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Total Synthesis of the Myxobacterial Macrolide Ripostatin B‡

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Abstract: This article describes the total synthesis of ripostatin B, which is a 14-membered macrolide of myxobacterial origin that inhibits *E. coli* RNA polymerase by a different mechanism of action than the first-line anti-tuberculosis drug rifampicin. Structurally, ripostatin B features a labile and synthetically challenging doubly skipped triene motif embedded in the macrolactone ring. Key steps in the synthesis were a Paterson aldol reaction, a low-temperature Yamaguchi esterification and an alkene metathesis reaction to close the macrolide ring. The natural product was synthesized in a longest linear sequence of 21 steps and 3.6% overall yield.

Keywords: Antibiotics · Ring-closing metathesis · Ripostatin · RNA polymerase · Total synthesis

Ripostatins A and B (**1**) (Fig. 1) were first isolated in 1995 by Reichenbach, Höfle and co-workers from the myxobacterium *Sorangium cellulosum* (strain So ce 377) found in a soil sample from Kenya.[1] Both compounds are 14-membered macrolides that differ only in the oxidation state at C(15) (ripostatin A exists as a hemiacetal/ketone mixture in a 4:3 ratio). The two ripostatins exhibit a similar, albeit narrow spectrum of antibiotic activity against gram-positive bacteria, with minimum inhibitory concentrations (MICs) of less than 1 μg/mL only against *S. aureus* and the hyperpermeable *E. coli* strain DH21tolC.[1a] Additionally, ripostatins A[1a] and B[2] both inhibit the DNA-dependent RNA polymerase (RNAP) from *E. coli* with sub-μM activity, while being inactive against eukaryotic RNA polymerase II.[1a]

RNA polymerase is a suitable target for antibacterial therapy.[3] It is an essential enzyme (permitting efficacy) and it is highly conserved across gram-positive and -negative bacteria (permitting broad-spectrum activity), while it differs significantly from mammalian RNA polymerases I, II and III (providing therapeutic selectivity).[4–6] RNAP is also a clinically validated target, since it is the target of the rifamycin-class of antibiotics (the most prominent member being rifampicin).[7,8] The rifamycins are clinically used for the treatment of both gram-positive and gram-negative bacteria.[9] They have proven particularly effective in the treatment of tuberculosis, where they are used as first-line drugs.[6,7] However, the number of bacterial strains resistant to the rifamycins is increasing.[7,10] Consequently, there is an urgent need for new RNAP-inhibitors that exhibit a different mode of action.

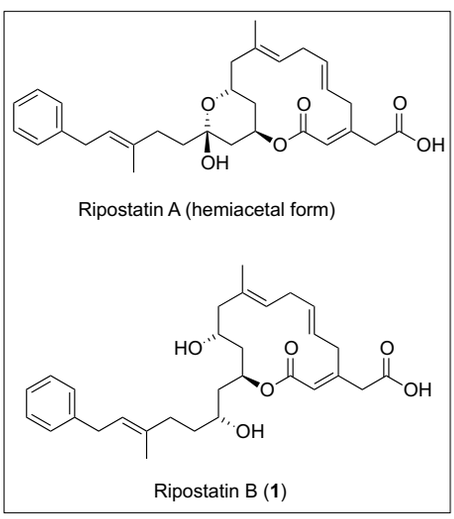

Fig. 1. Ripostatins A and B.

Ebright and co-workers have recently reported an X-ray crystal structure of a complex between *T. thermophilus* RNAP and myxopyronin, another myxobacterial RNA polymerase inhibitor.[4] The compound binds to the hinge that mediates opening and closing of the RNAP clamp, thus trapping the enzyme in a closed conformation and preventing access of the dsDNA to the active-site cleft.[4,11] This binding mode is fundamentally different from that of the rifamycin-type inhibitors, which prevent the extension of RNA beyond a length of 2–3 nucleotides by binding to a site close to the active-site cleft. Based on the location of RNAP mutations in myxopyronin- and ripostatin A-resistant *E. coli*, Ebright and co-workers have suggested that ripostatin A (as well as a number of other natural products) likewise inhibit(s) bacterial RNAP by binding to the hinge region of the enzyme.

