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SUPPORTING INFORMATION

Discovery of brevijanazines from *Aspergillus brevijanus* reveals the molecular basis for *p*-nitrobenzoic acid in fungi

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Supplementary Experimental Procedures

1. Strains and culture conditions

The wild-type *Aspergillus brevijanus* MST FP2436 obtained from the NRRL collection (NRRL 1935) was hydrated in sterile 0.2% carrageenan solution and recovered onto malt extract agar (MEA), with the remaining material preserved in 40% *v/v* glycerol solution at −80 °C. The culture was further sub-cultured and maintained on MEA at 24 °C, and also preserved as discs in 40% *v/v* glycerol solution at −80 °C. High molecular weight genomic DNA of *A. brevijanus* was prepared according to the protocol described previously.[1] *Saccharomyces cerevisiae* BJ5464-NpgA (*MATα ura3-52 his3-Δ200 leu2-Δ1trp1 pep4::HIS3 prb1 Δ1.6R can1 GAL*) was used for plasmid construction. *Aspergillus nidulans* LO8030[2] (a gift from Prof. Berl Oakley, University of Kansas) was used for heterologous expression of *bvj* genes. *Escherichia coli* 5α and 10β were used for standard DNA manipulation.

2. General experimental details

Optical rotations were acquired in MeOH on a Perkin-Elmer Model 341 polarimeter in a 50 × 5 mm cell or on a Jasco P-1010 polarimeter in a 100 × 3.5 mm cell. UV-vis spectra were acquired in MeCN on a Varian Cary 4000 spectrophotometer or a Jasco V-760 spectrophotometer in a 10 × 10 mm quartz cuvette. Analytical HPLC was performed on a gradient Agilent 1260 Infinity quaternary HPLC system. The column was an Agilent Zorbax SB-C18 (2.1 × 50 mm, 1.8 μm) eluted with a 0.6 mL/min gradient of 10-100% MeCN/H_2O (0.01% TFA) over 8.33 min. Preparative HPLC was performed on a gradient Shimadzu HPLC system comprising two LC-8A preparative liquid pumps with static mixer, SPD-M20A diode array detector and SCL-20AP system controller with standard Rheodyne injection port. The columns used in the purification of the metabolites were selected from either an Agilent Zorbax SB-C18 column (21.2 × 250 mm, 7 μm) or an Agilent Zorbax SB-C18 column (50 × 150 mm, 5 μm) eluted isocratically with acetonitrile/water mixtures with or without 0.01% TFA modifier, as described for each separation. LCMS was performed on an Agilent 1260 Infinity series HPLC equipped with an Agilent 6130 Infinity series single quadrupole mass detector in both positive and negative ion modes. High resolution electrospray ionization mass spectra (HRESIMS) were obtained on a Bruker Apex Qe 7T Fourier Transform Ion Cyclotron Resonance mass spectrometer equipped with an Apollo II ESI/MALDI Dual source or a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) by direct infusion. NMR data were recorded in DMSO-d_6 on either a Bruker Avance III 400 or a Bruker Avance II DRX-600K spectrometer. All NMR spectra were recorded at 25 °C, processed using Bruker Topspin 3.5 software and referenced to residual non-deuterated solvent signals (DMSO-d_6 : δ_H 2.49 / δ_C 39.5). Reactions were monitored by thin-layer chromatography on Merck silica gel 60 F_{254} TLC plates and visualized

by UV light and $KMnO_4$ or ninhydrin stains. Column chromatography was performed on silica gel (Flash Silica gel 230–400 mesh). Tetrahydrofuran and dichloromethane were freshly distilled from $Na_{(s)}$/benzophenone and CaH_2 respectively. Isopropanol was of HPLC standard and used without drying. Fmoc-L-Val-OH was obtained from Ontores Biotechnologies Inc (China). L-Val-OMe·HCl was obtained from Combi-Blocks (USA). All other reagents were obtained from Sigma Aldrich (Australia).

3. Collection and cultivation

Cultivation of *A. brevijanus* was optimized on a range of agar, liquid and grain-based media to identify the optimal conditions for production of the secondary metabolites. The agars, Czapek-dox agar (CZA), malt extract agar (MEA), yeast extract sucrose agar (YES), oatmeal agar (OMA) and casein glycerol agar (CGA), were prepared according to the recipes provided in Table S10. The liquid cultures were based on the agar recipes omitting agar. The grains, pearl barley, rice (jasmine and basmati) and cracked wheat, were prepared by hydration (50 g with 30 mL water in a 250 mL flask) during sterilization (121 °C for 40 min). The agar and grains were inoculated with a suspension of fungal spores and incubated at 24 °C for 14 days. The cultures were sub-sampled (1 g) and extracted with methanol (2 mL) 1 h on a wrist shaker, centrifuged (15,700 × *g* for 3 min) and analyzed by HPLC. The HPLC traces were accessioned into our in-house cometabolite database, COMET,[3] and the major metabolites were analyzed by retention time and UV-vis spectroscopic fit against known standards.

4. Cultivation and compounds isolation from *A. brevijanus*

A spore suspension of *A. brevijanus* from a 7-day-old MEA plate was used to inoculate 40 × 250 mL Erlenmeyer flasks, each containing 80 g of the hydrated and autoclaved basmati rice. The rice was incubated at 24 °C for 21 days, by which time the culture had grown extensively throughout the grain reaching maximal metabolite productivity. The grains were pooled, extracted with acetone (2 × 2 L), and the combined extracts were evaporated under reduced pressure to produce an aqueous slurry (1 L). The slurry was partitioned against ethyl acetate (2 × 1.5 L) and the combined ethyl acetate layer was reduced *in vacuo* to give a non-polar extract. The residue was dissolved in methanol (500 mL) and then defatted using hexanes (2 × 500 mL) to give the methanolic extract.

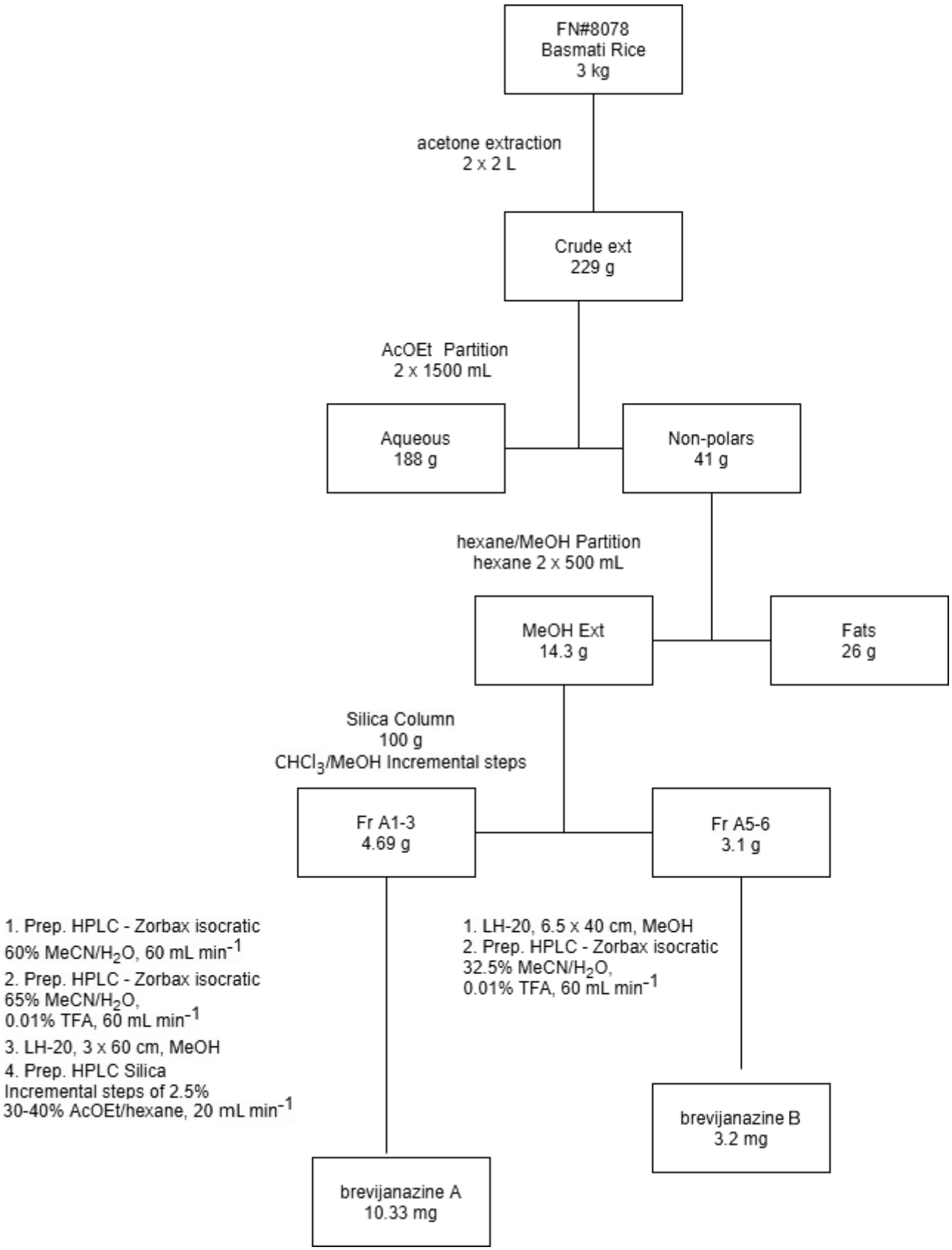
The methanolic extract was dissolved in $CHCl_3$ (300 mL) and absorbed onto silica gel (30 g) then dried *in vacuo* and loaded onto a silica gel column (100 g; 5 × 30 cm). The column was eluted with hexane, then 50% hexane/$CHCl_3$, 100% $CHCl_3$, followed by incremental steps of 1, 2, 4, 8, 16, 32, 64 and 100% MeOH/$CHCl_3$ (500 mL each fraction), to yield 11 fractions (Fr A1 to A11). Fractions

A1-A3 (4.69 g) were combined and further fractionated by preparative HPLC (Zorbax C_{18}, isocratic 60% MeCN/H_2O, 0.01% TFA, 60 mL/min, t_R 22.51 min) which yielded 367 mg of enriched material. This material was further enriched via preparative HPLC (Zorbax C_{18}, isocratic 65% MeCN/H_2O, 60 mL/min, t_R 15.23 min) producing 221 mg of enriched targets. The enriched targets were partially purified using Sephadex LH-20 size-exclusion chromatography with MeOH as the eluent (3 × 28 cm, 2 mL/min) yielding 182 mg. Final purification was achieved via preparative HPLC (Zorbax Si, stepwise in 2.5% increments from 30% to 40% EtOAc/hexane, 20 mL/min) yielding brevijanazine A (**1**; t_R 10.97 min; 10.3 mg).

Fractions A5-A6 (3.1 g) were combined and further fractionated using Sephadex LH-20 size-exclusion chromatography with MeOH as the eluent (6 × 48 cm, 10 mL/min) yielding a partially enriched fraction of 562 mg, which was purified by preparative HPLC (Zorbax C_{18}, isocratic 32.5% MeCN/H_2O, 0.01% TFA, 60 mL/min) yielding brevijanazine B (**2)** TFA salt (t_R 5.25 min; 3.2 mg).

Brevijanazine A (**1**): yellow solid; $[\alpha]_D^{23}$ +231 (*c* 0.12, MeOH); ^1H and ^{13}C NMR data, see Table S1; UV (MeCN) λ_{max} (log ε) 276 (4.29) nm; HR-ESI-MS: *m/z* 469.2071 $[M + H]^+$ (calcd. for $C_{24}H_{29}N_4O_6^+$, 469.2082); *m/z* 491.1890 $[M + Na]^+$ (calcd. for $C_{24}H_{28}N_4O_6Na^+$, 491.1901).

Brevijanazine B (**2**) TFA salt: yellow solid; $[\alpha]_D^{23}$ −6 (*c* 0.14, MeOH); ^1H and ^{13}C NMR data, see Table S2; UV (MeCN) λ_{max} (log ε) 279 (3.87) nm; HR-ESI-MS: *m/z* 320.1960 $[M + H]^+$ (calcd. for $C_{17}H_{26}N_3O_3^+$, 320.1969); *m/z* 342.1778 $[M + Na]^+$ (calcd. for $C_{17}H_{25}N_3O_3Na^+$, 342.1788).



Scheme S1. Fractionation and purification of brevijanazines A and B from *A. brevijanus*.

5. Total synthesis of brevijanazines A and B

Fmoc-L-Val-OSu (3). To a solution of Fmoc-L-Val-OH (20.0 g, 58.9 mmol) and *N*-hydroxysuccinimide (7.1 g, 61.9 mmol) in anhydrous CH_2Cl_2 (200.0 mL) was added *N,N′*-dicyclohexylcarbodiimide (12.8 g, 61.9 mmol). After 18 h, the resulting precipitate was filtered and the organic phase was washed with water (1 × 200 mL) and brine (1 × 200 mL), then dried over $MgSO_4$. Removal of the solvent under reduced pressure afforded the desired product **3** (95% purity) in quantitative yield. The material was used without further purification in the next step. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.14 (d, J = 8.6 Hz, 1H), 7.89 (d, J = 7.4 Hz, 2H), 7.74 (t, J = 8.4 Hz, 2H), 7.42 (t, J = 7.4 Hz, 2H), 7.33 (tt, J = 7.4, 1.3 Hz, 2H), 4.38 - 4.29 (m, 3H), 4.25 (br t J = 7.1 Hz, 1H), 2.81 (s, 4H), 2.20 (sept, J = 6.7 Hz, 1H), 1.02 (d, J = 6.7 Hz, 6H).

4-nitrobenzoic acid *N*-hydroxysuccinimide ester. To 4-nitrobenzoic acid (2.0 g, 12.0 mmol) and *N*-hydroxysuccinimide (1.45 g, 12.6 mmol) in anhydrous CH_2Cl_2 (40.0 mL) was added a solution of *N,N′*-dicyclohexylcarbodiimide (2.6 g, 12.6 mmol) in anhydrous CH_2Cl_2 (10.0 mL) dropwise. After 20 h the resulting precipitate was filtered and the solvent was removed under reduced pressure, affording the desired product as a white solid of 95% purity (1.86 g, 59%). The material was used without further purification in the next step. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.45 (d, J = 9.0 Hz, 2H), 8.35 (d, J = 9.0 Hz, 2H), 2.92 (s, 4H).

***cyclo*(L-Val-L-Val) (4)**. To a suspension of **3** (23.7 g, 54.4 mmol) and L-Val-OMe·HCl (10.0 g, 59.9 mmol) in HPLC grade *i*-PrOH (250.0 mL) was added Et_3N (7.65 mL, 55.5 mmol). The suspension was heated to 50 ºC for a period of 1 h 10 min, after which time the reaction mixture was diluted with *i*-PrOH (100.0 mL) and a second portion of Et_3N (30.4 mL, 218 mmol) was added. The reaction mixture was sealed in a pressure tube and heated to 120 ºC for a period of 32 h. The reaction mixture was cooled to room temperature and allowed to stand for a period of 18 h. The formed precipitate was filtered, washed with hexane (3×), water (1×), and finally hexane (3×), to afford **4** (3.0 g, 15.0 mmol, 28%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 7.94 (s, 1H), 3.70-3.67 (m, 1H), 2.18 (dsept, J = 3.5, 6.9 Hz, 1 H), 0.96 (d, J = 7.1 Hz, 3H), 0.84 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ_C 167.4, 59.1, 31.0, 18.7, 17.3.

(2*S*,5*S*)-2,5-diisopropylpiperazine (5). To a suspension of **4** (1.0 g, 5.0 mmol) in anhydrous THF (20.0 mL) at 0 ºC was added $LiAlH_4$ as a 2.0 M solution in THF (15.1 mL, 30.3 mmol) dropwise. After 20 min the solution was brought to room temperature for a period of 1 h and was then heated at

reflux for 24 h. The reaction mixture was cooled to 0 ºC and quenched by the addition of $Na_2SO_4 \cdot 10H_2O$. When no further effervescence was noted, the reaction mixture was stirred at room temperature for a period of 15 min followed by at reflux for 40 min. After cooling, the suspension was filtered and the solid was washed with THF. Evaporation of the solvent under reduced pressure afforded **5** (616.0 mg, 3.6 mmol, 71.7%) as a white semisolid. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 2.63 (dd, J = 5.9, 11.9 Hz, 1H), 2.57 (dd, J = 3.5, 11.9 Hz, 1H), 2.07 (ddd, J = 3.5, 5.9, 8.3 Hz, 1H), 1.81 (doct, J = 1.3, 6.7, 1H), 0.86 (d, J = 6.7 Hz, 3H), 0.81 (d, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ_C 59.9, 45.6, 27.5, 19.7, 19.5. HRESI(+)MS: *m/z* 171.1851 $[M + H]^+$ (calcd. for $C_{10}H_{23}N_2^+$, 171.1856).

Brevijanazine B (2).

Method A:

To piperazine **3** (610.0 mg, 3.6 mmol) and Et_3N (749.0 µL, 5.4 mmol) in anhydrous CH_2Cl_2 (15.0 mL) was added a solution of 4-nitrobenzoyl chloride (731.0 mg, 3.9 mmol) in CH_2Cl_2 (20.0 mL). After stirring for 1 h, the reaction mixture was quenched by the addition of 6 M HCl. The organic phase was extracted with 6 M HCl until all product had been extracted (3 × 35 mL). The acidic fractions were washed with EtOAc (2 × 50 mL) and basified using 6 M NaOH. The basic solution was extracted with EtOAc (2 × 50 mL) and the organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The crude material was purified by gravity column chromatography on silica gel, eluting with EtOAc (1% Et_3N), to afford **2** (358.0 mg, 1.1 mmol, 31.0% yield) as a white solid. R_f (EtOAc) 0.23.

Synthetic brevijanazine B (**2**): yellow solid; $[\alpha]_D^{23}$ −5 (*c* 0.16, MeOH); UV (MeCN) λ_{max} (log ε) 276 (3.93) nm; HR-ESI-MS: *m/z* 320.1961 $[M + H]^+$ (calcd. for $C_{17}H_{26}N_3O_3^+$, 320.1969)

Method B:

A suspension of **3** (360.0 mg, 2.1 mmol), 4-nitrobenzoic acid *N*-hydroxysuccinimide ester (1.4 g, 5.3 mmol) and Et_3N (737.0 µL, 5.3 mmol) in *i*-PrOH (12.0 mL) was heated to 80 ºC for a period of 20 h. The solvent was removed under reduced pressure and the crude residue was dissolved in EtOAc. The organic phase was extracted using 6 M HCl and the acidic aqueous phase was extracted with EtOAc. The acidic fraction was made basic using 6 M NaOH and extracted using EtOAc (2×). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure to afford 330.0 mg of a crude solid. The crude material was purified by gravity column chromatography on silica gel, eluting with EtOAc (2% Et_3N), to afford **2** (165.0 mg, 516.6 µmol, 24%) as a white solid. R_f (EtOAc) 0.23.

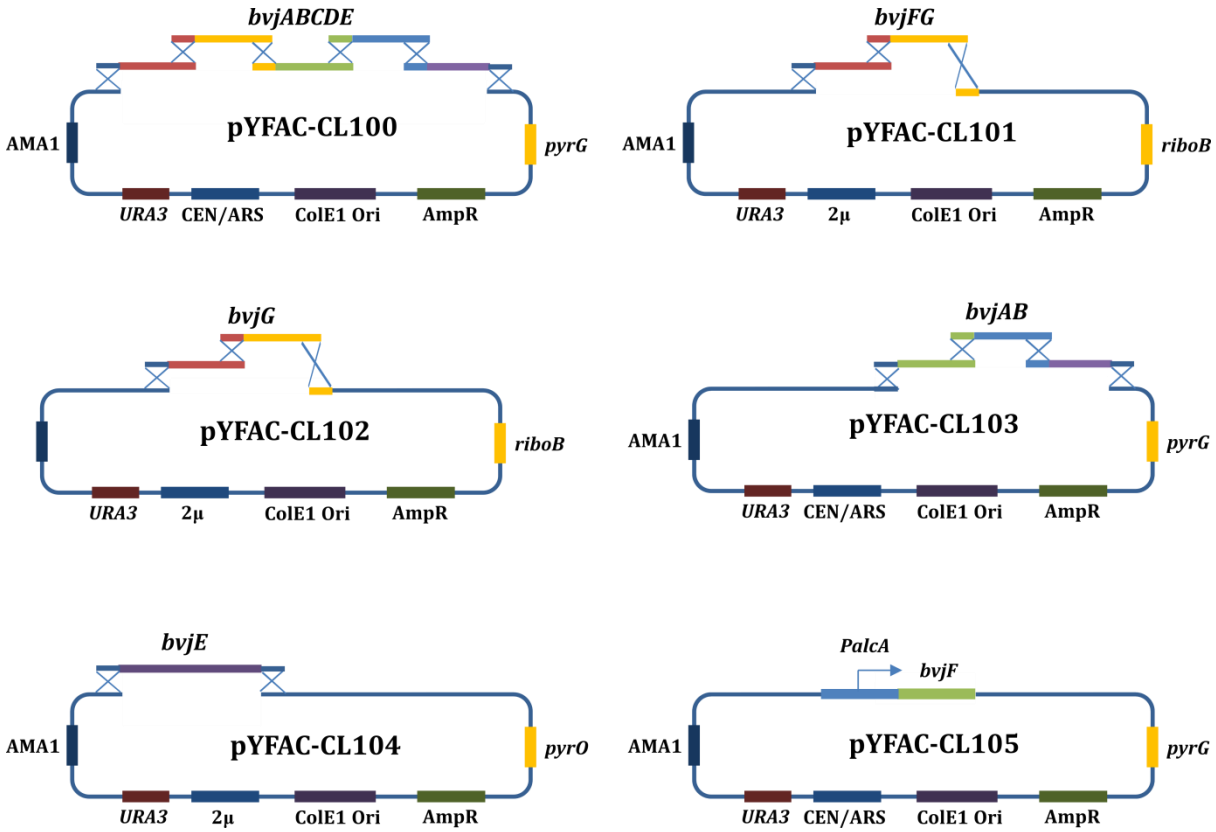
Brevijanazine A (1). To **3** (540.0 mg, 3.2 mmol) and Et_3N (972.0 µL, 7.0 mmol) in anhydrous CH_2Cl_2 (10.0 mL) was added a solution of 4-nitrobenzoyl chloride (1.236 g, 6.659 mmol) in CH_2Cl_2 (20.0 mL). After stirring for 3 h, the reaction mixture was diluted with CH_2Cl_2 and the organic phase was washed with 6 M HCl, 6 M NaOH, water, and brine. Drying over $MgSO_4$ and evaporation of the solvent under reduced pressure afforded 1.4 g of a white crude solid. The crude material was purified by gravity column chromatography on silica gel, eluting with EtOAc/hexane (4:5), to afford brevijanazine A (**1**) (930.0 mg, 2.0 mmol, 63%) as a white solid.

Synthetic brevijanazine A (**1**): yellow solid; $[\alpha]_D^{23}$ +224 (*c* 0.27, MeOH); UV (MeCN) λ_{max} (log ε) 275 (4.24) nm; HR-ESI-MS: *m/z* 491.1888 $[M + H]^+$ (calcd. for $C_{24}H_{28}N_4O_6Na^+$, 491.1901)

6. Heterologous expression of *bvj* pathway in *A. nidulans*

The entire *bvj* gene cluster was cloned as two separate fragments into three AMA1-based episomal vectors with two different auxotrophy markers (*pyrG* and *riboB*) under the native *bvj* gene promoters and terminators by yeast transformation-assisted recombination.[4] Briefly, the full length *bvjABCDE* fragment was amplified by PCR with three sets of primers from *A. brevijanus* gDNA (Table S6). Then *S. cerevisiae* strain BJ5464-NpgA competent cells were transformed by the three overlapping DNA fragments and NotI-digested pYFAC-pyrG to generate plasmid pYFAC-CL100 by yeast homologous recombination. The whole *BvjFG* region was amplified with the designed primers (Table S6) and ligated into the NotI site of pYFAC-ribo to get pYFAC-CL101 plasmid (see Scheme S2 below).

For the partial gene cluster expression, plasmids were constructed using the same method only with the difference in the gene fragment that amplified and cloned into the pYFAC vectors (see Table S7). For expression of *bvjF* in *A. nidulans*, the full length of *bvjF* was amplified with the designed primers (Table S6) and ligated into the PacI-digested pYFAC-CH1 linearized vector to generate plasmid pYFAC-CL105 by yeast homologous recombination. Extraction of the resulting pYFAC plasmids from the correct yeast transformants was performed with Zymoprep™ Yeast Plasmid Miniprep I Kit. The resulting heterologous expression plasmids were introduced into *A. nidulans* LO8030 by polyethylene glycol protoplast transformation as described previously.[5]



Scheme S2. Schematic diagram of plasmids constructed for heterologous expression.

7. *A. nidulans* feeding experiments

The *A. nidulans* transformant strain was grown on GMM liquid culture (50 mL) with necessary supplements (autotrophy supplements uridine 1.26 g/L and uracil 0.56 g/L, 0.025% riboflavin 10 mL/L, 0.2% pyridoxine-HCl 500 μL/L) at 37 °C at 180 rpm for 18 h. Then the culture was shaken at 25 °C at 180 rpm and cyclopentanone (125 μL) was added. After growth for 8 h, substrate (1-2 mg) dissolved in MeOH (50 μL) was added to the culture. After further incubation for 2-3 days, mycelium was collected by filtration and extracted with acetone, while the media was extracted with ethyl acetate/methanol/acetic acid (89.5 : 10 : 0.5). The crude extracts were reduced to dryness *in vacuo* and re-dissolved in methanol for LC-MS analysis.

8. Analytical methods

For the *A. nidulans bvj* expressing strains, extracts from both mycelium and media were analyzed. The crude extracts were reduced to dryness *in vacuo* and re-dissolved in methanol (200 μL) for LC-MS analysis. LC-DAD-MS metabolite profiles were acquired on an Agilent 1260 liquid chromatography (LC) system coupled to a diode array detector (DAD) and an Agilent 6130 single quadrupole mass spectrometer (MS) with an ESI source. Chromatographic separation was performed at 40 °C using a Kinetex C18 column (2.6 μm, 2.1 mm × 100 mm; Phenomenex). Chromatographic

separation was achieved with a linear gradient of 5–95% MeCN-H_2O (0.1% (*v/v*) formic acid) in 10 min followed by 95% MeCN for 3 min then 5% MeCN-H_2O for 3 min, with a flow rate of 0.75 mL/min. The MS data were collected in the *m/z* range 100–1000.

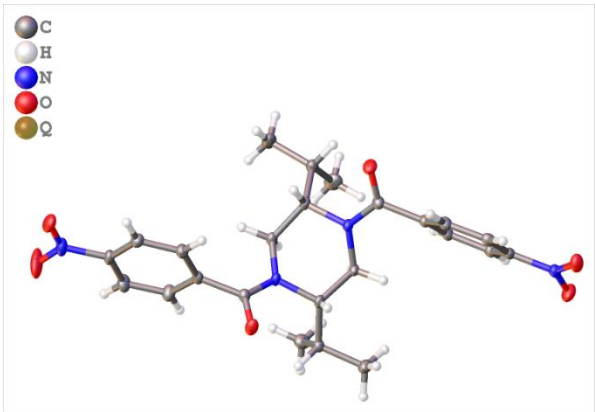
9. Preparation of microsomal lysate for BvjF from the *A. nidulans* transformant

The plasmid pYFAC-CL105 was introduced into *A. nidulans* LO8030 using the procedures described above. The *A. nidulans* transformant was then grown on the 100 mL selective GMM media with supplements (1 mL 0.25% riboflavin, 50 μL 0.2% pyridoxine-HCl) at 37 °C 180 rpm for 18 h. To induce the expression of BvjF, 250 mL of cyclopentanone was added. The cultures were incubated for another 3 day after induction at 25 °C 180 rpm. Mycelium was harvested after 3-days induction, frozen with liquid nitrogen and ground into fine powder with a mortar and pestle. The powder was resuspended in lysis and equilibration buffer (0.6 M sorbitol, 0.1 M KCl, 1.0 mM EDTA, 2.0 mM DTT, 50 mM Tris-HCl, pH 7.5) supplemented with 2 mM DTT and lysed by sonication on ice. Cellular debris was removed by centrifugation at 8,000 × *g* and 4 °C for 10 min, and the supernatant was further fractionated by ultracentrifugation at 100,000 × *g* and 4 °C for 1 h. The microsomal pellet was then resuspended in 1 mL of storage buffer (20% glycerol, 1.0 mM EDTA, 1.0 mM DTT, 50 mM Tris-HCl, pH 7.5) used directly for *in vitro* assays.

10. *In vitro* enzymatic assay of BvjF

A total volume of 200 μL reaction mixture, containing 150 μL BvjF microsome prepared from *A. nidulans*, 2 mM NADPH, and 1 mM of PABA was incubated at 30 °C for 24 h. Microsomal extracts from *A. nidulans* harboring empty pYFAC-CH1 were also prepared as a negative control. Subsequently, the reaction mixture was extracted twice with 200 μL EtOAc. After removal of solvent *in vacuo*, the extract was dissolved in methanol (200 μL) for HPLC-MS analysis.

11. X-ray crystallographic data for brevijanazine A (**1**)



Experimental. Single clear light yellow block crystals of brevijanazine A (**1**) were obtained by slow evaporation of a solution in acetonitrile. A suitable crystal with dimensions 0.23 × 0.12 × 0.10 mm^3 was selected and mounted on a XtaLAB Synergy, Single source at home/near, HyPix diffractometer. The crystal was kept at a steady T = 99.9(5) K during data collection. The structure was solved with the **ShelXT**[6] solution program using dual methods and by using **Olex2**[7] as the graphical interface. The model was refined with **ShelXL**[8] using full matrix least squares minimization on $\boldsymbol{F^2}$. Crystallographic data have been deposited in the CCDC (CCDC 2133461), and can be obtained free of charge via https://www.ccdc.cam.ac.uk/structures/, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (fax +441223336033; email deposit@ccdc.cam.ac.uk).

Crystal Data. $C_{24}H_{28}N_4O_6$, M_r = 468.50, orthorhombic, $P2_12_12_1$ (No. 19), a = 11.40330(10) Å, b = 12.51500(10) Å, c = 16.42590(10) Å, $\alpha = \beta = \gamma =$ 90°, V = 2344.18(3) Å^3, T = 99.9(5) K, Z = 4, Z' = 1, μ(Cu K$_\alpha$) = 0.801, 54321 reflections measured, 4852 unique (R_{int} = 0.0896) which were used in all calculations. The final wR_2 was 0.0856 (all data) and R_1 was 0.0330 (I≥2 σ(I)).

