Supporting information

Unprecedented 22,26-*seco* physalins from *Physalis angulata* and their anti-inflammatory potential

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Content

Experimental Section

General methods and materials.

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. UV spectra were measured with a Shimadzu UV 2201 spectrophotometer. ECD spectra were measured with a Bio-Logic Science MOS-450 spectrometer. IR spectra were recorded on a Bruker IFS 55 spectrometer. Bruker AV-600 spectrometer was used to record NMR spectra. Chemical shift values are expressed in δ (ppm) using the peak signals of the solvent DMSO-d_6 (δ_H 2.50 and δ_C 39.5) as reference, and coupling constants (J in Hz) are given in parentheses. HRESIMS data were acquired on an Agilent 6210 TOF mass spectrometer. Silica gel GF254 prepared for TLC was purchased from Qingdao Marine Chemical Factory (Qingdao, China). Silica gel (200−300 mesh, Qingdao Marine Chemical Factory, Qingdao, China) and octadecyl silica gel (Merck Chemical Company Ltd, German) were used for column chromatography (CC). RP-HPLC separations were conducted using an LC-6AD liquid chromatograph and a SPD-20A UV detector (Shimadzu, Kyoto, Japan) with a RP-C18 column (250 × 20 mm, 120 Å, 5 μm, YMC Co. Ltd). Spots were detected on TLC plates under UV light or by heating after spraying with anisaldehyde-H2SO4 reagent.

Plant material.

The stems and leaves of *P. angulata* were collected from Nanning, Guangxi Province, China, in July 2013, and identified by Pharmacist Jia-Fu Wei, Guangxi Institute for Food and Drug Control. A voucher specimen (PA-20130826) has been deposited in the herbarium of the Department of Natural Products Chemistry, Shenyang Pharmaceutical University.

Extraction and isolation.

The dried stems and leaves of *P. angulata* (9.5 kg) were extracted with 75% EtOH (110 L × 2 h × 2). The resulting extracts (1.3 kg) were concentrated *in vacuo*, suspended in H_2O (5 L), and partitioned with petroleum ether, EtOAc, and *n*-BuOH (5 L × 3), successively. The EtOAc extracts (116 g) were subjected to silica gel CC (10 × 80 cm) eluted with CH_2Cl_2/MeOH (100:1, 80:1, 60:1, 40:1, 20:1, 10:1, 8:1, 5:1, 3:1, 1:1, and 0:1 v/v) to obtain six fractions (E1−E6). Fraction E3 (35 g) was subjected to silica gel CC (6 × 80 cm) eluted with petroleum ether/acetone (10:1 to 0:1) to produce seven fractions (E31−E37). Fraction E33 (4.0 g) was separated by ODS CC (3 × 50 cm) with elution using a gradient of increasing MeOH (10–100%) in H_2O (1:9 to 1:0) to obtain three fractions (E331−E333). Fraction E331 (2 g) was chromatographed over silica gel CC (2 × 50 cm), eluted with a gradient of increasing acetone (0–100%) in petroleum ether, to obtain four fractions (E3311−E3314). The separation of fraction E3314 (1.1 g) by silica gel CC (2 × 50 cm) with $CHCl_3$/MeOH (80:1 to 1:1), preparative TLC ($CHCl_3$/acetone, 4:1) and preparative HPLC (65% MeOH/H_2O, 6 mL min^{-1}) yielded compound **2** (4 mg). Fraction E4 (15 g) was subjected to silica gel CC (5 × 70 cm), eluting with $CHCl_3$/acetone (80:1 to 1:1), to afford five fractions (E41−E45). Fraction E45 (4 g) was separated by ODS column (3 × 50 cm) eluted with MeOH/H_2O (1:9 to 1:0), preparative TLC (CH_2Cl_2/acetone, 2:1), and preparative HPLC (65% MeOH/H_2O) to obtain compound **1** (2 mg).

Physalin X (1): amorphous powder; $[\alpha]_D^{25}$ −28.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.5) nm; IR (KBr) ν_{max} 3395, 2921, 2850, 1647, 1469, 1384, 1120

cm^{-1}; ^{1}H (400 MHz, DMSO-d_6) and ^{13}C-NMR (100 MHz, DMSO-d_6) data, see Table 1; HRESIMS *m/z* 593.2234 [M – H]$^{-}$ (calcd for $C_{29}H_{37}O_{13}$, 593.2234).

Aromaphysalin B (2): amorphous powder; [α] − 38.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.9), 277 (3.1) nm; IR (KBr) ν_{max} 3395, 3189, 2920, 2849, 1646, 1469, 1420, 1384, 1119, 879, 803, 721 cm^{-1}; ECD ($\Delta\varepsilon$) 222 (+ 0.36), 242 (– 2.21), 334 (– 0.80) nm; ^{1}H (400 MHz, DMSO-d_6) and ^{13}C-NMR (100 MHz, DMSO-d_6) data, see Table1; HRESIMS *m/z* 543.1862 [M – H]$^{-}$, calcd for $C_{28}H_{31}O_{11}$ 543.1866.

ECD calculation.

In general, conformational analyses were carried out via random searching in the Sybyl-X 2.0 using the MMFF94S force field with an energy cutoff of 2.5 kcal/mol.[1] Due to the limitations of NOESY correlations, the results showed four lowest energy conformers for 6*S*, 11*S*, 13*S*, 15*S*, 16*S*, 17*R*, 20*S*, 22*R*, 24*S*-**2** and only one for 6*R*, 11*S*, 13*S*, 15*S*, 16*S*, 17*R*, 20*S*, 22*R*, 24*S*-**2**. Subsequently, the conformers were re-optimized using DFT at the B3LYP/6-31+G(d) level in gas phase by the GAUSSIAN 09 program. The energies, oscillator strengths, and rotational strengths (velocity) of the first 60 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, σ = 0.25 eV). To get the final spectra, the simulated spectra of three conformers with the proportion more than one percent were averaged according to the Boltzmann distribution theory and their relative Gibbs free energy (ΔG). Theoretical ECD spectra of the corresponding enantiomers were obtained by directly inverse of the ECD spectra of 6*S*, 11*S*, 13*S*, 15*S*, 16*S*, 17*R*, 20*S*, 22*R*, 24*S*-**2** and 6*R*, 11*S*, 13*S*, 15*S*, 16*S*, 17*R*, 20*S*, 22*R*, 24*S*-**2**.

NO Production Bioassay.

Compounds **1** and **2** were assayed for the inhibition of NO production according to the Griess method.[1-3] 1×10^6 Cells/well of RAW 264.7 cells were added into the 96-well plates, and incubated at 37 °C for 24 h by the stimulation of LPS (1 μg/mL) with or without test compounds. After the addition of Griess reagent [0.1% *N*-(1-naphthyl)-ethylenediamine (50 μL); 1% sulfanilamide in 5% H_3PO_4 (50 μL)], absorbance (540 nm) was recorded by using a microplate reader. The standard curve was used to calculate the NO concentrations and inhibitory rates.

Molecular Modeling.

The crystal structure of protein was taken from Protein Data Bank (PDB ID: 5FVX), chain A, HEME, and Zn^{2+} were used for docking according to our previous method.[4] The protein was modelled with CHARMM 22 force field. The 3D structure of compound was prepared and Gasteiger-Hückel charges was added using Sybyl software. Each ligand was subjected to energy minimization with Tripos force filed parameters. Blind docking was carried out using AutoDock 4 program. The 3D docking grid was sufficiently large to cover the protein. A total 100 conformations of each ligand were searched using Lamarckian generic algorithm, and the final docking conformations were clustered into different number of clusters in terms of the root-mean-square deviations of the ligand within the binding pocket of the receptor.

