Organic LETTERS
Letter
Cite This: Org. Lett. 2018, 20, 4854−4857
pubs.acs.org/OrgLett

AbmV Catalyzes Tandem Ether Installation and Hydroxylation during Neoabyssomicin/Abyssomicin Biosynthesis

Qinglian Li,*[†] Wenjuan Ding,[†,‡] Ziwei Yao,[†,‡] Jiajia Tu,[†] Liyan Wang,[§] Hongbo Huang,[†] Shengying Li,[∥] and Jianhua Ju*[†,‡]

[†]CAS Key Laboratory of Tropical Marine Bio-resources and Ecology, Guangdong Key Laboratory of Marine Materia Medica, RNAM Center for Marine Microbiology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, China

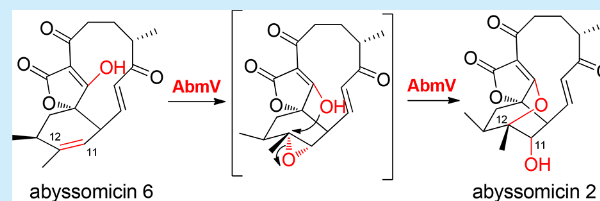
[‡]University of Chinese Academy of Sciences, 19 Yuquan Road, Beijing 100049, China

[§]College of Bio and Marine Sciences, Shenzhen University, 3688 Nanhai Ave, Shenzhen 518060, China

[∥]Shandong Provincial Key Laboratory of Synthetic Biology, CAS Key Laboratory of Biofuels, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, Shandong 266000 China

Supporting Information

ABSTRACT: Members of the abyssomicin class of natural products are characterized by a novel vinylic bridged ether ring. In this study, in vivo gene inactivation, structure elucidation of the accumulated intermediate abyssomicin 6, and in vitro enzyme assays enabled the identification of a cytochrome P450 enzyme, AbmV. AbmV carries out domino reactions involving bridged ether installation and C-11 hydroxylation during the biosynthesis of neoabyssomicins/abyssomicins in *S. koyangensis* SCSIO 5802.



Downloaded via SHANDONG UNIV on November 25, 2019 at 02:05:04 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

Spirotetronate natural products are diverse polyketide natural products that contain a signature spirotetronate moiety (tetronic acid spiro-linked to a cyclohexene/cyclohexane ring); examples include chlorothrin, kijanimicin, tetrocarcin A, lobophorin, and abyssomicin (Figure 1 and Figure S1).[1] Distinct from other spirotetronates, the abyssomicins possess an additional bridged ether ring (oxo-bridge) from the tetronate moiety (Figure 1, colored red). The abyssomicins have been isolated from both *Verrucosispora* and *Streptomyces* species.[2−9] For example, neoabyssomicins A (**1**) and B (**2**) and abyssomicins 2 (**3**) and 4 (**4**) were isolated from the deep sea derived *Streptomyces koyangensis* SCSIO 5802 by our group.[9] To date, ~30 abyssomicin derivatives have been isolated, and these can be classified as type I or "enantiomeric" counterparts type II (see Figure 1 for the chemical structures of the representatives for each type).[9]

Most abyssomicin derivatives possess attractive antibacterial and antiviral activities. Among them, abyssomicin C exhibits promising antibacterial activities against a number of Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA)[10] and *Mycobacterium tuberculosis*;[11] neoabyssomicin A (**1**) augments human immunodeficiency virus-1 (HIV-1) replication in a human lymphocyte model;[9] abyssomicin 2 (**3**) not only selectively reactivates latent HIV[8] but is also active against a panel of Gram-positive pathogens, including MRSA.[9] SAR studies have shown that the C8/C9 double bond is crucial for antibacterial activity.[2,4]