Formula	$C_{24}H_{28}N_4O_6$
$D_{calc.}$/ g cm^{-3}	1.327
μ/mm^{-1}	0.801
Formula Weight	468.50
Color	clear light yellow
Shape	block
Size/mm^3	0.23×0.12×0.10
T/K	99.9(5)
Crystal System	orthorhombic
Flack Parameter	−0.12(7)
Hooft Parameter	−0.11(6)
Space Group	$P2_12_12_1$
a/Å	11.40330(10)
b/Å	12.51500(10)
c/Å	16.42590(10)
α/°	90
β/°	90
γ/°	90
V/Å^3	2344.18(3)
Z	4
Z'	1
Wavelength/Å	1.54184
Radiation type	Cu K$_\alpha$
Θ_{min}/°	4.442
Θ_{max}/°	75.673
Measured Refl's.	54321
Indep't Refl's	4852
Refl's I≥2 σ(I)	4695
R_{int}	0.0896
Parameters	311
Restraints	0
Largest Peak	0.174
Deepest Hole	−0.240
GooF	1.057
wR_2 (all data)	0.0856
wR_2	0.0845
R_1 (all data)	0.0341
R_1	0.0330

12. *In vitro* bioassay methods for brevijanazines

The biological activity of **1** and **2** were tested in a series of *in vitro* bioassays to determine their antibacterial, antifungal, antiprotozoal, antitumor and herbicidal activities.

NS-1 (ATCC TIB-18) mouse myeloma cells and NFF (ATCC PCS-201) human fibroblast cells were inoculated into 96-well microtiter plates (190 µL) containing the test compounds, at 50,000 cells/mL in DMEM (Dulbecco's Modified Eagle Medium + 10% fetal bovine serum (FBS) + 1% penicillin/streptomycin (Life Technologies)) and incubated in a 37 °C (5% CO_2) incubator. At 48 h, resazurin (120 µg/mL; 10 µL) was added to each well and the plates were incubated for a further 48 h. MIC end points were determined visually by color change, and the absorbance of each well at 605 nm was measured using a Spectromax plate reader (Molecular Devices).

Bacillus subtilis (ATCC 6633) and *Staphylococcus aureus* (ATCC 25923) were used as indicative species for antibacterial activity. A bacterial suspension (50 mL in a 250 mL flask) was prepared in nutrient broth by cultivation for 24 h at 250 rpm, 28 °C. The suspension was diluted to 0.01 absorbance units per ml, and 10 µL aliquots were added to the wells of a 96-well microtiter plate, which contained the test compounds dispersed in nutrient broth (Amyl) with resazurin (120 µg/mL). The plates were incubated at 28 °C for 48 h during which time the positive control wells changed color from blue to light pink. MIC end points were determined visually, and absorbance was measured using a Spectromax plate reader (Molecular Devices) at 605 nm.

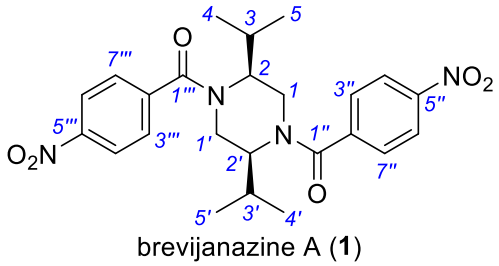
Candida albicans (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 9763) were used as indicative species for antifungal activity. A yeast suspension (50 mL in 250 mL flask) was prepared in 1% malt extract broth by cultivation for 24 h at 250 rpm, 24°C. The suspension was diluted to an absorbance of 0.005 and 0.03 absorbance units per ml for *C. albicans* and *S. cerevisiae*, respectively. Aliquots of *C. albicans* (20 µL) and *S. cerevisiae* (30 µL) were added to 96-well microtiter plates, which contained a 10-fold serial dilution of the test compounds dispersed in malt extract broth containing bromocrescol green (120 µg/mL). The plates were incubated at 24 °C for 48 h during which time the positive control wells changed color from blue to yellow. MIC end points were determined visually, and absorbance was measured using a Spectromax plate reader at 620 nm.

Tritrichomonas foetus (strain KV-1) was used as an indicative species for antiprotozoal activity. *T. foetus* was inoculated into 96-well microtiter plates (200 µL) at 4×10^4 cells/mL in *T. foetus* medium (0.2% tryptone, Oxoid; 0.1% yeast extract, Difco; 0.25% glucose; 0.1% L-cysteine; 0.1% K_2HPO_4; 0.1% KH_2PO_4; 0.1% ascorbic acid; 0.01% $FeSO_4\cdot7H_2O$; 1% penicillin/streptomycin (10,000 U/mL / 10,000 µg/mL, Life Technologies Cat. No. 15140122), 10% new born calf serum (NBCS), Life Technologies). The plates were incubated in anaerobic jars (Oxoid AG25) containing Anaerogen sachet (Oxoid AN25) in a 37 °C (5% CO_2) incubator. At 72 h MIC endpoints were determined visually and absorbance was measured using a Spectromax plate reader at 620 nm.

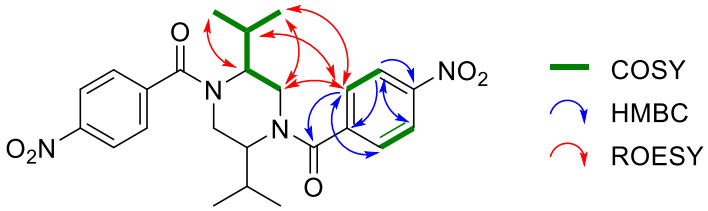
Eragrostis tef (teff) seed was used as indicative species for herbicidal discovery. Teff seeds (10 - 15) were dispensed using a LabTIE seed dispenser into the wells of a 96-well microtiter plate, which contained the test compounds dispersed in 200 µL of agar (1% *w/v*). The plates were placed in a tray wrapped with a semi-opaque bag, exposed to 1600 lux (inside the bag) using Power-Glo (20 W) and Sun-Glo (20 W) tubes, and incubated for 72 h at 24 °C. Inhibition of germination was determined visually.

Supplementary Tables

Table S1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for brevijanazine A (**1**) in DMSO-d_6

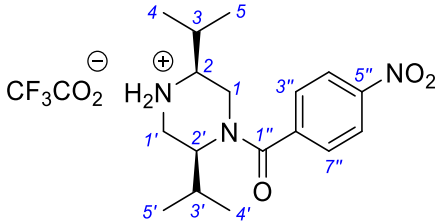


brevijanazine A (**1**)



Pos.	δ_H, mult. (*J* in Hz)	δ_C	HMBC	COSY	ROESY
1a	3.47, br d (13.0)	43.9		1b, 2	4, 5, 3″/7″
1b	3.23, br dd (13.0, 12.0)			1a, 2	4, 5, 3″/7″
2	4.17, br s	56.9		1a, 1b, 2	4, 5
3	1.95, br s	29.1		2, 4, 5	3″/7″
4	0.83, br s	17.9		3	1a, 1b, 2, 3″/7″
5	0.72, br s	19.4		3	1a, 1b, 2, 3″/7″
1″		170.1			
2″		142.4			
3″/7″	7.64, br d (8.3)	127.5	1″, 3″/7″, 5″	4″/6″	1a, 1b, 3, 4, 5
4″/6″	8.35, br d (8.3)	124.1	2″, 4″/6″, 5″	3″/7″	
5″		147.9			

Table S2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for the major and minor rotamers of natural brevijanazine B (**2**) TFA salt in DMSO-d_6



brevijanazine B (**2**) TFA salt

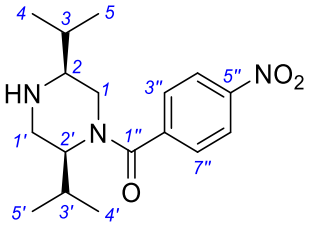
Pos.	Major rotamer		Minor rotamer		HMBC	COSY	ROESY
	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C			
1a	3.19 [a], m	[e]	4.38, m	[e]	2, 2′, 1″	1b, 2	3, 4, 5, 3″/7″
1b	2.52 [b], m		2.53 [b], m		2, 1″	1b, 2	3, 4, 5, 3″/7″
2	2.32 [c], ddd (9.3, 6.2, 3.0)	60.9 [d]	2.32 [c], m	60.2 [d]	1a, 1b, 3, 4, 5, 1′	1a, 1b, 3	4, 5, 3″/7″
3	1.47, m	29.9 [d]	1.67, m	30.6 [d]	2, 4, 5	2, 4, 5	1a, 1b
4	0.77, d (6.7)	18.2	0.96, d (6.7)	19.1	2, 3, 5	3	1a, 1b, 2, 3″/7″
5	0.66, d (6.8)	18.5	0.95, d (6.7)	18.5	2, 3, 4	3	1a, 1b, 2, 3″/7″
1′a	3.19 [a], m	[e]	3.03, m	[e]	2′	1′b, 2′	4′, 5′
1′b	2.77 dd (12.7, 3.3)		2.84, m		2	1′a, 2′	3′
2′	4.13, dd (10.7, 3.3)	54.1 [d]	2.93, dd (10.4, 3.1)	60.4	1, 1′, 3′, 4′, 5′, 1″	1′a, 1′b, 3′	4′, 5′
3′	2.32 [c], m	24.5	2.32 [a], m	25.0	2′	2′, 4′, 5′	1′b
4′	0.88, d (6.5)	18.8	0.70, d (6.5)	19.1	2′, 5′	3′	1′a, 2′, 5′
5′	0.98, d (6.8)	19.9	0.70, d (6.5)	19.5	2′, 4′	3′	1′a, 2′l 4′
1″		169.4		168.0			
2″		142.8 [d]		142.8 [d]			
3″/7″	7.62 (br d, 8.7)	127.8	7.61 (br d, 8.7)	128.1	1″, 3″/7″, 5″	4″/6″	2, 4, 5
4″/6″	8.28 (d, 8.7)	123.83	8.28 (d, 8.7)	123.84	2″, 4″/6″, 5″	3″/7″	
5″		147.6		147.5			

[a-c] Overlapping resonances.

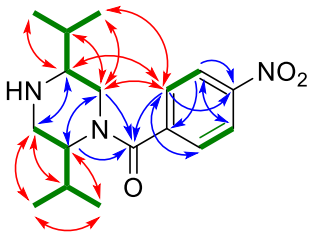
[d] Very broad resonances. Chemical shifts obtained from the HMBC spectrum.

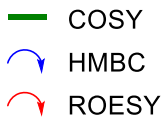
[e] Not observed due to significant peak broadening.

Table S3. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for the major and minor rotamers of synthetic brevijanazine B (**2**) free base in DMSO-*d*$_6$



brevijanazine B (**2**)





Pos.	Major rotamer		Minor rotamer		HMBC	COSY	ROESY
	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C			
1a	3.13[a], m	46.6	4.31, dd (12.6, 2.9)	40.5	2, 2′, 1″	1b, 2	3, 4, 5, 3″/7″
1b	2.75[b], m		2.46, dd (12.6, 11.2)		2, 1″	1a, 2	3, 4, 5, 3″/7″
2	2.23, ddd (9.3, 6.2, 3.0)	61.2	2.35[c], m	60.5	1a, 1b, 3, 4, 5, 1′	1a, 1b, 3	4, 5, 3″/7″
3	1.40, oct (6.7)	30.3	1.60, oct (6.7)	30.9	2, 4, 5	2, 4, 5	1a, 1b
4	0.77, d (6.7)	18.48	0.94, d (6.7)	18.9	2, 3, 5	3	1a, 1b, 2, 3″/7″
5	0.66, d (6.8)	18.31	0.93, d (6.8)	18.54	2, 3, 4	3	1a, 1b, 2, 3″/7″
1′a	3.12[a], m	46.1	2.97, dd (12.3, 1.4)	46.5	2′	1′b, 2′	4′, 5′
1′b	2.65, dd (12.7, 3.3)		2.75[b], m		2	1′a, 2′	3′
1′-NH	2.18, br s		2.18, br s				
2′	4.06, dd (10.7, 3.3)	54.7	2.85, dd (10.4, 3.1)	60.9	1, 1′, 3′, 4′, 5′, 1″	1′a, 1′b, 3′	4′, 5′
3′	2.35[a], m	24.4	2.35[c], m	24.8	2′	2′, 4′, 5′	1′b
4′	0.97, d (6.5)	20.0	0.68, d (6.5)	19.5	2′, 5′	3′	1′a, 2′, 5′
5′	0.88, d (6.8)	18.8	0.69, d (6.8)	19.1	2′, 4′	3′	1′a, 2′l 4′
1″		167.3		167.9			
2″		143.1		143.2			
3″/7″	7.60, d (8.7)	127.8	7.58, d (8.7)	128.1	1″, 3″/7″, 5″	4″/6″	2, 4, 5
4″/6″	8.274, d (8.7)	123.8	8.267, d (8.7)	123.8	2″, 4″/6″, 5″	3″/7″	
5″		147.51		147.45			

[a–c] Overlapping resonances

Table S4. *In vitro* bioassay results for brevijanazines.

Compound	MIC (μg/mL)							
	Bs [a]	*Sa* [b]	*Ca* [c]	*Sc* [d]	*Tf* [e]	NS-1 [f]	NFF [g]	*Teff* [h]
brevijanazine A (**1**)	–	–	–	–	–	25	–	–
brevijanazine B (**2**)	–	–	–	–	–	50	–	–

[a]*Bacillus subtilis* (ATCC 6633); [b]*Staphylococcus aureus* (ATCC 25923); [c] *Candida albicans* (ATCC 10231); [d]*Saccharomyces cerevisiae* (ATCC 9763); [e] *Tritrichomonas foetus* KV-1; [f] Mouse myeloma NS-1 cell line (ATCC TIB-18); [g] Human fibroblast NFF cell line (ATCC PCS-201); [h] *Eragrostis tef* (teff); – means no inhibition was observed up to 100 μg/mL.

Table S5. *A. brevijanus bvj* gene cluster in comparison to *A. tanneri* homologous gene cluster.

A. brevijanus ***bvj* gene cluster**[a] Gene name (JGI Protein ID)	***A. tanneri* homologous gene cluster** NCBI locus_tag (% id.)*	**Deduced function**
bvjA (278921)	***EYZ11-005793*** (61.4%)	NRPS (A-T-R)
bvjB (263911)	***EYZ11-005798*** (67.1%)	NmrA-like reductase
bvjC (275736)	***EYZ11-005803*** (56.9%)	*para*-Aminobenzoate synthase
bvjD (275735)	***EYZ11-005797*** (56.8%)	Aminodeoxychorismate synthase
bvjE (275735)	***EYZ11-005808*** (49.5%)	NRPS (A-T-C) PNBA transferase
bvjF (278922)	***EYZ11-005805*** (71.6%)	P450 (PNBA synthetase)
bvjG (278924)	***EYZ11-005795*** (50.0%)	NRPS (A-T-C) PNBA transferase

* Percentage values represent protein identity
a *bvj* gene cluster location in *Aspergillus brevijanus* CBS 111.46 genome on JGI MycoCosm database: https://mycocosm.jgi.doe.gov/cgi-bin/browserLoad/?db=Aspbrev1&position=scaffold_222:1-25000

Table S6. Primers used in this study.

Name	Sequence	Description
pKW-bvj_frag1-F	TAGTAACCTCGCGGGTGTTCTTGACGATGGCATCCTGCCATCAGGCTACTATTGGATACT	For the construction of pYFAC-CL100 plasmid
bvj_frag1-R1	CGACAGAGTTATGGTGCTTGT	
bvj_frag1-F2	GAGCCAAAGACGAGAGTAGC	
bvj_frag1-R2	GCATGTCTCACCGACATCAAG	
bvj_frag1-F3	GACGTGGACCATGTATACTCA	
bvj_frag1-R3	CGATGCATTCCTGACACATC	
bvj_frag1-F4	GTTGTTCTCGGCACTGATGTG	
pyrG-bvj_frag1-R	GACTTCAACACAGTGGAGGACATACCCGTAATTTTCTGGGCATTGATGAGTCGGTCGGTC	
pKW-bvj_frag2-F	CTTAGTAACCTCGCGGGTGTTCTTGACGATGGCATCCTGCGACCGACCGACTCATCAATT	For the construction of pYFAC-CL101 plasmid
bvj_frag2-R1	CGGTTAGGATATCCGCGAAC	
bvj_frag2-F2	TCCATCATCCATGTGCACG	
bvj_frag2-R	CTTCTGCTAAAGGGTATCATCGAAAGGGAGTCATCCAGCAGGGCTCGGAAGTCAGTAATA	
pKW-bvj_frag2_W/O P450-F	CCTTAGTAACCTCGCGGGTGTTCTTGACGATGGCATCCTGCTCGCGCTGGTTAATCAGAC	For the construction of pYFAC-CL102 plasmid with the primer bvj_frag2-R1
pKW-bvj_frag1 W/O PABA-F	ACTTCTGCTAAAGGGTATCATCGAAAGGGAGTCATCCAGCCAGCCAGAGTTTGCCTGACT	For the construction of pYFAC-CL103 plasmid with the primer bvj_frag1-R3
bvj_bvjE-R1	TGCTGCGCTTGTACATGC	For the construction of pYFAC-CLl04 plasmid
bvj_bvjE-F2	CGGATATCAGGACTGCTATCG	
pyrG-bvjE-R	GTAGGAGTGATGAGACCCAACAACCATGATACCAGGGGGCTGGCGATGGGCTACCAGTAA	
PalcA-bvjF-F	ATTAGAACTCTTCCAATCCTATCACCTCGCCTTAATATGGGATTCAGTACAATTGACACG	For the construction of pYFAC-CL105 plasmid
pyrG-bvjF-R	ACAGTGGAGGACATACCCGTAATTTTCTGGGCTTAATCTAATGCGTGGCTAGAGAGATCC	

Table S7. Plasmids constructed in this study.

Name	**Backbone**	**Description**
pYFAC-CL100	pYFAC-pyrG[4]	*Aspergillus nidulans* expression vector containing *bvjA*, *bvjB*, *bvjC*, *bvjD*, and *bvjE* under their native promoters and terminators.
pYFAC-CL101	pYFAC-ribo[4]	*Aspergillus nidulans* expression vector containing *bvjF* and *bvjG* under their native promoters and terminator.
pYFAC-CL102	pYFAC-ribo[4]	*Aspergillus nidulans* expression vector containing *bvjG* under its native promoter and terminator.
pYFAC-CL103	pYFAC-pyrG[4]	*Aspergillus nidulans* expression vector containing *bvjA* and *bvjB* under their native promoters and terminators.
pYFAC-CL104	pYFAC-pyrO[4]	*Aspergillus nidulans* expression vector containing *bvjE* under its native promoter and terminator.
pYFAC-CL105	pYFAC-CH1[4]	*Aspergillus nidulans* expression vector containing *bvjf* under alcohol-inducible *alcA* promoter.

Table S8. Strains used in this study.

Stains	**Organism**	**Description of Strains**
A. nidulans-bvjABCDEFG	*A. nidulans* LO8030	*A. nidulans* LO8030 expressing pYFAC-CL100 and pYFAC-CL101 plasmids.
A. nidulans-bvjABCDE	*A. nidulans* LO8030	*A. nidulans* LO8030 expressing pYFAC-CL100 plasmid.
A. nidulans-bvjABCDEG	*A. nidulans* LO8030	*A. nidulans* LO8030 expressing pYFAC-CL100 and pYFAC-CL102 plasmids.
A. nidulans-bvjABE	*A. nidulans* LO8030	*A. nidulans* LO8030 expressing pYFAC-CL103 and pYFAC-CL104 plasmids.
A. nidulans-bvjABG	*A. nidulans* LO8030	*A. nidulans* LO8030 expressing pYFAC-CL103 and pYFAC-CL102 plasmids.
A. nidulans-bvjE	*A. nidulans* LO8030	*A. nidulans* LO8030 expressing pYFAC-CL104 plasmid.
A. nidulans-bvjG	*A. nidulans* LO8030	*A. nidulans* LO8030 expressing pYFAC-CL102 plasmid.
A. nidulans-bvjF	*A. nidulans* LO8030	*A. nidulans* LO8030 expressing pYFAC-CL105 plasmid.

Table S9. Revised DNA and protein sequences encoded by *bvj* gene cluster in this study.

bvjA
DNA sequence: ATGACTATCATCCTTGAACACAAGAACGAGGCGGAGAGCAAGCAACACGGTCTAGCAGAGCATCTGGTCCAACG GGTCTTGCTGGAGGGCTCCAAGCCGGCAATTCAGGACGGGGACCTTGTCTTGAGTTATCGAGAACTTCATGCGA AAGCTTTATATCTTGCATGGCAGATCCATCAGCTGAGCCCCAAAAGCGACTCCCCCGTGGGAATCCTCGTTCCT CGGAGCCTGAACCATGTCCTGTCCCAGGTGGCCGTTATATACGCCGGACGGGCATGTGTTCCCCTGGATGACCG GCTTCCAGACAGTCATCTGGACGATGTGCTTCAAAAAGTGCGAGCTCAGCTAGTCATTACCACTGCAGCTCAGC AACTCCGGCTGCCATCTAGCCGCCGTCTCGTCGTCGACCAGCATACGCTCCCCAACACCGCAGAGAGCATCCAC TTTGAACCCGTCCGACGGAGCCCACAAGCCTGTTGTCATGTTCTTCACACCTCTGGGACGACGGGAAAACCCAA GGCGATTGAGGCCCGTGCTGAAGGGTTGATAAATCTATGTATAGATCCAGTGGATCTGGTACGCCGCGGCCAGC GCACGGCCCATGGGGCTGAGCCCATCTTTGACCTTTCTGTCCTCGAAATTTGGGGCCCCTTGCTTCGTGGGGGC ATGATTCTGGTCGTGCCACGGCAGACCATGCTCGACCCTCTGTCCCTCGAGCAATTCCTCCGGGCGCATCGAGT GGATGTGATGATGCTGACGACCTCCCTTCTCACAGTCACGGTTTACACTAGGCCCGGGGCATTCTCCACACTTG ACACCTTGGCCACTGGAGGCGAGGCCATCAATTTTCGGACAATTGAGGCCATGTTCCGCTCCGGGCCCCCAAGG CGCATCATCAACGGATATGGTCCTTCGGAAACTTCTGTGTTTGCGCTGCTACACGTCGTCACTAAAGAGGACGC CATCCGAGGACAAATACCCATTGGGAAGCCCCTGCAAAACGTCGAGACATTTATTGTAAACGAGGATATGGAGC CCGTGGAGCCAGGAGAGGTGGGCGAGCTCCTCATTGCGGGCATCGGGCTCGCTGGTGGTTATCTTCACGAGCCG GTCAAGACGGATCTTAGTTTTCTTTCCCTGTCCCATCTCCCTCGGACAGTGAAGCGTGGTACAGGCAAAGTGTA TCGCACGGGAGACCTGGTGCAACGAGATGCCCAGGGTGTCCATCACTACATCGGCCGGTTTGACCATCAGGTGA AGATCCGCGGCCAGCGCGTCGAGCTGGAGGCATTGGAAGACACTCTCTTGCAAACAAAGCTCGTGCGCGTCGCC GTCGTTATCAAAATCACTCCATTGGAGATTGATAAGGGGCAGTTCCTGCTGGCCTTCTGCATCCCTACTACCAC AACCATCACAGCCGGCGCCATCACCAAGGCTTATATCGAACAAGCACCGCACCACCTCGTGCCCCGAGTGGAGC TGATTGACAGTCTGCCTCTGGGACCAACTGGCAAGGCAGATCGCCGGCTGCTAGAGAGACAATATTTCGATCGC CTGGCCCTGGCACGGAGGAACCGCGTTCAAGTGACGAACAACAGTGGACCACGCGAGGTTGCCGCACAAATCCA GGATATCTGGACCGACGTCTTTGGTTTGACAACGGATCATCTCGCCGCGACGGATGACTTCGTGGCCATGGGAG GTACGTCACTGATGGCTGCGGTGATGATCGCACGCATCAATCAAATGTTCGGCATTTCCATCCGCTCGCAGATG GTGTATGAGAACACGACGGTCGAGGCTCTGACCCGGCTTGTCTTGCGCCTGCGTGATGAGGGCGAGGATCCGCA ATCGCTCAGCGAGACCGATGTCTGGCTGCAGGATTCCAAGCTGGCAGAGCAGCTTTGGCCTGAAGGGCACGCTA TTGCCGACTGGCAGGGACCTTCAGAAGGCCGTGTCTTTCTCACAGGGGCGACTGGCTTCATAGGGATCGTCATG CTGGCCTCCCTGGTGCAACGGCCGGACGTGAAGCAGGTAGCCTGCCTTGTGAGAGCCGCCAATAAGACCGCGGC TCAGCGTCGGCTACAGTCAACGTTGGCACAGTACGCCCTGGGCGCCGACTTTCAAAAGGTCATCGTGATTCCGG GTGACTTTCGCCGACCCCTGCTTGGCATGACAGAAGATCAGTACGACTACTACGCTCAATGGTCCAGCGTCGTC TTCCACCTCGGAGCTAAGGTCAGCTACGTGGCCCCGTATTCCTCTCACAGAGAGGAGAATGTCATGGGCACGCT CCATATGCTGCAGTTTACGAACCACCGCCGCCTTAAGCAGCTGCACTACACGTCAACGATCGCCGCATATGGGC CAACCGGGTACGTGACGGGGGCCAAGAAGGTGCTGGAAGATGAACGTCCGGCATCGCACCTGGCGGCGTTGTCG TACGATACGGGCTACTCGCAGAGCCAGTATGTGGCCGAGGCAATTGTATGGAACGCCATTGACAACGGCTTTCC CGTAGCTATCTACCGGCCAGGCTTCGTCATCGGCCACAGTCAATTTGGCGTATGCAATCCCAACGACTTCGTCT CGCGCCTTTACCGCAGTTGCATGGACATGGGTACATACCCATTGCTGCCAGATCAGCGGAAGGAGTTCGTTCCT GTGGACTTTGTGGTCAATGCTCTTGTGCACATCGCCGGTGACAGTCAGAATCTAGGCCATGCGTACAACCTCGT TCAGCCTGATGTTTCCAACGCCCTGGACATCAACACCTGCTTCGAACTTCTAAATCGTATCTCCCCGTATCGAA TGCGTGGGATTCCCTATCCTGAGTGGGTGAATTCCCTGTCTATGCGGCTGGATGACCCCCTGCAGCCGCTCACG CCCATGCTCAAGGAGCAAGTGCTTGGGAGCCGCTCTCGCTGGGAAGTTTACGAAGGAATGGTCGAGTATGGGCG CGAGAACCTCTGTCGAGCTCTAAGAAATGCGCCTGGTATTGCGCGGTGCGACCCGCTAGATATACTATTCCACA AACTGCTGTCGAGCTGGCTGCCAGCGAGAACAGTGCACGATGATTTCTCCAAGGAAAAGGCCACAGTGATAATC TCTTCGCACCCTATCAACCAAGAGGTTTGA
Protein sequence: MTIILEHKNEAESKQHGLAEHLVQRVLLEGSKPAIQDGDLVLSYRELHAKALYLAWQIHQLSPKSDSPVGILVP RSLNHVLSQVAVIYAGRACVPLDDRLPDSHLDDVLQKVRAQLVITTAAQQLRLPSSRRLVVDQHTLPNTAESIH FEPVRRSPQACCHVLHTSGTTGKPKAIEARAEGLINLCIDPVDLVRRGQRTAHGAEPIFDLSVLEIWGPLLRGG MILVVPRQTMLDPLSLEQFLRAHRVDVMMLTTSLLTVTVYTRPGAFSTLDTLATGGEAINFRTIEAMFRSGPPR RIINGYGPSETSVFALLHVVTKEDAIRGQIPIGKPLQNVETFIVNEDMEPVEPGEVGELLIAGIGLAGGYLHEP VKTDLSFLSLSHLPRTVKRGTGKVYRTGDLVQRDAQGVHHYIGRFDHQVKIRGQRVELEALEDTLLQTKLVRVA VVIKITPLEIDKGQFLLAFCIPTTTTITAGAITKAYIEQAPHHLVPRVELIDSLPLGPTGKADRRLLERQYFDR LALARRNRVQVTNNSGPREVAAQIQDIWTDVFGLTTDHLAATDDFVAMGGTSLMAAVMIARINQMFGISIRSQM VYENTTVEALTRLVLRLRDEGEDPQSLSETDVWLQDSKLAEQLWPEGHAIADWQGPSEGRVFLTGATGFIGIVM LASLVQRPDVKQVACLVRAANKTAAQRRLQSTLAQYALGADFQKVIVIPGDFRRPLLGMTEDQYDYYAQWSSVV FHLGAKVSYVAPYSSHREENVMGTLHMLQFTNHRRLKQLHYTSTIAAYGPTGYVTGAKKVLEDERPASHLAALS YDTGYSQSQYVAEAIVWNAIDNGFPVAIYRPGFVIGHSQFGVCNPNDFVSRLYRSCMDMGTYPLLPDQRKEFVP VDFVVNALVHIAGDSQNLGHAYNLVQPDVSNALDINTCFELLNRISPYRMRGIPYPEWVNSLSRLDDPLQPLTP

MLKEQVLGSRSRWEVYEGMVEYGRENLCRALRNAPGIARCDPLDILFHKLLSSWLPARTVHDDFSKEKATVIIS
SHPINQEV

bvjB

DNA sequence:

ATGACGTTCTATGAGAAGGTGATTCTTGTGTACGGCGCCACAGGTGAGCATAGCCCCCCCGATGTGGACAGAGC
TGCCACACTCACAAAATGGATATAGGCAATCAGGGGTTTGCCGTGGCTACGTCCTTGCTGAAGAGCCATAAGGG
CTTTTGTGTTCGTGCAATCACTCGGAATCCCCAGTCGGCAAAGGCACGCGAGCTGGCCGGCTTGGGTGCCGAGG
TGGTCAAGGCCGATGGACACAATGATGATGACATGAAGGGGGCTCTTGCTGGTGTCTGGGGTTTCTGGCTCAAC
ACCCACCACCACGACTCAGTATGTGGAACGAACTCTGCCACAGGTGTGCATTCGTATTGATGCTGATTTGAACA
TGTAGGCGGTTACCATTCCCGGTGGCCCAACTGATGAAGACTTCGGATGCCGGCTAGTGTCTCTTGCAGTCGCG
GCTGGAGTGAAGGTGTTCATCTACAGCACCTGCGAGTCTCCAGATGAGATAACCAAGGGCATGACTCCAGTCGA
GGGAATGGATGGTATGTGGCCCGTCATGTTTTCATAAAATCATATCACTGACATCAAGTCCCTCCATTCAGGAA
AGCATCGTGTTGAGATGTATGCTCGAGGCTTTAAGGAGTTTGACGCCGTGATTGGAGCCTTCCCCGGCTGGTAC
TTTGAGAATTTCATTGACTCAGAGTATGCACAGGCATTTGGCGGATTTCCAATCTTCCCGGATAAGGAGGGTTA
CTATACCCTGACCACGCCGGCCGTGGGTGGCAAGGGGAAAGTCCCAACCATTGCCGTCGCCGCTGACTTTGGGG
AAATGATCCATGGAATGTTCTTAAACCCTGCAAGATATCGAAACCAGACTATACAATGCGTCAGCGACACCTTC
ACCTTTAGTGATGTGGTCAAAACGTTTACCGAAGGCAGGGAGTCGTCTTTCTTTATCTATCTAGTGAAAGCTAA
CGATAGTTCAAAGTCACTGGTAAAAAGGCTCGCTATATCGAGATGGAGTCGTCCGATGACTTCCCAACTCACGG
CAGCAGTGTTTTGATAGAGCTGCAGAACGTCTTCACGTATACACAACACCAGAACGGATGGTTCTTTGGTCGTC
CCGAGGATCTTGCCACTTGTCAGTCTCTAAAGGATGACGCCCGCAAGGACAAAGGGCTGCCACTAGAGCGCGCC
ATGACCTTGAAAGGTTTTTTTGTCGATCACTATGGAAAGAAGCTGTAG

