

Supporting Information

Identification of the First Diketomorpholine Biosynthetic Pathway Using FAC-MS Technology

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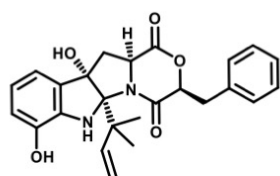
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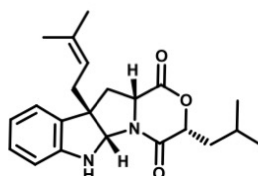
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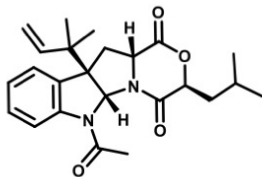
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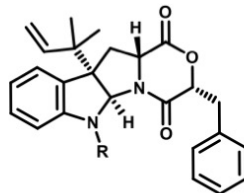
Shornephine A



Mollenine A



Javanicunine A



R = H (Acu-dioxomorpholine A)

R = Me (Acu-dioxomorpholine B)

Figure S1. Fungal diketomorpholine indole alkaloids.^{1,2,3,4,5,6} Until this work, the biosynthesis of diketomorpholines was unknown.

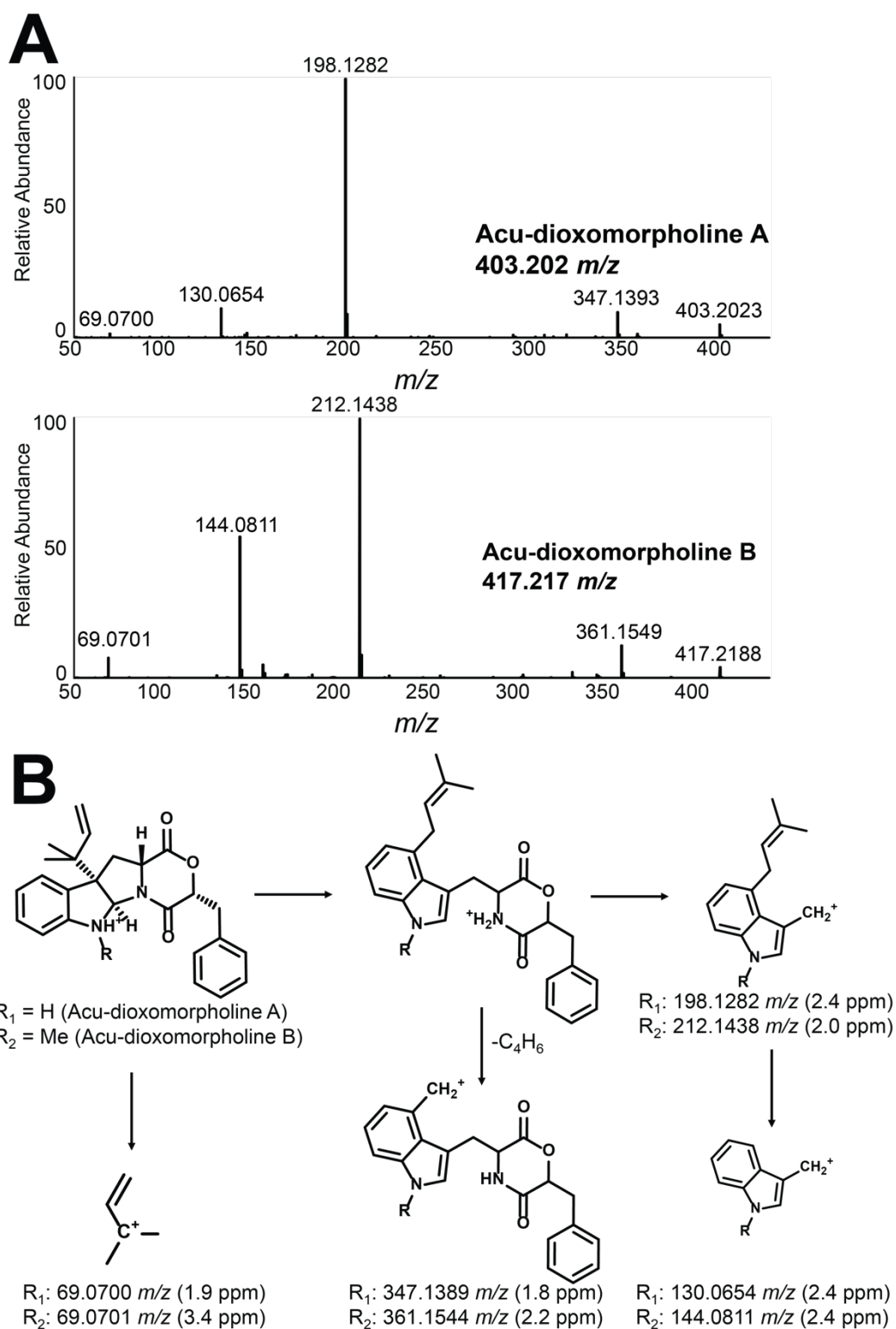


Figure S2. MS/MS structural data for acu-dioxomorpholine A and B. **A)** MS/MS spectra for acu-dioxomorpholine A and B are highly similar. Comparing the spectra for the two analogs, the three most intense peaks are related by CH_2 . **B)** A proposed fragmentation pathway for acu-dioxomorpholine A and B. A predicted alkyl migration of the prenyl group to the C4 position results in highly stable indole fragment ions (at right).

