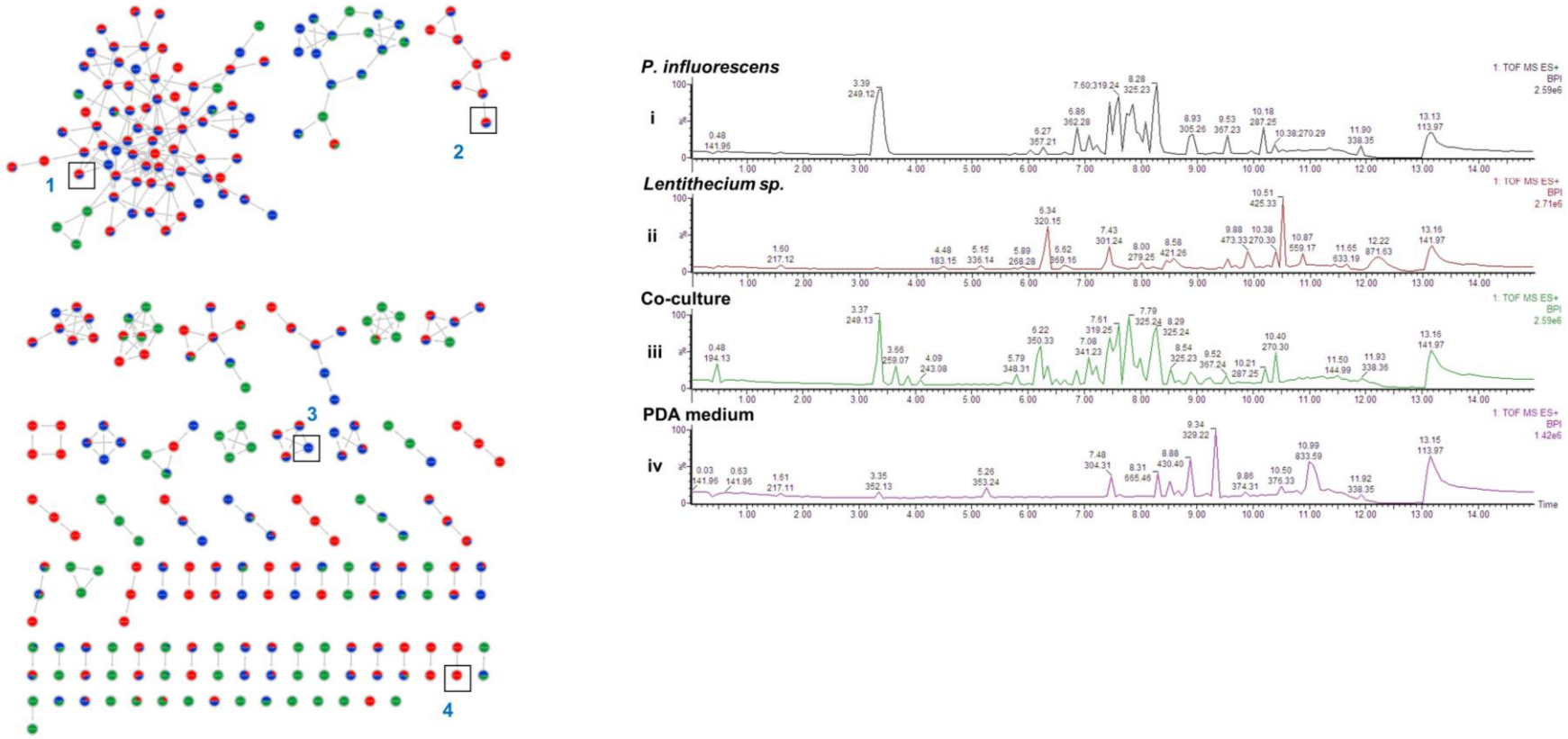

**(A)** **(B)**

**Figure S2**. (**A**) MN of mono-culture extracts of Helotiales sp. (red), *P. influorescens* sp. (green) and their co-culture (blue). Nodes were annotated putatively as: **1**- phomactin B2, **2**- dehydroxybisdethiobis(methylthio)gliotoxin, **3**- (5E)-4-hydroxy-12-methyl-1-oxacyclododec-5-ene-2,8-dione, **4**- cephalochromin, **5**- monodictysin B. (**B**) Base peak chromatograms of Helotiales sp. mono-culture (i), *P. influorescens* sp. mono-culture (ii), their co-culture (iii), blank PDA medium (iv).



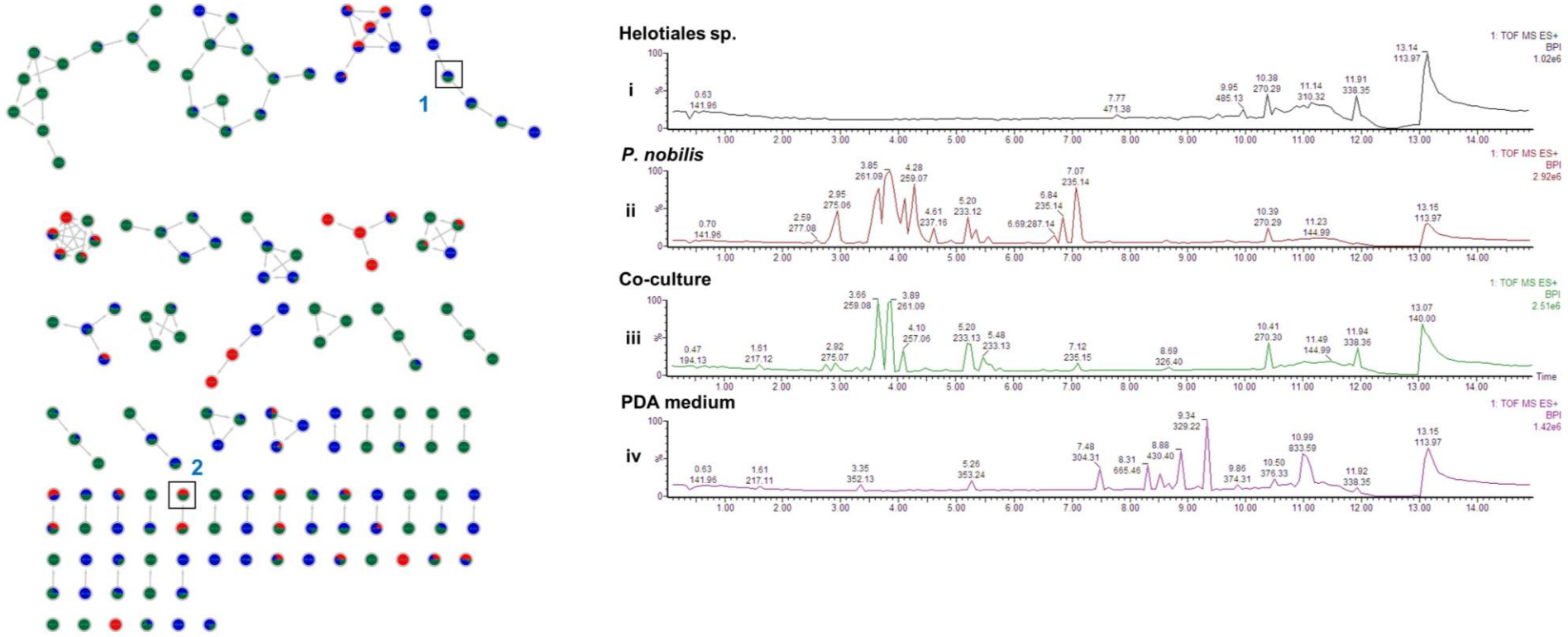

**(A)** **(B)**

**Figure S3**. (**A**) MN of mono-culture extracts of *P. nobilis* sp. (red), *Lentithecium* sp. (green) and their co-culture (blue). Nodes were annotated putatively as: **1**- 3-acetyl-5-isopropyl-pyrrolidine-2,4-dione, **2**- spiciferinone, **3**- truncatone. (**B**) Base peak chromatograms of *P. nobilis* mono-culture (i), *Lentithecium* sp. mono-culture (ii), their co-culture (iii), blank PDA medium (iv).




**(A)** **(B)**

**Figure S4**. (**A**) MN of mono-culture extracts of *P. influorescens* (red), *Lentithecium* sp. (green) and their co-culture (blue). Nodes were annotated putatively as: **1**- phomactin B2, **2**- (5E)-4-hydroxy-12-methyl-1-oxacyclododec-5-ene-2,8-dione, **3**- cephalochromin, **4**- fumonisin B4. (**B**) Base peak chromatograms of *P. influorescens* mono-culture (i), *Lentithecium* sp. mono-culture (ii), their co-culture (iii), blank PDA medium (iv).




**(A)** **(B)**

**Figure S5**. (**A**) MN of mono-culture extracts of Helotiales sp. (red), *P. nobilis*. (green) and their co-culture (blue). Nodes were annotated putatively as: **1**- spiciferinone, **2**- monodictysin B. (**B**) Base peak chromatograms of Helotiales sp. mono-culture (i), *P. nobilis* mono-culture (ii), their co-culture (iii), blank PDA medium (iv).

