

## Supplementary Materials for

### Lasiodipline G and other diketopiperazine metabolites produced by

#### *Lasiodiplodia chiangraiensis*

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## ABSTRACT

*Lasiodiplodia* fungi are known to colonize plants as both pathogens and/or endophytes; hence, they can be exploited for their beneficial roles. Many compound classes from the genus have exhibited their potential biotechnological applications. Herein, we report two new metabolites **1** and **2** together with three known cyclo-(D-Ala-D-Trp) (**3**), indole-3-carboxylic acid (**4**) and a cyclic pentapeptide clavatustide B (**5**), isolated from the submerged cultures of a recently described species *L. chiangraiensis*. Chemical structures of the isolated compounds were determined by extensive NMR spectroscopic analyses together with HRESIMS. The absolute configurations of the new compounds were established based on comparing experimental and calculated time-dependent density functional theory circular dichroism (TDDFT-ECD) spectra. Compound **1** exhibited significant cytotoxic activities against an array of cell lines with IC<sub>50</sub> values of 2.9-12.6 μM, as well as moderate antibacterial effects.

**Keywords:** *Lasiodiplodia*, new species, diketopiperazine, cytotoxic, antimicrobial.

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Table S1. 2D (COSY, HMBC and ROESY) NMR correlations of **1** in methanol-*d*<sub>4</sub> at 700 MHz.

Pos.	<sup>1</sup> H- <sup>1</sup> H COSY <sup>a</sup>	HMBC <sup>a</sup>	ROESY <sup>a</sup>
1			
2			
4			
5			
6-NH			
7	H-9w <sup>a</sup>	4, 5, 8, 9, 16	H-9, H-15
8			
9	H-7w <sup>a</sup>	7, 8, 11, 12w <sup>a</sup> , 15w <sup>a</sup> , 16	H-7
10-NH			
11			
12	H-13, H-14w <sup>a</sup>	14, 16	
13	H-12, H-14	11, 12w <sup>a</sup> , 15	
14	H-13, H-15	11w <sup>a</sup> , 12, 16	
15	H-14, H-13w <sup>a</sup>	8, 11, 13, 16w <sup>a</sup>	H-7
16			
2-Me		1, 2	3-NMe
3-NMe		2, 4	2-Me

<sup>a</sup> "w" denotes weak correlation.

Table S2. 2D (COSY, HMBC and ROESY) NMR correlations of **2** in methanol-*d*<sub>4</sub> at 700 MHz.

Pos.	<sup>1</sup> H- <sup>1</sup> H COSY	HMBC	ROESY
1			
2	2-CH <sub>3</sub>	C-1, 2-CH <sub>3</sub> , C-4	H-5
3-NOH			
4			
5		C-1, C-4, C-7, C-8,	H-2
6-NH			
7		C-1w <sup>a</sup> , C-4, C-5, C-8, C-9, C-16	H-15
8			
9	CH <sub>2</sub> -7w <sup>a</sup>	C-5w <sup>a</sup> , C-7, C-8, C-11, C-16	
10-NH			
11			
12	H-13, H-14w <sup>a</sup>	C-14, C-16	
13	H-12, H-14, H-15w <sup>a</sup>	C-11, C-12w <sup>a</sup> , C-14w <sup>a</sup> , C-15	
14	H-12w <sup>a</sup> , H-13, H-15	C-12, C-13w <sup>a</sup> , C-15w <sup>a</sup> , C-16	
15	H-13w <sup>a</sup> , H-14, H-15	C-8, C-11, C-13, C-16w <sup>a</sup>	CH <sub>2</sub> -7
16			
2-Me	H-2	C-1, C-2	

<sup>a</sup> "w" denotes weak correlation.

Table S3. Cytotoxicity (IC<sub>50</sub>) and antimicrobial activity (MIC) of 1-4.

Test Cell Line	IC <sub>50</sub> (μM)				Positive Control
	1	2	3	4	Epothilone B (μM)
Mouse fibroblast (L929)	5.8	n.a.	n.a.	87	0.65
Human endocervical adenocarcinoma (KB3.1)	8.4	n.a.	n.a.	n.a.	0.17
Human prostate carcinoma (PC-3)	6.3	n.d.	n.d.	n.d.	0.09
Human breast adenocarcinoma (MCF-7)	3.9	n.d.	n.d.	n.d.	0.07
Human ovarian cancer (SKOV-3)	3.9	n.d.	n.d.	n.d.	0.09
Human epidermoid carcinoma (A431)	2.9	n.d.	n.a.	n.a.	0.06
Human lung carcinoma (A549)	12.6	n.d.	n.d.	n.d.	0.05
Test Microorganism	MIC (μg/mL)				Positive Control (μg/mL)
<i>Staphylococcus aureus</i>	33.3	n.i.	n.i.	n.i.	0.21 <sup>G</sup>
<i>Escherichia coli</i>	n.d.	n.i.	n.i.	n.i.	0.83 <sup>G</sup>
<i>Bacillus subtilis</i>	n.i.	n.i.	n.i.	n.i.	16.6 <sup>O</sup>
<i>Pseudomonas aeruginosa</i>	n.d.	n.i.	n.i.	n.i.	0.22 <sup>G</sup>
<i>Pichia anomala</i>	n.d.	n.i.	66.6	n.i.	4.20 <sup>N</sup>
<i>Candida albicans</i>	n.d.	n.i.	n.i.	n.i.	2.20 <sup>N</sup>
<i>Acinetobacter baumannii</i>	n.d.	n.i.	n.i.	n.i.	0.52 <sup>C</sup>
<i>Chromobacterium violaceum</i>	n.d.	n.i.	n.i.	n.i.	1.70 <sup>G</sup>
<i>Schizosaccharomyces pombe</i>	n.d.	n.i.	n.i.	n.i.	2.10 <sup>N</sup>
<i>Mucor hiemalis</i>	n.d.	n.i.	n.i.	n.i.	2.10 <sup>N</sup>
<i>Rhodotorula glutinis</i>	n.d.	n.i.	n.i.	n.i.	2.30 <sup>N</sup>
<i>Mycobacterium smegmatis</i>	n.d.	n.i.	n.i.	n.i.	1.70 <sup>K</sup>

n.a.: No activity. n.i.: No inhibition up to 67 μg/mL. n.d.: Not determined.

G: Gentamycin; O: Oxytetracycline; N: Nystatin; C: Ciprofloxacin; K: Kanamycin.

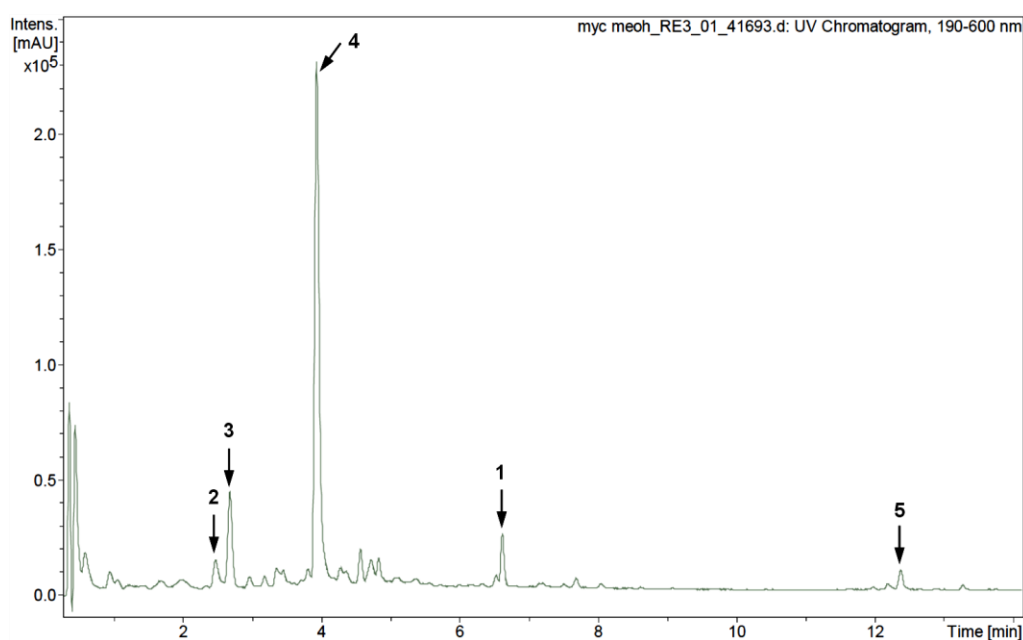


Figure S1. HPLC chromatogram of *Lasiodiplodia chiangraiensis* cultured on cotton seed (Q6) medium.

# Generic Display Report

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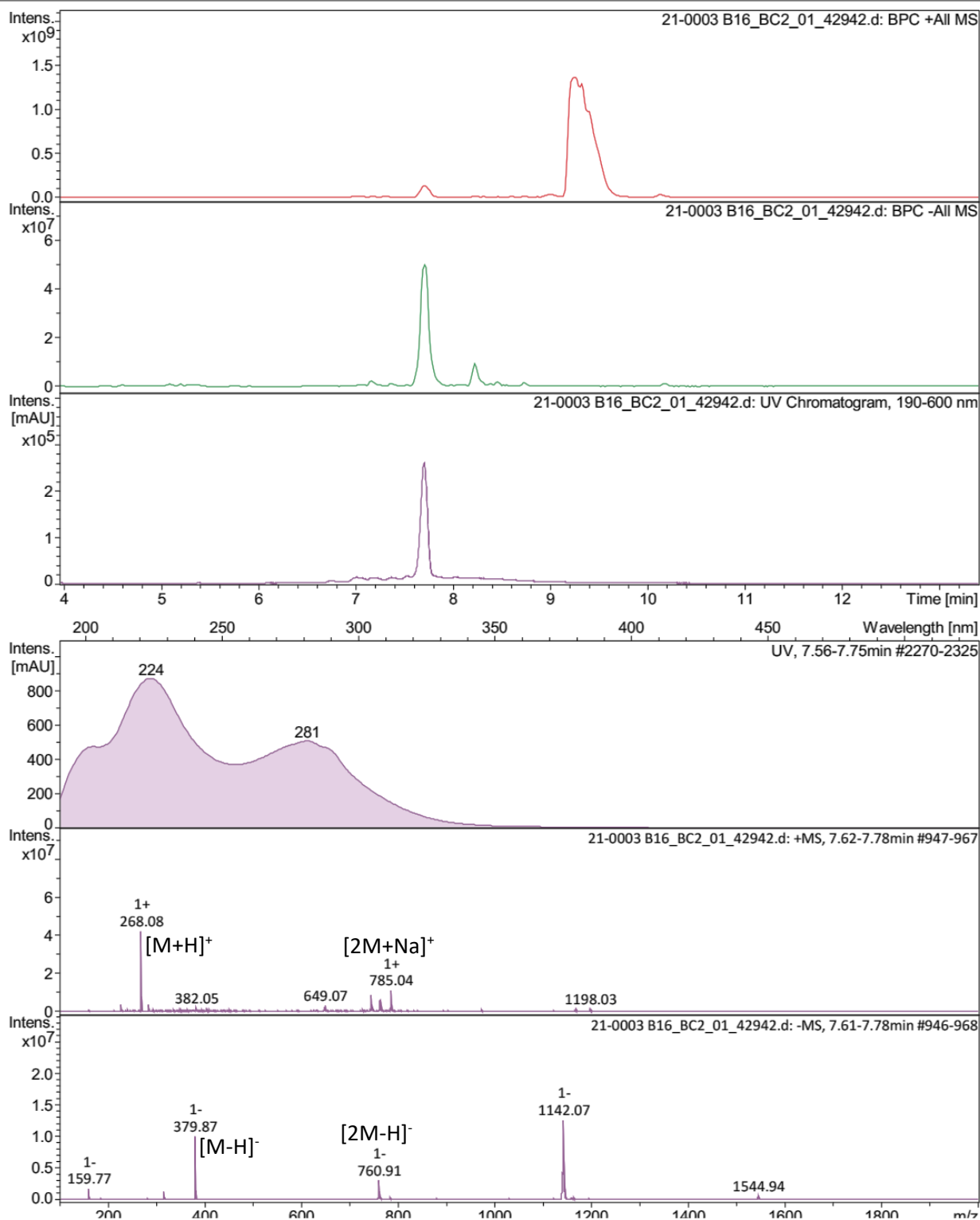


Figure S2. LRESIMS of **1**.

# Generic Display Report

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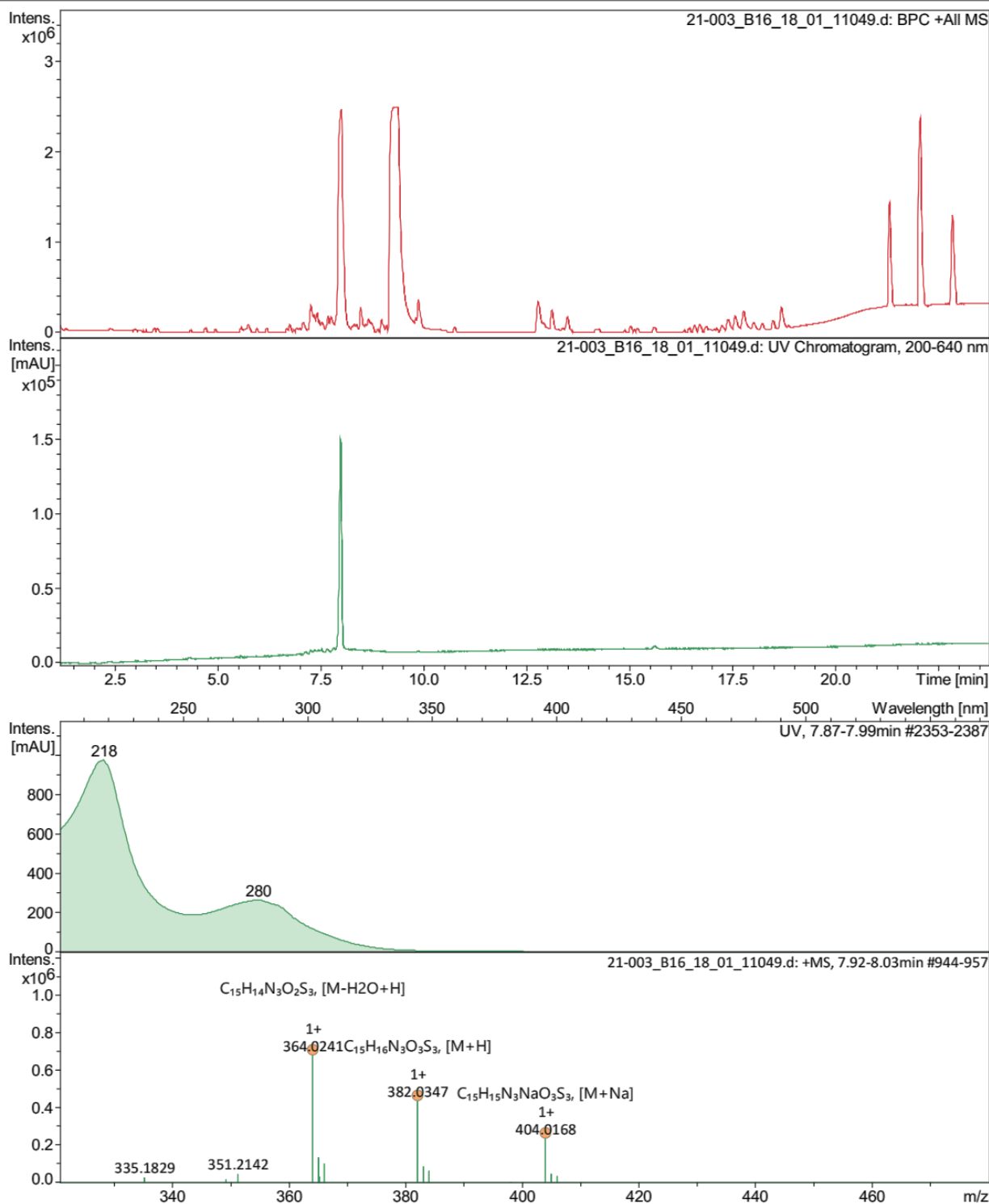


Figure S3. HRESIMS of 1.

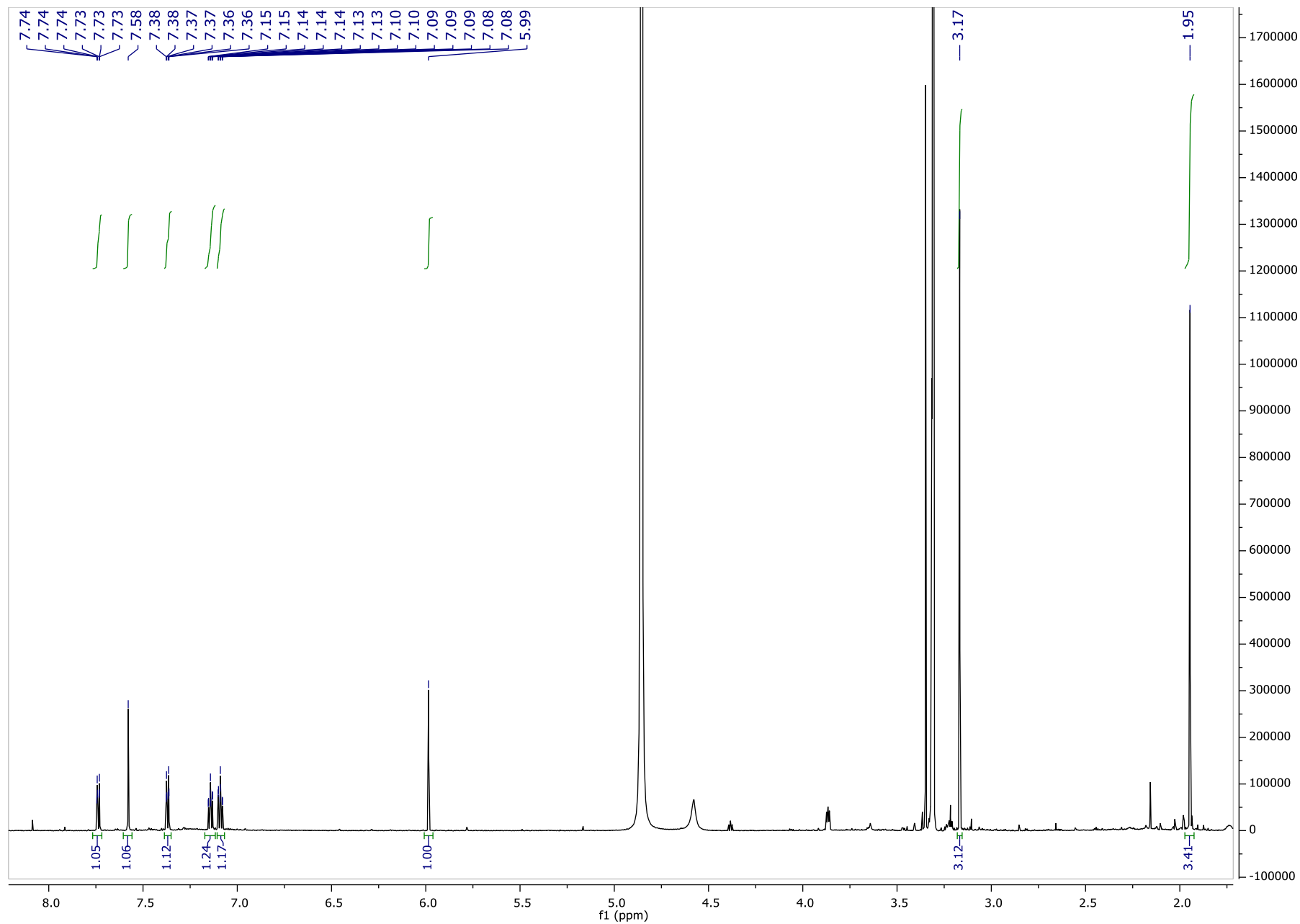


Figure S4.  $^1\text{H}$  NMR spectrum of **1** in methanol- $d_4$  at 700 MHz.



