Published in final edited form as:

J Am Chem Soc. 2019 February 13; 141(6): 2211–2214. doi:10.1021/jacs.8b12565.

Biosynthetic Origin of the Atypical Stereochemistry in the Thioheptose Core of Albomycin Nucleoside Antibiotics

Richiro Ushimaru and **Hung-wen Liu***

Department of Chemistry, and Division of Chemical Biology and Medicinal Chemistry, College of Pharmacy, University of Texas at Austin, Austin, Texas 78712, United States

Abstract

Albomycins are peptidyl thionucleoside natural products that display antimicrobial activity against clinically important pathogens. Their structures are characterized by a thioheptose with atypical stereo-chemistry including a D-xylofuranose ring modified with a D-amino acid moiety. Herein it is demonstrated that AbmH is a pyridoxal 5′-phosphate (PLP)-dependent transaldolase that catalyzes a *threo*-selective aldol-type reaction to generate the thioheptose core with a D-ribofuranose ring and an L-amino acid moiety. The conversion of L-to D-amino acid configuration is catalyzed by the PLP-dependent epimerase AbmD. The D-*ribo* to D-*xylo* conversion of the thiofuranose ring appears according to gene deletion experiments to be mediated by AbmJ, which is annotated as a radical *S*-adenosyl-L-methionine (SAM) enzyme. These studies establish several key steps in the assembly of the thioheptose core during the biosynthesis of albomycins.

Albomycins (**1–3**) are sulfur-containing sideromycin antibiotics isolated from several species of Streptomy-cetes.[1] The chemical structures of the albomycins consist of a 6′-amino-4′-thioheptose nucleoside (**4**) and an iron-chelating ferrichrome siderophore (**5**) that are connected via amide linkages to a serine residue (see Scheme 1).[2] This allows albomycins to be actively transported via the bacterial iron uptake system into bacterial cells,[3] where they are hydrolyzed by host peptidases liberating the thioheptose containing SB-217452 moiety (**6**) and the ferrichrome **5**.[4] SB-217452 (**6**) has been established as an inhibitor of bacterial seryl-*t*RNA synthetase due to its resemblance to seryl adenylate.[4] Hence, albomycins have potent antimicrobial activities against many Gram-negative and Gram-positive bacteria.

The gene cluster responsible for albomycin production in *Streptomyces* sp. ATCC 700974 has been sequenced.[5] Although several enzymes responsible for the tailoring modifications have been identified, little is known about how the 6′-amino-4′-thioheptose moiety (**4**) is biosynthesized. In particular, the **D**-configuration (6′*R*) of the *α*-amino acid and the D-*xylo*-

***Corresponding Author** h.w.liu@mail.utexas.edu.

Supporting Information

The Supporting Information is available free of charge on the ACSPublicationswebsite at DOI:10.1021/jacs.8b12565.

Details regarding experimental procedures and spectroscopic data (PDF)

Crystallographic data (CIF)

Notes

The authors declare no competing financial interest.

configuration (3′*R*) of the furanose ring in the thioheptose core (see **4** and 7) are less commonly found in nature. Whether these unusual stereochemistries are inherited directly from the biosynthetic precursors or introduced after the core has been assembled is unknown.

The *α*-amino-*β*-hydroxy acid moiety at C5′-C6′ of **4** is found in several peptidyl nucleoside natural products such as the liposidomycins, caprazamycins, and muraymycins.[6] It has been shown that this functional group is constructed in an aldol-type C—C bond forming reaction catalyzed by a PLP-dependent serine hydroxymethyl transferase homologue[7] using L-threonine and an aldehyde precursor as substrates.[8] A similar enzymatic conversion is also operative in the biosynthesis of obafluorin.[9] On the basis of BLAST analysis, *abmH* in the albomycin gene cluster exhibits sequence similarity to genes encoding L-threonine:aldehyde transaldolases.[5] Hence, the gene product AbmH was hypothesized to catalyze an aldol reaction between a 5′-oxo-4′-thionucleoside such as **8** and L-threonine (**9**) to form the C5′—C6′ bond (Scheme 1). The putative AbmH reaction product **10** may then be coupled with L-serine or a derivative thereof in the subsequent biosynthetic steps resulting in production of **11**, which is the predicted precursor of **1**.

