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The First Chemical Synthesis of the Core Structure of the Benzoylhydrazine−NAD Adduct, a Competitive Inhibitor of the *Mycobacterium tuberculosis* Enoyl Reductase

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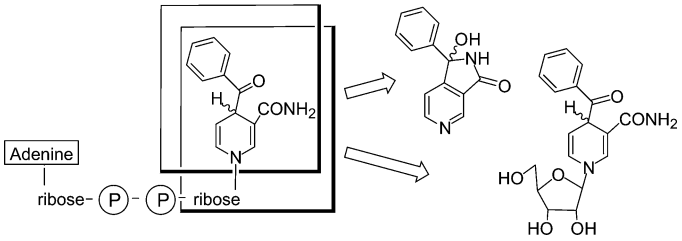

An isoniazid−NAD adduct has been recently proposed as the ultimate metabolite responsible for the antituberculous activity of isoniazid (INH). Its structure results from binding of the isonicotinoyl radical at C4 position of the nicotinamide ring of NAD with further possible and debated cyclization to form a cyclic hemiamidal derivative. Replacing the pyridine cycle of INH in INH−NAD adduct by a phenyl cycle (BH−NAD adduct) was shown previously to still retain the activity. On these bases, the core structure (4-benzoyl-1,4-dihydronicotinamide ribonucleoside) of the BH−NAD adduct and a series of analogues have been synthesized by using 3,4-pyridinedicarboximide as starting material. Depending on the nature of the substituent (pyridine or aryl) and on the oxidized or the reduced state of the nicotinamide nucleus, they were found either in a cyclized hemiamidal or an opened form or were shown to exist in equilibrium under cyclized or opened forms. Although none of these compounds could significantly inhibit activity of the InhA or MabA reductases (two possible targets of isoniazid), they represent attractive targets to develop potential second-generation inhibitors, including the total chemical synthesis of the bioactive BH−NAD adduct.

Introduction

Isoniazid (isonicotinic acid hydrazide, or INH) is one of the oldest synthetic antituberculous drugs which has been and is still widely used in prophylaxis and treatment of tuberculosis.[1,2] It is a prodrug[3−5] activated by the mycobacterial catalase-peroxidase KatG to generate an active form, most likely the isonicotinoyl radical, responsible for the lethal effect on bacterial cells. This active form binds covalently to the nicotinamide moiety of NAD(H)[6−8] and NADP(H),[9] coenzymes of the reductases InhA[10,11] and MabA,[9] respectively, two key enzymes involved in the biosynthesis of mycolic acids.[9,12,13] INH−

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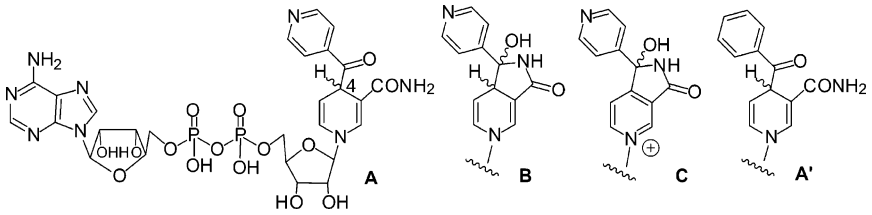

FIGURE 1. Structures of reduced (dihydropyridine) INH−NAD adducts (forms **A** and **B**), oxidized (pyridinium) INH−NAD adduct (form **C**), and benzoylhydrazine−NAD adduct (form **A′**).

NAD and INH−NADP adducts have been shown to be inhibitors of InhA and MabA.[9,14] The crystal structure of the INH−NAD adduct bound to InhA evidenced the covalent addition of an isonicotinoyl substituent at position 4 of the nicotinamide ring. It has been interpreted as the form **A** with configuration 4*S* (Figure 1).[6] However, our recent studies have shown that INH−NAD adducts prepared under biomimetic conditions (using manganese pyrophosphate as a mimic of KatG protein[8]) are present in solution as a mixture of opened keto-amide (form **A**; two epimers, both present as minor compounds) and cyclized hemiamidal structures (form **B**; four diastereoisomers, two of them are the major compounds),[8,15] raising the question about the effective active form(s) of INH−NAD adduct. One oxidized adduct (derived from oxidation of the dihydropyridine form to a pyridinium entity), initially proposed by Johnsson and Wilming as being a ketoamide structure,[7] was recently shown to have a hemiamidal structure (form **C**, two epimers).[16] Such an adduct has been found to be unable to inhibit InhA activity.[7,16]

A substantial fraction (more than one-third) of all clinical isolates that are resistant to INH results from KatG mutations.[17] Consequently, compounds able to inhibit InhA, the molecular target of INH, without requiring activation by KatG have tremendous promise as novel drugs to fight multidrug resistant tuberculosis. Recently, it was shown that use of benzoylhydrazine (BH) instead of isoniazid (i.e., isonicotinoylhydrazine) leads also to potent inhibitors of InhA, named BH−NAD adduct(s), through the covalent attachment of the benzoyl group in the place of the isonicotinoyl group, at position 4 of the nicotinamide ring of NAD (Figure 1, the opened structure **A′** has been proposed for such adduct).[14] In this work, we described the first chemical synthesis of the core structure of the BH−NAD adduct, i.e., the 4-benzoyl-1,4-dihydronicotinamide ribonucleoside **1** (Figure 2), and some related compounds as potential inhibitors of InhA. This approach could also give access to a total chemical synthesis of the BH−NAD adduct, currently only obtained by biochemical preparation in very low yield and without full chemical characterization. Furthermore, since the 4-aroylpyridine-3-carboxamide fragment can exist under opened (keto-amide) or cyclized (hemiamidal) debated forms (Figure 3), additional information on these structures and on factors governing the cyclization process are also presented throughout this work and will be discussed.

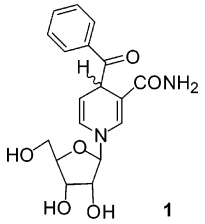

FIGURE 2. 4-Benzoyl-1,4-dihydronicotinamide ribonucleoside **1**: the core structure of the benzoylhydrazine−NAD adduct (form A′, Figure 1), a competitive inhibitor of the InhA reductase.

