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Ripostatin B (2)

Total Synthesis of RNA-Polymerase Inhibitor Ripostatin B and 15-Deoxyripostatin A**

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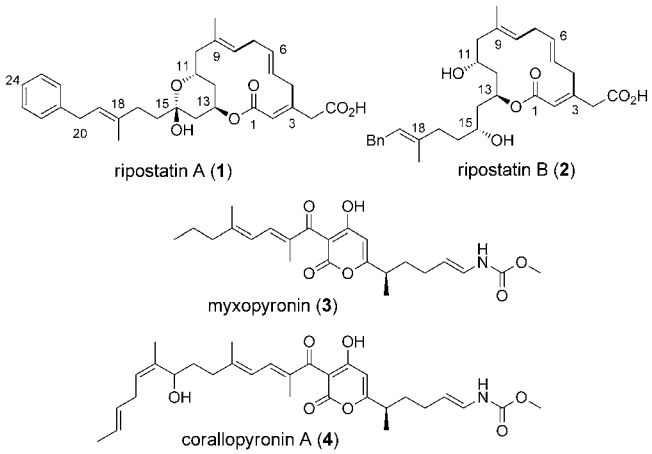
Natural products isolated from marine and terrestrial microorganisms, plants, and fungi play a crucial role in drug discovery and development in important areas, such as antibiotics research and cancer treatment.[1] The emergence of multi-drug-resistant strains over the past decades is a problem that plagues public health.[2] Consequently, the continuous search for new drug candidates that use a novel mode of action is essential for effective infection control measures.

The ripostatins A (**1**) and B (**2**; Scheme 1) are secondary metabolites isolated in 1994 by Höfle et al. from the fermentation broth of the gliding bacteria strain *Sorangium cellulosum* So ce 377.[3a] They are polyketide macrolides and characterized by a 14-membered macrolactone with an attached side chain. The synthetic challenge of these molecules is derived through three separated double bonds that easily isomerize into conjugation under acidic or basic conditions. In particular at the stage of synthetic intermediates, this problem generates a significant hurdle. Moreover, the C2–C3 *Z*-configured double bond is prone to isomerization into conjugation with the acid functionality.



Scheme 1. Natural products that are inhibitors of the bacterial RNA polymerase.

The initial biological evaluation of ripostatins revealed only modest activity against *Staphylococcus aureus* and *Escherichia coli* with minimal inhibitory concentration (MIC) values in the range of 1 $\mu g\,mL^{-1}$.[3b] At the same time, the ripostatins were found to have a cytostatic effect on L-929 mouse fibroblast cells.

However, more recently Ebright and co-workers reported the crystal structure of the bacterial RNA polymerase with myxopyronin (**3**); in that crystal structure the secondary metabolite is bound to the "switch region" of the polymerase.[4] Further studies with mutant bacterial strains provided strong evidence that corallopyronin A (**4**) and ripostatin A (**1**) bind to the same pocket. The fact that the amino acid sequence of the RNA polymerase is highly conserved among bacterial species but differs substantially from that of the mammalian enzyme makes the polymerase an appealing target for the development of new antibiotics.

Consequently, we became attracted to the ripostatins as a promising lead structure in our efforts to discover and develop novel antibiotics. Our aim was to provide a general synthetic access to the ripostatin framework and answer fundamental questions regarding its synthesis and structure–activity relationship by providing 15-deoxyripostatin A (**27**) and ripostatin B (**2**) as probes to answer the question whether the open-chain isomer or the cyclic hemiacetal are responsible for the biological activity of the ripostatins. Although preliminary studies towards the total synthesis of ripostatins were published by Kirschning and co-workers,[5] the construction of the macrocyclic lactone was proven to be rather difficult. The main challenge in the synthesis of ripostatins is to establish and maintain a so-called "skipped polyene" motif (C2–C9) within the macrocyclic ring; this motif is notorious for its lability and tendency to isomerize into the conjugated dienoate.[6]

We envisioned a retrosynthetic strategy (Scheme 2), which we hoped would overcome those difficulties by introducing the "skipped polyene" at a late stage in the synthesis and by performing the ring closure immediately afterwards. We argued that a double Stille cross-coupling directly followed by a ring-closing metathesis might be the key transformation for the successful construction of ripostatin B. The required precursor **5** can be derived by attachment of two allylic groups to the double vinyl iodide **6**, which,