To test the proposed role of AbmH, *N*-His_6-tagged AbmH was heterologously overexpressed and purified from *Escherichia coli* (Figure S2). Since attempts to synthesize **8** as a substrate for AbmH were unsuccessful due to complications involving the cross-coupling between the 4-amino and 5′-aldehyde groups, the uracil analogue **12** (existing as its hydrate form **13**) was synthesized as an alternative substrate (Figure 1A and Supporting Information). When **12/13** (0.6 mM) was incubated with AbmH (3.2 *μ*M) and L-threonine (**9**, 5 mM) in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (50 mM, pH 7.5), formation of several new compounds was detected by high-performance liquid chroma-tography (HPLC) (Figure 1B). On the basis of electrospray ionization-mass spectroscopy (ESI-MS) and nuclear Over-hauser effect spectroscopy (NOESY), the broad peak with a retention time at ~9 min was assigned as **15**, which was the nonenzymatically generated 4′*R*-epimer of **13** (Figures S28). In contrast, a product peak at ~8 min displayed a mass consistent with an aldol adduct, such as **16** (calcd *m/z* for $C_{11}H_{16}N_3O_7S^+$ $[M + H]^+$ 334.0703; found 334.0686, see Figure S29). A similar ESI-MS result was also obtained for the minor peak at 6 min revealing the formation of another aldol adduct with the same chemical composition. Moreover, ^1H NMR analysis indicated that the latter peak contained a mixture of two diastereomers of **16** (see Supporting Information).

To establish the stereochemistry of the newly formed stereogenic centers C5′/C6′, the major AbmH product was isolated and derivatized with phosgene to afford a cyclic carbamate (see Supporting Information).[10] ^1H NMR analysis of the carbamate (**17**) showed a value of 8.2 Hz for the $J_{5′,6′}$ coupling that was significantly different from the reported $J_{5′,6′}$ coupling (5.0 Hz) for **18**, which has *threo*-stereochemistry at C5′/C6′ (Figure 1C).[8a] This outcome suggested an *erythro*-configuration at C5′/C6′ of **16** (5′*S*,6′*R* or its 5′*R*,6′*S*-isomer, see Figure 1A). This assignment is supported by comparison with the carbamate derivative **19** derived from **1**, which has a $J_{5′,6′}$ coupling of 9.9 Hz.

However, compound **8** represented by its synthetically accessible analogue **12/13** may not be the natural substrate of AbmH because it is a D-xylose-based nucleoside, which is unusual, and several stereoisomers of **16** were also observed during the incubation of **12/13** with AbmH. An alternative candidate is the 3′*S*-epimer of **8** (i.e., $\mathbf{8_{ribo}}$, see Figure 2A), which has a D-ribose configuration. This would suggest that epimerization of C3′ is a necessary step prior to completion of the thioheptose core of **1**. The *abm* cluster contains a gene, *abmJ*, which is annotated as encoding a radical SAM enzyme. It is well-known that certain radical SAM enzymes are capable of catalyzing epimerization reactions at unactivated centers.[11,12] It was thus hypothesized that AbmH catalyzes the aldol-type reaction of $\mathbf{8_{ribo}}$ with L-threonine (**9**) to yield $\mathbf{10_{ribo}}$ prior to AbmJ-catalyzed C3′-epimerization to produce the thioheptose core in **1** (Figure 2A). To test this hypothesis, *abmJ* was in frame deleted in the producing strain (Figures 2B and S12). The Δ*abmJ* strain produced ferrichrome **5** as shown by HPLC and MS analysis; however, two new products were also observed. One had a mass consistent with that of **1** in its iron-chelated form (calcd *m/z* for $C_{37}H_{58}FeN_{12}O_{18}S^+$ $[M + H]^+$ 1046.3057; found 1046.2985, see Figure S29) and the other had a mass identical to that of SB-217452 (**6**, calcd *m/z* for $C_{16}H_{25}N_6O_9S^+$ $[M + H]^+$ 477.1398; found 477.1382, see Figure S29). However, neither of these two new products coeluted with **1** or **6** by HPLC, implying that they are epimers of **1** and **6** thereby implicating **22** and **21**, respectively.

