Supplementary Materials for

Anti-Inflammatory, Antiallergic and COVID-19 Main Protease (M^{pro}) Inhibitory Activities of Butenolides from a Marine-Derived Fungus *Aspergillus terreus*

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ABSTRACT

In December 2020, the UK authorities reported to the WHO that a new COVID-19 variant, considered to be a Variant Under Investigation from December 2020 (VUI-202012/01) was identified through viral genomic sequencing. However, several other mutants were previously reported, VUI-202012/01 proved to be about 70% more transmissible. Hence, the usefulness and effectiveness of the newly FDA-approved COVID-19 vaccines against these new variants are doubtfully questioned. As a result of these unexpected mutants from COVID-19 and due to lack of time, much research interest is directed toward assessing secondary metabolites as potential candidates for developing lead pharmaceuticals. In this study, a marine-derived fungus *Aspergillus terreus* was investigated affording two butenolide derivatives, butyrolactones I (**1**) and III (**2**), a meroterpenoid, terretonin (**3**) and 4-hydroxy-3-(3-methylbut-2-enyl)benzaldehyde (**4**). Chemical structures were unambiguously determined based on mass spectrometry and extensive 1D/2D NMR analyses experiments. Compounds (**1**-**4**) were assessed for their *in vitro* anti-inflammatory, antiallergic and *in silico* COVID-19 main protease (M^{pro}) and elastase inhibitory activities. Among the tested compounds, only **1** revealed significant activities comparable or even more potent than respective standard drugs that makes butyrolactone I (**1**) a potential lead entity for developing a new remedy to treat and/or control the currently devastating and deadly effects of COVID-19 pandemic and elastase-related inflammatory complications.

Keywords: *Aspergillus terreus*; butenolides; antiallergic; COVID-19 M^{pro}; elastase

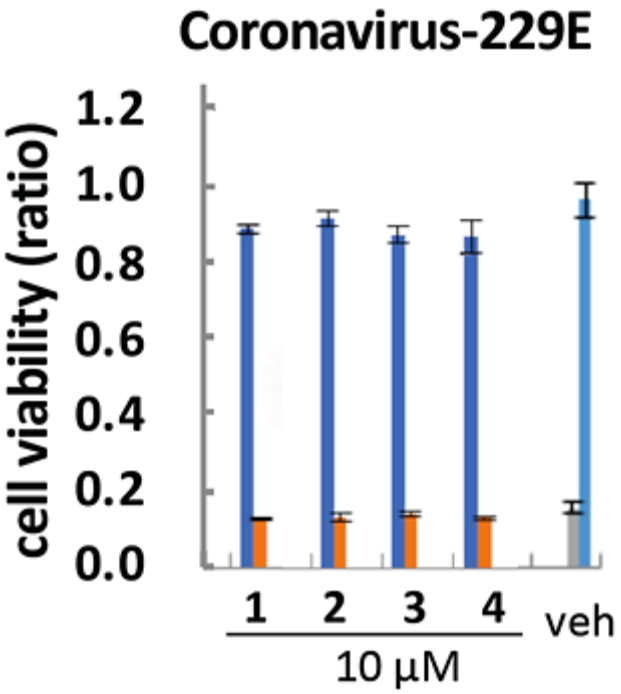
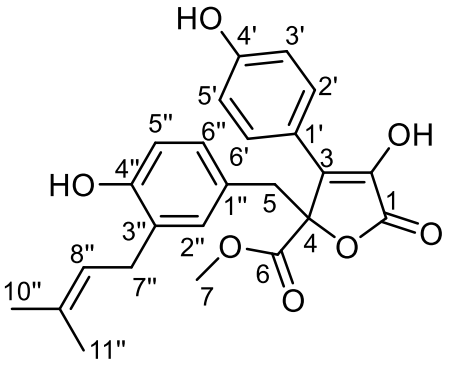


Figure S1. Human coronavirus 229E (HCoV-229E) protective activity. The cells infected by HCoV-229E were treated with the compounds (orange) or vehicle (gray). The difference would indicate protective effects against HCoV-229E infection. The uninfected cells were treated with the compounds (dark blue) or vehicle only (cyan), serving as control for toxicity of the samples and vehicle. Veh; vehicle

Table S1. ^{1}H and ^{13}C NMR Data of butyrolactone I (**1**).



Butyrolactone I (**1**)

Butyrolactone (**1**) in chloroform-*d*, 400 MHz		
#	δ_H (*J*[Hz])	δ_C, type
1		169.8, C
2		144.9, C
3		137.7, C
4		86.2, C
5	3.53, d (15.0)	38.7, CH_2
	3.47, d (15.0)	
6		170.1, C
7	3.75, s (3H)	53.7, CH_3
1'		121.8, C
2'	7.61, d (8.8)	129.6, CH
3'	6.90, d (8.8)	116.1, CH
4'		157.2, C
5'	6.90, d (8.8)	116.1, CH
6'	7.61, d (8.8)	129.6, CH
1"		124.6, C
2"	6.51, d (2.0)	131.8, CH
3"		128.8, C
4"		153.2, C
5"	6.52, d (8.1)	115.1, CH
6"	6.59, dd (8.1, 2.0)	129.2, CH
7"	3.12, d (7.2, 2H)	28.7, CH_2
8"	5.08, td (6.7, 5.7, 3.8)	121.8, CH
9"		133.9, C
10"	1.65, s (3H)	25.7, CH_3
11"	1.59, s (3H)	17.7, CH_3

