Supporting information

Biosynthesis of albomycin δ_2 provides a template for assembling siderophore and aminoacyl-tRNA synthetase inhibitor conjugates

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Materials and methods

Strains, plasmids, and reagents

Streptomyces sp. ATCC 700974 was routinely maintained on mannitol-soybean (MS) agar or R2YE agar. It grows well in TSB liquid medium and albomycin production medium: 20g starch, 5g L-ornithine-HC1, 1.8g KH_2PO_4, 10.2g $Na_2HPO_4 \cdot 2\ H_2O$, 2g $(NH_4)_2SO_4$, 2g NaCl, 2g $MgSO4 \cdot 7H_2O$, 0.8g $CaC1_2 \cdot 2H_2O$, 0.28g FeSO4.7 H_2O, 0.02g $ZnSO4 \cdot 7\ H_2O$, 1 L deionized water (1). Cloning plasmids, *E. coli* cells (Table 1S) and restriction enzymes were obtained from Life Technologies, Inc. and New England Biolabs. CopyControl Fosmid Library Production Kit was from Epicenter, an Illumina company. MS, DNA Agar, LB, R2YE, YEME and TSB media were reported by Kieser et al. (3). All chemical reagents used in this study were purchased from Sigma-Aldrich, TCI America, FisherScientific and Carbosynth Limited unless otherwise indicated. Apramycin 50 µg/ml, chloramphenicol 12–25 µg/ml, ampicillin 100µg/ml, kanamycin 50 µg/ml, thiostrepton 12 µg/ml and nalidixic acid 25 µg/ml were used for selection of *E. coli* or *Streptomyces* transformants.

DNA manipulations and targeted gene replacement

Manipulation of genomic DNA was performed by salting out procedure (3). Plasmid DNA was purified with a GenElute plasmid miniprep (Sigma). Fosmid DNA was purified with FosmidMAX DNA purification kit (Epicenter). DNA fragments were isolated from agarose gels with a QIAEX II gel extraction kit (Qiagen). PCR amplifications were done with SuperTaq DNA polymerase (Life Technologies), and were purified with a GenElute PCR Clean-up kit. Digestion with restriction endonucleases and ligation experiments were carried out by standard procedures under conditions recommended with New England Biolabs. DNA sequencing was performed by Ohio University Genomics Facility and Functional Biosciences, Inc. (Madison, WI). Gene replacement experiments used the PCR targeting REDIRECT technology (John Innes Center) (2). Primer pairs used to amplify the apramycin resistance [*aac (3)IV*] cassette from pIJ773 by PCR are described in Table S2.

Generation and screening of a cosmid library

A cosmid library of *S. sp* ATCC 700974 genomic DNA was constructed using the CopyControl Fosmid Library Production Kit. Enzymatically sheared large DNA fragments were ligated to pCC1FOS cloning-ready vector, *in vitro* packed with the supplied phage packing extracts and transduced into Phage T-1 Resistant EPI30-T1^R *E. coli* strain. About 2,200 of the resulting transductants were picked and transferred to 96-well microtiter plates containing LB medium and 12.5 μg/ml chloramphenicol to grow and freeze for long term storage. Clones were replica plated onto LB agar with antibiotics. After overnight growth at 37 ºC, colonies were transferred to nylon membrane filters for *in situ* colony hybridization analysis. The UV-cross linked membranes were screened using a labeled probe that was generated using the [^{32}P] dCTP DNA labeling and detection (Rediprime II Random prime labeling system, GE Healthcare).

DNA sequence analyses

Primary sequence alignments were performed using Vector NTI Advance 10.3.0 (Invitrogen). Assembly and contig editing were performed using SeqMan II (DNASTAR). ATCC700974 genome sequence was obtained by next-generation sequencing (Illumina HiSeq 2000) and analyzed using RAST (Rapid Annotation using Subsystem Technology) version 4.0 and CLC Genomics Workbench. Non-ribosomal peptide synthetase domains were analyzed by online program http://www.nii.res.in/nrps-pks.html. The *abm* biosynthetic cluster sequence reported in this paper is available in the GenBank database under accession no. JN252488.

Complementation of ∆*abmE* and ∆*abmI* mutants and the increased expression of downstream genes

First, the conjugation origin *oriT* was amplified from pIJ773 with primers oriT-EcoRI-F/ oriT-SacI-R to clone into the corresponding sites in pSE34 (4) to create pSE34-oriT. For ∆*abmE* complementation, five genes *abmA* to E were amplified with primers Alb4-pSE-XbaI-F/ Alb1-2-3-20-pSE-HindIII-R to clone into the corresponding sites in pSE34-oriT. For increasing the expression of *abmE* downstream genes, PCR primers Alb1-2-3-20-pSE-XbaI-F/ Alb1-2-3-20-pSE-HindIII-R were used. The two cloning steps resulted in two plasmids, pSE34-oriT-abmA-E and pSE34-oriT-abmA-D, which were introduced into the ∆*abmE* mutant to produce AbmE-1 and AbmE-2 strains, respectively. Gene *ctjE* was also amplified with Ctj5-pSE-Xba-F/ CTIJ5-pSE-Nhe-R, and cloned into pSE34-oriT-abmA-D digested with XbaI to generate pSE34-oriT-abmA-D that was introduced to ∆*abmE* to obtain strain AbmE-3. For complementation or increasing downstream gene expression in the ∆*abmI* mutant, an *abmE* to *abmH* (or *abmI*) fragment was amplified with primers Alb7(or Alb8)-pSE34-XbaI-F/ Alb4-NheI-R to clone into the

pSE34-oriT-abmA-D plasmid digested with XbaI. Plasmid pSE34-oriT-abmA-H (or pSE34-oriT-abmA-I) was resulted. This plasmid was first introduced into a Δ*abmH* strain to obtain AbmH-1, which showed restored albomycin production. The alternative plasmid plasmid was introduced into the Δ*abmI* mutant to create AbmI-1.

Overexpression of recombinant His$_6$-tagged AbmI and CtjF

Two NdeI/XhoI fragments harboring *abmI* gene from cosmid 8F8 and *ctjF* gene from *Streptomyces sp*. C were amplified with pET28-abmI-Nde-F/ pET28-abmI-Xho-R and pET28-ctjF-Nde-F/ pET28-ctjF-Xho-R primers. They were individually cloned into the corresponding sites of pET28a (Novagen) to obtain pET28-AbmI and pET28-CtjF. The plasmids were transformed to Rosetta 2(DE3)pLysS cells. The transformants were grown overnight in LB medium supplemented with kanamycin (50ug/ml) and chloramphenicol (25ug/ml) at 37°C, then diluted 100-fold into fresh Terrific Broth supplemented with antibiotics. The cultures were grown at 37°C until an A_{600} of ~0.6 was reached. The cultures were chilled on ice for 30 min. Then isopropyl-1-thio-β-D-galactopyranoside was added to a final concentration of 0.1mM (for AbmI and CtjF induction), and continued to grow with shaking at 20°C overnight. The cells were harvested by centrifugation at 5000 r/min for 15 min, and stored frozen at -70°C until further use.

Purification of His$_6$-tagged AbmI and CtjF

Cell pellet from 1 liter culture was re-suspended with 40ml B-PER protein extraction reagent (Thermo scientific) and PMSF was added to 1mM. The mixture was gently shaken at room temperature for 10min. Then lysozyme was added to 200uM and Benzonase nuclease added to 25 unit/ml. Continue shaking at 4°C for 30 min. Then the lysate was centrifuged at 12,000rpm for 30 min. Ni-NTA His·Bind Resin (Novagen) was added to the supernatant (1mL/L culture) and mixed gently at 4°C for 60min. The protein resin mixture was loaded onto a gravity flow column, and proteins were eluted with increasing concentrations of imidazole in elution buffer A (50mM NaH_2PO_4, 300 mM NaCl, pH8.0). Normally, 40 mM imidazole could wash the impurities from the column. Purified proteins could be eluted with 200 mM imidazole. The purified proteins were dialyzed and concentrated using Amicon ultrafiltration membrane filters (Millipore). The resulting protein was stored at -20 °C in elution buffer containing 15% glycerol and 10mM DTT. It is estimated His$_6$-AbmI and CtjF was produced at 2-3 mg/liter culture. Molecular weights under denature conditions were determined by SDS-PAGE. Concentrations

were determined by Bio-Rad Protein assay (Bio-Rad). Gel filtration was performed using a Sepharose 12 column on a Pharmacia Biotech FPLC with a flow rate of 0.3 ml/min in buffer C (50mM pH7.2 phosphate buffer,150mM NaCl). Bio-Rad gel filtration standards were used as markers for calculation of the molecular weight of the purified His_6-AbmI.

Figure S1. Putative *cchB* homologous gene clusters in *Streptomyces sp.* ATCC 700974 and *Streptomyces griesus* NBRC13350. Coelichelin gene cluster (*cchB*) has been characterized in *Streptomyces coelicolor* as in the reference. The vertical lines indicate the boundaries where the *cch* genes are conserved. *orfs1-9* are the homologs of *cchB-J*. Note that *cchA* and *cchK* are missing at the locus in the genome of ATCC 700974.

