Supporting Information

Nesteretal A, a Novel Class of Cage-like Polyketide from Marine-derived Actinomycete *Nesterenkonia halobia*

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1. Isolation Procedure

General Information

Optical rotations were measured with an Anton Paar MCP500 polarimeter at 20 °C. IR spectra (KBr disks, in cm^{-1}) were obtained on a Bruker Tensor 27 spectrometer. HRESIMS were performed on a Xevo G2 Q-TOF mass spectrometer (Wates). NMR spectra including ^{1}H, ^{13}C, Dept, HSQC, COSY, HMBC, and NOESY were recorded on a Bruker Avance 400 MHz spectrometer with TMS as an internal standard. Chemical shifts were reported in ppm using the signal of the residual solvent DMSO-d_5 (δ_H 2.50 for ^{1}H and δ_C 39.5 for ^{13}C) as an internal reference. Materials for column chromatography (CC) was performed on ODS (50 μm, Daiso, Japan), silica gel (200–300 mesh, Qingdao Marine Chemistry Co. Ltd. China), and Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala, Sweden).

Strain Material

The strain E5.1 was isolated from marine-derived scleractinian coral *Platygyra* from the Qiongdong of China Hainan and frozen immediately after collection. The 16S rDNA of producing strain E5.1 was amplified by polymerase chain reaction (PCR) and sequenced. It was closely related to *Nesterenkonia halobia* DSM 20541(T) with a 16S rRNA gene sequence similarity value of 99.85% based on the Basic Logic Alignment Search Tool (BLAST) sequence comparison.

Fermentation, Extraction and Isolation

The actinomycete *N. halobia* E5.1 was cultured on the 2216E (Shanghai Yuanye Bio-Technology Co., Ltd.) plate at 28 °C for 5 d. A large-scale fermentation (40 L) by shake fermentation was performed in 18×5 L Erlenmeyer flasks each containing 2 L of ISP2 medium (0.4% yeast extract, 1.0% malt extract, 0.4% glucose, 3.0% marine salt, pH 7.4). After incubating at 28 °C on a rotary shaker (200 rpm) for 10 days, the culture (40 L) was centrifuged to yield the supernatant and a mycelia cake. The supernatant and mycelia cake were extracted with EtOAc and Me_2CO for three times, respectively. The organic solvents were evaporated and combined to give a total residue (26.0 g). The extract was column chromatographed (CC) over silica gel using a stepwise gradient of PE-Me_2CO (1:0→0:1), followed by CC on ODS (MeOH-H_2O, 1:1) and silica gel (CH_2Cl_2-Me_2CO, 5:1). Final purification by CC over Sephadex LH-20 (MeOH) provided **1** (10.5 mg).

2. Biological Assay

2.1 RXRα Transcriptional Activity Assay

The human renal epithelial cells were seeded to 96-well plates in DMEM containing 10% FBS at 37 °C. After 24 h, two target plasmids (30 ng pBind RXRα LBD and 60 ng PG5 LUC) were transfected by Liposome 2000 into the cell. After the cells adhere to the wall, they were exposed to compound **1** for 16 h. Then the cells were rinsed with PBS and lysed by buffered solution on the oscillating platform for 15 min. According to the introduction of the Dual-Luciferase Reporter Assay System kit, the activities of firefly luciferase (FL) and rellina luciferase (RL) were checked. The activity fold was calculated as the relative luciferase activities ratio between sample and blank control.

$$\text{Relative luciferase activity (\%)} = FL / RL \times 100\%$$

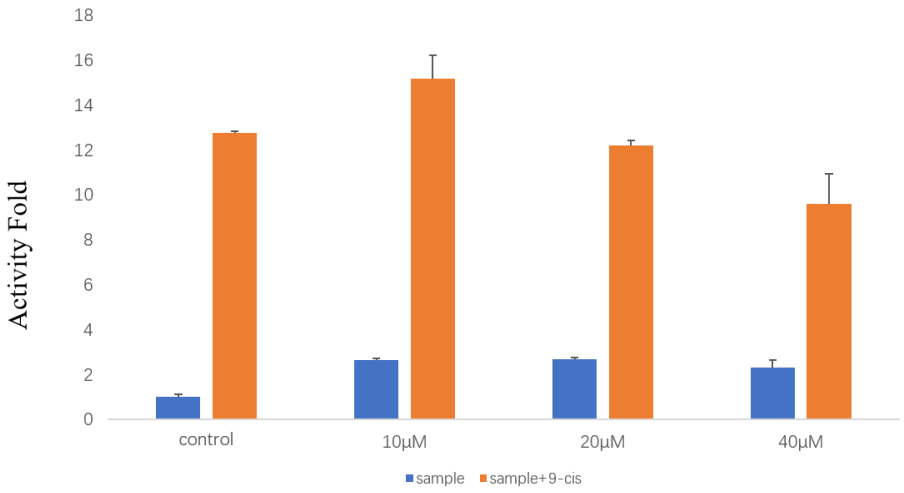

Figure S1. Effects of nesteral A on the transcriptional activities of RXRα

2.2 Cytotoxicity Assay

The *in vitro* cytotoxic bioassay was conducted using MTT method. Briefly, different cancer cells were incubated to 96-well cell plates and cultured for 24 h. Thereafter, the cells were treated with different concentrations of **1**. After 48h, 20 μL of MTT solution was added and the incubation continued for another 4 h. The supernatant was discarded softly, and the deposited formazan formed in the cells was dissolved with 100 μL of DMSO. The absorbencies were measured at 490 nm. The percentage of cell growth rate was calculated as follows：

$$\text{Growth Rate (\%)} = (OD_{sample} - OD_{blank}) / (OD_{control} - OD_{blank}) \times 100$$

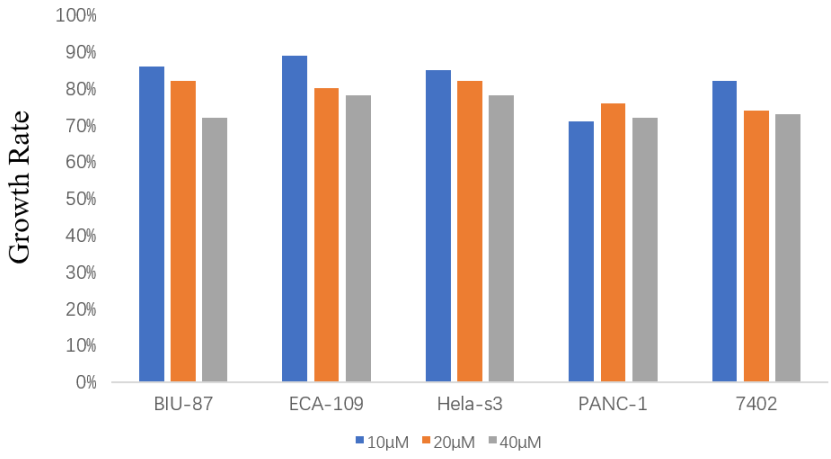

Figure S2. Cytotoxicity of nesteral A on the five different cancer cells

3. DFT Calculations for Biosynthetic Proposal

3.1 Computational Methods

All the structures were firstly optimized at the M06-2X/Def2-SVP level of DFT.[1-4] The frequency calculations were performed to confirm the characteristics of the calculated structures as minima. The calculation of single-point energies was then done at M06-2X/Def2-TZVP level[5] with a solvent method of SMD in water[6]. All the calculations were performed with the Gaussian 09 software package.[7]

