Electronic Supplementary Material (ESI) for Chemical Science

Supporting information

Discovery of a family of γ-aminobutyrate ureas via rational derepression of a silent bacterial gene cluster

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1 Strains and plasmids

1.1 Construction of plasmids and strains

The strains and plasmids used in this study are summarized in Table S1 and S2, respectively.

Table S1: Strains used in this study

Strains	Relevant properties	Reference
Streptomyces venezuelae		
ATCC 10712	Wild-type (gaburedin production almost undetectable)	Ref. 1, 2
gbnR::apr	*gbnR* replaced by *FRT-oriT-apr-FRT* cassette (gaburedin overproduction)	This study
gbnR::scar and gbnB::apr	*gbnR* deleted (81 bp left) and *gbnB* replaced by *oriT-apr* (gaburedin production abolished)	This study
E. coli		
DH5α	General cloning strain	Ref. 3
BW25113	Strain used for PCR-targeted mutagenesis	Ref. 4
ET12567	Nonmethylating strain used for conjugation with *Streptomyces*	Ref. 5

Table S2: Cosmids and plasmids used in this study

Cosmids/ Plasmids	Relevant properties	Reference
Cosmids		
SV4 F01	Cosmid from genomic library of S. venezuelae ATCC 10712; *neo* (contains the entire *gbn/sgn* gene cluster)	
pCC020	*gbnR* replaced by *FRT-oriT-apr-FRT* cassette in SV4_F01	This study
pCC021	*gbnR* replaced by 81 bp scar in SV4_F01	This study
pCC022	SV4_F01 with *gbnR*::scar and *gbnB*::*apr*	This study
Plasmids		
pIJ773	*FRT-oriT-apr-FRT*	Ref. 6
pIJ775	*oriT-apr*	Ref. 6
pIJ790	*gam bet exo cat*	Ref. 6
pUZ8002	Mobilization plasmid; *neo*	Ref. 7
BT340	FLP recombination plasmid; *flp bla cat repA101*	Ref. 4

Construction of plasmids pCC020-pCC022 and gene disruption in *S. venezuelae* to generate *S. venezuelae gbnR::apr* and *S. venezuelae gbnR::scar, gbnB::apr*

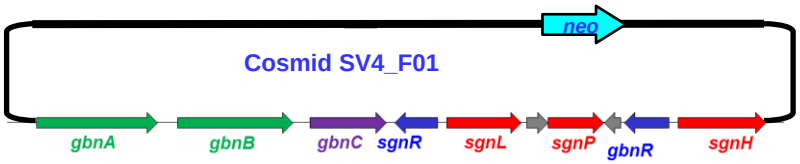


Figure S1. Map of the cosmid SV4_F01 used to construct the *S. venezuelae* mutant strains.

The *gbnR* gene within the SV4_F01 cosmid was first replaced with a cassette containing an *apr* apramycin resistance gene, *oriT* and FRT sequences using a PCR-targeting based gene replacement method to generate pCC020. The cassette was amplified from pIJ773 using the forward and reverse primers A and A' (Table 2) to disrupt *gbnR*. Correct replacement of *gbnR* with FRT-*apr*-*oriT*-FRT was confirmed by restriction digests and PCR using the primer pair C/C' which is complementary to regions upstream and downstream of *gbnR*. The plasmid pCC020 was then introduced into *S. venezuelae via* conjugation from *E. coli* ET12567/pUZ8002 to generate *S. venezuelae gbnR::apr* mutant strain following the procedure described by Gust *et al*. [6]

The FRT-*apr*-*oriT*-FRT cassette was then removed from plasmid pCC020 using a Flip recombinase as described by Gust *et al*. to generate pCC021.[6] This plasmid was then submitted to disruption of the *gbnB* gene using a disruption cassette amplified from pIJ775 that contain an *apr*-*oriT* cassette (without FRT sequences) using the forward and reverse primers B and B' to construct pCC022. Correct replacement of *gbnB* was confirmed by restriction digests and PCR using the primer pair D/D' which is complementary to regions upstream and downstream of *gbnB*. Plasmid pCC022 was then introduced into *S. venezuelae via* conjugation from *E. coli* ET12567/pUZ8002 to generate *S. venezuelae gbnR::scar, gbnB::apr* mutant strain following the procedure described by Gust *et al*. [6]

Table S3: Primers used in this study

Primers	**Sequence**
Disruption	
A	5'_AGGAACACGAACACAGCGACGGGAGGCGGACGCTCGGTGATTCCGGGGATCCGTCGACC_3'
A'	5'_GGGCGGGGTCGGTAGGCGTGGTCGGGGAGCGGCCGGTCATGTAGGCTGGAGCTGCTTC_3'
B	5'_CCGCCTCCGATCCCGACTTCGAGGGAGAACACCTCCATGATTCCGGGGATCCGTCGACC_3'
B'	5'_GTCTGCGGGTCGGACGGGTCGGCGGGGGCGGCCGGGTCATGTAGGCTGGAGCTGCTTC_3'
Screening	
C	5'_CCTTGAGTGGTGGTACTG_3'
C'	5'_GACGTCACACTCGTCATC_3'
D	5'_GCCACCAGCTGACCACAC_3'
D'	5'_TCCGGCTGGGAGTTGAGG_3'

Underlined sequences correspond to the 20 nucleotides P1 and 19 nucleotides P2 sequences.[6]

1.2 Sequence comparison of gaburedin biosynthetic gene clusters

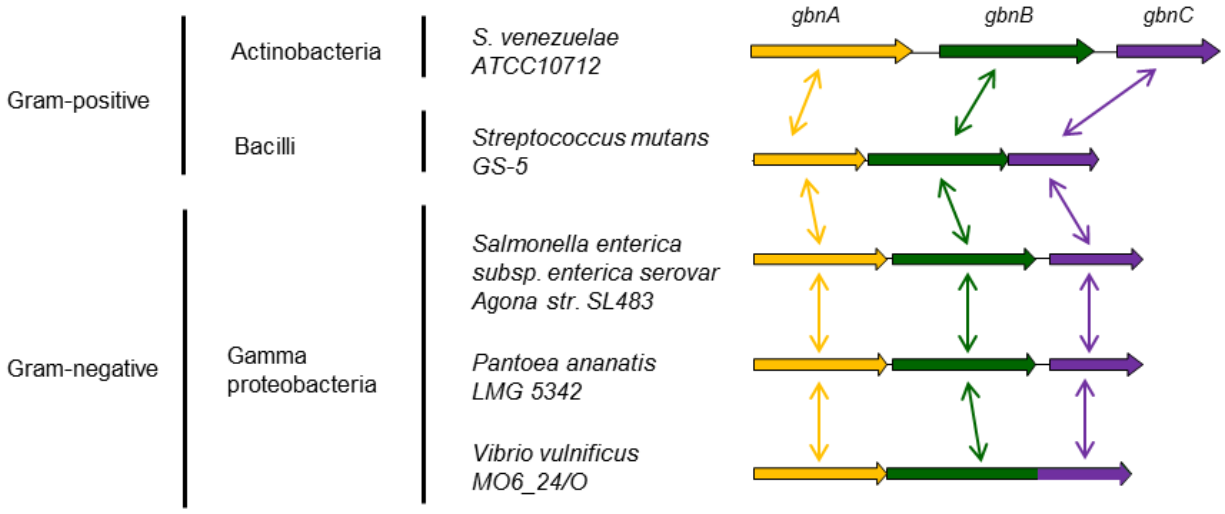


Figure S2. Gaburedin biosynthetic systems identified using comparative genomics.

2 Gaburedin production

Modified Supplemented Minimal Medium Solid (SMMS). Unless otherwise stated, modified Supplemented Minimal Medium Solid (SMMS) was made up in batches with the following ingredients added to final concentrations as follows: Difco casaminoacids (2 gL^{-1}), TES buffer (5.68 gL^{-1}) and Bacto agar (15 gL^{-1}) were dissolved in distilled water, the pH adjusted to 7.2 using 10 N NaOH and autoclaved.[8] At the time of use, the media was re-melted and the following ingredients were added to the stated concentrations: NaH_2PO_4 + K_2HPO_4 (50 mM each, 10 mL per litre of culture), $MgSO_4.7H_2O$ (1 M, 5 mL per litre of culture), glucose (50% w.v, 18 mL per litre of culture), trace element solution (2 mL per litre of culture). Trace elements solution contained 0.1 gL^{-1} each of $ZnSO_4.7H_2O$, $FeSO_4.7H_2O$, $MnCl_2.4H_2O$, $CaCl_2.6H_2O$ and NaCl. The solution was stored at 4°C in a refrigerator. With the exception of media used to grow the wild type *S. venezuelae* strain, apramycin was also added to a final concentration of 50 μg mL^{-1}.

Amino Acid Enriched Culture Media. Unless otherwise stated, modified Supplemented Minimal Medium Solid (SMMS) was prepared as above, with the addition of each amino acid to final concentration of 20 mM, and the pH adjusted to 7.2, with the exception of N-acetyl L-cysteine and L-glutamic acid, which were added to a final concentration of 10 mM. Culture media enriched with L-tyrosine and L-tryptophan were saturated due to their poor solubility in water. The prepared media was poured out (15 mL per plate) and inoculated with 10 μL of the *gbnR::apra* strain.

Unless otherwise stated, the *S. venezuelae* strains used in this study were incubated at 30 °C for 3 days. Plates were stored at 4 °C until the time of extraction, then ethyl acetate was added (in equal volume to the volume of culture media used) and acidified to pH 3 by the addition of 37% HCl. The ethyl acetate layer was removed and evaporated under reduced pressure and the remaining residue was redissolved in 500 μL 50:50 HPLC grade methanol/water for LC-MS analysis, with the exception of the 800 mL culture medium from which the gaburedins were purified, which was dissolved in 3 mL 50:50 HPLC grade methanol/water for HPLC purification (see section 4).

3 Synthesis of gaburedin A

To a stirred solution of mono-methyl glutarate (1.2 mL, 9.6 mmol, 1.4 Eq) in dry pyridine (6 mL), diphenylphosphorylazide (1.5 mL, 7 mmol, 1 Eq) was added and stirred at 90 °C for 1 h under argon. After 1 h, L-phenylalanine *O*-methyl ester hydrochloride (0.736 g, 3.41 mmol, 0.49 Eq) was added and the reaction mixture stirred at 90 °C for a further 4 h. After being allowed to cool, 30 mL ethyl acetate was added to the reaction mixture, the mixture washed with 2 M HCl (5 x 30 mL), and the ethyl acetate layer dried ($MgSO_4$) and removed under reduced pressure. The product was then purified by flash column chromatography (4:1 ethyl acetate/hexane eluent), yielding 331 mg (1.03 mmol, 30% yield) of protected gaburedin A, which was a white solid. This procedure is modified from the work of Takaya, *et al*.[9] The yield reported here is the overall yield from the two-step, one-pot reaction to yield the dimethyl ester from mono-methyl glutarate, treating the aminoacyl ester as the limiting reagent.

The dimethyl ester (28.8 mg, 8.94 x 10^{-5} mol) was redissolved in 17.5 mL of a stirred 1:1 mixture of methanol/aqueous NaOH to a final concentration of 2 M NaOH and heated to 50 °C. After 24 h, the methanol was removed and the remaining aqueous solution washed with ethyl acetate (3 x 20 mL), the pH of the mixture reduced to 1 using 2 M HCl, and ethyl acetate extractions (3 x 20 mL) performed. The ethyl acetate extracts were combined, dried ($MgSO_4$) and the ethyl acetate removed under reduced pressure. The remaining residue was dissolved in 3 mL of a 1:1 mixture of methanol/water and purified by reverse-phase HPLC (Section 4). Methanol was removed under reduced pressure and the water removed by lyophilization using a VirTis benchtop K freeze-dryer, furnishing 12.6 mg (4.27 x 10^{-5} mol, 48% yield) of gaburedin A, which was a white solid. The product was dissolved in d_6-DMSO for NMR analysis. ^{1}H, ^{13}C DEPT, COSY and HSQC NMR spectra were obtained using Bruker Avance DPX-400 and DRX-500 NMR spectrometers (see Section 7 for all NMR spectra).