Figure S2. (a) PCR analysis of a Δ*abmB* mutant strain. *abmB* was initially identified as a *cchB* homlog in a fosmid clone 8F8 along with other putative albomycin biosynthetic genes. (b) Bioactivities of the Δ*abmB* and wild-type strains against *E. coli* BW25113 measured by an agar diffusion bioassay, and HPLC profiles of the fermentation broth of the Δ*abmB* and wild-type.

Figure S3. PCR analysis of the *abmE* and *abmI* gene deletion mutants in comparison with the WT. The in-frame deletion mutants were generated with the REDIRECT technology.

Figure S4. ESI-MS of the purified compound from a pAbmA-D/Δ*abmE* strain grown on albomycin production agar medium. The spectrometer was set in positive mode to detect the albomycin related compounds in this work.

Figure S5. HPLC and MS analysis of the purified albomycin δ_2 from the Δ*abmE* mutant genetically complemented with *abmE* or *ctjE* genes, or the WT with the vector (pSE34) only. The HPLC conditions are stated in material and methods for the Synergi 4μ Fusion-RP 80Å column.

Figure S6. HPLC profile of the purified albomycins and partially purified bioactive metabolites of the *ΔabmE*. The HPLC conditions were like in Figure S3 except that the flow rate was 0.6 ml/min in this graph and each elution gradient was proportionally longer in order to resolve albomycin ε and δ_2. Each of the purified albomycin subtypes has been verified by ESI-MS analysis.

Figure S7. Bioassays of the bioactive compound produced by a Δ*abmI*/pAbmA-H mutant. *fhuA* is the transporter gene for ferrichrome siderophore. AbmK+ is an *E. coli* strain with abmK expressed on a plasmid previously published in this lab (Zeng, et al. 2009). AbmK- is the strain with a control plasmid.

Figure S8. Characterization of the His_6-AbmI. (a) Purified His_6-AbmI on SDS-PAGE. (b) Molecular weight determination of His_6-AbmI with gel filtration chromatography.

Figure S9. HPLC analysis of an extended AbmI *in vitro* reaction without *S*-adenosyl-L-homocysteine (SAH) nucleosidase. Notice that the major peak adjacent to cytidine in Figure 5 III and IV, which is adenine, did not exist here.

Figure S10. pH profile of the AbmI catalyzed *in vitro* reaction.

Figure S11. Extracted LC/ESI-MS ion chromatogram of the AbmI catalyzed *in vitro* reaction with various nucleoside substrates.

Figure S12. Side-by-side comparison of the AbmI and CtjF reactions with indicated substrates. S, substrate; P, product.

Figure S13. Albomycin δ_1 prepared by mild basic hydrolysis of albomycin δ_2. (a) HPLC chromatogram. It was run on a Phenomenex Sphereclone HPLC column (see materials and methods). (b) ESI-MS analysis.

Figure S14. AbmI catalyzed methylation of blasticidin S. (a) HPLC chromatogram. UV Detection: 270nm; (b) Extracted LC/MS ion chromatogram; (c) Stable isotope (deuterium) labeling of the *in-vitro* methylated blasticidin S.

Figure S15. Three hypothetical pathways for the formation of the C7N amino acid containing a 4'-thioxylofuranose-1-phosphate moiety. They start from three proposed reaction mechanisms of the PLP-dependent enzyme AbmD in transforming sulfur amino acids. Each pathways has its major pros (P) and cons (C). Pathway a, P: The AbmH reaction is like the proposed function of other homologs in uridyl peptide biosynthesis, C: a decarboxylation of Cys and installation of 5'-aldehyde is not clear. Pathway b, P: A smallest number of reactions are required and fewer intermediates possibly generated, C: no assignment for AbmH. Pathway c, P: closely agrees to the predicted function of the proteins and explains some stereochemistry, C: The AbmH reaction is distant from other antibiotic biosynthesis homologs. 2-Phosphoenolpyruvate is assumed to be a co-substrate in pathway a and b. But other C3 glycolytic metabolite such as 3-phosphoglyceraldehyde is not excluded. A possible phosphoryl migration catalyzed by AbmL (a phosphodiesterase homolog) might be involved if 2-phosphoenolpyruvate is the substrate.

Table S1. Strains, plasmids and cosmids used in this study

Strain/Plasmid /Cosmid	Relevant characteristics	Reference or Source
***S.sp* ATCC700974 strains**		
WT	Wild-type of albomycin producing strain	ATCC
ΔabmE	Non-producer for albomycin generated through disruption of AbmE by aac(3)IV	This study
ΔabmH	Non-producer for albomycin generated through disruption of AbmH by aac(3)IV	This study
ΔabmI	Non-producer for albomycin generated through disruption of AbmI by aac(3)IV	This study
AbmE-1	Producer for albomycinδ2 generated through expression of AbmE and four downstream genes of AbmA-D in ΔabmE	This study
AbmE-2	Producer for albomycinε generated through expression of four downstream genes of abmA-D in ΔabmE	This study
AbmE-3	Producer for albomycinδ2 generated through expression of CtjE and four downstream genes AbmA-D in ΔabmE	This study
AbmI-1	Non-producer generated through 8 downstream genes of AbmA-H in ΔabmI	This study
AbmH-1	Producer for albomycins generated through expression of 8 downstream genes of AbmA-H in ΔabmH	This study
***E. coli* strains**		
JM109	*recA*1, *endA*1, *gyrA*96, *thi*, *hsdR*17, *supE*44, *relA*1, Δ(*lac-proAB*)/F' [*traD*36, *proAB*$^+$, *lacI*^{q}, *lacZ*ΔM15]	This study
ET12567 /pUZ8002	dam dcm hsdS pUZ8002	John Innes
BW25113/pIJ790	*lacI*^{q}*rrnB*$_{T14}$*ΔlacZ*$_{WJ16}$*hsdR514 ΔaraBAD*$_{AH33}$*ΔrhaBAD*$_{LD78\ pIJ790}$	John Innes
EPI300TM-T1R	Host cell for construction of genomic cosmid library	EPICENTER
Rosetta™2(DE3)pLysS	F-*ompT hsdS*B(rB- mB-) *gal dcm* (DE3) pLysSRARE2 (CamR)	EMD
Plasmids		
pET28a	*Kan,T7 promotor*	Novagen
pSE34	*bla, tsr, ermE*promotor*	This study
pIJ773	*bla,aac(3)IV,oriT*	John Innes
pIJ790	*cat,oriR101,repA101ts,ParaBAD*	John Innes
pSE34-oriT	pSE34 derivative carrying 235 bp oriT fragment from pIJ773	This study
pET28-abmE	pET28a carrying complete abmE gene	This study
pET28-abmI	pET28a carrying complete abmI gene	This study
pET28-ctjF	pET28a carrying comlete CtjF gene	This study
pSE34-oriT-abmA-D	pSE34-oriT derivative carrying complete genes from abmA to abmD	This study
pSE34-oriT-abmA-H	pSE34-oriT derivative carrying complete genes from abmA to abmH	This study
pSE34-oriT-abmA-D-ctjE	pSE34-oriT derivative carrying complete genes from abmA to abmD and ctjE	This study
Cosmids		
8F8	A positive cosmid selected for sequencing	This study
5C3	A positive cosmid selected for sequencing	This study

22H10	A positive cosmid selected for sequencing	This study

oriT, origin of transfer of plasmid RK2; aac(3)IV, apramycin resistance gene; Kan, kanamycin resistance gene; tsr, thiostrepton resistance gene

Table S2 Primers used in this study (5'-3')

