

Supporting Information

Revised structural assignment of azalomycins based on genomic and chemical analysis

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Experimental section

General experimental procedures. Optical rotations were obtained utilizing a Jasco P-1020 polarimeter (Jasco, Easton, MD, USA). Experimental ECD spectra in MeOH were acquired in a quartz cuvette of 1 mm optical path length on a JASCO J-1500 spectropolarimeter (Tokyo, Japan). IR spectra were acquired on a Bruker IFS-66/S FT-IR spectrometer. NMR spectra, including ^1H - ^1H COSY, HSQC, HMBC, and ROESY experiments, were carried out using a Varian UNITY INOVA 800 NMR spectrometer operating at 800 MHz (^1H) and 200 MHz (^{13}C), with chemical shifts given in ppm (δ). Preparative high-performance liquid chromatography (HPLC) utilized a Waters 1525 Binary HPLC pump with a Waters 996 Photodiode Array Detector (Waters Corporation, Milford, CT, USA). Semi-preparative HPLC used a Shimadzu Prominence HPLC System with SPD-20A/20AV Series Prominence HPLC UV-Vis Detectors (Shimadzu, Tokyo, Japan). LC/MS analysis was carried out on an Agilent 1200 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector and a 6130 Series ESI mass spectrometer by using an analytical Kinetex (4.6 \times 100 mm, 3.5 μm). LC-ESI-HRMS based metabolomics were performed on a Dionex Ultimate3000 system coupled with a Luna Omega C18 column (100 \times 2.1 mm, particle size 1.6 μm , pore diameter 100 \AA , Phenomenex) combined with Q-Exactive Plus mass spectrometer (Thermo Scientific) equipped with an electrospray ion (ESI) source. Column oven was set to 40 $^\circ\text{C}$; scan range of full MS was set to m/z 150 to 2,000 with resolution of 70,000 and AGC target 3e6 and maximum IT 100 ms under positive and negative mode with centroid data type. MS² was performed to choose top10 intensive ions under positive mode with resolution of 17,500 and AGC target 1e5 and maximum IT 50 ms and (N)CE 28 with centroid data type. The spray voltage (+) was set to 4000 volt, and (-) was set to 3300 volt. The capillary temperature (+/-) was set to 340 $^\circ\text{C}$ and probe heater temperature (+/-) was set to 200 $^\circ\text{C}$. The sheath gas flow (+/-) was set to 35 L/min and Aux gas flow (+/-) to 5 L/min. Max spray current (+) and (-) was set to 100 volt. S-Lens RF level was set to 50. Merck precoated silica gel F254 plates and RP-18 F254s plates were used for thin layer chromatography (TLC). Spots were detected on TLC under UV light or by heating after spraying with anisaldehyde-sulfuric acid.

LC-HRMS/MS mediated molecular networking. *Streptomyces* sp. M56 was grown on 50 mL ISP-2 liquid (in 250 mL Erlenmeyer flask) for 7 days at 30 $^\circ\text{C}$ under shaking 180 rpm. The resultant culture broth was extracted twice by 50 mL of ethyl acetate (EtOAc), and evaporated under reduced pressure to give the EtOAc extract, which was dissolved into MeOH to reach the concentration of 0.1 mg/mL. The extract was submitted to LC-ESI-HRMS metabolomics analysis under standard condition; The metabolites were separated under the gradient: 0 – 0.5 min, 5% B; 0.5 – 18 min, 5% – 97% B; 18 – 23 min, 97% B; 23 – 25 min, 97% – 5% B; 25 – 30 min, 5% B (A: H₂O with 0.1% formic acid (FA); B:

MeCN with 0.1% FA), with flow rate of 0.3 mL/min and injection volume is 5 μ L. Metabolomics raw data acquired on a Thermo QExactive Plus mass spectrometer was converted to 32-bit mzXML files using MSConvert GUI (ProteoWizard) [1], in order to generate a mass spectral molecular networking using the GNPS platform (<https://gnps.ucsd.edu>) [2]. Data analysis used default parameters, except for the cosine threshold, set to 0.7, minimum matched fragment ions of 4, network TopK 10, and for the tolerances of the precursor- and fragment ion masses, both set to 0.02 Da. The mass spectral network was assembled and visualized using Cytoscape (www.cytoscape.org).

Cultivation and secondary metabolite extraction. *Streptomyces* sp. M56 was cultivated on 150 ISP-2 (ISP-2 medium and 2.0% Agar-Agar) agar plates (10 days, 30 $^{\circ}$ C) [3,4]. Mycelium covered agar was cut into small squares and extracted overnight with 100% MeOH. The MeOH soluble layer was filtered, and then the solvent was evaporated *in vacuo* to give the crude MeOH extract (30 g). The crude MeOH extract was suspended in 700 mL distilled water, and then solvent-partitioned with EtOAc, yielding 6 g of a residue.

Isolation of compounds. The EtOAc-soluble fraction (6 g) was fractionated by silica gel column chromatography eluted with CH₂Cl₂/MeOH (100:1–0:1 of gradient solvent system) to afford six fractions (A–F). The fraction F (730 mg) was loaded onto RP-C18 silica-gel column chromatography and fractionated using MeOH–H₂O (1:9–1:0 of gradient solvent system) to afford four subfraction (F1–F4). Subfraction F4 (80 mg) was purified by a semi-preparative HPLC applying a Phenomenex Luna C18 column (250 \times 10.0 mm i.d.) with 57% MeOH/H₂O of isocratic solvent system (flow rate = 2 mL/min) to yield compounds **1** (t_R = 22.0 min, 8.1 mg), **2** (t_R = 23.5 min, 3.3 mg), **3** (t_R = 46.0 min, 10.5 mg), and **4** (t_R = 51.0 min, 8.4 mg). All the isolation procedures were monitored by LC/MS analysis.

Physical constants and spectroscopic data of compounds 1-4.

Azalomycin F_{4b} (**1**). White amorphous powder; [α]_D²⁵ +21.3 (*c* 0.20 in MeOH); IR (KBr) ν_{\max} 3405, 2950, 2838, 1663, 1453, 1030 cm⁻¹; ECD (in MeOH) λ_{\max} ($\Delta\epsilon$) 231 (-1.2), 259 (3.9) nm; ¹H (800 MHz) and ¹³C NMR (200 MHz) see Table S1; HR-ESI-MS *m/z* 1082.6688 [M+H]⁺ (Calcd. for C₅₆H₉₆N₃O₁₇, 1082.6734).

Azalomycin F_{5b} (**2**). White amorphous powder; [α]_D²⁵ +15.3 (*c* 0.14 in MeOH); IR (KBr) ν_{\max} 3404, 2948, 2838, 1663, 1453, 1029 cm⁻¹; ECD (in MeOH) λ_{\max} ($\Delta\epsilon$) 232 (-1.0), 260 (3.6) nm; ¹H (800 MHz) and ¹³C NMR (200 MHz) see Table S1; HR-ESI-MS *m/z* 1096.6840 [M+H]⁺ (Calcd. for C₅₇H₉₈N₃O₁₇, 1096.6891).

Azalomycin F_{4a} (**3**). White amorphous powder; [α]D₂₀+19.7 (*c* 0.18 in MeOH); IR (KBr) ν_{\max} 3405, 2949, 2839, 1663, 1453, 1030 cm⁻¹; ECD (in MeOH) λ_{\max} ($\Delta\epsilon$) 232 (-1.3), 260 (3.8) nm; ¹H (800 MHz) and ¹³C NMR (200 MHz) see Table S1; HR-ESI-MS *m/z* 1082.6696 [M+H]⁺ (Calcd. for C₅₆H₉₆N₃O₁₇, 1082.6734).

Azalomycin F_{5a} (**4**). White amorphous powder; [α]D₂₀+23.1 (*c* 0.22 in MeOH); IR (KBr) ν_{\max} 3407, 2949, 2838, 1663, 1452, 1028 cm⁻¹; ECD (in MeOH) λ_{\max} ($\Delta\epsilon$) 230 (-1.5), 258 (4.1) nm; ¹H (800 MHz) and ¹³C NMR (200 MHz), see Table S1; HR-ESI-MS *m/z* 1096.6849 [M+H]⁺ (Calcd. for C₅₇H₉₈N₃O₁₇, 1096.6891).

Absolute configuration of 1,2-diols (C-18/C-19) in 1. Compound **1** (0.3 mg) and Mo₂(OAc)₄ (0.7 mg) were co-dissolved in DMSO (0.9 mL; ligand to metal ratio of approx. 1.0:1.2), and the mixture was directly subjected to ECD measurements. The first ECD spectrum was recorded immediately after mixing. Then, the mixture was kept for 30 min to enable complexation and then the ECD spectrum was acquired. The inherent ECD was subtracted and the diagnostic induced ECD curve was monitored at approx. 305 nm.

Gene cluster analysis. For the analysis the genome of *Streptomyces* sp. M56 was downloaded from NCBI (access number CP025018.1) and putative secondary metabolite-related biosynthetic gene clusters (BGC) were predicted by antiSMASH 5.0 [5]. Manual blast analysis resulted in the identification of a gene cluster (*azu*) with overall high homologies to the previously reported *azl* gene cluster (azalomycins F_{3a} biosynthesis in *Streptomyces* 211726) [6-8], and moderate homolog to *npm* gene cluster reported for the biosynthesis of niphimycins C-E in a marine-derived *Streptomyces* sp. IMB7-145 [9]. The putative functions of each gene were determined by comparing the deduced amino acid sequence with other bacterial homologues (Table S5).

Antifungal assay. Antifungal activity was tested against three major human pathogens including *Cryptococcus neoformans* H99, *Candida albicans* SC5314 and *Malassezia pachydermatis* CBS1879. Except for *M. pachydermatis*, minimum inhibitory concentrations (MIC) were measured using a standard broth serial dilution method from the CLSI (Clinical and laboratory standards institute) guideline [10]. MIC of *M. pachydermatis* was determined using the methods modified from the CLSI guideline [11]. The tested compounds were serially diluted 2-fold with mDixon medium and then 2.5 × 10³ CFU/mL of *M. pachydermatis* cells were inoculated into a 96 well plate and incubated at 34 °C for 3 days. Antifungal

drug concentrations tested ranged from 0.2 to 250 µg/mL for tested compounds; from 0.03 to 32.0 µg/mL for fluconazole (FLZ); and from 0.001 to 1.0 µg/mL for ketoconazole (KTZ). FLZ was used as a reference antifungal drug for *C. albicans*, *C. neoformans* and KTZ was used as a reference antifungal drug for *M. pachydermatis*.

Antiproliferative and cytotoxic properties. Antiproliferative and cytotoxic assays, and cells and culture conditions are described elsewhere [12]. Compounds were evaluated for the antiproliferative effects (GI₅₀) against human umbilical vein endothelial cells HUVEC (ATCC CRL-1730) and human chronic myeloid leukemia cells K-562 (DSM ACC 10) and for the cytotoxic effects (CC₅₀) against human cervix carcinoma cells HeLa (DSM ACC 57).

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Table S1. ¹H (800 MHz) and ¹³C (200 MHz) NMR data of compounds **1–4** in MeOH-*d*₄.^{a,b}

| C-Nr | R/S ^b | 1 (F _{4b}) | | 2 (F _{5b}) | | 3 (F _{4a}) | | 4 (F _{5a}) | |
|------|------------------|----------------------|--------------------------|----------------------|--------------------------|----------------------|--------------------------|----------------------|--------------------------|
| | | δ _C | δ _H (J in Hz) | δ _C | δ _H (J in Hz) | δ _C | δ _H (J in Hz) | δ _C | δ _H (J in Hz) |
| 1 | - | 170.3 s | - | 170.0 s | - | 170.1 s | - | 170.0 s | - |
| 2 | - | 126.9 s | - | 126.8 s | - | 126.7 s | - | 126.6 s | - |
| 3 | - | 140.4 d | 7.10 d (11.0) | 140.3 d | 7.09 d (11.0) | 140.7 d | 7.09 d (11.0) | 140.0 d | 7.08 d (11.0) |
| 4 | - | 127.9 d | 6.44 dd (15.0, 11.0) | 127.4 d | 6.43 dd (15.0, 11.0) | 128.3 d | 6.43 dd (15.0, 11.0) | 128.2 d | 6.43 dd (15.0, 11.0) |
| 5 | - | 146.2 d | 6.08 dd (15.0, 9.0) | 146.0 d | 6.07 dd (15.0, 9.0) | 146.7 d | 6.05 dd (15.0, 9.0) | 145.8 d | 6.05 dd (15.0, 9.0) |
| 6 | R | 44.7 d | 2.44 m | 44.6 d | 2.42 m | 44.9 d | 2.44 m | 44.2 d | 2.43 m |
| 7 | R | 75.7 d | 3.77 m | 75.7 d | 3.76 m | 75.8 d | 3.77 m | 75.0 d | 3.76 m |
| 8 | - | 39.3 t | 1.48 m, 1.76 m | 39.1 t | 1.46 m, 1.77 m | 39.8 t | 1.49 m, 1.77 m | 39.0 t | 1.45 m, 1.76 m |
| 9 | R | 75.2 d | 3.80 m | 75.1 d | 3.80 m | 75.7 d | 3.78 m | 75.2 d | 3.77 m |
| 10 | R | 44.6 d | 1.54 m | 44.5 d | 1.52 m | 44.6 d | 1.51 m | 44.5 d | 1.50 m |
| 11 | S | 72.5 d | 3.85 m | 72.1 d | 3.85 m | 72.6 d | 3.87 m | 72.2 d | 3.86 m |
| 12 | - | 33.6 t | 1.59 m, 1.97 m | 33.3 t | 1.56 m, 1.96 m | 33.8 t | 1.61 m, 1.99 m | 33.4 t | 1.60 m, 1.97 m |
| 13 | - | 30.4 t | 1.30 m, 1.41 m | 30.3 t | 1.29 m, 1.40 m | 30.7 t | 1.29 m, 1.40 m | 30.3 t | 1.28 m, 1.39 m |
| 14 | R | 40.6 d | 1.59 m | 40.4 d | 1.58 m | 41.1 d | 1.59 m | 40.6 d | 1.58 m |
| 15 | R | 72.3 d | 3.85 m | 72.5 d | 3.85 m | 72.6 d | 3.86 m | 72.1 d | 3.85 m |
| 16 | - | 41.9 t | 1.77 m | 41.6 t | 1.75 m | 42.2 t | 1.78 m | 42.0 t | 1.77 m |
| 17 | - | 100.1 s | - | 99.7 s | - | 99.7 s | - | 99.9 s | - |
| 18 | - | 77.6 d | 3.32 d (9.0) | 77.3 d | 3.33 d (9.0) | 77.7 d | 3.34 d (9.0) | 77.0 d | 3.34 d (9.0) |
| 19 | S | 69.8 d | 3.86 m | 69.6 d | 3.86 m | 70.1 d | 3.87 m | 69.5 d | 3.86 m |
| 20 | - | 41.4 t | 1.30 m, 1.88 m | 41.2 t | 1.29 m, 1.88 m | 41.7 t | 1.28 m, 1.89 m | 41.1 t | 1.27 m, 1.86 m |
| 21 | R | 65.7 d | 3.85 m | 65.5 d | 3.85 m | 66.2 d | 3.86 m | 65.6 d | 3.87 m |
| 22 | - | 44.4 t | 1.50 m | 44.3 t | 1.47 m | 44.7 t | 1.50 m | 44.1 t | 1.49 m |
| 23 | S | 66.2 d | 4.16 m | 66.1 d | 4.16 m | 71.4 d | 5.24 m | 71.0 d | 5.23 m |
| 24 | - | 44.6 t | 1.64 m | 44.4 t | 1.65 m | 44.3 t | 1.60 m | 43.8 t | 1.58 m |
| 25 | R | 71.0 d | 5.28 m | 70.5 d | 5.28 m | 66.0 d | 3.86 m | 65.5 d | 3.86 m |
| 26 | - | 44.0 t | 1.62 m, 1.77 m | 43.8 t | 1.61 m, 1.76 m | 44.3 t | 1.51 m, 1.70 m | 43.7 t | 1.50 m, 1.69 m |
| 27 | S | 65.8 d | 3.85 m | 65.5 d | 3.82 m | 66.6 d | 3.83 m | 65.8 d | 3.82 m |
| 28 | - | 44.2 t | 1.76 m | 44.1 t | 1.76 m | 44.3 t | 1.78 m | 43.5 t | 1.75 m |
| 29 | R | 74.3 d | 4.16 m | 73.9 d | 4.16 m | 74.6 d | 4.17 dd (9.0, 3.0) | 73.9 d | 4.17 dd (9.0, 3.0) |
| 30 | - | 140.2 s | - | 140.0 s | - | 140.2 s | - | 140.0 s | - |
| 31 | - | 125.6 d | 5.98 d (11.0) | 125.1 d | 5.97 d (11.0) | 125.6 d | 5.99 d (11.0) | 125.0 d | 5.98 d (11.0) |
| 32 | - | 128.7 d | 6.23 dd (15.0, 11.0) | 128.4 d | 6.22 dd (15.0, 11.0) | 129.0 d | 6.22 dd (15.0, 11.0) | 128.3 d | 6.22 dd (15.0, 11.0) |
| 33 | - | 136.4 d | 5.45 m | 136.1 d | 5.44 m | 136.7 d | 5.45 m | 136.0 d | 5.42 m |
| 34 | R | 41.0 d | 2.57 m | 40.8 d | 2.56 m | 41.3 d | 2.56 m | 40.8 d | 2.55 m |
| 35 | R | 81.0 d | 4.78 dd (7.5, 4.5) | 80.6 d | 4.78 dd (7.5, 4.5) | 81.2 d | 4.78 dd (7.5, 4.5) | 80.6 d | 4.78 dd (7.5, 4.5) |
| 36 | S | 34.9 d | 1.80 m | 35.0 d | 1.79 m | 35.6 d | 1.82 m | 35.0 d | 1.81 m |
| 37 | - | 34.3 t | 1.13 m, 1.34 m | 34.2 t | 1.13 m, 1.33 m | 34.7 t | 1.14 m, 1.34 m | 34.1 t | 1.15 m, 1.35 m |
| 38 | - | 28.0 t | 1.41 m | 27.7 t | 1.41 m | 28.3 t | 1.42 m | 27.7 t | 1.41 m |
| 39 | - | 33.6 t | 1.98 m | 33.3 t | 1.97 m | 33.9 t | 1.98 m | 33.2 t | 1.97 m |
| 40 | - | 132.8 d | 5.44 m | 132.5 d | 5.44 m | 133.0 d | 5.45 m | 132.3 d | 5.45 m |
| 41 | - | 130.4 d | 5.42 m | 130.0 d | 5.42 m | 130.7 d | 5.42 m | 130.2 d | 5.42 m |
| 42 | - | 30.6 t | 2.07 m | 30.4 t | 2.06 m | 30.9 t | 2.06 m | 30.4 t | 2.06 m |
| 43 | - | 29.9 t | 1.63 m | 29.8 t | 1.63 m | 30.2 t | 1.64 m | 29.7 t | 1.63 m |
| 44 | - | 42.0 t | 3.15 t (7.0) | 42.0 t | 3.17 t (7.0) | 42.4 t | 3.15 t (7.0) | 41.7 t | 3.16 t (7.0) |
| 45 | - | 12.9 q | 1.92 s | 12.8 q | 1.92 s | 13.2 q | 1.91 s | 12.5 q | 1.91 s |
| 46 | - | 17.1 q | 1.11 d (7.0) | 17.0 q | 1.11 d (7.0) | 17.3 q | 1.11 d (7.0) | 16.6 q | 1.11 d (7.0) |
| 47 | - | 10.4 q | 0.89 d (7.0) | 10.3 q | 0.89 d (7.0) | 10.8 q | 0.88 d (7.0) | 10.1 q | 0.87 d (7.0) |
| 48 | - | 15.1 q | 0.91 d (7.0) | 15.0 q | 0.91 d (7.0) | 15.0 q | 0.91 d (7.0) | 14.5 q | 0.91 d (7.0) |
| 49 | - | 13.1 q | 1.65 s | 12.8 q | 1.64 s | 13.5 q | 1.64 s | 13.0 q | 1.64 s |
| 50 | - | 17.8 q | 1.00 d (6.5) | 17.5 q | 1.01 d (6.5) | 18.0 q | 1.01 d (6.5) | 17.5 q | 1.01 d (6.5) |
| 51 | - | 14.6 q | 0.92 d (7.0) | 14.2 q | 0.93 d (7.0) | 15.0 q | 0.95 d (7.0) | 14.5 q | 0.95 d (7.0) |
| 52 | - | 158.0 s | - | 157.5 s | - | 158.0 s | - | - | - |
| 53a | - | 28.2 q | 2.84 s | 28.0 q | 2.84 s | 28.6 q | 2.84 s | 28.2 q | 2.85 s |
| 53b | - | - | - | 28.0 q | 2.84 s | - | - | 28.2 q | 2.85 s |
| 1' | - | 171.5 s | - | 171.4 s | - | 171.7 s | - | 171.2 s | - |
| 2' | - | 47.8 t | 3.23 s | 47.5 t | 3.24 s | 48.4 t | - | 47.3 t | - |
| 3' | - | 173.7 s | - | 173.5 s | - | 173.8 s | - | 173.1 s | - |

^a Coupling constants (in parentheses) are in Hz. ¹³C NMR data were assigned based on HSQC and HMBC experiments.

