Electronic Supplementary Information

Jadomycins, Put a Bigger Ring in it: Isolation of Seven- to Ten-Membered Ring Analogues

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Note added after first publication: This Supplementary Information file replaces that originally published on 12 August 2015.

The ESI has been altered from the original to reflect the revised structure of **3**. Additional data included are the HRMS (ESIp neg) data for **3** (Figure S27) and an overlay of the proton spectrum collected in 2015 and in 2017 (Figure S25f), which demonstrates that no sample changes or degradation were observed. The NMR characterization data for **3** has been updated (Figure S5). The proton NMR for 3(Figure S19) has been relabelled to correspond to the revised structure and a series of expansions that support the revision are shown in Figure S25ap e. All 2D spectra and the 13C spectrum for **3** are the same, therefore, these spectra have not been altered.

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Experimental Methods

All reagents were purchased from commercial sources and used without further purification. All solvents used for chromatographic methods were HPLC grade. Thin layer chromatography (TLC) plates were used to monitor jadomycin purification and calculate R_f values. TLC plates were glass backed, normal phase silica (250 μm thickness) purchased from Silicyle®. All jadomycins prepared did not require chemical or ultraviolet (UV) visualization, since they are colored compounds.

Compound Characterization Methods

Ultra-violet-visible spectroscopy

All UV-Vis spectroscopy on novel jadomycin compounds was carried out on a SpectraMax Plus Microplate Reader (Molecular Devices) and analyzed using SoftMax® Pro Version 4.8 Software. Samples were dissolved in methanol, placed in a quartz cuvette (1 cm path length), and scanned over a 280 nm – 700 nm range using 1 nm intervals. Two or three separate dilutions were used (concentrations are listed with the appropriate characterization data) to calculate a series of extinction coefficients (ε) from several maximal absorbance wavelengths (λ_{max}).

High-performance liquid chromatography

HPLC of purified jadomycin and crude extracts were performed using a Hewlett Packard Series 1050 instrument equipped with an Agilent Zorbax 5 μm Rx-C18 column (150 cm × 4.6 mm). Jadomycins were monitored at an absorbance of 254 nm. HPLC was conducted using a linear gradient from 90:10 A:B to 40:60 A:B over 8.0 min followed by a plateau at 40:60 A:B from 8.0 to 10.0 min and finally a linear gradient from 40:60 A:B to 90:10 A:B over the remaining 5.0 min with a flow rate of 1.0 $mLmin^{-1}$. Buffer A is an aqueous buffer containing 12 mM *n*-Bu_4NBr, 10 mM KH_2PO_4, and 5% HPLC

grade acetonitrile (CH_3CN), at pH 4.0 and B is 100% HPLC grade CH_3CN. Samples were analyzed by injecting 20 μL aliquots.

Liquid chromatography tandem mass spectrometry

Low-resolution mass spectra were obtained using an Applied Biosystems hybrid triple quadrupole linear ion trap (2000 *Qtrap*) mass spectrometer equipped with an electrospray ionization (ESI) source. The software for analysis of mass spectra was Analyst version 1.4.1 (Applied Biosystems). The capillary voltage was set to 4500 kV with a declustering potential of 60 V and the curtain gas was set to 10 (arbitrary units). For sample analysis an Agilent 1100 HPLC system was connected to the mass spectrometer fitted with a Phenomenex Kinetex 2.6u (150 × 2.10 mm) C18 column. The buffer system used for the isocratic method was 30% 5 mM ammonium acetate pH 5.5 and 70 % acetonitrile with a flow rate of 120 $\mu L min^{-1}$. Samples were diluted to a concentration of approximately 500 μM in 100% HPLC grade methanol prior to injection of 5 μL. Enhanced product ion (EPI) scans, in positive mode, were used to detect jadomycin ions along with their characteristic fragments, aglycone and phenanthroviridin, to confirm identities using a collision energy of -60 V. Typical EPI scans were conducted using two steps; 130.0 amu to 320.0 amu (0.005 sec) and 300.0 amu to 900.0 amu (0.150 sec) to obtain better resolution.

High-resolution mass spectrometry

HRMS traces of all jadomycins were recorded on a Bruker Daltonics MicroTOF Focus Mass Spectrometer using an ESI^+ source.

Nuclear magnetic resonance spectroscopy

NMR analyses of jadomycins were recorded using either a Bruker AV 500 MHz Spectrometer (^1H: 500 MHz, ^{13}C: 125 MHz) equipped with an auto-tune and match (ATMA) broadband observe (BBFO)

SmartProbe located at the Nuclear Magnetic Resonance Research Resource (NMR-3) facility (Dalhousie University) or a Bruker AV-III 700 MHz Spectrometer (^{1}H: 700 MHz, ^{13}C: 176 MHz) equipped with an ATMA 5 mm TCI cryoprobe located at the Canadian National Research Council Institute for Marine Biosciences (NRC-IMB) in Halifax, Nova Scotia. The use of each is specified next to the appropriate spectra. Spectra were recorded in CD_3OD with chemical shifts given in ppm after calibrating to the residual solvent peak (CD_3OD: 3.31 ppm). Structural characterization and signal assignments were accomplished using ^{1}H-NMR chemical shifts and multiplicities, and ^{13}C-NMR chemical shifts. In addition, ^{1}H-^{1}H correlated spectroscopy (COSY), ^{1}H-^{13}C heteronuclear single quantum coherence (HSQC), ^{1}H-^{13}C heteronuclear multiple bond correlation (HMBC), nuclear Overhauser effect spectroscopy (NOESY), and rotating frame nuclear Overhauser effect spectroscopy (ROESY) NMR experiments were used in the NMR analyses.

Purification Methods

Flash chromatography

Flash chromatography was performed using a Biotage SP1™ unit (Biotage®) using pre-packed normal phase silica columns (80 g) purchased from Silicyle®. The solvent systems used for eluting the natural product from the column are specified in the jadomycin purification methods and in each respective section.

Preparative thin layer chromatography

Preparative TLC was performed using 20 × 20 cm glass backed, normal phase silica TLC plates (Silicycle®, 1000 μm thickness). Preparative TLC was done by bringing up the natural product in minimal methanol in dichloromethane and spotting onto the plate. The plate was eluted in a specified solvent system. When the plate had been completely developed, it was removed from the solvent to

allow air-drying, at which point it was placed into the same solvent system to allow for further elution of the compounds. This was repeated until sufficient separation had occurred to allow for the band of interest to be successfully removed. The band was removed by scraping the silica off of the plate, and the compound was then eluted off of the silica using the same solvent system as was used for development. The solvent was then removed *in vacuo*.

General methods for size exclusion chromatography

Size exclusion chromatography was performed using Sephadex™ LH-20 resin (GE Healthcare). The column was run isocratically with methanol.

Media Preparation Methods

MYM growth media

MYM growth media was prepared by addition of maltose (0.4%, w/v), yeast extract (0.4%, w/v), and malt extract (1%, w/v) to distilled, deionized water (ddH_2O). The pH was adjusted to 7.0 with NaOH (5 M) or HCl (5 M) as necessary. The solution was then autoclaved.

MSM production media

MSM production media was prepared by addition of $MgSO_4$ (0.04%, w/v), MOPS (0.377%, w/v), salt solution (0.9%, v/v), trace mineral solution (0.45%, v/v), and 0.2% w/v $FeSO_4$ solution (0.45%, v/v) to ddH_2O. The pH was adjusted to 7.5 by NaOH (5 M) or HCl (5 M) as necessary. The solution was then autoclaved. The salt solution was made by addition of NaCl (1%, w/v) and $CaCl_2$ (1%, w/v) to ddH_2O. The trace mineral solution was made by addition of $ZnSO_4$ (0.088%, w/v), $CuSO_4$ (0.0039%, w/v), $MnSO_4$ (0.00061%, w/v), H_3BO_3 (0.00057%, w/v), and $(NH_4)_6Mo_7O_{24}$ (0.00037%, w/v) to ddH_2O.

General Jadomycin Production Methods

S. venezuelae ISP5230 VS1099 cultures were grown on MYM agar [maltose (0.4%, w/v), yeast extract (0.4%, w/v), malt extract (1%, w/v), agar (1.5%, w/v), apramycin (0.005% w/v)] incubated at 30°C for a period of two weeks. MYM growth media were inoculated with a loop of cells and incubated for a period of 16 – 24 hours with shaking (250 rpm) at 30°C. The cell growth period in the MYM media was established by observing cell growth under a microscope to determine whether cells were loosely packed and fibrous. The growth was terminated when cells reached this point. Once the initial growth period was complete, the bacteria were pelleted (8500 rpm, 4°C) and the supernatant decanted. The cell pellet from the 250 mL MYM cultures was re-suspended and washed with approximately 50 mL of MSM solution. This process was repeated twice to ensure removal of all traces of MYM. The cells were then re-suspended in minimal MSM solution, typically 15 mL for a cell pellet from a 250 mL culture.

The MSM production media was prepared by the addition of separately filter sterilized 30% glucose solution (2%, v/v), separately autoclaved 9 mM phosphate solution (0.54%, v/v), and separately filter sterilized 6 M aqueous amino acid solution (to a final concentration of 60 mM). The amino acid was added as a separately filter sterilized solution to avoid potential self-cyclization during autoclaving. The pH was then readjusted to 7.5 using NaOH (5 M) or HCl (5 M) as required. The re-suspended *S. venezuelae* was then added to the production media to an optical density of 0.6 at 600 nm (OD_{600}). The production media was immediately ethanol shocked (3%, v/v) to induce jadomycin production. The media was incubated with shaking (250 rpm) at 30°C while being monitored via OD_{600} and Abs_{526} for the duration of the production period. Every 24 hours the pH was readjusted to 7.5 using NaOH (5 M) or HCl (5 M) as required.

Jadomycin Purification Methods

Once production had been stopped, 48 – 144 hours for large-scale productions, the bacteria were removed from the solution by filtration through Whatman #5 filters, followed by 0.45 μm and 0.22 μm Millipore filters. The production media was then passed through a phenyl column (Silicycle®) that had been preconditioned with ddH_2O. The production media was loaded onto the column that was subsequently washed with ddH_2O (300 – 500 mL for a 2 L production) to remove all water-soluble material, and the crude natural product was eluted off of the column using 100% methanol (~250 mL for a 2 L production). The solvent was removed in vacuo, and the presence of the jadomycin of interest was examined using HPLC and LC-MS/MS analysis.

Jadomycin Hep (**1**) and jadomycin Non (**2**) were then further purified using an 80 g silica flash chromatography column preconditioned with dichloromethane. Both jadomycins were eluted using a 30 $mLmin^{-1}$ flow rate collecting 9 mL fractions. Purification was accomplished using a gradient system with solvent A (dichloromethane) and solvent B (methanol). The column was run with an increasing linear gradient of 0% to 10% solvent B (10 CV), an isocratic step of 10% solvent B (5 CV), an increasing linear gradient of 10% to 20% solvent B (5 CV), an isocratic step of 20% solvent B (10 CV), an increasing linear gradient of 20% to 50% solvent B (5 CV), an isocratic step of 50% solvent B (6 CV), an increasing linear gradient of 50% to 100% solvent B (1 CV), and a final isocratic step of 100% solvent B (2 CV). The majority of the natural product was eluted during both isocratic steps of 20% and 50% solvent B. The amount of material isolated for each compound is specified in each section, respectively.

Jadomycin Hep (**1**), jadomycin Non (**2**), and jadomycin 7-aminooctanoic acid(**3**) were then further purified using preparative TLC. The first preparative TLC was run using 15% methanol in dichloromethane as outlined above. The amount of material isolated for each compound is specified in each respective section. The three jadomycins were then purified using a second preparative TLC. This preparative TLC was initially eluted with 1:1 $EtOAc:CH_3CN$, allowed to air-dry, then eluted using

5:5:1 EtOAc:CH_3CN:H_2O. The amount of material isolated for each compound is specified in each respective section.

Final purification for all three jadomycins (**1**–**3**) was accomplished using Sephadex™ LH-20 resin run isocratically with methanol. This yielded the purified natural product and allowed for full characterization. The amount of natural product isolated and characterization data is presented with each compound, respectively.

Monitoring Jadomycin Production Methods

Jadomycin productions were monitored through the removal of 600 μL aliquots from the production media. These aliquots were analyzed at 600 nm (OD_{600}) for cell growth using a SpectraMax Plus Microplate Reader (Molecular Devices). The cells were then removed *via* centrifugation (10,000 rpm, 4°C). The supernatant was then analyzed, using the same instrument, at 526 nm (Abs_{526}) for the production of colored compounds, indicating the production of jadomycins, which are purple.

Jadomycin Hep (1)

Productions using 4-aminobutyric acid as the sole nitrogen source for *S. venezuelae* ISP5230 VS1099 for jadomycin incorporation, were monitored *via* OD_{600} for cell growth and Abs_{526} for jadomycin production (Figure S1). As seen in Figure S1, the cells appeared to be able to grow and appear to produce jadomycin in the presence of 4-aminobutyric acid. The productions were stopped after the typical 48-hour production period. HPLC and LC-MS/MS analysis was carried out on the crude material. The presence of jadomycin Hep was confirmed, and purification was carried forward.

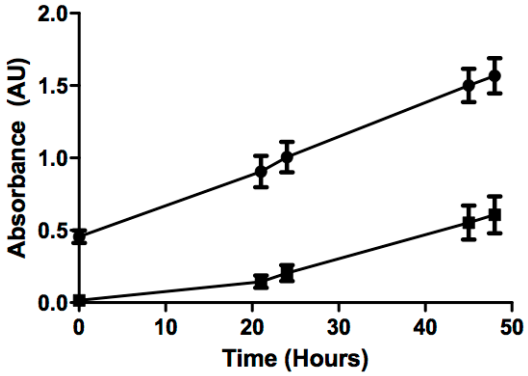


Figure S1. Monitoring cell growth at 600 nm (●) and production of colored compounds at 526 nm (■ in the presence of 4-aminobutyric acid. Error bars correspond to standard deviation between triplicates.

Following the initial phenyl column, 285.8 mg of crude natural product were isolated. This material was further purified using flash chromatography, yielding 219.2 mg of crude natural product. Following the 15% methanol in dichloromethane preparative TLC, 110.2 mg of crude natural product were isolated. The 1:1 EtOAc:CH_3CN/5:5:1 EtOAc:CH_3CN:H_2O preparative TLC then yielded 53.3 mg of crude natural product. The final purification was accomplished using a Sephadex™ LH-20 column, yielding 42.8 mg (21.4 mgL^{-1}) of jadomycin Hep (**1**) as a mixture of diastereomers (Mj:Mn 100:67) by ^{1}H-NMR spectroscopy. TLC R_f: 0.45 (10% CH_3OH in DCM); HPLC R_t = 8.27 min; UV-Vis (2.0×10^{-4} and 2.5×10^{-5} M, MeOH): λ_{max} (ε) = 387 (1384), 524 (1944), 316 (15864); LRMS (ESI^+): MS/MS (522) found 522 $[M+H]^+$, 392 $[M+H-digitoxose]^+$, 306 $[M+H-digitoxose-C_4H_6O_2]^+$; HRMS ($ESI^+$) for $C_{28}H_{27}NO_9$ $[M+Na]^+$: 544.1601 found, 544.1578 calculated; NMR spectra to follow, see characterization table for numbering.

