SUPPORTING INFORMATION

Oligomycins as inhibitors of K-Ras plasma membrane localisation

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Table of Contents

General experimental details

Fine chemicals were purchased from Merck or Sigma Aldrich 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 supplied by Labscan and filtered and degassed through an Alltech 0.45 μm polytetrafluoroethylene (PTFE) membrane prior to use. Deuterated solvents were supplied by Cambridge Isotopes (Andover, MA, USA). Commercial reagents were used without further purification.

Preparative HPLC was carried out on a system consisting of two Shimadzu LC-8A preparative liquid chromatographs with static mixer, Shimadzu SPD-M10 AVP diode array detector (DAD) and Shimadzu SCL-10 AVP system controller with a standard Rheodyne injection port. For all preparative HPLC the solvents were re-distilled before use. Semi-preparative HPLC was performed using an Agilent 1100 Series separations module equipped with Agilent 1100 Series Diode Array Detectors and Agilent 1100 Series fraction collector, controlled using ChemStation Agilent software.

Electrospray ionisation mass spectra (ESIMS) were acquired using an Agilent 1100 Series separations module equipped with an Agilent 1100 Series LC/MSD mass detector in both positive and negative ion modes. High-resolution (HR) ESIMS measurements were obtained on a Bruker micrOTOF with an ESI probe by direct infusion in acetonitrile at 3 μL/min using sodium formate clusters as an internal calibrant. Chiroptical measurements ($[\alpha]_D$) were obtained on a JASCO P-1010 polarimeter in a 100 × 2 mm cell at room temperature. UV-visible absorption spectra were obtained using a CARY50 UV-visible spectrophotometer in 1 cm quartz cells.

Nuclear magnetic resonance (NMR) experiments were carried out on a Bruker Avance 600 MHz spectrometer with 5 mm PASEL 1H/D-13C Z-Gradient probe, controlled by TopSpin II software. In all cases spectra were acquired at 25 °C (unless otherwise specified) in solvents as specified in the text, with referencing to residual ^{1}H signals in the deuterated solvent.

2. Taxonomy of microbial strain

2.1. MST-AS4799

The organism was isolated from a soil (Soil #3942) collected in 1997 from a roadside embankment near El Pont de Suert in Spain. The light reddish brown soil was neutral (pH 7.43) with low salinity (85 ppm NaCl), high colloidal (cloudiness) level and moderate levels of composted plant material. The culture grow readily on media commonly used for the cultivation of *Streptomyces* and was routinely passaged on International Streptomyces Project media 2 (ISP2) at 28 °C. An ISP2 agar plate was inoculated with the pure culture and incubated at 28 °C for 7 days, after which it was extracted with methanol to afford a crude extract for metabolite analysis. HPLC secondary metabolite analysis identified the strain as a producer of oligomycins A, B and C together with germicidins A and B, the most common oligomycin profile in nature (incidence of ~1 in 100 randomly isolated *Streptomyces*) and a lower producing replicate compared with MST-AS5339v11.

16S sequence of 721 BP (see below) identified the closest five sequence alignments belonged to the genus *Streptomyces*, accordingly the strain was classified as *Streptomyces* sp. MST-AS4799.

CACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTGGCGAACGGGTGAGTAACACG
TGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGAC
CACTGGGGGCATCTTCAGTGGTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAG
CTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGG
CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA
ATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTC
TTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCC
AGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGT
AGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGG
GCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATC
AGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGC
GTGGGGAGCGAACAGGATTAGATACCCTG

Organisms with five closest sequence alignments to *Streptomyces* sp. MST-AS4799 are as follows:

355557 EU841652.1 *Streptomyces hawaiiensis* str. HBUM174086	100%
313610 EU841653.1 *Streptomyces paradoxus* str. HBUM174056	100%
160428 AB249977.1 *Streptomyces arenae* str. NBRC 13016	100%
581927 GU227347.1 *Streptomyces hawaiiensis* str. HDJZ-ZWM-20	99.7%
276556 EU570675.1 *Streptomyces paradoxus* str. 173387	99.7%

2.2. MST-AS5339v11

The organism was isolated from a roadside embankment soil sample (Soil #2110) collected in 1995 near Hay in New South Wales, Australia. The greyish brown soil was weakly alkaline (pH 8.25) with low levels of salinity (222 ppm NaCl) and moderate levels of composted plant material. The culture grew readily on media commonly used for the cultivation of *Streptomyces* and was routinely passaged on ISP2 at 28 °C. HPLC secondary metabolite profile identified the strain as a high producer of oligomycins A-C, with germicidins A and B as co-metabolites. The culture was re-accessioned as MST-AS5339 in our talented strain library as a marker for this co-metabolite profile. The pattern is the most abundant profile of oligomycin producing strains found in nature with an incidence of ~1 in 100 randomly isolated *Streptomyces*. The parent strain showed erratic fermentation behaviour and was stabilised by mono-spore plating to generate strain MST-AS5339v11.

16S Reverse sequence (see below) was obtained after repeated efforts to obtain a forward sequence failed. 16S sequence of 543 BP identified the closest five sequence alignments belonged to the genus *Streptomyces*, accordingly the strain is identified as Streptomyces sp. MST-AS5339v11. Notably, the five closest species were identical to the strains identified as the same closest fits to *Streptomyces* sp. MST-AS4799, confirming that despite the geography disparity of the strains origin, the Spanish culture, *Streptomyces* sp. MST-AS4799 is a replicate of the Australian culture, *Streptomyces* sp. MST-AS5339v11.

GTCAGTATCGGCCCAGAGATCCGCCTTCGCCACCGGTGTTCCTCCTGATATCTGCGCATTTCACC
GCTACACCAGGAATTCCGATCTCCCCTACCGAACTCTAGCCTGCCCGTATCGACTGCAGACCCG
GGGTTAAGCCCCGGGCTTTCACAACCGACGTGACAAGCCGCCTACGAGCTCTTTACGCCCAATA
ATTCCGGACAACGCTCGCGCCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGCGCTT
CTTCTGCAGGTACCGTCACTTTCGCTTCTTCCCTGCTGAAAGAGGTTTACAACCCGAAGGCCGTC
ATCCCTCACGCGGCGTCGCTGCATCAGGCTTTCGCCCATTGTGCAATATTCCCCACTGCTGCCTC
CCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGGTCGCCCTCTCAGGCCGGCTACCC
GTCGTCGCCTTGGTGAGCCATTACCTCACCAACAAGCTGATAGGCCGCGGGCTCATCCTGCACC
GCCGGAGCTTTCGACCACTGAAGATGCC

Organisms with five closest sequence alignments to Streptomyces sp. MST-AS5339 are as follows:

GU227347.1 *Streptomyces hawaiiensis* str. HDJZ-ZWM-20	99.82%
EU841652.1 *Streptomyces hawaiiensis* str. HBUM174086	99.8%
EU841653.1 *Streptomyces paradoxus* str. BUM174056	99.8%
AB249977.1 *Streptomyces arenae* str. NBRC 13016	99.8%
EU570675.1 *Streptomyces paradoxus* str. 173387	99.6%

2.3. MST-AS5351

The organism was isolated for a roadside soil sample (Soil #1626) from shrub land near Carnarvon, Western Australia in 1995. The light orange brown soil was a weakly alkaline (pH 8.00) with high salinity (1,000 ppm NaCl) and low levels of composted plant material. The culture grew readily on media commonly used for the cultivation of *Streptomyces* and was routinely passaged on ISP2 at 28 °C. An ISP2 agar plate was inoculated with the pure culture and incubated at 28 °C for 7 days, after which it was extracted with MeOH for HPLC analysis. HPLC secondary metabolite analysis identified the strain as a producer of an unusual polar oligomycin with other co-metabolite containing a diene moiety, which was subsequently identified as nemadectin. The co-metabolite pattern of this oligomycin strain was rare, and was encountered only ~1 in

25,000 randomly sourced *Streptomyces*. The strain exhibited stable fermentation capability and was accessioned in the talented strain library as MST-AS5351.

16S sequence of 689 BP (see below) identified the closest five sequence alignments belonged to the genus Streptomyces, accordingly the strain was classified as *Streptomyces* sp. MST-AS5351.

CACATGCAAGTCGAACGATGAACCTCCTTCGGGAGGGGATTAGTGGCGAACGGGTGAGTAACA
CGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATATGA
CACTCTCGGGCATCCGATGAGTGTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATC
AGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACC
GGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCA
CAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACC
TCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTG
CCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTC
GTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACG
GGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATAT
CAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCG

Organisms with five closest sequence alignments to *Streptomyces* sp. MST-AS5351 are as follows:

FJ486461.1 *Streptomyces speibonae* str. HBUM173373	99.9%
EU593715.1 *Streptomyces piloviolofuscus* str. 174468	99.9%
EU570684.1 *Streptomyces variabilis* str.173370	99.9%
EU570415.1 *Streptomyces variabilis* str.173283	99.9%
EU841628.1 *Streptomyces variabilis* str. HBUM174897	99.9%

2.4. MST-AS5958

The organism was isolated from a soil (Soil #3246) collected at a light industrial site in Windsor, New South Wales in 1995. The orange brown soil was weakly acidic (pH 5.94) with very low saline levels (37 ppm NaCl) and high colloidal (cloudiness) levels containing low levels of plant materials. The culture grew readily on media commonly used for the cultivation of *Streptomyces* and was routinely passaged on ISP2 at 28 °C. An ISP2 agar plate was inoculated with the pure culture and incubated at 28 °C for 7 days, after which it was extracted with MeOH for HPLC analysis. HPLC secondary metabolite analysis identified the strain as a producer of an unusual oligomycin containing an additional non-polar co-metabolite (UV_{max} <205 nm) subsequently identified as venturicidin. The co-metabolite pattern of this oligomycin strain was extremely rare, and encountered only ~1 in 200,000 randomly sourced *Streptomyces*. The strain exhibited stable fermentation capability and was accessioned in the talented strain library as MST-AS5958.

16S sequence of 675 BP identified the closest five sequence alignments belonged to the genus *Streptomyces*, accordingly the strain was classified as *Streptomyces* sp. MST-AS5958.

TAGTGGCGAACGGGTGAGTAATACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAA
ACGGGGTCTAATACCGGATACTGACCTGCCGAGGCATCTTGGCGGGTCGAAAGCTCCGGCGGT
GCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGG
GTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGG
AGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGG
ATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCA
GAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCC
GGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTT
AACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGG
TGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCG
ATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAG

Organisms with five closest sequence alignments to *Streptomyces* sp. MST-AS5958 are as follows:

FJ972686.1 *Streptomyces fradiae* str. WF1	99.41%
DQ026631.1 *Streptomyces diastaticus* subsp. ardesiacus str. NRRL B-1773	99.41%
AB184068.2 *Streptomyces fradiae* str. NBRC12214	99.41%
AB184653.1 *Streptomyces diastaticus* subsp. ardesiacus str. NBRC 15402	99.41%
AB184069.1 *Streptomyces fradiae* str. NBRC12215	99.41%

Comparative analysis of the three oligomycin variants (AS5351, AS5958 and AS 4799) was carried out using NCBI Blast alignment tool based on 16S sequence data. This analysis demonstrated that the three cultures are taxonomically distinct and represent independent species within the genus *Streptomyces* (see table below).

	AS5351	**AS5958**	**AS4799**
AS5351	-	98% (636/651)	97% (674/692)
AS5958	98% (636/651)	-	98% (663/676)
AS4799	97% (674/692)	98% (663/676)	-

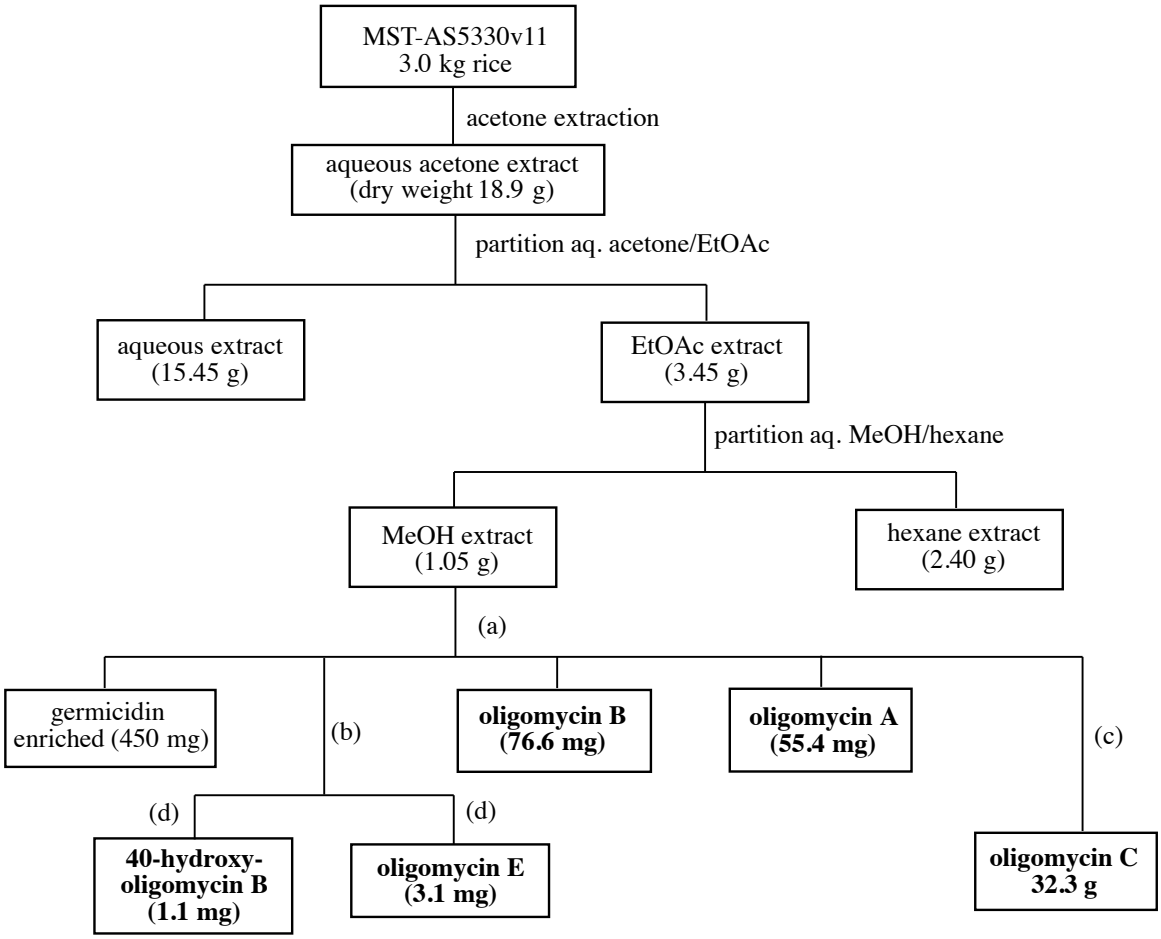
3. Cultivation and Isolation of compounds

3.1. MST-AS5339v11

Optimisation of MST-AS5339v11 on a range of liquid, agar and grain-based media demonstrated a broad substrate specificity with consistent yields of the oligomycins and the major co-metabolites on most media. Rice was selected as the preferred media for fermentation. Rice (1 kg) was hydrated in water (2 L), autoclaved (120 °C, 20 minutes) and allowed to cool on a horizontal surface to maximise surface area for aeration. The rice was inoculated with an aqueous spore suspension (100 mL) prepared from an ISP2 agar plate incubated at 28 °C for 7 days. The inoculated grain was mixed thoroughly and gently aerated with sterile air to hold the bag under positive pressure and was incubated at 28 °C for 10 days.

The grain (3 kg) was transferred to 3 × 5 L flasks and extracted with 1.5 volume of acetone for 2 h on a rotatory platform shaker at 125 rpm to ensure good maceration of the grain. The suspension was filtered under vacuum to remove the spent grains and mycelia. The combined acetone extracts were concentrated to an aqueous suspension (~1 L) *in vacuo* and partitioned against EtOAc (2 × 1 L) to provide a crude EtOAc extract (3.45 g), which was dissolved in 90% MeOH/H_2O (500 mL) and partitioned against hexane (2 × 500 mL) to remove the bulk of the grain fats and lipids. The MeOH layer was concentrated *in vacuo* to an aqueous residue and freeze-dried to provide MeOH extract (1.0 g), enriched with oligomycins and non-polar co-metabolites (Scheme S1).

The MeOH extract (1.05 g) was fractionated under isocratic conditions (82.5% MeCN/H_2O) on a preparative HPLC (Platinum EPS C_{18}, 150 × 50 mm, 5 μm, spring column, Grace Discovery) at 60 mL/min flow rate to afford pure oligomycin B (76.6 mg) and oligomycin A (55.4 mg), respectively. Fraction 6 (300 mg) was re-chromatograph on the same HPLC system (isocratic 87.5% MeCN/H_2O with 0.01% TFA) to afford pure oligomycin C (32.3 mg). Fraction 2 (127 mg) was re-chromatograph on the same HPLC system (isocratic 65% MeCN/H_2O) under neutral conditions to provide 14 fractions (2-1 to 2-14). Fraction 2-3- and 2-5 was subjected to preparative HPLC (Alltima C18 column, 150 × 22 mm, 5 μm, Grace Discovery, isocratic 77.5 % MeCN/H_2O) at 10 mL/min to yield 40-hydroxy-oligomycin B (1.1 mg) and oligomycin E (3.1 mg), respectively.