Leucine amino peptidase was used to cleave the thionucleoside moiety from the iron-chelating ferrichrome to characterize the products isolated from the Δ*abmJ* strain by NMR (Figure 2C). When the resulting thionucleoside was derivatized using phosgene, a $J_{5',6'}$ coupling of 9.7 Hz in the corresponding carbamate was noted, indicating an *erythro*-configuration at C5′/C6′ consistent with **24**. Moreover, ^{1}H NMR NOESY analysis of the thionucleoside revealed a correlation between H-6 and H-3′ (Figures 2C and S28). These observations support assignment of the thionucleoside as **23**. In contrast, a correlation between H-1′ and H-3′ was observed in **25** derived from hydrolysis of **1** (Figures 2C and S28). Collectively, these results indicate that the Δ*abmJ* metabolite is indeed **22**, which has the *S*-configuration at C3′. Hence, the annotated radical SAM enzyme AbmJ appears to be necessary for C3′ epimerization from the *S* to *R* stereochemistry during the biosynthesis of **1**. This hypothesis is also supported by the observation that complementation of the Δ*abmJ* mutant with *abmJ* partially restored production of **1** and **6** (Figure S13). While **1** showed antimicrobial activity against *E. coli* as previously reported,[1b] **22** exhibited significantly reduced bacterial growth inhibition based on disc-diffusion bioassays (Figure S26). These results imply the importance of the proposed C3′-epimerization for the biological activity of albomycins.

The proposed activity of AbmJ also implies that the biosynthetic substrate for AbmH is $\mathbf{8_{ribo}}$ rather than **8**. Therefore, to further test this hypothesis, the uracil analogue **26** (existing as its hydrate form **27**) was prepared (see Supporting Information). Upon incubation of **26/27** and L-threonine (**9**) with AbmH, a single aldol product was observed (Figures 2D and S14) in addition to the nonenzymatically generated 4′*R*-epimer of **27** (structure not shown). ^{1}H NMR analysis after phosgene derivatization of the aldol product demonstrated a coupling of $J_{5',6'}$ = 5.1 Hz indicating an unexpected *threo*-configuration at C5′/C6′ consistent with **28** (5′*S*,6′*S* or its 5′*R*,6′*R*-isomer). The formation of the *threo*-product from **26/27** by AbmH

is difficult to reconcile with the results from the *abmJ* deletion experiments because in the latter case the isolated product **22** has an *erythro*-configuration at C5′/C6′. Hence, in albomycin biosynthesis, the nascent product of the AbmH reaction (the **28** equivalent) must undergo isomerization to give the 5′*S*,6′*R*-configuration as seen in $\mathbf{10_{ribo}}$.

Epimerization of amino acids can be catalyzed by PLP-dependent enzymes.[13] The gene *abmD* in the *abm* gene cluster encodes another PLP-dependent enzyme, although it does not show high sequence similarity to known racemases/epimerases. While it has been reported that overexpression of *abmD* increases the level of **1** production,[14] the catalytic role of the gene product AbmD has not been characterized. To determine whether AbmD can catalyze the epimerization of the AbmH-product **28** (or its physiological equivalent), *N*-His_6-tagged AbmD was heterologously overexpressed and purified from *E. coli* (Figure S2). The UV absorption at 413 nm indicated the presence of PLP as an internal aldimine in AbmD (Figure S3). When the AbmH product (**28**, 0.056 mM) was incubated with AbmD (4.6 μM) in HEPES buffer (50 mM, pH 7.5), a new product was detected by HPLC (Figures S15 and S16). ESI-MS analysis indicated that the product has the same mass as **28** (Figure S29). The product structure was characterized to be **29** by NMR and coelution with the synthetic 5′*S*, 6′*R*-standard (Figures 2D, S18, and S66–S70).

To verify the position of epimerization (C5′ or C6′) resulting in **29**, the AbmD reaction with **28** was run in D_2O (92% D). Incorporation of a single deuterium into **29** (83% D) was observed, and no dideuteration was noted as would be expected for β-epimerization involving hydrogen exchange at C5′ (Figure S19). Likewise, when the reaction was conducted in $H_2{}^{18}O$ (90% ^{18}O), no ^{18}O-labeling was found in **29** as would be expected for β-epimerization involving hydroxyl-exchange at C5′ (Figure S19). These observations rule out β-epimerization (i.e., at C5′) as the process leading to **29** and thus indicate that AbmD-catalyzed epimerization only occurs at the α-carbon : (i.e., C6′) of the amino acid moiety (Figure S20). Hence, the AbmD-substrate, which is the AbmH-product, is (5′*S*,6′*S*)-**28**.

