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COMMUNICATION

Replication of biosynthetic reactions enables efficient synthesis of A-factor, a γ-butyrolactone autoinducer from *Streptomyces griseus*†

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We report a concise synthesis of A-factor, the prototypical γ-butyrolactone signalling compound of *Streptomyces* bacteria. In analogy to enzymatic reactions in A-factor biosynthesis, our synthesis features a tandem esterification–Knoevenagel condensation yielding a 2-acyl butenolide and a surprising, chemoselective conjugate reduction of this α,β-unsaturated carbonyl compound using sodium cyanoborohydride.

Streptomyces bacteria are best known as producers of half of the 10 000 known antibiotics and two-thirds of those used in clinical and veterinary medicine.[1] The biosyntheses of these molecules is often tightly regulated *via* the action of low-molecular weight pheromones. As inducers of antibiotic production, these molecules have potential applications in the production of drugs by fermentations,[2] in drug discovery,[3] and in studies of soil ecology.[4] The prototypical *Streptomyces* signalling compounds are the auto-inducing factor (A-factor), virginiae butanolide A (VB-A) and methylenomycin furan 1 (MMF1) that regulate streptomycin, virginiamycin, and methylenomycin production, respectively (Fig. 1). Because signalling molecules are usually produced in extremely small quantities, the only practical way to obtain quantities sufficient for use in basic and applied science is *via* chemical synthesis.

It is estimated that up to 60% of streptomycetes use 3-hydroxymethyl-γ-butyrolactones, like A-factor and VB-A for intra- and interspecies communication.[4] Fourteen 3-hydroxymethyl-γ-butyrolactones that differ with respect to stereochemistry and the substituent at C2 have been isolated from streptomycetes.[1]

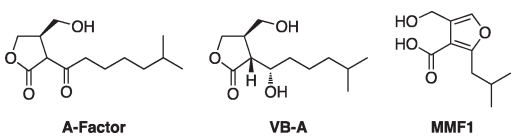

Fig. 1 Prototypical *Streptomyces* signalling molecules.

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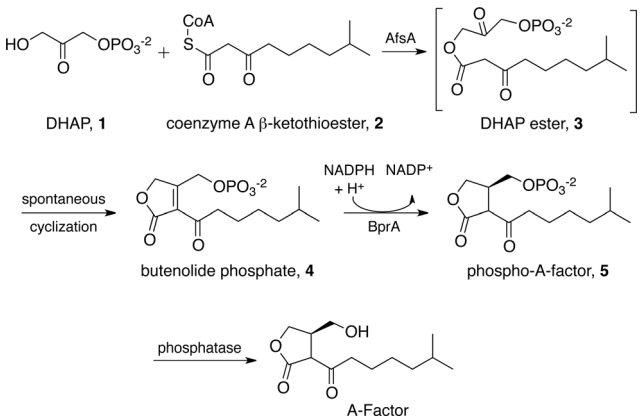
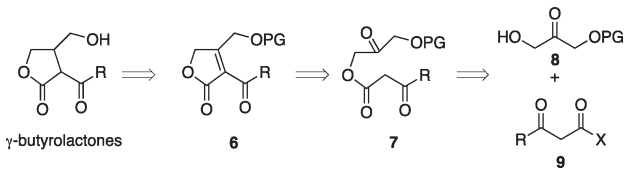
Differences in the structures of these molecules often account for their species-specific activities. A-factor, a metabolite of *Streptomyces griseus*, was the first γ-butyrolactone autoinducer identified.[5] Since its structure elucidation in the late 1960s, nine syntheses of A-factor have been published.[6] Seven of the nine routes arrive at A-factor through acylation of a 3-hydroxymethyl-γ-butyrolactone equivalent.[6a–g] While this approach is direct, the preparation of the 3-hydroxymethyl-substituted γ-butyrolactone is not trivial, especially in an enantiospecific fashion. We aimed to design an alternative route that would provide facile access to the core structure of any of the known streptomycete γ-butyrolactones using inexpensive and easily handled starting materials and reagents. We envisioned that an efficient synthesis could be realized by replicating the A-factor biosynthesis, which consists of three transformations[7] (Scheme 1).



