

Supporting Information

Photopiperazines A-D, photo-sensitive interconverting diketopiperazines with significant and selective activity against U87 glioblastoma cells, from a rare, marine-derived actinomycete of the family *Streptomycetaceae*.

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Collection and Phylogenetic Identification of strain AJS-327.

An unidentified sponge fragment was collected in approximately 200 m south of Scripps Pier in La Jolla, CA along the shoreline. The material was collected in a sterile 50 ml tube and transported to the laboratory within one hour. The sponge was chopped with sterile scissors without rinsing or surface-sterilizing, and fragments plated on A1 agar plates (10 g soluble potato starch, 4 g yeast extract, 2 peptone, 750 ml natural seawater, 250 ml distilled water, and 18 g agar). Colonies were removed and cultivated first in small scale for phylogenetic analysis and subsequently in larger scale for chemical examination.

Genomic DNA from strain AJS-327 was extracted using the Extract-N-Amp Plant DNA Extraction Kit (Sigma) according to the manufacturer's instructions. The partial 16S rDNA ribosomal gene was amplified using primers 27F and 1492R and the PCR product was submitted for sequencing at the UC Berkeley DNA Sequencing Facility. Chromatograms were analyzed by Finch TV (version 1.5.0). The sequence was submitted to GenBank (accession number MK17028). The sequence, (1378 bp), was analyzed by NCBI BLASTn search. The closest matching type strains were *Streptomyces cacaoi* strain NBRC 12748 (96% identity; accession number NR_041061.1), *Streptomyces oryzae* strain NBRC 109761 (96% identity; accession number NR_146025.1), *Streptomyces artemisiae* strain YIM 63135 (96% identity; accession number NR_116242.1), and *Streptomyces armeniacus* strain NBRC 12555 (96% identity; accession number NR_112247.1).

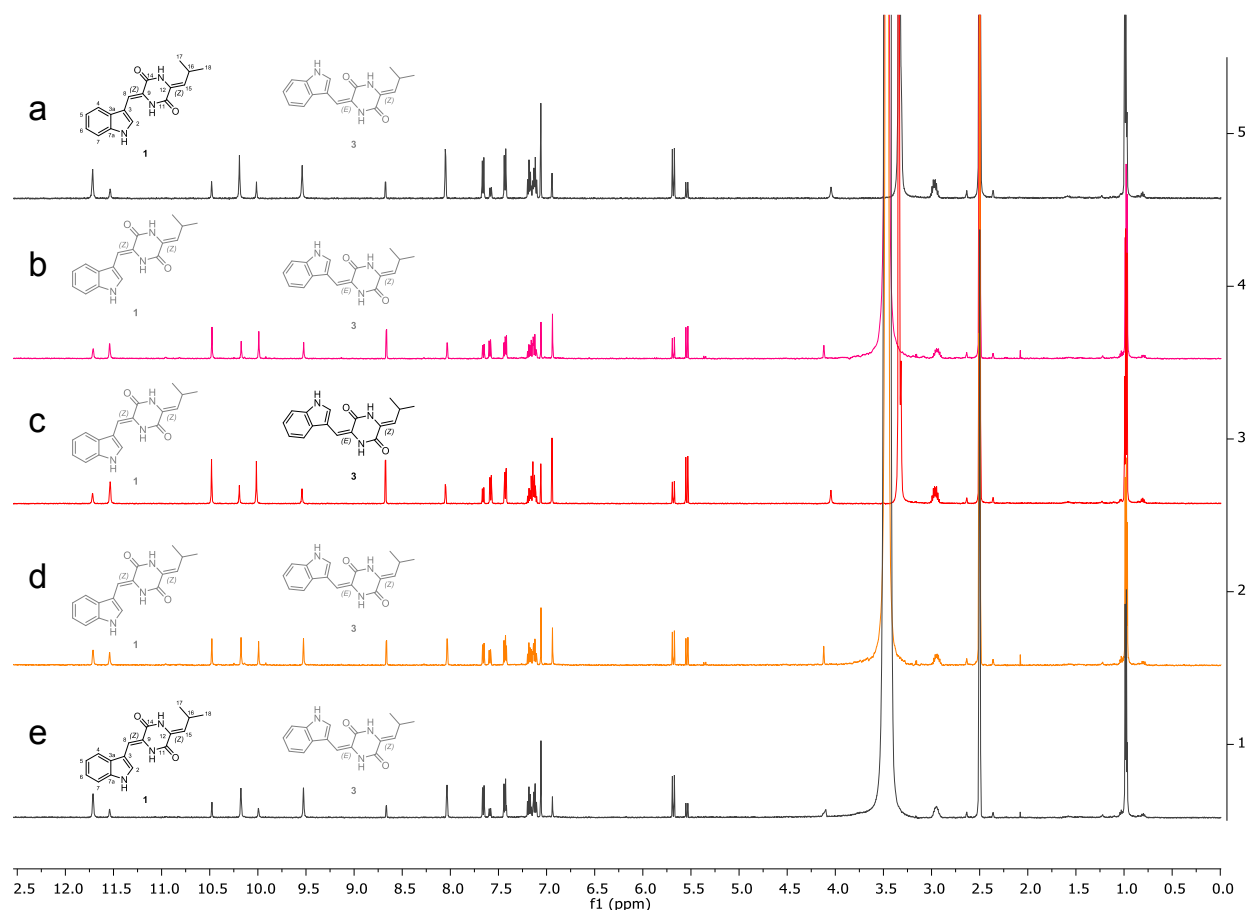
Cultivation and Extraction of strain AJS-327.

Strain AJS-327 was cultured in 8 x 1 L volumes using an A1 medium (10 g of starch, 4 g of yeast, 2 g of peptone, and 1 L seawater) while shaking at 180 rpm for 7 days at 27 °C. After 7 days, the broth was extracted with 16 L ethyl acetate, and the solvent was removed under vacuum to yield 1.3 g of organic extract.

Isolation of Photopiperazines A-D (1-4).

The organic extract (1.3 g) was subjected to silica vacuum flash chromatography, using a step gradient of dichloromethane (DCM) and MeOH (100% DCM to 100% MeOH) to afford seven fractions. Aliquots of fraction 5 (850 mg) were subjected to C-18 reversed phase semi-preparative scale HPLC (Phenomenex Luna C-18 column, 250 × 10, 10 μ m; 3 mL/min; 30% to 60% MeCN/H₂O over 30 min; UV detection at 210 nm) to yield peak 1 (3.7 mg, t_R 21.3 min), peak 2 (4.1 mg, t_R 22.3 min), and peak 3 (5.7 mg, t_R 23.2 min), see Figure 1. NMR analysis of each isolated peak indicated that peak 1 was a mixture of photopiperazines A and C in a ratio of 1.0 to 0.2. Peak 2 was found to be a mixture of photopiperazines B and D in a ratio of 1.0 to 0.4, while peak 3 was found to contain all 4 geometrical isomers in an integrated A to B to C to D ratio of 0.1:1.0:0.02:0.7.

Figure S1. ^1H NMR time course ($\text{DMSO-}d_6$) illustrating photopiperazine A (**1**) undergoing photoisomerization to photopiperazine C (**3**) and returning to its original mixture. a) initial sample of **1**. b) **1** was exposed to long wavelength light (365 nm) for 1 hr. c) **1** was exposed with long wavelength light (365 nm) for 2 hr. d) mixture under room light for 3 hr. e) converted **1** was under room light for 6 hr. The dark structures are the major components, while the lighter structures are minor or in close to equal concentrations.



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Figure S2. ^1H NMR spectrum of photopiperazine A (**1**) mix in $\text{DMSO}-d_6$.