References

1 C. P. Sun, A. G. Kutateladze, F. Zhao, L. X. Chen and F. Qiu, *Org. Biomol. Chem.,* 2017, **15**, 1110–1114.

2 C. P. Sun, C. Y. Qiu, T. Yuan, X. F. Nie, H. X. Sun, Q. Zhang, H. X. Li, L. Q. Ding, F. Zhao, L. X. Chen and F. Qiu, *J. Nat. Prod.,* 2016, **79**, 1586–1597.

3 C. P. Sun, T. Yuan, L. Wang, N. Kang, F. Zhao, L. X. Chen and F. Qiu, *RSC Adv.*, 2016, **6**, 76838–76847.

4 C. Y. Qiu, L. Tong, T. Yuan, F. Wang, F. Zhao and L. X. Chen, *J. Nat. Med.*, 2017, **71**, 292–298.

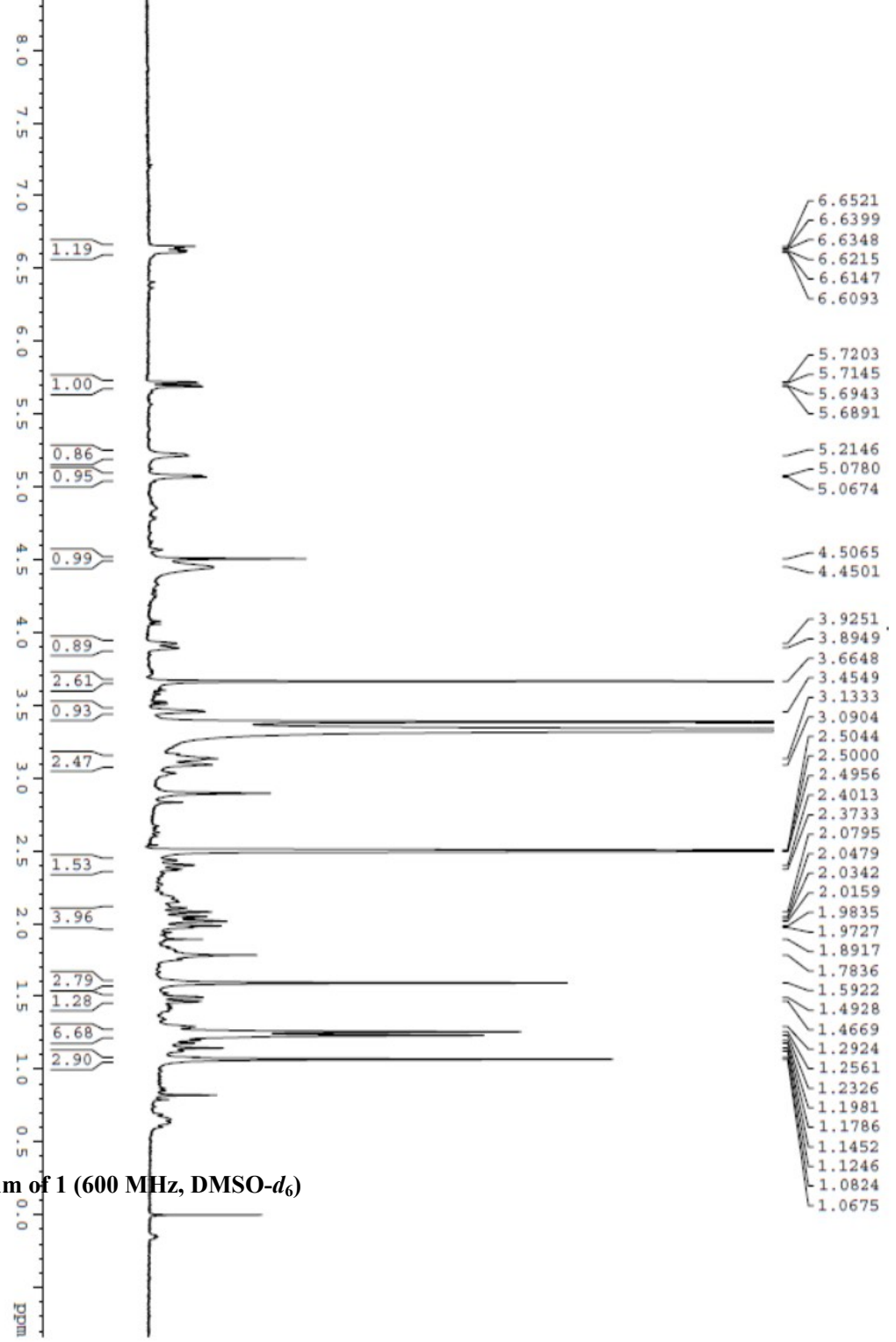


Figure S1. ^{1}H NMR spectrum of 1 (600 MHz, DMSO-d_6)