CchB-F2-tag,GTA AAA CGA CGG CCA GGG CGC SGG CCA GAG CGC SGC SGA

CchB-R2-tag, CAG GAA ACA GCT ATG ACG TCS ACC ACS GAG TAG TTS GT

AbmE-knockout-F,GCG GGA TGC TTC ACC GGC AAA CGC GTC TCC GGT TTC CGG GAG ATT CCG GGG ATC CGT CGA CC

AbmE-knockout-R,ACT GGA CCC GTG CCG TGC CGT CGA CGT GCA CCG CGC TCG GCG CTG TAG GCT GGA GCT GCT TC

AbmE-knockout-check-F, GCC ACG GTG CTC GAT CCG GGG CTC AGC

AbmE-knockout-check-R, AGA TCG ACC CCC AGT TCG GAA CCG AGC

AbmI-knockout-F, CGG GTC AAC GAG AAC GCC TGG GAC GTG CGC ACG CCC GTG ATT CCG GGG ATC CGT CGA CC

AbmI-knockout-R,CCT CCT GGG TCG CTG CGG GAG CTG TCG TCG TGT CGG TCA TGT AGG CTG GAG CTG CTT C

AbmI-knockout-check-F,CTG GAG TAC GAC GGC ACG GTG CGC CC

AbmI-knockout-check-R,ACG TTC TCG CTC GGC ACC AGG TTC AGC

pET28-abmE-NdeI-F, TTG ATC CAT ATG AAC AGC TAC TTC GAG CAC C

pET28-abmE-HindIII-R, GGA CCG AAG CTT TCA TGC CGT TCC CTC CGC

pET28-abmI-Nde-F, GCGAT CCG CAT ATG ACC GGG CTC CGG GCC GGT CAC C

pET28-abmI-Xho-R, TGT CGG CTC GAG TCA AGA CTT CCT CCG TGC GGT G

pSE-abmA-D-Xba-F, AAC GGC TCT AGA ATG ACG GTC CTT CCC CTC GGC GT

pSE-abmA-D-Hind-R, GGG TAA GCT TTC AGC GGT CGT GCC GGG CGG

pSE-abmI-XbaI-F,CTA GTC TAG AGT GGC CGC CCT CTT CGG CGC GCT CGG CCG CGA TCA GG

AbmE-NheI-R, ACC GGC TAG C TTCC CT CC T CAT GCC GTT CCC TCC GCC ACG

AbmA-H-check-F1, CGA TGG TCA TCA ACA CCT CGC TCA ACG

AbmA-H-check-R1, GCT CCT GGA CGT CAC CGC AGT ACA CG

AbmA-H-check-F2, GTA CAT GCT GTT CAC CAC CCG TGC

AbmA-H-check-R2, GAC CCA GCT CGA CGC TGC CGA GAA GAC

oriT-EcoRI-F,CGC TGC GAA TTC ATA ACC CTG CTT CGG GGT CA

oriT-SacI-R,TCG AAG GAG CTC TTC CCG CCA GCC TCG CAG AG

pSE-ctjE-Xba-F, ACG ATC TAG AGT GAT CGT CCT GGG AGT CAA CAG C

pSE-ctjE-Nhe-R, CGG AGC TAG CTC ACC CGT CCT TCT CGT GGA AGA ACC

pET28-ctjF-Nde-F, GGT GAT CCC ATA TGA CCG CCG ACA CCA CCC CCA CGA CG

pET28-ctjF-Xho-R, GGT GGG CTC GAG TCA CGT CGT CTT CCT CGT GGC GGT GAT

Apra-F1,CAG AAG GAA GGT CCA GTC GGT CAT GC

Apra-R1,TAC GGC ATC AGT TAC CGT GAG CTG C

Alb1-knock out-F,GGA AGG CTC ACC GAT TTC ATC AAC CAC AAG ATC ATC TTC CCC ATT CCG GGG ATC CGT CGA CC

Alb1-knock out-R,GCT GCC GGT CGT CGT CCG ACA GCT CTC CGT GAT GGC CCG GAG TGT AGG CTG GAG CTG CTT C

AbmB-KO-check-F, GAC TCG CCT GAG CGG ACG GGA ACA CGA

AbmB-KO-check-R, CGT GAT GAG GAA CGG CCG GTC GGA GCC

AbmC-knock-out-F,CTG GAC CGG TTT CGT CGT GTG GTG GAT GAG GAG CTG CCC GCC ATT CCG GGG ATC CGT CGA CC

AbmC -knock-out-R,CCA CGA GGG AAG GAC CGG CTC GTC CCT GGT CTG GAA CGC GAA TGT AGG CTG GAG CTG CTT C

AbmC -knock out-check-F, TGC CAC CGG TTC CTG ACG TCG GGC CC

AbmC -knock out-check-R, CCG CCA TCC CTC TGT CCT CCT TCT GC

AbmH-knock out-F, ATC GAA CAG GAG ACG CTG CTC CCC CTC CTG CGC GAC CAG GCC ATT CCG GGG ATC CGT CGA CC

AbmH-knock out-R, CCA GCG CGT CGT CCT CGA AGC AGT AGC GCG GGC GGT ACA GCC TGT AGG CTG GAG CTG CTT C

AbmH-knock out-check-F, CGA GGA GTA CCG CTG GCA GCA CCC GGT

AbmH-knockout-check-R, CGC CGG ACC AGG TTG TCC AGC AGC AG

AbmL -knock out-F,GTC TGG CTC CAC CCC GCG AAA GGG GAC GAG CCC GTG TTC CGG ATT CCG GGG ATC CGT CGA CC

AbmL -knock out-R,GGT GCG CCA CAC CGC CAG GGT GTC GAA GCG CGC CCG AAG CGG TGT AGG CTG GAG CTG CTT C

AbmL -knock out-check-F,CAG CTG GTT CAC CGA GGA GGA CCT CG

AbmL -knock out-check-R,CCC AAG CGT TAG GGC CTC GTC CGT CC

AbmM -knock out-F,GGC GTC GAG GTC CTC CTC GGT GAA CCA GCT GTC CCA GTC CCG ATT CCG GGG ATC CGT CGA CC

AbmM-knock out-R,CTG CTG CGC CAG GAC ATC GAC GAC TTC GTC CGG GTC GCT CGC TGT AGG CTG GAG CTG CTT C

AbmM -knock out-check-F,CGT TCC CGC AGG CGC TCG ACC TCG

AbmM -knock out-check-R,CAC CAG ACC CTC GCC GAC GCC ATC

AbmQ -knock out-F,CTG CTC GAC TGA CCT CAT CCC CCA TCG CCG GAG GAC CCC ATG ATT CCG GGG ATC CGT CGA CC

AbmQ -knock out-R,TCA TCG CGC CCG CCC GGT CCG CAG GGC CGC GAC CGC CGA GGC TGT AGG CTG GAG CTG CTT C

AbmQ -knock out-check-F,CTC TGG CCA CGA TGC CGA TGG CCC

AbmQ -knock out-check-R,CGA CGG TGA GAC GCA GCC GAT CAT

Orf4-knock out-F,GGA GGT TTC TGC AGG TTG CCG AAC GGC ACG GTG GTG GTC GCC ATT CCG GGG ATC CGT CGA CC

Orf4-knock out-R,GAG CAG GCC GTC CGG ATG GTG CAG CAC CGC CGT GAC CTC GTC TGT AGG CTG GAG CTG CTT C

Orf4-knock out-check-F,ACC GTC CTG GTC AGC CCC TCG GGT C

Orf4-knock out-check-R,TCA CCG CCC GGC ACG ACC GCT GAA C

oriT-EcoRI-F,CGC TGC GAA TTC ATA ACC CTG CTT CGG GGT CA

oriT-SacI-R,TCG AAG GAG CTC TTC CCG CCA GCC TCG CAG AG

Alb4-pSE-Xba-F, CGG GTC TAG AAT GAA CAG CTA CTT CGA GCA CC

Alb1-2-3-20-pSE-Xba-F, AAC GGC TCT AGA ATG ACG GTC CTT CCC CTC GGC GT

Alb1-2-3-20-pSE-Hind-R,GGG TAA GCT TTC AGC GGT CGT GCC GGG CGG

Alb7-pSE-XbaI-F, CTA GTC TAG AGT GGC CGC CCT CTT CGG CGC GCT CGG CCG CGA TCA GG

Alb4-NheI-R, ACC GGC TAG C TTCC CT CC T CAT GCC GTT CCC TCC GCC ACG

Alb8-pSE-Xba-F,TCC GTC TAG AGT GAC CGG GCT CCG GGC CGG TCA C

Alb8-pSE-Nhe-R, TCG G GC TAG C TC AAG ACT TCC TCC GTG CGG TGA TCG

Apra-F1, CAG AAG GAA GGT CCA GTC GGT CAT GC

Apra-R1, TAC GGC ATC AGT TAC CGTGAG CTG C

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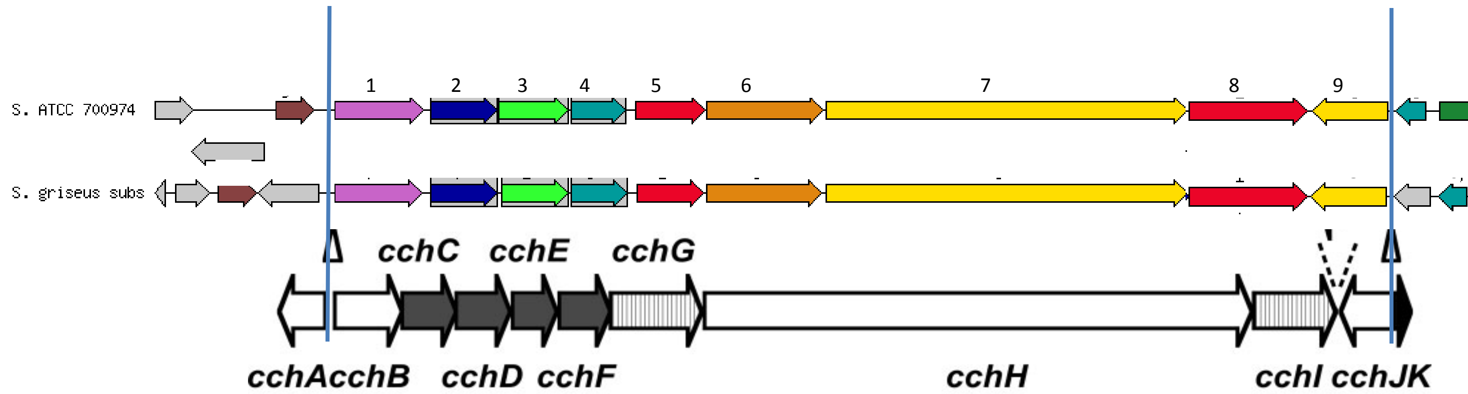
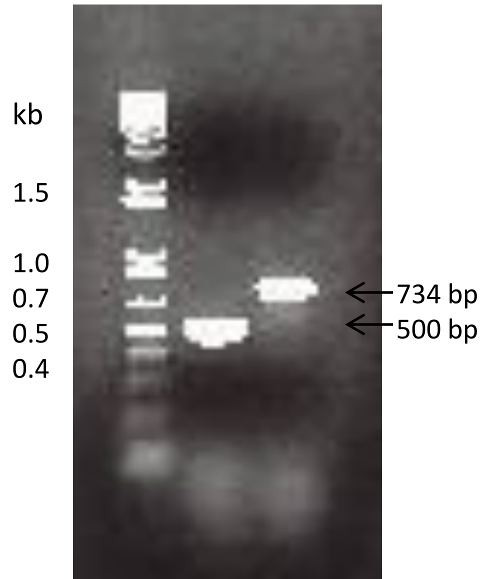
Supporting Figure S1 (Figure S1)

