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Biomimetic Synthesis of a New Class of Bacterial Signaling Molecules

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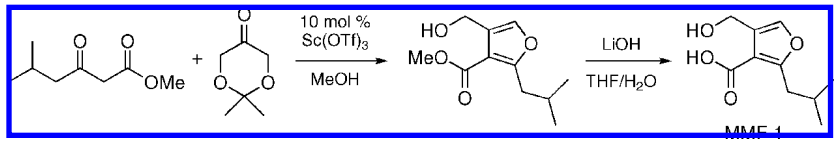
ABSTRACT



The first synthesis of a newly discovered class of bacterial signaling molecules from *Streptomyces coelicolor* has been developed. These molecules, known as the methylenomycin furans (MMFs), trigger production of the antibiotic methylenomycin. The synthesis features a scandium triflate-catalyzed domino reaction of β-ketoesters and dihydroxyacetone yielding 2,3,4-substituted furans. The proposed reaction sequence (aldol reaction, cyclization, and dehydrative aromatization) may be reminiscent of the biosynthetic reaction in which dihydroxyacetone phosphate and a β-ketothioester are condensed by an enzyme.

Bacteria commonly use low molecular weight signaling molecules to coordinate complex behaviors such as biofilm formation, differentiation, and antibiotic production.[1] *Streptomyces* bacteria are known to use signaling molecules to regulate antibiotic production. These soil-dwelling bacteria are the producers of two-thirds of the clinically used antibiotics.[2] Recently, Challis and co-workers reported the discovery of a

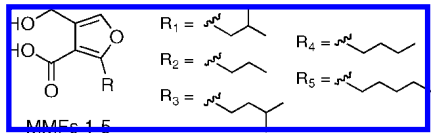
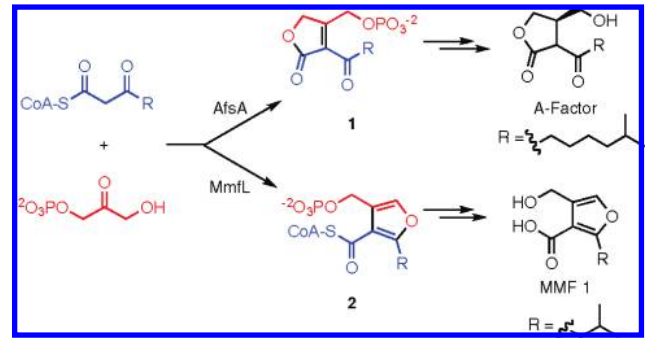


Figure 1. Methylenomycin furans (MMFs) from *S. coelicolor*.

new class of *Streptomyces* signaling molecules with a 2-alkyl-4-hydroxymethylfuran-3-carboxylic acid core structure.[3] Five compounds differing in the identity of the alkyl group at C2 were isolated from *S. coelicolor* and termed the methylenomycin furans (MMFs) as they induce the production of the antibiotic methylenomycin and share a furan core structure (Figure 1). These compounds are structurally distinct from the γ-butyrolactones, the major class of signaling molecules employed by the *Streptomyces* genus.[4] The prototypical γ-butyrolactone is A-Factor, an inducer of streptomycin biosynthesis in *Streptomyces griseus* (Scheme 1).

Scheme 1. Proposed Biosynthetic Pathways of γ-Butyrolactones and MMFs



(1) Bassler, B. L.; Losick, R. *Cell* **2006**, *125*, 237–246.
(2) Hopwood, D. A. *Streptomyces in Nature and Medicine: The Antibiotic Makers*; Oxford University Press: New York, 2007.
(3) Corre, C.; Song, J.; O'Rourke, S.; Chater, K. F.; Challis, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 17510–17515.
(4) Takano, E. *Curr. Opin. Microbiol.* **2006**, *9*, 287–294.

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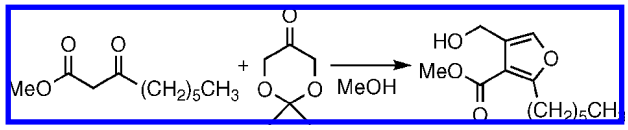
Interestingly, both the γ-butyrolactones and MMFs are biosynthetically derived from the same starting materials, dihydroxyacetone phosphate and a coenzyme A β-ketothioester (Scheme 1). These substrates are condensed by distinct enzymes belonging to the AfsA superfamily.[3] Based on the inherent reactivity of 1,3-dicarbonyl compounds and ketones, we suspected that catalysts used in synthetic organic chemistry could be to used to effect condensations reminiscent of the enzymatic reactions in the biosyntheses of the signaling molecules. In model studies, we tested this hypothesis using a β-ketoester (methyl 3-oxononanoate) in place of the coenzyme A β-ketothioester and ketal-protected dihydroxyacetone in place of dihydroxyacetone phosphate. Our expectation was that these starting materials would undergo a Knoevenagel-type reaction to give an α,β-unsaturated dicarbonyl compound that would subsequently lactonize upon ketal deprotection, yielding the butenolide intermediate (**1**) in γ-butyrolactone biosynthesis (Scheme 1).