Table S2. Comparison of the ^{13}C chemical shift of azalomycin F_{5a}/F_{5b} in $\text{MeOH}-d_4$.

| Nr | 4^a (F_{5a}) | | Chem. Pharm. Bull. 1982 (F_{5a}) ^{b,17,18} | | Mag. Res. Chem. 2011 ^d (F_{5a}) ³² | | Mar. Drug 2013 ^c (F_{5b}) ^{22,31} | | J. Antibiotics 1995 ^c (F_{5b}) ^{19,20} | |
|-----|-----------------------|----------------------|--|------------------------|---|------------------------|--|----------------------|---|----------------------------|
| | δ_C | δ_H (J in Hz) | δ_C | δ_H (J in Hz) | δ_C | δ_H (J in Hz) | δ_C | δ_H (J in Hz) | δ_C | δ_H (J in Hz) |
| 1 | 170.0 | - | 170.10 | - | 170.07 | - | 170.2 | - | 170.02 | - |
| 2 | 126.6 | - | 126.75 | - | 126.71 | - | 126.8 | - | 126.76 | - |
| 3 | 140.0 | 7.08 d (11.0) | 140.18 | 7.08 d (11.2) | 140.27 | 7.10 d (11.28) | 140.3 | 7.09 d (11.2) | 140.07 | 7.08 d (11.49) |
| 4 | 128.2 | 6.43 dd (15.0, 11.0) | 127.58 | 6.42 dd (14.7, 11.2) | 127.64 | 6.45 dd (14.58, 11.51) | 127.6 | 6.43 dd (11.5, 14.9) | 127.50 | 6.44 dd (14.58, 11.49) |
| 5 | 145.8 | 6.05 dd (15.0, 9.0) | 146.10 | 6.05 dd (14.7, 8.8) | 146.15 | 6.06 dd (14.91, 8.92) | 146.2 | 6.07 dd (15.1, 9.0) | 145.95 | 6.07 dd (14.80, 8.40) |
| 6 | 44.2 | 2.43 m | - | 2.43 m (8.8, 6.8, 4.8) | 44.48 | 2.45 m | 44.8 | 2.43 m | 40.64 | 2.44 m |
| 7 | 75.0 | 3.76 m | 75.77 | 3.76 m (4.8) | 75.68 | 3.78 m | 75.9 | 3.80 m | 75.78 | 3.76 t (3.8) |
| 8 | 39.0 | 1.45, 1.76 m | - | 1.45, 1.76 m | 39.27 | 1.50, 1.78 m | 39.3 | 1.50, 1.78 m | 39.27 | 1.55 m |
| 9 | 75.2 | 3.77 m | 75.03 | 3.77 m | 74.80 | 3.80 m | 75.4 | 3.80 m | 75.19 | 3.88 m |
| 10 | 44.5 | 1.50 m | 44.5 d | 1.50 m | 44.44 | 1.56 m | 44.7 | 1.54 m | 44.10 | 1.78 m |
| 11 | 72.2 | 3.86 m | - | 3.86 m | 72.27 | 3.92 m | 72.2 | 3.91 m | 72.33 | 3.88 m |
| 12 | 33.4 | 1.60, 1.97 m | - | 1.60, 1.97 m | 33.62 | 1.62, 1.35 m | 33.5 | 1.62, 1.38 m | 39.27 | 1.55 m |
| 13 | 30.3 | 1.28, 1.39 m | 30.62 | 1.28, 1.39 m | 30.67 | 1.33, 1.44 m | 30.7 | 1.30, 1.45 m | 29.77 | 1.55 m |
| 14 | 40.6 | 1.58 m | - | 1.58 m | 40.83 | 1.60 m | 40.6 | 1.60 m | 40.64 | 1.78 m |
| 15 | 72.1 | 3.85 m | - | 3.85 m | 72.27 | 3.86 m | 72.7 | 3.86 m | 72.49 | 3.88 m |
| 16 | 42.0 | 1.77 m | - | 1.77 m | 41.66 | 1.82 m | 41.9 | 1.80 m | 41.99 | 1.55 m |
| 17 | 99.9 | - | 99.79 | - | 99.72 | - | 99.9 | - | 99.79 | - |
| 18 | 77.0 | 3.34 d (9.0) | 77.33 | 3.33 d (10.2) | 77.16 | 3.35 d (9.10) | 77.5 | 3.34 d (9.2) | 77.39 | 3.35 d (8.00) |
| 19 | 69.5 | 3.86 m | - | 3.86 m | 69.74 | 3.88 m | 69.9 | 3.87 m | 69.69 | 3.88 m |
| 20 | 41.1 | 1.27, 1.86 m | - | 1.27, 1.86 m | 41.22 | 1.30, 1.91 m | 41.4 | 1.89, 1.30 m | 41.16 | 1.44 m |
| 21 | 65.6 | 3.87 m | - | 3.87 m | 65.39 | 3.410 m | 65.7 | 4.17 m | 65.68 | 4.08 m (10.83, 9.94) |
| 22 | 44.1 | 1.49 m | - | 1.49 m | 41.80 | 1.68, 1.78 m | 44.5 | 1.52 m | 41.89 | 1.44 m |
| 23 | 71.0 | 5.23 m | 70.75 | 5.21 m | 70.70 | 5.23 m | 66.3 | 4.03 m | 65.71 | 3.88 m |
| 24 | 43.8 | 1.58 m | - | 1.58 m | 43.63 | 1.72 m | 44.6 | 1.69 m | 44.55 | 1.55 m |
| 25 | 65.5 | 3.86 m | - | 3.86 m | 65.47 | 3.90 m | 70.8 | 5.28 m | 70.72 | 5.23 m |
| 26 | 43.7 | 1.50, 1.69 m | - | 1.50, 1.69 m | 46.57 | 1.49 m | 44.0 | 1.61, 1.83 m | 46.26 | 1.55 m |
| 27 | 65.8 | 3.82 m | 65.29 | 4.02 m | 65.99 | 4.04 m | 65.7 | 3.88 m | 66.32 | 4.02 m |
| 28 | 43.5 | 1.75 m | - | 1.75 m | 44.22 | 1.50, 1.57 m | 44.2 | 1.78 m | 44.02 | 1.55 m |
| 29 | 73.9 | 4.17 dd (9.0, 3.0) | 74.24 | 4.17 dd (8.8, 3.6) | 74.15 | 4.18 dd (8.81, 2.62) | 74.2 | 4.18 m | 74.28 | 4.17 dd (8.17, 3.09, 2.65) |
| 30 | 140.0 | - | 140.18 | - | 140.19 | - | 140.2 | - | 140.07 | - |
| 31 | 125.0 | 5.98 d (11.0) | 125.10 | 5.98 d (11.2) | 125.09 | 6.00 d (11.50) | 125.3 | 5.98 d (10.4) | 125.09 | 6.00 d (11.05) |
| 32 | 128.3 | 6.22 dd (15.0, 11.0) | 128.56 | 6.21 dd (14.9, 11.2) | 128.59 | 6.24 dd (14.9, 10.9) | 128.6 | 6.22 dd (10.9, 14.5) | 128.47 | 6.22 dd (14.0, 11.1) |
| 33 | 136.0 | 5.42 m | 136.15 | 5.42 m (14.9, 8.8) | 136.17 | 5.42 dd (13.51, 7.53) | 136.3 | 5.43 m | 136.11 | 5.44 m |
| 34 | 40.8 | 2.55 m | - | 2.55 m (8.8, 8.0, 6.8) | 41.05 | 2.57 m | 41.0 | 2.57 m | 40.84 | 2.56 dd (7.51, 7.07) |
| 35 | 80.6 | 4.78 dd (7.5, 4.5) | 80.81 | 4.78 dd (8.0, 4.0) | 80.71 | 4.80 dd (7.91, 3.68) | 80.9 | 4.78 dd (7.6, 4.0) | 80.85 | 4.78 dd (8.10, 4.19) |
| 36 | 35.0 | 1.81 m | - | 1.82 m (6.9, 4.0) | 35.05 | 1.84 m | 35.3 | 1.82 m | 33.52 | 1.82 m |
| 37 | 34.1 | 1.15, 1.35 m | - | 1.15, 1.35 m | 34.53 | 1.17, 1.35 m | 34.4 | 1.15, 1.35 m | 28.35 | 1.55 m |
| 38 | 27.7 | 1.41 m | - | 1.41 m | 27.91 | 1.43 m | 27.9 | 1.42 m | 27.85 | 1.55 m |
| 39 | 33.2 | 1.97 m | - | 1.98 m | 33.62 | 2.00 m | 33.6 | 1.99 m | 30.52 | 2.03 m |
| 40 | 132.3 | 5.45 m | 132.40 | 5.43 m (14.9) | 132.49 | 5.48 m | 132.6 | 5.44 m | 132.49 | 5.44 m |
| 41 | 130.2 | 5.42 m | 130.27 | 5.41 m (14.9) | 130.31 | 5.45 m | 130.3 | 5.44 m | 130.15 | 5.44 m |
| 42 | 30.4 | 2.06 m | - | 2.06 m | 30.67 | 2.08 m | 30.7 | 2.07 m | 29.77 | 2.03 m |
| 43 | 29.7 | 1.63 m | - | 1.63 m | 29.81 | 1.68 m | 29.9 | 1.67 m | 30.52 | 1.55 m |
| 44 | 41.7 | 3.16 t (7.0) | - | 3.15 t (6.8) | 42.09 | 3.18 t (7.30) | 42.2 | 3.17 t (7.3) | 42.14 | 3.15 t (6.85) |
| 45 | 12.5 | 1.91 s | 12.87 | 1.91 d (1.6) | 12.91 | 1.92 s | 12.9 | 1.92 s | 12.84 | 1.92 s |
| 46 | 16.6 | 1.11 d (7.0) | 17.05 | 1.10 d (6.8) | 17.17 | 1.12 d (6.75) | 17.1 | 1.11 d (6.8) | 16.89 | 1.10 d (6.85) |
| 47 | 10.1 | 0.87 d (7.0) | 10.52 | 0.87 d (7.0) | 10.43 | 0.89 d (6.91) | 10.5 | 0.89 d (6.9) | 10.54 | 0.86 d (6.84) |
| 48 | 14.5 | 0.91 d (7.0) | 14.93 | 0.91 d (7.0) | 14.78 | 0.92 d (6.72) | 15.2 | 0.91 d (6.7) | 14.94 | 0.88 d (6.63) |
| 49 | 13.0 | 1.64 s | 13.33 | 1.64 s | 13.28 | 1.65 s | 13.1 | 1.65 s | 13.33 | 1.65 s |
| 50 | 17.5 | 1.01 d (6.5) | 17.64 | 1.00 d (6.8) | 17.55 | 1.02 d (6.61) | 17.8 | 1.01 d (6.7) | 17.68 | 0.98 d (6.62) |
| 51 | 14.5 | 0.95 d (7.0) | 14.35 | 0.94 d (6.9) | 14.26 | 0.96 d (6.72) | 14.4 | 0.94 d (6.7) | 14.38 | 0.94 d (6.63) |
| 52 | 157.6 | - | 157.37 | - | 157.26 | - | 157.4 | - | 157.30 | - |
| 53a | 28.2 | 2.85 s | 28.40 | 2.84 s | 28.41 | 2.87 s | 28.4 | 2.85 s | 28.34 | 2.85 s |
| 53b | 28.2 | 2.85 s | 28.40 | 2.84 s | 28.41 | 2.87 s | 28.4 | 2.85 s | 28.34 | 2.85 s |
| 1' | 171.2 | - | 171.60 | - | 171.57 | - | 171.9 | - | 171.59 | - |
| 2' | 47.3 | - | 46.10 | 3.22 s | 46.08 | 3.22 s | 46.0 | 3.22 m | 46.26 | 3.24 s |
| 3' | 173.1 | - | 173.87 | - | 173.96 | - | 173.9 | - | 173.98 | - |

^a ^1H NMR was recorded at 800 MHz and ^{13}C NMR at 200 MHz; ^b ^1H NMR spectrum was recorded at 400 MHz and ^{13}C NMR at 100 MHz; ^c ^1H NMR was recorded at 500 MHz and ^{13}C NMR at 125 MHz; ^d ^1H NMR was recorded at 400 MHz and ^{13}C NMR at 100 MHz; ^e ^1H NMR was recorded at 400 MHz and ^{13}C NMR at 100 MHz.

Table S3. Azalomycin biosynthetic protein (Azu) annotations based on sequence homology. (137 Kbp, 40 regions)

| gene tag CFP59- | protein name | access. Nr. | size (aa) | annotation | closest homolog(s) ^a | identity (%)/- coverage (%) ^b | access. Nr. |
|--------------------|-----------------|-------------|--------------|--|------------------------------------|---|----------------------------------|
| 01156 | azu22 | AUA09068 | 185 | Carbon monoxide dehydrogenase small chain | | | |
| 01157 | azu21 | AUA09069 | 330 | 4-hydroxybenzoyl-CoA reductase subunit beta | | | |
| 01158 | azu20 | AUA09070 | 716 | Xanthine dehydrogenase molybdenum-binding subunit | | | |
| 01159 | azu19 | AUA09071 | 177 | DNA protection during starvation protein | | | |
| 01160 | azu18 | AUA09072 | 235 | Secreted effector protein pipB2 | | | |
| 01161 | azu17 | AUA09073 | 114 | hypothetical protein | | | |
| 01162 | azu16 | AUA09074 | 288 | Transposon Tn10 TetD protein | azl15 npm22 | 92/100 90/100 | ARM20296 AUO16420 |
| 01163 | azu15 | AUA09075 | 478 | Putrescine importer PuuP | azl14 npm21 | 85/100 91/100 | ARM20295 AUO16419 |
| 01164 | azu14 | AUA09076 | 267 | 4-guanidinobutyramide hydrolase/amidase | azl13 npm20 | 92/100 92/100 | ARM20294 AUO16418 |
| 01165 | azu13 | AUA09077 | 257 | HTH-type transcriptional regulator LuxR | azl12 npm19 | 90/100 89/100 | ARM20293 AUO16417 |
| 01166 | azu12 | AUA09078 | 307 | Fatty acyl-CoA reductase | | | |
| 01167 | azu11 | AUA09079 | 197 | Tetracycline repressor protein class A | | | |
| 01168 | azu10 | AUA09080 | 426 | putative inner membrane protein | azl11 npm13 | 94/100 89/100 | ARM20292 AUO16411 |
| 01169 | azu9 | AUA09081 | 475 | hypothetical protein | npm12 | 88/100 | AUO16410 |
| 01170 | azu8 | AUA09082 | 262 | Cellulose binding family II | azl10 | 91/100 | ARM20291 |
| 01171 | azu7 | AUA09083 | 145 | Endoribonuclease L-PSP | azl9 npm11 | 90/93 90/101 | ARM20290 AUO16409 |
| 01172 | azu6 | AUA09084 | 126 | putative HTH-type transcriptional regulator YtcD | azl8 npm10 | 92/100 94/98 | ARM20289 AUO16408 |
| 01173 | azu5 | AUA09085 | 339 | 4-guanidinobutyryl-CoA:ACP acyltransferase | azl5 npm7 | 94/94 90/94 | ARM20286 AUO16405 |
| 01174 | azu4 | AUA09086 | 478 | 4-guanidinobutanoate:CoA ligase | azl4 npm6 | 94/100 94/100 | ARM20285 AUO16404 |
| 01175 | azuA | AUA09087 | 2307 | Type I PKS | azlA npmA | 93/100 92/100 | ARM20284 AUO16423 |
| 01176 | azuH | AUA09088 | 2107 | Type I PKS | azlH npmI | 96/100 68/123 | ARM20283 AUO16403 |
| 01177 | azuG | AUA09089 | 3453 | Type I PKS | azlG npmH | 92/100 91/100 | ARM20282 AUO16422 |
| 01178 | azuF | AUA09090 | 3204 | Type I PKS | azlF npmG | 93/100 92/100 | ARM20281 AUO16402 |
| 01179 | azuE | AUA09091 | 8265 | Type I PKS | azlE npmF | 92/100 91/100 | ARM20280 AUO16401 |
| 01180 | azuD | AUA09092 | 4762 | Type I PKS | azlD npmE | 90/100 83/100 | ARM20279 AUO16400 |
| 01181 | azuC | AUA09093 | 3376 | Type I PKS | azlC npmD | 89/100 76/100 | ARM20278 AUO16399 |
| 01182 | azuB | AUA09094 | 5180 | Type I PKS | azlB npmC npmB | 91/100 82/66 85/52 | ARM20277 AUO16398 AUO16397 |
| 01183 | azu3 | AUA09095 | 68 | ferredoxin | azl3 npm5 | 91/100 90/100 | ARM20276 AUO16396 |
| 01184 | azu2 | AUA09096 | 399 | P450 | azl2 npm4 | 96/98 93/100 | ARM20275 AUO16421 |
| 01185 | azu1 | AUA09097 | 1579 | hypothetical protein | azl1 npm1 | 69/101 87/100 | ARM20274 AUO16393 |
| 01186 | | AUA09098 | 221 | hypothetical protein | | | |
| 01187 | | AUA09099 | 95 | hypothetical protein | | | |
| 01188 | | AUA09100 | 703 | ATP-dependent zinc metalloprotease FtsH | | | |
| 01189 | | AUA09101 | 628 | hypothetical protein | | | |
| 01190 | | AUA09102 | 176 | hypothetical protein | | | |
| 01191 | | AUA09103 | 378 | hypothetical protein | | | |
| 01192 | | AUA09104 | 693 | hypothetical protein | | | |
| 01193 | | AUA09105 | 418 | hypothetical protein | | | |
| 01194 | | AUA09106 | 675 | hypothetical protein | | | |
| 01195 | | AUA09107 | 695 | hypothetical protein | | | |

^a Mainly homologs from biosynthetic gene clusters encoding for characterized compounds were considered. Origin of gene clusters: *azl* = *Streptomyces* sp. 211726 (azalomycin F3a); *npm* = *Streptomyces* sp. IMB7-145 (niphimycins C-E). ^b Percent alignment and identify were determined using BLASTp, following default parameters. Percent alignment is the proportion of the Nam query sequence that aligns to each homolog.

