

A Concise Synthesis of Carolacton

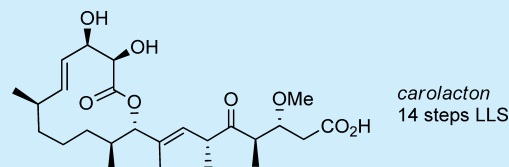
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S Supporting Information

ABSTRACT: A synthesis of carolacton, a myxobacterial natural product that has profound effects on *Streptococcus mutans* biofilms, is reported. The synthesis proceeds via a longest linear sequence of 14 steps from an Evans β -ketoimide and enabled preliminary evaluations of the effects of late-stage intermediates on *S. mutans* biofilms. These studies suggest that further investigations into carolacton's structure–function relationships are warranted.



Dental caries is a chronic health problem that impacts the majority of the global population.¹ Although culture-independent sequencing has illuminated the complexity of the human oral microbiome, *Streptococcus mutans* remains widely regarded as a primary etiological agent in caries.^{2,3} This facultatively anaerobic, microaerophilic, Gram-positive, bacterium excels in the complex environment of the oral cavity where stresses including low pH and an oxidative environment are coupled to variable salivary flow and carbohydrate supply.⁴ The physiologic adaptations of *S. mutans* to these pressures provide a competitive advantage versus non-cariogenic commensals that underpins the establishment and progression of caries.⁵

Broad-spectrum agents such as fluoride and chlorhexidine represent the state-of-the-art in anticariogenic preventative treatment, although the efficacy of chlorhexidine remains debated.⁶ Significant perturbation of the oral microbiome accompanies their use,⁷ and there is increasing evidence that such imbalances can favor pathogens versus commensals.⁸ Compounds that are species- or phenotype-selective would allow exploration of the hypothesis that targeting smaller elements of complex communities may be advantageous.⁹ In this light, we were attracted to a report describing the structure and activity of the myxobacterial metabolite carolacton (**1**, Figure 1).¹⁰ Initial characterizations showed that carolacton causes 35–66% cell death within *S. mutans* biofilms at concentrations ranging from 5 to 25 ng/mL. Remarkably, planktonic cultures of *S. mutans* were not affected by carolacton, nor were a variety of other bacteria.

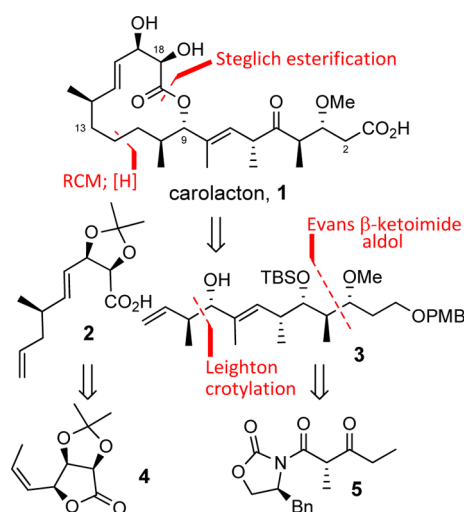


Figure 1. Carolacton structure and an overview of key features in the synthesis that traces to lactone **4** and β -ketoimide **5**.

The structure and activity of carolacton has attracted much scientific interest. Kirschning has completed a total synthesis, and several subunit syntheses have also been reported.¹¹ In addition, a number of investigations have pursued in more detail the biology of carolacton's effects on *S. mutans* and the question of its mode-of-action.¹² While there is accord on the differential activity of carolacton for biofilm versus planktonic cultures of *S. mutans*, coherency around mode-of-action has yet to emerge.

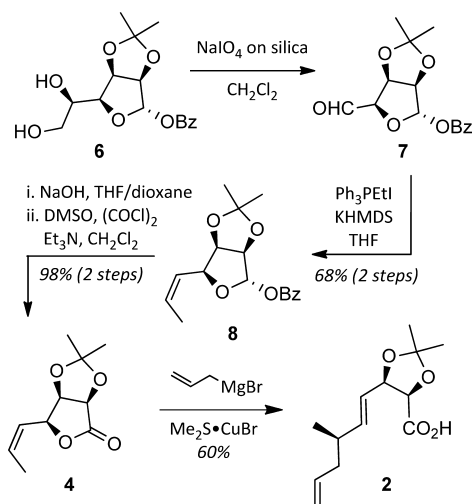
In this Letter, we describe a straightforward synthesis of carolacton that is amenable to an extensive exploration of structure–function relationships. Key elements of the synthesis are shown in Figure 1, including an assembly strategy that leverages macrocycle formation by esterification and a

Received: January 1, 2014

ring-closing metathesis, followed by a selective olefin hydrogenation.

