Re-evaluation of the Fijianolide/Laulimalide Chemotype Suggests an Alternate Mechanism of Action for C-15/C-20 analogs

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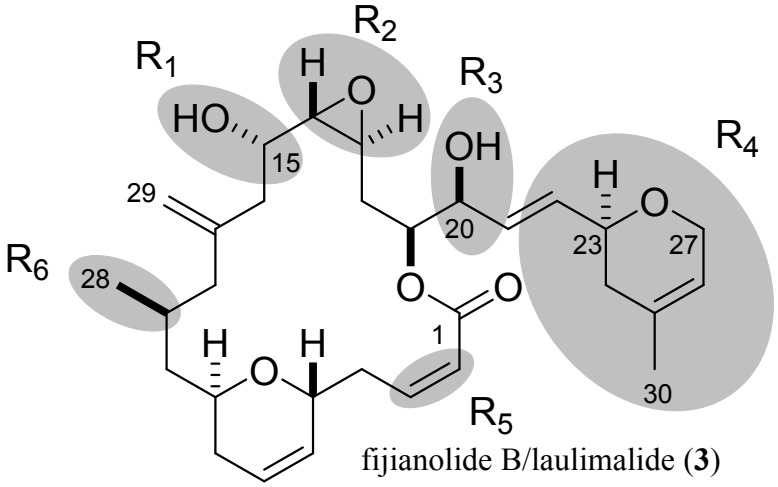
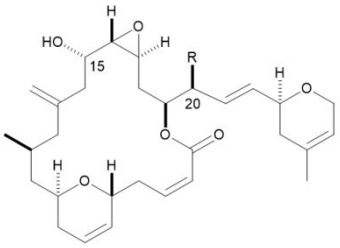
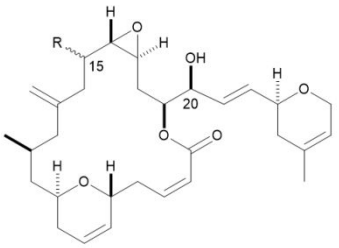


fijianolide B/laulimalide (**3**)

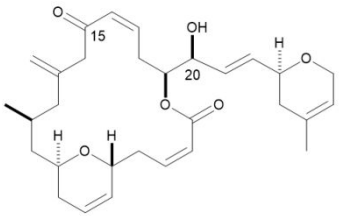
Selected synthetic analogs of **3** with modifications to C-15 or C-20



C-20, R = OTBS
C-20, R = OMe
C-20, R = OAc

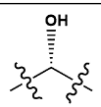
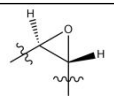
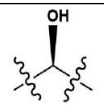
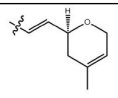
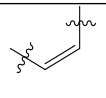
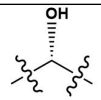
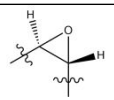
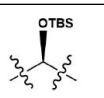
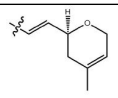
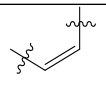
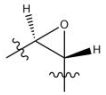
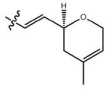
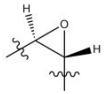
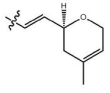
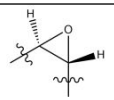
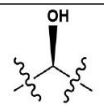
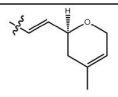
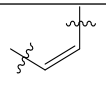
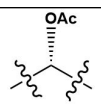
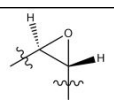
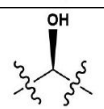
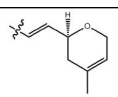
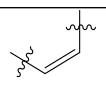


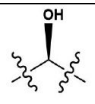
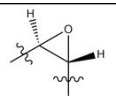
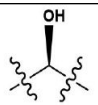
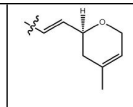
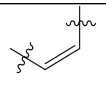
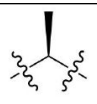
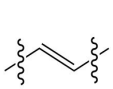
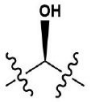
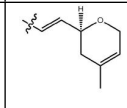
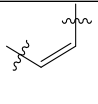
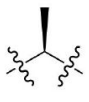
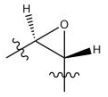
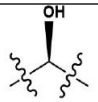
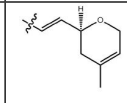
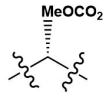
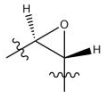
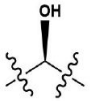
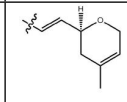
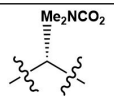
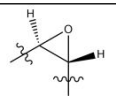
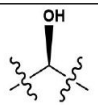
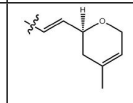
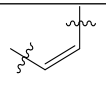
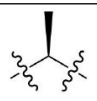
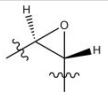
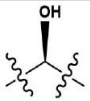
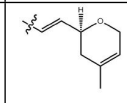
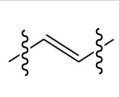
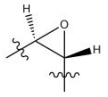
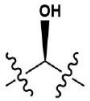
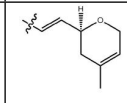
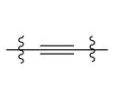
C-15, R = OMe, 15*S*
C-15, R = OAc, 15*S*
C-15, R = OH, 15*R*



C-15 oxo, C16/17 desepoxide

Table S1. Structural Modifications of Analogs of 37 Fijianolide B/Laulimalide (**1**) with Selected Potencies Against Selected Cancer Cell Lines.

entry	R1	R2	R3	R4	R5	R6	MDA-MB-435	NCI/ADR	MCF-7	HCT116	HT-29	A2780
										IC_{50} (nM)		
1							7.0[1] 2.3[4] 5.7[6]	36[2]	11.6[3] 3.8[2] 7.0[7]	3.0[1]	6.9[4]	3.4[5]
2							>1,000[4]					
3							242[4] 240[6]			590[4] 470[6]		
4							91±27[4]					
5							>1,000[4]					
6							23[4]					

7							176[4]					
8							>1,000[4]					
9	p-NO_2 (C_6H_4) CO_2						37[4]					
10							442[4]					
11							601[4]					
12									54[2]			
13							2,500[6]	13,700[6]				
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15							233[8]	433[8]				
16							1,170[9] 1,200[8]	1,200[8]				
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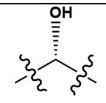
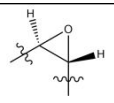
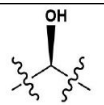
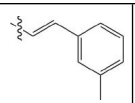
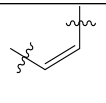
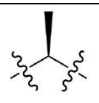
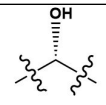
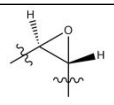
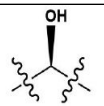
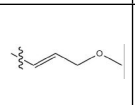
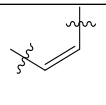
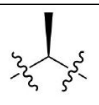
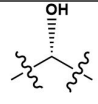
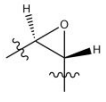
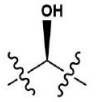
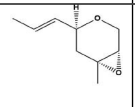
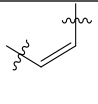
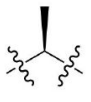
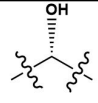
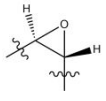
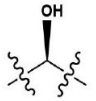
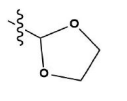
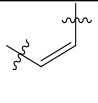
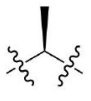
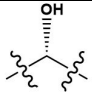
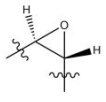
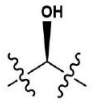
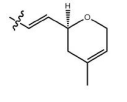
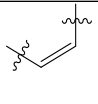
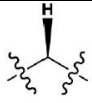
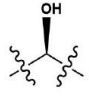
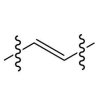
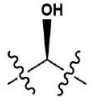
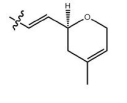
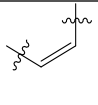
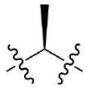
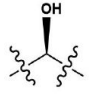
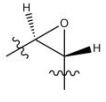
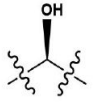
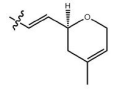
19							7,300[8]	12,400[8]				
20							7,900[8]	22,900[8]				
21							9,200[1]		1,400[1]			
22							>50,000[6]					
23							49.0[9]					50.0[5]
24							790[4]			2,700[4]		
25							176[4]					
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27							>1,000[4]				>1,000[4]	
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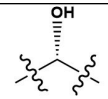
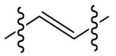
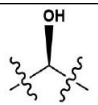
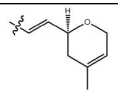
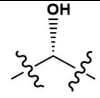
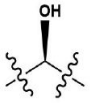
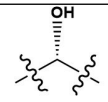
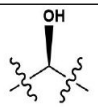
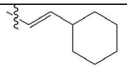
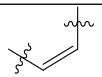
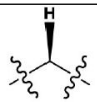
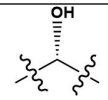
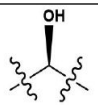
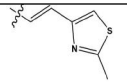
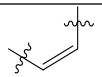
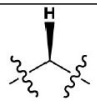
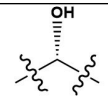
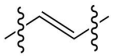
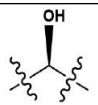
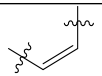
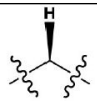
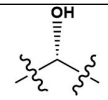
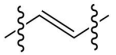
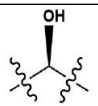
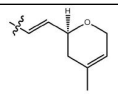
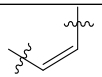
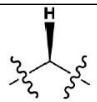
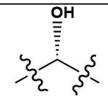
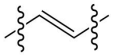
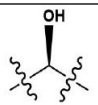
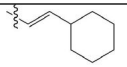
30							4,410[6]				22,000[6]	

31							16,500[6]	25,400[6]				
32												40,000[5]
33												9,000[5]
34												>50,000[5]
35												>50,000[5]
36												430[5]
37												>50,000[5]
38												>50,000[5]

1) Johnson, et al., **2007**, *J. Med. Chem. 50*, 3795-3803. 2) Mulzer et al., **2003**, *Chem. Rev.* 103, 3753-3786. 3) Gollner et al., **2009**, *Chem. Eur. J. 15,* 5979-5997. 4) Gallagher et al., **2004**, *Bioorg. Med. Chem. 14,* 575-579. 5) Pateson et al., **2005**, *Bioorg. Med. Chem. 15,* 2243-2247. 6) Wender et al., **2003**, *Org. Lett. 5,* 3507-3509. 7) Pryor et al., **2002**, *Biochemistry*, 41, 9109-9115. 8) Mooberry, et al., **2008**, *Mol. Pharmacol., 5* , 829-838. 9) Wender, et. al., **2006**, *Org. Lett.*, *8* , 1507-1510. 10) Quinoa et al., **1988**, *J. Org. Chem, 53,* 3642-3644. 11) Trost, B.M. et al., **2012** *Chem. Eur. J. 18*, 2961-2971.