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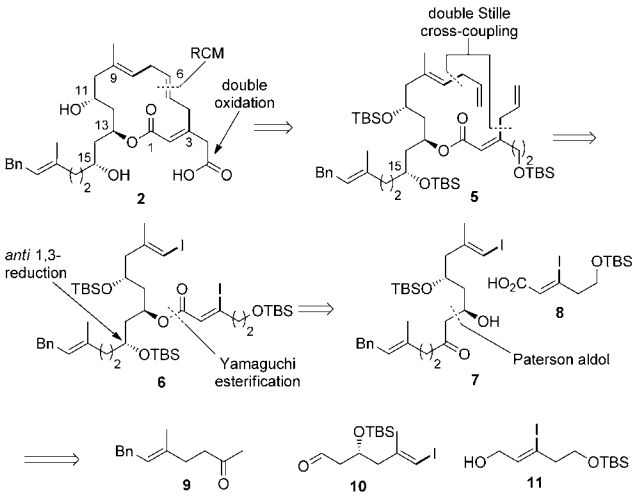
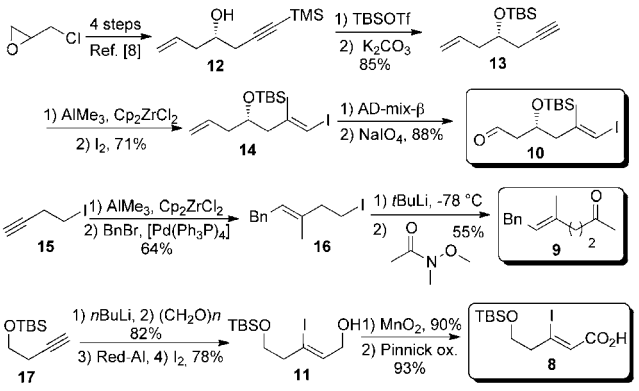


Scheme 2. Retrosynthetic analysis of ripostatin B. TBS = *tert*-butyldimethylsilyl, RCM = ring-closing metathesis.

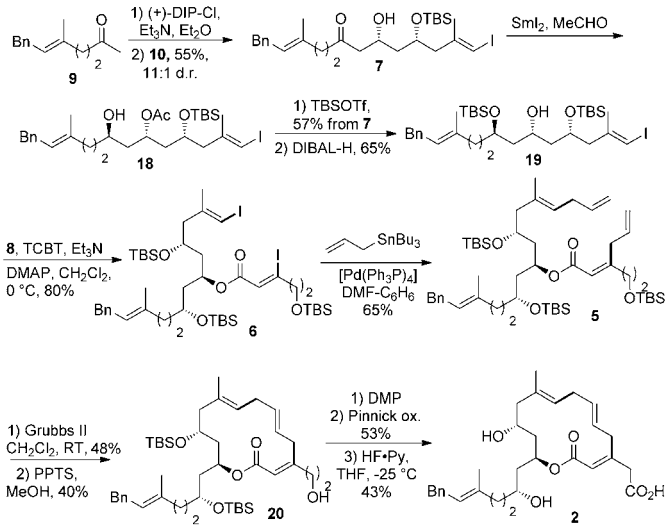
in turn, can be obtained through esterification of the iodoacrylic acid **8** with the corresponding alcohol fragment.

Our synthesis takes advantage of three equally complex building blocks and is outlined in Scheme 3. In the synthetic direction, (±)-epichlorohydrin was converted into the (*S*)-7-(trimethylsilyl)hept-1-en-6-yn-4-ol **12** in four steps according to the published procedure.[7] After simple protecting-group manipulations, the terminal vinyl iodide moiety was installed by carboalumination with subsequent iodine quench. Dihydroxylation of the double bond using the Sharpless protocol and subsequent periodate cleavage furnished aldehyde **10**. The intermediate for the Paterson aldol reaction **9** was readily synthesized from 3-butyn-1-iodide (**15**) using a one-pot carboalumination/cross-coupling reaction[8] with benzyl bromide followed by halogen–metal exchange and acylation with the Weinreb amide of acetic acid. The third segment, the carboxylic acid **8**, was prepared from TBS-protected 3-butyn-1-ol in five steps, including one carbon elongation, *cis*-selective reduction of triple bond with Red-Al followed by iodine quench and two consecutive oxidations with manganese oxide and sodium chlorite.[9]

With these building blocks in hand, the assembly of ripostatin B (**2**) was attempted (Scheme 4). At first, fragments **9** and **10** were connected using a Paterson aldol reaction[10]



Scheme 3. Synthesis of the main building blocks. *n*BuLi = n-butyllithium, *t*BuLi = *tert*-butyllithium, TBSOTf = *tert*-butyldimethylsilyl trifluoromethane sulfonate, Red-Al = sodium bis(2-methoxyethoxy)-aluminium hydride, Cp_2ZrCl_2 = bis(cyclopentadienyl) zirconium dichloride, Pinnick ox. = $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *tert*-BuOH/H_2O.



