Enzymatic Peroxidation in the Chemoenzymatic Synthesis of 13-Oxoverruculogen

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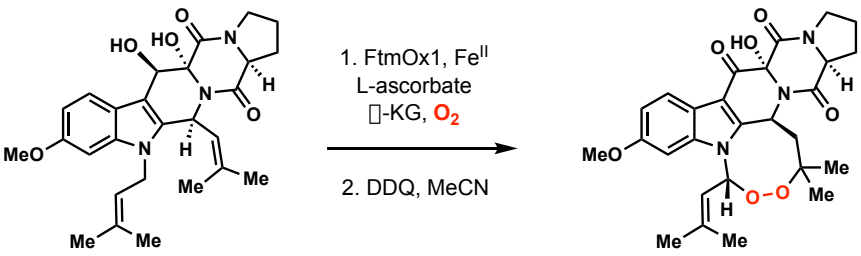

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Abstract Verruculogens are fumitremorgin alkaloids that contain an eight-membered endoperoxide ring. Due to their unusual structure and bioactivity, there has been much interest in these natural products since their discovery over forty years ago. Similarly, interest in their biosynthesis resulted in the discovery of verruculogen synthase (FtmOx1) that catalyzes endoperoxide formation in these natural products. Herein, we describe our work in this area through the chemoenzymatic synthesis of 13-oxoverruculogen by endoperoxidation of a substrate analog using FtmOx1.

Key words Biocatalysis, endoperoxides, fungal natural products, chemoenzymatic synthesis

Endoperoxide-containing natural products have seen considerable use in medicine due to their bioactive properties.[1,2] Perhaps the most notable is that of artemisinin, isolated from *Artemisia annua* in the 1970s, which is used as the frontline treatment for malaria disease.[3] The endoperoxide moiety has been found to be crucial for antimalarial activity of these compounds. Upon homolysis of the peroxide and β-scission, carbon centered radicals are formed that cause oxidative stress and inhibition of key enzymes in cell homeostasis.[4,5] While antimalarial activity is one of the most recognized properties of endoperoxide natural products, many other bioactivities have also been observed.[6] For example, verruculogen (**1**) isolated from *Aspergillus fumigatus* was found to possess tremorgenic properties.[6,7] These properties are common across the

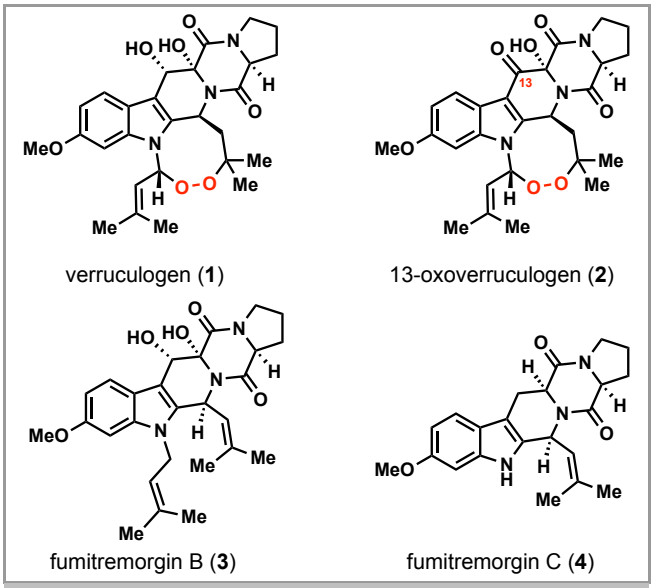


Figure 1 Fumitremorgin alkaloids and their endoperoxide congeners

fumitremorgin alkaloids (**1**-**4**, Figure 1).[8,9] More recently, 13-oxoverruculogen (**2**) was isolated and shown to be cytotoxic against multiple cancer cell lines (Figure 1).[10] Other fumitremorgin alkaloids, such as fumitremorgin C (**4**) have been reported to reverse drug resistance in cancer with potential applications in chemotherapy.[11]

Due to these biological properties, there is much interest in endoperoxide-containing compounds and how they originate in nature.[12-14] Artemisinin, despite its impact on society, still has unknown biosynthetic origins with regards to

