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Total Synthesis of Ripostatin A

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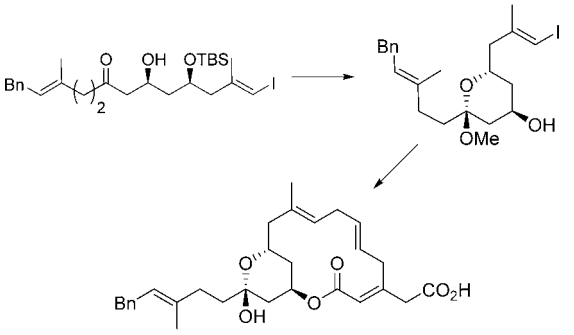
ABSTRACT



Ripostatin A, **1**

The first total synthesis of the bacterial RNA-polymerase inhibitor ripostatin A (1) was achieved. The route utilizes a cyclic methyl acetal intermediate and a sequence of a double Stille cross-coupling reaction followed by a ring-closing metathesis for the construction of the macrolactone ring. Additionally, an unprecedented formation of the 4-methoxy substituted tetrahydropyrans was observed during the acid catalyzed acetalization of the β,δ-dihydroxyketone.

Overcoming the emerging resistance of pathogenic bacteria to the antibiotics currently in use requires a continuous exploration of new cellular targets. In this regard, the bacterial RNA-polymerase is considered as an underexploited target, with only one marketed drug existing at present. Among several natural products with bacterial RNA-polymerase inhibitory activity (Figure 1), ripostatins[1] A (**1**) and B (**2**) attracted a considerable amount of synthetic efforts from high profile research groups.[2] Since the ripostatins, along with corallo- and myxopyronin (**4**), bind to the RNA-polymerase at a different binding site compared to rifampicin (**3**),[3] they were regarded as promising lead structures for the development of new antibiotic agents.[4]

The chemical challenges in the synthesis of these molecules arise from the sensitive nature of skipped polyene motifs within the macrocycle (C2−C9). Additionally, ripostatin A is inherently unstable under even slightly basic conditions and undergoes a ring-opening β-elimination leading to the inactive ripostatin C.[1b] Recently, three total syntheses of ripostatin B were independently accomplished.[5] We also reported the synthesis of 15-deoxyripostatin A, which was used as a molecular probe to determine the structural requirements for efficient binding to RNAP, and found it to be inactive against several bacterial strains, thus underlining the importance of the carbonyl or hydroxyl group in the C-15 position for the RNA-polymerase inhibitory activity. To make our SAR studies of ripostatin analogs more comprehensive, an access to the ripostatin A and its modified derivatives was required. Herein we report further development of our synthetic strategy and its application in the total synthesis of ripostatin A.

In our retrosynthetic analysis we opted to protect the ketone functionality of ripostatin A as a cyclic methyl acetal (Figure 2). Construction of the macrocyclic lactone would utilize a sequence of a double Stille cross-coupling

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(2) (a) Kujat, C.; Bock, M.; Kirschning, A. *Synlett* **2006**, 419–422. (b) Schleicher, K. D. PhD thesis, Massachusetts Institute of Technology, 2010.

(3) Mukhopadhyay, J.; Das, K.; Ismail, S.; Koppstein, D.; Jang, M.; Hudson, B.; Sarafianos, S.; Tuske, S.; Patel, J.; Jansen, R.; Irschik, H.; Arnold, E.; Ebright, R. H. *Cell* **2008**, *135*, 295–307.

(4) Häbich, D.; Nussbaum, F. *Angew. Chem., Int. Ed.* **2009**, *48*, 3397–3400.

(5) (a) Winter, P.; Hiller, W.; Christmann, M. *Angew. Chem., Int. Ed.* **2012**, *51*, 3396–3400. (b) Tang, W. F.; Prusov, E. V. *Angew. Chem., Int. Ed.* **2012**, *51*, 3401–3404. (c) Glaus, F.; Altmann, K. H. *Angew. Chem., Int. Ed.* **2012**, *51*, 3405–3409.