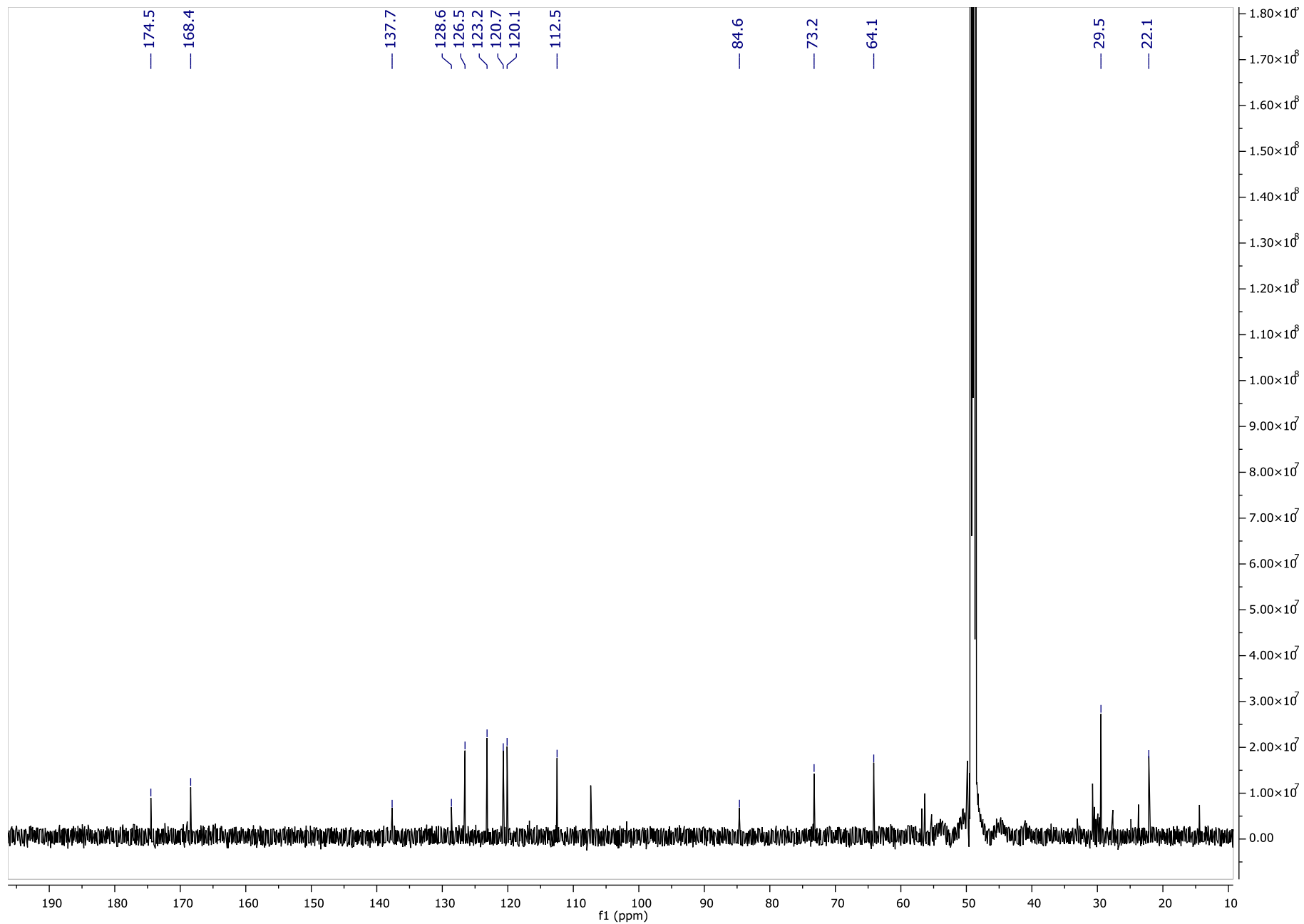


Figure S5.  $^{13}\text{C}$  NMR spectrum of **1** in methanol- $d_4$  at 175 MHz.

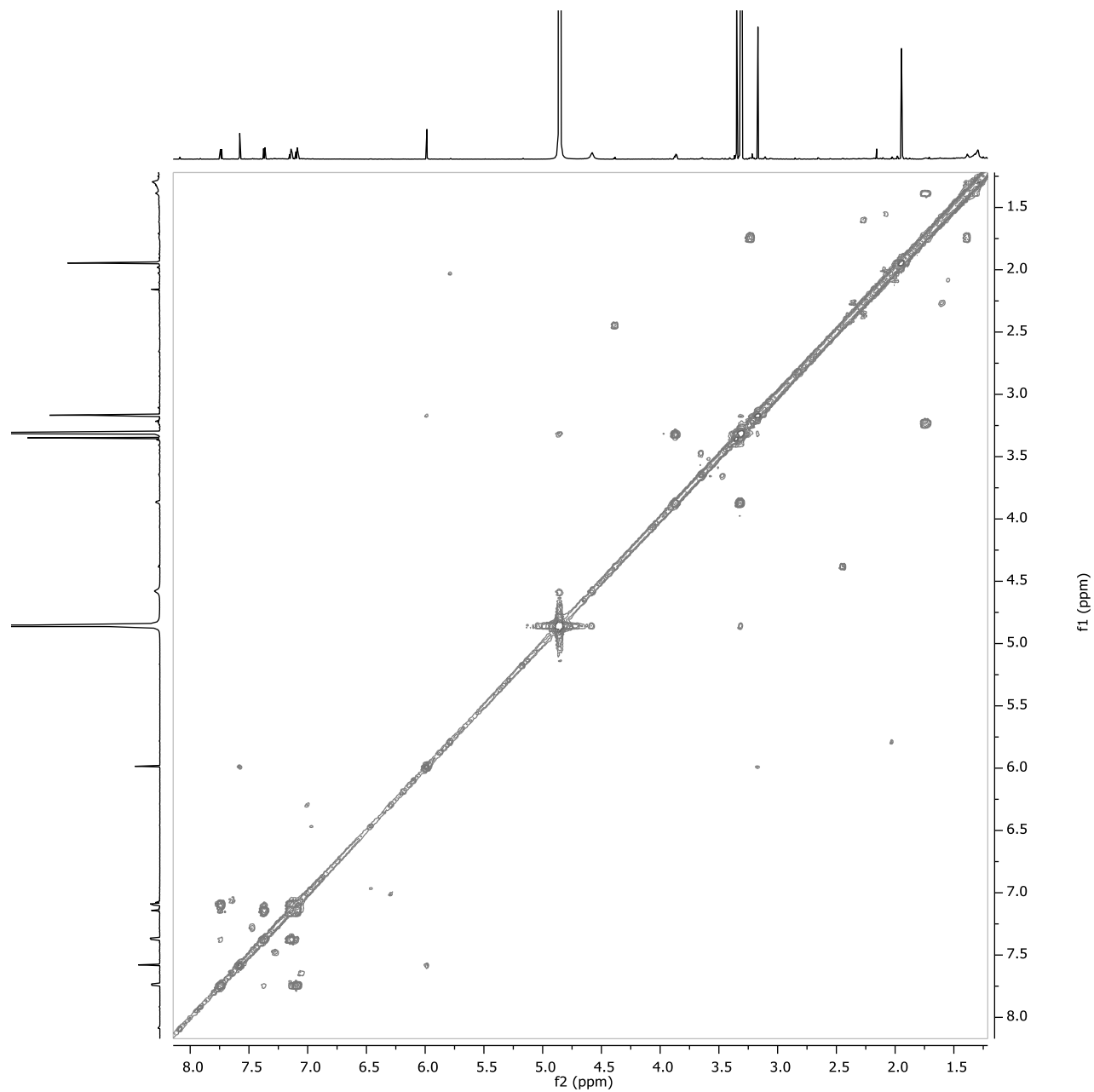


Figure S6.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **1** in methanol- $d_4$  at 700 MHz.

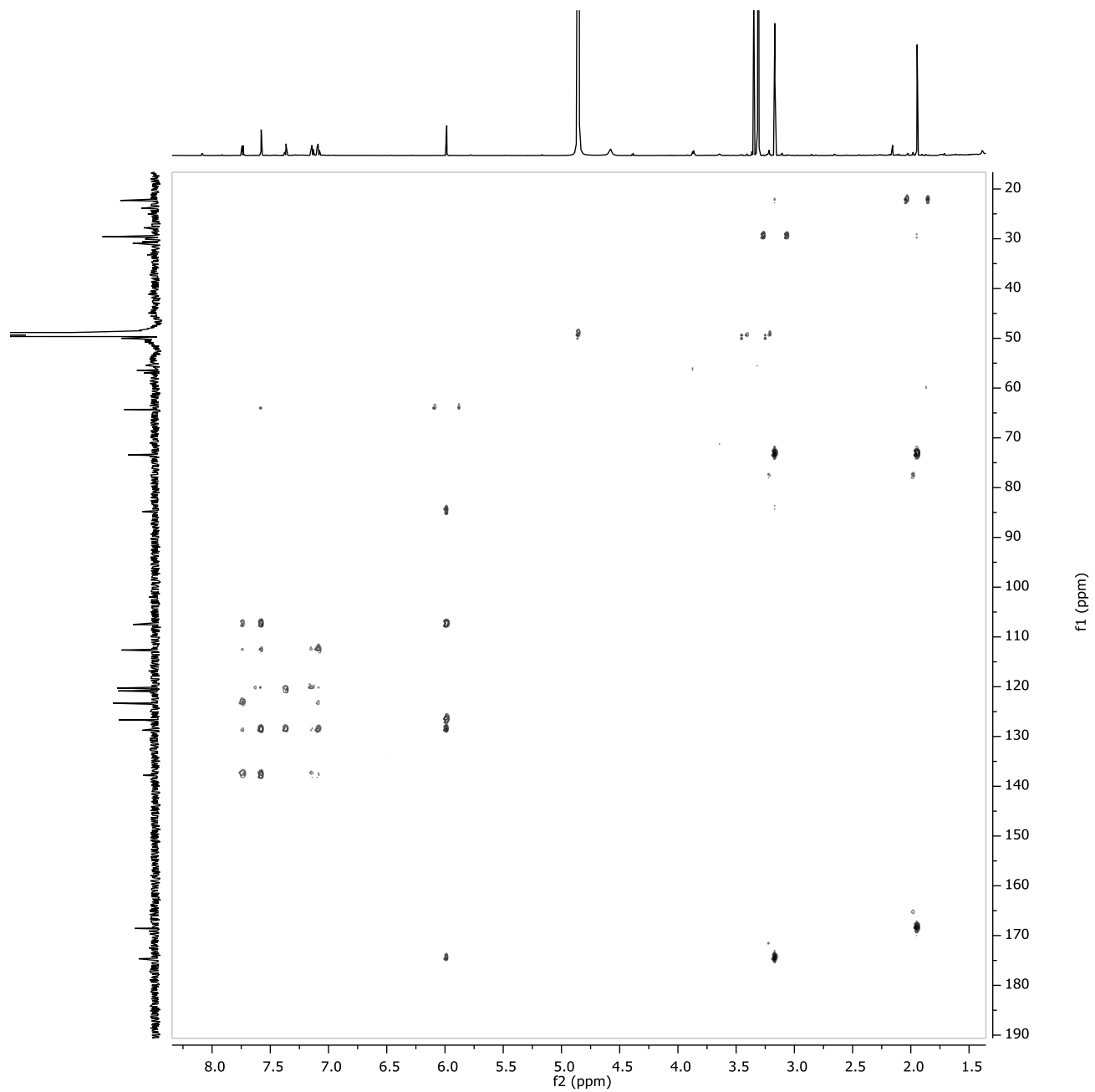


Figure S7. HMBC spectrum of **1** in methanol- $d_4$  at 700 MHz.

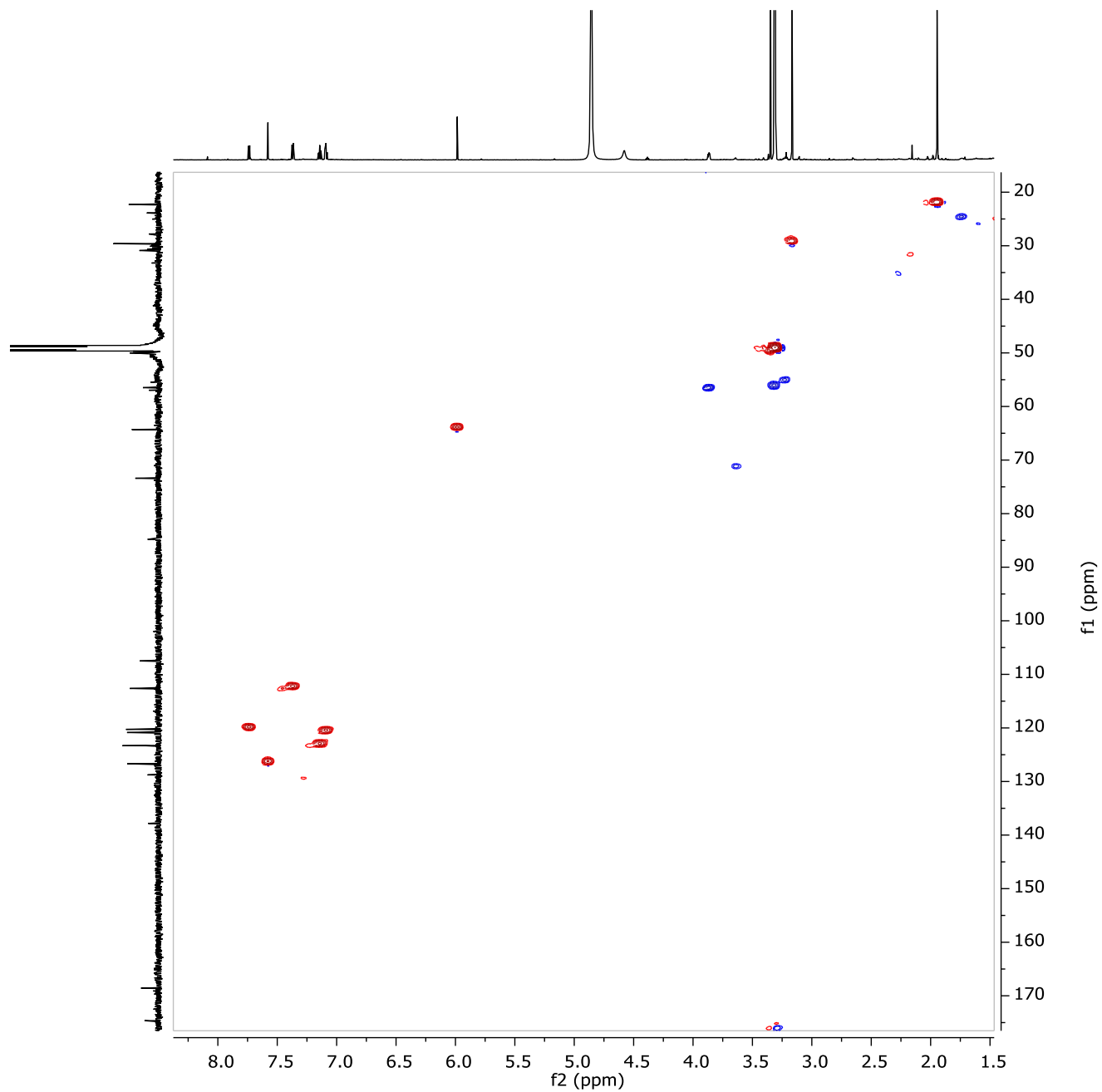


Figure S8. HSQC spectrum of **1** in methanol-*d*<sub>4</sub> at 700 MHz.

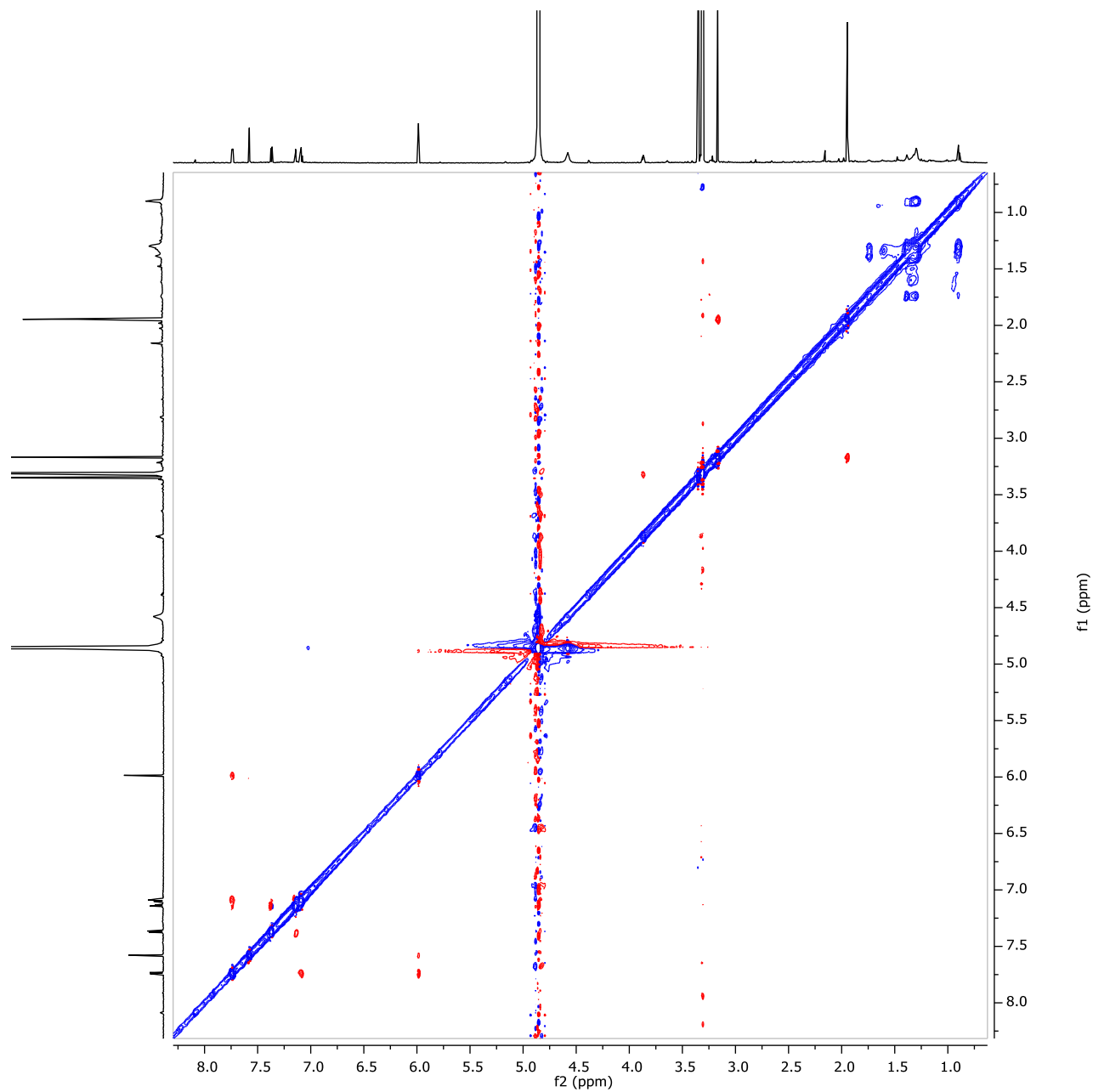


Figure S9. ROESY spectrum of **1** in methanol- $d_4$  at 700 MHz.

