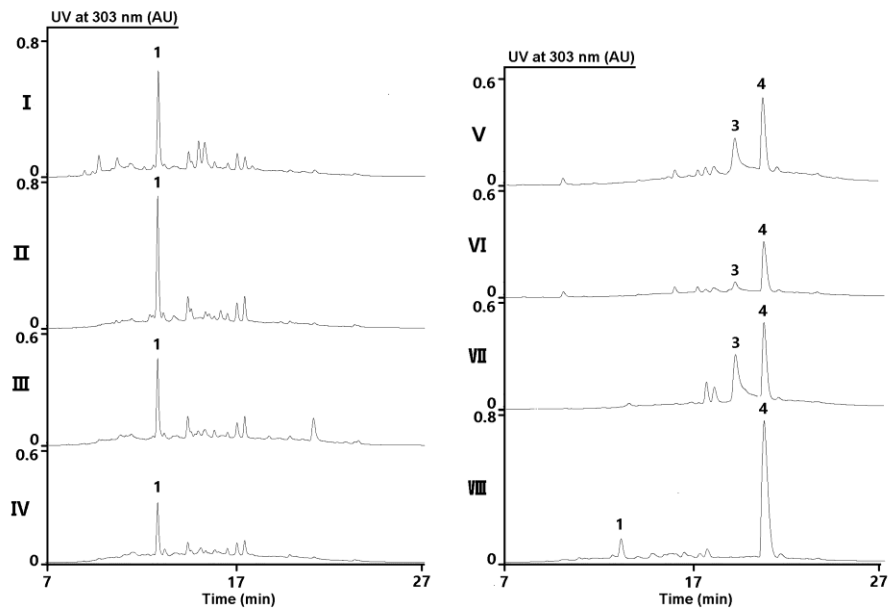
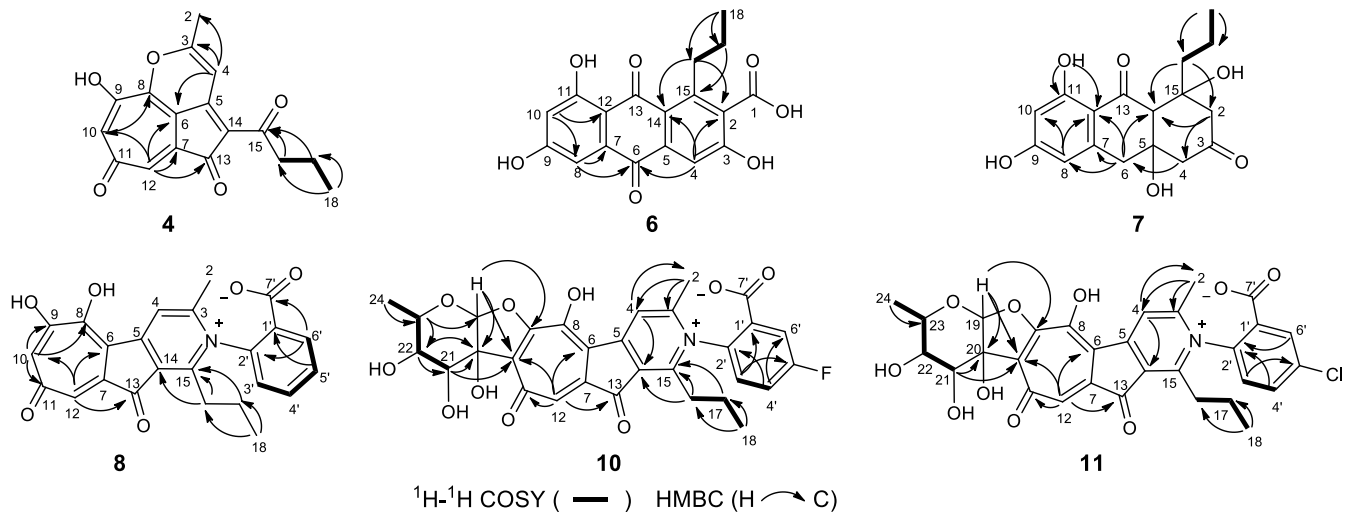


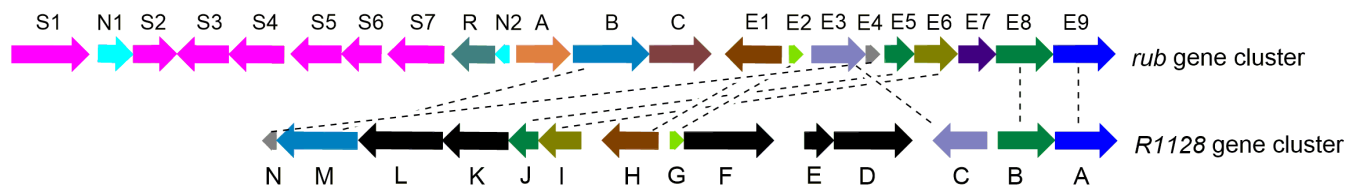
Supplementary Figure 1. The phenotype comparison of the engineered heterologous expression strains. (A) Front side of agar plate with strain *S. albus* 9B10; (B) Back side of agar plate with strain *S. albus* 9B10; (C) Front side of agar plate with strain *S. albus* pJTU2554; (D) Back side of agar plate with strain *S. albus* pJTU2554.



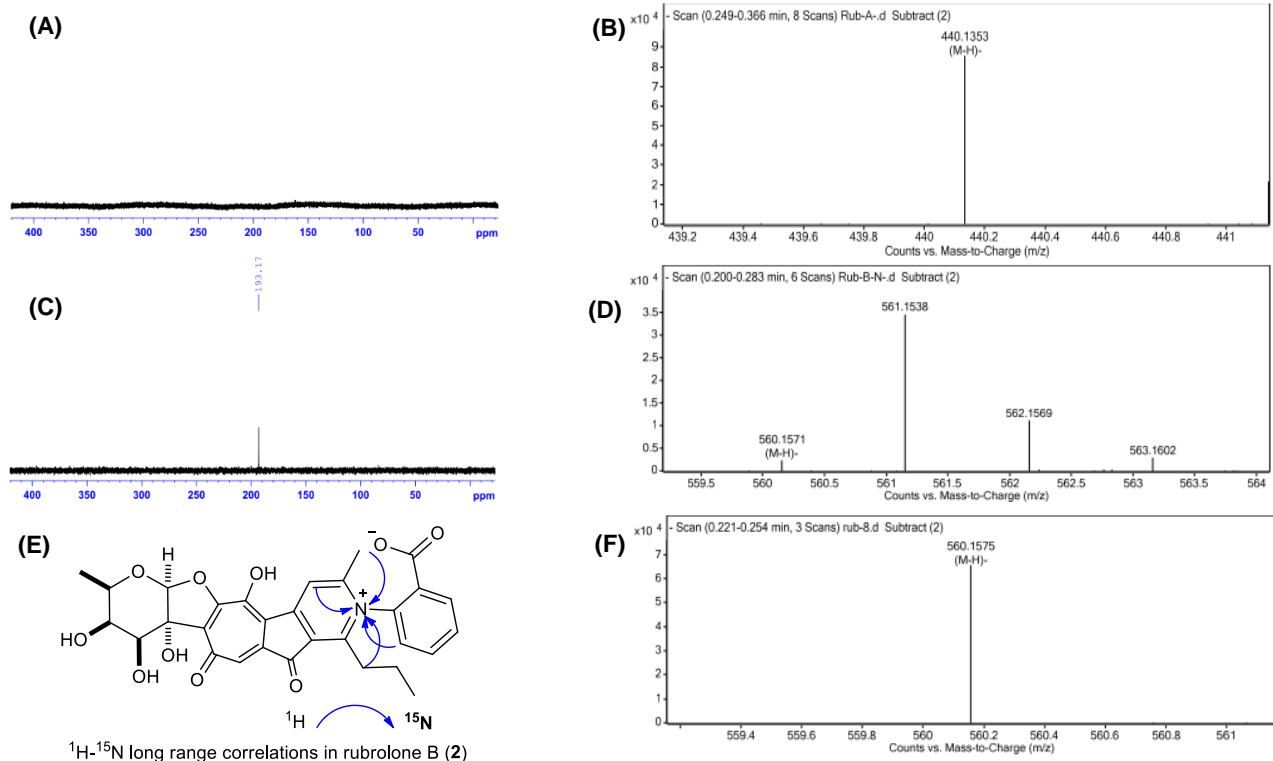
Supplementary Figure 2. HPLC profiles of the fermentation extracts of engineered heterologous expression strains. I: *S. albus* 9B10-1; II: *S. albus* 9B10-2; III: *S. albus* 9B10- $\Delta E7$; IV: *S. albus* 9B10- ΔA ; V: *S. albus* 9B10- $\Delta S2$; VI: *S. albus* 9B10- $\Delta S3$; VII: *S. albus* 9B10- $\Delta S7$; VIII: *S. albus* 9B10 with reduced nitrogen source (removing the ammonium acetate and decreasing the yeast extract to 1g/L in the fermentation medium).



Supplementary Figure 3. Selected COSY and HMBC correlations for compounds **4**, **6-8**, and **10-11**.

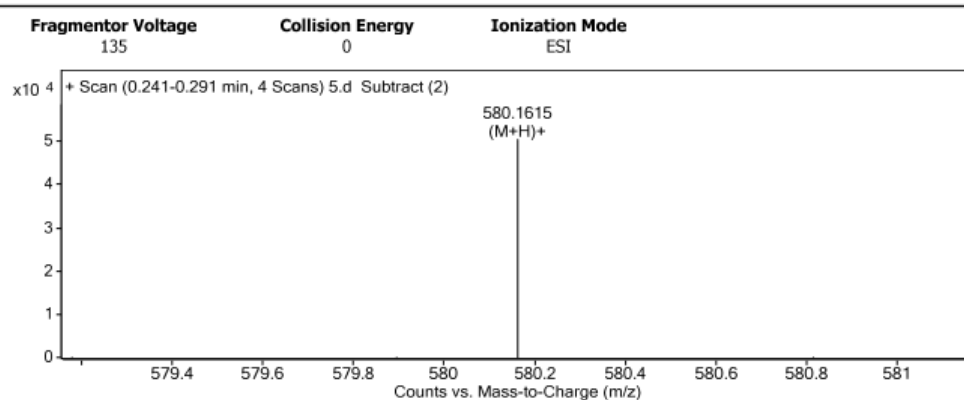


Supplementary Figure 4. Maps of the *rub* and *R1128* biosynthetic gene cluster.



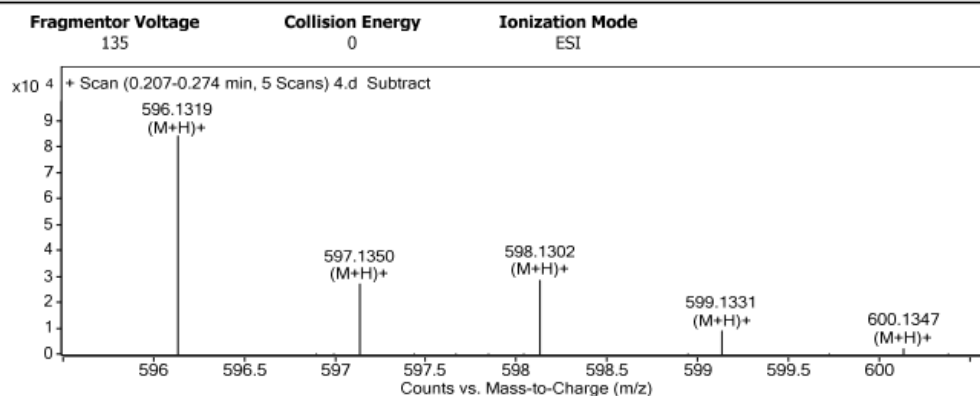
Supplementary Figure 5. MS and NMR data (measured in $\text{DMSO-}d_6$) of **1** and **2** with or without ^{15}N -labeled anthranilic acid feeding. (A) ^{15}N NMR spectrum of **1** obtained after feeding with ^{15}N -labeled anthranilic acid; (B) HRESIMS analysis of **1** obtained after feeding with ^{15}N -labeled anthranilic acid; (C) ^{15}N NMR spectrum of **2** obtained after feeding with ^{15}N -labeled anthranilic acid; (D) HRESIMS analysis of **2** obtained after feeding with ^{15}N -labeled anthranilic acid; (E) ^1H - ^{15}N long range correlations of **2** obtained after feeding with ^{15}N -labeled anthranilic acid; (F) HRESIMS analysis of **2** without feeding of ^{15}N -labeled anthranilic acid.

User Spectra



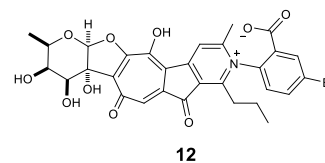
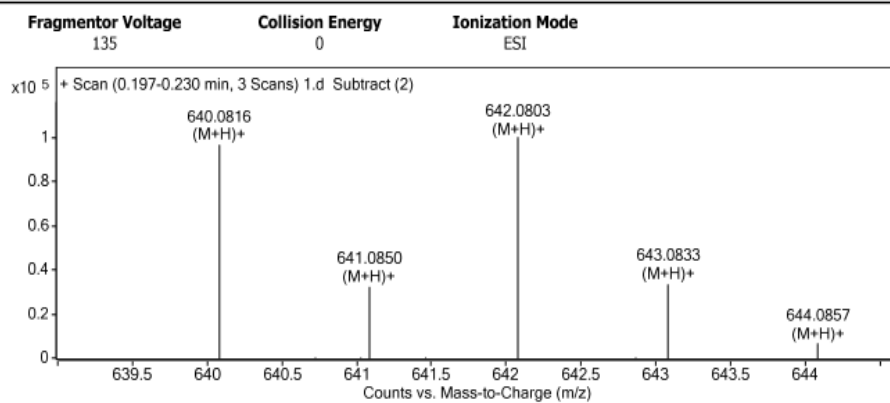
Supplementary Figure 6. HRESIMS analysis of compound **10**.

User Spectra



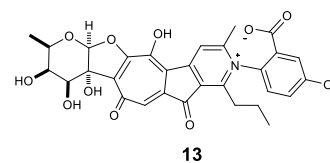
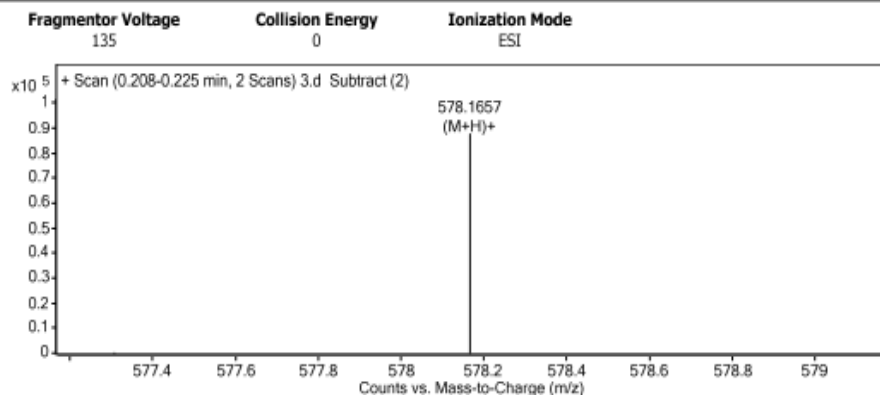
Supplementary Figure 7. HRESIMS analysis of compound 11.

User Spectra



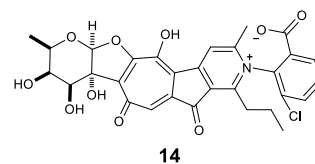
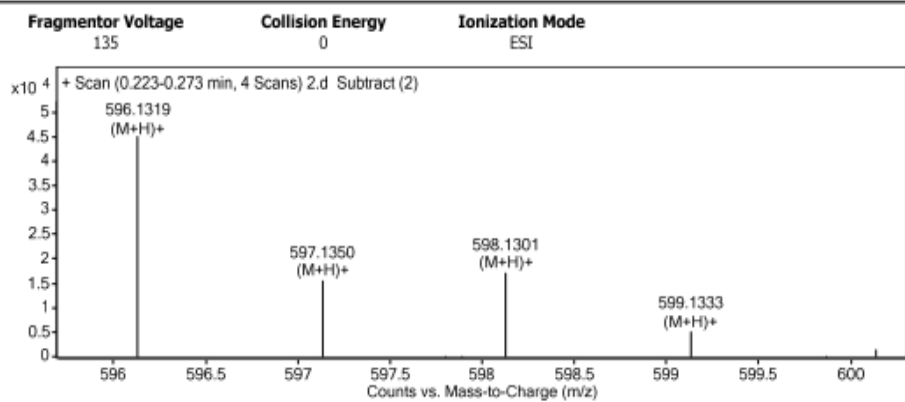
Supplementary Figure 8. HRESIMS analysis of compound 12.

User Spectra



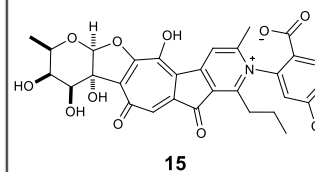
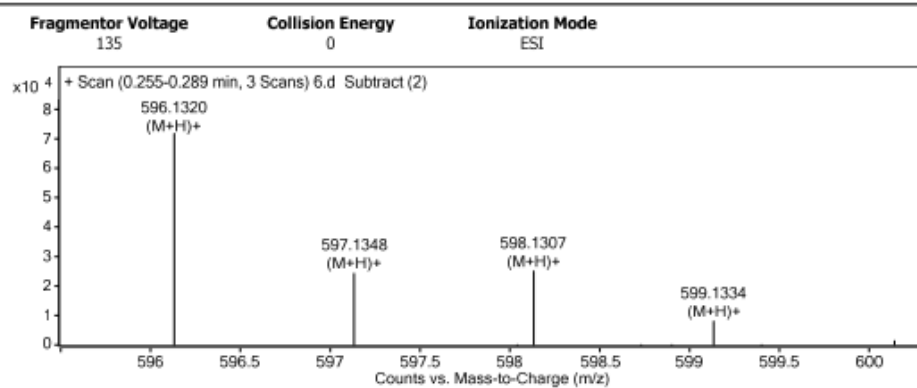
Supplementary Figure 9. HRESIMS analysis of compound 13.

User Spectra



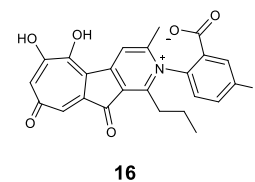
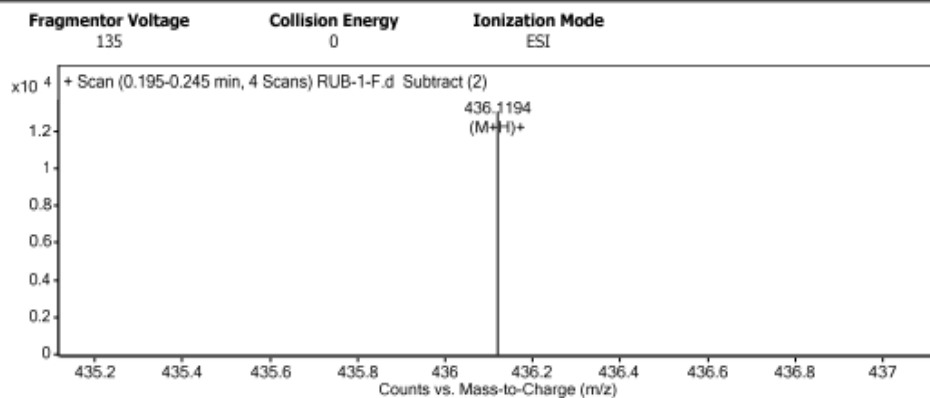
Supplementary Figure 10. HRESIMS analysis of compound 14.

User Spectra



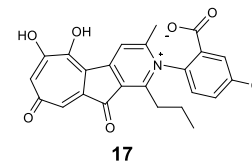
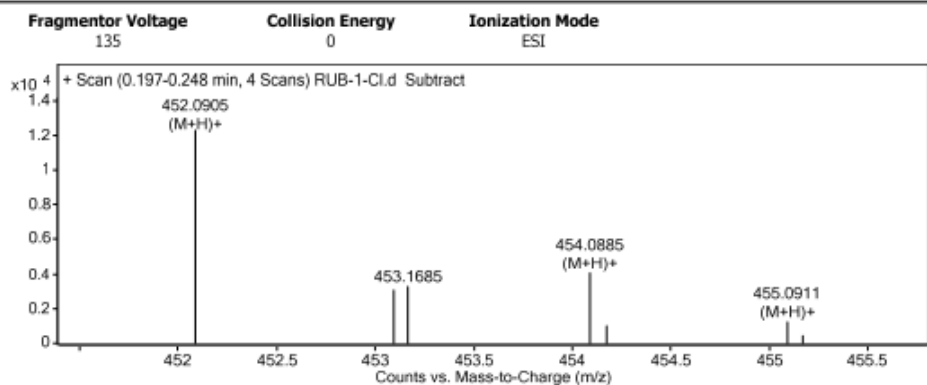
Supplementary Figure 11. HRESIMS analysis of compound 15.

User Spectra



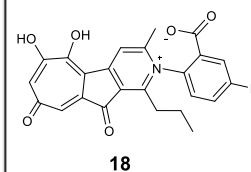
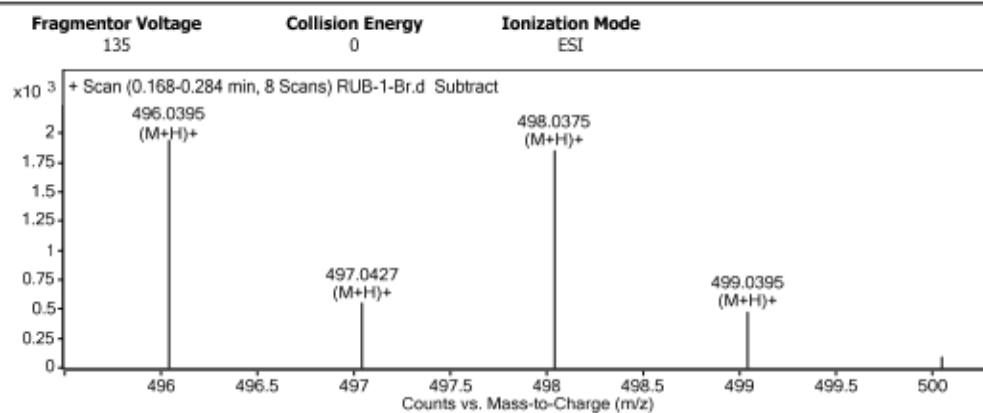
Supplementary Figure 12. HRESIMS analysis of compound 16.

User Spectra



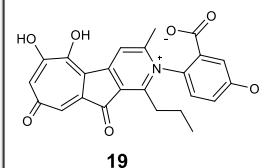
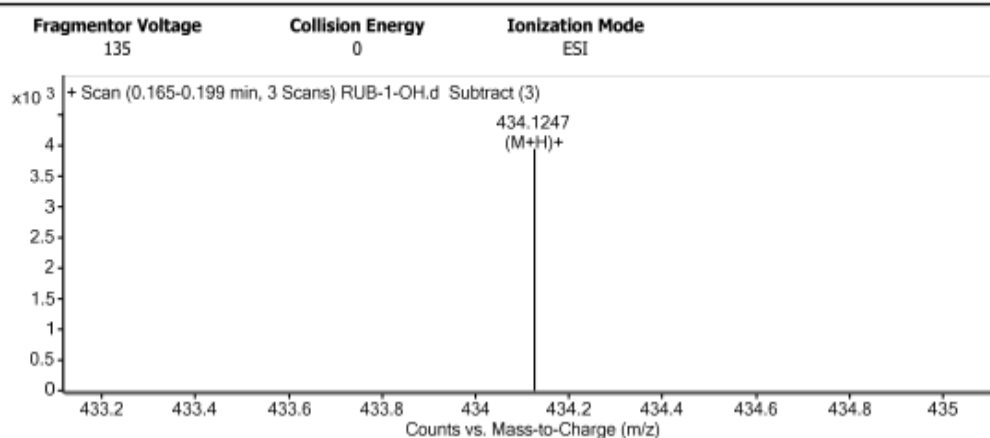
Supplementary Figure 13. HRESIMS analysis of compound 17.

User Spectra

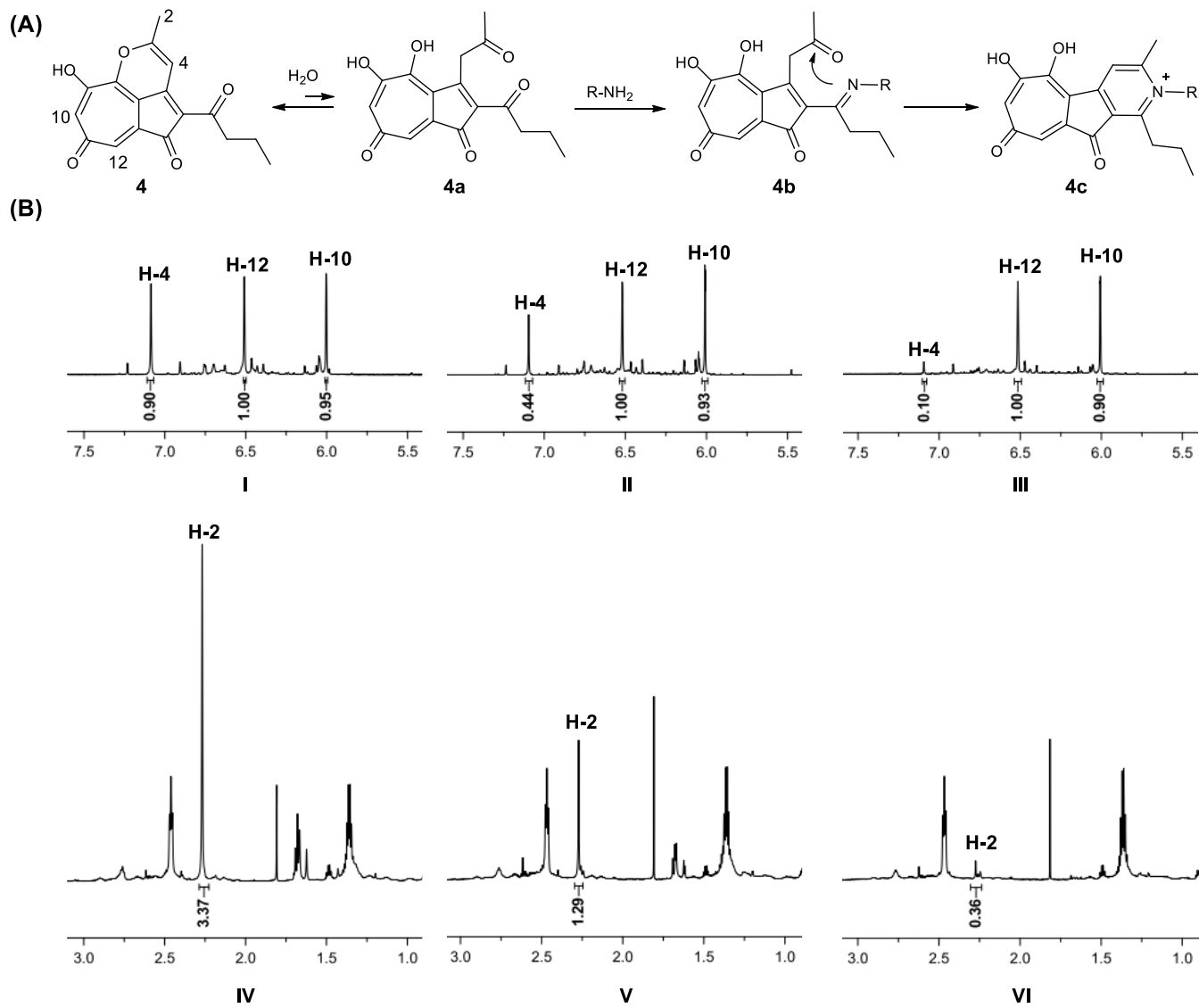


Supplementary Figure 14. HRESIMS analysis of compound 18.

