

Supporting information

Oxalactam A, a novel macrolactam with potent anti-*Rhizoctonia solani* activity from the endophytic fungus *Penicillium oxalicum*

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Fungus identification

The test endophytic fungus was identified as *Penicillium oxalicum* based on its morphological characteristics and 16S rRNA gene sequence by Shanghai Shengong Bioengineering Co., Ltd., the 16S rRNA gene sequence is TTGATATGCTTAAGTTCAGCGGGTATCCCTACCTGATCCGAGGTCAACCTGGTTAAGATTGATGGTGTTCGCCGGCGGGCGCCGGCCGGGCCTACAGAGCGGGTGACGAAGCCCCATACGCTCGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGCCCCCGGAAGCGGGGGGCGAGAGCCCAACACACAAGCCGTGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCGTTGTTGAAAGTTTAACTGATTTAGTCAAGTACTCAGACTGCAATCTTCAGACAAGAGTTCGTTTGTGTGTCTTCGGCGGGCGCGGGCCCCGGGGGCGGATGCCCCCGGCGGGCCGTGAGGCGGGCCCCGCCGAAGCAACAAGGTACGATAAACACGGGTGGGAGGTTGGACCCAGAGGGCCCTCACTCGGTAATGATCCTTCCGCAG (566 bp).

The identified result is as follows:

<u>Description</u>	<u>Max Score</u>	<u>Total Score</u>	<u>Query Cover</u>	<u>E value</u>	<u>Per. Ident</u>	<u>Accession</u>
Penicillium oxalicum strain 6-1F small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1046	1046	100%	0.0	100.00%	MW077100.1
Aspergillus aculeatus strain 3-18F small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1046	1046	100%	0.0	100.00%	MW077088.1
Penicillium oxalicum strain 3-16F small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1046	1046	100%	0.0	100.00%	MW077086.1
Nigrospora sphaerica strain 5-1F small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1046	1046	100%	0.0	100.00%	MW077068.1
Penicillium oxalicum strain 2-5F small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1046	1046	100%	0.0	100.00%	MW077050.1

<u>Description</u>	<u>Max Score</u>	<u>Total Score</u>	<u>Query Cover</u>	<u>E value</u>	<u>Per. Ident</u>	<u>Accession</u>
RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence						
Penicillium oxalicum strain 2-4F small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1046	1046	100%	0.0	100.00%	MW077049.1
Penicillium oxalicum strain 2-3F small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1046	1046	100%	0.0	100.00%	MW077048.1
Penicillium oxalicum strain 2-2F small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1046	1046	100%	0.0	100.00%	MW077047.1
Penicillium sp. isolate SZMC27118 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1046	1046	100%	0.0	100.00%	MT997202.1
Penicillium oxalicum isolate Z1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1046	1046	100%	0.0	100.00%	MT795727.1

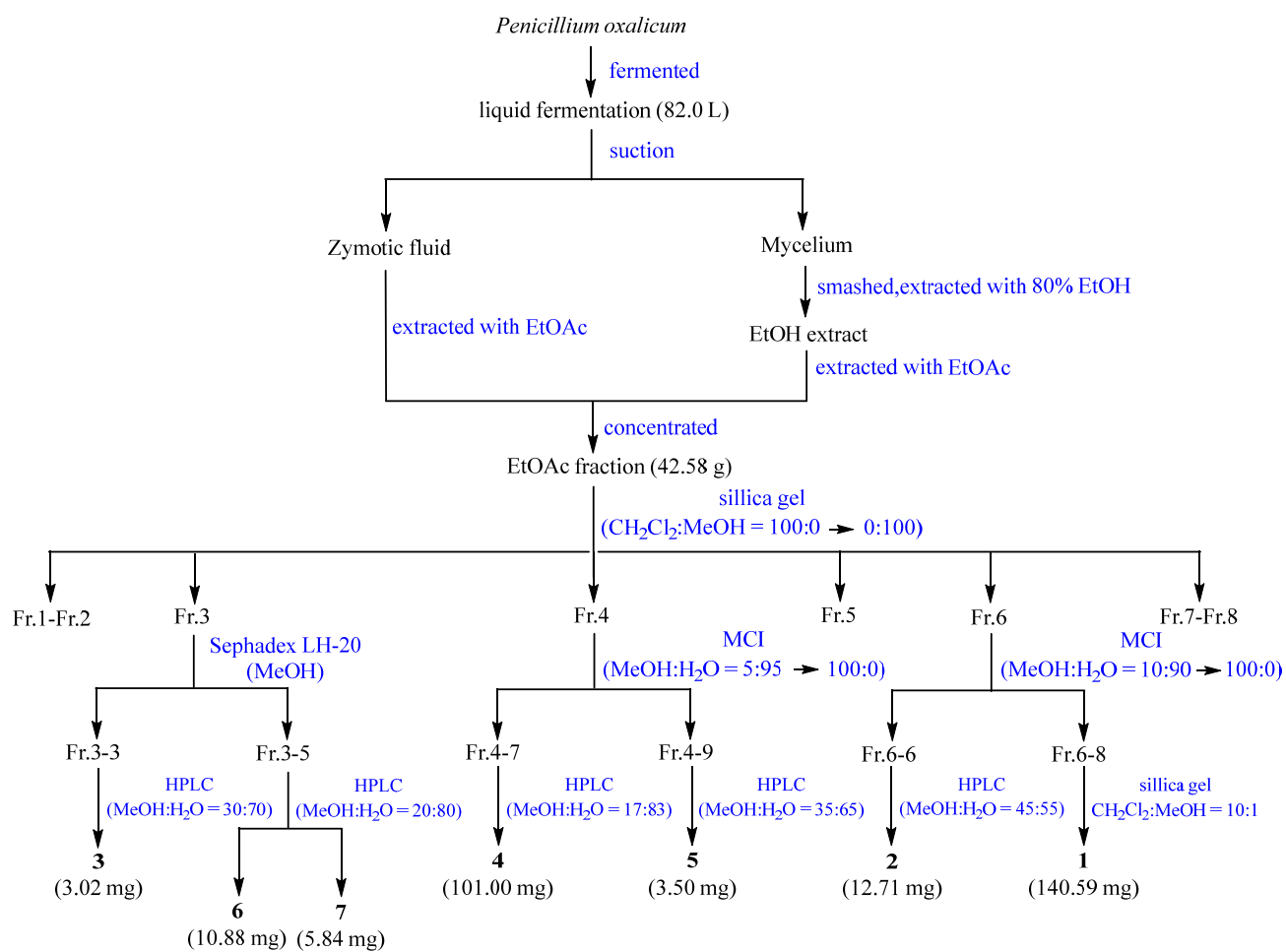


Figure S1. Flow chart of extraction and isolation.

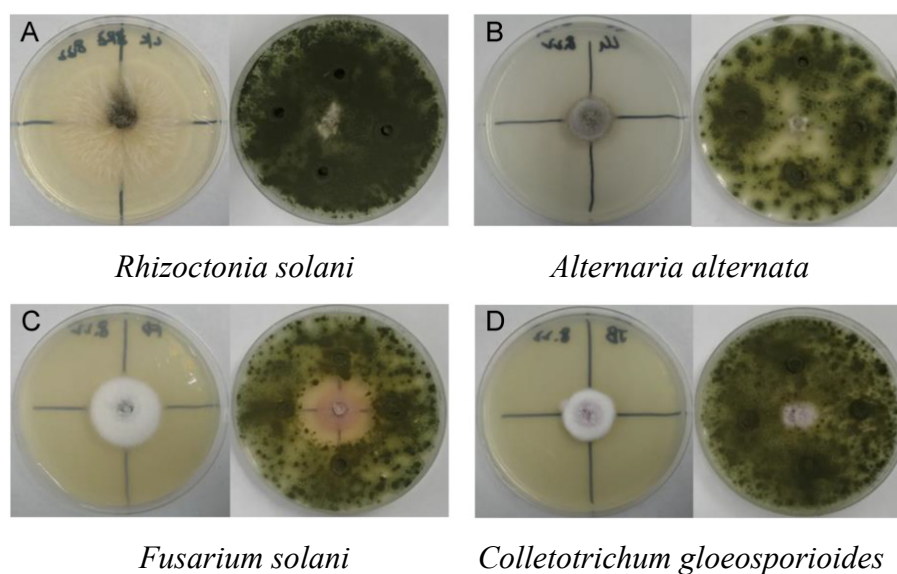


Figure S2. *Penicillium oxalicum* resisted the growth of four plant pathogenic fungi strains (Left of A–D: blank group; right of A–D: *Penicillium oxalicum*-treated group).

Table S1. *Penicillium oxalicum* resisted the growth of four plant pathogenic fungi strains.

Strain	Inhibition rate (%)
<i>Rhizoctonia solani</i>	96.83 ± 1.19
<i>Alternaria alternata</i>	83.33 ± 1.39
<i>Fusarium solani</i>	50.67 ± 4.99
<i>Colletotrichum gloeosporioides</i>	76.04 ± 1.47

Table S2. Cartesian coordinate for the lowest energy conformer of **1** calculated at TD/B3LYP/6-311G(d,p) level of theory in the gas phase.

Center number	Atomic number	Atomic type	Coordinates (Angstroms)		
			X	Y	Z
1	6	0	-0.9139	-2.73884	1.114038
2	6	0	-0.77555	-3.23078	-0.3526
3	6	0	-2.11285	-3.11669	-1.04262
4	6	0	-2.31535	-2.4007	-2.15141
5	7	0	-0.8874	-1.37729	1.215678
6	6	0	-3.65999	-2.12229	-2.77092
7	6	0	-4.21833	-0.74158	-2.34803
8	6	0	-3.31855	0.453999	-2.73833
9	6	0	-3.69691	1.739884	-2.02721
10	6	0	-2.93638	2.181279	-1.01094
11	6	0	-3.18887	3.343385	-0.08781
12	6	0	-3.77944	2.894613	1.28091
13	6	0	-2.84101	2.09065	2.149921
14	6	0	-2.75052	0.75579	2.146786
15	6	0	-1.89467	-0.06965	3.083638
16	6	0	-0.60933	-0.67227	2.457201
17	6	0	-4.96241	2.408002	-2.50874
18	8	0	-1.45992	0.659509	4.230495
19	6	0	0.494268	0.362572	2.222807
20	8	0	1.707668	-0.33157	1.89877
21	8	0	-1.04788	-3.50217	2.059975
22	8	0	0.181257	-2.46234	-1.06504
23	6	0	2.275283	-0.05938	0.673944
24	6	0	3.321475	-1.1331	0.364571
25	6	0	4.059023	-0.76686	-0.91759
26	6	0	4.665464	0.629018	-0.84227
27	6	0	3.561163	1.632114	-0.45616
28	8	0	2.924099	1.216681	0.749118
29	6	0	4.100948	3.029156	-0.15935
30	8	0	4.808709	3.4854	-1.31768
31	8	0	5.231616	0.885829	-2.11783
32	8	0	5.041047	-1.77349	-1.13176
33	8	0	2.692882	-2.40729	0.214725
34	1	0	-0.47408	-4.28637	-0.27297
35	1	0	-2.93681	-3.62479	-0.54097
36	1	0	-1.45001	-1.92763	-2.60885
37	1	0	-0.69813	-0.89634	0.344619
38	1	0	-4.38136	-2.89318	-2.47087
39	1	0	-3.58606	-2.16537	-3.86702
40	1	0	-5.22054	-0.61337	-2.7782

41	1	0	-4.33944	-0.74486	-1.25705
42	1	0	-2.27954	0.21174	-2.49121
43	1	0	-3.35969	0.59176	-3.82968
44	1	0	-2.05161	1.592933	-0.76584
45	1	0	-3.88452	4.061655	-0.53547
46	1	0	-2.25075	3.888168	0.088837
47	1	0	-4.09289	3.791592	1.831846
48	1	0	-4.68659	2.312254	1.079748
49	1	0	-2.20146	2.652382	2.832022
50	1	0	-3.37169	0.177624	1.463398
51	1	0	-2.49892	-0.93793	3.393617
52	1	0	-0.23054	-1.4136	3.167767
53	1	0	-5.17942	3.344843	-1.9897
54	1	0	-4.89718	2.626188	-3.58372
55	1	0	-5.83163	1.749251	-2.37924
56	1	0	-2.25365	1.01677	4.658835
57	1	0	0.222016	1.058687	1.41852
58	1	0	0.662766	0.943832	3.130693
59	1	0	1.050535	-2.55034	-0.61807
60	1	0	1.519179	-0.02418	-0.13162
61	1	0	4.025355	-1.1642	1.206856
62	1	0	3.333585	-0.76918	-1.74859
63	1	0	5.437531	0.639082	-0.05428
64	1	0	2.837095	1.688527	-1.28614
65	1	0	4.763298	2.969776	0.715877
66	1	0	3.26582	3.69892	0.085292
67	1	0	5.246876	4.321451	-1.10086
68	1	0	5.453743	1.835423	-2.13529
69	1	0	5.528399	-1.51461	-1.93159
70	1	0	3.364891	-2.98041	-0.19571

Table S3. Experimental and calculated chemical shifts of **1** for DP4+ probability statistical analysis.

