Biosynthesis of Fungal Drimane-Type Sesquiterpene Esters
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Abstract: Drimane-type sesquiterpenes exhibit various biological activities and are widely present in eukaryotes. Here, we completely elucidated the biosynthetic pathway of the drimane-type sesquiterpenes isolated from Aspergillus calidoustus and we discovered that it involves a drimenol cyclase having the same catalytic function previously only reported in plants. Moreover, since many fungal drimenol derivatives possess a γ-butyrolactone ring, we clarified the functions of the cluster-associated cytochrome P450 and FAD-binding oxidoreductase discovering that these two enzymes are solely responsible for the formation of those structures. Furthermore, swapping of the enoyl reductase domain in the identified polyketide synthase led to the production of metabolites containing various polyketide chains with different levels of saturation. These findings have deepened our understanding of how fungi synthesize drimane-type sesquiterpenes and the corresponding esters.

Introduction

Drimane-type sesquiterpenes are a large group of natural products with unique C15 bicyclic skeletons. They have been identified in various eukaryotes including plants,[1] liverwork,[2] molluscs,[3] sponges,[4] and fungi (primarily Aspergillus[5] and Penicillium[6] species). Many of them possess “drug-like” chemical properties and display diverse biological activities, including antimicrobial,[7] anti-inflammatory,[8] neurotransmission,[9] anti-diabetic,[10] and antihyperlipidemic[11] activity. Moreover, the well-known drimane dialdehydes act as antifeedants against insects and have potential to be used as alternative insecticides.[10]

Because of their interesting structural features and biological activities, fungi-derived drimanes have attracted increasing attention. Intriguingly, fungi-derived drimane-type sesquiterpenes can possess a γ-butyrolactone ring and are generally esterified (Figure 1). In some cases, esterification helps to increase the activities.[10b,11] However, so far the research on this class of compounds has been mainly focused on the isolation, structure elucidation, and bioactivity characterization, with the only exception of astellolides, which are partially characterized at a genetic level in Aspergillus oryzae.[12] The previous studies on the biosynthesis of astellolides suggested that drim-8-ene-11-ol is the used precursor, produced by the haloacid dehalogenase-like (HAD-like) terpene cyclase AstC and two dephosphorylases. Nonetheless, while astellolides harbor a Δ⁸-9 double bond, other isolated compounds like nanangenines, 22-hydroxyxylo- lodonin B and purpuride F contain a double bond at a different position (Δ⁷-8), suggesting a different biosynthesis (Figure 1).

As part of our program to discover novel bioactive molecules from Aspergilli, we performed a chemical investigation on Aspergillus calidoustus that led to the isolation of a series of drimane-type sesquiterpenes and their esters (1–16; Scheme 1), which were the dominant secondary metabolites. Meanwhile, we employed bioinformatics analysis and a gene deletion campaign to characterize the related biosynthetic gene cluster (BGC). Here, we demonstrated that the isolated drimane-type molecules originate from drimenol and that the identified terpene cyclase has the same activity observed in plant-derived cyclases.[13] Furthermore, to better clarify the

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Figure 1. Structures of representative fungal drimane-type sesquiterpene esters.
individual biosynthetic steps, the heterologous expressions of different combinations of the identified genes in *Aspergillus fumigatus* and *Saccharomyces cerevisiae* were conducted. Additionally, given the similarity between these compounds and nanangenines (Figure 1), we swapped the enoyl reductase (ER) domain of the identified polyketide synthase (PKS) with the ER domain from *Aspergillus nanangensis*, resulting in metabolites containing various polyketide chains. Lastly, because of the diversity of the ester moieties in drimane-type sesquiterpene esters, we determined the substrate specificity of the involved acyl transferase, demonstrating that this enzyme is able to use both ACP- and CoA-activated substrates.