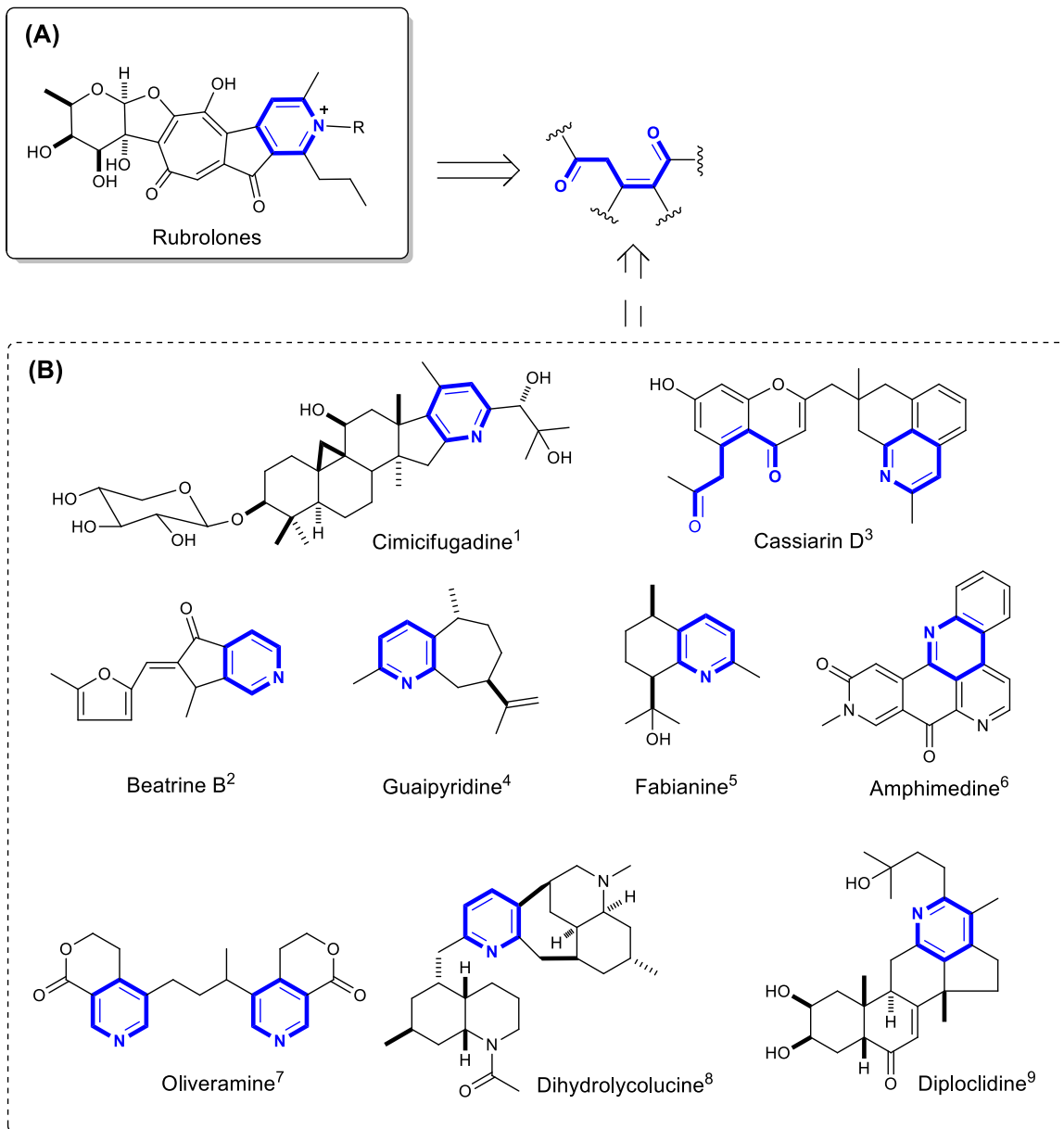
User Spectra



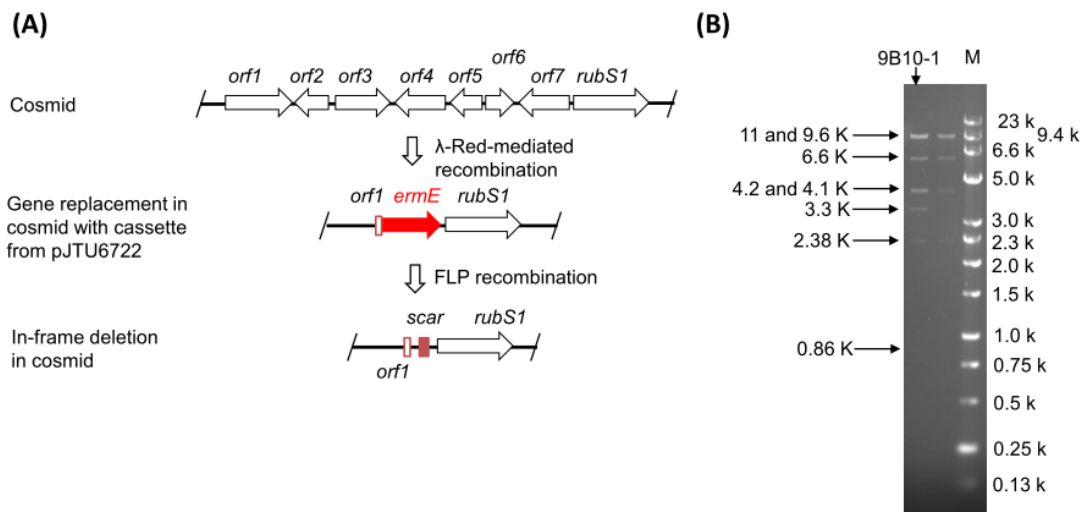
Supplementary Figure 15. HRESIMS analysis of compound 19.



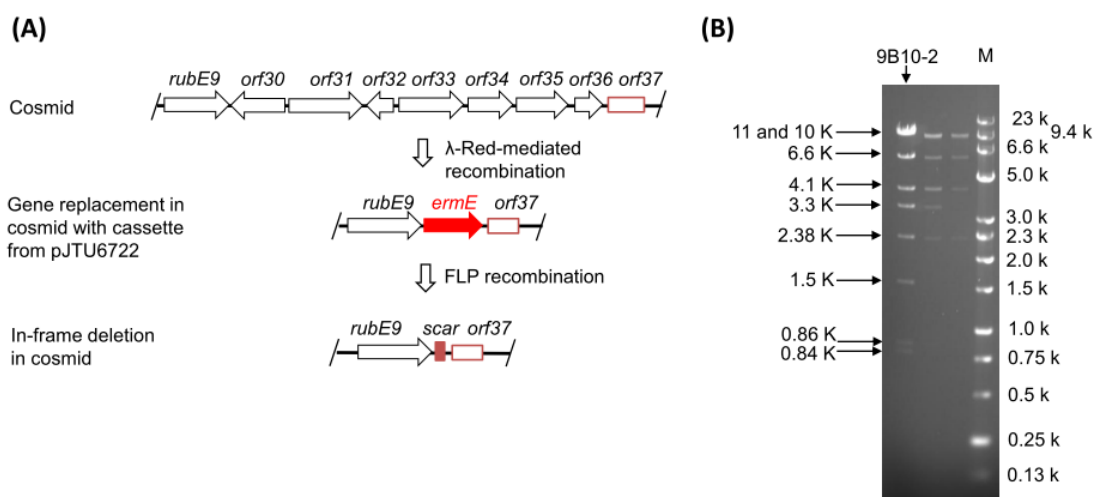
Supplementary Figure 16. (A) Proposed mechanism for the pyridyl moiety formation from intermediate **4**; (B) the enlarged ^1H NMR spectra of **4** in D_2O after 5 minutes (spectra I and IV, left), 2 hours (spectra II and V, middle) and 4 hours (spectra III and VI, right) incubation.



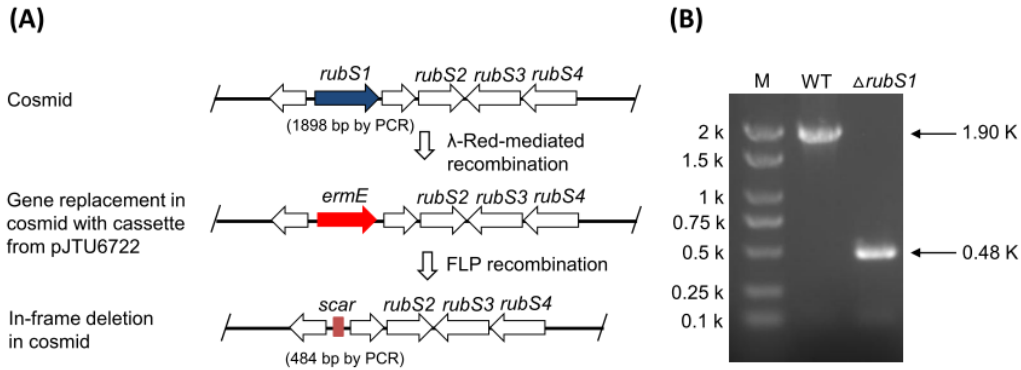
Supplementary Figure 17. The proposed central role of the reactive 1,5-dione moiety (blue) in accessing a diverse pyridine chemical universe including (A) rubrolones and (B) selected natural alkaloids for which are potentially involved in the non-enzymatical pyridine formation using the intermediacy of the 1,5-dione moiety.



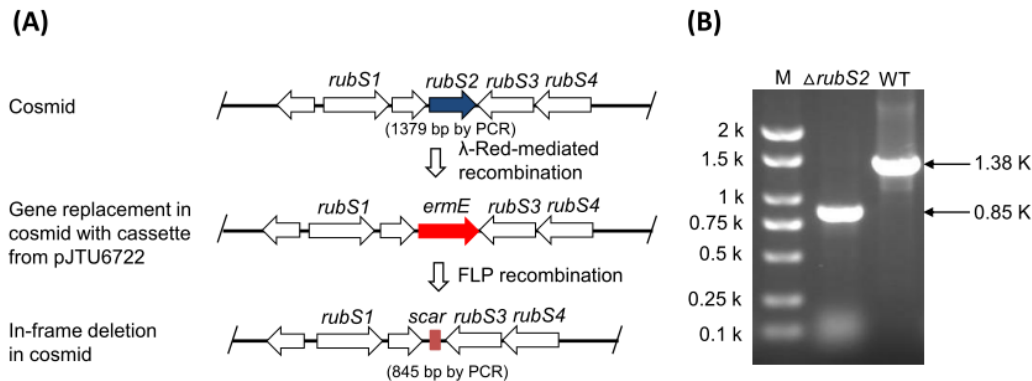
Supplementary Figure 18. Gene replacement of *orf1-7* using the PCR-targeting method. (A) Scheme for the construction of *orf1-7* replacement mutant. (B) Confirmation of plasmid p9B10-1 by restriction endonuclease BamHI. Lane M: DNA molecular ladder. Lane 9B10-1: Restriction fragments from the p9B10-1 (11259 bp, 9647 bp, 6601 bp, 4209 bp, 4123 bp, 3299 bp, 2376 bp, 858 bp, 20 bp).



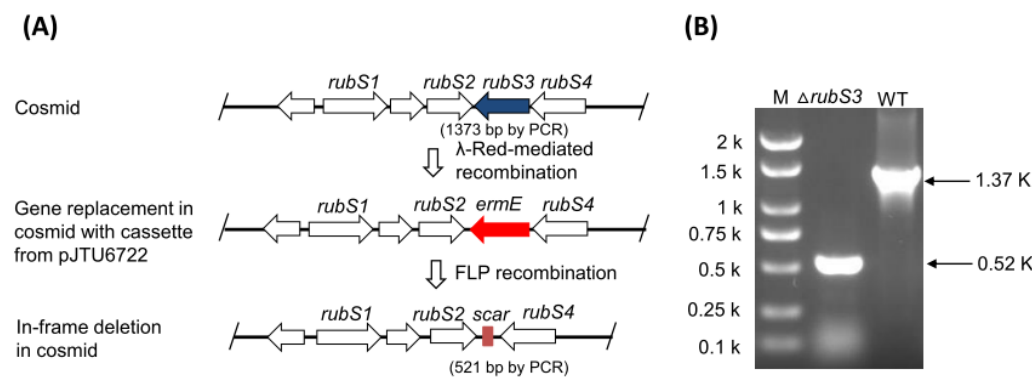
Supplementary Figure 19. Gene replacement of *orf30-37* using the PCR-targeting method. (A) Scheme for the construction of *orf30-37* replacement mutant. (B) Confirmation of plasmid p9B10-2 by restriction endonuclease BamHI. Lane M: DNA molecular ladder. Lane 9B10-2: Restriction fragments from the p9B10-2 (11259 bp, 10062 bp, 6601 bp, 4123 bp, 3299 bp, 2376 bp, 1473 bp, 858 bp, 844 bp, 37 bp).



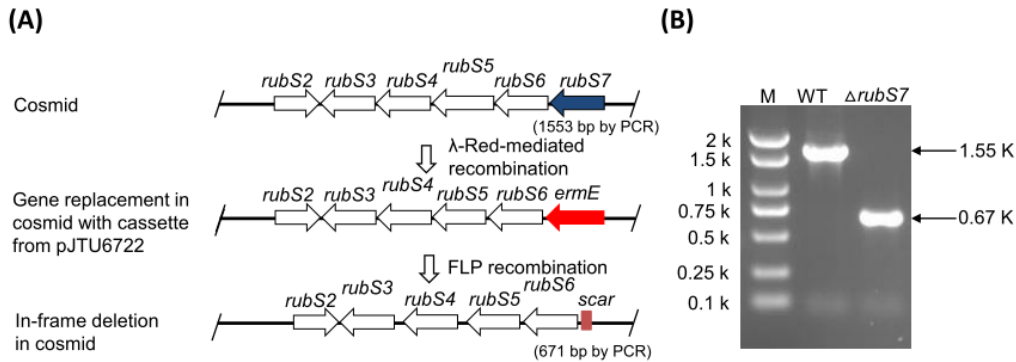
Supplementary Figure 20. Gene replacement of *rubS1* using the PCR-targeting method. (A) Scheme for the construction of *rubS1* replacement mutant. (B) Confirmation of mutant by PCR using the primers listed in supplementary Table 6. Lane M: DNA molecular ladder. Lane WT, PCR product from the *S. albus* 9B10. Lane $\Delta rubS1$, PCR product from *S. albus* 9B10- $\Delta S1$ mutant.



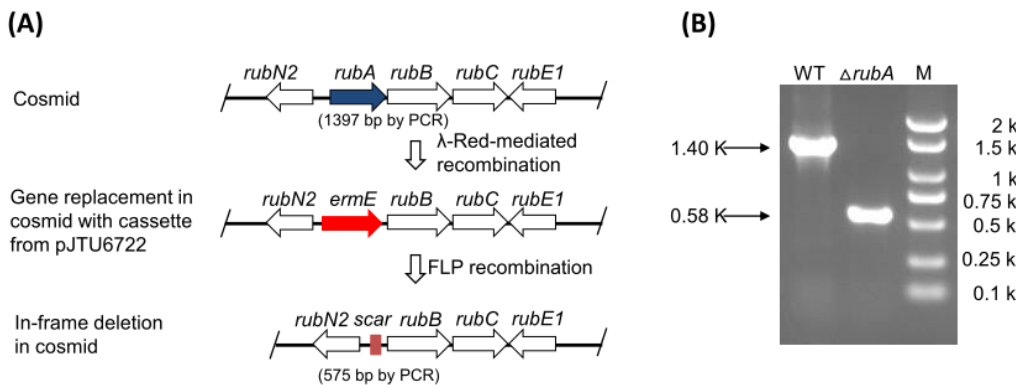
Supplementary Figure 21. Gene replacement of *rubS2* using the PCR-targeting method. (A) Scheme for the construction of *rubS2* replacement mutant. (B) Confirmation of mutant by PCR using the primers listed in supplementary Table 6. Lane M: DNA molecular ladder. Lane WT, PCR product from the *S. albus* 9B10. Lane $\Delta rubS2$, PCR product from *S. albus* 9B10- $\Delta S2$ mutant.



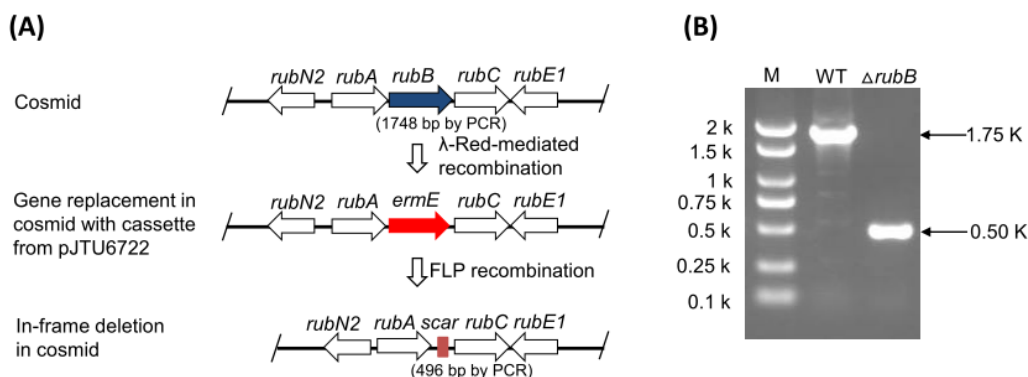
Supplementary Figure 22. Gene replacement of *rubS3* using the PCR-targeting method. (A) Scheme for the construction of *rubS3* replacement mutant. (B) Confirmation of mutant by PCR using the primers listed in supplementary Table 6. Lane M: DNA molecular ladder. Lane WT, PCR product from the *S. albus* 9B10. Lane $\Delta rubS3$, PCR product from *S. albus* 9B10- $\Delta S3$ mutant.



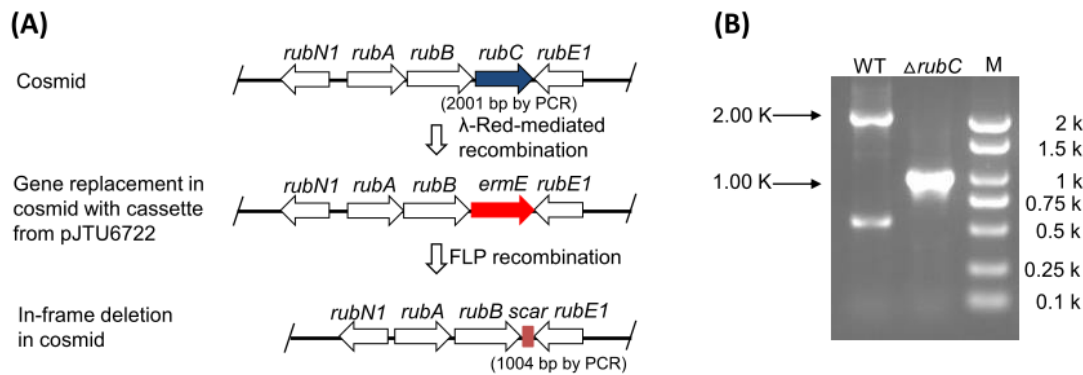
Supplementary Figure 23. Gene replacement of *rubS7* using the PCR-targeting method. (A) Scheme for the construction of *rubS7* replacement mutant. (B) Confirmation of mutant by PCR using the primers listed in supplementary Table 6. Lane M: DNA molecular ladder. Lane WT, PCR product from the *S. albus* 9B10. Lane $\Delta rubS7$, PCR product from *S. albus* 9B10- $\Delta S7$ mutant.



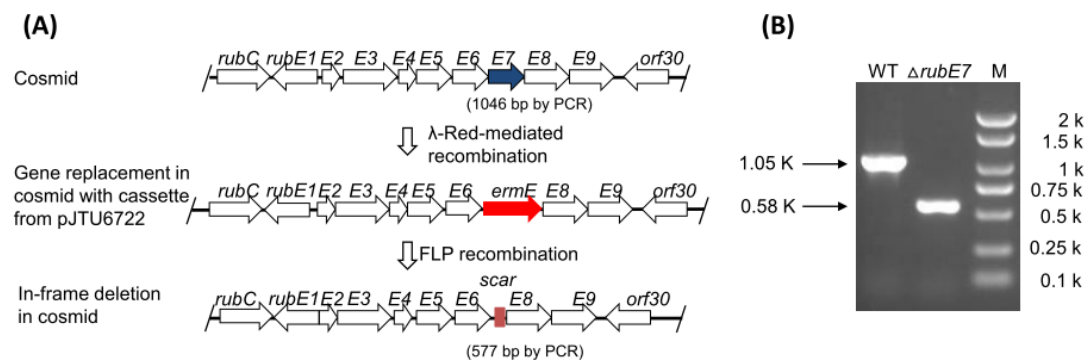
Supplementary Figure 24. Gene replacement of *rubA* using the PCR-targeting method. (A) Scheme for the construction of *rubA* replacement mutant. (B) Confirmation of mutant by PCR using the primers listed in supplementary Table 6. Lane M: DNA molecular ladder. Lane WT, PCR product from the *S. albus* 9B10. Lane $\Delta rubA$, PCR product from *S. albus* 9B10- ΔA mutant.



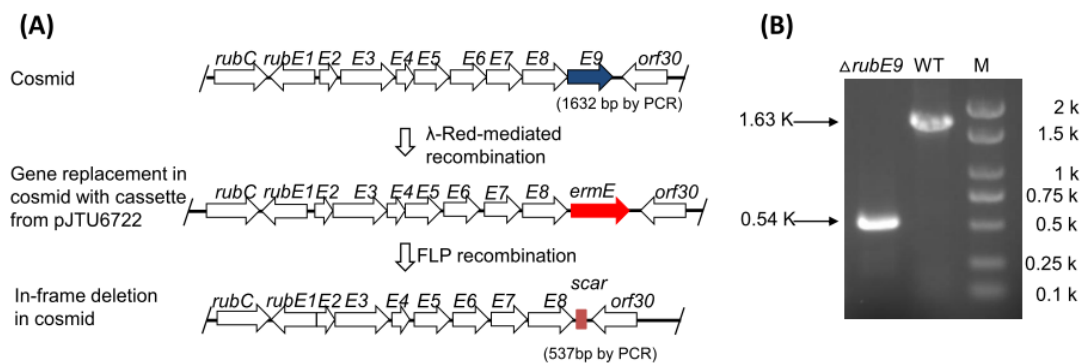
Supplementary Figure 25. Gene replacement of *rubB* using the PCR-targeting method. (A) Scheme for the construction of *rubB* replacement mutant. (B) Confirmation of mutant by PCR using the primers listed in supplementary Table 6. Lane M: DNA molecular ladder. Lane WT, PCR product from the *S. albus* 9B10. Lane $\Delta rubB$, PCR product from *S. albus* 9B10- ΔB mutant.



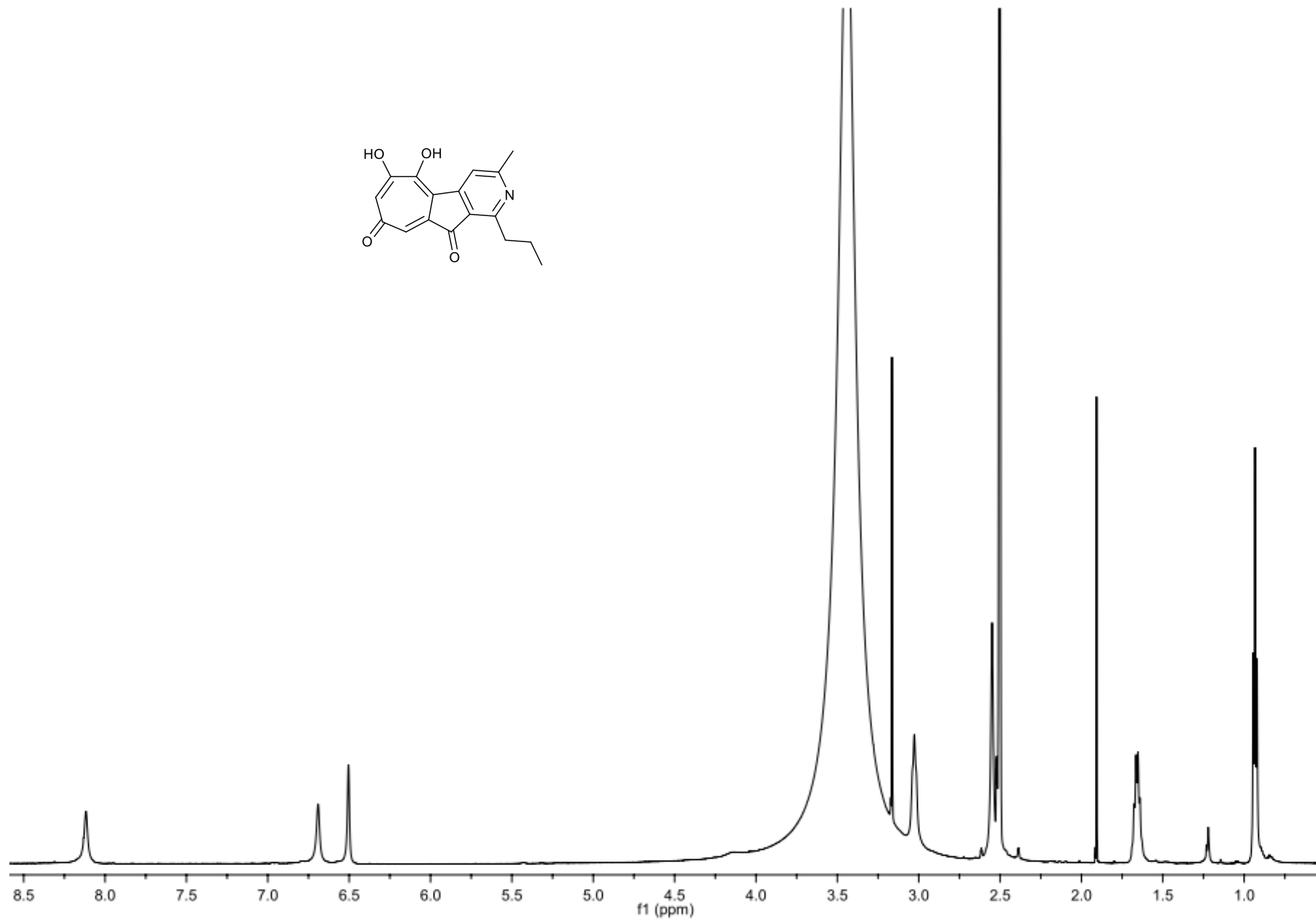
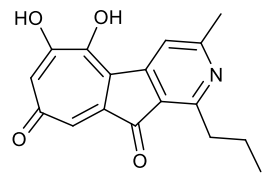
Supplementary Figure 26. Gene replacement of *rubC* using the PCR-targeting method. (A) Scheme for the construction of *rubC* replacement mutant. (B) Confirmation of mutant by PCR using the primers listed in supplementary Table 6. Lane M: DNA molecular ladder. Lane WT, PCR product from the *S. albus* 9B10. Lane $\Delta rubC$, PCR product from *S. albus* 9B10- ΔC mutant.



Supplementary Figure 27. Gene replacement of *rubE7* using the PCR-targeting method. (A) Scheme for the construction of *rubE7* replacement mutant. (B) Confirmation of mutant by PCR using the primers listed in supplementary Table 6. Lane M: DNA molecular ladder. Lane WT, PCR product from the *S. albus* 9B10. Lane $\Delta rubE7$, PCR product from *S. albus* 9B10- $\Delta E7$ mutant.

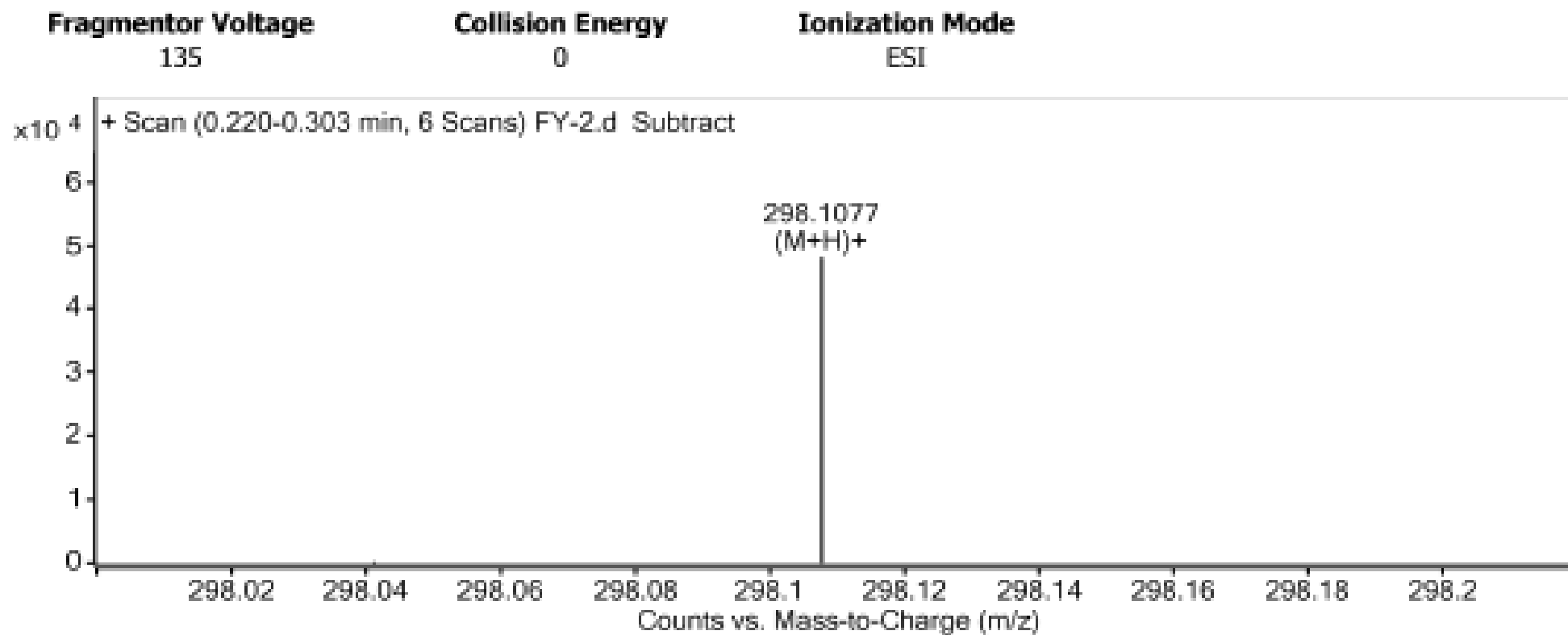


Supplementary Figure 28. Gene replacement of *rubE9* using the PCR-targeting method. (A) Scheme for the construction of *rubE9* replacement mutant. (B) Confirmation of mutant by PCR using the primers listed in supplementary Table 6. Lane M: DNA molecular ladder. Lane WT, PCR product from the *S. albus* 9B10. Lane $\Delta rubE9$, PCR product from *S. albus* 9B10- $\Delta E9$ mutant.