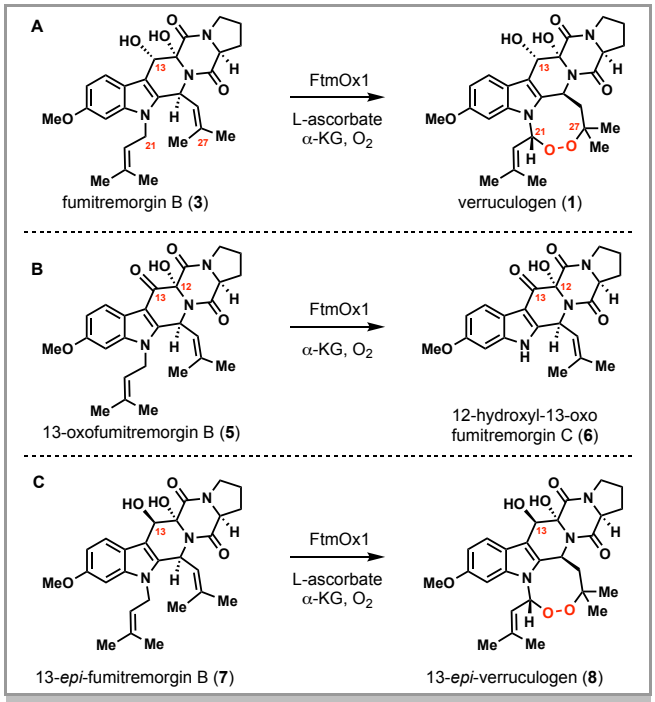
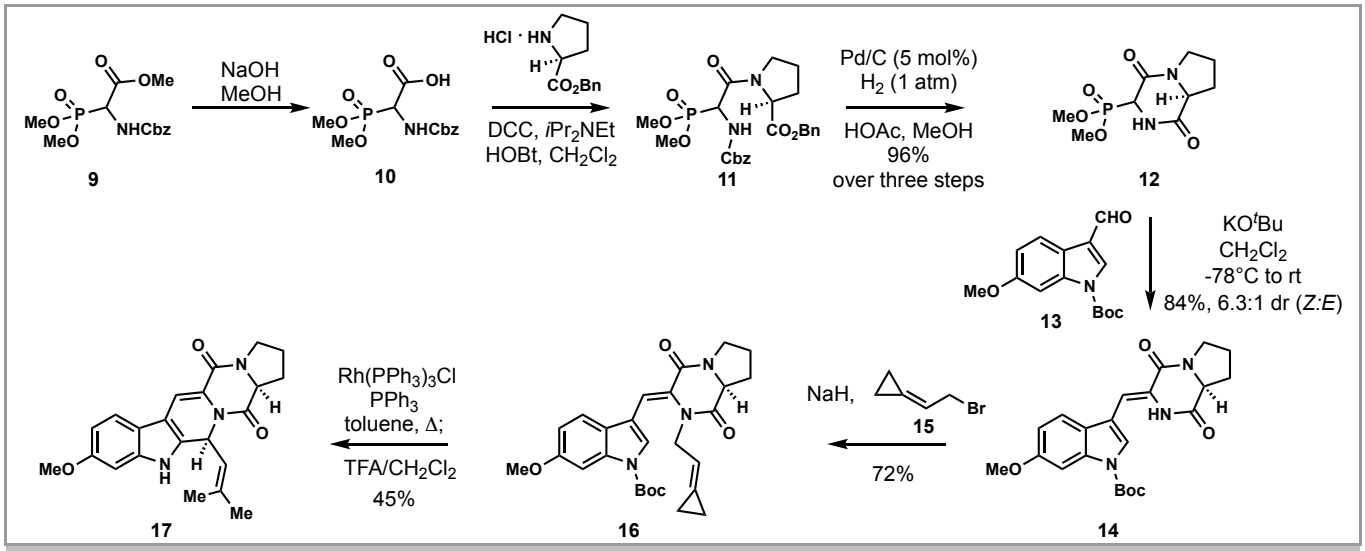


Figure 2 A) Native reactivity of FtmOx1. B) Deprenylation of 13-oxofumitremorgin B with FtmOx1 reported by Liu and co-workers.[20] C) Endoperoxidation of 13-*epi*-fumitremorgin B with FtmOx1.[21]

the formation of its endoperoxide ring.[15] Thus, the biosynthesis of endoperoxide-containing natural products remains poorly understood. While many endoperoxide natural products are known, only several endoperoxide-forming enzymes have been reported and characterized.[16] One of these enzymes is verruculogen synthase (FtmOx1), which is a part of the non-heme iron α-ketoglutarate dependent enzyme family.[17] Discovered in 2009, FtmOx1 was found to convert its substrate fumitremorgin B (**3**) to verruculogen (**1**).[17] The enzymatic reaction occurs by hydrogen atom abstraction of the C21 methylene to form an allylic radical. Afterwards, the radical reacts with molecular oxygen followed by 8-endo cyclization on to C27 to form the eight-membered endoperoxide ring (Figure 2A).[17] Since its discovery, the mechanism of FtmOx1 has been extensively studied by Bollinger-Krebs-Boal and co-workers.[18-19] To better understand the mechanism, researchers have also subjected substrate analogs to FtmOx1 to identify substrate tolerance.[20] Liu and co-workers found that subjecting 13-oxofumitremorgin B (**5**) to FtmOx1, α-ketoglutarate, and molecular oxygen resulted in exclusive deprenylation of the starting material to produce 12-hydroxyl-13-oxofumitremorgin C (**6**) with no endoperoxide formation (Figure 2B).[20] Prior to our work, no substrate analogs have undergone successful endoperoxide formation by FtmOx1.[21] While FtmOx1 has garnered interest from the biochemistry community due to its unusual reactivity, verruculogen and other endoperoxide-containing fungal alkaloids have been long-standing targets for chemical synthesis. Prior to our work, only Baran, through early-stage Mukaiyama peroxidation, reported the synthesis of endoperoxide-containing fumitremorgin alkaloids.[22]