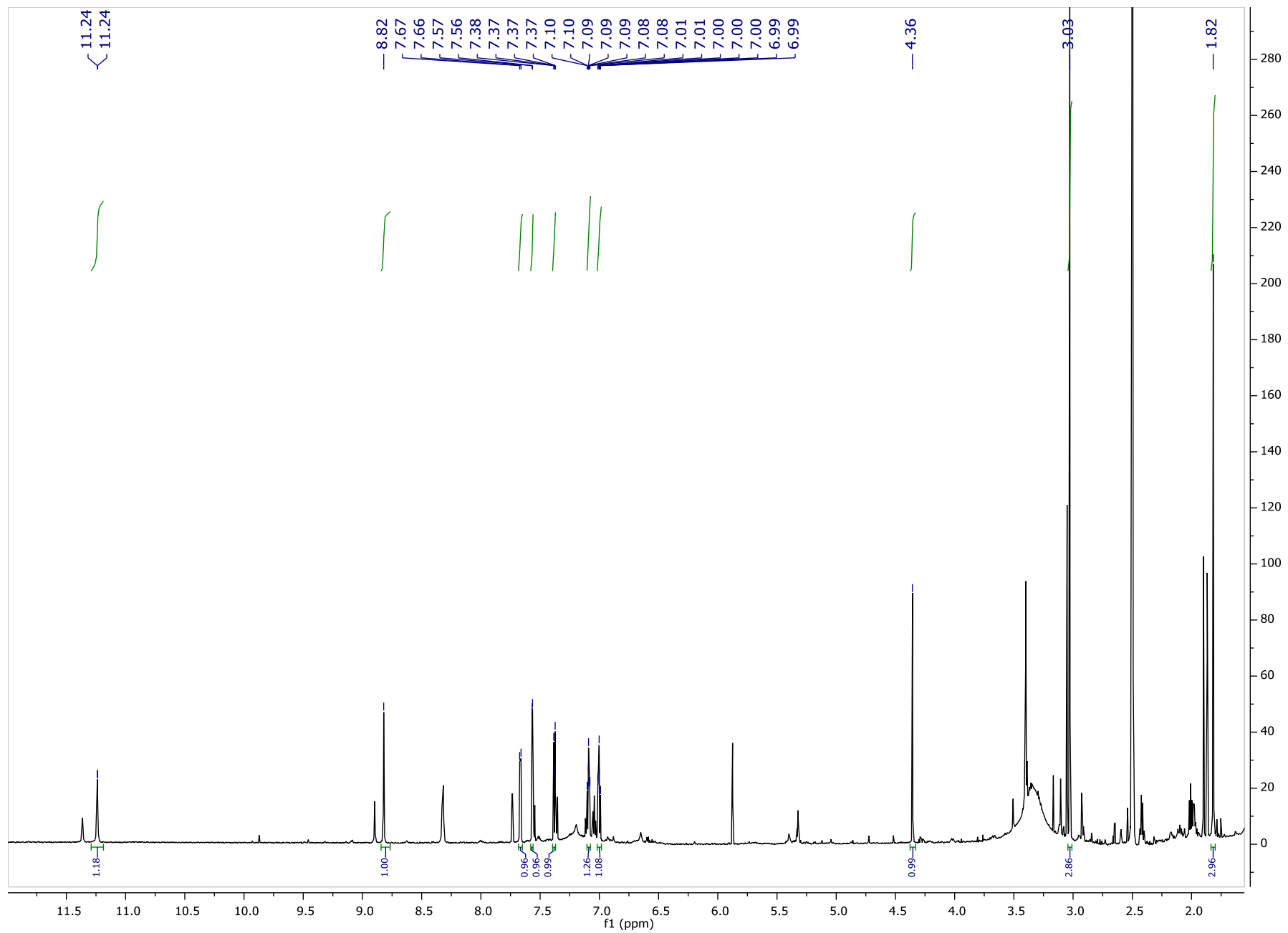


Figure S10.  $^1\text{H}$  NMR spectrum of **1** in  $\text{DMSO-}d_6$  at 700 MHz.

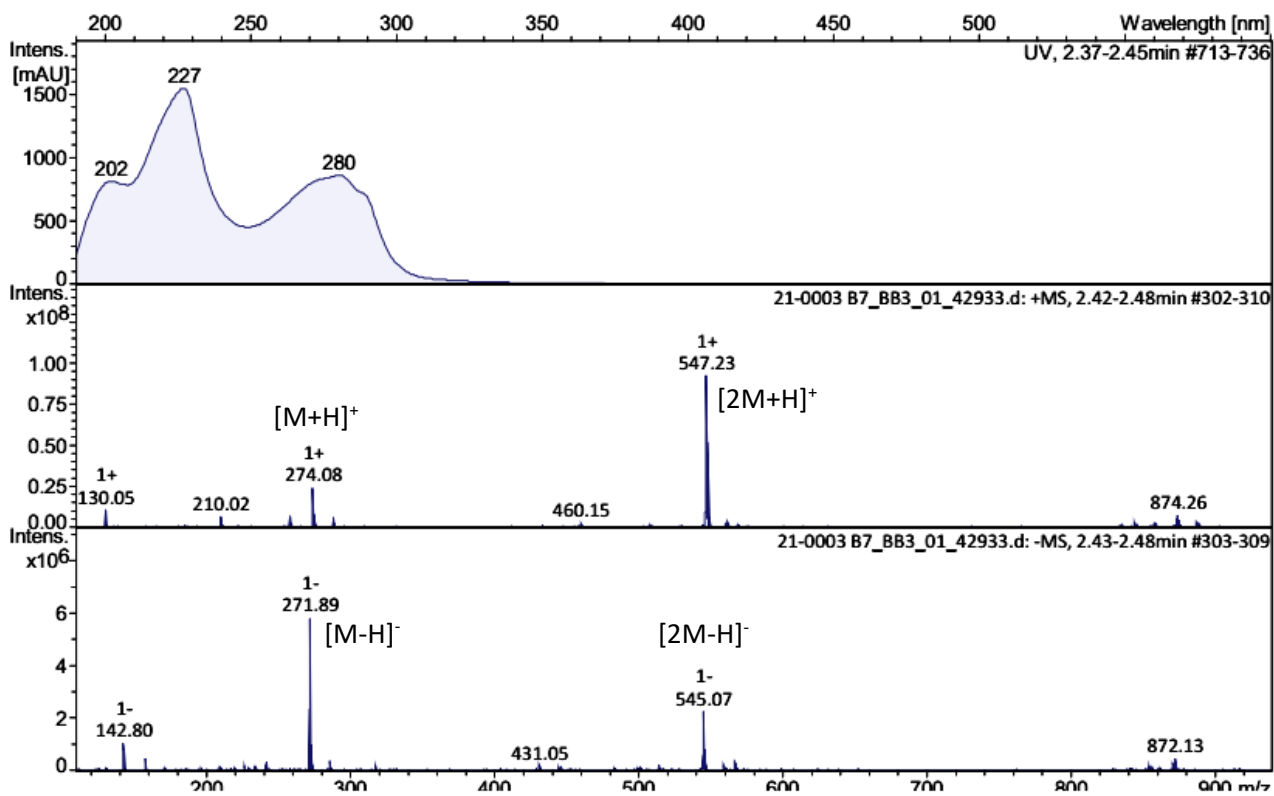


Figure S11. LRESIMS of 2.

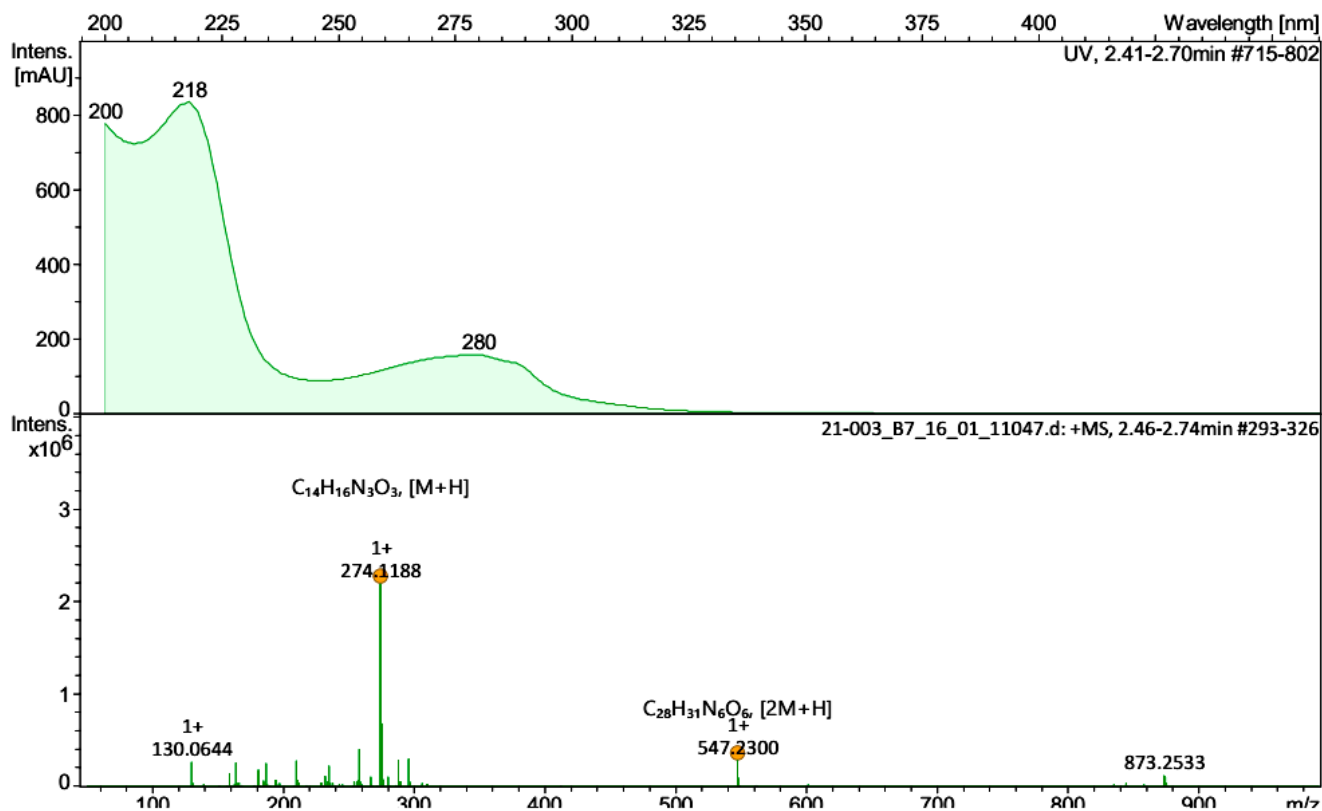


Figure S12. HRESIMS of 2.

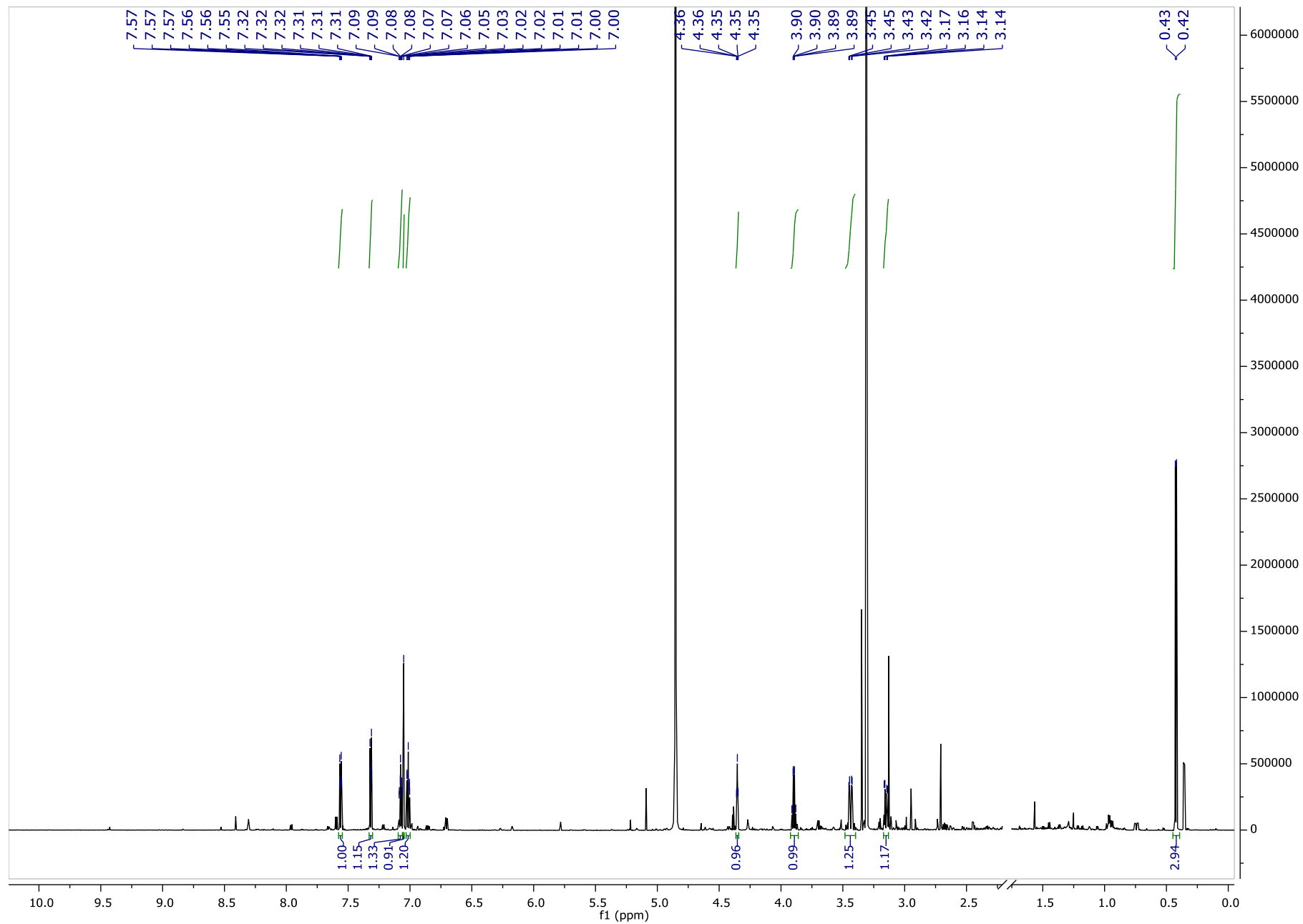


Figure S13.  $^1\text{H}$  NMR spectrum of **2** in methanol- $d_4$  at 700 MHz.



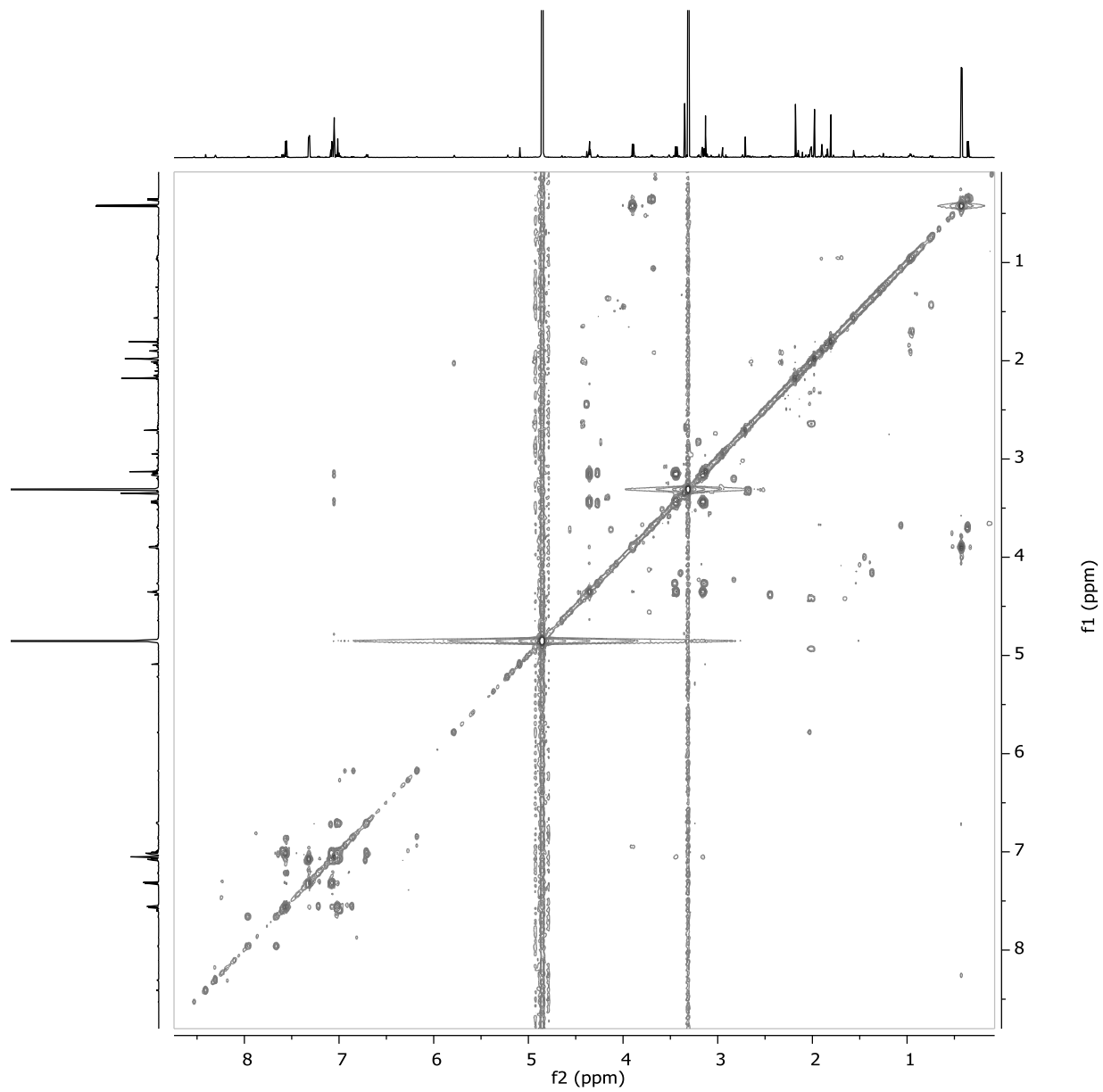


Figure S14.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **2** in methanol- $d_4$  at 700 MHz.

