

Supporting Information

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Two New 13-oxomilbemycins from a NTG-Induced Mutation Strain of *Streptomyces avermitilis* AVE-H39

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Table S1: SciFinder Search Report for 1 (13-oxomilbemycin β_3)

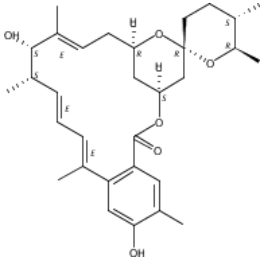
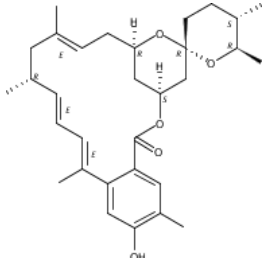
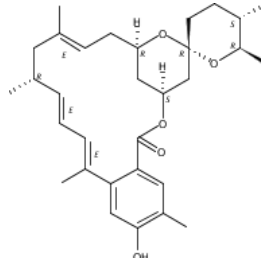
<p>Score: 96 1. 1884307-14-5</p>  <p>Absolute stereochemistry., Rotation (+)., Double bond geometry as shown.</p> <p>C₃₁ H₄₂ O₆ Milbemycin B, 2,5,6,7-tetrahydro-5-O-demethyl-7,28-dideoxy-13-hydroxy-25-methyl-, (13S,25R)-</p> <p>Key Physical Properties: Molecular Weight 510.66 Boiling Point (Predicted) Value: 708.1±60.0 °C Condition: Press: 760 Torr Density (Predicted) Value: 1.18±0.1 g/cm³ Condition: Temp: 20 °C Press: 760 Torr pKa (Predicted) Value: 8.37±0.70 Condition: Most Acidic Temp: 25 °C</p> <p>Related Info: ~ 1 References</p>	<p>Score: 95 2. 56198-39-1</p>  <p>Double bond geometry as shown., Absolute stereochemistry.</p> <p>C₃₁ H₄₂ O₅ Milbemycin B, 2,5,6,7-tetrahydro-5-O-demethyl-7,28-dideoxy-25-methyl-, (25R)-</p> <p>Key Physical Properties: Molecular Weight 494.66 Melting Point (Experimental) Value: 182-184 °C Condition: Solv: dichloromethane (75-09-2); hexane (110-54-3) Boiling Point (Predicted) Value: 674.3±55.0 °C Condition: Press: 760 Torr Density (Predicted) Value: 1.13±0.1 g/cm³ Condition: Temp: 20 °C Press: 760 Torr pKa (Predicted) Value: 8.38±0.70 Condition: Most Acidic Temp: 25 °C</p> <p>Related Info: ~ 40 References Reactions</p> <p>Experimental Properties</p>	<p>Score: 95 3. 82079-52-5</p>  <p>Double bond geometry as shown., Relative stereochemistry.</p> <p>C₃₁ H₄₂ O₅ Milbemycin B, 2,5,6,7-tetrahydro-5-O-demethyl-7,28-dideoxy-25-methyl-, (25R)-(±)- (9CI)</p> <p>Key Physical Properties: Molecular Weight 494.66 Boiling Point (Predicted) Value: 674.3±55.0 °C Condition: Press: 760 Torr Density (Predicted) Value: 1.13±0.1 g/cm³ Condition: Temp: 20 °C Press: 760 Torr pKa (Predicted) Value: 8.38±0.70 Condition: Most Acidic Temp: 25 °C</p> <p>Related Info: ~ 5 References Spectra</p>
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Table S2: SciFinder Search Report for **2** (25-ethyl-13-oxomilbemycin β_3)