**Results and Discussion**

**Biosynthesis of Drimane Sesquiterpenoids in A. calidoustus**

By fermenting *A. calidoustus* in V8 production medium, we observed the abundant production of different secondary metabolites. Large scale fermentation resulted in the isolation of sixteen compounds (1–16; Scheme 1 and Table S1). The extensive analysis of the HRESIMS and NMR data revealed that these molecules were all structurally related to drimane sesquiterpenoids with compounds 5–16 coupled to polyketide chains with different lengths (C₆ or C₈, Tables S2–S17). The here identified compounds 1–6, 8–10, 12–14 and 16, have already been reported in *Aspergillus ustus;*[^14] with 5 and 6 also having been identified in *Aspergillus flavus.*[^15] However, we also isolated a few novel derivatives, named calidoustene A (7), calidoustene B (11) and calidoustene C (15, Scheme 1).
As the identified compounds are structurally similar to the astellolides, drimane-type esters found in *A. oryzae*, we performed a genome mining analysis using the sequence of the characterized HAD-like terpene cyclase as probe,[23] and identified an orthologue on chromosome 2 (ASPCAL02978), confirming previous computational analysis.[11] Investigation of neighboring genes revealed the presence of six open reading frames, coding for potential proteins proposed to be likely involved in sesquiterpene ester biosynthesis (Figure 2A), specifically: a polyketide synthase (PKS; named DrtA) and the above mentioned HAD-like terpene cyclase (DrtB) theoretically involved in the biosynthesis of the C6/C8 polyketide chains and drimane backbone, respectively, an alpha/beta hydrolase (DrtE), a FAD-binding oxidoreductase (DrtC), a cytochrome P450 (DrtD), and a short-chain dehydrogenase (DrtF) possibly involved in further modifications of the obtained drimane sesquiterpene and the polyketide chain. To establish an efficient gene deletion campaign, for the confirmation of gene functions and cluster boundaries, we firstly deleted the gene coding for the AkuA DNA helicase (ASPCAL00120) in *A. calidoustus*, thereby suppressing the non-homologous end-joining repair mechanism.[24] Chemical analysis confirmed that the obtained *A. calidoustus ΔakuA* mutant still produced compounds 1–16 (Figure S1); thus, we used the obtained strain as the recipient for further deletions.

The deletion of the PKS coding gene *drtA* showed complete loss of all the sesquiterpene ester compounds, whereas 1–4 were still produced (Figure 2B). This confirmed that the identified DrtA was indeed responsible for the drimanesesquiterpenoids’ biosynthesis. Moreover, we observed the same chemical pattern by deleting the gene coding for the alpha/beta hydrolase DrtE. This implies that DrtE is responsible for the loading of the polyketide moiety on the drimane backbone. Additionally, conserved domain database (CDD)[17] analysis of the deduced DrtA amino acid sequence has shown that this PKS is missing a thiosterase (TE) domain, suggesting that DrtE mainly functions as an acyl-transferase but it likely executes an accessory thiosterase activity.[18]

The deletion of *drtB*, coding for the putative HAD-like terpene cyclase, as expected, completely abolished the drimanesesquiterpenes’ biosynthesis (Figure 2B), while deletion of *drtC* resulted in a large accumulation of 13, with all compounds harboring the γ-butyrolactone ring (5–11 and 14–16) or containing a carboxylic acid at C-11 (12) disappearing. This implies that DrtC is able to catalyze the formation of carboxylic acids at C-11 and C-12. Meanwhile, based on the feature of 13, the remaining two enzymes, DrtD and DrtE, are potential candidates involved in the formation of hydroxy groups at C-6, C-9 and C-12 in 13. Subsequently, deletion of *drtD* also led to complete absence of the drimanesesquiterpenes, confirming that DrtD plays a role in supplying oxidized drimane precursors in the biosynthesis.

Upon deletion of *drtF*, HPLC and LC–HRMS analyses showed that 5–11 disappeared, and 12–16 with fully unsaturated acyl chains could be detected (Figure 2B). This suggests that the short-chain dehydrogenase DrtF can catalyze the single or multiple oxidations occurring on the PKS chain. Interestingly, this strain yielded higher titters of 17 and 18, the former was previously identified in *A. ustus* [19] and permitted the isolation of two novel compounds, calidoustene D (19) and calidoustene E (20, Figure 2B and Scheme 1).

Lastly, we confirmed the BGC boundaries. We deleted genes ASPCAL02975 (coding for putative alpha/beta hydrolase) and ASPCAL02983 (coding for ankyrin repeats), and observed the same production pattern as for the wild type and the ΔakuA strain (Figure S1). We also attempted to delete the putative gene ASPCAL02976. However, the deletion of this locus probably failed due to its highly similarity to a second locus present in the genome (ASPCAL01670).

