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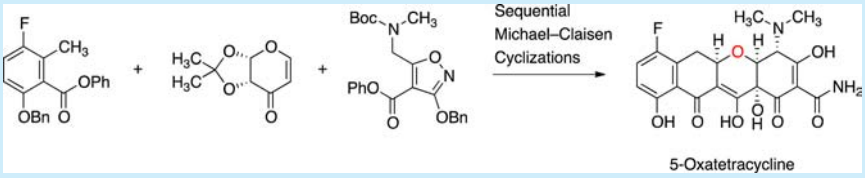
Diastereoselective Michael–Claisen Cyclizations of γ-Oxa-α,β-unsaturated Ketones en Route to 5-Oxatetracyclines

Fan Liu, Peter M. Wright, and Andrew G. Myers*

Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138, United States

Supporting Information



ABSTRACT: 5-Oxatetracyclines were synthesized from D-arabinose using sequential Michael–Claisen cyclization reactions via a 5-oxa-AB enone substrate. The 5-oxatetracyclines were found to have poor stability in aqueous buffer (pH 7.4, 37 °C) and showed little to no inhibition of bacterial growth (*S. aureus*, *E. coli*).

Coupling of various *o*-toluate phenyl esters (D-ring precursors) with enone **1** (AB enone)[1] by a highly diastereoselective Michael–Claisen (MC) cyclization reaction[2] provides the key step in a short and general route to tetracycline antibiotics, broadly defined (Figure 1).[3,4] As part of an

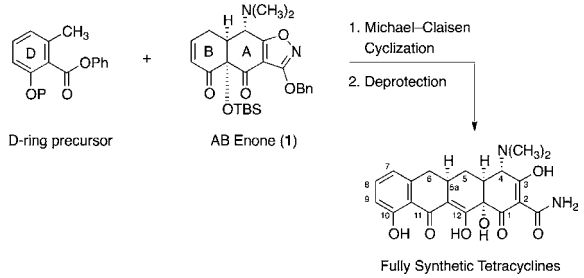
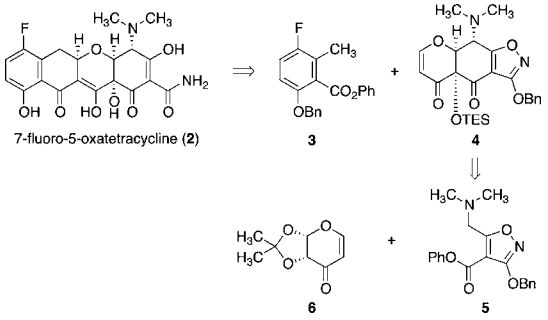


Figure 1. Michael–Claisen cyclization reaction of D-ring precursors and the AB enone (**1**) provides fully synthetic tetracycline antibiotic candidates with diverse substitution patterns; most are molecules that would be inaccessible through semisynthesis.

exploration of modifications of C5 within the B ring of tetracyclines, we were led to consider replacing the C5 methylene group with an oxygen atom. This was of interest because it would give rise to a molecule of nearly equal molecular weight but greater polar surface area (PSA = 161.4 Å^2 (5 = CH_2) vs PSA = 170.6 Å^2 (5 = O)), which has been suggested to correlate with improved antibiotic activity against infections caused by Gram-negative bacteria, an area of current unmet medical need.[5,6]

The 7-fluoro-5-oxatetracycline analogue **2** was identified as an interesting specific target for initial consideration in light of the favorable effects of 7-fluoro substitution upon antibiotic activity within fully synthetic (5-carba)tetracyclines.[3d] Applying a retrosynthetic disconnection paralleling that of Figure 1 to the novel target **2** gave rise to the known D-ring precursor **3** and the novel 5-oxa-AB enone **4** (Scheme 1). The proposed MC

Scheme 1. Retrosynthetic Disconnection of 5-Oxatetracycline Analogue 2 by Sequential MC Cyclizations



coupling of **3** and **4** was recognized to be potentially quite different from cyclization of the 5-carba-AB enone **1** in terms of both its feasibility and the stereochemical outcome, and it was therefore of interest to us to explore this transformation from a basic chemistry perspective. We imagined further simplification of subtarget **4** by a second MC transform, depicted in Scheme 1, giving rise to the chiral masked pyrone equivalent **6** and the known isoxazole phenyl ester **5** as proposed reactants.[7] The acetonide group within **6** was considered to provide a strong biasing element for trans nucleophilic addition (γ-pyrone itself, of course, would provide no stereochemical biasing element).