Reference

1. A. D. Becke. *J. Chem. Phys.* **1993**, *98*, 5648–5652.

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3. C. Lee, W. Yang, R. G. Parr. *Phys. Rev. B.* **1988**, *37*, 785.

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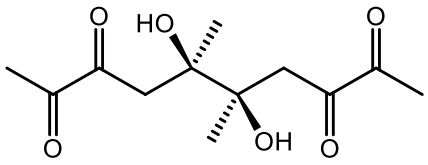
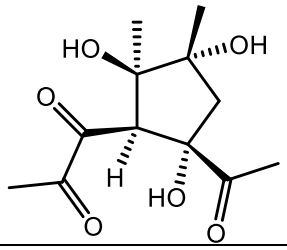
5. F. Weigend, M. Häser, H. Patzelt, R. Ahlrichs. *Chem. Phys. Lett.* **1998**, *294*, 143.

6. A. V. Marenich, C. J. Cramer, and D. G. Truhlar. *J. Phys. Chem. B*. **2009**, *113*, 6378.

7. M. J. Frisch, et al. Gaussian 09, Revision D.01 (Gaussian, Inc., Wallingford CT, 2016).

3.2 Cartesian Coordinates

Table S1. Optimized coordinates of compounds 5 and 6

Compound 5			Compound 6		
					
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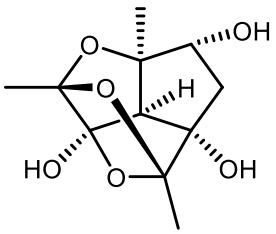
C 1.87180700	-0.44550700	-0.09014000	-1.38847000	-0.94947600	0.10228000
C 0.63969200	0.44694100	0.13558500	-0.08404900	-0.42588900	-0.53665100
C -0.63966600	-0.44702200	0.13566400	-0.03247300	1.10925000	-0.20131700
C -1.87183100	0.44542100	-0.08985200	-1.39193700	1.39223500	0.50049900
C 0.78216500	1.24154300	1.43780400	-2.30715000	0.30182500	-0.03539700
O 0.49614100	1.33210700	-0.95377300	-1.22226600	-1.21534500	1.47795100
C -0.78194700	-1.24165200	1.43788500	-1.95178200	-2.19857000	-0.55422500
O -0.49625100	-1.33213200	-0.95373900	-2.48665600	0.61886100	-1.41452000
C -3.15852900	-0.30882600	-0.24558700	-3.63563000	0.17709200	0.68209800
C -4.46405400	0.48041700	0.02698700	1.13160200	-1.27867000	-0.21170800
O -3.25253800	-1.46627100	-0.58795700	1.06262600	-2.40553700	0.20539900
C 3.15850400	0.30875400	-0.24583600	2.52659100	-0.70607400	-0.55275300
C 4.46403700	-0.48031500	0.02696100	3.68737800	-1.43511100	0.05220500
O 3.25244100	1.46616800	-0.58835800	2.61342200	0.26358700	-1.26178000
C 5.74012700	0.23809700	-0.30599300	1.07697300	1.41247100	0.79683300
O 4.40125800	-1.59129000	0.48257500	0.11302200	1.91002700	-1.33698100
C -5.74015000	-0.23768300	-0.30661400	1.27598400	0.61968400	1.69592600
O -4.40123500	1.59120000	0.48305600	1.83763800	2.68553000	0.62095200
H 1.71737200	-1.00308800	-1.02953100	-0.17672900	-0.46557200	-1.63312300
H 2.00547400	-1.20663200	0.69029500	-1.31700100	1.29659200	1.59068700
H -1.71747800	1.00319100	-1.02914500	-1.74463200	2.40011200	0.24356600
H -2.00551500	1.20639700	0.69073000	-2.87270700	-2.50269900	-0.03685100
H -0.13522000	1.80689700	1.65239400	-2.16810900	-2.03401700	-1.62014500
H 1.59985400	1.96835700	1.33266800	-1.22381300	-3.01290400	-0.46137600
H 1.00647700	0.59186200	2.29482200	-0.38602100	-0.83775200	1.78882800
H 1.32661900	1.82127000	-1.03331600	-3.06835600	-0.03930700	-1.81210800
H -1.59970400	-1.96840900	1.33289300	-4.27450300	-0.57189400	0.18796800
H 0.13544500	-1.80707200	1.65228300	-3.47048400	-0.14470200	1.71789900
H -1.00603400	-0.59196600	2.29495400	-4.16550300	1.13968700	0.67170100
H -1.32668700	-1.82137800	-1.03313900	3.59679600	-2.51379900	-0.12728600
H 5.78769500	1.18554000	0.24841000	4.62530200	-1.03512100	-0.34697200
H 6.59405200	-0.40121400	-0.05973300	3.64272900	-1.28698300	1.14274900
H 5.74838100	0.50197600	-1.37336300	-0.70031400	1.79735800	-1.85045500
H -5.78826700	-1.18498000	0.24800900	2.36890100	2.61509900	-0.34054700
H -6.59406100	0.40187400	-0.06095700	1.13864800	3.52854700	0.52751900
H -5.74785400	-0.50189300	-1.37389900	2.53459000	2.82945300	1.45308500