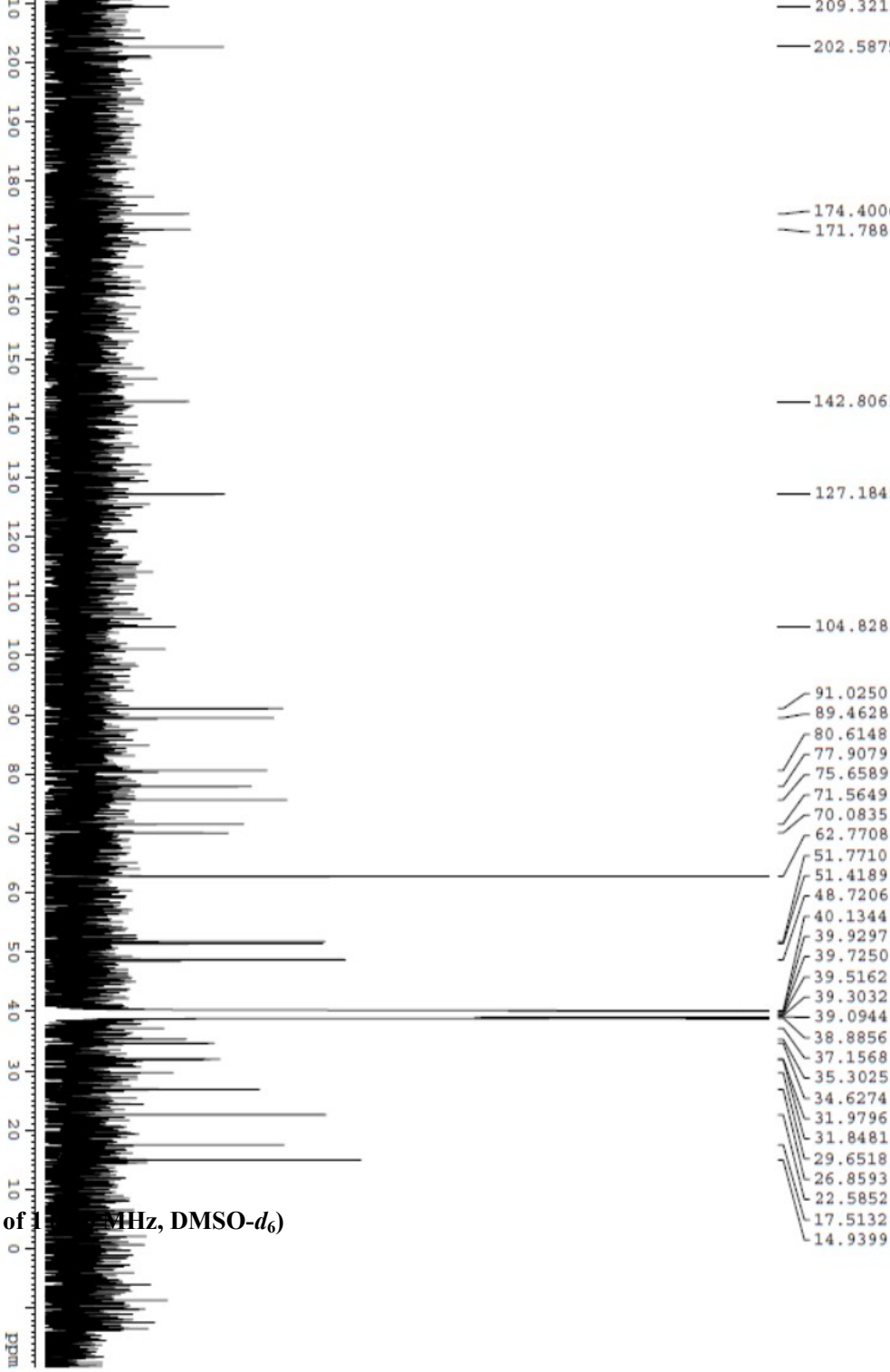


Figure S2. ^{13}C NMR spectrum of 1 (MHz, DMSO-d_6)

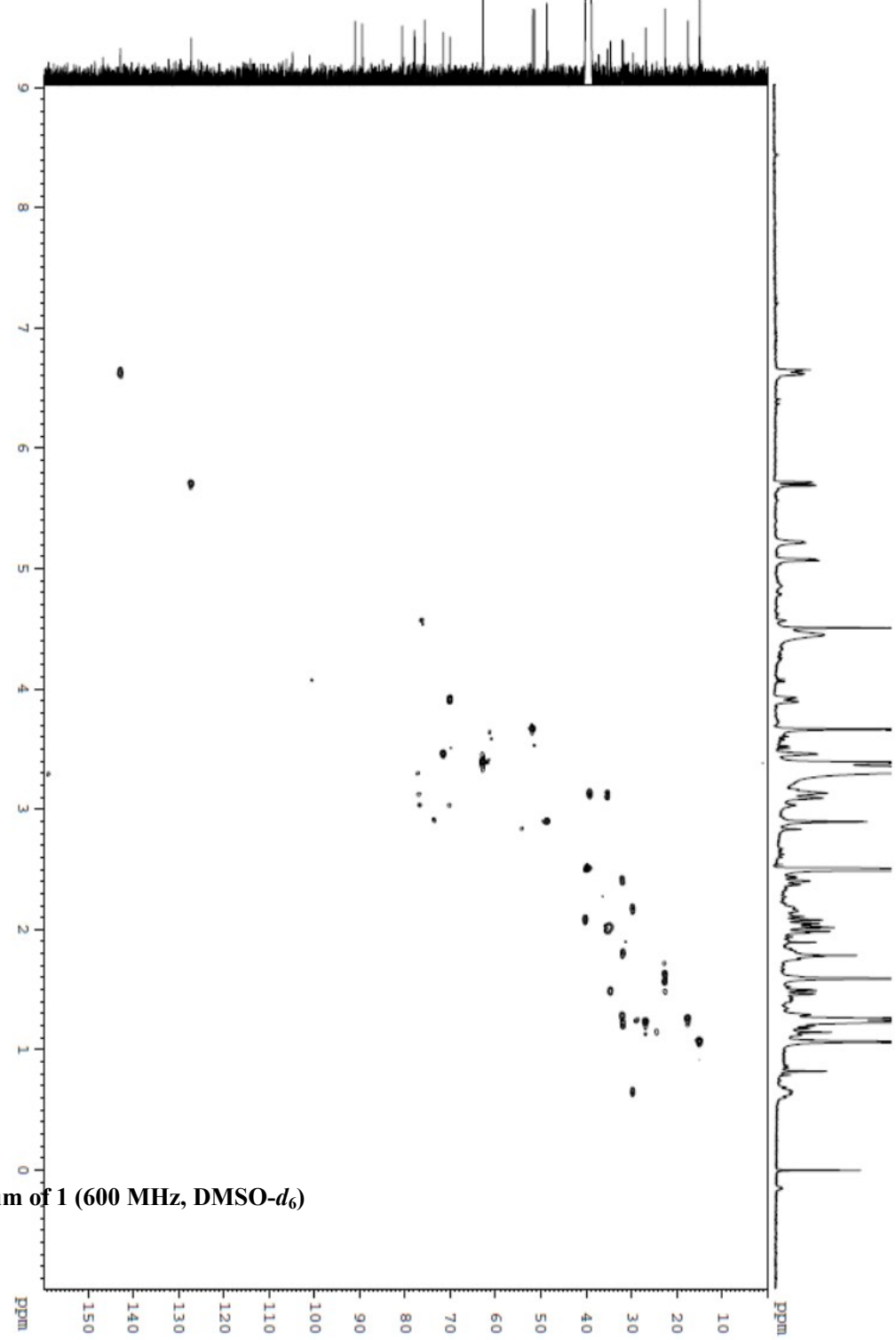


Figure S3. HSQC spectrum of 1 (600 MHz, DMSO-d_6)