Dihydropyrone **6** is available in optically pure form in three steps from D-arabinose.[8,9] Addition of the masked pyrone B-ring precursor **6** (1 equiv) to the sodium enolate of isoxazole ester **5** (2.00 equiv, formed using sodium hexamethyldisilazide as the base) at −78 °C followed by warming to −15 °C afforded the cyclized product **7** in 40% yield after purification by flash column chromatography (>20:1 dr; Scheme 2A). The use of phenyl ester **5** as the Claisen condensation partner was crucial to the success

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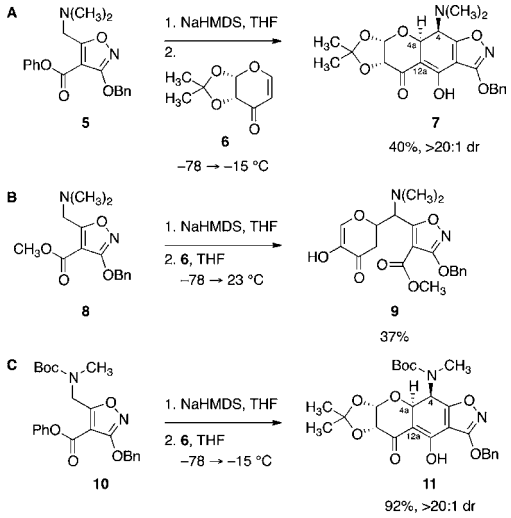
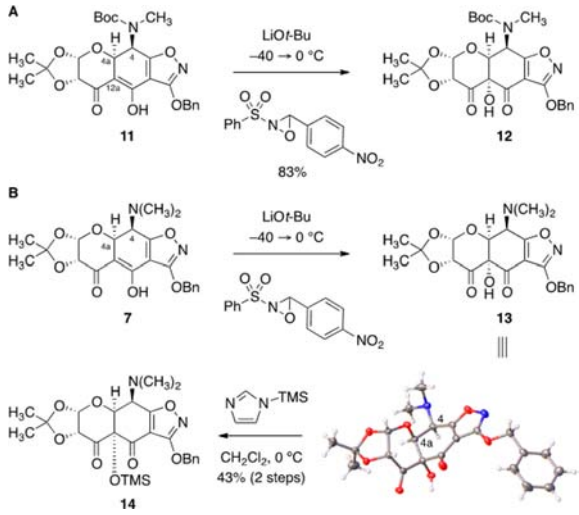
Scheme 2. MC Cyclization Reactions of 6



Scheme 3. (A) C12a Oxygenation of MC Adduct 11; (B) Synthesis of Oxa-AB Enone Precursor 14 from MC Adduct 7



of the reaction; when the corresponding methyl ester **8** was used instead, the noncyclized fragmentation product **9** (Scheme 2B; stereochemistry not determined) was observed to be the primary adduct (37% yield). A much more efficient MC cyclization reaction occurred when the masked pyrone **6** (7.00 g, 41.1 mmol, 1 equiv) was added to the sodium enolate of *N*-Boc phenyl ester **10**[9] (2.00 equiv, formed using sodium hexamethyldisilazide as the base) at −78 °C followed by warming after 1.5 h to −25 °C, affording the product **11** in 92% yield (19.5 g, >20:1 dr) after an aqueous workup and purification by flash column chromatography. Evidently, the *tert*-butyl carbamate function of the modified isoxazole coupling partner **10** greatly improved the efficiency of the cyclization reaction. This observation is consistent with our prior finding that *o*-toluates that bear anion-stabilizing groups typically undergo MC cyclization reactions more efficiently than those that do not.[3b,10] The observed facial selectivity is consistent with nucleophilic addition trans to the sterically demanding isopropylidene substituent. The adjacent stereogenic centers at C4 and C4a were formed with complete diastereocontrol, but the *N*,*N*-dimethylamino group was epimeric relative to natural tetracyclines, necessitating the development of an epimerization protocol (vide infra).