Table S1. Jadomycin Hep (**1**) $3a_{Mj}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity + Coupling Constants (*J*(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	2.29	bm	32.8		
2*			178.0		
3a	5.64	s	92.5		2', 3b, 4, 5, 13a
3b			130.0		
4	6.78	s	120.3	5-CH_3, 6	3a, 3b, 5, 5-CH_3, 6, 7a
5			141.3		
5-CH_3	2.37	s	30.0	4, 6	4, 5, 6
6	6.84	s	120.5	4, 5-CH_3	3a, 4, 5, 5-CH_3, 7a, 7b
7			154.8		
7a			114.0		
7b			117.3		
8			183.3		
8a*			114.8		
9	7.83	d(7.3)	121.5	10	8, 10, 11
10	7.72	t(8.1)	136.6	9, 11	9, 10, 12
11	7.56	d(6.7)	120.5	10	9, 10, 12, 13
12			155.7		
12a*			114.2		
13			185.7		
13a			153.4		
1'	1.98	bm	27.9	2'	
2'	4.09	bm	54.0	1'	
1"	5.97	m	96.3	2"	5", 12
2" (a)	2.42	m	36.3	1", 2"(b), 3"	1", 3", 5"
2"(b)	2.24	m	36.3	1", 2"(a), 3"	1", 3", 5"
3"	4.08	bs	68.4	2"(a), 2"(b)	
4"	3.28	obscured	74.0	5"	
5"	3.94	bm	66.4	4", 5"-CH_3	
5"-CH_3	1.22	d(5.8)	18.1	5"	4", 5"

(*) ^{13}C shifts were assigned by ^{13}C-NMR spectroscopy only and may be interchanged

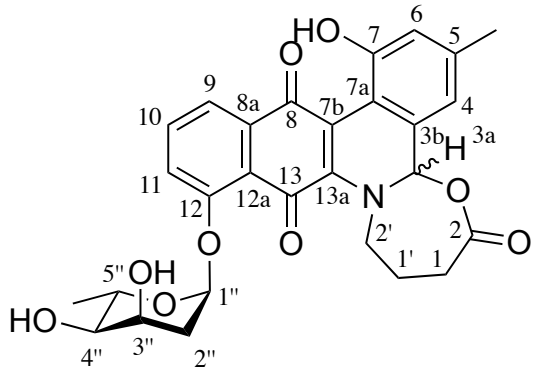
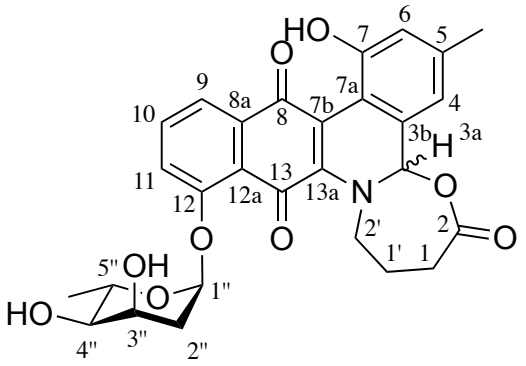


Table S2. Jadomycin Hep (**1**) $3a_{Mn}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity + Coupling Constants (*J*(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	2.29	bm	32.8		
2*			178.0		
3a	5.61	s	92.1		2', 3b, 4, 5, 13a
3b			131.0		
4	6.79	s	120.3	5-CH_3, 6	3a, 3b, 5, 5-CH_3, 6, 7a
5			141.7		
5-CH_3	2.37	s	30.0	4, 6	4, 5, 6
6	6.84	s	120.5	4, 5-CH_3	3a, 4, 5, 5-CH_3, 7a
7			154.8		
7a			114.0		
7b			117.3		
8			184.2		
8a			114.8		
9	7.85	d(7.4)	121.5	10	8, 10, 11
10	7.73	t(8.0)	136.5	9, 11	9, 10, 12
11	7.55	d(6.4)	120.3	10	9, 10, 12, 13
12			156.3		
12a			114.2		
13			184.0		
13a			151.2		
1'	2.08	bm	27.7	2'	
2'	4.10	bm	54.8	1'	
1"	5.97	m	96.4	2"	5", 12
2" (a)	2.42	m	36.3	1", 2"(b), 3"	1", 3", 5"
2"(b)	2.24	m	36.3	1", 2"(a), 3"	1", 3", 5"
3"	4.08	bs	68.1	2"(a), 2"(b)	
4"	3.28	obscured	74.0	5"	
5"	3.94	bm	66.4	4", 5"-CH_3	
5"-CH_3	1.22	d(5.8)	18.1	5"	4", 5"

(*) ^{13}C shifts were assigned by ^{13}C-NMR spectroscopy only and may be interchanged



Jadomycin Non (2)

Productions using 6-aminohexanoic acid as the sole nitrogen source for *S. venezuelae* ISP5230 VS1099 for jadomycin incorporation, were monitored *via* OD_{600} for cell growth and Abs_{526} for jadomycin production (Figure S2). As seen in Figure S2, the cells appeared to be able to grow and appear to produce jadomycin in the presence of 6-aminohexanoic acid. The productions were stopped after a 144-hour production period. HPLC and LC-MS/MS analysis was carried out on the crude material. The presence of jadomycin Non was confirmed, and purification was carried forward. Future productions were stopped after the typical 48-hour production period due to the fact that there was not a vast increase in the production of colored compounds after 48-hours (Figure S2).

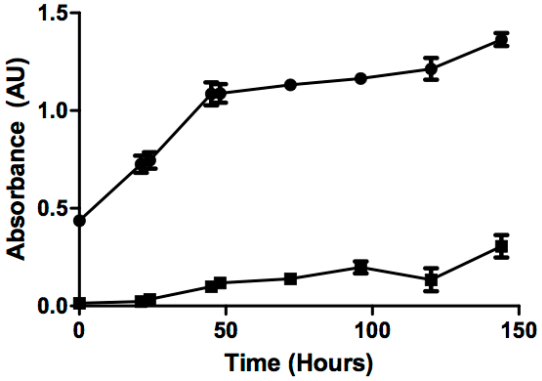


Figure S2. Monitoring cell growth at 600 nm (●) and production of colored compounds at 526 nm (■ in the presence of 6-aminohexanoic acid. Error bars correspond to standard deviation between triplicates.

Following the initial phenyl column, 282.4 mg of crude natural product were isolated. This material was further purified using flash chromatography, yielding 138.9 mg of crude natural product. Following the 15% methanol in dichloromethane preparative TLC, 35.6 mg mg of crude natural product were isolated. The 1:1 EtOAc:CH_3CN/5:5:1 EtOAc:CH_3CN:H_2O preparative TLC then yielded 29.8 mg of

crude natural product. The final purification was accomplished using a Sephadex™ LH-20 column, yielding 20.5 mg (10.3 mgL^{-1}) of jadomycin Non (**2**) as a mixture of diastereomers (Mj:Mn 100:63) by ^{1}H-NMR spectroscopy. TLC R_f: 0.45 (10% CH_3OH in DCM); HPLC R_t = 8.63 min; UV-Vis (1.3×10^{-4} and 3.3×10^{-5} M, MeOH): λ_{max} (ε) = 386 (4091), 522 (2791), 314 (20615); LRMS (ESI^+): MS/MS (550) found 550 $[M+H]^+$, 420 $[M+H-digitoxose]^+$, 306 $[M+H-digitoxose-C_6H_{10}O_2]^+$; HRMS ($ESI^+$) for $C_{30}H_{31}NO_9$ $[M+H]^+$: 572.1887 found, 572.1891 calculated; NMR spectra to follow, see characterization table for numbering.

Table S3. Jadomycin Non (**2**) $3a_{Mj}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity + Coupling Constants (*J*(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	2.24	obscured m	35.5	1'	
2*			178.6		
3a	5.62	s	92.6		4', 3b, 4, 6, 7a, 13, 13a
3b			129.9		
4	6.76	s	120.3	5-CH_3, 6	3a, 3b, 5-CH_3, 7, 7a
5			141.2		
5-CH_3	2.36	s	21.0	4, 6	3b, 4, 5, 6
6	6.83	s	120.5	4, 5-CH_3	3b, 5-CH_3, 7, 7a
7			154.7		
7a			114.3		
7b*			136.6		
8			183.0		
8a*			121.4		
9	7.81	d(7.6)	121.2	10	10, 11, 12, 13
10	7.71	t(8.1)	136.6	9, 11	9, 11, 12
11	7.55	d(6.5)	120.5	10	9, 10, 12, 13
12			155.6		
12a*			114.4		
13			185.8		
13a			153.7		
1'	1.61	m	26.0	1, 2'	
2'	1.33	m	27.3	1', 3'	
3'	1.77	m	31.9	2', 4'	1', 4'
4'	4.11, 4.01	m, m	54.6	3'	2', 3', 3a, 13a
1"	5.95	m	96.3	2"(a), 2"(b)	3", 5", 12
2" (a)	2.40	m	36.2	1", 2"(b), 3"	1", 4"
2" (b)	2.24	obscured m	36.3	1", 2"(a), 3"	1", 4"
3"	4.08	s	68.4	2"(a), 2"(b), 4"	
4"	3.28	obscured	74.0	3", 5"	
5"	3.91	m	66.4	4", 5"-CH_3	5"-CH_3
5"-CH_3	1.22	s	18.1	5"	4", 5"

(*) ^{13}C shifts were assigned by ^{13}C-NMR spectroscopy only and may be interchanged

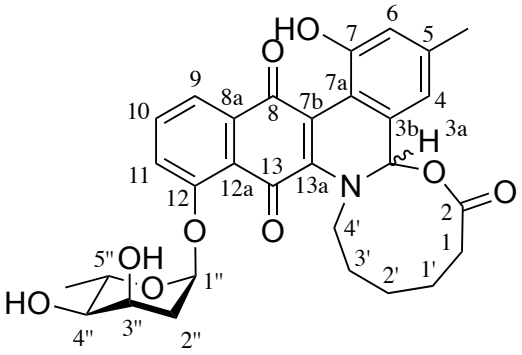
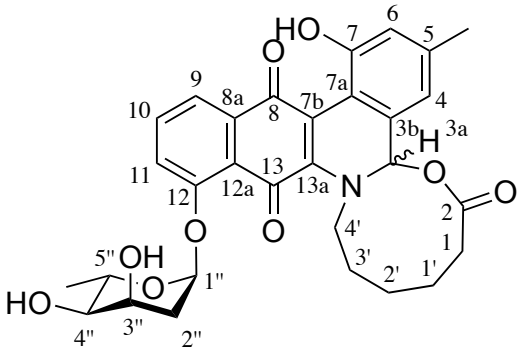


Table S4. Jadomycin Non (**2**) $3a_{Mn}$ diastereomer NMR data.

Position	δ ^{1}H (ppm)	Multiplicity + Coupling Constants (*J*(Hz))	δ ^{13}C (ppm)	COSY	HMBC
1	2.24	obscured m	35.5	1'	
2*			178.6		
3a	5.59	s	92.2		4', 3b, 4, 6, 7a, 13, 13a
3b			131.2		
4	6.78	s	120.2	5-CH_3, 6	3a, 3b, 5-CH_3, 7, 7a
5			141.7		
5-CH_3	2.36	s	21.0	4, 6	3b, 4, 5, 6
6	6.83	s	120.4	4, 5-CH_3	3b, 5-CH_3, 7, 7a
7			154.7		
7a			114.1		
7b*			136.6		
8			184.2		
8a*			121.4		
9	7.84	d(7.5)	121.5	10	10, 11, 12, 13
10	7.72	t(8.1)	136.5	9, 11	9, 11, 12
11	7.54	d(6.2)	120.6	10	9, 10, 12, 13
12			156.3		
12a*			117.7		
13			183.9		
13a			150.8		
1'	1.61	m	25.8	1, 2'	
2'	1.33	m	27.2	1', 3'	
3'	1.77	m	31.6	2', 4'	1', 4'
4'	4.37, 3.78	m, m	55.4	3'	2', 3', 3a, 13a
1"	5.95	m	96.5	2"(a), 2"(b)	3", 5", 12
2" (a)	2.40	m	36.2	1", 2"(b), 3"	1", 4"
2" (b)	2.24	obscured m	36.3	1", 2"(a), 3"	1", 4"
3"	4.08	s	68.1	2"(a), 2"(b), 4"	
4"	3.28	obscured	74.0	3", 5"	
5"	3.91	m	66.4	4", 5"-CH_3	5"-CH_3
5"-CH_3	1.22	d(1.4)	18.1	5"	4", 5"

(*) ^{13}C shifts were assigned by ^{13}C-NMR spectroscopy only and may be interchanged



Jadomycin 7-aminooctainoic acid (3)

Productions using 7-aminohexanoic acid as the sole nitrogen source for *S. venezuelae* ISP5230 VS1099 for jadomycin incorporation, were monitored by OD_{600} for cell growth and Abs_{526} for jadomycin production (Figure S3). As seen in Figure S3, the cells appeared to be able to grow and appear to produce jadomycin in the presence of 7-aminoheptanoic acid. The productions were stopped after a 144-hour production period. HPLC and LC-MS/MS analysis was carried out on the crude material.

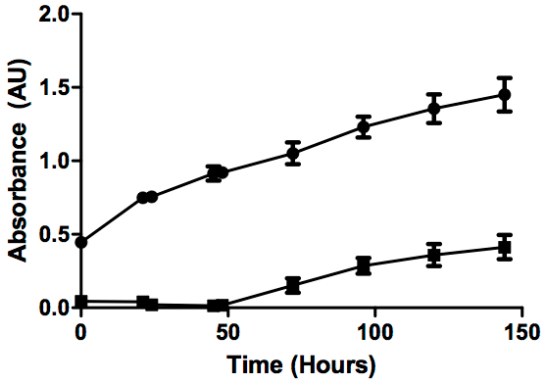


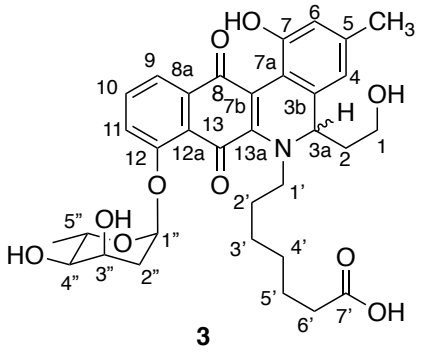
Figure S3. Monitoring cell growth at 600 nm (●) and production of colored compounds at 526 nm (■ in the presence of 7-aminoheptanoic acid. Error bars correspond to standard deviation between triplicates.

Following the initial phenyl column, 117.2 mg of crude natural product were isolated. Following the 15% methanol in dichloromethane preparative TLC, 15.6 mg of crude natural product were isolated. The 1:1 EtOAc:CH_3CN/5:5:1 EtOAc:CH_3CN:H_2O preparative TLC then yielded 8.4 mg of crude natural product. The final purification was accomplished using a Sephadex™ LH-20 column, yielding 5.6 mg (2.8 mgL^{-1}) of jadomycin 7-aminooctanoic acid **3** present with a mixture of compounds by ^{1}H-NMR spectroscopy. This jadomycin was different in color to the other jadomycins isolated, compounds **1** and **2**, as well as jadomycin AVA (Figure S4). TLC R_f: 0.43 (10% CH_3OH

in DCM); HPLC R_t = 8.75 min; UV-Vis (2.96×10^{-4}, 7.39×10^{-5} M, and 3.70×10^{-5} M, MeOH): λ_{max} (ε) = 551 (1944), 382 (2773), 327 (17151), 292 (15177); HRMS (ESI^-) for $C_{33}H_{38}NO_{10}$ $[M-H]^-$: 608.2505 found, 608.2501 calculated NMR spectra to follow, see characterization table for numbering.

Figure S4. NMR tubes showing the change in color between **1**, jadomycin AVA, and **2** as compared to **3.**

Table S5. Jadomycin 7-aminooctanoic acid (**3**), major species, NMR data.



3

			3 (major)	
position	δH(*J* in Hz)	δC, type	COSY	HMBC
1	3.48(1a), 3.40 (1b), (m)	58.4, CH_2	2	2, 3a
2	1.92(2a), 1.77(2b) (m)	35.5, CH_2	1, 3a	1
3a	4.72, (dd, 5.2, 8.6)	64.2, CH	2	2, 1',1, 4, 7a, 3b, 13a,8
3b	-	133.0, C		
4	6.57 (s)	119.2, CH		5-CH_3, 3a,7a, 6, 3b, 7
5*	-	140.8, C		
5-CH_3	2.31 (s)	21.0, CH_3		5a, 4, 6, 5
6	6.69 (s)	119.4, CH		5-CH_3, 7a, 4, 7
7	-	154.6, C		
7a	-	114.1, C		
7b	-	120.3, C		
8	-	179.7, C		
8a	-	111.7, C		
9	7.75, (d, 7.6)	120.9, CH		11, 10, 12, 8
10	7.67 (7, 7.7)	136.7, CH		9, 11, 12a, 12
11	7.48 (d, 7.6)	119.7,CH		9, 10, 12, 13
12	-	155.7, C		
12a	-	137.5, C		
13	-	185.9, C		
13a	-	156.7, C		
1'	4.17 (ddd, 5.5, 9.7, 14.0), 3.58 (ddd, 6.4, 9.1, 14.0)	54.3, CH_2	2'	4',3', 3a, 13a
2'	1.76 (obs)	31.5, CH_2	3'	3',4', 1'
3'	1.38 (obs)	30.1, CH_2**		4'
4'	1.37 (obs)	27.4, CH_2**	5'	3', 5'
5'	1.58 (m)	27.1, CH_2	4'. 6'	
6'	2.21 (obs)	36.2, CH_2	5'	
7'*	-	184.5, C		None observed
1"	5.89 (bd, 2.8)	96.3, CH	2"	5", 3", 12
2"	2.38 (bd), 2.21 (obs)	36.3, CH_2	1", 3"	3", 4", 1"
3"	4.07 (bs)	68.3, CH	2", 4"	5", 1"
4"	3.29 (m)	73.9, CH	3",5"	5"
5"	3.81 (m)	66.3, C	4", 5"-CH_3	4", 5"-CH_3
5"-CH_3	1.21 (d, 6.3)	18.1, CH_3	5"	4", 5", 1"

*Carbon shifts assigned by ^{13}C NMR only; **Proton signals overlap therefore carbon assignment from 2D spectra may be interchanged; obs: indicates obscured due to signal overlap with minor compound or solvent

Jadomycin 8-Aminooctanoic Acid

Productions using 8-aminooctanoic acid as the sole nitrogen source for *S. venezuelae* ISP5230 VS1099 for jadomycin incorporation were monitored *via* OD_{600} for cell growth and Abs_{526} for jadomycin production (Figure S5). As seen in Figure S5, the cells appeared to be able to grow in the presence of 8-aminooctanoic acid, however, there does not appear to be appreciable jadomycin production. The productions were stopped after a 144-hour production period. HPLC and LC-MS/MS analysis was carried out on the crude material. Jadomycin 8-aminooctanoic acid was not found, and purification was not continued.