δ_H (500 MHz, d_6-DMSO); 1.54 (2H, m, $CH_2CH_2CH_2$, J = 7.2 Hz), 2.17 (2H, t, CH_2CO_2H, J = 7.5 Hz), 2.84-3.01 (4H, m, CH_2Ph and $NHCH_2CH_2$, J = 5.3 Hz, 7.2 Hz, 7.3 Hz, 13.6 Hz), 4.29 (1H, m, CH, J = 5.3 Hz, 7.3 Hz), 5.96 (1H, d, CHNHCO, J = 7.8 Hz), 6.17 (1H, t, CH_2NHCO, J = 5.3 Hz), 7.16-7.25 (5H, m, ArH)

δ_C (125 MHz, d_6-DMSO); 25.5 ($CH_2CH_2CH_2$), 31.1 (CH_2CO_2H), 37.7 (CH_2Ph), 38.5 ($NHCH_2CH_2$), 54.1 (CH), 126.2 (ArH, *para*), 128.0 (ArH, *meta*), 129.3 (ArH, *ortho*), 137.8 (Ar, *ipso*), 157.5 (NHCONH), 174.0, (CO_2H), 174.3 (CO_2H)

m/z $[M+H]^+$ = 295.1283 (calculated 295.1288 for $C_{14}H_{19}N_2O_5$, error 2.0 ppm); $[M+Na]^+$ = 317.1103 (calculated 317.1108 for $C_{14}H_{18}N_2O_5Na$, error 1.6 ppm)

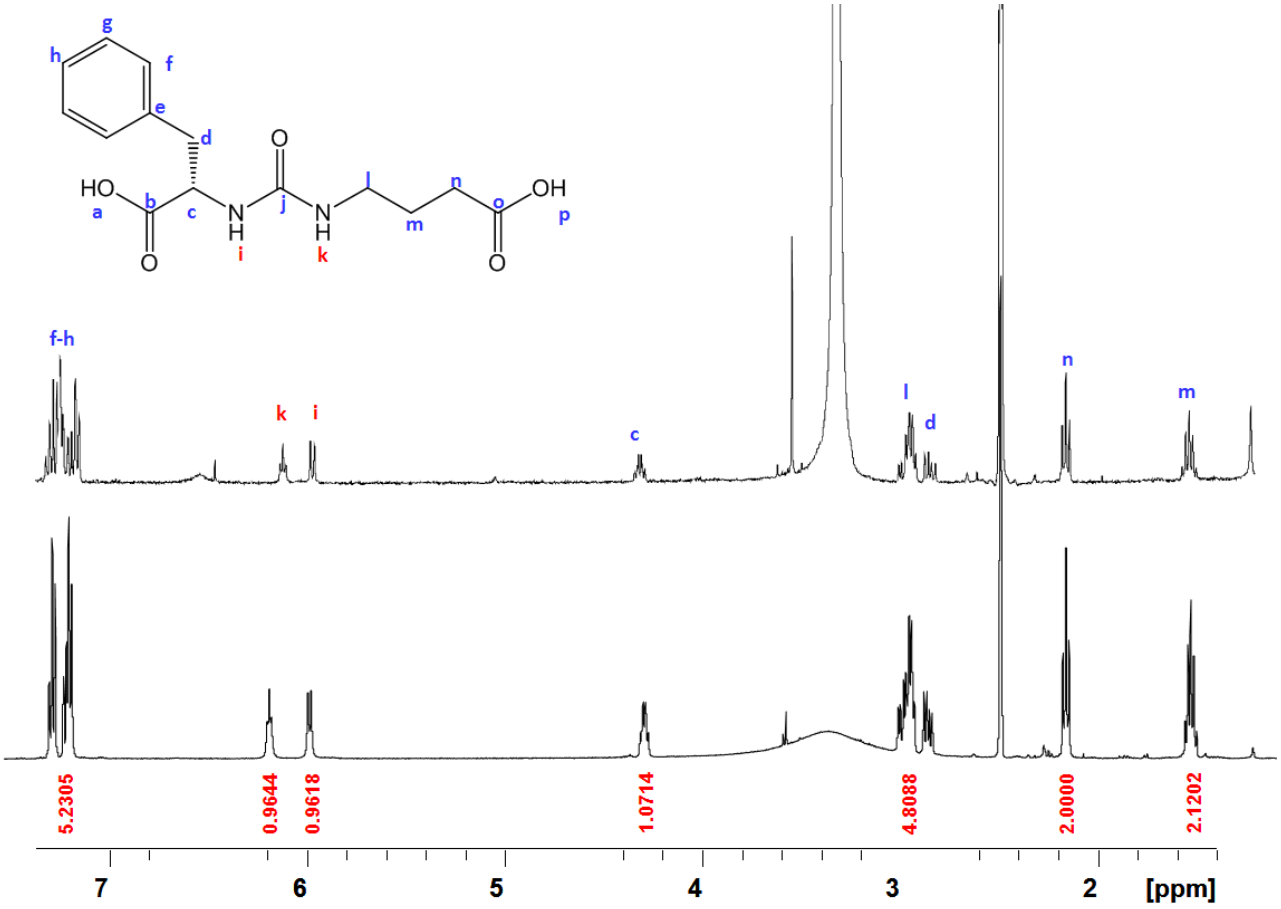


Figure S3. Comparison of the ^{1}H NMR spectra for natural (top) and synthetic (bottom) gaburedin A. Note that the small difference in the chemical shifts of protons k and i, which can participate in intermolecular hydrogen bonds, likely results from the differing concentrations of the two samples.

4. HPLC conditions

C_{18} HPLC:

Reverse-phase HPLC was performed using a Zorbax XBD-C_{18} column (21.2 x 150 mm with a 10 mm guard column, particle size 5 µm) connected to an Agilent 1200 HPLC equipped with a binary pump and DAD detector. Gradient elution was used (solvent A: water with 0.1 % HCOOH, solvent B: methanol) with a flow rate of 20 mL min^{-1}. Fractions were collected by time or absorbance at 210 nm using an automated fraction collector. The following elution profile was used: 80:20 solvent A/solvent B for 5 min, then 20:80 solvent A/solvent B over 25 min. Fractions were collected (at the retention time of interest), the fractions pooled, methanol removed under vacuum, and the metabolite of interest was re-extracted from the remaining water (2 x 50 mL ethyl acetate). The ethyl acetate was removed under reduced pressure and the sample re-dissolved in 50:50 methanol/water. Gaburedins B and C were not easily separable and were therefore co-purified. For gaburedin A, the sample was run through the HPLC as above, with the following gradient: 65:35 solvent A/solvent B for 5 min, then 35:65 solvent A/solvent B over 25 min.

Chiral HPLC:

Samples dissolved in 50:50 methanol/water were injected through an Agilent 1100 equipped with a quaternary pump through a Chiral-PAK IA column coupled to a Bruker High Capacity Trap (HCT) + ion trap mass spectrometer with an electrospray source, operating in positive ion mode. A 5 min isocratic elution (75:25 solventA/solvent B) was followed by gradient elution to 0:100 solvent A/solvent B over 25 min. Solvents A and B were water (0.1 % TFA) and methanol (0.1 % TFA), respectively.

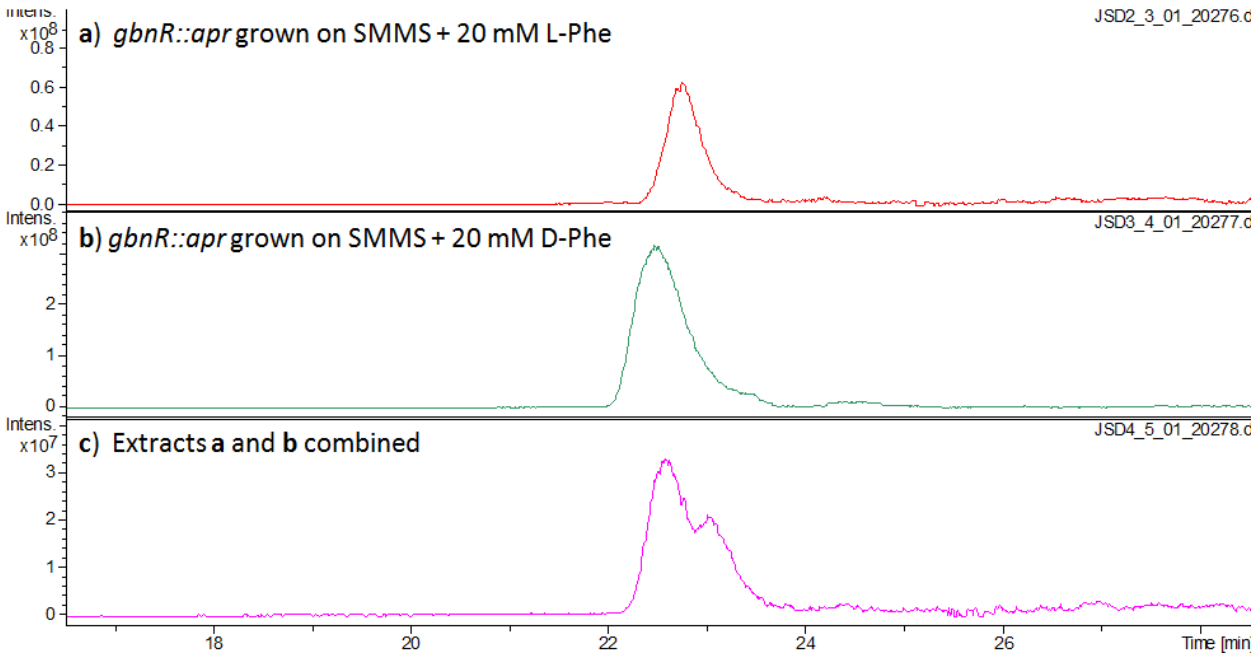


Figure S4. Extracted ion chromatograms for *m/z* = 295, corresponding to gaburedin A and its enantiomer, from chiral HPLC-MS analysis of samples prepared from the *S. venezuelae gbnR::apr* mutant grown on 20 mM L- or D-phenylalanine (panels **a** and **b**, respectively). Coinjection of extracts **a** and **b** (panel **c**) confirmed that the gaburedins produced upon supplementation with L- or D-phenylalanine are different; gaburedin A (panel **a**) and its enantiomer (panel **b**) have different retention times.

5. LC-MS data

20 µL of prepared extracts were injected through a reverse phase column (Zorbax C_{18}, size 4.6 x 150 mm, particle size 5 µm) connected to an Agilent 1100 HPLC. The outflow was routed to a Bruker High Capacity Trap (HCT) + ion trap mass spectrometer with an electrospray source, operating in positive ion mode. A 5 min isocratic elution (95:5 solventA/solvent B) was followed by gradient elution to 0:100 solvent A/solvent B over 25 min. Solvents A and B were water (0.1 % HCOOH) and methanol (0.1 % HCOOH), respectively.

The high-resolution data was obtained by performing UPLC-MS through a reverse phase column (Zorbax Eclipse Plus C_{18}, size 2.1 x 100 mm, particle size 1.8 µm) connected to a Dionex 3000RS UHPLC coupled to Bruker Ultra High Resolution (UHR) Q-TOF MS MaXis mass spectrometer with an electrospray source. Sodium formate (10 mM) was used for internal calibration and a *m/z* = 50 to 1500 scan range used with a gradient elution from 100:0 solvent A/solvent B to 0:100 solvent A/solvent B over 10 minutes. Solvents A and B were water (0.1 % HCOOH) and acetonitrile (0.1 % HCOOH), respectively.

