

Synthesis and Antimycobacterial Activity of 2,1'-Dihydropyridomycins

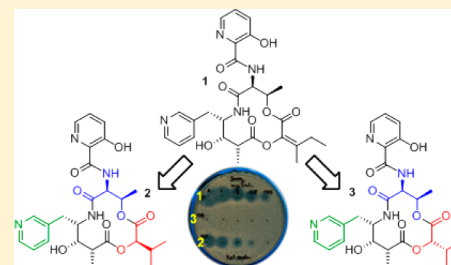
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Supporting Information

ABSTRACT: Dihydropyridomycins **2** and **3**, which lack the characteristic enol ester moiety of the potent antimycobacterial natural product pyridomycin (**1**), have been prepared from L-Thr, R- and S-hydroxy isovaleric acid, and 3-pyridinecarboxaldehyde. The 2R isomer **2** shows only 4-fold lower anti-*Mtb* activity than **1**, indicating that the enol ester moiety in the natural product is not critical for its biological activity. This finding establishes **2** as a potent and more practical lead for anti-TB drug discovery.



KEYWORDS: natural products, *InhA*, pyridomycin, total synthesis, tuberculosis

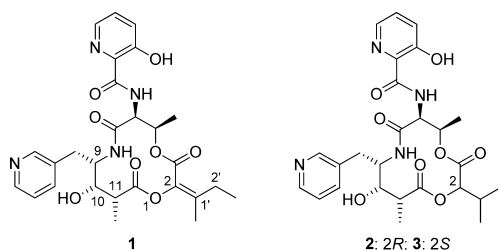
Tuberculosis (TB) is a frequently fatal infectious disease that causes more than 1.4 million deaths annually. TB was considered to be well contained in the 1960s, but recent decades have witnessed a resurgence of the disease, even in industrialized countries, due to comorbidity with AIDS and the emergence of multi- and extensively drug-resistant (MDR, XDR) strains of the causative pathogen *Mycobacterium tuberculosis* (*Mtb*).¹ These developments were paralleled by a decline in TB-directed drug discovery efforts; thus, as of today, the last TB drug with a novel mode of action was launched more than 40 years ago, and current combination therapy is insufficient to eliminate XDR *Mtb*.² As a consequence, there is an urgent need for the development of new anti-TB agents that can shorten the duration of treatment (current standard first line therapy of TB extends over 6 months) and/or are active against MDR and XDR bacteria.

Pyridomycin (**1**) (Chart 1) is a bacterial natural product that was first isolated from the *Streptomyces* strain 6706 in 1953.^{3,4} The compound was subsequently shown to exhibit significant in vitro antimycobacterial activity and low systemic toxicity in mice.⁵ While these findings were not further explored for

decades to come, we have recently confirmed the in vitro antimycobacterial activity of **1**, which we found to inhibit *Mtb* growth with a minimal inhibitory concentration (MIC) of 0.3 $\mu\text{g}/\text{mL}$.⁶ Moreover, we have identified the molecular target of **1** as the mycobacterial NADH-dependent enoyl-[acyl-carrier-protein] reductase (*InhA*),⁶ which is also the target of the clinical TB drug isoniazid (INH) (after metabolic activation and formation of a NADH adduct as the effective inhibitory species).^{7,8} Pyridomycin (**1**) is a competitive inhibitor at the NADH-binding site of *InhA* but has not shown cross-resistance with INH.⁶ This suggests that the exact molecular interactions of **1** with the NADH-binding site differ from those of the NADH adduct of INH. Together with the fact that the structure of **1** does not resemble any known TB drug, these findings render pyridomycin an auspicious starting point for TB drug discovery.

Only a single total synthesis of **1** has been reported in the literature; significant difficulties were encountered in that work⁹ with regard to the establishment of the enol ester double bond between C2 and C1',¹⁰ a problem that could not be solved in a fully satisfactory manner. In light of these difficulties, we felt that the broader exploration of the pyridomycin (or a pyridomycin-derived) scaffold for TB drug discovery would greatly benefit from saturation of the double bond between C2 and C1', provided that the enol ester moiety was not a critical prerequisite for antimycobacterial activity per se. To explore this question, 2'-desmethyl-2,1'-dihydropyridomycins **2** and **3** were targeted for synthesis and biological evaluation (Chart 1). As the target of **1** was unknown at the outset of this work, no

