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Asymmetric Total Synthesis of the Naturally Occurring Antibiotic Anthracimycin

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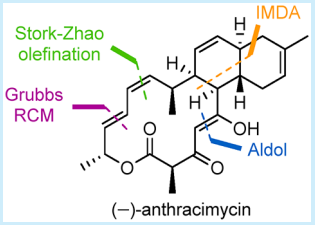
ABSTRACT: The first total synthesis of the potent antibiotic anthracimycin was achieved in 20 steps. The synthesis features an intramolecular Diels–Alder reaction to forge the *trans*-decalin moiety, and an unprecedented aldol reaction using a complex β-ketoester to provide the tricarbonyl motif. A Stork–Zhao olefination and Grubbs ring closing metathesis delivered the *E*/*Z*-diene and forged the macrocycle. The C2 configuration was set with a base-mediated epimerization, providing access to (−)-anthracimycin.



Methicillin resistant *Staphylococcus aureus* (MRSA) continues to present high infection and mortality rates globally.[1−3] Vancomycin has been a frontline antibiotic for treatment of MRSA infections for many years; however, vancomycin resistant *Staphylococcus aureus* (VRSA) and vancomycin-intermediate *Staphylococcus aureus* (VISA) infections are now relatively common.[1,2] Secondary antibiotic treatments are also demonstrating reduced efficacy due to the emergence of resistance, meaning that the discovery of antibiotics capable of tackling MRSA by novel mechanisms of action is a global health priority.[1,2] The lack of effective treatments, the high rate of resistance, and the diminished pipeline of novel antibiotics in development make MRSA a global health issue of the highest order.[2,3]

In 2013, anthracimycin ((−)-**1**) was isolated by bioassay-guided fractionation of the marine sediment derived *Streptomyces* sp. CNH365, collected off the coast of Santa Barbara, USA.[4] (−)-Anthracimycin ((−)-**1**) exhibited potent *in vitro* antibacterial activity against several MRSA strains (MIC 0.03−0.0625 μg/mL) alongside *Bacillus anthracis*, a major bioterrorism agent (UM23C1-1, MIC 0.031 μg/mL), and *M. tuberculosis* (H37Ra, MIC 1−2 μg/mL).[4−6] Anthracimycin ((−)-**1**) displayed minimal toxicity to human cells (IC_{50} 70 mg/L) and demonstrated protection against MRSA-induced mortality in mice following a single dose of 1 mg/kg.[5] Anthracimycin has also been found to suppress mTOR signaling and to be an effective inhibitor of hepatocellular carcinoma proliferation.[7] Anthracimycin ((−)-**1**) inhibits DNA and RNA synthesis through a process which does not involve DNA intercalation, though its exact mechanism of action has yet to be revealed.[5] However, the recent isolation of 2-*epi*-anthracimycin alongside (−)-**1** revealed that both natural products displayed cytotoxic activity against Jurkat cell lines through a mechanism involving G1 phase cell cycle arrest.[8] The potent *in vitro* and *in vivo* antibacterial activity of (−)-**1**, combined with its novel mechanism of action, make anthracimycin an ideal lead compound for development of novel antibiotics to combat MRSA.

Anthracimycin ((−)-**1**) is a 14-membered macrolide, whose structure was determined using a combination of NMR and X-ray crystallography.[4] Anthracimycin ((−)-**1**) possesses remarkable structural similarities to the antimalarial natural product chlorotonil A (**2**), which bears an additional C8 methyl group and C4 *gem*-dichloride moiety and exhibits the opposite configuration at all chiral centers that are common to anthracimycin (Figure 1).[9,10] Chlorotonil A (**2**) was synthesized in 2008 by Rahn and Kalesse,[11] and despite the structural similarities between natural products (−)-**1** and **2**, anthracimycin has not yet succumbed to total synthesis. Encouraged by our previous successful *endo*-selective intramolecular Diels–Alder (IMDA) route to the *trans*-decalin

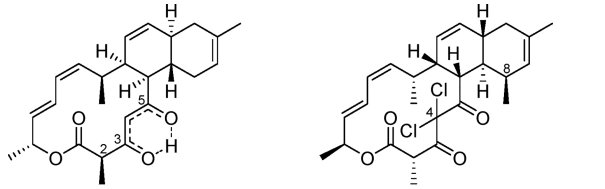


Figure 1. Structures of anthracimycin ((−)-**1**) and chlorotonil A (**2**).[4,9]

