

SUPPORTING INFORMATION

Reveromycins Revealed: New polyketide spiroketals from Australian marine-derived and terrestrial *Streptomyces* spp. A case of natural products vs artifacts.

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1. General Experimental Details

Fine chemicals were purchased from Merck, Sigma, Aldrich or Fluka unless otherwise specified. Analytical Grade solvent was used for solvent extractions and solid phase extractions (SPE). Solvents used for HPLC and HPLC-MS purposes were of HPLC grade (except methanol used in HPLC-MS which was of Mass Spectrometry grade) supplied by Labscan and filtered and degassed through an Alltech 0.45 µm polytetrafluoroethylene (PTFE) membrane prior to use. Water for HPLC purposes was filtered through an ELGA PurelabUltra system prior to degassing. Deuterated solvents were supplied by Cambridge Isotopes (Andover, MA, USA).

Solid phase extractions were carried out on cartridges as specified. Prior to use each cartridge was conditioned with methanol followed by water, before equilibration with the initial solvent. Elution was carried out under applied vacuum and fractions analyzed by HPLC-DAD-MS or FIA in ESI(±)MS mode.

High performance liquid chromatography (HPLC) was performed using:

- (1) A Waters 2790 separations module equipped with a Waters 996 photodiode array (PDA) detector, Waters Fraction Collector II (or Waters 2700 Sample Manager) and Alltech 500 evaporative light scatter detector (or Waters 410 Differential Refractometer), operated under the control of Waters Millennium 32® software.
- (2) An Agilent 1100 Series separations module equipped with six column switching capability (where required), six-position selection valve, quaternary or binary pump with vacuum degasser, well plate autosampler with thermostat control, diode array detector with analytical flow cell, Polymer Labs PL-ELS1000 ELSD connected through an Agilent 35900E analytical to digital interface, thermostated column compartment and fraction collector. Equipment was under the control of Agilent ChemStation software.
- (3) An Agilent 1100 Series preparative separations module equipped with two preparative pumps with gradient control, preparative autosampler, multiple wavelength detector with preparative flow cell, and preparative fraction collector. Equipment was under the control of Agilent ChemStation software.

Standard analytical HPLC conditions were 1 mL/min linear gradient elution from 90% H₂O/MeCN (0.01% TFA) to MeCN (0.01% TFA) over 15 min, followed by a 5 min flush with MeCN (0.01% TFA). The standard HPLC-DAD-MS gradient conditions were identical, however the TFA modifier was replaced with 0.05% HCO₂H in order to minimize ion suppression in the negative mode. The standard column used for both gradients was a Zorbax StableBond C₈ (150 × 4.6 mm, 5 µm) column, unless otherwise specified.

Chiroptical measurements ($[\alpha]_D$) were obtained on a Jasco P-1010 intelligent remote module polarimeter, using a 100 × 2 mm cell at room temperature (~22 °C). Circular dichroism (CD) spectra were acquired using a Jasco J-810 spectropolarimeter using a 1 mm quartz cell. Ultraviolet-visible (UV-vis) absorption spectra were obtained using a CARY3 UV-visible spectrophotometer using a 1 cm quartz cell. Infrared (IR) spectra were acquired on a Jasco FT/IR-460Plus spectrophotometer with samples examined as films on NaCl discs, or in a NaCl solution cell, or as KBr discs.

Nuclear magnetic resonance (NMR) experiments were carried out on:

- (1) A Varian Unity 400 MHz spectrometer with XYX probe, controlled by VNMRJ software.
- (2) A Bruker Avance 600 MHz spectrometer with either a 5 mm BBI 1H-BB Z-Gradient or 5 mm PASEL 1H/D-13C Z-Gradient probe, controlled by XWinNMR or TopSpin software.

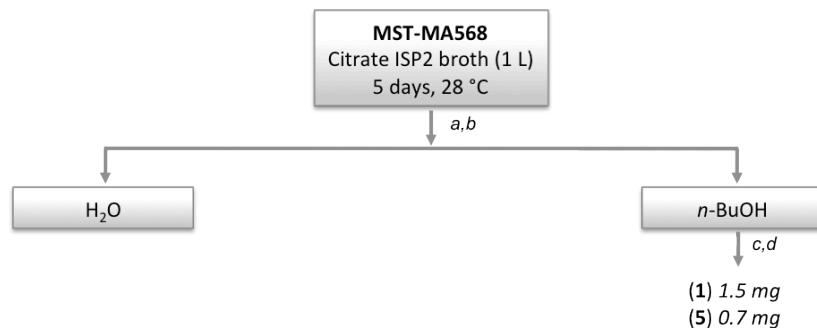
In all cases spectra were acquired at 25 °C (unless otherwise specified) in solvents as specified in the text, with referencing to residual ¹H signals in the deuterated solvent.

LC-NMR experiments were carried out on a Bruker Avance 600 MHz spectrometer with a 3 mm PASEI 1H/D-13C Z-Gradient probe, controlled by TopSpin software. The HPLC consisted of an Agilent 1100 Series separation module equipped with a quaternary pump, vacuum degasser and analytical autosampler, connected to a Bruker diode array detector, Bruker BSMS, Spark SPE robot and Foxy Jnr fraction collector, under the control of Bruker HyStar software.

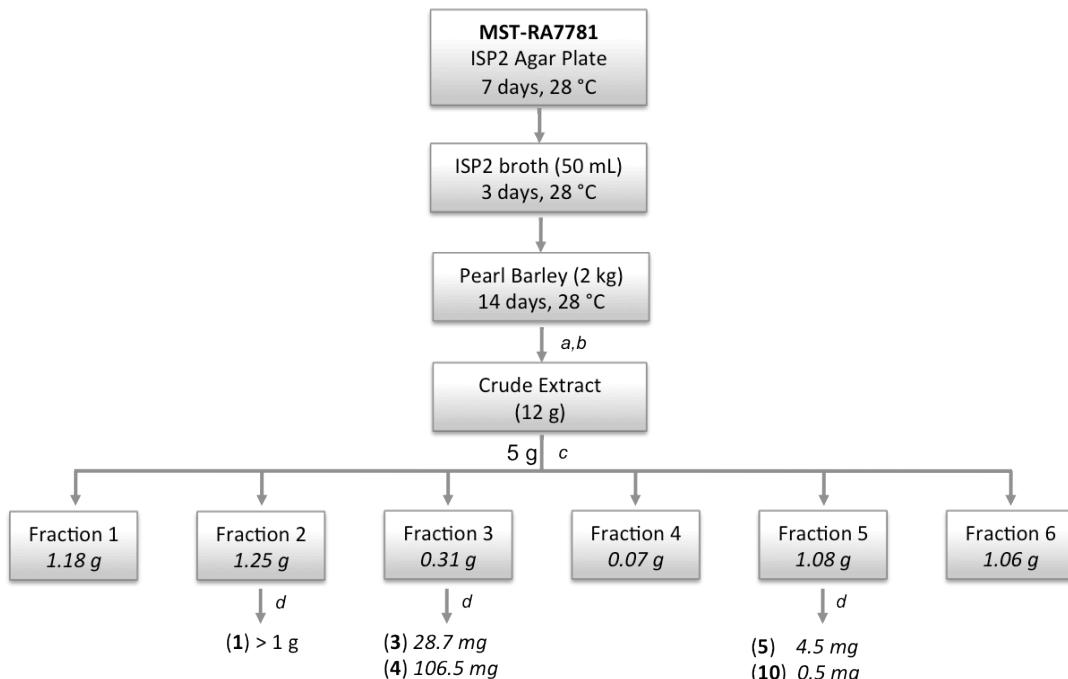
NMR chemical shift predictions were performed using ACD/Labs 7.0 HNMR and CNMR predictor modules.

Electrospray Ionisation Mass Spectra (ESI-MS) were acquired using both flow injection analysis (FIA) and liquid chromatography-diode array-mass spectrometry (HPLC-DAD-MS), were acquired on an Agilent 1100 series separation module with quaternary pump equipped with a vacuum degasser, an Agilent well plate autosampler with thermostat control, an Agilent diode array detector with micro flow cell, an Agilent thermostated column compartment and an Agilent single quadrupole mass detector. Equipment was under the control of Agilent ChemStation software. High Resolution Electrospray Ionisation Mass Spectra (HR-ESI-MS) measurements were obtained on either a Finnigan MAT 900 XL-Trap instrument with a Finnigan API III source or Bruker Daltonics micrOTOF-Q instrument with Apollo II ESI ion source. High-resolution electron-impact mass spectra (HR-EI-MS) measurements were obtained on a Kratos MS25RFA mass spectrometer at 70 eV.

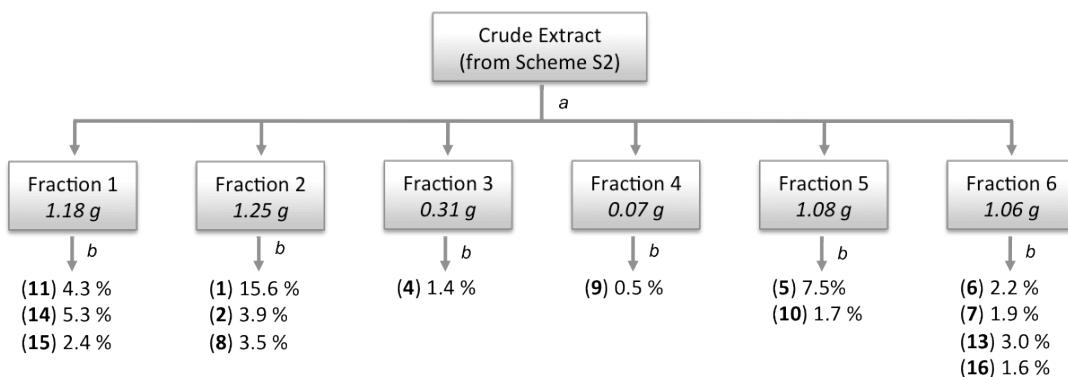
3. Cultivation and Fractionation



Scheme S1. Fractionation of reveromycins from MST-MA568. (a) fermentation supernatant dried *in vacuo*; (b) partitioning between H₂O and *n*-BuOH; (c) gel chromatography (Sephadex LH-20, MeOH); (d) C₈ HPLC.



Scheme S2. HPLC-DAD fractionation of reveromycins from MST-RA7781. (a) Acetone extraction (2×2.5 L) then concentration *in vacuo* to 1.2 L; (b) Ethyl acetate extraction (2×1.5 L) then concentration *in vacuo* to dryness; (c) Preparative C₁₈ HPLC; (d) Semi-preparative C₈ HPLC



Scheme S3. HPLC-DAD-SPE-NMR fractionation of reveromycins from MST-RA7781. (a) Preparative C₁₈ HPLC; (b) HPLC-DAD-SPE-NMR. Percent compositions are calculated on a mass-to-mass basis against the parent crude extract.

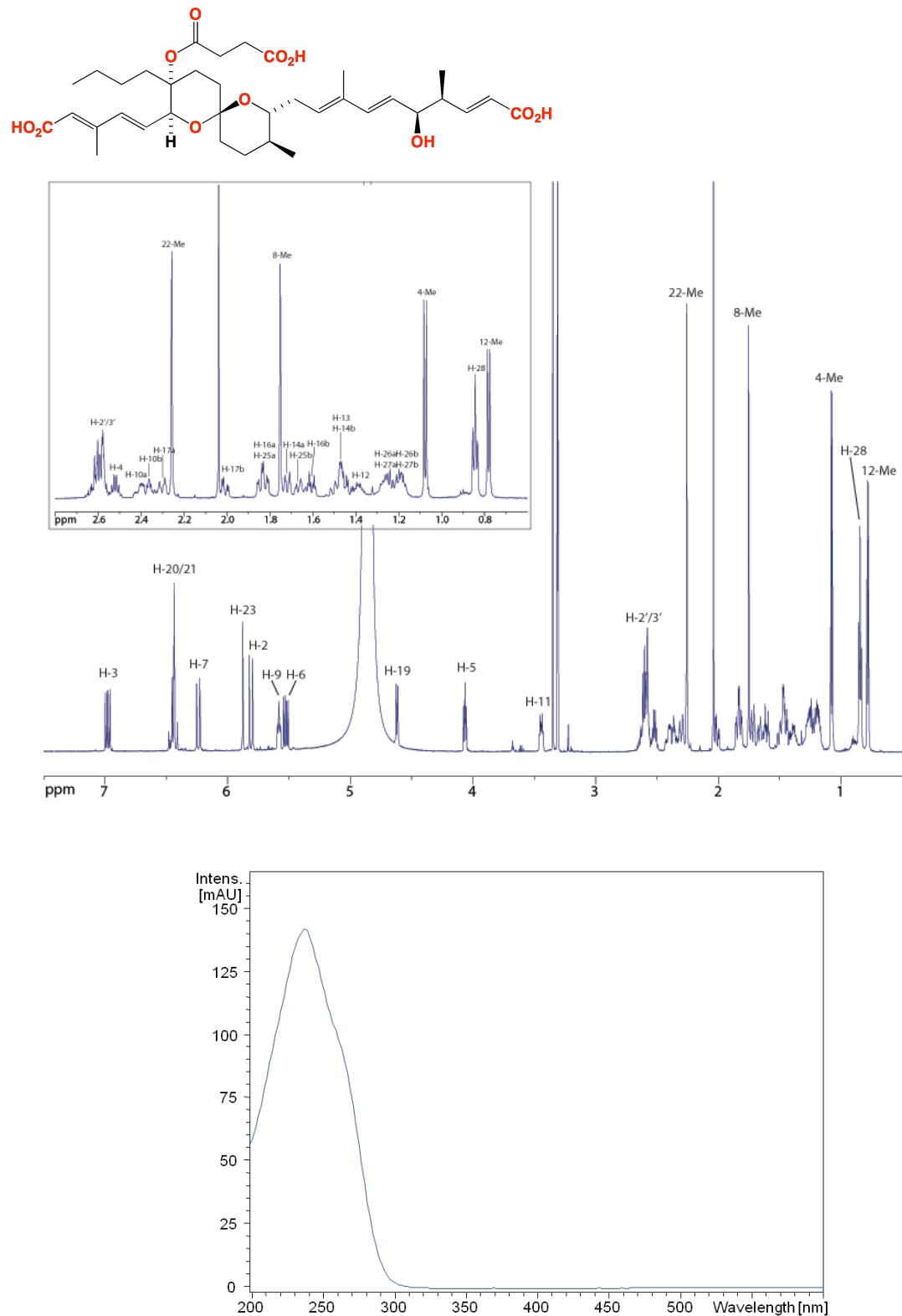


Figure S1. Upper panel: ¹H NMR (600 MHz, MeOH-*d*₄, HPLC-SPE-NMR) spectrum of reveromycin A (**1**), with inset showing expansion between 0.6 and 2.8 ppm. Lower panel: UV-vis spectrum of reveromycin A (**1**) extracted from HPLC diode array detector (MeCN:H₂O).

Table S1. NMR (600 MHz, MeOH-*d*₄, HPLC-SPE-NMR) data for reveromycin A (**1**).

Pos	δ_{H} (m, J (Hz))	$\delta_{\text{C}}^{\text{a}}$	COSY	TOCSY	HMBC (¹ H- ¹³ C)
1		169.8			
2	5.82 (dd, 15.7, 1.3)	122.3	3	3, 4, 4-Me	1, 4
3	6.98 (dd, 15.7, 7.7)	152.7	2, 4	2, 4, 4-Me	1, 2, 4, 5, 4-Me
4	2.53 (dqdd, 7.7, 6.8, 5.8, 1.3)	43.9	3, 5, 4-Me	2, 3, 5, 6, 7, 4-Me	2, 3, 5, 6, 4-Me
5	4.07 (ddd, 7.2, 5.8, 0.9)	76.7	4, 6	2, 4, 6, 7, 4-Me	3, 4, 6, 7, 4-Me
6	5.52 (dd, 15.7, 7.2)	127.7	5, 7	5, 7	4, 5, 8
7	6.25 (dd, 15.7, 0.9)	137.7	6	4, 5, 6, 4-Me	5, 6, 8, 8-Me
8		135.9			
9	5.58 (dd, 7.7, 6.8)	129.3	10a/b, 8-Me	10a/b, 11, 8-Me	7, 8-Me
10a	2.41 (ddd, 15.7, 7.7, 3.8)	32.8	9, 11	9, 11, 8-Me, 12-Me	9
10b	2.35 (ddd, 15.7, 6.8, 4.3)		9, 11	9, 11, 8-Me, 12-Me	8, 9, 11
11	3.45 (ddd, 10.1, 4.3, 3.8)	76.1	10a/b, 12	9, 10a/b, 12, 12-Me, 14a/b	9
12	1.39 (m)	36.2	12-Me	11, 12-Me, 14a/b	14
13	1.44 (m)	28.1	14a	14a	
14a	1.71 (ddd, 12.8, 2.7, 2.7)	36.7	13, 14b	13, 14b	12, 15
14b	1.47 (m)		14a	12, 14a, 12-Me	12
15		97.1			
16a	1.83 ^d (ddd, 13.6, 13.6, 4.3)	35.1	16b, 17b	16b, 17a/b	15, 17
16b	1.60 (ddd, 13.6, 4.3, 3.9)		16a	16a, 17a/b	15, 17
17a	2.29 (ddd, 14.2, 4.3, 3.9)	25.4	17b	16a/b, 17b	16
17b	2.02 (ddd, 14.2, 13.6, 4.3)		16a, 17a	16a/b, 17a	
18		84.2			
19	4.63 (d, 8.3)	79.5	20, 21	20, 21	15, 17, 18, 20, 21
20	6.46 (dd, 15.6, 8.3)	134.1	19	19	19, 20, 22, 23, 22-Me
21	6.44 (d, 15.6)	139.1	19	19	19, 21, 22, 23, 22-Me
22		152.6			
23	5.88 (d, 1.2)	121.3		22-Me	21, 24, 22-Me
24		170.0			
25a	1.83 ^d (ddd, 13.6, 13.6, 4.3)	34.8	25b, 26a/b	25b, 26a/b, 27a/b, 28	26, 27, 28
25b	1.66 (ddd, 13.6, 4.3, 3.9)		25a, 26a/b	25a, 26a/b, 27a/b, 28	
26a	1.24 (m)	23.8	25a/b	25a/b, 28	25, 27, 28
26b	1.22 (m)		25a/b	25a/b, 28	25, 27, 28
27a	1.26 (m)	23.3	28	25a/b, 28	25, 26, 28
27b	1.21 (m)		28	25a/b, 28	25, 26, 28
28	0.86 (t, 6.8)	14.6	27a/b	25a/b, 26a/b, 27a/b	26, 27
4-Me	1.08 (d, 6.8)	15.0	4	2, 3, 4, 5, 6	3, 4, 5
8-Me	1.75 (s)	12.5	9	9, 10a/b, 11	7, 8, 9
12-Me	0.79 (d, 6.5)	17.5	12	11, 12, 13, 14a/b	11, 13, 14
22-Me	2.25 (d, 1.2)	14.3		23	21, 22, 23
1'		173.8 ^c			
2'	2.62 ^b (m)	30.9			1', 3', 4'
3'	2.59 ^b (m)	29.7			1', 2', 4'
4'		176.4 ^c			

(a) ¹³C assignments obtained from gHSQC and gHMBC data. (b-c) Assignments interchangeable (d) Overlapping resonances