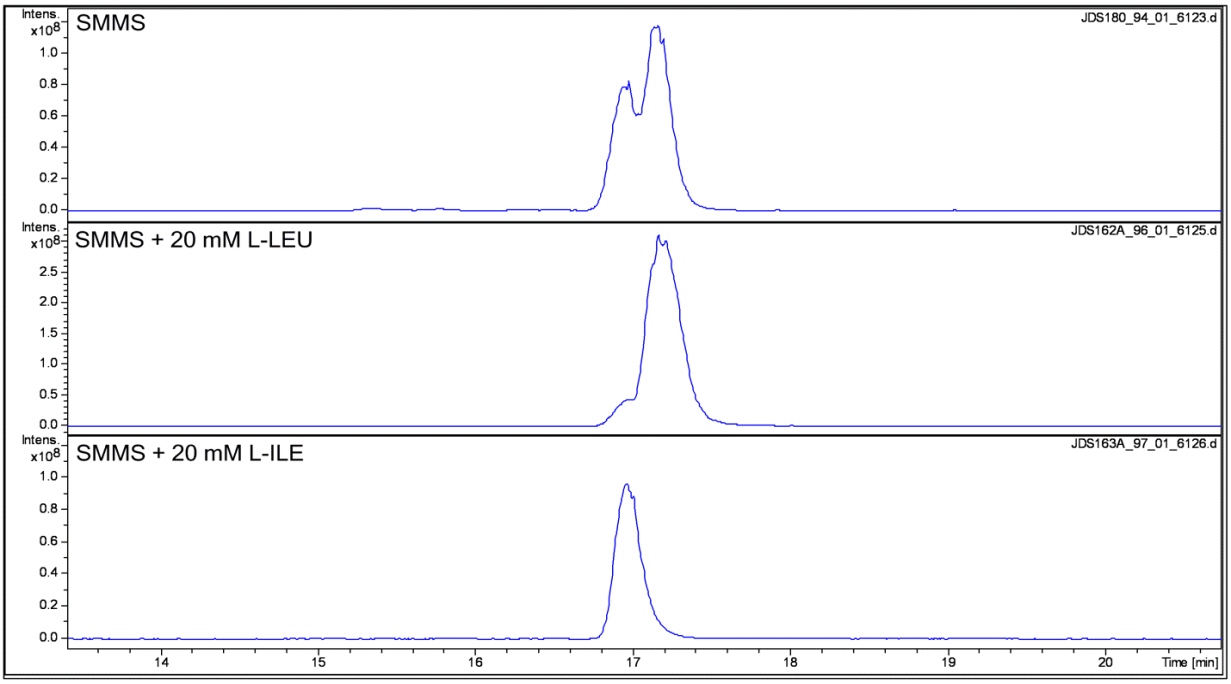


Figure S5. Extracted ion chromatogram for *m/z* = 261, corresponding to gaburedins B and C, from LC-MS analysis of the metabolites extracted at pH 3 from the *S. venezuelae gbnR::apra* strain when grown on SMMS for 3 days upon addition of 20 mM L-leucine or L-isoleucine to the media. The extracts were diluted by a factor of 10 using 50:50 HPLC water/methanol in order to achieve better resolution of the peaks.

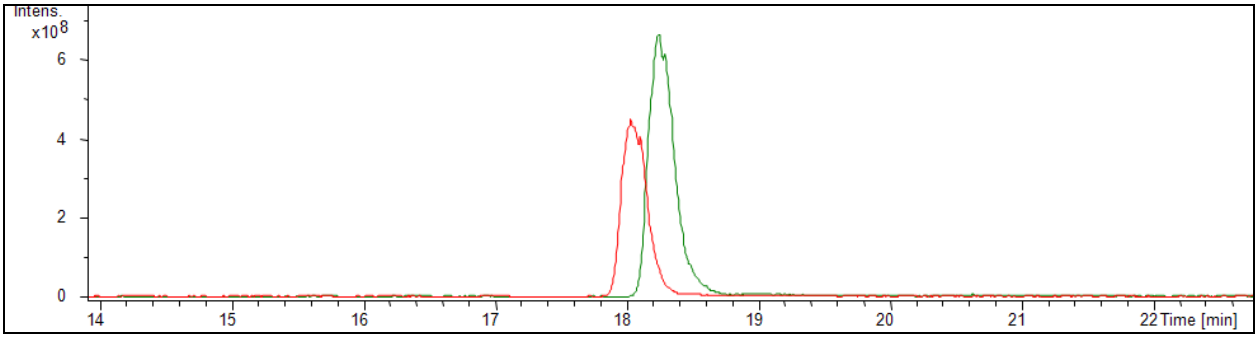


Figure S6. Extracted ion chromatogram for *m/z* = 271.0, corresponding to d_{10}-gaburedin B produced (green trace) and d_{10}-gaburedin C (red trace), from LC-MS analysis of the metabolites extracted at pH 3 from the *S. venezuelae gbnR::apra* strain when grown on SMMS for 3 days upon addition of 10 mM d_{10}-leucine (green trace) or d_{10}-isoleucine (red trace) to the media.

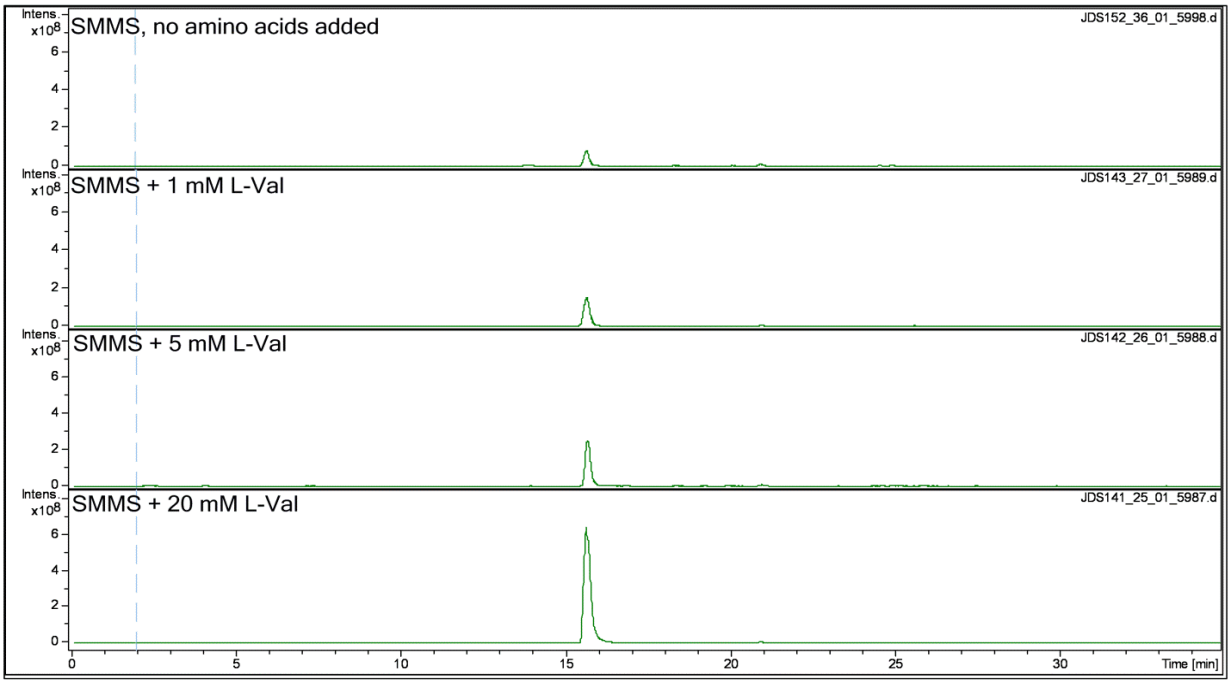
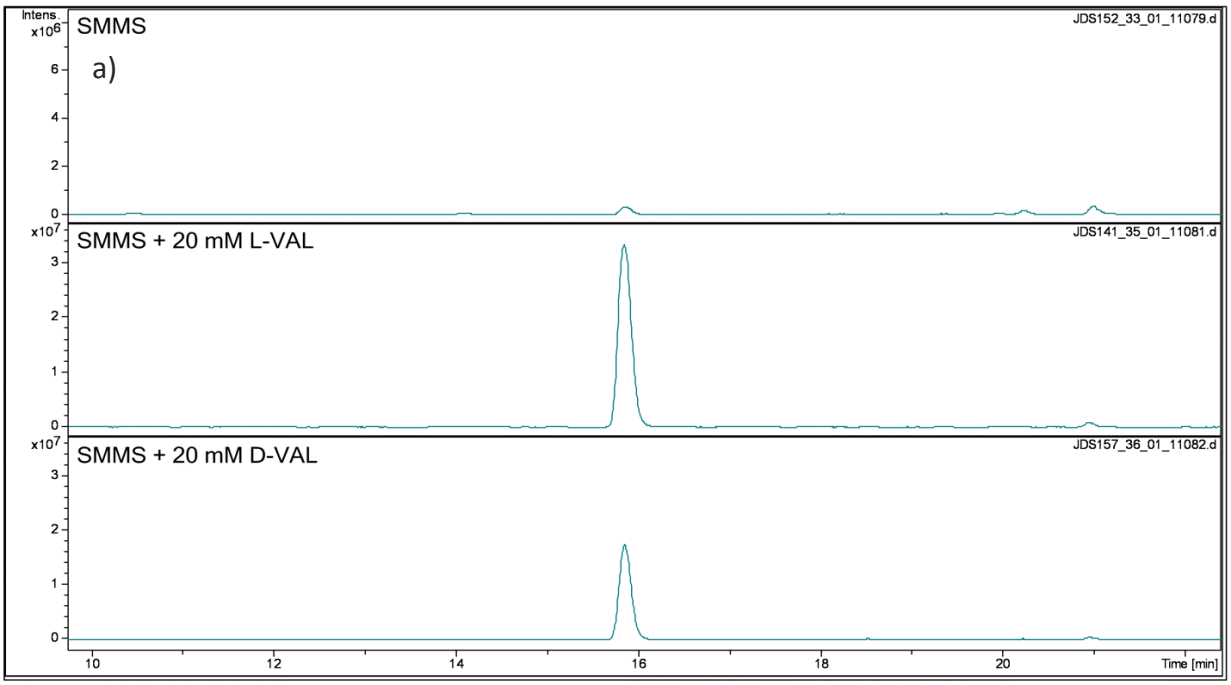


Figure S7. Extracted ion chromatogram for *m/z* = 247, corresponding to gaburedin D, from LC-MS analysis of the metabolites extracted at pH 3 from the *S. venezuelae gbnR::apra* strain when grown on SMMS for 3 days when different concentrations of L-valine were added to the media.