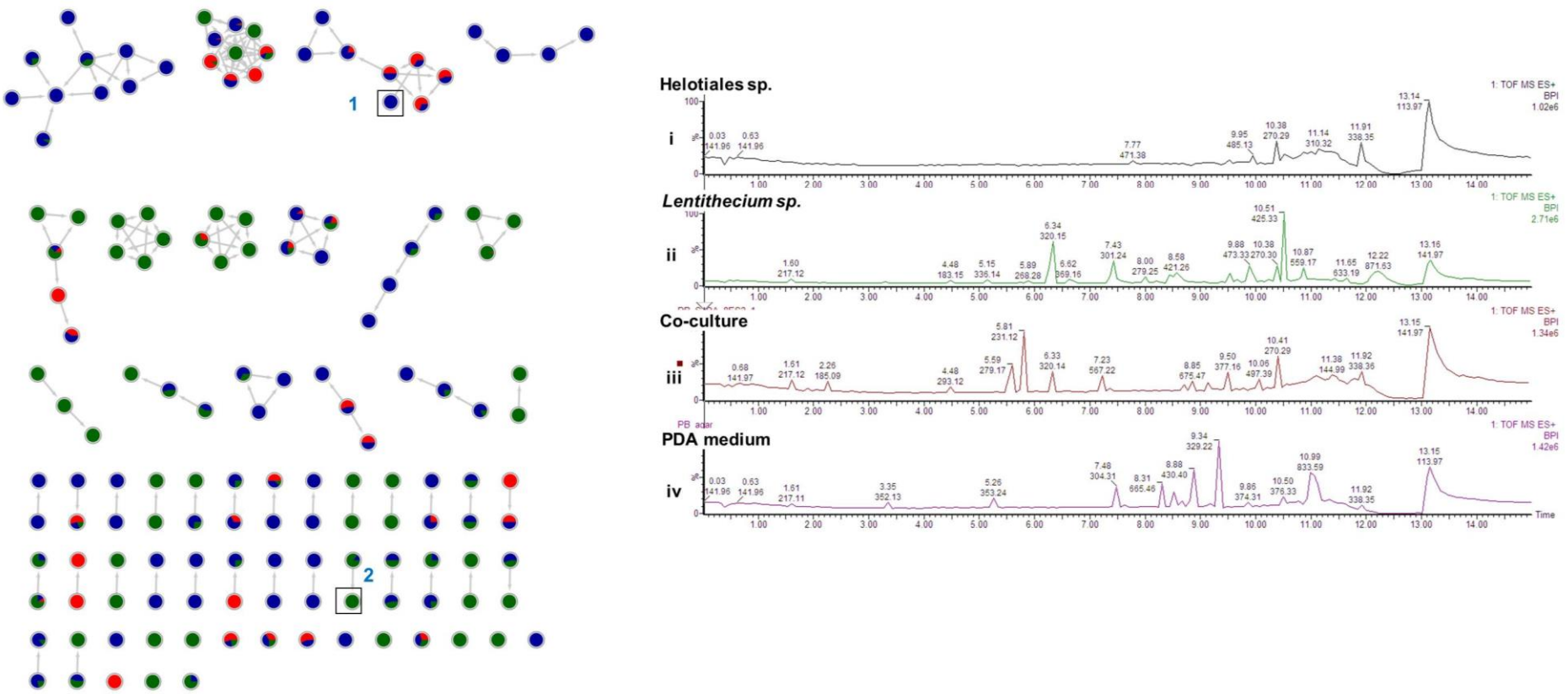



**Figure S6**. (**A**) MN of mono-culture extracts of Helotiales sp. (red), *Lentithecium* sp. (green) and their co-culture (blue). Nodes were annotated putatively as: **1**- truncatone, **2**- 3-acetyl-5-isopropyl-pyrrolidine-2,4-dione. (**B**) Base peak chromatograms of Helotiales sp. mono-culture (i), *Lentithecium* sp. mono-culture (ii), their co-culture (iii), blank PDA medium (iv).

