

# Synthetic Studies towards the Cadiolides

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## Abstract

A novel laboratory synthesis of cadiolides was studied. Cadiolides are secondary metabolites from marine ascidians and tunicates, and they were found to have potent activity against Gram positive bacteria like Methicillin Resistant *Staphylococcus Aureus* (MRSA). Previous laboratory synthesis of Cadiolide B has been accomplished by 6 steps and with a 42% overall yield. However, this approach was unable to provide Cadiolide C. In this research, a synthetic approach towards Cadiolide B was planned with cheaper and more readily-available reagents, which could in principle also be adapted to construct Cadiolides A, C, D, and E. The new approach involves a key intra-molecular 5-exo-trig-cyclization step on a beta-keto-ester of a dibenzylmethanol derivative. This research focused on to synthesis of the beta-keto-ester, but unexpected challenges were encountered along the way. The original approach involving acylated Meldrum's acid will be modified to a Claisen condensation. The synthesis of the dibenzylmethanol intermediate also needs to be optimized, and a Claisen condensation approach to this intermediate is also currently in progress.

## Introduction

Ascidians and Tunicates are marine invertebrate animals. Widespread in the world's oceans, there are thousands of species and most live in shallow water. They have been considered a rich source of secondary metabolites, which not only possess interesting chemical structures, but also exhibit a wide spectrum of pharmacological activities. Both ecteinascidin 743 (Yondelis) and dehydrodidemnin B (aplidine) are noteworthy examples (Wang, 2012).

Among these secondary metabolites, cadiolides possess potent activity against Gram Positive bacteria including MRSA. Figure 1 displays the structures of cadiolides; Table 1 shows their anti-MRSA activities. In 1998, cadiolides A and B were first isolated from Indonesian ascidians (*Botryllus sp.*) (Smith, 1998). Cadiolides C, D, E and F were isolated from the Korean tunicate *Pseudodistoma antiojoja* (Wang, 2012) and G, H and I were isolated from the dark red ascidian *Synoicum sp.* off the coast of southern Korea (Won, 2012).

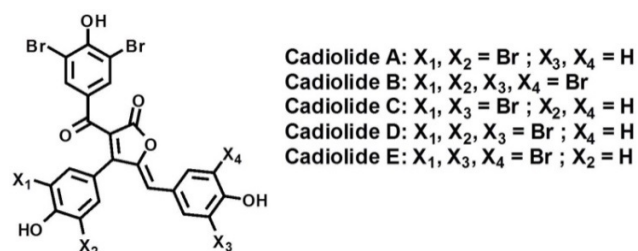


Figure 1. Structures of cadiolides.

As a Gram positive pathogen that has a significant impact on the world's public health, MRSA has been the most frequent cause of nosocomial bacteremia, skin/wound infections, and lower respiratory infections worldwide. Thus, there is a critical and ongoing need to explore new antibiotics against MRSA based on novel structural templates.

At the time of writing, we were only aware of one previous synthesis of cadiolides (Boukouvalas, 2005). Figure 2 describes Boukouvalas' synthesis of cadiolide B, which is centered on the sequential derivatization of a central butenolide ring, compound **1**.

Compound	MIC (ug/mL) against MRSA strains
Cadiolide B	0.5-1
Cadiolide C	<0.13-0.5
Vancomycin	0.5-1
Linezolid	2-4
Platensimycin	8

Table 1. Anti-MRSA activities of selected cadiolides.

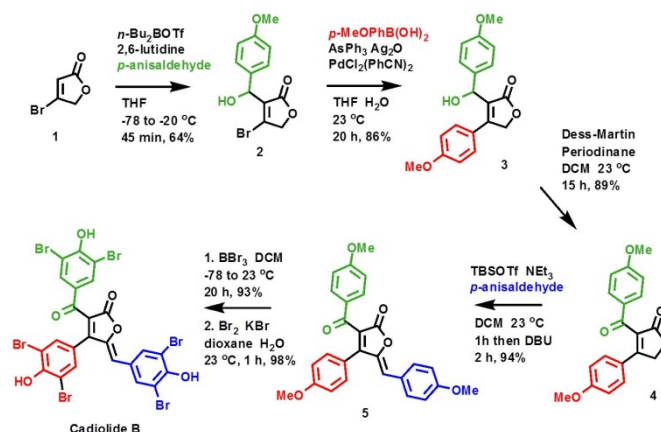


Figure 2. Boukouvalas' cadiolide B synthesis.