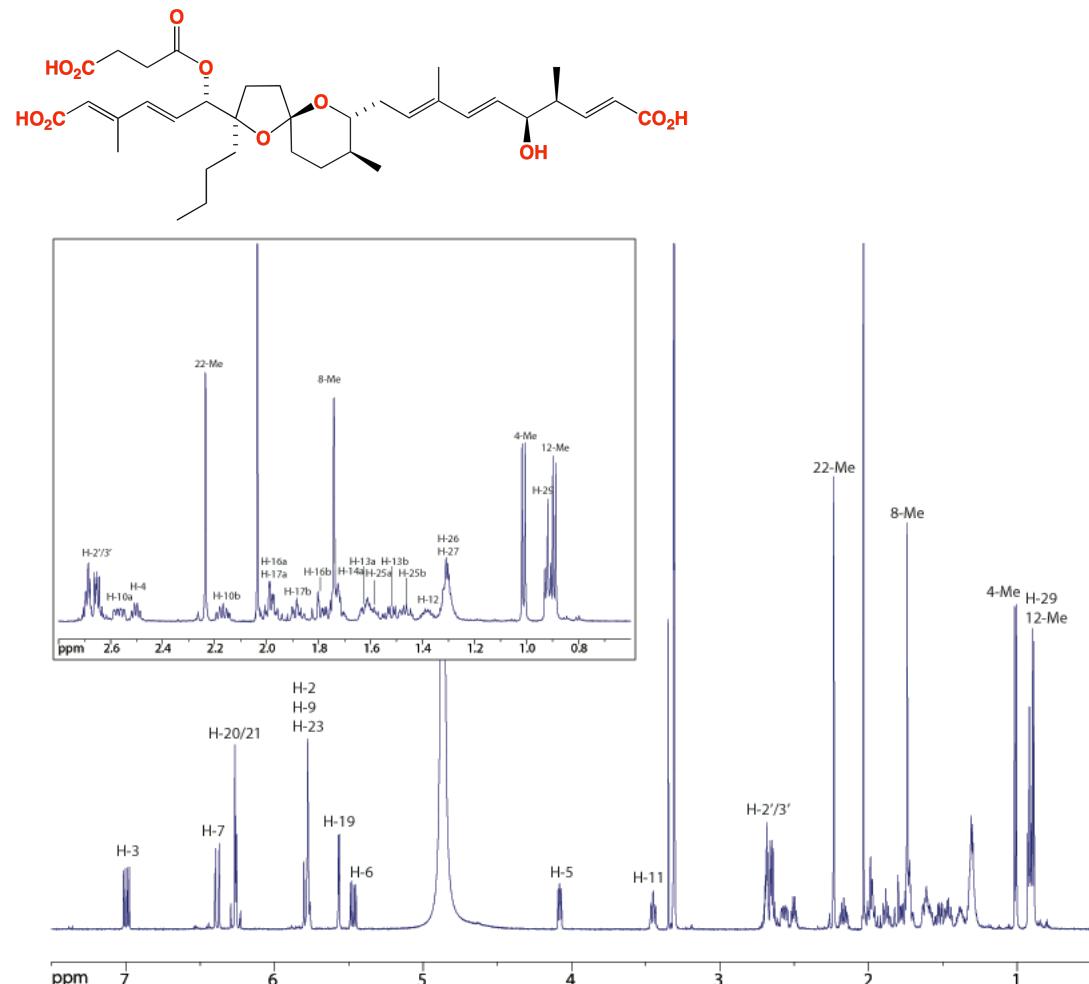


Figure S2. Upper panel: ^1H NMR (600 MHz, $\text{MeOH}-d_4$, HPLC-SPE-NMR) spectrum of reveromycin B (**2**), with inset showing expansion between 0.6 and 2.8 ppm. Lower panel: UV-vis spectrum of reveromycin B (**2**) extracted from HPLC diode array detector ($\text{MeCN}:\text{H}_2\text{O}$).

Table S2. NMR (600 MHz, MeOH-*d*₄, HPLC-SPE-NMR) data for reveromycin B (**2**).

Pos	δ_{H} (m, <i>J</i> (Hz))	$\delta_{\text{C}}^{\text{a}}$	COSY	TOCSY	HMBC (¹ H- ¹³ C)
1		169.7			
2	5.77 ^d (dd, 15.8, 1.2)	120.9	3	3, 4, 4-Me	1, 2, 4, 4-Me
3	6.98 (dd, 15.8, 7.5)	152.8	2, 4	2, 4, 4-Me	1, 2, 4, 4-Me
4	2.50 (dqdd, 7.5, 6.9, 5.2, 1.2)	43.8	3, 5, 4-Me	2, 3, 5, 4-Me	2, 3, 5, 6, 4-Me
5	4.08 (dd, 7.5, 5.2)	76.9	4, 6	4, 6, 7, 4-Me	3, 6, 7, 8
6	5.46 (dd, 15.7, 7.5)	126.9	5, 7	5	5, 8
7	6.39 (d, 15.7)	138.4	6	5	8, 9, 11, 8-Me
8		135.2			
9	5.77 ^d (m)	130.7	10a/b, 8-Me	10a/b, 11	
10a	2.57 (ddd, 15.3, 7.7, 2.7)	32.7	9, 10b, 11	9, 10b, 11	8, 9
10b	2.17 (ddd, 15.3, 8.6, 7.4)		9, 10a, 11	9, 10a, 11	8, 9, 11
11	3.45 (ddd, 10.0, 8.6, 2.7)	78.3	10a/b, 12	9, 10a/b, 12, 13b, 14, 12-Me	9
12	1.38 (m)	35.6	11, 12-Me	11, 13a/b, 14, 12-Me	
13a	1.61 (m)	30.1	13b, 14	11, 12, 13b, 14, 12-Me	
13b	1.51 (m)		13a, 14	12, 13a, 14, 12-Me	15
14	1.72 (m)	35.1	13a/b	11, 12, 13a/b, 12-Me	
15		108.7			
16a	1.99 (m)	39.5	16b, 17b	16b, 17b	15, 17, 18
16b	1.80 (m)		16a, 17a	16a, 17a	15
17a	1.99 (m)	32.8	16b, 17b	16b, 17b	15, 16, 18
17b	1.84 (m)		16a, 17a	16a, 17a	15, 16
18		88.5			
19	5.56 (d, 3.6)	80.2	20, 21	20, 21	16, 17, 18, 20, 21, 1'
20	6.25 (dd, 16.0, 3.6)	132.4	19, 21	19, 21	19, 20, 22, 23
21	6.28 (d, 16.0)	135.4	19, 20	19, 20	19, 21, 22, 23
22		152.3			
23	5.80 (d, 1.0)	122.2			19, 20, 21, 22, 24
24		169.7			
25a	1.59 (m)	35.4	25b, 26	25b, 26, 27, 28	26, 27
25b	1.47 (m)		25a, 26	25a, 26, 27, 28	26, 27
26	1.31 (m)	26.4	25a/b	25a/b, 28	25, 27, 28
27	1.31 (m)	24.1	28	25a/b, 28	25, 26, 28
28	0.92 (t, 6.9)	14.3	27	25a/b, 26, 27	26, 28
4-Me	1.02 (d, 6.9)	14.8	4	2, 3, 4	3, 4, 5
8-Me	1.74 (s)	12.6	9	9, 11	7, 8, 9
12-Me	0.89 (d, 6.5)	18.1	12	11, 12, 13a/b, 14	11, 12, 13, 14
22-Me	2.23 (d, 1.0)	13.7		23	21, 22, 23, 24
1'		172.7 ^c			
2'	2.60 ^b (m)	30.0			1', 3', 4'
3'	2.62 ^b (m)	29.8			1', 2', 4'
4'		175.7 ^c			

(a) ¹³C assignments obtained from gHSQC and gHMBC data. (b-c) Assignments interchangeable (d) Overlapping resonances

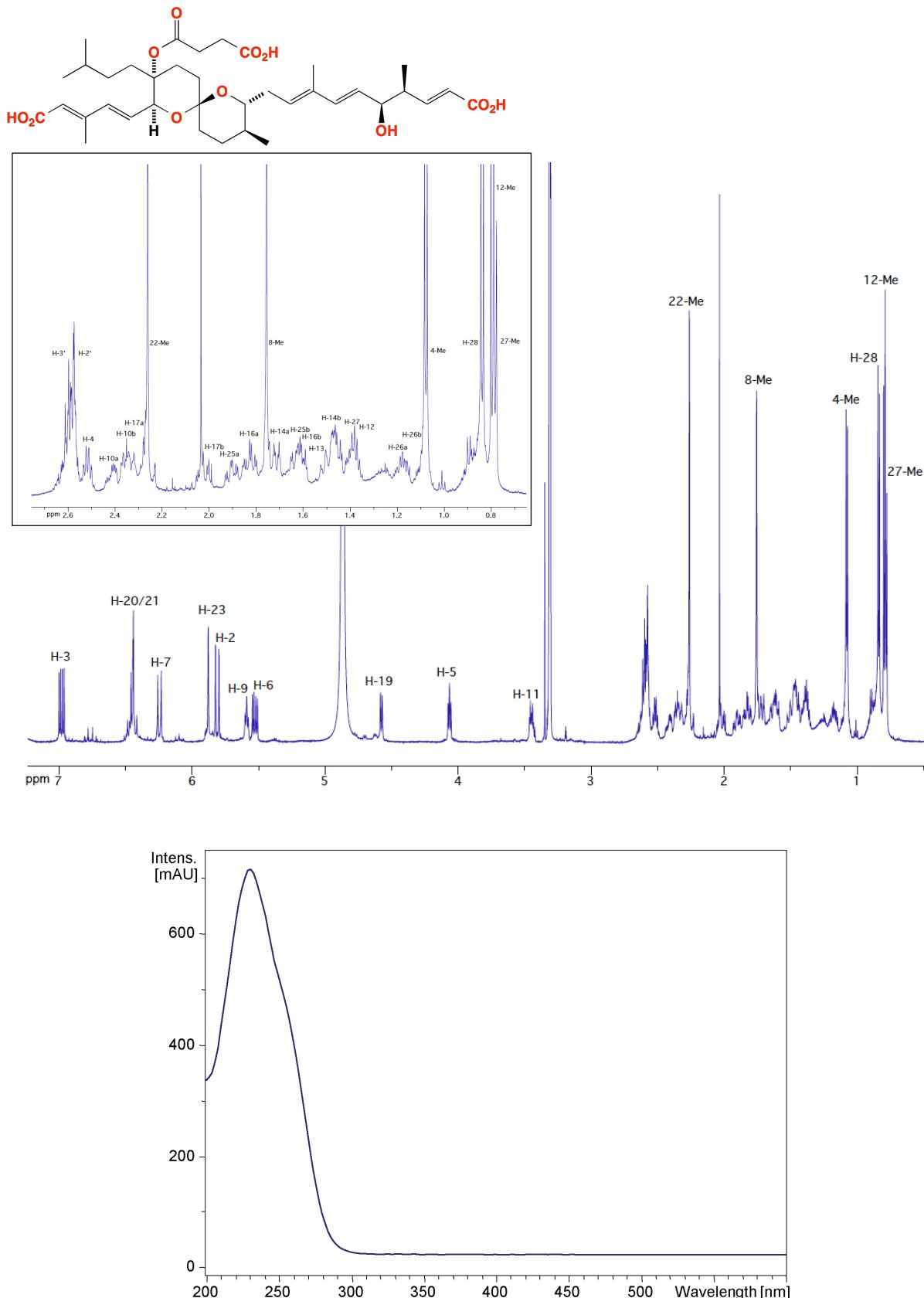


Figure S3. Upper panel: ¹H NMR (600 MHz, MeOH-d₄) spectrum of reveromycin C (**3**), with inset showing expansion between 0.6 and 2.8 ppm. Lower panel: UV-vis spectrum of reveromycin C (**3**) extracted from HPLC diode array detector (MeCN:H₂O).

Table S3. NMR (600 MHz, MeOH-*d*₄) data for reveromycin C (**3**).

Pos	δ_{H} (m, <i>J</i> (Hz))	$\delta_{\text{C}}^{\text{a}}$	COSY	HMBC (¹ H- ¹³ C)
1		169.9		
2	5.82 (dd, 15.7, 1.3)	122.3	3	1, 4
3	6.98 (dd, 15.7, 7.7)	152.6	2, 4	1, 2, 4, 5, 4-Me
4	2.53 (dqdd, 7.7, 6.8, 5.8, 1.3)	43.9	3, 5, 4-Me	2, 3, 5, 6, 4-Me
5	4.06 (dd, 7.3, 5.8)	76.7	4, 6	3, 4, 6, 7, 4-Me
6	5.52 (dd, 15.6, 7.3)	127.7	5, 7	4, 5, 8
7	6.25 (d, 15.6)	137.7	6	5, 6, 8, 8-Me
8		135.3		
9	5.58 (dd, 7.7, 6.8)	129.2	10a/b, 8-Me	7, 8-Me
10a	2.41 (ddd, 15.7, 7.7, 3.8)	32.8	9, 11	9
10b	2.35 ^d (m)		9, 11	8, 9, 11
11	3.45 (ddd, 10.1, 4.3, 3.8)	76.1	10a/b, 12	9
12	1.39 (m)	36.2	12-Me	14
13	1.44 (m)	28.1	14a	
14a	1.71 (ddd, 12.8, 2.7, 2.7)	36.8	13, 14b	12, 15
14b	1.47 (m)		14a	12
15		96.9		
16a	1.83 (ddd, 13.6, 13.6, 4.3)	35.1	16b, 17b	15, 17
16b	1.60 ^e (m)		16a	15, 17
17a	2.29 (ddd, 14.2, 4.3, 3.9)	25.2	17b	16
17b	2.02 (ddd, 14.2, 13.6, 4.3)		16a, 17a	
18		84.0		
19	4.63 (d, 8.3)	79.7	20, 21	15, 17, 18, 20, 21
20	6.46 (dd, 15.6, 8.3)	133.8	19	19, 20, 22, 23, 22-Me
21	6.44 (d, 15.6)	139.1	19	19, 21, 22, 23, 22-Me
22		152.6		
23	5.88 (d, 1.2)	121.4		21, 24, 22-Me
24		170.0		
25a	1.83 ^d (ddd, 13.6, 13.6, 4.3)	34.8	25b, 26a/b	26, 27
25b	1.63 ^e (m)		25a, 26a/b	26, 27
26a	1.17 (m)	31.7	25a/b	25, 27, 28, 27-Me
26b	1.09 (m)		25a/b	25, 27, 28, 27-Me
27	1.38 (m)	29.3	28, 27-Me	25, 26, 28, 27-Me
28	0.84 (d, 6.8)	22.9	27a/b	26, 27, 27-Me
4-Me	1.08 (d, 6.8)	15.0	4	3, 4, 5
8-Me	1.75 (s)	12.5	9	7, 8, 9
12-Me	0.79 (d, 6.5)	17.5	12	11, 13, 14
22-Me	2.25 (d, 1.2)	14.3		21, 22, 23
27-Me	0.80 (d, 6.8)	22.5	27	26, 27, 28
1'		173.1 ^c		
2'	2.58 ^b (m)	29.8		1', 3', 4'
3'	2.59 ^b (m)	30.9		1', 2', 4'
4'		175.6 ^c		

(a) ¹³C assignments obtained from gHSQC and gHMBC data. (b-c) Assignments interchangeable (d-e) Overlapping resonances

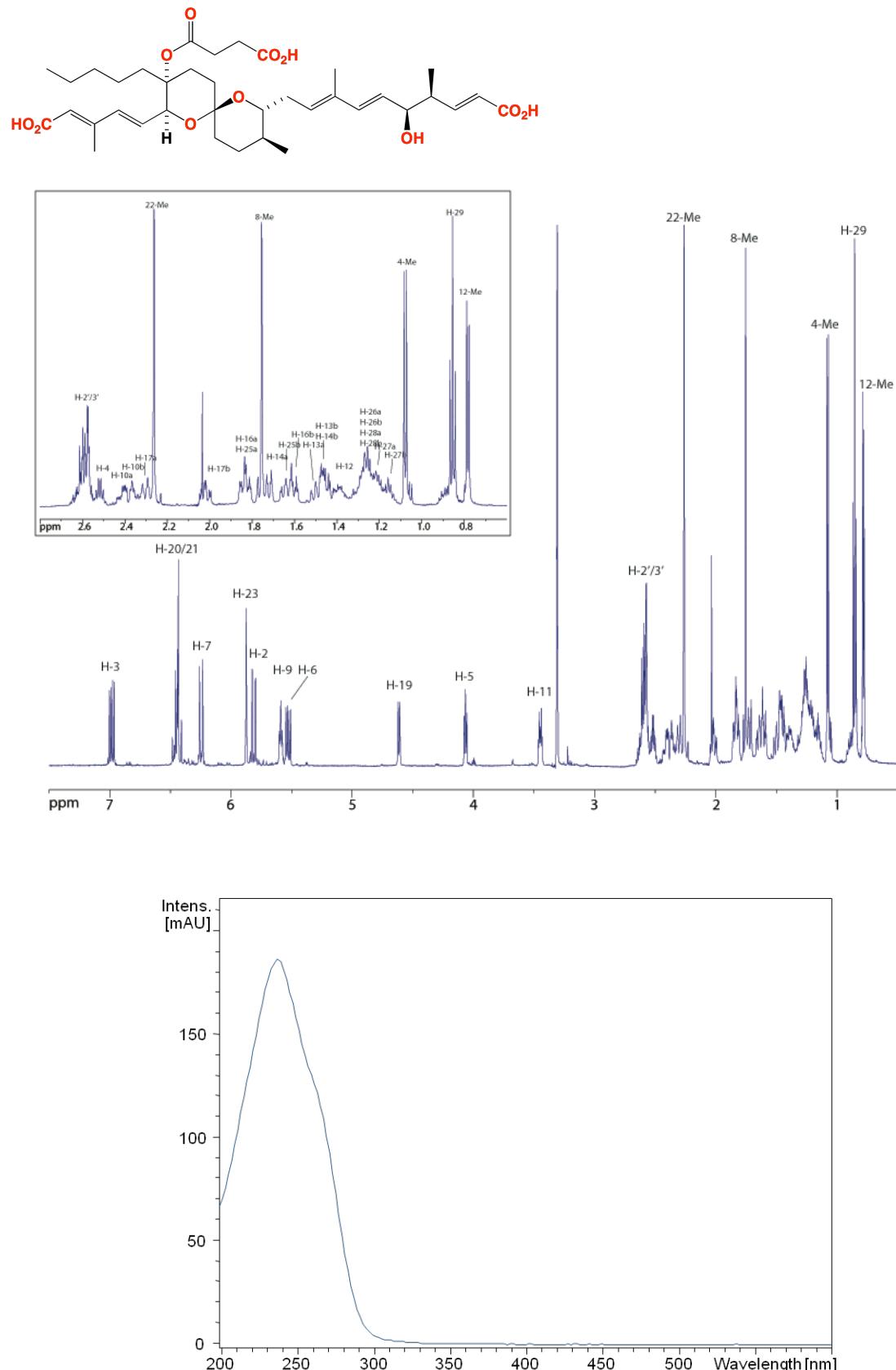


Figure S4. Upper panel: Presat ¹H NMR (600 MHz, MeOH-d₄, irr. δ_H 4.87, HPLC-SPE-NMR) spectrum of reveromycin D (**4**), with inset showing expansion between 0.6 and 2.8 ppm. Lower panel: UV-vis spectrum of reveromycin D (**4**) extracted from HPLC diode array detector (MeCN:H₂O)

Table S4. NMR (600 MHz, MeOH-*d*₄, HPLC-SPE-NMR) data for reveromycin D (**4**).