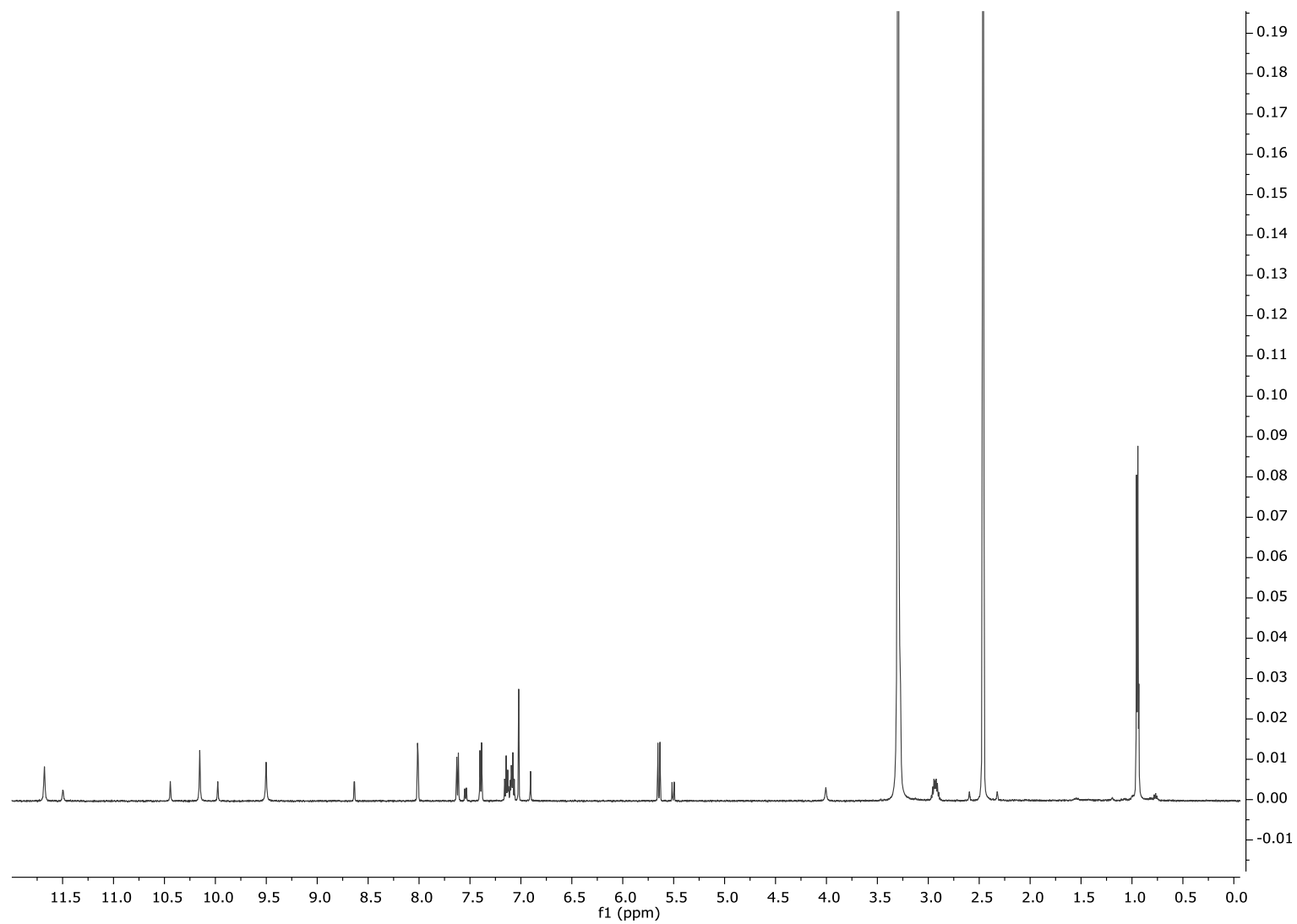


Figure S3. ^1H - ^1H COSY spectrum of photopiperazine A (**1**) mix in $\text{DMSO-}d_6$.

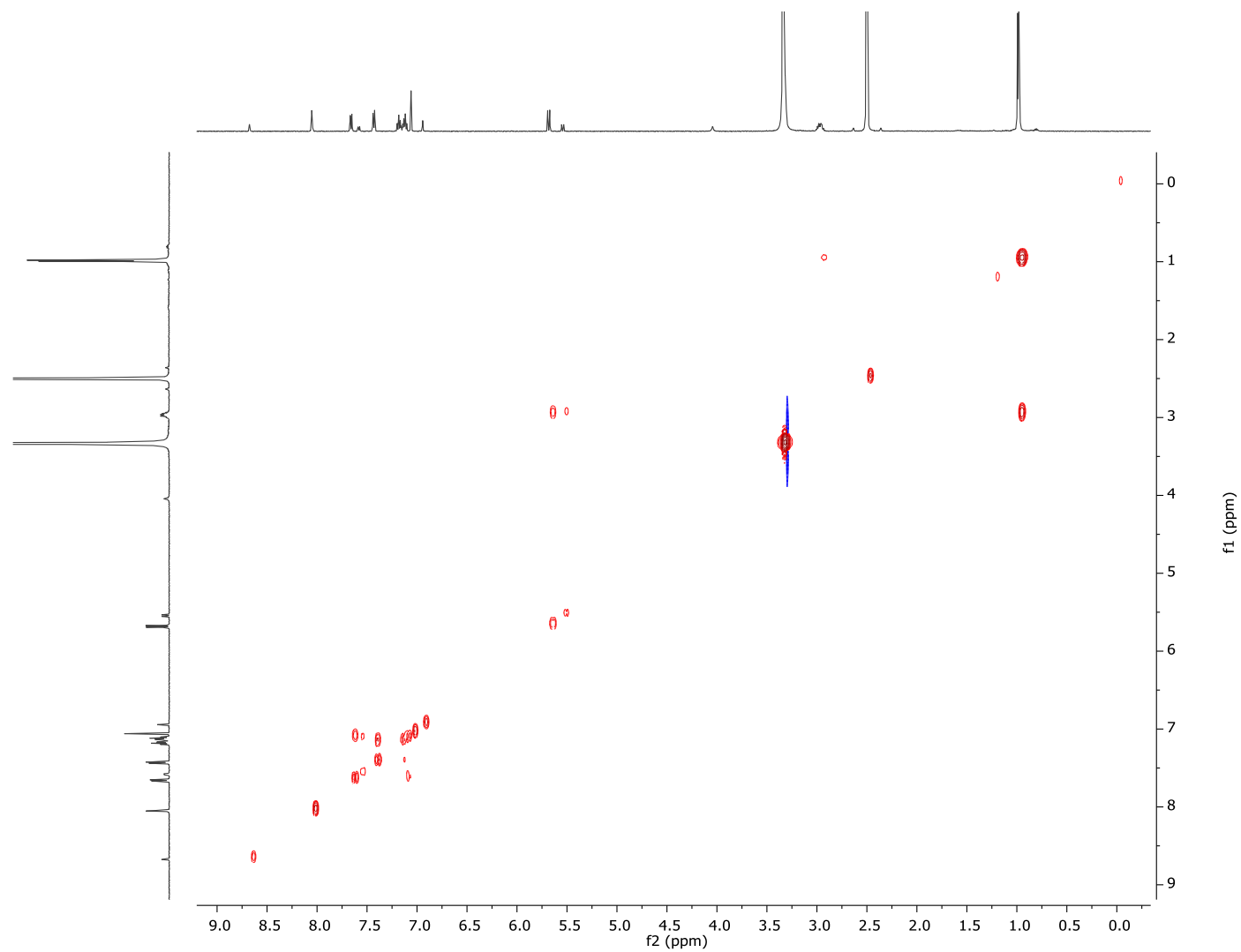


Figure S4. HSQC spectrum of photopiperazine A (**1**) mix in DMSO- d_6 .