Functional	Solvent	Basis Set				Type of Data			
mPW1PW91	Methanol	6-311G(d,p)				Scaled Shifts			
DP4+		3R*,15S*,16S*-1		3S*,15S*,16S*-1		3S*,15R*,16S*-1		3R*,15R*,16S*-1	
Bayes's theorem probability		97.08%		0%		2.92%		0%	
Nuclei	$\delta_{\text{experimental}}$	$\delta_{\text{calculated}}$	$\delta_{\text{corrected}}$	$\delta_{\text{calculated}}$	$\delta_{\text{corrected}}$	$\delta_{\text{calculated}}$	$\delta_{\text{corrected}}$	$\delta_{\text{calculated}}$	$\delta_{\text{corrected}}$
C-2	175.6	180.8	173.3	176.1	170.9	180.3	171.8	182.3	174.3
C-3	74.2	77.1	73.3	75.6	72.9	78.7	74.0	76.9	72.2
C-4	129.2	134.8	129.0	133.1	128.9	138.2	131.2	132.9	126.4
C-5	134.8	143.4	137.3	145.4	140.9	145.1	137.9	148.0	141.1
C-6	33.6	37.0	34.6	33.7	32.1	33.5	30.4	35.7	32.3
C-7	29.3	33.7	31.5	33.3	31.7	32.2	29.2	35.2	31.8
C-8	41.0	41.2	38.7	37.1	35.4	38.9	35.6	38.2	34.7
C-9	136.9	148.4	142.1	146.4	142.0	147.5	140.2	149.8	142.9
C-10	125.0	132.5	126.7	133.1	128.9	131.1	124.4	132.8	126.3
C-11	28.8	29.5	27.4	27.0	25.6	31.9	28.9	33.1	29.8
C-12	33.9	39.0	36.5	34.1	32.5	36.6	33.4	37.4	33.9
C-13	134.7	138.4	132.4	138.0	133.7	143.2	136.1	138.9	132.3
C-14	131.2	137.3	131.3	133.8	129.6	138.9	131.9	138.8	132.2
C-15	73.0	75.2	71.5	74.6	72.0	76.4	71.7	76.6	71.9
C-16	54.7	58.1	55.0	57.1	55.0	57.6	53.6	60.8	56.6
C-17	69.8	71.7	68.1	71.5	69.0	74.8	70.2	74.8	70.2
C-18	16.3	16.9	15.2	26.1	24.7	26.8	24.0	27.5	24.3
C-1'	104.9	107.1	102.3	105.0	101.5	108.6	102.7	110.0	104.2
C-2'	75.1	79.5	75.6	79.6	76.8	79.3	74.5	77.5	72.8
C-3'	78.1	80.1	76.2	81.2	78.4	81.9	77.0	79.2	74.4
C-4'	71.7	74.5	70.8	71.7	69.1	76.3	71.6	75.6	71.0
C-5'	78.0	79.9	76.0	80.7	77.9	79.7	74.9	78.9	74.1
C-6'	62.8	71.4	67.8	65.4	63.0	71.7	67.2	67.3	62.9

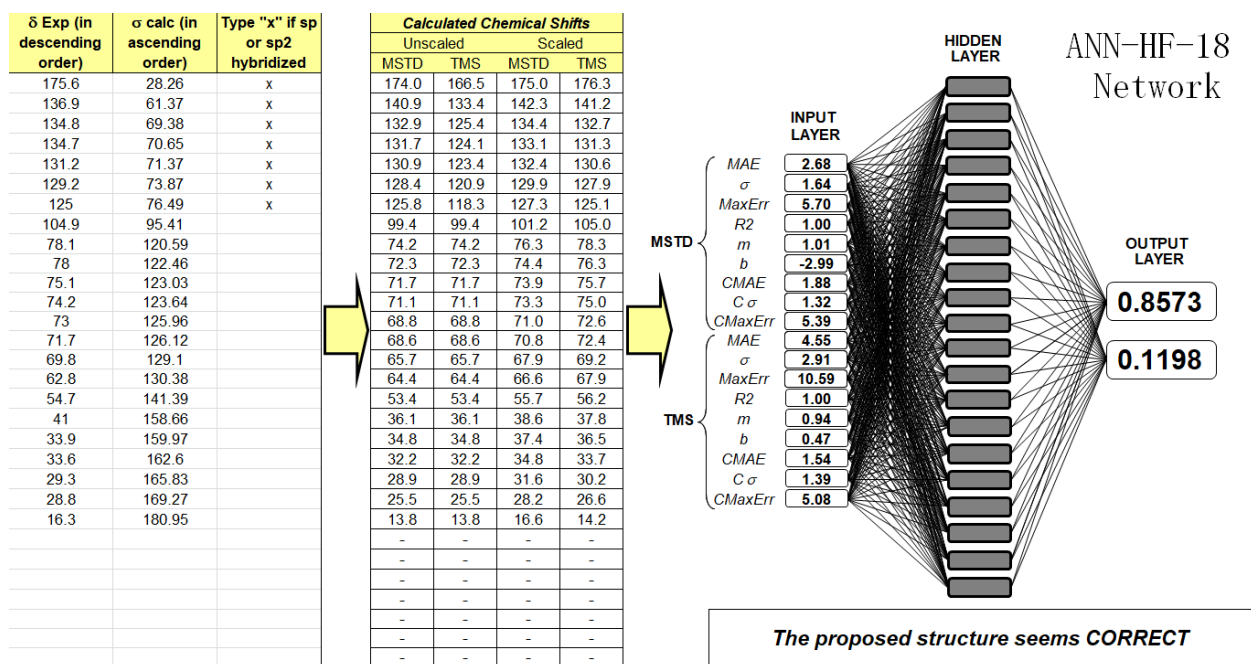


Figure S3. The result of ANNs analysis for (3R*,15S*,16S*)-1, and corresponding 18 parameters.

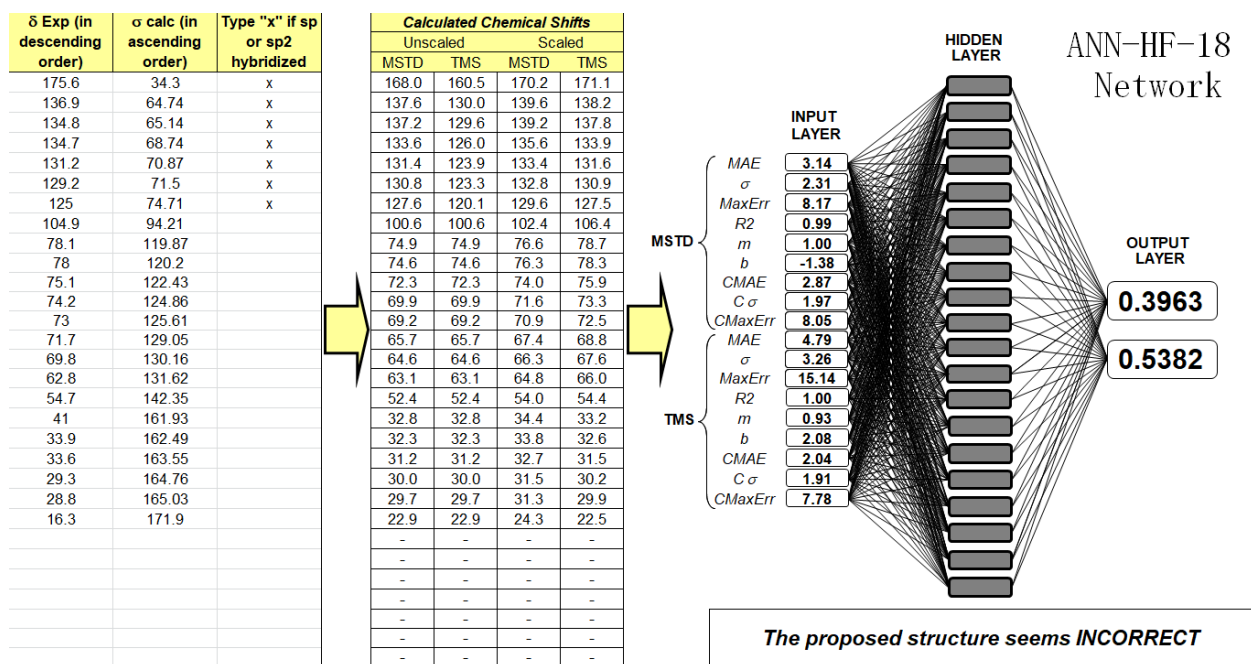


Figure S4. The result of ANNs analysis for (3R*,15R*,16S*)-1, and corresponding 18 parameters.

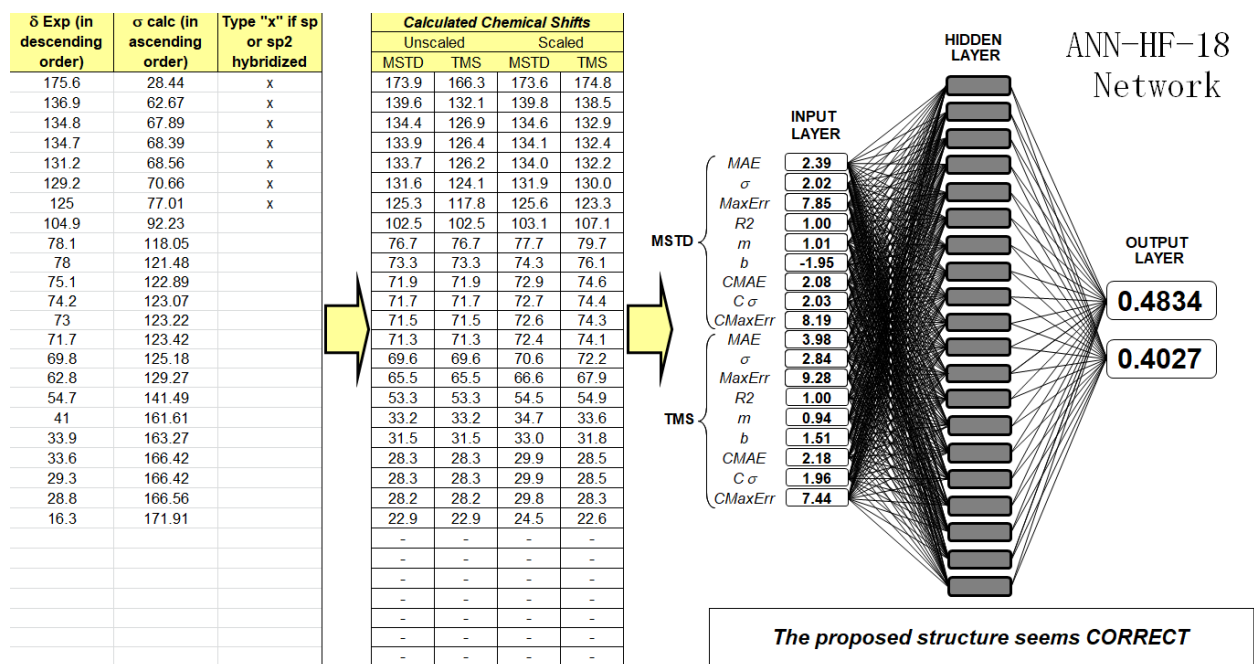


Figure S5. The result of ANNs analysis for (3S*,15S*,16S*)-1, and corresponding 18 parameters.

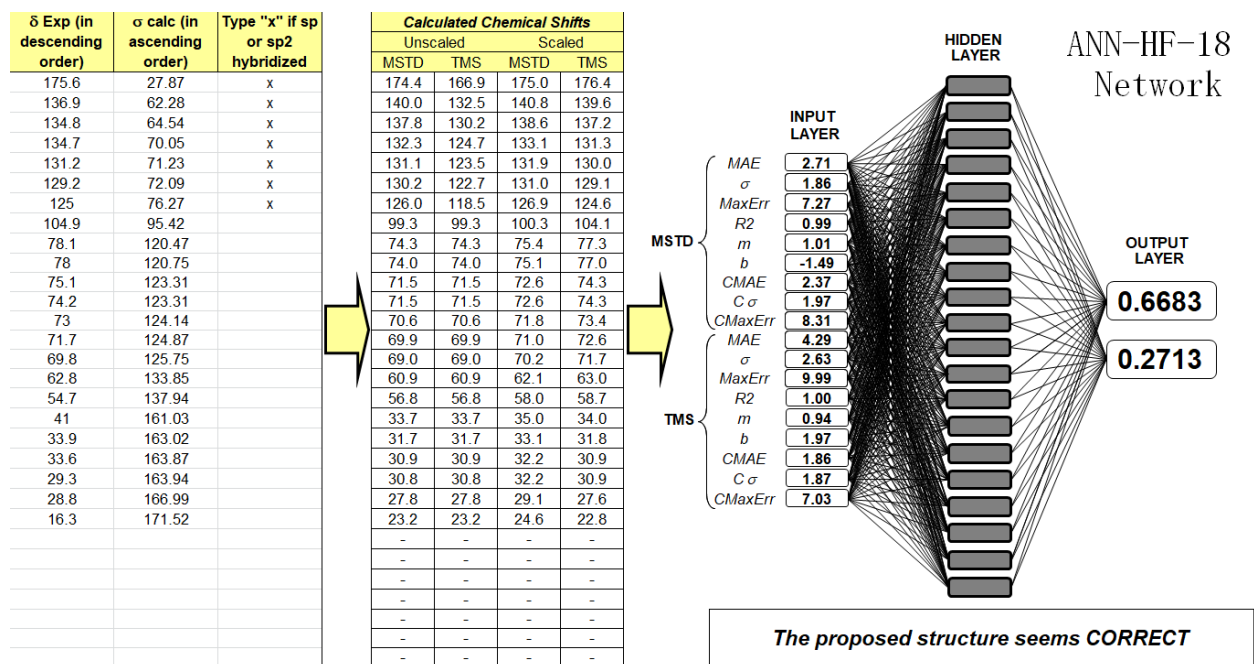


Figure S6. The result of ANNs analysis for (3S*,15R*,16S*)-1, and corresponding 18 parameters.

