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Studies on Antibiotics Active against Resistant Bacteria. Total Synthesis of MRSA-Active Tetarimycin A and Its Analogues

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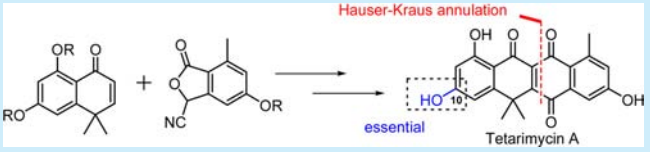
Supporting Information

ABSTRACT: Making use of the Hauser−Kraus annulation as a key step, the first total synthesis of tetarimycin A has been accomplished in a highly convergent and operationally simple manner. Preliminary SAR not only validated that tetarimycin A exhibited potent activity against MRSA and VRE at a low MIC value but also identified that the hydroxyl group at C-10 was essential for antibacterial activities.



Infections caused by multidrug-resistant bacteria have become a global public health crisis, and development of novel antibacterial drugs is one of the keys to combat increasing multidrug resistance. In clinical medicine, *Staphylococcus aureus* (*S. aureus*) is the most common Gram-positive pathogen responsible for a wide spectrum of infections.[1] Severe *S. aureus* infections are associated with poor prognosis and high mortality, especially in bacteremia caused by methicillin-resistant *Staphylococcus aureus* (MRSA).[2,3] For decades, vancomycin has been the drug of choice for treating serious MRSA infections. However, treatment failure due to MRSA isolates with reduced susceptibility to vancomycin has increased in recent years.[3,4] Thus, there is an urgent need for new antibiotics against these multidrug-resistant organisms. Tetarimycin A (**1**), recently reported as a MRSA-active agent (Figure 1), was isolated from the culture-broth extract of *Streptomyces albus* cobiosynthesized with an environmentally derived type-II polyketide gene cluster and its *Streptomyces* antibiotic regulatory protein (SARP); its structure was unambiguously identified by X-ray analysis.[5] During the course of antibiotic history, peripheral tailoring of a naturally occurring antibiotic substructure/pharmacophore is a common strategy to search for next generations with increased potency and varying the spectrum of antibacterial activities.[6] However, it is important to validate that the initial hit/lead compound (e.g., **1**) is not a false positive before any drug discovery projects gets started. Thus, developing an effective synthetic approach for target **1** to rapidly verify that its tetracyclic nucleus would be a useful substructure for seeking new antibiotics was our initial goal.

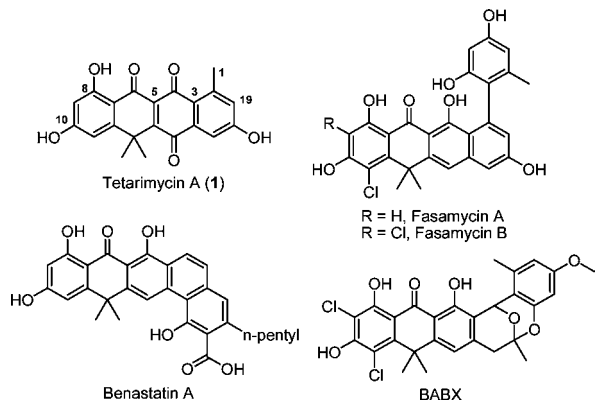
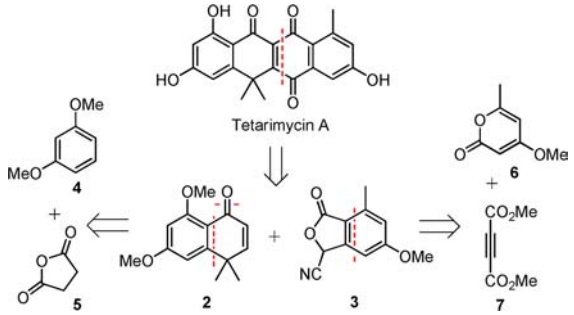


Figure 1. Tetarimycin A (**1**) and its congeners.

Herein, we report that the first total synthesis of tetarimycin A, and its closely related analogues have been experimentally realized. Our synthetic strategy and antibacterial screening results are presented as follows.