First derivatization involves an acylation with *p*-anisaldehyde via the borane enolate to afford intermediate **2**. A Palladium-catalyzed coupling to a boronic acid (Suzuki coupling) leads to compound **3**. Compound **4** is then made from oxidation, and is poised for reaction with another anisaldehyde unit after deprotonation to the silyl enolate. Finally, compound **5** is de-methylated and per-brominated

to afford cadiolide B. This total synthesis involves 6 steps with a very efficient 42% overall yield. However, earlier introduction of bromine atoms is incompatible with the use of a Suzuki coupling in the second step, which also involves expensive reagents. Moreover, the very late-stage perbromination of the advanced intermediate **5** makes it incapable to produce the partially brominated pattern found in other cadiolides such as cadiolide C. However, cadiolide C is the most active with MIC against MRSA in the range of <math><0.13\text{-}0.5\ \mu\text{g/mL}</math>.

To improve the two confines of Boukouvala's synthesis, a new approach to cadiolides was developed in this research with cheaper and readily-available reagents. In this approach the butenolide ring is constructed last, from an acyclic precursor possessing the phenol rings, which may have been pre-brominated in different ways. Therefore, it is adjustable for the construction of other cadiolides such as cadiolide C. Furthermore, the assembly of the ring is more likely to be more similar to the biogenetic pathway leading to such structures from tyrosines.

## Materials and Methods

The overall retrosynthetic analysis leading to our approach to cadiolides is depicted in Figure 3. The late-stage intra-molecular 5-exo-trig cyclization / Michael addition that occurs within structure **6** is the key step in this approach. The enolate from the beta-keto ester functionality is expected to add to the quinone methide obtained by selective oxidation (after deprotection) of the more electron-rich rings of structure **7**. The steps leading to compound **7** include a standard and straightforward beta-keto ester synthesis via an acylated Meldrum's acid and dibenzylmethanol intermediate **8** (Hogekamp, 2007; Yamamoto, 1987). In this research, the acylation of Meldrum's acid was conducted and explored by using different bases and under different conditions. The synthesis of

compound **8**, the dibenzylmethanol was

Figure 3: Our retrosynthetic analysis towards the cadiolides explored via four different approaches. The synthesis of compound **8** is illustrated in Figure 4. Each of these four approaches was tested under varied conditions in order to find the best method to optimize the yield.

The structures of the crude compounds were characterized by thin layer chromatography (TLC), gas chromatography–mass spectrometry (GC-MS), and  $^1\text{H}$  nuclear magnetic resonance spectroscopy ( $^1\text{H-NMR}$ ) analyses.

## Results and Discussions

The acylation reaction of Meldrum's acid as described in Figure 5 provided 85% yield of crude product using DMAP as the base in  $\text{CH}_2\text{Cl}_2$  as solvent via slow addition, and also the temperature has to be cooled down to  $0^\circ\text{C}$  at the beginning. Fast addition or allowing the reaction temperature to occur at room temperature resulted in crude mixtures containing large levels of impurities. The reaction with DMAP as base, toluene as solvent and reflux yielded no product at all. Moreover, after isolation, the acylated Meldrum's acid appears to be easily decomposed during purification by column chromatography. Therefore, it is better to use the crude acylated Meldrum's acid for beta-keto ester synthesis step directly without purification.

The first alkylation of the acetoacetate/Bayer-Villiger approach to synthesize the dibenzylmethanol intermediate was attempted with four variations of bases and conditions (Figure 6). Sodium methoxide in methanol, and sodium hydride in THF were tried at room temperature. LDA and DMAP in THF were tried at  $0^\circ\text{C}$ . Unfortunately, all results gave percent yields lower than 20%.

