

Supporting Information

for

Lanyamycin, a macrolide antibiotic from *Sorangium cellulosum*, strain Soce 481 (Myxobacteria)

Lucky S. Mulwa^{1,2}, Rolf Jansen¹, Dimas F. Praditya³, Kathrin I. Mohr^{1,2}, Patrick W. Okanya⁴, Joachim Wink², Eike Steinmann^{3,5} and Marc Stadler^{1,*§}

Address: ¹Department of Microbial Drugs, Helmholtz Centre for Infection Research and German Centre for Infection Research (DZIF), partner site Hannover/Braunschweig, Inhoffenstrasse 7, 38124 Braunschweig, Germany, ²Work group Microbial Strain Collection (MISG), Helmholtz Centre for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany, ³TWINCORE-Centre for Experimental and Clinical Infection Research (Institute of Experimental Virology) Hanover, Feodor-Lynen-Str. 7–9, 30625 Hannover, Germany. Tel: +49(0)511-220027-133, ⁴Department of Biochemistry and Biotechnology, The Technical University of Kenya, P.O. Box 52428 – 00200, Located along Haile Selassie Avenue, Nairobi, Kenya. Tel: +254(020) 2219929 and ⁵Department of Molecular and Medical Virology, Ruhr-University Bochum, 44801 Bochum, Germany

* Corresponding author

Email: Marc Stadler - marc.stadler@helmholtz-hzi.de

§Tel.: +49 531 6181-4240; Fax: +49 531 6181 9499

Tables of NMR data and figures of the ¹H and ¹³C NMR including the HCV infectivity results showing viability

Table S1. NMR data of lanyamycin 1 in CD₃OD (¹H/¹³C NMR at 500.3/125.8 MHz)

Pos.	δ_c	type	δ_H	H Mult. (<i>J</i> [Hz])	H in COSY	H in N/ROESY	H to C HMBC
1	166.06	C=O					15, 3
2	143.04	C					36, 3
3	133.63	CH	6.53	br s	5	5 >>37, 42	37, 5
4	132.61	C					6
5	147.62	CH	5.38	d (9.9)	37, 6, 3	3 >7 >38, 8 >10	38, 37, 6, 7, 3
6	39.67	CH	2.39	tq (10.0, 6.6)	38, 7, 5	37, (38) >8, 39	38
7	77.22	CH	3.38	br d (10.1)	6	5, 10 >9a, (8), 38 >11	39, 38, 9, 9, 6, 5
8	42.99	CH	1.48	m (9.8, 7.0, 3.1)	39, 9a, 9b	(39) >5, 6, (7) >11	39, 9, 9, 7
9a	38.78	CH ₂	2.03	m ^c	8, 9b, 10	39, 38, 9, 7, 11, 10	39, 7, 11
9b			1.88	dd (14.5, 6.4)	8, 9a, 10	39, 8, 9, 11, 10	39, 7, 11
10	138.29	CH	6.16	ddd (15.3, 9.5, 5.7)	9a, 9b, 11	8, 9, 9, 7, 5, 12	11, 12
11	132.75	CH	6.01	dd (15.1, 10.4)	10, 12	8, 9, 9, 7, 13	13, 10
12	139.79	CH	6.50	dd (15.2, 10.6)	13, 11	40, 14, 10	14, 11, 10
13	128.95	CH	5.17	dd (15.1, 9.3)	14, 12	40, 14, 11	11
14	85.75	CH	3.76	t (9.5)	15, 13	41, 16, 16, 40, 15, 13, 12, 17	40, 12
15	75.84	CH	4.81	ddd (9.8, 6.1, 4.1)	16a, 16b, 14	16, 16, 14, 17	16, 14
16a	33.21	CH ₂	2.86	m ^a	41, 16b, 15, 17	41, 14, 15, 17	14
16b			2.80	m ^b	41, 16a, 15, 17	41, 14, 15	14
17	140.25	CH	6.85	br t (7.0)	41, 16a, 16b	42, 20, 16, 20, 14, 21, 15	41, 16
18	140.58	C					41, 20
19	202.54	C=O					41, 20, 20, 17
20a	43.47	CH ₂	2.98	dd (15.5, 2.8)	20, 21	42, 20, 21, 17	22
20b			2.77	dd (15.6, 9.3)	20, 21	42, 24, 20, 17	22
21	72.40	CH	4.24	ddd (9.3, 8.0, 2.7)	20a, 20b, 22	42, 20, 17	42, 20, 22
22	51.55	CH	3.07	dq (8.0, 6.9) ^b	42, 21	43, 42, 24, 25	42, 20
23	217.46	C=O					43, 42, 24, 22
24	51.00	CH	2.89	qd (7.0, 5.5) ^a	43, 25	43, 42, 20, 22, 25, 26, 35, 31	43, 25
25	74.32	CH	3.89	t (5.5)	24, 26	43, 42, 24, 22, 26	43, 24
26	75.08	CH	5.19	br d (5.8)	25	43, 42, 24, 25	25
27	171.87	C=O					44, 28, 26
28	58.25	CH	4.29	dd (7.6, 6.6)	44a, 44b	45, 35, 31	45, 44, 44
29	198.10	C=O					44, 44, 28, 35, 31
30	138.12	C					34, 32
33	134.77	CH	7.60	tt (7.4, 1.2)	34, 32, 35, 31		35, 31
34, 32	130.10	2 CH	7.50	br t (7.8)	33, 35, 31	35, 31	
35, 31	129.55	2 CH	7.99	br d (7.5)	34, 32, 33	24, 28, 34, 32	33
36	60.79	OCH ₃	3.59	s		42, 37	
37	14.56	CH ₃	1.91	s	5	38, 6, 36, 3	5, 3
38	17.05	CH ₃	1.01	br d (6.6)	6	37, 9, 6, 7, 5	37, 6, 7
39	15.57	CH ₃	0.89	d (7.0)	8	8, 9, 9, 6, 7	7
40	56.65	OCH ₃	3.27	s		41, 14, 13, 12	14
41	11.74	CH ₃	1.79	s	16a, 16b, 17	16, 16, 40, 14	17
42	13.84	CH ₃	1.05	d (6.9)	22	20, 24, 20, 22, 36, 25, 21, 26, 3, 17	22
43	10.512	CH ₃	0.92	d (7.0)	24	24, 22, 25, 26	24, 25
44a	24.28	CH ₂	2.01	m ^{c, d}	45, 28		45, 28
44b			1.95	m ^d	45		45, 28
45	12.55	CH ₃	1.03	t (7.5)	44a, 44b	28	44, 44, 28

^{a, b, c, d} overlapping ¹H signals

Table S2. NMR data of lanyamycin **2** in CD₃OD (¹H/¹³C NMR at 700.44/176.14 MHz)