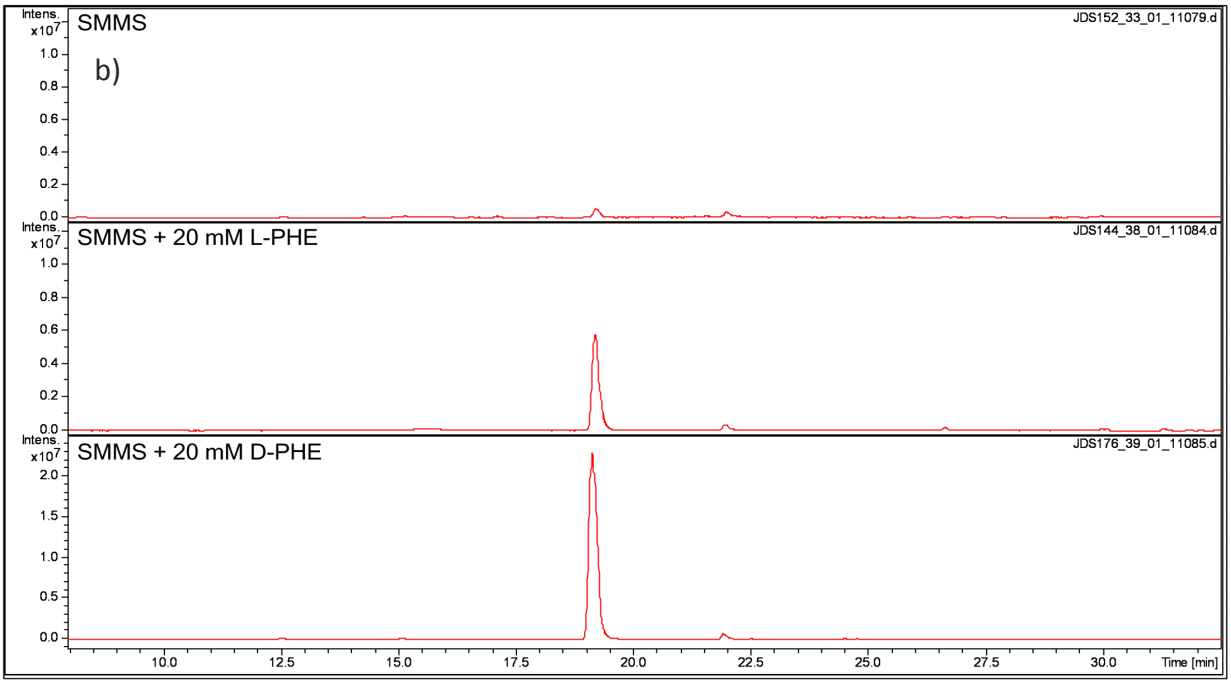


Figure S8. Extracted ion chromatograms for *m/z* = 247, corresponding to gaburedin D, from LC-MS analysis of the metabolites extracted at pH 3 from the *S. venezuelae gbnR::apra* strain when grown on SMMS for 3 days when 20 mM L- and D-valine were added to the media (panel **a**), and for *m/z* = 295, corresponding to gaburedin A, when 20 mM L- and D-phenylalanine were added (panel **b**). Production of gaburedin D is increased upon addition of either L- or D-valine to the media, and production of gaburedin A is increased upon addition L- or D-phenylalanine to the media.

Table S4: Gaburedin analogues produced by the *gbnR::apr* mutant when the medium is supplemented with diverse precursors

Precursor fed	*Observed m/z for $[M+H]^+$ of gaburedins*	*Gaburedin analogues*
D-phenylalanine	294.9	Enantiomer of gaburedin A
D-valine	247.1	Enantiomer of gaburedin D
d_8-DL-valine	255.1802 ($C_{10}H_{11}D_8N_2O_5$)	d_8-gaburedin D and d_8-enantiomer
L-glutamate	276.9	Gaburedin G
GABA	233.1134 ($C_9H_{17}N_2O_5$)	Gaburedin H
Isobutylamine	203.1391 ($C_9H_{18}N_2O_3$)	Gaburedin I
L-tyrosine	311.1235 ($C_{14}H_{19}N_2O_6$)	Gaburedin J
L-tryptophan	334.1401 ($C_{16}H_{20}N_3O_5$)	Gaburedin K
Glycine	205.0824 ($C_7H_{13}N_2O_5$)	Gaburedin L
L-alanine	218.8	Gaburedin M
L-proline	244.9	Gaburedin N
L-serine	235.0928 ($C_8H_{15}N_2O_6$)	Gaburedin O
L-threonine	249.1072 ($C_9H_{17}N_2O_6$)	Gaburedin P
L-lysine	-	gaburedin production decreased
L-cysteine	-	gaburedin production abolished
L-phenylalaninol	-	gaburedin production abolished
L-valinol	-	gaburedin production abolished

m/z values given to four decimal places and molecular formulae are given for samples analyzed by UHR-LC-MS. New species generated by the *gbnR::apr* strain upon addition of these amines to the media show the same loss of 129 Da upon in-source fragmentation that is observed in the gaburedins isolated from the *gbnR::apr* mutant grown on unmodified SMMS.

6 Mass spectrometry data

6.1 HRMS for gaburedins A-E and gaburedin analogues G-P

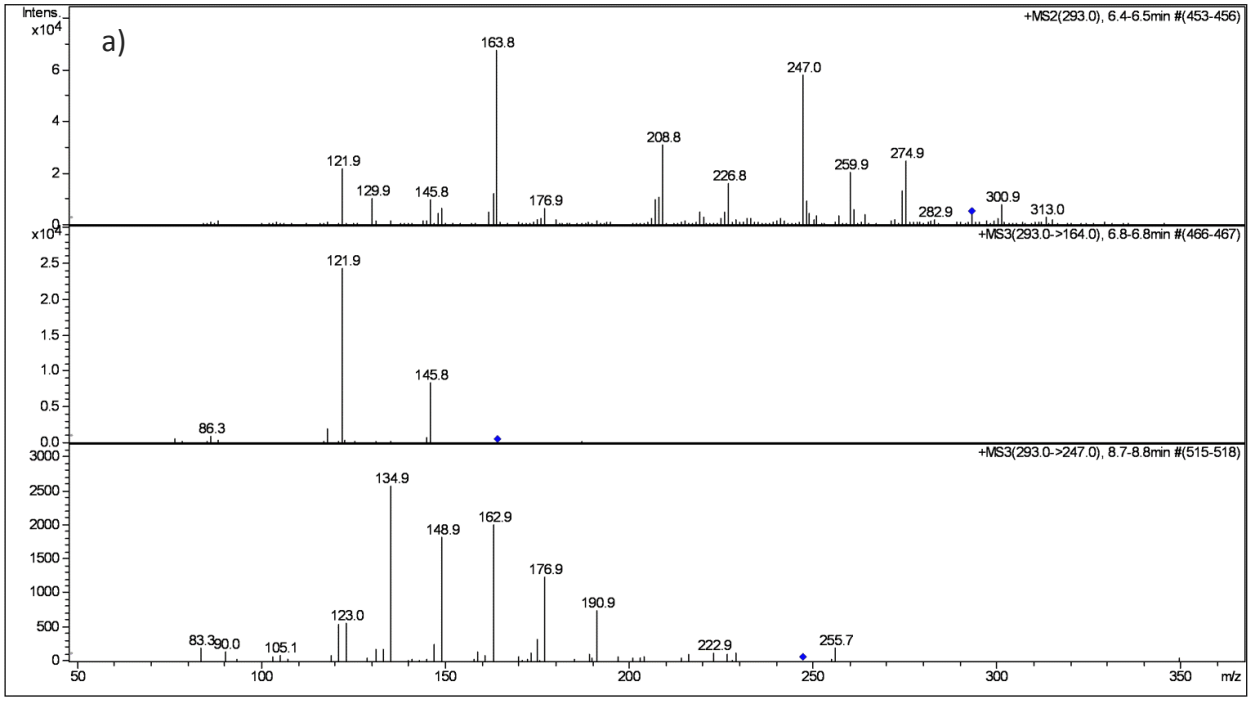
Table S5: UHR-MS errors and assignments of fragments observed for gaburedins and analogues

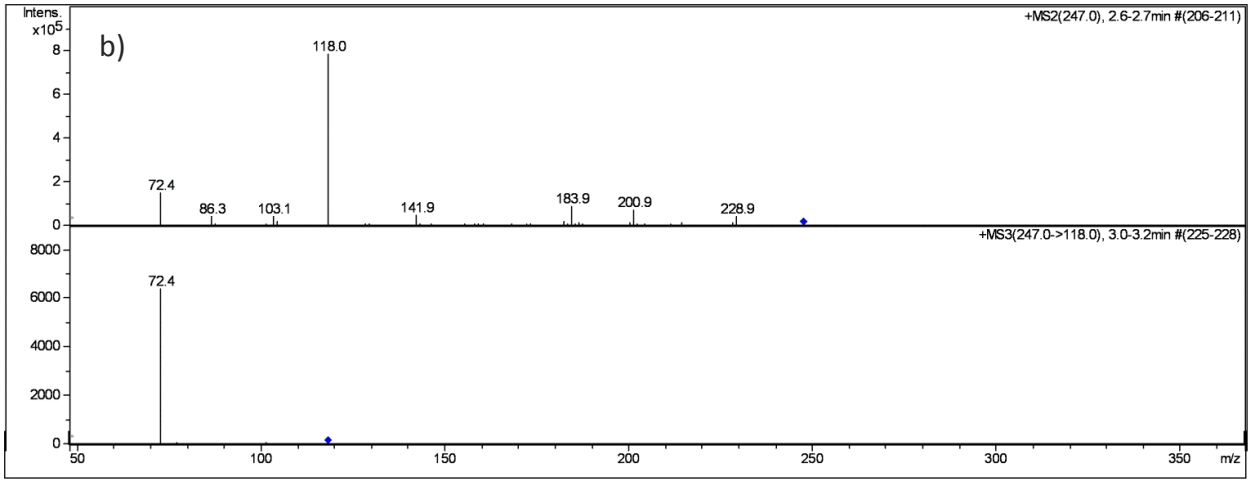
compound	Molecular formula	*Calculated* m/z	*Observed* m/z	*error / ppm*	*assignment*
Gaburedin A	**$C_{14}H_{19}N_2O_5$**	295.1288	295.1287	0.4	$[M+H]^+$
	$C_{14}H_{18}N_2O_5Na$	317.1108	317.1105	0.9	$[M+Na]^+$
	$C_9H_{12}NO_2$	166.0863	166.0864	-0.6	Phe
Gaburedin B/C	**$C_{11}H_{21}N_2O_5$**	261.1445	261.1440	1.8	$[M+H]^+$
	$C_{11}H_{20}N_2O_5Na$	283.1264	283.1259	1.9	$[M+Na]^+$
	$C_6H_{14}NO_2$	132.1019	132.1018	0.7	Ile/Leu
Gaburedin D	**$C_{10}H_{19}N_2O_5$**	247.1288	247.1291	-1.0	$[M+H]^+$
	$C_{10}H_{18}N_2O_5Na$	269.1108	269.1108	0.1	$[M+Na]^+$
	$C_5H_{12}NO_2$	118.0863	118.0867	-4.2	Val
Gaburedin E	**$C_{10}H_{17}N_2O_6S$**	293.0802	293.0800	0.8	$[M+H]^+$
	$C_{10}H_{16}N_2O_6SNa$	315.0621	315.0619	0.7	$[M+Na]^+$
	$C_5H_{10}NO_3S$	164.0376	164.0374	0.9	acetyl-Cys
Gaburedin F	**$C_{10}H_{19}N_2O_5S$**	279.1009	279.1017	-2.7	$[M+H]^+$
	$C_{10}H_{18}N_2O_5SNa$	301.0829	301.0834	-1.7	$[M+Na]^+$
	$C_5H_{12}NO_2S$	150.0583	150.0588	-3.0	Met
d_8-DL-Gaburedin D	**$C_{10}H_{11}D_8N_2O_5$**	255.1791	255.1802	-4.5	$[M+H]^+$
	$C_{10}H_{10}D_8N_2O_5Na$	277.1610	277.1617	-2.4	$[M+Na]^+$
	$C_5H_4D_8NO_2$	126.1365	126.1372	-6.0	d_8-Val
Gaburedin H	**$C_9H_{17}N_2O_5$**	233.1132	233.1134	-0.9	$[M+H]^+$
	$C_9H_{16}N_2O_5Na$	255.0951	255.0949	0.8	$[M+Na]^+$
	$C_4H_{10}NO_2$	104.0706	104.0714	-8.1	GABA
Gaburedin I	**$C_9H_{19}N_2O_3$**	203.1390	203.1391	-0.4	$[M+H]^+$
	$C_9H_{18}N_2O_3Na$	225.1210	225.1202	3.5	$[M+Na]^+$
	$C_4H_{12}N$	-	-	-	isobutylamine
Gaburedin J	**$C_{14}H_{19}N_2O_6$**	311.1238	311.1235	0.7	$[M+H]^+$
	$C_{14}H_{18}N_2O_6Na$	333.1057	333.1043	4.1	$[M+Na]^+$
	$C_9H_{12}NO_3$	182.0812	182.0806	3.4	Tyr
Gaburedin K	**$C_{16}H_{20}N_3O_5$**	334.1397	334.1401	-1.0	$[M+H]^+$
	$C_{16}H_{19}N_3O_5Na$	356.1193	356.1209	-4.5	$[M+Na]^+$
	$C_{11}H_{13}N_2O_2$	205.0972	205.0968	1.8	Trp
Gaburedin L	**$C_7H_{13}N_2O_5$**	205.0819	205.0824	-2.2	$[M+H]^+$
	$C_7H_{12}N_2O_5Na$	227.0638	227.0642	-1.6	$[M+Na]^+$
	$C_2H_6N_2O_5$	-	-	-	Gly
Gaburedin O	**$C_8H_{15}N_2O_6$**	235.0925	235.0928	-1.3	$[M+H]^+$
	$C_8H_{14}N_2O_6Na$	257.0744	257.0747	-1.2	$[M+Na]^+$
	$C_3H_8NO_3$	106.0499	106.0506	-6.8	Ser
Gaburedin P	**$C_9H_{17}N_2O_6$**	249.1081	249.1072	3.7	$[M+H]^+$
	$C_9H_{16}N_2O_6Na$	271.0901	271.0890	4.1	$[M+Na]^+$
	$C_4H_{10}NO_3$	120.0655	120.0651	3.7	Thr