In initial condensations, we examined the catalytic activity of piperidine, ammonium acetate, and diethylamine, which are known to catalyze carbon−carbon bond-forming reactions of 1,3-dicarbonyls.[5] No reaction was observed with any of the amine catalysts. These results can be ascribed to the lower reactivity of ketones relative to that of aldehydes in Knoevenagel condensations.[6] Next, we tested Lewis acidic metals as condensation catalysts because they have been shown to effect additions of 1,3-dicarbonyl compounds to aldehydes or ketones.[7] For practical and mechanistic considerations, we focused our efforts on air- and moisture-insensitive Lewis acid catalysts (Table 1). First, we reacted methyl 3-oxononanoate and ketal-protected dihydroxyacetone in methanol using scandium triflate as a catalyst. The expected α,β-unsaturated dicarbonyl compound product was not observed; however, a methyl 2-alkyl-4-hydroxymethylfuran-3-carboxylate product was isolated in good yield. This product has the identical skeleton observed in the *S. coelicolor* MMF signaling molecules. In subsequent experiments, we found that this reaction is also catalyzed by ytterbium triflate but not significantly by bismuth triflate or zinc chloride (Table 1). In the absence of scandium or ytterbium triflate, no reaction of the substrates was observed. Apparently, $Sc(OTf)_3$ and $Yb(OTf)_3$ catalyze the condensation and dehydrative aromatization of dihydroxyacetone and β-ketoesters.

Table 1. Condensation Catalyst Screening[a]



catalyst	loading (mol %)	% yield
$Sc(OTf)_3$	10	72
$Yb(OTf)_3$	10	56
$Bi(OTf)_3$	10	trace
$ZnCl_2$	10	trace

[a] Reactions were performed at room temperature for 15 h with equimolar substrates.

Given the mechanistic curiosity of the metal-catalyzed condensation and its potential use in the chemical synthesis of the MMFs, we studied the reaction in more detail. The structure of the product indicated that in situ deprotection of the dihydroxyacetone ketal took place prior to furan formation. Indeed, we observed rapid (<1 min) deprotection of the dihydroxyacetone by scandium triflate in methanol by thin-layer chromatography.[8] The rate of deprotection is clearly important because condensations in THF, where deprotection is markedly slower, yield much less product than those performed in methanol (42% vs 72% yield). Next, we examined the importance of the order of substrate and catalyst addition because both dihydroxyacetone and β-ketoesters are prone to self-condensation. We found that premixing scandium triflate with either of the substrates followed by addition of the other substrate led to an increase in side reactions and a ~30% decrease in furan yield. The optimal reaction conditions involved premixing of the substrates followed by addition of the catalyst. Then, we investigated the importance of reactant stoichiometry given the propensity of the β-ketoester and dihydroxyacetone to self-condense. It was found that a 1:1 stoichiometry was optimal. Finally, catalyst loadings were screened and the reaction was found to be highest yielding when 10 mol % of scandium triflate was used. Any more than 10 mol % catalyst resulted in side reactions that lowered the yield, while 5 mol % led to a slower reaction rate. With these optimized reaction conditions, we obtained methyl 2-hexyl-4-hydroxymethylfuran-3-carboxylate from ketal-protected dihydroxyacetone and methyl 3-oxononanoate in 72% yield.

Furan formation likely involves an aldol reaction of the β-ketoester and dihydroxyacetone. To examine the substrate scope of this reaction, we reacted a β-ketoester with two different substrates containing α-hydroxy groups, hydroxyacetone, and glycolaldehyde (Table 2). These substrates reacted to form the expected furans in 51 and 56% yields, respectively. In contrast, only starting materials were recovered from reactions with ketones lacking an α-hydroxy group (cyclohexanone, cyclopentanone, or 3-pentanone). The absence of aldol products in those reactions suggested that the α-hydroxy group is crucial for irreversible carbon−carbon bond formation. Thus, we propose that under these conditions β-ketoesters and α-hydroxycarbonyl compounds undergo a domino reaction.[5b,9] They first react in a reversible aldol