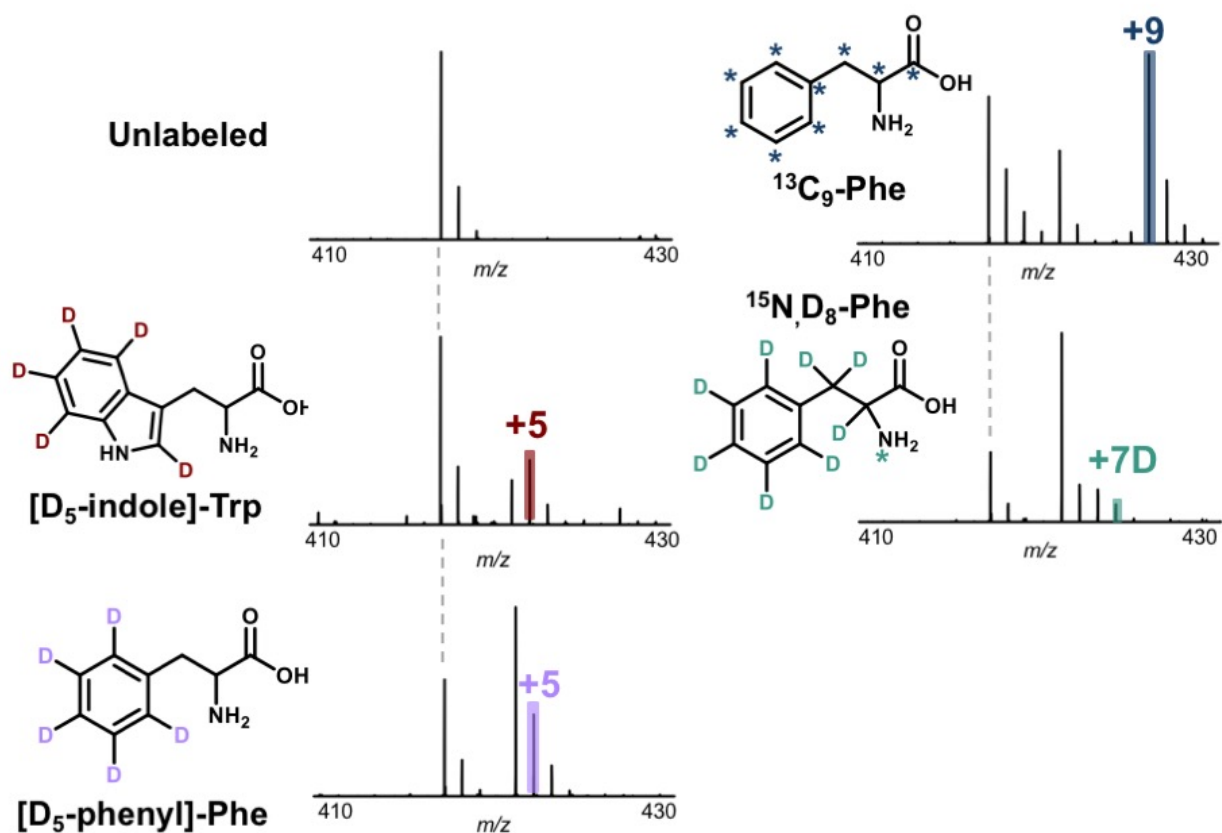


Figure S3. Atom tracking in acudioxomorpholine B biosynthesis using stable isotope labeling and MS. Data shown here corresponds to acudioxomorpholine A stable isotope labeling data in **Figure 3**. Feeding with (D₅-indole)-Trp resulted in a shift of +5 Da (422.2487 *m/z*, 0.09 ppm error), confirming Trp is a precursor. A +4 labeled species was also detected, likely corresponding to loss of the C2 deuterium during prenylation and ring closure. Feeding with (D₅-phenyl)-Phe resulted in a shift of +5 Da (422.24908 *m/z*, 1.0 ppm error), consistent with Phe being the source of the phenyl ring in acudioxomorpholine. Feeding with ¹³C₉-Phe resulted in a shift of +9 Da (426.2477 *m/z*, 0.4 ppm error), providing further support for phenylalanine incorporation. Feeding with D₈, ¹⁵N-Phe resulted in a shift of +7 Da (424.2615 *m/z*, 0.8 ppm error), indicating that phenylalanine is incorporated into acudioxomorpholine with loss of the nitrogen and a single deuterium. A +6 Da species was also detected from D₈, ¹⁵N-Phe labeling, likely resulting from phenylpyruvate tautomerization.

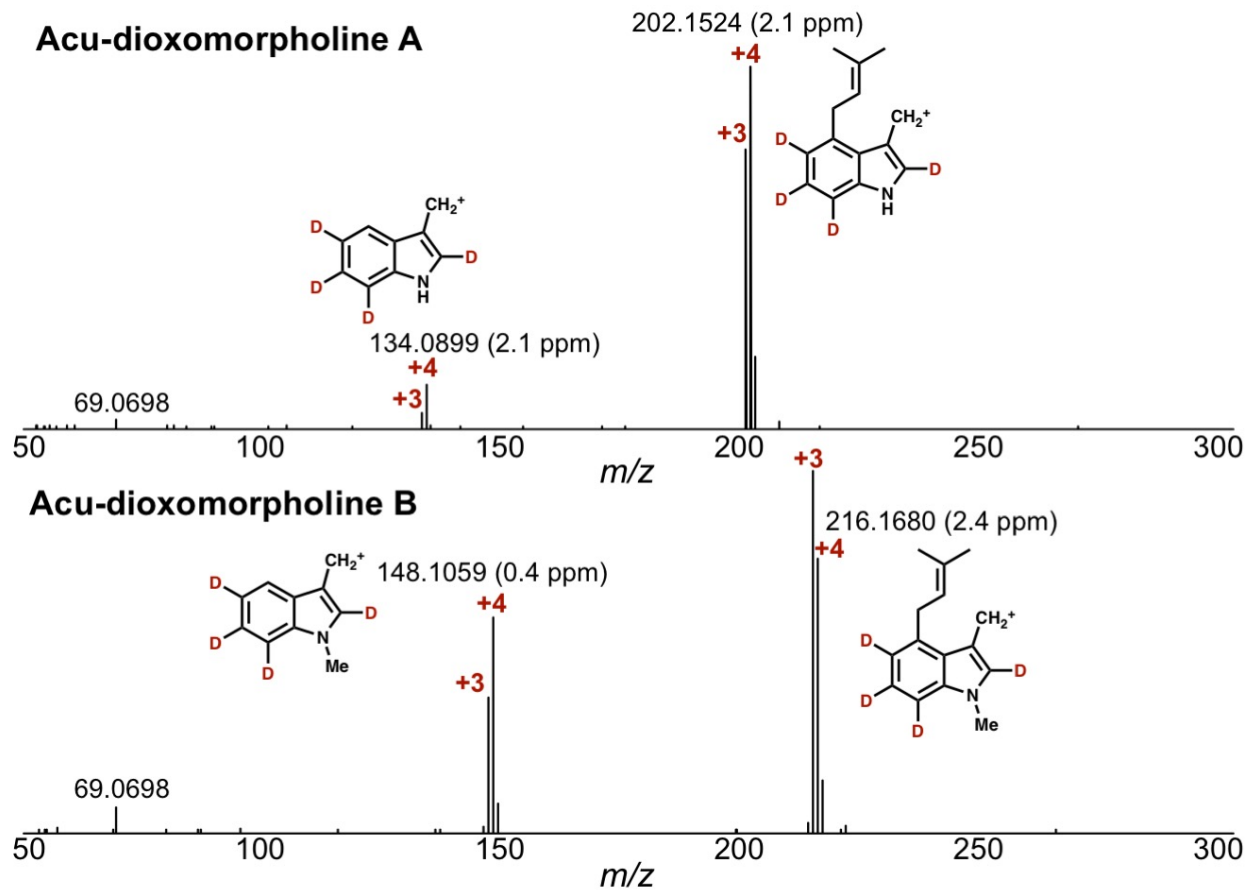


Figure S4. MS/MS spectra of [D₅-indole]-Trp labeled acu-dioxomorpholine A and B. The major species correspond to retention of four deuterons, consistent with an alkyl migration that results in loss of a deuteron. Species corresponding to three deuterons likely result from loss of the C2 deuteron during fragmentation.