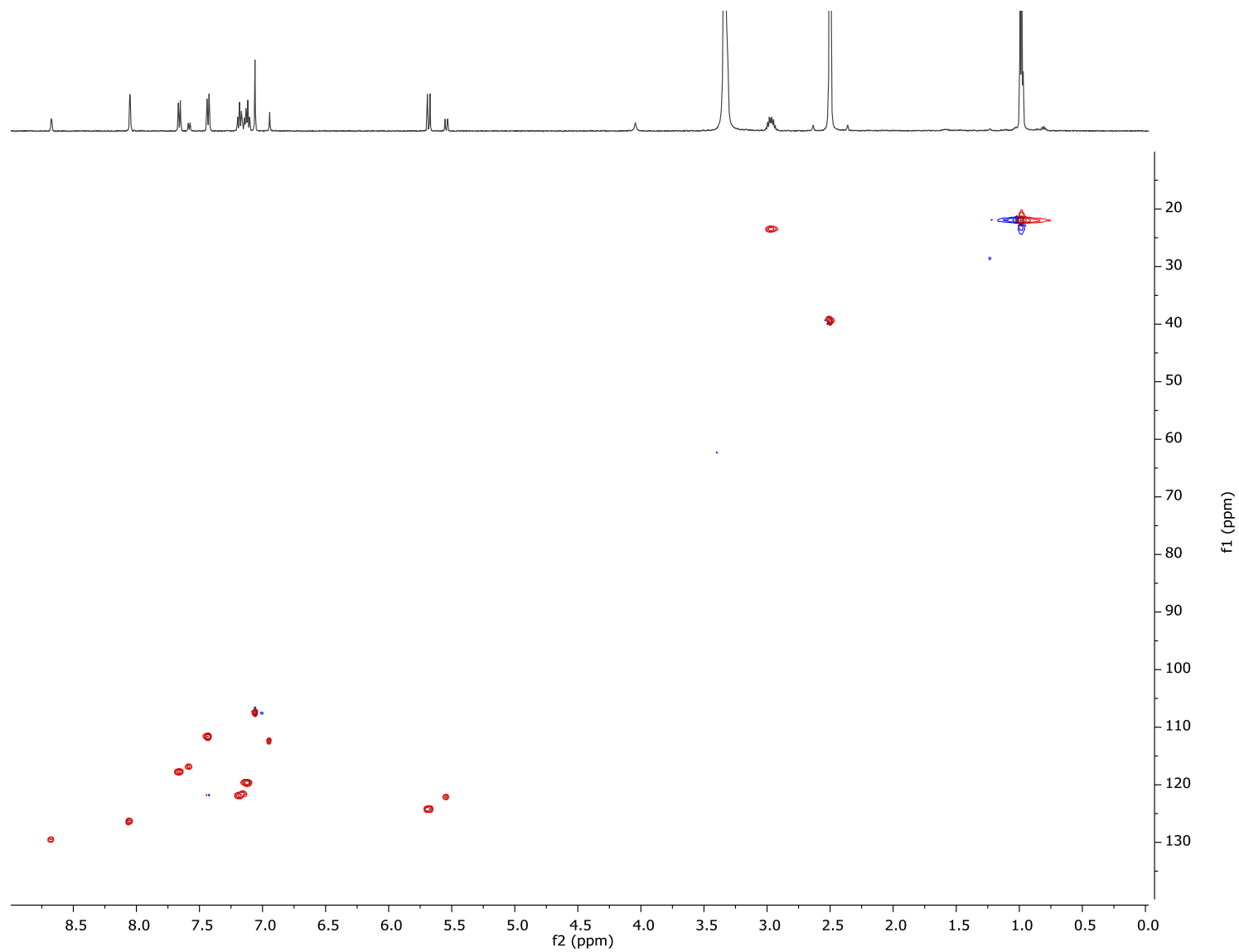


Figure S5. HMBC spectrum of photopiperazine A (**1**) mix in DMSO- d_6 .

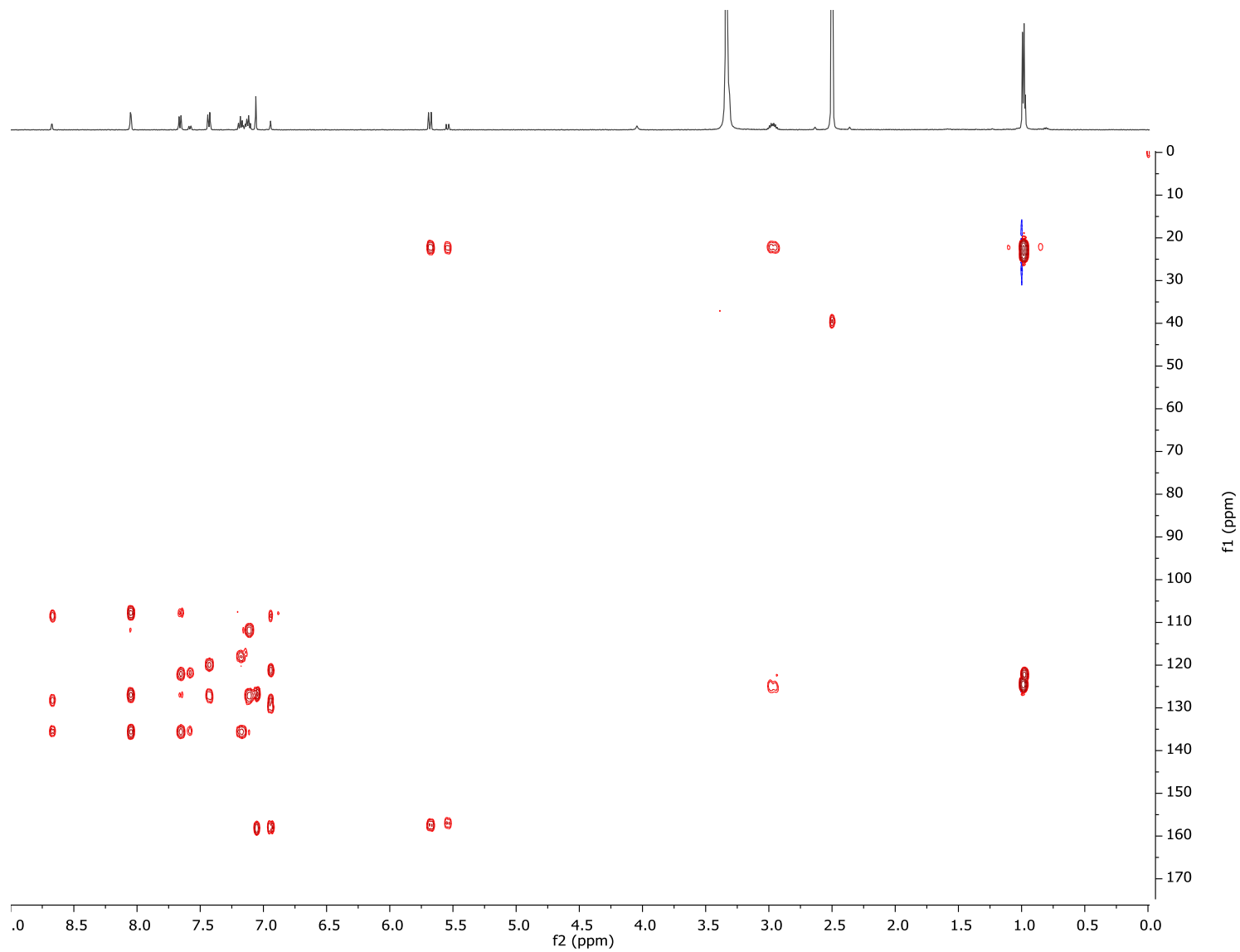


Figure S6. HR-ESI-TOFMS spectrum of photopiperazine A (**1**).