The demonstrated activities of AbmH and AbmD strongly suggest that $\mathit{8}_{ribo}$ is the substrate in the AbmH-catalyzed C5′—C6′ formation. However, there remains the possibility that the natural substrates of AbmH and AbmD are actually 4′-oxynucleosides rather than 4′-thionucleosides such that the sulfur atom is inserted after the AbmH/AbmD-catalyzed reactions. To investigate this possibility, compound **30** (existing as its hydrate form **31**) was chemically synthesized and incubated with AbmH under the standard assay conditions (Figure 2E). Two products were formed in ~1:1 ratio (Figure S21). One was identified as **32** with a *threo*-configuration (5′*S*,6′*S*) at C5′/C6′ ($J_{5',6'}$ = 5.2 Hz in the carbamate form) and the other was an *erythro*-heptose nucleoside (5′*R*,6′*S*)-*33* ($J_{5',6'}$ = 9.7 Hz in the carbamate form) based on the results of phosgene derivatization and comparison with standards (Supporting Information and Figure S22). These observations showed that the 4′-oxynucleoside can also be converted by AbmH, but with much reduced diastereoselectivity and catalytic efficiency (a 14-fold difference between k_{cat}/K_m for **26/27** vs **30/31**, see Table S1). Moreover, when the AbmH-products **32** and **33** were separately incubated with AbmD, no epimerization was observed in either case (Figure S23). These results indicate that AbmD can recognize the 4′-thiosugar **28** but not 4′-oxysugars **32/33**. Accordingly, the sulfur atom must be incorporated into the sugar skeleton early in the biosynthesis of **1**. However, the

apparent k_{cat}/K_m of AbmD with **29** was estimated to be only $(5.2 \pm 0.3) \times 10^{-4}$ min^{-1} μM^{-1} (Figure S25). While the poor catalytic efficiency of AbmD with **29** may be a consequence of **29** bearing a uracil rather than the biosynthetically more relevant cytosine nucleobase, the physiological substrate for the AbmD reaction ultimately remains ambiguous. Therefore, additional experiments will be needed to resolve this question and establish the timing between the AbmH and AbmD reactions.

In summary, the *in vitro* investigation of the substrate specificity and stereochemistry of the AbmH/AbmD-catalyzed reactions in conjunction with the *abmJ* deletion study establishes several key steps in the assembly of the thioheptose core during biosynthesis of albomycins. Although epimerizations catalyzed by AbmD and AbmJ do not result in major structural alterations, they appear to be essential for conferring the antimicrobial activity of **1**. The present study not only reveals the intricacy of the construction of a thionucleoside-containing natural product, but also enhances our knowledge regarding the correlation of structural variation and the biological activity important for the survival of the producing microorganisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (GM035906 and 1 S10 OD021508-01 for NMR) and the Welch Foundation (F-1511). We thank Jessi Cai for the assistance in the preparation of AbmH.

REFERENCES

(1) (a). Gause GF Recent studies on albomycin, a new antibiotic. Br. Med. J 1955, 2, 1177–1179. [PubMed: 13269824] (b)Stapley EO; Ormond RE Similarity of albomycin and grisein. Science 1957, 125, 587–589. [PubMed: 13409012] (c)Lin Z; Xu X; Zhao S; Yang X; Guo J; Zhang Q; Jing C; Chen S; He Y Total synthesis and antimicrobial evaluation of natural albomycins against clinical pathogens. Nat. Commun 2018, 9, 3445. [PubMed: 30181560]

(2) (a). Benz G Enzymatische spaltung der desferriform der albomycine δ_1, δ_2. Liebigs Ann. Chem 1984, 1984, 1399–1407.(b)Benz G; Born L; Brieden M; Grosser R; Kurz J; Paulsen H; Sinnwell V; Webber B Absolute konfiguration der desferriform der albomycine. Liebigs Ann. Chem 1984, 1984, 1408–1423.(c)Paulsen H; Brieden M; Benz G Synthese des sauersstoffanalogons der desferriform von δ_1-Albomycin. Liebigs Ann. Chem 1987, 1987, 565–575.

