Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Sporulaminals A and B: a Pair of Unusual Epimeric Spiroaminal Derivatives from a Marine-Derived Fungus *Paraconiothyrium sporulosum* YK-03

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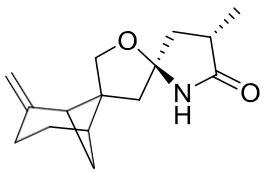
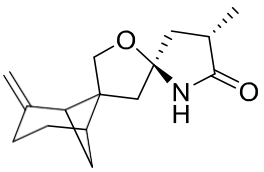
List of Supporting Information

Computational methods

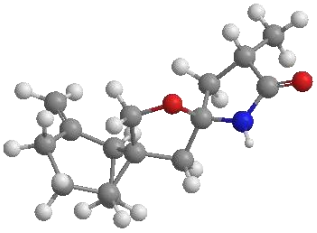
Relative configurations of **1** and **2** were deduced from their NOESY data.

Compound **1**: Stochastic conformational searches were firstly conducted under MMFF94 force field for 1a (2*S*, 4*R*, 6*S*, 7*S*, 9*S*), which gave two conformers. Their difference was that the orientation of methyl of the lactam ring was different. Conformers were optimized at the B3LYP/6-31G(d) basis set level in methanol and the frequencies were calculated by further time-dependent density functional theory (TDDFT) method, showing that these two conformers combined into one stable conformer, which was identical to the conformer of X-ray crystallographic analysis of compound **1**.

1	 sporulosine A (**1**)

1a	
1b	the enantiomer of ZHL4a

Stable conformer of **1a** in MeOH:



Using the conformers at the B3LYP/6-31G(d) basis set level in methanol, forty excitation states at the B3LYP/6-31G(d) basis set level were calculated, peak stretcher

was 0.4eV, and finally the calculation results were Boltzmann averaged to yield the depicted electronic circular dichroism (ECD) spectra of **1**, which was identical to the calculation results at the cam-B3LYP/6-31G(d)basis set level. All calculations were performed by Gaussian 09 program package (Version C.01).

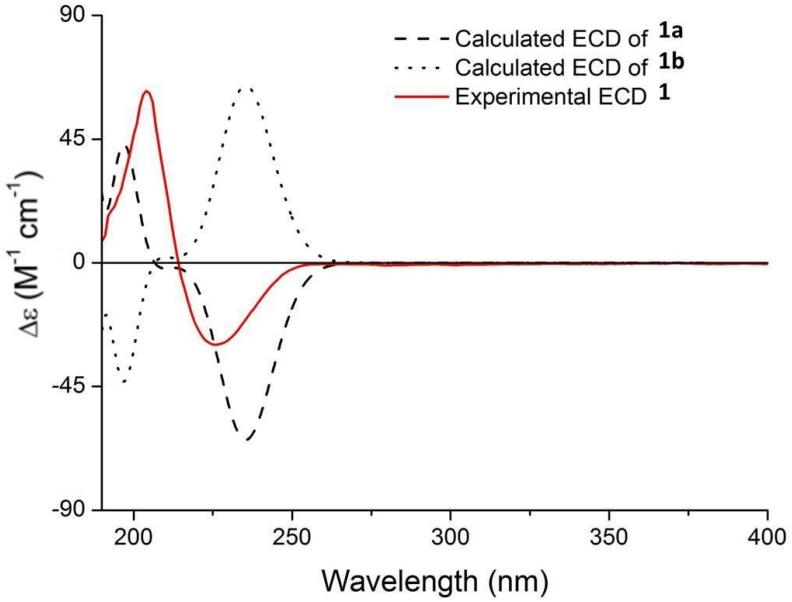


Figure S1. Calculated and experimental ECD spectra of **1**

Cpmpound **2**: The Spartan 14.0 (Wavefunction Inc., Irvine, CA, USA) search using molecular mechanics MMFF was performed for 2a (2S, 4S, 6S, 7S, 9S), which gave 100 conformers. The low-energy conformers of 2a accounting for more than 5% Boltzmann distribution were further optimized successively in the gas phase by semi-empirical method in Gaussian 09 program package, which were reoptimized and analysed frequency, orderly, using the density functional theory (DFT) at the B3LYP/6-31G (d, p) level and the same way in the methanol, resulted in no imaginary frequencies. Solvent effects were taken into consideration by using the conductor polarizable continuum model (CPCM). The conformers of 2a were calculated electronic circular dichroism (ECD) by the time-dependent density functional theory (TD-DFT) method at the B3LYP/6-31++G (d, p) level with the CPCM model in methanol solution. The overall calculated ECD curves of 2a were generated by Boltzmann weighting of their selected low-energy conformers using SpecDis 1.51 with σ = 0.12eV at -8nm shift.

Table S1 Energy analysis of **2a**

Label	MMFF		
	E(kJ/mol)	rel. E(kJ/mol)	Boltzmann Dist.
2a-1	-3.64	0.00	0.554
2a-2	-3.10	0.54	0.446

Table S2 Computational methods for ECD of **2a**

2a-1 Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	3.143606	0.845033	-0.593609
2	6	0	3.707414	-0.453570	-0.006880
3	6	0	2.519355	-1.429386	-0.107791
4	6	0	1.257225	-0.538567	-0.174019
5	8	0	0.762872	-0.243800	1.146697
6	6	0	0.057513	-1.134857	-0.929951
7	6	0	-1.179354	-0.630413	-0.167682
8	6	0	-0.626302	-0.589884	1.256840
9	6	0	4.213674	-0.208949	1.424057
10	8	0	3.774911	1.863295	-0.856479
11	7	0	1.797321	0.678435	-0.760576
12	6	0	-1.859827	0.647478	-0.805980
13	6	0	-2.734428	1.312146	0.228141
14	6	0	-3.875824	0.432442	0.720212
15	6	0	-3.560220	-1.085279	0.608303
16	6	0	-2.515529	-1.359407	-0.490538
17	6	0	-2.699397	-0.351880	-1.660416
18	6	0	-2.521355	2.555817	0.669831
19	1	0	4.547707	-0.773735	-0.631175
20	1	0	2.445190	-2.121968	0.733439
21	1	0	2.586965	-2.016359	-1.028736
22	1	0	0.104279	-2.228231	-0.858487
23	1	0	0.083475	-0.869985	-1.988894
24	1	0	-0.730035	-1.576943	1.733942
25	1	0	-1.107321	0.155316	1.895873

26	1	0	3.390022	0.084999	2.081852
27	1	0	4.966713	0.584213	1.434554
28	1	0	4.665631	-1.119197	1.829102
29	1	0	1.211617	1.477602	-0.966837
30	1	0	-1.216944	1.366308	-1.323570
31	1	0	-4.163319	0.703939	1.740336
32	1	0	-4.751058	0.647924	0.094038
33	1	0	-4.482312	-1.632475	0.383808
34	1	0	-3.195903	-1.476625	1.565247
35	1	0	-2.450380	-2.427085	-0.726831
36	1	0	-3.716593	-0.059078	-1.935641
37	1	0	-2.164792	-0.662446	-2.560487
38	1	0	-1.721706	3.171927	0.266835
39	1	0	-3.144061	3.002567	1.440813

2a-2 Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	3.143606	0.845033	-0.593609
2	6	0	3.707413	-0.453570	-0.006880
3	6	0	2.519355	-1.429386	-0.107792
4	6	0	1.257225	-0.538567	-0.174020
5	8	0	0.762872	-0.243800	1.146697
6	6	0	0.057512	-1.134858	-0.929951
7	6	0	-1.179354	-0.630413	-0.167682
8	6	0	-0.626302	-0.589884	1.256840
9	6	0	4.213674	-0.208949	1.424057
10	8	0	3.774910	1.863295	-0.856478
11	7	0	1.797321	0.678435	-0.760577
12	6	0	-1.859827	0.647478	-0.805981
13	6	0	-2.734428	1.312146	0.228141
14	6	0	-3.875823	0.432442	0.720213
15	6	0	-3.560219	-1.085279	0.608304
16	6	0	-2.515529	-1.359407	-0.490538
17	6	0	-2.699398	-0.351881	-1.660415
18	6	0	-2.521354	2.555817	0.669830
19	1	0	4.547707	-0.773735	-0.631175
20	1	0	2.445190	-2.121968	0.733438
21	1	0	2.586966	-2.016358	-1.028736
22	1	0	0.104279	-2.228232	-0.858487
23	1	0	0.083475	-0.869986	-1.988894

24	1	0	-0.730036	-1.576942	1.733942
25	1	0	-1.107322	0.155317	1.895872
26	1	0	3.390022	0.084999	2.081852
27	1	0	4.966712	0.584213	1.434554
28	1	0	4.665632	-1.119197	1.829102
29	1	0	1.211616	1.477601	-0.966837
30	1	0	-1.216944	1.366308	-1.323572
31	1	0	-4.163318	0.703940	1.740337
32	1	0	-4.751058	0.647924	0.094039
33	1	0	-4.482312	-1.632475	0.383810
34	1	0	-3.195902	-1.476624	1.565248
35	1	0	-2.450380	-2.427085	-0.726830
36	1	0	-3.716594	-0.059079	-1.935640
37	1	0	-2.164793	-0.662447	-2.560487
38	1	0	-1.721705	3.171927	0.266833
39	1	0	-3.144059	3.002568	1.440812

References

[1] Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Mo ro ku ma , K . ; Z a kr ze w sk i, V. G .; Vo th, G. A.; Sa lv a d or , P .; Dannenberg,J.J.; Dapprich,S.; Daniels,A.D.;Farkas,O; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian 09, Revision C1; Gaussian, Inc.: Wallingford, CT, 2010. [4]. Bruhn, T.; Hemberger, Y.; Schaumlöffel, A.; Bringmann, G. *Spec Dis*, version 1.51, University of Würzburg, Germany, 2010.

[2] Bruhn, T.; Schaumlöffel, A.; Hemberger, Y.; Bringmann, G. Quantifying the Comparison of Calculated and Experimental Electronic Circular Dichroism Spectra, Chirality 2013, 25, 243–249.

Table S3. 2D Structures of **2a**

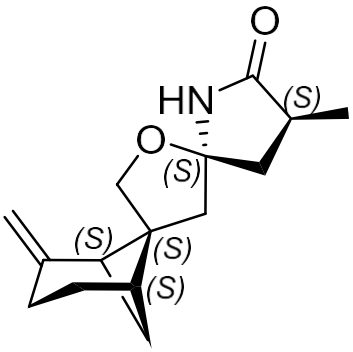
label	structure
2a	

Table S4 B3LYP/6-31++G (d, p) optimized lowest energy 3D conformers of **2a**.

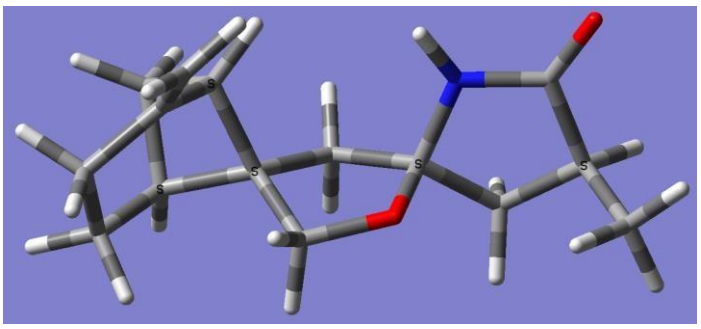
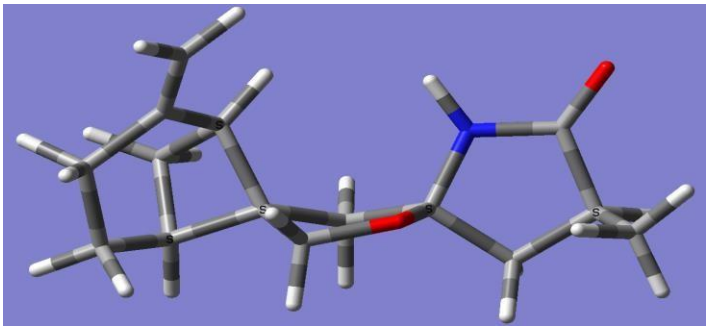
label	conformer	Boltzmann weighting factors
2a-1		50.00
2a-2		50.00

Figure S1 Calculated and experimental ECD spectra of **2a**.

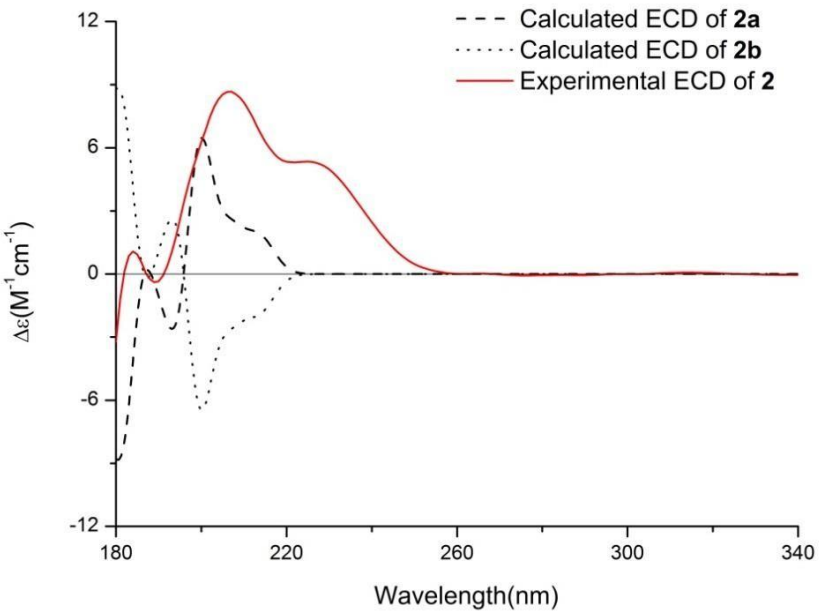


Figure S2. Calculated and experimental ECD spectra of **2**

pH-, H_2O- and temperature-dependent epimerization effect

pH-dependent epimerization effect:

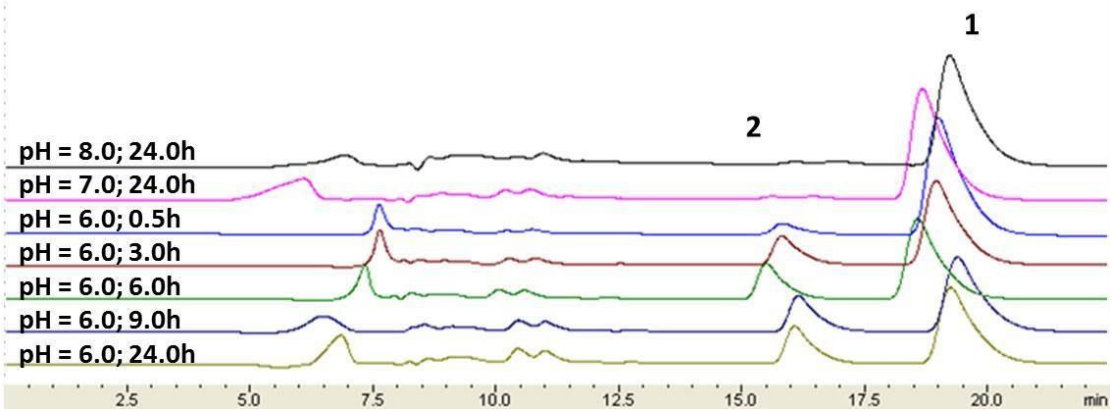


Figure S3. Dynamic chiral HPLC spectra of compound **1** dissolved in anhydrous ethanol at pH = 8.0, 7.0, 6.0.