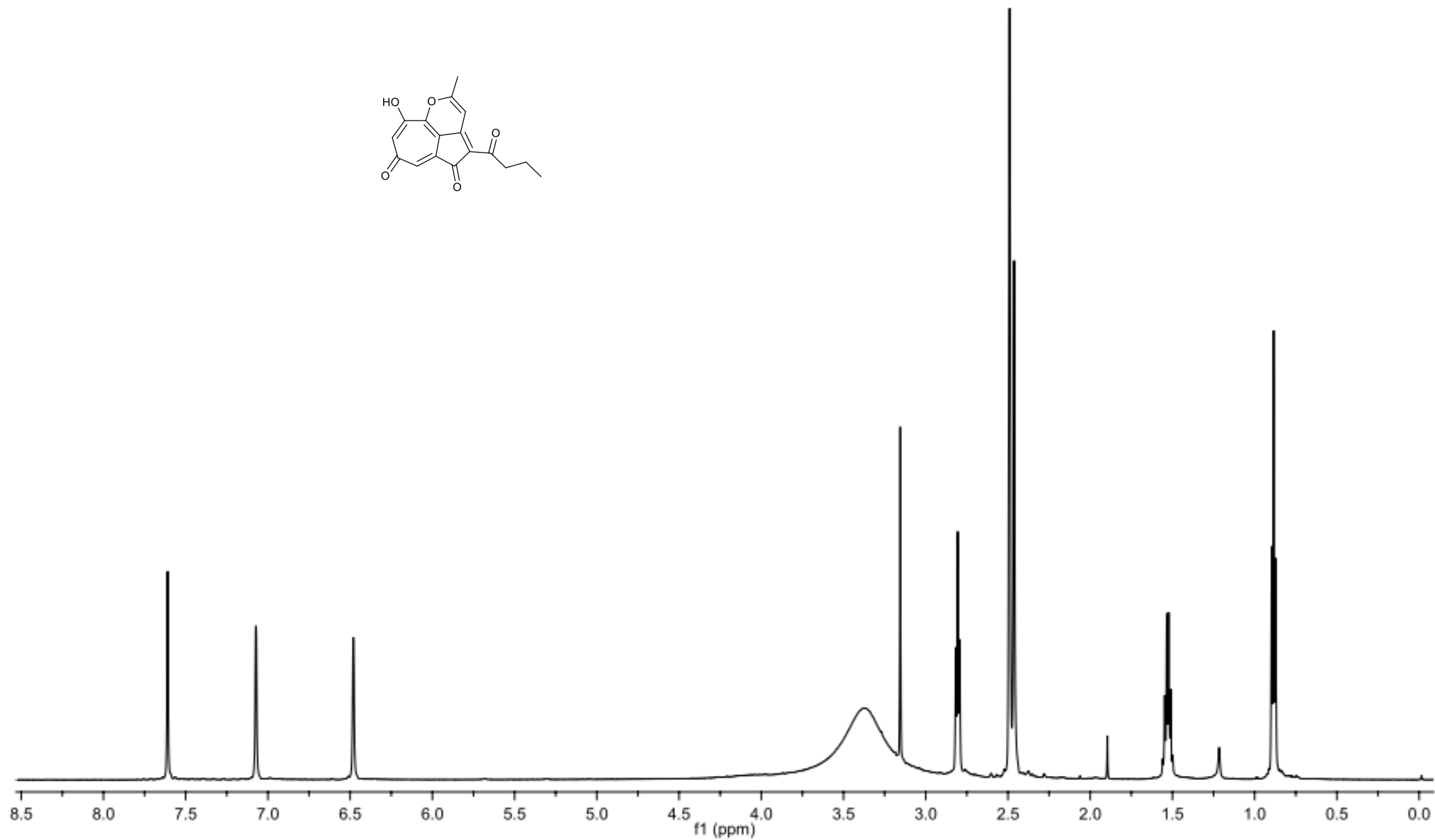
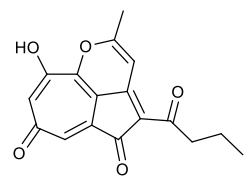


Supplementary Figure 29. ¹H NMR spectrum of compound **3** in DMSO-*d*₆.

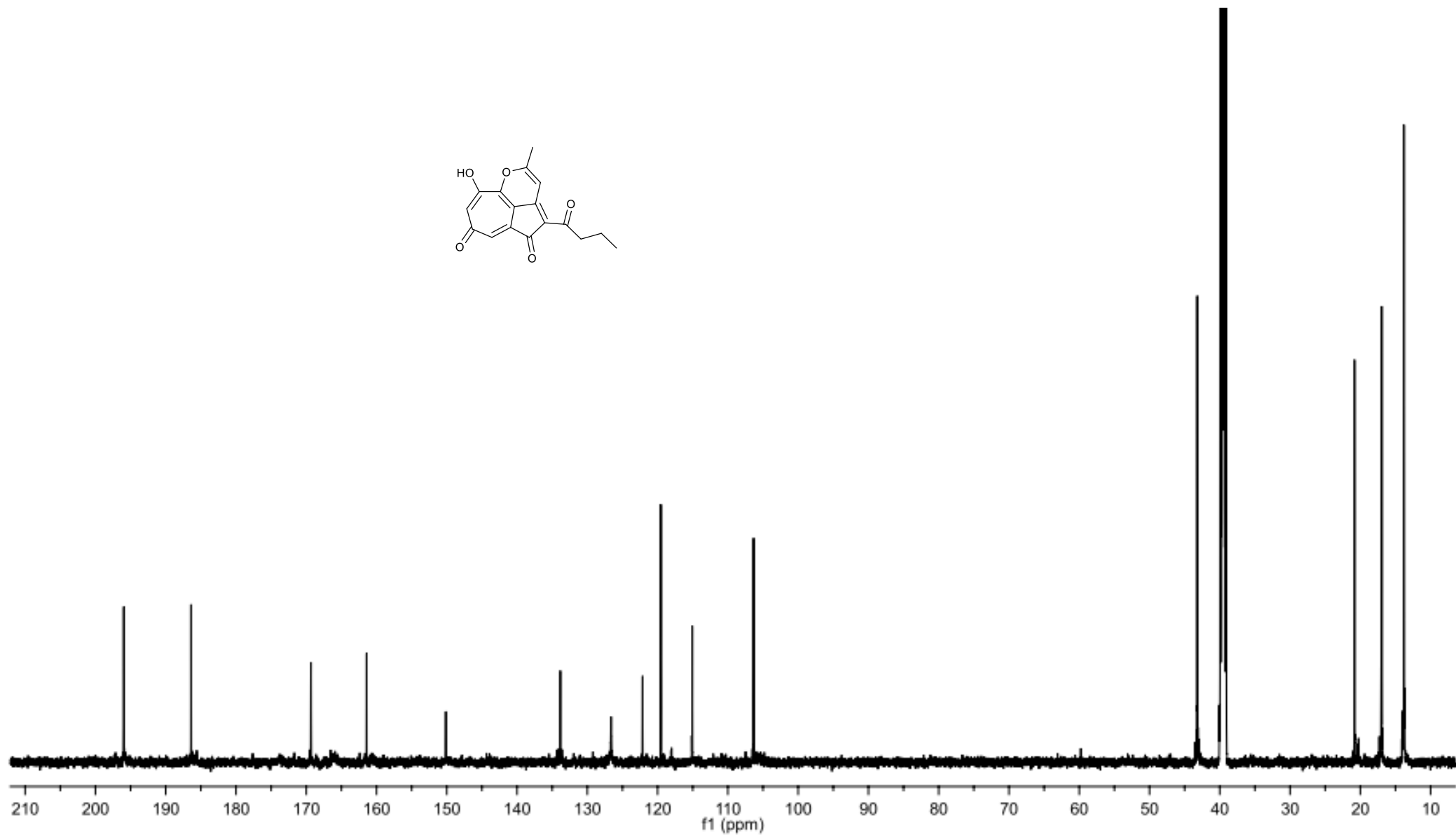
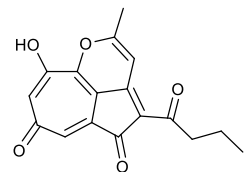
User Spectra



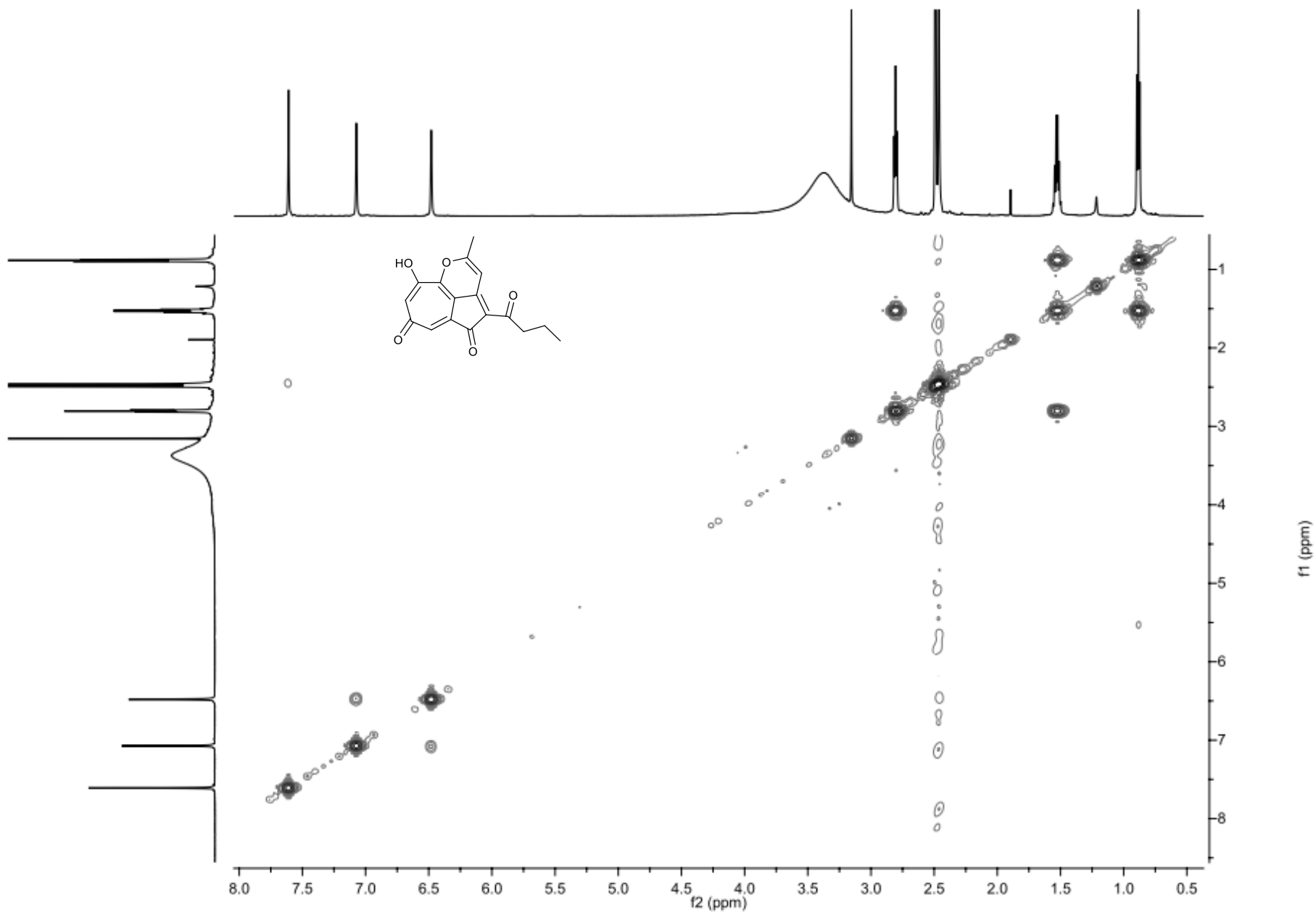
Supplementary Figure 30. HRESIMS analysis of compound 3.



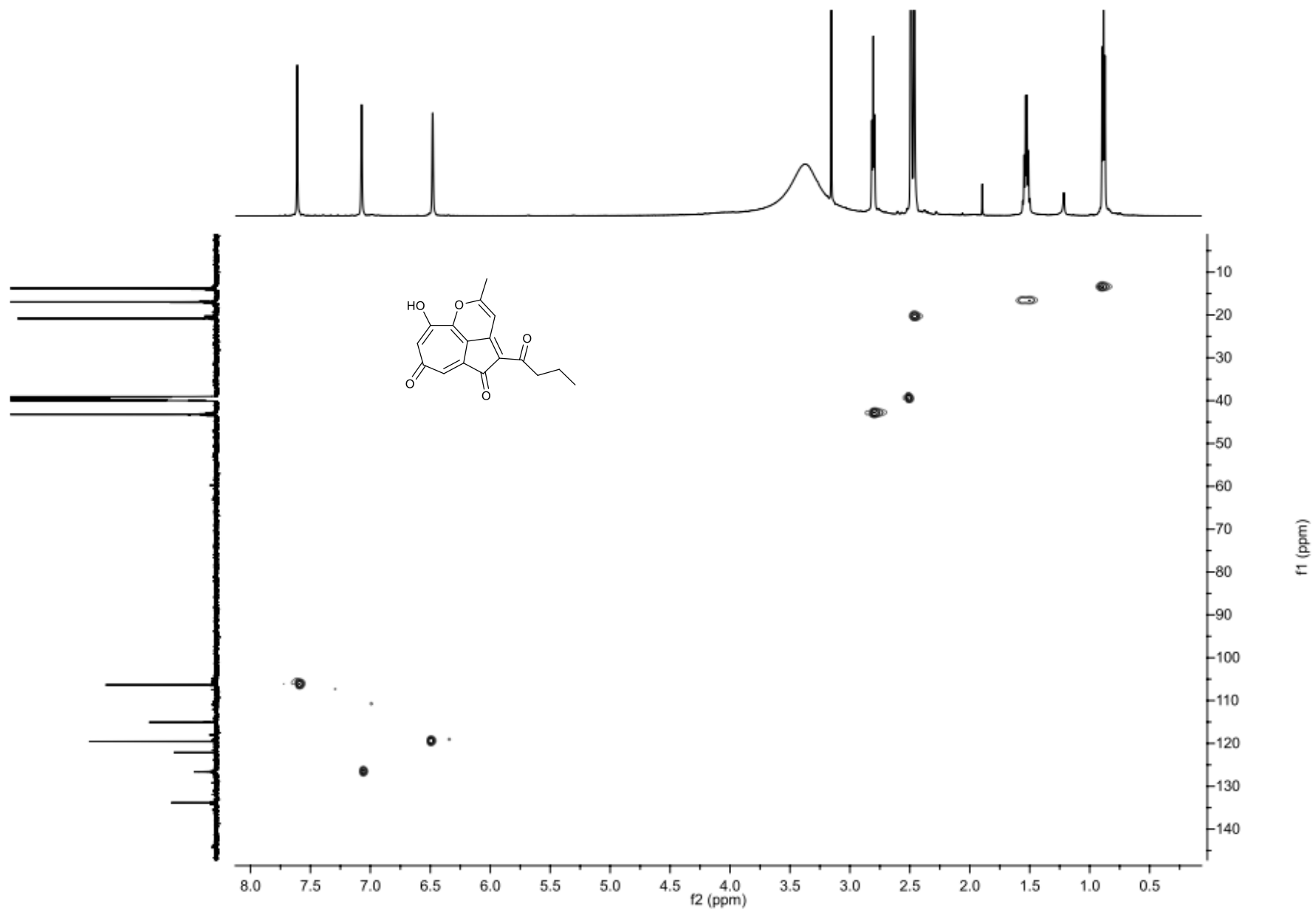
Supplementary Figure 31. ¹H NMR spectrum of compound **4** in DMSO-*d*₆.



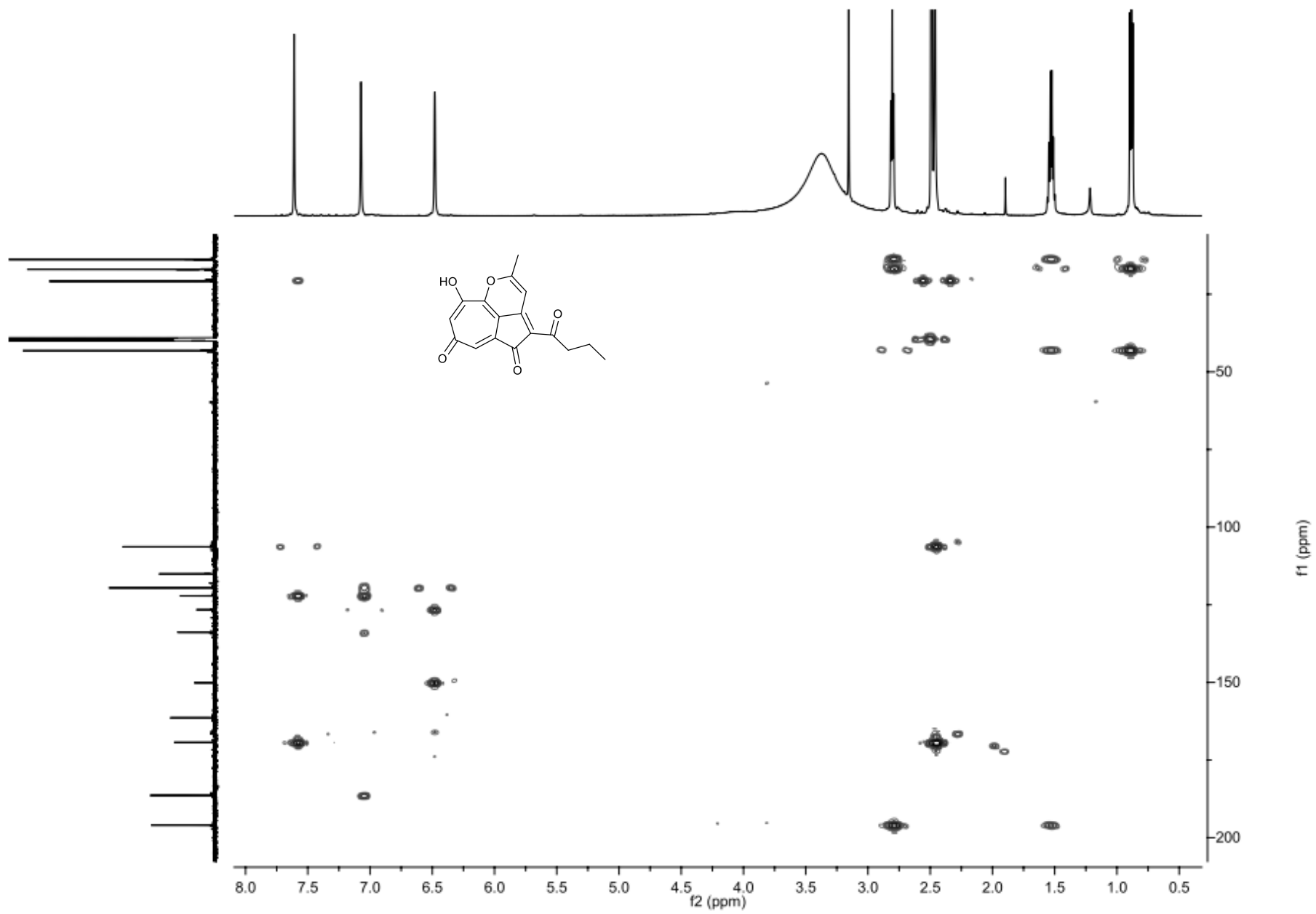
Supplementary Figure 32. ^{13}C NMR spectrum of compound 4 in $\text{DMSO-}d_6$.



Supplementary Figure 33. ^1H - ^1H COSY NMR spectrum of compound 4 in $\text{DMSO}-d_6$.

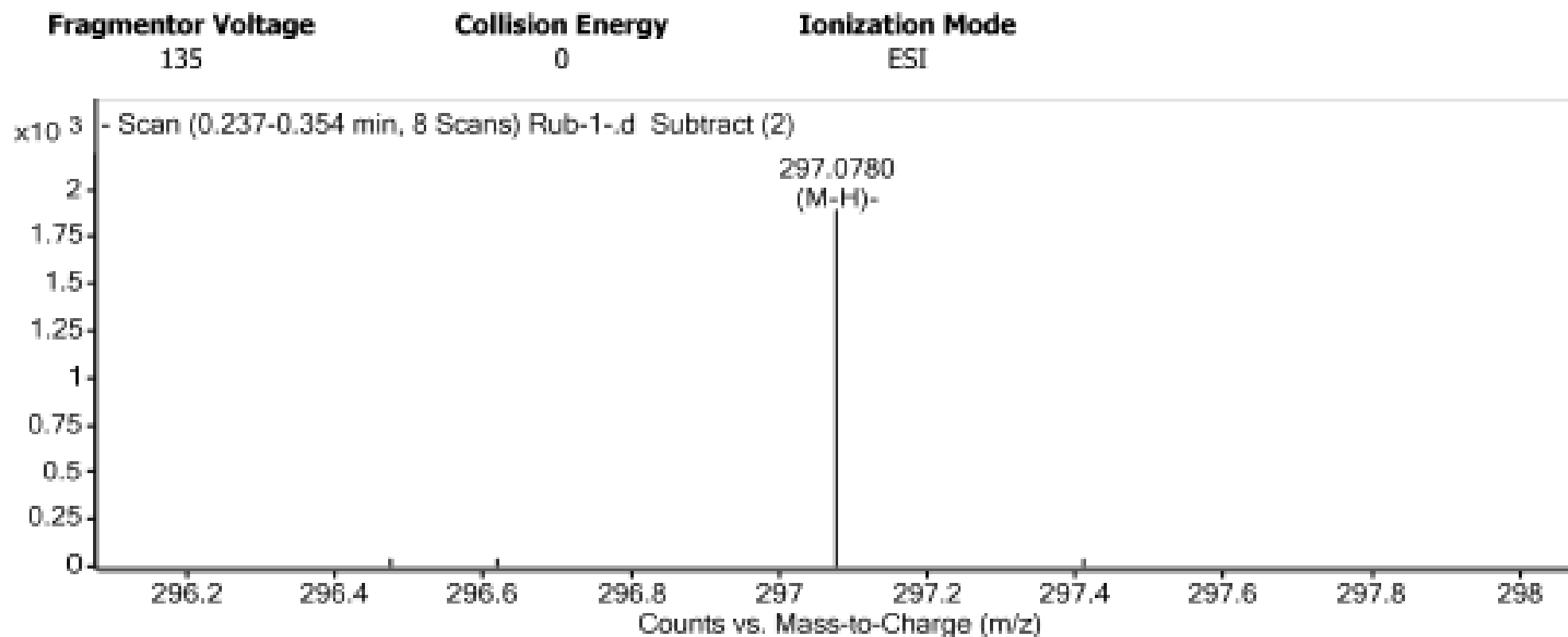


Supplementary Figure 34. HSQC NMR spectrum of compound 4 in DMSO-*d*₆.

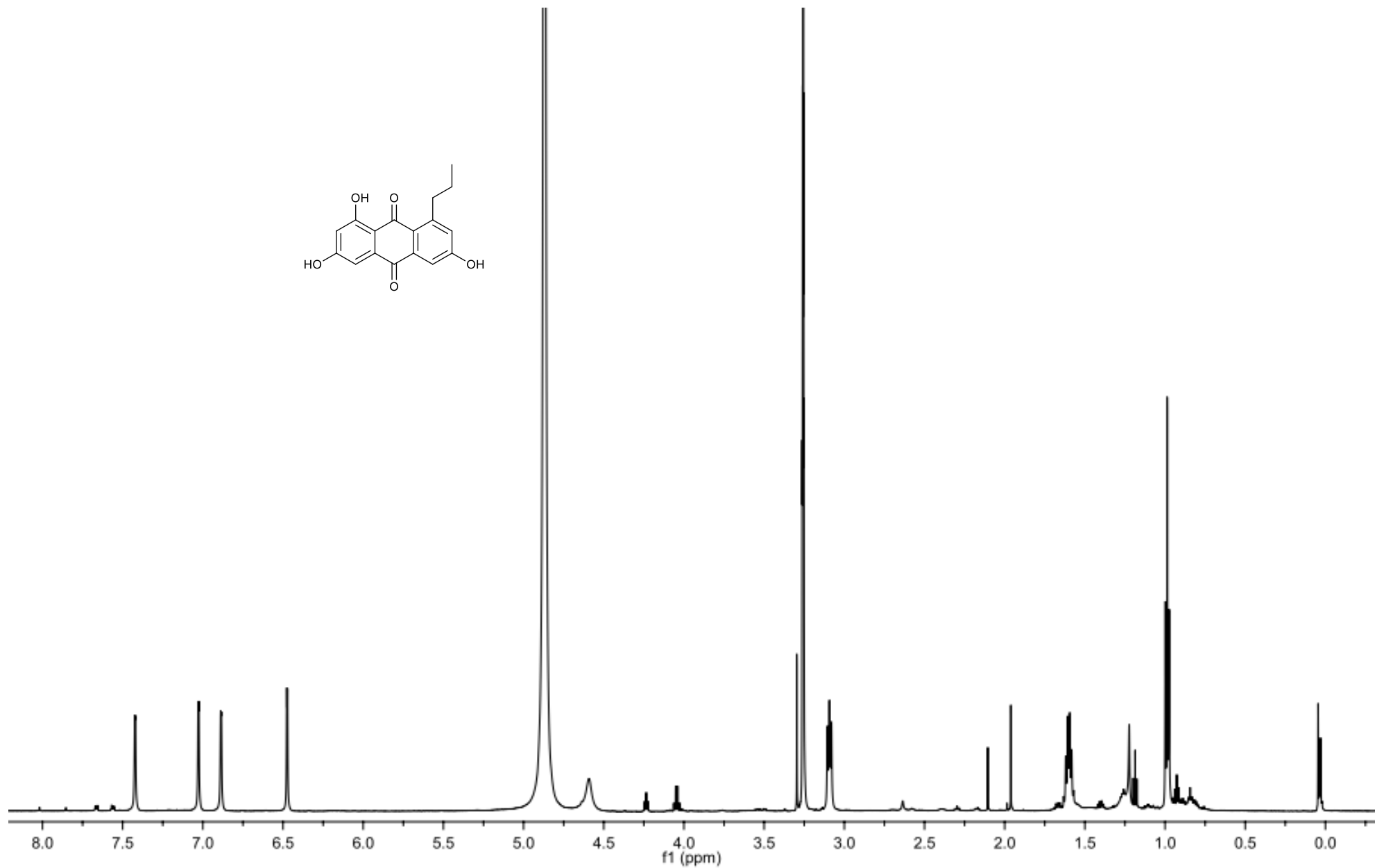
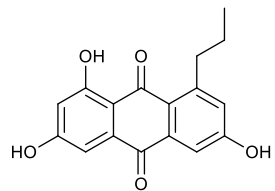


Supplementary Figure 35. HMBC NMR spectrum of compound 4 in DMSO-*d*₆.

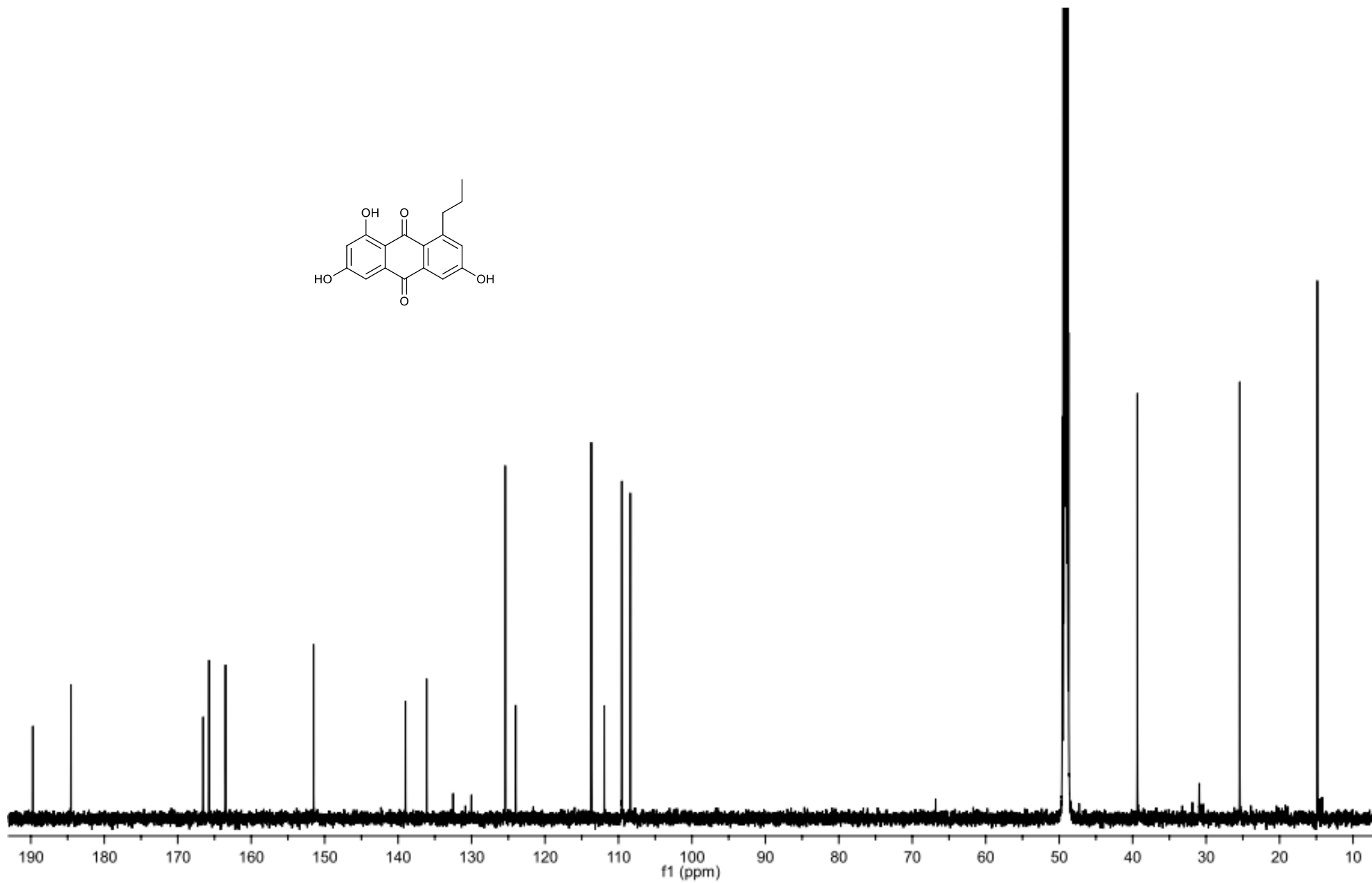
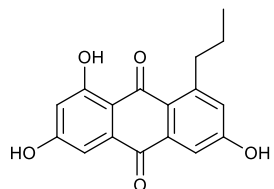
User Spectra



Supplementary Figure 36. HRESIMS analysis of compound 4.