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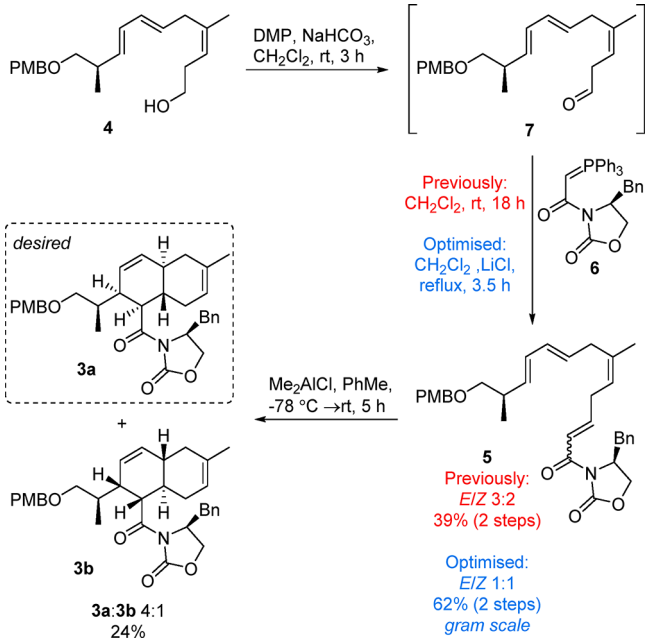
framework of anthracimycin ((−)-**1**),[12] we sought to complete the first synthesis of (−)-**1**. Completion of the total synthesis would also enable access to analogues of (−)-**1**, thereby facilitating structure−activity relationship studies of this important antibiotic.

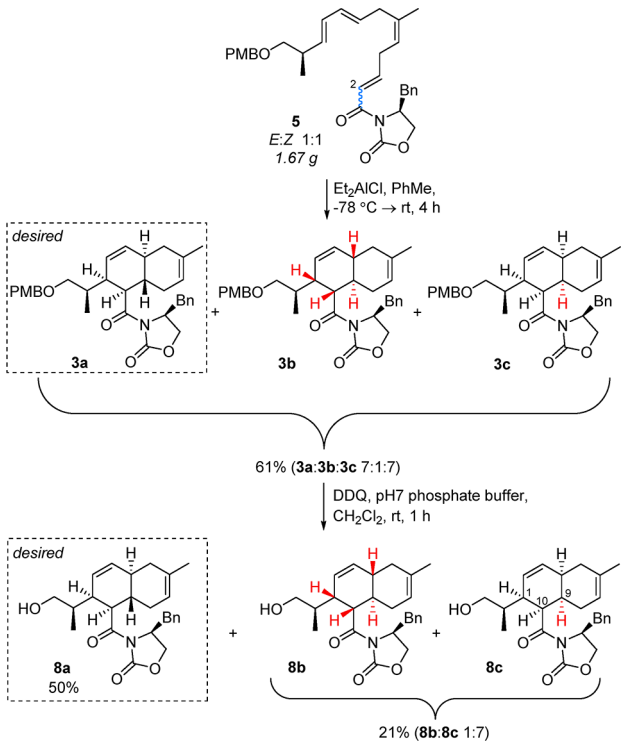
The Wittig−IMDA sequence used in our previously reported route to *trans*-decalin fragment **3a** presented a major bottleneck for our intended total synthesis, since **3a**/**b** was obtained in only 9% yield over three steps from alcohol **4**, and as a 4:1 mixture of diastereomers (**3a**:**3b**) (Scheme 1, red).[12] To obtain sufficient material to pursue construction of the macrocycle, and complete the synthesis of (−)-**1**, optimization of the Wittig−IMDA sequence was imperative. As such, Wittig reaction conditions were screened (Table S1, Supporting Information (SI)), and while the stereoselectivity of the reaction could not be improved, it was found that heating the reaction to reflux in the presence of lithium chloride provided **5** in 81% yield (6:5, *E*/*Z*). Pleasingly, these conditions were found to be amenable to gram scale [1.7 g (5.15 mmol) of alcohol **4**], yielding **5** in 62% yield over two steps (1:1, *E*/*Z*) (Scheme 1, blue). This material was then treated with diethylaluminum chloride (as opposed to dimethylaluminum chloride as used previously, owing to availability of the reagents), to provide 1.0 g of adduct **3** (61% combined yield) (Scheme 2). This gram-scale IMDA reaction of **5** yielded a partially separable mixture of three diastereomers; **3a**, **3b**, and **3c** (7:1:7). Adducts **3a** and **3b** were previously characterized and proposed to result from *endo* cycloaddition of *E*-**5** to the C2 *Si*-face and C2 *Re*-face of the dienophile moiety of **5**, respectively.[12]

