SI Appendix

# Selvamicin, an atypical antifungal polyene from two alternative genomic contexts

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## Supplementary methods

**General chemical analysis procedures:** UV-visible absorbance spectra were collected on an Amersham Biosciences Ultrospec 5300 Pro spectrophotometer. High resolution mass spectrometry analysis was performed on an Agilent 6530 ESI QTOF mass spectrometer interfaced with an Agilent 1290 Infinity Binary LC. COSY, TOCSY, ROESY, HSQC, H2BC, HMBC, and $^{1}$H NMR experiments were performed on either a Varian VNMRS 600 MHz spectrometer equipped with a triple resonance HCN inverse probe or on a Varian INOVA 500 MHz spectrometer equipped with a triple resonance HCN coldprobe. $^{13}$C NMR experiments were performed on a Varian 400 MHz spectrometer equipped with a Varian OneNMR probe. Chemical shifts were referenced to the residual solvent peak in DMSO-$d_6$. Optical rotation was measured on a Jasco P-2000 polarimeter fitted with a microcell (10 mm path length).

**Preparation of $Ac_9$-selvamicin:** Selvamicin (18 mg) was dissolved in anhydrous pyridine (0.5 mL) under nitrogen in an oven-dried vial containing a dry stir bar. This solution was cooled to 0 °C with stirring and a solution dimethylaminopyridine (1 mg) in anhydrous pyridine (100 µL) and acetic anhydride (100 µL) was added dropwise. After 5 min the reaction solution was warmed to room temperature and was stirred at room temperature under nitrogen for 5 h, at which point the reaction was complete by TLC. The reaction solution was evaporated to dryness *in vacuo* and $Ac_9$-selvamicin was purified by reversed-phase HPLC (Agilent 1200 series semipreparative HPLC equipped with a diode array detector; Phenomenex Luna 5 µm $C_{18}$ column, 250 × 10 mm, 3 mL/min) with an isocratic solvent mixture of 87% acetonitrile in water. $Ac_9$-selvamicin eluted at 8.4 min.

**$Ac_9$-selvamicin:** NMR spectral data, see **Table S2**; HR-ESI-TOFMS *m/z* 1329.5885 $[M+Na]^+$ (calcd for $C_{65}H_{94}NaO_{27}$: 1329.5875)

**Solubility determination:** Solubility for selvamicin and nystatin was measured with minor modifications from a previously reported protocol (1). Briefly, in microcentrifuge tubes, 20 µL 5 mM HEPES (pH = 7.4) was added to 2.5 mg of selvamicin and of nystatin and the resulting suspensions were vortexed vigorously for 30 min at 22 °C. The tubes were centrifuged, the resulting supernatants were diluted in HEPES buffer, and concentrations were determined by UV-vis absorbance (306 nm for nystatin and 335 nm for selvamicin).

**Isothermal calorimetry sterol binding assay:**
Large unilamellar vesicle (LUV) preparation: In a glass vial, a 25 mg/mL solution of palmitoyl oleoyl phosphatidylcholine (POPC) in chloroform (0.96 mL, Avanti Polar Lipids) was mixed with a freshly prepared 4 mg/mL solution of the appropriate sterol (ergosterol or cholesterol, Aldrich) in chloroform (0.35 mL). The sterol solution was omitted for preparation of sterol-free POPC LUVs. The resulting solution was evaporated to dryness *in vacuo* to yield a lipid film, which was placed under high vacuum for at least 5 h. To this film was added 1 mL 5 mM HEPES (pH adjusted to 7.4 with KOH) and the resulting suspension was vortexed for 3 min. This lipid suspension was loaded into a syringe and passed through a 0.1 µM filter (Whatman) 21 times using an Avanti Polar Lipids Mini-Extruder to yield an LUV suspension (32 mM POPC, 11 mol % sterol; assumed no loss during extrusion).

Isothermal calorimetry (ITC) experiments: Solutions of polyene (150 μM selvamicin or nystatin) in 1% DMSO/ 5 mM HEPES (pH = 7.4) were prepared by dilution from a 15 mM solution in DMSO. 8 mM POPC LUV suspensions in 1% DMSO/ 5 mM HEPES (pH = 7.4) were prepared by dilution of the above LUV suspensions with HEPES buffer and DMSO. ITC experiments were performed on a MicroCal $iTC_{200}$ instrument (Malvern Instruments) with the 150 μM polyene solution in the sample cell (200 μL) and the LUV suspension injected by pipette. Experiments were performed at 25 °C and consisted of an initial injection of 0.4 μL followed by 18 injections of 2 μL each at intervals of 150 s. Experiments were performed for both nystatin and selvamicin with sterol-free LUVs, cholesterol-containing LUVs, and ergosterol-containing LUVs, with a minimum of two replicates for each condition. Robust binding, as indicated by heats evolved, was observed only for nystatin with ergosterol-containing vesicles. A dissociation constant for the nystatin-ergosterol interaction was estimated with the MicroCal ITC-ORIGIN analysis software in which the integrated heat for the last injection was subtracted from all of the data and a single binding site was assumed.

**Induction with propionate and butyrate:** Spores of each *Pseudonocardia* isolate were diluted into sterile double distilled water ($ddH_2O$) and spread onto ISP2 agar (BD Difco™ ISP2; 1.5 mL agar per well in 12-well plates) supplemented with the appropriate inducer (sodium butyrate or sodium propionate, Aldrich; 1-$^{13}$C-sodium butyrate or 1-$^{13}$C-sodium propionate, Cambridge Isotope Labs; 0, 25, or 150 mM final concentration with all conditions in duplicate; added after autoclaving), which were incubated at 30 °C for 14 d. The agar was cut out of each well and soaked in 2 mL ethyl acetate for 48 h. The ethyl acetate extract was evaporated to dryness *in vacuo*, redissolved in 0.1 mL methanol, and analyzed by HPLC (Agilent 1200 series, equipped with a diode array detector). The selvamicin peak in the 375 nm absorbance chromatogram was integrated for each sample. Samples were also analyzed by HPLC-high resolution ESI-MS.

**Sequence comparison and analysis:** Conserved replicons in the two chromosomes were compared using an average nucleotide identity (ANI) calculator (2), which provided a two-way ANI value of 83.3% from 8071 genomic fragments. The selvamicin gene cluster annotations were performed using antiSMASH2 (3) and blastp (nonredundant proteins db). The Geneious aligner (4) was used for pairwise alignment with proteins from the nystatin biosynthetic gene cluster from *S. noursei* ATCC 11455 (accession no. AF263912). Polyketide synthase domains were detected by antiSMASH2 (3), and the translated protein sequences were aligned using Clustal W. Extractions from these domain alignments are displayed in **Fig. S10**.

## Supplementary data

**Figure S1.** UV spectrum of selvamicin in methanol

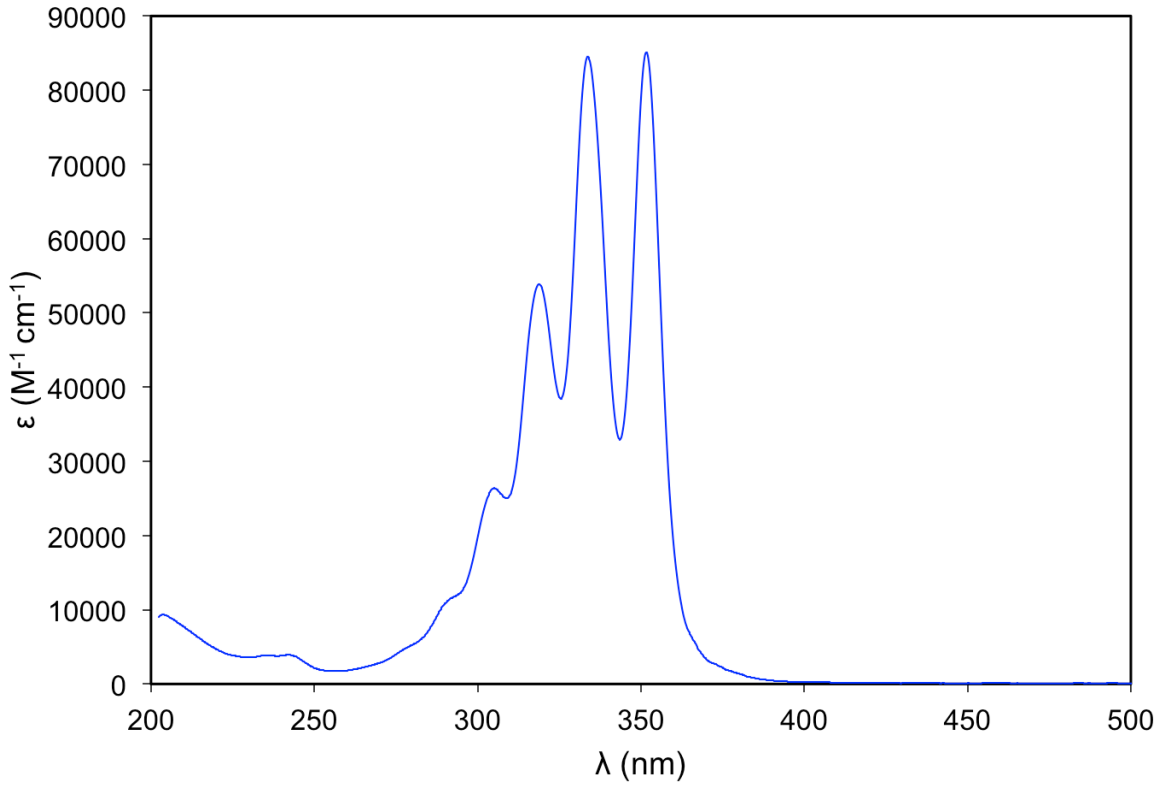


**Table S1.** NMR spectral data for selvamicin in DMSO-$d_6$.

| Position | $\delta_H$ | mult (*J* in Hz) | $\delta_C$ | |
|---|---|---|---|---|
| 1 | | | 172.68 | C |
| 2 | $H_a$ 2.52 | obs | 30.40 | $CH_2$ |
| | $H_b$ 2.10 | ddd (17.4, 11.6, 5.7) | | |
| 3 | 1.81 | obs | 27.79 | $CH_2$ |
| | 1.36 | obs | | |
| 4 | 3.10 | obs | 72.00 | CH |
| 4-OH | 4.36 | d (7.0) | | |
| 5 | 3.46 | m | 74.17 | CH |
| 5-OH | 4.78 | d (5.5) | | |
| 6 | $H_a$ 1.63 | dt (14.6, 10.4, 10.4) | 39.17* | $CH_2$ |
| | $H_b$ 1.28 | d (13.8) | | |
| 7 | 4.26 | m | 68.27 | CH |
| 7-OH | 5.51 | s | | |
| 8 | $H_a$ 1.53 | obs | 46.16 | $CH_2$ |
| | $H_b$ 1.53 | obs | | |
| 9 | | | 97.32 | C |
| 9-OH | 5.89 | s | | |
| 10 | $H_a$ 1.56 | obs | 40.39 | $CH_2$ |
| | $H_b$ 1.52 | obs | | |
| 11 | 3.53 | ddd (12, 7.2, 4.8) | 68.27 | CH |
| 11-OH | 4.28 | d (7.2) | | |
| 12 | | | 71.18 | C |
| 12-OH | 3.61 | s | | |
| 13 | 3.96 | d (9.1) | 69.57 | CH |
| 14 | $H_a$ 1.42 | dd (14.6, 9.3) | 33.38 | $CH_2$ |
| | $H_b$ 2.18 | dd (15.1, 3.8) | | |
| 15 | 4.34 | d (7.6) | 76.29 | CH |
| 16 | 5.98 | dd (15.3, 9.1) | 136.38 | CH |
| 17 | 6.06 | dd (15.2, 10.4) | 128.35 | CH |
| 18 | 6.36 | dd (14.8, 10.5) | 132.88 | CH |
| 19 – 24 | 6.08 - 6.46 | | 131.5 - 133.5 | 6 CH |
| 25 | 5.35 | br s | 135.57* | CH |
| 26 | 2.50 | obs | 42.85* | CH |
| 27 | 3.10 | obs | 73.50* | CH |
| 28 | 1.82 | obs | 39.30* | CH |
| 29 | 5.22 | br s | 73.53 | CH |
| 30 | $H_a$ 1.35 | obs | 22.68 | $CH_2$ |
| | $H_b$ 2.06 | obs | | |
| 31 | 0.75 | t (7.3, 7.3) | 10.78* | $CH_3$ |
| 32 | 1.01 | s | 21.44 | $CH_3$ |
| 33 | 1.01 | obs | 17.87* | $CH_3$ |
| 34 | 0.93 | d (7.1) | 12.17 | $CH_3$ |
| 1' | 4.40 | s | 96.80 | CH |
| 2' | 3.57 | dd (5.2, 3.4) | 70.89 | CH |
| 2'-OH | 4.29 | d (5.2) | | |
| 3' | 3.18 | ddd (9.1, 6.0, 3.3) | 73.65 | CH |
| 3'-OH | 4.50 | d (6.2) | | |
| 4' | 3.08 | obs | 72.00 | CH |
| 4'-OH | 4.71 | d (4.9) | | |
| 5' | 3.06 | obs | 72.13 | CH |
| 6' | 1.14 | d (5.6) | 17.93 | $CH_3$ |
| 1'' | 4.64 | br s | 98.66 | CH |
| 2'' | $H_a$ 1.79 | obs | 35.38 | $CH_2$ |
| | $H_b$ 1.99 | dd (12.9, 4.7) | | |
| 3'' | 4.05 | dt (7.9, 4.1, 4.1) | 61.41 | CH |
| 3''-OH | 4.17 | br s | | |
| 4''-OMe | 3.28 | s | 55.79 | $CH_3$ |
| 4'' | 2.81 | dd (8.6, 2.9) | 81.80 | CH |
| 5'' | 4.02 | obs | 63.10 | CH |
| 6'' | 1.10 | d (6.4) | 17.61 | $CH_3$ |