Protein sequence:

MTFYEKVILVYGATGNQGFAVATSLLKSHKGFCVRAITRNPQSAKARELAGLGAEVVKADGHNDDDM
KGALAGVWGFWLNTHHHDSAVTIPGGPTDEDFGCRLVSLAVAAGVKVFIYSTCESPDEITKGMTPVEGMDGKHR
VEMYARGFKEFDAVIGAFPGWYFENFIDSEYAQAFGGFPIFPDKEGYYTLTTPAVGGKGKVPTIAVAADFGEMI
HGMFLNPARYRNQTIQCVSDTFTFSDVVKTFTEVTGKKARYIEMESSDDFPTHGSSVLIELQNVFTYTQHQNGW
FFGRPEDLATCQSLKDDARKDKGLPLERAMTLKGFFVDHYGKKL

bvjC

DNA sequence:

ATGGCTCAACCAAGCGTTCTCTTTATTGACGCCTATGACTCTTTCTCCAATAACATCATCACCTTATTGGAAGA
GCTATTGAATGTCTATGTGGTAAAAATCACAATCGACTGTCCCCTTCCCGCGGACCTTCGTGCCTCTTACGCCG
GTATCATCATCGGCCCAGGGCCGGGCAGCCCATACAAAGCCACCGACGTGGGCTGTATCAATGACGTCTGGAAA
CTATCACCCGAGGAGATGGTTCCTGTTCTGGGCGTCTGCCTTGGATTCCAGAGCCTTGTTGCACACCATGGTGG
CAGCATTCAACGTCTGCCGTACCCACGTCATGGCATTGAGACCGAGGTTCAAACCTGTAATGCCTCAATCTTCC
AGGACATCCCATCGCTCAGTTCGGTGCAATACCATTCTCTGTACGGAAGTATCGGTGATCGATCTGCCAGTGAC
GAAATGTGGACTGCCAGCGAAACGTGTCCGAATCTTGTCCCGCTAGCCTGGGACGTGGACCATGTATACTCACG
AAGACCCCCAACTGCCAATGATGCTCGTGCATCGCTGGGGGTTCTCAACCCTCGTCATATCTTGATGTCGGTGA
GACATGCAAGCAAACCATACTACGGCGCTCAGTTCCACCCAGAGTCCGTGTGTTCGGACGCTAACGCTCGTCAG
ATGATCCTTAACTGGTGGCAAACGGCCTTGGATTGGCATCGCGAAGCTGGCAACGTTCGTTGGTGCCAGATAGT
CGATGCAGTTAAGGTTTTCGCATCCAAGATTCCCCTGGGAATGTTGAATGTCGCGATGATTCGGGATCATTGCC
GCACTGCCGGCCCAGAGATCGTAGTGCTGGACTCGGAGATGAATCAAGCATCCCCCTTGGGTAACCACAGTATT
ATTGGACTTGTCTATCCCAACAGCCTGAAGATCTGCTATACCGCCGGAGACAAGCAGGTCTTTCTGGTTCGGGG
CGCGGAAGCCACTGCTGAAAGCCTGGAGCCATACAACGACAATATCTTCGAGTTCCTCAAAGCTTTCATAAAGA
AGCATCACCGACGCCATACTGTGCCAGACTCCCCTTTTTGGGGAGGACTCATGGGCTATATCTCCTATGAGGCC
TGCCTGGAAACACTGAATATCACTGGGAAGAACGTCAATGCCAACCGTCCAGACATTCAGTTTGCATTTGTAGA
GCGCAGCGTGGTTATAAATCATACCACCAACCACGTCCACATCCAGAGTATCATGCCTGAAGATTTTCACTGGG
TCAAGGATACGGCGTCCAGCCTGGCTCGTCTGGTTTATGTTCCCTCCCGCCCGGTGAGCCACGGCGAGGGAGTA
TCGCCAACATCGCTCTCTGTGCATTCCTCTGGCGACGATAGTTCCATAGGCCCTGGCTCCTCTACTGACAGTCG
CAGCTCTTACCCGACATCAGTGGAGTCCGTCGGGGTGACTCACTCTCACCCCAAGTACCACGTCCCCAGCGAGG
CGAGCTACAGCCGGAAGATTGTCGAGTGTCAACAGGAGATCAGGGAAGGCAACTCCTACGAACTATGTCTGACT
GGCCAGACGGAGATTGAGATCCCGGCCTATCCCTCAATGCTATCGTCCTGGTCGCGATACCTGCGCCTGAGGCG
CGTGAACCCGGCTCCTTTCGGGGCCTATGTGCGCCTAGGACCGGTTACTGTGATGTCTTCGTCGCCGGAGCGCT
TCATGTCGTGGAGCAGGCCGGATTCACACCATCGTGTTACTTGCCAGTTCCGGCCCATCAAAGGAACCGTCAAG
AAAGAGCGTGTTCATCCCGACGGCCTTGTTGAGGCGGTAGATCGCGCAACTGCCACAAGGATCCTGTCGACCCC
CAAGGAGCGGGCGGAGAACCTGATGATCGTCGACCTCATCCGCCATGATCTGGCCGGAGCGGTAGAGCCTGGTA
GCGTCATAACCAAGTCCCTGATGCAGGTGGAAGAGTATCACAGTGTCTATCAGCTAGTAACAGTTATTGAGGGT
ACACTGAGCACCCAGCGCCCTTCTCCCCGGCCGAATCATGGCATCGACGTGTTGGCAGTGAGCCTACCACCAGG
CAGTATGACTGGAGCGCCCAAGAAAAGGTCCTGCGAGCTTTTGCAGAATATTGAGGATGGGGCGCCGCGGTCGA
TTTACTCCGGGGTACTCGGATACATGGATGTCGGTGGAGGCGGGGATTTCTCGGTTGTGATTCGCACTGCATAT
CGATGGGATGATGAATGCAACGAAAATGGCGAAGTGTGGAGGGTAGGCGCAGGGGGAGCCATCACTGACATGAG

CAATGAGCGTGATGAGTGGGATGAGATGCATACCAAGGTGGATGCAGTGCTTCGGTCCTTCAAGCAGGTGCATG
TGATCTGA

Protein sequence:
MAQPSVLFIDAYDSFSNNIITLLEELLNVYVVKITIDCPLPADLRASYAGIIIGPGPGSPYKATDVGCINDVWK
LSPEEMVPVLGVCLGFQSLVAHHGGSIQRLPYPRHGIETEVQTCNASIFQDIPSLSSVQYHSLYGSIGDRSASD
EMWTASETCPNLVPLAWDVDHVYSRRPPTANDARASLGVLNPRHILMSVRHASKPYYGAQFHPESVCSDANARQ
MILNWWQTALDWHREAGNVRWCQIVDAVKVFASKIPLGMLNVAMIRDHCRTAGPEIVVLDSEMNQASPLGNHSI
IGLVYPNSLKICYTAGDKQVFLVRGAEATAESLEPYNDNIFEFLKAFIKKHHRRHTVPDSPFWGGLMGYISYEA
CLETLNITGKNVNANRPDIQFAFVERSVVINHTTNHVHIQSIMPEDFHWVKDTASSLARLVYVPSRPVSHGEGV
SPTSLSVHSSGDDSSIGPGSSTDSRSSYPTSVESVGVTHSHPKYHVPSEASYSRKIVECQQEIREGNSYELCLT
GQTEIEIPAYPSMLSSWSRYLRLRRVNPAPFGAYVRLGPVTVMSSSPERFMSWSRPDSHHRVTCQFRPIKGTVK
KERVHPDGLVEAVDRATATRILSTPKERAENLMIVDLIRHDLAGAVEPGSVITKSLMQVEEYHSVYQLVTVIEG
TLSTQRPSPRPNHGIDVLAVSLPPGSMTGAPKKRSCELLQNIEDGAPRSIYSGVLGYMDVGGGGDFSVVIRTAY
RWDDECNENGEVWRVGAGGAITDMSNERDEWDEMHTKVDAVLRSFKQVHVI

bvjD

DNA sequence:
ATGGCAGAGCAGATTGGTGTGTTTACCACTTTGCGGTACGACCCAGCACTACCAGACGAGGTTCACACGAATGC
GGTTGATTCCTATCCGGATACCCCCGTCACGCCGTTTTACCTCCTCCGCTATCATCAGGACCGCCTGCTCCATG
CTGCCAAGTGCCTTGAGTGGGCAGACGCGGTCCTGTTCTTAGAGAAGCCCACCAGCCAATTTGTCGAGTTCCTA
GATGGTTCGATTCCGGATAAGACAACACCGTGGCGGCTCCGGGTTGTCGTTGATAGCTGTGGAAATGGGACGGC
CGAGGCATTCCCAGCCAGCACAGTGGAGACCACCAGTCTTTTGATTTCGAGTGCTTTAGGAATTTCGTCGGAGG
CATGGAGTGTATATGTCGACTCCGAGCCTACCATCCCATCAAAGTACACCACCCATAAGACGACTGCGCGAGAT
CATTATACAGCCGCACGACACAGATGTGGCATCTTGTCTCTACAAGCCCAGGCCGAGGTACTTCTGATAAATCC
AGAGGGCGAGGTGATGGAGGGAAGTATAACAAGTGTGTATTTGAGGTGCCGTCGCTCTGTCGCCCATGACGATC
AAAGCGAATGTCCTGAATGGGTCACACCTCCACTATCCAGTGGAGGCAATGCAGGAACAACACGACGGTACGCG
TTGGGACACGGCTTCTGCTCGGAACGACAGATTAGCGCGGCTGAATTGGTTGACGGAGAAGAATGTTGGCTCAG
TAACGCAGTTCGAGGGTTCATAAGGGGCACAATTGTTCTCAAGAGGTAA

Protein sequence:
MAEQIGVFTTLRYDPALPDEVHTNAVDSYPDTPVTPFYLLRYHQDRLLHAAKCLEWADAVLFLEKPTSQFVEFL
DGSIPDKTTPWRLRVVVDSCGNGTAEAFPASTVETTSLLISSALGISSEAWSVYVDSEPTIPSKYTTHKTTARD
HYTAARHRCGILSLQAQAEVLLINPEGEVMEGSITSVYLRCRRSVAHDDQSECPEWVTPPLSSGGNAGTTRRYA
LGHGFCSERQISAAELVDGEECWLSNAVRGFIRGTIVLKR

bvjE

DNA sequence:
ATGACCAAGTCAATCCCTGAGCCCCAAGAGCCGTCTCATCGCAGCAGTGTCAGTGAGATAGTGGGTGGTCCAGT
TGAATTCGTTATTAGGGGCCCATTGGCCTGTTTCGAACATATAGGCAACACTCATCCGGACGCCGTGGCATTGA
TTTGTGGCCATCAACGCCCTGGAGTATATGGGCTGGTGAGCTCAAGTGGGTCACATAAAGAAGATGCGTCACCT
GGCCCGGCACGGTGGCCATATGGTGTTTTATTGAAAGCACTGAAACAGCTGTCCTTGTCGTTGAACCGATTGGG
TCTCTTGCCCAATAGTTCGCTGTTCGTCATTCTGGGTAACTCTGTCGAACACGTCCTGGTATCCCTGGCGGCGT
ATCGGATTGGCTGTGTCCATGTGTCAATGAATCCTCAAAATCTCGCCAACCCGTCGGTAGCTAGTCATATGGTC
ACTACGGTATTGGAGCATCGCAAGACGTCGAGAGTGGGCTTCGTAGCCCAGGACAAGCGGGCAGCAGCACGGTT
TGATGCTCTATTTCCAGACTTAAACTGCGTAAGGATTTCTGTAGAGAACGGTATACCAGGCTGGATCCCTTTTG
AAACGTTGCTCCACTCCGAGTGTGAAAGTGCTGGAACGAAGGAGGAAGACCAGTGCCACAGGCAGCAGCATTCA
GAAACGTCCATCTTCTTCACAAGCGGGACGACATCAATGCCCAAAGCATGCAGCATGAATTTATCCGCGTGGAT
GACAGCCCTTGGCAGCCGCTCCAGCTTAGCATTGACTTCGCCCGGCGACAGAGTTATGGTGCTTGTACCCACCA
GCCATTCTTTTGGGTACATGGGACAAATGTTTGCCTTGACGCGTGGGGCTACTCTCGTCTTTGGCTCCTACGGA
GAGTTCGACCCCAAGATGTCGATCAATCTGTTGCGCAGTGATGGCTGCACCCACCTGGTCATGGTGGCCGCCTT
GGCCAATGCCTTCATCTCCGCCGACCTAAGCGGTATTAAGCCGCTGCGGGCAGCTGTCCTATCCGGAATGGGCC
TGCCAATAGCGGTAGCAAAGGAGTTGCAGAGAGCCCTTGCAATGCCGCAAATAGAAAACATCTATGGGATGATG
GAGGGTGTGCATTTTTCTACGGGGCCAGTCAATGATATTCAGTCCATTTGTACAGGTGGCTATATCTCCGCAGG
ATTTCCCTCGTGTGGCGCCACAGCCCGCATCTGTGCTCCTGGGACACGGCTTCCCCTTCGTCGAGGCGAGCCTG
GTGAACTCCATTATAGTGGCTTCCAGCTCACAAAGGGATACGTTGGAATGGAGACGAACGACTTTTTCGCTGAT
GAAGATGGTAAACGCTGGTTCAATACTGGTGACCAAGCCATTATCCATACGGACGGTCAACTCTATATTGTAGG
CAGATACAAGGACGTTATCGTACGTGGGGGAAAGAATGTCTCCCCCTCTGCCATTGAGTCAGTCTTGAATGAGC
AGCACCACCTACAAACGCTAAATCCGCAGGTCGTTGCGTGCCCTGACCCTATCGCTGGGGAGGTTCCAGTGGTC
GTCGTCAACCAACCCATTGATAAAGACACAATACTACACGTTATCGATACCATACGTGTGAAGATGGGGCCTAT
GTACGTCCCAAGCAAGGTTGTGTCAATTGATATGCTCGGTCAGCCAGATTATCCACGAACAACATCCGGGAAAA
TTCAAAAAAGCAAGCTTGCTGCGCTTGTACATGCGGAGGAGGAATCTCATTCCTCGTCCACTCCCGATAGCAGT
CCTGATATCCGGTCCACCGCCAGCAACAAGATAAAGGAGCTCTGGGCAAGAACACTTGGAATTTCCGACGTGGA

CCTTGATATACACTCTCGTTTGGCGGAGCGAACGGACAGTATCACTTTGATGAGGGTGCATTCCAAAGCCCGCA
GAGCGACGGGTAAGGATGTGTCGTATACAGCGTGGGCCTCGGTTGAGACAATTGCGGATCAAATGGATTTATTC
GCACAATCTCCCCACATGCGACATATTGGTATCAAATGCCCTCGACCACCGTTGTTACGTCCACGGGGACCACG
TCCAGAAGATATGGTCCACCTATCGGGAGACCCGAACGGGTTCTGGGATACTAAGGACCTCATCGCACGAAAAA
TTGCTCCGTTCAACCTAGATTGGGATGATGTTGAATCTGTTTTCCCATCAACTGACACAATTGATCTATTGCAT
CGACACAACGTCCTCAATAGCTGGGAGATGTTCATTTCGGTGATCACTACGAATTCAGATAAGAACGTGTGTTT
TGTATCTTGTCTCCAGAAAATGATTGAGCTGACATTCCCAGGCGCTGCGCTCGGCAATGGAAAGGACACTATCC
GTACACCCAATGCTGCTTTCTTTCCTTGTCTCTGGCGATCTCAATGTTGCACATGGCCGTACGCTTCATGTCAA
TATTCATCCCTGCAGAAACATGCTGGATGAGTGCATCGTTGACTACGGTGAGGTTGACAACCTCCATGAGCTGC
GATATCTCAGGGAAAATTTCCCCAAAGAACACCGGATAGTGCCGACGGGCCTTGCTTTCAAAGGACTTGTTATA
TTTGTCAAGGAAACAAACAGTGCAGCACTGGTGTCCAATAGTAAGTATATGAGACAAGAATTATTGAGGTCGAA
TTACTGAGATTCTGACTACGACAGTCTCCCACGCCTCCCTGGATGGGACCTATCAAGCATTATTTATGGACGAC
CTCAATCACGCTCTTGGTGGCGACGAGCAACTGGAGTCTCATCTTCCATACAAAGCGTGGGCAGAAGCCTATTA
TCTCTTCGCCGATTCTGCCGCTGCCAGGCCAGCCCTTGACTATCACCTCGCTGAGATAGAGAAGCTTCAGGACC
GGAGACCCAAAATCTGGCCTGGTCCTCCCACTATGCCGGAGGCTACCACCAAGGAAGCCAATAACGAACTAAAA
CAGTTTGAGTTCCAGCTCCCGGGCATTTTAAGCCTCCGCCGCCATCACCCCACCCTTACTTCGTCTGTTGTCGT
TAAGGTGGCTCTCGCGCTTCTAGTGATGCATAGGACTCAGCAGCCAAGGGCAATCTTTATGAACACCGAGTCGG
CGCGGTCCTCATTTCCGTTTCTCCCTCCATCAGTTTCTGACTTGGGTTCCTTTCAAGCGATCGATGTTGCTGGT
CCAACTATCACCATCAACATTGATATTATTGACTACCAGTCTACGCAAACTGTTCTCGACCTGCTGCAACAGGT
TCAGGAGCAGCAGGAAAGCACTACCAAGCATGCAAATGCACCATGGCACAAGATCTTCGAGCAAGCTCCGTGGG
CTCAAGATCTCTTTTGCGGGCTTGCAGACACCCTGATATTCAACTGGACAGGTGCAAGCACTTTCCAAGAAGAC
ACCGCTCACAGTGTTCATGAGAATATTAAGATCAAACAGCTCTTCTTTCGCCCAAATACCCGACTTATGGTAGA
CGCGGGAATGGCGGGGAAGGATGGCACAGACGTGGTCATCTGCCTAAGCGGGCTGATTTCAAGGATATCGGTAG
ACTCAATCAATAGCATTGCGACGGATCTTAAGCAAATCACTACCTGGCTTGTGGAAGGGAACAATTGGGAACGA
CCTGTTCGACACTATATCGAGTGTCTGTCCTACTTCCTACAATGA

Protein sequence:

MTKSIPEPQEPSHRSSVSEIVGGPVEFVIRGPLACFEHIGNTHPDAVALICGHQRPGVYGLVSSSGSHKEDASP
GPARWPYGVLLKALKQLSLSLNRLGLLPNSSLFVILGNSVEHVLVSLAAYRIGCVHVSMNPQNLANPSVASHMV
TTVLEHRKTSRVGFVAQDKRAAARFDALFPDLNCVRISVENGIPGWIPFETLLHSECESAGTKEEDQCHRQQHS
ETSIFFTSGTTSMPKACSMNLSAWMTALGSRSSLALTSPGDRVMVLVPTSHSFGYMGQMFALTRGATLVFGSYG
EFDPKMSINLLRSDGCTHLVMVAALANAFISADLSGIKPLRAAVLSGMGLPIAVAKELQRALAMPQIENIYGMM
EGVHFSTGPVNDIQSICTGGYISAGFPSCGATARICAPGTRLPLRRGEPGELHYSGFQLTKGYVGMETNDFFAD
EDGKRWFNTGDQAIIHTDGQLYIVGRYKDVIVRGGKNVSPSAIESVLNEQHHLQTLNPQVVACPDPIAGEVPVV
VVNQPIDKDTILHVIDTIRVKMGPMYVPSKVVSIDMLGQPDYPRTTSGKIQKSKLAALVHAEEESHSSSTPDSS
PDIRSTASNKIKELWARTLGISDVDLDIHSRLAERTDSITLMRVHSKARRATGKDVSYTAWASVETIADQMDLF
AQSPHMRHIGIKCPRPPLLRPRGPRPEDMVHLSGDPNGFWDTKDLIARKIAPFNLDWDDVESVFPSTDTIDLLH
RHNVLNSWEMFISVITTNSDKNALRSAMERTLSVHPMLLSFLVSGDLNVAHGRTLHVNIHPCRNMLDECIVDYG
EVDNLHELRYLRENFPKEHRIVPTGLAFKGLVIFVKETNSAALVSNSHASLDGTYQALFMDDLNHALGGDEQLE
SHLPYKAWAEAYYLFADSAAARPALDYHLAEIEKLQDRRPKIWPGPPTMPEATTKEANNELKQFEFQLPGILSL
RRHHPTLTSSVVVKVALALLVMHRTQQPRAIFMNTESARSSFPFLPPSVSDLGSFQAIDVAGPTITINIDIIDY
QSTQTVLDLLQQVQEQQESTTKHANAPWHKIFEQAPWAQDLFCGLADTLIFNWTGASTFQEDTAHSVHENIKIK
QLFFRPNTRLMVDAGMAGKDGTDVVICLSGLISRISVDSINSIATDLKQITTWLVEGNNWERPVRHYIECLSYF
LQ

bvjF

DNA sequence:

ATGGGATTCAGTACAATTGACACGCTCAAGGCCGCGCTGCCATGGGTTTTGATCGCGATGGTAGGAATTATGCT
AAGGAGAAGATACTTTTCGCCCATTGCGAAGATACGAGGCCCCTGGATAGCCTCGTTTTCTACACTCTGGCATA
TGTGGCATGTCGCCCGTGGTGATCTGGAATATGTCGTGCTGCGGGAACACCGAAAGCACGGTATGATTATTATG
GACCGGCCGGCCAGGGTTGTGGTAGGAGGTTCACCTAGCTAATAGGCGTTGCAGGCGATTTTGTTCGGATTGGA
AACAACGAGGTCTCTTGTAGCCATCCTGATAGCATTACAGCGGTCCTTGCACCGCCCCATCGAAAGGTAAAGTC
GAGGATGTTCCATTTTCTTCTGTCATTGACAAGATGGCGGTGCTGAATAAACTTCTAGGCTGAATGGTATCAGG
CTCTGGCCGTGCCTGACAAACACCACCAGACCCCAATGTCCGAGTGTGACCCAAAGCGCCACCGGGAGCGATCA
TCCATCACGGGTAGCAGCTACTCACTGTCCAATCTGATCAAATCAGAGGACGAGGTGGATGGCTGTATCAGGGA
GCTTCGCGAGAAGCTTCAGGAAATTGCCAAGGCCGGGCAGGCCTTCAACTTGGACCACTGGCTGAACTATGTAG
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Note: The regions predicted as introns in this study are highlighted in red.

Table S10. Media recipes

Glycerol Casein Agar (CGA) *Ingredient*	*Quantity*
Glycerol (Chem-Supply)	30 g
Casein peptone (Amyl)	2 g
K_2HPO_4(Chem-Supply)	1 g
NaCl (Chem-Supply)	1 g
$MgSO_4.7H_2O$ (AnalaR)	0.5 g
Trace element solution*	5 mL
Distilled H_2O	1000 mL
Bacteriological agar (Amyl)	20 g
Autoclave	
***Trace element solution**	
$CaCl_2.2H_2O$	3 g
$FeC_6O_7H_5$	1 g
$MnSO_4$	0.2 g
$ZnCl_2$	0.1 g
$CuSO_4.5H_2O$	0.025 g
$Na_2B_4O_7.10H_2O$	0.02 g
$CoCl_2$	0.004 g
$Na_2MoO_4.2H_2O$	0.01 g
Distilled H_2O	1000 mL
Filter sterilize	

Czapeks Agar (CZA) *Ingredient*	*Quantity*
Czapeks Dox Media (Oxoid)	45.4 g
Distilled H_2O Autoclave	1000 mL

Malt Extract Agar (MEA) *Ingredient*	*Quantity*
Bacteriological peptone (Difco)	3 g
Malt Extract (Amyl)	60 g
Bacteriological glucose (Amyl)	60 g
Distilled H_2O	1000 mL
Adjust pH to 5.5	
Bacteriological agar (Amyl)	20 g
Autoclave	

Oatmeal Agar (OMA) *Ingredient*	*Quantity*
Oatmeal Agar (Amyl)	72.5 g
Distilled H_2O	1000 mL
Autoclave	

Oatmeal Liquid (OML) *Ingredient*	*Quantity*
Oat flakes (Coles)	20 g
Trace element solution* $CaCO_3$ (Univar) Distilled H_2O	5 mL 1 g 1000 mL
***Trace element solution**	
$CaCl_2.2H_2O$	3 g
$FeC_6O_7H_5$	1 g
$MnSO_4$	0.2 g
$ZnCl_2$	0.1 g
$CuSO_4.5H_2O$	0.025 g
$Na_2B_4O_7.10H_2O$	0.02 g
$CoCl_2$	0.004 g
$Na_2MoO_4.2H_2O$	0.01 g
Distilled H_2O	1000 mL
Filter sterilize	

Yeast Extract Sucrose Agar (YES) *Ingredient*	*Quantity*
Yeast Extract (Difco)	20 g
Sucrose (Amyl)	150 g
Bacteriological Agar (Amyl)	20 g
Distilled H_2O	1000 mL
Autoclave	

Supplementary Figures

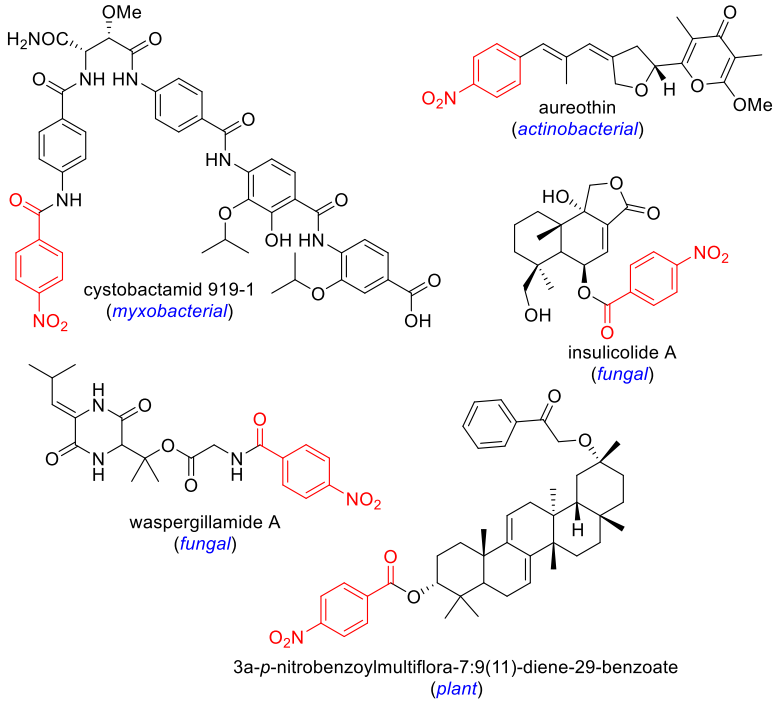

Fig. S1 Selected examples of natural products derived from the para-nitrobenzoic acid building block (highlighted in red).

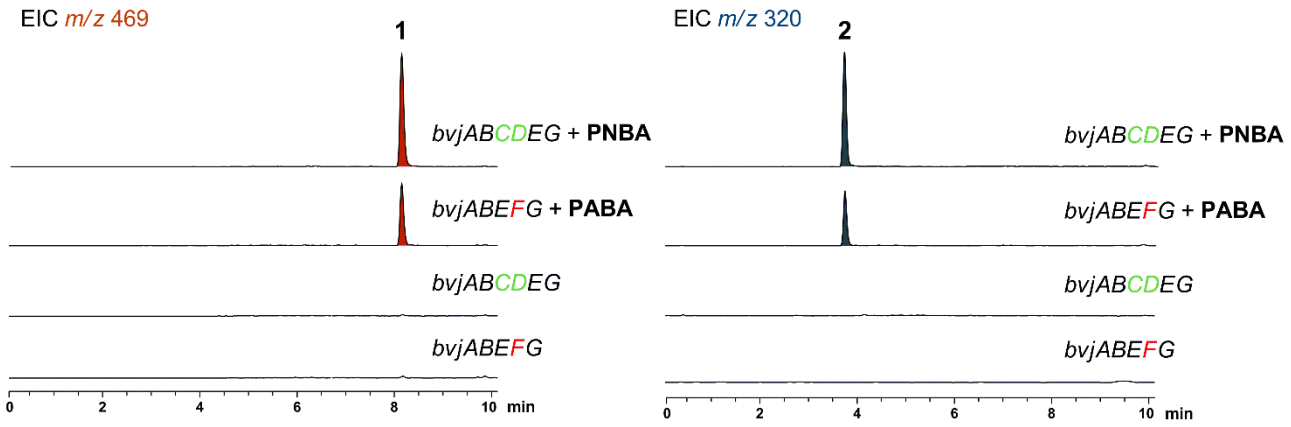

Fig. S2. LC-MS chromatograms showing the production of **1** and **2** from extracts of *A. nidulans* heterologous expression strains in combination with precursor feeding.

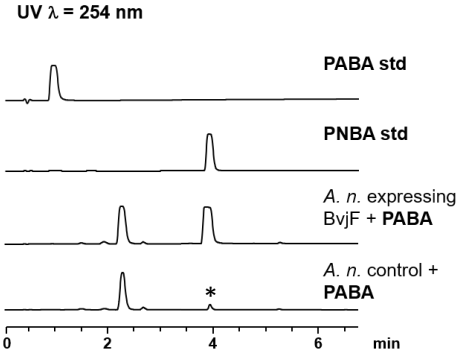

Fig. S3. LC-MS chromatograms showing the production of **PNBA** from extracts of *A. nidulans* heterologous expression strains fed with the precursor PABA.