The gene deletion campaign was very useful for assigning gene functions to all open reading frames composing the *drt* BGC. However, we could not fully elucidate the structure of the HAD-like terpene cyclase product. During astellolide biosynthesis, the terpene cyclase is responsible for the formation of drimanyl pyrophosphate, which is then dephosphorylated leading to the synthesis of drim-8-ene-11-ol (Figure 1).[25] Since the hydroxy group at C-9 can potentially cause the migration of the double bond to Δ15, drim-8-ene-11-
was also predicted to be the nanangenines’ precursor (Figure 1).[11] However, we observed that not all of the intermediates isolated from *A. calidoustus* harbor a hydroxy group at C-9, such as 3, 4 and 18. Also, previously isolated compounds, such as 22-hydroxyxylodonin B and purpuride F, are also missing the hydroxy group at C-9 (Figure 1). Based on these observations, we postulated that drimenol is the likely upstream precursor and the product of the terpene cyclase DrtB. Next, *A. calidoustus* *drtB* mutant strain was treated with drimenol, resulting in the re-detection of drimane-type sesquiterpenes and their esters (Figure 2C), which validates our hypothesis.

To verify the activity of DrtB, we performed heterologous expression of the isolated cDNA in *Escherichia coli*. Expression of the native open reading frame resulted in no accumulation of the recombinant protein. Subsequently, we deleted the highly hydrophobic C-terminal part of the enzyme, which led to an observable production of drimenol in vivo (Figure 3A and Figure S2). Furthermore, the incubation of the purified DrtB with farnesyl pyrophosphate (FPP) led to the in vitro synthesis of drimenol (Figure S3), thus confirming that DrtB is a drimenol cyclase.

To better characterize the role played by DrtC and DrtD in modifying drimenol, we heterologously expressed three genes, namely *drtB*, *drtD* and *drtC*, in *S. cerevisiae* using yeast expression plasmids. The individual expression of *drtB* or *drtD* produced no detectable drimenol or drimenol derivatives (Figure 3B, ii and iii), suggesting that the drimenol produced by DrtB might be further metabolized in *S. cerevisiae*. The bicistronic expression of *drtB* and *drtD* however led to the production of 17 and 18 (Figure 3B, iv). Unexpectedly, this strain also produced 21, which possesses the same molecular weight as 18. Co-expression of *drtB*, *DrtD* and *drtC* produced 22 and 23, in which C-11 is oxidized into carboxylic acid and condensed to a γ-butyrolactone ring (Figure 3B, v). Therefore, the results confirmed that DrtD catalyzes the hydroxylation at C-6 and C-9 position, and it is also able to oxidize the hydroxy group at C-11 to an aldehyde. Additionally, DrtD seems to further oxidize the hydroxy group at C-6 to a ketone.

To further confirm this hypothesis, drimenol was fed to *S. cerevisiae* expressing *drtD* alone, with a control strain containing the empty plasmid (Figure 3C). With the feeding experiments, the production of 17, 18 and 21 was confirmed, and a small amount of 18 was also produced by the control. Additionally, the production of 24 further confirmed that DrtD could oxidize the alcohol at C-11 to an aldehyde, while the presence of 25 and 26 confirmed that this P450 also catalyzes the hydroxylation at C-12. Taken together, the heterologous expression in yeast demonstrated that the P450 DrtD is responsible for the hydroxylations at C-6, C-9 and C-12, as well as the oxidation of hydroxy groups at C-6 and C-11 to a ketone and an aldehyde, respectively; then, the C-11 aldehyde can be further oxidized into a carboxylic acid by DrtC. Moreover, these results show that DrtB, DrtD and DrtC are solely responsible for the formation of the different drimane structures observed during drimane sesquiterpene biosynthesis in *A. calidoustus*, and that the different degree of oxidation at C-11 and C-12 determines the divergent γ-butyrolactone conformations observed in 15, 16 and 20.

