Electronic Supplementary Information (ESI) to

Spontaneous oxidative cyclisations of 1,3-dihydroxy-4-dimethylallylnaphthalene to tricyclic derivatives

Jinglin Wang, [‡a,b] Huomiao Ran, [‡a] Xiulan Xie, [c] Kaiping Wang [d] and Shu-Ming Li [*a]

[a] Institut für Pharmazeutische Biologie und Biotechnologie, Philipps-Universität Marburg, Robert-Koch-Straße 4, 35037 Marburg, Germany. E-Mail: shuming.li@staff.uni-marburg.de.

[b] Union Hospital of Huazhong University of Science and Technology, Department of Pharmacy, No. 1227, Jiefang Road, 430030 Wuhan, China

[c] Fachbereich Chemie, Philipps-Universität Marburg, Hans-Meerwein-Straße 4, 35032 Marburg, Germany

[d] Hubei Key Laboratory of Nature Medicinal Chemistry and Resource Evaluation, Tongji Medical College of Pharmacy, Huazhong University of Science and Technology, 430030 Wuhan, China

Corresponding to Shu-Ming Li, Telephone: +49-6421-28-22461; FAX: +49-6421-28-26678; E-mail: shuming.li@staff.uni-marburg.de

Table of Contents

Experimental Procedures

1. Chemicals

Dimethylallyl diphosphate (DMAPP) was synthesized according to the method reported previously.[1] 1,3-dihydroxynaphthalene (**1**) was obtained from Fluka. Oxygen-18 ($^{18}O_2$, 97 %) and ^{18}O-enriched water ($H_2{}^{18}O$, 97 %) were purchased from Eurisotop. All other chemicals used in this study were of analytical grade.

2. Overproduction and purification of recombinant proteins

Overproduction and purification of FgaPT2,[2] CdpNPT,[3] FtmPT1,[4] and AnaPT[5] were carried out as described in the literature.

3. Enzyme assays with different prenyltransferases

The enzymatic reaction mixtures (50 µl) contained 50 mM Tris-HCl (pH 7.5), 10 mM $CaCl_2$, 1 mM 1,3-dihydroxynaphthalene (**1**), 2 mM DMAPP, 0.15–1.5% (*v/v*) glycerol, 5% (*v/v*) dimethyl sulfoxide (DMSO) and 20 µg of the purified recombinant proteins. These mixtures were incubated at 37°C for 30 min or 16 h and terminated by addition of one volume acetonitrile (CH_3CN) and subsequently centrifuged at 17,000 $\times g$ for 30 min before further analysis on HPLC. For structure elucidation, products were isolated from large-scale incubations of 10 ml with 4 mg protein.

4. Time and pH dependent assays with 1

To determine the nonenzymatic formation, a time dependent assay was performed. 1 mM 1,3-dihydroxynaphthalene (**1**) was incubated with 10 mM $CaCl_2$, 2 mM DMAPP, 0.15–1.5% (*v/v*) glycerol, 5% (*v/v*) DMSO and 20 µg of denatured FgaPT2 in 50 mM Tris-HCl (pH 7.5) at 37°C for 0, 0.5, 4 and 24h. pH dependence assays were carried out by incubation in phosphate buffer at pH 2.5, 6.0, 7.5, 8.5 and 10 for 1 h. The products were monitored on LC-HRMS.

5. Enzyme assays under $^{18}O_2$-enriched atmosphere and in buffer with ^{18}O-enriched water

For incubation with FgaPT2 and 1,3-dihydroxynaphthalene (**1**) under $^{18}O_2$-enriched atmosphere, a 500 µL assay contained the same components as in the standard reaction mixture. $^{16}O_2$ in the reaction mixture was removed by application of vacuum followed by flushing with argon for three times. Argon was then removed by vacuum and finally $^{18}O_2$ was allowed to enter the reaction mixture, as reported previously.[6,7] After incubation at 37 °C for 3 h, the reaction was terminated by addition of 500 µL CH_3CN, and subjected to LC-HRMS analysis as described below. One assay was carried out under normal condition as a control. For incubation with FgaPT2 and 1,3-dihydroxynaphthalene (**1**) in buffer with ^{18}O-enriched

water, a 50 µL reaction mixture contained the same components as in the standard assay in a mixture of $H_2{}^{18}O$ and $H_2{}^{16}O$ with a ratio of 4:1.

6. HPLC and LC-HRMS conditions for analysis and isolation of products

Separation was performed on an Agilent series 1200 HPLC (Agilent Technologies, Böblingen, Germany) with an Agilent Eclipse XDB-C18 column (150 × 4.6 mm, 5 µm). H_2O (A) and CH_3CN (B), both with 0.1 % (*v/v*) trifluoroacetic acid, were used as solvents at a flow rate of 0.5 mL/min. The substances were eluted with a linear gradient from 15–80 % B in 50 min. The column was then washed with 100 % (*v/v*) solvent B for 10 min and equilibrated with 5 % (*v/v*) solvent B for 10 min. Product isolation was performed on the same equipment with an Agilent Eclipse XDB-C18 column (9.4 × 250 mm, 5 µm) column, and a linear gradient from 35–80 % B in 20 min at a flow rate of 2.5 ml/min.

LC-HRMS analysis was performed on an Agilent 1260 HPLC system equipped with a microTOF-Q III spectrometer (Bruker, Bremen, Germany) by using a Multospher 120 RP18-5µ column (250 × 2 mm, 5 µm) (CS-Chromatographie Service GmbH, Langerwehe, Germany). H_2O (A) and CH_3CN (B), both with 0.1% (*v/v*) formic acid, were used as solvents at a flow rate of 0.25 mL/min and the same gradient for separation. Electrospray positive or negative ionization mode was selected for determination of the exact masses. The capillary voltage was set to 4.5 kV and a collision energy of 8.0 eV. Sodium formate was used in each run for mass calibration. The masses were scanned in the range of *m/z* 100–1500. Data were evaluated with the Compass DataAnalysis 4.2 software (Bruker Daltonik, Bremen, Germany).

7. NMR analysis

For structural elucidation, the isolated products were dissolved in DMSO-*d6* or CD_3CN and subjected to NMR analysis. The spectra were recorded at room temperature on a Bruker Avance III 500 MHz (^1H) or 125 MHz (^{13}C) spectrometer installed with a cryo probe 5 mm Prodigy for Broad Band Observation. All spectra were processed with MestReNova 6.0.2 (Metrelab Research) and the chemical shifts were referenced to those of the solvents. The NMR data are given in Tables S2–S5 and spectra as Figures S1–S23.

8. Structure elucidation

Compound **2** was obtained as beige amorphous solid. The ^1H and ^{13}C NMR of **2** showed signals of one methylene, one olefin and two tertiary methyl units. In addition, the HMBC correlations of H-1´/C-3, C-4 and C-10 suggested that a dimethylallyl residue was attached to position C4.

Compound **3** was isolated as creamy white solid. The HMBC correlations of H-5/C-4, H-8/C-1, H-2/C-4, H-2/C-3´, H-2/C-2´, H-1´/C-3, H-1´/C-3´ as well as ^1H-^1H COSY correlations of H-1´/H-2´/2´-OH indicated that a bicyclo[3.3.1]nonane system was fused with an aromatic ring through C-9 and C-10. Two additional hydroxyl groups were confirmed to be at C-4 and C-2´

by the HMBC correlation of 4-OH/C-4, C-1´ and C-10 as well as of 2´-OH/C-1´, C-2´ and C-3´. The relative configuration of **3** was determined by NOESY analysis. Strong correlations of ´H-2´/H-4´ with H-1´/H-5´ as well as weak cross peak between 4-OH and H-2´ suggest that 4-OH and 3´-OH are located with opposite orientations.

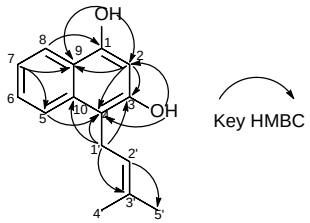
Compound **4** and **5** was obtained as creamy white solids. **4** and **5** are two isomers with the same molecular formula, $C_{15}H_{16}O_4$, deduced from HR-ESI-MS data. The ^{1}H, ^{13}C, and HMBC (Tables S4 and S5) showed the same planar structures for **4** and **5**, namely 4,3´-dihydroxyl tetrahydrofuran derivatives with two chiral centers. The relative configuration of **4** as shown in Figure S18 was confirmed by the NOESY correlations of 4-OH to H-2´. In comparison, the NOESY spectrum of **5** suggested an α-orientated 4-OH and β-orientated 2´-H as shown in Figure S23.

Tables

Table S1 HR-ESI-MS data of the reported compounds

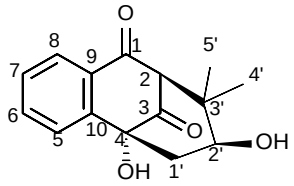
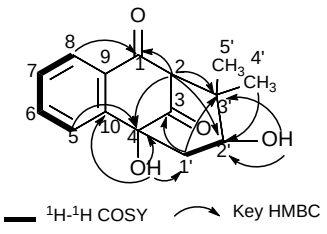
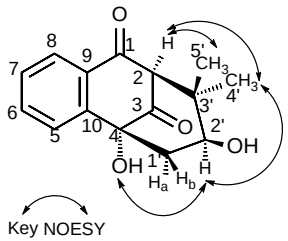
Compound	Formula	$[M + H]^+$		Deviation [ppm]	$[M - H]^-$		Deviation [ppm]
		Calculated	Measured		Calculated	Measured	
2	$C_{15}H_{16}O_2$	229.1223	229.1224	-0.4	227.1078	227.1088	-4.4
3	$C_{15}H_{16}O_4$	261.1121	261.1126	-1.9	259.0976	259.0974	0.8
4	$C_{15}H_{16}O_4$	261.1121	261.1129	-3.1	259.0976	259.0972	1.5
5	$C_{15}H_{16}O_4$	261.1121	261.1127	-2.3	259.0976	259.0983	-2.7

Table S2 NMR data of compound **2** (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR)



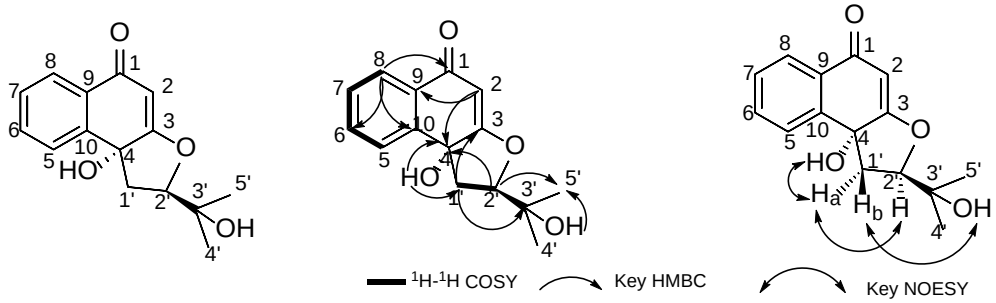
Position	δ_H, multi., *J* in Hz	δ_C, type	HMBC correlations
1	-	152.2, C	-
2	6.62, s	100.6, CH	C-1, 3, 4, 9
3	-	152.2, C	-
4	-	109.5, C	-
5	7.67, dd, 8.5, 1.0	122.6, CH	C-1, 4, 7, 9, 10
6	7.37, ddd, 8.5, 6.7, 1.6	126.3, CH	C-8, 10
7	7.16, ddd, 8.3 6.7, 1.0	120.7, CH	C-5, 6, 9
8	7.98, dd, 8.3, 1.6	122.4, CH	C-1, 6, 10
9	-	120.1, C	-
10	-	133.8, C	-
1´	3.52, d, 6.7	23.1, CH_2	C-2´, 3´, 3, 4, 10
2´	5.09, m	124.5, CH	C-4´, 5´
3´	-	129.7, C	-
4´	1.80, s	17.9, CH_3	C-2´, 3´, 5´
5´	1.61, s	25.5, CH_3	C-2´, 3´, 4´
1-OH	9.88, s	-	C-1, 2, 9
3-OH	9.30, s	-	C-2, 3, 4

Table S3 NMR data of compound **3** (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR)

								
Position	**δ_H, multi., *J* in Hz**		**δ_C, type**		**HMBC correlations**		**COSY correlations**	**NOESY correlations (s:strong, w:weak)**
solvent	**DMSO-*d6***	**CD_3CN**	**DMSO-*d6***	**CD_3CN**	**DMSO-*d6***	**CD_3CN**	**DMSO-*d6***	**CD_3CN**
1	-	-	194.2, C	193.7, C	-	-	-	-
2	3.25, s	3.30, s	73.6, CH	73.1, CH	C-1, 3, 4, 9, 2′, 3′,4′, 5′	C-1, 3, 4, 9, 2′, 3′,4′, 5′	-	H-4′, 5′
3	-	-	205.2, C	204.7, C	-	-	-	-
4	-	-	77.6, C	77.6, C	-	-	-	-
5	7.83, m[a]	7.83, ddd, 7.9, 1.3, 0.5	125.5, CH	125.1, CH	C-4, 7, 9	C-4, 7, 9	H-6	-
6	7.82, m[a]	7.78, ddd, 7.9, 7.2, 1.4	135.9, CH	135.5, CH	C-10	C-8, 10	H-5, 7	H-7
7	7.55, m	7.51, ddd, 7.9, 7.2, 1.3	128.4, CH	128.1, CH	C-5, 9	C-5, 9	H-6, 8	H-6, 8
8	7.90, d, 7.9	7.94, ddd, 7.9, 1.4, 0.5	125.2, CH	124.8, CH	C-1, 6, 10	C-1, 6, 10	H-7	H-7
9	-	-	131.2, C	129.8, C	-	-	-	-
10	-	-	146.8, C	146.1, C	-	-	-	-
1′a	1.98, dd, 12.8, 5.2	2.17, dd, 12.7, 5.4	46.8, CH_2	46.5, CH_2	C-3, 4, 10, 2′, 3′	C-3, 4, 10, 2′, 3′	H-2′	H-2′, 2′-OH, H-5′(s), H-4′(w)
1′b	2.11, dd, 12.8, 11.3	2.21, dd, 12.7, 11.3			C-3, 4, 10, 2′, 3′	C-3, 4, 10, 2′, 3′	H-2′	H-2′, 2′-OH
2′	3.09, ddd, 11.3, 5.5, 5.2	3.25, ddd, 11.3, 5.6, 5.4	69.6, CH	69.5, CH	C-3′, 4′, 5′	C-1′, 4′, 5′	H-1′a, 1′b, 2′-OH	H-4′, 1′a, 1′b
3′	-	-	44.8, C	44.7, C	-	-	-	-
4′	1.01, s	1.08, s	18.8, CH_3	18.5, CH_3	C-2, 2′, 3′, 5′	C-2, 2′, 3′, 5′	-	H-2′, 1′a(w)
5′	0.91, s	0.91, s	24.8, CH_3	24.5, CH_3	C-2, 2′, 3′, 4′	C-2, 2′, 3′, 4′	-	H-2, 1′a(s)
4-OH	6.37, s	4.37, s	-	-	C-4, 1′, 10	C-4, 1′, 10	-	H-2′
2′-OH	4.94, d, 5.5	2.98, d, 5.6	-	-	C-2′, 3′	C-1′, 2′, 3′	H-2′	H-1′a, 1′b

[a] Signals are overlapping with each other.