**Figure S7**. $^{1}H$ NMR spectrum of compound **1** ($CDCl_3$, 600 MHz).

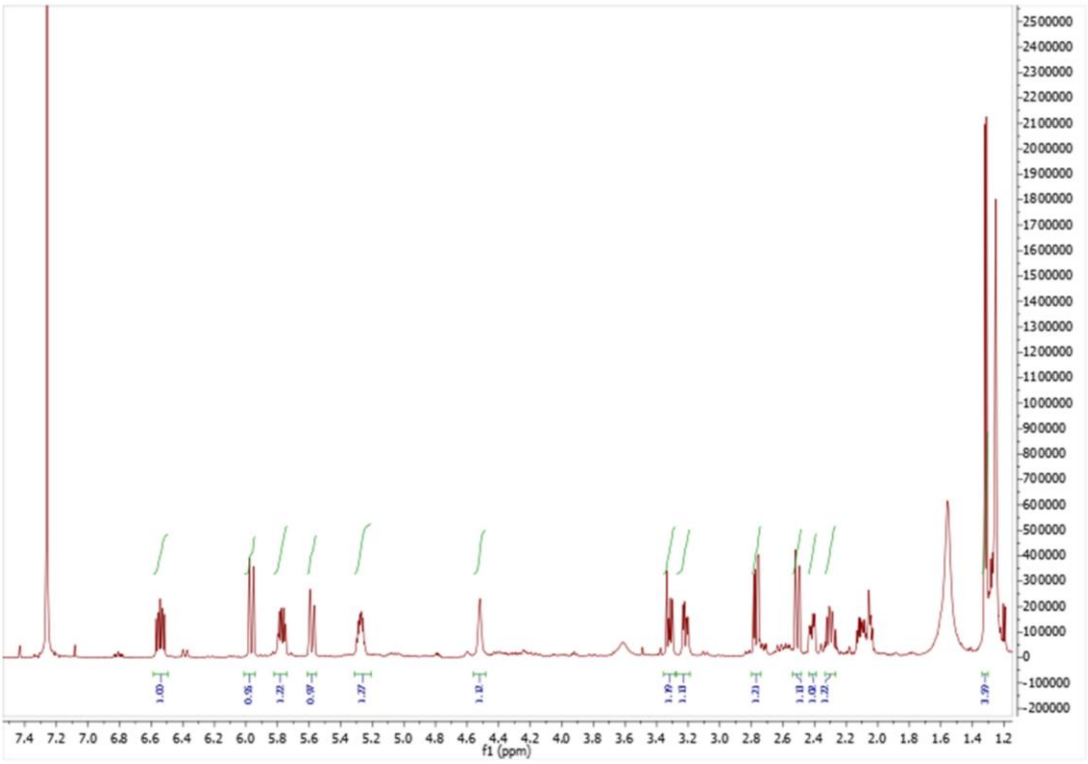



**Figure S8**. $^{13}C$ NMR spectrum of compound **1** ($CDCl_3$, 150 MHz).

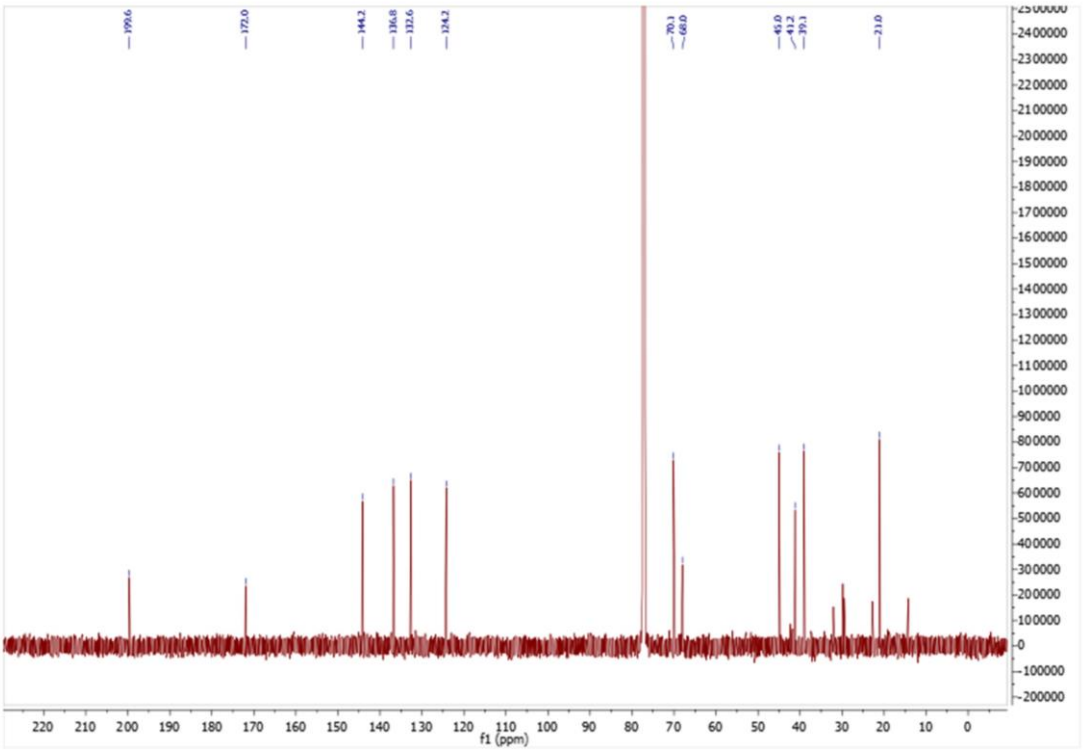


**Figure S9**. DEPT-135 spectrum of compound **1** ($CDCl_3$, 150 MHz).

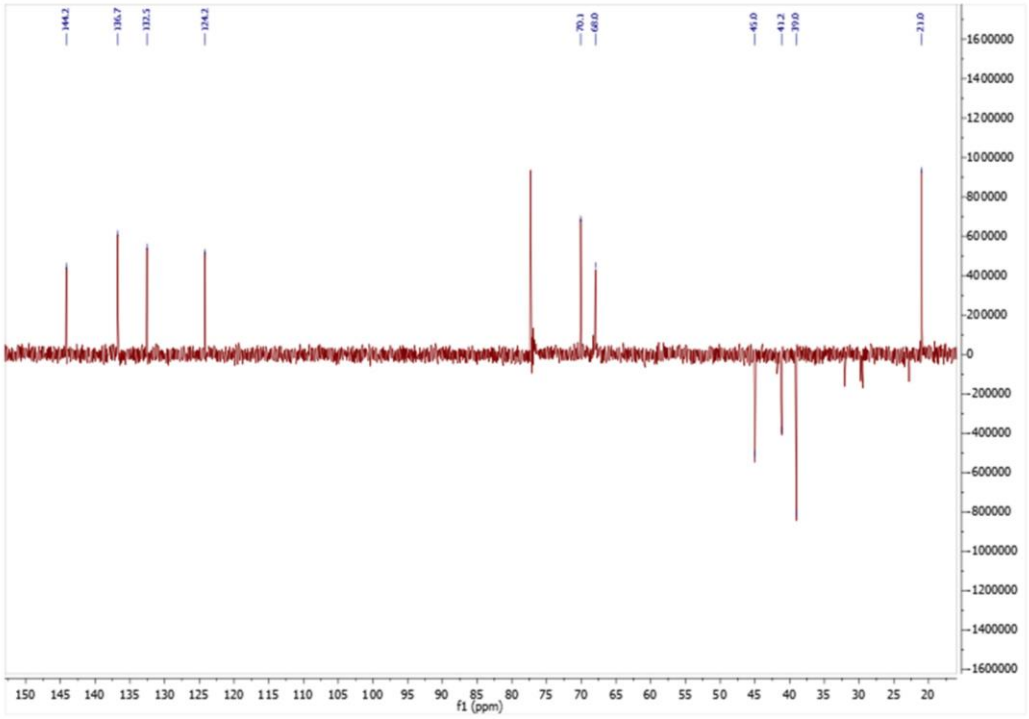



**Figure S10**. HR-ESIMS spectrum of compound **1.**

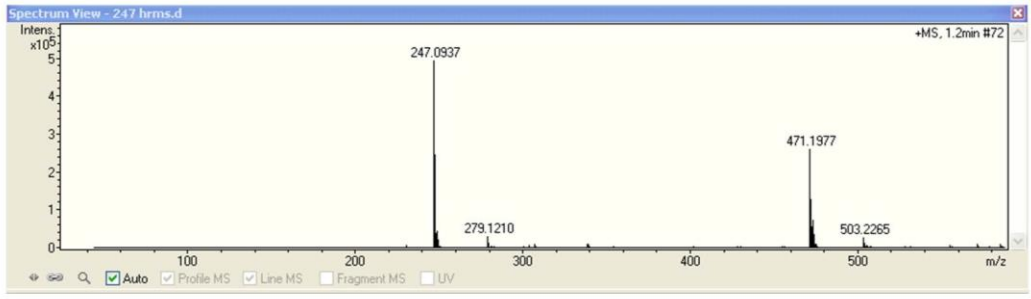


**Figure S11**. $^{1}$H NMR spectrum of compound **2** ($CD_3OD$, 600 MHz).

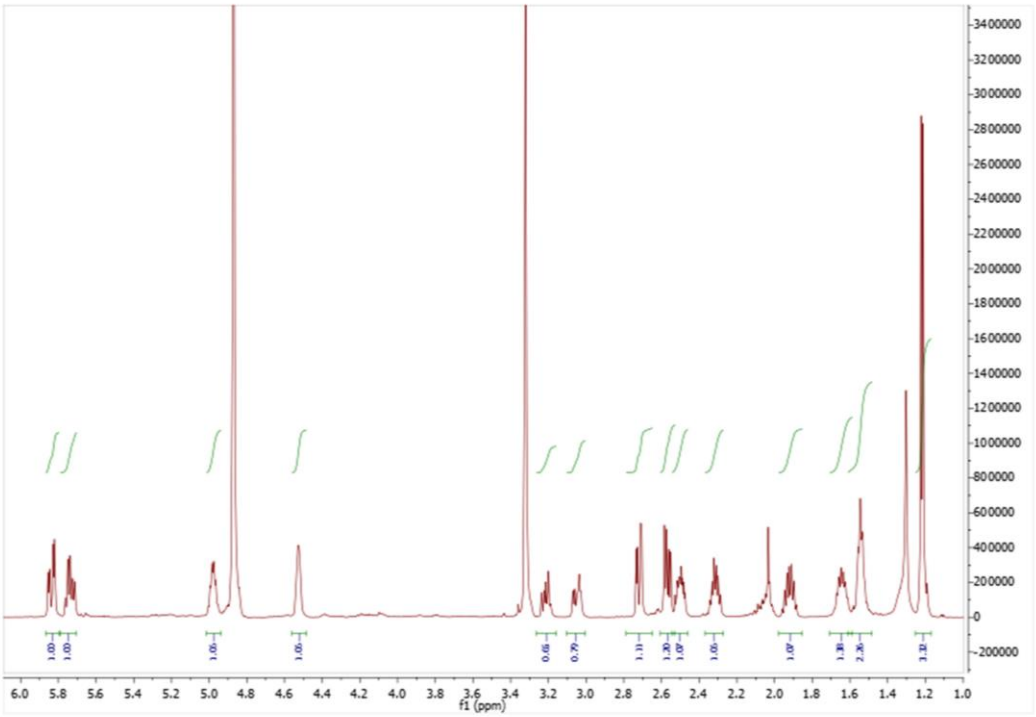