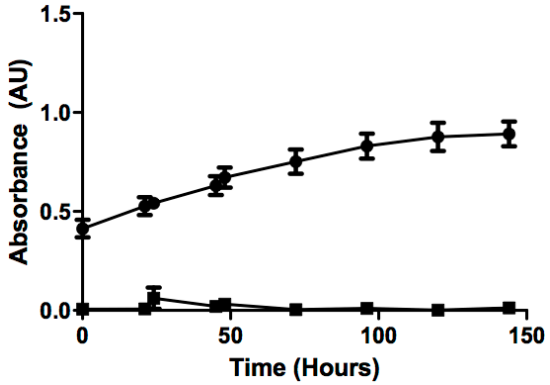


Figure S5. Monitoring cell growth at 600 nm (●) and production of colored compounds at 526 nm (■ in the presence of 8-aminooctanoic acid. Error bars correspond to standard deviation between triplicates.

NMR Spectra for Compounds 1 – 3

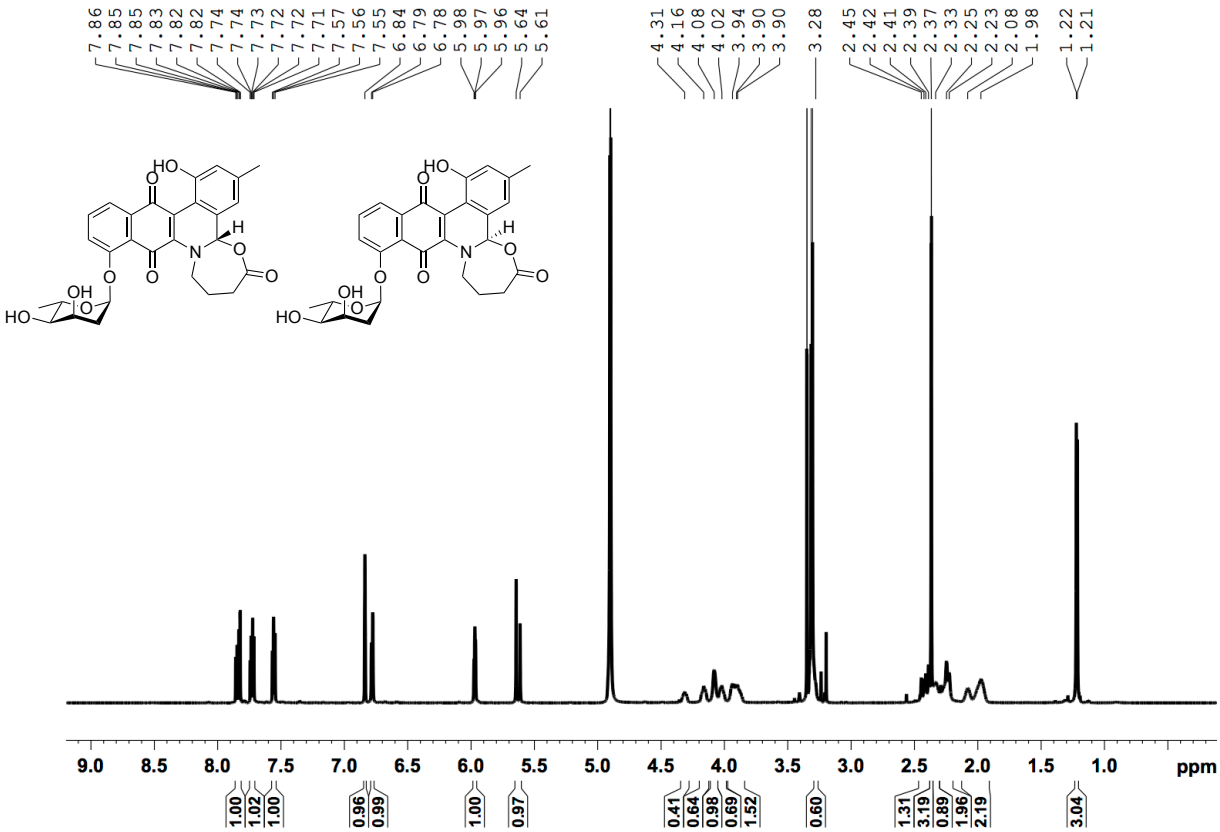


Figure S6. 1H-NMR spectrum of **1** (diastereomeric mixture) in CD3OD (1H: 700 MHz).

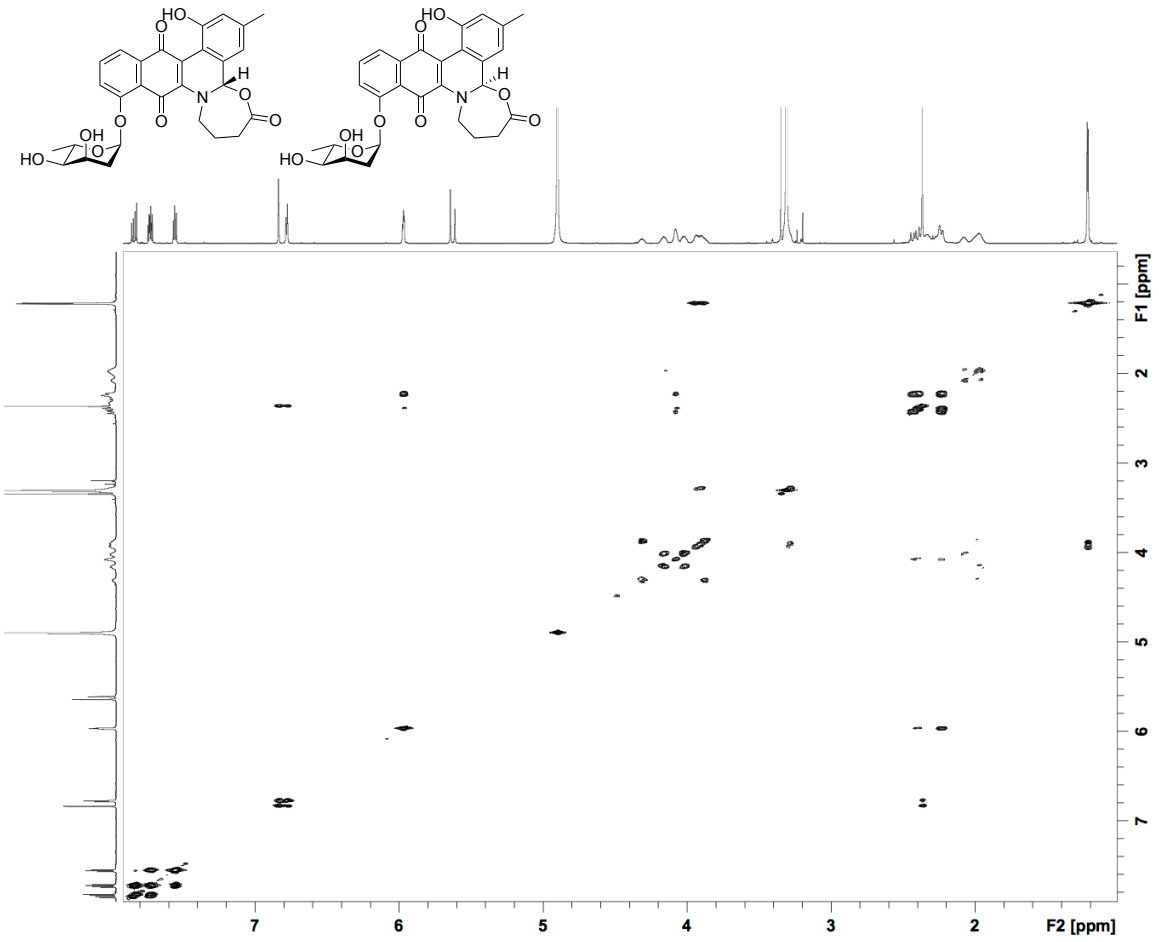


Figure S7. ^{1}H-^{1}H COSY spectrum of **1** (diastereomeric mixture) in CD_3OD.

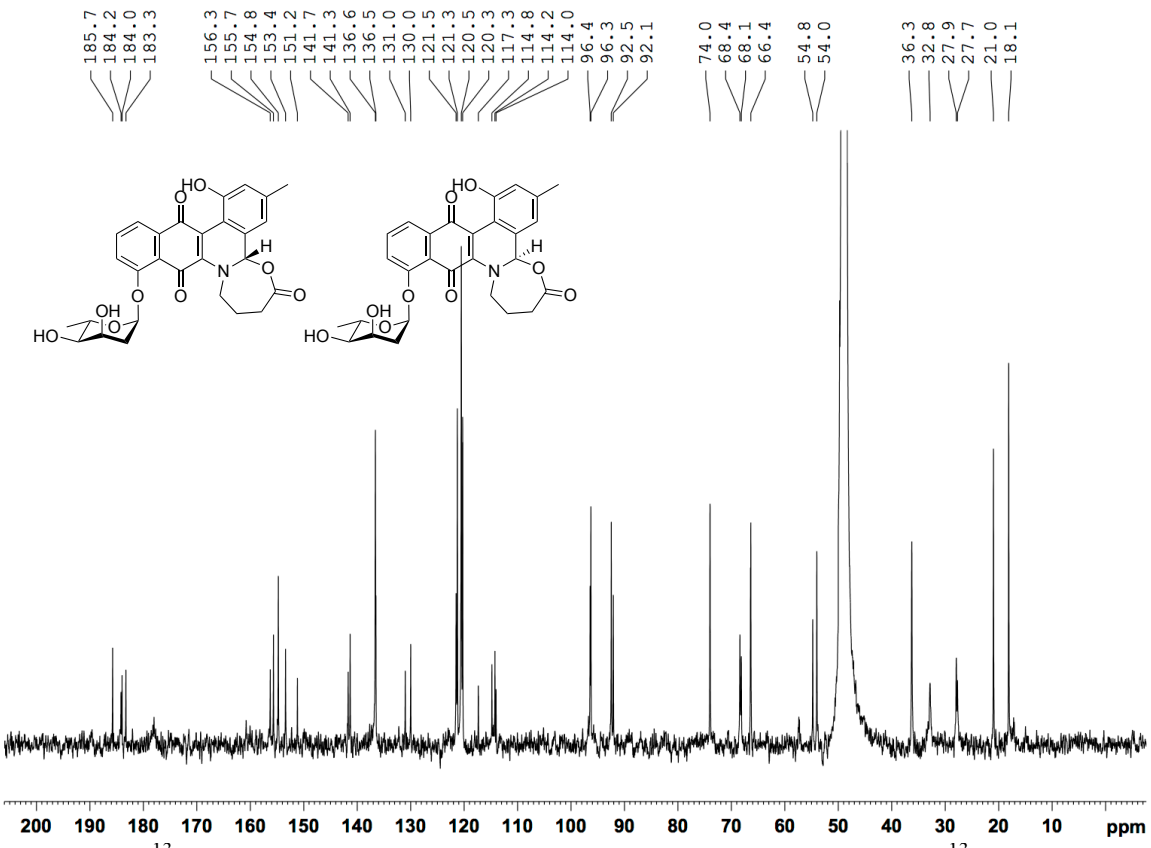


Figure S8. ^{13}C-NMR spectrum of **1** (diastereomeric mixture) in CD_3OD (^{13}C: 176 MHz).

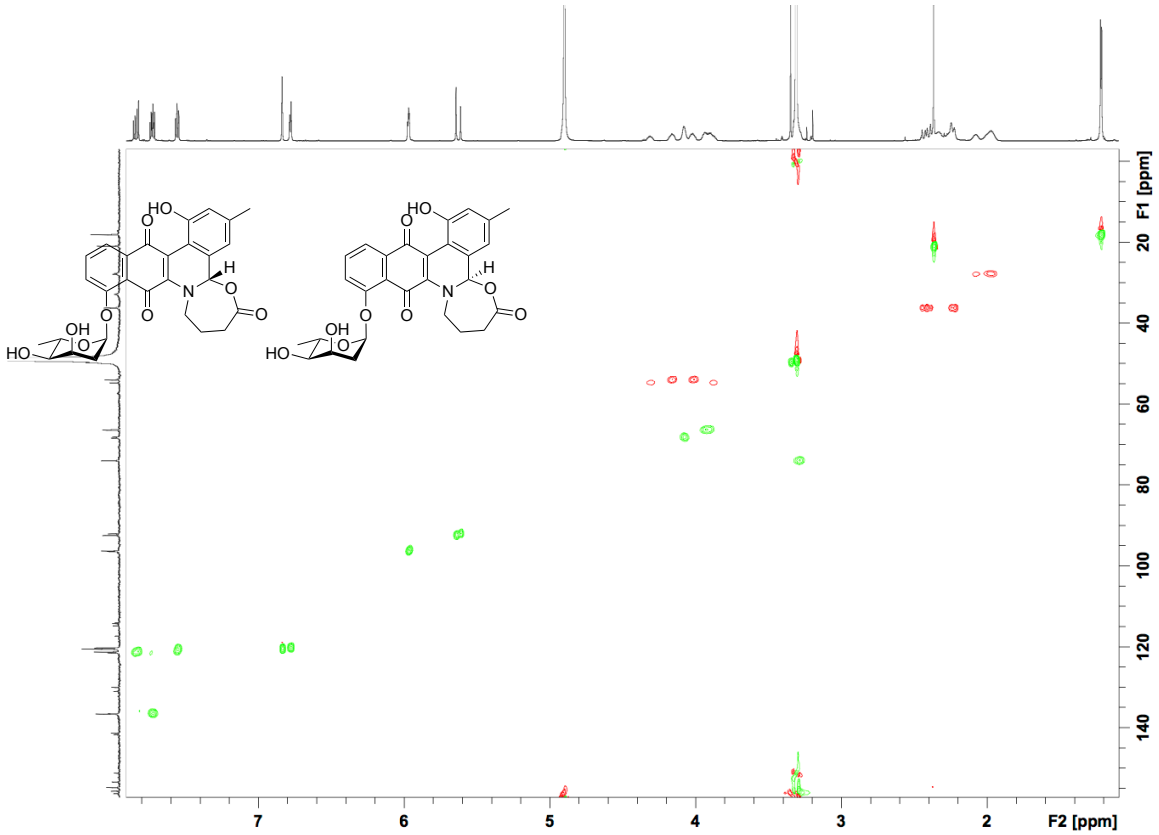


Figure S9. Edited-HSQC (^{1}H-^{13}C) spectrum of **1** (diastereomeric mixture) in CD_3OD.

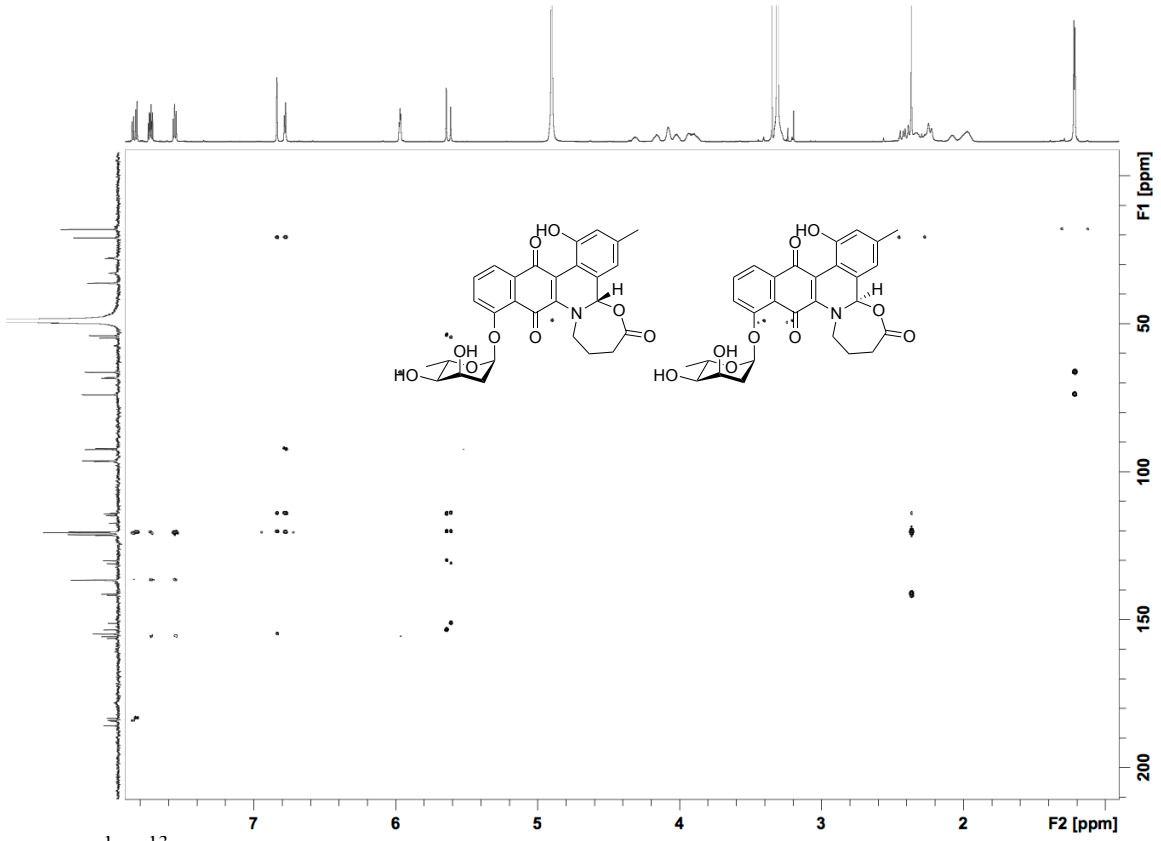


Figure S10. ^{1}H-^{13}C HMBC (6.5 μs mixing time) spectrum of **1** (diastereomeric mixture) in CD_3OD.