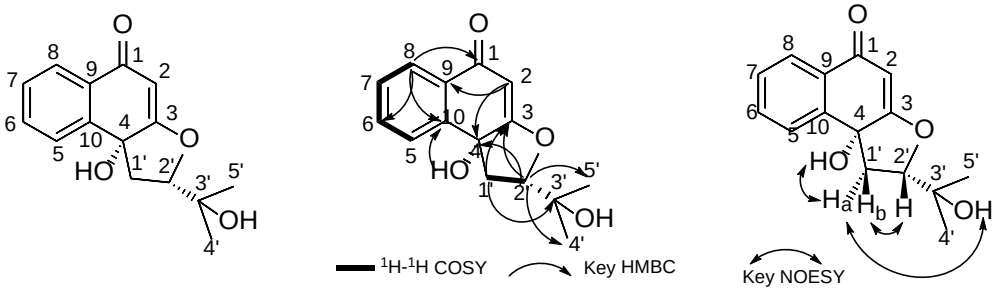
Table S4 NMR data of compound **4** (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR)



Position	δ_H, multi., *J* in Hz	δ_C, type	HMBC	COSY correlations	NOESY correlations
1	-	185.1, C	-	-	-
2	5.55, s	98.5, CH	C-1, 3, 4, 9	-	H-2´, 4-OH
3	-	179.6, C	-	-	-
4	-	73.2, C	-	-	-
5	7.62, m[a]	126.6, CH	C-1, 4, 7, 9	H-6	H-1´a, 1´b, 4-OH, 7
6	7.61, m[a]	132.1, CH	C-8, 10	H-5, 7	H-7
7	7.49, ddd, 7.7, 6.2, 2.4	128.3, CH	C-5, 9, 10	H-6, 8	H-5, 6, 8
8	7.89, d, 7.7	125.4, CH	C-1, 6, 10	H-7	H-7
9	-	130.6, C	-	-	-
10	-	141.2, C	-	-	-
1´a	2.70, dd, 12.6, 4.6		C-3, 4, 2´	H-1´a, 2´	H-5, 2´, 4-OH, 1´b, 5´
1´b	2.05, ddd, 12.6, 10.1, 1.1	35.9, CH_2	C-10, 2´, 3´	H-1´b, 2´	H-5, 3´-OH, 1´a, 4´, 5´
2´	4.77, dd, 10.1, 4.6	91.5, CH	C-1´, 4´, 5´	H-1´a, 1´b	H-1´a, 4´, 5´, 4-OH
3´	-	69.2, C	-	-	-
4´	1.22, s	26.1, CH_3	C-2´, 3´, 5´	-	H-5´,1´a, 1´b, 3´-OH, 2´, 4-OH
5´	1.11, s	25.6, CH_3	C-2´, 3´, 4´	-	H-4´, 1´a, 3´-OH, 2´, 4-OH
4-OH	6.27, d, 1.1	-	C-1´, 3, 4	-	H-4´, 5´, 1´a, 2´, 2, 5
3´-OH	4.68, s	-	C-2´, 3´, 4´, 5´	-	H-4´, 5´, 1´b, 5

[a] Signals are overlapping with each other.

Table S5 NMR data of compound **5** (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR)



Position	δ_H, multi., *J* in Hz	δ_C, type	HMBC correlations	NOESY correlations
1	-	185.2, C	-	-
2	5.60, s	98.5, CH	C-3, 4, 9	H-2´
3	-	180.1, C	-	-
4	-	72.1, C	-	-
5	7.65, m[a]	126.8, CH	C-4, 7, 9	H-1´a, 1´b, 6
6	7.64, m[a]	132.1, CH	C-8	H-5, 7
7	7.50, ddd, 7.6, 6.2, 2.5	128.3, CH	C-5, 9	H-6, 8
8	7.90, d, 7.6	125.3, CH	C-1, 6, 10	H-7
9	-	130.6, C	-	-
10	-	141.2, C	-	-
1´a	2.91, dd, 13.9, 1.1	34.7, CH_2	C-3, 4, 3´	H-1´b, 5, 5´, 3´-OH, 4-OH
1´b	2.54, dd, 13.9, 10.0		C-2´, 10, 4, 3´	H-1´a, 5, 2´
2´	4.71, dd, 10.0, 1.1	91.7, CH	C-1´, 4´, 5´, 3, 4	H-1´b, 4´, 5´, 2
3´	-	70.0, C	-	-
4´	1.35, s	26.4, CH_3	C-2´, 3´, 5´	H-2´, 5´, 3´-OH, 4-OH
5´	1.26, s	27.0, CH_3	C-2´, 3´, 4´	H-2´, 4´, 1´a, 3´-OH, 4-OH
4-OH	6.98, br s	-	C-1´, 4, 10	1´a, 4´, 5´
3´-OH	6.11, br s	-	C-2´	1´a, 4´, 5´

[a] Signals are overlapping with each other.

Figures

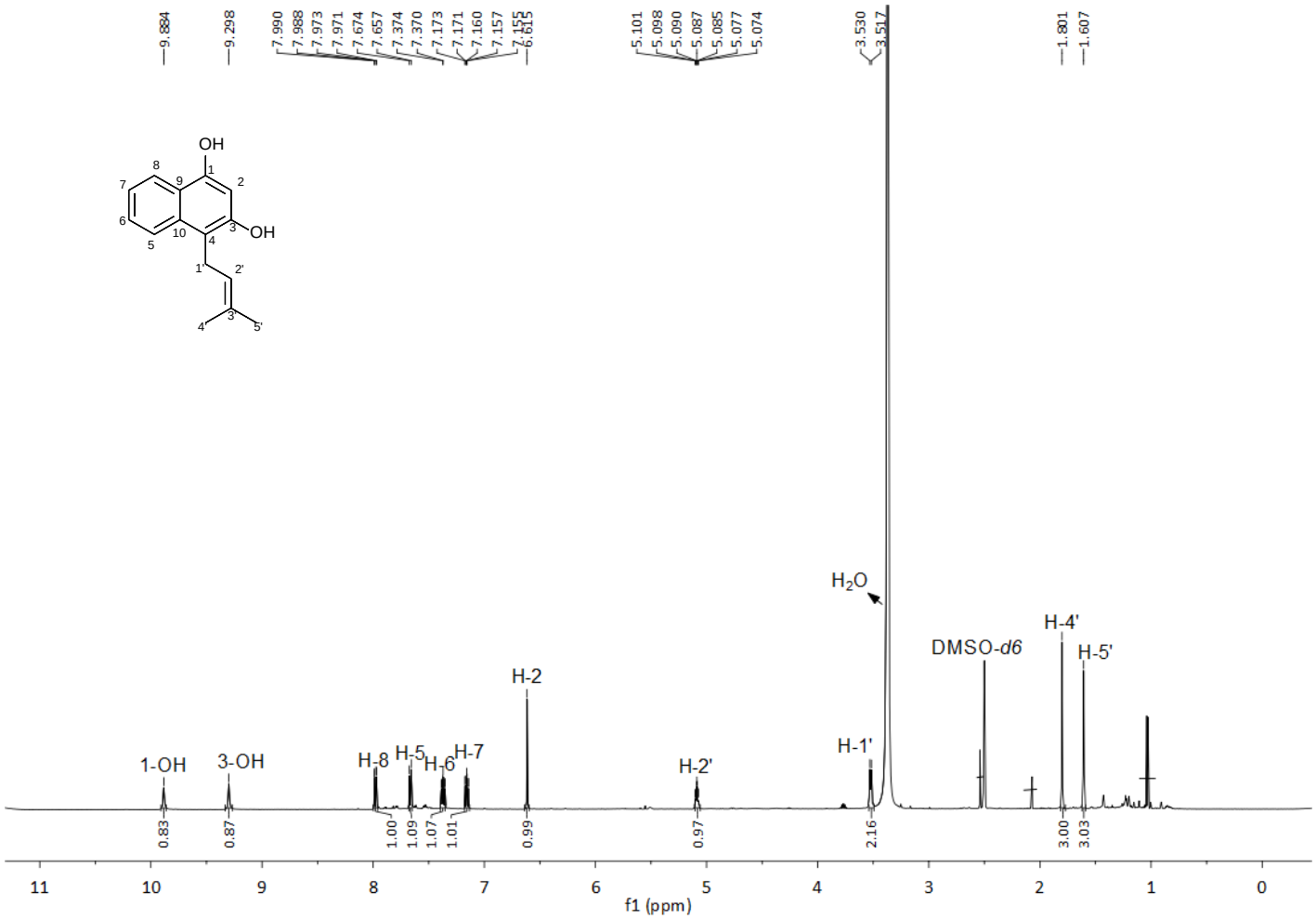


Figure S1. ^{1}H NMR spectrum of compound **2** in DMSO-*d6* (500 MHz)

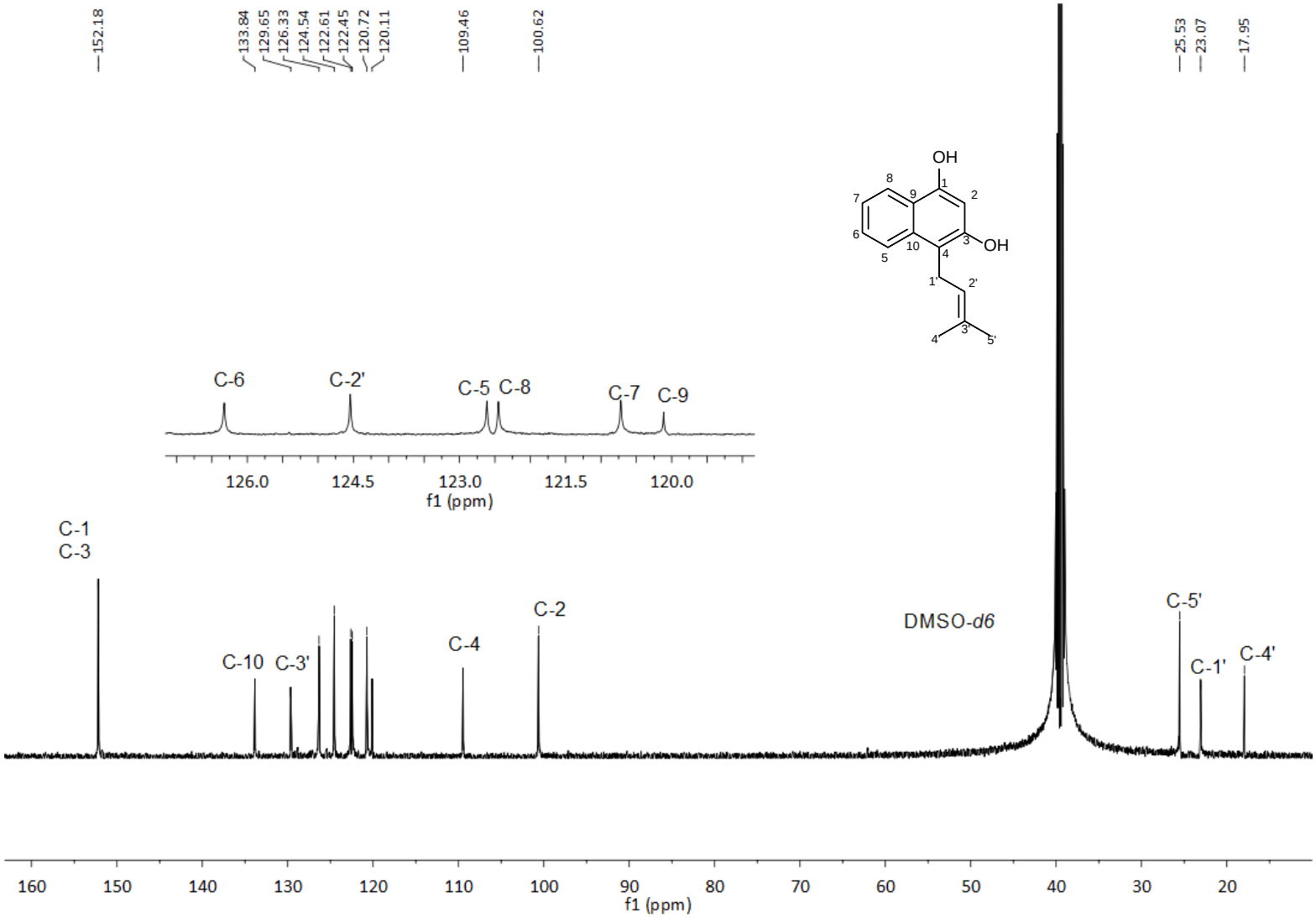


Figure S2. ^{13}C NMR spectrum of compound **2** in DMSO-*d6* (125 MHz)