*Chemical shift extracted from HSQC spectrum

**Table S2.** NMR spectral data for $Ac_9$-selvamicin in DMSO-$d_6$.

| Position | $\delta_H$ | mult (*J* in Hz) | $\delta_C$* | |
|---|---|---|---|---|
| 1 | | | 171.43‡ | C |
| 2 | $H_a$ 2.33 | ddd (17.4, 11.5, 4.6) | 29.38 | $CH_2$ |
| | $H_b$ 2.08 | obs | | |
| 3 | $H_a$ 1.70 | obs | 25.14 | $CH_2$ |
| | $H_b$ 1.57 | obs | | |
| 4 | 4.81 | obs | 72.34 | CH |
| 5 | 4.92 | dt (9.8, 2.2, 2.2) | 70.38 | CH |
| 6 | $H_a$ 1.75 | obs | 34.33 | $CH_2$ |
| | $H_b$ 1.67 | obs | | |
| 7 | 5.05 | obs | 67.26 | CH |
| 8 | 2.56 | dd (15.9, 9.9) | 45.93 | $CH_2$ |
| | 2.47 | obs | | |
| 9 | | | 204.20‡ | C |
| 10 | $H_a$ 2.47 | obs | 41.98 | $CH_2$ |
| | $H_b$ 2.77 | dd (18.3, 9.4) | | |
| 11 | 5.12 | dd (9.4, 2.5) | 70.28 | CH |
| 12-OH | 4.81 | s | | |
| 12 | | | 73.95‡ | C |
| 13 | 4.47 | d (9.7) | 71.08 | CH |
| 14 | $H_a$ 1.50 | t (12.7, 12.7) | 36.01 | $CH_2$ |
| | $H_b$ 1.73 | obs | | |
| 15 | 3.90 | t (9.8, 9.8) | 77.10 | CH |
| 16 | 5.46 | dd (14.2, 8.9) | 133.01 | CH |
| 17 - 23 | 6.14 - 6.43 | | 131.0 - 134.0 | 7 CH |
| 24 | 6.01 | m | 130.17 | CH |
| 25 | 5.54 | dd (14.8, 9.8) | 132.95 | CH |
| 26 | 2.41 | m | + | CH |
| 27 | 3.30 | obs | + | CH |
| 28 | 1.98 | obs | 39.25 | CH |
| 29 | 5.00 | br s | 74.48 | CH |
| 30 | $H_a$ 1.43 | dt (15.3, 7.9, 7.9) | 22.93 | $CH_2$ |
| | $H_b$ 1.82 | obs | | |
| 31 | 0.78 | t (7.3, 7.3) | 9.41 | $CH_3$ |
| 32 | 0.95 | s | 17.77 | $CH_3$ |
| 33 | 0.95 | obs | 17.0 | $CH_3$ |
| 34 | 0.95 | obs | 11.45 | $CH_3$ |
| 1' | 4.89 | d (1) | 95.51 | CH |
| 2' | 5.25 | obs | 68.88 | CH |
| 3' | 5.08 | dd (10.2, 3.6) | 70.22 | CH |
| 4' | 4.75 | t (9.9, 9.9) | 70.19 | CH |
| 5' | 3.53 | dq (9.5, 6.4, 6.4, 6.4) | 68.95 | CH |
| 6' | 1.06 | d (6.1) | 17.05 | $CH_3$ |
| 1" | 4.72 | d (4.66) | 97.64 | CH |
| 2" | $H_a$ 1.93 | obs | 32.58 | $CH_2$ |
| | $H_b$ 2.10 | obs | | |
| 3" | 5.25 | obs | 64.28 | CH |
| 4" | 2.98 | dd (9.4, 3.0) | 79.33 | CH |
| 4"-OMe | 3.24 | s | 55.96 | $CH_3$ |
| 5" | 3.98 | dq (9.4, 6.4, 6.4, 6.4) | 62.63 | CH |
| 6" | 1.11 | d (6.2) | 17.28 | $CH_3$ |
| Ac | 1.91 - 2.09 | | 20.3 - 20.7 | 9 $CH_3$ |
| Ac | | | 168.5 - 170.5‡ | 9 C |

* Chemical shifts extracted from HSQC spectrum, except where noted

‡ Chemical shift extracted from HMBC spectrum

+ not observed

**Figure S2.** Selvamicin NMR correlations. **(a)** Key H2BC correlations supporting the planar structure of selvamicin. **(b)** Key ROESY correlations supporting the relative stereochemistry of selvamicin from C4-C13.

