SUPPLEMENTARY MATERIAL

Penicindopene A, a New Indole Diterpene from the Deep-Sea Fungus *Penicillium* sp. YPCMAC1

Lijun Liu[a, #], Wei Xu[b, #], Shumin Li[a], Mengying Chen[a], Yongjun Cheng[a], Wangjun Yuan[a], Zhongbin Cheng[a, c*] and Qin Li[a, d *]

[a] *College of Pharmacy, Henan University, Kaifeng 475004, P. R. China.*

[b] *Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, State Oceanic Administration, Xiamen 361005. P. R. China.*

[c] *College of Chemistry and Chemical Engineering, Henan University, Kaifeng 475004, P. R. China.*

[d] *Eucommia ulmoides Cultivation and Utilization of Henan Engineering Laboratory, Kaifeng 475004, P. R. China.*

(Corresponding Author, *E-mail: czb360@126.com; liqin6006@163.com)

[#]They contributed equally to this work.

ABSTRACT

A new indole diterpene, named penicindopene A (**1**), together with seven known compounds (**2**–**8**), was isolated from the deep-sea fungus *Penicillium* sp. YPCMAC1. The structure of penicindopene A was elucidated by extensive spectroscopic analyses (1D and 2D NMR, and HRESIMS data), in addition to the ECD calculations for the assignments of its absolute configuration. Penicindopene A represented the first example of indole diterpenes possessing a 3-hydroxyl-2-indolone moiety, and it exhibited moderate cytotoxicities against A549 and HeLa cell lines with IC_{50} values of 15.2 and 20.5 μM, respectively.

Keywords: Indole diterpene; deep-sea fungus; *Penicillium* sp. YPCMAC1; cytotoxicity

Content

Experimental

Cytotoxic bioassays

Cells (A549 or HeLa) were seeded in 96-well microtiter plates at 5000 cells/well. After 24 h, test compounds were added to the wells. After incubation for 48 h, 20 μL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Solarbio) was added, and the incubation was continued for 4 h at 37 °C. The cell viability was determined by measuring the metabolic conversion of MTT into purple formazan precipitate by viable cells. The medium was then removed, and cells were then lysed with 100 μL of triplex solution of 10% sodium dodecyl sulfate, 5% isopropyl alcohol, and 12 mM HCl. The MTT assay results were obtained using a microplate spectrophotometer (SpectraMax I3, Molecular Devices, USA) plate reader at 570 nm. Compounds were tested at five concentrations (50, 25, 12.5, 6.25, and 3.12 μM) in 100% DMSO with a final concentration of DMSO of 0.5% (v/v) in each well. Each concentration of the compounds was tested in three parallel experiments. IC_{50} values were calculated using the means ± SEM using GraphPad Prism 5.

Computational details.

In general, conformational analyses were carried out via random searching in the Sybyl-X 2.0 (Tripos Associates Inc.: St. Louis) using the MMFF94S force field with an energy cutoff of 2.5 kcal/mol. The results showed seven lowest energy conformers for both 3*S*, 5*S*, 8*S*, 9*S*, 10*R*, 21*S*-**1** and 3*S*, 5*S*, 8*S*, 9*S*, 10*R*, 21*R*-**1**. Subsequently, the conformers were re-optimized using DFT at the b3lyp/6-31+g(d) level in gas phase by the GAUSSIAN 09 program (Gaussian, Inc., Wallingford CT). The energies, oscillator strengths, and rotational strengths (velocity) of the first 30 electronic excitations were calculated using the TDDFT methodology at the b3lyp/6-311++g(d,p) level in vacuum. The ECD spectra were simulated by the overlapping Gaussian function (half the bandwidth at 1/e peak height, $\sigma = 0.30$) (Stephens P J et al. 2010). To get the final spectra, the simulated spectra of the conformers were averaged according to the Boltzmann distribution theory and their relative Gibbs free energy (ΔG). By comparing the experiment spectrum with the calculated ECD spectra, the absolute configuration of **1** was determined to be 3*S*, 5*S*, 8*S*, 9*S*, 10*R*, 21*S*-**1**.

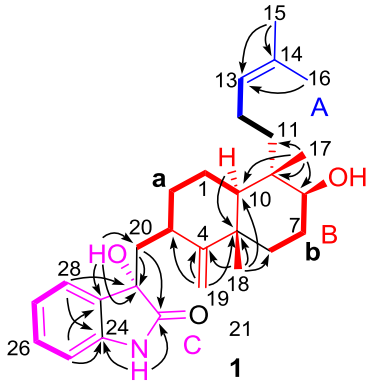


Figure S1. Key ^{1}H-^{1}H COSY and HMBC correlations of **1**.

