SUPPORTING INFORMATION

Interkingdom Genetic Mix-and-Match to Produce Novel Sunscreens

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Supporting References

SupportingTables

Table S1. The *Rhodococcus fascians* D188 MAA operon.

Gene name	Locus	Function
mysA	a3L23_Rs04425	2-*O*-desmethyl-4-deoxygadusol synthase
mysB	a3L23_Rs04430	*O*-methyltransferase
mysC	a3L23_Rs04435	ATP-Grasp ligase-like
mysD	a3L23_Rs04440	D-Ala D-Ala ligase-like

Table S2. Bacterial strains used in this study.

Strains	Relevant genotype/comments	Source/Ref
Escherichia coli DH10B	*F⁻ mcrA Δ(mrr-hsdRMS-mcrBC)ϕ80lacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara, leu)7697 galU galK λ⁻ rspL nupG*	GibcoBRL
E. coli ET12567(pUZ8002)	*dam dcm hsdS,* pUZ8002	[1]
Rhodococcus fascians D188	Wild-type strain	[2]
Streptomyces coelicolor M1152	*Δact Δred Δcpk Δcda rpoB[*C1298T*]*	[3]
S. coelicolor pTMAO-RF	*S. coelicolor* M1152 plus pTMAO-RF	This study
S. coelicolor pTMAO-G1	*S. coelicolor* M1152 plus pTMAO-G1	This study
S. coelicolor pTMAO-G2	*S. coelicolor* M1152 plus pTMAO-G2	This study
S. coelicolor pTMAO-G3	*S. coelicolor* M1152 plus pTMAO-G3	This study

Table S3. Plasmids used in this study.

Plasmid	Description	Source/Ref
pBlueScript II SK(-)	ColE1-based phagemid vector with f1 (-) and pUC origins, T3, T7 and lac promoters; *bla*.	Stratagene
pRSET B	N- terminal his-tag, T7-promoter	Novagen
pRSET B-*valA*	pRSET B containing the gene *valA* from *S. hygroscopicus*	[4]
pXY201-eGFP	Multicopy *Streptomyces* expression vector containing eGFP, thiostrepton promoter, N-terminal His tag, Amp, Apr, ColE1, *oriT*	Ref[5] and this study
pTMAO-RF	pXY201 containing the *R. fascians* MAA operon	This study
pTMAO-G1	pXY201 containing synthetic operon of *valA* and *drMT-Ox*	This study
pTMAO-G2	pXY201 containing synthetic operon of *valA, drMT-Ox,* and *mysC*	This study
pTMAO-G3	pXY201 containing synthetic operon of *valA, drMT-Ox, mysC*, and *mysD*	This study

Table S4. Primers used in this study.

Primer	5´-Sequence*-3´
pRSET-B FWD	AGAAAGCTTGATCCGGCTGCTAA
pRSET-B REV	CATCCGACCCATTTGCTGTCCAC
MysA-pRSETB-FWD	GTGGACAGCAAATGGGTCGGATGAGTAATCTTCAGGCCCAGGTC
MysD-pRSETB-REV	GCCGGATCAAGCTTTCTAGATCATGACGACGACTCCTTCACC
T7leader-FWD	ATGGCTAGCATGACTGGTGGACAGCAAATGGGT
T7leader-REV	ACCCATTTGCTGTCCACCAGTCATGCTAGCCAT
eGFPpXY201-FWD	CTCAGAAGGGAGCGGACATACATGGTGAGCAAGGGCGAGGAGCTG
ValAcpcr-F	ATGACCATGACCAAGCAGAGTTCCTTATCCC
ValAcpcr-R	TCACACCCCCATGTCCACGGC
MTOxstrep-cpcr-F	ATGCAGACCGCGAAGGTCTCG
MTOxstrep-cpcr-R	TCACCAGAGGCTCTGCCCG
ValA-R-MT-Ox-assembly	CCGCTCCCTTCTCTCTAGATCACACCCCCATGTCCACGGC
p201egfp-dalalink-F	TGAAGGAGTCGTCGTCATGAGTGAGCAAGGGCGAGGAGCT
GadATP-F	TGACTCAGAAGGGAGCGGACATACATGATCCGCACCGTCGCGGC
GadDala-R	AGCTCCTCGCCCTTGCTCACTCATGACGACGACTCCTTCACCG
GadATP-R	AGCTCCTCGCCCTTGCTCACTCAGTCGCCTCCGTTTTCCACGAG
p201AddMTOX-F	AAGCTTCTCAGAAGGGAGCGGACATACATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGG

*nucleotides underlined refer to restriction sites

Supporting Figures

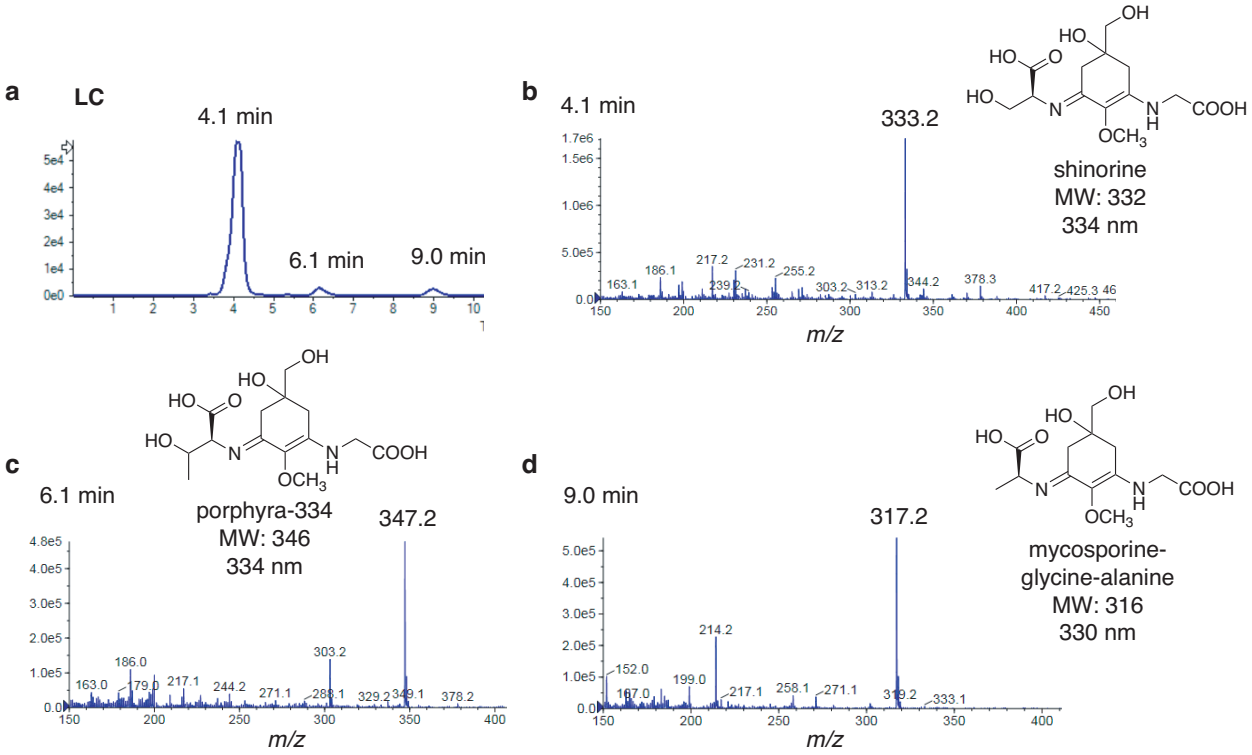

Figure S1. MAAs identified by LC-MS during purification of shinorine from *S. coelicolor.*