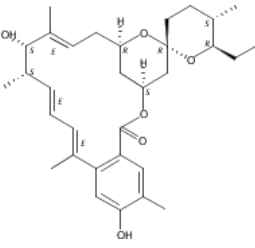
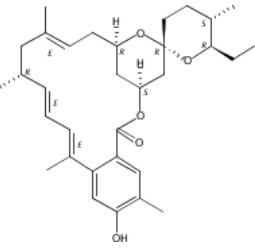
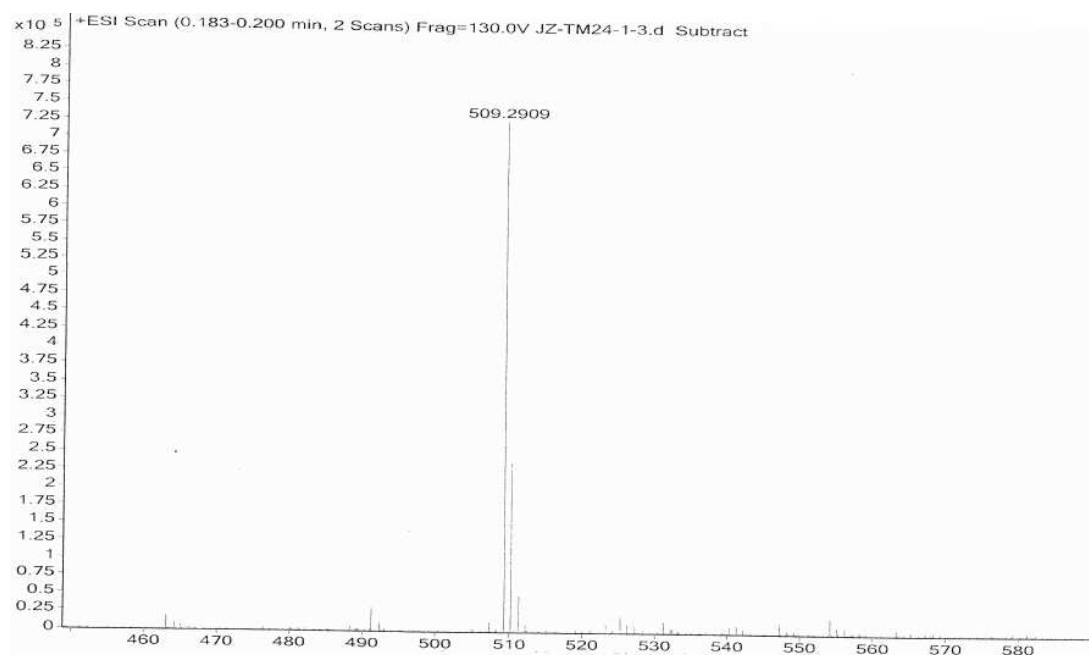
<p>Score: 96</p> <p>1.</p> <p>1884307-13-4</p>  <p>Absolute stereochemistry., Rotation (+)., Double bond geometry as shown.</p> <p>C₃₂H₄₄O₆ Milbemycin B, 2,5,6,7-tetrahydro-5-O-demethyl-7,28-dideoxy-25-ethyl-13-hydroxy-, (13S,25R)-</p> <p>Key Physical Properties:</p> <p>Molecular Weight 524.69</p> <p>Boiling Point (Predicted) Value: 715.8±60.0 °C Condition: Press: 760 Torr</p> <p>Density (Predicted) Value: 1.16±0.1 g/cm³ Condition: Temp: 20 °C Press: 760 Torr</p> <p>pKa (Predicted) Value: 8.37±0.70 Condition: Most Acidic Temp: 25 °C</p> <p>Related Info: ~ 1 References</p>	<p>Score: 95</p> <p>2.</p> <p>948576-51-0</p>  <p>Double bond geometry as shown., Absolute stereochemistry.</p> <p>C₃₂H₄₄O₅ Milbemycin B, 2,5,6,7-tetrahydro-5-O-demethyl-7,28-dideoxy-25-ethyl-, (25R)-</p> <p>Key Physical Properties:</p> <p>Molecular Weight 508.69</p> <p>Boiling Point (Predicted) Value: 682.9±55.0 °C Condition: Press: 760 Torr</p> <p>Density (Predicted) Value: 1.12±0.1 g/cm³ Condition: Temp: 20 °C Press: 760 Torr</p> <p>pKa (Predicted) Value: 8.38±0.70 Condition: Most Acidic Temp: 25 °C</p> <p>Related Info: ~ 2 References Reactions</p>
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Table S3: ^1H and ^{13}C -NMR Data for 13-oxomilbemycin β_3 (**1**) and 13 α -hydroxy milbemycin β_3

position	δ_{H} (mult., J in Hz)		δ_{C} (ppm)	
	1	13 α -hydroxy milbemycin β_3	1	13 α -hydroxy milbemycin β_3
1			168.4	169.4
2			122.8	123.5
3	7.50 (s)	7.34 (s)	132.8	131.9
4			123.3	122.8
5			156.2	156.1
6	6.59 (s)	6.63 (s)	114.4	114.2
7			144.8	144.1
8			137.7	134.9
9	5.68 (d, 10.9)	5.69 (d, 10.8)	126.9	128.5
10	6.46 (dd, 15.0, 10.9)	6.16 (dd, 15.2, 10.8)	128.9	127.3
11	5.41 (dd, 15.0, 9.5)	5.43 (dd, 15.2, 9.5)	135.5	134.3
12	3.93 (m)	2.61 (m)	46.0	40.5
13		4.02 (br s)	202.1	79.0
14			137.7	137.8
15	6.71 (t, 7.5)	5.24 (d, 10.4)	139.1	117.9
16	2.38 (m)	2.38 (m)	33.6	33.4
	2.64 (m)	2.32 (m)		
17	3.83 (m)	3.76 (m)	65.7	67.6
18	1.18 (m)	0.77 (q, 11.8)	35.3	36.7
	1.96 (m)	1.97 (m)		
19	5.38 (m)	5.47 (m)	68.4	68.0
20	1.41 (t, 11.9)	1.42 (t, 12.0)	40.8	41.2
	2.03 (m)	1.97 (m)		
21			97.7	97.8
22	1.55 (m)	1.55 (m)	35.7	35.8
	1.70 (m)	1.67 (m)		
23	1.55 (m)	1.54 (m)	27.7	27.8
24	1.27 (m)	1.26 (m)	36.5	36.6
25	3.27 (m)	3.31 (m)	71.5	71.3
26	2.25 (s)	2.22 (s)	15.3	15.4
27	2.12 (d, 0.9)	2.06 (s)	19.3	18.1
28	1.22 (d, 6.7)	1.21 (d, 6.8)	16.5	18.3
29	1.81 (br s)	1.64 (br s)	12.2	15.1
30	0.85 (d, 6.5)	0.84 (d, 6.5)	17.9	17.9
31	1.14 (d, 6.2)	1.14 (d, 6.2)	19.3	19.4

Table S4: ^1H and ^{13}C -NMR Data for 25-ethyl-13-oxomilbemycin β_3 (**2**) and 13 α -hydroxy-25-ethyl milbemycin β_3