Pos	δ _H (m, J (Hz))	δ _C ^a	COSY	TOCSY	HMBC (¹ H- ¹³ C)
1		169.9			
2	5.82 (dd, 15.8, 1.3)	122.4	3	3, 4, 5, 4-Me	1, 4
3	6.98 (dd, 15.8, 7.7)	152.9	2, 4	2, 4, 5, 4-Me	1, 2, 4, 5, 4-Me
4	2.52 (ddqd, 7.7, 6.9, 6.8, 1.3)	44.2	3, 4-Me	2, 3, 5, 7, 4-Me	2, 3, 5, 6, 4-Me
5	4.07 (dd, 7.3, 6.9)	76.8	4, 6	4, 6, 7, 4-Me	3, 4, 6, 7, 4-Me
6	5.54 (dd, 15.7, 7.3)	128.0	5, 7	4, 5, 7, 4-Me	5, 7
7	6.26 (d, 15.7)	137.9	6	4, 5, 6	5, 6, 8, 8-Me
8		135.3			
9	5.59 (dd, 7.7, 6.8)	129.5	10a/b	10a/b, 11, 8-Me	7, 10, 8-Me
10a	2.41 (ddd, 15.7, 7.7, 3.8)	32.9	9, 11	9, 11, 12, 8-Me	9, 11
10b	2.37 (ddd, 15.7, 6.8, 4.3)		9, 11	9, 11, 12, 8-Me	8, 9
11	3.45 (ddd, 10.1, 4.3, 3.8)	76.2	10a/b, 12	9, 10a/b, 12, 13a/b, 14a/b, 12-Me	9
12	1.40 (m)	34.8	11, 13a/b, 12-Me	11, 13a, 14a, 12-Me	11
13a	1.54 (m)	28.7	12, 14a/b	14a	14
13b	1.45 (m)		12, 14a/b	14a/b, 12-Me	12
14a	1.72 (ddd, 12.8, 2.7, 2.7)	36.8	13a/b	13a/b, 14b	12, 15
14b	1.47 (m)		13a/b, 14a	13a, 14a, 12-Me	15
15		96.9			
16a	1.83 ^d (ddd, 13.6, 13.6, 4.3)	32.8	16b, 17b	16b, 17a/b	14, 15, 17, 18
16b	1.61 (ddd, 13.6, 4.3, 3.9)		16a, 17a	16a, 17a/b	15, 17, 18
17a	2.30 (ddd, 13.8, 4.3, 3.9)	25.3	16b, 17b	16a/b, 17b	
17b	2.02 (ddd, 13.8, 13.6, 4.4)		16a, 17a	16a/b, 17a	16
18		83.9			
19	4.62 (d, 8.3)	79.7	20, 21	20, 21	15, 18, 20, 21
20	6.46 (dd, 15.6, 8.3)	134.2	19	19	19, 21, 22, 23, 22-Me
21	6.44 (d, 15.6)	139.1	19	19	19, 20, 22, 23, 22-Me
22		152.5			
23	5.88 (d, 1.0)	121.6		22-Me	21, 22, 24, 22-Me
24		169.7			
25a	1.83 ^d (ddd, 13.6, 13.6, 4.3)	39.3	25b, 26a/b	25b, 26a/b, 27a/b, 28a/b, 29	
25b	1.65 (ddd, 13.6, 4.3, 3.9)		25a, 26a/b	25a, 26a/b, 27a/b, 28a/b, 29	
26a	1.29 (m)	22.9	25a/b	25a/b, 29	27
26b	1.23 (m)		25a/b	25a/b, 29	
27a	1.21 (m)	33.0	28a/b	25a/b, 29	29
27b	1.16 (m)		28a/b	25a/b, 29	26, 28
28a	1.29 (m)	22.9	27a/b, 29	25a/b, 29	27
28b	1.23 (m)		27a/b, 29	25a/b, 29	
29	0.85 (t, 7.1)	14.2	28a/b	25a/b, 26a/b, 27a/b, 28a/b	26, 27, 28
4-Me	1.08 (d, 6.8)	14.9	4	2, 3, 4, 5	3, 4, 5
8-Me	1.76 (s)	12.9		9, 10a/b	7, 8, 9
12-Me	0.79 (d, 6.5)	17.2	12	10a/b, 11, 12, 13a/b, 14b	11, 12, 13
22-Me	2.26 (d, 1.0)	14.4		23	21, 22, 23, 24
1'		173.2 ^c			
2'	2.58 ^b (m)	31.3			1', 3', 4'
3'	2.62 ^b (m)	29.7			1', 2', 4'
4'		175.8 ^c			

(a) ¹³C assignments obtained from gHSQC and gHMBC data. (b-c) Assignments interchangeable. (d) Overlapping resonances

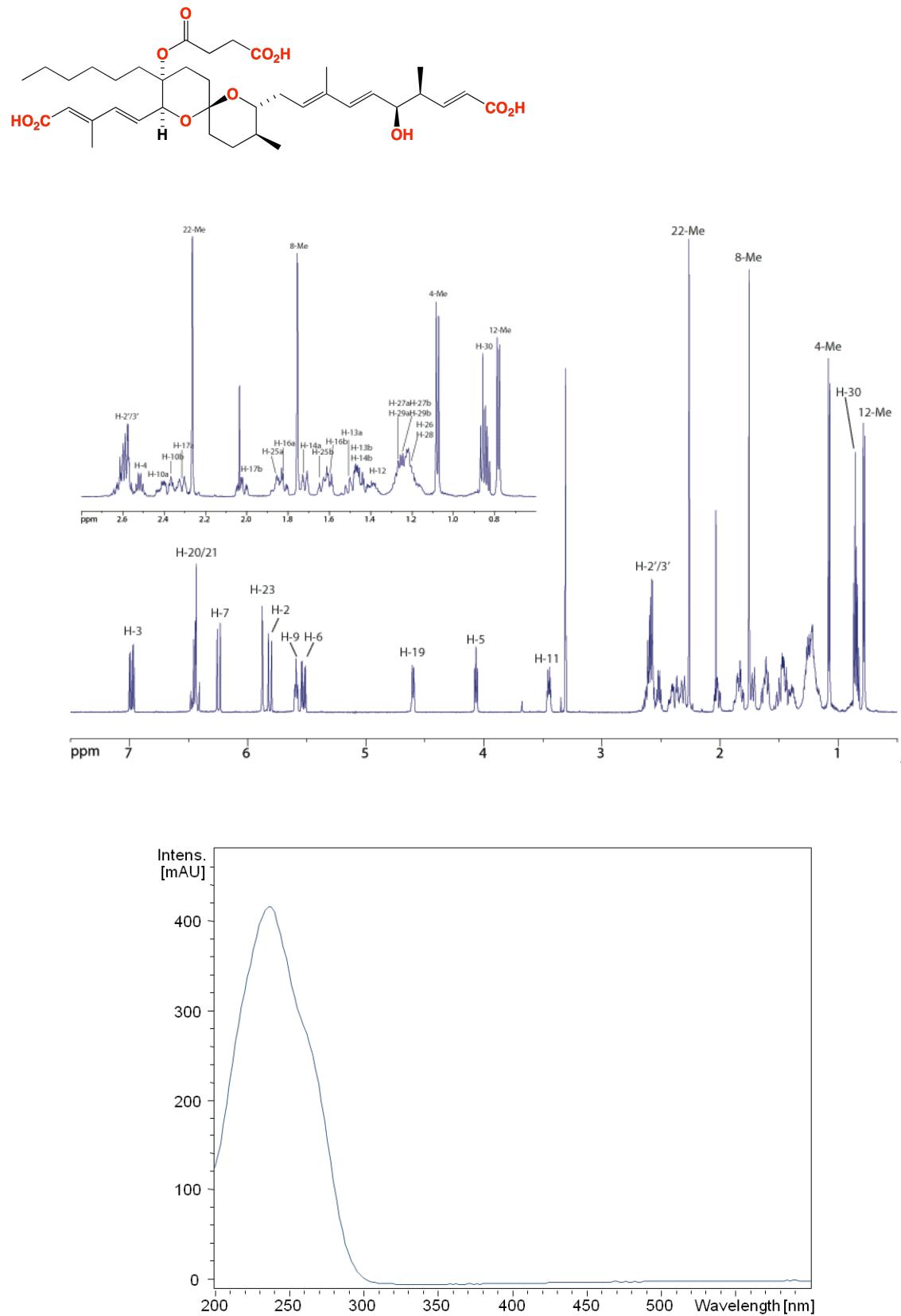


Figure S5. Upper panel: Presat ¹H NMR (600 MHz, MeOH-*d*₄, irr δ_H 4.84, HPLC-SPE-NMR) spectrum of reveromycin E (**5**), with inset showing expansion between 0.6 and 2.8 ppm. Lower panel: UV-vis spectrum of reveromycin E (**5**) extracted from HPLC diode array detector (MeCN:H₂O).

Table S5. NMR (600 MHz, MeOH-*d*₄, HPLC-SPE-NMR) data for reveromycin E (**5**).

Pos	δ_{H} (m, <i>J</i> (Hz))	$\delta_{\text{C}}^{\text{a}}$	COSY	TOCSY	HMBC (¹ H- ¹³ C)
1		170.2			
2	5.82 (dd, 15.8, 1.2)	122.5	3	3, 4, 5, 4-Me	1, 4
3	6.99 (dd, 15.8, 7.7)	152.8	2, 4	2, 4, 5, 4-Me	1, 2, 4, 5, 4-Me
4	2.52 (ddqd, 7.7, 6.8, 6.8, 1.2)	44.1	3, 5, 4-Me	2, 3, 5, 7, 4-Me	2, 3, 5, 6, 4-Me
5	4.07 (dd, 7.3, 6.8)	76.9	4, 6	4, 6, 7, 4-Me	3, 4, 6, 7, 4-Me
6	5.54 (dd, 15.6, 7.3)	127.8	5, 7	4, 5, 7, 4-Me	5, 8
7	6.26 (d, 15.6)	137.8	6	4, 5, 6	5, 8, 9, 8-Me
8		135.2			
9	5.59 (dd, 7.1, 6.9)	129.4	10a/b	10a/b, 11, 8-Me	7, 10, 11, 8-Me
10a	2.41 (ddd, 15.7, 7.7, 3.8)	32.9	9, 11	9, 11, 12, 8-Me	11
10b	2.37 (ddd, 15.7, 6.8, 4.3)		9, 11	9, 11, 12, 8-Me	8, 9, 11, 8-Me
11	3.45 (ddd, 10.1, 4.3, 3.8)	76.2	10a/b, 12	9, 10a/b, 12, 13a/b, 14a/b, 12-Me	9, 10
12	1.40 (m)	34.6	11, 13a, 12-Me	11, 13a, 14a, 12-Me	
13a	1.51 (m)	28.6	12, 14a	12, 14a	11, 14
13b	1.44 (m)		14a	11, 14a, 12-Me	14, 15
14a	1.72 (ddd, 12.8, 2.7, 2.7)	36.8	13a/b, 14b	12, 13a/b, 14b	12
14b	1.47 (m)		14a	11, 14a, 12-Me	13, 15
15		97.1			
16a	1.82 (ddd, 13.6, 13.6, 4.3)	32.6	16b, 17b	16b, 17a/b	14, 15, 17, 18
16b	1.60 (ddd, 13.6, 4.2, 3.9)		16a	16a, 17a/b	14, 15, 17, 18
17a	2.31 (ddd, 13.8, 4.3, 3.9)	25.3	17b	16a/b, 17b	15
17b	2.02 (ddd, 13.8, 13.6, 4.2)		16a, 17a	16a/b, 17a	15, 16
18		84.1			
19	4.61 (d, 8.3)	79.8	20, 21	20, 21	15, 17, 20, 21
20	6.46 (dd, 15.6, 8.3)	134.1	19	19	19, 21, 22, 23, 22-Me
21	6.44 (d, 15.6)	139.1	19	19	19, 20, 22, 23, 22-Me
22		152.3			
23	5.88 (d, 0.9)	121.5		22-Me	21, 22, 24, 22-Me
24		169.9			
25a	1.85 (ddd, 13.6, 13.6, 4.3)	35.3	25b, 26	25b, 26, 27a/b, 28, 29a/b	18, 27, 29
25b	1.64 (ddd, 13.6, 4.2, 3.9)		25a, 26	25a, 26, 27a/b, 28, 29a/b	18, 27, 29
26	1.20 (m)	30.4	25a/b	25a/b, 30	
27a	1.27 (m)	23.5		25a/b, 30	28, 30
27b	1.25 (m)			25a/b, 30	28
28	1.22 (m)	32.4		25a/b, 30	26
29a	1.27 (m)	22.6	30	25a/b, 30	28, 30
29b	1.25 (m)		30	25a/b, 30	28
30	0.86 (t, 6.9)	14.3	29a/b	26, 27a/b, 28, 29a/b	27, 28, 29
4-Me	1.08 (d, 6.8)	15.1	4	2, 3, 4, 5, 7	3, 4, 5
8-Me	1.75 (s)	12.8	9	9, 10a/b	7, 8, 9
12-Me	0.78 (d, 6.5)	17.8	12	10a/b, 11, 12, 13a/b, 14a/b	11, 12, 13
22-Me	2.26 (d, 0.9)	14.5		20, 21, 23	21, 22, 23, 24
1'		172.9 ^c			
2'	2.61 ^b (m)	31.1			1', 3', 4'
3'	2.57 ^b (m)	29.7			1', 2', 4'
4'		175.4 ^c			

(a) ¹³C assignments obtained from gHSQC and gHMBC data. (b-c) Assignments are interchangeable.

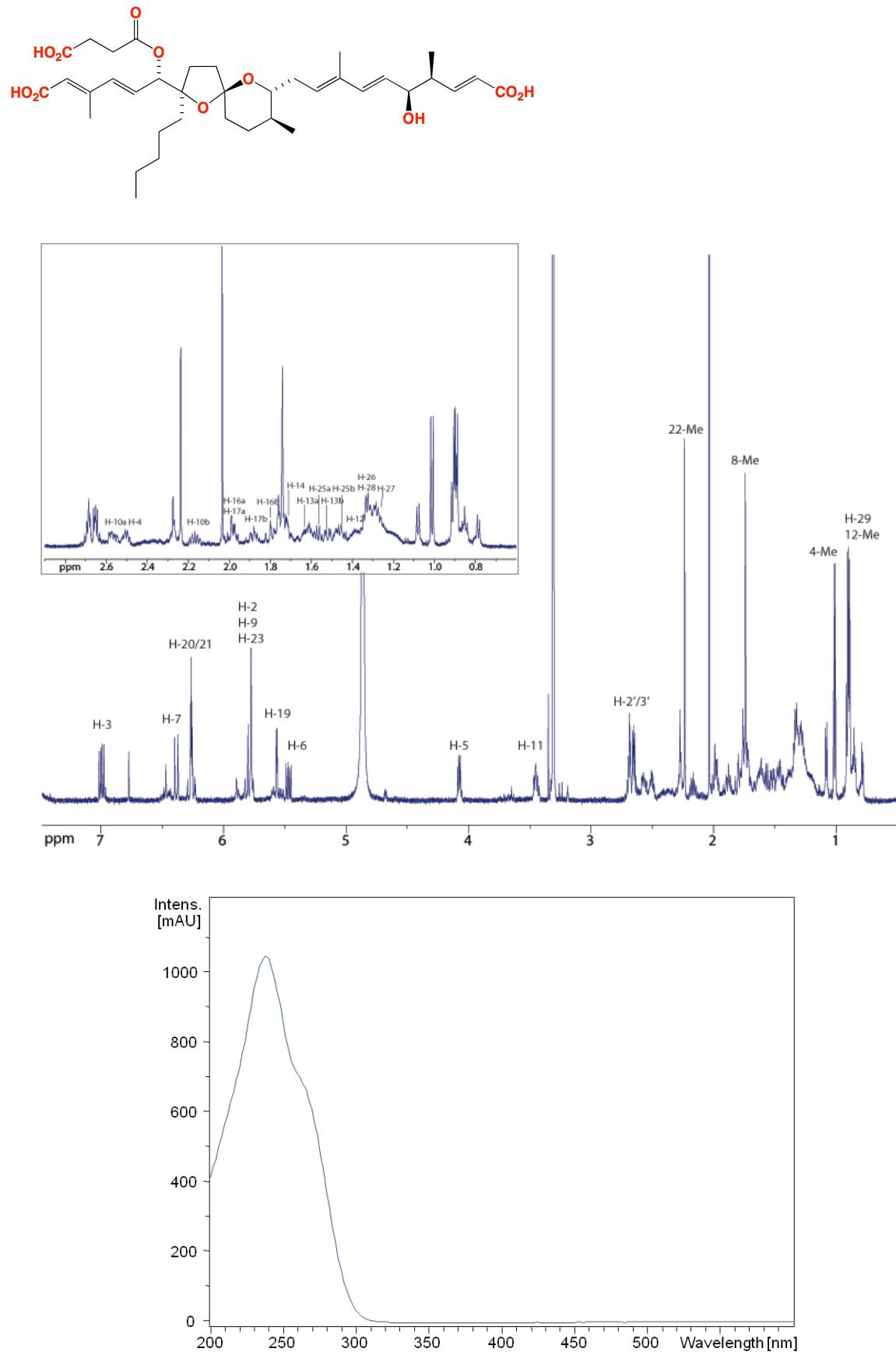


Figure S6. Upper panel: ¹H NMR (600 MHz, MeOH-d₄, HPLC-SPE-NMR) spectrum for reveromycin F (**6**), expansion between 0.6 and 2.8 ppm. Lower panel: UV-vis spectrum of reveromycin F (**6**) extracted from HPLC diode array detector (MeCN:H₂O).

Table S6. NMR (600 MHz, MeOH-*d*₄, HPLC-DAD-SPE-NMR) data for reveromycin F (**6**).