Table S2. Optimized coordinates of compound 1

	Compound 1		
			
	E = -918.425340943		
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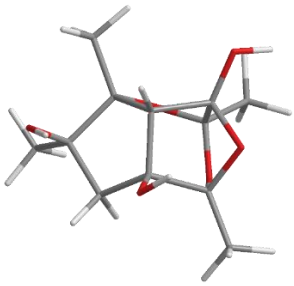
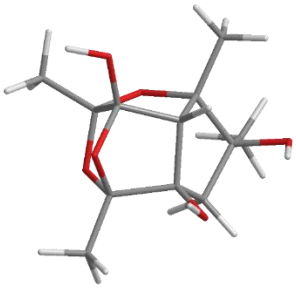
C	-0.13925100	-0.02428400	-1.06047900
C	0.07743500	1.31989300	-0.30492000
C	-1.03768500	1.40720100	0.75678500
C	-2.05851400	0.34712800	0.34300200
O	-0.39578400	-1.36677900	0.82878700
C	0.97917000	-1.11417300	0.68962400
C	1.20149000	-0.72238900	-0.80408500
C	-2.99213100	-0.10320300	1.44867500
O	-2.76846800	0.93538200	-0.74278900
C	-1.90152200	-1.89565100	-0.95658300
O	0.14440200	2.43001700	-1.15346900
C	1.49933000	1.05640300	0.28702600
O	2.14280400	0.32522600	-0.73101900
O	1.35757700	0.09428900	1.32535100
C	1.78198600	-2.26754100	1.22190400
O	1.58809300	-1.76420600	-1.59513400
C	2.27506000	2.25177500	0.74371400
H	-0.38558300	0.12146300	-2.11902700
H	-1.50287300	2.40203000	0.75724500
H	-0.64713400	1.18733700	1.75745500
H	-3.75261400	-0.79990600	1.06056500
H	-3.50972500	0.76237900	1.88521200
H	-2.42073600	-0.62049800	2.23051100
H	-3.46952900	0.33298200	-1.01596500
H	-1.16812700	-2.57988700	-1.40636600
H	-2.51884600	-2.47029700	-0.25162900
H	-2.53662300	-1.49570600	-1.76005100
H	-0.74325700	2.55636200	-1.51325400
H	1.50119400	-3.18670600	0.69438000
H	2.84838500	-2.06369000	1.06634000
H	1.58427200	-2.38089400	2.29576700
H	1.67887600	-1.44289900	-2.50111900
H	2.38060100	2.95029300	-0.09363900
H	1.73389100	2.74760900	1.56018900
H	3.25992200	1.92892900	1.10274600

4. Optimized coordinates for NMR and OR calculations

Table S3. Optimized coordinates of 1 in the gas phase at b3lyp/6-31G* level for ^{13}C NMR chemical shifts calculation

		1	
C	2.99080100	-0.09518400	1.48105900
C	2.08671600	0.34350700	0.33913600
C	1.07267100	1.44768100	0.71234000
O	2.94672900	0.81849700	-0.71251800
C	-0.07363400	1.32193000	-0.30903500
C	1.18743100	-0.79391700	-0.25129900
C	0.14105000	-0.03343700	-1.07038900
C	1.93868300	-1.89984400	-0.97700700
O	0.39658500	-1.40961300	0.81494100
O	-0.06765900	2.44828100	-1.17778000
C	-0.97903500	-1.13355100	0.67922800
C	-1.78535700	-2.28892900	1.22782100
C	-1.20162100	-0.71470400	-0.81932500
O	-1.55594400	-1.67753200	-1.73418500
O	-1.35199800	0.07974200	1.35382800
C	-1.48957300	1.06466000	0.32186100
O	-2.15564000	0.35880400	-0.72808300
C	-2.27067500	2.24836800	0.82354500
H	0.37711400	0.09723800	-2.12820300
H	3.75423700	-0.79085200	1.11786700
H	3.50458500	0.77272000	1.90732600
H	2.40678100	-0.58773200	2.26317300
H	1.53944800	2.43586100	0.66150200
H	0.69553800	1.29402000	1.72565700
H	2.43747600	1.43753800	-1.26116800
H	2.62811300	-1.47561100	-1.71086400
H	2.51431700	-2.49603100	-0.26150600
H	1.23232900	-2.56461300	-1.48456700
H	-0.76479600	2.29817500	-1.83963400
H	-1.56444300	-2.41940800	2.29099200
H	-2.85589100	-2.08415800	1.12350800
H	-1.52801500	-3.21400700	0.70376700
H	-2.39621200	-2.07358400	-1.44705400
H	-3.26403000	1.92480400	1.14472300
H	-1.75188300	2.69629700	1.67605700
H	-2.36598900	3.00442500	0.04118700

Table S4. Optimized coordinates (2*R*,4*S*,5*R*,8*R*,9*R*,10*R*,11*R*)-1 and (2*S*,4*R*,5*S*,8*S*,9*S*,10*S*,11*S*)-1 at the b3lyp/6-31g(d) level in MeOH for OR calculations

(2*R*,4*S*,5*R*,8*R*,9*R*,10*R*,11*R*)-**1** (**1a**)			(2*S*,4*R*,5*S*,8*S*,9*S*,10*S*,11*S*)-**1** (**1b**)		
					
C	-2.96597100	-0.09546200	1.50172500	2.96598200	-0.09546800	1.50171700
C	-2.08487200	0.34330900	0.34123800	2.08487300	0.34330500	0.34123900
C	-1.07587600	1.45981500	0.69550700	1.07587500	1.45980500	0.69551900
O	-2.97605900	0.80806200	-0.69136400	2.97604800	0.80806300	-0.69137200
C	0.07658500	1.32730400	-0.31878800	-0.07658100	1.32730200	-0.31878200
C	-1.18642200	-0.79004300	-0.25872100	1.18642100	-0.79004600	-0.25871800
C	-0.13590700	-0.03035100	-1.07508400	0.13590800	-0.03035100	-1.07508200
C	-1.93740400	-1.89259400	-0.99071300	1.93740000	-1.89259900	-0.99071000
O	-0.40071900	-1.41357800	0.80820300	0.40071500	-1.41357800	0.80820600
O	0.07934500	2.45428200	-1.18495700	-0.07932500	2.45428400	-1.18494900
C	0.97858600	-1.13721500	0.67893000	-0.97859100	-1.13721300	0.67892900
C	1.78257900	-2.29074200	1.23421600	-1.78258700	-2.29074000	1.23421100
C	1.20532100	-0.71747000	-0.81731600	-1.20532100	-0.71746800	-0.81731600
O	1.55870800	-1.67740800	-1.73367400	-1.55870700	-1.67740500	-1.73367600
O	1.33955300	0.07964800	1.35556800	-1.33955800	0.07964900	1.35556600
C	1.48799200	1.06755200	0.32151300	-1.48799400	1.06755500	0.32150900
O	2.16051400	0.35575800	-0.71838500	-2.16051200	0.35576200	-0.71838800
C	2.26856800	2.24931100	0.82795100	-2.26857000	2.24931500	0.82794400
H	-0.36631700	0.09653400	-2.13447800	0.36632000	0.09653500	-2.13447400
H	-3.71945300	-0.81487700	1.16398100	3.71947200	-0.81486900	1.16396100
H	-3.48851700	0.76946200	1.92366600	3.48851900	0.76945700	1.92366700
H	-2.36286200	-0.56149800	2.28504800	2.36288300	-0.56152200	2.28503600
H	-1.55088300	2.44241600	0.62506600	1.55087900	2.44240900	0.62509300
H	-0.70415600	1.33128500	1.71416600	0.70415200	1.33125900	1.71417500
H	-2.46287300	1.34975200	-1.31421700	2.46286200	1.34979000	-1.31419400
H	-2.54584400	-2.47090800	-0.28735100	2.59629800	-1.46899700	-1.75226200
H	-1.22825300	-2.57443600	-1.47062300	2.54583400	-2.47091800	-0.28734700
H	-2.59629700	-1.46899200	-1.75226900	1.22824800	-2.57443800	-1.47062400
H	0.76164500	2.30143400	-1.86192200	-0.76162000	2.30144500	-1.86192000
H	1.55745400	-2.42128200	2.29693700	-1.55747000	-2.42127900	2.29693400
H	2.85314100	-2.08860100	1.13215100	-2.85314800	-2.08859900	1.13214000
H	1.53227200	-3.21749800	0.71013900	-1.53227500	-3.21749600	0.71013600
H	2.37263600	-2.11736800	-1.43149400	-2.37263700	-2.11736300	-1.43149900
H	3.25240900	1.92269600	1.17509400	-3.25242800	1.92270900	1.17504600
H	1.73588200	2.71431100	1.66270200	-1.73590500	2.71429100	1.66272300
H	2.39020500	2.99297900	0.03754000	-2.39016600	2.99300100	0.03754300