We first used to advantage the improper stereochemistry at C4 to direct oxygenation at C12a with natural stereochemistry. Thus, addition of a catalytic amount of lithium *tert*-butoxide (0.20 equiv) to a solution of 3-(4-nitrophenyl)-2-(phenylsulfonyl)oxaziridine (1.30 equiv) and adduct **11** (1.98 g, 3.85 mmol, 1 equiv) at −40 °C followed by warming to 0 °C afforded amino alcohol **12** as the only observable product in 83% yield after purification by flash column chromatography (Scheme 3A).[7,11] Oxygenation at C12a of the MC adduct **7** could also be carried out with similar efficiency to give compound **13**, which upon treatment with trimethylsilylimidazole (5.00 equiv) afforded trimethylsilyl ether **14** in 43% yield over two steps. The intermediate amino alcohol **13** could be recrystallized from ethyl acetate−hexanes, providing crystals suitable for X-ray analysis. The solid-state structure we obtained (Scheme 3B) confirmed the stereochemical outcomes of both the MC cyclization and C12a-oxygenation reactions.

To complete the synthesis of 5-oxa-AB enone **4**, we next investigated epimerizations of the C4 stereogenic center within intermediates **12** and **14**. Prior studies have shown that the 4-position of tetracyclines is prone to epimerization, often under mild conditions.[12,7] In all examples of which we are aware, tetracyclines and their analogues with the natural C4-*S* configuration are thermodynamically more stable than the C4-*R* epimers (tetracycline itself exists as a 1.5:1 mixture of 4*S* and 4*R* epimers, respectively, at equilibrium in 1 M aqueous sodium dihydrogen phosphate/methanol at 25 °C[12a,b]). When we attempted epimerization of intermediate **14** under similar conditions, however, we obtained solely quinone **15** (70% yield; Scheme 4A). Use of *N*-Boc-methylamine **12** as the substrate likewise led to hydroquinone **16** (50% yield). Inspection of the X-ray structure of intermediate **13** (depicted in Scheme 3B) reveals that the molecule adopts a conformation in which the C4a−O and C4−H bonds are antiperiplanar, an alignment that potentially facilitates an eliminative fragmentation that is not possible in the 5-carba analogues; this is likely the case for its trimethylsilyl ether **14** and *N*-Boc methylamine **12** as well, since comparison of coupling constants from ^{1}H NMR spectra of **13**, **14**, and **12** suggest that they adopt similar conformations. Of equal or greater concern is the fact that computational analyses of **13** and **14** and their "natural" C4-*S* epimers suggested that we may have been attempting a contrathermodynamic epimerization; i.e., **13** was calculated to be more stable than its 4*S* epimer (ΔG_{calc} = +0.6 kcal/mol).[9] This is in contrast to 5-carba-AB enone **1**, which was computed and observed to be significantly more stable than its 4*R* epimer (the observed equilibrium ratio of des-TBS-(4*S*)-**1** and its 4*R* epimer is 13:1, respectively, in 4 M NaH_2PO_4/methanol/THF solution at 60 °C;[7] ΔG_{calc} = −2.9 kcal/mol[9]). Therefore, to effect the desired epimerization, we developed an alternative strategy, which is depicted in Scheme 4B. Treatment of *N*-Boc methylamine **12** with trifluoroacetic acid at 0 °C afforded amino alcohol **17** in 72% yield. Heating of a solution of **17** in benzene at 45 °C in the presence of benzaldehyde (10.0 equiv) and acetic acid (2.00 equiv) afforded three products, which were separated by flash column chromatography: recovered **17** (38%), its 4*S* epimer **18** (31%), and benzylidene aminal **19** (~17%, impure). Thus, the ratio of the desired 4*S* epimer **18** to the undesired 4*R* epimer **17** was 1:1.2, which we believe to be the equilibrium distribution of products. Separately, benzylidene aminal **19** could be hydrolyzed to give a 10:1 mixture of **18** and **17**, respectively (81% combined isolated yield). A simpler and improved epimerization protocol was developed, employing a larger excess of acetic acid (20.0

