OL | Organic Letters

pubs.acs.org/OrgLett Letter

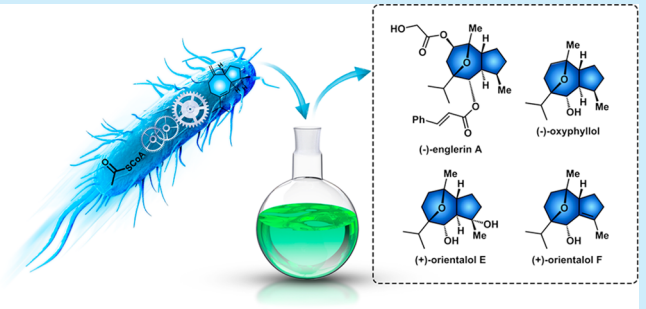
Syntheses of Epoxyguaiane Sesquiterpenes (−)-Englerin A, (−)-Oxyphyllol, (+)-Orientalol E, and (+)-Orientalol F: A Synthetic Biology Approach

Shu-Bin Mou,[†] Wen Xiao,[†] Hua-Qi Wang, Su-Jing Wang, and Zheng Xiang*

Cite This: https://dx.doi.org/10.1021/acs.orglett.0c00325 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: A combined approach toward syntheses of epoxyguaiane sesquiterpenes is presented. By use of a fungus sesquiterpene cyclase, guaian-6,10(14)-diene was produced through metabolic engineering of the isoprenoid pathway in *E. coli*. (−)-Englerin A, (−)-oxyphyllol, (+)-orientatol E, and (+)-orientalol F have been synthesized in two to six steps. This strategy provided rapid access to the epoxyguaiane core structure and would facilitate syntheses of (−)-englerin A and its analogues for evaluation of their therapeutic potentials in drug discovery.



Since its isolation by Beutler and co-workers from *Phyllanthus engleri* found in East Africa, englerin A (**1**) has attracted extensive attention from chemists, biologists, and physicians.[1] Englerin A demonstrates potent and selective inhibitory activity toward renal cancer cell lines, whereas englerin B (**2**), lacking the glycolic ester moiety, shows no significant cell growth inhibition.[1a] Members of the NCI Urologic Oncology Branch reported that englerin A induces glucose addiction through activation of protein kinase C-θ (PKCθ), while simultaneously starving the cell of glucose through inhibition of transport.[2] Additionally, Waldmann and co-workers discovered that englerin A is a potent and selective activator of transient receptor potential canonical (TRPC) calcium channels and it induces cell death by elevated Ca^{2+} influx and Ca^{2+} overload.[3] Englerin A has a complex architecture, which consists of a rare epoxyguaiane skeleton and seven stereocenters. Its structure and antirenal cancer ability make it an appealing and however challenging synthetic target. Based on the structure proposed by Beutler and co-workers, the Christmann group synthesized the enantiomeric (+)-englerin A for the first time using a ring-closing metathesis (RCM) strategy from *trans,cis*-nepetalactone and established the absolute configuration of (−)-englerin A.[4] Several research groups have reported the syntheses of englerin A via different approaches, including gold-catalyzed cyclization,[5] [4 + 3] cycloaddition,[6] [3 + 2] cycloaddition, and so on.[7] Besides englerins A and B, some epoxyguaiane sesquiterpenes (Figure 1a) isolated from *Phyllanthus oxyphyllus* bearing the same scaffold have been synthesized using the similar above-mentioned strategies.[6b,7k,8] Until now, all these published syntheses are totally based on chemical synthetic approaches. Herein, we report an efficient strategy, which combines