According to the retrosynthetic analysis illustrated in Scheme 1, it was envisioned that tetarimycin A should be effectively constructed by Hauser−Kraus annulation of Michael acceptor **2** and donor **3**. Subsequently, three methyl protecting groups are supposed to be removed simultaneously under treatment with BBr_3 to afford the final product **1**.[7] Fragments **2** and **3**, accounting for building the central tetracyclic unit, are extremely critical for the success of the design. In principle,

Scheme 1. Retrosynthetic Analysis of Tetarimycin A



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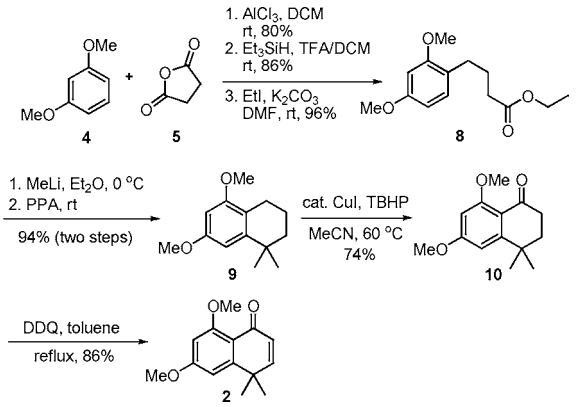
they could be individually prepared by making use of Friedel–Crafts acylation via commercially available substrates **4** and **5** and Diels–Alder cycloaddition of diene **6** (2-pyrone) and dienophile **7** (DMAD) as a key operation.

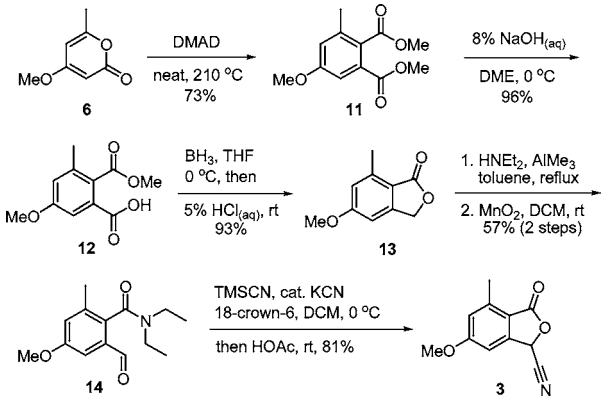
On the basis of this scenario, the total synthesis began with the preparation of enone **2** as shown in Scheme 2. Succinic

Scheme 2. Preparation of Methyl-Protected Enone 2



anhydride **5** underwent Friedel–Crafts acylation with 1,3-dimethoxylbenzene **4** in the presence of $AlCl_3$ to afford aryl ketone in 80% yield. The ketone carbonyl thus formed was reduced with Et_3SiH to give a saturated carboxylic acid in 86% yield, which was further alkylated with ethyl iodide to furnish ester **8** in almost quantitative yield (96%).[8] Compound **8** was subjected to excess MeLi to form tertiary alcohol, which subsequently underwent intramolecular Friedel–Crafts alkylation under PPA acidic catalysis to give tetralin **9** in 94% yield over two steps.[9] To our delight, the benzylic carbonyl group could be smoothly generated via oxidation of **9** with *tert*-butyl hydroperoxide and CuI as catalyst to afford the corresponding tetralone **10** in good yield (74%), which was further oxidized by DDQ to achieve the desired enone **2** in 86% yield.[10] Cyanophthalide **3** was also successfully synthesized as a donor according to Scheme 3. 2-Pyrone **6** was heated in neat

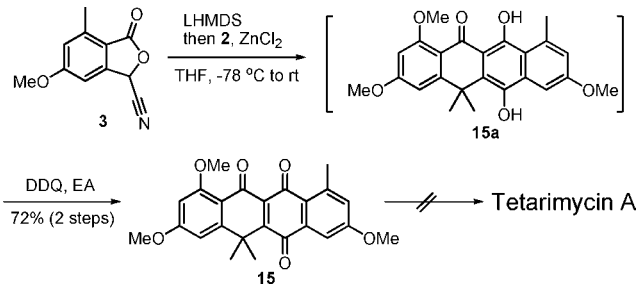
Scheme 3. Preparation of Methyl-Protected Donor 3