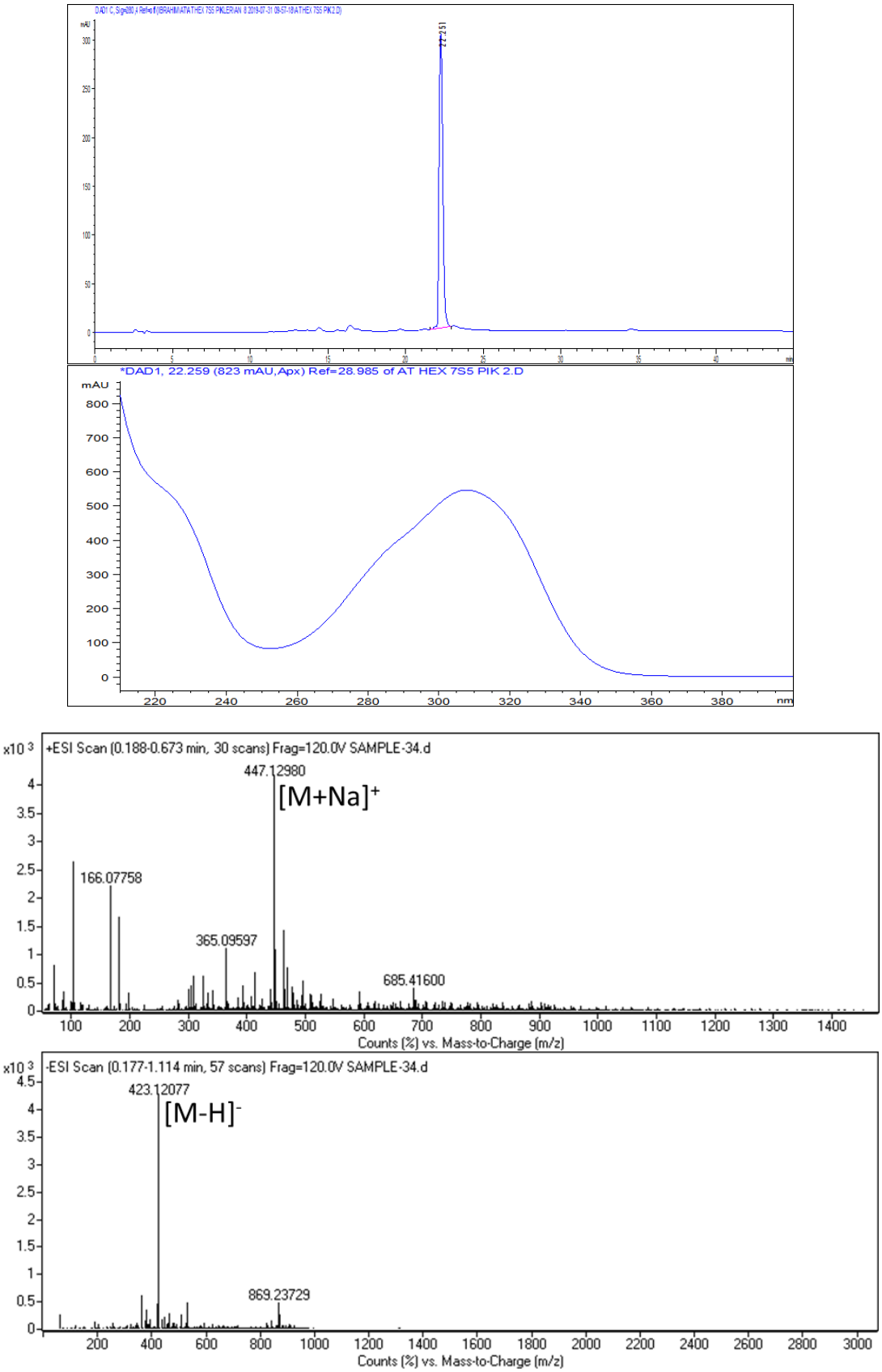


Figure S2. HPLC chromatogram and HRESIMS of **1**

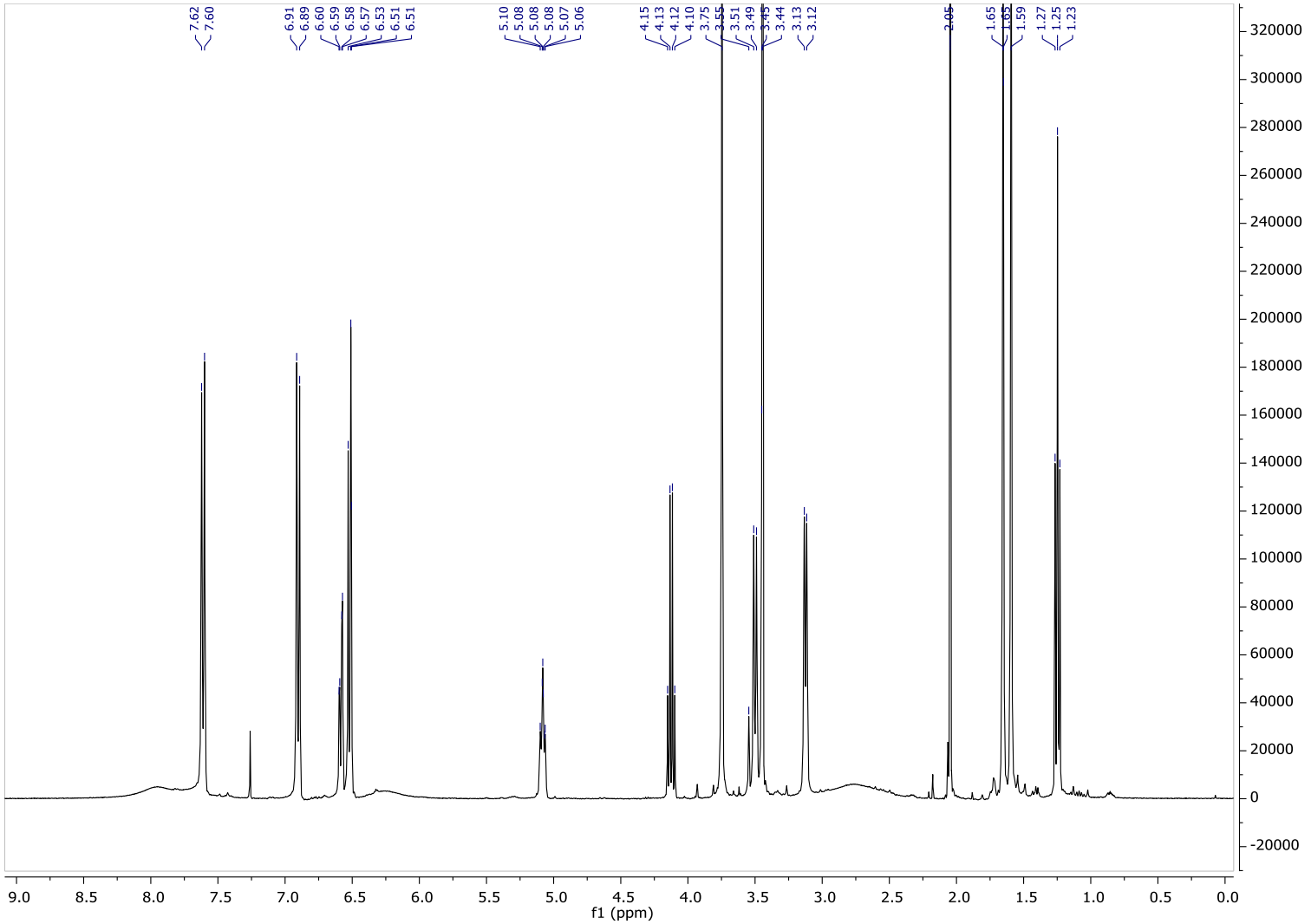


Figure S3. ^{1}H-NMR spectrum of **1** in chloroform-*d*

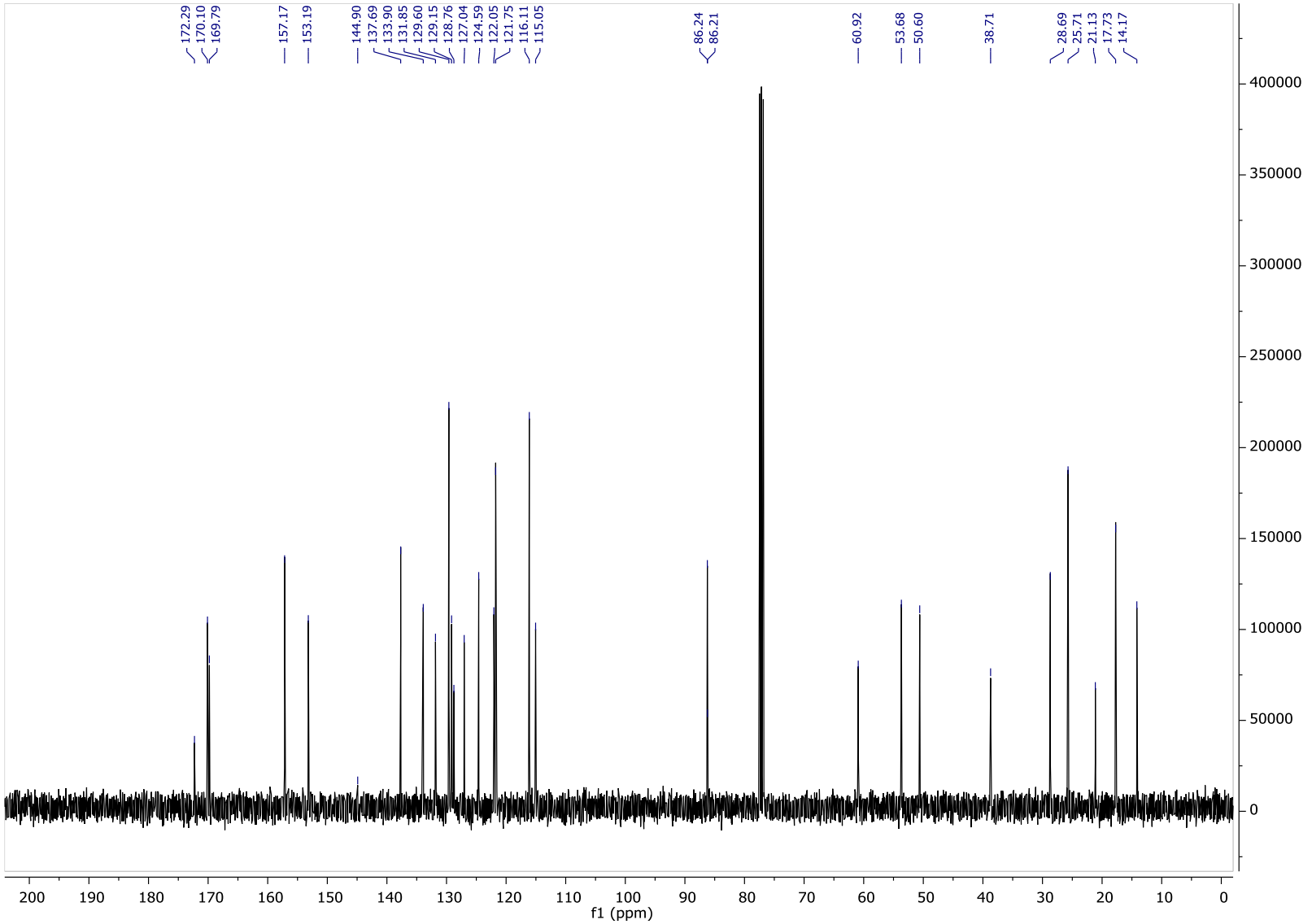


Figure S4. ^{13}C-NMR spectrum of **1** in chloroform-*d*

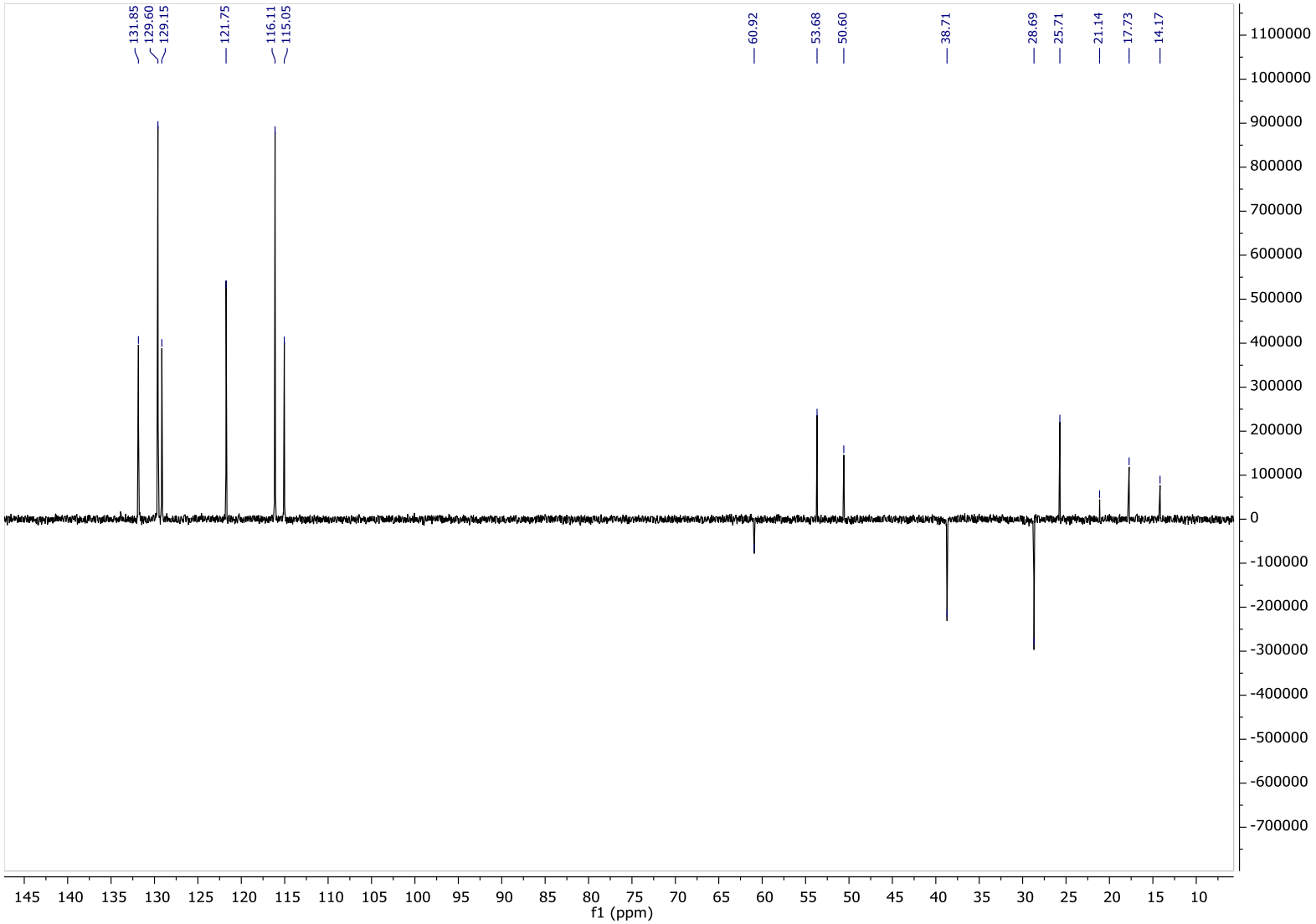


Figure S5. DEPT spectrum of **1** in chloroform-*d*

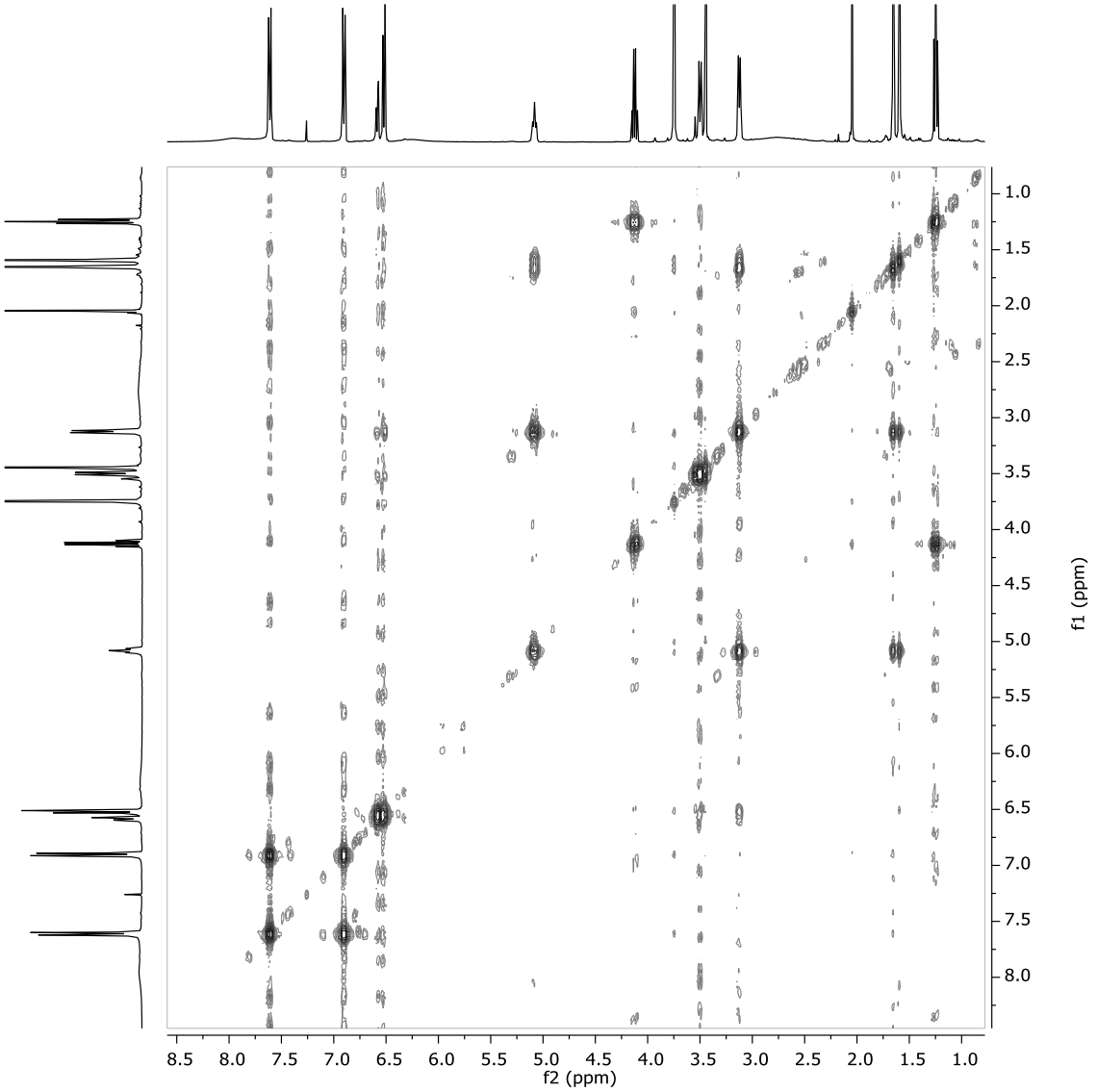


Figure S6. ^{1}H–^{1}H COSY spectrum of **1** in chloroform-*d*

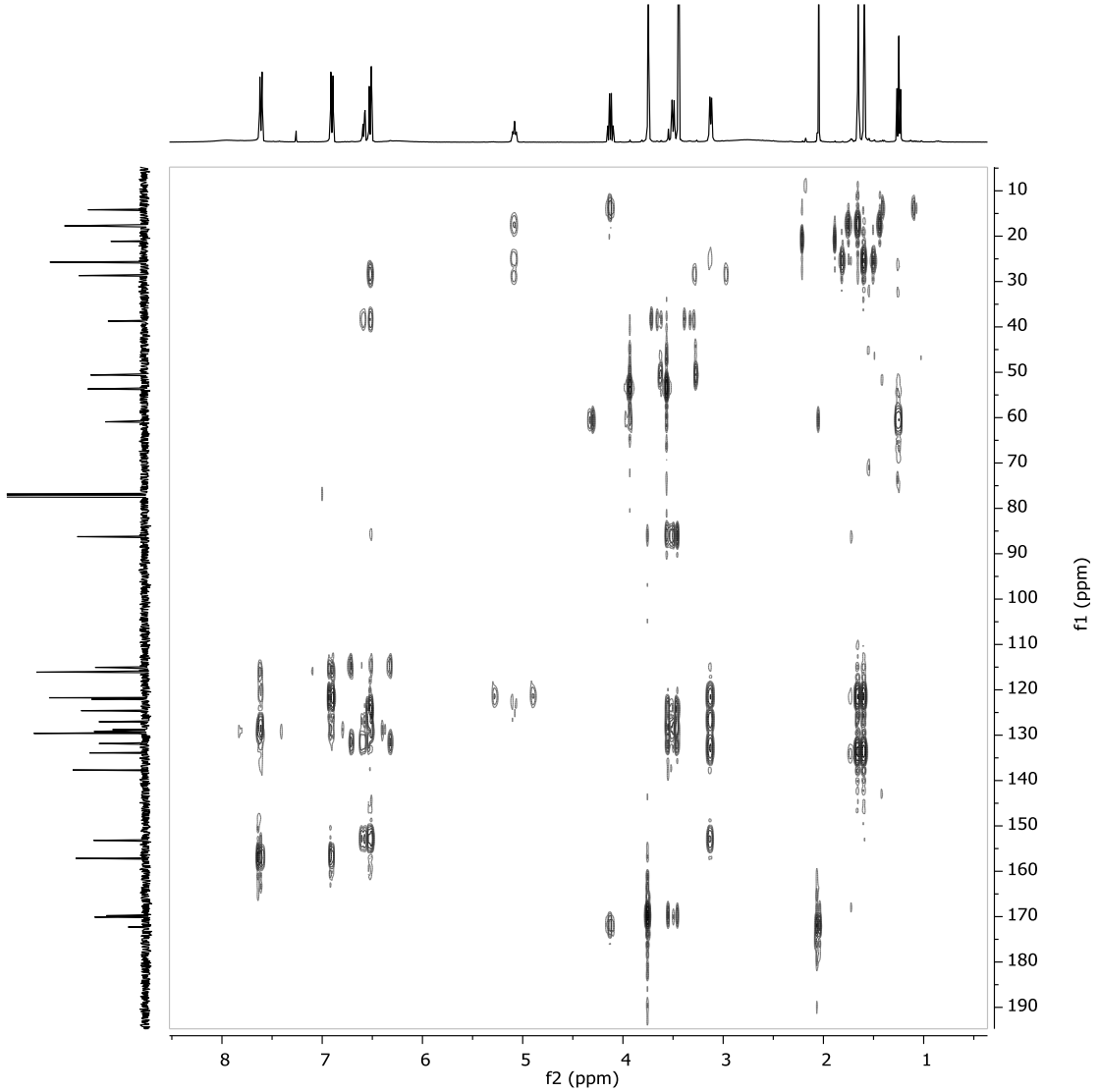


Figure S7. *g*HMBC spectrum of **1** in chloroform-*d*

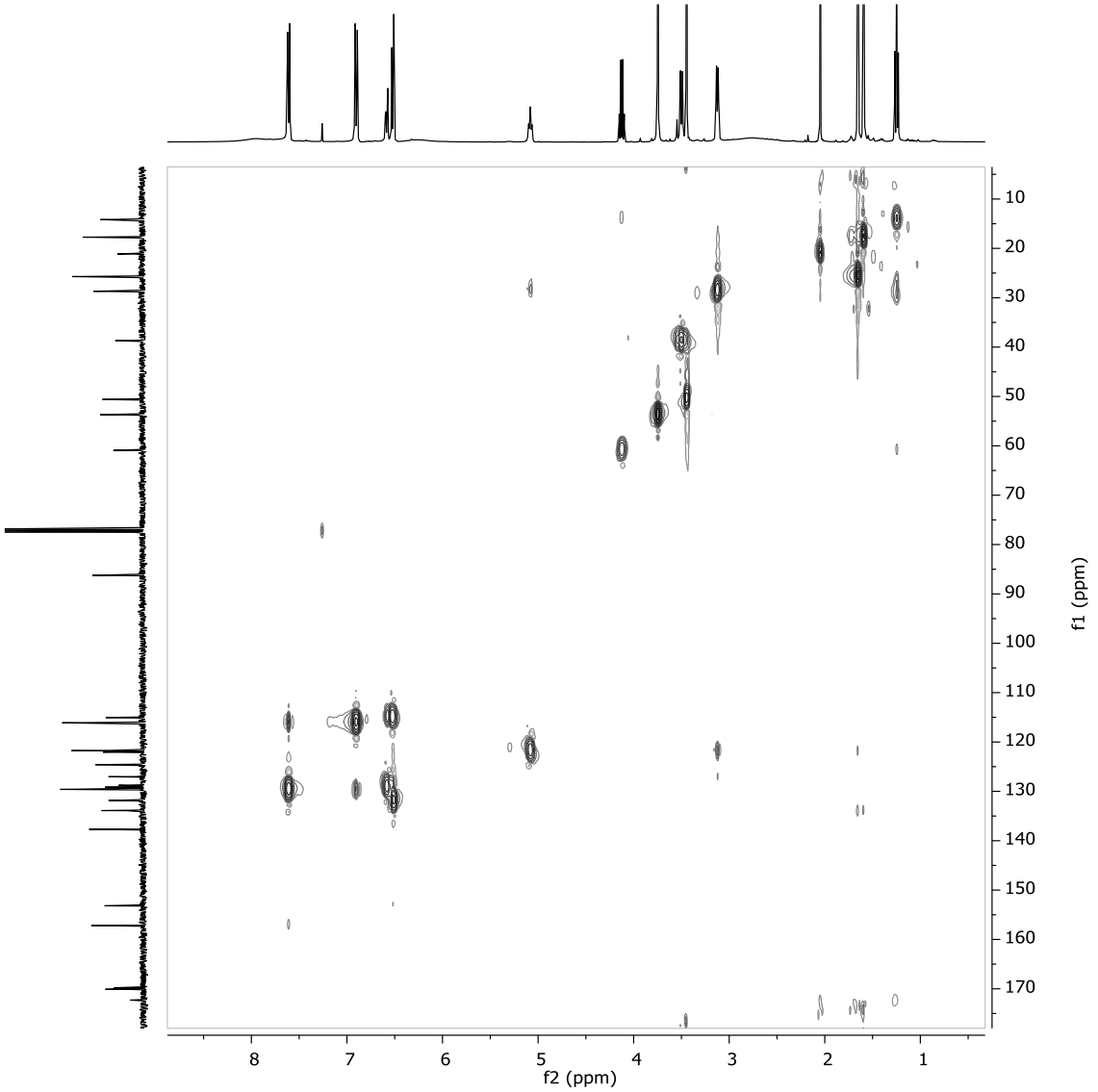


Figure S8. *g*HMQC spectrum of **1** in chloroform-*d*

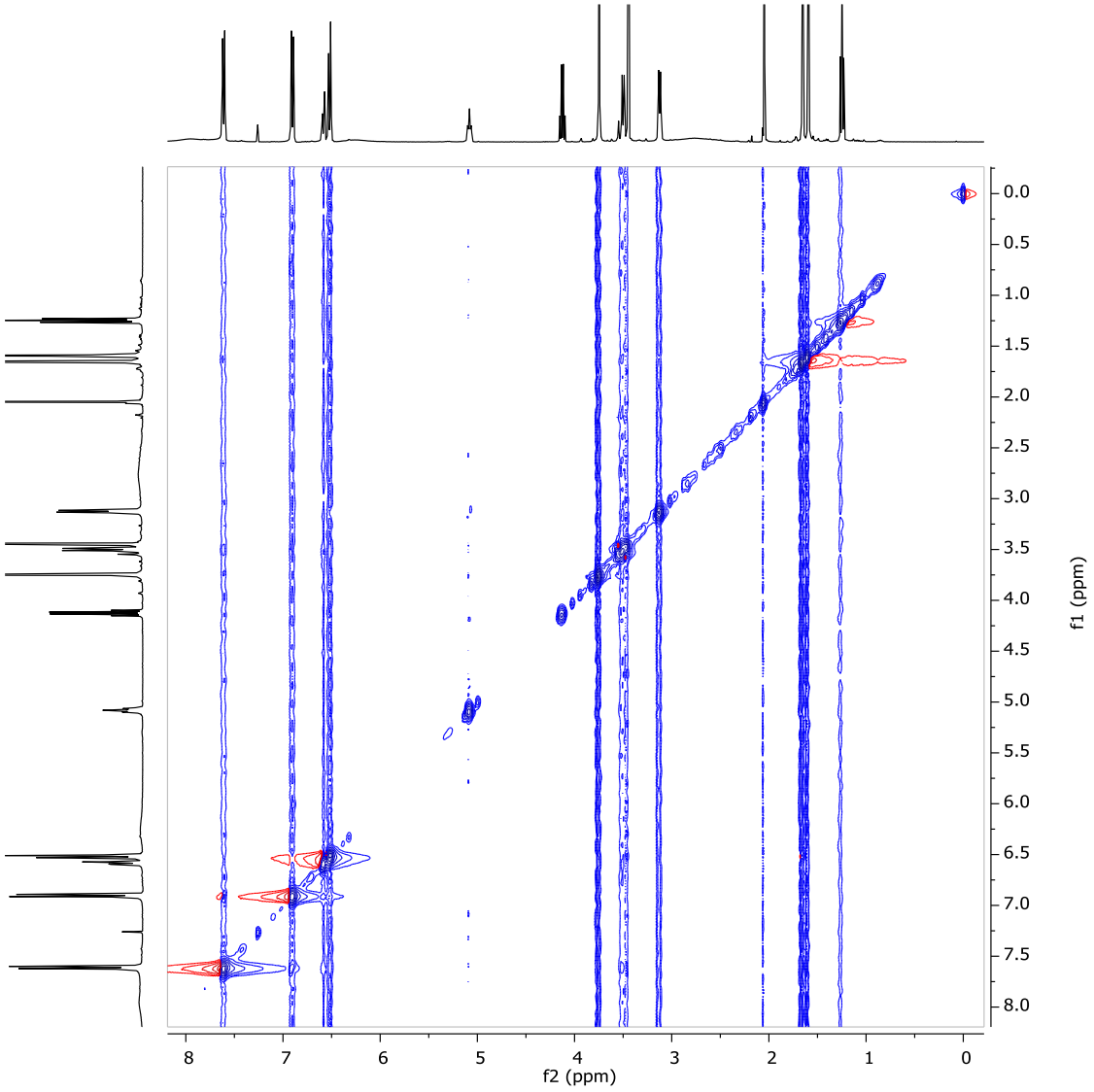
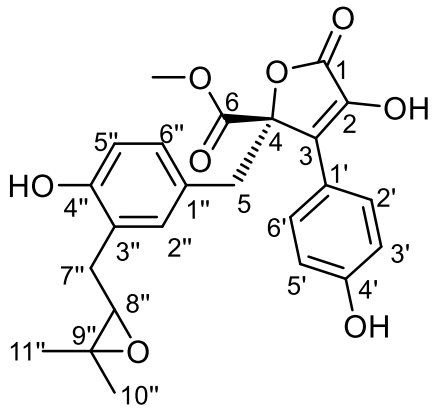


