Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

New Cytotoxic Natural Products from the Marine Sponge-Derived Fungus *Pestalotiopsis* sp. by Epigenetic Modification

Hui Lei [1], Dan Zhang [1], Nan Ding[3], Siwei Chen [1], Can Song [1], Yu Luo [1], Xiujuan Fu [1], Xiaoxu Bi [2*], and Hong Niu [1*]

[1] School of Pharmacy, Southwest Medical University, Luzhou, Sichuan 646000, China;

[2] College of Agriculture and Life Sciences, Kunming University, Kunming, Yunnan 650241, China;

[3] Institute of Pathogenic Biology, University of South China, Hengyang 421001, China;

*To whom correspondence should be addressed.

Tel/fax: +86-0830-316 2291. E-mail: dawn@nwsuaf.edu.cn

Qualitative Analysis Report

Data Filename	LH-4-NEG.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-A2
Instrument Name	Instrument 1	**User Name**	
Acq Method	default-20191128-neg.m	**Acquired Time**	7/14/2020 8:55:04 AM
IRM Calibration Status	Success	**DA Method**	analysis.m
Comment			

Sample Group **Info.**

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
75	0	ESI

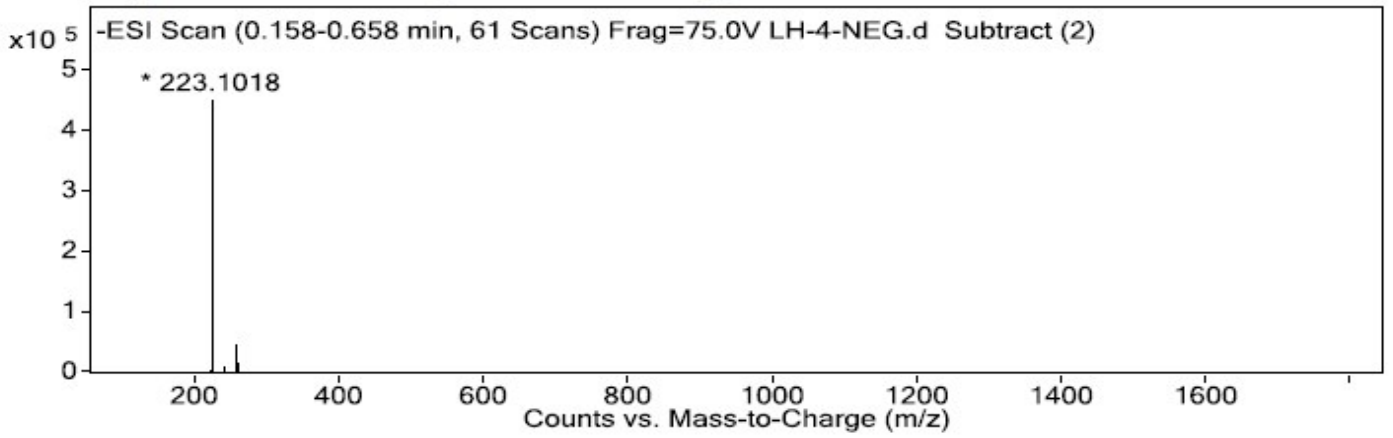


Figure S1. HRESI-MS spectrum of the new compound **1**

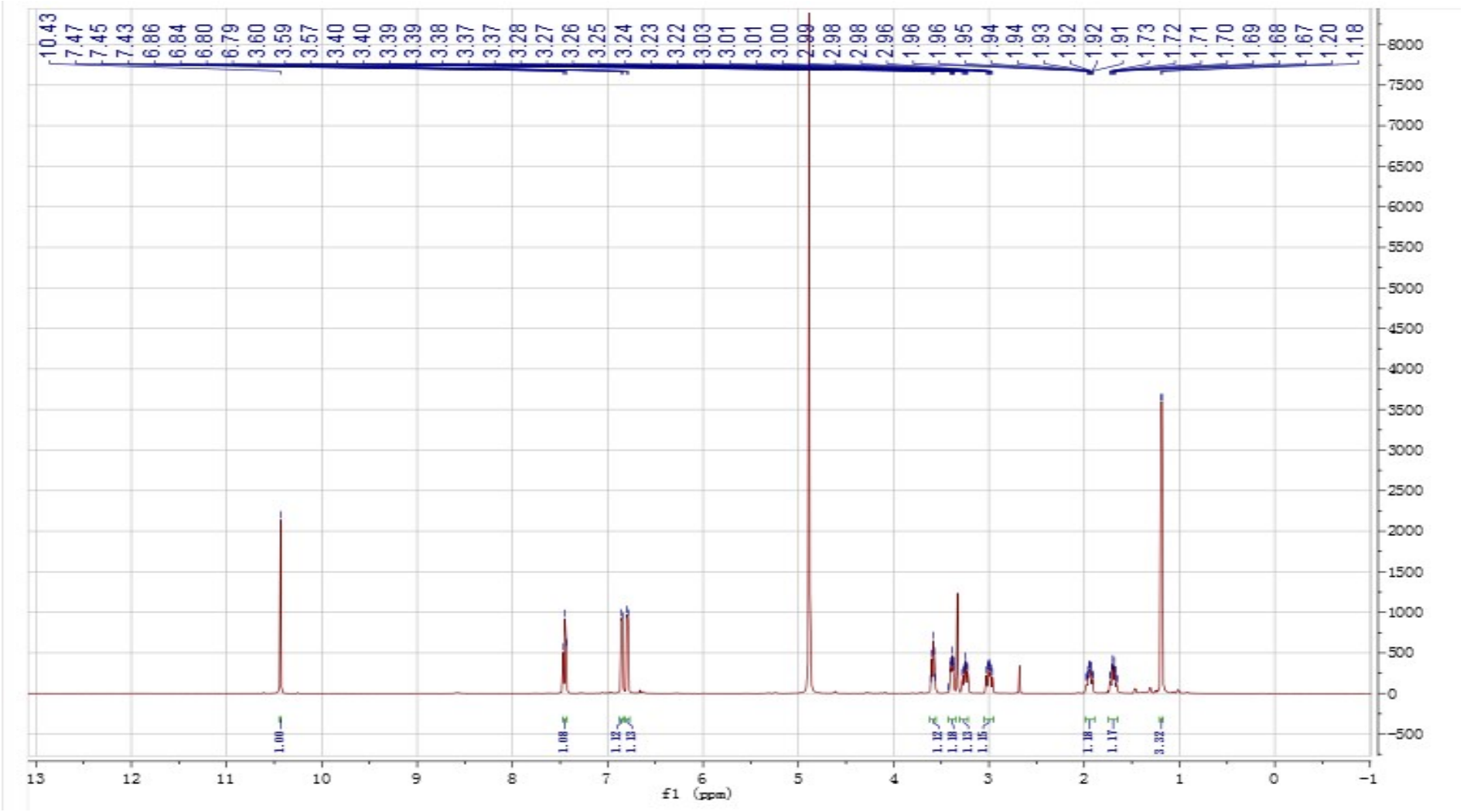


Figure S2. ^{1}H NMR (600 MHz, CD_3OD) spectrum of the new compound **1**

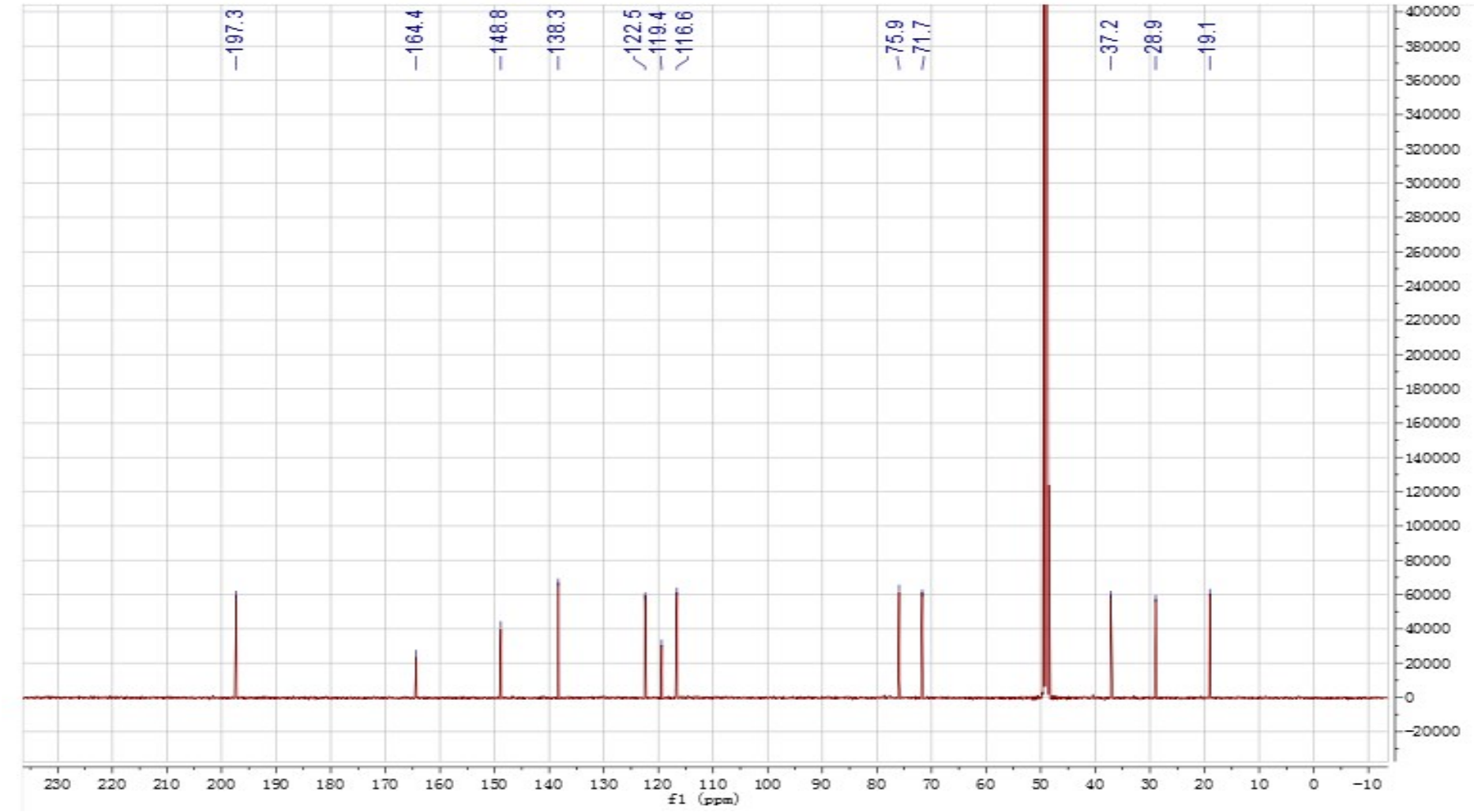


Figure S3. ^{13}C NMR (150 MHz, CD_3OD) spectrum of the new compound **1**

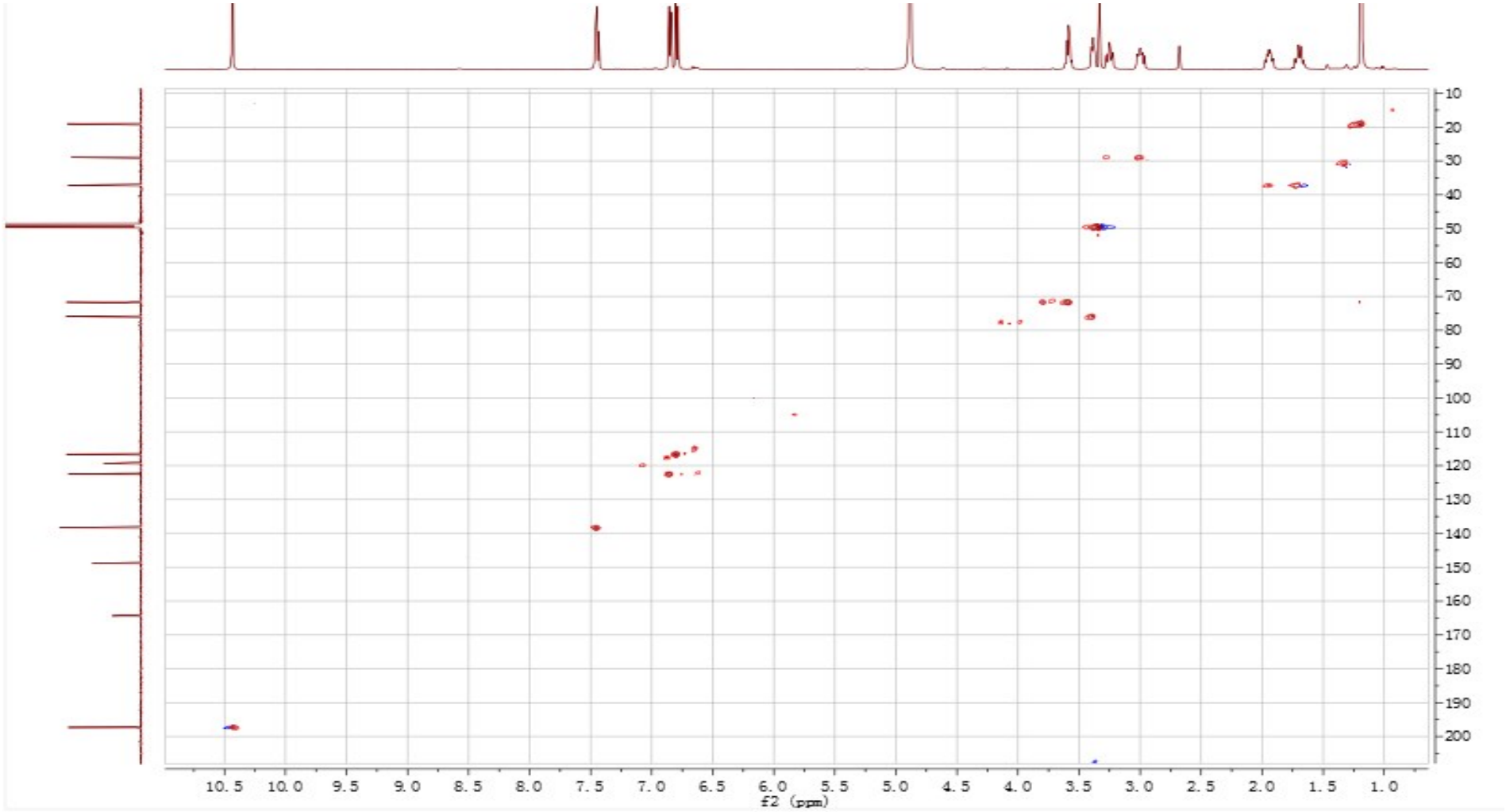


Figure S4. HSQC spectrum of the new compound **1**

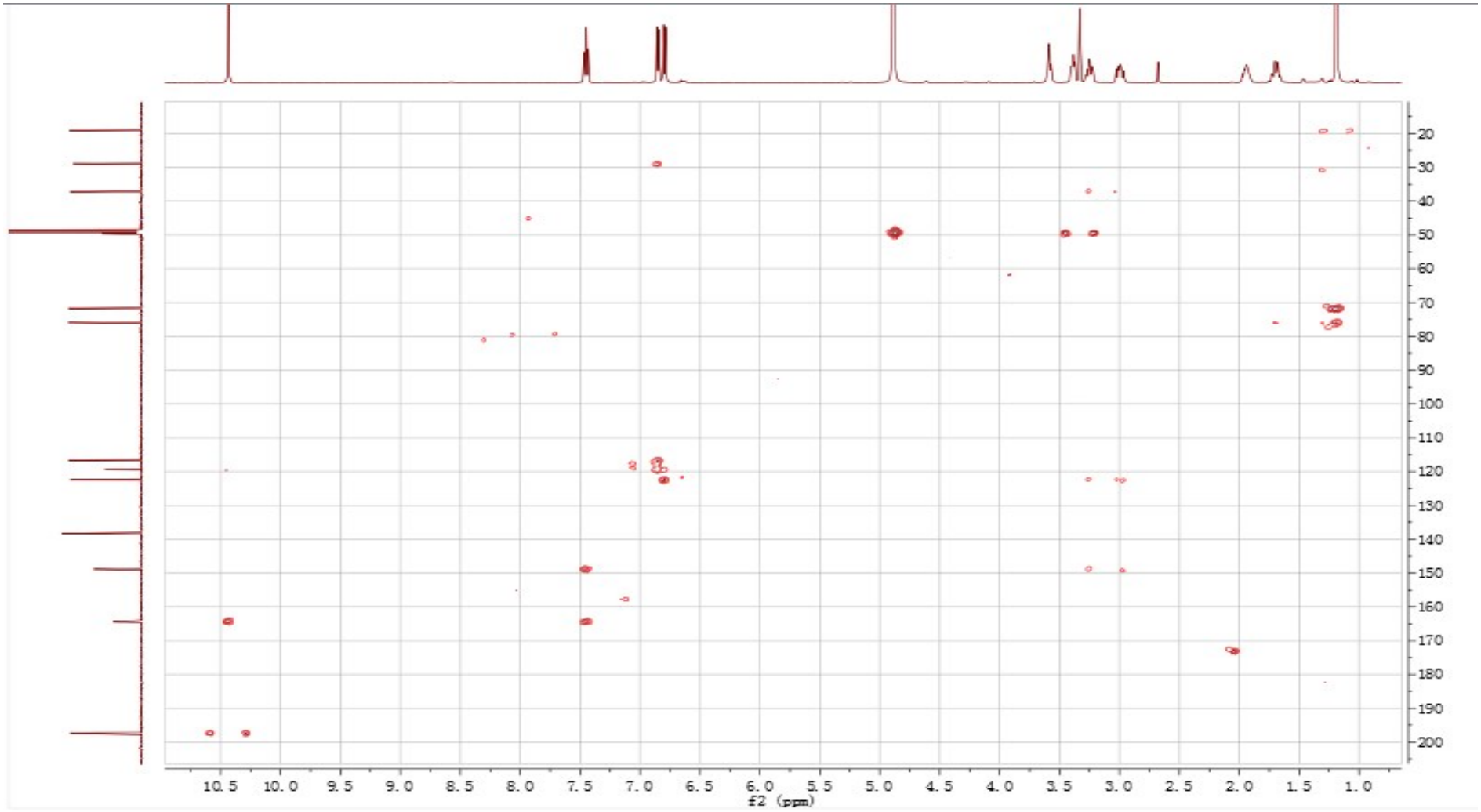


Figure S5. HMBC spectrum of the new compound **1**

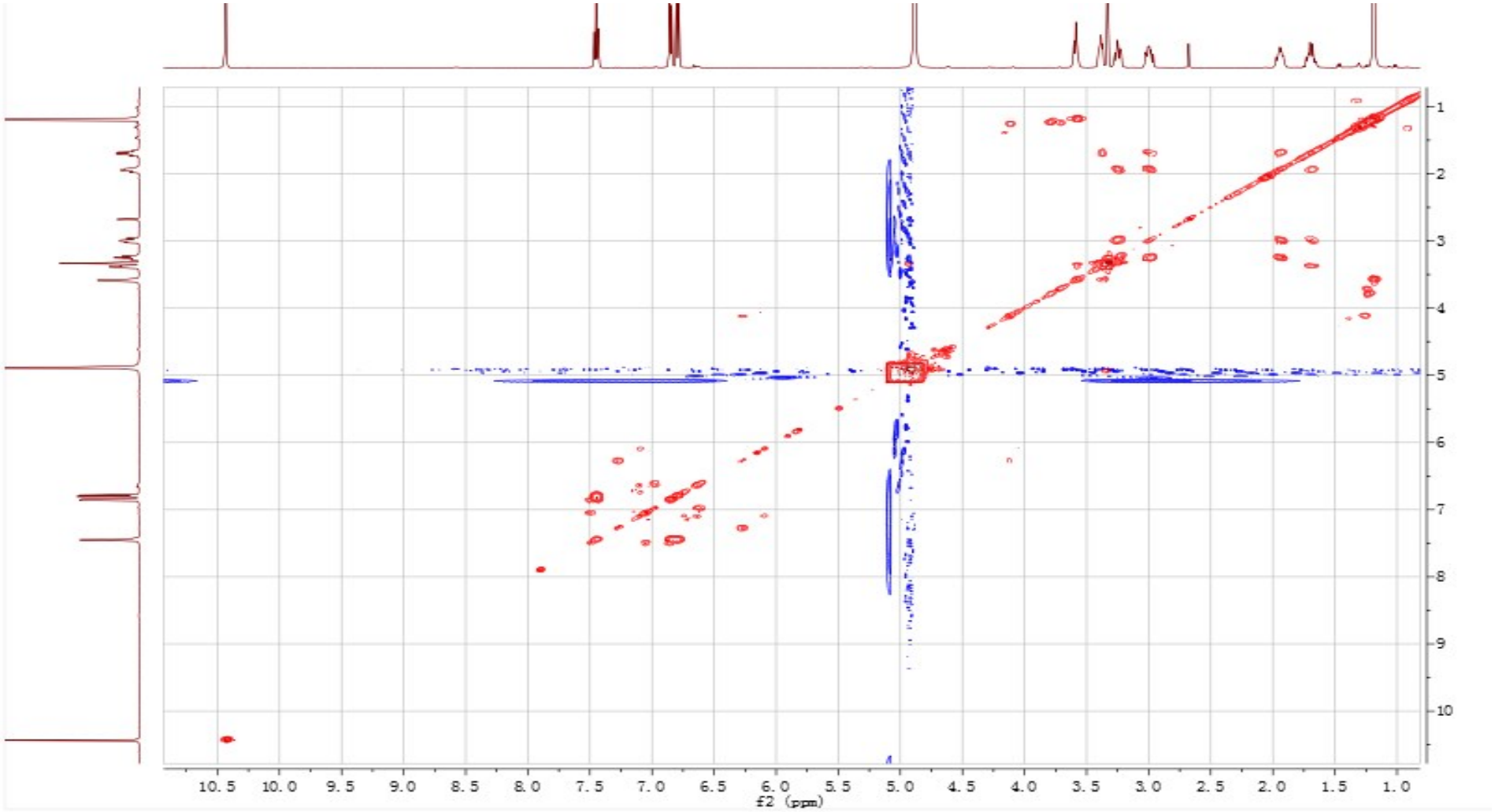


Figure S6. COSY spectrum of the new compound **1**

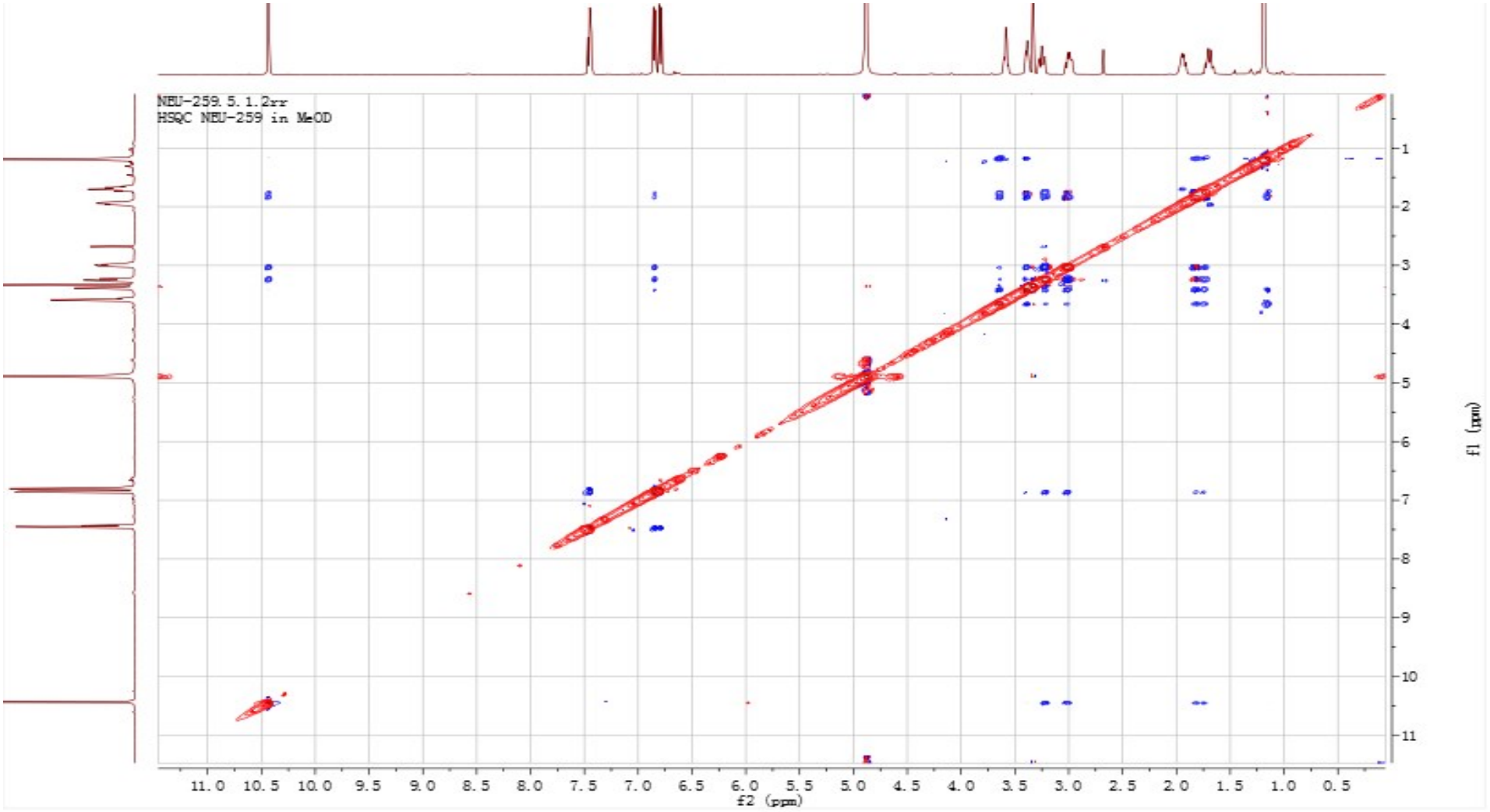


Figure S7. NOESY spectrum of the new compound **1**

Qualitative Analysis Report

Data Filename	LH-2-NEG.d	**Sample Name**	Unavailable
Sample Type	Unavailable	**Position**	Unavailable
Instrument Name	Unavailable	**User Name**	Unavailable
Acq Method		**Acquired Time**	Unavailable
IRM Calibration Status	Success	**DA Method**	analysis.m
Comment	Sample information is unavailable		