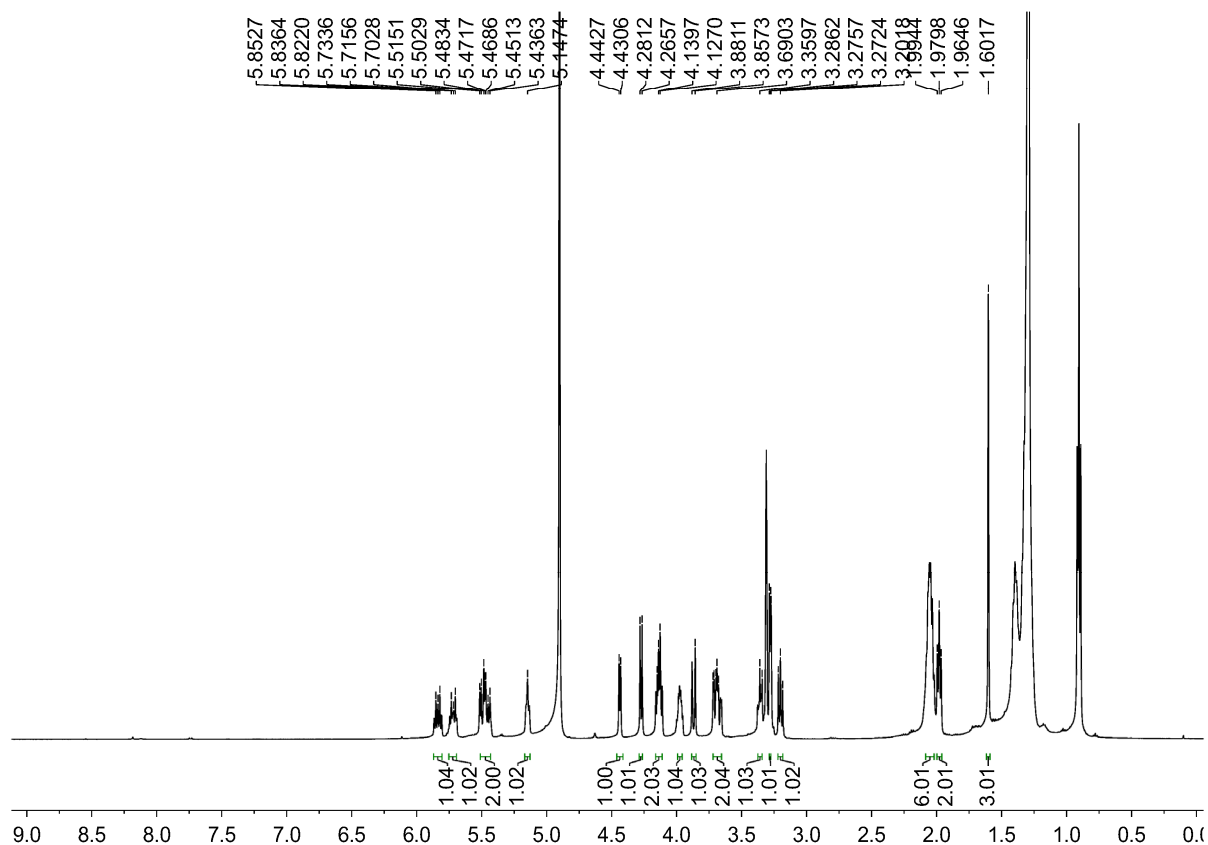


Figure S7. ^1H NMR (500 MHz, methanol- d_4) of compound **1**.

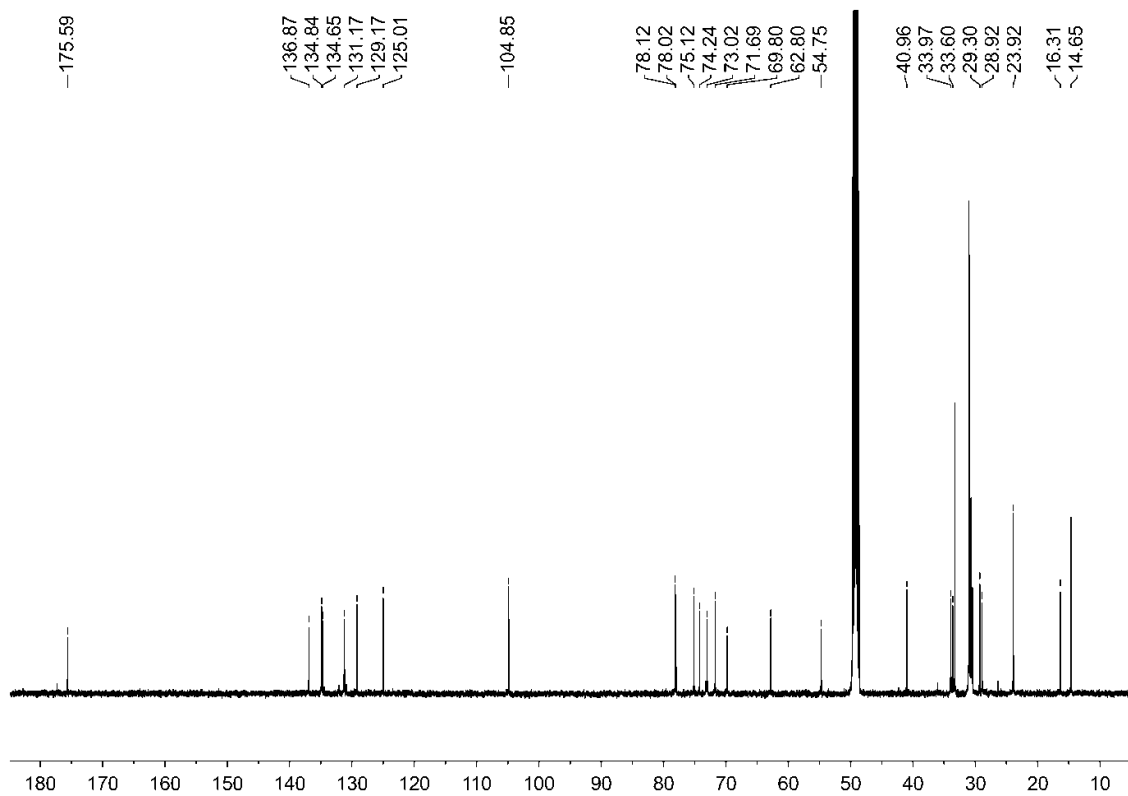


Figure S8. ^{13}C NMR (125 MHz, methanol- d_4) of compound **1**.

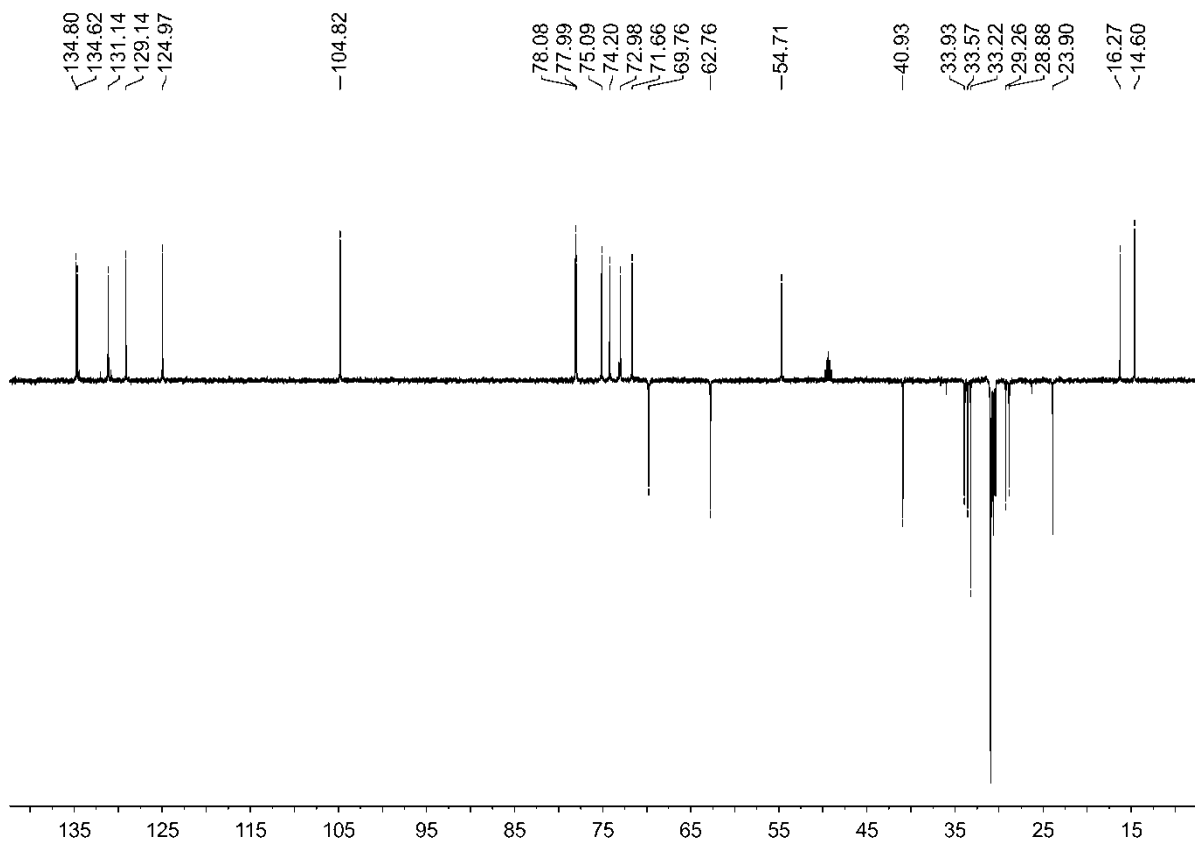


Figure S9. DEPT135 (125 MHz, methanol- d_4) of compound **1**.

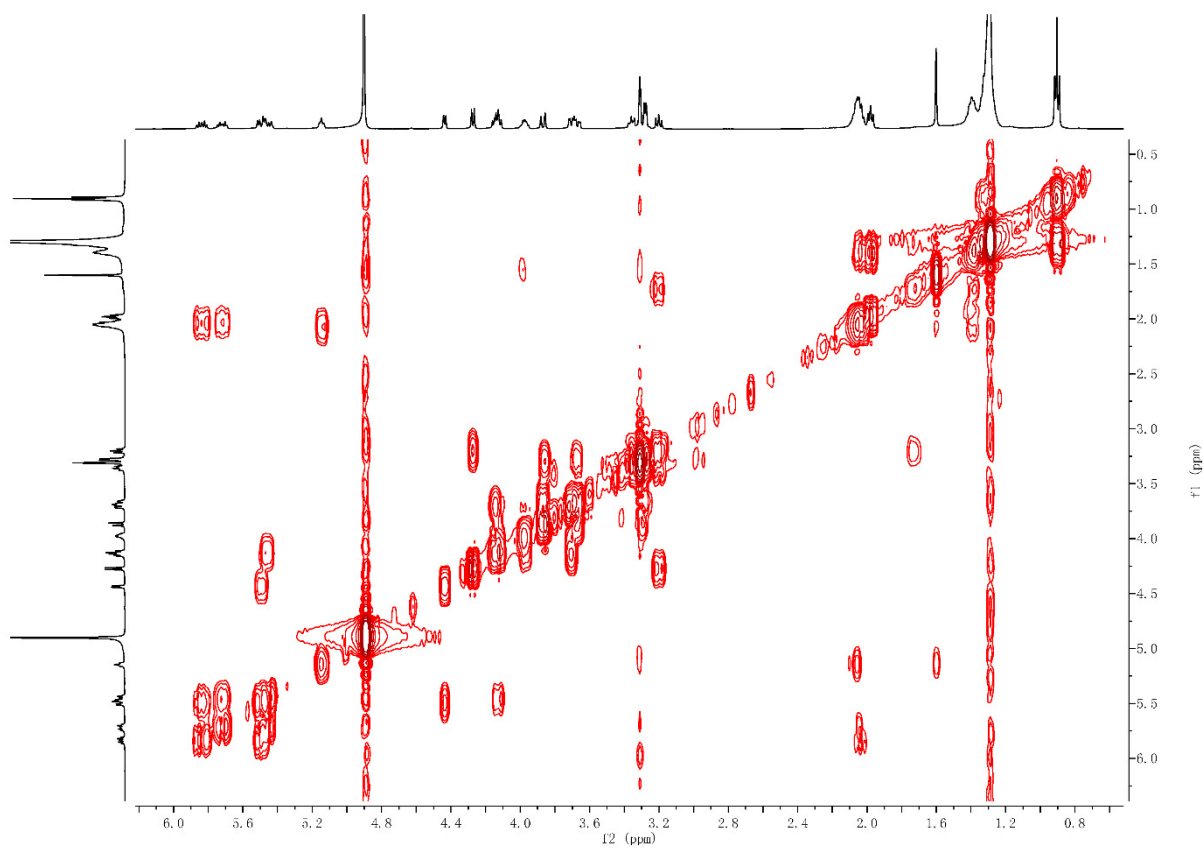


Figure S10. ^1H - ^1H COSY (500 MHz, methanol- d_4) of compound **1**.

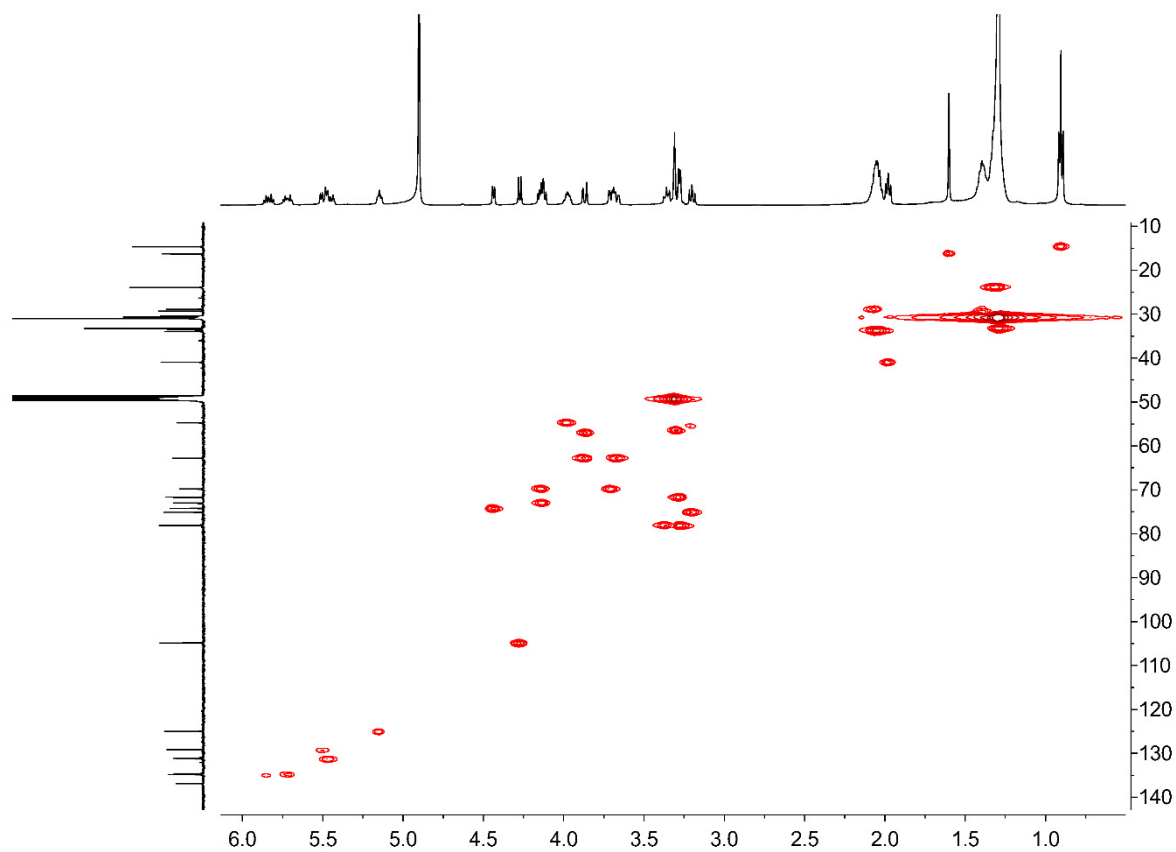


Figure S11. HSQC (methanol- d_4) of compound **1** (^1H : 500 MHz, ^{13}C : 125 MHz).