5. NMR Spectra

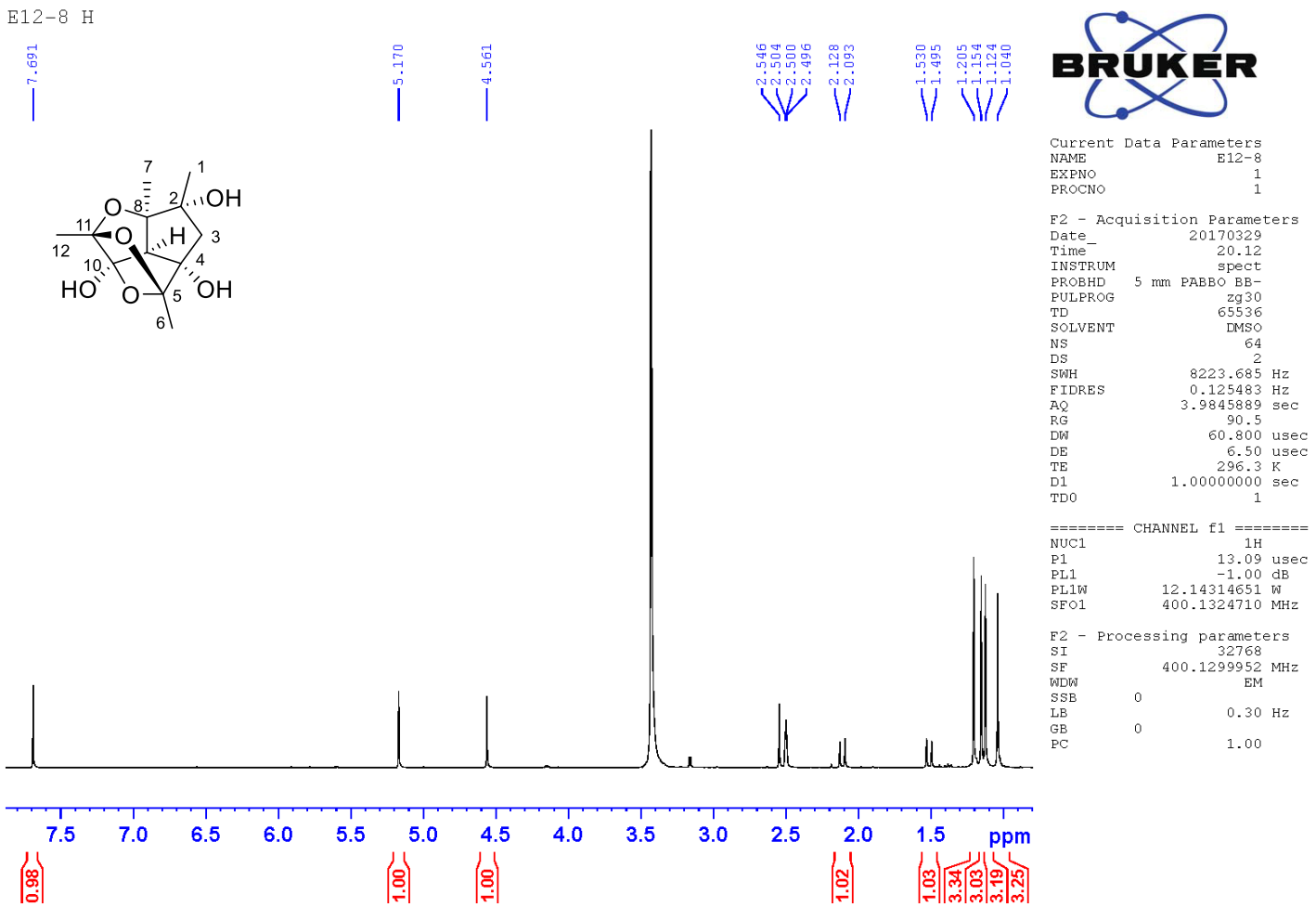


Figure S3. ^{1}H NMR spectrum of **1** in DMSO-d_6

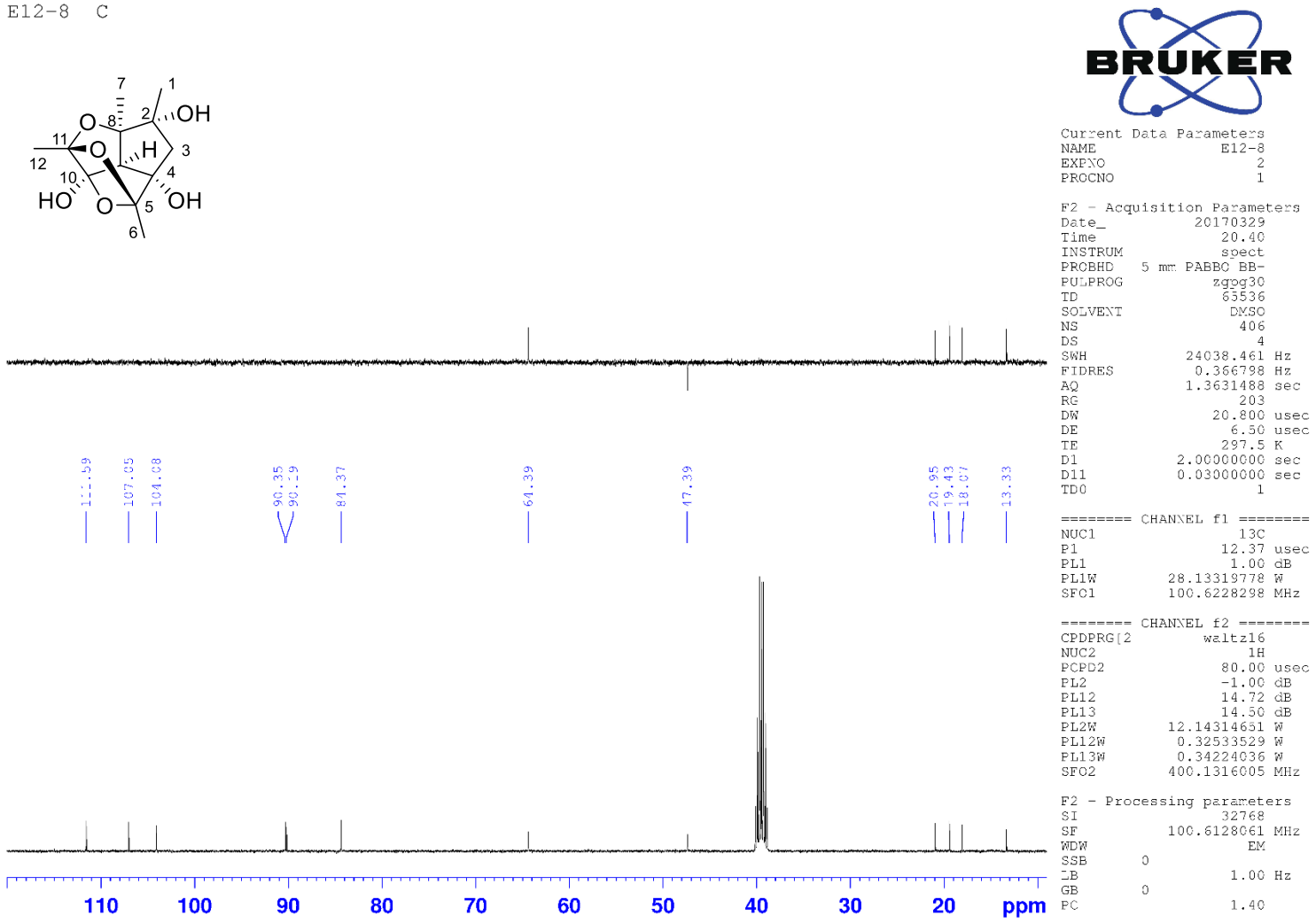