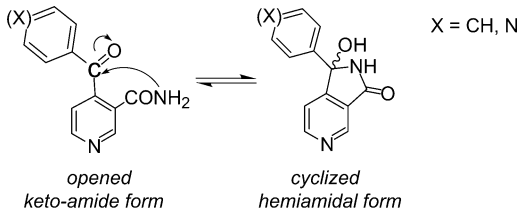

FIGURE 3. Possible equilibrium between opened and cyclized forms of 4-aroylpyridine-3-carboxamides.

Results and Discussion

Syntheses of Hemiamidal Precursors. In a previously reported synthesis of 4-benzoylnicotinamide (Figure 3, left, X = CH) by an ortho-metalation−electrophilic substitution sequence on *N*,*N*-diisopropylnicotinamide, we have shown that the equilibrium between the opened form is completely shifted toward the cyclized form (Figure 3, X = CH).[18] This initial observation led us to consider a one-step synthesis of this cyclized hemiamidal from the commercially available 3,4-pyridine dicarboximide **2** (Scheme 1). Addition of phenyllithium at −80 °C in THF proceeded in good yield furnishing a mixture of para and meta adducts (**3** and **3′**, $R^1 = C_6H_5$, Scheme

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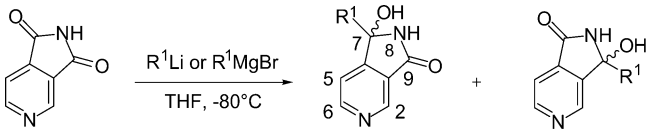
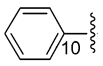
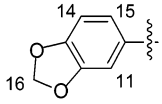
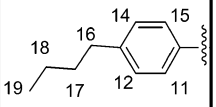
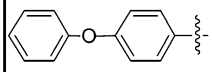
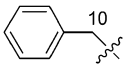
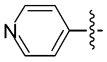
SCHEME 1. Synthesis of Hemiamidal Precursors[a]



[a] The pyridine carboxamide ring is numbered in the usual way; IUPAC nomenclature is indicated for each prepared compound in the Experimental Section.

TABLE 1. Various Hemiamidal Precursors (See General Formula in Scheme 1)

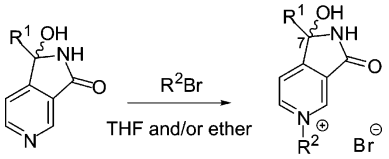
Compd.	R^1	Overall yield	Ratio *para*/*meta* regioisomers	Yield of **3-8***
3 / 3'		89 %	4 / 1	47%
4 / 4'		65 %	2 / 1	26%
5 / 5'		66 %	5 / 1	42%
6 / 6'		90 %	4 / 1	40%
7 / 7'		60 %	2 / 1	as crude product (85/15)
8 / 8'		0 %	/	/

* Isolated para compounds **3**−**8** containing less than 5% of the meta regioisomer.

1 and Table 1; meta and para refer to the position of the carbonyl group that undergoes nucleophilic attack). The desired and major product **3** was separated from the minor one **3′** by selective recrystallization from acetone. The regioisomer adduct **3** was unambiguously identified because its spectroscopic data were identical to the ones described previously for the hemiamidal **3** obtained by an unequivocal synthetic approach.[18]

The versatility and efficiency of this method allowed us to prepare other derivatives by using different aromatic Grignard reagents or lithio-aromatic nucleophiles (see the Experimental Section). The reaction generally proceeded in good yield to the formation of a mixture of para (**4**−**7**) and meta (**4′**−**7′**) compounds, in favor of the first ones (the ratio para/meta was always more than 2; Table 1). Unfortunately, in the case of the arylation with 4-lithiopyridine, prepared as previously described from 4-bromopyridine,[19] no addition product (**8** and/or **8′**) could be detected, probably due to the instability of 4-bromopyridine (Table 1).[20] From the mixture of the regioisomers meta and para, compounds **4**−**7** were purified by silica gel column chromatography or, more easily, by recrystallization from acetone or ethanol. The structures of the para isomers **4**−**6** were attributed by analogy with the NMR chemical shifts of H2 and H6 previously observed for compounds **3** (H2 was more deshielded than H6)[18] and **3′** (this work; H6 was more deshielded than H2). Surprisingly, in the case of the pair of regioisomers **7/7′** (with the phenyl linked to C7 through a methylene unit), H6 was more deshielded than H2 in the major product. HMQC, HMBC, and Jmod NMR experiments allowed a complete attribution of protons and carbons of this molecule. A ^3J correlation between C7 and H5 in the HMBC spectrum (instead of an unlikely ^4J correlation) and the spatial proximity between H5 and protons of the methylene linker (revealed by NOE experiments) showed that the major isomer was the para regioisomer **7**, as for compounds **4**−**6**.

SCHEME 2. Alkylation of Hemiamidal Compounds with Alkyl Bromides



Syntheses of Pyridinium Derivatives (Scheme 2 and Table 2). Alkylation of compounds **3**−**5** by various alkyl bromides in THF and/or ether afforded the pyridinium derivatives **9**−**17**. Condensation of **3** with (2-acetoxyethoxy)methyl bromide in THF gave instantaneously **9** as a white precipitate. Products **14** and **16** were obtained in the same way. A similar reactivity was also observed with 1-bromo-(2,3,5)-tri-*O*-acetyl-*β*-D-ribofuranose giving the compound **12**. However, all of these compounds were rather unstable in solution at ambient temperature due to their possible reaction with nucleophiles, giving back in 24 h substantial amounts of the starting products **3**−**5** through *N*-side-chain or *N*-glycosydic bond cleavage. So, we tried to lower the electrophilic nature of carbon C1′ of the chain by replacing the adjacent ether oxygen atom with an alkoxycarbonyl group. The reactivity of ethyl bromoacetate and ethyl 2-bromopropionate with the corresponding hemiamidals **3**−**5** in THF at reflux was sufficient to obtain the products **10**, **11**, **15**, and **17** in good yield. The stability of these last pyridinium derivatives is highly improved. In contrast, despite considerable efforts including variations of solvents (DMF, CH_3CN) and temperature conditions, neither the 1-bromo-2,3-propanediol nor the protected 1-bromo-2,3-*O*-acetylpropanediol reacted with **3** to give **13** or its diacetylated derivative (Table 2).