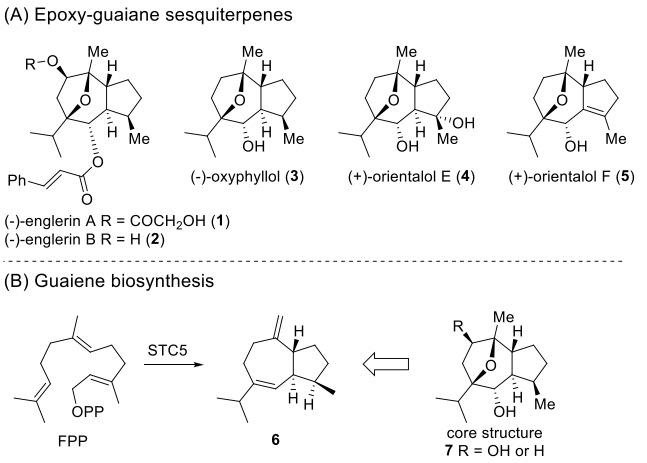


Figure 1. (A) Epoxyguaiane sesquiterpene natural products. (B) Products of guaiene synthase STC5.

microbial production of the guaiane skeleton in *Escherichia coli* with concise chemical transformations.[9]

The key to efficient syntheses of epoxyguaiane natural products is how to construct the molecular scaffold in a stereoselective and efficient manner. We envisioned that by use of a guaiene sesquiterpene synthase, the guaiane skeleton can

Received: January 23, 2020

be produced through metabolic engineering of the isoprenoid pathway in microbes and further be converted to (−)-englerin A and other epoxyguaiane natural products.[10] However, the biosynthetic machinery of these natural products from *P. engleri* and *P. oxyphyllus* has not been elucidated, and the cyclase gene has not been cloned. In this scenario, we proposed using a guaiene cyclase from other species to fulfill this purpose. In 2010, Kumeta and Ito characterized three synthases from *Aquilaria*, which could produce α-guaiene and δ-guaiene.[11a] In 2016, Dickschat and co-workers reported STC5, a guaiane sesquiterpene synthase identified from plant pathogenic fungus *Fusarium fujikuroi*.[11b] The absolute configuration of its product (**6**) (Figure 1b), guaian-6,10(14)-diene was confirmed by enantioselective synthesis. Comparing the structures of these enzymatic products and epoxyguaiane natural products, we noticed that the STC5 product, compound **6** consists of the same *trans*-fused bicyclo[5.3.0]guaiane-type moiety and three stereocenters with the same configurations as (−)-englerin A. Retrosynthetically, the epoxy group and two secondary hydroxyl ester groups of the core structure can be transformed from the two C═C bonds. Therefore, we planned our syntheses using compound **6** as a synthetic precursor.

We began with heterologous production of compound **6** through engineering the isoprenoid pathway in *E. coli* (Figure S1). Previously, some groups have reported the microbial production of monoterpenes, sesquiterpenes and diterpenes in *E. coli* and *Saccharomyces cerevisiae*.[12] By modulating the expression of the terpene synthase gene and the isoprenoid pathway, several terpenoids, including the precursor of artemisinin and Taxol, have been produced in *E. coli* or *S. cerevisiae* with titers in the g/L range. Inspired by these studies, we divided the mevalonate pathway into two operons. The first synthetic operon consists of an acetoacetyl-CoA thiolase (*atoB, E. coli*), a HMG-CoA synthase (*mvaS, Staphylococcus aureus*), and a truncated HMG-CoA reductase (*mvaA, S. aureus*). The second synthetic operon consists of a mevalonate kinase (*mvaK1*), a phosphomevalonate kinase (*mvaK2*), a phosphomevalonate decarboxylase (*mvaD*), and an isopentenyl diphosphate isomerase (*fni*) from *Streptococcus pneumoniae*. All the genes were codon-optimized and the two operons were subcloned into two multiple-cloning sites of pACYCDuet-1. The first operon converted acetyl-CoA to mevalonate, which was further transformed to isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) by the second operon. The genes encoding farnesyl pyrophosphate synthase (ERG20, *S. cerevisiae*) and the sesquiterpene synthase (STC5, *F. fujikuroi*) were subcloned into two multiple-cloning sites of pETDuet-1. The expression of all the genes was under the control of a bacteriophage T7 promoter. The strain containing both plasmids was tested for compound **6** production under two-phase flask cultivation. To our delight, a peak with molecular mass of *m*/*z* 204.2 was observed from the organic phase after 72 h of induction (Figure 2). The compound was purified and its ^{1}H NMR, ^{13}C NMR, and mass spectra were identical to the spectra reported by Dickschat and co-workers.[11b] A guaian-6,10(14)-diene titer of 119.4 ± 24.0 mg/L was obtained.