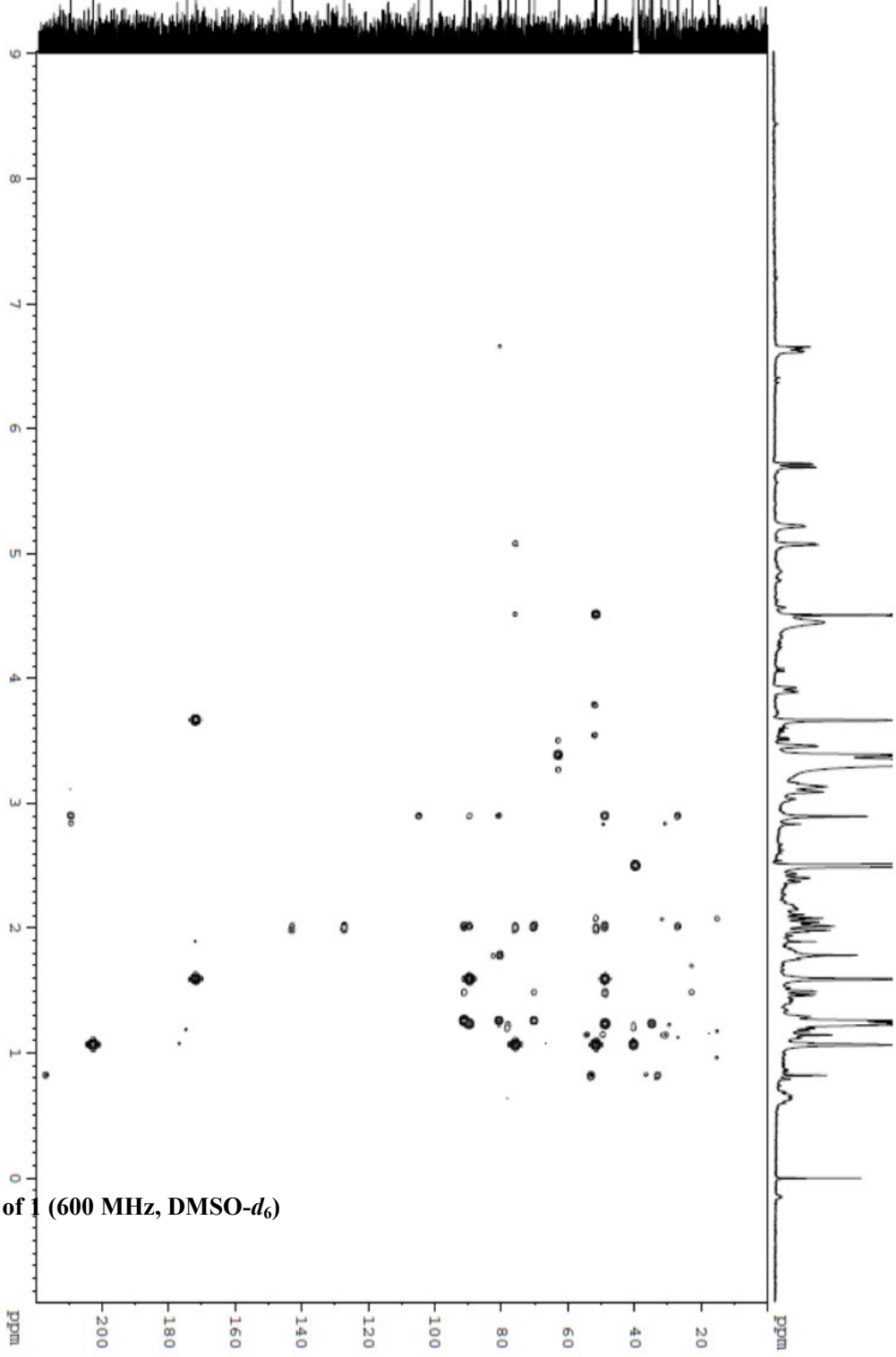


Figure S4. HMBC spectrum of 1 (600 MHz, DMSO-d_6)

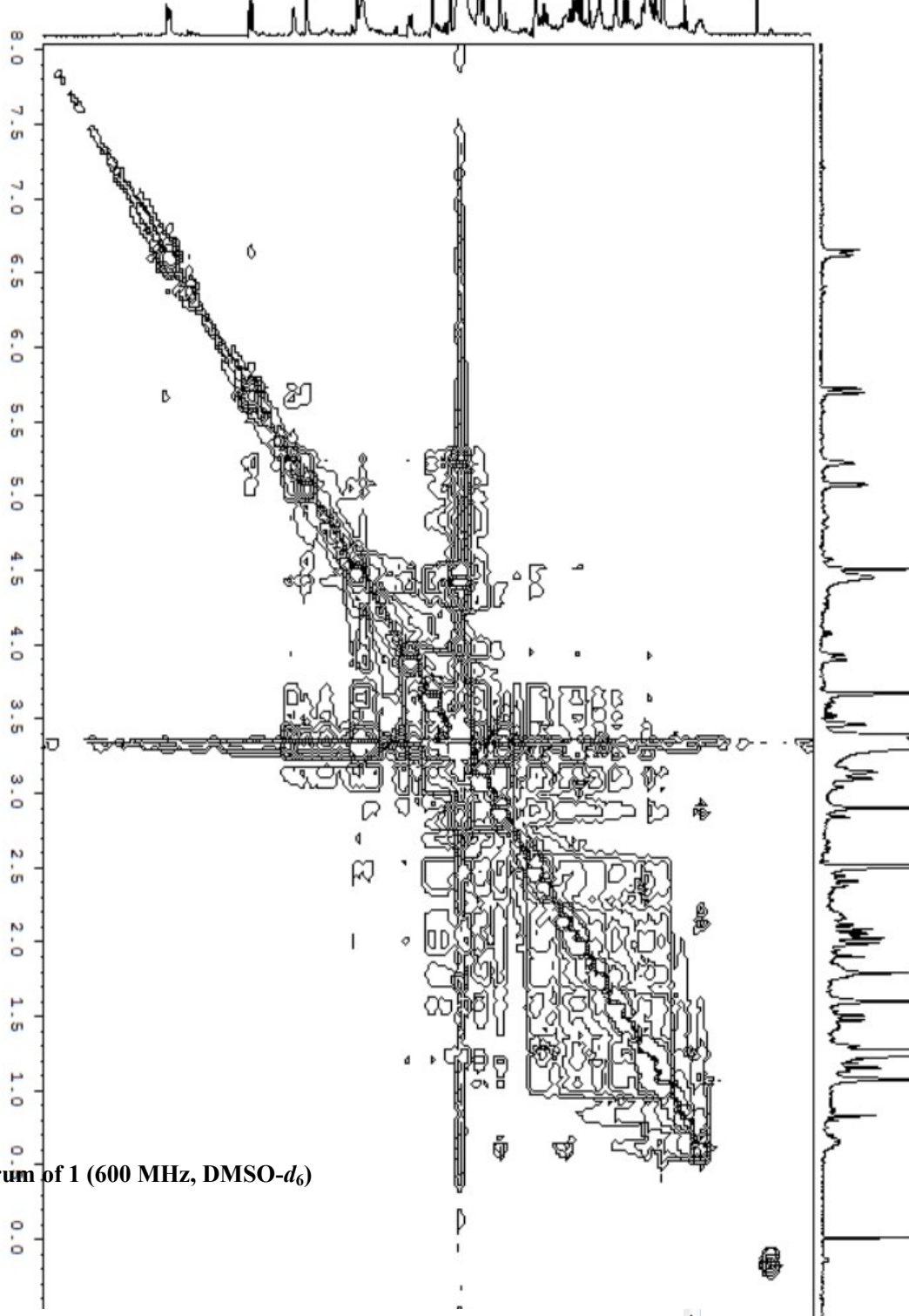


Figure S5. NOESY spectrum of 1 (600 MHz, DMSO-d_6)

Tolerance = 1.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
247 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:

Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	O	Na
593.2234	100.00	593.2234	0.0	0.0	11.5	C29 H37 O13	512.5	n/a	n/a	29	37	13	