6.2 MS/MS fragmentation patterns observed for purified gaburedins A-E

For samples analyzed in positive ion mode, fractions were taken directly from the HPLC and injected into a Bruker High Capacity Trap (HCT) + ion trap mass spectrometer with an electrospray source, and ions isolated in the high capacity trap. For samples analyzed in negative ion mode, NH_4OH was added to the samples before use to a final concentration of 0.01% NH_4OH.

Positive ion mode: $[M+H]^+$ ions





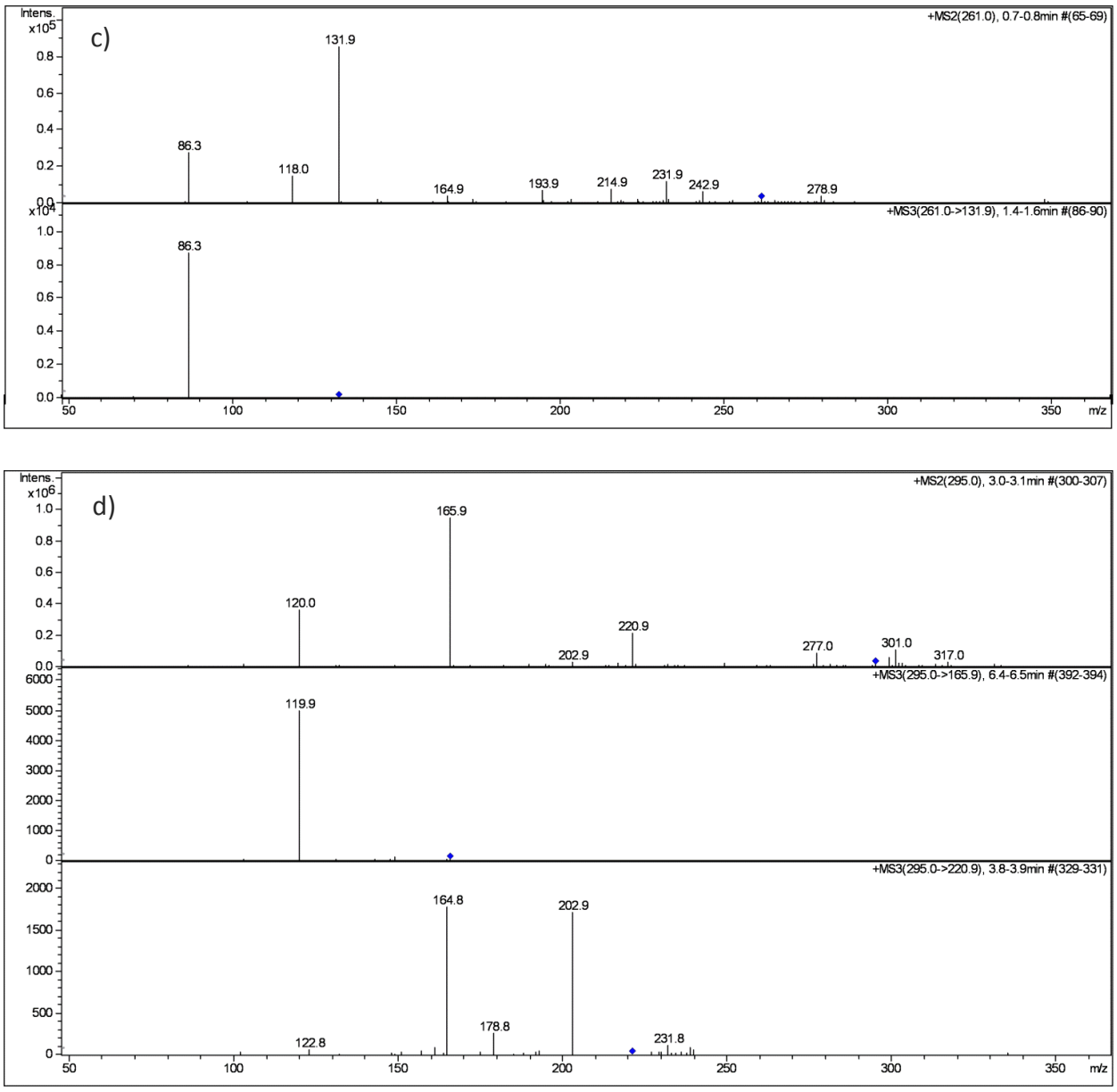
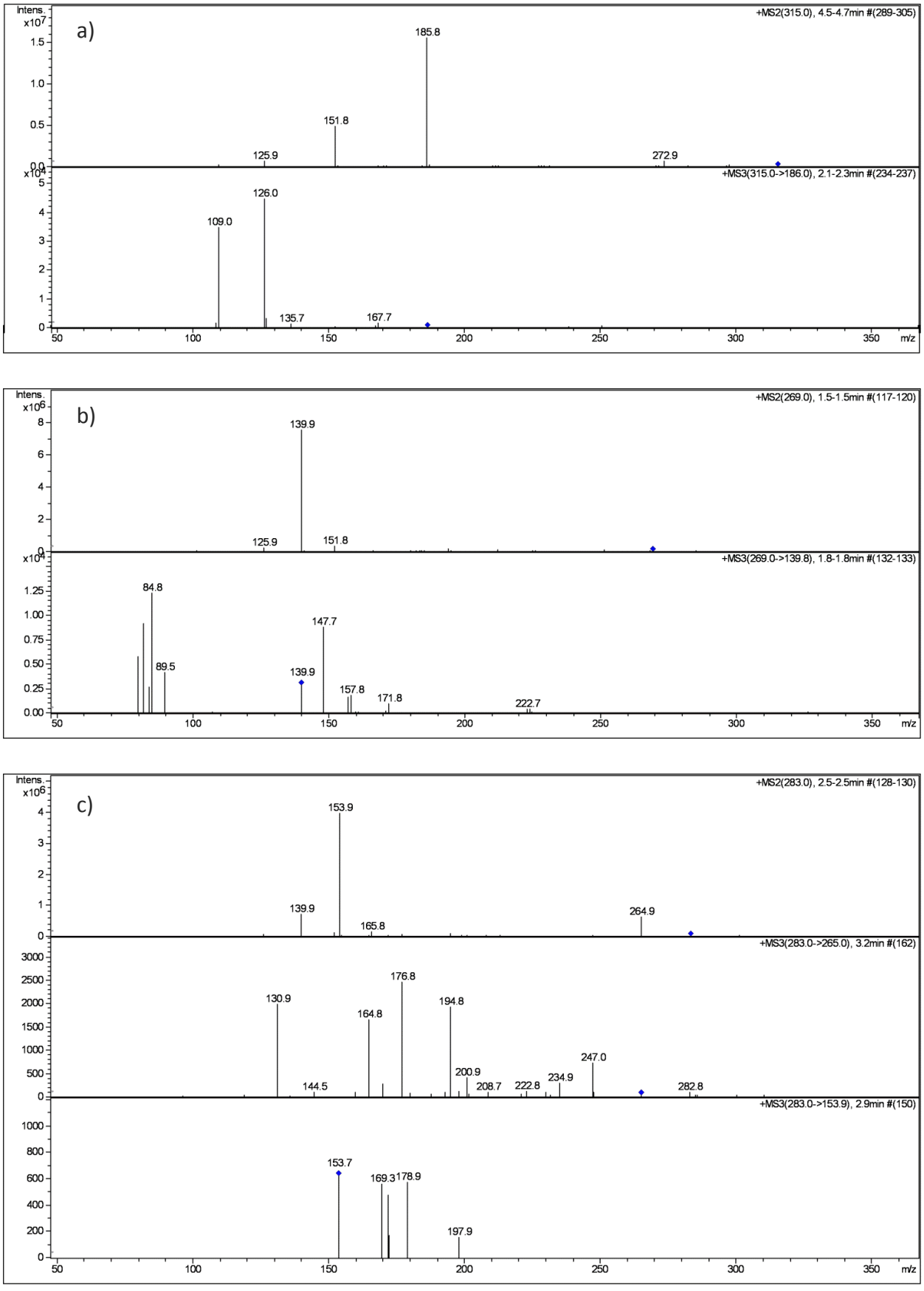


Figure S9. Tandem mass spectra for the $[M+H]^+$ ions of isolated gaburedins E (panel **a**), D (panel **b**), B/C (panel **c**) and A (panel **d**). The same mass loss of 129 Da and further mass loss of 46 Da for gaburedins A-D are observed, whilst gaburedin E loses the same 129 Da fragment, followed by a 42 Da fragment. The *m/z* values for these fragments are in agreement with ions assigned in the high resolution mass spectra shown in Table S4.