In our synthesis of 13-oxoverruculogen (**2**), we envisioned the use of FtmOx1 for late-stage enzymatic endoperoxidation. Initially, we planned to convert the native substrate fumitremorgin B (**3**) to verruculogen (**1**), and then oxidize the C13 alcohol to form 13-oxoverruculogen (**2**). However, we were cognizant that the stereochemical configuration of the C13 alcohol was inconsequential as the stereocenter is removed upon oxidation of the alcohol. Hence, an alternative strategy to synthesis 13-oxoverruculogen (**2**) would involve the endoperoxidation of the diastereomer, 13-*epi*-fumitremorgin B (**7**), to access the unnatural endoperoxide 13-*epi*-verruculogen (**8**, Figure 2C). Subsequent alcohol oxidation of **8** would also produce 13-oxoverruculogen (**2**). By using enzymatic C-H bond functionalization to form the endoperoxide ring, this strategy allowed us to circumvent the need for unstable peroxide intermediates and install the endoperoxide in the penultimate step of the synthesis.[21]



Scheme 1 Formation of the pentacyclic core

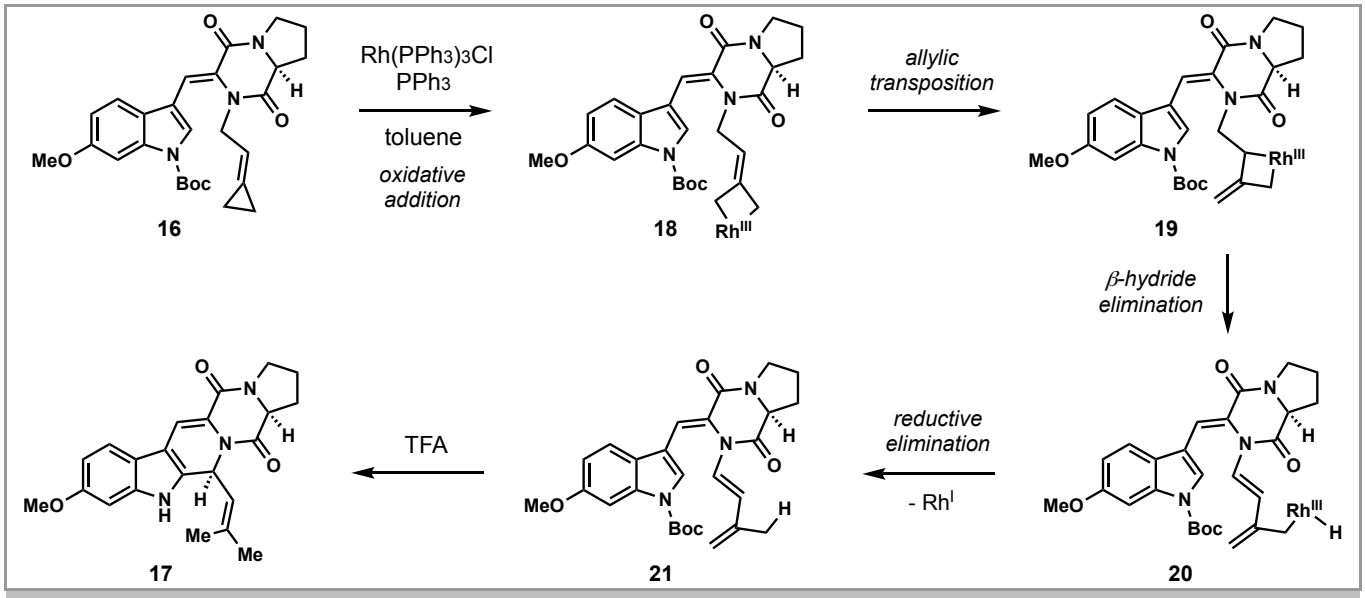
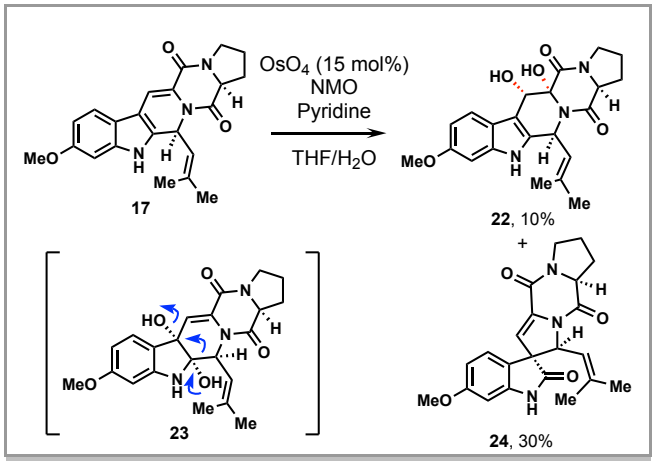


Figure 3 Mechanism of rhodium-catalyzed isomerization of alkylidenecyclopropanes by C–C bond activation

Pentacyclic core formation

Our synthesis of 13-oxoverruculogen begins with commercially available phosphonoglycine methyl ester **9** which underwent saponification to form carboxylic acid **10** (Scheme 1). Compound **10** was subjected to carbodiimide-mediated (DCC) coupling with L-proline benzyl ester hydrochloride to form dipeptide **11**. The carbamate of **11** was removed by hydrogenolysis (Pd/C, H_2) and the resulting amine cyclized onto the benzyl ester using acetic acid. This resulted in the formation of diketopiperazine **12** in 96% yield over 3 steps. Compound **12** was then subjected to Horner–Wadsworth–Emmons reaction with aldehyde **13** to form product **14**, as a 6.3:1 ratio of *Z*/*E* geometric isomers. The major isomer *Z*-**14** was isolated in 72% yield. With dehydroamino acid derivative **14** in hand, we set out to install the central six-membered ring in the pentacyclic core of the fumitremorgin alkaloids. Alkylation of the amide in **14** with sodium hydride and dehydroprenylbromide **15** afforded **16** in 72% yield (Scheme 1).