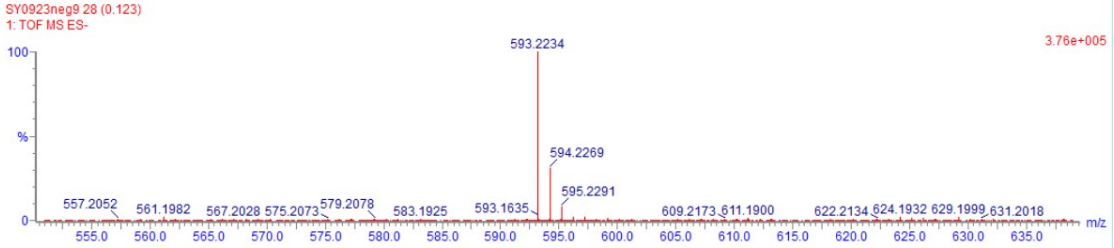
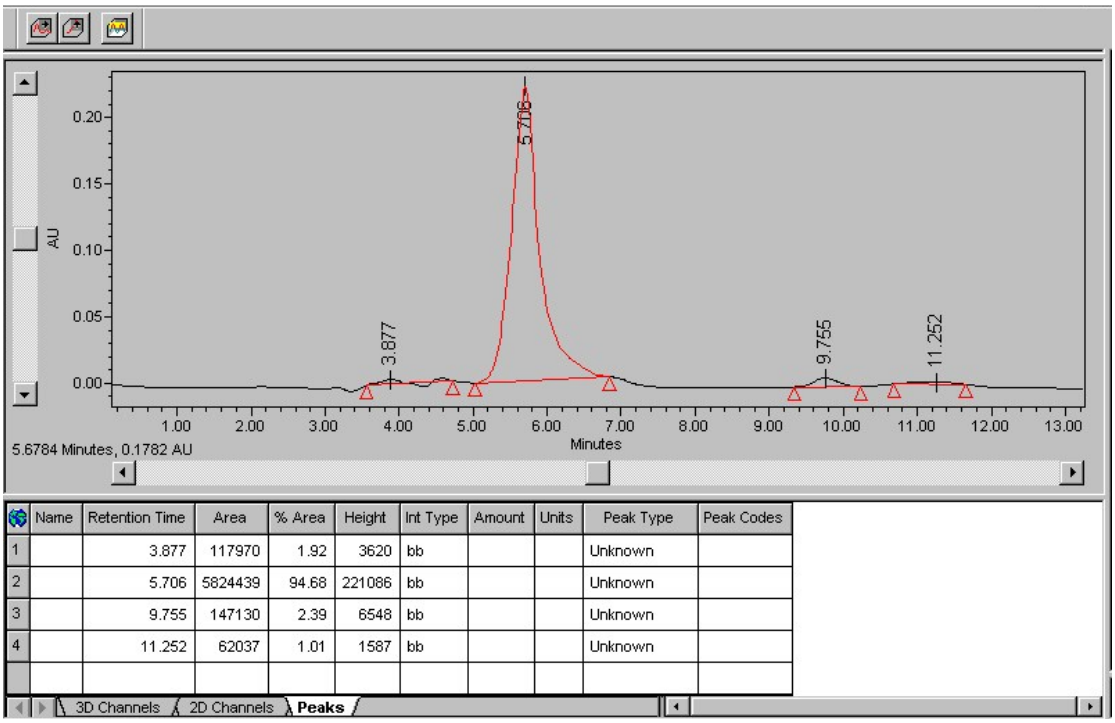


Figure S6. HRESIMS spectrum of 1



	Name	Retention Time	Area	% Area	Height	Int Type	Amount	Units	Peak Type	Peak Codes
1		3.877	117970	1.92	3620	bb			Unknown	
2		5.706	5824439	94.68	221086	bb			Unknown	
3		9.755	147130	2.39	6548	bb			Unknown	
4		11.252	62037	1.01	1587	bb			Unknown	

3D Channels | 2D Channels | Peaks

Figure S7. HPLC chromatogram of 1. The purity of 1 is 94.68% according to the HPLC analysis result.

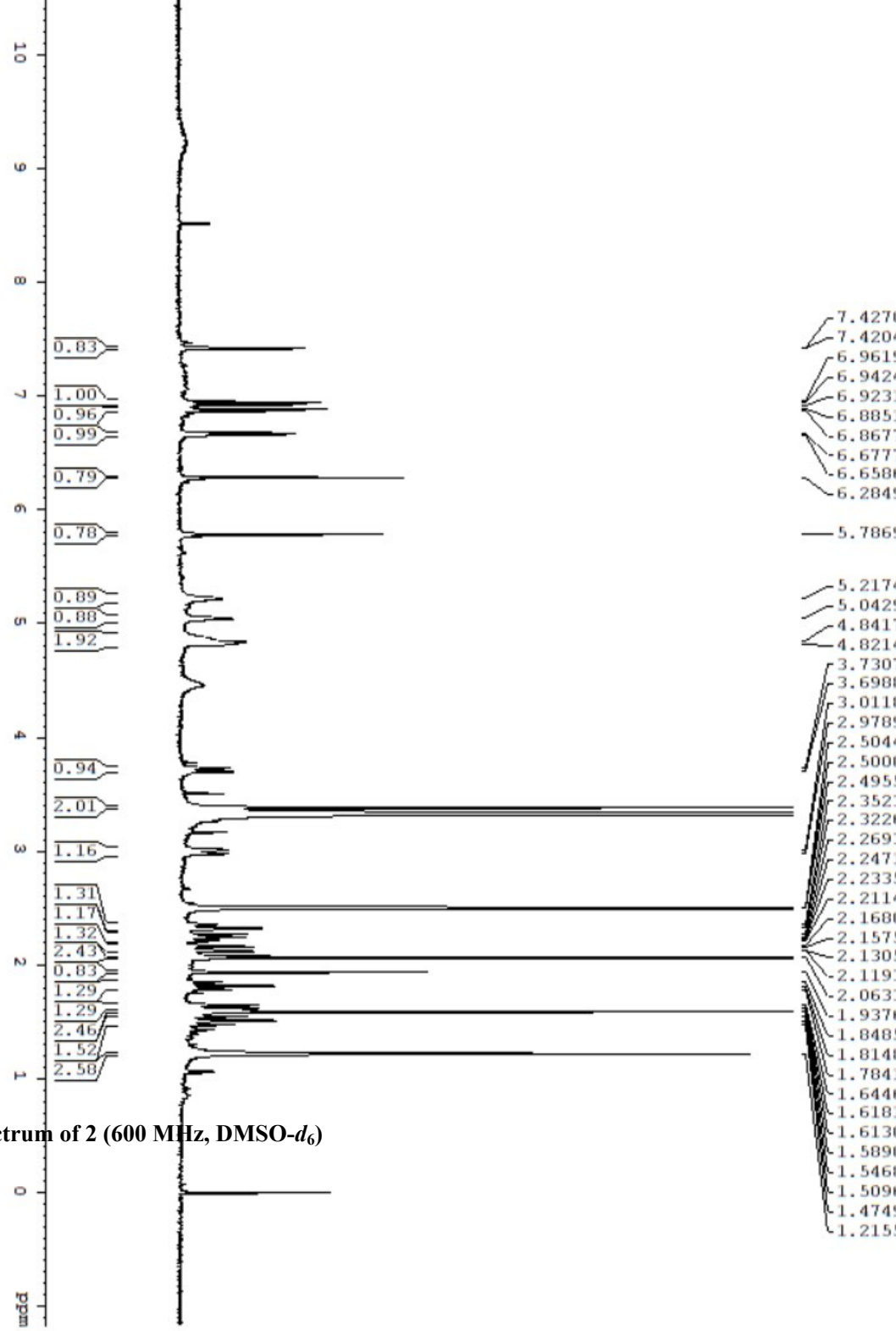


Figure S8. ^{1}H NMR spectrum of 2 (600 MHz, DMSO-d_6)