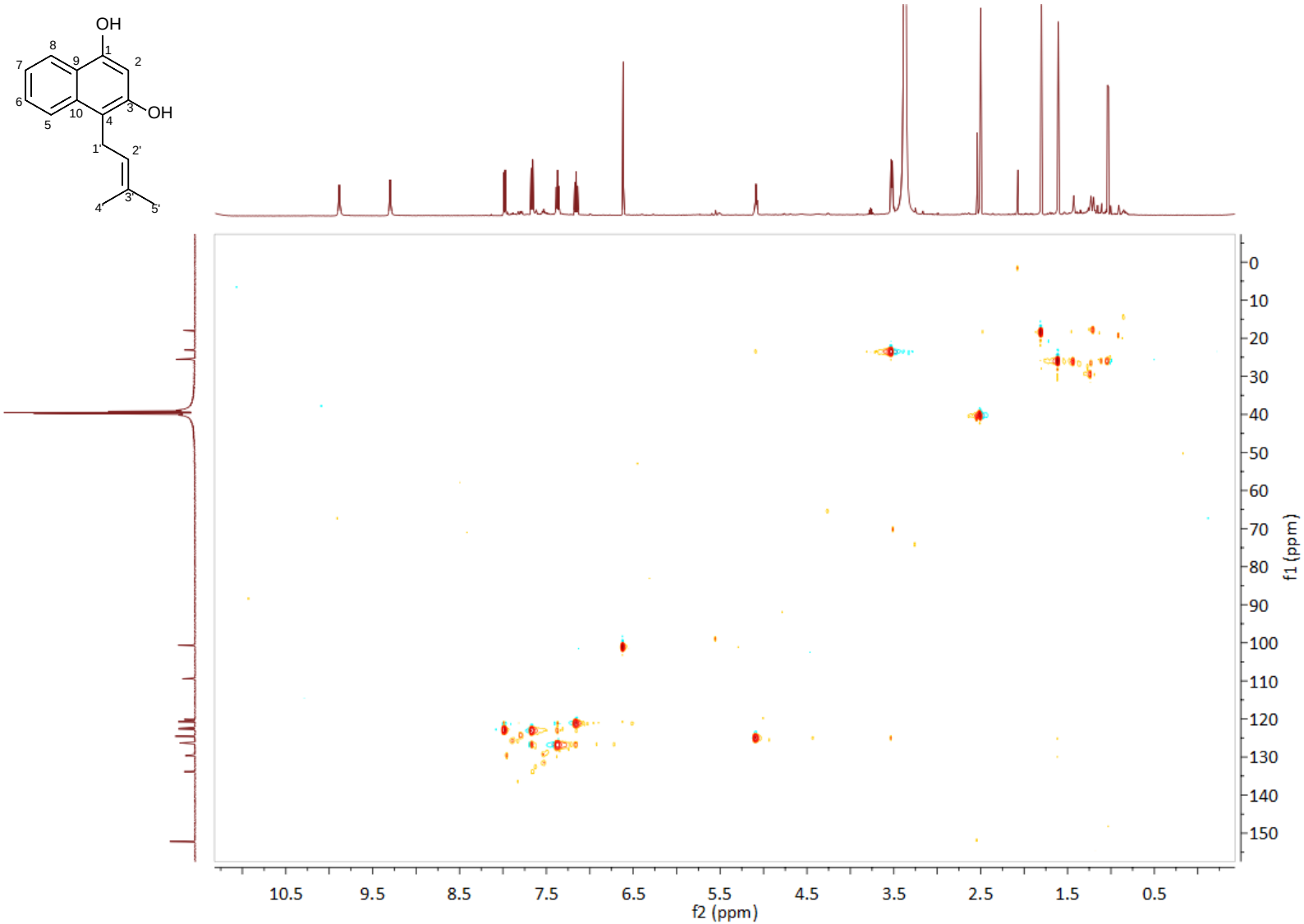


Figure S3. HSQC NMR spectrum of compound **2** in DMSO-*d6*

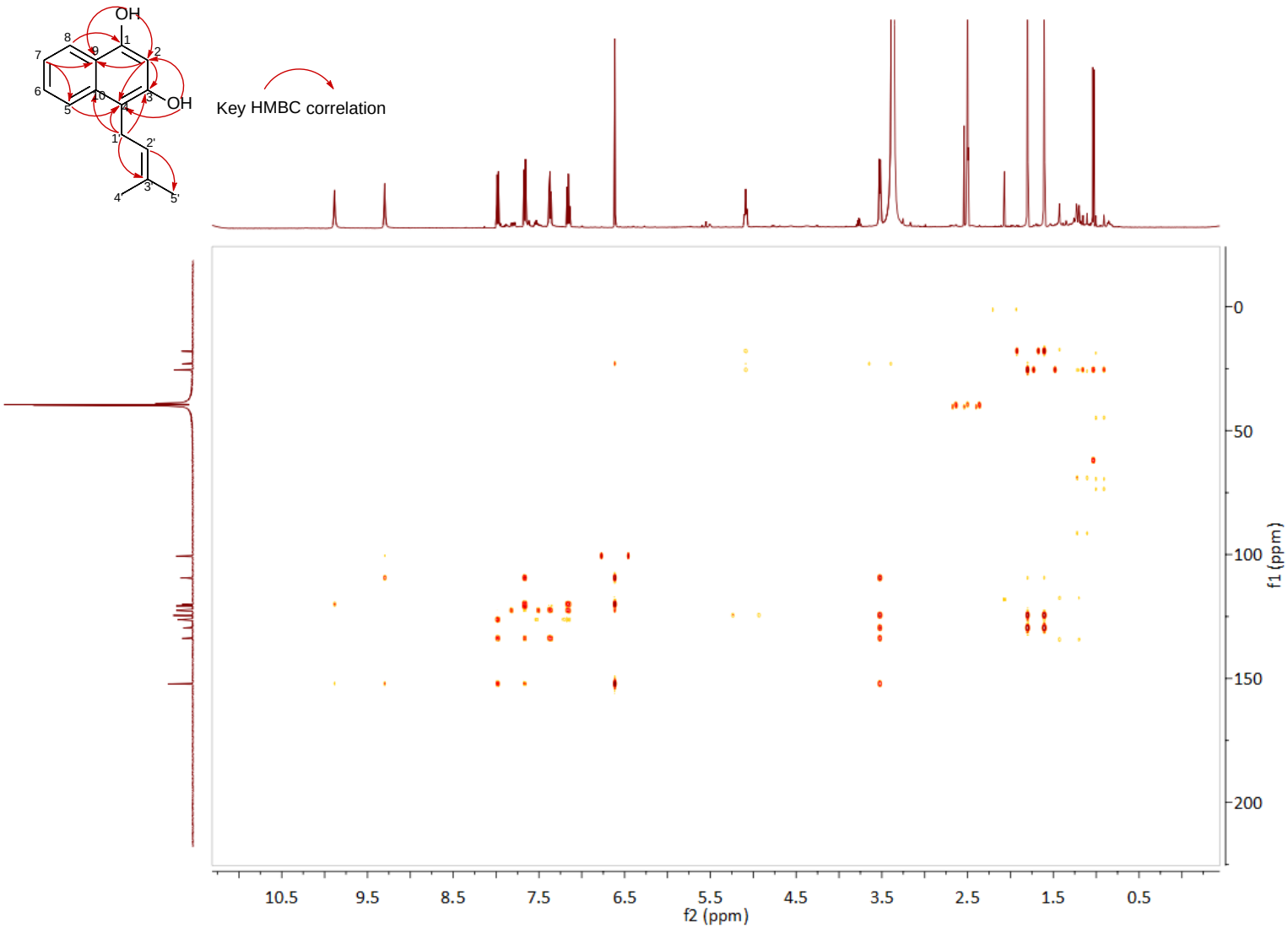


Figure S4. HMBC NMR spectrum of compound **2** in DMSO-*d6*

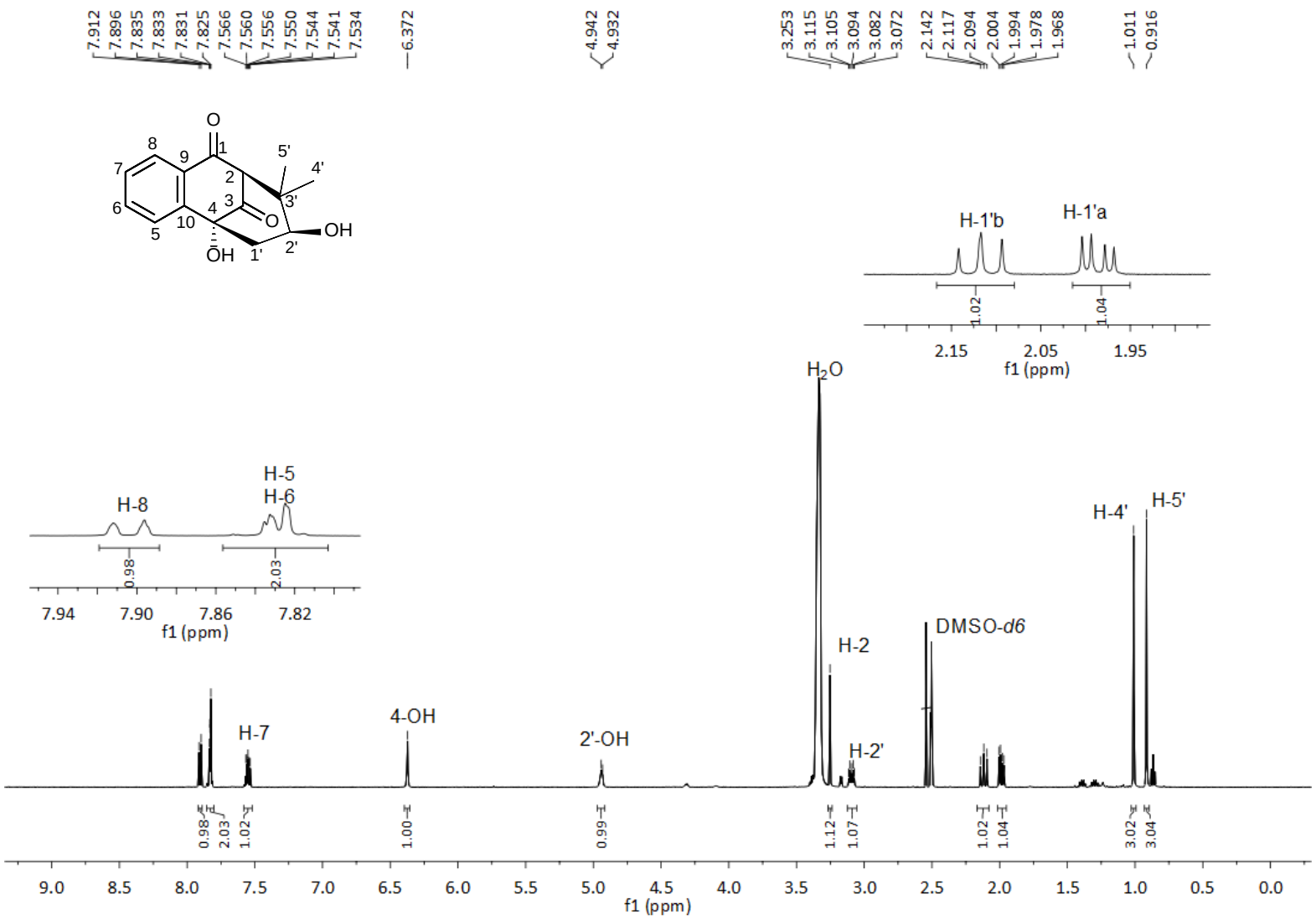


Figure S5. ^{1}H NMR spectrum of compound **3** in DMSO-*d6* (500 MHz)

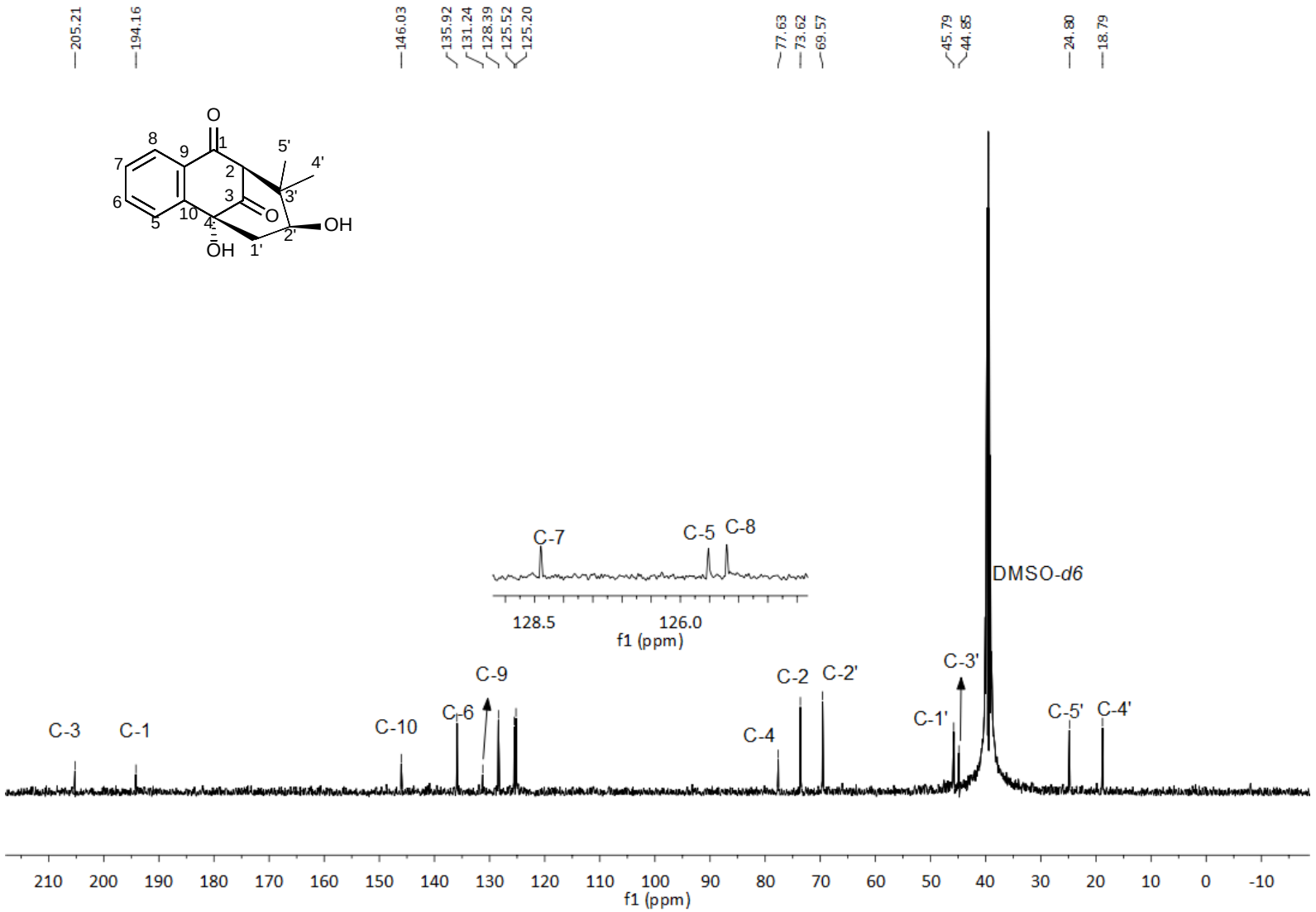


Figure S6. ^{13}C NMR spectrum of compound **3** in DMSO-*d6* (125 MHz)