Table S2. ^{1}H NMR Data of Fijianolide B/Laulimalide (**3**) in Benzene-*d6*

No.	Type	δ_H[a]	mult.	*J* (Hz)
1	C			
2	CH	5.70	d	11.6
3	CH	6.01	ddd	11.4, 9.5, 3.6
4	CH_2	3.84	m	
		1.89	m	
5	CH	3.92	m	
6	CH	5.40	dq	10.2, 3.0
7	CH	5.61	ddd	10.1, 5.3, 2.5
8	CH_2	1.83	m	
		1.74	m	
9	CH	3.66	dd	9.0, 3.3
10	CH_2	1.52	m	
		1.15	ddd	14.3, 4.5, 3.1
11	CH	1.80	m	
12	CH_2	2.57	dd	13.1, 4.3
		1.85	m	
13	C			
14	CH_2	2.11	m	
		2.09	m	
15	CH	3.96	m	
16	CH	2.71	dd	3.3, 2.5
17	CH	2.88	ddd	9.3, 3.6, 2.4
18	CH_2	2.15	ddd	13.8, 3.0, 1.5
		1.43	ddd	14.1, 8.4, 3.6
19	CH	5.09	ddd	10.0, 4.9, 1.6
20	CH	4.10	m	
21	CH	4.71	ddd	16.2, 5.4, 1.7
22	CH	5.79	ddd	16.1, 4.8, 1.2
23	CH	3.90	m	
24	CH_2	1.96	m	
		1.61	m	
25	C			
26	CH	5.11	bs	
27	CH_2	4.07	bs	
28	CH_3	0.84	d	6.5
29	CH_2	4.89	s	
		4.87	s	
30	CH_3	1.47	s	

[a] Measured at 400 MHz (^{1}H) in benzene-*d6*.

Table S3. ^{1}H NMR Data of Fijianolide B Di-Acetate (**4**) in benzene-*d6*

No.	Type	δ_H[a]	mult.	*J* (Hz)
1	C			
2	CH	5.77	d	10.8
3	CH	5.96	ddd	10.9, 10.9, 3.8
4	CH_2	3.96	m	
		2.06	m	
5	CH	4.13	bd	8.7
6	CH	5.43	bd	10.8
7	CH	5.64	m	
8	CH_2	1.76	m	
9	CH	3.73	m	
10	CH_2	1.53	m	
		1.16	ddd	14.3, 4.2, 3.0
11	CH	1.78	m	
12	CH_2	2.54	dd	14.8, 6.0
		1.84	m	
13	C			
14	CH_2	2.23	dd	15.9, 9.6
		1.69	m	
15	CH	5.63	m	
16	CH	2.78	dd	3.2, 2.8
17	CH	2.89	dq	8.9, 2.0
18	CH_2	2.13	m	
19	CH	5.35	ddd	11.5, 5.7, 1.6
20	CH	5.53	dd	
21	CH	5.75	dd	15.8, 5.0
22	CH	5.88	ddd	15.9, 4.7, 0.6
23	CH	3.82	ddd	8.6, 3.3, 3.3
24	CH_2	1.94	m	
		1.50	m	
25	C			
26	CH	5.12	bs	
27	CH_2	4.08	bs	
		3.92	bs	
28	CH_3	0.82	d	6.0
29	CH_2	4.97	bs	
		4.86	bs	
30	CH_3		bs	
	OAc	1.77	s	
	OAc	1.65	s	

[a]Measured at 500 MHz (^{1}H) in benzene-*d6*.

Table S4. ^{13}C, ^{1}H, HMBC, and COSY NMR Data of Fijianolide J (**5**) in Benzene-*d6*.

No.	Type[a]	δ_C[b]	δ_H[c]	mult.	*J* (Hz)	gHMBC[c]	gCOSY[c]
1	C	165.5				2, 4	
2	CH	120.4	5.76	d	11.4	4	3, 4
3	CH	150.3	5.93	ddd	11.3, 10.2, 3.6	4	3, 4
4	CH_2	34.2	4.72	m		3, 6	2, 3, 5
			2.04	m			
5	CH	73.4	4.09	bd	10.2	3, 4	4, 6, 7
6	CH	129.1	5.36	bd	10.2	4	5, 7
7	CH	125.2	5.57	m		8	5, 6, 8
8	CH_2	33.5*	1.73	m			9, 10
			1.55				
9	CH	68.2	3.54	m		8	8, 9, 10, 11
10	CH_2	44.0	1.46	m		8, 28	8, 9, 11
			1.10	ddd	14.3, 4.7, 2.4		
11	CH	30.0	1.86	m		10, 12, 28	10, 12
12	CH_2	46.7	2.53	dd	13.4, 5.1	11, 28, 29	10, 11, 29
			1.86	dd	13.9, 10.1		
13	C	145.5				11, 12	
14	CH_2	37.7	2.09	m		12, 29	15, 29
15	CH	66.4	3.97	m		14, 16	14, 16
16	CH	60.9	2.67	dd	3.0, 2.3	14, 18	15, 17
17	CH	51.4	2.87	ddd	8.8, 4.2, 2.2	16, 18, 19	16, 18
18	CH_2	33.5*	2.23	ddd	14.1, 4.2, 2.1	17	17, 19
			1.44	m			
19	CH	74.8	5.40	dd	11.6, 2.1	18	18
20	C	194.0				18, 19, 21, 22	
21	CH	122.5	6.65	dd	15.6, 2.0	22	22, 23
22	CH	147.9	6.96	dd	15.6, 3.5		21, 23
23	CH	72.4	3.68	m		21, 22	21, 22, 24
24	CH_2	34.8	1.63	m		22, 30	23
			1.28	m			
25	C	131.1				24, 30	
26	CH	120.1	5.02	bs			24, 27, 30
27	CH_2	65.8	4.07	bd	15.9		26
			3.85	bd	15.9	30	
28	CH_3	21.2	0.86	bd	6.7	10, 12	11
29	CH_2	112.0	4.93	s		12, 14, 15	12, 14
			4.89	s			
30	CH_3	22.7	1.37	s			26, 27

[a] Carbon type determined by DEPT, HSQC, and HMBC experiments (see Figures S5, S7, and S8 in Supporting Information). [b]Measured at 175 MHz. [c]Measured at 700MHz. * Interchangeable assignments.

Table S5. ^{13}C and ^{1}H NMR Data of Fijianolide L (**6**) in Benzene-*d6*.

No.	Type[a]	δ_C[b]	δ_H[c]	mult.	*J* (Hz)	gHMBC[c]	gCOSY[c]
1	C	164.9					
2	CH	121.0	5.82	d	11.1	3	3, 4
3	CH	147.7	5.78	m			2, 4
4	CH_2	34.3	3.34	m		3, 5	3, 5
			1.78	m			
5	CH	72.7	4.23	dd	10.2, 6.3	4	6, 4
6	CH	128.7	5.38	bd	10.1		7, 8
7	CH	125.1	5.58	m			6, 8
8	CH_2	31.6	1.73	m		10	
			1.55				
9	CH	65.9	3.46	m			7, 8, 10
10	CH_2	44.9	1.44	m		11, 12, 28	11, 12
			0.84	ddd	14.1, 9.3, 2.0		
11	CH	27.4	1.86	m		10, 12, 28	
12	CH_2	46.7	2.83	q	16.6		29
			1.73	dd	13.9, 10.1		
13	C	140.8					
14	CH_2	43.2	1.98	m		12	16, 17
15	C	202.2					
16	CH	54.7	2.96	d	1.9	14, 17, 18, 19	14
17	CH	65.4	3.05	ddd	6.5, 6.0, 1.8	18	18
18	CH_2	33.2	2.08	m		17, 19	17, 19
			1.57	m			
19	CH	74.6	5.41	dd	7.8, 5.7	16, 17, 18	18
20	C	193.4					
21	CH	122.2	6.60	dd	15.7, 1.9	18	22
22	CH	147.0	6.96	dd	15.7, 3.6		21
23	CH	72.0	3.66	m		22, 27	
24	CH_2	34.8	1.63	m			30
			1.27	m			
25	C	130.6					
26	CH	119.7	5.00	bs		24	27, 30
27	CH_2	59.7	3.99	bd	15.8		26, 30
			3.83	bd	15.8		
28	CH_3	19.2	0.74	bd	6.6	10, 11, 12	10, 12
29	CH_2	116.0	4.88	s		12, 14	
			4.80	s			
30	CH_3	22.3	1.35	s		24	24, 26, 27

[a] Carbon type determined by HSQC and HMBC experiments (see Figures S12 and S13 in Supporting Information). [b]Measured at 100 MHz. [c]Measured at 400MHz.

Figure S1. ^{1}H NMR spectrum of Fijianolide B/Laulimalide (**3**) in C_6D_6 (400 MHz).