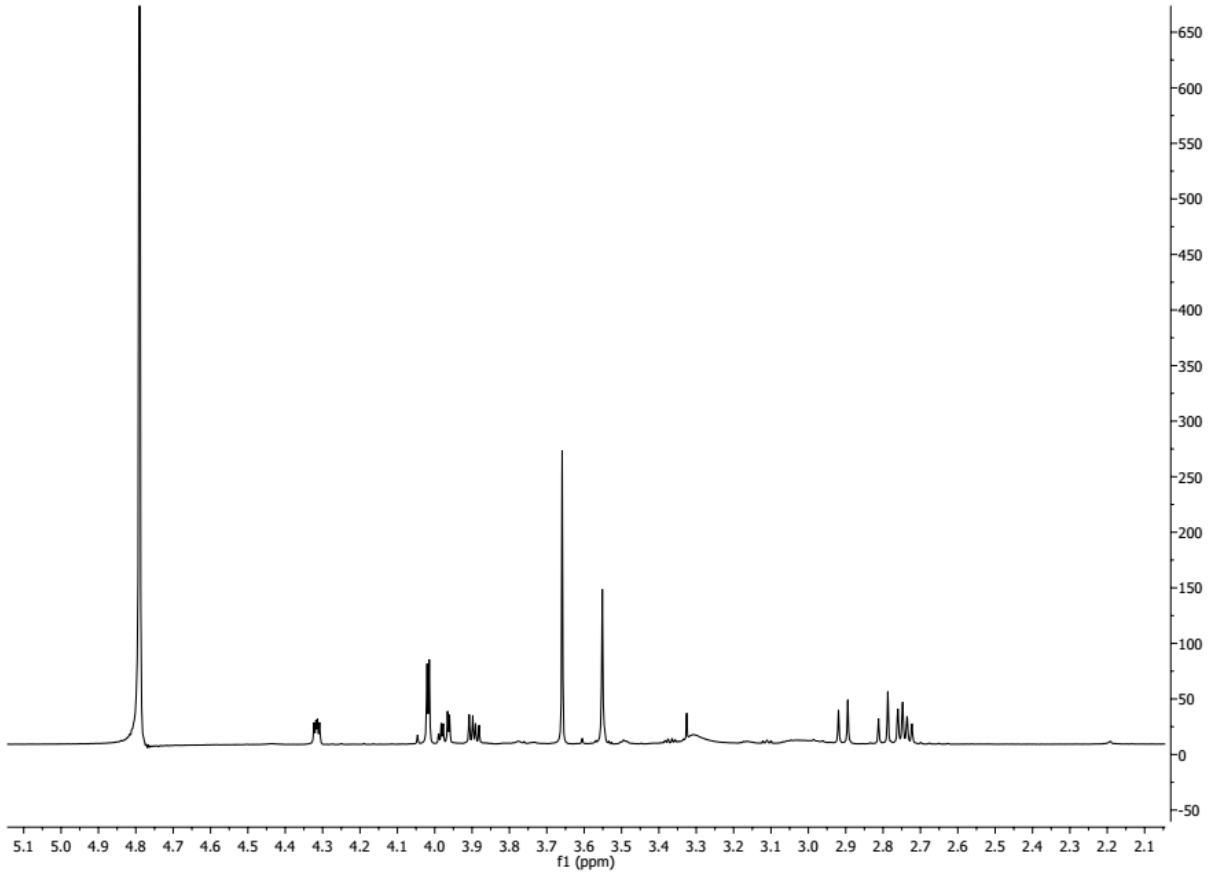

Figure S2. ^{1}H NMR spectrum of shinorine from the culture broth of pTMAO-RF.

ATGCAGACGGCCAAGGTCTCCGACACGCCCGTGGAATTCATCGTGGAACACCTGCTGAAG
GCGAAGGAAATCGCGGAAAACCACGCGTCGATCCCCGTGGAACTCCGCGACAACCTGCA
GAAGGCGCTCGACATCGCGTCGGGGCTCGACGAATACCTCGAACAGATGTCGAGCAAGG
AATCGGAACCCCTCACGGAACTCTACCGCAAGTCGGTGTCGCACGACTGGAACAAGGTGC
ACGCGGACGGGAAGACGCTCTTCCGGCTCCCCGTGACGTGTATCACGGGTCAGGTCGAA
GGGCAGGTGCTCAAGATGCTCGTGCACATGTCGAAGGCGAAGCGGGTGCTCGAAATCGG
AATGTTCACGGGTTACGGGGCGCTCAGCATGGCGGAAGCGCTCCCGGAAAACGGTCAGC
TCATCGCGTGTGAACTCGAACCGTACCTCAAGGACTTCGCGCAGCCCATCTTCGACAAGA
GCCCCCACGGGAAGAAGATCACGGTGAAGACGGGACCCGCGATGGACACGCTCAAGGAA
CTCGCGGCGACGGGTGAACAGTTCGACATGGTGTTCATCGACGCGGACAAGCAGAACTAC
ATCAACTACTACAAGTTCCTGCTCGACCACAACCTGCTCCGGATCGACGGTGTGATCTGTG
TGGACAACACGCTCTTCAAGGGTCGCGTGTACCTCAAGGACTCGGTCGACGAAATGGGTA
AGGCGCTCCGGGACTTCAACCAGTTCGTGACGGCGGACCCCCGGGTCGAACAGGTGATC
ATCCCGCTCCGCGACGGTCTCACGATCATCCGCCGGGTGCCGTACACGCCCCAGCCGAA
CTCGCAGAGCGGGACGGTGACGTACGACGAAGTGTTCCGGGGTGTGCAGGGGAAGCCC
GTGCTCGACCGGCTCCGCCTCGACGGGAAGGTGGCGTACGTCACGGGTGCGGGGCAGG
GGATCGGTCGCGCGTTCGCGCACGCGCTCGGTGAAGCGGGCGCGAAGGTGGCGATCAT
CGACATGGACCGCGGGAAGGCGGAAGACGTCGCGCACGAACTCACGCTCAAGGGGATCA

GCTCGATGGCGGTGGTGGCGGACATCAGCAAGCCCGACGACGTGCAGAAGATGATCGAC
GACATCGTGACGAAGTGGGGCACGCTCCACATCGCGTGTAACAACGCGGGGATCAACAA
GAACTCGGCGTCGGAAGAAACCAGCCTCGAAGAATGGGACCAGACGTTCAACGTGAACCT
GCGGGGTACGTTCATGTGTTGTCAGGCGGCGGGTCGCGTGATGCTCAAGCAGGGGTACG
GGAAGATCATCAACACGGCGTCGATGGCGTCGCTCATCGTGCCGCACCCCCAGAAGCAG
CTCTCGTACAACACGTCCAAGGCGGGTGTGGTGAAGCTCACGCAGACGCTCGGGACGGA
ATGGATCGACCGGGGAGTGCGCGTGAACTGTATCTCGCCGGGTATCGTCGACACGCCCC
TCATCCACAGCGAATCGCTCGAACCGCTCGTGCAGCGGTGGCTCAGCGACATCCCCGCG
GGACGCCTCGCGCAGGTGACGGACCTGCAGGCGGCGGTCGTGTACCTCGCGTCGGACG
CCTCGGACTACATGACGGGACACAACCTCGTCATCGAAGGTGGGCAGTCGCTCTGGTAG