position	δ_{H} (mult., J in Hz)		δ_{C} (ppm)	
	2	13 α -hydroxy-25-ethyl milbemycin β_3	2	13 α -hydroxy-25-ethyl milbemycin β_3
1			168.4	170.0
2			122.8	121.6
3	7.49 (s)	7.32 (s)	132.9	131.6
4			123.2	123.0
5			156.2	157.6
6	6.60 (s)	6.60 (s)	114.5	113.5
7			144.8	144.5
8			137.7	134.6
9	5.67 (d, 10.9)	5.60 (d, 10.7)	126.8	127.9
10	6.45 (dd, 15.0, 10.9)	6.22 (dd, 15.1, 10.7)	129.0	127.0
11	5.41 (dd, 15.0, 9.8)	5.45 (dd, 15.1, 10.0)	135.5	134.6
12	3.94 (m)	2.62 (m)	46.0	40.7
13		3.97 (br s)	202.1	78.6
14			137.7	138.1
15	6.73 (t, 7.2)	5.30 (d, 8.6)	139.1	117.4
16	2.37 (m)	2.33 (m)	33.6	33.0
17	2.65 (m)			
17	3.84 (m)	3.77 (m)	65.8	67.9
18	1.19 (m)	0.79 (q, 12.2)	35.5	36.6
18	1.95 (m)	2.00 (m)		
19	5.38 (m)	5.40 (m)	68.5	68.0
20	1.41 (t, 11.9)	1.37 (t, 12.0)	41.0	41.2
20	2.04 (m)	1.93 (m)		
21			97.6	97.5
22	1.53 (m)	1.54 (m)	35.6	35.3
22	1.69 (m)	1.66 (m)		
23	1.53 (m)	1.54 (m)	27.9	27.6
24	1.33 (m)	1.32 (m)	34.3	34.3
25	3.06 (m)	3.14 (m)	76.3	76.1
26	2.24 (s)	2.17 (s)	15.3	14.3
27	2.11 (br s)	2.06 (s)	19.2	17.1
28	1.22 (d, 6.6)	1.18 (d, 6.6)	16.5	17.7
29	1.80 (br s)	1.66 (br s)	12.1	13.9
30	0.84 (d, 6.5)	0.85 (d, 6.4)	17.8	16.7
31	1.33 (m)	1.35 (m)	25.7	25.5
31	1.69 (m)	1.74 (m)		
32	0.96 (t, 7.3)	1.01 (t, 7.3)	10.2	9.2



Scan	Retention Time (min)	Mass (m/z)	Abundance	Charge	Formula	Mass (m/z)	Abundance	Charge	Formula
MFG	509.2909	C31 H40 O6	(M+H) ⁺	97.42	-2.04	97.42	508.2825	12	
(M+H) ⁺	509.2909	98.7	95.53			97.42	97.42	99.66	725733.2 C31 H41 O6
	719460.3	70.3	100			509.2898	-1.1	725733.2	100 71 509.2909 -2.15
	246263.1	24.1	34.2			510.2932	-0.8	237552.2	32.7 23.2 510.2939 -1.54
	49712.4	4.9	6.9			511.2961	-1.2	50181.2	6.9 4.9 511.2973 -2.28
	7404.5	0.7	1			512.2989	-2.4	9373.8	1.3 0.9 512.3013 -4.59
MFG	509.2909	C24 H44 O11	(M+H) ⁺	65.38	9.46	65.38	508.2884	3	
MFG	507.2762	C24 H42 O11	(M+H) ⁺	57.69	6.06	57.69	506.2727	4	
MFG	507.2762	C31 H38 O6	(M+H) ⁺	54.67	-5.55	54.67	506.2668	13	

Figure S1: HR-ESI-MS Spectrum of 1 (13-oxomilbemycin β_3)

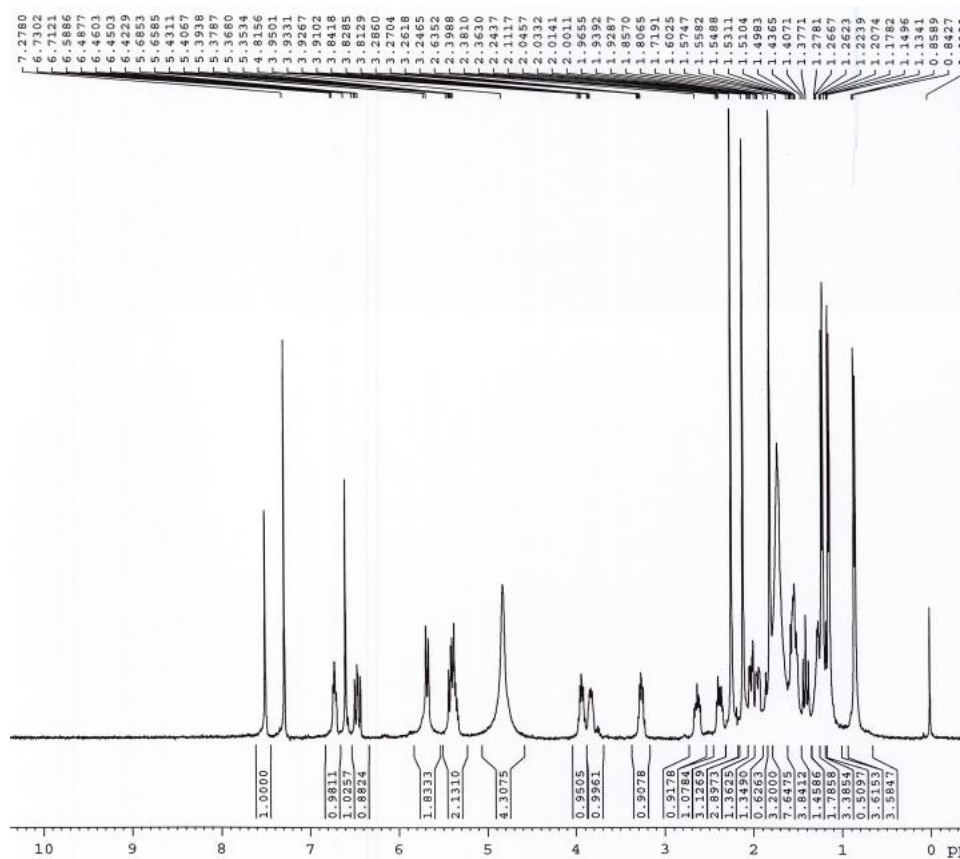


Figure S2: $^1\text{H-NMR}$ (400 MHz, CDCl_3) Spectrum of **1** (13-oxomilbemycin β_3)