The attractive biological profile of the ripostatins as well as their interesting chemical structure (notably the C(2)–C(9) doubly skipped triene motif) prompted us to embark on the total synthesis of ripostatin B[12] with the goal to provide a chemical basis for future SAR studies. No total synthesis of either ripostatin had been published prior to our initial communication. However, concurrent with our own work total syntheses of ripostatin B were independently developed by Christmann and co-workers, and Prusov and Tang.[13a,b] In mid-2012, Prusov and Tang also published a total synthesis of ripostatin A.[13c]

Due to the anticipated tendency of the skipped triene-motif (C(2)–C(9)) for double-bond migration, we based our retrosynthetic analysis on a ring-closing metathesis (RCM), which, due to the absence of base in the ring-closing step, should minimize problems with the double-bond framework (Scheme 1).[14] After RCM, the primary hydroxyl group would be deprotected selectively and oxidized, thereby

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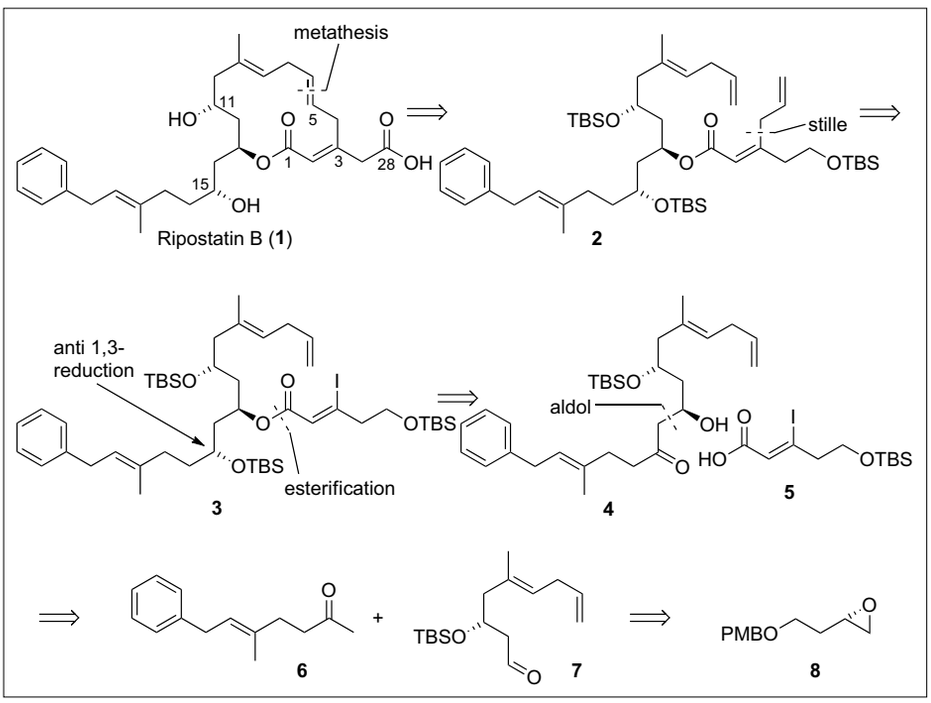
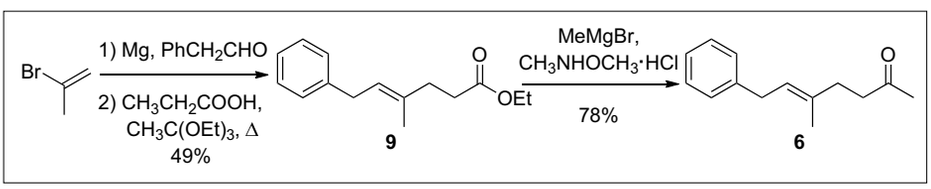
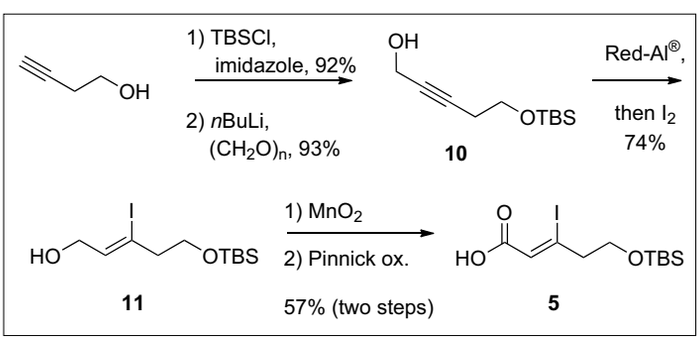
installing the vinylogous malonate-type C(1)-C(3)-C(28) framework. The RCM-precursor **2** could be accessible by Stille-coupling between an allylstannane and vinyl iodide **3**. The Stille reaction was specifically chosen because it is usually performed at neutral pH, which would minimize double-bond migration in the dienoic ester. Vinyl iodide **3** should be accessible by esterification between alcohol **4** and iodoacrylic acid **5**.[15] In principle, performing the Stille coupling with acid **5** (or a suitably protected derivative thereof) prior to esterification should lead to a more convergent approach toward the natural product. This approach, however, had failed in the hands of Kirschning and co-workers, due to the pronounced tendency for double-bond migration in the dienoic acid under a variety of basic esterification conditions (under neutral or acidic conditions, yields were generally unsatisfactory).[16] We intended to set the C(13)- and C(15)-stereocenters by an aldol reaction between ketone **6** and aldehyde **7** (stereocenter at C(13)), followed by an *anti* 1,3-reduction (stereocenter at C(15)). Compound **7** would in turn be accessible from epoxide **8**[17] by epoxide opening and subsequent elaboration of the diene moiety.



