Concise Chemoenzymatic Synthesis of Fasamycin A

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ABSTRACT: We report the development of a chemoenzymatic approach towards 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 via Michael–Dieckmann condensation 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.

Antibiotic resistance is becoming one of the most pressing threats to human health, especially with the emergence of multi-drug 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 twelve '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.[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 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.

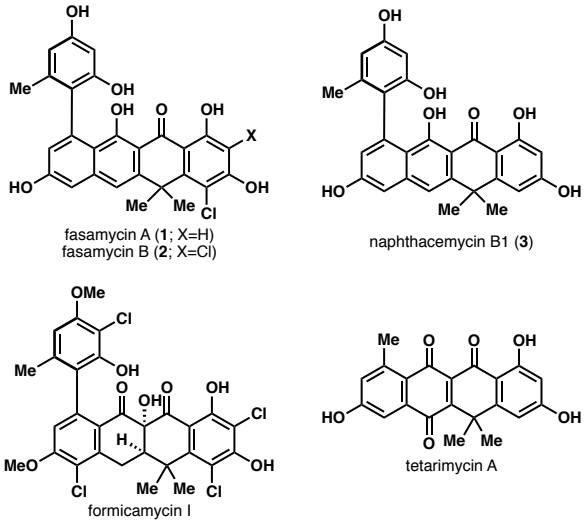
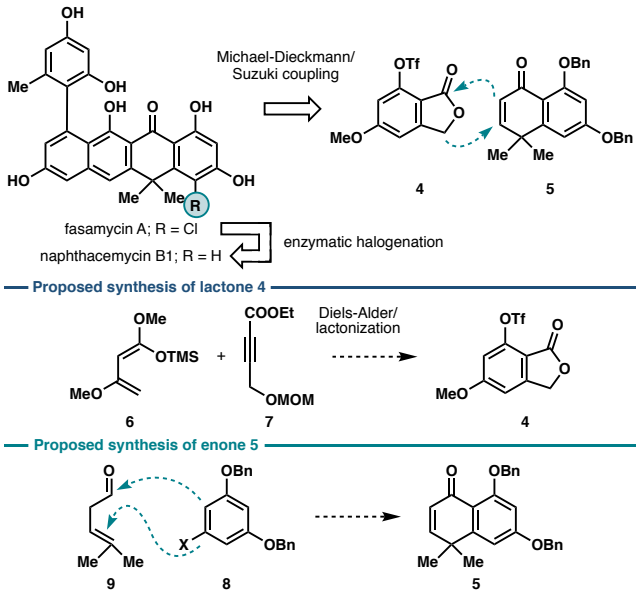


Figure 1. Structures of fasamycins A and B and related natural products.

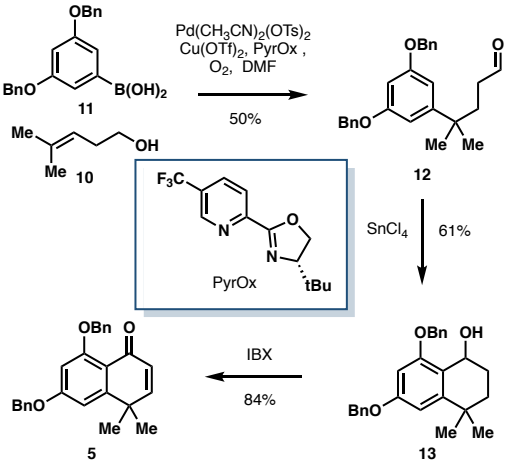
The medicinal potential and the unique framework of this natural product family have stimulated two elegant chemical syntheses by the groups of Shia and Kraus.[7,8] Additionally, Shia has also reported the first synthesis of a related natural product, BE-24566B, also known as ABX.[9] Inspired by these 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. Naphthacemycin B1 could be assembled convergently through a Michael-Dieckmann annulation featuring lactone **4** and enone **5**, and the pendant arene could be installed 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**).

