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Chemoenzymatic Total Synthesis of the Antiproliferative Polyketide (+)-(*R*)-Aureothin

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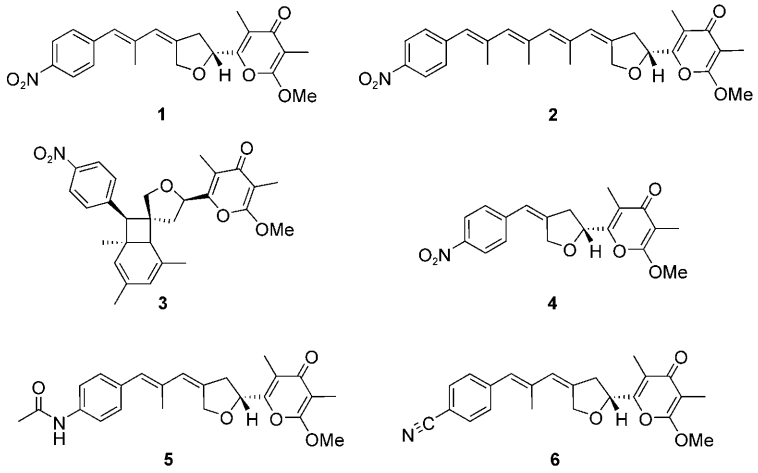
Aureothin (**1**) is a densely functionalized polyketide metabolite of *Streptomyces thioluteus* that efficiently stalls proliferation of various tumor cells.[1] The unusual nitroaryl and tetrahydrofuryl-pyrone moieties are shared by a number of related *Streptomyces* natural products, such as the HIV protease inhibitor neoaureothin (spectinabilin, **2**),[2] and the immunosuppressant SNF 4435C (**3**) and its diastereomer SNF 4435D.[3] The latter pair are derived from **2** by a unique photoinduced rearrangement cascade, which eventually yields the lower homologue orinocin (**4**) and mesitylene by "polyene splicing".[4] The natural (*Streptomyces*) and semisynthetic derivatives *N*-acetyl aureothamine (**5**)[5] and aureonitrile (**6**)[6] proved to be potent anti-*Helicobacter* and antiproliferative agents, respectively.



The structures and bioactivities of **1** and **3** have triggered great interest in their total synthesis.[7] However, the preparation of the chiral furyl segment proved to be most challenging due to facile racemization, and as of yet no enantioselective total synthesis of (+)-(*R*)-**1** has been reported.

We started the synthesis of the polyketide backbone with a modified Julia olefination of the known aldehyde **7**[11] with the 1-phenyl-1*H*-tetrazol-5-yl sulfone (PT-sulfone) **8a** (Scheme 1, Supporting Information).[12] Compound **8a** was in turn prepared by Williams-type thioetherification of ethyl-4-bromopentanoate with 1-phenyl-1*H*-tetrazole-5-thiol and oxidation of the resulting sulfide to the corresponding sulfone with ammonium molybdate tetrahydrate in H_2O_2.[13] Attempts to synthesize **8a** by nucleophilic substitution of 5-ethylsulfonyl-1-phenyl-1*H*-tetrazole with ethyl 3-bromopropionoate were not satisfactory, probably due to the less reactive methylene group in the α-position of the sulfone. The best results for the modified Julia olefination with **8a** were obtained with DME as solvent and KHMDS as base, yielding **9** in moderate yield (48%) and *E*/*Z* ratio (73:27). Yields were raised to 60% (*E*/*Z* = 63:37) with the *tert*-butyl derivative **8b** (TBT-sulfone). Ethyl ester **9** was reduced to aldehyde **10** by treatment with DIBAH, prior to chain extension in analogy with the synthesis of cyercene A and the placidenes.[14] Compound **10** was then first subjected to an aldol reaction with the dianion of methyl 2-methyl-3-oxopentanoate (**11**). Subsequent oxidation of the β-hydroxy alcohol **12** with DMP afforded diketoester **13**, which was cyclized to α-pyrone **14** with the aid of DBU. Finally, methyl fluorosulfonate was used to methylate the prototropic ambident nucleophile **14** regioselectively at the γ-position.[15]

As an alternative to chemical methylation we also employed the *S*-adenosyl methione (SAM) dependent regiospecific pyrone methyltransferase AurI. To test its potential for transforming the unnatural substrate **14**, AurI was heterologously produced in *S. lividans* and *S. albus* host strains by use of the expression plasmid pHJ95. Pyrone **14** (1.3 μmol in DMSO) was administered to cultures of these strains, and the course of the biotransformation was monitored by LC-MS analysis of the crude extracts. We observed the formation of the desired γ-pyrone **15** together with an uncharacterized side product in the broth of *S. albus*/pHJ95 (Supporting Information). While this approach is generally viable, we continued the synthesis with the chemically methylated pyrone because of the higher yields.