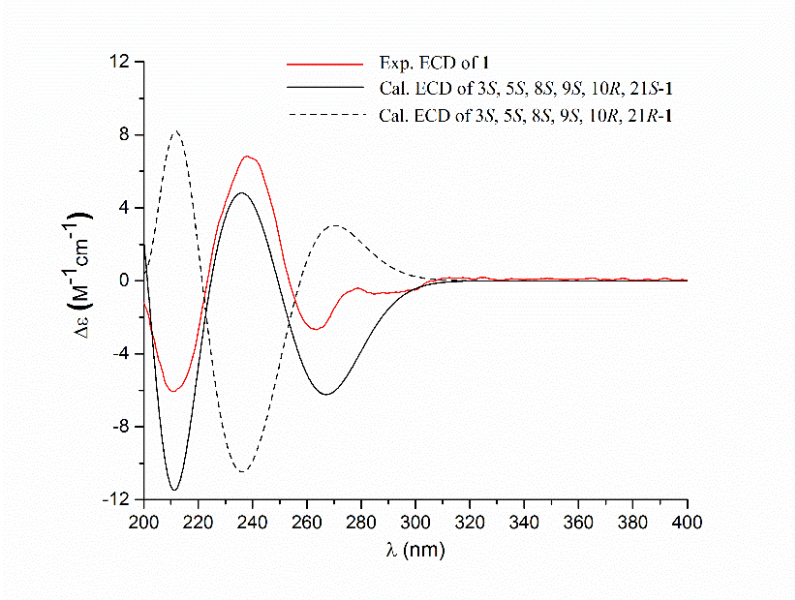


Figure S2. Experimental spectrum of **1** and calculated ECD spectra of 3*S*, 5*S*, 8*S*, 9*S*, 10*R*, 21*S*-**1** and 3*S*, 5*S*, 8*S*, 9*S*, 10*R*, 21*R*-**1** at the B3LYP/6-311++G(d,p) level.

Table S1. NMR Data of **1** in DMSO-d_6 (J in Hz, δ in ppm)

No.	**1**	
	δ_H [a]	δ_C [b]
1	1.28, m	21.5
2	1.67, m; 0.65, m	35.4
3	2.18, m	34.1
4		161.8
5		39.8
6	1.37, dt (13.0, 3.3, 3.3); 1.45, m	35.4
7	1.57, m	27.5
8	3.24, m	71.1
9		41.0
10	0.88, dd (11.8, 3.6)	47.1
11	0.98, m; 1.45, m	36.7
12	1.74, m; 1.56, m	20.9
13	4.98, tt (7.0, 7.0, 1.2, 1.2)	125.1
14		129.8
15	1.59, s	25.5
16	1.48, s	17.4
17	0.65, s	17.0
18	0.84, s	20.6
19	4.55, s; 4.46, s	100.2
20	1.73, dd (13.7, 8.1) 2.05, dd (13.7, 3.7)	40.0
21		75.7
22		179.5
23	10.19, s	
24		141.7
25	6.79, d (7.7)	109.5
26	7.19, td (7.7, 7.7, 1.2)	128.8
27	6.95, td (7.7, 7.4, 0.9)	121.4
28	7.25, d (7.4)	124.3
29		132.4
OH-21	5.79, s	
OH-7	4.27, d (5.2)	

[a]Recorded at 600 MHz. [b]Recorded at 150 MHz. Chemical shifts are in ppm, coupling constants J in Hz

Table S2. Cytotoxic Activity for Compound **1** against A549 and HeLa (μM).

compound	A549	HeLa
1	15.2 ± 0.8	20.5 ± 1.4
5-Fluoracil[a]	35.3 ± 1.3	26.7 ± 1.6

[a]Positive control

Figure S3 ^{1}H NMR Spectrum of Penicindopene A (**1**) in DMSO-d_6 (600 MHz)