Temperature-dependent epimerization effect:

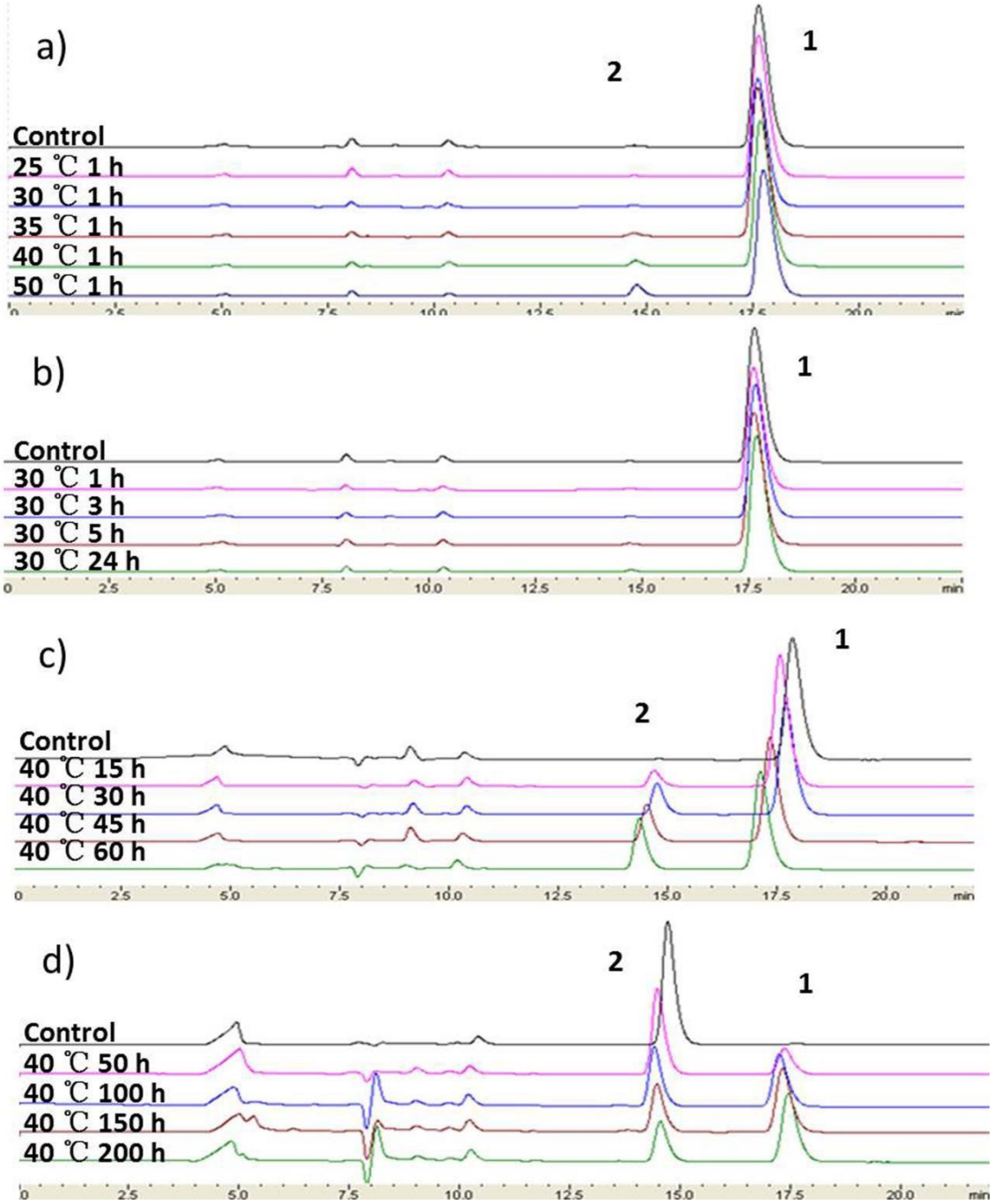


Figure S4. Dynamic chiral HPLC spectra: a) **1** dissolved in anhydrous ethanol heating at 25, 30, 35, 40, 50 °C; b) **1** dissolved in anhydrous ethanol heating at 30 °C; c) **1** dissolved in anhydrous ethanol heating at 40 °C; d) **2** dissolved in anhydrous ethanol heating at 40 °C

H_2O-dependent epimerization effect:

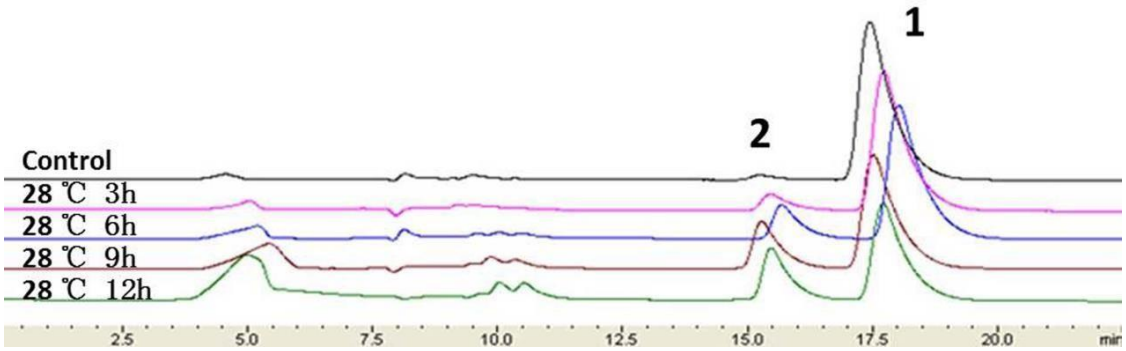


Figure S5. Dynamic chiral HPLC spectra of **1** dissolved in H_2O heating at 28 °C

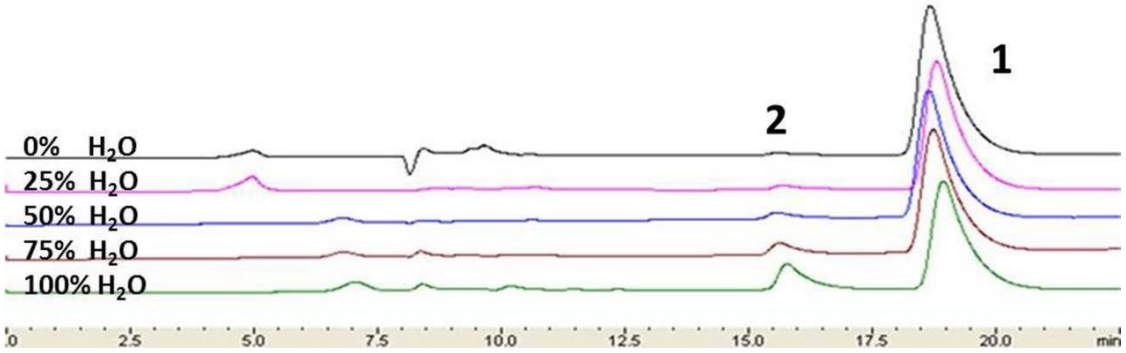
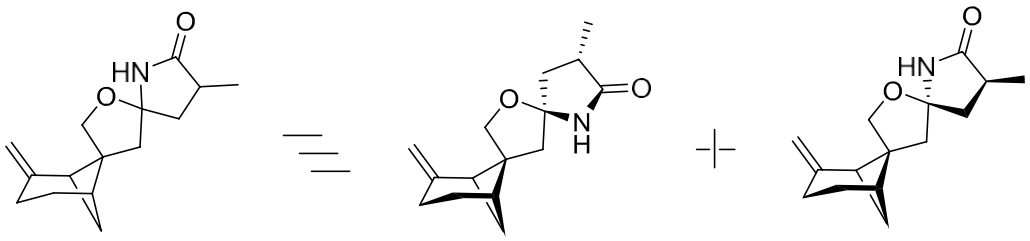
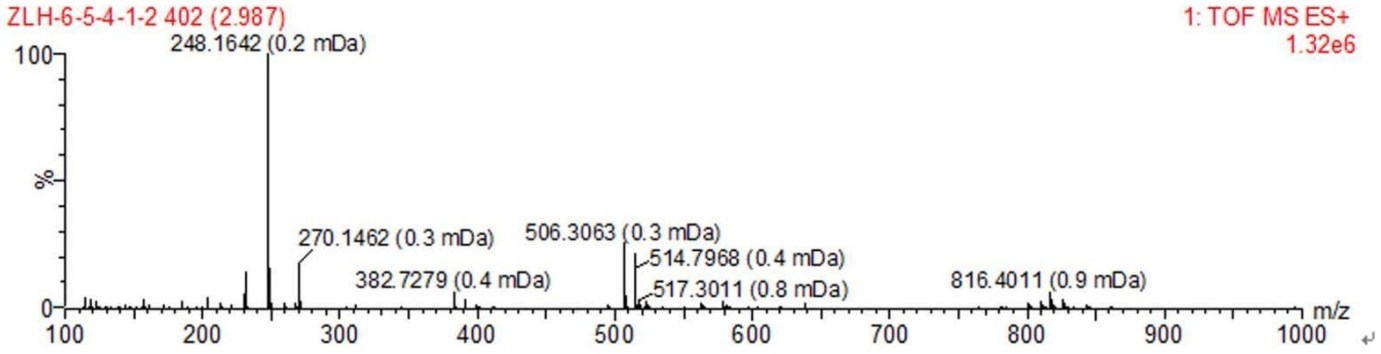


Figure S6. Chiral HPLC chromatogram of 1 dissolved in the mixed solution of Ethanol and H2O heating at 30 °C for 4h

Figure S7. HR ESI-TOF MS spectrum for the mixture of 1 and 2 in CH_3OH

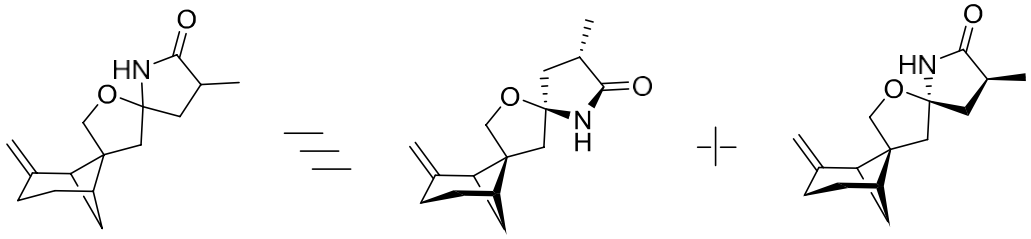


The **1**/**2** mixture (5:2) sporulosine A (**1**) sporulosine B (**2**)



m/z	248.1642	$C_{15}H_{22}NO_2$	$[M+H]^+$
m/z	270.1462	$C_{15}H_{21}NO_2Na$	$[M+Na]^+$

Figure S8. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum for the mixture of 1 and 2



The **1**/**2** mixture (5:2) sporulosine A (**1**) sporulosine B (**2**)