Figure S3. Codon optimized *drMT-Ox* sequence for expression in *Streptomyces.*

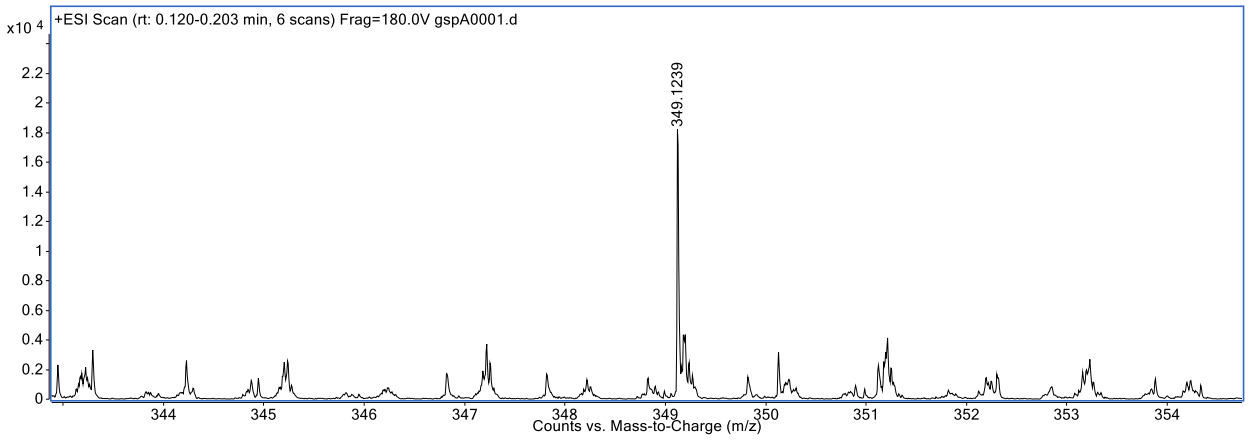

Figure S4. HR-MS spectrum of gadusporine A.

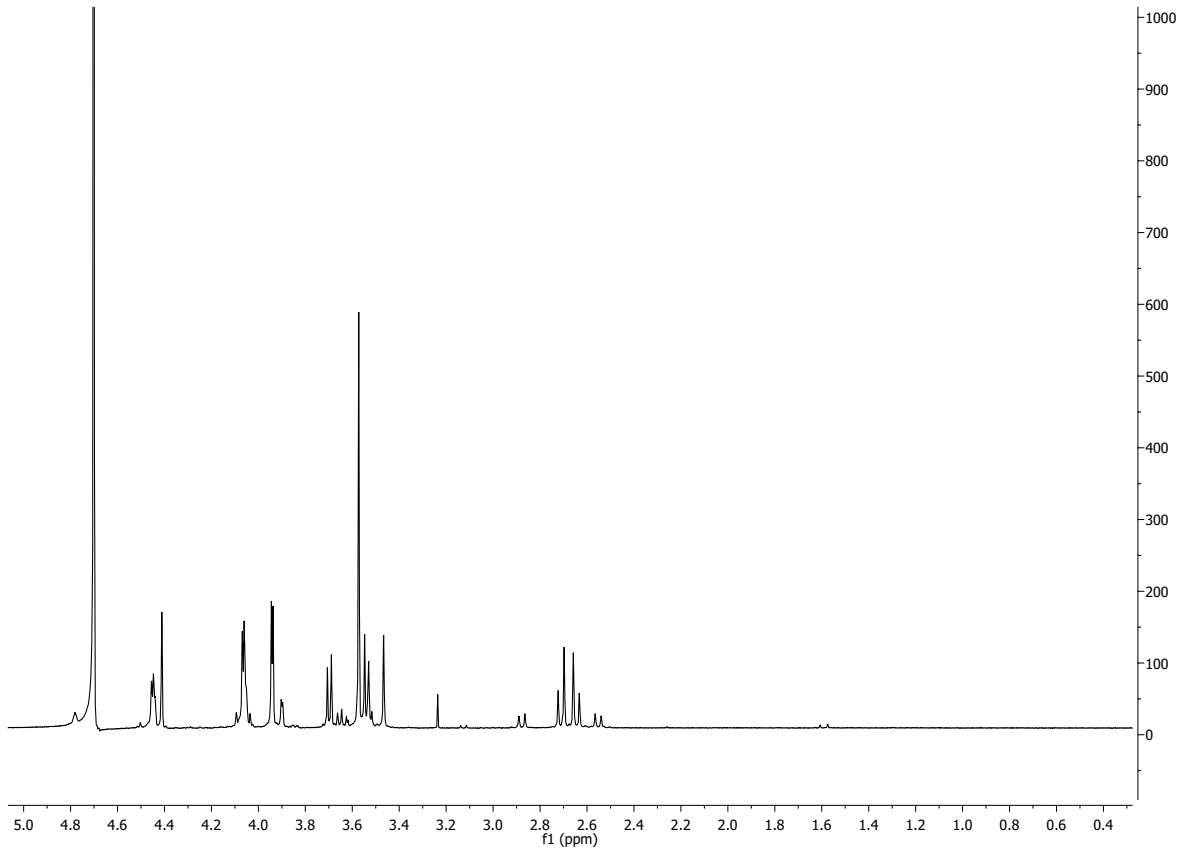

Figure S5. ^{1}H NMR spectrum of gadusporine A (700 MHz, D_2O).