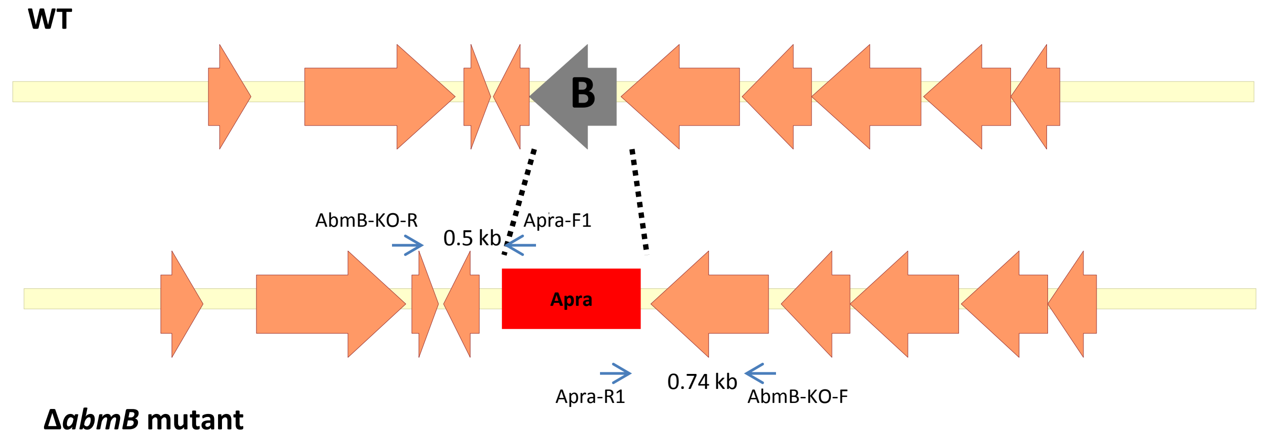


Coelichelin *cch* gene cluster from *S. coelicolor.* Microbiology (2006) 152: 3355-3366

Supporting Figure S2 (Figure S2)

a.





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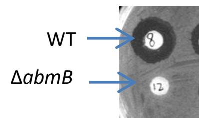
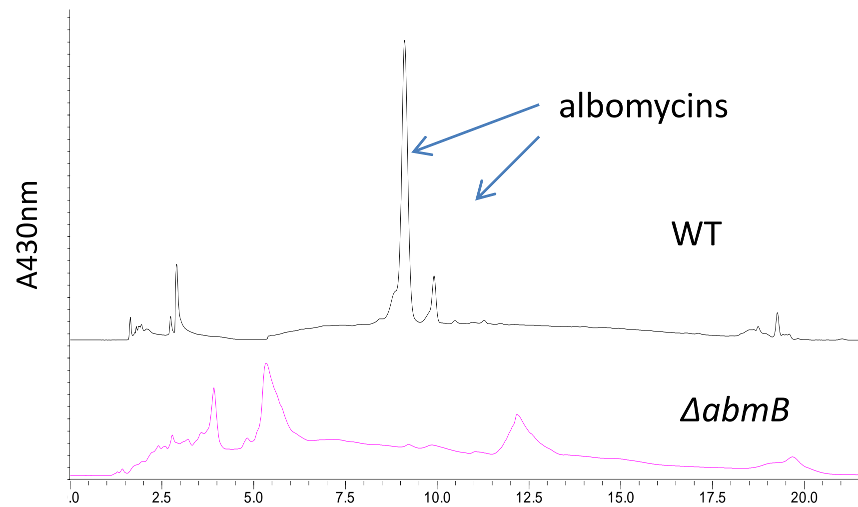
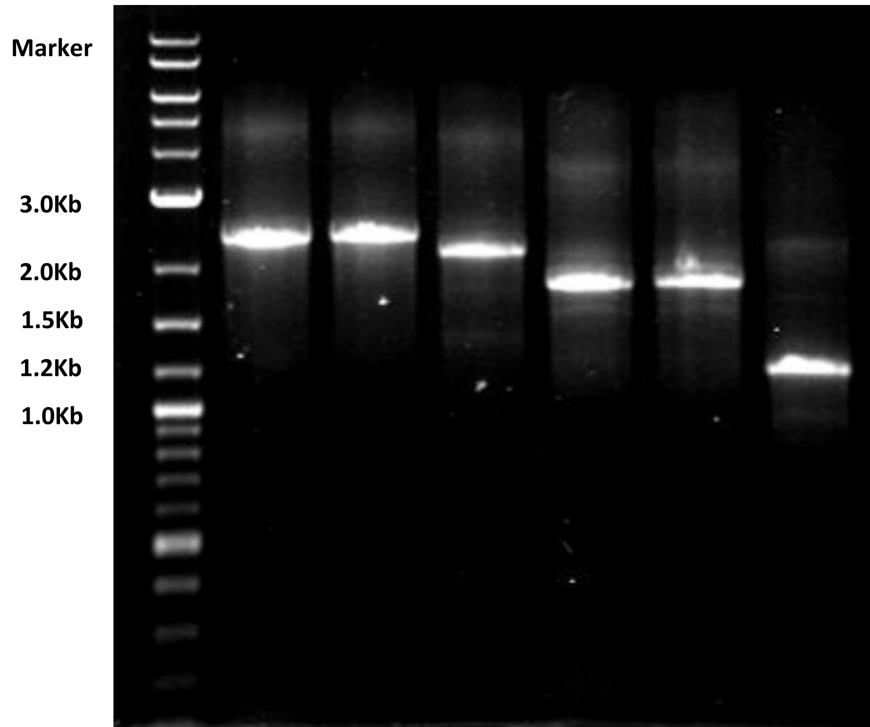
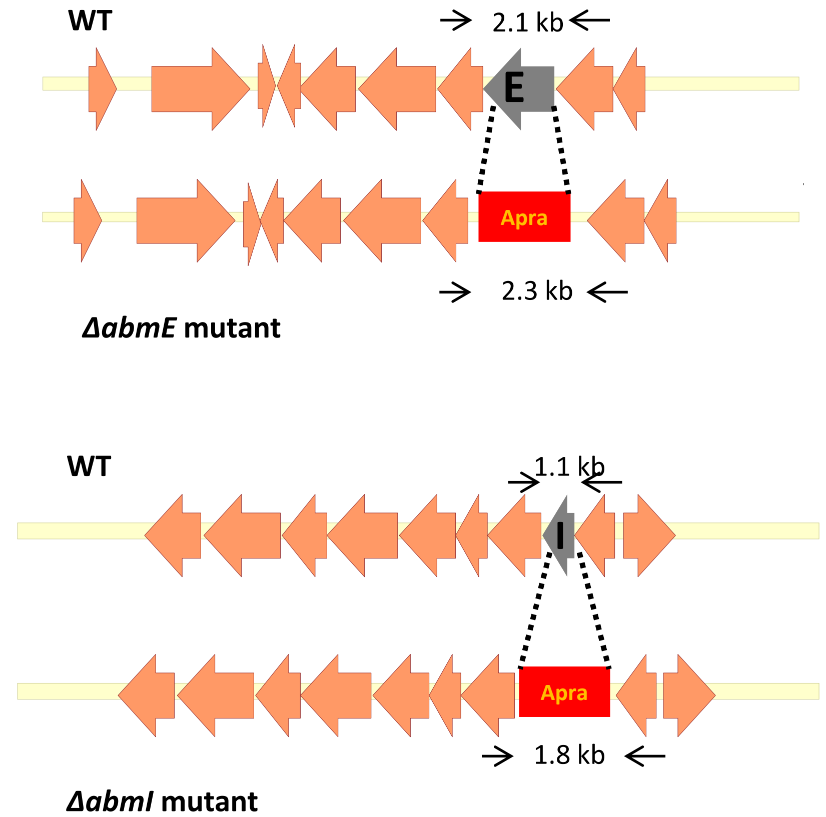