HPLC conditions:
(a) Platinum EPS C_{18} column (150x50 mm, 5 μm), 82.5% MeCN/H_2O isocratic elution, 60 mL/min flow rate
(b) Platinum EPS C_{18} column (150x50 mm, 5 μm), 65% MeCN/H_2O isocratic elution, 60 mL/min flow rate
(c) Platinum EPS C_{18} column (150x50 mm, 5 μm), 87.5% MeCN/H_2O (0.01% TFA), isocratic elution, 60 mL/min flow rate
(d) Alltima C_{18} column (150x22 mm, 5 μm), 77.5% MeCN/H_2O isocratic elucion, 10 mL/min flow rate)

Scheme S1. Isolation scheme for compounds from MST-AS5330v11

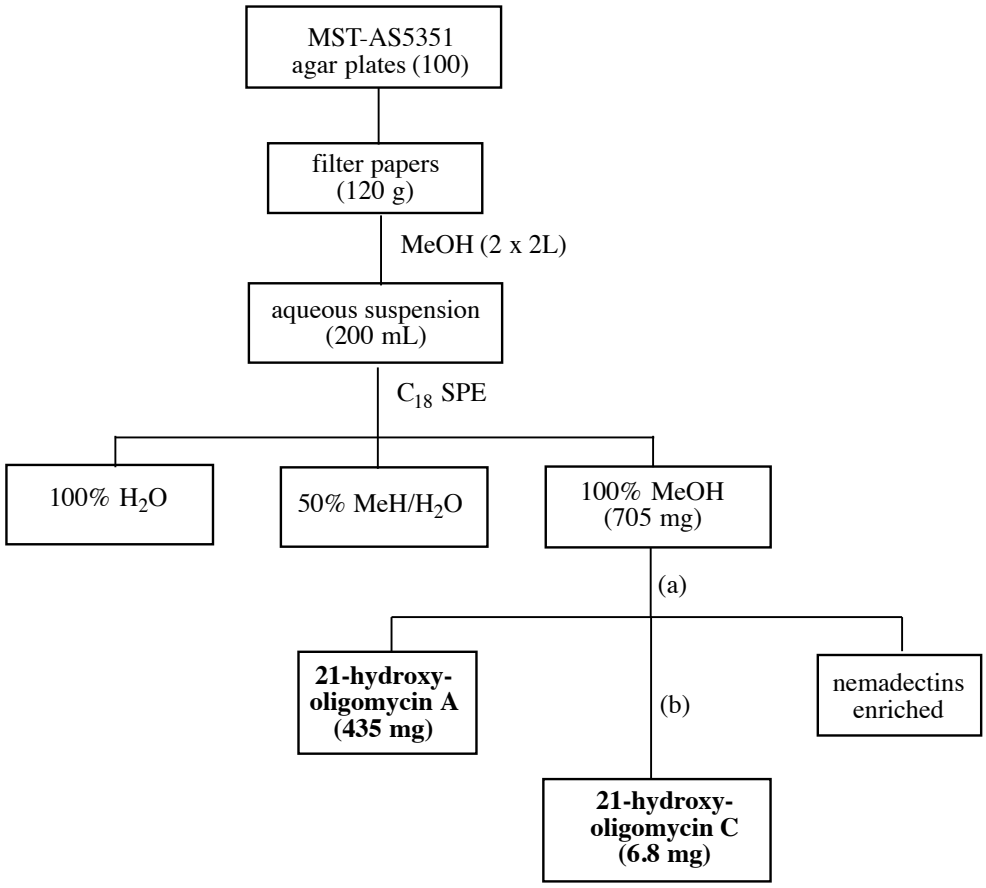
3.2. MST-AS5351

Optimisation of MST-AS5351 on a range of liquid, agar and grain based media demonstrated a highly media dependent co-metabolite patterns, with the oligomycins preferred the ISP2 agar using a "filter paper raft" method[1] to improve the yield and simplify the isolation strategy. ISP2 agar plates (100 plates, 9 cm, 15 mL media each) were carefully layered with individual sheets of moistly dampened and sterilized filter papers (8.5 cm, Whatman). The paper rafts were inoculated with a spore suspension prepared by stirring an inoculated ISP2 agar plate (previously incubated at 28 °C for 7 days) in 100 mL of sterile water. The inoculated plates were incubated at 28 °C for 7 days.

The paper (120 g) was removed from the plates, pooled in a flask (5 L) and extracted with MeOH (2 × 2L) for 2 h on a rotatory platform shaker at 125 rpm. The suspension was filtered under vacuum to remove the spent mycelia and paper fibres. The MeOH extract were concentrated to an aqueous suspension (200 mL), diluted to 2 L with deionised water then applied to 2 × 10 g C_{18} SPE tubes (Bond-elut, Varian), washed with deionised water (40 mL) followed by 50% MeOH/H_2O (40 mL). The SPEs were eluted with 100% MeOH (80 mL) and evaporated to dryness *in vacuo* to afford the enriched oligomycin extract (705 mg) (Scheme S2).

The oligomycin enriched extract was fractionated on a preparative HPLC (Platinum EPS C_{18} column, 150 × 50 mm, 5 μm, Grace Discovery, 30% MeCN/H_2O to 100% MeCN over 25 min, held at 100% MeCN for 5

min, with constant 0.01% triethylamine modifier) at 60 mL/min flow rate (collected at 20 mL/fraction) to afford pure 21-hydroxy-oligomycin A (435 mg), nemadectins mixture and enriched 21-hydroxy-oligomycin C fraction (55 mg), which was further purified using a preparative Waters Symmetry C_{18} column (150 × 19 mm) at 10 mL/min under isocratic conditions (60% MeCN/H_2O) to afford pure 21-hydroxyoligomycin C (6.8 mg).



HPLC conditions:
(a) Platinum EPS C_{18} column (150 x 50 mm, 5 μm), 30% MeCN/H_2O to 100% MeCN over 25 min, held at 100% MeCN for 5 min (constant 0.01% triethylamine modifier), 60 mL/min flow rate
(b) Symmetry C_{18} column (150 x 19 mm, 7 μm), isocratic 60% MeCN/H_2O, 10 mL/min flow rate

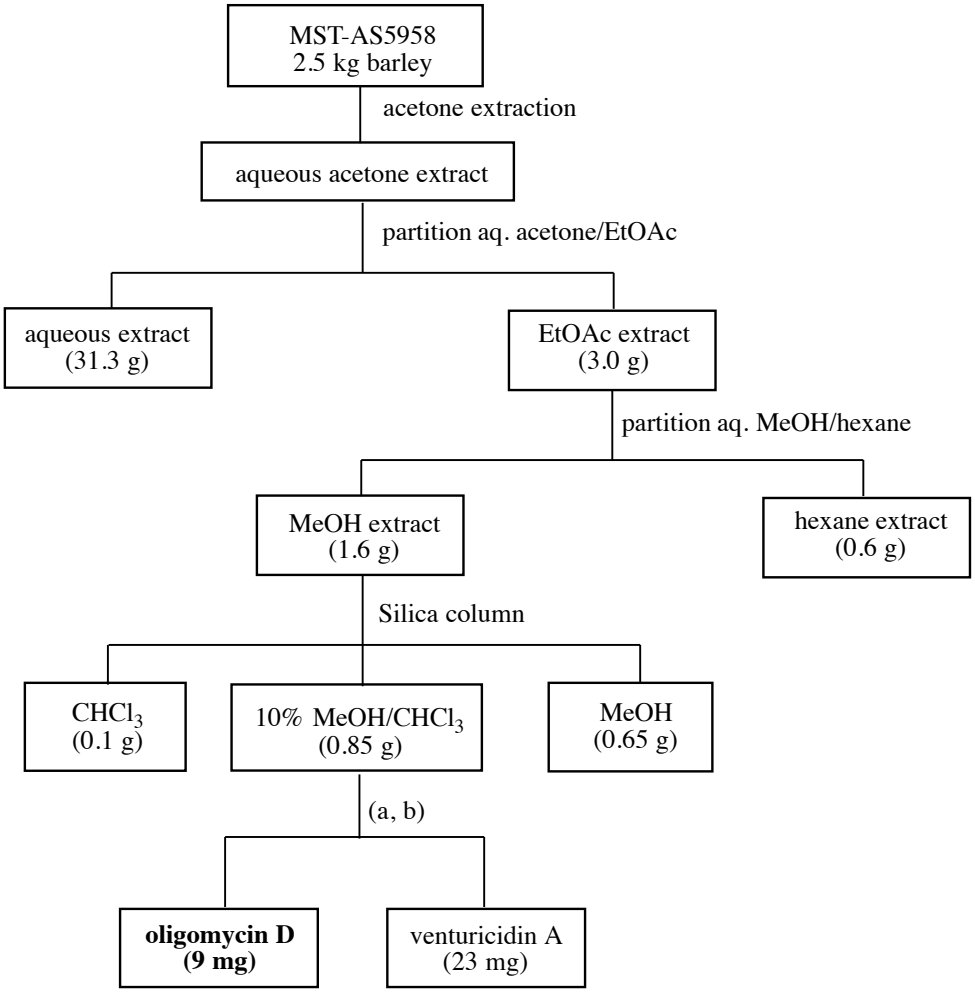
Scheme S2. Isolation scheme for compounds from MST-AS5351

3.3. MST-AS5958

Optimisation of MST-AS5958 on a range of liquid, agar and grain based media demonstrated low production in liquid and on agar based media, with higher levels noted using the grains barley and wheat. Barley was selected as preferred media for fermentation. Barley (1 kg) was hydrated in water (2 L), autoclaved (120 °C, 20 minutes), and cooled on a horizontal surface to maximise aeration. The grain was inoculated with an aqueous spore suspension (100 mL) prepared from an ISP2 agar plate incubated at 28 °C for 7 days. The inoculated grain was mixed thoroughly and gently aerated with sterile air to hold the bag under positive pressure then incubated at 28 °C for 10 days.

The grain (2.5 kg) was transferred to 3 × 5 L flasks and extracted with 1.5 volume of acetone for 2 h on a rotatory platform shaker at 125 rpm to ensure good maceration of the grain. The suspension was filtered under vacuum to remove the spent grains and mycelia. The pooled acetone extract were concentrated to an aqueous suspension (~1 L) *in vacuo* and partitioned against EtOAc (2 × 500 mL) to provide a crude EtOAc extract (3.0 g), which was dissolved in 90% MeOH/H_2O (250 mL) and partitioned against hexane (350 mL) to remove the bulk of the grain fats and lipids. The methanol layer was concentrated *in vacuo* to an aqueous residue and freeze-dried to 1.6 g of MeOH extract (Scheme S3).

The MeOH extract was dissolved in $CHCl_3$ and passed through a silica column (120 g) to remove fats and lipids, followed by 10% MeOH/$CHCl_3$ to elute the oligomycin fraction (850 mg) and washed with 100% MeOH. The enriched oligomycin fraction was fractionated under isocratic conditions (55% MeCN/H_2O) using a preparative Platinum EPS C_{18} column (150 × 50 mm, 5 μm, spring column, Grace Discovery) at 60 mL/min (collected at 20 mL/fraction) to afforded enriched oligomycin D fraction (50 mg), which was further chromatographed on a preparative HPLC (Luna C_{18} column, 100 × 20 mm, 5 μm, Phenomenex, isocratic 70% MeCN/H_2O at 10 mL/min) afforded pure oligomycin D (9 mg) and venturicidin A (23 mg).



HPLC conditions:
(a) Platinum EPS C_{18} column (150 x 50 mm, 5 μm), 55% MeCN/H_2O isocratic elution, 60 mL/min flow rate
(b) Luna C_{18} column (150 x 20 mm, 5 μm), 70% MeCN/H_2O isocratic elution, 10 mL/min flow rate

Scheme S3. Isolation scheme for compounds from MST-AS958

4. Biological assays

4.1. K-Ras bioassay

K-Ras bioassay was carried out as previously described.[2] Briefly, Madin-Darby canine kidney (MDCK) cells stably co-expressing monomeric green fluorescence protein (mGFP) coupled to the N-terminus of oncogenic K-Ras (K-RasG12V) and mCherry-CAAX, a red fluorescence fusion protein that decorates endomembranes, were plated at 150,000 cells/well on 12-well plates. After 24 h, cells were treated with compounds and incubated for another 48 h. Each compound was tested in 3 independent experiments. At the end of incubation time, cells were fixed with 4% paraformaldehyde and imaged in a Nikon A1R confocal microscope. Ras mislocalisations from the plasma membranes were calculated using Manders coefficients, by measuring the fraction of mCherry-CAAX co-localizing with mGFP-K-RasG12V.[1] IC_{50} values and two-tailed t-tests were calculated using Prism software (Ver. 5.0c, GraphPad).

4.2. Resazurin cytotoxicity assay

The resazurin assay was modified from that previously described,[3] using adherent cell line SW620 and its P-gp over-expressing daughter cell lines SW620 Ad300.[4] Briefly, cells were harvested with trypsin and dispensed into 96-well microtitre assay plates at 2,000 cells/180μL/well, followed by incubation for 18 h at 37 °C with 5% CO_2 (to allow cells to attach). Oligomycins were dissolved in 5% DMSO in PBS (v/v) and aliquots (20 μL) tested over a series of concentrations. Control wells were treated with 5% aqueous DMSO. After 68 h incubation at 37 °C with 5% CO_2 an aliquot (20 μL) of resazurin in PBS was added to each well (5 μM/well), and the microtitre plates incubated for a further 4 h in dark at 37 °C in 5% CO_2 to allow blue and non-fluorescent resazurin to be converted to bright red and fluorescent resofulin. After this final incubation, resofulin fluorescence was detected using a POLARstar Omega plate reader at an excitation wavelength of 550 nm and emission of 584 nm. IC_{50} values were calculated using Prism 5.0 (GraphPad Software Inc., La Jolla, CA), as the concentration of analyte required for 50% inhibition of cancer cell growth (compared to negative controls). All experiments were performed in duplicate.

4.3. Flow cytometry

The ability of oligomycins to inhibit P-gp mediated efflux of calcein AM was measured using flow cytometry, based on a previously described method.[4,5] Briefly, P-gp overexpressing SW620 Ad300 cells were harvested with trypsin and re-suspended in RPMI 1640 (phenol red-free with 10% FBS) to give a final concentration of 50×10^4 cells/mL. Cells were then pre-incubated with 20 μM of oligomycins, or positive control 20 μM verapamil for 15 min at 37°C in 5% CO_2. Subsequently, cells were incubated with 0.25 μM calcein AM for 30 min followed by washing twice with cold medium. Intracellular fluorescence intensity of calcein was then directly measured on a BD FACSCanto™ II flow cytometer (Becton Dickinson, San Jose, CA). Calcein was detected with a 480 nm laser and a 530/30 nm bandpass filter and data were analyzed through FlowJo (Tree Star, Inc, Ashland, OR). By increasing threshold value on forward scatter versus side scatter, debris was eliminated. Dead cells were excluded based on propidium iodide staining.

Reference

1. Cottoquinazoline A and Cotteslosins A and B, Metabolites from an Australian Marine-Derived Strain of Aspergillus versicolor Fremlin L. J. et al J. Nat. Prod. 72:666-670 (2009
2. K.-J. Cho, J.-H. Park, A. M. Piggott, A. A. Salim, A. A. Gorfe*, et al.*, *J. Biol. Chem.* **2012**, *287*, 43573.
3. J. O'brien, I. Wilson, T. Orton, F. Pognan, *Eur. J. Biochem*. **2000**, *267*, 5421.
4. X.-C. Huang, Y.-L. Sun, A.A. Salim, Z.-S. Chen, R.J. Capon, *Biochem. Pharmacol*. **2013**, *85*,1257.
5. C.J. Henrich, H.R. Bokesch, M. Dean, S.E. Bates, Robey RW, *et al*., *J. Biomol. Screen.* **2006**, *11*, 176.

Table S1. NMR (600 MHz, DMSO-d_6) data for 21-hydroxy-oligomycin C (**7**)

Position	δ_C	δ_H, multiplicity (*J* in Hz)	COSY	HMBC (^{1}H to ^{13}C)
1	164.7	-	-	-
2	120.9	5.84, d (15.7, 0.7)	3	1, 4
3	150.8	6.77, dd (15.7, 8.5)	2, 4	1, 2, 4, 5, 35
4	41.1	2.38, ddq (8.5, 7.4, 6.8)	3, 5, 35	2, 3, 5, 6, 35
5	72.0	3.72, ddd (7.4, 4.8, 4.2)	4, 6, 5-OH	35, 36
6	46.4	2.67, qd (7.1, 4.2)	5, 36	7, 36
7	215.5	-	-	-
8	47.5	2.50, m[b]	9, 37	7, 9, 37
9	70.8	3.90, m[a]	8, 10, 9-OH	10, 37, 38
10	51.2	2.71, dq (7.0, 7.0)	9, 38	8, 9, 11, 38
11	216.8	-	-	-
12	49.4	2.75, dq (9.3, 6.8)	13, 39	11, 13, 39
13	72.5	3.45, ddd (9.3, 6.2, 1.2)	12, 14, 13-OH	11, 12, 15, 40
14	33.7	1.57, m[a]	13, 15ab, 40	12, 16
15a	36.7	2.04, m[a]	14, 15b, 16	13, 14, 17
b		1.95, m[a]	14, 15a, 16	13, 14, 17
16	130.8	5.44, ddd (14.6, 10.1, 4.7)	15ab, 17	15, 17, 18
17	132.1	6.04, dd (14.6, 10.5)	16, 18	15, 19
18	132.0	5.98, dd (14.8, 10.5)	17, 19	16, 17, 20
19	132.7	5.37, dd (14.8, 9.3)	18, 20	17, 18, 20
20	49.9	2.03, m[a]	19, 21, 41a	
21	70.5	3.62, m	20, 22, 21-OH	
22a	35.3	1.46, m	21, 23	21, 23
b		1.42, m	21, 23	
23	66.6	3.91, m[a]	22, 24	21, 22, 25, 27, 43
24	34.4	1.94, m[a]	23, 25, 43	25, 26, 43
25	74.8	4.88, dd (11.3, 5.0)	24, 26	1, 43, 44, 26
26	37.4	1.73, dq (11.3, 6.6)	25, 44	25, 27, 28, 44
27	98.9	-	-	-
28a	25.2	1.84, ddd (13.7, 13.7, 4.8)	28b, 29ab	27, 29
b		1.16, ddd (13.7, 2.8, 2.8)	28a, 29ab	
29a	26.1	1.94, m[a]	28a, 29b, 30	28
b		1.37, m	28ab, 29a	27
30	29.7	1.53, m[a]	29a, 31, 45	
31	67.2	3.88, m[a]	30, 32ab	45
32a	42.5	1.42, m[a]	31, 32b	
b		1.25, m[a]	31, 32a, 33	33
33	62.8	3.78, m	32b, 34, 33-OH	31
34	24.9	1.11, d (6.2)	33	32, 33
35	16.2	1.00, d (6.8)	4	3, 4, 5
36	10.7	0.98, d (7.1)	6	5, 6, 7
37	10.1	0.95, d (6.8)	8	7, 8, 9
38	12.6	0.99, d (7.0)	10	9, 10, 11
39	13.3	0.74, d (6.8)	13	11, 12, 13
40	12.5	0.75, d (6.9)	14	13, 14, 15
41a	21.8	1.53, m[a]	20, 41b, 42	21, 40
b		1.25, m[a]	41a, 42	
42	12.4	0.80, t (7.4)	41ab	20, 41
43	5.4	0.77, d (6.9)	24	23, 24, 25
44	11.5	0.86, d (6.6)	26	25, 26, 27
45	11.0	0.85, d (6.7)	30	29, 30, 31
5-OH	-	4.62, d (4.8)	5	4, 5, 6
9-OH	-	4.74, d (6.7)	9	8, 9, 10
13-OH	-	4.09, d (6.2)	13	12, 13
21-OH	-	4.50, d (3.1)	21	20, 21, 22
33-OH	-	4.41, d (5.8)	33	32, 33, 34

[a]Coupling constant cannot be determined due to signals overlap.[b]Signal is obscured under DMSO-d_6 solvent peak.