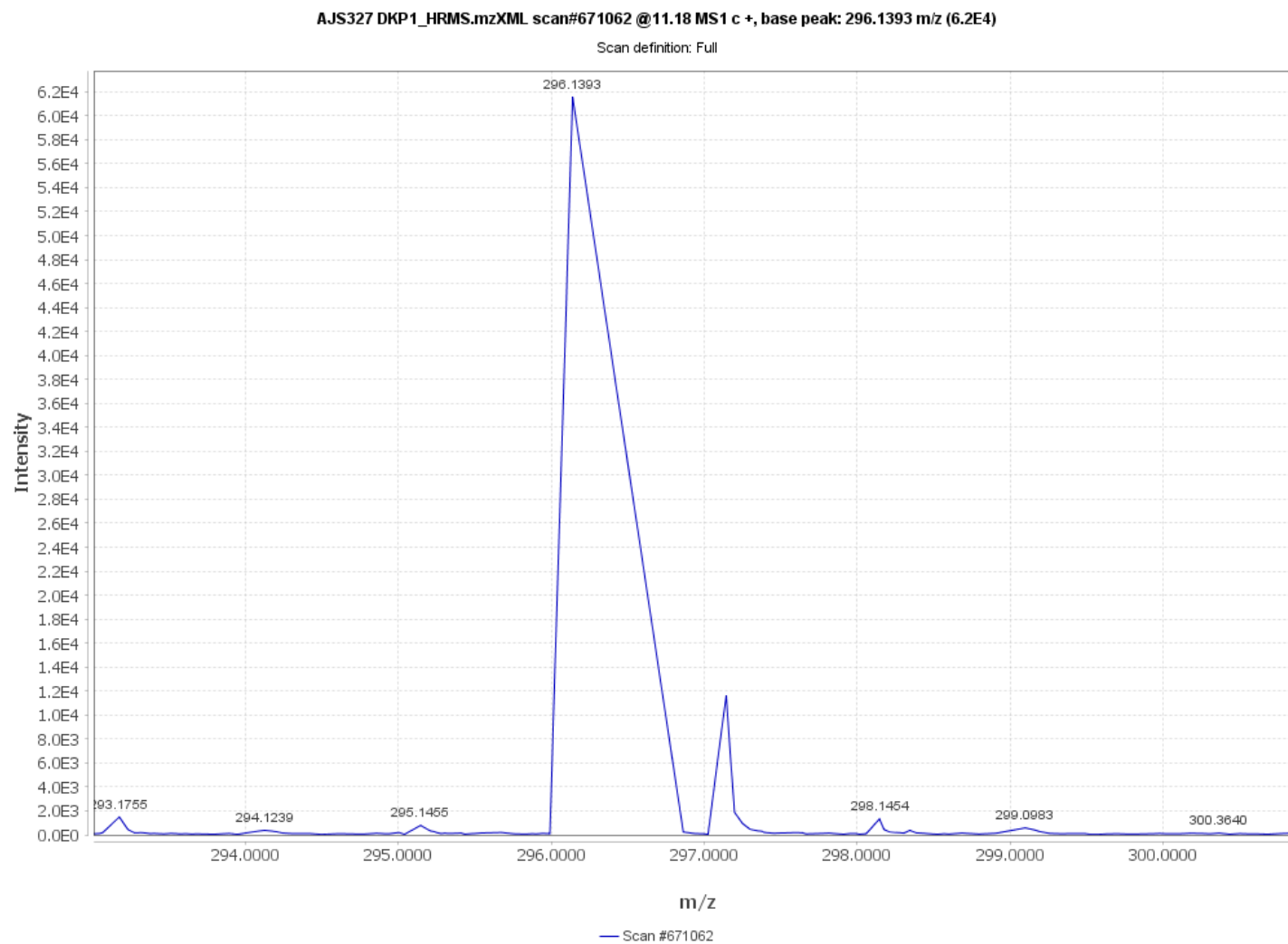


Figure S7. ^1H NMR spectrum of photopiperazine B (**2**) mix in $\text{DMSO}-d_6$.

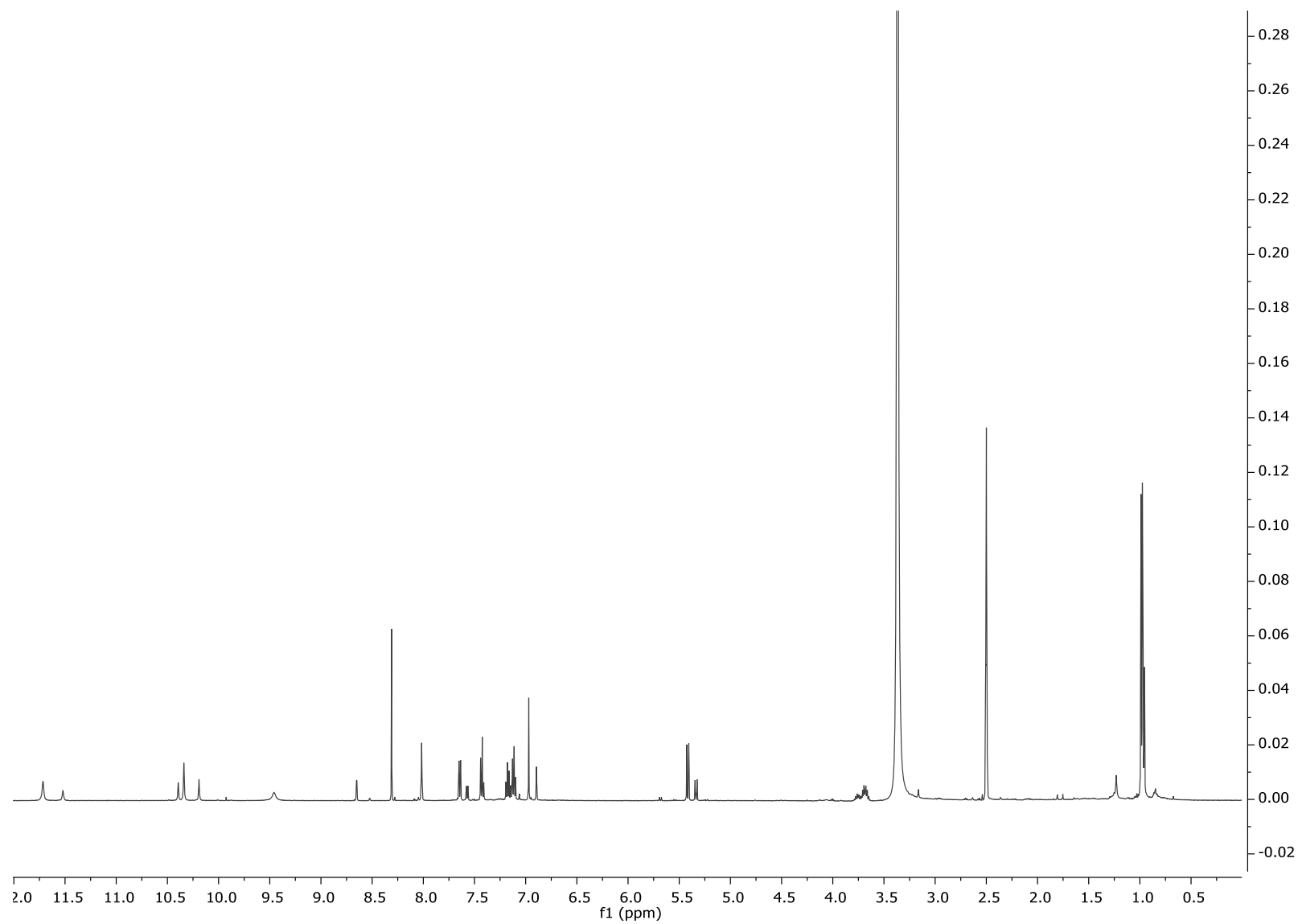


Figure S8. ^{13}C NMR spectrum of photopiperazine B (**2**) mix in $\text{DMSO-}d_6$.