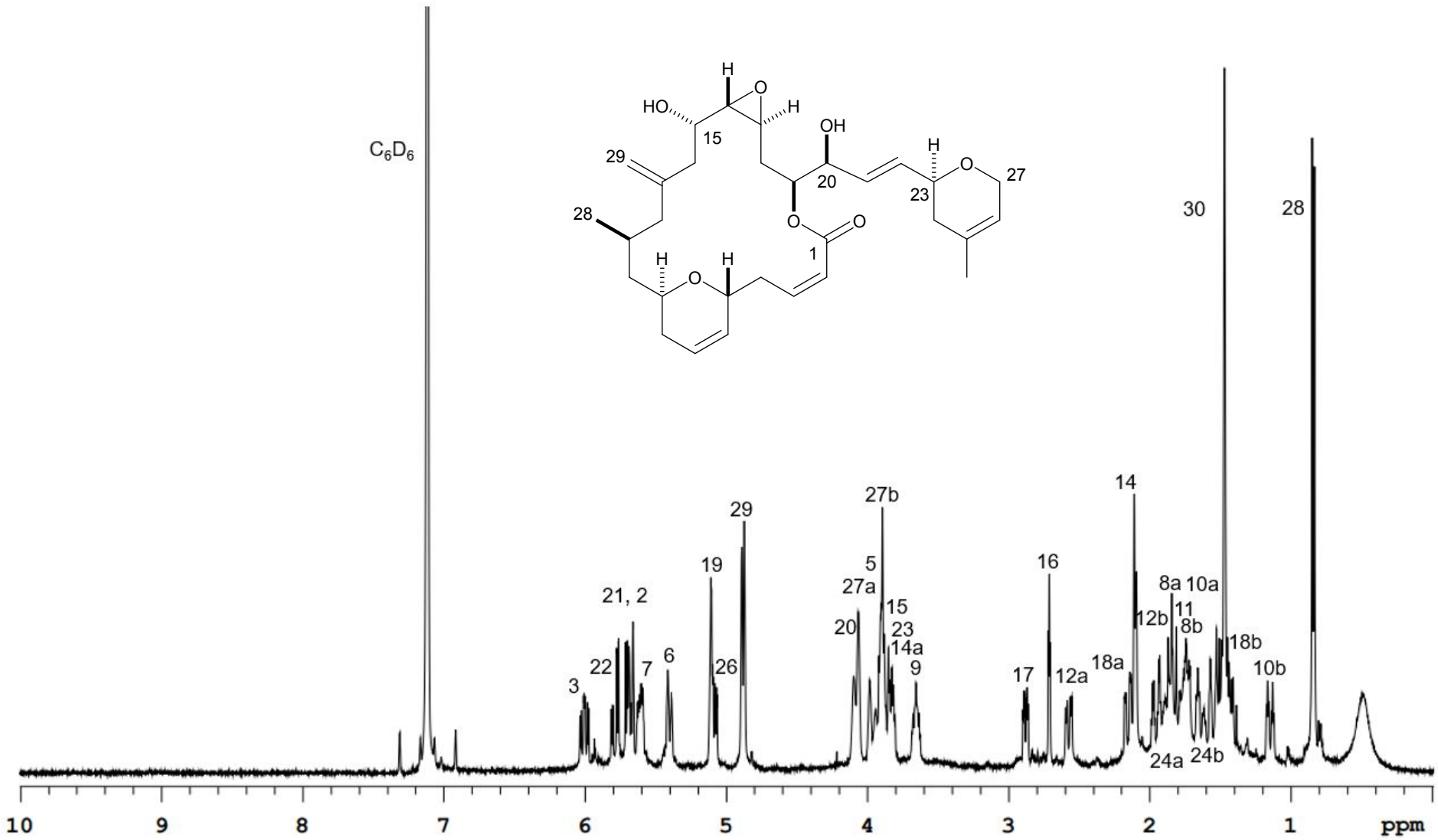


Figure S2. ^{1}H NMR Spectrum of Fijianolide B di-acetate (**4**) in C_6D_6 (500 MHz)

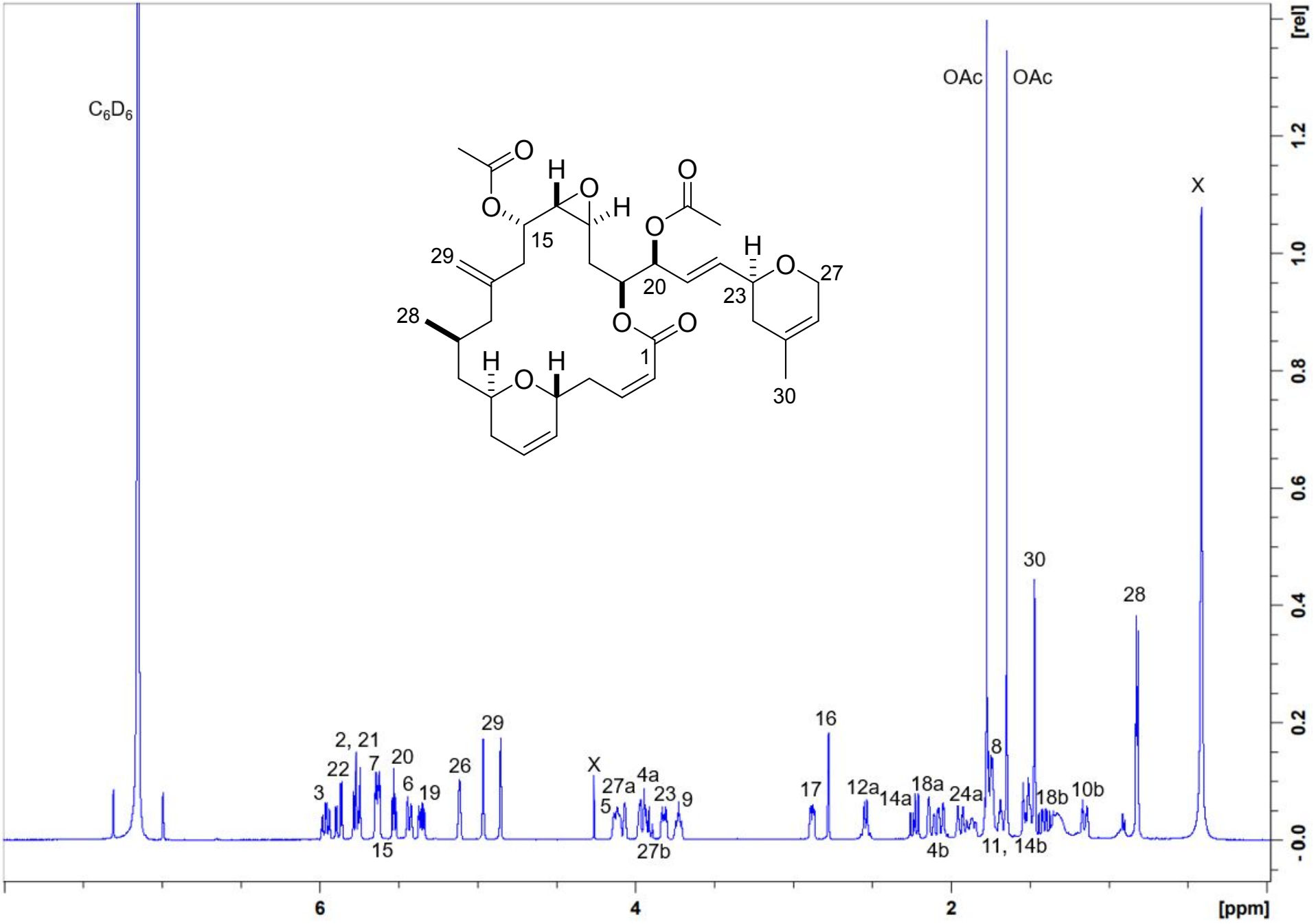


Figure S3. ^{1}H NMR spectrum of Fijianolide J (**5**) in C_6D_6 (400 MHz).

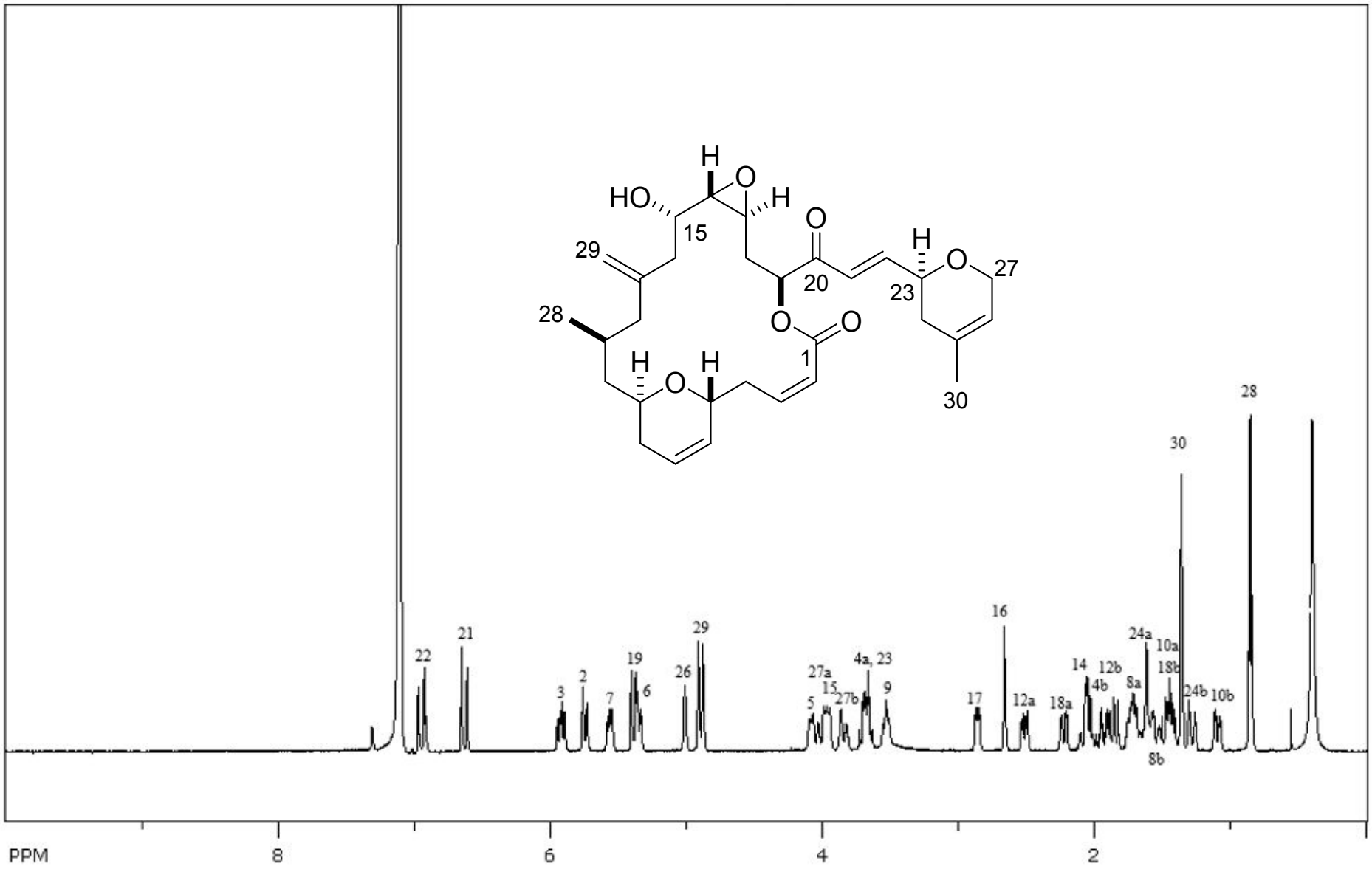


Figure S4. ^{13}C NMR spectrum of Fijianolide J (**5**) in C_6D_6 (175 MHz).