Scheme 4. Completion of the synthesis. (+)-DIP-Cl = (+)-B-chlorodiisopinocampheylborane, DIBAL-H = diisobutylaluminum hydride, TCBT = 2,4,6-trichlorobenzoyl chloride, DMP = Dess–Martin periodinane, DMAP = 4-dimethylaminopyridine, PPTS = pyridinium *p*-toluenesulfonate, Grubbs II = (1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(phenylmethylene)(tricyclohexylphosphine)ruthenium, py = pyridine.

that proceeds in a good yield and diastereoselectivity. No attempts were made to improve either the yield or selectivity since both starting materials were readily available. Then, hydroxyketone **7** was subjected to an *anti* 1,3-reduction according to the Evans–Hoveyda[11] protocol to give the acetate-protected diol **18**. Its absolute configuration was confirmed to be 15-*R* by Mosher ester analysis.[12] After careful examination of different condensation protocols, the esterification under Yamaguchi[13] conditions was found to be the method of choice for linking the iodoacrylic acid fragment **8** to the alcohol **19**. In the next step, the simultaneous introduction of two terminal allylic groups was performed by the Stille cross-coupling[14] with excess of allyltributylstannane. The reaction proceeded without any difficulties at elevated temperatures in a mixture of benzene and DMF in the presence of 5 mol% tetrakis(triphenylphosphin)palladium as catalyst. At this stage we realized that attachment of the allylic group to the acrylate fragment by cross-coupling reaction after the esterification step is crucial for the success of the synthesis; otherwise a rapid shift of the double-bond position under esterification conditions would occur.

In the last stage of the synthesis, the macrolactone ring was formed by exposure of a 1 mM solution of the diene **5** in dichloromethane to the second-generation Grubbs catalyst at room temperature, whereas reaction at reflux temperature

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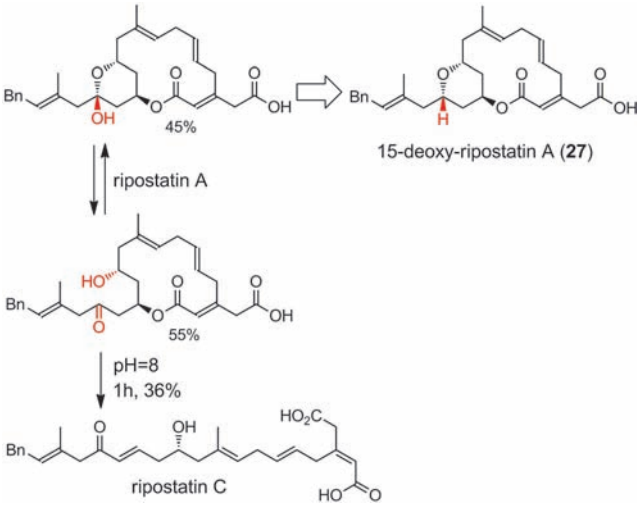
produced a substantial number of side products. Gratifyingly, only the *trans*-alkene product was isolated from the metathesis reaction.[15] Finally, the exocyclic carboxylic acid functionality was secured by selective cleavage of the primary TBS ether followed by two consecutive oxidations with Dess–Martin periodinane and sodium hypochlorite, respectively. To protect the rather electron rich "skipped polyene" motif from the electrophilic chlorine species during the Pinnick oxidation, it was imperative to add dimethyl sulfoxide (DMSO) to the reaction mixture as a co-solvent and scavenger;[16] otherwise, a number of unidentified by-products were produced. Finally, the global deprotection was achieved by treatment of the penultimate intermediate with the Olah reagent at −25 °C to give ripostatin B in 43 % yield.[17]

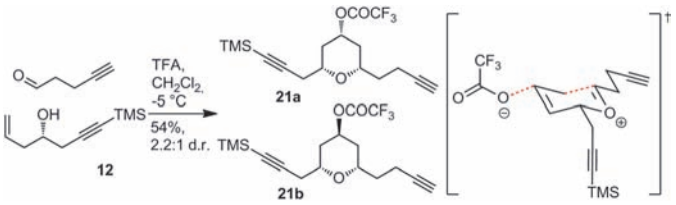
To gain a deeper understanding of the binding mode of the ripostatins, we decided to clarify whether the hemiketal or ketone form of ripostatin A (Scheme 5) is responsible for its



