JOC The Journal of Organic Chemistry

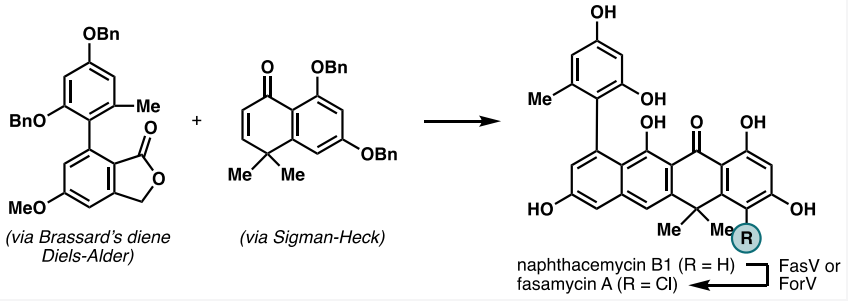
pubs.acs.org/joc Article

Concise Chemoenzymatic Synthesis of Fasamycin A

Jian Li and Hans Renata*

Cite This: https://doi.org/10.1021/acs.joc.1c00526 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: We report the development of a chemoenzymatic approach toward fasamycin A, a halogenated naphthacenoid that exhibits activities against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis*. The synthesis was accomplished in a convergent manner: two fragments were combined together in a Sammes annulation to afford a dimethylnaphthacenone system. Finally, an enzymatic halogenation was employed to introduce the requisite chlorine substituent of the natural product at a late stage.

INTRODUCTION

Antibiotic resistance is becoming one of the most pressing threats to human health, especially with the emergence of multidrug resistant (MDR) pathogens that prove recalcitrant to existing treatments.[1] Highlighting the urgency of the matter, the World Health Organization recently released a list of 12 "priority pathogens" that exhibit resistance to a number of antibiotics, including carbapenem-resistant *Acinetobacter baumanii* and *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant *Enterococcus faecalis* (VRE). Given their involvement in "biological warfare" among bacteria, many bacterial secondary metabolites are known to be effective antimicrobials, and these natural products make up the majority of antibiotics that are used in the clinic today.[2] Despite this success, pathogenic bacteria constantly evolve new modes of resistance, and new antibiotic candidates need to be constantly advanced to address this challenge.

In 2011, Brady and co-workers discovered two chlorinated polyaromatic natural products, namely fasamycins A (**1**) and B (**2**), through heterologous expression of an environmental DNA gene cluster (Figure 1).[3] Fasamycin A was noted to exhibit potent antibacterial activities against MRSA (MIC = 3.1 μg/mL) and VRE (MIC = 0.8 μg/mL) by inhibiting FabF, a key enzyme in the biosynthesis of type II fatty acid in bacteria.[4] FabF has been shown to be indispensable for bacterial cell viability and currently represents an underdeveloped target in antibacterial drug discovery.[5] To date, only a few small molecules, mostly natural products, have been

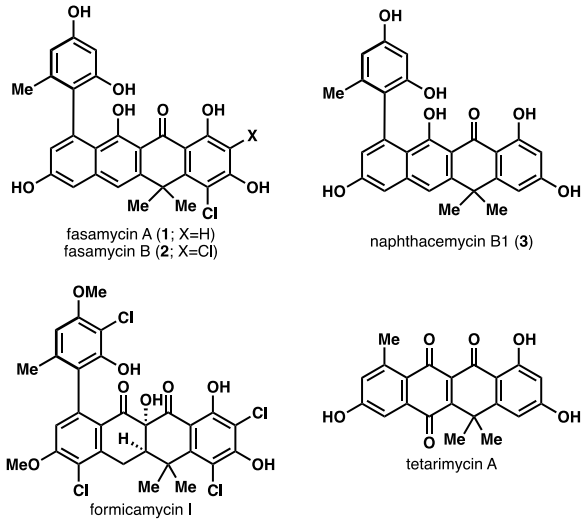


Figure 1. Structures of fasamycins A and B and related natural products.

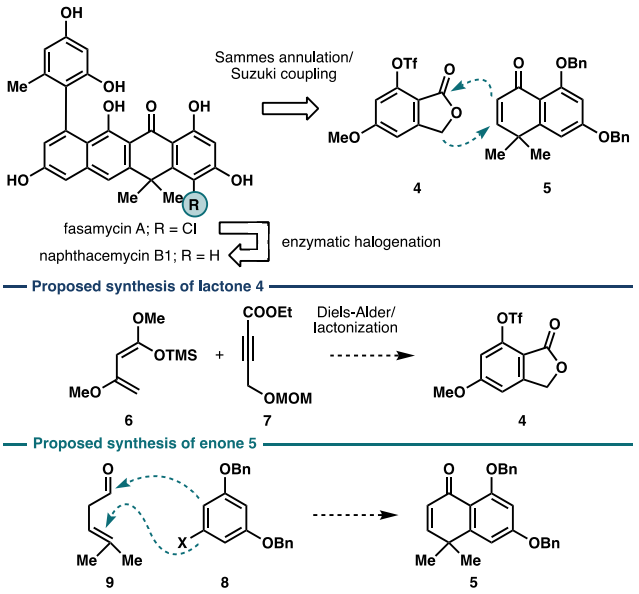
Special Issue: Natural Products: An Era of Discovery in Organic Chemistry

Received: March 4, 2021

reported to act as inhibitors of this enzyme. By analyzing the organization of the gene cluster, Brady and co-workers also proposed a late stage enzymatic C−H chlorination reaction catalyzed by a flavin-dependent halogenase (FDH) on naphthacemycin B1[6] to furnish the fasamycins. Based on its unique biogenesis and its potential to be a lead compound for antibiotic development, we set out to develop a chemoenzymatic synthesis of fasamycin A.