Positive ion mode: $[M+Na]^+$ adducts



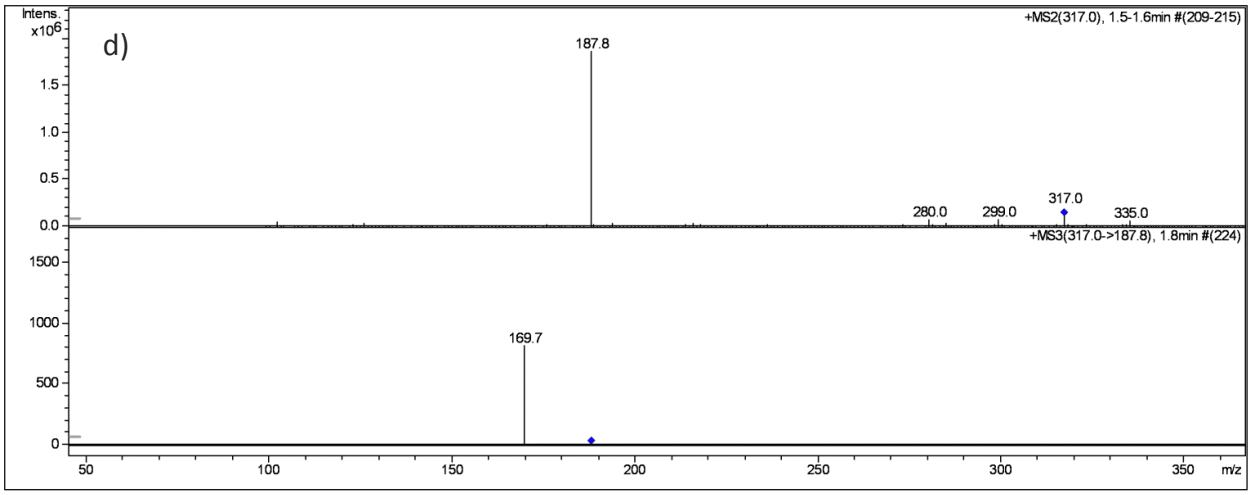
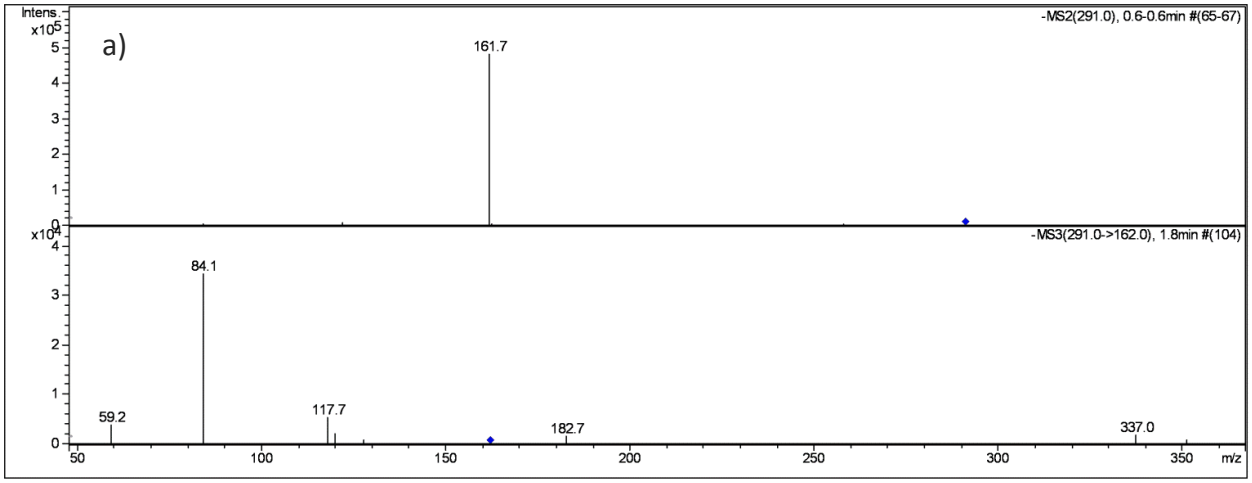


Figure S10. Fragmentation pattern of the $[M+Na]^+$ adducts of gaburedins E (panel **a**), D (panel **b**), B/C (panel **c**) and A (panel **d**). The same mass loss of 129 Da for gaburedins A-E are observed. The *m/z* values for these observed ions are in agreement with ions assigned in the high resolution mass spectra shown in Table S4.

Negative ion mode: $[M-H]^-$ ions



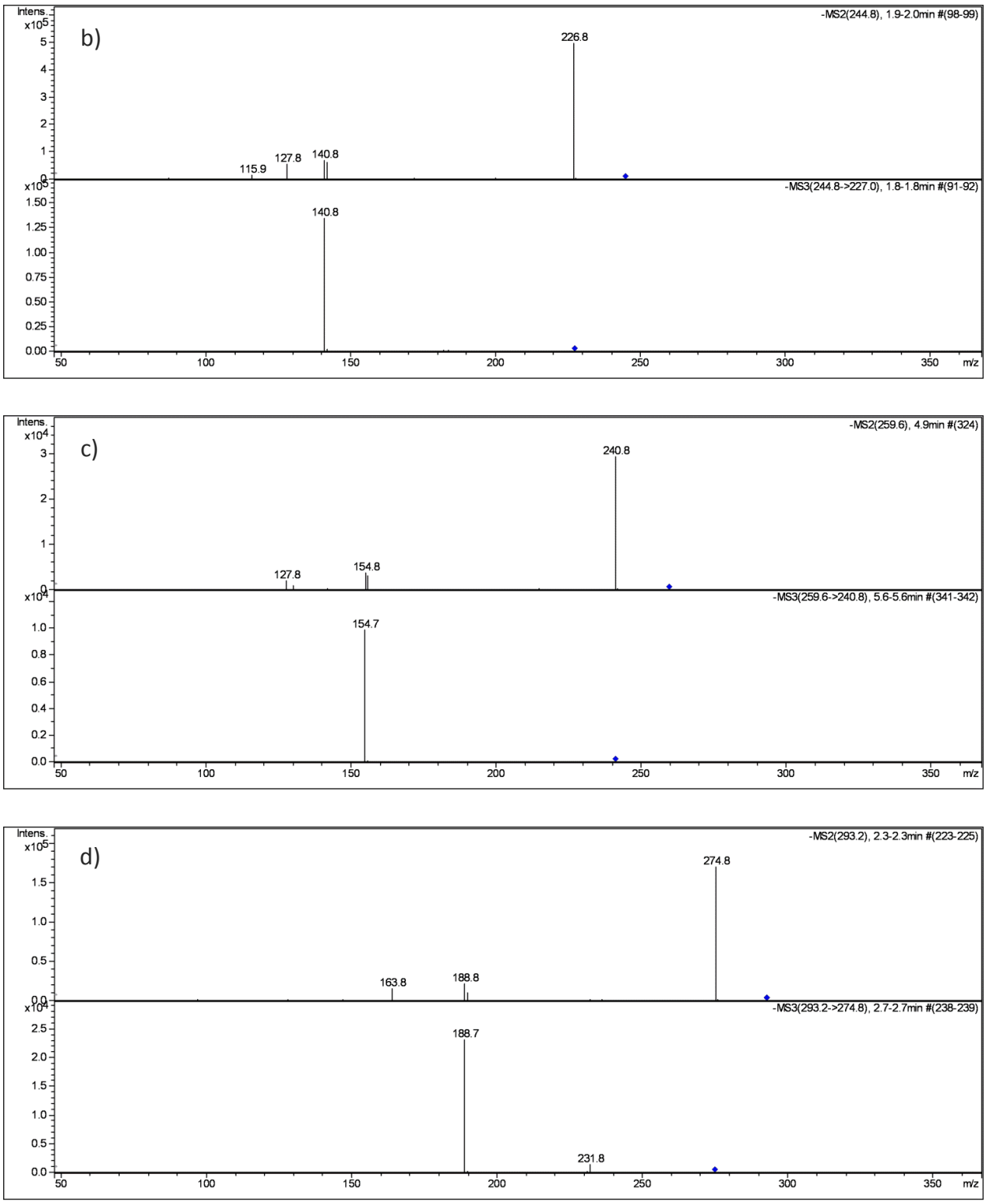


Figure S11. Fragmentation pattern of the $[M–H]^-$ ion of gaburedins E (panel **a**), D (panel **b**), B/C (panel **c**) and A (panel **d**). The same loss of a 18 Da fragment for gaburedins A-D is observed, followed by loss of a 86 Da, whilst gaburedin E loses a129 Da fragment, and a subsequent 76 Da fragment.