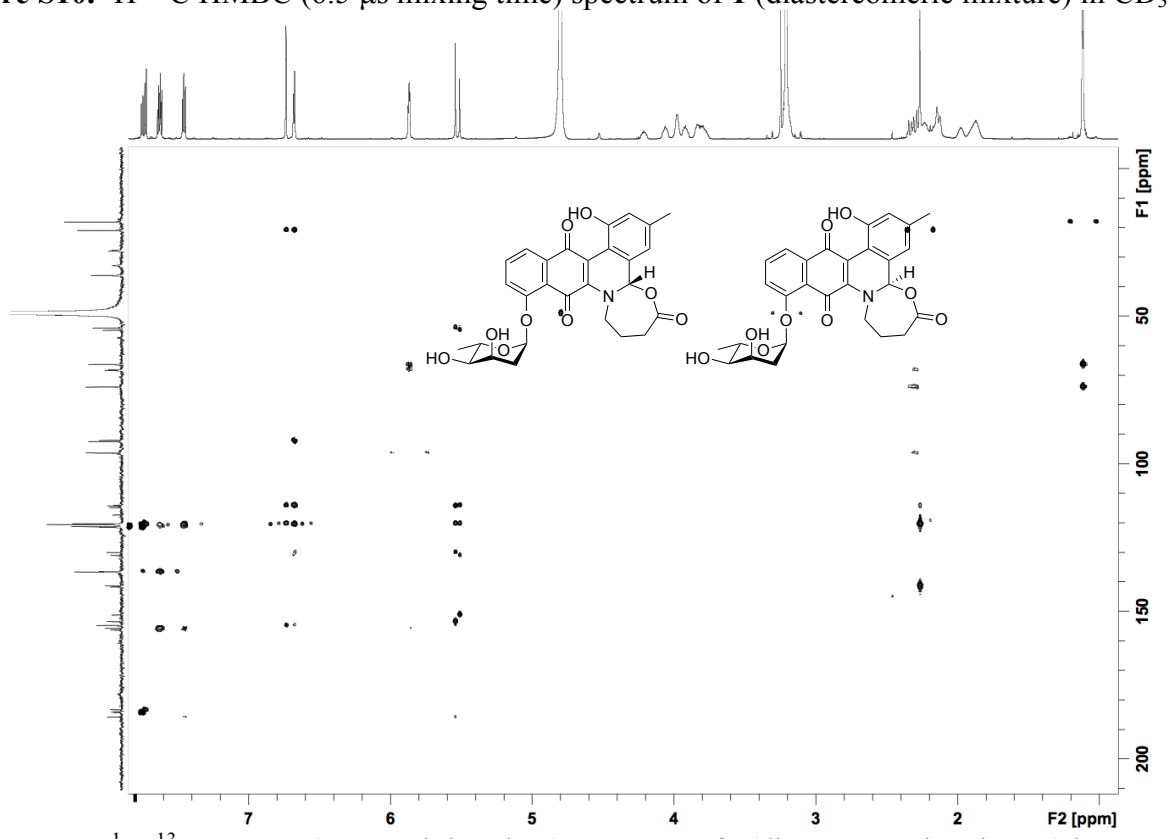


Figure S11. ^{1}H-^{13}C HMBC (25 μs mixing time) spectrum of **1**(diastereomeric mixture) in CD_3OD.

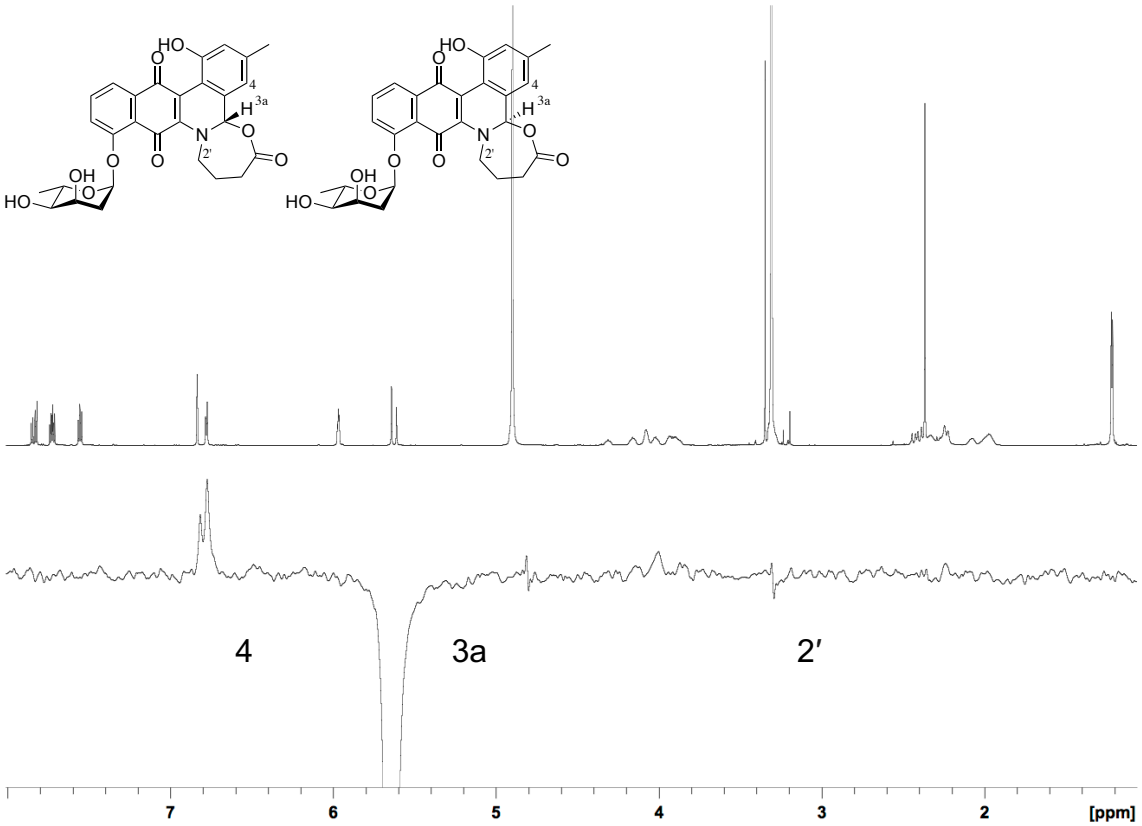


Figure S12. Overlay of ^{1}H-NMR spectrum of **1** (top) and with ROESY showing simultaneous irradiation of $H3a_{Mj}$ and $H3a_{Mn}$ (bottom) in CD_3OD (^{1}H: 500 MHz).

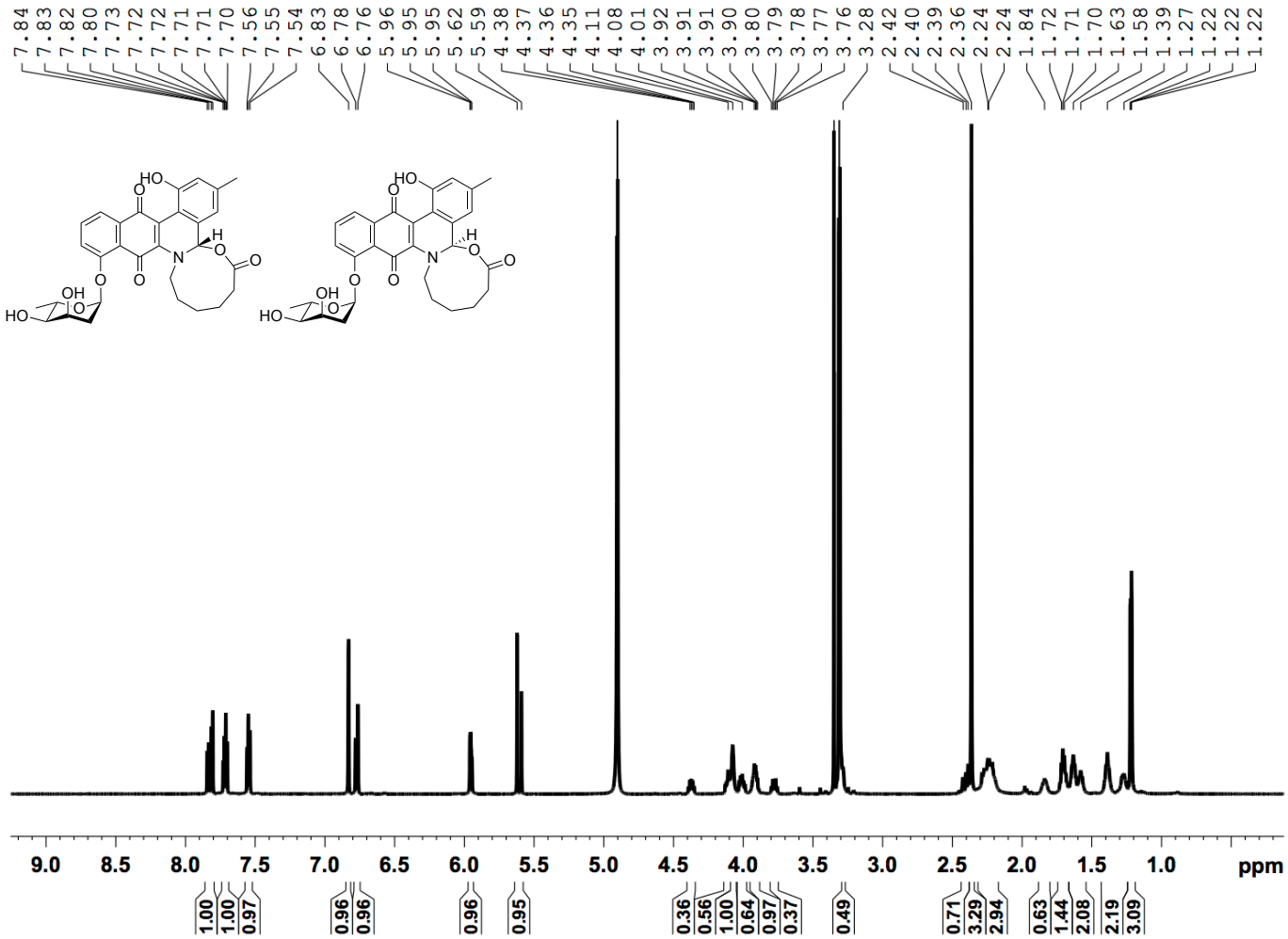


Figure S13. ^{1}H-NMR spectrum of **2** (diastereomeric mixture) in CD_3OD (^{1}H: 700 MHz).

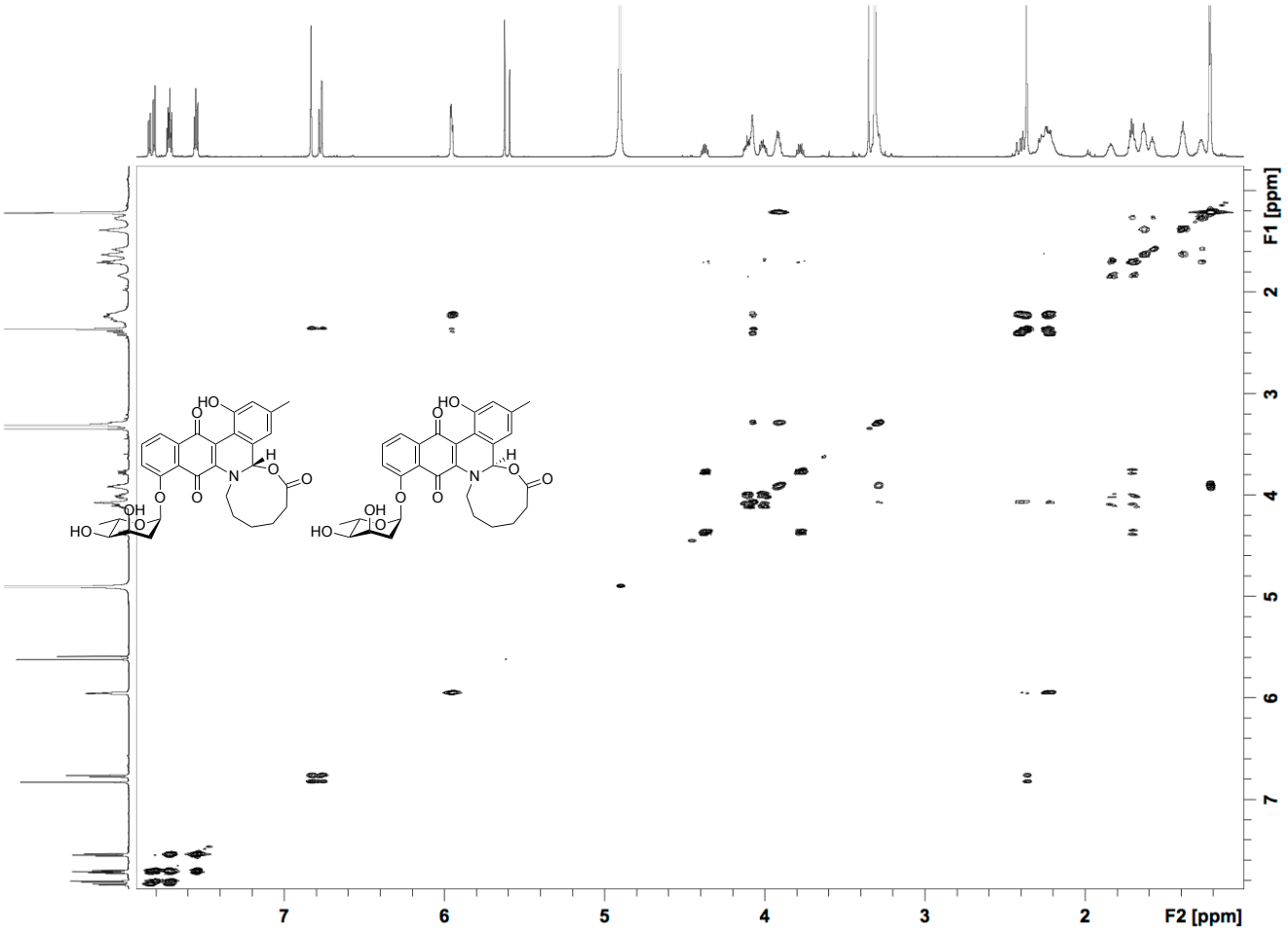


Figure S14. ^{1}H-^{1}H COSY spectrum of **2** (diastereomeric mixture) in CD_3OD.

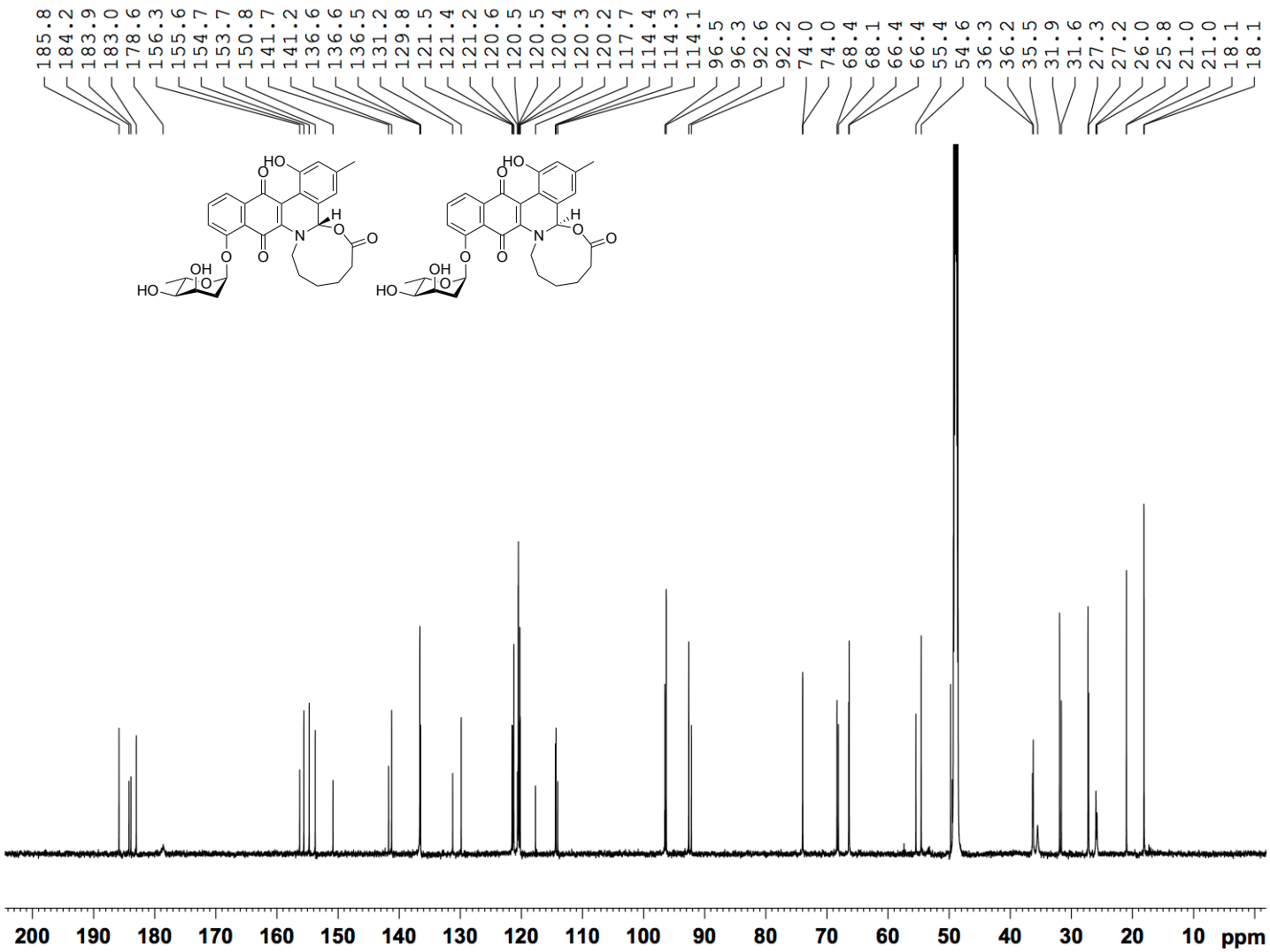


Figure S15. ^{13}C-NMR spectrum of **2** (diastereomeric mixture) in CD_3OD (^{13}C: 176 MHz).