Chart 1



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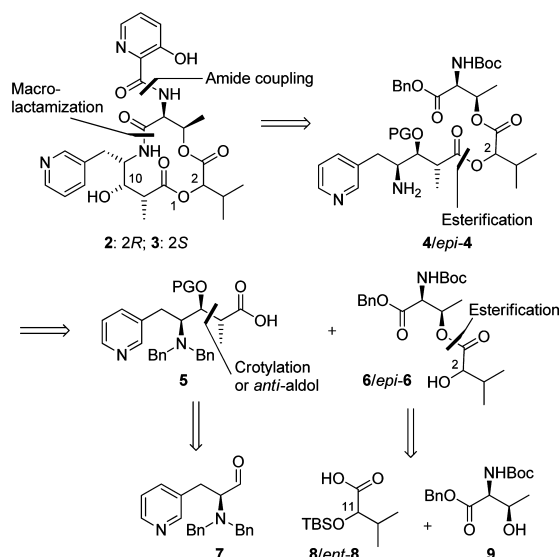
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predictions were possible with regard to the more favorable configuration of the new chiral center at C2 of dihydropyridomycins (if any), and both configurations needed to be addressed; in addition, **2** and **3** are not formal reduction products of **1** itself but incorporate a symmetrical *iso*-propyl substituent at C2, thus avoiding uncertainties about the preferred configuration of a chiral *sec*-butyl substituent.

As illustrated in Scheme 1, the synthesis of analogues **2** and **3** was to be based on macrolactamization of amino acids **4/epi-4**,

Scheme 1. Retrosynthesis of **2** and **3**^a

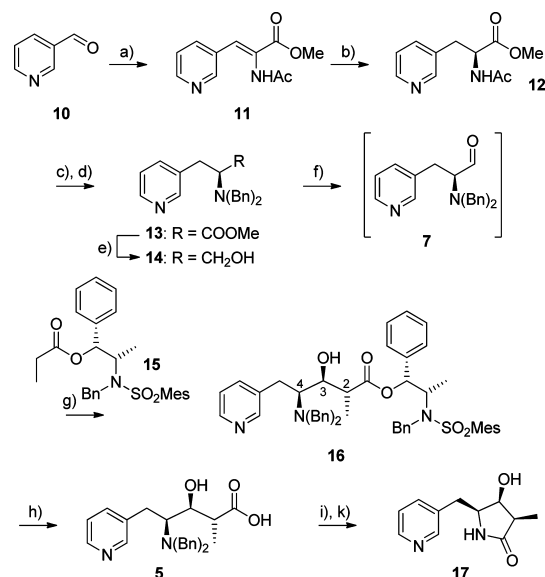


^aAc, acetyl; Bn, benzyl; TBS, *t*-butyldimethylsilyl; and PG, protecting group or H.

which would be followed by deprotection of the exocyclic amino group, coupling with 3-hydroxypicolinic acid, and final deprotection of the hydroxyl group on C10. The cyclization precursors **4/epi-4** can be further disconnected into acid **5** and alcohols **6/epi-6** (Scheme 1), of which the latter would be the result of the esterification of commercially available *t*-butyloxycarbonyl (Boc)-L-Thr benzyl ester (**9**) with TBS-protected hydroxy acids **8/ent-8**, respectively. Acid **5** would be obtained from aldehyde **7** either via auxiliary-based *anti*-selective aldol reaction according to Masamune¹¹ or by stereoselective crotylboration,¹² followed by appropriate functional group manipulations. Lastly, aldehyde **7** was envisioned to be accessed by enantioselective hydrogenation of a 3-pyridyl-2-amino acrylic acid derivative.

The synthesis of acid **5** was initiated by Erlenmeyer azlactone synthesis¹³ from 3-pyridinecarboxaldehyde (**10**) and *N*-acetyl-glycine, which produced, after azlactone opening with NaOAc/MeOH, the *Z*-configured ester **11** as the sole isomer in 78% yield (Scheme 2). The subsequent stereoselective hydrogenation was best performed with [Rh(COD)(*R,R*-DIPAMP)]-BF₄^{14,15} as a catalyst in the presence of HBF₄ as a noncomplexing acid.¹⁶ These conditions provided the desired *S*-configured amino acid with high selectivity (87% ee) in 85% isolated yield at a catalyst load of only 0.1%.¹⁷ While reactions performed with [Rh(COD)(*R*-propranolol)]BF₄^{18,19} in combination with HBF₄ (3% catalyst, 55 °C) or with [Rh(COD)-(*R,R*-DIPAMP)]BF₄ in the absence of HBF₄ (2% catalyst, 55 °C) likewise gave good selectivities (ee values of 85 and 87%,