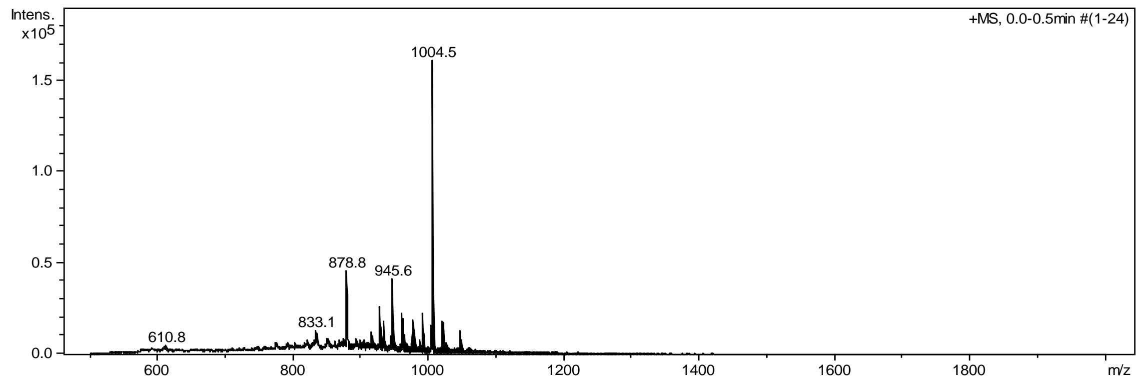
Supporting Figure S3



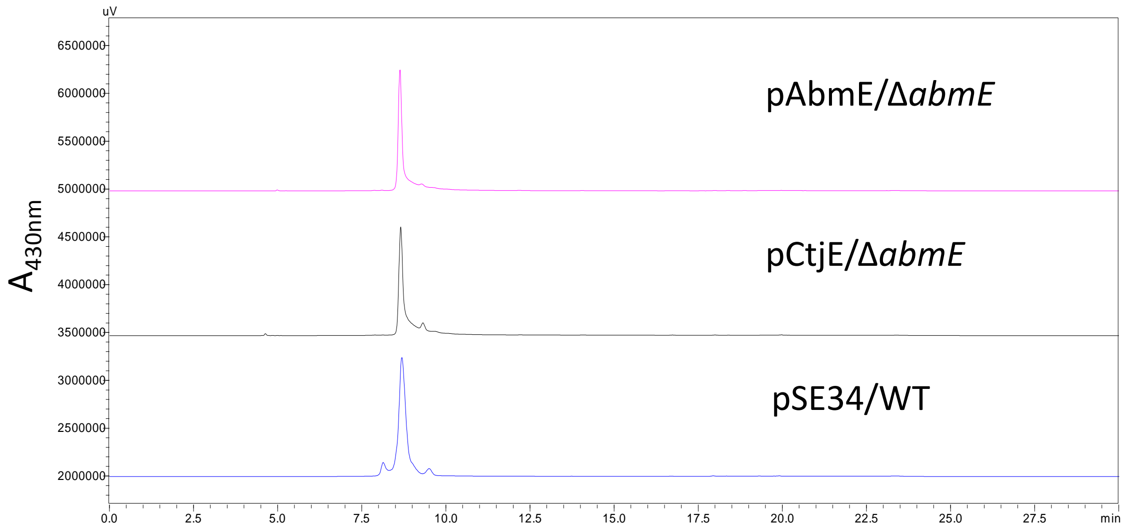
Stains	*ΔabmE*_1	*ΔabmE* _2	WT	*ΔabmI*_1	*ΔabmI*_2	WT
Size(Kb)	2.3	2.3	2.1	1.8	1.8	1.1
Primers	AbmE-check-F/AbmE-check-R			AbmI-check-F/AbmI-check-R		