Our early biosynthetic studies as well as those of others unveiled a common pathway relevant to the abyssomicin class of natural products.[11−13] The pathway is composed of the following steps: (i) assembly of the carbon chain by a modular type I polyketide synthase (PKS); (ii) termination of the carbon chain elongation by incorporation of a glycerol-derived three-carbon unit, leading to formation of the tetronate moiety; (iii) generation of the double bond exocyclic to the tetronate moiety via an acetylation−elimination process and ensuing intramolecular [4 + 2] cyclization to yield the spirotetronate moiety-containing polyketide core backbone; and (iv) further tailoring reactions to produce the final structures of abyssomcin derivatives (Figure 2B). In particular, the first post-tailoring step is proposed to be the oxidation of the spirotetronate moiety-containing polyketide core backbone to generate the unique bridged ether ring (Figure 2B);[11,12] however, the specific enzyme(s) and the precise details for how this enzyme(s) carry out the relevant chemistry have eluded characterization. In our early studies of neoabyssomcins/abyssomicins (**1**−**4**) biosynthesis in *S. koyangensis* SCSIO 5802, we identified the biosynthetic gene cluster that codes for production of **1**−**4** (termed *abm* gene cluster). Heterologous expression of the *abm* cluster in *S. coelicolor*[11] revealed that all the genes responsible for construction of **1**−**4**, including those for formation of the bridged ether ring, are embedded within the *abm* gene cluster. In this work, we report the in vivo and in vitro characterization of a cytochrome P450 enzyme, AbmV; this enzyme catalyzes the epoxidation and subsequent

Received: June 26, 2018
Published: August 2, 2018

ACS Publications © 2018 American Chemical Society

DOI: 10.1021/acs.orglett.8b01997
Org. Lett. 2018, 20, 4854−4857

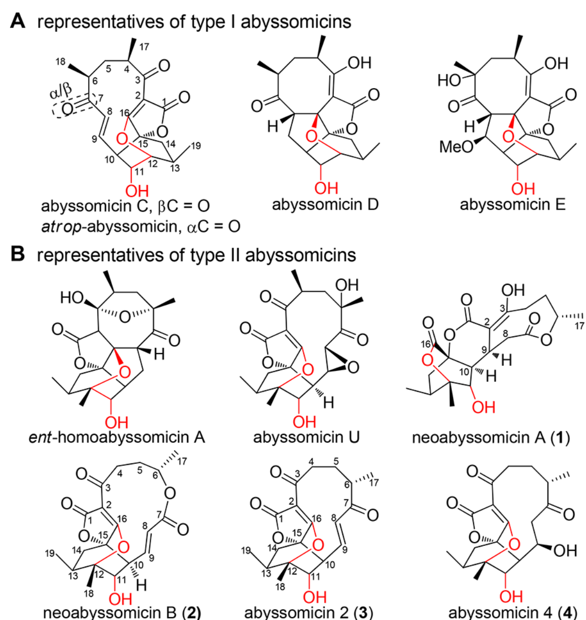


Figure 1. Chemical structures of representative abyssomicins; **1**–**4** from *S. koyangensis* SCSIO 5802. The characteristic bridging ether is indicated in red.

nucleophilic substitution process to construct the bridged ether ring and install the C-11 OH group during biosynthesis of neoabyssomicins/abyssomicins in *S. koyangensis* SCSIO 5802.

Based on the hypothesis that bridged ether generation might be initiated by oxidation of the C11–C12 olefin polyketide backbone, we speculated that a cytochrome P450 enzyme(s) might be involved in this process due to the diverse roles of cytochrome P450 enzymes in catalyzing various oxidation reactions.[14−16] Bioinformatics analysis revealed that only one cytochrome P450 enzyme, AbmV, is encoded within the *abm* gene cluster (Figure 2A). Sequence alignments of AbmV with the well-characterized cytochrome P450s involved in secondary metabolism revealed that AbmV contains the conserved heme-binding Cys, EXXR motif in the Helix-K, and Thr in the Helix-I (Figure S2). Notably, *abmG*, encoding a P450 redox partner protein, ferredoxin, was observed directly downstream of *abmV* in the *abm* gene cluster (Figure 2A). Thus, AbmV was selected as the preferred candidate responsible for installing the bridged ether of the abyssomicin-like natural products.