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
75	0	ESI

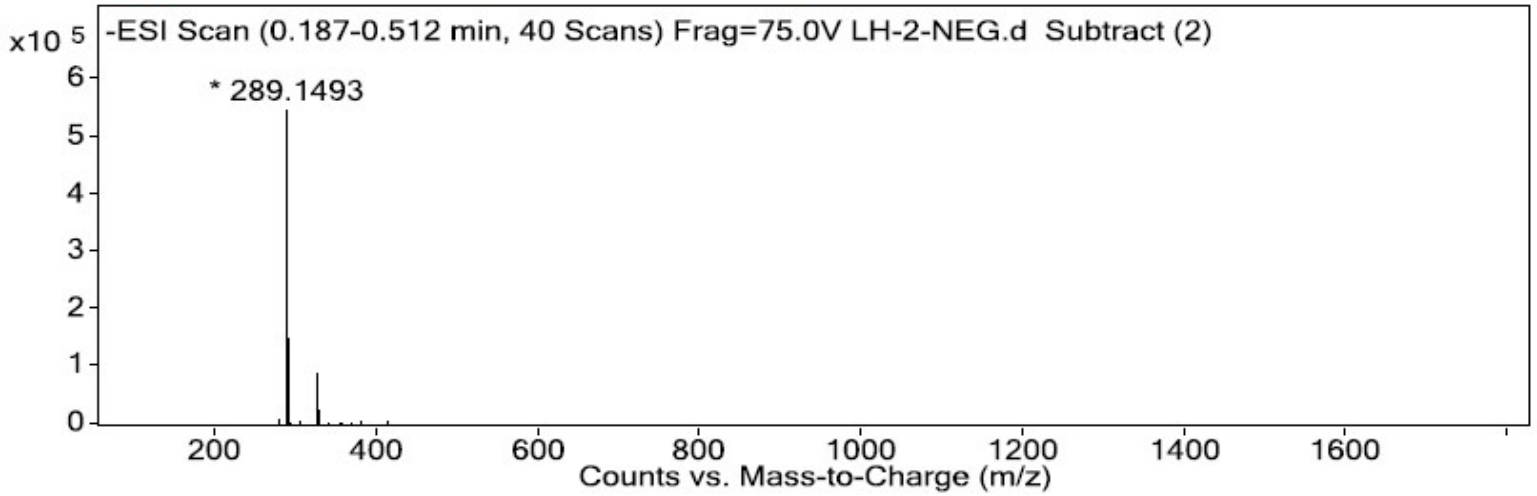


Figure S8. HRESI-MS spectrum of the new compound **2**

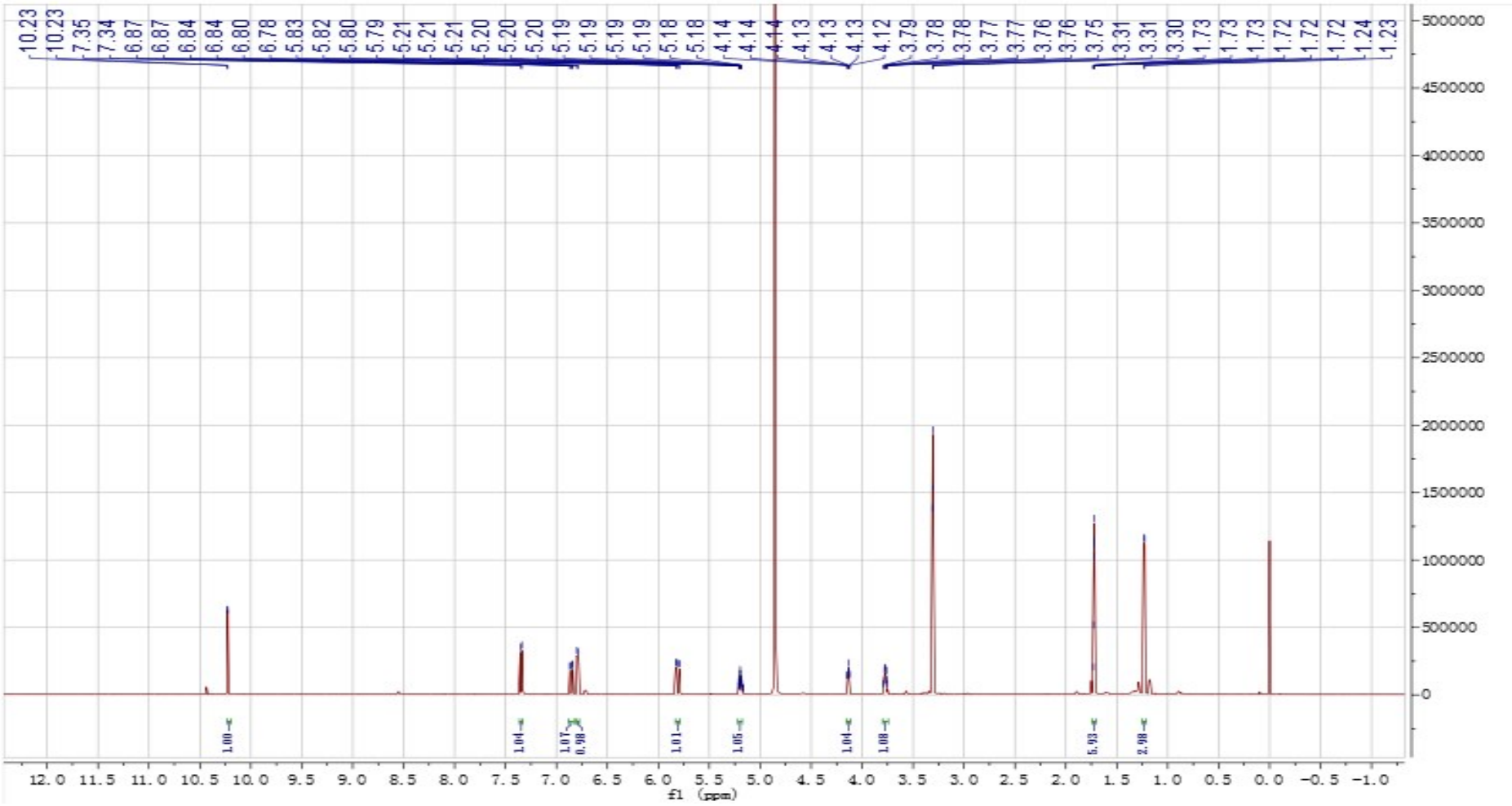


Figure S9. ^{1}H NMR (600 MHz, CD_3OD) spectrum of the new compound **2**

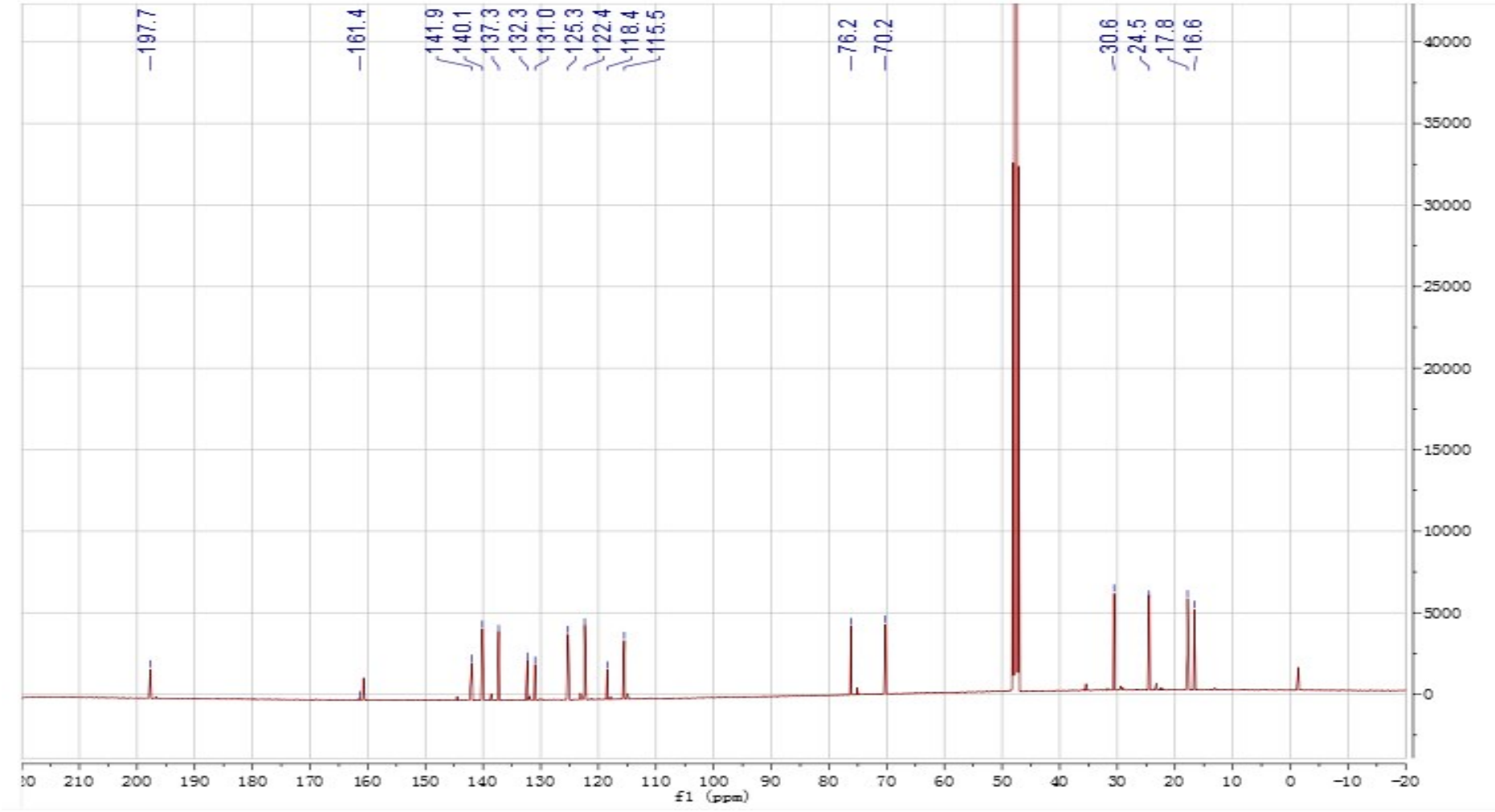


Figure S10. ^{13}C NMR (150 MHz, CD_3OD) spectrum of the new compound **2**

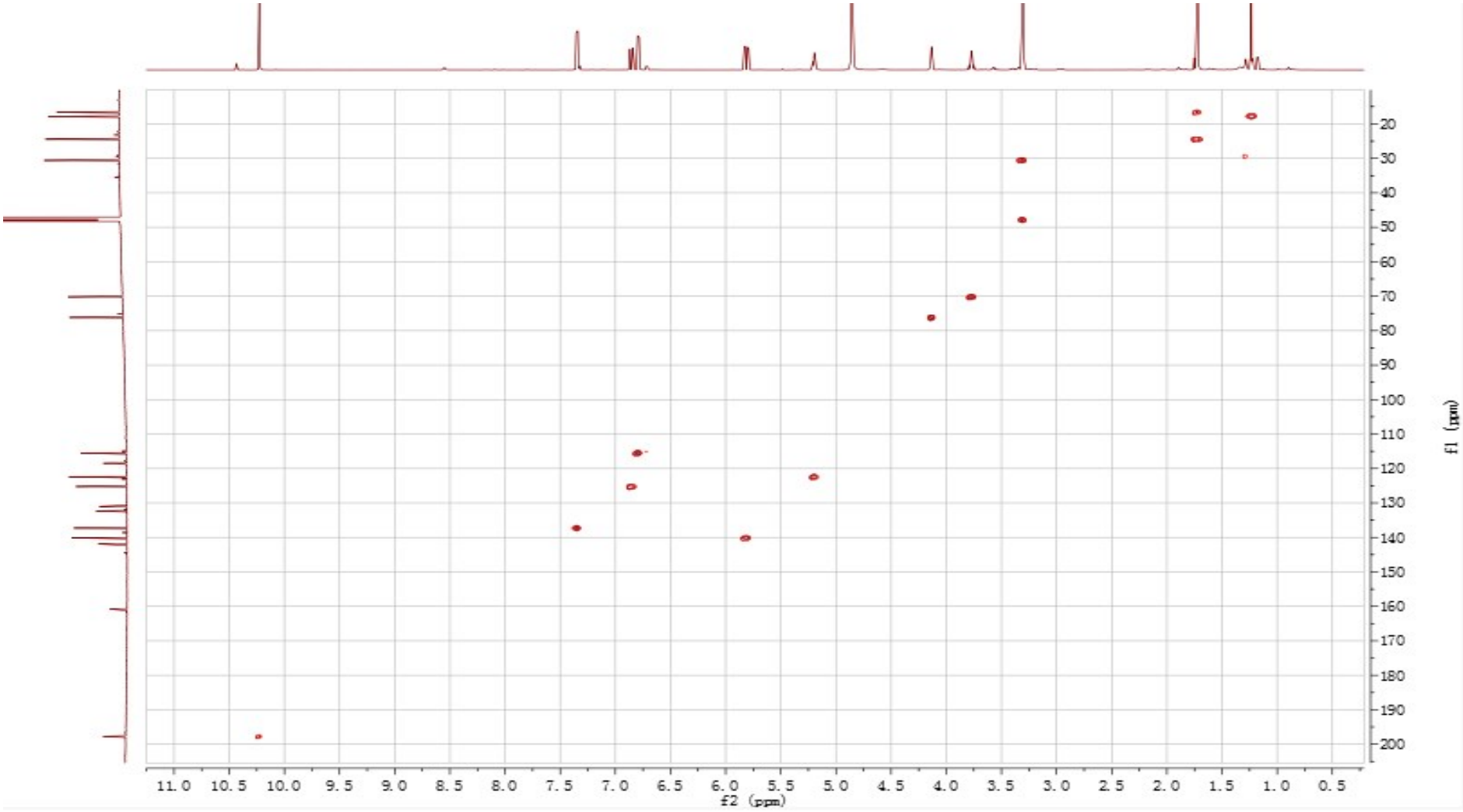


Figure S11. HSQC spectrum of the new compound **2**

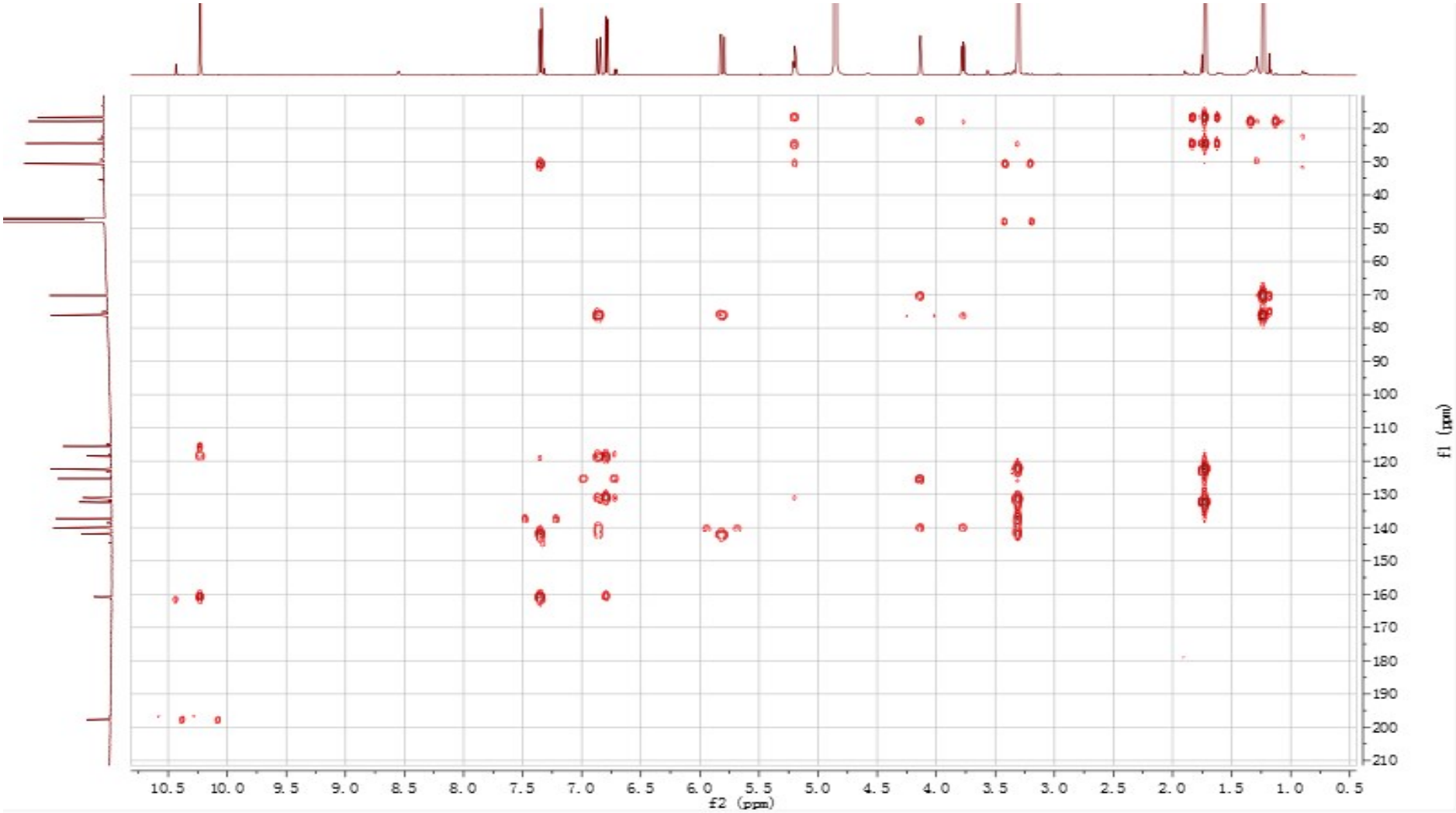


Figure S12. HMBC spectrum of the new compound **2**

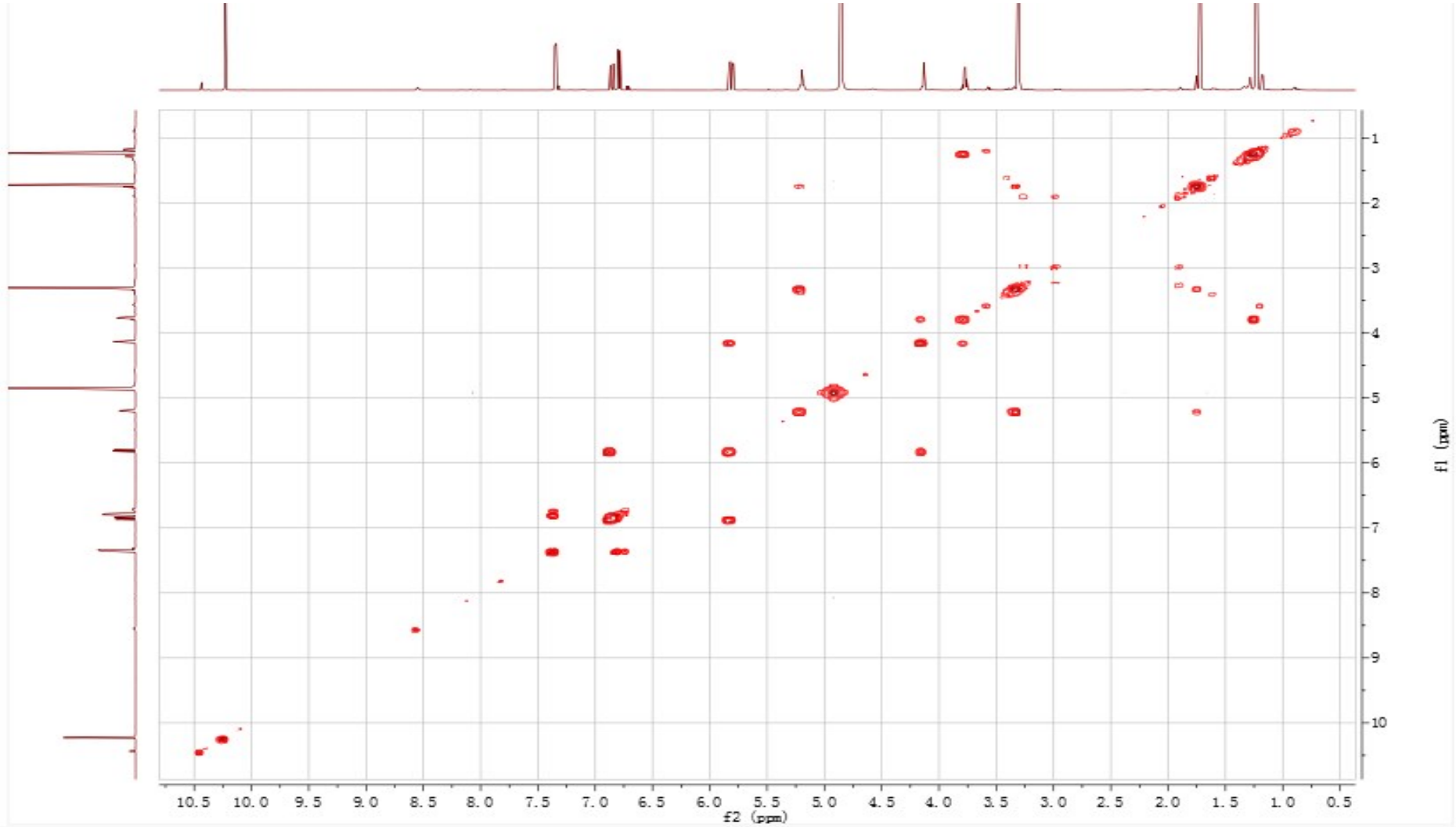


Figure S13. COSY spectrum of the new compound **2**

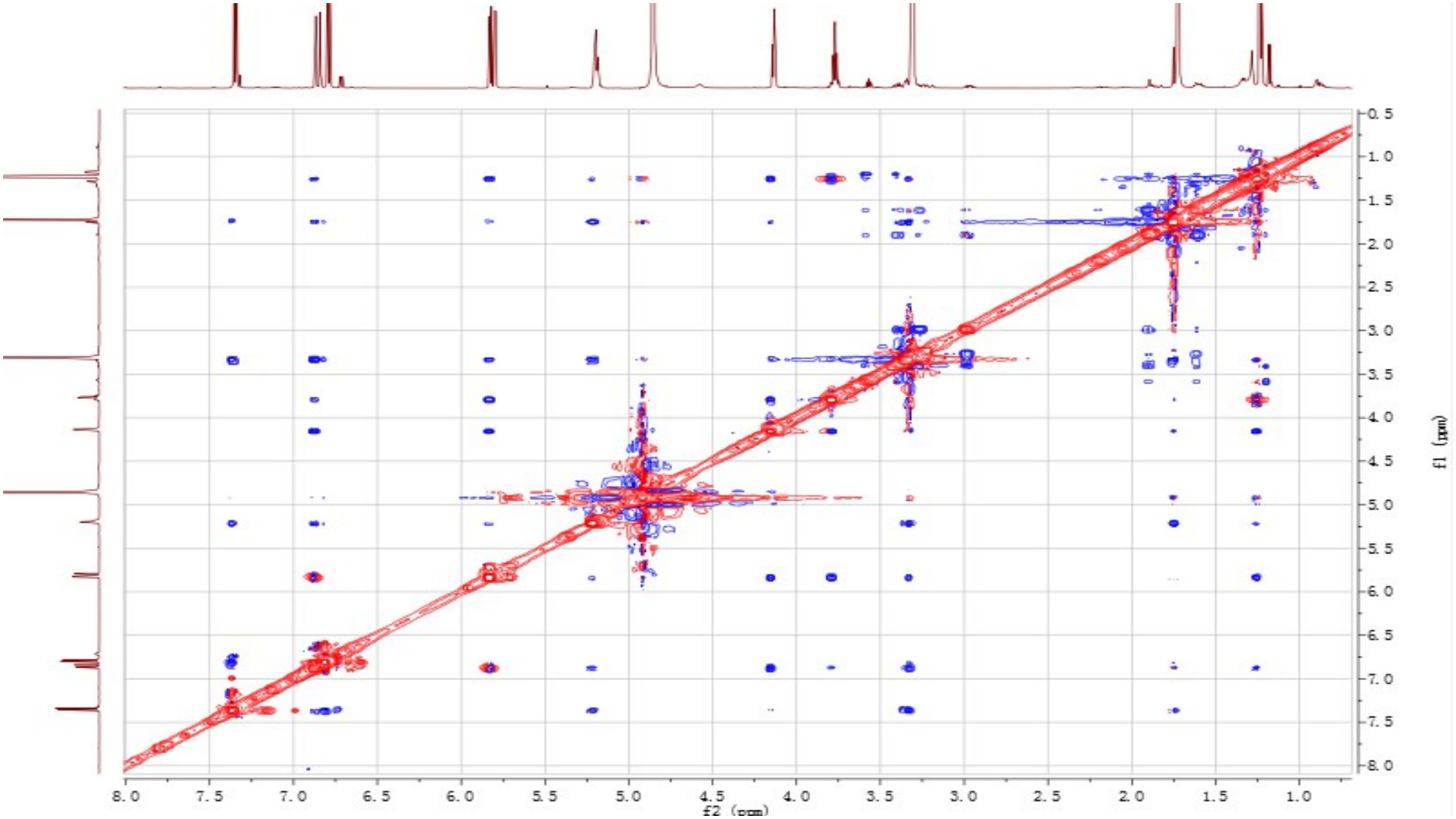


Figure S14. NOESY spectrum of the new compound **2**

Qualitative Analysis Report

Data Filename	LH-482-POS.d	**Sample Name**	
Sample Type	Sample	**Position**	P1-A2
Instrument Name	Instrument 1	**User Name**	
Acq Method	default-20191128-pos.m	**Acquired Time**	7/14/2020 9:35:28 AM
IRM Calibration Status	success	**DA Method**	analysis.m
Comment			

