Expanding the pleuromutilin class of antibiotics by *de novo* chemical synthesis

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Abstract

New pleuromutilin-like compounds were synthesized in approximately 11 steps from 3-allylcyclopent-2-enone by a strategy featuring sequential carbonyl addition reactions. Several analogs possessing the C14 tiamulin ester side chain displayed activity in a *Mycobacterium tuberculosis* mc[2]7000 assay. The results described herein provide a basis for further efforts to expand the structural and stereochemical diversity of the pleuromutilin class of bacterial protein synthesis inhibitors through advances in chemical synthesis.

Introduction

The rate of new antibiotic approvals is declining in an era when drug resistant and extremely drug resistant strains of bacteria are posing greater threats to human health.[1–3] This already serious situation is worsened by the dearth of new antibiotic drug candidates in the pipelines of pharmaceutical companies.[4] Historically, a handful of families of natural products, such as the penicillins, cephalosporins, erythromycins, and tetracyclines have given rise to a large fraction of the approved antibiotics, and the process of semi-synthesis continues to be the predominant avenue to new ones.[4] In a noteworthy departure from the tried-and-true strategy of producing new antibiotics through systematic synthetic tailoring of the structures of known antibacterial natural products, the Myers laboratory demonstrated the power of *de novo* chemical synthesis in their expansion of the tetracycline class of antibiotics;[5] their efforts led to the discovery of new tetracyclines that are unavailable from the naturally occurring tetracyclines.

Our laboratory was drawn to the problem of achieving an analogous expansion of the pleuromutilin class of antibiotics through the development of direct syntheses of functionalized, pleuromutilin-like molecular scaffolds. Pleuromutilin (**1**), the flagship member of the antibiotic class bearing its name, possesses eight stereogenic centers and an intriguing "propellane-like" fusion of 5-, 6-, and 8-membered rings wherein the three rings share a common carbon-carbon bond.[6] This unique and rather rigid molecular architecture permits the C11 hydroxyl group and the C3 and C21 carbonyl oxygens to make bonding interactions within the peptidyl transferase center of the 50S subunit of bacterial ribosomes.[7] Through this molecular association, which is reinforced by hydrophobic bonding

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interactions, pleuromutilin (**1**) and its relatives retapamulin (**2**) and tiamulin (**3**) inhibit bacterial protein synthesis and display activity against bacterial strains that have become resistant to the macrolide class. Retapamulin (**2**) and tiamulin (**3**) were invented by scientists at GlaxoSmithKline and Sandoz Pharma, respectively, in the course of a substantial effort to develop new antibiotics from the pleuromutilin class by the strategy of semi-synthesis.[8, 9] Nabriva Therapeutics is also pursuing the development of new, semi-synthetic derivatives of pleuromutilin.[10] The aim of the research described herein was the development of a complementary strategy for expanding the depth and diversity of the pleuromutilins that would be founded on direct laboratory syntheses of the structurally complex molecular scaffold that defines this underdeveloped class of antibiotics.[11–13] This report addresses our continued advances in this area,[13] with a specific focus on application to tuberculosis – a disease presenting significant challenges to the discovery of novel therapeutics.[14]

Design for synthesis

In the planning phase, we conceived tricyclic scaffolds lacking the methyl groups found in compounds **1–3** since their contribution to the antibacterial properties of the pleuromutilins was unclear and their inclusion would lengthen our synthesis effort. However, the designed scaffolds would retain the pleuromutilin-like hydroxyl group at C11 as well as the C3 and C21 carbonyl functions since these groups make bonding interactions with the bacterial ribosome.[7b] This reasoning led to the conception of the pleuromutilin-like compound **4** (Scheme 1) as a target for synthesis, and we were especially interested in the prospects for a direct annulation of the 8-membered ring system through chemoselective additions of a hypothetical dianion to the aldehyde functions in a compound of type **5**. While there were concerns about the stereochemical outcomes of these carbonyl addition events, we embraced the possibility that this strategy for synthesis might provide direct access to an expanded set of screening candidates and that compounds with unnatural stereochemical configurations may still show useful margins of antibacterial activity. Ultimately, we were able to approximate the "linchpin" tactic outlined in Scheme 1 through the sequential application of the Nozaki-Hiyama-Kishi (NHK) bond construction method.[15] The established utility of Cr(II) or Cr(II)/Ni(II)-mediated bond formations in syntheses of challenging medium-sized rings was the principal justification for our decision to rely on this process in this context.