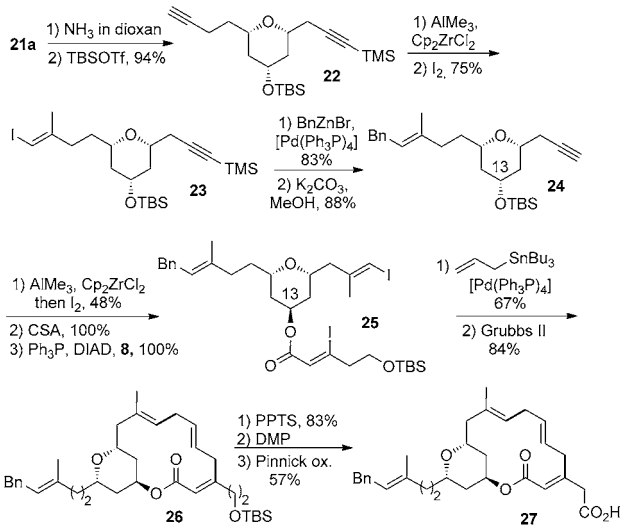
Scheme 5. Keto–hemiketal equilibrium in ripostatin A, decomposition pathway, and proposed stabilized 15-deoxy-analogue.

biological activity. It is also known that ripostatin A is not stable under slightly basic conditions and decomposes into inactive ripostatin C by β-acetoxy elimination from the ketone form.[3a] Therefore, the 15-deoxyripostatin A was proposed as a stable structural analogue, in which the tetrahydropyran moiety would represent the locked keto–hemiketal equilibrium.

The tetrahydropyrane core of the ripostatin A analogue **27** was easily accessed through a Prins cyclization[18] between the previously described homoallylic alcohol **12** and 4-pentynal (Scheme 6). Under optimized conditions, this transformation produced a mixture of two separable diastereomers in a 2.2:1 ratio and 54 % combined yield. As expected, the all-*syn* diastereomer **21a** was the major product. The poor diastereoselectivity at this step was not an issue, because both diastereomers were taken further in the synthesis and converted later into ester **25** (Scheme 7) employing an appropriate esterification method, either using the Yamaguchi protocol or Mitsunobu[19] conditions with concomitant inversion at carbon atom C13.



Scheme 6. Synthesis of the tetrahydropyrane core by Prins cyclization.



Scheme 7. Synthesis of the 15-deoxyripostatin A. CSA = 10-camphorsulfonic acid, DIAD = diisopropyl azodicarboxylate.

According to our plan, the benzyl side chain was to be introduced through regioselective elaboration of the left-hand triple bond in the presence of the other trimethylsilyl-protected one. Unfortunately, despite numerous attempts, we were unable to perform carboalumination and cross-coupling reactions in a one-pot manner as it was used earlier for 3-butyn-1-iodide (**15**). We propose that this failure may be attributed to coordination effects of the vinylaluminum species with the oxygen atom of the tetrahydropyrane. Nevertheless, conversion into vinyl iodide **23** followed by Negishi cross-coupling with benzylzinc bromide[8a] provided us with the desired product. Except for removal of the trimethylsilyl group from the left-hand triple bond and Mitsunobu esterification, the remaining steps in the synthesis parallel those described for ripostatin B.

The biological validation identified 15-deoxyripostatin A as inactive against several bacterial strains. Among several possible explanations, we rationalize that the keto form is necessary for binding to the RNA polymerase. Alternatively, a functional group with strong hydrogen-bond donor–acceptor properties must be present near or within the tetrahydropyrane cycle.

In summary, a short and efficient total synthesis of ripostatin B is described in which the longest linear sequence includes 18 steps and 0.22 % overall yield. Because of its convergent nature the synthesis route enables the rapid assembly of further analogues to improve the pharmacolog-

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ical properties of the ripostatins required to advance this promising lead into medicinal applications. The key steps to avoid double-bond isomerization of the skipped triene are a double Stille cross-coupling reaction and a ring closing metathesis. Furthermore, a stable and conformationally locked analogue of ripostatin A was prepared and tested in vivo. Further work to produce optimized analogues is currently in progress and will be reported in due course.[20]

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