Scheme 1. Retrosynthetic Analysis of Fasamycin A



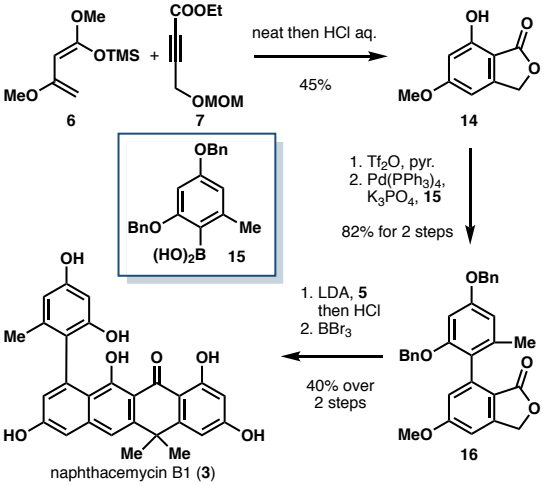
We first focused our efforts towards identifying an efficient route to enone **5**. The same fragment has been synthesized previously by Shia through the use of hydrogen-atom-transfer(HAT)-based Giese coupling, followed by Friedel-Crafts acylation and oxidation state adjustment. Seeking to establish 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 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**.

Scheme 2. Synthesis of Enone 5



Towards 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. Towards 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 group into arene side chain via standard Suzuki coupling conditions provided lactone **16** in 82% overall yield. LDA-induced Michael-Dieckmann cyclization between **5** and **16**, followed by treatment with HCl furnished the desired dimethylnaphthacenone ring system. Finally, global deprotection with BBr_3 proceeded in 40% yield (over two steps) to complete our synthesis of naphthacemycin B1.

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

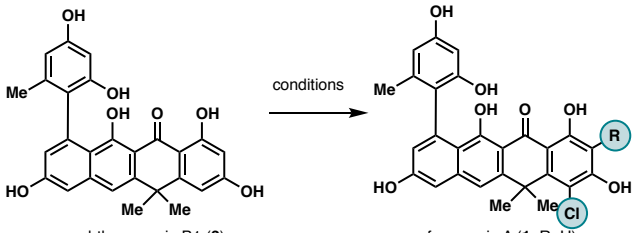


With naphthacemycin B1 in hand, we set out to functionally characterize FasV and investigate its use in the final C–H chlorination step. FasV shares 59.8% and 55.4% sequence identity with ForV, a FDH from formicamycin biosynthesis, and AbxH, a FDH from BE-24566B biosynthesis. While no biosynthetic studies 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 non-chlorinated 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, 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 homolog from *S. formicae*, the native producer of the formicamycins), SsuE,[18] CtcQ[19] and HpaC[20] (Table 1). 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. 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. Though the low conversion was disappointing, future enzyme engineering efforts or genome mining for enzyme homologs could potentially address this issue. Additionally, 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]

Table 1. Enzymatic Halogenation Screening[a]



Halogenase	Reductase	Conversion
FasV	Fre	0%
FasV	SfFre	0%
FasV	SsuE	3% as a mixture
FasV	CtcQ	7% as a mixture
FasV	HpaC	3–5% as a mixture
ForV	Fre	0%
ForV	SfFre	0%
ForV	SsuE	3% as a mixture
ForV	CtcQ	5% as a mixture
ForV	HpaC	0%

[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.

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 towards 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 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, KMnO4, 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 x 50 mm, 2.7 micron). 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).