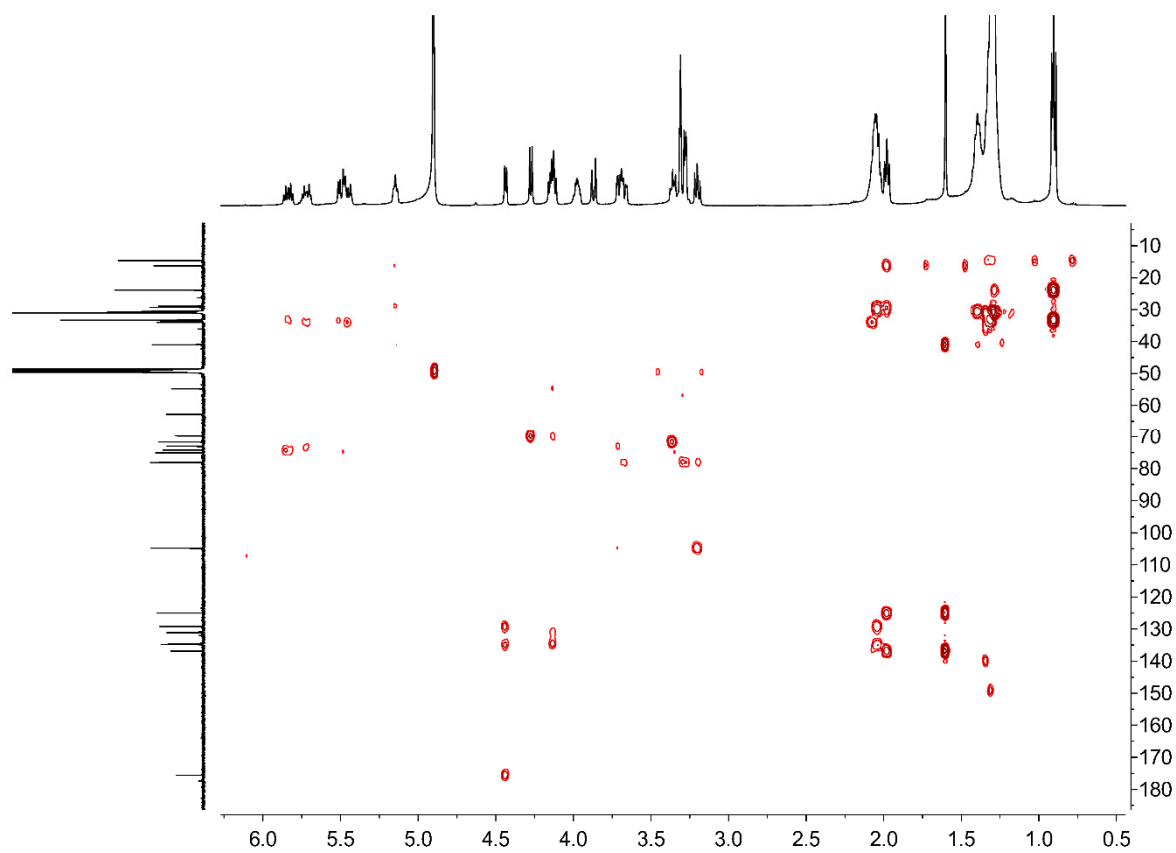


Figure S12. HMBC (methanol- d_4) of compound **1** (^1H : 500 MHz, ^{13}C : 125 MHz).

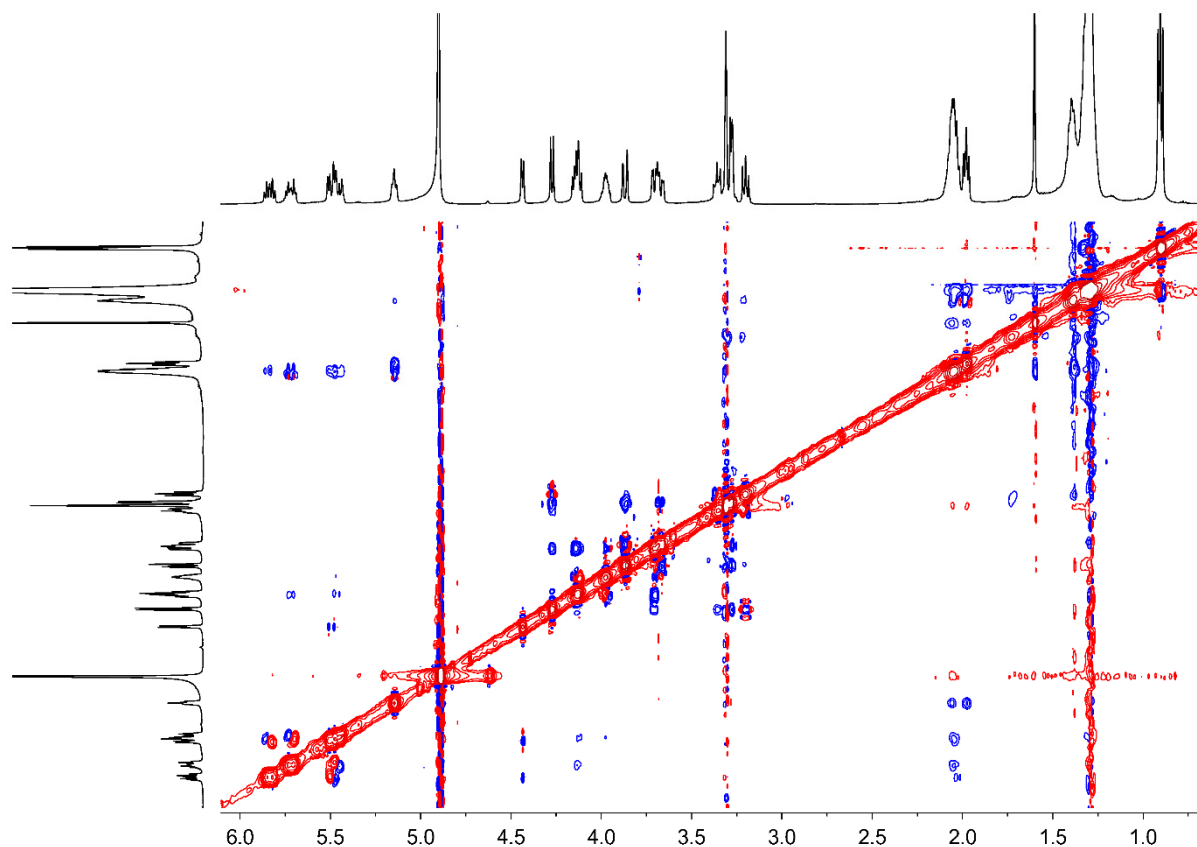
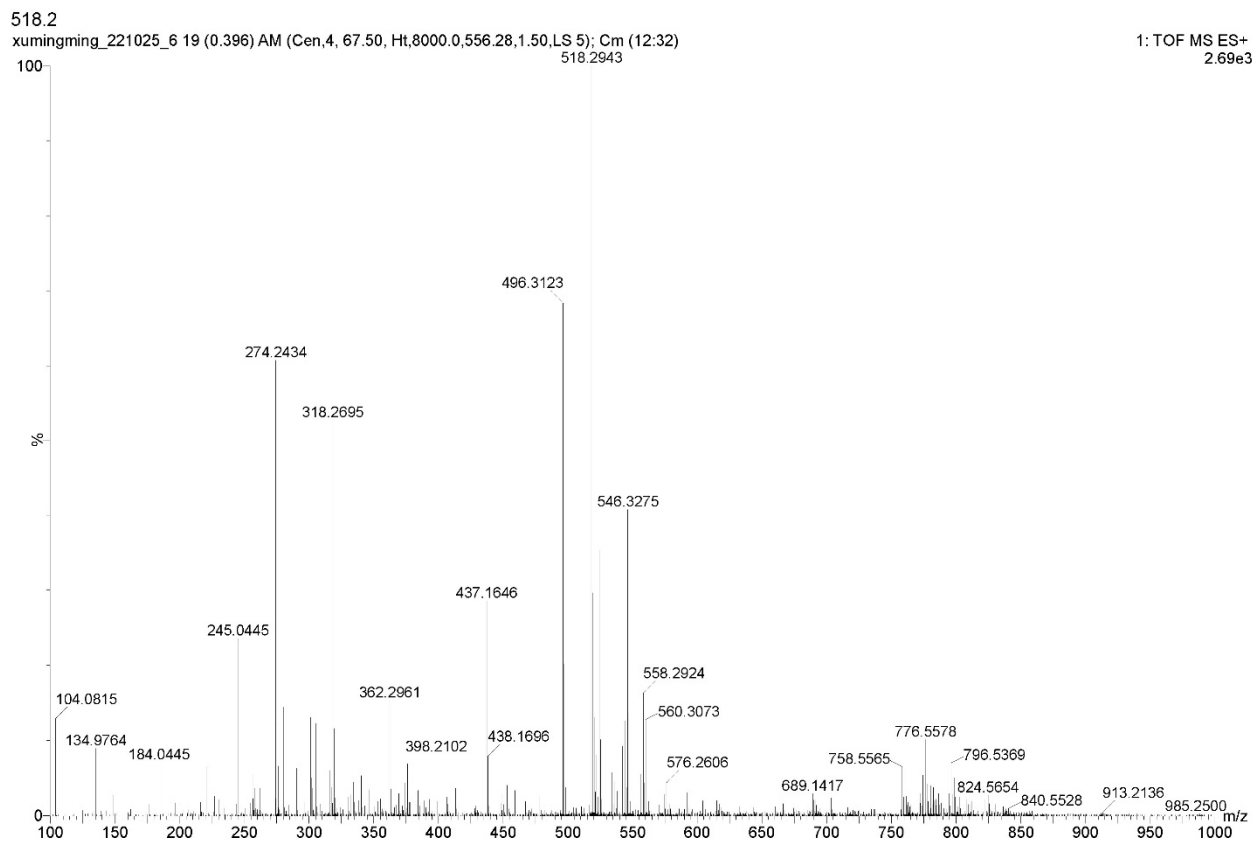


Figure S13. NOESY (500 MHz, methanol- d_4) of compound **1**.



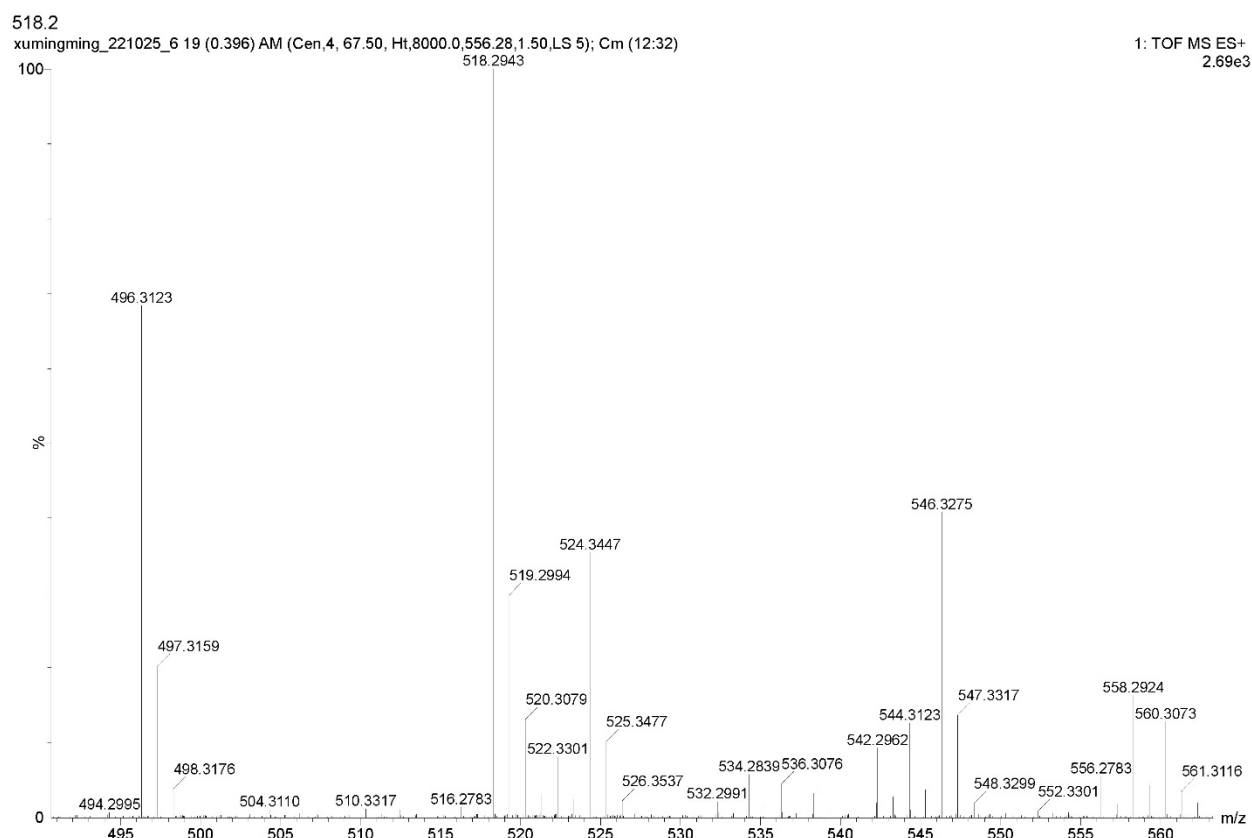


Figure S14. (+)-HR-ESI-MS of compound **1**.