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
75	0	ESI

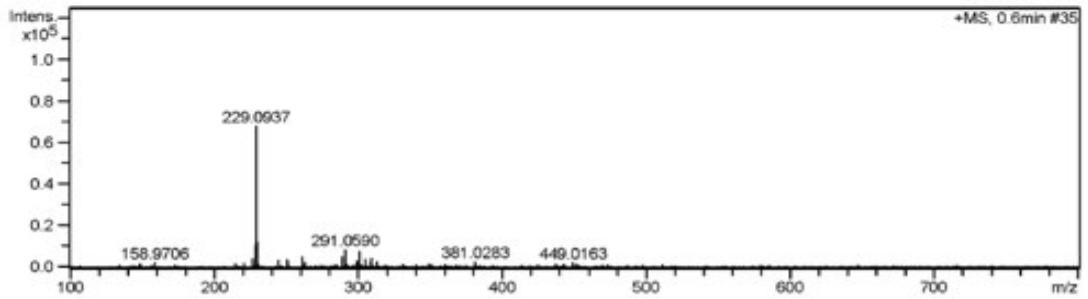


Figure S15. HRESI-MS spectrum of the new compound **3**

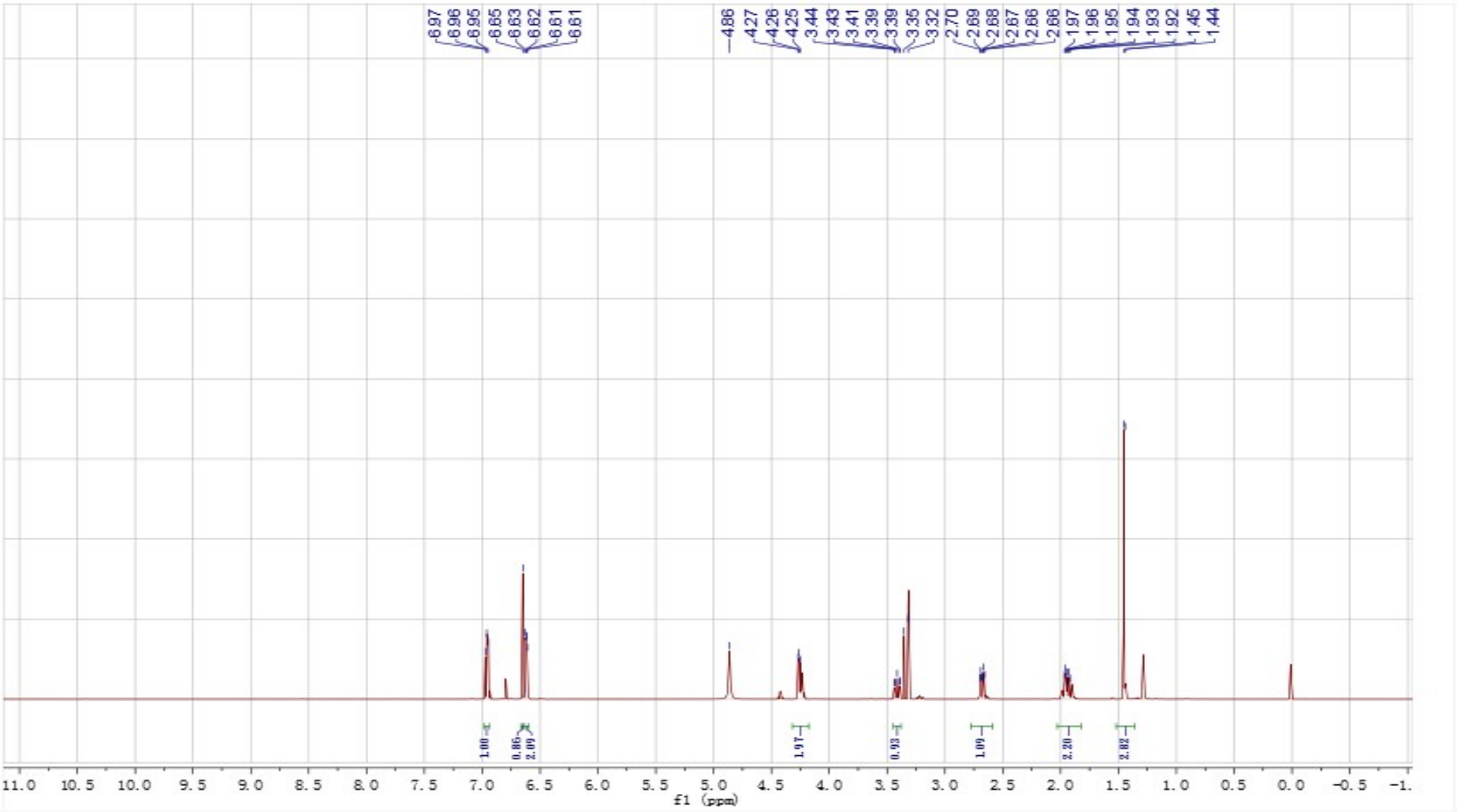
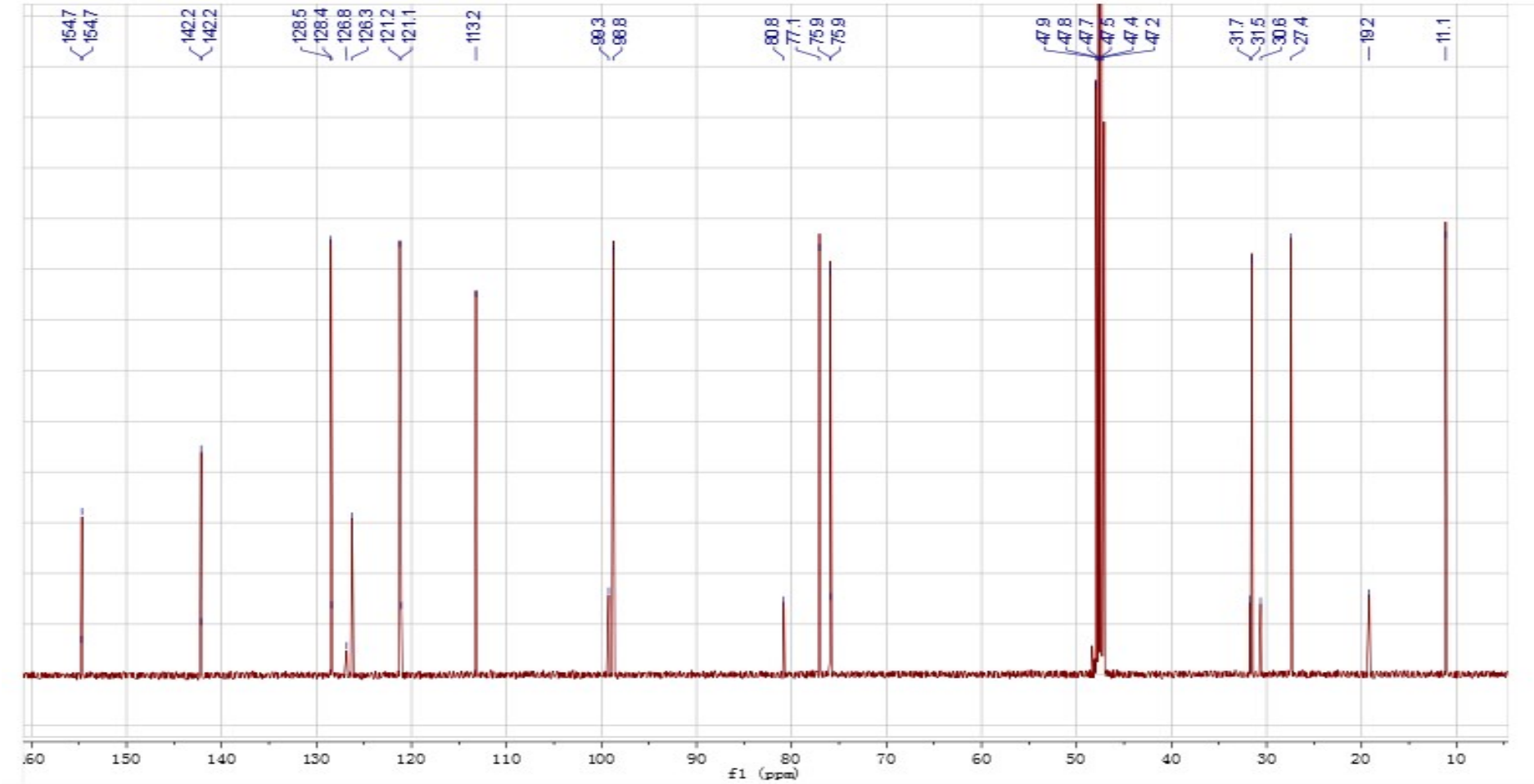
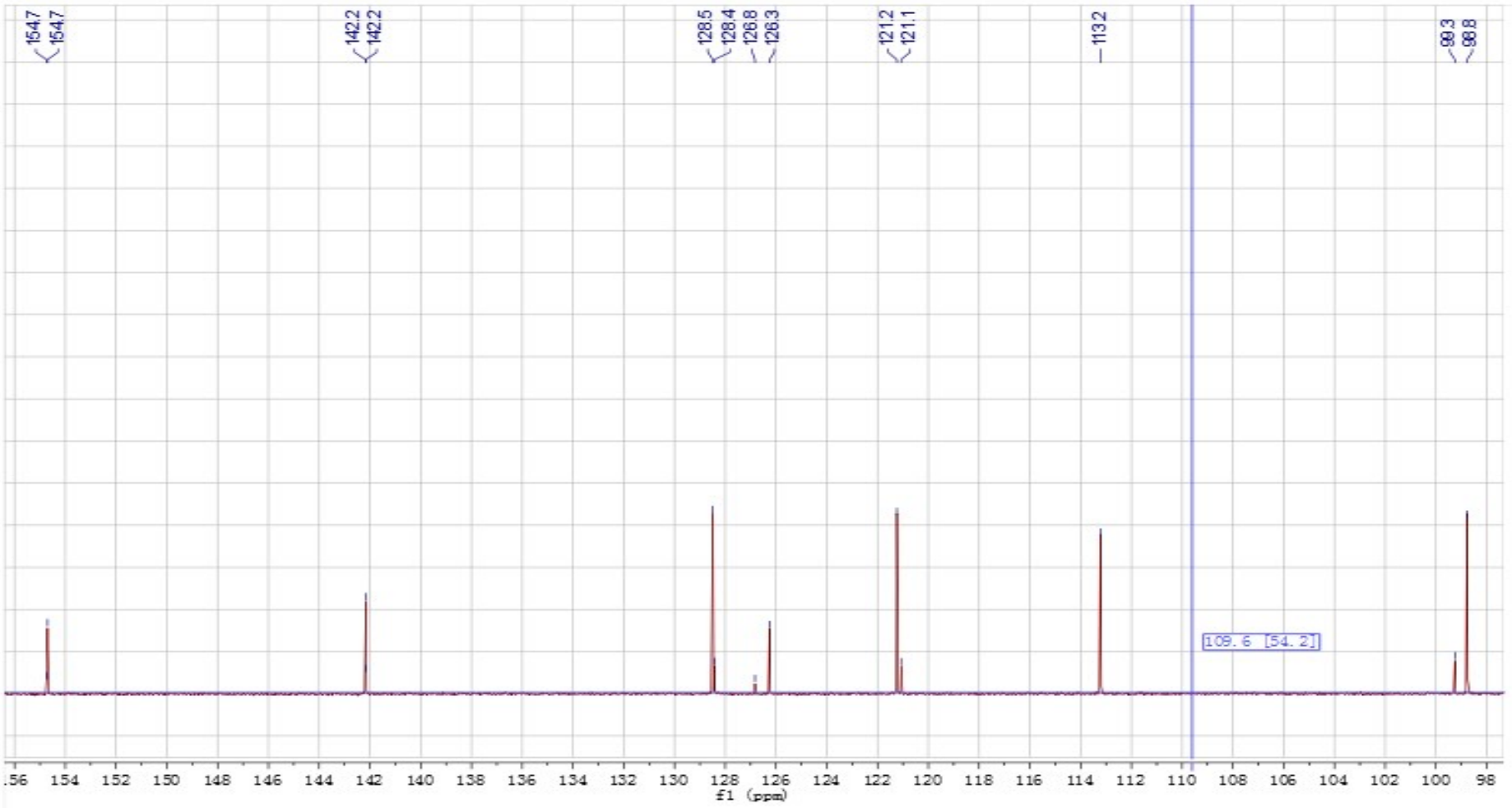


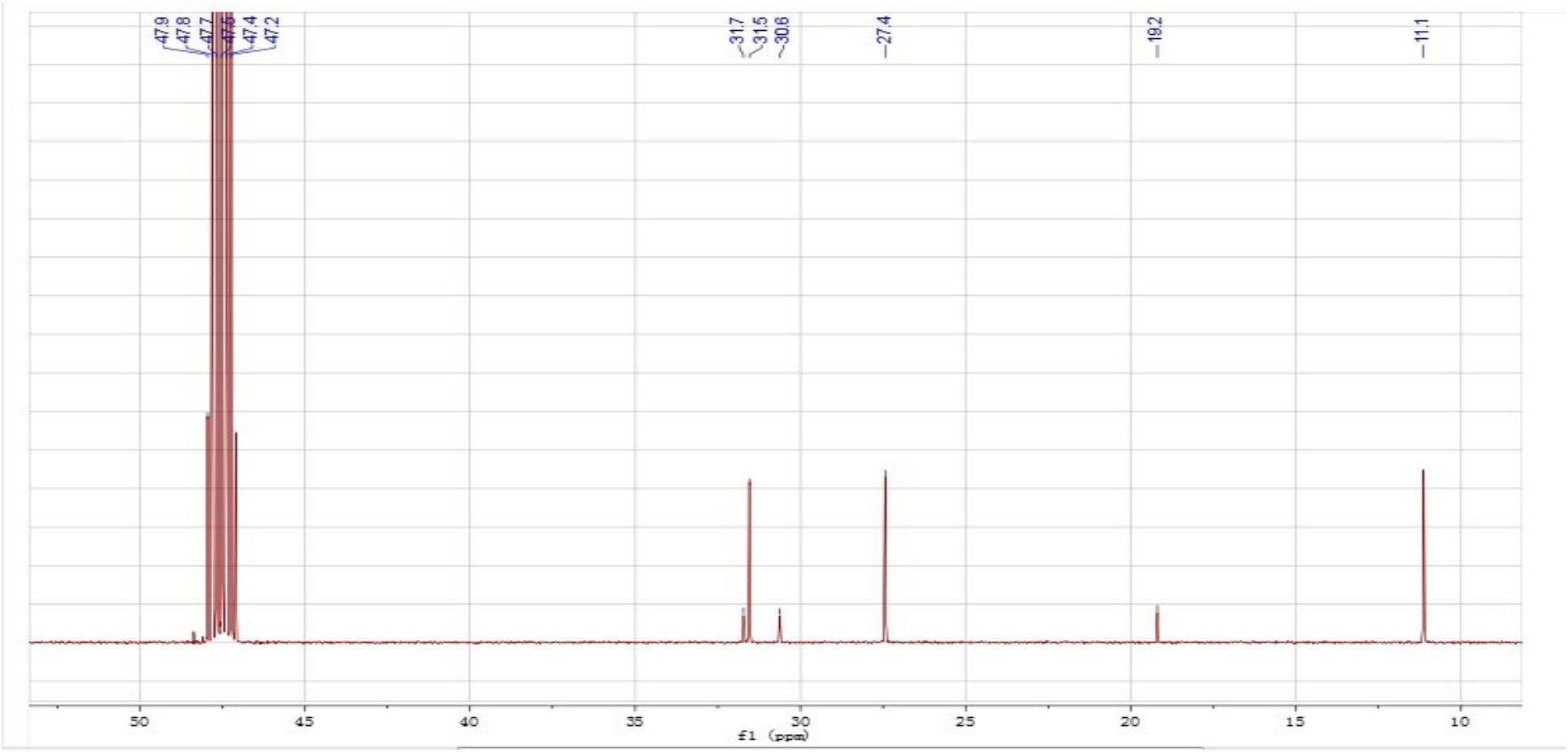
Figure S16. ^{1}H NMR (600 MHz, CD_3OD) spectrum of the new compound **3**



Fiure S17. ^{13}C NMR (150 MHz, CD_3OD) spectrum of the new compound **3**



Fiure S18. Enlarged-1 ^{13}C NMR (150 MHz, CD_3OD) spectrum of the new compound **3**



Fiure S19. Enlarged-2 ^{13}C NMR (150 MHz, CD_3OD) spectrum of the new compound **3**