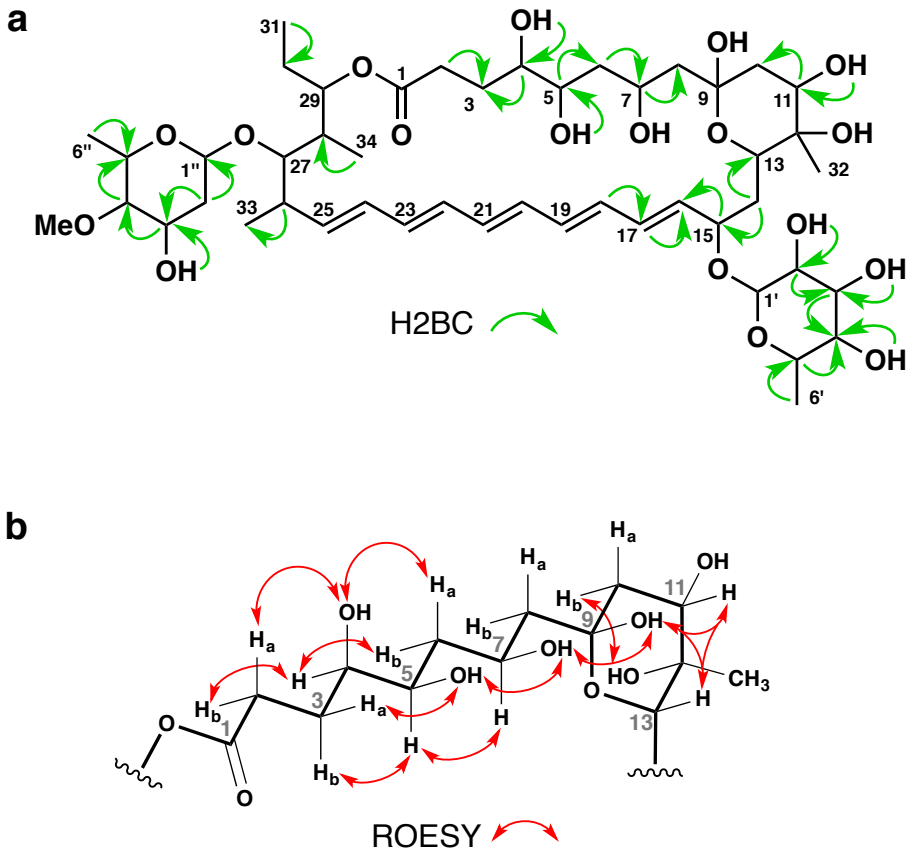



**Figure S3.** Key $Ac_9$-selvamicin NMR correlations supporting its planar structure

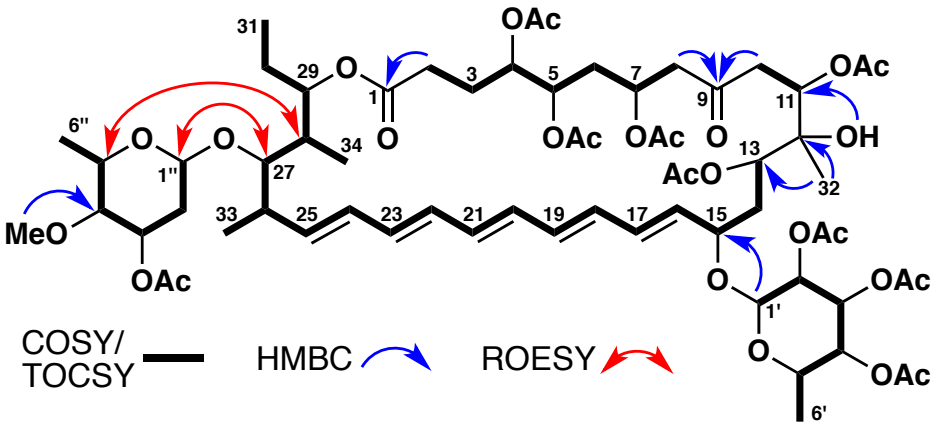


**Figure S4.** NMR correlations and coupling constants supporting sugar stereochemistry

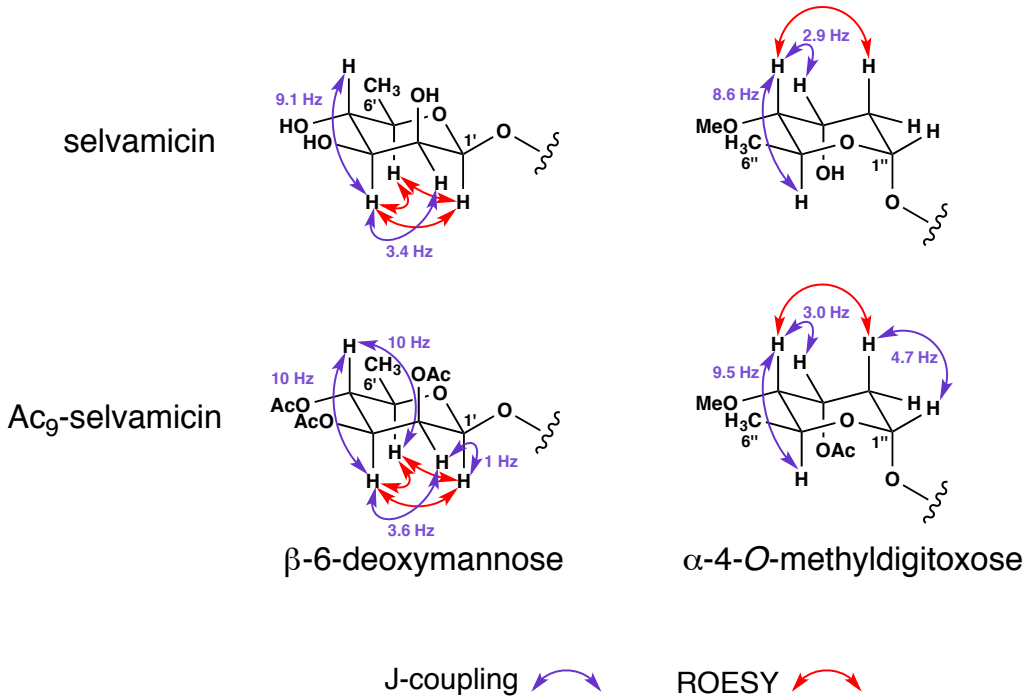
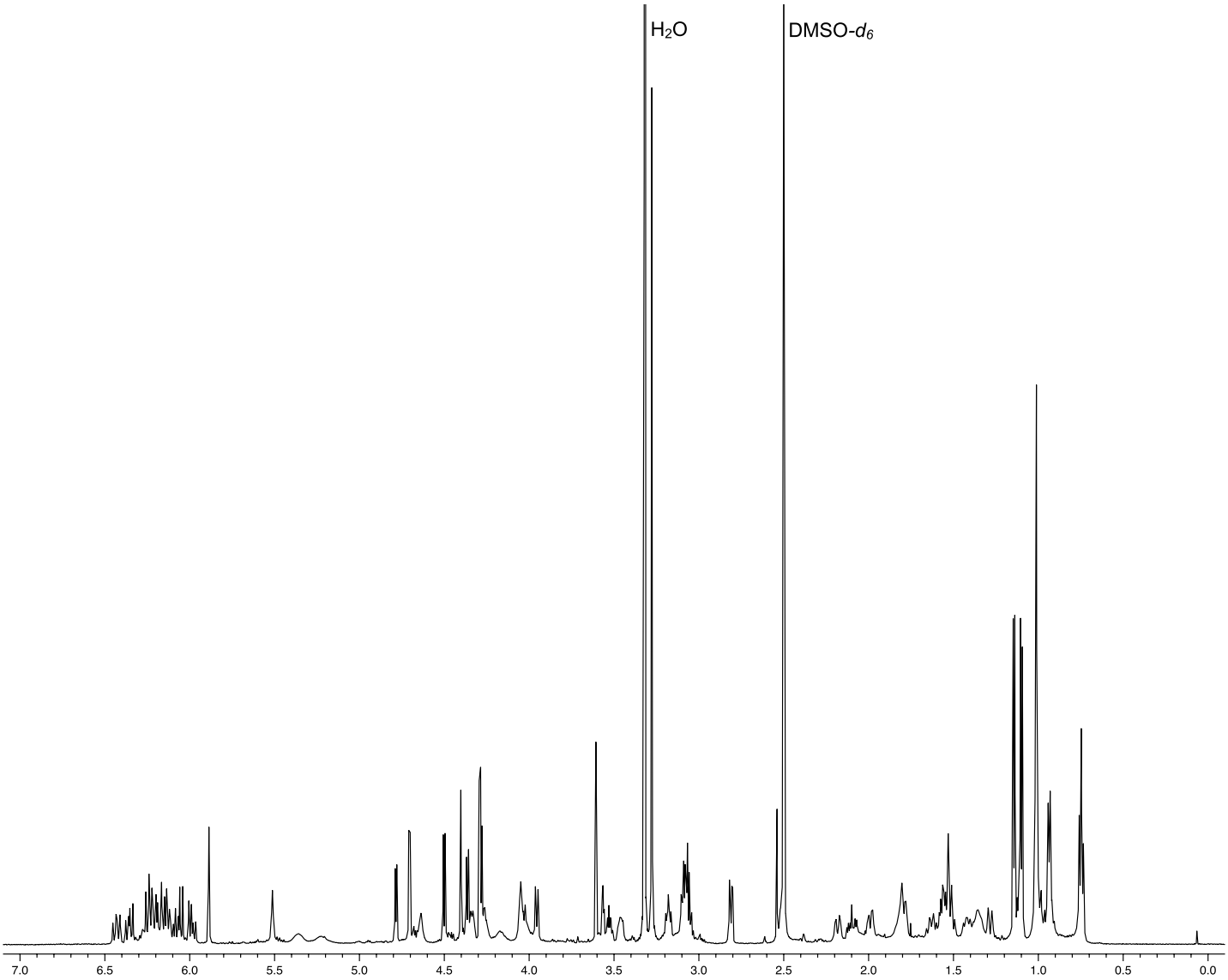
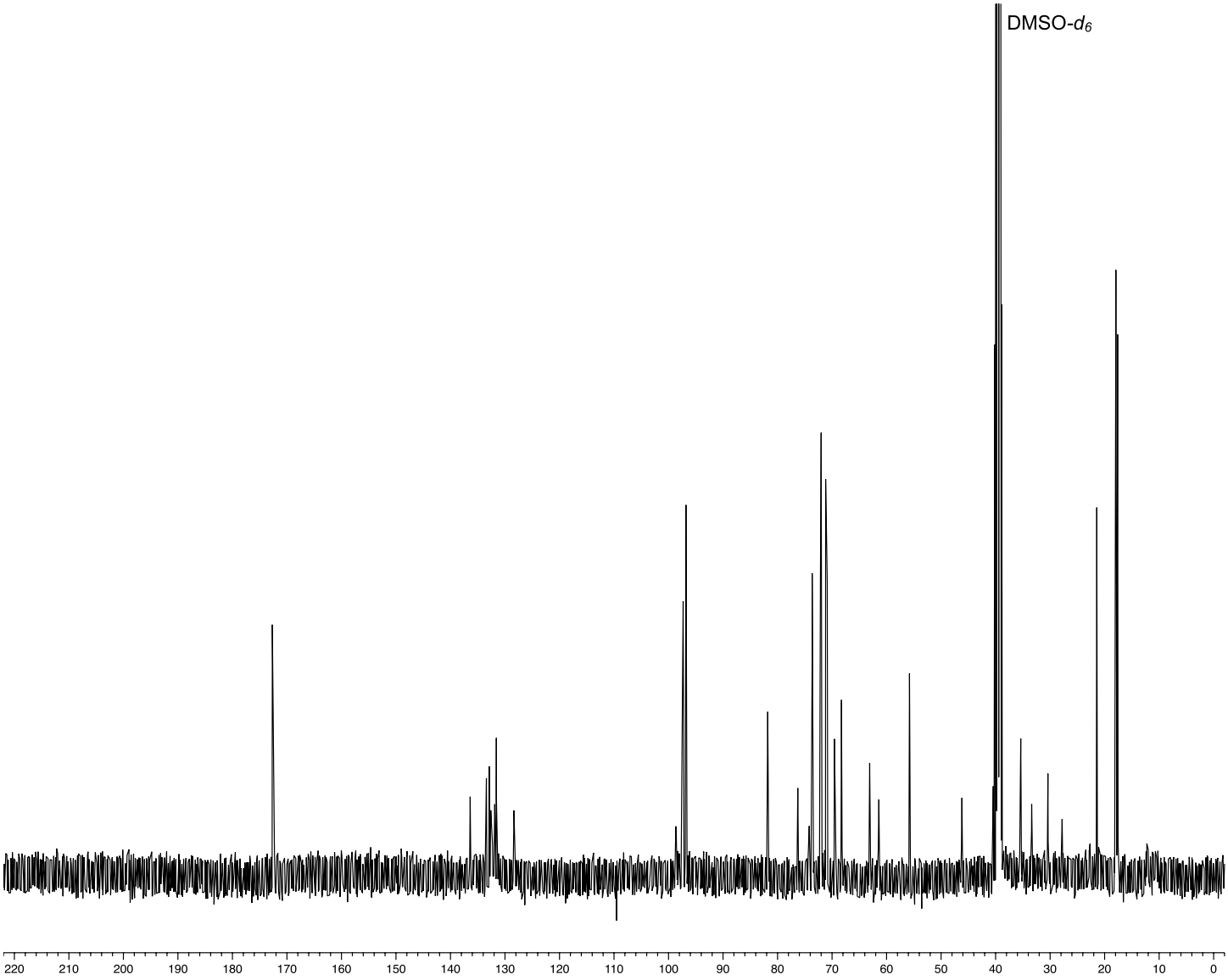


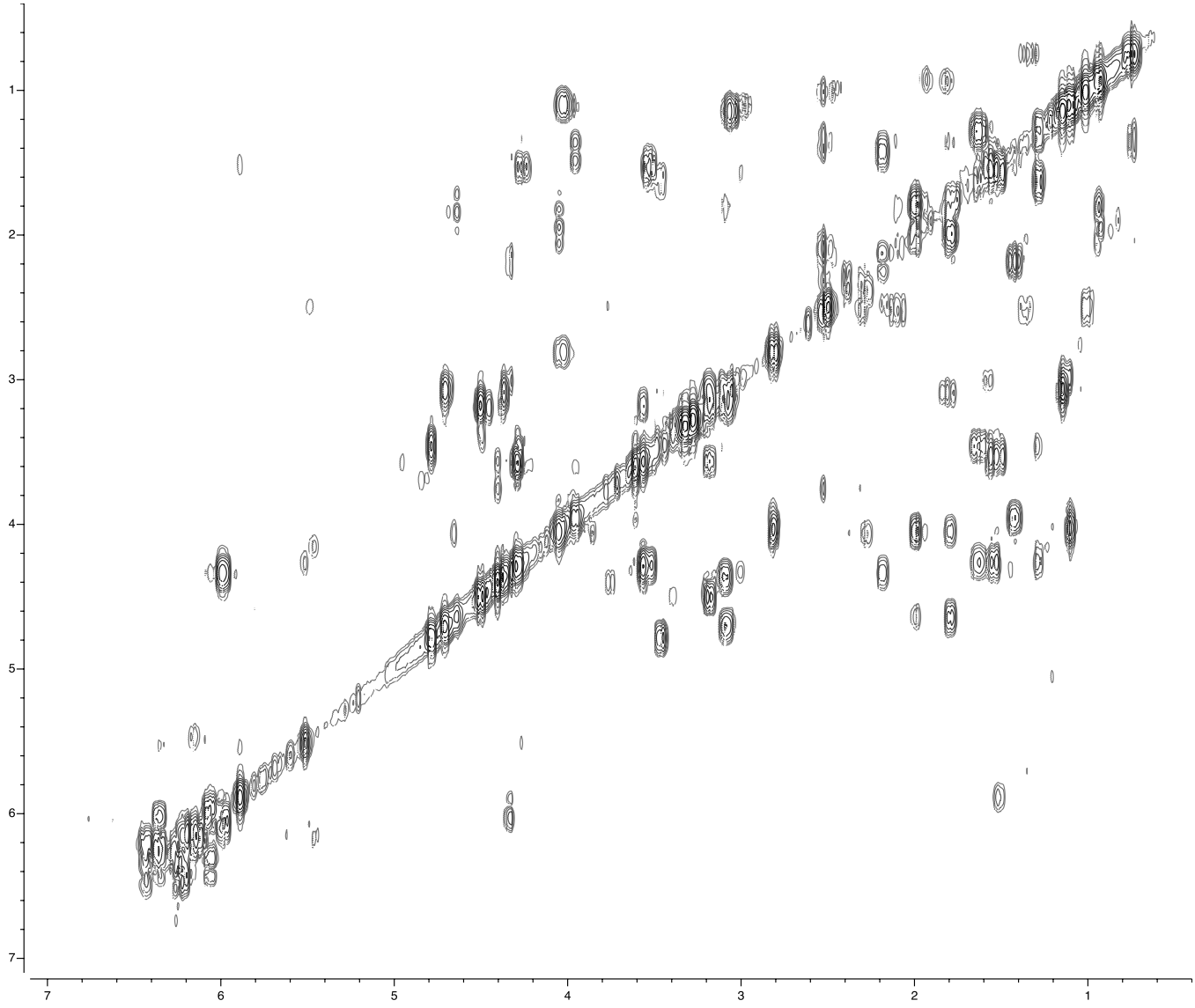
**Figure S5.** Selvamicin NMR spectra in DMSO-$d_6$