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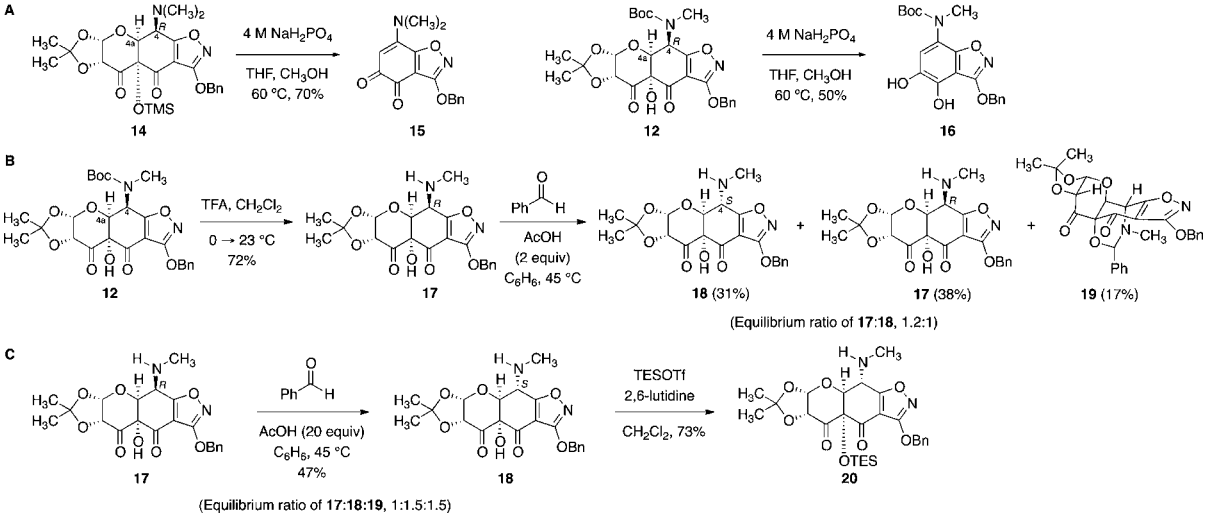
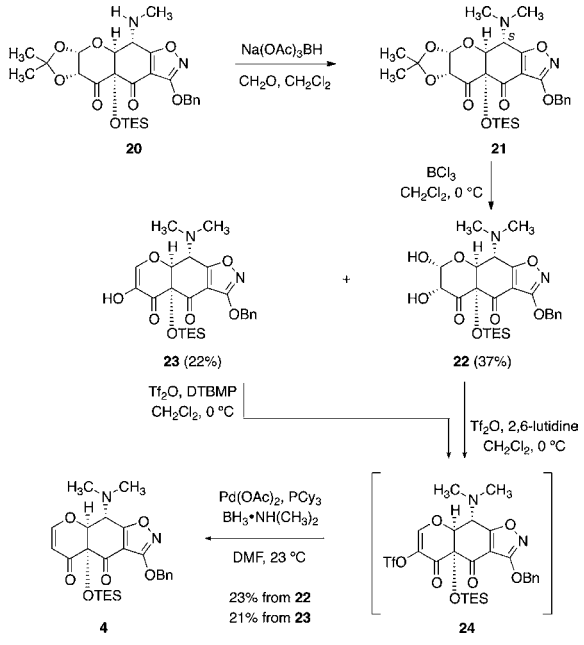
Scheme 4. (A) Attempted Epimerizations of 14 and 12 Led to Fragmentation To Give Quinone 15 and Hydroquinone 16, Respectively; (B) Epimerization of the C4 Stereogenic Center under Mildly Acidic Conditions in the Presence of Benzaldehyde; (C) Epimerization of 17 on a Gram Scale and Triethylsilyl Ether Protection of the Tertiary Hydroxyl Group