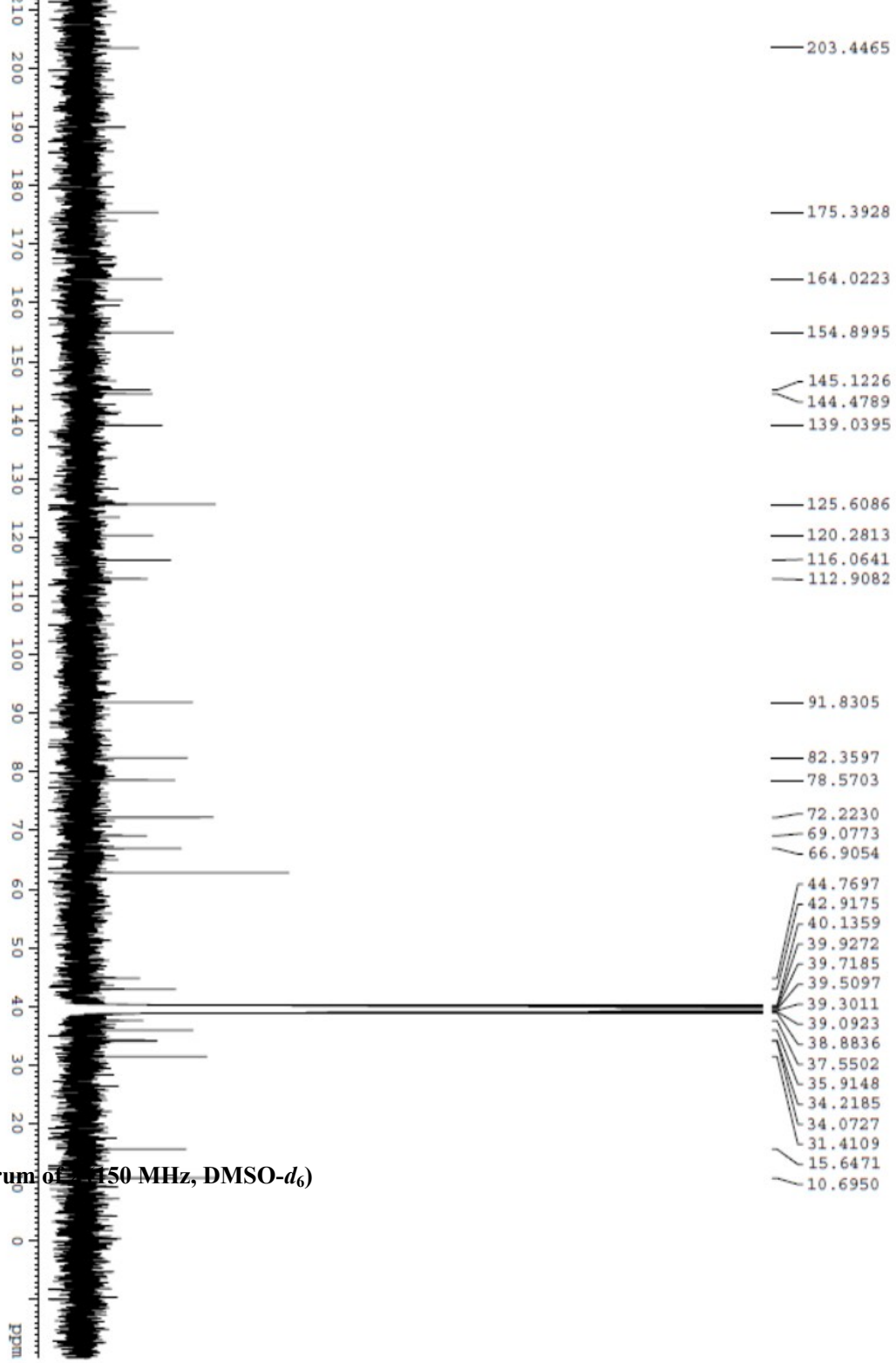


Figure S9. ^{13}C NMR spectrum of (150 MHz, DMSO-d_6)

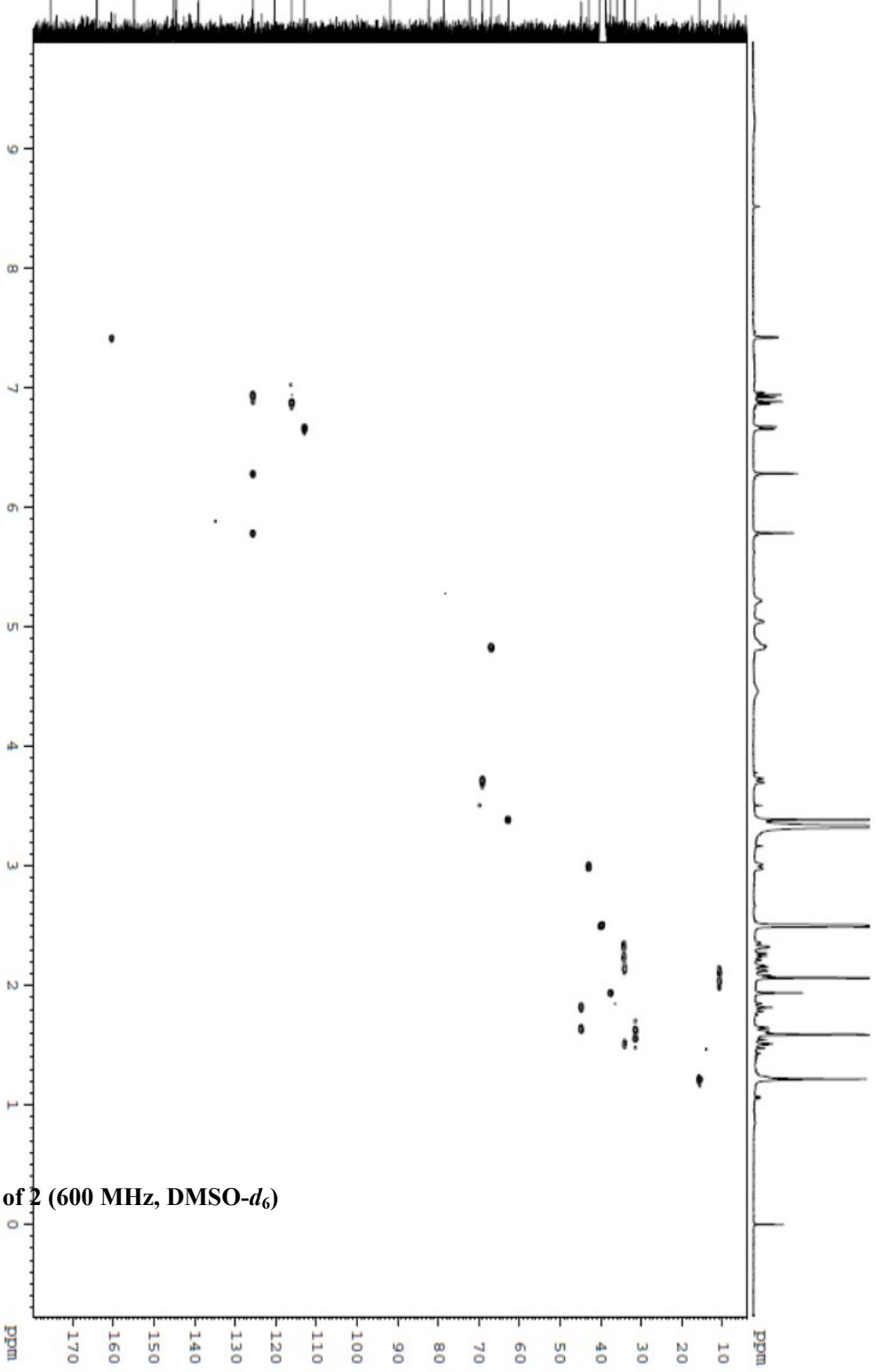


Figure S10. HSQC spectrum of 2 (600 MHz, DMSO-d_6)