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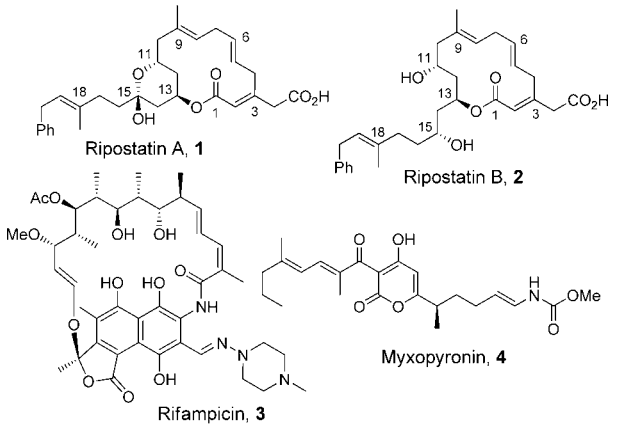


Figure 1. Natural products with bacterial RNA-polymerase inhibitory activity.

reaction and ring-closing metathesis. The required double vinyliodide precursor can be obtained via esterification of the β-idodoacrylic acid with a 4-hydroxy-THP-intermediate, which, in turn, should be easily accessible from the corresponding polyol fragment.

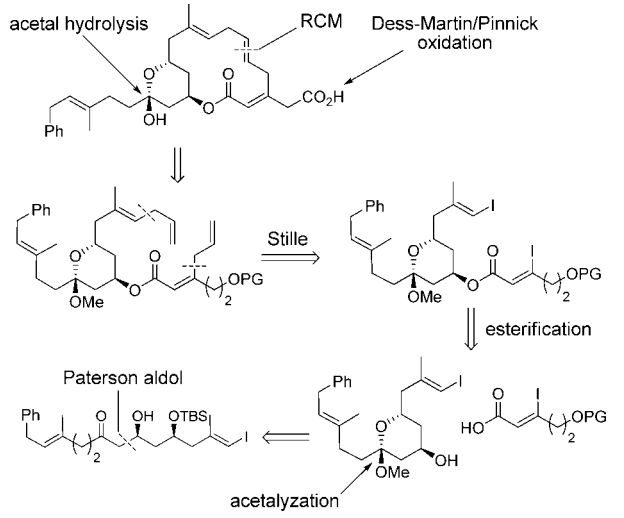
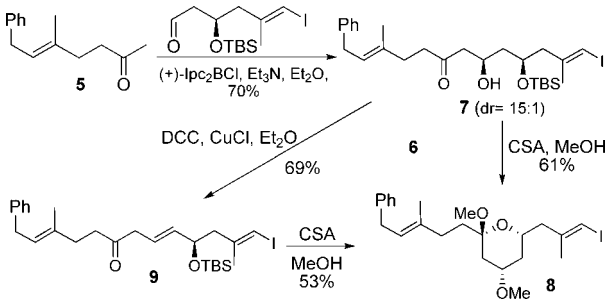


Figure 2. Retrosythetic analysis of ripostatin A.

Taking advantage of the correctly positioned carbonyl group in the Paterson aldol[6] adduct **7**,[5b] a direct conversion to the corresponding cyclic acetal was attempted. Whereas no reaction occurred with PPTS as a catalyst, exposure of the hydroxyketone **7** to CSA in methanol resulted in complete consumption of the starting material and formation of a new product. However, analysis of its NMR spectra revealed the presence of a second methoxy group in the C-13 position of the tetrahydropyrane ring (Scheme 1, ripostatin A numbering). Except for the original report on ripostatin A isolation,[1a] we found no evidence in the literature regarding such concomitant formation of the methyl ether during the acetalization of β,δ-dihydroxyketones. We speculate that the ether **8** forms via the acid catalyzed water elimination followed by the addition of methanol. In a control experiment, exposure of the separately prepared α,β-unsaturated ketone **9** to the reaction conditions resulted in formation of **8** in 53% yield.[7]

Scheme 1. CSA-Catalyzed Deprotection/Acetalization[a]



[a] CSA = D,L-10-camphorsulfonic acid, DCC = dicyclohexylcarbodiimide.