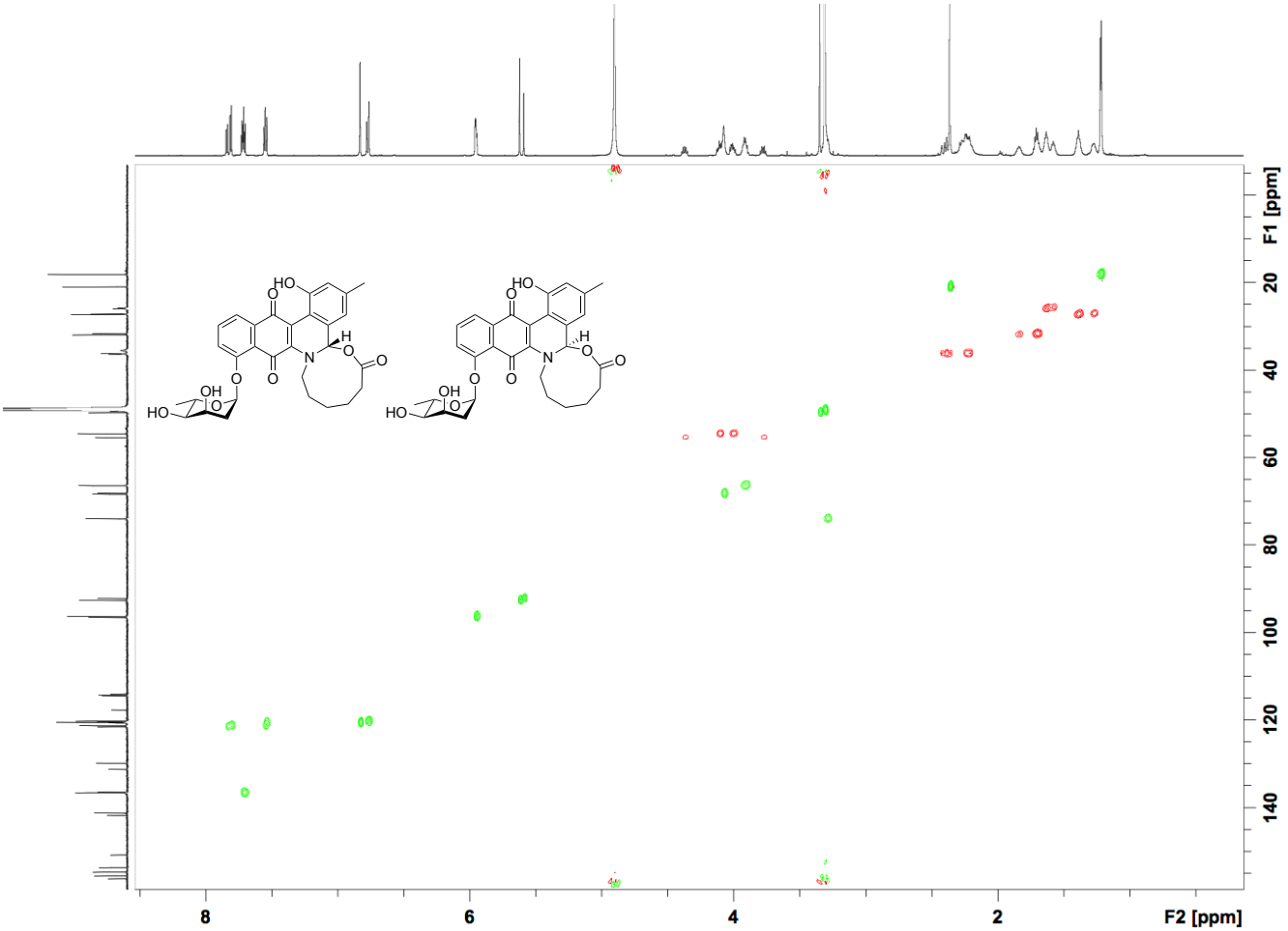


Figure S16. Edited-HSQC (^{1}H-^{13}C) spectrum of **2** (diastereomeric mixture) in CD_3OD.

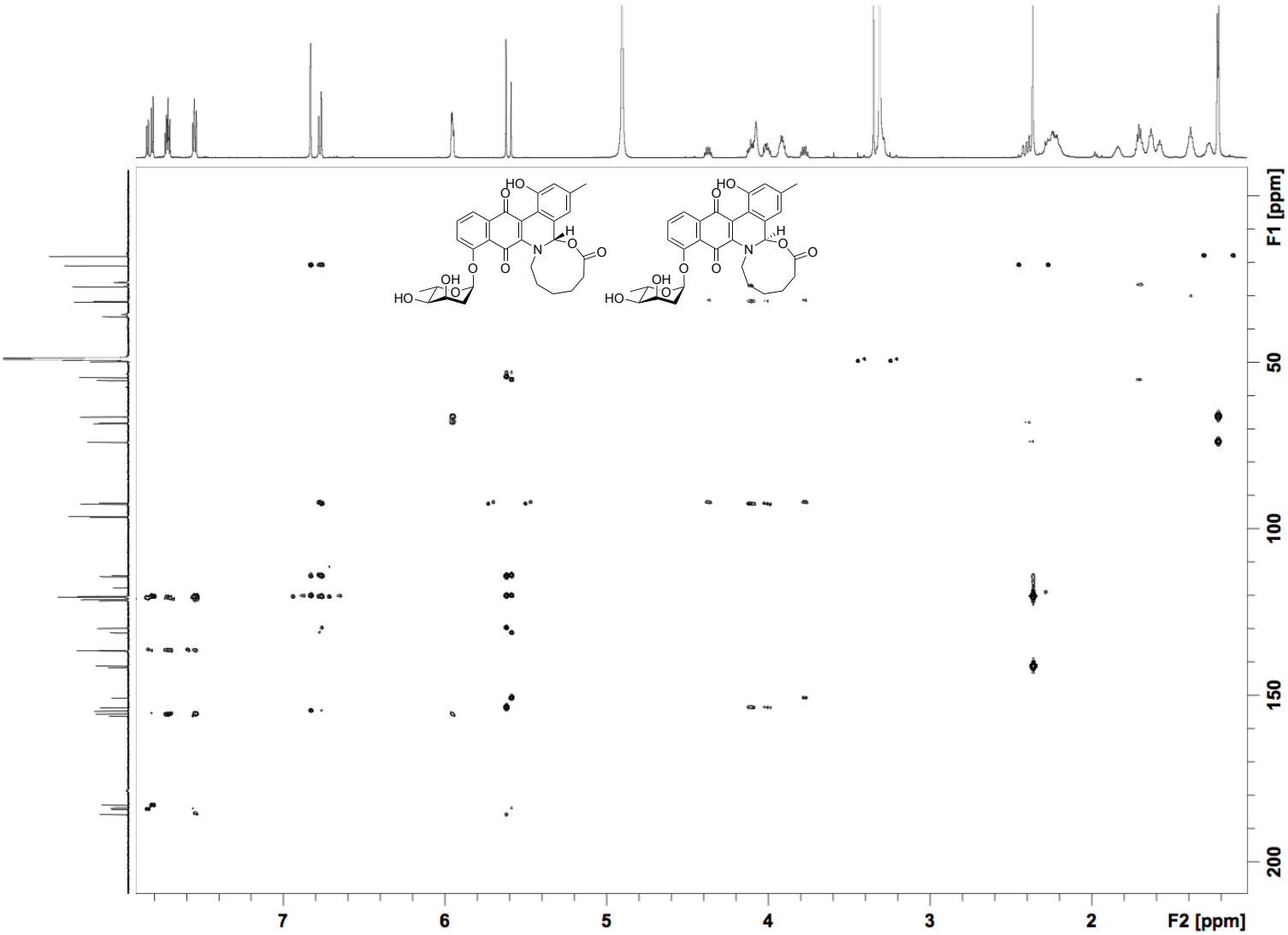


Figure S17. ^{1}H-^{13}C HMBC (25 μs mixing time) spectrum of **2** (diastereomeric mixture) in CD_3OD.

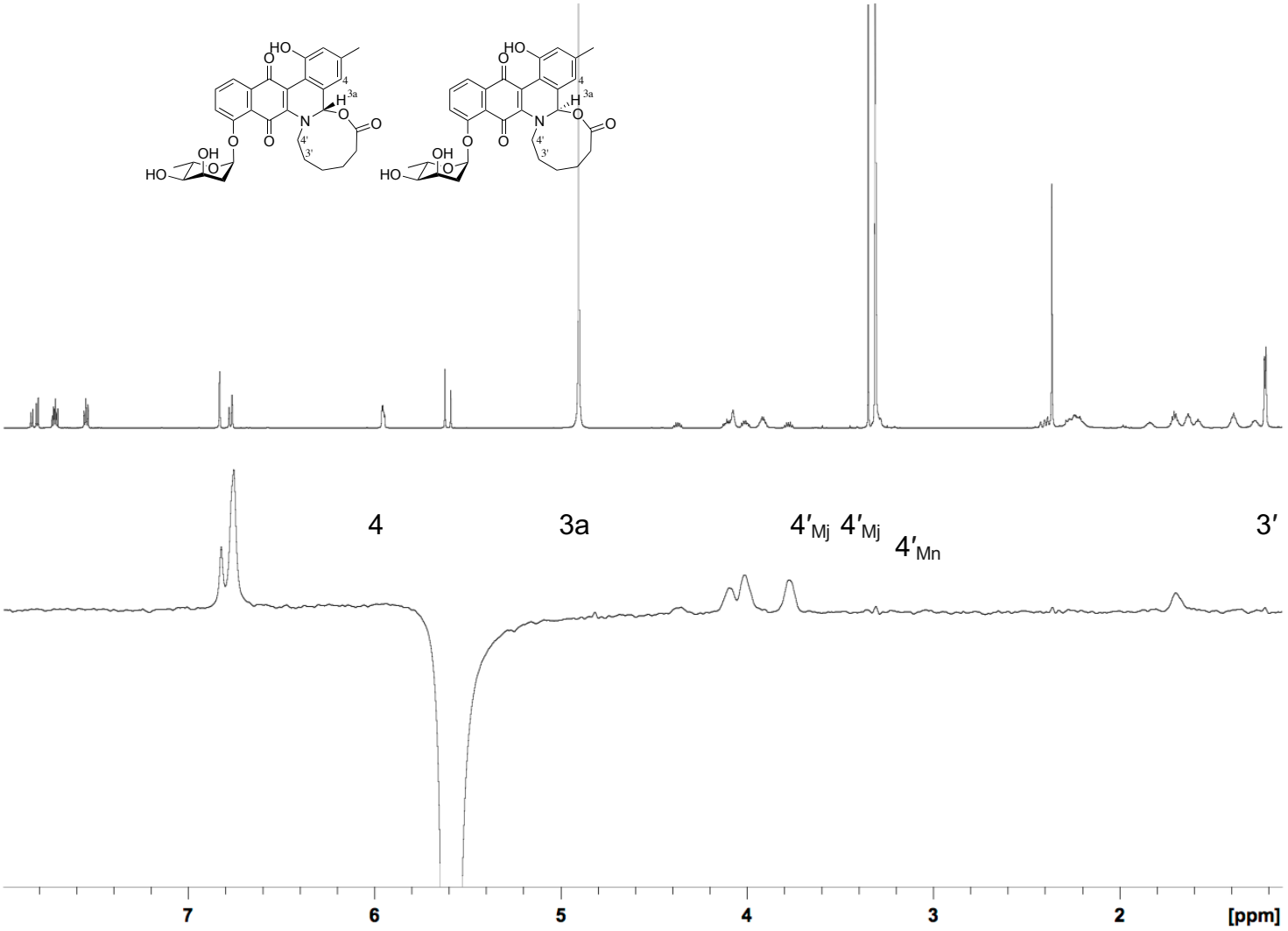


Figure S18. Overlay of ^{1}H-NMR spectrum of **2** (top) and with ROESY showing simultaneous irradiation of H3a$_{Mj}$ and H3a$_{Mn}$ (bottom) in CD_3OD (^{1}H: 500 MHz).

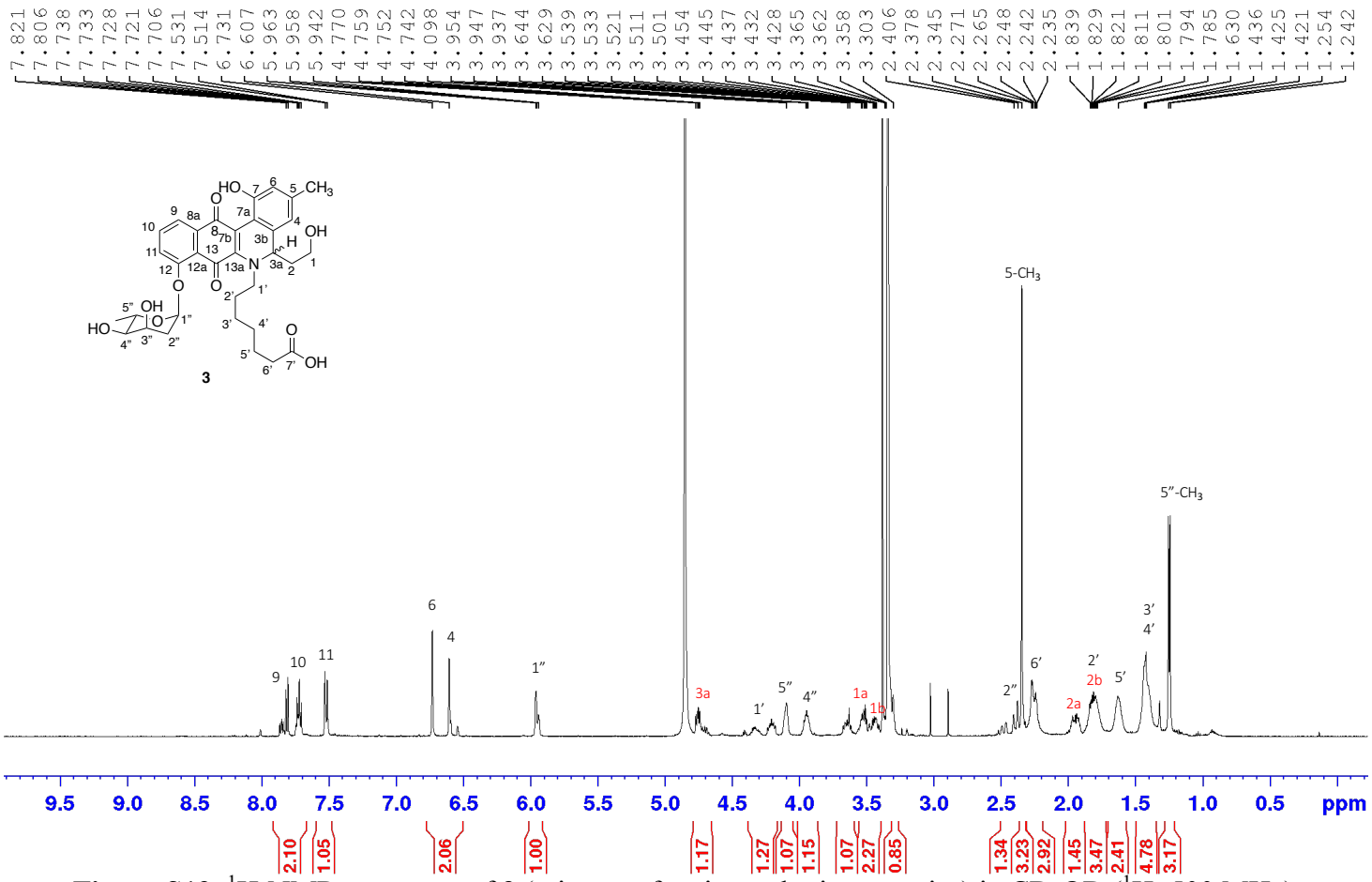


Figure S19. ^{1}H-NMR spectrum of **3** (mixture of major and minor species) in CD_3OD (^{1}H: 500 MHz).