All these pyridinium compounds were shown to exist only under a cyclized hemiamidal form on the basis of ^{13}C NMR analyses since the chemical shift of C7 is characteristic of a tetrahedral carbon (around 88 ppm).

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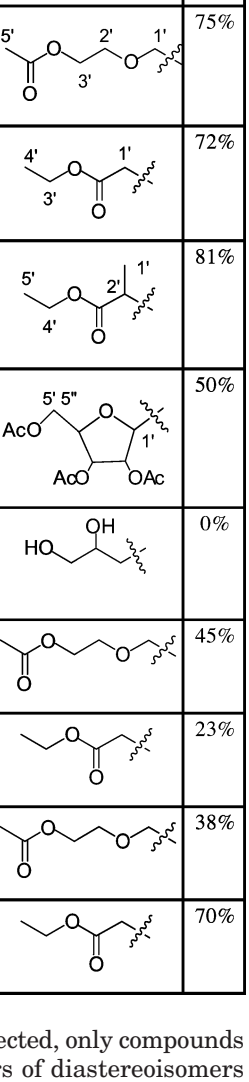
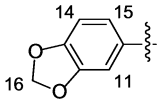
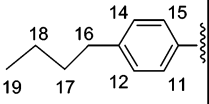
(20) Karig, G.; Spencer, J. A.; Gallagher, T. *Org. Lett.* **2001**, *3*, 835−838.

TABLE 2. Pyridinium Compounds (See General Formula in Scheme 2)

Starting compd	Pyridinium derivative	R^1	R^2	Yield
3	9			75%
	10			72%
	11			81%
	12			50%
	13			0%
4	14			45%
	15			23%
5	16			38%
	17			70%

It is interesting to note that, as expected, only compounds **11** and **12** are present as two pairs of diastereoisomers since they have additional asymmetric(s) carbon(s) in the alkyl chain. This feature results in a splitting of ^{1}H and ^{13}C NMR signals. Such a splitting of NMR signals has been previously observed in the case of oxidized INH−NAD adduct[7] and interpreted as a consequence of intramolecular stacking of the two aromatic moieties present in the opened form of the INH−NAD adduct. In the present case, stacking interactions cannot explain the NMR data, and only the cyclization process with creation of a second chiral center at C7 and formation of two pairs of diastereoisomers can explain the splitting of the NMR signals for **11** and **12**.[16]

Syntheses of Dihydropyridine Derivatives (DHP). One main difficulty in the route to the BH−NAD adduct of dihydropyridine-type appeared to be the regioselective reduction of the pyridinium ring. Indeed, few reagents are available to achieve 1,4-reduction, and moreover, 1,4-dihydropyrine products are known to be rather unstable. In particular, they are sensitive to air oxidation[21] and to protonation under acidic conditions.[22,23]

We first chose to study the reduction of **10** which is simple and quite easy for preparation (it has no additional chiral center in the chain). The most widely used reagent for selective 1,4-reduction of pyridinium salts is sodium dithionite[24,25] but by treating **10** with this reductant in the presence of sodium carbonate and in heterogeneous water/dichloromethane solution, we could only obtained an unstable product which was not the expected 1,4-dihydropyridine/hemiamidal derivative **21** (Scheme 3). Attempts to realize the reduction step by electrochemistry failed also (only unknown compounds were detected by NMR) and reduction methods involving hydride transfer from transition-metal hydride are not yet preparative methods.[26−28] As expected, the use of a hydride reagent like $NaBH_4$ provided in majority the 1,2-DHP isomer **18** (85% yield, Scheme 3) which was characterized by ^{13}C NMR as an exclusively cyclized structure (C7 carbon signal at 87.6 ppm). Only traces of the target 1,4-DHP compound could be obtained in these conditions.[29] Thus, we tried two other reducing agents derived from $NaBH_4$: $NaBH_3CN$ and $NaBH(OAc)_3$. The former led to the formation of tetrahydropyridines (identified by mass spectrometry and ^{1}H NMR) as major products, even when used in default. The latter, which is a weaker reducing agent,[30] led to the formation of a 80:20 mixture of 1,4-DHP **21** and 1,2-DHP derivatives in 80% yield. The regioselectivity can be improved up to more than 95% in favor of the desired 1,4-DHP isomer versus the 1,2-DHP isomer by adding $NaBH(OAc)_3$ in solution in acetic acid. In this way, other intermediate reducing species are probably created, as proposed by Nutaitis.[30] The 1,4-DHP compound **21** formed initially had a cyclized structure as the starting material but, interestingly, it was then slowly and spontaneously converted into the open structure **23**. This conversion can be efficiently base-catalyzed ($NaHCO_3$ or NH_3). The following ^{1}H and ^{13}C NMR data supported a cyclized structure for **21** and an opened one for **23**: H4 resonance is deshielded in **23** (δ = 5.05 ppm) with respect to **21** (δ = 3.79 ppm), NH_2 resonance is a broad singlet (δ = 6.67 ppm) in **23** whereas NH and OH are two distinct singlets (δ = 8.03 and 6.35 ppm, respectively) in **21**, and finally, the chemical shift of C7 is characteristic of a carbonyl group for **23** (δ = 198.4 ppm) and of a tetrahedral carbon (δ = 92.5 ppm) for **21**. In addition, only one set of signals was obtained in the ^{1}H and ^{13}C spectra of the cyclized structure **21**. These results indicate that the reduction of the pyridinium ring was completely stereoselective, giving only one pair of enantiomers. To confirm the stereoselectivity and to find the relative configuration of the two carbons C4 and C7, we have realized NOE coupling experiments, which showed a spatial proximity between H4 and the hydroxy-