In the present work, as an additional observation to our previously reported study, *Z*-**5** was completely consumed, and an additional diastereomer, **3c**, of the Diels−Alder adduct was observed. The configuration of this new diastereomer **3c** could not be determined unequivocally by 2D NMR spectroscopy due to unresolved bridgehead protons. However, it was

Scheme 1. Previously Reported Wittig−IMDA Sequence to *trans*-Decalin Fragment 3a[12]



Scheme 2. Gram Scale IMDA and PMB Deprotection Sequence



postulated that this diastereomer results from cycloaddition of *Z*-**5** proceeding via a similar mechanism to **3a** (*endo*, C2 *Si*-face addition) affording *cis*-fused decalin **3c**.[13,14] It remains unclear why the IMDA reaction of *Z*-**5** was not observed originally. While diastereomers **3a**−**c** were only partially separable, it was found that following *p*-methoxybenzyl (PMB) deprotection of the mixture, desired alcohol **8a** could be separated from diastereomers **8b** and **8c**, thus providing a pure sample with the same stereochemical configuration as anthracimycin ((−)-**1**).

With gram scale access to alcohol **8a** established, attention was turned to its elaboration to the macrocycle of anthracimycin ((−)-**1**). It was envisaged that the tricarbonyl moiety could be forged using an aldol reaction or Claisen condensation with *β*-ketoester **9**, while the *E*,*Z*-diene motif could feasibly be installed using a Grubbs ring closing metathesis (RCM) (Scheme 3). As the C2 methyl group of (−)-**1** was expected to be readily epimerizable due to the different conformational stabilities of the C2 epimers, the configuration at this position would be set following construction of the macrocycle.[11] The requisite diene **10** or **11** could be installed by Stille coupling of tributyl(vinyl)tin with *Z*-vinyl iodide **12** or **13** which in turn could be obtained via a Stork−Zhao olefination of aldehyde **14** or **15**. Access to **14** or **15** could feasibly be achieved following auxiliary cleavage and functional group manipulations of alcohol **8a**.

Given that our planned construction of the macrocycle hinged on the coupling of *β*-ketoester **9** to a decalin containing fragment, it was first deemed prudent to explore its accessibility.

Known *β*-ketoester **9** was found to be inaccessible via transesterification of ethyl 2-methylacetoacetate with commercially available (*R*)-3-buten-2-ol using Gilbert and Kelly's procedure.[15] It was suspected that the volatility of (*R*)-3-

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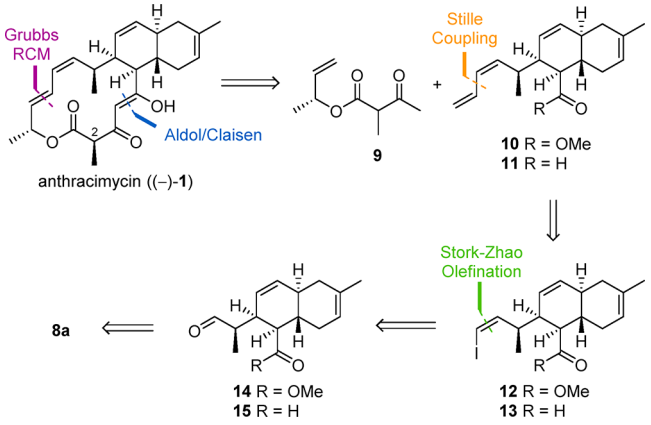
Scheme 3. Retrosynthetic Analysis of the Macrocycle of (−)-1