7 NMR spectroscopy

7.1 NMR characterization of gaburedin A isolated from culture extract

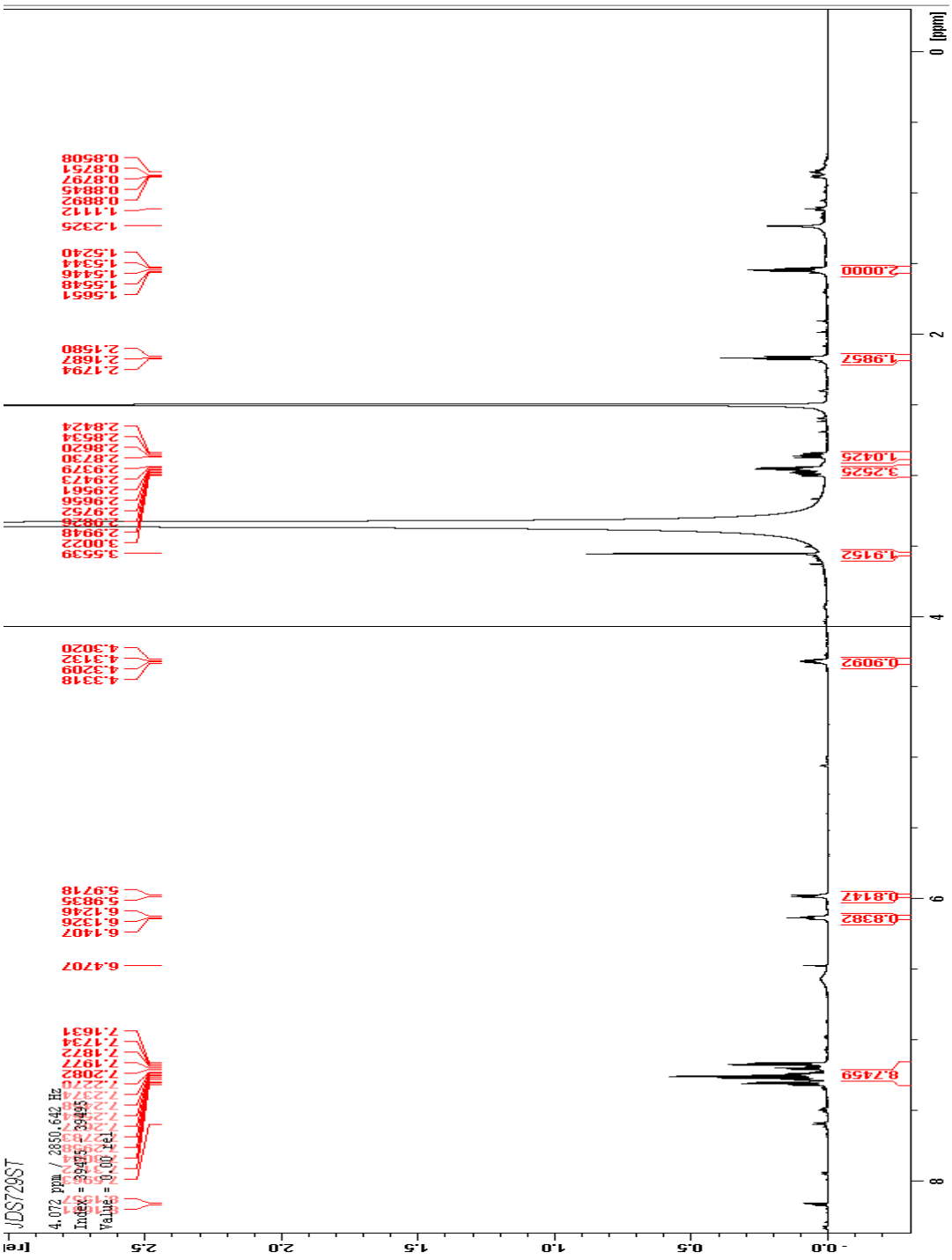


Figure S12. 400 MHz ^{1}H NMR spectrum of gaburedin A in d_6-DMSO

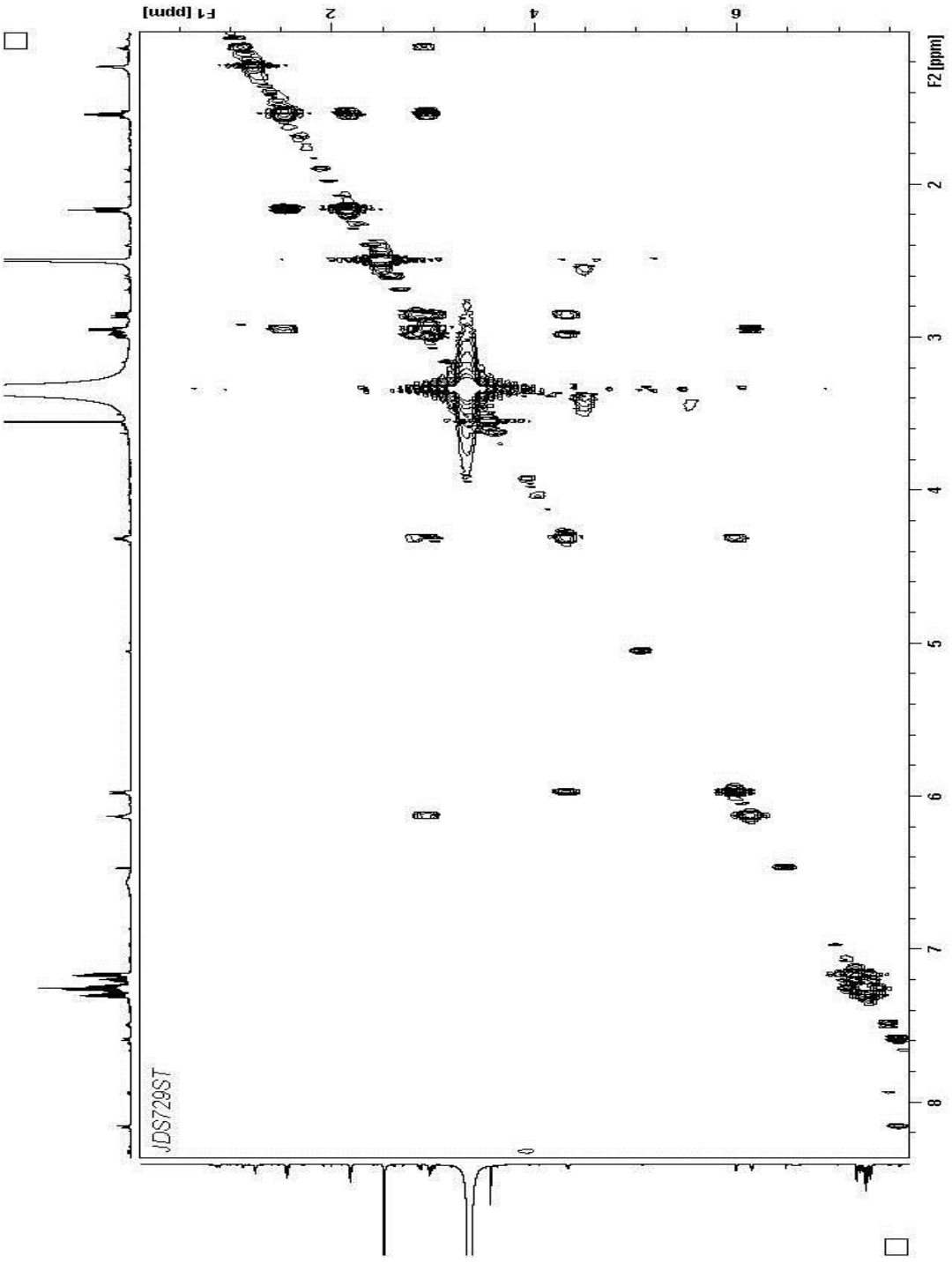


Figure S13. 400 MHz COSY spectrum of gaburedin **6** in d_6-DMSO

7.2 NMR characterization of synthetic standard of gaburedin A

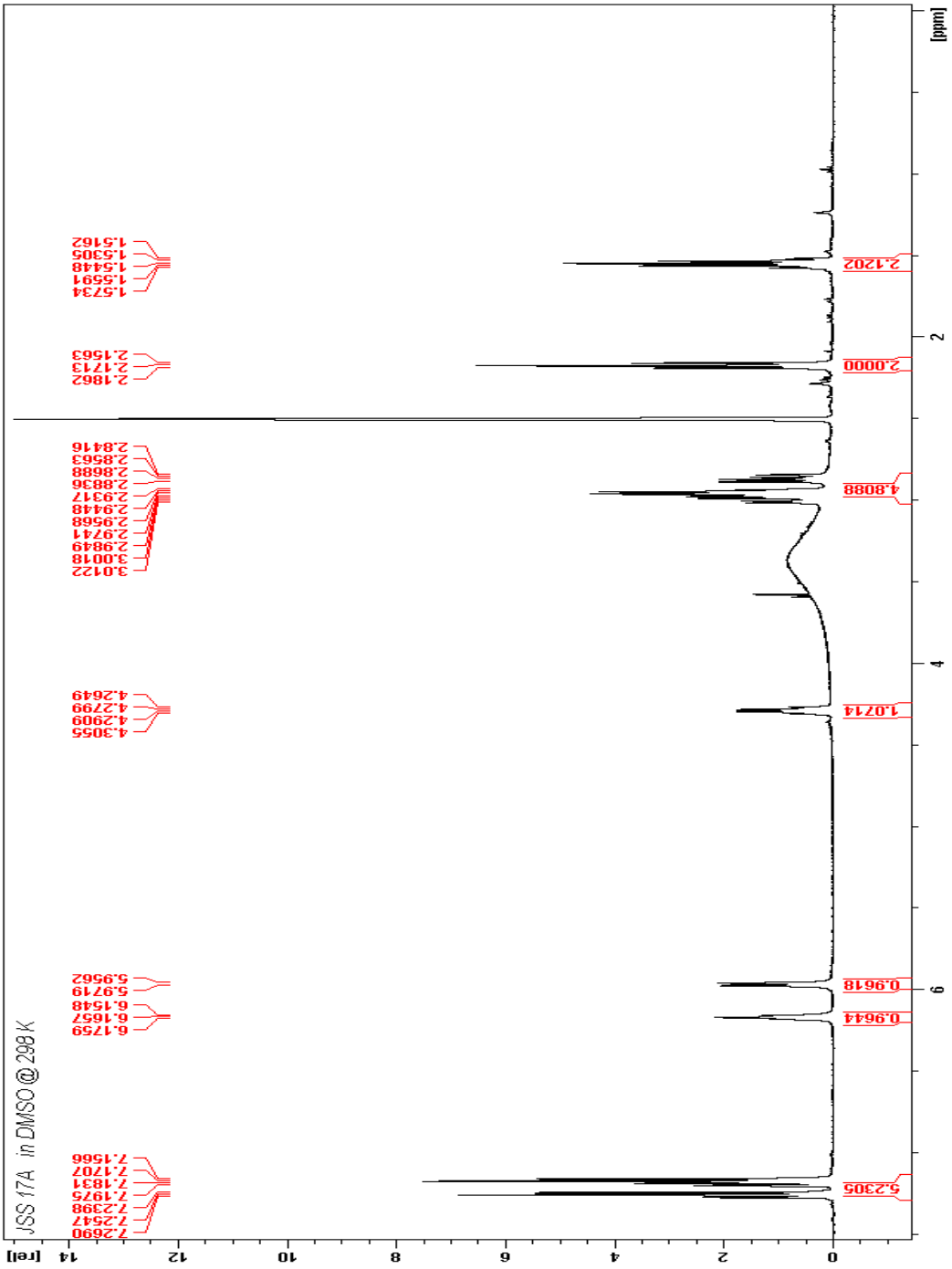


Figure S14. 500 MHz ^{1}H NMR spectrum of authentic standard of gaburedin A in d_6-DMSO

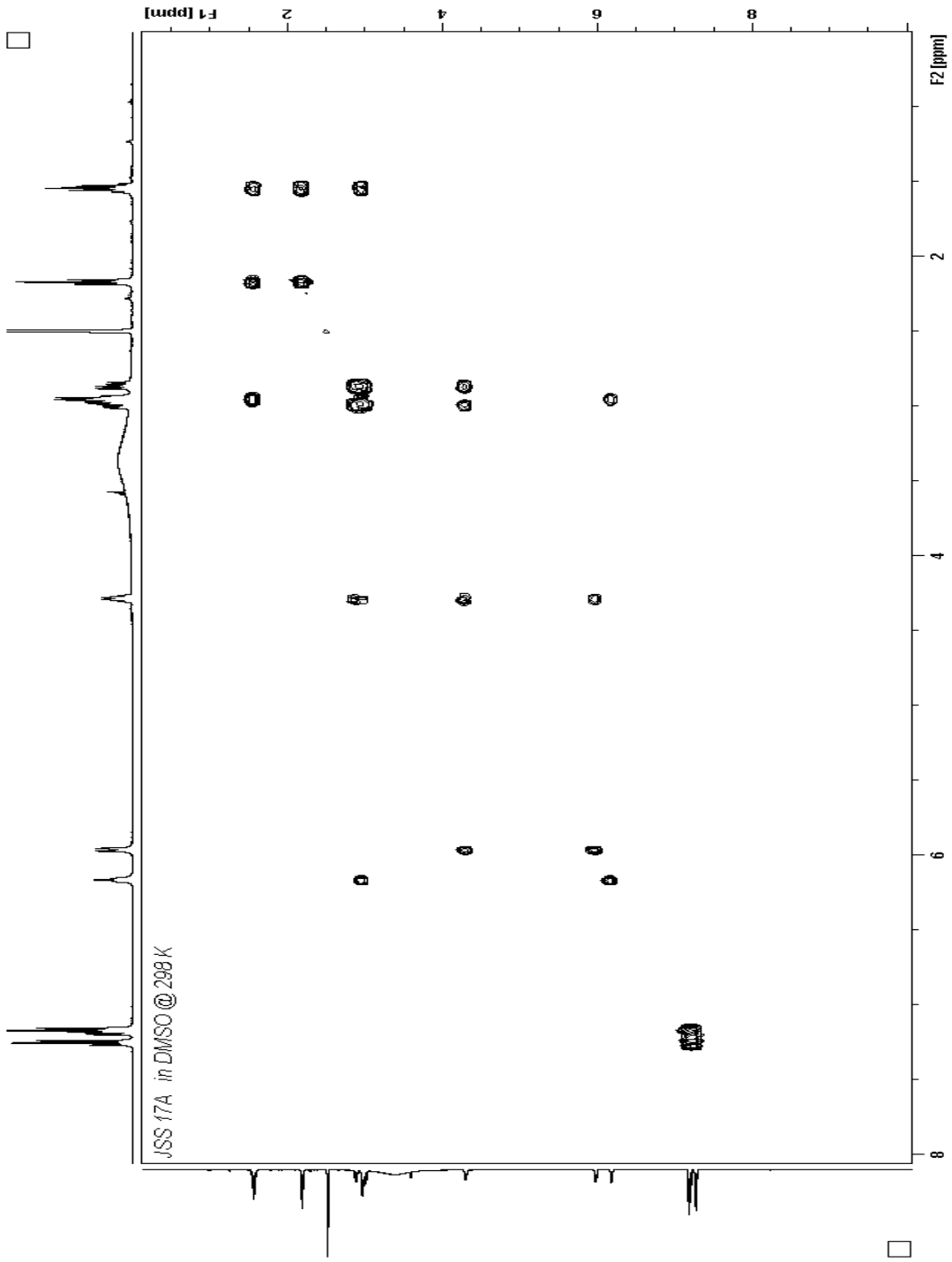


Figure S15. 500 MHz COSY spectrum of authentic standard of gaburedin A in d_6-DMSO