The medicinal potential and the unique framework of this natural product family have stimulated two elegant chemical syntheses by the groups of Shia and Kraus.[7,8] Additionally, Shia has also reported the first synthesis of a related natural product, BE-24566B, also known as ABX.[9] Inspired by these chemical syntheses and the biosynthetic proposal, our retrosynthesis for **1** starts from disconnection of its C−Cl bond via a late stage enzymatic chlorination to reveal naphthacemycin B1 (**3**). Here, we envisioned an opportunity to functionally characterize the FDH (hereby termed FasV) from fasamycin biosynthesis for the first time and perform initial investigations into its biocatalytic utility (Scheme 1). Similar to Shia's approach,[7]

Scheme 1. Retrosynthetic Analysis of Fasamycin A

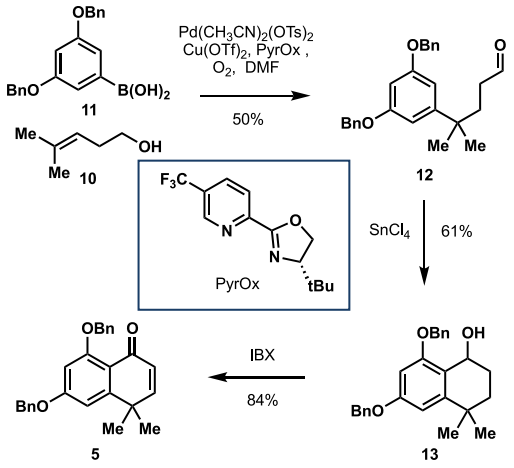


we deemed the use of an annulation strategy to construct the central ring of **3** to be the most convergent and strategic. To this end, a convergent assembly of **3** was envisioned through a Sammes annulation featuring lactone **4** and enone **5** and installation of the pendant arene by a Suzuki coupling. Lactone **4** could be traced back to Brassard's diene **6** and ynoate **7** via a Diels−Alder and lactonization sequence. Finally, enone **5** could be synthesized through a formal annulation involving resorcinol derivative **8** and an appropriate 6C coupling partner (e.g., **9**).

RESULTS AND DISCUSSION

We first focused our efforts toward identifying an efficient route to enone **5**. The same fragment has been synthesized previously by Shia through the use of hydrogen-atom-transfer (HAT)-based Giese coupling, followed by Friedel−Crafts acylation and oxidation state adjustment. Seeking to establish a complementary disconnection with reduced overall step-count, we envisioned the use of Sigman's redox-relay Heck reaction[10] to forge the key quaternary carbon of **5**. Gratifyingly, union of alkene **10** and boronic acid **11** under Sigman's conditions proceeded uneventfully (Scheme 2) to provide aldehyde **12** in

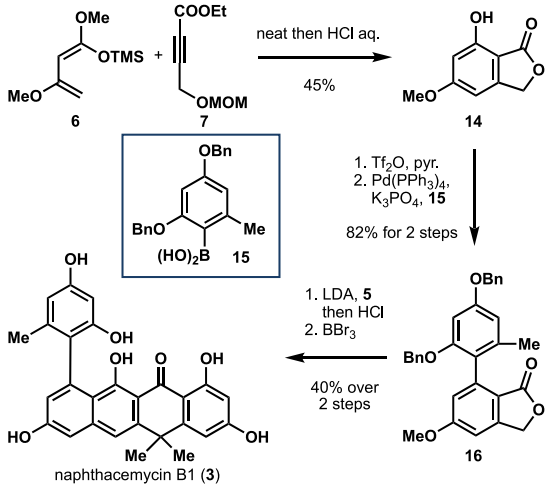
Scheme 2. Synthesis of Enone 5



50% yield. A $SnCl_4$-induced intramolecular Friedel−Crafts[11] cyclization gave benzylic alcohol **13** in 61% yield and this alcohol was directly oxidized to the corresponding enone in one pot by using IBX as an oxidant[12] to complete the synthesis of enone **5**.

Toward lactone **4**, we noted that a related structure had been prepared by Shia through the use of ring substitution/functionalization of a resorcinol derivative. We envisioned that a ring annulation strategy would allow for a more expedient access. Toward this goal, Brassard's diene **6**[13] and alkyne **7**[14] were utilized to construct lactone **14** through a sequential Diels−Alder reaction and acid-induced lactonization (Scheme 3). Triflation of the phenol group and conversion of the triflate

Scheme 3. Synthesis of Lactone 16 and Completion of the Synthesis of Naphthacemycin B1



group into arene side chain via standard Suzuki coupling conditions provided lactone **16** in 82% overall yield. LDA-induced Sammes annulation between **5** and **16**, followed by treatment with HCl furnished the desired dimethylnaphthacenone ring system. Finally, global deprotection with BBr_3

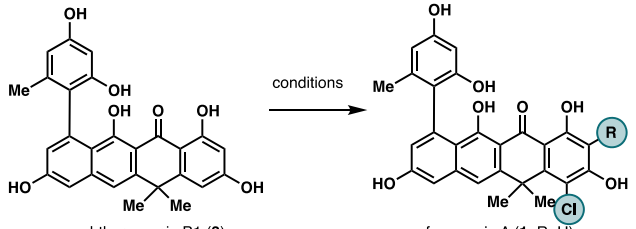
https://doi.org/10.1021/acs.joc.1c00526
J. Org. Chem. XXXX, XXX, XXX−XXX

proceeded in 40% yield (over two steps) to complete our synthesis of naphthacemycin B1.