Figure S9. NOESY spectrum of **1** in chloroform-*d*

Table S2. ^{1}H and ^{13}C NMR Data of butyrolactone III (**2**).



Butyrolactone III (**2**)

Butyrolactone III (**2**) in chloroform-*d*, 600 MHz		
#	δ_H (*J*[Hz])	δ_C, type
1		169.6, C
2		137.6, C
3		129.7, C
4		86.1, C
5	3.51, d (14.7)	38.8, CH_2
	3.41, d (14.7)	
6		169.9, C
7	3.73, s (3H)	53.7, CH_3
1'		122.0, C
2'	7.56, d (9.0)	129.6, CH
3'	6.90, d (9.0)	116.2, CH
4'		157.3, C
5'	6.90, d (9.0)	116.2, CH
6'	7.56, d (9.0)	129.6, CH
1"		118.4, C
2"	6.51, d (2.1)	132.1, CH
3"		124.8, C
4"		152.0, C
5"	6.53, d (8.1)	116.7, CH
6"	6.51, dd (8.1, 2.1)	128.9, CH
7"	2.82, dd (17.0, 5.0)	30.9, CH_2
	2.58, dd (17.0, 6.0)	
8"	3.74, m	69.7, CH
9"		76.8, C
10"	1.24, s (3H)	22.0, CH_3
11"	1.21, s (3H)	24.8, CH_3

