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A modular and enantioselective synthesis of the pleuromutilin antibiotics

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Abstract

The tricyclic diterpene fungal metabolite (+)-pleuromutilin has served as a starting point for antibiotic development. Semisynthetic modification of its glycolic acid subunit at C14 provided the first analogs fit for human use, and derivatization at C12 led to 12-*epi*-pleuromutilins with extended-spectrum antibacterial activity, including activity against Gram-negative pathogens. Given the inherent limitations of semisynthesis, however, accessing derivatives of (+)-pleuromutilin with full control over their structure presents an opportunity to develop derivatives with improved antibacterial activities. Here we disclose a modular synthesis of pleuromutilins by the convergent union of an enamide with a bifunctional iodoether. We illustrate our approach through synthesis of (+)-12-*epi*-mutilin, (+)-11,12-di-*epi*-mutilin, (+)-12-*epi*-pleuromutilin, (+)-11,12-di-*epi*-pleuromutilin, and (+)-pleuromutilin itself in 17 to 20 steps.

The diterpene fungal metabolite (+)-pleuromutilin (**1** in Fig. 1A) (1–4) inhibits the growth of predominantly Gram-positive pathogens (GPPs). Because of its unique mechanism of action, which involves binding to the highly conserved peptidyl transferase center of the bacterial ribosome, resistance to pleuromutilin is slow to develop, and it displays minimal cross-resistance with existing antibiotics (5, 6). Extensive efforts have been devoted to improving the pharmacological profile of (+)-pleuromutilin (**1**) by derivatization (7). Whereas the primary binding interactions of (+)-pleuromutilin (**1**) with the ribosome arise from its tricyclic core, the majority of semisynthetic efforts have focused on modification of its C14 side chain (6, 7). More than 3000 C14 derivatives have been prepared. These efforts culminated in the approval of retapamulin (**3**) in 2007 for the treatment of topical methicillin-resistant *Staphylococcus aureus* (MRSA) infections (8) and the development of lefamulin (currently in phase 3 clinical trials) for the treatment of community-acquired bacterial pneumonia (6).

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SUPPLEMENTARY MATERIALS

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Materials and Methods

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Despite progress in the treatment of GPPs, the lack of antibiotics for the treatment of Gram-negative pathogens (GNPs) remains a global health concern. Various C14-modified pleuromutilins exhibit poor uptake into Gram-negative bacteria and are efficiently transported out of target cells by efflux pumps (6). Berner reported in 1986 that the C12 quaternary stereocenter of pleuromutilin could be epimerized (to a ~1:1 mixture of C12 epimers) by an unusual zinc-mediated retroallylation-allylation reaction (see **4**, Fig. 1A) (9). Capitalizing on this discovery, researchers at Nabriva Therapeutics reported in 2015 that functionalization of the transposed alkene provides 12-*epi*-pleuromutilins with extended-spectrum activity. These compounds exhibit activity against various GNPs, including carbapenem-resistant *Enterobacteriaceae* (CRE) (10). Given the increasing occurrence of drug-resistant GNPs (11) and the emerging crisis of CRE (12), a flexible synthetic strategy to access 12-*epi*-pleuromutilins was deemed valuable. Such a strategy would overcome the inherent limitations of semisynthetic modification of (+)-pleuromutilin (**1**) and provide an opportunity to develop pleuromutilins with varied architecture and potentially improved antibacterial properties.

To maximize the scope of accessible derivatives, we conceived a modular approach to the 12-*epi*-mutilin scaffold. Our design involved late-stage construction of the macrocycle by using a conjunctive reagent that could be easily modified at positions 11 to 13 (see **5** and **6**, Fig. 1B). After considerable experimentation, the neopentyl iodide **8** and the enamide **7** were developed as the conjunctive reagent and electrophile, respectively. Three remarkable total syntheses of pleuromutilin (27 to 34 steps) have been reported (13–15), and many synthetic approaches to the molecule have been disclosed (16–23). Our synthesis is modular in nature, which we expect will facilitate analog production. We illustrate our approach through the enantioselective synthesis of (+)-12-*epi*-mutilin (**4**), (+)-11,12-di-*epi*-mutilin (**26**), (+)-12-*epi*-pleuromutilin (**29**), (+)-11,12-di-*epi*-pleuromutilin (**32**), and (+)-pleuromutilin (**1**) itself in 17 to 20 steps.