Cluster Name	GenBank Accession	No. of Amino Acids	Closest Genbank Homolog	Amino Acid Identity (%)
	XP_020052745	827	Calpain Family Cysteine Protease (<i>Colletotrichum simmondsii</i>) [KXH31552]	417/896 (47%)
	XP_020052746	451	Hypothetical Protein (<i>Aspergillus bombycis</i>) [XP_022388258]	278/461 (62)
	XP_020052747	197	TM2 Domain-Containing Protein 2 (<i>Fusarium oxysporum f. sp. Cubense race 4</i>) [EMT69206]	56/116 (28)
	XP_020052748	343	Dihydroflavonol-4-Reductase (<i>Capronia coronata CBS 617.96</i>) [XP_007727020]	203/338 (59)
	XP_020052749	115	Tyrosinase (<i>Aspergillus nomius NRRL 13137</i>) [XP_015404592]	44/64 (38)
	XP_020052750	407	Flavin-Containing Monooxygenase 1 (<i>Diplodia corticola</i>) [XP_020127287]	115/352 (28)
	XP_020052751	368	Alpha-1,6-Mannosyltransferase (<i>Aspergillus niger</i>) [GAQ45755]	215/350 (58)
	XP_020052752	172	Envelope Glycoprotein D (<i>Talaromyces marneffeii PM1</i>) [KFX48112]	22/34 (13)
	XP_020052753	430	Flavoheмоprotein (<i>Aspergillus fumigatus var. RP-2014</i>) [KEY80579]	378/433 (88)
	XP_020052754	734	Ankyrin Repeat Protein (<i>Aspergillus fumigatus Z5</i>) [KMK56345]	204/721 (28)
	XP_020052755	682	Sensor Histidine Kinase/Response Regulator (<i>Aspergillus niger CBS 513.88</i>) [XP_001398645]	323/681 (47)
	XP_020052756	706	Glucan 1,3-Beta-Glucosidase D (<i>Aspergillus niger CBS 513.88</i>) [XP_001394735]	549/708 (78)
	XP_020052757	267	AhpC/TSA Family Thioredoxin Peroxidase (<i>Aspergillus kawachii IFO 4308</i>) [GAA85921]	240/266 (90)
	XP_020052758	435	Peptidase M20 (<i>Penicillium italicum</i>) [KGO68526]	277/437 (64)
	XP_020052759	467	Pantothenate Transporter (<i>Aspergillus oryzae RIB40</i>) [XP_003190219]	406/467 (87)
	XP_020052760	223	Acetyltransferase GNAT Domain Protein (<i>Aspergillus parasiticus SU-1</i>) [KJK67501]	85/223 (38)
	XP_020052761	157	Mannose-Binding Lectin (<i>Penicillium expansum</i>) [XP_016596221]	61/140 (39)
	XP_020052762	176	Cytochrome P450 (<i>Aspergillus parasiticus SU-1</i>) [KJK61402]	58/93 (33)
	XP_020052763	367	Transaldolase (<i>Talaromyces islandicus</i>) [CRG84235]	241/364 (66)
	XP_020052764	765	Transcription Factor, Fungi (<i>Penicillium camemberti</i>) [CRL25087]	506/733 (66)
	XP_020052765	136	RutC Family Protein YjgH (<i>Aspergillus udagawae</i>) [GAO81378]	110/136 (80)
	XP_020052766	368	N-Ethylmaleimide Reductase (<i>Aspergillus kawachii IFO 4308</i>) [GAA92790]	274/361 (74)
	XP_020052767	505	60S Ribosomal Export Protein NMD3 (<i>Aspergillus terreus NIH2624</i>) [XP_001212120]	384/492 (76)
	XP_020052768	469	Protein Ycf2 (<i>Talaromyces islandicus</i>) [CRG87194]	188/423 (44)
	XP_020052769	349	Arrestin-Like, N-Terminal (<i>Penicillium camemberti</i>) [CRL18225]	101/323 (33)
	XP_020052770	162	Hypothetical Protein (<i>Talaromyces islandicus</i>) [CRG87192]	83/147 (56)
	XP_020052771	356	Integral Membrane Protein (<i>Aspergillus kawachii IFO 4308</i>) [GAA92009]	182/350 (52)
	XP_020052772	390	Alcohol Dehydrogenase (<i>Aspergillus kawachii IFO 4308</i>) [GAA87592]	285/391 (73)

Cluster Name	GenBank Accession	No. of Amino Acids	Closest Genbank Homolog	Amino Acid Identity (%)
adxA	XP_020052773	2413	Nonribosomal Peptide Synthetase 10 (<i>Penicillium subrubescens</i>) [OKP06700]	810/2095 (39)
adxB	XP_020052774	378	NADPH-Dependent Methylglyoxal Reductase (<i>Aspergillus clavatus</i> NRRL 1) [XP_001276060]	174/372 (47)
adxC	XP_020052775	422	Aromatic Prenyltransferase, DMATS Type (<i>Penicillium expansum</i>) [XP_016600775]	224/428 (52)
	XP_020052776	135	Protein of Unknown Function DUF4267 (<i>Penicillium occitanis</i>) [PCG98689]	64/126 (47)
	XP_020052777	1379	ABC Transporter, Integral Membrane Type 1 (<i>Penicillium expansum</i>) [XP_016594290]	864/1423 (63)

Table S1. Predicted Open Reading Frames on FAC AaFAC30-6A16.