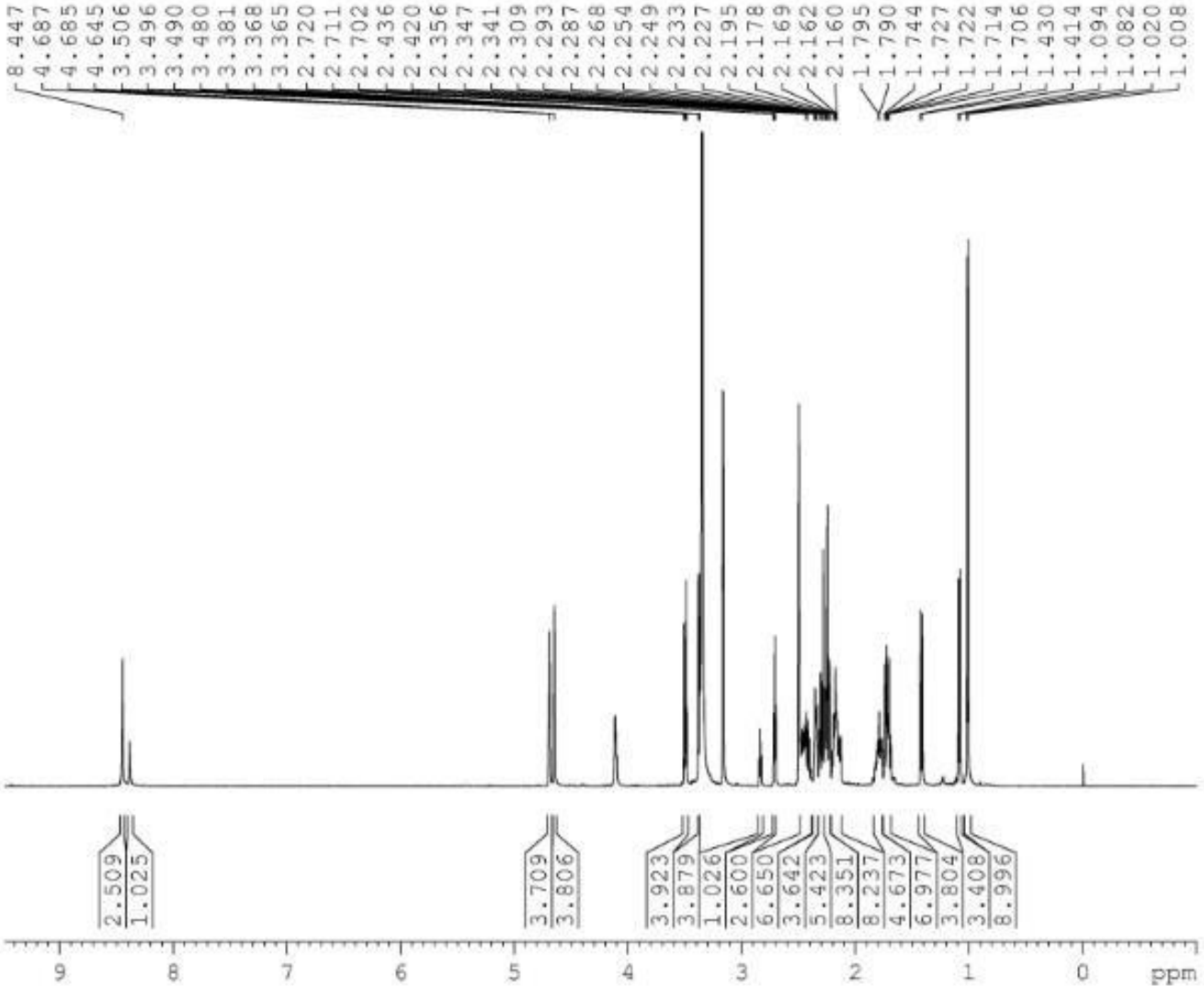
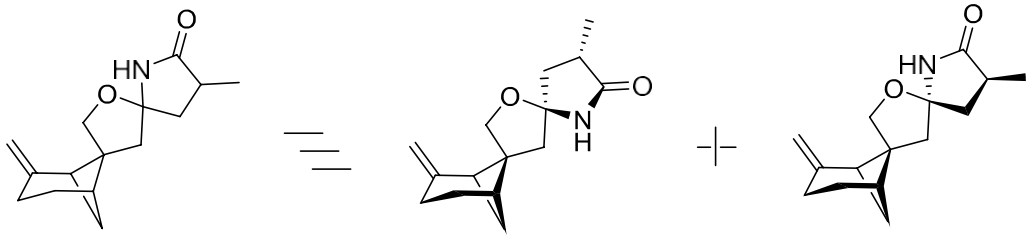


Figure S9. ^{13}C NMR (100 MHz, DMSO-d_6) spectrum for the mixture of 1 and 2



The **1**/**2** mixture (5:2) sporulosine A (**1**) sporulosine B (**2**)

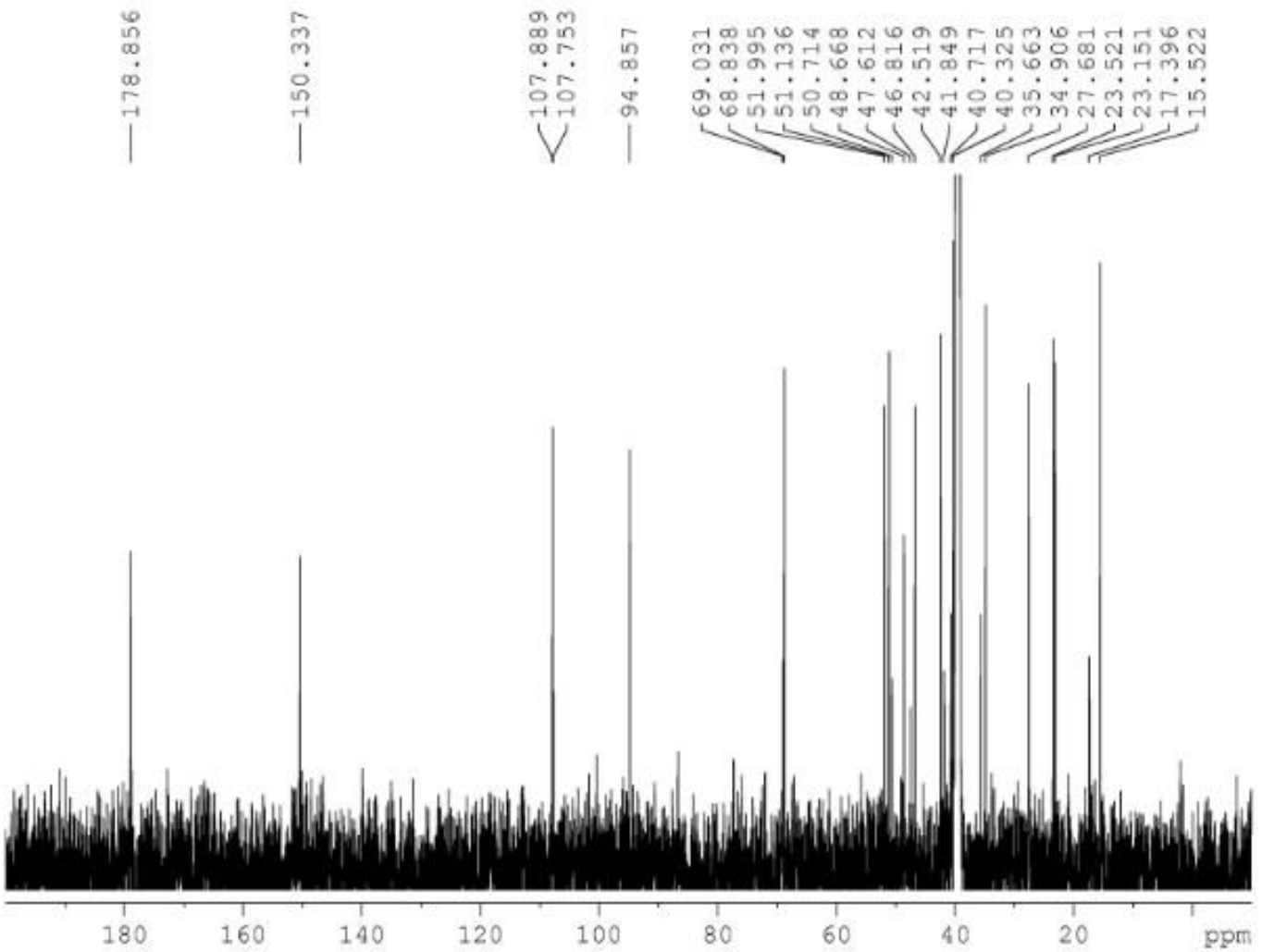
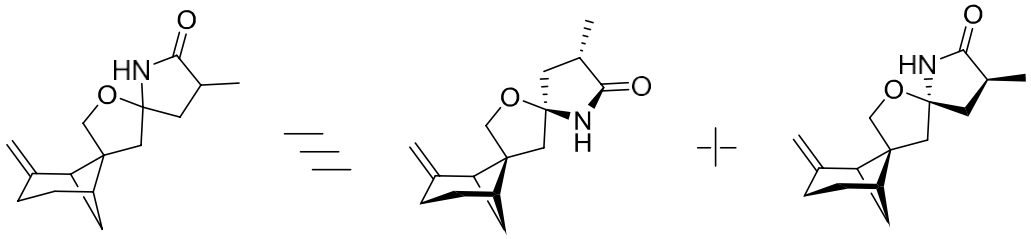


Figure S10. HSQC (600 MHz, DMSO-d_6) spectrum for the mixture of 1 and 2



The **1**/**2** mixture (5:2) sporulosine A (**1**) sporulosine B (**2**)

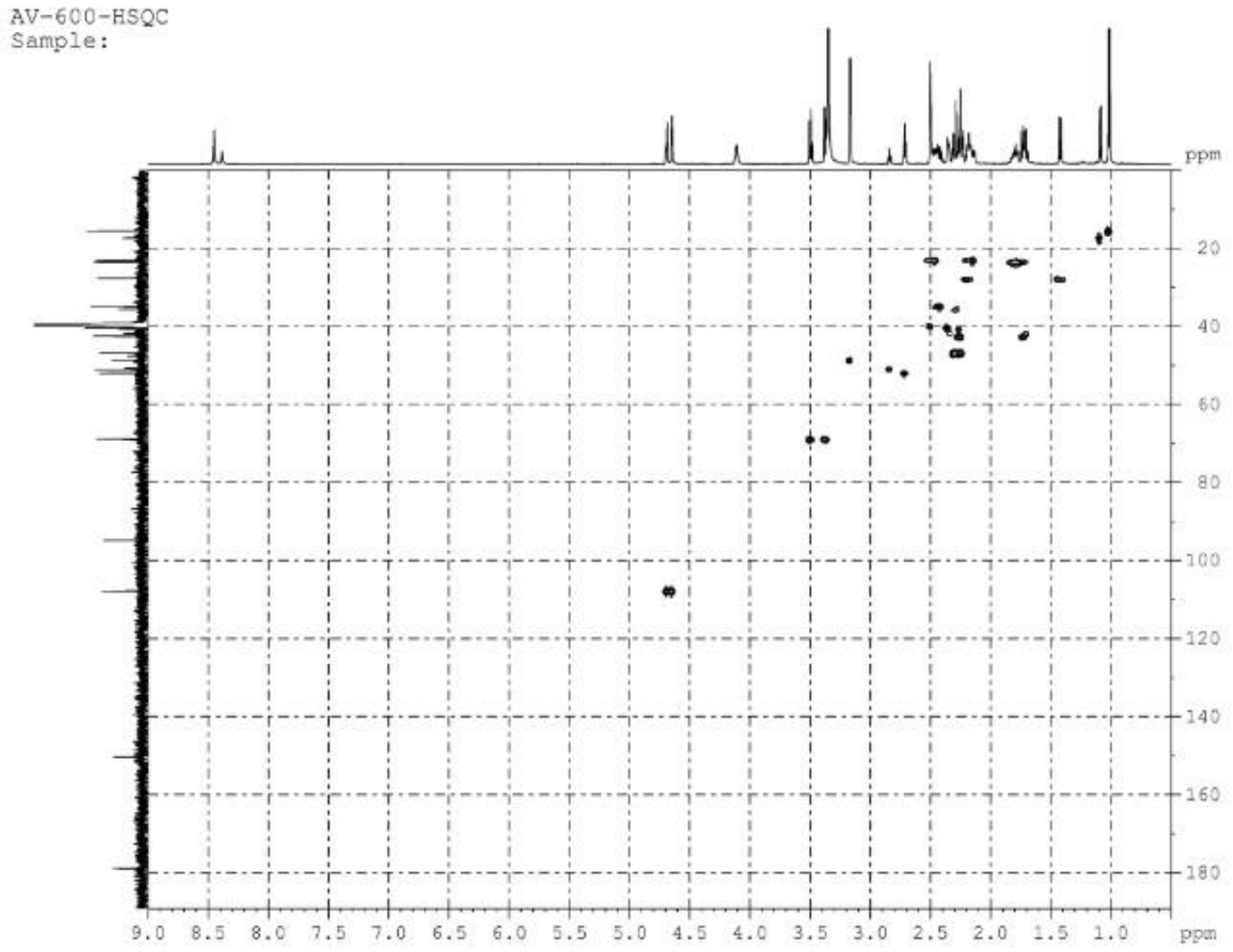
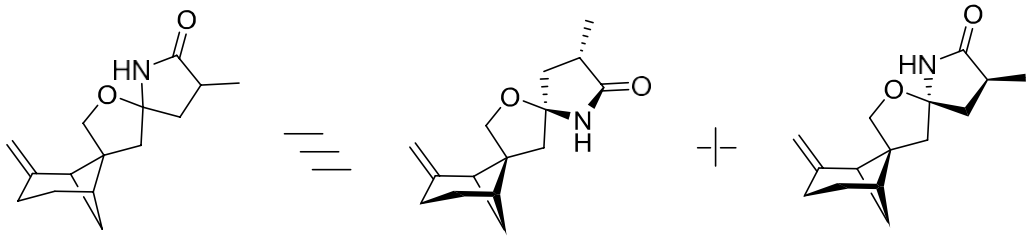


Figure S11. HMBC (600 MHz, DMSO-d_6) spectrum for the mixture of 1 and 2



The **1**/**2** mixture (5:2) sporulosine A (**1**) sporulosine B (**2**)