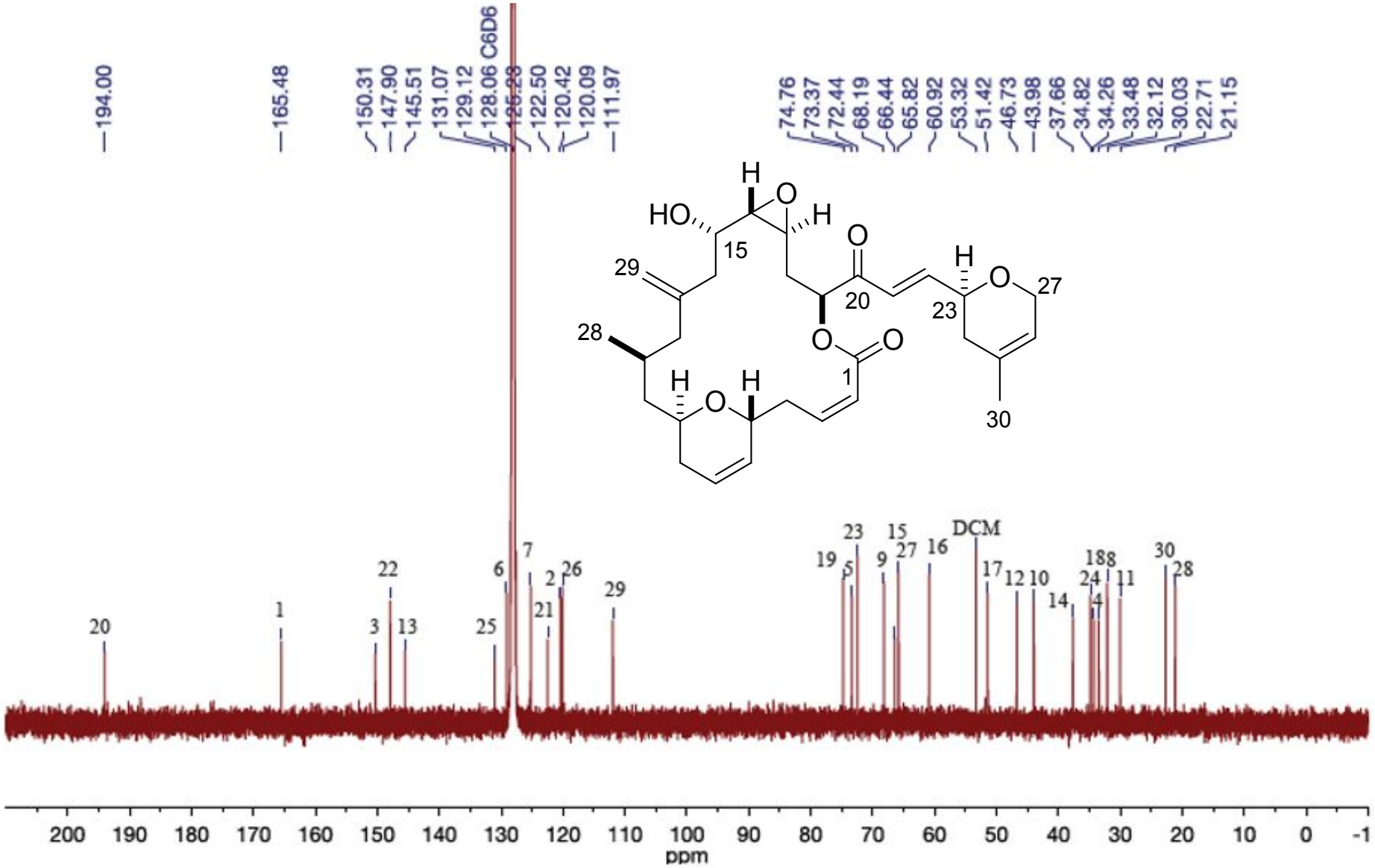


Figure S5. DEPT NMR spectra of Fijianolide J (**5**) including a) DEPT-45 b) DEPT-90 and c) DEPT-135.

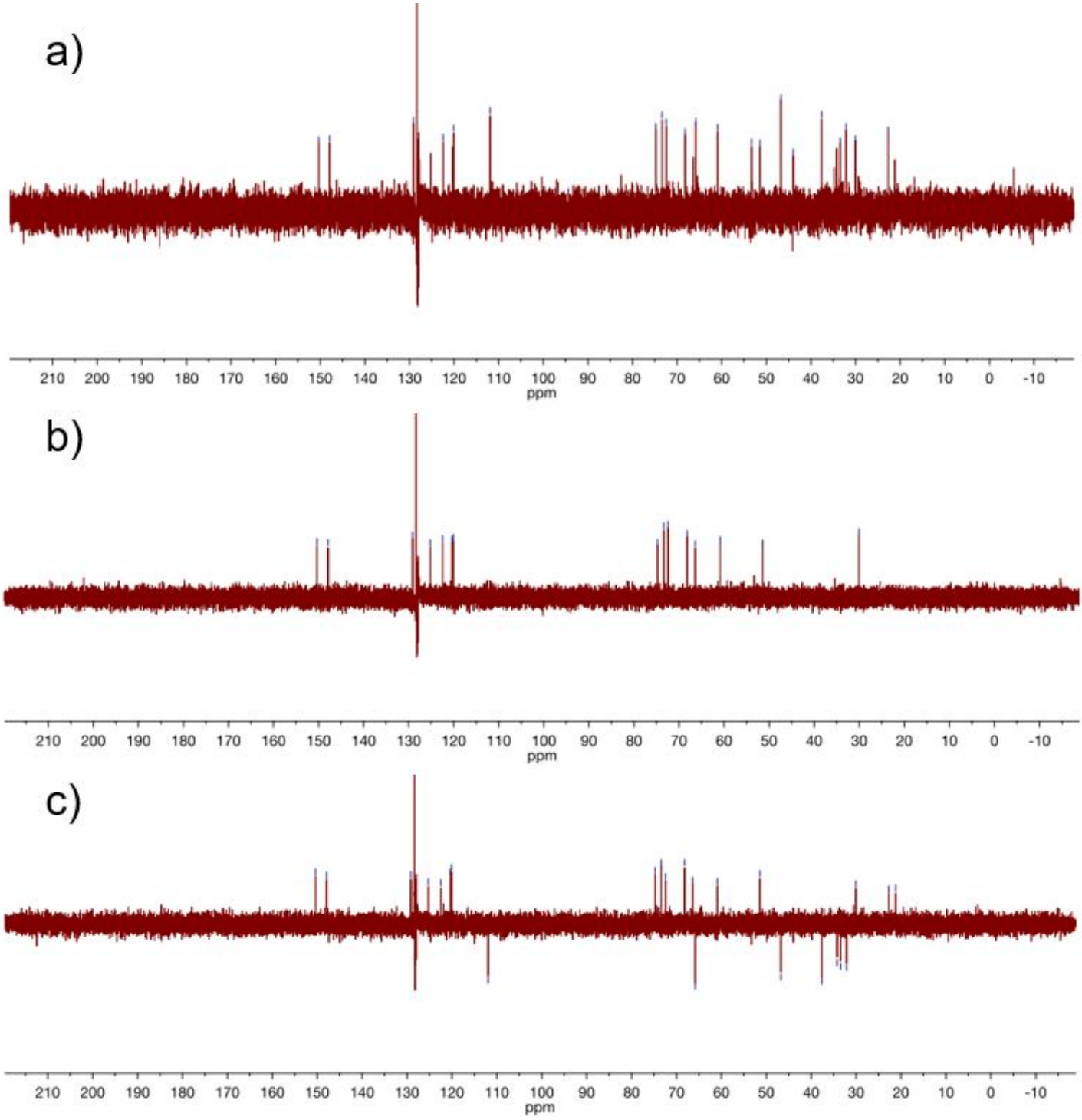


Figure S6. gCOSY spectrum of Fijianolide J (**5**) in C_6D_6 (700 MHz)

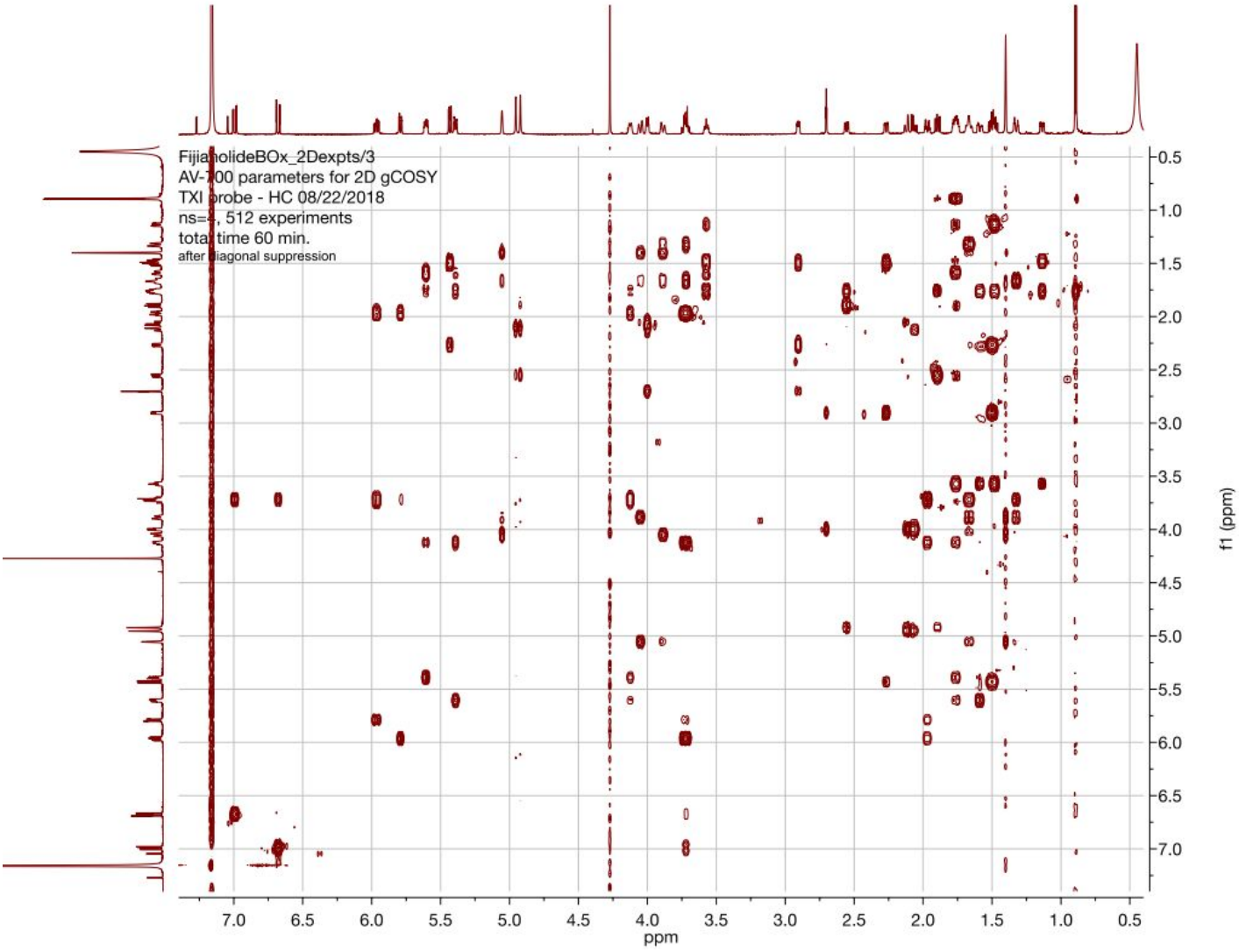


Figure S7. HSQC spectrum of Fijianolide J (**5**) in C_6D_6 (700 MHz)