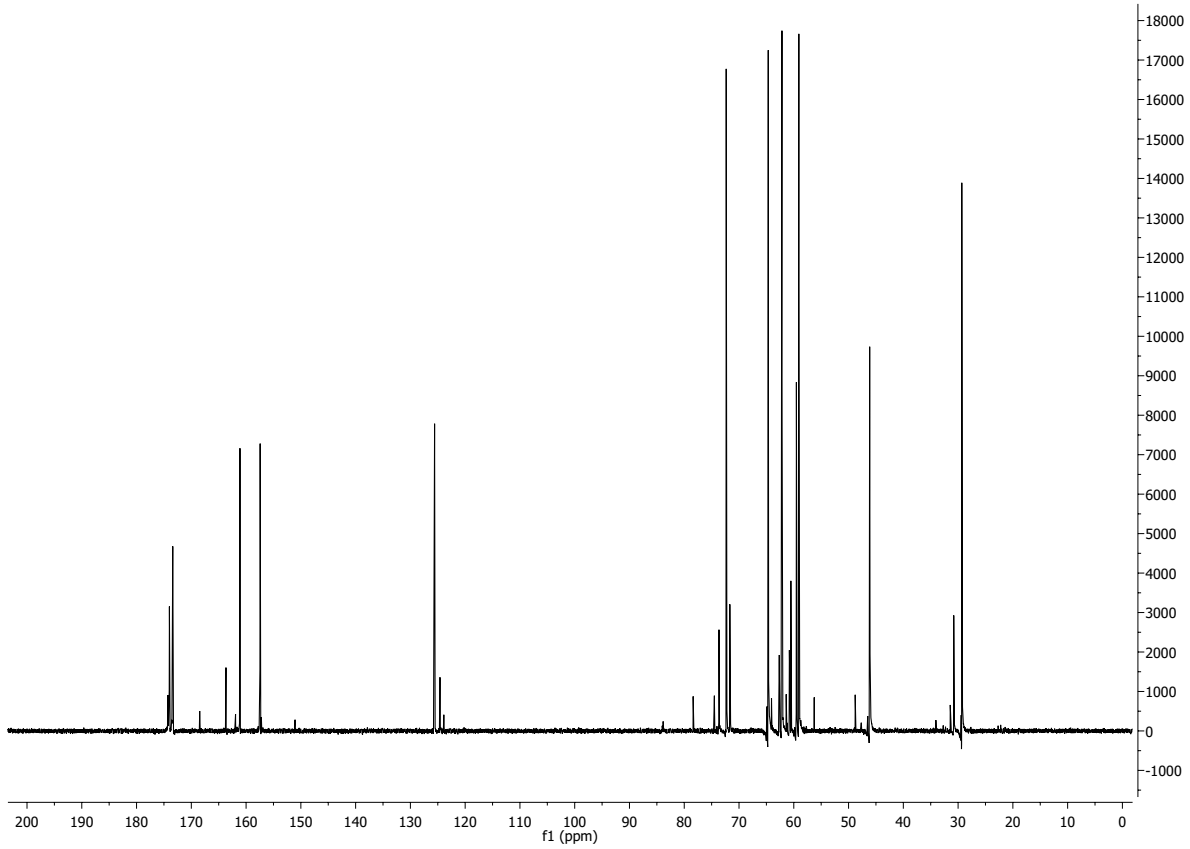

Figure S6. ^{13}C NMR spectrum of gadusporine A (175 MHz, D_2O).

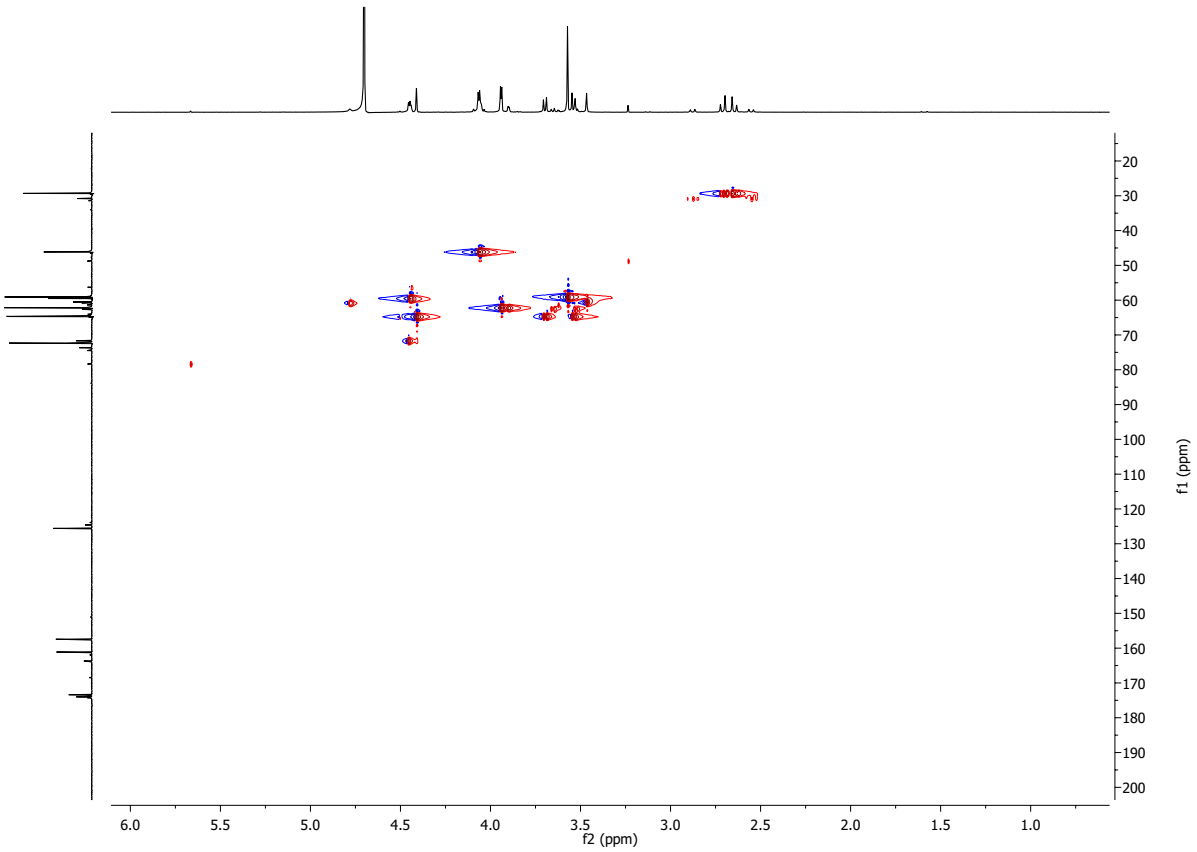

Figure S7. HSQC spectrum of gadusporine A (700 MHz, D_2O).

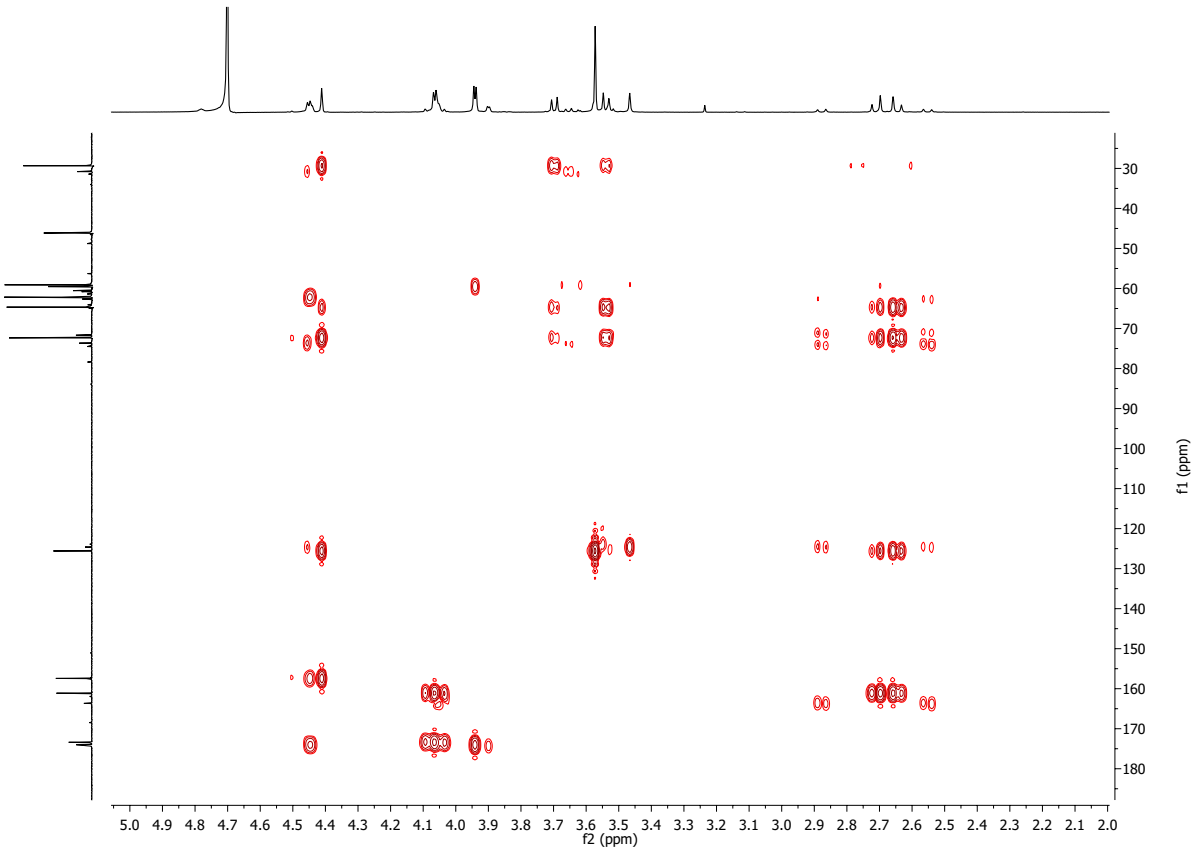

Figure S8. HMBC spectrum of gadusporine A (700 MHz, D_2O).