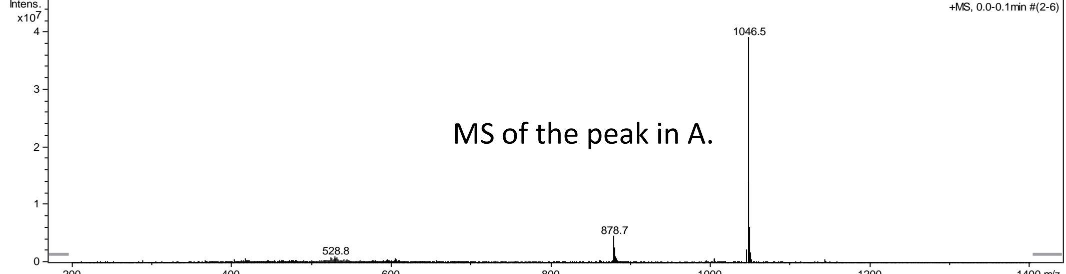


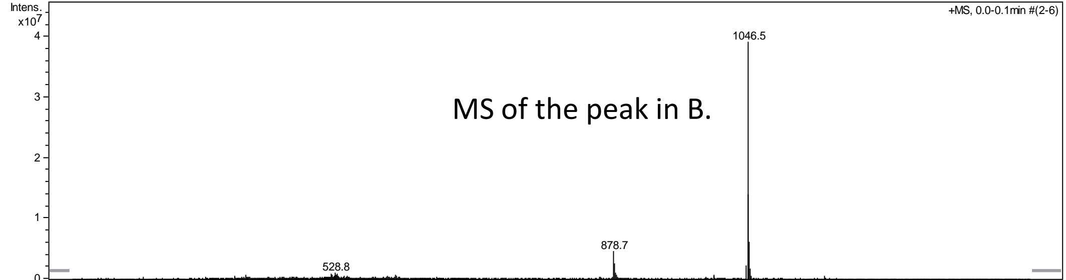
Supporting Figure S4

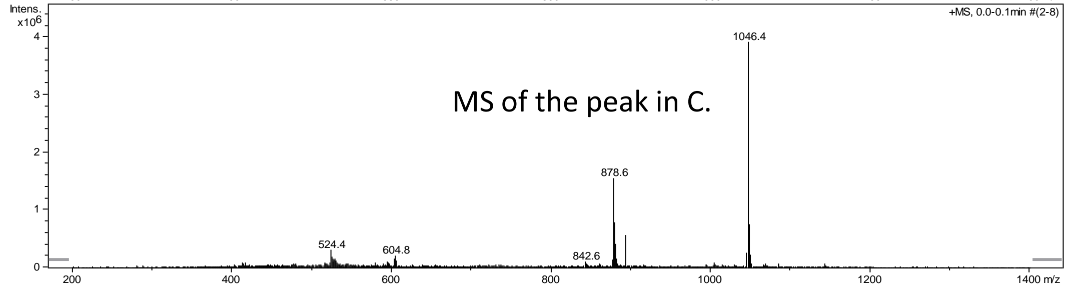


Supporting Figure S5



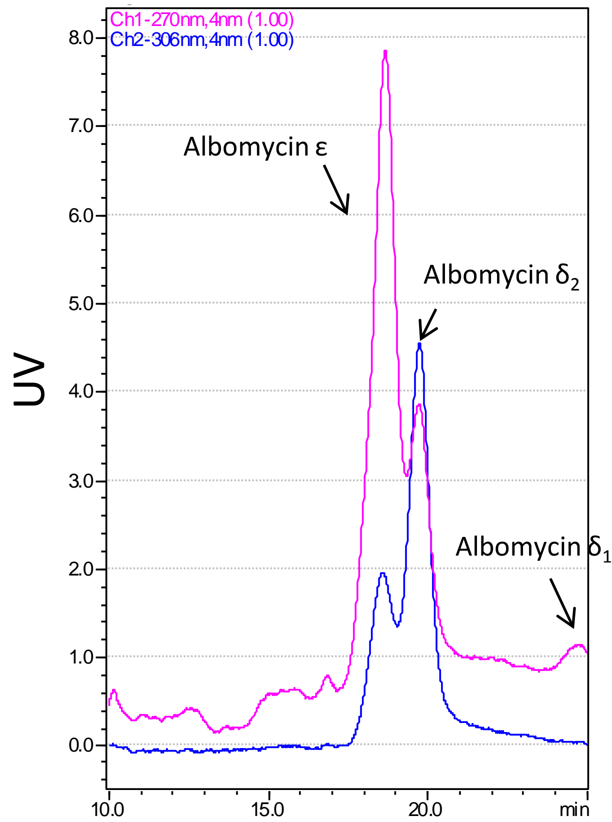




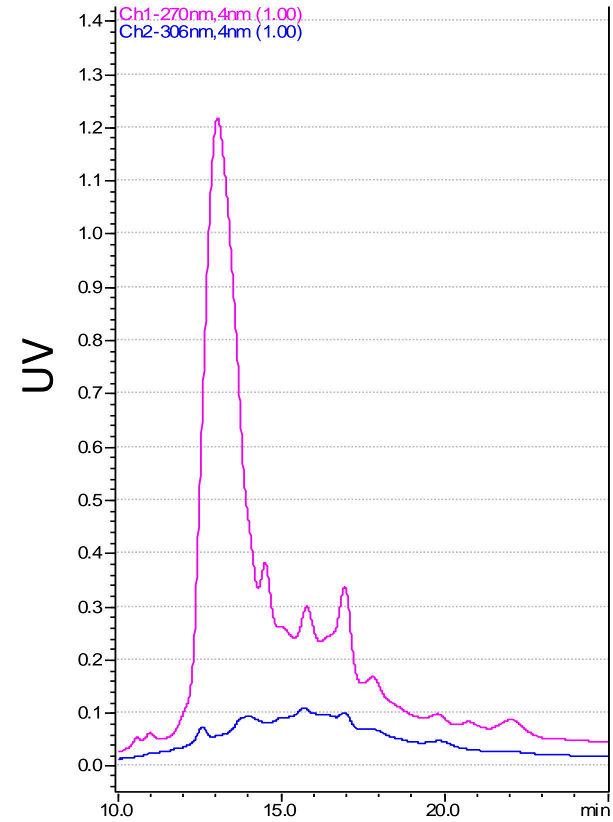


Supporting Figure S6

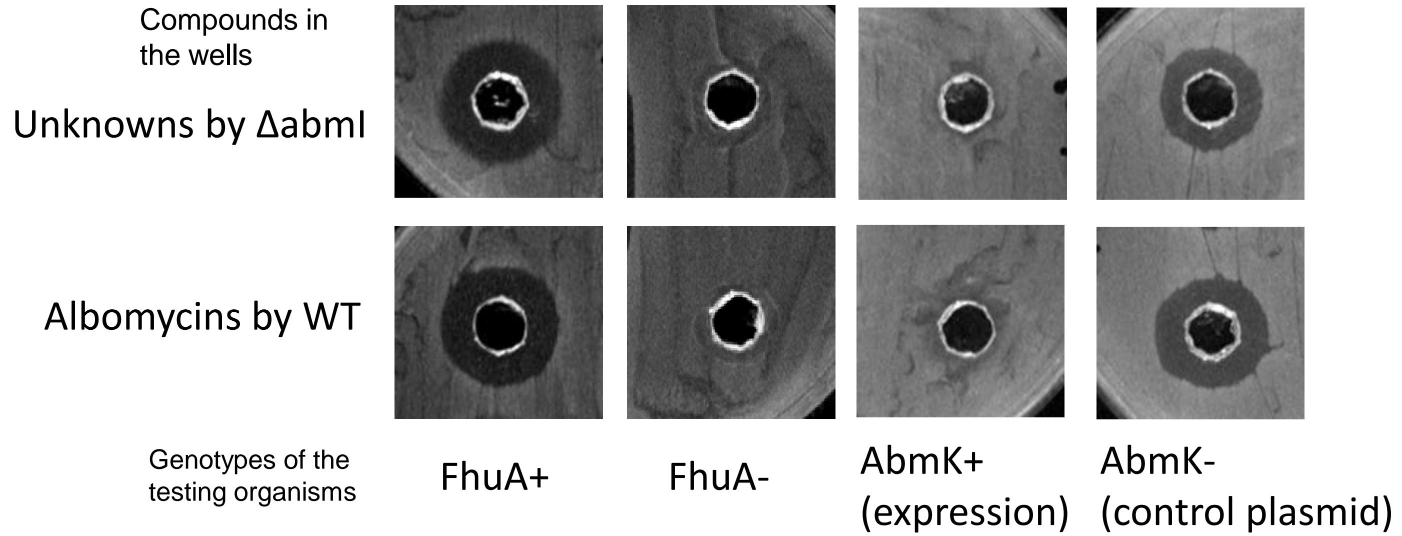
HPLC analysis of purified albomycins



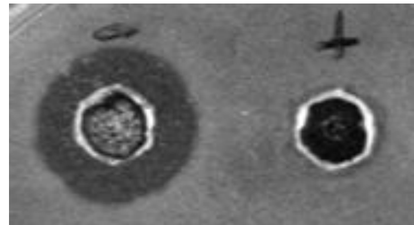
HPLC analysis of an active faction extracted from ΔabmI mutant



Supporting Figure S7

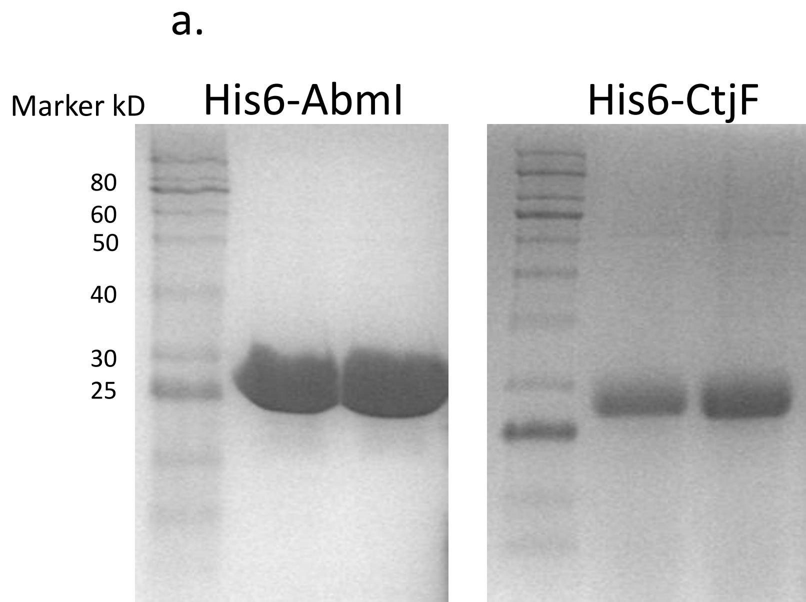


ΔabmI extracts treated
by protease K

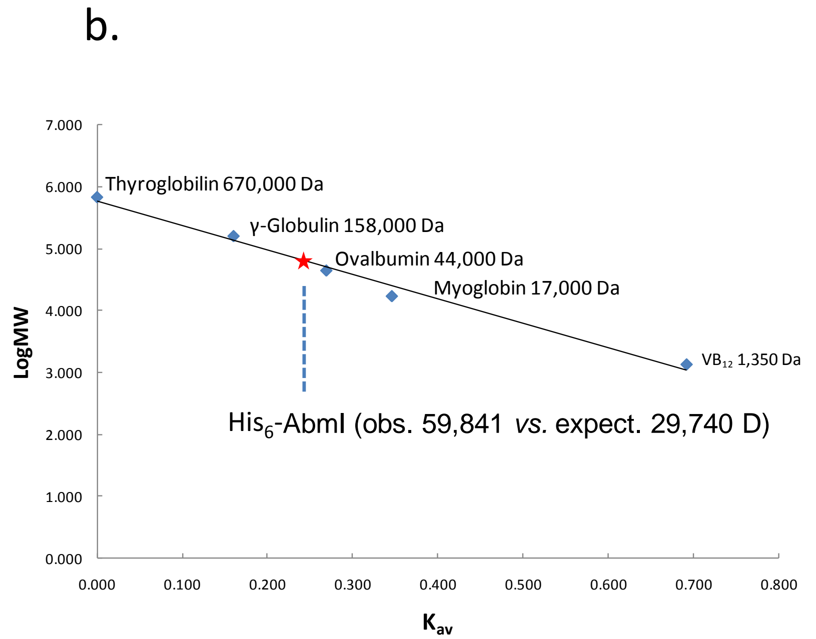


Supporting Figure S8

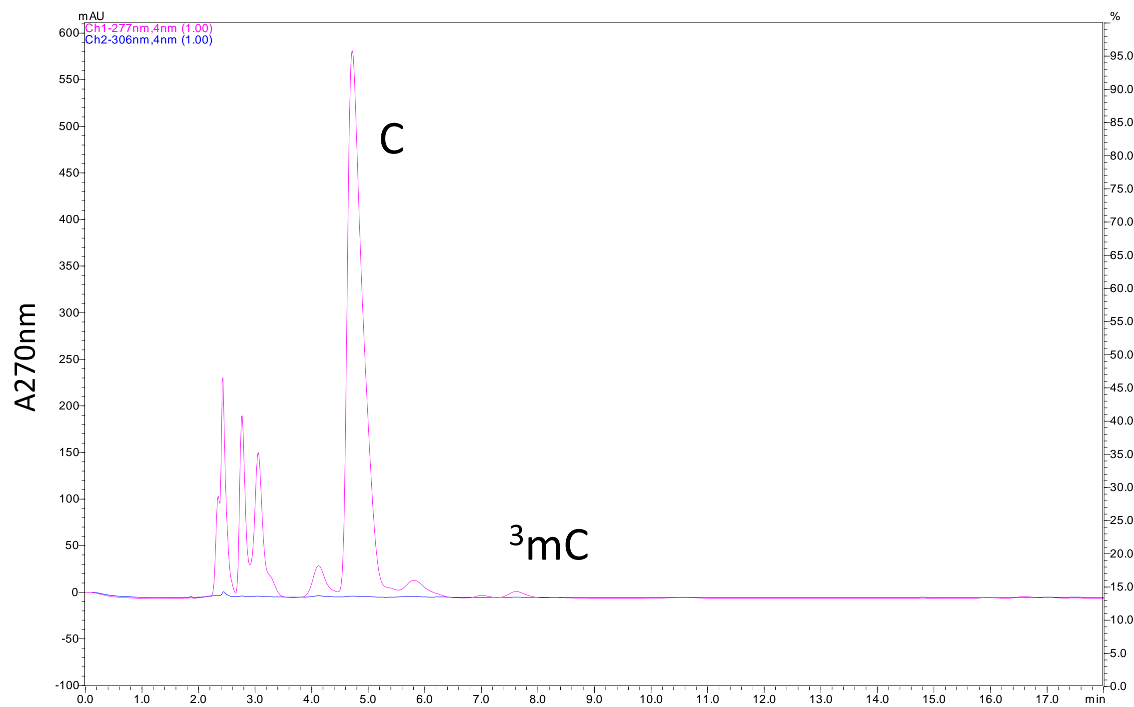
a.