Scheme 2 Osmium-catalyzed dihydroxylation of **17**.

Compound **16** contains an alkylidenecyclopropane (ACP) which was subjected to Shi's conditions for C–C bond activation and cycloisomerization to form C2 prenylated indoles.[23] In their proposed mechanism the rhodium catalyst undergoes an oxidative addition into the ACP to form a four-membered rhodacyclobutane (**18**, Figure 3). Intermediate **18** can then isomerize to **19** through a trimethylenemethane (TMM)-like transition state. Rhodacycle **19** undergoes β-hydride elimination to form rhodium hydride **20**. Reductive elimination of **20** produces diene **21** and regenerates the rhodium(I) catalyst. In the rhodium-catalyzed reaction, diene **21** is formed as the major product as opposed to the desired compound **17**, the product from cycloisomerization. In Shi's report, the cyclization is proposed to occur due to adventitious acid.[23] Therefore, diene **21** was subjected to trifluoroacetic acid (TFA) which resulted in the formation of compound **17**. This reaction occurs through protonation of the extended enamide in **21** followed by a Pictet–Spengler reaction to forge the six-membered ring. Much to our delight, the highly acidic conditions also removed the Boc group in the same pot to form pentacycle **17** (Figure 3).[23]

While the pentacyclic core of the fumitremorgin alkaloids was prepared in six steps, the installation of the *cis*-1,2-diol found in fumitremorgin B proved challenging. Hino and co-workers reported the *cis*-1,2-diol of fumitremorgin B can be formed with osmium tetroxide under Upjohn conditions, with the desired product being formed in only 10% yield (**22**).[24] We obtained similar results when we tried to form the *cis*-1,2-diol and only a 10% yield of the desired product was observed (**22**, Scheme 2). Rather than dihydroxylation of the C12–C13 double bond, the use of OsO_4 primarily results in dihydroxylation of the indole C2-C3 double bond to form intermediate **23**. After dihydroxylation, a pinacol rearrangement involving a [1,2]-alkyl shift results in the formation of a spirocyclic oxindole **24** in 30% yield. A similar rearrangement was reported previously by Li and co-workers in their synthesis of spirotryptostatins.[25] Various conditions were attempted to improve the selectivity of this reaction such as different ligands, stoichiometric oxidants

and solvents. Despite numerous attempts, we were unable to increase the yield of **22**. It is well known that OsO_4 will preferentially attack the more electron-rich double bond. Because the indole contains a 6-methoxy group, the indole C2-C3 double bond is very reactive. Meanwhile, the C12–C13 is electronically deactivated due to conjugation with the carbonyl in the diketopiperazine ring. Dihydroxylation of related systems lacking the 6-methoxy group of the indole resulted in much higher yields of the desired diol from functionalization of the C12–C13 double bond.[24] *Cis*-1,2-diol **22** can be converted to fumitremorgin B (**3**) by prenylation in 49% yield using potassium hydroxide, 18-crown-6 and prenyl chloride. While FtmOx1 can convert **3** to verruculogen (**1**) in 62% yield and the reported alcohol oxidation by Liu and co-workers can be used to obtain 13-oxoverruculogen (**2**),[20] the low yielding dihydroxylation step lowers the overall efficiency of this route (Figure 4a). As such, we investigated alternative strategies to access 13-oxoverruculogen (**2**).

Promiscuous enzymatic peroxidation

The difficulty in forming the *cis*-1,2-diol of fumitremorgin B (**3**) led us to examine if FtmOx1 could accept the *trans*-diol for endoperoxidation (Figure 4a). As previously mentioned, the last step in the synthesis would involve oxidation of the C13 alcohol, so the stereocenter at this position would be inconsequential in the synthesis of 13-oxoverruculogen. In 2022, the Zhou group reported a crystal structure of wild-type FtmOx1 with its substrate and cofactors bound (Figure 4b).[26] Utilizing this crystal structure, we observed an empty pocket in the active site of FtmOx1, which could potentially accommodate the *trans*-diol in the substrate analog (**7**). Moreover, the surface model of the FtmOx1-fumitremorgin B-αKG ternary complex shows fumitremorgin B (**3**) is partially solvent exposed which could allow for modified substrate analogs to fit in the active site (Figure 4b). With this thought in mind, we decided to investigate the use of a *trans*-dihydroxylation reaction.