Pos	δ_{H} (m, <i>J</i> (Hz))	$\delta_{\text{C}}^{\text{a}}$	COSY	TOCSY	HMBC (¹ H- ¹³ C)
1		169.8			1, 4, 4-Me
2	5.80 ^d (dd, 15.8, 1.2)	120.9	3	3, 4, 4-Me	1, 2, 4, 5, 4-Me
3	6.99 (dd, 15.8, 7.6)	152.8	2, 4	2, 4, 4-Me	2, 3, 5, 6, 4-Me
4	2.51 (dqdd, 7.6, 6.9, 5.4, 1.2)	44.0	3, 5	2, 3, 5, 4-Me	3, 4, 6, 7, 4-Me
5	4.08 (dd, 7.5, 5.4)	76.9	4, 6	4, 6, 7, 4-Me	4, 5, 8
6	5.46 (dd, 15.7, 7.5)	127.0	5, 7	5, 7	5, 8, 9, 8-Me
7	6.37 (d, 15.7)	138.4	6	5, 6	
8		135.8			7, 8, 10, 8-Me
9	5.80 ^d (m)	130.7	10a/b, 8-Me	10a/b, 11	8, 9
10a	2.57 (ddd, 15.3, 7.7, 2.6)	32.8	9, 10b	9, 10b, 11	8, 9, 11
10b	2.17 (ddd, 15.3, 8.6, 7.4)		9, 10a, 11	9, 10a, 11	9
11	3.45 (ddd, 10.0, 8.6, 2.6)	78.3	10a/b, 12	9, 10a/b, 12, 13b, 14	1, 4, 4-Me
12	1.39 (m)	35.6	11	11, 13a/b, 14, 12-Me	1, 2, 4, 5, 4-Me
13a	1.61 (m)	30.2	13b, 14	12, 13b, 14, 12-Me	
13b	1.53 (m)		13a	12, 13a, 14, 12-Me	
14	1.72 (m)	35.2	13a	12, 13a/b, 12-Me	
15		108.8			
16a	1.99 (m)	39.6	16b, 17a/b	16b, 17a/b	15, 18
16b	1.80 (m)		16a, 17a/b	16a, 17a/b	15, 17
17a	1.99 (m)	32.7	16a/b, 17b	16a/b, 17b	15, 18
17b	1.88 (m)		16a/b, 17a	16a/b, 17a	14, 15
18		88.6			
19	5.56 (d, 4.1)	80.3	20, 21	20, 21	17, 18, 20, 21, 25, 1'
20	6.25 (dd, 16.0, 4.1)	132.4	19, 21	19, 21	19, 21, 22, 23, 22-Me
21	6.28 (d, 16.0)	135.8	19, 20	19, 20	19, 20, 22
22		152.2			
23	5.80 ^d (m)	122.2	22-Me	22-Me	24
24		170.1			
25a	1.57 (m)	35.6	25b, 26	25b, 26, 27, 28	
25b	1.48 (m)		25a, 26	25a, 26, 27, 28	
26	1.34 (m)	23.7	25a/b, 27	25a/b, 27, 28, 29	
27	1.29 (m)	33.5	26, 28	25a/b, 26, 29, 29	
28	1.34 (m)	23.7	27, 29	25a/b, 26, 27, 29	
29	0.87 (m)	14.2	28	25a/b, 26, 27, 28, 29	26, 27, 28
4-Me	1.02 (d, 6.9)	14.9	4	2, 3, 4, 5	3, 4, 5
8-Me	1.74 (s)	12.6	9	11	7, 8, 9
12-Me	0.87 (m)	18.1	12	11, 12, 13a/b, 14	11, 12, 13
22-Me	2.24 (d, 1.0)	13.8	23	23	20, 21, 22, 23, 24
1'		172.9			
2'	2.68 ^b (m)	30.3			1', 4'
3'	2.65 ^b (m)	29.6			1', 4'
4'		175.9			

(a) ¹³C assignments obtained from gHSQC and gHMBC data. (b) Assignments interchangeable. (c) Overlapping resonances

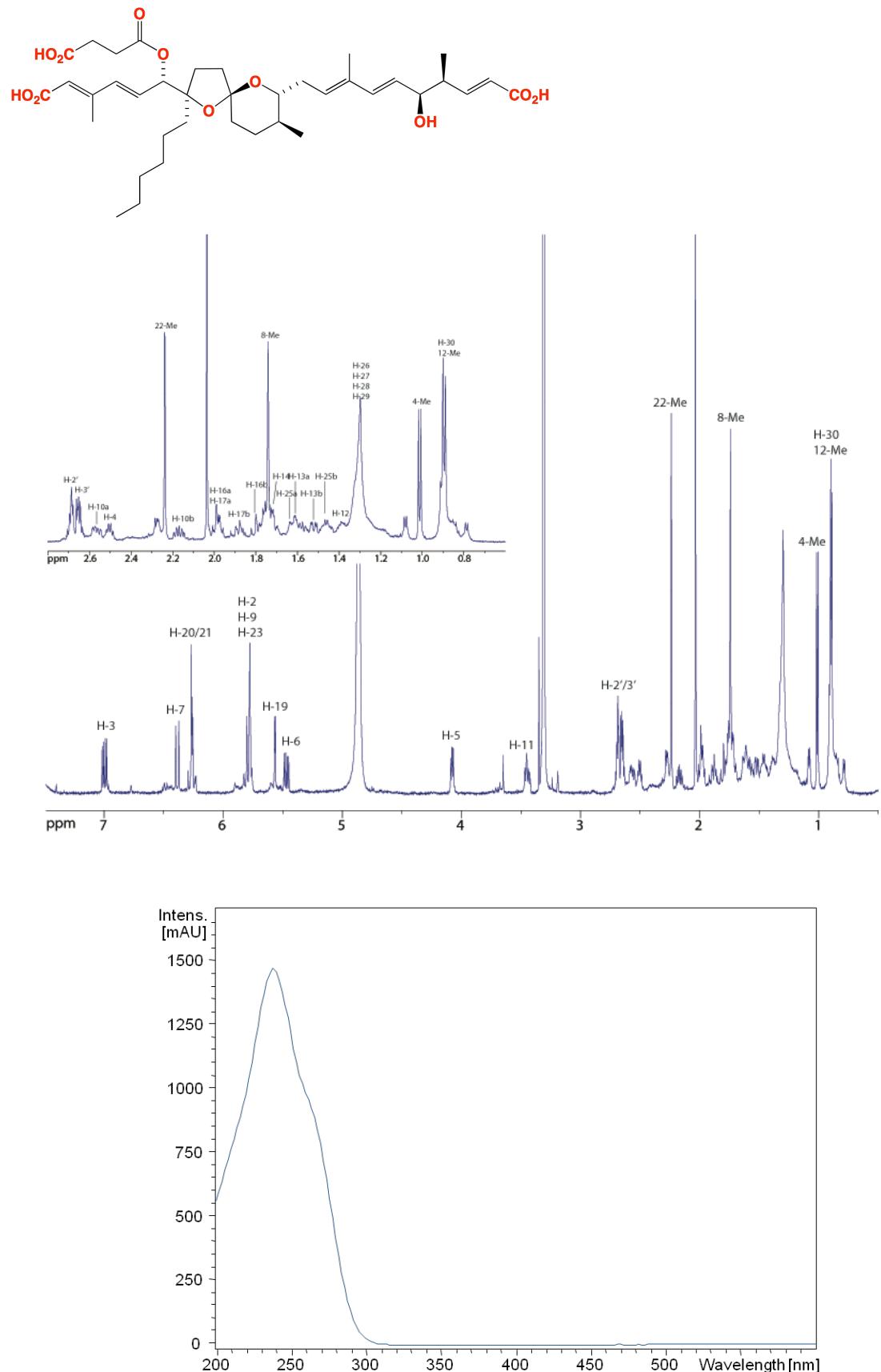


Figure S7. Upper panel: ¹H NMR (600 MHz, MeOH-d₄, HPLC-SPE-NMR) spectrum for reveromycin G (7), expansion between 0.6 and 2.8 ppm. Lower panel: UV-vis spectrum of reveromycin G (7) extracted from HPLC diode array detector (MeCN:H₂O).

Table S7. NMR (600 MHz, MeOH-*d*₄, HPLC-SPE-NMR) data for reveromycin G (**7**).

Pos	δ_{H} (m, <i>J</i> (Hz))	$\delta_{\text{C}}^{\text{a}}$	COSY	TOCSY	HMBC (¹ H- ¹³ C)
1		169.7			
2	5.78 ^d (dd, 15.7, 1.2)	120.9	3	3, 4, 4-Me	
3	6.99 (dd, 15.7, 7.5)	152.8	2, 4	2, 4, 4-Me	1, 4
4	2.49 (ddqd, 7.5, 6.8, 5.4, 1.2)	43.9	5, 4-Me	2, 3, 5, 4-Me	
5	4.08 (dd 7.5, 5.4)	76.9	4, 6	4, 6, 7, 4-Me	6
6	5.46 (dd, 15.7, 7.5)	127.1	5, 7	5, 7	
7	6.37 (d, 15.7)	138.4	6	5, 6	5
8		135.1			
9	5.78 ^d (m)	130.7	10a/b, 8-Me	10a/b, 11	
10a	2.57 (m)	32.5	9, 10b	9, 10b, 11	12
10b	2.17 (m)		9, 10a, 11	9, 10a, 11	
11	3.45 (m)	78.4	10a/b, 12	9, 10a/b, 12, 13b, 14, 12-Me	
12	1.39 (m)	35.5	11, 12-Me	11, 13a/b, 14, 12-Me	
13a	1.62 (m)	30.2	13b, 14	12, 13b, 14, 12-Me	
13b	1.52 (m)		13a, 14	12, 13a, 14, 12-Me	
14	1.72 (m)	35.2	13a/b	12, 13a/b, 12-Me	12
15		108.9			
16a	1.98 (m)	39.5	16b, 17b	16b, 17a/b	18
16b	1.78 (m)		16a, 17a/b	16a, 17a/b	15
17a	1.98 (m)	32.8	16b, 17b	16a/b, 17b	18
17b	1.88 (m)		16a/b, 17a	16a/b, 17a	15
18		88.7			
19	5.56 (d, 3.6)	80.2	20, 21	20, 21	18, 21
20	6.25 (dd, 15.9, 3.6)	132.4	19, 21	19, 21	
21	6.28 (d, 15.9)	135.7	19, 20	19, 20	
22		152.7			
23	5.78 (d, 0.9)	122.3	22-Me	22-Me	
24		170.3			
25a	1.57 (m)	35.7	25b, 26	25b, 26, 27, 28, 29, 30	
25b	1.46 (m)		25a, 26	25a, 26, 27, 28, 29, 30	
26	1.30 (m)	30.7	25a/b	25a/b, 30	
27	1.30 (m)		23.8	25a/b, 30	
28	1.30 (m)		32.9	25a/b, 30	
29	1.30 (m)	23.8	30	25a/b, 30	
30	0.90 (m)	14.2	29	26, 27, 28, 29	27, 28, 29
4-Me	1.01 (d, 6.8)	15.0	4	2, 3, 4, 5	3, 4, 5
8-Me	1.74 (s)	12.6	9	10a/b, 11, 12	7, 8, 9
12-Me	0.90 (m)	18.0	12	10a/b, 11, 12, 13a/b, 14	11, 12, 13
22-Me	2.24 (d, 0.9)	13.7	23	23	21, 22, 23
1'		176.0 ^c			
2'	2.68 ^b (m)	30.1			1', 4'
3'	2.65 ^b (m)	29.6			1', 4'
4'		173.4 ^c			

(a) ¹³C assignments obtained from gHSQC and gHMBC data. (b-c) Assignments interchangeable. (d) Overlapping resonances

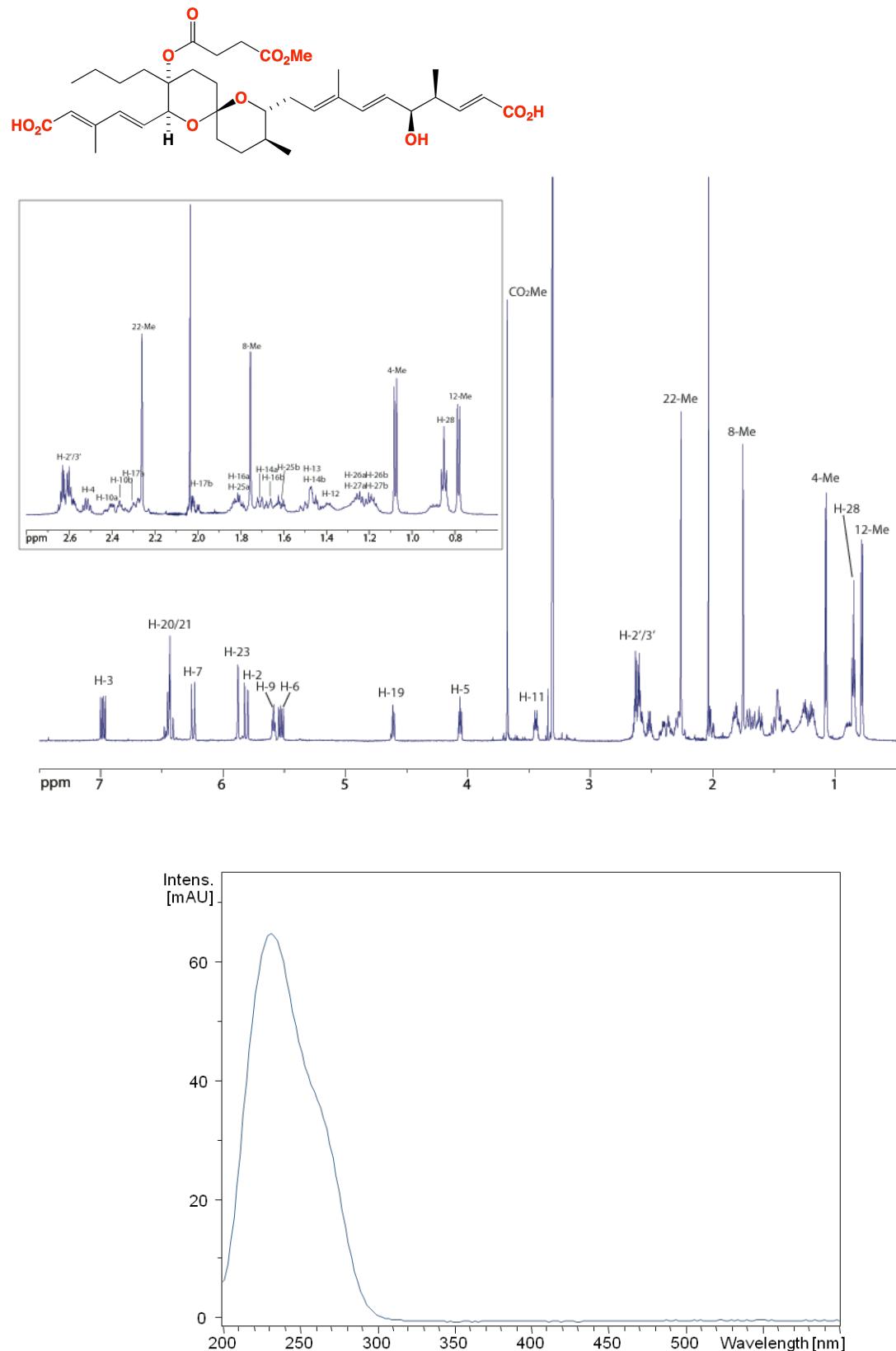


Figure S8. Upper panel: Presat ¹H NMR (600 MHz, MeOH-*d*₄, irr δ_H 4.87, HPLC-SPE-NMR) spectrum for reveromycin A 4'-methyl ester (**8**), expansion between 0.6 and 2.8 ppm. Lower panel: UV-vis spectrum of reveromycin A 4'-methyl ester (**8**) extracted from HPLC diode array detector (MeCN:H₂O)

Table S8. NMR (600 MHz, MeOH-*d*₄, HPLC-SPE-NMR) data for reveromycin A 4'-methyl ester (**8**).

Pos	δ_{H} (m, J (Hz))	$\delta_{\text{C}}^{\text{a}}$	COSY	TOCSY	HMBC (^1H - ^{13}C)
1		169.5			
2	5.81 (dd, 15.7, 1.2)	122.3	3	3, 4, 4-Me	1, 4
3	6.98 (dd, 15.7, 7.7)	152.8	2, 4	2, 4, 5, 4-Me	1
4	2.53 (dqdd, 7.7, 6.8, 5.8, 1.2)	43.9	3, 5, 4-Me	2, 3, 5, 4-Me	3, 5, 4-Me
5	4.07 (dd, 7.3, 5.8)	76.7	3, 6	4, 6, 7, 4-Me	4, 6, 7
6	5.53 (dd, 15.7, 7.3)	127.8	5, 7	4, 5, 6, 4-Me	4, 8
7	6.24 (d, 15.7)	137.7	6	4, 5, 6	5, 8, 9, 8-Me
8		135.1			
9	5.59 (dd, 7.7, 6.8)	129.4	10a/b	10a/b, 11	8, 8-Me
10a	2.41 (ddd, 15.7, 7.7, 3.8)	32.7	9, 11	9, 11, 8-Me	
10b	2.37 (ddd, 15.7, 6.8, 4.3)		9, 11	9, 11, 8-Me	
11	3.45 (ddd, 10.1, 4.3, 3.8)	76.2	10a/b, 12	9, 10a/b, 13, 12-Me	9
12	1.39 (m)	34.6	13, 12-Me	13, 14a/b	
13	1.48 (m)	28.5	12, 14a	12, 14a, 12-Me	
14a	1.72 (m)	36.8	13, 14b	13, 14b	
14b	1.46 (m)		14a	12, 14a, 12-Me	
15		96.7			
16a	1.82 (m)	35.1	16b, 17b	16b, 17a/b	
16b	1.61 (ddd, 13.6, 4.3, 3.9)		16a	16a, 17a/b	
17a	2.28 (m)	25.1	17b	16a/b, 17b	
17b	2.02 (m)		16a, 17a	16a/b, 17a	
18		84.2			
19	4.62 (d, 8.3)	79.5	20, 21	20, 21	15, 18, 20, 21
20	6.46 (dd, 15.6, 8.3)	133.9	19	19	19, 22, 23, 22-Me
21	6.44 (d, 15.6)	139.2	19	19	19, 20, 22, 23, 22-Me
22		151.6			
23	5.88 (d, 1.1)	121.4		22-Me	21, 24, 22-Me
24		169.5			
25a	1.81 (m)	32.8	25b, 26b	25b, 26a/b, 27a/b	
25b	1.66 (m)		25a, 26a	25a, 26a/b, 27a/b	
26a	1.26 (m)	23.8	25b	25a/b, 27a/b, 28	
26b	1.20 (m)		25a	25a/b, 28	
27a	1.26 (m)	23.4	28	25a/b, 28	
27b	1.20 (m)		28	25a/b, 28	
28	0.86 (t, 6.9)	14.0	27a	25a/b, 26a/b, 27a/b	26, 27
4-Me	1.08 (d, 6.8)	15.0	4	2, 3, 4, 5	3, 4, 5
8-Me	1.76 (s)	12.8		10a/b	7, 8, 9
12-Me	0.78 (d, 6.5)	17.7		11, 12, 13, 14a/b	11, 12, 13
22-Me	2.26 (d, 1.1)	14.4		23	21, 22, 23, 24
1'		176.9			
2'	2.60 ^b (m)	30.9			1', 4'
3'	2.62 ^b (m)	29.6			1', 4'
4'		174.4			
CO ₂ Me	3.68 (s)	51.8			4'

(a) ^{13}C assignments obtained from gHSQC and gHMBC data. (b) Assignments are interchangeable.