Table S4. Determination of KR domain specificity (CLUSTAL multiple sequence alignment by MUSCLE (3.8))

| Module | Loop | Catalytic Region |
|---------------|-------------|------------------------|
| Ery2_A1 | HAAGLPQQVAI | SSGAGVWGSARQGAYAAANA |
| Meg6_A1 | HAAGVPQSTPL | SSGAGVWGSANLGAYAAANA |
| Ole6_A1 | HTAGVPDSRPL | SSNAGVWGS GGQAVYAAANA |
| Pik5_A1 | HTAGAPGGDPL | SSNAGVWGS GWQGVYAAANA |
| Sor6_A1 | HAGGIEPHAPL | SSGAVVWGGGQGGYAAANA |
| Tyl6_A1 | HTAGTPHSAEF | SSGAAVWGS GGQTAYGAANA |
| azuD_module7 | HAAGVEQAAEL | SSIAGVWGS GGQAAYGAANA |
| azuD_module9 | HAAGVEQAAEL | SSIAGVWGS GGQAAYGAANA |
| azuE_module11 | HAAGANAAGPL | SSIAGVWGS GGQAAYGAANA |
| azuF_module15 | HAAGVTLAASL | SSI SGVWGGGS QGVYGSNA |
| azlD_KR1 | HAAGVEQAAEL | SSIAGVWGS GGQAAYGAANA |
| azlD_KR3 | HAAGVEQAAEL | SSIAGVWGS GGQAAYGAANA |
| azlE_KR2 | HAAGANAAGPL | SSIAGVWGS GGQAAYGAANA |
| azlF_KR1 | HAAGVTIAASL | SSI SGVWGGGS QGVYGSNA |
| Amp1_A2 | HTAAVIELAAL | SSTAGMWGSGVHAAYVAGNA |
| Can13_A2 | HTAAVIELQSI | SSTAGMWGSGRHAAYVAANA |
| Ela4_A2 | HIAGAGVLVPL | SSISAVWGS GEH GAYAAANA |
| Nys1_A2 | HAAAAIELSAL | SSTAGMWGSGVHAAYVAGNA |
| Pim7_A2 | HTAVTIELAPL | SSTAGMWGSGAHAAYVAGNA |
| Ave1_B1 | HTAGILDDATL | SSVTGTWGNAGQGAYAAANA |
| Tyl1_B1 | HTAGILDDAVI | SSAAATFGAPGQANYAAANA |
| Asc8_B1 | HTAATLDDGIL | SSAAAVLGS PGQGN YAAANA |
| Ave7_B1 | HAAGVLDDATI | SSAAGILGSAGQANYAAANA |
| Ave9_B1 | HAAGVLDDATI | SSAAGILGSAGQGN YAAANA |
| Rap10_B1 | HTAGVLDDGVV | SSAAGVLGSAGQGN YAVANA |
| azuA_module1 | HAAGVLDDGVI | SSVAGVFGSPGQGN YAAANS |
| azuB_module2 | HAAGVLDDGLL | SSATGVLGGAGQSN YAAANV |
| azuB_module3 | HTAGVLDDGVV | SSAAGTLGGPGQGS YAAAGNA |
| azuB_module4 | HTAGVLDDGVV | SSLSGTLGGTGQAN YAAANA |
| azuC_module6 | HTAGVLDDGVL | SSAAGTLGGPGQGS YAAAGNA |
| azuD_module8 | HAAGVLDDGVL | SSFAGVVGAGQGAY AAANA |
| azuE_module10 | HAAGILDDGVL | SSFAGAIGGAGQAAY AAANA |
| azuE_module12 | HAAGVLDDGLI | SSYAGTVGGAGQGS YAAANA |
| azuE_module13 | HAAGVLDDGVV | SSVSGTFGGAGQAN YAAAGNA |
| azuF_module16 | HAAGVLDDGVL | SSLAGAIGGAGQGS YAAANA |
| azuG_module17 | HTAGVLDDGVV | SSGAATLGGPGQGS YAAAGNA |
| azuG_module18 | HAAGVLDDGVV | SSASSNFGGGGQAN YAAANA |
| azuH_module19 | HATGVLDDGLF | SSAAGVFGSAGQSN YAAANV |
| azuC_module5 | HAAGVLDDGLL | SSAAGTLGGPGQGS YAAANV |
| azlA_KR | HAAGVLDDGVI | SSVAGVFGSPGQGN YAAANS |
| azlB_KR1 | HAAGVLDDGLL | SSAAGVLGSAGQSN YAAANV |
| azlB_KR2 | HTAGVLDDGVL | SSAAGTLGGPGQGS YAAAGNA |
| azlB_KR3 | HTAGVLDDGVV | SSLSGTLGGTGQAN YAAANA |
| azlC_KR1 | HAAGVLDDGLL | SSAAGTLGGPGQSN YAAANV |
| azlC_KR2 | HTAGVLDDGVL | SSAAGTIGGPGQGS YAAAGNA |
| azlD_KR2 | HAAGVLDDGVL | SSFAGVVGAGQGAY AAANA |
| azlE_KR1 | HAAGILDDGVL | SSFAGAVGSAGQAAY AAANA |
| azlE_KR4 | HAAGVLDDGLI | SSYAGTVGGAGQGS YAAANA |
| azlE_KR5 | HAAGVLDDGVV | SSVSGTFGGAGQAN YAAAGNA |
| azlF_KR2 | HAAGVLDDGVL | SSLAGAIGGAGQGS YAAANA |
| azlG_KR1 | HTAGVLDDGVV | SSGAGTLGGPGQGS YAAAGNA |
| azlG_KR2 | HAAGVLDDGVV | SSASSNFGGGGQAN YAAANA |
| azlH_KR | HATGVLDDGLF | SSAAGAFGAAGQSN YAAANV |
| Ery1_B2 | HAAATLDDGTV | SSFASAFGAPLGGYAGNA |
| Lan1_B2 | HTAATLDDGTL | SSFASAFGAPLGCYAGNA |
| Meg1_B2 | HVAATLDDGTV | SSSTAFAFGAPLGGYVGNNA |
| Pik1_B2 | HTAGALDDGIV | SSVSSTLGIPIGQGN YAHNA |
| Oli14_C1 | HTAGVAGHGPL | SSGAAVWGS GNGANAAAGG |
| Ery3_C2 | HAGTLTNFGSI | SSVAGIWWGAGMAAY AAGSA |
| Lan3_C2 | HAATRTEFGPV | SSVAGVWGGAGMAGY AAGSA |
| Meg3_C2 | HAETLTNFAGV | SSVAGVWGGVGMAY AAGSA |
| Nid4_C2 | HAPPLVPLAPL | SSVSGVWGGAAQGA YAAATA |
| Pik3_C2 | HLPPTVDSEPL | SSVAAIWWGAGQGA YAAAGTA |
| Tyl4_C2 | VAPPVAPPTPL | SSVAGVWGGAGQGGY AAGTA |

Table S5. Determination of ER domain specificity (CLUSTAL multiple sequence alignment by MUSCLE (3.8))

| Module | catalytic region |
|---------------|--|
| OleER4_2S | (38 `)-VNFRDVLLALGMYPD-EGLMGAEAAAGVV-67aa-RGGESVLVHSAAGGVGMAAVQLARHWD |
| MegER4_2S | (38 `)-VNFRDVLLALGMYPE-PAEMGTEASGVV-67aa-QAGQSVLVHAAAGGVGMAAVALARRAG |
| LkmER4_2S | (38 `)-VNFRDVLLALGMYPE-PAEMGTEASGVV-67aa-QAGQSVLVHAAAGGVGMAAVALARRAG |
| EryER4_2S | (38 `)-VNFRDVLLALGMYPQ-KADMGTEAAGVV-67aa-RAGQSVLIHAAAGGVGMAAVALARRAG |
| FKbER6_2S | (38 `)-LNFRDVLIALGTYPG-QGVLGGEEAAGIV-67aa-RPGEKVLIIHAAATGGVGSAAARQIARHLG |
| FKbER7_2S | (38 `)-LNFRDVLIALGTYDG-ATALGGEEAAGVV-67aa-RAGEKVLVHAAATGGVAMAATQVARHLQ |
| azlA_ER_2S | (38 `)-LNFRDALIAIGMYPDDHATMGEGAGVV-67aa-QAGESILVHTATGGVGMMAAVQLARHLG |
| azlE_ER_2S | (38 `)-LNFRDVLNALGMYPGEAGPLGGEGAGVV-67aa-KKGQSVLVHSAAGGVGMATLQLARHFG |
| azuA_module1 | (38 `)-LNFRDALIAIGMYPEDDATMGEGAGVV-67aa-RAGESILVHTATGGVGMMAAVQLARHLG |
| azuE_module14 | (38 `)-LNFRDVLNALGMYPGEAGPLGGEGAGVV-67aa-KAGQSVLVHSAAGGVGMATLQLARHLG |
| GdmER1_2R | (38 `)-QNFRDVLVALGGVAG-QEGLGGEGAGVV-67aa-QPGETVLVHAAAGGVGMAAVQLARHFG |
| HbmER1_2R | (38 `)-QNFRDVLVALGGVAG-QEGLGGEGAGVV-67aa-QPGETVLVHAAAGGVGMAAVQLARHFG |
| NigER8_2R | (38 `)-VNFRDVLVGLMYPG-QTGLGGEGAGVV-67aa-RPGESVLIIHAAATGGVGTAAVRIARHLG |
| FKbER9_2R | (38 `)-LNFRDDTVALGVVAD-DRPLGSEAAAGVV-67aa-RPGEKVLIIHAAATGGVAAAQIARHLD |
| RapER13_2R | (38 `)-LNFRDVVALGMVND-NRPTGGEEAAGVV-67aa-SEGESVLIIHAAAGGVGMAATQIARHLG |
| RapER1_2R | (38 `)-LNFRDVVALGMVDD-KRLAGGEAAGVV-67aa-SAGESVLIIHAAAGGVGMAATQIARHLG |
| | **** .:* * *.:*: * :.:*:*: *: * : :**. |

The sequences from this study (*azu*) are highlighted in blue, and the sequences of the most closely related homologs are highlighted in red (*azl*). The LDD consensus regions are highlighted in yellow and PN and W consensus regions are highlighted in green and blue respectively.

The PKSs and sequence accession numbers are: DEBS, erythromycin, L07626, X62569; OLE, oleandomycin, AF220951; PIK, pikromycin, AF079138; TYL, tylosin, U78289; NID, niddamycin, AF016585; RAPS, rapamycin, X86780; FK506, AF082100, y10438; FK520, AF235504; AVE, avermectin, AB032367; RIF, rifamycin, AF040570; SOR, soraphen, U24241; EPO, epothilone, AF217189; MXA, myxalamid, AF319998; MTA, mxyathiazole, AF188287; PIM, pimaricin, J278573; NYS, nystatin, AF263912; AMPH, amphotericin, AF357202, VinP3, vicenistatin, BAD08359; as well as:

azl = *Streptomyces* sp. 211726 (azalomycin F3a);

npm = *Streptomyces* sp. IMB7-145 (niphimycins C-E)

Table S6. Antifungal activity of compounds **1-4**.

| compd. | <i>M. pachydermatis</i> CBS 1879 | <i>C. albicans</i> SC5314 | <i>C. neoformans</i> H99 |
|----------|-------------------------------------|------------------------------|-----------------------------|
| | ($\mu\text{g/mL}$) | ($\mu\text{g/mL}$) | ($\mu\text{g/mL}$) |
| 1 | > 250 | 7.81 | 3.91 |
| 2 | > 250 | > 250 | 31.25 |
| 3 | > 250 | 3.91 | 1.95 |
| 4 | > 250 | 7.81 | 3.91 |
| KTZ | 0.125 | n.d. | n.d. |
| FLZ | n.d. | 0.5 | 4.0 |

n.d – not determined.

Table S7. Antiproliferative and cytotoxicity activity of compounds **1-4**.

| compd. | <i>Antiproliferative effect (GI_{50})</i> | | <i>Cytotoxicity (CC_{50})</i> |
|-------------|--|------------------------------------|--|
| | HUVEC | K-562 | HeLa |
| | ($\mu\text{g/mL}$) | ($\mu\text{g/mL}$) | ($\mu\text{g/mL}$) |
| 1 | 39.0 (\pm 0.6) | 32.8 (\pm 1.5) | 40.5 (\pm 0.05) |
| 2 | 10.4 (\pm 0.3) | 11.9 (\pm 0.2) | 12.7 (\pm 0.2) |
| 3 | 9.3 (\pm 0.2) | 10.1 (\pm 0.7) | 13.4 (\pm 0.3) |
| 4 | 13.1 (\pm 0.5) | 15.6 (\pm 0.9) | 11.2 (\pm 0.3) |
| imatinib | 10.9 (\pm 1.2) | 0.1 (\pm 6.7×10^{-3}) | 38.8 (\pm 1.4) |
| doxorubicin | 0.1 | 1.0 (\pm 0.6) | 2.0 (\pm 0.8) |

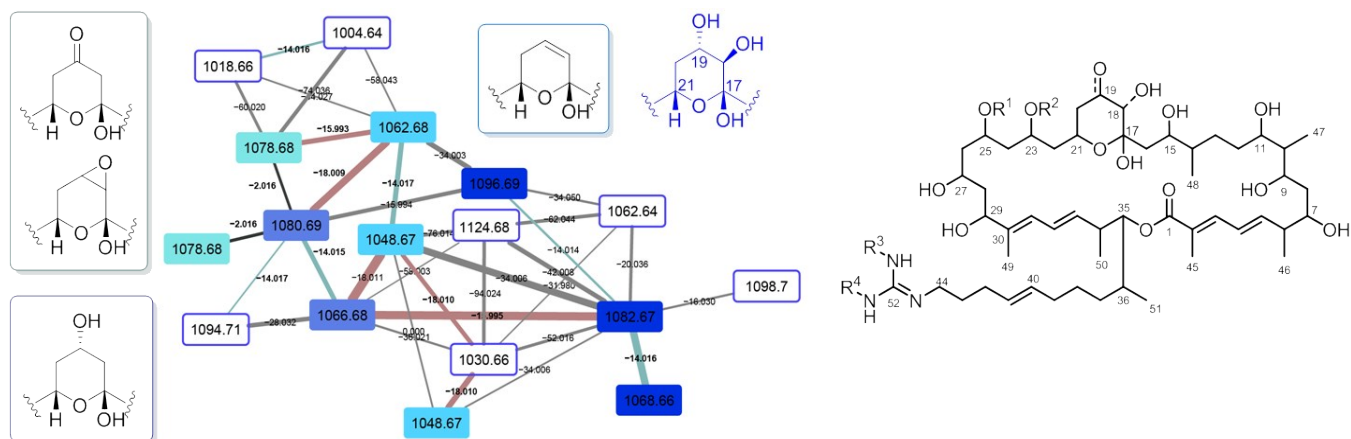


Figure S1. HRMS²-based GNPS analysis depicting molecular ion cluster putatively assigned as an azalomycin cluster with putative structural features assigned to *m/z* 1096.69, 1082.67 and 1068.66 (diol moiety C-18 and C-19); *m/z* 1080.69 and 1066.68 (C-19 alcohol); *m/z* 1062.68 and 1048.67 (enoyl derivatives); *m/z* 1078.68 (C-19/C-18 epoxy). Data obtained from HRMS² measurements of EtOAc extract (7 d, ISP2 liquid broth) in positive mode ESI-HRMS.

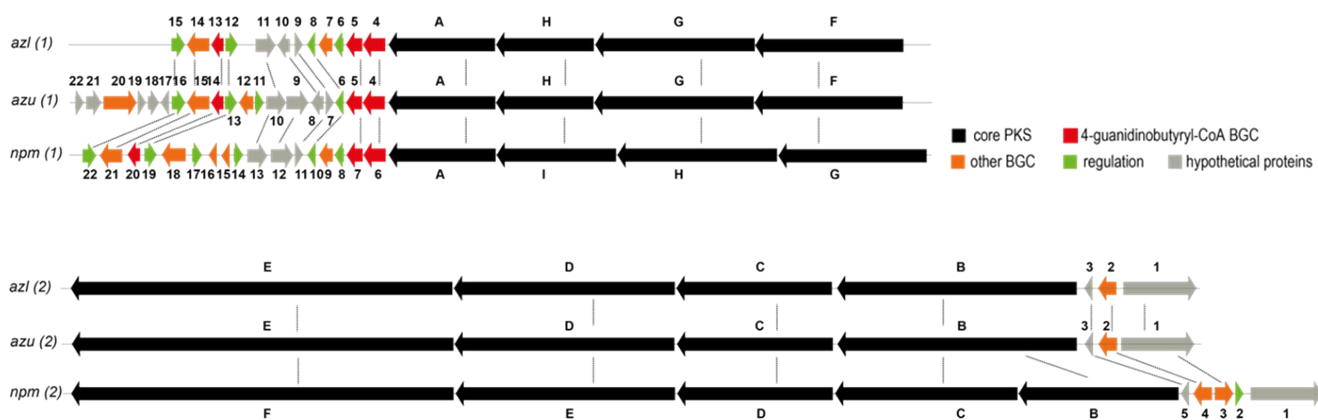


Figure S2. Graphical comparison of azalomycins biosynthesis gene cluster (*azu*) from *Streptomyces* sp. M56 and azalomycin F_{3a} biosynthesis gene cluster (*azl*) from *Streptomyces* sp. 211726, and niphimycin C biosynthesis gene cluster (*npm*) from *Streptomyces* sp. IMB7-145.