With compound **6** in hand, we embarked on synthesis of (−)-englerin A (Scheme 1). Regio- and stereoselective epoxidation of compound **6** with *m*-CPBA provided compound **8** in 49% yield. We also tested Jacobsen's catalysts to improve the diastereoselectivity. However, neither (*R,R*)-

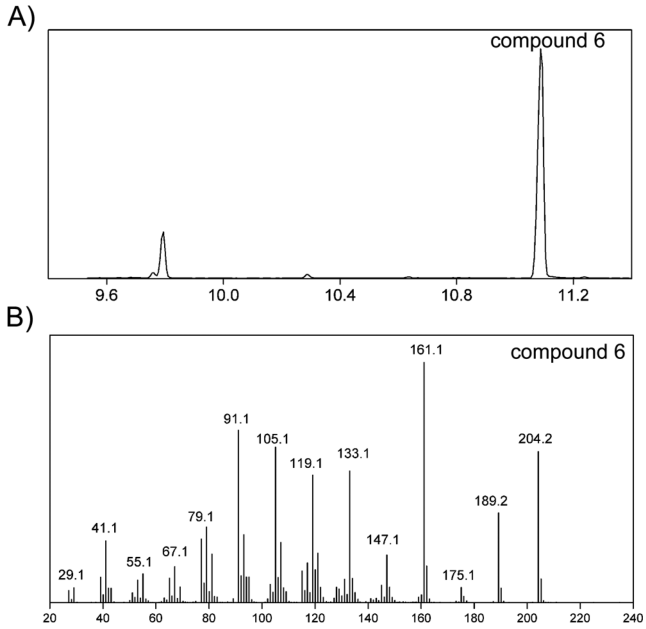
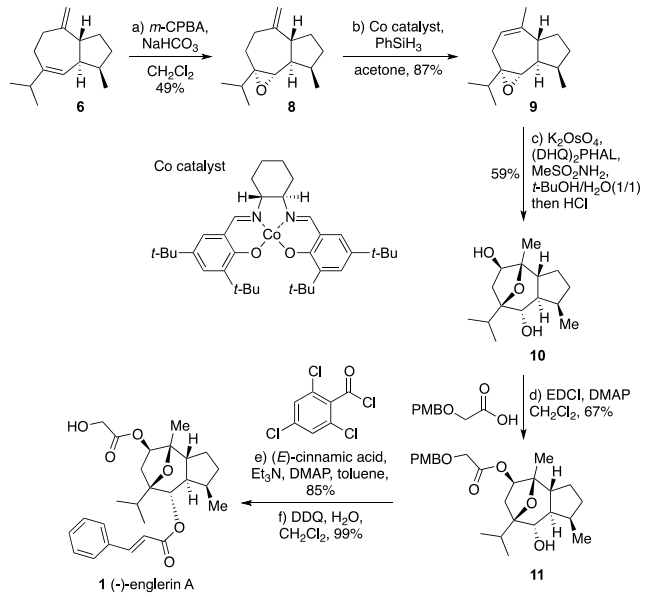


Figure 2. (A) GC−MS analysis of compound **6** produced by *E. coli*. (B) Mass spectrum of compound **6** produced by *E. coli*.