When PABA was fed to *A. nidulans* containing an empty plasmid control, PABA was not detected in the LC-MS chromatogram, while a peak eluting at 2.3 min, corresponding to anthranilic acid, was observed. Likewise, in *A. nidulans* expressing *bvjF* fed with PABA, the peak corresponding to anthranilic acid (t_R 2.3 min) was also detected as a major peak. We propose that metabolic enzymes in *A. nidulans* convert PABA to anthranilic acid.

* unrelated background peak from the heterologous host *A. nidulans* LO8030, which coelutes with PNBA.

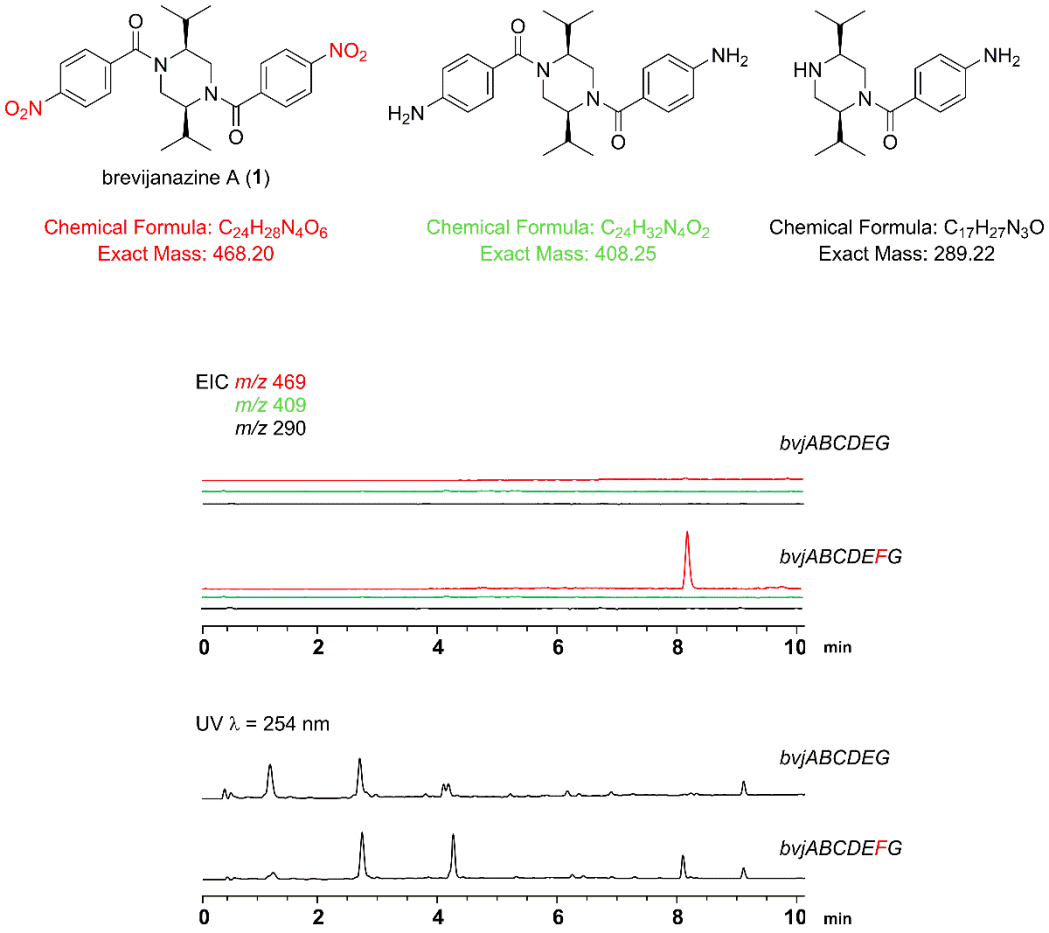

Fig. S4. LC-MS chromatograms showing the putative PABA-derived derivatives of **1** and **2** not produced by *A. nidulans* expressing *bvjABCDEG*.

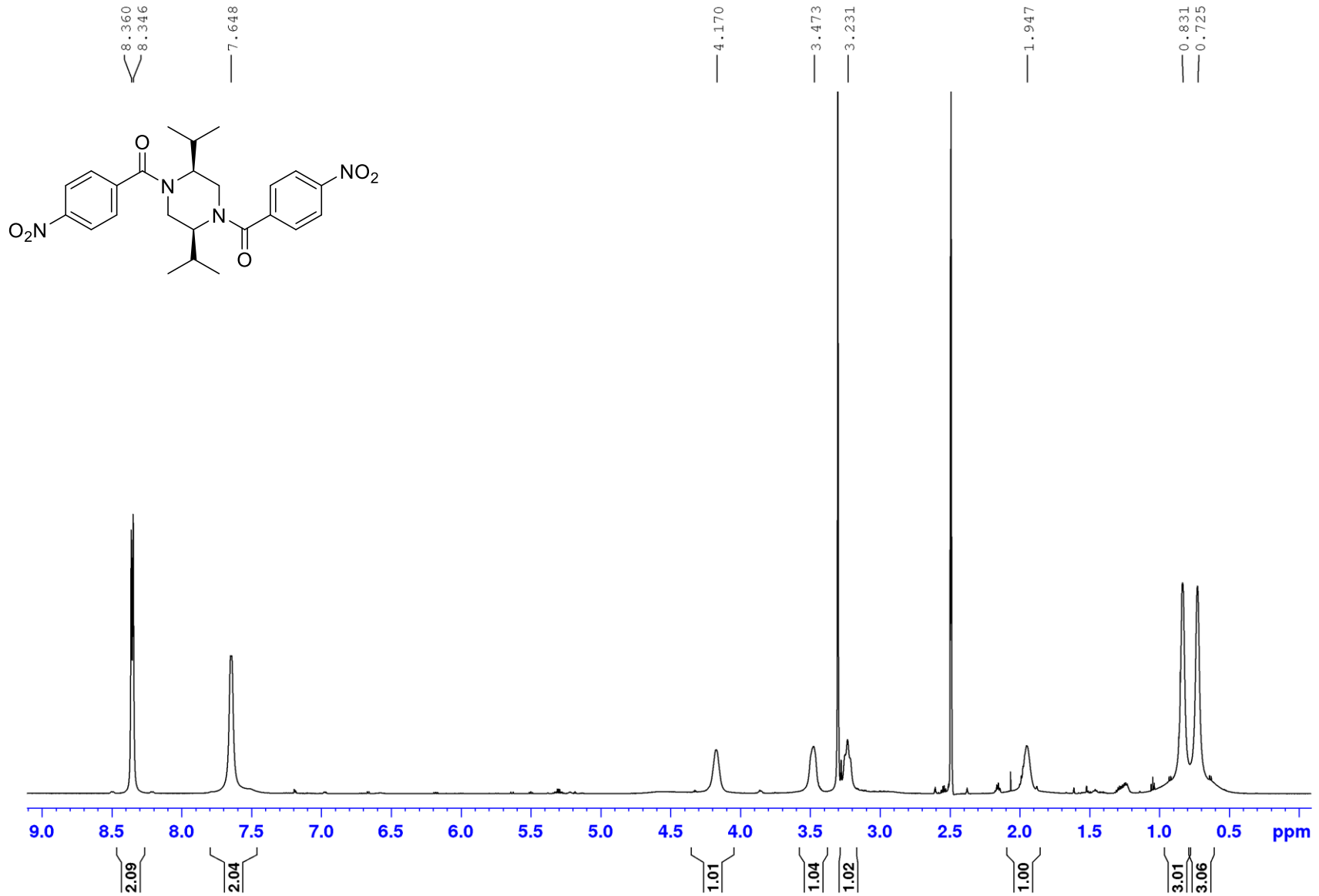

Fig. S5. ^{1}H NMR spectrum (600 MHz) of natural brevijanazine A (**1**) in DMSO-d_6.

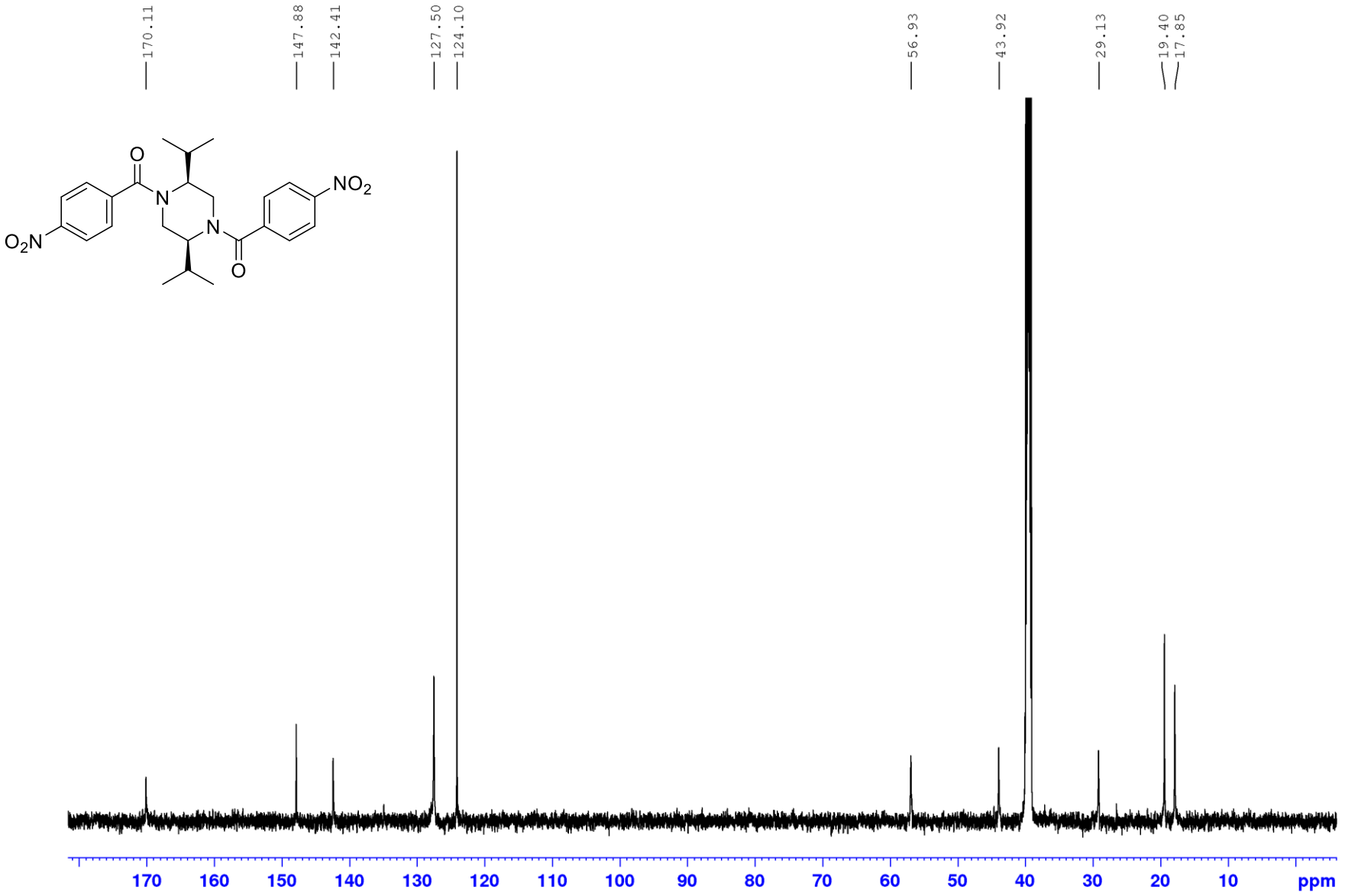

Fig. S6. ^{13}C NMR spectrum (150 MHz) of natural brevijanazine A (**1**) in DMSO-d_6.

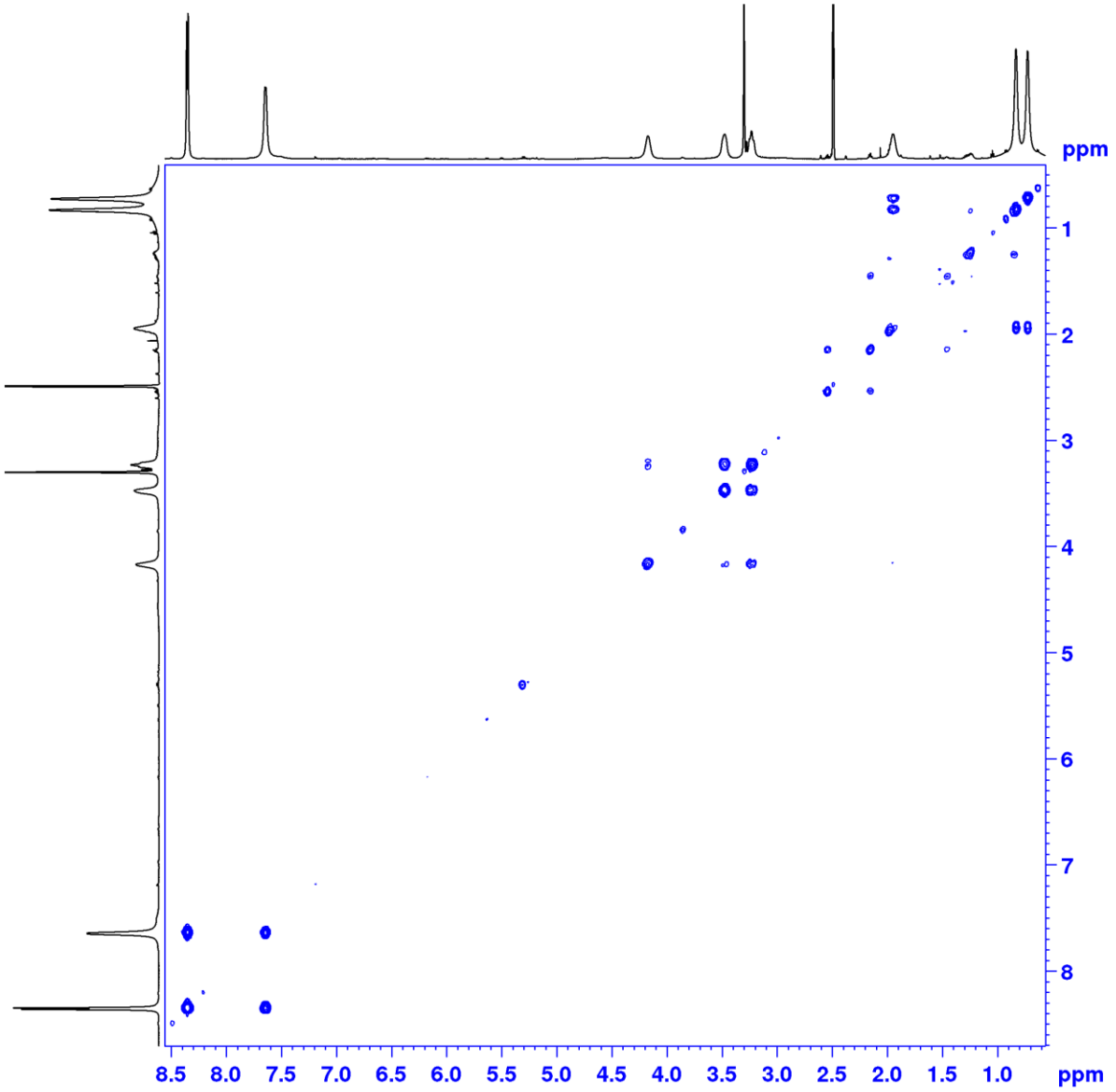

Fig. S7. ^{1}H-^{1}H COSY spectrum (600 MHz) of natural brevijanazine A (**1**) in DMSO-d_6.

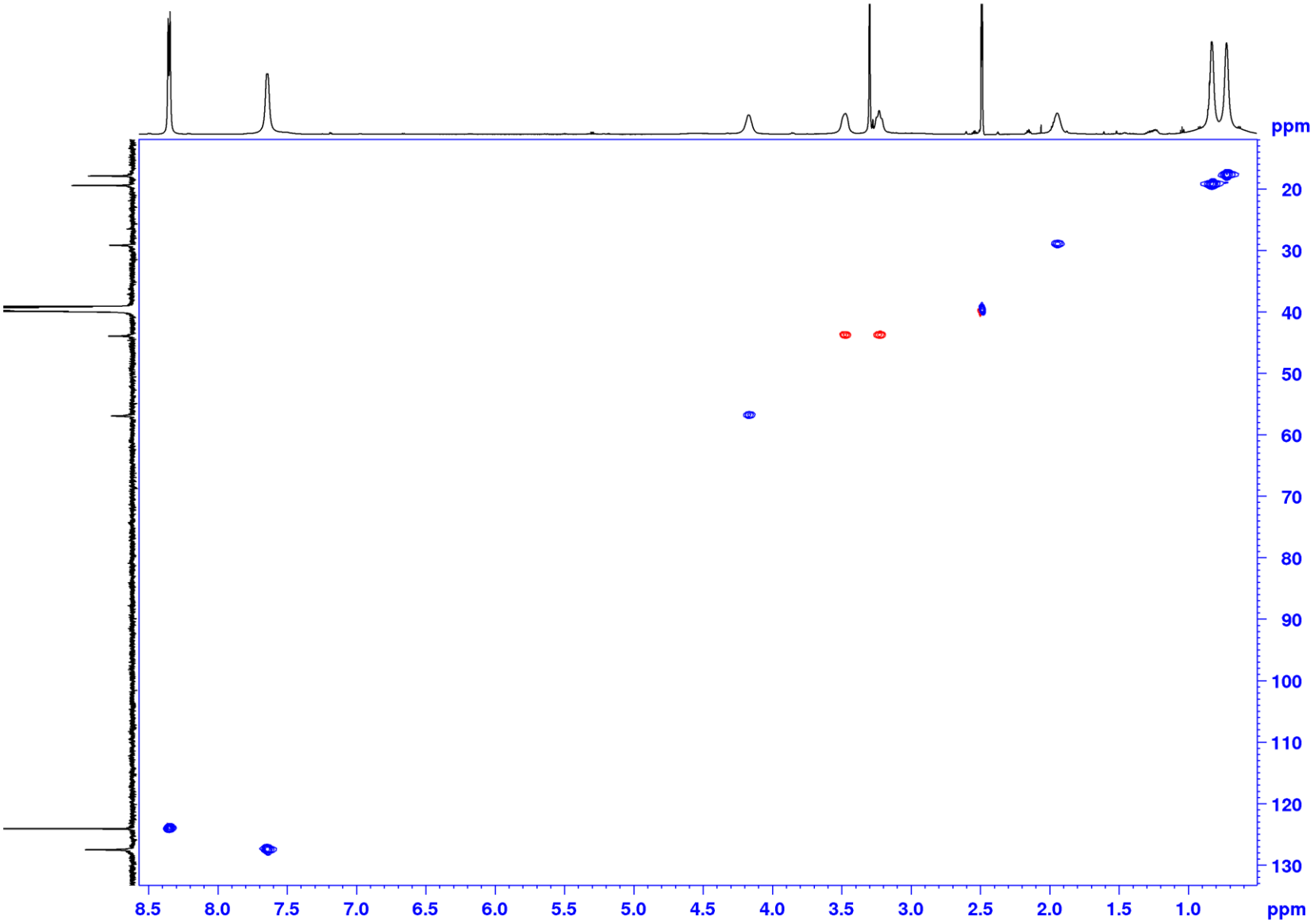

Fig. S8. HSQC spectrum (600 MHz) of natural brevijanazine A (**1**) in DMSO-d_6.

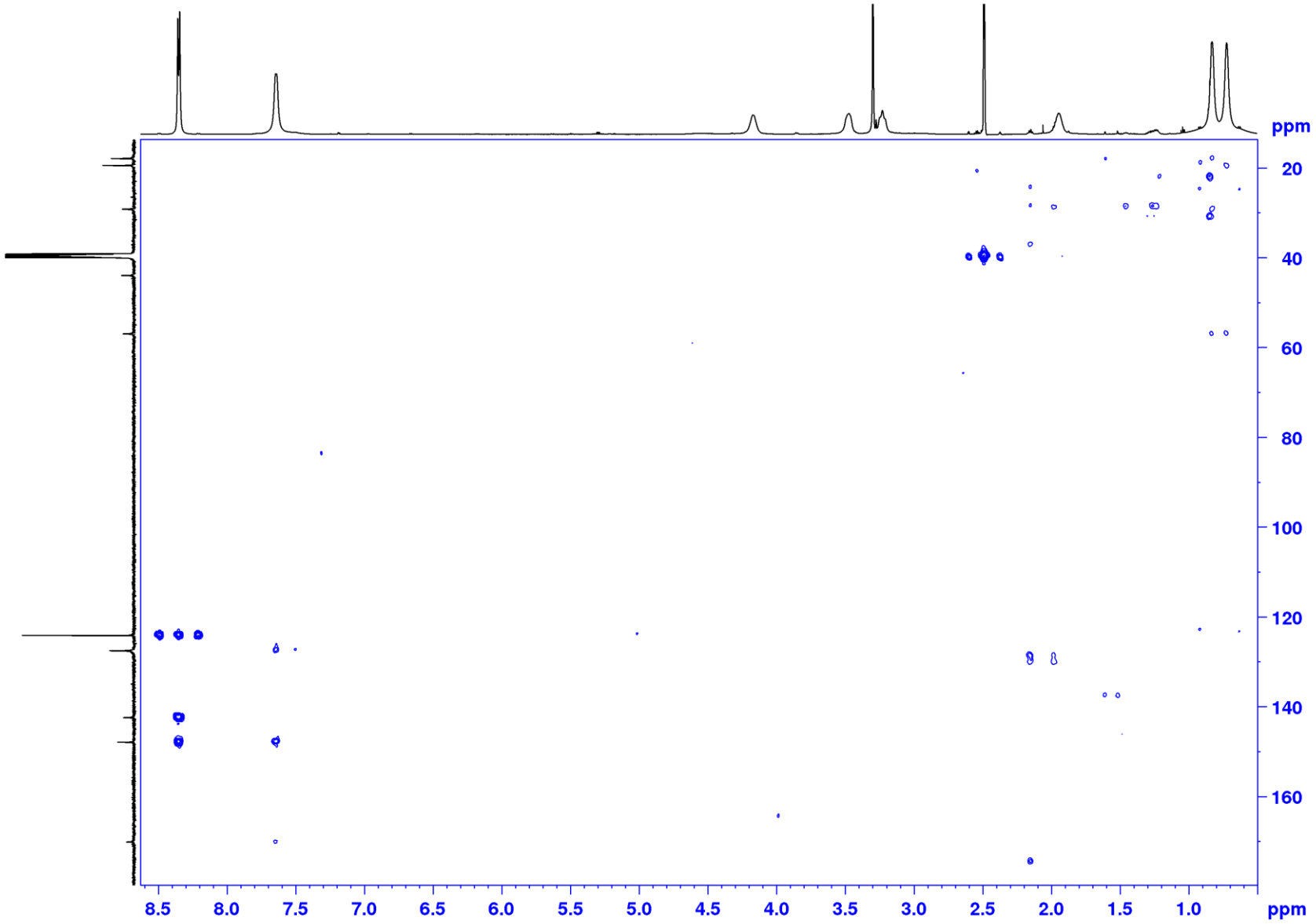

Fig. S9. HMBC spectrum (600 MHz) of natural brevijanazine A (**1**) in DMSO-d_6.

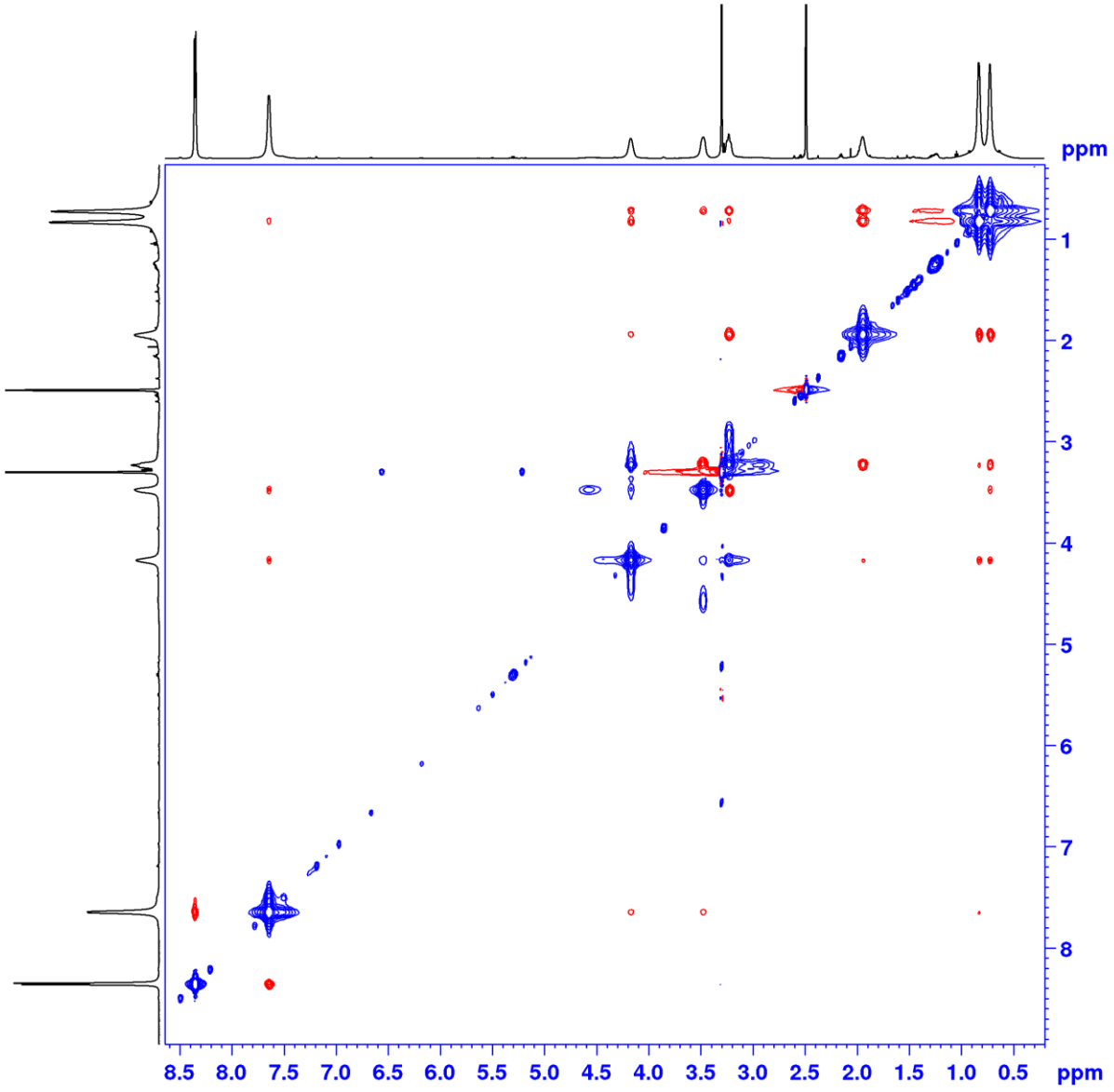

Fig. S10. ROESY spectrum (600 MHz) of natural brevijanazine A (**1**) in DMSO-d_6.