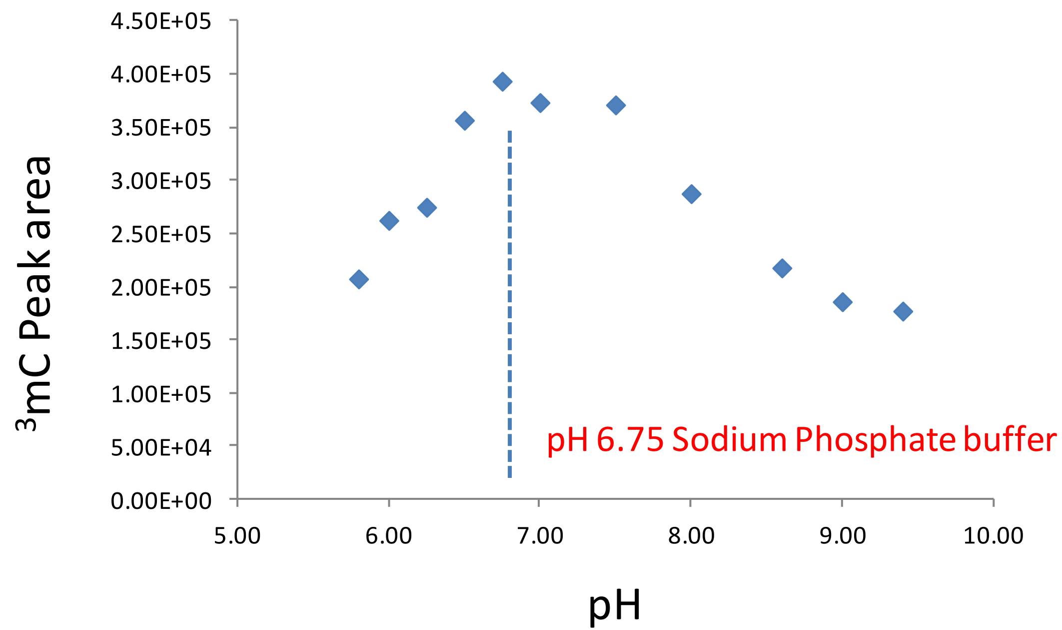
b.