(3) (a). Pramanik A; Braun V Albomycin uptake via a Ferric hydroxamate transport system of *Streptococcus pneumoniae* R6. J. Bacteriol 2006, 188, 3878–3886. [PubMed: 16707680] (b)Ferguson AD; Braun V; Fiedler HP; Coulton JW; Diederichs K; Welte W Crystal structure of the antibiotic albomycin in complex with the outer membrane transporter FhuA. Protein Sci. 2000, 9, 956–963. [PubMed: 10850805] (c)Clarke TE; Braun V; Winkelmann G; Tari LW; Vogel HJ X-ray crystallographic structures of the *Escherichia coli* periplasmic protein FhuD bound to hydroxamate-type siderophores and the antibiotic albomycin. J. Biol. Chem 2002, 277, 13966–13972. [PubMed: 11805094]

(4). Stefanska AL; Fulston M; Houge-Frydrych CSV; Jones JJ; Warr SR A potent seryl tRNA synthetase inhibitor SB-217452 isolated from a *Streptomyces* species. J. Antibiot 2000, 53, 1346–1353. [PubMed: 11217799]

(5). Zeng Y; Kulkarni A; Yang Z; Patil PB; Zhou W; Chi X; Van Lanen S; Chen S Biosynthesis of albomycin δ_2 provides a template for assembling siderophore and aminoacyl-tRNA synthetase inhibitor conjugates. ACS Chem. Biol 2012, 7, 1565–1575. [PubMed: 22704654]

(6) (a). Winn M; Goss RJ; Kimura K; Bugg TD Antimicrobial nucleoside antibiotics targeting cell wall assemblyrecent advances in structure-function studies and nucleoside biosynthesis. Nat. Prod. Rep 2010, 27, 279–304. [PubMed: 20111805] (b)Walsh CT; Zhang W Chemical logic and enzymatic machinery for biological assembly of peptidyl nucleoside antibiotics. ACS Chem. Biol 2011, 6, 1000–1007. [PubMed: 21851099]

(7) (a). Schirch L; Gross T Serine transhydroxymethylase. Identification as the threonine and allothreonine aldolases. J. Biol. Chem 1968, 243, 5651–5655. [PubMed: 5699057] (b)Gutierrez ML; Garrabou X; Agosta E; Servi S; Parella T; Joglar J; Clapeś P Serine hydroxymethyl transferase from *Streptococcus thermophilus* and l-threonine aldolase from *Escherichia coli* as stereocomplementary biocatalysts for the synthesis of β-hydroxy-α,ω-diamino acid derivatives. Chem. - Eur. J 2008, 14, 4647–4656. [PubMed: 18384024]

(8) (a). Barnard-Britson S; Chi X; Nonaka K; Spork AP; Tibrewal N; Goswami A; Pahari P; Ducho C; Rohr J; Van Lanen SG Amalgamation of nucleosides and amino acids in antibiotic biosynthesisdiscovery of an l-threonine:uridine-5′-aldehyde transaldolase. J. Am. Chem. Soc 2012, 134, 18514–18517. [PubMed: 23110675] (b)Cai W; Goswami A; Yang Z; Liu X; Green KD; Barnard-Britson S; Baba S; Funabashi M; Nonaka K; Sunkara M; Morris AJ; Spork AP; Ducho C; Garneau-Tsodikova S; Thorson JS; Van Lanen SG The biosynthesis of capuramycin-type antibioticsidentification of the A-102395 biosynthetic gene cluster, mechanism of self-resistance, and formation of uridine-5′-carboxamide. J. Biol. Chem 2015, 290, 13710–13724. [PubMed: 25855790]

(9) (a). Scott TA; Heine D; Qin Z; Wilkinson B An L-threonine transaldolase is required for l-*threo*-β-hydroxy-α-amino acid assembly during obafluorin biosynthesis. Nat. Commun 2017, 8, 15935. [PubMed: 28649989] (b)Schaffer JE; Reck MR; Prasad NK; Wencewicz TA β-Lactone formation during product release from a nonribosomal peptide synthetase. Nat. Chem. Biol 2017, 13, 737–744. [PubMed: 28504677]

(10). Futagawa S; Inui T; Shiba T Nuclear magnetic resonance study of the stereoisomeric 2-oxazolidone and 2-phenyl-2-oxazoline derivatives of α-amino-β-hydroxy acids. Bull. Chem. Soc. Jpn 1973, 46, 3308–3310.