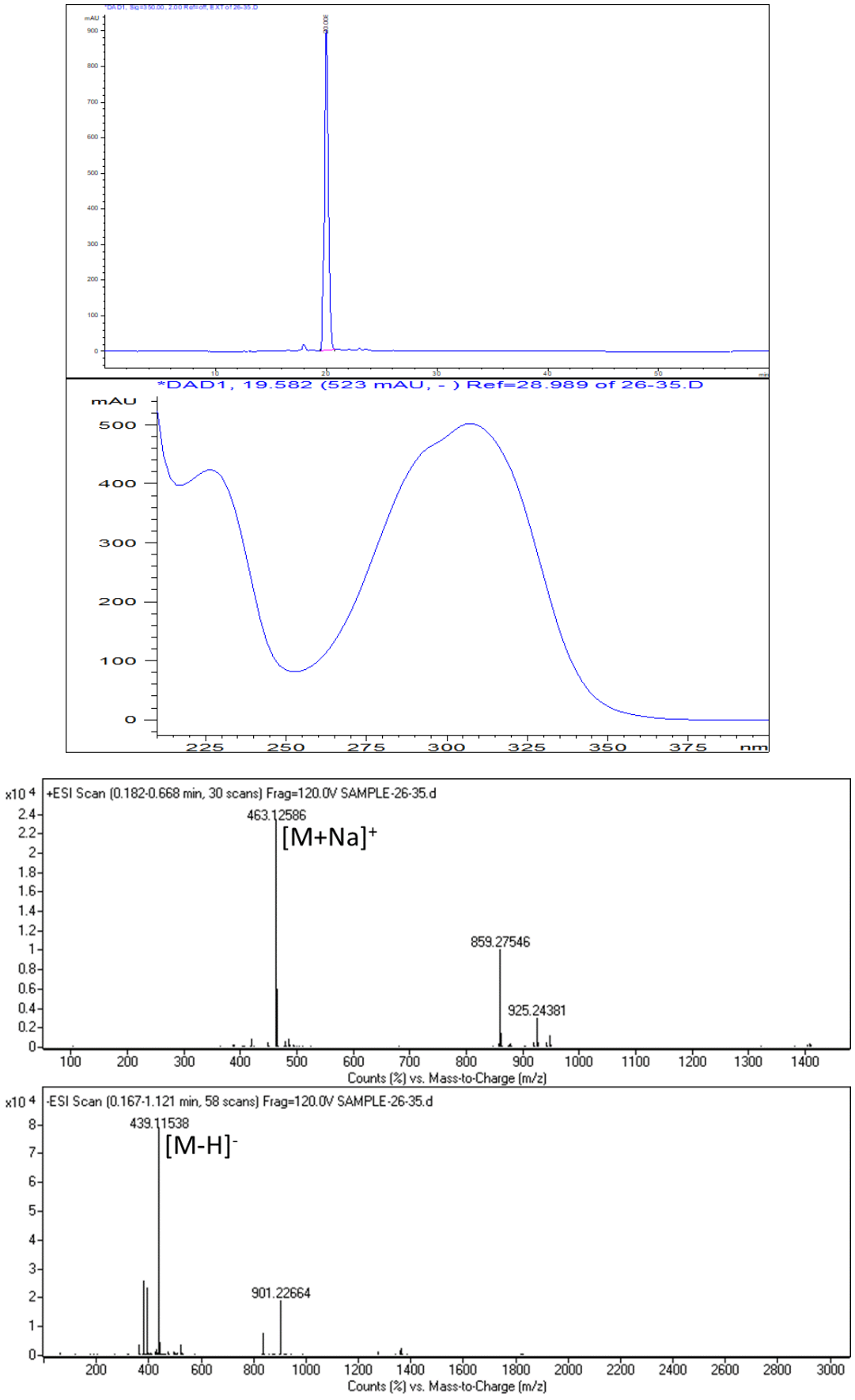


Figure S10. HPLC chromatogram and HRESIMS spectrum of **2**

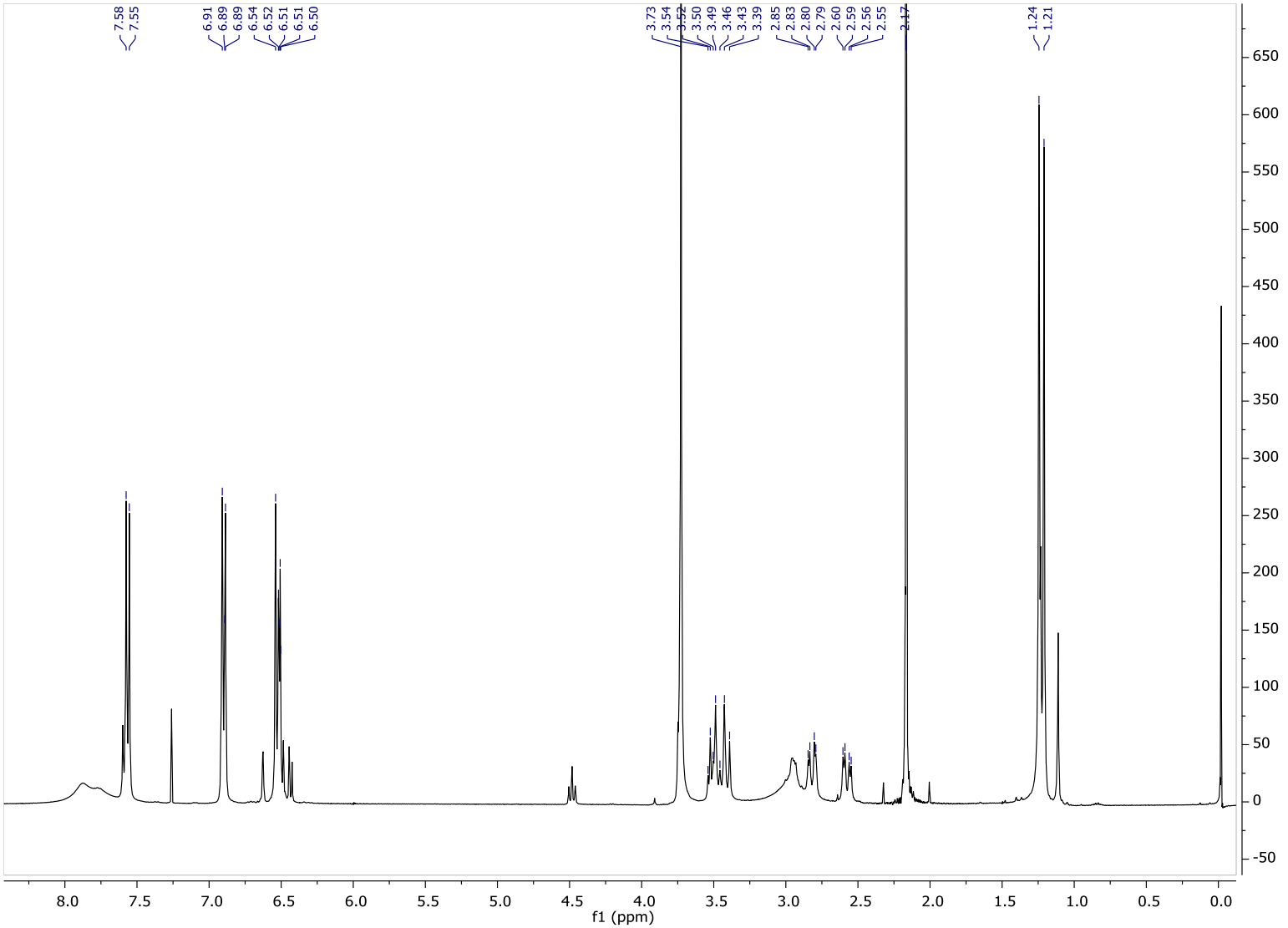


Figure S11. ^{1}H-NMR spectrum of **2** in chloroform-*d*

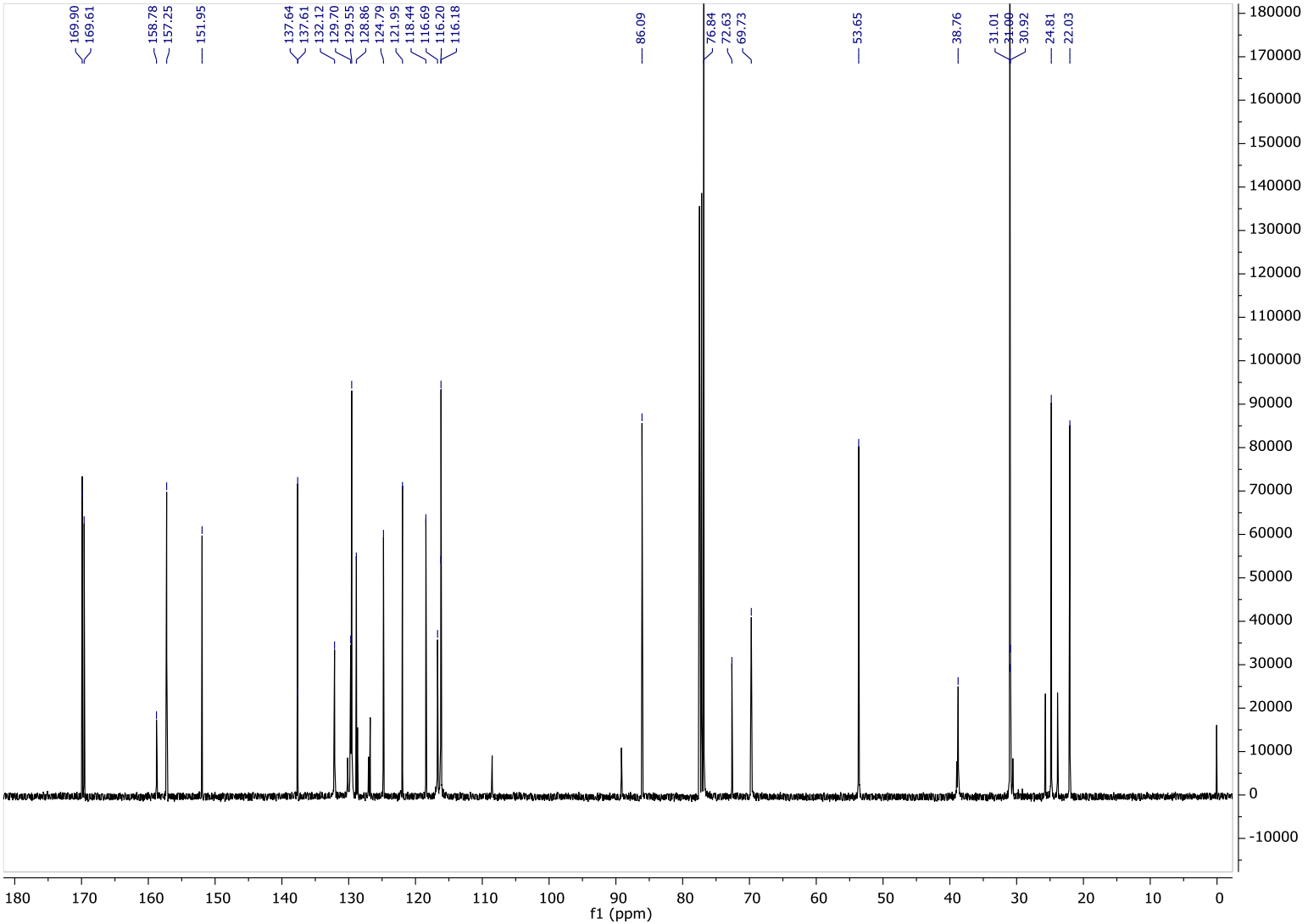


Figure S12. ^{13}C-NMR spectrum of **2** in chloroform-*d*

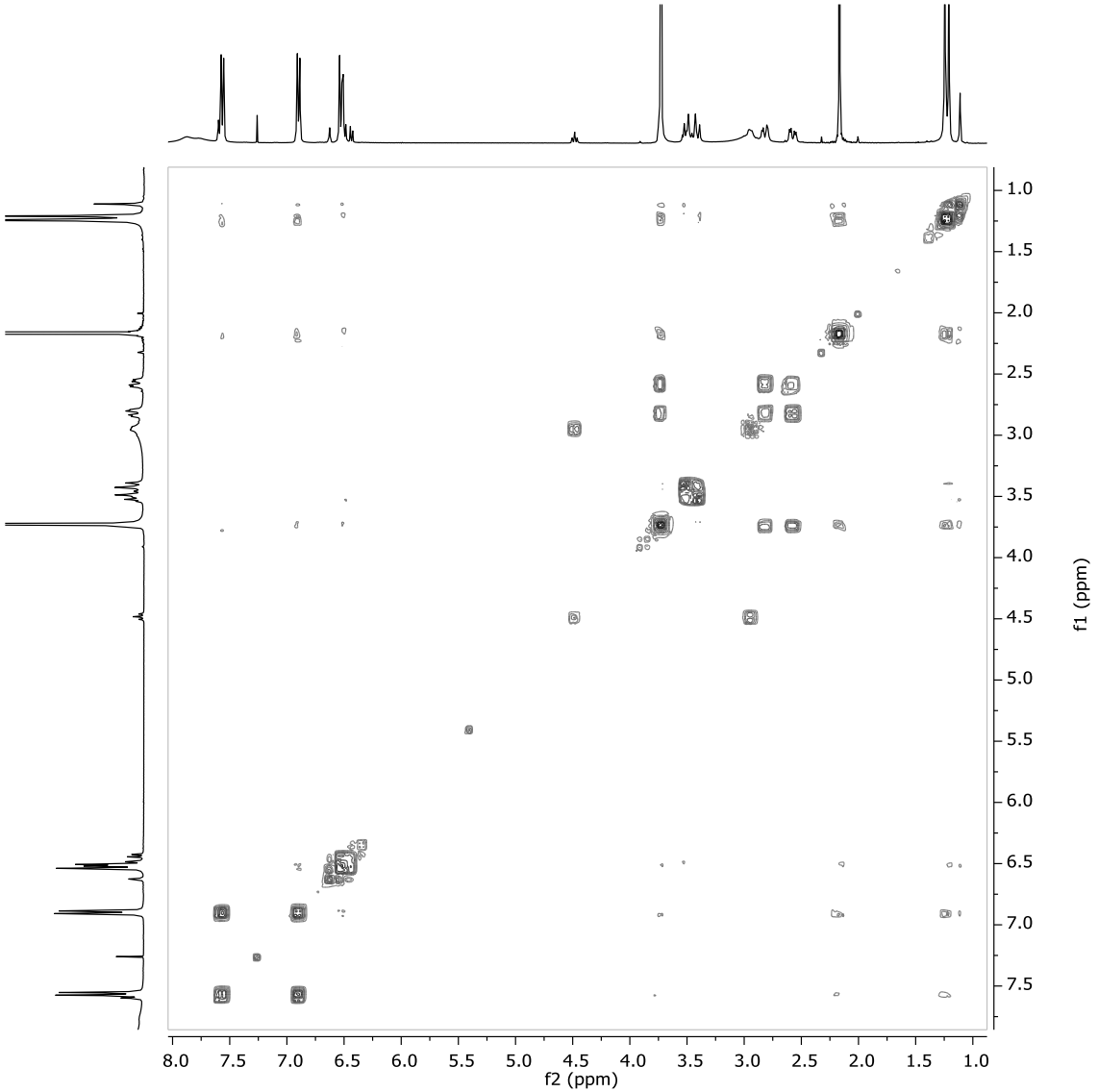


Figure S13. ^{1}H–^{1}H COSY spectrum of **2** in chloroform-*d*

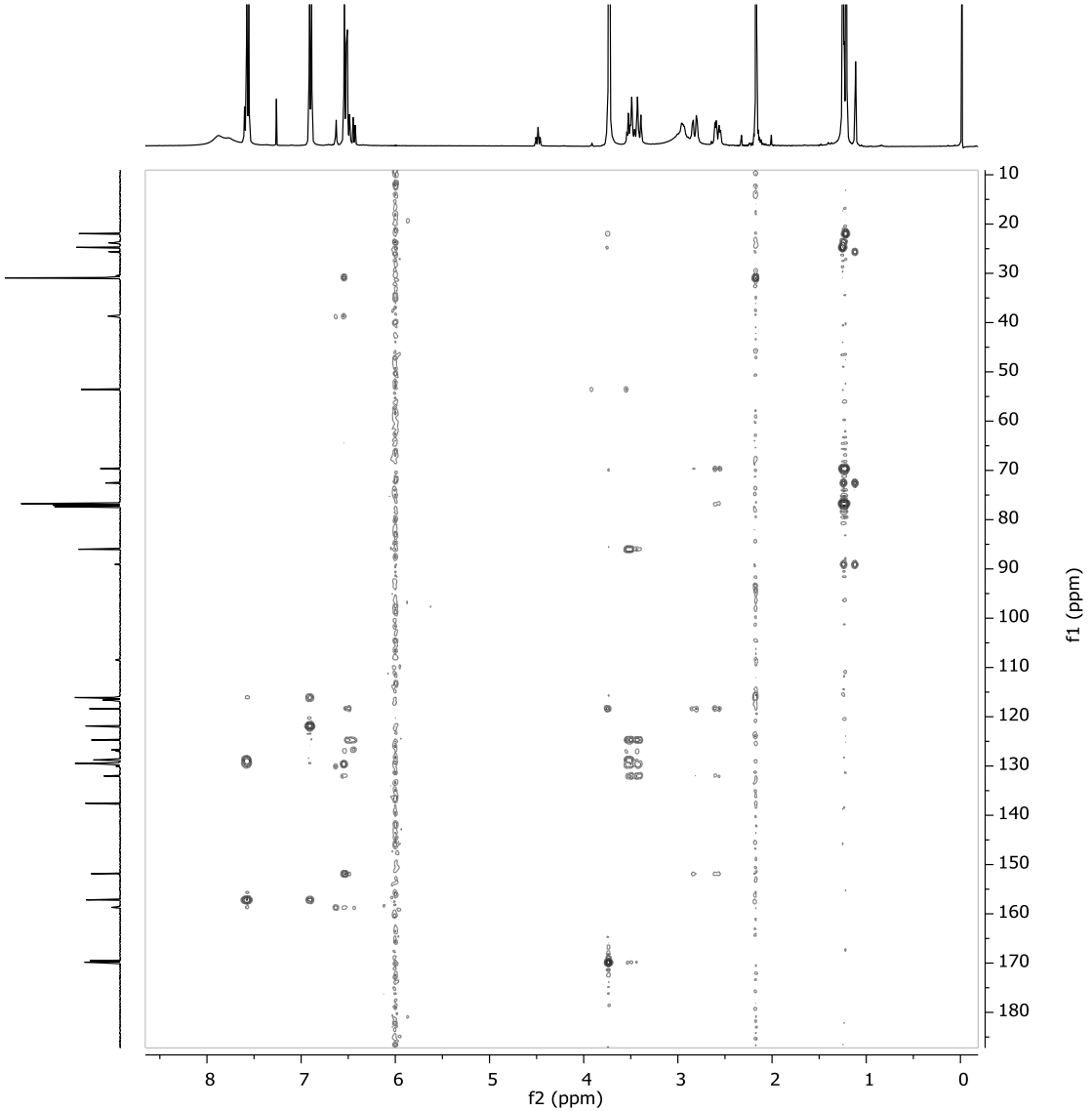


Figure S14. *g*HMBC spectrum of **2** in chloroform-*d*

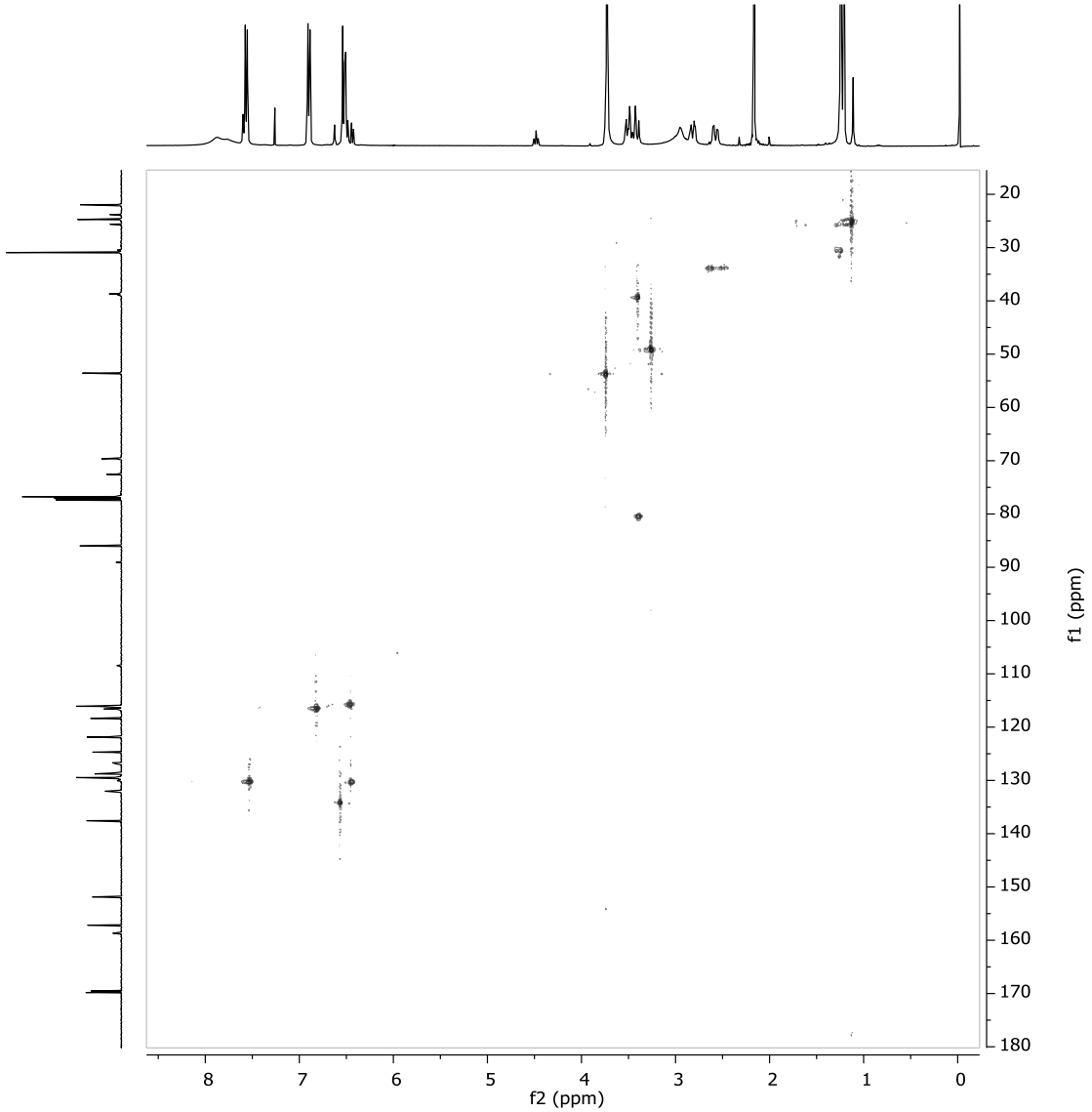


Figure S15. *g*HMQC spectrum of **2** in chloroform-*d*

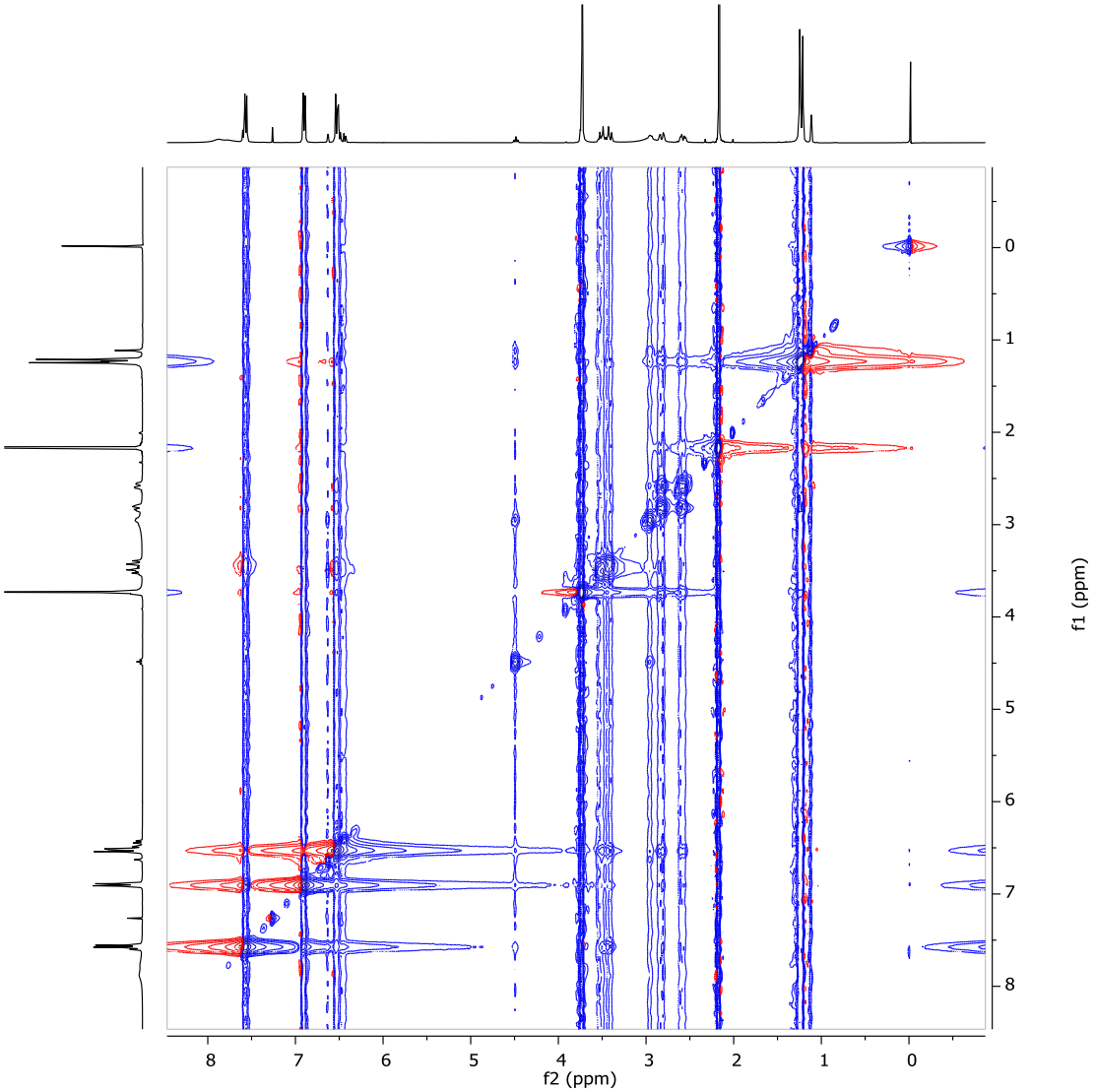
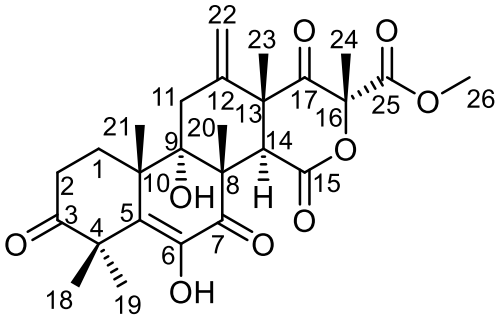


Figure S16. NOESY spectrum of **2** in chloroform-*d*