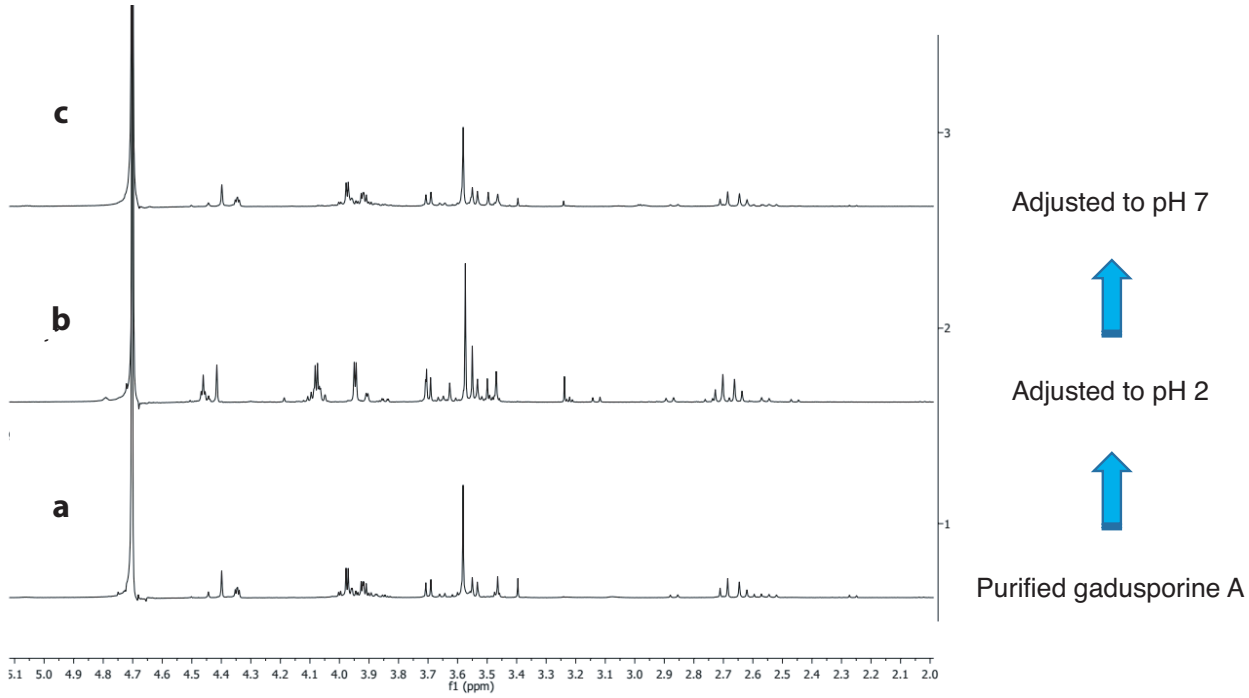

Figure S9. ^{1}H NMR spectra of gadusporine A in different pHs (700 MHz, D_2O).

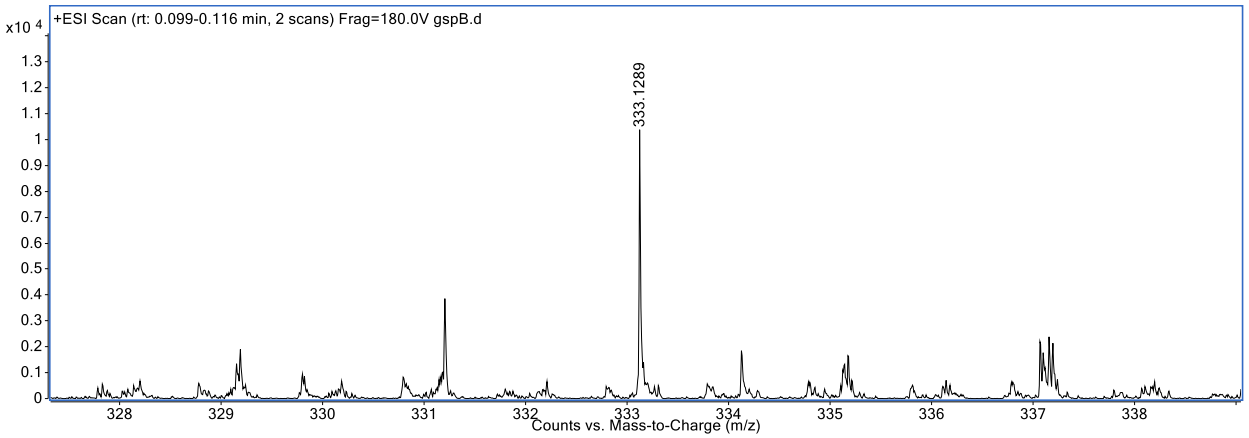

Figure S10. HR-MS spectrum of gadusporine B.