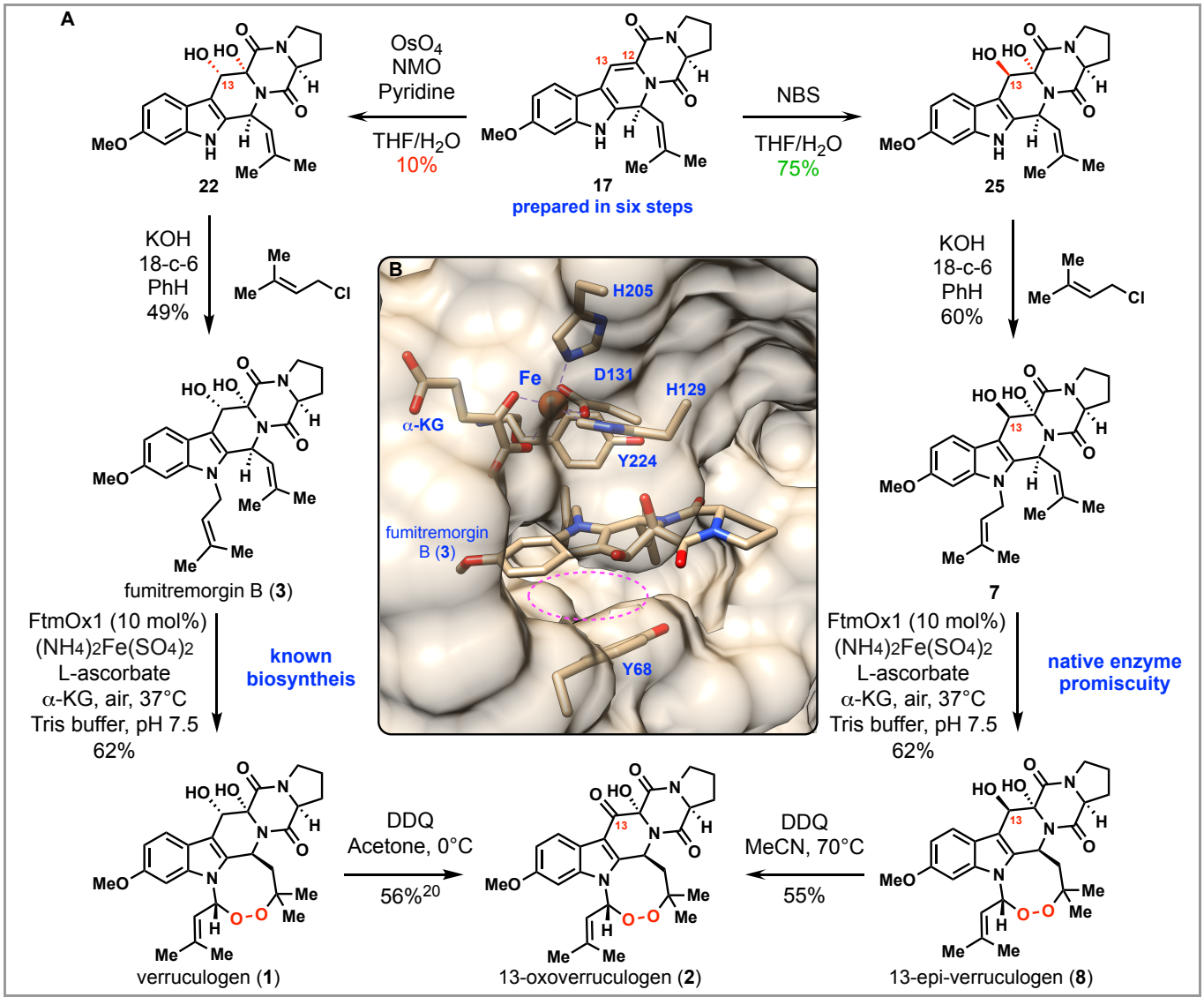


Figure 4 A) Chemoenzymatic synthesis of 13-oxoverruculogen B) X-ray crystal structure of FtmOx1-fumitremorgin B-α-KG ternary complex reported by Zhou and co-workers (PDB: 7ETK).[26] Surface model of FtmOx1 is shown with fumitremorgin B and α-KG bound. Key amino acids such as active site ligands (D131, H129, H205) and Y68 and Y224 are highlighted in blue. The empty pocket that would allow for 13-*epi*-fumitremorgin B to fit in the active site is highlighted in purple.

Using *N*-bromosuccinimide (NBS) in aqueous tetrahydrofuran resulted in the formation of **25** with 75% yield. While installation of the *cis*-1,2-diol by OsO_4 occurs through a concerted mechanism, the *trans*-diol is formed through a stepwise mechanism. NBS reacts from the less hindered back side of the molecule forming the bromonium intermediate. Ring opening of the bromonium ion by the amido group of the diketopiperazine generates an iminium ion on C12. The presence of water readily attacks this intermediate, resulting in a *cis*-bromo alcohol. The C13 bromide is then displaced through an SN2 reaction with the water in solution forming **25** as the primary product.

Prenylation of the indole was accomplished using prenyl chloride in benzene to make the substrate analog 13-*epi*-fumitremorgin B (**7**), in analogy to the synthesis of fumitremorgin B (**3**) from *cis*-1,2-diol **22**. With the substrate analog in hand, we subjected it to the optimized conditions we had found for the synthesis of verruculogen. Initial LC/MS analysis of the enzyme assay showed the formation of a small peak with identical mass as verruculogen but with a different retention time. This indicated that **7** was accepted by FtmOx1 for endoperoxidation to form 13-*epi*-verruculogen (**8**). Thus, **7** was the first substrate analog found to be endoperoxidized by FtmOx1. Though the initial yield was only 9%, increasing the temperature to 37°C and adding ferrous iron in the form of iron ammonium sulfate increased the isolated yield to 62%, comparable to our reported yield of verruculogen from subjecting fumitremorgin B to FtmOx1 (Figure 4a). The addition of iron (II) and higher temperature were essential to obtain synthetically useful yields of 13-*epi*-verruculogen (**8**). With our optimized enzymatic peroxidation in hand, the C13 alcohol of **8** was oxidized using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to complete the first *de novo* synthesis of 13-oxoverruculogen (**2**).[21]