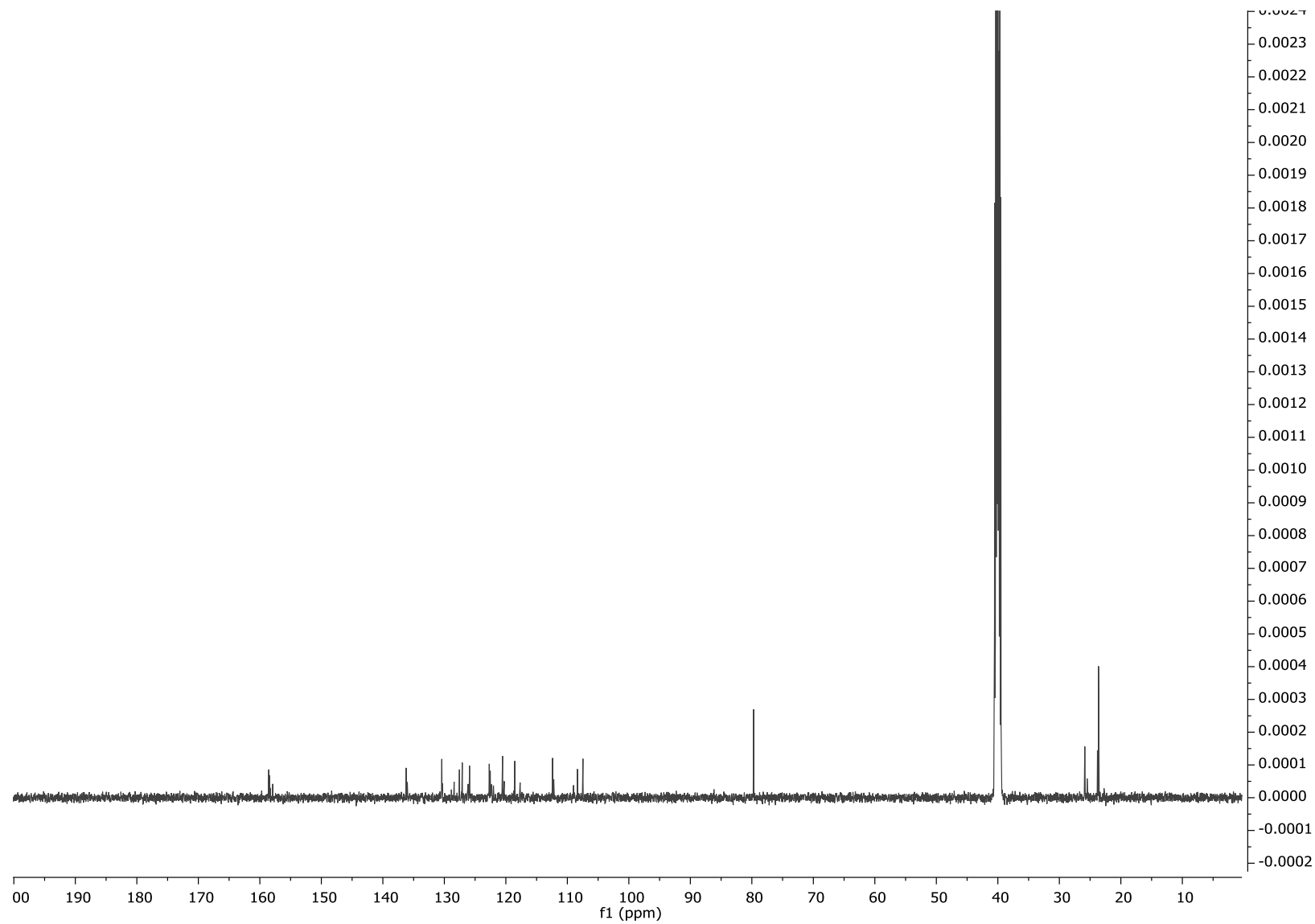


Figure S9. ^1H - ^1H COSY NMR spectrum of photopiperazine B (**2**) mix in $\text{DMSO}-d_6$.

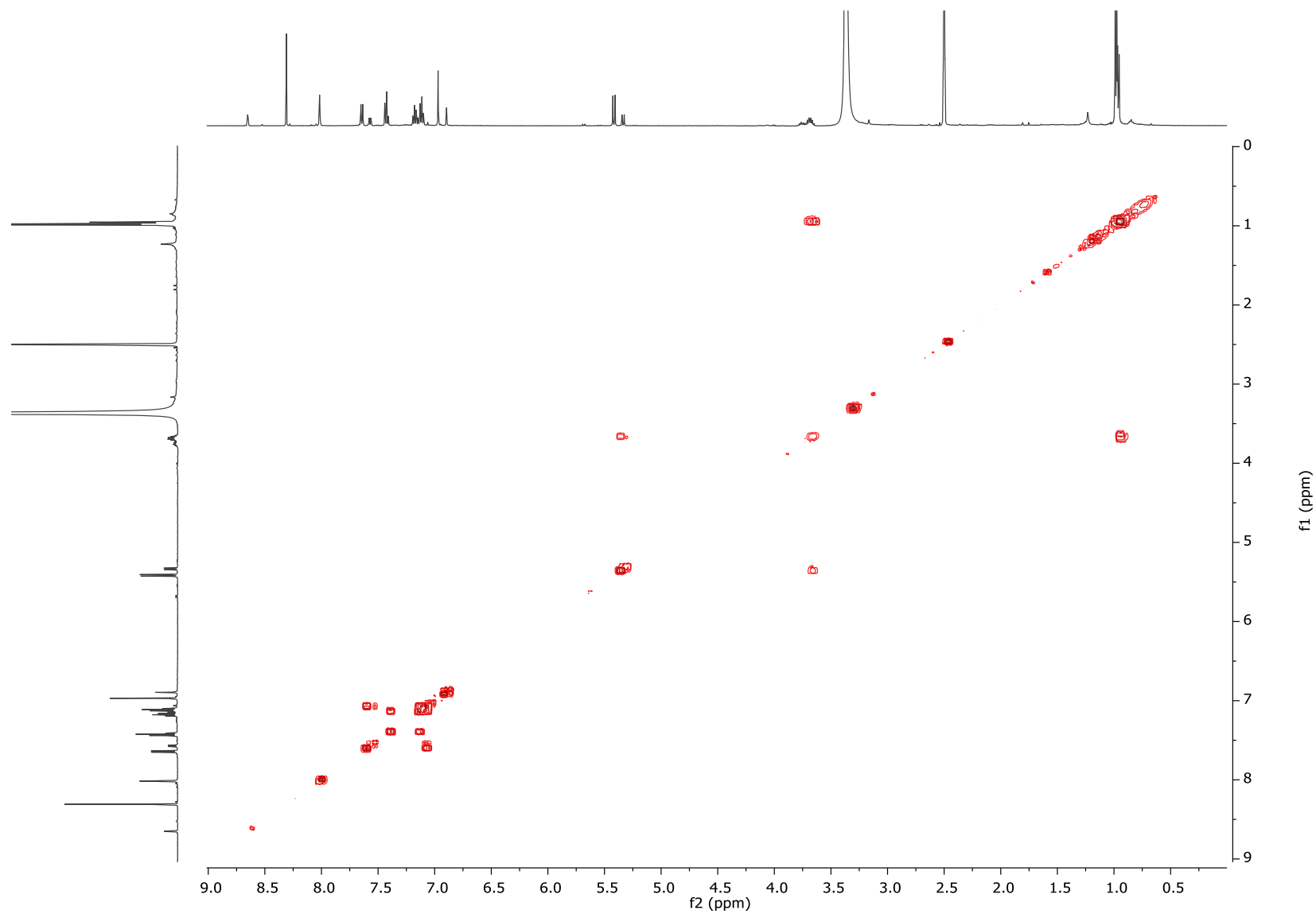


Figure S10. HSQC NMR spectrum of photopiperazine B (**2**) mix in DMSO- d_6 .