To probe the exact role(s) of AbmV in the biosynthesis of neoabyssomicins/abyssomicins, we inactivated *abmV* in wild-type *S. koyangensis* SCSIO 5802 using established λ-RED-mediated PCR-targeting mutagenesis methods. The resulting Δ*abmV* mutant was confirmed by PCR. HPLC analysis of the fermentation extract of the Δ*abmV* mutant revealed that production of main metabolites **2**–**4** was abolished. In turn, a new metabolite peak (Figure 3, trace (ii)) was clearly present in the HPLC traces, and its molecular weight of 330.14 was determined by LCMS analysis. The accumulated intermediate was termed abyssomicin 6 (**5**) herein. Complementation of the Δ*abmV* mutant using *abmV* in the trans position restored the

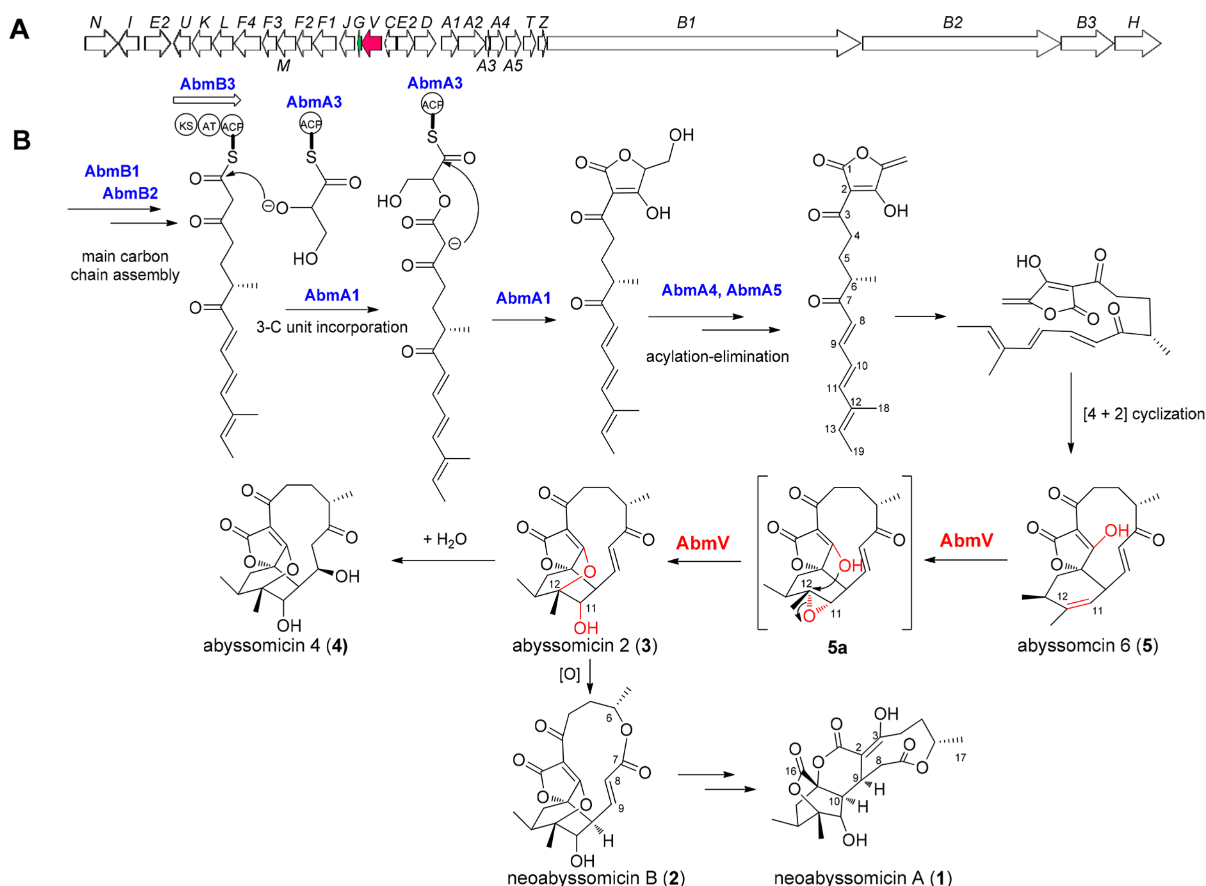


Figure 2. (A) Genetic organization of the neoabyssomicin/abyssomicin BGC in *S. koyangensis* SCSIO 5802. The cytochrome P450 gene *abmV* and the ferredoxin gene *abmG* are shaded in red and green, respectively. (B) Proposed pathway for post-tailoring steps in neoabyssomicin/abyssomicin biosynthesis in *S. koyangensis* SCSIO 5802; the AbmV-catalyzed step has been validated in this work.