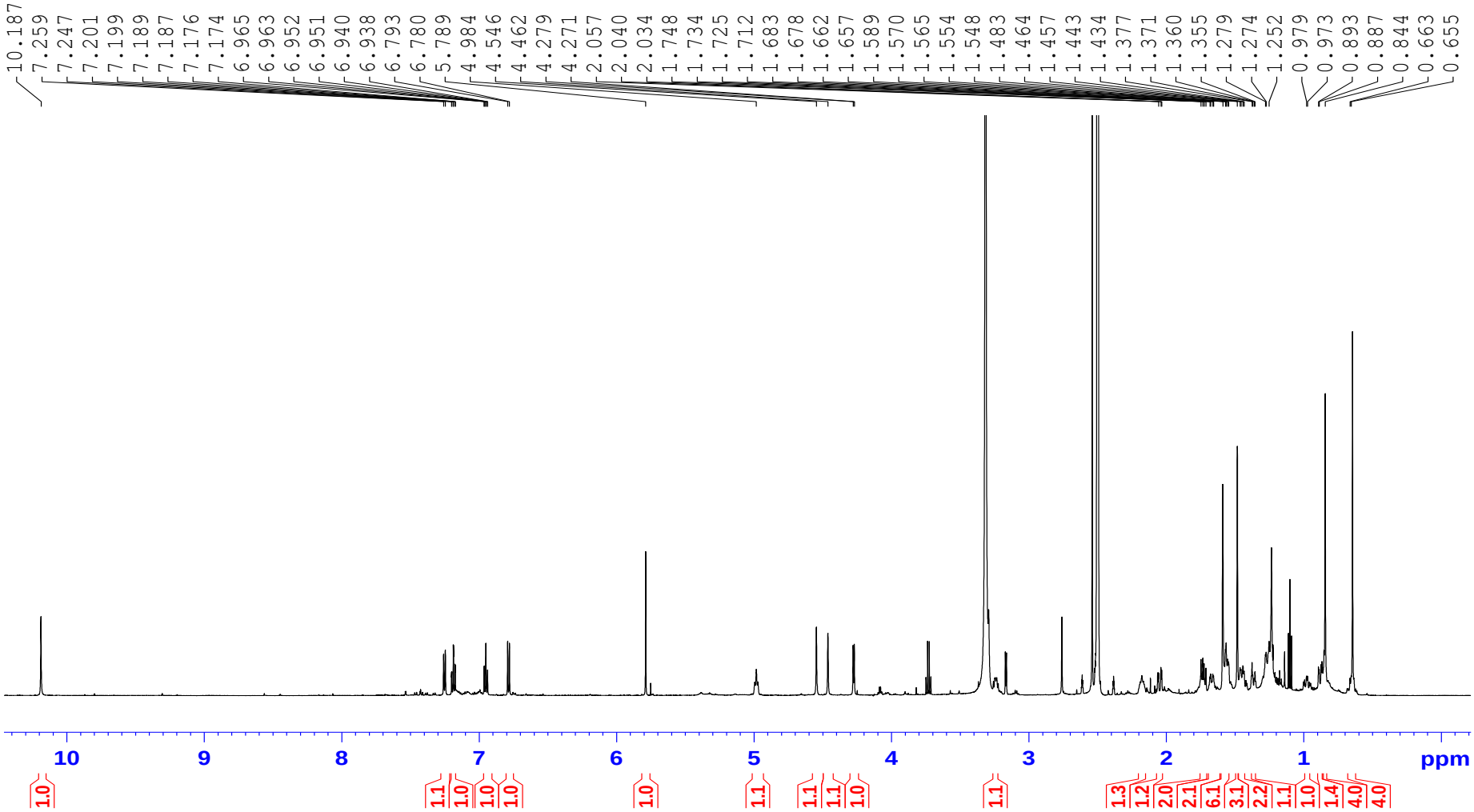


Figure S4 ^{13}C NMR Spectrum of Penicindopene A (**1**) in DMSO-d_6 (150 MHz)

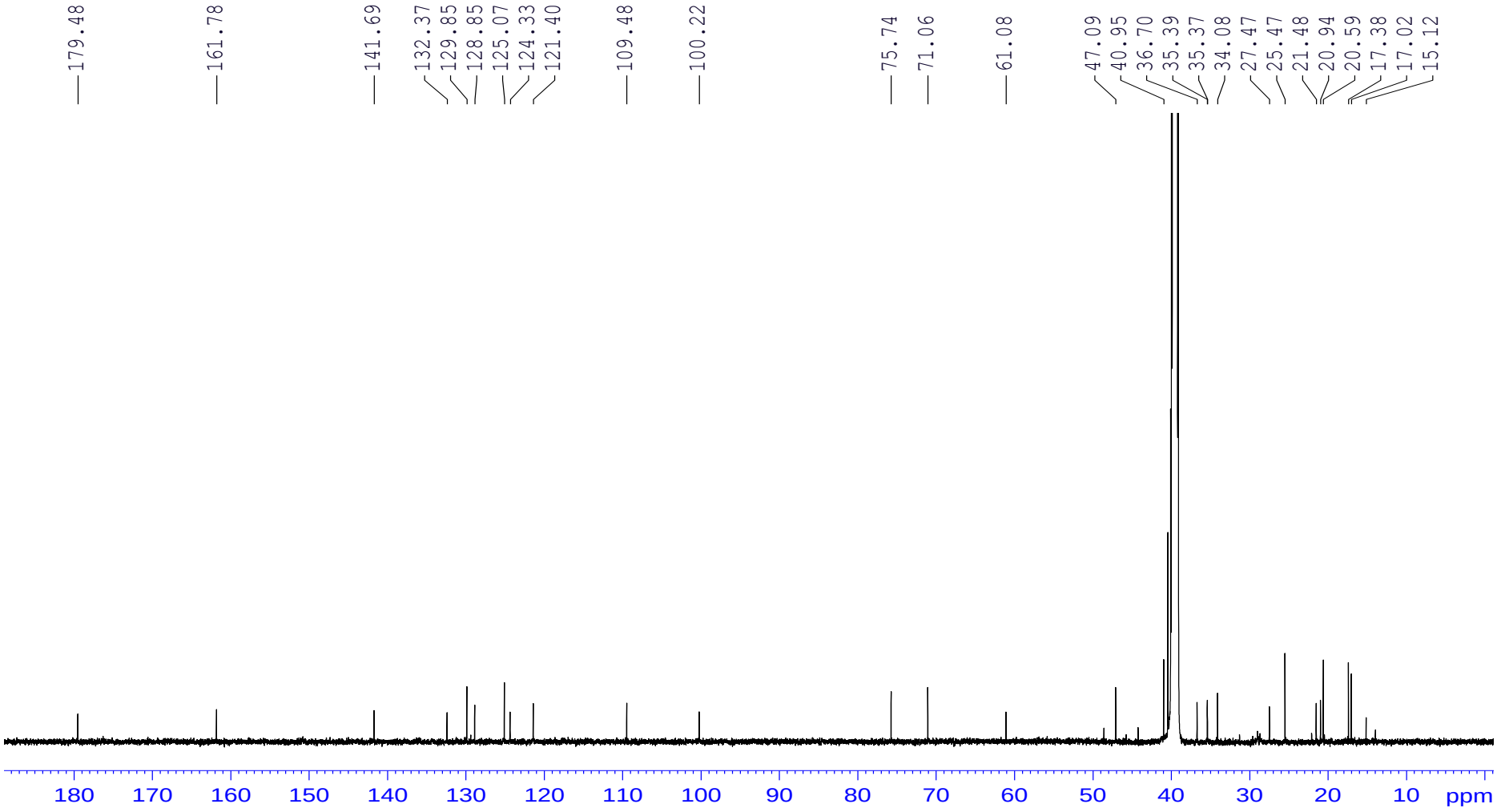


Figure S5 HSQC Spectrum of Penicindopene A (**1**) in DMSO-d_6 (600 MHz)