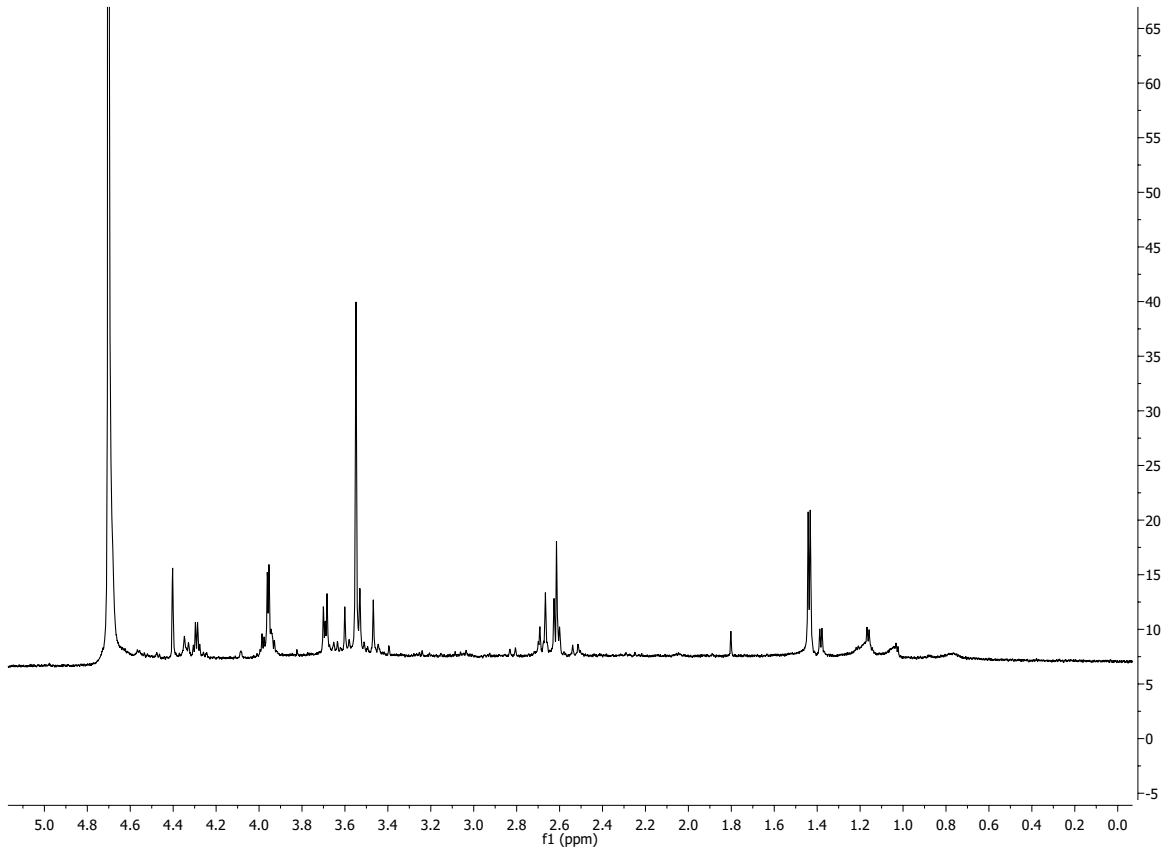

Figure S11. ^{1}H NMR spectrum of gadusporine B (700 MHz, D_2O).

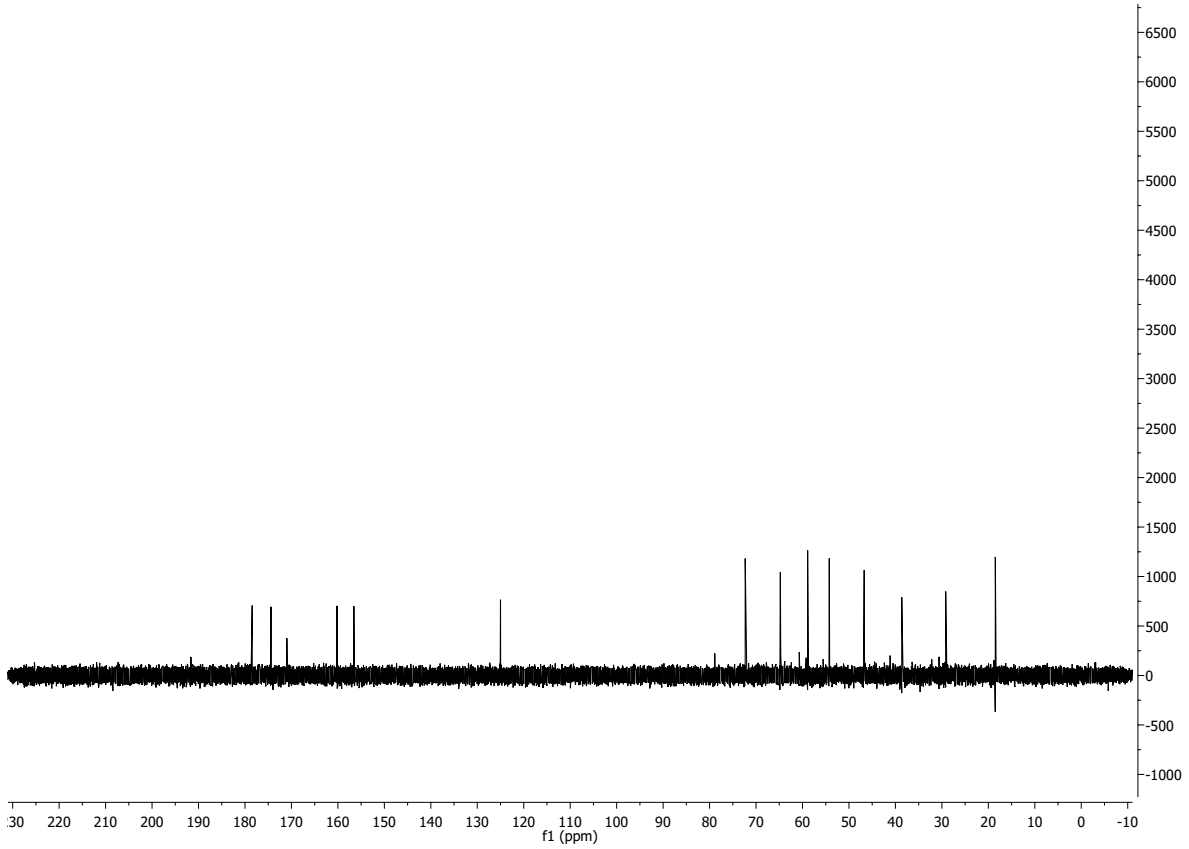

Figure S12. ^{13}C NMR spectrum of gadusporine B (175 MHz, D_2O).

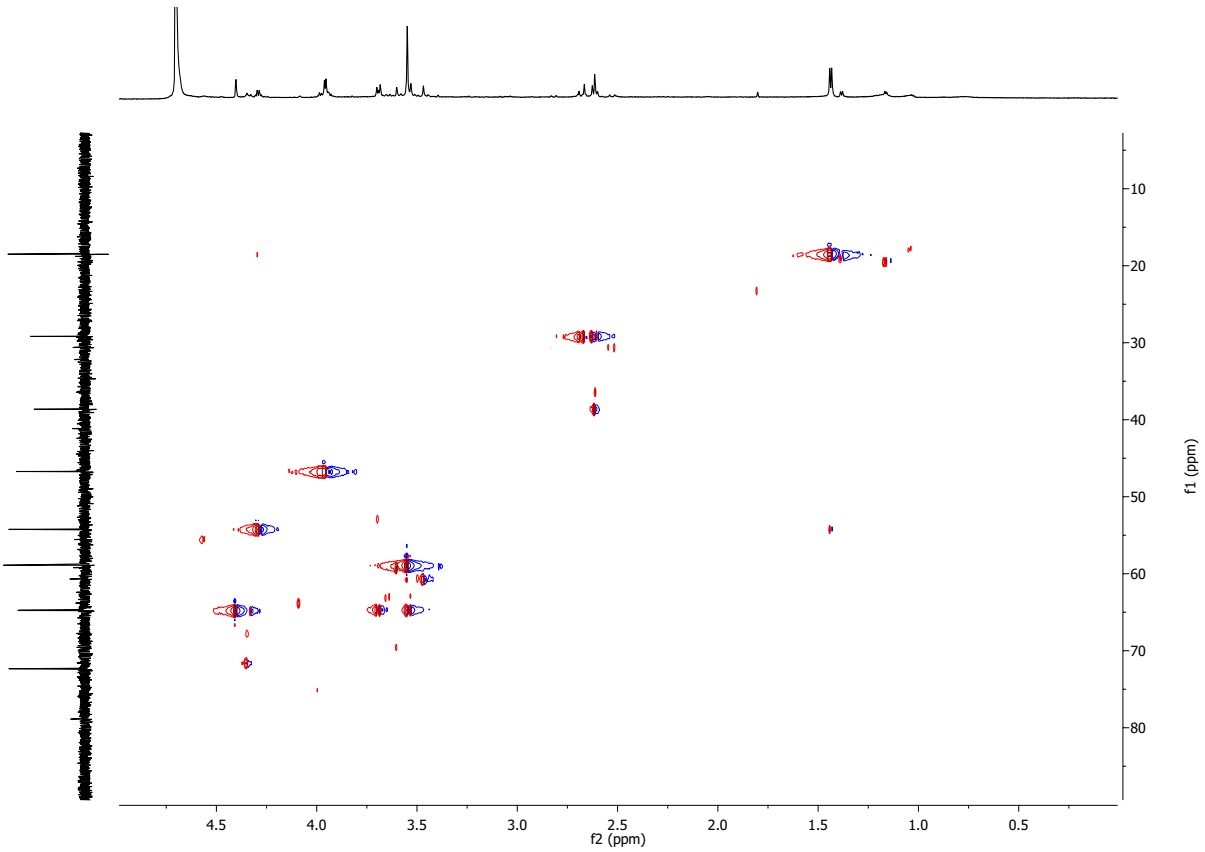

Figure S13. HSQC spectrum of gadusporine B (700 MHz, D_2O).