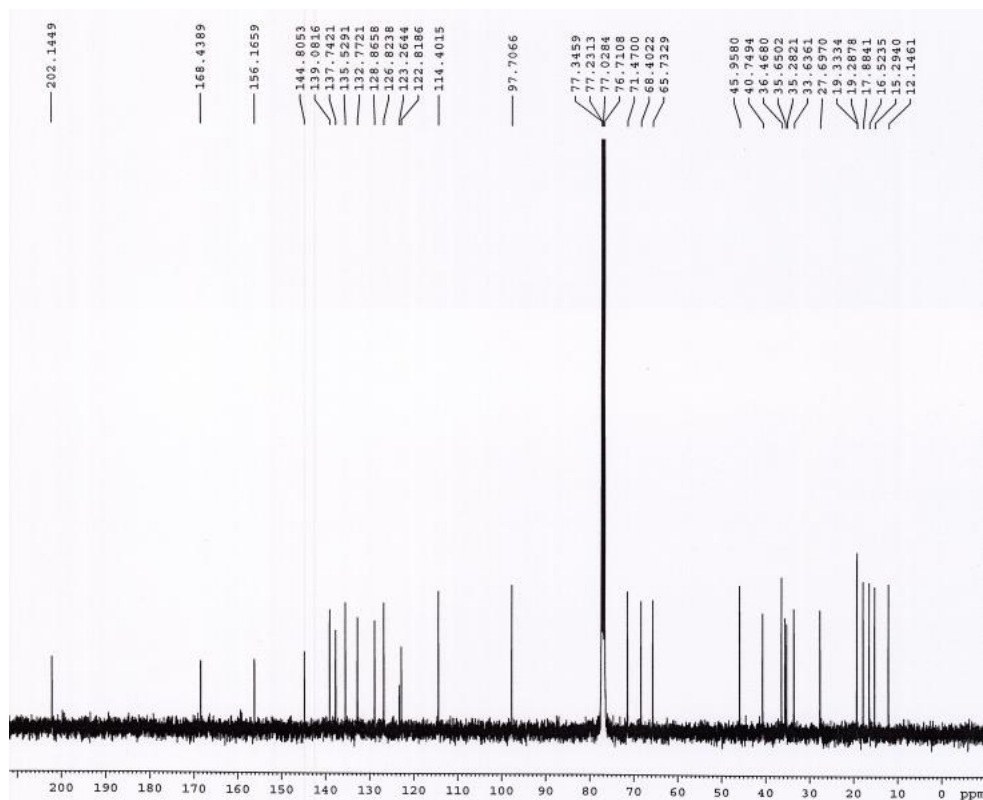


Figure S3: $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) Spectrum of **1** (13-oxomilbemycin β_3)

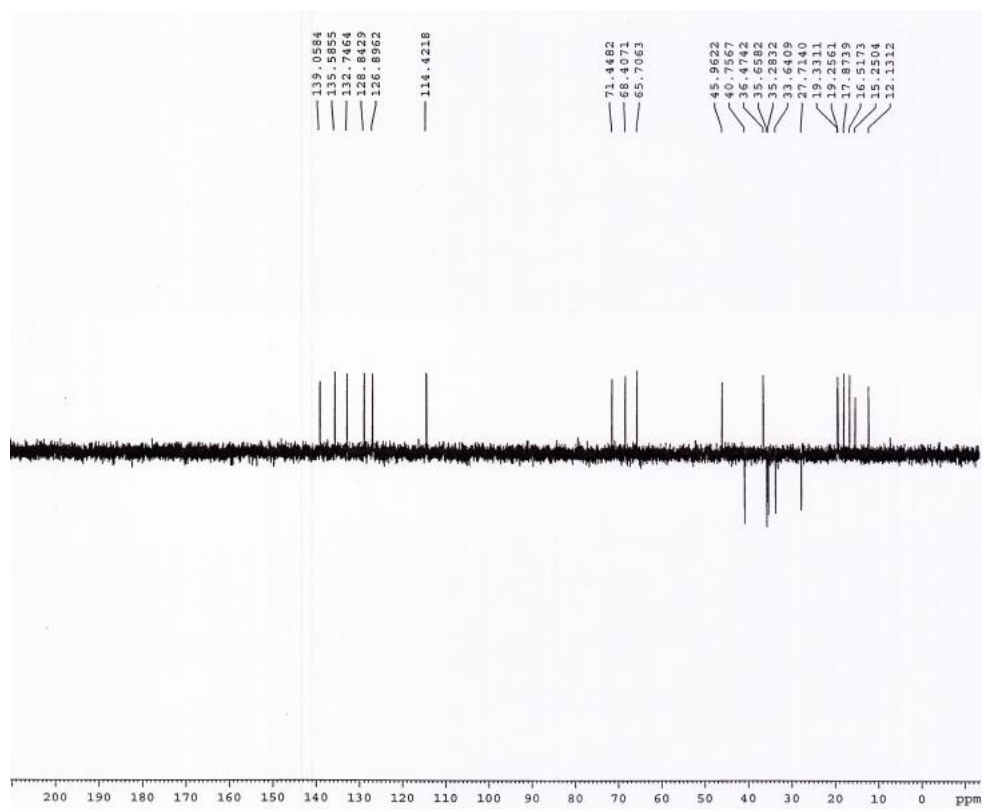


Figure S4: DEPT135 (100 MHz, CDCl₃) Spectrum of **1** (13-oxomilbemycin β₃)