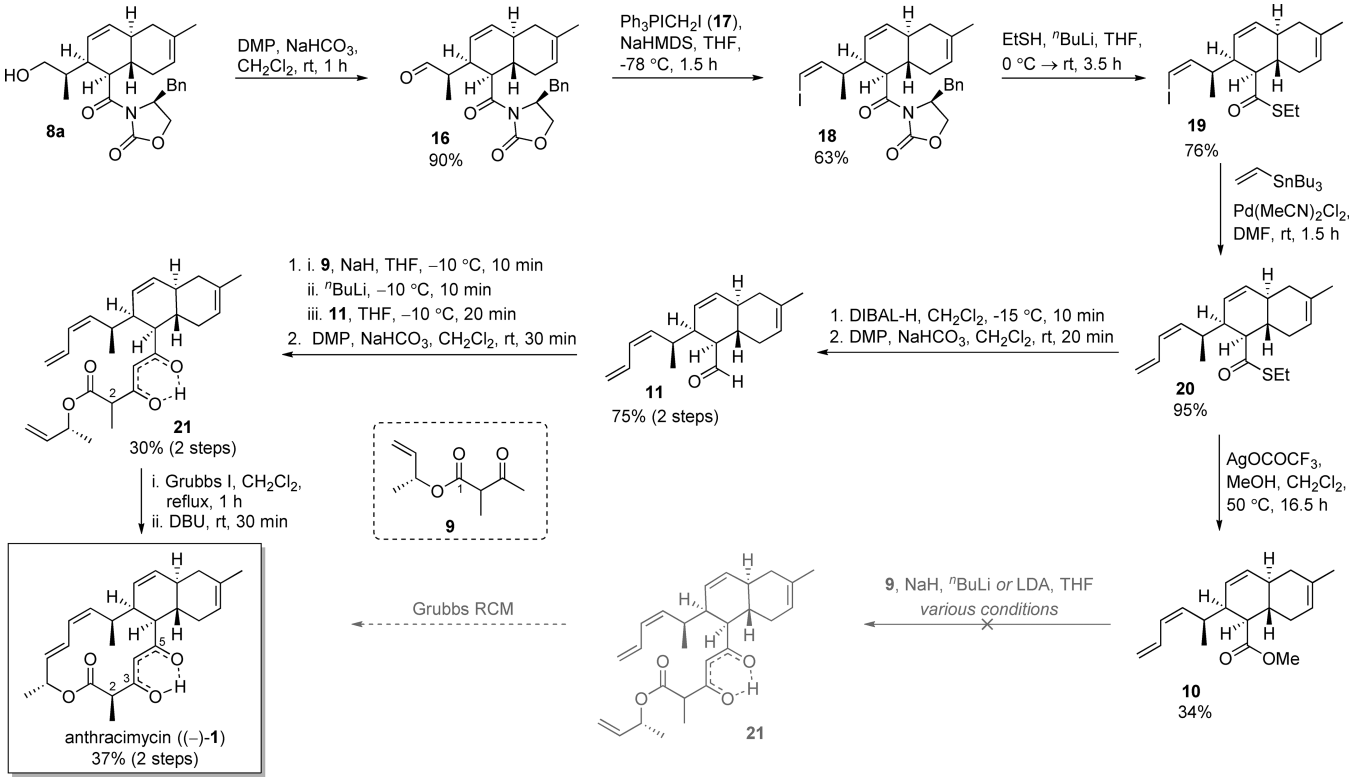


buten-2-ol was impeding smooth transesterification under the high temperatures required; thus, an alternative method to obtain **9** was sought. Ethyl 2-methylacetoacetate was therefore hydrolyzed to the crude carboxylic acid, which was then directly coupled with (*R*)-3-buten-2-ol at room temperature to provide ester **9** in moderate yield (see SI for further details).