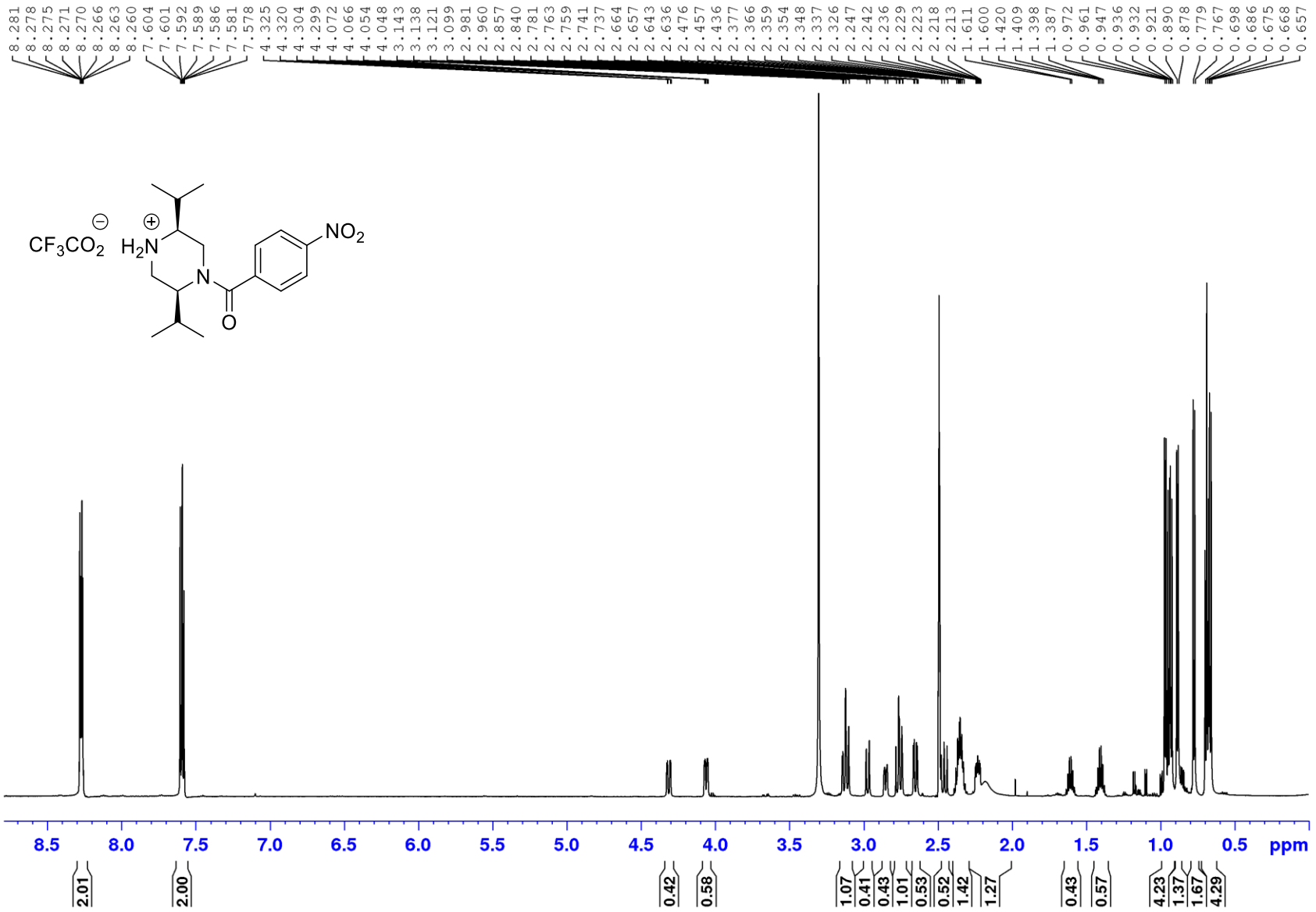

Fig. S11. ^{1}H NMR spectrum (600 MHz) of natural brevijanazine B (**2**) TFA salt in DMSO-d_6

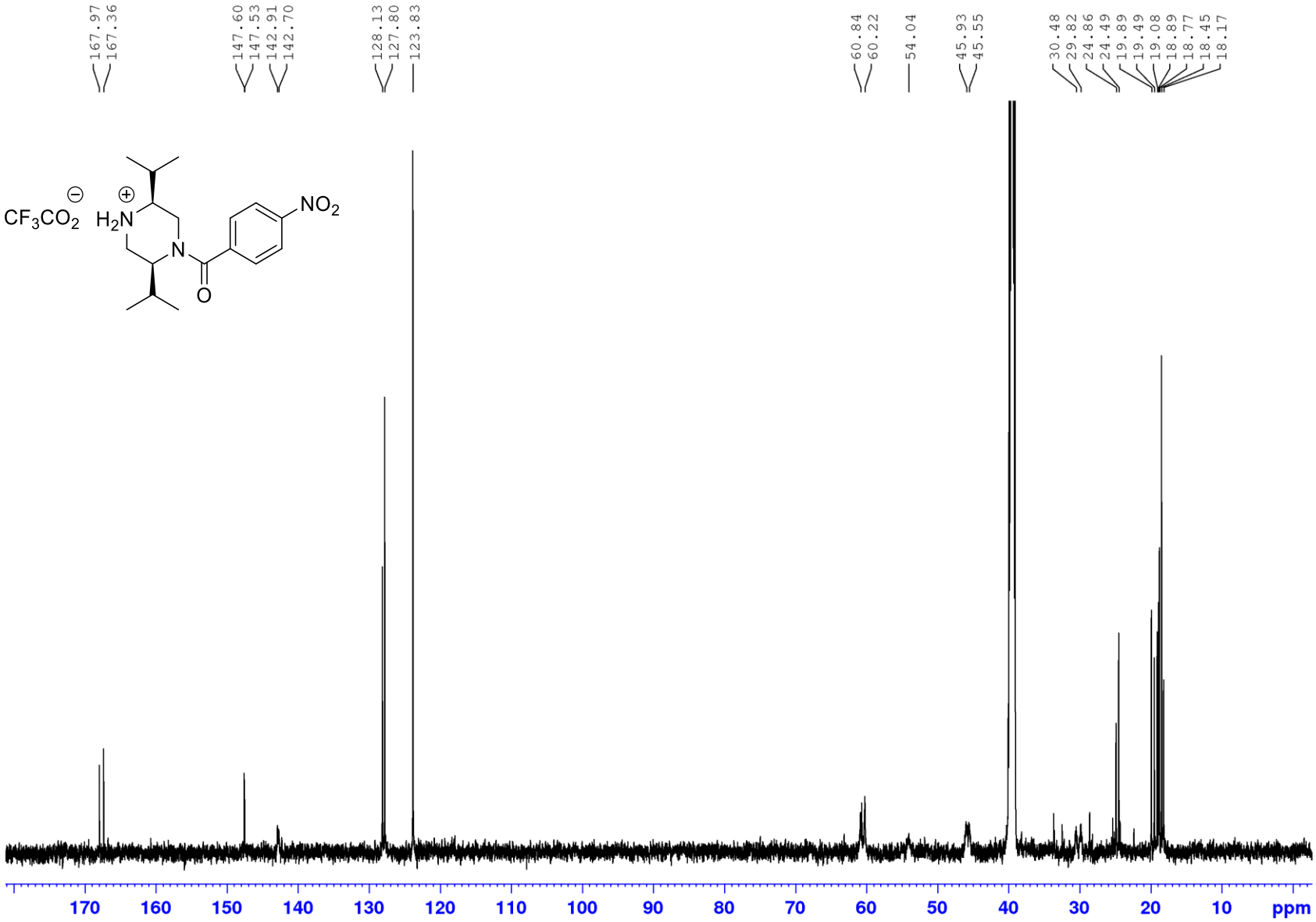

Fig. S12. ^{13}C NMR spectrum (150 MHz) of natural brevijanazine B (**2**) TFA salt in DMSO-d_6.

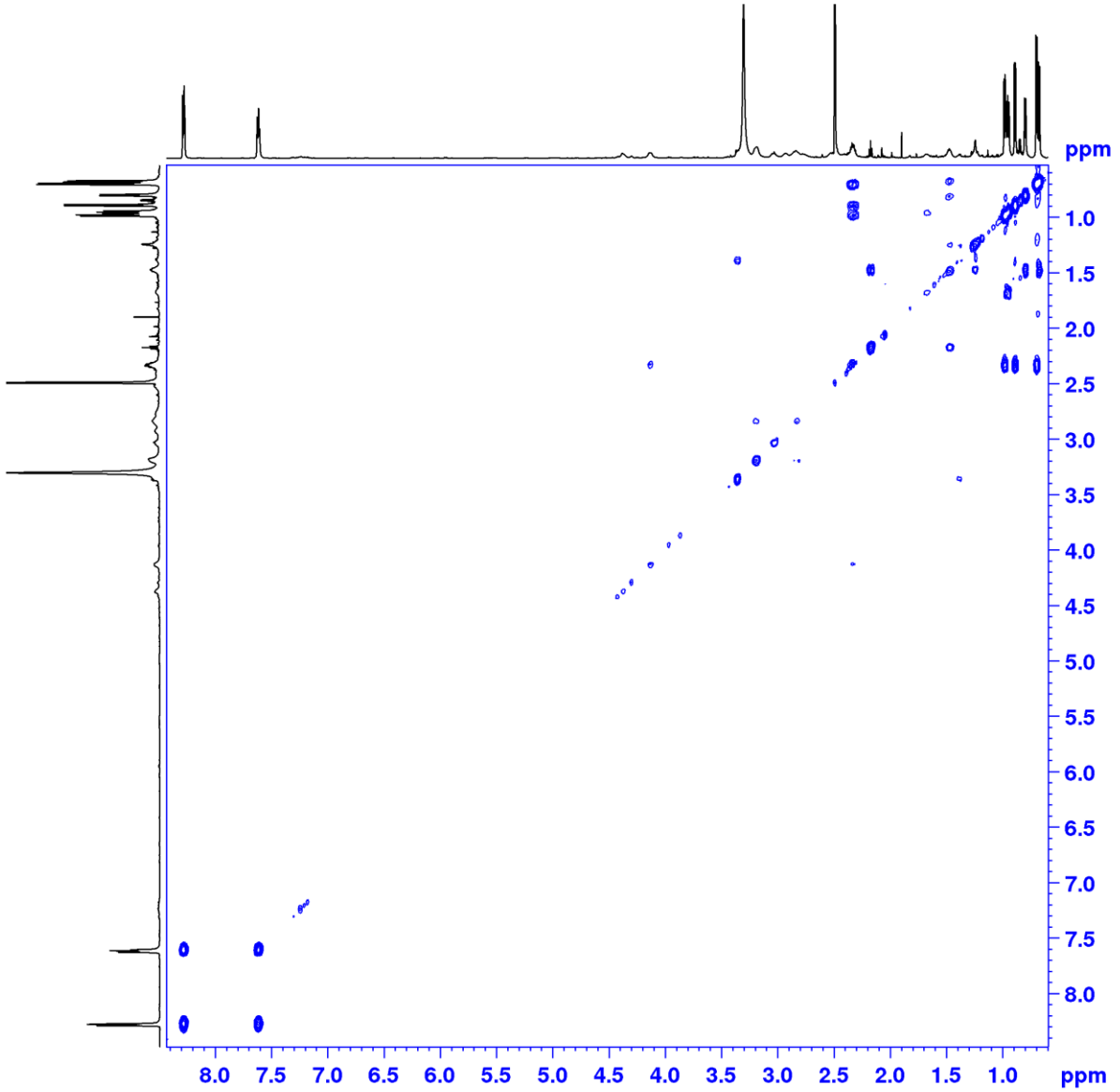

Fig. S13. ^{1}H-^{1}H COSY spectrum (600 MHz) of natural brevijanazine B (**2**) TFA salt in DMSO-d_6.

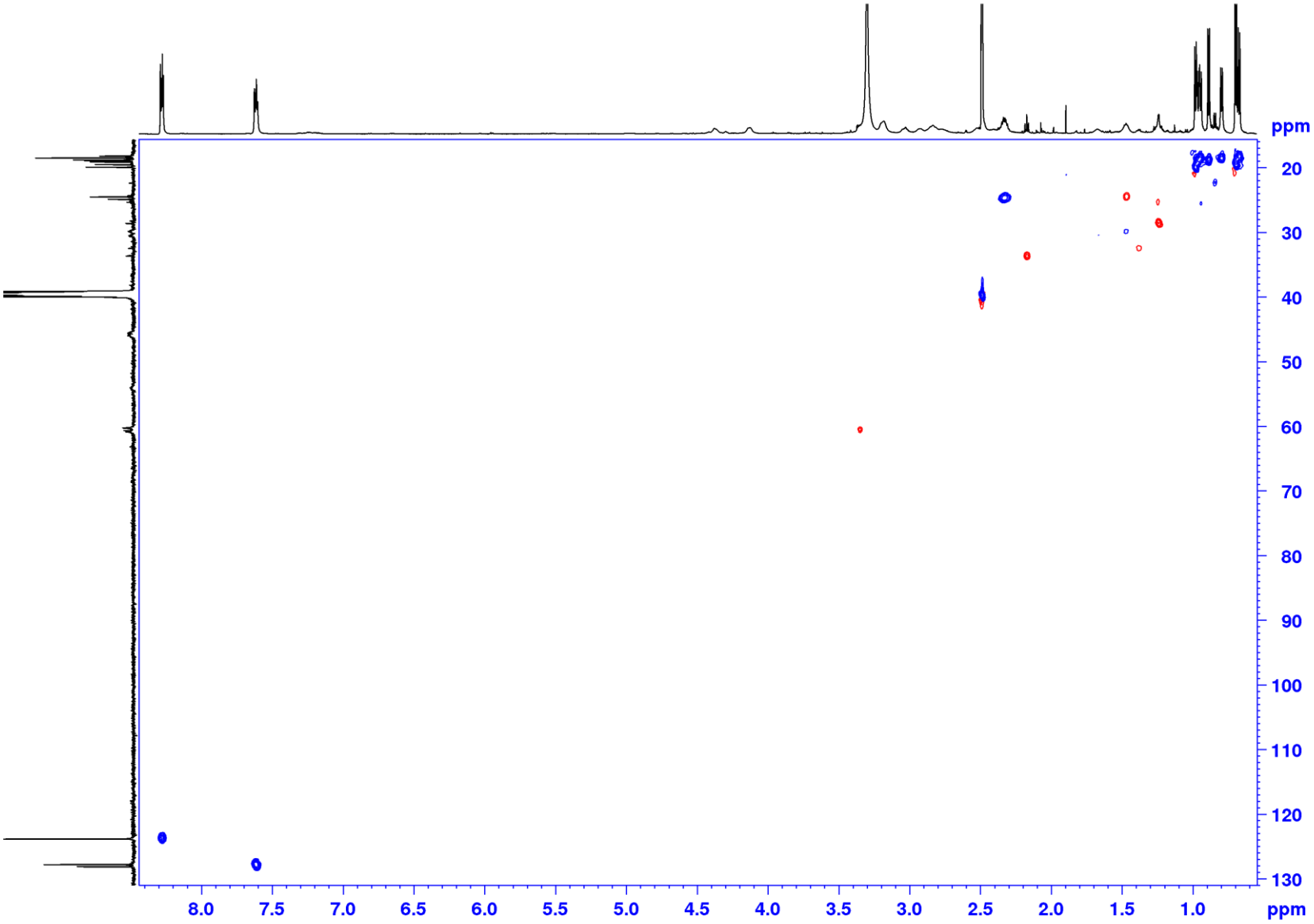

Fig. S14. HSQC spectrum (600 MHz) of natural brevijanazine B (**2**) TFA salt in DMSO-d_6.

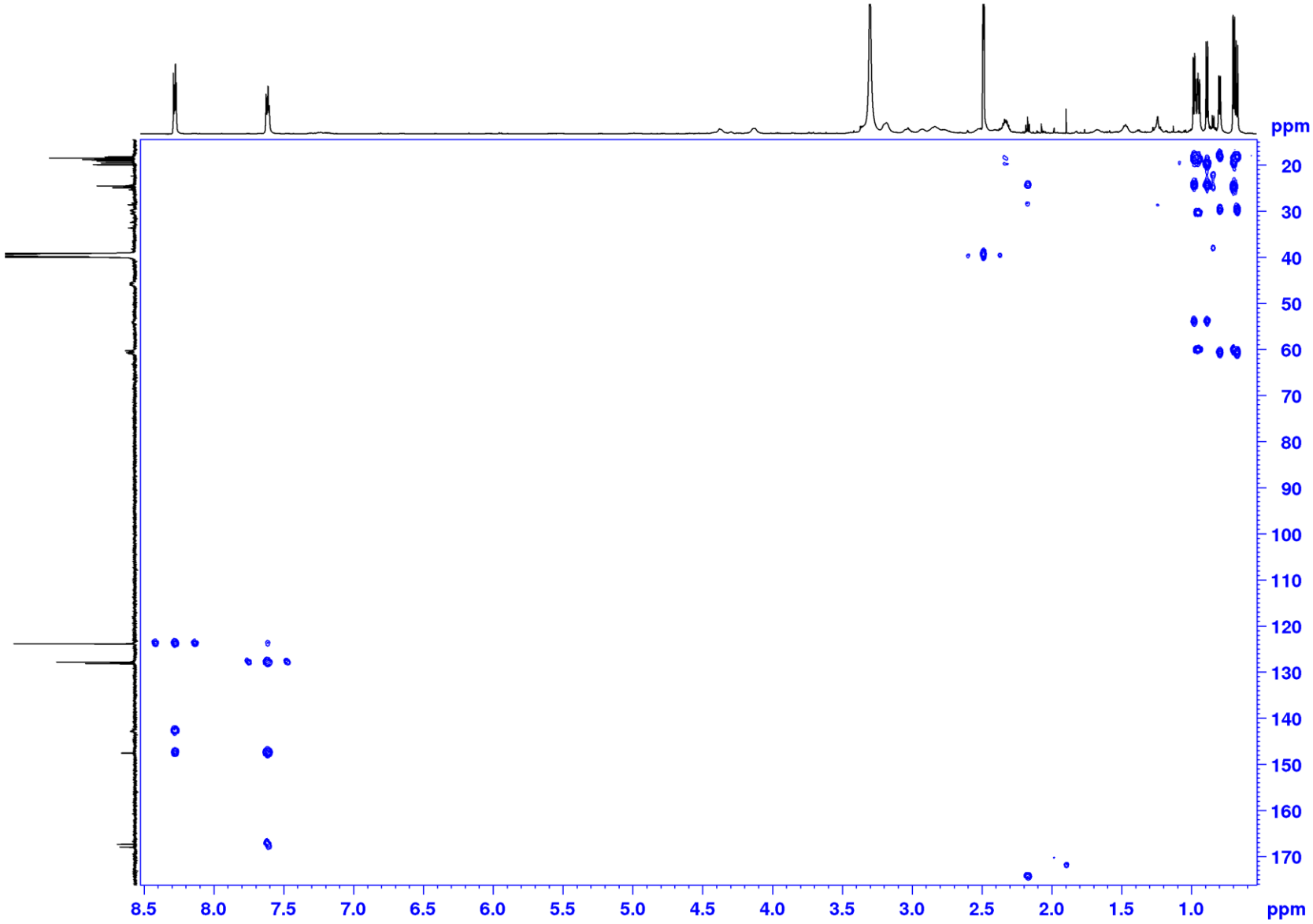

Fig. S15. HMBC spectrum (600 MHz) of natural brevijanazine B (**2**) TFA salt in DMSO-d_6.

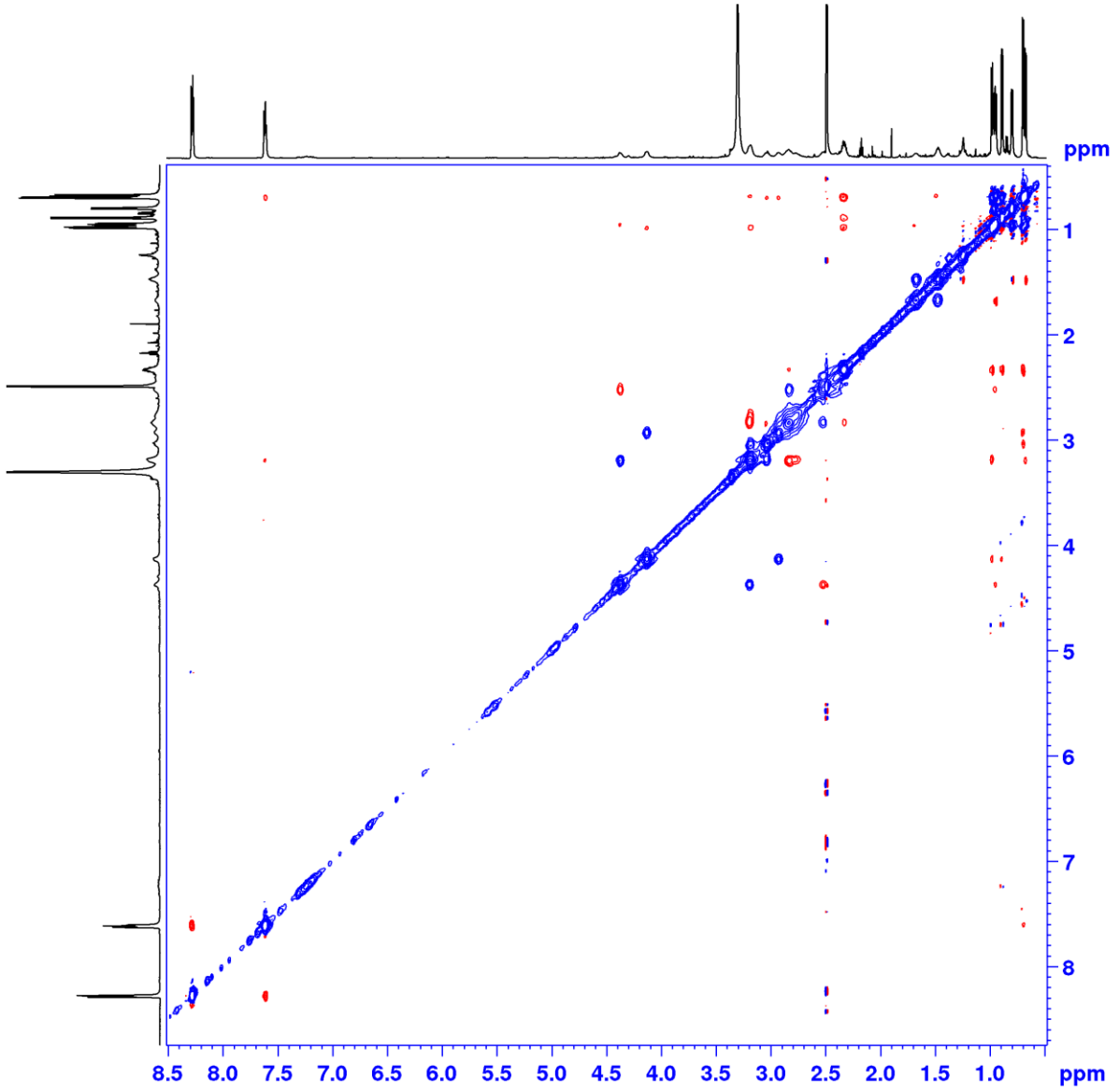

Fig. S16. ROESY spectrum (600 MHz) of natural brevijanazine B (**2**) TFA salt in DMSO-d_6.

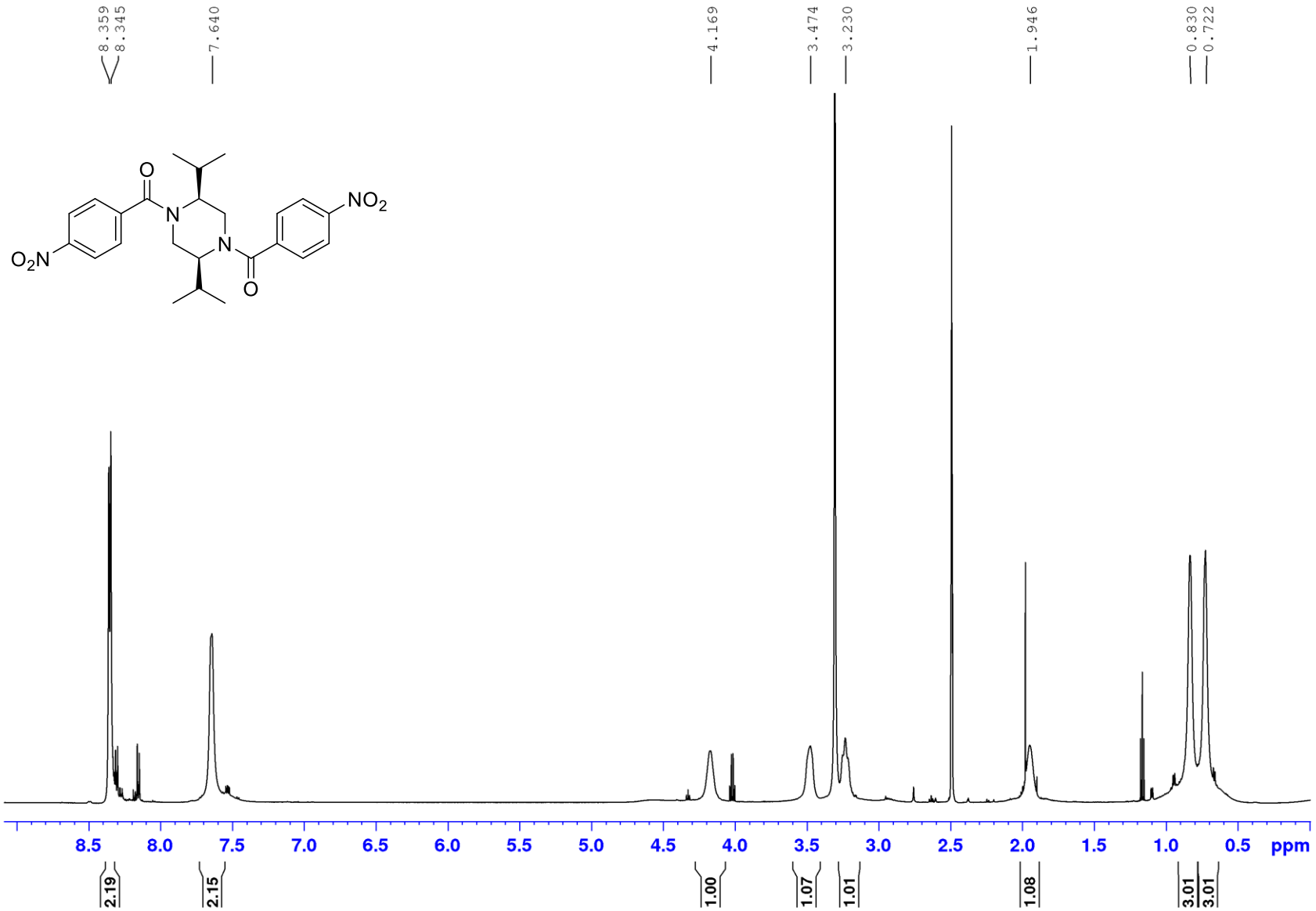

Fig. S17. ^{1}H NMR spectrum (600 MHz) of synthetic brevijanazine A (**1**) in DMSO-d_6.

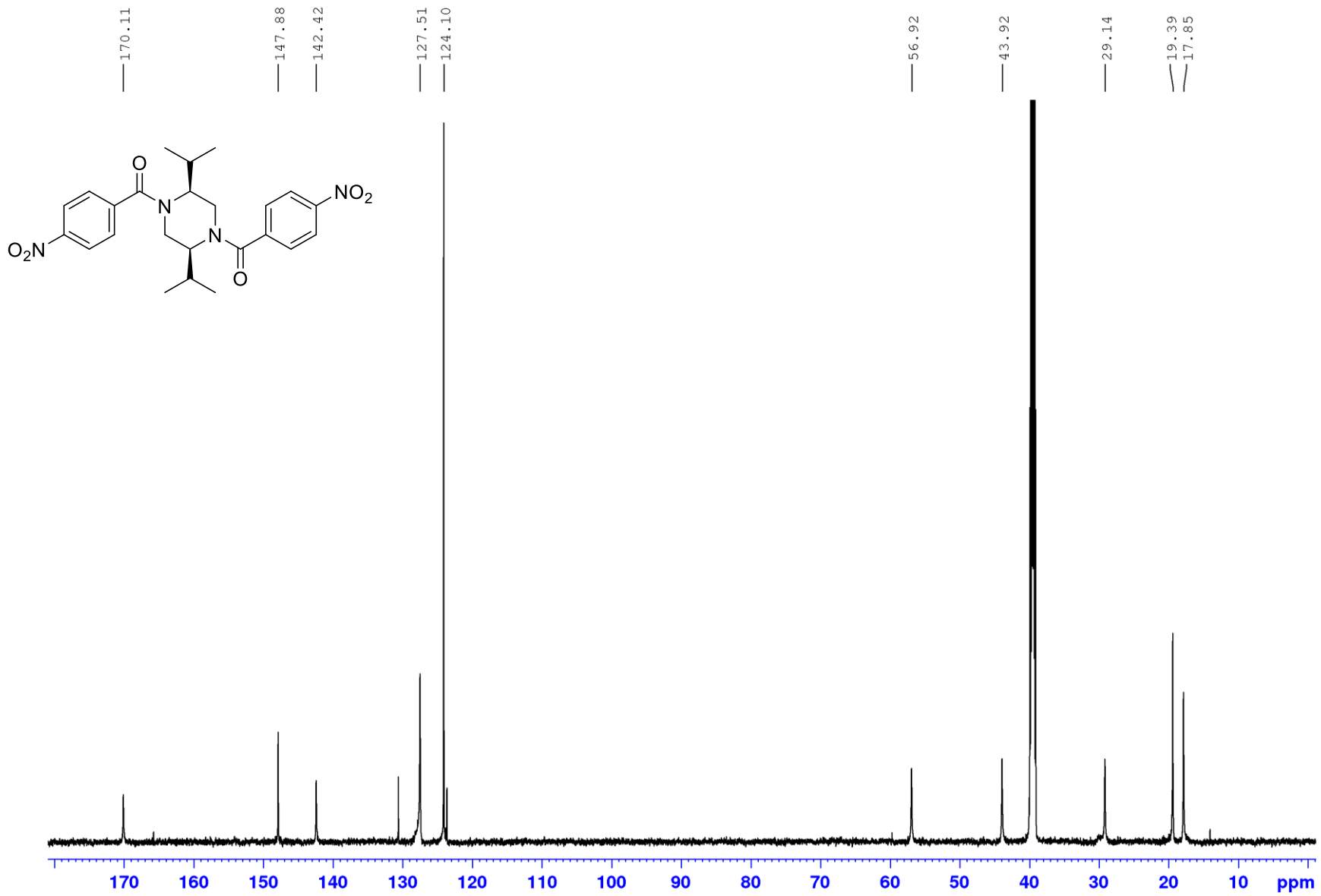

Fig. S18. ^{13}C NMR spectrum (150 MHz) of synthetic brevijanazine A (**1**) in DMSO-d_6.

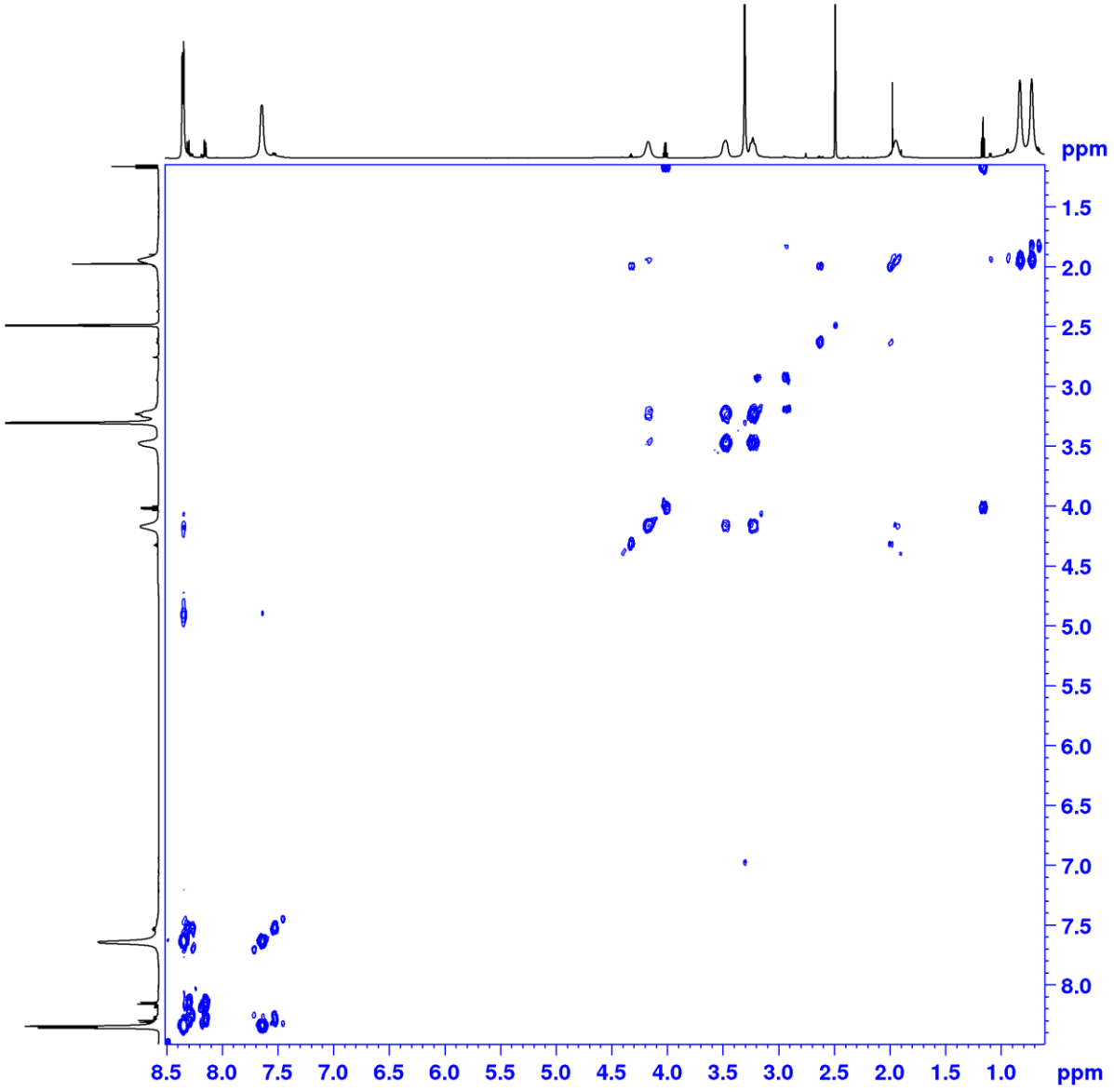

Fig. S19. ^{1}H-^{1}H COSY spectrum (600 MHz) of synthetic brevijanazine A (**1**) in DMSO-d_6.