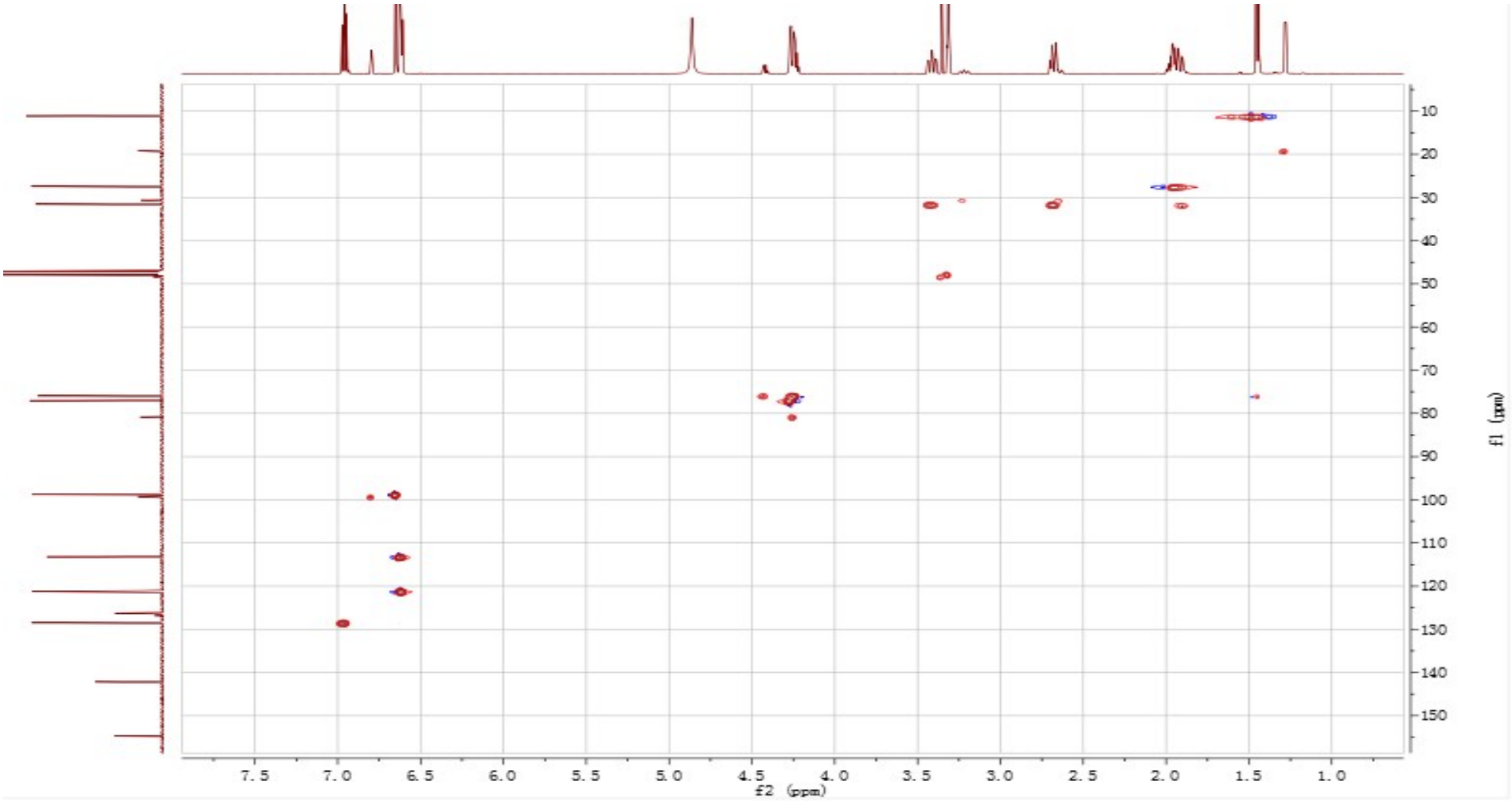


Figure S20. HSQC spectrum of the new compound **3**

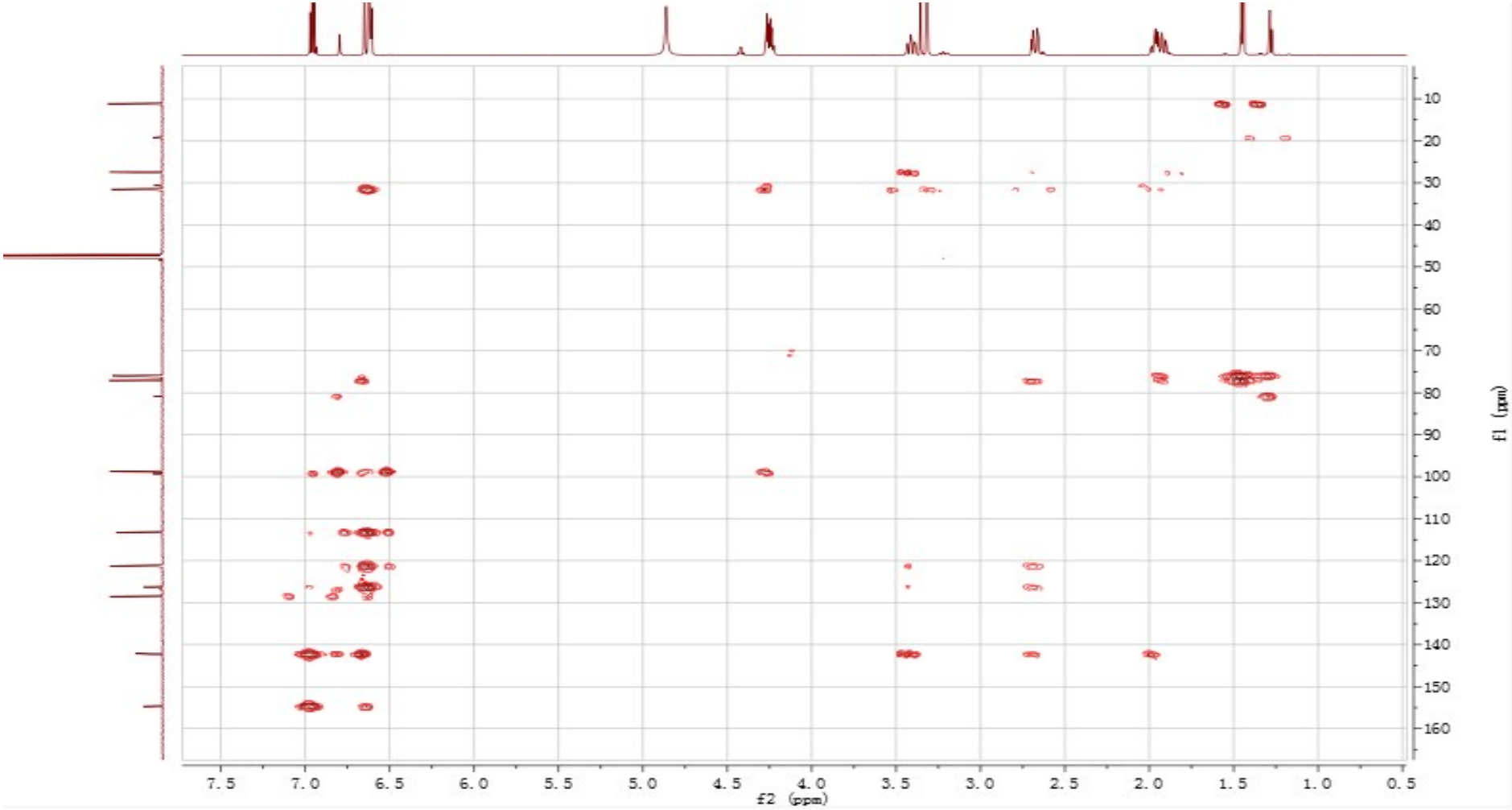


Figure S21. HMBC spectrum of the new compound **3**

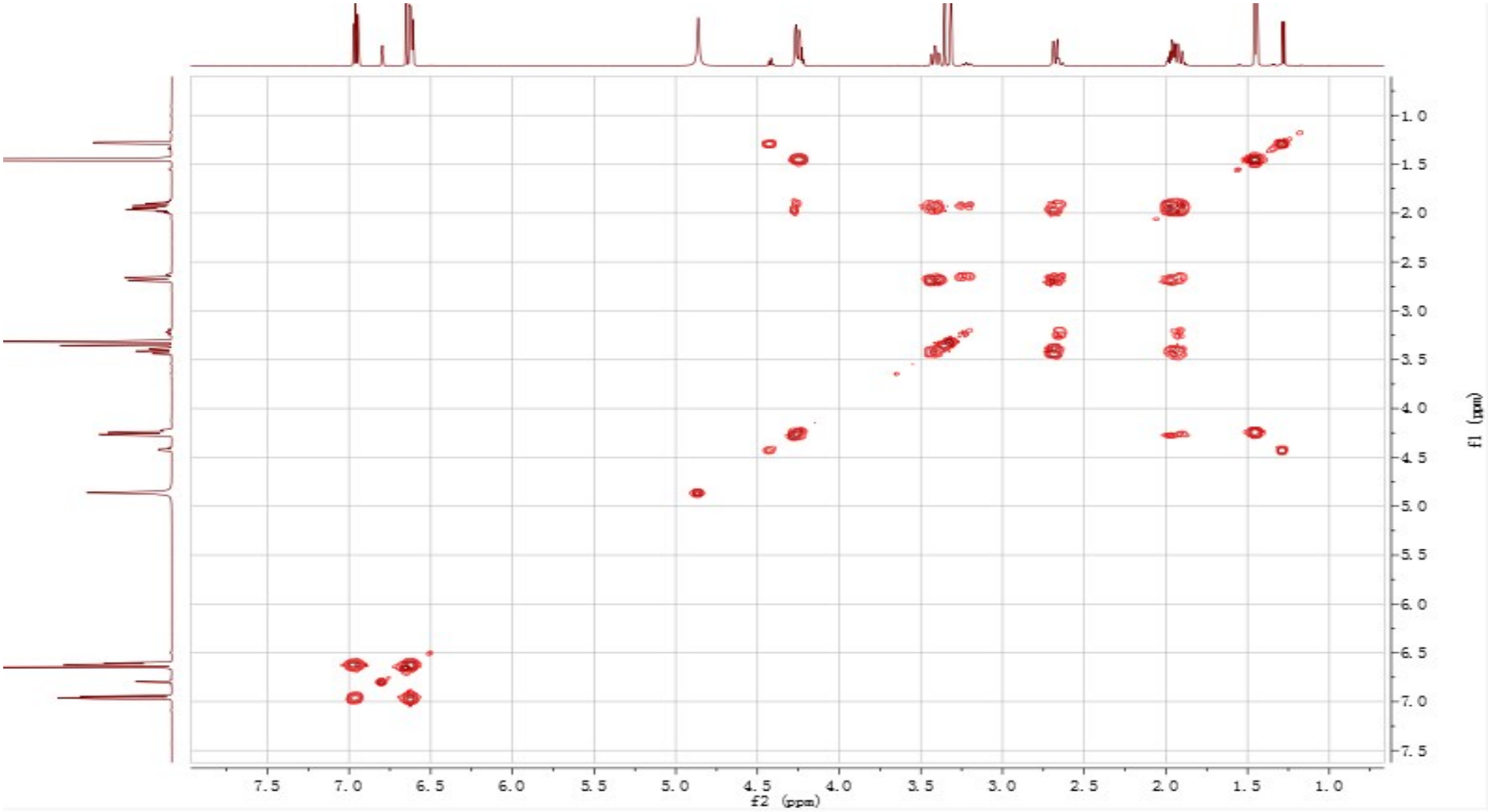


Figure S22. COSY spectrum of the new compound **3**

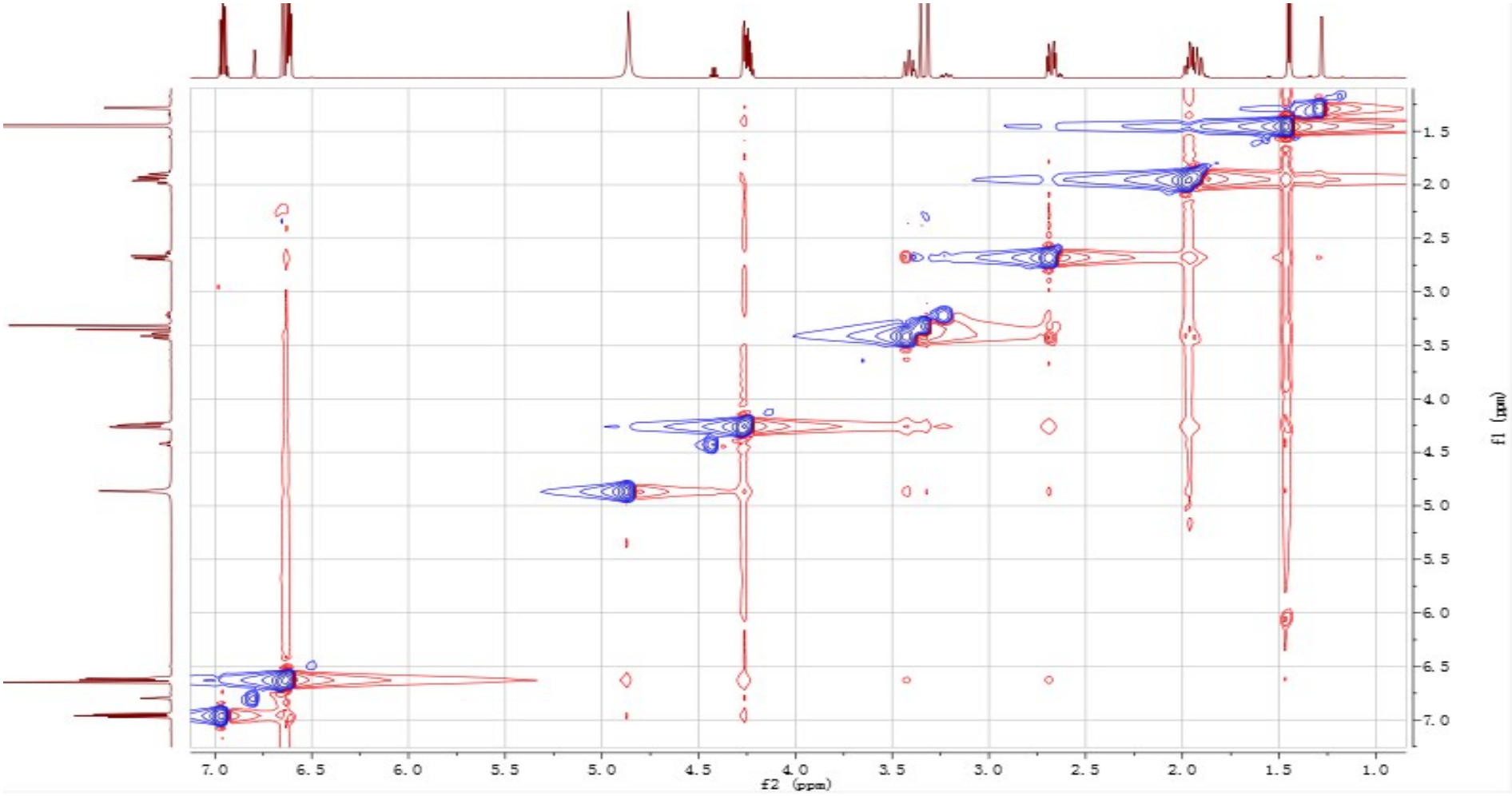


Figure S23. NOESY spectrum of the new compound **3**

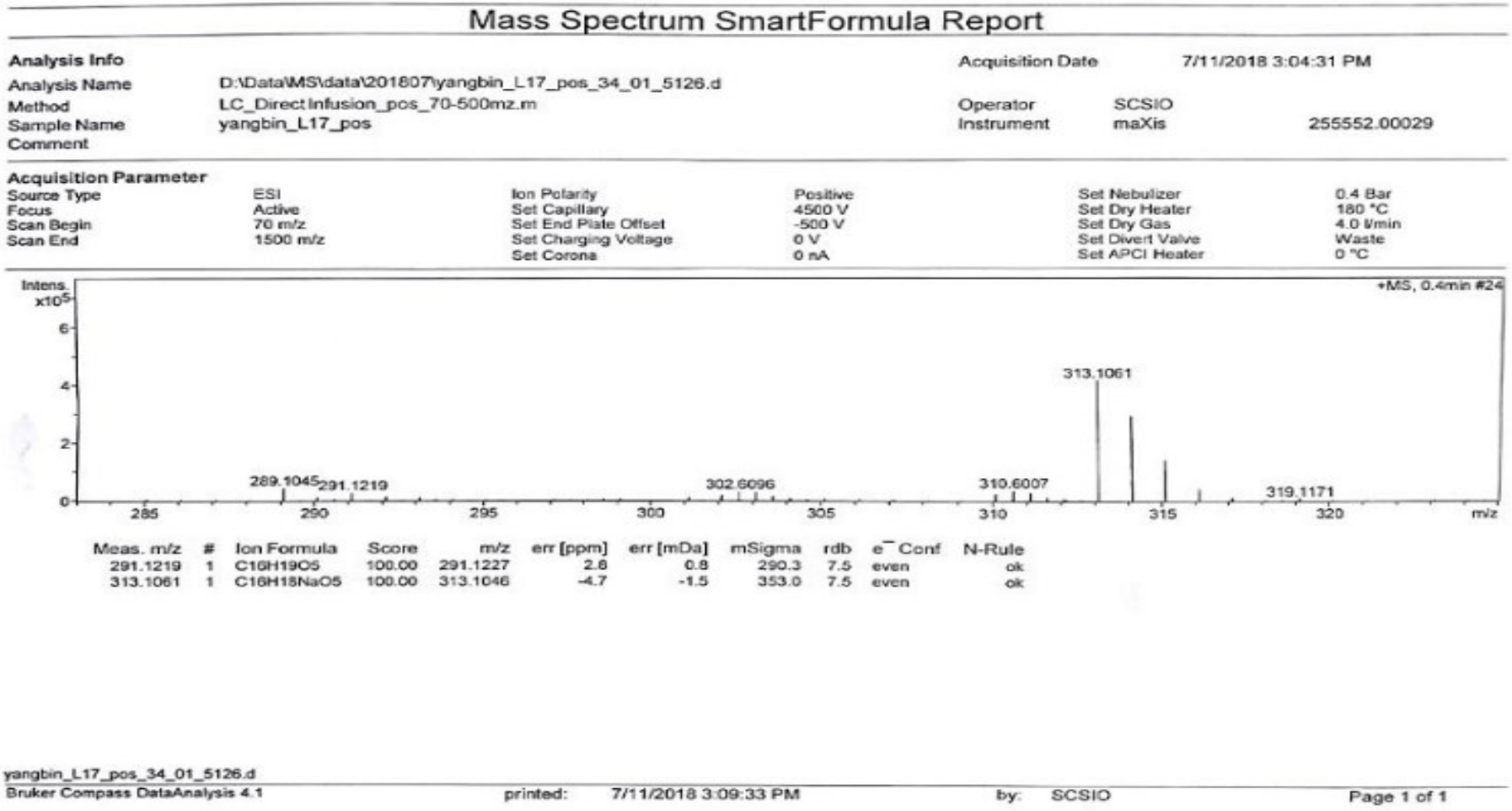


Figure S24. HRESI-MS spectrum of the new compound **4**

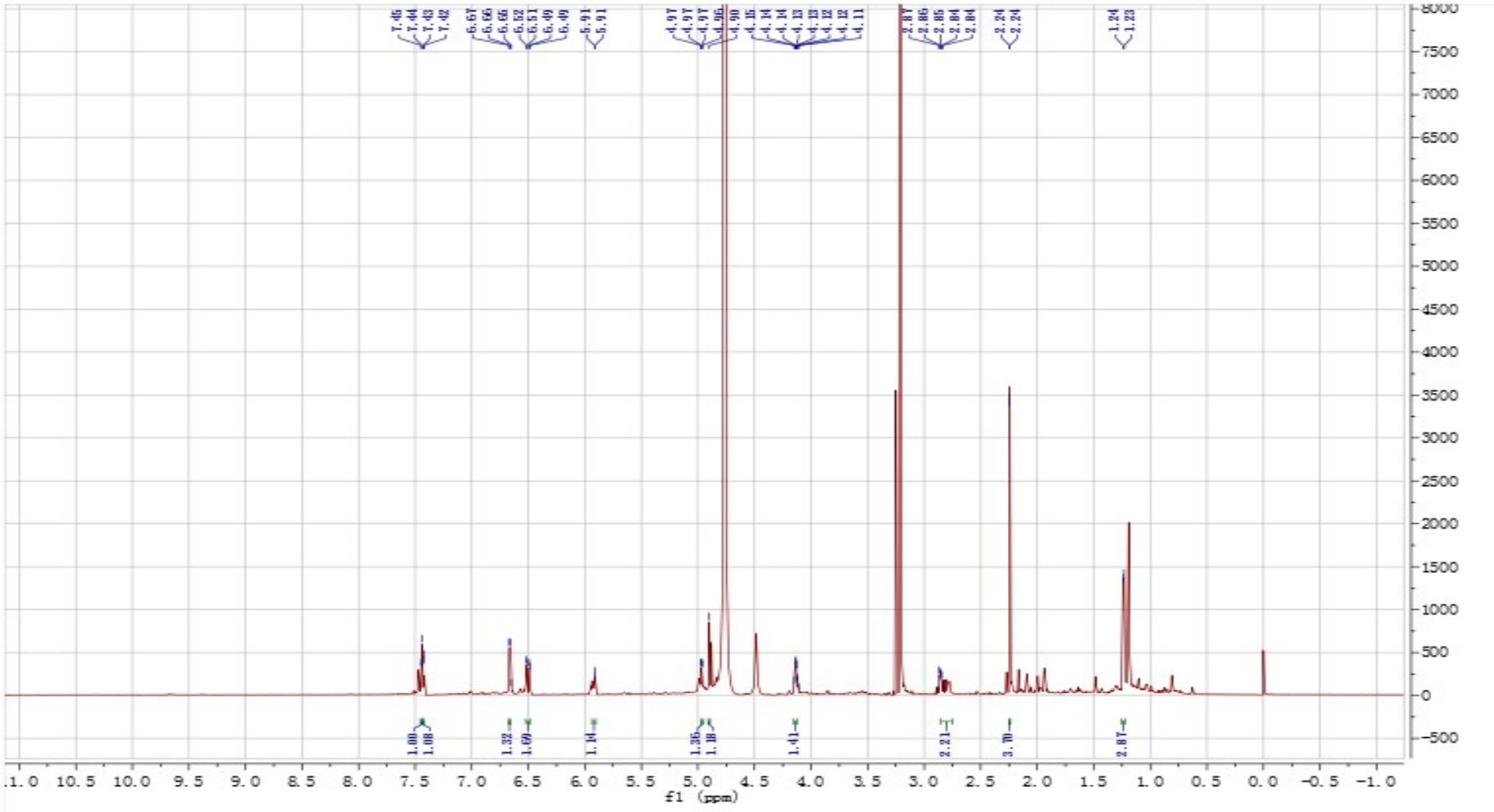


Figure S25. ^{1}H NMR (600 MHz, CD_3OD) spectrum of the new compound **4**

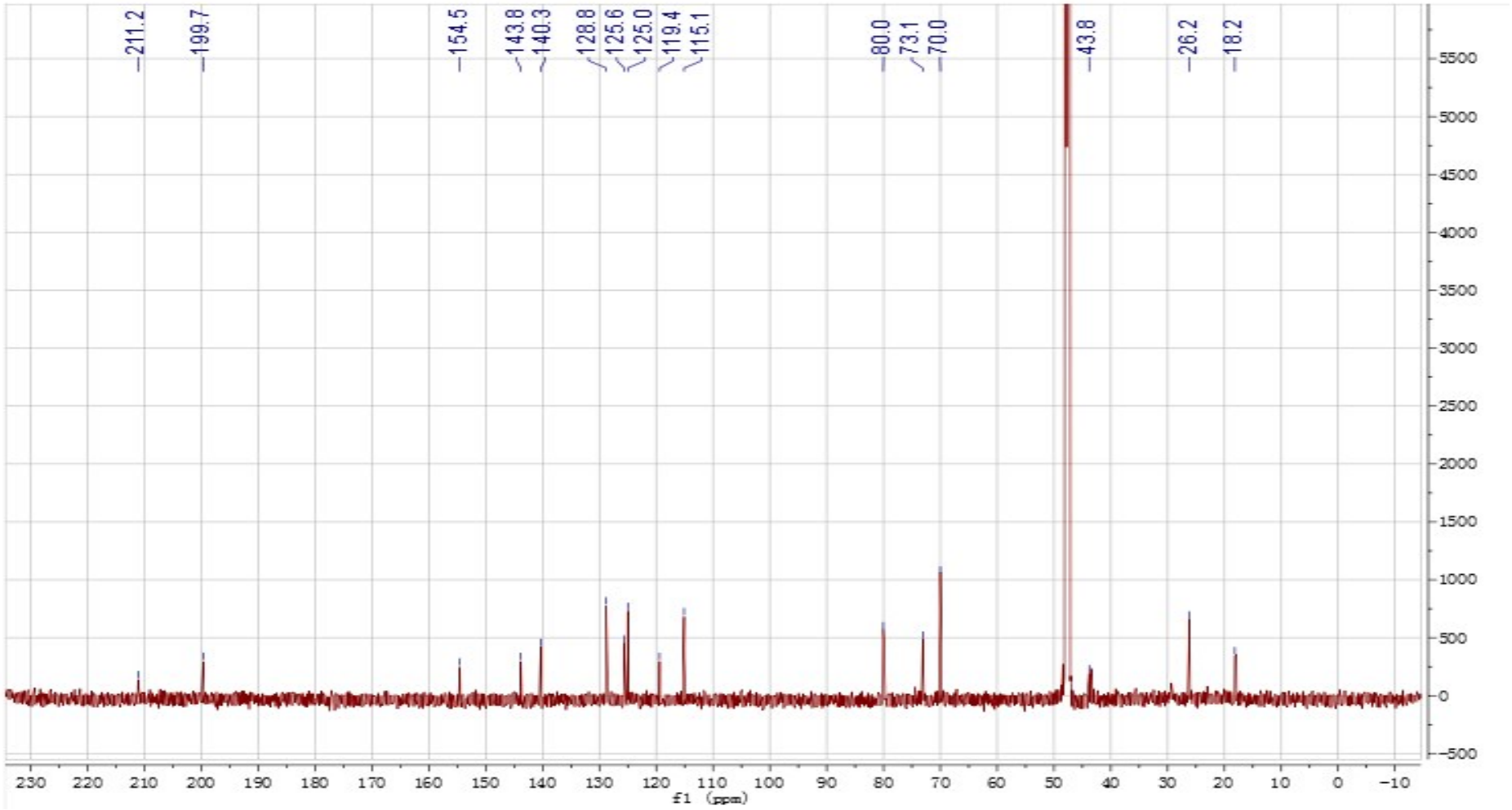


Figure S26. ^{13}C NMR (150 MHz, CD_3OD) spectrum of the new compound **4**

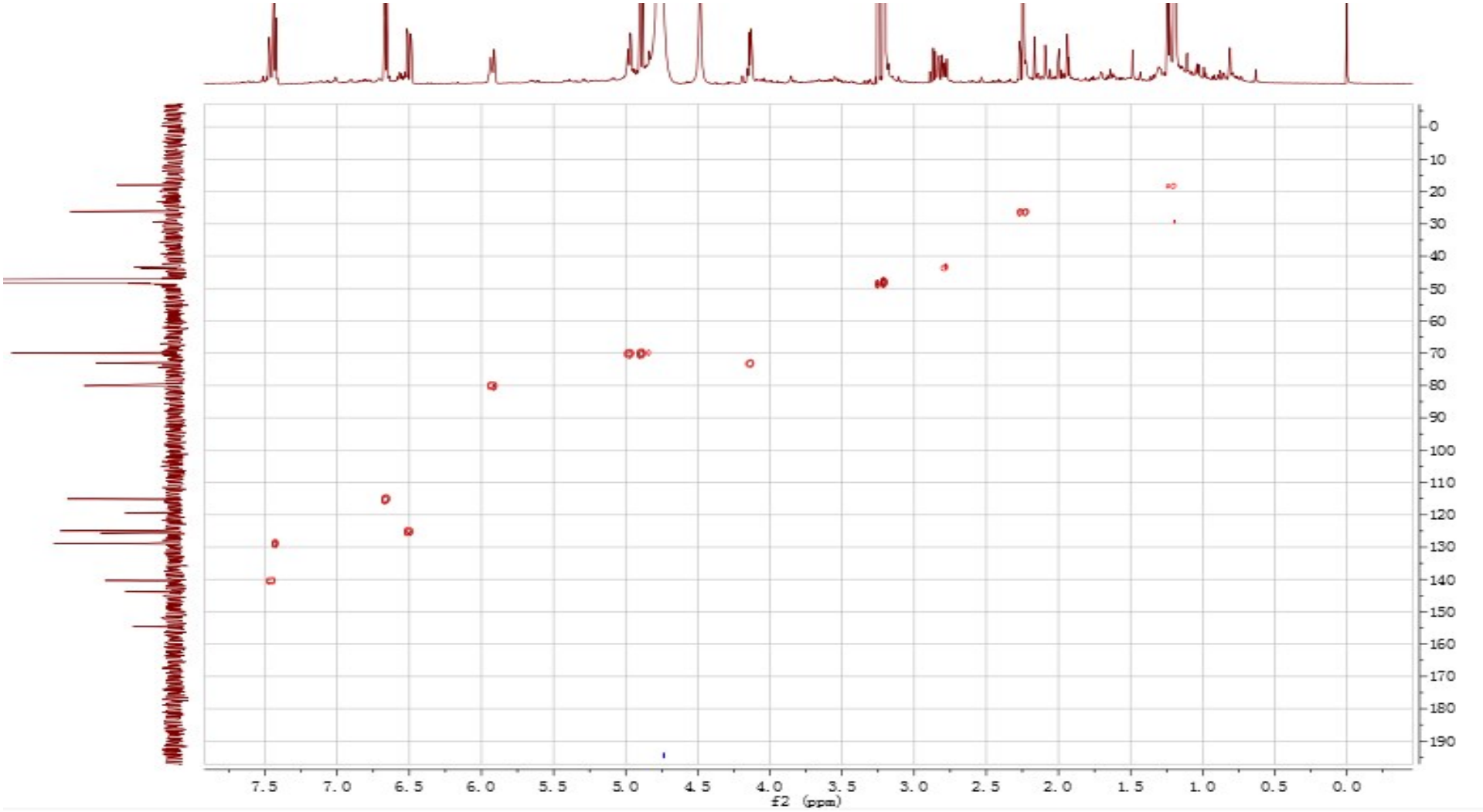


Figure S27. HSQC spectrum of the new compound **4**

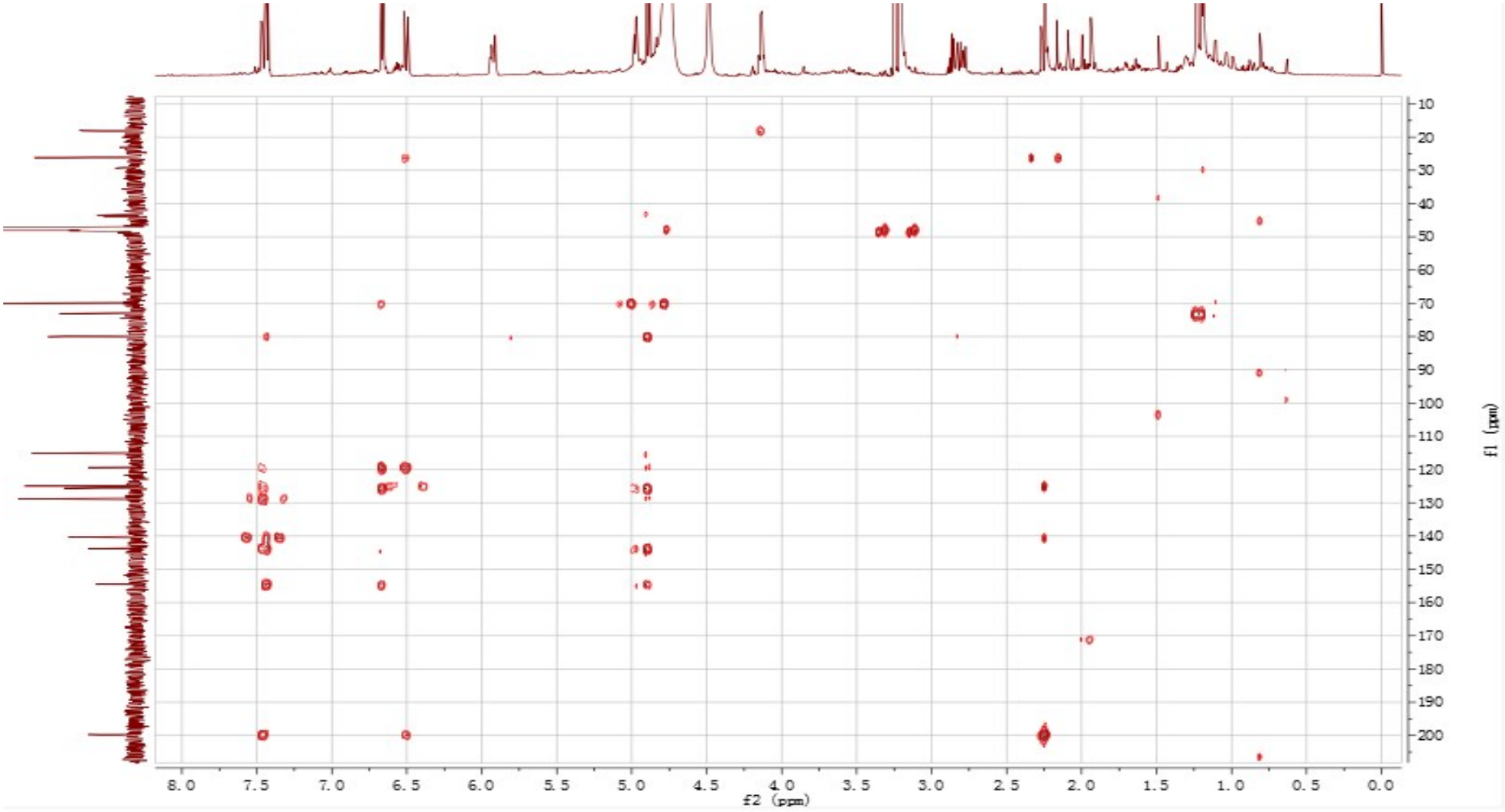


Figure S28. HMBC spectrum of the new compound **4**

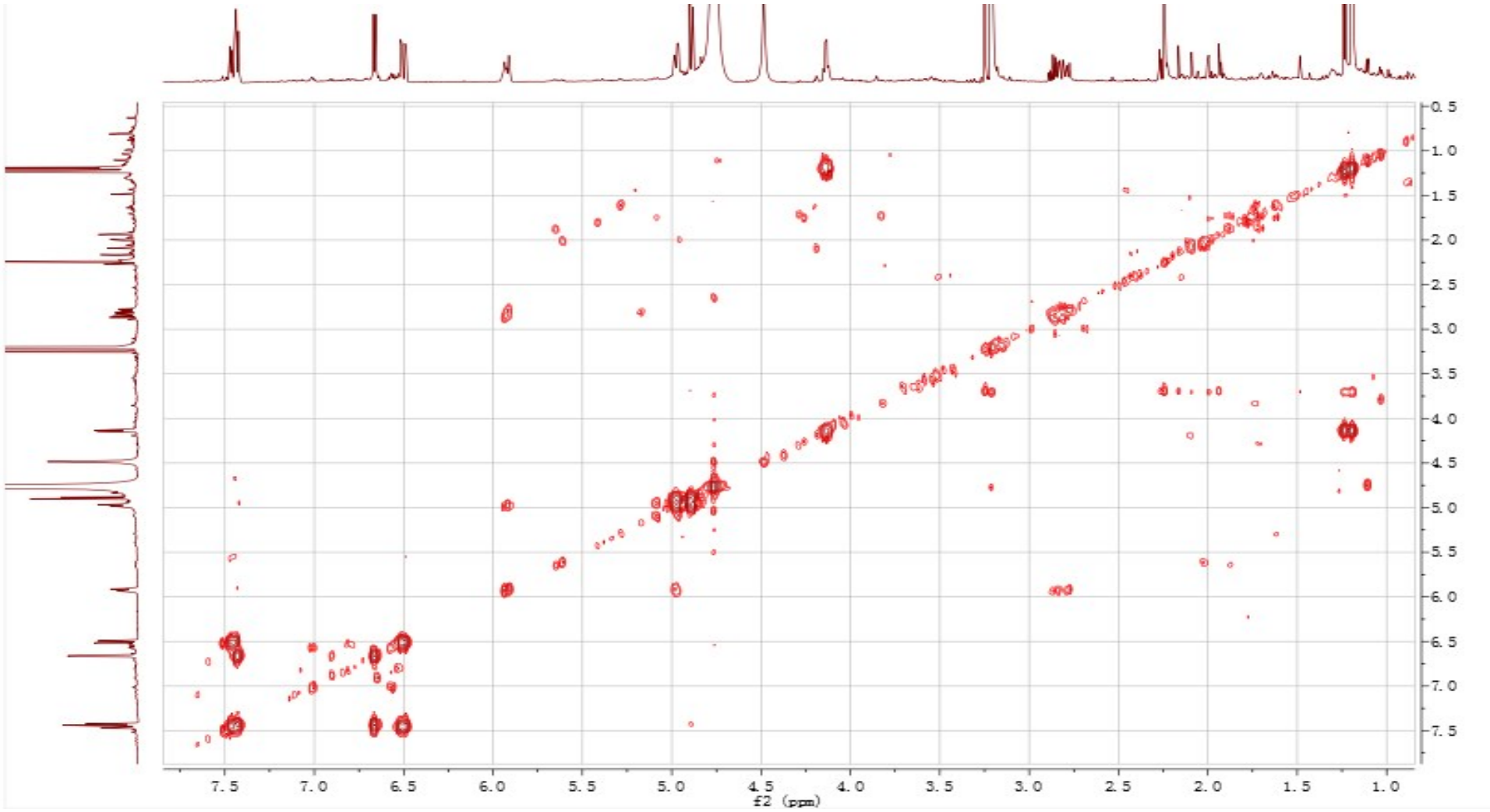


Figure S29. COSY spectrum of the new compound **4**

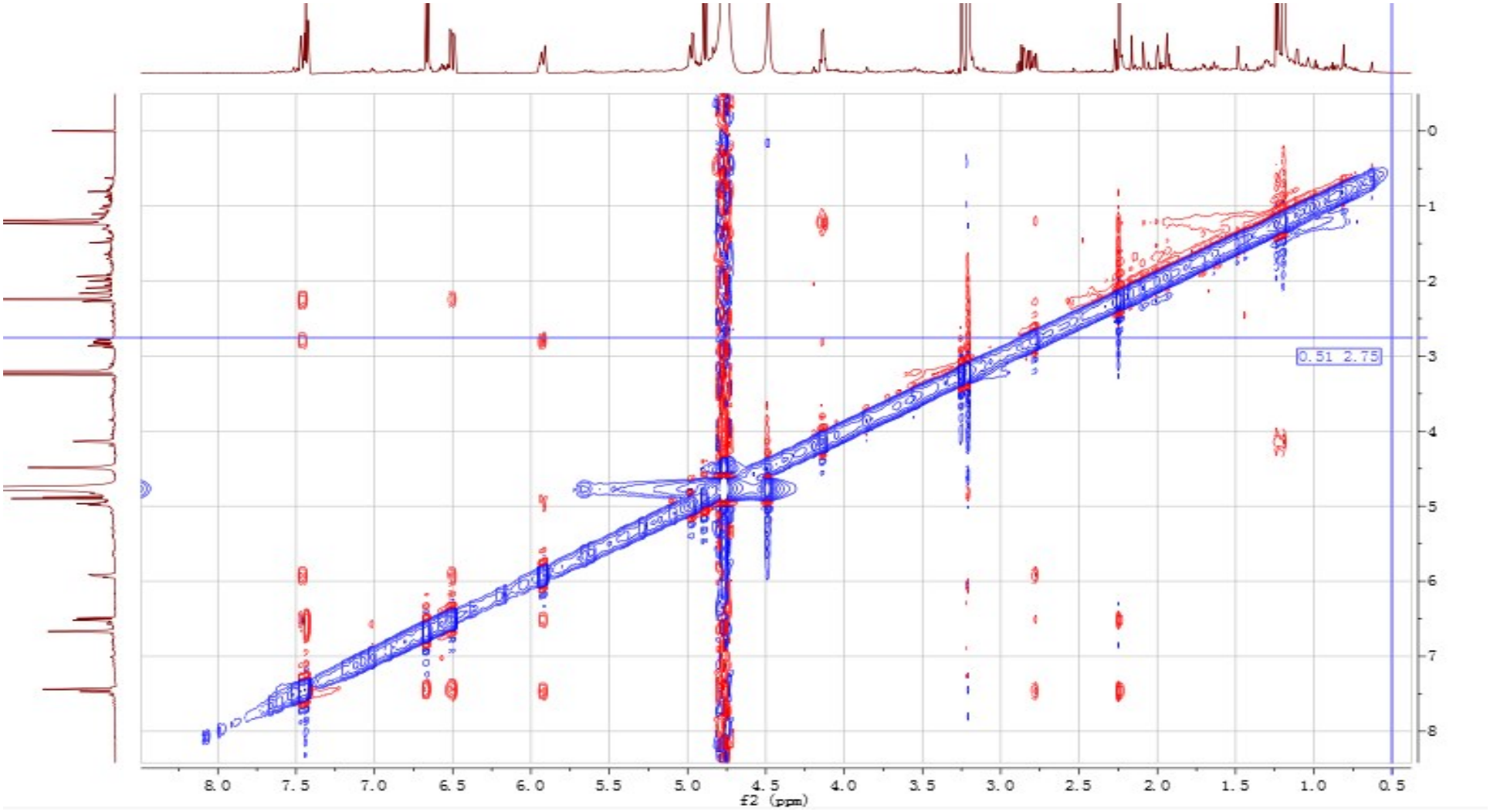


Figure S30. NOESY spectrum of the new compound **4**

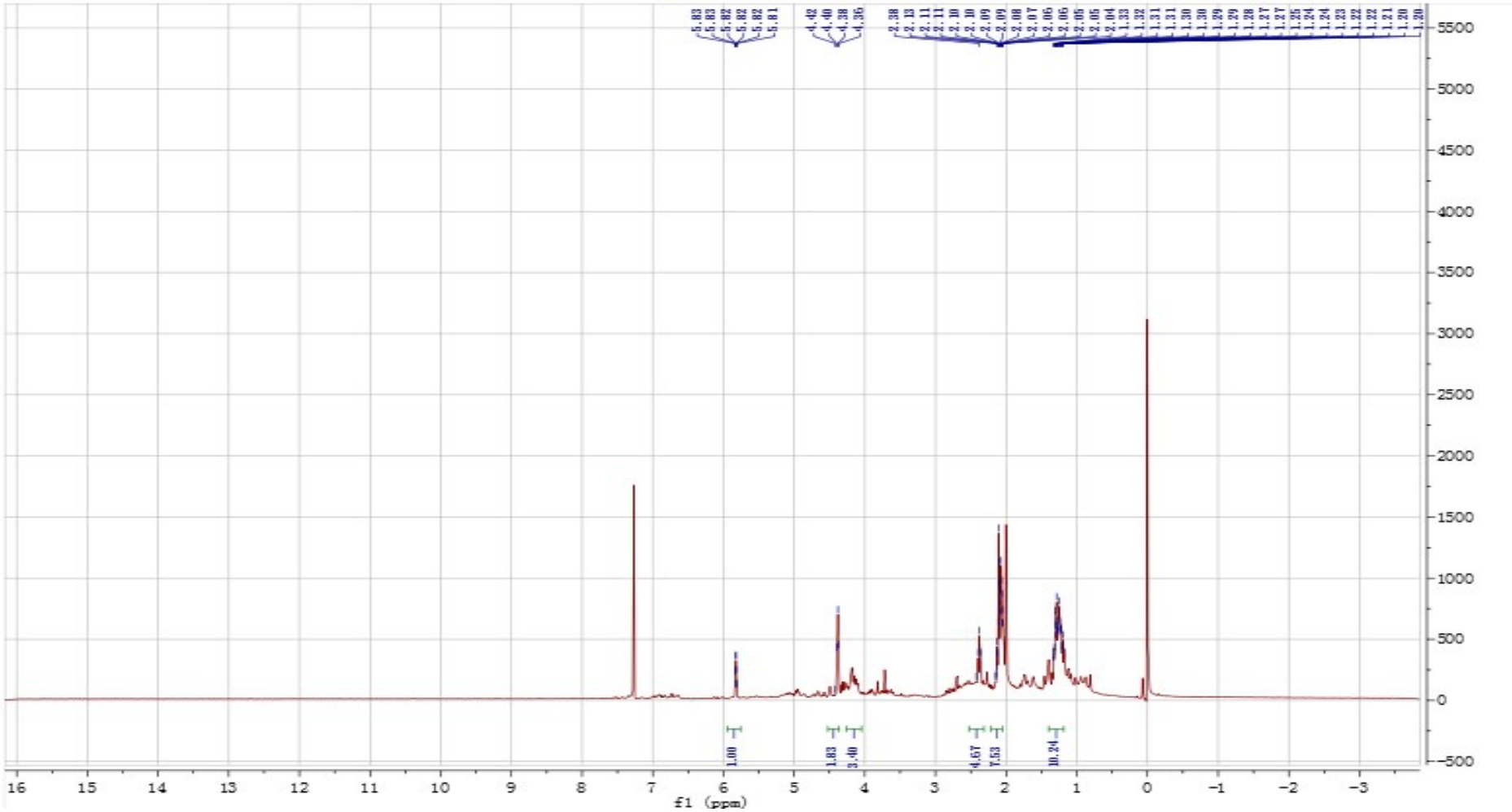


Figure S31. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the compound **5**