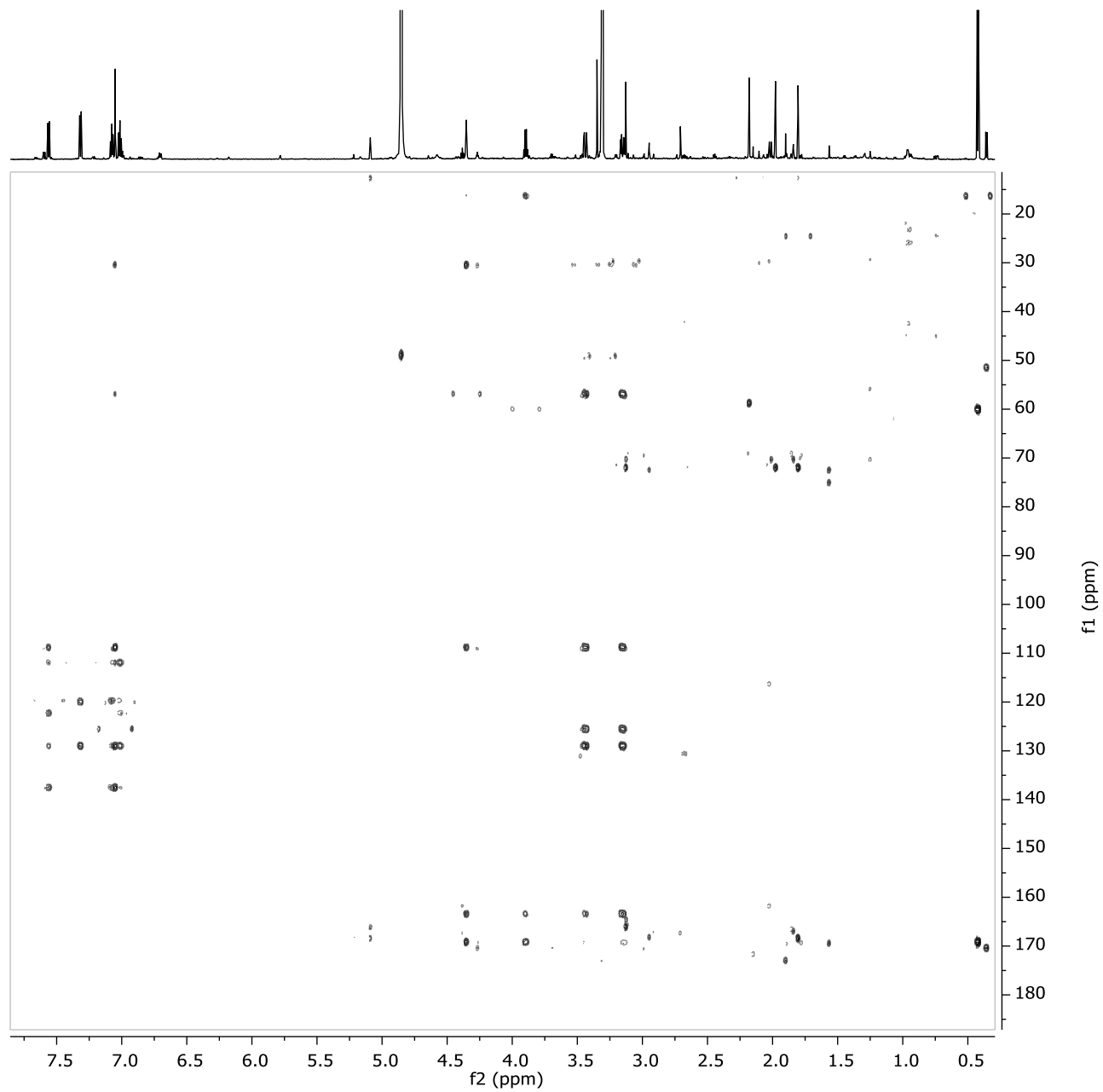


Figure S15. HMBC spectrum of **2** in methanol- $d_4$  at 700 MHz.

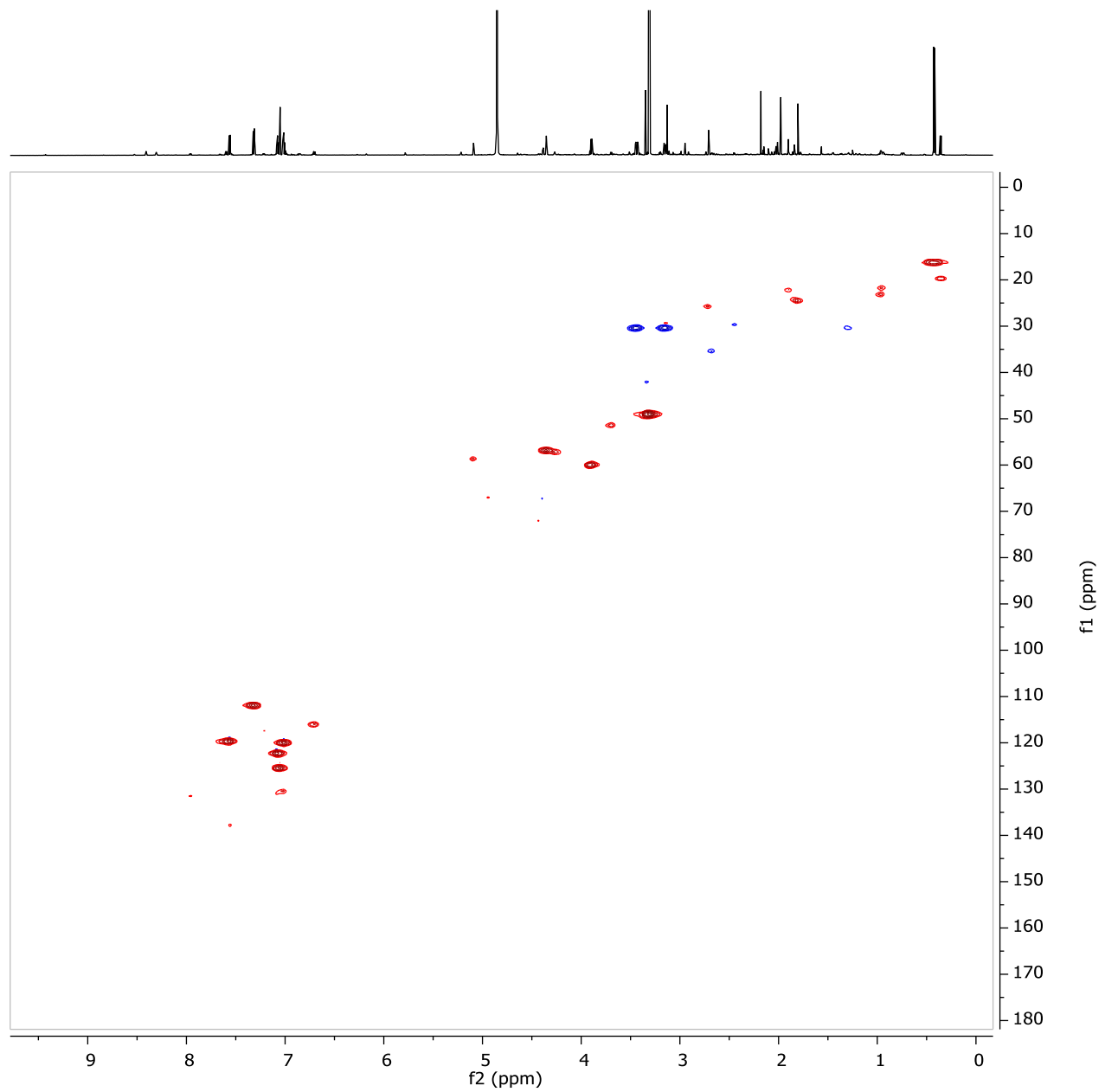


Figure S16. HSQC spectrum of **2** in methanol- $d_4$  at 700 MHz.

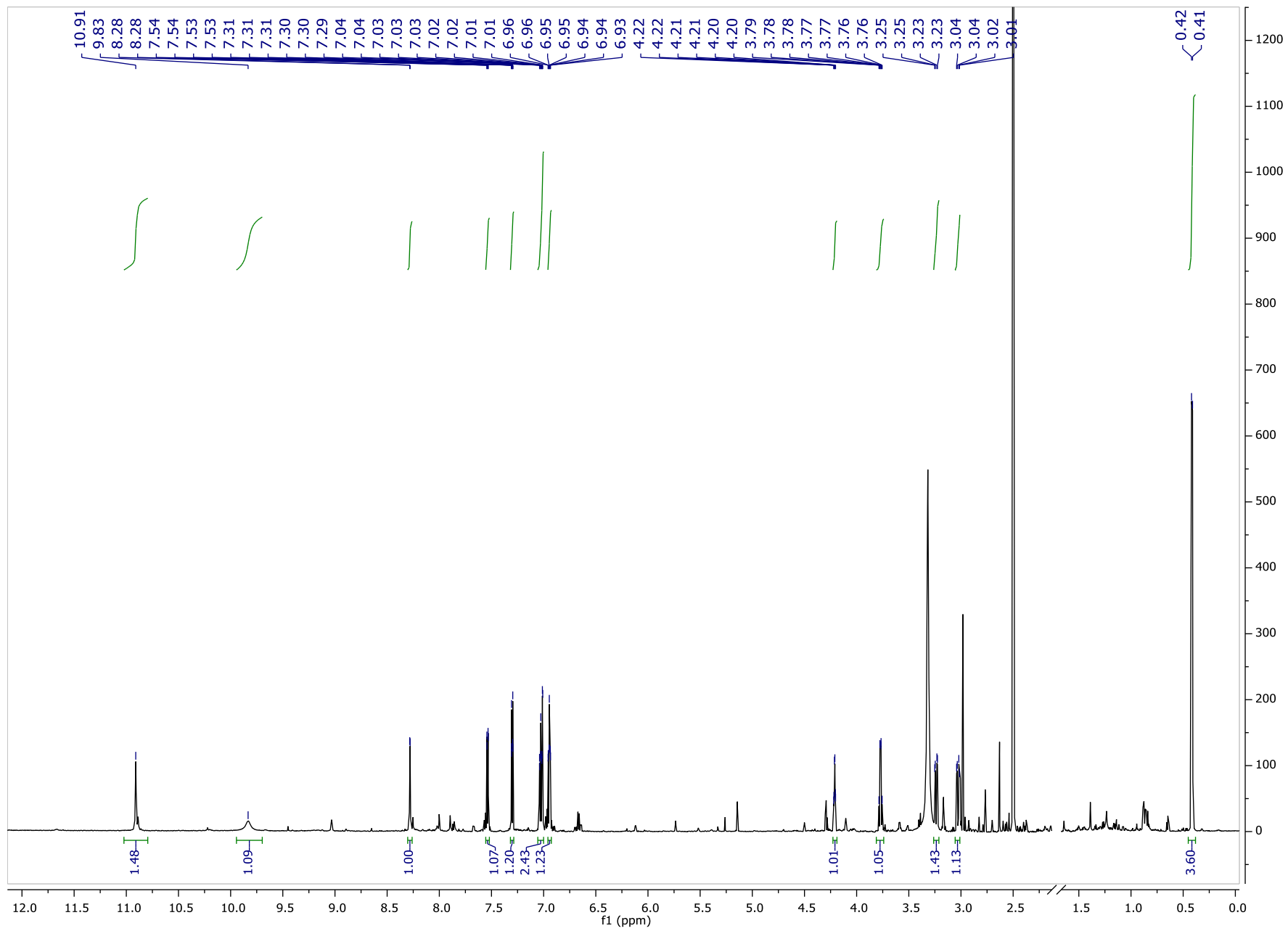


Figure S17.  $^1\text{H}$  NMR spectrum of **2** in  $\text{DMSO-}d_6$  at 700 MHz.

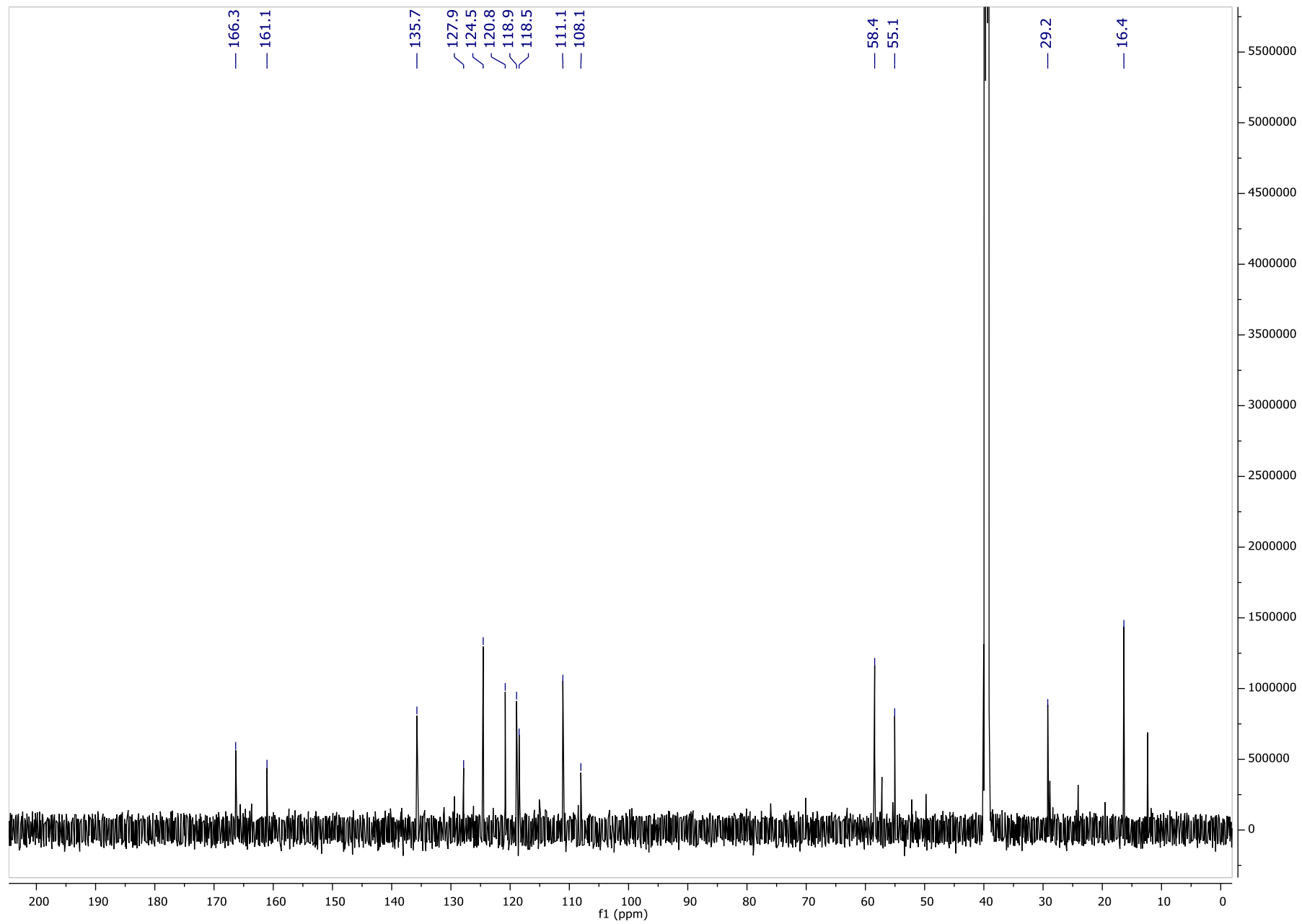


Figure S18.  $^{13}\text{C}$  NMR spectrum of **2** in  $\text{DMSO-}d_6$  at 175 MHz.

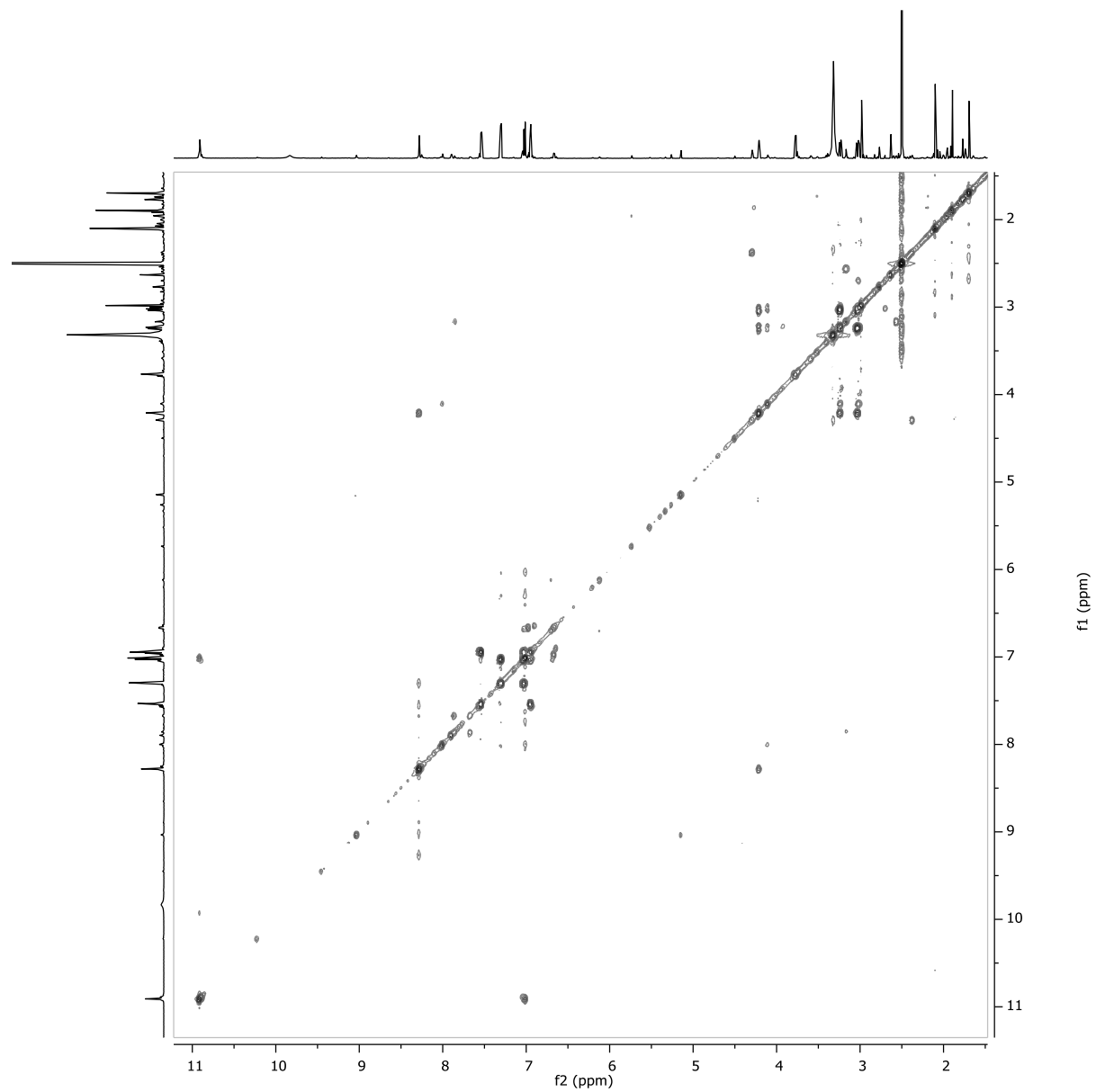


Figure S19.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **2** in  $\text{DMSO-}d_6$  at 700 MHz.