Results and discussion

Our path to the desired tricyclic scaffolds utilized bicyclic cyano alkene **9**, a substance that was readily available from 3-allylcyclopent-2-enone (**6**) and Grignard reagent **7** by the reaction sequence shown in Scheme 2.[13] The introduction of the nitrile function via a conjugate addition to enone **8** was both efficient and highly diastereoselective. After a high-yielding ozonolysis of the alkene in **9** (Scheme 3), the C11–C12 bond was constructed by a union of aldehyde **10** with vinyl bromide **11** under the conditions of a NHK reaction. This procedure afforded compound **12** as a 1:1 mixture of C11 epimers. A *tert*-butyldimethylsilylation of the epimeric mixture of alcohols produced **13**[16] and a subsequent, selective desilylation under mildly acidic conditions gave rise to compounds **14a** and **14b** as a separable mixture of diastereoisomers.

Interestingly, it was also possible to achieve a one-flask synthesis of **14a** and **14b** from aldehyde **10** and vinyliodide **15** by an *in situ tert*-butyldimethylsilylation of the intermediate chromium alkoxides and a simple quench with aqueous HCl to cleave the primary trimethylsilyl ether. This direct strategy for simultaneously achieving a key bond formation and a solution to the hydroxyl group differentiation problem was enabled by Fürstner and Shi's method[17] for performing *in situ* σ-bond metatheses of chromium alkoxides and trialkylsilyl chlorides; it also served our effort by providing access to both epimers at C11.

Epimer **14a** was transformed to the pleuromutilin-type tricyclic architecture by the following three-step sequence of reactions (Scheme 4): (1) conversion of the alcohol to the corresponding bromide by the action of carbon tetrabromide and triphenylphosphine; (2) reduction of the nitrile function with diisobutylaluminum hydride (DIBAL-H) and subsequent hydrolysis to afford the desired aldehyde **16a**; and (3) the pivotal chromium-mediated cyclization of **16a**, which afforded a 3:2 ratio of diastereoisomers **17a** and **17b** in 73% yield.

Epimer **14b** was advanced in an analogous fashion, and it was observed that the reductive cyclization of **16b** to a 2:1 mixture of compounds **17c** and **17d** was significantly slower than the cyclization of **16a**; at room temperature, the reductive cyclization of **16a** required only 5 minutes, while compound **16b** required 24 hours. After chromatographic separations of the two sets of diastereoisomers (i.e. **17a/17b** and **17c/17d**), NOESY spectroscopic analyses were performed to establish the relative stereochemistry of each stereoisomer. The nOe correlations that supported our assignments are indicated by the two-headed arrows.

Since prior SAR studies established the importance of the C14 ester side chain to antibacterial efficacy within the pleuromutilin class,[7a, 9a,b, 10] we conducted the two-stage transformations shown in Scheme 5. The couplings of compounds **17a** and **17c** with either the pleuromutilin-like glycolic acid derivative **18** or the tiamulin-like carboxylic acid **19** were mediated by dicyclohexylcarbodiimide (DCC) and followed by straightforward acid-induced deprotections to yield the novel screening candidates **4, 20a**, and **20c**.

Our previously developed synthesis of the molecular framework of pleuromutilin featuring the method of ring-closing olefin metathesis[13] invited an opportunity to produce additional antitubercular screening candidates from tricyclic alkenes **21a** and **21b** (Scheme 6). A straightforward, two-fold deprotection of compound **21a** afforded tricyclic alcohol **24**, which was subsequently joined with carboxylic acid **19** in the presence of DCC to give the novel, tiamulin-like ester **25**. It was also possible to epoxidize the trisubstituted alkenes in compounds **21a** and **21b** with high margins of diastereoface selectivity; however, our subsequent effort to effect a base-induced epoxide ring opening[18] of **22a** to compound **23a** was unsuccessful and an attempt to achieve an analogous conversion of **22b** to **23b** was only partially successful.[19] In our experience, the chromium-mediated, reductive cyclization strategy described herein is especially effective at producing compounds with the type of constitution embodied in compound **4** and its relatives.