Scheme 2^a



^aReagents and conditions: (a) (i) Ac-Gly-OH, NaOAc, Ac₂O, 115 °C, 18 h; (ii) NaOAc, MeOH, rt, 72 h, 78%. (b) [Rh(COD)(*R,R*-DIPAMP)]BF₄ (0.1 mol %), H₂ (5 bar), HBF₄, MeOH, 50 °C, 18 h, 85% (87% ee). (c) SOCl₂, MeOH, reflux, 18 h, 86% (crude). (d) Benzaldehyde, NaCNBH₃, molecular sieves 4 Å, MeOH/AcOH 10:1, rt, 24 h, 88%. (e) LAH, Et₂O, 0 °C, 30 min, 99%. (f) Dess–Martin periodinane, CH₂Cl₂, 0 °C, 30 min. (g) *c*-Hex₂BOTf, **15**, Et₃N, CH₂Cl₂, -78 → 0 °C, 73% (2 steps), dr 5:1. (h) LiOH, THF/MeOH/H₂O 3:2:2, rt, 24 h, 96%. (i) (Trimethylsilyl)diazomethane, MeOH/toluene 1:2, 0 °C, 81%. (k) H₂ (1 bar), Pd/C, MeOH, rt, 67%. Ac, acetyl; COD, 1,5-cyclooctadiene; *R,R*-DIPAMP, (1*R*,2*R*)-bis[(2-methoxyphenyl)-phenylphosphino]ethane; and *c*-Hex₂BOTf, dicyclohexylboron trifluoromethanesulfonate.

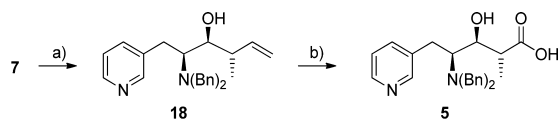
respectively), neither of these conditions led to full substrate turnover.²⁰

Ester **12** was then transformed into the corresponding *N,N*-bis-benzyl derivative **13** by acetamide cleavage with MeOH/SOCl₂ and reductive amination of the resulting free amino ester with benzaldehyde in 76% overall yield. Reduction of the ester moiety with lithium aluminum hydride (LAH) followed by Dess–Martin oxidation²¹ of the ensuing alcohol then provided aldehyde **7**; to minimize the risk of epimerization, the latter was not purified but used crude in subsequent reactions.²² Aldol reaction of **7** with the Masamune reagent **15**¹¹ and dicyclohexylboron trifluoromethanesulfonate (*c*-Hex₂BOTf) as the Lewis acid proceeded with good stereoselectivity (5:1 in favor of the desired isomer over the sum of all other isomers) and provided ester **16** in 52% yield (from **14**) as a single isomer after flash column chromatography. Cleavage of the auxiliary under basic conditions then cleanly delivered acid **5** in excellent yield (95%).

The predicted absolute configuration of the C10 stereocenter in **5** (pyridomycin numbering) was confirmed by Mosher ester analysis²³ of its methyl ester (prepared with TMS-diazomethane in 81% yield), while the relative configuration of the C9, C10, and C11 stereotriad was validated by X-ray crystallography of lactam **17** (which formed spontaneously upon debenzoylation of the methyl ester of **5** by catalytic hydrogenation in MeOH; Scheme 2).

As an alternative to the aldol-based strategy depicted in Scheme 2, we have also investigated the installation of the

stereocenters at C10 and C11 by means of substrate-directed crotylation of aldehyde **7**, employing the crotyl chromium reagent derived from CrCl₂ and crotyl bromide¹² (Scheme 3).

Scheme 3^a

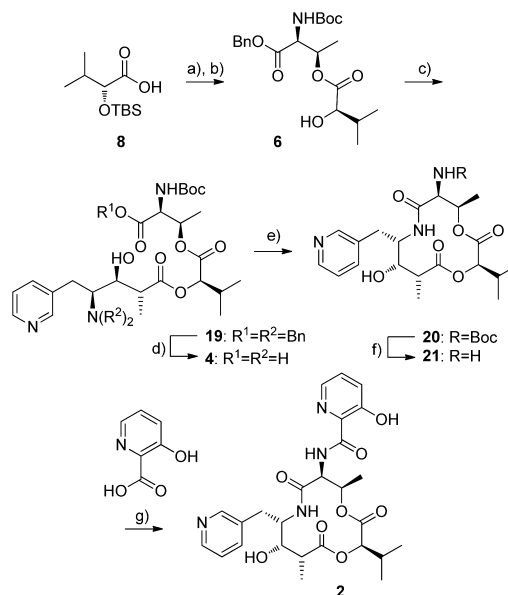
^aReagents and conditions: (a) Crotyl bromide, CrCl₂, THF, rt, 3 h, 67% from **14**, dr 15:1. (b) (i) AD-mix α , *t*-BuOH/H₂O 1:1, 3 days; (ii) NaIO₄, CH₂Cl₂/H₂O 3:2, rt, 2 h; (iii) NaClO₂, NaH₂PO₄, *t*-BuOH/H₂O 4:1, rt, 2 h, 45%. rt, room temperature.