Pos.	δ _C	XHn	δ _H	H Mult. (J [Hz])	H in COSY	H in N/ROESY	H to C HMBC
1	166.03	C					3
2	143.07	C					36, 3
3	133.57	CH	6.55	d (0.7)	-	5	37, 5
4	132.62	C					37
5	147.55	CH	5.39	br d (10.1)	6 >37	3, 7 >10, 38, 6, 8	38, 37, 6, 7, 3
6	39.67	CH	2.40	tq (10.0, 6.6)	38, 7, 5	37 >39, 5, 8	38, 5
7	77.24	CH	3.39	br d (10.5)	6	5, 10 >9a, 8, 38, 6	39, 38, 9, 9, 6, 5
8	42.99	CH	1.50	dqd (9.9, 6.8, 3.0)	39, 9a	39, 7 >6, 10, 11 >5	39, 9, 9, 7
9a	38.78	CH ₂	2.05	m ^c	8, 9b, 10	39 >7, 10, 11	39, 8, 7, 11, 10
9b			1.89	ddd (12.4, 9.9, 2.0)	9a, 10	39 >11, 10	39, 8, 7, 11, 10
10	138.26	CH	6.17	ddd (15.3, 9.8, 5.7)	9b, 11 >9a	7, 12 >5 >8, 9a	9, 9, 12
11	132.78	CH	6.03	dd (15.3, 10.5)	10, 12	13 >9b	9, 9, 13, 12
12	139.76	CH	6.51	dd (15.2, 10.4)	13, 11	14, 10 >40	14, 11, 10
13	128.99	CH	5.20	br dd (15.1, 9.5)	12 >14	11 >14, 40	11
14	85.77	CH	3.77	t (9.5)	15, 13	40 >12, 16a, 16b, 17	16, 40, 15, 12
15	75.84	CH	4.83	ddd (10.1, 6.5, 3.7)	14 >16a, 16b	16a, 16b, 14, 17	16, 16, 14, 13, 17
16a	33.20	CH ₂	2.88	m ^a	16, 15, 17	15, 41, 14, 17	14, 17
16b			2.81	ddd (15.7, 8.8, 6.9) ^b	16, 15, 17	41, 14, 17, 15	14, 17
17	140.16	CH	6.85	ddq (8.6, 6.2, 1.3)	16a, 16b >41	20a, 20b >15, 21, 14, 16a	16
18	140.58	C					41, 16
19	202.53	C					41, 20, 20, 21, 17
20a	43.35	CH ₂	2.98	dd (15.5, 3.0)	20b, 21	20b, 21, 17 >42	22
20b			2.78	dd (15.7, 9.3) ^b	20a, 21	20a, 24 >17 >21	22
21	72.10	CH	4.25	ddd (9.2, 8.0, 3.0)	20a, 20b, 22	42, 20a, 24, 22, 17	42, 20, 20, 22
22	51.98	CH	3.06	dq (7.8, 7.0)	42 >21	42 >24, 25, 21	42, 20
23	217.63	C					42, 24, 22, 25, 21
24	50.73	CH	2.91	qd (6.9, 5.8) ^a	43, 25	42 >22, 25 >26	25
25	74.52	CH	3.85	t (5.5)	24, 26	43, 24, 22, 26	43, 24, 26
26	75.03	CH	5.22	d (5.2)	25	43, 25 >24	25
27	171.66	C					44, 44, 28, 26
28	58.29	CH	4.34	dd (7.7, 6.7)	44, 44	31/35, 44a, 44b >45	45, 44, 44
29	198.19	C					44, 44, 28, 31, 35
30	138.15	C					32, 34
31, 35	129.76	CH	8.06	dd (8.4, 1.3)	32/34, 33	32/34 >28	33
32, 34	130.06	CH	7.53	td (7.5, 1.6)	33, 31/35	31/35, 33	
33	134.81	CH	7.63	tt (7.3, 1.3)	32/34 >31/35	32/34	32, 34, 31, 35
36	60.77	CH ₃	3.61	s	-	37	3
37	14.57	CH ₃	1.92	d (1.1)	5	38, 6, 3	5, 3
38	17.06	CH ₃	1.03	br d (6.7)	6	37, 7, 5	37, 6, 7, 5
39	15.57	CH ₃	0.90	d (7.1)	8	8 >9a, 9b, 6	8, 7
40	56.67	CH ₃	3.29	s	-	14 >13, 12, 41	14
41	11.74	CH ₃	1.81	br s	17	16a, 16b, 40	17
42	13.71	CH ₃	1.06	d (7.1)	22	24, 20', 22, 21	22, 21
43	11.28	CH ₃	1.07	d (6.9)	24	25, 26	24, 25
44a	24.62	CH ₂	2.03	m ^c	45, 44b, 28	28	45, 28
44b			1.97	s	45, 44b, 28	28	45, 28
45	12.57	CH ₃	1.02	t (7.2)	44, 44	28	44, 44, 28
			4.59	s			

^{a, b, c, d} overlapping ¹H signals

Table S3. NMR data of lanyamycin **1/2** in DMSO-*d*₆. (¹H/¹³C/¹⁵N = 700/176/70.99 MHz)