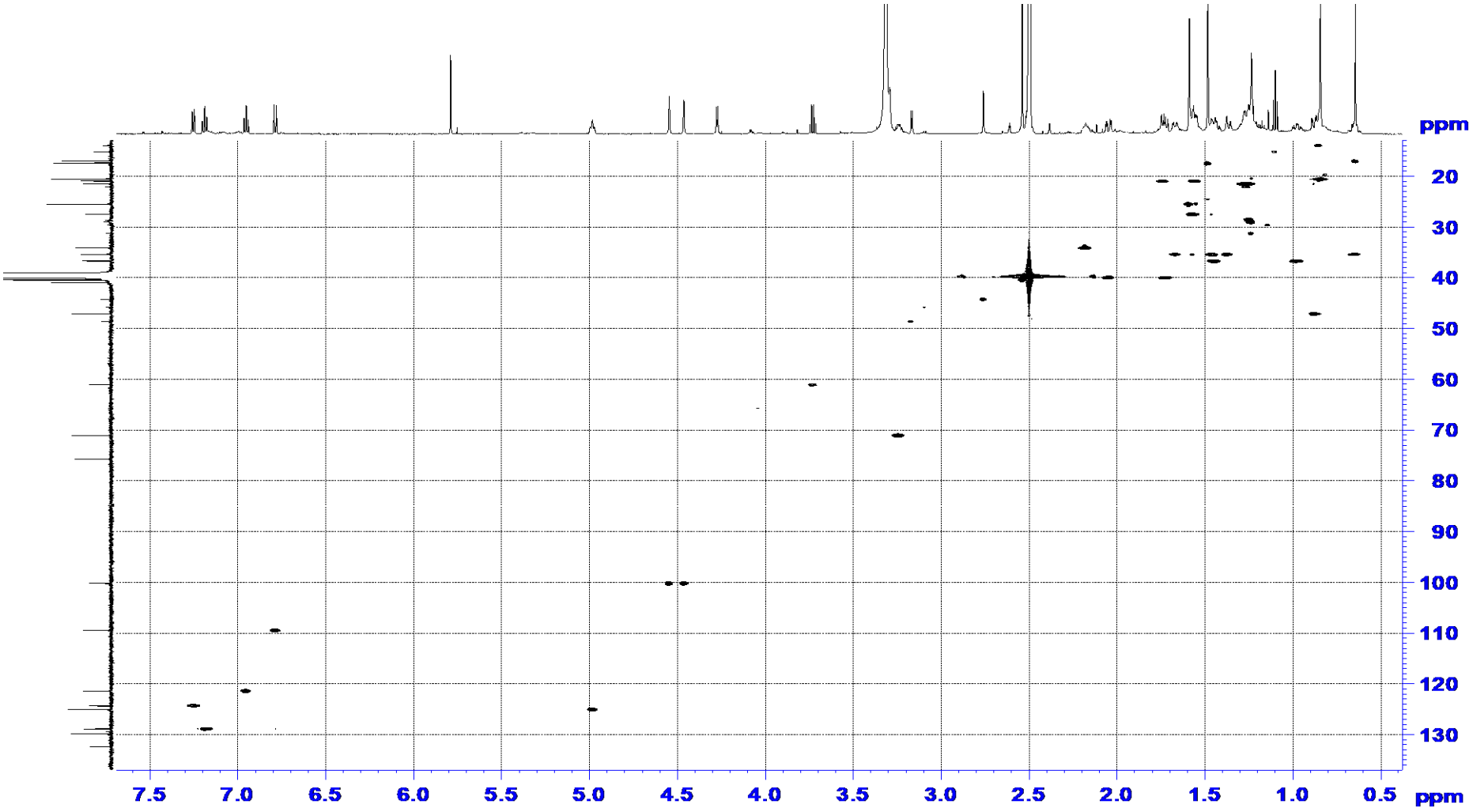


Figure S6 ^{1}H-^{1}H COSY Spectrum of Penicindopene A (**1**) in DMSO-d_6 (400 MHz)