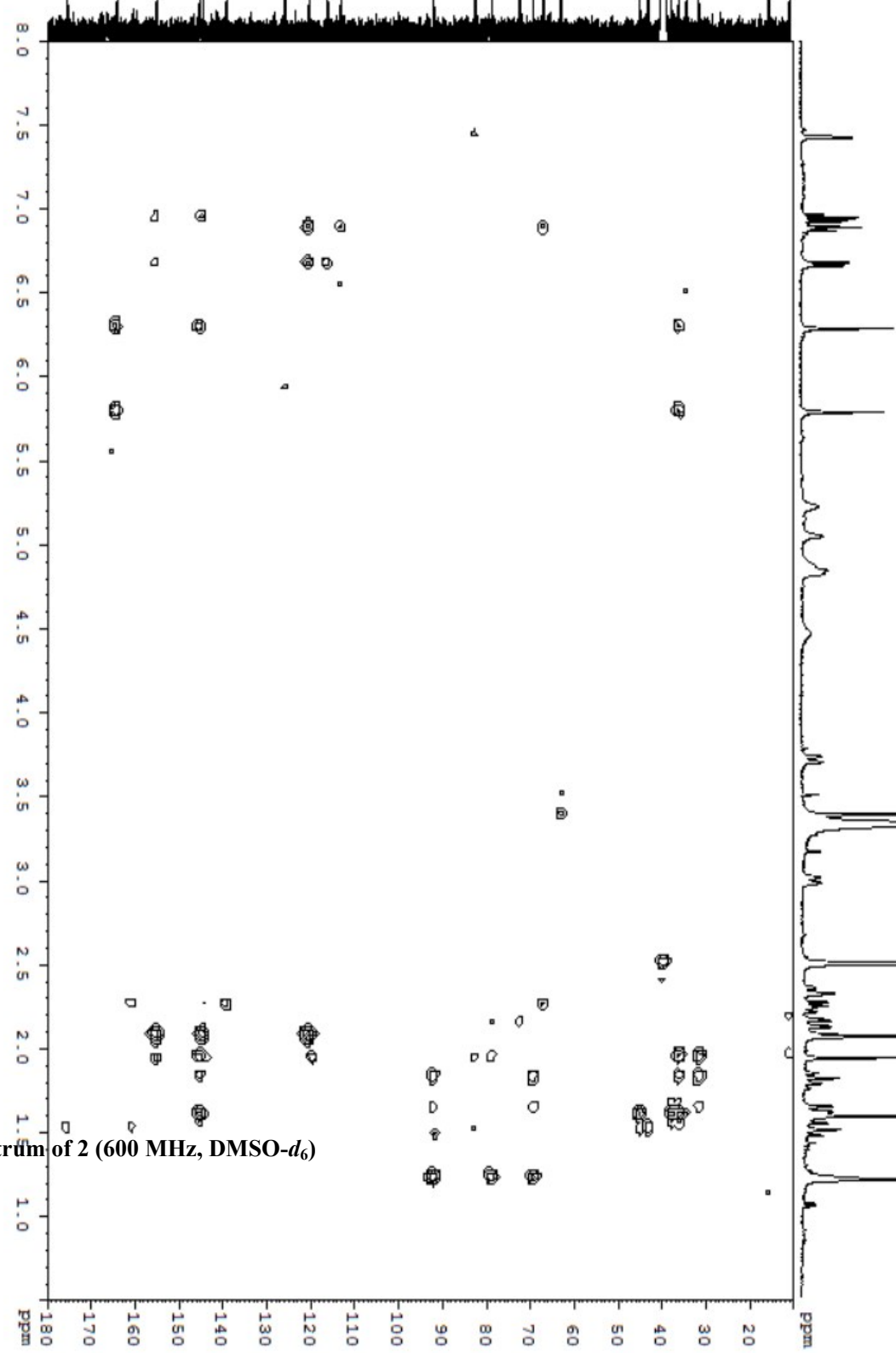


Figure S11. HMBC spectrum of 2 (600 MHz, DMSO-d_6)

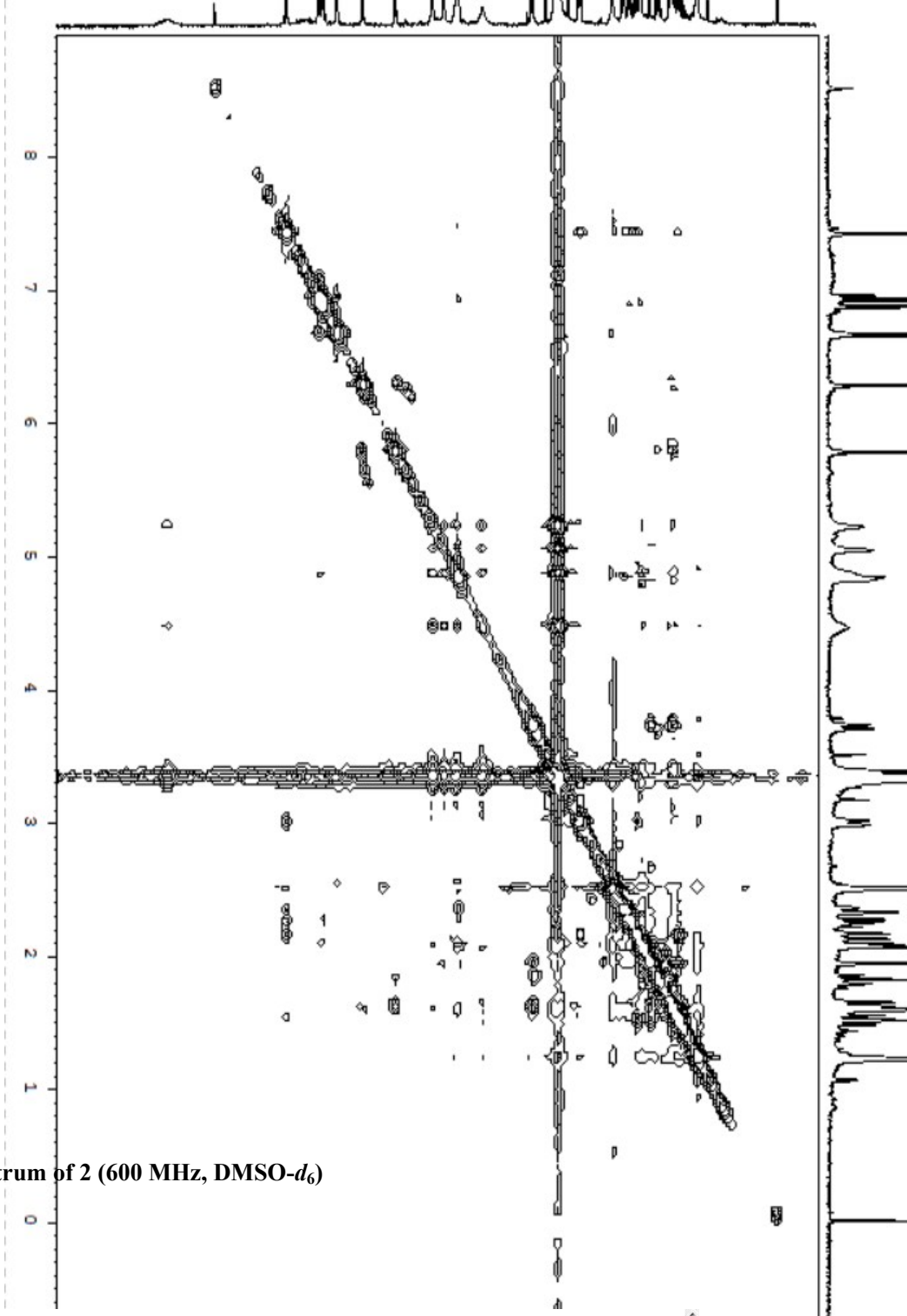


Figure S12. NOESY spectrum of 2 (600 MHz, DMSO-d_6)

Tolerance = 1.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
229 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:

Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	O	Na
543.1862	100.00	543.1866	-0.4	-0.7	13.5	C28 H31 O11	75.5	n/a	n/a	28	31	11	

