DOI: 10.1002/ange.201200871

Ripostatin B (3)

Total Synthesis of the Bacterial RNA Polymerase Inhibitor Ripostatin B**

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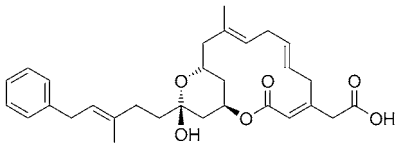
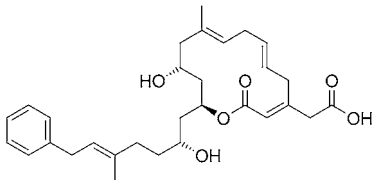
The resistance of bacterial pathogens to antibiotic treatment has emerged as one of the central threats to public health in the 21st century.[1] At the same time, the surge of bacterial drug resistance has been paralleled by a decline in antibacterial drug discovery research in most of the pharmaceutical industry,[1,2] a decline which has led to a general current paucity of agents (or the complete absence thereof) with satisfactory activity against major drug-resistant bacteria.[1,3] Thus, there is an urgent need for the development of new types of antibiotics, including the continuous chemical and biological assessment of new structural leads with promising antibacterial activity.

Bacterial RNA polymerase (RNAP) is the target of the rifamycin class of antibiotics, with rifamycin derivatives such as rifampicin, rifabutin, and rifapentin being essential drugs for the treatment of tuberculosis (TB).[3] RNAP is highly conserved across gram-positive and gram-negative bacteria, but differs significantly from mammalian RNA polymerases I, II, and III, thus providing the basis for the selective inhibition of bacterial growth and survival.[4] Thus, RNAP is a highly relevant, if underexploited,[5,6] target for antibacterial drug discovery, especially in light of the recent discovery of a new mode of RNAP inhibition by a number of natural products,[4a,7] including the myxobacterial macrolide ripostatin A.[8,9] The binding of these compounds to the "switch region" of RNAP stabilizes a closed conformation of the enzyme, thus preventing access of the dsDNA substrate to the active-site cleft. This inhibition mode is fundamentally different from that of rifamycin-type inhibitors;[4a,7] as a result, switch-region binders exhibit very limited cross-resistance with rifampicin.[7,10]

Ripostatins A and B are 14-membered macrolides that were first isolated in 1995 from the myxobacterium *Sorangium cellulosum* (strain So ce 377) at the Helmholtz Centre for Infection Research (formerly the GBF).[8] Both compounds show a narrow spectrum of antibiotic activity with MIC's of less than 1 $\mu g\,mL^{-1}$ only against *S. aureus* and the hyperpermeable *E. coli* strain DH21tolC; ripostatin A inhibits RNAP with submicromolar activity with no effect on eukaryotic RNA polymerase II.[8] No explicit data for the inhibition of RNAP by ripostatin B have been reported, but based on its structural relationship with ripostatin A and its similar antibiotic spectrum, an identical mode of action can be inferred for both compounds.



ripostatin A



ripostatin B

Natural ripostatins are not suitable drug candidates themselves, owing to both their limited antibacterial activity and their unfavorable biopharmaceutical properties, but these issues may be addressed by appropriate structural modifications.[5] To provide the chemical basis for structure–activity relationship (SAR) studies with ripostatins and concurrent lead optimization efforts, we embarked on the stereoselective total synthesis of ripostatin B. We used a strategy that was built around the construction of the macrolide ring by ring-closing olefin metathesis (RCM). Herein we report on the total synthesis of ripostatin B (**1**) through the successful implementation of this approach. No total synthesis of either ripostatin A or B had been reported in the literature prior to our own work and the concurrent independent work by Christmann and co-workers, and Prusov and Tang, which is also described in this issue of *Angewandte Chemie*.[11]

Our complete retrosynthesis of ripostatin B (**1**) is summarized in Scheme 1. Among the various possible target sites for RCM-based ring closure,[12] the C5–C6 double bond was