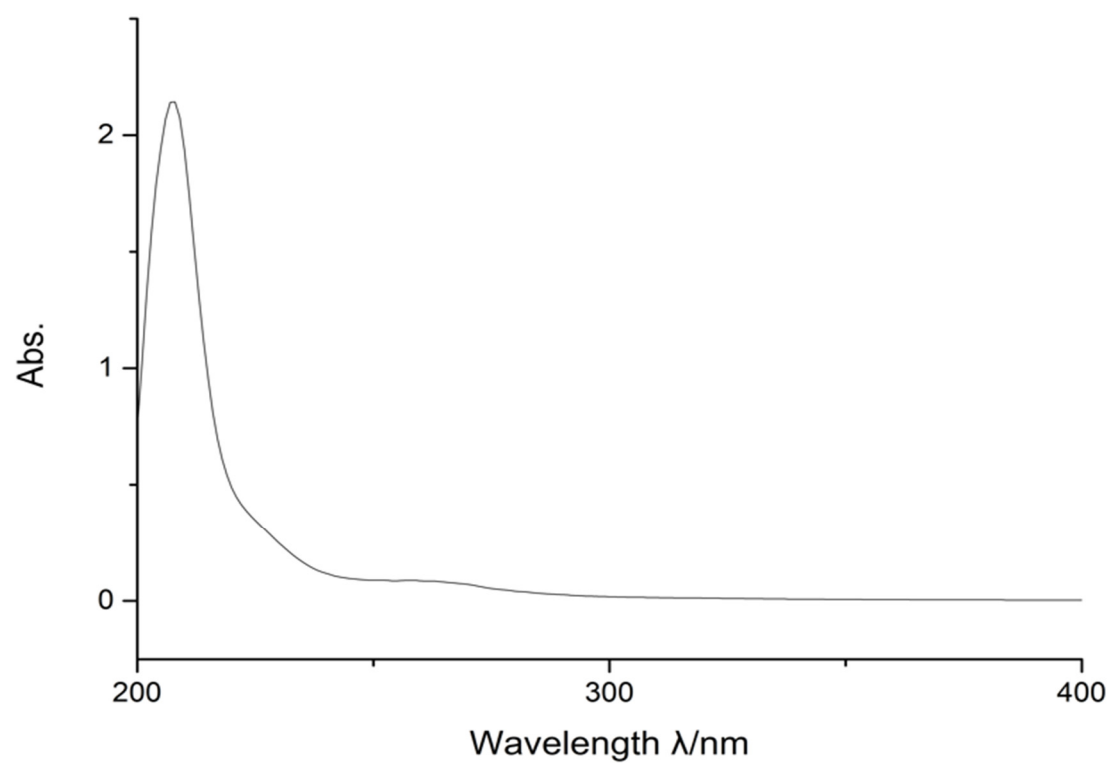


Figure S15. UV of compound **1**.

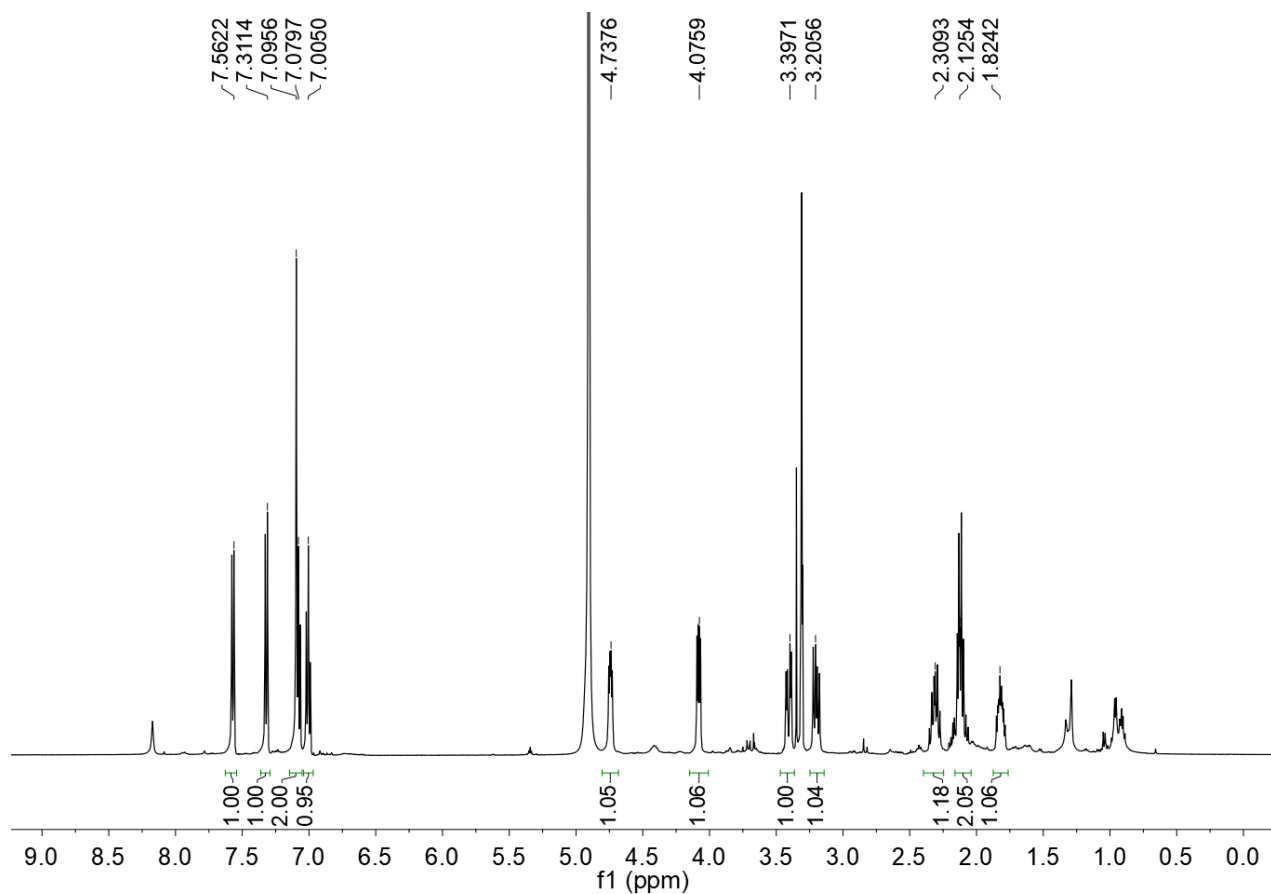


Figure S16. ¹H NMR (500 MHz, methanol-*d*₄) of compound 2.

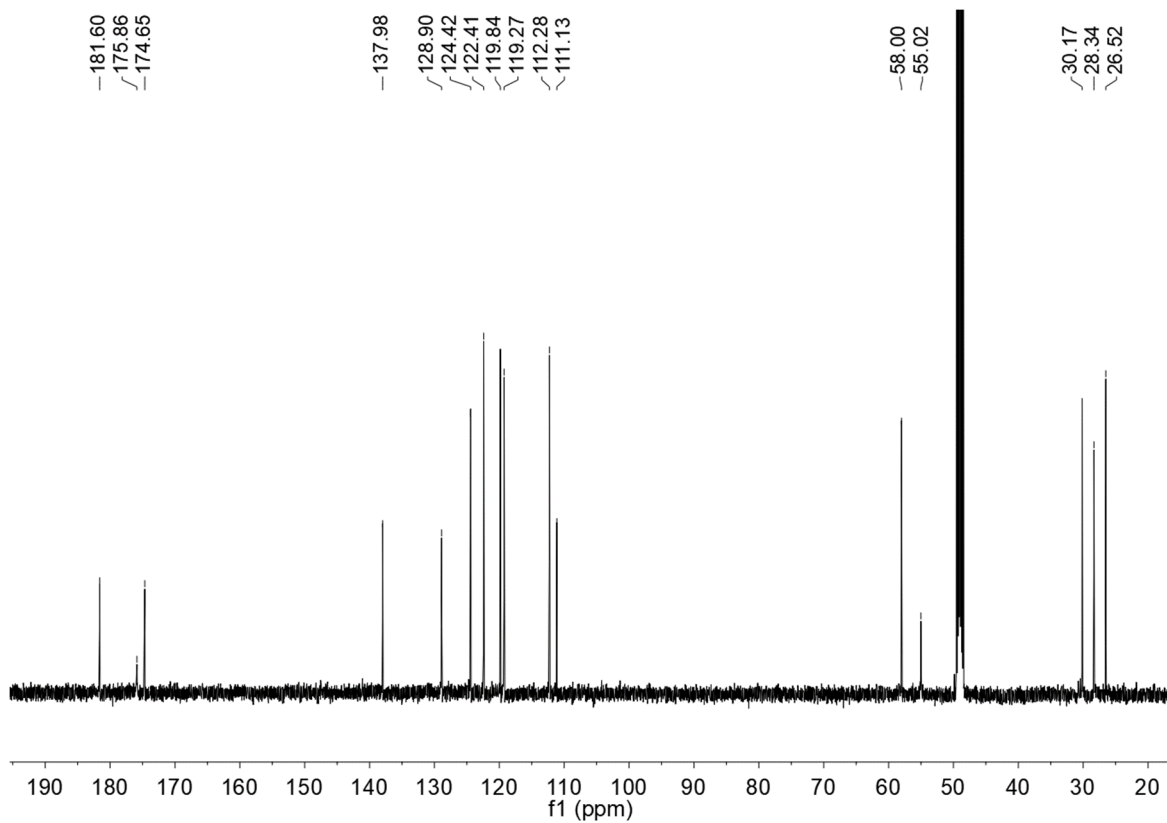


Figure S17. ¹³C NMR (125 MHz, methanol-*d*₄) of compound 2.

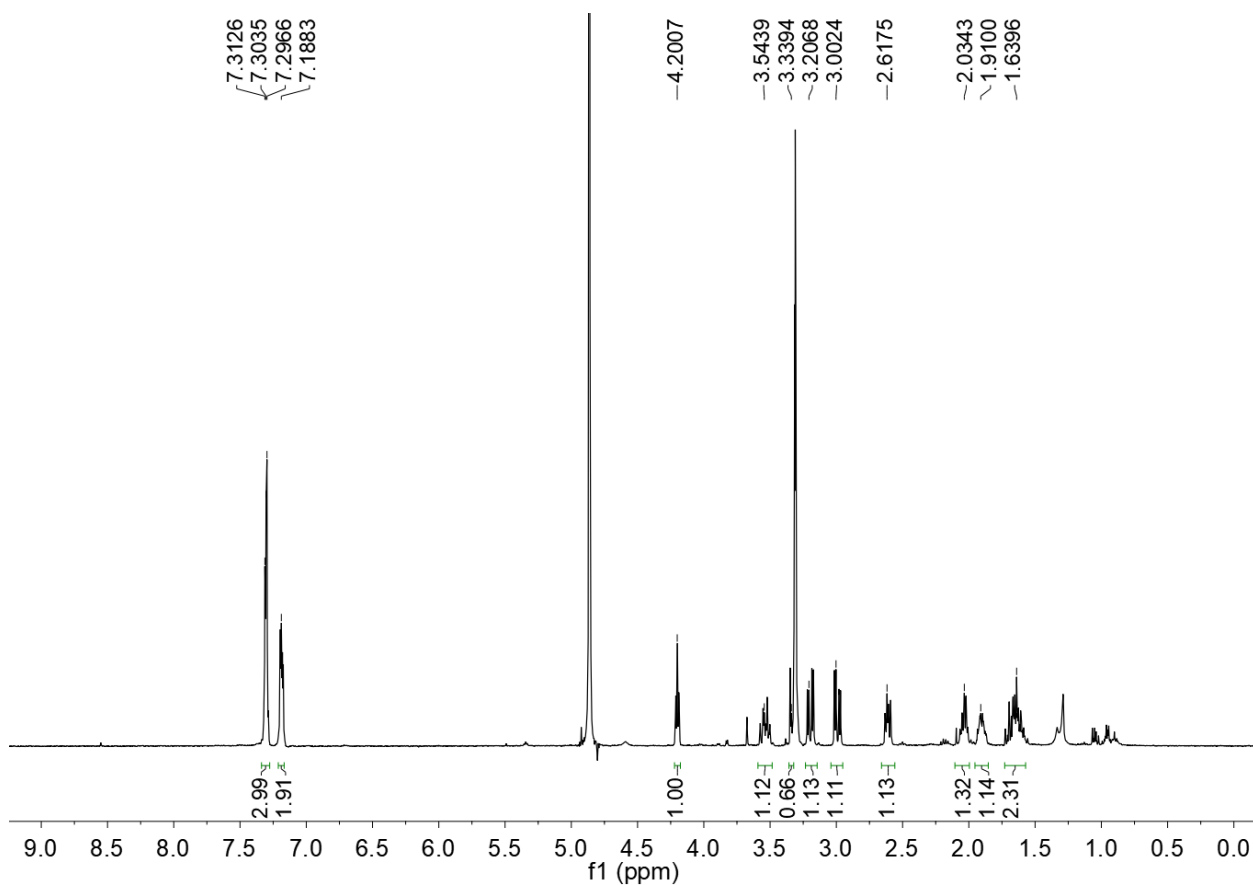


Figure S18. ¹H NMR (500 MHz, methanol-*d*₄) of compound **3**.