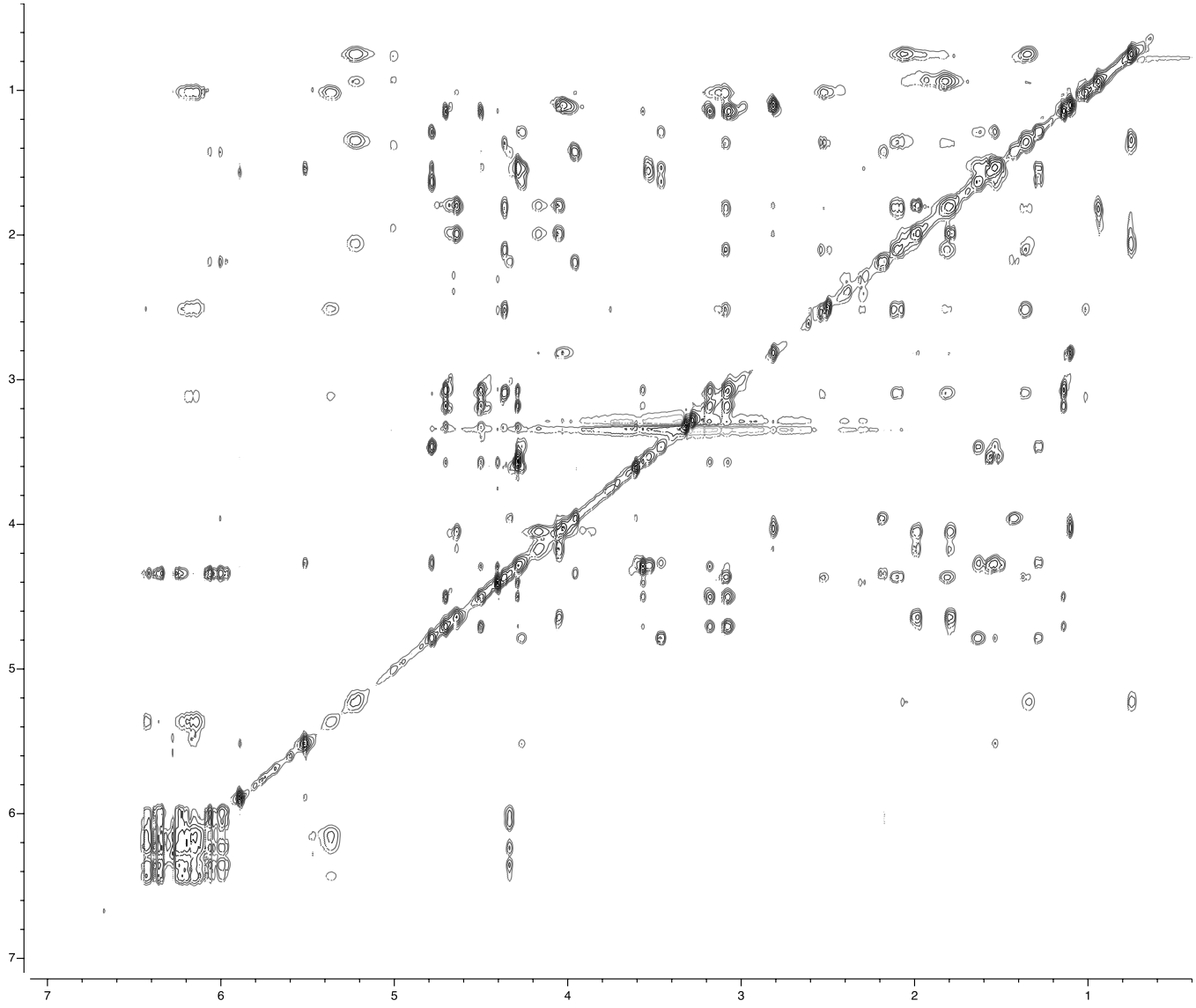

**(a)** 600 MHz $^1$H NMR spectrum of selvamicin in DMSO-$d_6$



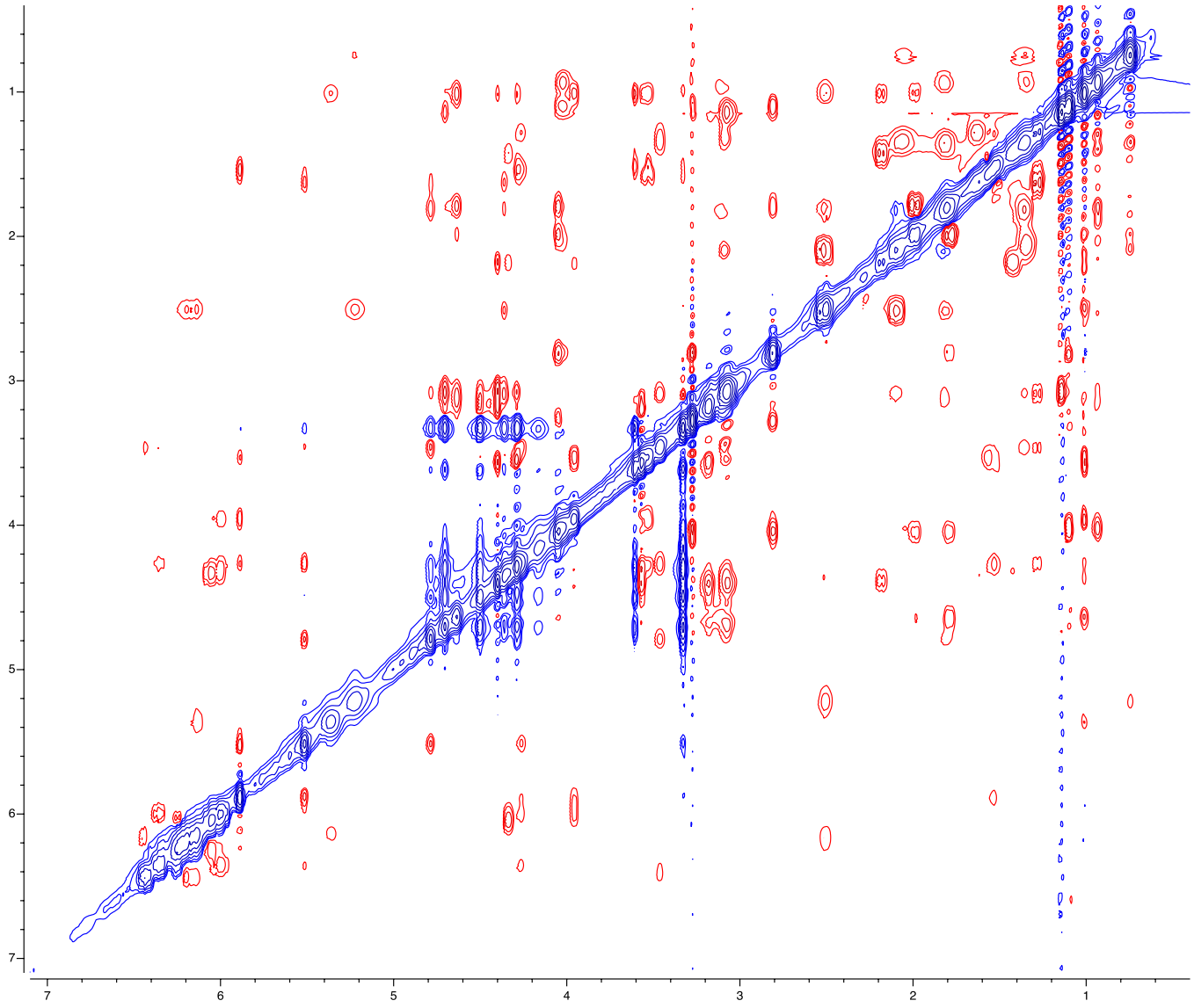

**(b)** 100 MHz $^{13}$C NMR spectrum of selvamicin in DMSO-$d_6$



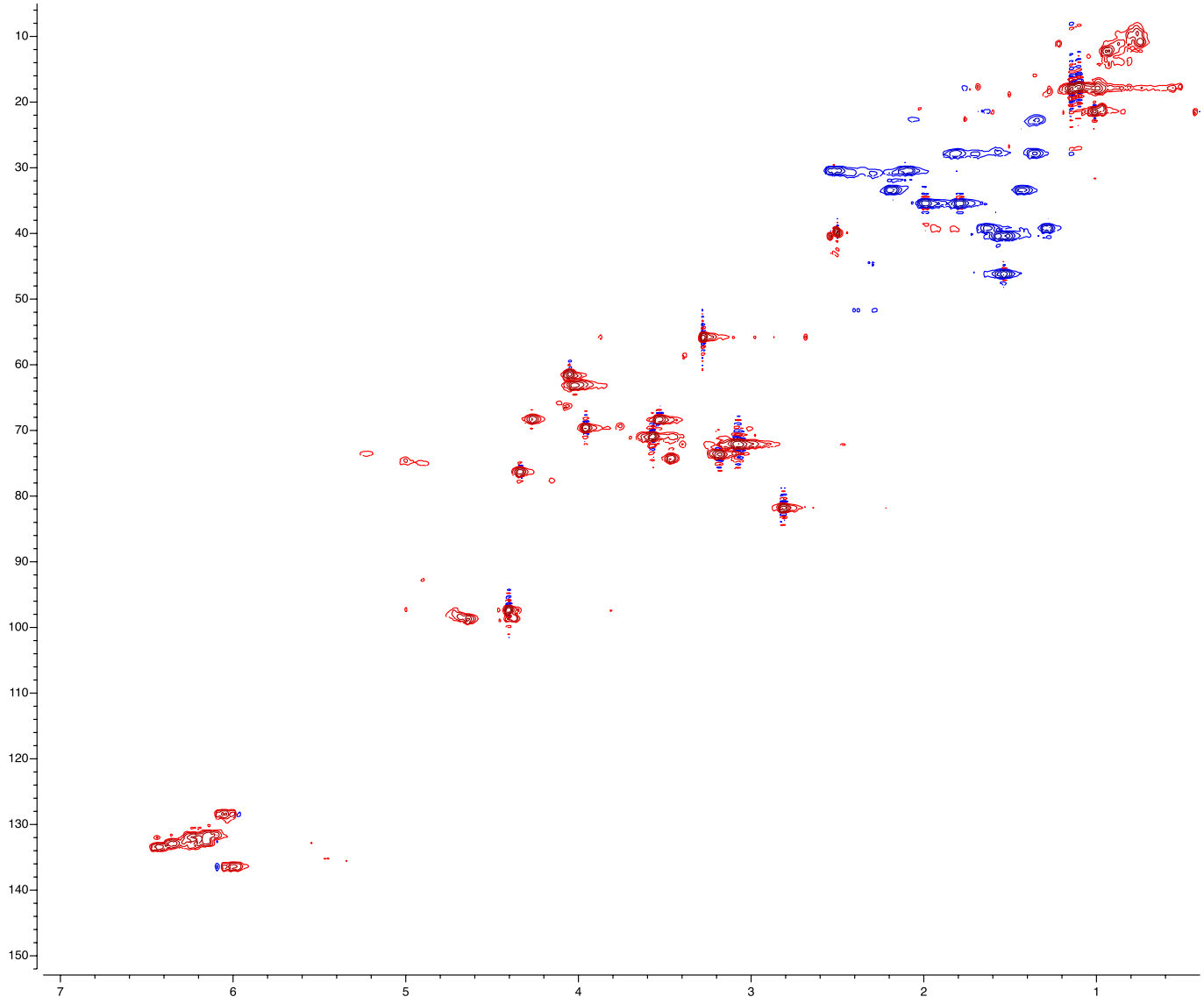

**(c)** 600 MHz COSY spectrum of selvamicin in DMSO-$d_6$



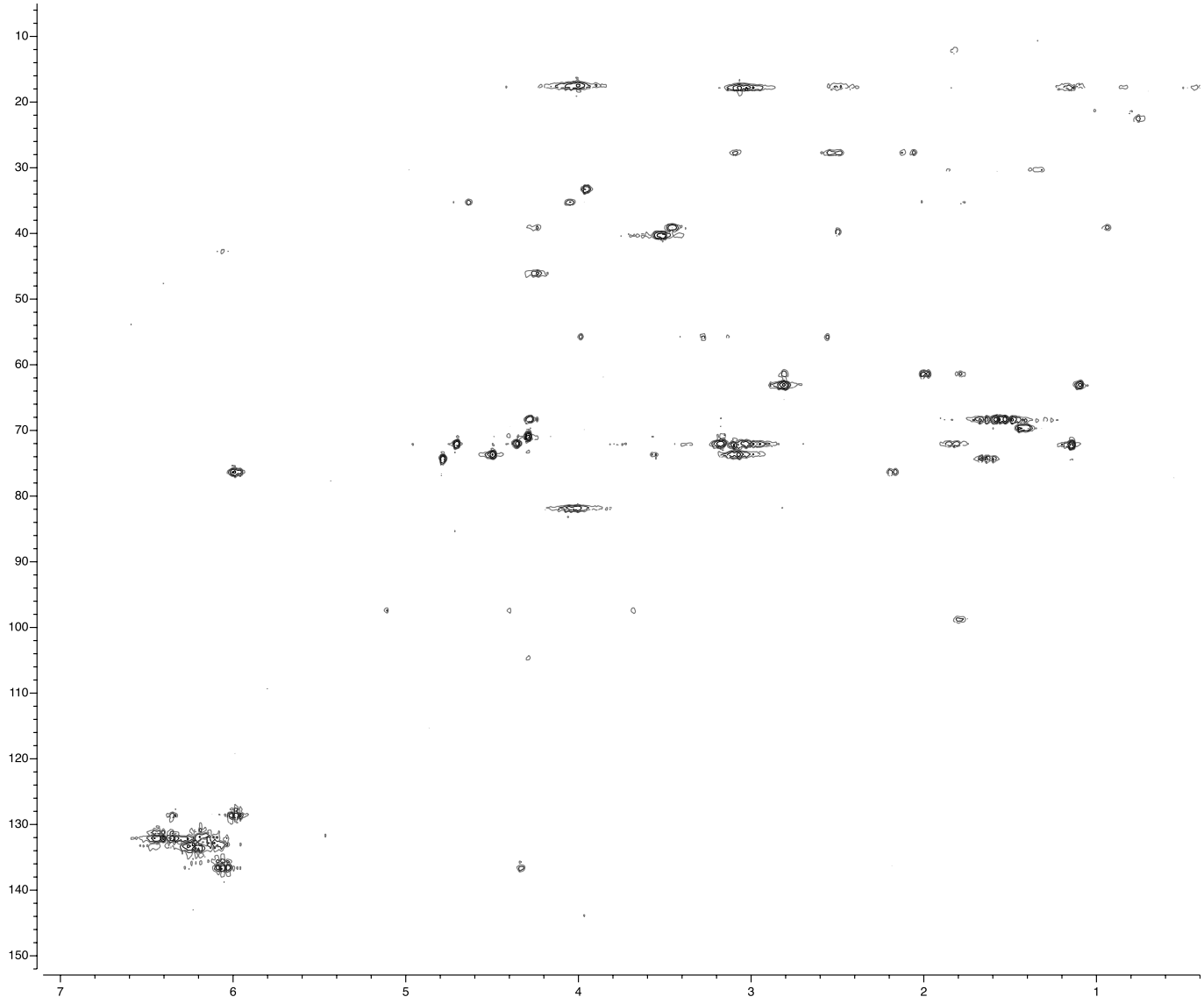

**(d)** 600 MHz TOCSY spectrum of selvamicin in DMSO-$d_6$



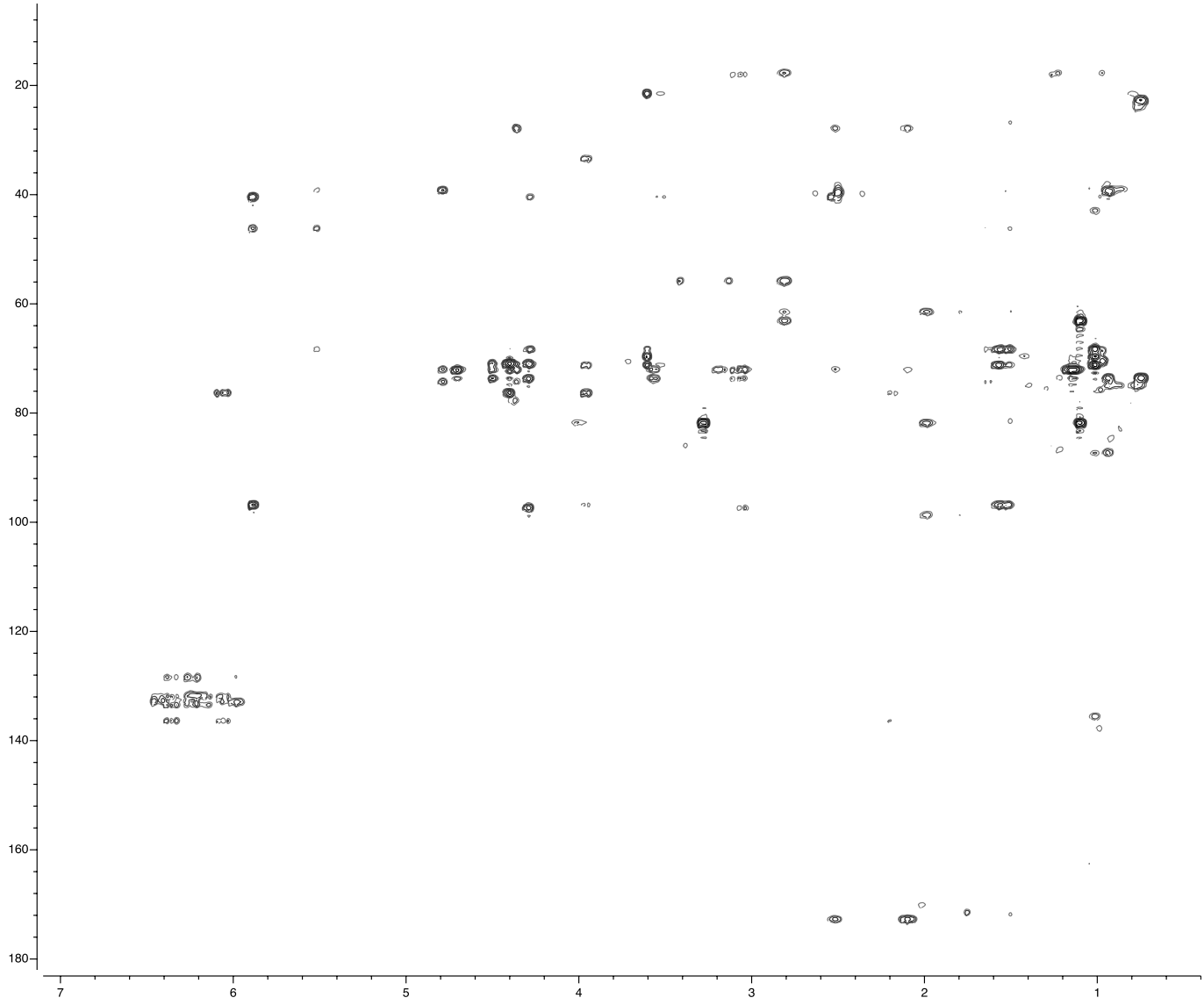

**(e)** 500 MHz ROESY NMR spectrum of selvamicin in DMSO-$d_6$




**(f)** 600 MHz multiplicity-edited HSQC NMR spectrum of selvamicin in DMSO-$d_6$. CH and $CH_3$ group correlations are shown in red and $CH_2$ group correlations are shown in blue.