Conclusion

Central to our strategy was the use of enzymatic peroxidation by FtmOx1 to avoid the need to prepare reactive peroxide intermediates. Using a substrate analog, we avoided the need to synthesize the *cis*-1,2-diol found in fumitremorgin B (**3**), which would have severely hampered the synthetic efficiency of our route. The use of 13-*epi*-fumitremorgin B (**7**) allowed for the preparation of 13-*epi*-verruculogen (**8**), an alcohol diastereomer that also allowed for the synthesis of 13-oxoverruculogen (**2**). Our work highlights the utility of biocatalysis and the use of native enzyme promiscuity in the synthesis of natural products. Using biocatalysis, enzymatic reactions that are unprecedent with small molecule catalysts can be incorporated into multistep synthesis.[27] This strategy could greatly improve the efficiency of natural product synthesis. Native enzyme promiscuity broadens the use of biocatalysts by examining alternative intermediates other than the native substrate that can be functionalized by the enzyme.[28,29] Given the use of α-ketoglutarate dependent iron enzymes in natural product synthesis,[30-31] we expect that the strategies described herein would result in additional flexibility in retrosynthetic design. As a result, synthetic routes can be orchestrated to avoid impractical and unstable intermediates that would otherwise be difficult to prepare. Applications of enzymatic reactions in multistep synthesis are expanding rapidly and will only continue to grow with advances in biotechnology.[32,33]

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Conflict of Interest

The authors declare no conflict of interest.

References

(1) Coghi, P.; Yaremenko, I. A; Prommana, P.; Radulov, P. S.; Syroeshkin, M. A.; Wu, Y. J.; Gao, J. Y.; Gordillo, F. M.; Mok, S.; Wong, V. K. W.; Uthaipibull, C.; Terent'ev, A. O. Novel Peroxides as Promising Anticancer Agents with Unexpected Depressed Antimalarial Activity. *ChemMedChem* **2018**, *13*, 902-908.

(2) Vil', V. A.; Yaremenko, I. A.; Ilovaisky, A. I.; Terent'ev, A. Peroxides with Anthelmintic, Antiprotozoal, Fungicidal and Antiviral Bioactivity: Properties, Synthesis and Reactions. *Molecules* **2017**, *22*, 1881.

(3) Tu, Y. The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. *Nature Medicine* **2011**, *17*, 1217-1220.

(4) Meshnick, S. R. Artemisinin: Mechanisms of Action, Resistance and Toxicity. *Int. J. Parasitol.* **2002**, *32*, 1655–1660.

(5) (a) Haynes, R. K.; Cheu, K.-W.; N'Da, D.; Coghi, P.; Monti, D. Considerations on the Mechanism of Action of Artemisinin Antimalarials: Part 1--the "carbon Radical" and "Heme" Hypotheses. *Infect. Disord. Drug Targets* **2013**, *13*, 217–277. (b) Bridgford, J. L.; Xie, S. C.; Cobbold, S. A.; Pasaje, C. F. A.; Herrmann, S.; Yang, T.; Gillett, D. L.; Dick, L. R.; Ralph, S. A.; Dogovski, C.; Spillman, N. J.; Tilley, L. Artemisinin kills malaria parasites by damaging proteins and inhibiting the proteasome. *Nat. Commun.* **2018**, *9*, 3801.

(6) For a review on the anticancer properties of artemisinin, see: Slezakova, S.; Ruda-Kucerova, J. Anticancer Activity of Artemisinin and its Derivatives. *Anticancer Res.* **2017**, *37*, 5995-6003

(7) Cole, R. J.; Kirksey, J. W. The Mycotoxin Verruculogen: a 6-*O*-Methylindole. *J. Agric. Food Chem.* **1973**, *21*, 927-929.

(8) Fayos, J.; Lokensgard, D.; Clardy, J.; Cole, R. J.; Kirksey, J. W. Structure of Verruculogen, a Tremor Producing Peroxide from *Penicillium verruculosum*. *J. Am. Chem Soc.* **1974**, *96*, 6785-6787.

(9) Cole, R. J.; Kirksey, J. W.; Moore, J. H.; Blankenship, B. R.; Diener, U. L.; Davis, N. D. Tremorgenic Toxin from *Penicillium verruculosum*. *Appl. Microbiol.* **1972**, *24*, 248-250.

(10) Li, X.-J.; Zhang, Q.; Zhang, A.-L.; Gao, J.-M. Metabolites from Aspergillus Fumigatus, an Endophytic Fungus Associated with Melia Azedarach, and Their Antifungal, Antifeedant, and Toxic Activities. *J. Agric. Food Chem.* **2012**, *60*, 3424–3431.

(11) Wang, F.; Fang, Y.; Zhu, T.; Zhang, M.; Lin, A.; Gu, Q.; Zhu, W. Seven new prenylated indole diketopiperazine alkaloids from holothurian-derived fungus *Aspergillus fumigatus*. *Tetrahedron* **2008**, *64,* 7986.

(12) Rabindran, S. K.; Ross, D. D.; Doyle, L. A.; Yang, W.; Greenberger, L. M.. Fumitremorgin C reverses multidrug resistance in cells transfected with the breast cancer resistance protein. *Cancer Res* **2000**, 47-50.