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. Cell were harvested by centrifugation (4 °C, 15 min, 3,000xg), 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 (3x1 min, 50% duty cycle). The lysate was centrifuged at 15,000xg 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 cut-off 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 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) *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 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) *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 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: 1H NMR (400 MHz, $CDCl_3$) δ 7.74–7.59 (m, 2 H), 7.48–7.27 (m, 8H), 6.70–6.63 (m, 2 H), 6.56 (d, *J* = 2.3 Hz, 1 H), 6.24 (d, *J* = 10.1 Hz, 1 H), 5.19 (s, 2 H), 5.10 (s, 2 H), 1.42 (s, 6 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 184.0, 162.5, 161.4, 154.9, 153.0, 136.9, 136.2, 128.9, 128.7, 128.5, 128.4, 127.8, 127.7, 126.8, 115.2, 105.0, 99.5, 70.8, 70.3, 38.1, 30.7; HRMS (ESI) *m/z* [M + Na]$^+$ calcd for $C_{26}H_{24}O_3Na$ 407.1618, found 407.1625.

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 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: 1H NMR (400 MHz, Acetone-d_6) δ 8.59 (s, 1 H), 6.69–6.62 (m, 1 H), 6.48–6.42 (m, 1 H), 5.26 (S, 2 H), 3.88 (s, 3 H); ^{13}C NMR (101 MHz, Acetone-d_6) δ 171.2, 168.0, 158.6, 151.4, 105.5, 102.1, 100.5, 70.5, 56.5; HRMS (ESI) *m/z* [M + Na]+ calcd for $C_9H_8O_4Na$ 203.0315, found 203.0315.

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 was obtained as a pale yellow solid (1.56 g, 99%), which 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 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: 1H NMR (400 MHz, $CDCl_3$) δ 7.52–7.07 (m, 10 H), 6.86 (s, 2 H), 6.61–6.56 (m, 1 H), 6.54 (d, *J* = 2.3 Hz, 1 H), 5.18 (dd, *J* = 7.9, 0.8 Hz, 2 H), 5.05 (s, 2 H), 4.96 (*d*, J = 1.9 Hz, 2 H), 3.88 (s, 3 H), 2.09 (s, 3 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.7, 164.1, 159.6, 157.1, 149.8, 139.7, 138.0, 137.3, 137.1, 128.7, 128.4, 128.1, 127.8, 127.5, 126.9, 119.1, 118.9, 117.0, 108.0, 105.1, 98.6, 70.5, 70.1, 68.2, 55.9, 20.6; HRMS (ESI) *m/z* [M + Na]+ calcd for $C_{30}H_{26}O_5Na$ 489.1672, found 489.1679.

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 pre-naphthacemycin B1 as yellow oil. To a stirred solution of this compound 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 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) *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 20 mL scintillation vial was 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 ºC, 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 was 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 ºC, 200 rpm. The mixture was extracted with ethyl acetate (5 mL). To provide sufficient material for isolation, twenty reactions were performed in parallel and combined at the end. The combined organic layers were concentrated under vacuum and purified with preparative thin layer chromatography (EtOAc/petroleum ether 1:2) to give fasamycin A (1.3 mg, 5.2%) as a yellow solid: 1H NMR (400 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 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) *m/z* [M + Na]+ calcd for $C_{27}H_{21}ClO_7Na$ 515.0868, found 515.0877. $[\alpha]_D^{24}$ = +5.7 (c = 0.20 in MeOH).

ASSOCIATED CONTENT

Supporting Information

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

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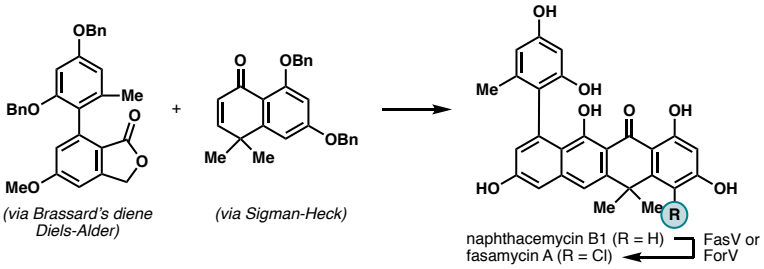
TOC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fasamycin A (R = Cl)
FasV or ForV