VIP Natural Product Synthesis

DOI: 10.1002/anie.201204259

Convergent Asymmetric Synthesis of (+)-Aureothin Employing an Oxygenase-Mediated Resolution Step**

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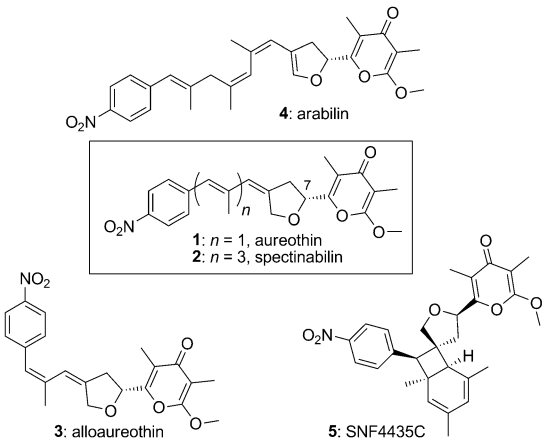
Aureothin (**1**) is an unusual polyketide metabolite of *Streptomyces thioluteus* that is endowed with antitumor, antifungal, antiparasitic, and pesticidal activities (Scheme 1).[1] Recent studies also demonstrated that **1** combines high efficiency and selectivity against trypanosome strains.[2] The molecule accumulates in a dense framework a stereodefined 1,3-diene with a 4-nitroaryl group and a chiral tetrahydrofuran moiety connected to the α′-methoxy-γ-pyrone scaffold. As a representative of rare natural nitro-substituted pyrones, the structure of **1** is closely related to other bioactive polypropionates, such as (+)-spectinabilin (also known as neoaureothin, **2**),[3] their respective isomers (−)-alloaureothin (**3**)[4] and (−)-arabilin (**4**),[5] and cycloisomer SNF4435C (**5**).[6] Only recently has *rac*-**1** become the target of total syntheses,[7] along with a renewed general interest in unsaturated polypropionates of this class.[8] The synthetic route to (+)-**1** is paved with hurdles, such as the stereodefinition and the conservation of the 1,3-diene and the stereocenter at C7. Indeed, both were reported to be prone to isomerization under sunlight and to epimerization in an acidic or basic environment.[9]



Scheme 1. Aureothin and related nitro-substituted pyrone metabolites.

The aureothin biosynthesis involves a regime of enzymes that assemble the molecule from nitrobenzoyl, malonyl, and methylmalonyl units in a highly specific way and under mild conditions.[10] As the final step, an unusual multifunctional cytochrome P450 monooxygenase (AurH) introduces the homochiral THF ring.[11] Notably, this oxygen heterocycle is an important pharmacophore for antifungal activity. AurH could be employed in the semisynthesis of (+)-**1** from deoxyaureothin as well as in the combinatorial biosynthesis of aureothin analogues.[12] Yet, from a synthetic point of view, the bottleneck preparation of the α′-methoxy-γ-pyrone scaffold has remained a challenge.[13] Synthetic strategies were designed accordingly, impairing the convergent character of the routes. In response, we sought to develop a new strategy to circumvent the situation by allowing 1) rapid access to the α′-methoxy-γ-pyrone scaffold and 2) convergence to the whole synthetic plan.

Herein, we report the successful implementation of our strategy for the construction of the aureothin skeleton, culminating in a short asymmetric synthesis of this natural product after an unprecedented regiodivergent and parallel kinetic resolution promoted by a cytochrome P450 monooxygenase.

Our approach relies on the conjugate addition of a lithiated nucleophile for the desymmetrization of α,α′-dimethoxy-γ-pyrone **6** (Scheme 2). This symmetric substrate, left unexploited since it was first reported as a side product by Woodward,[14] turned out to be a versatile building block for the preparation of α′-methoxy-γ-pyrones.[15] Hence, **8**, which exhibits the full carbon backbone of aureothin, was expected to arise from the desymmetrization of **6** by conjugate addition of a nucleophilic carbon center, followed by coupling with **7**, an electrophilic precursor of the side chain of the target. Our synthetic plan takes advantage of the two fragments **6** and **7**, which are readily available. While **6** can be obtained without any chromatography on multigram scale by the acid-promoted cyclization of dimethyl 2,4-dimethyl-3-oxopentanedioate in 60% yield,[15a] **7** is prepared stereoselectively in 58% yield from 4-nitrobenzaldehyde using a four-step procedure that relies on conventional and robust reactions (see the Supporting Information).