To evaluate the antitubercular efficacies of the tricyclic esters **4, 20a, 20c**, and **25**, we measured their respective effects on the growth of *M. tuberculosis* mc[2]7000 (*M. tuberculosis* H37Rv Δ*RD1* Δ*panCD*),[20] utilizing the Microplate Alamar Blue assay (Table 1).[21] Our results with pleuromutilin and tiamulin showed low micromolar MIC values, in accord with a previous report with *Mtb* strains.[22] Expectedly, the compounds with the tiamulin-like ester side chain displayed higher inhibitory activity against *Mtb* mc[2]7000 (**1** vs. **3**; **4** vs. **20a**). In relation to tiamulin (**3**), the tiamulin-like tricycle **20a** displayed an attenuated activity. However, we were intrigued by the promising efficacy of analog **20c**, a compound with the tiamulin-type C14 ester side chain and unnatural relative stereochemistry at C11; this scaffold also lacks the peripheral methyl groups as well as the C12 quaternary stereocenter and yet it compares favorably to tiamulin (**3**) in its inhibitory action against *Mtb* mc[2]7000. Another unanticipated finding was the moderate, yet significant, level of activity of compound **25**, a substance that lacks the polar hydroxyl function at C11 and possesses unnatural relative stereochemistry at C14.

The behaviors of compounds **20c** and **25** in this whole-cell assay suggest that it is difficult to extrapolate the antibacterial potentials of simplified, pleuromutilin-like compounds from a

knowledge of the structural details of the pleuromutilin/50S ribosome complex.[23] Further studies are needed to determine if the new members of the pleuromutilin family described herein are targeting the peptidyl transferase center of the 50S subunit of bacterial ribosomes and to evaluate their activities against a broader range of bacterial strains.