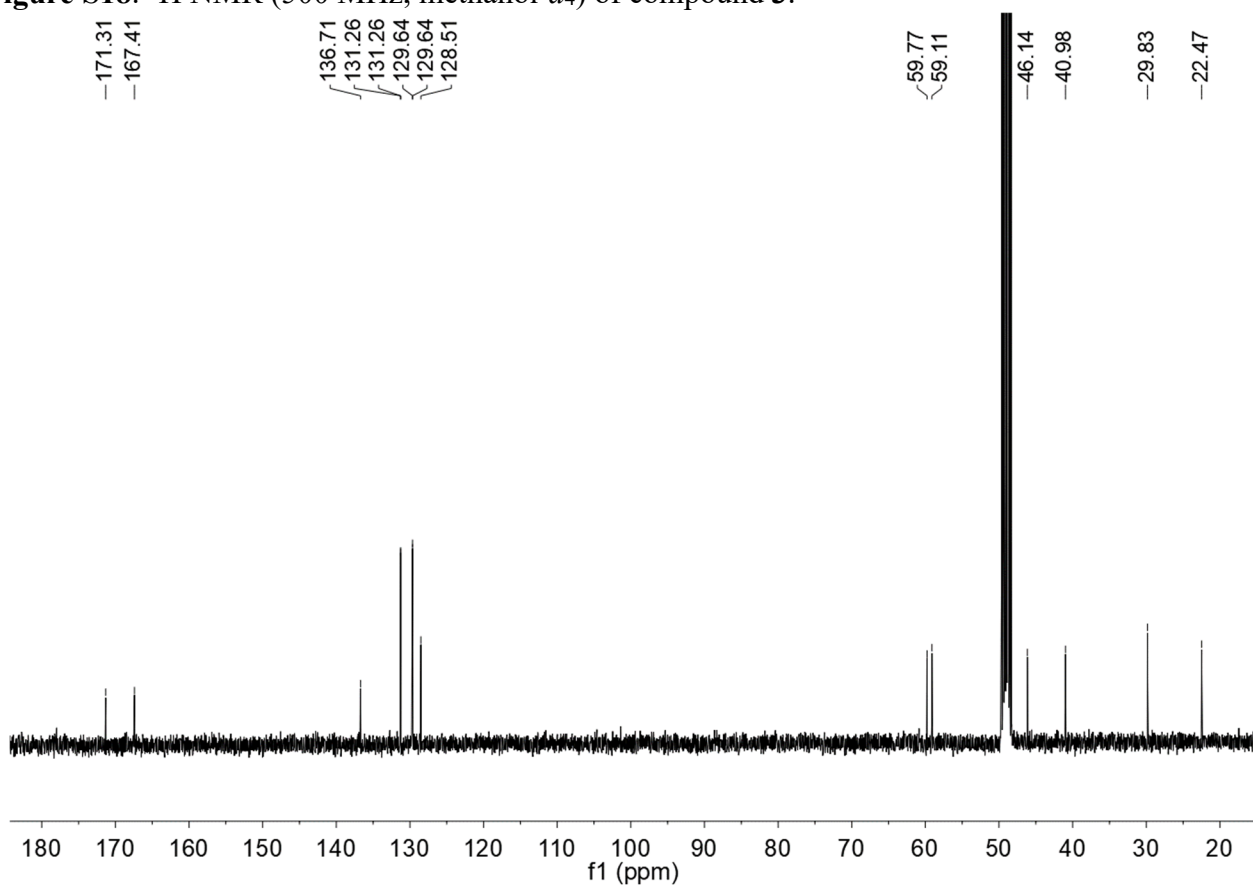


Figure S19. ¹³C NMR (125 MHz, methanol-*d*₄) of compound **3**.

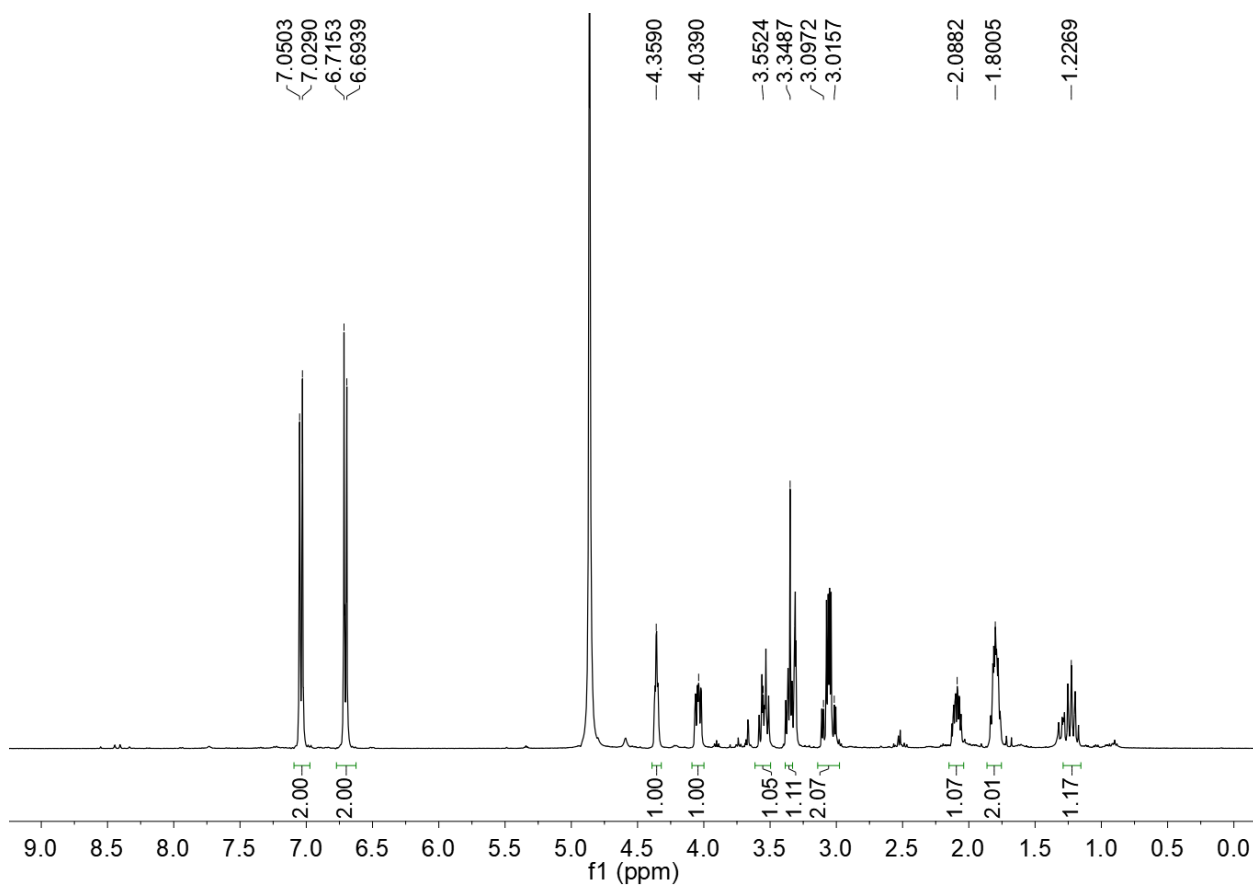


Figure S20. ¹H NMR (500 MHz, methanol-*d*₄) of compound 4.

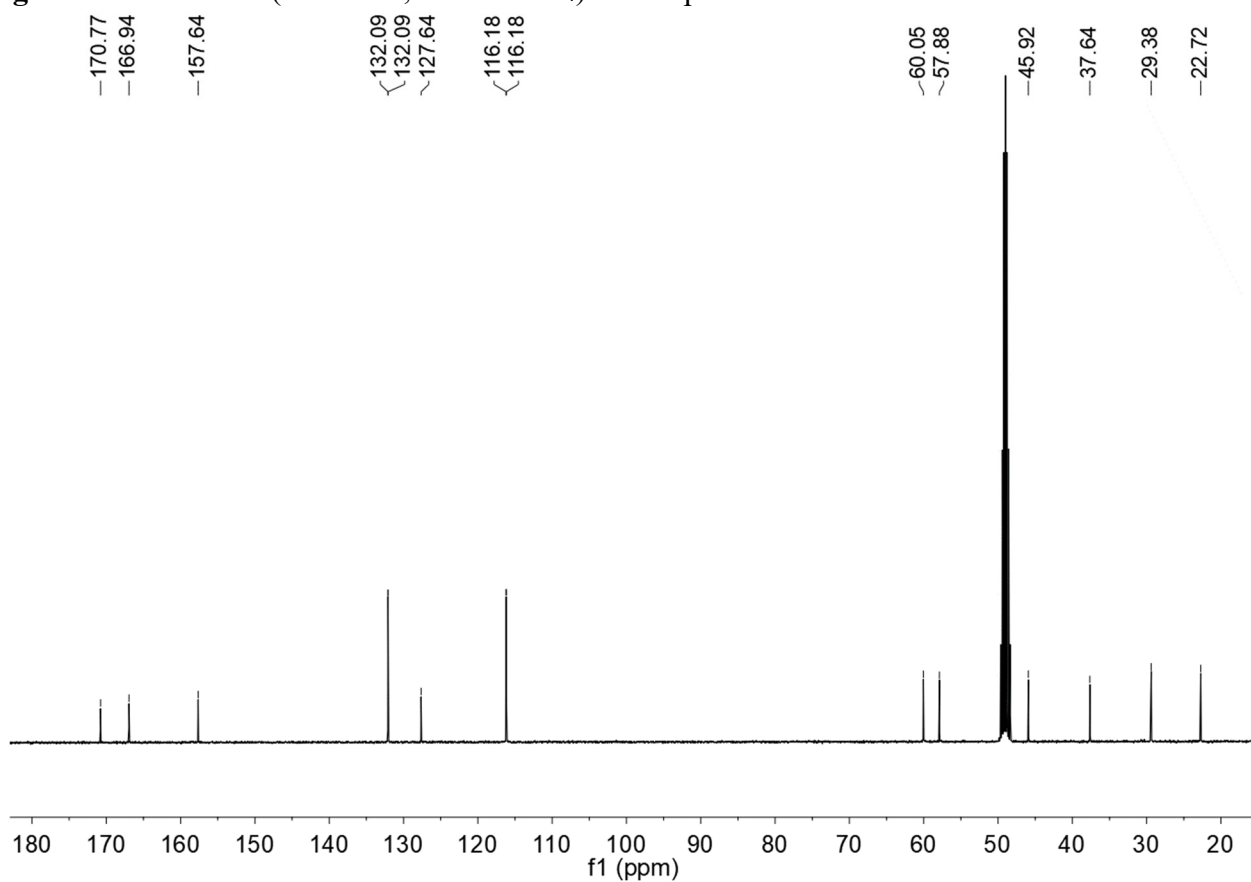


Figure S21. ¹³C NMR (125 MHz, methanol-*d*₄) of compound 4.

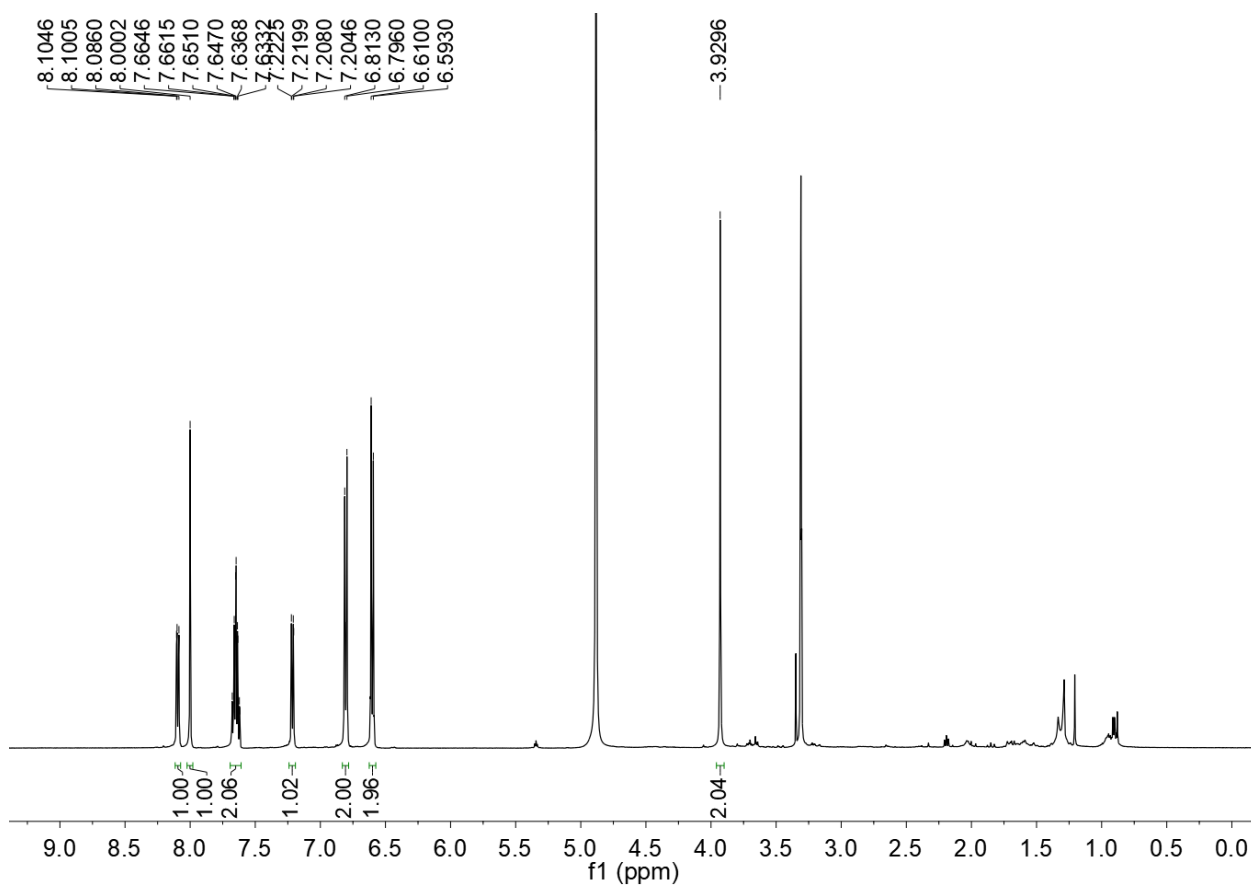


Figure S22. ¹H NMR (500 MHz, methanol-*d*₄) of compound **5**.