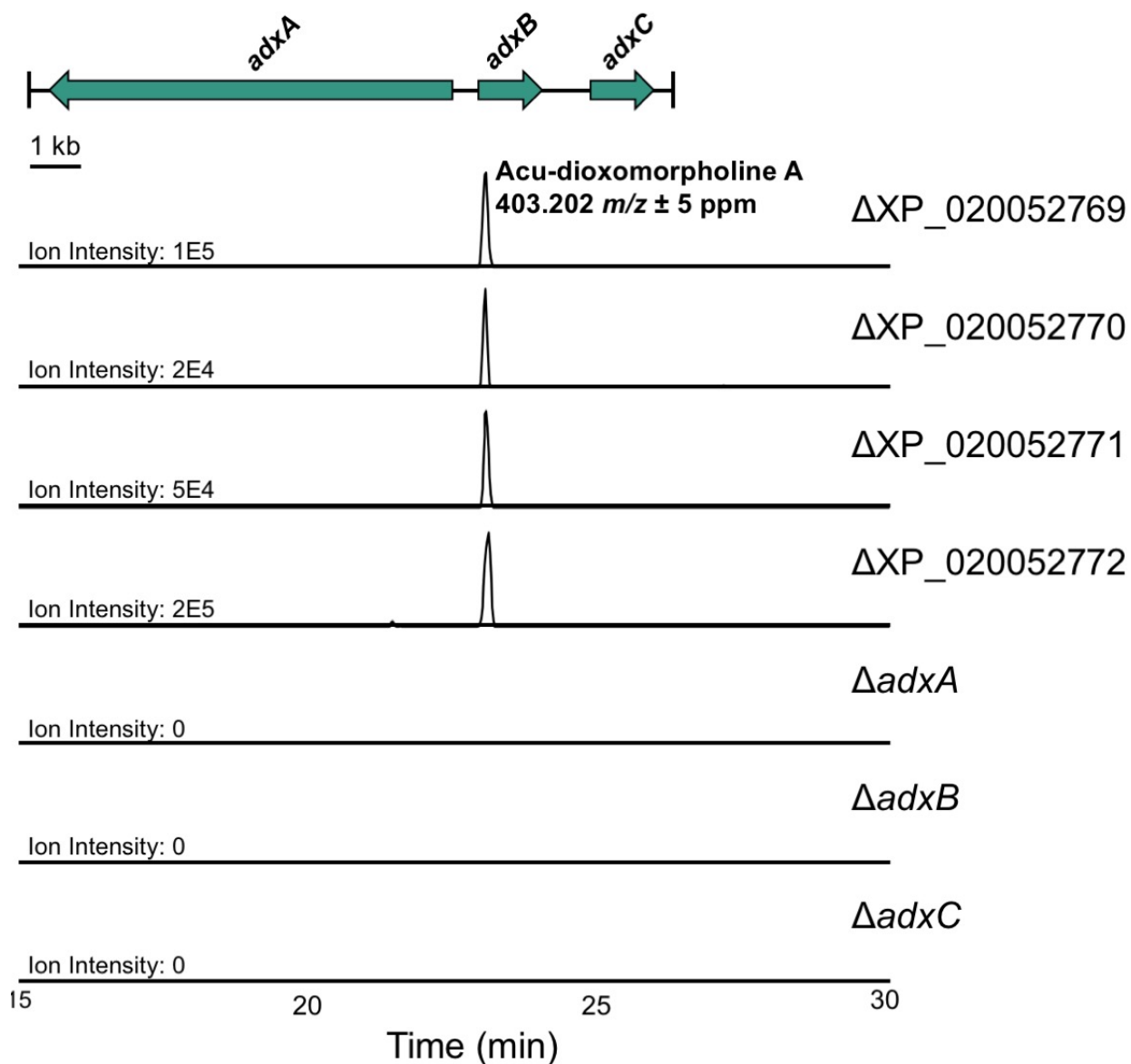


Figure S5. Genetic deletions of predicted acu-dioxomorpholine BGC genes. Acu-dioxomorpholine A production was completely abolished upon deletion of *adxA*, nonribosomal peptide synthetase; *adxB*, phenylpyruvate reductase; and *adxC*, prenyltransferase.

NAD(P)H-Binding Motif

AdxB		1	-----MNLALPPGSTVVV	TCAN	YLA	SHTSNE	27
sp Q9UUN9 ALD2_SPOSA	2	--AKIDNAVL	PEGSLVLV	TCAN	FV	SHVVEQ	31
sp Q0D1P7 TERH_ASPTN	1	--MTVSSSIV	PPGGLVLV	TCVT	FIC	SYIANG	30
sp Q03049 YD541_YEAST	1	-----	MSNTVLV	SCAS	FIAL	LHILSQ	21
sp Q9UT59 YKJ7_SCHPO	1	-----	MSGKLVLV	TCVT	FIC	AHVAEQ	22
sp Q9S9N9 CCR1_ARATH	1	---MPVDVAS	PAGKTVCV	TCAG	YIA	SWIVKI	29
sp Q9SAH9 CCR2_ARATH	1	-----	MLVDGKLV	CVTCAG	YIA	SWIVKL	24
sp Q6K9A2 CCR1_ORYSJ	3	SSFEANNNN	NGEKQLV	CVTCAG	FIC	SWVKE	35
sp P53111 ARI1_YEAST	1	-----	MTTDTTVFV	SCAT	FIAL	HIMND	23
sp Q500U8 TKPR1_ARATH	1	-----	MDQAKGKVCV	TCAS	FIA	SWLVKR	24
sp O94563 YGD4_SCHPO	1	-----	MSELVLI	TCIT	FV	SHSAEA	21
sp Q9SEV0 BAN_ARATH	1	---MDQTLT	HTGSKKACV	TCGT	NLA	SILIKH	29
sp Q12068 GRE2_YEAST	1	-----	MSVVF	SCAN	FIA	AQHVDL	19
sp P51104 DFRA_DIACA	8	TLGKHDINK	VQGGETVCV	TCAS	FIC	SWLMR	41
sp Q9CA28 TKPR2_ARATH	1	-----	MSEYLV	TCGT	FIA	SYIIKS	20
sp D7U6G6 ANRPN_VITVI	1	---MATQHP	IGKKTACV	VTG	FV	SLLVKL	28
sp Q5FB34 ANRCS_VITVI	1	---MATQHP	IGKKTACV	VTG	FV	SLLVKL	28
sp Q9XE55 DFRA_MALDO	1	-----	MGSESESV	CVTCAS	FIC	SWLVMR	24
sp Q7PCC4 ANRCH_VITVI	1	---MATQHP	IGKKTACV	VTG	FV	SLLVKL	28
sp P53183 YGD9_YEAST	1	-----	MTTEKTVFV	SCAT	FIAL	HVVDD	24