With naphthacemycin B1 in hand, we set out to functionally characterize FasV and investigate its use in the final C−H chlorination step. FasV shares 59.8% and 55.4% sequence identity with ForV, a FDH from formicamycin biosynthesis, and AbxH, a FDH from BE-24566B biosynthesis. While no biosynthetic studies have been performed on the fasamycins, some of the late-stage tailoring steps in formicamycin and BE-24566B have begun to be elucidated.[15,16] Notably, in frame deletion of *forV* resulted in the production of nonchlorinated precursors to the formicamycins and *in trans* complementation rescued the production of the formicamycins. Additionally, *in vitro* assays had been performed to verify the role of AbxH in BE-24566B biosynthesis.

Heterologous expressions of FasV and ForV as N-His_6-tagged proteins resulted in satisfactory yields of soluble proteins, particularly in the presence of cochaperones GroES/EL, allowing us to start screening for an optimal set of C−H chlorination conditions. The two purified FDHs were tested in combination with five FAD reductases, namely Fre,[17] SfFre (a Fre homologue from *S. formicae*, the native producer of the formicamycins), SsuE,[18] CtcQ,[19] and HpaC[20] (Table 1).

Table 1. Enzymatic Halogenation Screening[a]



naphthacemycin B1 (**3**)

fasamycin A (**1**; R=H)
fasamycin B (**2**; R=Cl)

halogenase	reductase	conversion	isolated yield of **1** (%)
FasV	Fre	0%	ND
FasV	SfFre	0%	ND
FasV	SsuE	3% as a mixture	ND
FasV	CtcQ	7% as a mixture	5
FasV	HpaC	3−5% as a mixture	ND
ForV	Fre	0%	ND
ForV	SfFre	0%	ND
ForV	SsuE	3% as a mixture	ND
ForV	CtcQ	5% as a mixture	ND
ForV	HpaC	0%	ND
FasV	CtcQ	4% as a mixture[b]	ND
FasV	CtcQ	4% as a mixture[c]	ND
N.A.	CtcQ	0%	ND
FasV K75A	CtcQ	0%	ND

[a]Standard conditions for screening: naphthacemycin B1 (0.5 mM, 1 equiv), NADH/NADPH (1 mM, 2 equiv), FAD (100 μM, 20 mol %), halogenase (5 μM, 1 mol %), Reductase (5 μM, 1 mol %), buffer (20 mM phosphate pH 7.4, 100 mM NaCl, 5 mL total volume), 12 h. [b]Reaction was performed in the presence of Opt13 (5.0 μM) and $Na_2HPO_3{\cdot}5H_2O$ (10 mM). [c]Reaction was performed at 30 °C.

Despite extensive screening, only low conversions to the chlorinated products could be observed and several reductases (Fre and SfFre) failed to give any desired products at all. Additionally, a mixture of monochlorination regioisomers was consistently detected, though LC/MS analyses and comparison with authentic product standard showed that fasamycin A was always the major chlorinated product. Among the combinations tested, the FasV/CtcQ pair gave the highest conversion though the overall chlorination yield was still unsatisfactory. While the presence of bischlorinated products could be detected through HPLC extracted ion chromatogram analysis of the crude reaction, the amounts detected were too low to allow further isolation and structural determination. At this stage, we also tested a range of different pHs and temperatures for the reaction (Table 1 and Supplementary Table S1) and found that a pH range of 7.4−8.0 was optimal for the reaction and that temperature had minimal effect on the outcome of the reaction. The use of Opt13[21] to regenerate the NADH cofactor did not lead to any improvement in the reaction conversion. Chitnumsub and Chaiyen[22] and Lewis[23] had discussed the possibility of hypohalous acid leakage as an alternative mechanism for FDH-catalyzed halogenation. In light of the low efficiency of our reaction, we sought to determine if this pathway was operative with FasV. To this end, we tested the halogenation reaction in the absence of FasV and in the presence of a variant of FasV containing mutation at its active-site lysine residue (K75A). In both cases, no formation of **1** could be observed, suggesting that **1** was not formed from the reaction of **3** with free HOCl in our system.

The enzymatic reaction was next performed on preparative scale, and purification of the reaction mixture by preparative TLC afforded fasamycin A in pure form. To benchmark the enzymatic chlorination strategy, naphthacemycin B1 was submitted to electrophilic chlorination with several small-molecule reagents. However, intractable mixtures were consistently observed across all conditions tested. This observation suggests that, despite its low efficiency, the enzymatic chlorination proceeds under much milder conditions than conventional chemical halogenations. While the low conversion was disappointing, it is worth noting that during the characterization of the halogenase from BE-24566B biosynthesis, Lei, Qu, and co-workers also observed only low conversion to the halogenated products despite the use of high enzyme loading.[16] Furthermore, this issue could potentially be addressed with future enzyme engineering efforts or genome mining for enzyme homologues. In light of the challenges associated with evolving inefficient enzymes for improved activities, we anticipate that the latter strategy would facilitate the identification of a better starting point for an evolution campaign. A recent report from Lewis and co-workers[24] on the utility of family-wide activity profiling of FDHs to uncover new enzymes with unique substrate preferences bodes well for the realization of this idea.