Figure S3. Sequence alignment of arginine monooxygenase. AUA13754 and AUA09953: tryptophan 2-monooxygenase from M56; ORF_6127: an arginine monooxygenase from *Streptomyces* sp. IMB7-145; AAX98202: arginine monooxygenase in ECO-02301 biosynthetic gene cluster from *S. aizunensis*; AEM87306: amine oxidase from azalomycin F from *S. violaceusniger* Tu 4113; AQW50864: amine oxidase from *S. hygrosopicus*; KUL53773: amino oxidase from *S. violaceusniger*; SEB92316: tryptophane 2-monooxygenase from *S. melanosporofaciens*.

```

AAX98202      MTSFSPAPTTMLVPDFPFPSYDGWLRHPAGLGALPPERAGTPVAVVGGGMAGMTAAAYELMR
AUA13754      MTCATASATTMLVPDFPFPSYDRWLSHPAGLGSLPPAMHGTEVAVIGGGMSGLTAAAYELLR
AQW50864      MTCATASATTMLVPDFPFPSYDRWLSHAAGLGALPAAMHGTEVAVIGGGMSGLTAAAYELLR
AEM87306      MTCATASATTMLVPDFPFPSYDRWLSHPAGLGSLPAAMHGTEVAVIGGGMSGLTAAHELLR
AUA09953      MTCATASATTMLVPDFPFPSYDRWLSHPAGLGALPAAVHGTEVAVIGGGMSGLTAAAYELLR
ORF_6127      MTCATASATTMLVPDFPFPSYDRWLSHPAGLGALPAAVHGTEVAVIGGGMSGLTAAAYELLR
KUL53773      MTCATASATTMLVPDFPFPSYDRWLSHPAGLGALPAAAHGTEVAVIGGGMSGLTAAAYELLR
SEB92316      MTCATASATTMLVPDFPFPSYDRWLSHPAGLGALPAAAHGTEVAVIGGGMSGLTAAAYELLR
** . :.: .*****:*** ** *.****:* . ** ***:****:*:****:*:*

AAX98202      LGLRPVVEAEQLGGRMRSPVFPQPGLVAEMGAMRFPVSARSLEFHYIDLGLRTPSPFPN
AUA13754      LGLSPVLYEAEQLGGRMRSLPFPNPEYKAEMGAMRFPVIAARSLFHYIDLGLPTRPFPN
AQW50864      LGLSPVLYEAEQLGGRMRSTPFPNPEYKAEMGAMRFPVSARSLEFHYIDLGLSTRPFPN
AEM87306      LGLSPVLYEAEQLGGRMRSTPFPNPEYKAEMGAMRFPVSARSLEFHYIDLGLSTHPFPN
AUA09953      LGLSPVLYEAEQLGGRMRSTPFPNPEYKAEMGAMRFPVSARSLEFHYIDLGLSTRPFPN