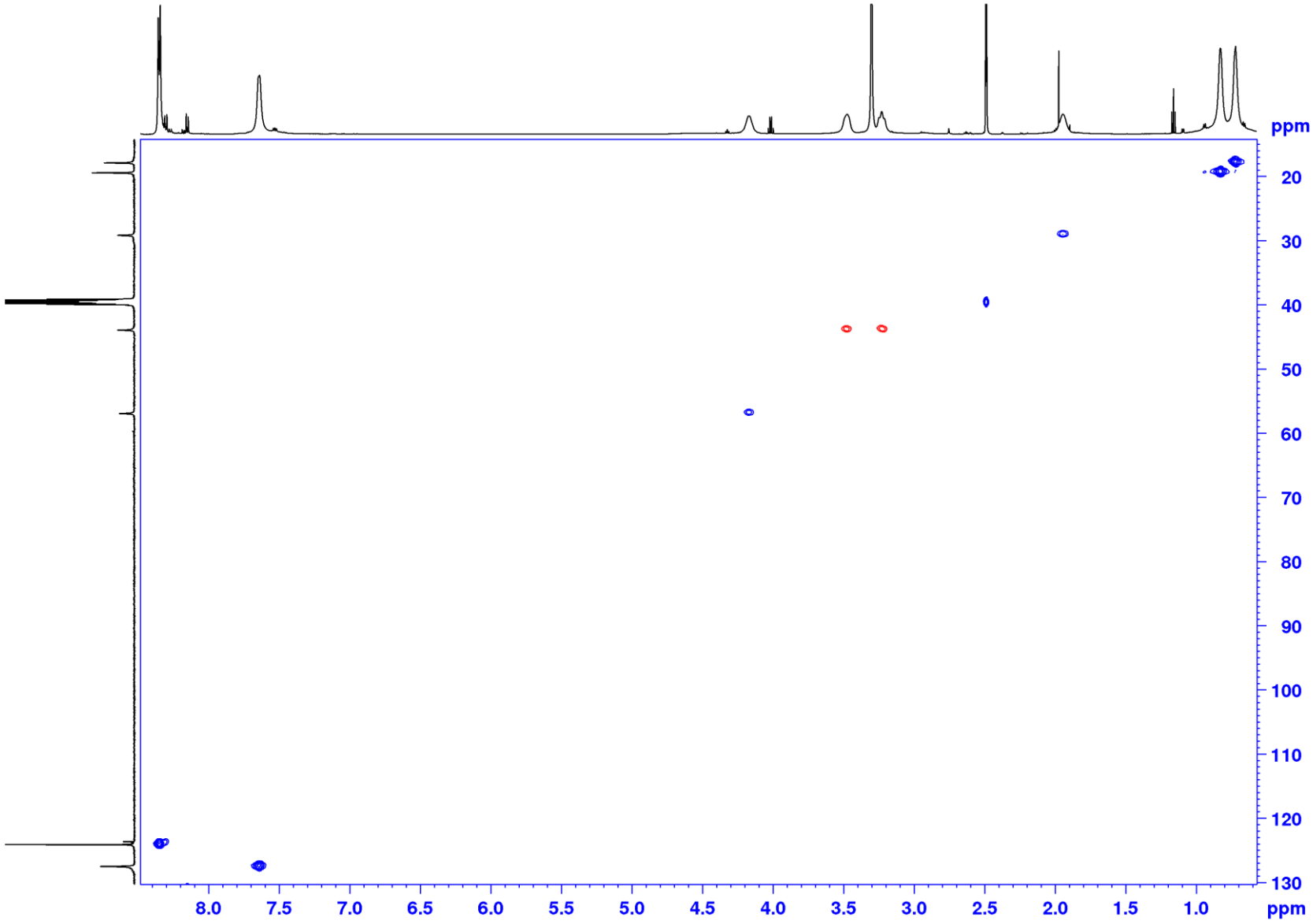

Fig. S20. HSQC spectrum (600 MHz) of synthetic brevijanazine A (**1**) in DMSO-d_6.

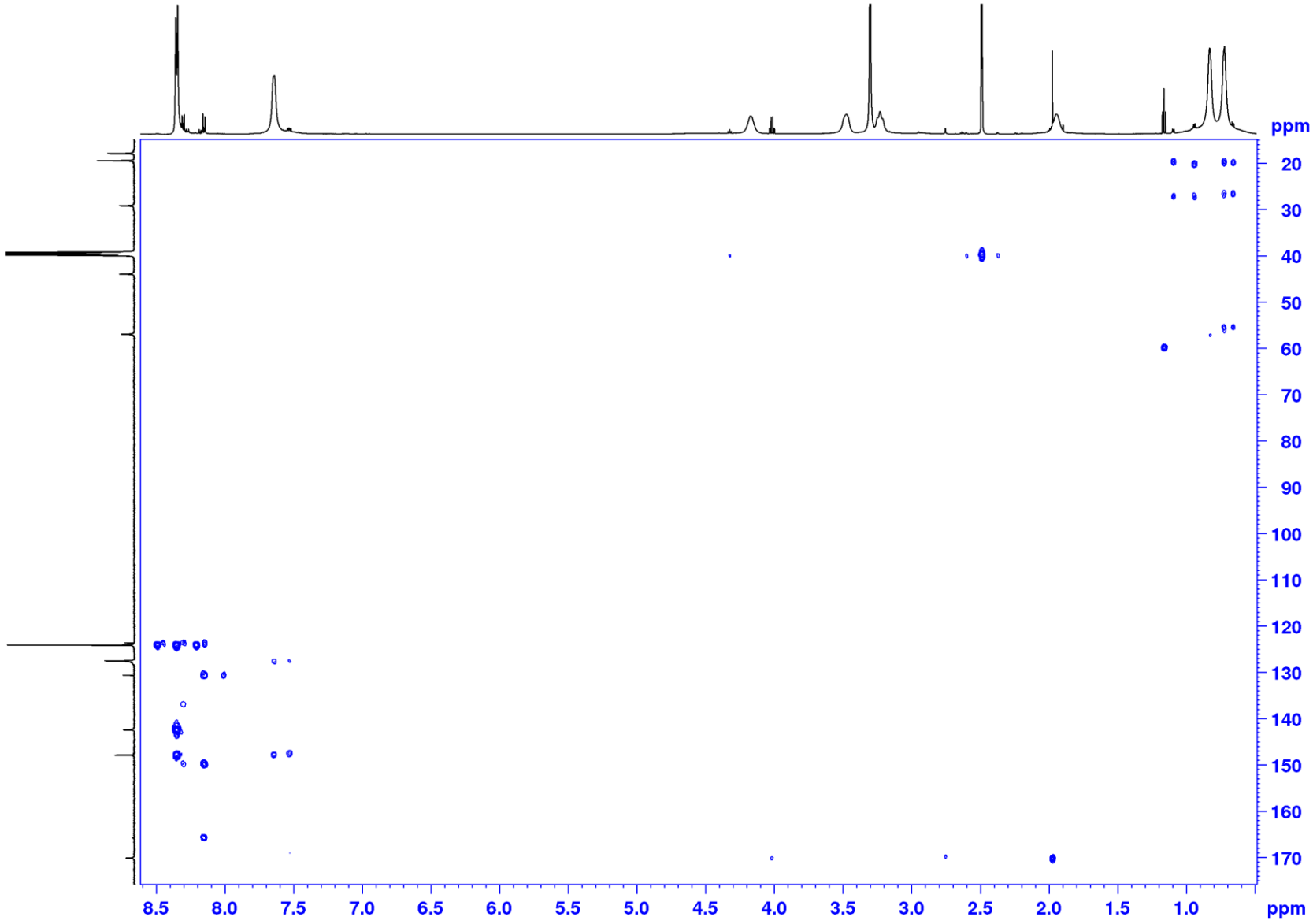

Fig. S21. HMBC spectrum (600 MHz) of synthetic brevijanazine A (**1**) in DMSO-d_6.

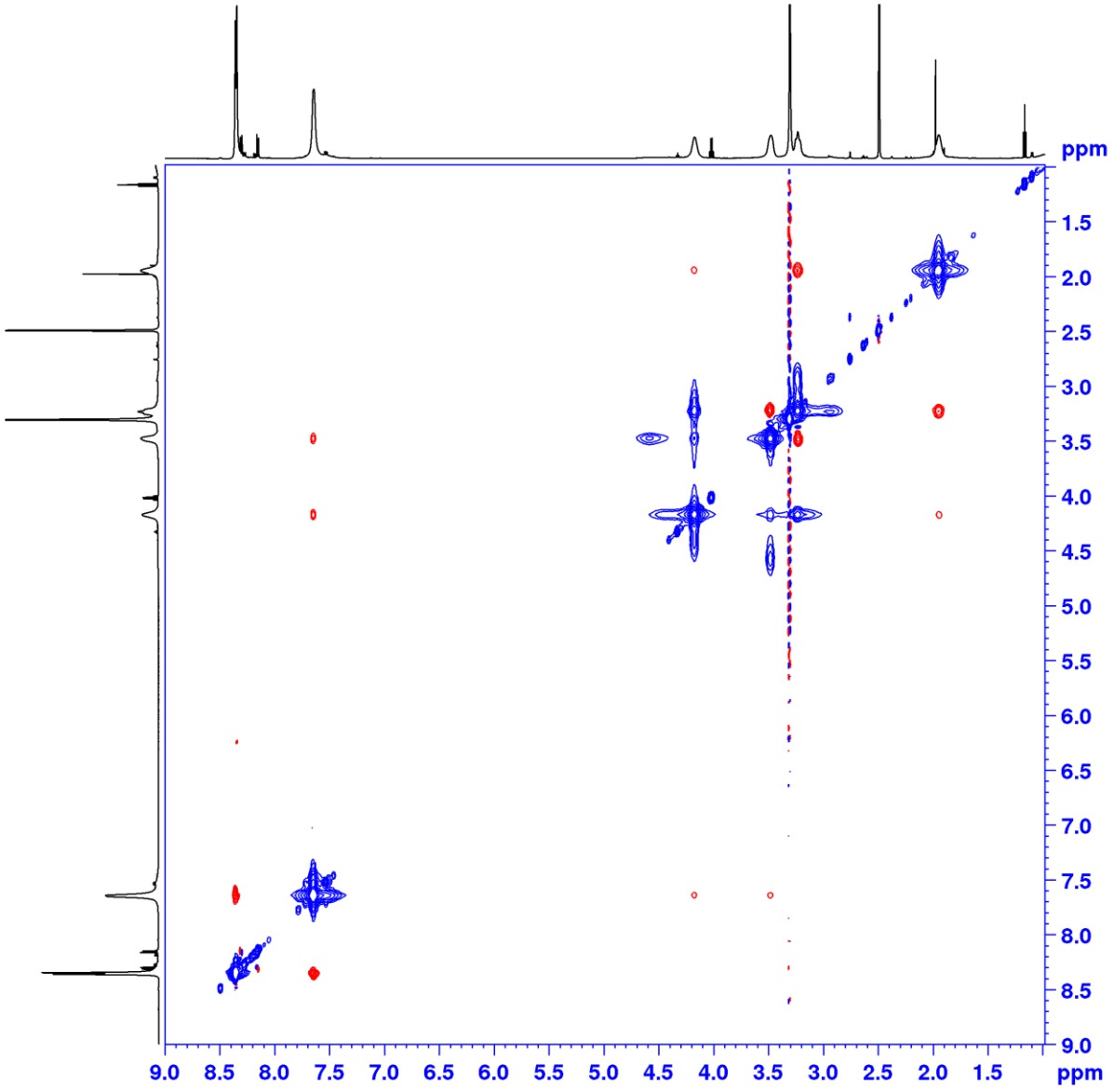

Fig. S22. ROESY spectrum (600 MHz) of synthetic brevijanazine A (**1**) in DMSO-d_6.

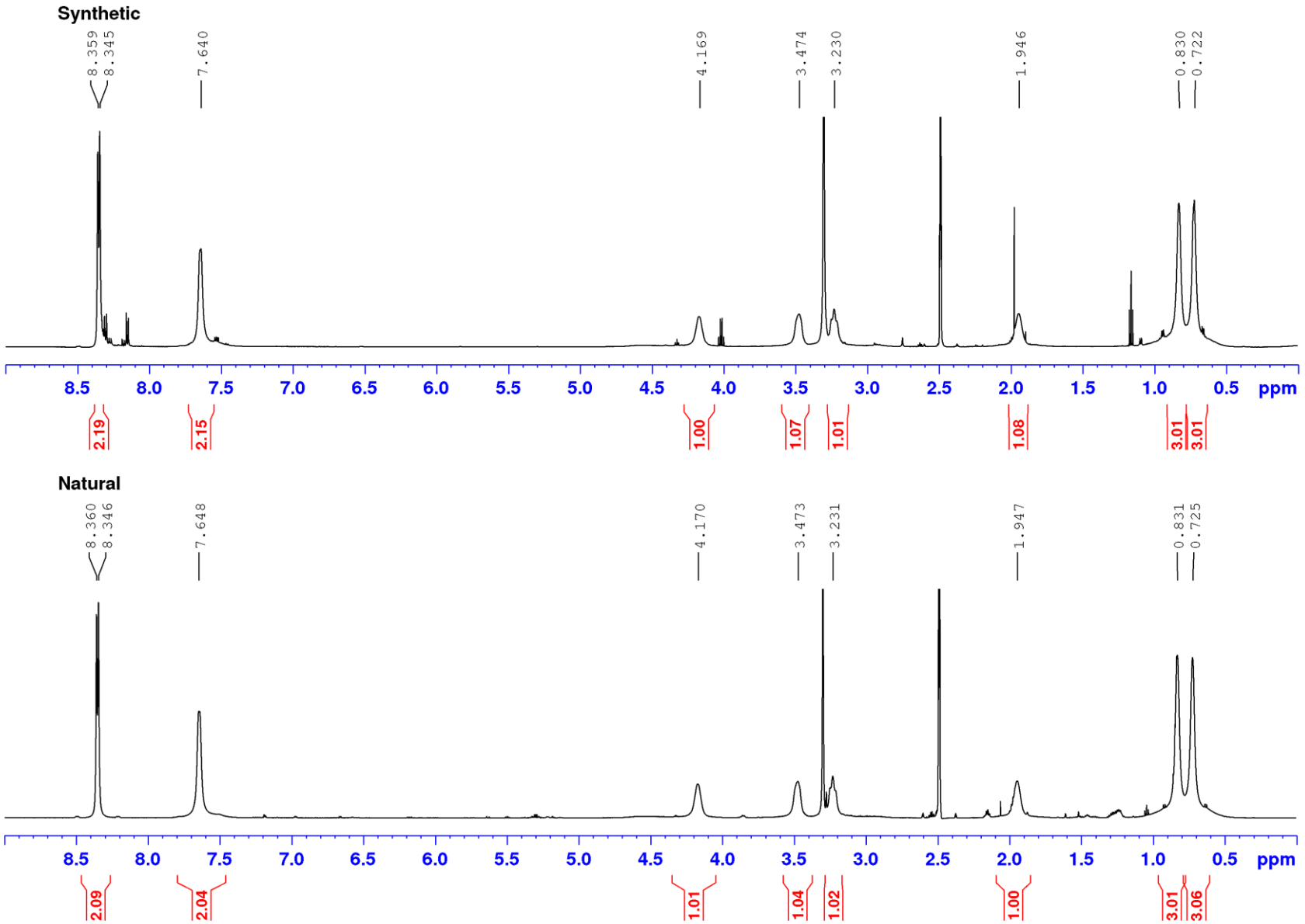

Fig. S23. Comparison of ^{1}H NMR spectra (600 MHz) of natural and synthetic brevijanazine A (**1**) in DMSO-d_6.

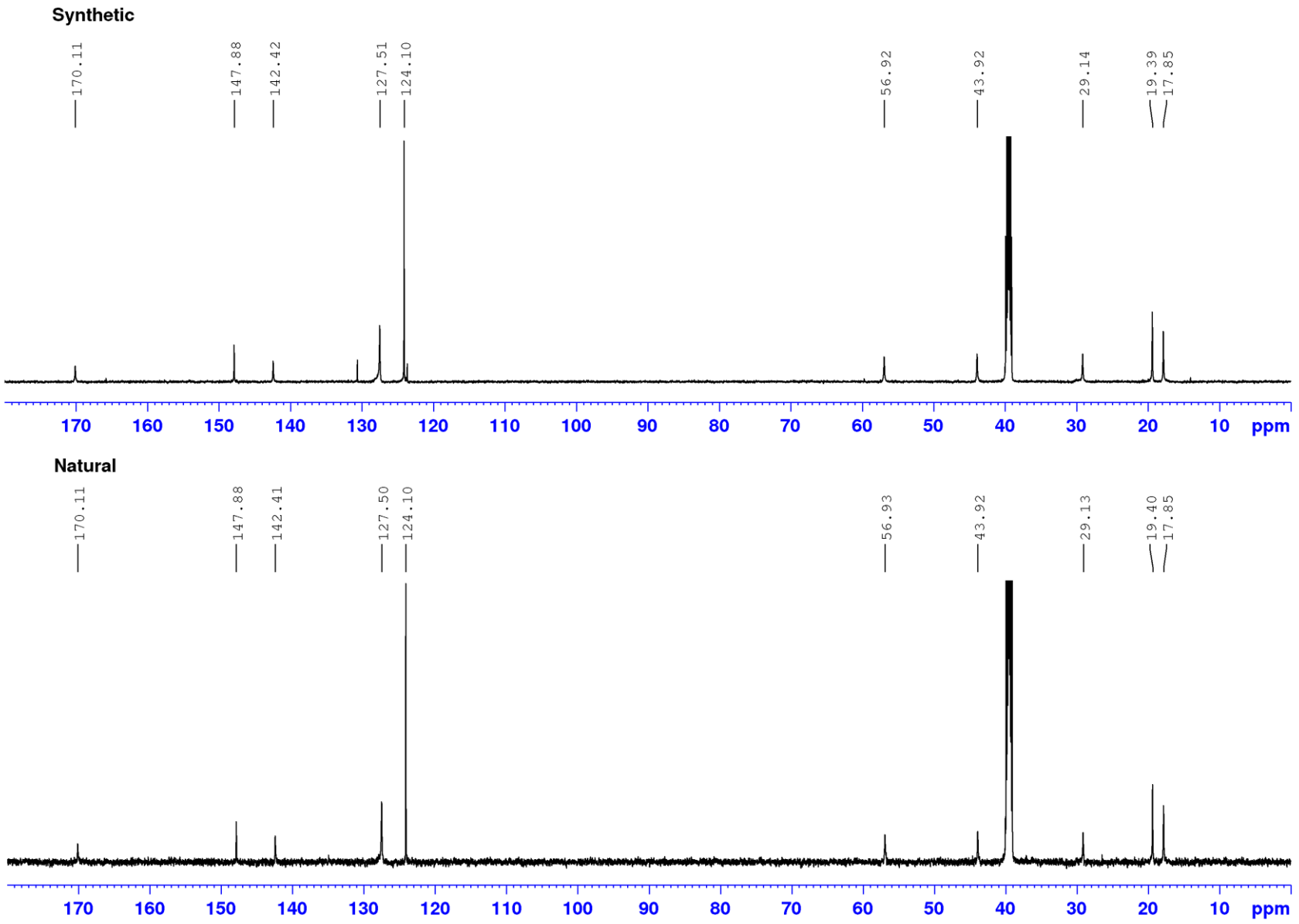

Fig. S24. Comparison of ^{13}C NMR spectra (150 MHz) of natural and synthetic brevijanazine A (**1**) in DMSO-d_6.

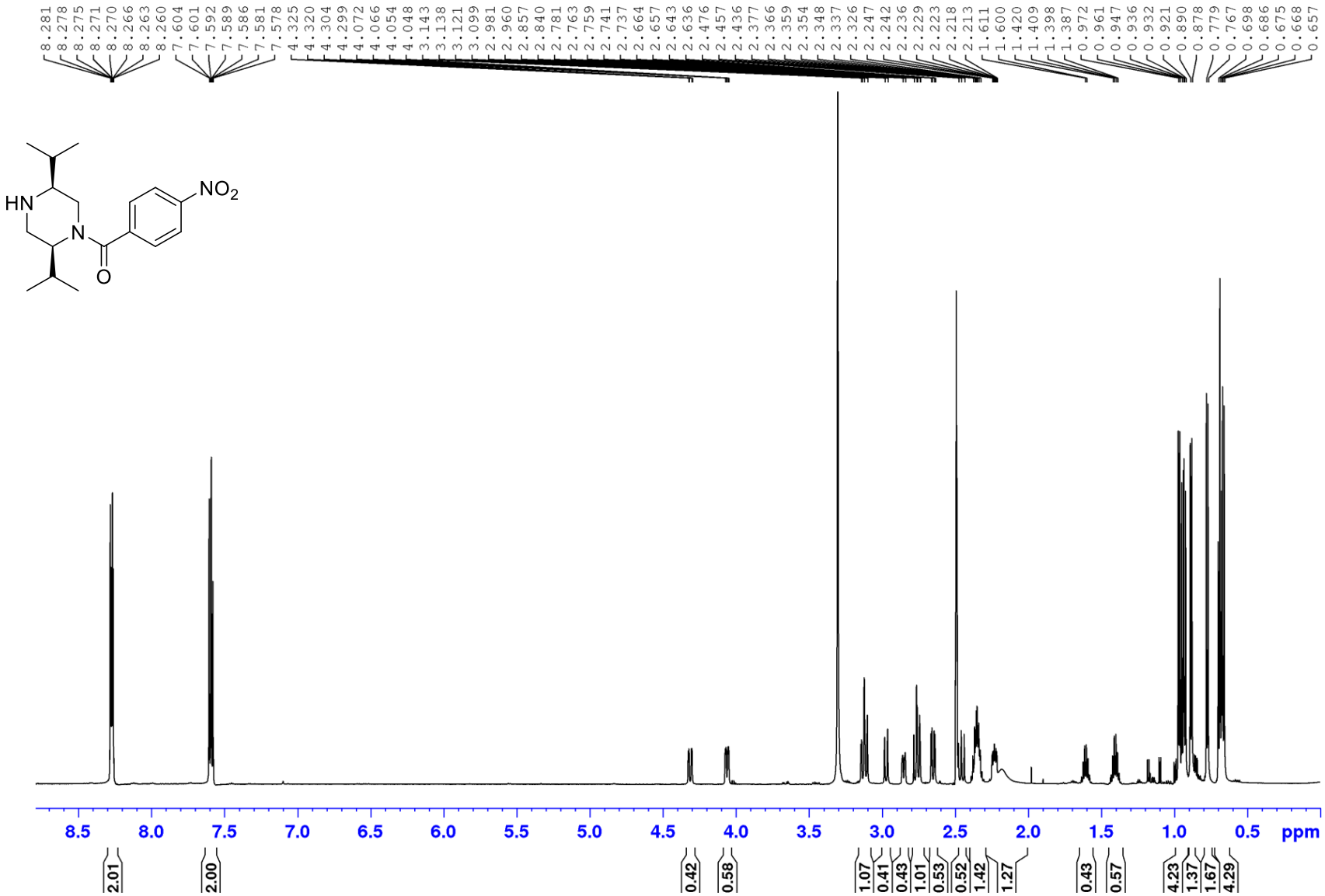

Fig. S25. ^{1}H NMR spectrum (600 MHz) of synthetic brevijanazine B (**2**) free base in DMSO-d_6.

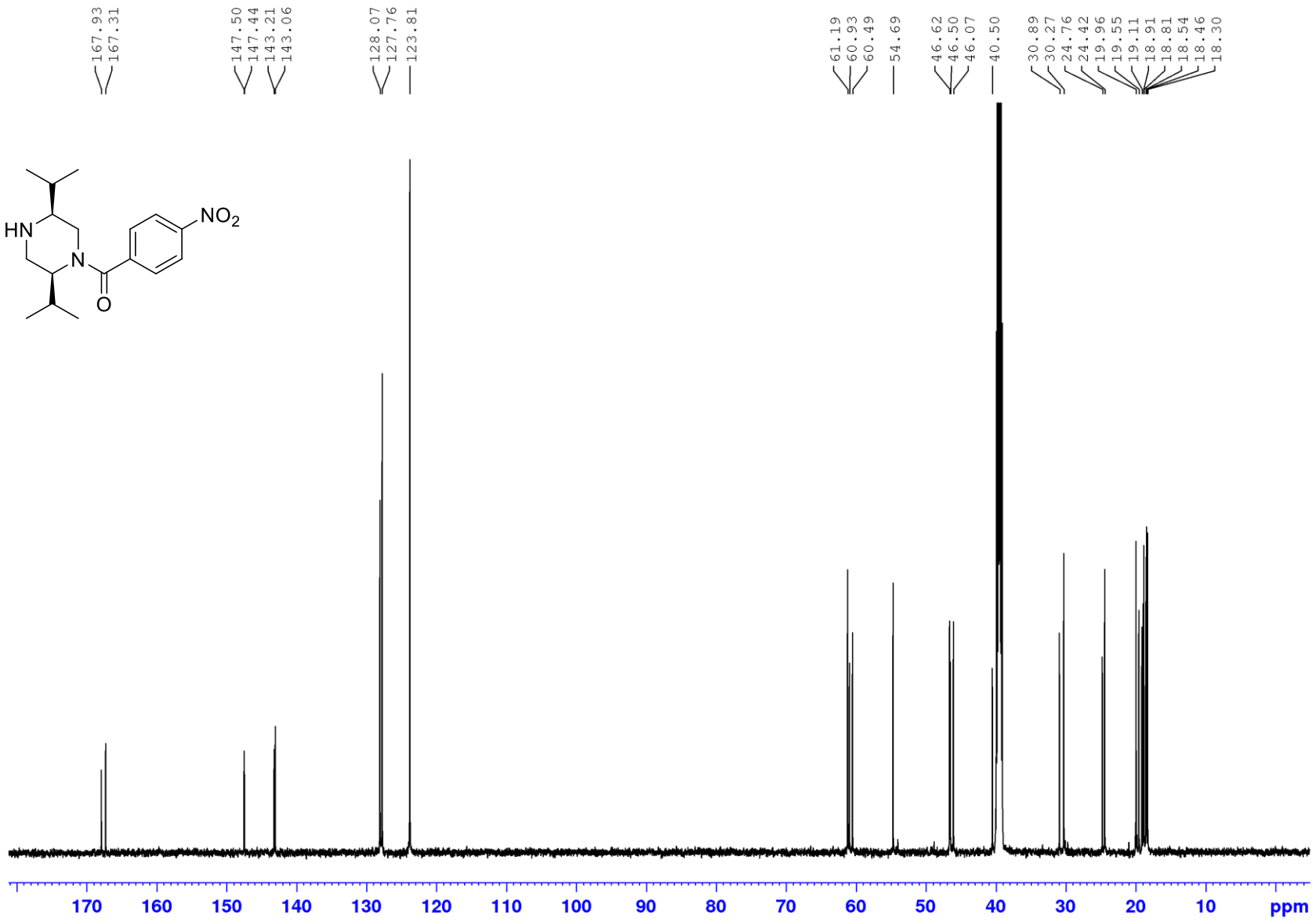

Fig. S26. ^{13}C NMR spectrum (150 MHz) of synthetic brevijanazine B (**2**) free base in DMSO-d_6.

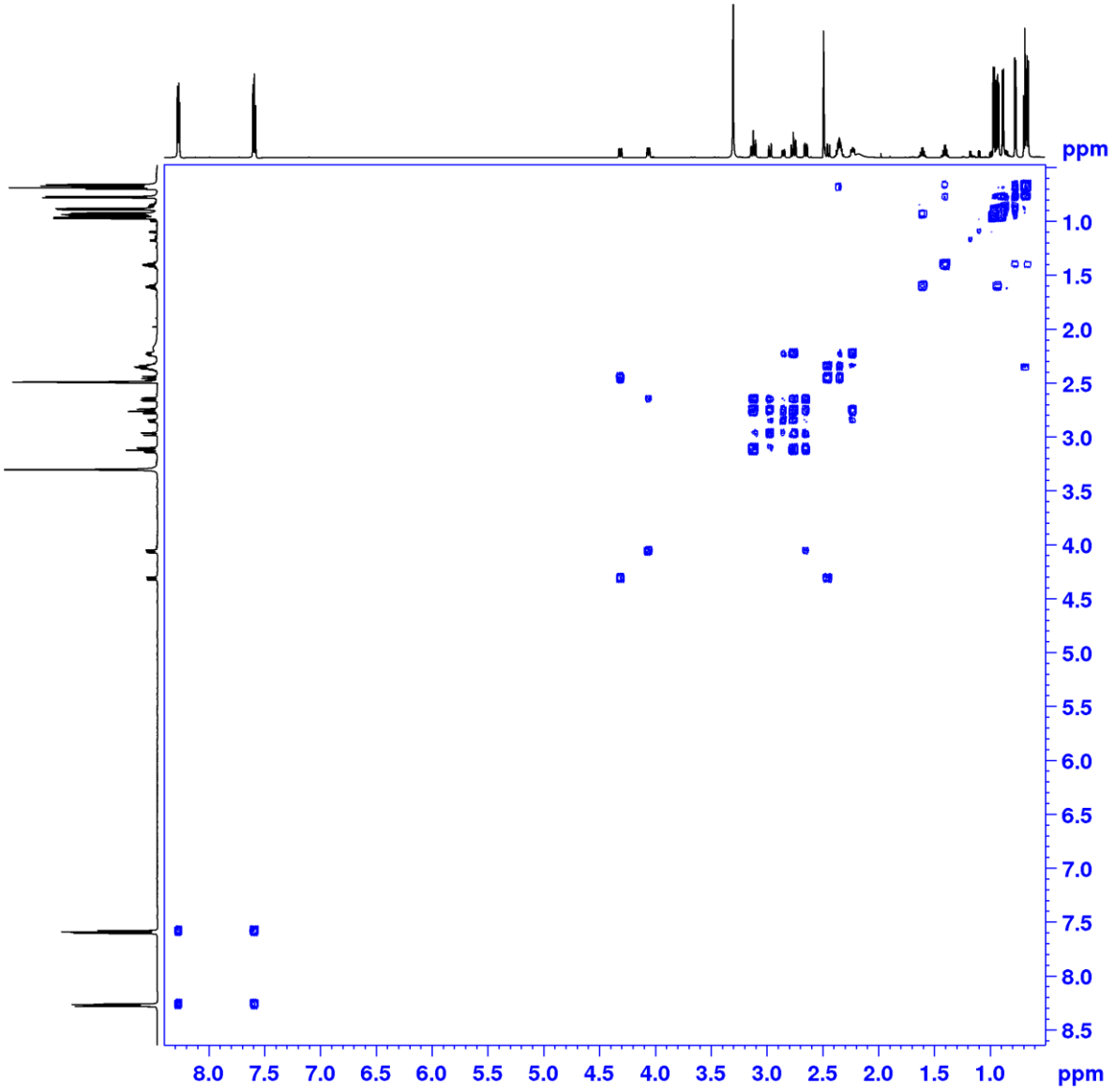

Fig. S27. ^{1}H-^{1}H COSY spectrum (600 MHz) of synthetic brevijanazine B (**2**) free base in DMSO-d_6.