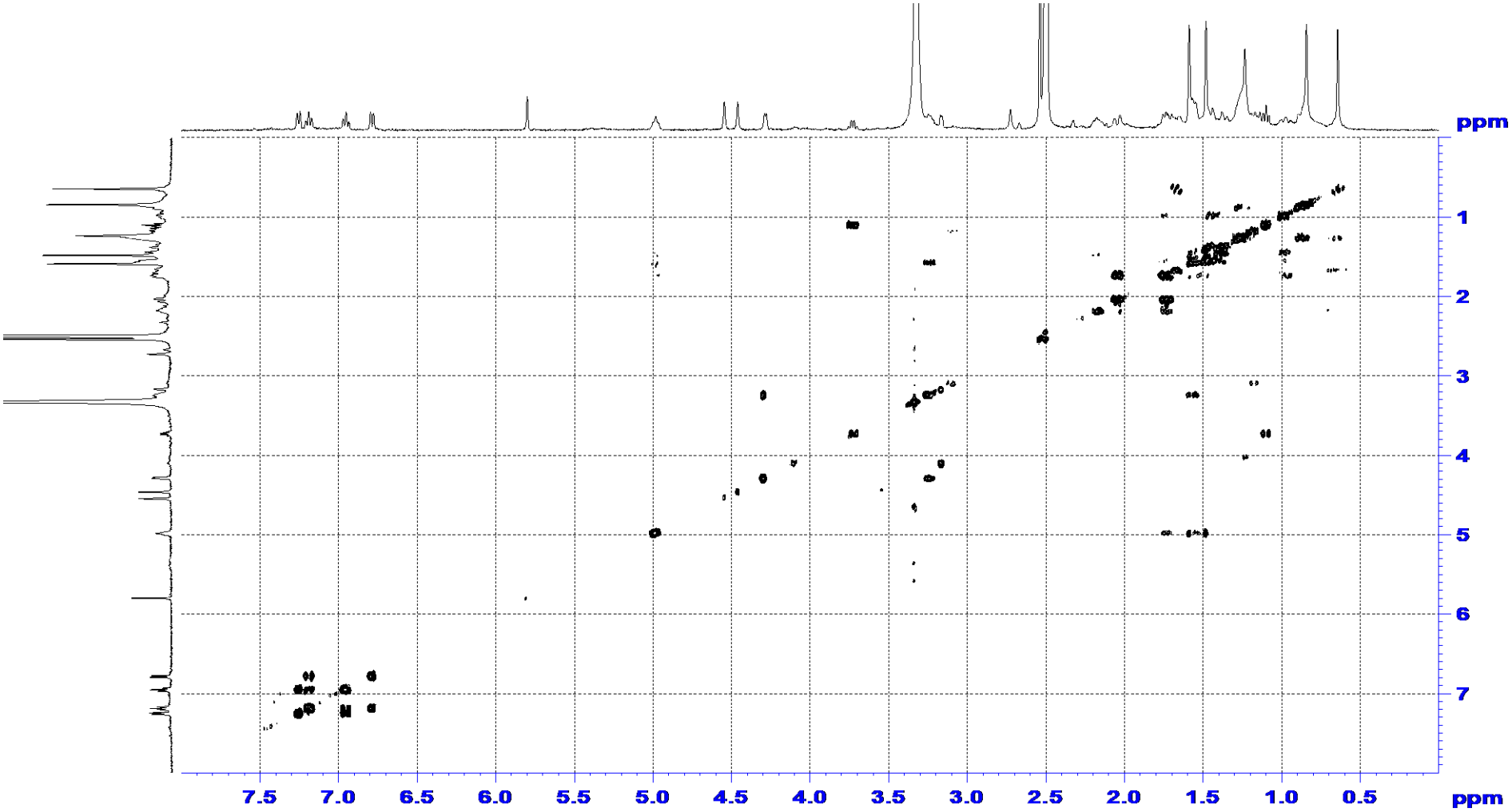


Figure S7 HMBC Spectrum of Penicindopene A (**1**) in DMSO-d_6 (600 MHz)