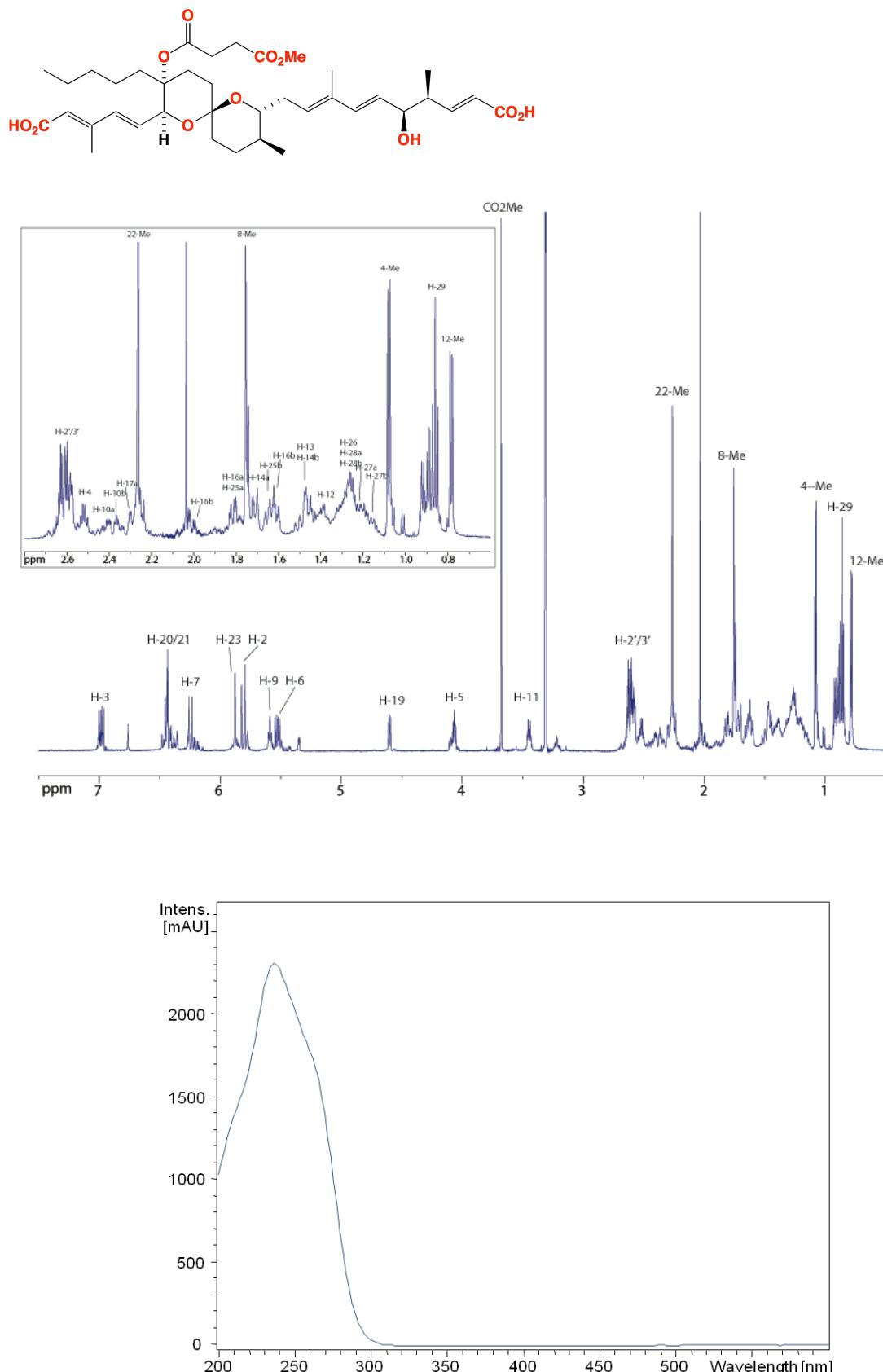


Figure S9. Upper panel: Presat ^1H NMR (600 MHz, $\text{MeOH}-d_4$, irr. δ_{H} 4.87, HPLC-SPE-NMR) spectrum for reveromycin D 4'-methyl ester (**9**), expansion between 0.6 and 2.8 ppm. Lower panel: UV-vis spectrum of reveromycin D 4'-methyl ester (**9**) extracted from HPLC diode array detector ($\text{MeCN}:\text{H}_2\text{O}$).

Table S9. NMR (600 MHz, MeOH-*d*₄, HPLC-SPE-NMR) data for reveromycin D 4'-methyl ester (**9**).

Pos	δ_{H} (m, <i>J</i> (Hz))	$\delta_{\text{C}}^{\text{a}}$	COSY	TOCSY	HMBC (¹ H- ¹³ C)
1		170.3			
2	5.82 (dd, 15.7, 1.2)	122.7	3	3, 4, 4-Me	1, 4, 4-Me
3	6.98 (dd, 15.7, 7.7)	152.5	2, 4	2, 4, 4-Me	1, 2, 4, 5, 4-Me
4	2.52 (ddqd, 7.7, 6.9, 6.8, 1.2)	44.3	3, 5, 4-Me	2, 4-Me	2, 3, 5, 4-Me
5	4.07 (dd, 7.3, 6.9)	77.1	4, 6		3, 4, 6, 7, 4-Me
6	5.54 (dd, 15.7, 7.3)	128.1	5, 7	7	4, 5, 8
7	6.25 (d, 15.7)	137.9	6	6	5, 6, 8, 8-Me
8		135.6			
9	5.59 (dd, 7.7, 6.8)	129.6	10a/b, 8-Me	10a/b, 11, 8-Me	7, 8-Me
10a	2.41 (ddd, 15.7, 7.7, 3.8)	32.9	9, 11	9, 11, 8-Me	9
10b	2.37 (ddd, 15.7, 6.8, 4.3)		9, 11	9, 11, 8-Me	8, 9, 11
11	3.45 (ddd, 10.1, 4.3, 3.8)	76.4	10a/b, 12	9, 10a/b, 12, 12-Me	9, 29
12	1.39 (m)	34.9	13, 12-Me	13, 14a, 12-Me	
13	1.47 (m)	28.8	14a	12, 14a, 12-Me	12, 15
14a	1.71 (m)	37.1	13, 14b	12, 13, 14b, 12-Me	12, 15
14b	1.45 (m)		14a	14a, 12-Me	12, 15
15		97.1			
16a	1.80 (ddd, 13.6, 13.6, 4.3)	33.2	16b, 17a/b	16b, 17a/b	15, 17, 18
16b	1.62 (ddd, 13.6, 4.3, 3.9)		16a, 17a/b	16a, 17a/b	15, 17, 18
17a	2.31 (m)	25.6	16a/b, 17b	16a/b, 17b	16
17b	2.02 (ddd, 13.8, 13.6, 4.3)		16a/b, 17a	16a/b, 17a	15, 16
18		84.5			
19	4.61 (d, 8.2)	79.9	20, 21	20, 21	15, 18, 20, 21
20	6.46 (dd, 15.6, 8.2)	134.2	19	19	19, 21, 22, 23
21	6.44 (d, 15.6)	139.4	19	19	19, 20, 22, 23
22		152.9			
23	5.88 (d, 0.9)	121.9	22-Me	21, 22-Me	21, 24, 22-Me
24		170.4			
25a	1.81 (ddd, 13.6, 13.6, 4.3)	35.6	25b, 26	25b, 26, 27a/b	
25b	1.64 (ddd, 13.6, 4.3, 3.9)		25a, 26	25a, 26, 27a/b	27
26	1.22 (m)	22.6	25a/b	25a/b, 29	
27a	1.20 (m)	33.1		25a/b, 29	
27b	1.16 (m)			25a/b, 26, 28a/b, 29	28
28a	1.28 (m)	23.5	29	25a/b, 29	27
28b	1.25 (m)		29	25a/b, 27a/b, 29	27
29	0.87 (t, 7.2)	14.4		26, 27a/b, 28a/b	27, 28
4-Me	1.08 (d, 6.8)	15.4	4	2, 3, 4	3, 4, 5
8-Me	1.76 (s)	13.2	9	9, 10a/b	7, 8, 9
12-Me	0.79 (d, 6.5)	18.2		11, 12, 13	11, 12, 13
22-Me	2.26 (d, 0.9)	14.8	23		20, 21, 22, 23
1'		173.3			
2'	2.63 ^b (m)	31.2			1', 3', 4'
3'	2.60 ^b (m)	29.9			1', 2', 4'
4'		174.7			
CO ₂ Me	3.68 (s)	52.4			4'

(a) ¹³C assignments obtained from gHSQC and gHMBC data. (b) Assignments are interchangeable.

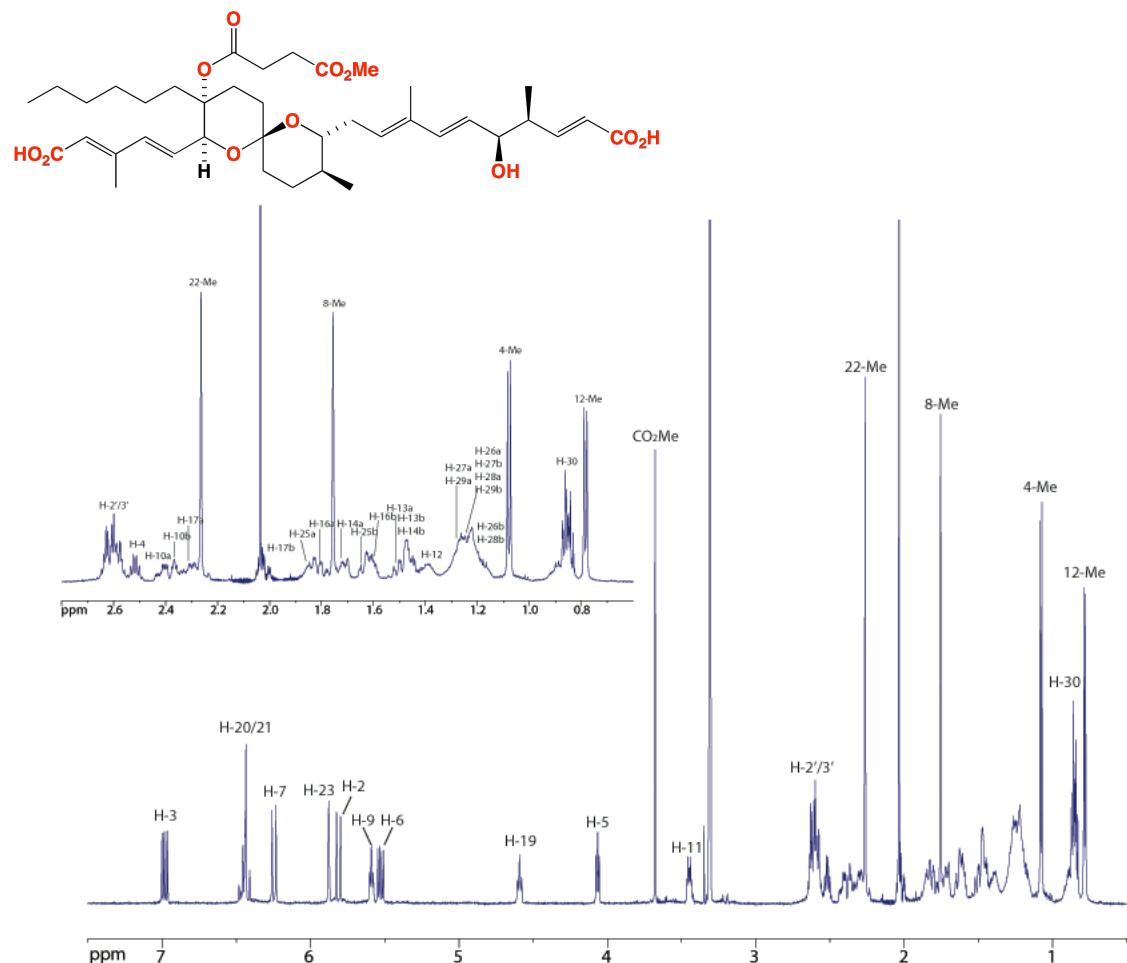


Figure S10. Upper panel: Presat ¹H NMR (600 MHz, MeOH-*d*₄, irr δ_H 4.84, HPLC-SPE-NMR) spectrum for reveromycin E 4'-methyl ester (**10**), expansion between 0.6 and 2.8 ppm. Lower panel: UV-vis spectrum of reveromycin E 4'-methyl ester (**10**) extracted from HPLC diode array detector (MeCN:H₂O)

Table S10. NMR (600 MHz, MeOH-*d*₄, HPLC-SPE-NMR) data for reveromycin E 4'-methyl ester (**10**).

Pos	δ_{H} (m, J (Hz))	$\delta_{\text{C}}^{\text{a}}$	COSY	TOCSY	HMBC (¹ H- ¹³ C)
1		170.2			
2	5.81 (dd, 15.7, 1.1)	122.3	3	3, 4, 5, 4-Me	
3	6.98 (dd, 15.7, 7.7)	152.9	2, 4	2, 4, 5, 4-Me	1, 4
4	2.52 (dqdd, 7.7, 6.8, 5.9, 1.1)	44.0	3, 5, 4-Me	2, 3, 5, 7, 4-Me	2, 5, 4-Me
5	4.07 (dd, 7.3, 5.9)	76.8	4, 6	4, 6, 7, 4-Me	3, 7
6	5.54 (dd, 15.6, 7.3)	127.8	5, 7	4, 5, 7, 4-Me	5, 8, 8-Me
7	6.26 (d, 15.6)	137.7	6	4, 5, 6	5, 8, 9, 8-Me
8		135.6			
9	5.59 (dd, 7.7, 6.8)	129.3	10a/b	10a/b, 11, 8-Me	7, 10, 8-Me
10a	2.43 (ddd, 15.7, 7.7, 3.8)	32.8	9, 11	9, 11	
10b	2.37 (ddd, 15.7, 6.8, 4.3)		9, 11	9, 11	
11	3.45 (ddd, 10.1, 4.3, 3.8)	76.2	10a/b, 12	10a/b, 12, 13a/b, 14a, 12-Me	10, 12
12	1.39 (m)	34.8	11	13a/b, 14a/b, 12-Me	11
13a	1.52 (m)	28.3	14a	12, 14a/b, 12-Me	
13b	1.44 (m)		14a/b	12 14a/b, 12-Me	
14a	1.71 (m)	36.7	13b, 14b	13a/b, 14b	
14b	1.48 (m)		13b, 14a	12, 13a/b, 14a, 12-Me	
15		96.3			
16a	1.82 (m)	32.5	16b, 17b	16b, 17a/b	
16b	1.62 (m)		16a	16a, 17a/b	
17a	2.30 (m)	25.3	17b	16a/b, 17b	
17b	2.02 (m)		16a, 17a	16a/b, 17a	
18		84.0			
19	4.59 (d, 8.4)	79.7	20, 21	20, 21	15, 18, 20, 21
20	6.46 (dd, 15.6, 8.4)	134.2	19	19	19, 21, 22
21	6.44 (d, 15.6)	139.2	19	19	19, 20, 22
22		152.5			
23	5.88 (s)	121.6		22-Me	21, 22, 24
24		169.3			
25a	1.86 (m)	35.8	25b, 26a/b	25b, 26a/b, 27a/b	26
25b	1.60 (m)		25a, 26a/b	25a, 26a/b, 27a/b	
26a	1.25 (m)	30.3	25a/b	25a/b, 30	28
26b	1.21 (m)		25a/b	25a/b, 30	
27a	1.29 (m)	23.8		25a/b, 30	
27b	1.25 (m)			25a/b, 30	
28a	1.25 (m)	32.4		25a/b, 30	
28b	1.21 (m)			25a/b, 30	
29a	1.29 (m)	23.8	30	25a/b, 30	
29b	1.25 (m)		30	25a/b, 30	
30	0.85 (m)	14.3	29a/b	26a/b, 27a/b, 28a/b, 29a/b	
4-Me	1.07 (d, 6.8)	15.0	4	2, 3, 4, 5, 7	3, 4, 5
8-Me	1.76 (s)	12.6	9	9, 10a/b	7, 8, 9
12-Me	0.78 (d, 6.5)	17.8	12	10a/b, 11, 12, 13a/b, 14a	11, 12, 13
22-Me	2.26 (s)	14.3	23	23	21, 22, 23, 24
1'		174.0			
2'	2.58 ^b (m)	30.9			1', 3'
3'	2.63 ^b (m)	29.5			1', 2'
4'		174.2			
CO ₂ Me	3.68 (s)	51.9			4'

(a) ¹³C assignments obtained from gHSQC and gHMBC data. (b) Assignments are interchangeable.

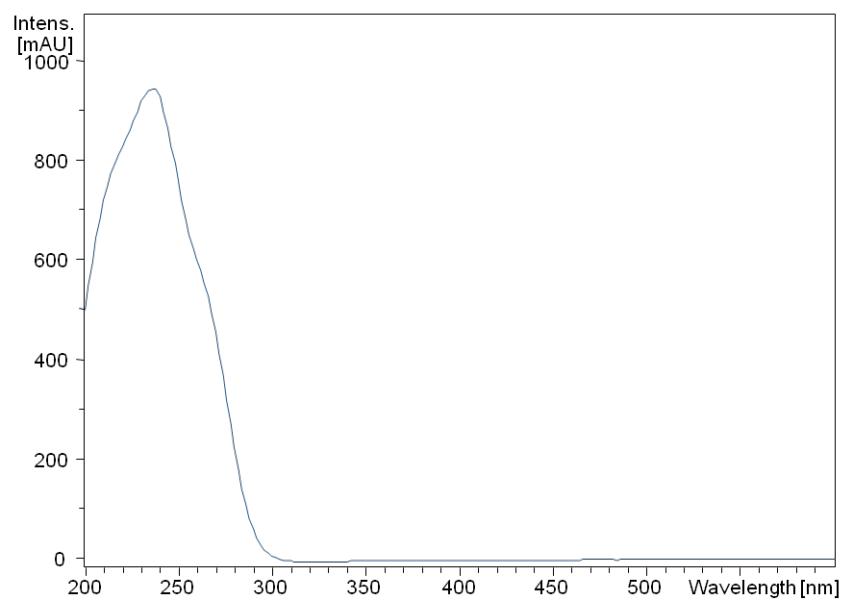
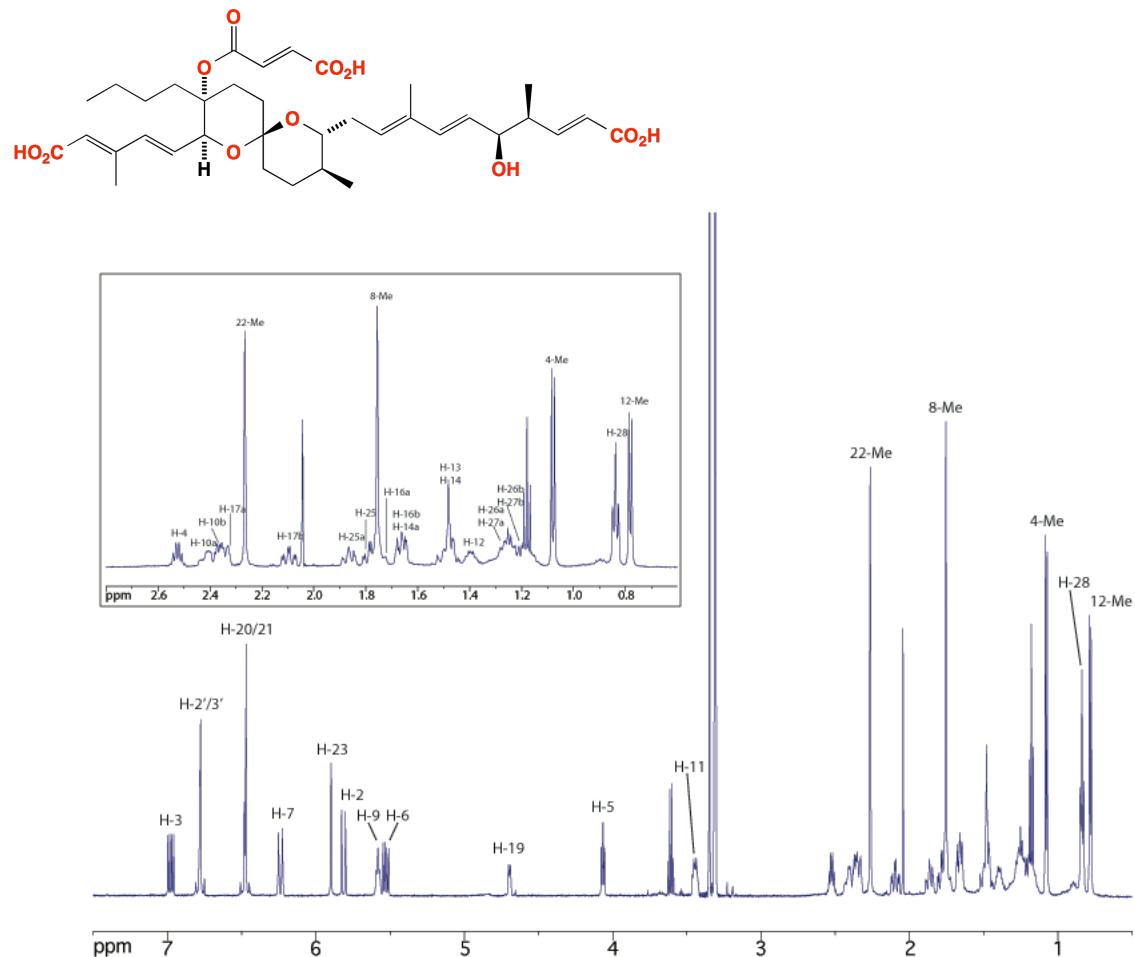


Figure S11. Upper panel: Presat ^1H NMR (600 MHz, $\text{MeOH}-d_4$, irr δ_{H} 4.87, HPLC-SPE-NMR) spectrum for reveromycin H (11), expansion between 0.6 and 2.8 ppm. Lower panel: UV-vis spectrum of reveromycin H (11) extracted from HPLC diode array detector ($\text{MeCN}:\text{H}_2\text{O}$)

Table S11. NMR (600 MHz, MeOH-*d*₄, HPLC-SPE-NMR) data for reveromycin H (**11**).