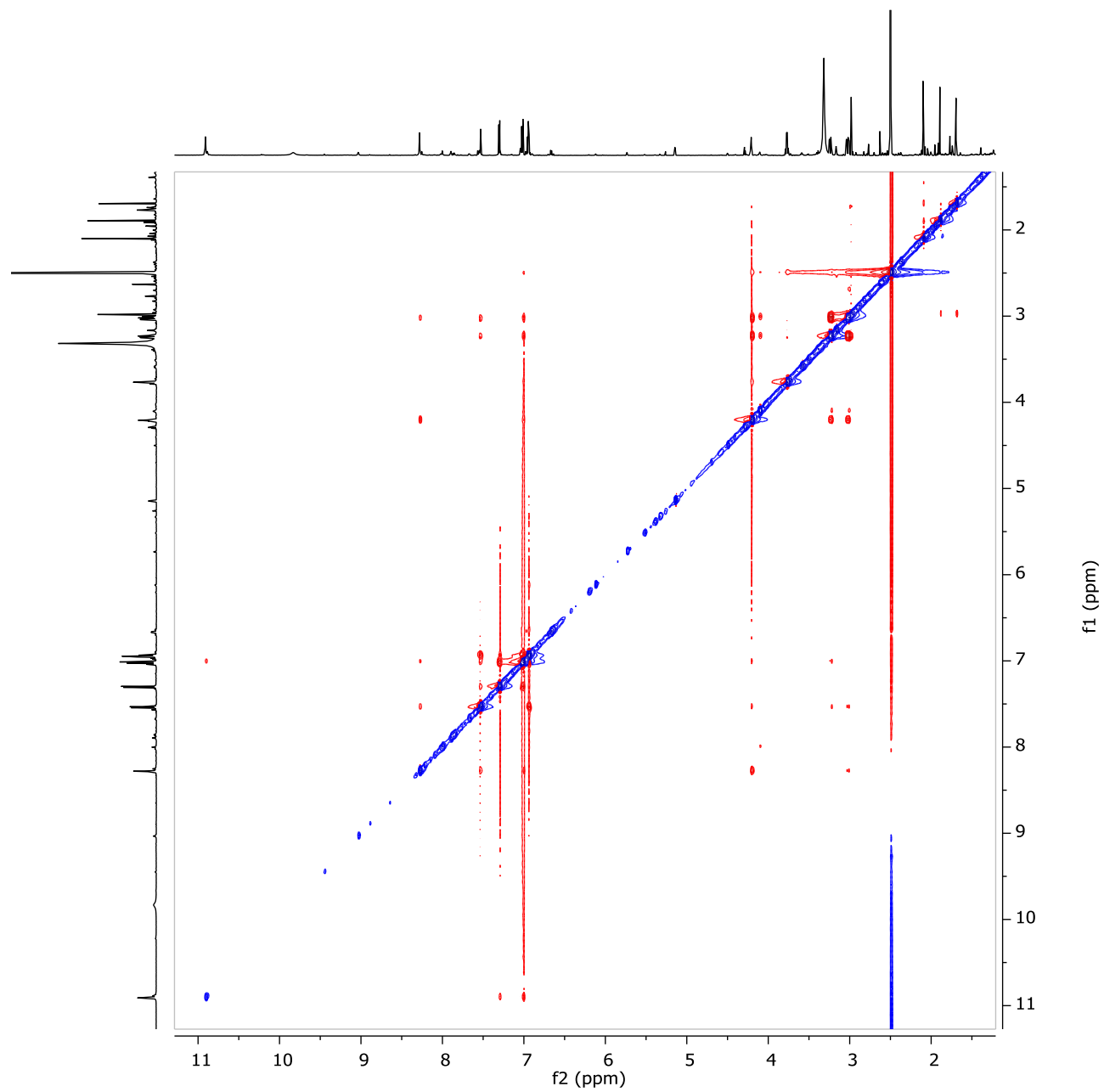


Figure S20. ROESY spectrum of **2** in DMSO-*d*<sub>6</sub> at 700 MHz.

# Generic Display Report

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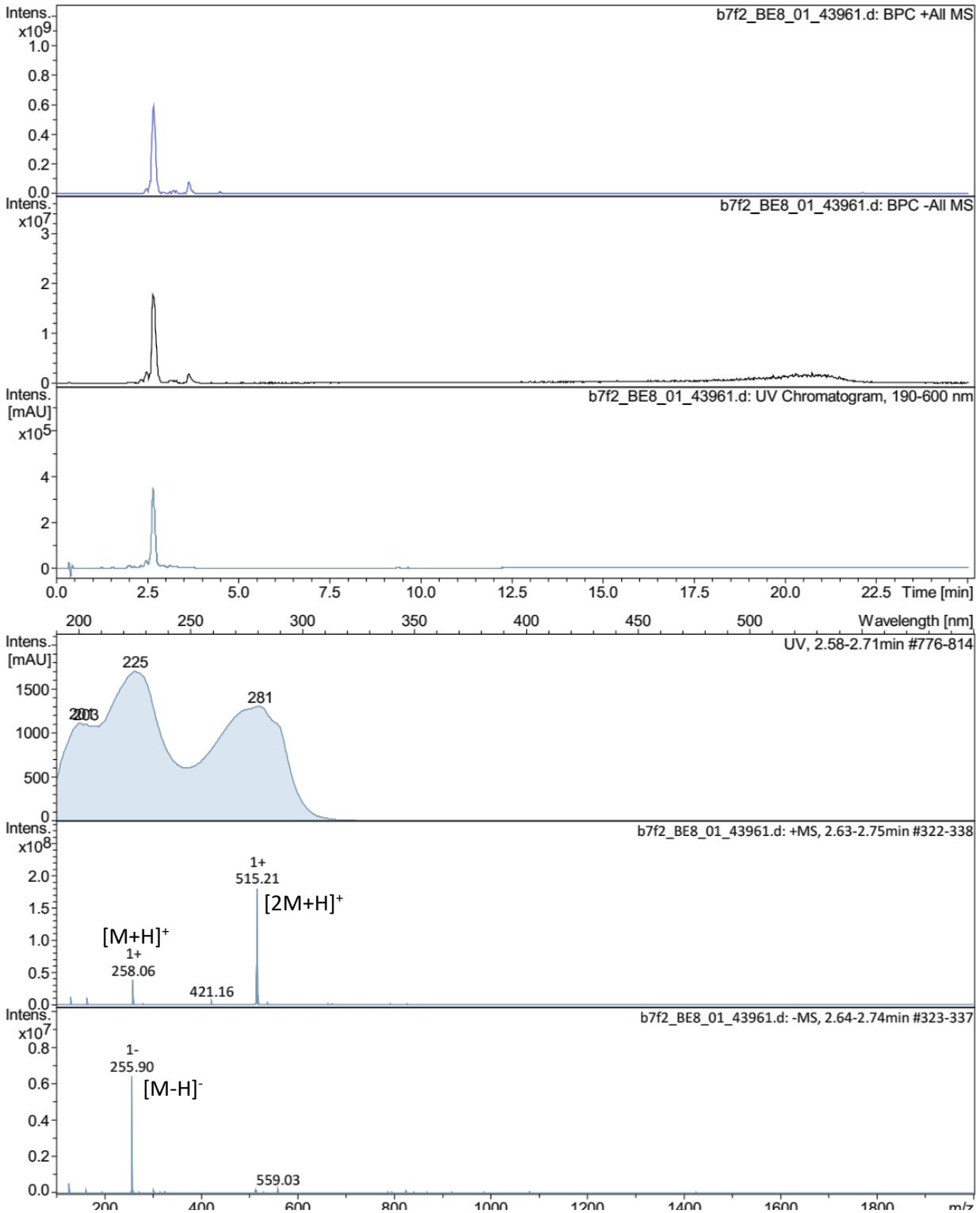


Figure S21. LRESIMS of 3.



# Generic Display Report

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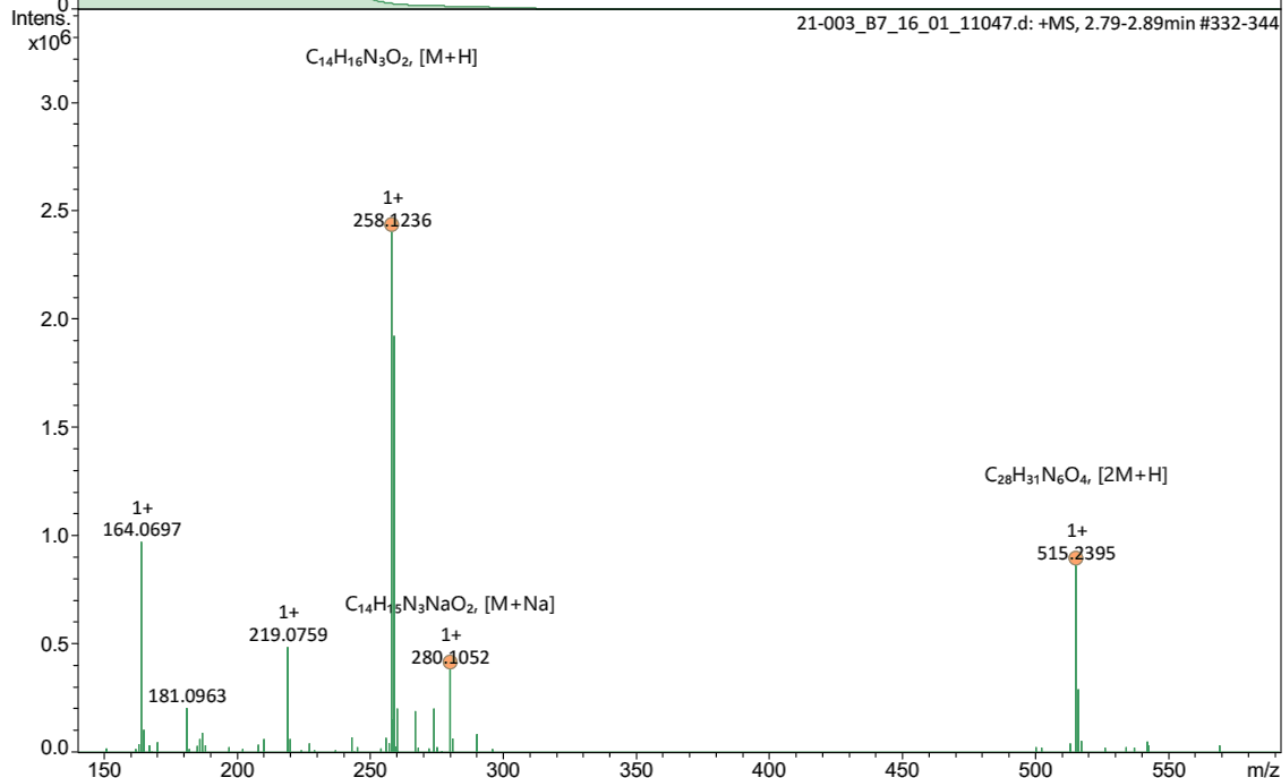
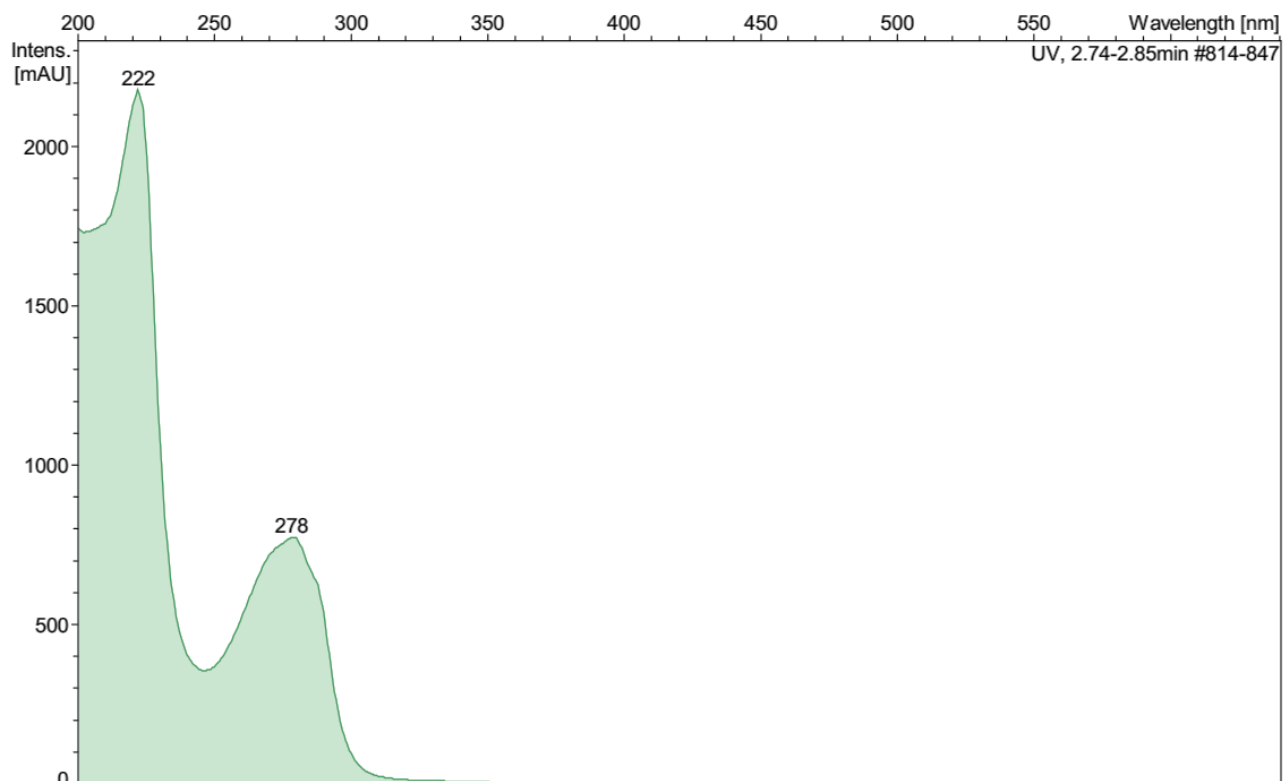


Figure S22. HRESIMS of 3.

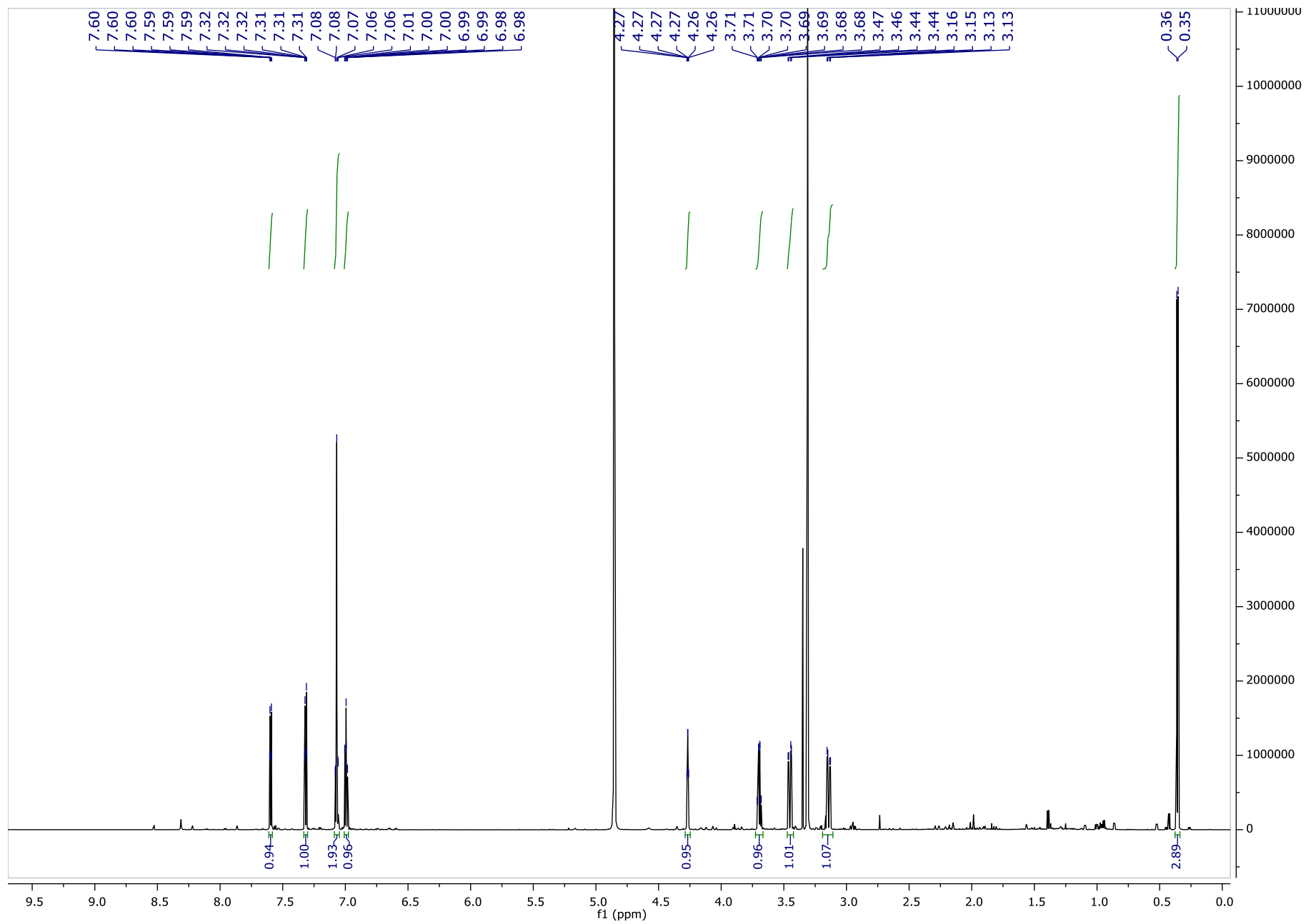


Figure S23.  $^1\text{H}$  NMR spectrum of **3** in methanol- $d_4$  at 700 MHz.