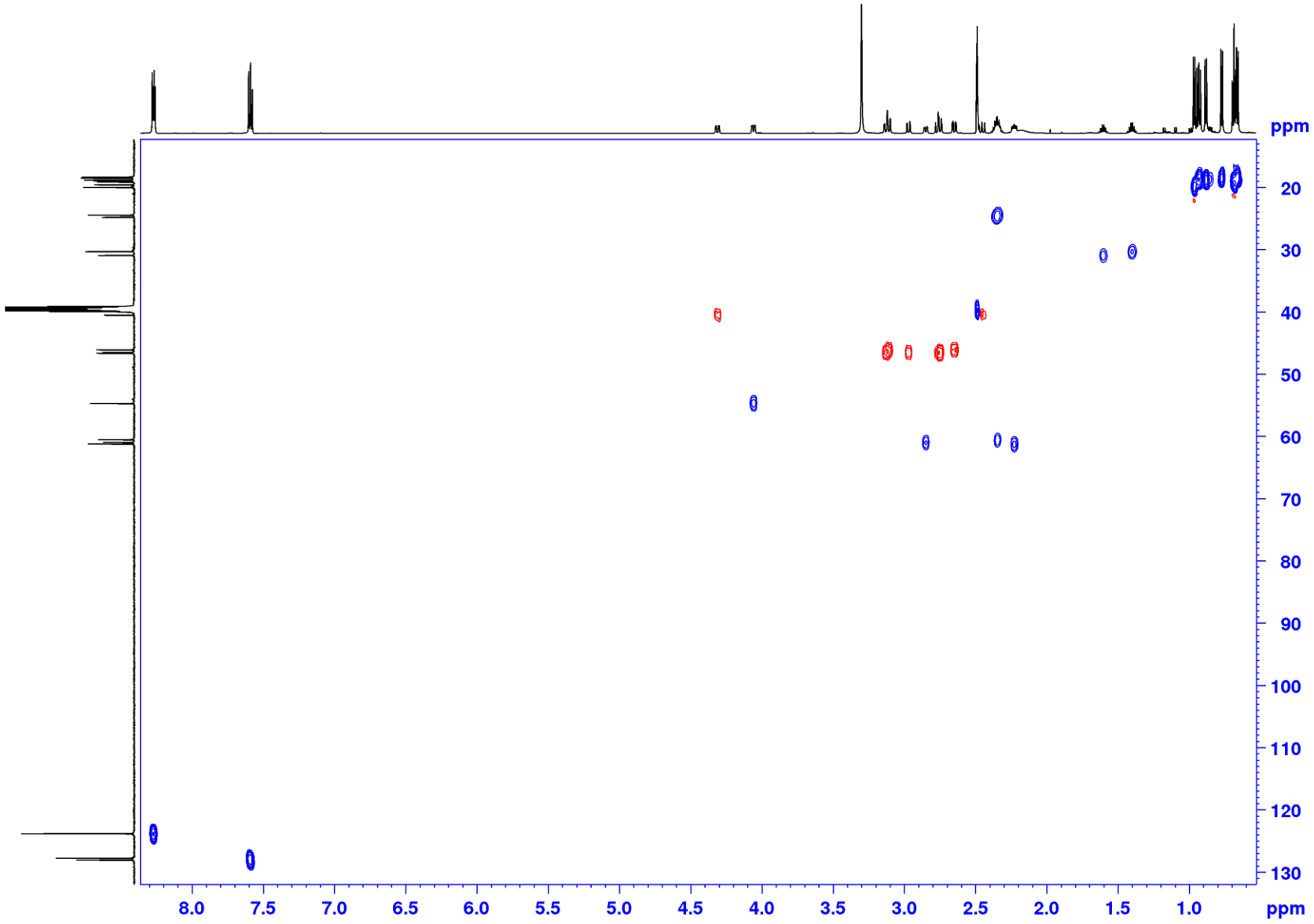

Fig. S28. HSQC spectrum (600 MHz) of synthetic brevijanazine B (**2**) free base in DMSO-d_6.

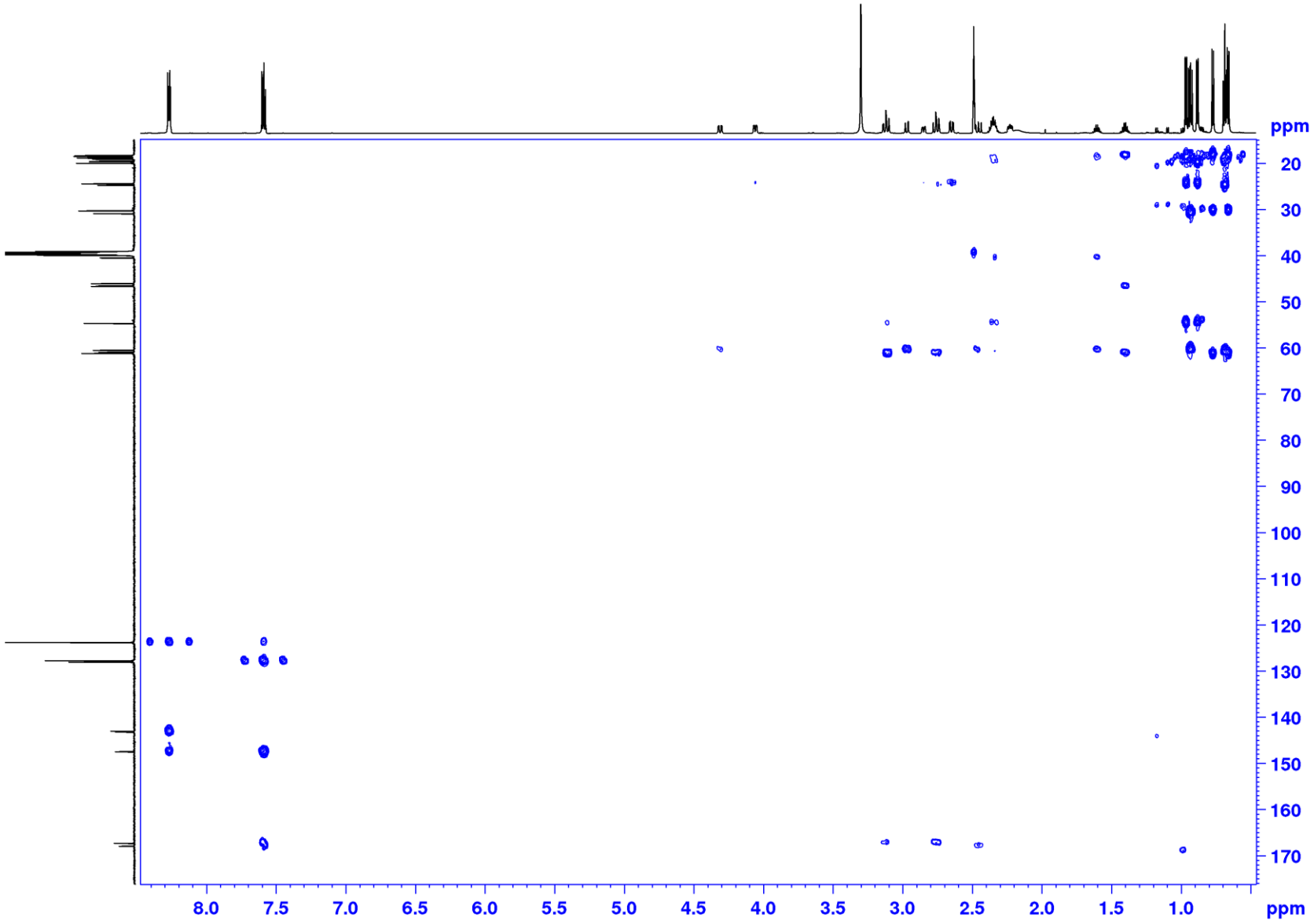

Fig. S29. HMBC spectrum (600 MHz) of synthetic brevijanazine B (**2**) free base in DMSO-d_6.

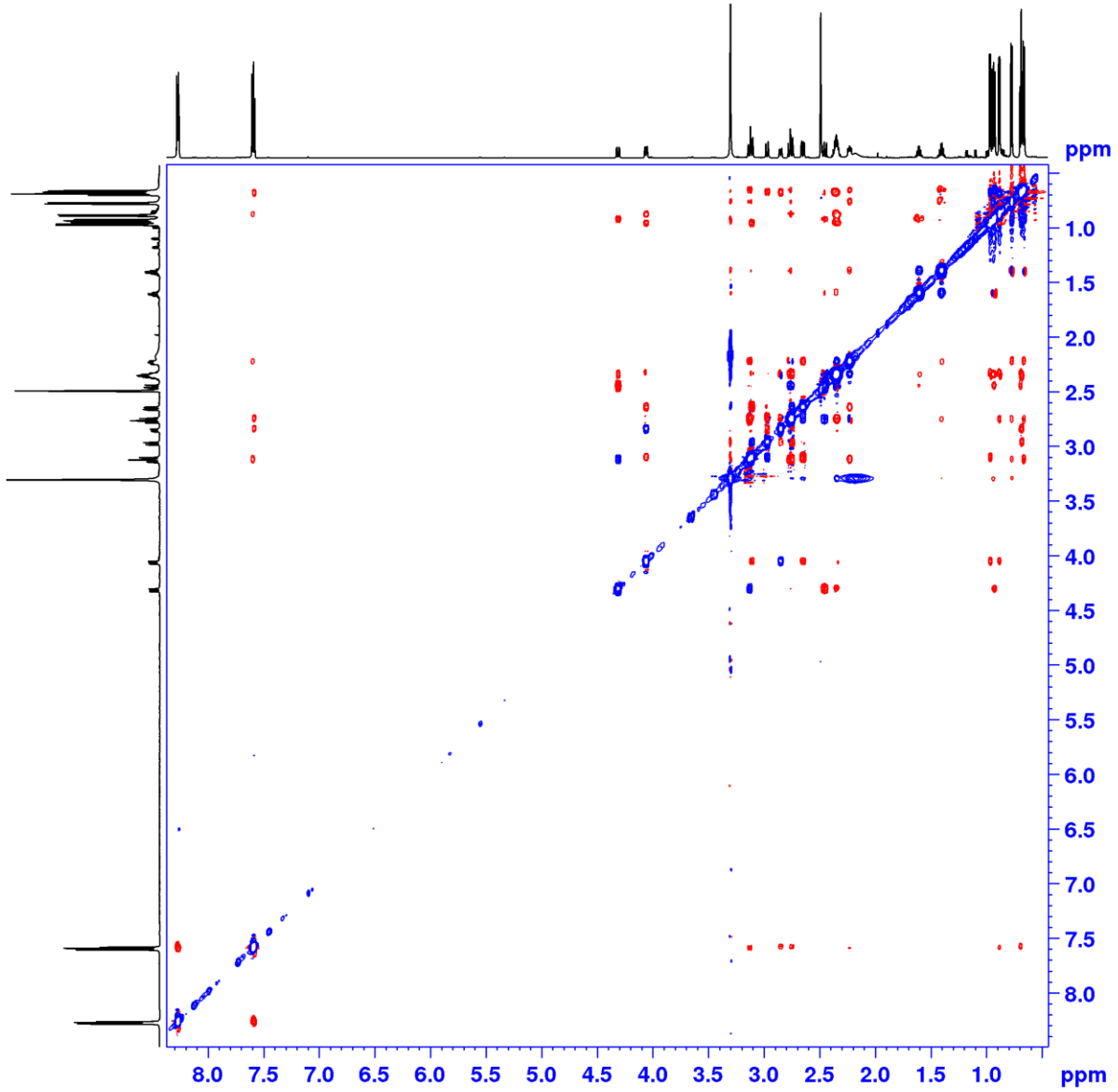

Fig. S30. ROESY spectrum (600 MHz) of synthetic brevijanazine B (**2**) free base in DMSO-d_6.

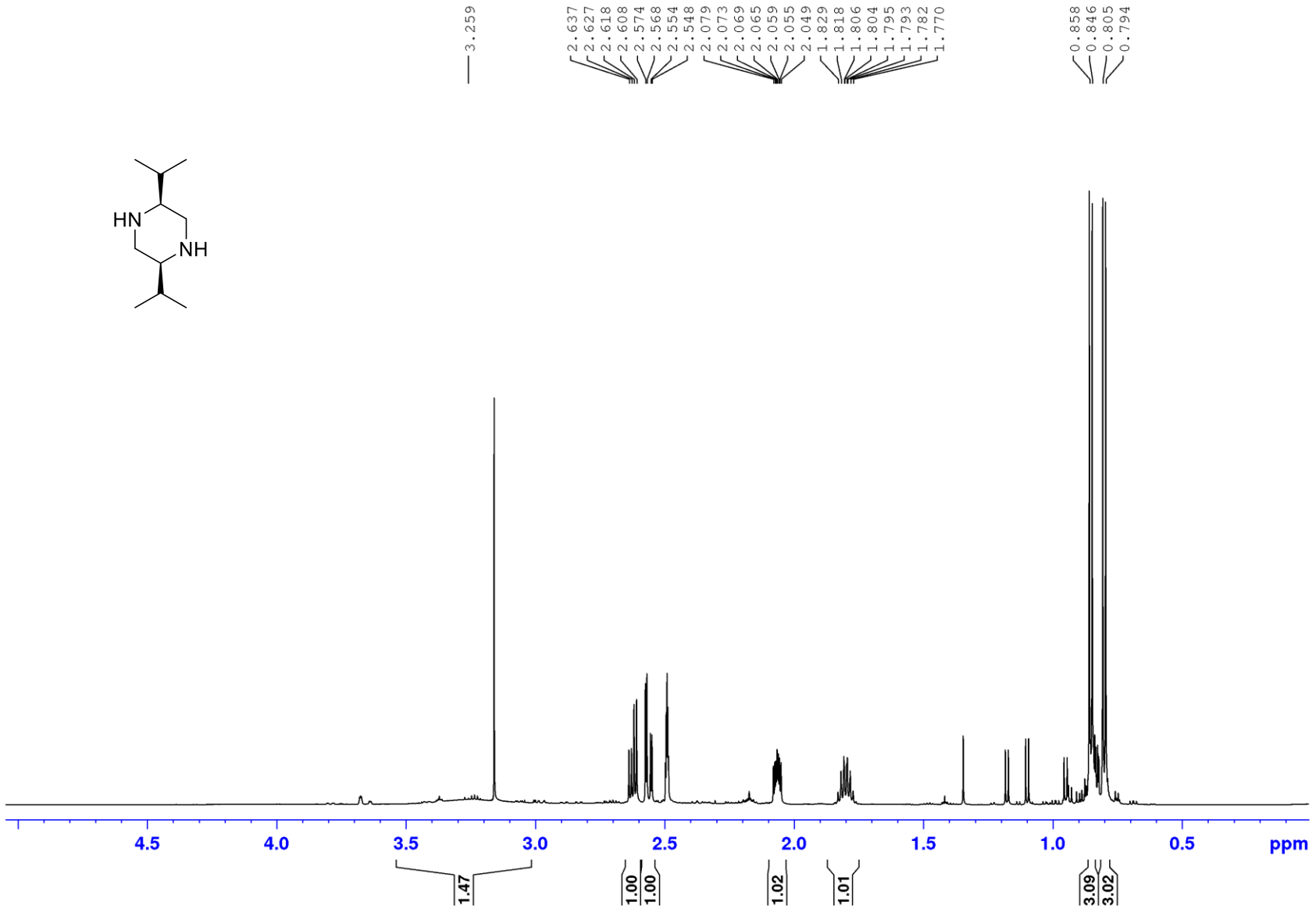

Fig. S31. ^{1}H NMR spectrum (600 MHz) of synthetic (2*S*,5*S*)-2,5-diisopropylpiperazine (**5**) in DMSO-d_6.

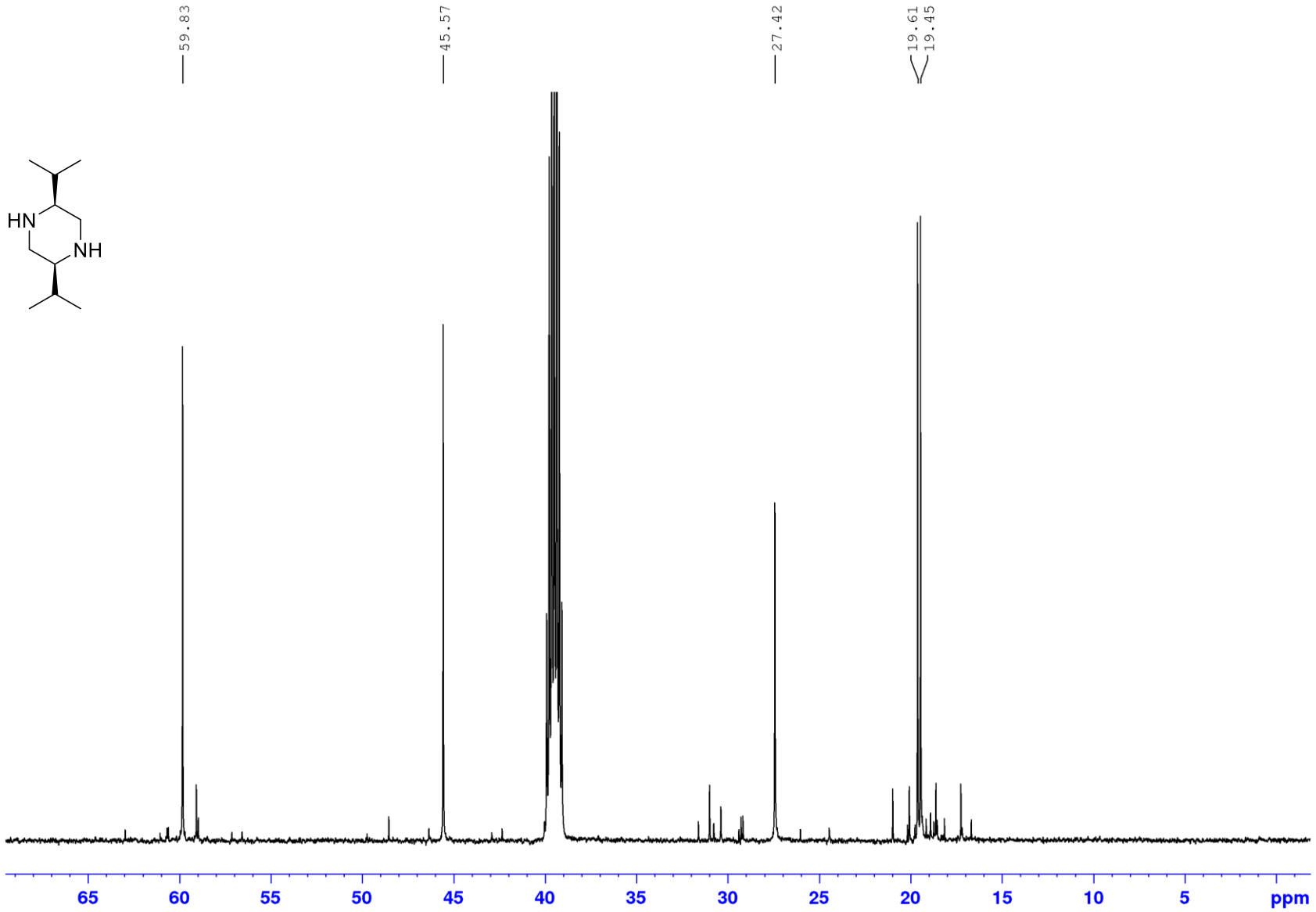

Fig. S32. ^{13}C NMR spectrum (150 MHz) of synthetic (2*S*,5*S*)-2,5-diisopropylpiperazine (**5**) in DMSO-d_6.

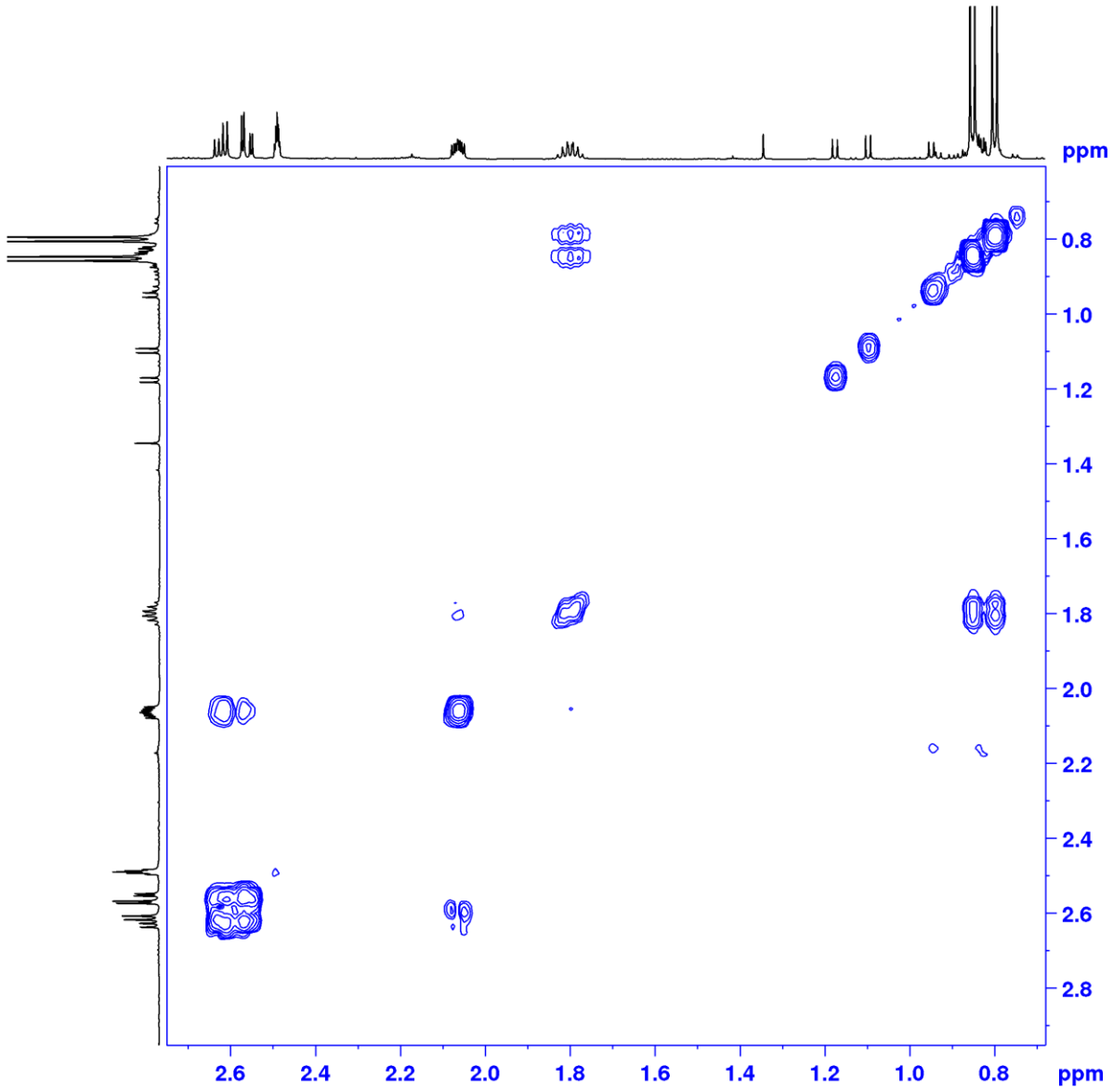

Fig. S33. ^{1}H-^{1}H COSY spectrum (600 MHz) of synthetic (2*S*,5*S*)-2,5-diisopropylpiperazine (**5**) in DMSO-d_6.

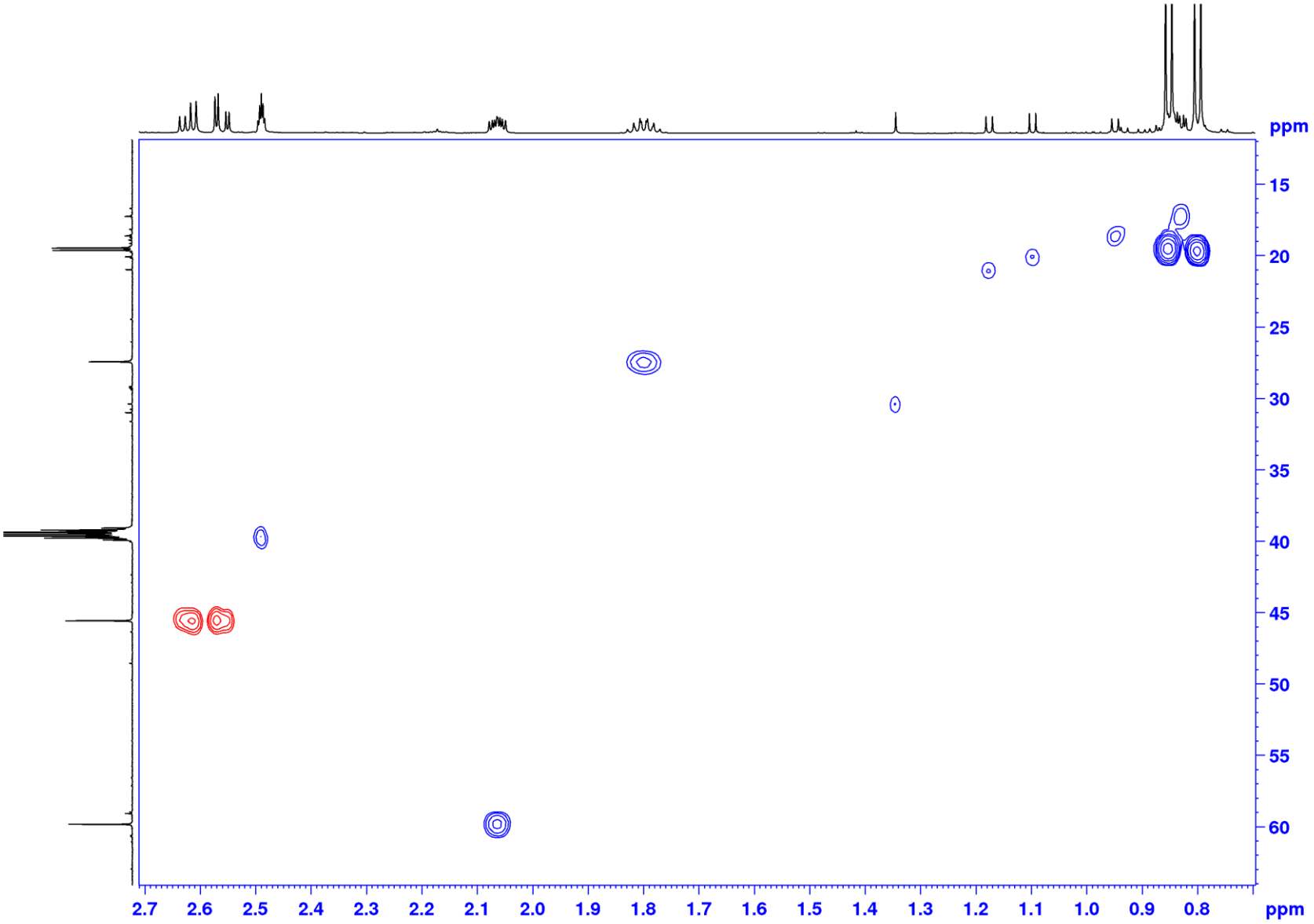

Fig. S34. HSQC spectrum (600 MHz) of synthetic (2*S*,5*S*)-2,5-diisopropylpiperazine (**5**) in DMSO-d_6.

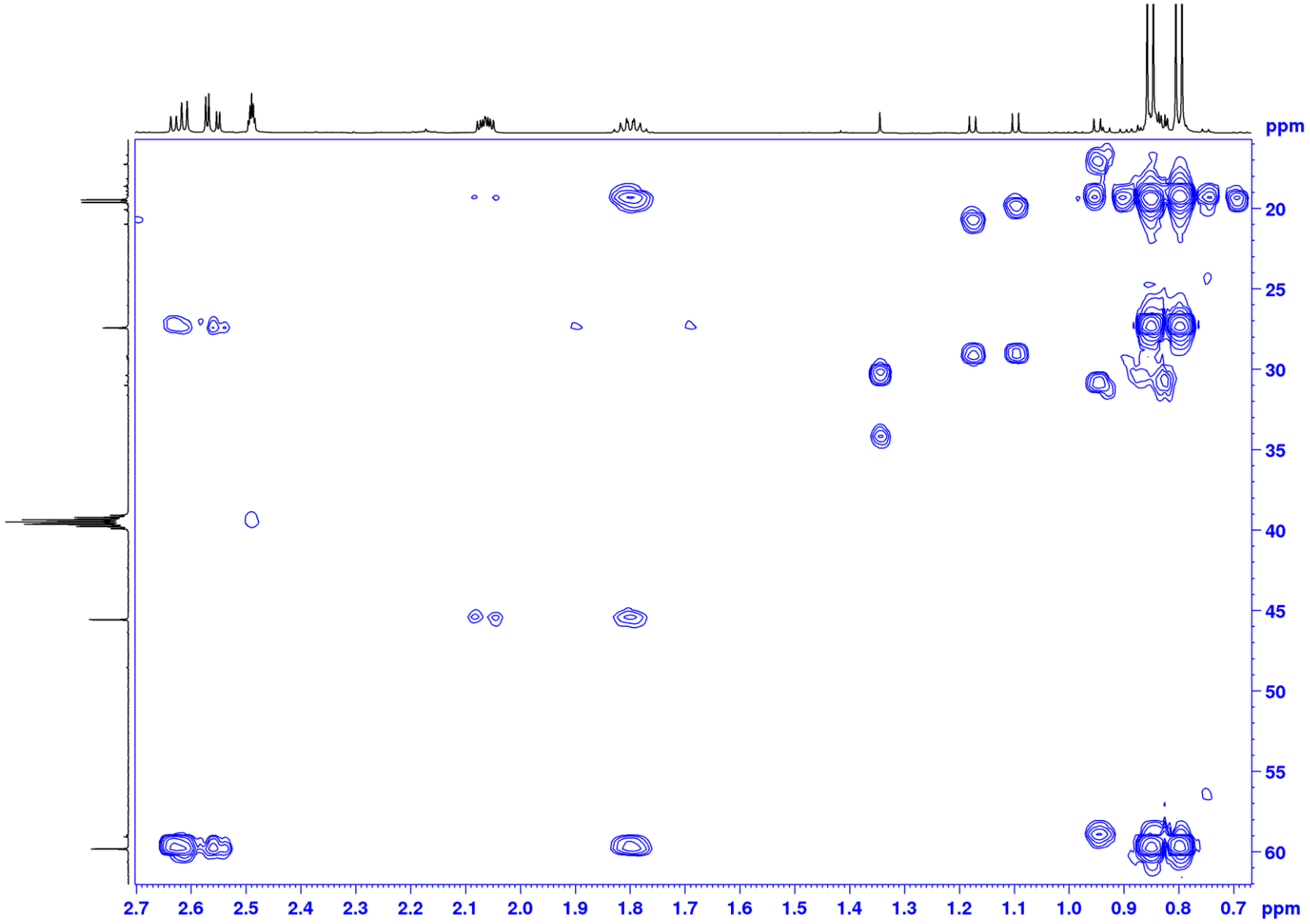

Fig. S35. HMBC spectrum (600 MHz) of synthetic (2*S*,5*S*)-2,5-diisopropylpiperazine (**5**) in DMSO-d_6.