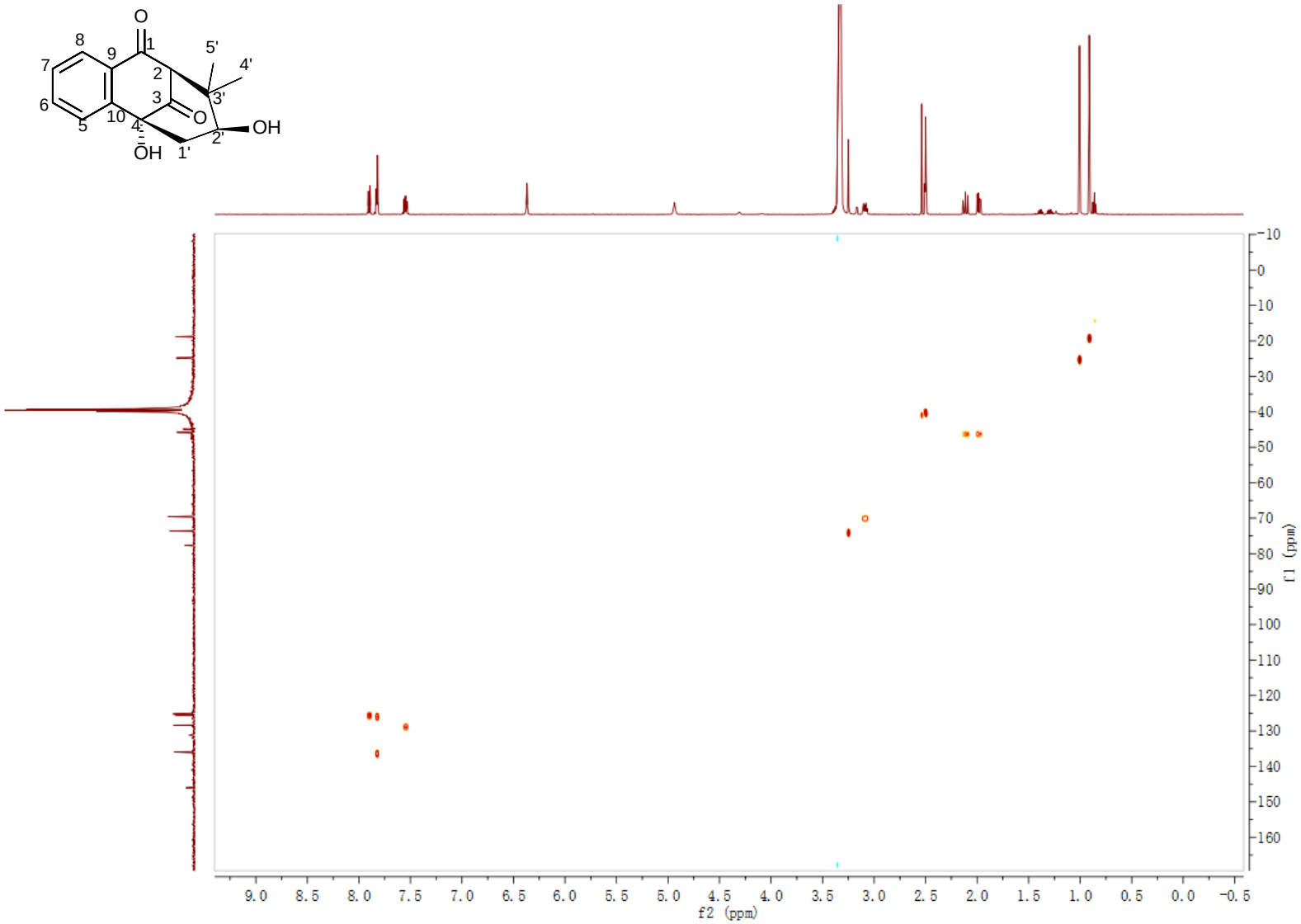


Figure S7. HSQC NMR spectrum of compound **3** in DMSO-*d6*

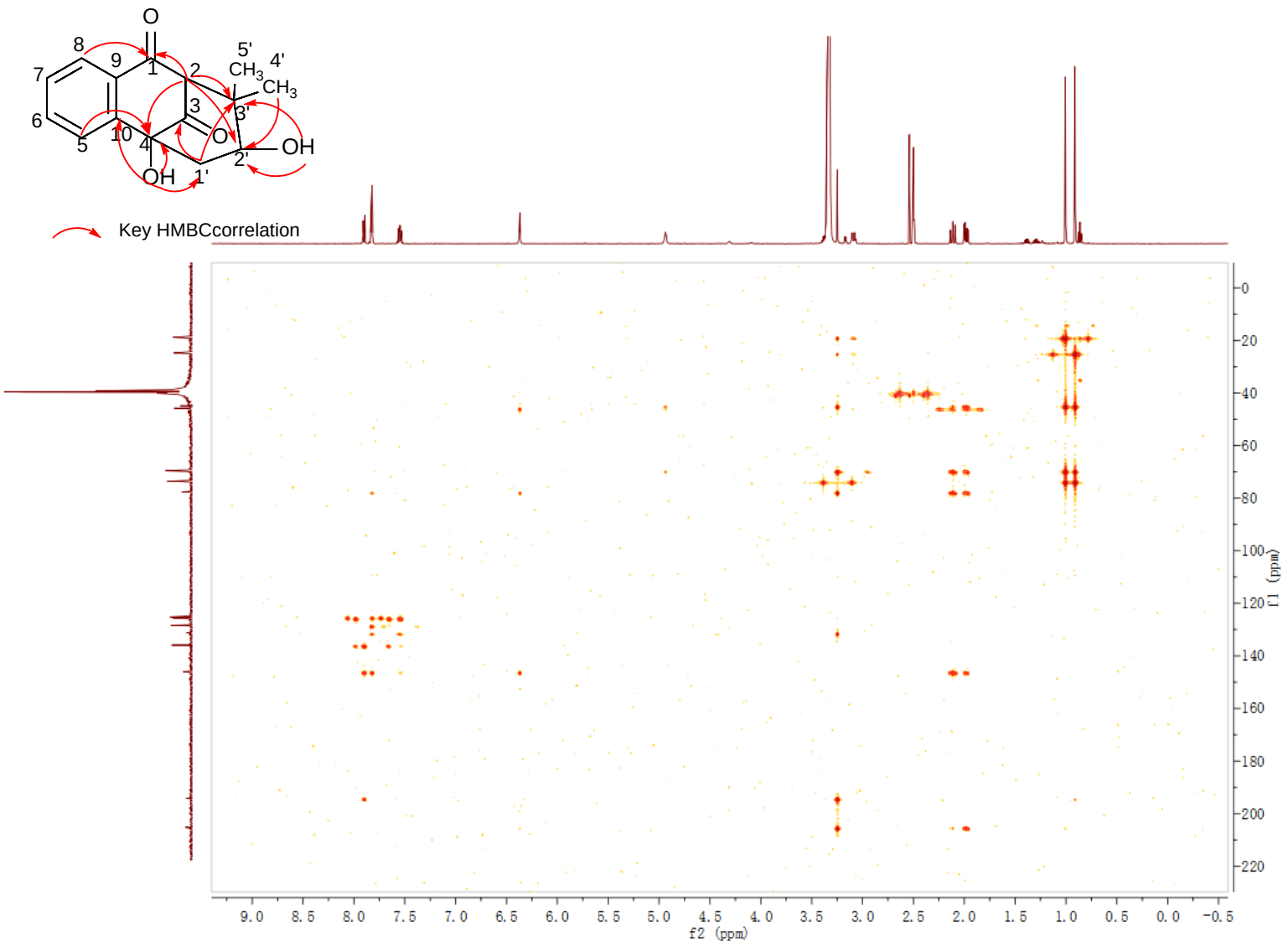


Figure S8. HMBC NMR spectrum of compound **3** in DMSO-*d6*

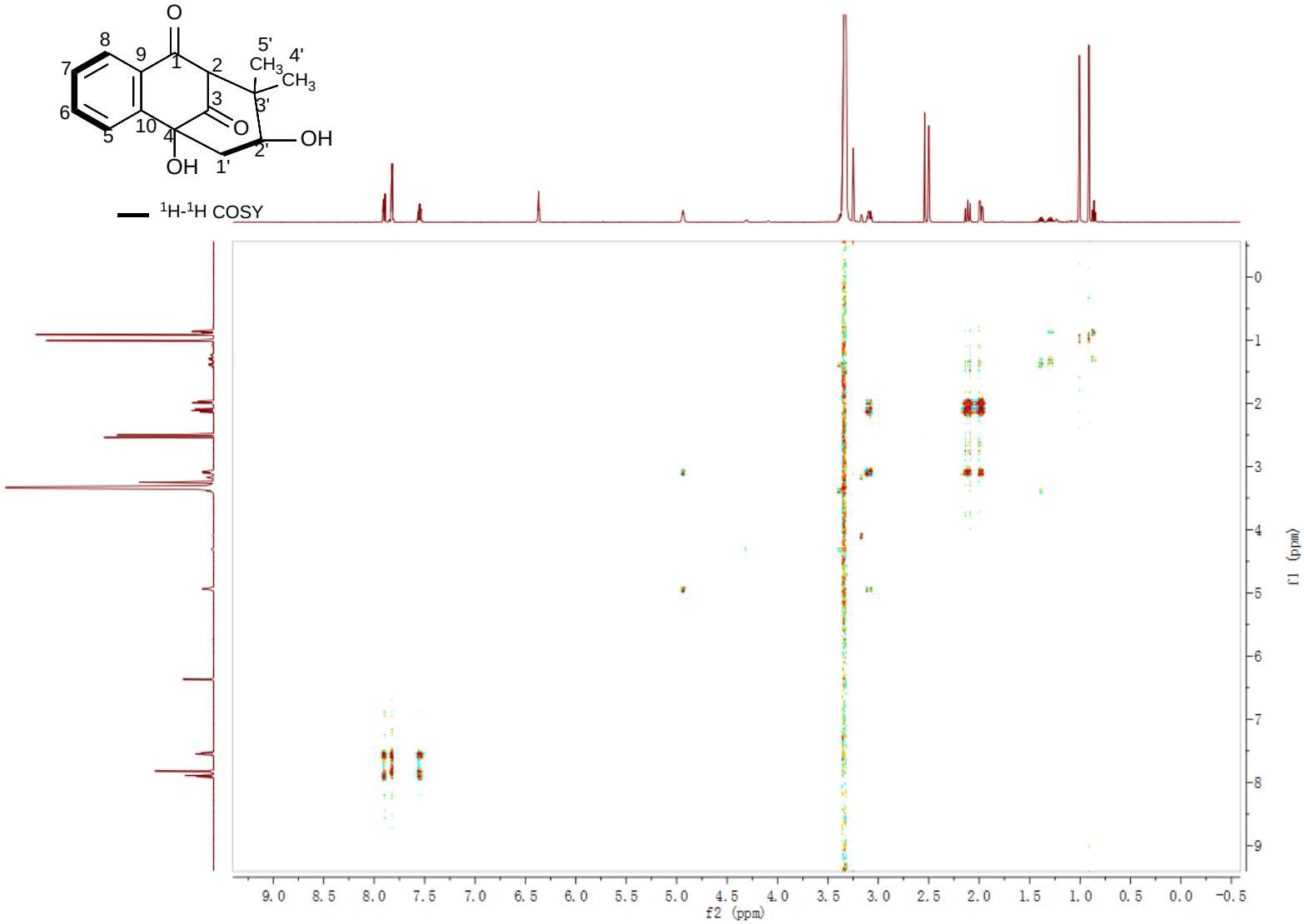


Figure S9. ^{1}H-^{1}H COSY NMR spectrum of compound **3** in DMSO-*d6*

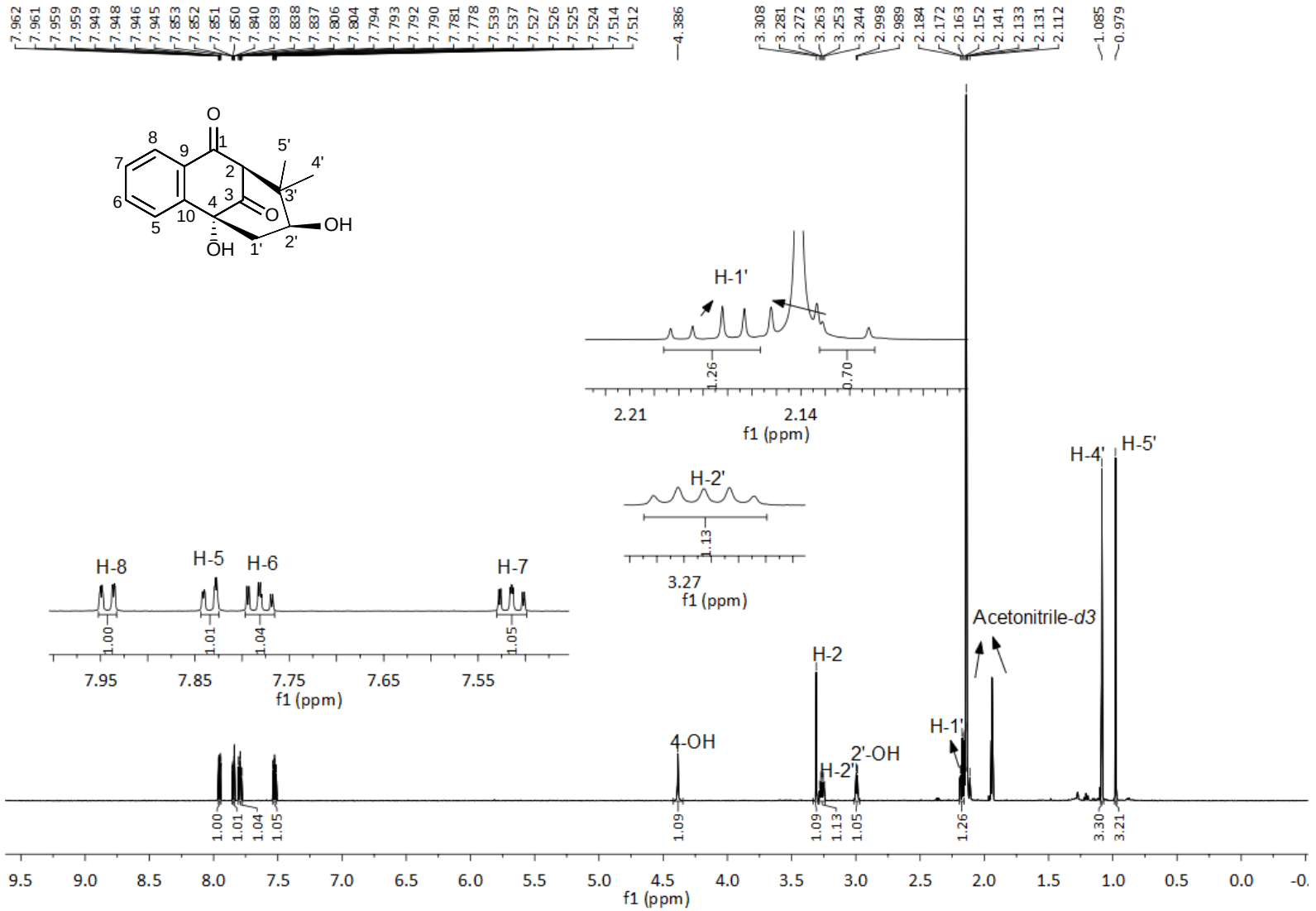