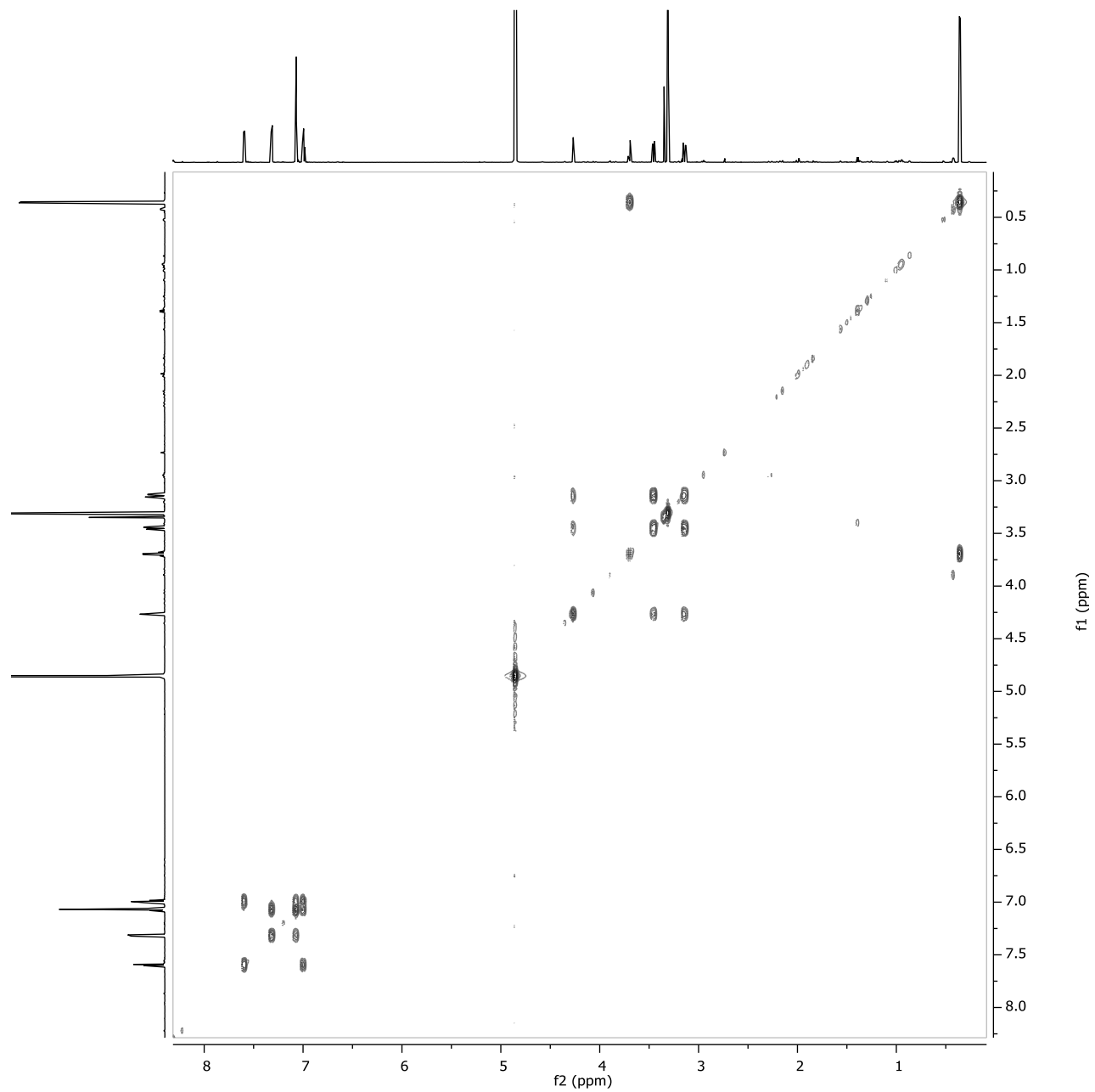


Figure S24.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **3** in methanol- $d_4$  at 700 MHz.

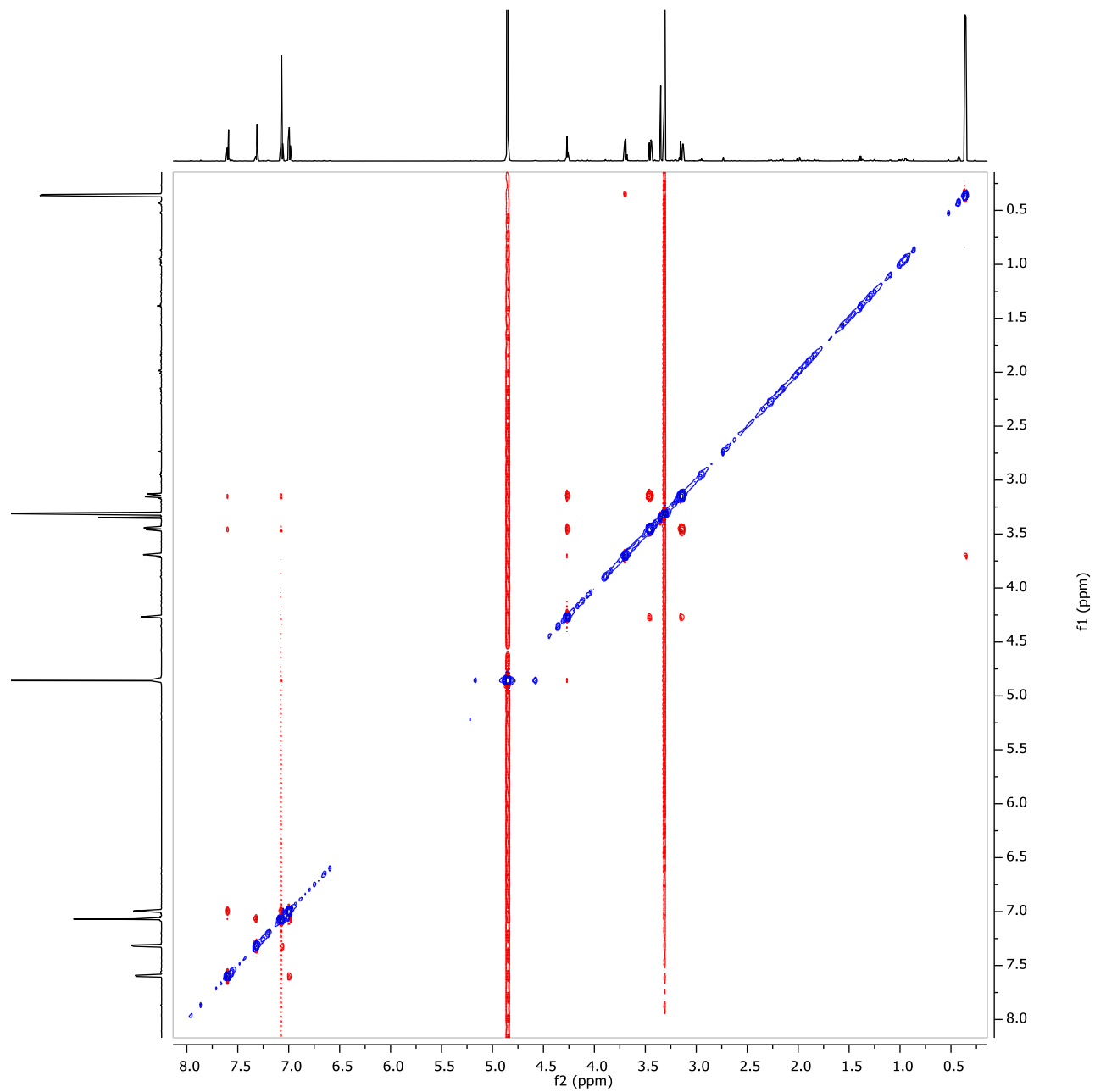


Figure S25. ROESY spectrum of **3** in methanol-*d*<sub>4</sub> at 700 MHz.

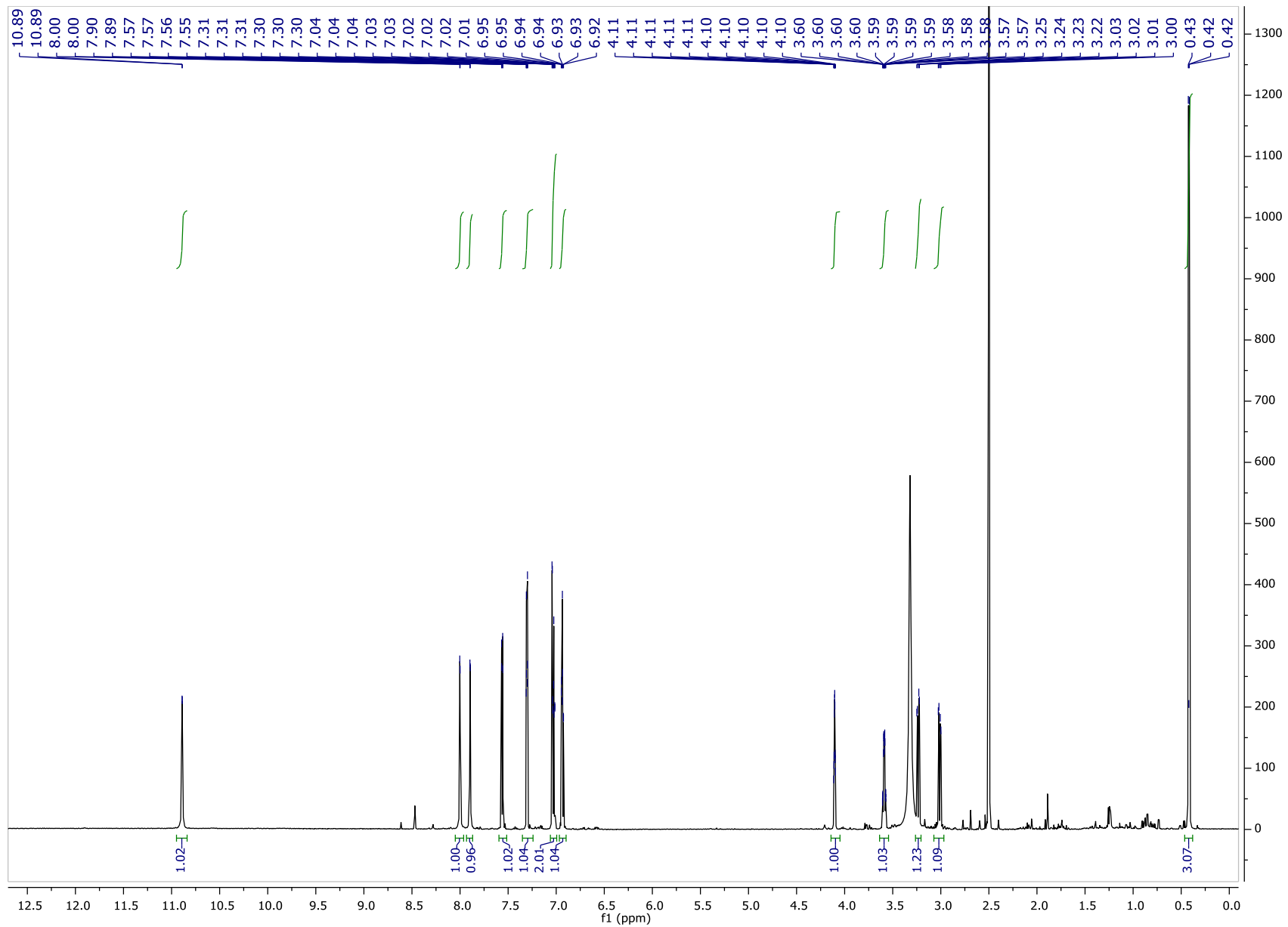


Figure S26.  $^1\text{H}$  NMR spectrum of **3** in  $\text{DMSO-}d_6$  at 700 MHz.

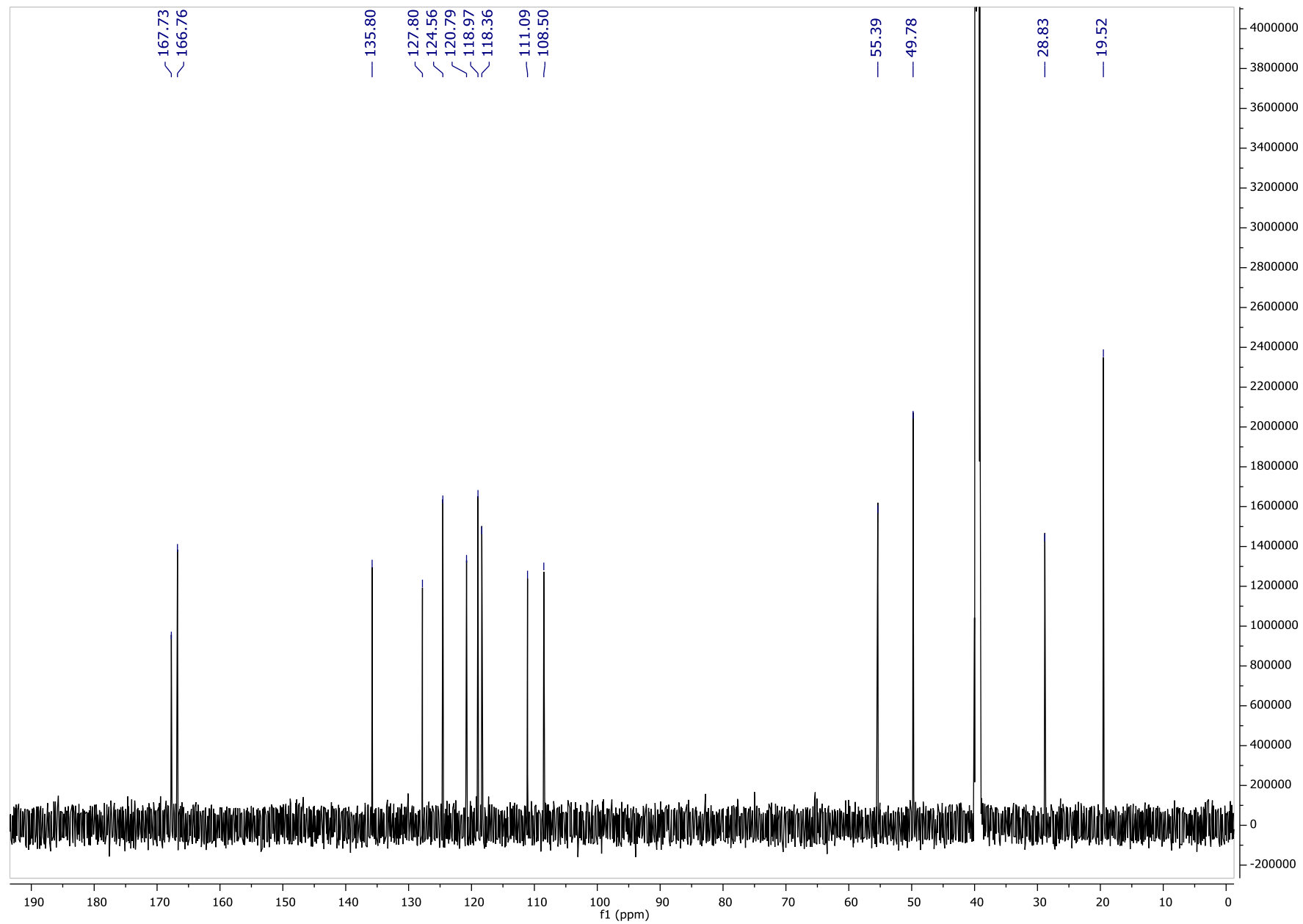


Figure S27.  $^{13}\text{C}$  NMR spectrum of **3** in  $\text{DMSO-}d_6$  at 175 MHz.

# Generic Display Report

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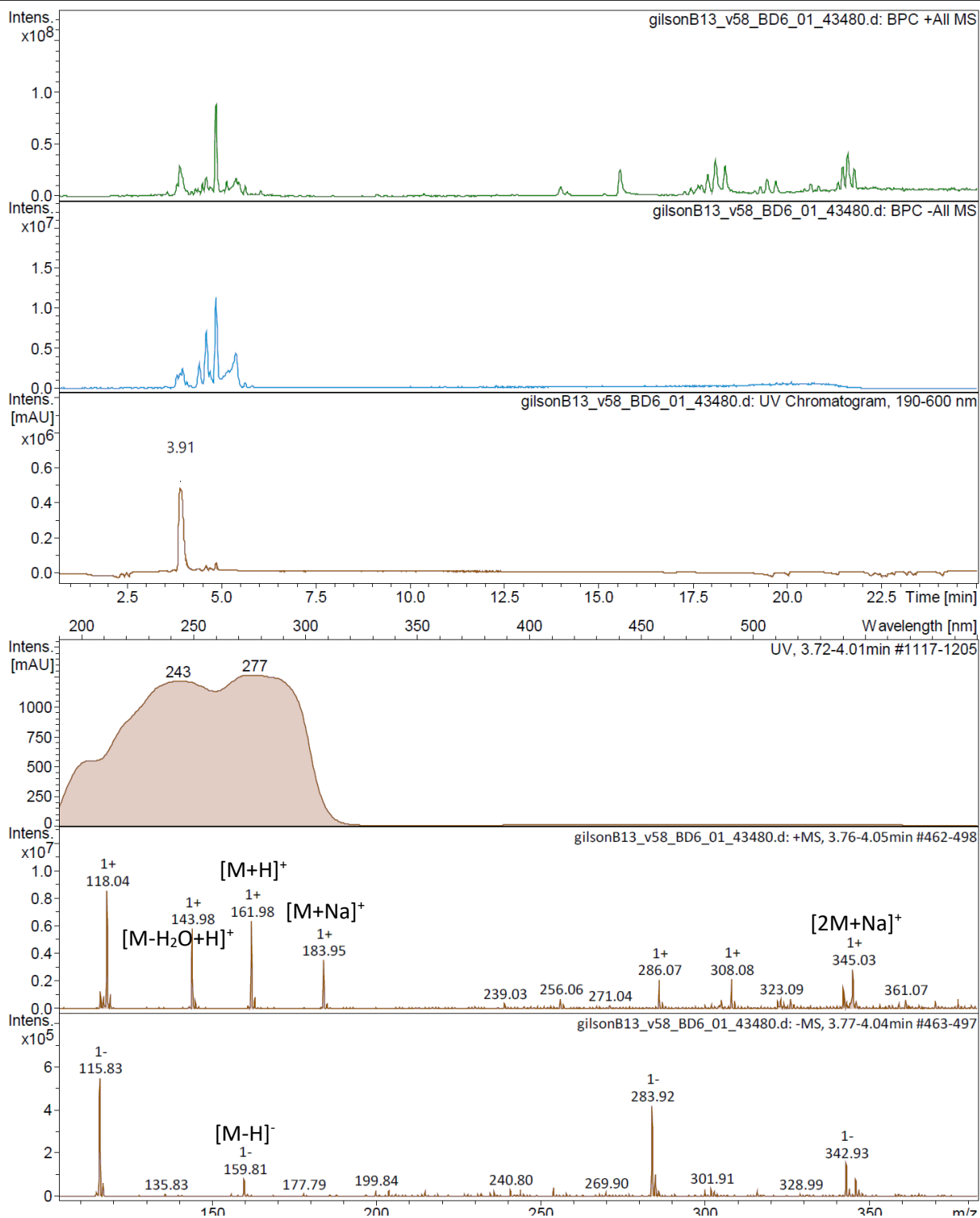


Figure S28. LRESIMS of 4.