**Enoyl Reductase Domain Swapping of the Polyketide Synthase DrtA**

Among all the drimane-type sesquiterpene esters isolated from fungi, the structures of nanangenines (Figure 1), with the acyl chains fully saturated, suggest a divergent evolution of
the involved PKSs. It is known that the \(\alpha-\beta\) double bond formed by a PKS dehydratase (DH) domain is reduced by an enoyl reductase (ER) domain to generate a single bond in the nascent polyketide.\(^{[18]}\) Therefore, we assume that fungal PKSs involved in drimane-type sesquiterpene ester biosynthesis would present variations in their ER domains. Genome mining on the available fungal genomes identified that the \(\text{drt}\) BGC is conserved in twelve different \textit{Aspergillus} species (Figure 4A). Phylogenetic analysis based on the deduced amino acid sequences of the identified PKS–ER domains revealed the presence of two distinct clades: clade I includes \textit{A. calidoustus} and \textit{A. ustus}, both producing sesquiterpene esters with different levels of desaturation in the polyketide chain, and clade II, which includes \textit{A. nanangensis}. To validate the phylogenetic analysis, we aimed to swap the ER domain present in DrtA with the PKS–ER potentially

![Figure 4](image-url)
involved in nanogenine biosynthesis. The ER region to be swapped was determined based on the amino acid sequence alignment of the closely related PKS–ER domains (Figure S4), and the modified PKS was named DrtA* (Figure 4B).

Because of the lack of available selection markers, any attempt to introduce the \textit{drtA} gene into the \textit{A. calidoustus} \textit{DdrtA} mutant failed. Therefore, we firstly expressed the synthetic \textit{drtA} gene in the \textit{A. calidoustus} wild-type strain under the control of a tetracycline-inducible promoter (\textit{tetON}) [20].

Although the overall production of drimane sesquiterpenes in the \textit{wt/drtA*} mutant was lower than that of the wild type, the expression of the modified DrtA* led to the identification of an novel metabolite, calidoustene F (27, Figure 4C, D). This compound was successfully isolated from a large-scale cultivation, and, as expected, it was a drimane-type sesquiterpene ester having the PKS chain with a reduced terminal double bond (Figure 4D). However, with this experiment we did not identify any molecules harboring a fully saturated polyketide. To further examine the function of the ER-swapped \textit{drtA*} gene we performed heterologous expression (Figure 5A). We first expressed each PKS gene, \textit{drtA} or \textit{drtA*}, in \textit{A. fumigatus} to yield mutant strains CEA17/\textit{drtA} and CEA17/\textit{drtA*}. Afterwards, different combinations of additional \textit{drt} genes were polycistronically expressed in CEA17/\textit{drtA} and CEA17/\textit{drtA*}. The mutant strains missing the \textit{drtC} gene mainly produced 13 (Figure 5A, ii, iv, vi and viii), as observed in the \textit{A. calidoustus} \textit{DdrtC} strain (Figure 2B). However, those strains lacking DrtC but expressing the ER-swapped PKS, namely \textit{drtA*BDEF} (Figure 5A, i) and \textit{drtA*BDE} (Figure 5A, iii), also produced metabolite 30, with a partially saturated polyketide tail. The addition of \textit{drtC} to the polycistrone led to the production of 12, 16 and 28 (Figure 5A, i, iii, v and vii), intermediates with highly unsaturated polyketide chains. Nonetheless, in mutants containing the swapped ER-domain (Figure 5A, i and iii), we identified compounds 27 and 29, with partially reduced acyl chains, and as well 31–33 containing fully saturated acyl chains (Figure 5B). These results confirmed that, upon ER domain swap, the obtained PKS could produce fully saturated acyl chains. However, we also observed partially saturated and fully unsaturated acyl chains, indicating that the specificity of the ER domain is influenced by other structural domains. It is known that the reductive steps during polyketide elongation are optional, suggesting that the ER reductive step is always skipped by DrtA. [24] Nevertheless, the ER-domain in the \textit{drtA*} mutants is able to optionally skip the reducing steps during polyketide chain synthesis (Figure S5). Moreover, the heterologous expression in \textit{A. fumigatus} revealed another interesting aspect of the biosynthesis: since we did not detect compounds 1–4, we assume that hydroxylations at C-2 and C-3 are catalyzed by endogenous enzymes in \textit{A. calidoustus} not associated to the \textit{drt} BGC (Scheme 1).