The reaction provided the desired homoallylic alcohol **18** with excellent diastereoselectivity (dr 15:1) in 67% yield over two steps from alcohol **14**. Dihydroxylation of the double bond with AD-mix- α ,²⁴ periodate cleavage of the resulting diol, and Pinnick–Kraus oxidation of the ensuing aldehyde then gave the desired acid **5** in 45% overall yield as a single isomer (from **18**). Overall, this approach gave acid **5** in 30% yield from alcohol **14**; it is thus somewhat less efficient than the aldol-based route via ester **16**, which gave **5** in 50% overall yield.

The elaboration of acid **5** into dihydropyridomycin **2** commenced with its esterification with alcohol **6**; the latter was obtained by Yamaguchi type esterification²⁵ of TBS-protected (*R*)-hydroxy isovaleric acid (**8**) with Boc-Thr-OBn (**9**) followed by TBS removal with HF-pyridine in 63% overall yield (Scheme 4).

Unexpectedly, ester formation between **5** and **6** was found to be highly challenging, with virtually no reaction being observed for a number of common condensing agents [1-*tert*-butoxy-2-*tert*-butoxycarbonyl-1,2-dihydroisoquinoline (BBDI), *N*-cyclohexyl-*N'*-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulfonate (CMC), *N,N'*-dicyclohexyl-carbodiimide (DCC), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDCI), and *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU)]. Efficient ester formation occurred only under highly optimized reaction conditions, which entailed reaction of **5** with 2,4,6-trichlorobenzoyl chloride²⁵ at -78 °C (to form the mixed anhydride), subsequent simultaneous addition of alcohol **6** and 4-dimethylaminopyridine (DMAP) in toluene, and slow warming of the reaction mixture to -35 °C. Using this procedure, the desired ester **19** could be obtained in yields of up to 50% (Scheme 4) (64% for *epi*-**19**).²⁶ Gratifyingly, subsequent global debenzoylation to amino acid **4** proceeded smoothly, which set the stage for the crucial ring-closing reaction. Efficient cyclization of **4** was achieved by treatment of the compound with *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and *N,N*-diisopropylethylamine (DIEA) in 1% DMF/dichloromethane at high dilution, which delivered depsipeptide **20** in 63% yield (79% for *epi*-**20**). In light of the efficiency of this process, no other coupling reagents were investigated for this transformation. Cleavage of the Boc-group and coupling of 3-hydroxypicolinic acid to the free amine **21** (HATU/DIEA) finally furnished (2*R*)-2'-desmethyl-2,1'-dihydropyridomycin (**2**) in 52% yield over two steps. The 2*S* diastereomer **3** was obtained from (*S*)-hydroxy isovaleric acid (*ent*-**8**) in a completely analogous way.

Intriguingly, the assessment of the antimycobacterial activity of dihydropyridomycins **2** and **3** revealed that 2*R* isomer **2**

Scheme 4^a

^aReagents and conditions: (a) Boc-*L*-Thr-OBn (**9**), 2,4,6-trichlorobenzoyl chloride, Et₃N, toluene, DMAP, rt, 18 h, 73%. (b) HF-pyridine, THF, 0 °C \rightarrow rt, 16 h, 86%. (c) Acid **5**, 2,4,6-trichlorobenzoyl chloride, Et₃N, toluene, -78 °C, 5 min, then alcohol **6**, toluene, DMAP, -78 \rightarrow -35 °C, 43 h, 50%. (d) H₂ (1 bar), Pd/C, MeOH, rt, 5 h, quantitative. (e) HATU, DIEA, CH₂Cl₂, DMF (1%), 10⁻³ M, rt, 18 h, 63%. (f) TFA, CH₂Cl₂, 0 °C \rightarrow rt, 3 h, quantitative (crude). (g) 3-Hydroxypicolinic acid, HATU, DIEA, acetonitrile, rt, 18 h, 52% (2 steps). DIEA, *N,N*-diisopropylethylamine; DMAP, 4-dimethylaminopyridine; HATU, *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; and rt, room temperature.

retains most of the activity of natural **1** (4-fold loss in potency; Table 1). On the basis of this finding, the presence of an enol

Table 1. Antimycobacterial Activity of 1–3 and InhA Inhibition

compd	MIC (μ g/mL) ^a	K _i (μ M) ^b
pyridomycin (1)	0.39	4.8 \pm 1.1
2	1.56	71.0 \pm 7.9
3	12.5	ND