(13) Wen, W.; Yu, R. Artemisinin biosynthesis and its regulatory enzymes: Progress and perspective. *Pharmacogn Rev.* **2011**, 5, 189-194.

(14) Hu, X.; Maimone, T. J. Four-Step Synthesis of the Antimalarial Cardamom Peroxide via an Oxygen Stitching Strategy. *J. Am. Chem. Soc.* **2014**, *136*, 5287-5290.

(15) Hu, X.; Lim, P.; Fairhurst, R. M.; Maimone, T. J. Synthesis and study of the antimalarial cardamom peroxide. *Tetrahedron* **2018**, *74*, 3358-3369.

(16) Varela, K.; Arman, H. D.; Yoshimoto, F. K. Synthesis of [15,15,15-2H3]-Dihydroartemisinic Acid and Isotope Studies Support a Mixed Mechanism in the Endoperoxide Formation to Artemisinin. *J. Nat. Prod.* **2021**, *84*, 1967–1984.

(17) Mori, T.; Abe, I. Structural Basis for Endoperoxide-Forming Oxygenases. *Beilstein J. Org. Chem.* **2022**, *18*, 707–721.

(18) Steffan, N.; Grundmann, A.; Afiyatullov, S.; Ruan, H.; Li, S.-M. FtmOx1, a non-heme Fe(II) and α-ketoglutarate-dependent dioxygenase, catalyses the endoperoxide formation of verruculogen in *Aspergillus fumigatus*. *Org. Bio. Chem.* **2009**, *7*, 4082-4087.

(19) Dunham, N. P.; Del Río Pantoja, J. M.; Zhang, B.; Rajakovich, L. J.; Allen, B. D.; Krebs, C.; Boal, A. K.; Bollinger, J. M. Hydrogen Donation but not Abstraction by a Tyrosine (Y68) during Endoperoxide Installation by Verruculogen Synthase (FtmOx1). *J. Am. Chem Soc.* **2019**, *141*, 9964-9979.

(20) Lin, C.-Y.; Hernández, A. L. M.; Laramore, T. N.; Silakov, A.; Krebs, C.; Boal, A. K.; Bollinger, J. M., Jr. Use of Noncanonical Tyrosine Analogues to Probe Control of Radical Intermediates during Endoperoxide Installation by Verruculogen Synthase (FtmOx1). *ACS Catal*. **2022**, *12*, 6968-6979.

(21) Zhu, G.; Yan, W.; Wang, X.; Cheng, R.; Naowarojna, N.; Wang, K.; Wang, J.; Song, H.; Wang, Y.; Liu, H.; Xia, X.; Costelloe, C. E.; Liu, X.; Zhang, L.; Liu, P. Dissecting the Mechanism of the Nonheme Iron Endoperoxidase FtmOx1 Using Substrate Analogues. *JACS Au* **2022**, *2*, 1686-1698.

(22) Yang, J.; Singh, B.; Cohen, G.; Ting, C. P. Chemoenzymatic Synthesis of 13-Oxoverruculogen. *J. Am. Chem. Soc.* **2023**, *145*, 19189–19194.

(23) Feng, Y.; Holte, D.; Zoller, J.; Umemiya, S.; Simke, L. R.; Baran, P. S. Total Synthesis of Verruculogen and Fumitremorgin A Enabled by Ligand-Controlled C-H Borylation. *J. Am. Chem Soc.* **2015,** *137*, 10160-10163.

(24) Zhang, D.-H.; Tang, X.-Y.; Wei, Y.; Shi, M. Rhodium(I)-Catalyzed Cycloisomerization of Nitrogen-Tethered Indoles and Alkylidenecyclopropanes: Convenient Access to Polycyclic Indole Derivatives. *Chem. Eur. J.* **2013,** *19*, 13668-13673.

(25) Kodato, S.-i.; Nakagawa, M.; Hongu, M.; Kawate, T.; Hino, T. Total synthesis of (+)-fumitremorgin B, its epimeric isomers, and demethoxy derivatives. *Tetrahedron* **1988**, *44*, 359-377.

(26) Xi, Y.-K.; Zhang, H.; Li, R.-X.; Kang, S.-Y.; Li, J.; Li, Y. Total Synthesis of Spirotryprostatins through Organomediated Intramolecular Umpolung Cyclization. *Chem. Eur. J.* **2019**, *25*, 3005-3009.

(27) Wu, L.; Wang, Z.; Cen, Y.; Wang, B.; Zhou, J. Structural Insight into the Catalytic Mechanism of the Endoperoxide Synthase FtmOx1. *Angew. Chem. Int. Ed.* **2022**, *61*, e202112063.

(28) Tanifuji, R.; Koketsu, K.; Takakura, M.; Asano, R.; Minami, A.; Oikawa, H.; Oguri, H. *J. Am. Chem. Soc.* **2018**, *140*, 34, 10705-10709.