(11) (a). Freeman MF; Gurgui C; Helf MJ; Morinaka BI; Uria AR; Oldham NJ; Sahl HG; Matsunaga S; Piel J Metagenome mining reveals polytheonamides as posttranslationally modified ribosomal peptides. Science 2012, 338, 387–390. [PubMed: 22983711] (b)Benjdia A; Guillot A; Ruffie P; Leprince J; Berteau O Post-translational modification of ribosomally synthesized peptides by a radical SAM epimerase in Bacillus subtilis. Nat. Chem 2017, 9, 698–707. [PubMed: 28644475]

(12). Kudo F; Hoshi S; Kawashima T; Kamachi T; Eguchi T Characterization of a radical *S*-adenosyl-l-methionine epimerase, NeoN, in the last step of neomycin B biosynthesis. J. Am. Chem. Soc 2014, 136, 13909–13915. [PubMed: 25230155]

(13) (a). Yoshimura T; Esak N Amino acid racemasesfunctions and mechanisms. J. Biosci. Bioeng 2003, 96, 103–109. [PubMed: 16233494] (b)Du Y-L; Ryan KS Pyridoxal phosphate-dependent reactions in the biosynthesis of natural products. Nat. Prod. Rep 2019, DOI:10.1039/C8NP00049B.

(14). Kulkarni A; Zeng Y; Zhou W; Van Lanen S; Zhang W; Chen S A branch point of *Streptomyces* sulfur amino acid metabolism controls the production of albomycin. Appl. Environ. Microbiol 2016, 82, 467–477. [PubMed: 26519385]

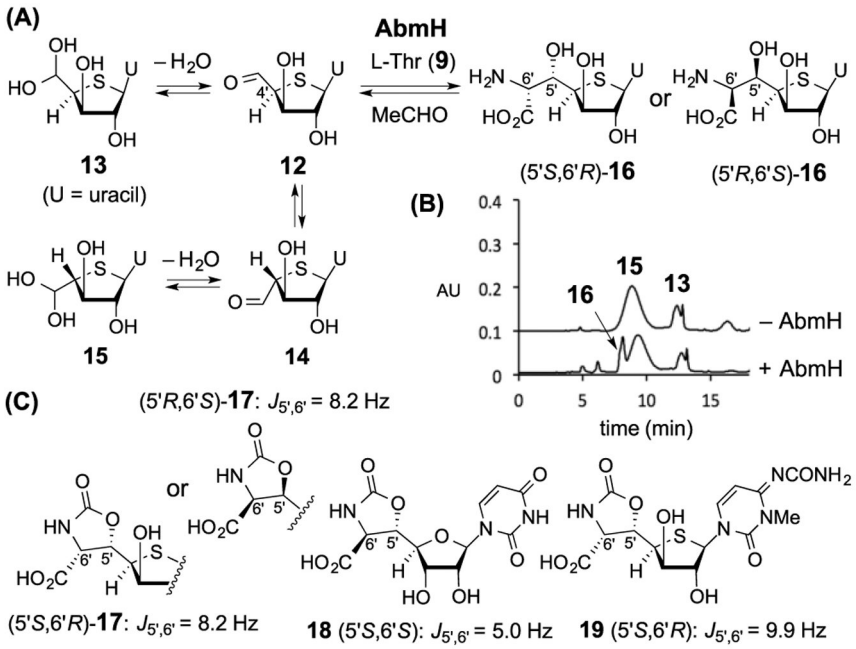


Figure 1.

(A) AbmH reaction of **12**/**13**. (B) HPLC-UV analysis. (C) Phosgene-derivatized AbmH-product **17** (5′*S*,6′*R* or its 5′*R*,6′*S*-isomer) and its analogues.