DOI: 10.1021/acs.orglett.8b01997
Org. Lett. 2018, 20, 4854–4857

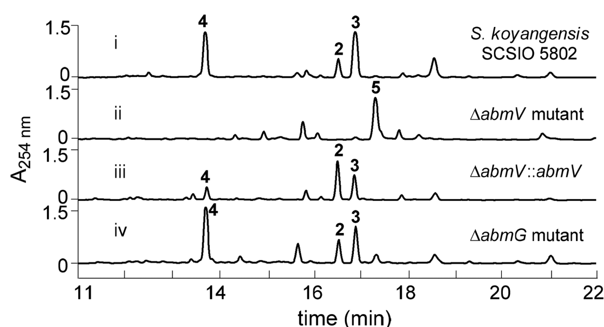


Figure 3. HPLC analyses of fermentation broths: (i) wild-type *S. koyangensis* SCSIO 5802; (ii) Δ*abmV*; (iii) Δ*abmV*:*abmV*; (iv) Δ*AbmG*.

production of the full set of metabolites, as shown in the wild-type strain (Figure 3, trace (iii)). These results demonstrate the involvement of *abmV* in neoabyssomcin/abyssomicin construction and suggest that **5** might be the direct precursor of AbmV. However, inactivation of *abmG* did not impact the biosynthesis of **2**–**4** (Figure 3, trace (iv)); this observation may be explained by the presence of a compensatory ferredoxin elsewhere within the *S. koyangensis* SCSIO 5802 genome.

To elucidate the structure of accumulated intermediate **5**, a 12 L scale fermentation of the Δ*abmV* mutant was performed. The culture broth was extracted with butanone, and the mycelial cake was extracted with acetone. Silica gel column chromatography and subsequent preparative HPLC enabled the purification of abyssomicin 6 as a white solid (yield: ~6 mg/L). HR-ESI-MS revealed that **5** has a molecular formula of $C_{19}H_{21}O_5$ ($[M - H]^-$, calcd 329.1394; found 329.1398), 16 Da smaller than that of abyssomicin 2 (**3**), probably indicating the lack of an oxygen atom. The ^1H and ^{13}C NMR data (Table S3) revealed the presence of two ketone carbonyls (δ_C 196.1, C-3; 207.1, C-7), one ester carbonyl (δ_C 173.7, C-1), as well as four nonprotonated carbons, six methines, three methylenes, and three methyls. The 1D NMR data of **5** resembled those reported for **3**, indicating that **5** and **3** share structural similarities. Detailed comparisons revealed that two oxygen-bearing carbons at δ_C 75.4 and δ_C 90.4 in **3** were replaced by two sp^2-hybridized carbons at δ_C 118.0 (C-11) and δ_C 140.8 (C-12) in **5**, thus proving the existence of the C-11/C-12 double bond in **5**. This structural feature was further supported by COSY and HMBC correlations (Figure 4). The ^{13}C NMR

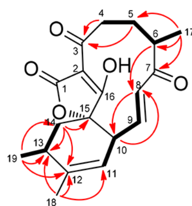


Figure 4. Selected COSY (bold line) and HMBC (arrow line) correlations of abyssomicin 6 (**5**).