Determining the Substrate Specificity of the Acyltransferase DrtE

Motivated by the diversity of the ester moieties in fungi-derived drimane-type sesquiterpene esters, we determined the substrate specificity of the involved acyl transferase DrtE by feeding different potential substrates together with drimenol to \textit{S. cerevisiae} expressing \textit{drtD}, \textit{drtE} and two different CoA ligases. The 4-coumaroyl-CoA ligase from \textit{Nicotiana tabacum} (4CL) [21] and the long-chain-fatty-acid-CoA ligase from \textit{E. coli} (FadD) [22] were used to esterify selected substrates, namely hexanoic, octanoic, and cinnamic

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**Figure 5.** Heterologous expression in \textit{A. fumigatus}. Genes of \textit{drtA} and \textit{drtA*} were expressed in \textit{A. fumigatus} in combination with the other \textit{drt} genes. A) LC–HRMS EIC of the metabolites from \textit{A. fumigatus} transformants. B) The structures of compounds 28–33 (marked with *) were deduced based on their HRESIMS spectra.
acid. Interestingly, DrtE was able to use all tested CoA-activated substrates, not only the different lengths of fatty acyl-CoA (C₆ and C₈), but also the cinnamoyl-CoA (Figure 6).

**Conclusion**

Here, we elucidated the complete biosynthetic pathway of the fungal natural products drimane-type sesquiterpenes and their esters (1–16) isolated from *A. calidoustus*, established through gene inactivation, heterologous expression and feeding experiments. Firstly, the backbone of these compounds, drimenol, is produced by the terpene cyclase DrtB. Next, the P450 DrtD catalyzes the hydroxylation at C-6, C-9 and C-12, and it is also responsible for the oxidation of hydroxy groups at C-6 and C-11 to ketone and aldehyde, respectively. Then, the biosynthesis can go in two directions, either the hydroxylated drimenol is further hydroxylated at C-2 and C-3 by an enzyme(s) not associated with the dtB BGC, or the FAD-binding oxidoreductase DrtC further oxidizes C-11 or C-12 obtaining a carboxylic acid, which is then condensed with the γ-OH to form the butyrolactone ring. The polyketide synthase DrtA synthesizes different lengths (C₆ and C₈) of PKS chains, which are then oxidized to varying degrees by the short-chain dehydrogenase DrtF. Finally, these PKS chains are transferred onto drimane sesquiterpenes by the acyltransferase DrtE, forming the sesquiterpene esters. Which could be an efficient tool for further applications, such as the substrate-driven derivatization of drimane-type sesquiterpene esters.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords**: Aspergillus calidoustus · biosynthesis · drimane · natural products · terpenoids

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[1] a) S. Belhadj, H. Keskes, C. Apel, F. Roussi, M. Litaudon, O. Hentati, N. Allouche, *Chem.-Biol. Interact.* 2020, 330, 109167; b) D. He, C. Sulebodnick, L. H. Rakotondraibe, *Bioorg. Med. Chem. Lett.* 2017, 27, 1754–1759; c) Y. Hu, L. Tao, H. Tan, M. Zhang, K. Shimizu, F. Zhang, C. Zhang, *Inflammation* 2017, 40, 1204–1213; d) S. Karmahapatra, C. Kientz, S. Shetty, J. C. Yalowich, L. H. Rakotondraibe, *J. Nat. Prod.* 2018, 81, 625–629.

[2] a) B. J. M. Jansen, A. de Groot, *Nat. Prod. Rep.* 2004, 21, 449–477; b) B. J. M. Jansen, A. de Groot, *Nat. Prod. Rep.* 1991, 8, 309–318.
[3] a) L. Rahbar, C. Christophersen, J. Frisvad, H. S. Bengaard, S. Larsen, B. R. Rassing, J. Nat. Prod. 1997, 60, 811–813; b) A. N. Yurchenko, P. T. H. Trinh, O. F. Smetanina, A. B. Rasín, R. S. Popov, S. A. Dyshlyovoy, G. von Amsberg, E. S. Menchinskaya, T. T. Than Van, S. S. Afiayarullow, Mar. Drugs 2019, 17, 579;

[4] a) M. Ma, H. Ge, W. Yi, B. Wu, Z. Zhang, Tetrahedron Lett. 2020, 61, 151504;

[5] a) C. Intaraudom, W. Punyain, N. Bunbamrung, A. Dramae, T. Boonruanggrapa, P. Pittayakajonwut, Fitoterapia 2019, 138, 104533;

[6] a) C. Chen, W. Sun, M. Wei, Y. Liang, J. Wang, H. Zhu, Y. Zhang, Bioorg. Chem. 2019, 91, 103166;

[7] a) W. Fang, X. Lin, X. Zhou, J. Wan, X. Lu, B. Yang, W. Ai, J. Lin, T. Zhang, Z. Tu, MedChemComm 2014, 5, 701–705;

[8] a) K. Xu, Q. Zhou, X. Q. Li, T. Luo, M. C. Li, Y. Z. Zhang, J. Zhang, Chem. Biol. 2020, 104, 104252;

[9] Y. Li, C. Wu, D. Liu, P. Proksch, P. Guo, W. Lin, J. Nat. Prod. 2014, 77, 138–147.