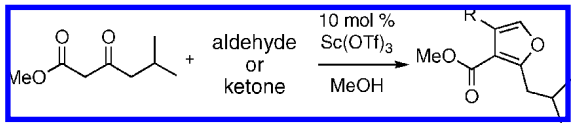
(5) (a) Tietze, L. F.; Beifuss, U. The Knoevenagel Reaction. In *Comprehensive Organic Syntheses*; Trost, B. M., Fleming, I., Eds.; Pergamon: Oxford, 1991; Vol. 2, pp 341−394. (b) Tietze, L. F.; Rackelmann N. The Domino Knoevenagel−Hetero-Diels−Alder Reaction and Related Transformations. In *Multicomponent Reactions*; Zhu J., Bie, H., Eds.; Wiley-VCH: Weinheim, 2005; Vol. 12, pp 1−168.

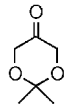
(6) Smith, M., March, J., Eds. *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, 5th ed.; John Wiley & Sons, Inc.: New York, 2001.

(7) (a) Rao, P. S.; Venkataratnam, R. V. *Tetrahedron Lett.* **1991**, *32*, 5821–5822. (b) Narsaiah, A. V.; Nagaiah, K. *Synth. Commun.* **2003**, *33*, 3825–3832.

(8) The deprotection of acetals by scandium triflate is known. Oriyama, T.; Watahiki, T.; Kobayashi, Y.; Hirano, H.; Suzuki, T. *Synth. Commun.* **2001**, *31*, 2305–2311.

Table 2. Condensation Substrate Scope[a]



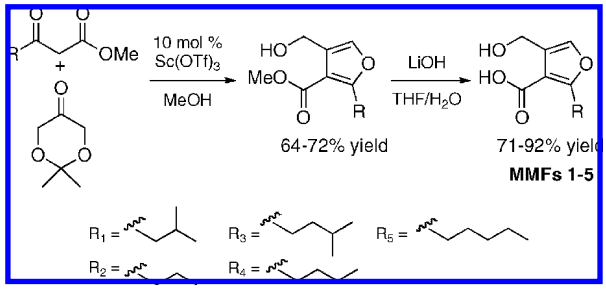
aldehyde/ketone	% yield
	60, R = -CH_2OH
	51, R = -CH_3
	56, R = -H

[a] Reactions were performed at room temperature for 18 h with equimolar substrates.

reaction, followed by addition of the α-hydroxy group to the ketone yielding a ketal intermediate that undergoes dehydrative aromatization to give the furan. The equilibrium most likely favors starting materials, but product formation is driven by irreversible aromatization. Curiously, the nucleophilic primary alcohol of the aldol product does not react with the electrophilic carbonyl of the methyl ester; a butenolide product, which would result from such a reaction, was not isolated.

The carbon skeleton of the condensation product was the same as in the proposed structures of the *S. coelicolor*

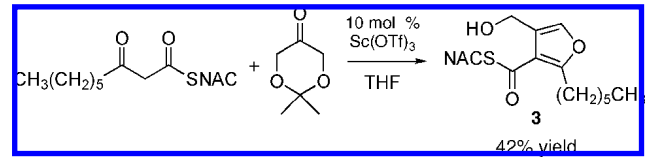
Scheme 2. Synthesis of the *S. coelicolor* MMFs



MMFs.[3] To validate those structures, we synthesized all five MMFs using the appropriate β-ketoester substrates and ketal-protected dihydroxyacetone. The furan methyl esters were formed in good yields (60−72%) by the scandium-catalyzed condensation of the β-ketoester and ketal-protected dihydroxyacetone in methanol at room temperature within 18 h. We obtained the *S. coelicolor* MMFs 1−5 in 71−92% yield by saponification of the corresponding furan methyl esters using 2.5 equiv of LiOH in 1:1 THF/water (Scheme 2). The NMR spectra and the masses of the compounds were identical to those reported for the natural MMFs (see the Supporting Information).[3]

The dihydroxyacetone and β-ketoesters used in our chemical synthesis are structurally reminiscent of the enzymatic

Scheme 3. Condensation with a β-Ketothioester Substratea



[a] The reaction was performed at room temperature for 18 h with equimolar substrates.

substrates, dihydroxyacetone phosphate and coenzyme A β-ketothioesters. To directly test whether these structural similarities translate into similar reactivities, we carried out a scandium-catalyzed condensation using ketal-protected dihydroxyacetone and a coenzyme A thioester mimic, the *N*-acetylcysteamine β-ketothioester (SNAC).[10] This reaction gave the expected furan thioester, **3**, in 42% yield (Scheme 3).[11]