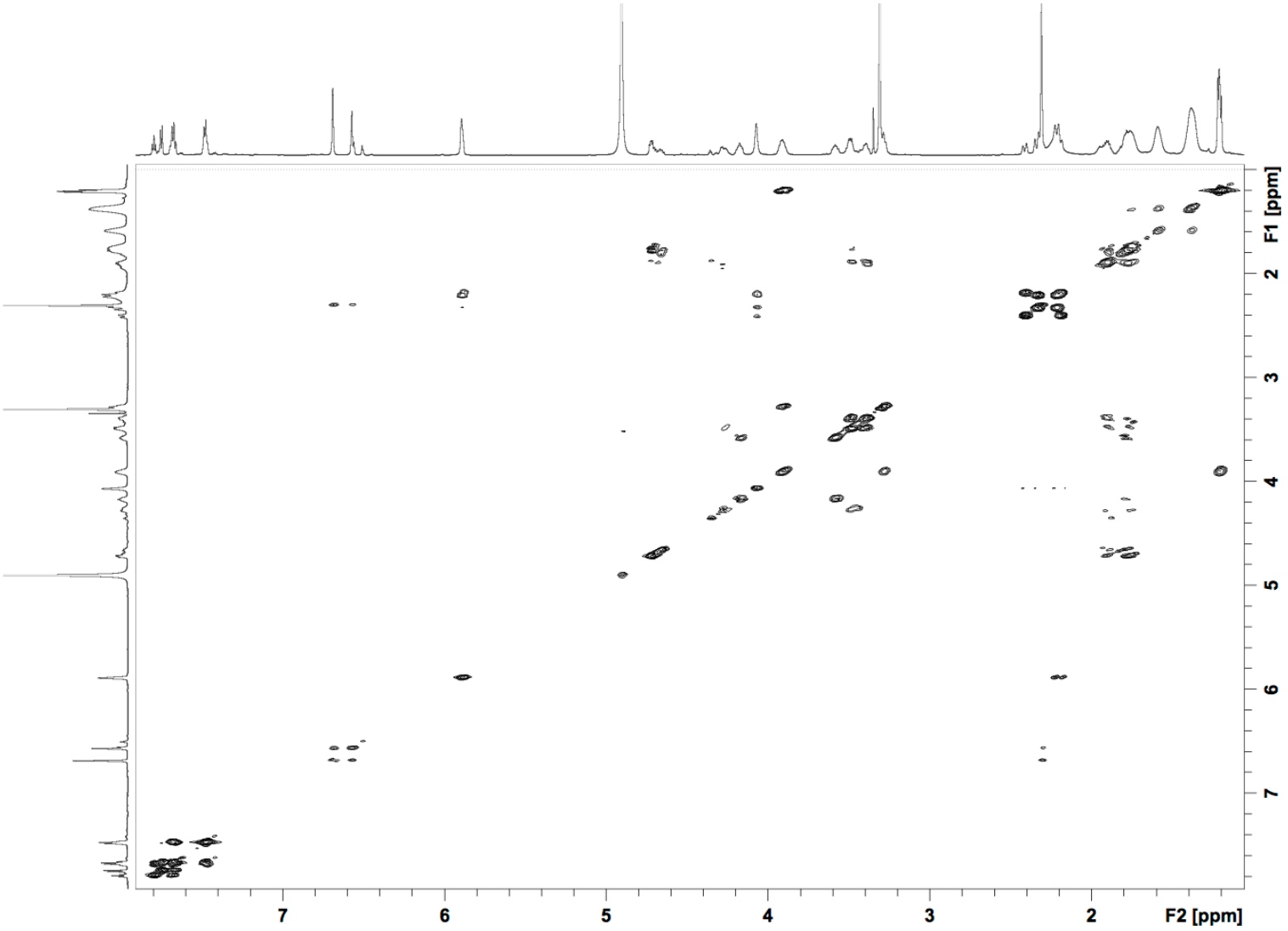


Figure S20. ^{1}H-^{1}H COSY spectrum of **3** (mixture of major and minor species) in CD_3OD (700 MHz).

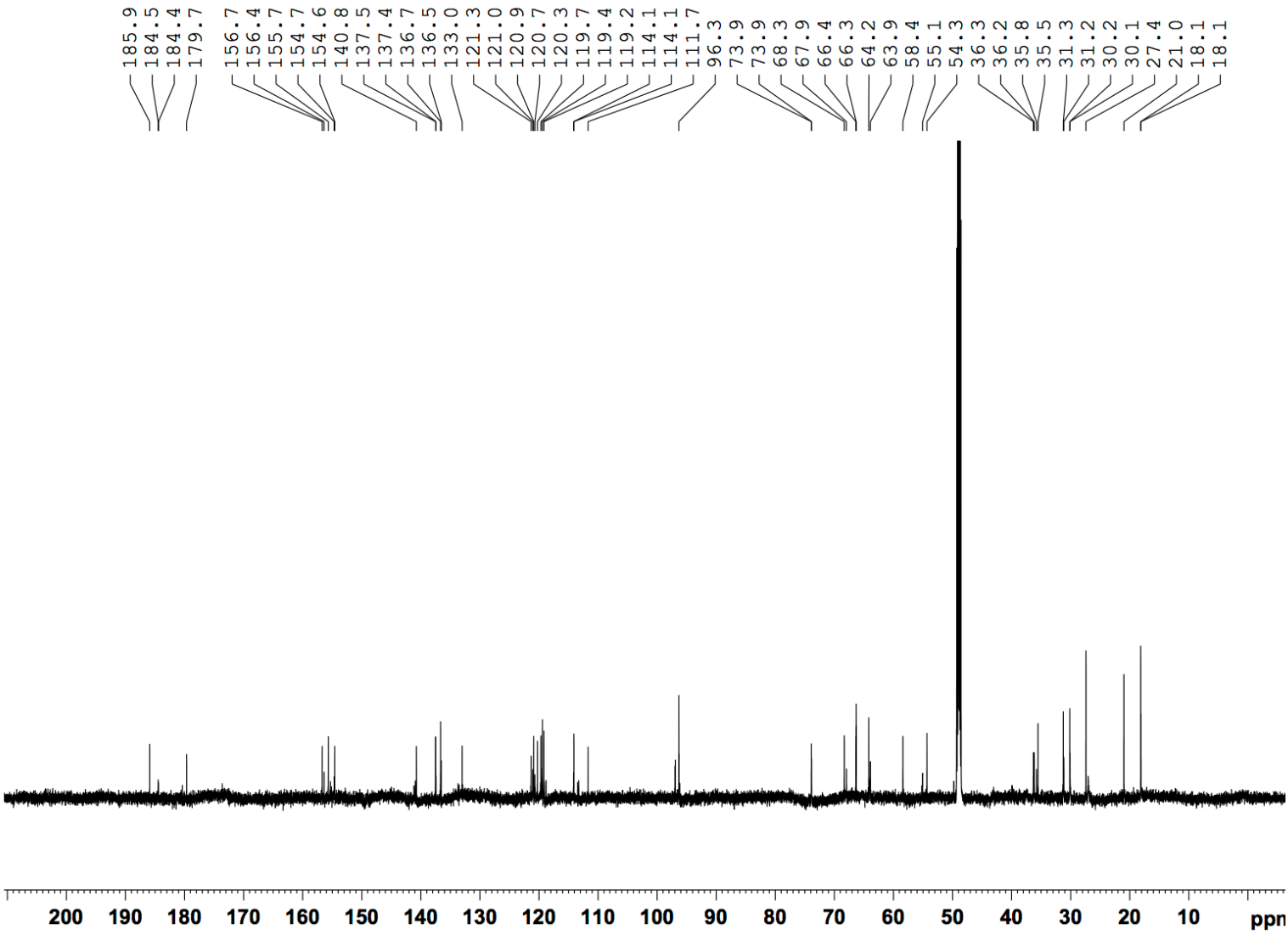


Figure S21. ^{13}C-NMR spectrum of **3** (mixture of major and minor species) in CD_3OD (^{13}C: 176 MHz).

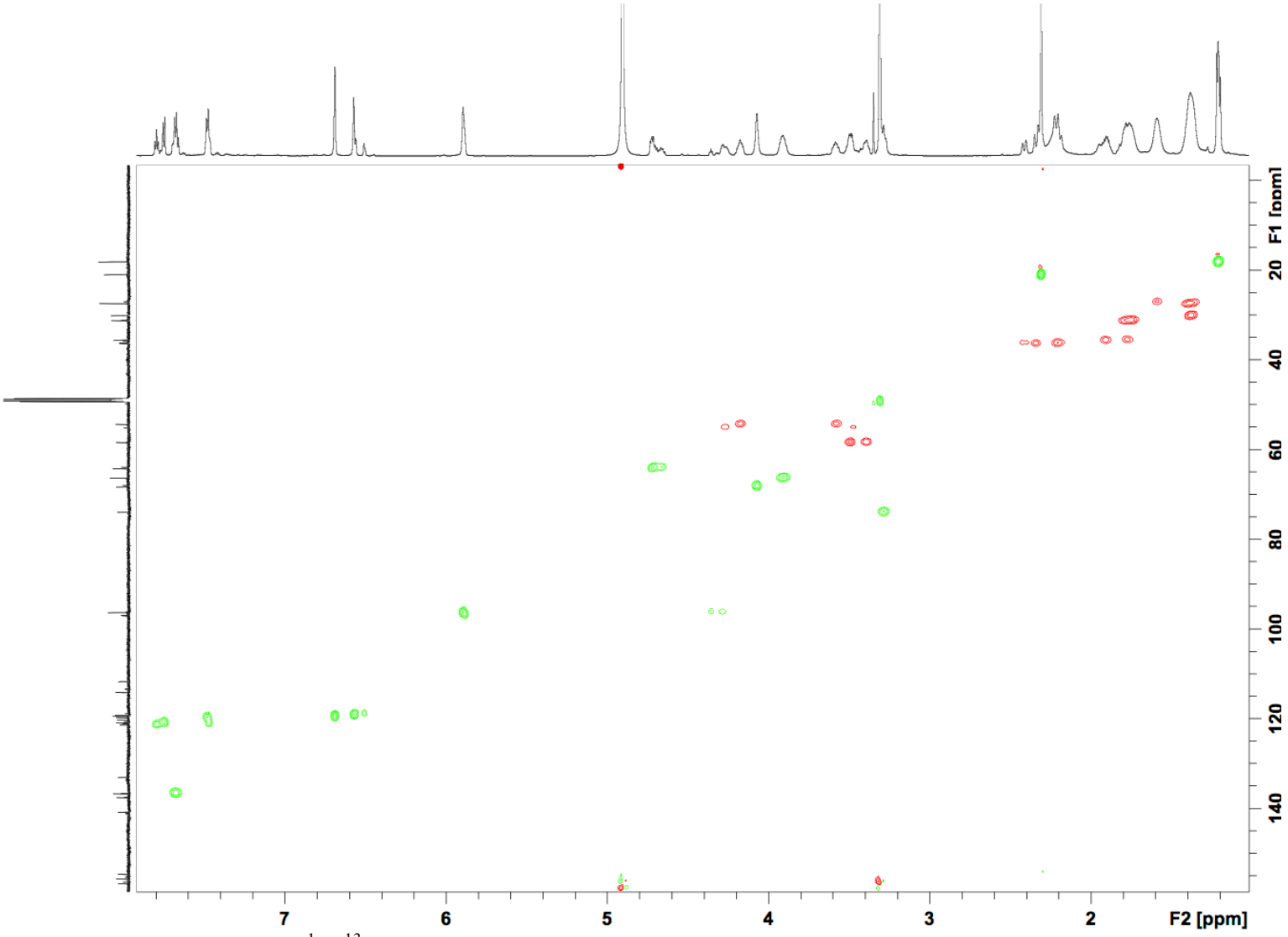


Figure S22. Edited-HSQC (^{1}H-^{13}C) spectrum of **3** (mixture of major and minor species) in CD_3OD.

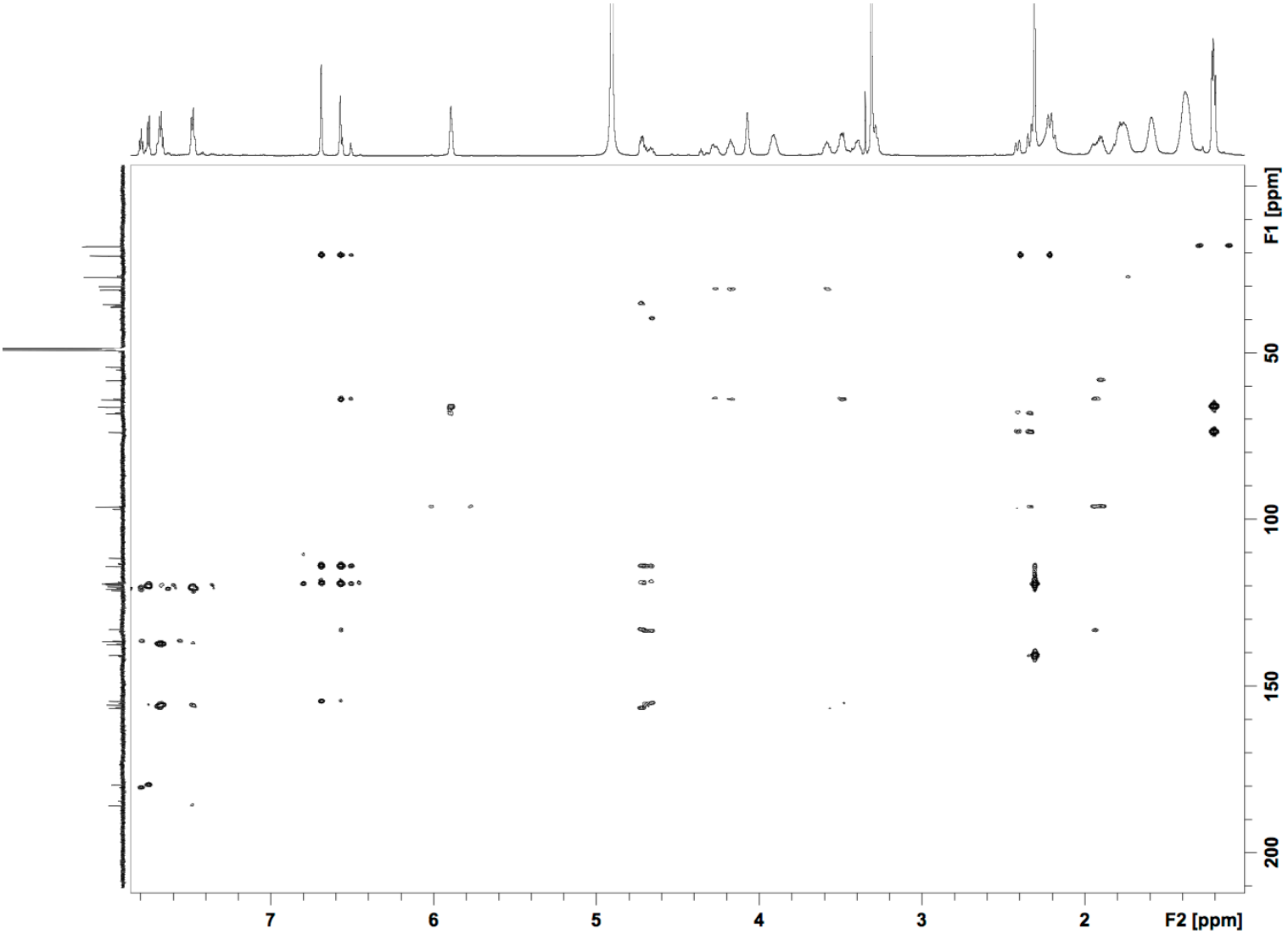


Figure S23. ^{1}H-^{13}C HMBC (6. 5 μs mixing time) spectrum of **3** (mixture of major and minor species) in CD_3OD.

