OL | Organic Letters

Letter

Cite This: Org. Lett. 2019, 21, 2330−2334

pubs.acs.org/OrgLett

Multidomain P450 Epoxidase and a Terpene Cyclase from the Ascochlorin Biosynthetic Pathway in *Fusarium* sp.

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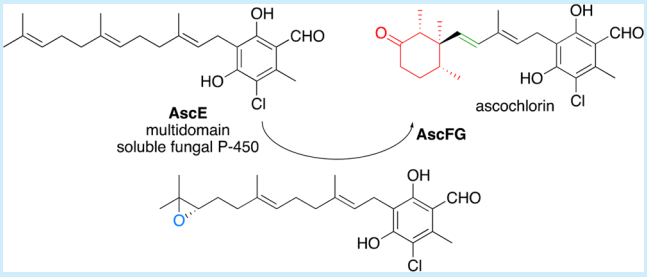
Supporting Information

ABSTRACT: Ascochlorin is a medicinally important fungal meroterpenoid. Its biosynthetic pathway in *Fusarium* sp. was identified, and the stereoselective epoxidation of the farnesyl group by the multidomain, soluble P450 monooxygenase AscE and the subsequent formation of the unique time-thylcyclohexanone ring by the membrane-bound cyclase AscF were investigated. Precursor-directed biosynthesis generated novel bromo-substituted derivatives, which exhibited potent cytotoxic activities. This study paves the way for the future metabolic engineering of medicinally important meroterpenoids for drug discovery.



Meroterpenoids are hybrid natural products partially derived from terpenoid pathway and include a variety of structurally diverse and biologically active compounds.[1] Several types of fungal meroterpenoid biosynthetic pathways have recently been characterized. These pathways typically involve the synthesis of a prenyl acceptor by an iterative type I polyketide synthase (PKS), prenyltransfer by a UbiA-type prenyltransferase (PT), epoxidation of the prenyl group by a flavin-dependent oxidase (FMO), and cyclization of the epoxide by a membrane-bound terpene cyclase (TPC), followed by various postcyclization modification reactions (e.g., oxidation, reduction, and acetylation).[2] In this way, complex molecular scaffolds are generated from simple building blocks in relatively short steps. This is a potential synthetic biology platform for the further production of medicinally important molecules for drug discovery.

Ascochlorin (**1a**) was first isolated as an antivirus agent from fungus *Ascochyta viciae* (Figure 1).[3a] In addition to the antivirus activity, **1a** exhibits various biological activities, such as antitumor, antiinflammatory, and immunosuppressive activities.[4] A number of ascochlorin derivatives have been isolated from various fungi, including *Fusarium*, *Colletotrichum*, *Cylindrocladium*, and *Verticillium*,[3] illustrating the wide distribution of the ascochlorin-type biosynthetic pathway. We have recently reported the biosyntheses of LL-Z1272β (**2**) in *Stachybotrys bisbyi* PYH05-7,[5] and ascofuranone (**3**) in *Acremonium egyptiacum*,[6] which share the early stage in their biosynthetic pathways. Thus, in both cases, **2** is generated by the orsellinic acid-producing PKS, the nonribosomal peptide synthetase-like reductase, and the UbiA-type PT.[5] Furthermore, the epoxidation of **2** by a P450 monooxygenase and the subsequent cyclization of the resultant epoxide by a membrane-bound TPC lead to the formation of the monocyclic terpene scaffold of **1a**.[6] However, the detailed mechanisms of these enzyme reactions and their catalytic properties have not been rigorously investigated. In this study, we identified the ascochlorin biosynthetic enzymes AscA−G from the fungus *Fusarium* sp. NBRC 100844 and characterized the catalytic machineries that generate the stereoselective epoxidation of the farnesyl group by the multidomain soluble fungal P450 monooxygenase AscE and the subsequent formation of the unique trimethylcyclohexanone ring by the membrane-bound TPC AscF. Furthermore, novel brominated analogs were produced by feeding experiments, and the effect of the halogen atom substitution on the cytotoxicity was evaluated.