Figure S4. ^{13}C NMR spectrum of **1** in DMSO-d_6

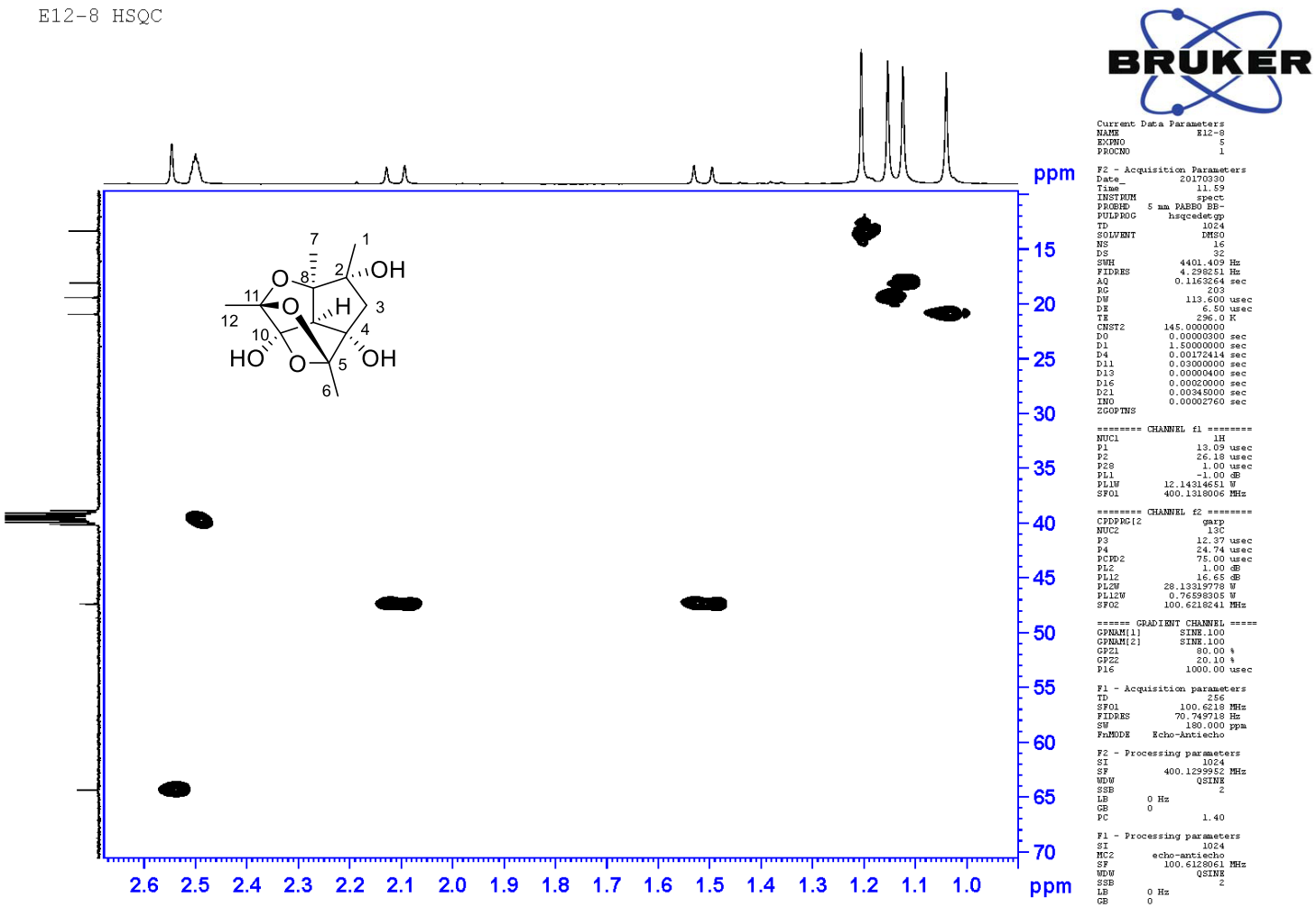


Figure S5. HSQC spectrum of **1** in DMSO-d_6