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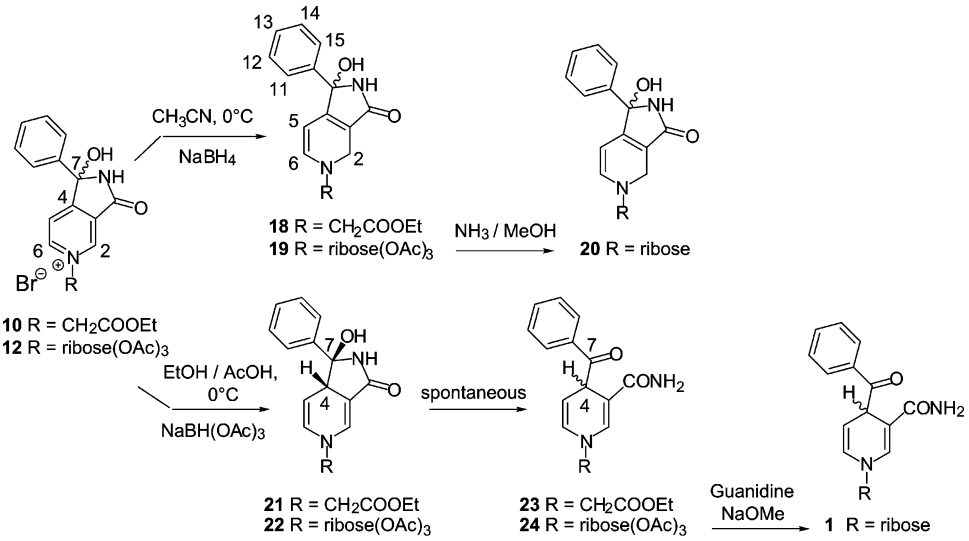
(29) Büchi, G.; Coffen, D. L.; Kocsis, K.; Sonnet, P. E.; Ziegler, F. E. *J. Am. Chem. Soc.* **1966**, *88*, 3099−3109 and references therein.
(30) Nutaitis, C. F. *J. Chem. Educ.* **1989**, *66*, 673−675.

SCHEME 3. Reduction of Pyridinium Derivatives 10 and 12 by Sodium Borohydride and Related Reducing Agents



lic proton (7-OH) and not between H4 and the phenyl protons. Thus, we concluded that the phenyl ring and the dihydropyridine nucleus were on the same side of the pyrrolidinone ring.

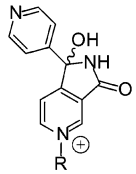
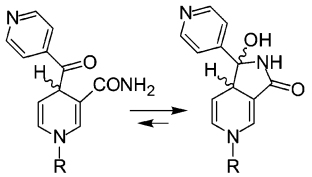
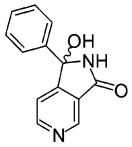
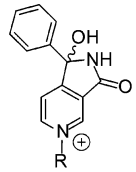
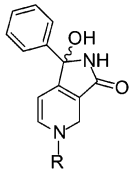
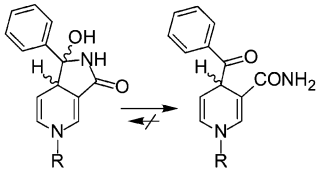
This synthetic pathway was then employed to synthesize the compounds bearing a ribose substituent. The introduction of the ribose moiety was based on a protocol described for the synthesis of nicotinamide mononucleoside which goes through a brominated intermediate.[31,32] It required the use of acetate protecting group for the following reasons: this protecting group is readily removable in the presence of the reactive pyridinium function, it is stable to the bromination conditions and it is able to provide neighboring group participation during the glycosylation step for a high β-diastereoselectivity.[31] We first considered to deprotect the ribose of **12** prior to the reduction step, because of the instability of the DHP compounds. However, the NH_3/MeOH conditions used for this deprotection (3 equiv of NH_3 1 M in MeOH, -3 to -5 °C for 20 h) gave only traces of the fully deprotected compound (the product was isolated and identified by TLC and mass spectrometry: ESI, positive mode, *m/z* 359 (M^+)) and a very large amount of the *N*-glycosyl bond cleavage product **3**. We then tried to avoid this side reaction by reducing the pyridinium prior to deprotection. The reduction step with $NaBH(OAc)_3$ was complicated by partial cleavage of the *N*-glycosidic bond (regeneration of hemiamidal **3**) and formation of undesired 1,2-DHP isomer (less than 10%). However, the crude product could be characterized by NMR as the cyclized structure **22**, but it could not be isolated in pure form since the purification step by silica gel column chromatography gave a substantial amount of the open product **24**. Since this one is the stable isomer, it was isolated and fully characterized. Further deprotection in standard conditions with NH_3 in MeOH for 4 h gave the desired product **1** in poor yield (10%), due to the instability of the DHP compound in solution at ambient temperature and during the purification process (column chromatography on silica gel). The use of guanidinium/sodium methylate,[33] a more potent deprotecting system for acetates, allowed to shorten the reaction time (less than 10 min) and thus to improve the yield of **1** (25%, two epimers). In the case of the 1,2-DHP derivative **19**, the NH_3/MeOH standard method was employed to give the 1,2-DHP ribonucleoside **20** (two epimers) in an exclusively cyclized structure (checked by ^1H and ^{13}C NMR).

From a structural point of view concerning the possible cyclization involving the 4-aroyl and 3-carboxamide substituents of the pyridine nucleus to give cyclized hemiamidals, we can conclude that such process is depending on several factors (Table 3): (i) 4-benzoylnicotinamide and various derivatives substituted on the phenyl ring (column 2) are stable in cyclized hemiamidal structure (unfortunately, we did not succeed up to now to prepare the 4-isonicotinoylnicotinamide to check the influence of the replacement of the phenyl ring by a pyridine nucleus); (ii) compounds of pyridinium type (column 3) exist as unique cyclized hemiamidal structure both in the case of 4-isonicotinoyl- and of 4-benzoyl-substituted compounds; (iii) in the case of 1,2-dihydropyridine adducts (column 4), the cyclized hemiamidal was the only structure observed, possibly by stabilization of the pyrrolinone ring formed when a 3,4-double bond is present; (iv) in the case of 1,4-dihydropyridine adducts (column 5), the nature of the 4-aroyl substituent plays an important role: in INH−NAD adducts (4-isonicotinoyl substituent), a dynamic equilibrium exists between the opened form (minor form) and the cyclized hemiamidal structure (main form),[8,15] whereas in BH−NAD models (4-benzoyl substituent), the cyclized hemiamidal structure initially present is progressively converted to 100% of opened form.