Pos.	δ _{C(N)}	m	δ _H	m (J [Hz])	ROESY ^b	H to C HMBC
1	163.34	C				3 >15
1*	163.36	C				3 >15
2	141.12	C				36, 3 >37
3	130.99	CH	6.39	s	5 >	37, 5
3*	130.96	CH	6.40	s	5 >	37, 5
4	130.07	C				37 >6
5	146.54	CH	5.37	br d (9.9)	3 >7 >38, 8, 10	38, 37, 3 >6, 7
6	38.04	CH	2.23	tq (9.9, 6.7)	38, 37 >39, 8 >7OH	38, 7, 5, 7OH
7	74.31	CH	3.25	2x dd (9.9, 6.2)	7OH >5, 10, 38 >9a	39, 38, 9a >5 >7OH
7OH		OH	4.49	2x d (6.7)	7 >39, 38 >6	-
8	41.35	CH	1.34	m	39 >9b, 6 >5, 11	39, 9a, 9b, 7OH
9a	37.28	CH ₂	1.95	m	9b, 39, 10 >7, 7OH	39, 7, 11 >10
9b		CH ₂	1.79	M ^f	9a, 11, 39, 8	
10	136.93	CH	6.13	br ddt (15.3, 9.7, 5.9)	12, 9a >5	11, 12 >9a, 9b
11	130.93	CH	6.01	dd (15.3, 10.5)	9b, 13 >8	13 >9a, 9b
12	137.74	CH	6.48	dd (15.3, 10.5)	14, 10	14 >10
13	127.47	CH	5.18	dd (15.3, 9.5)	11, 15 >40	11
14*	83.59	CH	3.72	dd (9.3, 8.0)	40, 12 >15, 16b	40, 12 >16*b
14	83.54	CH	3.74	dd (9.3, 8.0)	40, 12 >15, 16b	40, 12 >16b
15*	73.69	CH	4.71	m	16*a, 16*b, 13 >17*	14, 16b >13, 17, 17*
15	73.67	CH	4.71	m	16a, 16b, 13 >17	
16a, 16*b	31.69	CH ₂	2.77	m ^d	overlap 40a/40b	14, 17, 17*
16b, 16*b		CH ₂	2.69	m ^w	overlap 40a/40b	-
17	137.48	CH	6.69	ddq (8.2, 6.8, 1.2)	20a, 20b, 15 >21	41 >20
17*	137.55	CH	6.72	ddq (8.0, 6.8, 1.2)	20*a, 20*b, 15/15* >21*	41* >20*
18/18*	138.59	C, C			-	41, 41*, 16b; 16*b
19	199.87	C			-	41 >17 >20b
19*	199.91	C			-	41* >17* >20*b
20a	42.22	CH ₂	2.78	m ^d	overl. with 16a/16b, 24	22, 21OH
20b		CH ₂	2.69	m	overl. with 16a/16b, 24	22, 21OH
20*a	42.40	CH ₂	2.79	m	overl. with 16a/16b, 24	22*, 21*OH
20*b		CH ₂	2.73	m ^e	overl. with 16a/16b, 24	22*, 21*OH
21	69.78	CH	4.10	dddd (8.8, 8.0, 5.8, 3.3)	21OH, 22, 20b >20a	42 >20b, 22 >21OH
21*	70.14	CH	4.13	dddd (8.4, 8.0, 6.0, 3.4)	21*OH, 22*, 20*a	42* >20*, 22* >21*OH
21OH		OH	4.77	d (5.6)	21 >22	-
21*OH		OH	4.88	d (6.0)	21*	-
22	49.91	CH	2.99	quin (7.0)	42, 25 >21, 21OH	42 >21OH >20b
22*	49.20 ¹	CH	3.06	quin (7.0)	42*, 25* >21*, 21*OH	42* >21*OH, 20*b
23	214.61	C			-	43, 42 >22 >24
23*	214.29	C			-	43* >42*, 22*
24	49.20 ¹	CH	2.77	m ^d	overl. with 24*, 20a, 16a and 20a*, 16*a	43, 25OH
24*	49.58	CH	2.78	m ^d	overl. with 24, 20a, 16a and 20*a, 16 a *	43*, 25*OH
25	72.55	CH	3.70	br q (5.6)	25OH, 22, 24 >43, 28, 26OH, NH	43, 26OH >25OH
25*	72.12	CH	3.83	br dt (4.4, 6.2)	25*OH, 22*, 24* >26*, 26*OH, NH*	43*, 26*OH >25*OH,
25OH		OH	4.78	d (6.0)	25, 43	-
25*OH		OH	4.72	d (5.6)	25* >43*	-
26	73.15	CH	4.96	dt (9.1, 5.8)	26OH, 43 >25 >>NH	26OH
26*	73.28	CH	4.91	ddd (9.2, 6.7, 5.4)	26*OH, 43* >25* >>NH*	26*OH
26OH		OH	5.89	d (5.6)	26	-
26*OH		OH	5.95	d (5.4)	26*	-
NH	(137.82)	NH	8.64	d (9.3)	28 >31/35, 25 >24, 26	-
NH*	(138.56)	NH	8.75	d (9.5)	28* >31*/35*, 25* >24*, 26*	-
27	168.33	C			-	28, NH, 44a >26*
27*	168.60	C			-	28*, NH*, 44*a >26*
28	56.01	CH	4.32	br t (7.0)	31/35, NH >45	45 >44a, 44b
28*	56.14	CH	4.27	dd (7.3, 6.7)	31*/35*, NH* >45	45* >44*b, 44*b
29	195.51	C			-	35/31, 28, >44b
29*	195.48	C			-	35*/31*, 28* >44*b
30	136.45	C			-	34/32
30*	136.26	C			-	34*/32*
33	133.17	CH	7.63	m		34/32, 35/31

33*	133.08 CH	7.55 m		34*/32*, 35*/31*
34/32	128.68 CH	7.51 m	35/31	34/32
34*/32*	128.68 CH	7.47 m	35*/31*	34*/32*
35/31	128.20 CH	8.00 m	32/34, 28	33, 35/31
35*/31*	127.87 CH	7.94 m	32*/34*, 28* >24*	33*, 35*/31*
36	59.41 CH ₃	3.51 2x s	37	>>3
37	13.91 CH ₃	1.82 br s		5, 3
38	16.56 CH ₃	0.93 d (6.5) ^g	6, 37, >7OH >7, 5	7
39	15.30 CH ₃	0.79 d (7.1)	8, 7OH >9b >6,	7
40	55.61 CH ₃	3.19 2x s	14 >13 >12	14
41	11.32 CH ₃	1.69 s	16a/16b, 40	17
41*	11.35 CH ₃	1.71 s	16*a/16*b, 40	17*
42	12.49 CH ₃	0.88 d (6.9) ^{ac,h}	22, 22' overl. 24	22
42*	12.68 CH ₃	0.89 d (6.9) ^{a,c,t}	22*, 20*a overl. 24*	22*
43	9.83 CH ₃	0.87 d (6.9) ^{a,h}	25OH, 26, 24	24, 25
43*	8.59 CH ₃	0.57 d (6.9) ^a	26*, 24* >25*OH	24*, 25*
44*a	21,80 CH ₂	1.88 m	45*, 28*	45*, 28*
44*b	CH ₂	1.77 M ^f	45*, 28*	
44a	22.29 CH ₂	1.85 M ^f	45, 28	45, 28
44b	CH ₂	1.78 m	45, 28	
45	11.79 CH ₃	0.88 t (7.4) ^{a,h}	28	28, 44
45*	11.83 CH ₃	0.94 t (7.3) ^{a,g}	28*	28*, 44*

^a from *J*res-Spectrum; ^b sorted with decreasing intensity; ^c interchangeable ¹H; ^{d,e,f,g,h} overlapping ¹H signals