**Figure S12**. $^{13}$C NMR spectrum of compound **2** ($CD_3OD$, 150 MHz).

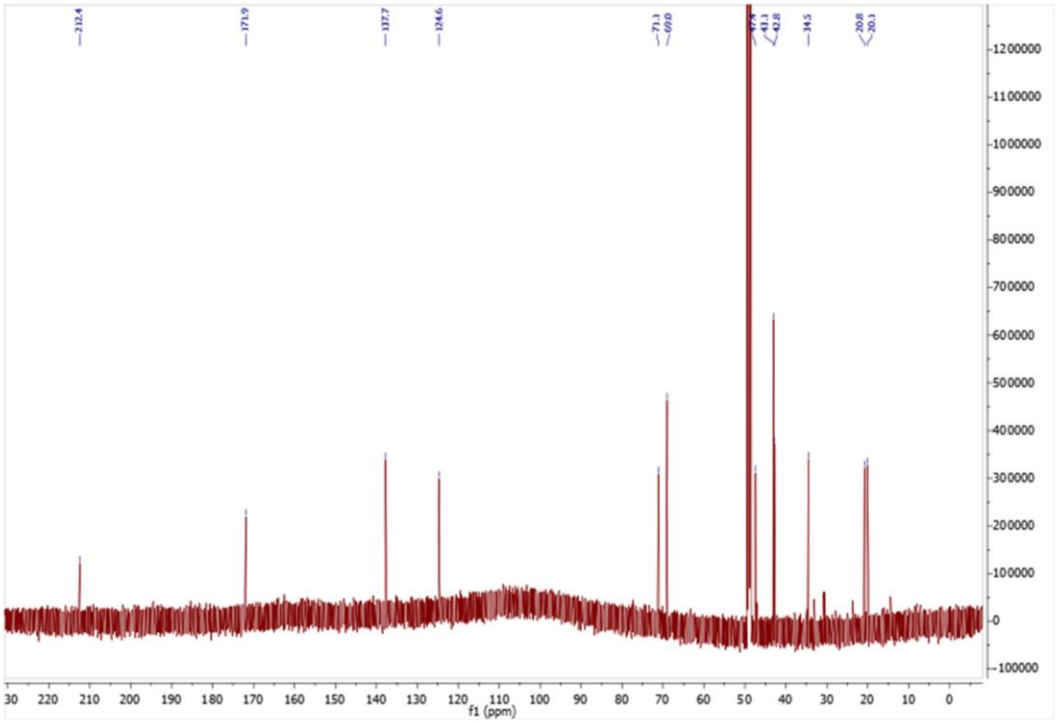


**Figure S13**. COSY spectrum of compound **2** ($CD_3OD$, 600 MHz).

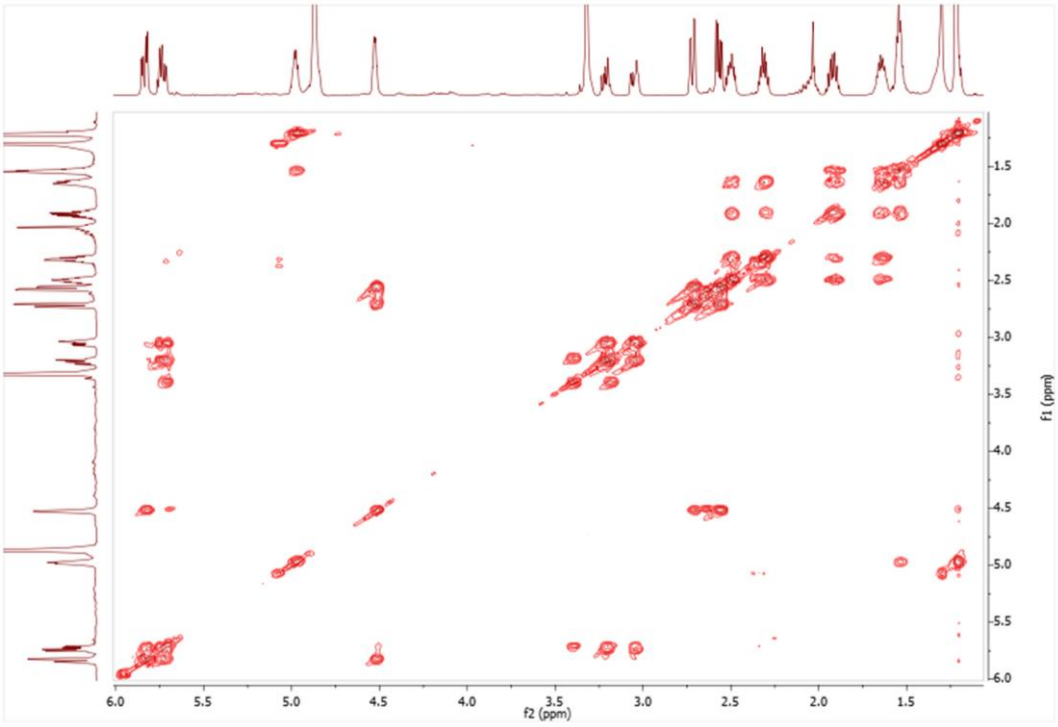



**Figure S14**. HSQC spectrum of compound **2** ($CD_3OD$, 600 MHz).

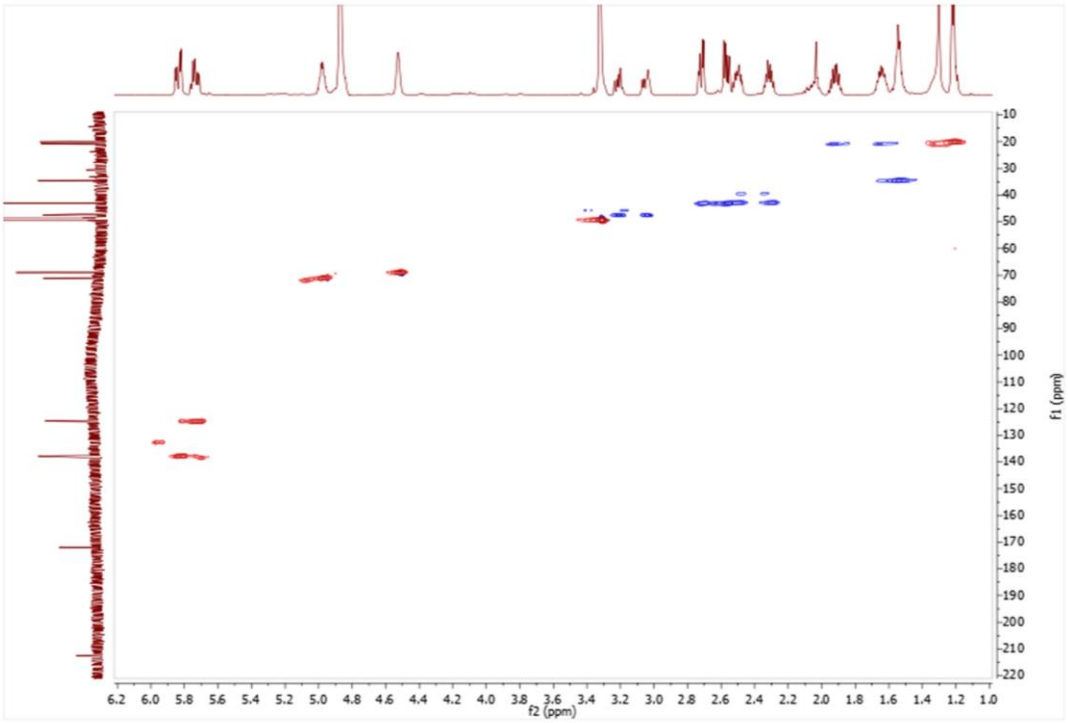


**Figure S15**. HMBC spectrum of compound **2** ($CD_3OD$, 600 MHz).

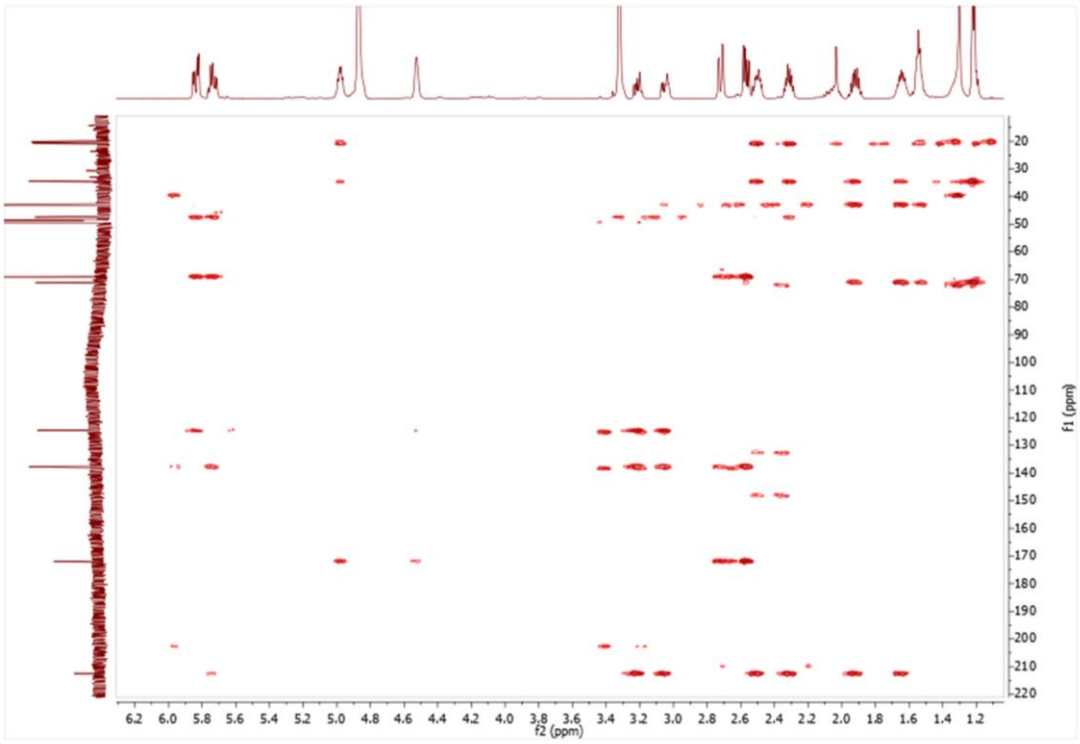



**Figure S16**. NOESY spectrum of compound **2**, ($CD_3OD$, 600 MHz).

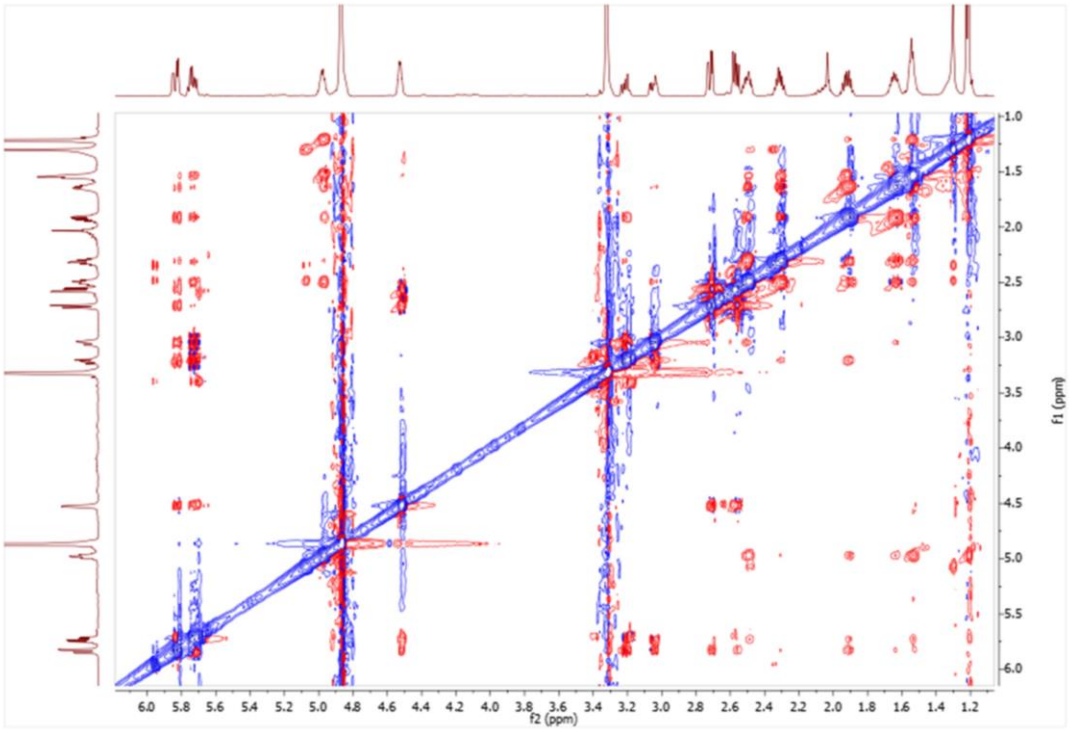