Scheme 1 A-factor biosynthetic pathway.

The mechanism of A-factor biosynthesis has recently been elucidated.[7] In the first step, an enzyme called AfsA catalyzes the condensation of dihydroxyacetone phosphate (DHAP), **1**, and a coenzyme A β-ketothioester, **2**, yielding an ester intermediate, **3**.[7] (Scheme 1) This intermediate is proposed to undergo a spontaneous intramolecular Knoevenagel condensation to give a butenolide, **4**. The butenolide is transformed to γ-butyrolactone **5** by BprA *via* stereospecific conjugate reduction using NADPH as a hydride donor. Finally, the phosphate ester of **5** is hydrolyzed



Scheme 2 Biologically inspired retrosynthesis of A-factor.

by an unknown phosphatase to give A-factor as the final product. All of the streptomycete γ-butyrolactone signalling compounds are thought to be biosynthesized *via* this pathway.

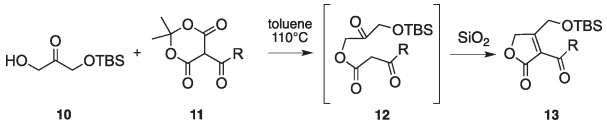
We predicted that the key transformations in the biosynthesis could be replicated in the laboratory (Scheme 2). The first transformation in A-factor biosynthesis is the formation of a butenolide *via* the condensation of DHAP with a coenzyme A β-ketothioester. Given the incompatibility of DHAP and the coenzyme A β-ketothioester with organic solvents, we anticipated that readily soluble and less reactive analogs of these molecules would be needed, **8** and **9** (Scheme 2). As a surrogate for DHAP, we selected monosilylated dihydroxyacetone, **10**. As a mimic of the reactive coenzyme A β-ketothioester, we chose acyl Meldrum's acid, **11**. Analogous β-ketothioesters were not selected because previous studies in our group demonstrated that these compounds undergo furan formation in reactions with dihydroxyacetone.[8] In contrast, acyl Meldrum's acids are known to undergo alcoholysis yielding β-ketoesters[9,10] in a manner that is reminiscent of the reaction of the coenzyme A β-ketothioester with DHAP. As is the case in the A-factor biosynthesis, we proposed that the silyl-protected dihydroxyacetone and the acyl Meldrum's acid could be induced to undergo a tandem esterification–intramolecular Knoevenagel condensation yielding a 2-acyl butenolide. In further analogy to the biosynthesis, we envisioned that the butenolide could be converted to the γ-butyrolactone *via* a conjugate reduction using reagents commonly used in preparative organic chemistry. The preparation of A-factor *via* the synthesis and conjugate reduction of a butenolide is distinct from all published routes.[6]

Initially, we sought conditions for the condensation of the acyl Meldrum's acid and the monosilylated dihydroxyacetone in a biomimetic esterification–Knoevenagel reaction sequence. Using conditions for alcoholysis of acyl Meldrum's acids based on those reported in the literature,[9] **10** and **11** were heated to 90 °C at a 2 : 1 molar ratio in toluene (Table 1, entry a). Upon consumption of the acyl Meldrum's acid, we observed a mixture of the butenolide **13** and uncyclized β-ketoester **12** as products. In contrast to the biosynthesis, the β-ketoester **12** did not spontaneously cyclise; it was the major product as determined by TLC analysis of the crude reaction. Interestingly, purification of the reaction products by silica gel chromatography yielded butenolide **13** as the major product. It was apparent that cyclization of the ester was promoted by the weakly acidic silica gel. Accordingly, we found that the yield of the butenolide was significantly enhanced when excess silica gel (>200 : 1 by weight SiO_2 : crude product) was used in the chromatographic purification of the reaction. These reaction and purification conditions provided the butenolide **13** in 21% yield.