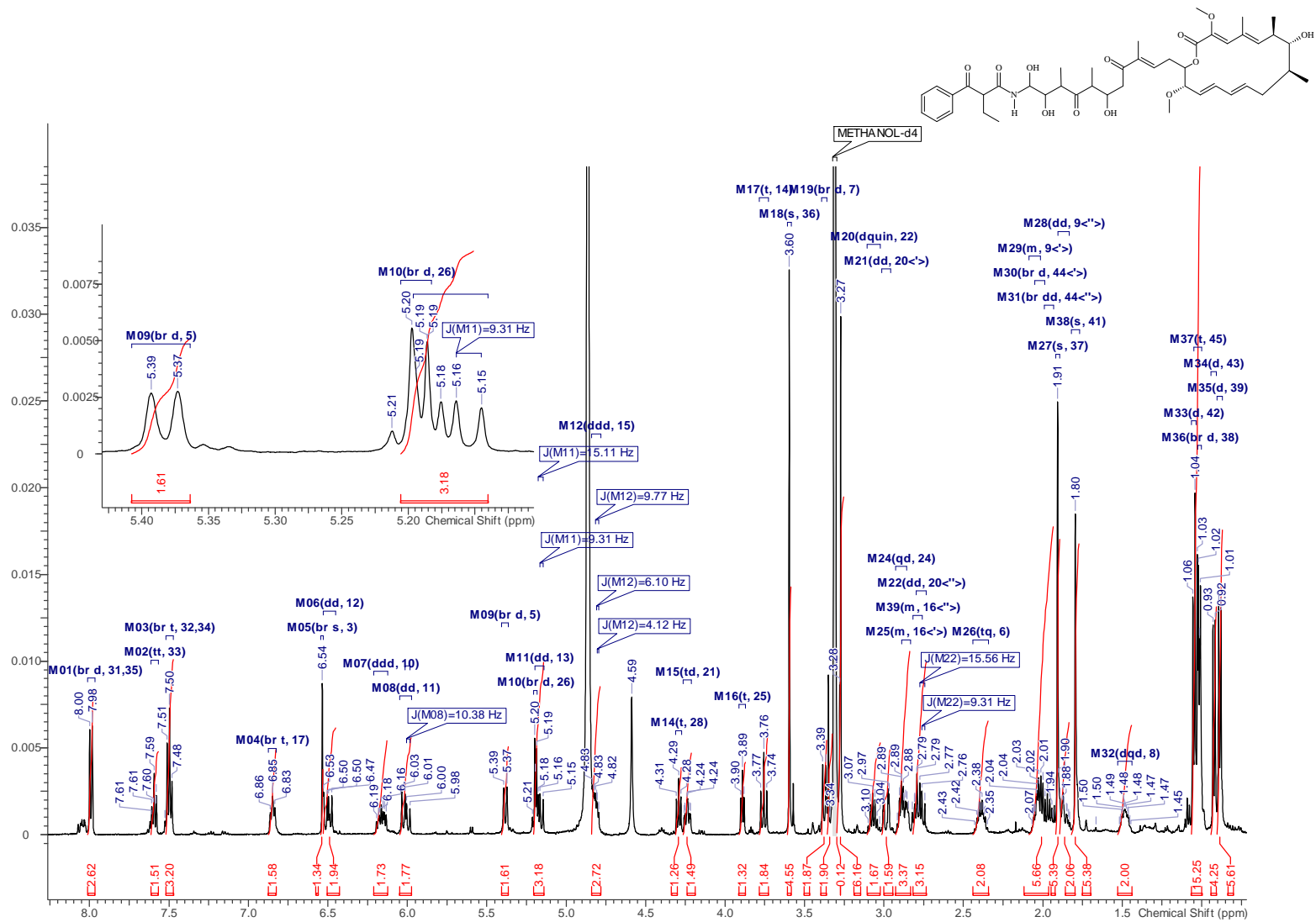


Figure S1. ¹H NMR spectrum of lanyamycin (1) in CD₃OD (500.3 MHz)

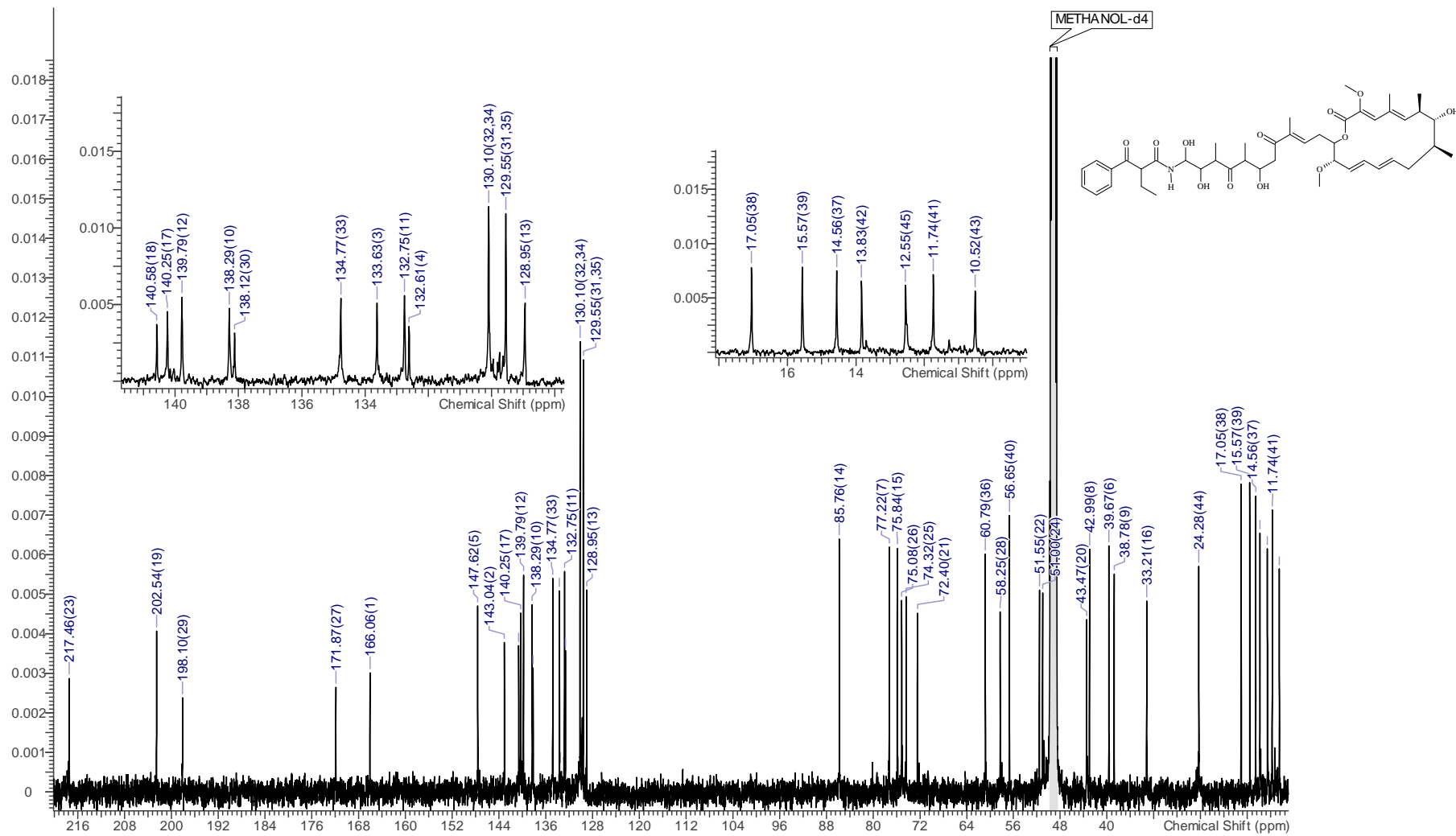


Figure S2. ^{13}C NMR spectrum of lanyamycin (1) in CD_3OD (125.8 MHz)