The synthesis of the C12–C19 acid **2** began with known gulonolactone-derived diol **6**¹³ (available in 4 steps, 62% overall from D-gulonolactone, Scheme 1). Oxidative cleavage of the diol with sodium periodate on silica¹⁴ gave aldehyde **7**, which was subjected to a Z-selective Wittig olefination to yield alkene **8**. Removal of the benzoate, followed by Swern oxidation, provided the key lactone **4**. In accord with Trost's studies,¹⁵ exposing **4** to allylmagnesium bromide in the presence of CuBr·SMe₂ resulted in S_N2' opening of the lactone to produce acid **2** in 60% yield and as a single diastereomer.

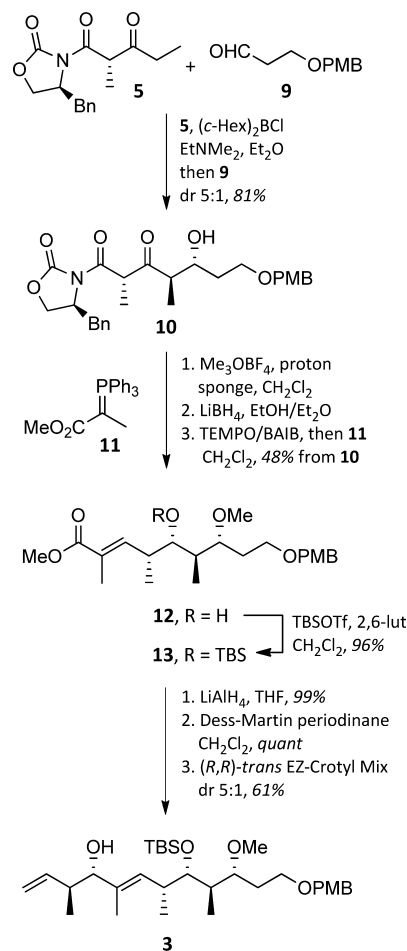
Scheme 1. Synthesis of the C12–C19 Subunit 2 from Gulonolactone-Derived Diol 6



The stereochemically complex C1–C11 domain was assembled as shown in Scheme 2. Enolization of the Evans β -ketoimide **5** under conditions favoring formation of the (*E*)-enol borane, followed by reaction with aldehyde **9**, produced **10** in 81% yield and with a dr of 5:1.¹⁶ Separation of the diastereomers was not possible. Conveniently, the subsequent three-step sequence of methylation, reduction, and oxidation–Wittig olefination not only proceeded in 48% overall yield but also allowed for isolation of enoate **12** as a single diastereomer. Silylation of the alcohol gave TBS ether **13**. Introduction of the final two stereocenters was readily achieved by conversion of the ester to an aldehyde and application of Leighton's (*R,R*)-*trans* EZ-CrotylMix to give alcohol **3** (61%, 5:1 dr).¹⁷

With routes to the two key subunits secured, it was possible to investigate the end-game for the synthesis, which began with a Steglich esterification to connect acid **2** and alcohol **3**, producing a near-quantitative yield of **14** (Scheme 3). Reaction of **14** with 5.5 mol % of the second-generation Grubbs catalyst was unsurprisingly selective for the terminal olefins and led to lactone **15** in 76% yield. Our plans demanded a selective hydrogenation of the $\Delta^{11,12}$ olefin at this juncture, a strategy predicated on conformational analysis of **15** suggesting peripheral addition would favor the desired reduction.¹⁸ Pleasingly, this was borne out under operationally simple conditions: subjecting **15** to 5% Pd/C in EtOH under a hydrogen balloon resulted in selective reduction of the $\Delta^{11,12}$ olefin as well as removal of the PMB group (70%