Our proposed mechanism for furan formation has interesting implications for the biosyntheses of both the γ-butyrolactones and MMF signaling molecules in *Streptomyces* bacteria. Both classes of signaling molecules are derived from dihydroxyacetone phosphate and a coenzyme A β-ketothioester via the action of homologous enzymes. It is not clear how homologous enzymes generate different molecular skeletons from the same substrates. In contrast to the proposed biosyntheses of γ-butyrolactones and MMFs,[3,12] we suspect that an intermediate, **4**, resulting from the aldol reaction of a coenzyme A β-ketothioester and dihydroxyacetone phosphate substrates is common to the biosynthesis of both classes of signaling molecules (Scheme 4). Although only the MMF-like furan product is observed in the laboratory, it is conceivable that an enzyme could generate either the furan (**2**) or butenolide (**1**) product via different chemoselective ring-closing reactions (Scheme 4). Reaction of the primary alcohol with the thioester will yield the butenolide intermediate of γ-butyrolactone biosynthesis. Reaction of the primary alcohol with the ketone will yield a furan product leading to formation of the MMFs. The true test of this hypothesis awaits biochemical characterization of the enzymes involved in MMF biosynthesis.

The scandium-catalyzed condensation and dehydrative aromatization of β-ketoesters and α-hydroxy ketones under mild conditions is a remarkable domino reaction. This reaction is a variant of the Garcia Gonzalez reaction, which is a metal-catalyzed condensation of ring-opened sugars and

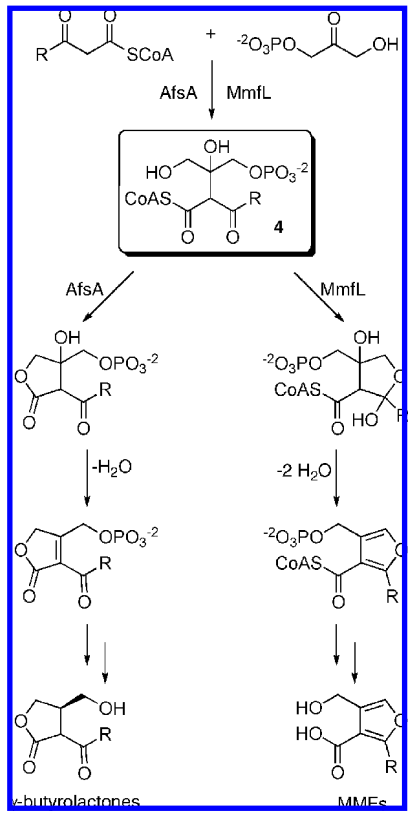
(9) Tietze, L. F. *Chem. Rev.* **1996**, *96*, 115–136.

(10) (a) Cane, D. E.; Yang, C. *J. Am. Chem. Soc.* **1987**, *109*, 1255–1257. (b) Gilbert, I. H.; Ginty, M.; O'Neill, J. A.; Simpson, T. J.; Staunton, J.; Willis, C. L *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1587–1590.

(11) All reactions with the SNAC substrate were perfomed using THF as a solvent as the thioester was not sufficiently stable in methanol.

(12) Kato, J.; Funa, N.; Watanabe, H.; Ohnishi, Y.; Horinouchi, S. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 2378–2383.

Scheme 4. Proposed Biosynthetic Pathways of the γ-Butyrolactones and MMFs



dicarbonyl compounds.[13,14] In contrast to the Garcia Gonzalez reaction, the key reaction reported in the MMF synthesis uses an α-hydroxy ketone as a substrate with only 10 mol % catalyst, and it proceeds at room temperature in good yield. Our findings are of further interest because a metal catalyst appears to mimic a reaction catalyzed by an enzyme. In any case, this work highlights the potential for using synthetic organic chemistry to provide mechanistic insight into complex enzymatic reactions.[15]

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Supporting Information Available: Procedures and spectroscopic data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) (a) Rodrigues, F.; Canac, Y.; Lubineau, A. *Chem. Commun.* **2000**, 2049–2050. (b) Bartoli, G.; Fernandez-Bolaos, J. G.; Di Antonio, G.; Foglia, G.; Giuli, S.; Gunella, R.; Mancinelli, M.; Marcantoni, E.; Paoletti, M. *J. Org. Chem.* **2007**, *72*, 6029–6036.

(14) A furan methyl ester product was isolated from a zinc chloride-catalyzed reaction of hydroxyacetone and ethyl acetoacetate. Garcia Gonzalez, F. *Adv. Carbohydr. Chem.* **1956**, *11*, 97–143.

(15) Kim, J.; Ashenhurst, J. A.; Movassaghi, M. *Science* **2009**, *324*, 238–241.