Figure S10. ^{1}H NMR spectrum of compound **3** in CD_3CN (500 MHz)

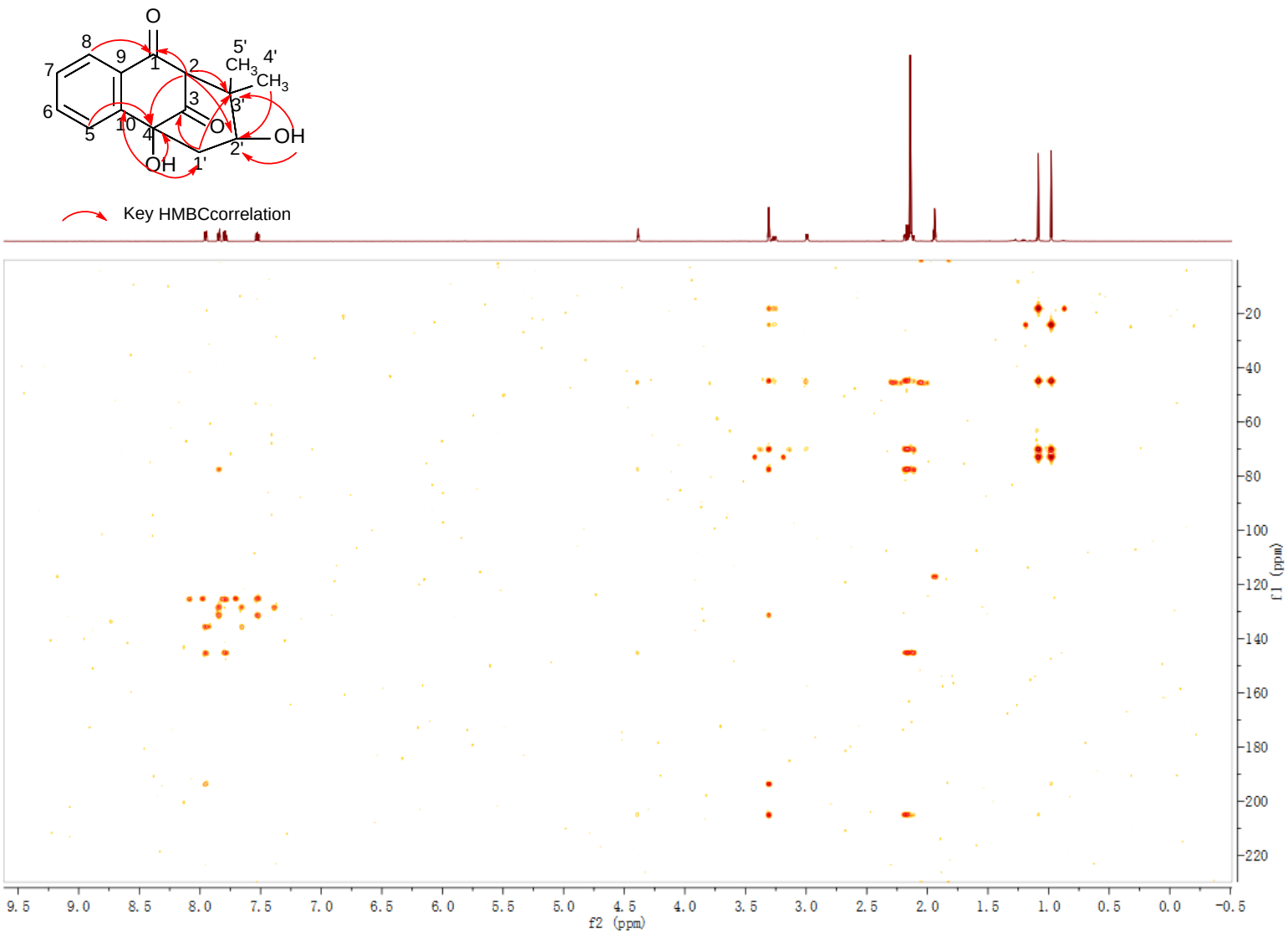


Figure S11. HMBC NMR spectrum of compound **3** in CD_3CN

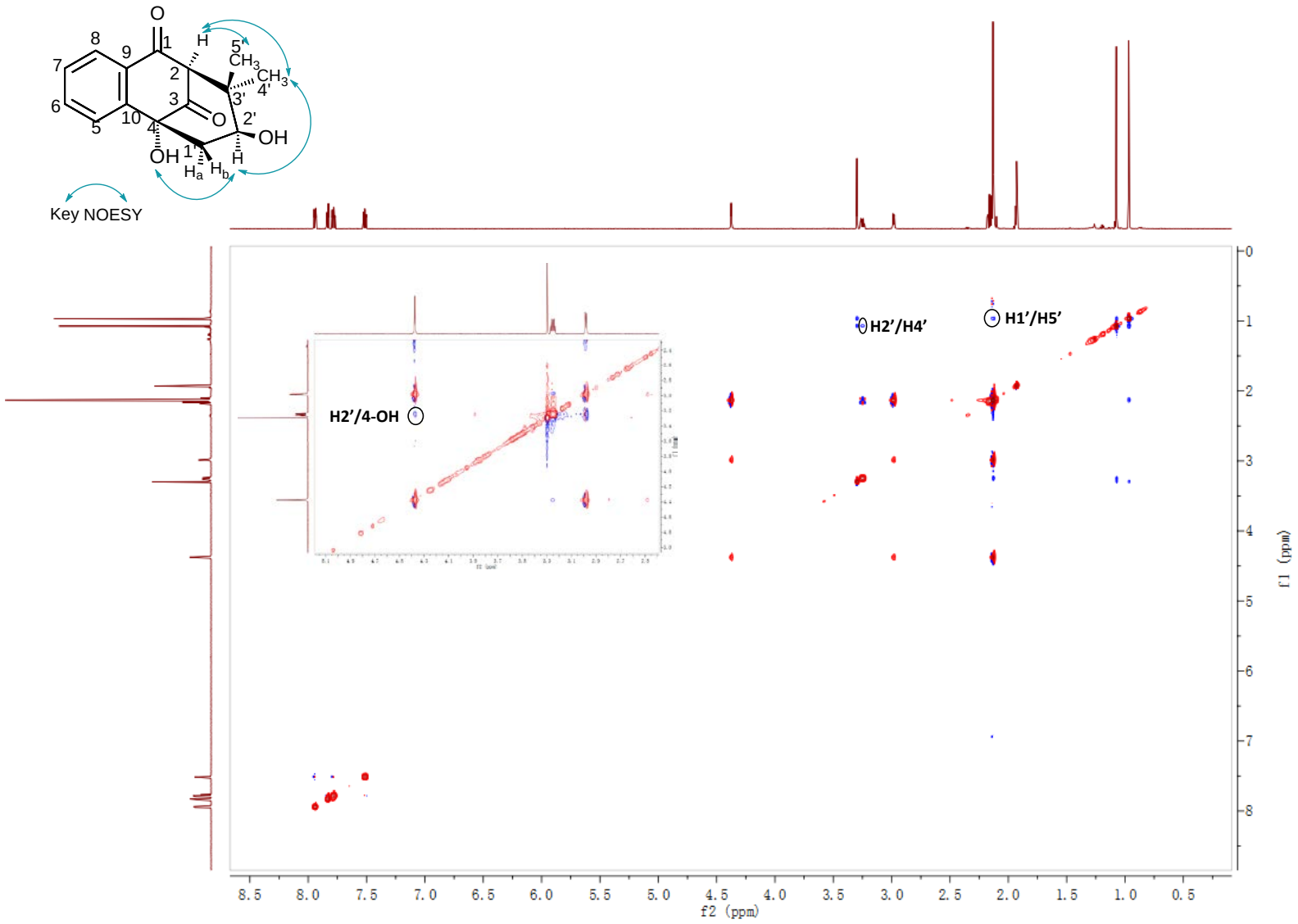


Figure S12. NOESY NMR spectrum of compound **3** in CD_3CN

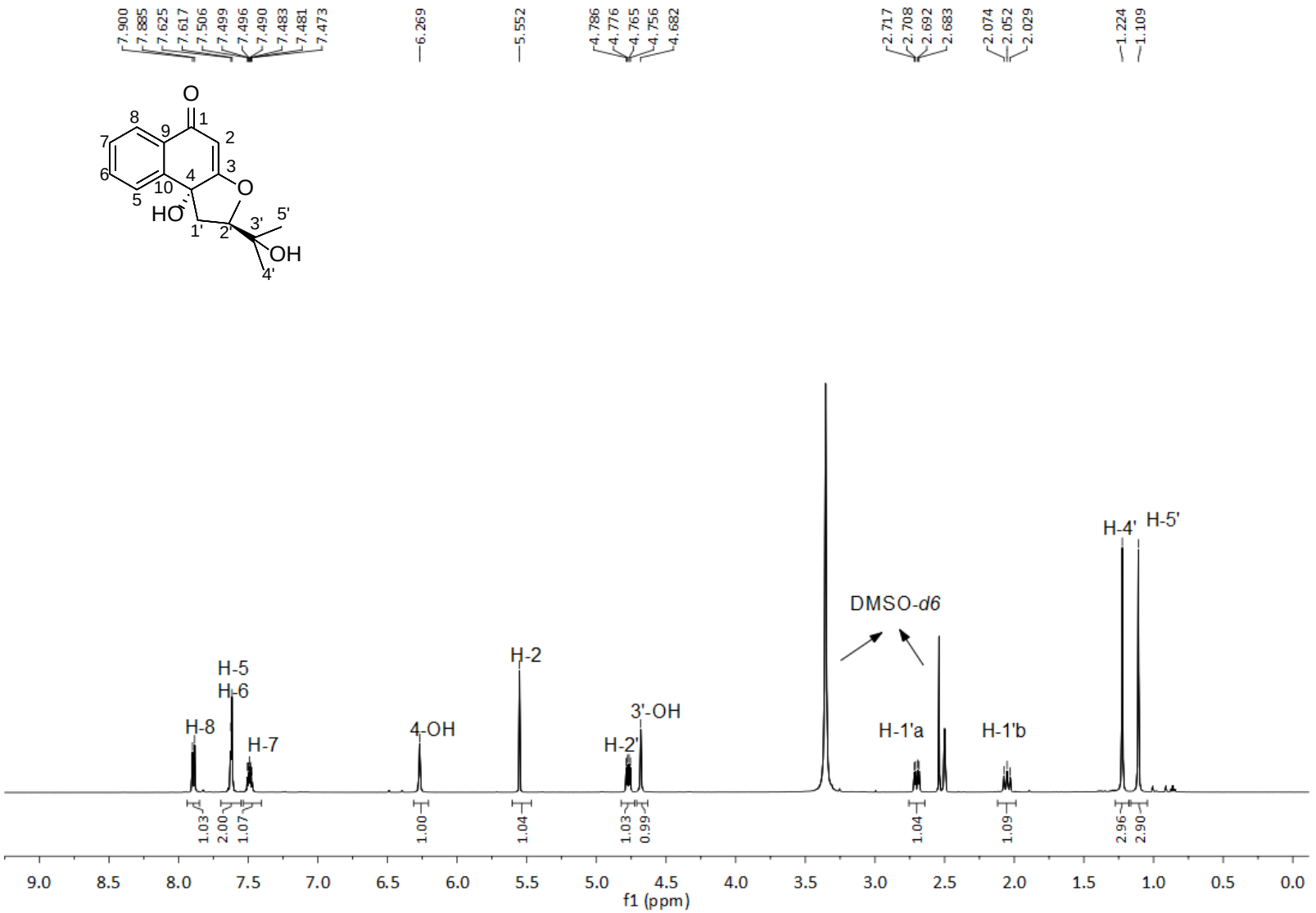


Figure S13. ^{1}H NMR spectrum of compound **4** in DMSO-*d6* (500 MHz)