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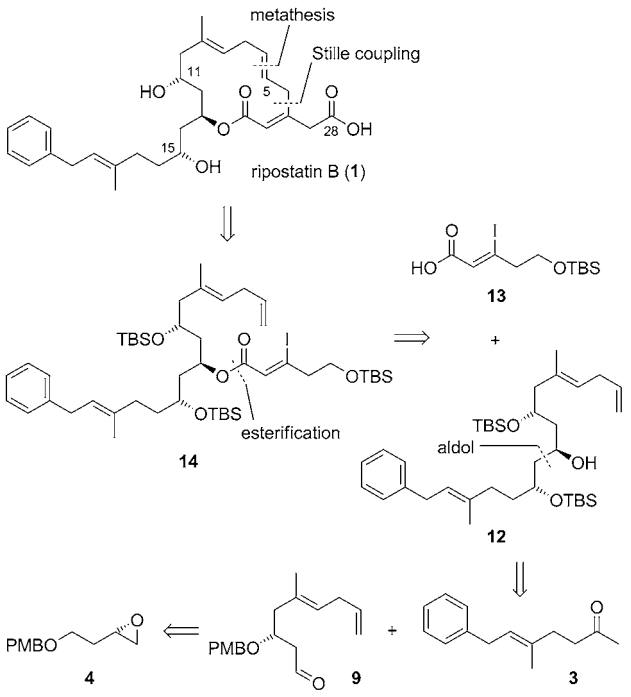
[**] This work was supported by the European Community Framework Programme 7, More Medicines For Tuberculosis (MM4TB), grant agreement no. 260872. We are indebted to Dr. Bernhard Pfeiffer (ETH Zürich) for NMR spectroscopy support and to Louis Bertschi from the ETHZ LOC MS Service for HRMS spectra. We are particularly grateful to Dr. Evgeny Prusov, Helmholtz Centre for Infection Research, Braunschweig (Germany), for sharing with us spectroscopic data for ripostatin B prior to publication and to Dr. Evgeny Prusov and Prof. Mathias Christmann, Technical University Dortmund, for agreeing to delay publication of their accepted manuscripts on the total synthesis of ripostatins until our own work, as described in this paper, had been reviewed.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201200871.

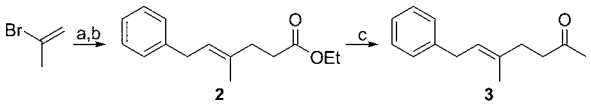


Scheme 1. Retrosynthesis of ripostatin B (**1**). PMB = *para*-methoxybenzyl, TBS = *tert*-butyldimethylsilyl.

selected for disconnection, as this provides the only diene precursor with two unencumbered terminal double bonds (which, therefore, was assumed to be the most reactive variant). This diene was envisioned to be accessed from vinyl iodide **14** by Stille coupling with $Bu_3SnCH_2CH{=}CH_2$; after RCM, the primary hydroxy group would be deprotected selectively and then converted into the C28 carboxylate prior to final deprotection of the secondary hydroxy groups at C11 and C15. Vinyl iodide **14** can be further disconnected into acid **13**[13] and the partially protected triol **12**, which could be obtained through a stereoselective aldol reaction between methyl ketone **3** and protected β-hydroxy aldehyde **9**, followed by stereoselective *anti* 1,3-reduction of the resulting aldol product. Aldehyde **9** was projected to be derived from chiral epoxide **4**[14] by epoxide ring opening with an appropriate carbon nucleophile and subsequent elaboration of the diene moiety.