DMAD (**7**) at 210 °C to give Diels–Alder adduct **11** (73%), which was selectively hydrolyzed under basic conditions to afford **12** in 96% yield.[11] Chemoselective reduction of compound **12** was effected with BH_3 to give an ester alcohol intermediate, which without purification underwent cyclization under acidic conditions to form **13** in 93% yield.[12] Lactone **13** was smoothly coupled with diethylamine under catalysis with $AlMe_3$ to form the corresponding amide alcohol (65%), which was oxidized with MnO_2 to afford amide aldehyde **14** in 88% yield. Compound **14** thus obtained was allowed to react with TMSCN/KCN (cat.) in the presence of 18-crown-6-ether to form cyano alcohol intermediate, which underwent acidic cyclization with HOAc to provide fragment **3** in 81% yield.[7a,13]

With Michael acceptor **2** and donor **3** in hand, Hauser–Kraus annulation was then carried out (Scheme 4), giving rise

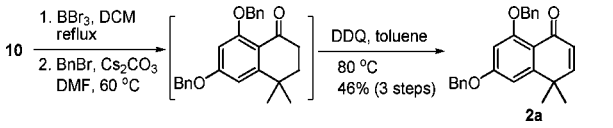
Scheme 4. Incomplete Synthesis of Tetarimycin A



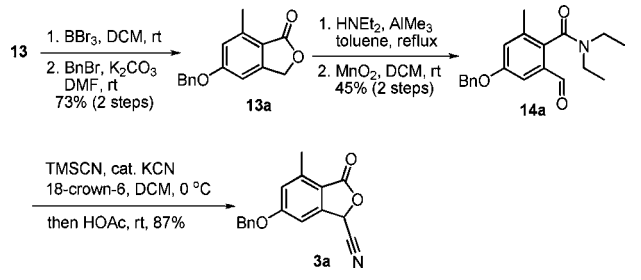
to a mixture of *p*-hydroquinone **15a** and the autoxidized *p*-benzoquinone **15**, which without purification was further oxidized to afford product **15** exclusively in 72% over two steps.[7h]

Unfortunately, attempts to deprotect the methyl groups of **15** by many conventional methods, including treatment with BBr_3, TMSI, EtSNa, etc., turned out to be fruitless. In all cases examined, a complex mixture was obtained along with compound **16** as a major side product (13–44%). As such, this 15-step synthetic approach was abandoned. Instead, we decided to change the methyl into the benzyl as a new protecting group to continue the total synthesis. As illustrated in Schemes 5 and 6 starting from advanced intermediates **10**

Scheme 5. Preparation of Benzyl-Protected Enone 2a



Scheme 6. Preparation of Benzyl-Protected Donor 3a



and **13**, respectively, a benzyl-protected acceptor **2a** and donor **3a** were effectively prepared in moderate yields. All reaction conditions in Schemes 5 and 6 were merely adapted from the previous methyl-protected route except that demethylation and benzylation were additionally carried out.

This newly designed approach turned out to be successful in that, unlike the methyl group, the benzyl group was found to be cleanly and easily removed under hydrogenolysis. As depicted

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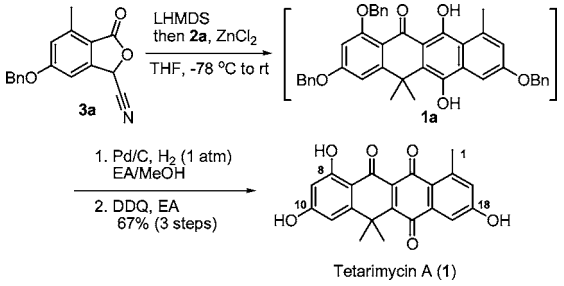
in Scheme 7, the coupling of **2a** and **3a** in the presence of LHMDS and $ZnCl_2$ afforded a mixture of a Hauser–Kraus

Scheme 7. Completed Synthesis of Tetarimycin A (1)



adduct **1a** and the corresponding autoxidized *p*-benzoquinone, which without purification were subjected to debenzylation with $Pd/C/H_2$ followed by oxidation with DDQ to accomplish the desired target **1** in 67% yield over three steps. The spectral data (^1H NMR, ^{13}C NMR, and HRMS) of synthetic molecule **1** are in good agreement with those of tetarimycin A reported in the literature.[5a]

During the course of the total synthesis, we also took advantage of some intermediates to synthesize structurally closely related analogues (Figure 2), synthetic procedures of