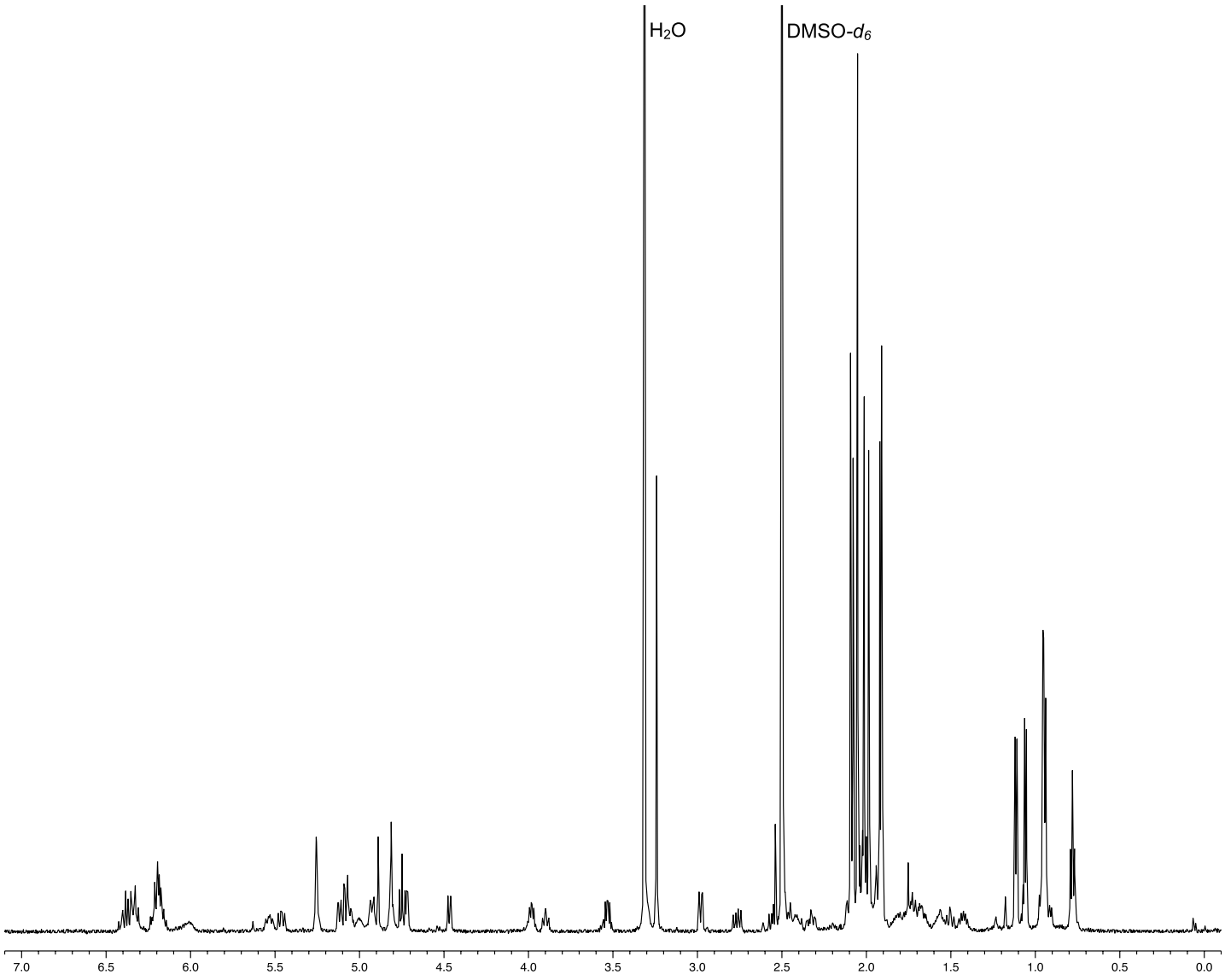



**(g)** 500 MHz H2BC NMR spectrum of selvamicin in DMSO-$d_6$

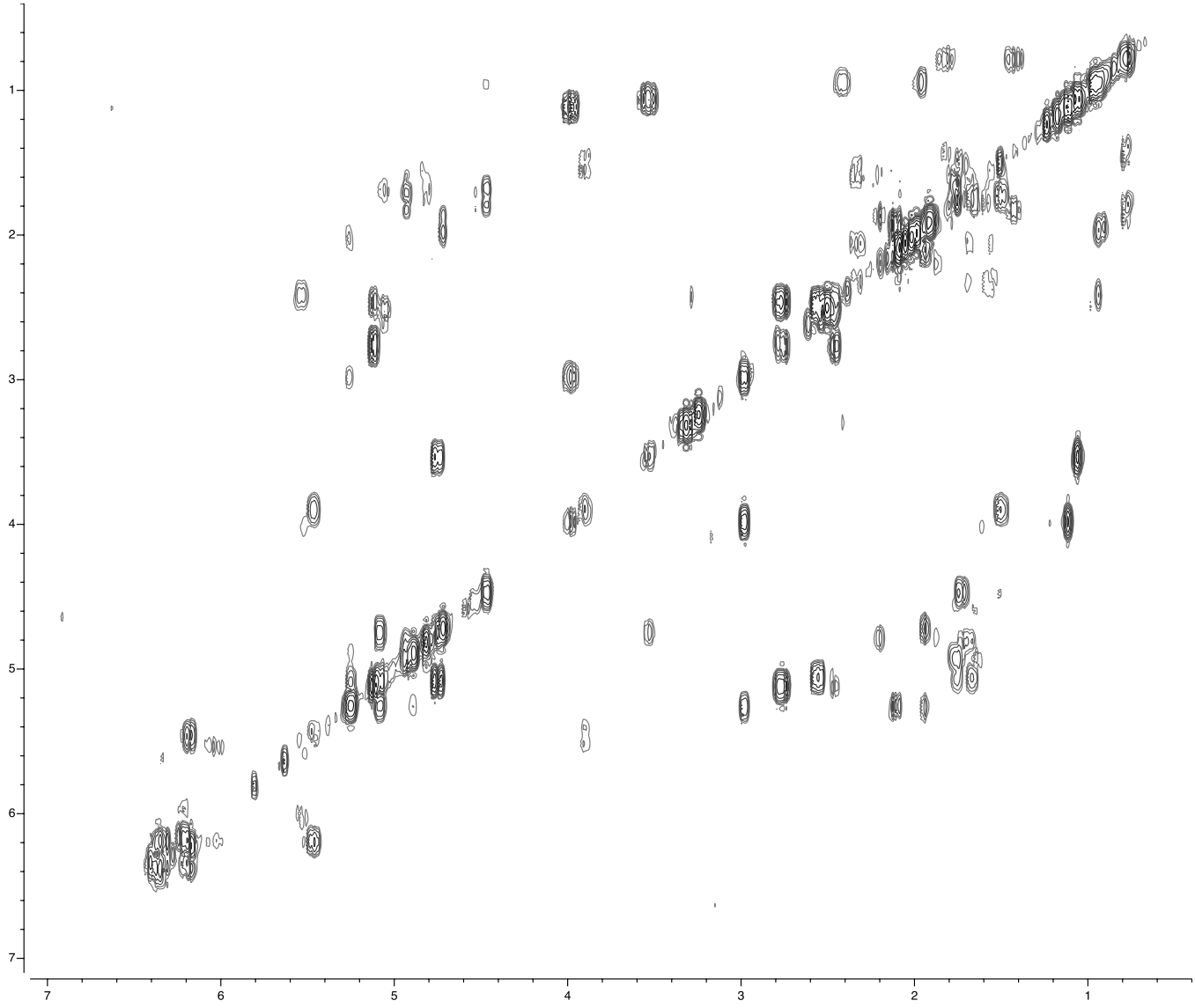



**(h)** 500 MHz HMBC spectrum of selvamicin in DMSO-$d_6$

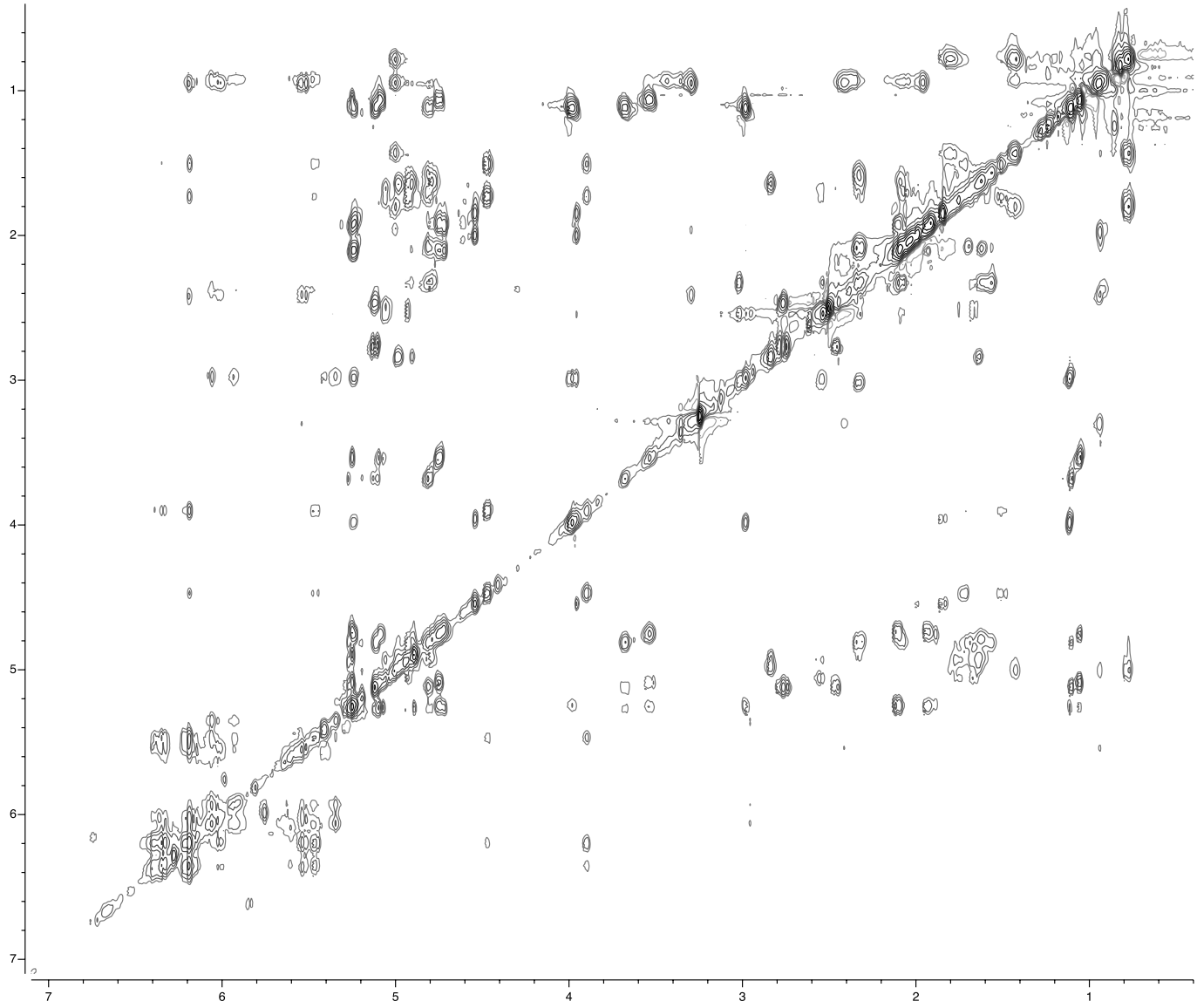
**Figure S6.** $Ac_9$-selvamicin NMR spectra in DMSO-$d_6$