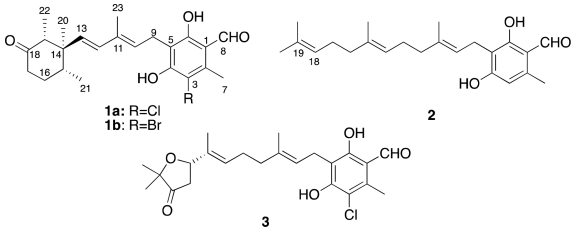


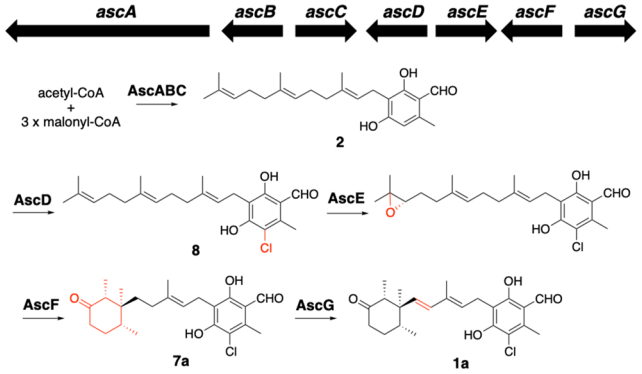
Figure 1. Chemical structures of ascochlorin (**1a**), its brominated derivative (**1b**), LL-Z1272β (**2**), and ascofuranone (**3**).

Received: February 18, 2019
Published: March 22, 2019

DOI: 10.1021/acs.orglett.9b00616
Org. Lett. 2019, 21, 2330−2334

To search for the biosynthetic gene cluster, we performed a tBlastn analysis on the draft genome sequence of the ascochlorin producer *Fusarium* sp. NBRC 100844, by using the amino acid sequence of StbC[5] as a query. The obtained *ascA-G* cluster contains seven genes, encoding StbABC homologues: a PKS (AscA), an NRPS-like reductase (AscB), and a UbiA-type PT (AscC), as well as an FMO enzyme (AscD), two P450 enzymes (AscE, AscG), and a membrane-bound TPC (AscF) (Scheme 1 and Figures S1,S2). In fungal

Scheme 1. Gene Cluster and Proposed Biosynthetic Pathway of 1a



meroterpenoid biosyntheses,[2] FMOs usually catalyze the epoxidation of a prenyl side chain prior to the terpene cyclization reaction; however, the FMO AscD shares 50 and 51% identity with the known tryptophan halogenases, RadH and Rdc2, respectively,[7] suggesting that AscD catalyzes the halogenation of the arylaldehyde moiety of **2**. Indeed, as previously reported, the coexpression of *ascD* with the biosynthetic genes for the plant meroterpenoid daurichromenic acid (DCA) in *Aspergillus oryzae* led to the production of unnatural novel halogenated derivatives of DCA,[8] indicating that AscD functions as a halogenase but not as an epoxidase. Then we hypothesized that a P450, either AscE or AscG, is involved in the epoxidation of the C-18-C-19 double bond of **2** for the biosynthesis of ascochlorin. Notably, AscE shares 36% amino acid sequence identity with the soluble P450 alkene epoxidase/alkane hydroxylase P450BM-3 from *Bacillus megaterium*,[9] and homology modeling revealed that AscE also forms an unusual multidomain structure consisting of the heme-binding P450 domain and flavin-NADP$^+$ binding domain, which reduces the heme iron to turn over the reaction as in the case of P450BM-3 (Figure S3).[10] Actually, TMHMM analysis[11] (prediction of transmembrane helices in proteins) suggested that AscE is a soluble protein without any transmembrane (TM) region. In contrast, most of the characterized fungal P450 enzymes contain an N-terminal TM region.[12] On the basis of these observations, we predicted that the unusual and novel multidomain P450 AscE catalyzes the epoxidation of the terminal C-18-C-19 double bond and the other P450 AscG desaturates at C-12−C-13.

First, to connect the *asc* cluster with ascochlorin biosynthesis, we heterologously expressed *ascABC* in *A. oryzae* NSAR1[13] and analyzed the metabolites that accumulated in the mycelia of each transformant by high-performance liquid chromatography (HPLC). As a result, **2** was detected as a product of AscABC (yield 7.0 mg/L) (Figure 2A, lane i), thus confirming that the PKS AscA, the NRPS-like reductase AscB,