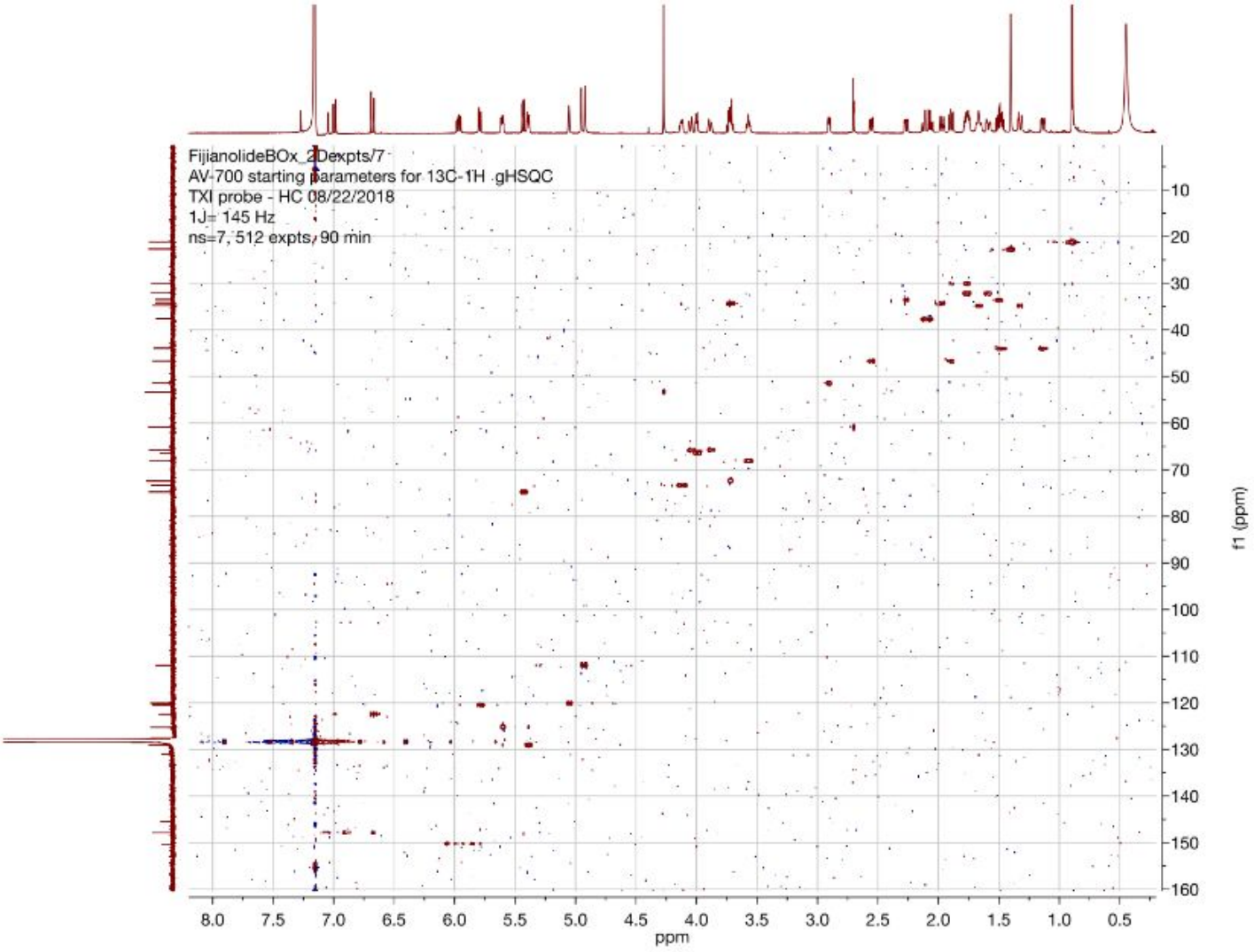


Figure S8. HMBC spectrum of Fijianolide J (**5**) in C_6D_6 (700 MHz)

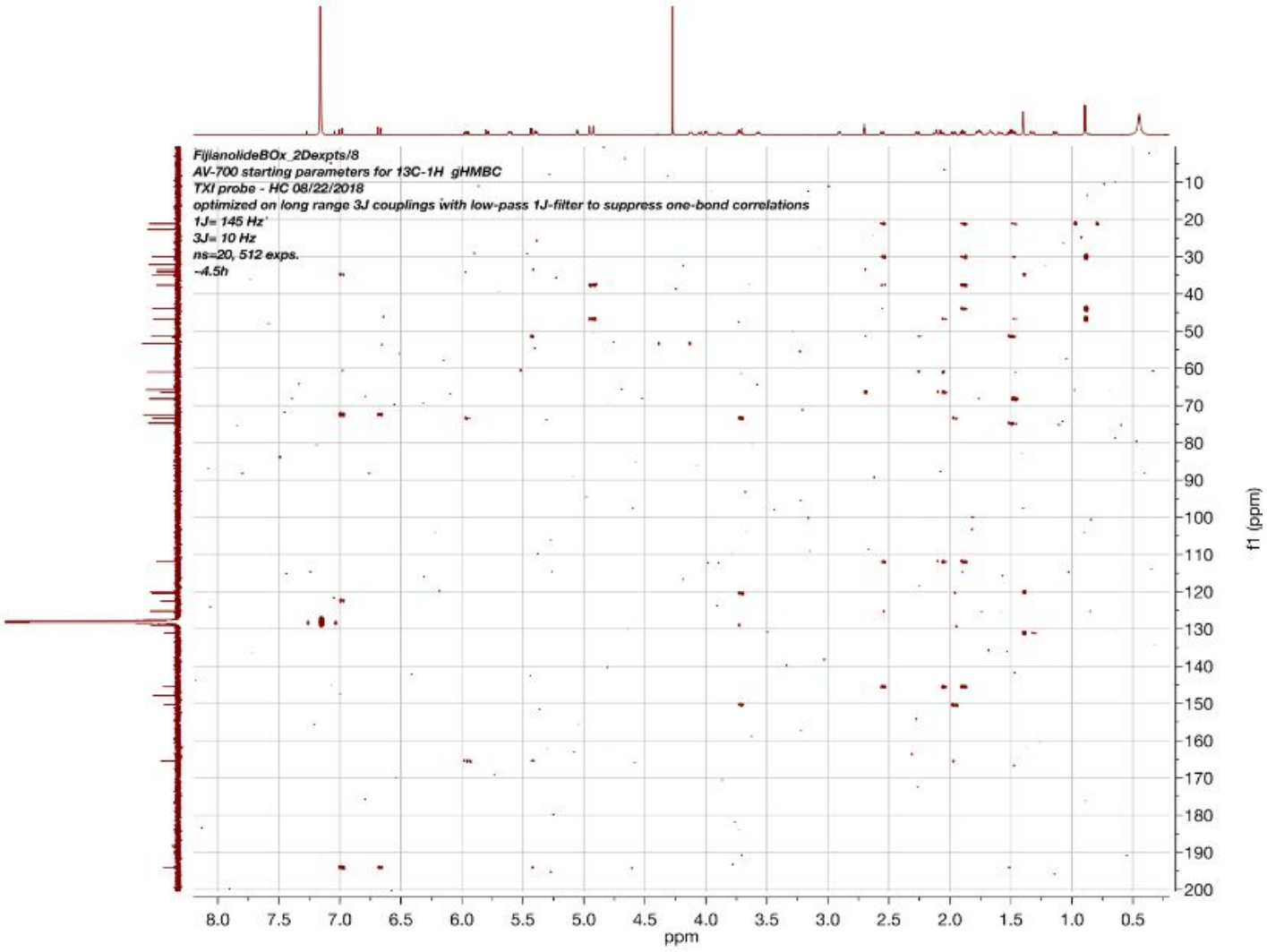


Figure S9. ^{1}H NMR spectrum of Fijianolide L (**6**) in C_6D_6 (400 MHz)

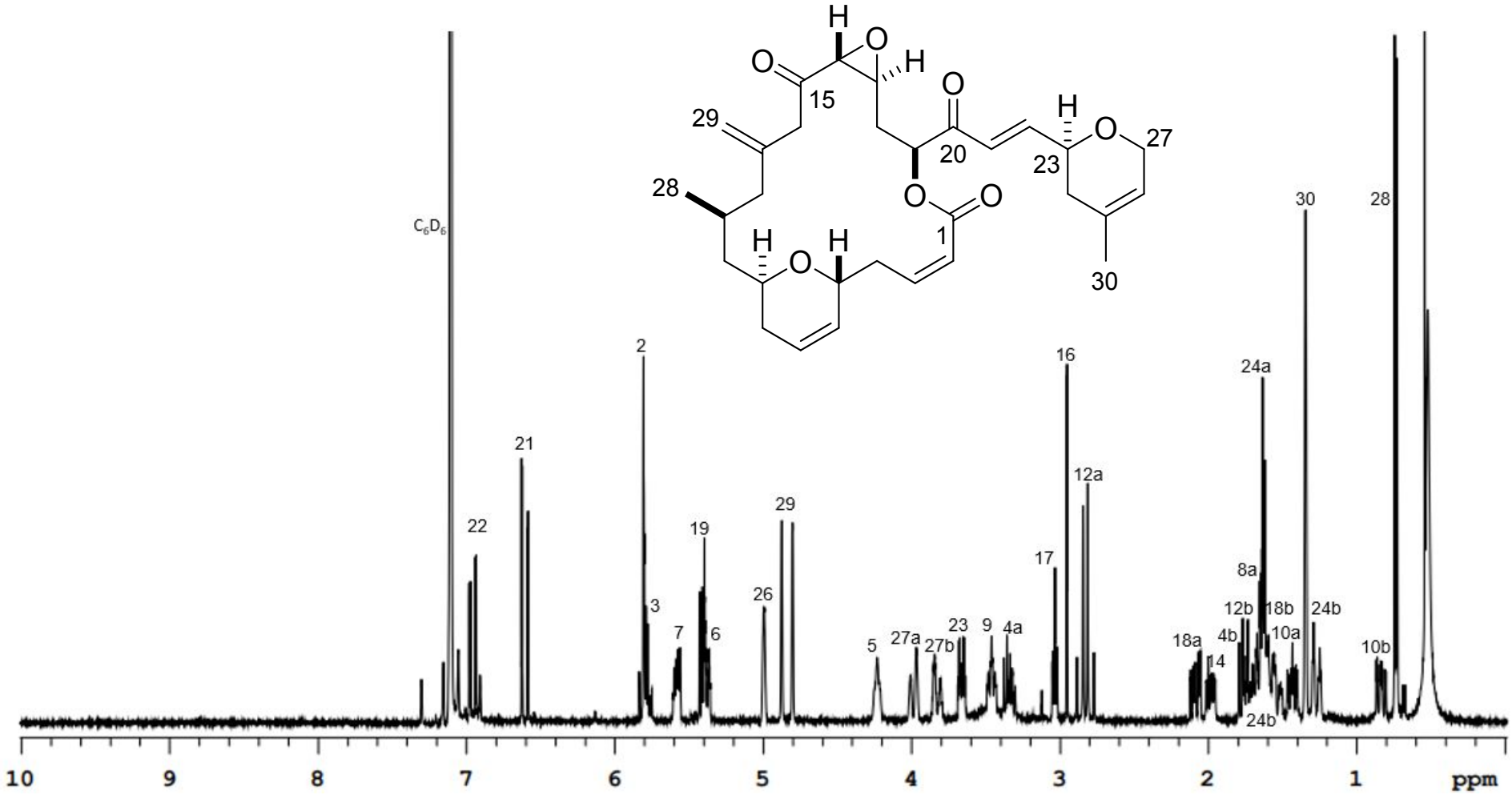


Figure S10. ^{13}C NMR spectrum of Fijianolide L (**6**) in C_6D_6 (100 MHz)