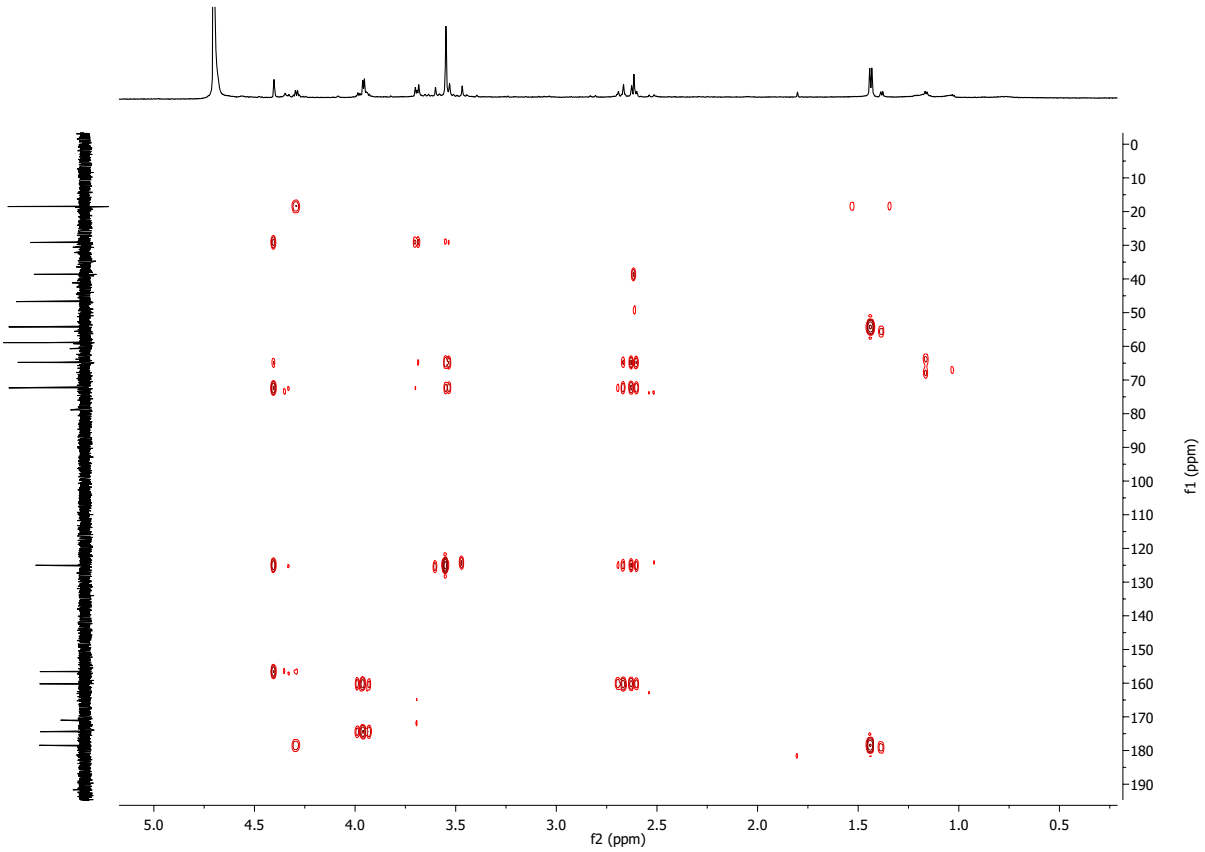

Figure S14. HMBC spectrum of gadusporine B (700 MHz, D_2O).

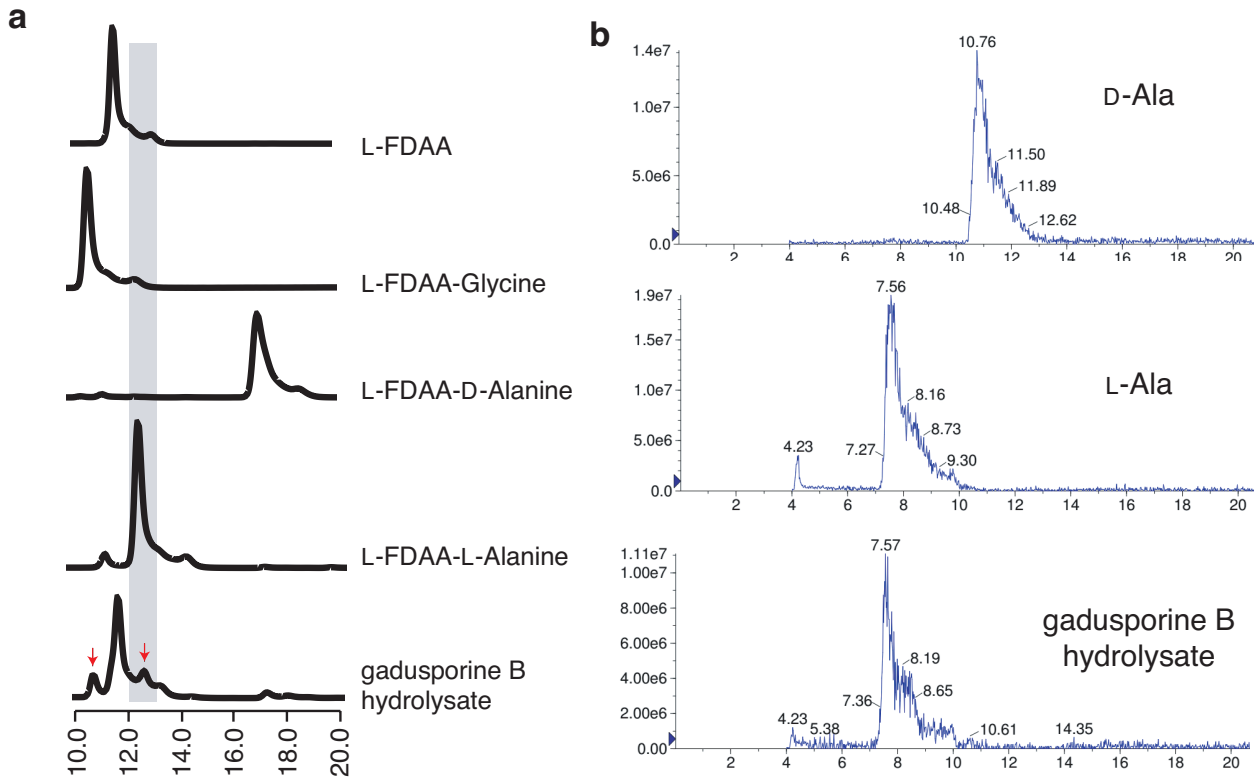

Figure S15. Marfey's analysis of gadusporine B. **a**. Partial HPLC chromatograms of L-FDAA-derivatized gadusporine B hydrolysate and amino acid standards; **b**. Extracted ion chromatograms of gadusporine B hydrolysate and amino acid standards confirming the Marfey's analysis results.