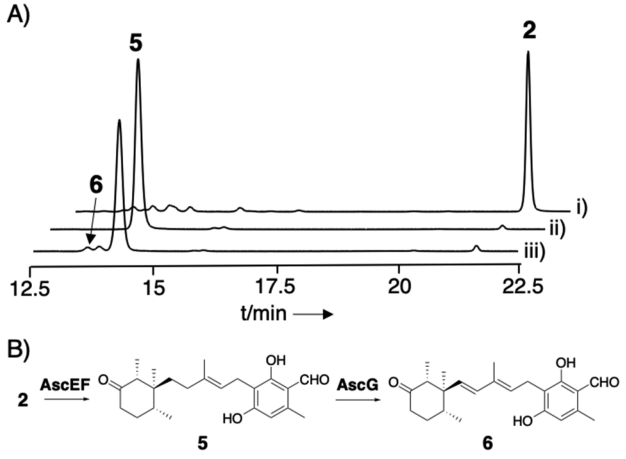


Figure 2. HPLC profile of the mycelia extract of *A. oryzae* transformants (A) and reactions by AscEFG (B). The metabolites from the transformants harboring (i) *ascABC*, (ii) *ascABCEF*, and (iii) *ascABCEFG* were analyzed. The chromatograms were monitored at 295 nm.

and the UbiA-type PT AscC are definitely involved in the biosynthesis of **2**, as observed in the *A. egyptiacum* pathway.[6] Next, to elucidate each step of ascochlorin biosynthesis, *ascE*, *F*, and *G* were additionally coexpressed in the host with *ascABC*. HPLC analyses of the products from each transformant showed that the *A.oryzae*/*ascABCEF* strain produced **5** (5.2 mg/L) and the *A.oryzae*/*ascABCEFG* strain produced **6** (0.1 mg/L) (Figure 2A, lanes ii and iii). Comparisons of the NMR and MS data with the literature identified **5** as dechloro-12,13-dihydroascochlorin and **6** as dechloroascochlorin (Figure 2B, Table S6, and Figures S4, S5, S23, S24, S29, and S30).[3c] The optical rotation value of **5** ($[\alpha]^{25}_{D}$ +7.60 (*c* 0.43, CH_3OH)) matched the literature data well.[3c] This data indicated that the absolute configurations were 14*S*,15*R*,19*R*, which is identical to that of **1a**. The above data clearly demonstrated that the farnesyl group of **2** is converted to **5** by AscE and AscF and then further oxidized into **6** by AscG (Figure 2B).

Next, in order to investigate the catalytic mechanisms of the P450 monooxygenase AscE and the TPC AscF, we conducted in vitro characterizations of these two enzymes (Figure 3). Since, as mentioned above, the rare multidomain fungal P450 AscE was predicted to lack the TM region, we expected that AscE could be produced as a soluble enzyme in *Escherichia coli* and analyzed by an in vitro enzyme reaction. Thus, we expressed the *ascE* cDNA by using the pColdII expression system in Rosetta2 strain, extracted the protein from the transformant with buffer, and checked its soluble and insoluble protein fractions by SDS−PAGE. As expected, the protein band corresponding to AscE was detected in the soluble fraction (Figure S6), indicating that the AscE protein was solubilized in the buffer. We also confirmed that the heme-binding domain of AscE was in the active form by a CO-binding spectral assay (Figure S7). Therefore, we utilized the soluble protein fraction for the assay with **2** as a substrate. HPLC analyses of the reaction products revealed the conversion of **2** to **4**, specifically in the AscE reaction (Figure 3A lanes i and (ii). The HR-MS of **4** was 371.2228, indicating that its molecular formula should be $C_{23}H_{32}O_4$ (calcd 371.2205), corresponding to the epoxidated **2**. With the prediction that **4** should be the precursor of **5**, we added the

DOI: 10.1021/acs.orglett.9b00616
Org. Lett. 2019, 21, 2330−2334

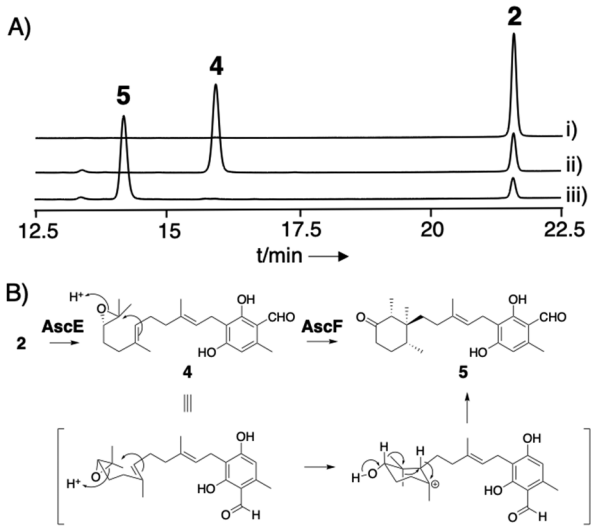


Figure 3. HPLC profile of the products from AscEF in vitro assay (A) and their reactions (B). Compound **2** was incubated with soluble protein extract from Rosetta2/pColdII (i) and the extract from Rosetta2/pColdII-AscE (ii). The AscE reaction was further incubated with the membrane fraction from INVSCII/pESCUra-AscF (iii). The chromatograms were monitored at 295 nm.