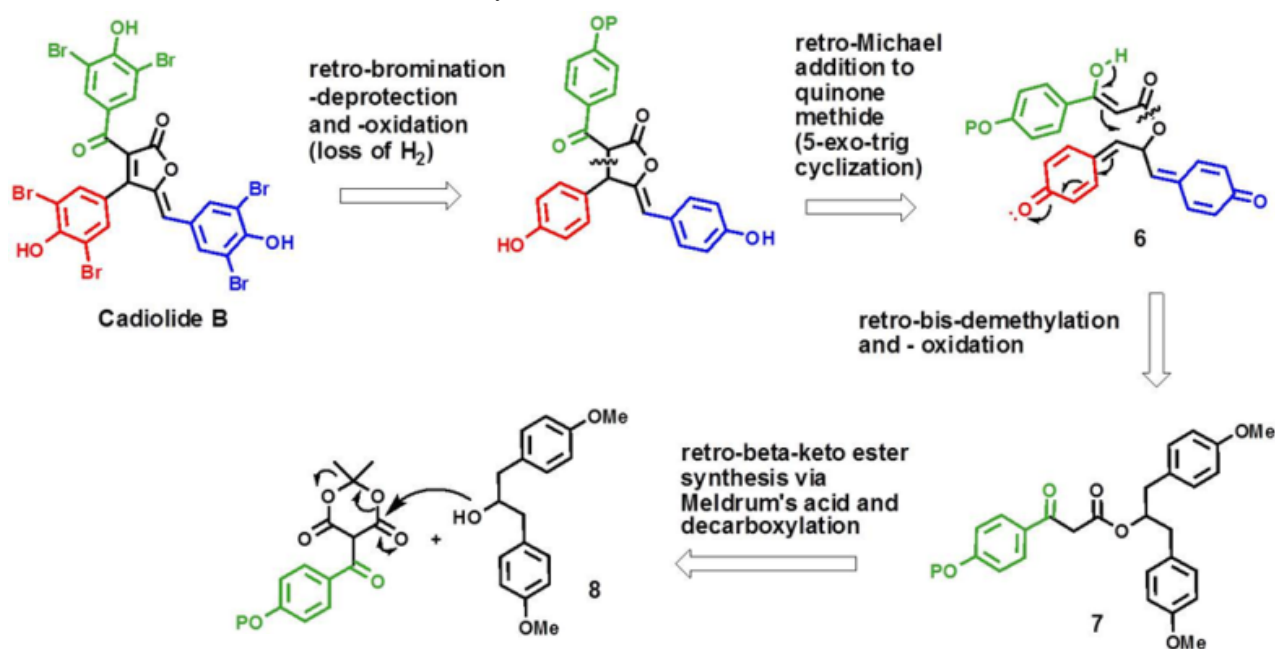


Figure 3. Our retrosynthetic analysis towards the cadiolides.

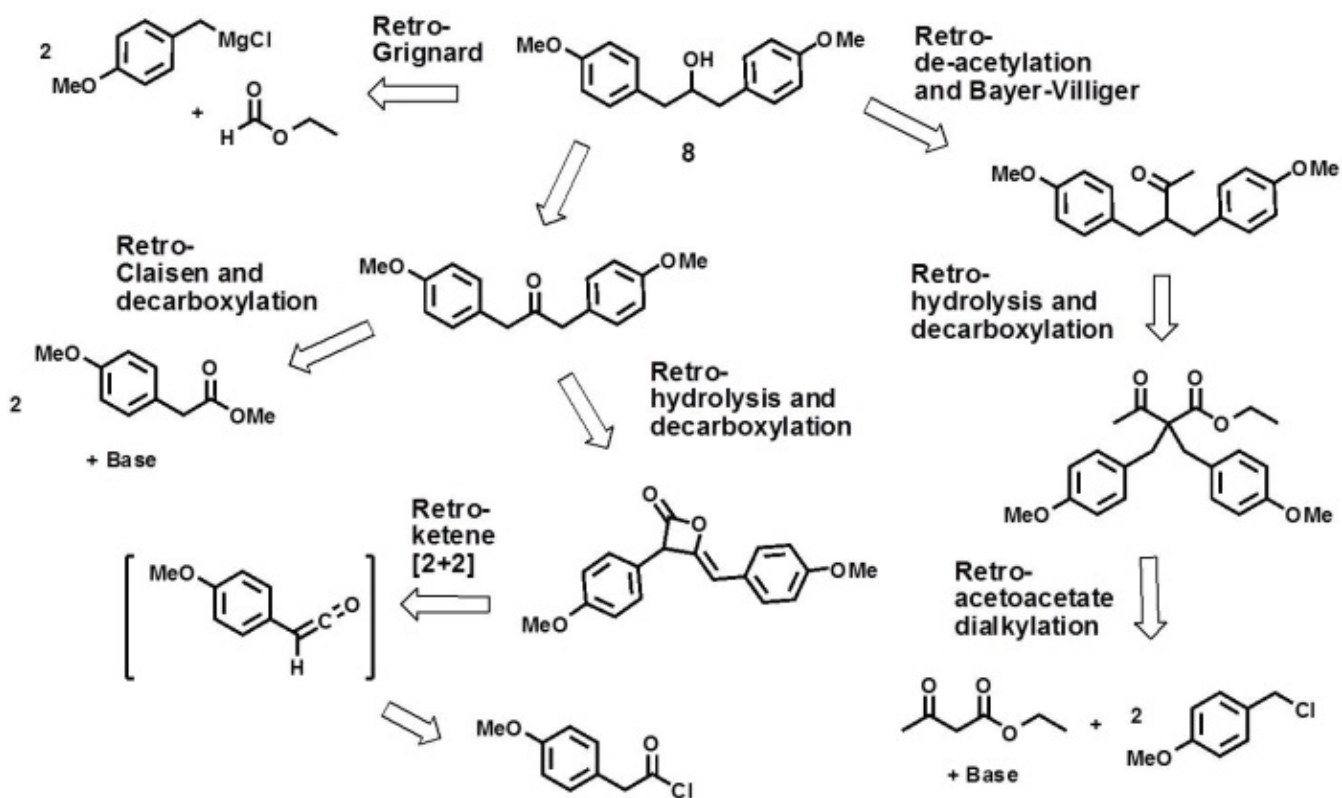


Figure 4. Four different synthetic approaches to dibenzylmethanol 8.