**Figure S17**. $^{1}H$ NMR spectrum of compound **2** ($CDCl_3$, 600 MHz).

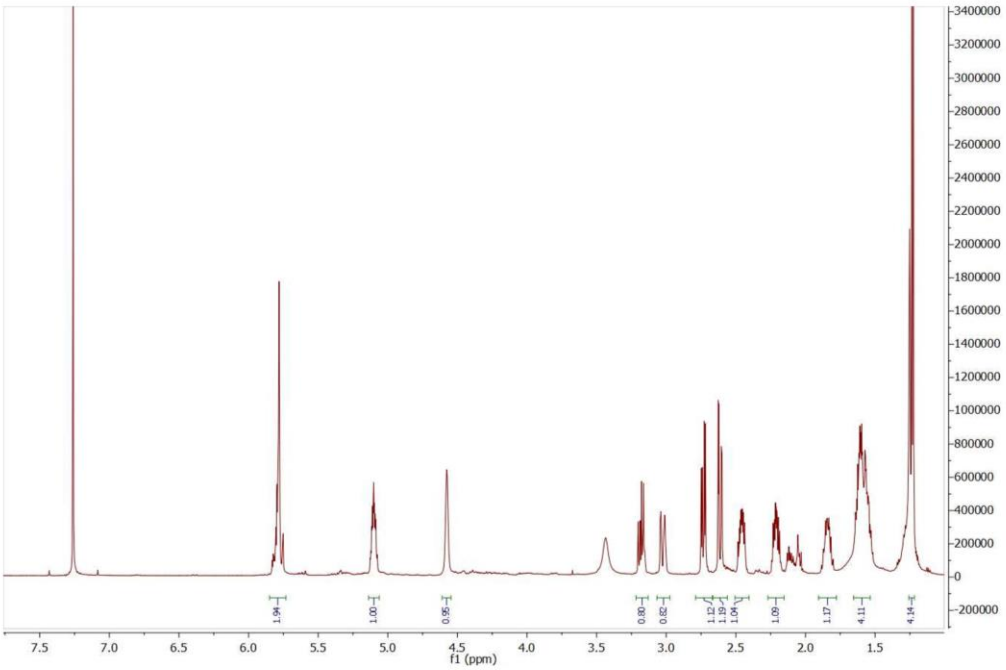



**Figure S18**. HR-ESIMS spectrum of compound **2.**

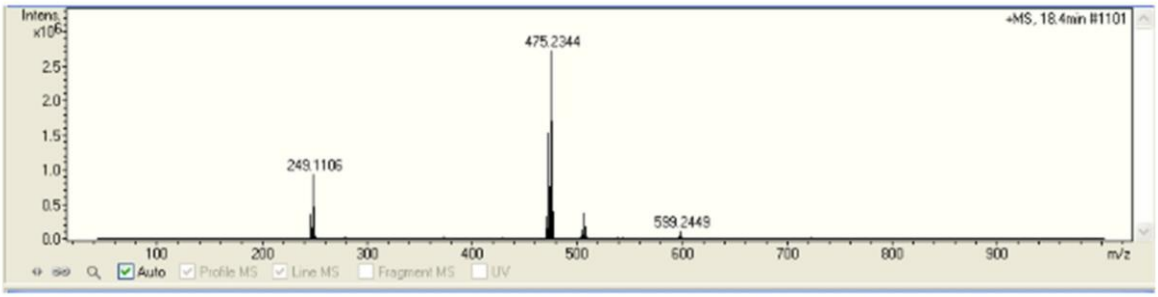



**Figure S19.** FT-IR spectrum of compound **2.**

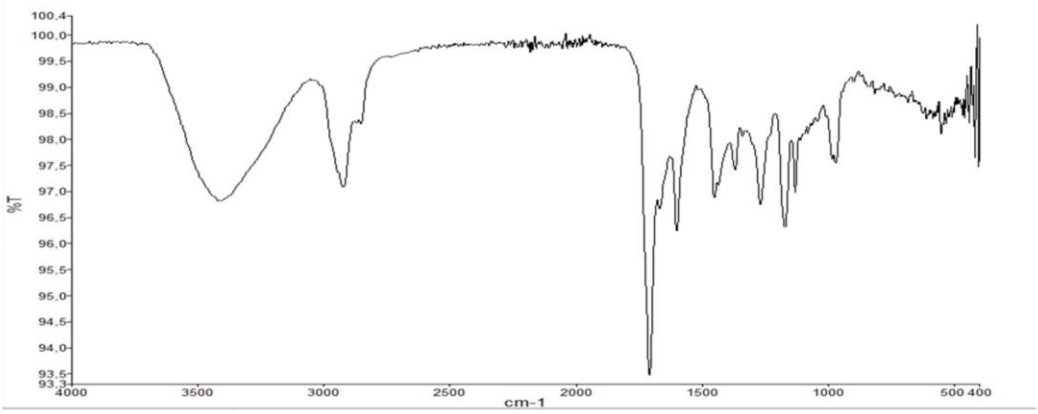


**Figure S20**. $^{1}H$ NMR spectrum of compound **3** ($CDCl_3$, 600 MHz).

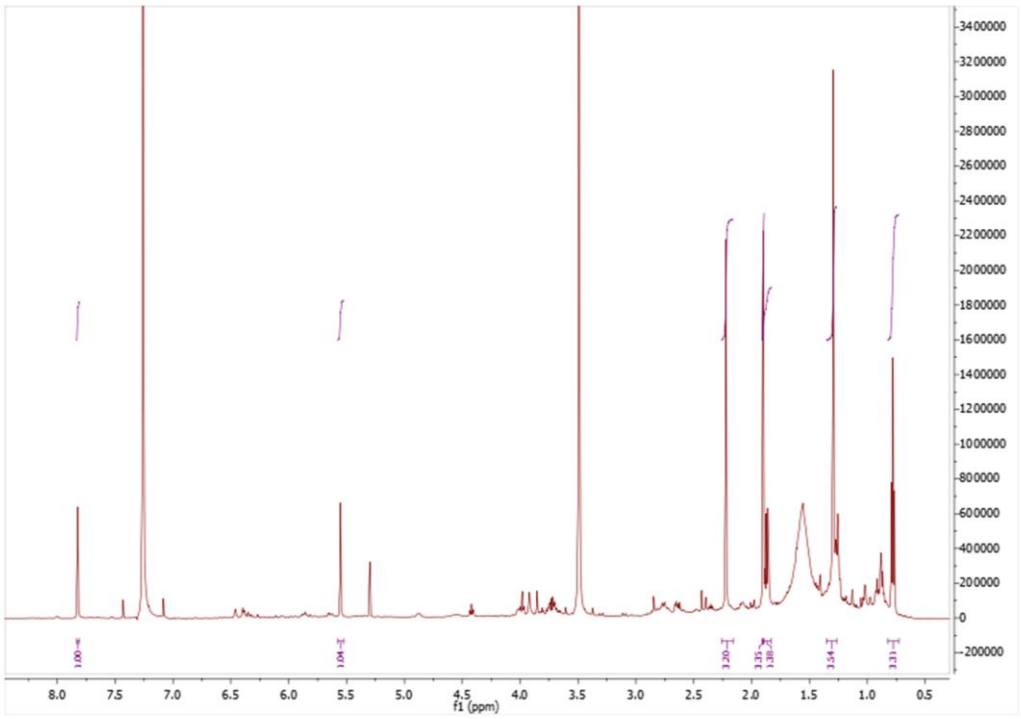



**Figure S21**. COSY spectrum of compound **3** ($CDCl_3$, 600 MHz).

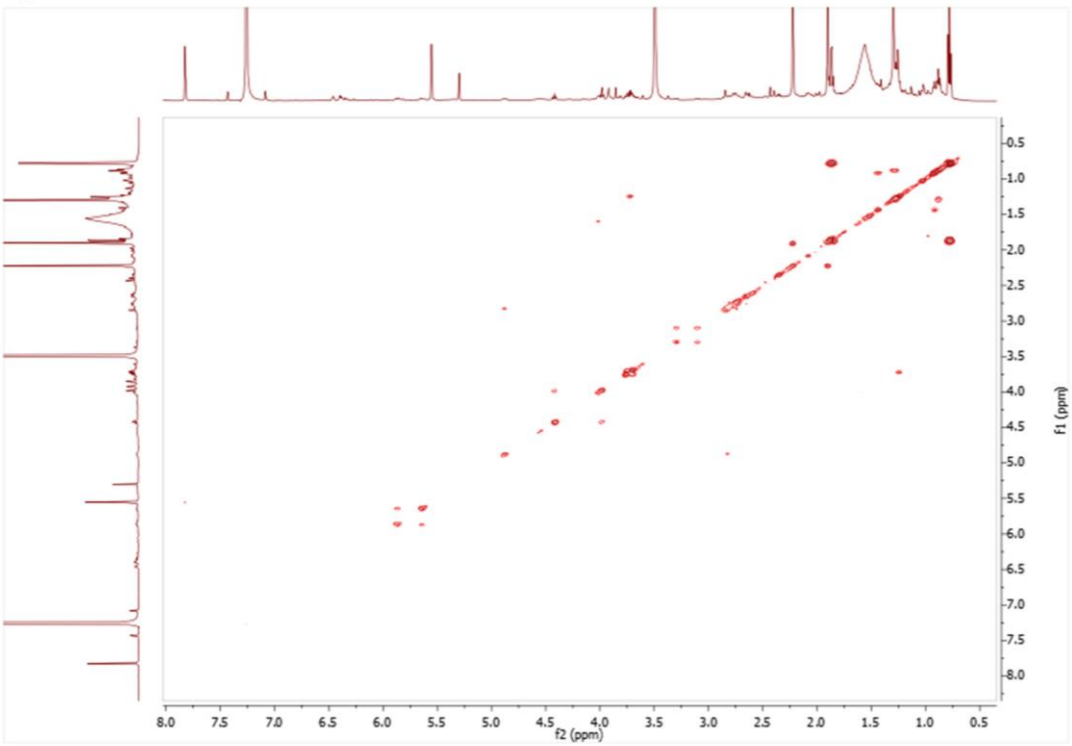


**Figure S22**. HSQC spectrum of compound **3** ($CDCl_3$, 600 MHz).

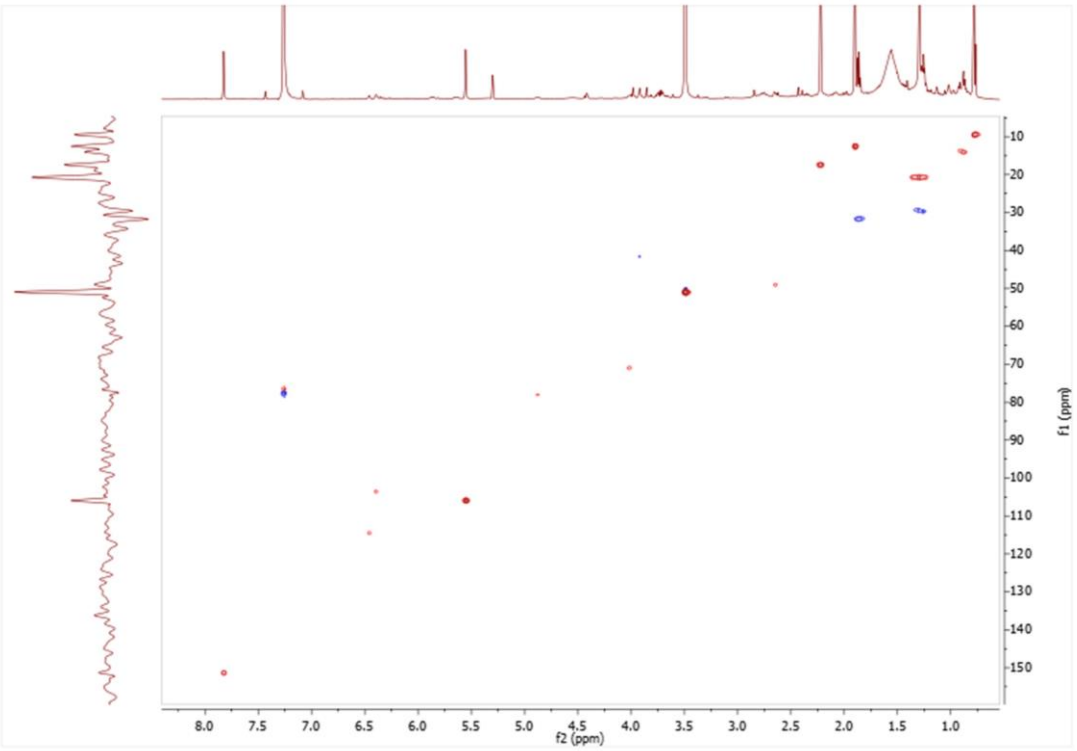