yeast microsomal fraction containing AscF into the AscE reaction and incubated the mixture. As expected, **4** was converted into **5** in the AscEF reaction (Figure 3A, lane iii). These data clearly showed that **4** was cyclized into **5** by AscF, indicating that **4** arises from the 18,19-epoxidation of **2**. The hydrolyzed product of **4**, cylindrocarpol[3b] (**4′**), can be isolated from an extract from the mycelia of the *Aspergillus oryzae* transformant harboring *ascABCEF* incubated on a large scale as well as **5**. A Mosher analysis performed on **4′** revealed that the stereochemistry of C-18 on **4′** was *R*. Thus, **4** was deduced to be hydrolyzed to **4′** in an S_N2 manner in *A. oryzae* (Figure S8), as reported in the literature.[14] Therefore, the stereochemistry of C-18 in **4** was determined as *S*. As reported previously, the ascochlorin derivative produced by the hydrolysis of the 18,19-epoxide of **2** was isolated from *Microcera* sp., and the C-18 stereochemistry was identified as *S*.[3g] This indicates that the stereoselectivity of the epoxidation is conserved in the ascochlorin-type biosynthesis. We also investigated the substrate specificity of AscE and found that it also accepts 5-farnesyl-6-hydroxymellein, an intermediate of the verruculide biosynthetic pathway (Figure S9),[15] indicating that AscE also possesses relaxed substrate specificity. When racemic-**4**, which was prepared through the bromohydrin formation of **2** by *N*-bromosuccinimide and the dehydrohalogenation under alkaline conditions, was reacted with the TPC AscF as a substrate, half of the racemic **4** was consumed, suggesting that AscF only accepts the *S*-enantiomer as a substrate (Figure S10).

We also performed mutation studies to understand the catalytic mechanism of terpene cyclization by AscF. AscF is classified as a distinct clade that includes no characterized enzymes in the phylogenetic tree of meroterpenoid TPCs (Figure S11). As compared with known terpene cyclases, it contains a relatively large amount of Trp (14/267aa), a larger fourth TM helix, and a linker between the fourth and fifth TM regions, compared with known TPCs[16] (Figure S2). The aromatic residues in TPCs are thought to stabilize the cation on the intermediate via cation−π interactions, and mutations of these residues could alter the terpene cyclization pattern.[17] Thus, we expected that the tryptophan residues, including the ones in the fourth TM domain, are involved in the catalysis to form the unique monocyclic terpene ring. Accordingly, we mutated four sets of aromatic residues into alanine, and each mutant was analyzed in vivo by coexpression in *A. oryzae* with *ascABCE*. When AscF W131A, W132A (in the fourth TM region), or W228A (in the seventh TM region) was expressed, significant decreases in the cyclization activity were observed (Figure S11), suggesting that either the conformation of these two TM regions or the cation-stabilizing effect is important for the activity. To assign the function of each Trp residue, we are currently solving the X-ray crystal structure. Similarly, the conserved acidic residues E61 and D235 in AscF, corresponding to the active site residues E63 and D218 in Pyr4, were also shown to be important for the activity through site-directed mutagenesis (Figure S10).

When we additionally expressed *ascD* in the *A. oryzae* harboring *ascABCEF*, we observed **7a** in the presence of chloride and **7b** in the presence of bromide as a product, respectively (Figure S12). Their *m*/*z* values clearly suggested that **7a** and **7b** were generated through the halogenation of **5** by AscD (Figure S4). To prepare large amounts of **7a** and **7b**, we incubated *Fusarium* sp. with KCl or KBr and isolated **1b**, **7a**, and **7b** (3.5, 10.1, 17.5 mg/L, respectively) (Figure 4). The