Table S2. NMR (600 MHz, DMSO-d_6) data for 40-hydroxy-oligomycin B (**8**)

Position	δ_C	δ_H, multiplicity (*J* in Hz)	COSY	HMBC (^{1}H to ^{13}C)
1	164.4	-	-	-
2	121.3	5.88, d (15.6)	3	1, 4
3	150.3	6.79, dd (15.6, 9.7)	2, 4	1, 4, 5, 35
4	41.6	2.39, m[a]	3, 5, 35	2, 3, 5, 35
5	71.6	3.79, m[a]	4, 6, 5-OH	4, 36
6	43.4	2.62, qd (7.0, 2.3)	5, 36	7, 36
7	215.9	-	-	-
8	47.9	2.36, m[a]	9, 37	7, 9, 37
9	72.3	3.93, dd (8.4, 8.4)	8, 10, 9-OH	37
10	42.0	3.57, m[a]	9, 38	9, 11, 38
11	220.6	-	-	-
12	81.9	-	-	-
13	73.3	3.58, m[a]	14, 13-OH	14, 15, 40
14	40.6	1.82, m	13, 15b, 40ab	
15a	32.2	2.09, m[a]	15b, 16	
b		1.89, m[a]	14, 15a, 16	
16	130.3	5.37, ddd (14.5, 10.5, 3.6)	15ab, 17	17
17	132.6	6.04, dd (14.5, 10.5)	16, 18	15, 19
18	130.8	5.97, dd (14.8, 10.5)	17, 19	16, 20
19	136.4	5.12, dd (14.8, 9.7)	18, 20	17, 20
20	45.2	1.86, m[a]	19, 21ab, 41ab	
21a	30.4	1.66, m	20, 21b, 22ab	
b		1.31, m[a]	20, 21a, 22ab	
22a	30.1	1.55, m[a]	21ab, 22b, 23	
b		1.07, m[a]	21ab, 22a, 23	
23	68.9	4.04, br d (10.7)	22ab, 24	43
24	35.4	1.99, qdd (6.2, 6.2, 6.2)	23, 25, 43	
25	74.7	4.84, dd (11.6, 5.1)	24, 26	1, 3, 26, 44
26	30.9	2.36, m[a]	25, 44	25, 27, 44
27	99.3	-	-	-
28	202.7	-	-	-
29a	43.7	2.95, dd (14.6, 5.7)	29b, 30	45
b		2.10, d (14.6)	29a, 30	
30	36.1	2.21, m[a]	29ab, 31, 45	
31	67.1	4.45, br d (9.7)	30, 32ab	30, 45
32a	41.3	1.53, m	31, 32b	
b		1.37, m	31, 32a, 33	
33	62.7	3.80, m[a]	32b, 34, 33-OH	
34	24.9	1.14, d (6.2)	33	32, 33
35	17.6	1.03, d (6.5)	4	3, 4, 5
36	10.0	0.89, d (7.0)	6	5, 6, 7
37	7.9	0.95, d (6.7)	8	7, 8, 9
38	15.0	1.00, d (6.8)	10	9, 10, 11
39	23.5	0.94, s		11, 12, 13
40a	60.3	3.67, m	14, 40b, 40-OH	
b		3.27, dd (11.2, 4.8)	14, 40a, 40-OH	
41a	28.2	1.36, m[a]	20, 41b, 42	
b		1.25, m[a]	20, 41a, 42	
42	12.1	0.82, t (7.5)	41ab	20, 41
43	6.03	0.84, d (6.8)	24	22, 23, 24
44	11.5	0.75, d (6.6.)	26	25, 26, 27
45	12.6	0.86, d (6.9)	30	29, 30, 31, 32
5-OH	-	4.41, d (3.0)	5	4
9-OH	-	4.85, d (7.7)	9	8, 9
12-OH	-	5.90, s		11
13-OH	-	4.36, d (8.5)	13	12, 13
33-OH	-	4.48, d (5.0)	33	32
40-OH	-	5.46, br s	40ab	

[a] Coupling constant cannot be determined due to signals overlap

Table S3a. ^{1}H NMR (600 MHz, DMSO-d_6) data for known oligomycins **1**, **2** and **6**

Position	oligomycin A (**1**)	21-hydroxy-oligomycin A (**6**)	oligomycin B (**2**)
2	5.88, d (15.6)	5.85, d (15.7)	5.89, d (15.6)
3	6.75, dd (15.6, 9.4)	6.73, dd (15.7, 9.2)	6.81, dd (15.6, 9.6)
4	2.39, m[a]	2.39, m	2.39, m[a]
5	3.80, ddd (8.3, 3.2, 2.2)	3.72, m[a]	3.80, ddd (8.4, 2.6, 2.1)
6	2.62, qd (7.1, 2.2)	2.60, qd (7.3, 3.5)	2.63, qd (7.1, 2.1)
8	2.39, m[a]	2.32, qd (6.7, 1.9)	2.37, m[a]
9	3.94, m overlap	3.85, ddd (9.7, 7.3, 1.9)	3.95, m[a]
10	3.54, m, overlap	3.54, qd (9.7, 6.8)	3.55, qd (6.8, 5.7)
13	3.54, m[a]	3.52, d (7.8)	3.52, dd (7.7, 1.1)
14	1.73, m[a]	1.71, m[a]	1.72, qdd
15a	2.04, m[a]	2.03, m[a]	2.01, m[a]
b	1.92, m[a]	1.91, m[a]	1.91, br d, (13.0)
16	5.39, ddd, 1(4.8, 10.7, 3.9)	5.41, ddd (14.5, 10.8, 4.0)	5.39, ddd (14.8, 10.8, 4.0)
17	6.04, ddd (14.8, 10.6, 1.4)	6.02, dd (14.5, 10.7)	6.03, ddd (14.8, 10.5, 1.4)
18	5.94, dd (14.9, 10.6)	5.97, dd (14.3, 10.7)	5.95, dd (14.9, 10.5)
19	5.16, dd (14.9, 9.6)	5.37, dd (14.3, 9.7)	5.16, dd (14.9, 9.7)
20	1.85, m[a]	2.02, m[a]	1.85, m[a]
21a	1.62, dddd (12.0, 12.0, 4.0, 4.0)	3.69, m[a]	1.66, dddd (12.3, 12.3, 3.6, 3.6)
b	1.31, m[a]	-	1.31, m[a]
22a	1.52, m[a]	1.50, m[a]	1.53, m[a]
b	0.98, m[a]	1.37, m[a]	1.06, m[a]
23	3.77, m[a]	3.94, ddd (7.0, 7.0, 1.9)	4.04, ddd (10.5, 2.2, 2.2)
24	1.91, m[a]	1.96, m[a]	1.99, m[a]
25	4.86, dd (11.3, 5.0)	4.89, dd (11.3, 5.0)	4.84, dd (11.7, 4.9)
26	1.71, m[a]	1.72, m[a]	2.35, m[a]
28a	1.82, m[a]	1.83, ddd 13.0, 13.0, 4.4)	-
b	1.15, ddd (12.1, 3.2, 2.5)	1.16, ddd 13.0, 3.0, 3.0)	
29a	1.98, dddd (13.1, 13.1, 4.1, 4.1)	1.94, m[a]	2.94, dd (14.6, 5.6)
b	1.34, m[a]	1.36, m[a]	2.10, dd (14.6, 2.2)
30	1.52, m[a]	1.54, m[a]	2.20, m[a]
31	3.92, m[a]	3.89, ddd (9.7, 2.2, 2.2)	4.45, m[a]
32a	1.39, ddd (13.6, 10.0, 3.5)	1.42, ddd (13.5, 9.7, 3.5)	1.53, m[a]
b	1.24 m[a]	1.25, m[a]	1.37, m[a]
33	3.74, m[a]	3.78, m	3.80, m[a]
34	1.09, d (6.1)	1.11, d (6.1)	1.14, d (6.1)
35	1.01, d (6.7)	1.00, d (6.7)	1.02, d (6.7)
36	0.90, d (7.1)	0.92, d (7.3)	0.89, d (7.1)
37	0.92, d (7.2)	0.95, m[a]	0.92, d (6.6)
38	1.00, d (6.7)	0.98, d (6.8)	0.99, d (6.8)
39	0.93, s	0.92, s	0.92, s
40	0.92, d (6.8)	0.92, d (7.3)	0.91, d (7.0)
41a	1.32, m[a]	1.51, m[a]	1.35, m[a]
b	1.23, m[a]	1.27, m[a]	1.25, m
42	0.80, t (7.2)	0.79, t (7.4)	0.82, t (7.2)
43	0.83, d (7.0)	0.81, d (6.6)	0.82, d (6.8)
44	0.88, d (6.6)	0.86, d (7.4)	0.75, d (6.5)
45	0.85, d (7.0)	0.85, d (6.5)	0.86, d (7.1)
5-OH	4.41, d (3.2)	4.48, d (3.8)	4.49, d (5.0)
9-OH	4.83, d (7.3)	4.76, d (7.3)	4.89, d (7.2)
12-OH	4.99, s	4.97, s	4.96, s
13-OH	4.22, d (7.8)	4.22, d (7.8)	4.25, d (7.7)
21-OH	-	4.40, m[a]	-
33-OH	4.30, d (5.1)	4.40, m[a]	4.40, d (3.2)

[a] Coupling constant cannot be determined due to signals overlap

Table S3b. ^{1}H NMR (600 MHz, DMSO-d_6) data for known oligomycins **3**-**5**

Position	oligomycin C (**3**)	oligomycin D (**4**)	oligomycin E (**5**)
2	5.87, d (15.6)	5.86, d (15.6)	5.90, d (5.6)
3	6.74, dd (15.6, 9.3)	6.74, dd (15.7, 9.3)	6.91, dd (15.6)
4	2.39, ddq (9.3, 8.0, 6.6)	2.39, m[a]	2.42, m[a]
5	3.81, dd (8.0, 2.3)	3.79, m[a]	3.87, ddd (7.5, 3.3, 2.4)
6	2.56, qd (7.1, 2.3)	2.62, ddd (14.3, 6.9, 2.3)	2.63, qd (6.9, 2.4)
8	2.51, m[b]	2.40, m[a]	2.41, m[a]
9	3.97, m[a]	3.93, m[a]	3.94, ddd (9.8, 7.3, 2.3)
10	2.74, m[a]	3.55, m[a]	3.52, dd (9.8, 7.0)
12	2.75, m[a]	-	-
13	3.48, br d (8.4)	3.54, m[a]	3.55, br d (7.3)
14	1.57, m[a]	1.72, m	1.71, m[a]
15a	2.04, ddd (13.4, 10.7, 10.7)	2.05, ddd (13.0, 10.9, 10.9)	2.03, m[a]
b	1.97, m[a]	1.92, m[a]	1.90, m[a]
16	5.41, ddd (14.8, 10.9, 4.4)	5.40, ddd (14.8, 10.9, 4.1)	5.40, ddd (14.7, 10.9, 3.8)
17	6.07, dd (14.8, 10.7)	6.04, dd (14.8, 10.6)	6.03, dd (14.7, 10.6)
18	5.95, dd (15.0, 10.7)	5.95, dd (15.0, 10.6)	5.97, dd (14.7, 10.6)
19	5.17, dd (15.0, 9.7)	5.17, m[a]	5.20, dd (14.7, 9.8)
20	1.86, m[a]	1.86, m	1.93, m[a]
21a	1.59, m[a]	1.62, m	1.65, m[a]
b	1.33, m[a]	1.30, m[a]	1.37, m[a]
22a	1.58, m[a]	1.52, m[a]	1.63, m[a]
b	1.00, m[a]	1.00, m[a]	1.12, m[a]
23	3.77, m[a]	3.77, m[a]	4.13, ddd (9.5, 2.8, 2.8)
24	1.92, m	1.92, m[a]	1.92, m[a]
25	4.85, dd (11.3, 4.9)	5.17, m[a]	4.89, dd (5.5, 1.0)
26a	1.71, dq (11.3, 6.5)	1.65, dd (12.5, 5.2)	-
b	-	1.57, dd (12.5, 12.5)	
28a	1.82, ddd (13.5, 13.5, 4.5)	1.55, m[a]	-
b	1.16, ddd (13.5, 3.5, 3.5)	1.35, m[a]	
29a	1.98, m[a]	1.98, dddd (13.3, 8.9, 4.0, 4.0)	3.11, dd (13.3, 5.6)
b	1.34, m[a]	1.32, m[a]	2.03, m[a]
30	1.52, m	1.53, m[a]	2.89, m
31	3.92, ddd (9.8, 2.2, 2.2)	3.92, m[a]	4.42, ddd (9.8, 2.1, 2.1)
32a	1.39, ddd (13.6, 9.8, 3.3)	1.40, ddd (13.4, 10.0, 3.0)	1.50, ddd (13.6, 9.8, 2.6)
b	1.24, m[a]	1.22, m[a]	1.36, m[a]
33	3.74, m[a]	3.75, m[a]	3.76, m
34	1.09, d (6.2)	1.09, d (6.1)	1.13, d (5.7)
35	1.02, d (6.9)	1.01, d (6.6)	1.02, d (6.8)
36	0.96, d (7.1)	0.91, d (7.5)	0.93, m[a]
37	0.88, d (6.5)	0.92, d (6.9)	0.91, m[a]
38	1.02, d (6.9)	1.01, d (6.6)	0.92, m[a]
39	0.72, d (6.9)	0.94, s	0.93, s
40	0.77, d (6.8)	0.92, d (6.9)	0.99, d (6.8)
41a	1.33, m[a]	1.33, m[a]	1.34, m[a]
b	1.24, m[a]	1.23, m[a]	1.23, m[a]
42	0.80, t (7.5)	0.80, t (7.5)	0.93, t (7.3)
43	0.79, d (6.8)	0.81, d (6.9)	1.00, d (7.2)
44	0.88, d (6.5)	-	1.13, s
45	0.85, d (6.8)	0.86, d (6.9)	0.87, d (6.9)
5-OH	4.50, br s	4.43, d (3.4)	4.47, d (3.3)
9-OH	4.77, br s	4.82, d (7.4)	4.79, d (7.3)
12-OH	-	5.00, s	4.95, s
13-OH	3.97, m[a]	4.23, d (7.5)	4.28, d (7.3)
26-OH	-	-	4.11, d (1.0)
33-OH	4.29, br s	4.29, d (5.2)	4.55, d (4.5)

[a] Coupling constant cannot be determined due to signals overlap. [b]Signal is obscured under DMSO-d_6 solvent peak

Table S4. ^{13}C NMR (150 MHz, DMSO-d_6) data for known oligomycins **1-6**

Position	**1**	**6**	**2**	**3**	**4**	**5**
1	164.5	164.6	164.4	164.6	164.2	164.7
2	121.5	121.1	121.4	121.4	121.6	121.3
3	149.8	150.3	150.3	150.1	150.0	150.5
4	41.4	41.7	41.5	41.3	41.4	41.6
5	71.4	71.8	71.5	71.7	71.5	71.4
6	43.4	44.2	43.3	45.3	43.3	43.5
7	215.6	215.4	215.8	215.3	215.4	215.1
8	47.5	47.9	47.6	47.1	47.5	47.3
9	72.1	72.1	72.1	71.0	72.1	72.1
10	41.9	42.0	41.8	49.9	41.9	42.0
11	221.7	221.6	221.9	217.1	221.7	221.3
12	82.6	82.6	82.6	51.2	82.6	82.6
13	72.5	72.5	72.4	71.6	72.5	72.0
14	33.4	33.2	33.3	33.7	33.3	33.3
15	38.0	38.1	38.0	36.7	38.0	38.0
16	130.7	130.9	130.7	130.5	130.7	131.0
17	132.0	132.3	132.0	132.0	132.0	131.9
18	130.8	132.1	130.9	130.8	130.8	131.0
19	136.2	132.8	136.2	136.1	136.2	135.9
20	45.3	50.0	45.3	44.9	45.1	44.9
21	30.5	70.4	30.4	30.5	30.4	30.3
22	30.6	35.8	30.2	30.2	30.4	29.7
23	67.7	66.7	69.9	67.6	68.5	69.4
24	35.6	34.6	35.3	35.2	35.1	35.4
25	75.1	74.7	74.8	75.2	69.8	73.3
26	37.2	37.4	30.9	37.2	35.1	72.7
27	98.3	98.9	99.3	98.3	96.5	97.6
28	25.5	25.2	202.7	25.5	29.2	206.0
29	26.2	26.1	43.7	26.2	26.1	44.7
30	29.7	29.7	36.1	29.7	29.8	37.7
31	66.9	67.3	67.1	66.9	67.0	67.8
32	42.5	42.5	41.3	42.6	42.4	41.2
33	62.5	62.8	62.6	62.5	62.5	62.5
34	25.0	24.9	24.9	25.0	24.9	24.8
35	17.4	17.2	17.4	17.1	17.3	17.2
36	9.9	10.7	9.8	9.9	10.0	10.2
37	8.0	8.1	8.0	9.5	8.0	8.1
38	14.9[a]	14.9	14.9[a]	13.4	14.9	14.7[a]
39	22.1	22.0	22.1	12.7	22.1	22.0
40	14.8[a]	14.9	14.8[a]	12.0	14.9	14.9[a]
41	28.3	22.5	28.2	28.1	28.2	28.1
42	12.0	12.3	12.1	11.6	12.0	12.0
43	6.2	5.6	6.0	6.0	5.4	8.5
44	11.6	11.5	11.5	11.1	-	21.0
45	11.0	11.1	12.6	13.3	11.2	12.3

[a-b] values with the same superscript within the same column are interchangeable.

Table S5. ^{1}H NMR (600 MHz, DMSO-d_6) data for oligomycins **3**, **7** and **6**

Position	oligomycin C (**3**)	21-hydroxy-oligomycin C (**7**)	21-hydroxy-oligomycin A (**6**)
2	5.87, d (15.6)	5.84, d (15.7, 0.7)	5.85, d (15.7)
3	6.74, dd (15.6, 9.3)	6.77, dd (15.7, 8.5)	6.73, dd (15.7, 9.2)
4	2.39, ddq (9.3, 8.0, 6.6)	2.38, ddq (8.5, 7.4, 6.8)	2.39, m
5	3.81, dd (8.0, 2.3)	3.72, ddd (7.4, 4.8, 4.2)	3.72, m[a]
6	2.56, qd (7.1, 2.3)	2.67, qd (7.1, 4.2)	2.60, qd (7.3, 3.5)
8	2.51, m[b]	2.50, m[b]	2.32, qd (6.7, 1.9)
9	3.97, m[a]	3.90, m[a]	3.85, ddd (9.7, 7.3, 1.9)
10	2.74, m[a]	2.71, dq (7.0, 7.0)	3.54, qd (9.7, 6.8)
12	2.75, m[a]	2.75, dq (9.3, 6.8)	
13	3.48, br d (8.4)	3.45, ddd (9.3, 6.2, 1.2)	3.52, d (7.8)
14	1.57, m[a]	1.57, m[a]	1.71, m[a]
15a	2.04, ddd (13.4, 10.7, 10.7)	2.04, m[a]	2.03, m[a]
b	1.97, m[a]	1.95, m[a]	1.91, m[a]
16	5.41, ddd (14.8, 10.9, 4.4)	5.44, ddd (14.6, 10.1, 4.7)	5.41, ddd (14.5, 10.8, 4.0)
17	6.07, dd (14.8, 10.7)	6.04, dd (14.6, 10.5)	6.02, dd (14.5, 10.7)
18	5.95, dd (15.0, 10.7)	5.98, dd (14.8, 10.5)	5.97, dd (14.3, 10.7)
19	5.17, dd (15.0, 9.7)	5.37, dd (14.8, 9.3)	5.37, dd (14.3, 9.7)
20	1.86, m[a]	2.03, m[a]	2.02, m[a]
21a	1.59, m[a]	3.62, m	3.69, m[a]
b	1.33, m[a]		-
22a	1.58, m[a]	1.46, m	1.50, m[a]
b	1.00, m[a]	1.42, m	1.37, m[a]
23	3.77, m[a]	3.91, m[a]	3.94, ddd (7.0, 7.0, 1.9)
24	1.92, m	1.94, m[a]	1.96, m[a]
25	4.85, dd (11.3, 4.9)	4.88, dd (11.3, 5.0)	4.89, dd (11.3, 5.0)
26	1.71, dq (11.3, 6.5)	1.73, dq (11.3, 6.6)	1.72, m[a]
28a	1.82, ddd (13.5, 13.5, 4.5)	1.84, ddd (13.7, 13.7, 4.8)	1.83, ddd 13.0, 13.0, 4.4)
b	1.16, ddd (13.5, 3.5, 3.5)	1.16, ddd (13.7, 2.8, 2.8)	1.16, ddd 13.0, 3.0, 3.0)
29a	1.98, m[a]	1.94, m[a]	1.94, m[a]
b	1.34, m[a]	1.37, m	1.36, m[a]
30	1.52, m	1.53, m[a]	1.54, m[a]
31	3.92, ddd (9.8, 2.2, 2.2)	3.88, m[a]	3.89, ddd (9.7, 2.2, 2.2)
32a	1.39, ddd (13.6, 9.8, 3.3)	1.42, m[a]	1.42, ddd (13.5, 9.7, 3.5)
b	1.24, m[a]	1.25, m[a]	1.25, m[a]
33	3.74, m[a]	3.78, m	3.78, m
34	1.09, d (6.2)	1.11, d (6.2)	1.11, d (6.1)
35	1.02, d (6.9)	1.00, d (6.8)	1.00, d (6.7)
36	0.96, d (7.1)	0.98, d (7.1)	0.92, d (7.3)
37	0.88, d (6.5)	0.95, d (6.8)	0.95, m[a]
38	1.02, d (6.9)	0.99, d (7.0)	0.98, d (6.8)
39	0.72, d (6.9)	0.74, d (6.8)	0.92, s
40	0.77, d (6.8)	0.75, d (6.9)	0.92, d (7.3)
41a	1.33, m[a]	1.53, m[a]	1.51, m[a]
b	1.24, m[a]	1.25, m[a]	1.27, m[a]
42	0.80, t (7.5)	0.80, t (7.4)	0.79, t (7.4)
43	0.79, d (6.8)	0.77, d (6.9)	0.81, d (6.6)
44	0.88, d (6.5)	0.86, d (6.6)	0.86, d (7.4)
45	0.85, d (6.8)	0.85, d (6.7)	0.85, d (6.5)
5-OH	4.50, br s	4.62, d (4.8)	4.48, d (3.8)
9-OH	4.77, br s	4.74, d (6.7)	4.76, d (7.3)
12-OH			4.97, s
13-OH	3.97, m[a]	4.09, d (6.2)	4.22, d (7.8)
21-OH		4.50, d (3.1)	4.40, m[a]
33-OH	4.29, br s	4.41, d (5.8)	4.40, m[a]