ORF_6127      LGLSPVLYEAEQLGGRMRSTPFPNPEYKAEMGAMRFPVSARSLEFHYIDLGLSTRPFPN
KUL53773      LGLSPVLYEAEQLGGRMRSTPFPNPEYKAEMGAMRFPVSARSLEFHYIDLGLSTRPFPN
SEB92316      LGLSPVLYEAEQLGGRMRSTPFPNPEYKAEMGAMRFPVSARSLEFHYIDLGLSTRPFPN
** : ** :***** *****:* *****:*****:***** * *****

AAX98202      PLAANTPSTLIDLGGERTARSAEQLPDVYQEVASAWEKALQERAEATMRDAIQRRDVE
AUA13754      PLAPGTPSTLIDLNGDRDRARTTEELPGVYQEVADAWDKALQERADLATLRDAIQRRDVT
AQW50864      PLAPATASTLIDLNGGQDRVRTAAELPGVYQEVADAWDKALQERADLATLRDAIQRRDVT
AEM87306      PLAPATASTLIDLNGGQDRARTADELPGVYQEVADAWDKALQERADLATLRDAIQRRDAT
AUA09953      PLAPATASTLIDLNGGQDRARTASELPDVYQEVADAWDKALQERADLATLRDAIQRRDVS
ORF_6127      PLAPATASTLIDLNGGQDRARTASELPDVYQEVADAWDKALQERADLATLRDAIQRRDVS
KUL53773      PLAPATTSTLIDLNGGQDRARTAGELPDVYQEVADAWDKALQERADLATLRDAIQRRDVS
SEB92316      PLAPATASTLIDLNGGQDRARTAGELPDVYQEVADAWDKALQERADLATLRDAIQRRDVS
*** . *.*****.* . .*: :*.*****.*:*****:*****:*****.*

AAX98202      TVKQIWNRLVREFDDQSFYGFLLATSSAFQSFHRHREIFGQVGFGTGGWDTDFPNSVLEILR
AUA13754      TLKTIWNNSLVKEFDDQSFYGFLLATSPTFQSFHRHREIFGQVGFGTGGWDTDFPNSVLEILR
AQW50864      TLKTIWNNSLVKEFDDQSFYGFLLATSSTFQSFHRHREIFGQVGFGTGGWDTDFPNSVLEILR
AEM87306      TLKTIWNNSLVKEFDDQSFYGFLLATSSAFQSFHRHREIFGQVGFGTGGWDTDFPNSVLEILR
AUA09953      TLKTIWNNSLVREFDDQSFYGFLLATSSAFQSFHRHREIFGQVGFGTGGWDTDFPNSVLEILR
ORF_6127      TLKTIWNNSLVREFDDQSFYGFLLATSSAFQSFHRHREIFGQVGFGTGGWDTDFPNSVLEILR
KUL53773      TLKTIWNNSLVREFDDQSFYGFLLATSSAFQSFHRHREIFGQVGFGTGGWDTDFPNSVLEILR
SEB92316      TLKTIWNNSLVREFDDQSFYGFLLATSSAFQSFHRHREIFGQVGFGTGGWDTDFPNSVLEILR
*: * :** ** .*****:*****:*****:*****:*****:*****