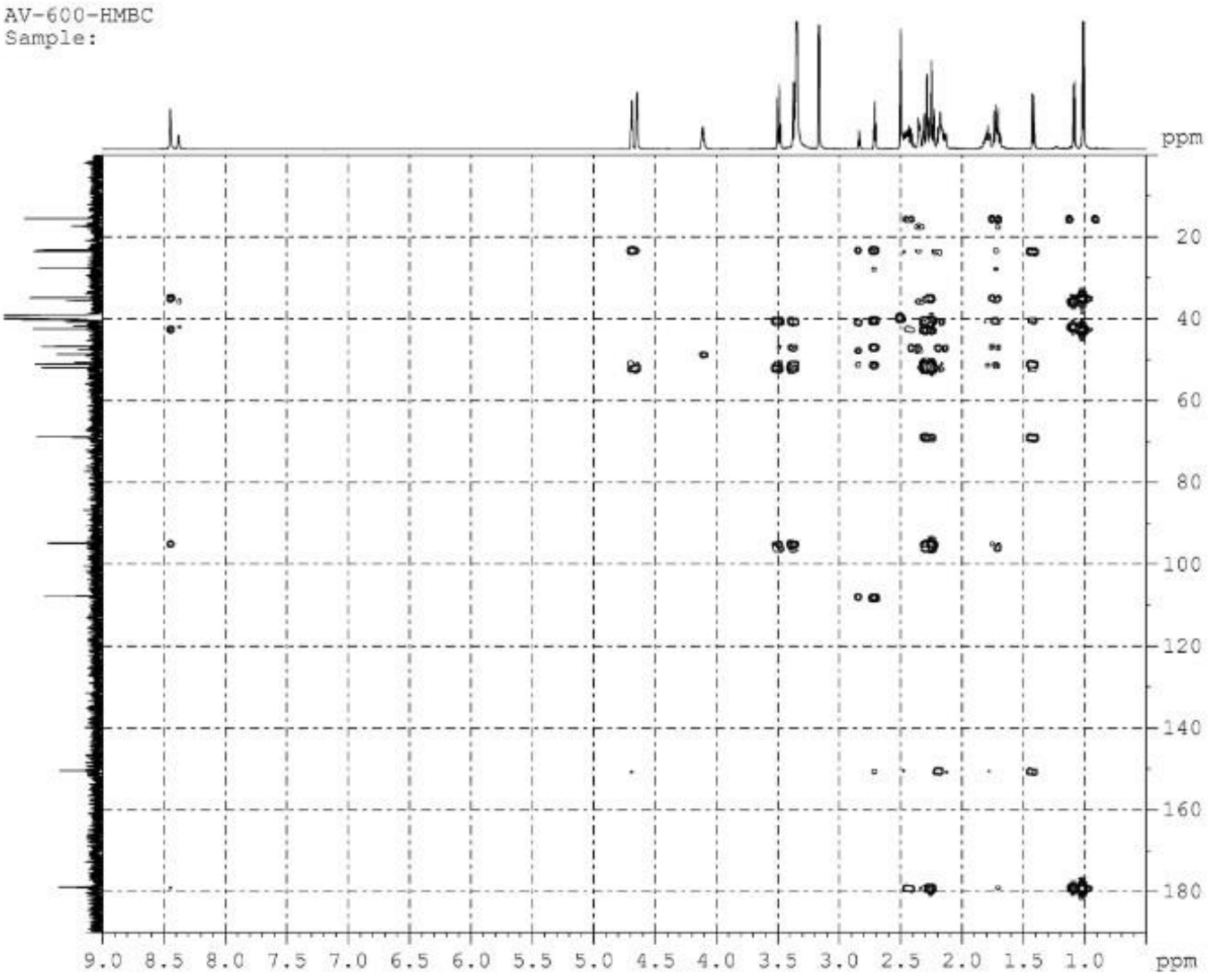
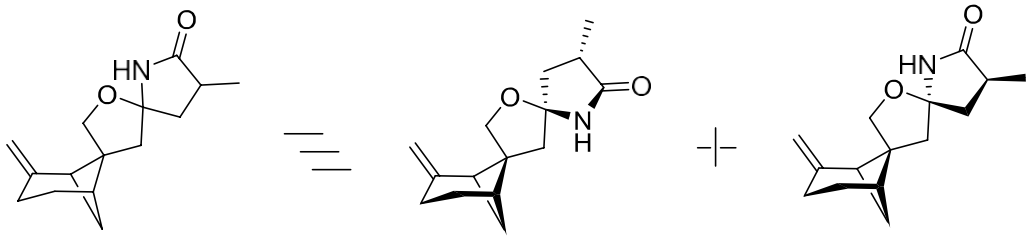


Figure S12. NOESY (600 MHz, DMSO-d_6) spectrum for the mixture of 1 and 2



The **1**/**2** mixture (5:2) sporulosine A (**1**) sporulosine B (**2**)

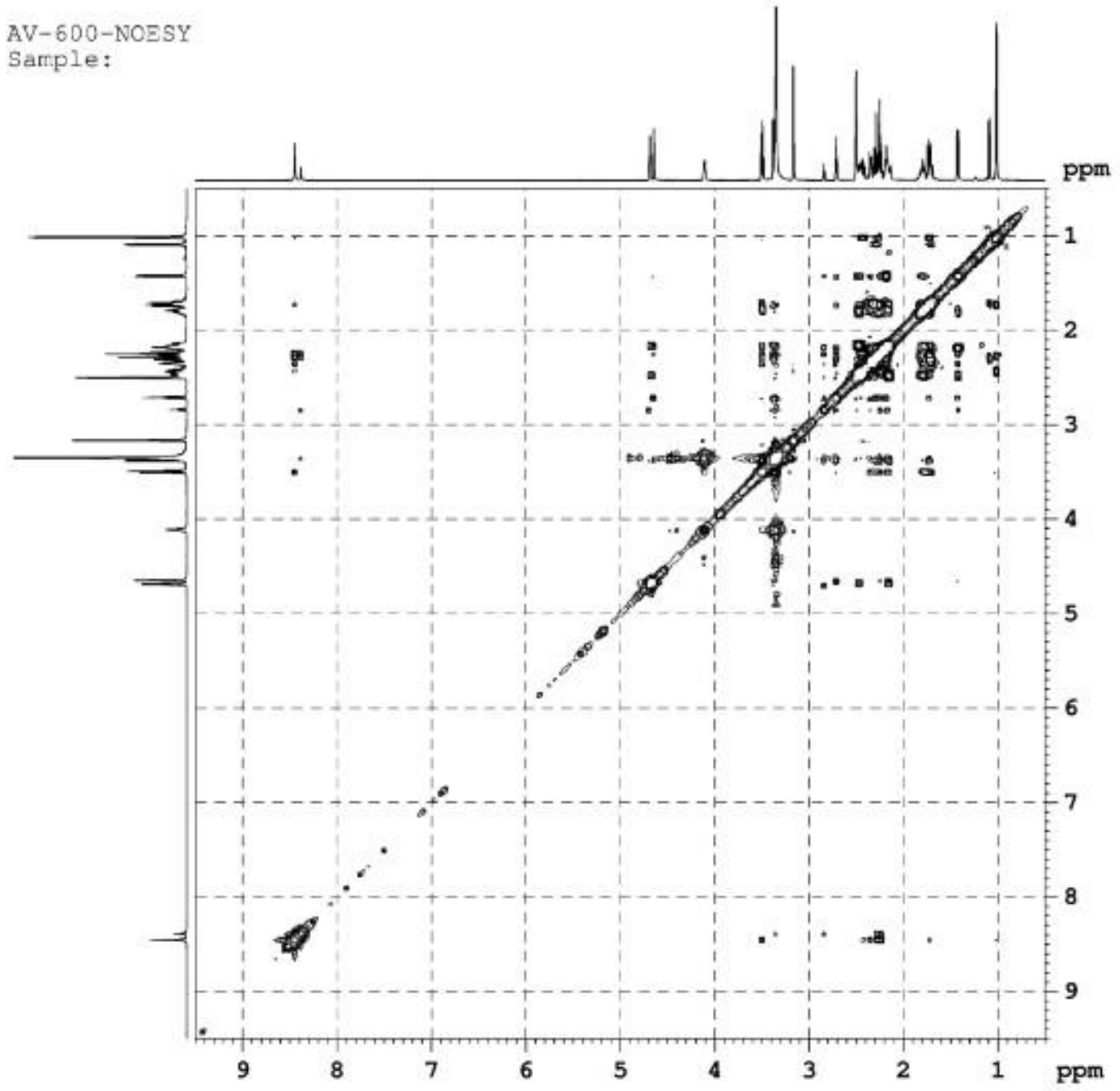
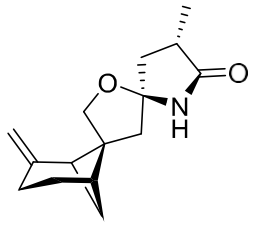


Figure S13. HR ESI-TOF MS spectrum of compound 1 in CH_3OH



sporulosine A (**1**)

Mass Spectrum Molecular Formula Report

Analysis Info

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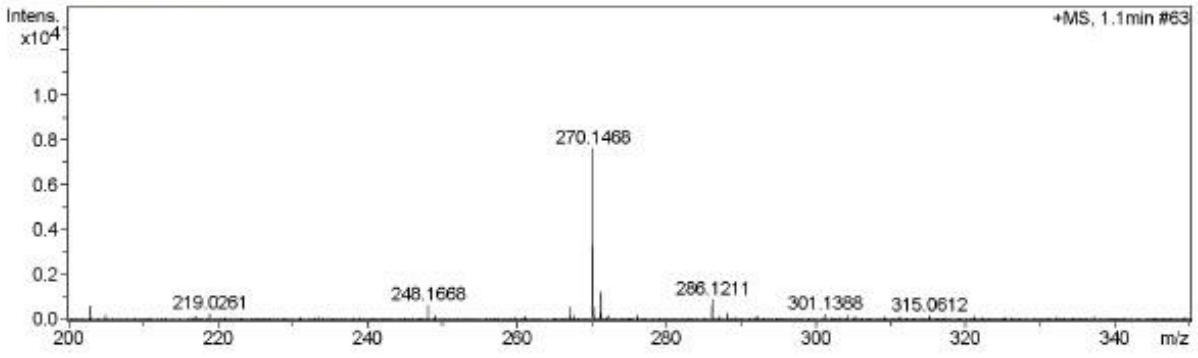
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source

Generate Molecular Formula Parameter

Formula, min.	C15H21N1O2Na						
Formula, max.							
Measured m/z	270.147	Tolerance	5	ppm	Charge	1	
Check Valence	no	Minimum	0		Maximum	0	
Nirogen Rule	yes	Electron Configuration	both				
Filter H/C Ratio	yes	Minimum	0		Maximum	3	
Estimate Carbon	yes						



Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 15 H 21 N 1 Na 1 O 2	0.007	270.1465	-1.42	-1.09	-0.38	5.50	ok	even

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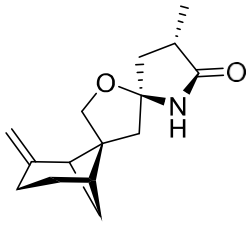
Figure S14. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound 1



sporulosine A (**1**)

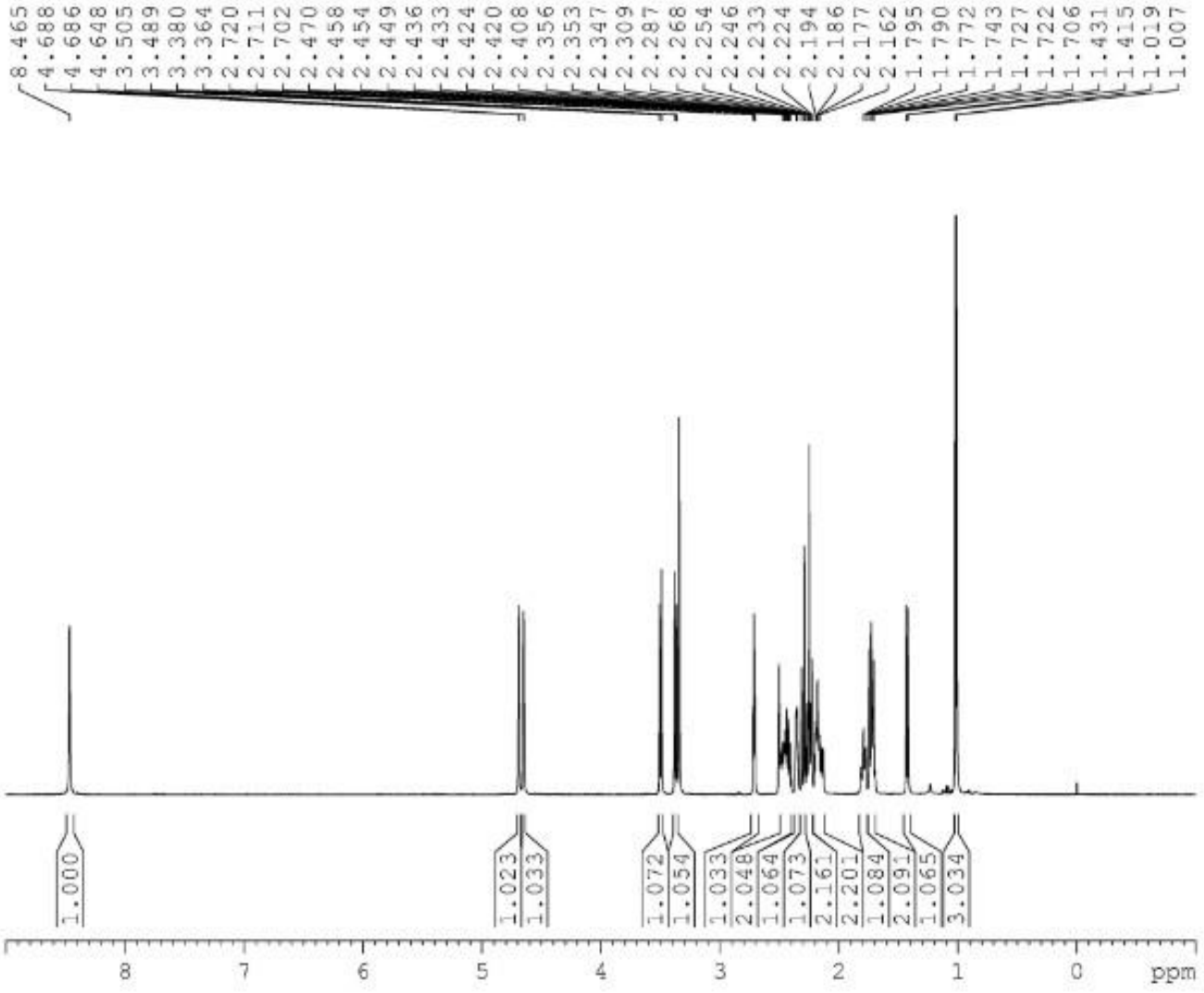
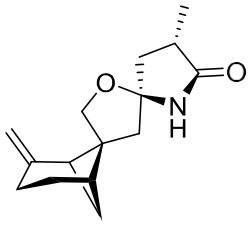


Figure S15. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of compound 1



sporulosine A (**1**)