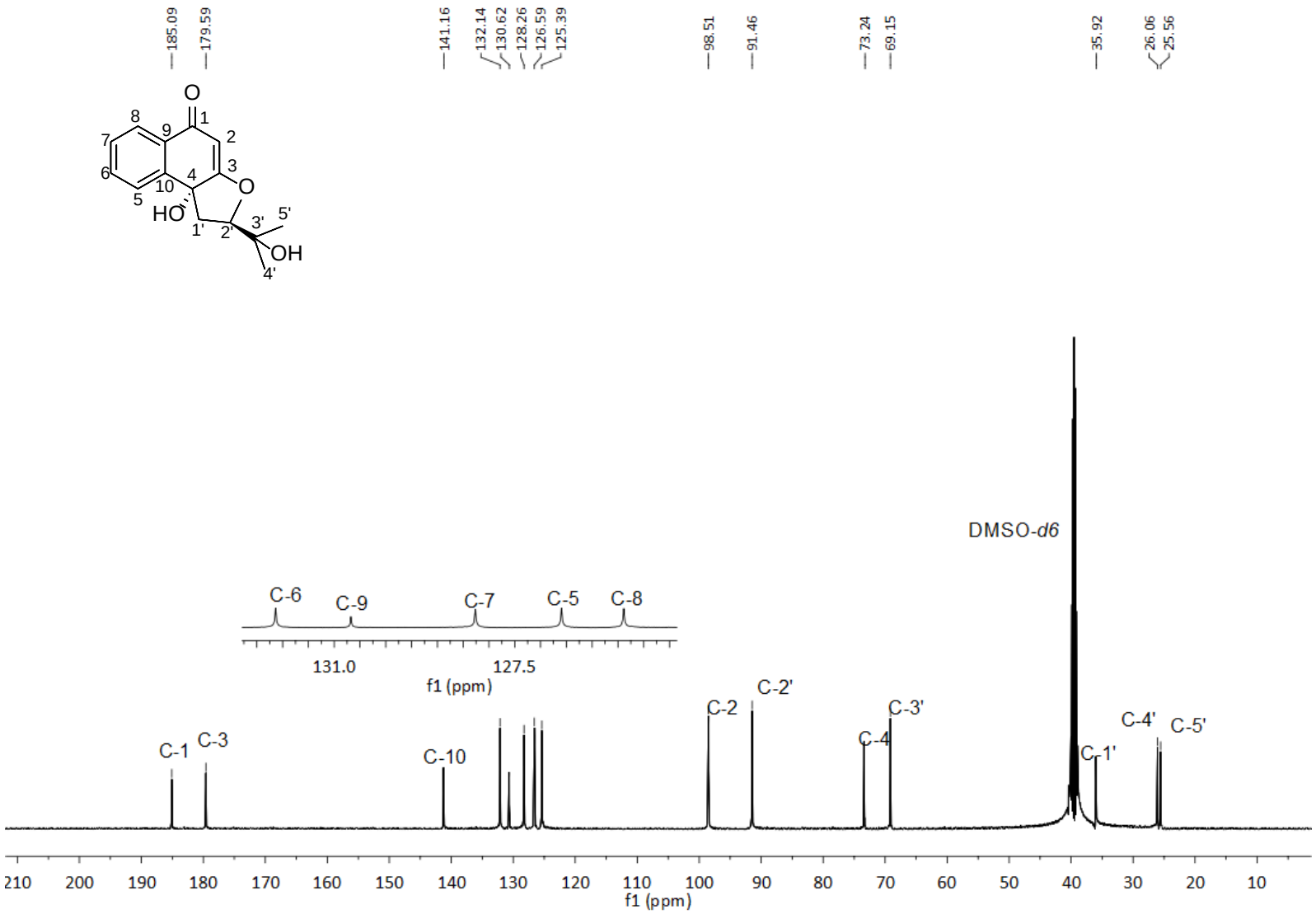


Figure S14. ^{13}C NMR spectrum of compound **4** in DMSO-*d6* (125 MHz)