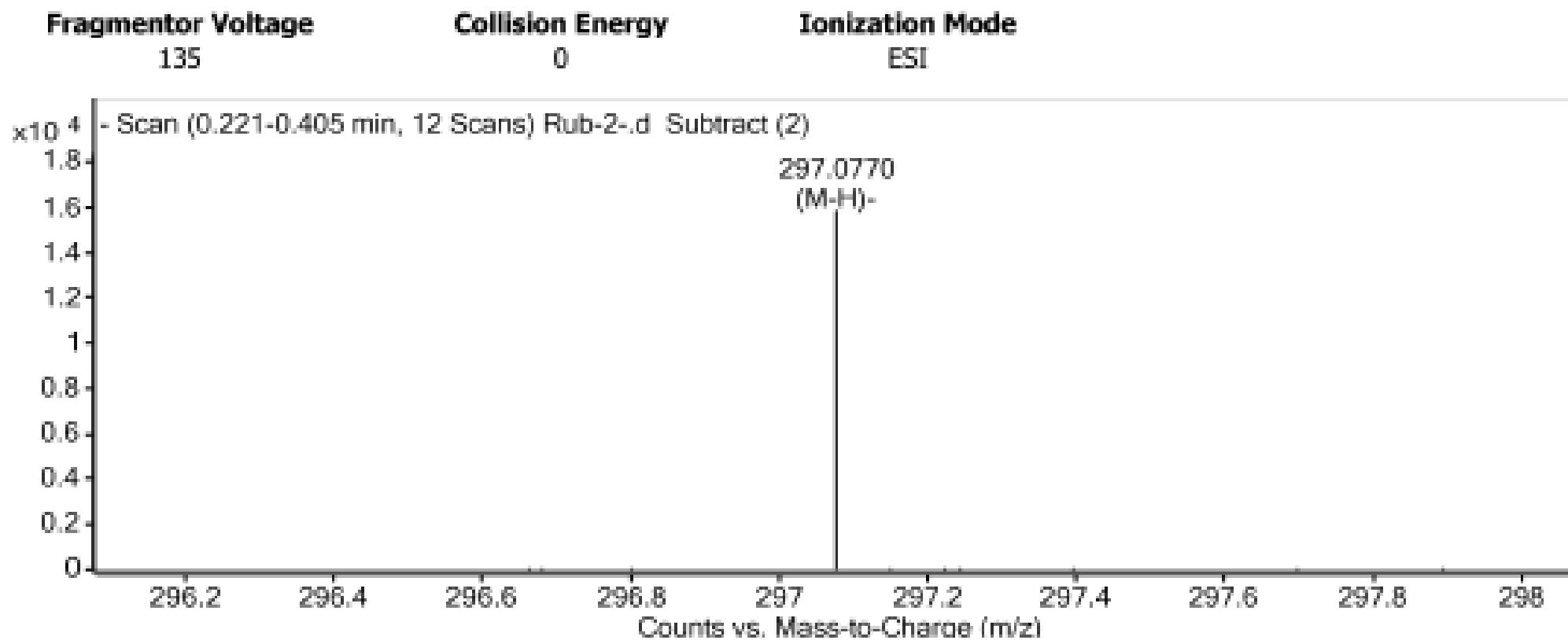


Supplementary Figure 37. ¹H NMR spectrum of compound 5 in CD₃OD.

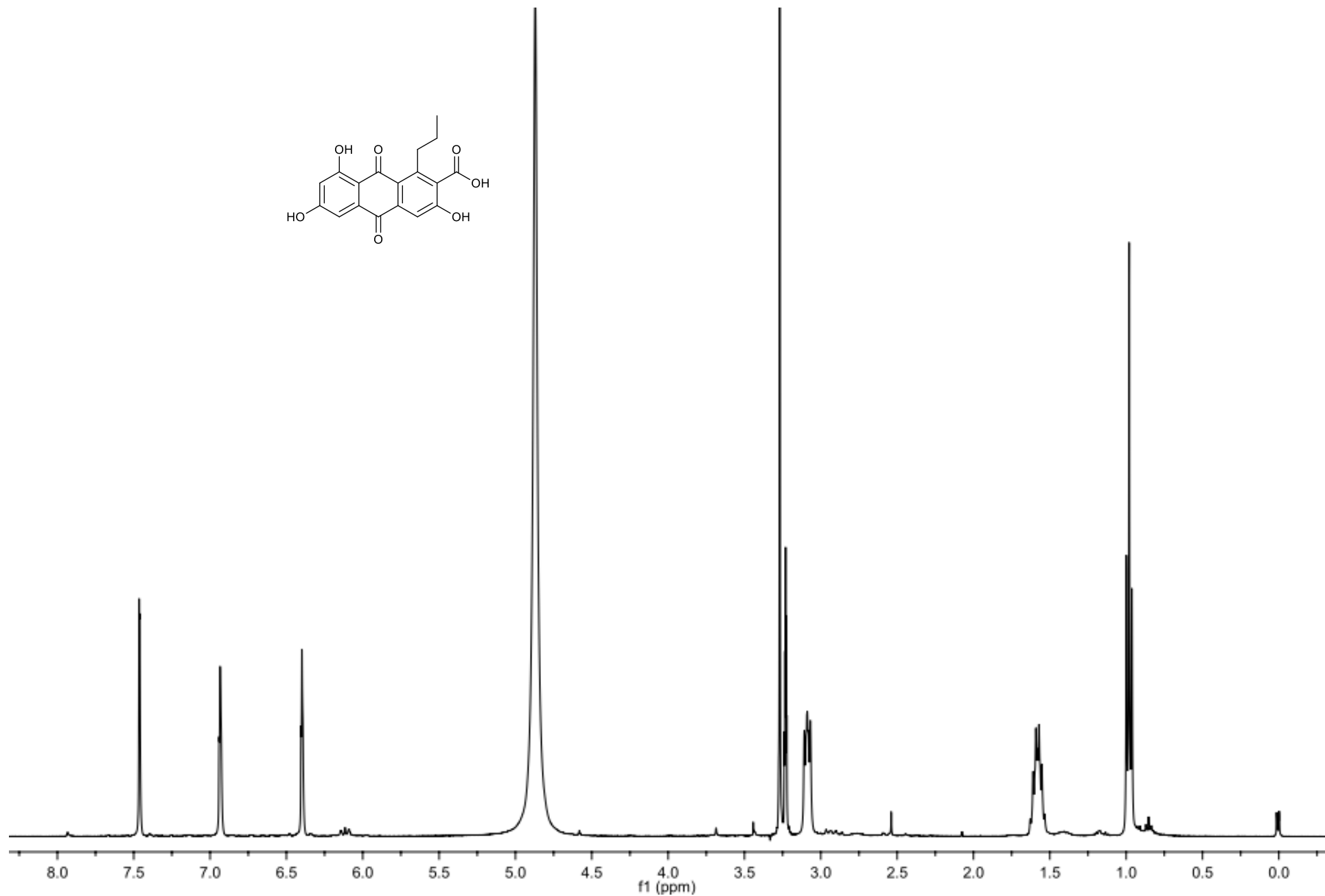
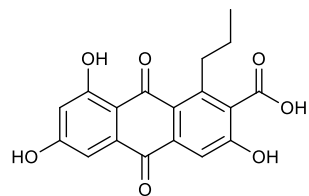


Supplementary Figure 38. ¹³C NMR spectrum of compound 5 in CD₃OD.

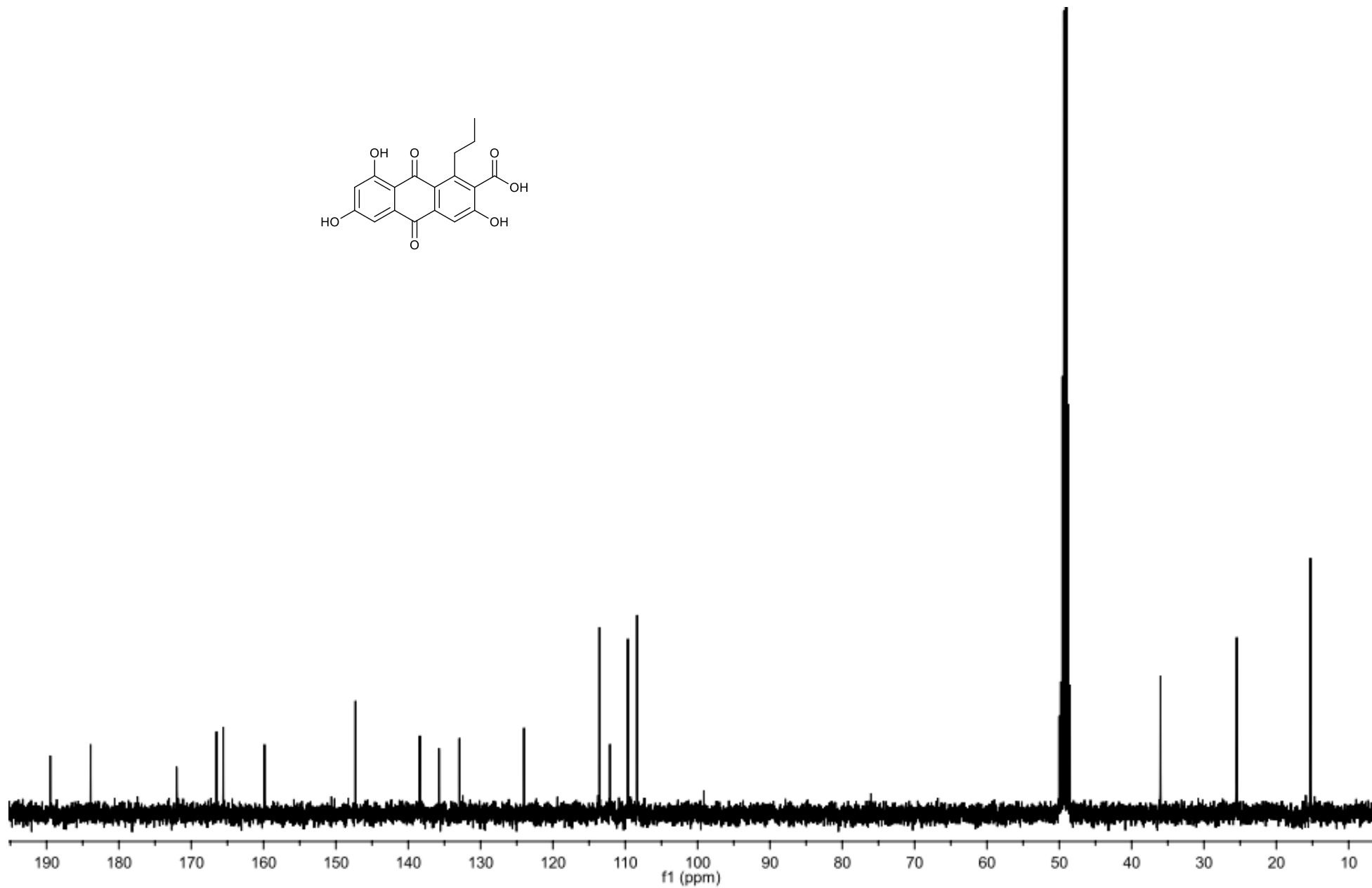
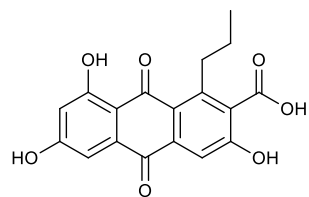
User Spectra



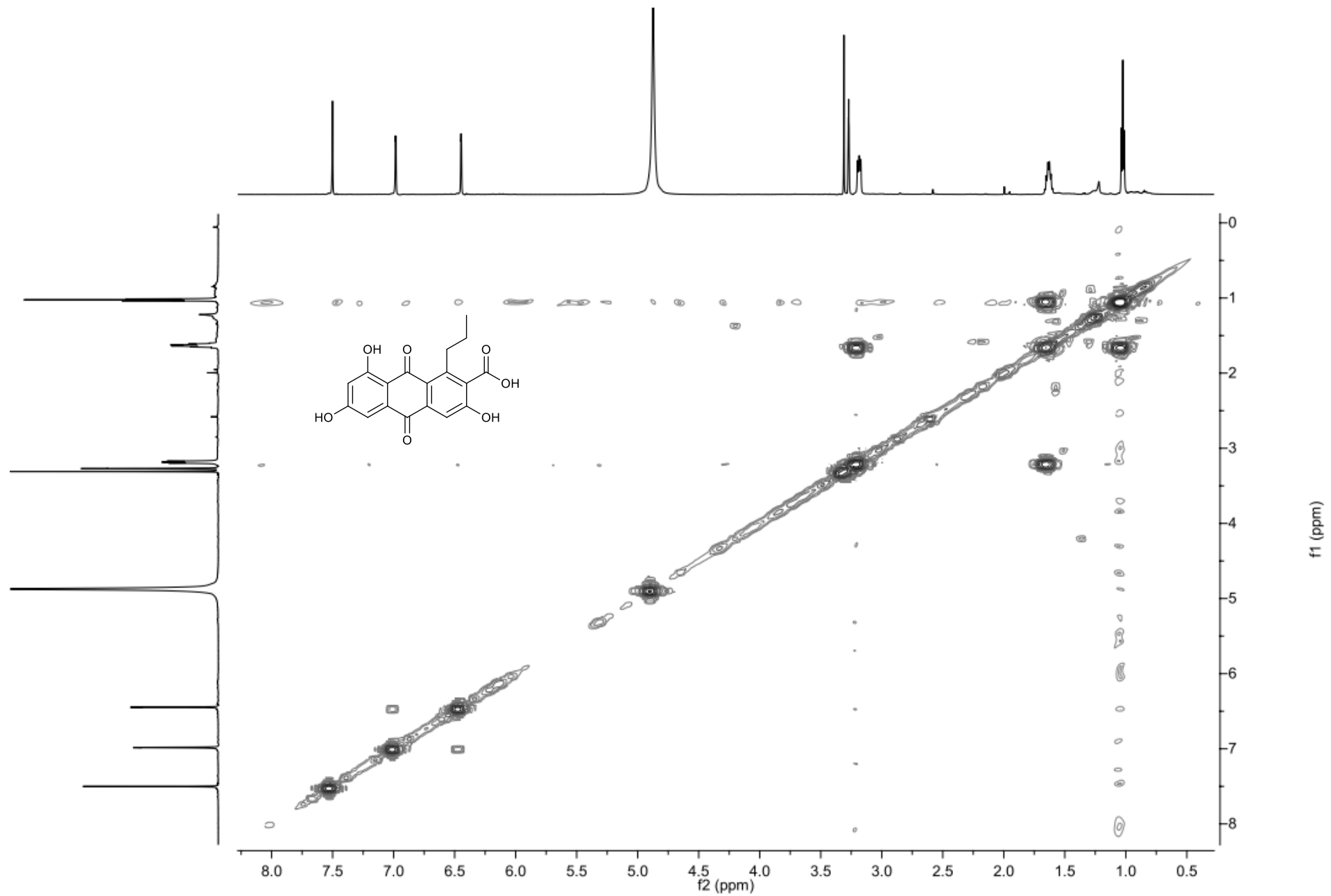
Supplementary Figure 39. HRESIMS analysis of compound 5.



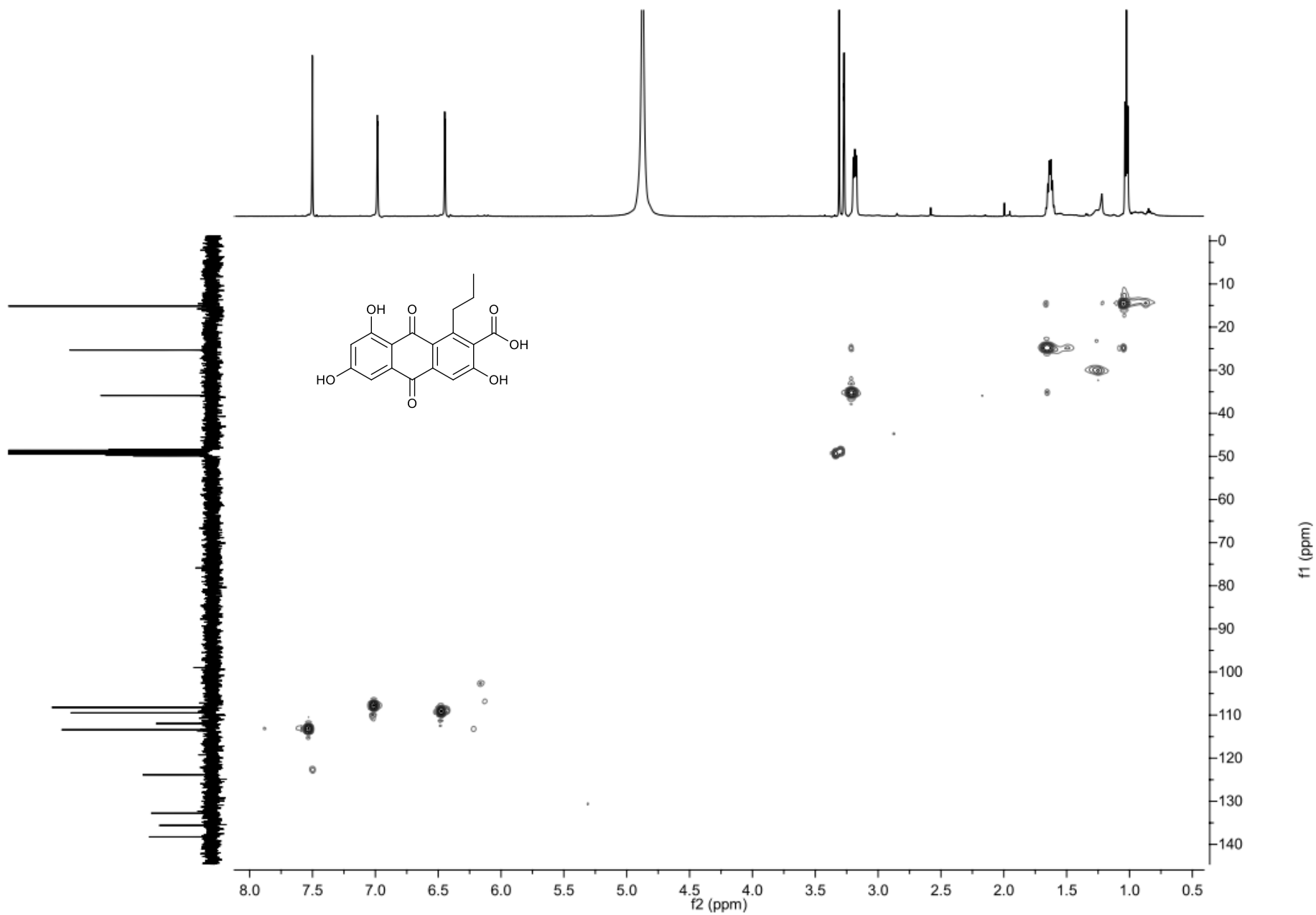
Supplementary Figure 40. ¹H NMR spectrum of compound 6 in CD₃OD.



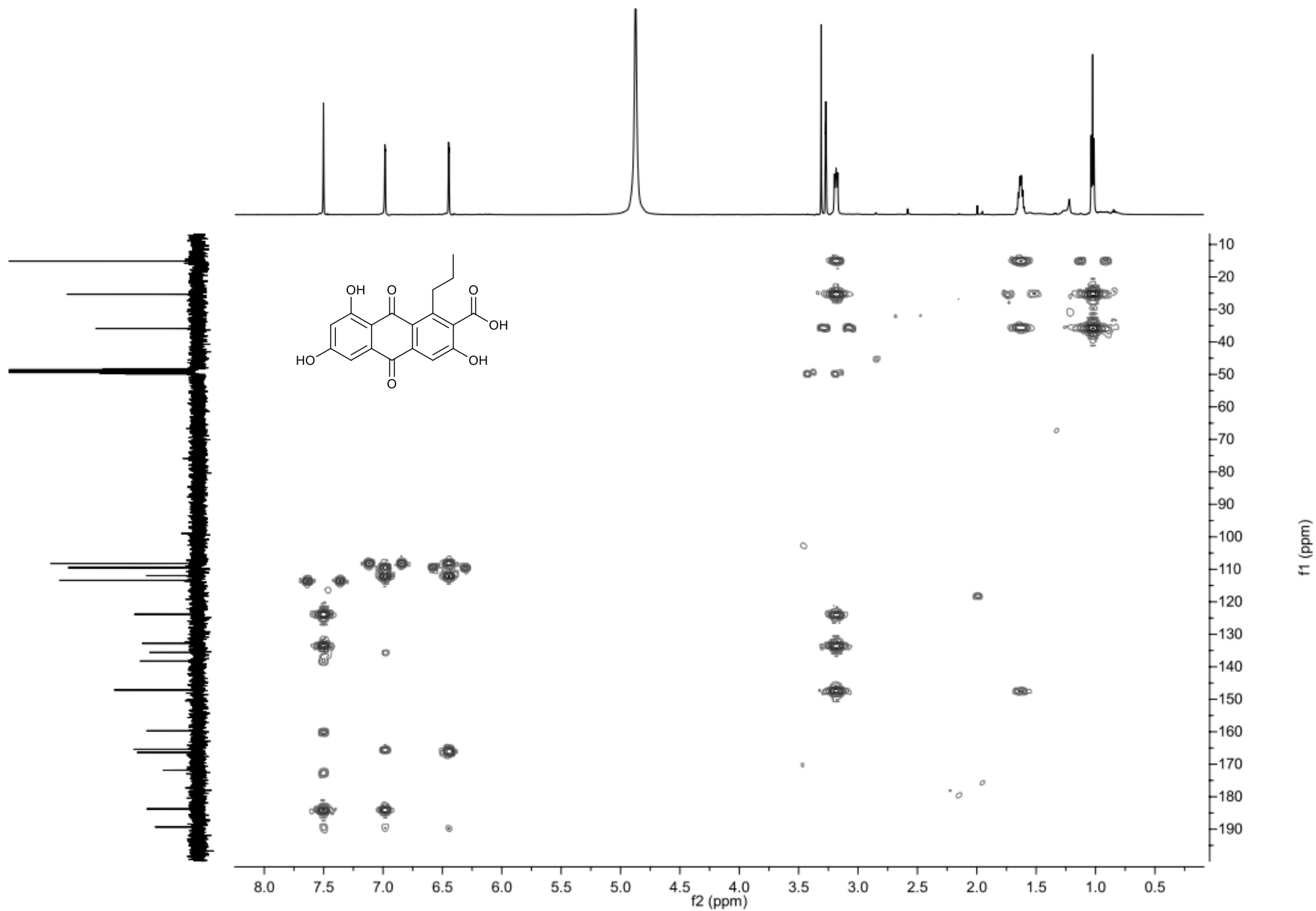
Supplementary Figure 41. ¹³C NMR spectrum of compound 6 in CD₃OD.



Supplementary Figure 42. ^1H - ^1H COSY NMR spectrum of compound **6** in CD_3OD .

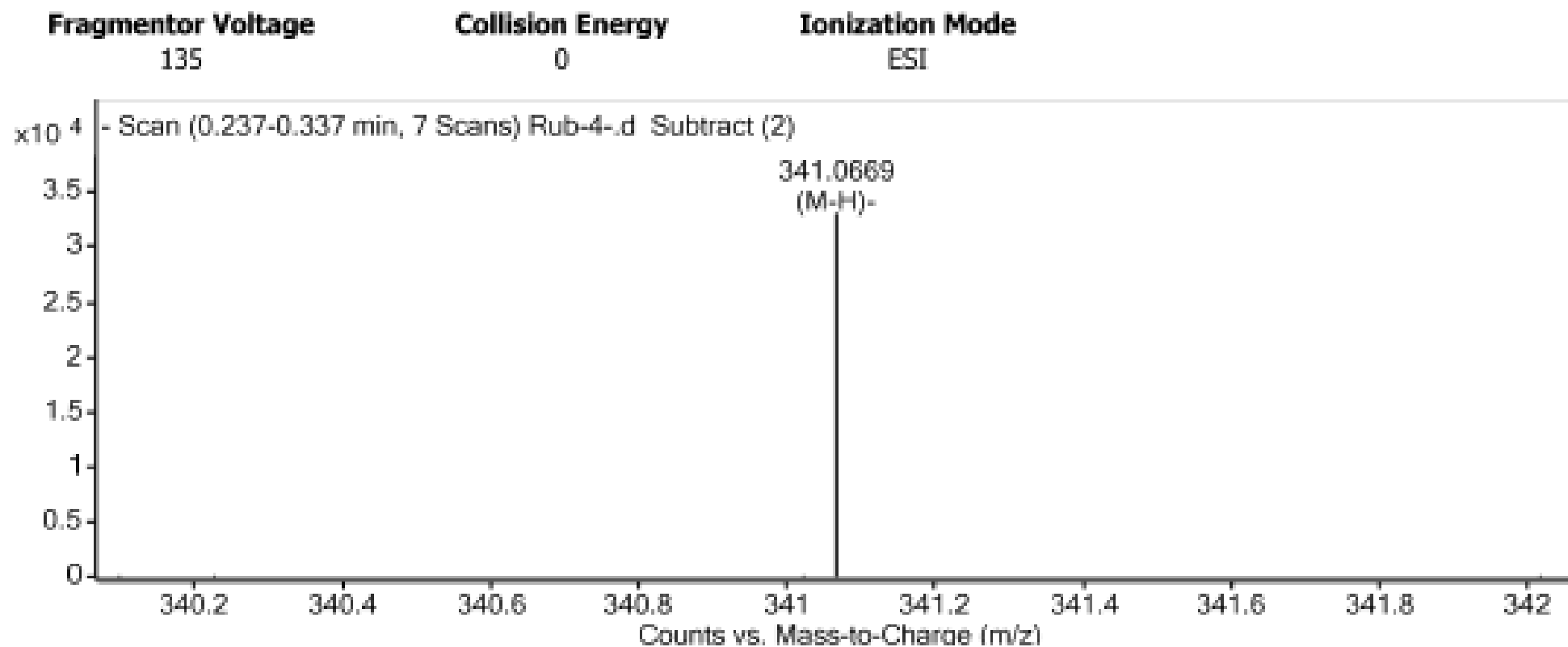


Supplementary Figure 43. HSQC NMR spectrum of compound 6 in CD₃OD.

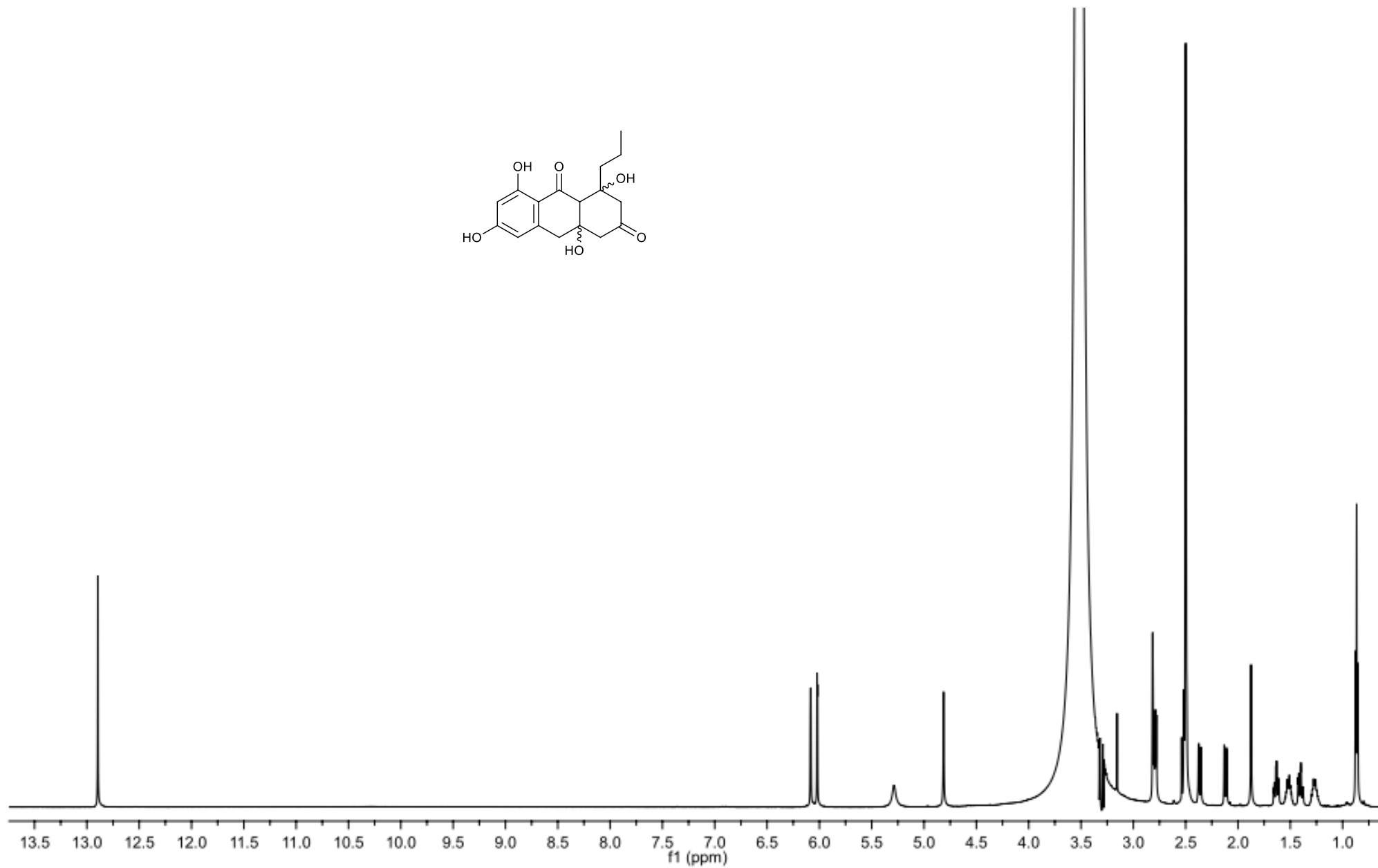
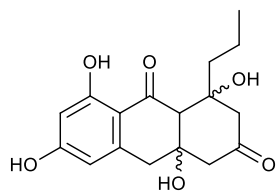


Supplementary Figure 44. HMBC NMR spectrum of compound **6** in CD₃OD.