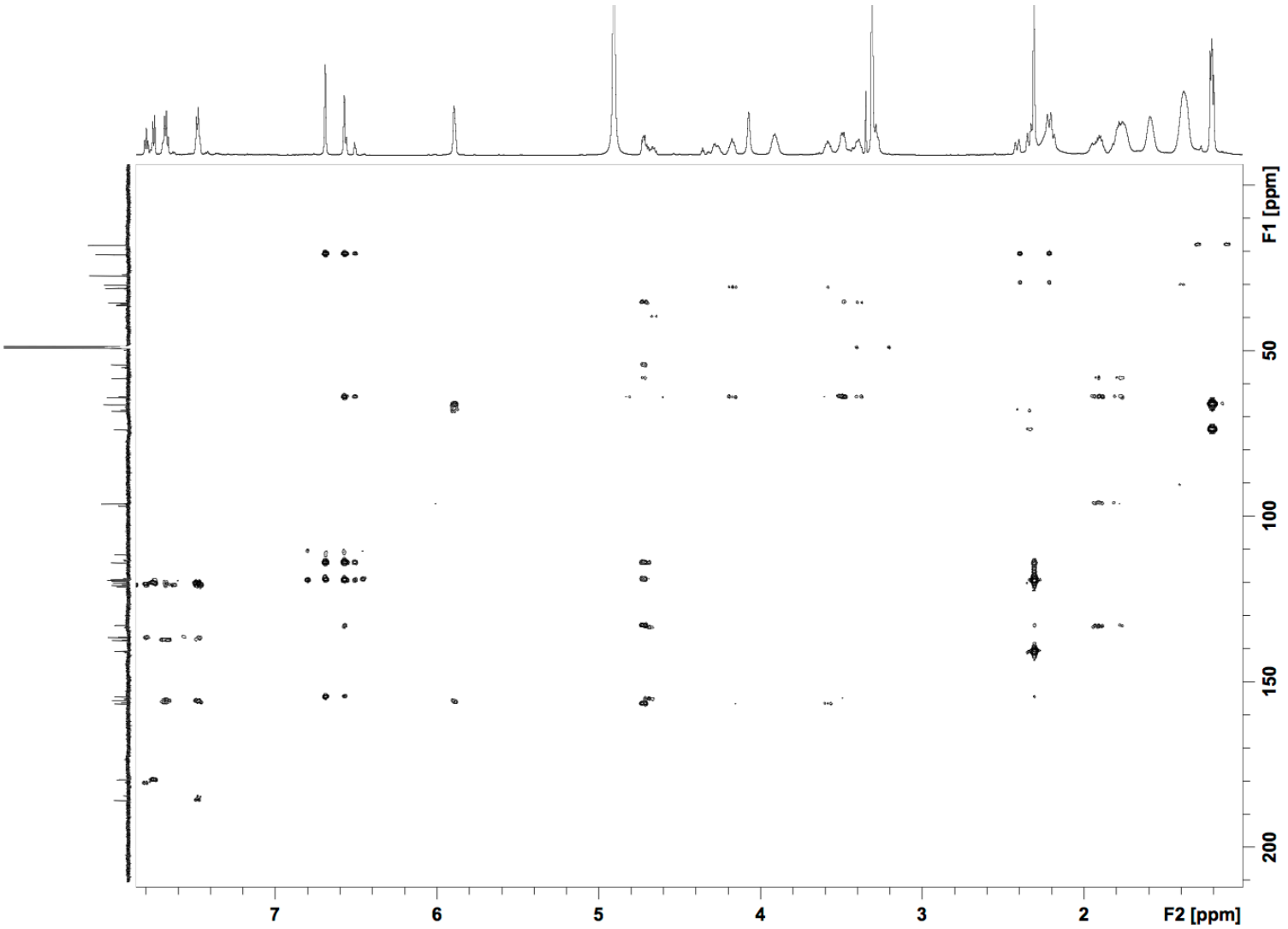


Figure S24. ^{1}H-^{13}C HMBC (25 μs mixing time) spectrum of **3** (mixture of major and minor species) in CD_3OD.

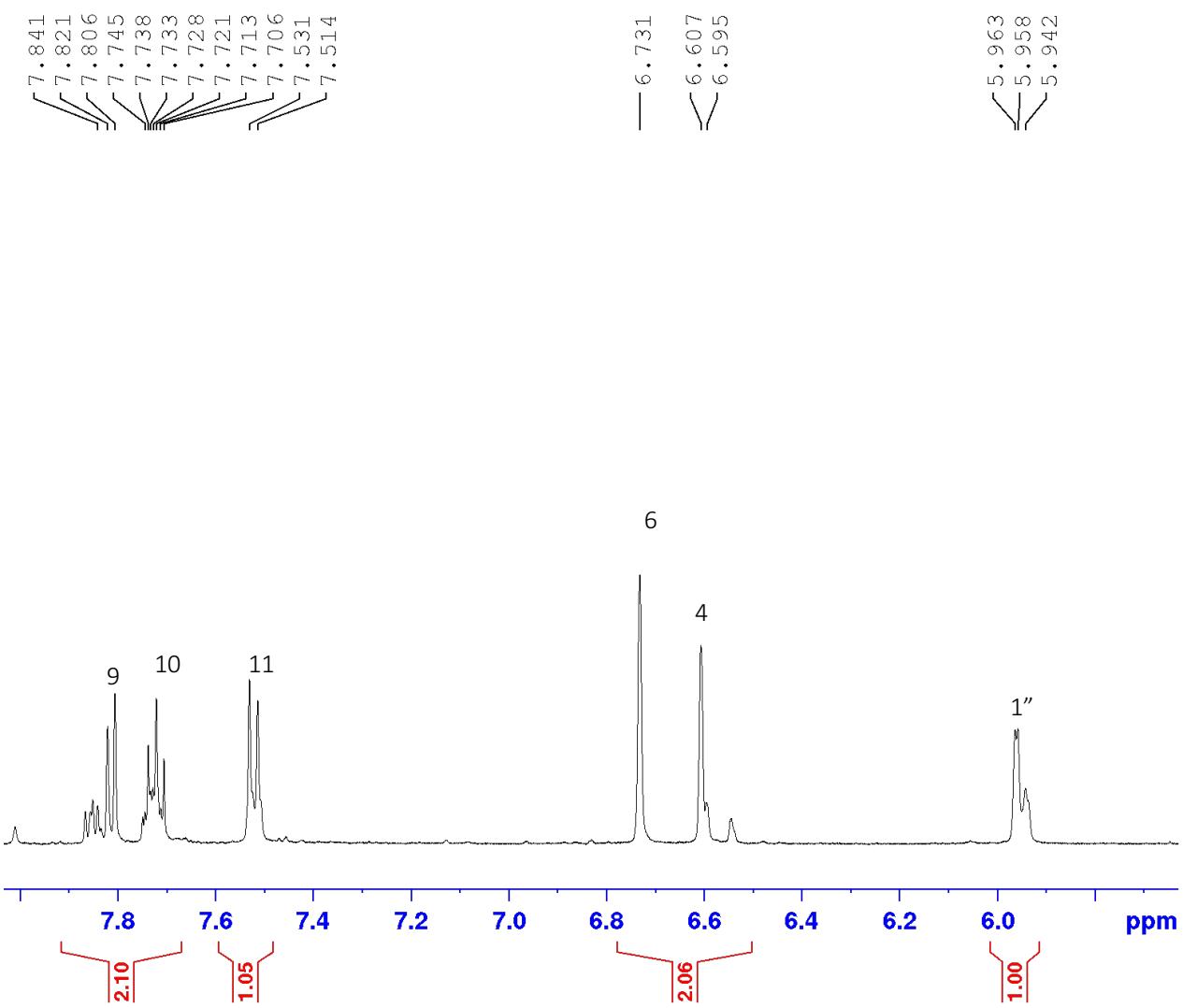


Figure S25a. An expansion of the ^{1}H NMR spectrum validating revised structure of **3** (500 MHz, methanol-d_4). Labelling is for major compound unless otherwise indicated. In red are the protons corresponding to the -CH2CH2OH substituent.

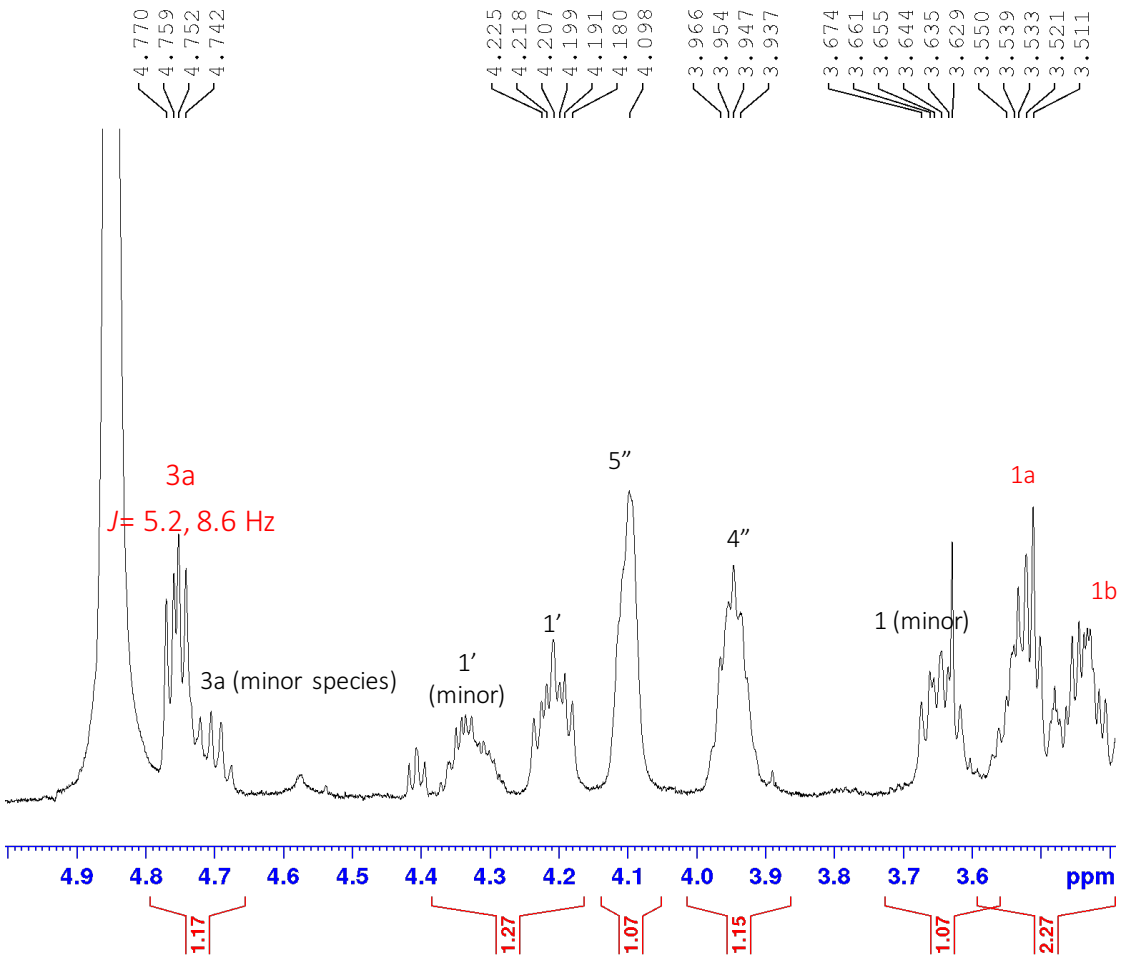


Figure S25b. An expansion of the ^{1}H NMR spectrum validating revised structure of **3** (500 MHz, methanol-d_4). Labelling is for major compound unless otherwise indicated. In red are the protons corresponding to the -CH2CH2OH substituent.

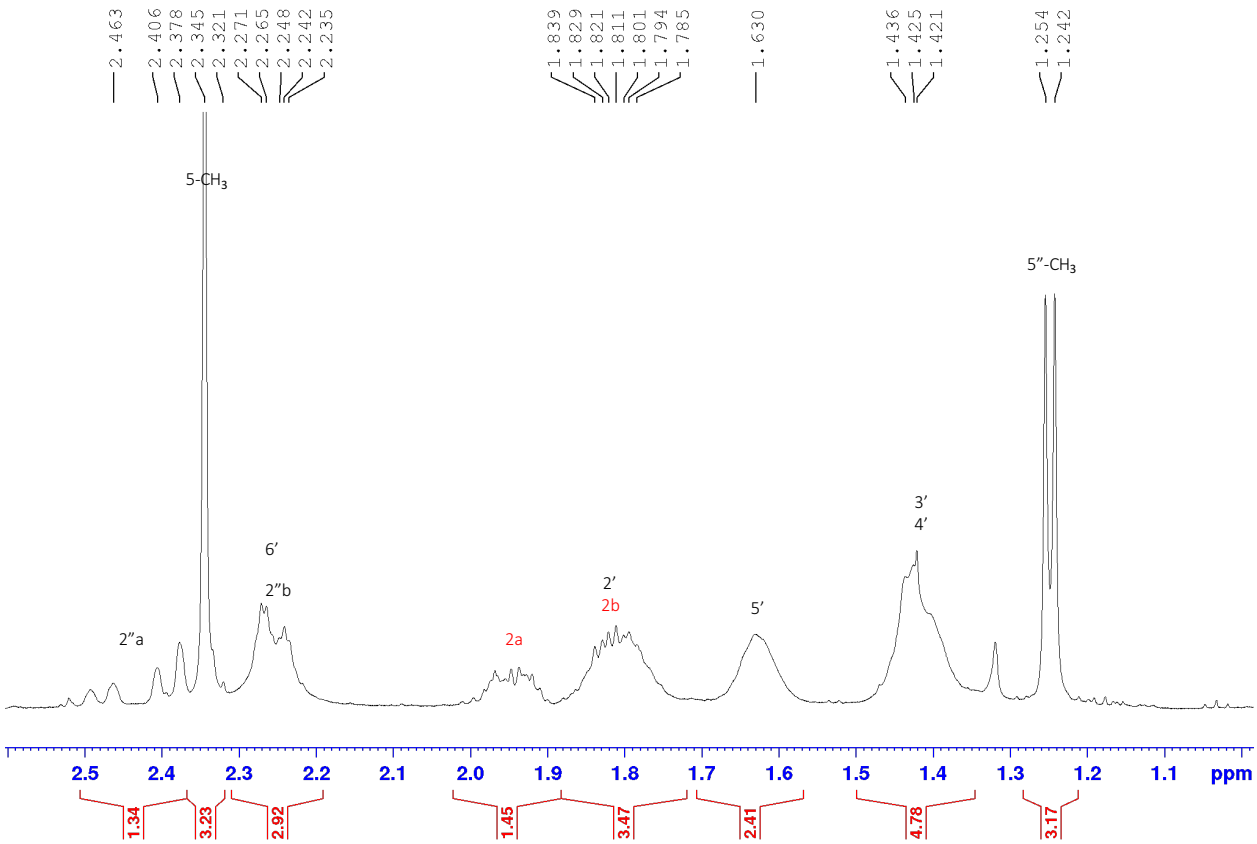


Figure S25c. An expansion of the ^{1}H NMR spectrum validating revised structure of **3** (500 MHz, methanol-d_4). Labelling is for major compound unless otherwise indicated. In red are the protons corresponding to the -CH2CH2OH substituent.

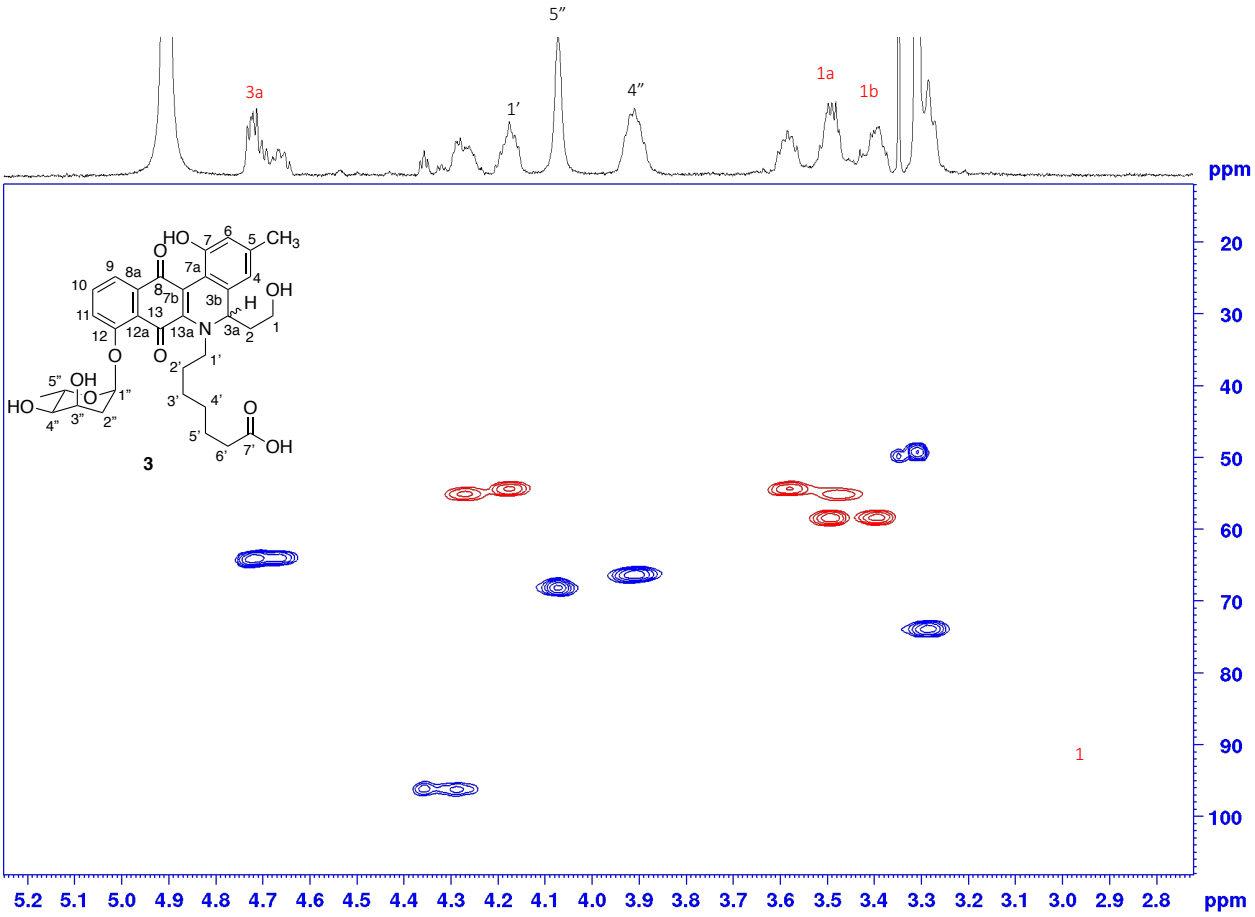


Figure S25d. Expansions of Edited HSQC experiment with opposite phasing of CH and CH_3 (blue) and CH_2 (red)
spectrum validating revised structure of **3** (500 MHz, methanol-d_4). Labelling is for major compound unless otherwise indicated. In red are the protons corresponding to the -CH2CH2OH substituent.