Being unable to achieve a direct synthesis of methyl acetal, we decided to employ a two-step protocol. Treatment of the aldol adduct **7** with TBAF in DMF[8] led to a clean conversion into the corresponding β,δ-dihydroxyketone, which upon exposure to PPTS in a mixture of MeOH and $HC(OMe)_3$ undergoes clean cyclization into the desired intermediate **10** (Scheme 2). We next examined the use of a modified Yamaguchi protocol[9] for esterification of the iodoacrylic acid **11** as it was done in the synthesis of ripostatin B. However, a direct application of the earlier established conditions afforded the target ester **12** with yields varying between 45% and 51%, along with several byproducts which arise from HI elimination (**13**) and subsequent water addition (**14**).

Seeking to improve this step, we studied the acylation of sodium alkoxide[10] derived from the alcohol **10** with the corresponding acid chloride but obtained a significantly lower yield of the desired ester.

During our synthesis of 15-deoxyripostatin A we established that Mitsunobu reaction[11] provides superior results

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(7) Complete details of this investigation will be published in a separate communication.

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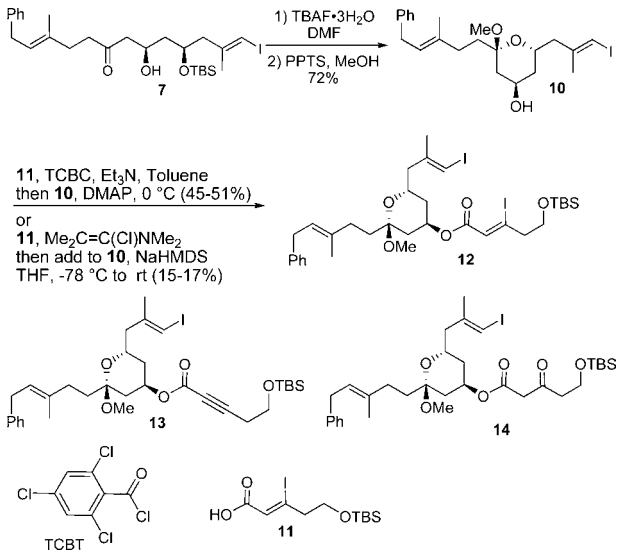
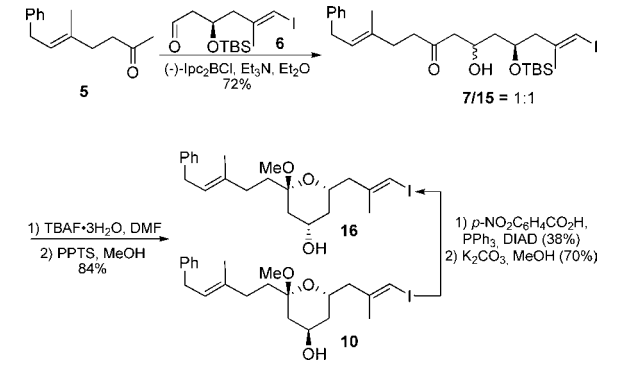
Scheme 2. Esterification of the Cyclic Acetal Core



for the ester formation in the case of the 4-hydroxytetrahydropyran system. Since Mitsunobu esterification proceeds with the inversion of configuration at the alcohol position, preparation of the diastereomeric β-hydroxyketone utilizing an enantiomeric enolization agent in the Paterson aldol reaction was investigated (Scheme 3). Unexpectedly, a nearly 1:1 mixture of diastereomeric adducts was obtained, which implies the existence of a strong substrate facial selectivity bias in this particular reaction.[12] The aldol adducts were carried through two following steps and separated at the stage of cyclic methyl acetals **10** and **16**.