Table S3. ^{1}H and ^{13}C NMR Data of terretonin (**3**).



Terretonin (**3**)

Terretonin (**3**) in chloroform-*d*, 400 MHz		
#	δ_H (*J*[Hz])	δ_C, type
1	2.36, dd (13.1, 9.3) 1.76, dd (13.1, 8.3)	28.3, CH_2
2	2.70, dd (19.0, 8.6) 2.52, ddd (19.2, 11.3, 8.5)	32.8, CH_2
3		214.2, CO
4		47.9, C
5		131.7, C
6		138.8, C
7		197.1, CO
8		52.5, C
9		77.7, C
10		43.3, C
11	2.96, dt (14.2, 1.6) 2.27, d (14.2)	35.0, CH_2
12		139.9, C
13		49.6, C
14	3.54, s	44.7, CH
15		167.9, CO
16		85.6, C
17		201.6, CO
18	1.46, s (3H)	23.7, CH_3
19	1.46, s (3H)	23.7, CH_3
20	1.94, s (3H)	19.9, CH_3
21	1.21, s (3H)	18.7, CH_3
22	5.08, d (1.5) 5.45, d (1.5)	117.2, CH_2
23	1.43, s (3H)	23.5, CH_3
24	1.72, s (3H)	21.4, CH_3
25		168.7, CO
26	3.79, s (3H)	53.8, CH_3
6-OH	6.14, s	