To begin our synthesis of the enamide **7**, we developed a method for asymmetric conjugate addition-C-acylation, which relies on the unique reactivity of zincate enolates (Fig. 2A) (24). This reaction entails copper-catalyzed enantioselective 1,4-addition of dimethylzinc to cyclohex-2-ene-1-one (**9**), in situ activation of the resulting zinc enolate with methyllithium, and C-acylation with methylcyanoformate. The β -ketoester product undergoes diastereoselective α -methylation (25) to provide **10** with 71% yield (two steps), 97:3 enantiomeric ratio (er), and >20:1 diastereomeric ratio (dr). Deprotonation of **10** and trapping of the resulting enolate with *N*-phenyltriflimide provided the vinyl triflate **11** (88%), which was converted to the dienone **12** (83%) via a palladium-catalyzed carbonylative coupling (26). Copper-catalyzed Nazarov cyclization (27) then formed the hydrindanone **13** as a single double-bond regioisomer (88%). In agreement with earlier studies (19), extensive experimentation was required to productively functionalize the hydrindanone **13**. Conjugate addition of diethylaluminum cyanide (**28**) to **13** proceeded with 3:1 selectivity at C9 (pleuromutilin numbering), but these epimers were difficult to separate on preparative scales. Fortunately, we found that the undesired minor epimer could be selectively reduced in situ with di-*iso*-butylaluminum hydride. Treatment of the unpurified product mixture with dilute sodium hydroxide then effected quantitative inversion of the C4

stereocenter to yield the desired *cis*-hydrindanone **15**, which was isolated as a single diastereomer (65% yield from **13**). The relative stereochemistry of **15** was confirmed by x-ray crystallography. Protection of the ketone as the ethylene glycol ketal then provided **16** (84%) (29). Selective functionalization of the nitrile substituent in **16** was challenging because of its steric congestion and the similar reactivity profile of the ester substituent. Ultimately, we found that the addition of excess methyllithium to **16**, followed by di-*tert*-butyl dicarbonate, provided the cyclic enamide **7** (80%). This cascade reaction likely comprises the addition of methyllithium to the nitrile, intramolecular cyclization of the resulting anion **17**, deprotonation to form **18**, and N-acylation. In support of this mechanistic scenario, the intermediacy of **18** was confirmed by N-protonation in the absence of di-*tert*-butyl dicarbonate and isolation. This cascade accomplishes both C–C bond formation at C10 and activation of the C14 methyl ester for fragment coupling. The conjunctive reagent, iodoether **8**, was prepared by site- and stereoselective α -alkylation of the chiral tigloyl imide **19** with *para*-methoxybenzyl chloromethyl ether (60%, 7:1 dr) (30), imide reduction (71%), and deoxyiodination (74%) (Fig. 2B).

In the key fragment-coupling step, an organolithium reagent derived from **8** by lithium-halogen exchange was added to the enamide **7** (Fig. 3). In situ hydrolysis of the resulting lithioenamine provided the methyl ketone **21** (48%). Essential to the success of this twofold neopentyl fragment coupling was the electronic activation of the C14 carbonyl group and the minimization of nearby nonbonded interactions via construction of the cyclic enamide functional group. Before this achievement, >15 different fragment-coupling strategies were examined by using reactants derived from compounds **10** to **15**, but each strategy was far inferior to the enamide-organolithium coupling described here. For example, imide N-substituents that were less electron-withdrawing or more sterically hindered than the *tert*-butoxy carbonyl group diminished the electrophilicity of the C14 carbonyl, which led to little or no detectable coupling products. In addition, carbonyl electrophiles that lacked the cyclic enamide functional group, such as those derived from compounds **10** to **15**, could not be successfully elaborated to related fragment-coupling products. Next, formal dehydration of the methyl ketone **21** via base-induced elimination of a transient vinyl triflate provided the alkyne **22** (81%). Removal of the *para*-methoxybenzyl group followed by oxidation of the resulting alcohol with the Dess-Martin periodinane (31) generated the alkynyl aldehyde **23** (83%, two steps).