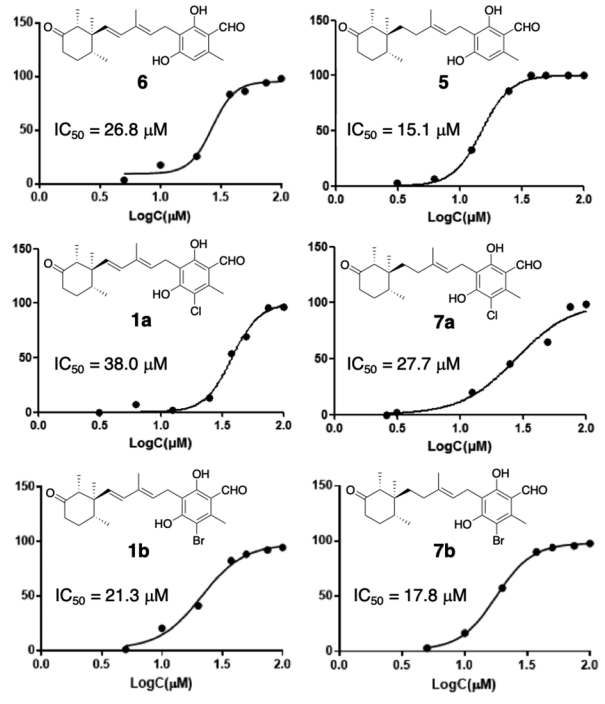


Figure 4. Cytotoxicity of **1a** and its analogues on MCF-7 breast cancer cell line.

spectroscopic analyses revealed that **1b**, **7a**, and **7b** were identified as 3-bromoascochlorin,[3h] 12,13-dihydroascochlorin,[3c] and 3-bromo-12,13-dihydroascochlorin (Table S6, Figures S4, S5, S20, S21, S24, S25, S26, and S27). These data indicated that AscD possesses relaxed halogen atom specificity and can accept both chloride and bromide. Its substrate specificity on halogen acceptor was also tested in vitro by using **2** or **5** as a substrate, with the crude protein extract from the *E. coli* expressing AscD (Figure S6), so that it can utilize the endogenous flavin reductase.[18] As a result, AscD halogenated 47% of **2** and 8% of **5** to produce **8** and **7a**,

DOI: 10.1021/acs.orglett.9b00616
Org. Lett. 2019, 21, 2330−2334

respectively (Figure S15). As judged from its m/z 389.1877 (calcd 389.1899, $C_{23}H_{30}ClO_3^-$) (Figure S4), **8** was deduced to be ilicicolin A.[19] The halogenation on **2** by AscD was also observed in the ascofuranone biosynthesis,[6] but this is the first insight on the substrate specificity obtained from in vitro assay. Since **7b** is a novel compound, we tested its cytotoxicity on the MCF-7 breast cancer cell line, along with **1a**, **1b**, **5**, **6**, and **7a**. Compound **7b** exhibited potent cytotoxicity, comparable to that of **5** and higher than that of **7a**, and **1b** exhibited higher activity than **6** and **1a** (Figure 4). These results indicated that the bromination at the C-3 position of **6** is more effective for the cytotoxicity, as compared with the chlorination at this position.

In this study, by using an *A. oryzae* expression system and in vitro assay, we proposed the ascochlorin biosynthetic pathway from **2** to **1a**, including the biosynthesis of **2** by AscABC, the halogenation at C-3 of orcylicaldehyde of **2** by the flavin-dependent halogenase AscD, the epoxidation of the terminal olefin by the multidomain P450 AscE, the cyclization by the membrane-bound TPC AscF, and the dehydrogenation of H-12 and -13 by the P450 AscG (Scheme 1). The halogenation likely occurs before AscE-catalyzed epoxidation, as shown in the ascofuranone pathway.[6] This is the first report for the substrate and stereospecificities of the rare multidomain fungal P450 monooxygenase and the membrane-bound TPC, which construct the monocyclic terpene structure of the meroterpenoid. We employed **2** as a substrate for AscEF reactions, as we did not obtain the sufficient amount of the halogenated intermediate **8** in this study. As the multidomain structure of AscE is identical to that of the bacterial P450 BM3,[9,10] it might have been horizontally transferred from the bacterial genome. The stereochemistry of C-18 of **4** is determined as *S*, indicating that AscE reacts with the olefin from the same face as the flavin-dependent meroterpenoid epoxidases Pyr5, AndE, and squalene epoxidase but opposite to AdrH, AusM, PrhM, and Trt8 (Figure S11).[2,15,20] VrtK is the P450 monooxigenase responsible for monocyclic terpene ring formation in viridicatumtoxin biosynthesis,[21] but it catalyzes the cyclization via the dehydrogenation of the geranyl group, in contrast to the epoxidation and cyclization by AscEF.