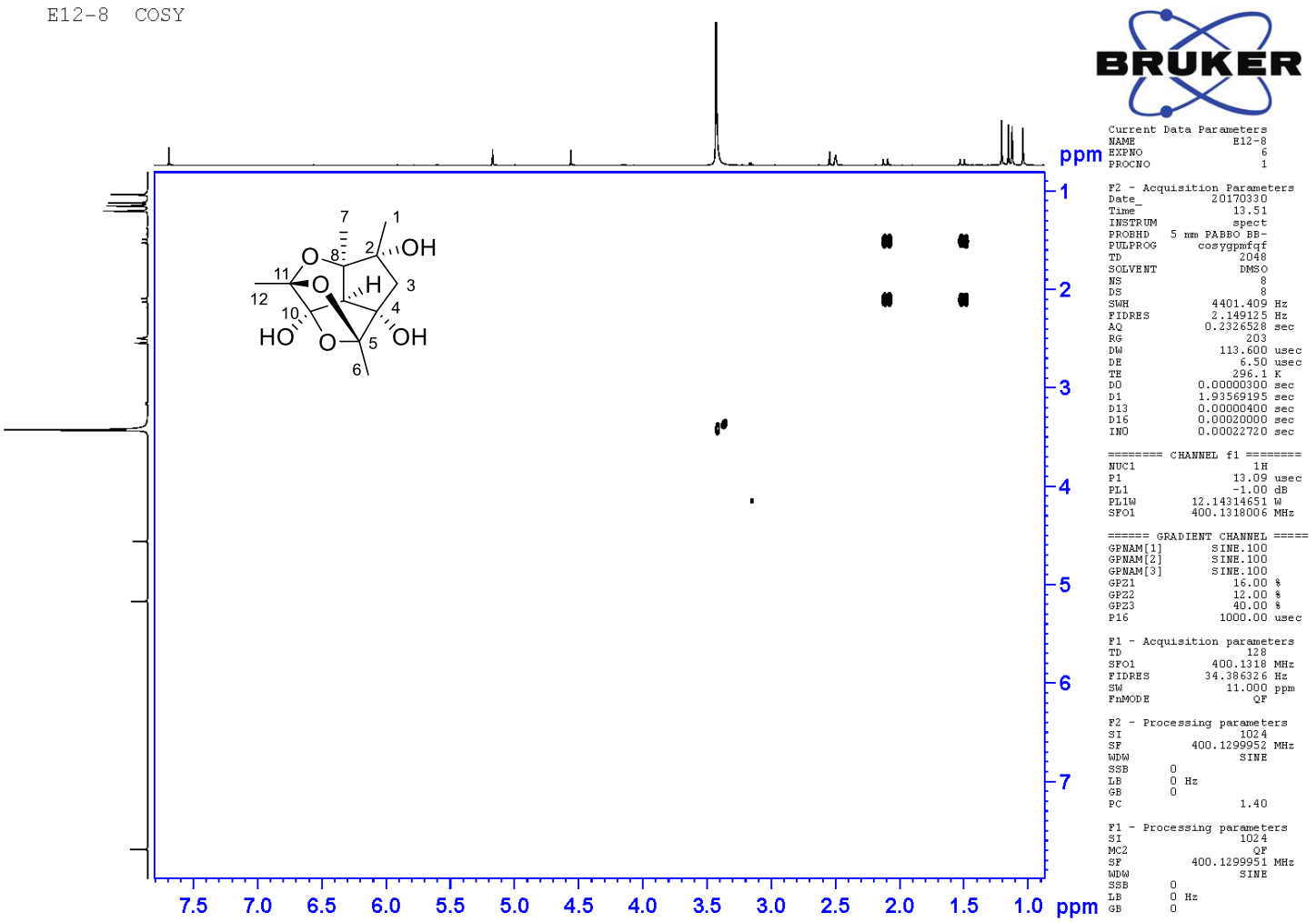


Figure S6. COSY spectrum of **1** in DMSO-d_6

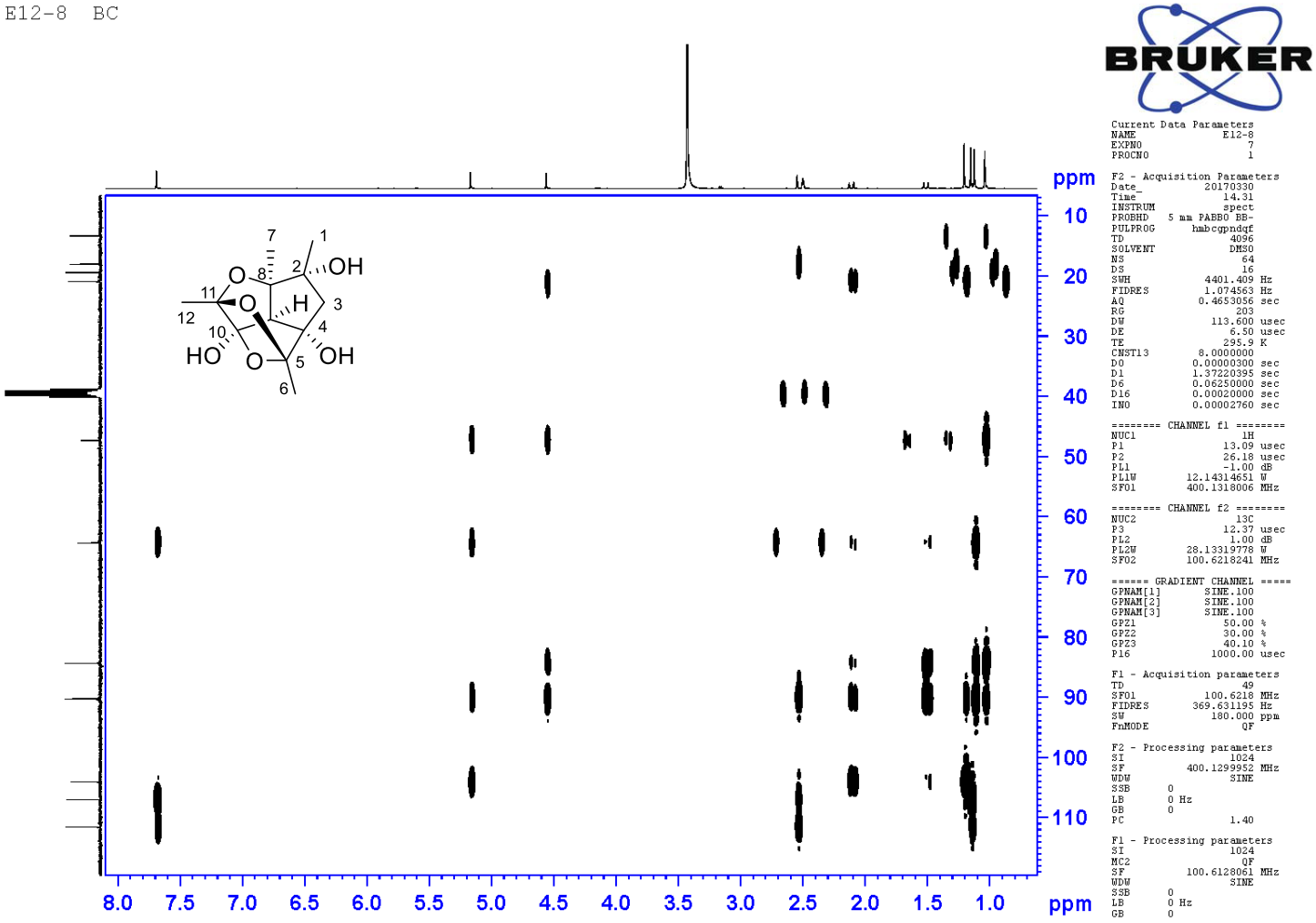