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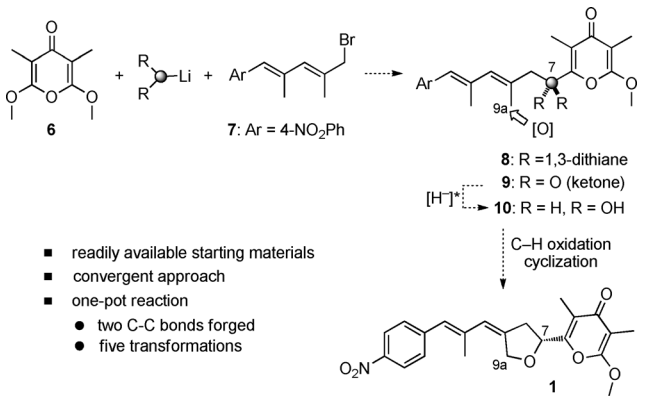
[**] We are grateful to the Région Haute-Normandie for generous financial support of this work. Prof. Sidney Hecht (Arizona State University) is thanked for helpful discussion.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201204259.



Scheme 2. Synthetic strategy to synthesize (+)-**1**.

The coupling of these two fragments requires a nucleophile that can eventually become C7 at the right level of oxidation. Using 2-lithio-1,3-dithiane as a nucleophile is expected to afford a direct precursor of ketone **9** and alcohol **10** after oxidative cleavage and reduction. Next, the ideal pathway to **1** would involve the hydroxy-directed C–H bond oxidation of the allylic position at C9a of **10**, followed by cyclization of the resulting diol.

Literature precedents in this regard were encouraging since the mono-oxidation at C9a of (*R*)-**10** by cytochrome P450 monooxygenase AurH has been reported on an analytical scale.[11b] With regards to the installation of the stereocenter at C7, an enantioselective reduction of ketone **9** could presumably be envisaged to prepare alcohol **10** with a significant *ee* value. Setting the stereocenter at such a late stage of the synthesis is intended to minimize the risk of epimerization.

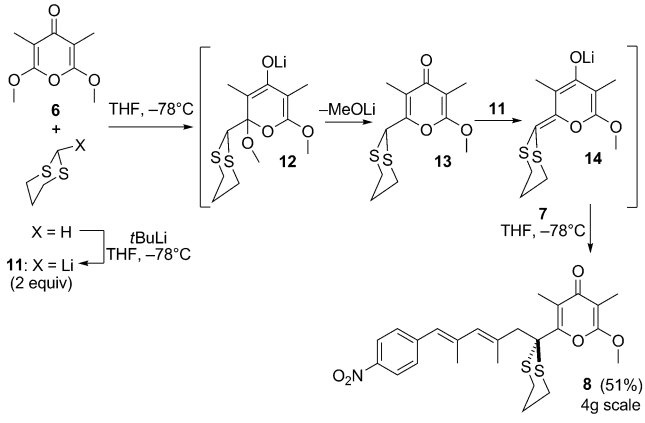
The one-pot procedure for the preparation of **8** was attempted with 2-lithio-1,3-dithiane **11** as a nucleophile and base (Scheme 3). Pleasingly, the exposure of **6** to two equivalents of **11** at −78 °C followed by addition of electrophile **7** led to the desired product **8** in 51 % yield and 90 % conversion without isomerization of the 1,3-diene system. This yield is to be regarded as satisfying in light of the five-step sequential process performed on a four-gram scale and involving the formation of **11**, 1,4-addition to **6**, then elimination of MeOLi from **12**, deprotonation of **13** with **11**, and chemoselective alkylation of **14** with **7**.[16]

With the full skeleton of the aureothin at hand, our efforts turned to the dethioacetalization of **8**. This step was much more challenging than anticipated because of the sensitivity of the 1,3-dienes and the electron-withdrawing effect of the nitro group. When formed, ketone **9** also proved to be rather unstable at room temperature probably because of the facile enolization into a trienic system, thus preventing any attempts of isolation.