CONCLUSION

In summary, we have accomplished the chemoenzymatic synthesis of fasamycin A. The western and eastern segments were concisely prepared and convergently assembled to furnish the naphthacemycin core skeleton. By relying on the native FDH from fasamycin biosynthesis, a late-stage enzymatic chlorination reaction was performed to install the chlorine substituent of the natural product. This work also provided the first direct biochemical confirmation of the halogenation activity of FasV and ForV and presented our initial foray toward evaluating their performance and viability as late-stage halogenation biocatalysts. Finally, the route described herein lays down the blueprint for future chemoenzymatic preparation of other halogenated naphthacenone natural products and will

https://doi.org/10.1021/acs.joc.1c00526
J. Org. Chem. XXXX, XXX, XXX−XXX

facilitate the discovery of new antibiotics and their SAR studies.

EXPERIMENTAL SECTION

General Materials and Methods. Unless otherwise noted, all chemicals and reagents for chemical reactions were purchased at the highest commercial quality and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) and liquid chromatography/mass spectrometry (LC/MS). TLC was performed with 0.25 mm E. Merck silica plates (60F-254) using short-wave UV light as the visualizing agent and ninhydrin, $KMnO_4$, or phosphomolybdic acid and heat as developing agents. LC/MS was performed with Agilent 1260 Infinity System equipped with Poroshell 120 EC-C18 column (3.0 × 50 mm, 2.7 μm). NMR spectra were recorded on a Bruker spetrometer and calibrated using residual undeuterated solvent. Optical rotations were measured on Autopol IV polarimeter (Rudolph Research Analytical). Electrocompetent *E. coli* BL21(DE3) were purchased from Lucigen.

Plasmid Generation for Enzyme Expression. With the exception of HpaC, expression vectors for all enzymes used in this work were obtained via DNA synthesis from Twist Bioscience. Briefly, the codon-optimized DNA sequences of the respective enzymes were inserted between the NdeI and XhoI restriction sites within the commercial pET28a(+) vector. The resulting vectors were used directly to transform electrocompetent *E. coli* BL21(DE3).

Generation of FasV K75A Mutant. Site-directed mutagenesis to generate FasV K75A mutant was performed by using standard Quikchange PCR method with primers containing the desired K75A mutation and wild-type FasV as the PCR template. The resulting PCR product was digested with DpnI, gel purified, repaired using NEBuilder HiFi DNA Assembly kit (NEB product no.: 2621) and used directly to transform electrocompetent *E. coli* BL21(DE3).

Enzyme Expression and Purification. Expression vectors were used directly to transform electrocompetent *E. coli* strain BL21(DE3). Sonication was performed using a Qsonica Q500 sonicator. Purified enzymes were accessed via immobilized metal ion affinity chromatography with HisTrap HP column. Recombinant *E. coli* BL21(DE3) cells harboring plasmids that encode for the appropriate enzyme variants were cultured under standard conditions with IPTG induction. Optionally, soluble expression of FasV and ForV could be improved through coexpression of the chaperones GroES/EL under previously reported conditions.[25] Cell were harvested by centrifugation (4 °C, 15 min, 3000*g*), and the cell pellet was stored at −20 °C or below for at least 2 h. Purification was performed with an AKTA pure FPLC system (GE Healthcare). The thawed cell pellet was resuspended in Ni−NTA buffer A (25 mM Tris·HCl, 200 mM NaCl, 25 mM imidazole, pH 9.0, 4 mL/g of cell wet weight) and lysed by sonication (3 × 1 min, 50% duty cycle). The lysate was centrifuged at 15000*g* for 30 min at 4 °C to remove cell debris. The collected supernatant was subjected to a Ni-NTA chromatography step using a Ni sepharose column (HisTrap-HP, GE Healthcare, Piscataway, NJ). The protein was eluted from the Ni sepharose column using 25 mM Tris·HCl, 200 mM NaCl, 300 mM imidazole, pH 8.0. Ni-purified protein was buffer exchanged into 0.05 M phosphate buffer (pH = 7.0) using a 30 kDa MW cutoff centrifugal filter. Protein concentrations were determined by A280 with calculated extinction coefficients as obtained at https://web.expasy.org/protparam. For storage, proteins were portioned into 100 μL aliquots, flash frozen on liquid N_2, and stored at −80 °C.

*4-(3,5-Bis(benzyloxy)phenyl)-4-methylpentanal (**12**).* A stirred solution of $Pd(CH_3CN)_2(OTs)_2$ (159 mg, 0.30 mmol), $Cu(OTf)_2$ (54 mg, 0.15 mmol), PyrOx (123 mg, 0.045 mmol), and 3 Å molecular sieves (750 mg) in DMF (400 mL) was stirred under O_2 for 10 min at 22 °C. To the resulting mixture were sequentially added alcohol **10** (500 mg, 4.99 mmol) and boronic acid **11** (1.67 g, 5.00 mmol) at rt. The reaction mixture was stirred at room temperature for 24 h before it was quenched with saturated aq $NaHCO_3$(400 mL). The mixture so obtained was extracted with EtOAc (3 × 200 mL). The combined organic phases were washed with brine (400 mL), dried over anhydrous Na_2SO_4, and filtered. After removal of the solvent under vacuum, the residue was subjected to flash column chromatography for purification using EtOAc/petroleum ether (1:10 → 1:4) as eluent to give aldehyde **12** (971 mg, 50%) as a pale yellow oil: ^{1}H NMR (400 MHz, acetone-d_6) δ 9.59 (s, 1 H), 7.51−7.23 (m, 10 H), 6.64−6.58 (m, 2 H), 6.54 (s, 1 H), 5.10 (s, 4 H), 2.25−2.11 (m, 2 H), 1.93−1.83 (m, 2 H); ^{13}C{^{1}H} NMR (101 MHz, acetone-d_6) δ 202.5, 160.7, 151.5, 138.3, 129.1, 128.4, 128.3, 106.3, 99.9, 70.3, 40.4, 37.9, 36.2, 32.1; HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{26}H_{28}O_3Na$ 411.1931, found 411.1933.