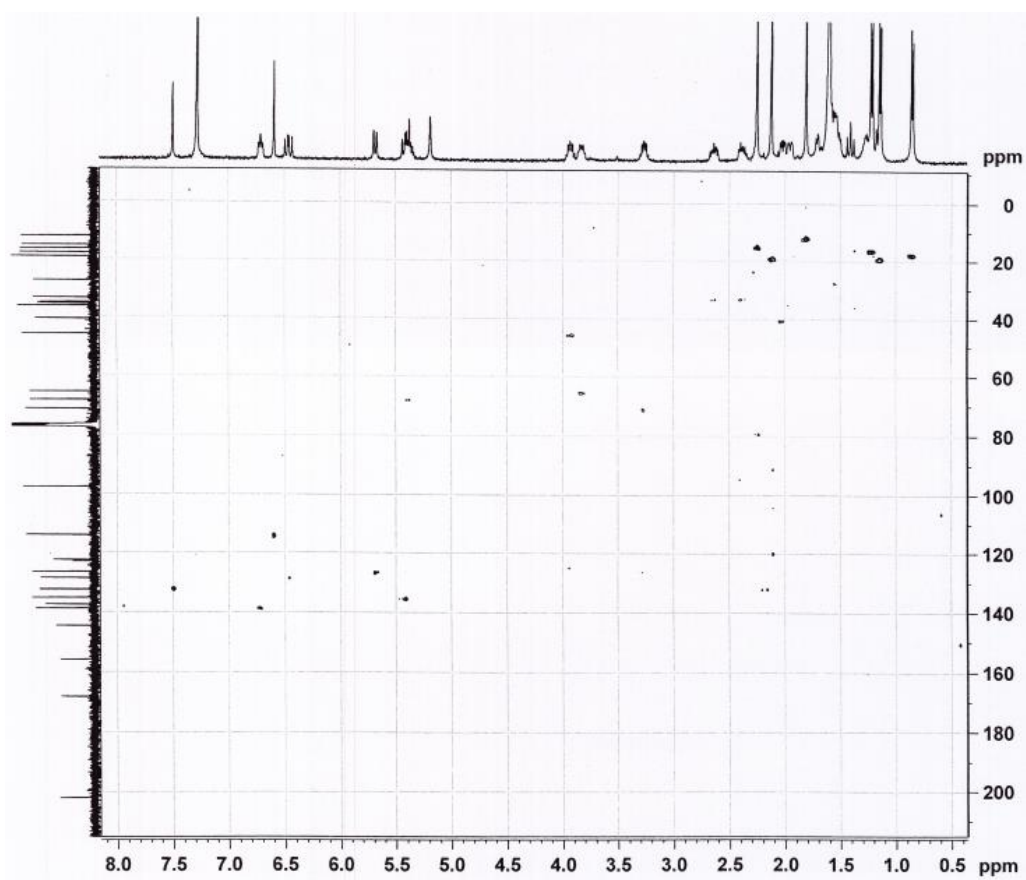


Figure S5: HMQC Spectrum of 1 (13-oxomilbemycin β_3)

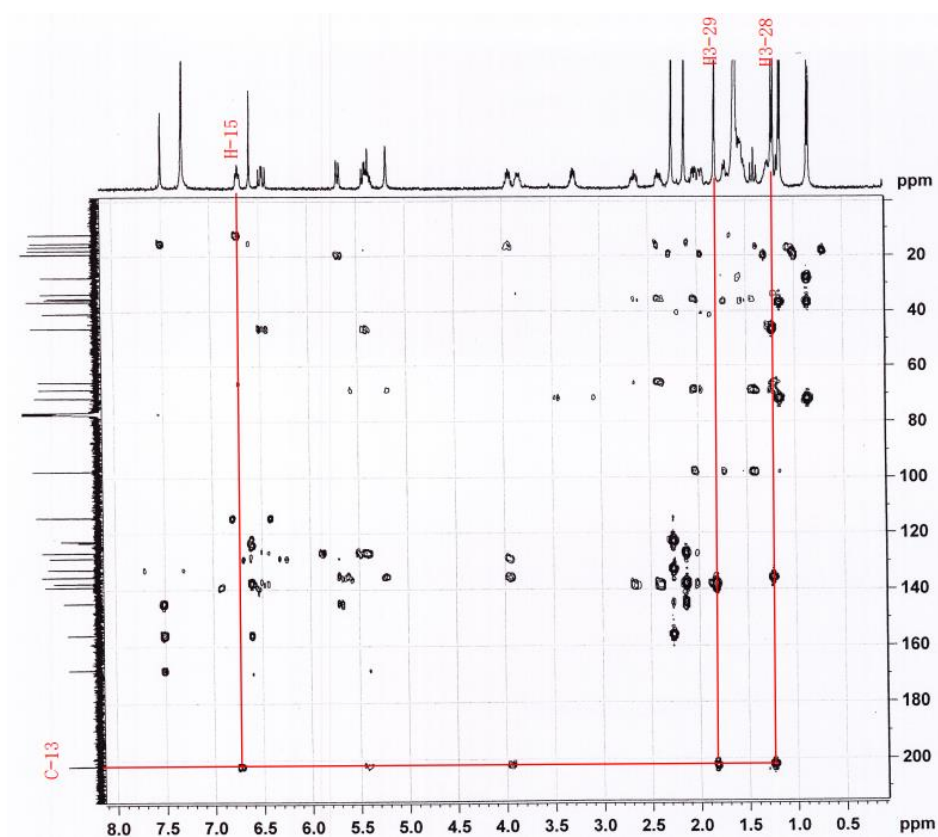


Figure S6: HMBC Spectrum of 1 (13-oxomilbemycin β_3)