The knowledge obtained in this study will contribute toward investigations of the biosyntheses of other related meroterpenoids, such as **3**,[6] SMTPs,[22] and siccanochromenes (Figure S16).[23] Ascofuranone biosynthesis includes **4** as an intermediate, but C-16 of **4** is oxidized and the oxidized intermediate is cyclized in a different manner from AscF (Figure S16A).[6] Siccanochromenic acid contains a monocyclic terpene ring, but its cyclization reaction is different from that of AscF. Its biosynthesis includes terpene cyclization with the direct protonation of the terminal olefin, in a manner similar to that for the MacJ reaction in macrophorin biosynthesis (Figure S16B).[24] Stachybotrydial, a fungal meroterpenoid with a bicyclic terpenoid structure, which is derived from the same precursor as ascochlorin, was isolated from *Stachybotrys* sp.[25] The TPC involved in stachybotrydial biosynthesis may share the substrate binding site with AscF but apparently possesses a different reactivity for terpene cyclization (Figure S17). Comparison of the TPCs involved in the ascochlorin-related biosyntheses with AscF would yield valuable knowledge for understanding their catalytic mechanisms. Through the characterizations of terpene epoxidase and cyclase involved in ascochlorin biosynthesis, this study paves the way toward deepening our understanding of meroterpenoid biosynthesis. The modification enzymes identified in this study, such as the halogenase and P450 enzymes, can be utilized for future biosynthetic manipulations of medicinally important fungal meroterpenoids.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b00616.

Experimental details, spectroscopic data, and supplementary figures and tables (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Prof. K. Gomi (Tohoku University) and Prof. K. Kitamoto (The University of Tokyo) for kindly providing the expression vectors and the fungal strain. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (JSPS KAKENHI Grant Nos. JP16H06443, JP17H04763, JP17KT0095, and JP18K19139), JST/NSFC Strategic International Collaborative Research Program, and Kobayashi International Scholarship Foundation.

■ REFERENCES

(1) Geris, R.; Simpson, T. J. *Nat. Prod. Rep.* **2009**, *26*, 1063−1094.
(2) Matsuda, Y.; Abe, I. *Nat. Prod. Rep.* **2016**, *33*, 26−53.
(3) (a) Tamura, G.; Suzuki, S.; Takatsuki, A.; Ando, K.; Arima, K. *J. Antibiot.* **1968**, *21*, 539−544. (b) Zhang, P.; Bao, B.; Dang, H. T.; Hong, J.; Lee, H. J.; Yoo, E. S.; Bae, K. S.; Jung, J. H. *J. Nat. Prod.* **2009**, *72*, 270−275. (c) Singh, S. B.; Ball, R. G.; Bills, G. F.; Cascales, C.; Gibbs, J. B.; Goetz, M. A.; Hoogsteen, K.; Jenkins, R. G.; Liesch, J. M.; Lingham, R. B.; Silverman, K. C.; Zink, D. L. *J. Org. Chem.* **1996**, *61*, 7727−7737. (d) Ellestad, G. A.; Evans, R. H., Jr.; Kunstmann, M. P. *Tetrahedron* **1969**, *25*, 1323−1334. (e) Takamatsu, S.; Rho, M. C.; Masuma, R.; Hayashi, M.; Komiyama, K.; Tanaka, H.; Omura, S. *Chem. Pharm. Bull.* **1994**, *42*, 953−956. (f) Hosono, K.; Ogihara, J.; Ohdake, T.; Masuda, S. *J. Antibiot.* **2009**, *62*, 571−574. (g) Isaka, M.; Yangchum, A.; Supothina, S.; Laksanacharoen, P.; Luangsa-ard, J. J.; Hywel-Jones, N. L. *J. Antibiot.* **2015**, *68*, 47−51. (h) Aldridge, D. C.; Borrow, A.; Foster, R. G.; Large, M. S.; Spencer, H.; Turner, W. B. *J. Chem. Soc., Perkin Trans. 1* **1972**, *1*, 2136−2141.
(4) (a) Sakaguchi, K.; Nakajima, H.; Mizuta, N.; Furukawa, C.; Ozawa, S.; Ando, K.; Chang, Y.; Yamagishi, H.; Magae, J. *Biochem. Biophys. Res. Commun.* **2005**, *329*, 46−50. (b) Berry, E. A.; Huang, L.; Lee, D.; Daldal, F.; Nagai, K.; Minagawa, N. *Biochim. Biophys. Acta, Bioenerg.* **2010**, *1797*, 360−370. (c) Shen, W.; Ren, X.; Zhu, J.; Xu, Y.; Lin, J.; Li, Y.; Zhao, F.; Zheng, H.; Li, R.; Cui, X.; Zhang, X.; Lu, X.; Zheng, Z. *Eur. J. Pharmacol.* **2016**, *791*, 205−212. (d) Min-Wen, J. C.; Yan-Jiang, B. C.; Mishra, S.; Dai, X.; Magae, J.; Shyh-Chang, N.;