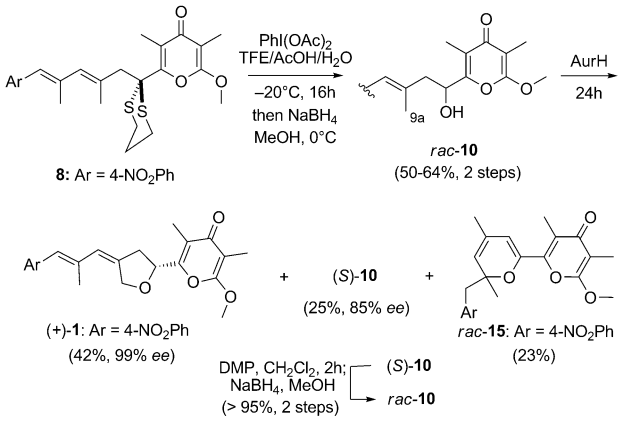
Among the various reagents tested for the dethioacetalization of **8**, $PhI(OAc)_2$ in CH_3CN/H_2O generated **9** without isomerization of the 1,3-diene group, as observed by ^1H NMR analysis of the crude reaction mixture. Treatment of the crude ketone with $NaBH_4$ allowed the isolation of the more stable alcohol **10**, but only in 15–20 % yields (2 steps). We therefore sought to optimize the procedure by ensuring a fast hydrolysis step of the 1,3-dithiane at low temperature. Retaining the mild oxidant $PhI(OAc)_2$, experiments were carried out in media of increasing polarity and acidity in order to stabilize the electron-deficient species formed during the activation of the 1,3-dithiane (see the Supporting Information for detailed conditions). Gratifyingly, when the reaction with $PhI(OAc)_2$ was run in a mixture of trifluoroethanol (TFE), acetic acid (AcOH), and water ($v/v/v = 6:3:1$) at −20 °C, **8** was completely converted into **9**, which was then reduced to give *rac*-**10** in 50–64 % yields over the two steps (Scheme 4). Notably, this is one of the rare examples for dethioacetalization at low temperature with mild and available reagents.

Having secured access to the direct precursor of aureothin, we undertook the aerobic oxidation of the allylic position at C9a of *rac*-**10** with the P450-monooxygenase AurH.

Whereas a previous report mentioned the oxidation of alcohol (*R*)-**10** into aureothin by treatment with AurH, at least on an analytical scale, the directed oxidation on a synthetically useful scale was still unexplored. The stereochemical issue of the C–H bond oxidation of *rac*-**10** was also unknown. The biotransformation was carried out on a preparative scale with 200 mg of *rac*-**10** and a cultivation broth of



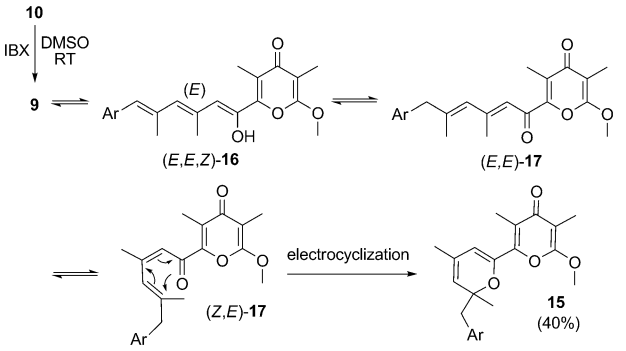
Scheme 3. One-pot preparation of **8**.



Scheme 4. Synthesis of (+)-**1** from **8**. DMP = Dess–Martin periodinane.