InhA and MabA Activity Assays in the Presence of the INH−NAD Analogues. Based on the scaffold of the isoniazid−NAD adduct, we have synthesized 4-benzoyl-1,4-dihydronicotinamide ribonucleoside and a series

(31) Lee, J.; Churchil, H.; Choi, W.-B.; Lynch, J. E.; Roberts, F. E.; Volante, R. P.; Reider, P. J. *Chem. Commun.* **1999**, 729−730.
(32) Mickhailopulo, I. A.; Pricota, T. I.; Timoshchuk, V. A.; Akhrem, A. A. *Synthesis* **1981**, 388−389.
(33) Adapted from Ellerwik, U.; Magnusson, G. *Tetrahedron Lett.* **1997**, *38*, 1627−1628.

TABLE 3. Opened Structure or Cyclized Hemiamidal Structure Depending on Structural Features

	4-Aroylnicotinamide precursor	Pyridinium type	1,2-Dihydropyridine type	1,4-Dihydropyridine type
INH-NAD adducts	-		-	 minor major
BH-NAD models				 intermediate end product

of related compounds. Since the K_d value of NADH for InhA is 2 μM[34] while the K_i estimated (and debated) values for the INH−NAD adduct vary from ~1 nM[14,35] to 100 nM,[7] binding of the 4-isonicotinoyl fragment to NAD, and probably also binding of the benzoyl fragment (the adduct BH−NAD has also been reported to give inhibition of InhA[14]) could be responsible for an increase of affinity for InhA of about 20−2000-fold between the natural cofactor NADH and the INH−NAD inhibitor (and probably also the BH−NAD inhibitor). Our hopes were that truncated analogues of these inhibitors still retain sufficient affinity to inhibit the InhA or/and MabA targets.

All of the synthesized analogues were tested as potential inhibitors of InhA and MabA enzymes using procedures previously described.[9,36] A pool of INH−NAD adducts prepared by oxidation of INH with manganese pyrophosphate in the presence of NAD^+ was used as reference[36] and displayed high inhibition of InhA activity (50% inhibition at 10 nM). Products **5** and **17** behave like InhA activators (activity was increased by a factor around 2.5), but at the moment, no evident explanation could be proposed. All the other BH−NAD models tested at 100 μM, including the 4-benzoyl-1,4-dihydronicotinamide ribonucleoside **1**, did not significantly affect InhA nor MabA activities. These results showed that the lack of ADP moiety found in the NAD adduct is a critical point and that simple addition of the benzoyl radical on truncated adducts is not sufficient to compensate for this absence. So we must consider these compounds only as the first of a series of compounds that need further structural modulation to improve their interaction with the target(s) and to give access to potential inhibitors. As example, especially in the case of the simplest molecule **3**, the replacement of the phenyl group by a pyridyl group (like in the INH−NAD inhibitor), or/and the addition of a hydrophobic substituent able to interact with the neighboring site of the enoyl acyl substrate in InhA might considerably increase the affinity and consequently the inhibiting activity. Such syntheses are currently in progress in our group, as also the full chemical synthesis of the biochemical adduct BH−NAD.

Conclusion

In the context of reemergence of tuberculosis during the past two decades, there is an urgent need to develop new and better treatments, particularly for tuberculosis infections resistant to traditional antibiotics. Variations of the existing TB drugs or design of drugs acting at new targets would substantially improve the actual TB control programs.[36−43] In our search of potential inhibitors of InhA and MabA derived from the isoniazid-NAD adduct, we achieved synthesis of the 4-benzoyl-1,4-dihydronicotinamide ribonucleoside **1**, in only four steps by a simple and versatile method using 3,4-pyridinedicarboximide as starting material. To our knowledge, no other synthetic pathway towards this original structure and some other related ones has been previously developed (without using the expensive NAD^+ or NADH as part of the

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(36) Nguyen, M.; Quémard, A.; Broussy, S.; Bernadou, J.; Meunier, B. *Antimicrob. Agents Chemother.* **2002**, *46*, 2137−2144.
(37) Velezheva, V. S.; Brennan, P. J.; Marshakov, V. Y.; Gusev, D. V.; Lisichkina, I. N.; Pergudov, A. S.; Tchernousova, L. N.; Smirnova, T. G.; Andreevskaya, S. N.; Medvedev, A. E. *J. Med. Chem.* **2004**, *47*, 3455−3461.
(38) Pasqualoto, K. F. M.; Ferreira, E. I.; Santos-Filho, O. A.; Hopfinger, A. J. *J. Med. Chem.* **2004**, *47*, 3755−3764.
(39) Sacchettini, J. InhA crystals and three-dimensional structure. United States Patent 5,882,878, March 16, 1999.
(40) Staveski, M. M.; Sneddon, S. F.; Yee, C.; Janjigian, A. (Applicant Genzyme corp.). InhA inhibitors and methods of use thereof. PCT/US01/40045, WO 01/56974 A2, August 9, 2001.
(41) Yatvin, M. B.; Pederson, R. L. (Applicant Enzrel, Inc.). Antimycobacterial compounds. PCT/US01/21640, WO 02/04478 A1, January 17, 2002.
(42) Quémard, A.; Daffé, M.; Ducasse, S.; Marrakchi, H.; Labesse, G.; Cohen-Gonsaud, M.; Doughet, D. (Applicants CNRS/University Montpellier 1/INSERM). Use of the protein MabA (FABG1) of *Mycobacterium tuberculosis* for designing and screening antibiotics. FP 02 04018, March 29, 2002; WO 03/082911, October 9, 2003.
(43) Andries, K.; Verhasselt, P.; Guillemont, J. et al. *Science* **2005**, *307*, 223−227.

molecule). Although none of these compounds showed significant inhibition of InhA and MabA enzymes, the core structure of BH−NAD adduct can be seen as an attractive target to develop new synthetic analogues as possible antitubercular agents and also as a key intermediate in the total synthesis of the bioactive BH−NAD adduct. We are currently working to improve binding affinity and selectivity of these analogues of the isoniazid−NAD adduct.