# Generic Display Report

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Acquisition Date 07.12.2022 17:08:01  
Operator ate06  
Instrument maXis

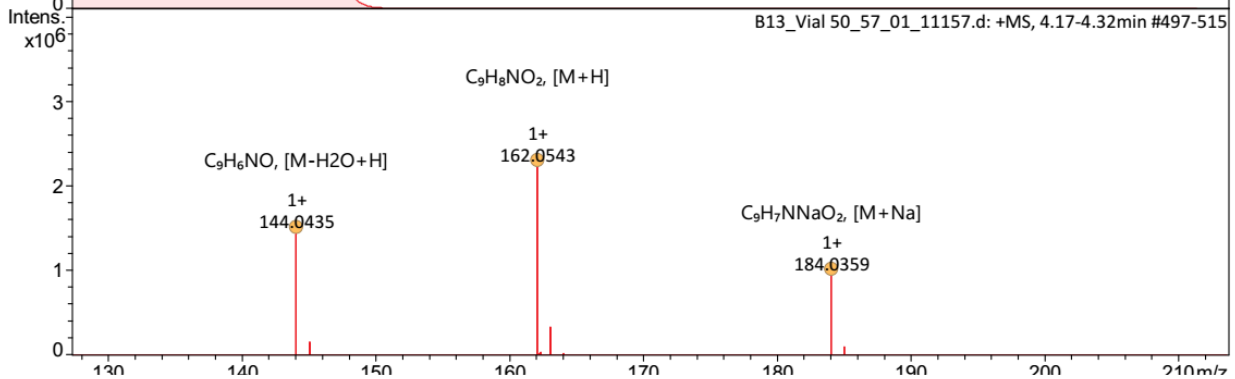
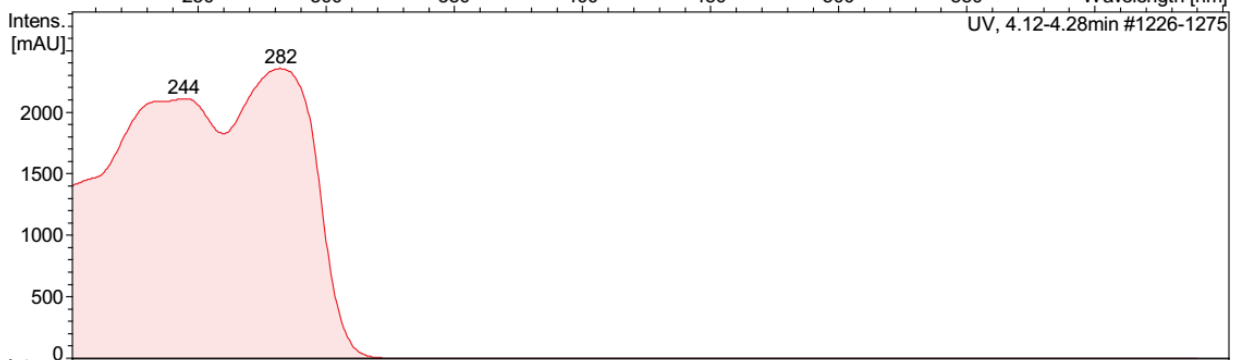
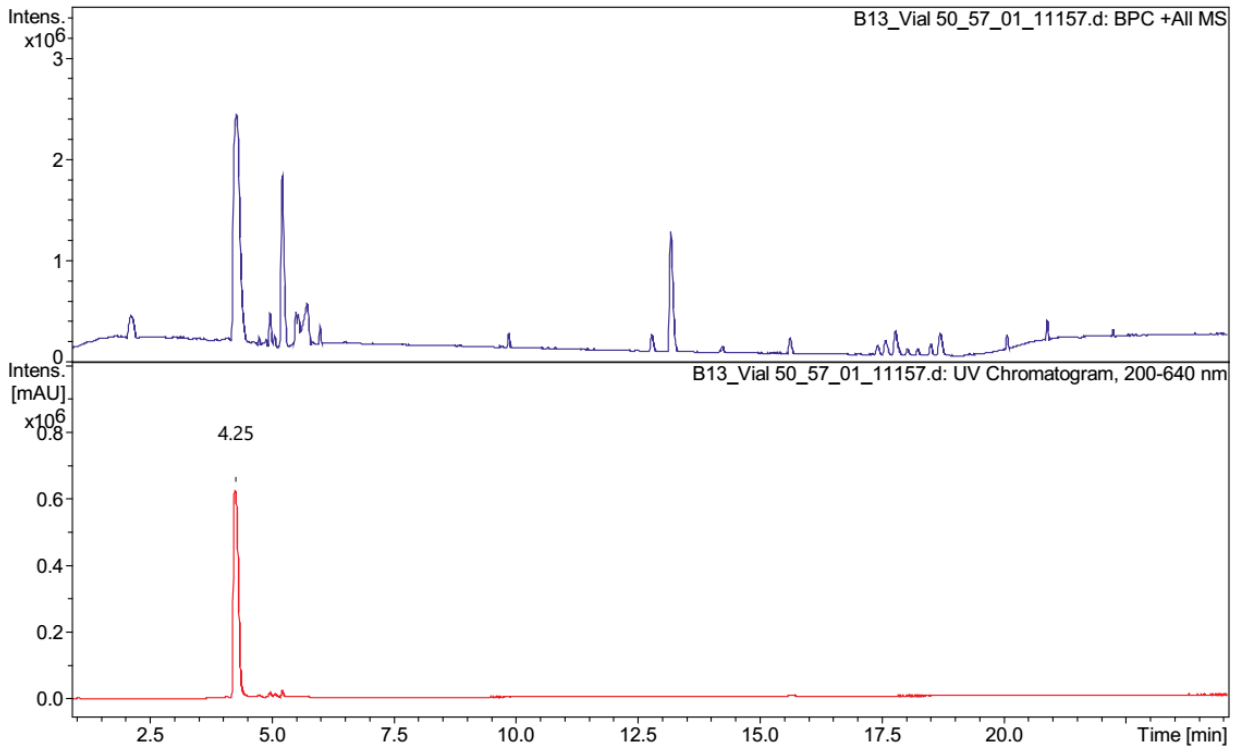


Figure S29. HRESIMS of 4.



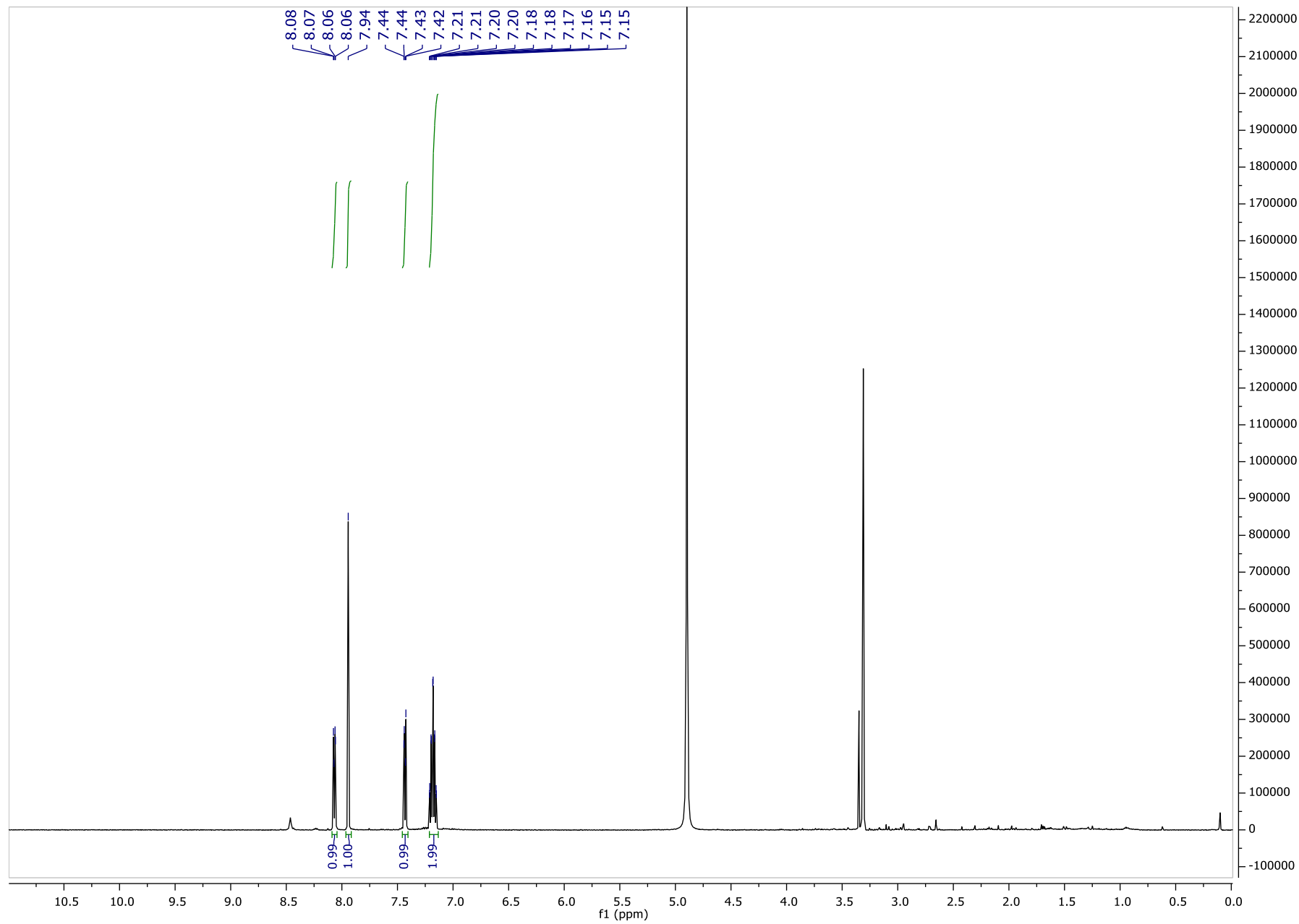


Figure S30.  $^1\text{H}$  NMR spectrum of **4** in  $\text{methanol-}d_4$  at 500 MHz.

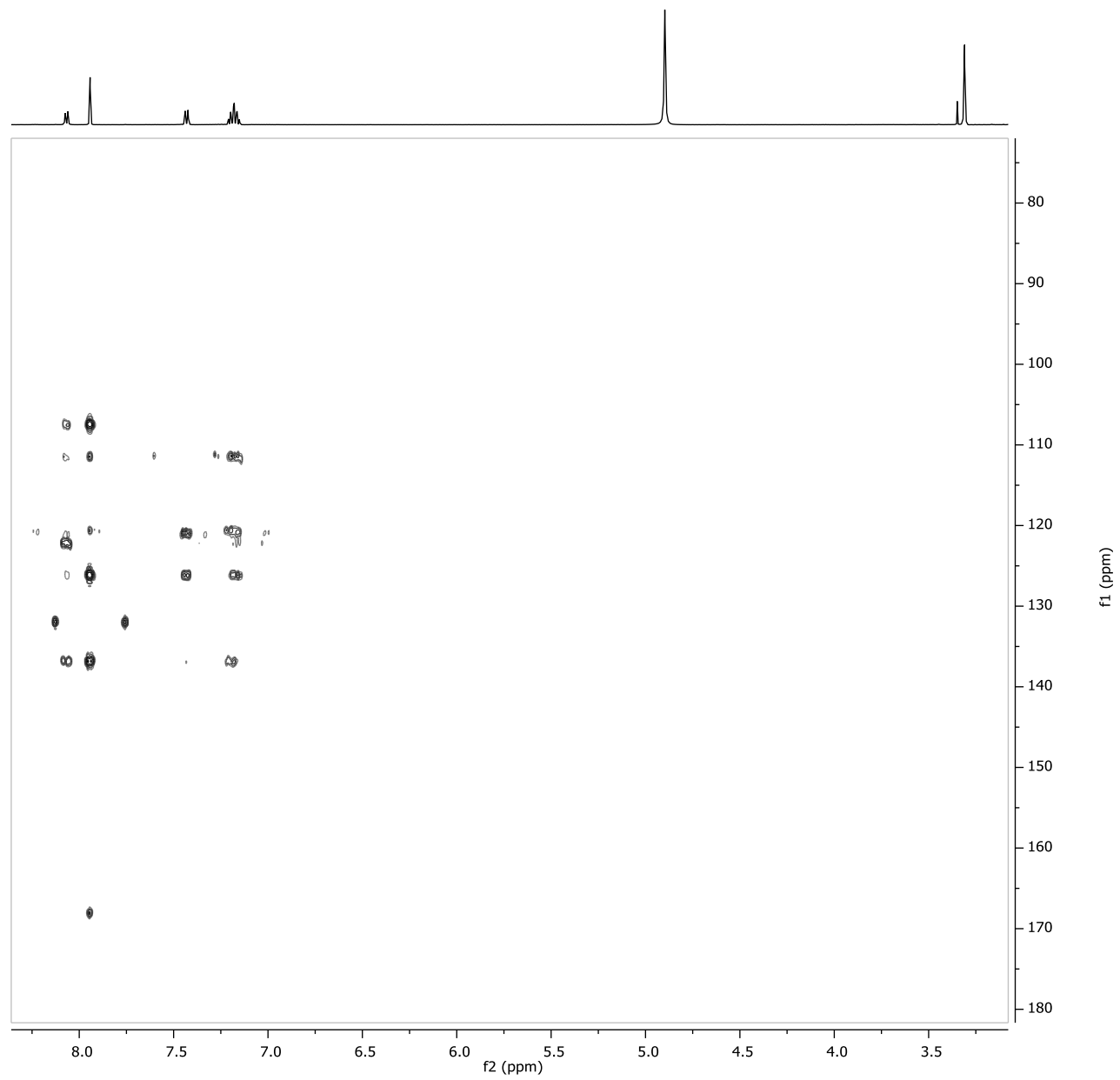


Figure S31. HMBC spectrum of **4** in methanol-*d*<sub>4</sub> at 500 MHz.

# Generic Display Report

## Analysis Info

Analysis Name S:\DATA\AmaZon\sel22\_Sherif Elsayed\21-00003 gillson\_2nd\21-0003 B18\_BC4\_01\_42944.d  
Method 42944.m  
Sample Name 21-0003 B18  
Comment

Acquisition Date 11.11.2022 01:53:49  
Operator esu  
Instrument amaZon speed

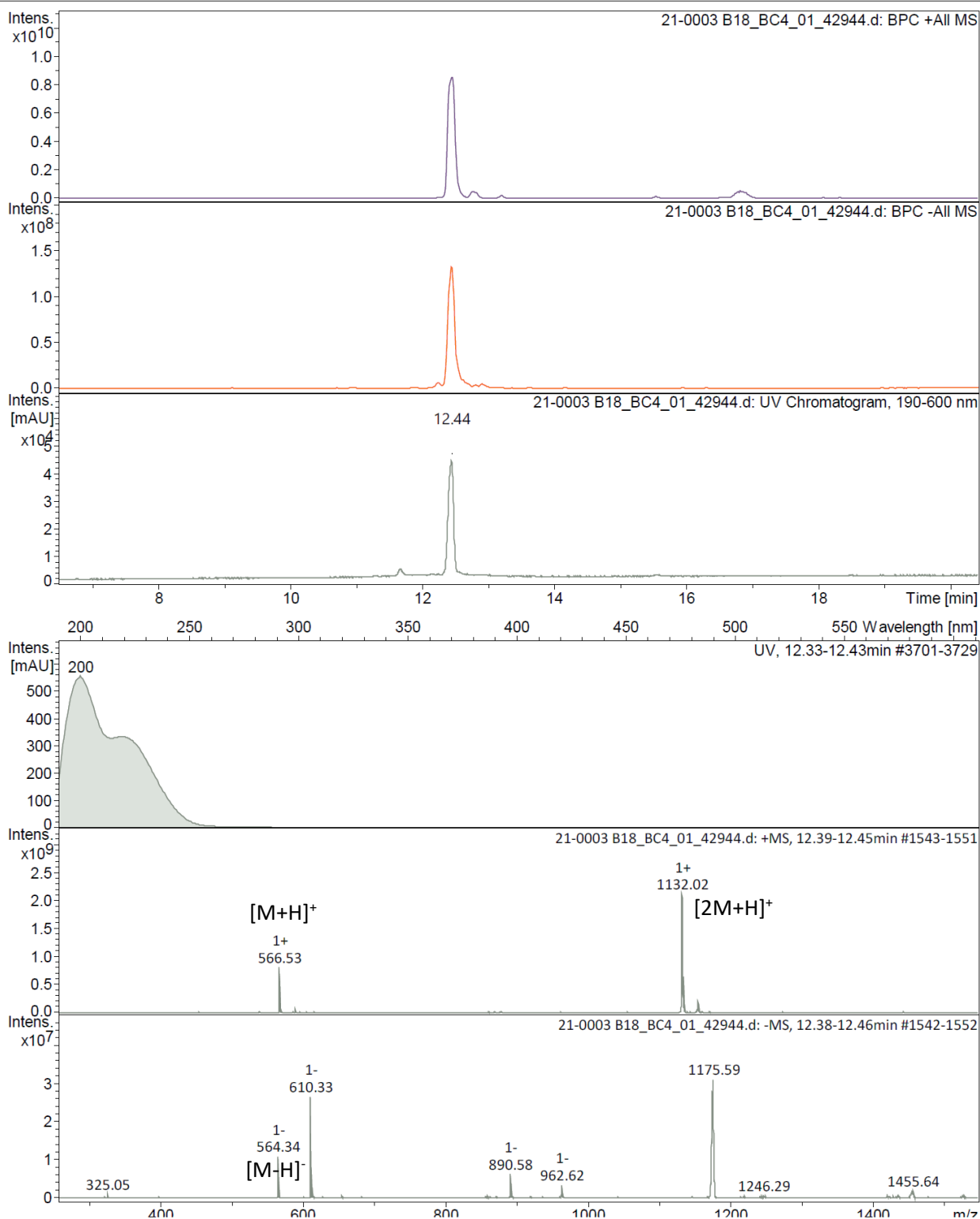


Figure S32. LRESIMS of 5.