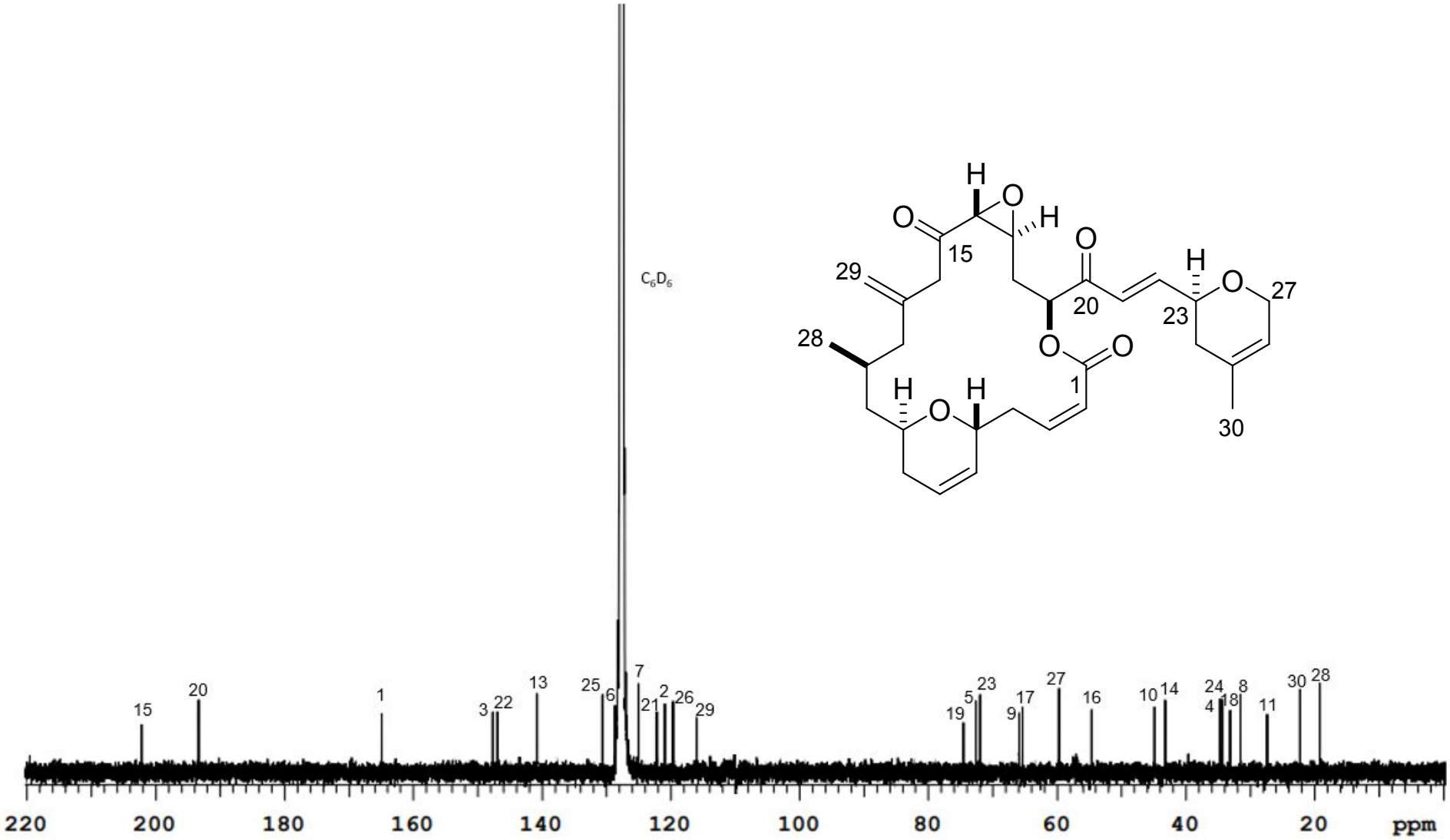


Figure S11. gCOSY spectrum of Fijianolide L (**6**) in C_6D_6 (800 MHz)

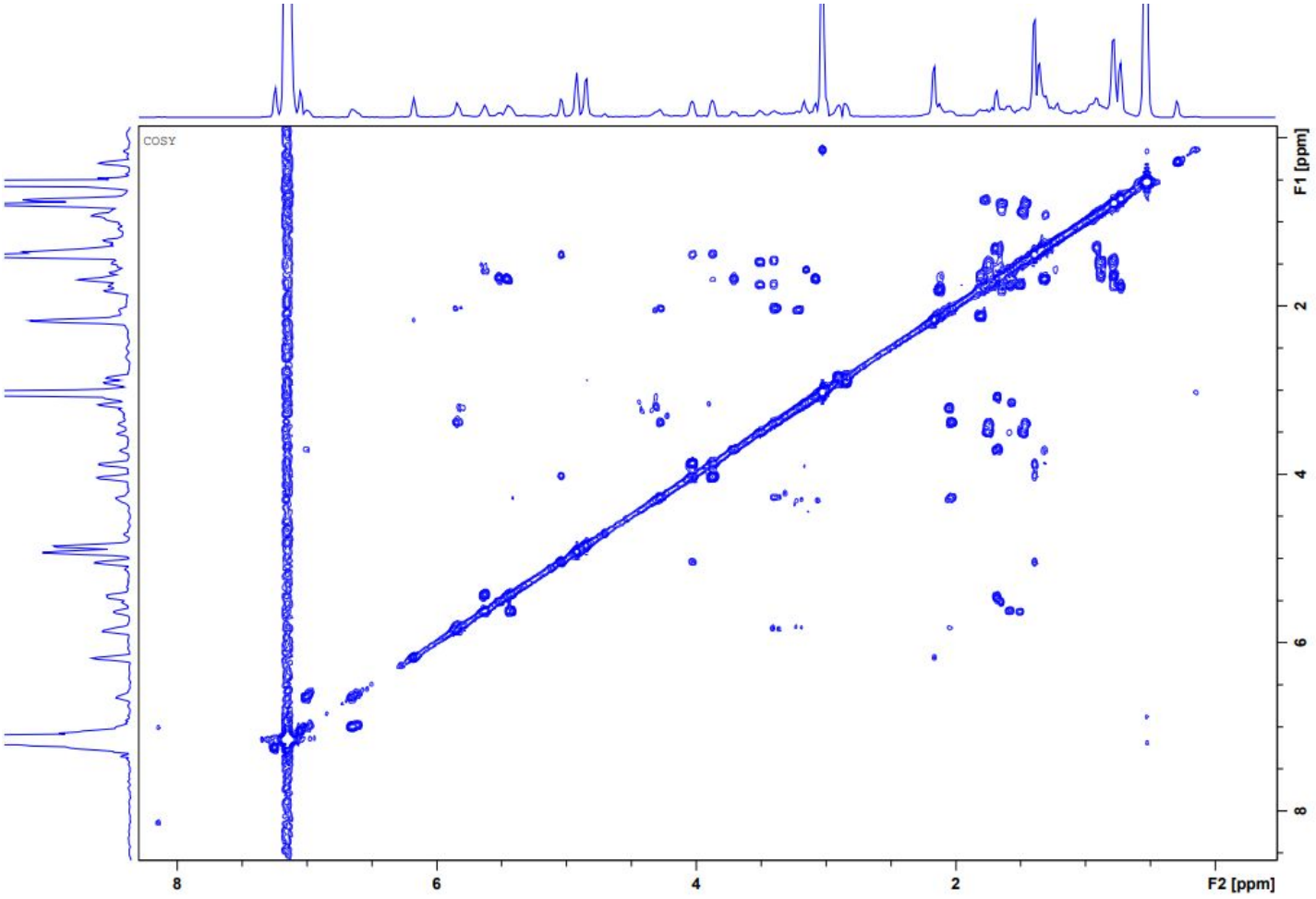


Figure S12. HSQC spectrum of Fijianolide L (**6**) in C_6D_6 (800 MHz)