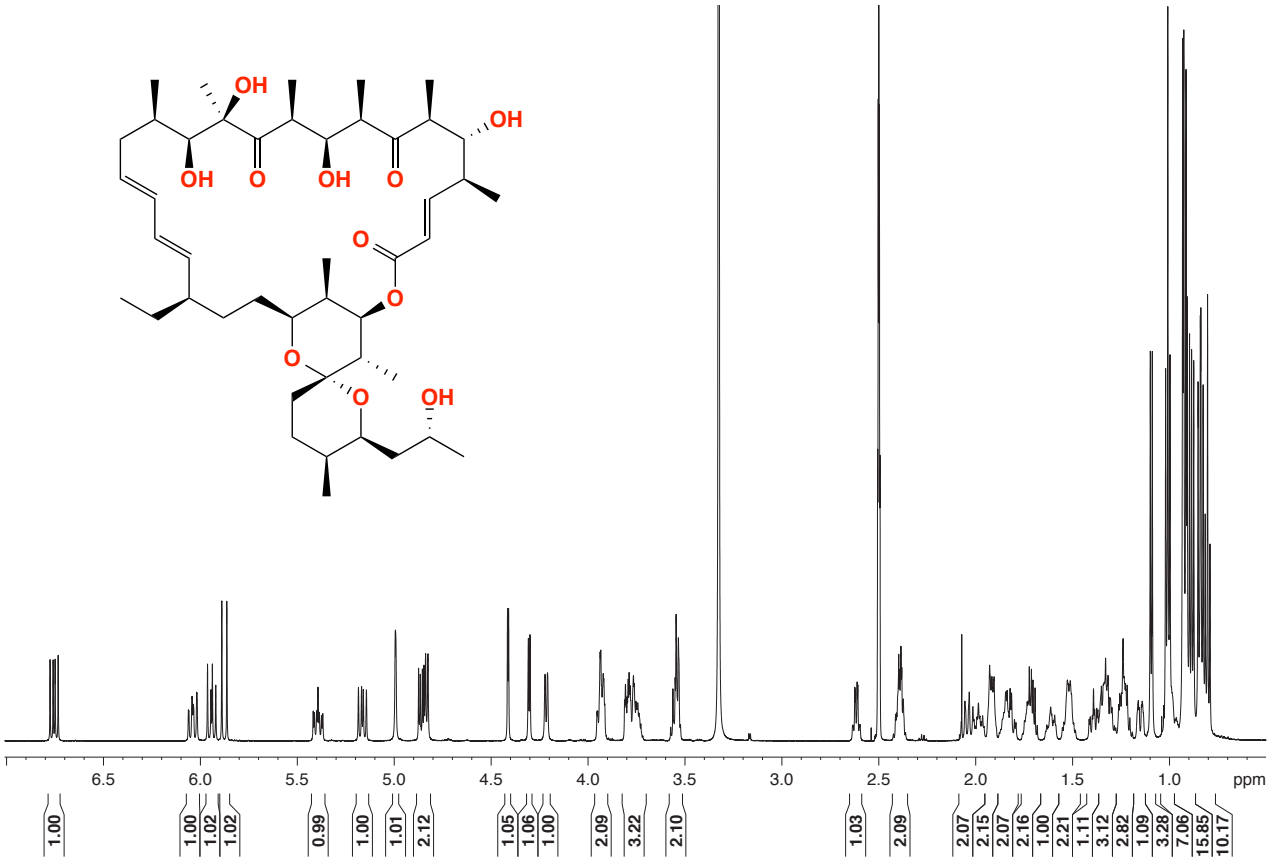


Figure S1a. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of oligomycin A (**1**)

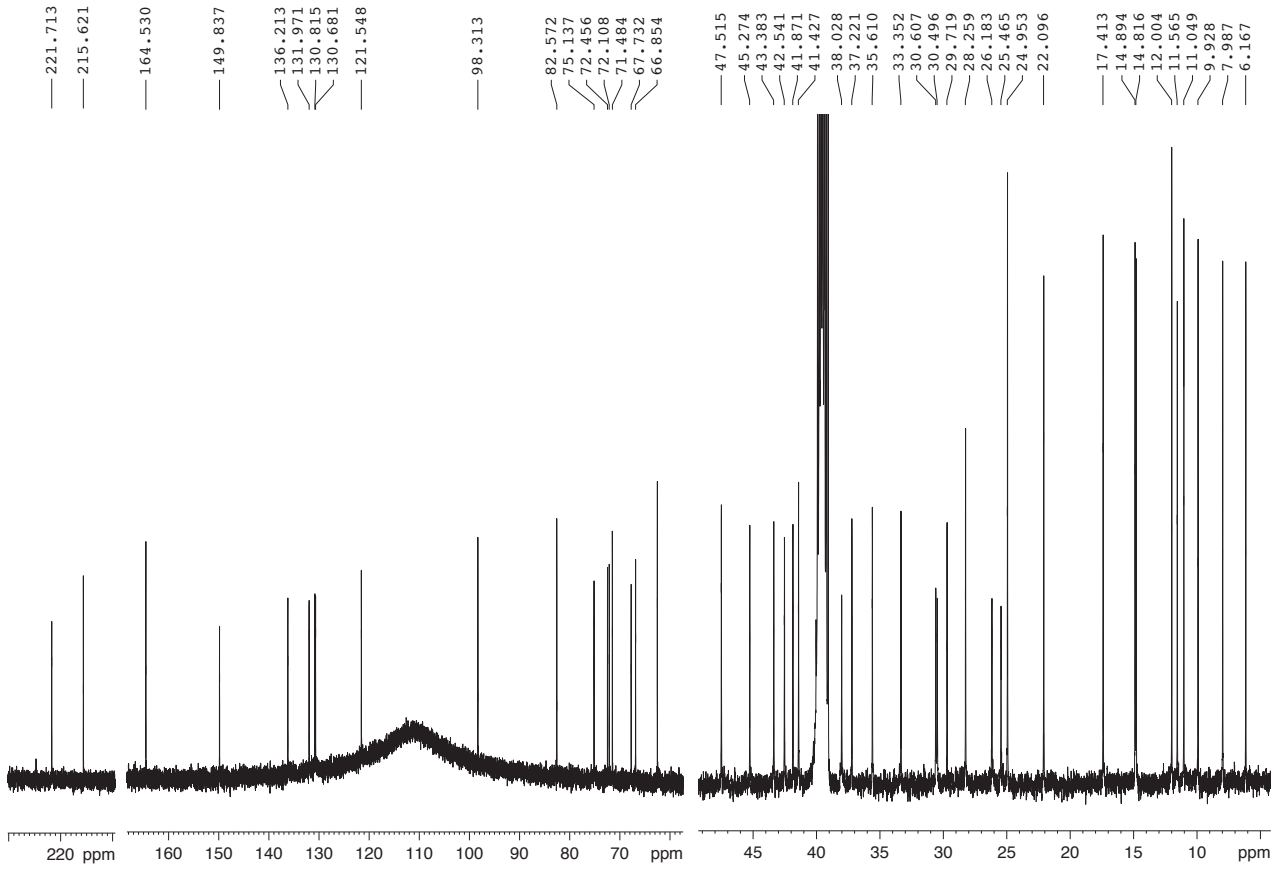


Figure S1b. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of oligomycin A (**1**)

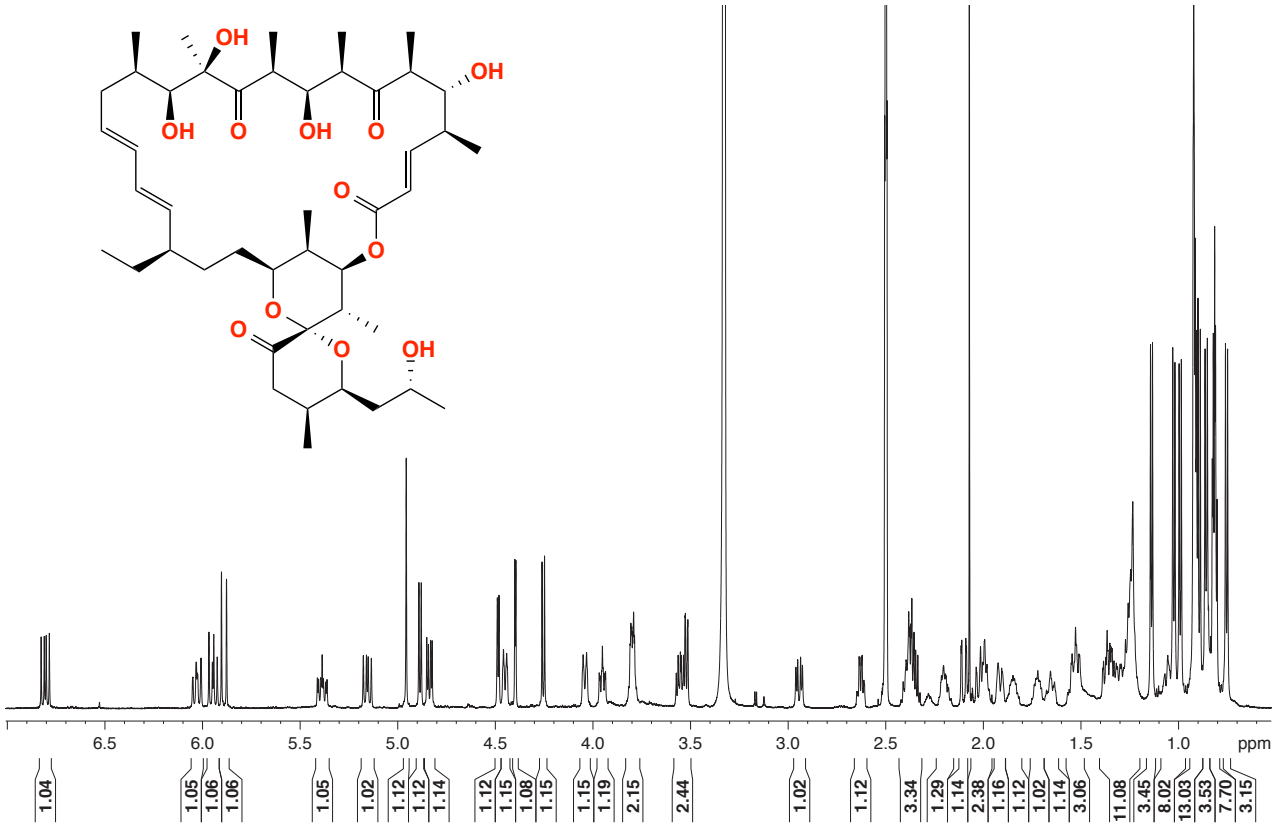


Figure S2a. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of oligomycin B (**2**)

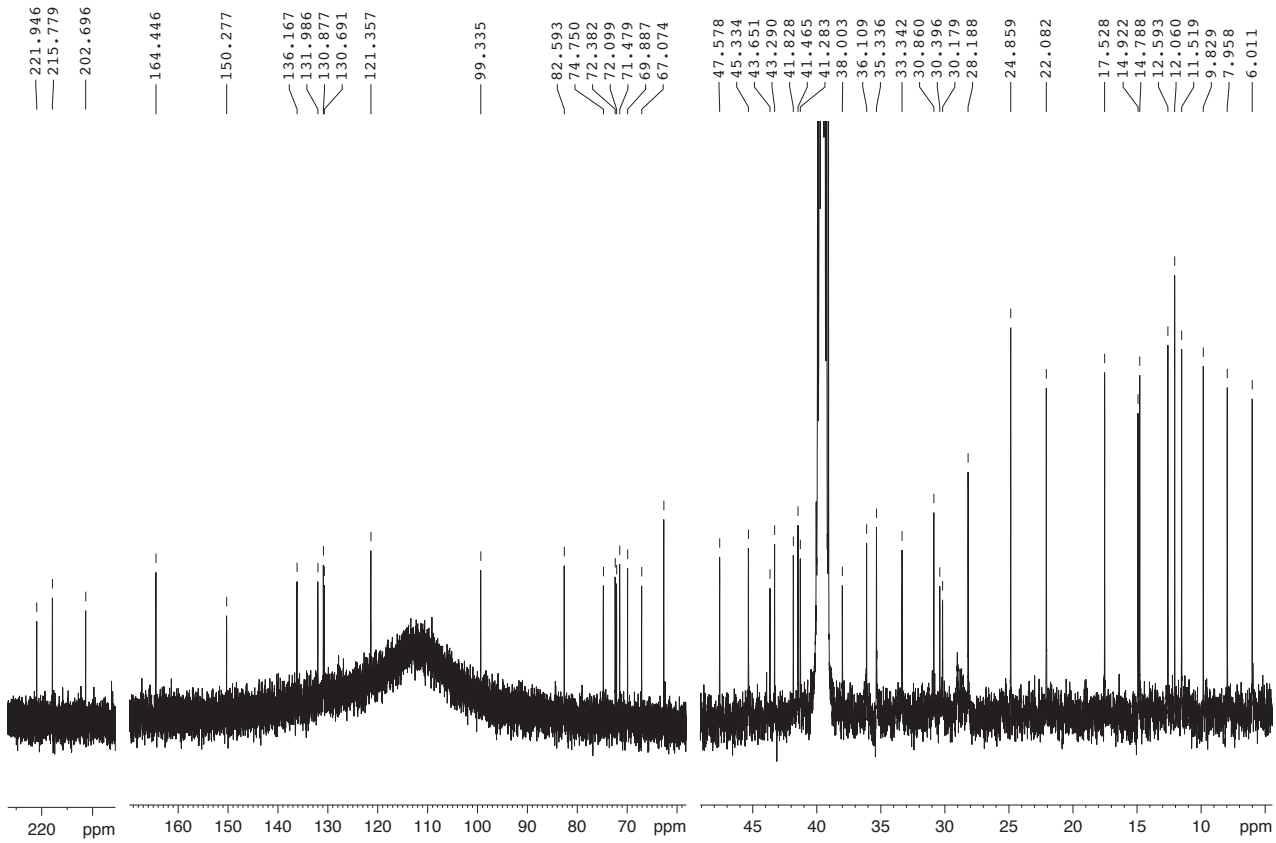


Figure S2b. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of oligomycin B (**2**)

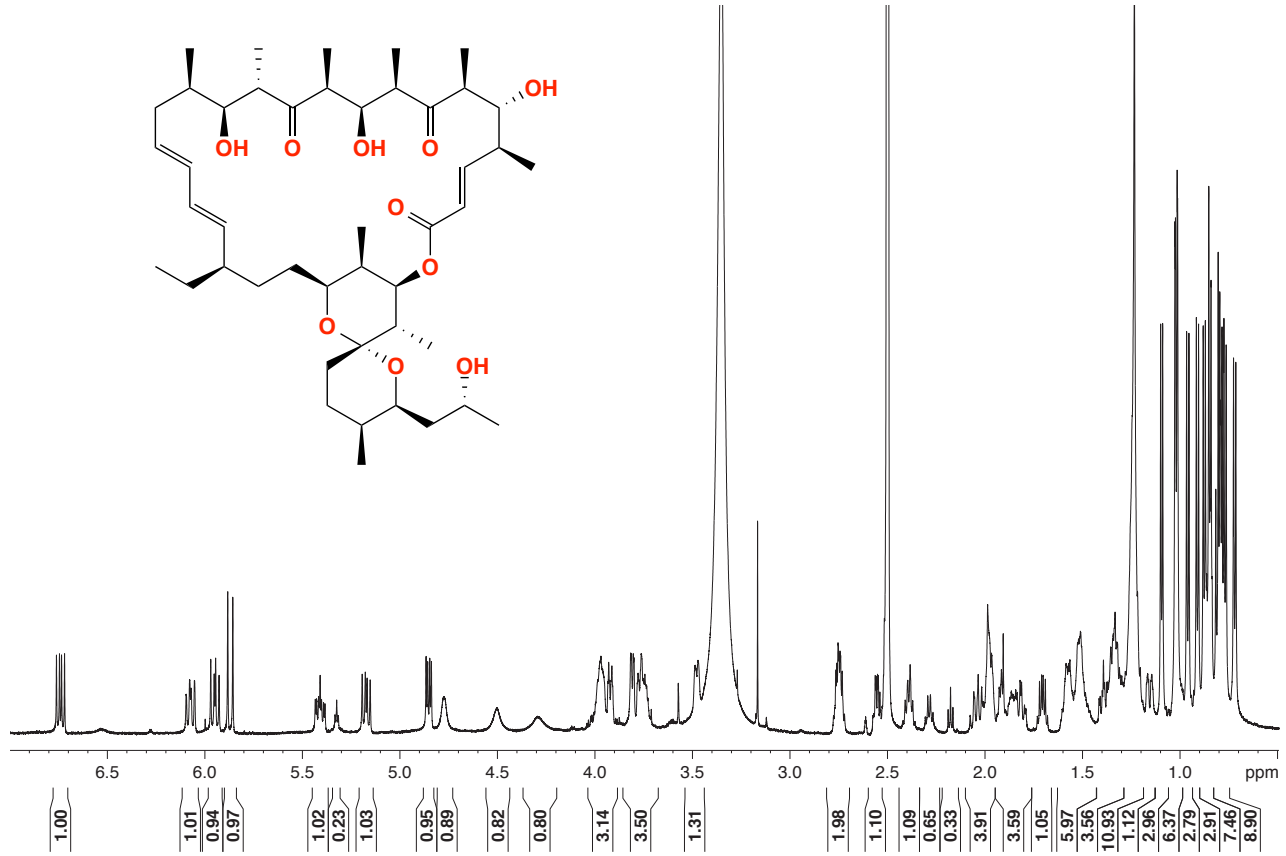


Figure S3a. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of oligomycin C (**3**)