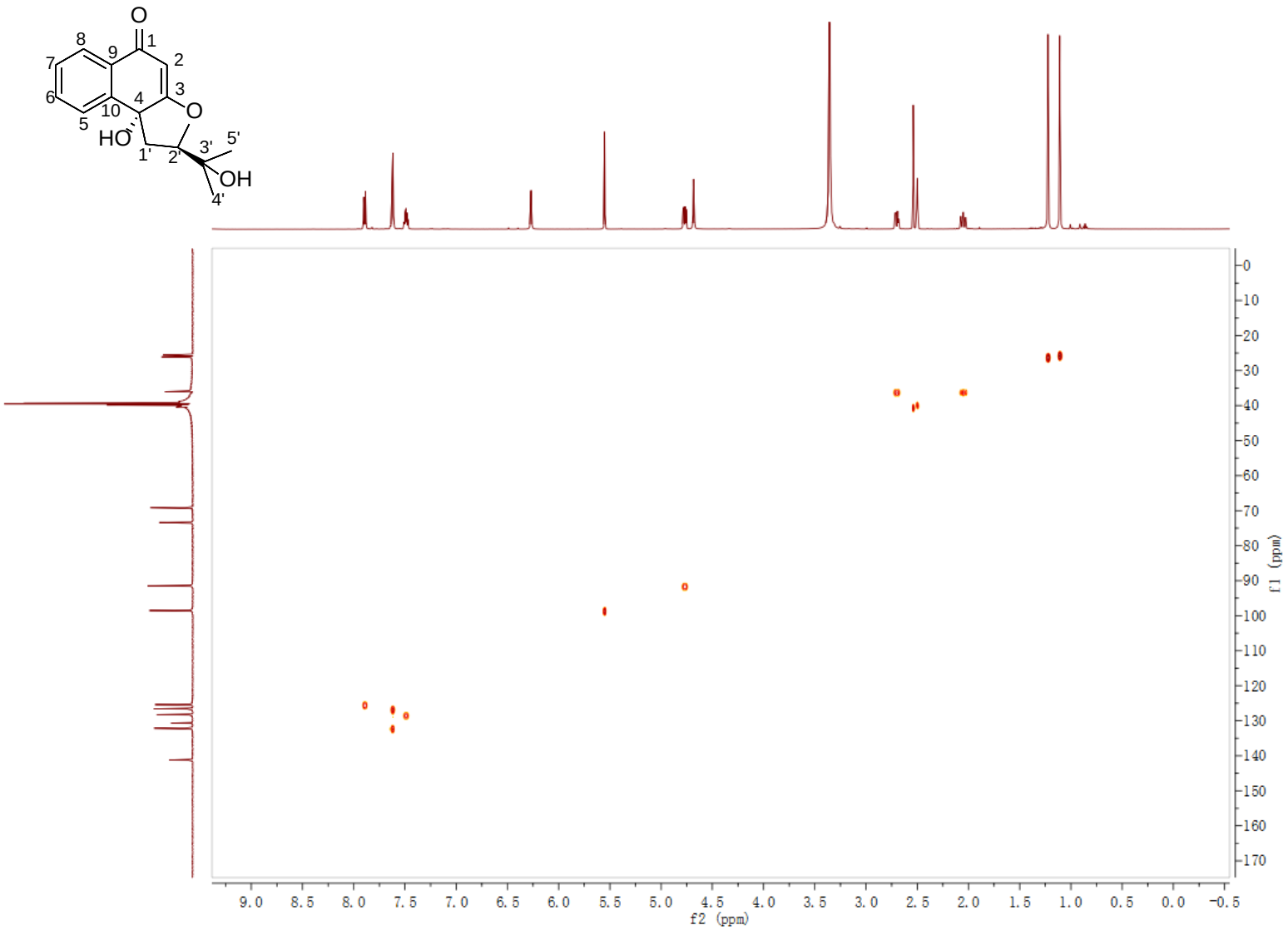


Figure S15. HSQC NMR spectrum of compound **4** in DMSO-*d6*

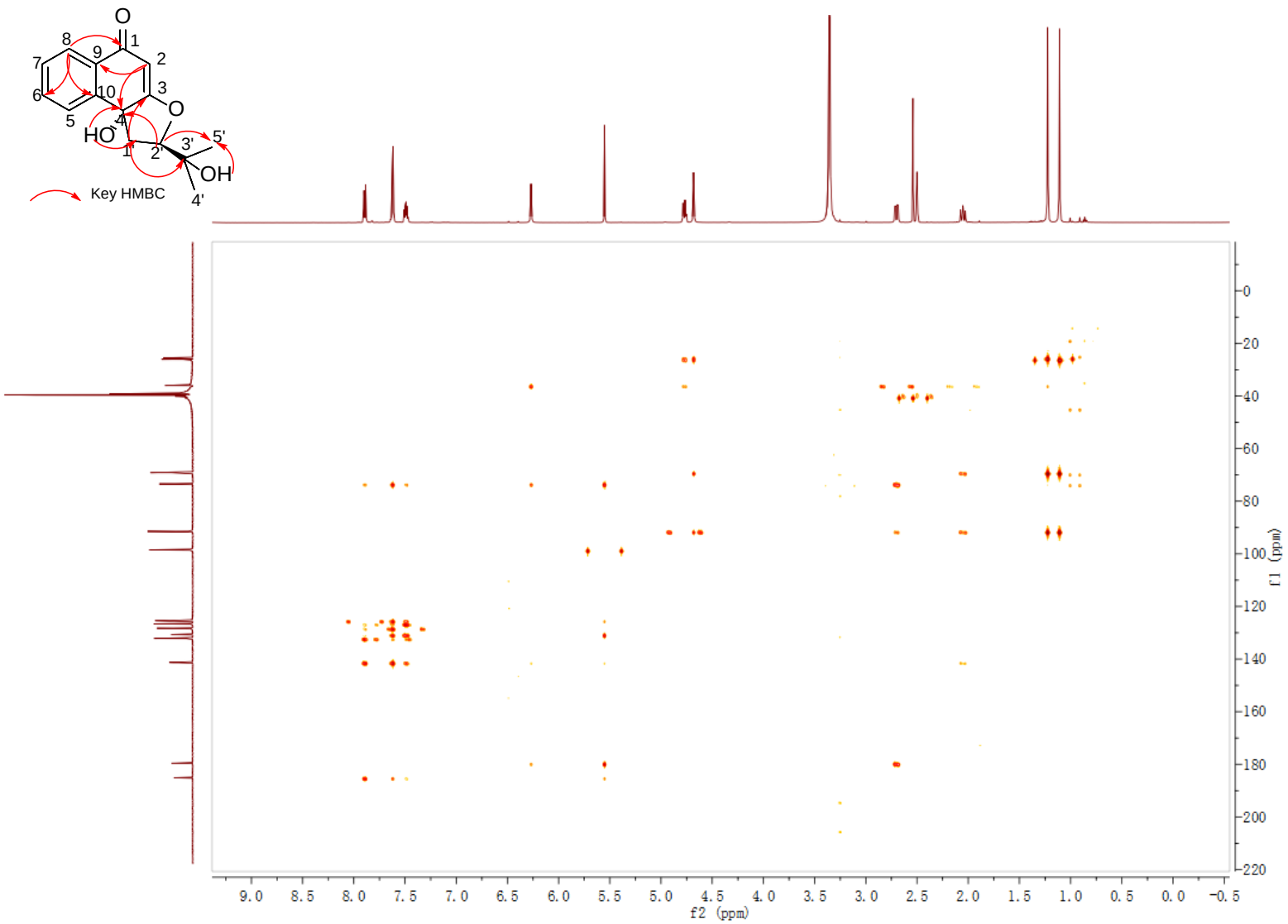


Figure S16. HMBC NMR spectrum of compound **4** in DMSO-*d6*

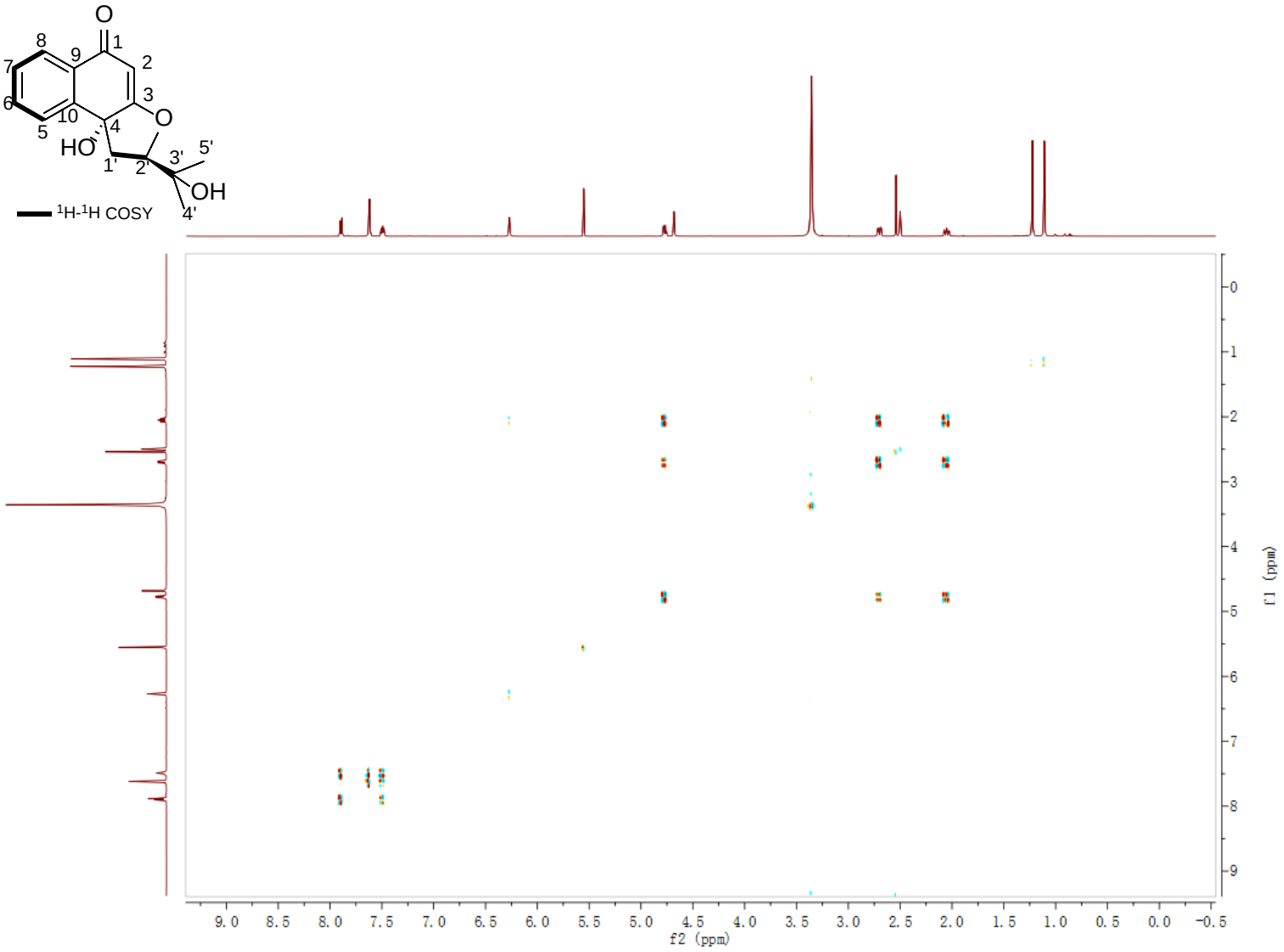


Figure S17. ^{1}H-^{1}H COSY NMR spectrum of compound **4** in DMSO-*d6*

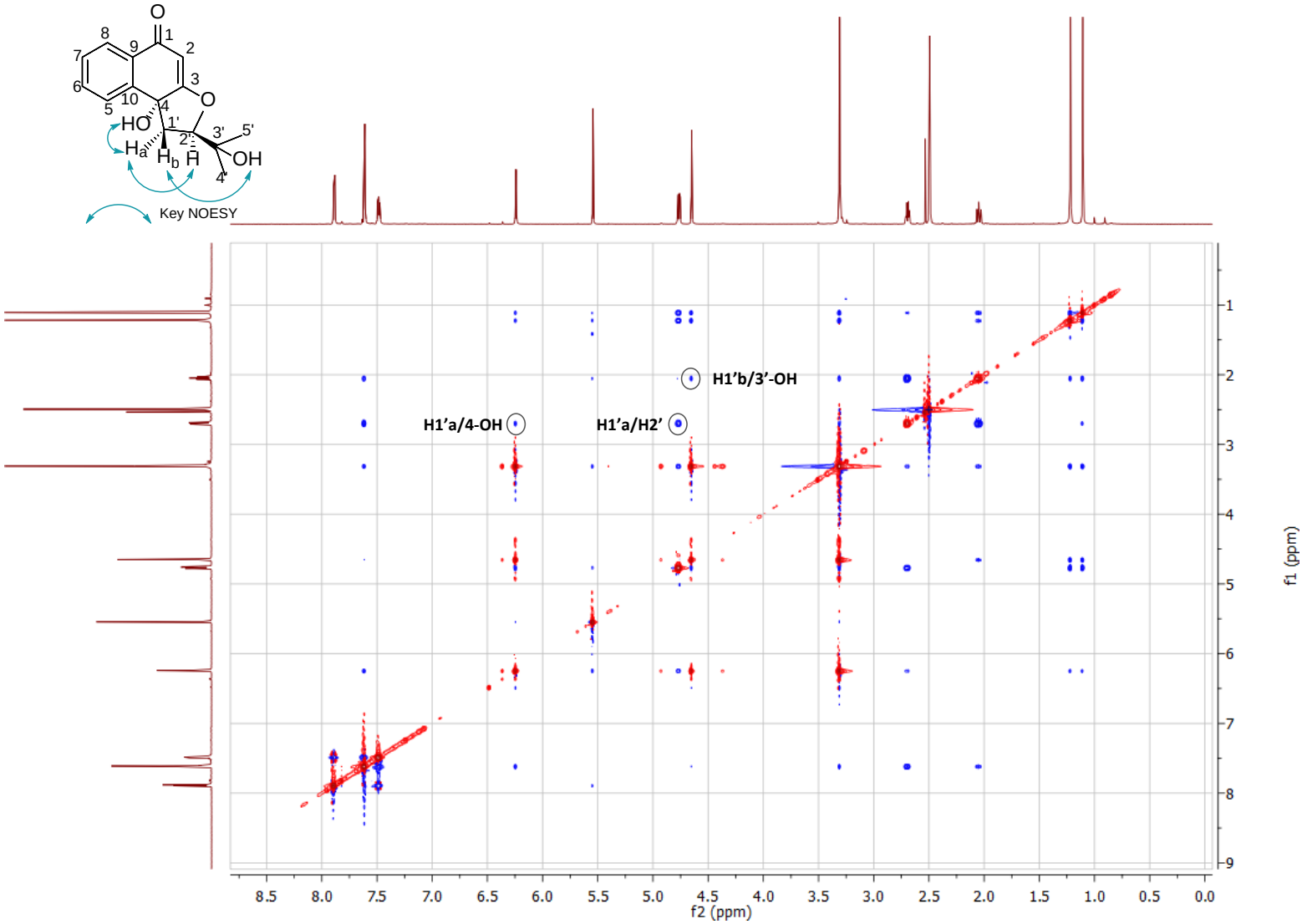


Figure S18. NOESY NMR spectrum of compound **4** in DMSO-*d6*

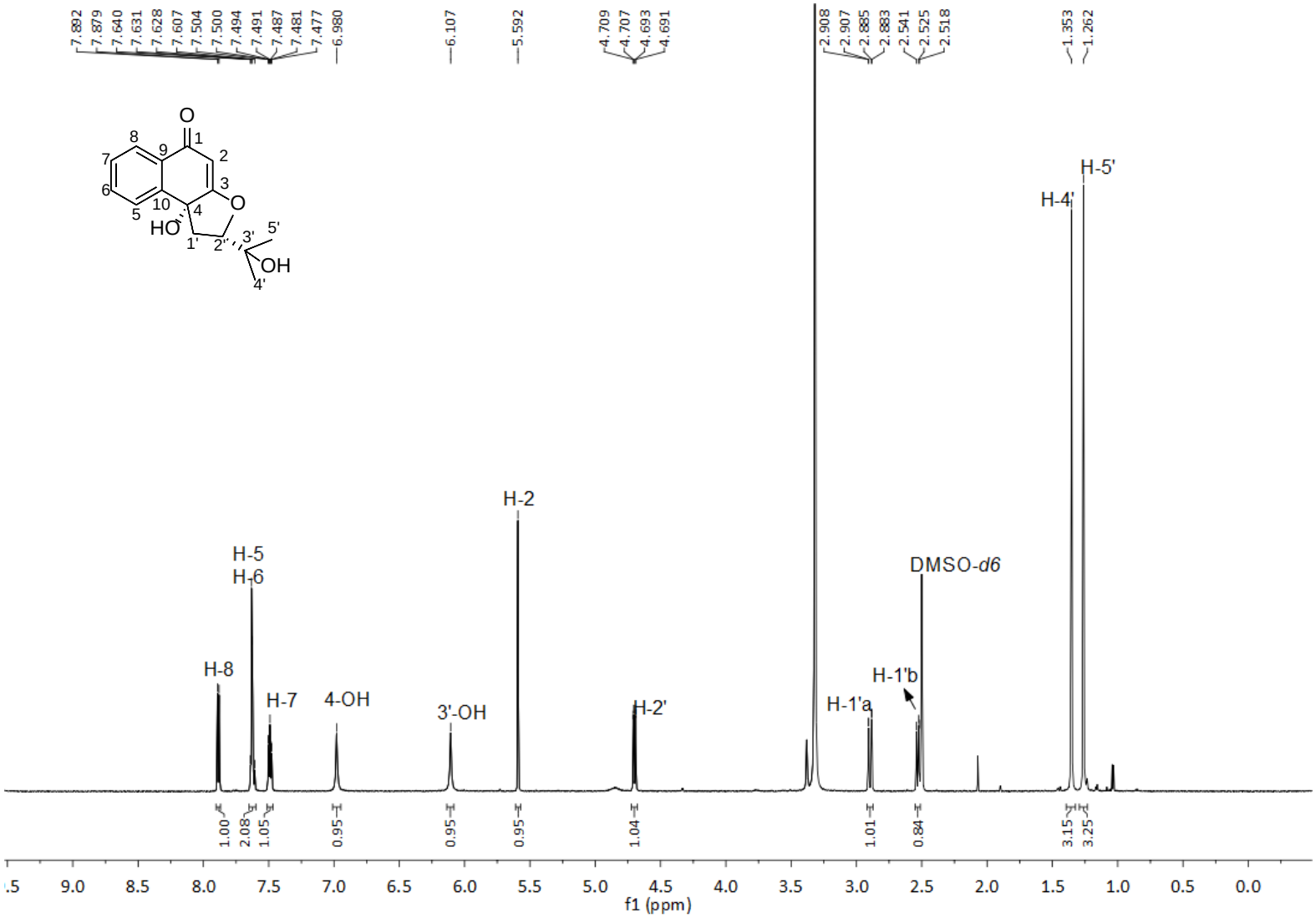


Figure S19. ^{1}H NMR spectrum of compound **5** in DMSO-*d6* (500 MHz)

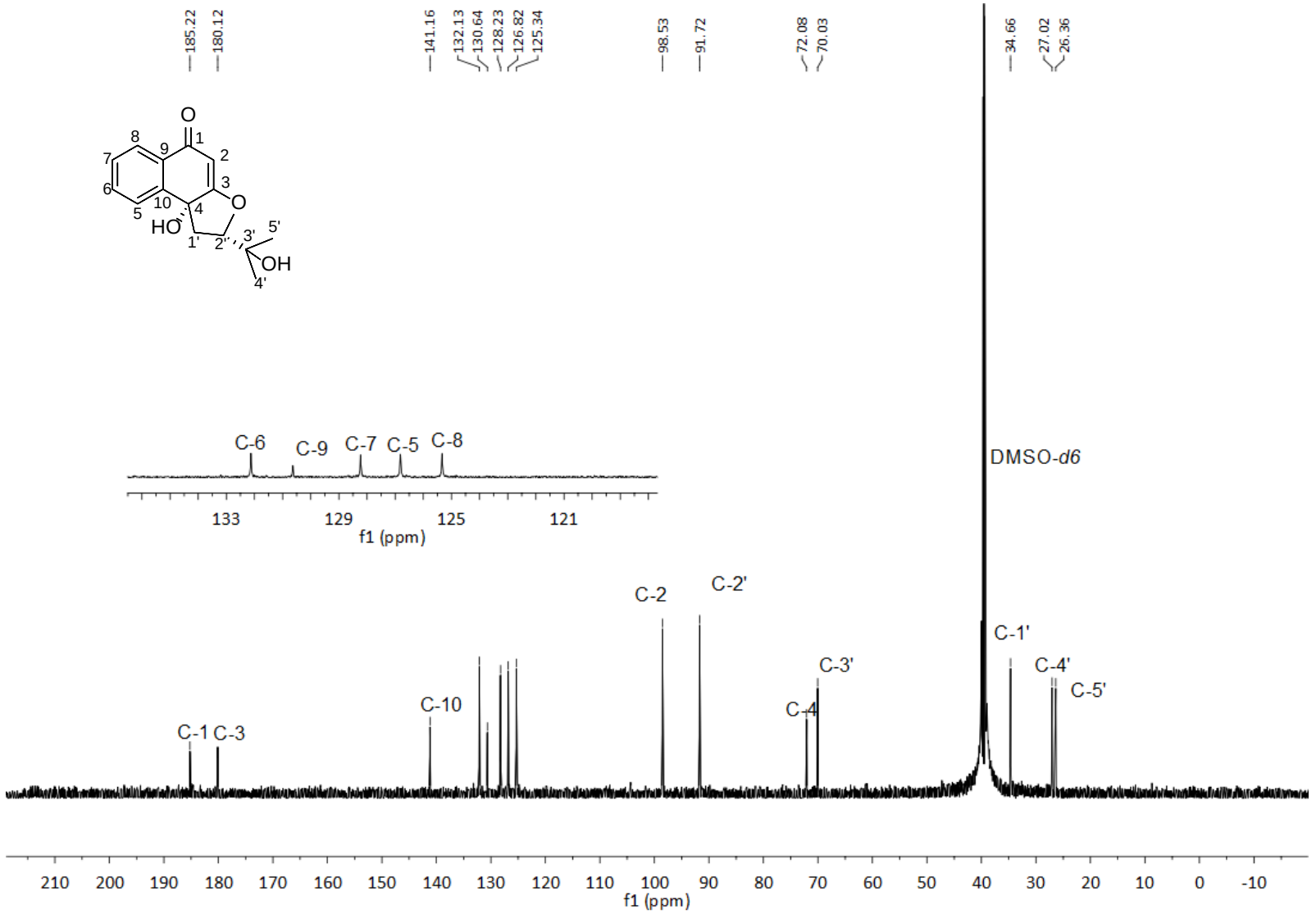


Figure S20. ^{13}C NMR spectrum of compound **5** in DMSO-*d6* (125 MHz)