(29) (a) Li, J.; Liao, H.-J.; Tang, Y.; Huang, J.-L.; Cha, L.; Lin, T.-S.; Lee, J.-L.; Kurnikov, I. V.; Kurnikova, M. G.; Chang, W.-c.; Chan, N. L.; Guo, Y. Epoxidation catalyzed by the nonheme iron (II)-and 2-oxoglutarate-dependent oxygenase, AsqJ: Mechanistic elucidation of oxygen atom transfer by a ferryl intermediate. *J. Am. Chem. Soc.* **2020**, 142, 6268-6284. (b) Tang, H.; Tang, Y.; Kurnikov, I. V.; Liao, H.-J.; Chen, N.-L.; Kurnikova, M. G.; Guo, Y.; Chang, W.-c. Harnessing the Substrate Promiscuity of Dioxygenase AsqJ and Developing Efficient Chemoenzymatic Synthesis for Quinolones. *ACS Catal.* **2021**, *11*, 12, 7186–7192. (c) Mader, S.L., Bräuer, A., Groll, M.; Kaila, V. R. I. Catalytic mechanism and molecular engineering of quinolone biosynthesis in dioxygenase AsqJ. *Nat. Commun.* **2018**, *9*, 1168. (d) Einsiedler, M.; Jamieson, C. S.; Maskeri, M. A.; Houk, K. N.; Gulder, T. A. M. Fungal Dioxygenase AsqJ Is Promiscuous and Bimodal: Substrate-Directed Formation of Quinolones versus Quinazolinones. *Angew. Chem. Int. Ed.* **2021**, *60*, 8297–8302. (e) Einsiedler, M.; Gulder, T. A. M. Discovery of extended product structural space of the fungal dioxygenase AsqJ. *Nat. Commun.* **2023**, *14*, 3658.

(30) Cheng, L.; Li, D.; Mai, B. K.; Bo, Z.; Cheng, L.; Liu, P.; Yang, Y. Stereoselective Amino Acid Synthesis by Synergistic Photoredox-Pyridoxal Radical Biocatalysis. *Science* **2023**, *381*, 444–451.

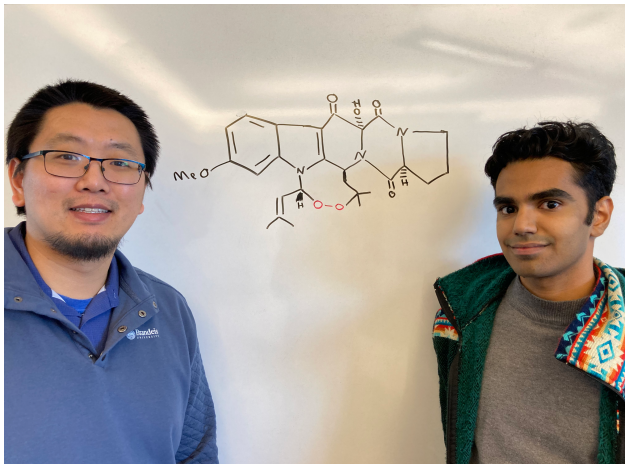
(31) (a) Doyon, T. J.; Perkins, J. C.; Dockrey, S. A. B.; Romero, E. O.; Skinner, K. C.; Zimmerman, P. M.; Narayan, A. R. H. Chemoenzymatic o-Quinone Methide Formation. *J. Am. Chem. Soc.* **2019**, *141*, 20269-20277. (b) Romero, E. O.; Perkins, J. C.; Burch, J. E.; Delgadillo, D. A.; Nelson, H. M.; Narayan, A. R. H. Chemoenzymatic Synthesis of (+)-Xyloketal B. *Org. Lett.* **2023**, *25*, 1547-1552.

(32) (a) Zwick, C. R. III; Sosa, M. B.; Renata, H. Characterization of a Citrulline 4-Hydroxylase from Nonribosomal Peptide GE81112 Biosynthesis and Engineering of Its Substrate Specificity for the Chemoenzymatic Synthesis of Enduracididine. *Angew. Chem. Int. Ed.* **2019**, *58*, 18854-18858. (b) Zwick, C. R. III; Renata, H. Remote C–H Hydroxylation by an α-Ketoglutarate-Dependent Dioxygenase Enables Efficient Chemoenzymatic Synthesis of Manzacidin C and Proline Analogs. *J. Am. Chem. Soc.* **2018**, *140*, 1165-1169.

(33) Brandenberg, O. F.; Chen, K.; Arnold, F. H., Directed Evolution of a Cytochrome P450 Carbene Transferase for Selective Functionalization of Cyclic Compounds. *J. Am. Chem. Soc.* **2019,** *141*, 8989-8995.

(34) Fasan, R. Tuning P450 Enzymes as Oxidation Catalysts. *ACS Catalysis* **2012**, *2*, 647-666.

Biosketches



Chi Pan Ting (left) completed his BS in chemistry at the University of Illinois at Urbana-Champaign in 2012 working under the direction of Professor Steven Zimmerman. He attended the University of California, Berkeley where he pursued PhD studies with Professor Tom Maimone. For his PhD, he completed the total synthesis of aryltetralin lignans and complex polycyclic polyprenylated acylphloroglucinols. In 2017, he returned to his *alma mater* at the University of Illinois where he studied biochemistry in the lab of Professor Wilfred van der Donk as a NIH postdoctoral researcher. In 2020, he started his independent career at Brandeis University where his research group is focused on natural products total synthesis and biocatalysis.

Brandon Singh (right) was born and raised on Long Island, New York. He completed his undergraduate studies in biochemistry at Hofstra University under Dr. Emily Mundorff. In 2021, he began his PhD studies in biochemistry at Brandeis University where he joined the Ting group, focusing on the chemoenzymatic synthesis of natural products.