The poor yield of butenolide formation warranted further optimization.[11] Our finding that the silyl-protected dihydroxyacetone **10** was never completely consumed in the reaction led us to increase the reaction temperature to 110 °C and to use a 0.5 molar excess of the acyl Meldrum's acid (Table 1, entry b). Promisingly, these reaction conditions provided **13** in 51% yield. While the yield was improved, TLC analysis of the reaction still revealed the presence of unconsumed **10**. We reasoned that the thermal instability of acyl Meldrum's acid was at least partially responsible for this yield.[12] On this basis, we added **11** to the reaction in two portions. The addition of the acyl Meldrum's acid in two 0.75 equivalents provided the butenolide in 55% yield (Table 1, entry c). In the optimized reaction that provided the desired product in 70% yield (Table 1, entry d), we initiated the reaction with 1.2 equivalents of acyl Meldrum's acid and added 0.5 equivalents more after three hours. While preparation of a butenolide *via* an intramolecular Knoevenagel condensation has been reported,[13] our preparative method is of particular interest because it uses a tandem reaction sequence rather than effecting the reactions in separate steps.

The reaction following butenolide formation in the A-factor biosynthesis is the enzyme catalyzed conjugate reduction.[7] Again, our objective was to effect this transformation in the laboratory. While there are several precedents for conjugate reduction of enones and simple enoates, selective reductions of α,β-unsaturated-1,3-dicarbonyl compounds (*e.g.*, a 2-acylbutenolide) are a challenging proposition.[14,15] In such a molecule, one can easily envision competing 1,4- and 1,2-reductions. To mimic the enzyme-catalyzed reaction, we sought reagents that could

Table 1 Optimization of butenolide formation[a]



Reaction	Equiv of monosilyl DHA	Equiv of acyl MA	Solvent	*T*/°C	Time /h	% Yield
a	2	1	toluene	90	2.5	21
b	1	1.5	toluene	110	2.5	51
c	1	0.75 × 2	toluene	110	2.5	55
d	1	1.2 + 0.5	toluene	110	3, then 5	70

[a] For the purposes of optimization, we used **11** R = $-(CH_2)_5CH_3$ which is easier to prepare than the A-factor side chain where R = $-(CH_2)_4CH(CH_3)_2$ DHA = dihydroxyacetone, MA = Meldrum's acid

Table 2 Reduction of the 2-acyl butenolide

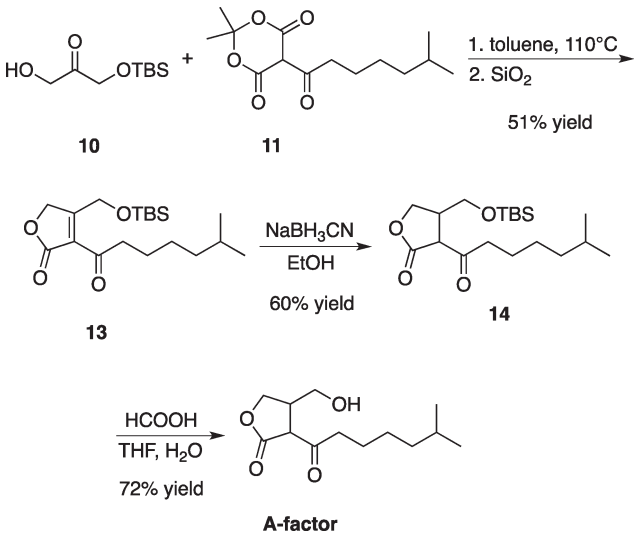
Catalyst	Mol (%)	Hydrogen source	Solvent	Time/h	% Yield
Stryker's Reagent	1	PMHS	toluene	24	0
DBTM-segphos(CuH)	1	PMHS	toluene	24	0
(BDP)CuH	1	PMHS	toluene	24	0
$[Rh(NBD)_2BF_4]$/JOSIPHOS	1	H_2	MeOH	24	0
none	N/A	$NaBH_3CN$	EtOH	30	66