The particular order of steps projected for the assembly of the precursor diene for the ring-closure reaction from **12**, **13**, and $Bu_3SnCH_2CH{=}CH_2$ was chosen based on the results of the only prior study on the synthesis of ripostatins by Kirschning and co-workers (which, however, did not lead to a total synthesis).[15] Thus, although a Stille coupling of **13** (or a suitable ester thereof) with $Bu_3SnCH_2CH{=}CH_2$ prior to esterification with **12** would represent a more convergent approach to the RCM substrate, Kirschning and co-workers had been unable to implement this strategy, partly owing to the pronounced tendency of the dienoic acid for double-bond migration under basic conditions.[15]

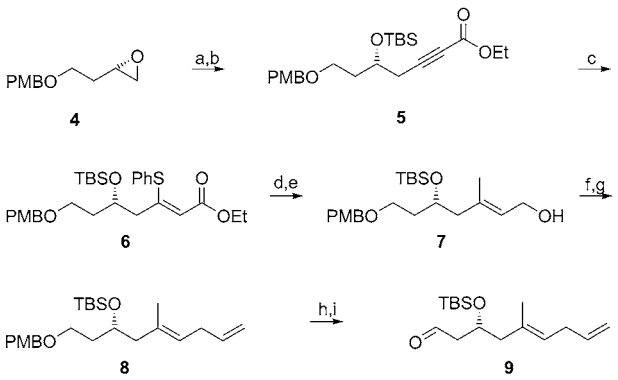
As illustrated in Scheme 2, the synthesis of ketone **3** started from 2-bromopropene, which was converted into the



Scheme 2. a) Mg, THF, reflux, 15 min, then RT, 1.5 h, then $PhCH_2CHO$, 0 °C, 1 h; b) $CH_3C(OEt)_3$, 145 °C, 3 h, 49% (two steps); c) *N*,*O*-dimethylhydroxylamine·HCl, CH_3MgBr, THF, −5 °C, 1.5 h, then RT, 14 h, 78%.

corresponding Grignard reagent; reaction of the latter with phenyl acetaldehyde gave a secondary alcohol that was directly transformed into ester **2** by an *ortho*-ester Claisen rearrangement in 49% overall yield (based on 2-bromopropene).[16] Treatment of **2** with CH_3MgBr and *N*,*O*-dimethylhydroxylamine hydrochloride, in accordance with the procedure of Williams et al.,[17] directly furnished the desired ketone **3** in 78% yield (via the corresponding Weinreb amide).

The elaboration of epoxide **4** (obtained from D-aspartic acid in 3 steps and 60% overall yield)[14] into aldehyde **9** is outlined in Scheme 3 and was initiated by epoxide opening with ethyl propiolate; subsequent protection of the resulting



Scheme 3. a) HC≡CC(O)OEt, *n*BuLi, $BF_3{\cdot}OEt_2$, THF, −78 °C, 45 min, 86%; b) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 30 min, 96%; c) PhSH, NaOMe (5 mol%), MeOH, RT, 14 h, 85%; d) CuI, CH_3MgBr, THF, −78 °C → −30 °C, 30–40 min, 95%; e) DIBAL-H, CH_2Cl_2, −78 °C, 30 min, 87%; f) CBr_4, PPh_3, 2,6-lutidine, CH_2Cl_2, −78 °C, 2 h, 85%; g) $Bu_3SnCH{=}CH_2$, $AsPh_3$, $[Pd_2(dba)_3]$ (12 mol%), DMF, RT, 75 h, 79%; h) TMSI, NEt_3, CH_2Cl_2, 0 °C, 1.5 h, then K_2CO_3, MeOH, RT, 5 min, 92%; i) $(COCl)_2$, DMSO, NEt_3, CH_2Cl_2, −78 °C, 1 h, 98%. dba = dibenzylideneacetone, DIBAL-H = diisobutylaluminium hydride, DMF = *N*,*N*-dimethylformamide, DMSO = dimethylsulfoxide, *n*BuLi = *n*-butyllithium, Tf = trifluoromethanesulfonyl, TMS = trimethylsilyl.