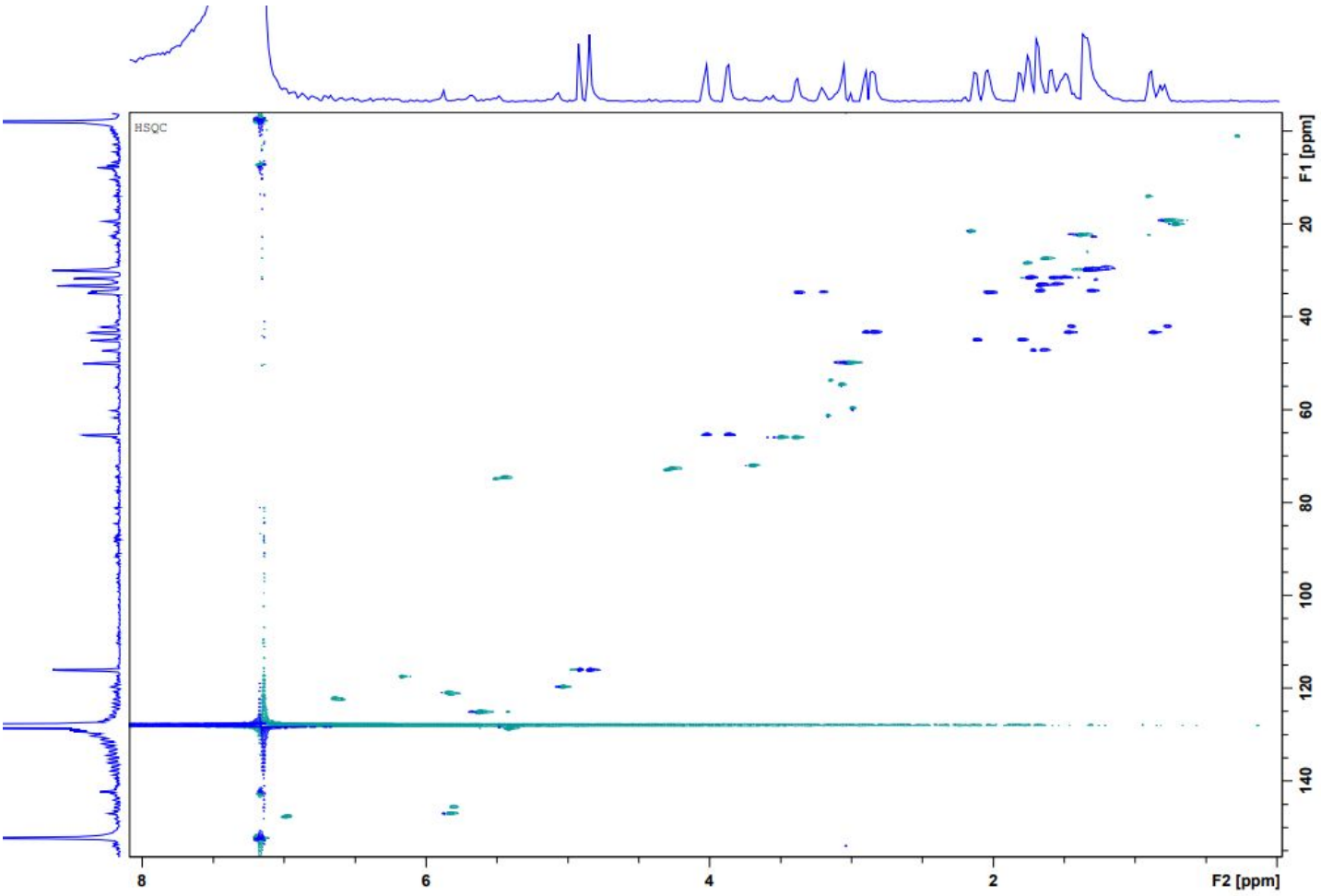


Figure S13. HMBC spectrum of Fijianolide L (**6**) in C_6D_6 (800 MHz)

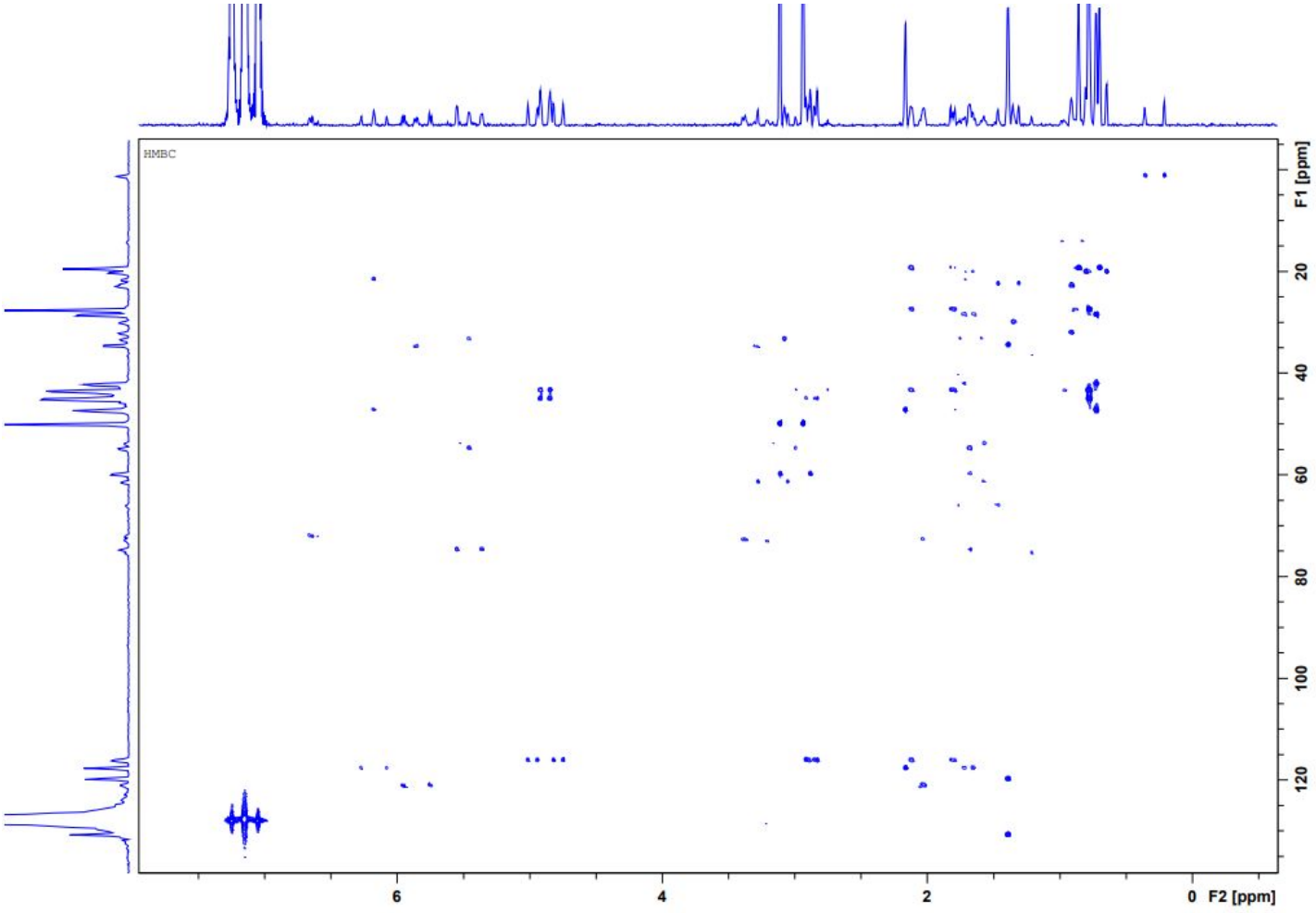


Figure S14. ESI-HAMS spectrum of Fijianolide B/Laulimalide (**3**)

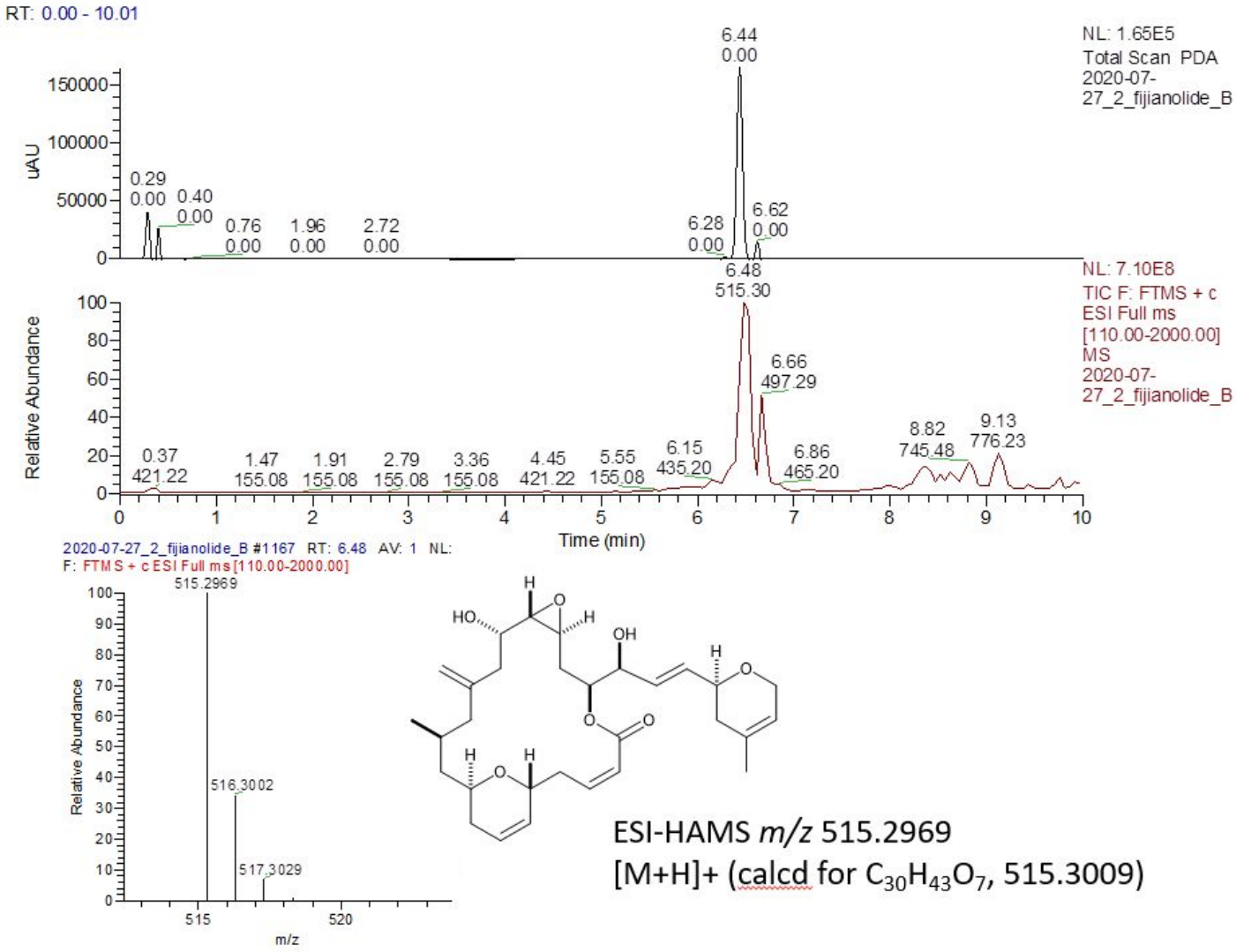


Figure S15. ESI-HAMS spectrum of Fijianolide J (**5**)