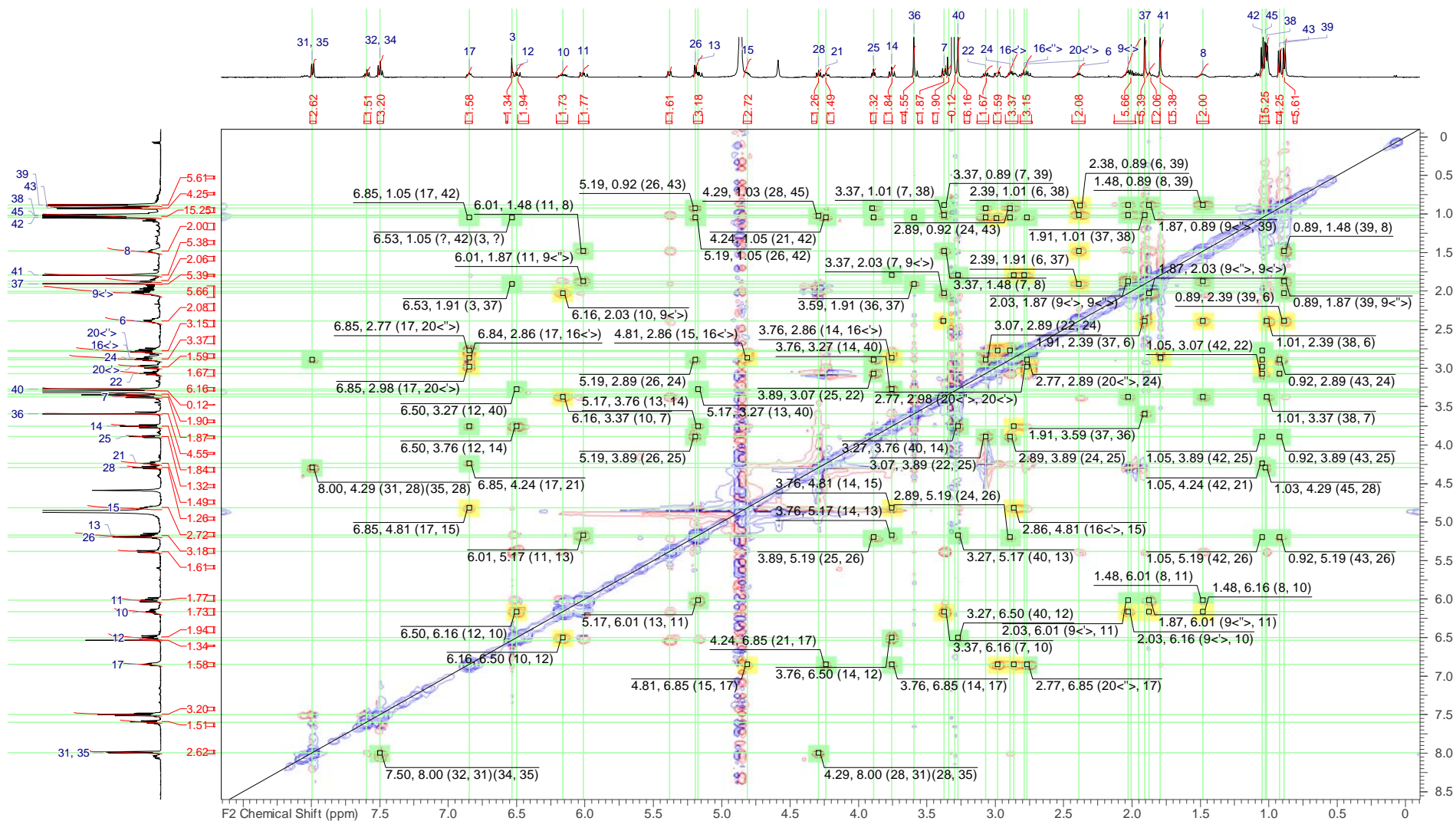


Figure S3. ROESY NMR spectrum of lanyamycin (1) (1H/13C/15N NMR at 500.3/125.8/50.7 MHz)