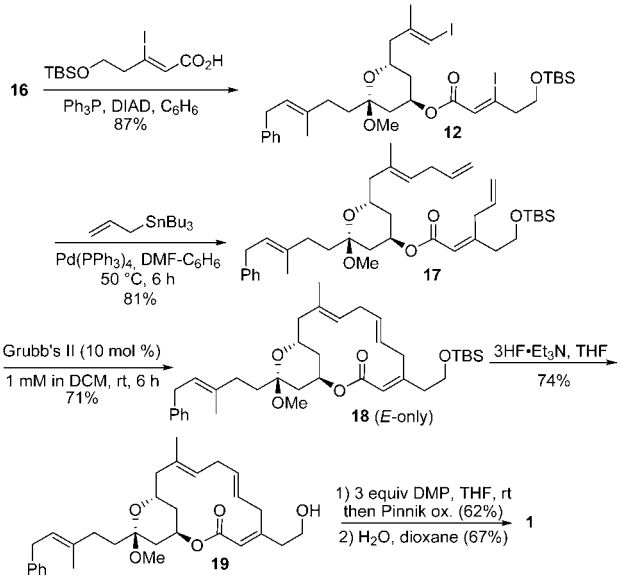
Scheme 3. Alternative Route to the Cyclic Acetal Core



Here, several attempts were made to perform the isomerization of **10** to **16** by ester formation with *p*-nitrobenzoic or acetic acid and following ester cleavage. However, the overall yield of these sequences was rather low. Therefore, the methyl acetals **10** and **16** were separately converted to the ester **12** via Yamaguchi or Mitsunobu esterification methods, respectively.

According to our plan, two terminal allylic groups were simultaneously introduced via Stille cross-coupling[13] with allyltributylstannane and the resulted diene **17** was subjected to the ring-closing metathesis reaction[14] under standard conditions to give the macrocyclic lactone **18** in 71% yield as a single geometrical isomer (Scheme 4). Deprotection of the primary TBS group using the HF•Et_3N complex in THF followed by implementation of the one-pot Dess-Martin oxidation/Pinnick oxidation protocol reported by Altmann[5c] provided us with the earlier described ripostatin A methyl acetal. The last challenge was to achieve a mild hydrolysis of the cyclic acetal **19** in the presence of a rather sensitive skipped polyene motif. Several conditions were investigated in order to liberate the ketone group of ripostatin A, and it was found that simple exposure of **19** to the aqueous dioxane resulted in a clean conversion to the target compound.

Scheme 4. Endgame of the Synthesis



From our previous experience with ripostatin B and some other natural products we learned that the appearance of ^1H and ^{13}C NMR spectra of these compounds can be greatly affected by exact isolation and purification procedures. In order to perform a correct comparison of spectroscopic data of our material with those reported in the literature, a predominantly keto-form of ripostatin A was obtained by slow evaporation of its water–methanol

(12) This result not only contradicts the expected outcome of the Paterson aldol reaction, but also, according to the polar Cornforth model, an 1,3-*anti* aldol adduct should be favorable in the case of β-silyloxy aldehydes. At present, we have no explanation for this phenomenon.

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solution. The resulted amorphous solid was dried *in vacuo*, and both ^{1}H and ^{13}C NMR spectra were recorded immediately after sample preparation. The measurements were repeated after 48 h and revealed, as expected, the presence of a 4:3 equilibrium mixture of keto- and cyclic acetal forms.[15]

In summary, the first total synthesis of the bacterial RNA-polymerase inhibitor ripostatin A was accomplished. The longest linear sequence consist of 14 steps (starting from *S*-epichlorohydrine) and proceeds with 5% overall yield. The key steps in the synthesis are a Paterson aldol reaction, Mitsunobu esterification, double Stille cross-coupling reaction, and ring-closing metathesis. The success of this synthesis confirmed the applicability of our strategy for the preparation of macrocyclic skipped trienes. Additionally, a rather interesting and earlier undescribed formation of methyl ethers during acid catalyzed acetalization was discovered. Further applications of this general approach to the synthesis of other stabilized analogs of ripostatin A and B are currently underway in our laboratory and will be reported in due course.

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

(15) The spectroscopic properties of synthetic **1** were in good agreement with the literature data for ripostatin A (^{1}H and ^{13}C NMR, HRMS, UV, and optical rotation).[1b] The ^{1}H NMR spectrum of our material corresponds exactly with that of the natural product (a graphical comparison is provided in the Supporting Information). Deviation of some ^{13}C-resonances can be explained by concentration effects and residual amounts of buffer components present in the original specimen. $[\alpha]_D^{20} = +15.2$ ($c = 0.5$ in MeOH); lit. $[\alpha]_D^{20} = 15.1$ ($c = 1$ in MeOH).

The authors declare no competing financial interest.