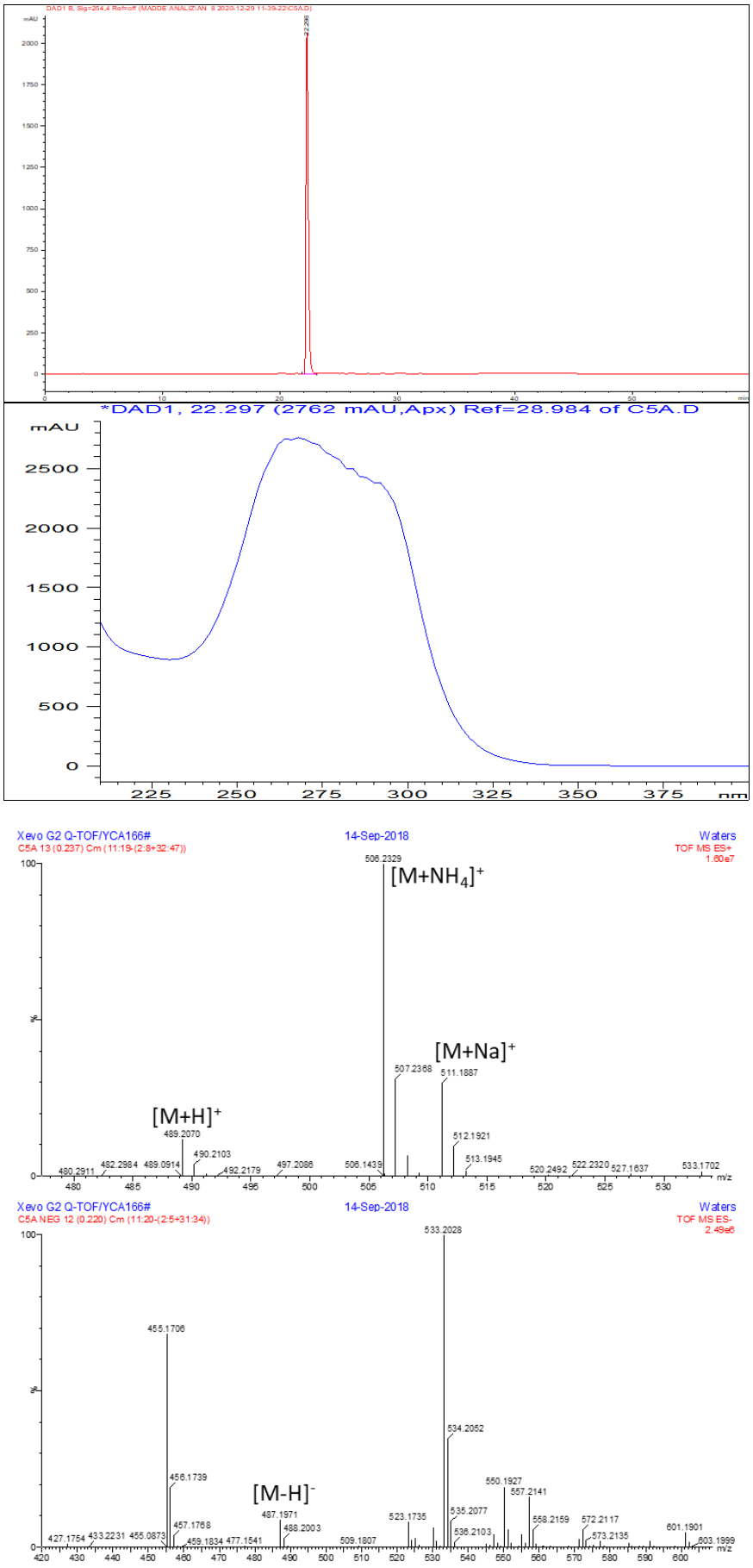


Figure S17. HPLC chromatogram and HRESIMS spectrum of **3**

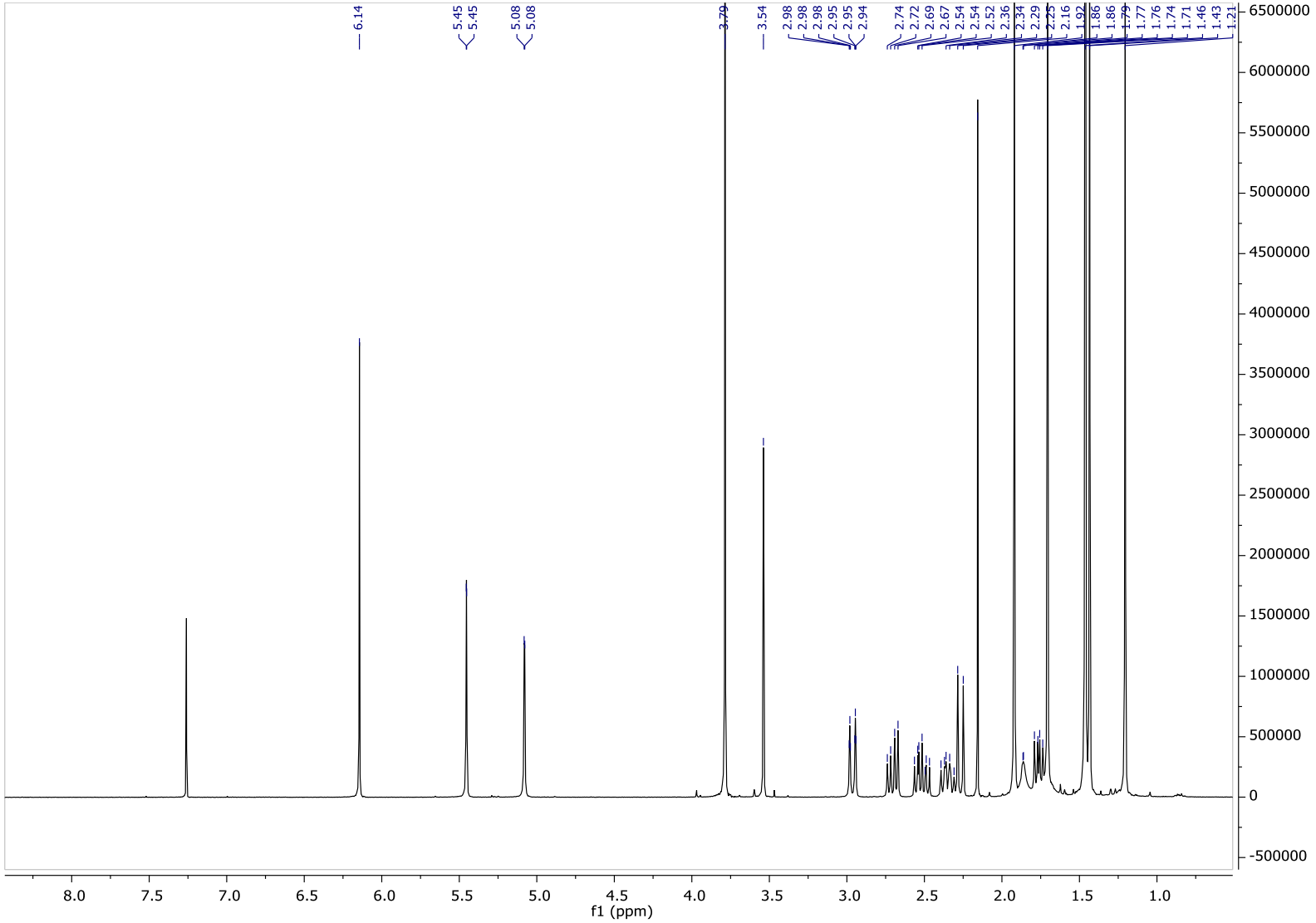


Figure S18. ^{1}H-NMR spectrum of **3** in chloroform-*d*

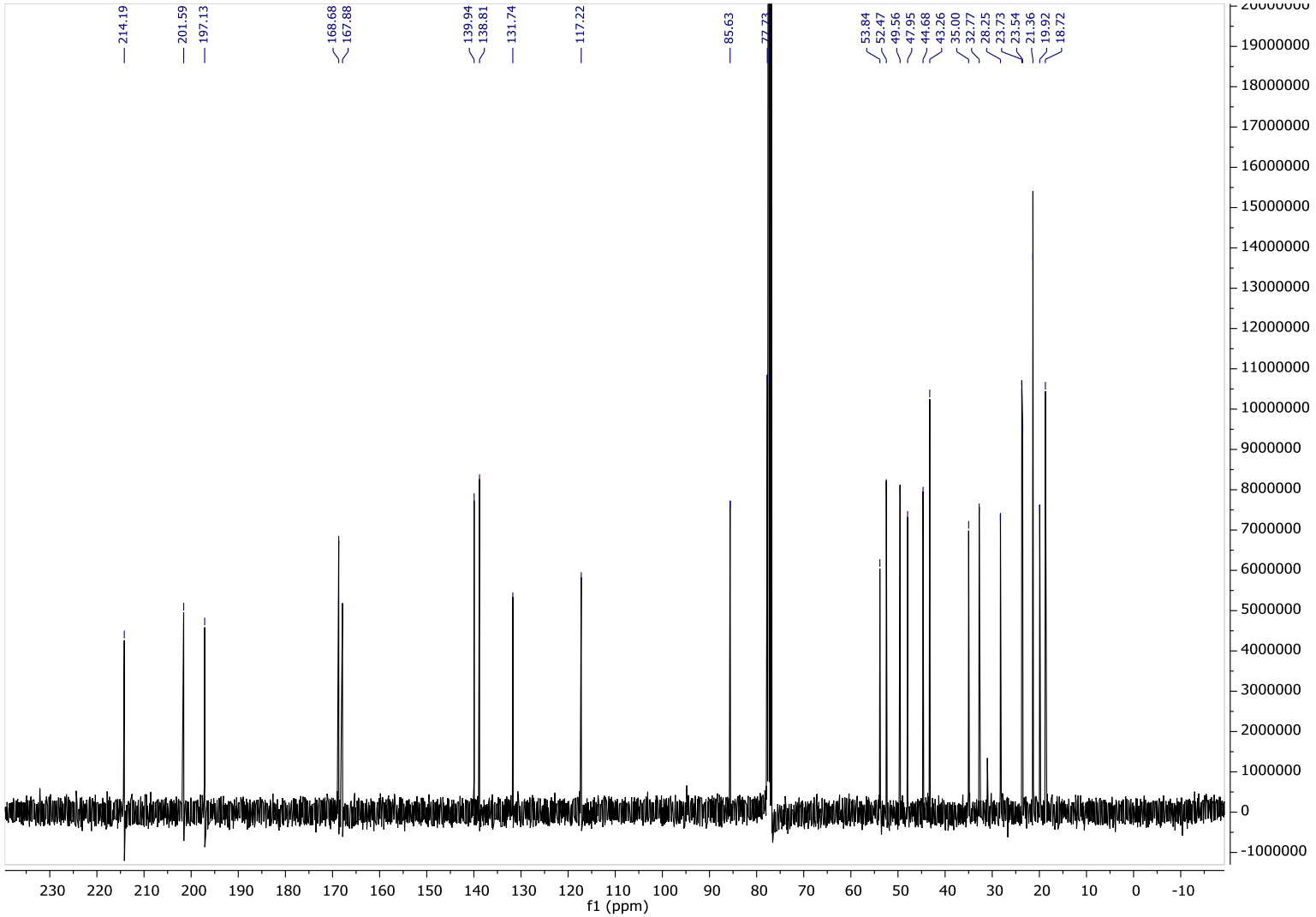


Figure S19. ^{13}C-NMR spectrum of **3** in chloroform-*d*

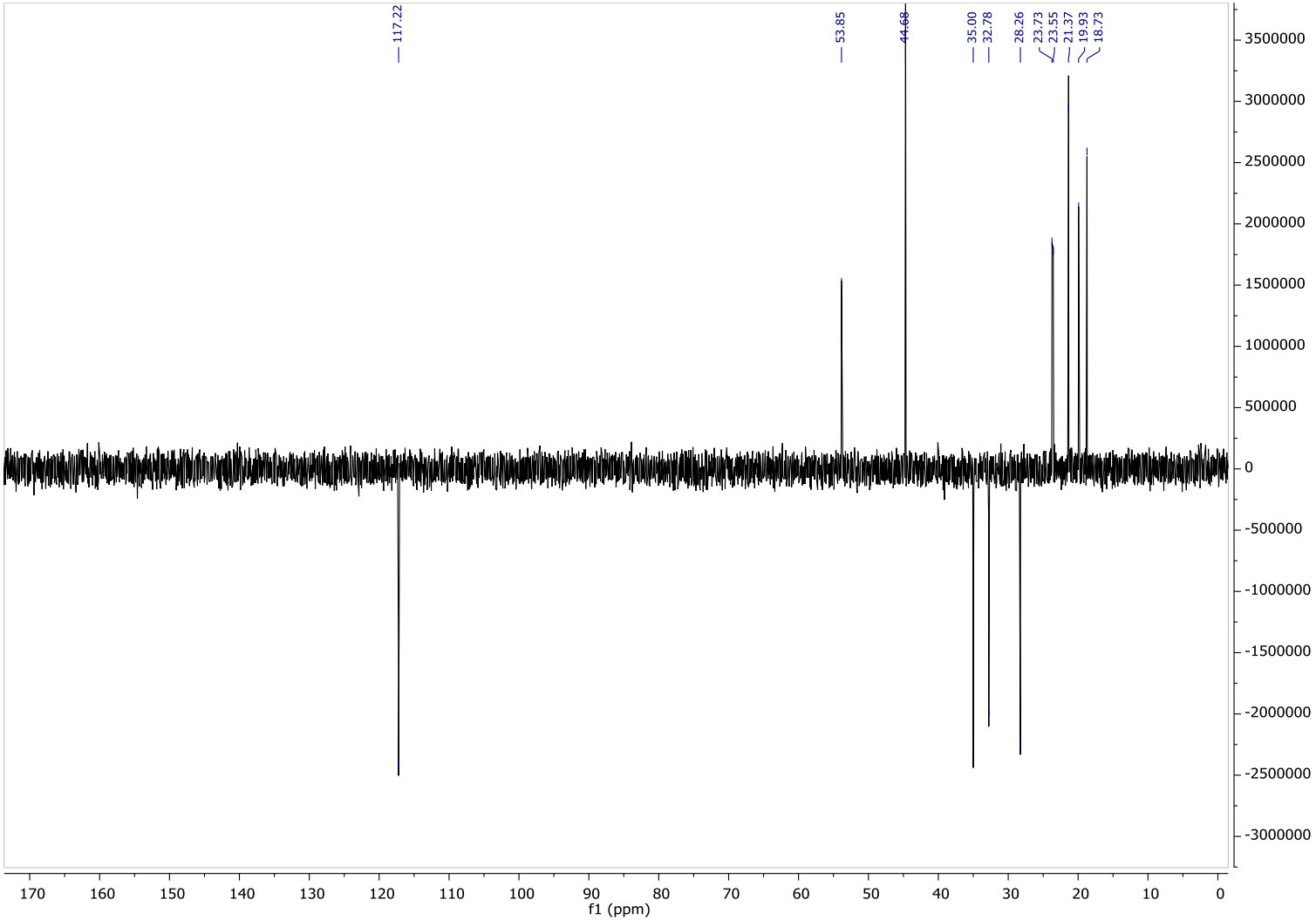


Figure S20. DEPT spectrum of **3** in chloroform-*d*

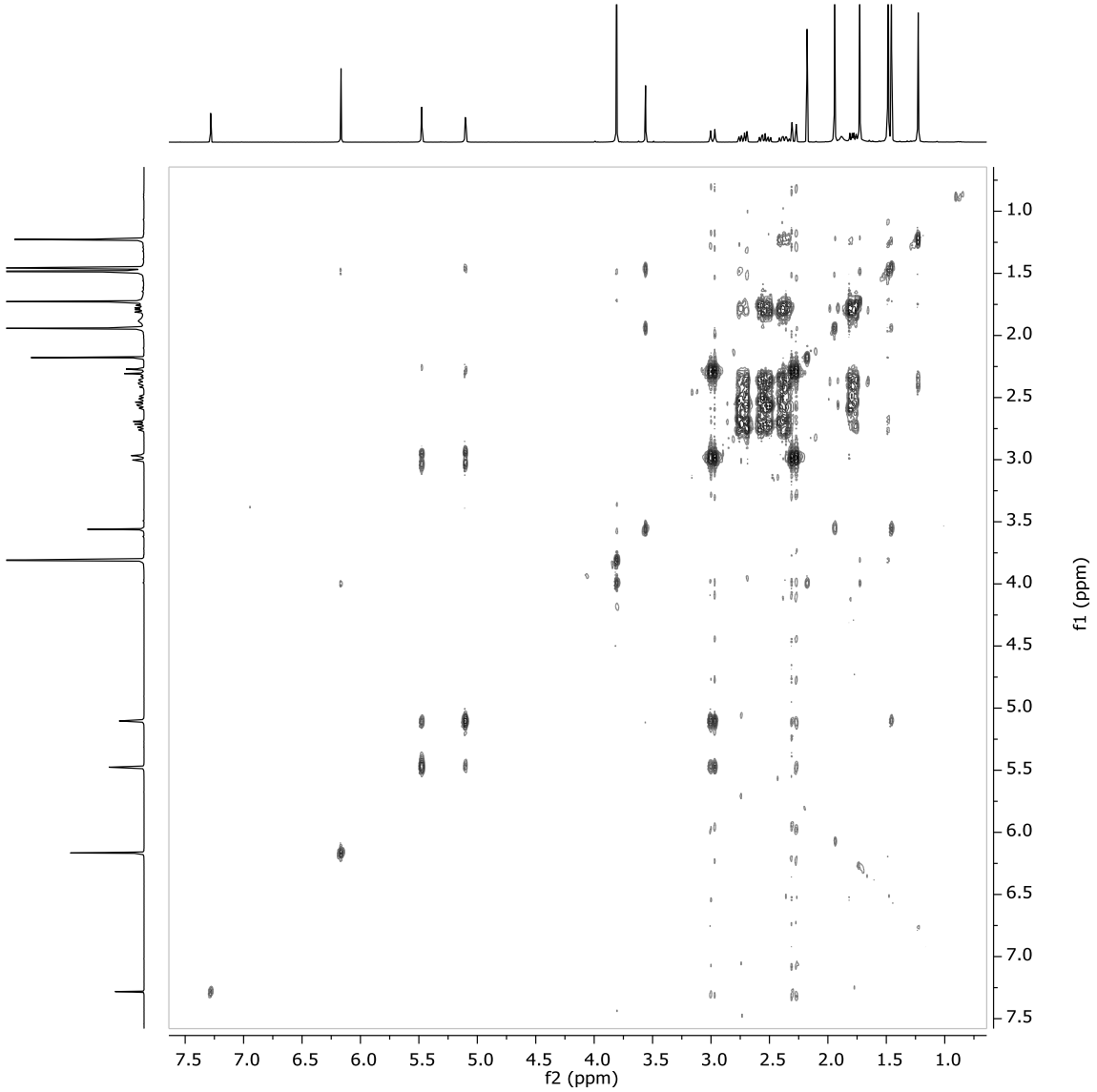


Figure S21. ^{1}H–^{1}H COSY spectrum of **3** in chloroform-*d*

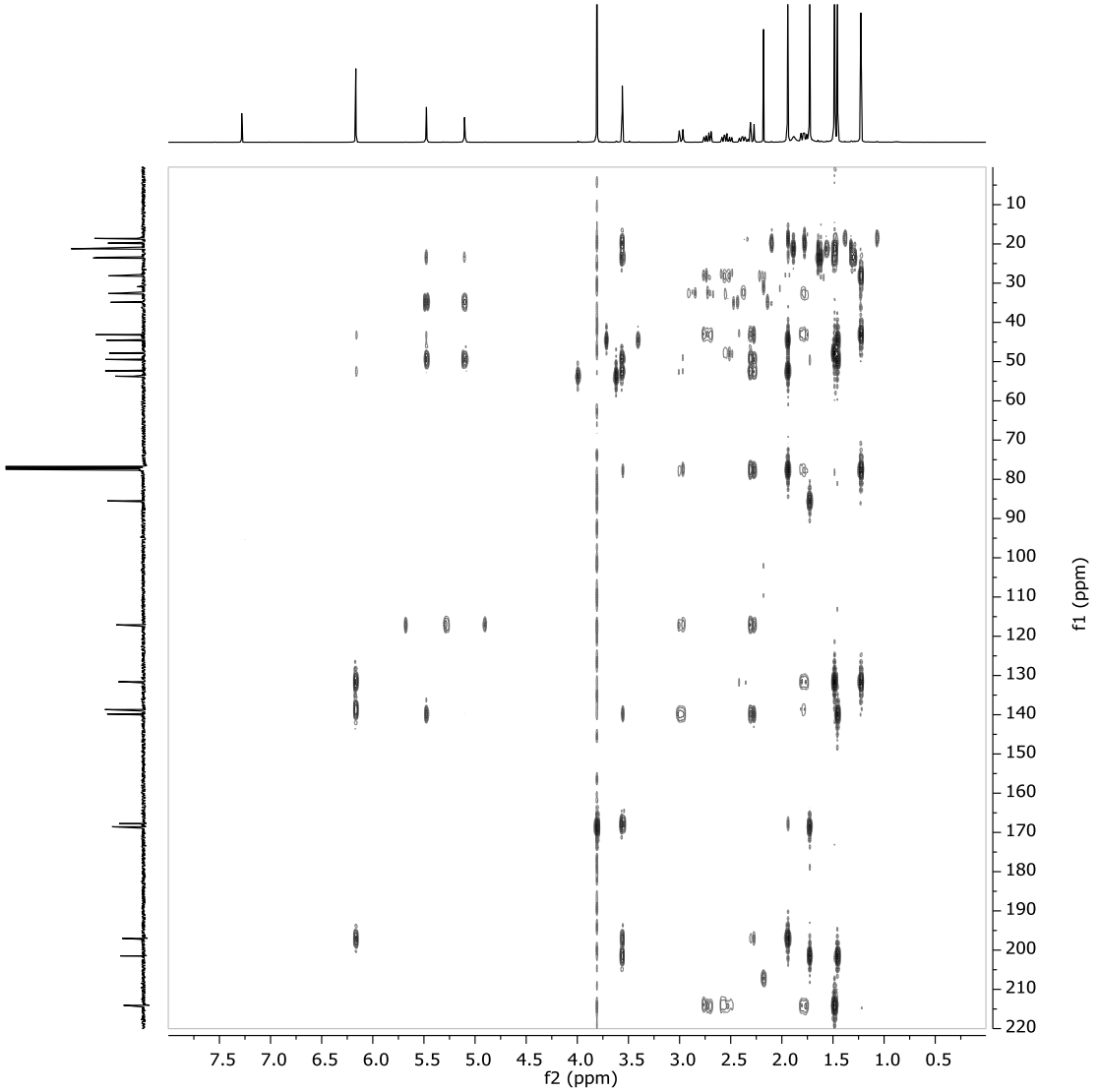


Figure S22. *g*HMBC spectrum of **3** in chloroform-*d*

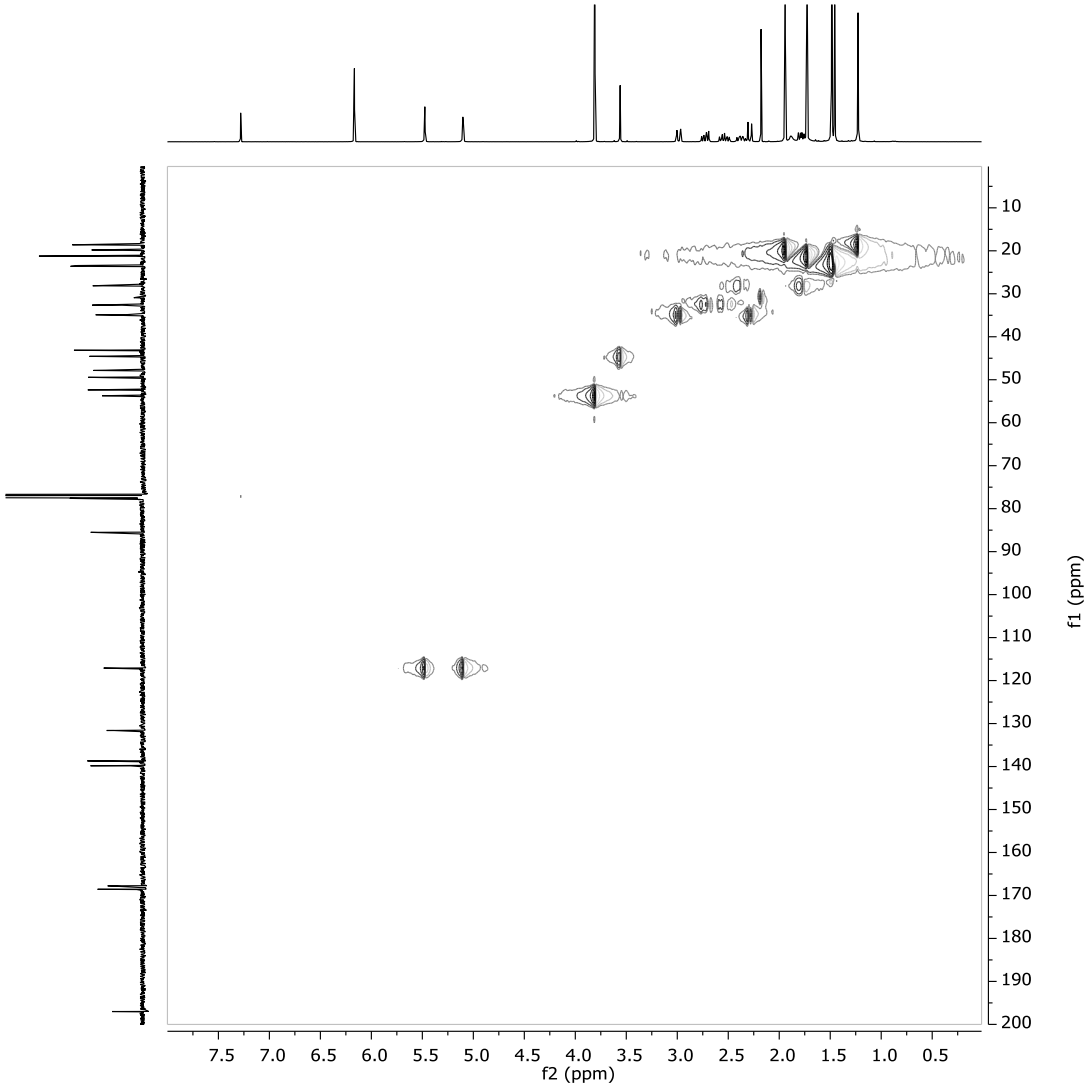


Figure S23. *g*HMQC spectrum of **3** in chloroform-*d*