chemical shift value of C-16 in **5** changed from δ_C 185.8 to δ_C 175.7, indicating the ether bridge between C-12 and C-16 was absent. The coupling constant of $J_{H\text{-}8/H\text{-}9}$ = 15.8 Hz indicated an *E*-olefin for the C-8/C-9 double bond. The NOE of H-10/H-13 was observed, demonstrating that H-10 and H-13 were oriented on the same side of the cyclohexene moiety in **5**. Consequently, **5** is a spirotetronate-containing intermediate in neoabyssomicin/abyssomicin biosynthesis that lacks the bridged ether ring (Figure 4). The absolute configurations of asymmetric centers in **5** are biogenetically presumed to be the same as those in abyssomicin 2 (**3**). We speculate that abyssomicin 6 (**5**) is formed by an enzyme-catalyzed intramolecular [4 + 2] cyclization. As a consequence, AbmV may convert abyssomicin 6 into a product with a bridged ether ring. We specifically envisioned a domino sequence in which substrate epoxidation by AbmV would be followed by etherification and C-11 hydroxylation, the result of epoxide scission.

To validate this hypothesis, we overexpressed and purified AbmV from *E. coli* (SI, Figure S3) and assayed its activity with **5** as substrate in vitro. Bacterial cytochrome P450 enzymes usually require a ferredoxin and a ferredoxin reductase as the redox partner proteins to transfer two electrons from NADPH to their heme-iron reactive center for dioxygen activation. Since the native ferredoxin reductase for AbmV was not identified in the *abm* gene cluster, the ferredoxin Fdx_1499 and the ferredoxin reductase FdR_0978 from the cyanobacterium type strain *Synechococcus elongatus* PCC 7942[17] were prepared as surrogate redox partner proteins to reconstitute AbmV activity in vitro. When AbmV was incubated with the purified **5** in the presence of Fdx_1499, FdR_0978, and NADPH at 30 °C for 2 h, the enzyme readily converted **5** to a new product with the same HPLC retention time and UV profile as the authentic standard abyssomicin 2 (**3**) (Figure 5, traces i, ii, and vi). The enzymatic product **3**

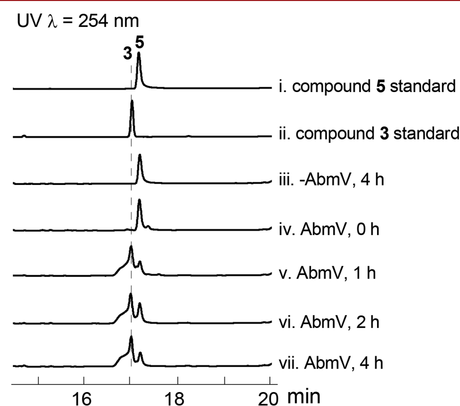


Figure 5. HPLC analyses of in vitro assays with (traces iv–-vii) or without (trace (iii) AbmV.

contains a shoulder peak. The product peak as well as the shoulder peak all showed identical mass (m/z = 347.1495) to that of abyssomicin 2 (Figure S4). We noticed that an EtOAc extract of standard **3** in the enzymatic Tris–HCl buffer (50 mM, pH 8.0) for 2 h also showed the same HPLC profile, suggesting the shoulder peak might be tautomer of abyssomicin 2. In addition, the time-course experiments revealed the formation of **3** concomitant with consumption of **5** in a time-dependent manner (Figure 5, traces iii–vii). These results provide clear evidence that AbmV is responsible for catalyzing formation of the bridged ether ring and C-11 hydroxylation of the spirotetronate scaffold during the course of neoabyssomicin/abyssomicin biosynthesis.

Carbon center hydroxylations and C=C double bond epoxidations are the most frequently reported reactions catalyzed by the majority of biosynthetic cytochrome P450s.[14−16] A small number of cytochome P450s that catalyze unusual intramolecular

DOI: 10.1021/acs.orglett.8b01997
Org. Lett. 2018, 20, 4854−4857

etherifications have also been identified.[14−16] Such enzymes include AurH from *S. thioluteus* in aureothin biosynthesis,[18] PtmO5 from *S. platensis* in platensimycin biosynthesis,[19] AtmQ from *Aspergillus flavus* in paspalicine biosynthesis,[20] and CYP71A32 from peppermint *Mentha piperita* in menthofuran biosynthesis.[21] However, bifunctional etherification and hydroxylation at the adjacent carbon have been undocumented thus far. Ether ring formation and hydroxylation catalyzed by AbmV likely results from a domino reaction sequence involving (i) epoxidation of the cyclohexene C11−C12 double bond in absyssomin 6 to afford intermediate **5a** followed by (ii) subsequent epoxide ring-opening via nucleophilic attack of the tetronate OH upon C12 to afford abyssomicin 2 (**3**) (Figure 2B).