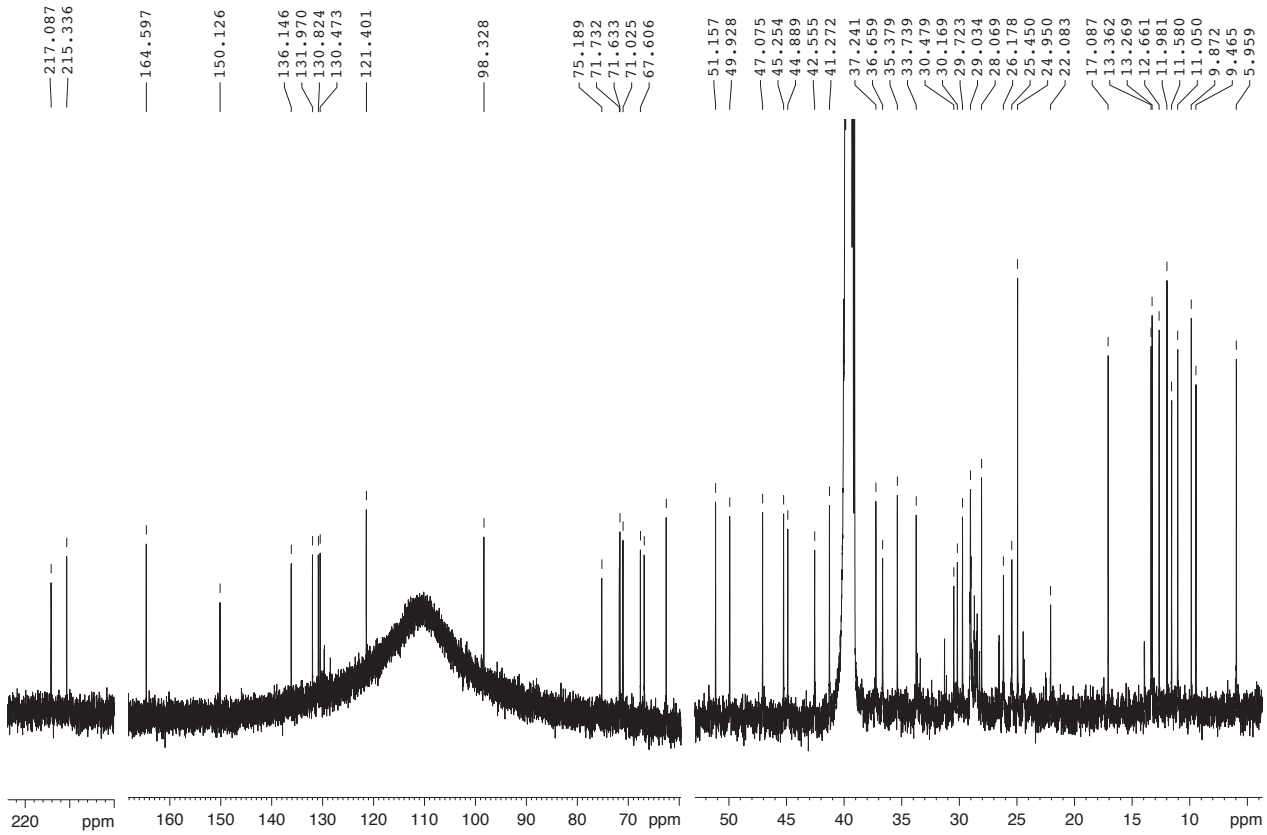


Figure S3b. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of oligomycin C (**3**)

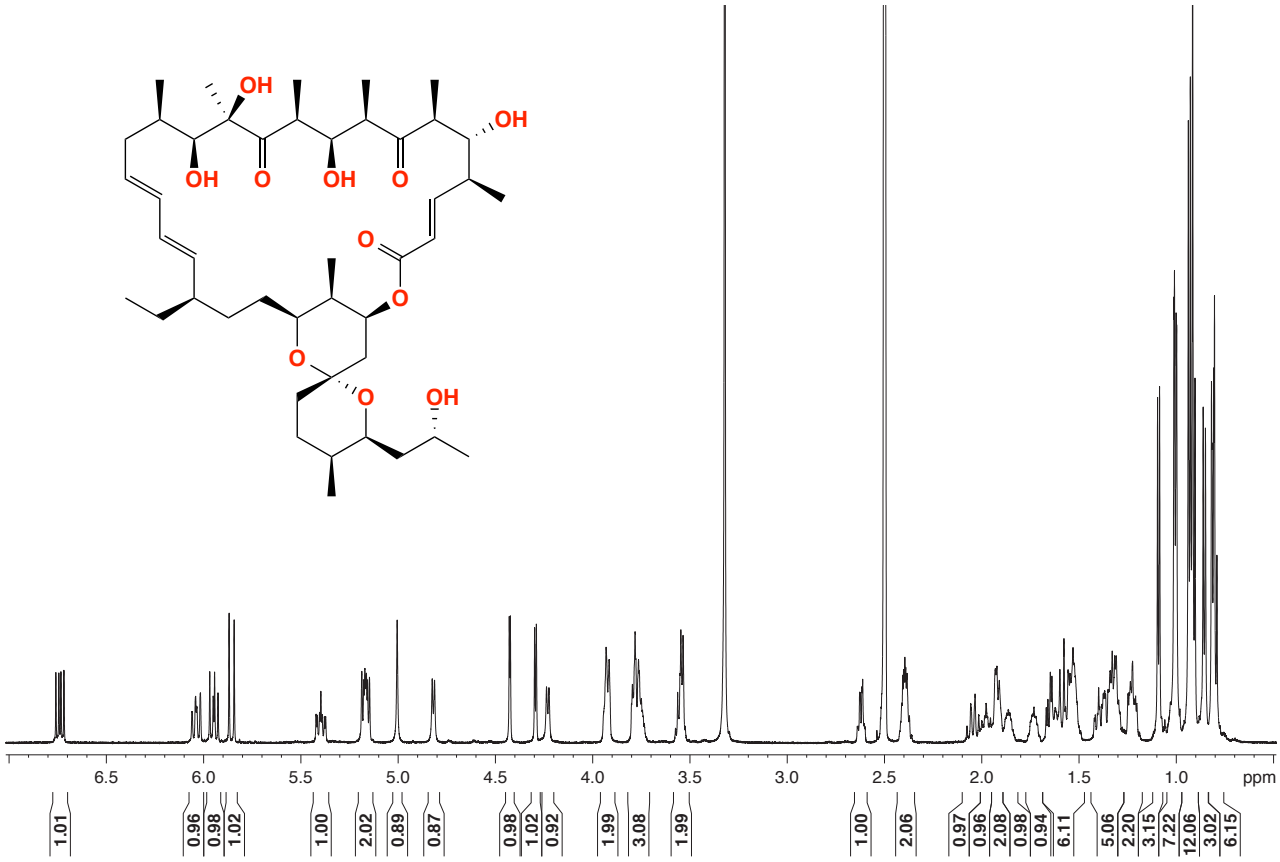


Figure S4a. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of oligomycin D (**4**)

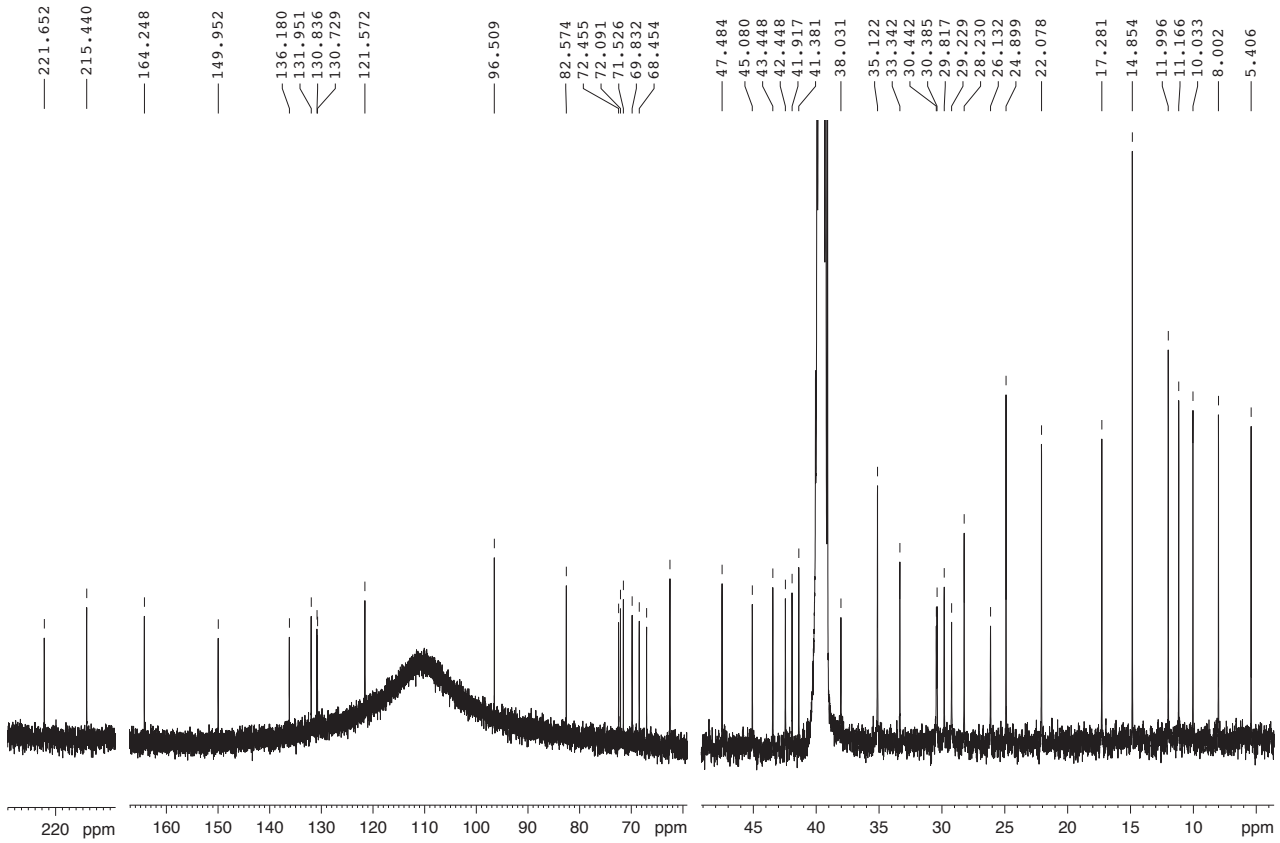


Figure S4b. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of oligomycin D (**4**)

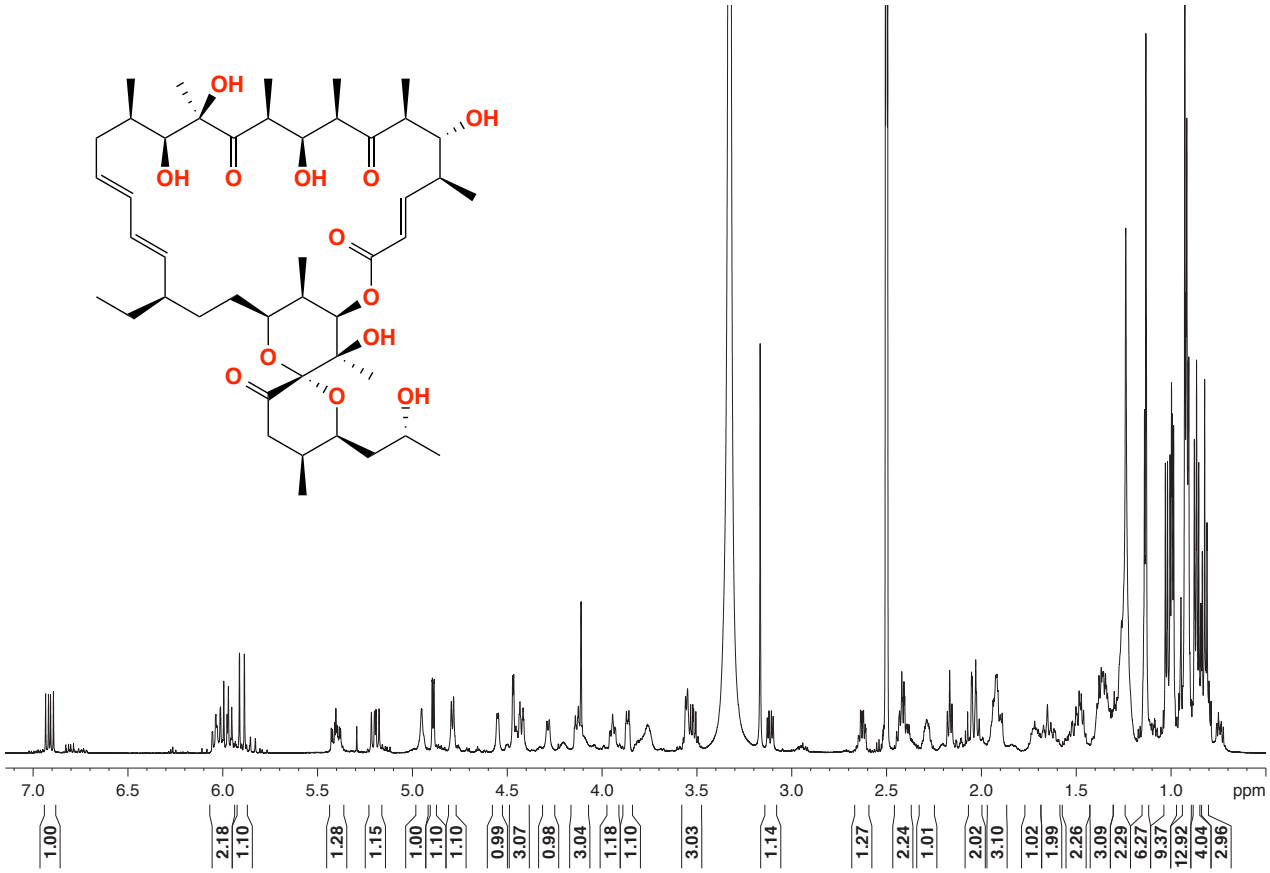


Figure S5a. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of oligomycin E (**5**)

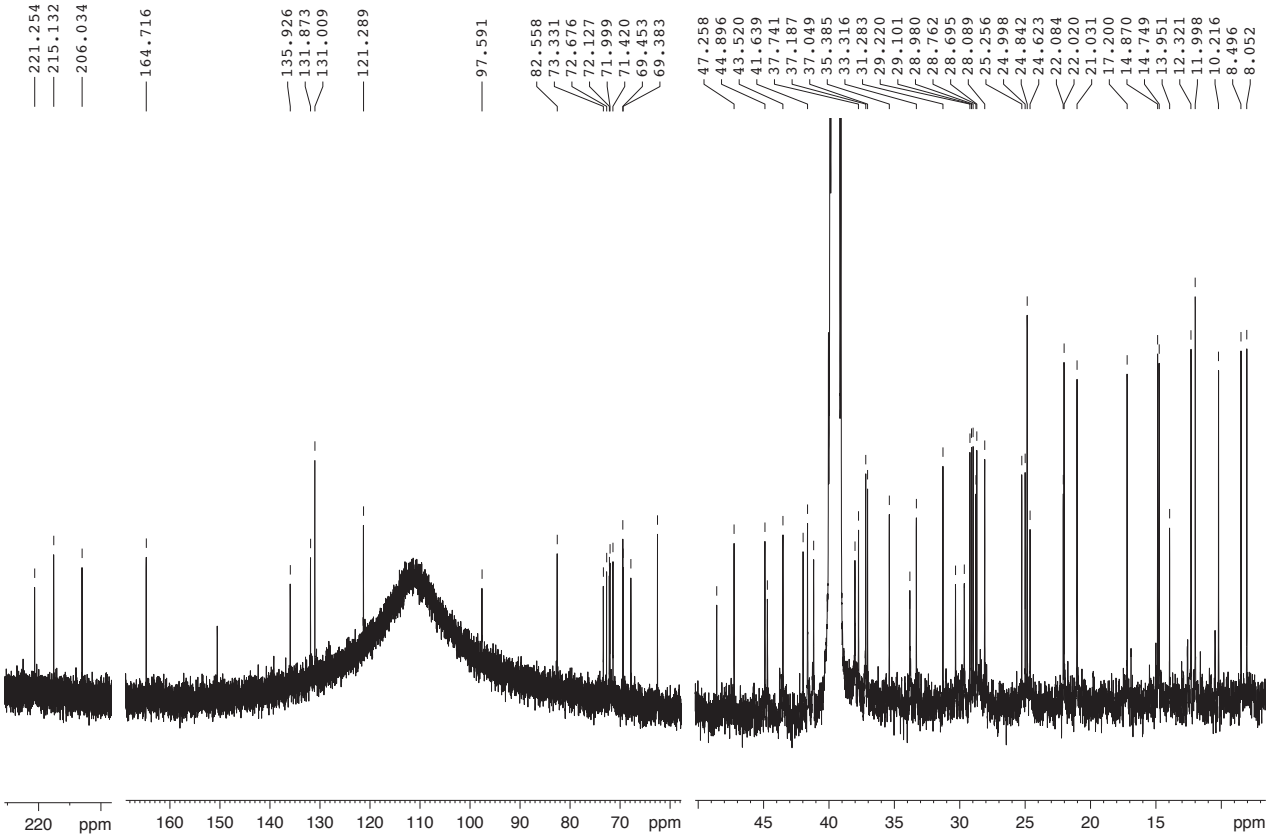


Figure S5b. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of oligomycin E (**5**)

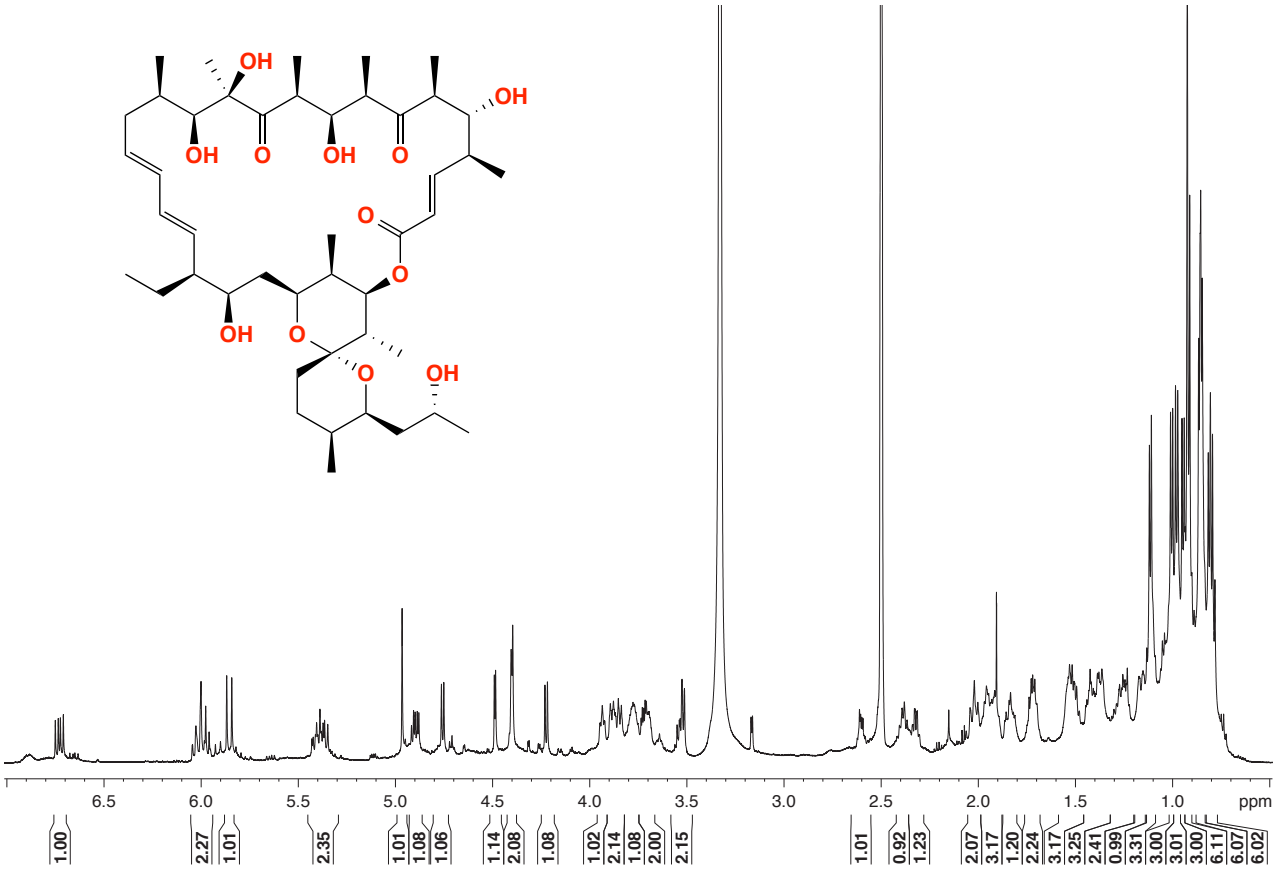


Figure S6a. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of 21-hydroxy-oligomycin A (**6**)