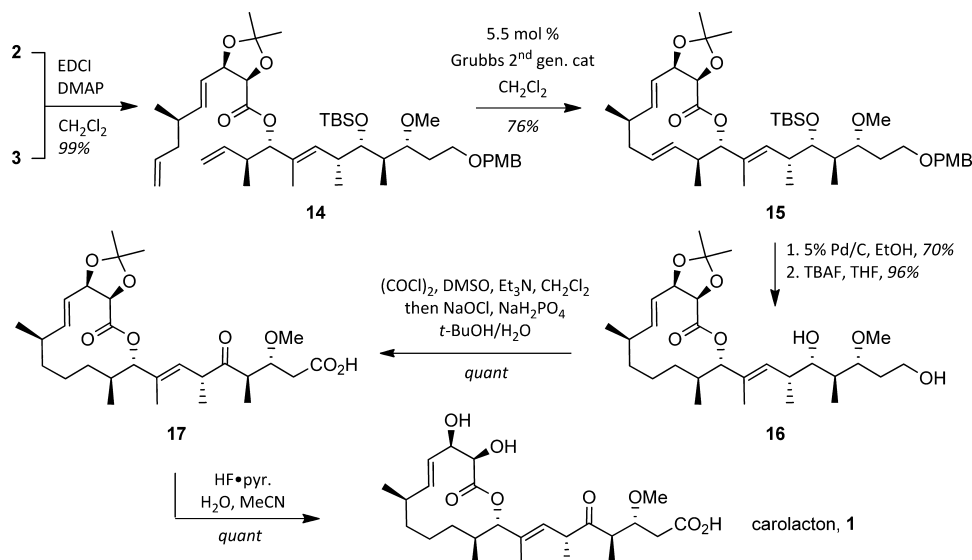
Scheme 2. Synthesis of the C1–C11 Subunit 3



yield). Removal of the TBS ether with TBAF produced diol **16** in 96% yield and set the stage for a simultaneous oxidation of the primary and secondary alcohols under Swern conditions and subsequent Lindgren–Pinnick oxidation to yield keto-acid **17**. Removal of the acetonide yielded synthetic carolacton (**1**). Analytical characteristics of this material were in full accord with data reported for the natural product.

Our initial synthetic venture provided multimilligram amounts of intermediates in the late stages of the synthesis. We capitalized on this to perform a preliminary biological evaluation of compounds **16**, **17**, and carolacton against *S. mutans* UA159 grown planktonically and also within a biofilm. None of the compounds displayed any inhibitory activity against planktonic cells at concentrations <250 μ M. In the case of carolacton, this is in accord with previous reports.^{12b} However, when the compounds were incubated with *S. mutans* in the presence of biofilm-inducing media, dramatic morphological and architectural changes to the biofilm matrix were observed (Figure 2). Confocal microscopy with LIVE/DEAD staining demonstrated that carolacton (>500 nM) and **16** (>62.5 μ M) significantly affected both the integrity and morphology of the biofilm matrix when compared to the DMSO control. In contrast, compound **17** had no observable effect at concentrations <250 μ M. While the reasons for the significant difference in activity between compound **16** and **17** remain unclear at this time, our preliminary investigations provide credence to the suggestion that a minimal set of features needed for activity in the carolacton family might be

Scheme 3. Subunit Coupling and Completion of the Synthesis



readily identified by synthesis. Future efforts will focus on expanding the understanding of structure–function relationships in the carolacton class, as well as obtaining a detailed understanding of the mechanism of action of carolacton on *S. mutans* biofilms.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by Yale University (A.J.P.), in part by the NIH (CA110246, A.J.P.), and Temple University (W.M.W.). We thank Professor Bettina A. Buttaro (Temple University) for advice and assistance with confocal microscopy of *S. mutans* biofilms, and Dr. Jennifer A. Poole (Yale University) for mass spectrometry assistance.

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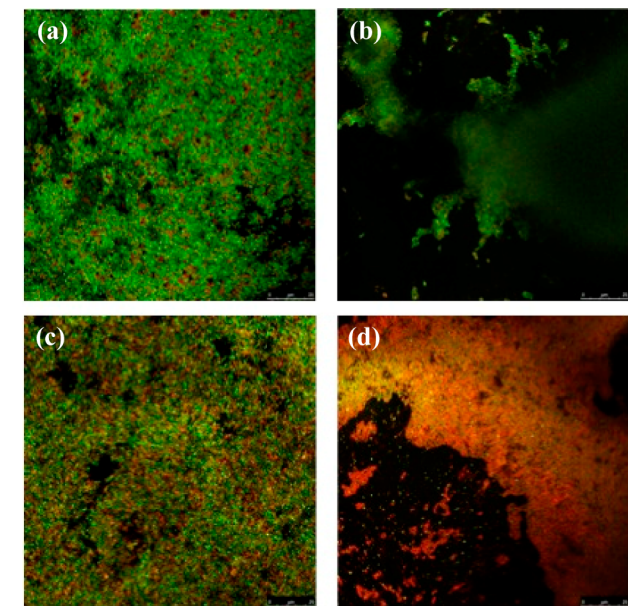


Figure 2. Confocal microscopy imaging of *S. mutans* UA159 biofilm cells treated with 250 μ M (a) DMSO, (b) carolacton, (c) 17, and (d) 16.

ASSOCIATED CONTENT

Supporting Information

Experimental methods and data, including spectra, for new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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