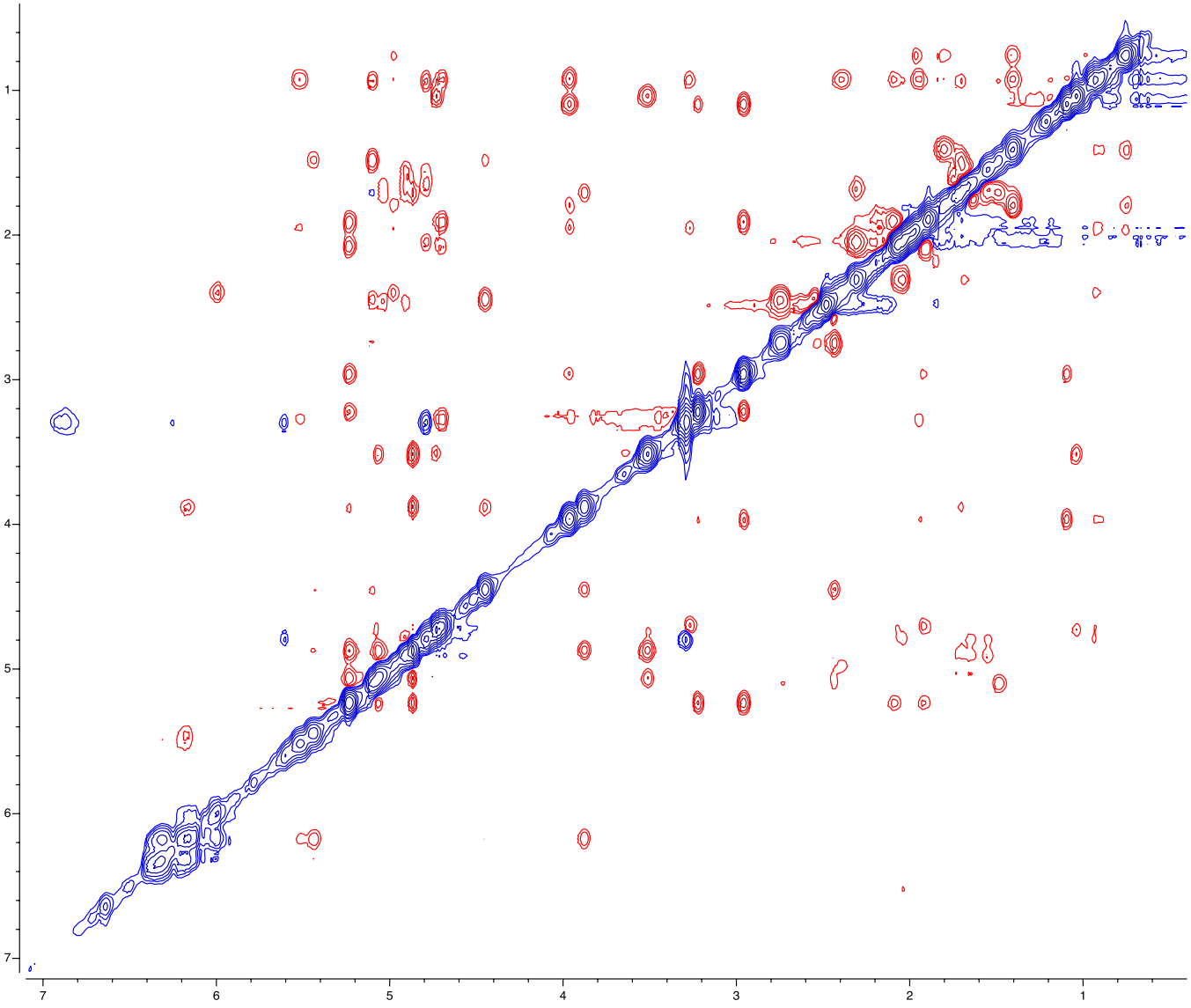

**(a)** 600 MHz $^1$H NMR spectrum of $Ac_9$-selvamicin in DMSO-$d_6$



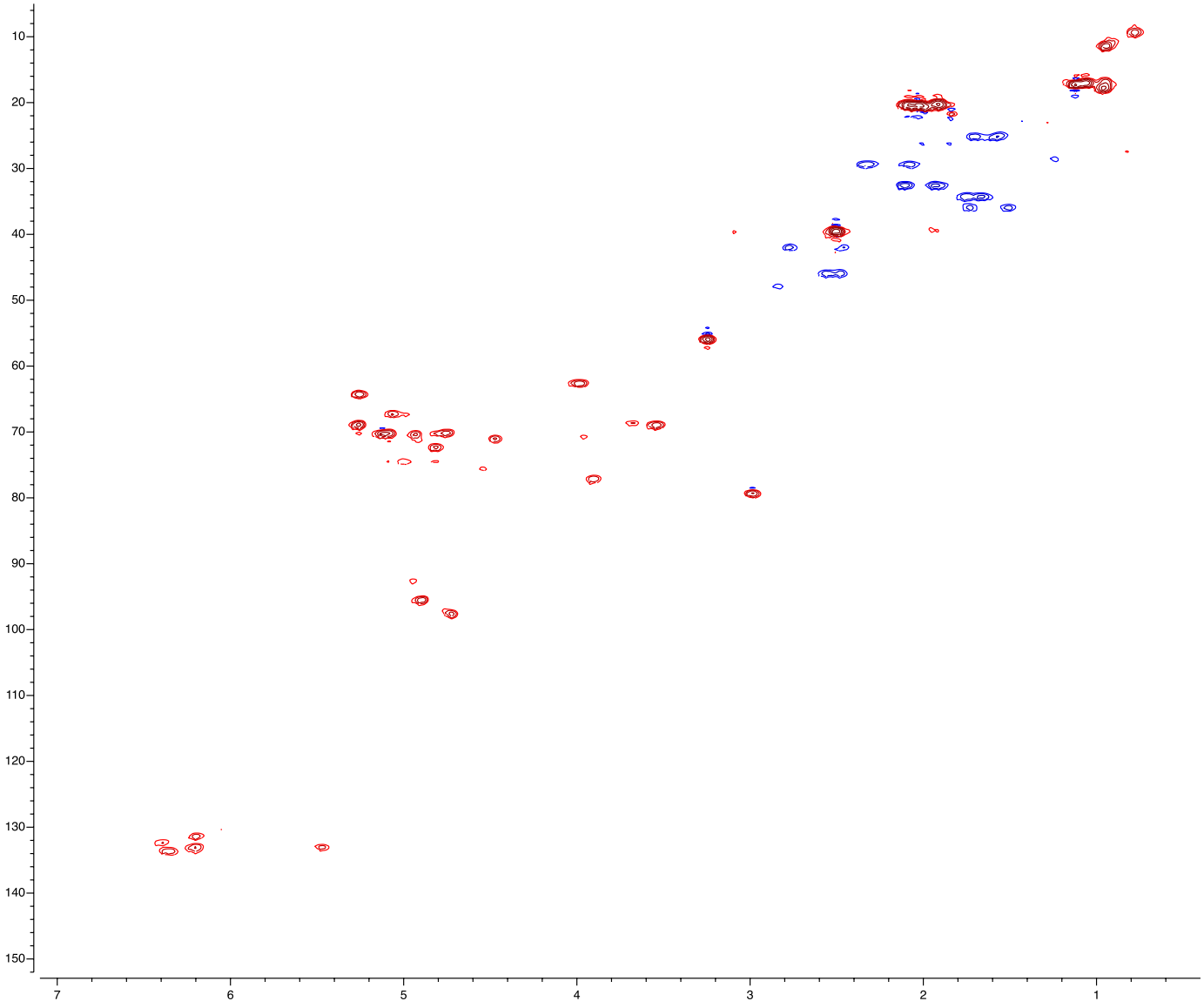

**(b)** 600 MHz COSY spectrum of $Ac_9$-selvamicin in DMSO-$d_6$



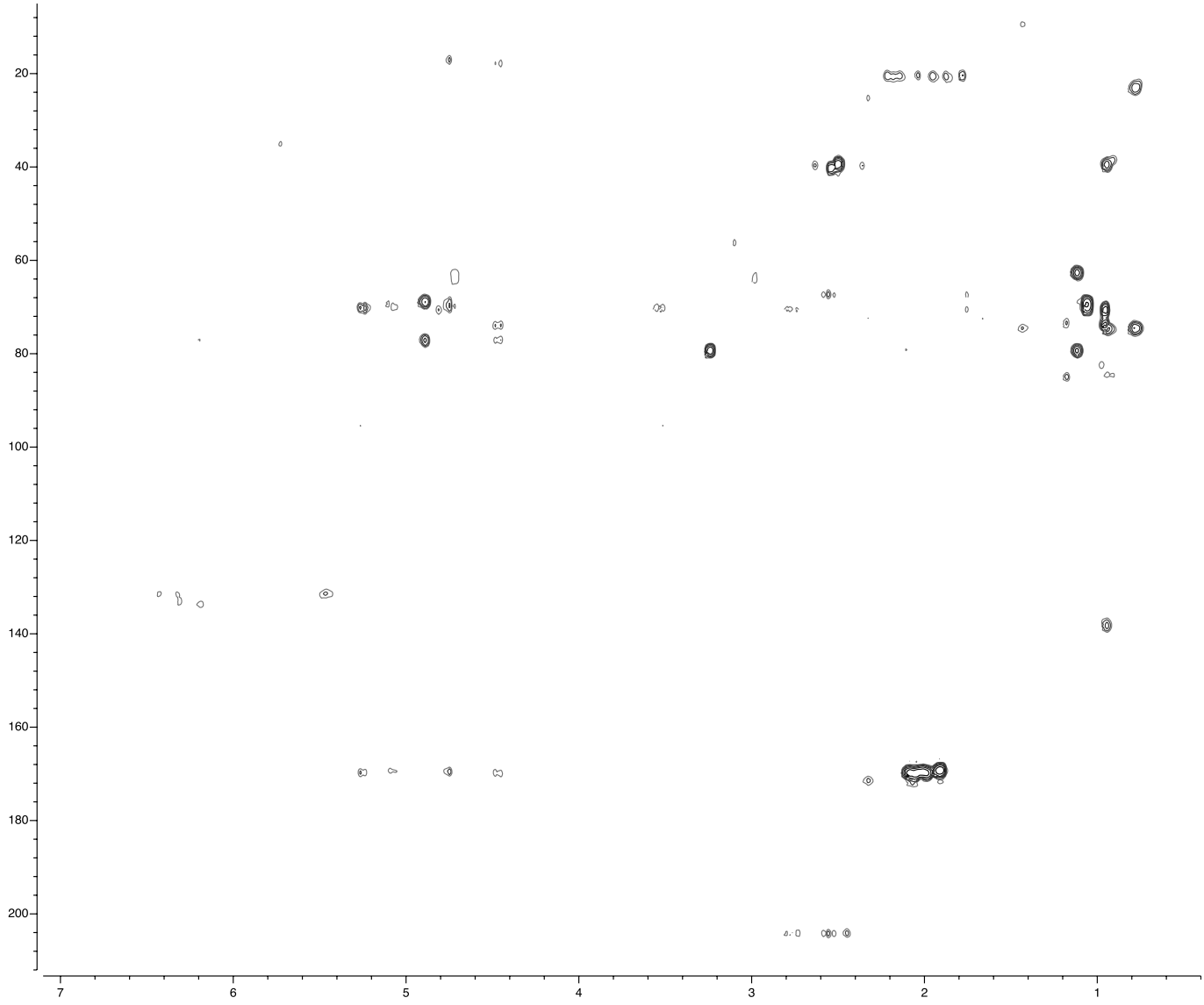

**(c)** 600 MHz TOCSY spectrum of $Ac_9$-selvamicin in DMSO-$d_6$




**(d)** 600 MHz ROESY NMR spectrum of $Ac_9$-selvamicin in DMSO-$d_6$




**(e)** 600 MHz multiplicity-edited HSQC NMR spectrum of $Ac_9$-selvamicin in DMSO-$d_6$. CH and $CH_3$ group correlations are shown in red and $CH_2$ group correlations are shown in blue.