Experimental Section

Preparation of Compounds 3−7. To a solution of 3,4-pyridinedicarboximide **2** (2.0 g, 13.5 mmol) in dry THF (100 mL), under argon at −80 °C, was added phenyllithium (2.0 M in dibutyl ether, 2 equiv) dropwise, and the mixture was stirred at −80 °C for 1 h and then allowed to warm to room temperature over 1 h. Water was added (50 mL), and the product was extracted with ethyl acetate (6 × 100 mL). The combined organic phase was dried over Na_2SO_4, and the solvent was evaporated to give a yellow solid, which was recrystallized in acetone to give hemiamidal **3** as a white solid, yield 47%. Changing phenyllithium by 4-phenoxyphenylmagnesium bromide (0.5 M in THF) or benzylmagnesium bromide (20% in THF) allowed us to obtain **6** in 29% yield (as a white solid after recrystallization in acetone) or **7** in 60% yield (as a white solid 85:15 mixture of para and meta isomers after purification by silica gel chromatography with dichloromethane/MeOH 97:3 as eluent), respectively. Compound **4** or **5** was prepared in the same way by addition to a dry THF solution of 3,4-pyridinedicarboximide **2** (2.0 g, 13.5 mmol in 50 mL) of a solution obtained by dropwise addition of butyllithium (2.0 M in cyclohexane, 2.5 equiv) to a solution of 1-bromo-3,4-methylenedioxybenzene or 1-bromo-4-butylbenzene in dry THF (50 mL), under argon at −80 °C, and the mixture was stirred at −80 °C for 30 min. The following steps are the same as for compounds **3**, **6**, and **7** and afforded **4** in 26% yield (as a yellow solid after rectristallization in ethanol) and **5** in 42% yield (as a white powder after purification by silica gel chromatography with dichloromethane/MeOH 97:3 as eluent or recrystallization in acetone).

1-Hydroxy-1-phenyl-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridin-3-one (3). Analytical data obtained were identical to those previously described.[18]

1-(Benzo-1,2-dioxol-4-yl)-1-hydroxy-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridin-3-one (4): IR (KBr, ν_{max}/cm^{-1}) 3314, 3086, 1689, 1607, 1450, 1256, 1040; ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.46 (s, 1H), 8.85 (s, 1H), 8.71 (d, J = 5.1 Hz, 1H), 7.42 (d, J = 5.0 Hz, 1H), 7.16 (s, 1H), 7.03 (s, 1H), 6.90 (m, 2H), 6.01 (s, 2H); MS (EI) *m/z* 270 (M^+), 253.

1-(4-Butylphenyl)-1-hydroxy-1,2-dihydro-3*H*-pyrrolo-[3,4-*c*]pyridin-3-one (5): mp 177−178 °C; IR (KBr, ν_{max}/cm^{-1}) 3300, 3051, 2951, 2922, 1694, 1606, 1432, 1293, 1083; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.45 (s, 1H), 8.86 (s, 1H), 8.71 (d, J = 4.8 Hz, 1H), 7.39 (d, J = 4.8 Hz, 1H; d, J = 8.4 Hz, 2H), 7.18 (d, J = 8.4 Hz, 2H), 7.11 (s, 1H), 2.55 (t, J = 7.5 Hz, 2H), 1.51 (quintuplet, J = 7.5 Hz, 2H), 1.27 (sextuplet, J = 7.3 Hz, 2H), 0.87 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO d_6) δ 167.9 (Cq), 159.5 (Cq), 153.8 (CH), 145.4 (CH), 143.3 (Cq), 138.7 (Cq), 129.2 (2CH), 127.0 (Cq), 126.3 (2CH), 118.7 (CH), 88.1 (Cq), 35.3 (CH_2), 33.9 (CH_2), 22.6 (CH_2), 14.9 (CH_3); MS (EI) *m/z* 282 (M^+), 265 (M^+ − OH), 161, 149.

1-Hydroxy-1-(4-phenoxyphenyl)-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridin-3-one (6): IR (KBr, ν_{max}/cm^{-1}) 3306, 3165, 3068, 1697, 1609, 1591, 1254; ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.86 (d, J = 0.8 Hz, 1H), 8.71 (d, J = 5.0 Hz, 1H), 7.49 (m, 2H), 7.42 (dd, J = 4.9 Hz and J = 1.0 Hz, 1H), 7.39 (m, 2H), 7.20 (s, 1H), 7.15 (t, J = 7.4 Hz, 1H), 6.99 (m, 4H); MS (EI) *m/z* 318 (M^+), 301 (M^+ − OH), 197.

1-Hydroxy-1-benzyl-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridin-3-one (7): mp 233 °C; IR (KBr, ν_{max}/cm^{-1}) 3425 (broad), 1701, 1613, 1084, 703; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.17 (s, 1H), 8.76 (d, J = 5.2 Hz, 1H), 8.60 (s, 1H), 7.64 (d, J = 4.8 Hz, 1H), 7.01−7.11 (m, 5H), 6.70 (s, 1H), 3.41 (d, J = 13.6 Hz, 1H), 3.22 (d, J = 13.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, attributions from HMQC-GS and HMQC-GS LR experiments) δ 167.1 (Cq, C9), 157.2 (C4), 153.1 (C2), 144.9 (C6), 135.8 (C11), 131.3 (2CH, C12 and C16), 128.4 (Cq, C13 and C15), 128.3 (C3), 127.3 (CH, C14), 119.0 (CH, C5), 88.6 (Cq, C7), 44.5 (CH_2, C10); MS (EI) *m/z* 222 (M^+ − H_2O).

Preparation of Compounds 9, 14, and 16. To a stirred solution of hemiamidal **3**, **4**, or **5** (0.5 mmol) in dry THF (20−100 mL, **3** and **4**) or THF/ether (50 mL, **5**) was added 2-(acetoxyethoxy)methyl bromide (2 equiv)[15,44] was added dropwise at rt under argon. After 30 min, the white precipitate (cases of **9** and **14**) was filtered, washed with THF and ether, and dried under vacuum to give **9** in 80% yield or **14** in 49% yield. In the case of **16**, spontaneous precipitation did not occur, and only after addition of 10 mL ether could the gel/precipitate be filtered, washed with ether, centrifuged (ether), and quickly desiccated under vacuum to give **16** in 45% yield.