Ser-Tyr-Lys Catalytic Triad

AdxB		144	SMRRVVFIS	SMAL	YTPN	-PGQ	QGLV	168	204	RQVEV	Y	SACK	MIC	EW	AWEW	223
sp Q9UUN9 ALD2_SPOSA	124	SVKRFVLT	STVS	ALIPK	-PN	VEGY		148	172	KSLWV	Y	AASK	TE	EL	AAWKF	191
sp Q0D1P7 TERH_ASPTN	124	SVKRFVFT	S	DQAAS	NR	--TTREIL		147	171	RGWDV	Y	SAL	KAQ	VE	KEMWRF	190
sp Q03049 YD541_YEAST	120	TVERVVVTS	SCTAI	ITLAK	MDD	PSV		145	162	DGINA	Y	FASK	KA	FAE	KAWEF	181
sp Q9UT59 YKJ7_SCHPO	127	SIKRIVITS	S	FAAVGN	FQID	PHNNKV		142	161	NGIVA	Y	CASK	KL	AE	EAREY	180
sp Q9S9N9 CCR1_ARATH	128	KVKRVVITS	S	IGAVY	MDPN	-RDPEAV		142	159	---NW	Y	CYK	M	VAE	QAAWET	175
sp Q9SAH9 CCR2_ARATH	113	KVKRVVFTS	S	IGAVY	MNP	-RDTQAI		137	154	---NW	Y	CYK	M	LA	EQSAWET	170
sp Q6K9A2 CCR1_ORYSJ	122	GVRRVVFTS	S	YGAVH	MNP	-RSPDAV		146	153	---NL	Y	CCAK	M	MAE	MATEE	179
sp P53111 ARI1_YEAST	123	TVEKVIVTS	S	TAA	LVTP	DMNKGDLV		147	164	NAVA	Y	CGSK	K	FAE	KTAWEF	183
sp Q500U8 TKPR1_ARATH	119	SLKRVVLT	SS	STVR	IRDD	-FDPKIP		143	159	--QVW	Y	ALSK	T	LA	EQAAWK	176
sp O94563 YGD4_SCHPO	118	KVKRFVYIS	S	EAA	LKGP	VNYFGDGHV		143	162	DELLN	Y	TVCK	K	LGER	AMHAF	181
sp Q9SEV0 BAN_ARATH	123	SVKRVIIYTS	S	AAAVS	INNL	-SGTGIV		147	164	PFNWG	Y	PISK	V	LA	EKTAWEF	183
sp Q12068 GRE2_YEAST	119	SVERVVLTS	S	YAAV	FDMA	KENDKSLT		143	160	DPVNA	Y	CGSK	K	FAE	KAWEF	179
sp P51104 DFRA_DIACA	135	KLRRVVFTS	S	GGT	VNVE	--ATQKPV		157	174	MTGWM	Y	FVSK	I	LAE	QAAWKY	193
sp Q9CA28 TKPR2_ARATH	117	TLKRIVLT	SS	CS	SIRY	FD-ATEASP		141	157	--NLW	Y	GYAK	T	LGER	EAWRI	174
sp D7U6G6 ANRPN_VITVI	122	SVKRVILT	S	AAAV	TINQL	-DGTGLV		146	163	PPTWG	Y	PASK	T	LA	EKAAWKF	182
sp Q5FB34 ANRCS_VITVI	122	SVKRVILT	S	AAAV	TINQL	-DGTGLV		146	163	PPTWG	Y	PASK	T	LA	EKAAWKF	182
sp Q9XE55 DFRA_MALDO	119	TVRKLVT	S	AGT	VNVE	--EHQKPV		141	158	MTGWM	Y	FVSK	T	LA	EQAAWKY	177
sp Q7PCC4 ANRCH_VITVI	122	SVKRVILT	S	AAAV	TINQL	-DGTGLV		146	163	PPTWG	Y	PASK	T	LA	EKAAWKF	182
sp P53183 YGD9_YEAST	123	TVEKVIVTS	S	VAA	ALSP	GMKDTSFV		148	165	NAVSA	Y	CGSK	K	FAE	KTAWDF	184

Figure S6. Multiple sequence alignment of AdxB, phenylpyruvate reductase. AdxB contains an NAD(P)H binding motif and a Ser-Tyr-Lys catalytic triad characteristic of short chain dehydrogenase family enzymes.

A	NRPS	Substrate	Specificity Code
	NRPS13-A1 (BAH23995)	Trp	D-V-M-F-I-G-A-V-N-K
	RoqA-A1 (XP_002568558)	Trp	D-S-L-E-L-V-A-V-V-K
	AnaPS-A2 (XP_001258077)	Trp	D-V-M-F-V-G-E-V-A-K
	AdxA-A1	Trp	D-A-G-I-V-G-A-C-A-K

B	NRPS	Substrate	Specificity Code
	Destruxin Synthetase-A2 (XP_007826232)	α -Hydroxy- isocaproate	G-A-N-L-I-G-A-T-V-K
	Beauvericin Synthetase-A1 (AFJ44691)	α -Hydroxy- isovalerate	G-A-L-M-I-V-G-S-I-K
	Bassianolide Synthetase-A1 (AFP96785)	α -Hydroxy- isovalerate	G-A-L-M-V-V-G-S-I-K
	Enniatin Synthetase-A1 (CAA79245)	α -Hydroxy- isovalerate	G-A-L-H-V-V-G-S-I-K
	PF1022 Synthetase-A1	Phenyllactate	G-A-V-I-V-V-A-T-M-K
	AdxA-A2	Phenyllactate	G-G-F-I-Y-A-A-V-M-K

Figure S7. Adenylation domain substrate predictions for AdxA, nonribosomal peptide synthetase. (A) AdxA-A1 is proposed to activate Trp. Substrate binding residues are hydrophobic and similar to those of other Trp-activating adenylation domains. (B) AdxA-A2 is proposed to activate phenyllactate. Substrate binding residues are hydrophobic and similar to those of PF1022 synthetase which also activates phenyllactate. Residues are colored according to the Taylor coloring scheme.⁷