equiv) to give a 1:1.5:1.5 mixture of **17**, **18**, and **19** (Scheme 4C). This was directly concentrated and subjected to purification by column chromatography (leading to lysis of **19** in situ), providing **18** in 47% yield (20:1 mixture with **17**, 3.70 g scale). The trace amount of **17** was removed in the next step, where only the desired 4*S* epimer **18** underwent triethylsilylation (TESOTf, 2,6-lutidine) to afford pure triethylsilyl ether **20** in 73% yield.

Triethylsilyl ether **20** was transformed into 5-oxa-AB enone **4** in four steps (Scheme 5). *N*-Methylation was achieved upon

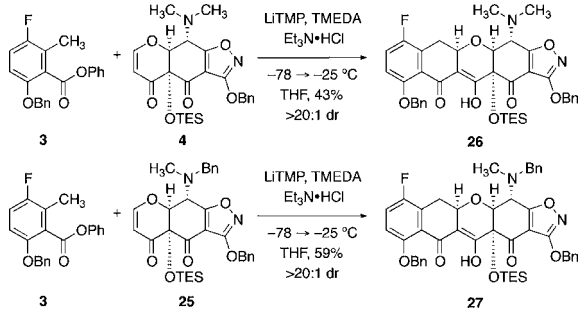
Scheme 5. Synthesis of 5-Oxa-AB Enone 4



subjection of a solution of **20** in dichloromethane to aqueous formaldehyde (4.00 equiv) and sodium triacetoxyborohydride (12.0 equiv) at 0 °C to afford the (4*S*)-*N*,*N*-dimethylamino product **21**, contaminated with ∼6% of its 4*R* epimer (not shown). The mixture was treated with boron trichloride (1.50 equiv) in dichloromethane at 0 °C for 0.5 h. The acetonide protective group was cleaved while preserving the triethylsilyl ether function, affording separately, after purification by flash column chromatography, hemiacetal **22** (37%) and its dehydration product **23** (22%). Both products were transformed into the common enol triflate **24** in the presence of triflic anhydride (3.50 equiv for **22** and 2.00 equiv for **23**) and an appropriate base (5.00 equiv of 2,6-lutidine for **22** and 4.00 equiv of 2,6-di-*tert*-butyl-4-methylpyridine for **23**) (Scheme 5). Enol triflate **24** was reduced directly, without purification, using borane−dimethylamine complex[13] (5.00 equiv) in the presence of palladium acetate (0.80 equiv) to afford 5-oxa-AB enone **4** (23% yield from hemiacetal **22** and 21% yield from enol **23**).

We next investigated construction of the C ring of 5-oxatetracyclines by the proposed MC cyclization reaction (Scheme 6). Deprotonation of the D-ring precursor **3** (2.10

Scheme 6. MC Cyclizations of 4 and 25 with D-Ring Precursor 3 To Afford 5-Oxatetracycline Derivatives 26 and 27



equiv) with lithium tetramethylpiperidide (2.40 equiv) in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (7.00 equiv) and triethylamine hydrochloride (0.03 equiv)[3d] at −78 °C produced a bright-red solution. Addition of enone **4** at −78 °C followed by warming to −25 °C over 1.5 h provided the desired cyclization product **26** as a single diastereomer in 43% yield. The analogous transformation was also achieved using 5-oxa-AB enone **25**[9] as the substrate to afford the cyclization product **27** (59%). Both cyclization products were stereochemically homologous with natural tetracyclines, as indicated by NOE