We envisioned using the alkynyl aldehyde **23** in a reductive cyclization reaction to forge the macro-cycle of the targets. Although aldehyde- and alkyne-reductive cyclizations have enabled the synthesis of 5,6, and >10-membered rings (32–35), the synthesis of medium rings using this method is conspicuously absent. This is likely due to unfavorable reaction kinetics—for example, C–O bond-forming ring closures to make five-membered cyclic ethers are $\sim 10^5$ times as fast as those to make eight-membered cyclic ethers (36). In the reductive cyclization of **23**, we envisioned that the limited number of rotatable bonds along the nascent macrocycle would lower the entropic penalty of ring closure while enhancing regio- and stereocontrol. Furthermore, the presence of sp^2 -hybridized carbon atoms at locations C10 and C14 of the allylic alcohol product **24** would alleviate transannular nonbonded interactions in the eight-membered ring. After extensive experimentation with

iridium, rhodium, ruthenium, titanium, and nickel-based catalysts, we found that treatment of **23** with bis(1,5-cyclooctadiene)nickel (30 mol %), 1,3-bis-(2,6-di-*iso*-propylphenyl)imidazol-2-ylidene (IPr, 30 mol %), and triethylsilane (3 equivalents) at 20°C resulted in smooth reductive cyclization (35). A single diastereomer of the allylic silyl ether product was formed, which was desilylated to provide allylic alcohol **24** [60% yield, >20:1 dr, >20:1 regiomer ratio (rr)].

To complete the synthesis, the allylic alcohol **24** was subjected to a high-yielding two-step isomerization comprising oxidation with the Dess-Martin periodinane followed by site- and stereoselective reduction of the resulting enone with samarium diiodide (98% yield over two steps, >20:1 dr at C10) (31). The boat-chair conformation and absolute stereochemistry of **25** were confirmed by x-ray crystallography (see inset image, Fig. 3). Single electron reduction of the diketone **25** with an excess of sodium proceeded with thermodynamic selectivity (>20:1 equatorial-axial at C14, 3:1 equatorial-axial at C11) to provide, after ketal hydrolysis, (+)-12-*epi*-mutilin (**4**) and (+)-11,12-di-*epi*-mutilin (**26**). Stepwise methods for reduction of diketone **25** were also investigated. Reduction of **25** with samarium diiodide in tetrahydrofuran and water provided a 1.3:1 mixture of the C11 epimers **28** and **27** (37). Alternatively, lithium triethylborohydride reduction of **25** provided the C11 axial alcohol **27** with 1:>20 diastereoselectivity. Reduction of the remaining C14 ketone in **27** or **28** with sodium in ethanol (13) followed by ketal hydrolysis then formed **4** and **26**, respectively, both with >20:1 dr at C14 (92% and 74% for **4** and **26**, respectively).

(+)-12-*Epi*-mutilin (**4**) and 11,12-di-*epi*-mutilin-ketal (**31**) were easily elaborated to pleuromutilins (Fig. 4). Adapting Procter's protocol (15), (+)-12-*epi*-mutilin (**4**) was converted to (+)-12-*epi*-pleuromutilin (**29**) via stepwise acylation of the C11 and C14 alcohols with trifluoroacetylimidazole and *O*-trifluoroacetyl glycolic acid, respectively, followed by in situ methanolysis of the trifluoroacetyl esters (59%, two steps, 1.1% overall from **9**). Related conditions provided access to *O*-trityl-12-*epi*-pleuromutilin (**30**, 64% two steps). The C12 quaternary stereocenter of **30** was epimerized via the retroallylation-allylation reaction developed by Berner (9) to afford, after in situ removal of the trityl protecting group, a separable 1:1.7 mixture of (+)-pleuromutilin (**1**, 33%, 0.4% overall from **9**) and (+)-12-*epi*-pleuromutilin (**29**, 56%). Finally, the C14-alcohol of 11,12-di-*epi*-mutilin-ketal (**31**) was selectively acylated with *O*-trityl-glycolic acid and then deprotected to afford (+)-11,12-di-*epi*-pleuromutilin **32** (66%, two steps, 2.3% overall from **9**).

We can envision adapting the strategy outlined herein to create additional derivatives with optimized structures (ring sizes, atomic substitution, and/or substitution patterns) that may further address constraints inherent to semisynthetic approaches and may be accessible by shorter, higher-yielding sequences. Recent successes in the development of convergent routes to tetracycline (38) and macrolide (39) antibiotics underscore the potential to generate new clinical antibacterial candidates by total synthesis (40).

Supplementary Material

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Fig. 1. Pleuromutilins and 12-*epi*-pleuromutilins are promising classes of antibiotics.

(A) Structures of selected pleuromutilins and 12-*epi*-pleuromutilins. Pleuromutilins are active against predominantly Gram-positive pathogens, whereas 12-*epi*-pleuromutilins display extended spectrum activity, including activity against Gram-negative pathogens. (B) Retrosynthetic analysis of the mutilin scaffold. The synthetic fragments **7** and **8** were used in the synthesis of pleuromutilins.