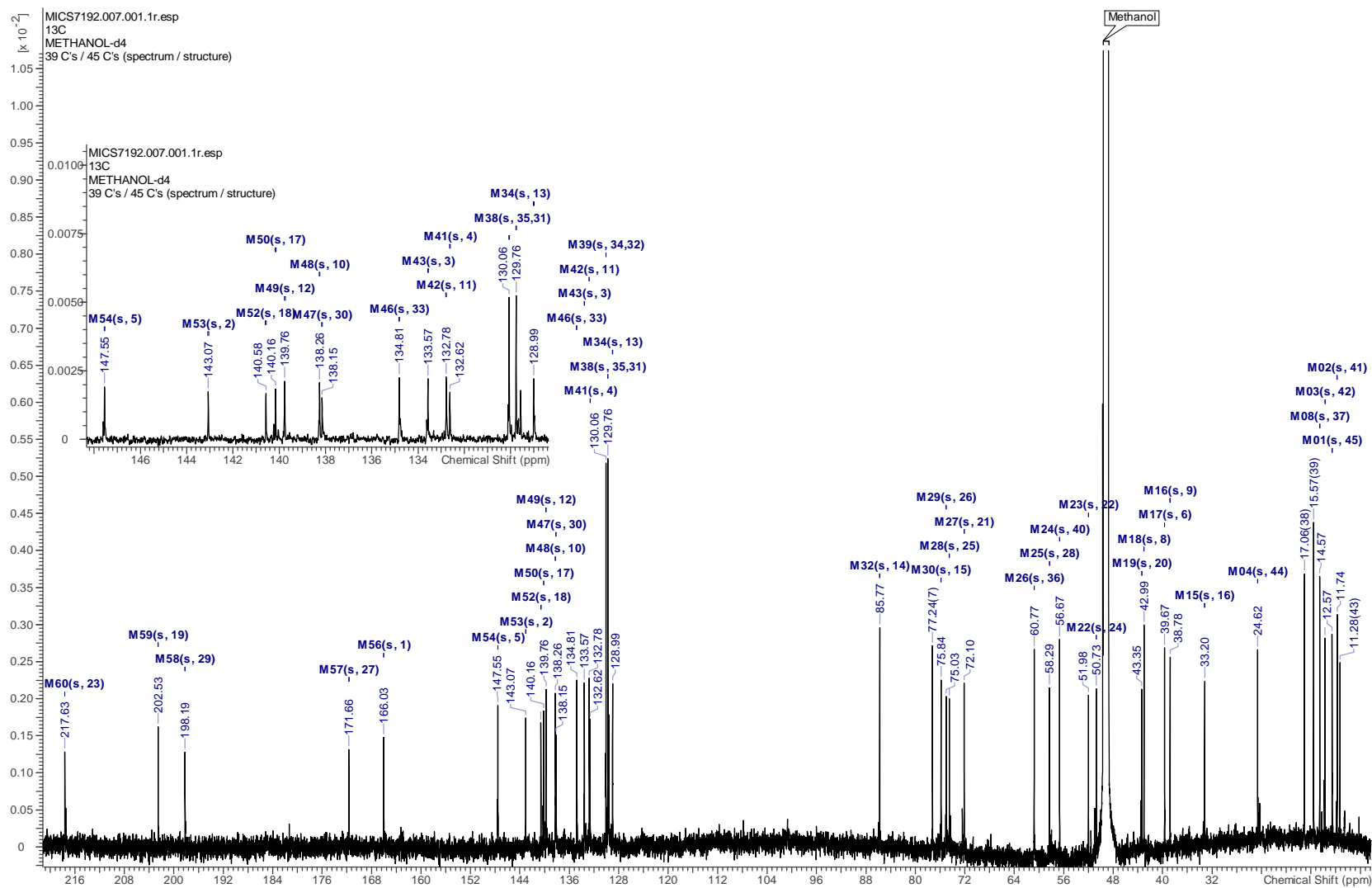


Figure S4. ^{13}C NMR spectrum of lanyamycin (**2**) in CD_3OD (125.8 MHz)

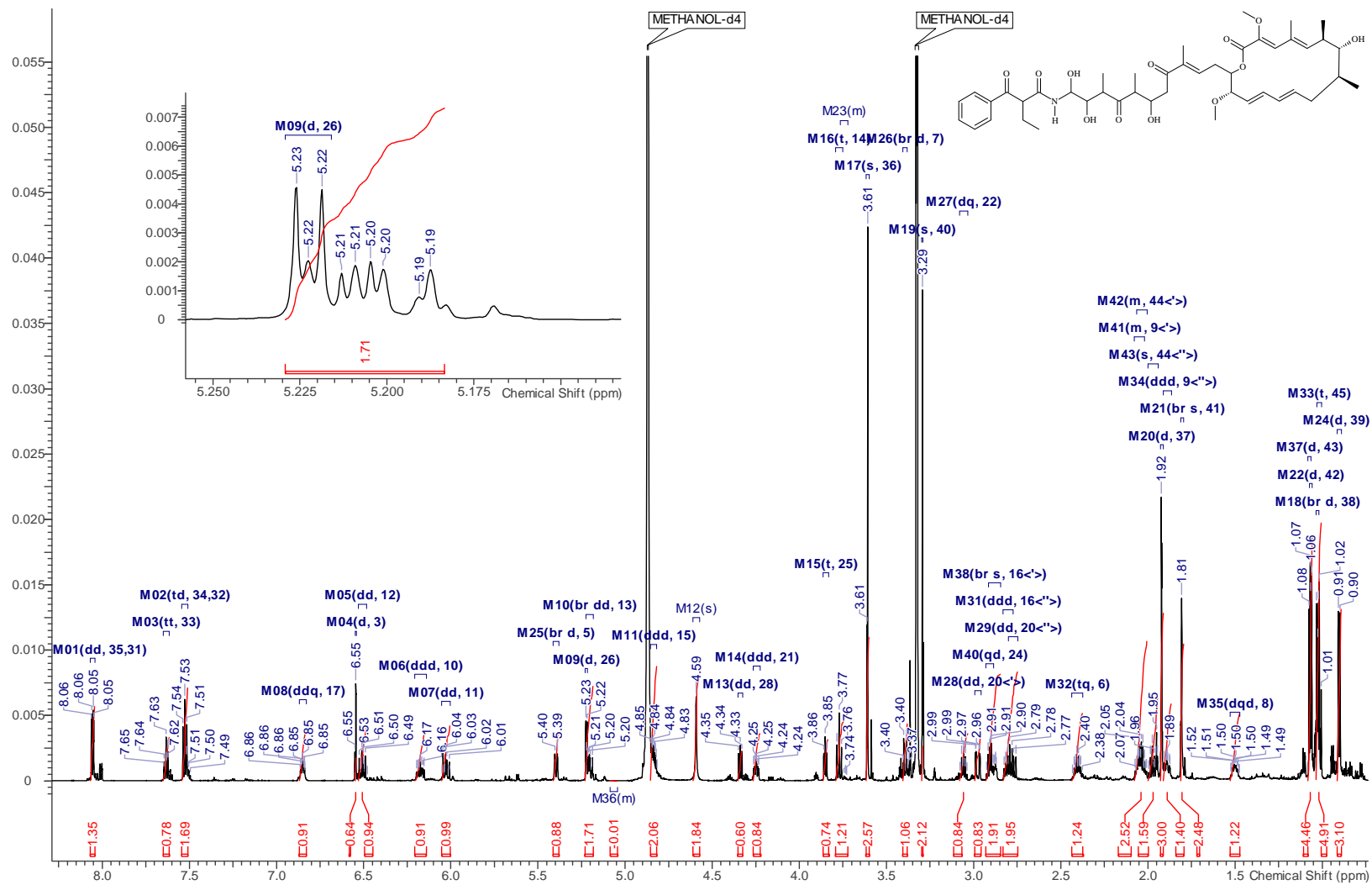


Figure S5. ¹H NMR spectrum of lanyamycin (2) in CD₃OD (500.3 MHz)