Figure S7. HMBC spectrum of **1** in DMSO-d_6

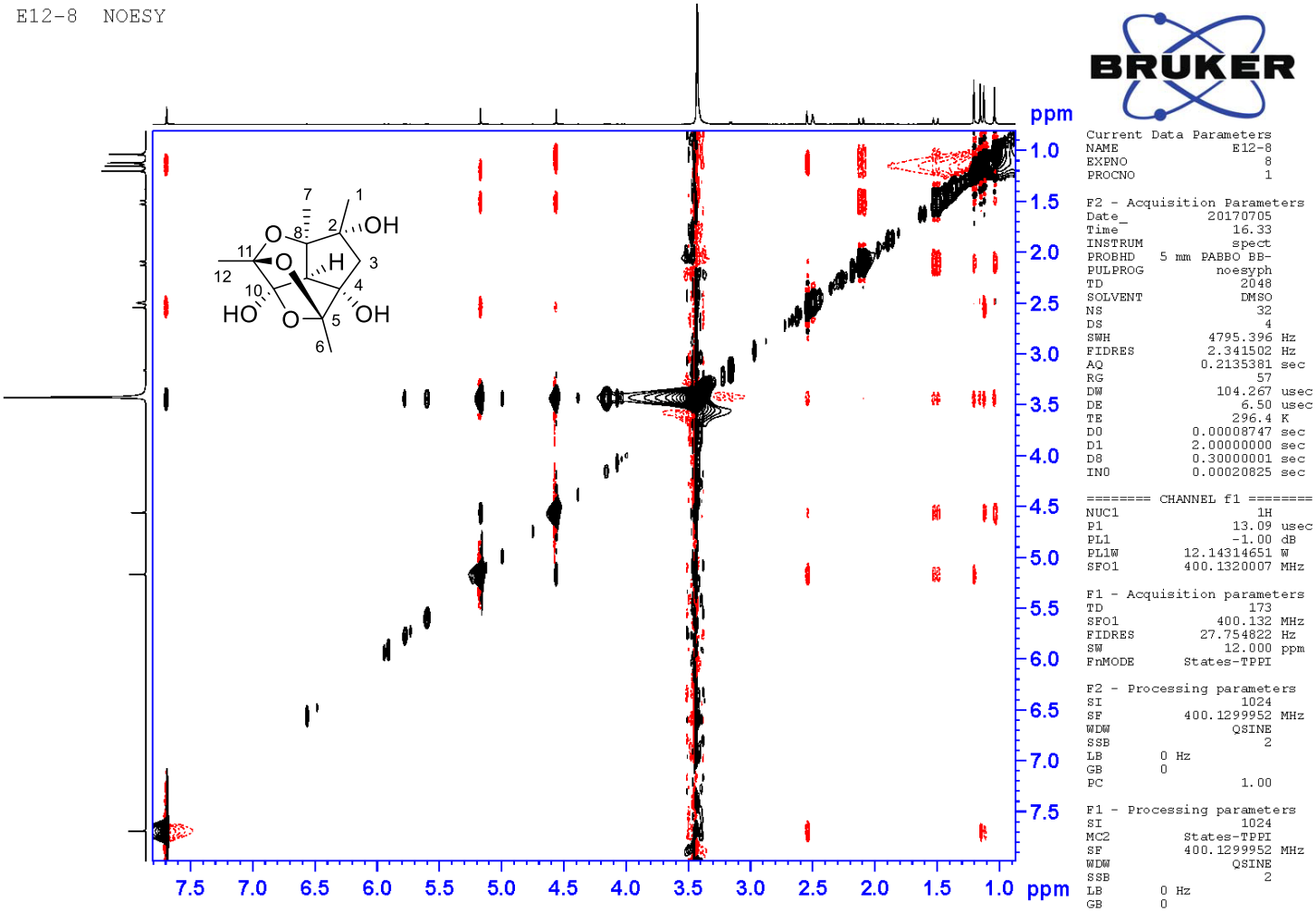


Figure S8. NOESY spectrum of **1** in DMSO-d_6