Through the development of a flexible design for synthesis, we accomplished concise syntheses of new compounds that approximate the unique structure of the well known natural product pleuromutilin and show promise as leads for the development new, pleuromutilin-like antibiotics. The tricyclic molecular scaffold that defines the pleuromutilin family is complex and yet our preliminary studies show that effective syntheses of biologically active compounds having this scaffold are possible. As we move forward in our effort to expand the structural and stereochemical diversity of the pleuromutilin class of antibiotics, there is a high likelihood that we will discover additional, new compounds with promising activity against *M. tuberculosis* and possibly resistant strains of TB as well as other bacteria.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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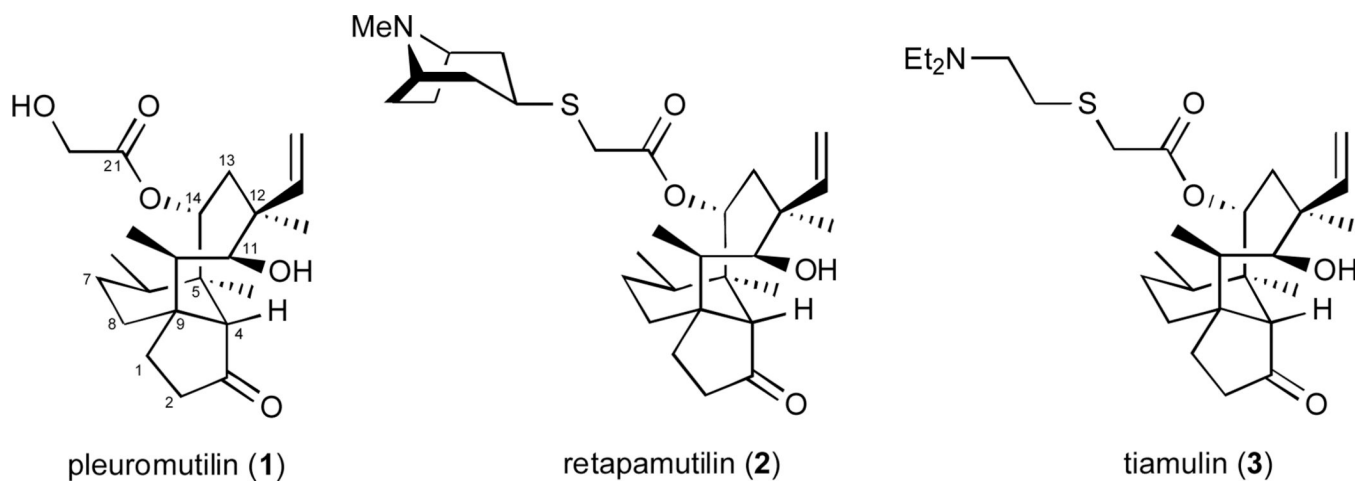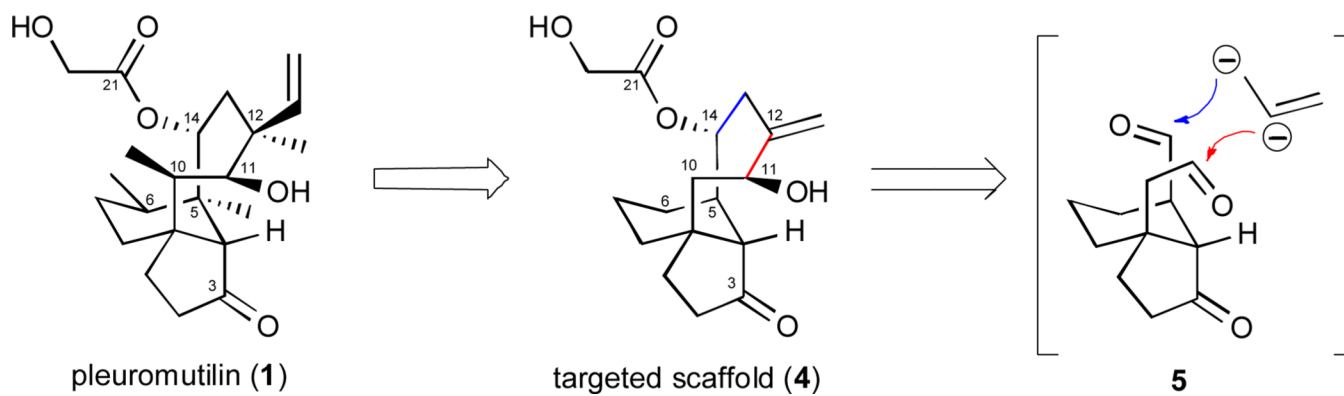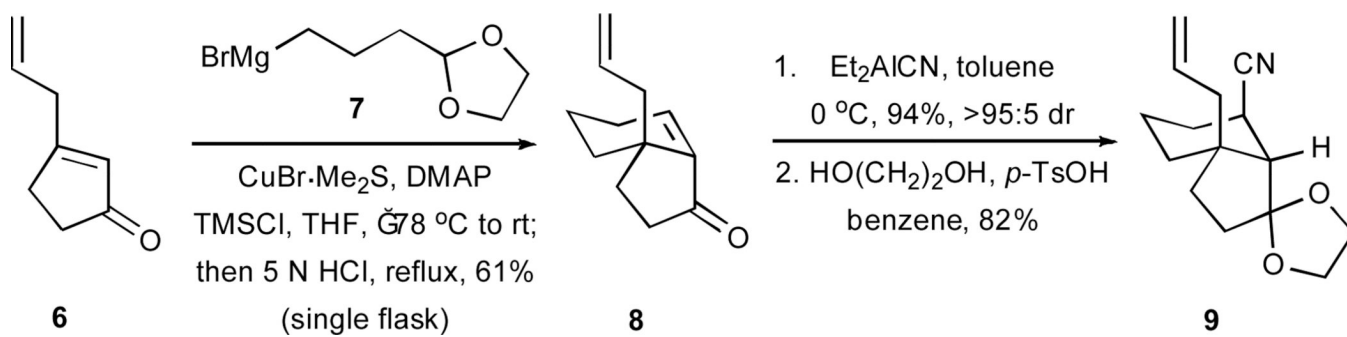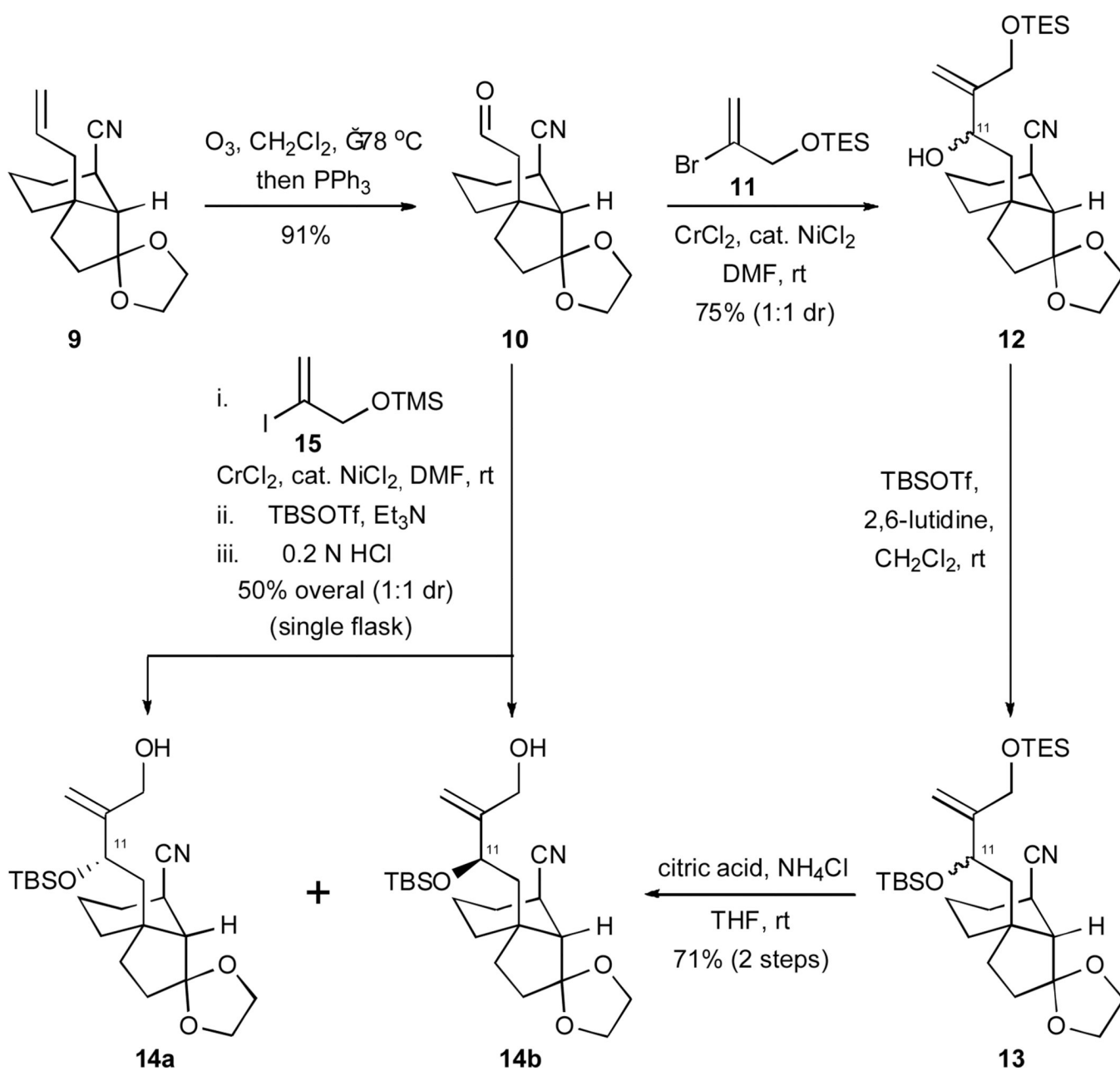
Fig. 1.