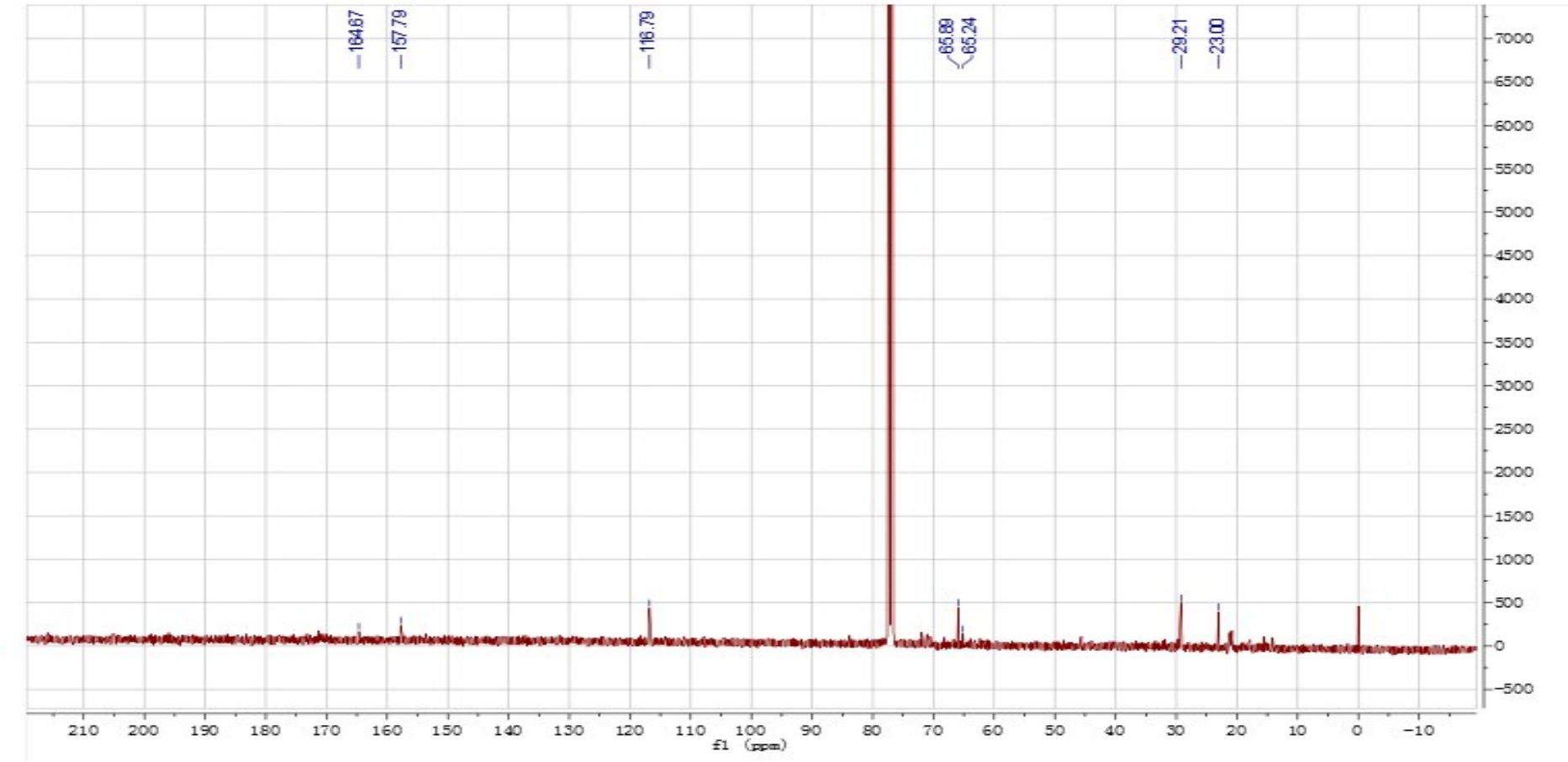


Figure S32. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of the compound **5**