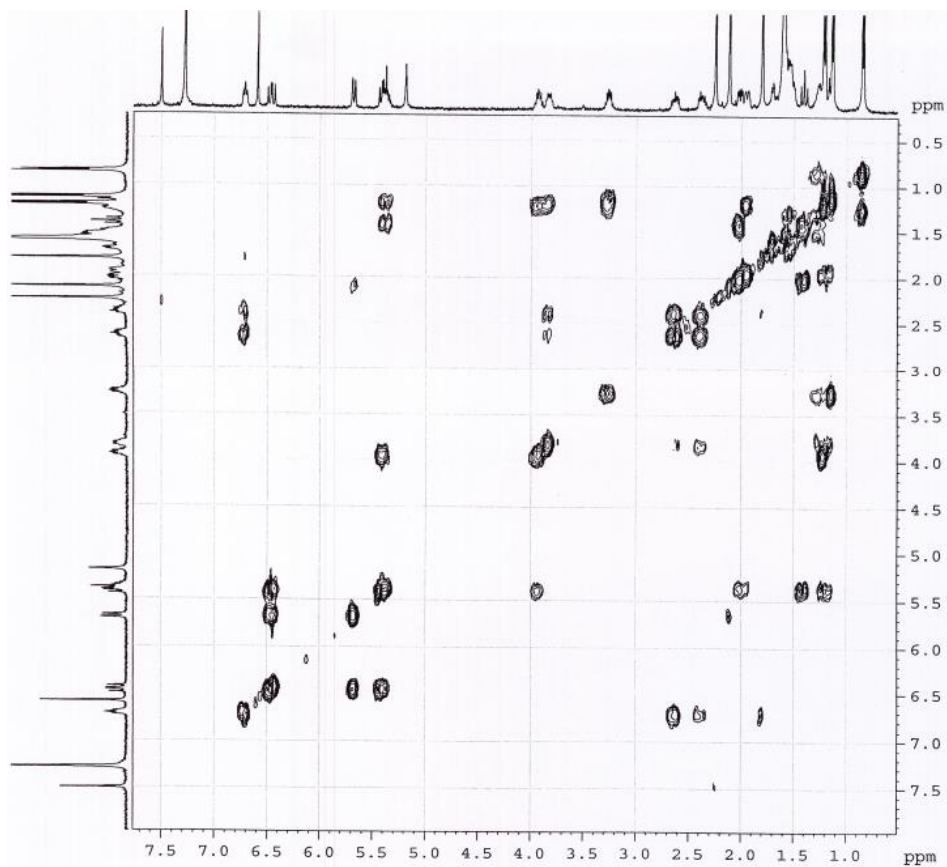
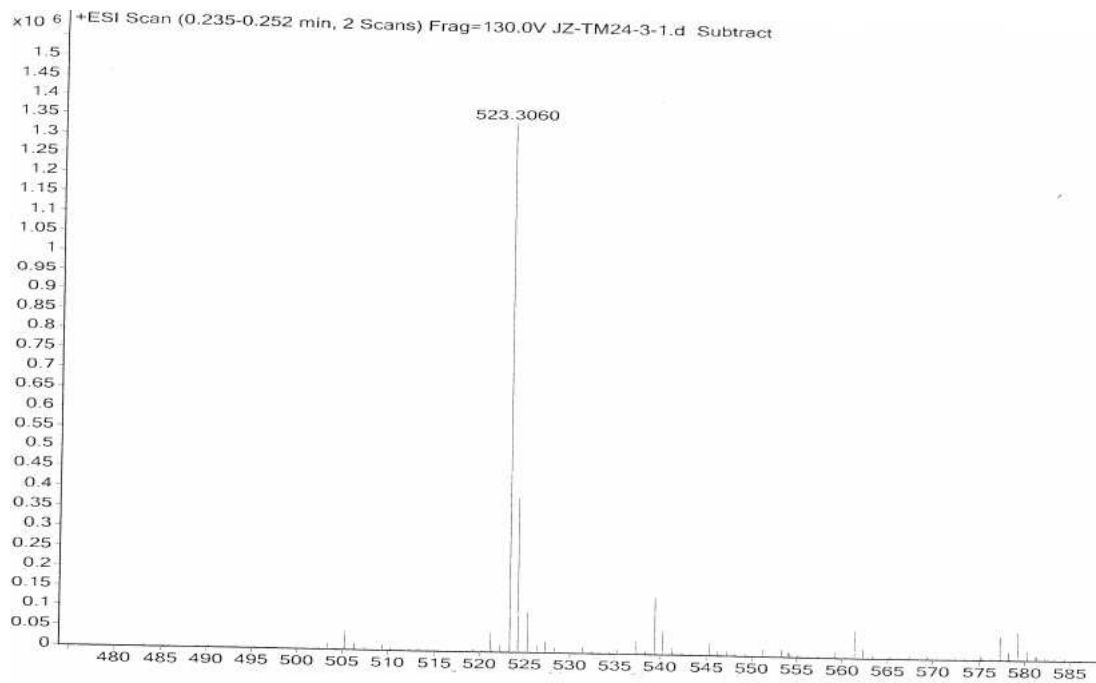


Figure S7: ^1H - ^1H COSY Spectrum of **1** (13-oxomilbemycin β_3)