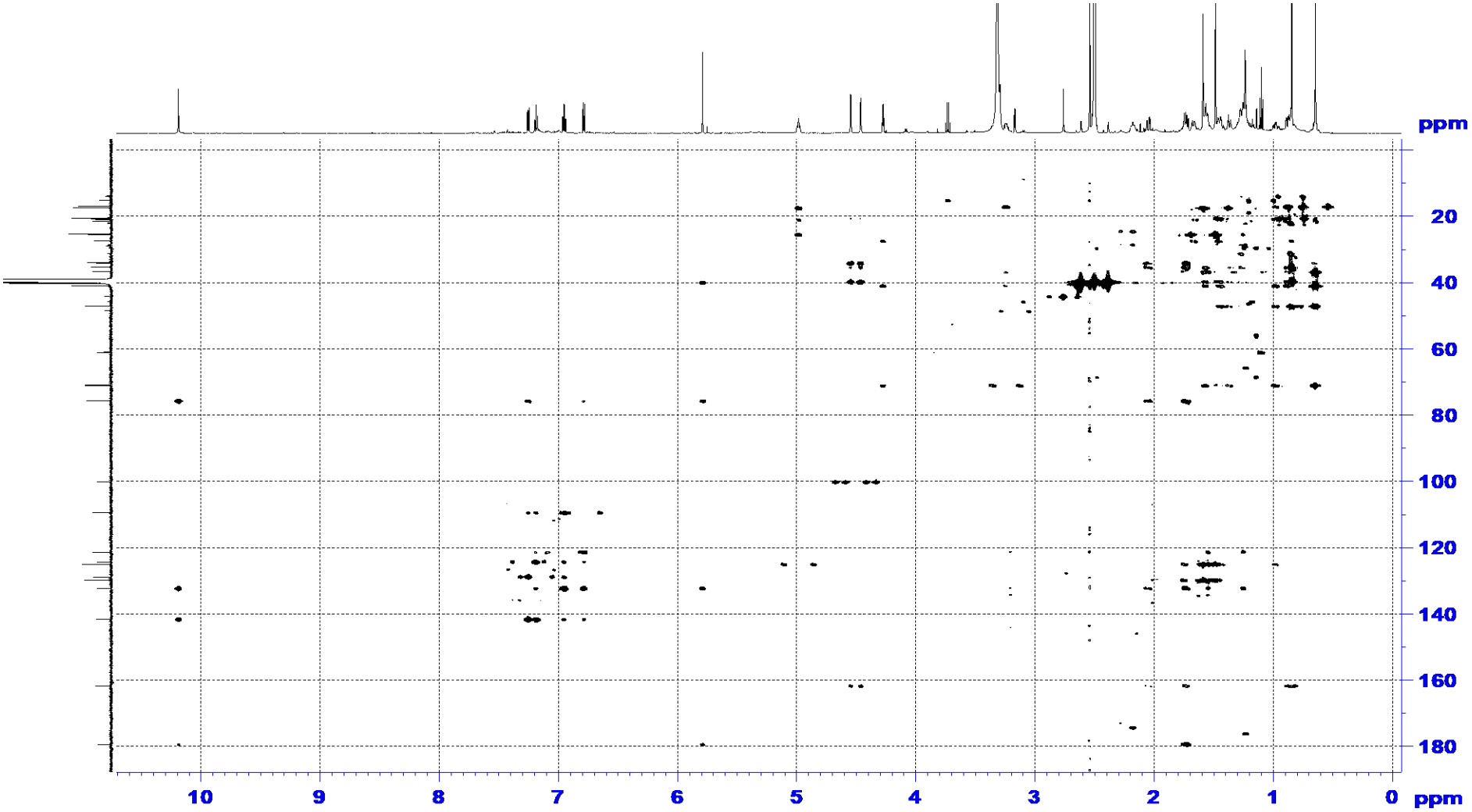


Figure S8 NOESY Spectrum of Penicindopene A (**1**) in DMSO-d_6 (600 MHz)

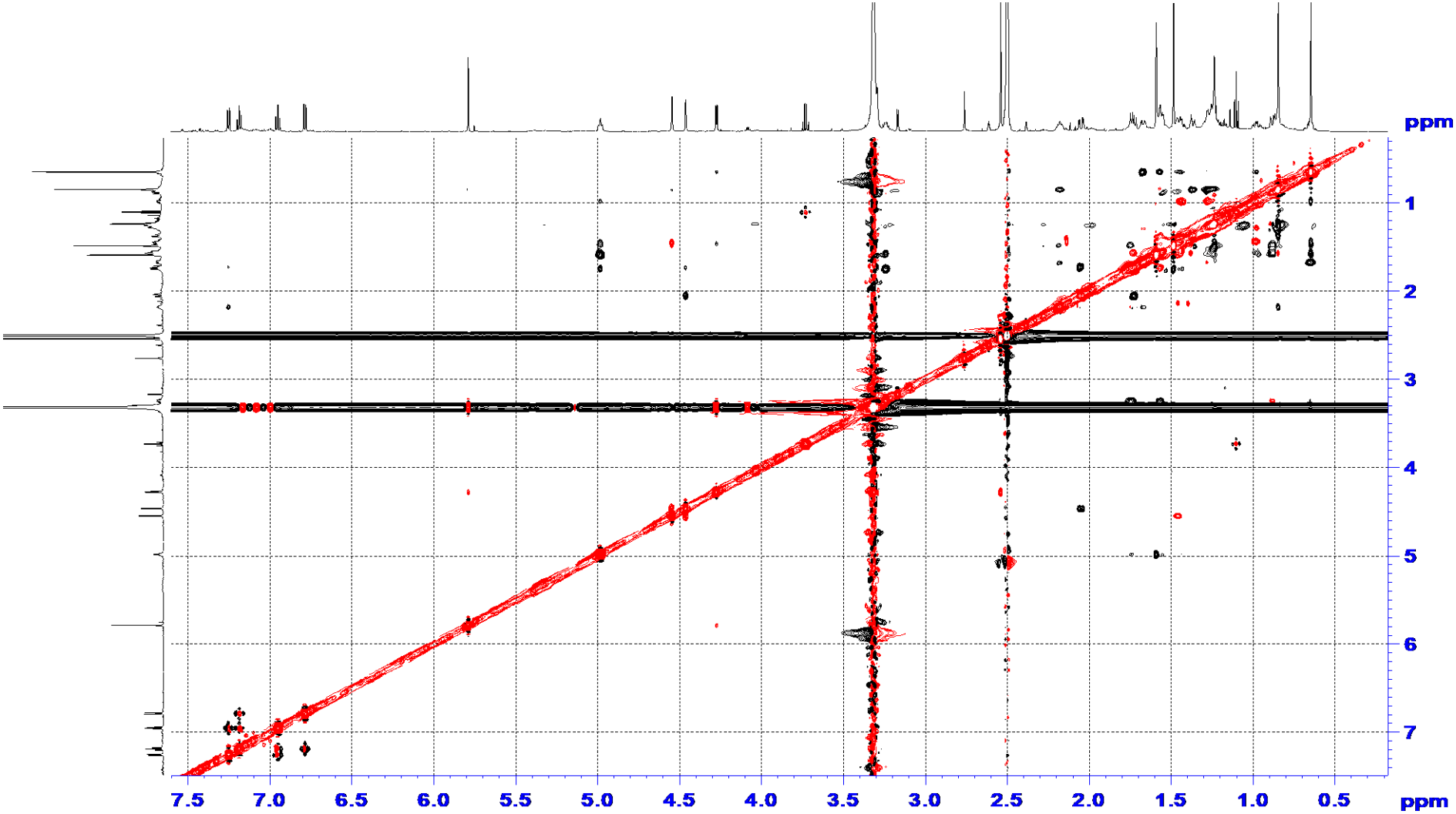


Figure S9 HRESI Spectrum of Penicindopene A (**1**)