Pos	δ_{H} (m, <i>J</i> (Hz))	$\delta_{\text{C}}^{\text{a}}$	COSY	TOCSY	HMBC (¹ H- ¹³ C)
1		170.3			
2	5.83 (d, 15.7)	122.7	3	3, 4, 4-Me	1
3	6.99 (dd, 15.7, 7.7)	153.8	2, 4	2, 4, 4-Me	1
4	2.53 (dq, 7.7, 6.8, 6.8)	44.3	3, 5, 4-Me	2, 3, 5, 4-Me	
5	4.07 (dd, 7.3, 6.8)	77.1	4, 6	4, 6, 7, 4-Me	
6	5.55 (dd, 15.6, 7.3)	128.1	5, 7	5, 7	
7	6.25 (d, 15.6)	138.0	6	5, 6	5, 6, 8, 12-Me
8		135.4			
9	5.58 (dd, 7.3, 6.8)	129.5	10a/b	10a/b, 11	
10a	2.40 (m)	34.9	9	9, 11	
10b	2.36 ^d (m)		9	9, 11	
11	3.43 (m)	76.5	12	9, 10a/b, 12, 12-Me	
12	1.38 (m)	34.9	11	11	
13a	1.53 (m)	29.7			
13b	1.49 (m)			10b, 11, 14a, 12-Me	
14a	1.65 (m)	37.3		11, 14b, 12-Me	
14b	1.47 (m)				
15		97.1			
16a	1.78 (m)	33.0	16b, 17a/b	17a/b	
16b	1.69 (m)		16a, 17a/b	17a/b	
17a	2.33 ^d (m)	25.6	16a/b, 17b	16a/b, 17b	
17b	2.09 (ddd, 14.2, 13.6, 4.4)		16a/b, 17a	16a/b, 17a	
18		85.4			
19	4.70 (d, 7.0)	79.9	20, 21	20, 21	20, 21
20	6.48 (dd, 15.5, 7.0)	134.1	19	19	22
21	6.46 (d, 15.5)	139.8	19	19	22
22		153.0			
23	5.89 (s)	122.0		22-Me	1
24		170.3			
25a	1.86 (m)	35.3	26b, 27b	26a/b, 27a/b	
25b	1.80 (m)			26a/b, 27a/b	
26a	1.25 (m)	25.4		26b, 27b, 28	
26b	1.19 (m)			26a, 27a, 28	
27a	1.25 (m)	24.3		26b, 27b, 28	
27b	1.19 (m)				
28	0.85 (t, 7.0)	14.3	26a, 27a	25a/b, 26a/b, 27a/b	26, 27
4-Me	1.08 (d, 6.8)	15.4	4	2, 3, 5	3, 4, 5
8-Me	1.75 (s)		13.2	9	7, 8, 9
12-Me	0.78 (d, 6.6)	18.1	12	11, 12	11, 12, 13
22-Me	2.26 (s)	14.8		23	21, 22, 23, 24
1'		165.9 ^c			
2'	6.81 ^b (d, 16.0)	135.7			1', 2', 3', 4'
3'	6.78 ^b (d, 16.0)	135.7			1', 2', 3', 4'
4'		169.4 ^c			

(a) ¹³C assignments obtained from gHSQC and gHMBC data. (b-c) Assignments are interchangeable. (d) Overlapping resonances

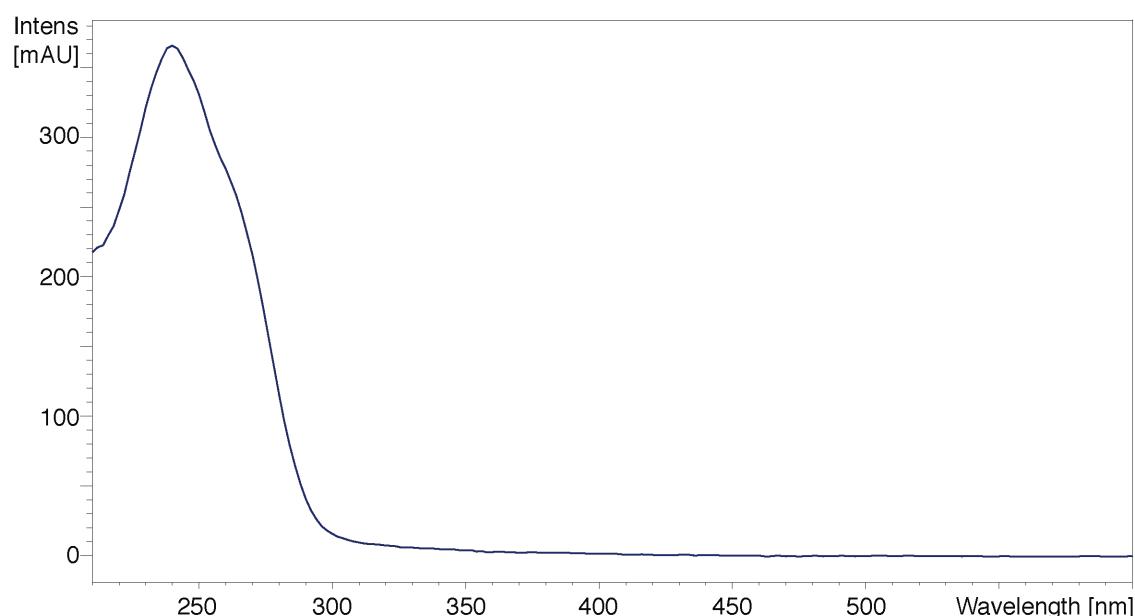
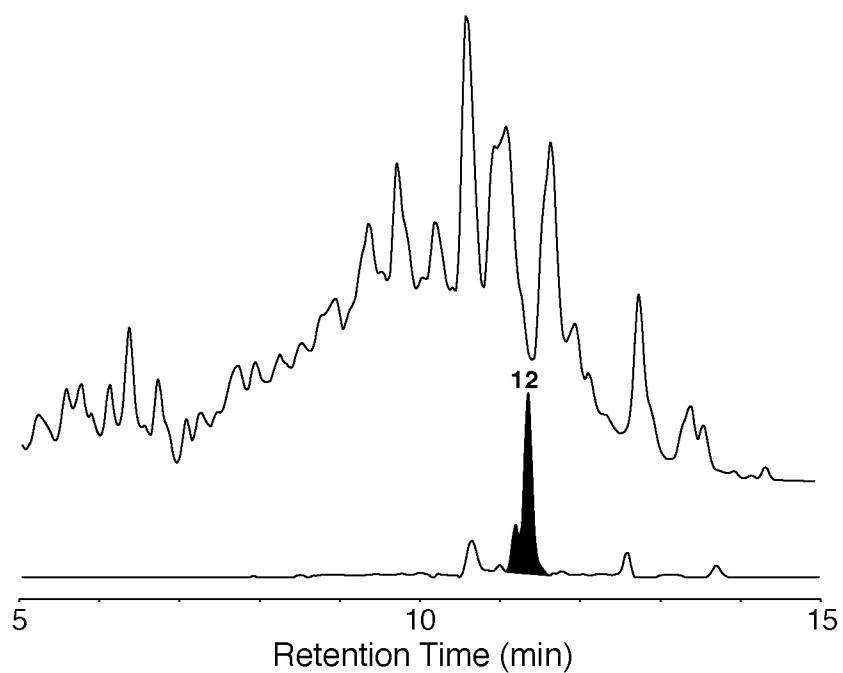


Figure S12. Upper panel: Total negative ion chromatogram from HPLC-MS separation of crude extract of *Streptomyces* sp. MST-RA7781 overlaid with extracted negative ion chromatogram (m/z 671) corresponding to reveromycin I (**12**). Lower panel: UV-vis spectrum of reveromycin I (**12**) extracted from HPLC diode array detector (MeCN:H₂O).

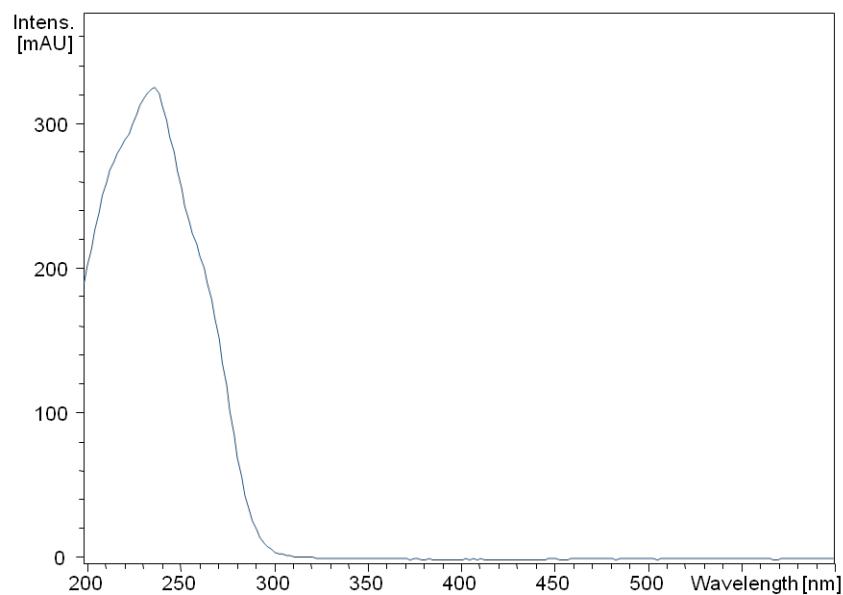
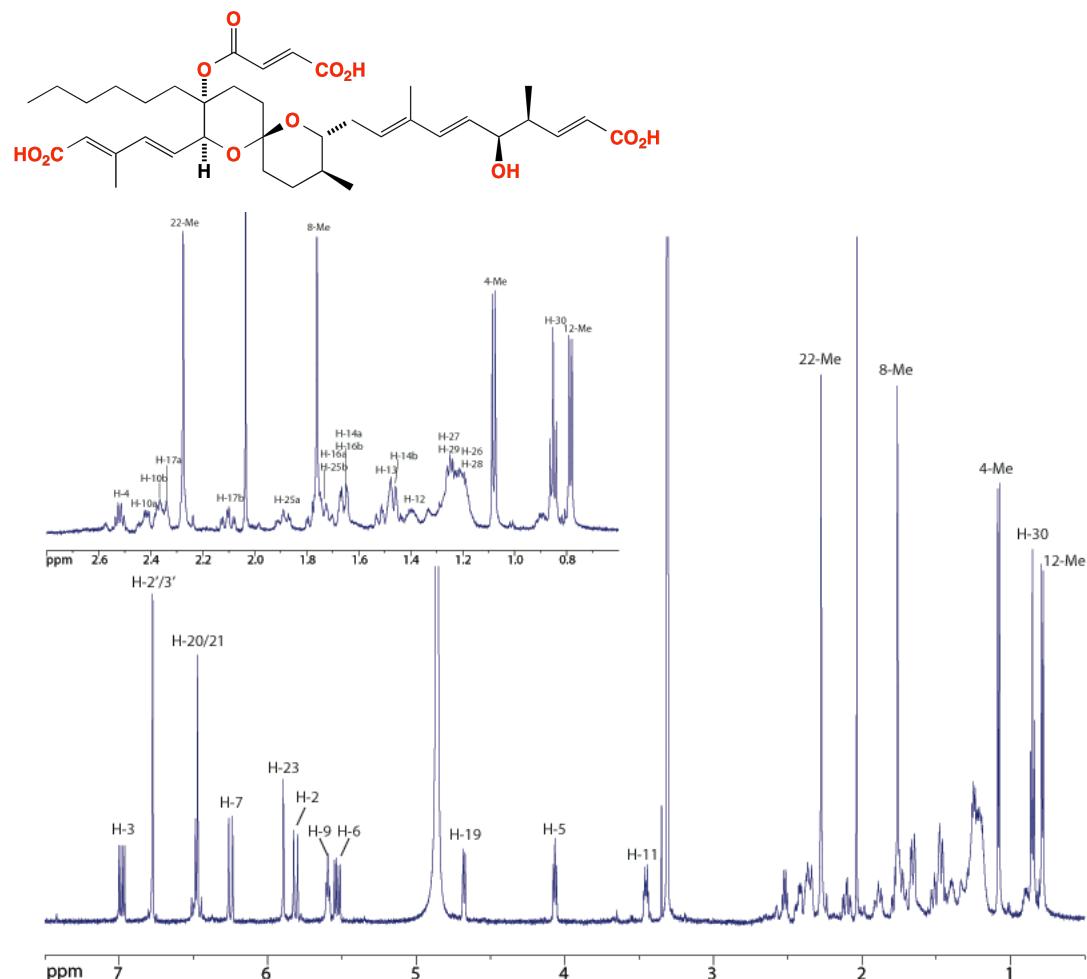


Figure S13. Upper panel: ¹H NMR (600 MHz, MeOH-*d*₄, HPLC-SPE-NMR) spectrum of reveromycin J (**13**), expansion between 0.6 and 2.8 ppm. Lower panel: UV-vis spectrum of reveromycin J (**13**) extracted from HPLC diode array detector (MeCN:H₂O).

Table S13. NMR (600 MHz, MeOH-*d*₄, HPLC-SPE-NMR) data for reveromycin J (**13**).

Pos	δ_{H} (m, <i>J</i> (Hz))	$\delta_{\text{C}}^{\text{a}}$	COSY	TOCSY	HMBC (¹ H- ¹³ C)
1		169.3			
2	5.80 (dd, 15.8, 1.1)	122.5	3	3, 4, 5, 4-Me	3
3	6.99 (dd, 15.8, 7.7)	152.5	2, 4	2, 4, 4-Me	1
4	2.56 (ddqd, 7.7, 6.8, 5.8, 1.1)	44.0	3, 5, 4-Me	2, 6, 7, 4-Me	
5	4.07 (dd, 7.2, 5.8)	76.7	4, 6		
6	5.53 (dd, 15.6, 7.2)	127.8	5, 7	5, 7	
7	6.26 (d, 15.6)	137.6	6	4, 5, 6	5, 6
8		135.0			
9	5.60 (dd, 7.7, 6.8)	129.1	10a/b, 8-Me	8, 10a/b, 11	
10a	2.41 (ddd, 15.7, 7.7, 3.9)	32.8	9, 10b, 11	9, 10b, 11	
10b	2.38 ^c (m)		9, 10a, 11	9, 10a, 11	
11	3.45 (ddd, 10.0, 4.3, 3.9)	76.2	10a, 12	9, 10a/b, 13, 14b, 12-Me	
12	1.40 (m)	34.6	11, 12-Me	11, 13, 14a/b	
13	1.48 (m)	28.5	14a/b	11, 12, 14a/b	
14a	1.67 (m)	36.9	13, 14b	11, 12, 13, 14b	
14b	1.45 (m)		13, 14a	11, 12, 13, 14a	
15		N.O.			
16a	1.77 (m)	32.7	16b, 17a/b	16b, 17a/b	
16b	1.67 (m)		16a	16a, 17a/b	
17a	2.35 ^c (m)	25.2	17b	16a/b, 17b	
17b	2.09 (ddd, 14.2, 13.6, 4.3)		16a/b, 17a	16a/b, 17a	
18		N.O.			
19	4.68 (d, 7.9)	79.6	20, 21	20, 21	
20	6.47 (dd, 15.6, 7.9)	133.5	19, 21	19, 21	
21	6.49 (d, 15.6)	139.5	19, 20	19, 20	
22		151.9			
23	5.90 (d, 0.9)	121.5	22-Me	22-Me	
24		N.O.			
25a	1.87 (m)	35.3	25b, 26	25b, 26, 27, 28, 29, 30	
25b	1.72 (m)		25a, 26	25a, 26, 27, 28, 29, 30	
26	1.23 (m)	30.2	25a/b	25a/b, 30	
27	1.26 (m)	23.0		25a/b, 30	
28	1.20 (m)	32.5		25a/b, 30	
29	1.26 (m)	23.0	30	25a/b, 30	
30	0.85 (t, 6.9)	14.1	29	25a/b, 26, 27, 28, 29	
4-Me	1.08 (d, 6.8)	14.9	4	2, 3, 4, 5	3, 4, 5
8-Me	1.76 (s)	12.8	9	9	7, 8, 9
12-Me	0.79 (d, 6.5)	17.8	12	10a/b, 11, 12, 13, 14a/b	11
22-Me	2.27 (d, 0.9)	14.5	23	23	21, 22, 23
1'		165.2 ^b			
2'	6.78 (s)	134.9			1', 4'
3'	6.78 (s)	134.9			1', 4'
4'		168.1 ^b			

(a) ¹³C assignments obtained from gHSQC and gHMBC data. (b) Assignments are interchangeable. (c) Overlapping resonances. N.O. = not observed