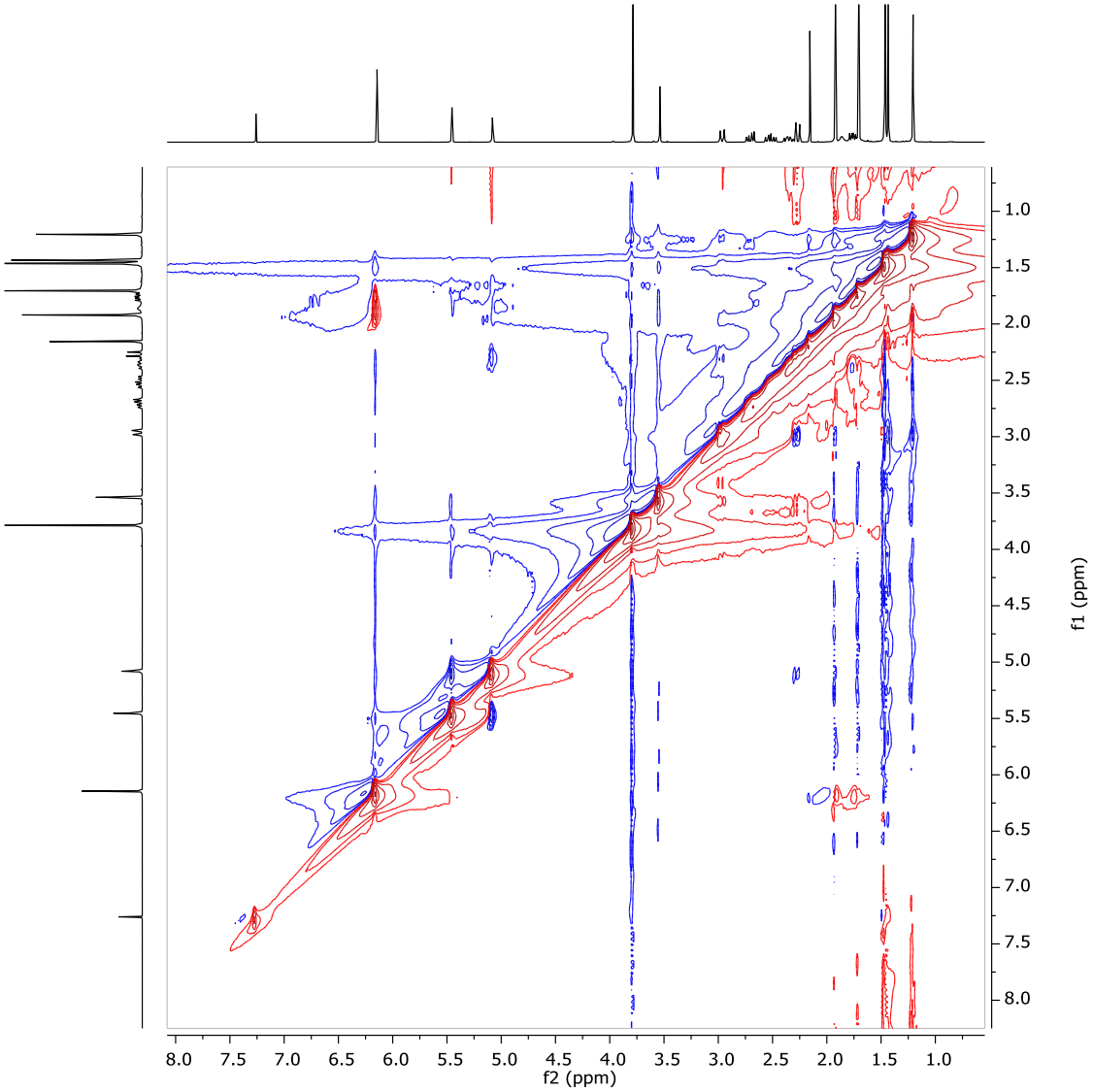
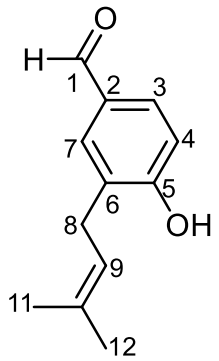


Figure S24. NOESY spectrum of **3** in chloroform-*d*

Table S4. ^{1}H and ^{13}C NMR Data of 4-hydroxy-3-(3-methylbut-2-enyl)benzaldehyde (**4**).



4-Hydroxy-3-(3-methylbut-2-enyl)benzaldehyde (**4**)

4-Hydroxy-3-(3-methylbut-2-enyl)benzaldehyde (**4**) in DMSO-d_6, 600 MHz					
#	δ_H (J[Hz])	δ_C, type	#	δ_H (J[Hz])	δ_C, type
1	9.75, s	191.1, CO	7	7.58, d (2.2)	130.7, CH
2		128.4, C	8	3.26, d (7.4, 2H)	27.7, CH_2
3	7.60, dd (8.2, 2.2)	130.1, CH	9	5.29, td (7.4,1.5)	121.9, CH
4	6.95, d (8.2)	115.1, CH	10		132.2, C
5		161.1, CO	11	1.71, d (1.5, 3H)	25.5, CH_3
6		128.5, C	12	1.68, d (1.5, 3H)	17.6, CH_3
5-OH	10.58, s				