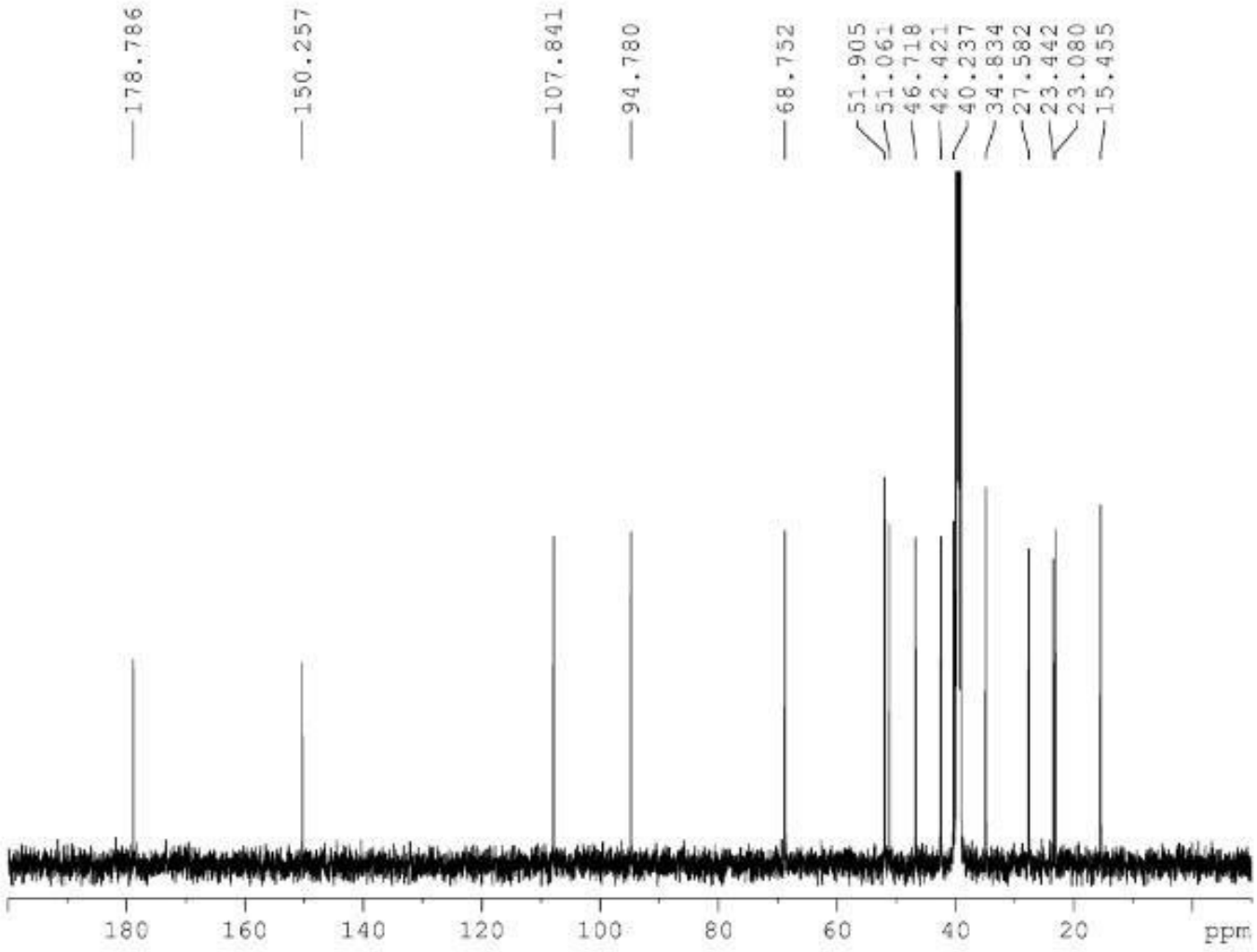
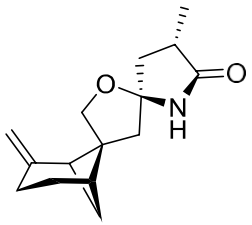


Figure S16. The HSQC correlations of compound 1 in HSQC (600 MHz, DMSO-d_6) spectrum of the 1/2 mixture



sporulosine A (**1**)

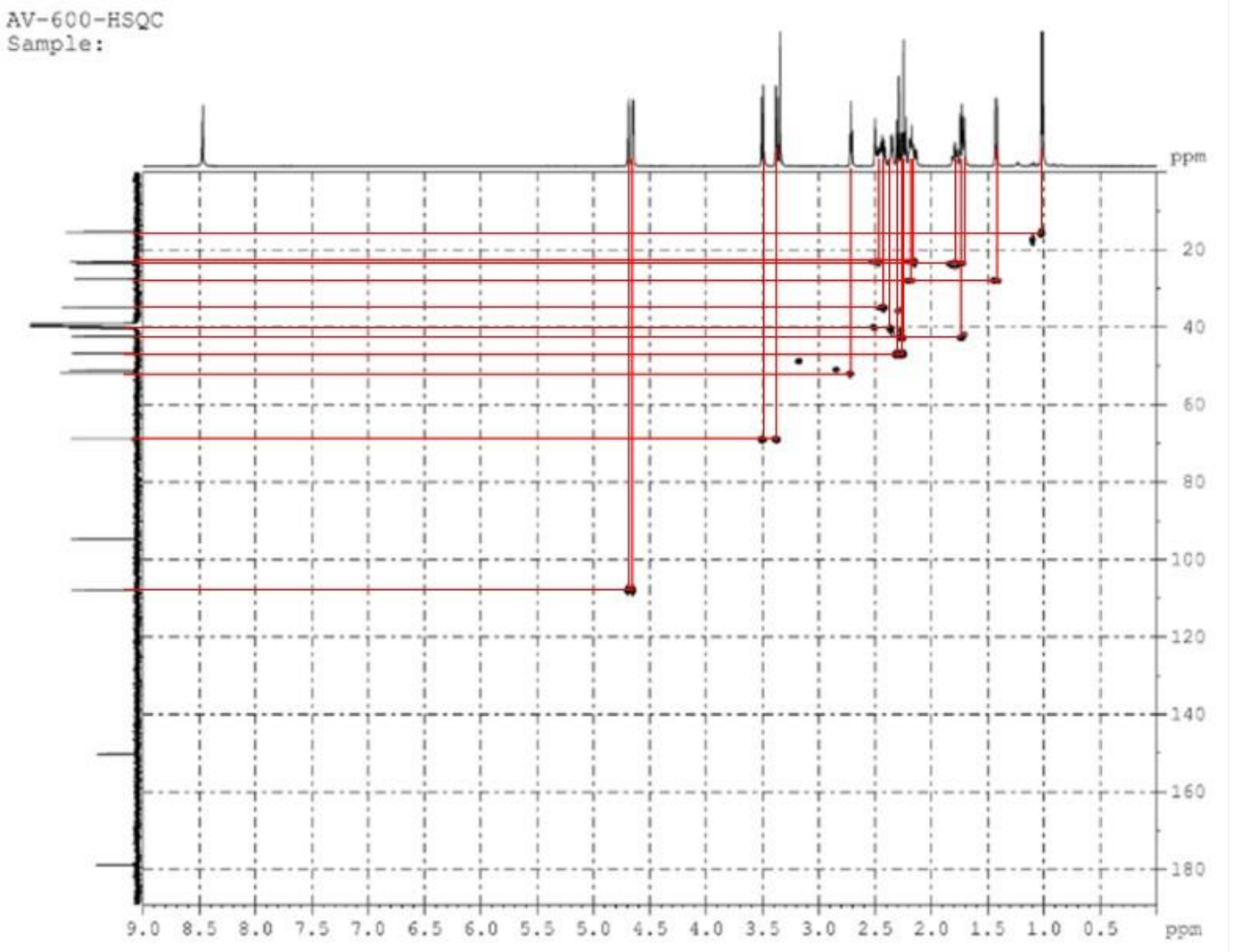
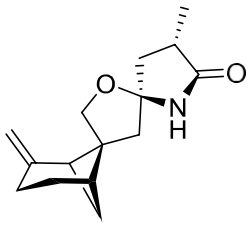


Figure S17. The HMBC correlations of compound 1 in HMBC (600 MHz, DMSO-d_6) spectrum of the 1/2 mixture



sporulosine A (**1**)

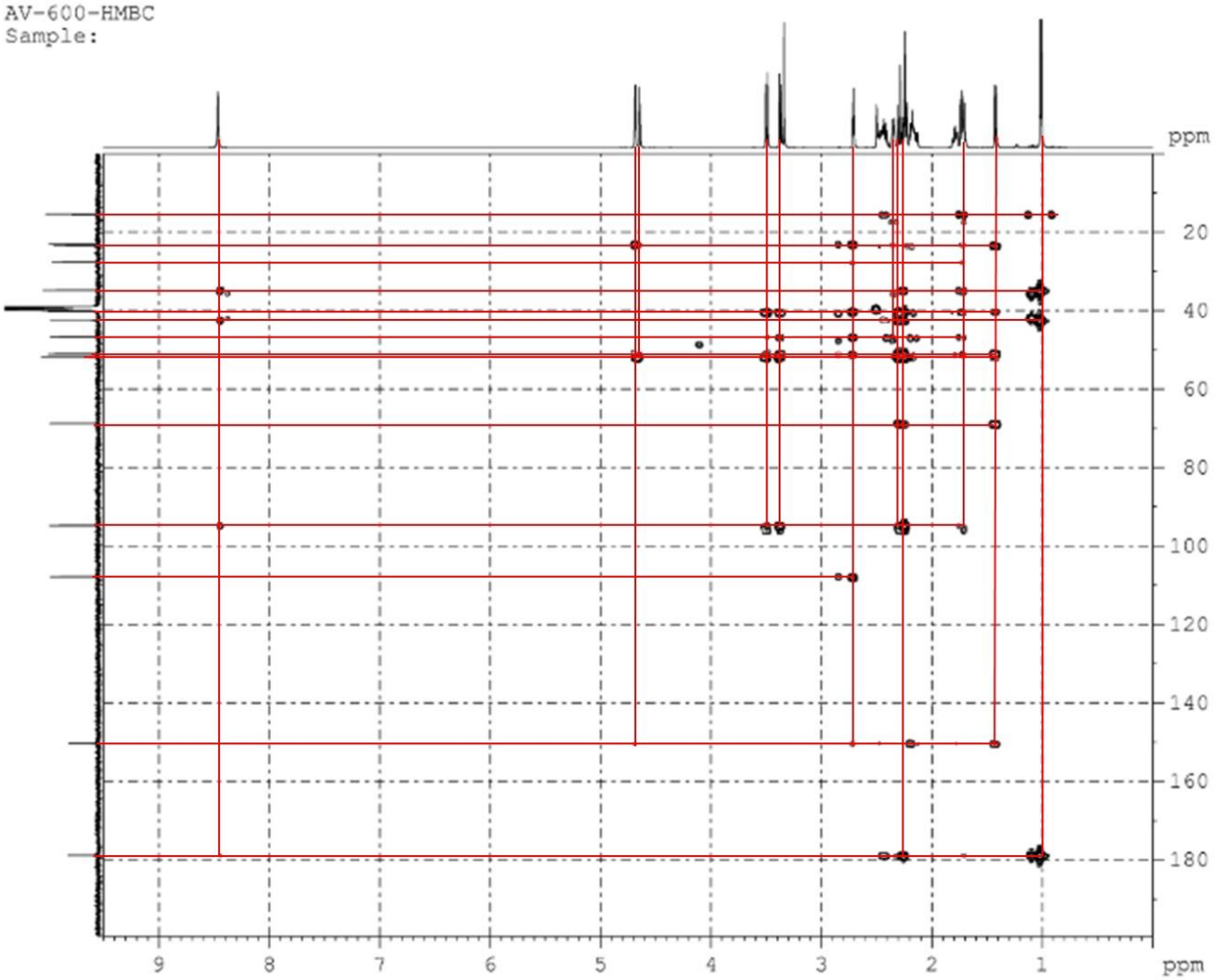
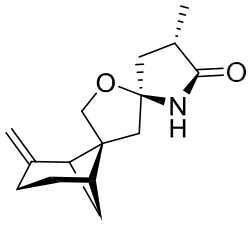


Figure S18. NOESY (600 MHz, DMSO-d_6) spectrum of compound 1



sporulosine A (**1**)

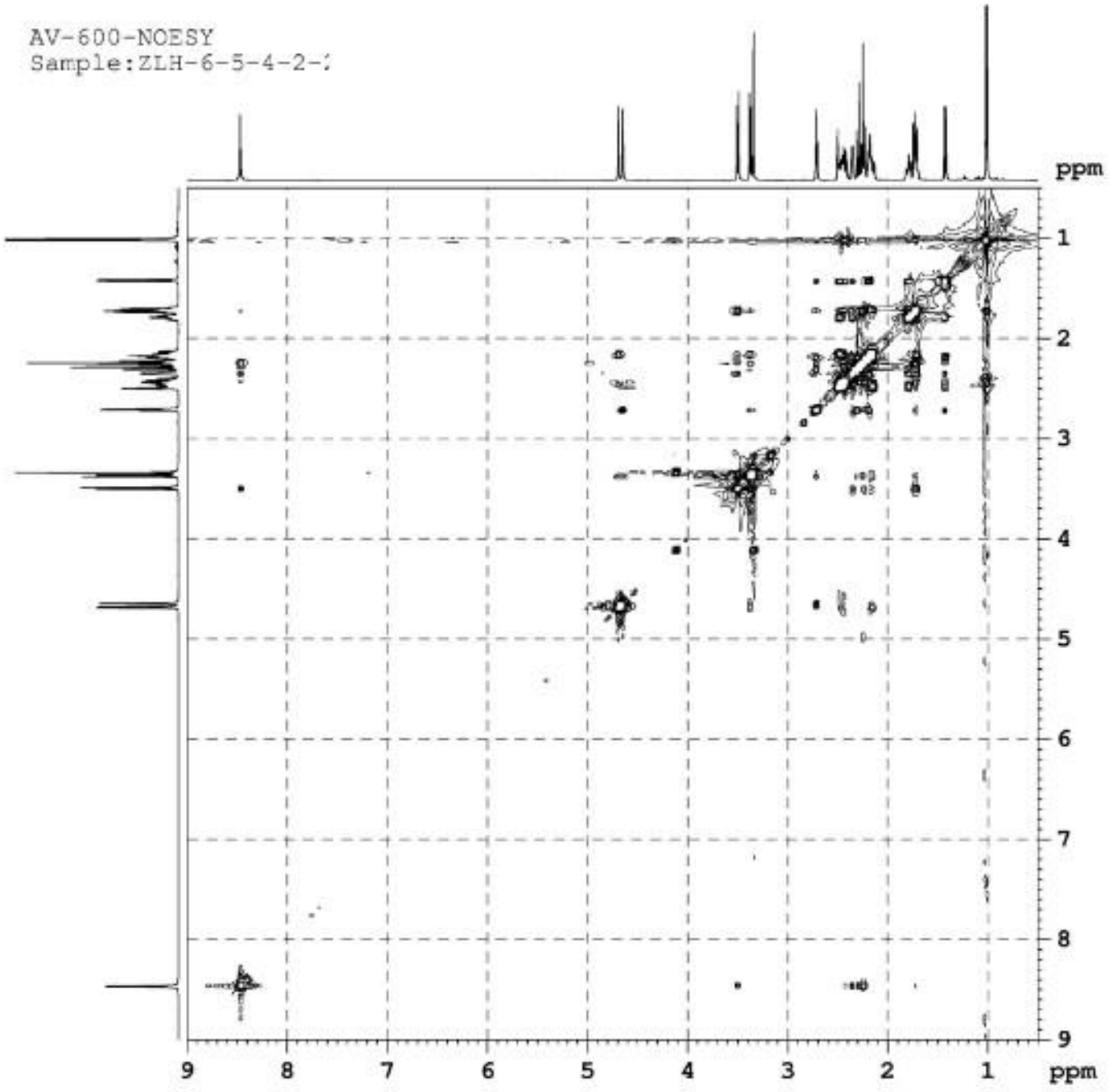
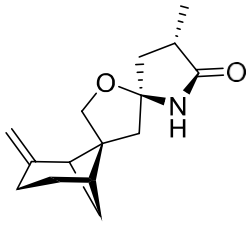