AAX98202      VVYTEADDNQVAIDGGSSQVPRGLWEHRPRGCAHWPAGTSLASLHGGTARPRVRAVARDG
AUA13754      VVVTEADDHQVIGVGGSSQVPNGLWEHRPETLAHWPRGTSLSLHGGRRPRAVTRLRRTA
AQW50864      VVVTEADDNQVIGVGGSSQVPNGLWEHQPETLAHWPRGTSLSLHGGRRPRAVTRLRRTA
AEM87306      VVVTEADDNQVIGVGGSSQVPNGLWEHQPETLAHWPRGTSLSLHGGRRPRAVTRLRRTA
AUA09953      VVVTEADDNQVIGVGGSSQVPNGLWGHQPETLAHWPRGTSLSLHGGRRPRAVTRLRRTA
ORF_6127      VVVTEADDNQVIGVGGSSQVPNGLWGHQPETLAHWPRGTSLSLHGGRRPRAVTRLRRTA
KUL53773      VVVTEADDNQVIGVGGSSQVPNGLWGHQPETLAHWPRGTSLSLHGGRRPRAVTRLRRTA
SEB92316      VVVTEADDNQVIGVGGSSQVPNGLWEHQPETLAHWPRGTSLSLHGGRRPRAVTRLRRTA
** *****:***.* ** .***.* ** *. * ***** *****:***** .** * : * .

AAX98202      DGFLVTDADGHRERFASVVYTPHVWTLNLRVACDPALLTQPLWTAVERTHYMGASKLFVL
AUA13754      DGVRVTDESGEEREFPAVIFTPHVWTLNLRIDCDPALLSTPLWTAVERTHYMGASKLFVL
AQW50864      DGVRVTDESGEEREFPAVIYSPHLWTLNLRVDCDPDLLSTPLWTAVERTHYMGASKLFVL
AEM87306      DGVRVTDESGEEREFPAVVYSPHVWTLNLRVDCDPDLLSTPLWTAVERTHYMGASKLFVL
AUA09953      DGVRVTDESGEEREFPAVVYSPHVWTLNLRVDCDPDLLSTPLWTAVERTHYMGASKLFVL
ORF_6127      DGVRVTDESGEEREFPAVVYSPHVWTLNLRVDCDPDLLSTPLWTAVERTHYMGASKLFVL

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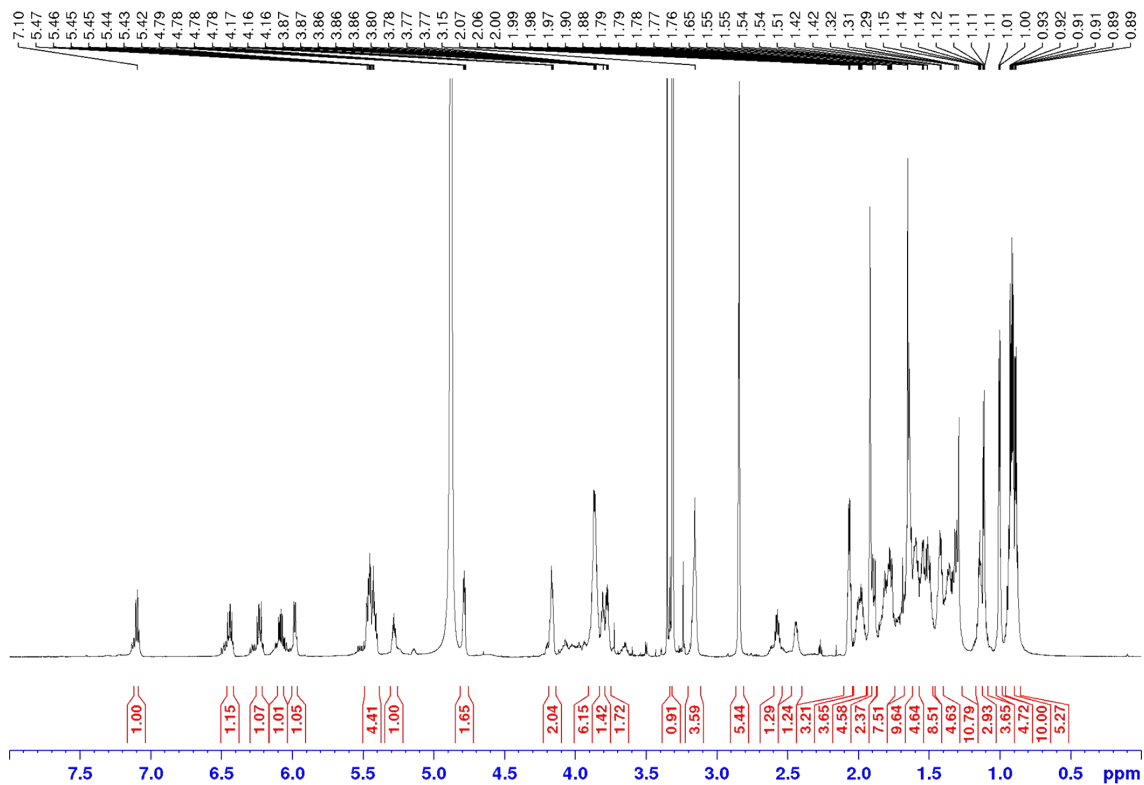



Figure S4. ^1H NMR spectrum of azalomycin F_{4b} (**1**) (CD_3OD , 300K, 800 MHz).

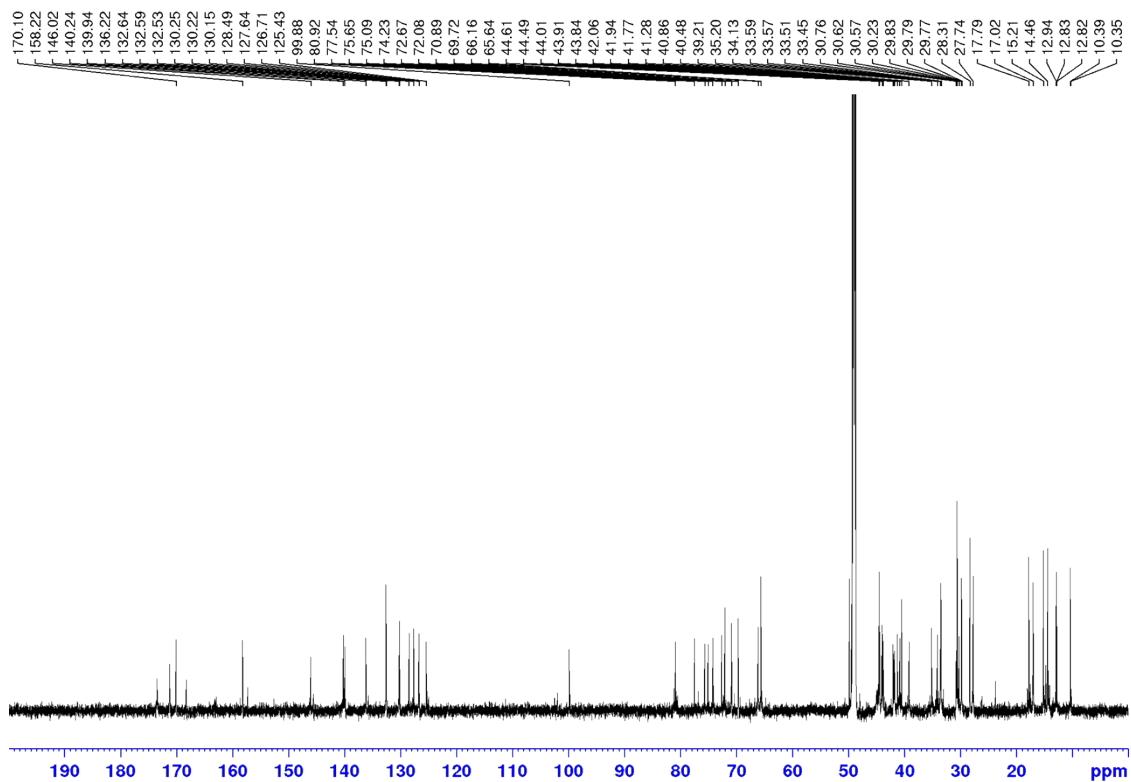


Figure S5. ^{13}C NMR spectrum of azalomycin F_{4b} (**1**) (CD_3OD , 300K, 200 MHz).

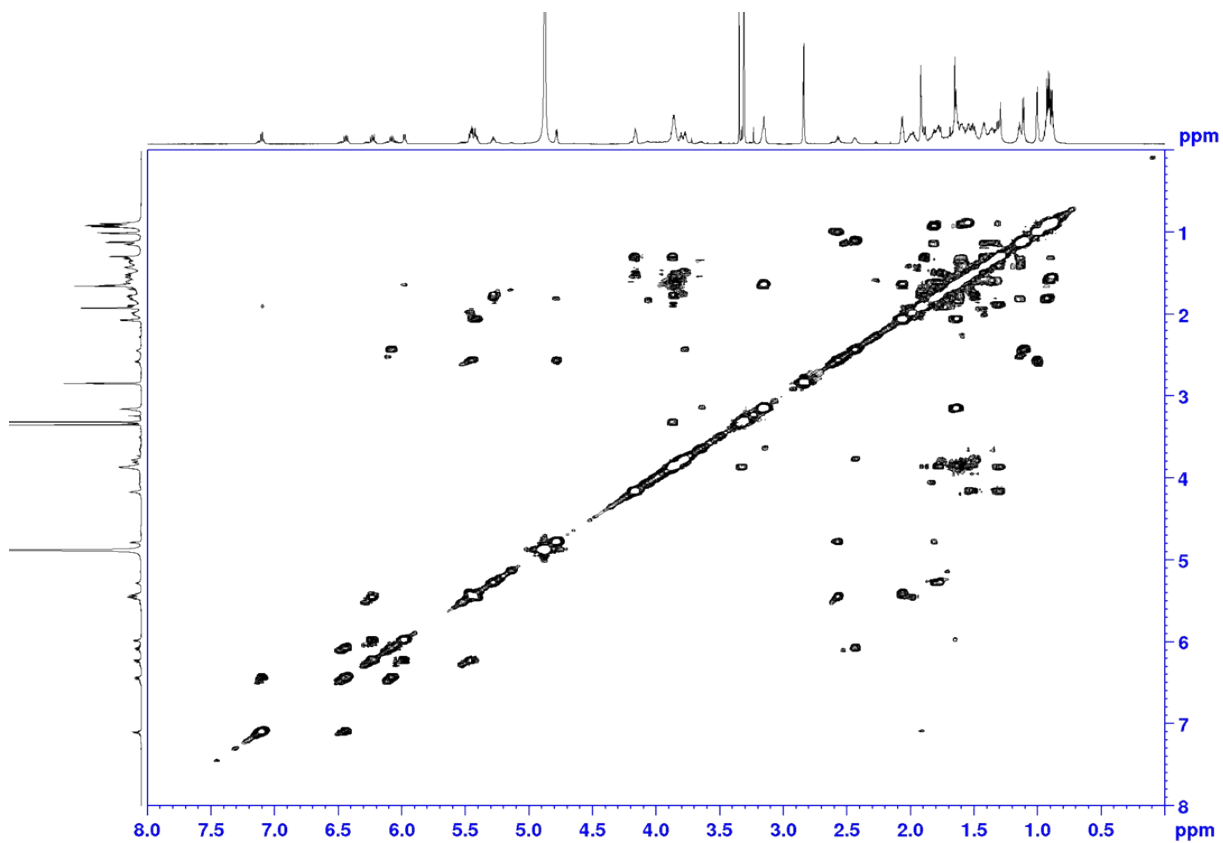


Figure S6. ^1H - ^1H COSY spectrum of azalomycin F_{4b} (**1**) (CD_3OD , 300K, 800 MHz).