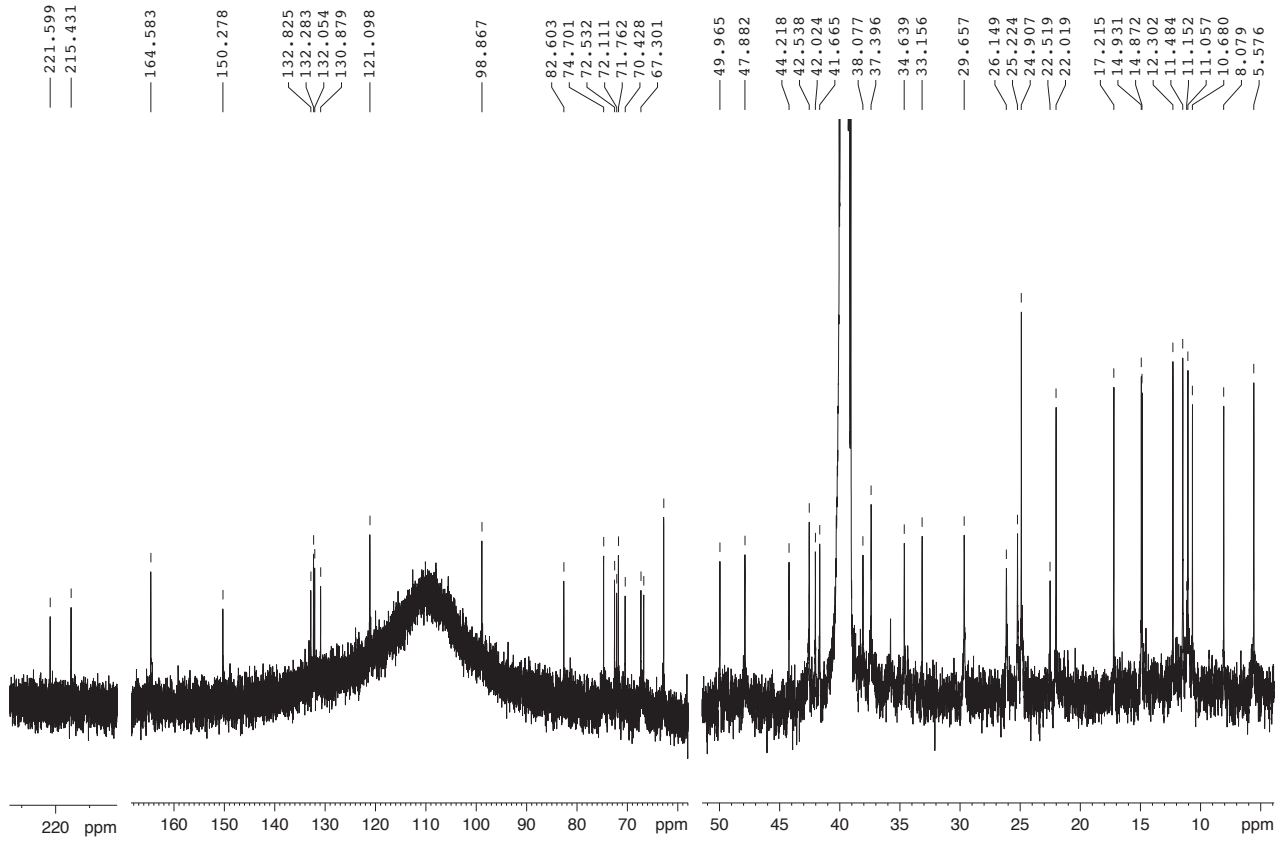


Figure S6b. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of 21-hydroxy-oligomycin A (**6**)

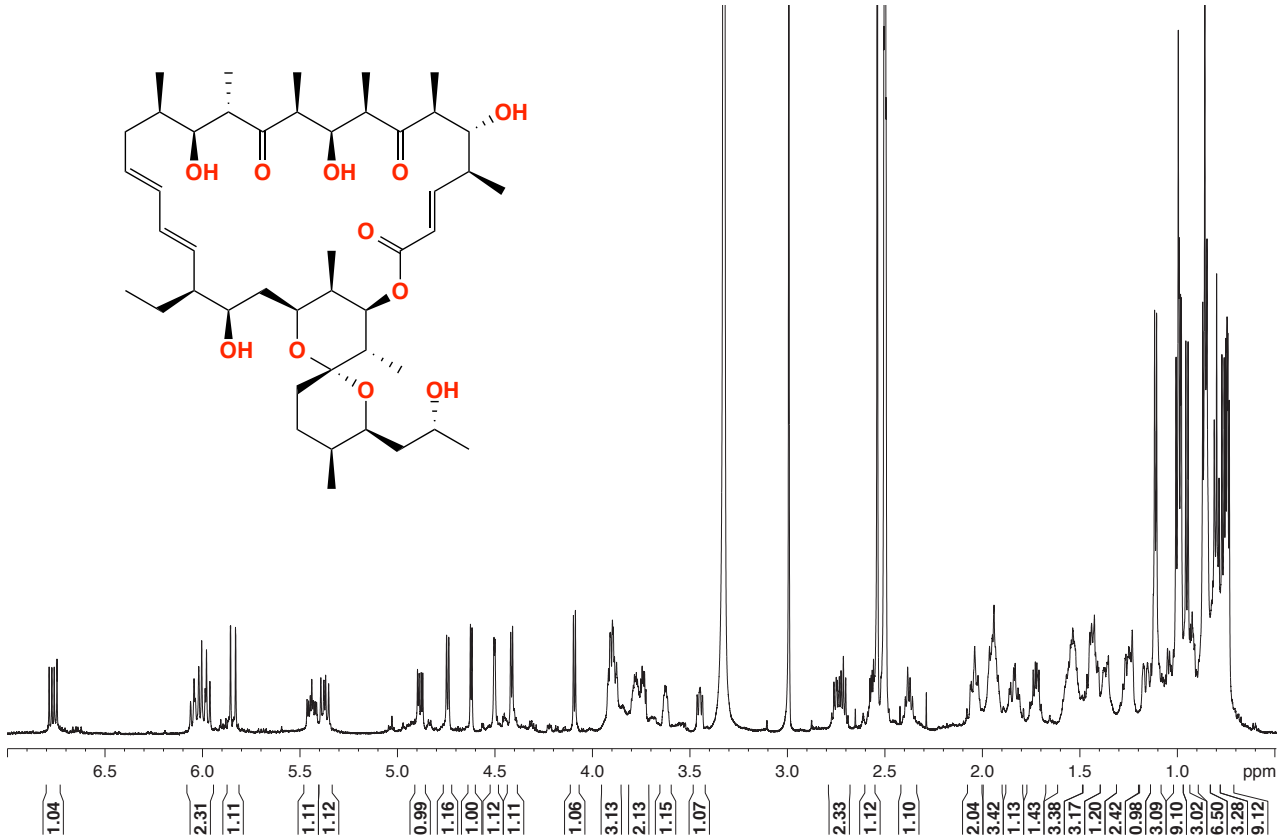


Figure S7a. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of 21-hydroxy-oligomycin C (**7**)

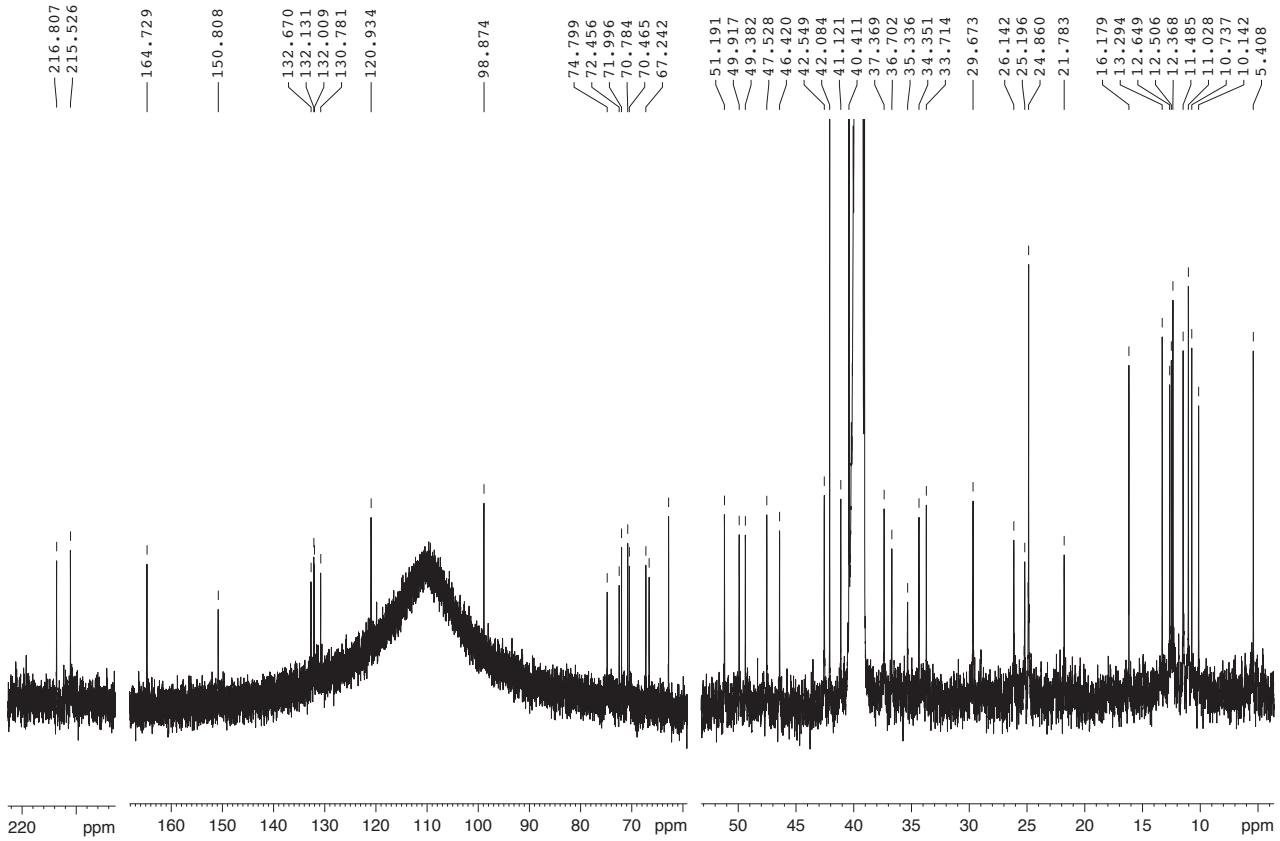


Figure S7b. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of 21-hydroxy-oligomycin C (**7**)

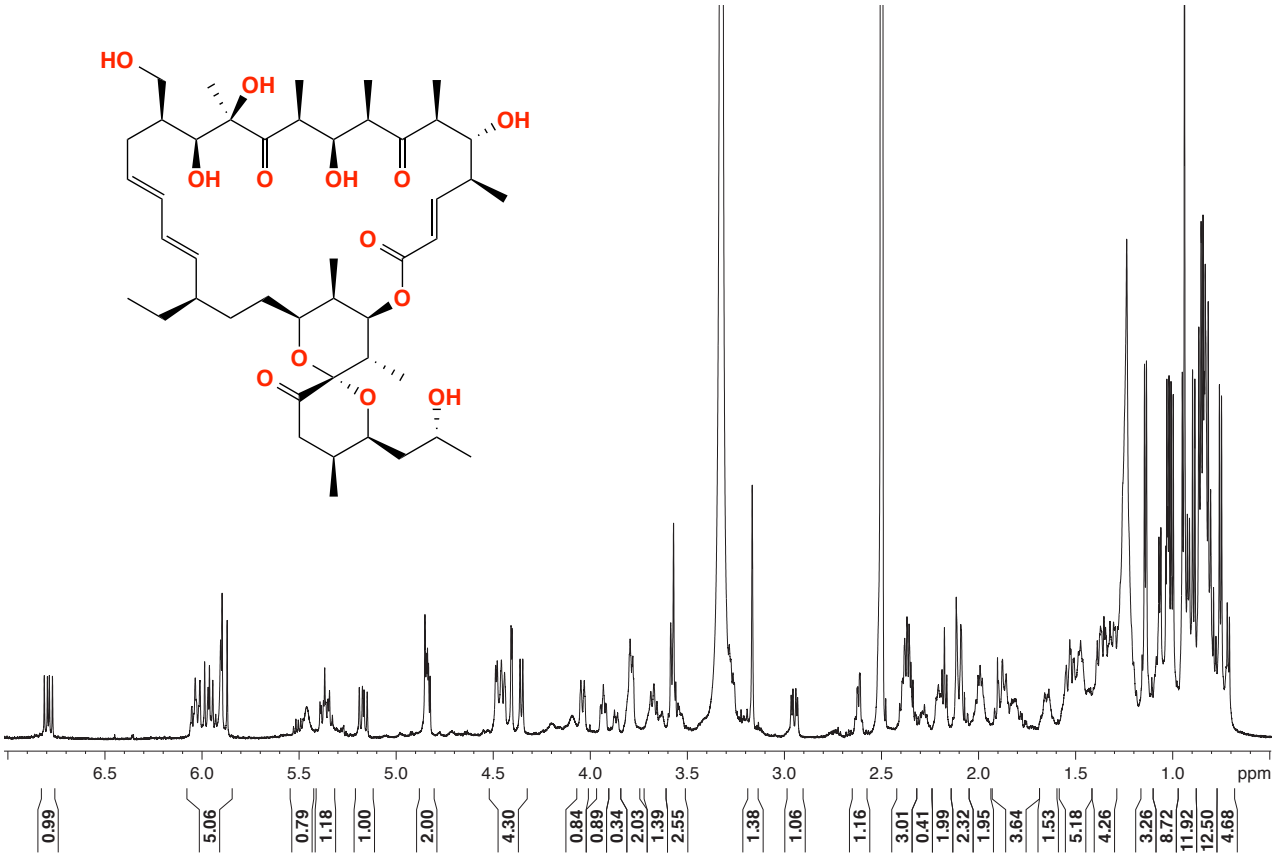


Figure S8a. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of 40-hydroxy-oligomycin B (**8**)

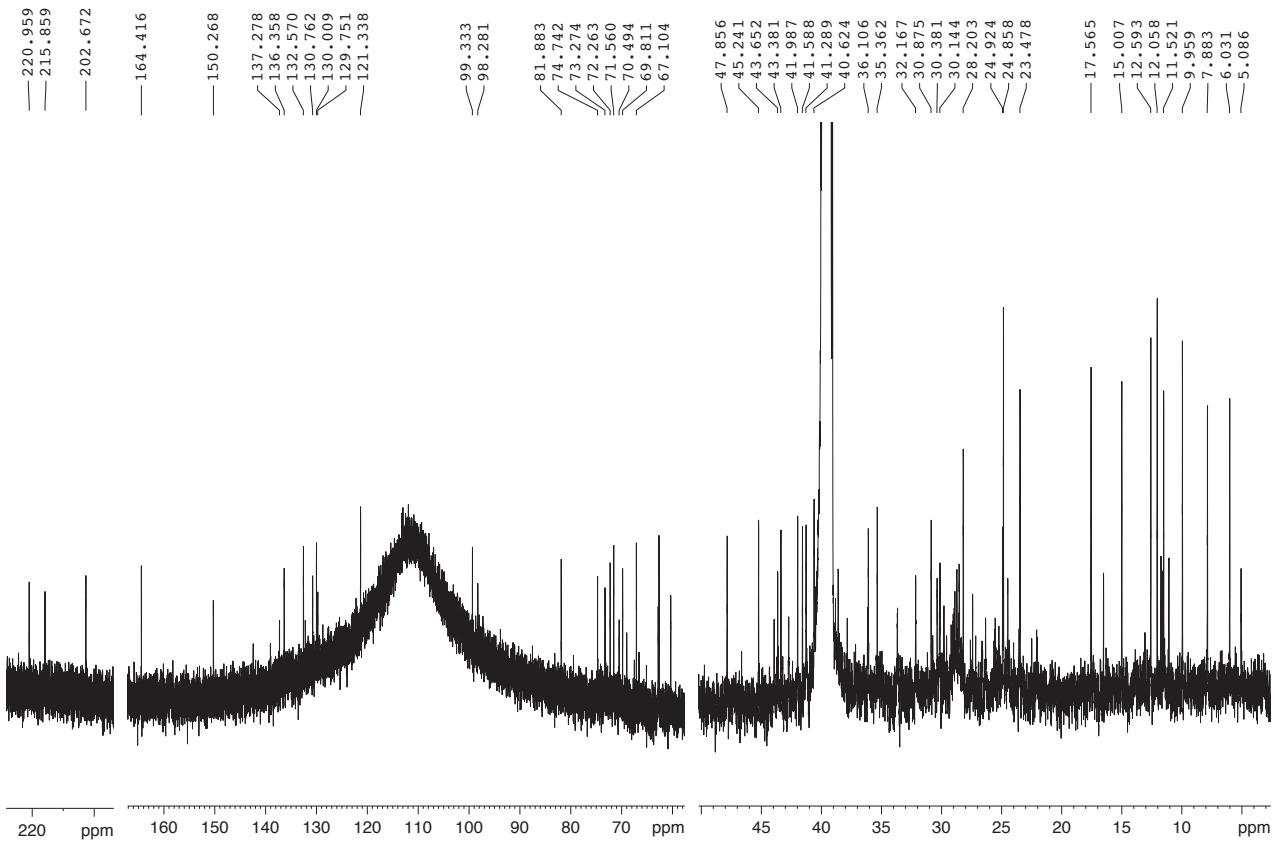


Figure S8b. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of 40-hydroxy-oligomycin B (**8**)