5-[(2-Acetoxyethoxy)methyl]-1-hydroxy-3-oxo-1-phenyl-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridinium Bromide (9): IR (KBr, ν_{max}/cm^{-1}) 3003 (broad), 1735, 1726, 1647, 1254, 1053; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.27 (s, 1H), 9.59 (s, 1H), 9.28 (d, ^3J = 6.0 Hz, 1H), 8.28 (d, ^3J = 6.0 Hz, 1H), 7.78 (s, 1H), 7.59 (m, 2H), 7.44 (m, 3H), 6.08 (s, 2H), 4.13 (m, 2H), 3.89 (m, 2H), 1.96 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.1 (Cq), 167.0 (Cq), 164.0 (Cq), 148.3 (CH), 140.9 (CH), 139.0 (Cq), 130.8 (Cq), 130.1 (CH), 129.6 (2CH), 126.8 (2CH), 122.9 (CH), 89.3 (CH_2), 88.5 (Cq), 69.5 (CH_2), 63.3 (CH_2), 21.5 (CH_3); MS (ESI) *m/z* 343 (M^+), 327, 227.

5-[(Ethoxycarbonyl)methyl]-1-hydroxy-3-oxo-1-phenyl-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridinium Bromide (10). This compound was prepared from hemiamidal **3** as previously reported, and all analytical data obtained were identical to those previously described.[18]

5-[1-(Ethoxycarbonyl)ethyl]-1-hydroxy-3-oxo-1-phenyl-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridinium Bromide (11). To a refluxed solution of hemiamidal **3** (180 mg, 0.66 mmol) in dry THF (30 mL) was added racemic ethyl 2-bromopropionate (2.40 g, 133 mmol) by aliquots (6 × 0.4 g) every 20 h. After 120 h of reaction, the white precipitate was filtered, washed three times with THF and ether, and dried under vacuum to give 200 mg of **11**: yield 78% (mixture of two racemic diastereoisomers); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.28 (s, 1H), 9.69 and 9.66 (2s, 1H), 9.39 and 9.35 (2d, ^3J = 6 Hz, 1H), 8.32 and 8.30 (2d, ^3J = 6 Hz, 1H), 7.78 (2s, 1H), 7.60 (m, 2H), 7.44 (m, 3H), 6.14 (q, ^3J = 6 Hz, 1H), 4.22 (2q, ^3J = 6 Hz, 2H), 1.95 and 1.93 (2d, ^3J = 6 Hz, 3H), 1.23 (2t, ^3J = 9 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.0 (Cq), 166.6 (Cq), 164.0 and 163.9 (Cq), 150.4 and 149.8 (CH), 143.1 and 142.5 (CH), 139.0 (Cq), 130.9 and 130.6 (Cq), 130.1 (CH), 129.6 (2CH), 126.8 (2CH), 122.8 and 122.6 (CH), 88.5 and 88.4 (Cq), 68.6 (CH), 63.5 (CH_2), 18.4 (CH_3), 14.7 (CH_3); MS (ESI) *m/z* 327 (M^+).

5-(Tri-*O*-acetyl-β-D-ribofuranose)-1-hydroxy-3-oxo-1-phenyl-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridinium Bromide (12). To a solution of tetra-*O*-acetyl-β-D-ribofuranose (1.13 g, 3.54 mmol) in dichloromethane (20 mL) at 0 °C was added dropwise hydrogen bromide 30 wt % in acetic acid (1.12 mL, 5.31 mmol). After 10 min, the solvent was evaporated, and the residual acetic acid was coevaporated with toluene (5 mL). The orange oily residue was dried under vaccum (1 h), dissolved in dry acetonitrile (40 mL), and added to a suspension of hemiamidal **3** (400 mg, 1.77 mmol) in dry acetonitrile (160 mL). The mixture was stirred for 4 h at room temperature, under argon and in the dark. Afterward, the solvent was evaporated, and the oily residue was dissolved in dichloromethane (low volume), precipitated with ether,and filtered

(44) Robins, M. J.; Hatfield, P. W. *Can. J. Chem.* **1982**, *60*, 547−553.

to give 900 mg of a white powder. This crude product could be used directly for the next step or purified by column chromatography on silica gel (dichloromethane/MeOH 98:2 to 95:5) to give 500 mg of **12** as a white powder: yield 50% (two epimers); IR (KBr, ν_{max}/cm^{-1}) 3392, 3135,1734, 1652, 1232, 1062; ^{1}H NMR (300 MHz, DMSO-d_6, a splitting of most of the signals was observed because of the presence of two epimers) δ 10.34 (s, 1H), 9.53 and 9.48 (s, 1H), 9.28 and 9.26 (d, J = 6.0 Hz, 1H), 9.36 and 9.32 (d, J = 6.0 Hz, 1H), 7.83 and 7.80 (s, 1H), 7.61 and 7.44 (m, 5H), 6.74 (s, 1H), 5.64 (m, 1H), 5.45 and 5.41 (d, J = 6.0 Hz, 1H), 4.67 (m, 1H), 4.46 (m, 2H), 2.16 (m, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.9 (Cq), 170.4 (Cq), 170.1 (Cq), 167.5 (Cq), 163.9 (Cq), 153.8 (CH), 145.4 (CH), 139.0 (Cq), 131.1 (Cq), 130.1 (CH), 129.6 (2CH), 126.9 (2CH), 123.2 (CH), 98.4 (CH), 88.5 (Cq), 82.4 (CH), 76.0 (CH), 69.0 (CH), 62.8 (CH_2), 21.4, 21.3, 21.1 ($3CH_3$); MS (ESI) *m/z* 485 (M^+); HRMS (FAB^+) found 485.1557, calcd 485.1560.