With *β*-ketoester **9** in hand, attention turned to elaboration of alcohol **8a**. Oxidation of **8a** with Dess-Martin periodinane (DMP) afforded aldehyde **16** (Scheme 4). Pleasingly, Stork–Zhao olefination of **16** with iodomethylphosphonium salt **17**[16] at −78 °C yielded the desired *Z*-vinyl iodide **18** in 63% yield. Direct cleavage of the auxiliary of **18** to an ester or aldehyde moiety was then attempted, however, the desired methyl ester could not be obtained using dimethyl carbonate and sodium methoxide under several conditions, returning only starting material **18**. Meanwhile, treatment of **18** with diisobutylaluminum hydride (DIBAL-H) failed to provide direct access to an aldehyde, instead yielding an intractable mixture of products. Carboxylic acid and Weinreb amide derivatives were also inaccessible under numerous conditions; however, ethanethiolate was pleasingly found to mediate auxiliary cleavage of **18**, providing thioester **19** in good yield.[17] Stille coupling of **19** with tributyl(vinyl)tin proceeded smoothly, affording diene **20** in excellent yield. Diene **20** was then converted to methyl ester **10** using silver trifluoroacetate;[18] however, treatment of methyl ester **10** with the dianion of **9** (generated either using NaH and ^{n}BuLi, or lithium diisopropylamide) resulted only in returned starting material **10** under a range of conditions.

It was conceived that a more electrophilic coupling partner, such as aldehyde **11**, could enable union of these two fragments. Fukuyama reduction[17] of thioester **20** gave rise to a complex, inseparable mixture, presumably resulting from undesired reduction of the diene moiety. Treatment of thioester **20** with DIBAL-H also failed to directly provide aldehyde **11**, although the corresponding alcohol was delivered as the major product, with trace formation of desired aldehyde **11**. This was deemed inconsequential however, since treatment of the crude mixture with DMP pleasingly afforded desired aldehyde **11** in 75% yield over two steps. Aldol reaction of aldehyde **11** with the dianion of **9** resulted in consumption of the starting materials; however, NMR and TLC analysis of the crude material were complicated by the presumed presence of four diastereomeric aldol products. Pleasingly, immediate treatment of this crude mixture of diastereomers with DMP afforded tricarbonyl **21** as a 1:1 mixture of C2 epimers, albeit in moderate yield (30% over two steps). Most examples of aldol reactions with dienolates in the academic literature

Scheme 4. Synthesis of Anthracimycin ((−)-1) from Alcohol 8a



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employ simple β-ketoesters, such as ethyl 2-methylacetoacetate and ethyl acetoacetate, or their methyl or *tert*-butyl ester counterparts.[19−23] To the best of our knowledge, this is the sole example of an aldol reaction using a β-ketoester with a more complex C1 substituent in peer-reviewed literature. While attempts to improve the yield of **21** were disappointing, sufficient material was nevertheless obtained to attempt the final metathesis.

Treatment of triketone **21** with Grubbs I catalyst in dichloromethane under reflux resulted in rapid consumption of the starting material. NMR analysis of the crude material indicated the presence of anthracimycin ((−)-**1**) as a 1:1 mixture with its C2 epimer (see SI for details). Pleasingly, subsequent treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) effected epimerization of 2-*epi*-**1**. Additionally, it was found this could be conducted as a one-pot procedure, whereby addition of DBU to the metathesis reaction mixture following consumption of **21** provided anthracimycin ((−)-**1**) in 37% yield as a single diastereomer. Comparison of the ^{1}H and ^{13}C NMR data of synthetic (−)-**1** showed excellent agreement with those reported for authentic anthracimycin ((−)-**1**) (Tables S2 and S3, SI).

It is worth noting that 2-*epi*-**1** was stable in organic solvents and to simple aqueous workup under pH neutral conditions. As Tomoda and co-workers reportedly detected 2-*epi*-**1** following extraction (without altering the pH) of the *Streptomyces* sp. culture broth with organic solvents,[8] we do not have any reason to believe 2-*epi*-**1** is an artifact of accidental epimerization of (−)-**1** during the isolation process.

To conclude, the first asymmetric total synthesis of anthracimycin ((−)-**1**) was achieved in 20 steps, using an unprecedented aldol reaction with a complex C1 substituted β-ketoester to furnish the tricarbonyl fragment, and a one-pot Grubbs RCM/base-mediated epimerization to forge the macrocycle and set the C2 configuration. Studies of the antibacterial activity and mechanism of action of (−)-**1** are ongoing and will be disclosed in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c01913.

Detailed experimental procedures, full spectroscopic data for all new compounds and NMR comparison tables for authentic and synthetic anthracimycin (PDF)

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Notes

The authors declare no competing financial interest.

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