DOI: 10.1021/acs.orglett.9b00616
Org. Lett. 2019, 21, 2330−2334

Kumar, A. P.; Sethi, G. *Adv. Protein Chem. Struct. Biol.* **2017**, *108*, 199−225.
(5) Li, C.; Matsuda, Y.; Gao, H.; Hu, D.; Yao, S.; Abe, I. *ChemBioChem* **2016**, *17*, 904−907.
(6) Araki, Y.; Awakawa, T.; Matsuzaki, M.; Cho, R.; Matsuda, Y.; Hoshino, S.; Shinohara, Y.; Kido, Y.; Inaoka, D. K.; Nagamune, K.; Ito, K.; Abe, I.; Kita, K. *Proc. Natl. Acad. Sci. U.S.A.* **2019**, in press.
(7) (a) Wang, S.; Xu, Y.; Maine, E. A.; Wijeratne, E. M. K.; Espinosa-Artiles, P.; Gunatilaka, A. A. L.; Molnàr, I. *Chem. Biol.* **2008**, *15*, 1328−1338. (b) Zhou, H.; Qiao, K.; Gao, Z.; Vederas, J. C.; Tang, Y. *J. Biol. Chem.* **2010**, *285*, 41412−41421. (c) Zeng, J.; Zhan, J. *ChemBioChem* **2010**, *11*, 2119−2123.
(8) Okada, M.; Saito, K.; Wong, C. P.; Li, C.; Wang, D.; Iijima, M.; Taura, F.; Kurosaki, F.; Awakawa, T.; Abe, I. *Org. Lett.* **2017**, *19*, 3183−3186.
(9) (a) Boddupalli, S. S.; Estabrook, R. W.; Peterson, J. A. *J. Biol. Chem.* **1990**, *265*, 4233−4239. (b) Ost, T. W. B.; Munro, A. W.; Mowat, C. G.; Taylor, P. R.; Pesseguiero, A.; Fulco, A. J.; Cho, A. K.; Cheesman, M. A.; Walkinshaw, M. D.; Chapman, S. K. *Biochemistry* **2001**, *40*, 13430−13438. (c) Glieder, A.; Farinas, E. T.; Arnold, F. H. *Nat. Biotechnol.* **2002**, *20*, 1135−1139. (d) Farinas, E. T.; Alcalde, M.; Arnold, F. *Tetrahedron* **2004**, *60*, 525−528.
(10) (a) Ruettinger, R. T.; Wen, L. P.; Fulco, A. J. *J. Biol. Chem.* **1989**, *264*, 10987−10995. (b) Sevrioukova, I. F.; Li, H.; Zhang, H.; Peterson, J. A.; Poulos, T. L. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 1863−1868. (c) Munro, A. W.; Girvan, H. M.; McLean, K. J. *Biochim. Biophys. Acta, Gen. Subj.* **2007**, *1770*, 345−359.
(11) (a) Krogh, A.; Larsson, B.; von Heijne, G.; Sonnhammer, E. L. L. *J. Mol. Biol.* **2001**, *305*, 567−580. (b) Sonnhammer, E. L. L.; von Heijne, G.; Krogh, A. *Proceedings of the Sixth International Conference on Intelligent Systems for Molecular Biology*; Glasgow, J., Littlejohn, T., Major, F., Lathrop, R., Sankoff, D., Sensen, C., Eds.; AAAI Press, 1998; pp 175−182.
(12) Durairaj, P.; Hur, J.; Yun, H. *Microb. Cell Fact.* **2016**, *15*, 125−140.
(13) Jin, F. H.; Maruyama, J.; Juvvadi, P. R.; Arioka, M.; Kitamoto, K. *FEMS Microbiol. Lett.* **2004**, *239*, 79−85.
(14) Zhang, X. M.; Archelas, A.; Furstoss, R. *J. Org. Chem.* **1991**, *56*, 3814−3817.