We next employed vinyl iodide **15** for a $Pd(PPh_3)_4$-catalyzed Stille coupling for completion of the polyketide backbone. Iodine/tin exchange provided a stannane that was immediately coupled with 1-iodo-4-nitrobenzene, yielding isomerically pure deoxyaureothin (**16**, 82%; Figure 1).

Compound **16** represents the last intermediate in the aureothin pathway. The final biosynthetic steps are catalyzed by the unique bifunctional cytochrome P450 monooxygenase AurH. To circumvent challenges in electron transport and cofactor regeneration we performed the biotransformation in a whole-cell system. AurH was heterologously produced in *S. livid-*

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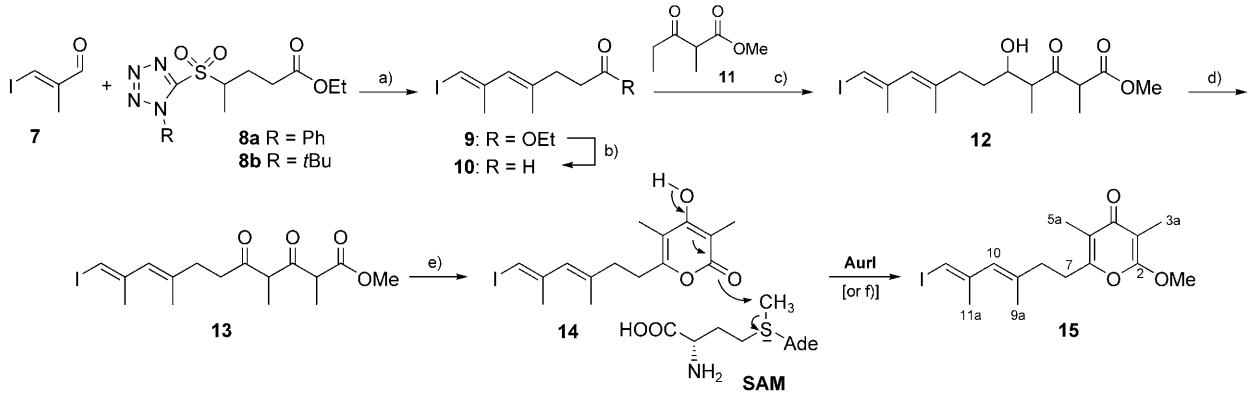

Scheme 1. Synthetic route to vinyl iodide **15**. a) KHMDS, DME, −60 °C, 48%, *E*/*Z* 73:27; b) DIBAH, toluene, −78 °C, 88%; c) NaH, *n*BuLi, THF, 0 °C, 55%; d) DMP, CH_2Cl_2, 0 °C to RT; 46%; e) DBU, benzene, 80 °C, 62%; f) either biotransformation by AurI (Ade = adenosyl) or treatment with $MeOSO_2F$, CH_2Cl_2, 0 °C to RT, 80%.