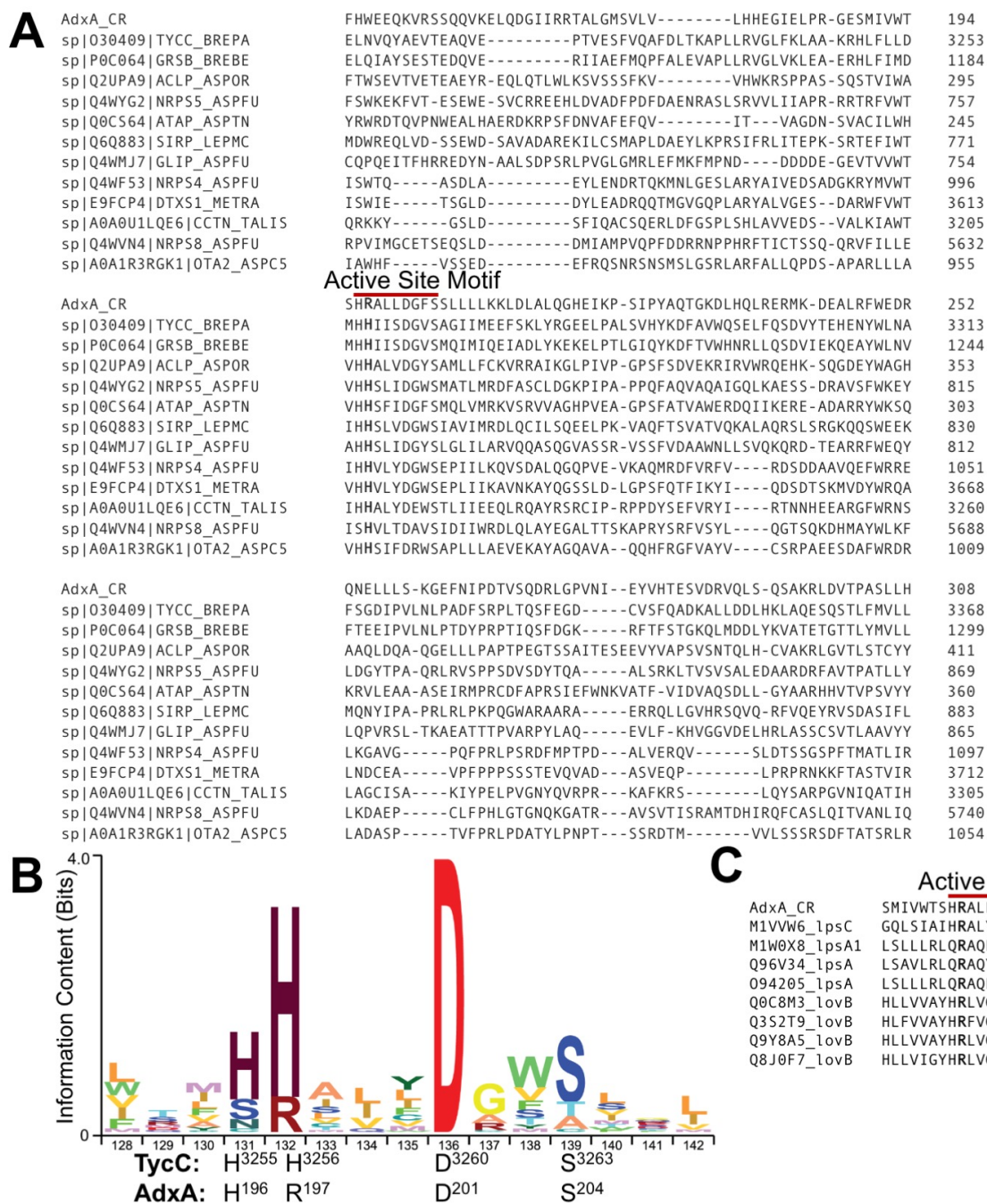


Figure S8. Bioinformatics analysis of the first condensation domain of AdxA. (A) AdxA-C1 has an arginine in place of the catalytic histidine observed in bacterial and fungal condensation domains. Sequences include AdxA-C1, the bacterial NRPS proteins TycC and GrsB, and the ten closest experimentally-characterized fungal homologs of AdxA-C1 from SwissProt. (B) A profile HMM generated from 240 fungal condensation domains in MIBiG reveals that eight other fungal condensation domains also contain an arginine in place of the usual catalytic histidine (H3256 for TycC). (C) Multiple sequence alignment of these eight condensation domains and AdxA-C1. The LpsA and LpsC condensation domains catalyze amide formation in the biosynthesis of D-lysergic acid peptides.⁸ LovB does not catalyze amide formation; however, its precise role in lovastatin biosynthesis is unknown.⁹

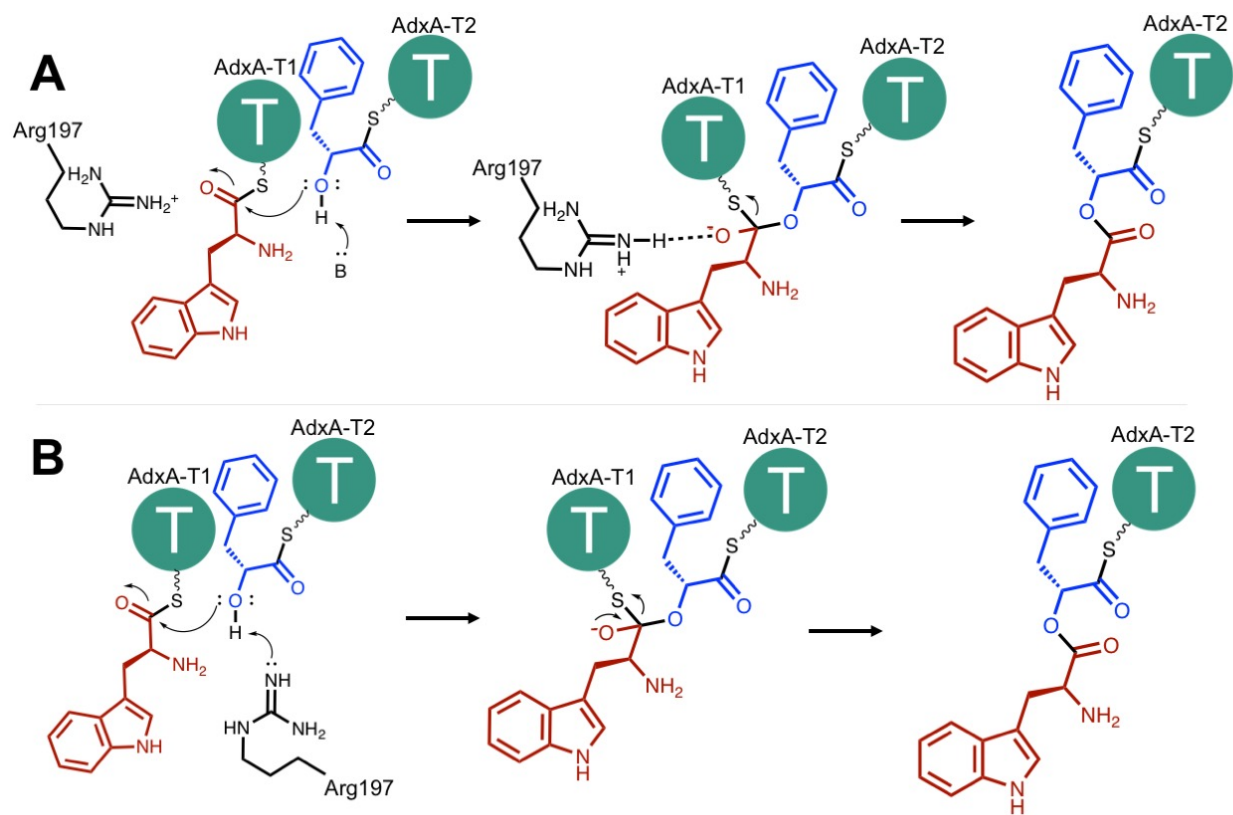


Figure S9. Two possible roles for AdxA Arg197. (A) Arg197 could act to stabilize the tetrahedral intermediate. Additional active site residues bind to the hydroxyl group of the phenyllactate-S-T intermediate to aid in nucleophilic attack. (B) Arg197 could act as a catalytic base, deprotonating the hydroxyl group of the phenyllactate-S-T intermediate for nucleophilic attack. Additional active site residues would bind to Arg197 to promote its availability as a Lewis base to deprotonate the hydroxyl of phenyllactate.