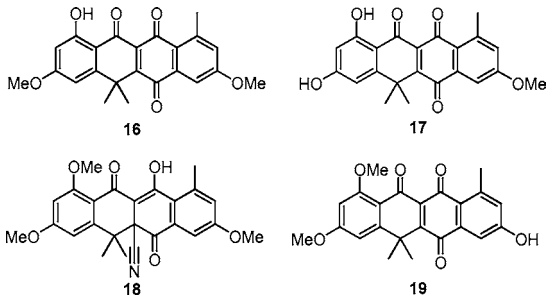


Figure 2. Synthetic analogues of tetarimycin A (**1**).

which are detailed in Supporting Information. As compiled in Table 1, preliminary structure–activity relationships (SAR) revealed that a free hydroxyl group at the C-10 position (i.e., **1** and **17**) was required for potent anti-MRSA and anti-VRE activity. As evidenced by derivatives **16**, **18**, and **19**,[14] a complete loss of activity was observed when this hydroxyl group at C-10 was methylated. The SAR also suggest that as demonstrated by many historical cases,[6] the tetracyclic skeleton of **1** might have great potential for further peripheral modifications at variable C-1, C-8, and C-18 positions to generate more druglike molecules as the free hydroxyl moiety at C-10 is retained. Also emphasized is the fact that target **1** and its analogue **17** exhibited equally potent activity against MRSA and VRE strains at a MIC = 1 and 2 μg/mL, respectively, again implying that they could serve as valuable hit compounds for novel antibiotics against drug-resistant bacteria. All tested compounds possessed a MIC > 8 μg/mL against a Gram-negative strain (ATCC 25922), and thus, we tentatively assumed that natural product **1** and its structurally related analogues should be Gram-positive-specific inhibitors. Mechanistically, tetarimycin A might inhibit an enzyme such as FabF, which has been identified as a molecular target of its congeners fasamycins A and B.[5b] Nevertheless, further studies on its mechanism of action are necessary before any conclusions can be derived.

Table 1. In Vitro Activities of Tetarimycin A (1) and Its Derivatives against Different Bacterial Strains

	MIC[b] (μg/mL) of compound					
organism[a]	**1**	**15**	**16**	**17**	**18**	**19**
Escherichia coli						
ATCC 25922	>8	>8	>8	>8	>8	>8
Staphylococcus aureus						
ATCC 29213 (MSSA)	1	>8	>8	1	>8	>8
ATCC 43300 (MRSA)	1	>8	>8	1	>8	>8
M056 (MSSA)	1	>8	>8	1	>8	>8
N216 (MRSA)	1	>8	>8	1	>8	>8
Enterococcus faecalis						
ATCC 51299 VRE	2	>8	>8	2	>8	>8

[a]*E. coli*, Gram-negative bacteria; *S. aureus* and *E. faecalis*, Gram-positive bacteria; ATCC, American Type Culture Collection; M056 and N216, clinical isolates; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; VRE, vancomycin-resistant enterococci. [b]MIC, minimum inhibitory concentration.[15]

In conclusion, the first total synthesis of tetarimycin A has been accomplished in a highly convergent manner by making use of the Hauser–Kraus annulation as a key operation. The synthetic approach demonstrated is highly flexible and promises general utility for development of natural and/or unnatural antibiotics containing a linear tetracyclic pharmacophore as seen with tetarimycin A.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02039.

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

Crystallographic data for **18** (CIF)

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Notes

The authors declare no competing financial interest.

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(14) Crystallographic data for CCDC 1406153 (**18**) also lend support to the structural elucidation of natural product **1**: $C_{25}H_{23}N_1O_6$, M_w = 433.44, monoclinic, a = 31.5153(16) Å, b = 8.7548(5) Å, c = 18.0436(10) Å, V = 4154.5(4) Å^3, space group $C2/c$, Z = 8, a total of 16928 reflections were collected in the range 1.55−26.38°. Of these, 4254 were independent; for the observed data, wR2 = 0.0996, R1 = 0.0434.
(15) The minimum inhibitory concentration (MIC) of each compound was determined using a broth microdilution method following the guidelines of the Clinical and Laboratory Standards Institute. Briefly, a 0.5 McFarland standard suspension of each test isolate was prepared using colonies from an overnight sheep blood agar plate and then diluted 1:100 in cation-adjusted Mueller−Hinton broth (CAMHB) to obtain an final inoculum of 1−1.5 × 10^6 CFU/mL. A 50 μL portion of the inoculum was then dispensed into wells containing 50 μL of test compound prepared in CAMHB containing 0.2% DMSO. The plates were then incubated at 35 °C in ambient air overnight and read at 20 and 24 h after incubation.

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