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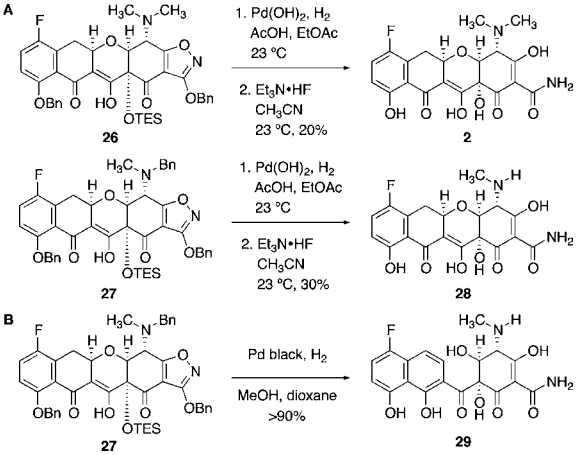
analysis. This stereochemistry arises from addition of the *o*-toluate anion along a pseudoaxial trajectory, opposite the bulky triethylsilyl ether substituent, and is the same stereochemical outcome we had observed in the earlier "5-carba" series.

After considerable experimentation, a workable if modest-yielding sequence for deprotection of the 5-oxatetracycline precursors **26** and **27** was found (Scheme 7A). Hydrogenolysis

Scheme 7. (A) Synthesis of 5-Oxatetracyclines 2 and 28; (B) Debenzylation and B-Ring Fragmentation of 27



of **26** and **27** in ethyl acetate containing acetic acid (20.0 equiv) in the presence of palladium hydroxide (4.00 equiv) and hydrogen gas (1 atm) at 23 °C for 10 min afforded the intermediate *O*- and *N*-debenzylated triethylsilyl ether products (not depicted). These were not purified but were treated with triethylammonium fluoride (75.0 equiv) in acetonitrile at 23 °C for 15 min to afford 7-fluoro-5-oxaminocycline **2** in 20% yield (two steps) and 7-fluoro-4-methylamino-5-oxaminocycline **28** in 30% yield (two steps) after purification by reversed-phase HPLC. After its isolation, 5-oxatetracycline **2** was found to have poor stability in aqueous phosphate buffer (pH 7.4); the half-life of **2** was measured by ^{1}H NMR analysis to be ca. 6.5 h in 1 M aqueous KD_2PO_4 solution at 37 °C (no major decomposition products were discernible). Both compounds **2** and **28** were tested against wild-type *Escherichia coli* and *Staphylococcus aureus* strains and exhibited antibiotic activities that were markedly inferior to those of their 5-carba analogues: the methylamine analogue **28** was found to be inactive (MIC > 32 μg/mL) against both *S. aureus* (Newman) and *E. coli* (MC 4100); 5-oxatetracycline **2** was inactive against *E. coli* (MC 4100) but showed modest activity (MIC = 16 μg/mL) against *S. aureus* (Newman).[9] At this point, we believe that the poor antibiotic activities of 5-oxatetracyclines **2** and **28** may reflect in part their inherent instabilities under aqueous conditions. Parenthetically, when hydrogenolysis of the MC annulation product **27** was conducted in methanol without the addition of acetic acid, dihydroxynaphthalene **29** was obtained as the only observable product (>90%), the result of an apparent eliminative fragmentation reaction (Scheme 7B).

We have shown that cross-conjugated vinylogous ester substrates undergo stereocontrolled Michael−Claisen cyclization reactions with *o*-toluate phenyl esters. We also discovered a novel means to effect epimerization of the C4 stereogenic center of 5-oxa-AB enones, overcoming an inherent propensity for fragmentation under basic conditions. This enabled a stereoselective synthesis of 5-oxatetracyclines, which were found to have poor stability under physiological conditions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03491.

Procedures and spectroscopic data (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*myers@chemistry.harvard.edu

ORCID

Andrew G. Myers: 0000-0001-9602-6915

Notes

The authors declare no competing financial interest.

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