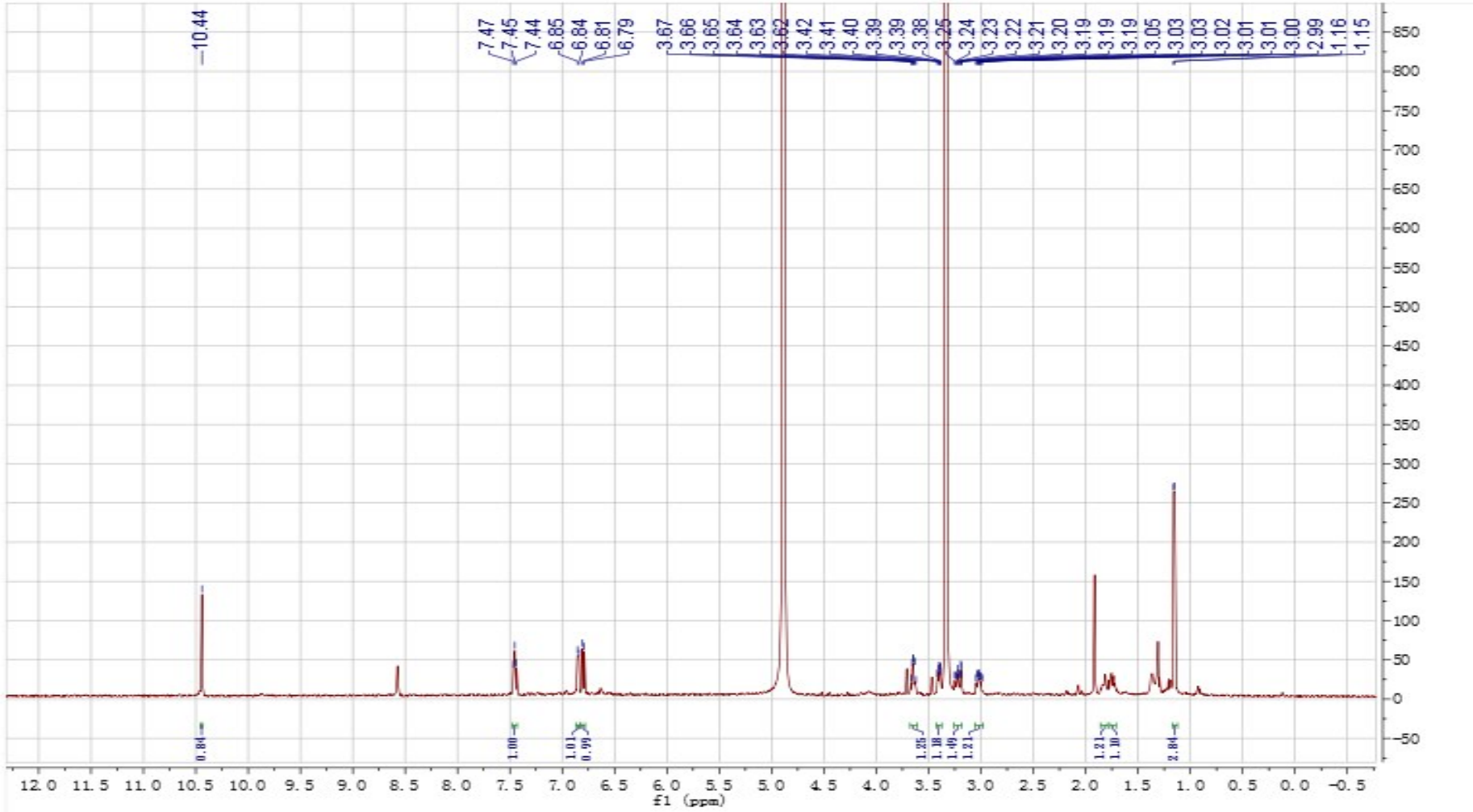


Figure S33. ^{1}H NMR (600 MHz, CD_3OD) spectrum of the compound **6**

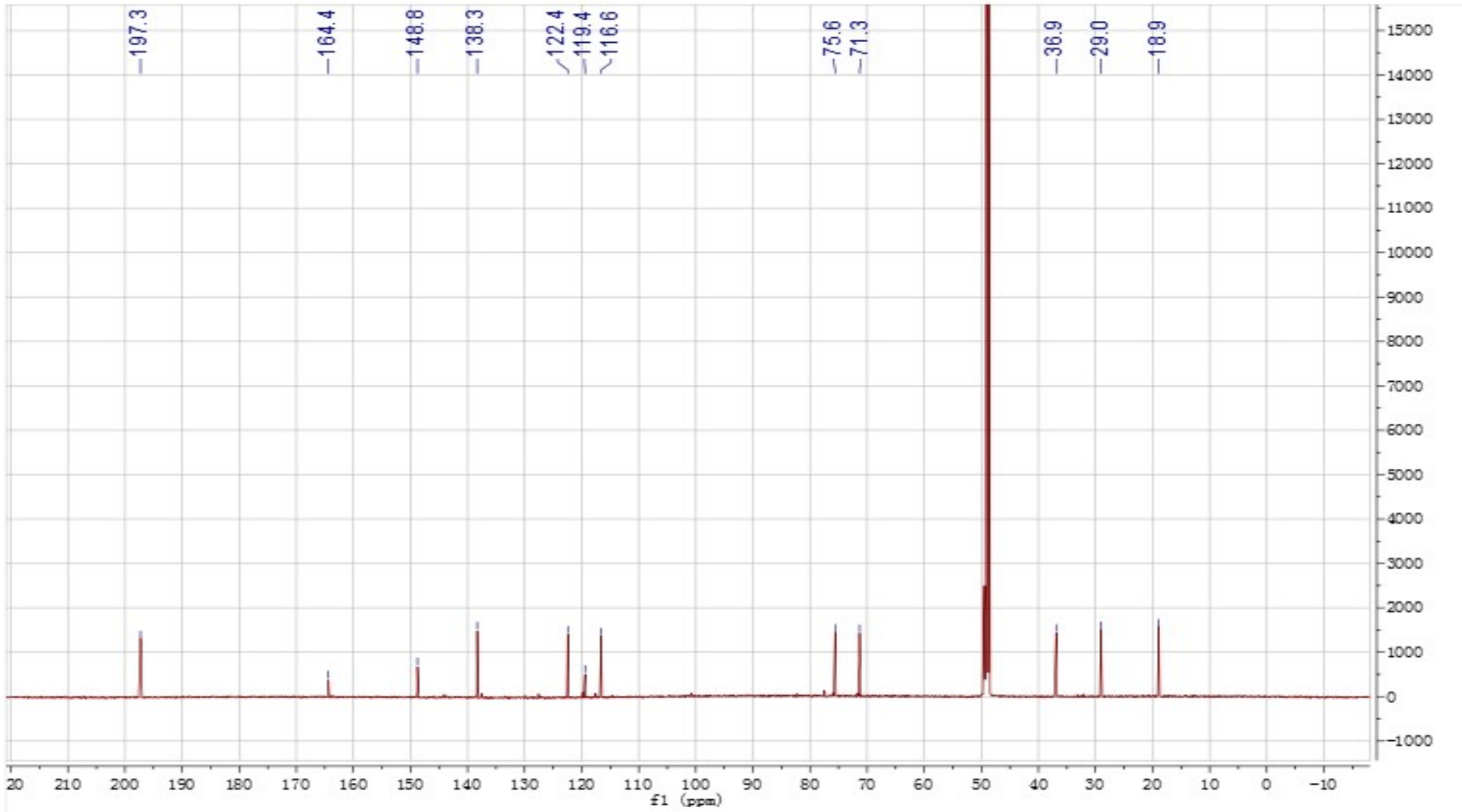


Figure S34. ^{13}C NMR (150 MHz, CD_3OD) spectrum of the compound **6**

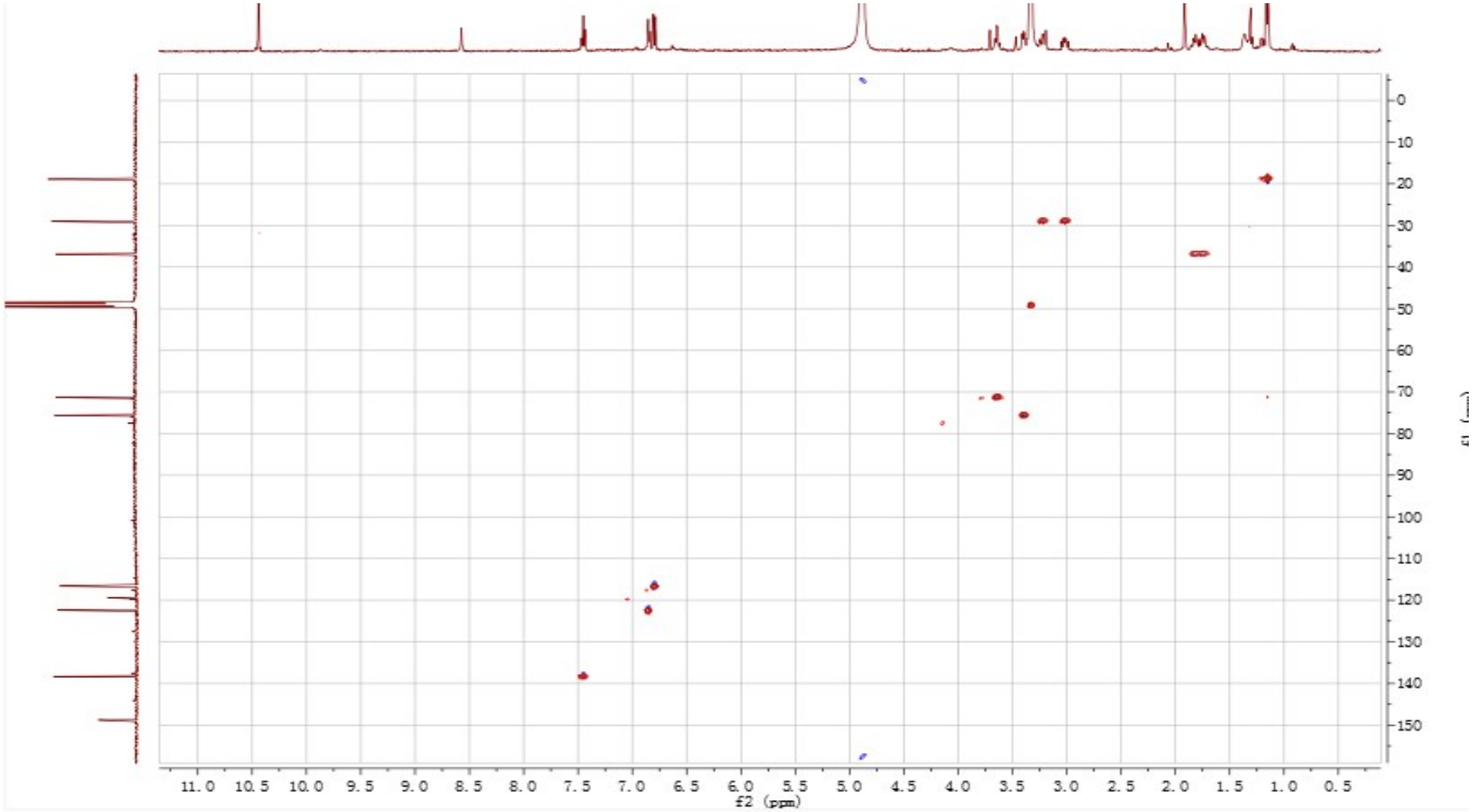


Figure S35. HSQC spectrum of the compound **6**

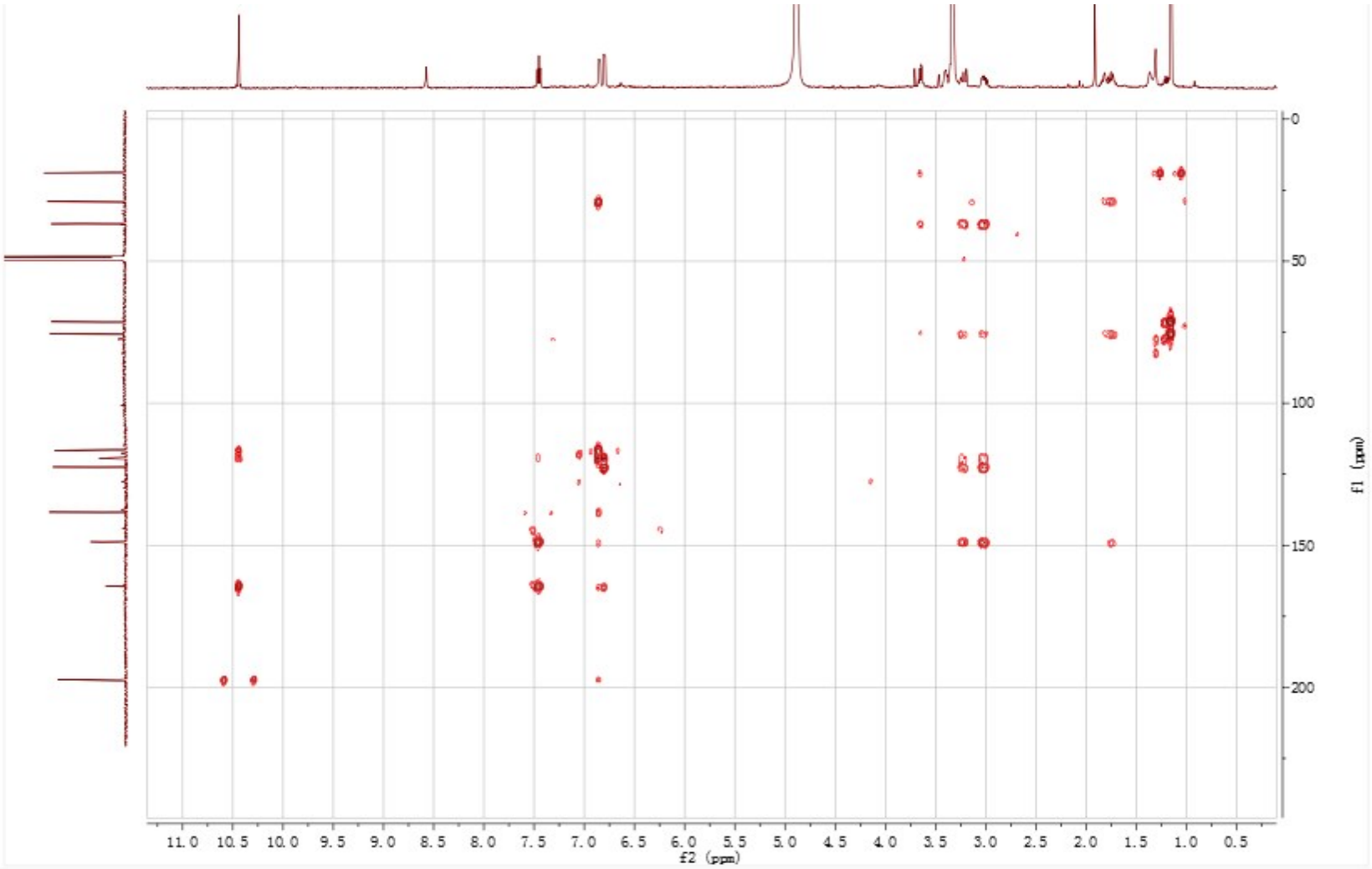


Figure S36. HMBC spectrum of the compound **6**

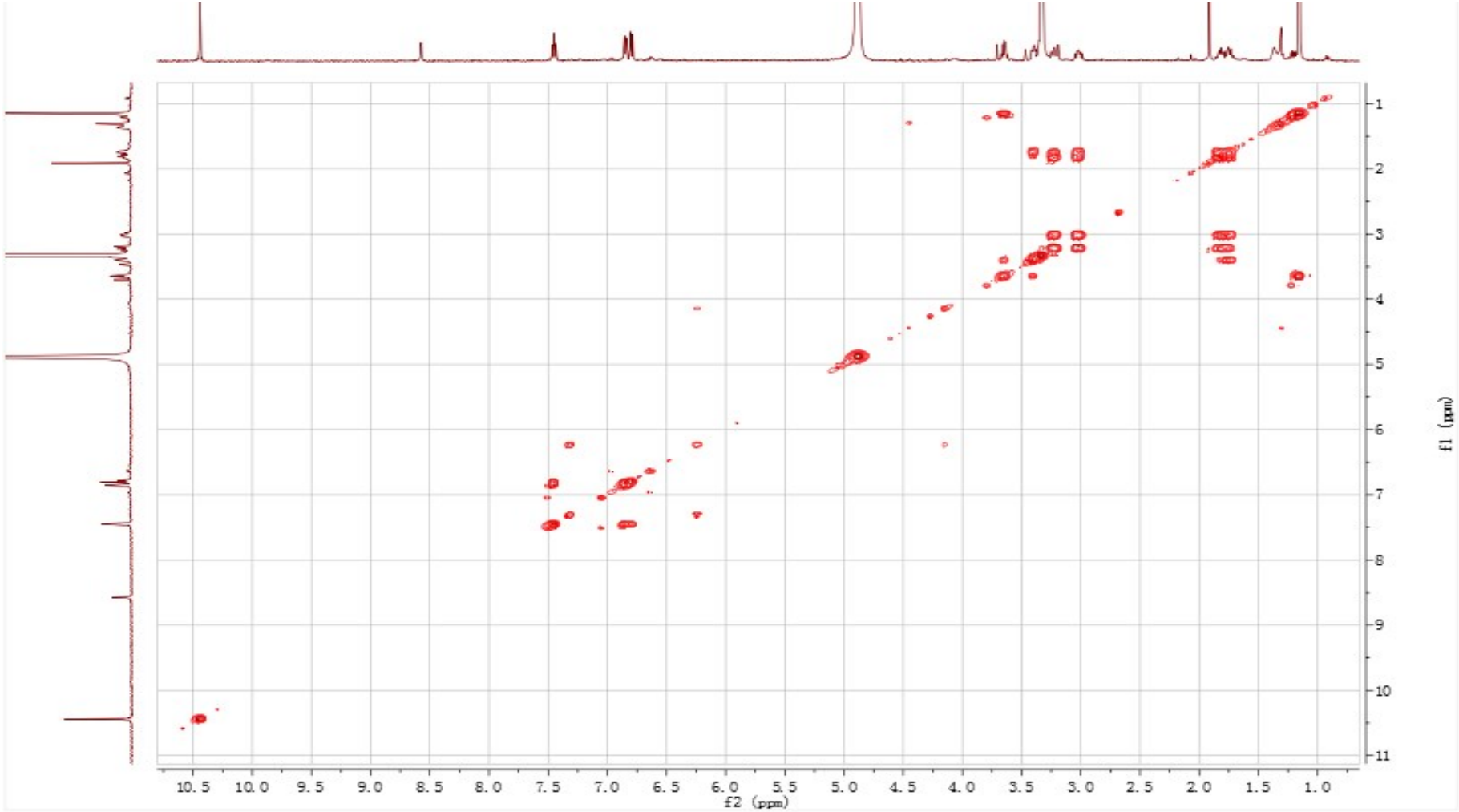


Figure S37. COSY spectrum of the compound **6**

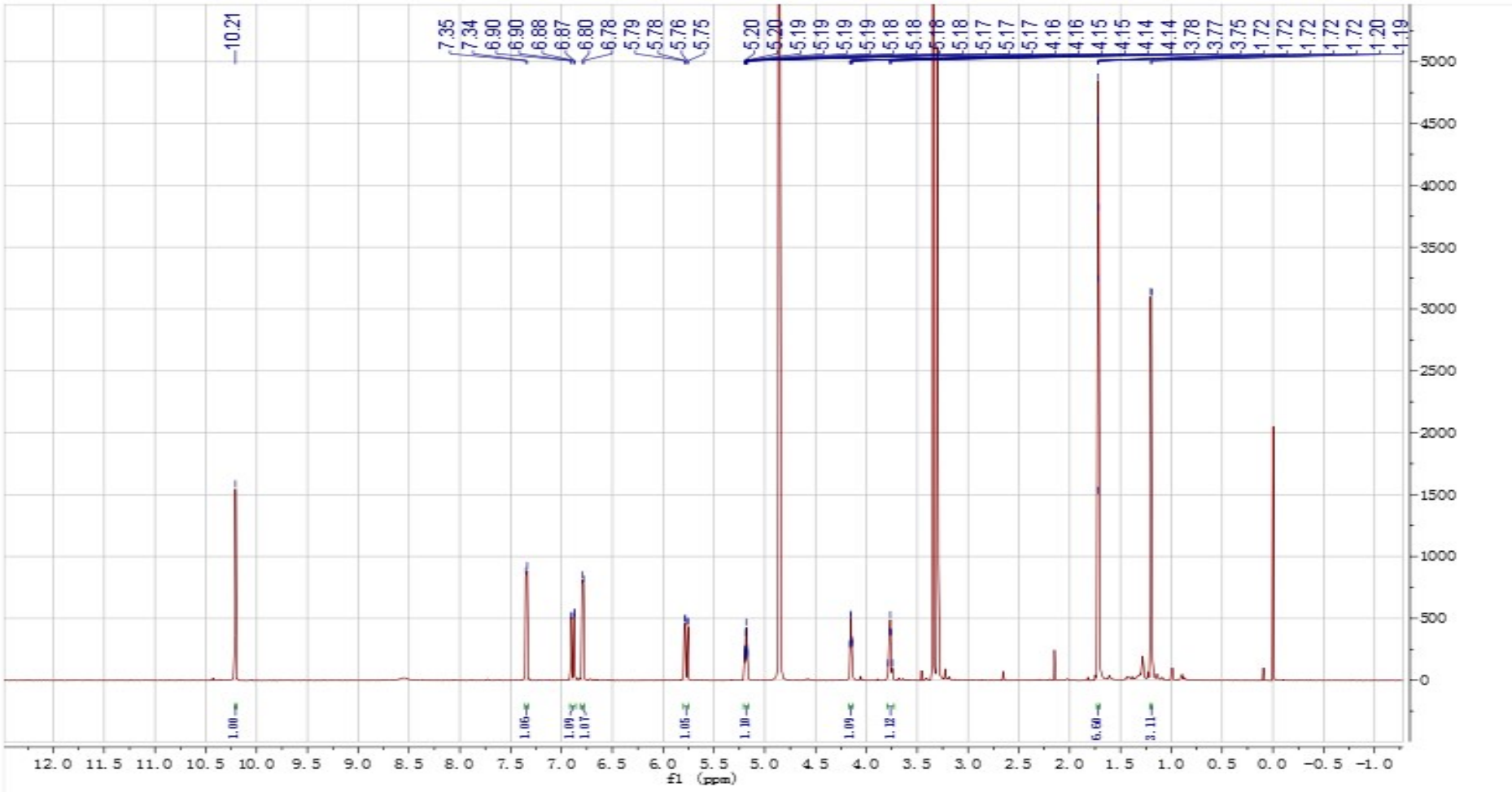


Figure S38. ^{1}H NMR (600 MHz, CD_3OD) spectrum of the compound **7**

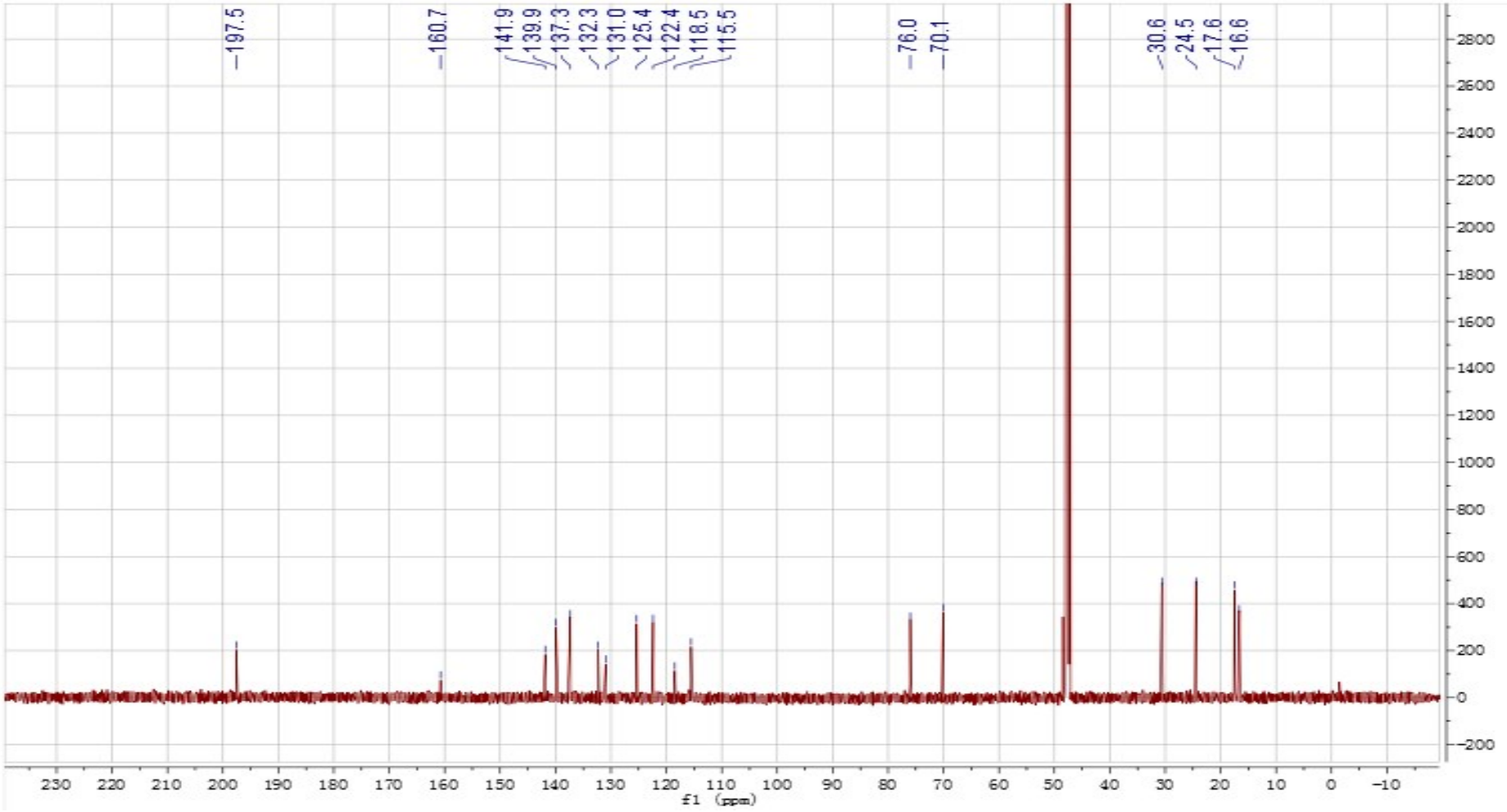


Figure S39. ^{13}C NMR (150 MHz, CD_3OD) spectrum of the compound **7**

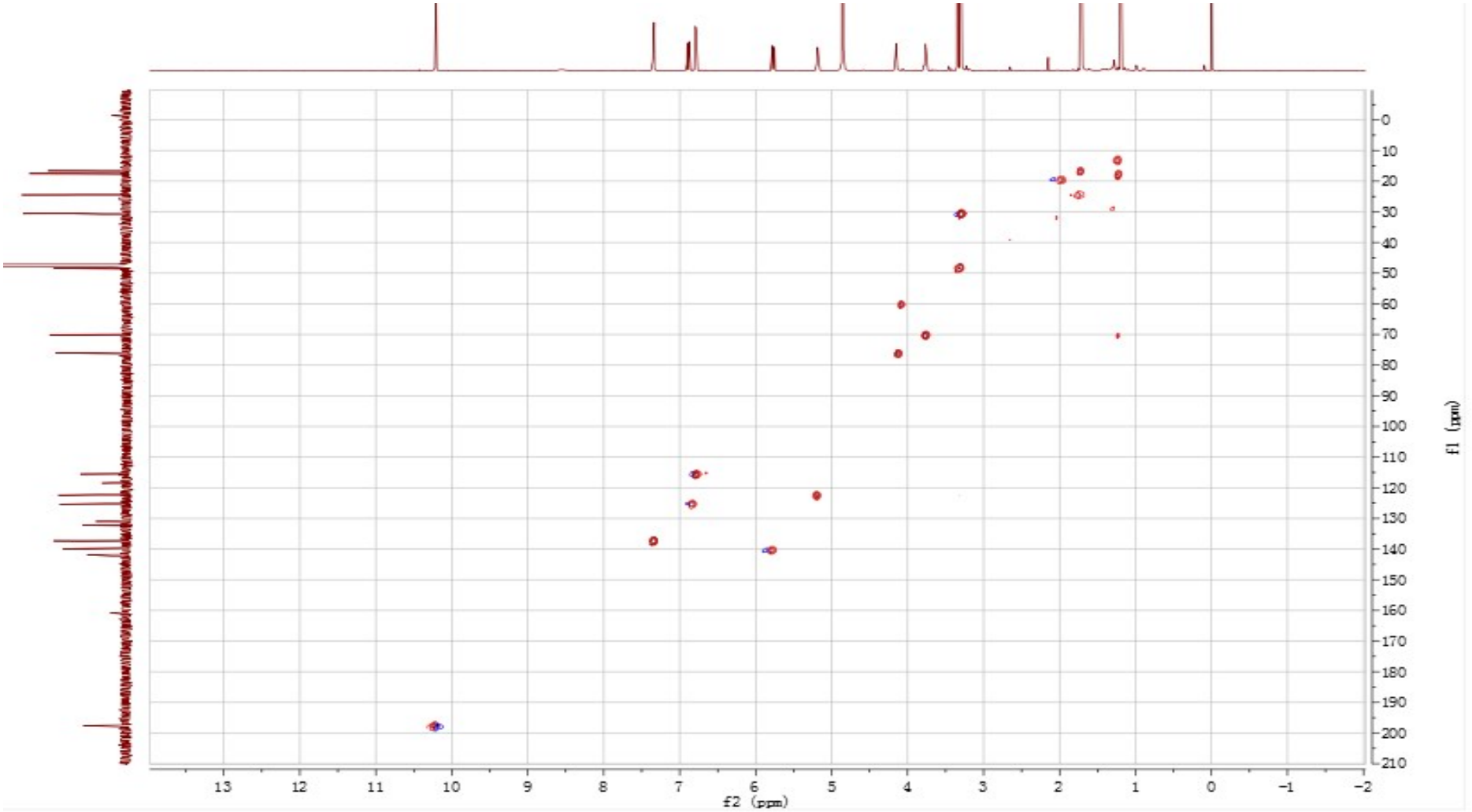


Figure S40. HSQC spectrum of the compound **7**

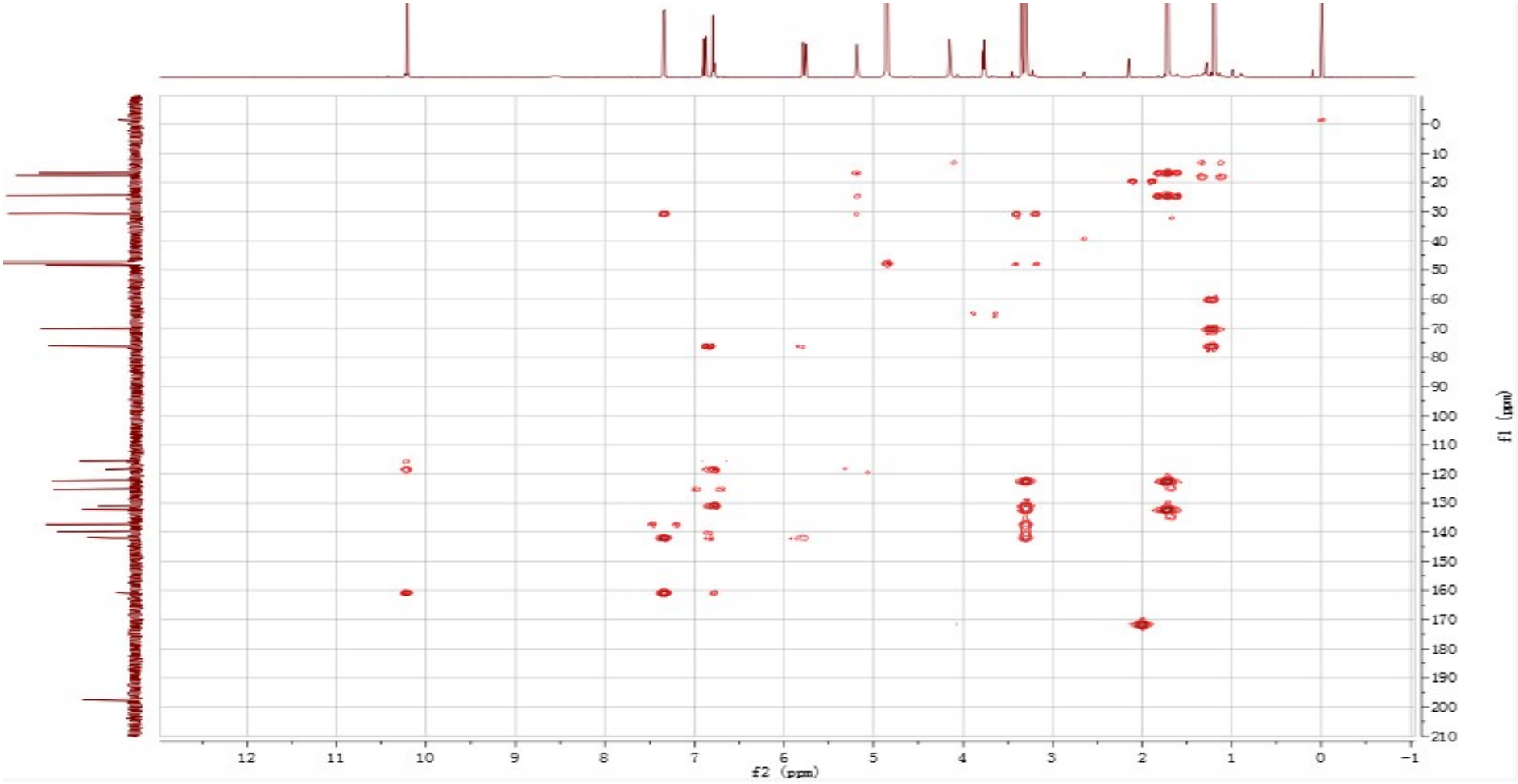


Figure S41. HMBC spectrum of the compound **7**

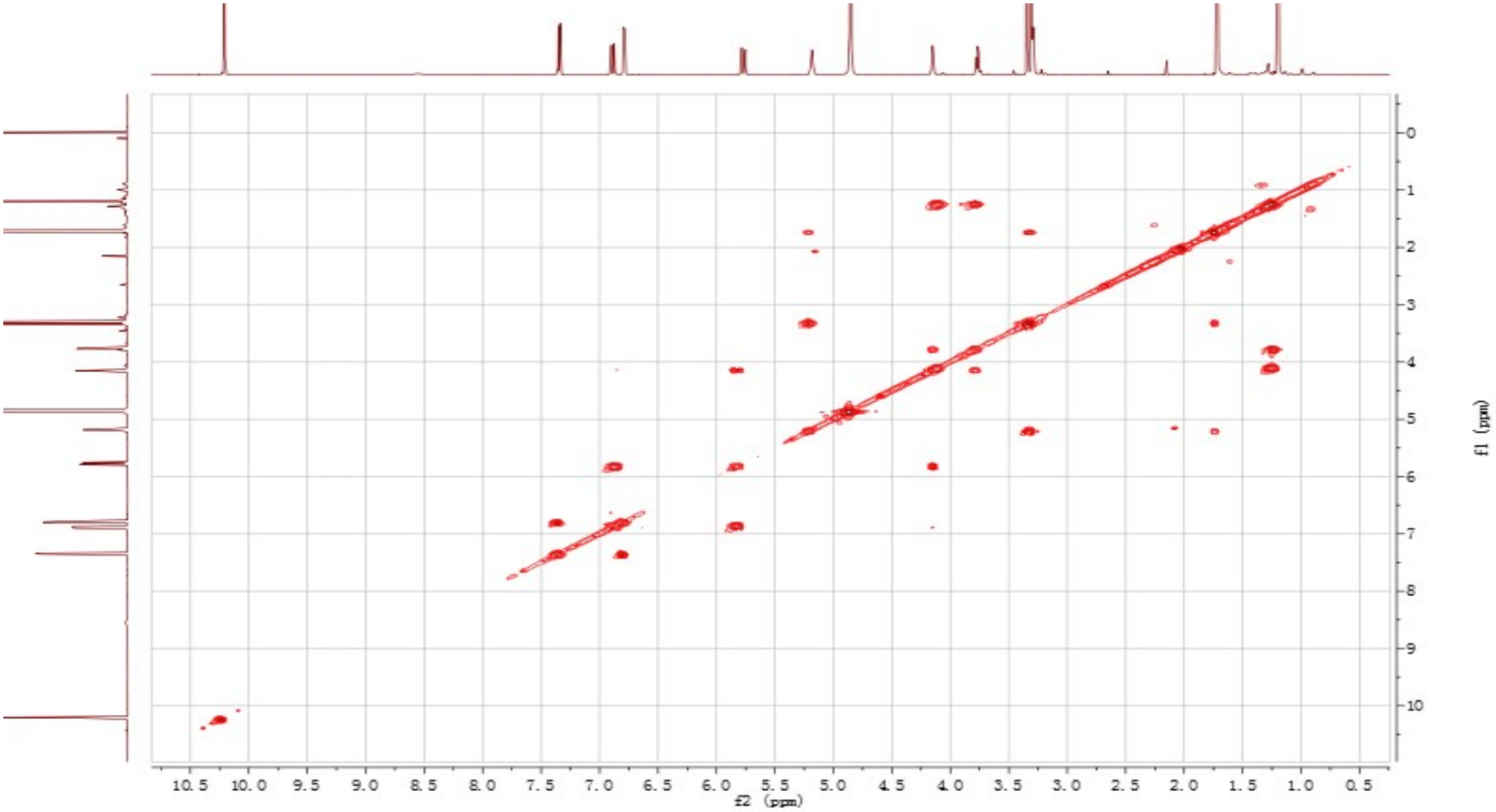


Figure S42. COSY spectrum of the compound **7**

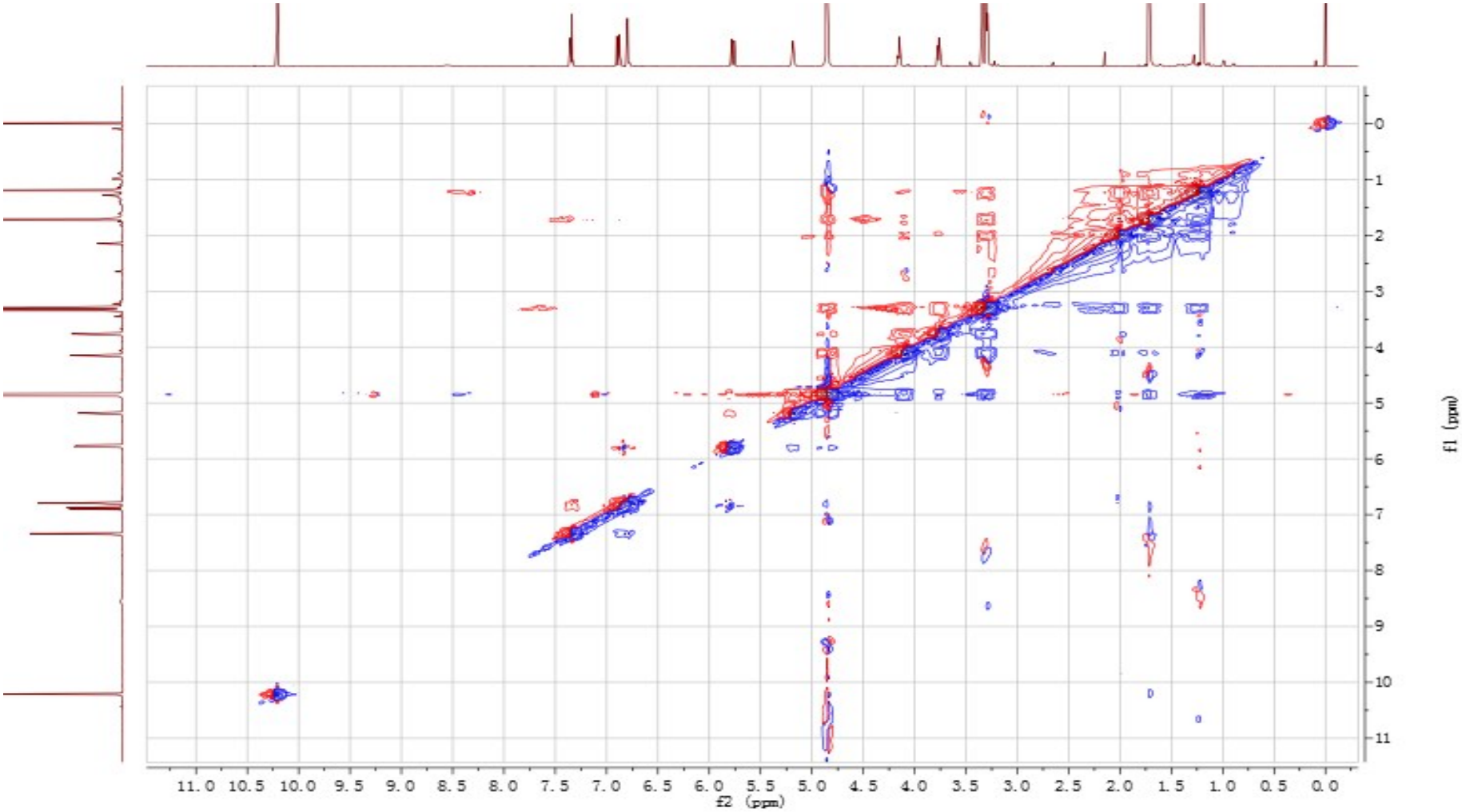


Figure S43. NOESY spectrum of the compound **7**

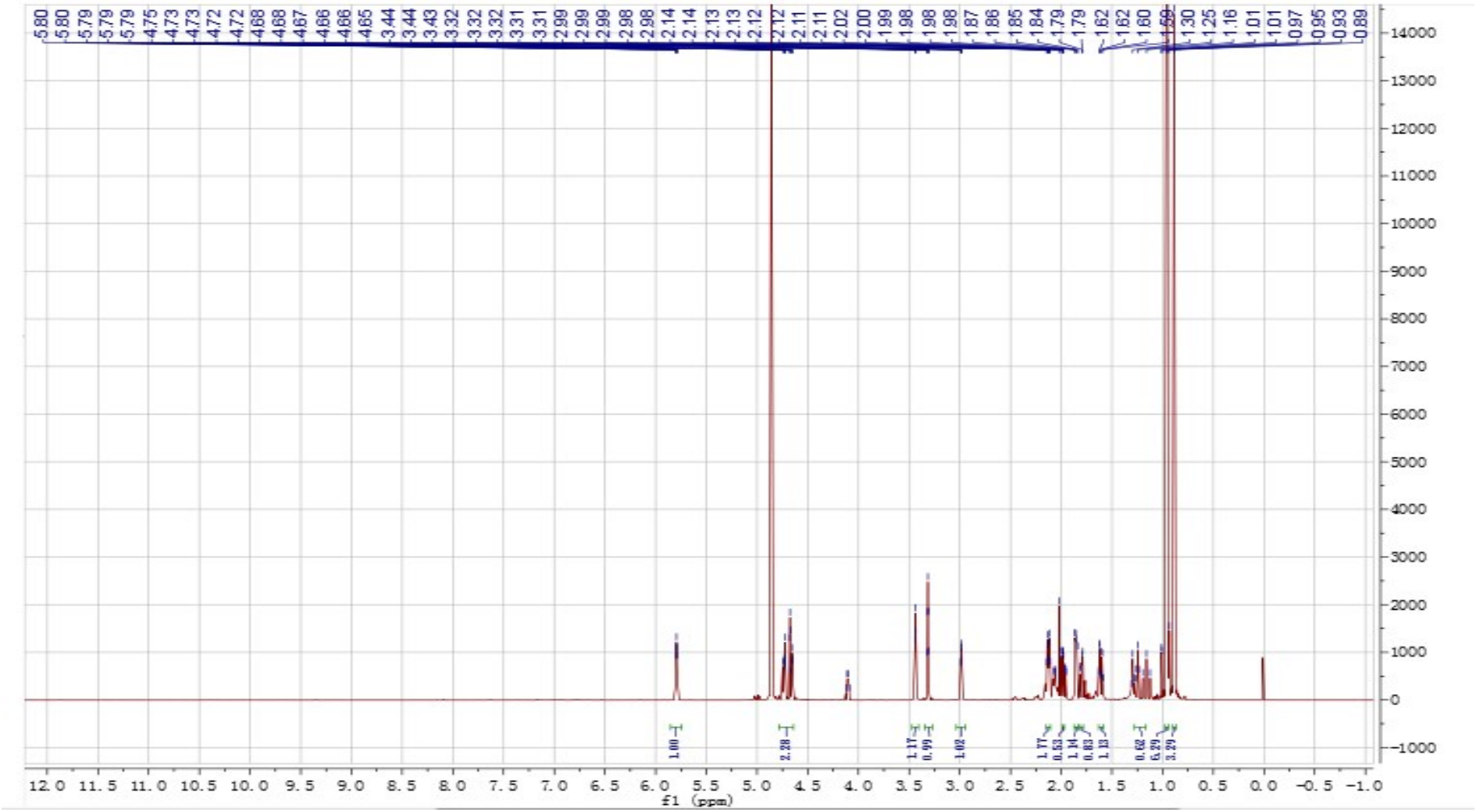


Figure S44. ^{1}H NMR (600 MHz, CD_3OD) spectrum of the compound **8**

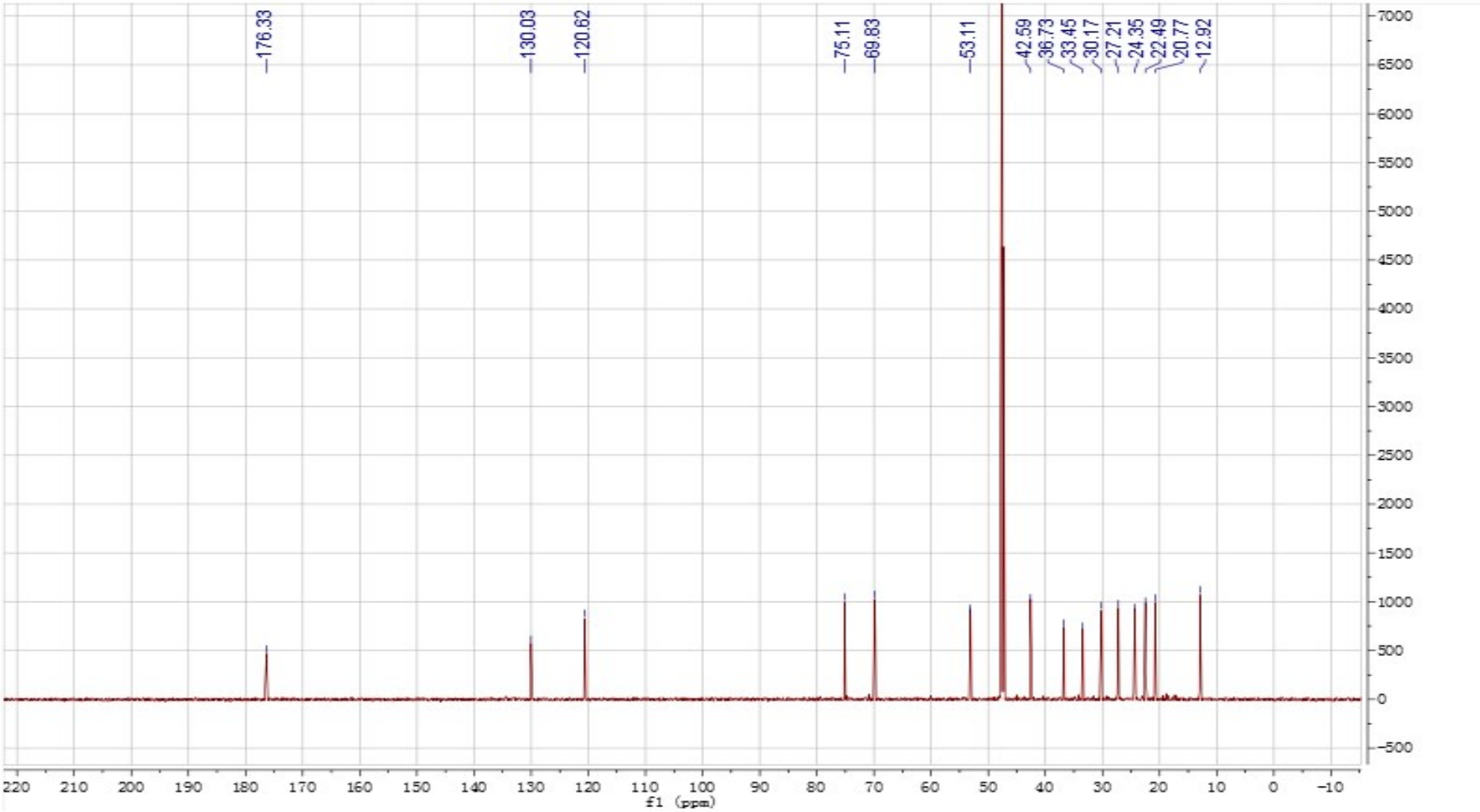


Figure S45. ^{13}C NMR (150 MHz, CD_3OD) spectrum of the compound **8**

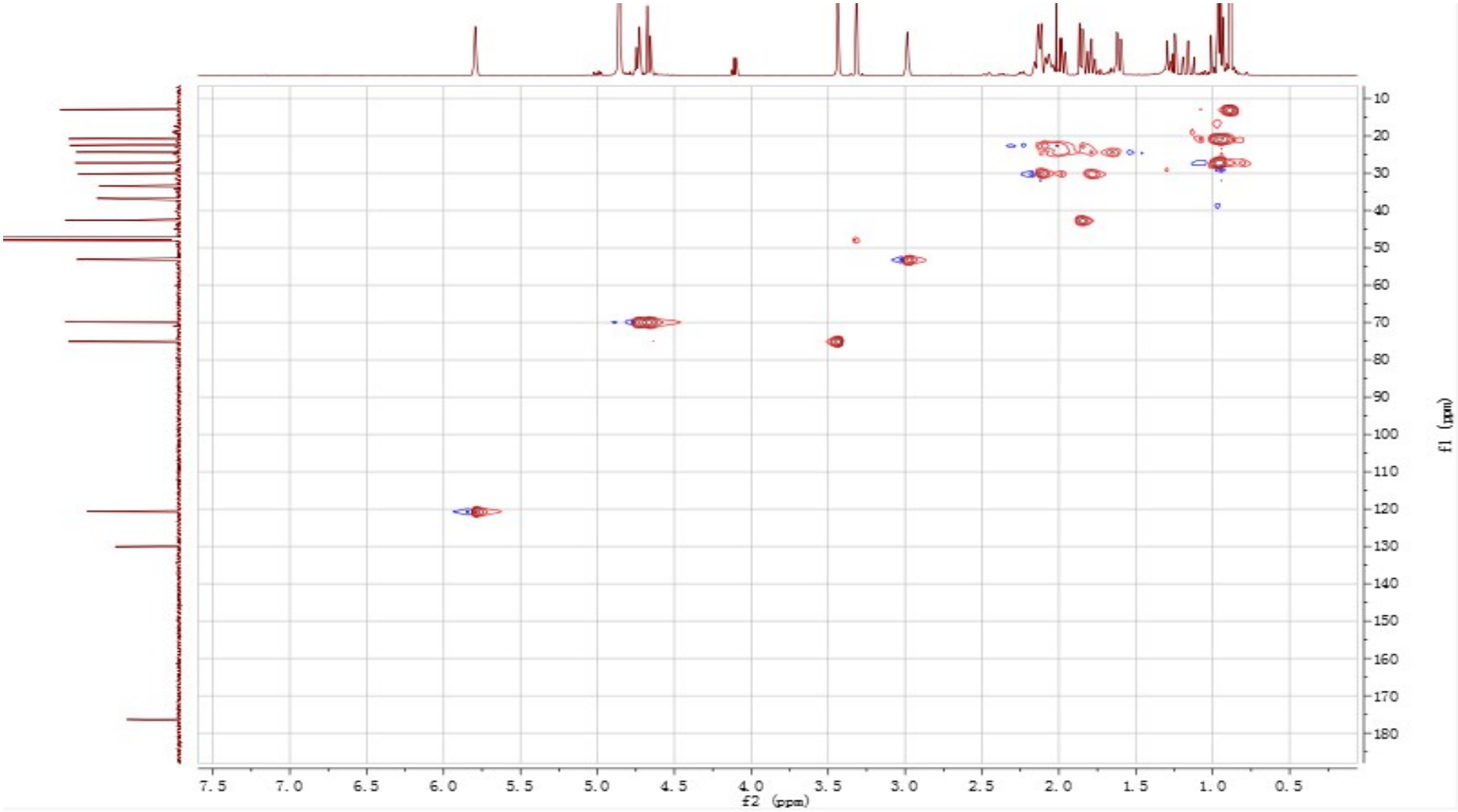


Figure S46. HSQC spectrum of the compound **8**

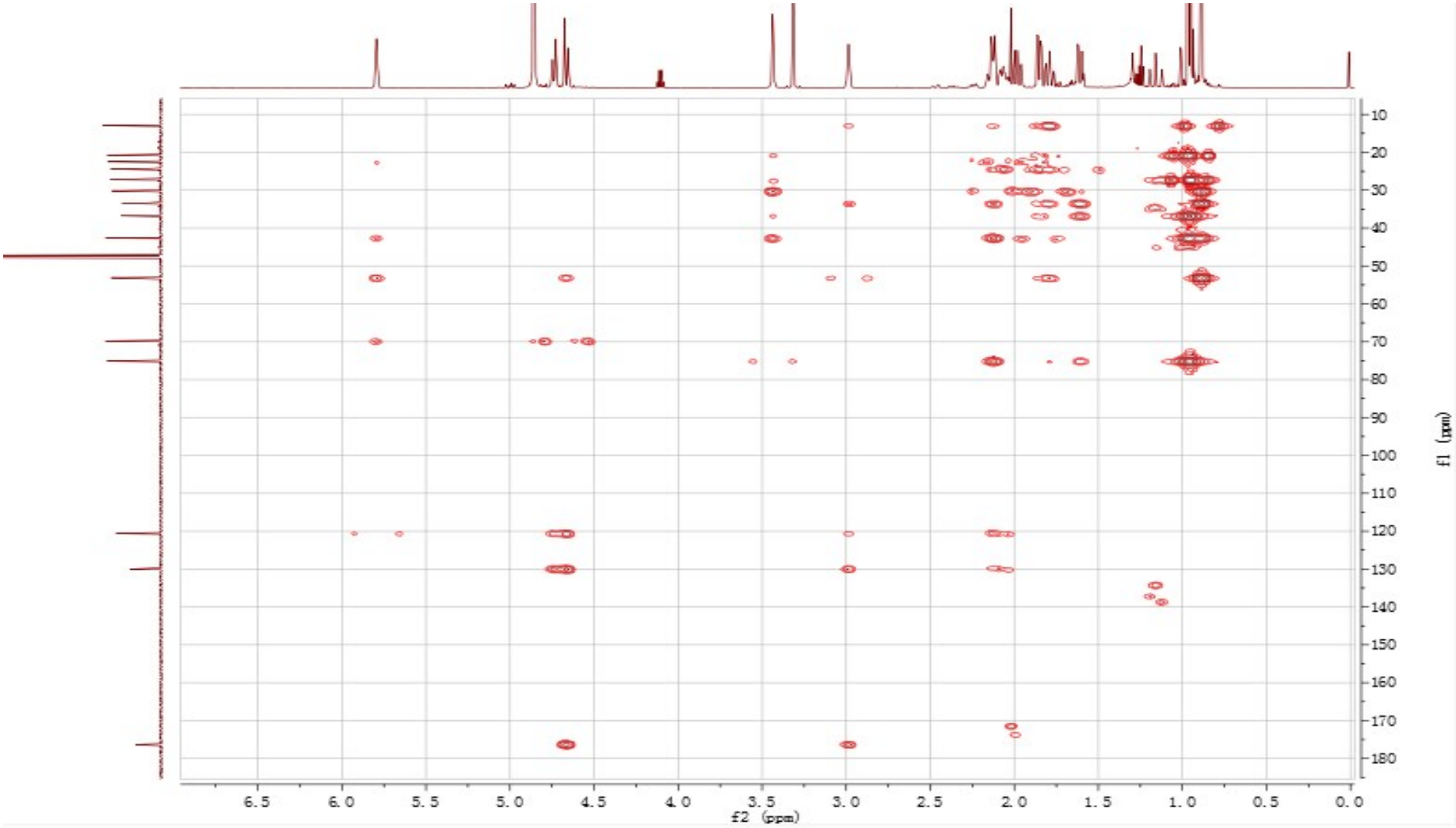


Figure S47. HMBC spectrum of the compound **8**

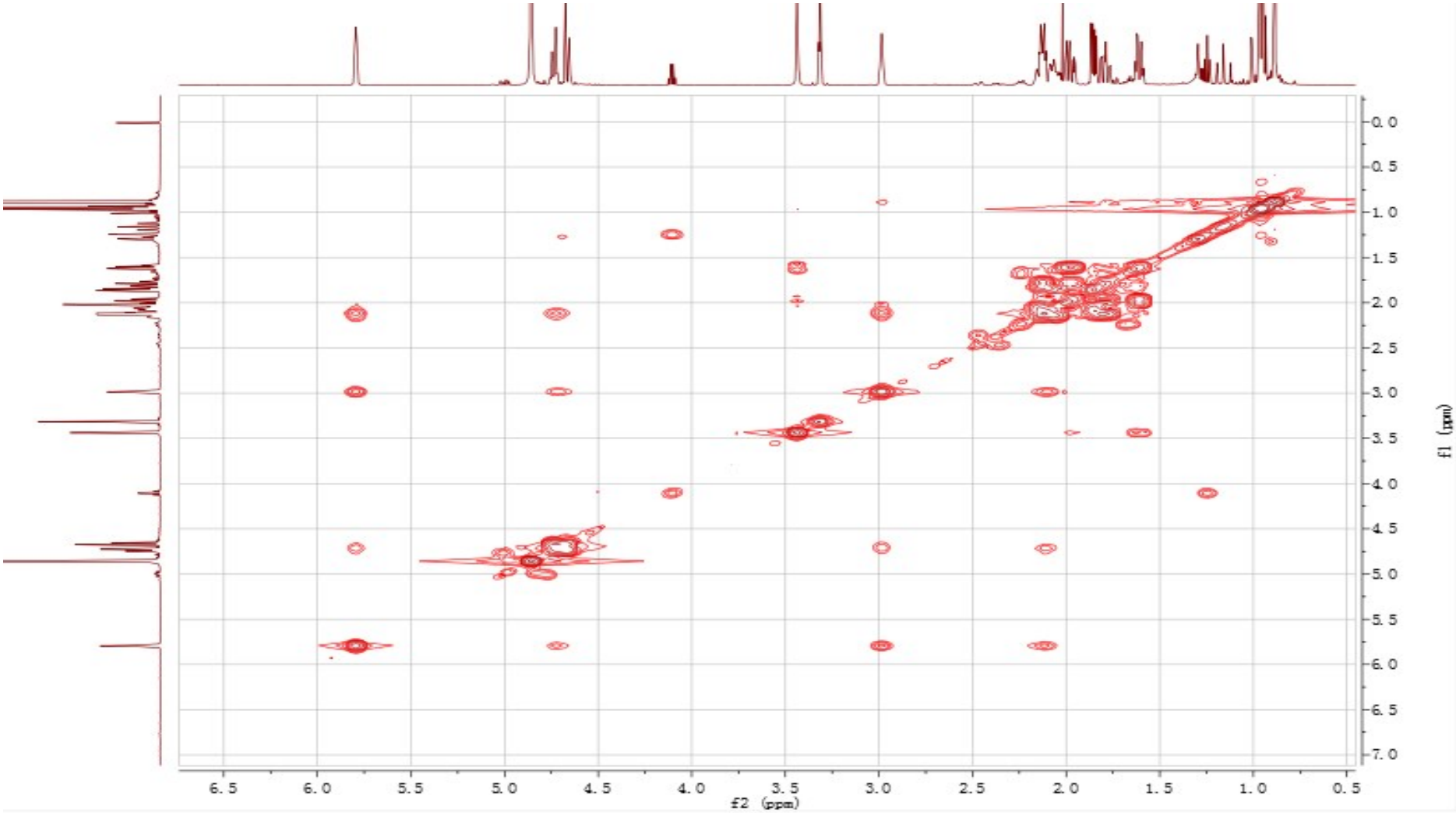


Figure S48. COSY spectrum of the compound **8**

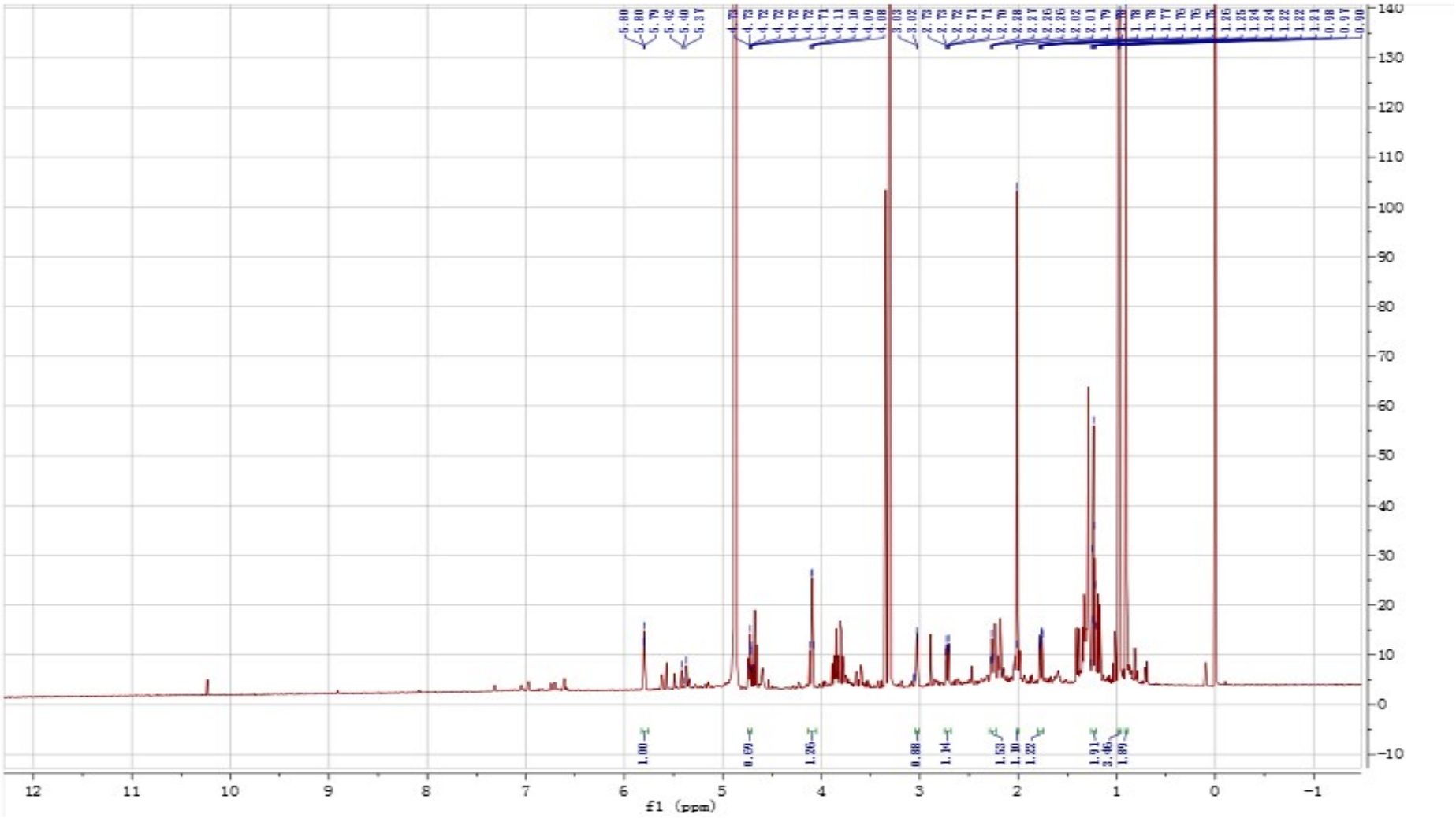


Figure S49. ^{1}H NMR (600 MHz, CD_3OD) spectrum of the compound **9**

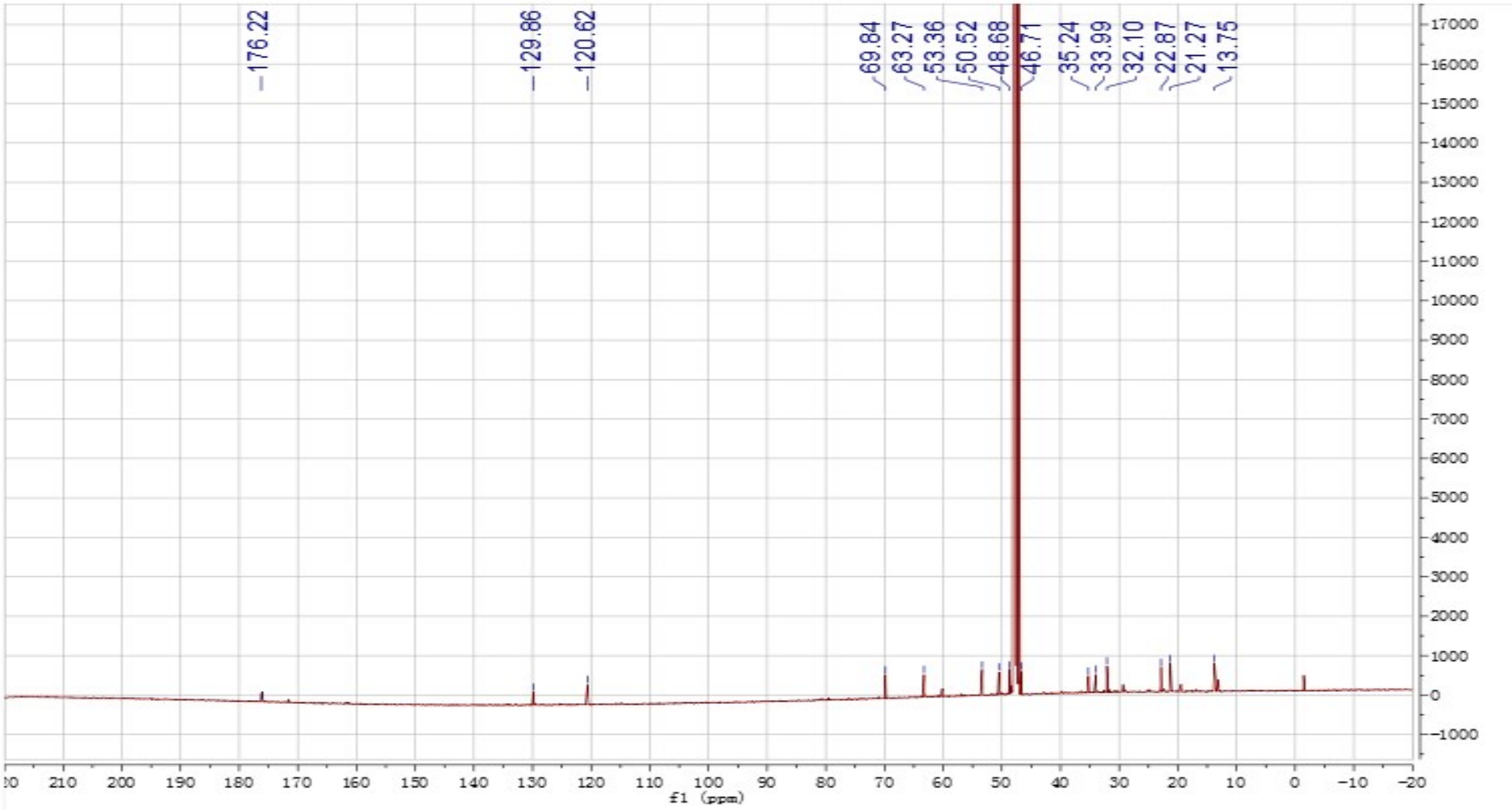


Figure S50. ^{13}C NMR (150 MHz, CD_3OD) spectrum of the compound **9**

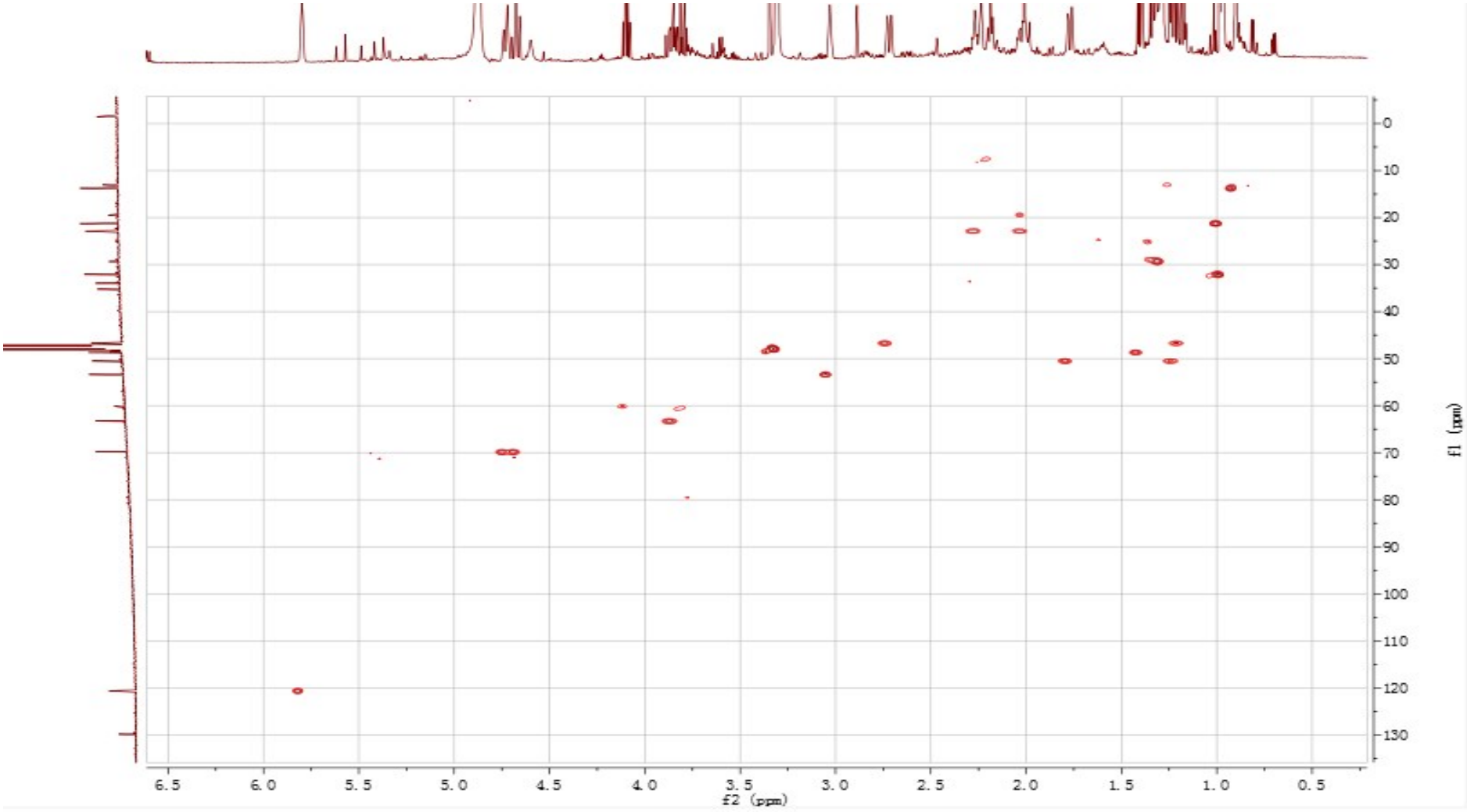


Figure S51. HSQC spectrum of the compound **9**

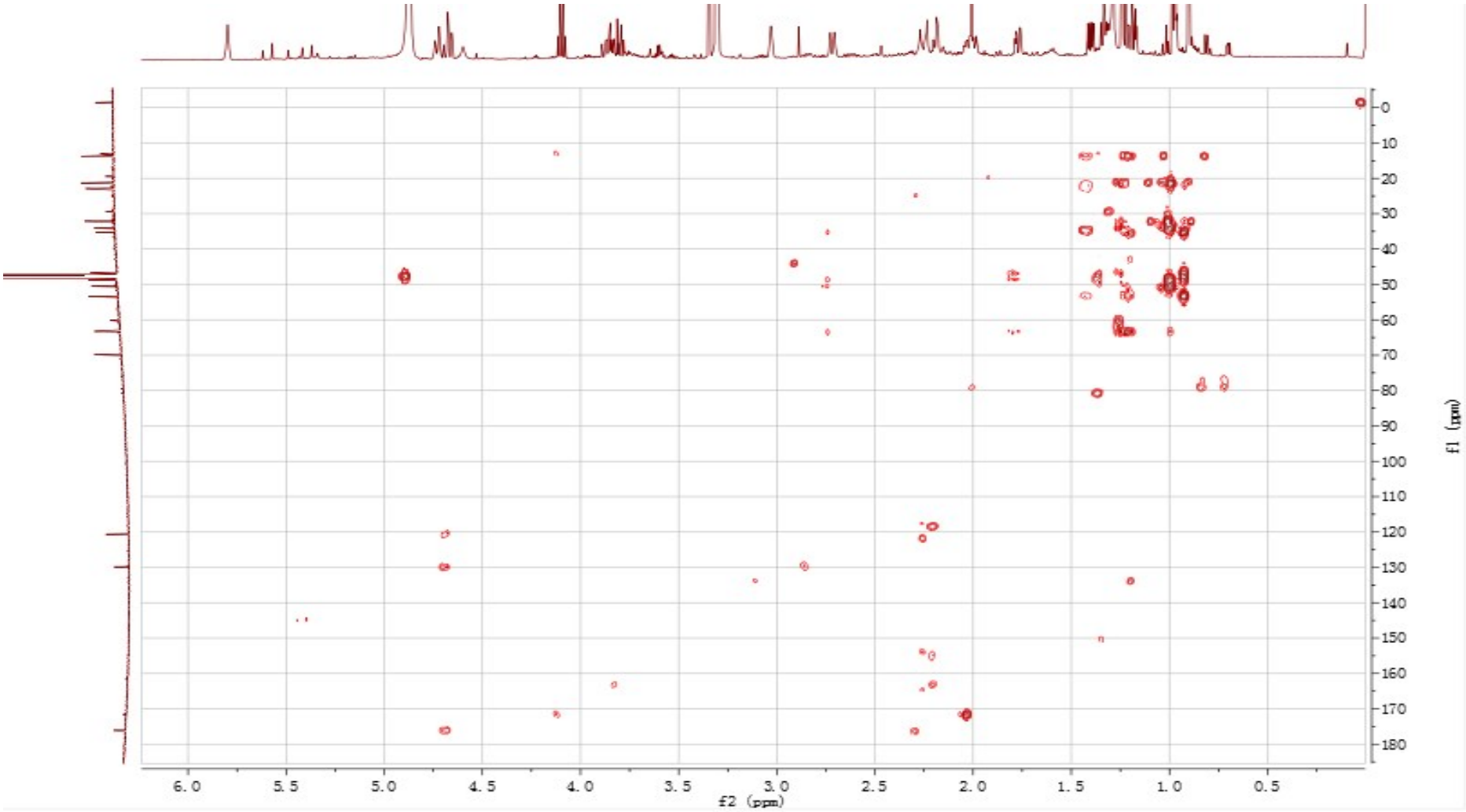


Figure S52. HMBC spectrum of the compound **9**

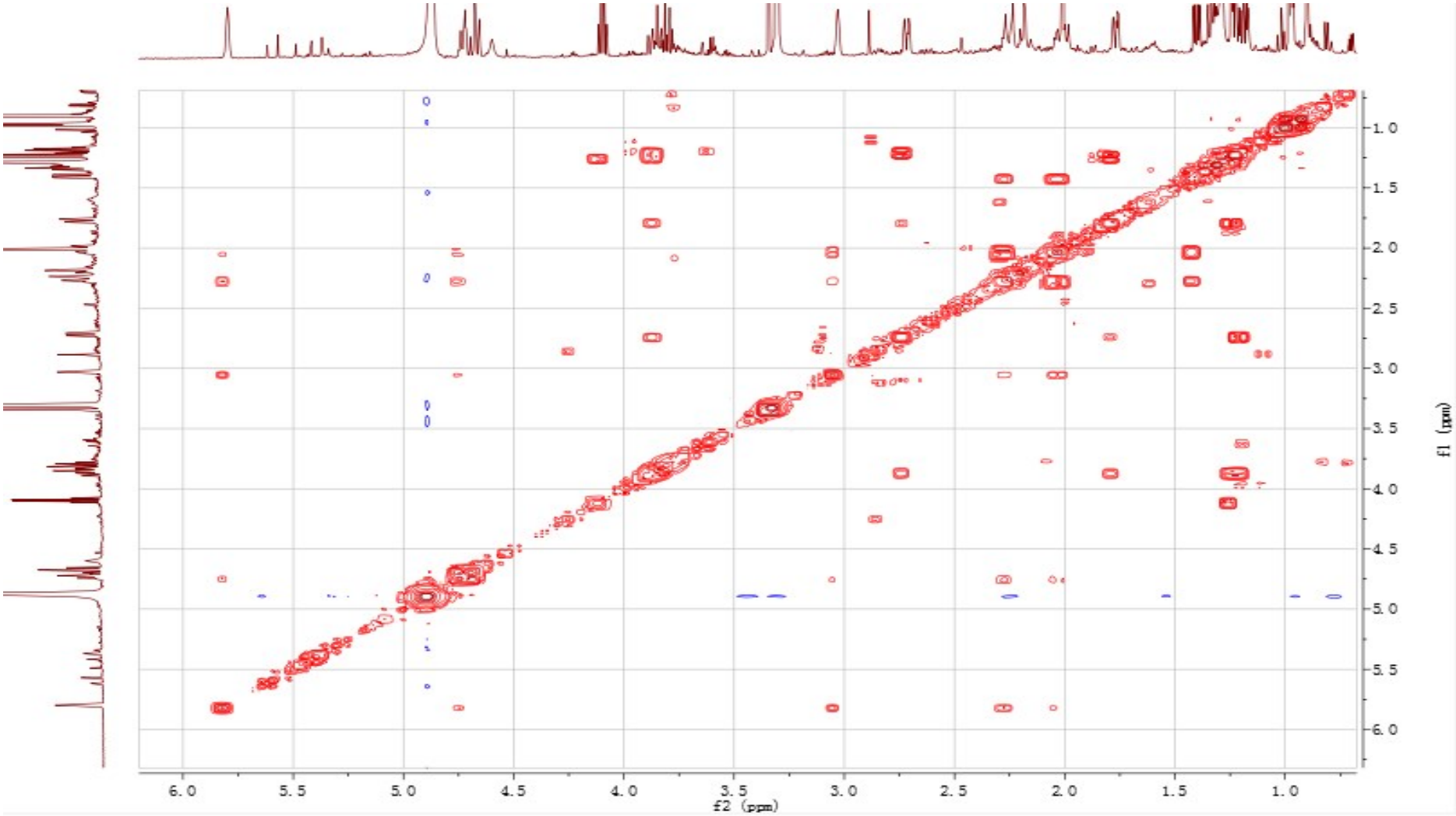


Figure S53. COSY spectrum of the compound **9**

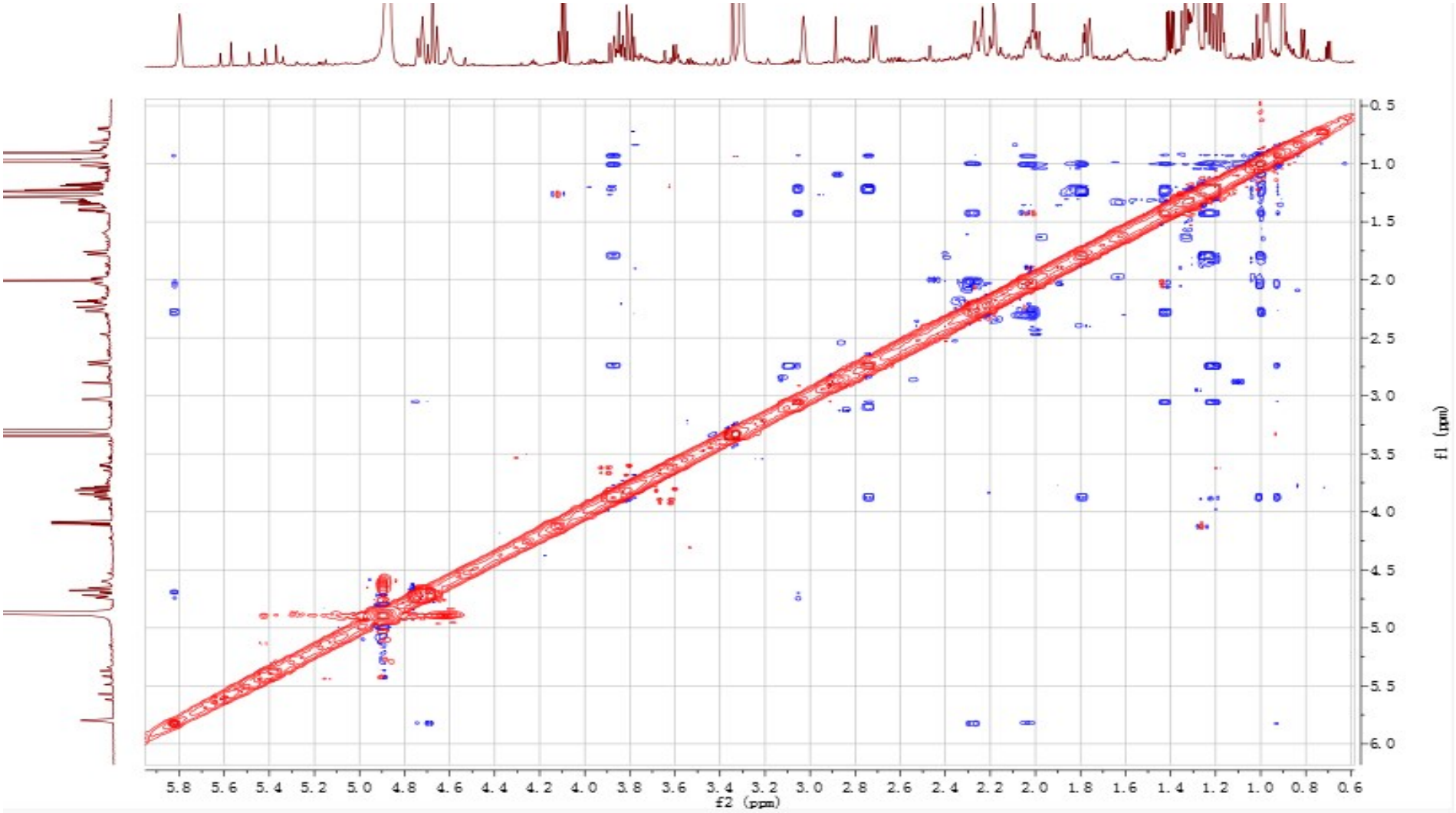


Figure S54. NOESY spectrum of the compound **9**