Fig. 2. Stereoselective syntheses of the coupling fragments.

(A) Synthesis of the enamide **7**. Reagents and conditions: (1) $\text{Zn}(\text{CH}_3)_2$, copper(II) triflate [$\text{Cu}(\text{OTf})_2$] (0.5 mol %), L^* (1.0 mol %), toluene, 0°C , then CH_3Li , -78°C , then methylcyanoformate, -78°C ; (2) iodomethane, sodium *tert*-butoxide ($t\text{-BuONa}$), CH_3OH , 0°C , 71%; (3) potassium bis(trimethylsilyl) amide (KHMDS), *N*-phenyl triflimide, tetrahydrofuran (THF), -78°C ; (4) CO (1 atm), tetravinyltin, LiCl , tetrakis(triphenylphosphine)palladium(0) [$\text{Pd}(\text{PPh}_3)_4$] (5 mol %), dimethylformamide (DMF), 40°C ; (5) $\text{Cu}(\text{OTf})_2$ (5 mol %), $(\text{CH}_2\text{Cl})_2$, 70°C ; (6) diethylaluminum cyanide (Et_2AlCN), THF, 0°C , then di-*iso*-butylaluminum hydride (DIBALH), -78°C , then 0.01 M NaOH, CH_3OH – H_2O (5:1), 0°C ; (7) trimethylsilyl trifluoromethanesulfonate (TMSOTf), bis(trimethylsilyl)ethylene glycol, CH_2Cl_2 , 30°C ; and (8) CH_3Li , toluene, 0°C , then di-*tert*-butyl dicarbonate, 0°C . (B) Synthesis of the bifunctional iodoether **8**. Reagents and conditions: (1) sodium bis(trimethylsilyl)amide (NaHMDS), *para*-methoxybenzyl chloromethyl ether (PMBOCH_2Cl), THF, -78°C to 20°C ; (2) LiAlH_4 , Et_2O , 0°C ; and (3) PPh_3 , I_2 , imidazole, THF, 70°C .

Fig. 3. Synthesis of (+)-12-*epi*-mutilin (4) and (+)-11,12-di-*epi*-mutilin (26) from 7 and 8.

Reagents and conditions: (1) *t*-BuLi, **8**, Et₂O, -45°C, then **7** -45°C, then HCl, THF, 0°C; (2) KHMDS, *N*-(5-chloro-2-pyridyl)bis(trifluoromethanesulfonimide) (Comins' reagent), THF, -78°C; (3) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), CH₂Cl₂, pH 7 buffer, 20°C; (4) Dess-Martin periodinane (DMP), CH₂Cl₂, 20°C; (5) bis(cyclooctadiene)nickel(0) [Ni(cod)₂] (30 mol %), 1,3-bis-(2,6-di-*iso*-propylphenyl)imidazol-2-ylidene (IPr, 30 mol %), triethylsilane, THF, 20°C, then tetra-*n*-butylammonium fluoride (TBAF), 20°C; (6) DMP, CH₂Cl₂, 20°C; (7) Sml₂, CH₃OH, 20°C; (8) Na, EtOH, 20°C; (9) HCl, H₂O, CH₃OH, THF, 20°C; (10) (i) Sml₂, THF, H₂O, 20°C; (ii) lithium triethylborohydride (LiEt₃BH), THF, 20°C; (11) Na, EtOH, 20°C, then HCl, H₂O, CH₃OH, THF, 20°C.

Fig. 4. Synthesis of (+)-12-*epi*-pleuromutilin (29), (+)-pleuromutilin (1), and (+)-11,12-di-*epi*-pleuromutilin (32).

Reagents and conditions: (1) 1-(trifluoroacetyl)imidazole, ethyl acetate (EtOAc), -78°C ; (2) *O*-trifluoroacetyl glycolic acid, 1-ethyl-3-(dimethylaminopropyl)carbodiimide (EDC), 4-dimethylaminopyridine (DMAP), 20°C , then CH_3OH , NaHCO_3 , 20°C ; (3) 1-(trifluoroacetyl)imidazole, EtOAc, -78°C ; (4) *O*-trityl glycolic acid, EDC, DMAP, 20°C , then CH_3OH , NaHCO_3 , 20°C ; (5) Et_2Zn , DMF, 100°C , then HCl, 20°C ; (6) *O*-trityl glycolic acid, EDC, DMAP, 20°C ; (7) HCl, 20°C .