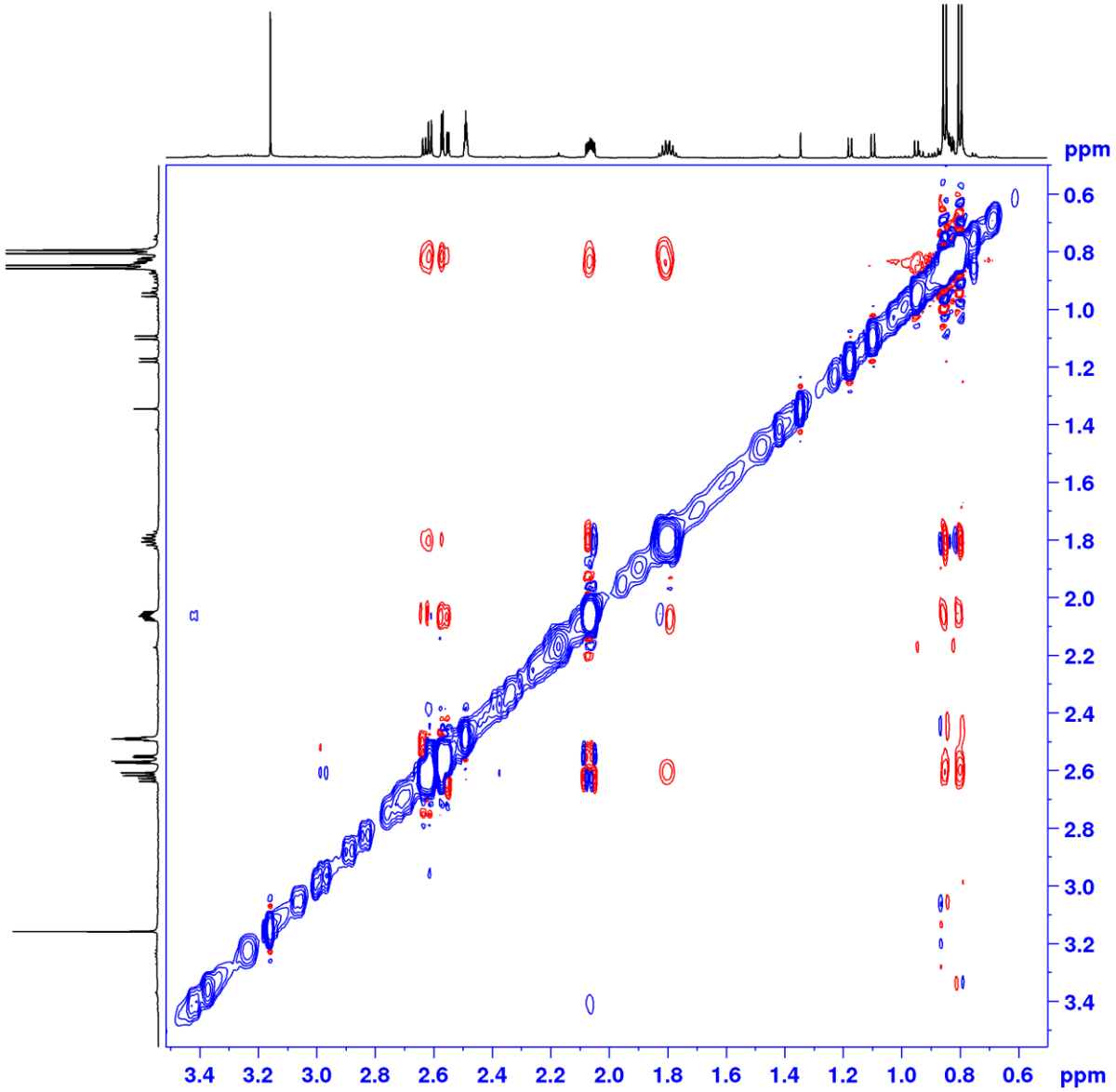

Fig. S36. ROESY spectrum (600 MHz) of synthetic (2*S*,5*S*)-2,5-diisopropylpiperazine (**5**) in DMSO-d_6.

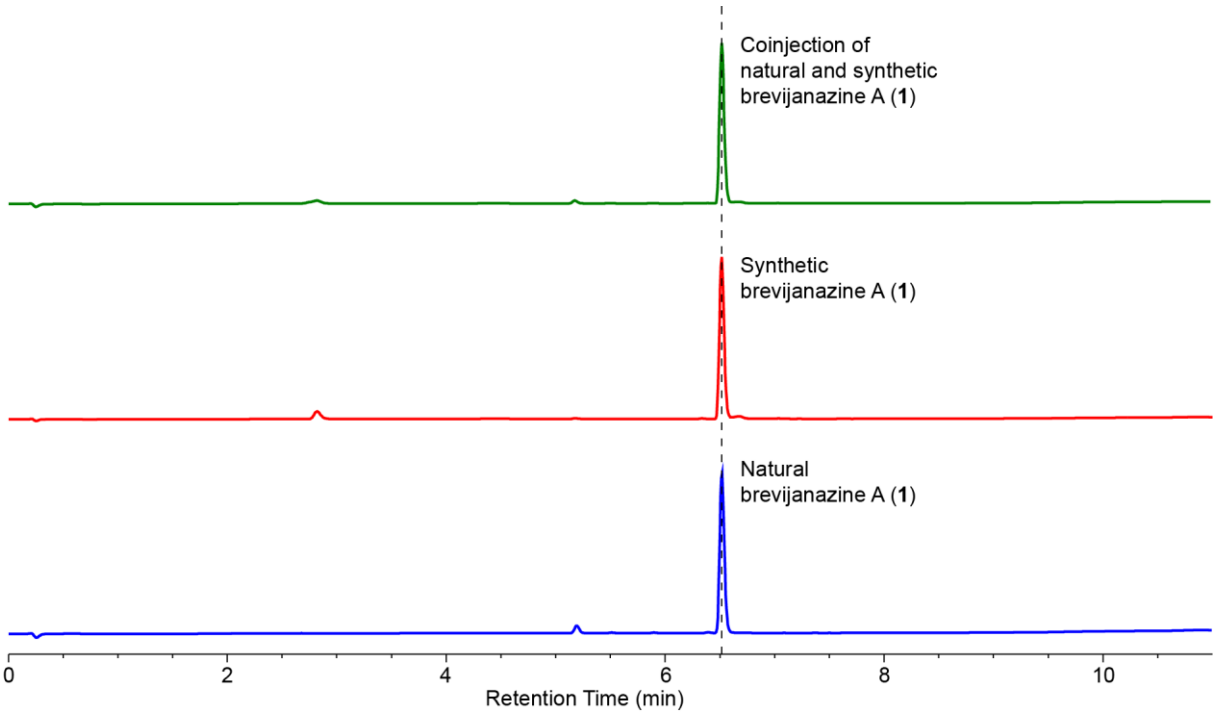

Fig. S37. HPLC traces (254 nm) comparing natural and synthetic brevijanazine A (**1**)

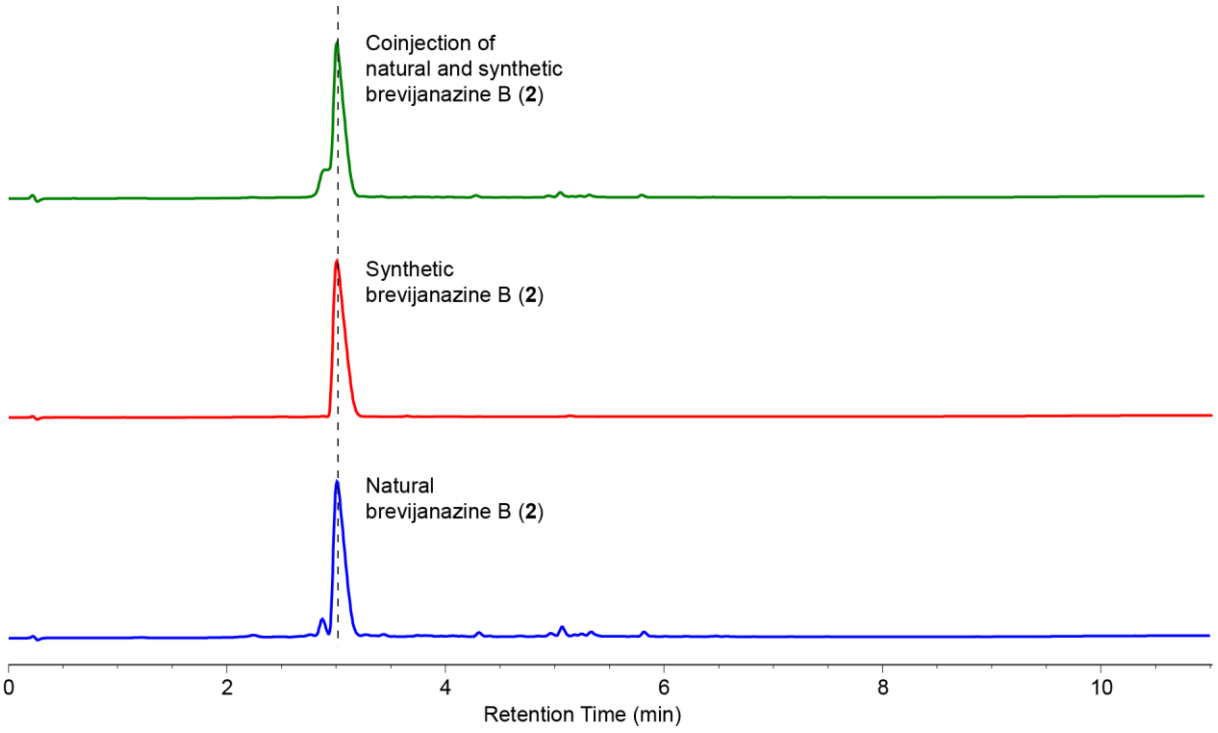

Fig. S38. HPLC traces (254 nm) comparing natural and synthetic brevijanazine A (**2**)

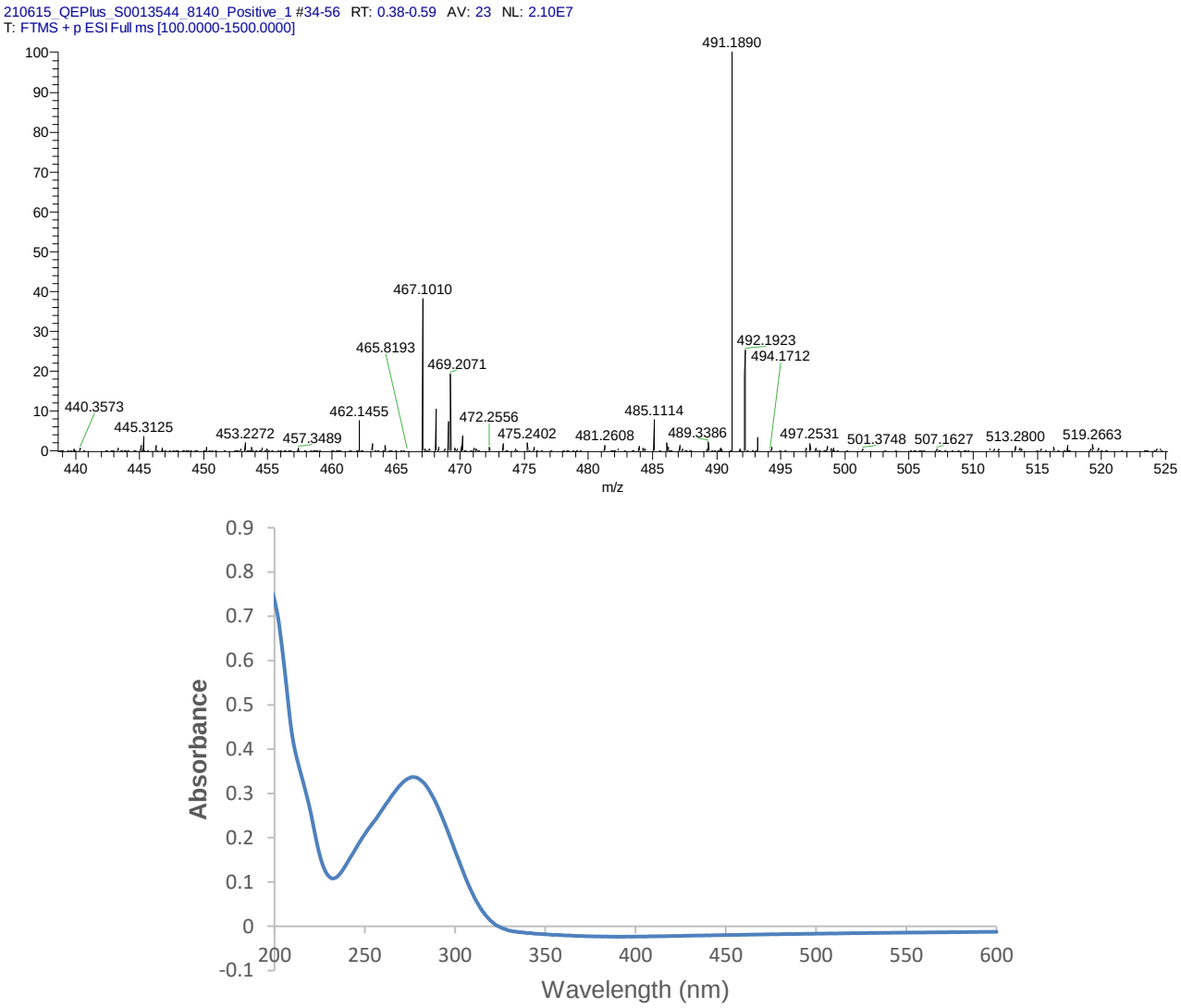

Fig. S39. HRMS and UV-vis (MeCN) spectra of natural brevijanazine A (**1**).

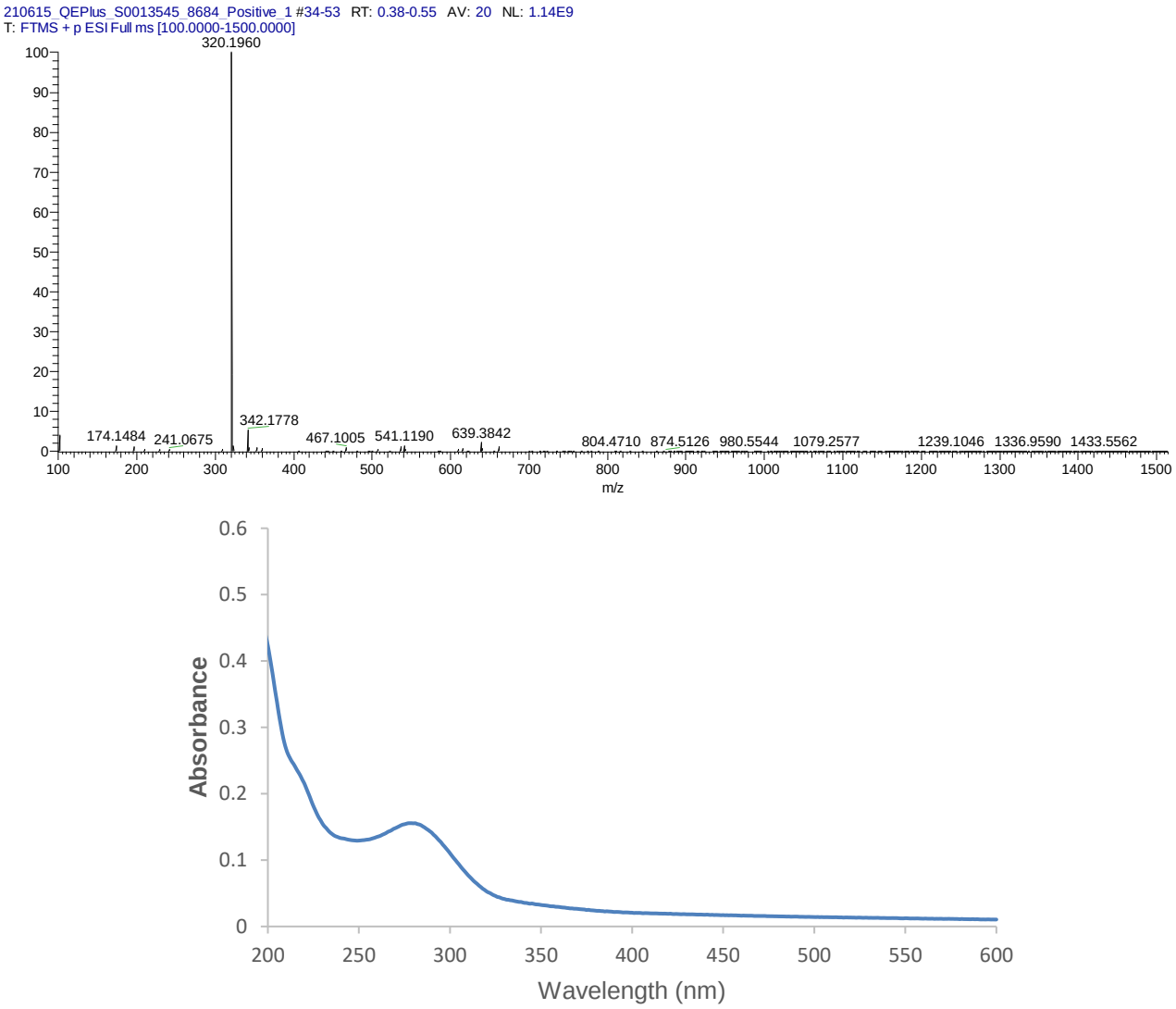

Fig. S40. HRMS and UV-vis (MeCN) spectra of natural brevijanazine B (**2**) TFA salt.

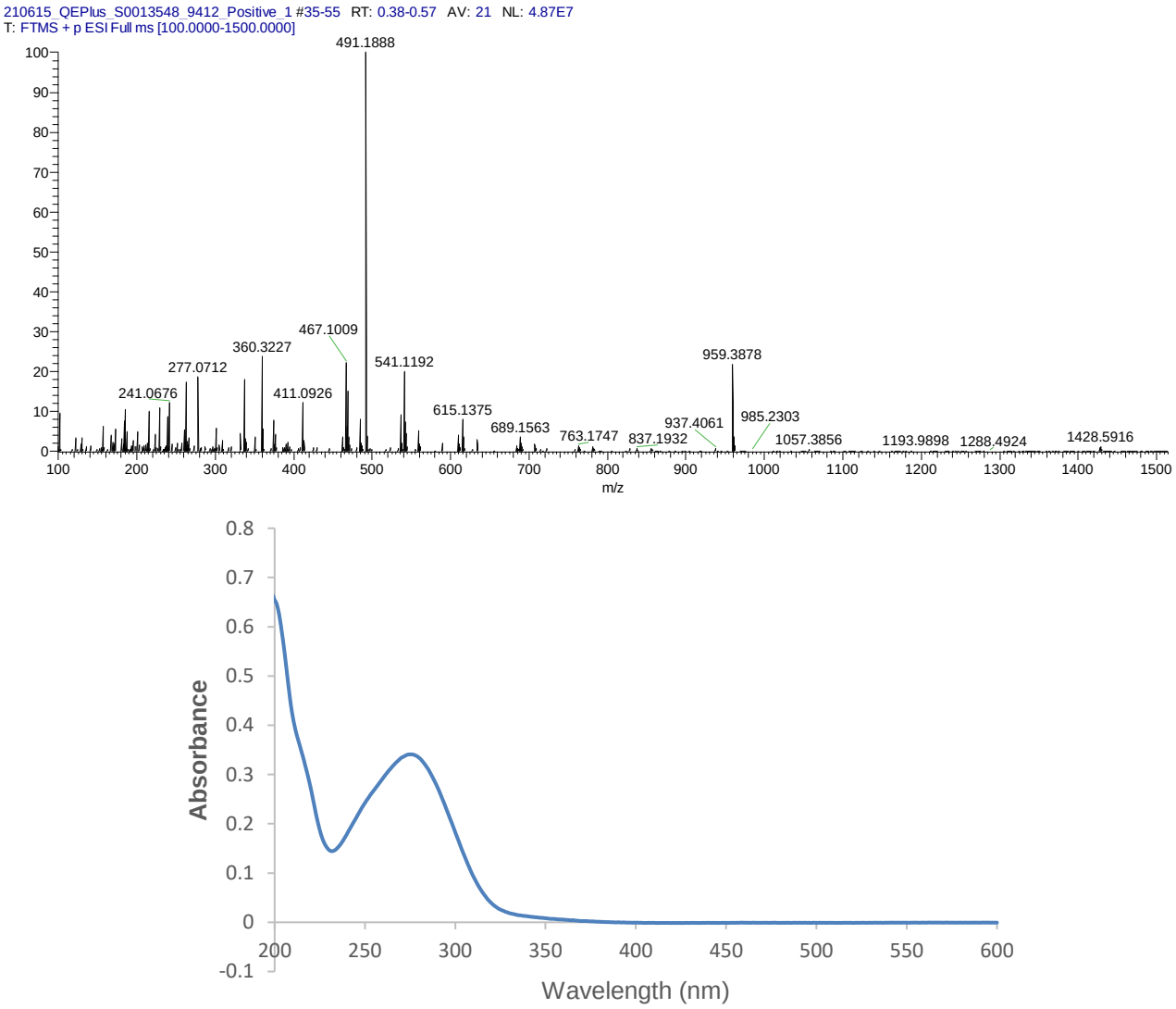

Fig. S41. HRMS and UV-vis (MeCN) spectra of synthetic brevijanazine A (**1**).

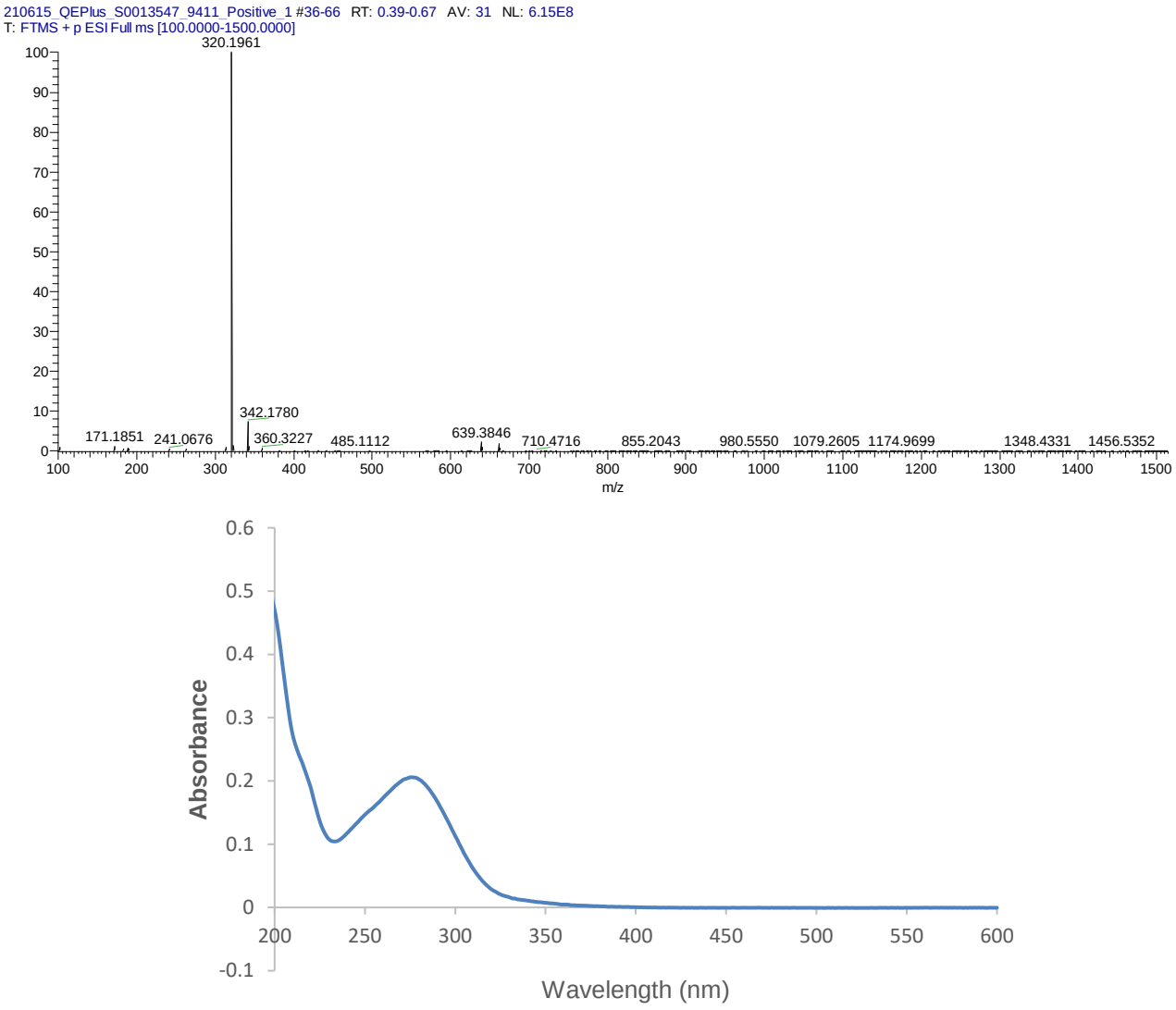

Fig. S42. HRMS and UV-vis (MeCN) spectra of synthetic brevijanazine B (**2**) free base.

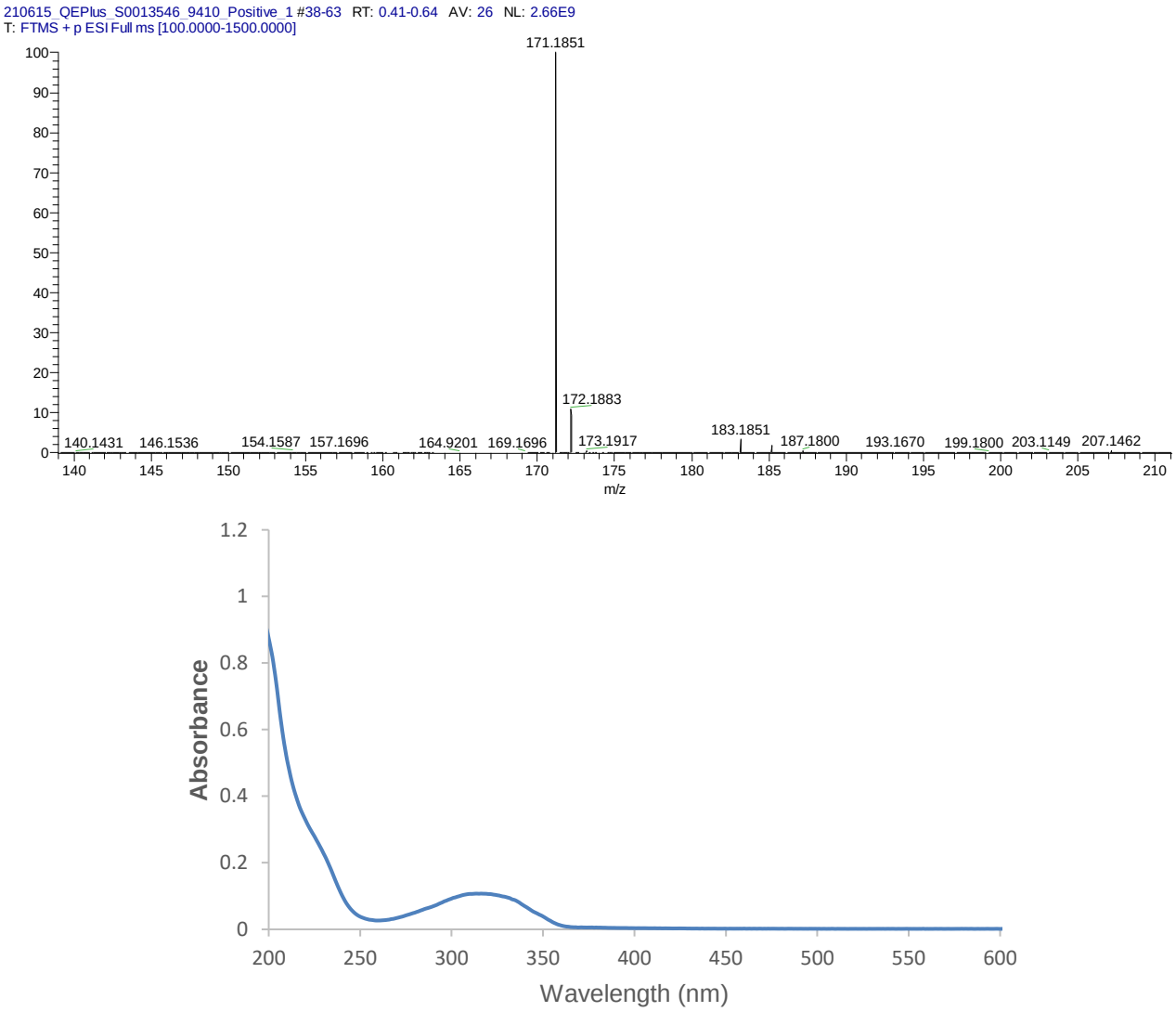

Fig. S43. HRMS and UV-vis (in MeCN) spectra of synthetic (2*S*,5*S*)-2,5-diisopropylpiperazine (**5**).

Supplementary references

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