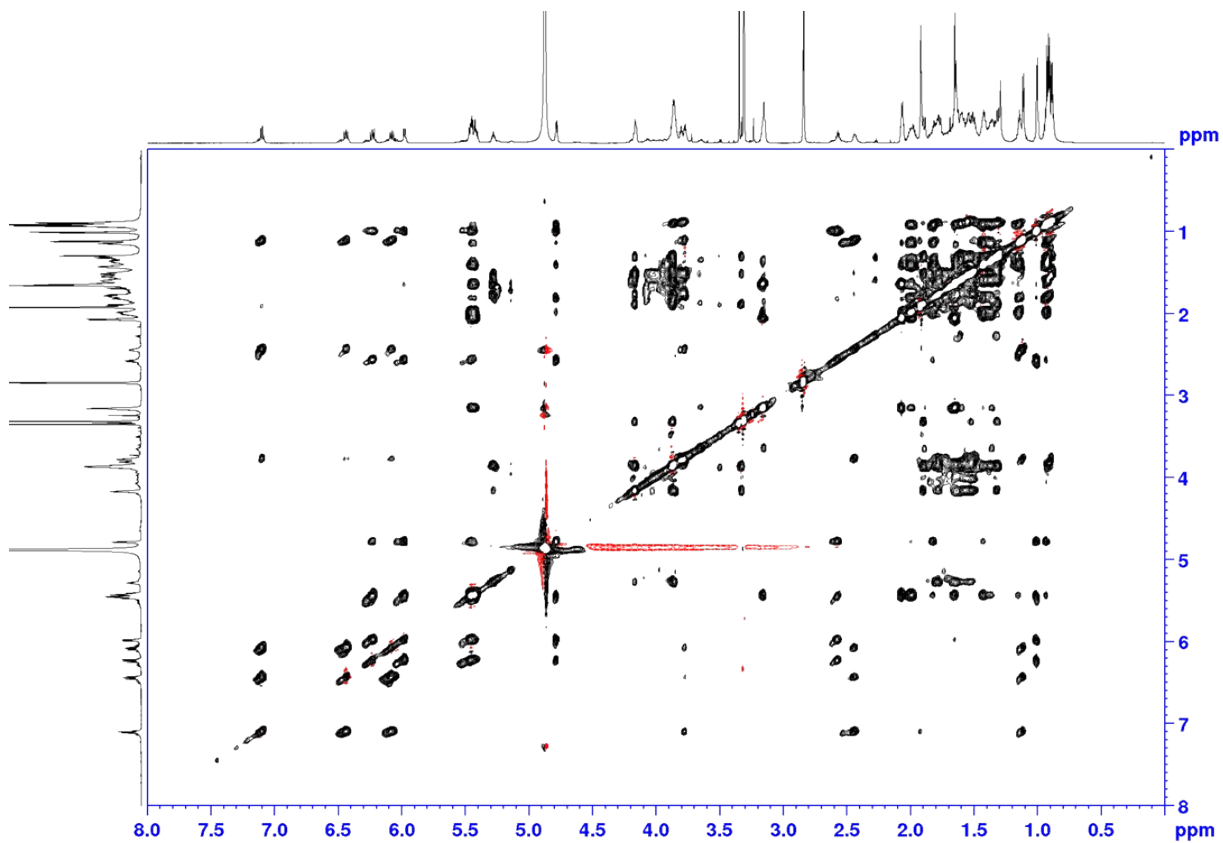


Figure S7. ^1H - ^1H TOCSY spectrum of azalomycin F_{4b} (**1**) (CD_3OD , 300K, 800 MHz).

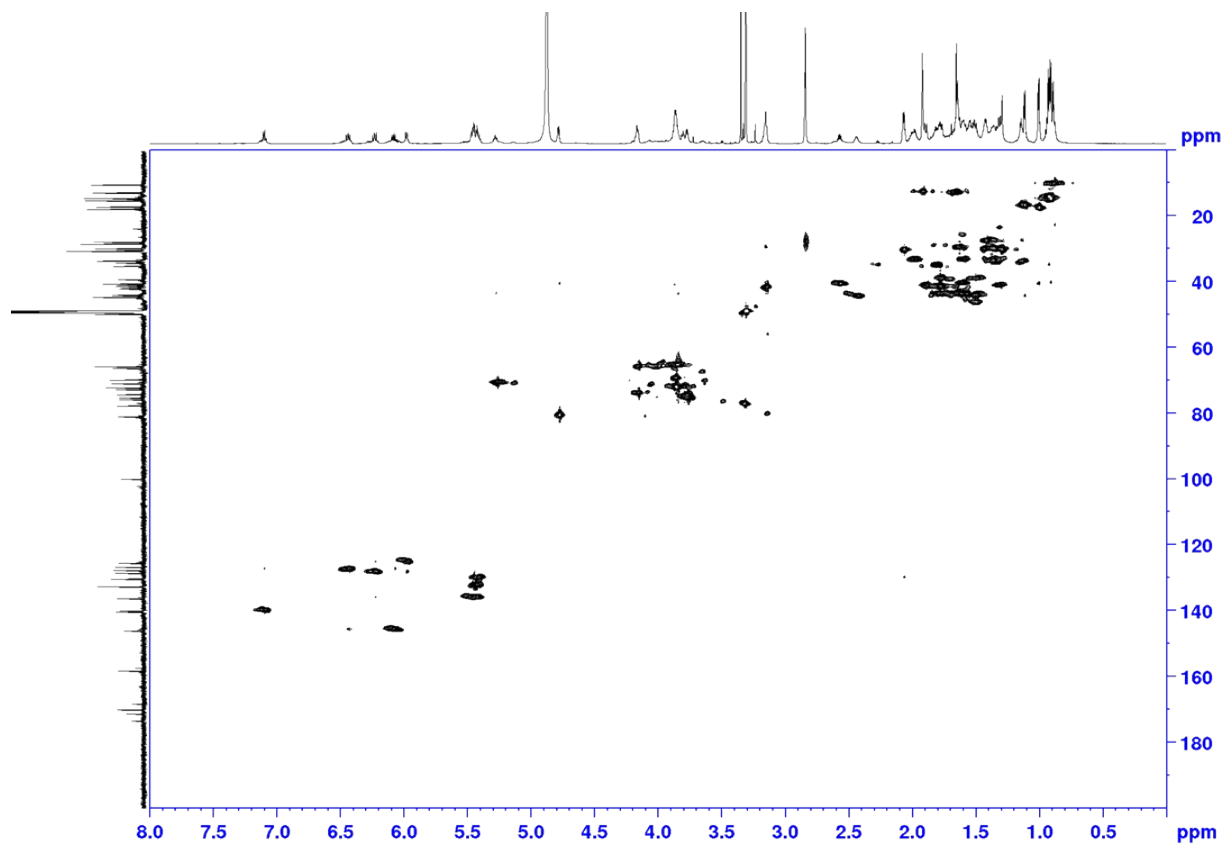


Figure S8. HSQC spectrum of azalomycin F_{4b} (**1**) (CD₃OD, 300K, 800 MHz).

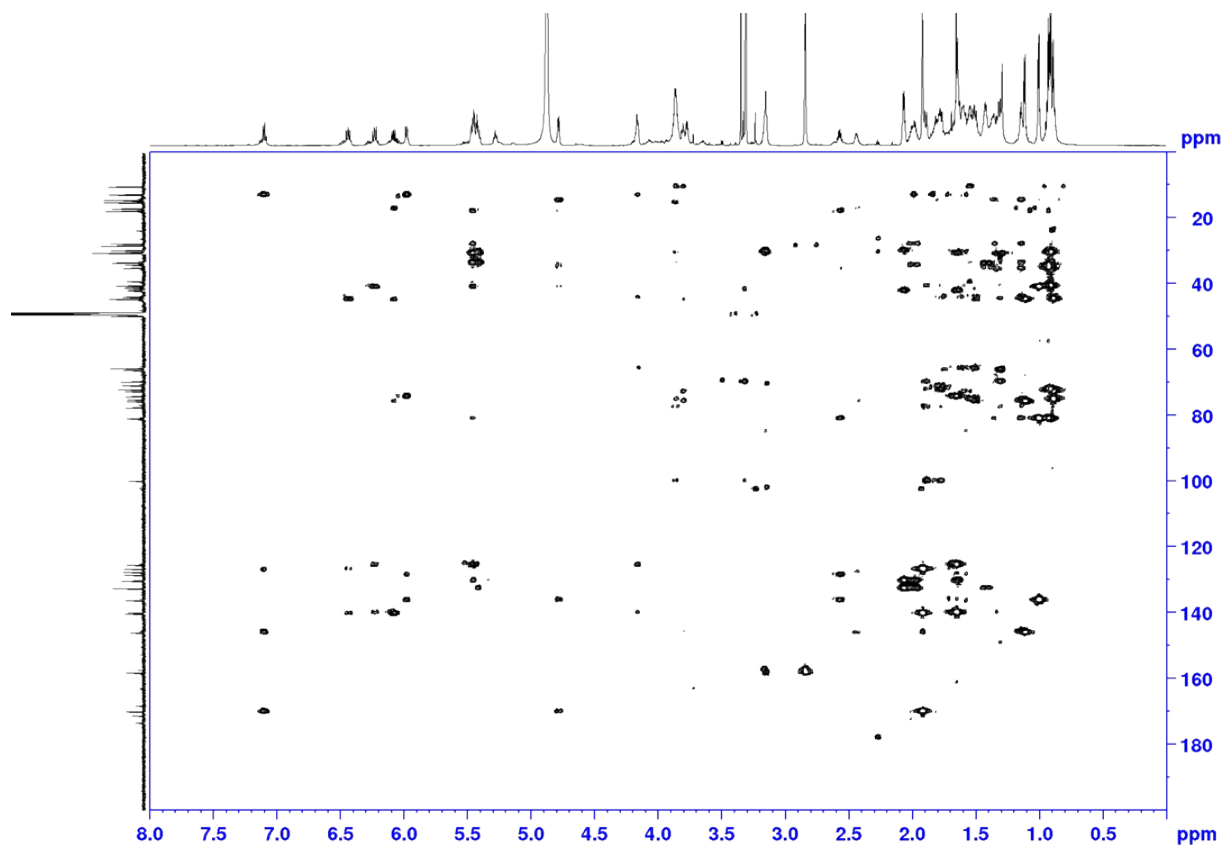


Figure S9. HMBC spectrum of azalomycin F_{4b} (**1**) (CD₃OD, 300K, 800 MHz).

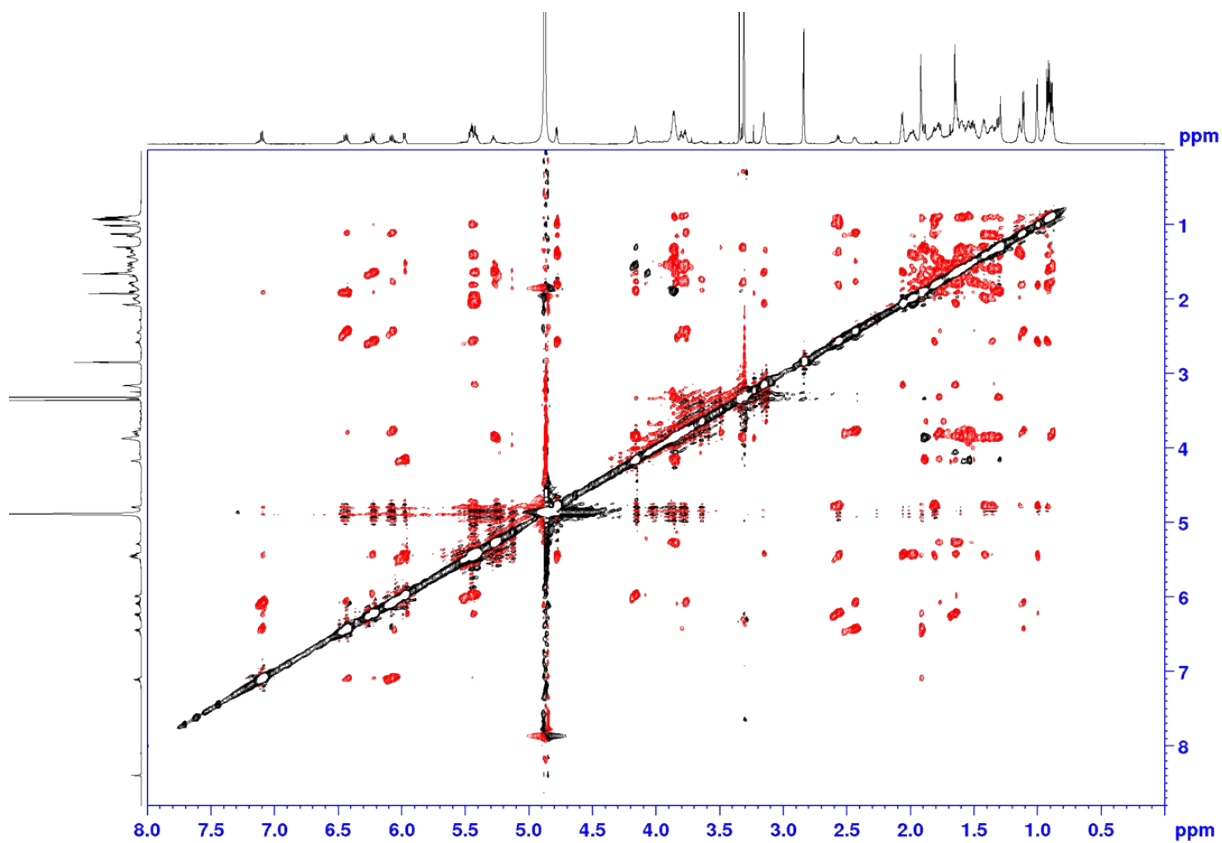


Figure S10. ROESY spectrum of azalomycin F_{4b} (**1**) (CD₃OD, 300K, 800 MHz).

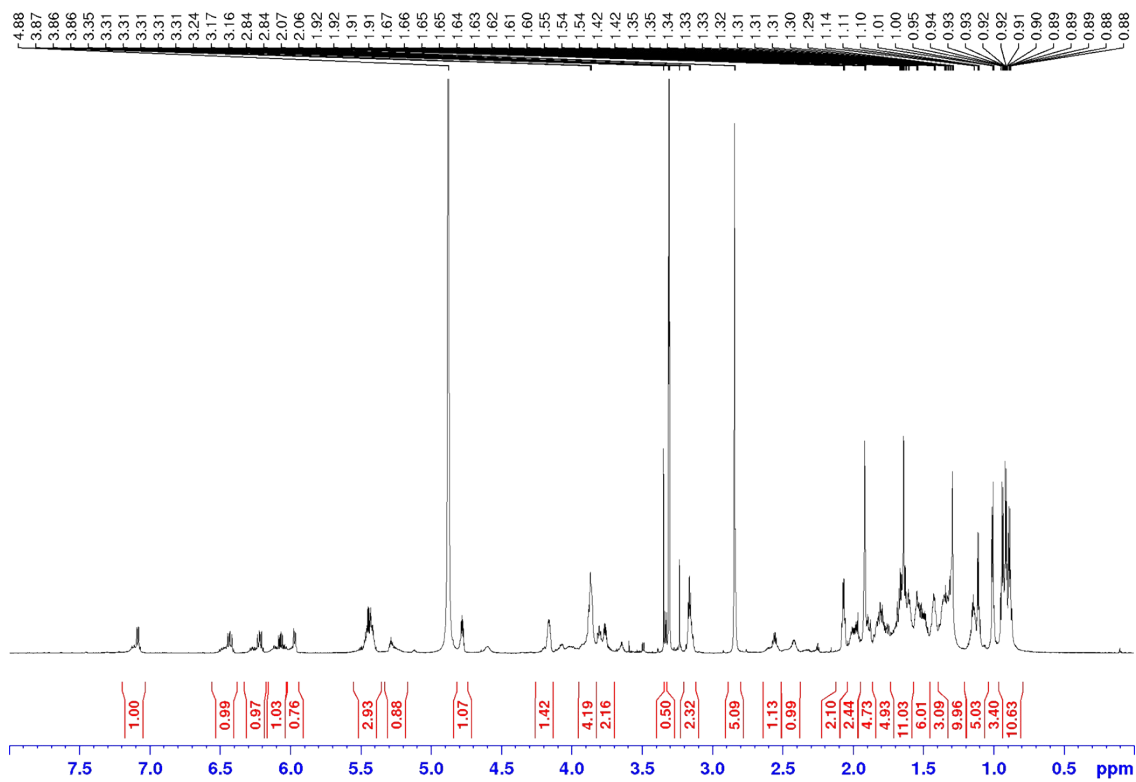


Figure S11. ¹H NMR spectrum of azalomycin F_{5b} (**2**) (CD₃OD, 300K, 800 MHz).

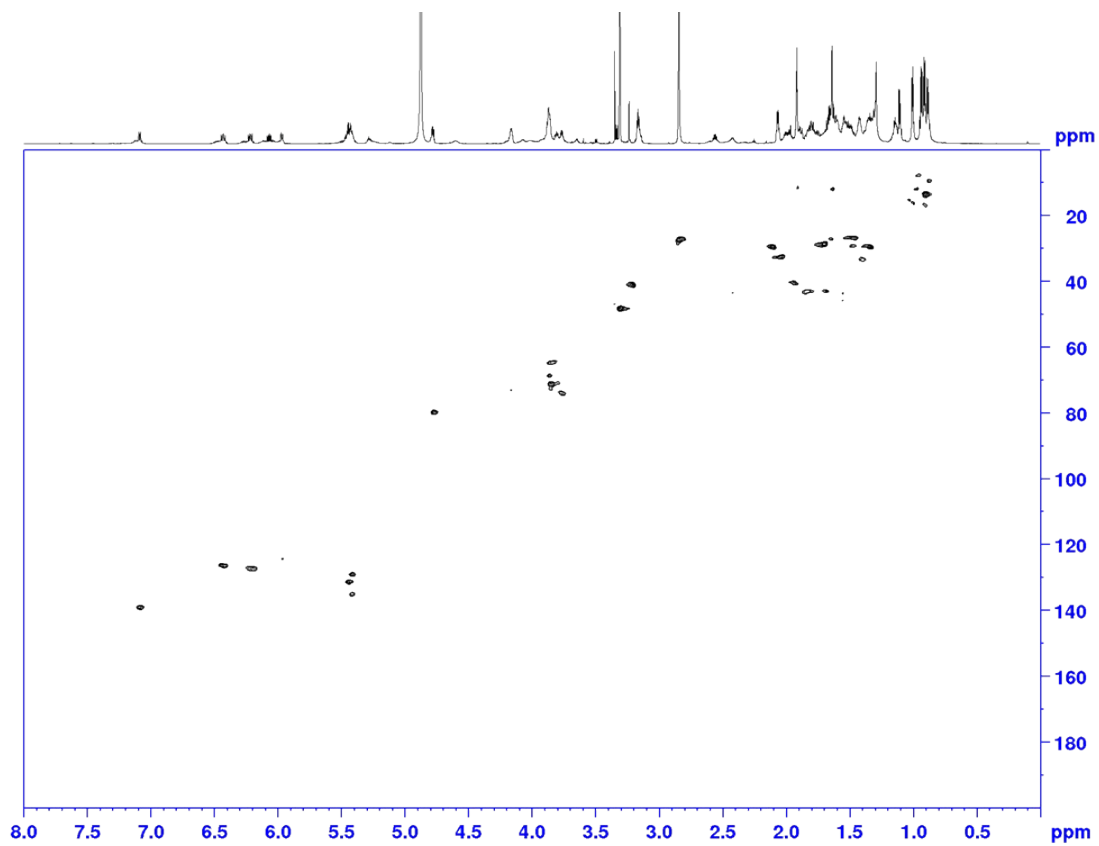


Figure S12. HSQC spectrum of azalomycin F_{5b} (**2**) (CD₃OD, 300K, 800 MHz).

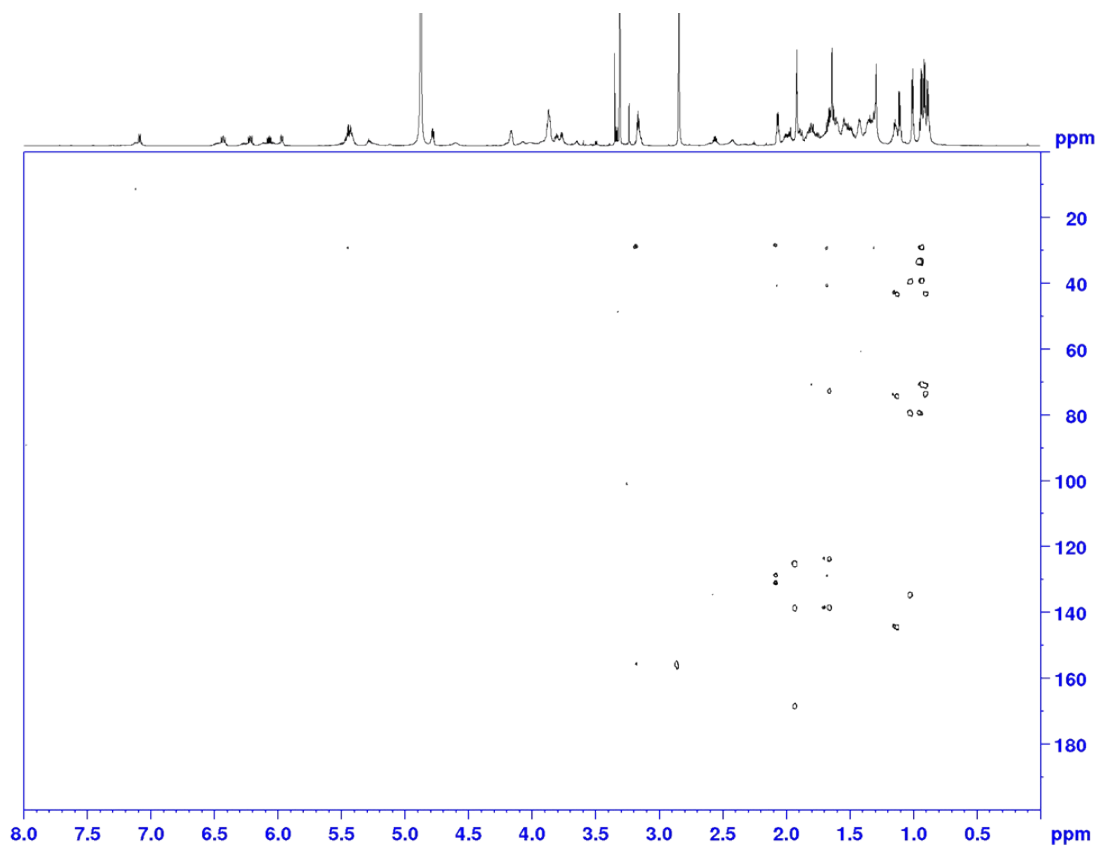


Figure S13. HMBC spectrum of azalomycin F_{5b} (**2**) (CD₃OD, 300K, 800 MHz).

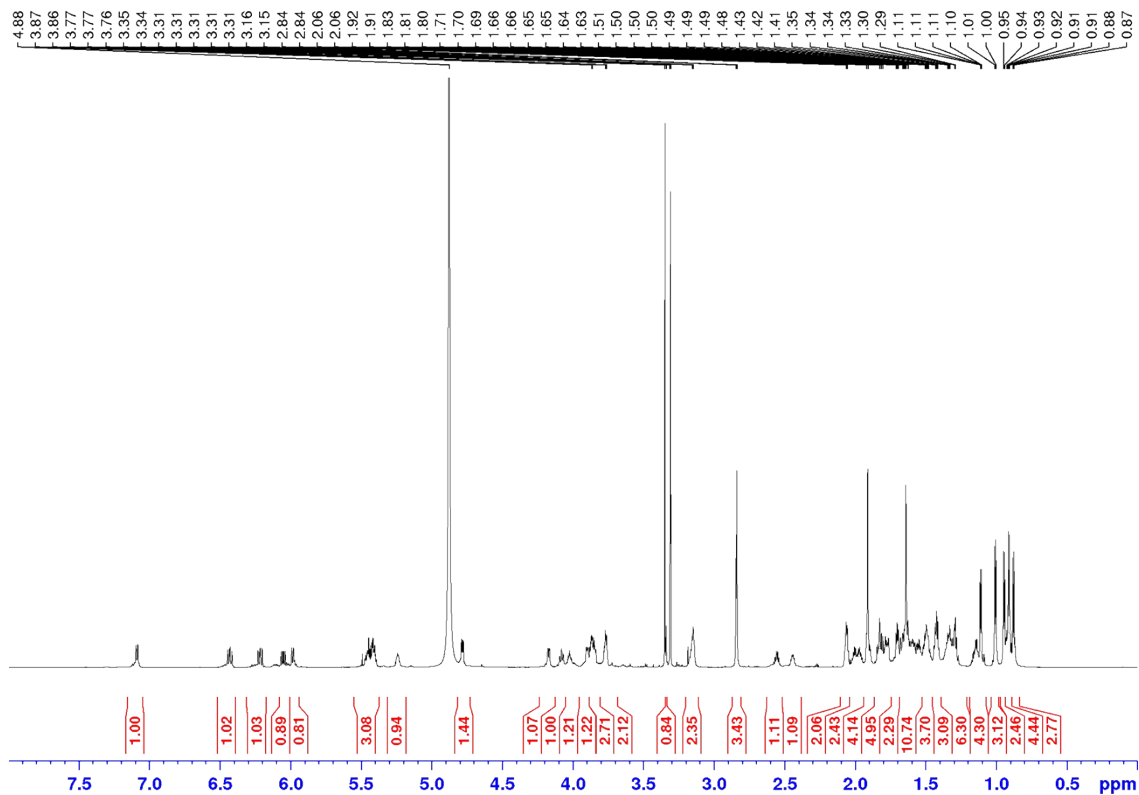


Figure S14. ^1H NMR spectrum of azalomycin F_{4a} (**3**) (CD_3OD , 300K, 800 MHz).

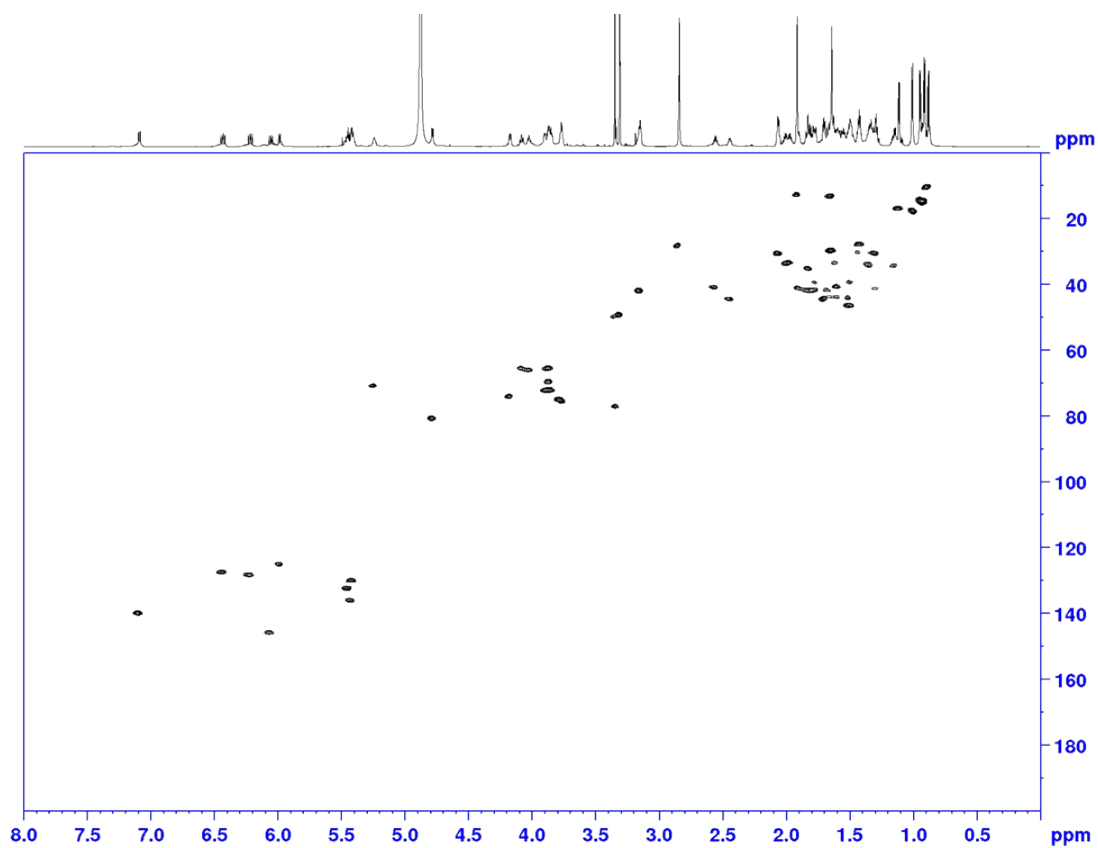


Figure S15. HSQC spectrum of azalomycin F_{4a} (**3**) (CD_3OD , 300K, 800 MHz).

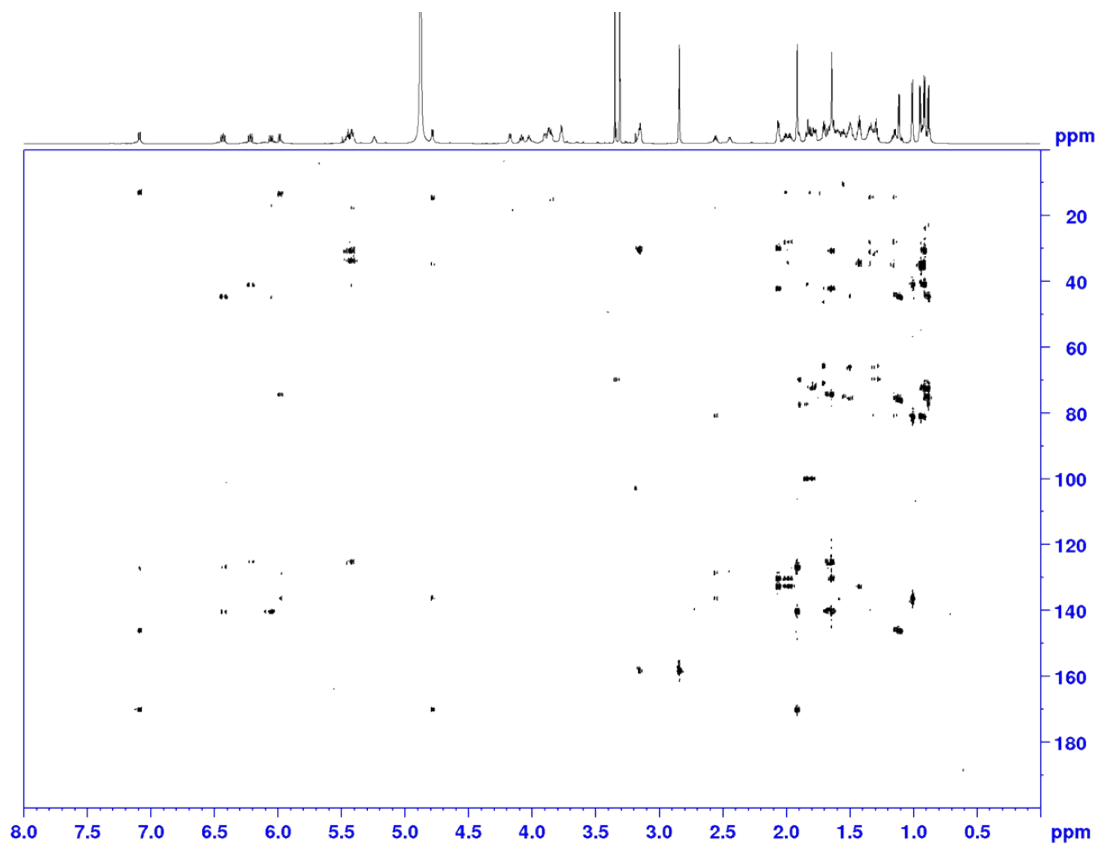


Figure S16. HMBC spectrum of azalomycin F_{4a} (**3**) (CD₃OD, 300K, 800 MHz).

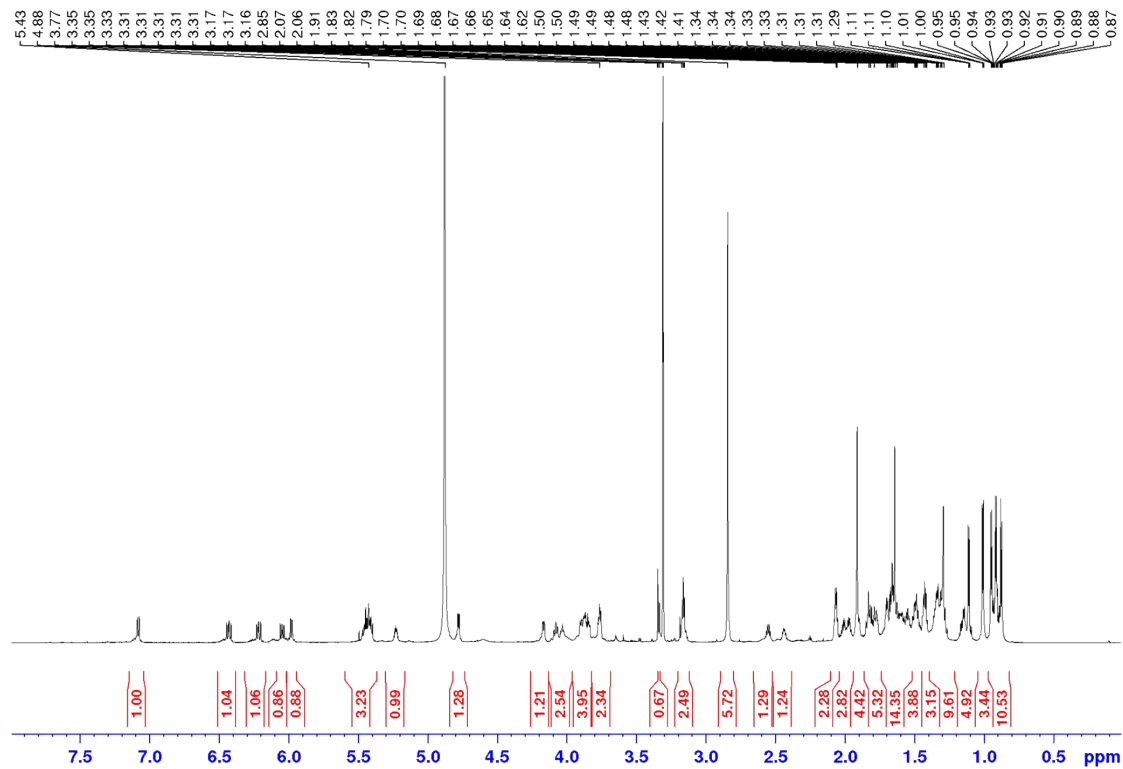


Figure S17. ¹H NMR spectrum of azalomycin F_{5a} (**4**) (CD₃OD, 300K, 800 MHz).

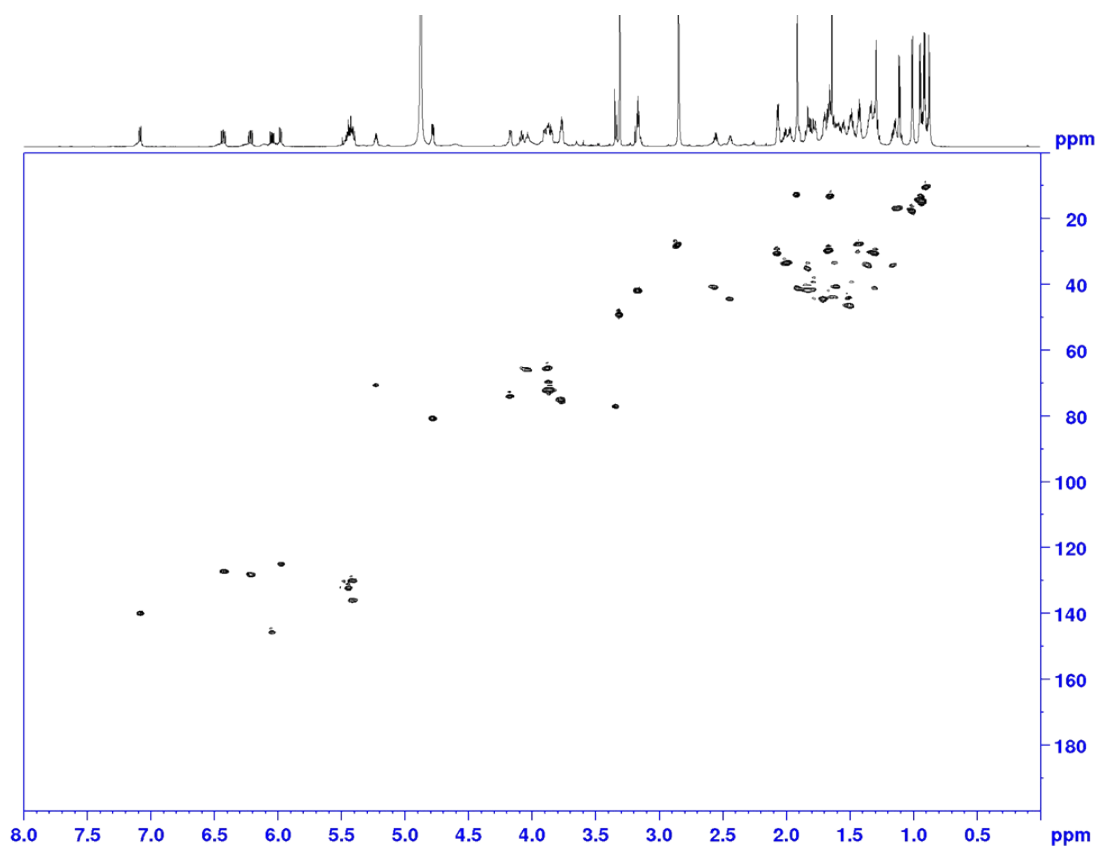


Figure S18. HSQC spectrum of azalomycin F_{5a} (**4**) (CD₃OD, 300K, 800 MHz).

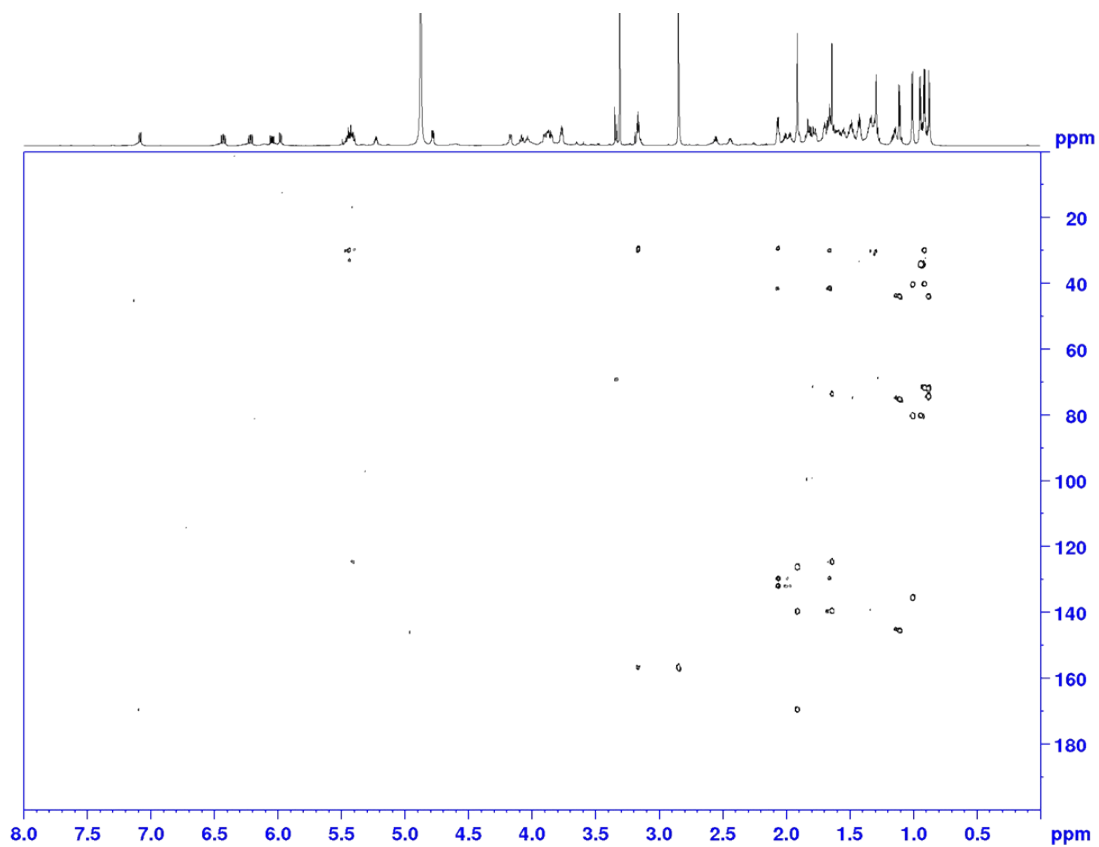