*6,8-Bis(benzyloxy)-4,4-dimethyl-1,2,3,4-tetrahydronaphthalen-1-ol (**13**).* To a stirred solution of aldehyde **12** (971 mg, 2.50 mmol) in CH_2Cl_2 (10 mL) was added $SnCl_4$ (3 mL, 1.0 M in CH_2Cl_2) at −78 °C. The reaction mixture was stirred at the same temperature for 4 h and then quenched with saturated aq $NaHCO_3$(20 mL). The resulting mixture was extracted with EtOAc (3 × 20 mL), and the combined organic phases were washed with brine (150 mL) and dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent under vacuum, the residue was subjected to flash column chromatography for purification using EtOAc/petroleum ether (1:3 → 1:1) as eluent to give alcohol **13** (592 mg, 61%) as a pale yellow oil: ^{1}H NMR (400 MHz, acetone-d_6) δ 7.54−7.29 (m, 10 H), 6.65 (d, *J* = 2.3 Hz, 1 H), 6.58 (d, *J* = 2.4 Hz, 1 H), 5.20−5.07 (m, 4 H), 5.05−4.94 (m, 1 H), 1.93−1.76 (m, 2 H), 1.53−1.38 (m, 1 H), 1.29 (s, 3 H), 1.17 (s, 3H); ^{13}C{^{1}H} NMR (101 MHz, acetone-d_6) δ 159.7, 158.7, 148.7, 138.4, 138.3, 129.1, 129.1, 128.4, 128.4, 128.3, 128.1, 120.7, 104.7, 98.5, 70.5, 70.2, 62.1, 34.7, 33.7, 31.5, 30.8, 27.9; HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{26}H_{28}O_3Na$ 411.1931, found 411.1937.

*6,8-Bis(benzyloxy)-4,4-dimethylnaphthalen-1(4H)-one (**5**).* To a stirred solution of alcohol **13** (300 mg, 0.772 mmol) in DMSO (3 mL) was added IBX (476 mg, 1.70 mmol) at 22 °C. The resulting mixture was heated to 100 °C in an oil bath and stirred at that temperature for 48 h before it was cooled to 22 °C and diluted with EtOAc (20 mL). The resultant mixture was sequentially washed with saturated aq $Na_2S_2O_3$ (10 mL) and brine (10 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under vacuum, and the residue was rapidly purified by flash column chromatography with EtOAc/petroleum ether (1:10 → 1:2) to give enone **5** (249 mg, 84%) as a colorless oil. Characterization data for this compound are in agreement with previous literature report.[7]

*7-Hydroxy-5-methoxyisobenzofuran-1(3H)-one (**14**).* To a stirred solution of alkyne **7** (1.72 g, 10.0 mmol) in toluene (10 mL) was added Brassard's diene **6** (2.43 g, 12.0 mmol) at 22 °C. The resulting mixture was heated to 110 °C in an oil bath and stirred at the same temperature for 12 h before it was cooled to 22 °C. HCl was added (10 mL, 1.0 M in ethyl acetate), and the resulting mixture was stirred for 3 h. The organic phase was diluted with 100 mL ethyl acetate, washed with brine (100 mL), dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated under vacuum, and the residue was purified by flash column chromatography with EtOAc/petroleum ether (1:10 → 1:1) to give lactone **14** (810 mg, 45%) as a white powder. Characterization data for this compound are in agreement with previous literature report.[7]

*7-(2,4-Bis(benzyloxy)-6-methylphenyl)-5-methoxyisobenzofuran-1(3H)-one (**16**).* To a stirred solution of lactone **14** (901 mg, 5.0 mmol) in CH_2Cl_2 (15 mL) were sequentially added pyridine (804 μL, 10 mmol) and Tf_2O (1.01 mL, 6.0 mmol) at −20 °C. The reaction mixture was stirred at the same temperature for 2 h before it was quenched with saturated aq $NaHCO_3$(20 mL). The resulting mixture was extracted with EtOAc (3 × 20 mL), and the combined organic phases were washed with brine (150 mL) and dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent under vacuum, the triflated lactone **4** was obtained as a pale yellow solid (1.56 g, 99%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (dd, *J* = 1.8, 0.9 Hz, 1 H), 6.92−6.85 (m, 1 H), 5.33−5.16 (m, 2H), 3.94 (s, 3H); ^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$) δ 166.3, 166.1, 151.2, 147.0, 118.9 (q, *J* = 320.8 Hz), 111.0, 110.2, 106.3, 68.9, 56.7; HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{10}H_7F_3O_6SNa$ 334.9808, found 334.9812.

https://doi.org/10.1021/acs.joc.1c00526
J. Org. Chem. XXXX, XXX, XXX−XXX

Characterization data for this compound are in agreement with the previous literature report.[7]