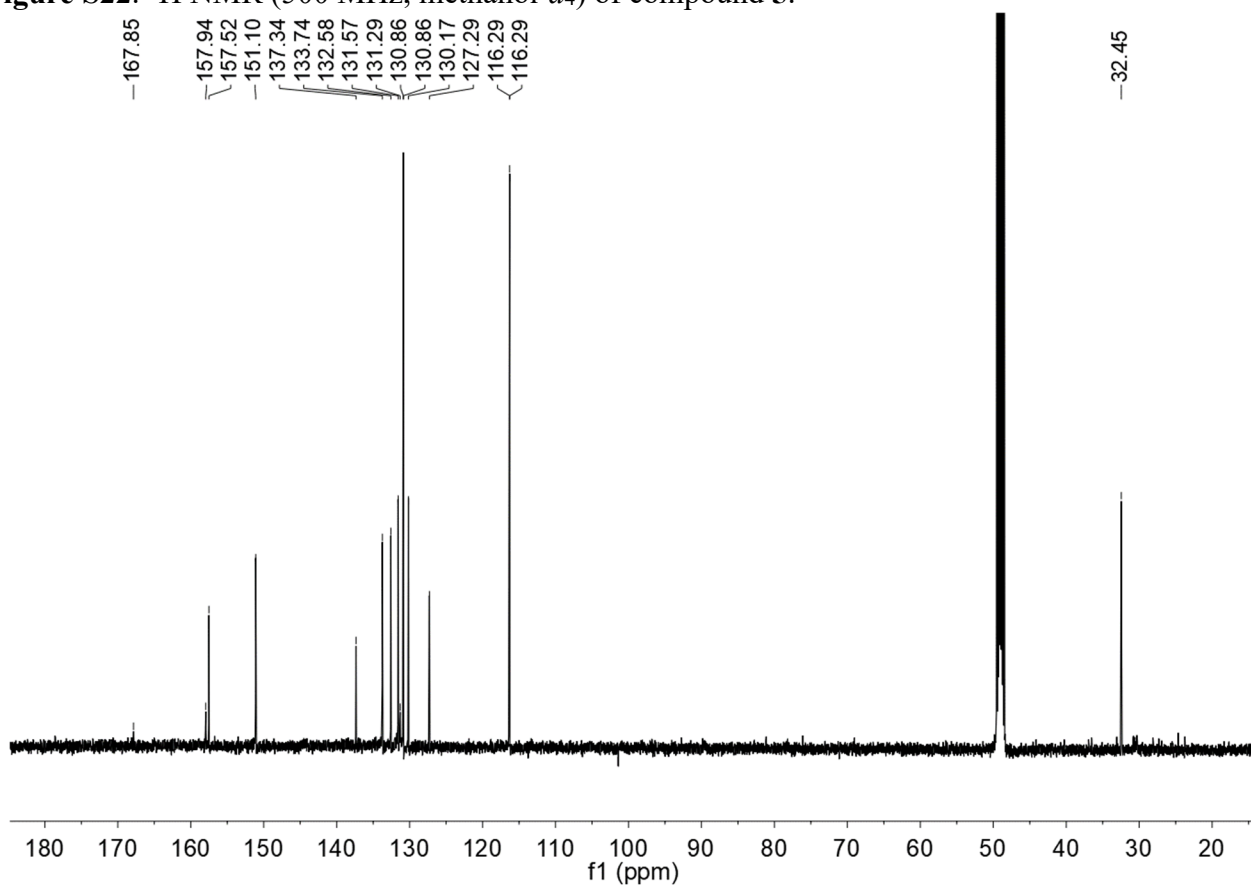


Figure S23. ¹³C NMR (125 MHz, methanol-*d*₄) of compound **5**

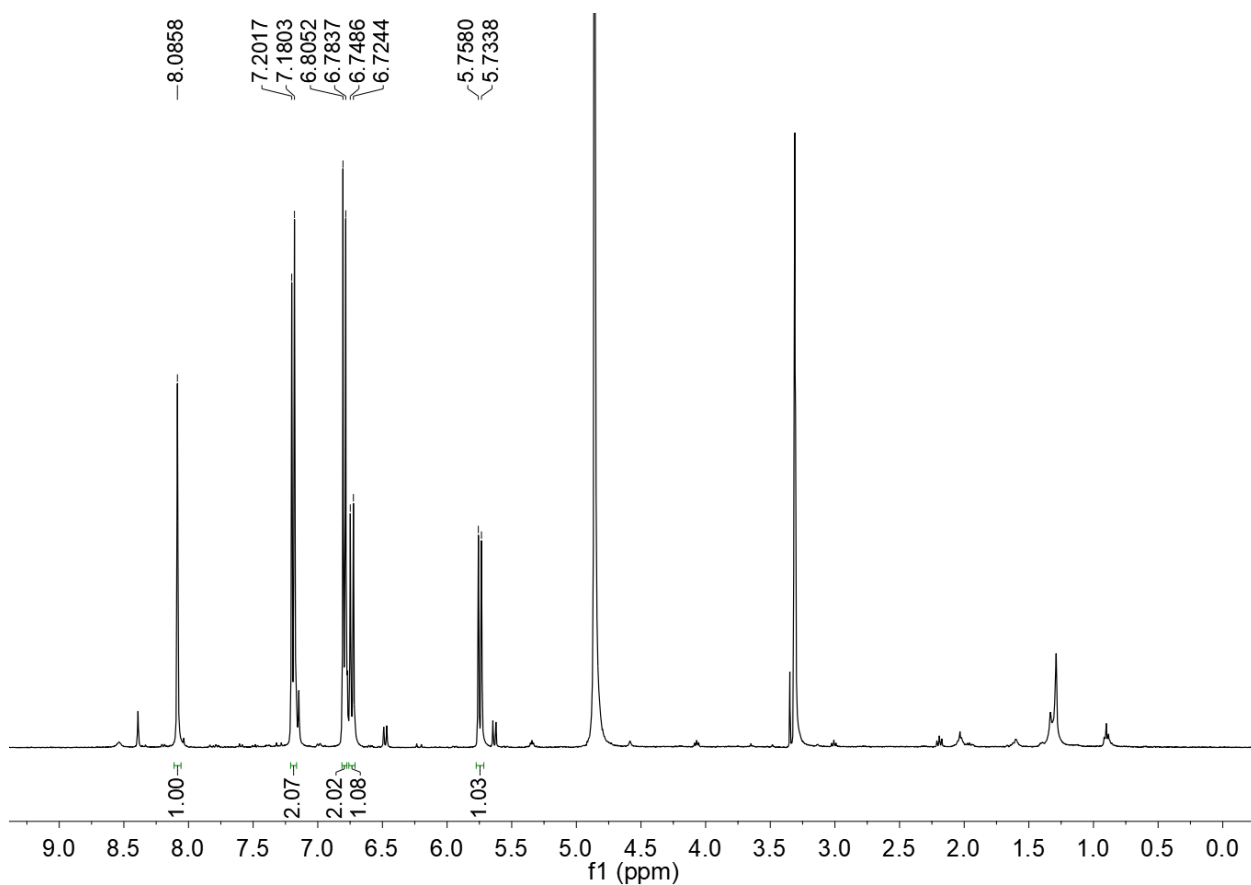


Figure S24. ¹H NMR (500 MHz, methanol-*d*₄) of compound **6**.

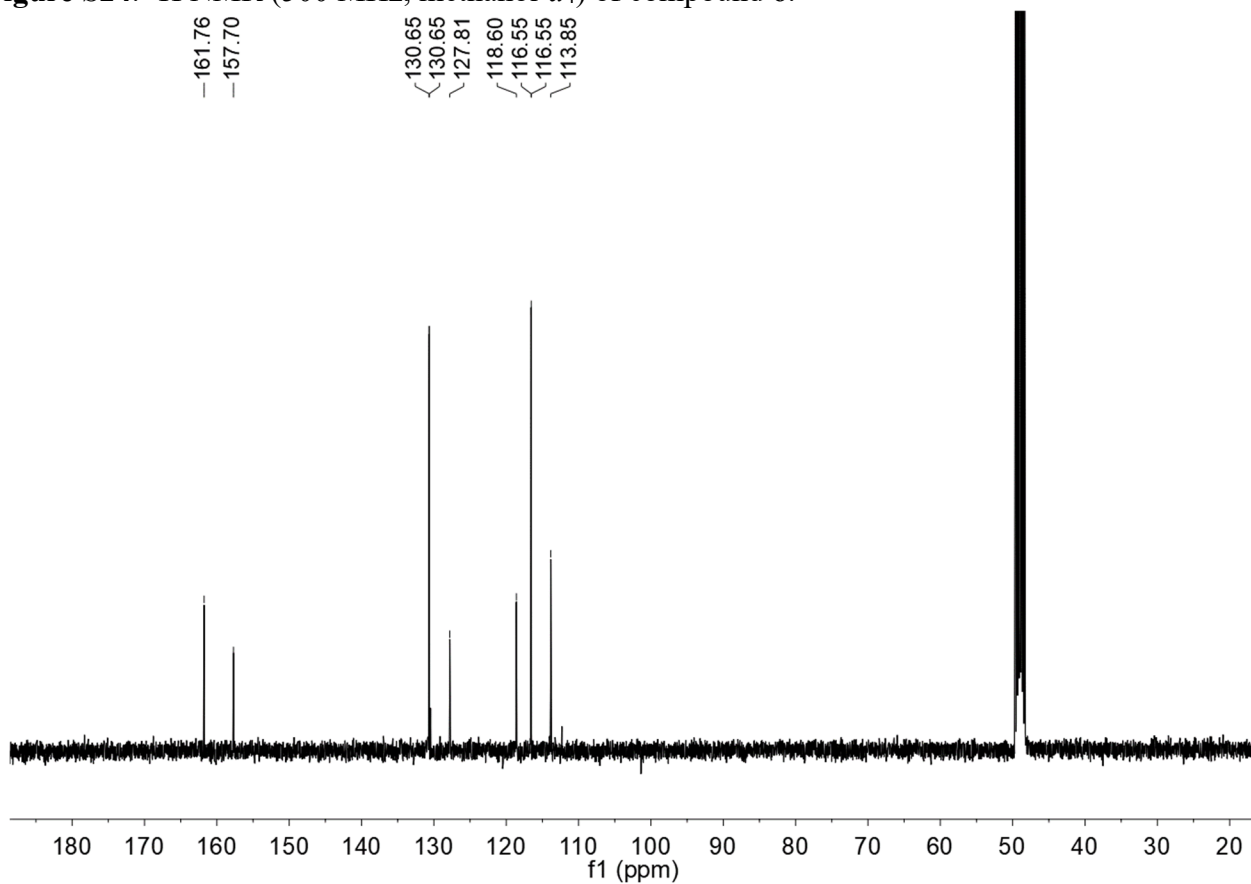


Figure S25. ¹³C NMR (125 MHz, methanol-*d*₄) of compound **6**.

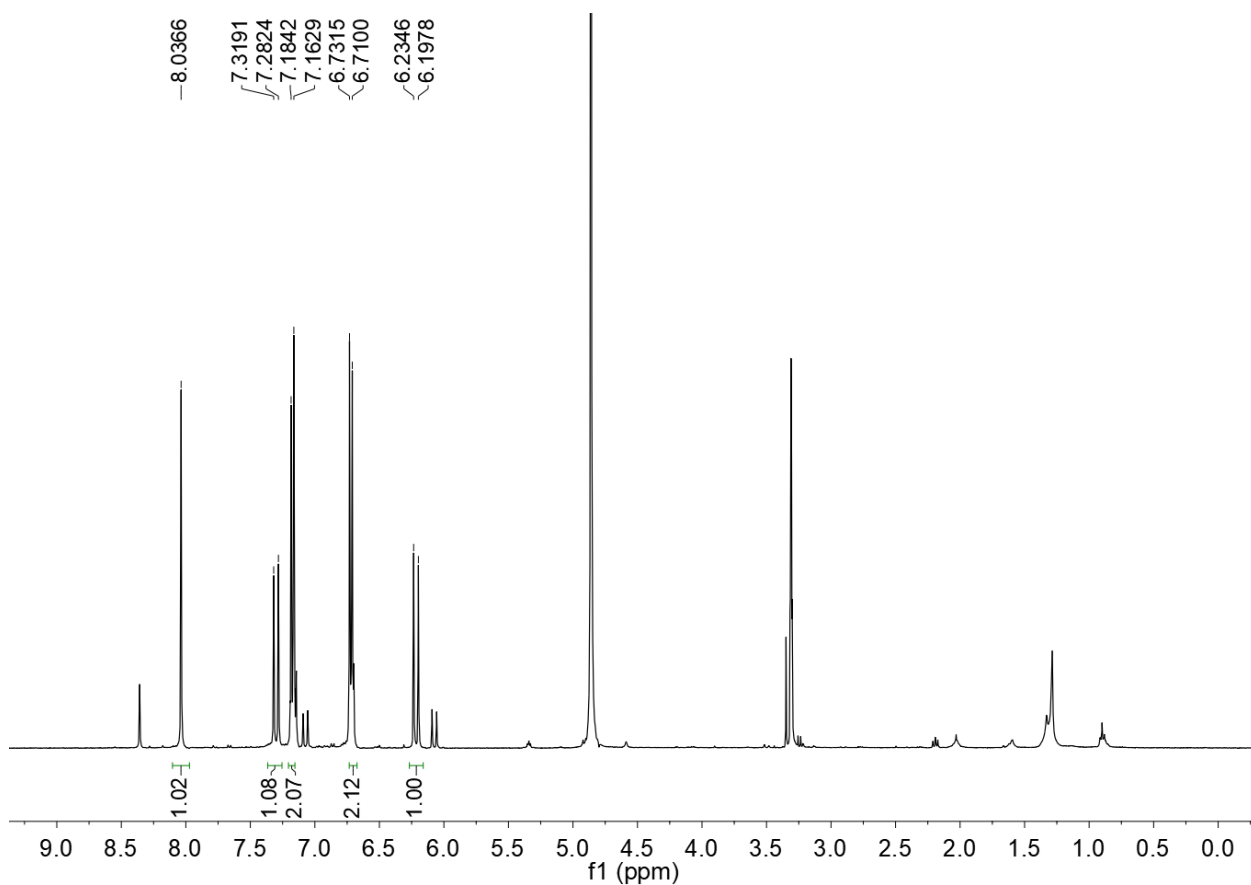


Figure S26. ^1H NMR (500 MHz, pyridine- d_5) of compound **7**.