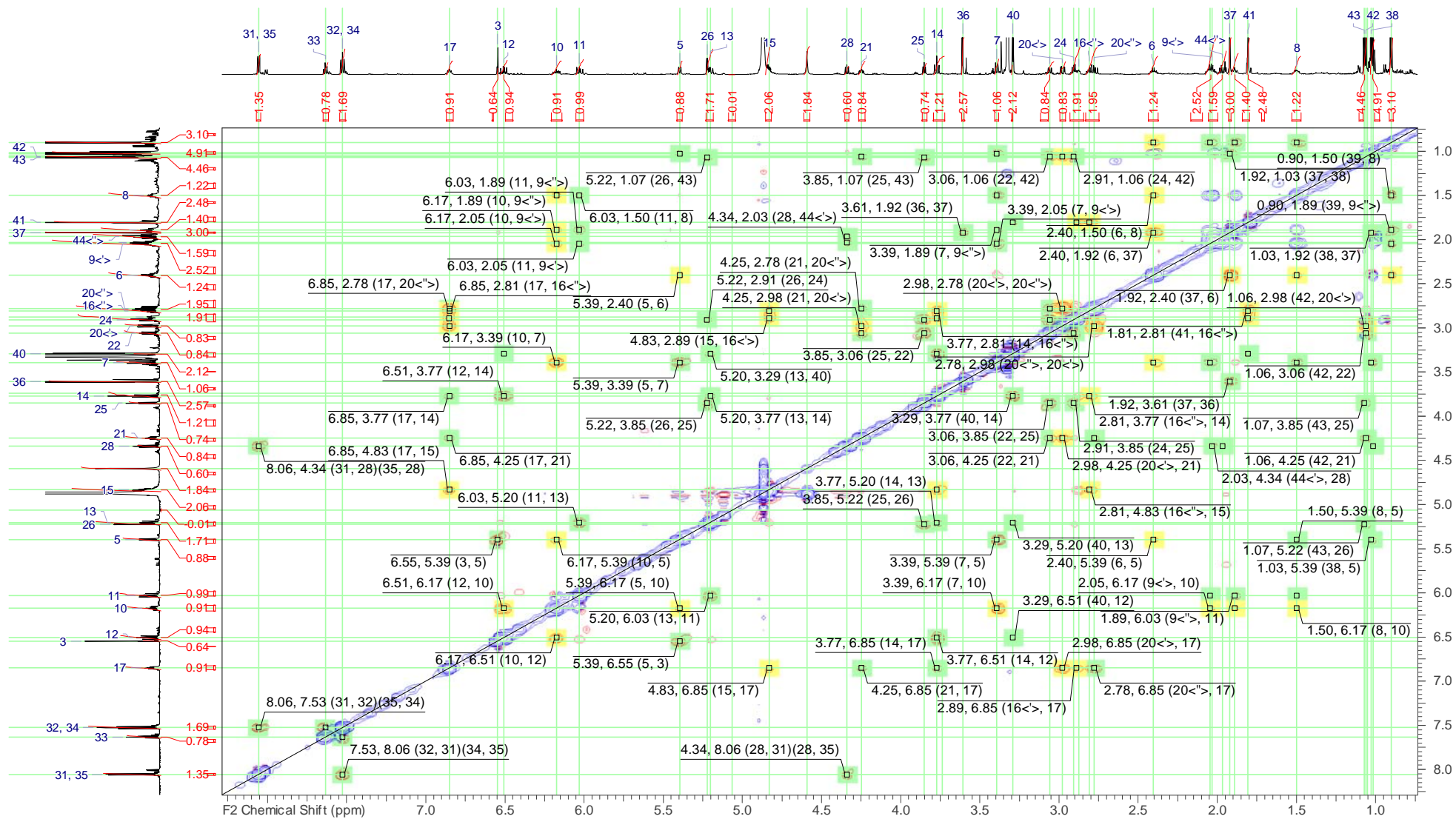


Figure S6. ROESY NMR spectrum of lanyamycin (**2**)

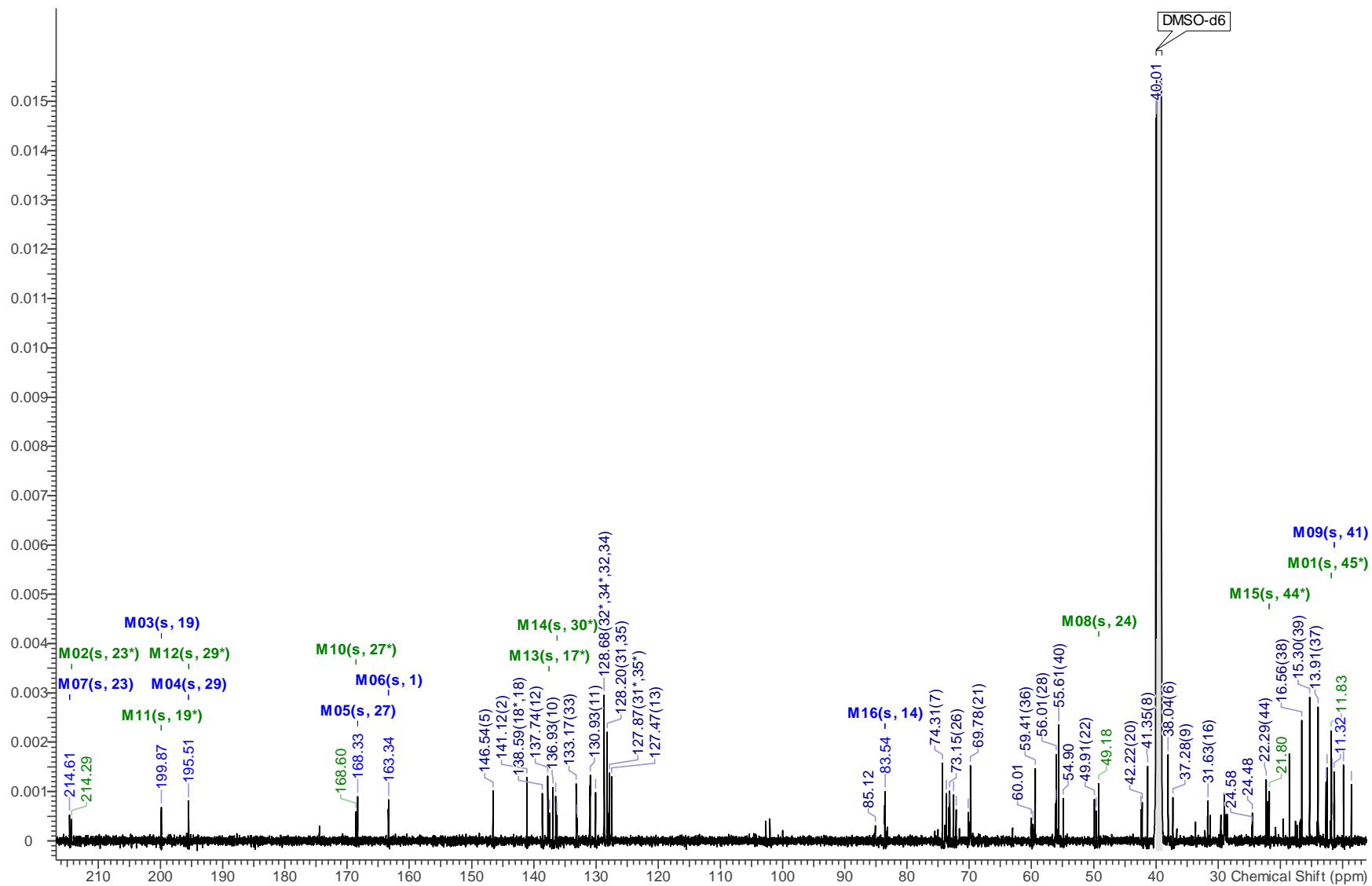
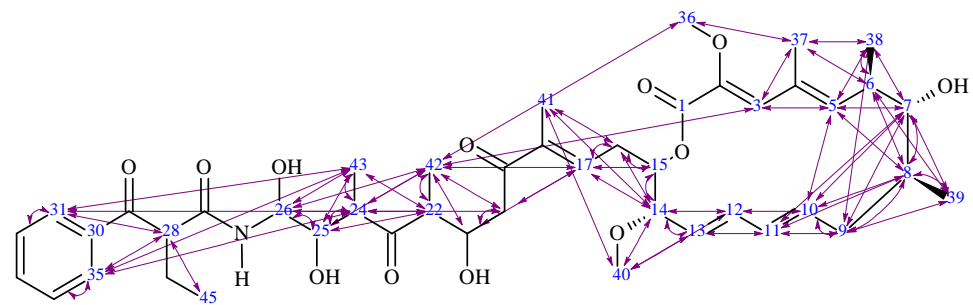
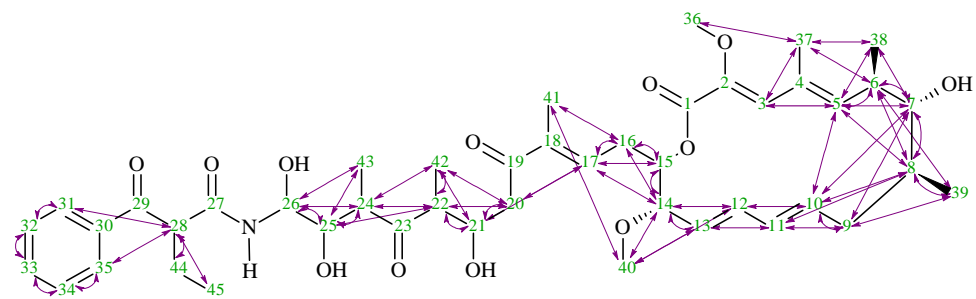