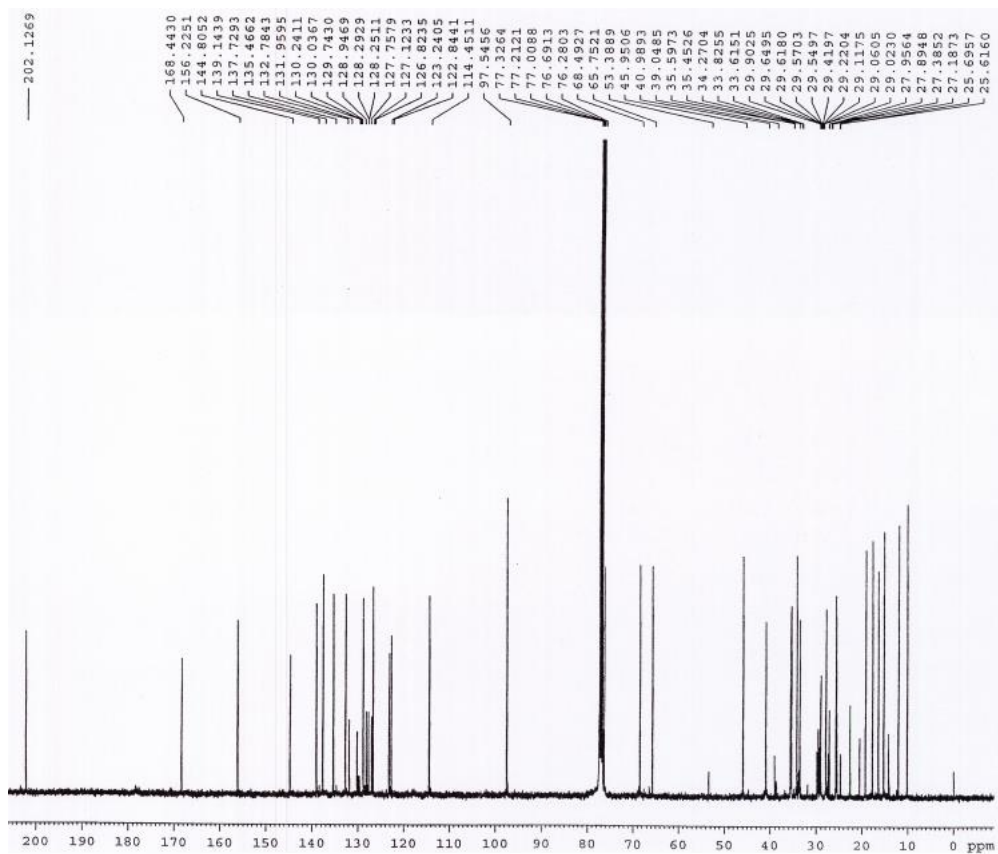


Figure S10: ^{13}C -NMR (100 MHz, CDCl_3) Spectrum of **2** (25-ethyl-13-oxomilbemycin β_3)

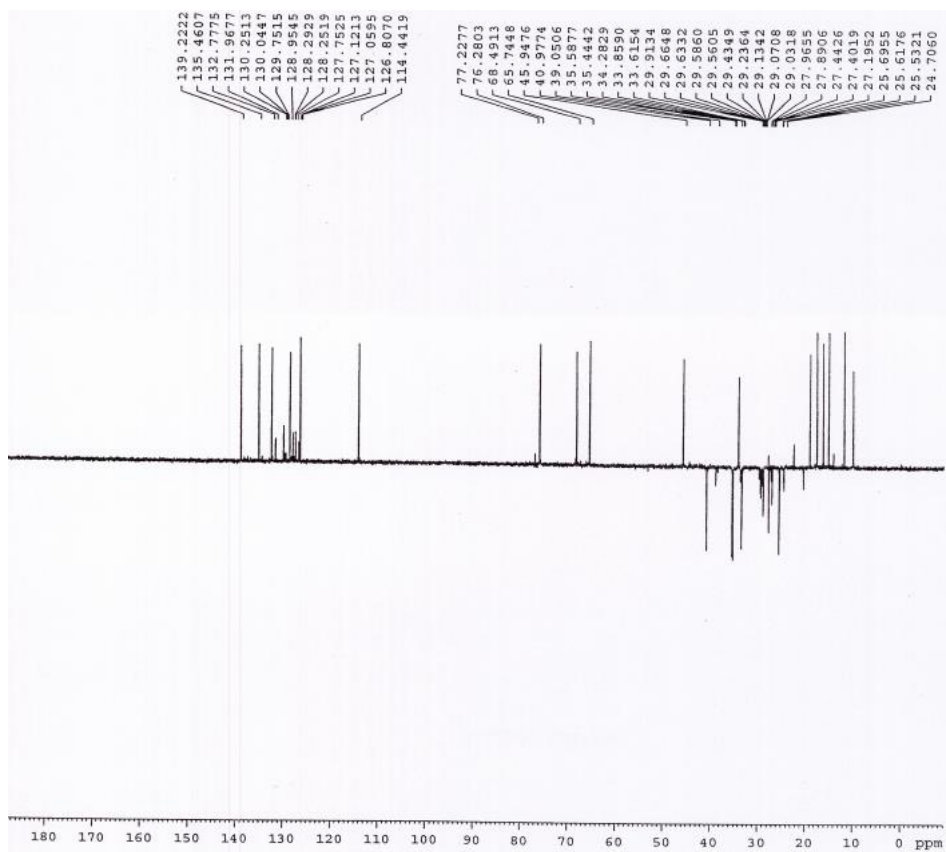


Figure S11: DEPT135 (100 MHz, CDCl₃) Spectrum of **2** (25-ethyl-13-oxomilbemycin β₃)

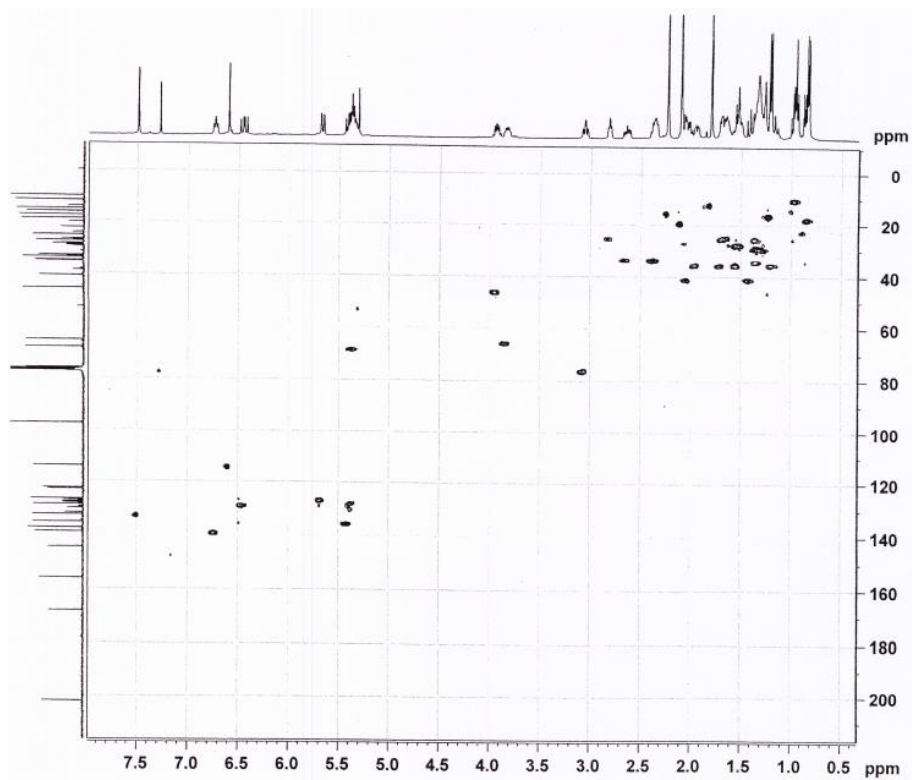


Figure S12: HMQC Spectrum of **2** (25-ethyl-13-oxomilbemycin β₃)