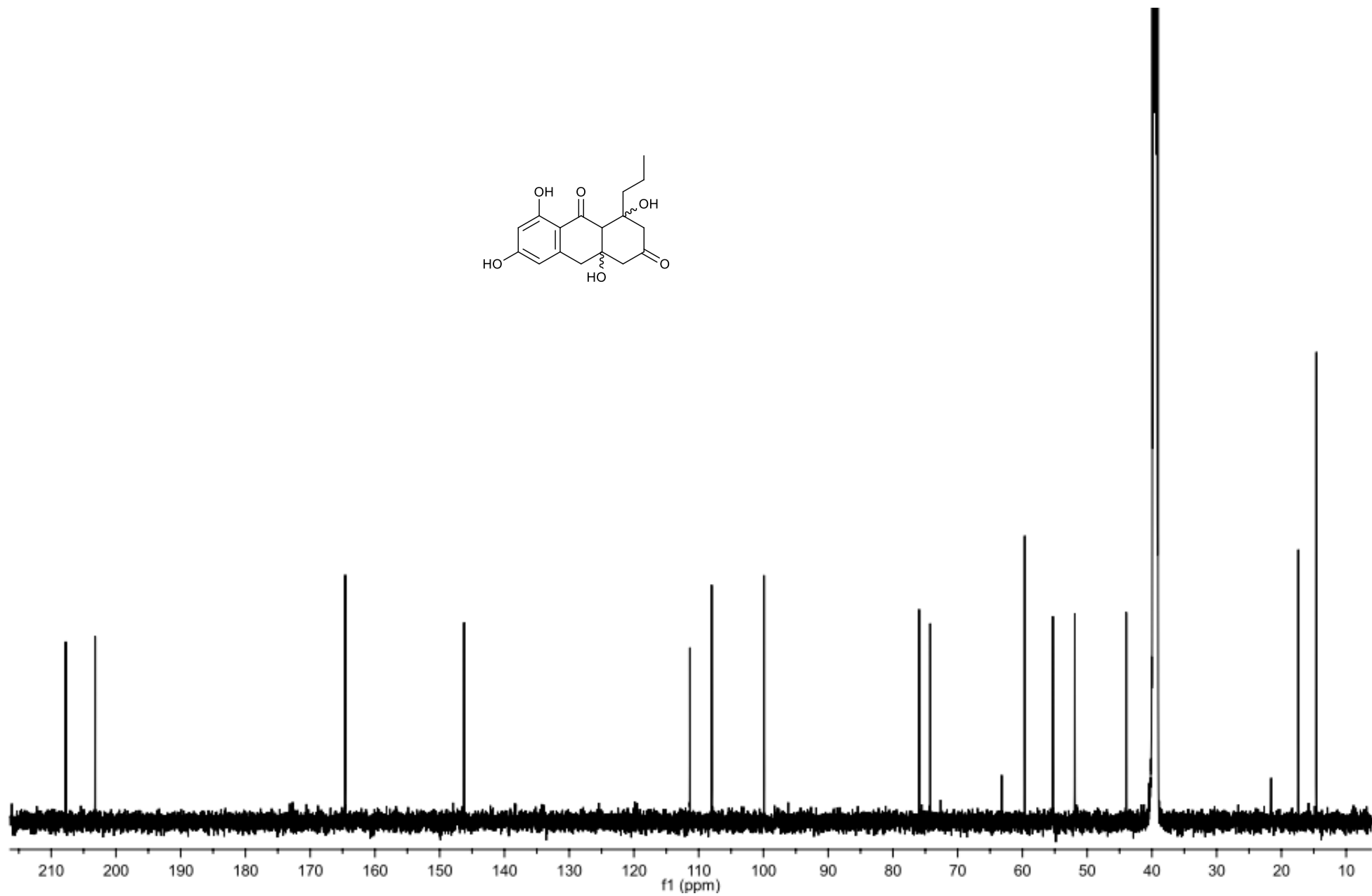
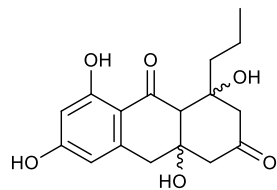
User Spectra



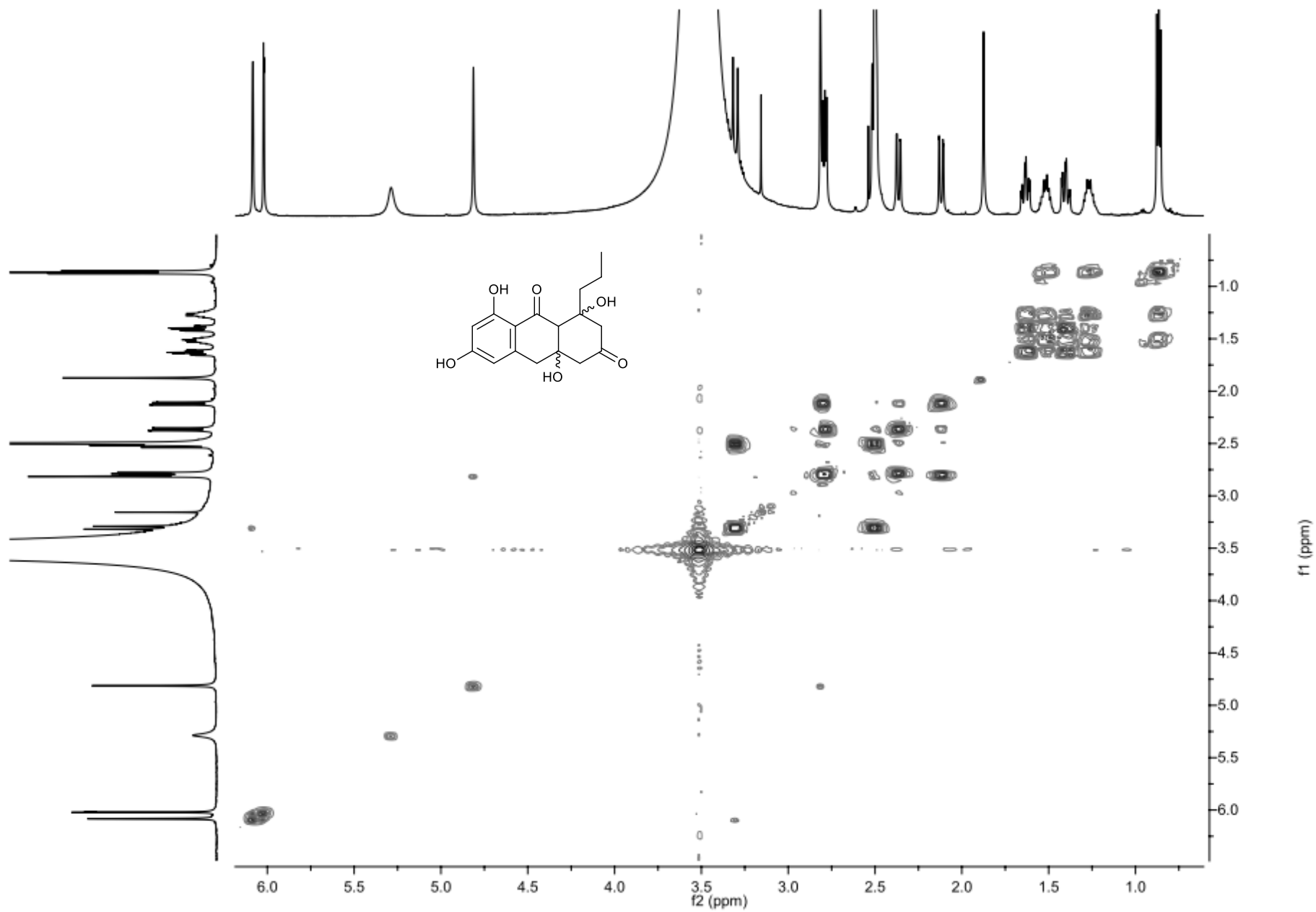
Supplementary Figure 45. HRESIMS analysis of compound 6.



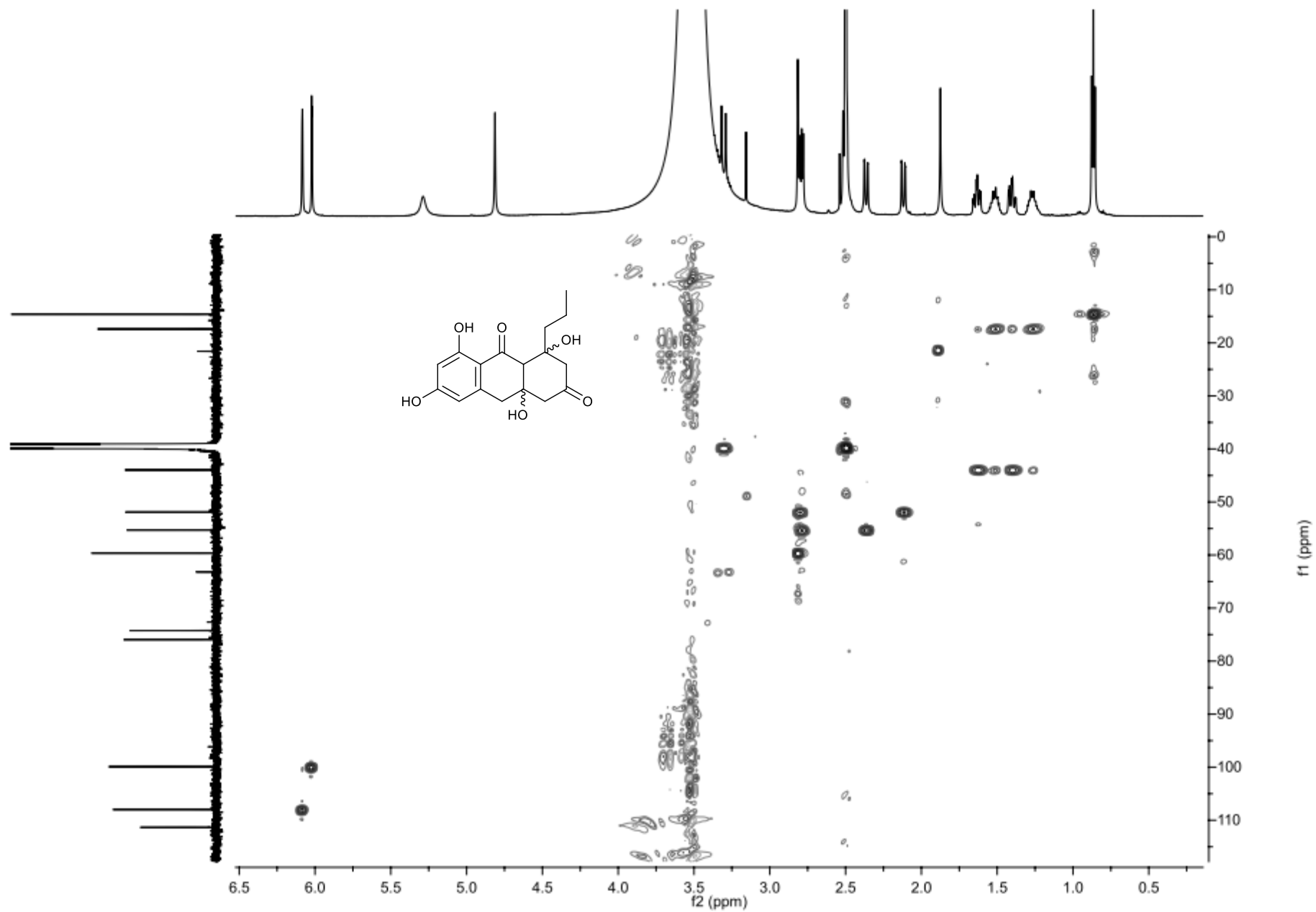
Supplementary Figure 46. ^1H NMR spectrum of compound 7 in $\text{DMSO-}d_6$.

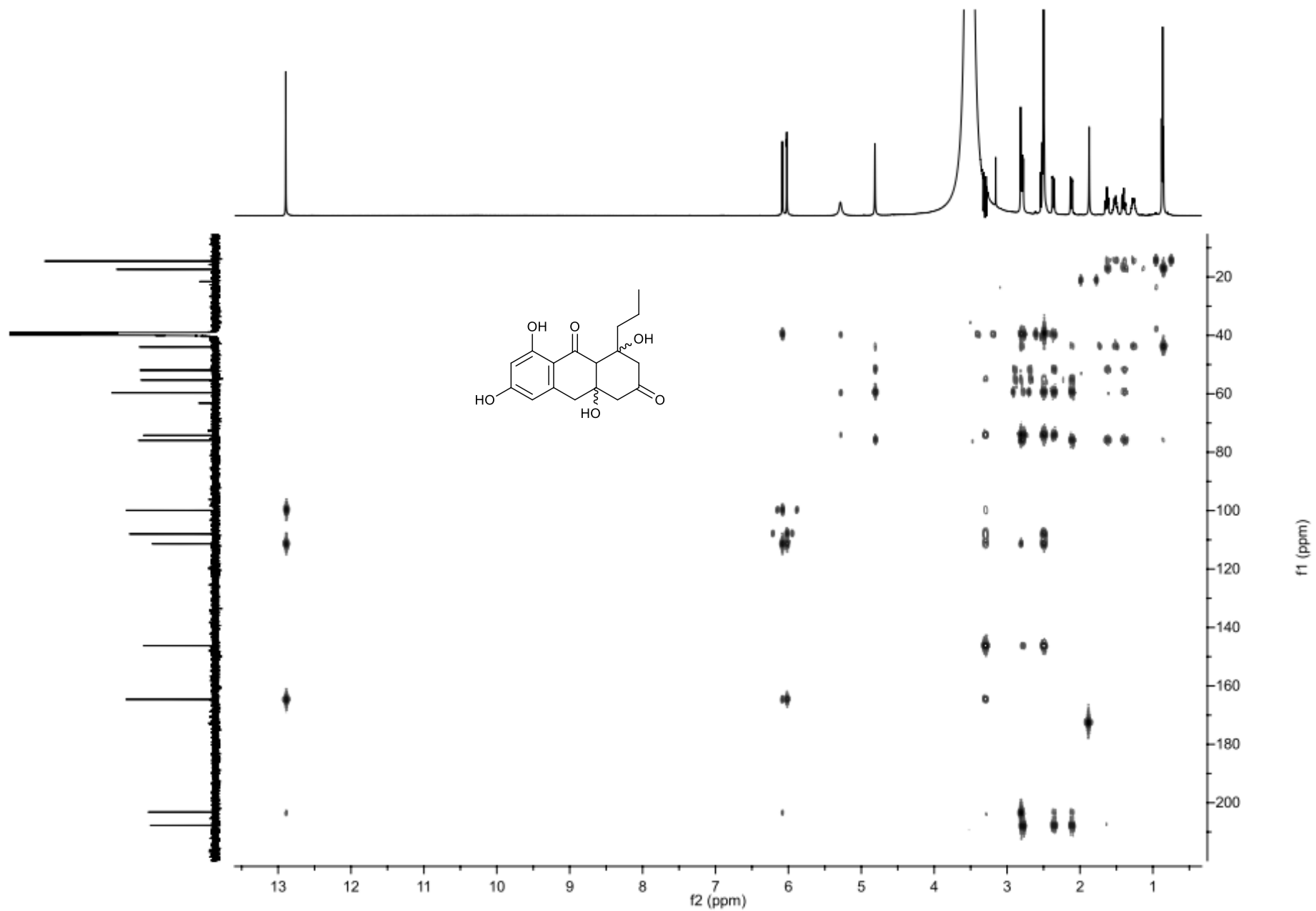


Supplementary Figure 47. ^{13}C NMR spectrum of compound 7 in $\text{DMSO-}d_6$.



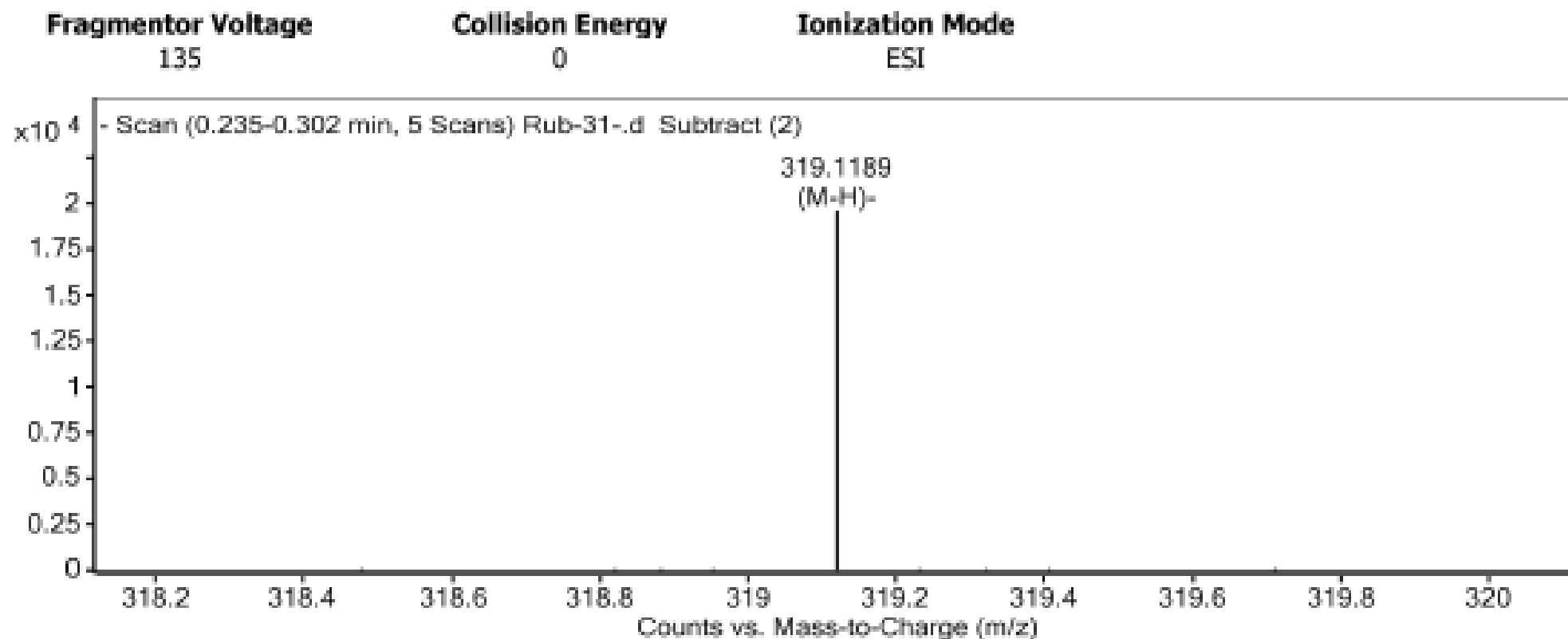
Supplementary Figure 48. ^1H - ^1H COSY NMR spectrum of compound 7 in $\text{DMSO-}d_6$.



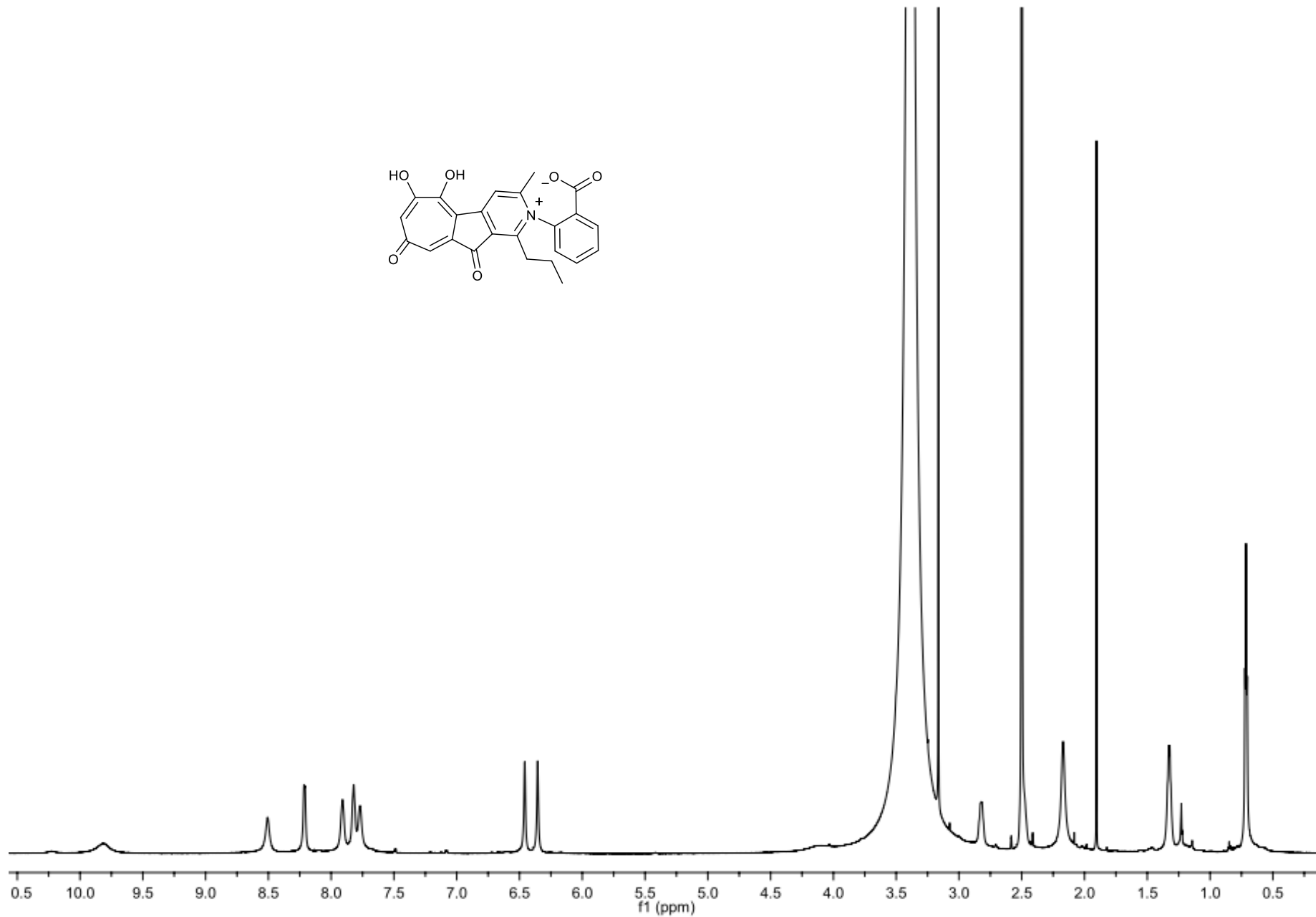
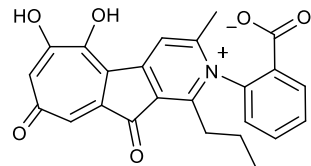


Supplementary Figure 50. HMBC NMR spectrum of compound 7 in DMSO-*d*₆.

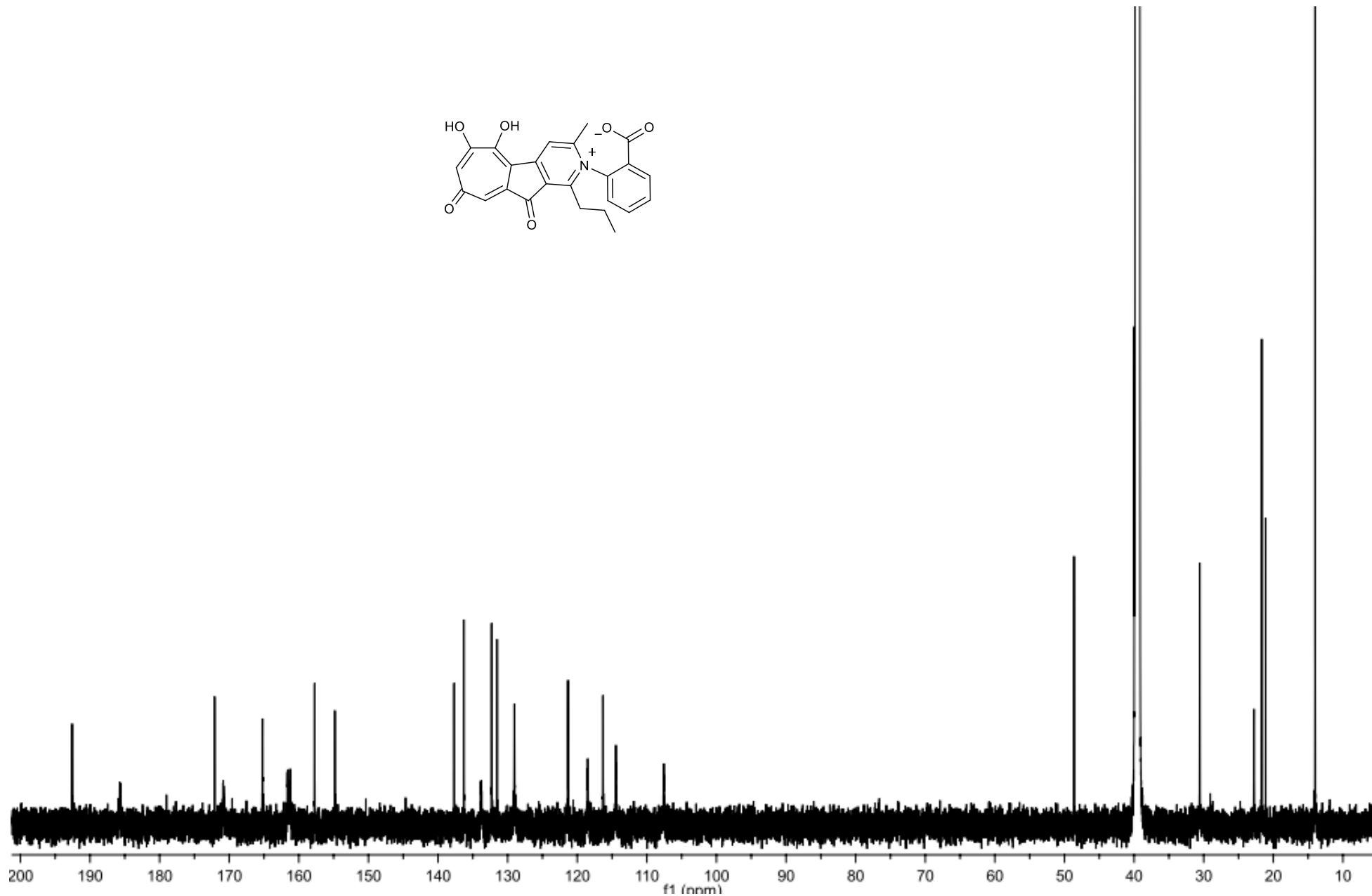
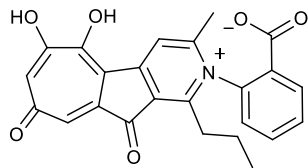
User Spectra



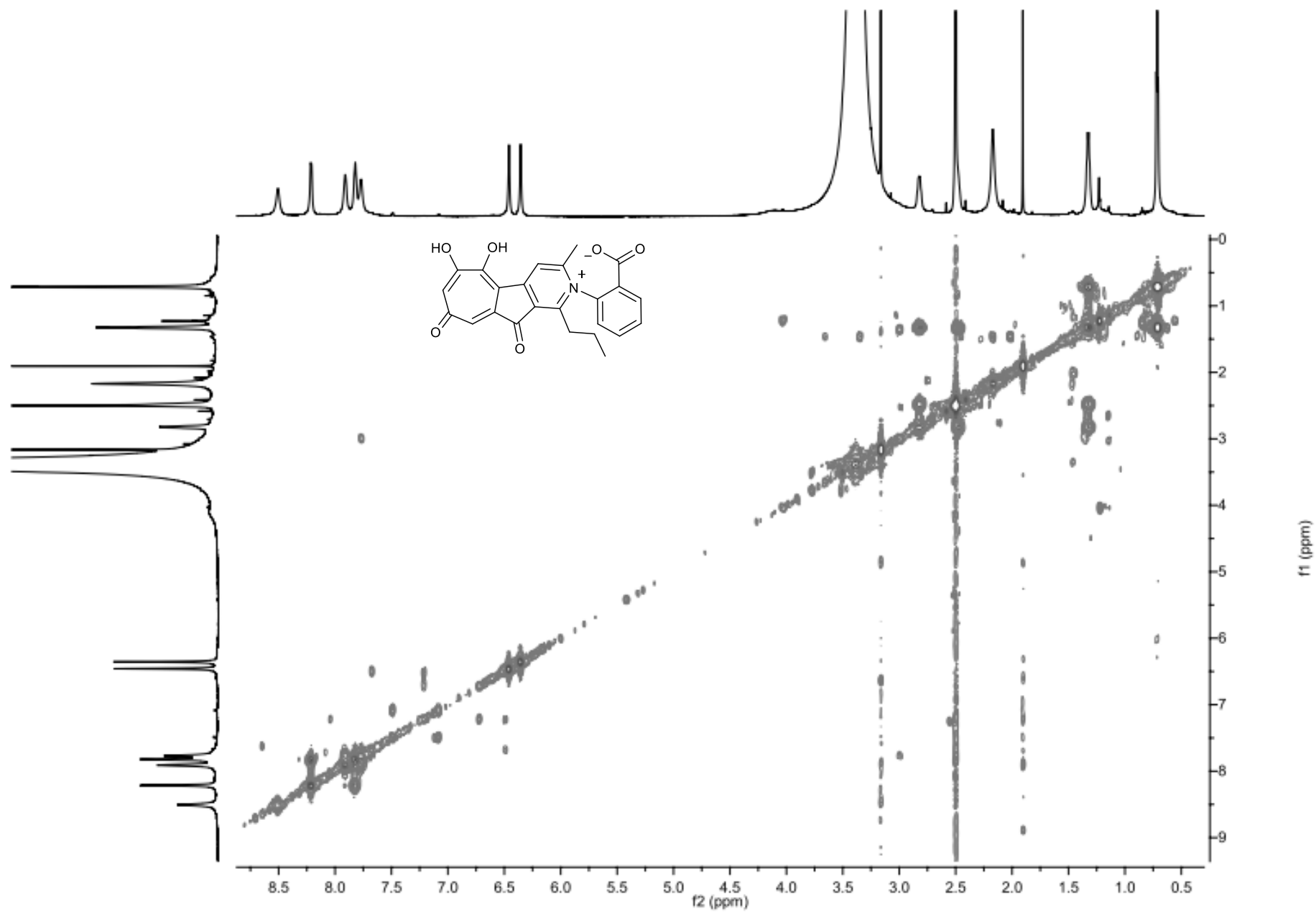
Supplementary Figure 51. HRESIMS analysis of compound 7.