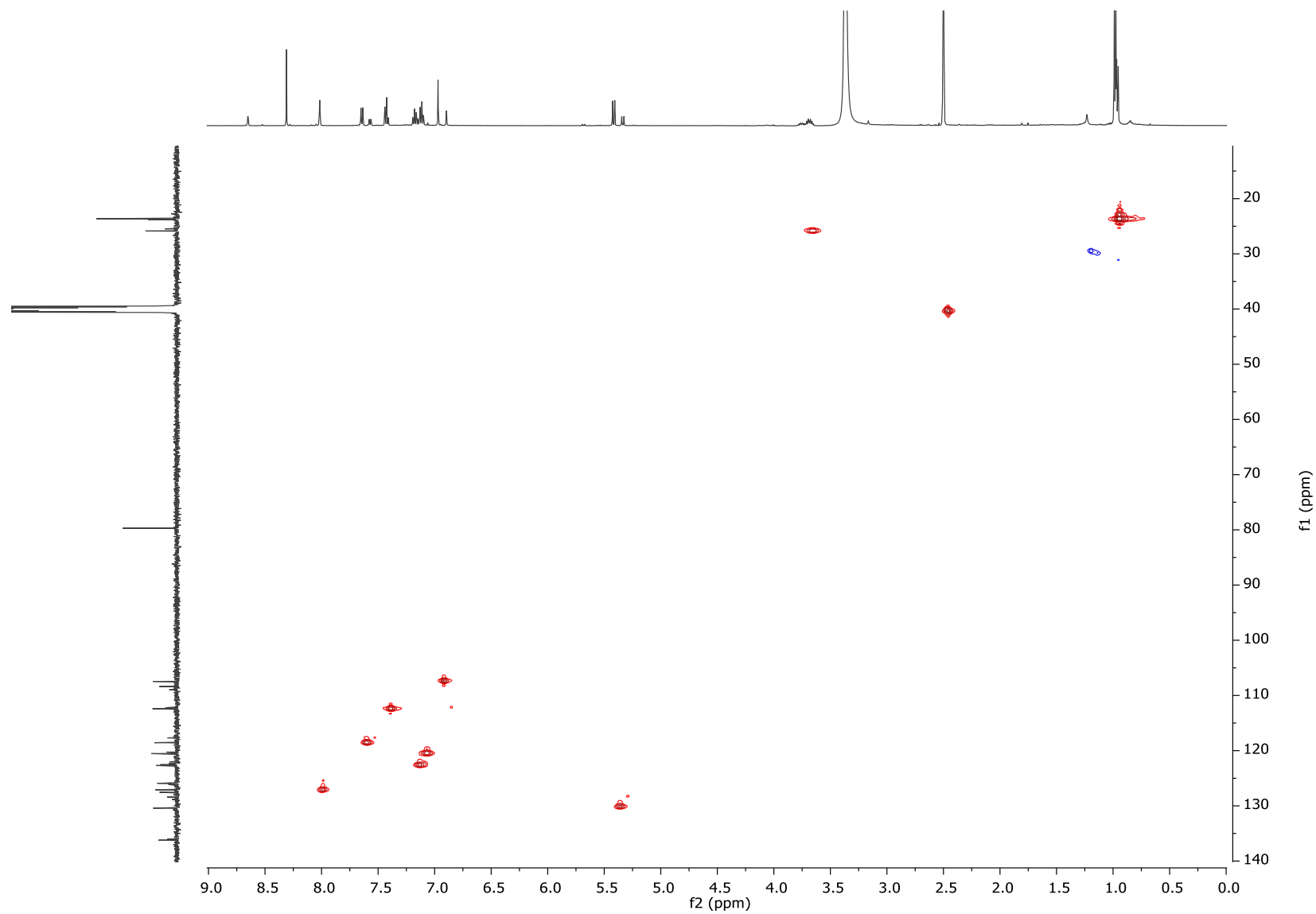


Figure S11. HMBC NMR spectrum of photopiperazine B (**2**) mix in DMSO- d_6 .

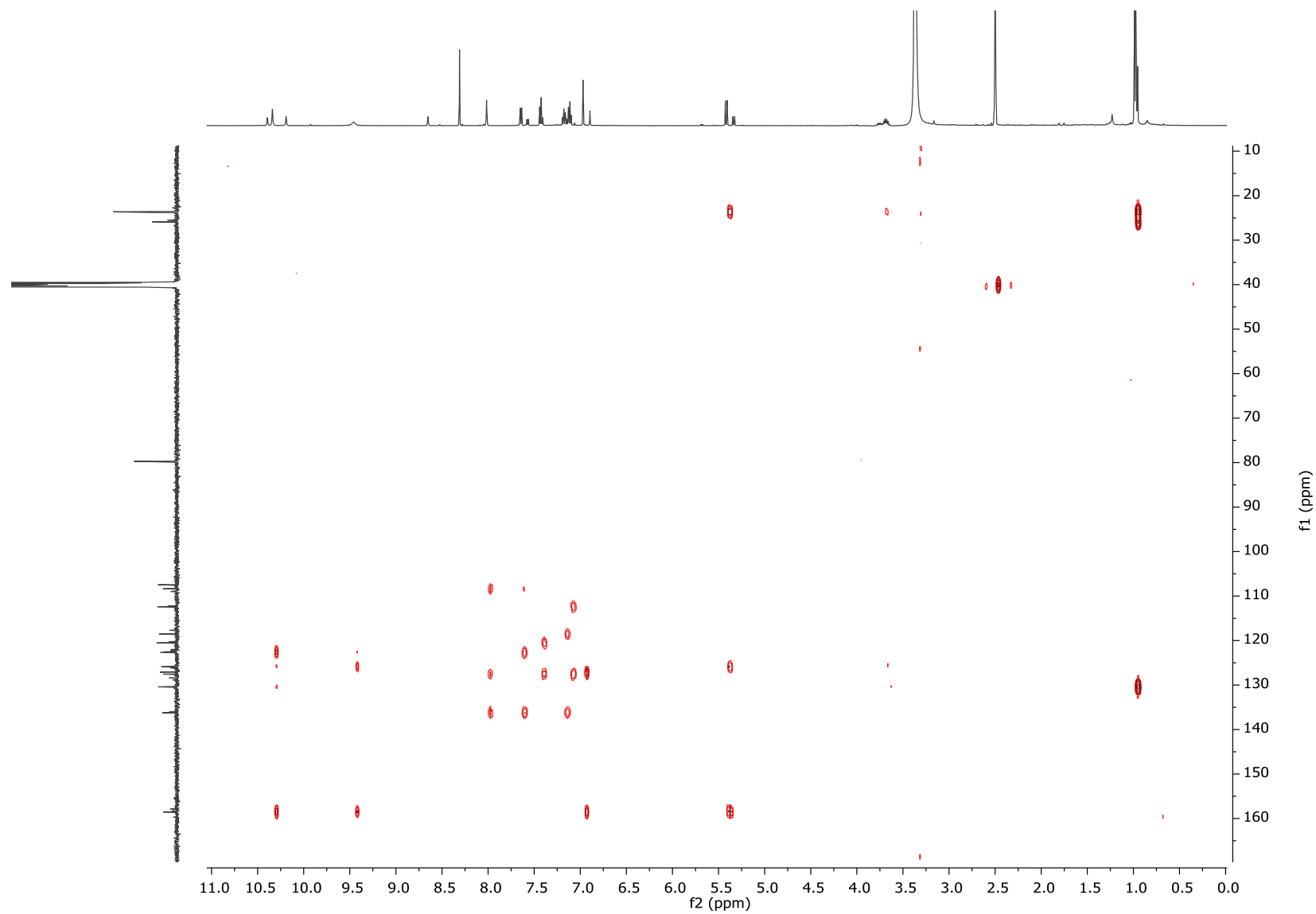


Figure S12. HR-ESI-TOFMS spectrum of photopiperazine B (**2**) mix.