Scheme 1. Retrosynthetic analysis of ripostatin B. PMB = *para*-methoxybenzyl,TBS = *tert*-butyldimethylsilyl.



Scheme 2. Synthesis of ketone **6**.

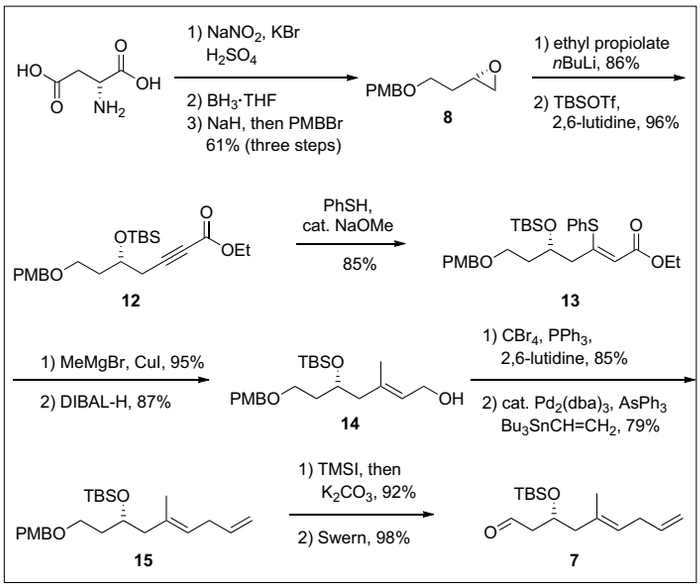


Scheme 3. Synthesis of acid **5**. Red-Al® = sodium bis(2-methoxyethoxy)aluminum dihydride.

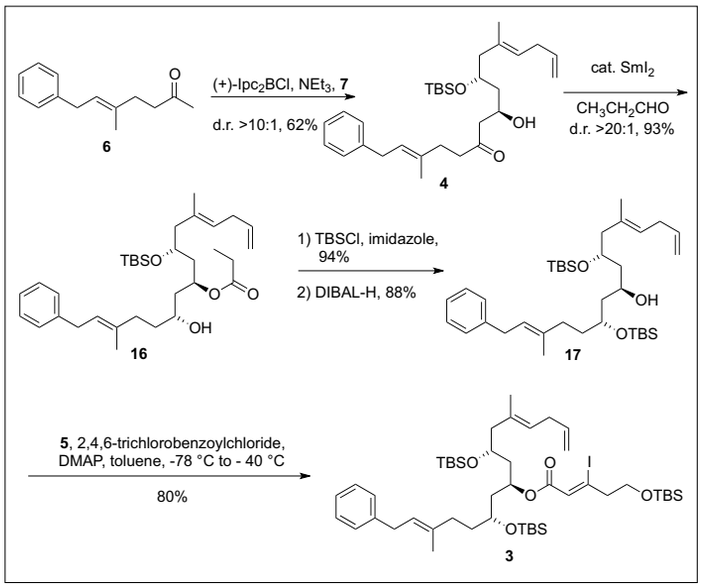
The synthesis of ketone **6** started with the addition of the Grignard reagent derived from 2-bromopropene to phenylacetaldehyde (Scheme 2). The resulting secondary alcohol was submitted to a Johnson-Claisen rearrangement with triethyl orthoacetate in the presence of a catalytic amount of propionic acid at 145 °C, giving γ,δ-unsaturated ester **9**[18] in 49% yield for the two-step sequence. By using Williams' procedure, which involves treatment of the ester with an excess of the Grignard-reagent in presence of *N*,*O*-dimethylhydroxylamine hydrochloride, **9** could be transformed into methyl ketone **6** in one pot.[19]