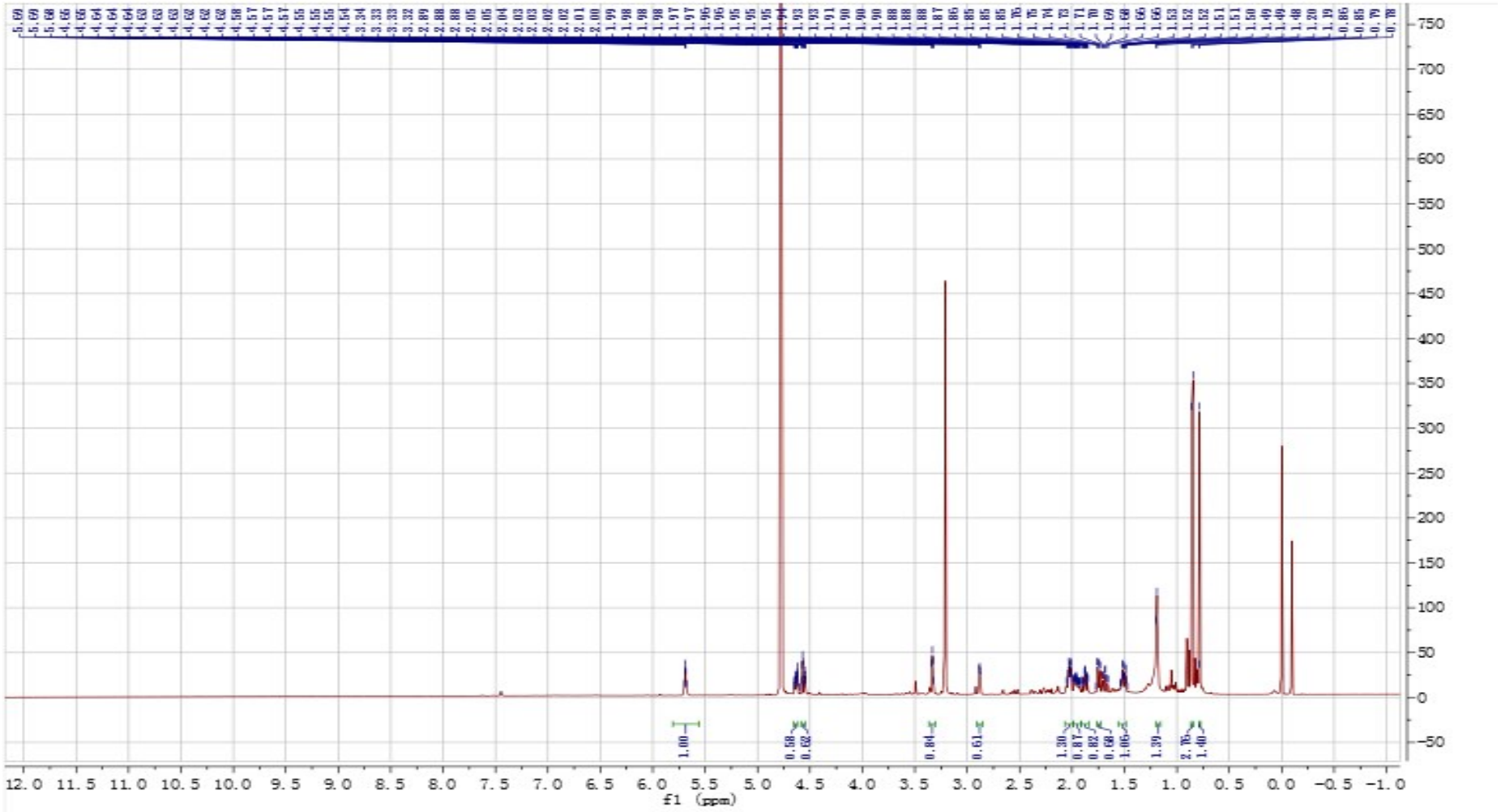


Figure S55. ^{1}H NMR (600 MHz, CD_3OD) spectrum of the compound **10**

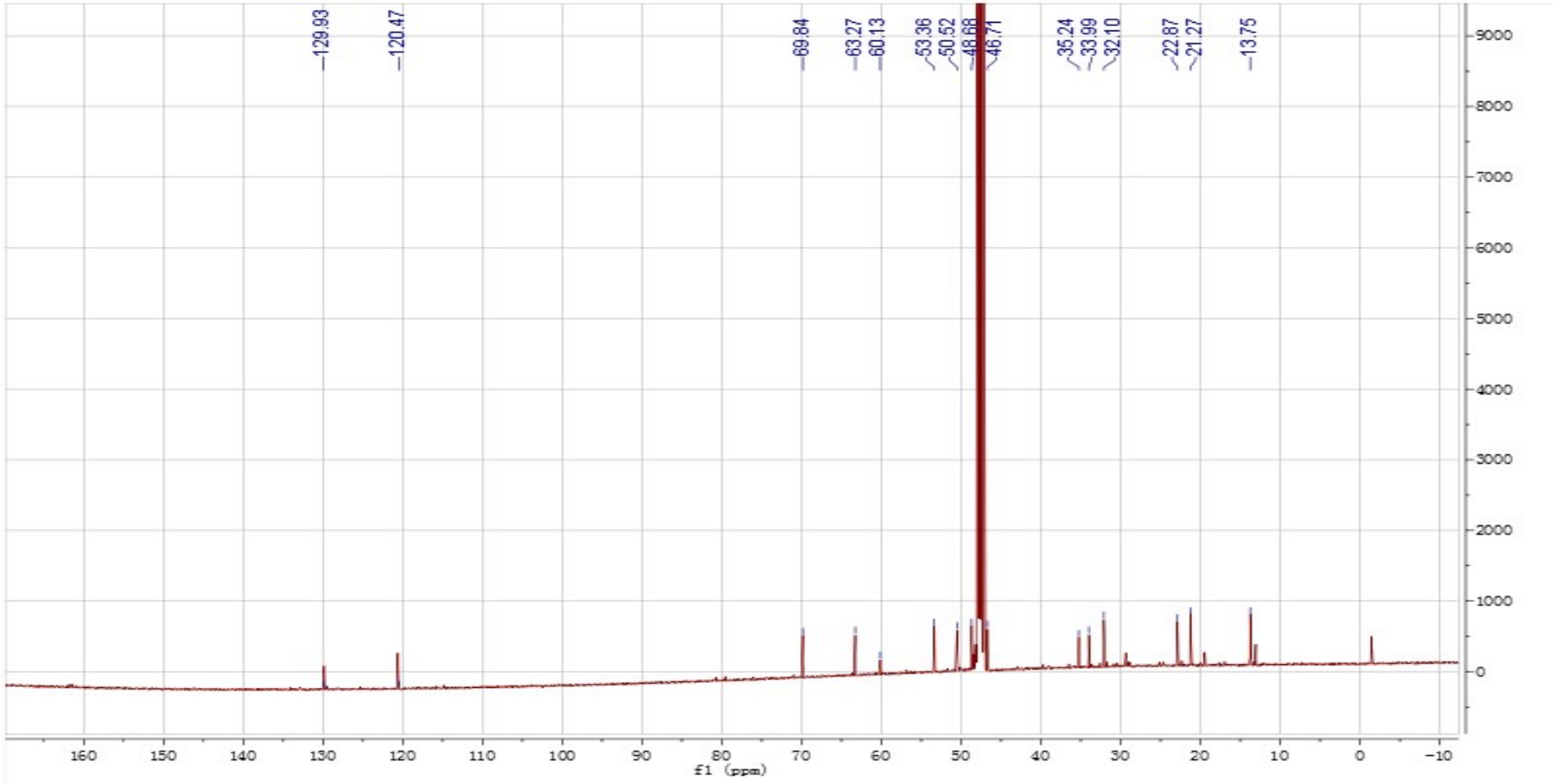


Figure S56. ^{13}C NMR (150 MHz, CD_3OD) spectrum of the compound **10**

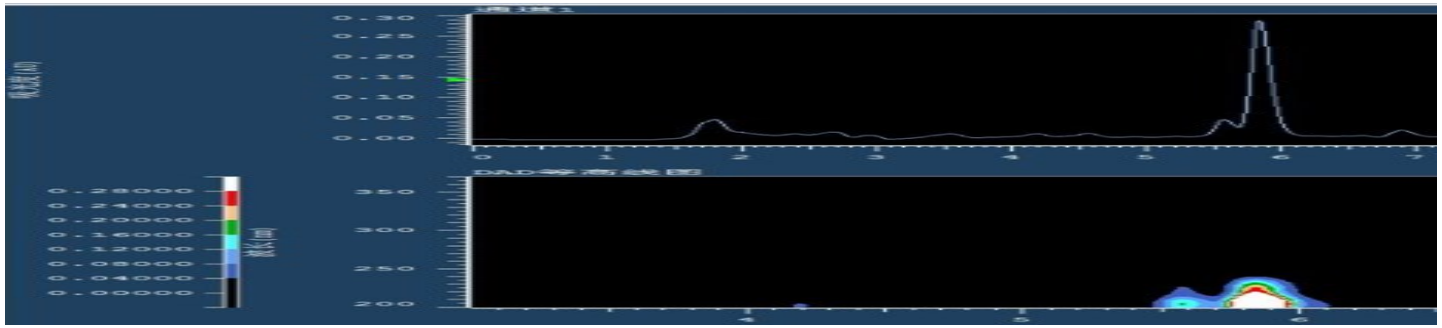


Figure S57. Chromatogram of the chiral HPLC separation of **3** and **11.**

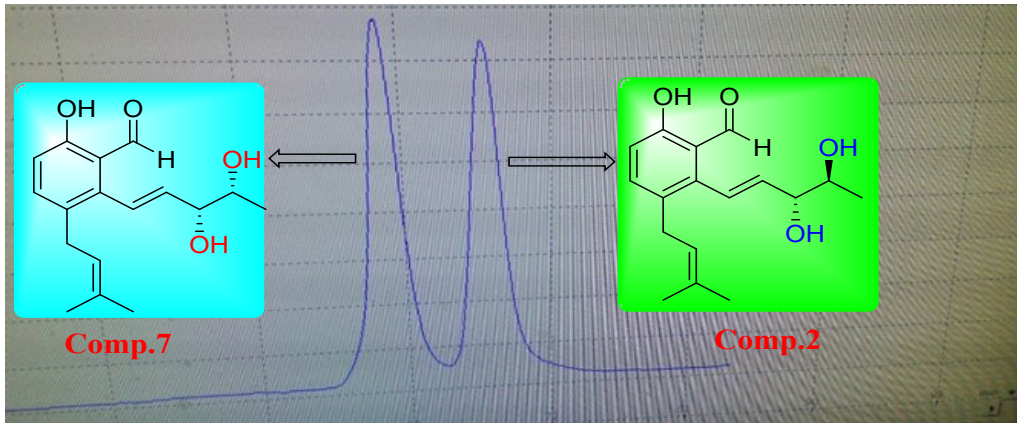


Figure S58. Chromatogram of the HPLC separation of **2** and **7.**

Table S1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data for compounds **1** and **6** in CD_3OD

	1		**6**	
No.	d_C, type	d_H (J in Hz)	d_C, type	d_H (J in Hz)
1	197.3, CH	10.43, s	197.3, CH	10.44, s
2	119.4, C		119.4, C	
3	164.4, C		164.4, C	
4	116.6, CH	6.79, d (8.0)	116.6, CH	6.80, d (8.0)
5	138.3, CH	7.45, t (8.0)	138.3, CH	7.45, t (8.0)
6	122.5, CH	6.85, d (8.0)	122.5, CH	6.85, d (8.0)
7	148.8, C		148.8, C	
8	28.9, CH_2	3.25, ddd (14.5, 10.4, 4.6)	29.1, CH_2	3.23, ddd (13.8, 10.3, 4.8)
		3.00, ddd (14.5, 10.1, 6.7)		3.03, ddd (13.8, 10.1, 6.8)
9	37.2, CH_2	1.94, ddd (13.4, 10.1, 6.6)	36.9, CH_2	1.82, ddd (14.1, 6.8, 3.4)
		1.69, ddd (13.4, 9.8, 4.6)		1.76, ddd (14.1, 9.4, 4.6)
10	75.9, CH	3.39, ddd (9.0, 5.8, 2.4)	75.6, CH	3.41, ddd (9.4, 5.1, 3.1)
11	71.7, CH	3.59, dd (6.2, 5.8)	71.3, CH	3.65, qd (6.4, 5.1)
12	19.1, CH_3	1.19, d (6.2)	18.9, CH_3	1.16, d (6.4)

Table S2. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data for compounds **2** and **7** in CD_3OD

	2		**7**	
No.	d_C, type	d_H (J in Hz)	d_C, type	d_H (J in Hz)
1	197.7, CH	10.23, s	197.6, CH	10.21, s
2	118.4, C		118,5, C	
3	161.4, C		160.7, C	
4	115.5, CH	6.79, d (8.6)	115.5, CH	6.79, d (8.6)
5	137.3, CH	7.35, d (8.6)	137.3, CH	7.34, d (8.6)
6	131.0, C		131.0, CH	
7	141.9, C		141.9, C	
8	125.3, CH	6.85, dd (16.1, 1.3)	125.4, CH_2	6.89, dd (16.0, 1.4)
9	140.1, CH	5.81, dd (16.1, 6.1)	139.9, CH_2	5.77, dd (16.0, 5.1)
10	76.2, CH	4.13, ddd (6.4, 5.2, 1.4)	76.0, CH	4.15, dq (5.8, 1.4)
11	70.2, CH	3.77, qd (6.2, 5.2)	70.1, CH	3.77, qd (6.4, 5.8)
12	17.8, CH_3	1.23, d (6.2)	17.6, CH_3	1.20, d (6.4)
1'	30.6, CH_2	3.31, d (7.1)	30.6, CH_2	3.30, d (5.7)
2'	122.4, CH	5.20, t (7.1)	122.4, CH	5.18, t (5.7)