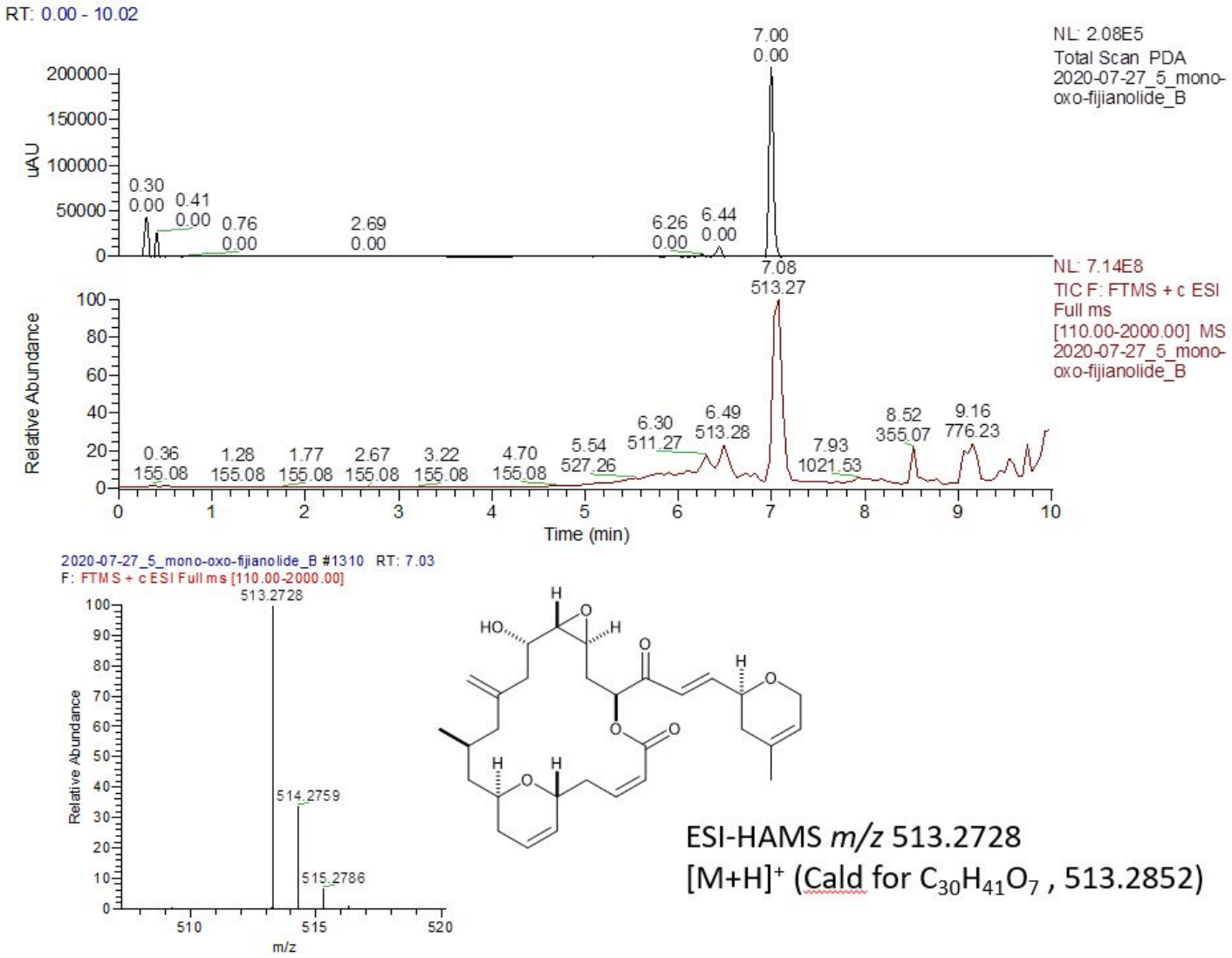
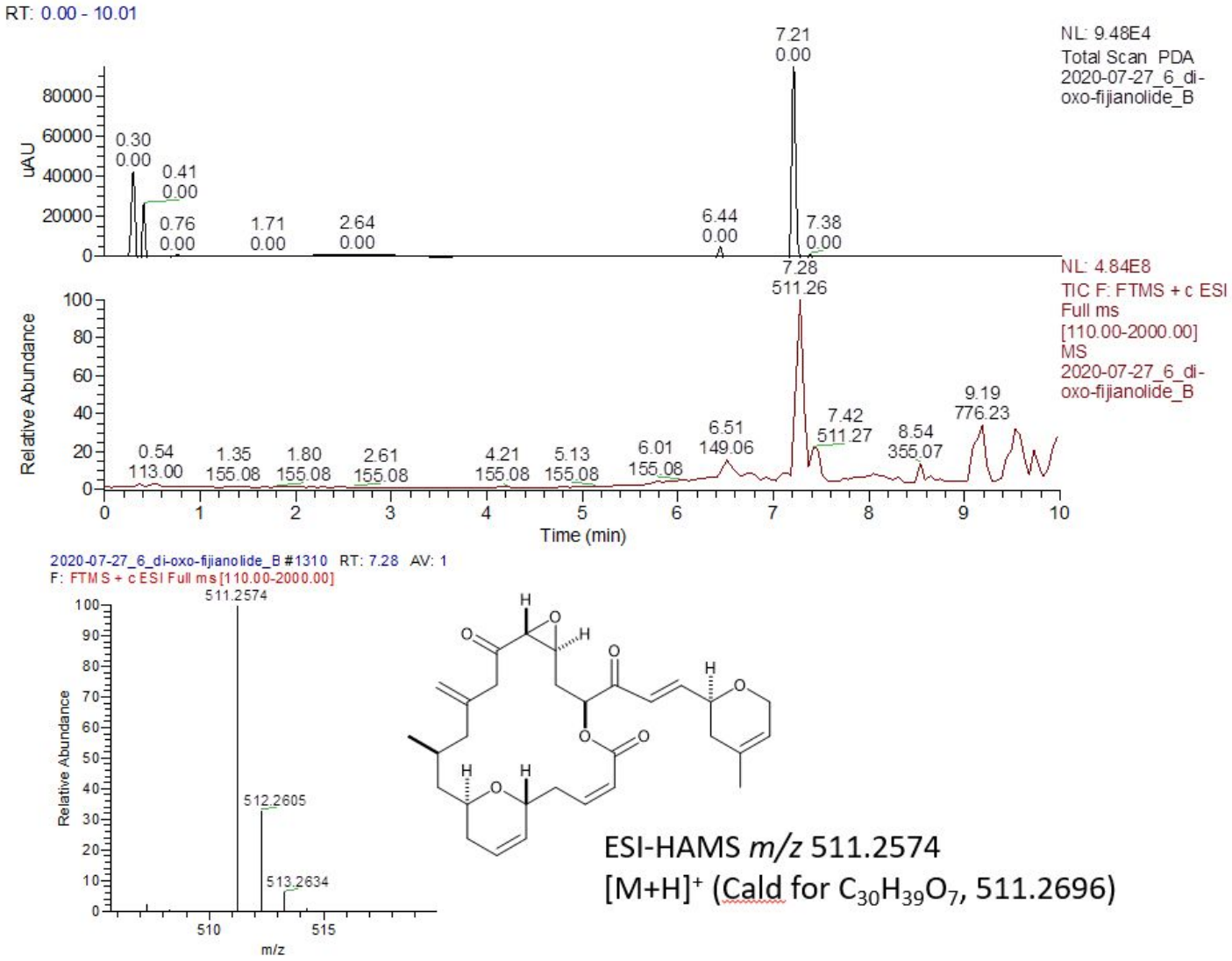


Figure S16. ESI-HAMS spectrum of Fijianolide L (**6**)



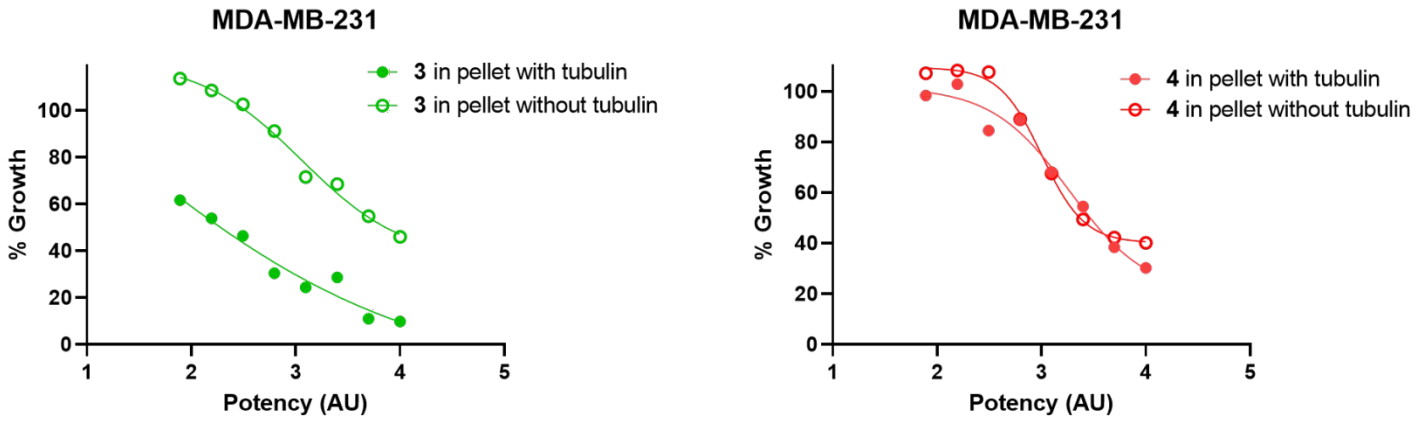


Figure S17. Cosedimentation of antiproliferative activity with purified tubulin. Compounds **3**, **4**, or **5** were incubated with purified tubulin protein at a 5-fold molar excess of drug (100 µM) to protein (20 µM) in GPEM buffer for one hour at 37˚C. Control reactions lacking tubulin protein were performed side-by-side. Microtubules were then pelleted from each reaction along with any microtubule-associated compound by centrifugation at 21,000g for 30 min at room temperature to avoid any microtubule depolymerization. The supernatant was removed and pellets resuspended in DMSO. To take into account the difference in potency of the original compounds, the pellets containing the less potent compounds **4** and **5** were resuspended in 10 µL DMSO while reactions with the most potent compound **3** were resuspended in 100 uL DMSO. Two-fold serial dilutions of these pellet fractions were prepared and 1 µL of each was added to MDA-MB-231 cells for 48 h. The resulting cellular growth over the 48 h treatment period was determined using the SRB assay and graphed as a percent of growth compared to the time of drug addition (y = 0) and vehicle-treated controls (y = 100). The resulting concentration response to growth inhibition was graphed as arbitrary units (AU) comparing the effects of compound in the pellet fraction with or without tubulin to provide an indication of whether a potent compound was able to specifically coprecipitate with purified tubulin. We found that a potent, antiproliferative compound specifically coprecipitated with the microtubule pellet from our stocks of **3**, but not **4** or **5**, demonstrating that the antiproliferative effects of the latter two compounds do not appear to be due to contamination with a small amount of **3**. No antiproliferative activity was observed in the undiluted pellet fraction of **5** with tubulin.