Scheme 1. Synthesis of (−)-Englerin A

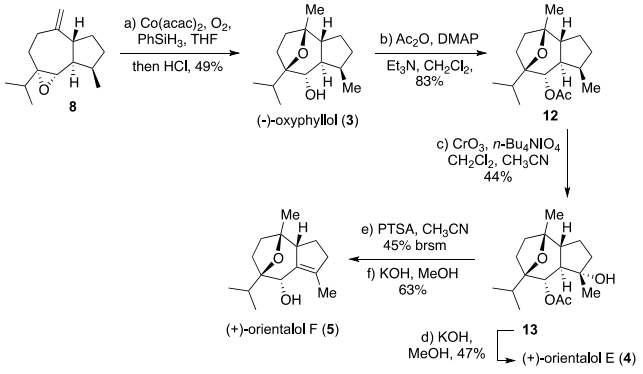


nor (*S,S*)-Jacobsen's catalyst[13] gave higher yield with better diastereoselectivity. Compound **8** was subjected to Co(II) catalyst with $PhSiH_3$ and offered the alkene isomerization product compound **9**.[14] We also tested Grotjahn's catalyst[15] but did not observe any isomerization product. Compound **9** underwent the Sharpless asymmetric dihydroxylation reaction,[16] followed by treatment with acid to give the epoxyguaiane intermediate **10** in one pot. We followed López's procedure to complete the transformations from compound **10** to (−)-englerin A in three steps.[6c] Herein, the synthesis takes only six steps with 14.2% overall yield.

We then used compound **8** to synthesize (−)-oxyphyllol, (+)-orientalol E, and (+)-orientalol F (Scheme 2). Compound **8** was subjected to Mukaiyama hydration[17] conditions followed by acid treatment to provide (−)-oxyphyllol (**3**). After

https://dx.doi.org/10.1021/acs.orglett.0c00325
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 2. Syntheses of (−)-Oxyphyllol, (+)-Orientalol E, and (+)-Orientalol F



protection of the hydroxyl group as an acetate, compound **12** was selectively oxidized under Fuchs' condition[18,6b] to give intermediate **13**. Deprotection of the acetyl group furnished (+)-orientalol E (**4**). Compound **13** underwent elimination under acidic conditions and was further deprotected to give (+)-orientalol F (**5**).

In summary, we have developed a combined biosynthetic and chemosynthetic approach toward collective syntheses of (−)-englerin A, (−)-oxyphyllol, (+)-orientalol E, and (+)-orientalol F. Our strategy enabled rapid access to epoxyguaiane natural products in a step- and redox-economical manner[19] and highlighted the power of combining microbial production and organic synthesis.[20] Syntheses and evaluation of potent and bioavailable englerin analogues via this strategy is undergoing in our laboratory and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c00325.

Experimental procedures and characterization data for all new compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Zheng Xiang − *State Key Laboratory of Chemical Oncogenomics, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Shenzhen 518055, China;* orcid.org/0000-0003-2925-842X; Email: xiangzheng@pkusz.edu.cn

Authors

Shu-Bin Mou − *State Key Laboratory of Chemical Oncogenomics, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Shenzhen 518055, China*

Wen Xiao − *State Key Laboratory of Chemical Oncogenomics, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Shenzhen 518055, China*

Hua-Qi Wang − *State Key Laboratory of Chemical Oncogenomics, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Shenzhen 518055, China*

Su-Jing Wang − *State Key Laboratory of Chemical Oncogenomics, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Shenzhen 518055, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.0c00325

Author Contributions

†S.-B.M. and W.X. contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Prof. Zhen Yang (Peking University Shenzhen Graduate School) for chemical reagents. We thank Prof. Jing Xu (Southern University of Science and Technology), Prof. Chen Xu (SUSTech), Dr. Zhihong Huang (Genomics Institute of the Novartis Research Foundation), and Dr. Jun Huang (PKUSZ) for helpful discussions. This work was financially supported by Shenzhen Science and Technology Program (JCYJ20170307100832684, KQTD20170330155106581, and JCYJ20180302153611416).