The above triflated lactone **4** was taken to the next step without further purification. To a stirred solution of the triflate and boronic acid **15** (2.44 g, 7.0 mmol) in toluene (20 mL) were sequentially added $Pd(PPh_3)_4$ (289 mg, 0.25 mmol) and K_3PO_4 (10 mL, 1.0 M in water). The resultant mixture was heated to 110 °C in an oil bath and stirred at the same temperature for 8 h before it was cooled to 22 °C. The reaction mixture was diluted with 50 mL ethyl acetate, washed with brine (100 mL), dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated under vacuum, and the residue was purified by flash column chromatography with EtOAc/petroleum ether (1:50 → 1:10) to give lactone **16** (1.91 g, 82%) as a colorless oil. Characterization data for this compound are in agreement with previous literature report.[7]

*Naphthacemycin B1 (**3**).* To a stirred solution of lactone **16** (446 mg, 1.00 mmol) and enone **5** (384 mg, 1.00 mmol) in THF (4 mL) was added LDA (0.6 mL, 2.0 M in THF/heptane/ethylbenzene, 1.2 mmol) at −78 °C. The reaction mixture was warmed to 22 °C and stirred at that temperature for 48 h before it was quenched with HCl (3.0 mL, 1.0 M in ethyl acetate). The resulting mixture was extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with brine (150 mL) and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent under vacuum, the residue was purified by flash column chromatography with EtOAc/petroleum ether (1:60 → 1:10) to give 374 mg of prenaphthacemycin B1 (**17**) as yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66−7.54 (m, 2 H), 7.52−7.29 (m, 15 H), 7.13−7.00 (m, 4 H), 6.99−6.90 (m, 2 H), 6.88 (d, J = 2.3 Hz, 1 H), 6.83 (d, J = 2.5 Hz, 1 H), 6.64−6.56 (m, 2 H), 6.51 (d, J = 2.3 Hz, 1 H), 5.30 (s, 2 H), 5.18 (s, 2 H), 5.14 (s, 2 H), 5.12−5.01 (m, 2 H), 4.93 (s, 2 H), 3.94 (s, 3 H), 2.08 (s, 3 H), 1.75 (s, 3 H), 1.73 (s, 3 H); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 187.8, 165.8, 163.4, 162.2, 160.0, 158.5, 156.8, 155.4, 145.0, 140.3, 138.9, 137.7, 137.4, 137.2, 136.8, 136.2, 128.9, 128.8, 128.7, 128.6, 128.2, 128.1, 127.9, 127.8, 127.8, 127.1, 126.9, 126.9, 126.7, 120.7, 118.2, 114.4, 113.5, 109.5, 107.4, 106.1, 104.9, 99.4, 98.5, 71.1, 70.4, 70.4, 70.2, 55.4, 39.2, 34.5, 33.8, 21.0; HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{56}H_{48}O_7Na$ 855.3293, found 855.3301.

To a stirred solution of prenaphthacemycin B1 (**17**) in CH_2Cl_2 (3 mL) was added BBr_3 (2.0 mL, 1.0 M in CH_2Cl_2) at −78 °C. The resulting mixture was warmed to 22 °C and stirred at the same temperature for 8 h before it was quenched with saturated aq $NaHCO_3$(20 mL). The resultant mixture was extracted with EtOAc (3 × 20 mL), and the combined organic phases were washed with brine (50 mL) and dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent under vacuum, the residue was subjected to flash column chromatography for purification using EtOAc/petroleum ether (1:3 → 1:1) as eluent to give naphthacemycin B1 **3** (183 mg, 40%) as a yellow solid: ^{1}H NMR (600 MHz, methanol-d_4) δ 7.36 (s, 1 H), 7.05 (d, J = 2.5 Hz, 1 H), 6.72 (d, J = 2.5 Hz, 1 H), 6.65 (d, J = 2.3 Hz, 1 H), 6.27 (dd, J = 2.4, 0.8 Hz, 1 H), 6.24 (dd, J = 2.3, 0.6 Hz, 1 H), 6.21 (d, J = 2.2 Hz, 1 H), 1.88 (d, J = 0.7 Hz, 3 H), 1.71 (s, 3 H), 1.69 (s, 3 H); ^{13}C{^{1}H} NMR (151 MHz, Methanol-d_4) δ 210.5, 191.6, 166.9, 166.6, 166.3, 160.1, 157.5, 155.8, 155.4, 146.5, 142.9, 140.8, 138.3, 124.5, 122.5, 118.2, 116.1, 110.1, 108.8, 108.7, 107.6, 106.9, 102.0, 100.8, 39.7, 34.7, 34.5, 20.7; HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{27}H_{22}O_7Na$ 481.1258, found 481.1264.

General Condition for Enzymatic Halogenation Screening. To a 5 mL phosphate-buffered saline solution (20 mM phosphate pH 7.4, 100 mM NaCl) in a 20 mL scintillation vial were added NADH or NADPH (1 mM final concentration), FAD (100 μM final concentration), 0.25 mL of naphthacemycin B1 stock solution (10 mM in methanol, ca. 0.5 mM final concentration), halogenase (5.0 μM final concentration), and reductase (5.0 μM final concentration). The vial was sealed and shaken for 12 h at 22 °C, 200 rpm. The mixture was extracted with ethyl acetate (5 mL). The organic layer was concentrated under vacuum and analyzed by reverse phase liquid chromatography.