Figure S19. IR spectrum of compound 1



sporulosine A (**1**)

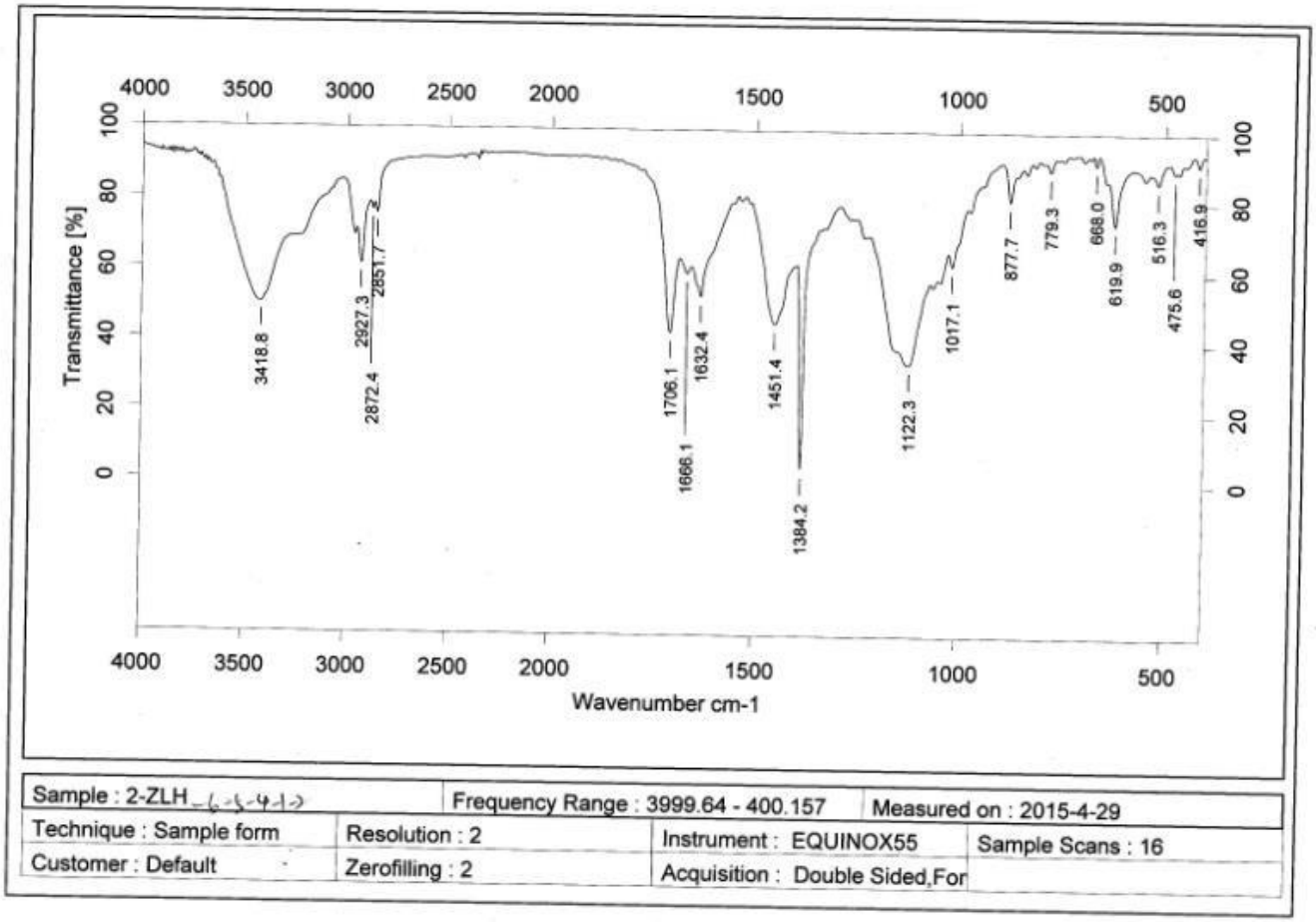
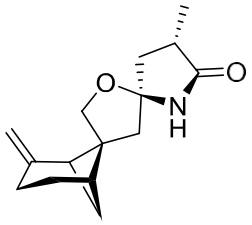
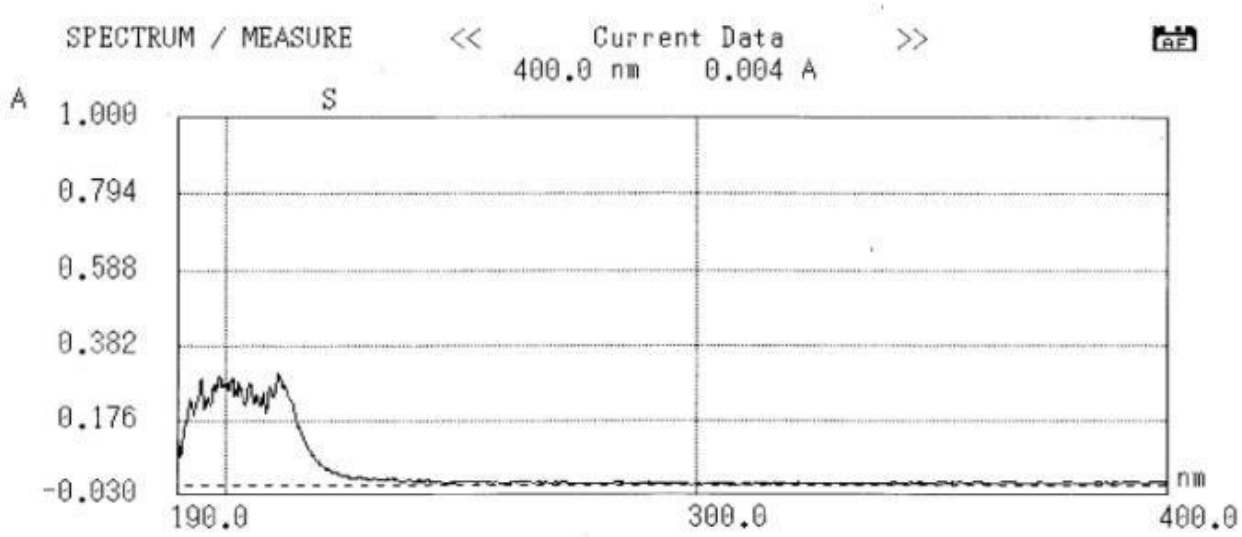


Figure S20. UV spectrum of compound 1



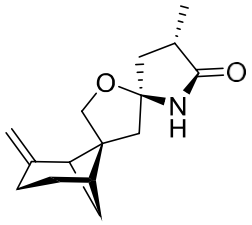
sporulosine A (**1**)



> Set sample then press [Start Scan].
eEnlarge/Reduce File (SP) Rec. Output Set Parameters Start Scan

NO.	ABSCISSA	PEAK	HEIGHT	ABSCISSA	VALLEY	HEIGHT
1	211.0	0.3096	0.0816	208.6	0.1987	-0.0726
2	205.2	0.2783	0.0807			
3	192.4	0.2341	0.0690			

Figure S21. CD spectrum of compound 1



sporulosine A (**1**)

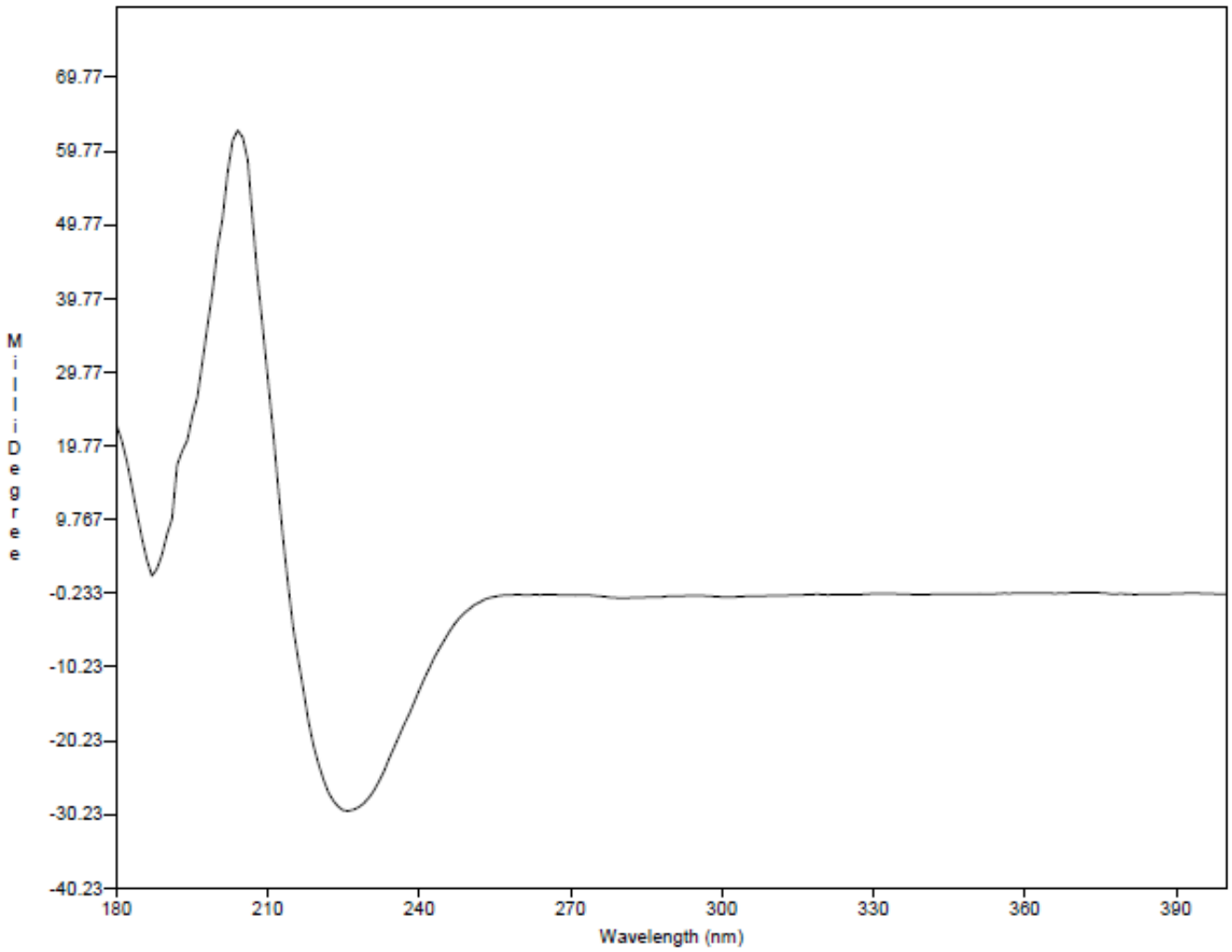
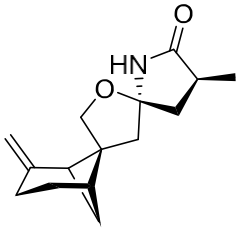


Figure S22. HR ESI-TOF MS spectrum of compound 2 in CH_3OH



sporulosine B (**2**)

Mass Spectrum Molecular Formula Report

Analysis Info

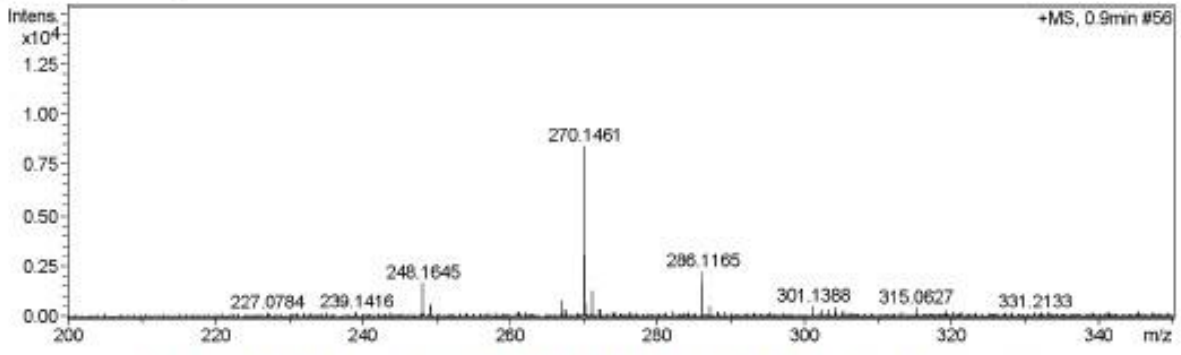
Analysis Name	D:\Data\20151230CEYANG\ZLH-6-X.d	Acquisition Date	12/30/2015 2:16:51 PM
Method	tune_wide_pos.m	Operator	Bruker Customer
Sample Name	ZLH-6-X	Instrument / Ser#	micrOTOF-Q 125
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source