■ REFERENCES

(1) (a) Ratnayake, R.; Covell, D.; Ransom, T. T.; Gustafson, K. R.; Beutler, J. A. *Org. Lett.* **2009**, *11*, 57−60. (b) Wu, Z.; Zhao, S.; Fash, D. M.; Li, Z.; Chain, W. J.; Beutler, J. A. *J. Nat. Prod.* **2017**, *80*, 771−781. (c) Hagihara, S.; Hanaya, K.; Sugai, T.; Shoji, M. *Asian J. Org. Chem.* **2019**, *8*, 48−62.

(2) Sourbier, C.; Scroggins, B. T.; Ratnayake, R.; Prince, T. L.; Lee, S.; Lee, J. M.; Trepel, J. B.; Beutler, J. A.; Linehan, W. M.; Neckers, L. M. *Cancer Cell* **2013**, *23*, 228−237.

(3) Akbulut, Y.; Gaunt, H. J.; Muraki, K.; Ludlow, M. J.; Amer, M. S.; Bruns, A.; Vasudev, N. S.; Radtke, L.; Willot, M.; Hahn, S.; Seitz, T.; Ziegler, S.; Christmann, M.; Beech, D. J.; Waldmann, H. *Angew. Chem., Int. Ed.* **2015**, *54*, 3787−3791.

(4) (a) Willot, M.; Radtke, L.; Könning, D.; Fröhlich, R.; Gessner, V. H.; Strohmann, C.; Christmann, M. *Angew. Chem., Int. Ed.* **2009**, *48*, 9105−9108. (b) Radtke, L.; Willot, M.; Sun, H.; Ziegler, S.; Sauerland, S.; Strohmann, C.; Frohlich, R.; Habenberger, P.; Waldmann, H.; Christmann, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 3998−4002.

(5) (a) Zhou, Q.; Chen, X.; Ma, D. *Angew. Chem., Int. Ed.* **2010**, *49*, 3513−3515. (b) Molawi, K.; Delpont, N.; Echavarren, A. M. *Angew. Chem., Int. Ed.* **2010**, *49*, 3517−3519.

(6) (a) Xu, J.; Caro-Diaz, E. J.; Theodorakis, E. A. *Org. Lett.* **2010**, *12*, 3708−3711. (b) Wang, J.; Chen, S. G.; Sun, B. F.; Lin, G. Q.; Shang, Y. J. *Chem. - Eur. J.* **2013**, *19*, 2539−2547. (c) Nelson, R.; Gulias, M.; Mascarenas, J. L.; Lopez, F. *Angew. Chem., Int. Ed.* **2016**, *55*, 14359−14363. (d) Guo, L.; Plietker, B. *Angew. Chem., Int. Ed.* **2019**, *58*, 8346−8350.

(7) (a) Nicolaou, K. C.; Kang, Q.; Ng, S. Y.; Chen, D. Y. *J. Am. Chem. Soc.* **2010**, *132*, 8219−8222. (b) Li, Z.; Nakashige, M.; Chain, W. J. *J. Am. Chem. Soc.* **2011**, *133*, 6553−6556. (c) Lee, J.; Parker, K. A. *Org. Lett.* **2012**, *14*, 2682−2685. (d) Gao, P.; Cook, S. P. *Org. Lett.* **2012**, *14*, 3340−3343. (e) Takahashi, K.; Komine, K.; Yokoi, Y.; Ishihara, J.; Hatakeyama, S. *J. Org. Chem.* **2012**, *77*, 7364−7370. (f) Zahel, M.; Kessberg, A.; Metz, P. *Angew. Chem., Int. Ed.* **2013**, *52*, 5390−5392. (g) Zhang, J.; Zheng, S.; Peng, W.; Shen, Z. *Tetrahedron Lett.* **2014**, *55*, 1339−1341. (h) Hanari, T.; Shimada, N.; Kurosaki, Y.; Thrimurtulu, N.; Nambu, H.; Anada, M.; Hashimoto, S. *Chem. - Eur. J.* **2015**, *21*, 11671−11676. (i) Kusama, H.; Tazawa, A.; Ishida, K.; Iwasawa, N. *Chem. - Asian J.* **2016**, *11*, 64−67. (j) Morisaki, K.; Sasano, Y.; Koseki, T.; Shibuta, T.; Kanoh, N.; Chiou, W. H.; Iwabuchi, Y. *Org. Lett.* **2017**, *19*, 5142−5145. (k) Liu, P.; Cui, Y.;