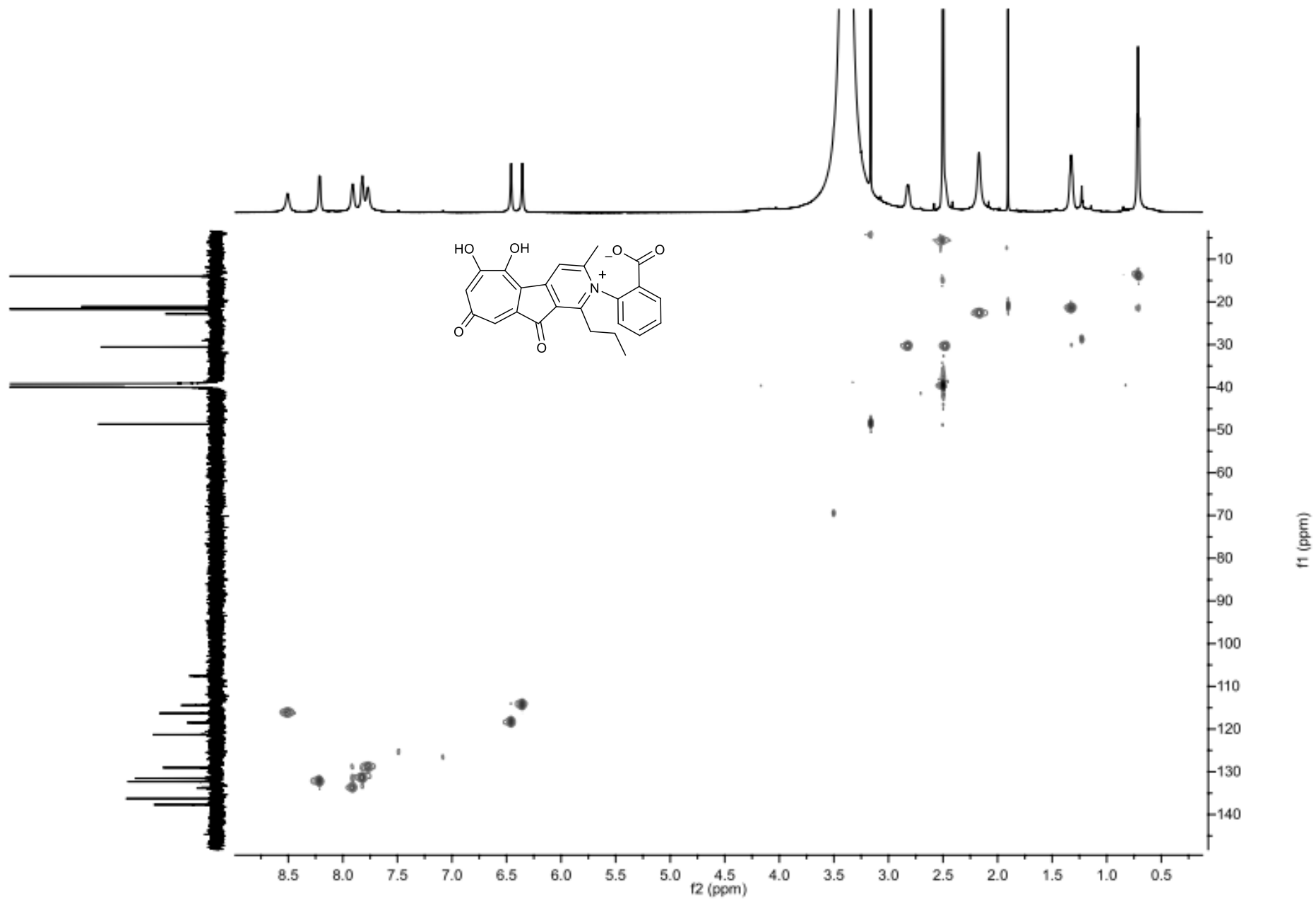
Supplementary Figure 52. ¹H NMR spectrum of compound 8 in DMSO-d₆.



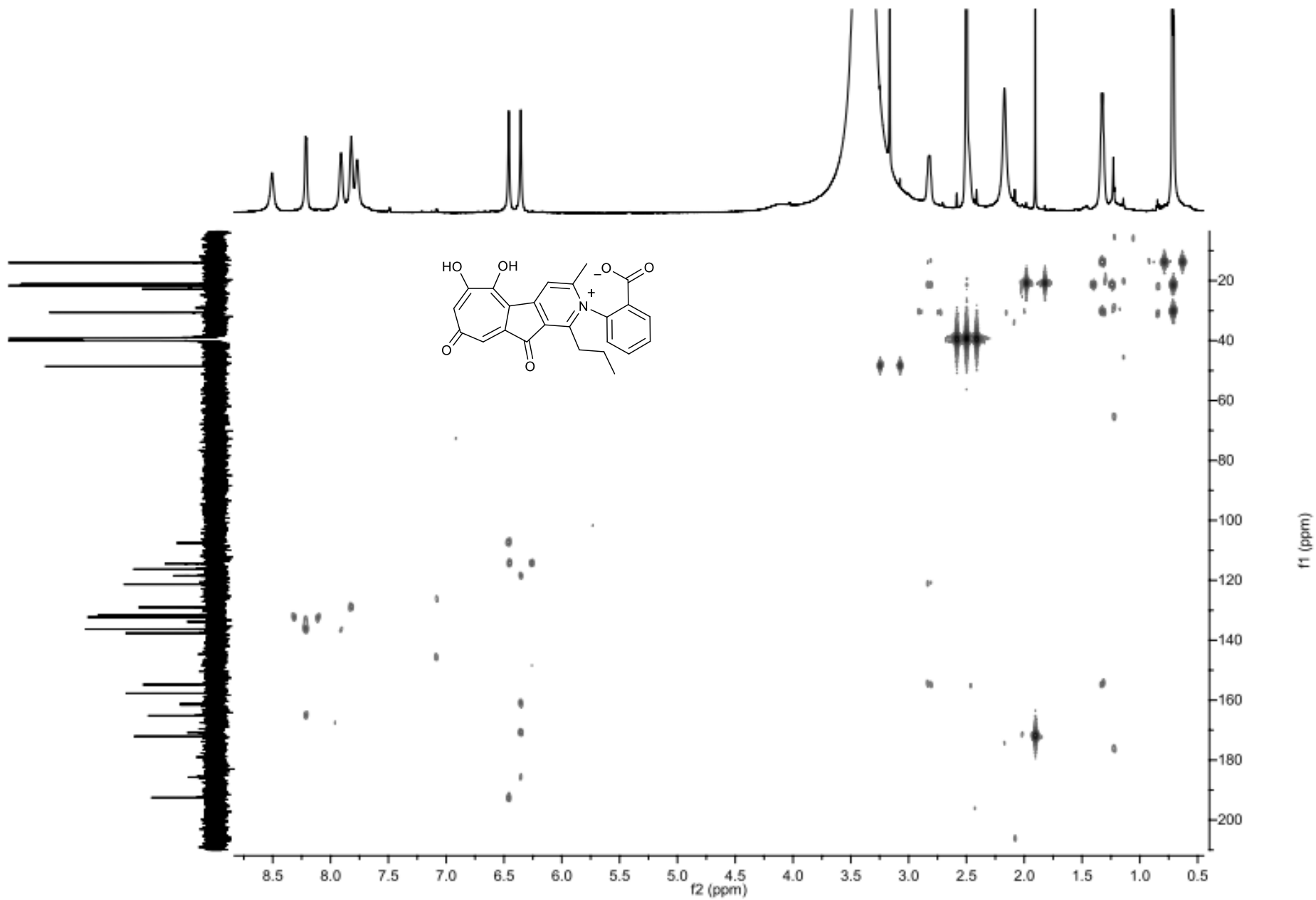
Supplementary Figure 53. ^{13}C NMR spectrum of compound **8** in $\text{DMSO-}d_6$.



Supplementary Figure 54. ¹H-¹H COSY NMR spectrum of compound **8** in DMSO-*d*₆.

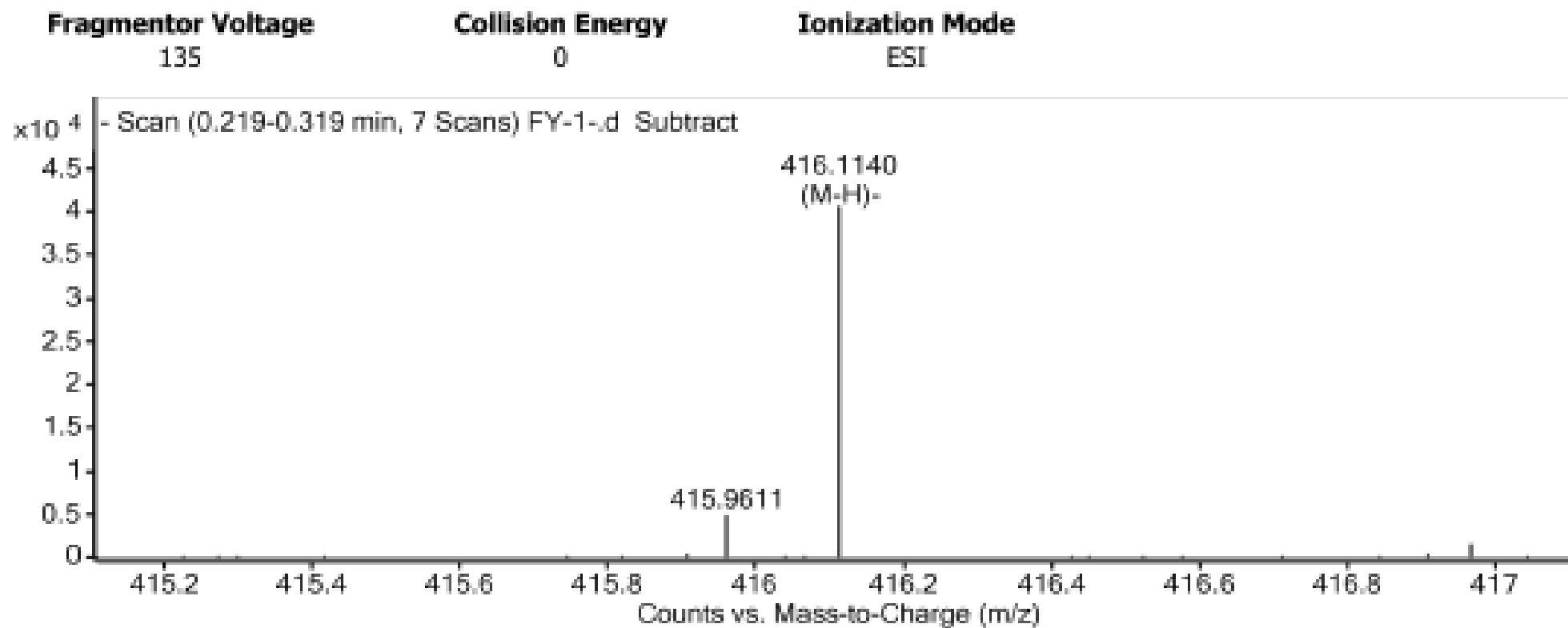


Supplementary Figure 55. HSQC NMR spectrum of compound 8 in DMSO-*d*₆.

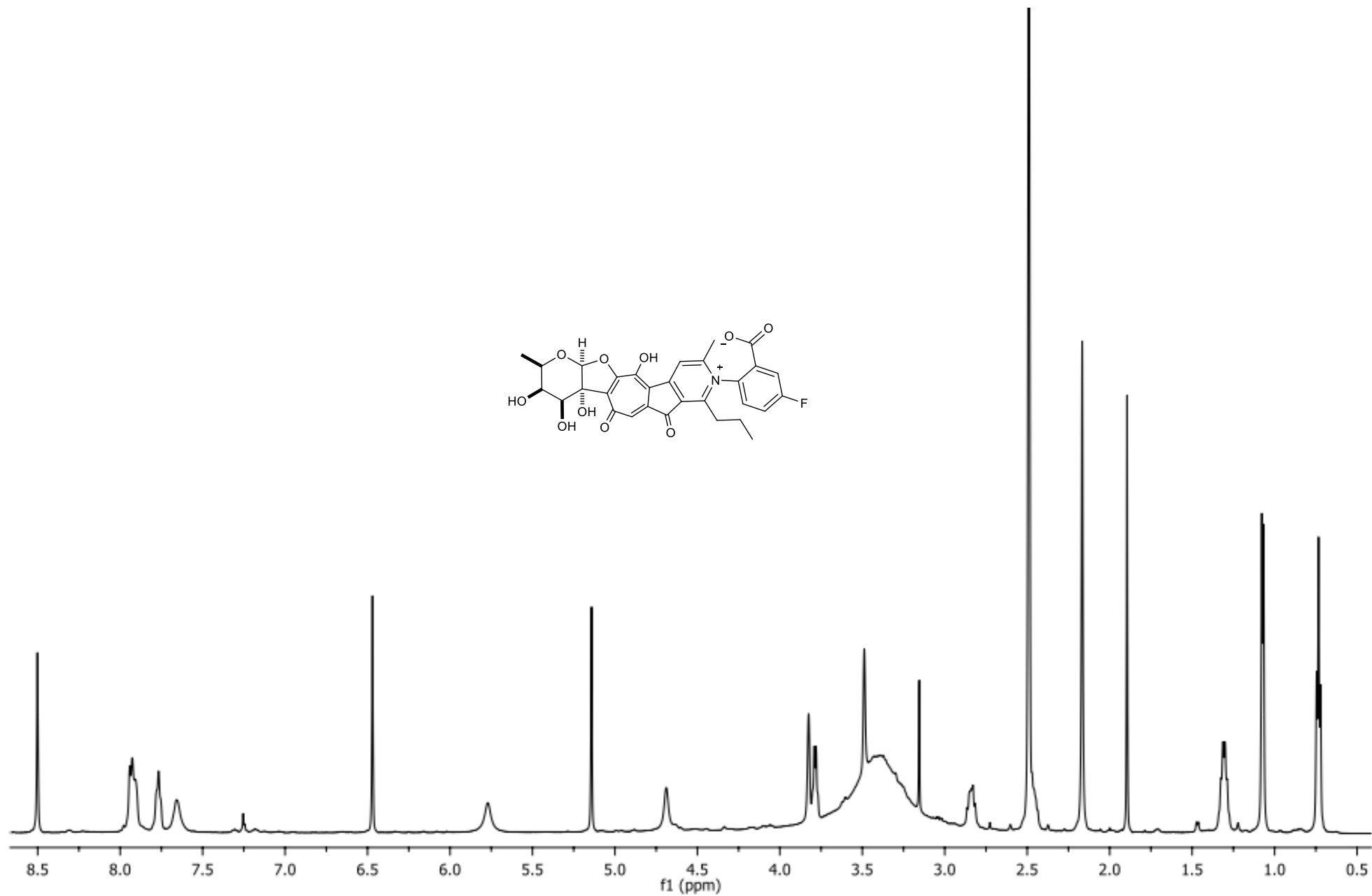


Supplementary Figure 56. HMBC NMR spectrum of compound 8 in DMSO-*d*₆.

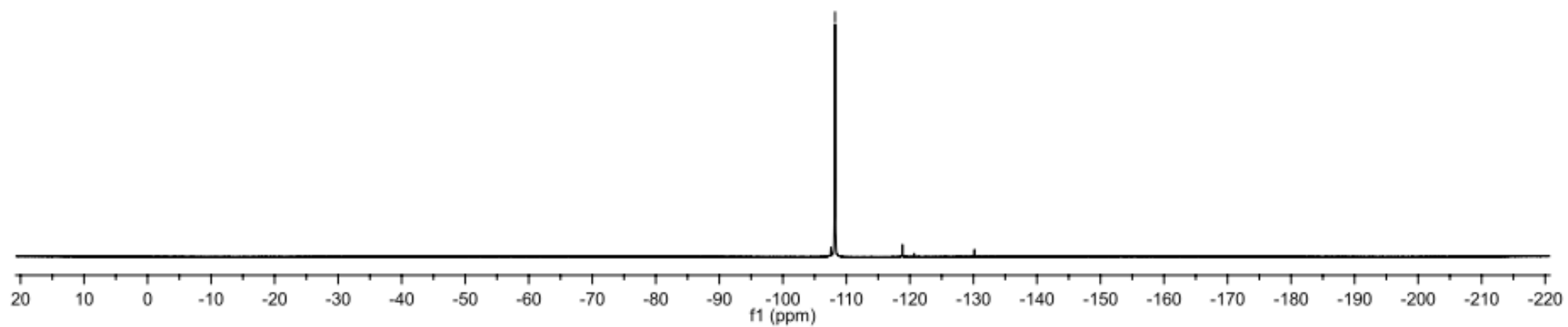
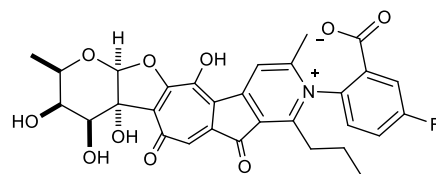
User Spectra



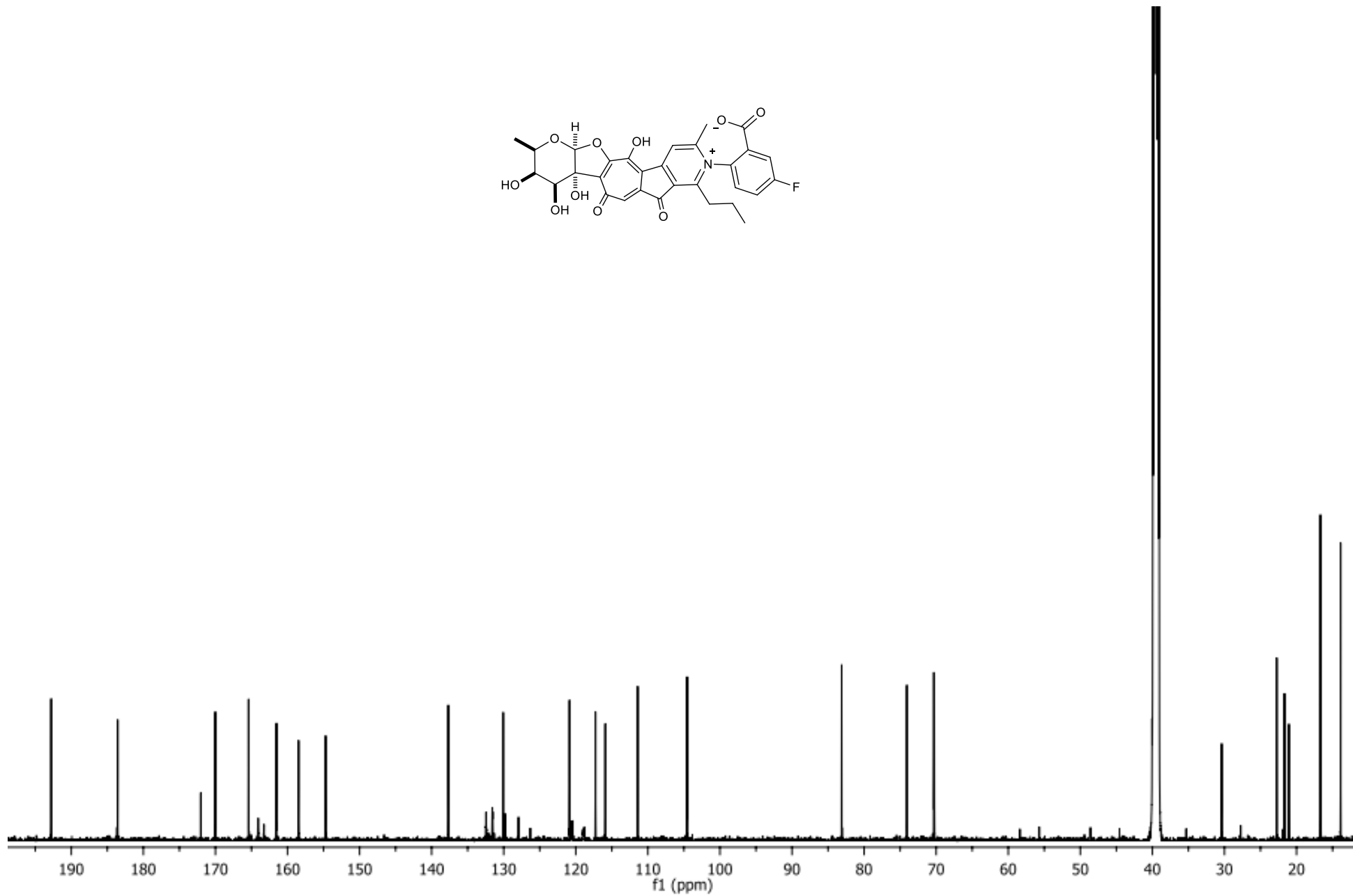
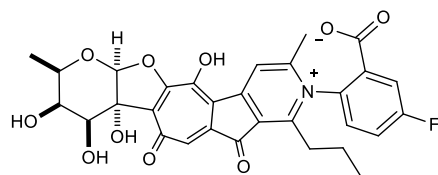
Supplementary Figure 57. HRESIMS analysis of compound 8.



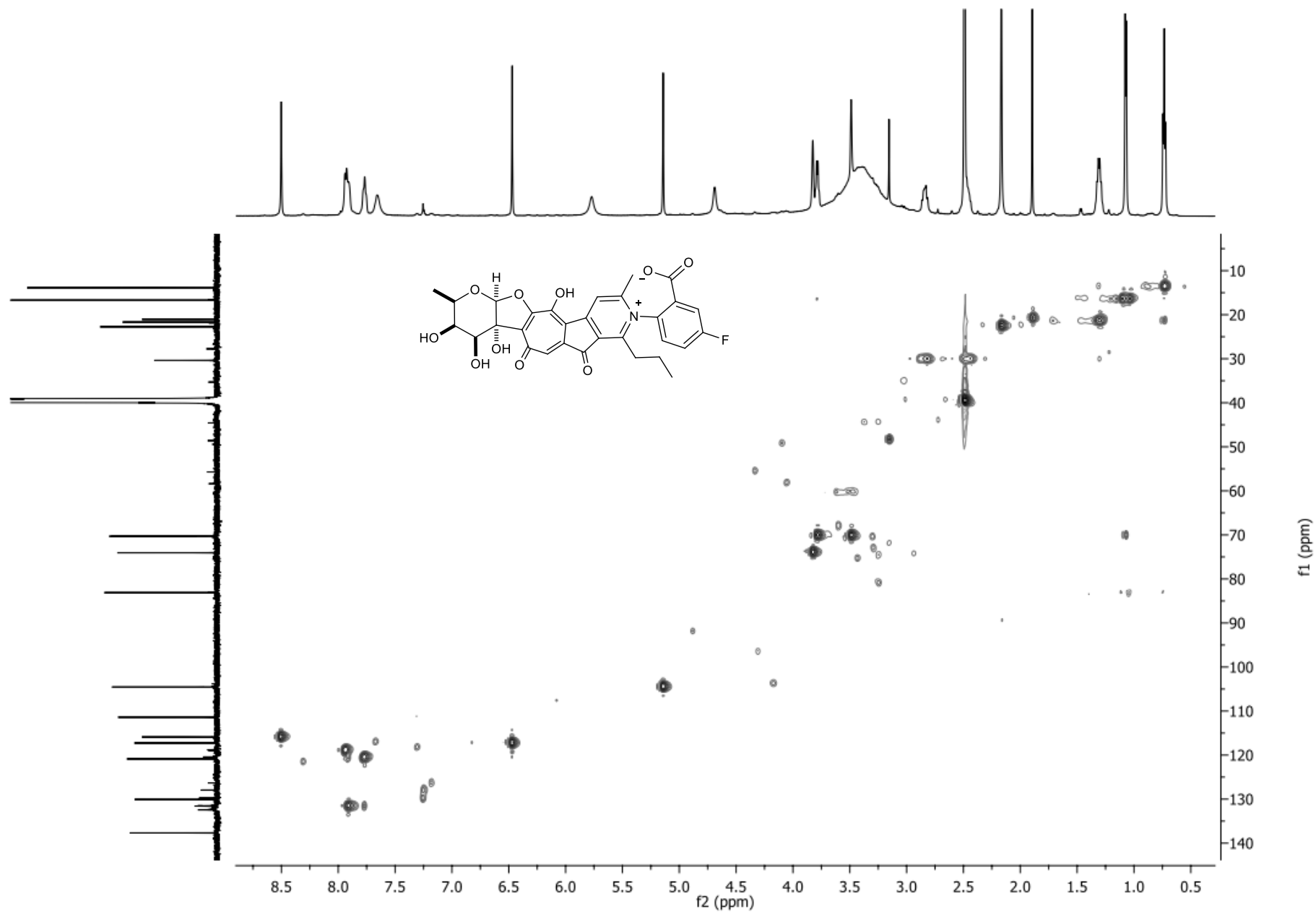
Supplementary Figure 58. ¹H NMR spectrum of compound **10** in DMSO-d₆.



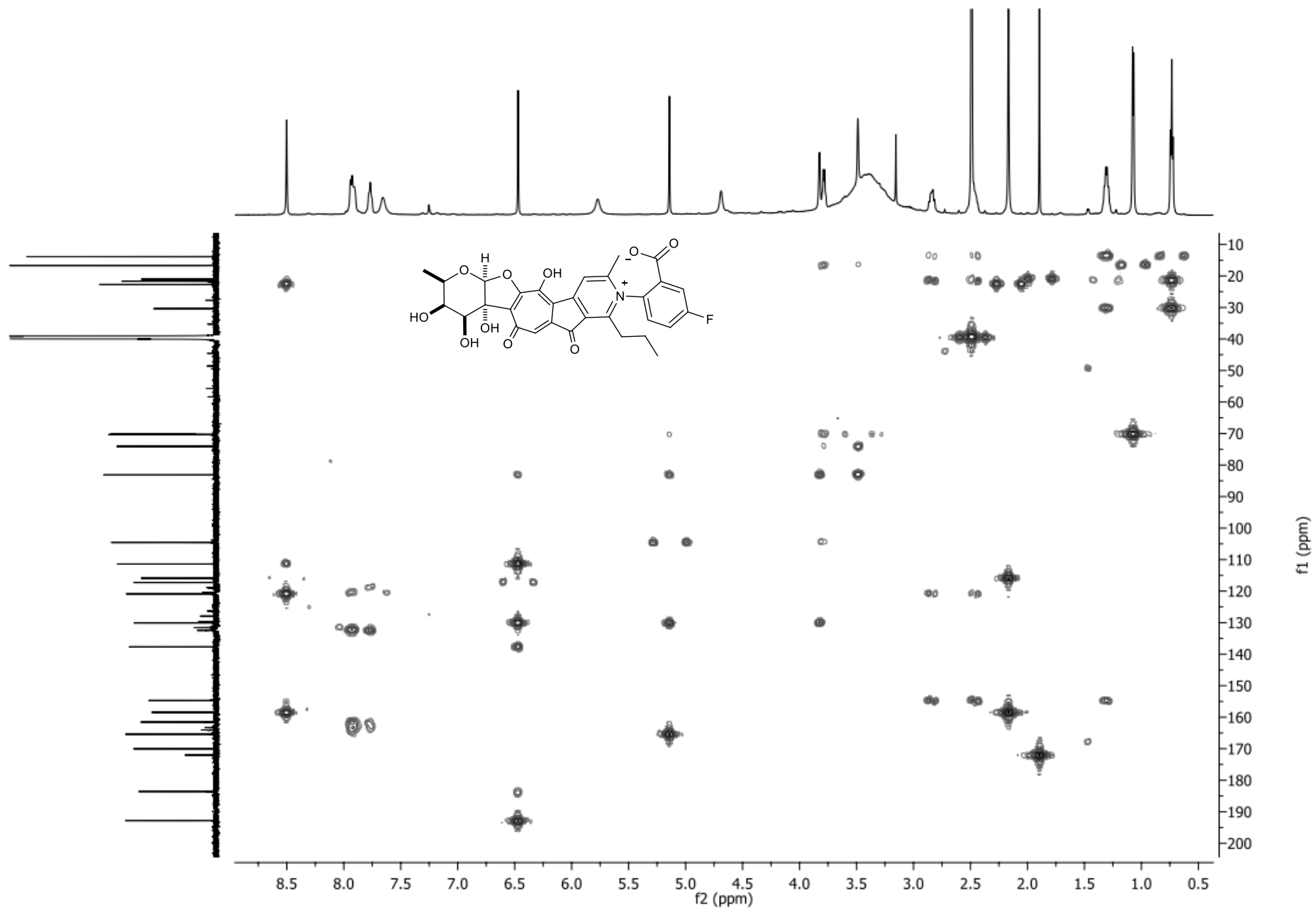
Supplementary Figure 59. ^{19}F NMR spectrum of compound **10** in $\text{DMSO-}d_6$.