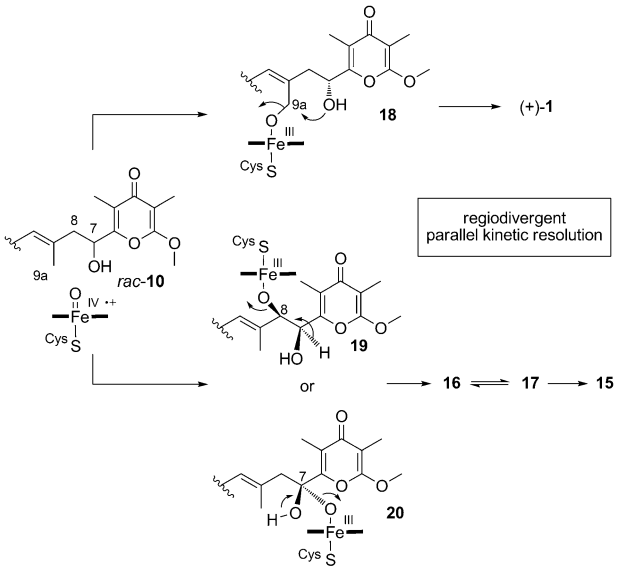
only 400 mL containing 7.84 g wet biomass (1.18 g dry biomass; Scheme 4). After 24 hours of exposure to AurH in vivo, (+)-**1** was successfully generated in 42% yield and excellent *ee* values of 99% without isomerization of the 1,3-diene group. Interestingly, (*S*)-**10** was recovered in 19% yield (85% *ee*) contaminated with 6% of isomerized 1,3-diene. An unexpected side product, *rac*-2*H*-pyran **15** was also isolated in 23% yield. In view of this preliminary data, we assumed that (*R*)-**10** was transformed into (+)-**1** by kinetic resolution of *rac*-**10** with a maximum yield of 50%. In order to further improve the efficiency of the transformation, (*S*)-**10** was easily recycled and converted into *rac*-**10** by oxidation into **9** followed by reduction (> 95% yield, 2 steps). The asymmetric total synthesis of aureothin was then accomplished in four steps from readily available building blocks, such as **6** and **7**, employing for the first time AurH for the resolution of a racemate.

We next investigated the origin of **15**, which was suspected to be formed during the oxidation of (*S*)-**10** into ketone **9**. As mentioned above, **9** is relatively unstable, probably because of the tautomeric equilibrium with enol **16** (Scheme 5). This equilibrium would enable the formation of the α,β-unsaturated ketone (*E*,*E*)-**17**, which after isomerization into (*E*,*Z*)-**17** would undergo 6π-electrocyclization to generate **15**. To support this hypothesis, the transformation of **9** into **15** was attempted, and monitored by ^{1}H NMR analysis. Ketone **9** was generated by oxidation of **10** with 2-iodoxybenzoic acid (IBX) in dimethylsulfoxide (DMSO). During the first three hours, **9** was the only product observed, then **15** started to appear. After 19 hours, ketone **9** was completely converted into **15** (40% yield, not optimized), thus indicating that **9** is a likely intermediate en route to **15** during the AurH-promoted biooxidation.



Scheme 5. Possible pathway to **15** from ketone **9**.

This data suggests that AurH promotes the regiodivergent and parallel kinetic resolution (PKR) of *rac*-**10**, thus allowing the preparation of (+)-**1** in excellent enantiopurity (Scheme 6).[17] Oxidation of the methyl group at C9a of (*R*)-**10** would probably generate intermediate **18**, which provides (+)-**1** after cyclization. On the other hand, the enantiorecognition between AurH and (*S*)-**10** resulted in the formation of enol **16** through intermediates **19** or **20**, which were obtained by oxidation of the C8 or C7 positions, respectively. Importantly, to the best of our knowledge, this is the first example of a P-450 cytochrome mediated parallel and regiodivergent kinetic resolution, with the site-selectivity of the C–H bond oxidation being completely directed by enantiodiscrimination.[18] The fact that the enzymatic system overrides the electronic preference for oxidation of secondary versus primary C–H bonds is also noteworthy. The next steps of the mechanism likely involve tautomerization of **16** into **17** and isomerization followed by 6π-electrocyclization to provide the complex non-natural α′-methoxy-γ-pyrone **15**.