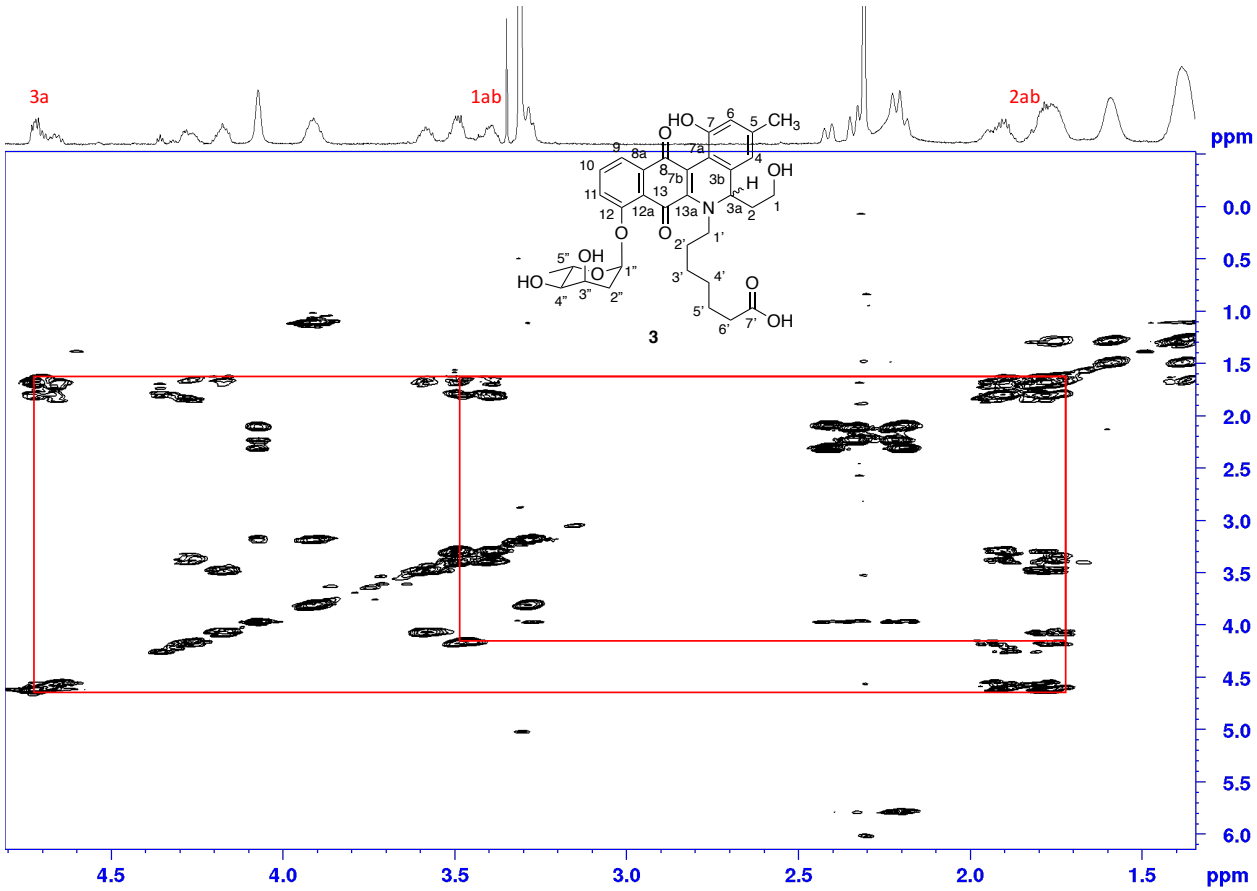


Figure S25e. Expansions of COSY NMR spectrum validating revised structure of **3** (500 MHz, methanol-d_4). Labelling is for major compound unless otherwise indicated. In red are the protons corresponding to the -CH2CH2OH substituent. ; red box shows (ethanolic) H1, H2, H3 coupling system

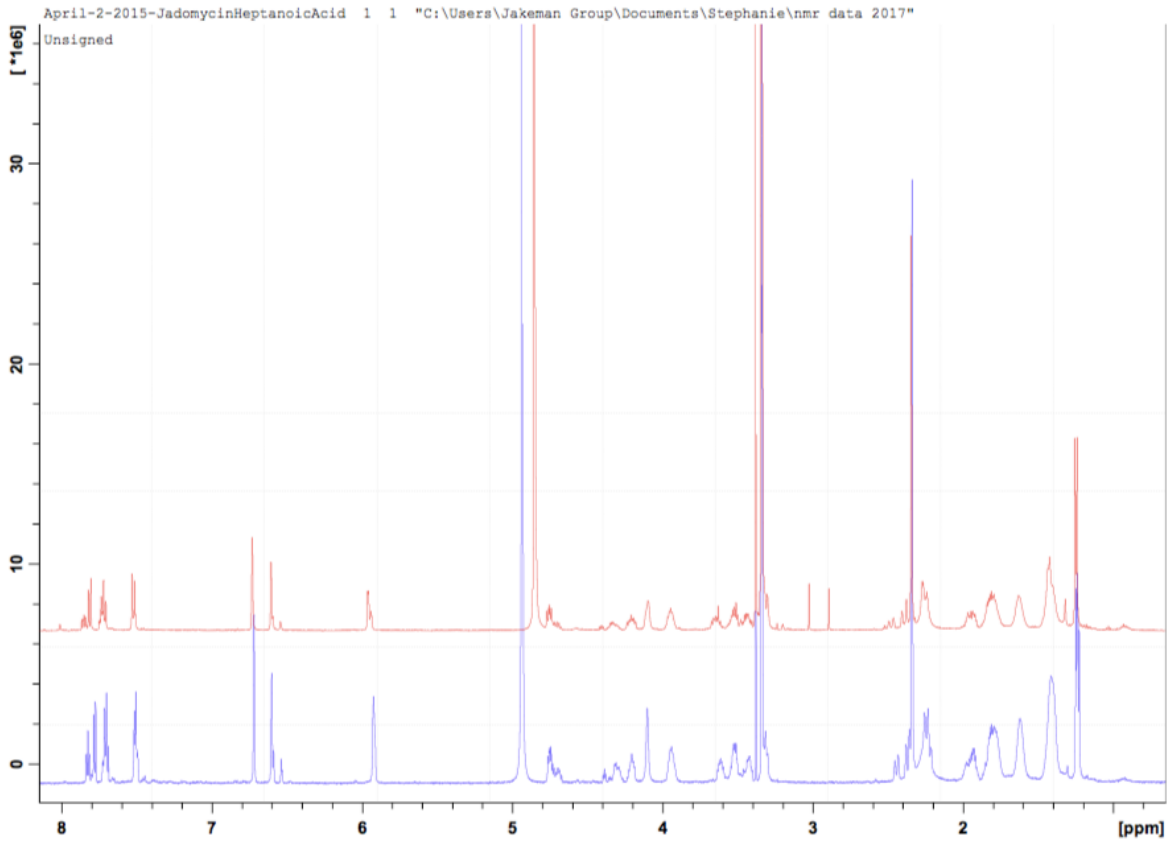


Figure S25f. ^{1}H NMR spectra of **3** recorded in 2015 (blue) and 2017 (red). No significant degradation or changes to the sample were observed.

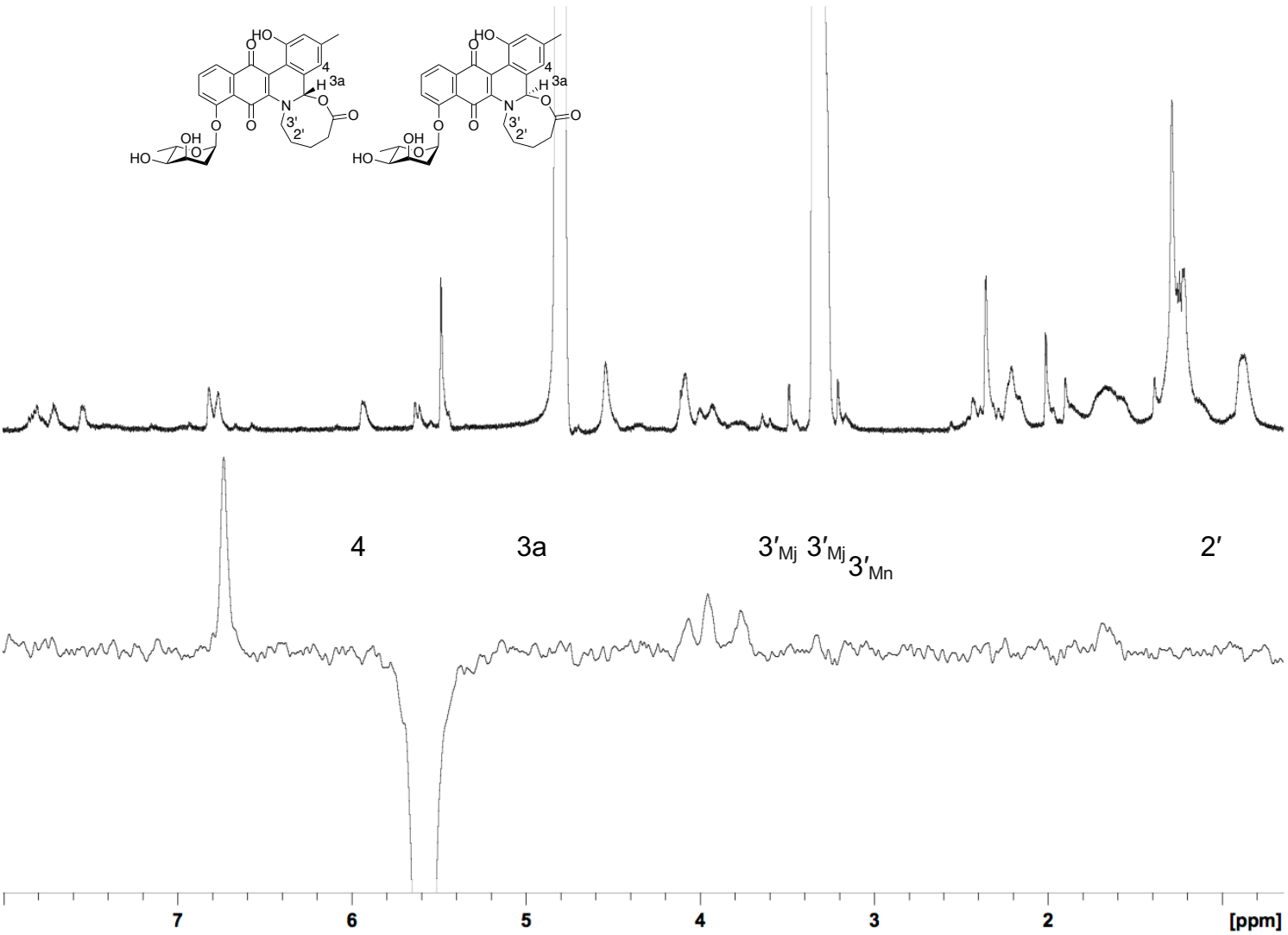
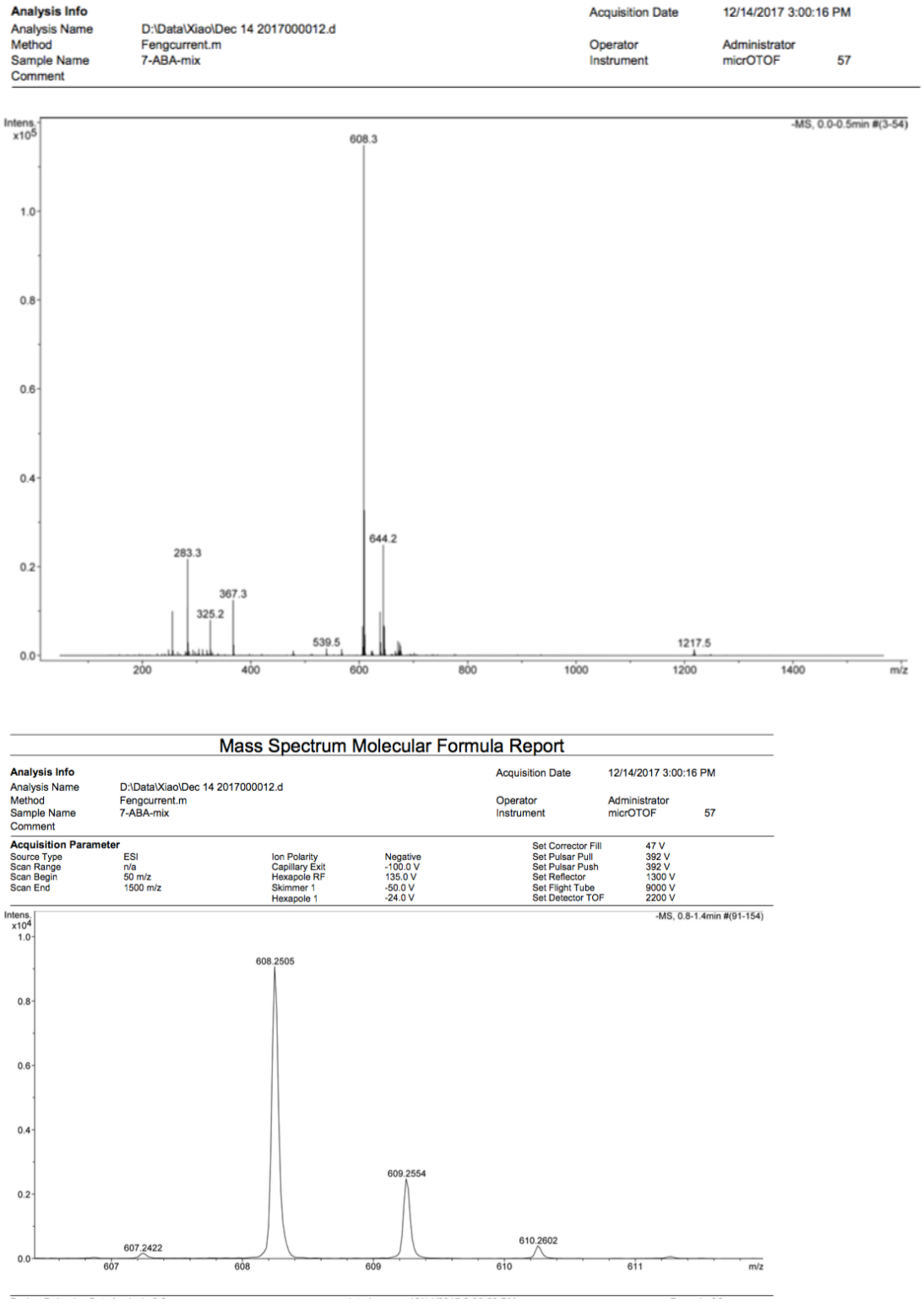


Figure S26. Overlay of ^{1}H-NMR spectrum of jadomycin AVA (top) and with NOESY showing simultaneous irradiation of H3a$_{Mj}$ and H3a$_{Mn}$ (bottom) in CD_3OD (^{1}H: 500 MHz).

HRMS data for Compound 3



Analysis Info

Analysis Name	D:\Data\Xiao\Dec 14 2017000012.d	Acquisition Date	12/14/2017 3:00:16 PM
Method	Fengcurrent.m	Operator	Administrator
Sample Name	7-ABA-mix	Instrument	micrOTOF 57
Comment			

Mass Spectrum Molecular Formula Report

Analysis Info

Analysis Name	D:\Data\Xiao\Dec 14 2017000012.d	Acquisition Date	12/14/2017 3:00:16 PM
Method	Fengcurrent.m	Operator	Administrator
Sample Name	7-ABA-mix	Instrument	micrOTOF 57
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Corrector Fill	47 V
Scan Range	n/a	Capillary Exit	-100.0 V	Set Pulsar Pull	392 V
Scan Begin	50 m/z	Hexapole RF	135.0 V	Set Pulsar Push	392 V
Scan End	1500 m/z	Skimmer 1	-50.0 V	Set Reflector	1300 V
		Hexapole 1	-24.0 V	Set Flight Tube	9000 V
				Set Detector TOF	2200 V

Figure S27. MS data (ESI-neg) for compound 3.