Figure S7. ¹³C NMR spectrum of lanyamycin (1/2) in DMSO-d6 (125.8 MHz)

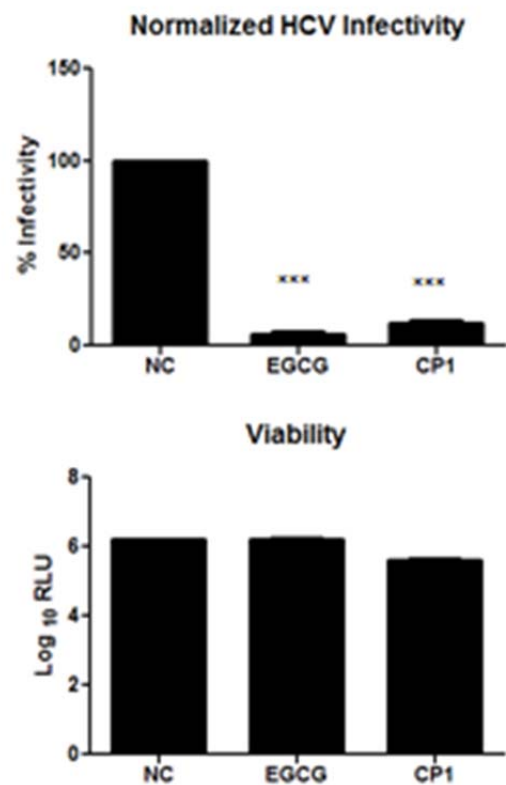


Lanyamycin 1



Lanyamycin 2

Figure S8. Comparison of the NOE intensities of lanyamycin 1 and 2



The Assay was performed in quadruplicate and is presented as the mean \pm standard deviation. *** $P \leq 0.05$

Figure S9. Lanyamycin (1/2) effects on HCV infectivity including viability assay

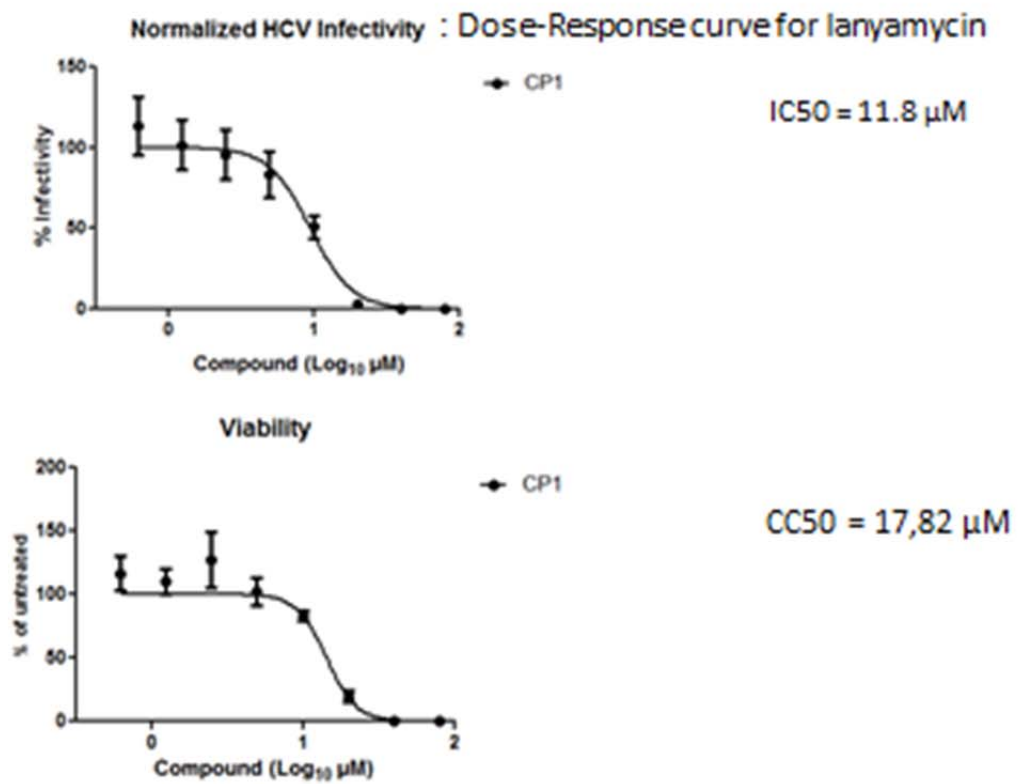


Figure S10. Dose-Response curve for lanyamycin (1/2) HCV infectivity