effect 1,4-conjugate addition enantioselectively. Due to their utility in stereospecific reductions of enoates, we first screened CuH reagents including Stryker's reagent, and [DBTM-segphos(CuH)], and (BDP)CuH.[14] Unfortunately, none of the desired product **14** was recovered from reactions with these catalysts (Table 2). Experiments indicate that the low yield was due to catalyst poisoning by the reduction substrate[16] (Table 2). Next, we tried $[Rh(NBD)_2]BF_4$/JOSIPHOS as the reaction catalyst because it has also been reported to effect conjugate reductions with high % ee.[15] Unfortunately, we observed no activity of this catalyst towards our substrates.

Given the failure of the CuH and Ru based-catalysts, we turned our attention to other reductants. While sodium borohydride is known to effect both 1,2 and 1,4 reductions of α,β-unsaturated compounds,[17] sodium cyanoborohydride was reported by Hutchins and co-workers to effect only the conjugate reduction of an α,β-unsaturated-1,3-dicarbonyl compound (*i.e.* 6-nitro-3-benzoyl-3,4-dihydrocoumarin).[18] This group hypothesized that the chemoselectivity of the reduction was based on the slow rate of ketone and aldehyde reductions by sodium cyanoborohydride at pHs above 4. On this basis, we examined the capacity of sodium cyanoborohydride to effect the desired reduction. We were gratified to find that sodium cyanoborohydride reduced the butenolide in ethanol within 30 min, providing the racemic γ-butyrolactone **14** in an isolated yield of 66% (Table 2). Interestingly, we did not observe any pH dependence of this reaction as the yield and reaction times were the same when the reaction was carried out in the presence of HCl (data not shown). Although the reaction is not enantioselective like the enzyme-catalyzed reduction, it is noteworthy because it highlights the utility of sodium cyanoborohydride for conjugate reductions in the presence of functional groups that are sensitive to reduction.

The last step in A-factor biosynthesis is hydrolysis of the phosphate ester.[7] Analogously, the last step in our A-factor synthesis was cleavage of the silyl ether of **14**. This was easily effected in 72% yield by stirring the substrates in 6 : 3 : 1 THF : HCOOH : H_2O for 24 h.[19] Using this biomimetic strategy, we were able to prepare racemic A-factor in three steps and 22% overall yield (Scheme 3).

In conclusion, we report the shortest synthesis of A-factor published to date. The brevity of this synthesis is based on our replication of the enzyme-catalyzed reactions. Our observations have implications for both synthetic organic chemistry and



Scheme 3 Synthesis of A-factor.

biological chemistry. With respect to the synthesis of 2-acyl butenolides, we are the first to report preparation of these molecules *via* a tandem reaction sequence of an esterification–intramolecular Knoevenagel condensation. This route is of interest because 2-acyl butenolides can be transformed into complex natural products, including the syringolides,[20] methylenomycin,[21] the acyl α-L-rhamnopyranosides,[22] and cyclophostin.[23] Interestingly, our butenolide-forming reaction requires high temperature and acid-catalysis while the corresponding biological reaction is reported to be spontaneous.[7] Based on these differences, it is tempting to speculate that the phosphate moiety of the DHAP ester may have an unappreciated role as a catalytic base in the intramolecular Knoevenagel condensation.[24] With respect to the butenolide reduction, this is one of only a few instances in which sodium cyanoborohydride has been reported to chemoselectively effect a conjugate reduction. This observation is noteworthy because the reductant is generally thought to be useful in synthetic chemistry only for reductive aminations and for reductions of aldehyde and ketones at low pH.[18] Taken together, the details of this concise synthesis shed new light on pervasive reactions in synthetic organic chemistry and biochemistry.

Acknowledgements

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