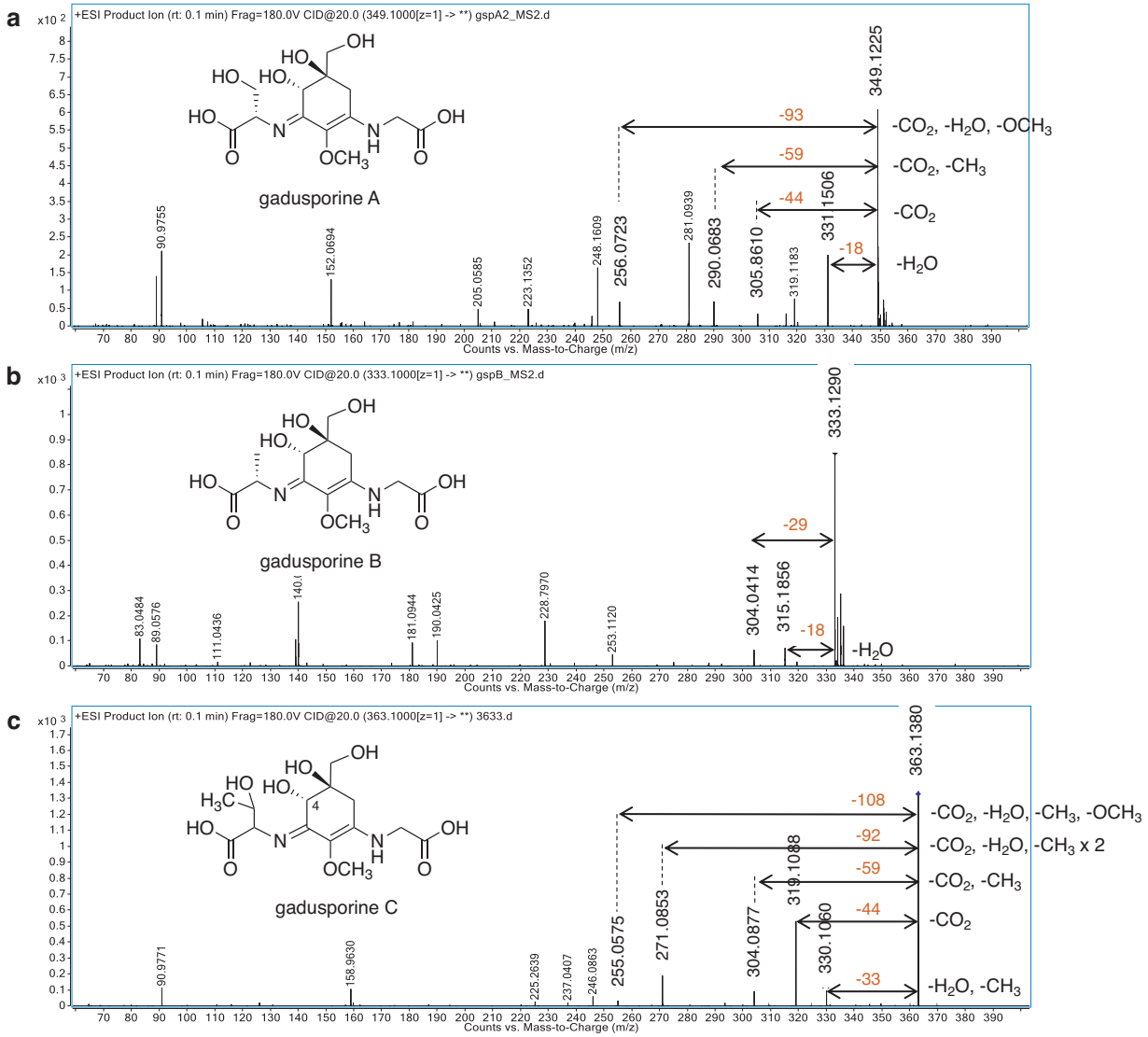

Figure S16. HR-MS fragmentations of the gadusporines: **a**. gadusporine A; **b**. gadusporine B; and **c**. gadusporine C. Gadusporines A and C gave similar MS/MS fragmentation patterns, whereas gadusporine B gave a different pattern. They are consistent with those of their corresponding MAA analogs, shinorine, mycosporine-glycine-alanine, and porphyra-334, respectively.[6]

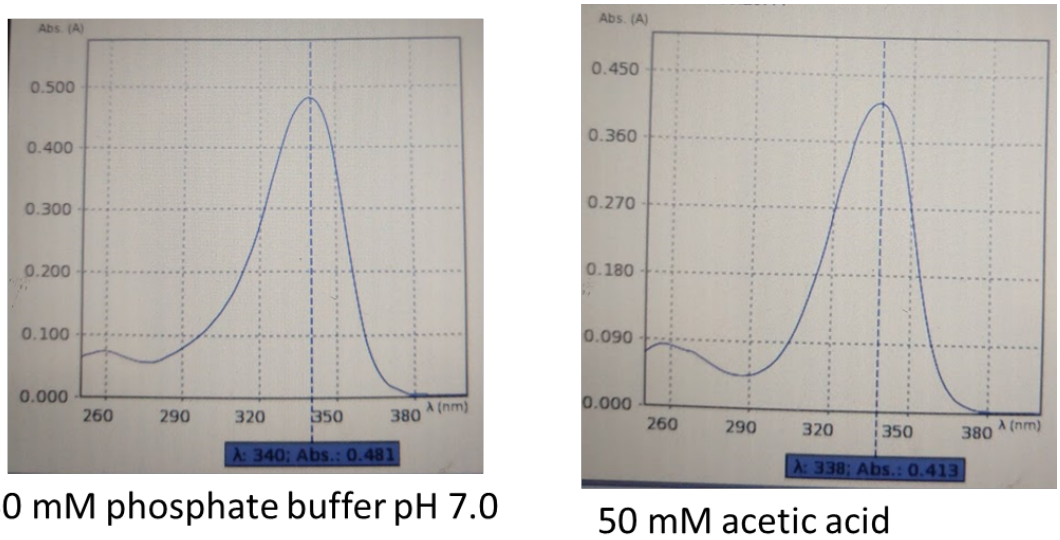

Figure S17. UV absorbance of gadusporine A at different pH values.

Supporting References

(1) Paget, M. S., Chamberlin, L., Atrih, A., Foster, S. J., and Buttner, M. J. (1999) Evidence that the extracytoplasmic function sigma factor sigmaE is required for normal cell wall structure in *Streptomyces coelicolor* A3(2), *J. Bacteriol. 181*, 204-211.

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(3) Gomez-Escribano, J. P., and Bibb, M. J. (2011) Engineering *Streptomyces coelicolor* for heterologous expression of secondary metabolite gene clusters, *Microb. Biotechnol. 4*, 207-215.

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(6) Miyamoto, K. T., Komatsu, M., and Ikeda, H. (2014) Discovery of gene cluster for mycosporine-like amino acid biosynthesis from Actinomycetales microorganisms and production of a novel mycosporine-like amino acid by heterologous expression, *Appl. Environ. Microbiol. 80*, 5028-5036.