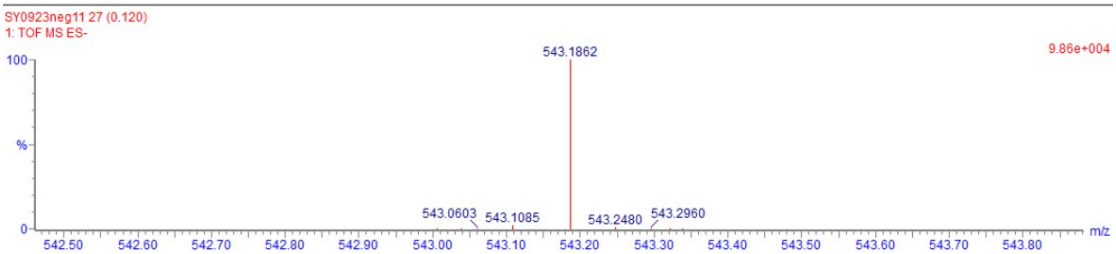


Figure S13. HRESIMS spectrum of 2

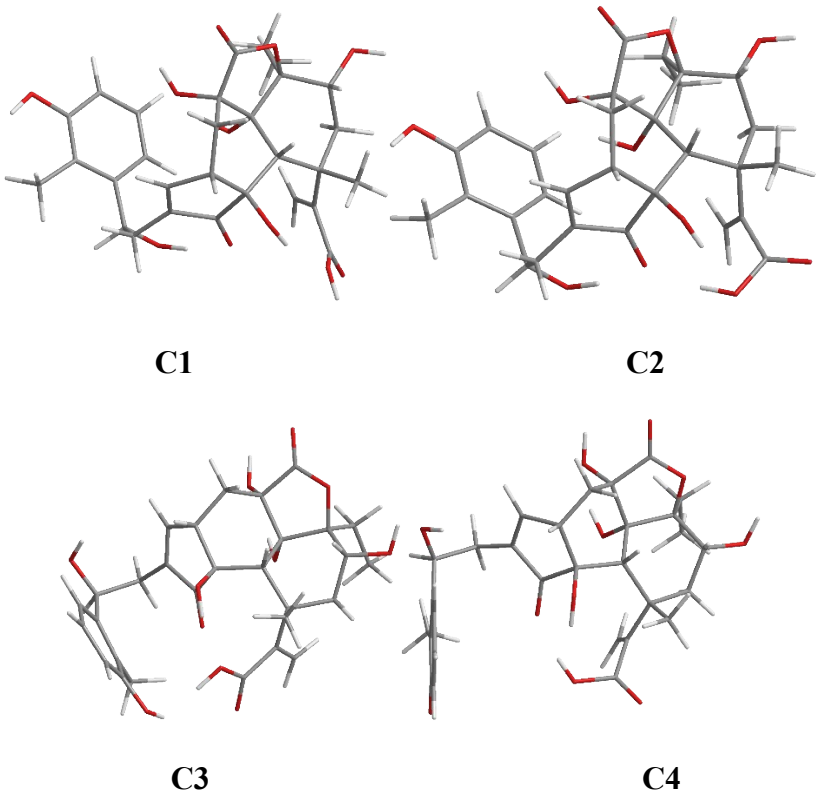


Figure S14. The stable conformers of compound 6*S*, 11*S*, 13*S*, 15*S*, 16*S*, 17*R*, 20*S*, 22*R*, 24*S*-2

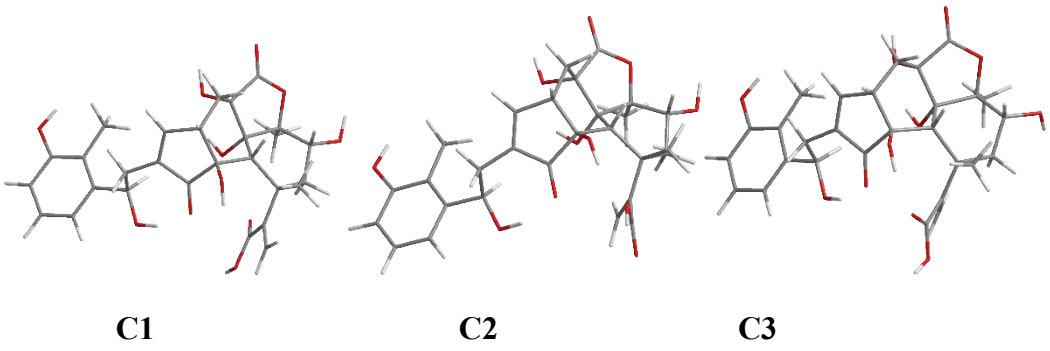


Figure S15. The stable conformers of compound 6*R*, 11*S*, 13*S*, 15*S*, 16*S*, 17*R*, 20*S*, 22*R*, 24*S*-2

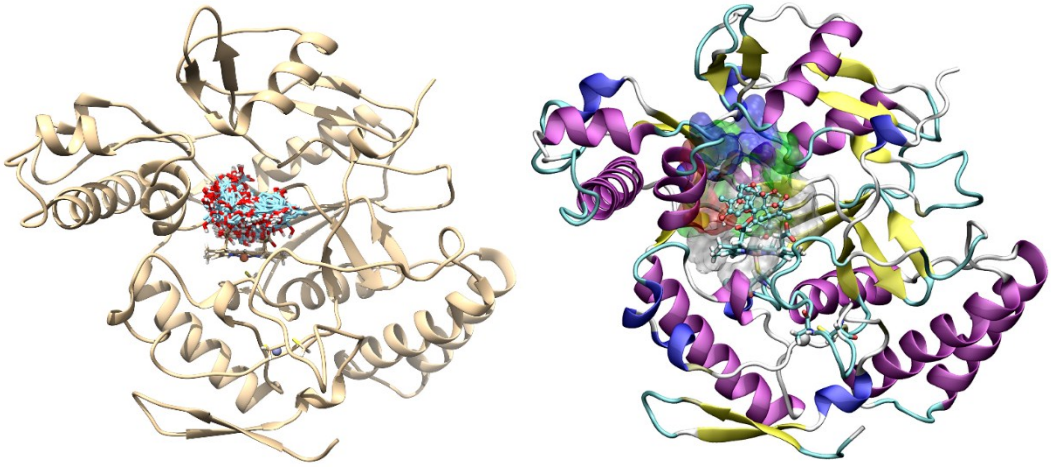


Figure S16. The molecular docking result of compound 2 with NOS

Table S1. B3LYP-calculated relative energies (Kcal/mol) and conformational population (%) for the most stable conformers of 6*S*, 11*S*, 13*S*, 15*S*, 16*S*, 17*R*, 20*S*, 22*R*, 24*S*-2

conf	$\Delta E_{6\text{-}31+G(d)}$[a]	%[b]
C1	0.00	84.75
C2	0.0022904	7.48
C3	0.0025292	5.80
C4	0.0035472	1.97

[a]Relative to **C1** with $E_{6\text{-}311++G(d,p)}$ = -1953.5332102 kcal/mol. [b]Calculated using free energy values from Gaussian 03W according to $\Delta G = -RT \ln K$.

Table S2. B3LYP-calculated relative energies (Kcal/mol) and conformational population (%) for the most stable conformers of 6*R*, 11*S*, 13*S*, 15*S*, 16*S*, 17*R*, 20*S*, 22*R*, 24*S*-2

conf	$\Delta E_{6\text{-}31+G(d)}$[a]	%[b]
C1	0.00	93.16
C2	0.0029556	4.06
C3	0.003313	2.78

[a]Relative to **C1** with $E_{6\text{-}311++G(d,p)}$ = -1914.22289 kcal/mol. [b]Calculated using free energy values from Gaussian 03W according to $\Delta G = -RT \ln K$.