Generate Molecular Formula Parameter

Formula, min.	C8H21N1Na1O2						
Formula, max.							
Measured m/z	270.146	Tolerance	5	ppm	Charge	1	
Check Valence	no	Minimum	0		Maximum	0	
Nitrogen Rule	yes	Electron Configuration	both				
Filter H/C Ratio	yes	Minimum	0		Maximum	3	
Estimate Carbon	yes						



Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 15 H 21 N 1 Na 1 O 2	0.017	270.1465	1.44	2.95	0.39	5.50	ok	even

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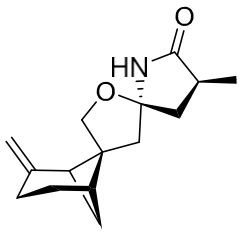
Figure S23. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound 2



sporulosine B (**2**)

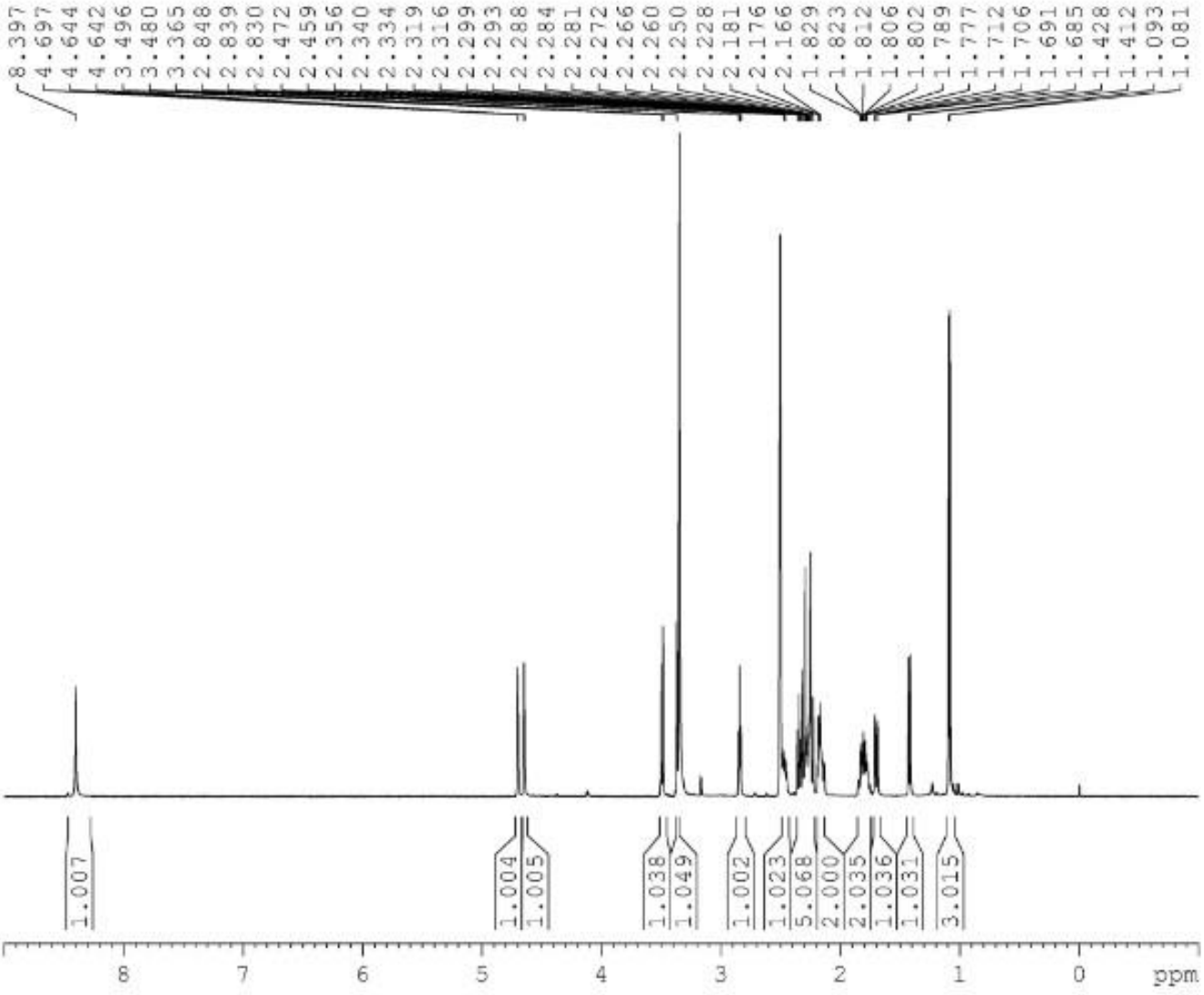
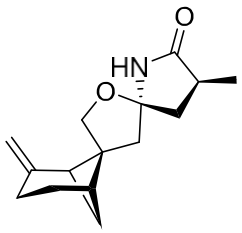


Figure S24. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of compound 2



sporulosine B (**2**)

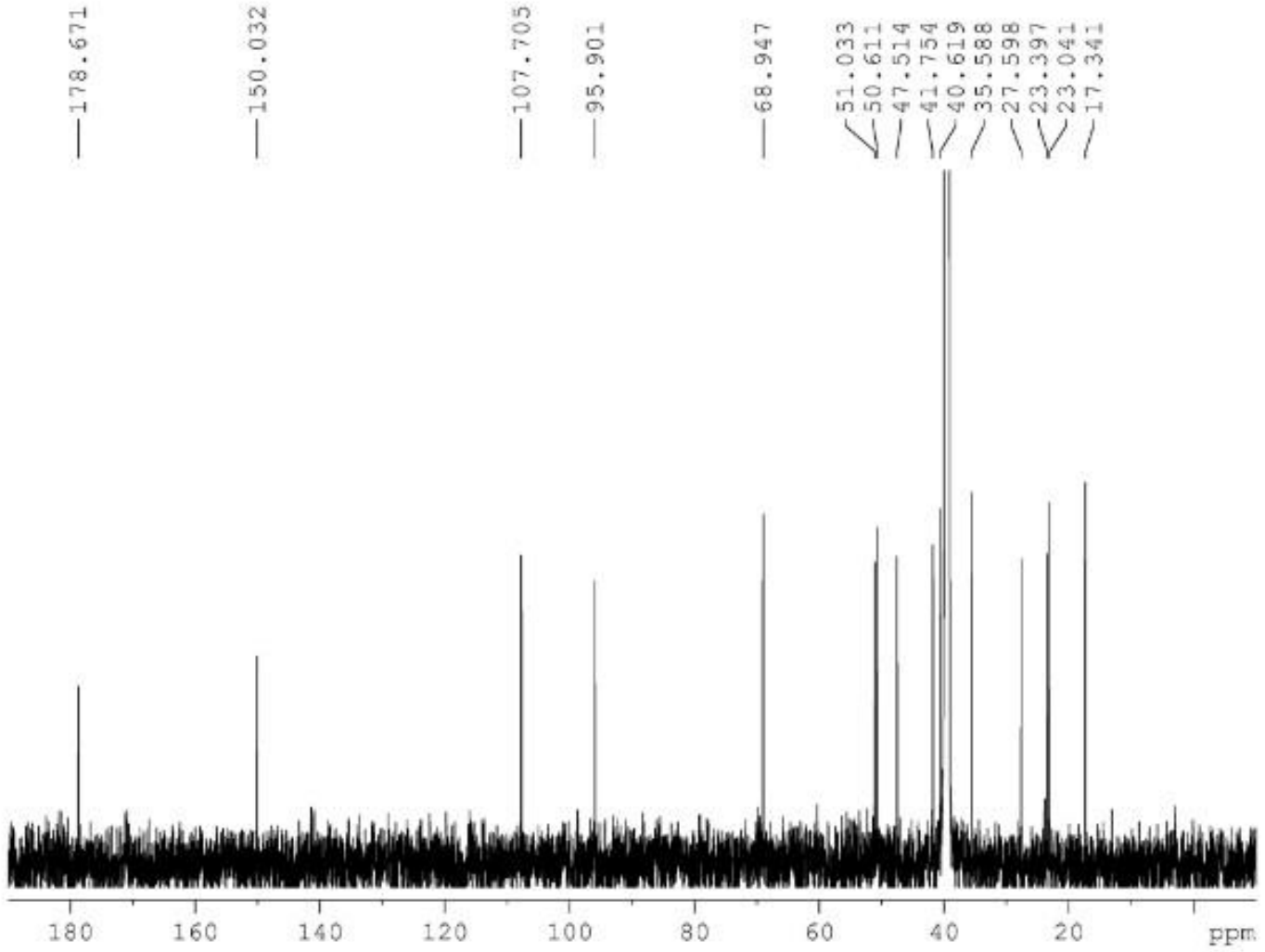
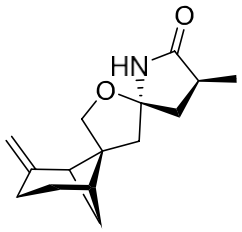


Figure S25. The HSQC correlations of compound 2 in HSQC (600 MHz, DMSO-d_6) spectrum of the 1/2 mixture



sporulosine B (**2**)

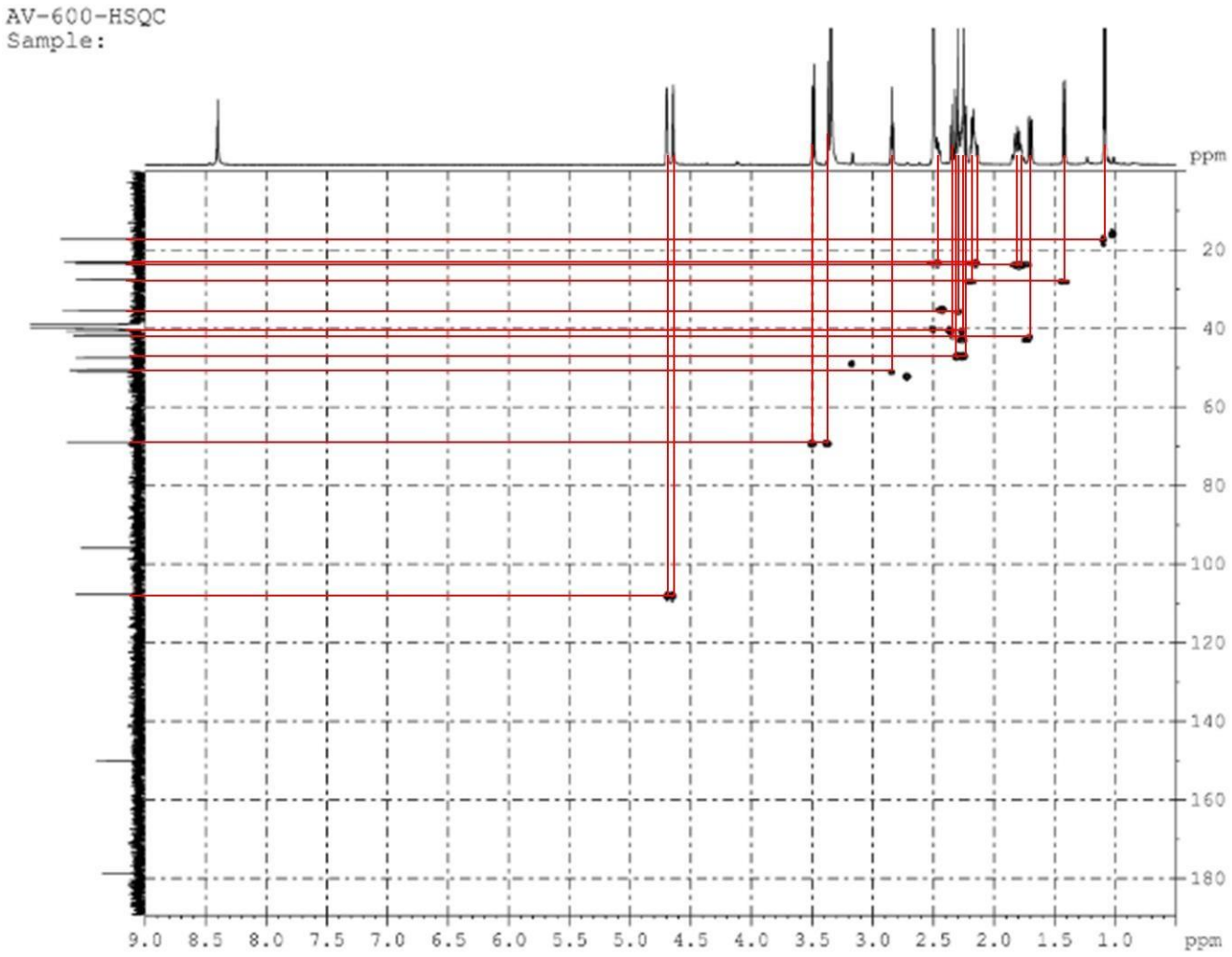
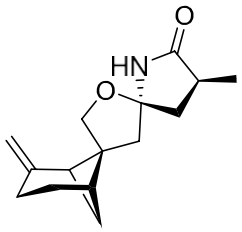


Figure S26. The HMBC correlations of compound 2 in HMBC (600 MHz, DMSO-d_6) spectrum of the 1/2 mixture



sporulosine B (**2**)