The known carboxylic acid **5**[15] was synthesized from 3-butyn-1-ol, which was TBS-protected and homologated with paraformaldehyde in 86% yield (two steps, Scheme 3). The triple bond was then reduced stereoselectively with Red-Al® and the intermediate aluminate was quenched with iodine to give vinyl iodide **11** in 74% yield. A two-step oxidation involving MnO_2-mediated conversion of the allylic alcohol into the aldehyde, followed by a Pinnick reaction afforded then iodoacrylic acid **5** in 57% yield from **11**.

The synthesis of aldehyde **7** departed from D-(−)-aspartic acid; this was converted into epoxide **8** *via* a known three-step sequence,[17,20] which involved displacement of the amino group by bromide under retention of configuration, reduction of both carboxylic acid groups to the alcohol stage, and finally epoxide formation by treatment of the diol with base (NaH) and PMBBr (Scheme 4). PMB-protected epoxy alcohol **8** was obtained in 61% yield for the three-step sequence. Epoxide opening with the anion derived from ethyl propiolate followed by TBS-protection of the resulting secondary alcohol then gave TBS-ether **12** in 83% yield (two steps). The trisubstituted *E*-configured double bond was installed next by treatment of **12** with thiophenol and a catalytic amount of sodium methoxide,[21] to provide thioether **13** (85%), followed by displacement of the phenylsulfenyl moiety by a methyl group with full retention of configuration by treatment with dimethylcuprate at −78 °C and warming to −30 °C over 30–40 minutes.[21] After DIBAL-H reduction, the resulting primary alcohol **14** was converted into the corresponding allylic bromide using Appel's conditions (CBr_4, PPh_3) in the presence of 2,6-lutidine;[22] this bromide was then converted into 1,4-diene **15** by Stille reaction with $Bu_3SnCH{=}CH_2$ using $Pd_2(dba)_3$ (12 mol%) as palladium source and 0.5 equiv. $AsPh_3$.[23] PMB-removal from **15** then turned out to be rather challenging. Deprotection could not be achieved under standard DDQ conditions, which might be attributed to the presence of the 1,4-diene moiety.[24] After the investigation of a range of methods, we finally found that TMSI[25] cleaves the PMB-ether very efficiently, leading, after cleavage of the ensuing TMS-ether with K_2CO_3 in methanol, to the desired primary alcohol



Scheme 4. Synthesis of aldehyde **7**. dba = dibenzylideneacetone, DIBAL-H = diisobutylaluminium hydride, DMF = *N*,*N*-dimethylformamide, DMSO = dimethylsulfoxide, *n*BuLi = *n*-butyllithium, Tf = trifluoromethanesulfonyl, TMS = trimethylsilyl.



Scheme 5. Assembly of **5**, **6** and **7**. DMAP = 4-dimethylaminopyridine, (+)-Ipc_2BCl = (+)-diisopinocamphenyl chloroborane.

in 92% yield. This alcohol was then oxidized under standard Swern conditions to aldehyde **7** in 98% yield.