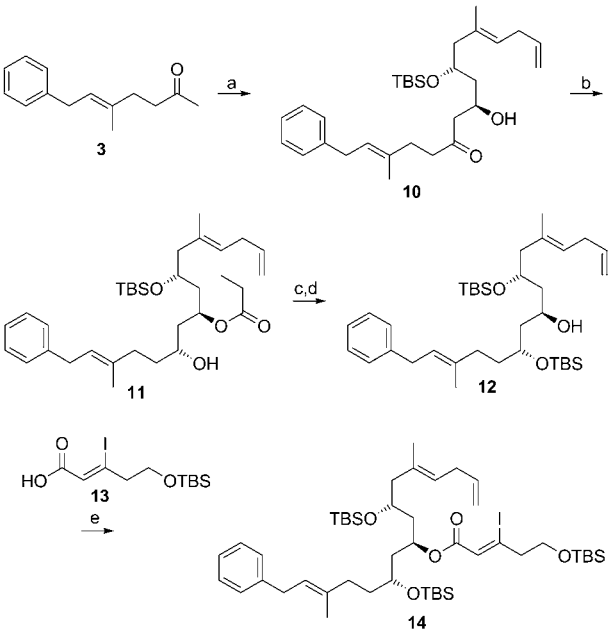
secondary hydroxy group with TBS then furnished TBS ether **5** in 83% overall yield. Treatment of **5** with thiophenol and a catalytic amount of sodium methoxide in MeOH[18] gave (*Z*)-thioether **6** in 85% yield. Treatment of **6** with CH_3MgI/Cu resulted in replacement of the thioether moiety by a methyl group in excellent yield (95%) with complete retention of configuration.[18] The reaction proved extremely sensitive to the exact experimental conditions, such that the desired product was only obtained if the reaction mixture was allowed to warm to −30 °C in the cooling bath over a period of 30–

40 min, after addition of **6** to the suspension of dimethylcuprate at −78 °C. No conversion was observed upon slow warming to room temperature or if **6** was added at −40 °C or 0 °C. The coupling product was then reduced to alcohol **7** with DIBAL-H in CH_2Cl_2 at −78 °C; subsequent conversion of **7** into the corresponding bromide with CBr_4/Ph_3P in the presence of 2,6-lutidine[19] followed by Stille coupling with $Bu_3SnCH{=}CH_2$ gave diene **8** in 55 % overall yield (from **6**).

The elaboration of **8** into the desired aldehyde **9** turned out to be more challenging than expected, because the attempted cleavage of the primary PMB ether under standard oxidative conditions with DDQ resulted in complete decomposition. Among the various other methods investigated, the most efficient approach to the clean removal of the PMB group from **8** involved treatment with TMSI[20] followed by cleavage of the ensuing silyl ether with K_2CO_3 in MeOH, to give the free primary alcohol in an excellent yield of 92 %. Oxidation of the primary hydroxy group under Swern conditions then furnished the desired aldehyde **9** in 98 % yield.

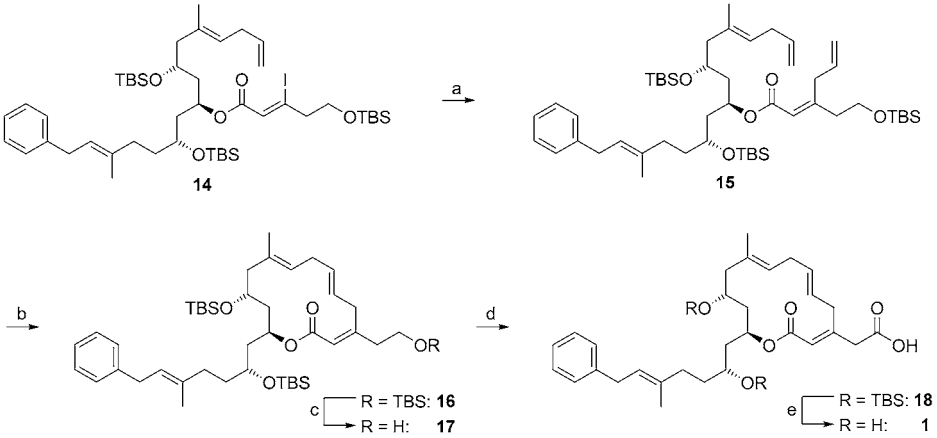
With building blocks **3** and **9** in hand, the crucial aldol reaction was then investigated under Paterson conditions with the chiral boron enolate derived from methyl ketone **3** by treatment with Et_3N and (+)-Ipc_2BCl in CH_2Cl_2[21] (Scheme 4). When a 1.5-fold excess of the enolate was employed at −78 °C to −20 °C, the reaction proceeded with good diastereoselectivity (d.r. > 10:1) and the desired stereoisomer could be isolated in 62 % yield and 94 % *de*.