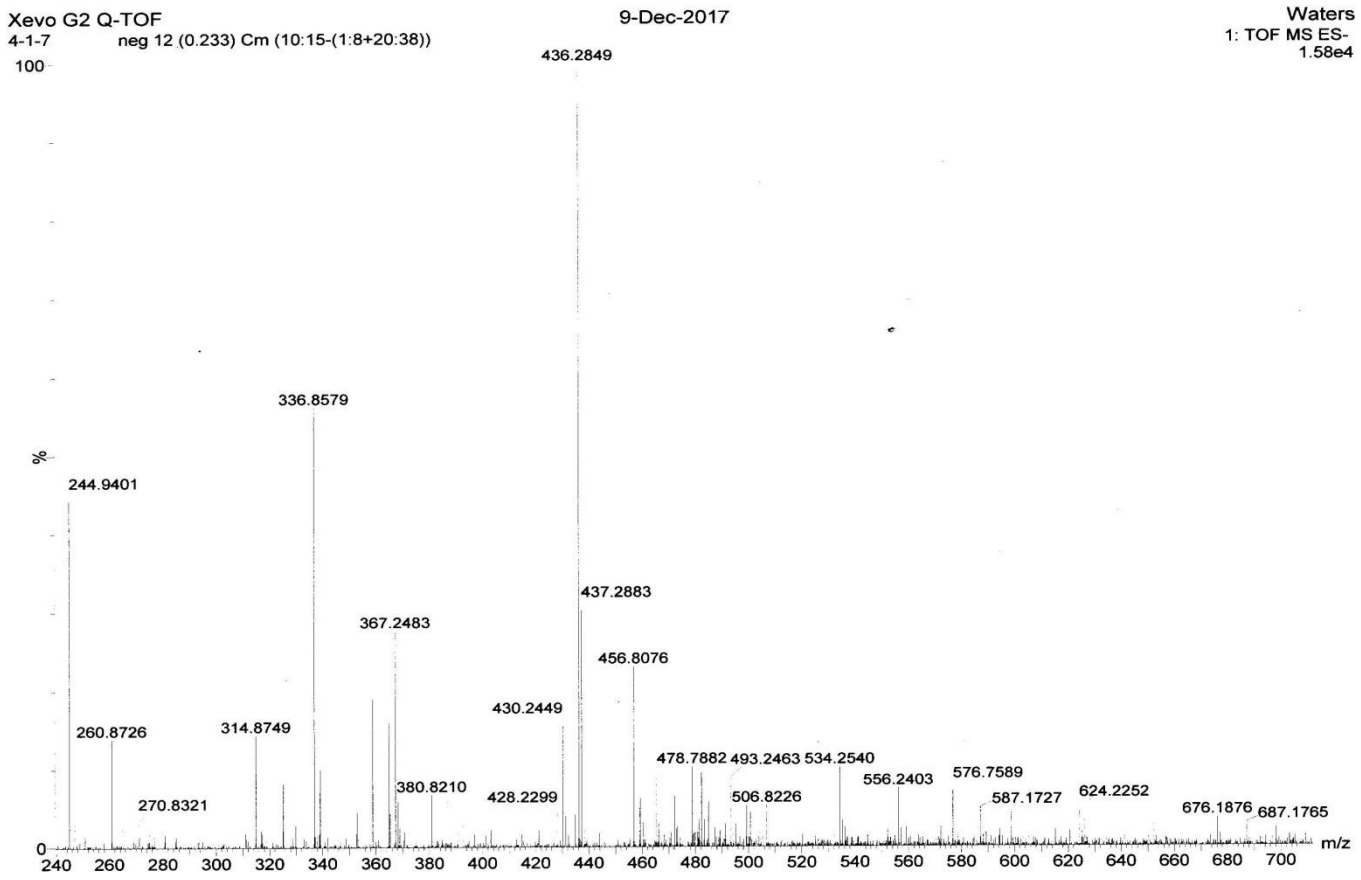


Table S3. The optimized conformers of 3*S*, 5*S*, 8*S*, 9*S*, 10*R*, 21*S*-**1**.

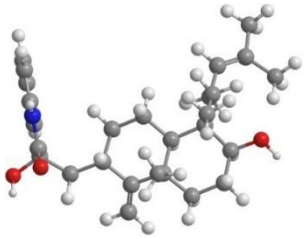
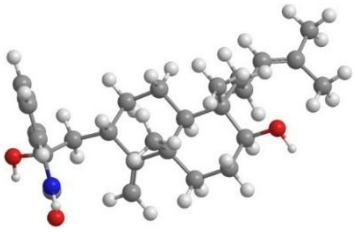
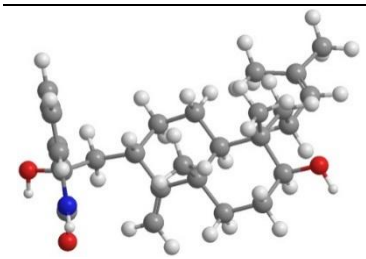
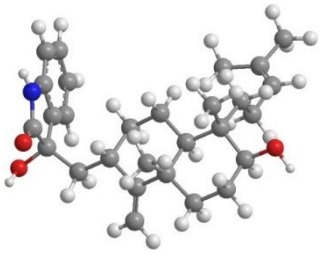
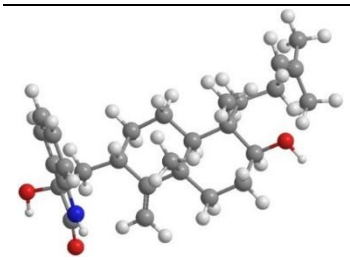
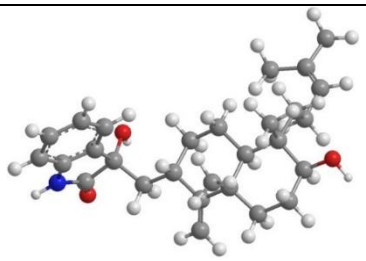
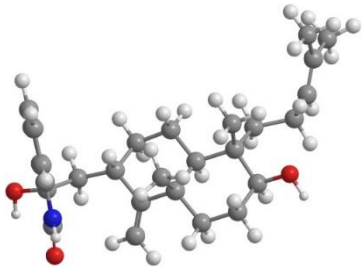
C1	C2
	