**(f)** 500 MHz HMBC spectrum of $Ac_9$-selvamicin in DMSO-$d_6$

**Figure S7.** Induction of selvamicin production by sodium propionate and sodium butyrate.

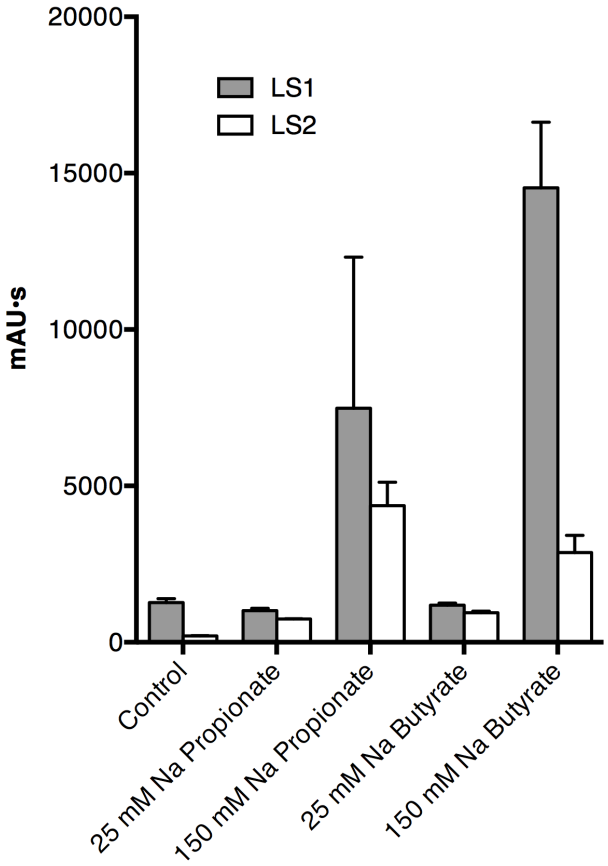


**Figure S8.** Selvamicin mass spectra from HPLC-ESI-HRMS of *Pseudonocardia* culture extracts

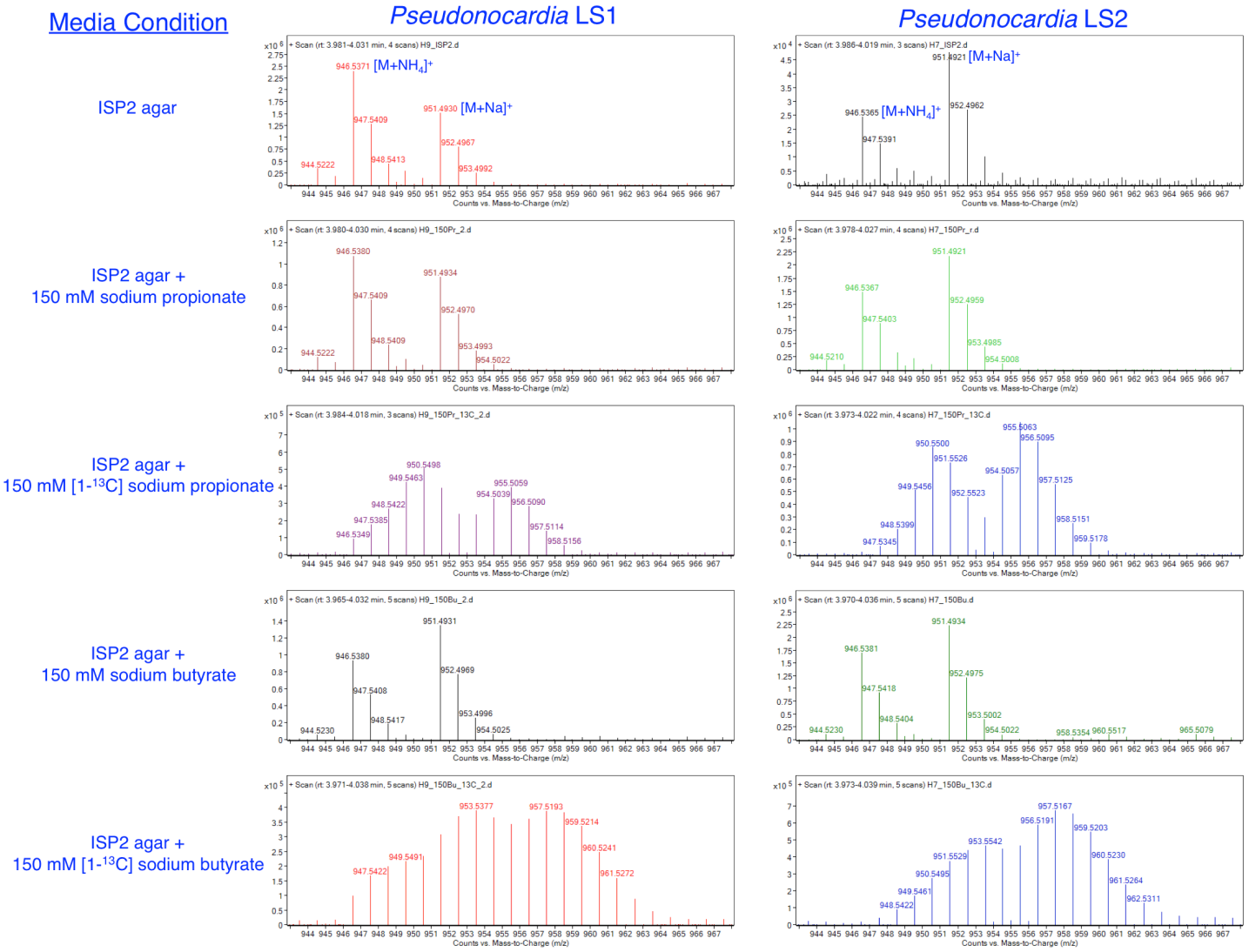



**Table S3.** MIC values (μM) for selvamicin and nystatin against a panel of fungi

| | selvamicin | nystatin |
|---|---|---|
| *Candida albicans* SC5314 | 23 | 1.0 |
| *Saccharomyces cerevisiae* | 21 | 1.1 |
| *Trichoderma harzianum* T22 | 26 | 2.1 |
| *Aspergillus fumigatus* ATCC 1028 | 40 | 1.2 |

**Figure S9.** Isothermal calorimetry traces assaying polyene-sterol interactions

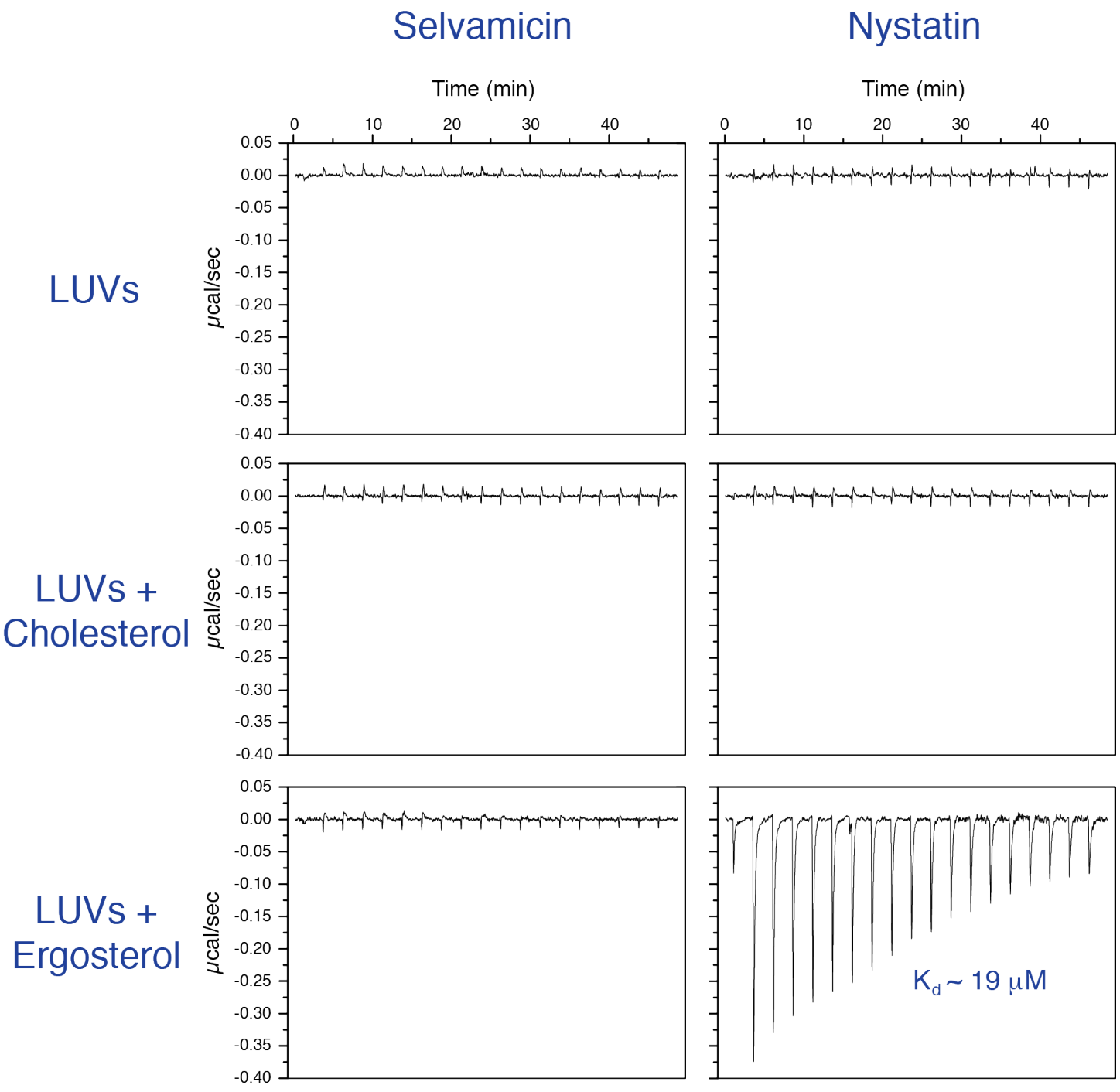


**Table S4.** Predicted proteins of the selvamicin BGC[a]

| | Putative Protein | Putative Function | LS1 top blastp hit *v.* nr proteins (% identity) | Nys BGC[b] homolog (% identity) |
|---|---|---|---|---|
| **SelE** | Thioesterase | Proofreading thioesterase | oleoyl-ACP hydrolase [Streptomyces sp. NRRL S-1868] (60%) | NysE (48%) |
| **SelDIII** | GDP-mannose-4,6-dehydratase | 6-deoxymannose biosynthesis | GDP-mannose 4,6-dehydratase [Streptomyces natalensis] (79%) | NysDIII (78%) |
| **SelI** | Type I PKS | PKS modules 7-12 | beta-ketoacyl synthase [Streptomyces sp. NRRL B-24891] (61%) | NysI (60%) |
| **SelJ** | Type I PKS | PKS module 13 | hypothetical protein VR41_12010 [Streptomyces sp. NRRL B-1568] (61%) | NysJ (58%) |
| **SelSI** | *O*-methyltransferase | 4-*O*-methyldigitoxose biosynthesis | macrocin O-methyltransferase [Streptomyces sp. 769] (58%) | --- |
| **SelSII** | dTDP-4-dehydrorhamnose 3,5-epimerase | 4-*O*-methyldigitoxose biosynthesis | dTDP-4-dehydrorhamnose 3,5-epimerase [Actinobacteria bacterium OK006] (76%) | --- |
| **SelSIII** | glucose-1-phosphate thymidylyltransferase | 4-*O*-methyldigitoxose biosynthesis | glucose-1-phosphate thymidylyltransferase [Streptomyces aureofaciens] (73%) | --- |
| **SelSIV** | dTDP-glucose 4,6-dehydratase | 4-*O*-methyldigitoxose biosynthesis | dTDP-glucose 4,6-dehydratase [Actinokineospora enzanensis] (76%) | --- |
| **SelSV** | Glycosyltransferase | 4-*O*-methyldigitoxose glycosyltransfer | protein IroB [Streptomyces sp. NRRL F-5126] (49%) | --- |
| **SelSVI** | dTDP-hexose 3-ketoreductase | 4-*O*-methyldigitoxose biosynthesis | oxidoreductase [Streptomyces stelliscabiei] (55%) | --- |
| **SelSVII** | dTDP-hexose 2,3-dehydratase | 4-*O*-methyldigitoxose biosynthesis | NDP-hexose 2,3-dehydratase [Sciscionella sp. SE31] (58%) | --- |
| **SelA** | Type I PKS | PKS loading module | modular polyketide synthase [Streptomyces himastatinicus] (47%) | NysA (46%) |
| **SelB** | Type I PKS | PKS modules 1-2 | polyketide synthase [Streptomyces scopuliridis] (62%) | NysB (61%) |
| **SelC** | Type I PKS | PKS modules 3-6 | type I polyketide synthase [Streptomyces sp. NRRL B-24891] (58%) | NysC (56%) |
| **SelK** | Type I PKS | PKS module 14 + thioesterase | type I polyketide synthase [Streptomyces sp. TAA204] (57%) | NysK (51%) |
| **SelL** | P450 monooxygenase | hydroxylation | cytochrome P450 [Streptomyces roseoverticillatus] (54%) | NysL (53%) |
| **SelP** | 2-oxoglutarate and Fe(II)-dependent oxygenase | hydroxylation | phytanoyl-CoA dioxygenase [Streptomyces himastatinicus] (68%) | --- |
| **SelDI** | Glycosyltransferase | 6-deoxymannose glycosyltransfer | MGT family glycosyltransferase [Streptomyces sp. AcH 505] (66%) | NysDI (63%) |
| **SelG** | ABC transporter | Efflux | ABC transporter permease [Saccharothrix syringae] (51%) | --- |
| **SelH** | ABC transporter | Efflux | ABC transporter [Saccharothrix sp. NRRL B-16348] (67%) | NysH (28%) |
| **SelRI** | Transcriptional regulator | Regulation | CppRI [Pseudonocardia autotrophica] (73%) | NysRI (46%) |
| **SelRII** | Transcriptional regulator | Regulation | CppRII [Pseudonocardia autotrophica] (57%) | NysRII (32%) |
| **SelRIII** | Transcriptional regulator | Regulation | hypothetical protein WY02_00420 [Pseudonocardia sp. AL041005-10] (60%) | NysRIII (38%) |
| **SelO** | Decarboxylase | Unknown | CppO [Pseudonocardia autotrophica] (90%) | --- |
| **SelRIV** | Transcriptional regulator | Regulation | CppRIV [Pseudonocardia autotrophica] (74%) | ORF4 (42%) |
| **SelRV** | Transcriptional regulator | Regulation | CppRV [Pseudonocardia autotrophica] (54%) | --- |
| **SelRVI** | Transcriptional regulator | Regulation | hypothetical protein [Pseudonocardia sp. EC080625-04] | --- |

[a]Predicted genes/pseudogenes and the gene products derived from sequences < 250 bp are omitted from the table
[b]Nystatin BGC from *S. noursei* ATCC 11455 (accession no. AF263912)

**Figure S10.** Extractions from PKS domain alignments. Active site residues (5) and AT specificity motifs (6) are in bold.