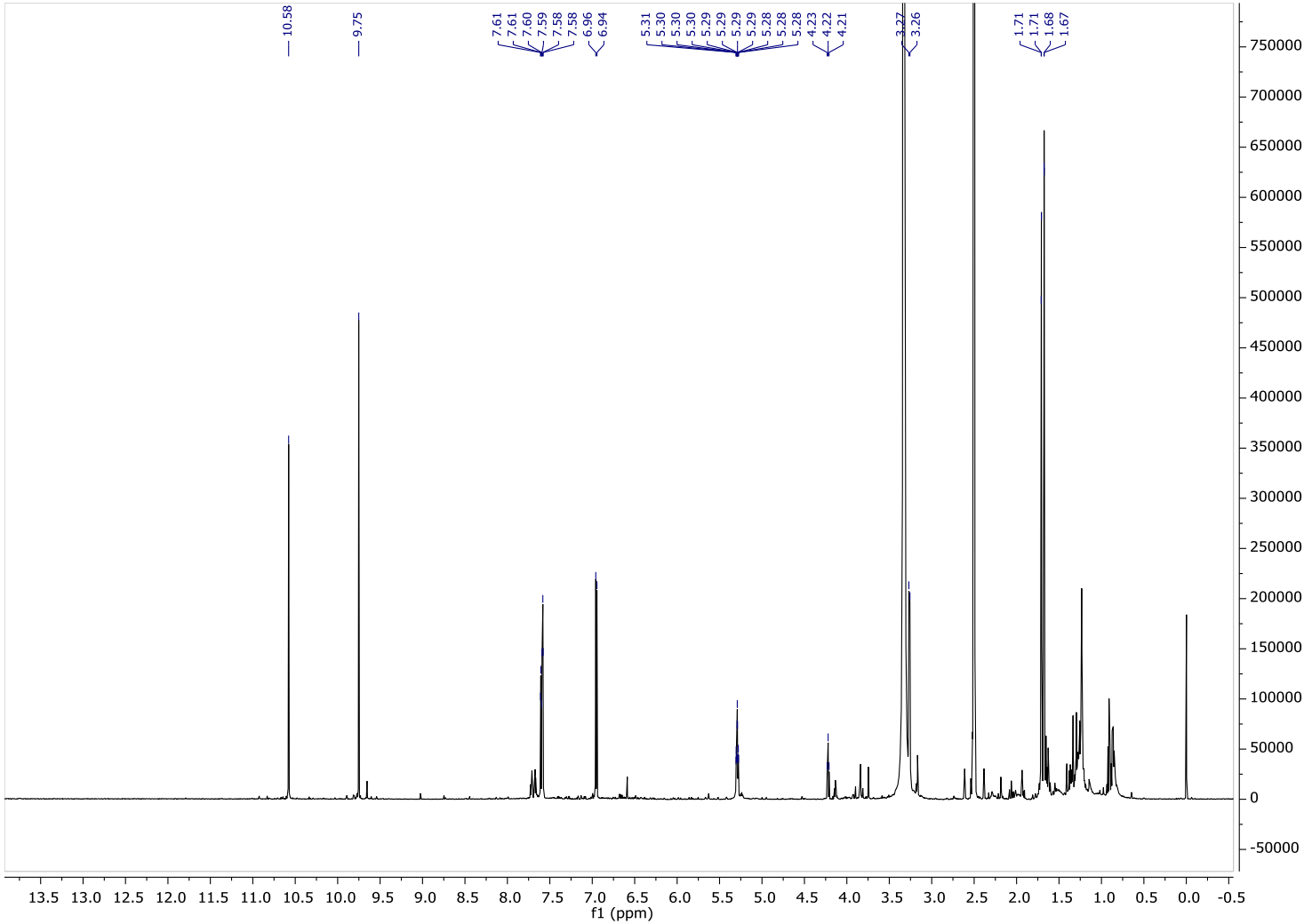


Figure S25. ^{1}H-NMR spectrum of **4** in DMSO-d_6

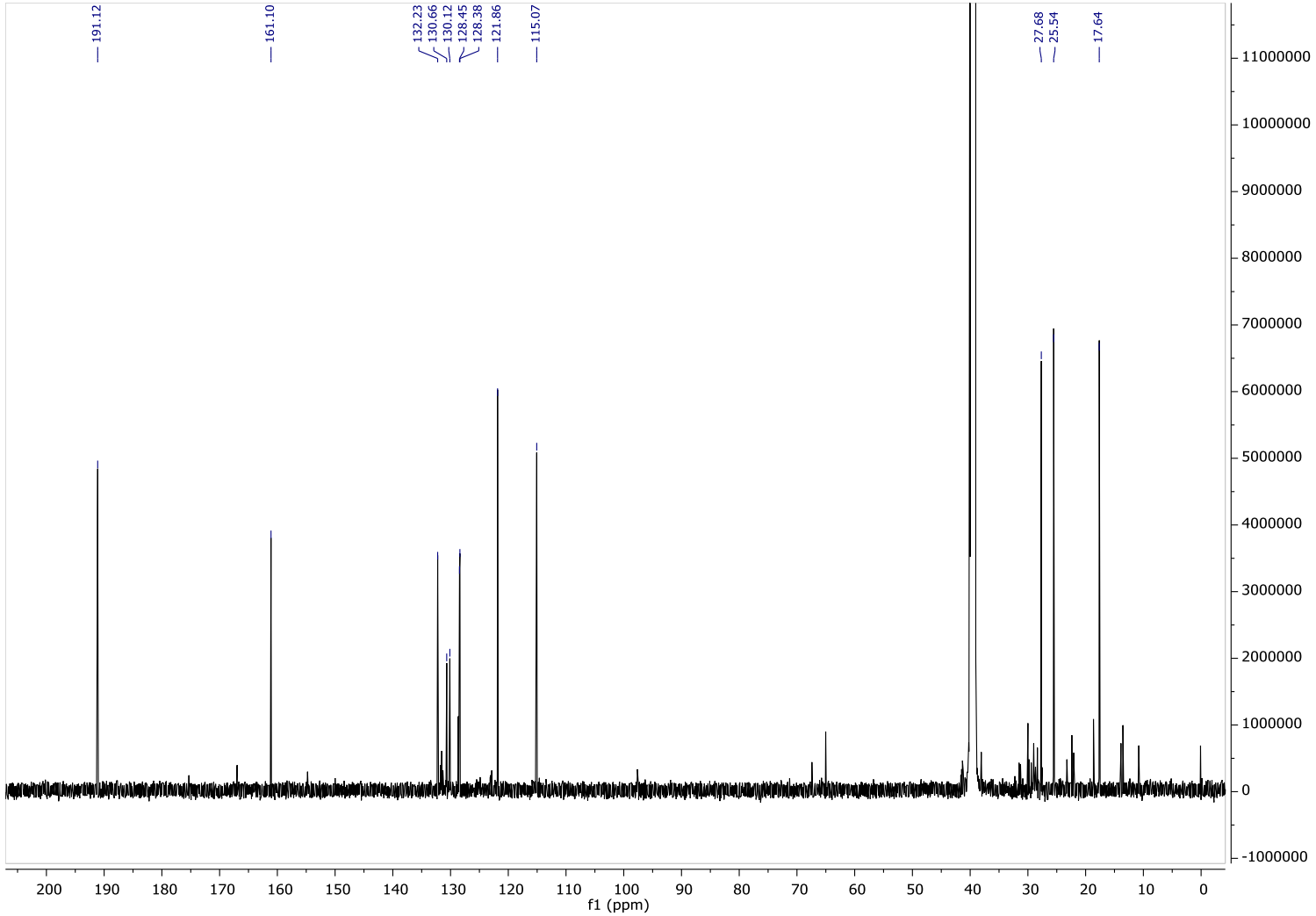


Figure S26. ^{13}C-NMR spectrum of **4** in DMSO-d_6

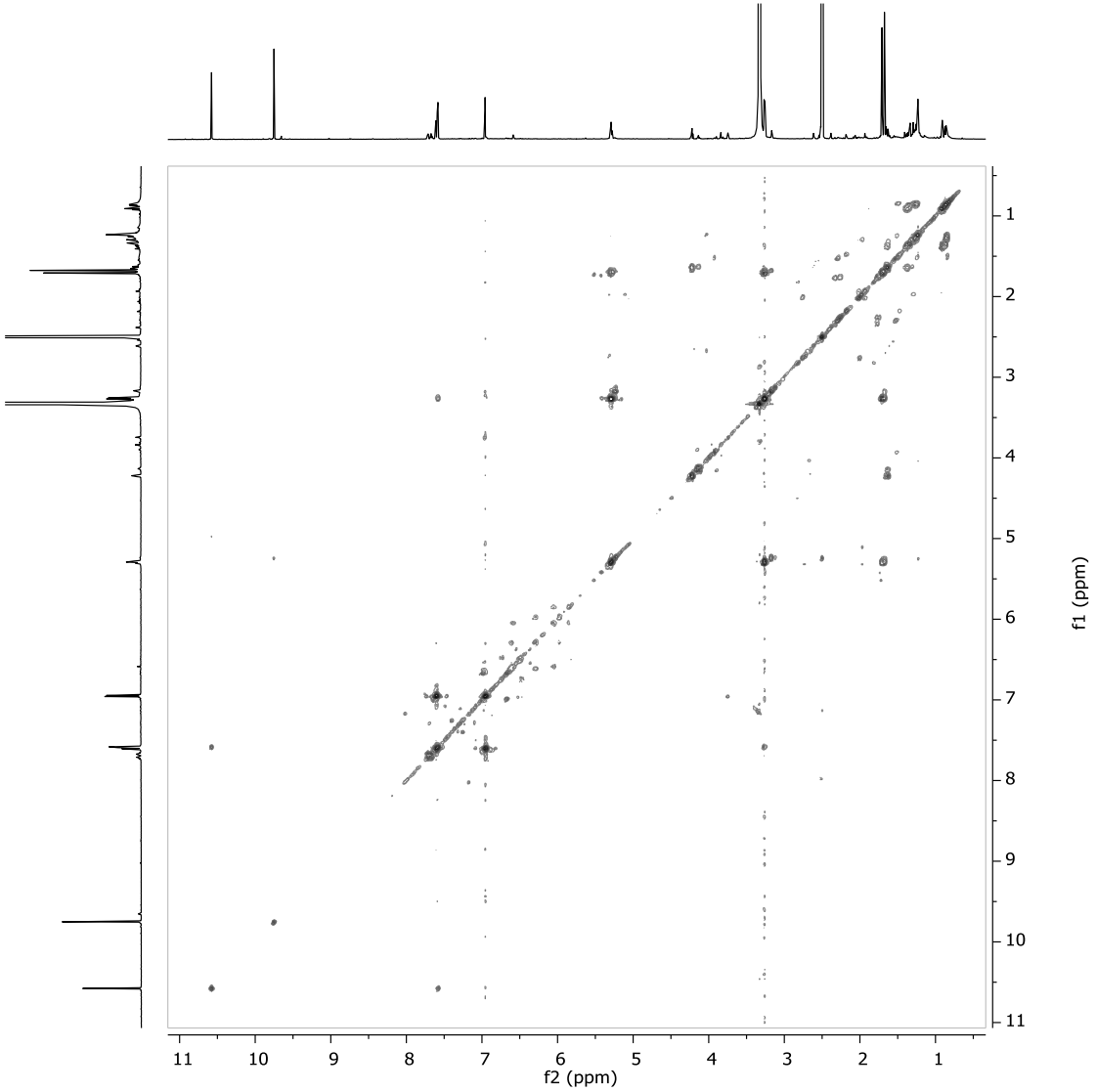


Figure S27. ^{1}H–^{1}H COSY spectrum of **4** in DMSO-d_6

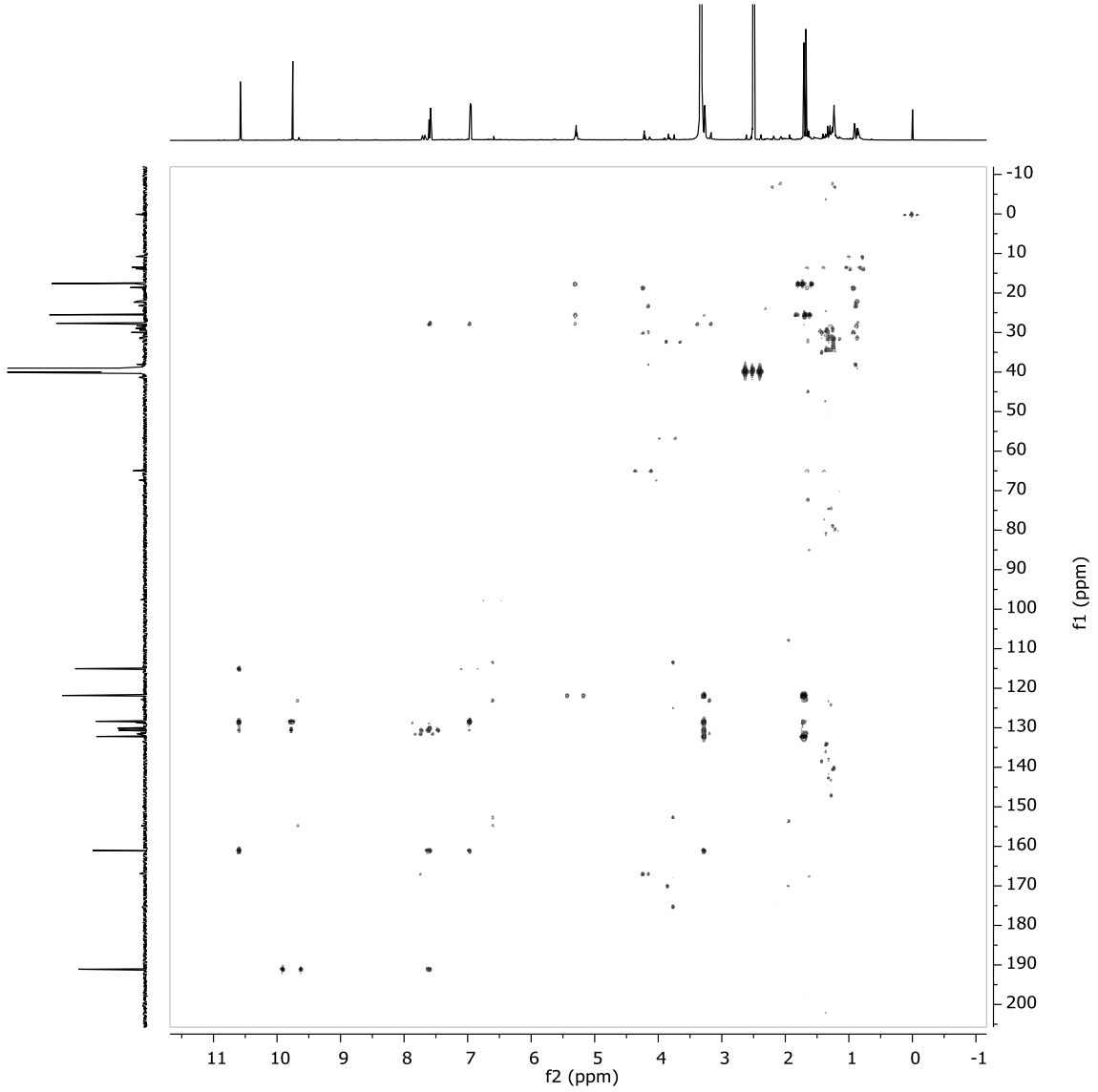


Figure S28. *g*HMBC spectrum of **4** in DMSO-d_6

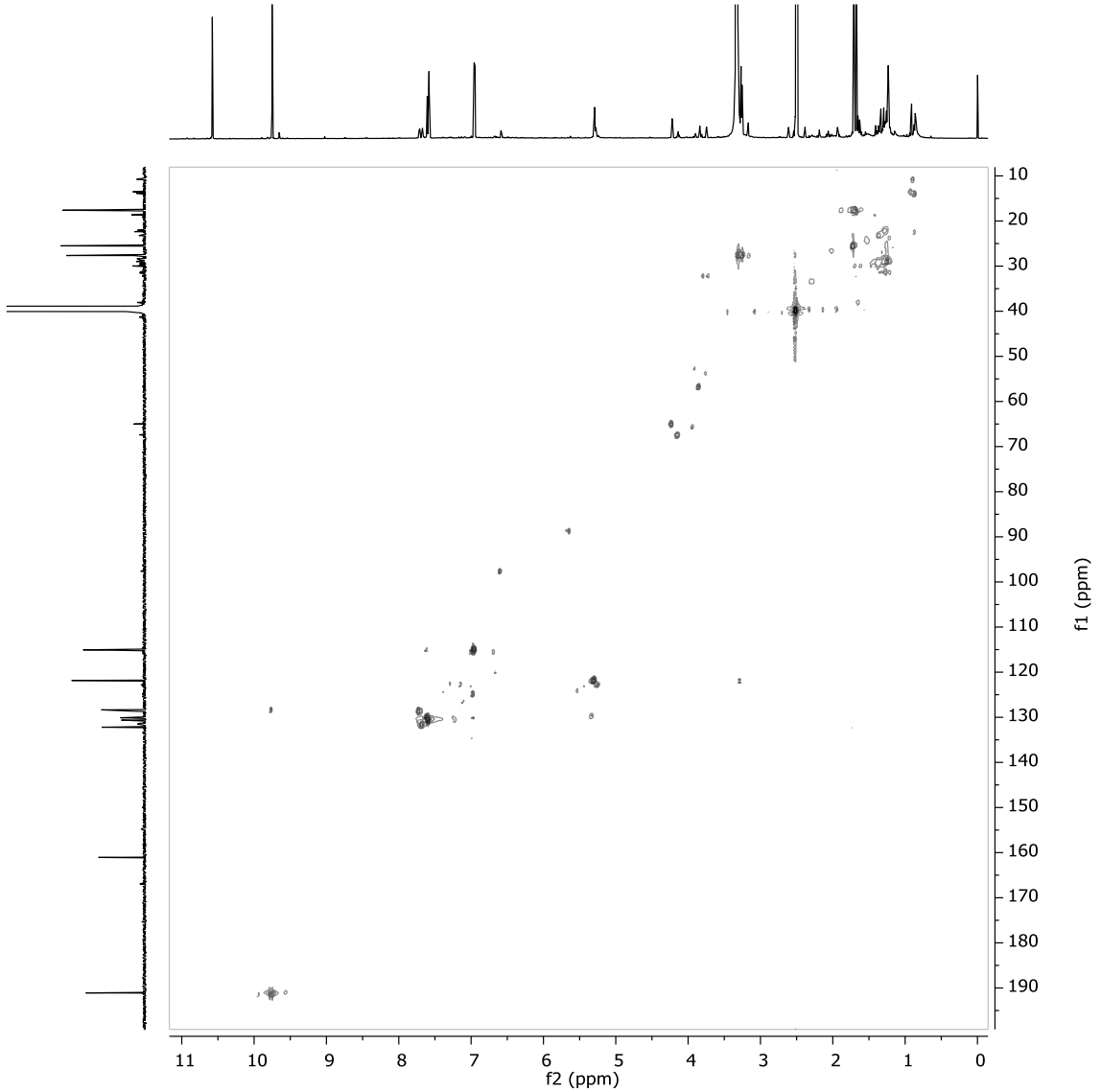


Figure S29. *g*HMQC spectrum of **4** in DMSO-d_6