Chen, K.; Zhou, X.; Pan, W.; Ren, J.; Wang, Z. *Org. Lett.* **2018**, *20*, 2517−2521. (l) Reagan, C.; Trevitt, G.; Tchabanenko, K. *Eur. J. Org. Chem.* **2019**, *2019*, 1027−1037.
(8) (a) Jiménez-Núñez, E.; Molawi, K.; Echavarren, A. M. *Chem. Commun.* **2009**, 7327−7329. (b) Wang, C.-L.; Sun, B.-F.; Chen, S.-G.; Ding, R.; Lin, G.-Q.; Xu, J.-Y.; Shang, Y.-J. *Synlett* **2012**, 263−266. (c) Zahel, M.; Metz, P. *Beilstein J. Org. Chem.* **2013**, *9*, 2028−2832. (d) Zahel, M.; Wang, Y.; Jäger, A.; Metz, P. *Eur. J. Org. Chem.* **2016**, *2016*, 5881−5886. (e) Gu, Y.; Huang, J.; Gong, J.; Yang, Z. *Org. Chem. Front.* **2017**, *4*, 2296−2300.
(9) Siemon, T.; Wang, Z.; Bian, G.; Seitz, T.; Ye, Z.; Lu, Y.; Cheng, S.; Ding, Y.; Huang, Y.; Deng, Z.; Liu, T.; Christmann, M. While this paper was under review, a similar approach was reported. *J. Am. Chem. Soc.* **2020**, DOI: 10.1021/jacs.9b12940.
(10) (a) Chang, M. C.; Keasling, J. D. *Nat. Chem. Biol.* **2006**, *2*, 674−681. (b) Keasling, J. D. *ACS Chem. Biol.* **2008**, *3*, 64−76.
(11) (a) Kumeta, Y.; Ito, M. *Plant Physiol.* **2010**, *154*, 1998−2007. (b) Burkhardt, I.; Siemon, T.; Henrot, M.; Studt, L.; Rosler, S.; Tudzynski, B.; Christmann, M.; Dickschat, J. S. *Angew. Chem., Int. Ed.* **2016**, *55*, 8748−8751.
(12) (a) Martin, V. J.; Pitera, D. J.; Withers, S. T.; Newman, J. D.; Keasling, J. D. *Nat. Biotechnol.* **2003**, *21*, 796−802. (b) Ro, D. K.; Paradise, E. M.; Ouellet, M.; Fisher, K. J.; Newman, K. L.; Ndungu, J. M.; Ho, K. A.; Eachus, R. A.; Ham, T. S.; Kirby, J.; Chang, M. C.; Withers, S. T.; Shiba, Y.; Sarpong, R.; Keasling, J. D. *Nature* **2006**, *440*, 940−3. (c) Tsuruta, H.; Paddon, C. J.; Eng, D.; Lenihan, J. R.; Horning, T.; Anthony, L. C.; Regentin, R.; Keasling, J. D.; Renninger, N. S.; Newman, J. D. *PLoS One* **2009**, *4*, No. e4489. (d) Paddon, C. J.; Westfall, P. J.; Pitera, D. J.; Benjamin, K.; Fisher, K.; McPhee, D.; Leavell, M. D.; Tai, A.; Main, A.; Eng, D.; Polichuk, D. R.; Teoh, K. H.; Reed, D. W.; Treynor, T.; Lenihan, J.; Fleck, M.; Bajad, S.; Dang, G.; Dengrove, D.; Diola, D.; Dorin, G.; Ellens, K. W.; Fickes, S.; Galazzo, J.; Gaucher, S. P.; Geistlinger, T.; Henry, R.; Hepp, M.; Horning, T.; Iqbal, T.; Jiang, H.; Kizer, L.; Lieu, B.; Melis, D.; Moss, N.; Regentin, R.; Secrest, S.; Tsuruta, H.; Vazquez, R.; Westblade, L. F.; Xu, L.; Yu, M.; Zhang, Y.; Zhao, L.; Lievense, J.; Covello, P. S.; Keasling, J. D.; Reiling, K. K.; Renninger, N. S.; Newman, J. D. *Nature* **2013**, *496*, 528−532. (e) Ajikumar, P. K.; Xiao, W.