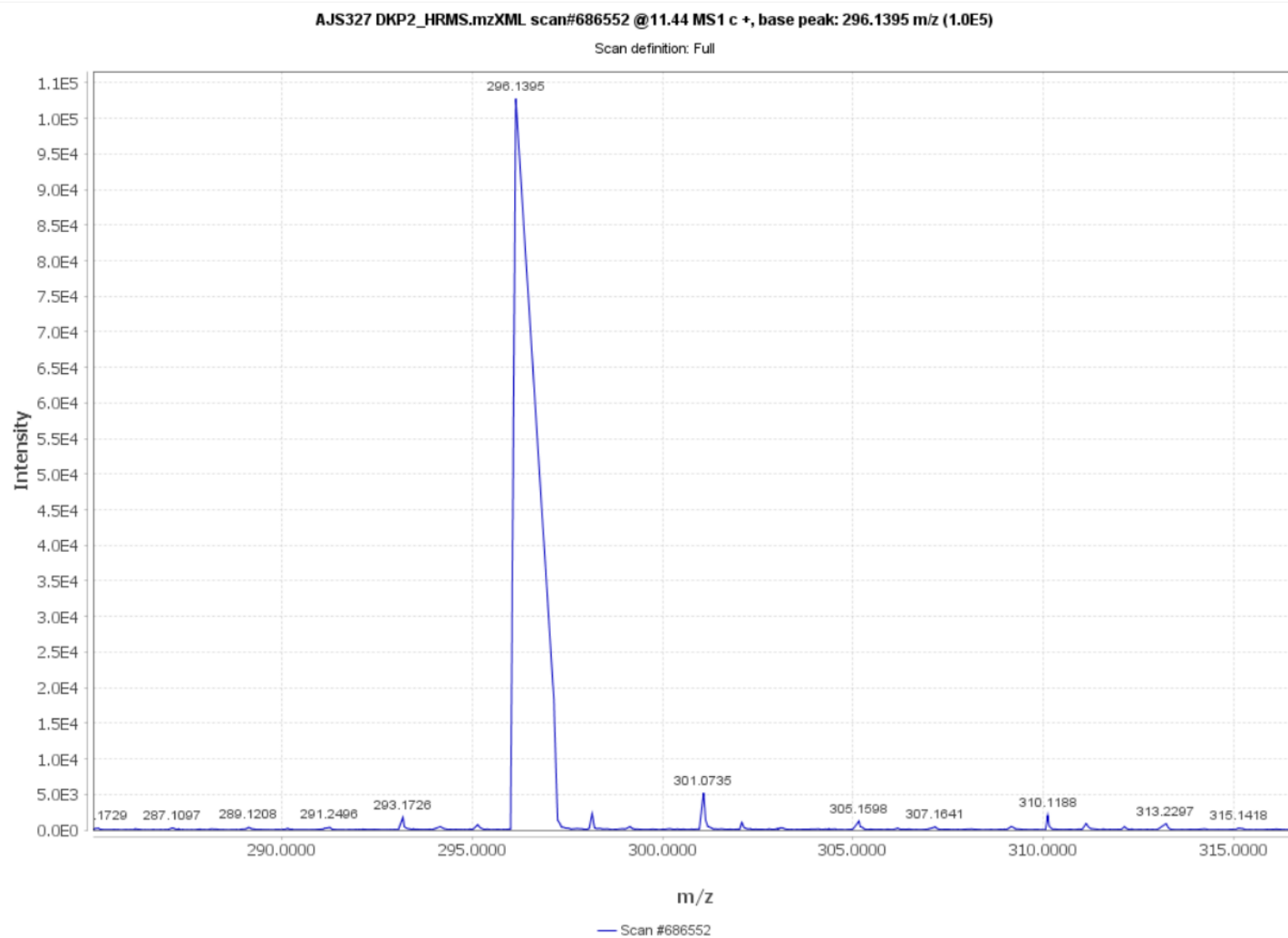


Figure S13. ^1H NMR spectrum of photopiperazines A (**1**, top) and B (**2**, bottom) in $\text{DMSO-}d_6$.

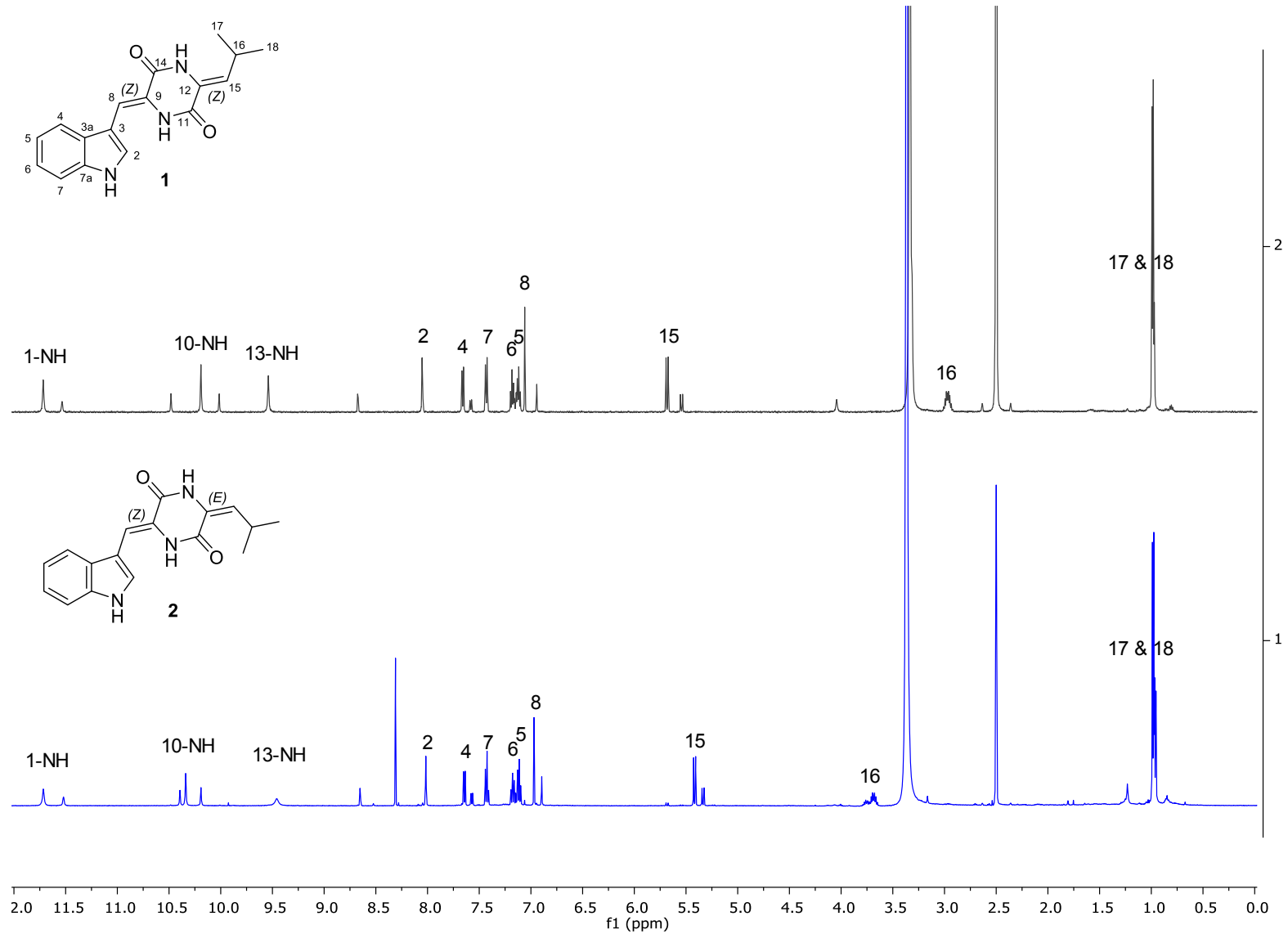


Figure S14. ^1H NMR spectrum of photopiperazines **1** (top) and **3** (bottom) in $\text{DMSO}-d_6$.

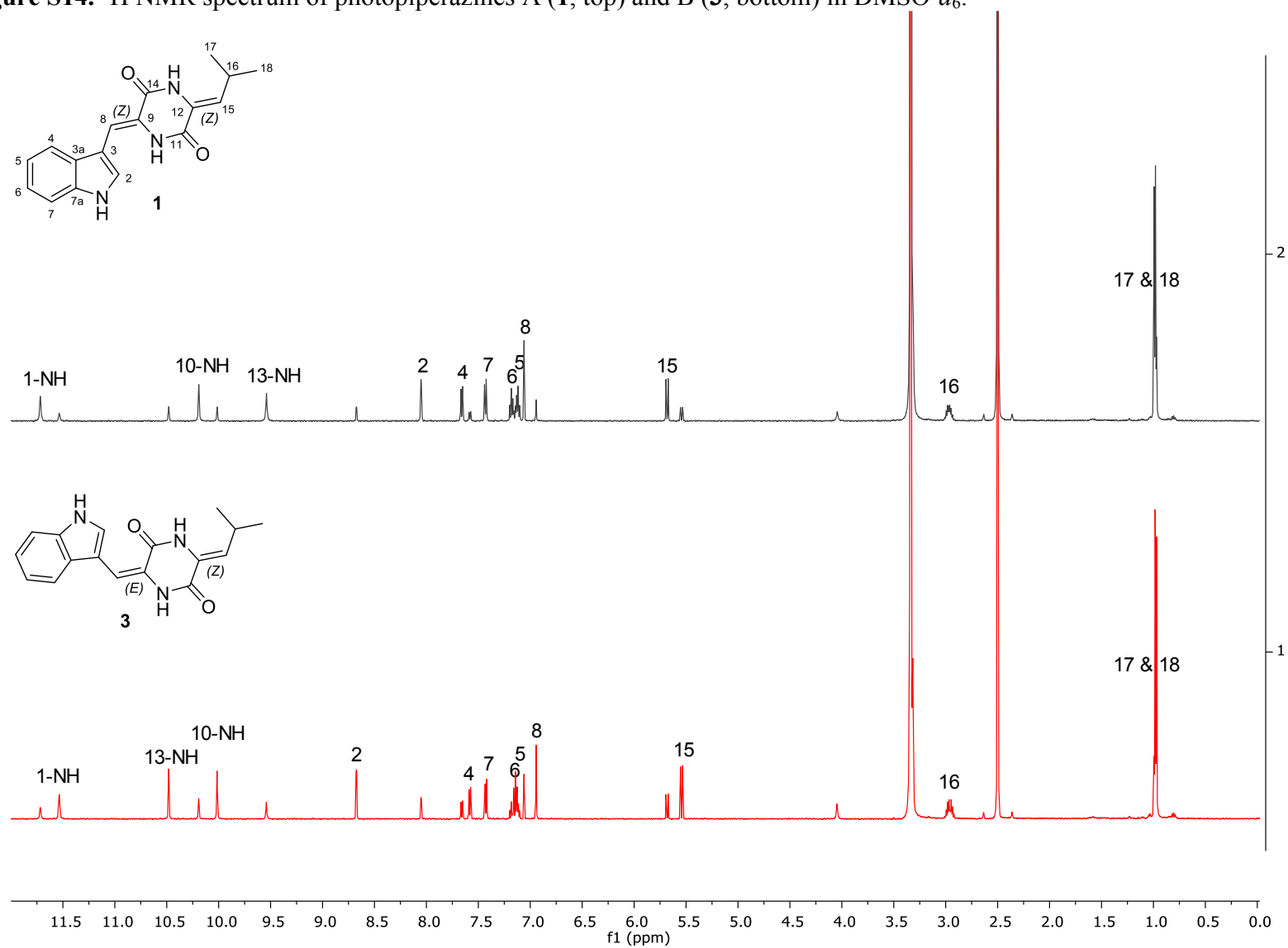


Figure S15. ^1H NMR spectrum of photopiperazines A (**1**, black), B (**2**, blue), C (**3**, red), and D (**4**, pink) in $\text{DMSO-}d_6$.