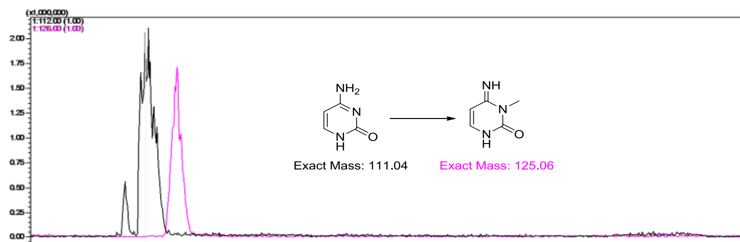
Supporting Figure S9

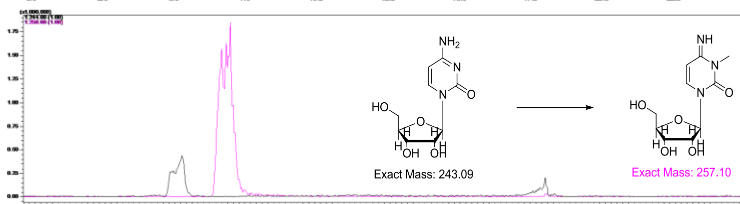


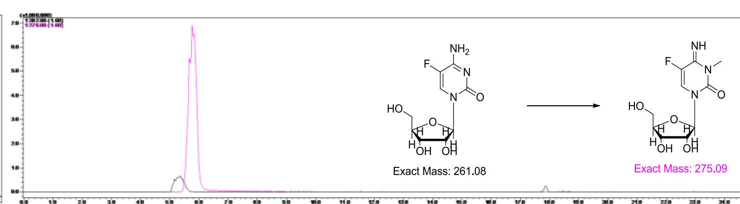
Supporting Figure S10

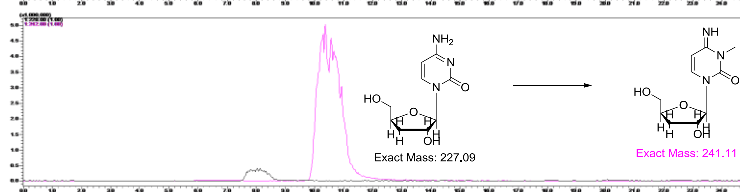


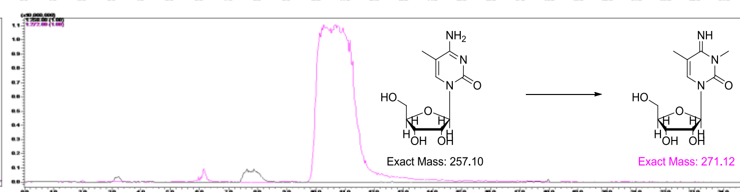
Supporting Figure S11

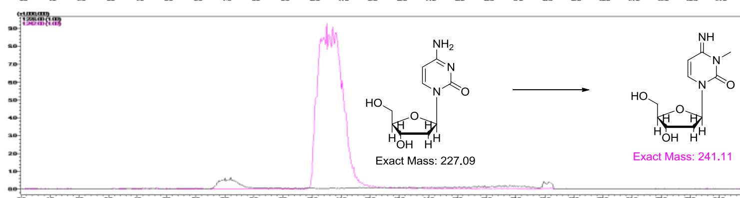


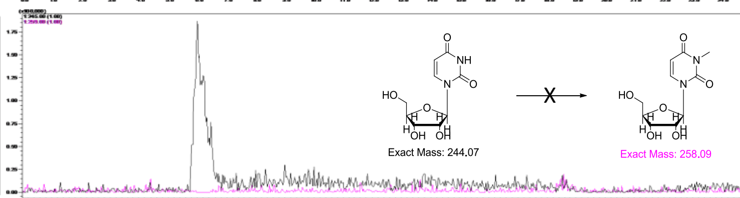




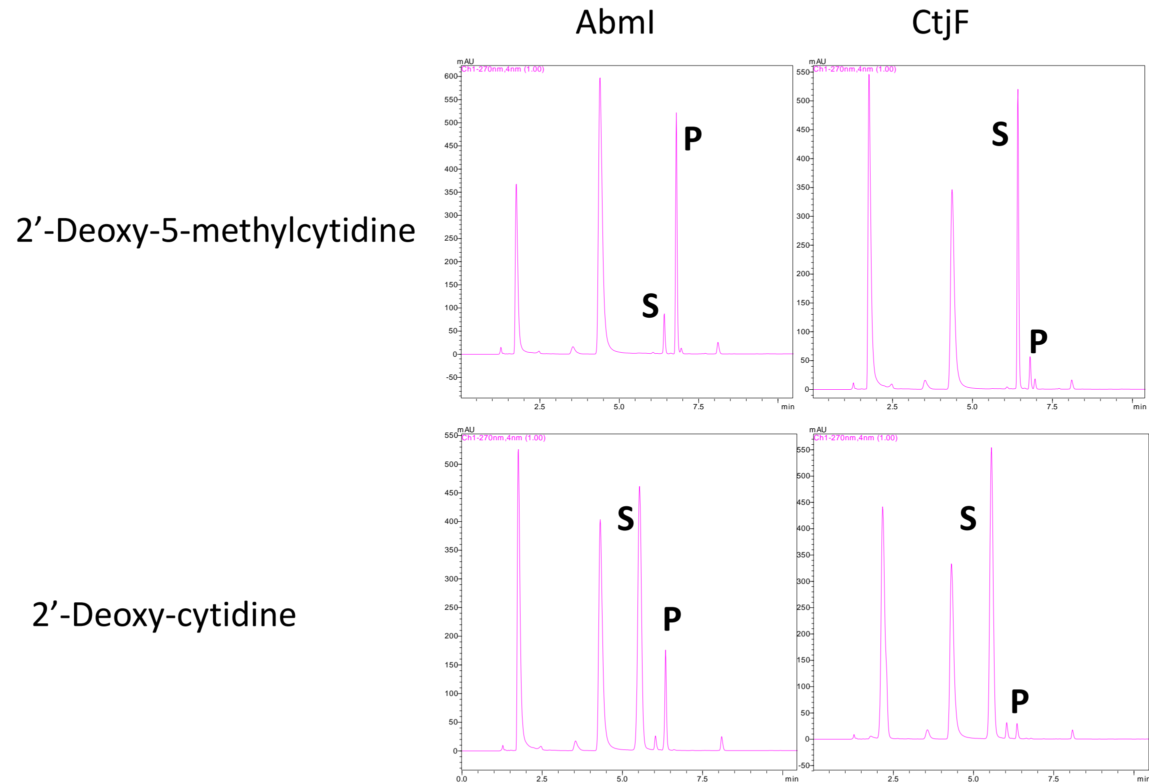






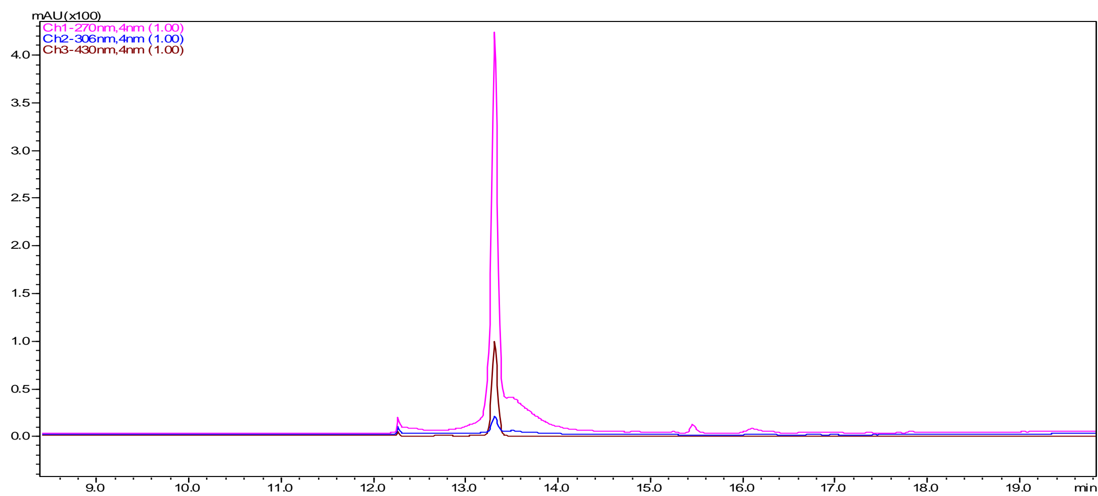


Supporting Figure S12

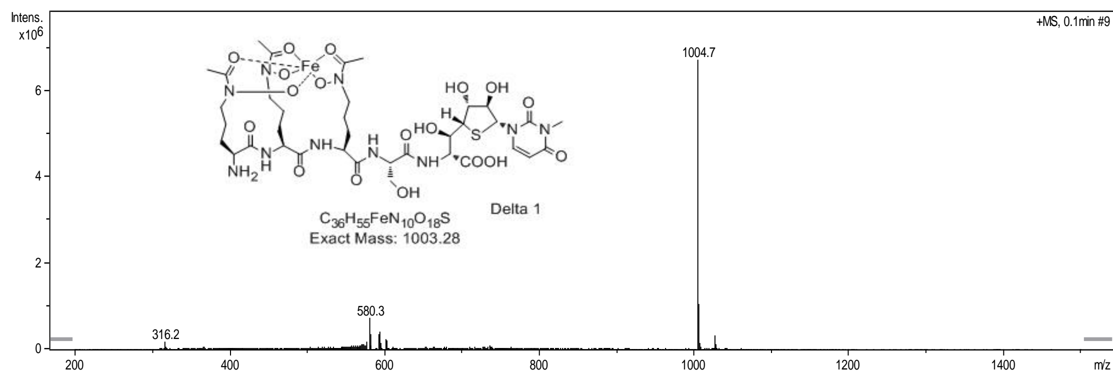


Supporting Figure S13

a.

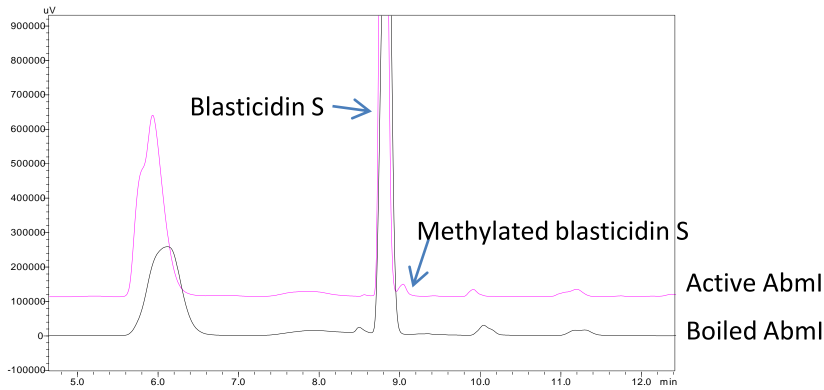


b.

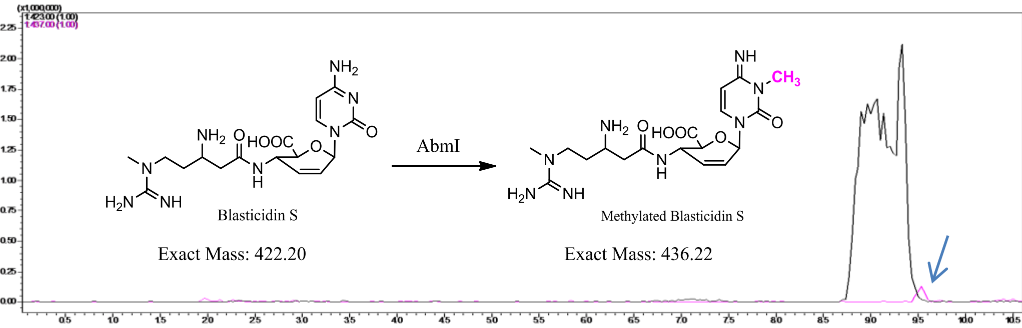


Supporting Figure S14

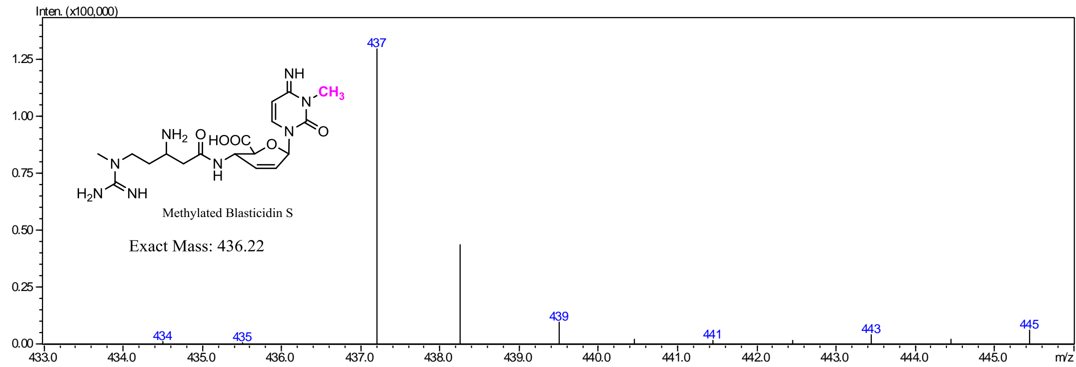
a.

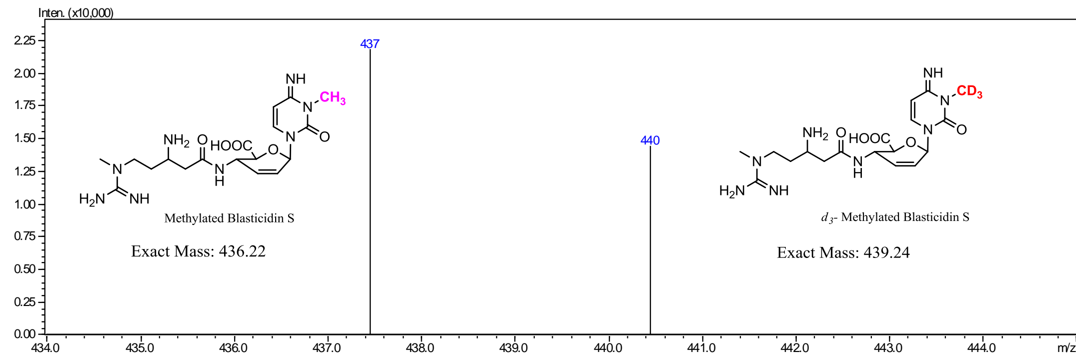


b.



c.





Supporting Figure S15