-H.; Tyo, K. E. J.; Wang, Y.; Simeon, F.; Leonard, E.; Mucha, O.; Phon, T. H.; Pfeifer, B.; Stephanopoulos, G. *Science* **2010**, *330*, 70−74. (f) Kong, M. K.; Kang, H. J.; Kim, J. H.; Oh, S. H.; Lee, P. C. *J. Biotechnol.* **2015**, *214*, 95−102. (g) Zhou, Y. J.; Gao, W.; Rong, Q.; Jin, G.; Chu, H.; Liu, W.; Yang, W.; Zhu, Z.; Li, G.; Zhu, G.; Huang, L.; Zhao, Z. K. *J. Am. Chem. Soc.* **2012**, *134*, 3234−3241. (h) Schalk, M.; Pastore, L.; Mirata, M. A.; Khim, S.; Schouwey, M.; Deguerry, F.; Pineda, V.; Rocci, L.; Daviet, L. *J. Am. Chem. Soc.* **2012**, *134*, 18900−18903.
(13) Jacobsen, E. N.; Zhang, W.; Muci, A. R.; Ecker, J. R.; Deng, L. *J. Am. Chem. Soc.* **1991**, *113*, 7063−7064.
(14) Crossley, S. W. M.; Barabé, F.; Shenvi, R. A. *J. Am. Chem. Soc.* **2014**, *136*, 16788−16791.
(15) Grotjahn, D. B.; Larsen, C. R.; Gustafson, J. L.; Nair, R.; Sharma, A. *J. Am. Chem. Soc.* **2007**, *129*, 9592−9593.
(16) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483−2547.
(17) Isayama, S.; Mukaiyama, T. *Chem. Lett.* **1989**, *18*, 1071−1074.
(18) Lee, S.; Fuchs, P. L. *J. Am. Chem. Soc.* **2002**, *124*, 13978−13979.
(19) (a) Newhouse, T.; Baran, P. S.; Hoffmann, R. W. *Chem. Soc. Rev.* **2009**, *38*, 3010−21. (b) Wender, P.; Verma, V. A.; Paxton, T. J.; Pillow, T. H. *Acc. Chem. Res.* **2008**, *41*, 40−49. (c) Burns, N. Z.; Baran, P. S.; Hoffmann, R. W. *Angew. Chem., Int. Ed.* **2009**, *48*, 2854−67.
(20) (a) Westfall, P. J.; Pitera, D. J.; Lenihan, J. R.; Eng, D.; Woolard, F. X.; Regentin, R.; Horning, T.; Tsuruta, H.; Melis, D. J.; Owens, A.; Fickes, S.; Diola, D.; Benjamin, K. R.; Keasling, J. D.; Leavell, M. D.; McPhee, D. J.; Renninger, N. S.; Newman, J. D.; Paddon, C. J. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, E111−E118. (b) Hsu, S. Y.; Perusse, D.; Hougard, T.; Smanski, M. J. *ACS Synth. Biol.* **2019**, *8*, 2397−2403.

https://dx.doi.org/10.1021/acs.orglett.0c00325
Org. Lett. XXXX, XXX, XXX−XXX