AT Domains:

| | Specificity motif | Active Site |
|---|---|---|
| *Mal-CoA* | **HAFH** | |
| *MeMal-CoA* | **YASH** | **GHS G** |
| LM_LS1 | ----------DPELD | TPRRVAG**SA**V**G**EVAAAHV |
| LM_LS2 | ----------DPELD | TPRRVAG**AA**V**G**EVAAAHV |
| M1_LS1 | MIAVD**YASH**SAHVEAIEQ | HPDAVL**GHS**Q**G**EIAAAVV |
| M1_LS2 | MIAVD**YASH**SAHVEAIEQ | HPDAVL**GHS**Q**G**EIAAAVV |
| M2_LS1 | RVDVD**YASH**GTHVEAVRD | EPAAVV**GHS**Q**G**EIAAAHV |
| M2_LS2 | RVDVD**YASH**GTHVEAVRD | EPAAVV**GHS**Q**G**EIAAAHV |
| M3_LS1 | RLATS**HAFH**SPLMAPMIE | APDYLV**GHS**I**G**EIAAAHV |
| M3_LS2 | RLATS**HAFH**SPLMAPMVE | APDYLV**GHS**I**G**EIAAAHV |
| M4_LS1 | RLATS**HAFH**SPSMAPMLD | TPERVV**GHS**I**G**EIAAAHV |
| M4_LS2 | RLATS**HAFH**SPSMAPMLD | TPERVV**GHS**I**G**EIAAAHV |
| M5_LS1 | RLATS**HAFH**SPLMAPMME | VPDHLV**GHS**I**G**EIAAAHV |
| M5_LS2 | RLATS**HAFH**SPLMAPMME | VPDHLV**GHS**I**G**EIAAAHV |
| M6_LS1 | RLRTS**HAFH**SPLMAPMME | VPDHLV**GHS**I**G**EIAAAHV |
| M6_LS2 | RLATS**HAFH**SPLMAPMME | RPDRLV**GHS**I**G**EIAAAHV |
| M7_LS1 | RLRVS**HAFH**SPLMEPMLA | RPTQLI**GHS**I**G**EIAAAHV |
| M7_LS2 | RLRVS**HAFH**SPLMEPMLA | RPTQLI**GHS**I**G**EIAAAHV |
| M8_LS1 | RLRTS**HAFH**SPLMAPMLD | VPDRLA**GHS**I**G**EIAAAHV |
| M8_LS2 | RLRTS**HAFH**SPLMAPMLD | VPDRLA**GHS**I**G**EIAAAHV |
| M9_LS1 | RIAVD**YASH**SAYVEAVEE | TPDAVL**GHS**Q**G**EIAAAVV |
| M9_LS2 | RIAVD**YASH**SAYVEAVEE | TPDAVL**GHS**Q**G**EIAAAVV |
| M10_LS1 | ELTVS**HAFH**SPLMDPMLA | HPDQVA**GHS**I**G**ELAAAHV |
| M10_LS2 | ELTVS**HAFH**SPLMDPMLA | HPDQVA**GHS**I**G**ELAAAHV |
| M11_LS1 | RLRVS**HAFH**SPLMDPMLD | VPDVLA**GHS**V**G**EIAAAHV |
| M11_LS2 | RLRVS**HAFH**SPLMDPMLD | VPDVLA**GHS**V**G**EIAAAHV |
| M12_LS1 | RLSVS**HAFH**SPLMDPITE | TPAFVA**GHS**V**G**EIAAAHV |
| M12_LS2 | RLPVS**HAFH**SPLMDPITE | TPAFVA**GHS**V**G**EIAAAHV |
| M13_LS1 | RLATS**HAFH**SPLMAPMME | VPDHLV**GHS**I**G**EIAAAHV |
| M13_LS2 | RLATS**HAFH**SPLMAPMME | VPDHLV**GHS**I**G**EIAAAHV |
| M14_LS1 | RLSVS**HAFH**SPLMDPMLE | RPGMLA**GHS**V**G**EIAAAHV |
| M14_LS2 | RLSVS**HAFH**SPLMDPMLE | RPGMLA**GHS**V**G**EIAAAHV |

DH Domains:

| | H G P | Y |
|---|---|---|
| LM_LS1 | WIAD**H**RPG**G**GGATL**P**VPA | GERLDGAG--**F**GPDLAGL |
| LM_LS2 | WIAD**H**RPG**G**GGATL**P**VPA | GERLDGAG--**F**GPDLAGL |
| M3_LS1 | WLAD**H**EVA**G**-RALL**P**GTA | YERLTDLGFR**Y**GPTFRGL |
| M3_LS2 | WLAD**H**EVA**G**-RALL**P**GTA | YERLTDLGFR**Y**GPTFRGL |
| M4_LS1 | WLVD**H**AVS**G**-TVLL**P**GSA | YQRFADDGFD**Y**GPVFRGL |
| M4_LS2 | WLVD**H**AVS**G**-TVLL**P**GSA | YQRFADDGFD**Y**GPVFRGL |
| M5_LS1 | WLAD**H**VVG**G**-RVLL**P**GTA | YDRLAETGLA**Y**GPAFRGL |
| M5_LS2 | WLAD**H**VVG**G**-RVLL**P**GTA | YDRLAETGLA**Y**GPAFRGL |
| M6_LS1 | WLAD**H**TVG**G**-RVLL**P**GTA | YDRFAEAGFG**Y**GPAFRGL |
| M6_LS2 | WLAD**H**TVG**G**-RVLL**P**GTA | YDRFAEAGFG**Y**GPAFRGL |
| M7_LS1 | WLAD**H**AVH**G**-RVLL**P**GTA | YDSLAAAGLE**Y**GTTFQGL |
| M7_LS2 | WLAD**H**AVY**G**-RVLL**P**GTA | YDSLAAAGLE**Y**GATFQGL |
| M13_LS1 | WLAD**H**VVG**G**-AVAL**P**GTG | YA--TDTGVQ**Y**GPVFRGL |
| M13_LS2 | WLAD**H**VVG**G**-AVAF**P**GTG | YA--TDTGVQ**H**GPVFRGL |
| M14_LS1 | WLAD**H**VVG**G**-HVIM**P**GAA | YERYAETGLQ**Y**GPAFRGL |
| M14_LS2 | WLAD**H**VVG**G**-HVIM**P**GAA | YERYAETGLQ**Y**GPAFRGL |

ER Domains:

| | K D |
|---|---|
| M13_LS1 | QVLDLG**P**TD--DPVGQPGTTDAAS**A**LDTVD |
| M13_LS2 | QVLDLG**P**TD--DPVGQPGTTDAAS**A**LDTVD |
| M14_LS1 | RFVEMG**K**TDVRDPDALPGVAYRAF**D**LMEAG |
| M14_LS2 | RFVEMG**K**TDVRDPDALPGVAYRAF**D**LMEAG |

KR Domains:

| | S Y N |
|---|---|
| M1_LS1 | TFVLYT**S**TAGMWGSGRHAA**Y**AAG**N**AYLSAL |
| M1_LS2 | TFVLYT**S**TAGMWGSGRHAA**Y**AAG**N**AYLSAL |
| M2_LS1 | AFVLFS**S**GASAWGSGGQPG**Y**AAA**N**AWLDAL |
| M2_LS2 | AFVLFS**S**GASAWGSGGQPG**Y**AAA**N**AWLDAL |
| M3_LS1 | AFVLFS**S**VAAVVGSPGQGN**Y**AAG**N**AALDAL |
| M3_LS2 | AFVLFS**S**VAAVVGSPGQGN**Y**AAG**N**AALDAL |
| M4_LS1 | AFVLFS**S**LAGTLGSAGQAN**Y**AAA**N**AFLDGL |
| M4_LS2 | AFVLFS**S**LAGTLGSAGQAN**Y**AAA**N**AFLDGL |
| M5_LS1 | GFVLFS**S**VAGTLGAAGQAN**Y**AAA**N**AFLDAL |
| M5_LS2 | GFVLFS**S**VAGTLGAAGQAN**Y**AAA**N**AFLDAL |
| M6_LS1 | GFVLFS**S**VAGTAGSAGQAN**Y**AAG**N**AFLDAL |
| M6_LS2 | GFVLFS**S**VAGTAGSAGQAN**Y**AAG**N**AFLDAL |
| M7_LS1 | AFVLFS**S**VAGTAGAAGQGN**Y**AAA**N**AALDSL |
| M7_LS2 | AFVLFS**S**VAGTAGAAGQGN**Y**AAA**N**AALDSL |
| M8_LS1 | LFVLFS**S**IAGVWGSRGQAA**Y**AAG**N**AALDAL |
| M8_LS2 | LFVLFS**S**IAGVWGSRGQAA**Y**AAG**N**AALDAL |
| M9_LS1 | AFVLFS**S**TAGMWGSGAHAA**Y**VAG**N**AYLAAL |
| M9_LS2 | AFVLFS**S**TAGMWGSGAHAA**Y**VAG**N**AYLAAL |
| M10_LS1 | AFVLFS**S**VAGTIGAAGQGN**Y**AAA**N**AVLDAA |
| M10_LS2 | AFVLFS**S**VAGTIGAAGQGN**Y**AAA**N**AVLDAA |
| M11_LS1 | AFVLCT**T**IAATWGVRGQDA**D**AETG**A**AYTAI |
| M11_LS2 | AFVLCT**T**IAATWGVRGQDA**D**AETG**A**AYTAI |
| M12_LS1 | AFVLFA**S**ASAAVGNAGQAN**Y**AAA**N**AVLDAL |
| M12_LS2 | AFVLFA**S**ASAAVGNAGQAN**Y**AAA**N**AVLDAL |
| M13_LS1 | AFVLYS**S**TAGVIGSPGQSN**Y**AAA**N**AGLDAL |
| M13_LS2 | AFVLYS**S**TAGVIGSPGQSN**Y**AAA**N**AGLDAL |
| M14_LS1 | AFVLFS**S**ISGLIGGLGQGN**Y**SAA**N**TFLDAL |
| M14_LS2 | AFVLFS**S**ISGLIGGLGQGN**Y**SAA**N**TFLDAL |

**Figure S11.** Schematic of selvamicin PKS domain architecture. Putative inactive domains are shaded gray.

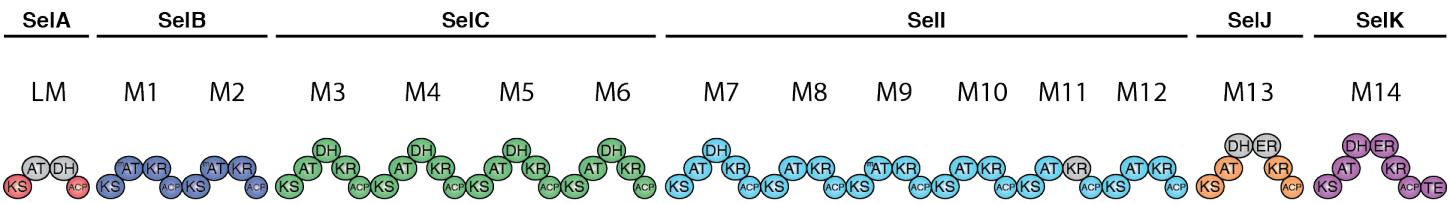
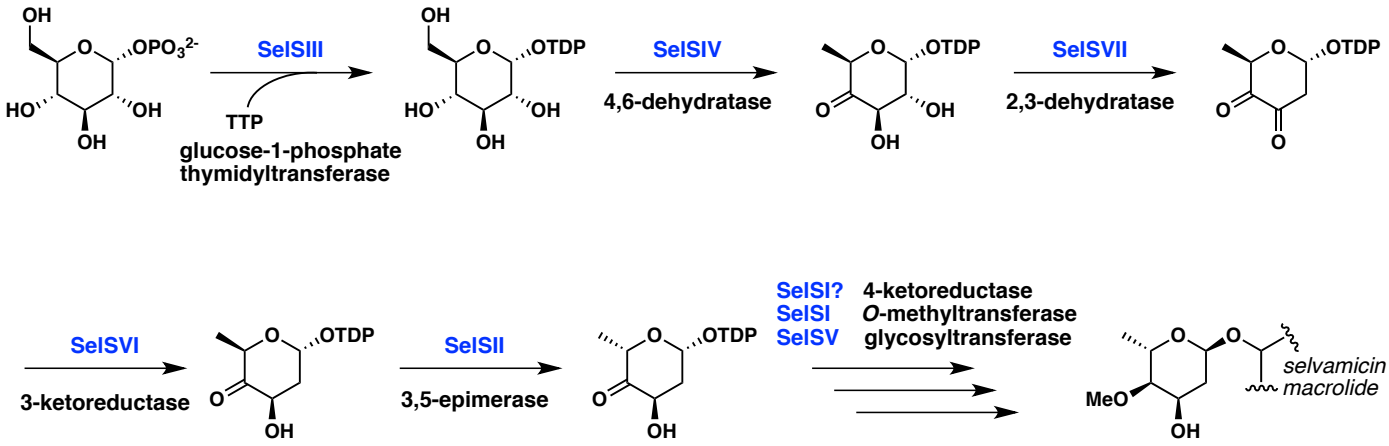



**Figure S12.** Proposed reactions carried out by the selvamicin 4-*O*-methyldigitoxose sugar subcluster



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