C3	**C4**
	
C5	**C6**
	
C7	
	

Table S4. B3LYP-calculated relative energies (Kcal/mol) and conformational population (%) for the most stable conformers of 3*S*, 5*S*, 8*S*, 9*S*, 10*R*, 21*S*-**1**

conformer	ΔE (kcal/mol)[a]	Population (%)[b]
C1	0	36.29
C2	0.000364	24.67
C3	0.000657	18.09
C4	0.001104	11.25
C5	0.001391	8.31
C6	0.003609	0.79
C7	0.003873	0.6

[a]Relative to conformer C1 with $E_{6\text{-}311++g(d,p)}$ = -1371.246224 Kcal/mol. [b]Calculated using free energy values from Gaussian 03W according to ΔG = -RT In K.

Table S5. The optimized conformers of 3*S*, 5*S*, 8*S*, 9*S*, 10*R*, 21*R*-**1**

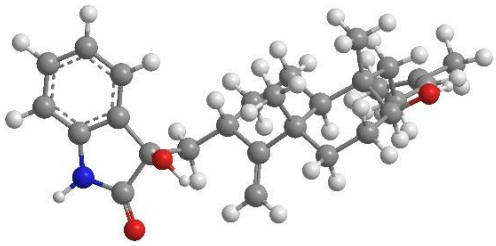
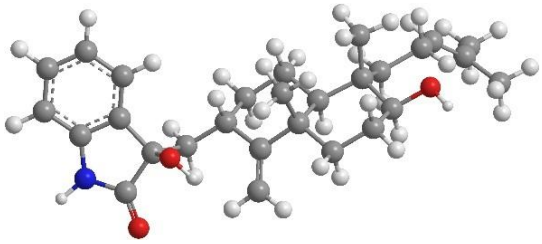
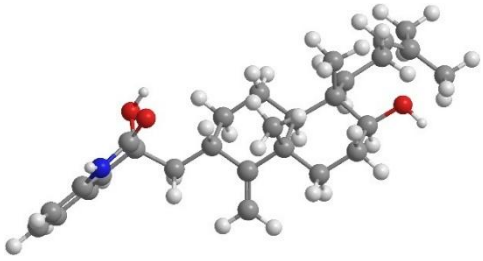
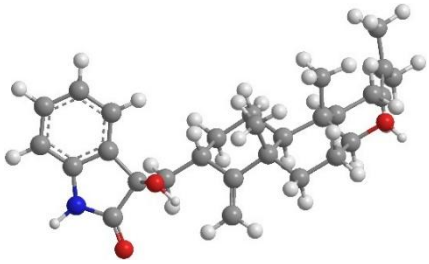
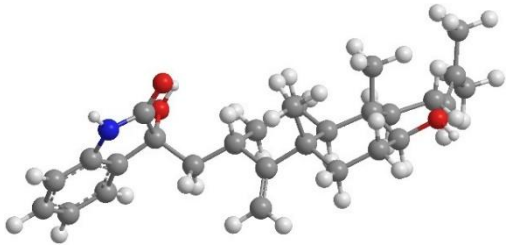
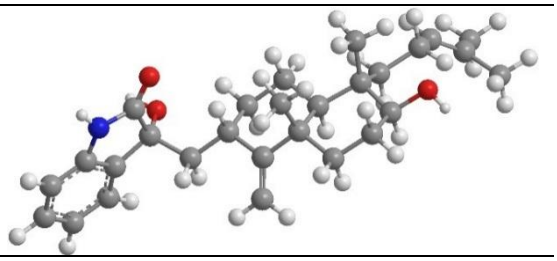
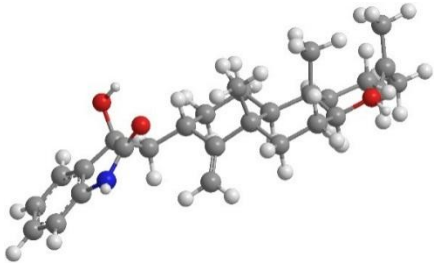
C1	C2
	
C3	**C4**
	
C5	**C6**
	
C7	
	

Table S6. B3LYP-calculated relative energies (Kcal/mol) and conformational population (%) for the most stable conformers of 3*S*, 5*S*, 8*S*, 9*S*, 10*R*, 21*R*-**1**

conformer	ΔE (kcal/mol)[a]	Population (%)[b]
C1	0	57.58
C2	0.001693	9.57
C3	0.001715	9.34
C4	0.001947	7.31
C5	0.001974	7.1
C6	0.002084	6.32
C7	0.002858	2.78

[a]Relative to conformer C1 with $E_{6\text{-}311++g(d,p)}$ = -1371.24433356 Kcal/mol. [b]Calculated using free energy values from Gaussian 03W according to ΔG = -RT In K.

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 Jr J A; 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; Morokuma K; Zakrzewski V G; Voth G A; Salvador P; Dannenberg J J; Dapprich S; Daniels A D; Farkas Ö; Foresman J B; Ortiz J V; Cioslowski J; Fox D J. Gaussian 09, Rev. C 01; Gaussian, Inc., Wallingford CT, 2009.

Stephens P J; Harada N. **2010**. ECD cotton effect approximated by the Gaussian curve and other methods. *Chirality*, 22:229–233.

Sybyl Software, version X 2.0; Tripos Associates Inc.: St. Louis, MO, 2013.