The subsequent Evans–Tishchenko reduction[22] of the aldol product **10** proceeded smoothly and in the presence of 35 mol % of SmI_2 furnished the desired β-hydroxy ester **11** in excellent yield (93 %). The latter was then elaborated into the bis(TBS)-protected triol **12** by reaction with TBSCl and subsequent reduction of the ester moiety with DIBAL-H; **12** was obtained in 83 % yield from **11**.[23] Initial attempts at the esterification of alcohol **12** with acid **13**[13] under standard Yamaguchi conditions at room temperature[24] produced disappointingly low yields (< 30 %), with significant amounts of starting alcohol remaining unreacted. A range of alternative esterification methods was then investigated, none of which provided ester **14** in yields significantly in excess of 30 % (if any product was formed at all). However, a careful reassessment of the reaction conditions for the Yamaguchi esterification showed that the reaction was significantly more efficient at lower temperature. Based on this finding, an optimized protocol for the esterification of **12** with **13** was developed; this protocol involved mixing all the components (**12**, **13**, trichlorobenzoylchloride, Et_3N, DMAP) in toluene at −78 °C followed by slow warming to −40 °C, but not above. With this protocol, **14** could finally be obtained in 80 % yield.



Scheme 4. a) (+)-Ipc_2BCl, NEt_3, CH_2Cl_2, −78 °C, then **9**, −78 °C → −20 °C, 12 h, then H_2O_2, MeOH, pH 7 buffer, 0 °C, 90 min, 62 % (94 % *de*); b) CH_3CH_2CHO, SmI_2 (35 mol %), THF, −20 °C, 1 h, 93 % (d.r. > 20:1); c) TBSCl, imidazole, DMF, RT, 20 h, 94 %; d) DIBAL-H, CH_2Cl_2, −78 °C, 1 h, 88 %; e) **13**, 2,4,6-trichlorobenzoylchloride, DMAP, NEt_3, toluene, −78 °C → −40 °C, 3 h, 80 %. DMAP = 4-dimethylaminopyridine, (+)-Ipc_2BCl = (+)-diisopinocamphenyl chloroborane.

Stille coupling between vinyl iodide **14** and $Bu_3SnCH_2CH{=}CH_2$ proceeded smoothly to furnish diene **15** in 86 % yield (Scheme 5), thus setting the stage for ring closure by RCM. The efficiency of the ring-closure reaction was initially assessed in a series of small-scale experiments



Scheme 5. a) $Bu_3SnCH_2CH{=}CH_2$, $AsPh_3$, $[Pd_2(dba)_3]$ (15 mol %), DMF, RT, 16 h, 86 %; b) Grubbs I (12 mol %), CH_2Cl_2, RT, 3.5 h, then DMSO, RT, 17 h, 77 %; c) HF·py, pyridine, THF, 0 °C, 4 h, 81 %; d) DMP, THF, RT, 30 min, then $NaClO_2$, $NaH_2PO_4{\cdot}H_2O$, *t*BuOH, H_2O, 2-methyl-2-butene, RT, 20 min, 81 %; e) 5 % aq. HF in CH_3CN, 0 °C, 3 h, 88 %. DMP = Dess–Martin periodinane, Grubbs I = bis(tricyclohexylphosphine)benzylidene ruthenium(IV)dichloride.