*Fasamycin A (**1**).* To a 5 mL phosphate-buffered saline solution (20 mM phosphate pH 7.4, 100 mM NaCl) in 20 mL scintillation vial were added NADH (1 mM final concentration), FAD (100 μM final concentration), 0.25 mL of naphthacemycin B1 stock solution (10 mM in methanol, ca. 0.5 mM final concentration), FasV (5.0 μM final concentration), and CtcQ (5.0 μM final concentration). The vial was sealed and shaken for 12 h at 22 °C, 200 rpm. The mixture was extracted with ethyl acetate (5 mL). To provide sufficient material for isolation, 20 reactions were performed in parallel and combined at the end. The combined organic layers were concentrated under vacuum and purified by preparative thin-layer chromatography (EtOAc/petroleum ether 1:2) to give fasamycin A (1.3 mg, 5.2%) as a yellow solid: ^{1}H NMR (600 MHz, methanol-d_4) δ 7.34 (s, 1 H), 7.05 (d, J = 2.5 Hz, 1 H), 6.72 (d, J = 2.5 Hz, 1 H), 6.45 (d, J = 1.0 Hz, 1 H), 6.26 (dd, J = 2.4, 0.8 Hz, 1 H), 6.23 (d, J = 2.3 Hz, 1 H), 2.06 (s, 3 H), 2.04 (s, 3 H), 1.88 (s, 3 H); ^{13}C{^{1}H} NMR (151 MHz, methanol-d_4) δ 191.6, 166.6, 165.5, 160.3, 157.5, 155.5, 150.0, 149.2, 143.5, 140.9, 138.2, 124.4, 122.6, 117.8, 117.0, 109.9, 108.8, 106.3, 103.6, 100.8, 40.7, 30.3, 30.2, 20.7; HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{27}H_{21}ClO_7Na$ 515.0868, found 515.0877; $[\alpha]^{24}_D$ = +5.7 (c = 0.20 in MeOH).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c00526.

Protein and DNA sequences, ^{1}H and ^{13}C NMR data, and HPLC traces (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Hans Renata − *The Scripps Research Institute, Department of Chemistry, Jupiter, Florida 33458, United States;* orcid.org/0000-0003-2468-2328; Email: hrenata@scripps.edu

Author

Jian Li − *The Scripps Research Institute, Department of Chemistry, Jupiter, Florida 33458, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.joc.1c00526

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Funding for this work is generously provided by the National Institutes of Health grants GM128895 (H.R.) and S10 OD021550 (for 600 MHz NMR instrumentation). We thank Prof. David H. Sherman and Dr. Amy E. Fraley for providing plasmid pET11a-HpaC (encoding for HpaC flavin reductase). We acknowledge the Roush, Bannister, and Shen laboratories for generous access to their reagents and instrumentation.

■ REFERENCES

(1) Pearson, H. 'Superbug' hurdles key drug barrier. *Nature* **2002**, 418−469.

(2) Clardy, J.; Fischbach, M. A.; Walsh, C. T. New Antibiotics from Bacterial Natural Products. *Nat. Biotechnol.* **2006**, *24*, 1541−1550.

(3) Feng, Z.; Kallifidas, D.; Brady, S. F. Functional Analysis of Environmental DNA-derived Type II Polyketide Synthases Reveals Structurally Diverse Secondary Metabolites. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 12629−12634.

(4) Feng, Z.; Chakraborty, D.; Dewell, S. B.; Reddy, B. V.; Brady, S. F. Environmental DNA-Encoded Antibiotics Fasamycins A and B Inhibit FabF in Type II Fatty Acid Biosynthesis. *J. Am. Chem. Soc.* **2012**, *134*, 2981−2987.