[10] a) I. Kubo, I. Ganjian, Experientia 1981, 37, 1063–1064; b) J. Escalera, C. A. von Hahn, B. F. Besseu, M. Sivula, S. E. Jordt, J. Biol. Chem. 2008, 283, 24136–24144; c) E. A. Inocente, M. Shaya, N. Acosta, L. H. Rakotondrainibe, P. M. Piermarini, PLoS Neglected Trop. Dis. 2018, 12, e0006265; d) I. Montenegro, A. Madrid, M. Cuellar, M. Seeger, J. F. Alfaro, X. Besoain, J. P. Martín, I. Ramírez, Y. Olguín, M. Valenzuela, Molecules 2018, 23, 2053; e) I. Montenegro, L. Pino, E. Werner, A. Madrid, L. Espinoza, L. Moreno, J. Villena, M. Cuellar, Molecules 2013, 18, 4192–4208.

[11] H. J. Lacey, C. L. Gilchrist, A. Crombie, J. A. Kalaitzis, D. Vuong, P. J. Rutledge, P. Turner, J. I. Pitt, E. Lacey, Y. H. Choo, Beilstein J. Org. Chem. 2019, 15, 2631–2643.

[12] Y. Shinohara, S. Takahashi, H. Osada, Y. Koyama, Sci. Rep. 2016, 6, 32865.

[13] a) M. G. L. Henquet, N. Prota, J. J. van der Hooft, M. Varbanova-Herde, R. J. Hulzink, M. de Vos, M. Prins, M. T. de Both, M. C. Franssen, H. Bouwmeester, Plant J. 2017, 90, 1052–1063; b) M. Kwon, S. A. Cochrane, J. C. Vederas, D. K. Ro, FEBS Lett. 2014, 588, 4597–4603.

[14] a) Z. Lu, Y. Wang, C. Miao, P. Liu, K. Hong, W. Zhu, J. Nat. Prod. 2009, 72, 1761–1767; b) G. F. Neuhaus, S. Loesgen, J. Nat. Prod. 2021, 84, 37–45; c) H. Zhou, T. Zhu, S. Cai, Q. Gu, D. Li, Chem. Pharm. Bull. 2011, 59, 762–766; d) H. Liu, R. Edrada-Ebel, R. Ebel, Y. Wang, B. Schulz, S. Draeger, W. E. Muller, V. Wray, W. Lin, P. Proksch, J. Nat. Prod. 2009, 72, 1585–1588.

[15] Y. F. Liu, Y. F. Yue, L. X. Feng, H. J. Zhu, F. Cao, Mar. Drugs 2017, 15, 550.

[16] Y. Ninomiya, K. Suzuki, C. Ishii, H. Inoue, Proc. Natl. Acad. Sci. USA 2004, 101, 12248–12253.

[17] a) Marcher-Bauer, M. K. Derbyshire, N. R. Gonzales, S. Lu, F. Chitsaz, L. Y. Geer, R. C. Geer, J. He, M. Gwadz, D. I. Hurwitz, Nucleic Acids Res. 2015, 43, D222–D226.

[18] C. Hertweck, Angew. Chem. Int. Ed. 2009, 48, 4688–4716; Angew. Chem. 2009, 121, 4782–4811.

[19] M. A. Hayes, S. K. Wrigley, I. Chetland, E. E. Reynolds, A. M. Ainsworth, D. V. Renno, M. A. Latif, X. M. Cheng, D. I. Hupe, P. Charlton, J. Antibiot. 1996, 49, 505–512.

[20] V. Meyer, F. Wanka, J. van Gent, M. Arentshorst, C. A. van den Hond, A. F. Ram, Appl. Microbiol. 2011, 77, 2975.

[21] Z. Li, S. K. Nair, Structure 2015, 23, 2032–2042.

[22] J. W. Campbell, R. M. Morgan-Kiss, J. E. Cronan, Jr., Mol. Microbiol. 2003, 47, 793–805.

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