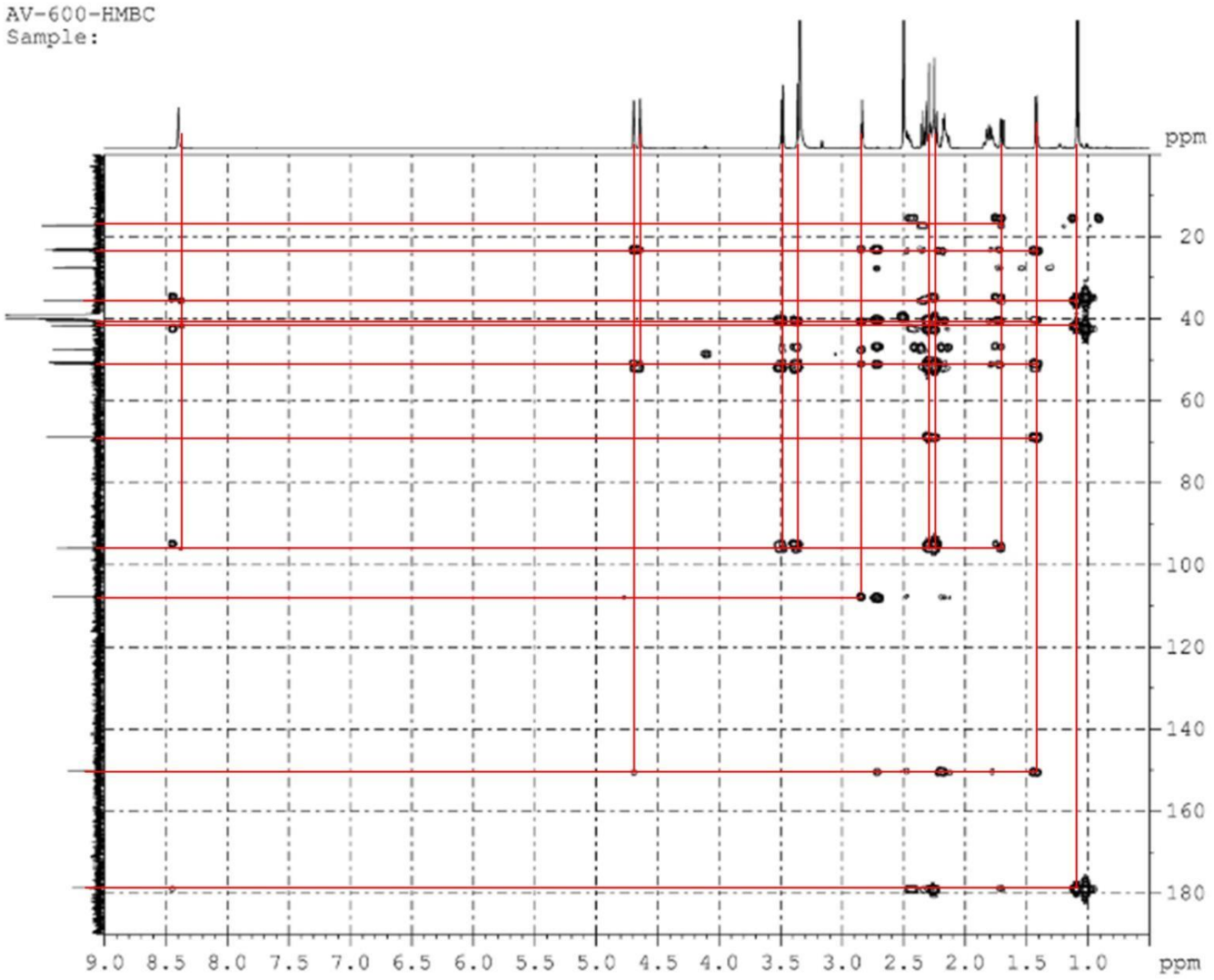
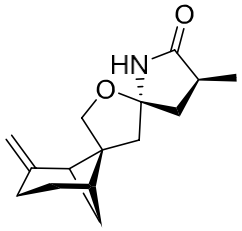


Figure S27. NOESY spectrum (600 MHz, DMSO-d_6) of compound 2



sporulosine B (**2**)

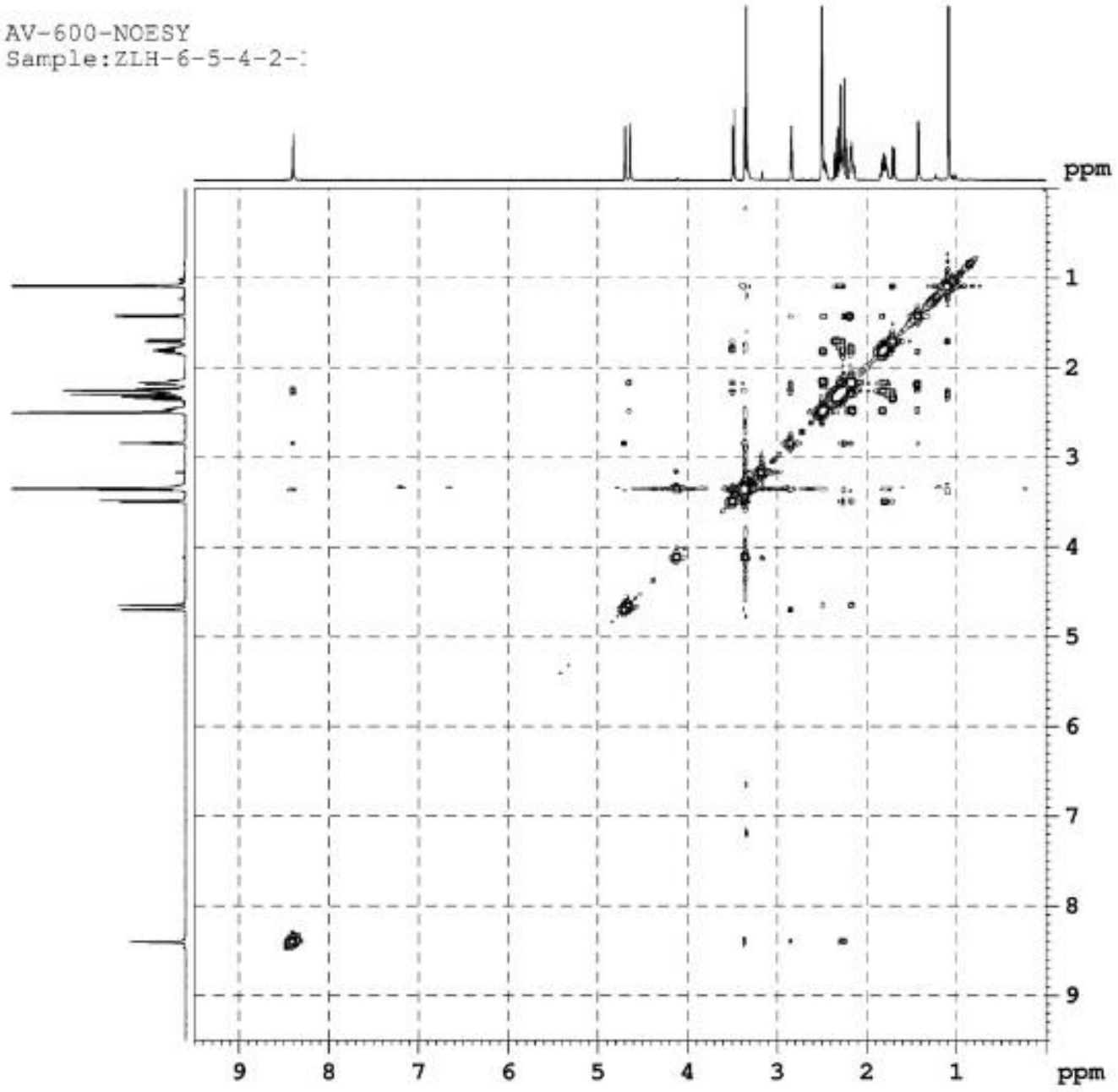
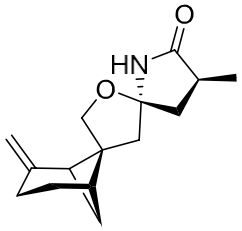


Figure S28. IR spectrum of compound 2



sporulosine B (**2**)

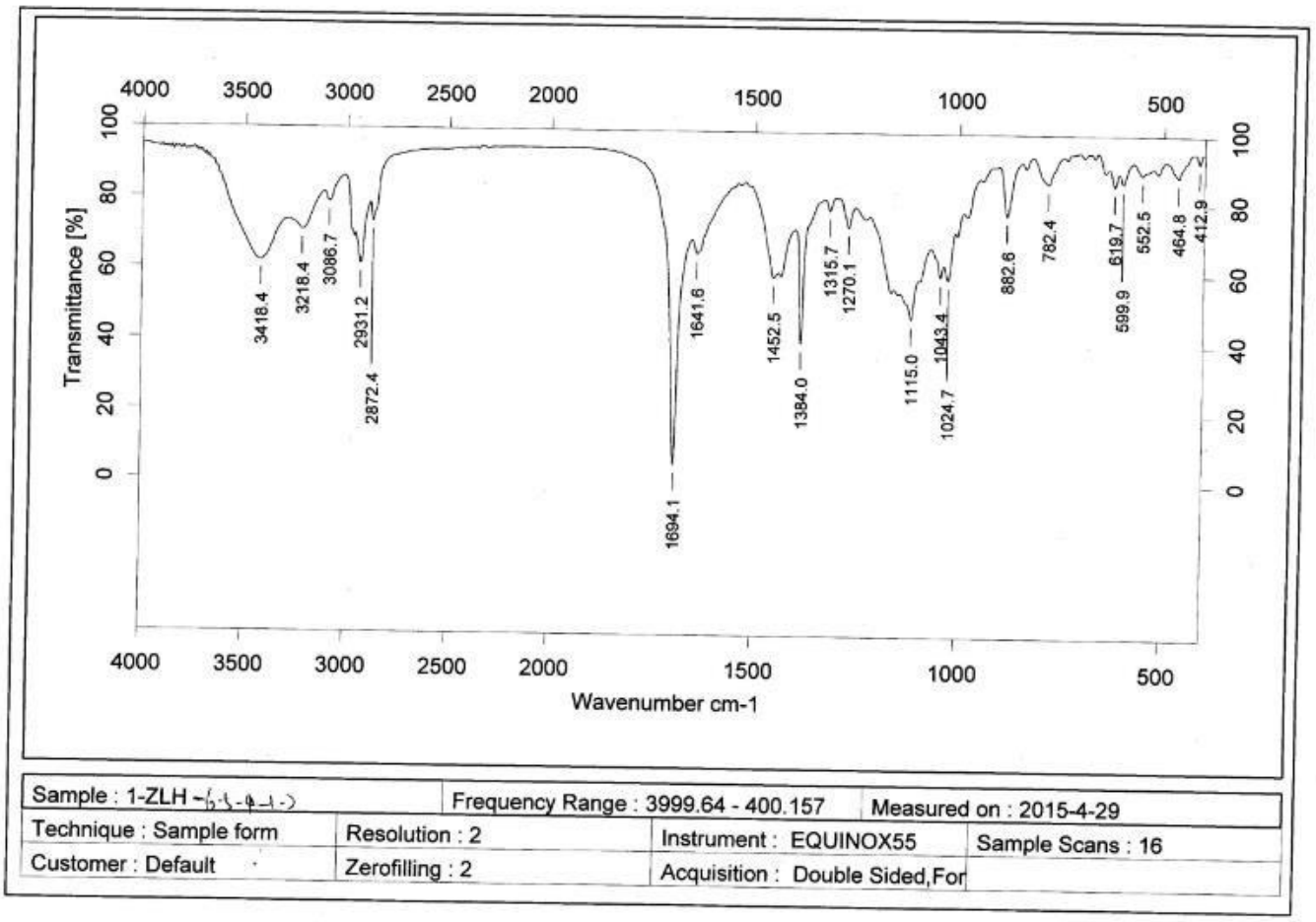
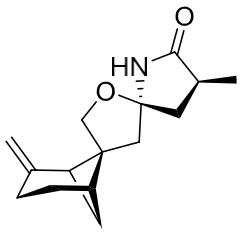
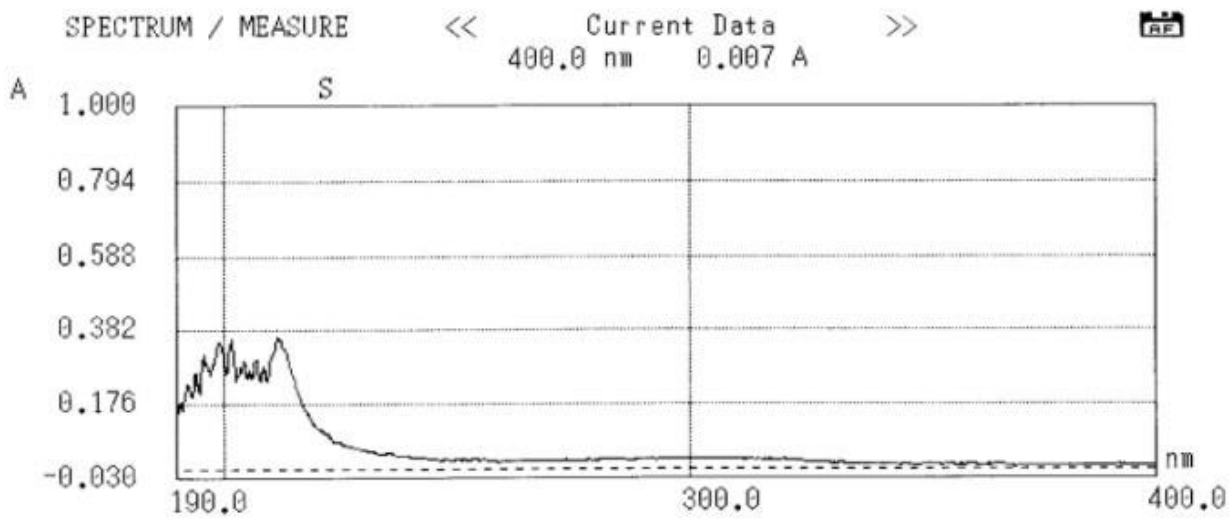


Figure S29. UV spectrum of compound 2



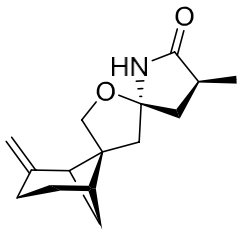
sporulosine B (**2**)



> Set sample then press [Start Scan].
Enlarge/Reduce File (SP) Rec. Output Set Parameters Start Scan

NO.	ABSCISSA	PEAK	HEIGHT	ABSCISSA	VALLEY	HEIGHT
1	211.4	0.3604	0.1474	207.8	0.2423	-0.1171

Figure S30. CD spectrum of compound 2



sporulosine B (**2**)