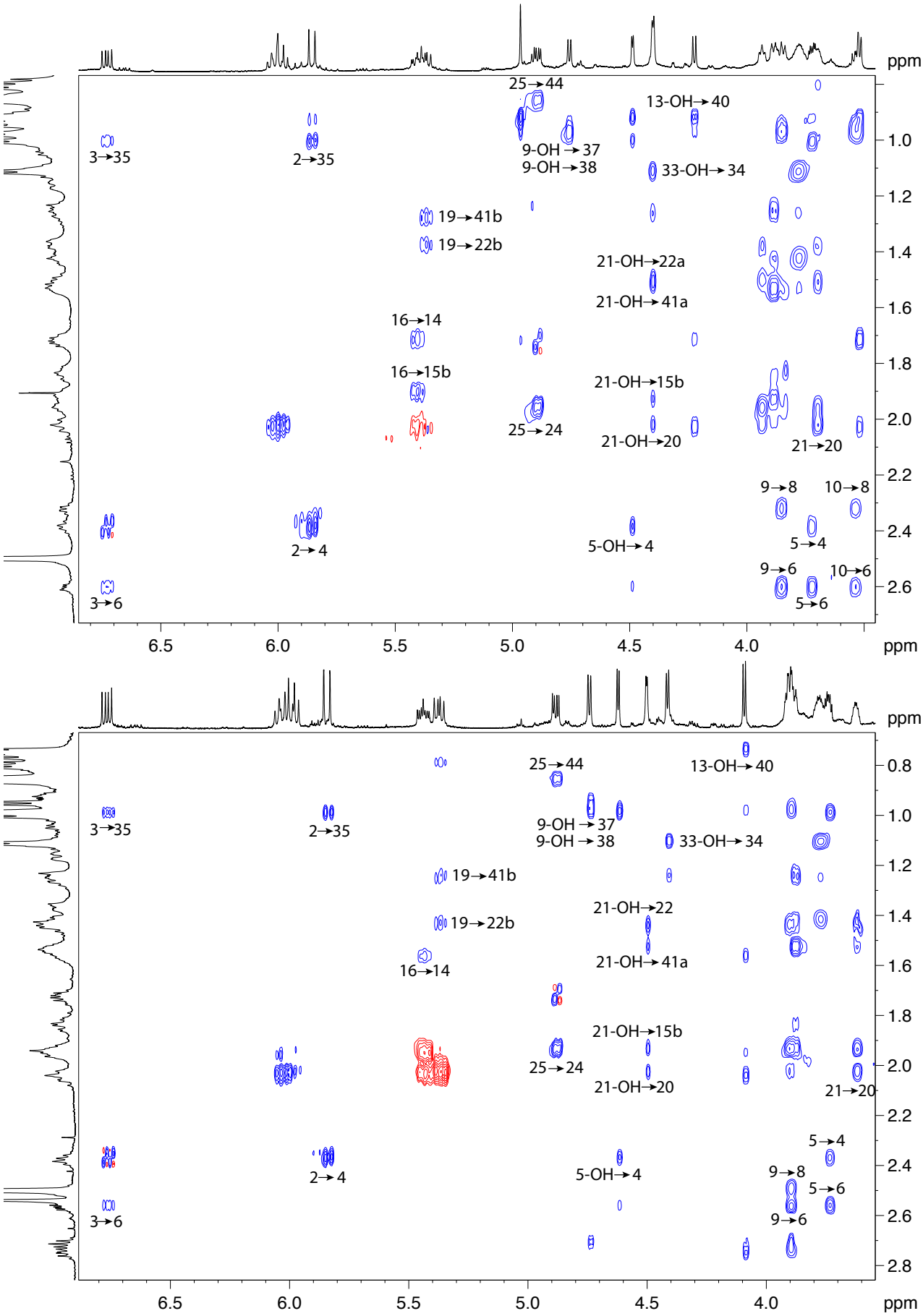


Figure S9a. ROESY (600 MHz, DMSO-d6) spectra of 21-hydroxy-oligomycin A (**6**) (top) and 21-hydroxy-oligomycin C (**7**) (bottom), with key correlations annotated.

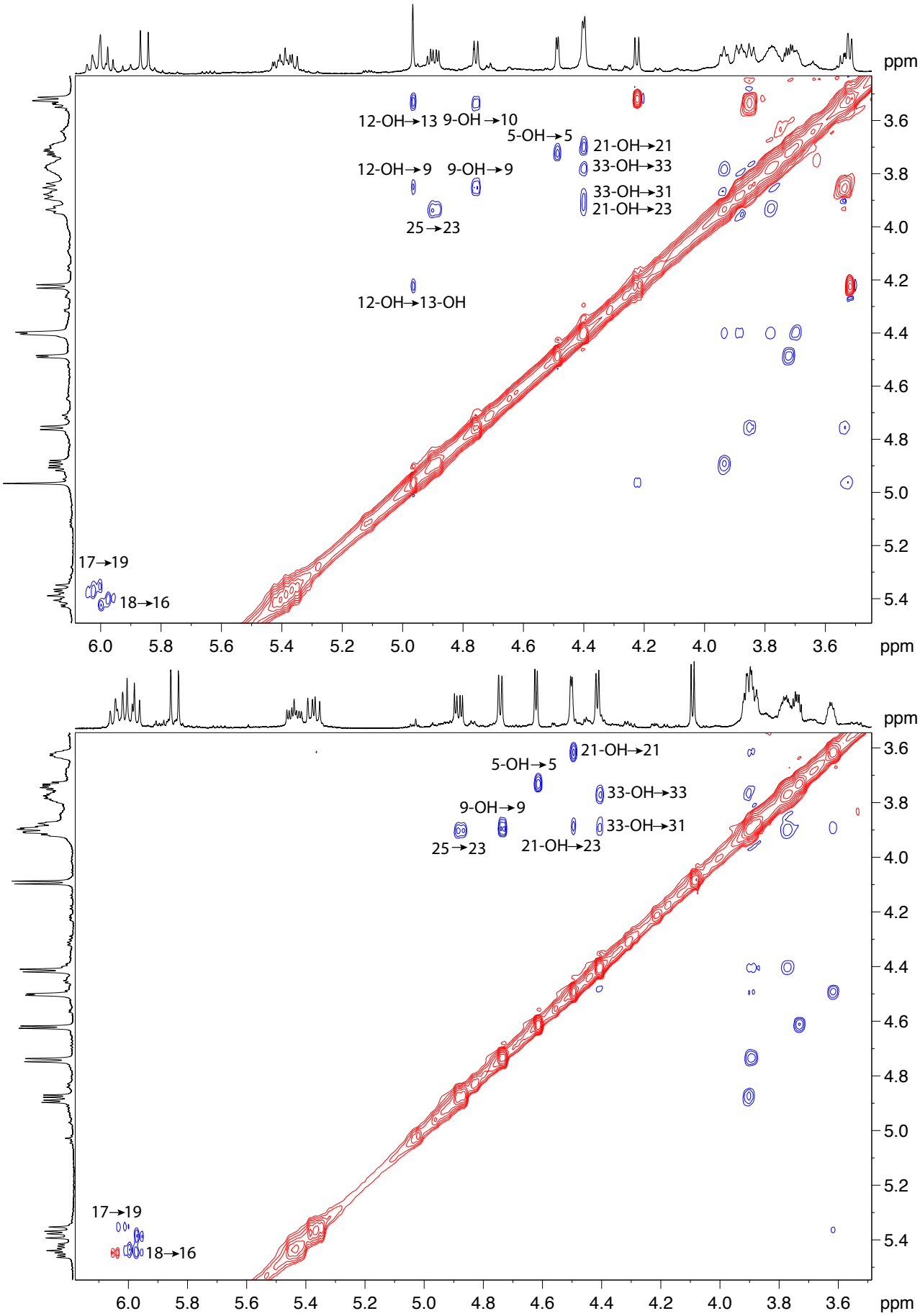


Figure S9b. ROESY (600 MHz, DMSO-d_6) spectra of 21-hydroxy-oligomycin A (**6**) (top) and 21-hydroxy-oligomycin C (**7**) (bottom), with key correlations annotated.

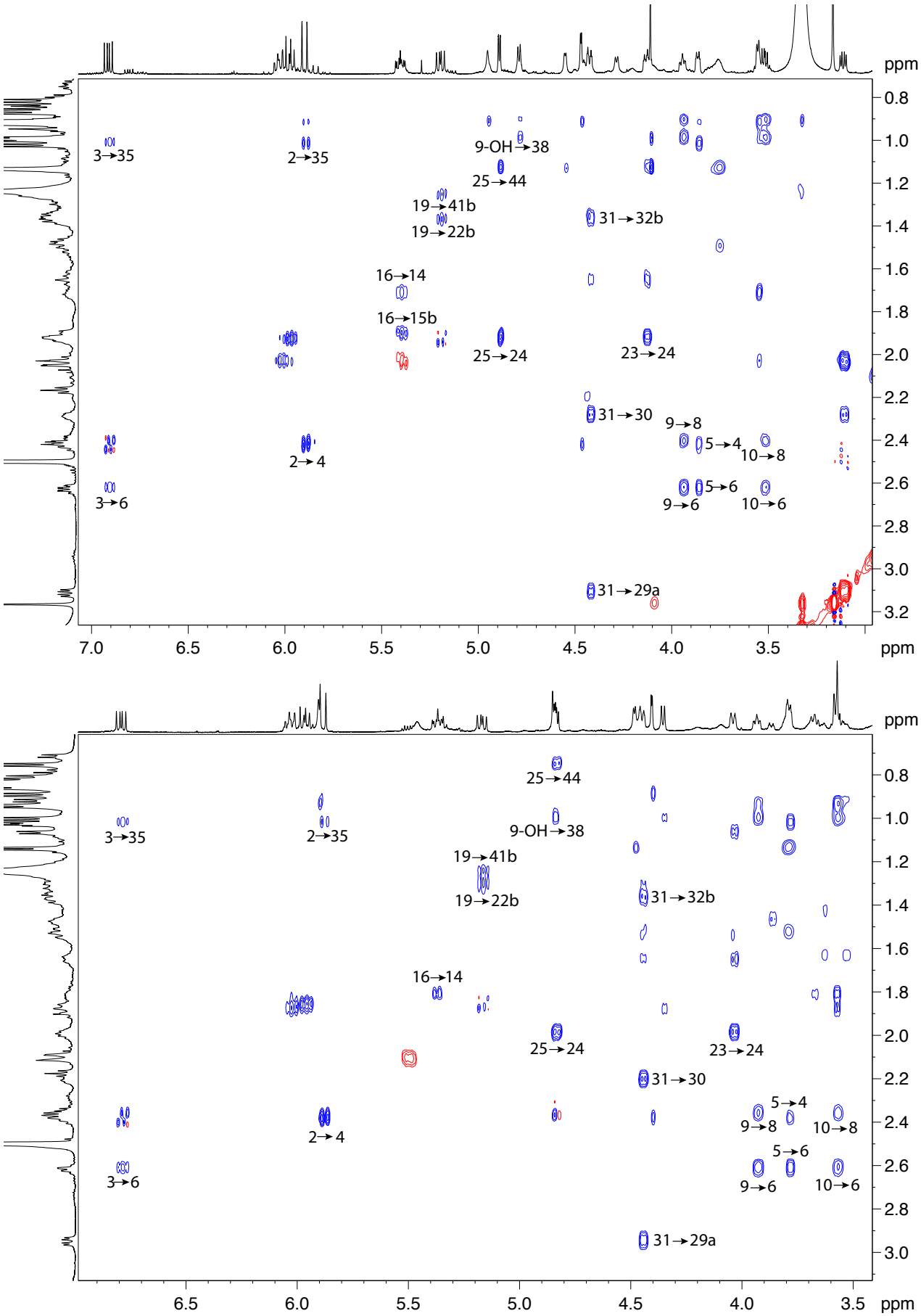


Figure S10a. ROESY (600 MHz, DMSO-d_6) spectra of oligomycin E (**5**) (top) and 40-hydroxy-oligomycin B (**8**) (bottom), with key correlations annotated.

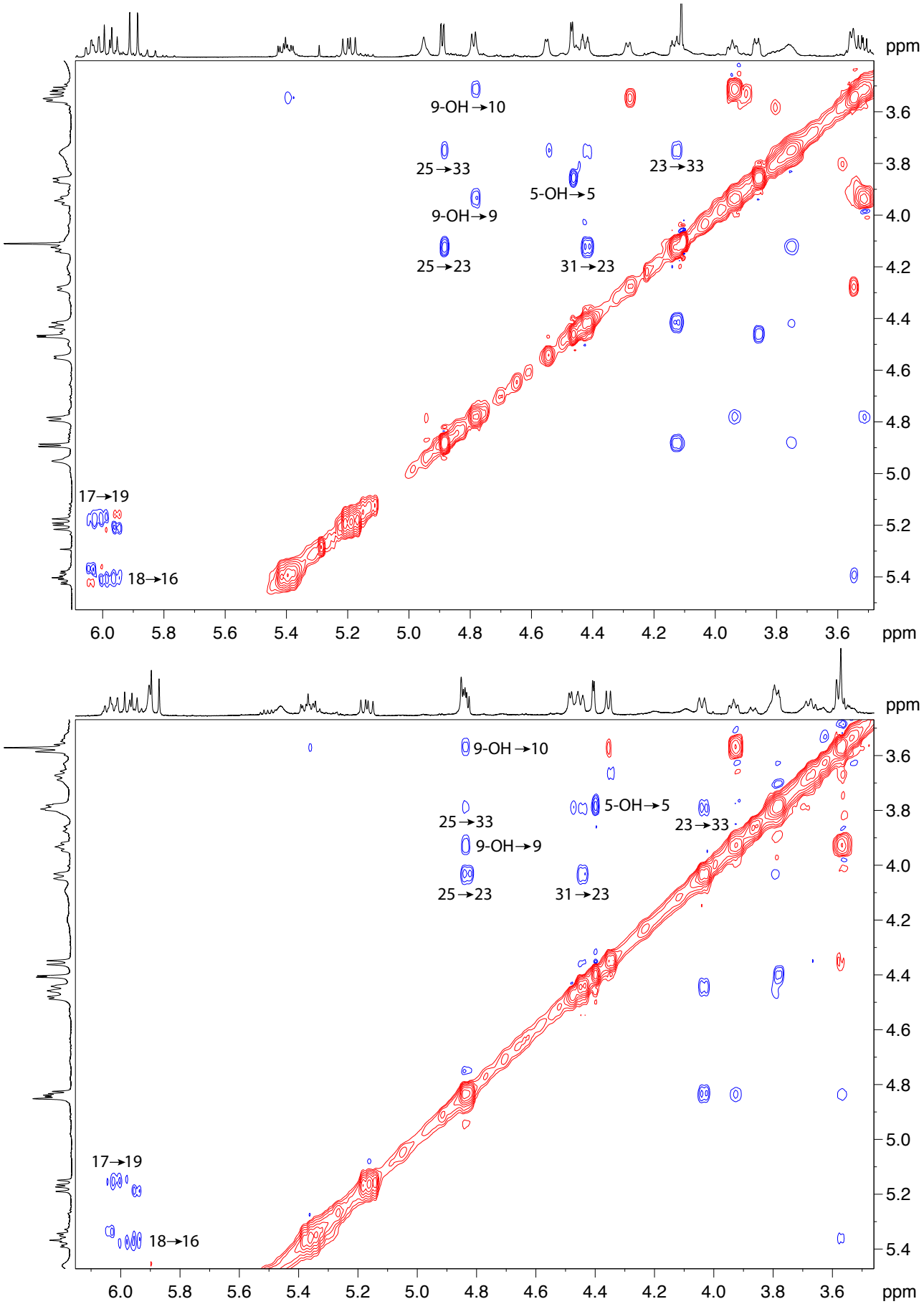


Figure S10b. ROESY (600 MHz, DMSO-d_6) spectra of oligomycin E (**5**) (top) and 40-hydroxy-oligomycin B (**8**) (bottom), with key correlations annotated

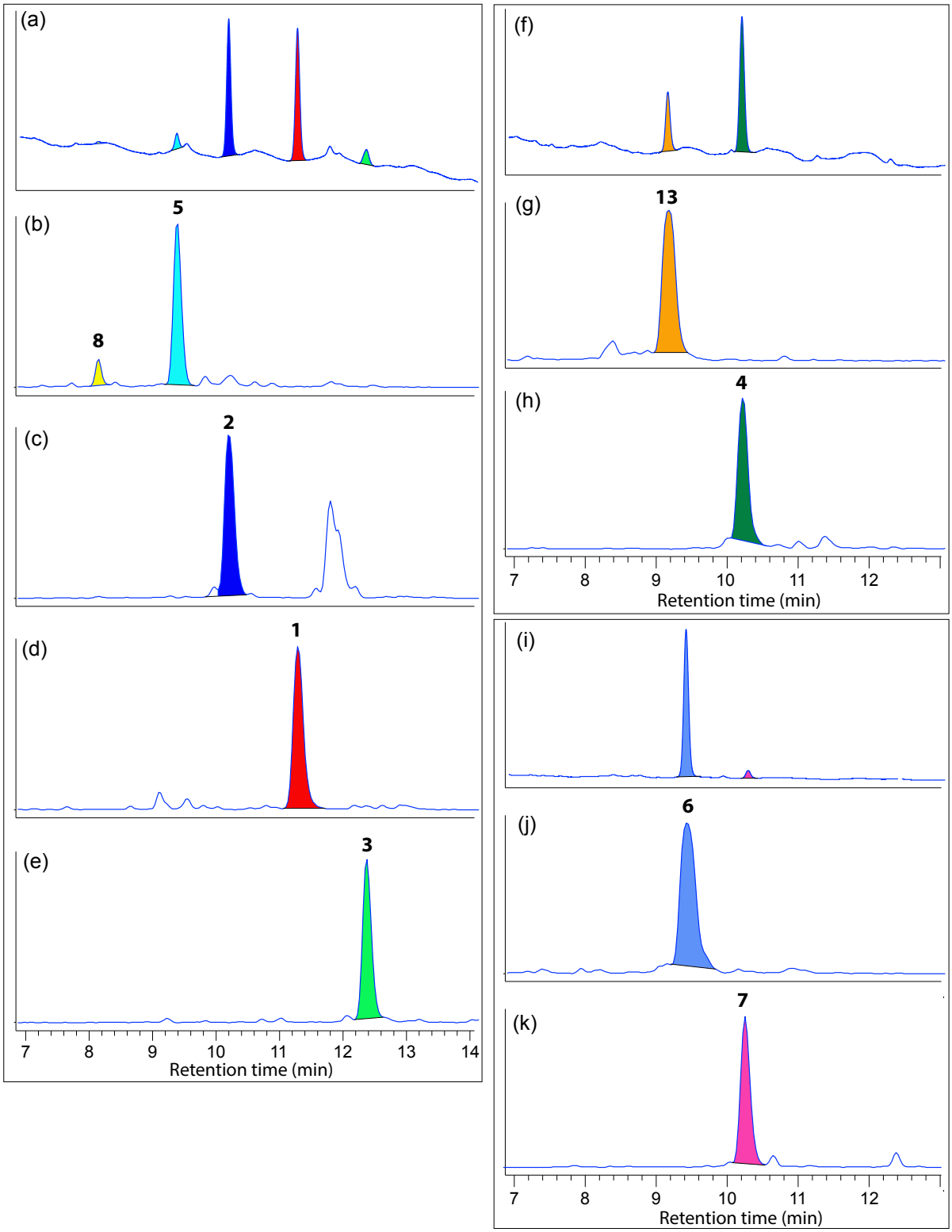


Figure S11. HPLC-DAD-ESIMS profile of crude extracts of AS5339v11, AS5958 and AS5351. (a) DAD (210 nm) chromatogram of AS5339v11 crude extract and extracted ion chromatograms at (b) 843 $[M+Na]^+$, (c) 827 $[M+Na]^+$, (d) 813 $[M+Na]^+$, (e) 797 $[M+Na]^+$; (f) DAD (210 nm) chromatogram of AS5958 crude extract and extracted ion chromatograms at (g) 772 $[M+Na]^+$, (h) 799 $[M+Na]^+$; (i) DAD (210 nm) chromatogram of AS5351 crude extract and extracted ion chromatograms at (j) 829 $[M+Na]^+$, (k) 813 $[M+Na]^+$. HPLC-DAD-ESIMS conditions: 1 mL/min elution through a Zorbax Eclipse XDB-C_8, 4.6 × 150 mm, 5 μm column, using a gradient from 90-40% H_2O/MeCN over 3 min, followed by 40% H_2O/MeCN to MeCN over 10 min, with an isocratic 0.05% formic acid/MeCN modifier .