**Figure S23**. HMBC spectrum of compound **3** ($CDCl_3$, 600 MHz).

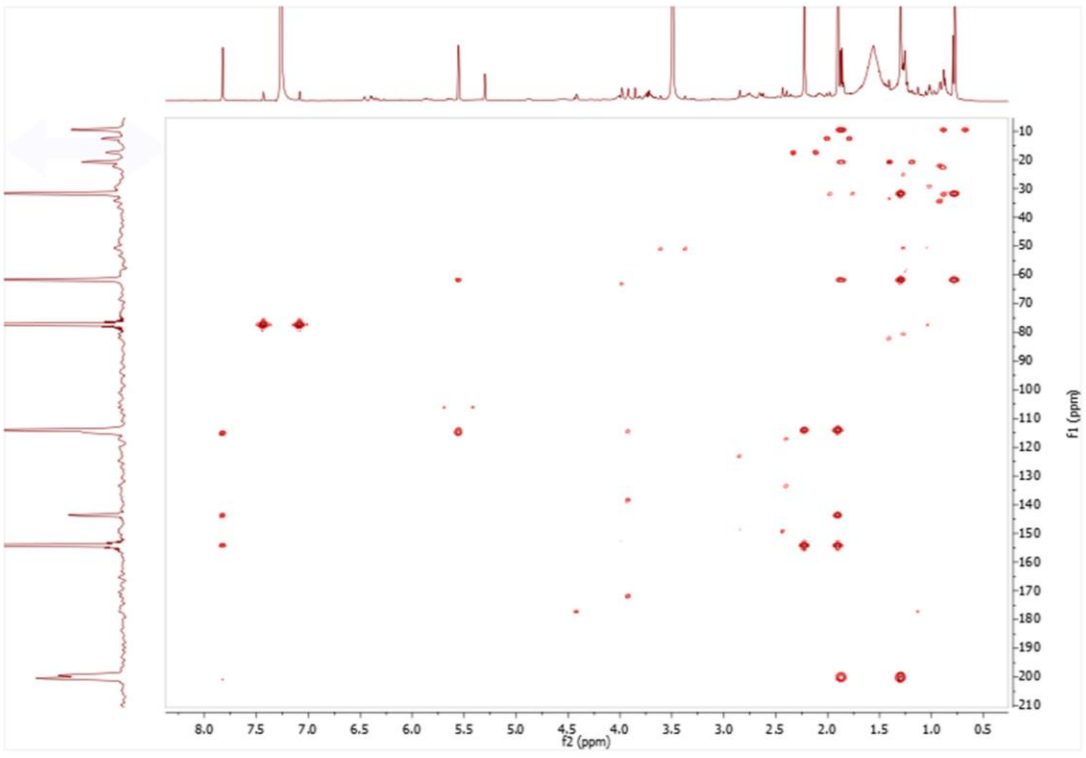


**Figure S24**. NOESY spectrum of compound **3** ($CDCl_3$, 600 MHz).

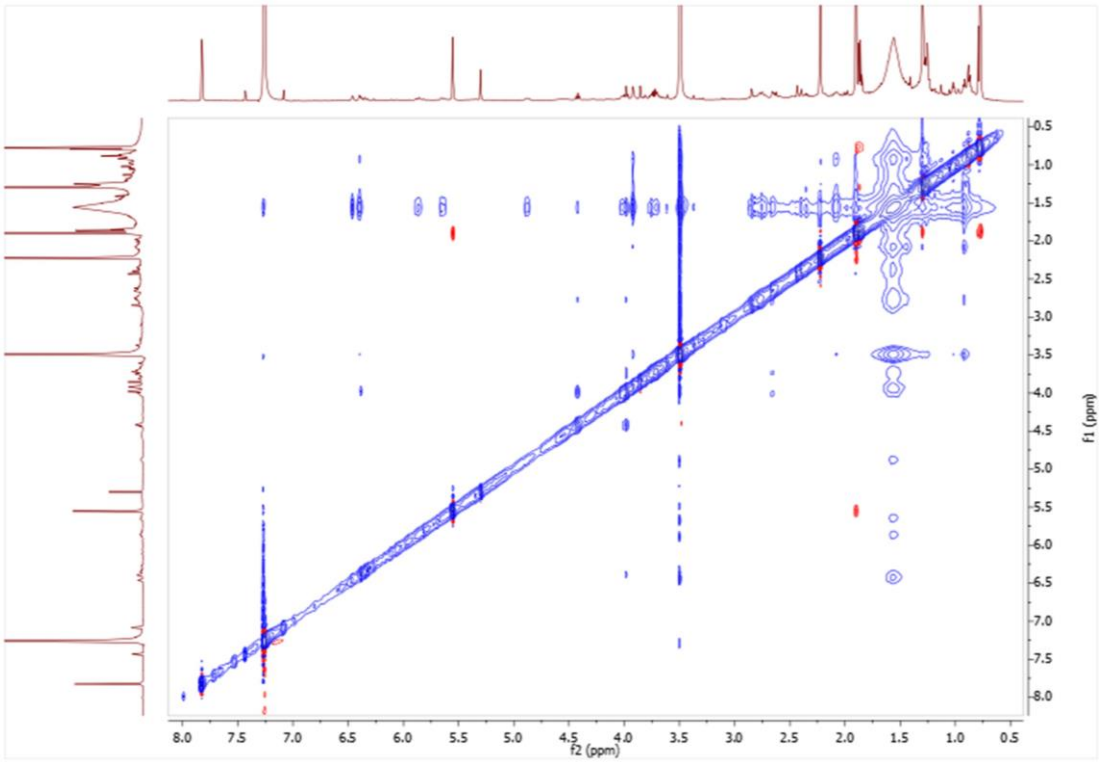



**Figure S25**. HR-ESIMS spectrum of compound **3.**

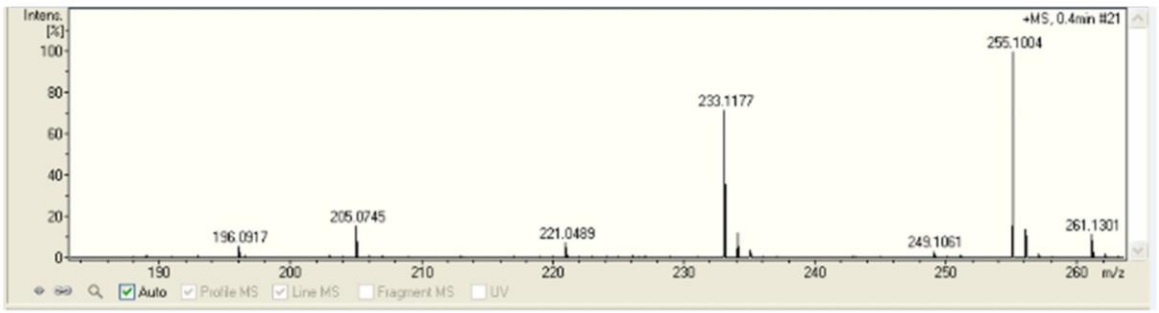


**Figure S26**. $^{1}H$ NMR spectrum of compound **4** ($CD_3OD$, 600 MHz).

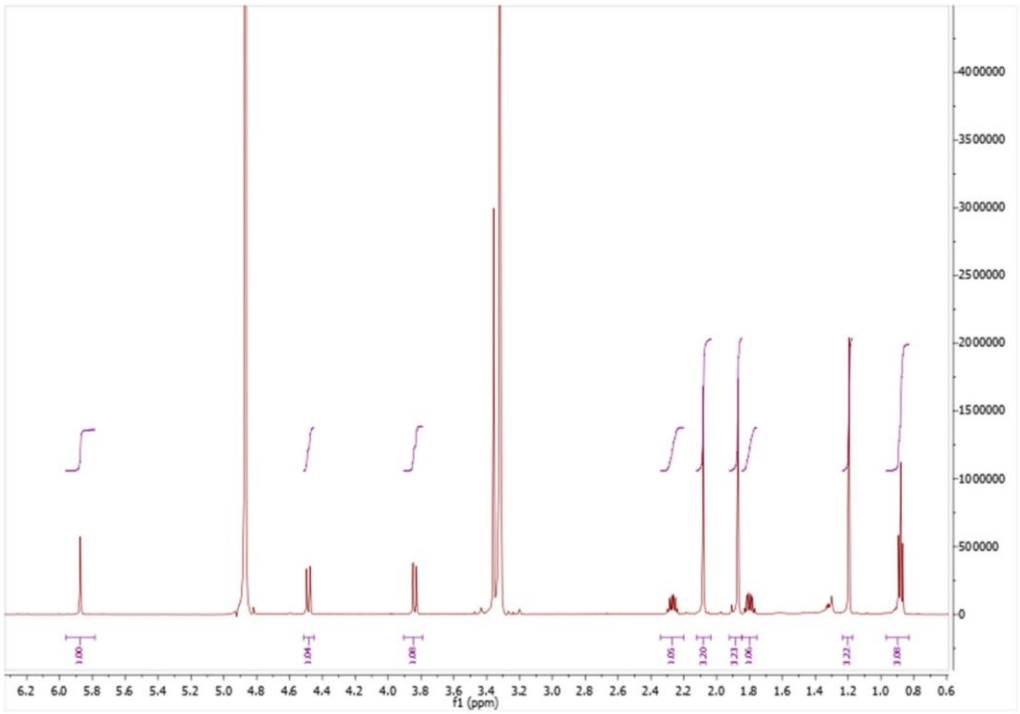



**Figure S27**. $^{13}C$ NMR spectrum of compound **4** ($CD_3OD$, 150 MHz).

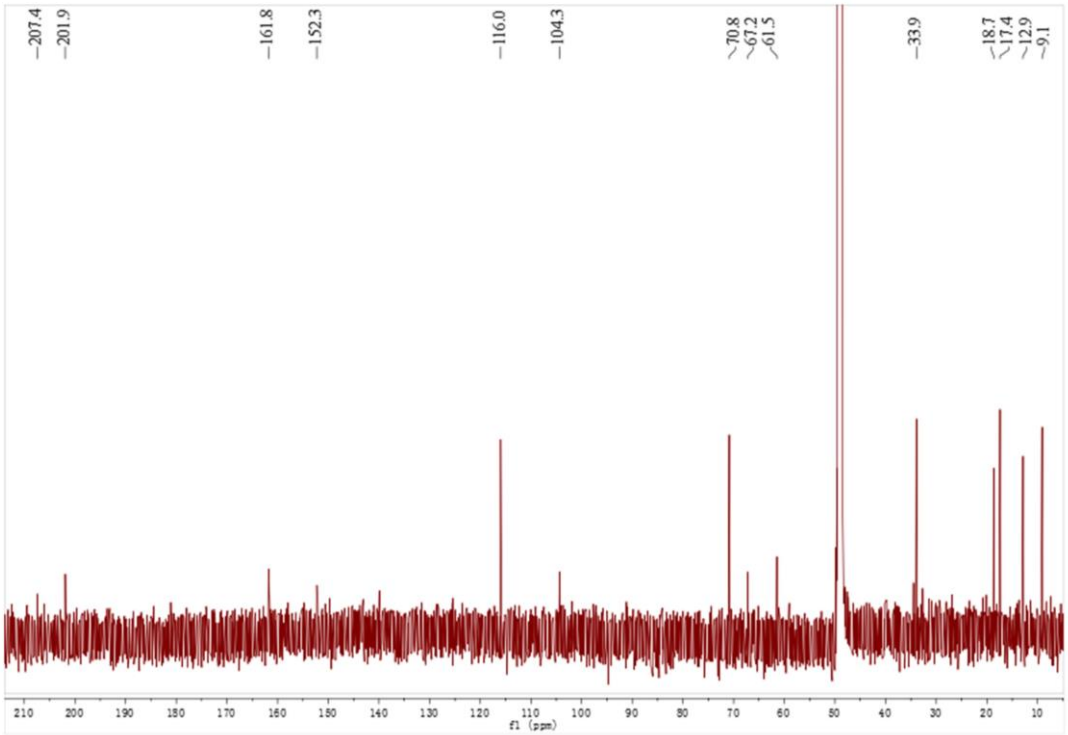