In summary, we have applied in vivo gene inactivation, structure elucidation of intermediate **5**, and in vitro biochemical assays to identify and characterize the cytochrome P450 AbmV. This unique P450 enzyme catalyzes bridged ether installation and C-11 hydroxylation in tandem during the course of neoabyssomicin/abyssomicin biosynthesis in *S. koyangensis* SCSIO 5802. AbmV represents the first example of a cytochrome P450 that catalyzes such a set of domino reactions during secondary metabolism. Consequently, this work provides important insight into the post-tailoring steps involved in abyssomicin-like biosynthesis but also provides a glimpse of previously unrecognized cytochrome P450 functional diversities.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b01997.

Experimental details, NMR data, and 1D and 2D NMR spectra of compound **5** (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: liql@scsio.ac.cn.

*E-mail: jju@scsio.ac.cn.

ORCID

Hongbo Huang: 0000-0002-5235-739X

Shengying Li: 0000-0002-5244-870X

Jianhua Ju: 0000-0001-7712-8027

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported in part by the China NSF (31670087, 81425022, U1501223, U1706206, and 41476133), the CAS (XDA11030403 and XDA13020302-2), Guangdong NSF (2016A030312014), the Pearl River S&T Nova Program of Guangzhou (201806010109), and the Special Support Program for Training High-Level Talents in Guangdong (No. 2016TQ03R288).

■ REFERENCES

(1) Vieweg, L.; Reichau, S.; Schobert, R.; Leadlay, P. F.; Sussmuth, R. D. *Nat. Prod. Rep.* **2014**, *31*, 1554−1584.

(2) Bister, B.; Bischoff, D.; Ströbele, M.; Riedlinger, J.; Reicke, A.; Wolter, F.; Bull, A. T.; Zähner, H.; Fiedler, H. P.; Süssmuth, R. D. *Angew. Chem., Int. Ed.* **2004**, *43*, 2574−2576.

(3) Keller, S.; Nicholson, G.; Drahl, C.; Sorensen, E.; Fiedler, H. P.; Süssmuth, R. D. *J. Antibiot.* **2007**, *60*, 391−394.

(4) Wang, Q.; Song, F.; Xiao, X.; Huang, P.; Li, Li.; Monte, A.; Abdel - Mageed, W. M.; Wang, J.; Guo, H.; He, W.; Xie, F.; Dai, H.; Liu, M.; Chen, C.; Xu, H.; Liu, M.; Piggott, A. M.; Liu, X.; Capon, R. J.; Zhang, L. *Angew. Chem., Int. Ed.* **2013**, *52*, 1231−1234.

(5) Niu, X. - M.; Li, S. - H.; Görls, H.; Schollmeyer, D.; Hilliger, M.; Grabley, S.; Sattler, I. *Org. Lett.* **2007**, *9*, 2437−2440.

(6) Abdalla, M. A.; Yadav, P. P.; Dittrich, B.; Schüffler, A.; Laatsch, H. *Org. Lett.* **2011**, *13*, 2156−2159.

(7) Wang, X.; Elshahawi, S.; Cai, W.; Zhang, Y.; Ponomareva, L. V.; Chen, X. *J. Nat. Prod.* **2017**, *80*, 1141−1149.

(8) León, B.; Navarro, G.; Dickey, B. J.; Stepan, G.; Tsai, A.; Jones, G. S.; Morales, M. E.; Barnes, T.; Ahmadyar, S.; Tsiang, M.; Geleziunas, R.; Cihlar, T.; Pagratis, N.; Tian, Y.; Yu, H.; Linington, R. G. *Org. Lett.* **2015**, *17*, 262−265.