Scheme 6. Mechanistic proposal for the regiodivergent parallel kinetic resolution of *rac*-**10**.

In order to gain insight into the possible binding mode of the non-natural (*S*)-**10**, we modeled the substance into the crystal structures of AurH (Figure 1).[19] We employed both three-dimensional AurH structures that account for the oxygenation step of the reaction sequence (PDB entries 3P3X and 3P3L, respectively), which leads to the naturally occurring tetrahydrofuran ring. Using SwissDock[20] for the docking calculations, we found a ligand orientation in the

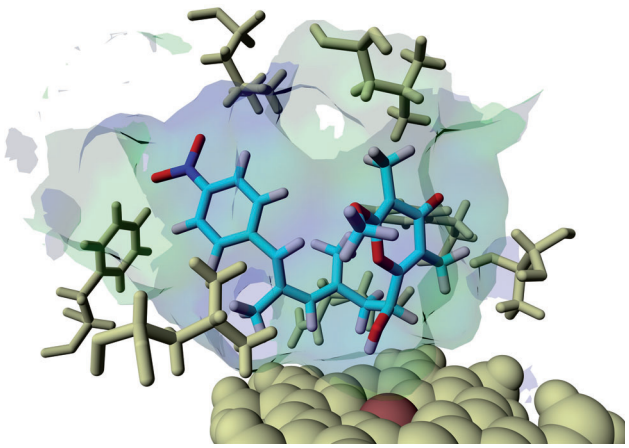


Figure 1. Model of substrate binding and AurH-mediated oxidation of (*S*)-**10** to the corresponding ketone (EADock DSS).

3P3X-based model that renders a reaction at C7 in intermediate **20** most plausible (binding energy $-8.76\ kcal\,mol^{-1}$). The 7-hydroxy group is directed toward the heme iron (distance heme iron and C7: 5.08 Å, distance Fe-O7 3.84 Å; Fe-C7-O-angle = 25.8°) whereas the (7*R*)-proton points away from the Fe-C7 axis (distance Fe-(7*R*)H 4.98 Å; Fe-C7-(7*R*)H-angle = 78.8°). This model supports the hypothesis of an oxidative attack of the (7*S*)-hydroxy group. In contrast, an oxidative attack of the remotely positioned C8 is unlikely (distance Fe-C8 5.92 Å; distance Fe-H8 4.49 Å) as it is shielded by the 7-hydroxy group. Furthermore, the carbon backbone of the substrate is twisted in a way that the side chain at C9a points away from the active site (distance Fe-C9a = 8.02 Å). Consequently, an oxidative attack of C9a as in (*R*)-**10** is impossible in this state. To support this hypothesis, we also modeled intermediate **18** into both template structures using the same approach. In this case, we found a model with the 3P3L structure as template, thus explaining the natural reaction sequence. It shows the highest of all binding energies ($-9.34\ kcal\,mol^{-1}$) observed here. Remarkably, the distance between heme iron and C9a (5.72 Å) is smaller than between heme iron and C7 (6.01 Å). The model described shows a possible binding mode of (*S*)-**10**, which can explain the formation of the unusual pyran structure of **15** through intermediate **20**.

In conclusion, the umpolung strategy we have designed and applied to the desymmetrization of **6** allowed the one-pot construction of the complete carbon backbone **8** of aureothin with maximized convergence. The last steps include the regioselective aerobic C–H bond oxidation and cyclization of *rac*-**10**, which proceeded according to an unprecedented regiodivergent PKR pathway promoted at a synthetically relevant scale by AurH, a multifunctional cytochrome P450 monooxygenase. This transformation completed the asymmetric stereoselective synthesis of aureothin in eight steps from 4-nitrobenzaldehyde and 8% overall yield with *ee* values as high as 99%. On this basis, the extension of the strategy to the synthesis of other natural products is ongoing.

Received: June 1, 2012
Published online: July 16, 2012

Keywords: aureothin · cytochrome P450 · natural products · oxidation · parallel kinetic resolution

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