# Generic Display Report

## Analysis Info

Analysis Name S:\DATA\Maxis\sel22\_SherifElsayed\22\_11\_MAxis\21-003\_B18\_19\_01\_11052.d  
Method pos\_säure\_10000\_screening\_ms\_100\_2500\_line.m  
Sample Name 21-003\_B18  
Comment Screening01  
Waters Acquity UPLC BEH C\_18 1,7um 2.1x50mm

Acquisition Date 23.11.2022 09:01:14  
Operator ate06  
Instrument maXis

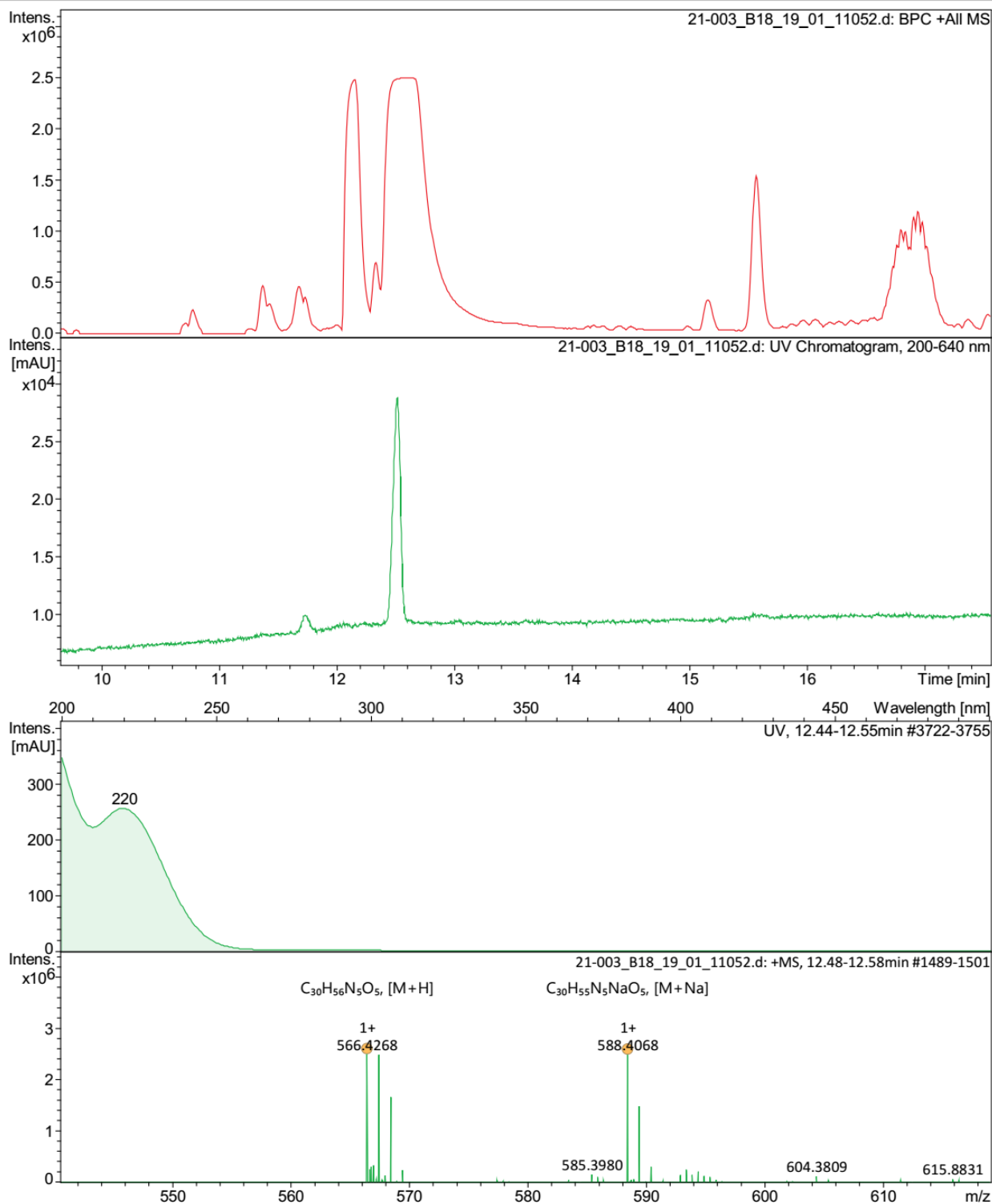


Figure S33. HRESIMS of 5.

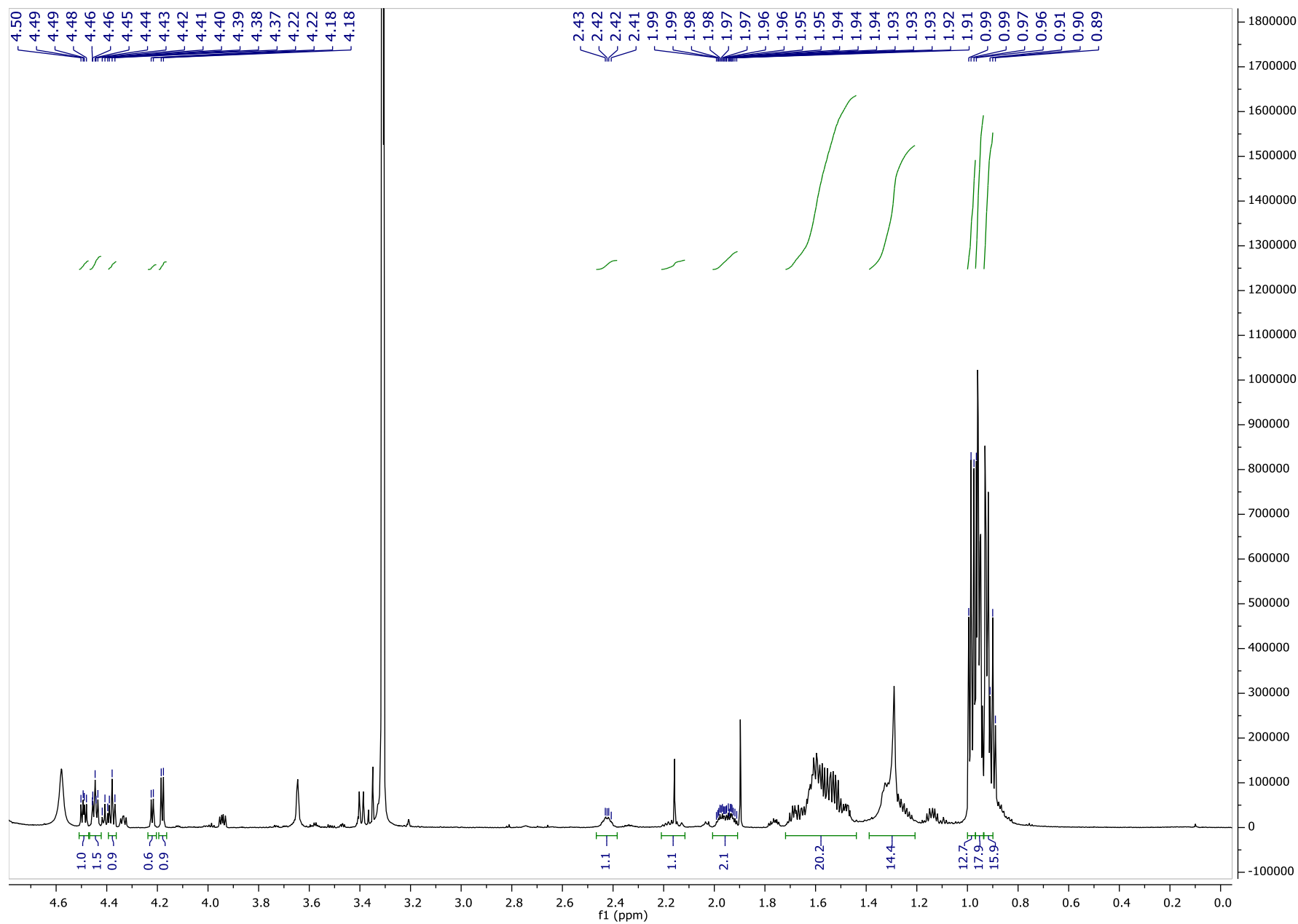


Figure S34.  $^1\text{H}$  NMR spectrum of **5** in methanol- $d_4$  at 700 MHz.

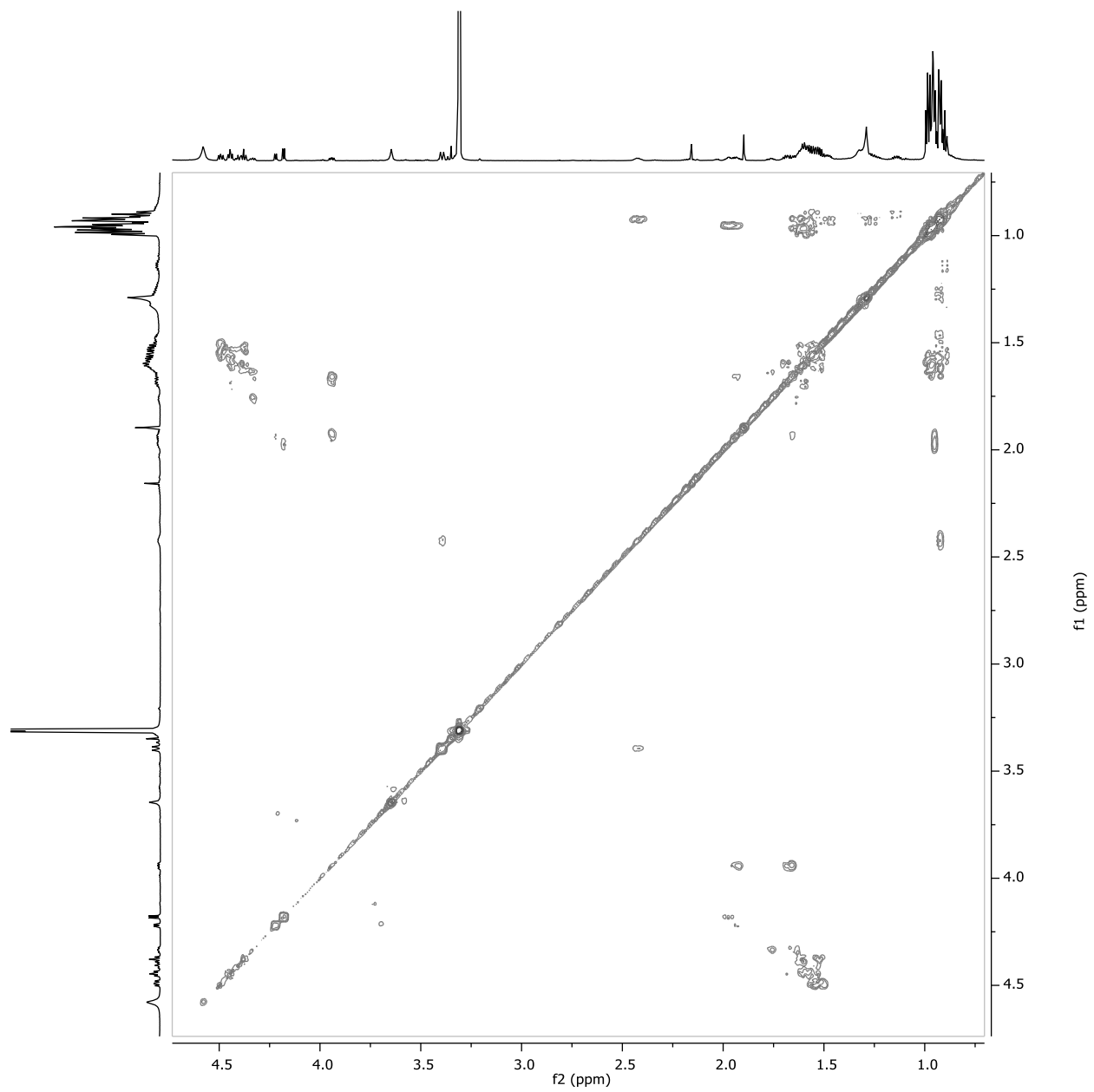


Figure S35.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **5** in methanol- $d_4$  at 700 MHz.

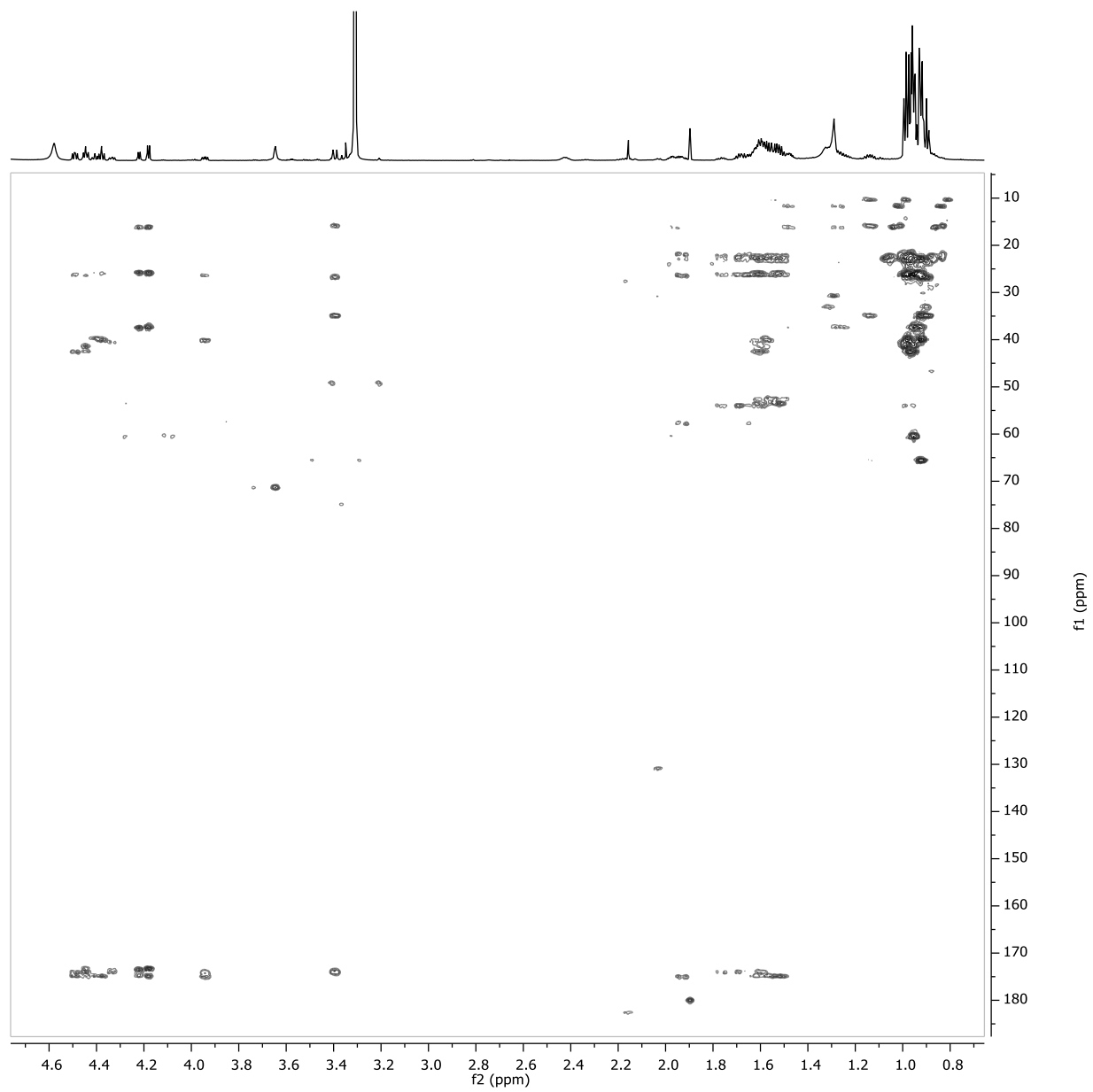


Figure S36. HMBC spectrum of **5** in methanol- $d_4$  at 700 MHz.

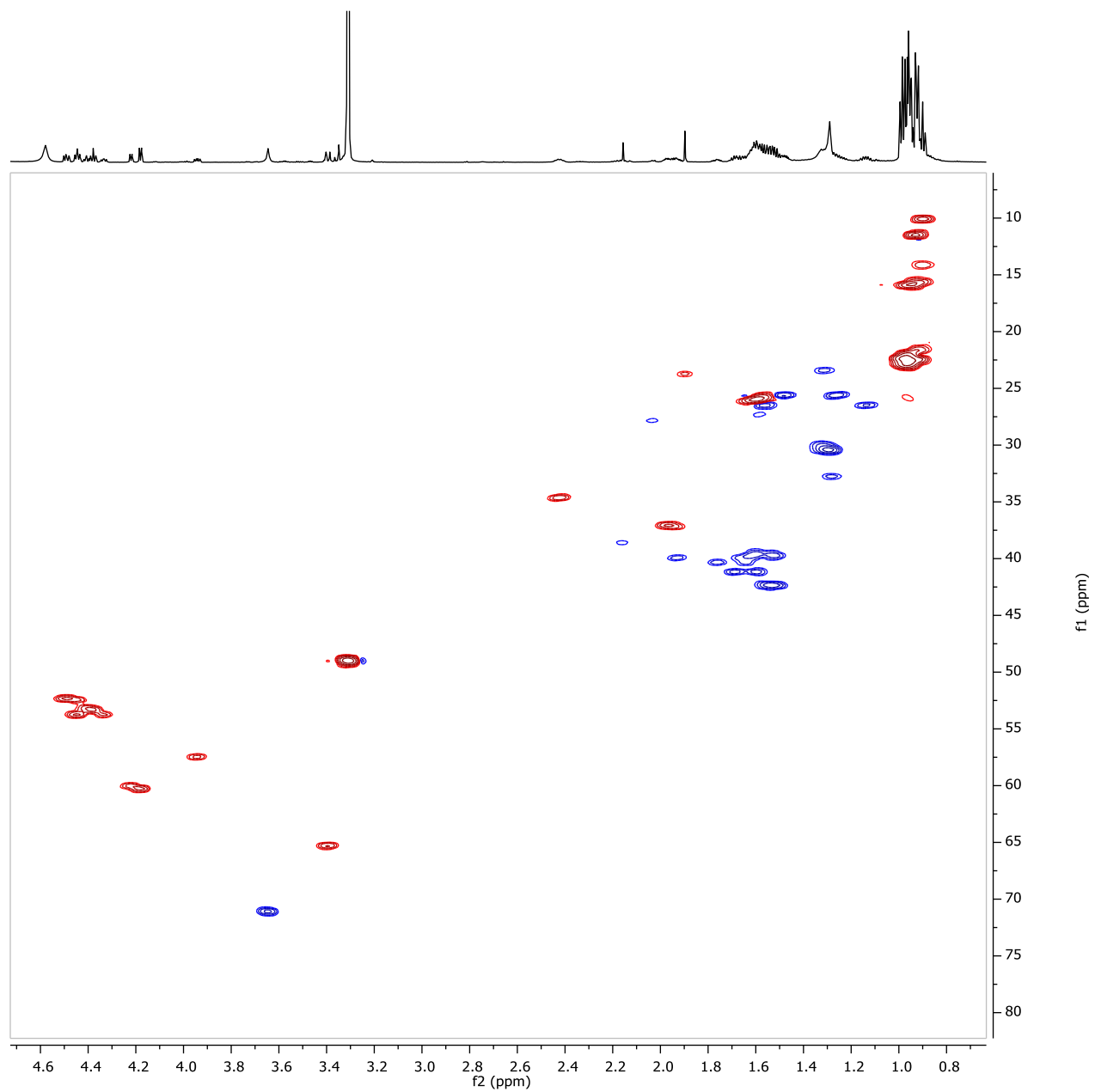


Figure S37. HSQC spectrum of **5** in methanol-*d*<sub>4</sub> at 700 MHz.