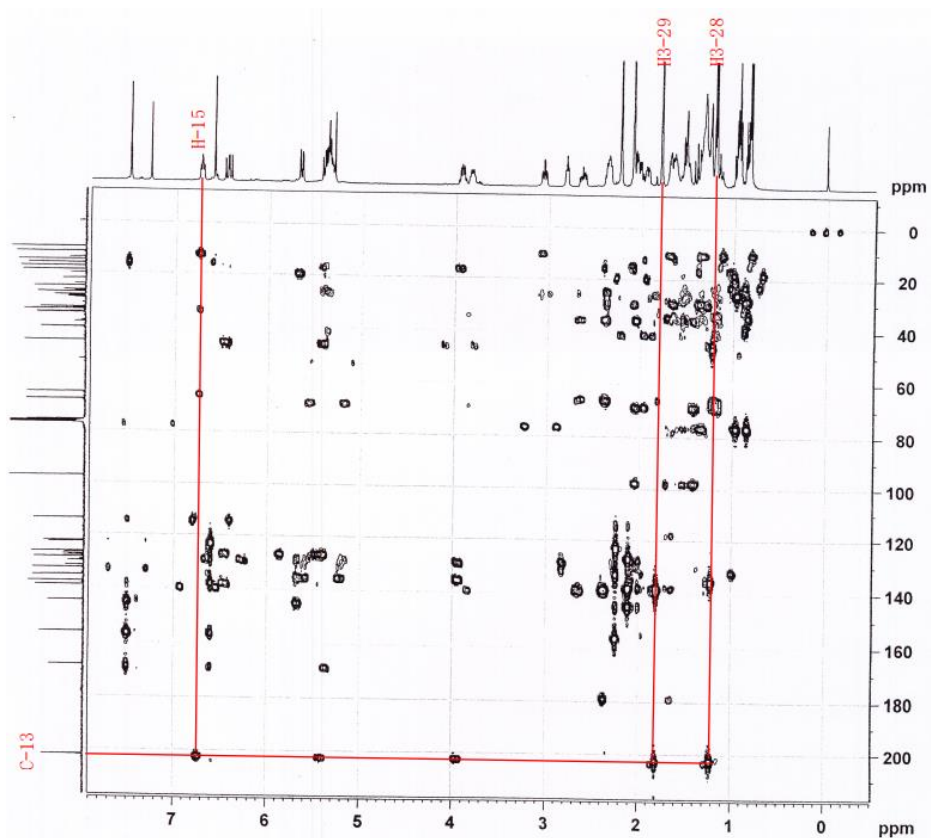


Figure S13: HMBC Spectrum of **2** (25-ethyl-13-oxomilbemycin β_3)

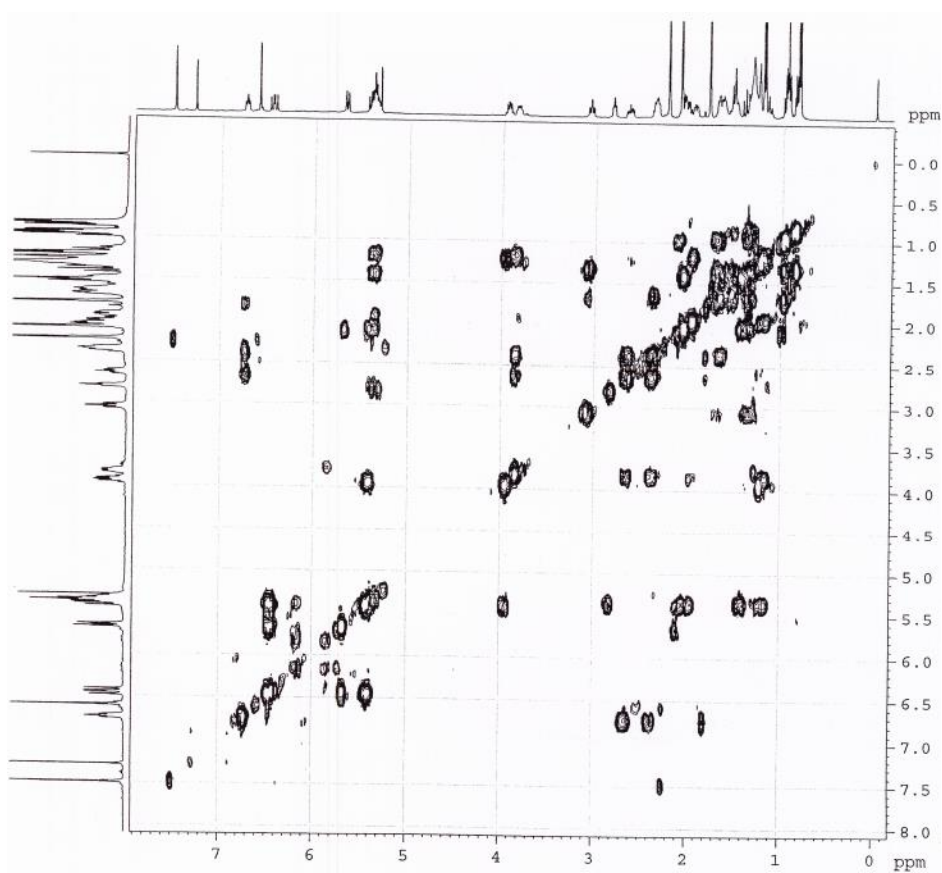


Figure S14: ^1H - ^1H COSY Spectrum of **2** (25-ethyl-13-oxomilbemycin β_3)