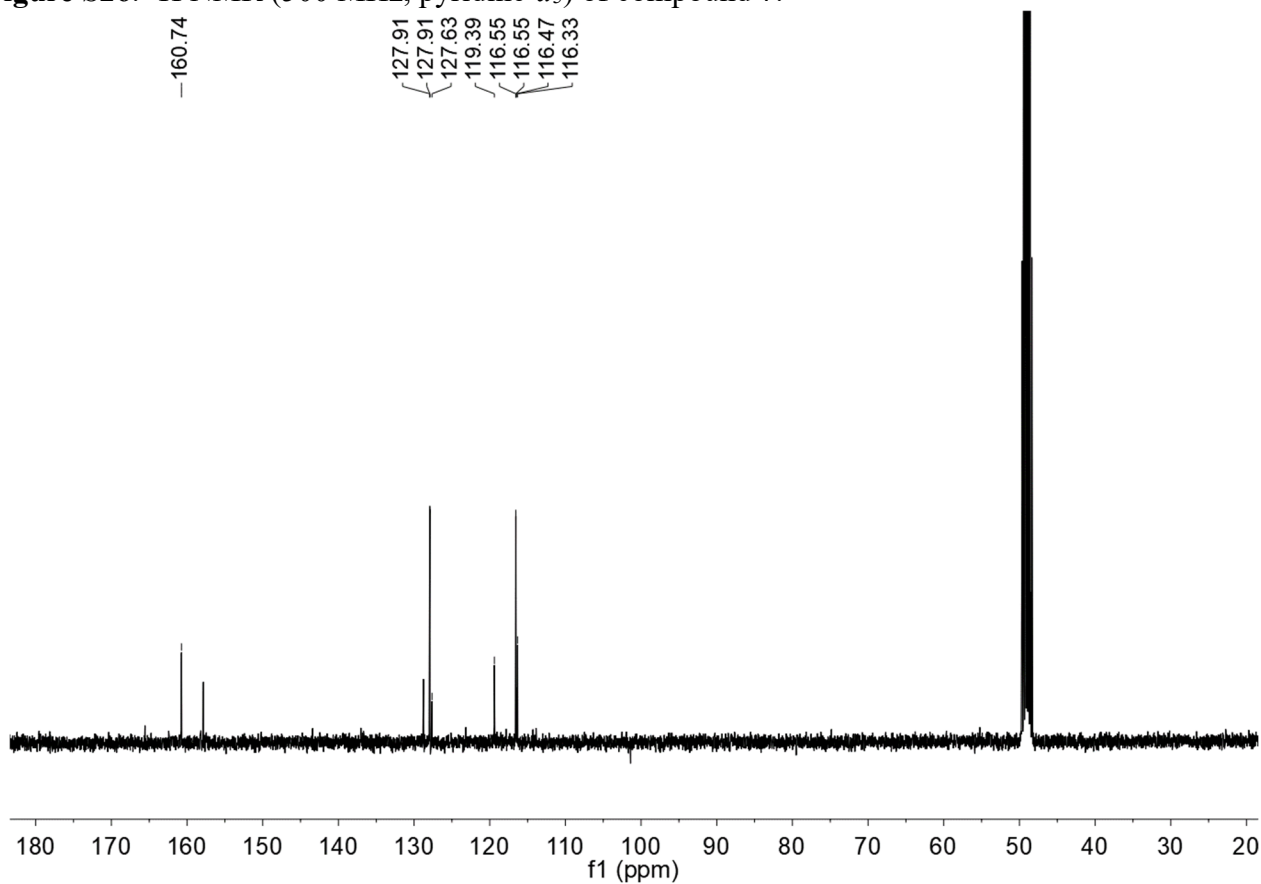


Figure S27. ^{13}C NMR (125 MHz, pyridine- d_5) of compound **7**.

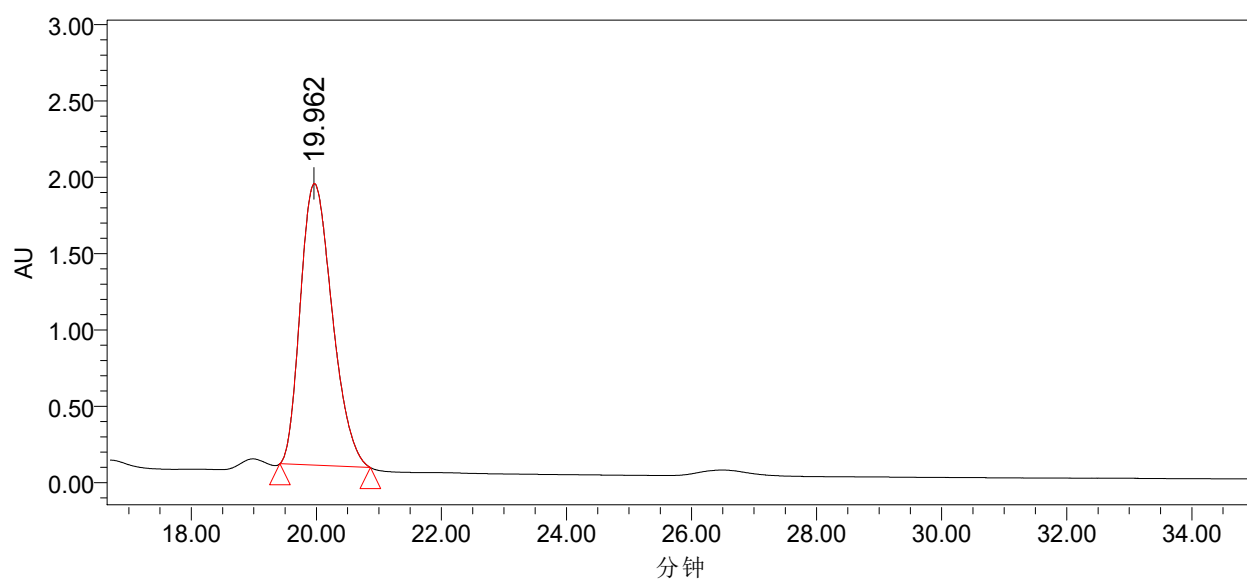


Figure S28. HPLC chromatogram of the derivative of the standard *L*-Glucose.

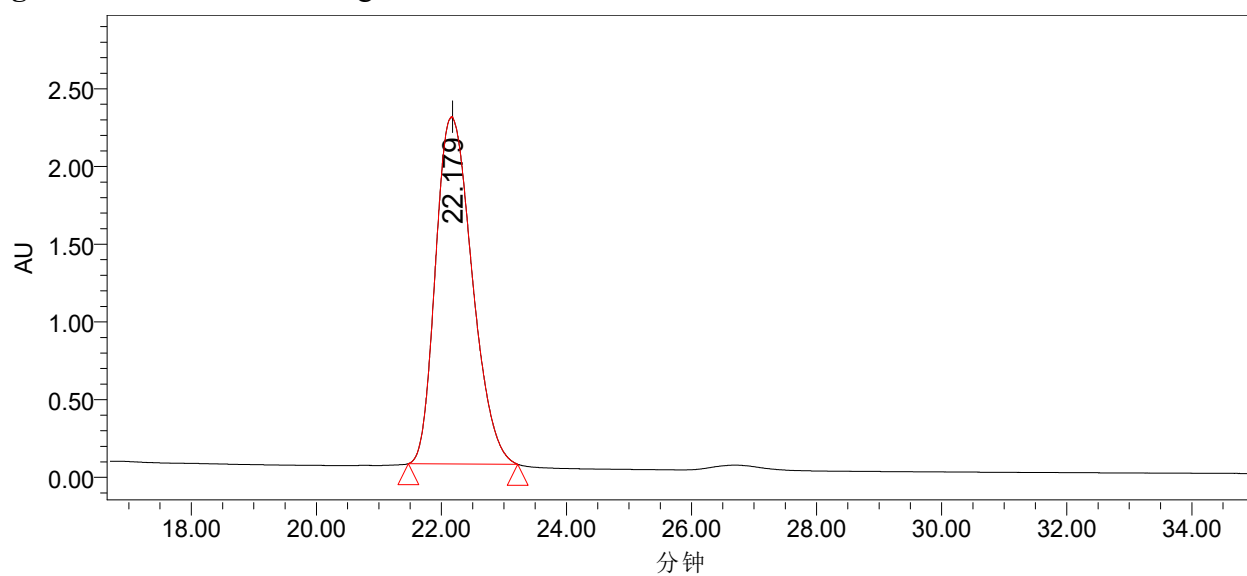


Figure S29. HPLC chromatogram of the derivative of the standard *D*-Glucose.

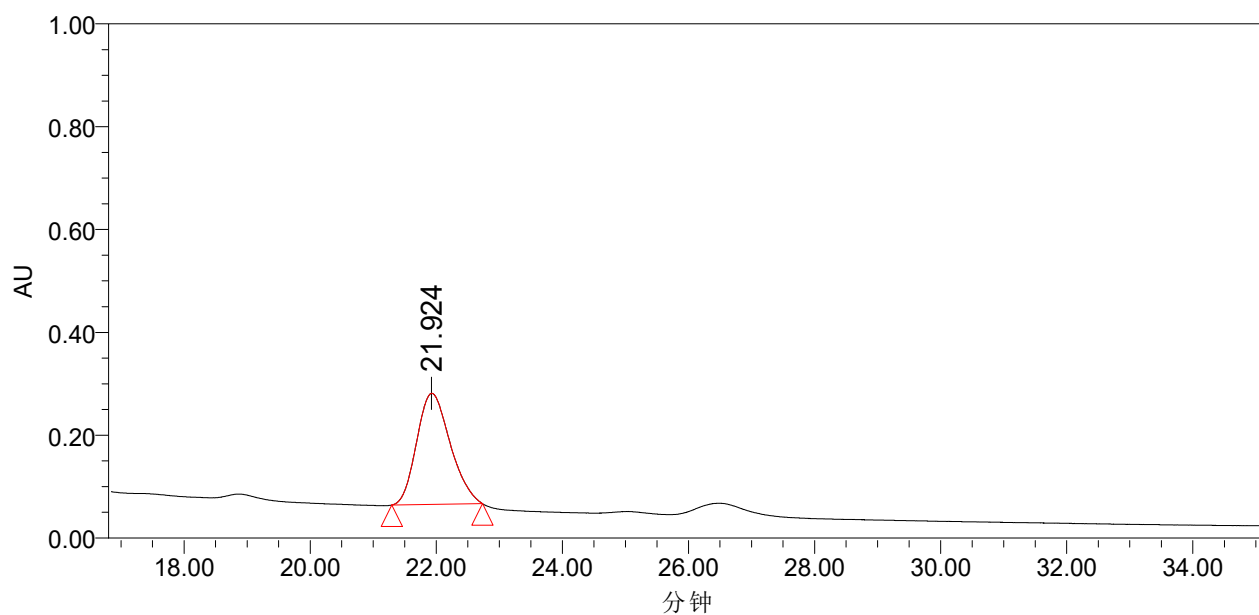


Figure S30. HPLC chromatogram of derivative of the hydrolysate of compound **1**.

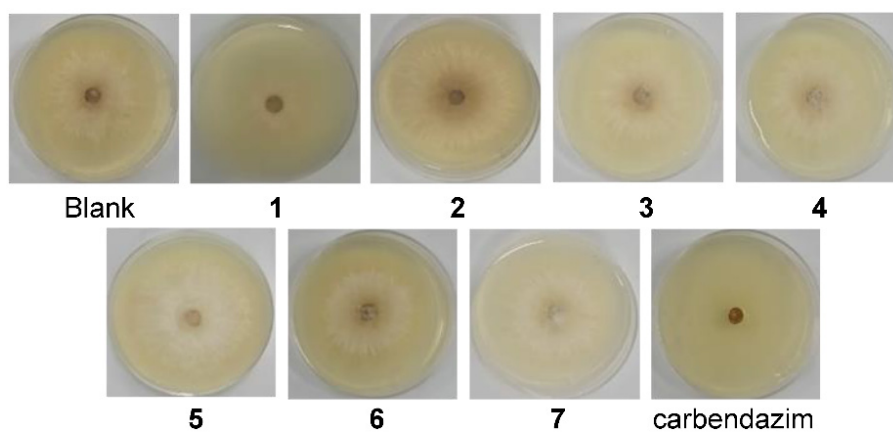


Figure S31. Compounds **1**–**7** and carbendazim (100 μ M) inhibit the growth of *Rhizoctonia solani*.

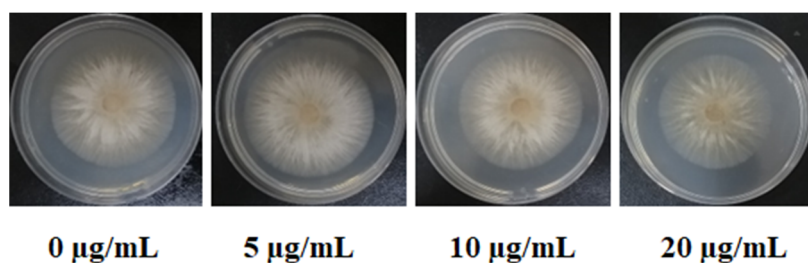


Figure S32. Growth inhibition of *Rhizoctonia solani* with different concentrations of compound **1**.