(5) Heath, R.; White, S.; Rock, C. Inhibitors of Fatty Acid Synthesis as Antimicrobial Chemotherapeutics. *Appl. Microbiol. Biotechnol.* **2002**, *58*, 695−703.
(6) Fukumoto, A.; Kim, Y.-P.; Matsumoto, A.; Takahashi, Y.; Suzuki, M.; Onodera, H.; Tomoda, H.; Matsui, H.; Hanaki, H.; Iwatsuki, M.; Ōmura, S.; Shiomi, K. Naphthacemycins, Novel Circumventors of β-lactam Resistance in MRSA, Produced by *Streptomyces* sp. KB-3346−5. I. The Taxonomy of the Producing Strain, and the Fermentation, Isolation and Antibacterial Activities. *J. Antibiot.* **2017**, *70*, 562−567.
(7) (a) Huang, J.-K.; Yang Lauderdale, T.-L.; Lin, C.-C.; Shia, K.-S. Total Synthesis of Tetarimycin A, (±)-Naphthacemycin A9, and (±)-Fasamycin A: Structure-Activity Relationship Studies against Drug-Resistant Bacteria. *J. Org. Chem.* **2018**, *83*, 6508−6523. (b) Huang, J.-K.; Yang Lauderdale, T.-L.; Shia, K.-S. Studies on Antibiotics Active against Resistant Bacteria. Total Synthesis of MRSA-Active Tetarimycin A and Its Analogues. *Org. Lett.* **2015**, *17*, 4248−4251.
(8) Wang, S.; Kraus, G. A. Synthesis of (±)-Naphthacemycin A_9. *J. Org. Chem.* **2018**, *83*, 15549−15552.
(9) Huang, J.-K.; Shia, K.-S. Development of a Cross-Conjugated Vinylogous [4+2] Anionic Annulation and Application to the Total Synthesis of Natural Antibiotic (±)-ABX. *Angew. Chem., Int. Ed.* **2020**, *132*, 6602−6607.
(10) (a) Mei, T.-S.; Werner, E. W.; Burckle, A. J.; Sigman, M. S. Enantioselective Redox-Relay Oxidative Heck Arylations of Acyclic Alkenyl Alcohols using Boronic Acids. *J. Am. Chem. Soc.* **2013**, *135*, 6830−6833. (b) Mei, T.-S.; Patel, H. H.; Sigman, M. S. Enantioselective Construction of Remote Quaternary Stereocentres. *Nature* **2014**, *508*, 340−344.
(11) Genot, A.; Florent, J. C.; Monneret, C. Anthracyclinone. 3. Chiral pool synthesis of anthracyclinones via tetralin intermediates. *J. Org. Chem.* **1987**, *52*, 1057−1063.
(12) Nicolaou, K. C.; Montagnon, T.; Baran, P. S.; Zhong, Y.-L. Iodine(V) Reagents in Organic Synthesis. Part 4. o-Iodoxybenzoic Acid as a Chemospecific Tool for Single Electron Transfer-Based Oxidation Processes. *J. Am. Chem. Soc.* **2002**, *124*, 2245−2258.
(13) Savard, J.; Brassard, P. Reactions of ketene acetals-14 The use of simple mixed vinylketene acetals in the annulation of quinones. *Tetrahedron* **1984**, *40*, 3455−3464.
(14) Piers, E.; Tillyer, R. D. Dilithium (trimethylstannyl)(2-thienyl)(cyano)cuprate, a synthetically useful higher-order cuprate reagent. *J. Org. Chem.* **1988**, *53*, 5366−5369.
(15) Qin, Z.; Munnoch, J. T.; Devine, R.; Holmes, N. A.; Seipke, R. F.; Wilkinson, K. A.; Wilkinson, B.; Hutchings, M. I. Formicamycins, Antibacterial Polyketides Produced by *Streptomyces formicae* Isolated from African Tetraponera Plant-ants. *Chem. Sci.* **2017**, *8*, 3218−3227.
(16) Mei, X.; Yan, X.; Zhang, H.; Yu, M.; Shen, G.; Zhou, L.; Deng, Z.; Lei, C.; Qu, X. Expanding the Bioactive Chemical Space of Anthrabenzoxocinones through Engineering the Highly Promiscuous Biosynthetic Modification Steps. *ACS Chem. Biol.* **2018**, *13*, 200−206.
(17) Spyrou, G.; Haggård-Ljungquist, E.; Krook, M.; Jörnvall, H.; Nilsson, E.; Reichard, P. Characterization of the Flavin Reductase Gene (fre) of *Escherichia coli* and Construction of a Plasmid for Overproduction of the Enzyme. *J. Bacteriol.* **1991**, *173*, 3673−3679.
(18) van Der Ploeg, J. R.; Iwanicka-Nowicka, R.; Bykowski, T.; Hryniewicz, M. M.; Leisinger, T. The *Escherichia coli* ssuEADCB Gene Cluster is Required for the Utilization of Sulfur from Aliphatic Sulfonates and is Regulated by the Transcriptional Activator Cbl. *J. Biol. Chem.* **1999**, *274*, 29358−29365.
(19) Zhu, T.; Cheng, X.; Liu, Y.; Deng, Z.; You, D. Deciphering and Engineering of the Final Step Halogenase for Improved Chlortetracycline Biosynthesis in Industrial *Streptomyces aureofaciens*. *Metab. Eng.* **2013**, *19*, 69−78.
(20) Prieto, M. A.; Garcia, J. L. Molecular Characterization of 4-hydroxyphenylacetate 3-hydroxylase of *Escherichia coli*. A Two-protein Component Enzyme. *J. Biol. Chem.* **1994**, *269*, 22823−22829.
(21) McLachlan, M. J.; Johannes, T. W.; Zhao, H. Further Improvement of Phosphite Dehydrogenase Thermostability by Saturation Mutagenesis. *Biotechnol. Bioeng.* **2008**, *99*, 268−274.
(22) Phintha, A.; Prakinee, K.; Jaruwat, A.; Lawan, N.; Visitsatthawong, S.; Kantiwiriyawanitch, C.; Songsungthong, W.; Trisrivirat, D.; Chenprakhon, P.; Mulholland, A.; van Pee, K.-H.; Chitnumsub, P.; Chaiven, P. Dissecting the Low Catalytic Capability of Flavin-Dependent Halogenases. *J. Biol. Chem.* **2021**, *296*, 100068.
(23) Mondal, D.; Fisher, B.; Jiang, Y.; Lewis, J. C. Flavin-Dependent Halogenases Catalyze Enantioselective Olefin Halocyclization. *ChemRxiv* **2021**, DOI: 10.26434/chemrxiv.12982433.v1.
(24) Fisher, B. F.; Snodgrass, H. M.; Jones, K. A.; Andorfer, M. C.; Lewis, J. C. Site-Selective C-H Halogenation Using Flavin-Dependent Halogenases Identified via Family-Wide Activity Profiling. *ACS Cent. Sci.* **2019**, *5*, 1844−1856.
(25) Zhang, X.; King-Smith, E.; Renata, H. Total Synthesis of Tambromycin by Combining Chemocatalytic and Biocatalytic C-H Functionalization. *Angew. Chem., Int. Ed.* **2018**, *57*, 5037−5041.

https://doi.org/10.1021/acs.joc.1c00526
J. Org. Chem. XXXX, XXX, XXX−XXX