(2 mg of **15**) with either Grubbs II or Hoveyda–Grubbs II catalyst.[12] Rapid consumption of diene **15** was observed in all cases (in CH_2Cl_2, $ClCH_2CH_2Cl$, and toluene), with MS analysis of the reaction mixtures indicating the presence of a product with the mass of the desired macrolactone **16** (Scheme 5); however, TLC analysis after concentration consistently showed the formation of three different products with similar R_f values that were not characterized further. In contrast, the use of Grubbs I catalyst in CH_2Cl_2 at room temperature gave clean conversion of **15** into **16** and eventually provided **16** in 77% yield.[25] The best results were obtained when the reaction was terminated by addition of DMSO before full consumption of the starting material; the latter could be readily separated from **16** by flash chromatography.

The selective cleavage of the primary TBS ether in **16** was first attempted with sodium periodate in aqueous THF,[26] but the reaction was found to be concentration and scale dependent; the yield of alcohol **17** varied between 50% and 70%, with significant amounts of the doubly deprotected species being formed with the larger-scale reactions. Gratifyingly, however, treatment of **16** with pyridine-buffered HF·py at 0°C led to clean formation of primary alcohol **17** in 81% yield, with only trace amounts of doubly deprotected material being detected. Subsequent attempts at the direct oxidation of **17** to the corresponding carboxylic acid **18** under a variety of reaction conditions met with complete failure, thus leading us to resort to a two-step sequence that involved the initial conversion of **17** into the aldehyde followed by further oxidation to the carboxylic acid with a different oxidant. Thus, **17** was submitted to Dess–Martin oxidation conditions, which resulted in spot-to-spot conversion to a less-polar product. Unfortunately, however, smearing was observed on TLC after an aq. $Na_2S_2O_3$/$NaHCO_3$ work-up, and subsequent Pinnick–Kraus oxidation of this material gave the desired carboxylic acid **18** in varying yields of only less than 5 to 34%. At the same time, however, by NMR spectroscopy the aldehyde was found to be stable under the conditions of the Dess–Martin oxidation for at least one hour, thus suggesting that its further oxidation without isolation might lead to cleaner and higher yielding conversion into the carboxylic acid. This approach could be successfully implemented, such that the addition of $NaClO_2$, $NaH_2PO_4{\cdot}H_2O$, *t*BuOH, H_2O, and 2-methyl-2-butene to the Dess–Martin oxidation mixture furnished the desired carboxylic acid **18** in an excellent yield of 81%.[27] Cleavage of the two secondary TBS ethers in **18** with 5% aqueous HF in CH_3CN then gave ripostatin B (**1**) in 88% yield.

Although the ^1H and ^{13}C NMR spectra of synthetic **1** deviated from those reported for natural **1**[9] in some of the signals, the ^1H NMR spectrum fully matches with the one that has recently been obtained for natural ripostatin B by the group of Prusov.[11b]

In conclusion, we have developed an efficient modular total synthesis of the bacterial RNAP inhibitor ripostatin B. The longest linear sequence in the synthesis comprised 21 steps (from D-aspartic acid; as the starting material for epoxide **4**) and furnished the target molecule in 3.6% overall yield. The modular nature of our approach lends itself to the synthesis of analogues with independent modifications in different parts of the ripostatin structure and thus provides a sound basis for future SAR studies and lead optimization efforts. Work along these lines has been initiated in our laboratory.

Received: February 1, 2012
Published online: February 29, 2012

Keywords: antibiotics · ring-closing metathesis · ripostatin · RNA polymerase · total synthesis

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