AdxC	84	ELFQCILGSTG-PFELLSQNFTKS-RSTTRIAFEPTSHRA	119			
sp B6HJU1 ROQD_PENRW	88	DIYKCLLGGTG-SVELLSQNVQKL-GLTARVAFEPYSYIA	123			
sp I4AY86 BREPT_ASPVE	84	-KWRSTISRSLPIEFSLNFQKGSRRLLRIGFEPVNFLS	120			Indole -NH
sp Q9C141 DMAW2_CLAP2	76	ERWLSILTRCGLPLELSLNCTD---SVVRYAYEPINEMT	110			Binding
sp A1DJ20 FTMT3_NEOFI	84	D-----YH-PLFISQNFQRS-GSTIRLGFQPRAYSS	112			
sp M1WA41 DMAW_CLAP2	76	ERWLSILTRCGLPLELSLNCTD---SVVRYTYEPINEVT	110			
sp Q50EL0 DMAW_ASPFU	76	PRWLSILTRYGTPFELSLNCSN---SIVRYTFEPINQHT	110			
sp P0CT20-2 DMAW_CLAPU	92	ERWLSILTRCGLPLELSLNCTD---SVVRYTYEPINEVT	126			DMAPP Binding
sp P0CT20 DMAW_CLAPU	76	ERWLSILTRCGLPLELSLNCTD---SVVRYTYEPINEVT	110			
sp Q6X2E3 DMAW_EPINE	76	ERWLSILTRYGTPFELSLNCSN---SIVRYTYEPINAAT	110			
sp Q6X2E1 DMAW2_EPICN	76	ERWLSILTRYGTPFELSLNCSN---SIVRYTYEPINAAT	110			C4 Proton
sp C5FTN3 DMAW_ARTOC	79	NRWLSILTRYGTPFELSLNCSQ---SLVRYTYEPINSAT	123			Abstraction
sp Q6X2E2 DMAW1_EPICN	76	ERWLSILTRYGTPFELSLNCSN---SVVRYTYEPINAAT	110			
sp Q6X1E1 DMAW_BALOB	76	ERWLSILTRYGTPFELSLNCSN---SVVRYTYEPINEAT	110			
sp Q12594 DMAW_CLAFS	75	HTWPSVLTRYGIPFELSLNCLD---SVVRYTFEPTTEHT	109			
sp D4AK48 DMAW_ARTBC	79	NRWLSILTRYGTPFELSLNCSQ---SLVRYTYEPINSAT	113			
sp D4D449 DMAW_TRIVH	79	NRWLSILTRYGTPFELSLNCSQ---SLVRYTYEPINSAT	113			
sp A1DA62 FTMB_NEOFI	88	TQWRSGMVLTLGLPIEFSNNVAR---ALIRIGVDPVTADS	122			
sp Q4WAW7 FTMB_ASPFU	89	TQWRSGMVLTLGLPIEFSNNVAR---ALIRIGVDPVTADS	123			
sp B9WZX3 FTMB_ASPFM	89	TQWRSGMVLTLGLPIEFSNNVAR---ALIRIGVDPVTADS	123			
AdxC	163	IAKLPFKQTQILALDLDLPAG-ISVKEYFYFYP	198	254	DLVDPHRTRLKFIYLAEF	270
sp B6HJU1 ROQD_PENRW	168	ISGTAWKTQILLALDLGQTG-ITIKKEYFYFYP	203	258	DLVDPAAATRIKLYLMEL	274
sp I4AY86 BREPT_ASPVE	165	---QLKSQGAFGDFNPDGAILVKGYVFP	198	255	DLVEMSRQRVKIYGAHT	271
sp Q9C141 DMAW2_CLAP2	154	LVKEQIKTQNKLALDLKESQ-FALKVYFYFYP	189	254	DLIDPAKSRVKIYLQEQ	270
sp A1DJ20 FTMT3_NEOFI	158	SLSPSFKTQGLLAVELPRTGSITLKGWFL	197	263	DHVDMSRTRFKLYLYEC	289
sp M1WA41 DMAW_CLAP2	154	LVKEQIKTQNKLALDLKESQ-FALKVYFYFYP	189	254	DLIDPAKSRVKIYLLEK	270
sp Q50EL0 DMAW_ASPFU	154	LVGGTIRTQNKLALDLKQGR-FALKTYIYYP	190	249	DLTSPAKSRKIYILLEQ	266
sp P0CT20-2 DMAW_CLAP	170	LVKEQIKTQNKLALDLKESQ-FALKVYFYFYP	205	270	DLIDPAKSRVKIYLLEK	286
sp P0CT20 DMAW_CLAPU	154	LVKEQIKTQNKLALDLKESQ-FALKVYFYFYP	189	254	DLIDPAKSRVKIYLLEK	270
sp Q6X2E3 DMAW_EPINE	154	LVKEQIKTQNKLALDLKQGR-FALKTYIYYP	189	256	DLVSPKSRVKIYLLEK	272
sp Q6X2E1 DMAW2_EPICN	154	LVKEQIKTQNKLALDLKQGR-FVLKTYIYYP	189	256	DLVSPKSRVKIYLLER	272
sp C5FTN3 DMAW_ARTOC	157	LVGGQIRTQNKLALDLKGGN-FVLKTYIYYP	192	251	DLIDPSKSRVKIYILEL	267
sp Q6X2E2 DMAW1_EPICN	154	LVKEQIKTQNKLALDLKQGR-FVLKTYIYYP	189	256	DLISPTKSRVKIYLLER	272
sp Q6X1E1 DMAW_BALOB	154	LVNQCIRTQNKLALDLKQGR-FVLKTYIYYP	189	254	DLMDPHKSRVKIYLHER	270
sp Q12594 DMAW_CLAFS	153	VNQQPIRTQNKLALDLKQGR-FALKVYLYYP	189	261	DLVDPKSRVKIYILLEQ	287
sp D4AK48 DMAW_ARTBC	157	LVGGQIRTQNKLALDLKGGN-FVLKTYIYYP	192	251	DLIDPSKSRVKIYILEL	277
sp D4D449 DMAW_TRIVH	157	LVGGQIRTQNKLALDLKGGN-FVLKTYIYYP	192	251	DLIDPSKSRVKIYILEL	277
sp A1DA62 FTMB_NEOFI	166	LFKSPWKSQILTAMDQKSGTVLVKAYFYFYP	203	283	DLVEPGKSRVKFYASER	299
sp Q4WAW7 FTMB_ASPFU	167	LFRSPWKSQILTAMDQKSGTVLVKAYFYFYP	204	284	DLVEPGKSRVKFYASER	300
sp B9WZX3 FTMB_ASPFM	167	LFRSPWKSQILTAMDQKSGTVLVKAYFYFYP	204	284	DLVEPGKSRVKFYASER	300
AdxC	342	GRRAPIPKFIYFYP	353	400	HHQAWLSFSYARSKGPLYLTVYYH	422
sp B6HJU1 ROQD_PENRW	344	GEALPKPKFIYFYP	355	400	DHQAWLSFSYTKKGPYLTMYH	422
sp I4AY86 BREPT_ASPVE	345	GSSFPVPKFIYLP	356	403	RLQSWISYSYAKKGVYMSVYFH	435
sp Q9C141 DMAW2_CLAP2	341	NDPMPPEPQVYFT	352	399	YLHSLVSFSYRR-NKPYLSVYLH	448
sp A1DJ20 FTMT3_NEOFI	369	GEKWPQPKVYFYP	380	227	GLQHVLVSFSYRPKTGPTTYVYVW	453
sp M1WA41 DMAW_CLAP2	341	NNPMPPEPQVYFT	352	399	YLHAYISFSYRR-NKPYLSVYLH	448
sp Q50EL0 DMAW_ASPFU	336	NDPVPEPQVYFT	347	398	YLHAYISFSYRD-RTPYLSVYLQ	459
sp P0CT20-2 DMAW_CLAPU	357	NDPMPPEPQVYFT	368	415	YLHAYISFSYRR-NKPYLSVYLH	464
sp P0CT20 DMAW_CLAPU	341	NDPMPPEPQVYFT	352	399	YLHAYISFSYRR-NKPYLSVYLH	448
sp Q6X2E3 DMAW_EPINE	343	NDPIPEPQVYFT	354	401	YIHSYISFSYRN-NKPYLSVYLH	450
sp Q6X2E1 DMAW2_EPICN	343	NDPIPEPQVYFT	354	401	YIHSYISFSYRN-NKPYLSVYLH	450
sp C5FTN3 DMAW_ARTOC	338	NQAMPEPQVYFT	349	396	YLHAYISFSYRK-GTPYLSVYLQ	446
sp Q6X2E2 DMAW1_EPICN	343	NDPIPEPQVYFT	354	401	YIHSYISFSYRK-NKPYLSVYLH	448
sp Q6X1E1 DMAW_BALOB	341	GDPMPPEPQVYFT	352	399	YLHTYISFSYRK-NKPYLSVYLH	465
sp Q12594 DMAW_CLAFS	348	DQMPPEPQVYFT	359	406	YLHTYVSFSYRK-NKPYLSVYLH	455
sp D4AK48 DMAW_ARTBC	338	DQAMPEPQVYFT	349	385	-----	385
sp D4D449 DMAW_TRIVH	338	DQAMPEPQVYFT	349	375	-----	375
sp A1DA62 FTMB_NEOFI	371	QSPFPDPQMYVC	383	430	HLCAVVSFAYKD-GGAYVTLVNH	463
sp Q4WAW7 FTMB_ASPFU	373	QSPFPDPQMYVC	384	431	HLCAVVSFAYKN-GGAYVTLVNH	464
sp B9WZX3 FTMB_ASPFM	360	-----	360	360	-----	360