(9) Song, Y.; Li, Q.; Qin, F.; Sun, C.; Liang, H.; Wei, X.; Wong, N.; Ye, L.; Zhang, Y.; Shao, M.; Ju, J. *Tetrahedron* **2017**, *73*, 5366−5372.

(10) Riedlinger, J.; Reicke, A.; Zähner, H.; Krismer, B.; Bull, A. T.; Maldonado, L. A.; Ward, A. C.; Goodfellow, M.; Bister, B.; Bischoff, D.; Süssmuth, R. D.; Fiedler, H. P. *J. Antibiot.* **2004**, *57*, 271−279.

(11) Gottardi, E. M.; Krawczyk, J. M.; von Suchodoletz, H.; Schadt, S.; Mühlenweg, A.; Uguru, G. C.; Pelzer, S.; Fiedler, H. P.; Bibb, M. J.; Stach, J. E.; Süssmuth, R. D. *ChemBioChem* **2011**, *12*, 1401−1410.

(12) Tu, J.; Li, S.; Chen, J.; Song, Y.; Fu, S.; Ju, J.; Li, Q. *Microb. Cell Fact.* **2018**, *17*, 28.

(13) Wang, X.; Elshahawi, S. I.; Cai, W.; Zhang, Y.; Ponomareva, L. V.; Chen, X.; Copley, G. C.; Hower, J. C.; Zhan, C. G.; Parkin, S.; Rohr, J.; Van Lanen, S. G.; Shaaban, K. A.; Thorson, J. S. *J. Nat. Prod.* **2017**, *80*, 1141−1149.

(14) Rudolf, J. D.; Chang, C. Y.; Ma, M.; Shen, B. *Nat. Prod. Rep.* **2017**, *34*, 1141−1172.

(15) Greule, A.; Stok, J. E.; De Voss, J. J.; Cryle, M. J. *Nat. Prod. Rep.* **2018**, DOI: 10.1039/C7NP00063D.

(16) Zhang, X.; Li, S. *Nat. Prod. Rep.* **2017**, *34*, 1061−1089.

(17) (a) Sun, Y.; Ma, L.; Han, D.; Du, L.; Qi, F.; Zhang, W.; Sun, J.; Huang, S.; Kim, E. S.; Li, S. *J. Ind. Microbiol. Biotechnol.* **2017**, *44*, 161−166. (b) Qi, F.; Lei, C.; Li, F.; Zhang, X.; Wang, J.; Zhang, W.; Fan, Z.; Li, W.; Tang, G. L.; Xiao, Y.; Zhao, G.; Li, S. *Nat. Commun.* **2018**, *9*, 2342.

(18) (a) Zocher, G.; Richter, M. E. A.; Mueller, U.; Hertweck, C. *J. Am. Chem. Soc.* **2011**, *133*, 2292−2302. (b) He, J.; Muller, M.; Hertweck, C. *J. Am. Chem. Soc.* **2004**, *126*, 16742−16743. (c) Henrot, M.; Richter, M. E. A.; Maddaluno, J.; Hertweck, C.; De Paolis, M. *Angew. Chem., Int. Ed.* **2012**, *51*, 9587−9591. (d) Richter, M. E. A.; Traitcheva, N.; Knupfer, U.; Hertweck, C. *Angew. Chem., Int. Ed.* **2008**, *47*, 8872−8875.

(19) Rudolf, J. D.; Dong, L. B.; Manoogian, K.; Shen, B. *J. Am. Chem. Soc.* **2016**, *138*, 16711−16721.

(20) Nicholson, M. J.; Koulman, A.; Monahan, B. J.; Pritchard, B. L.; Payne, G. A.; Scott, B. *Appl. Environ. Microbiol.* **2009**, *75*, 7469−7481.

(21) Bertea, C. M.; Schalk, M.; Karp, F.; Maffei, M.; Croteau, R. *Arch. Biochem. Biophys.* **2001**, *390*, 279−286.

DOI: 10.1021/acs.orglett.8b01997
Org. Lett. 2018, 20, 4854−4857