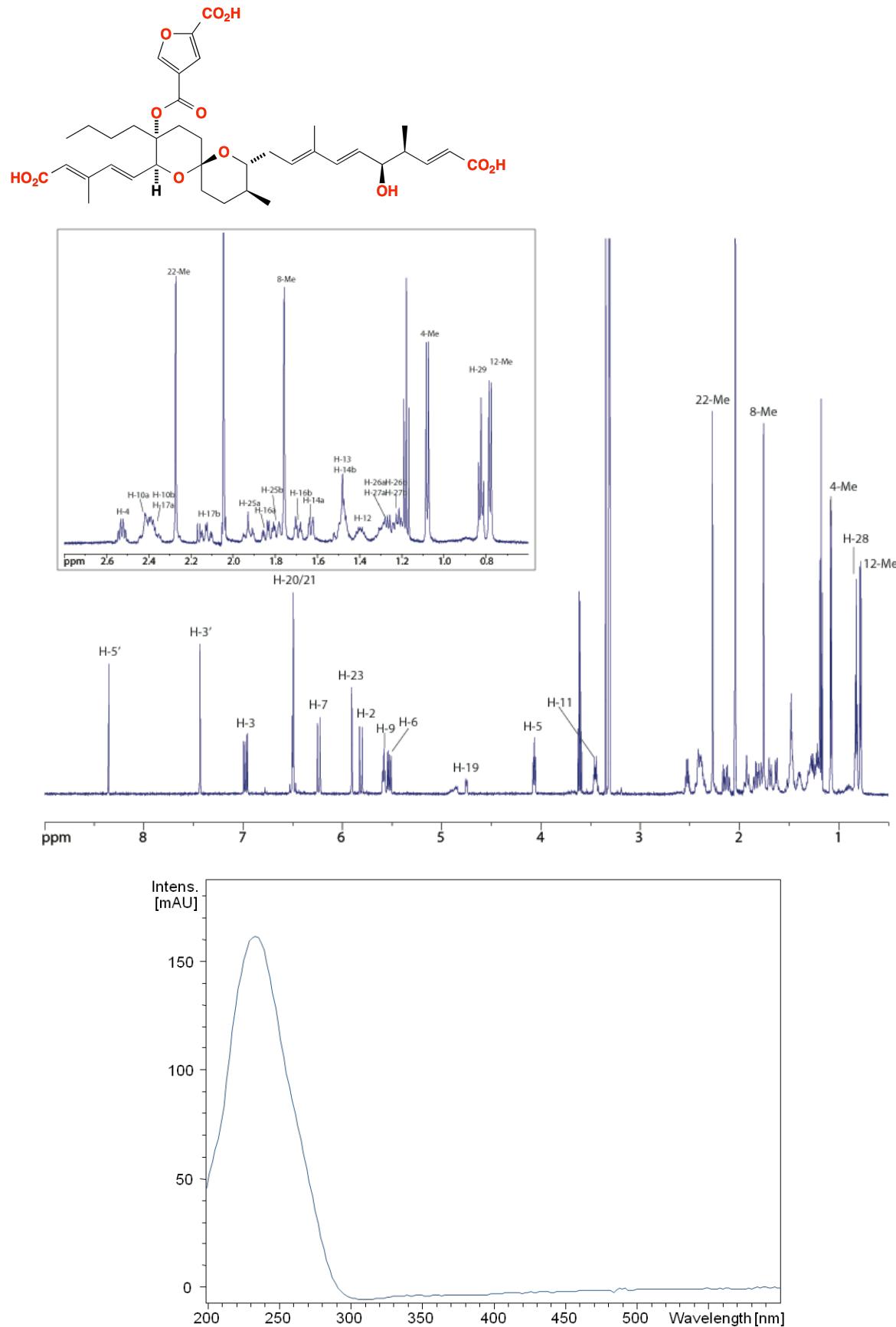


Figure S14: Upper panel: Presat ^1H NMR (600 MHz, MeOH- d_4 , irr δ_{H} 4.87, HPLC-SPE-NMR) spectrum of reveromycin K (**14**), expansion between 0.6 and 2.8 ppm. Lower panel: UV-vis spectrum of reveromycin K (**14**) extracted from HPLC diode array detector (MeCN:H₂O).

Table S14. NMR (600 MHz, MeOH-*d*₄, HPLC-SPE-NMR) data for reveromycin K (**14**).

Pos	δ_{H} (m, J (Hz))	$\delta_{\text{C}}^{\text{a}}$	COSY	TOCSY	HMBC (¹ H- ¹³ C)
1		169.9			
2	5.83 (dd, 15.7, 1.2)	122.7	3	3, 4, 5, 4-Me	1, 4
3	6.98 (dd, 15.7, 7.7)	153.0	2, 4	2, 4, 5, 4-Me	1, 4-Me
4	2.53 (dqdd, 7.8, 6.8, 6.8, 1.2)	44.3	3, 4-Me	2, 3, 5, 4-Me	3
5	4.07 (dd, 7.4, 6.8)	77.1	6	4, 6, 7, 4-Me	3, 7, 4-Me
6	5.54 (dd, 15.6, 7.4)	128.1	5, 7	4, 5, 7, 4-Me	6, 7
7	6.25 (d, 15.6)	138.0	6	4, 5, 6	5, 6, 8-Me
8		134.2			
9	5.58 (dd, 7.7, 6.8)	129.5	10a/b	7, 10a/b, 11	8-Me
10a	2.42 ^c (m)	33.1	9	9, 10b, 11	
10b	2.38 ^c (m)		9	9, 10a, 11	
11	3.46 (ddd, 10.1, 4.3, 3.8)	76.5	12	9, 10a/b, 13, 14a/b, 12-Me	
12	1.40 (m)	34.5	8-Me	14a	
13	1.48 (m)	28.9	14a/b, 12	12, 14a, 12-Me	
14a	1.63 (m)	37.3	13, 14b	13, 14b	
14b	1.48 (m)		13	12, 14a, 12-Me	
15		97.1			
16a	1.82(ddd, 13.7, 13.6, 4.4)	32.7	16b, 17b	16b, 17a/b	
16b	1.69 (ddd, 13.7, 4.4, 3.7)		16a, 17b	16a, 17a/b	
17a	2.41 ^c (m)	25.4	17b	16a/b, 17b	
17b	2.13 (ddd, 14.2, 13.7, 4.4)		16a, 17a	16a/b, 17a	
18		85.2			
19	4.67 (d, 6.8)	80.1	20, 21	20, 21	21
20	6.49 (dd, 15.5, 6.8)	134.1	19, 21	19, 21	
21	6.50 (d, 15.5)	139.8	19, 20	19, 20	
22		152.3			
23	5.91 (d, 1.0)	121.9		22-Me	22, 22-Me
24		170.2			
25a	1.93 (m)	35.7	25b, 26a/b	25b, 26a/b, 27a/b, 28	
25b	1.79 (m)		25a, 26a/b	25a, 26a/b, 27a/b, 28	
26a	1.27 (m)	24.3	25a/b	25a/b, 28	
26b	1.25 (m)		25a/b	25a/b, 28	
27a	1.27 (m)	23.9	28	25a/b, 28	
27b	1.25 (m)		28	25a/b, 28	
28	0.84 (t, 6.8)	14.1	27a/b	25a/b, 26a/b, 27a/b	26, 27
4-Me	1.09 (d, 6.8)	15.4	4	2, 3, 4, 5, 6	3, 4, 5
8-Me	1.76 (s)	13.2		9, 10a/b	7, 8, 9
12-Me	0.78 (d, 6.4)	18.1	12	11	11, 12, 13
22-Me	2.27 (d, 1.0)	14.3		23	21, 22, 23, 24
1'		162.7 ^b			
2'		147.8			
3'	7.44 (s)	117.8		5'	2', 4', 5'
4'		123.4			
5'	8.36 (s)	152.2		3'	2', 3', 4'
6'		161.1 ^b			

(a) ¹³C assignments obtained from gHSQC and gHMBC data. (b) Assignments are interchangeable. (c) Overlapping resonances.

Table S14a. NMR (400 MHz, MeOH-*d*₄) data for reveromycin K trimethyl ester (**14a**).

Pos	δ_{H} (m, J (Hz))	$\delta_{\text{C}}^{\text{a}}$	COSY	HMBC (¹ H- ¹³ C)
1		168.0		
2	5.88 (d, 1.0, 15.8)	122.0	3	1, 4
3	7.02 (dd, 7.6, 15.8)	151.0	2, 4	1
4	2.58 (m)	43.0	3, 5, 4-Me	
5	4.11 (m)	77.0	4, 6	
6	5.51 (dd, 7.6 15.9)		5, 7	
7	6.22 (d, 15.9)	138.0	6	5, 9, 8-Me
8		135.0		
9	5.54 (m)	130.0	10, 8-Me	8
10	2.39 (m)		9, 11	
11	3.41 (m)	76.0	10, 12	
12	1.37 (m)	34.0	11, 14b, 12-Me	
13	1.30-1.50 (m)	28.0	14b	
14a	1.45 (m)		14b	
14b	1.63 (m)		12, 13/14a	
15				
16	1.67-1.90 (m)		17a, 17b	
17a	2.02 (m)		16, 17b	
17b	2.35 (m)		16, 17a	
18				
19	4.72 (d, 8.2)	79.0	20/21	
20	6.39 (m)	133.0	19	19, 22
21	6.39 (m)	139.0	19	19, 22
22		152.0		
23	5.85 (d, 0.9)	120.0	22-Me	22-Me
24		168.0		
25a	1.77 (m)		26, 27	
25b	1.81 (m)		26, 27	
26	1.07-1.30 (m)	25.0	25a/b, 2	
27	1.07-1.30 (m)	24.0	25a/b, 28	
28	0.80 (t, 7.0)	14.0	26, 27	26, 27
1'				
2'		145.0		
3'	7.44 (s)	117.0		2', 4', 5'
4'		122.0		
5'	8.08 (s)	150.0		2', 3', 4'
6'		159.0		
4-Me	1.09 (d, 7.0)	15.0	4	3, 4, 5
8-Me	1.72 (s)	13.0		7, 8, 9
12-Me	0.76 (d, 6.4)	18.0	12	11, 12, 13
22-Me	2.25 (d, 0.9)	14.5	23	21, 22, 23
CO ₂ Me	3.74 (s)	51.0		1/24
CO ₂ Me	3.73 (s)	51.0		1/24
4'-CO ₂ Me	3.92 (s)	52.0		4'-CO ₂ Me

(a) ¹³C assignments obtained from gHSQC and gHMBC data.

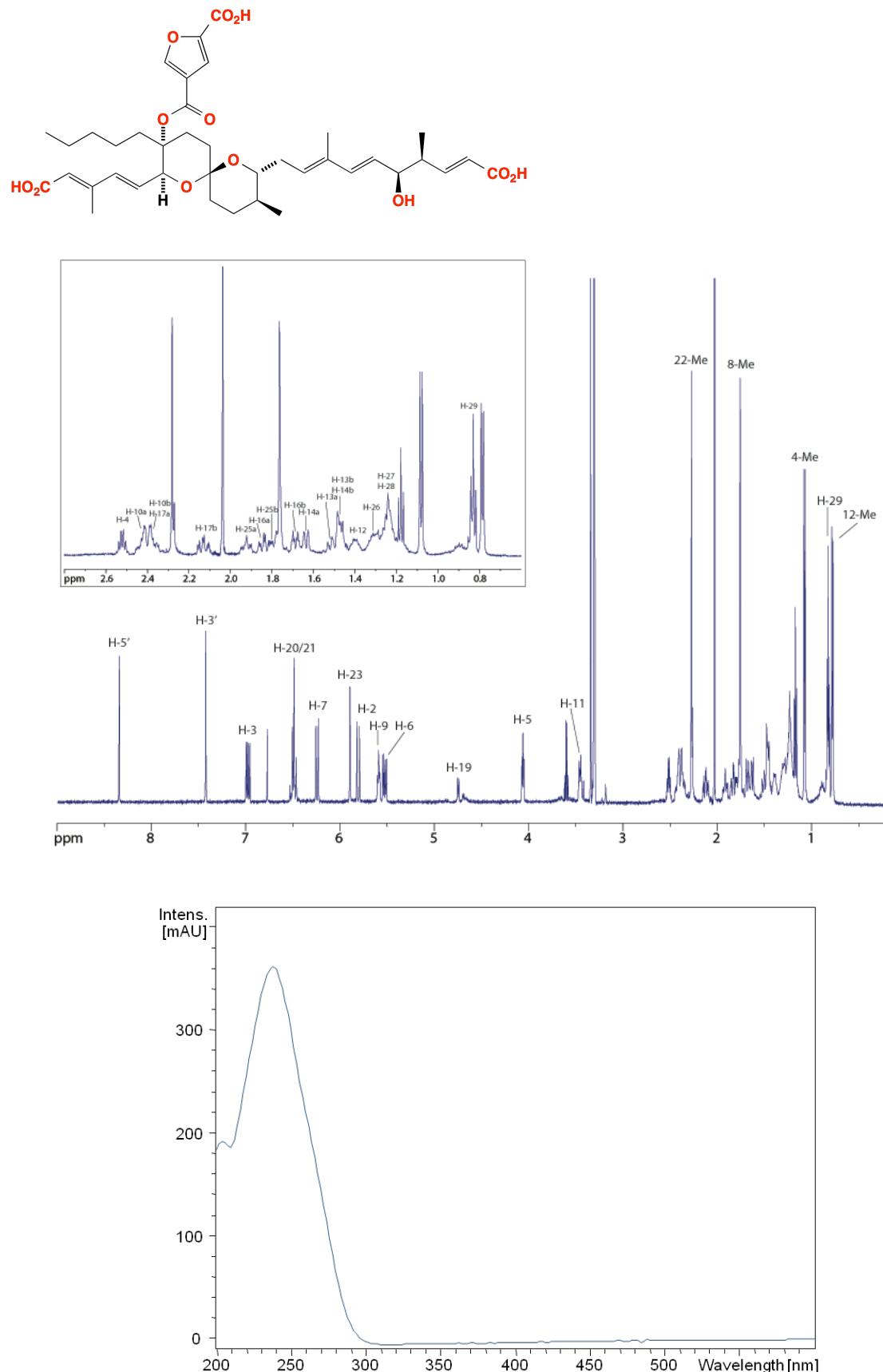


Figure S15. Upper panel: Presat ¹H NMR (600 MHz, MeOH-d₄, irr. δ_H 4.87, HPLC-SPE-NMR) spectrum of reveromycin L (**15**), expansion between 0.6 and 2.8 ppm. Lower panel: UV-vis spectrum of reveromycin L (**15**) extracted from HPLC diode array detector (MeCN:H₂O).

Table S15. NMR (600 MHz, MeOH-*d*₄, HPLC-SPE-NMR) data for reveromycin L (**15**).

Pos	δ_{H} (m, J (Hz))	$\delta_{\text{C}}^{\text{a}}$	COSY	TOCSY	HMBC (¹ H- ¹³ C)
1		170.1			
2	5.83 (dd, 15.7, 1.2)	122.4	3	3, 4, 5, 4-Me	1,
3	6.98 (dd, 15.7, 7.8)	152.6	2, 4	2, 4, 4-Me	1, 4-Me
4	2.53 (ddqd, 7.8, 6.8, 6.8, 1.2)	44.0	4-Me	2, 3, 5, 6, 4-Me	5
5	4.07 (dd, 7.3, 6.8)	76.8	4, 6	2, 3, 4, 6, 7, 4-Me	2, 4, 6, 4-Me
6	5.54 (dd, 15.6, 7.3)	127.9	7, 5	4, 5, 7, 4-Me	
7	6.26 (d, 15.6)	137.5	6	4, 5, 6	9, 5, 8-Me
8		135.4			
9	5.60 (dd, 7.7, 6.8)	129.1	10a/b	11, 10a/b	
10a	2.42 ^b (m)	32.7	9	9, 11	
10b	2.38 ^b (m)		9		
11	3.46 (ddd, 10.1, 4.3, 3.8)	76.1	12	9, 10a/b, 12, 13a/b, 14a/b, 12-Me	
12	1.40 (m)	34.7		11, 12, 13a/b, 14a/b, 12-Me	
13a	1.53 (m)	28.4		11, 12, 13b, 14a/b, 12-Me	
13b	1.51 (m)			11, 12, 13a, 14a/b, 12-Me	
14a	1.64 (m)	37.1		11, 12, 13a/b, 14b, 12-Me	
14b	1.51 (m)			11, 12, 13a/b, 14a, 12-Me	
15		N.O.			
16a	1.84 (ddd, 13.5, 13.4, 4.2)	32.9	16b, 17b	16b, 17a/b	
16b	1.69 (ddd, 13.5, 4.4, 3.7)		16a, 17b	16a, 17a/b	
17a	2.39 ^b (m)	25.3	17b	16a/b, 17b	
17b	2.15 (ddd, 14.2, 13.4, 4.4)		16a/b	16a/b, 17a	
18		N.O.			
19	4.76 (d, 8.0)	79.8	20, 21	20, 21	21
20	6.49 (dd, 15.4, 8.0)	133.8	19	19	24, 23, 22, 19, 22-Me
21	6.50 (d, 15.4)	139.4	19	19	24, 23, 22, 19, 22-Me
22		152.1			
23	5.90 (d, 1.0)	121.4		22-Me	21, 22-Me
24		171.5			
25a	1.92 (m)	35.4	25b, 26a/b	25b, 27a/b, 28a/b, 29	20
25b	1.79 (m)		25a, 26a/b	25a, 27a/b, 28a/b, 29	
26a	1.32 (m)	23.5	25a/b	25a/b, 29	
26b	1.30 (m)		25a/b	25a/b, 29	
27a	1.27 (m)	33.0		27b, 28a/b, 29	
27b	1.23 (m)			27a, 28a/b, 29	
28a	1.27 (m)	23.0	29	27a/b, 28b, 29	
28b	1.23 (m)		29	27a/b, 28a, 29	
29	0.83 (t, 6.9)	14.0	28a/b	25a/b, 26a/b, 27a/b, 28a/b	26, 27, 28
4-Me	1.08 (d, 6.8)	15.0	4	2, 3, 4, 5, 6	3, 4, 5
8-Me	1.76 (s)	12.9		9, 10a/b	7, 8, 9
12-Me	0.78 (d, 6.5)	17.8	12	11	11, 12, 13
22-Me	2.28 (d, 1.0)	14.5		20, 21, 23	21, 22, 23
1'					
2'		148.2			
3'	7.43 (s)	117.2		5'	2', 4', 5'
4'		123.6			
5'	8.35 (s)	151.3		3'	2', 3', 4'
6'					

(a) ¹³C assignments obtained from gHSQC and gHMBC data. (b) Overlapping resonances. N.O. = not observed