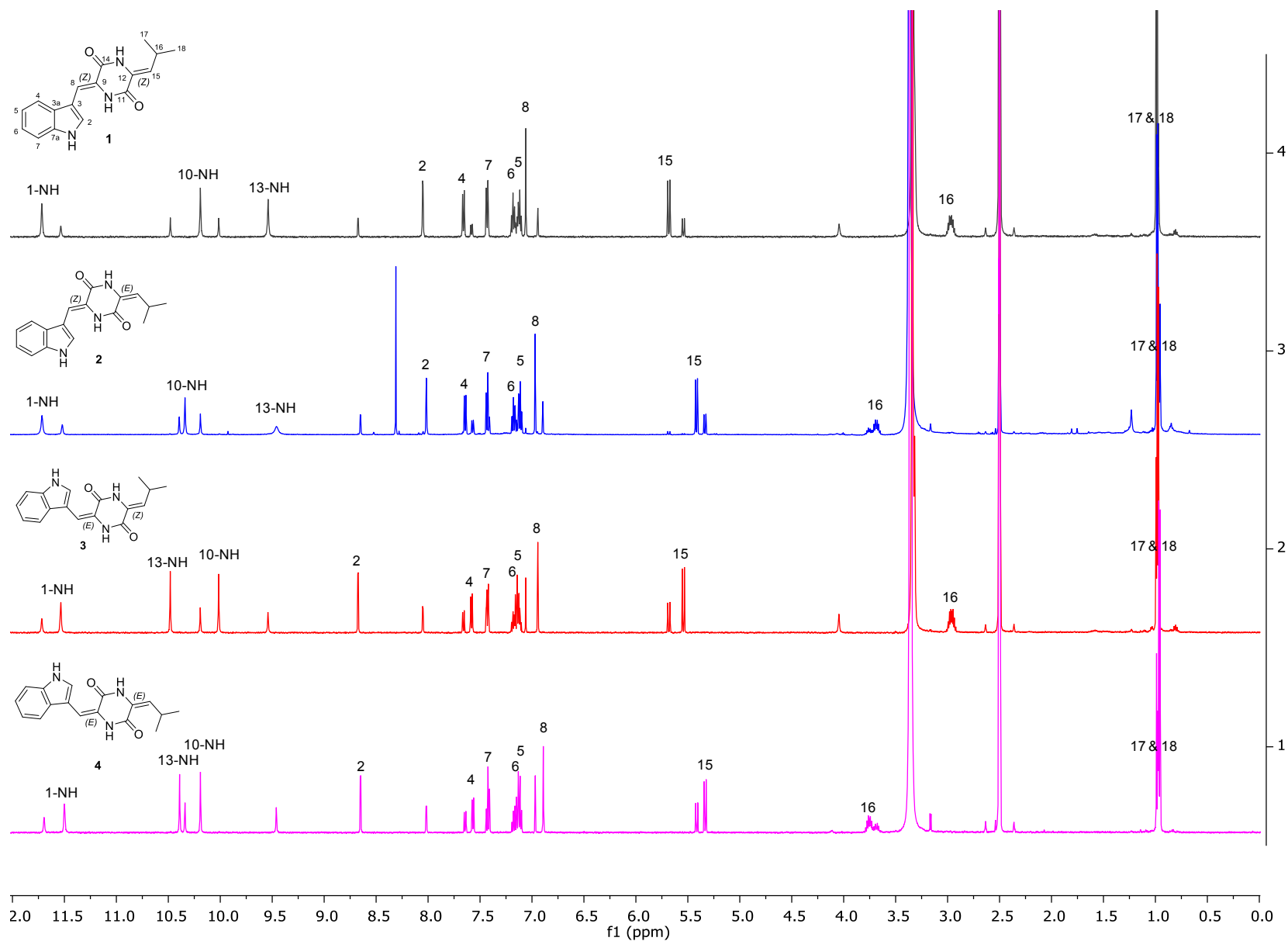


Figure S16. Conformations of **1-4** illustrating spatial interactions with the carbonyl groups.

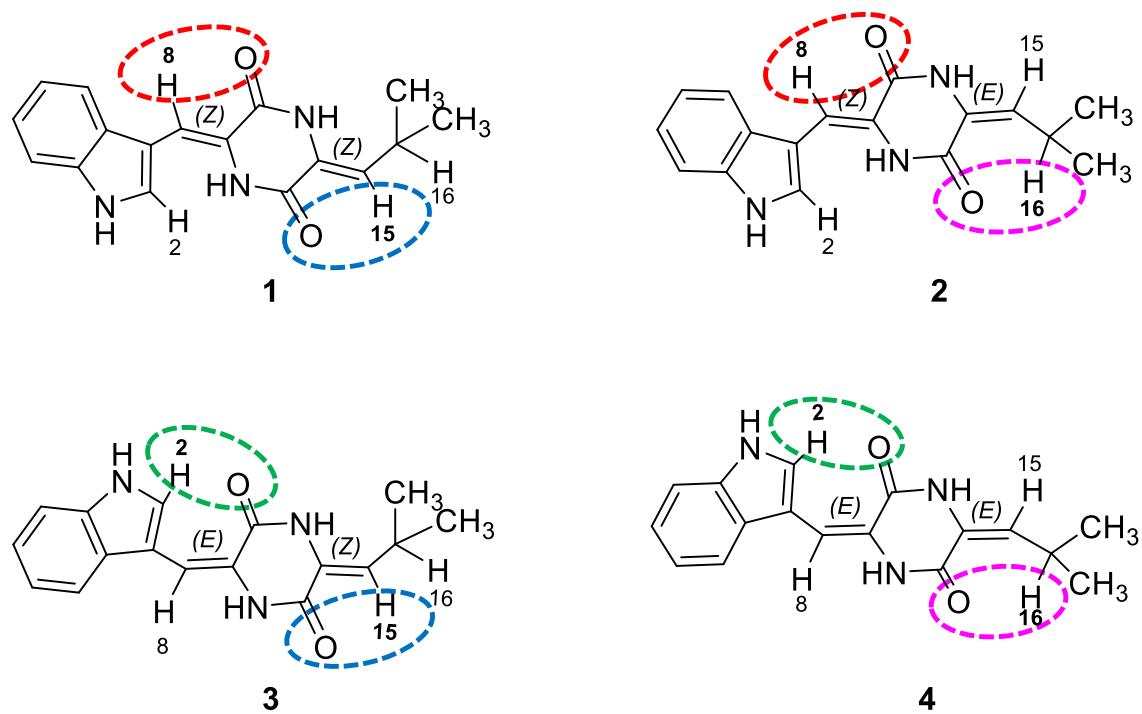


Figure S17. A listing showing the percent identity and percent similarity of the proteins in the AJS-327 gene clusters in comparison with those of *AlbA-AlbC*.

<i>Albonoursin protein</i>	<i>AJS-327 protein locus</i>	<i>% Identity</i>	<i>% Similarity</i>
<i>AlbA</i>	<i>E0L36_00395</i>	38.4	49.3
<i>AlbB</i>	<i>E0L36_00400</i>	38.1	55.7
<i>AlbC</i>	<i>E0L36_00405</i>	42.5	55.2

Figure S18. Alignment of the amino acid sequence of AlbC and its homologous in strain AJS-327, with pockets P1 and P2 highlighted.