**Figure S28**. COSY spectrum of compound **4** ($CD_3OD$, 600 MHz).

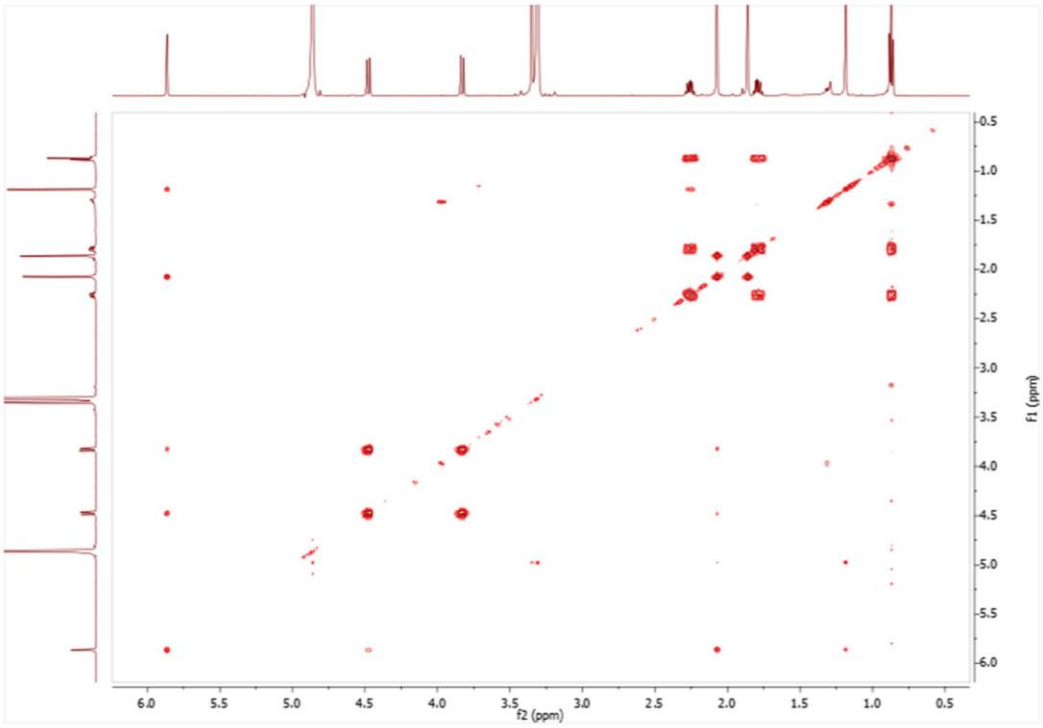



**Figure S29**. HSQC spectrum of compound **4** ($CD_3OD$, 600 MHz).

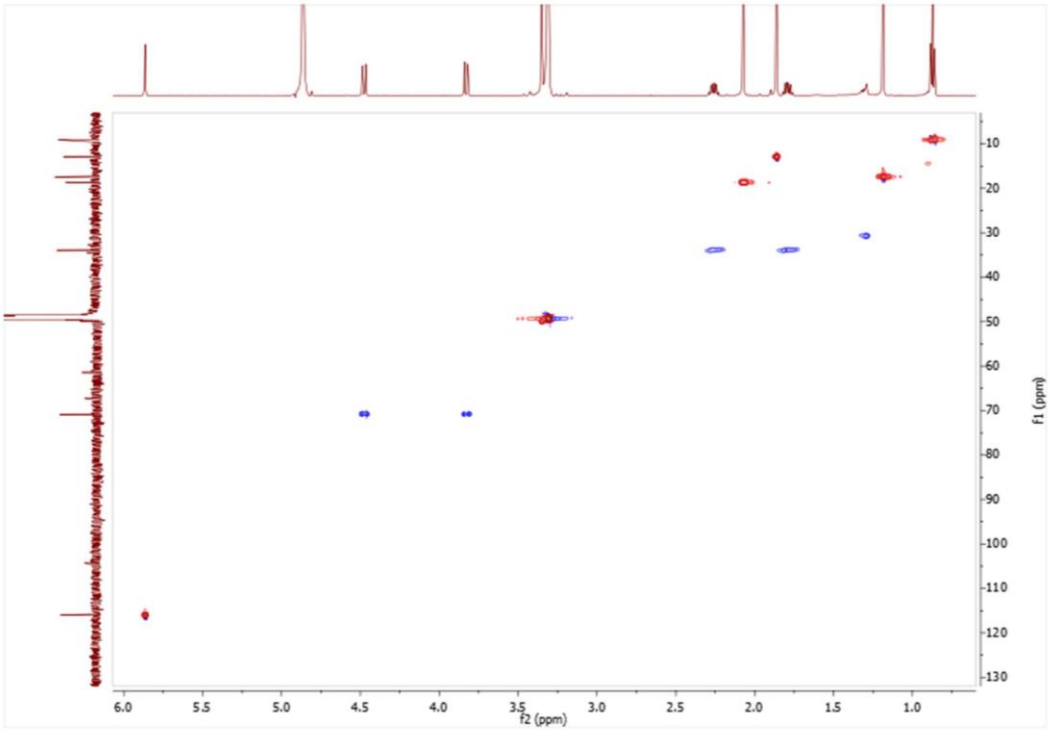


**Figure S30**. HMBC spectrum of compound **4** ($CD_3OD$, 600 MHz).

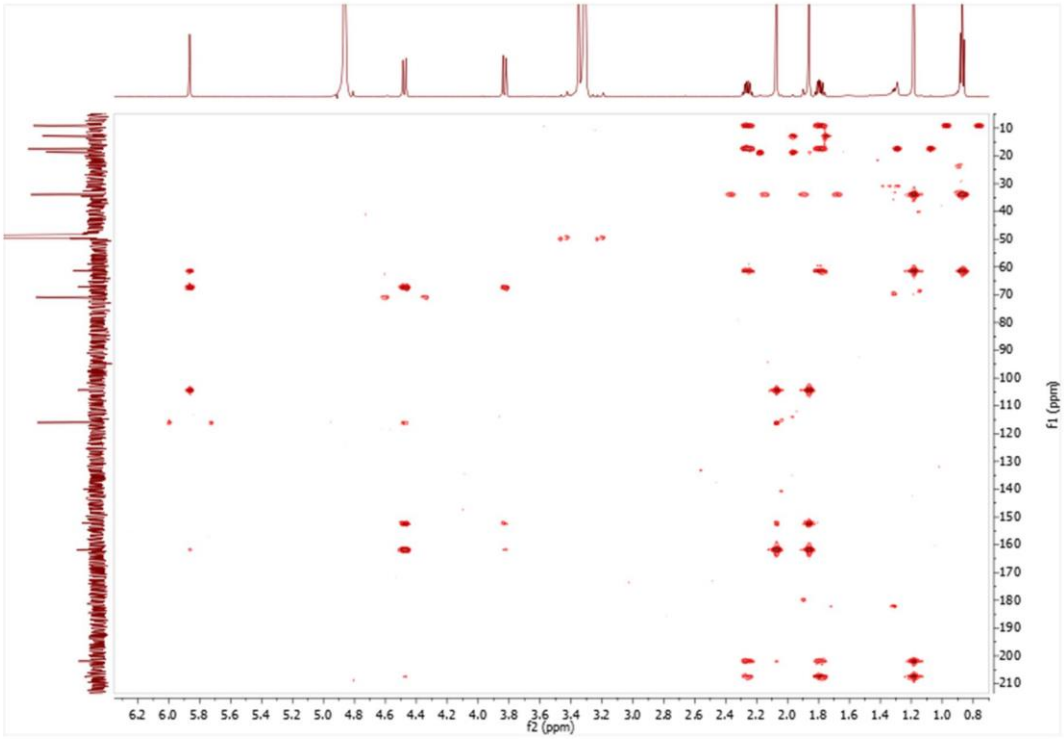



**Figure S31**. NOESY spectrum of compound **4** ($CD_3OD$, 600 MHz).

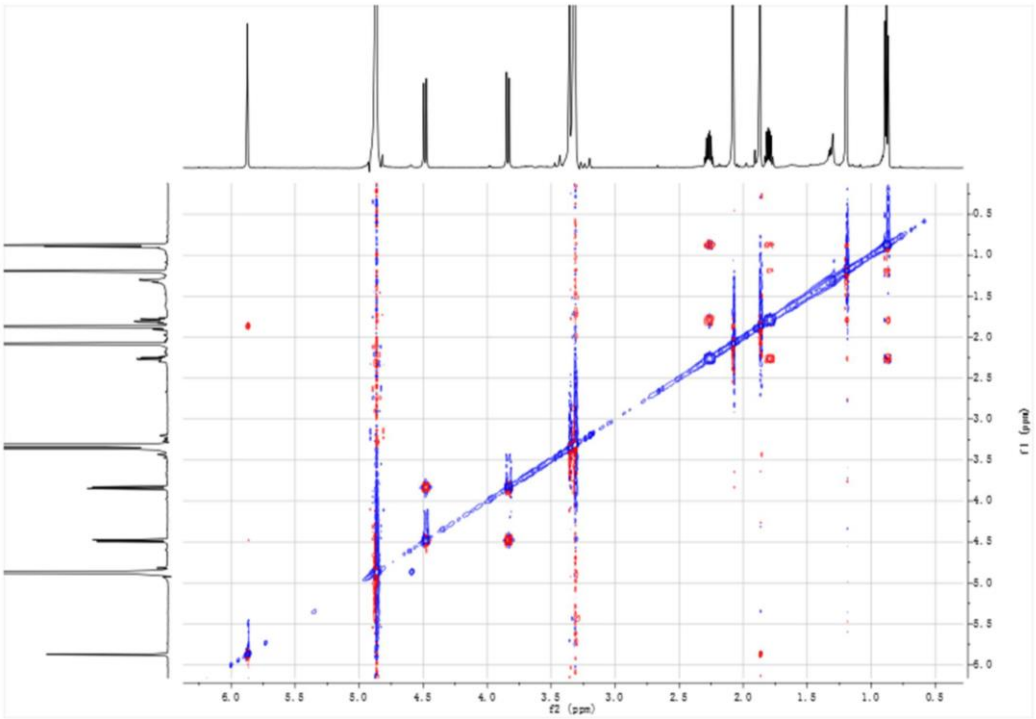


**Figure S32**. $^{1}H$ NMR spectrum of compound **4** ($CDCl_3$, 600 MHz).

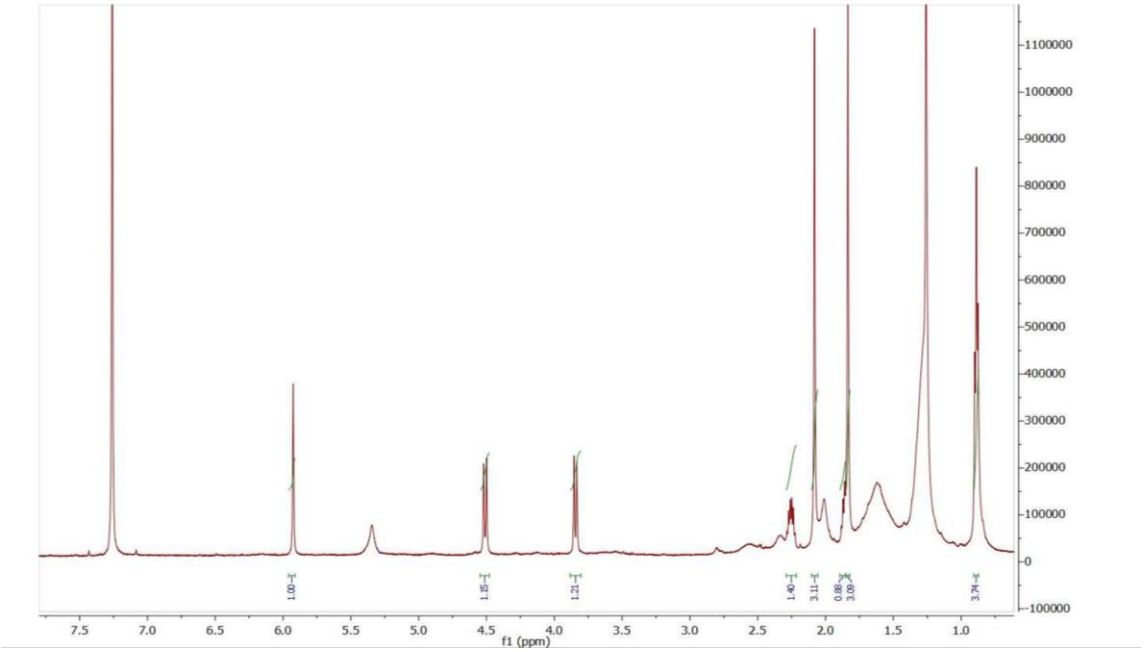