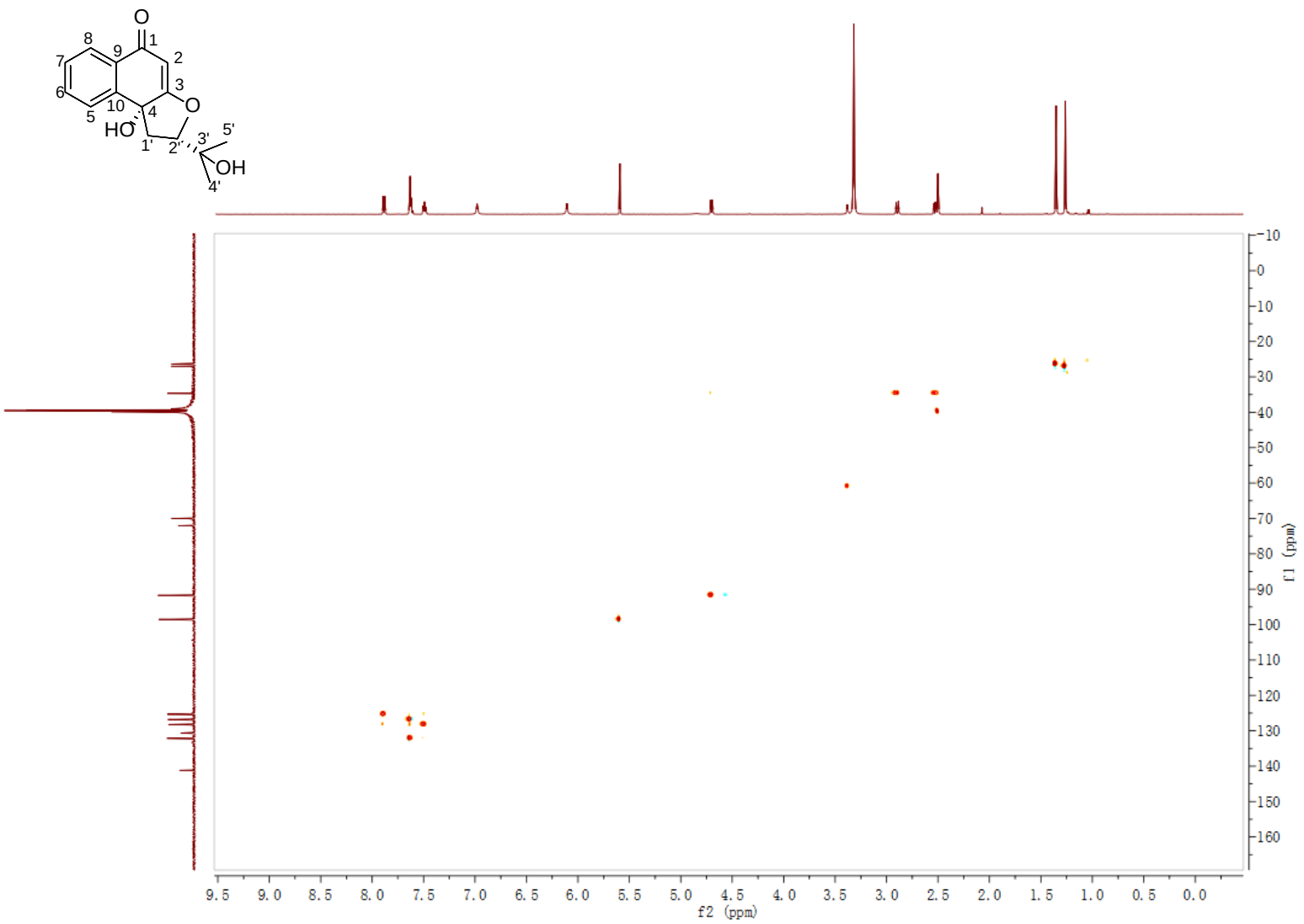


Figure S21. HSQC NMR spectrum of compound **5** in DMSO-*d6*

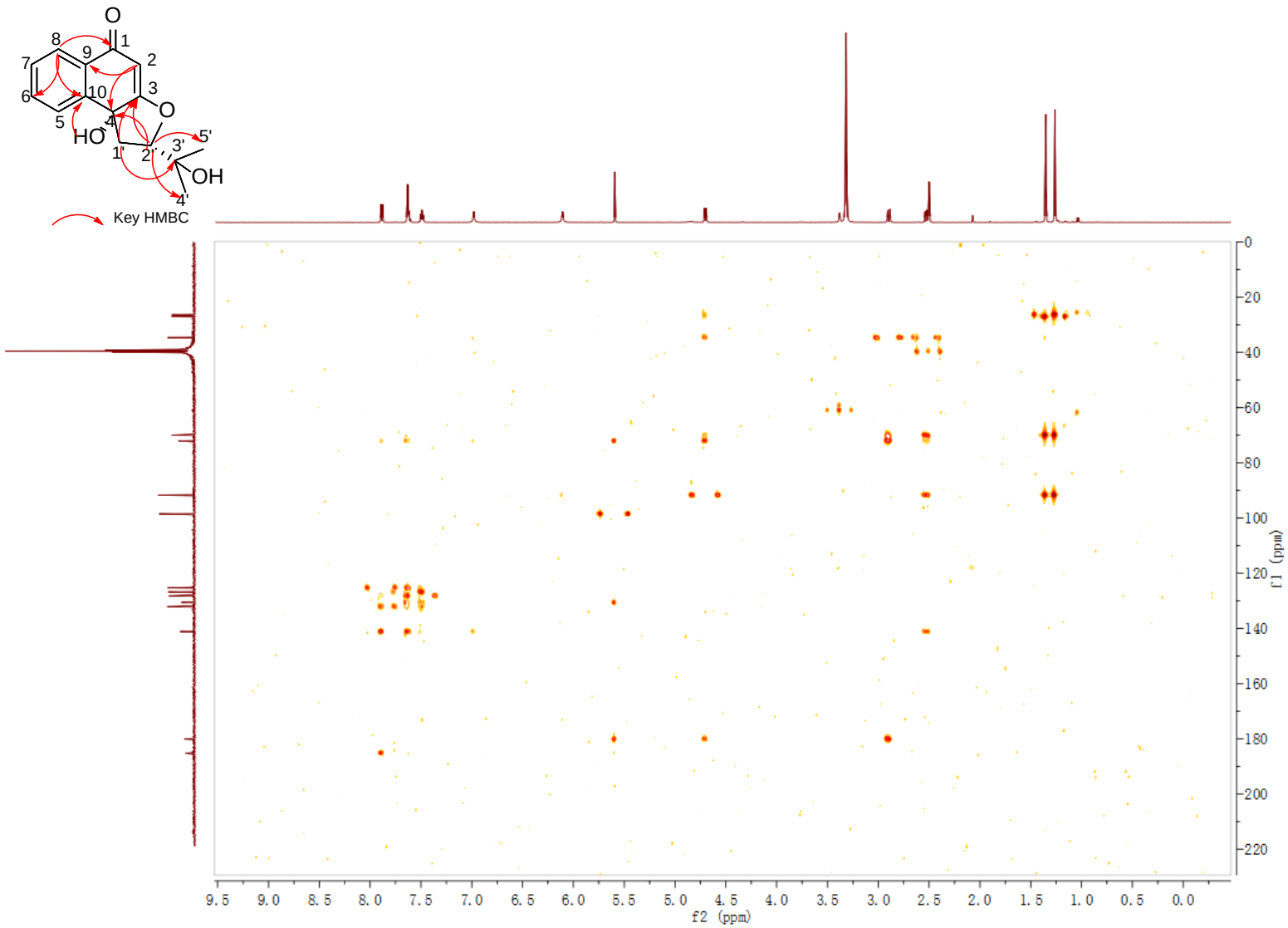


Figure S22. HMBC NMR spectrum of compound **5** in DMSO-*d6*

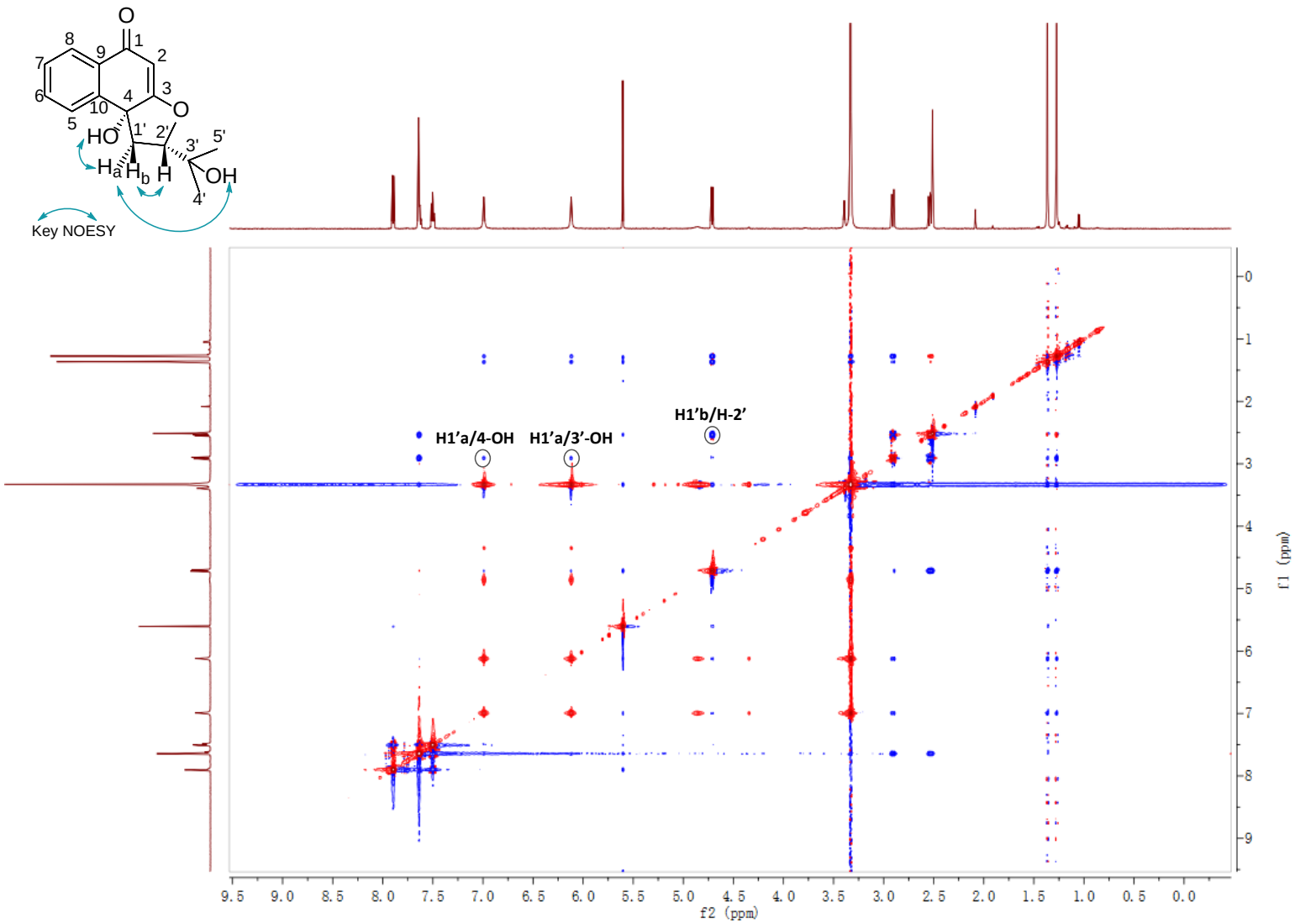


Figure S23. NOESY NMR spectrum of compound **5** in DMSO-*d6*

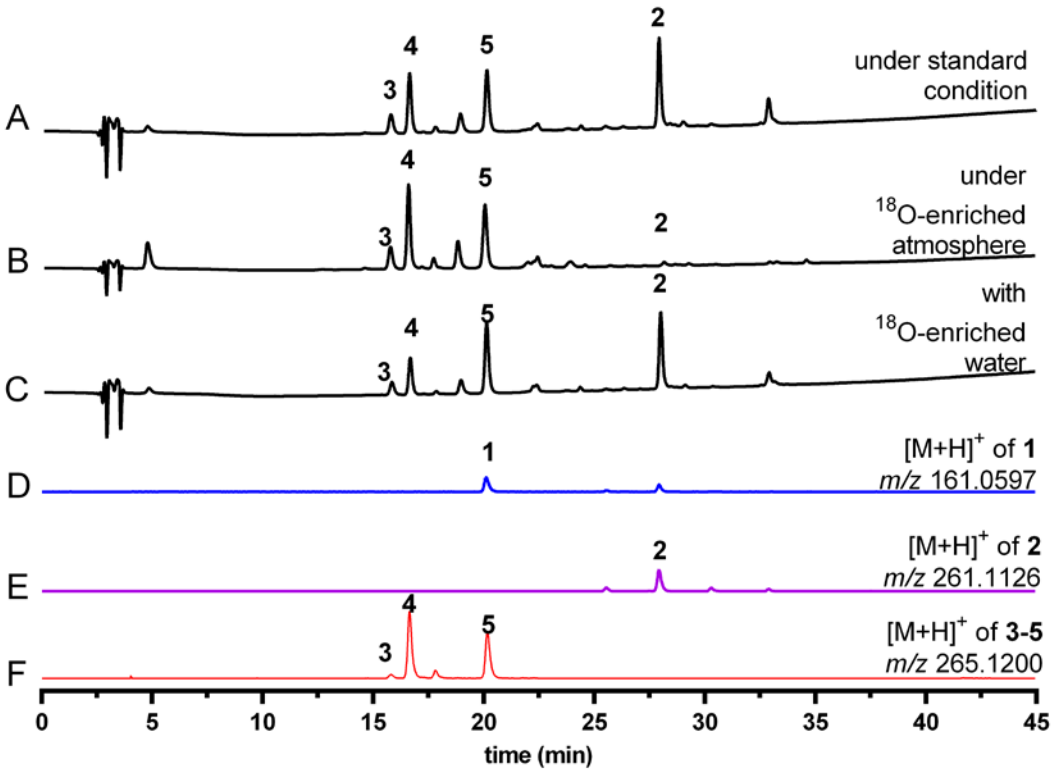


Figure S24. LC-HRMS analysis of the incubation mixtures with FgaPT2

The enzyme assays were incubated under standard condition (A), under ^{18}O-enriched atmosphere (B) or in buffer with ^{18}O-enriched water (C) at room temperature for 3h. Extracted Ion Chromatograms (EICs) refer $[M + H]^+$ ions of **1** (D), **2** (E) and **3**−**5** (F) with a tolerance range of ± 0.005.

Referece list

1 A. B. Woodside, Z. Huang, and C. D. Poulter, *Org. Synth.*, 1988, **66**, 211-215.

2 N. Steffan, I. A. Unsöld, and S.-M. Li, *Chembiochem*, 2007, **8**, 1298-1307.

3 W.-B. Yin, H.-L. Ruan, L. Westrich, A. Grundmann, and S.-M. Li, *Chembiochem*, 2007, **8**, 1154-1161.

4 A. Grundmann and S.-M. Li, *Microbiology*, 2005, **151**, 2199-2207.

5 W.-B. Yin, A. Grundmann, J. Cheng, and S.-M. Li, *J. Biol. Chem.*, 2009, **284**, 100-109.

6 H. Ran, V. Wohlgemuth, X. Xie, and S.-M. Li, *ACS Chem. Biol.*, 2018, **13**, 2949-2955.

7 N. Steffan, A. Grundmann, A. Afiyatullov, H. Ruan, and S.-M. Li, *Org. Biomol. Chem.*, 2009, **7**, 4082-4087.