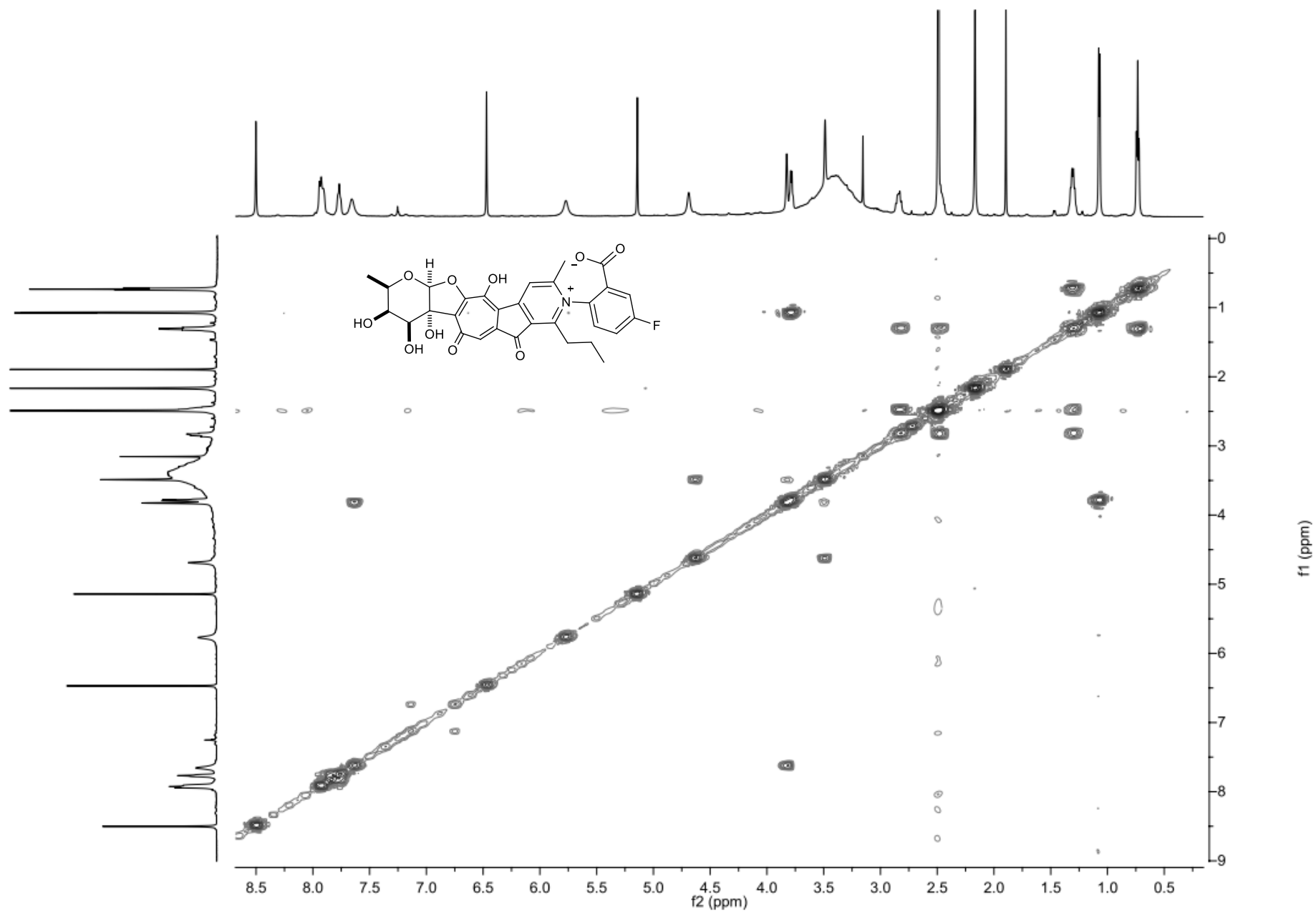
Supplementary Figure 60. ¹³C NMR spectrum of compound 10 in DMSO-*d*₆.



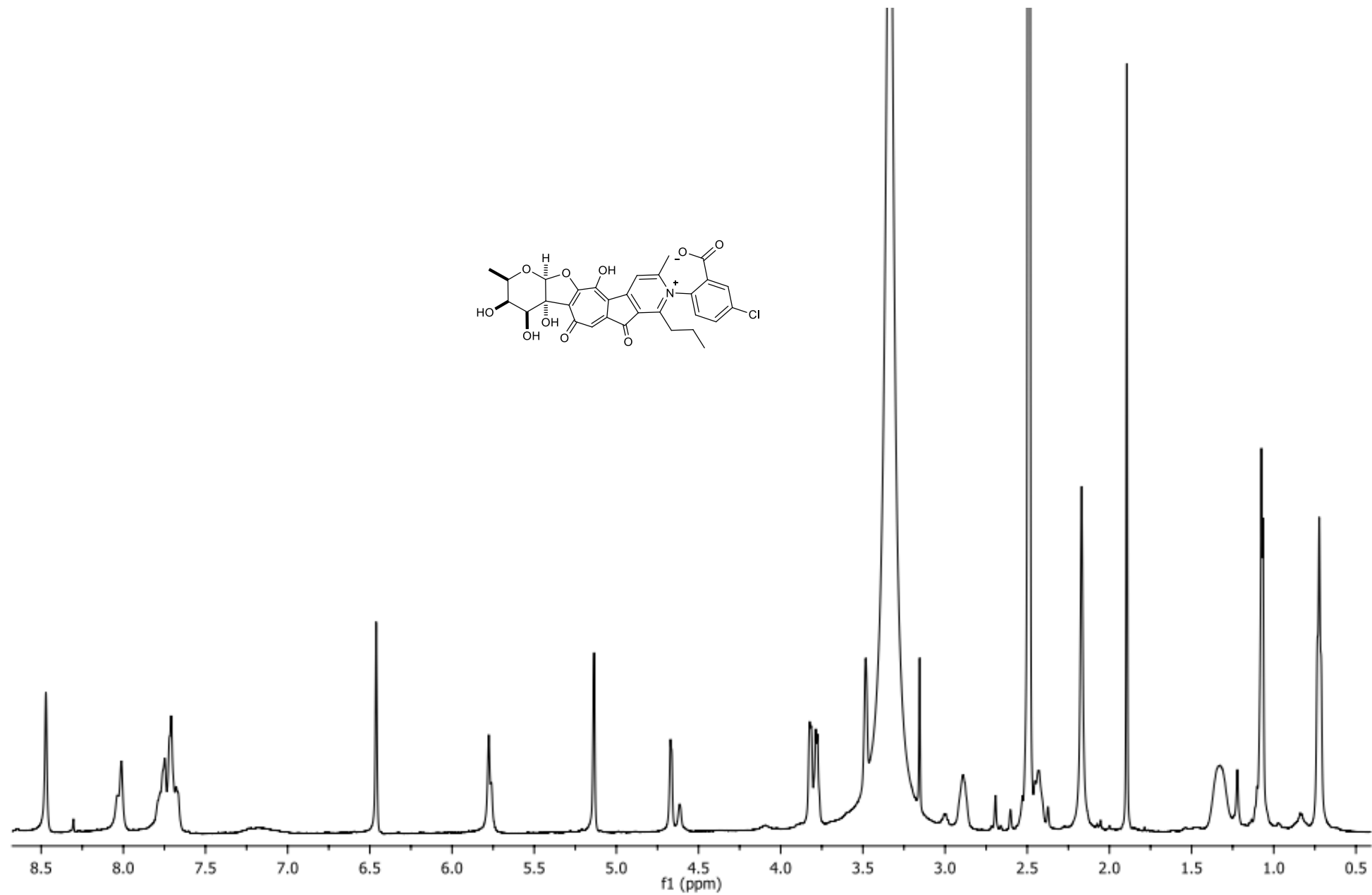
Supplementary Figure 61. HSQC NMR spectrum of compound 10 in DMSO-*d*₆.



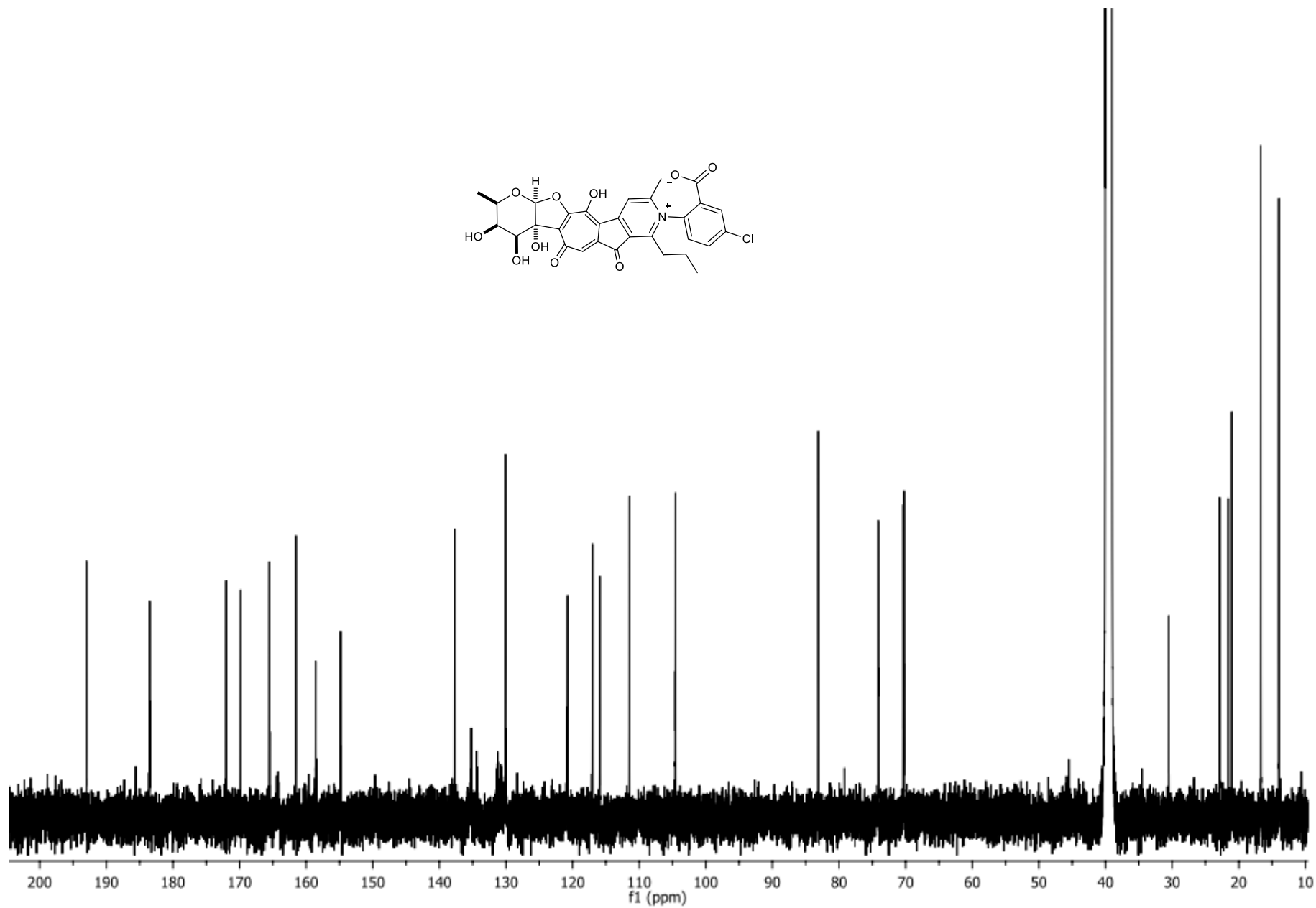
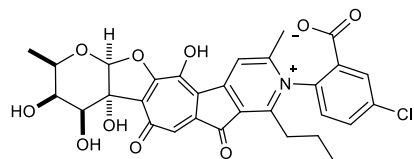
Supplementary Figure 62. HMBC NMR spectrum of compound **10** in DMSO- d_6 .



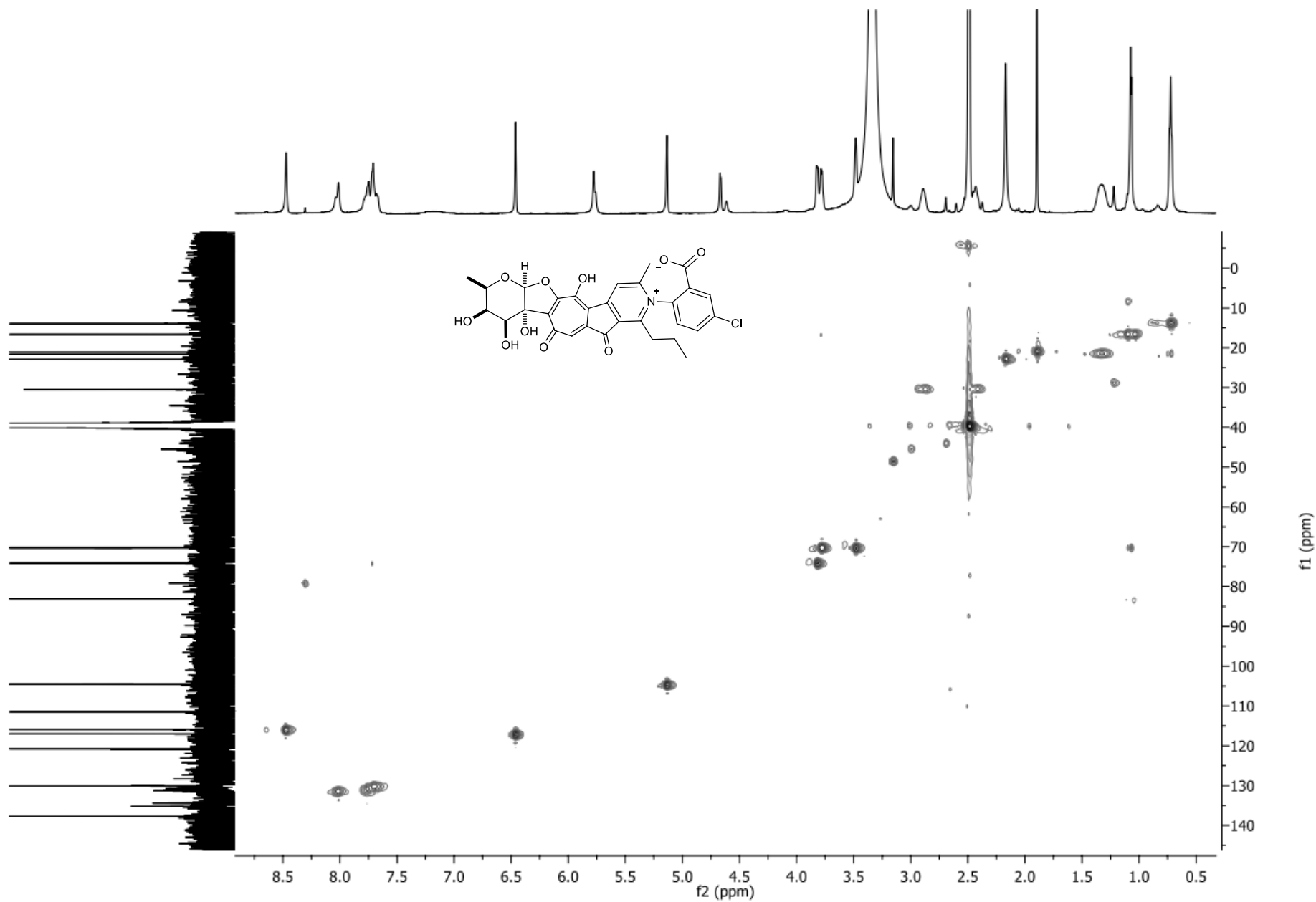
Supplementary Figure 63. ^1H - ^1H COSY NMR spectrum of compound **10** in $\text{DMSO-}d_6$.



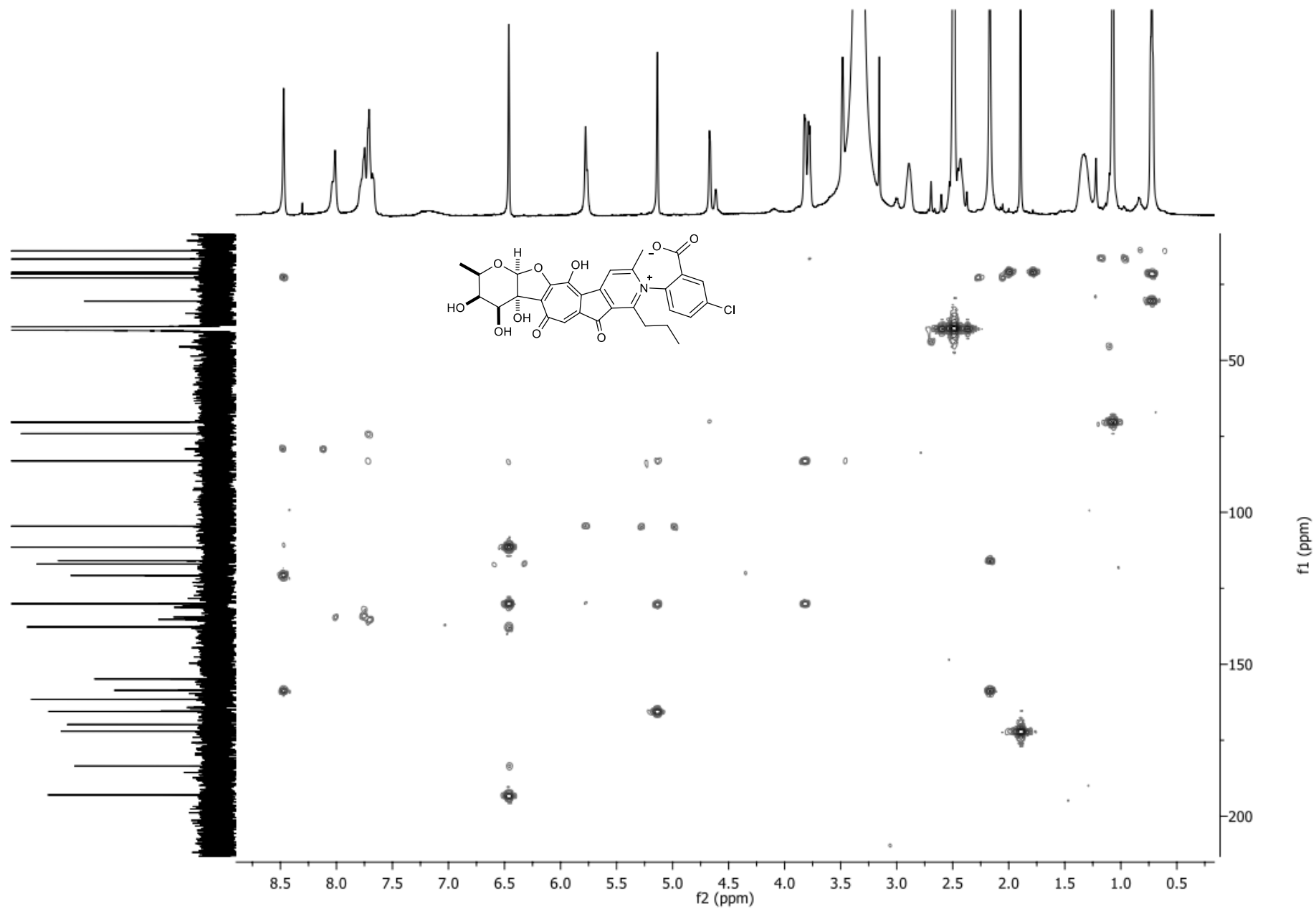
Supplementary Figure 64. ¹H NMR spectrum of compound 11 in DMSO-*d*₆.



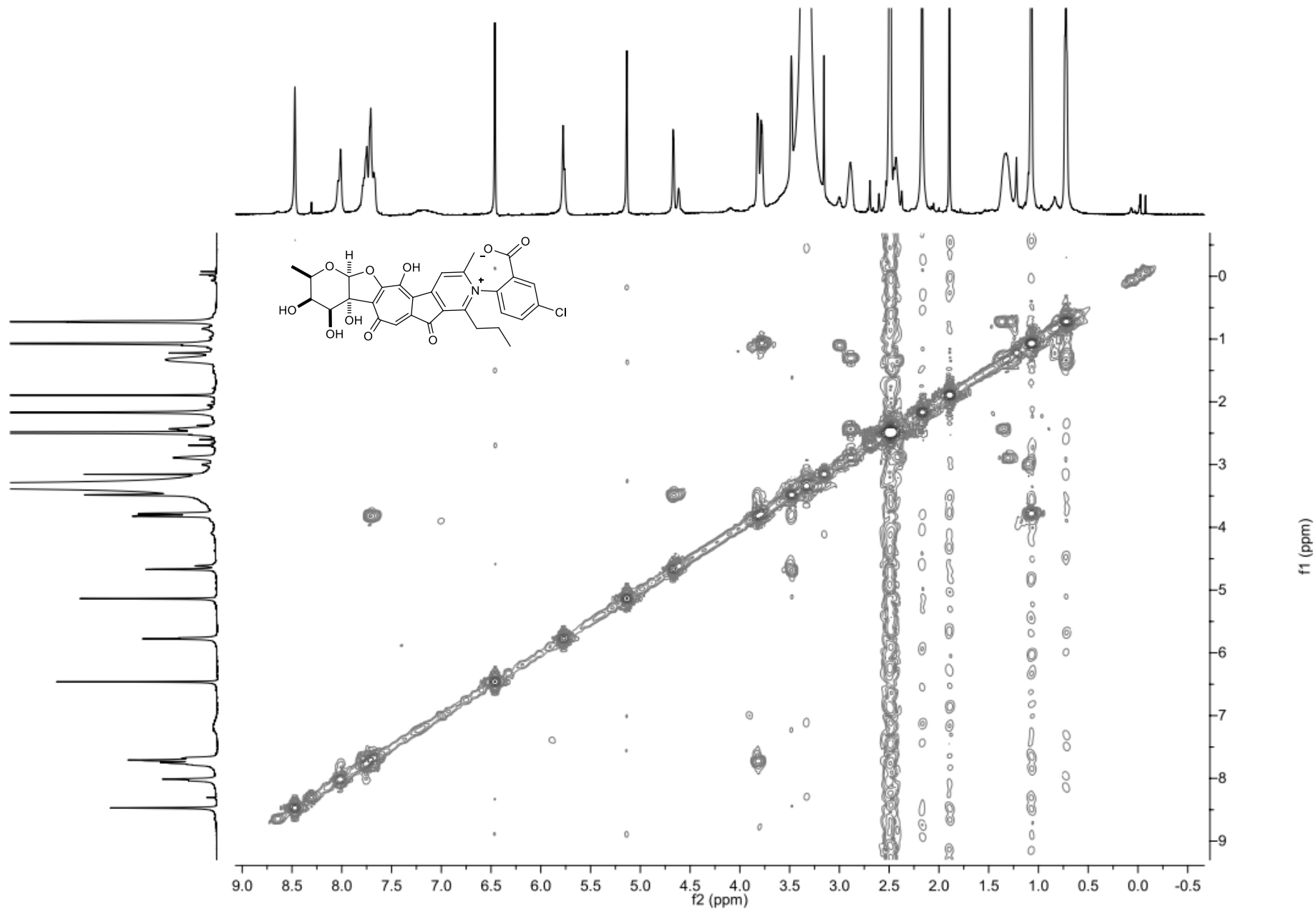
Supplementary Figure 65. ¹³C NMR spectrum of compound 11 in DMSO-*d*₆.



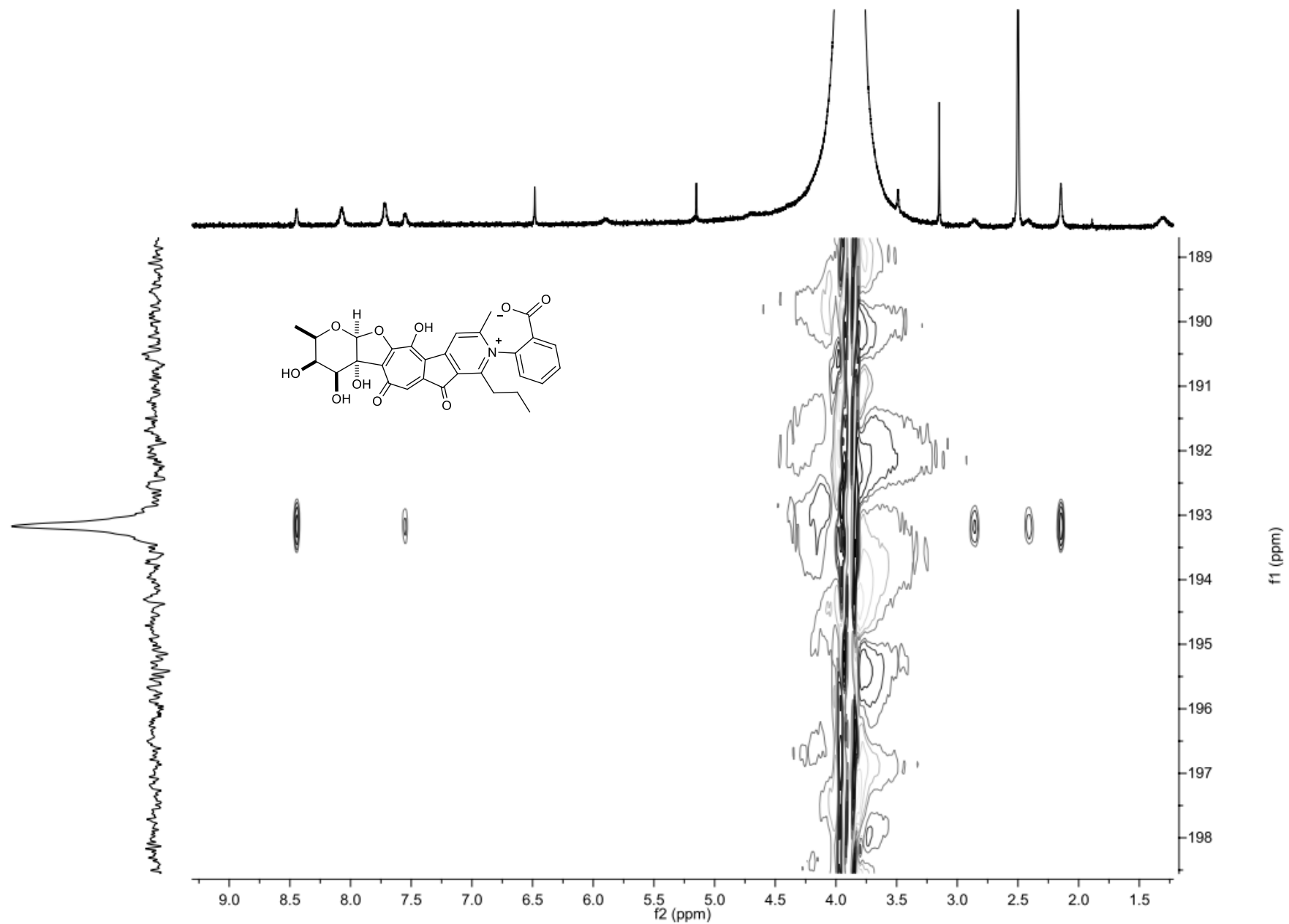
Supplementary Figure 66. HSQC NMR spectrum of compound 11 in DMSO-*d*₆.