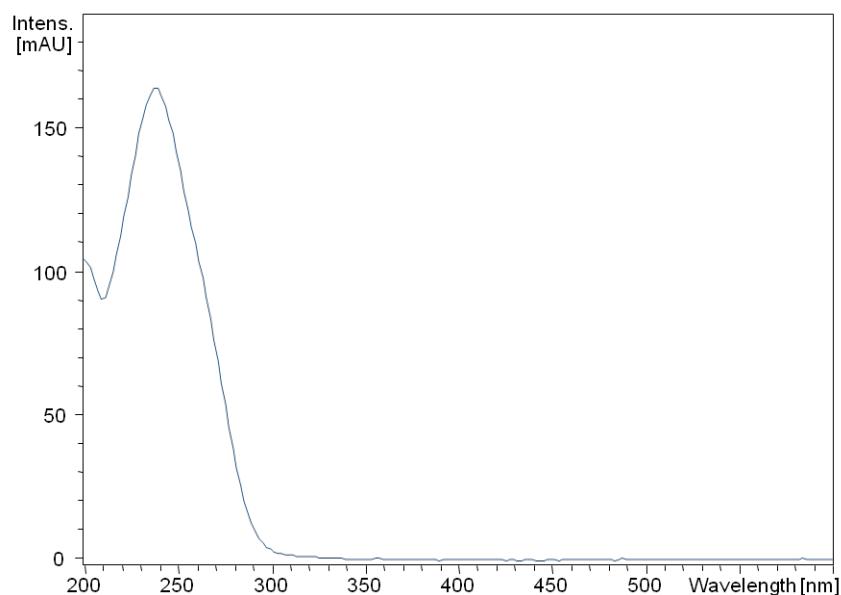
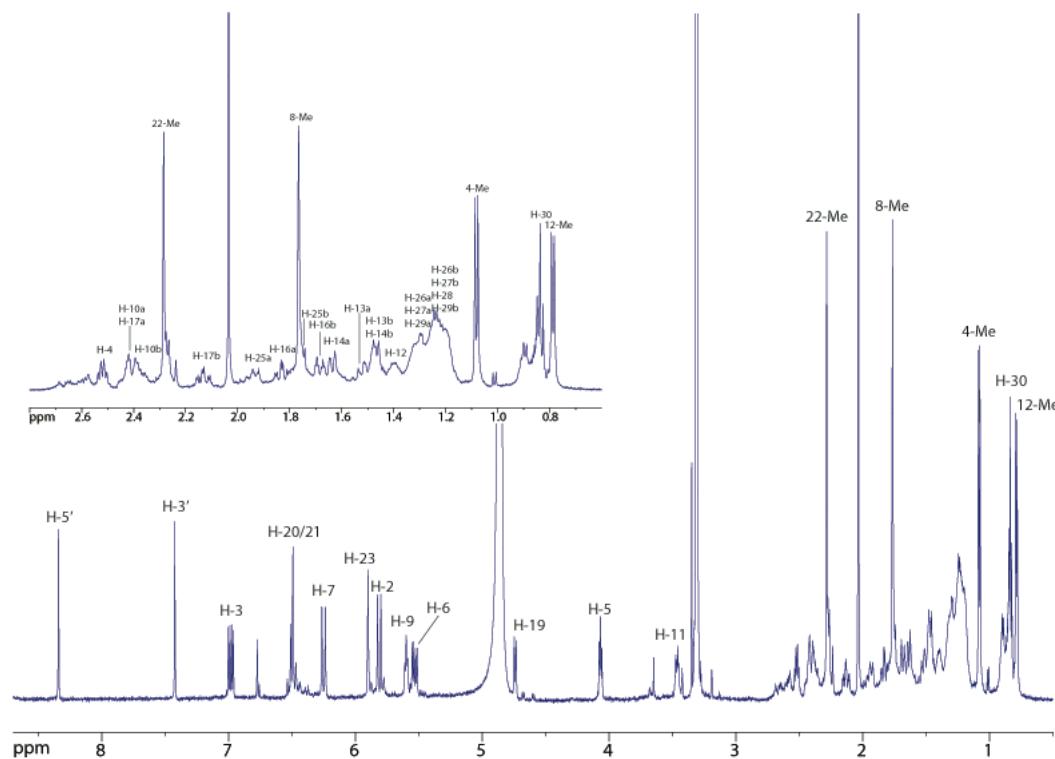
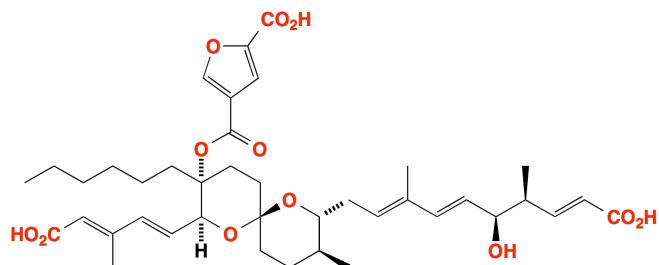


Figure S16. Upper panel: ^1H NMR (600 MHz, MeOH- d_4 , HPLC-SPE-NMR) spectrum for reveromycin M (**16**), expansion between 0.6 and 2.8 ppm. . Lower panel: UV-vis spectrum of reveromycin M (**16**) extracted from HPLC diode array detector (MeCN:H₂O).

Table S16. NMR (600 MHz, MeOH-*d*₄, HPLC-DAD-SPE-NMR) data for reveromycin M (**16**).

Pos	δ _H (m, J (Hz))	δ _C	COSY	TOCSY	HMBC (¹ H- ¹³ C)
1		170.1			
2	5.80 (dd, 15.7, 1.2)	122.1	3	3, 4	4
3	6.98 (dd, 15.7, 7.7)	152.4	2, 4	2, 4, 4-Me	1
4	2.56 (ddqd, 7.7, 6.8, 6.8, 1.2)	43.9	5, 4-Me	2, 3, 5, 7, 4-Me	
5	4.07 (dd, 7.3, 6.8)	76.6	4, 6	4, 6, 7, 4-Me	
6	5.53 (dd, 15.4, 7.3)	127.6	5, 7	5, 7	8
7	6.24 (d, 15.7)	137.4	6	4, 5, 6	5, 6
8		135.7			
9	5.60 (dd, 7.7, 6.8)	129.0	10a/b	10a/b	
10a	2.40 ^b (m)	32.7	9, 10b	9, 10b, 11	
10b	2.37 (m)		9, 10a	9, 10a, 11	
11	3.46 (ddd, 10.1, 4.3, 3.8)	75.9	12	9, 10a/b, 12, 13a/b, 14b, 12-Me	
12	1.39 (m)	34.5	13a/b, 12-Me	11, 13a/b, 14a/b, 12-Me	
13a	1.52 (m)	28.4	12, 13b, 14a/b	11, 12, 13b, 14a/b, 12-Me	
13b	1.47 (m)		12, 13a, 14a/b	11, 12, 13a, 14a/b, 12-Me	
14a	1.64 (m)	37.0	13a/b, 14b	11, 12, 13a/b, 14b, 12-Me	
14b	1.46 (m)		13a/b, 14a	11, 12, 13a/b, 14a, 12-Me	
15		N.O.			
16a	1.83 (m)	32.6	16b, 17a/b	16b, 17a, 17b	
16b	1.69 (m)		16a	16a, 17a, 17b	
17a	2.41 ^b (m)	25.3	17b, 16a	16a/b, 17b	
17b	2.13 (ddd, 14.2, 13.4, 4.4)		16a/b, 17a	16a/b, 17a	
18		N.O.			
19	4.73 (d, 8.0)	79.8	20, 21	20, 21	
20	6.49 (dd, 15.4, 8.0)	133.6	19, 21	19, 21	19
21	6.50 (d, 15.4)	139.4	19, 20	19, 20	19
22		152.4			
23	5.90 (d, 1.0)	121.5	22-Me	22-Me	21, 22-Me
24		N.O.			
25a	1.94 (m)	35.6	25b, 26a/b	25b, 26a/b, 27a/b, 28, 29a/b	
25b	1.79 (m)		25a, 26a/b	25a, 26a/b, 27a/b, 28, 29a/b	
26a	1.28 (m)	30.2	25a/b	25a/b, 30	
26b	1.21 (m)		25a/b	25a/b, 30	
27a	1.28 (m)	23.2		25a/b, 30	
27b	1.21 (m)			25a/b, 30	
28	1.21 (m)	32.4		25a/b, 30	
29a	1.28 (m)	23.2	30	25a/b, 30	
29b	1.21 (m)		30	25a/b, 30	
30	0.84 (t, 6.8)	14.1	29a/b	26a/b, 27a/b, 28a/b, 29a/b	27, 28, 29
4-Me	1.08 (d, 6.8)	15.0	4	2, 3, 4, 5, 7	3, 4, 5
8-Me	1.77 (s)	12.7	9	9	7, 8, 9
12-Me	0.78 (d, 6.5)	17.7	12	10a/b, 11, 12, 13a/b, 14a/b	11, 12, 13
22-Me	2.28 (d, 1.0)	14.4	23	23	21, 22, 23
1'		N.O.			
2'		147.8			
3'	7.42 (s)	117.4		5'	2', 5'
4'		123.1			
5'	8.34 (s)	152.1		3'	2', 3', 4'
6'		N.O.			

(a) ¹³C assignments obtained from gHSQC and gHMBC data. (b) Overlapping resonances. N.O. = not observed

5. Bioassay

Cytotoxicity Testing (MTT assay)

Cells were seeded in a 96-well microtitre plate at 2×10^5 cells/mL in 100 μL of DMEM supplemented with 10% FBS and the plate was incubated for 3 – 5 h (37 °C; 5% CO₂). A solution of each compound in DMSO (300 μM) was serially diluted to 300 nM with 10% aqueous DMSO. Aliquots (10 μL) of each dilution (or of 10% aqueous DMSO for control wells) were added the plate in duplicate and the plate was incubated for 24 h (37 °C; 5% CO₂). A solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma, USA) in PBS was added to each well to a final concentration of 0.5 mg/mL and the plate was incubated for a further 2 h (37 °C; 5% CO₂). The medium was then carefully aspirated from each well and precipitated formazan crystals were dissolved in DMSO (100 μL). Finally, the absorbance of each well was measured at 600 nm spectrophotometrically.

The results of the cytotoxicity testing are summarised in Table S17.

Table S17. Cytotoxicity of reveromycins

Compound	Cytotoxicity – IC ₅₀ (μM)			
	AGS ^a	HeLa ^b	HT29 ^c	HFF-1 ^d
Reveromycin A (1)	30	30	>30	>30
Reveromycin B (2)	>30	>30	30	>30
Reveromycin D (4)	>30	>30	>30	>30
Reveromycin E (5)	17	20	30	>30
Reveromycin F (6)	>30	>30	>30	>30
Reveromycin G (7)	>30	>30	>30	>30
Reveromycin A-4'Me (8)	2	10	>30	>30
Reveromycin D-4'Me (9)	2	2	30	>30
Reveromycin E-4'Me (10)	>30	>30	>30	>30
Reveromycin H (11)	>30	>30	>30	>30
Reveromycin K (14)	>30	>30	>30	>30
Reveromycin L (15)	>30	>30	>30	>30
Reveromycin M (16)	>30	>30	>30	>30

^aAGS - gastric adenocarcinoma (ATCC CRL-1739)

^bHeLa - cervical adenocarcinoma (ATCC CCL-2)

^cHT29 - colorectal adenocarcinoma (ATCC HTB-38)

^dHFF-1 = human foreskin fibroblast (ATCC SCRC-1041)

Antibacterial testing

The bacterium to be tested was streaked onto a tryptic soy agar plate and was incubated at 37 °C for 24 h. One colony was then transferred to fresh tryptic soy broth (15 mL) and the cell density was adjusted to 10^4 - 10^5 cfu/mL. The compounds to be tested were dissolved in DMSO and diluted with H₂O to give 300 μM stock solutions (10% DMSO). The stock solutions were then serially diluted with 10% DMSO to give final concentrations of 30 μM to 0.01 μM in 1% DMSO. An aliquot (20 μL) of each dilution was transferred to a 96-well microtiter plate and freshly prepared microbial broth (180 μL) was added to each well. The plates were incubated at 37 °C for 24 h and the optical density of each well was measured spectrophotometrically at 600 nm.

No significant antibacterial activity was observed against *Escherichia coli* (ATCC 11775), *Pseudomonas aeruginosa* (ATCC 10145), *Staphylococcus aureus* (ATCC 9144, ATCC 25923) or *Bacillus subtilis* (ATCC 6051, ATCC 6633).

Antifungal testing

The fungus to be tested was streaked onto a Sabouraud agar plate and was incubated at 26.5 °C for 48 h. One colony was then transferred to fresh Sabouraud broth (15 mL) and the cell density was adjusted to 10^4 - 10^5 cfu/mL. The pH of the broth was adjusted to either pH 3 (with 1 M HCl) or pH 7 (with 1 M NaOH). The compounds to be tested were dissolved in DMSO and diluted with H₂O to give 300 μM stock solutions (10% DMSO). The stock solutions were then serially diluted with 10% DMSO to give final concentrations of 30 μM to 0.01 μM in 1% DMSO. An aliquot (20 μL) of each dilution was transferred to a 96-well microtiter plate and freshly prepared microbial broth (180 μL) was added to each well. The plates were incubated at 26.5 °C for 48 h and the optical density of each well was measured spectrophotometrically at 600 nm.

The results of the antifungal testing are summarised in Table S18.

Table S18. Antifungal activities of reveromycins against three *Candida* sp. at pH 3 and pH 7

Compound	<i>C. albicans</i> ^a				<i>C. krusei</i> ^b				<i>C. parapsilosis</i> ^c			
	MIC (μM)		IC ₅₀ (μM)		MIC (μM)		IC ₅₀ (μM)		MIC (μM)		IC ₅₀ (μM)	
	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7
Reveromycin A (1)	3.7	—	0.9	—	3.7	—	2.7	—	3.7	—	2.6	—
Reveromycin B (2)	30	—	10.1	—	30	—	9.4	—	30	—	10.4	—
Reveromycin E (5)	1.8	—	0.3	—	3.7	—	1.6	—	3.7	—	1.4	—
Reveromycin F (6)	15	—	6.1	—	30	—	15	—	30	—	15.3	—
Reveromycin G (7)	15	—	7.5	—	30	—	10.1	—	30	—	10.6	—
Reveromycin A-4'Me (8)	0.9	—	0.2	—	1.8	—	0.9	—	1.8	—	0.9	—
Reveromycin D-4'Me (9)	0.9	—	0.05	—	1.8	—	0.9	—	1.8	—	1.1	—
Reveromycin L (15)	30	—	2.3	—	15	—	10.1	—	15	—	8.5	—
Reveromycin M (16)	7.5	—	2.2	—	7.5	—	2.5	—	7.5	—	2.1	—

^aATCC 10231. ^bATCC 6258. ^cATCC 22019. — = no activity

6. Taxonomy

Strain MST-MA568 16S rRNA sequence

AACGGTGAAGCCTTCGGGGTGGATCAGTGGCGAACGGGTGAGTAACACGTGGCAATGTGCCCTGCACTCTGGG
ACAAGCCCTGGAAACGGGTCTAATACCGGATATGACCTGGGGCGCATGCTTCCGGTGGAAAGCTCCGGCGT
GCAGGATGAGCCCCGCGGCCTATCAGCTTGGTGGGTGATGGCTACCAAGGCAGCAGCGACTGGTAGCCGGCCTG
AGAGGGCGACCAGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGAATATTGCAC
AATGGGCGCAAGCCTGATGCAGCGACGCCGTGAGGGATGACGCCCTCGGGTTGTAAACCTTTCAGCAGGG
AAGAAGCGTGAGTGACGGTACCTGCAGAAGAACGGCTAAACTACGTGCCAGCAGCGCGTAATACGTAGGG
TGCAGCGTGTCCGAAATTATTGGCGTAAAGAGCTCGTAGGCAGCTTGTGCGTCGGATGTGAAAGCCGGGG
CTTAACCTCCGGTCTGCATTGATACGGCAGGCTAGAGTCGGTAGGGAGATCGGAATTCTGGTGTAGCGGT
GAAATGCGCAGATATCAGGAGGAACACCGGTGGCAAGGCAGATCTGGGCCGATACTGACGCTGAGGAGCGAA
ACGTGGGAGCGAACAGGATTAAATACCGTGGAA

Organisms with >98% identity

Code	Organism Description
EU159564.1	<i>Streptomyces</i> sp. 919 16S ribosomal RNA gene
DQ347907.1	Uncultured bacterium clone ST60 16S ribosomal RNA gene
AY897204.1	<i>Streptomyces</i> sp. 123 16S ribosomal RNA gene
AY882020.1	<i>Streptomyces yanglinensis</i> strain 913 16S ribosomal RNA gene
AY882019.1	<i>Streptomyces yanglinensis</i> strain 317 16S ribosomal RNA gene
AY876940.1	<i>Streptomyces yanglinensis</i> strain 1307 16S ribosomal RNA gene
EU722758.1	<i>Streptomyces</i> sp. M-MN-1 16S ribosomal RNA gene
AY897205.1	<i>Streptomyces</i> sp. 157 16S ribosomal RNA gene
AY876942.1	<i>Streptomyces guanduensis</i> strain 701 16S ribosomal RNA gene

Strain MST-RA7781 16S rRNA sequence

ACATGCAAGTCGAACGGTGAAGCCTTCGGGGTGGATCAGTGGCGAACGGGTGAGTAACACGTGGCAATCTGCC
CTGCACTCTGGACAAGCCCTGGAAACGGGTCTAATACCGGATATGACCTGGGGCGCATGCTTCTGGGTGGAA
AGCTCCGGCGGTGCAGGATGAGCCCGGGCTATCAGCTTGGTGGGTGATGGCTACCAAGGCAGCAGCG
GTAGCCGGCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGG
GGAATATTGCACAATGGCGCAAGCCTGATGCAGCGACGCCGTGAGGGATGACGCCCTCGGGTTGTAAACCT
CTTCAGCAGGAAAGAAGCGTGAGTGACGGTACCTGCAGAAGAACGGCTAAACTACGTGCCAGCAGCCCG
TAAATACGTAGGGTGCAGCGTTGCCGAAATTATTGGCGTAAAGAGCTCGTAGGCAGCTTGTGCGTCGGATGT
GAAAGCCGGGCTTAACCTCCGGTCTGCATTGATACGGCAGGCTAGAGTCGGTAGGGAGATCGGAATTCC
TGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCAAGGCAGATCTGGGCCGATACTGACG
CTGAGGAGC

Organisms with >98% identity

Code	Organism Description
HM449821.1	<i>Streptomyces</i> sp. SUK09 16S ribosomal RNA gene
HM449820.1	<i>Streptomyces</i> sp. SUK08 16S ribosomal RNA gene
EU159564.1	<i>Streptomyces</i> sp. 919 16S ribosomal RNA gene
AY897204.1	<i>Streptomyces</i> sp. 123 16S ribosomal RNA gene
AY882020.1	<i>Streptomyces yanglinensis</i> strain 913 16S ribosomal RNA gene
AY882019.1	<i>Streptomyces yanglinensis</i> strain 317 16S ribosomal RNA gene
AY876940.1	<i>Streptomyces yanglinensis</i> strain 1307 16S ribosomal RNA gene
FJ418898.1	<i>Streptomyces</i> sp. FXJ1.139 16S ribosomal RNA gene
DQ347907.1	Uncultured bacterium clone ST60 16S ribosomal RNA gene
EU214970.1	<i>Streptomyces</i> sp. CNS-780 SF06 16S ribosomal RNA gene
AY897205.1	<i>Streptomyces</i> sp. 157 16S ribosomal RNA gene
EU683612.1	<i>Streptomyces axinellae</i> strain Pol001 16S ribosomal RNA gene
EU722758.1	<i>Streptomyces</i> sp. M-MN-1 16S ribosomal RNA gene
AY876942.1	<i>Streptomyces guanduensis</i> strain 701 16S ribosomal RNA gene