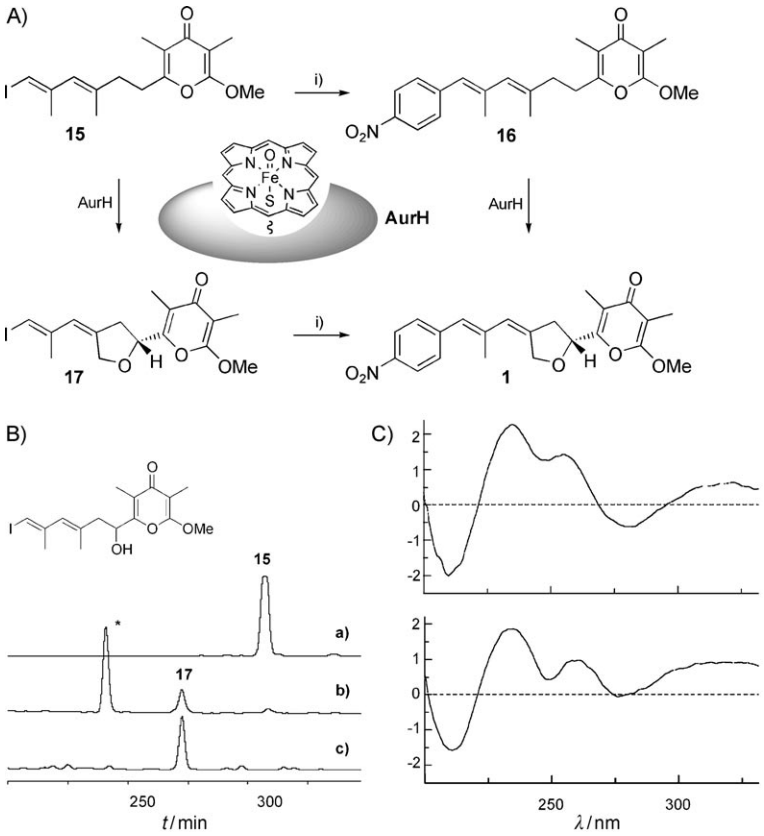

Figure 1. A) Synthesis of **1** from vinyl iodide **15**. a) Stille coupling; Sn_2Me_6, $Pd(PPh_3)_4$, then 1-iodo-4-nitrobenzene, CuI, Pd_2dba_3, THF, reflux, (85%). B) HPLC profile: in vivo biotransformation of vinyl iodide **15** with AurH (32–75%); a) reference **15**, b) after 1 d, c) after 3 d. C) CD spectra of semisynthetic **1** (top) and of reference (+)-(*R*)-**1** from *S. thioluteus* (bottom).

ans ZX1 by use of the expression plasmid pHJ110.[10b] HPLC analysis documented the complete transformation of **15** into enantiomerically pure **17** (Figure 1).

This reaction in itself completes the first chemoenzymatic and biomimetic synthesis of **1**. However, we also sought to modify the route to make it more flexible for aureothin analogues, testing earlier synthetic intermediates such as **13**, **14**, and **15** as substrates for AurH. Compounds **13** and **14** either were not or were only partially converted, possibly because of the lacking O-methylated pyrone moiety. In sharp contrast, the transformation of **15** gave a major product with the expected mass (M: 402) of the heterocyclic compound, so the iodine moiety seems to be bioisosteric with the nitroaryl group of deoxyaureothin. Interestingly, during the early stage of the biotransformation another peak occurred, decreasing over time. HR-MS data pointed towards a monohydroxylated intermediate, most likely the 7-OH derivative (Figure 1).

A larger scale biotransformation of **15** with *S. lividans* ZX1/pHJ110 was carried out in order to provide enough material for a full structure elucidation and further synthetic steps. The crude extract was subjected to silica gel column chromatography, and final purification by semipreparative HPLC yielded pure **17** (31%). NMR, HR-MS, and IR data unequivocally corroborated the structure of **17** (Figure 1). Future in vitro work will be directed towards improving the currently mediocre yields, which are likely due to microbial degradation. However, having enantiomerically pure vinyl iodide **17** to hand, we probed it for the Stille coupling. After iodine/tin exchange the intermediary stannane was readily coupled with 1-iodo-4-nitrobenzene as shown for **16** and in the previous racemic syntheses of **1** and **4**.[5,7c] For the determination of the absolute configuration we compared the CD spectra of the chemoenzymatically synthesized product with an authentic sample of (+)-(*R*)-**1** (Figure 1). The identity of both spectra clearly indicated that the two samples share the same stereochemistry.

The synthesis of therapeutics by taking advantage of synergism of synthetic and enzymatic transformations is an emerging field.[16] Here we report the first asymmetric synthesis of the antiproliferative agent (+)-(*R*)-aureothin by a chemoenzymatic approach. The polyketide backbone was assembled in a modular fashion by chemical synthesis and tailored enzymati-

cally by the regioselective pyrone methyl transferase AurI and the bifunctional cytochrome P450 monooxygenase AurH, which is capable of installing two C–O bridges. Besides deoxyaureothin we also succeeded in introducing the oxa heterocycle into a bioisosteric vinyl iodide by asymmetric enzymatic oxygenation. The resulting synthetic building block was successfully employed for a Stille coupling yielding (+)-(*R*)-**1**, as confirmed by CD spectroscopy. Although cytochrome P450 enzymes have been used extensively in biotransformations, to the best of our knowledge these results represent the first example of the use of an asymmetric cytochrome P450-mediated furan heterocyclization in synthesis. Our modular approach should provide the basis for the synthesis of various analogues of the aureothin family of polyketides.

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Keywords: antitumor agents · biotransformations · natural products · oxygenases · polyketides

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