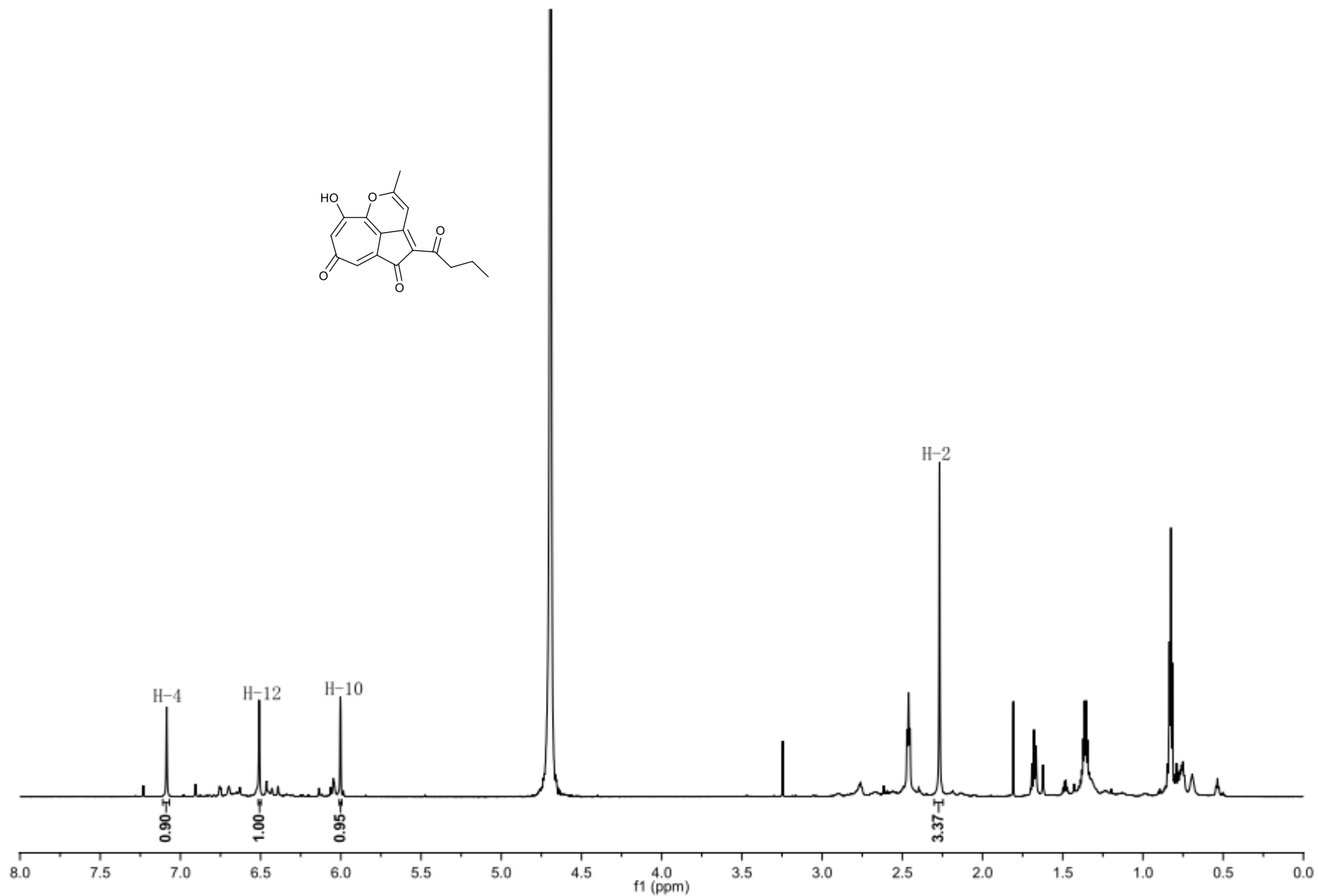
Supplementary Figure 67. HMBC NMR spectrum of compound 11 in DMSO-*d*₆.



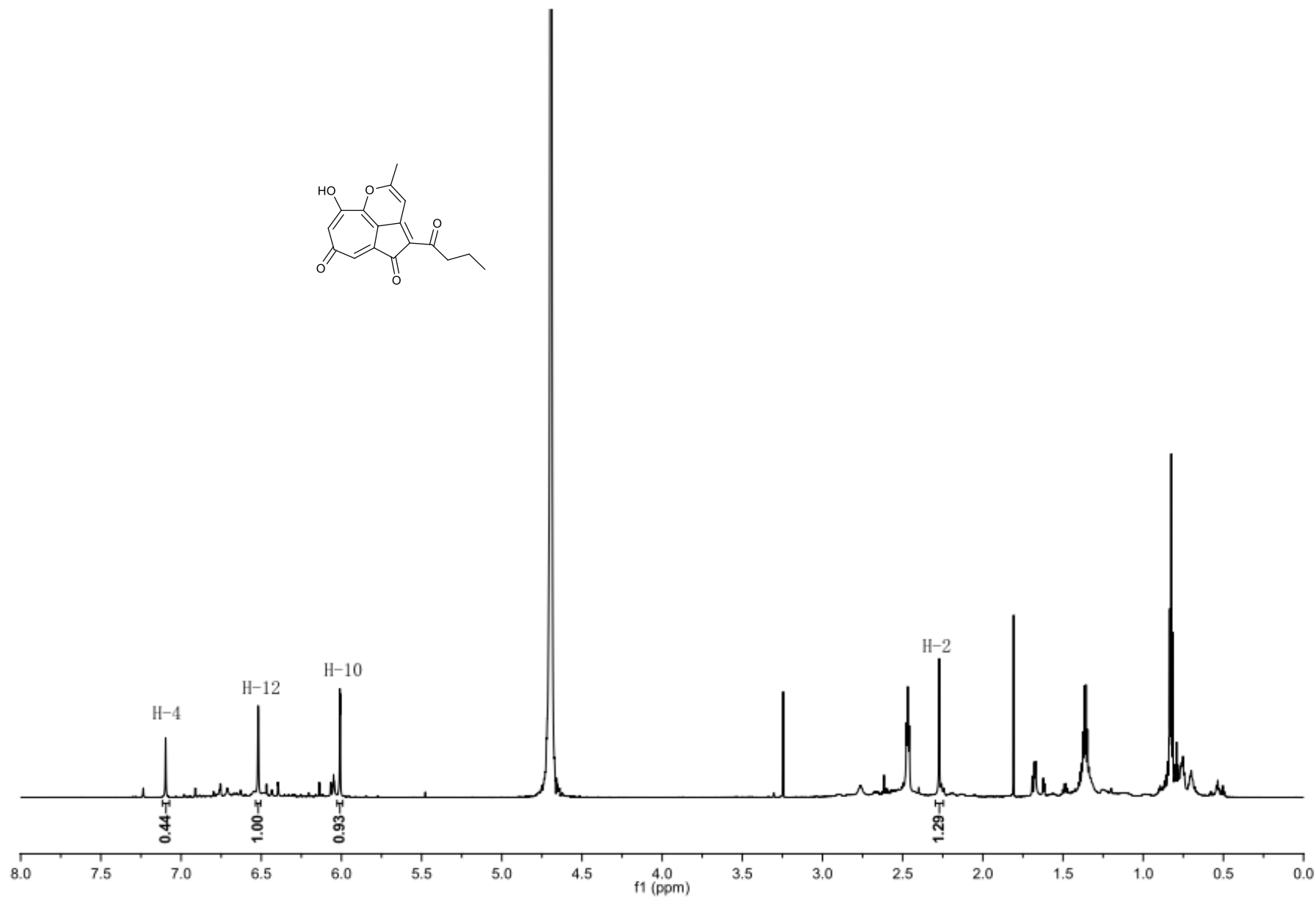
Supplementary Figure 68. ^1H - ^1H COSY NMR spectrum of compound **11** in $\text{DMSO-}d_6$.



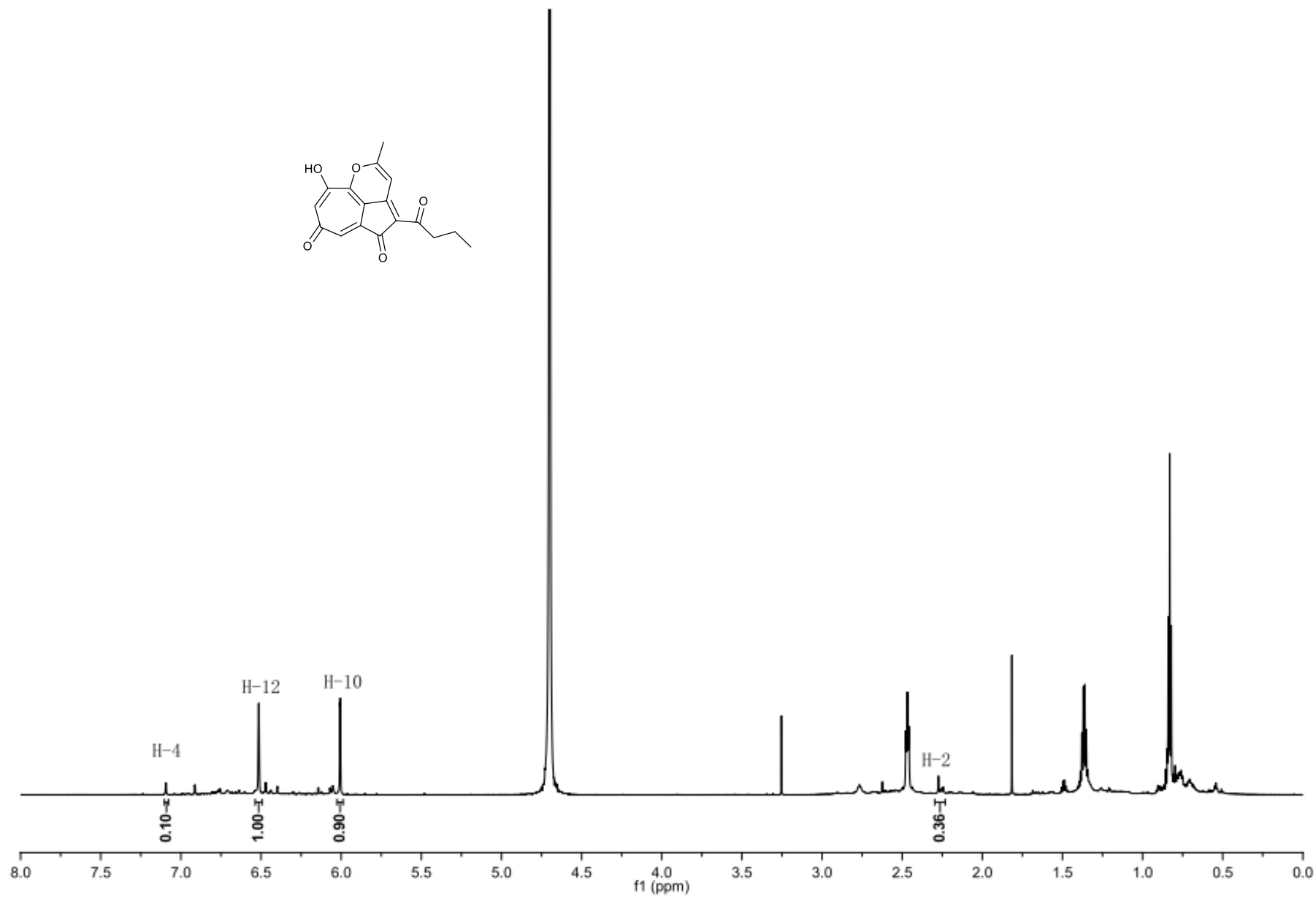
Supplementary Figure 69. Enlarged ^1H - ^{15}N long range correlations NMR spectrum of ^{15}N -labeled **2** in $\text{DMSO-}d_6$



Supplementary Figure 70. ¹H NMR spectrum of compound 4 in D₂O after 5 minutes incubation.



Supplementary Figure 71. ¹H NMR spectrum of compound 4 in D₂O after 2 hours incubation.



Supplementary Figure 72. ¹H NMR spectrum of compound 4 in D₂O after 4 hours incubation.

Supplementary Tables

Supplementary Table 1. Deduced roles of genes in *rub* gene cluster based on sequence homology.

Protein	Size (aa)	Category	Function	Closest sequence similarity		
				Protein, Origin	Positives/Identities	Accession No.
RubS1	492	sugar	FAD-linked oxidoreductase	AknOx, <i>Streptomyces galilaeus</i>	65/52	Q0PCD7
RubN1	135	hypothetical protein	Unknown	Sgcj, <i>Streptomyces carzinostaticus</i>	56/43	4OVM
RubS2	204	sugar	dTDP-4-keto-6-deoxy-D-glucose 3,5-epimerase	StrM, <i>Streptomyces griseus</i>	63/46	P29783
RubS3	310	sugar	dTDP-glucose-4-ketoreductase	NovS, <i>Streptomyces niveus</i>	56/44	Q9L9E9
RubS4	343	sugar	dTDP-glucose 4,6 dehydratase	NovT, <i>Streptomyces niveus</i>	75/63	Q9L9E8
RubS5	289	sugar	Glucose-1-phosphate thymidyltransferase	RfbA, <i>Shigella flexneri</i>	75/58	P37779
RubS6	278	sugar	NAD dependent epimerase/dehydratase	AzoB, <i>Xenophilus azovorans</i>	45/29	Q8KU07
RubS7	384	sugar	Glycosyltransferase	ElmGT, <i>Streptomyces olivaceus</i>	48/36	Q9F2F9
RubR	345	regulation	SARP family transcriptional regulator	Dnrl, <i>Streptomyces peucetius</i>	54/39	P25047
RubN2	115	hypothetical protein	Unknown	AcrR, <i>Sciscionella marina</i>	69/51	WP_020501920
RubA	311	oxygenase	F420-dependent oxidoreductase	SsuD, <i>Amycolatopsis methanolica</i> 239	79/68	AIJ25240
RubB	518	oxygenase	Oxygenase	ZhuM, <i>Streptomyces</i> sp. R1128	67/55	AAG30200.1
RubC	389	oxygenase	naphthocyclinone hydroxylase	ActVA 5, <i>Streptomyces coelicolor</i> A3(2)	59/45	CAA41641
RubE1	319	PKS	β -ketoacylsynthase III (KSIII)	ZhuH, <i>Streptomyces</i> sp. R1128	78/61	AAG30194.1
RubE2	91	PKS	Acyl carrier protein (ACP)	ZhuG, <i>Streptomyces</i> sp. R1128	73/53	AAG30194.1
RubE3	306	PKS	Acyl transferase (AT)	ZhuC, <i>Streptomyces</i> sp. R1128	67/56	AAG30190.1
RubE4	84	PKS	Acyl carrier protein (ACP)	ZhuN, <i>Streptomyces</i> sp. R1128	82/68	AAG30201.1
RubE5	164	PKS	Bifunctional cyclase/dehydratase	ZhuJ, <i>Streptomyces</i> sp. R1128	76/69	AAG30197.1
RubE6	251	PKS	Cyclase	ZhuI, <i>Streptomyces</i> sp. R1128	88/79	AAG30196.1
RubE7	206	PKS	NADH dehydrogenase	McyH1, <i>Streptomyces flaveolus</i>	80/69	AFG19428
RubE8	409	PKS	β -ketoacylsynthase II (KS α)	ZhuB, <i>Streptomyces</i> sp. R1128	88/79	AAG30189.1
RubE9	400	PKS	Chain length factor (CLF/KS β)	ZhuA, <i>Streptomyces</i> sp. R1128	81/71	AAG30188.1

Supplementary Table 2. NMR data of **3** and **4** in DMSO-*d*₆, and **5** in CD₃OD (δ in ppm, *J* in Hz).

No.	3		4			5	
	δ_{H}	δ_{C}	δ_{H}	COSY	HMBC	δ_{C}	δ_{H}
2	2.55 (s)	20.82	2.46 (s)		4, 3	125.42	6.89 (s)
3		169.33				163.49	
4	8.12 (s)	106.34	7.61 (s)		6, 3, 2	113.71	7.42 (s)
5		161.41				139.00	
6		122.14				184.54	
7		133.84				136.11	
8		150.12				108.40	7.03 (s)
9		nd				165.73	
10	6.50 (s)	119.54	6.48 (brs)	12	12, 8	109.53	6.48 (s)
11		nd				166.55	
12	6.69 (s)	126.62	7.07 (brs)	10	13, 10, 7, 6	111.94	
13		186.39				189.72	
14		115.07				124.01	
15		195.96				151.51	
16	3.03 (t, 7.1)	43.20	2.81 (t, 7.2)	17	18, 17, 15	39.36	3.09 (t, 7.6)
17	1.65 (m)	16.96	1.53 (m)	18, 16	18, 16, 15	25.42	1.60 (m)
18	0.93 (t, 7.3)	13.80	0.89 (t, 7.4)	17	17, 16	14.86	0.99 (t, 7.3)

^and means not detected in the NMR spectra.

Supplementary Table 3. NMR data of **6** in CD₃OD and **7-8** in DMSO-*d*₆ (δ in ppm, *J* in Hz).

No.	6				7				8			
	δ_C	δ_H	COSY	HMBC	δ_C	δ_H	COSY	HMBC	δ_C	δ_H	COSY	HMBC
1	171.86											
2	132.77				51.91	2.12 (dd,13.6, 1.7), 2.80 (overlapped)	4	16, 15, 14, 4, 3	22.77	2.17 (s)		
3	159.72				207.86				157.73			
4	113.41	7.50 (s)		14,13, 6, 5, 3, 2, 1	55.29	2.36 (dd,13.0, 1.6), 2.79 (overlapped)	2	14, 6, 5, 3, 2	116.31	8.51 (s)		
5	138.25				74.25				161.61			
6	183.76				40.04	2.52 (overlapped) 3.30 (d, 16.5),		14, 12, 8, 7, 5, 4	107.54			
7	135.60				146.23				137.69			
8	108.22	6.98 (d, 1.9)	10	13, 12, 10, 7, 6	107.98	6.08 (brs)	10	12, 10, 6	170.87			
9	166.36				164.59				161.22			
10	109.49	6.45 (d, 1.9)	8	12, 9, 8	99.91	6.02 (d, 1.9)	8	9, 8	114.45	6.36 (s)		12, 11, 9, 8
11	165.42				164.60				185.74			
12	111.97				111.35				118.51	6.46 (s)		13, 10, 6
13	189.33				203.21				192.57			
14	123.85				59.65				121.32			
15	147.15				75.95				154.82			
16	35.86	3.18 (t, 7.6)	17	18, 17, 15, 14, 2	43.97	1.40 (td, 3.7, 13.0), 1.63 (td, 4.3, 13.0)	17	18, 17, 15, 14, 2,	30.57	2.82 (m), 2.49 (m)	17	18, 17, 15, 14
17	25.32	1.63 (m)	18, 16	18, 16, 15	17.40	1.26 (m), 1.52 (m)	18, 16	18, 16	21.66	1.32 (m)	18, 16	18, 16, 15
18	15.13	1.07 (t, 7.3)	17	17, 16	14.60	0.86 (t, 7.2)	17	17, 16	13.99	0.71 (t, 6.9)	17	17, 16
1'									129.02			
2'									136.30			
3'									129.03	7.77	4'	
4'									133.80	7.91	3'	
5'									131.53	7.82	6'	1'
6'									132.30	8.21	5'	2', 7'
7'									165.21			
OH-5						5.29 (brs)		14, 6, 5				
OH-11						12.89 (s)		13, 12, 11, 10				
OH-15						4.81 (s)		15, 14, 2				

Supplementary Table 4. NMR data of compounds **10** and **11** in DMSO-*d*₆ (δ in ppm, *J* in Hz).

No.	10				11				
	δ_C	δ_H	δ_F	HMBC	COSY	δ_C	δ_H	HMBC	COSY
2	22.75	2.17 (s)		4, 3		22.87	2.17 (s)	4, 3	
3	158.46					158.57			
4	115.90	8.50 (s)		14, 3, 2		115.90	8.47 (s)	14, 3, 2	
5	161.53					161.53			
6	111.41					111.45			
7	137.69					137.69			
8	170.02					169.83			
9	165.39					165.53			
10	130.07					130.08			
11	183.56					183.49			
12	117.27	6.47 (s)		13, 11, 10, 7, 6		117.00	6.46 (s)	13, 11, 10, 7, 6	
13	192.79					192.98			
14	120.88					120.82			
15	154.70					154.83			
16	30.38	2.48 (m) 2.84 (m)		18, 17, 14	H-17	30.51	2.48 (m) 2.89 (m)		H-17
17	21.67	1.30 (m)		18, 16	H-18, H-16	21.59	1.33 (m)		H-18, H-16
18	13.88	0.73 (t, 7.1)		17, 16	H-17	13.97	0.72 (t, 6.1)	17, 16	H-17
19	104.55	5.14 (s)		20, 10, 9		104.55	5.13 (s)	20, 10, 9	
20	83.11					83.09			
21	74.07	3.82 (brs)		20, 10	H-22, OH-21	74.10	3.82 (brs)	20, 10	H-22, OH-21
22	70.31	3.49 (brs)		24, 21, 20	H-23, OH-22, H-21	70.35	3.48 (brs)		OH-22, H-21
23	70.26	3.78 (m)		24, 22, 21, 19	H-24, H-22	70.23	3.78 (m)		H-24, H-22
24	16.69	1.07 (d, 6.2)		23, 22	H-23	16.69	1.07 (d, 5.9)	23, 22	H-23
1'	129.79					128.31			
2'	132.45					134.44			
3'	131.58	7.91 (m)		5', 1'	H-4'	129.97	7.71 (m)	5'	H-4'
4'	120.44	7.77 (m)		6', 2'	H-3'	131.23	8.01 (m)	2'	H-3'
5'	162.39		-108.22			135.19			
6'	118.82	7.94 (m)		4', 2'		130.75	7.75 (m)	4', 2'	
7'	164.06					165.43			
OH-20		5.77 (s)					5.78 (s)	19	
OH-21		7.66 (brs)			H-21		7.68 (brs)	21, 20	H-21
OH-22		4.69 (s)			H-22		4.67 (s)	23	H-22

Supplementary Table 5. Strains and plasmids used and generated in this study.

Strains/Plasmid	Purpose	Sources
Strains		
<i>E. coli</i>		
DH10B	Host strain for cloning	Invitrogen
BW25113/pIJ790	Host strain for PCR targeting	Ref. 10
ET12567/pUZ8002	Donor strain for conjugation	Ref. 11
XL1-blue MR	Host strain for genomic library	Agilent Technologies
DH5a/BT340	Host strain for in-frame deletion	Ref. 12
<i>Streptomyces</i>		
<i>Streptomyces</i> sp. KIB-H033	Rubrolones wild type producing strain	This study
<i>Streptomyces albus</i> J1074	Host strain for heterologous expression	Ref. 13
<i>S. albus</i> 9B10	<i>Streptomyces albus</i> J1074 integrated with plasmid 9B10 which contains <i>rub</i> biosynthetic gene cluster	This study
<i>S. albus</i> 9B10-1	<i>orf1-orf7</i> inactivation mutant of <i>S. albus</i> 9B10	This study
<i>S. albus</i> 9B10-2	<i>orf30-orf37</i> inactivation mutant of <i>S. albus</i> 9B10	This study
<i>S. albus</i> 9B10-Δ <i>S1</i>	<i>rubS1</i> inactivation mutant of <i>S. albus</i> 9B10	This study
<i>S. albus</i> 9B10-Δ <i>S2</i>	<i>rubS2</i> inactivation mutant of <i>S. albus</i> 9B10	This study
<i>S. albus</i> 9B10-Δ <i>S3</i>	<i>rubS3</i> inactivation mutant of <i>S. albus</i> 9B10	This study
<i>S. albus</i> 9B10-Δ <i>S7</i>	<i>rubS7</i> inactivation mutant of <i>S. albus</i> 9B10	This study
<i>S. albus</i> 9B10-Δ <i>A</i>	<i>rubA</i> inactivation mutant of <i>S. albus</i> 9B10	This study
<i>S. albus</i> 9B10-Δ <i>B</i>	<i>rubB</i> inactivation mutant of <i>S. albus</i> 9B10	This study
<i>S. albus</i> 9B10-Δ <i>C</i>	<i>rubC</i> inactivation mutant of <i>S. albus</i> 9B10	This study
<i>S. albus</i> 9B10-Δ <i>E7</i>	<i>rubE7</i> inactivation mutant of <i>S. albus</i> 9B10	This study
<i>S. albus</i> 9B10-Δ <i>E9</i>	<i>rubE9</i> inactivation mutant of <i>S. albus</i> 9B10	This study
Plasmids		
pJTU2554	Apr ^r , Cosmid vector for genomic library construction	Ref. 10
pJTU6722	Ery ^r , Vector for PCR targeting	Constructed by Prof. Meifeng Tao
p9B10	Apr ^r , Cosmid which contains <i>rub</i> biosynthetic gene cluster	This study
p9B10-1	Apr ^r , gene inactivation clone used for <i>orf1-orf7</i> mutant	This study
p9B10-2	Apr ^r , gene inactivation clone used for <i>orf30-orf37</i> mutant	This study
p9B10-Δ <i>S1</i>	Apr ^r , gene inactivation clone used for <i>rubS1</i> mutant	This study
p9B10-Δ <i>S2</i>	Apr ^r , gene inactivation clone used for <i>rubS2</i> mutant	This study
p9B10-Δ <i>S3</i>	Apr ^r , gene inactivation clone used for <i>rubS3</i> mutant	This study
p9B10-Δ <i>S7</i>	Apr ^r , gene inactivation clone used for <i>rubS7</i> mutant	This study
p9B10-Δ <i>A</i>	Apr ^r , gene inactivation clone used for <i>rubA</i> mutant	This study
p9B10-Δ <i>B</i>	Apr ^r , gene inactivation clone used for <i>rubB</i> mutant	This study
p9B10-Δ <i>C</i>	Apr ^r , gene inactivation clone used for <i>rubC</i> mutant	This study
p9B10-Δ <i>E7</i>	Apr ^r , gene inactivation clone used for <i>rubE7</i> mutant	This study
p9B10-Δ <i>E9</i>	Apr ^r , gene inactivation clone used for <i>rubE9</i> mutant	This study

Supplementary Table 6. Primers used for constructing and confirming the mutants.

Primers	Targeted genes	Sequences
For PCR targeting		
Tar-H2517-F	<i>orf1-orf7</i>	5'-CTCCGACGCCGTGCGGTGAAATCTTTTCAGGAGGAAGTCCattccggggatccgctgacc-3'
Tar-H2511-R		5'-GTGCACCCGCAACAGCGAGTACTGGATCCTGCGCACCGGtgtaggctggagctgcttc-3'
Tar-H2518-F	<i>rubS1</i>	5'-ACCGTCCGCCCCGGCGACCCGCGCTACGACGACCTGGCGattccggggatccgctgacc-3'
Tar-H2518-R		5'-CCGCGCTCTCGATCTCCGTGCGGGCGGCATCGGTGCTCGtgtaggctggagctgcttc-3'
Tar-H2549-F	<i>orf30-orf37</i>	5'-CCGGCACCCTCGCGGGCGGCATCTTCGCGGTCCGGCACCCattccggggatccgctgacc-3'
Tar-H2542-R		5'-TTCGGTTCCGACCGAGGCAGGACAGCGGGCCGGTCCCGtgtaggctggagctgcttc-3'
Tar-H2541-F	<i>rubE9</i>	5'-CCGATGAGGCCGCCCGCAGTCGTACCCGGGATCGGGGTGattccggggatccgctgacc-3'
Tar-H2541-R		5'-GGGTCCCCTTCGGTTCCGACCGAGGGCGGGGGTCAtgtaggctggagctgcttc-3'
Tar-H2539-F	<i>rubE7</i>	5'-GCAACGACGGCCGGCACGGCTCCTGGCGCTGGACGCGGCCattccggggatccgctgacc-3'
Tar-H2539-R		5'-AGCGCAAACCCGTGCACGAGGTGCCCCAGGCGGAGGTCAtgtaggctggagctgcttc-3'
Tar-H2528-F	<i>rubA</i>	5'-CTGCGCAAACCGATCTCTGAAATGGGGTGTGGGCAATGattccggggatccgctgacc-3'
Tar-H2528-R		5'-GCCCGGGCCCGCGGGTCCGGGCACCCGGCCCGCGGTCAtgtaggctggagctgcttc-3'
Tar-H2530-F	<i>rubB</i>	5'-ACCCGCGTCGGCTGCCGTGCCGATGGGAGTGGGAGATGattccggggatccgctgacc-3'
Tar-H2530-R		5'-GGTCACCACGTCGACGCGTCCGGCCAGCCGCGCGCCGTtgtaggctggagctgcttc-3'
Tar-H2531-F	<i>rubC</i>	5'-CCGAAGGAGACTGATGTGACGCACCCAGTAGCCCGCGGattccggggatccgctgacc-3'
Tar-H2531-R		5'-TGCCGGGGACGGCAGGGCGGGCGGGTGCACGGGTCAtgtaggctggagctgcttc-3'
Tar-H2520-F	<i>rubS2</i>	5'-ACAACGTGCGGCCGCGCTCGCCCCGGAGCCGAAGACCattccggggatccgctgacc-3'
Tar-H2520-R		5'-GCGGCCGGCACGGCGGACGGGCGCCGGGGGGCGGTGTGCGtgtaggctggagctgcttc-3'
Tar-H2521-F	<i>rubS3</i>	5'-GCCACAGGGCGAGGTCCGCGTCGGAGGGGCCGCTGTCCGattccggggatccgctgacc-3'
Tar-H2521-R		5'-GGCAGTCGGCGGTGCGGTCACCGCCCCGGCCCGGGGGCGtgtaggctggagctgcttc-3'
Tar-H2525-F	<i>rubS7</i>	5'-ATTGCCGACTCCTCGACAAAGGGCGTATGCGCAGCCATGattccggggatccgctgacc-3'
Tar-H2525-R		5'-CTCCGCGGTCAGTTGTCTCCTGTCCGGCGGCGACACCAGtgtaggctggagctgcttc-3'
For gene validation		
H2518-check-F	<i>rubS1</i>	5'-GTGAGTGTTCCCTTGAGCAA-3'
H2518-check-R		5'-GAACCTTGC GGATGTCCTC-3'
H2541-check-F	<i>rubE9</i>	5'-CAACCTGCACGAACCGGATC-3'
H2541-check-R		5'-AGCACATGACGAAGGGCCTG-3'
H2539-check-F	<i>rubE7</i>	5'-TGCGCGAGATGGTTCGAGGAGTA-3'
H2539-check-R		5'-CGGAAGTCAGCAGGTCCACGAAT-3'
H2528-check-F	<i>rubA</i>	5'-CATGGTTCGATTCTGTCTCTCAT-3'
H2528-check-R		5'-GCGTTCATCGAGCACTGGAAGT-3'
H2530-check-F	<i>rubB</i>	5'-CTGCCTCAGCCATTGCTCAAGA-3'
H2530-check-R		5'-GGTGCGTCACATCAGTCTCCTTC-3'
H2531-check-F	<i>rubC</i>	5'-AGCGTGACCGAAGCGATGCA-3'
H2531-check-R		5'-AACACCTCAGCGGCGTCGAT-3'
H2520-check-F	<i>rubS2</i>	5'-GGACATCCGCAAGTTCAT-3'
H2520-check-R		5'-CTGCACTACGTCAACACT-3'
H2521-check-F	<i>rubS3</i>	5'-GCCTGAAGCTGATCCTGT-3'
H2521-check-R		5'-GATCGACGACAGGAT-3'
H2525-check-F	<i>rubS7</i>	5'-GCGGCTACTTGACGATGTCCTC-3'
H2525-check-R		5'-GCTACCACGAGTACCTGATGTTCT-3'

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