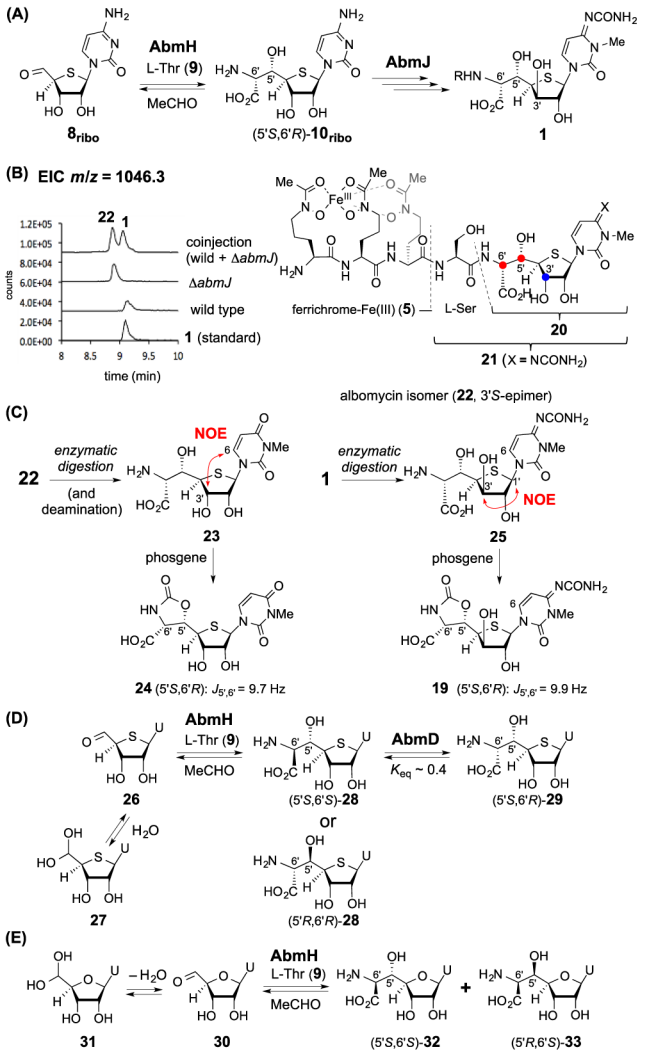
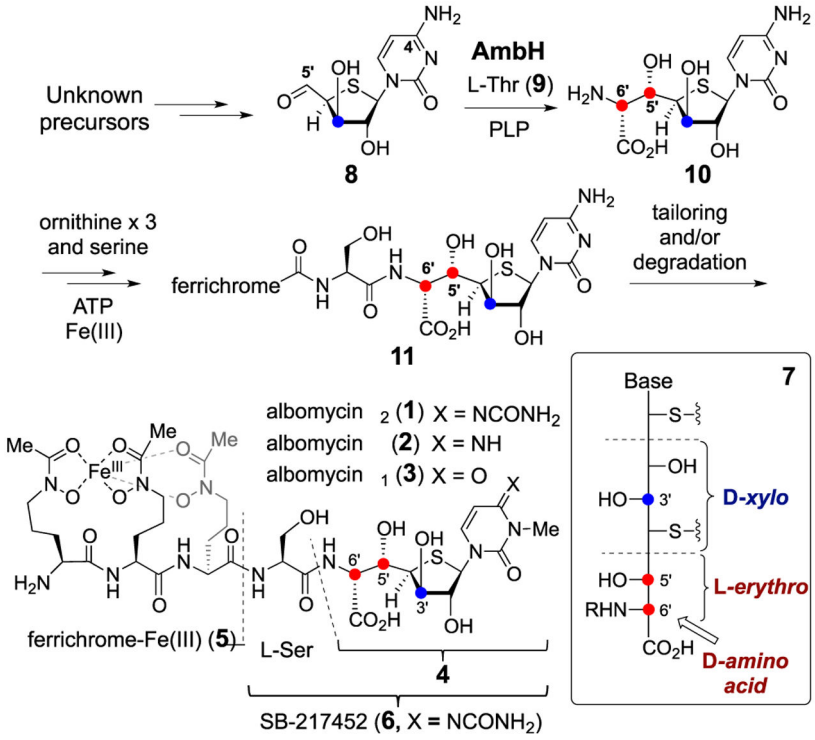


Figure 2.

(A) Alternative proposal of the albomycin biosynthesis. (B) LC–MS analysis of the metabolites from the Δ*abmJ* strain. Extracted ion chromatogram (EIC) traces corresponding to $[M + H]^+$ signals from **1** or **22** are shown. (C) NOESY analysis and phosgene derivatization of the digested compounds. (D) Reaction of **26**/**27** with AbmH and AbmD to afford **28** and **29**. (E) Reaction of **30**/**31** with AbmH.



Scheme 1.
Proposed Biosynthetic Pathway of Albomycins