**Figure S33**. HR-ESIMS spectrum of compound **4.**

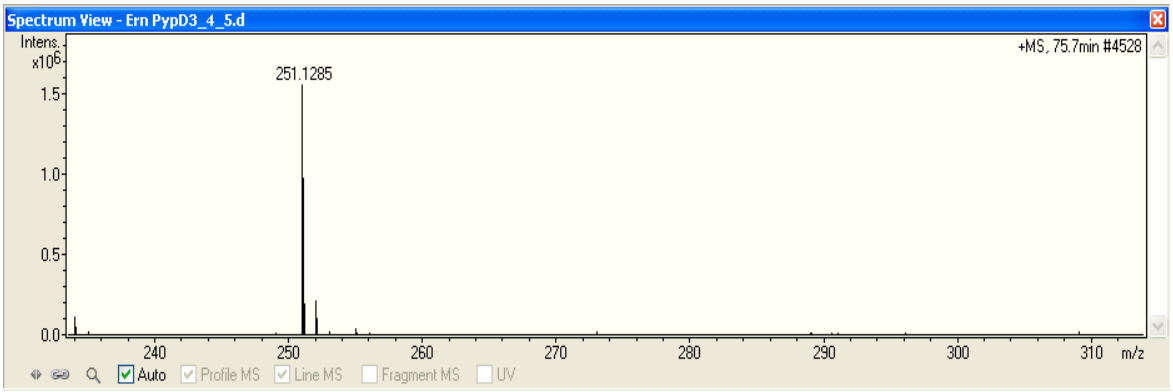



**Figure S34.** FT-IR spectrum of compound **4.**

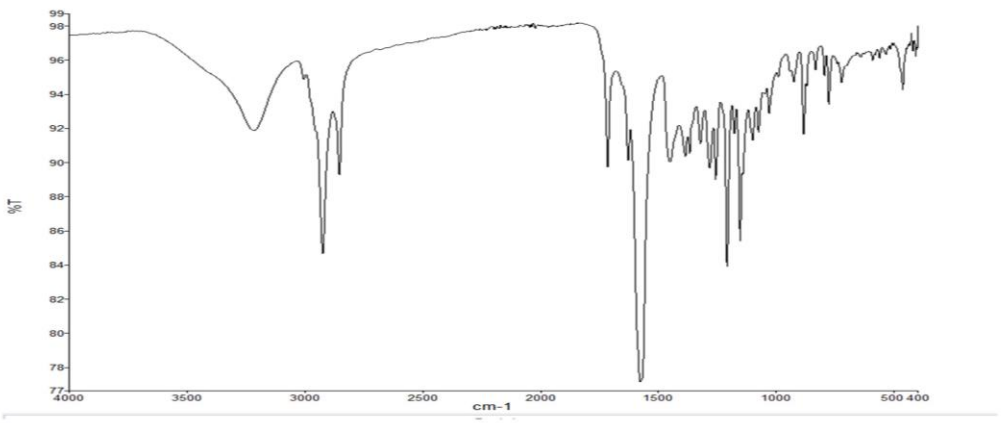


**Figure S35**. $^{1}H$ NMR spectrum of compound **5** ($CDCl_3$, 600 MHz).

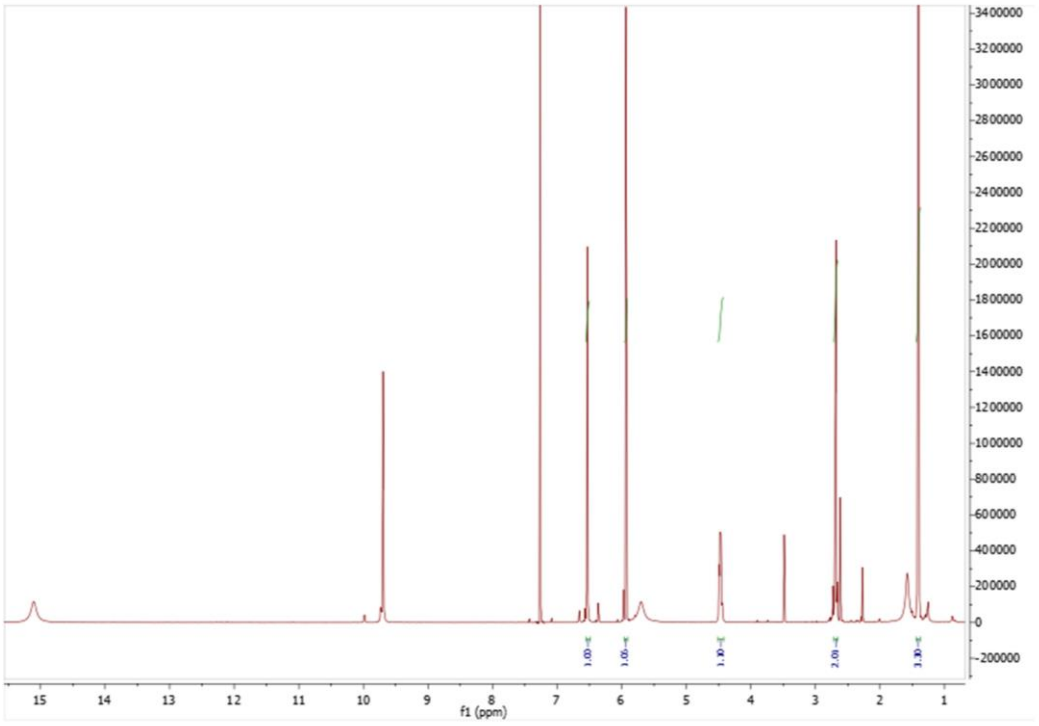



**Figure S36**. $^{13}C$ NMR spectrum of compound **5** ($CDCl_3$, 150 MHz).

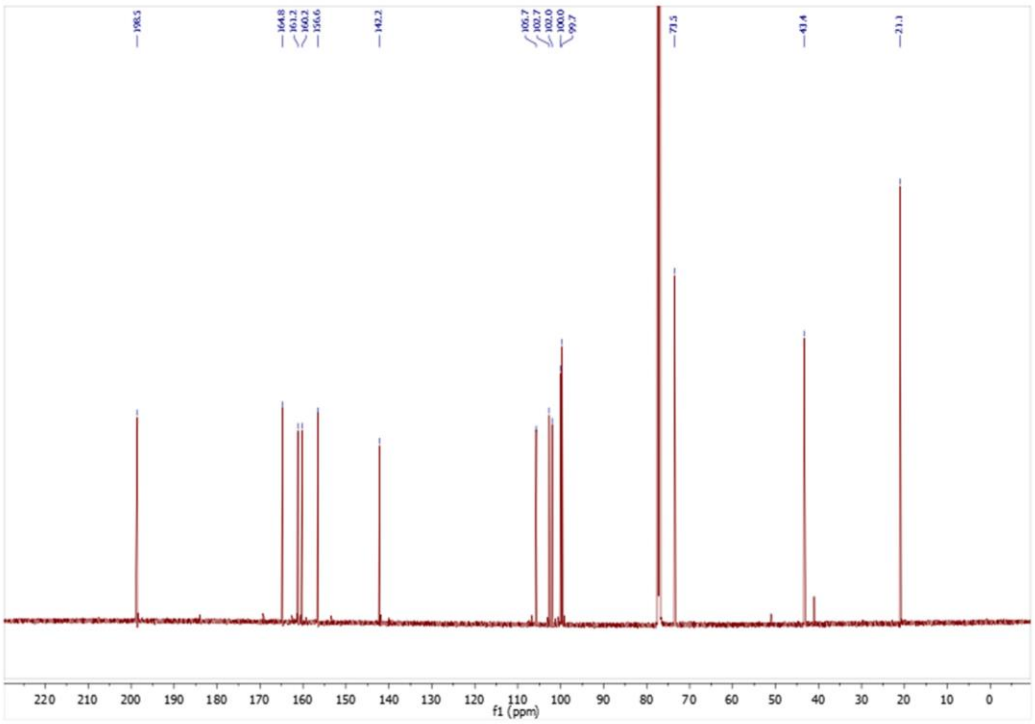


**Figure S37**. DEPT-135 spectrum of compound **5** ($CDCl_3$, 150 MHz).

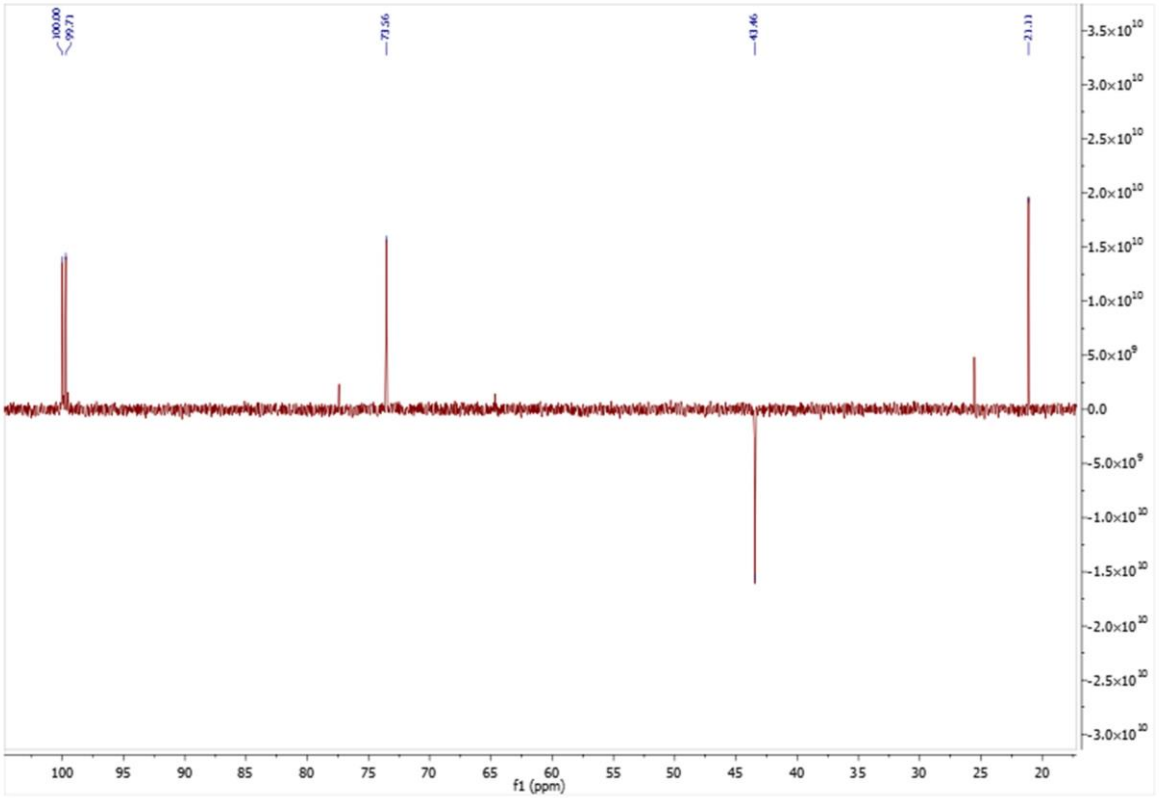



**Figure S38**. HR-ESIMS spectrum of compound **5.**