(15) (a) Yamazaki, H.; Nakayama, W.; Takahashi, O.; Kirikoshi, R.; Izumikawa, Y.; Iwasaki, K.; Toraiwa, K.; Ukai, K.; Rotinsulu, H.; Wewengkang, D. S.; Sumilat, D. A.; Mangindaan, R. E.; Namikoshi, M. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 3087−3090. (b) Bai, T.; Quan, Z.; Zhai, R.; Awakawa, T.; Matsuda, Y.; Abe, I. *Org. Lett.* **2018**, *20*, 7504−7508.
(16) (a) Itoh, T.; Tokunaga, K.; Matsuda, Y.; Fujii, I.; Abe, I.; Ebizuka, Y.; Kushiro, T. *Nat. Chem.* **2010**, *2*, 858−864. (b) Matsuda, Y.; Wakimoto, T.; Mori, T.; Awakawa, T.; Abe, I. *J. Am. Chem. Soc.* **2014**, *136*, 15326−15336. (c) Matsuda, Y.; Awakawa, T.; Itoh, T.; Wakimoto, T.; Kushiro, T.; Fujii, I.; Ebizuka, Y.; Abe, I. *ChemBioChem* **2012**, *13*, 1738−1741. (d) Matsuda, Y.; Awakawa, T.; Abe, I. *Tetrahedron* **2013**, *69*, 8199−8204. (e) Matsuda, Y.; Iwabuchi, T.; Fujimoto, T.; Awakawa, T.; Nakashima, Y.; Mori, T.; Zhang, H.; Hayashi, F.; Abe, I. *J. Am. Chem. Soc.* **2016**, *138*, 12671−12677.
(17) (a) Tomita, T.; Kim, S. Y.; Teramoto, K.; Meguro, A.; Ozaki, T.; Yoshida, A.; Motoyoshi, Y.; Mori, N.; Ishigami, K.; Watanabe, H.; Nishiyama, M.; Kuzuyama, T. *ACS Chem. Biol.* **2017**, *12*, 1621−1628. (b) Christianson, D. W. *Chem. Rev.* **2017**, *117*, 11570−11648. (c) Dickschat, J. S. *Nat. Prod. Rep.* **2016**, *33*, 87−110. (d) Baunach, M.; Franke, J.; Hertweck, C. *Angew. Chem., Int. Ed.* **2015**, *54*, 2604−2626.
(18) Spyrou, G.; Haggard-ljungquist, E.; Krook, M.; Jornvall, H.; Nilsson, E.; Reichard, P. *J. Bacteriol.* **1991**, *173*, 3673−3679.
(19) Hayakawa, S.; Minato, H.; Katagiri, K. *J. Antibiot.* **1971**, *24*, 653−654.
(20) Abe, I.; Rohmer, M.; Prestwich, G. D. *Chem. Rev.* **1993**, *93*, 2189−2206.
(21) Chooi, Y.-H.; Hong, Y. J.; Cacho, R. A.; Tantillo, D. J.; Tang, Y. *J. Am. Chem. Soc.* **2013**, *135*, 16805−16808.
(22) (a) Shinohara, C.; Hasumi, K.; Hatsumi, W.; Endo, A. *J. Antibiot.* **1996**, *49*, 961−966. (b) Hasumi, K.; Ohyama, S.; Kohyama, T.; Ohsaki, Y.; Takayasu, R.; Endo, A. *J. Antibiot.* **1998**, *51*, 1059−1068.
(23) (a) Nozoe, S.; Suzuki, K. T.; Okuda, S. *Tetrahedron Lett.* **1968**, *9*, 3643−3646. (b) Nozoe, S.; Suzuki, K. *Tetrahedron Lett.* **1969**, *10*, 2457−2460. (c) Nozoe, S.; Suzuki, K. *Tetrahedron* **1971**, *27*, 6063−6071.
(24) Tang, M. C.; Cui, X.; He, X.; Ding, Z.; Zhu, T.; Tang, Y.; Li, D. *Org. Lett.* **2017**, *19*, 5376−5379.
(25) Lin, T.; Chang, X.; Chen, C.; Tsai, Y. *Biochem. Biophys. Res. Commun.* **2005**, *331*, 953−957.

DOI: 10.1021/acs.orglett.9b00616
Org. Lett. 2019, 21, 2330−2334