The molecular structures of pleuromutilin (**1**), retapamulin (**2**), and tiamulin (**3**).
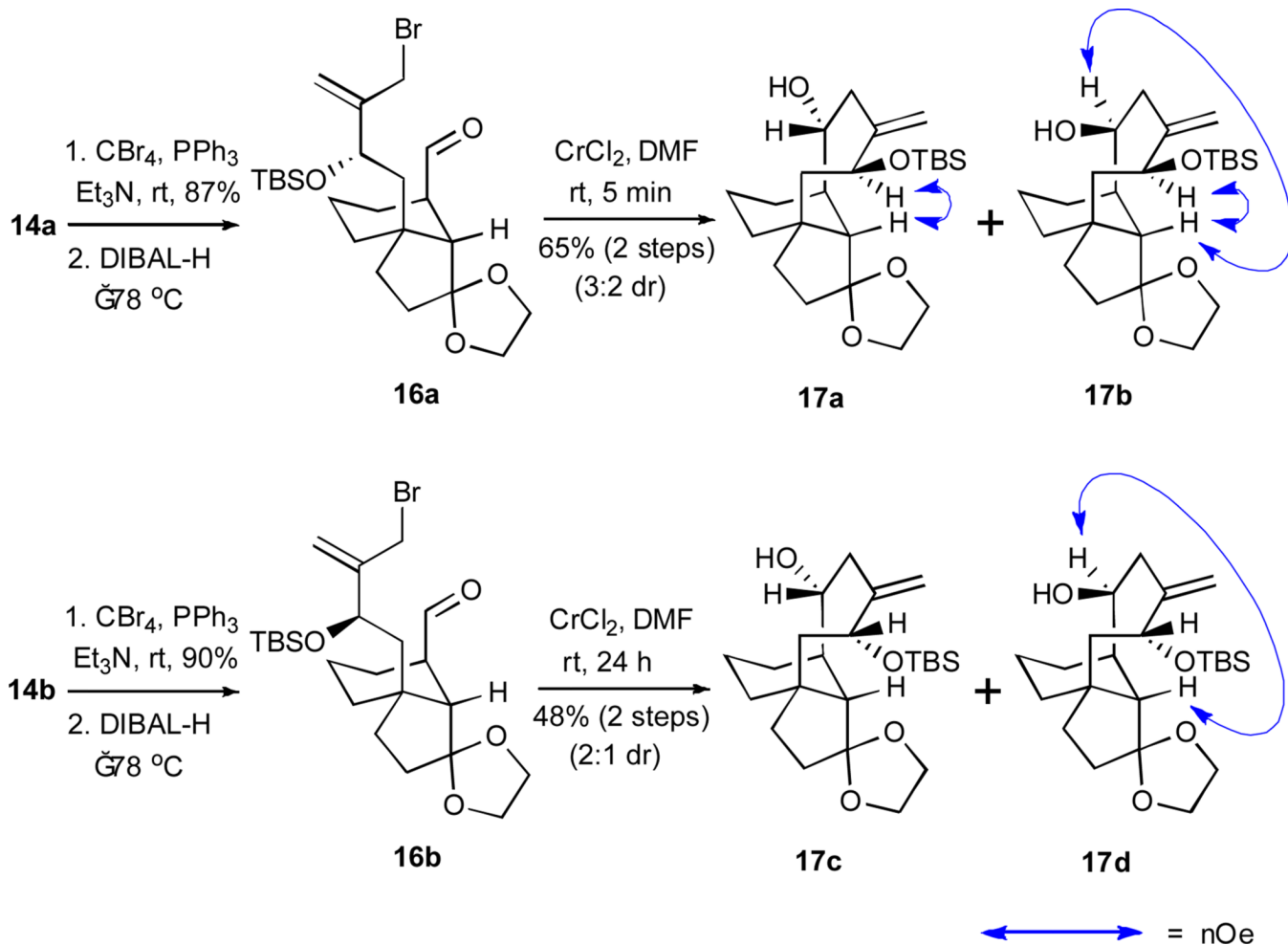
Scheme 1.
Derivation of a simplified, pleuromutilin-like scaffold **4** and the concept of a linchpin cyclization strategy.
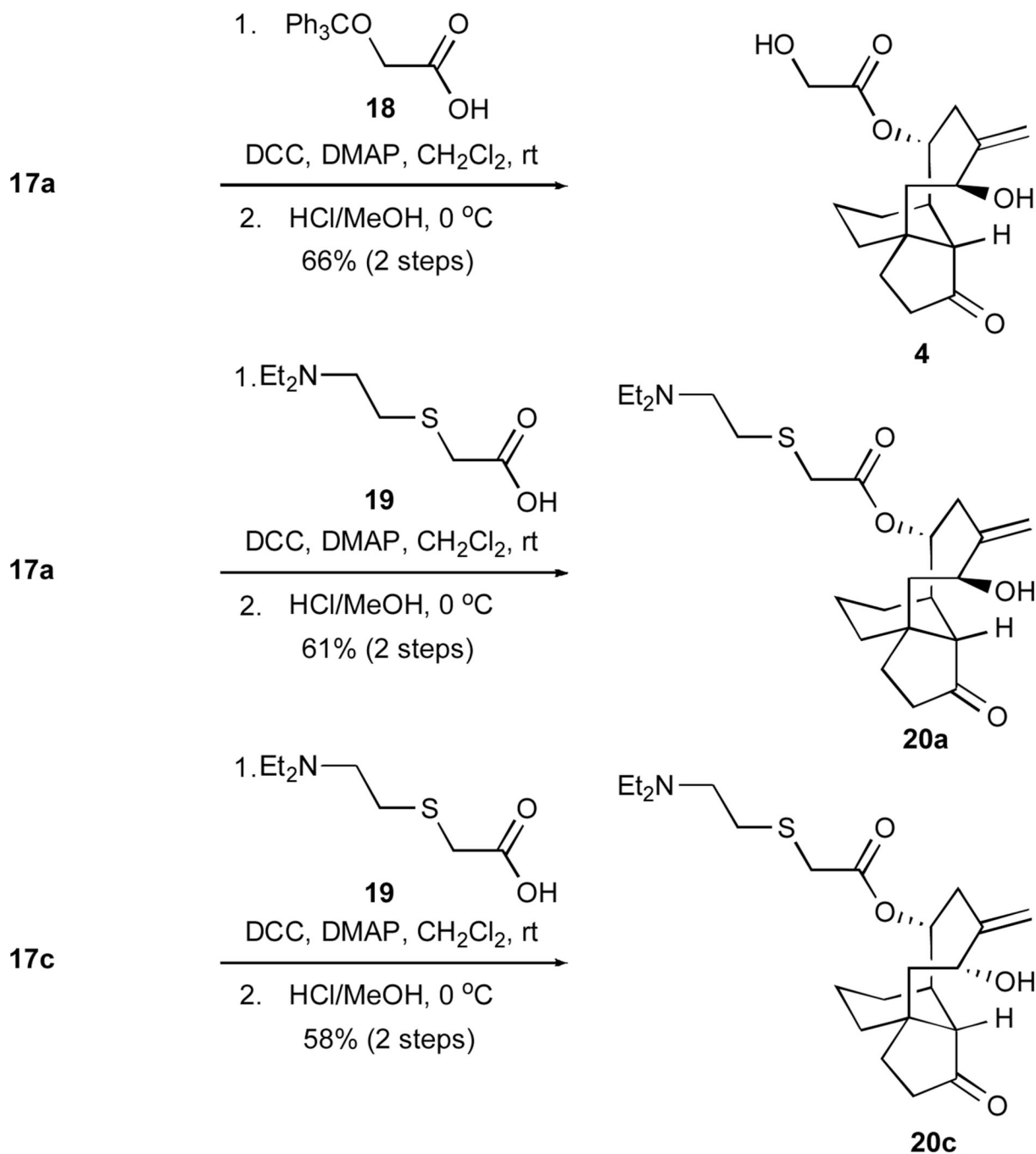
Scheme 2.
Synthesis of precursor **9**. DMAP = 4-dimethylaminopyridine; *p*-TsOH = *p*-toluene sulfonic acid.