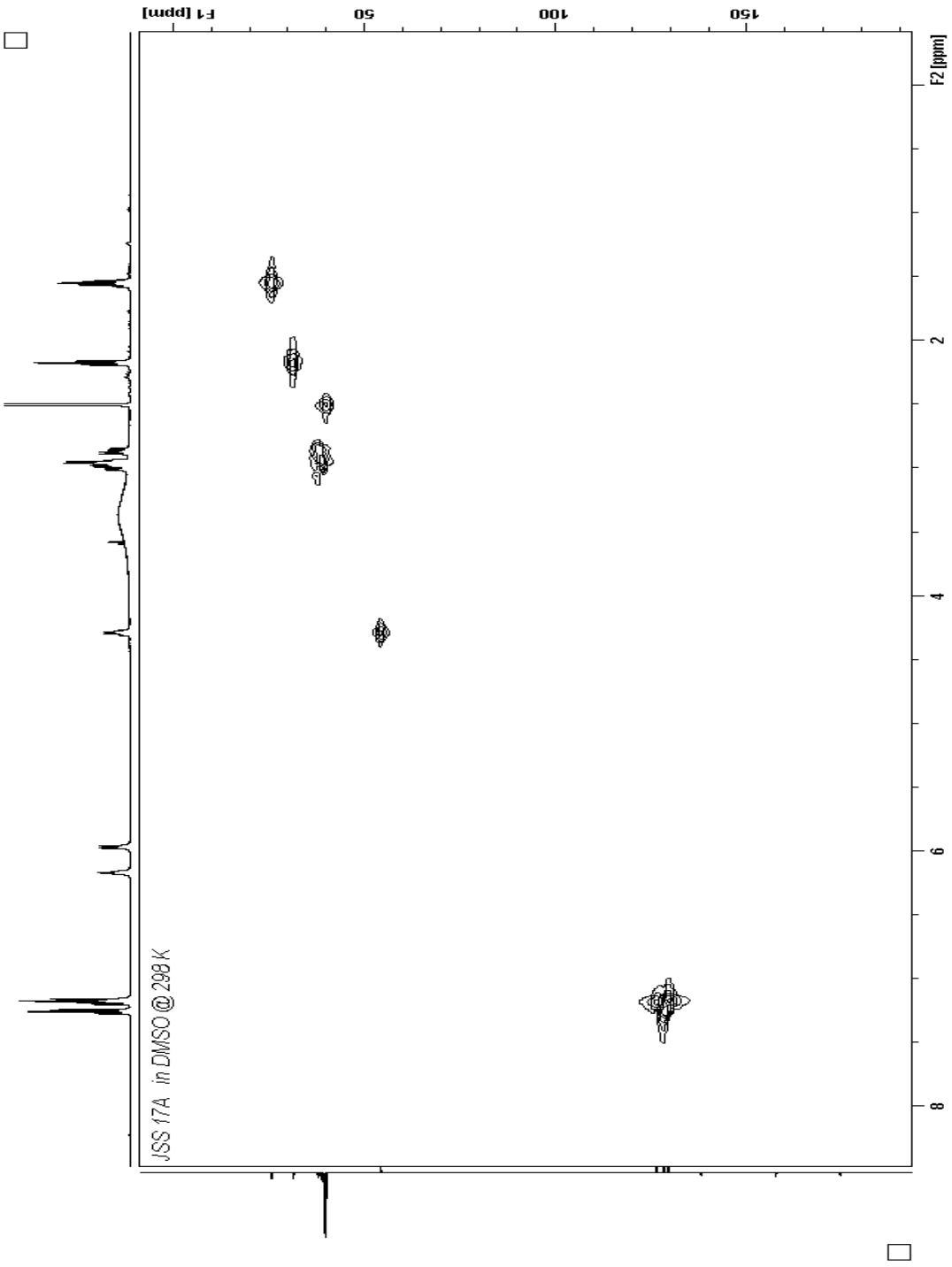


Figure S16. 500 MHz HMQC spectrum of authentic standard of gaburedin A in d_6-DMSO

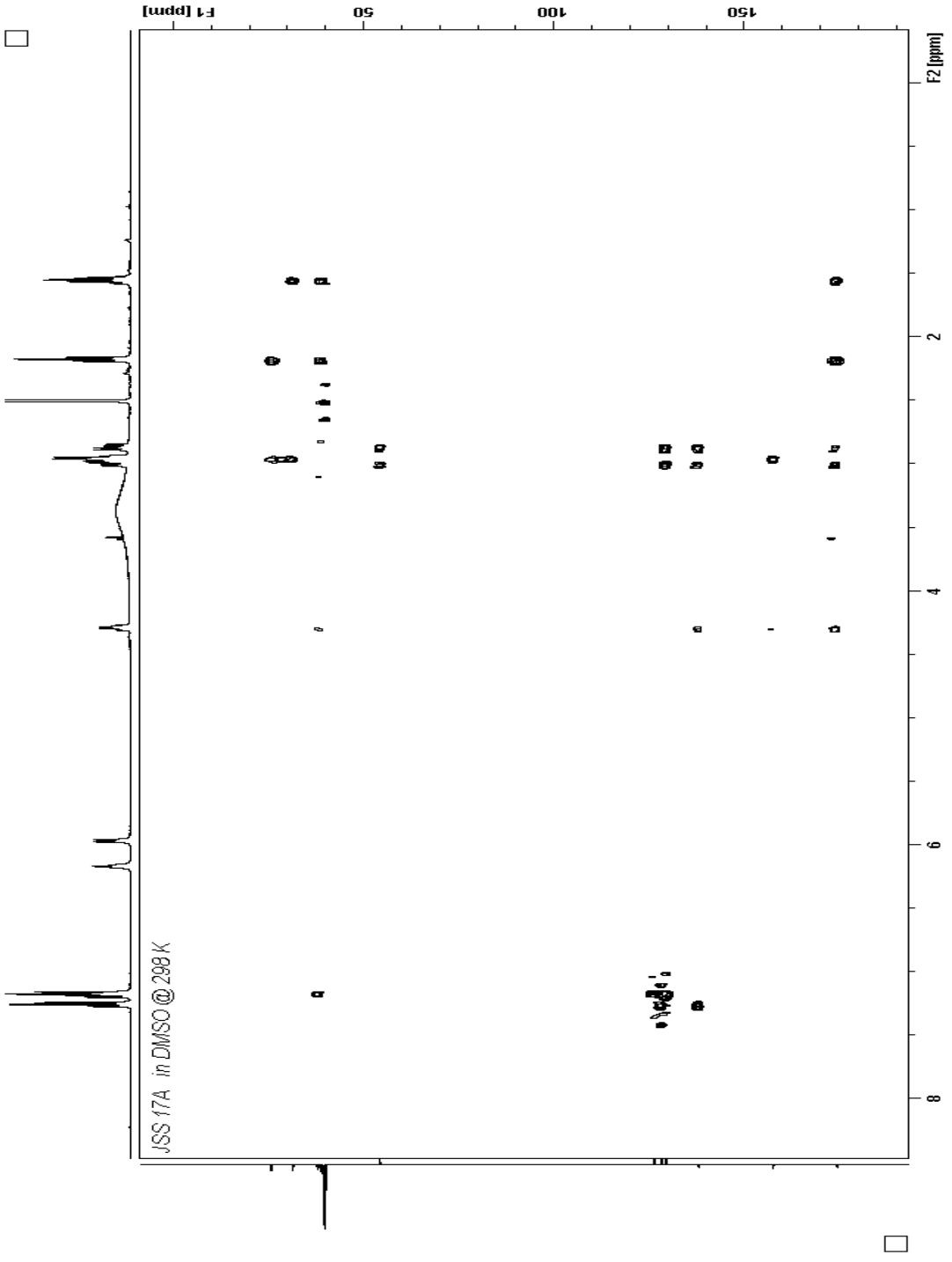


Figure S17. 500 MHz HMBC spectrum of authentic standard of gaburedin A in d_6-DMSO

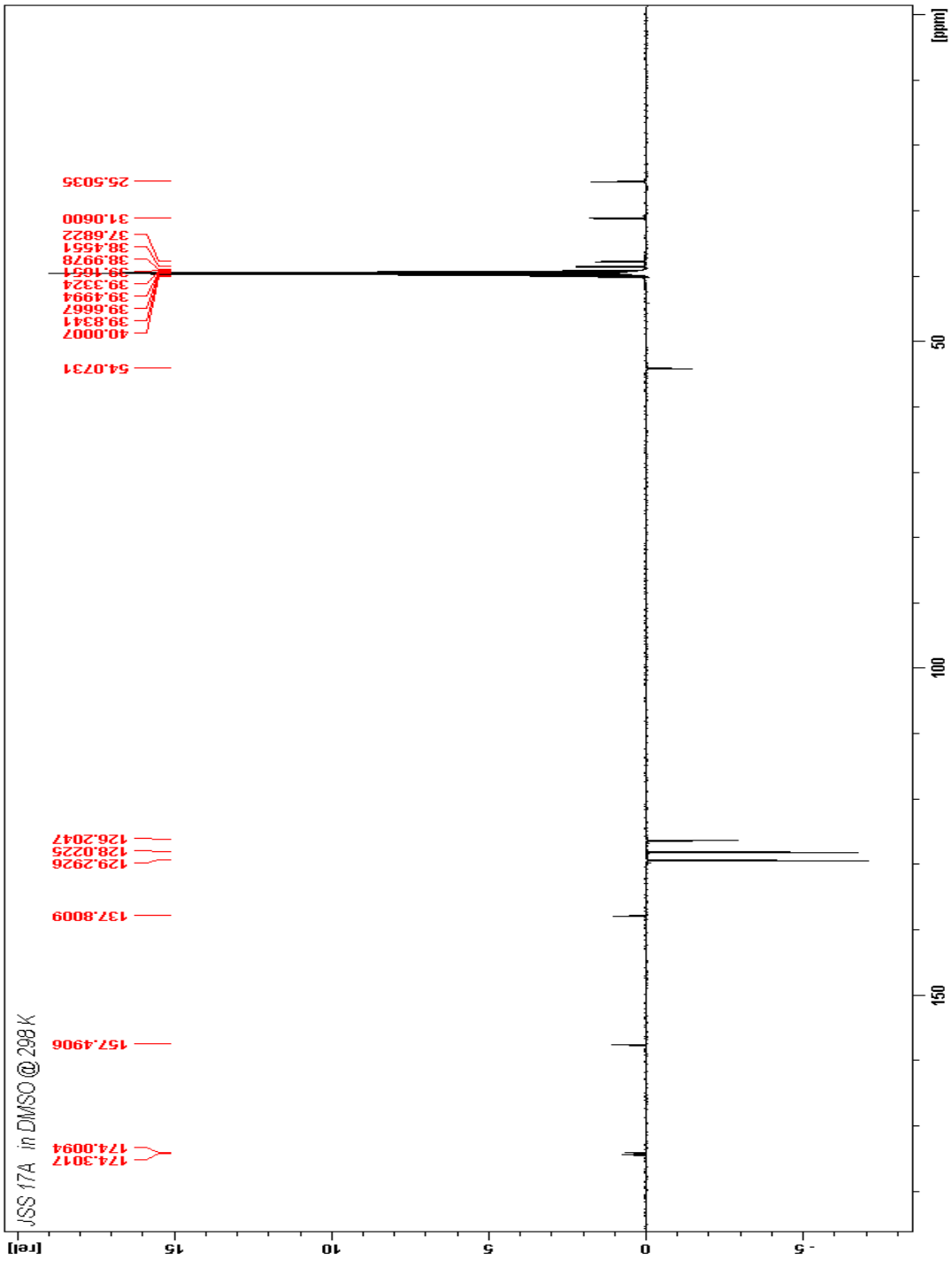


Figure S18. 500 MHz PENDANT spectrum of authentic standard of gaburedin A in d_6-DMSO

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