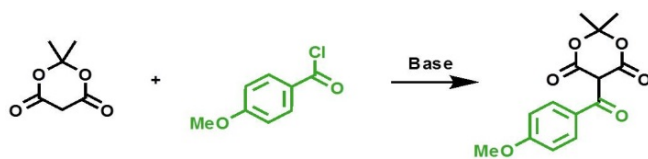


Figure 5. Acylation of Meldrum's acid

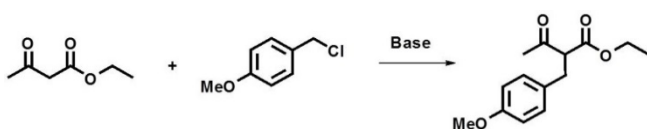


Figure 6. Benzylation of ethyl acetoacetate

In addition, the second alkylation with a very hindered enolate still needed to be performed to afford the dibenzylmethanol intermediate to be used for the next key cyclization reaction. Thus, the acetoacetate/Bayer-Villiger approach is not considered as a desirable one.

Figure 7 depicts the Grignard approach to afford the dibenzylmethanol intermediate. As shown by the results summarized in Table 2, the Grignard reaction proved to be extremely sensitive to the conditions used. The reaction has the highest yield when formate was added around 1 hour after the addition of Mg, though the actual percent yield was still under 20%. Best conditions still needs to be identified, and will probably require cooler temperatures throughout the

reaction. For these reasons, the Grignard approach is also not an ideal method.

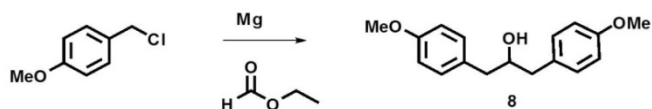


Figure 7. Grignard approach to dibenzylmethanol 8.

Conditions	Results
Mg powder, THF, RT, Barbier	No desired product by GC/MS
Mg chip, THF, RT to 0 °C then RT; formate added 40 min later	Major products are 2-phenylacetaldehyde and 1,2-diphenylethane
Mg chip, THF, RT; formate added 1h later	<20% yield by GC/MS
Mg chip, THF, 0 °C to RT; formate added 40 min later	No improvement over above (GC/MS)
Mg chip, THF, RT, Barbier	No desired product by GC/MS

Table 2. Results from Grignard approach synthesizing dibenzylmethanol 8.

Finally, the known ketene [2+2] approach to the dibenzylmethanol derivative 8 was investigated as shown in Figure 8. The percent yield of the second step is desirable but the percent yield of the first step is very low (11.6%).

Furthermore, the requirement of a laborious purification by column chromatography makes this approach not synthetically useful. The low reported yield of first step was also confirmed by the published literature source.

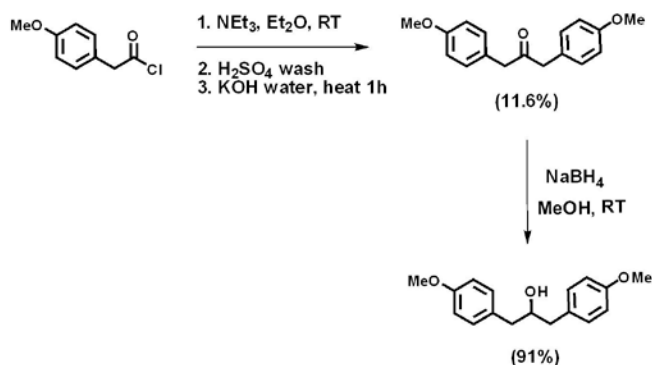


Figure 8. Ketene [2+2] Approach synthesizing Dibenzylmethanol

Unexpected challenges along the route prevented the exploration of the key cyclisation step. Synthesis of dibenzylmethanol intermediate still needs to be optimized, and focus should be shifted to the Claisen Condensation approach.

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### Biography

Shican Li is from Zhengzhou, China. After graduating from Henan Experimental High School in China, she came to study at University of New Haven when she was 17. She is now a senior majoring in Biology, pre-med, with a minor in chemistry. Shican plans to be a doctor and a poet in the future.