Scheme 3.
The elaboration of compounds **14 a** and **14b** from **9** featuring an intermolecular NHK bond construction. TBS = *tert*-butyldimethylsilyl; TES = triethylsilyl; TMS = trimethylsilyl; DMF = *N,N*-dimethylformamide; THF = tetrahydrofuran.
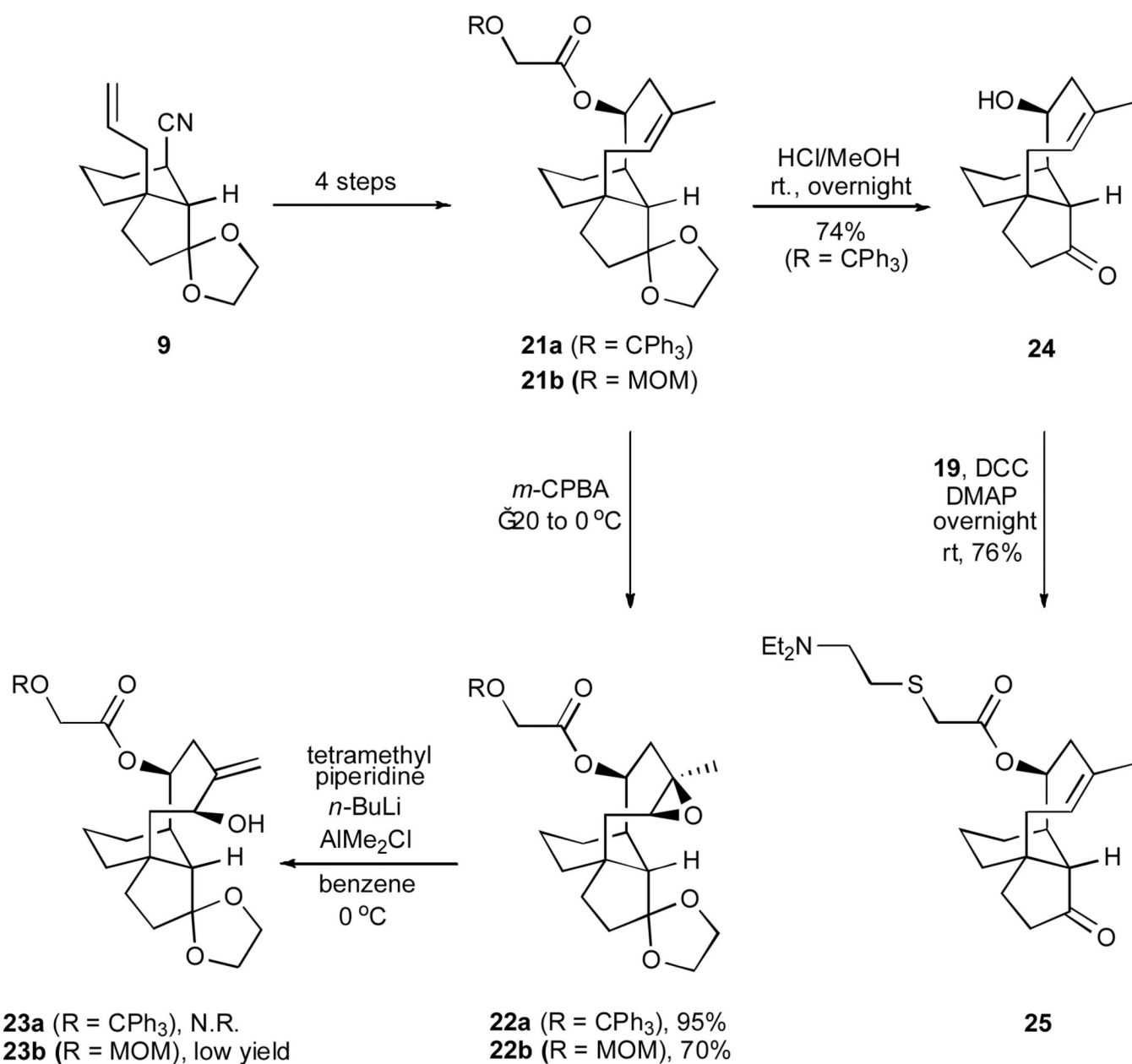
Scheme 4.
Construction of functionalized pleuromutilin-like scaffolds featuring reductive cyclizations.
DIBAL-H = diisobutylaluminum hydride; TBS = *tert*-butyldimethylsilyl; nOe = nuclear
Overhauser effect.

Scheme 5.
Syntheses of pleuromutilin-like compound **4** and tiamulin-like compounds **20a** and **20c**.
DCC = *N, N*'-dicyclohexylcarbodiimide; DMAP = 4-dimethylaminopyridine.

Scheme 6.
A synthesis of the simplified, tiamulin-like ester **25**. *m*-CPBA = 3-chloroperoxybenzoic acid; N.R. = no reaction; MOM = methoxymethyl.

Table 1

MIC values for pleuromutilin family members against *Mtb* mc^{2}7000.

Compound	mc^{2}7000 7H9 MIC (ug/mL)^a
Pleuromutilin (**1**)	25–50
tiamulin (**3**)	12–25
4	>200
20a	50–100
20c	12–25
25	50

aEach MIC value was reported as a range of experimentally assayed compound concentrations that bounds the mean for three independent determinations. Compounds **4**, **20a**, **20c**, and **25** were tested as racemates.