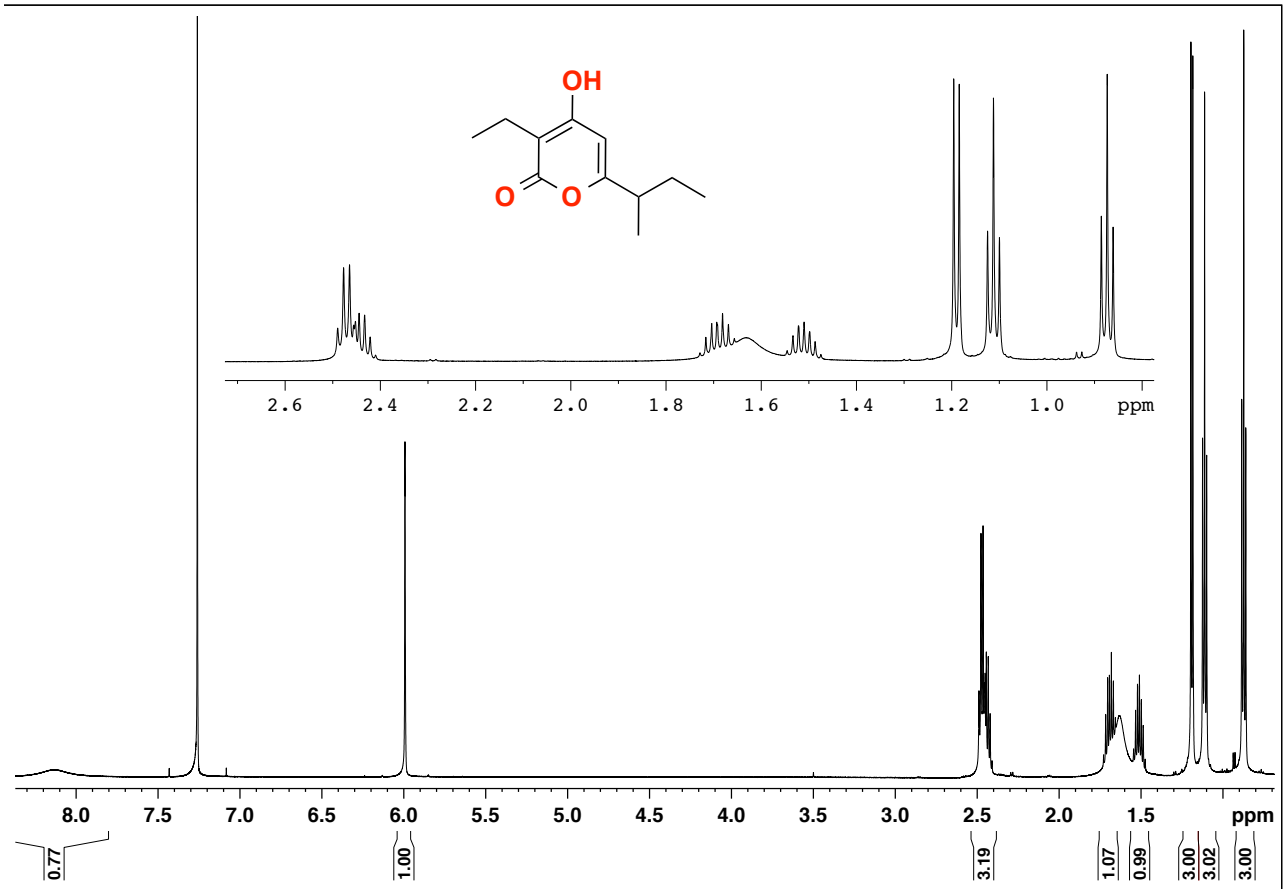


Figure S12. ^{1}H NMR (600 MHz, CDCl3) spectrum of germicidin A (**9**)

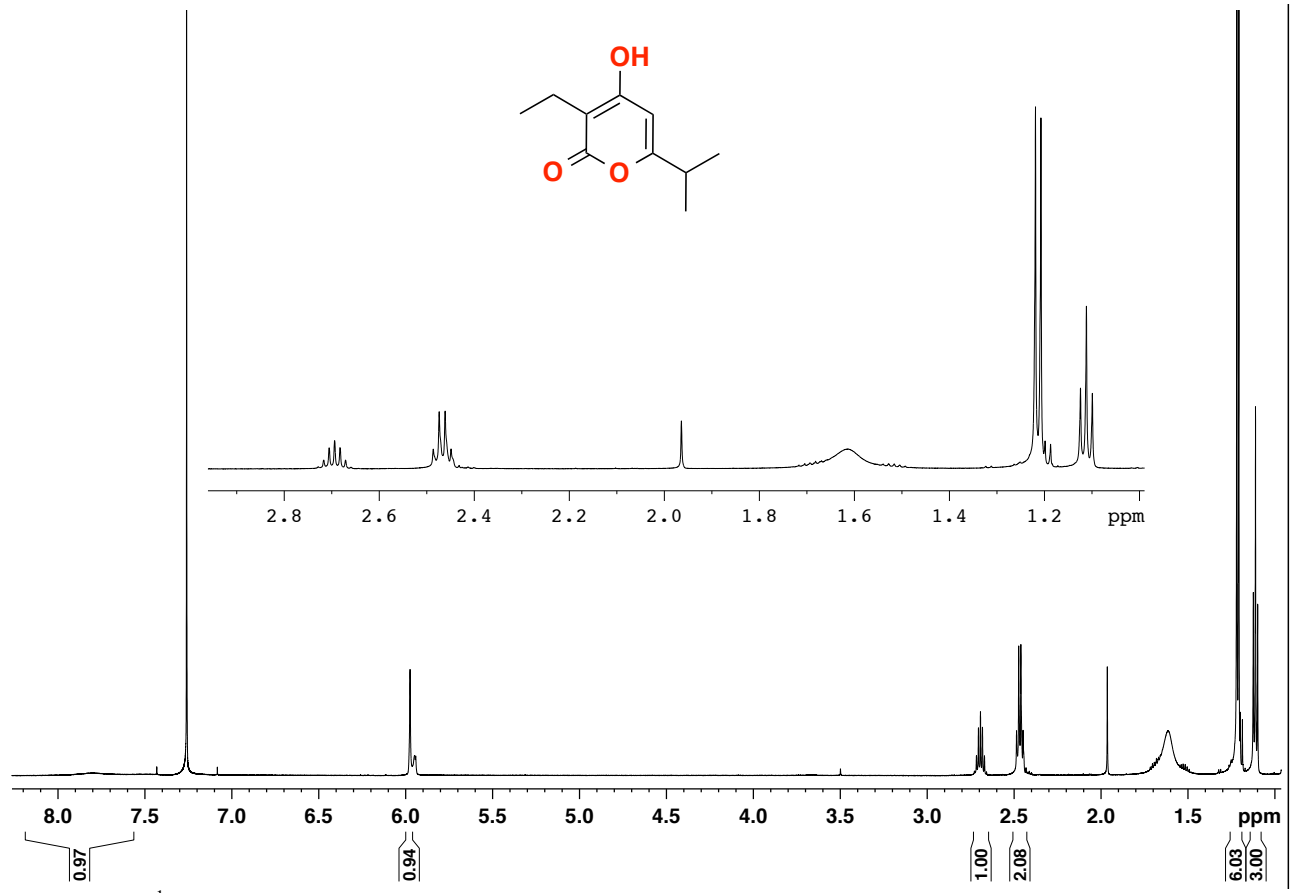


Figure S13. ^{1}H NMR (600 MHz, CDCl3) spectrum of germicidin B (**10**)

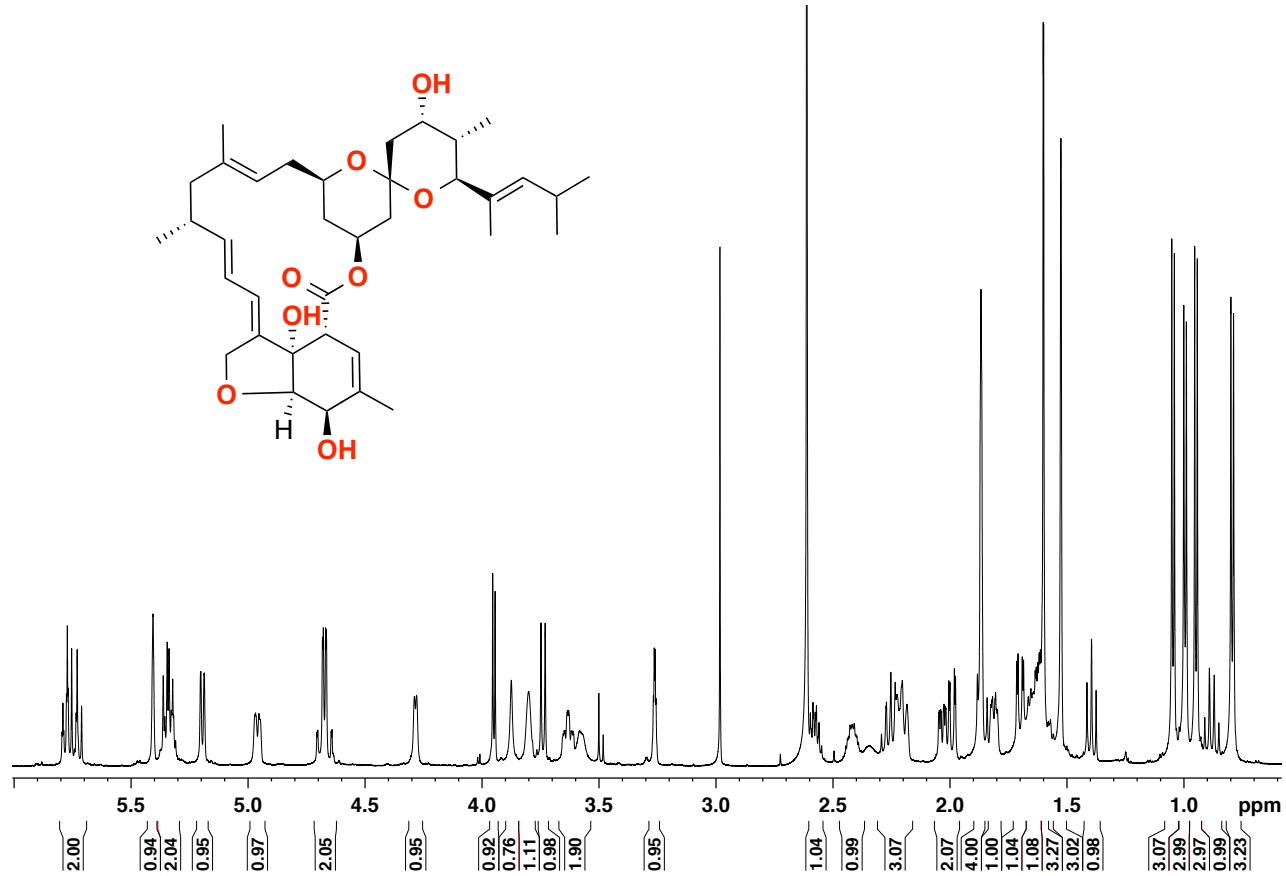


Figure S14. ^{1}H NMR (600 MHz, CDCl3) spectrum of nemadectin α (**11**)

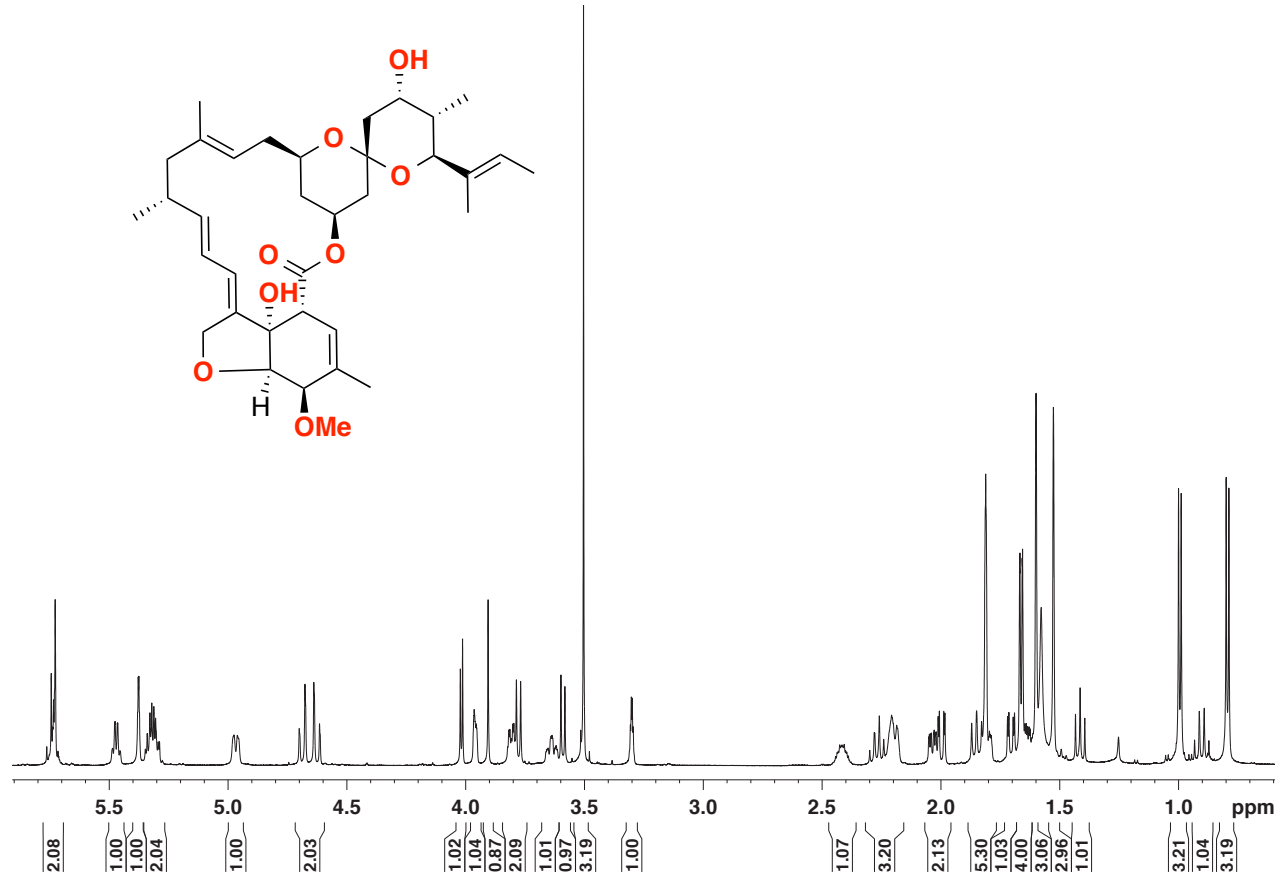


Figure S15. ^{1}H NMR (600 MHz, CDCl3) spectrum of nemadectin γ (**12**)

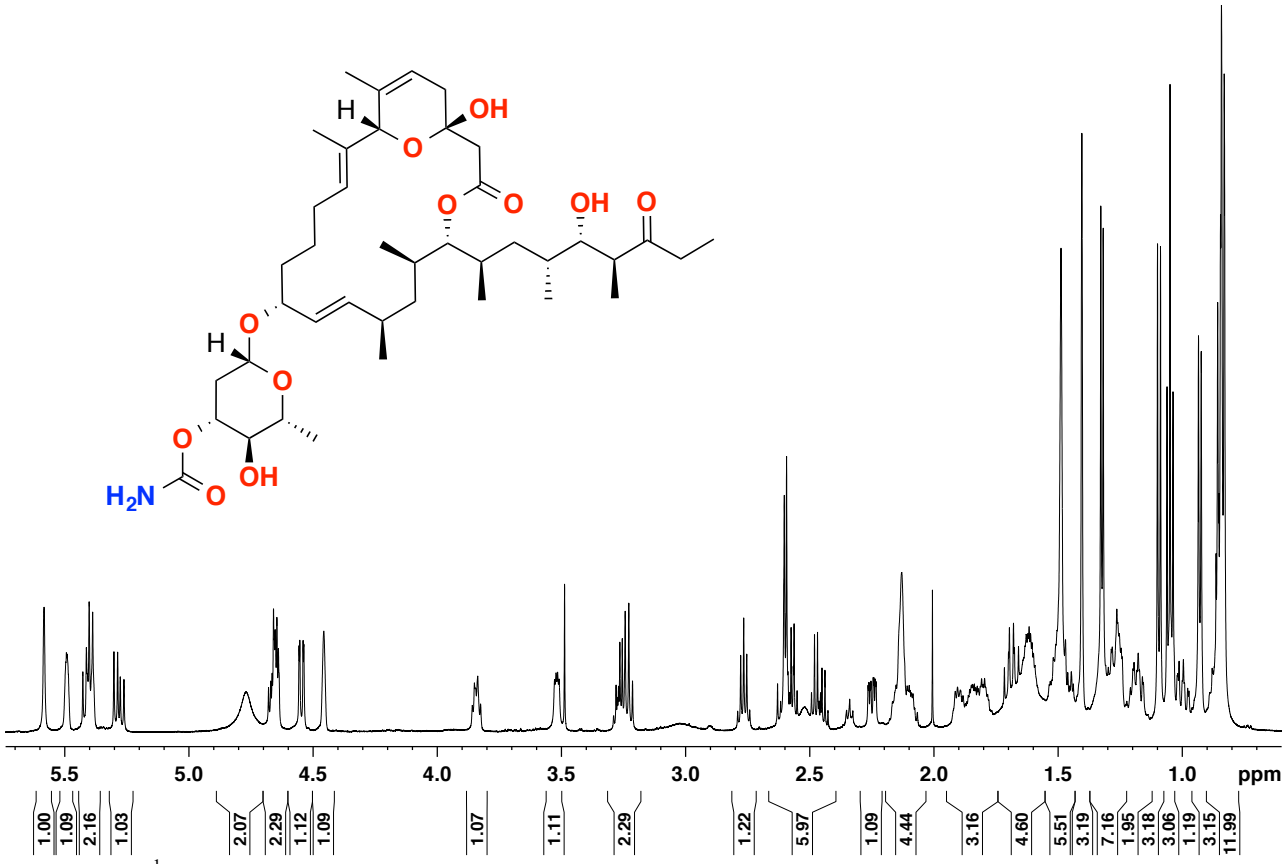


Figure S16. ^{1}H NMR (600 MHz, CDCl3) spectrum of venturicidin A (**13**)