3'	132.3, C		132.3, C	
4'	16.6, CH_3	1.72, s	16.6, CH_3	1.72, s
5'	24.5, CH_3	1.72, s	24.5, CH_3	1.72, s

Computational details for compound 1-3 (ECD)

The calculations of new compounds **1-3** were performed by using the density functional theory (DFT) as carried out in the Gaussian 09. Conformation search were performed with MMFF94S force fields using Maestro 10.2 software. All these conformers were further optimized by the density functional theory method at the B3LYP/6-31G(d) level. The ECD were calculated using density functional theory (TDDFT) at B3LYP/6-31+G(d) level. The calculated ECD curves were all generated using SpecDis v 1.53 software.

Figure S59. Comparison of the calculated ECD spectra for **1** with the experimental spectrum of **1**

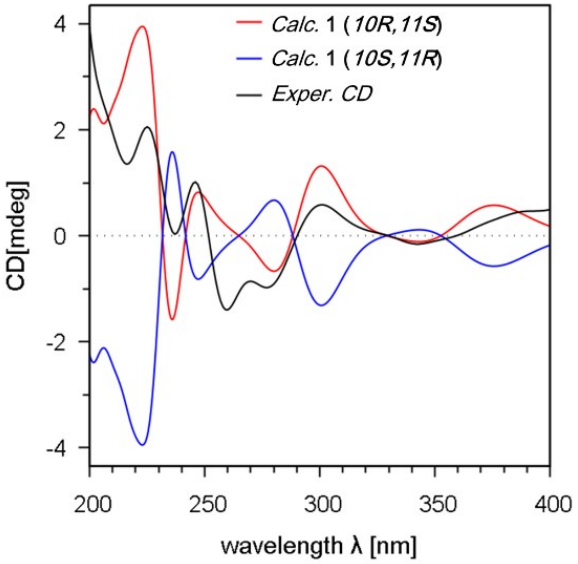


Table S3. Energy analysis for conformers of **1** at B3LYP/6-31G(d) level

Conformer	**Energy（hartree）**	**Ratio（%）**
2	-767.7954334	8.62
3	-767.7942924	2.57
4	-767.7959113	14.31
5	-767.7955714	9.98
8	-767.7951577	6.44
9	-767.7948101	4.45
10	-767.7945882	3.52

12	-767.7947665	4.25
13	-767.7954791	9.05
14	-767.7951098	6.12
15	-767.7941229	2.15
16	-767.7951059	6.09
21	-767.7946171	3.63
22	-767.7942295	2.41
25	-767.7946866	3.91
27	-767.7942942	2.58
29	-767.7949942	5.41
30	-767.7948158	4.48

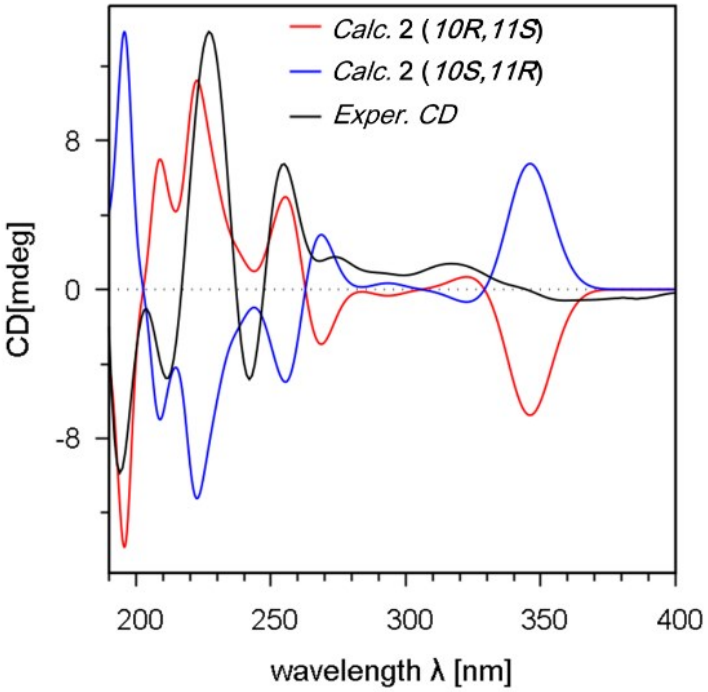


Figure S60. Comparison of the calculated ECD spectra for **2** with the experimental spectrum of **2**

Table S4. Energy analysis for conformers of **2** at B3LYP/6-31G(d) level

Conformer	**Energy**（hartree）	**Ratio**（%）
1	-961.9075567	1.71
2	-961.9072757	1.27
6	-961.908038	2.85
8	-961.9081127	3.09
10	-961.9110437	69.09
14	-961.9073732	1.41
16	-961.9073852	1.43
30	-961.9083516	3.98
37	-961.9078313	2.29
42	-961.9081663	3.27
69	-961.9072664	1.26

71	-961.9084103	4.24
81	-961.9080239	2.81
85	-961.9072888	1.29

Figure S61. Comparison of the calculated ECD spectra for **3** with the experimental spectrum of **3**

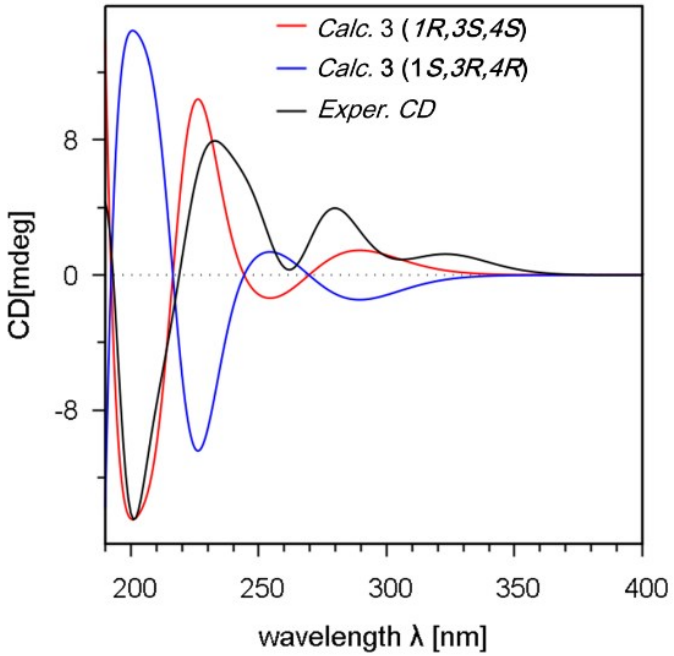


Table S5. Energy analysis for conformers of **1** at B3LYP/6-31G(d) level

conformer	**Energy（hartree）**	**Ratio（%）**
1	-691.5644337	66.82
2	-691.5636597	29.42
4	-691.5610731	1.90
5	-691.5610583	1.87