Figure S10. Multiple sequence alignment of AdxC, aromatic prenyltransferase. AdxC has a glutamate predicted to bind to the substrate indole nitrogen and residues predicted to bind to DMAPP. AdxC lacks a lysine proposed to abstract the proton from the C4 position in 4-dimethylallyl tryptophan synthases, which is consistent with its proposed prenylation at C3.

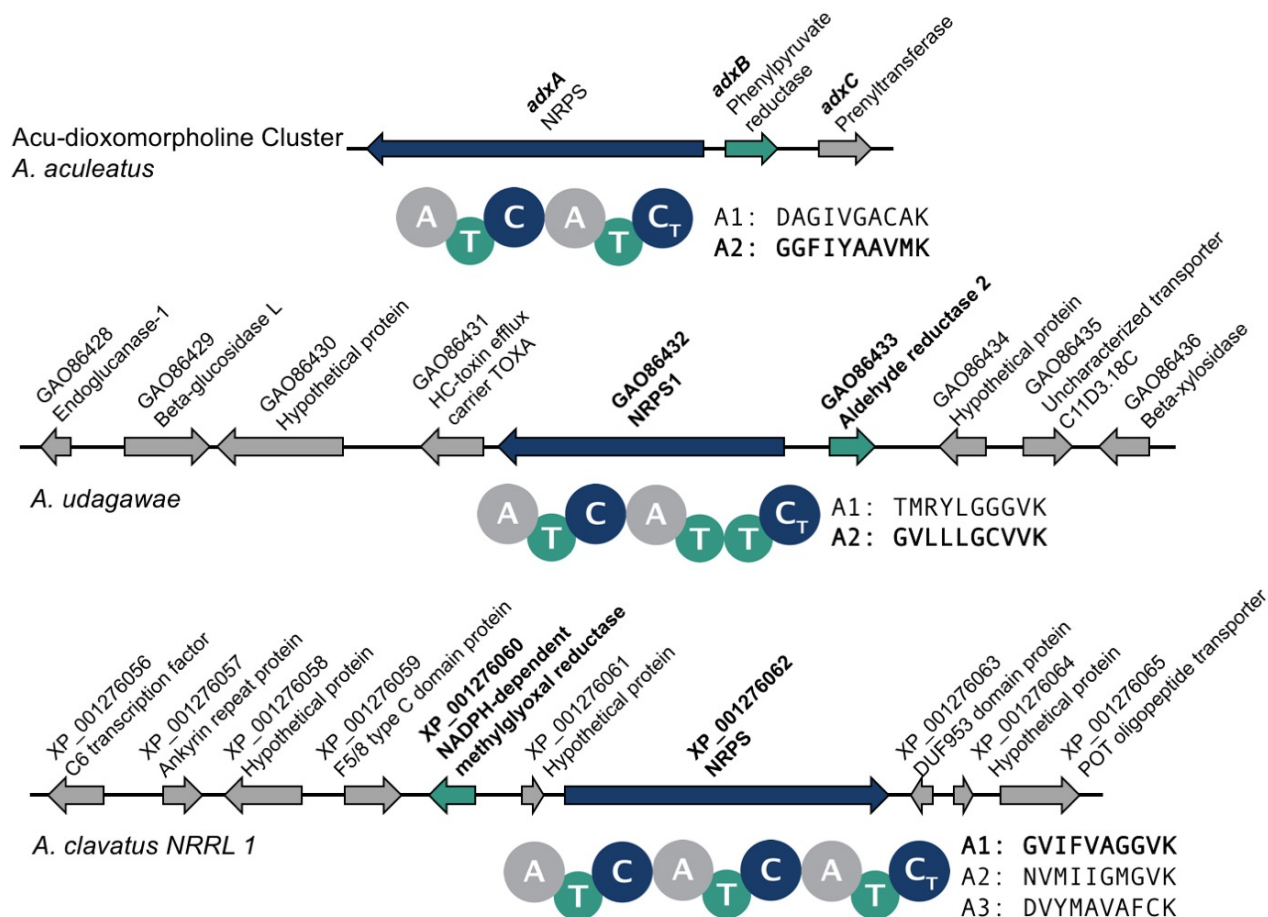


Figure S11. Putative diketomorpholine or α -hydroxy acid BGCs. These clusters were identified by BLASTP searches of AdxB. Genes are labeled with their GenBank accession numbers and functional annotations. The *A. udagawae* and *A. clavatus* clusters each contain an NRPS adenylation domain with substrate-binding residues similar to those of AdxA-A2.

Supporting Information References

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