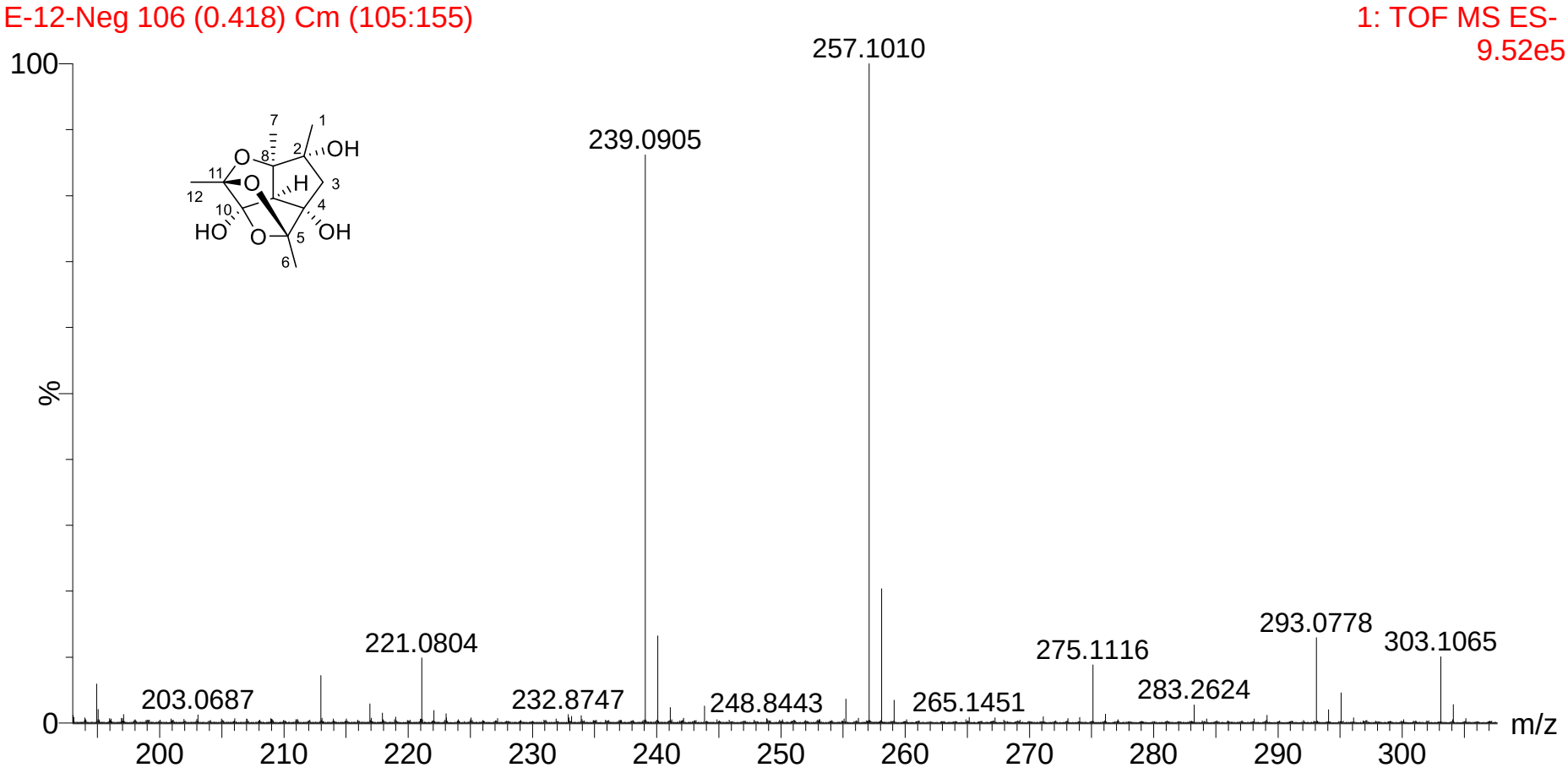


Figure S9. The HR-ESIMS spectrum of **1**

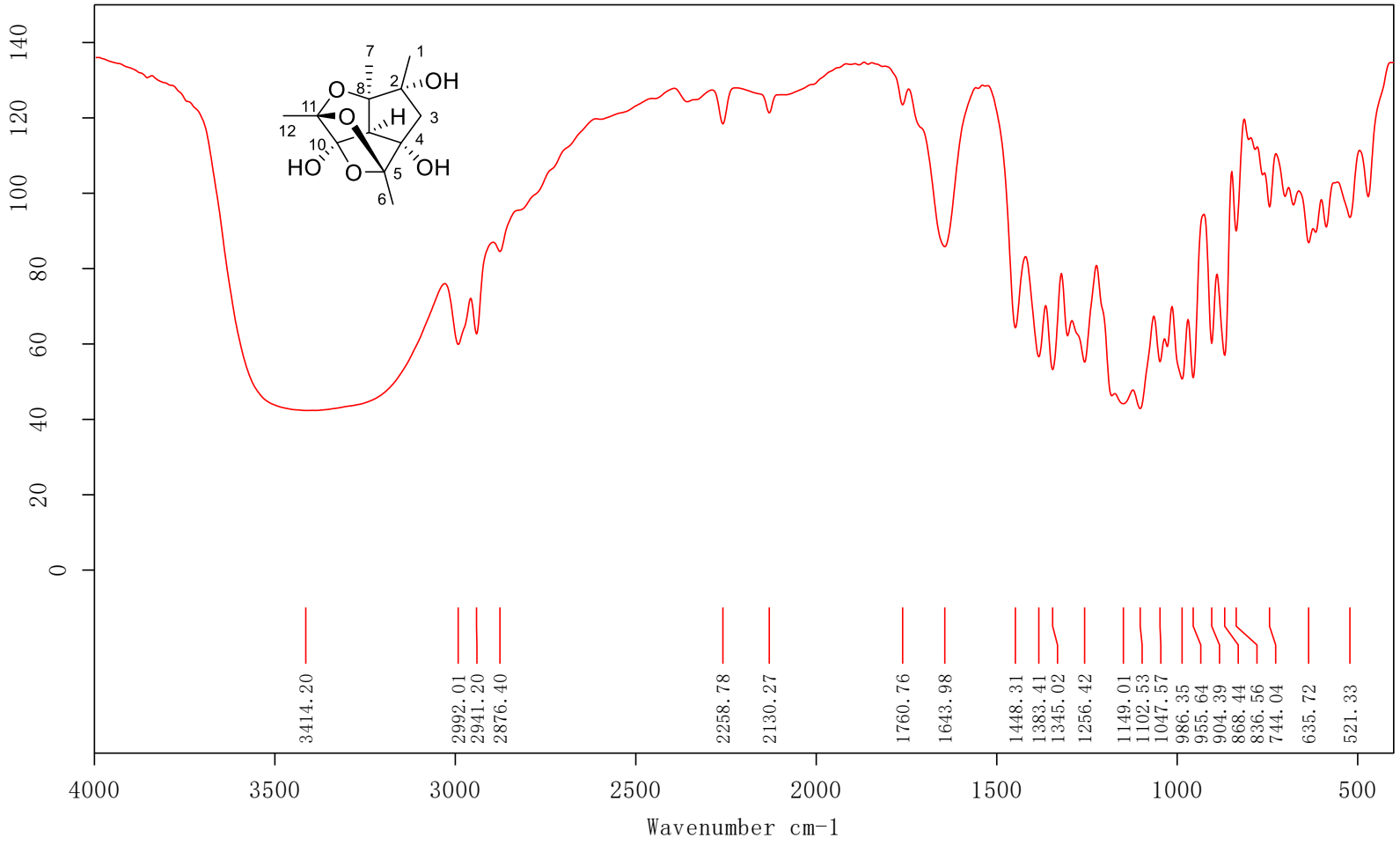


Figure S10. The IR spectrum of **1**