Figure S19. HMBC spectrum of azalomycin F_{5a} (**4**) (CD₃OD, 300K, 800 MHz).

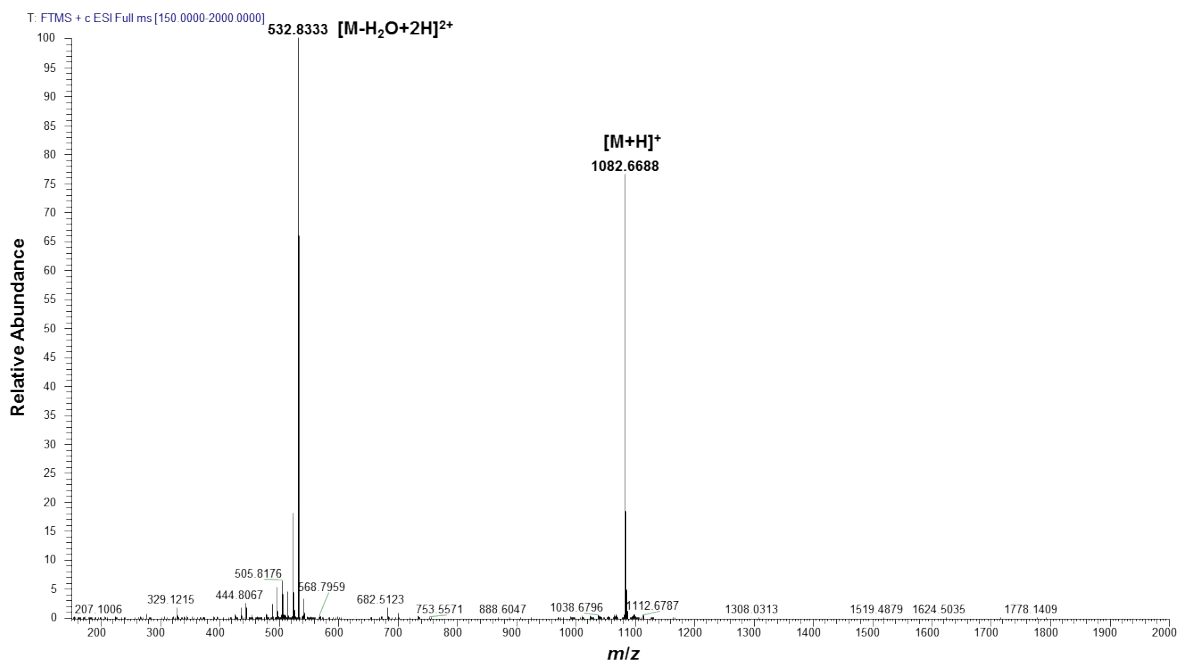


Figure S20. ESI-HRMS (+) spectrum of azalomycin F_{4b} (**1**).

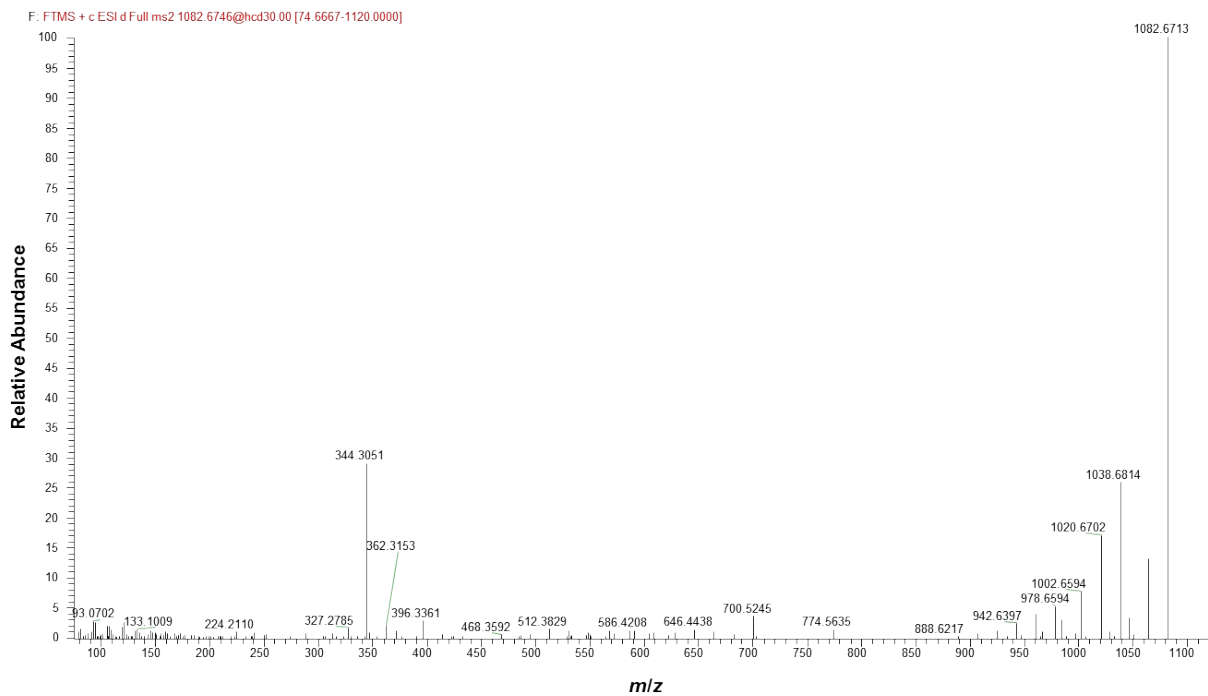


Figure S21. ESI-HRMS² (+) spectrum of azalomycin F_{4b} (**1**).

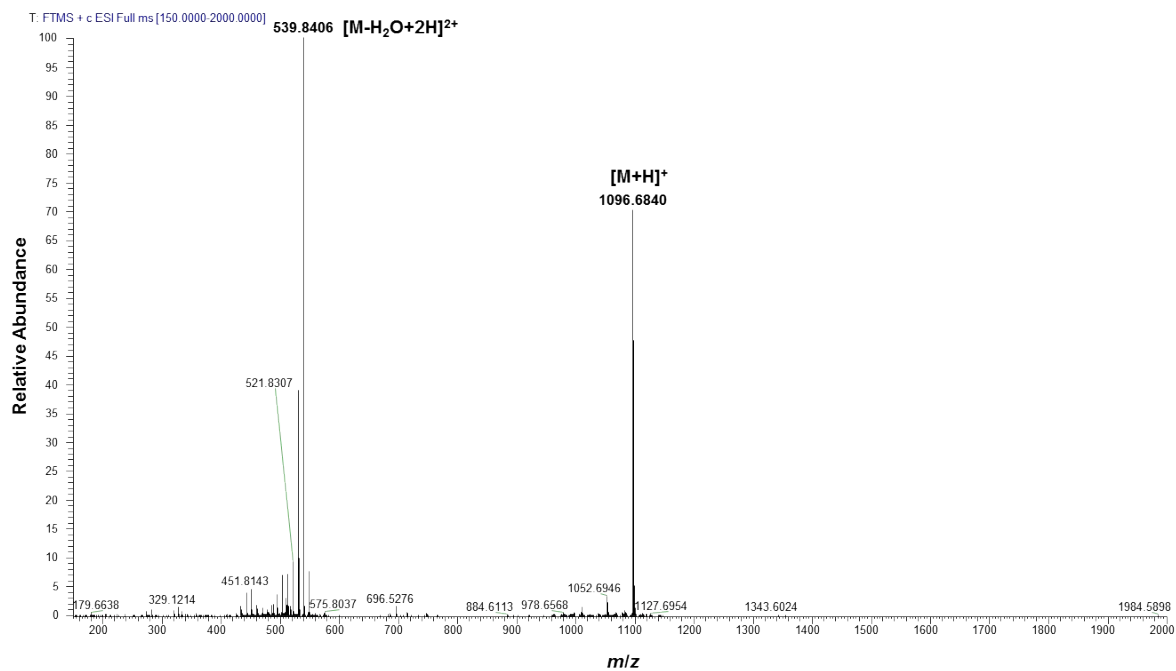


Figure S22. ESI-HRMS (+) spectrum of azalomycin F_{5b} (2).

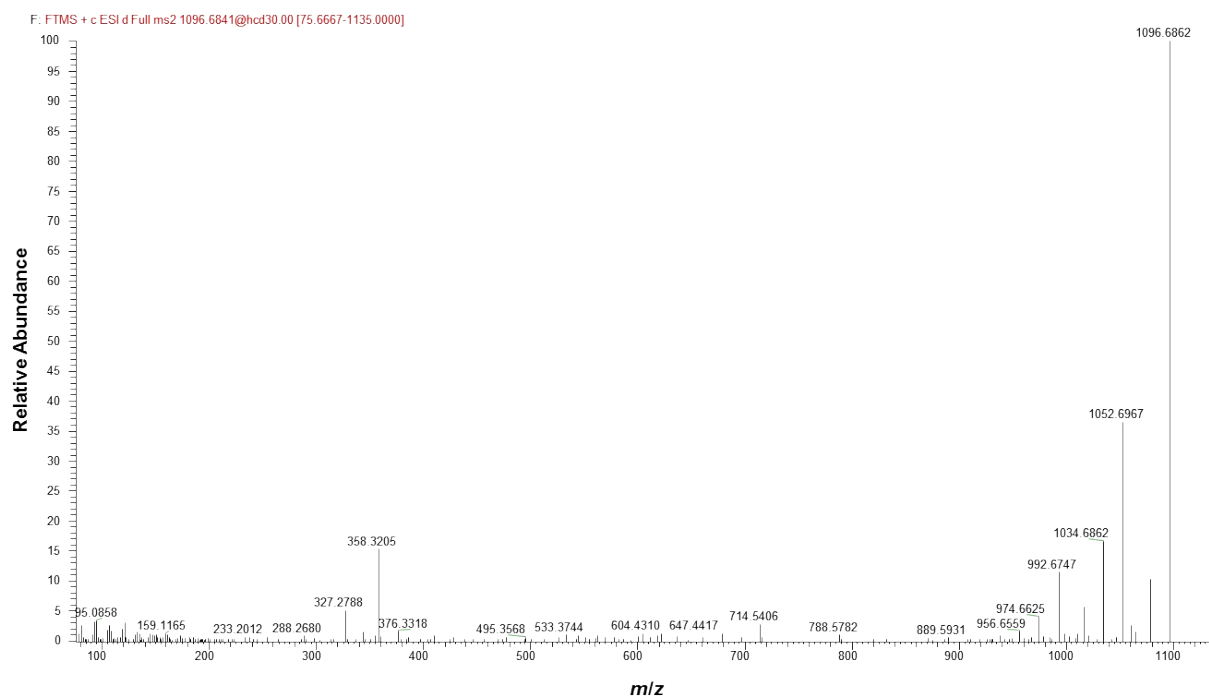


Figure S23. ESI-HRMS² (+) spectrum of azalomycin F_{5b} (2).

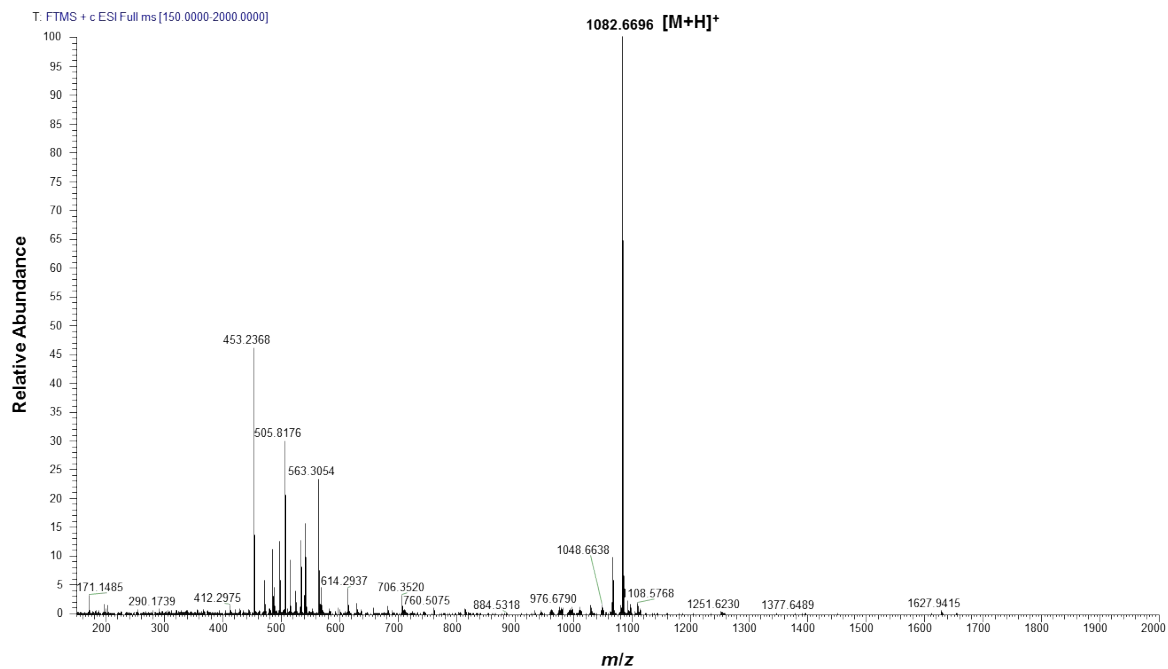


Figure S24. ESI-HRMS (+) spectrum of azalomycin F_{4a} (**3**).

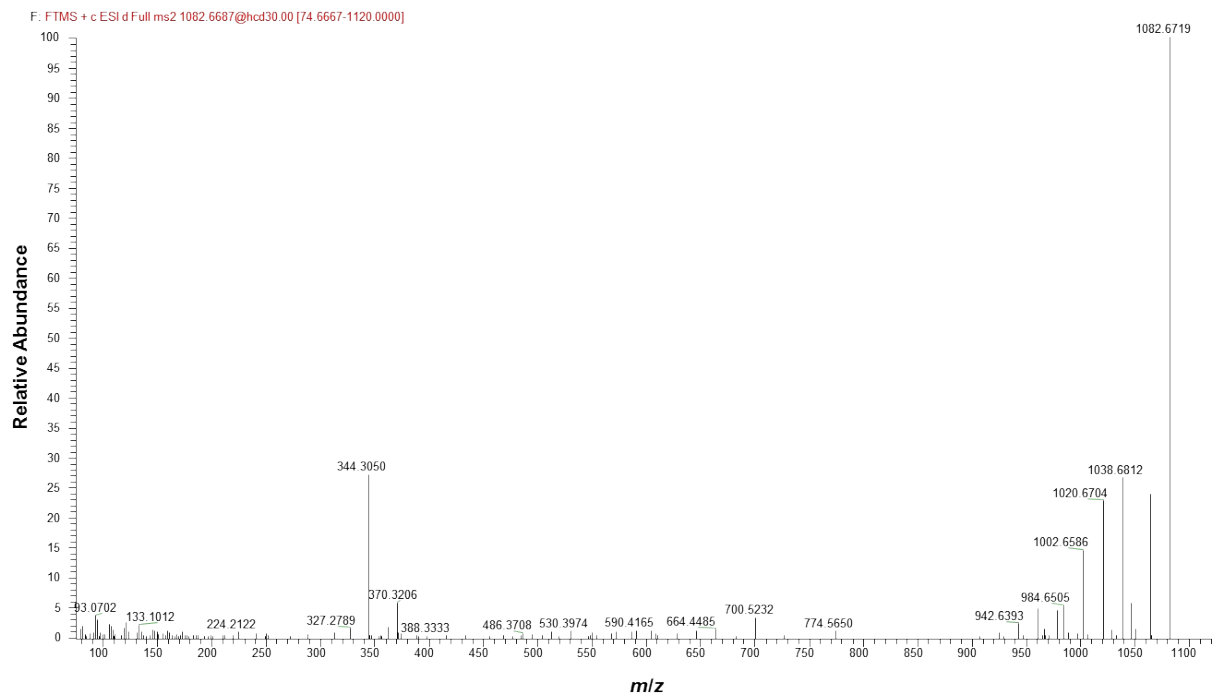


Figure S25. ESI-HRMS² (+) spectrum of azalomycin F_{4a} (**3**).

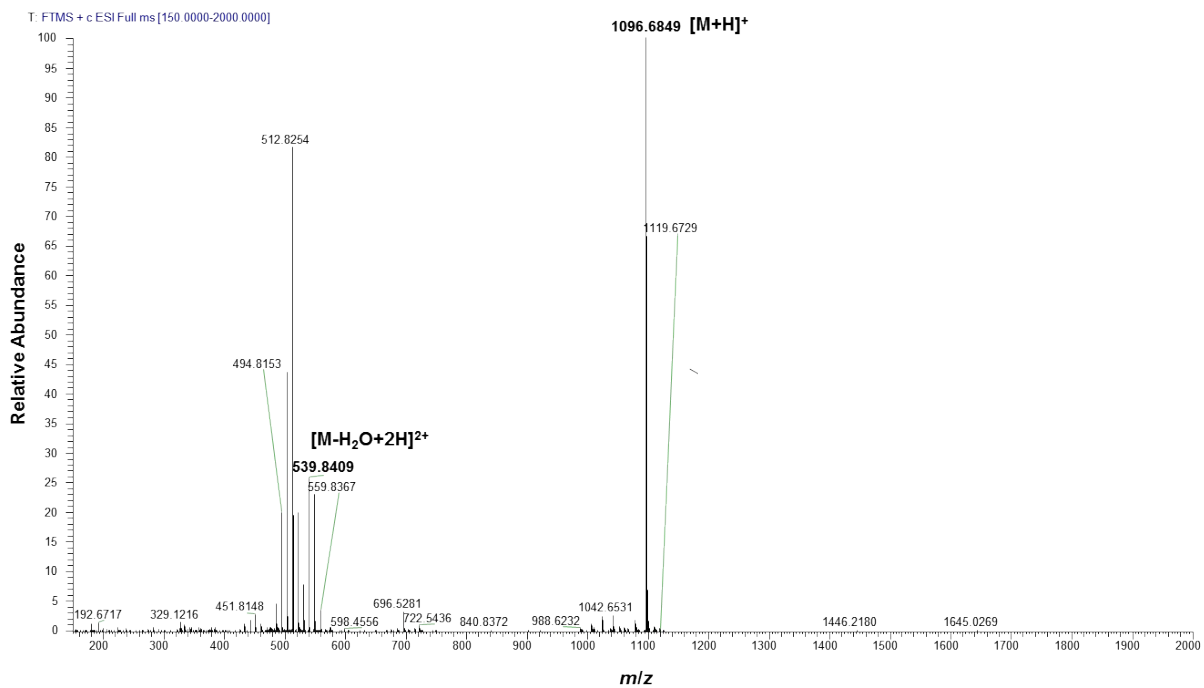


Figure S26. ESI-HRMS (+) spectrum of azalomycin F_{5a} (4).

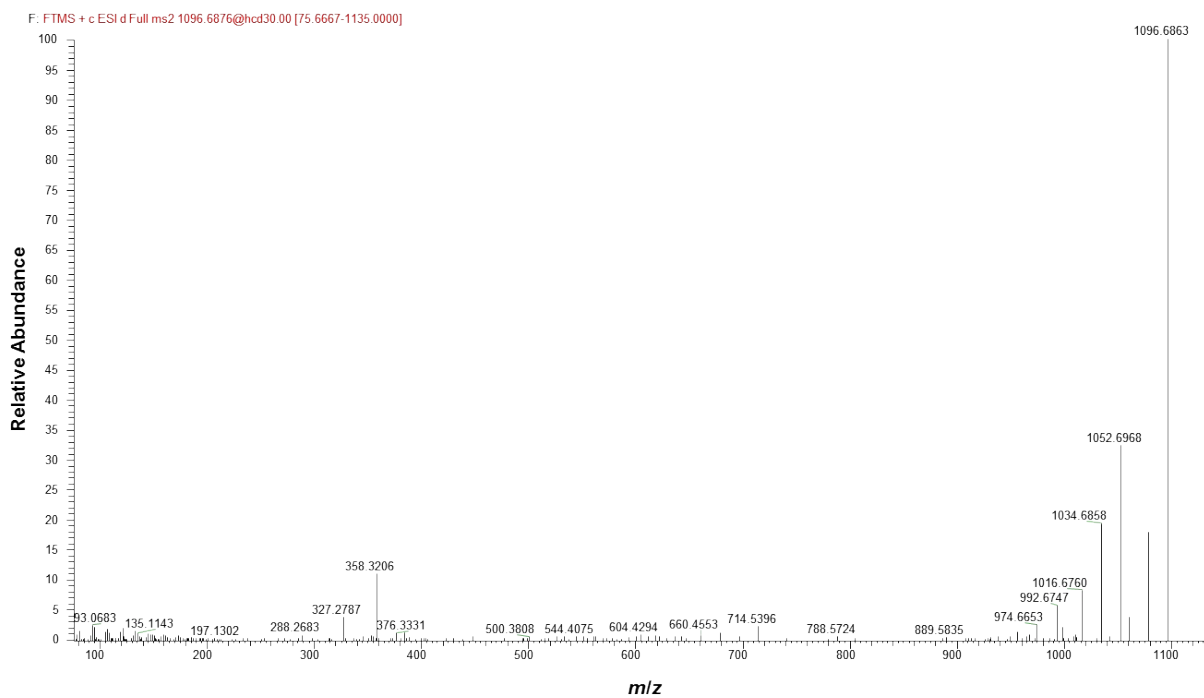


Figure S27. ESI-HRMS² (+) spectrum of azalomycin F_{5a} (4).

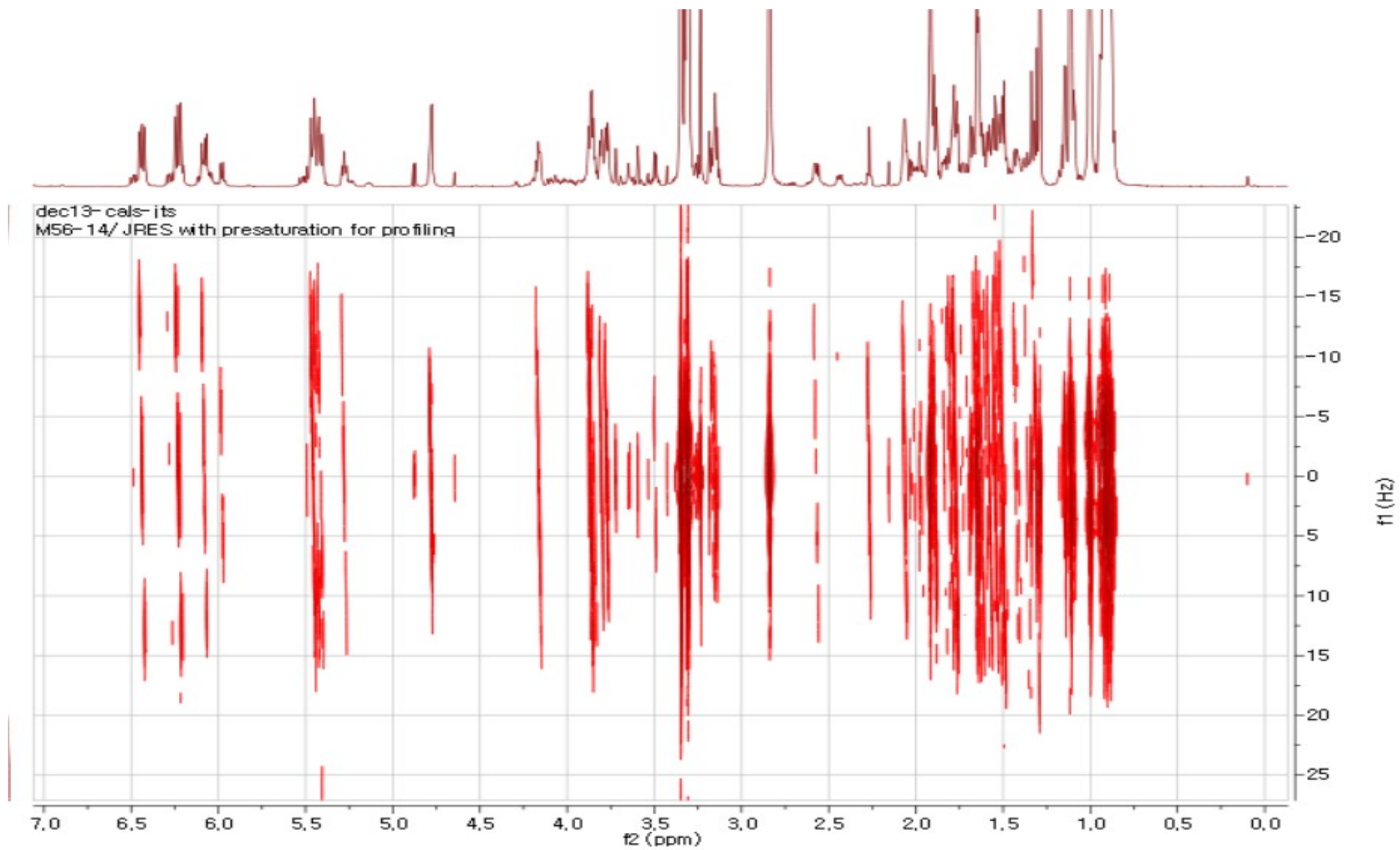


Figure S28. The homonuclear J -resolved spectroscopy (JRES) of compound **1**.

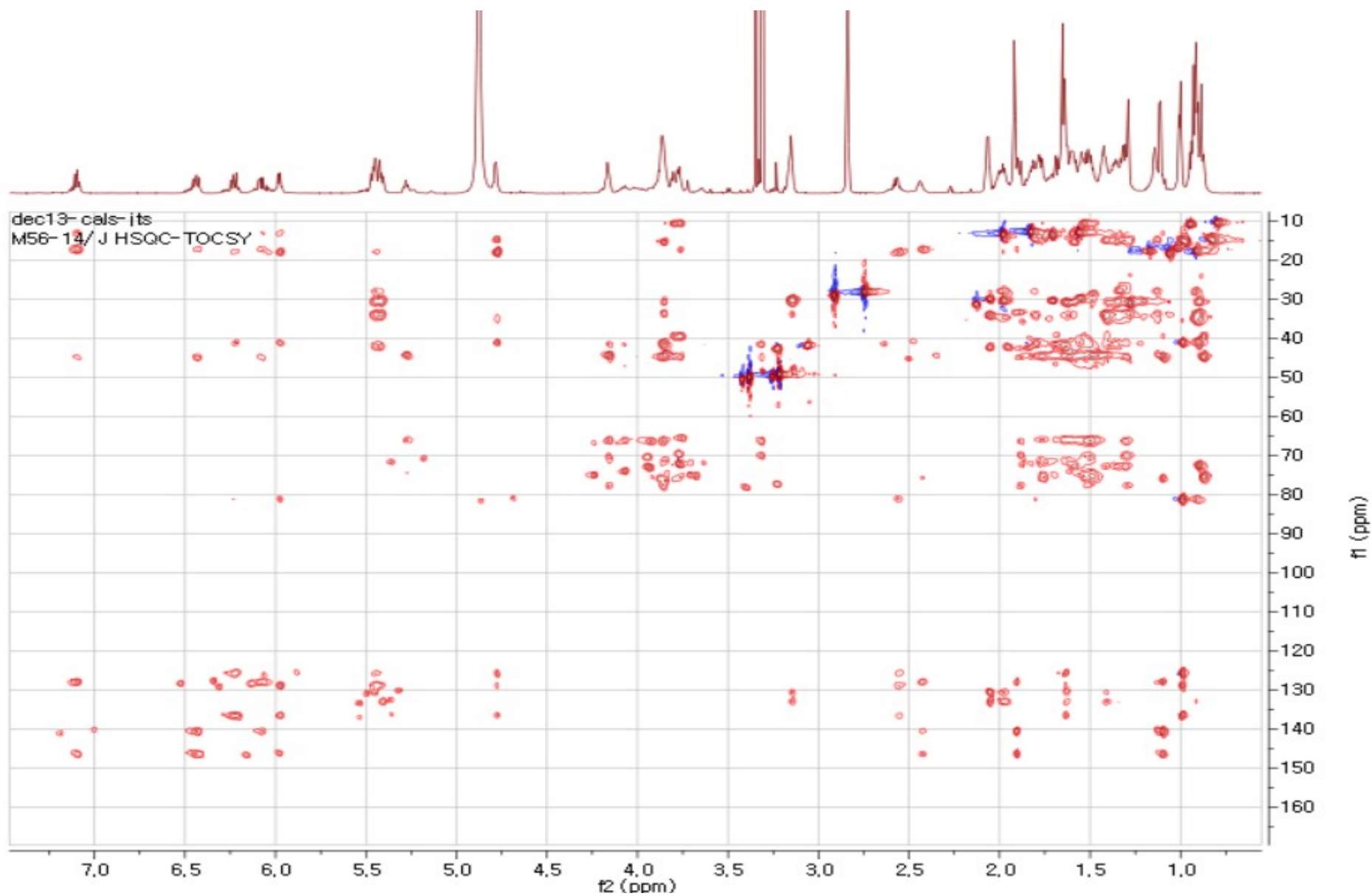


Figure S29. The HSQC-HECADE data of compound 1.

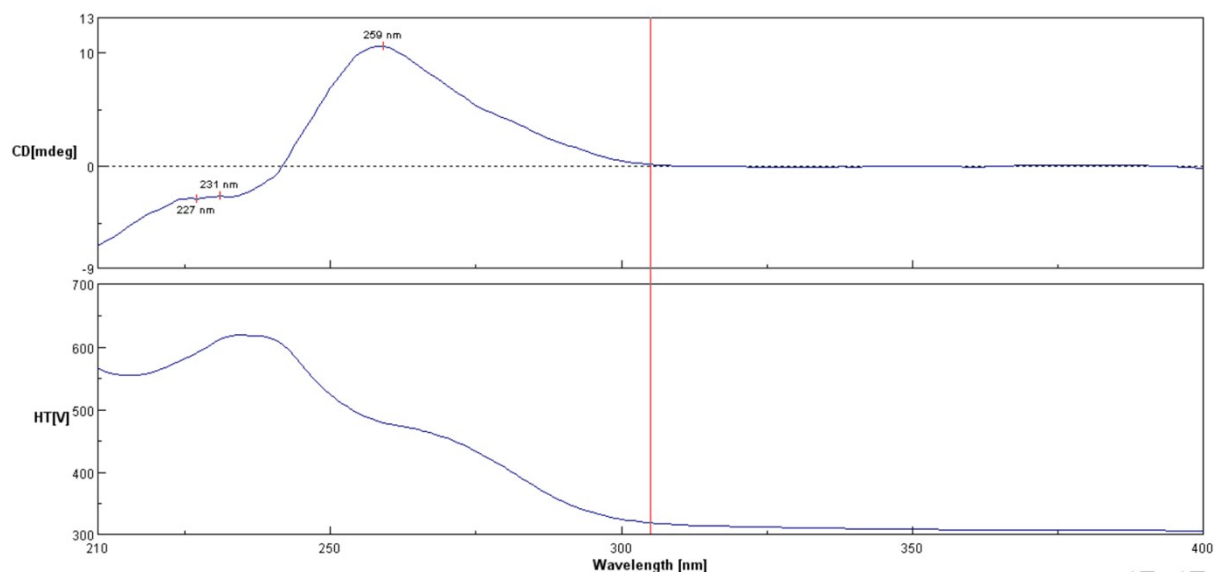


Figure S30. The ECD data of compound 1.

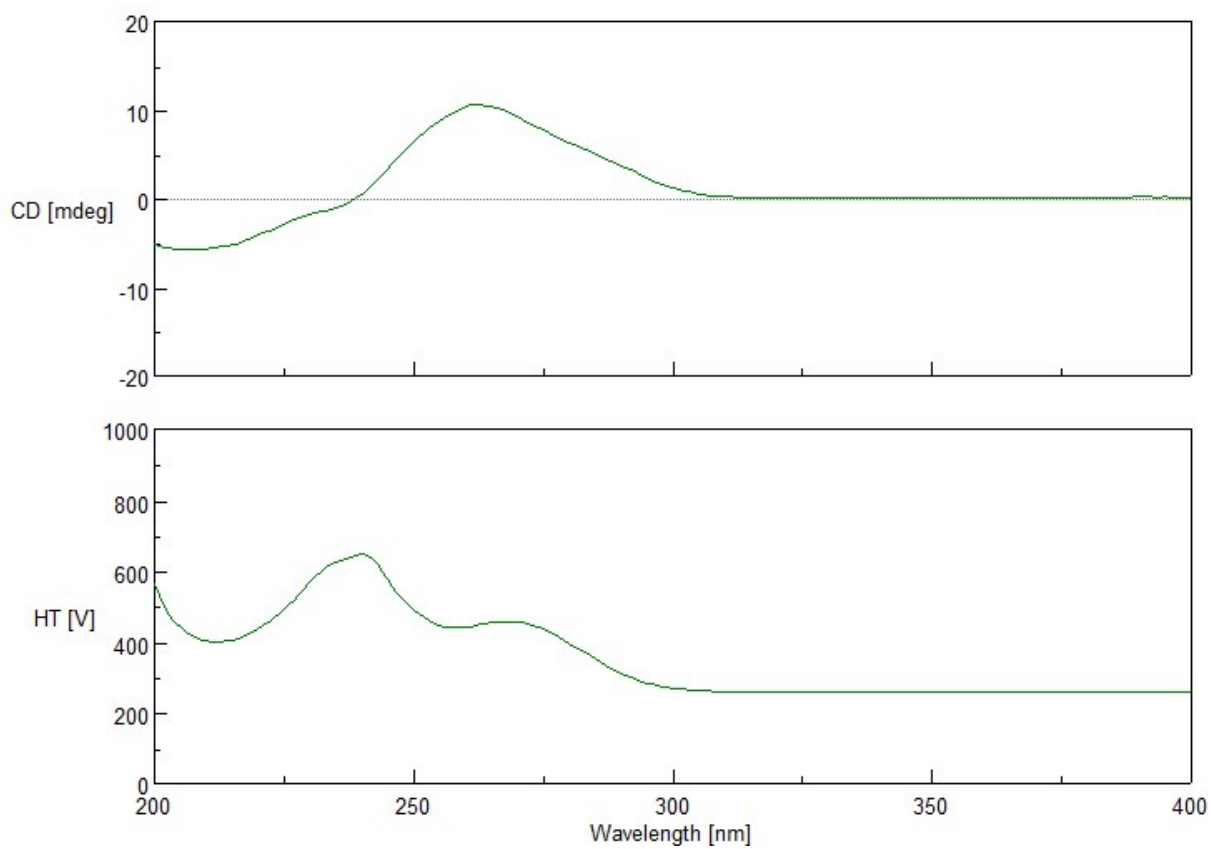


Figure S31. The ECD data of compound 2.

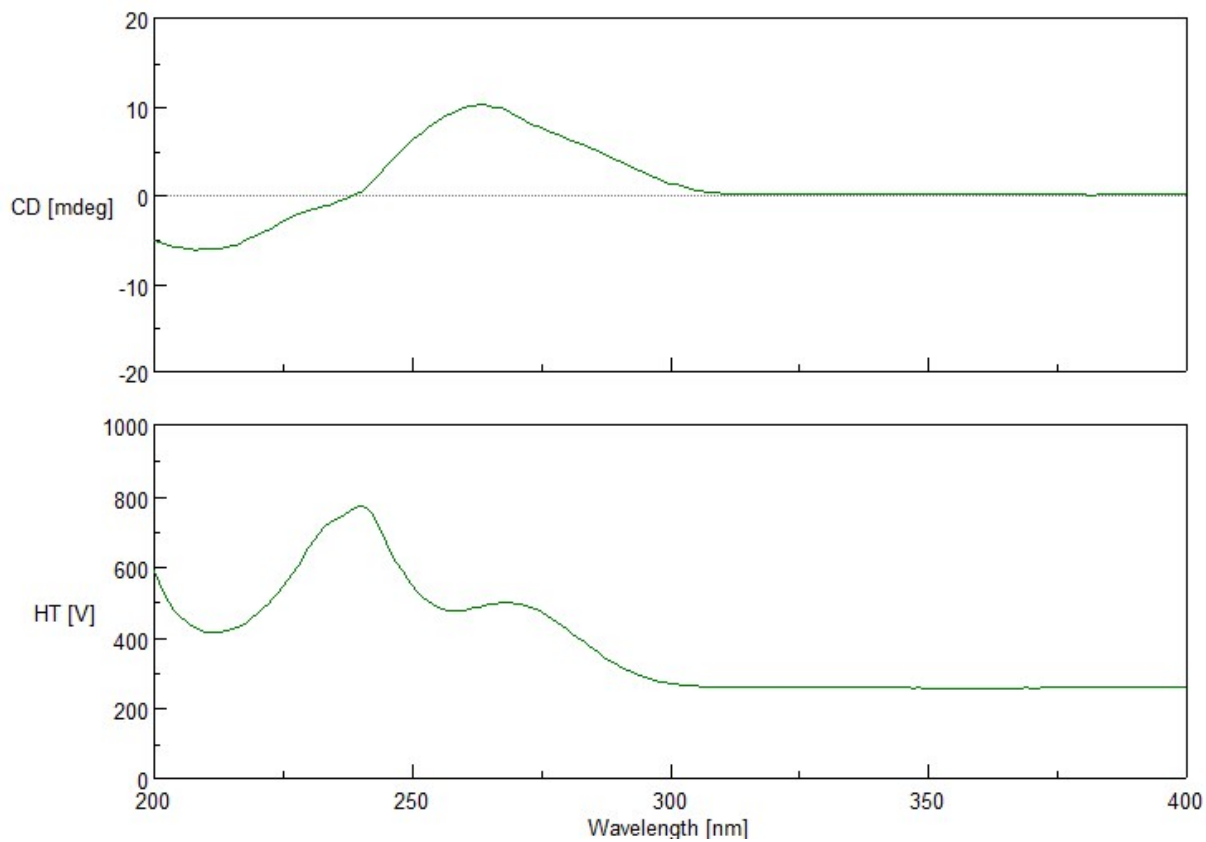


Figure S32. The ECD data of compound **3**.

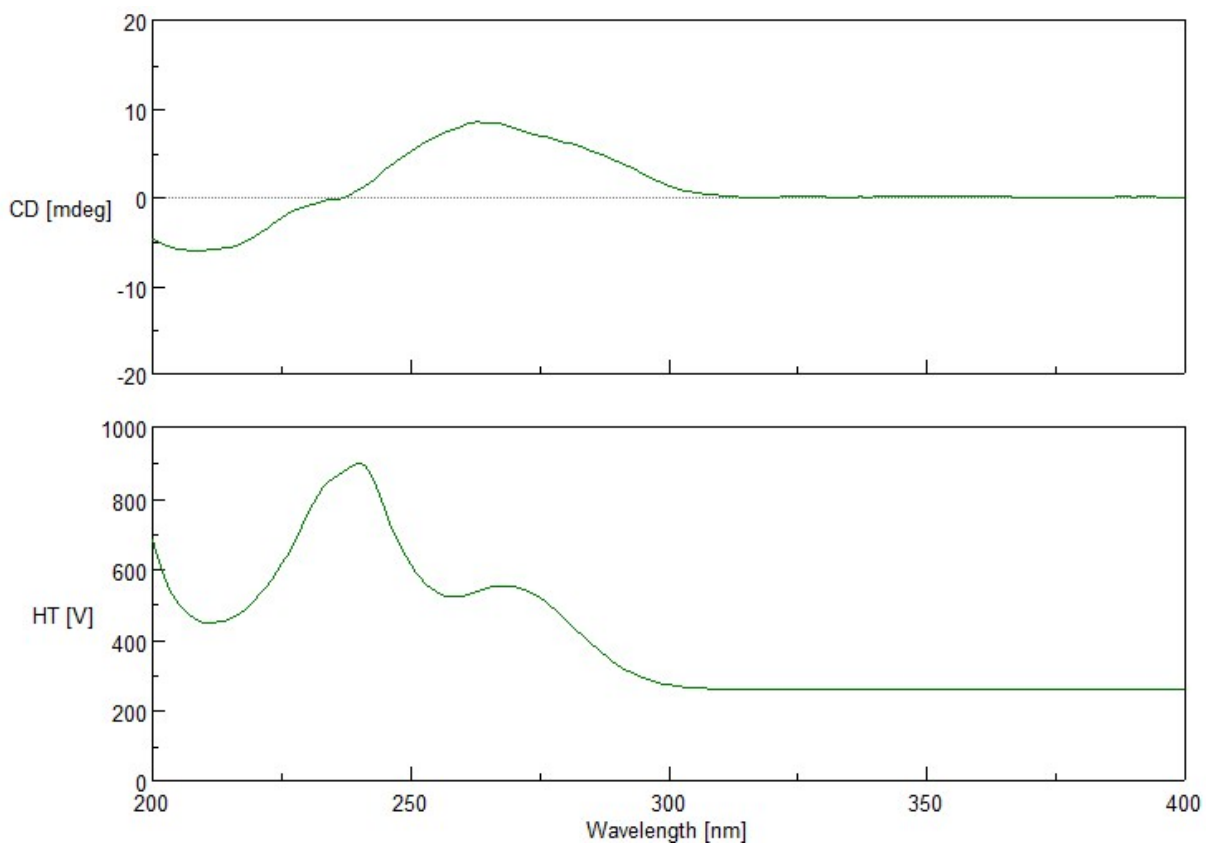


Figure S33. The ECD data of compound **4**.

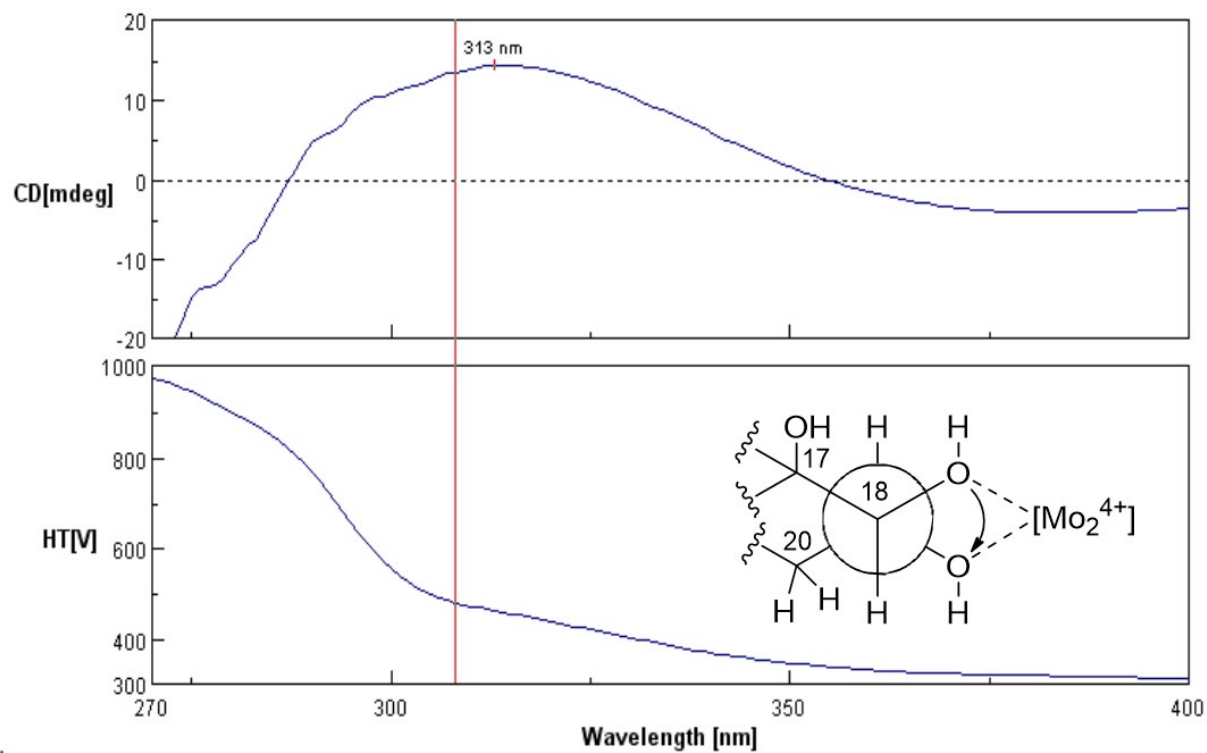


Figure S34. The induced circular dichroism (ICD) data of compound **1**.