^aMinimal inhibitory concentrations (MIC) were measured against *Mtb* strain H37Rv using the resazurin method. Data are average values from two independent experiments. ^bInhibition constants (K_i) were measured against InhA (S94A) with 2-*trans*-octenoyl-CoA or 2-*trans*-dodecenoyl-CoA as the substrate. Data are average values from three independent experiments \pm SDs (with each experiment carried out in triplicate). ND, no activity detectable up to a concentration of 75.7 μ M. For details, see ref 6 and the Supporting Information.

ester moiety is not a critical requirement for the antimycobacterial activity of pyridomycin-derived structures. At the same time, a clear activity difference is apparent between **2** and **3**, with the latter being 32-fold less potent against *Mtb* H37Rv than **1** (8-fold potency difference to **2**), thus pointing to the importance of the configuration of the newly created chiral center at C2. The difference in antibacterial activity between **2** and **3** is also reflected in their differential ability to inhibit the pyridomycin target InhA *in vitro* (Table 1). Unfortunately, no K_i value against InhA could be determined for **3**, due to its

limited solubility; no inhibition of InhA was detectable at the solubility limit for the compound (ca. 75 μM). The difference in K_i values between **2** and pyridomycin (ca. 15-fold) significantly exceeds the difference in antibacterial activity (4-fold difference in MIC values). The reasons for this apparent discrepancy are unknown at this point but may be related to target-independent parameters such as differences in bacterial cell wall penetration or intracellular metabolism.

To rationalize the differences in InhA inhibition between **2** and **3** at the structural level, we have performed preliminary docking studies.²⁷ However, in the absence of structural information on the InhA–pyridomycin complex, attempts to dock the compounds into the extensive NADH-binding pocket of InhA did not produce any conclusive results.

In summary, we have achieved the total synthesis of two close structural analogues of the bacterial metabolite **1** that lack the characteristic enol ester moiety of the natural product. While both analogues **2** and **3** retain the ability of **1** to inhibit *Mtb* growth, 2*R* isomer **2** is more potent, with its MIC for *Mtb* H37Rv being increased only 4-fold relative to **1**. Because of the improved synthetic accessibility of **2** over natural **1**, this discovery should facilitate future SAR work and, potentially, the development of pyridomycin-derived drug candidates for TB treatment. The synthesis and biological evaluation of new pyridomycin variants that are based on the macrocyclic scaffold of analogue **2** are currently ongoing in our laboratories.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures and analytical data for all new compounds. Crystallographic information on **17**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

BBDI, 1-*tert*-butoxy-2-*tert*-butoxycarbonyl-1,2-dihydroisoquinoline; Bn, benzyl; Boc, *t*-butyloxycarbonyl; CMC, *N*-cyclohexyl-*N'*-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulfonate; COD, 1,5-cyclooctadiene; DCC, *N,N'*-dicyclohexylcarbodiimide; DIEA, *N,N*-diisopropylethylamine; *R,R*-DI-PAMP, (1*R*,2*R*)-bis[(2-methoxyphenyl)-phenyl-phosphino]-ethane; DMAP, 4-dimethylaminopyridine; EDCl, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; HATU, *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; *c*-Hex₂BOTf, dicyclohexylboron trifluoromethanesulfonate; LAH, lithium aluminum hydride; TBS, *t*-butyldimethylsilyl; TBTU, *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate

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(26) The difficulties encountered for the ester formation between **5** and **6** are unlikely to be related to the presence of the free hydroxyl group on C10 of **5** (pyridomycin numbering). In general, **5** could be reisolated unchanged from unsuccessful esterification experiments, except for an attempted DCC-mediated coupling, which gave the corresponding *N*-acyl urea. The inherently low reactivity of the C10 hydroxyl group is also reflected in the fact that the conversion of **5** into the corresponding *t*-butyldimethylsilyl (TBS) or TMS ether was not possible in preparatively viable yields.

(27) Docking was performed using LibDock in Discovery Studio 3.5 (Accelrys, San Diego). The wild-type InhA (InhADWTdec11_refmac19_coot) was obtained as a PDB file and then run through the “Prepare proteins” protocol in Discovery Studio 2.5.5 (Accelrys). A binding site sphere of 13.99 Å was generated around NADH. The docking tolerance was 0.25, the docking preference was High Quality, the conformation method was Fast, and the minimization algorithm was steepest descent (RMSD cutoff 1, max steps 1000, RMS gradient 0.001, force field CHARMM, and nonbond list radius = 14). Pyridomycin (**1**), **2**, and **3** were docked into the protein structure.