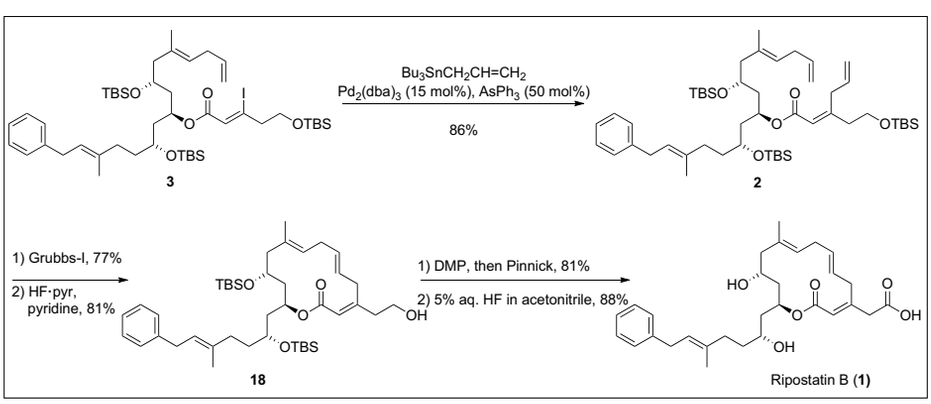
With all three building blocks in hand, the first step in their assembly was the Paterson-aldol reaction[26] between aldehyde **7** and ketone **6** (Scheme 5). The reaction proceeded in moderate yield (62%) but gave the desired aldol product **4** with reasonable stereoselectivity (d.r. >10:1). The C(15) stereocenter was then set by a samarium-mediated 1,3-*anti* reduction (Evans-Tishchenko reduction[27]) that furnished **16** in high yield (93%) and with excellent stereoselectivity (d.r. >20:1). The C(15) hydroxyl group was next protected with TBS before the propionate ester was reductively removed with DIBAL-H giving **17** in 83% yield for the two-step sequence.

A range of different esterification protocols was then examined for the condensation between **17** and iodoacrylic acid **5**, including the standard Yamaguchi[28] protocol, none of which provided reasonable amounts of ester **3**; in general, the major part of alcohol **17** was re-isolated unchanged. Best results were finally obtained using a modified Yamaguchi protocol that involved mixing of all components in toluene at −78 °C, before the reaction was allowed to warm to −40 °C over 2–3 h (Scheme 5). Following this approach **3** was obtained in 80% yield if two equivalents of iodoacrylic acid **5** were employed. Stille coupling between **3** and $Bu_3SnCH_2CH{=}CH_2$ then afforded diene **2** (Scheme 6),[23] thus setting the stage for the key ring-closing metathesis reaction.

While the Grubbs II and Hoveyda-Grubbs II catalysts both led to the formation of unidentified side products in the metathesis reaction, Grubbs I catalyst in dichloromethane afforded a much cleaner reaction, which furnished 77% of the macrocycle as a single isomer (Scheme 6). NMR analysis revealed the newly formed double bond to be *E*-configured. It proved important to terminate the reaction by addition of DMSO[29] before full conversion was reached, otherwise side products appeared (TLC analysis) and cyclization yields were lower.

After cleavage of the primary TBS-ether using pyridine buffered HF·pyridine, the free hydroxyl group needed to be oxidized to the carboxylic acid state, thereby installing the potentially labile vinylogous malonate-type system. Attempts at the direct oxidation of alcohol **18** to the corresponding carboxylic acid met with complete failure. Oxidation of **18** with Dess-Martin periodinane gave an aldehyde that was found to be stable in the reaction medium, but partly decomposed upon work-up (saturated aqueous $Na_2S_2O_3$/$NaHCO_3$), thus leading to low and erratic yields in a subsequent Pinnick-oxidation. Fortunately, a one-pot procedure, involving DMP-mediated oxidation in THF followed by Pinnick oxidation gave the desired carboxylic acid in 81% yield.[30] Finally, removal of the two remaining TBS protecting groups with 5% aqueous HF in acetonitrile afforded ripostatin B in 88% yield.

In summary, an efficient and modular total synthesis of ripostatin B (**1**) is described. The longest linear sequence included 21 steps and provided the target molecule in 3.6% overall yield. Key steps were a Paterson aldol reaction, a modified Yamaguchi-type esterification and a ring-closing metathesis reaction. This strategy enabled the incorporation of the sensitive doubly skipped triene motif. The synthesis of analogs and their biological evaluation is currently ongoing in our laboratories.



Scheme 6. Endgame toward ripostatin B. DMP = Dess–Martin periodinane, Grubbs I = bis(tricyclohexylphosphine)benzylidene ruthenium(IV)dichloride.

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