5-[(2-Acetoxyethoxy)methyl]-1-(benzo-1,2-dioxol-4-yl)-1-hydroxy-3-oxo-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridinium bromide (14): IR (KBr, ν_{max}/cm^{-1}) 3393 (broad), 3138, 1725, 1648, 1250, 1034; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.21 (s, 1H), 9.55 (s, 1H), 9.24 (d, ^3J = 6.0 Hz, 1H), 8.29 (d, ^3J = 6.0 Hz, 1H), 7.73 (s, 1H), 7.08 (s, 1H), 7.05 and 6.95 (2d, J = 6 Hz, 2H), 6.05 (2s, 4H), 4.15 (m, 2H), 3.87 (m, 2H), 1.97 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) 171.1 (Cq; weak signal), 167.0 (Cq), 164.0 (Cq), 148.9 (Cq), 148.5 (Cq), 148.2 (CH), 141.9 (CH), 132.7 (Cq), 130.8 (Cq), 122.8 (CH), 120.5 (CH), 109.0 (CH), 107.5 (CH), 102.4 (CH_2), 89.3 (CH_2), 88.3 (Cq), 69.5 (CH_2), 63.3 (CH_2), 21.4 (CH_3); MS (DCI) *m/z* 387 (M^+), 371, 369, 271.

1-(Benzo-1,2-dioxol-4-yl)-5-[(ethoxycarbonyl)methyl]-1-hydroxy-3-oxo-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridinium Bromide (15). To a refluxed solution of hemiamidal **4** (100 mg, 0.37 mmol) in THF (100 mL) was added ethyl bromoacetate (164 μL, 1.48 mmol). After 78 h, the white precipitate was filtered, washed three times with THF and ether, and dried under vacuum to give 74 mg of **15**: yield 46%; IR (KBr, ν_{max}/cm^{-1}) 3145, 3009, 1755, 1719 (and shoulder on the left (ester)), 1654, 1488, 1242, 1037; ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.24 (s, 1H), 9.53 (s, 1H), 9.16 (d, ^3J = 6.1 Hz, 1H), 8.33 (d, ^3J = 6.2 Hz, 1H), 7.76 (s, 1H), 7.14 (s, 1H), 7.04 and 6.95 (2d, J = 8.2 Hz, 2H), 6.05 (s, 2H), 5.69 (s, 2H), 4.24 (q, ^3J = 7.0 Hz, 2H), 1.26 (t, ^3J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, DMSO d_6) δ 167.1 (Cq), 166.5 (Cq), 163.8 (Cq), 151.2 (CH), 148.9 (Cq), 148.6 (Cq), 143.7 (CH), 132.6 (Cq), 130.4 (Cq), 122.5 (CH), 120.4 (CH), 109.1 (CH), 107.4 (CH), 102.4 (CH_2), 88.4 (Cq), 63.3 and 61.2 ($2CH_2$), 14.8 (CH_3); MS (ESI) *m/z* 357 (M^+); HRMS (FAB^+) found 357.1085, calcd 357.1087.

5-[(2-Acetoxyethoxy)methyl]-1-(4-butylphenyl)-1-hydroxy-3-oxo-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridinium bromide (16): IR (KBr, ν_{max}/cm^{-1}) 3152, 2957, 2930, 1728, 1652, 1249, 1064; ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.26 (s, 1H), 9.57 (s, 1H), 9.22 (d, ^3J = 6.3 Hz, 1H), 8.26 (d, ^3J = 6.2 Hz, 1H), 7.72 (bs, 1H), 7.49 and 7.25 (2d, J = 8.2 Hz, 4H), 6.02 (s, 2H), 4.15 (m, 2H), 3.87 (m, 2H), 2.58 (t, J = 7.5 Hz, 2H), 1.95 (s, 3H). 1.52 (quintuplet, J = 7.5 Hz, 2H), 1.27 (sextuplet, J = 7.4 Hz, 2H), 0.88 (t, J = 7.2 Hz, 3H); MS (DCI) *m/z* 283 (hemiamidal + H^+), no M^+ detected.

1-(4-Butylphenyl)-5-[(ethoxycarbonyl)methyl]-1-hydroxy-3-oxo-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridinium Bromide (17). To a refluxed solution of hemiamidal **5** (100 mg, 0.35 mmol) in THF/ether (1/1, 10 mL) was added ethyl bromoacetate (58 μL, 0.53 mmol). After 20 h, the white precipitate was filtered, washed two times with ether, and dried under vacuum to give 100 mg of **22**: yield 70%; IR (KBr, ν_{max}/cm^{-1}) 3385, 3040, 2958, 2931, 1748, 1724, 1654, 1374, 1215; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.28 (s, 1H), 9.55 (s, 1H), 9.14 (d, ^3J = 6.3 Hz, 1H), 8.30 (d, ^3J = 6.2 Hz, 1H), 7.75 (s, 1H), 7.47 and 7.26 (2d, J = 8.3 Hz, 4H), 5.68 (s, 2H), 4.24 (q, ^3J = 7.0 Hz, 2H), 2.58 (t, J = 7.5 Hz, 2H), 1.53 (quintuplet, J = 7.5 Hz, 2H), 1.27 (m, 2H), 1.26 (t, ^3J = 7.0 Hz, 3H), 0.88 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.1 (Cq), 166.7 (Cq), 163.8 (Cq), 151.2 (CH), 144.5 (Cq), 143.7 (CH), 136.2 (Cq), 130.5 (Cq), 129.6 (2CH), 126.7 (2CH), 122.6 (CH), 88.5 (Cq), 63.3 and 61.3 ($2CH_2$), 35.3, 33.9 and 22.6 ($3CH_2$), 14.8 and 14.6 ($2CH_3$); MS (DCI) *m/z* 369 (M^+), 353, 283 (hemiamidal + H^+).

Ethyl (1-Hydroxy-3-oxo-1-phenyl-1,2,4,5-tetrahydro-3*H*-pyrrolo[3,4-*c*]pyridin-5-yl)acetate (18). To a solution of **10** (50 mg, 0.13 mmol) in acetonitrile (10 mL) was added sodium borohydride (5 mg, 0.13 mmol) with cooling in an ice−water bath. After 10 min at 0 °C, water was added, the product was extracted with dichloromethane and dried over Na_2SO_4, and the solvent was evaporated under vacuum. The product was precipitated from dichoromethane/hexane (9:1) to give 35 mg of **18** as a yellow powder: yield 85%; IR (KBr, ν_{max}/cm^{-1}) 3370, 3210, 1736, 1674, 1548, 1193; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.23 (s, 1H), 7.30 (m, 5H), 6.41 (s, 1H), 6.39 (d, J = 6.9 Hz, 1H), 4.45 (d, J = 7.2 Hz, 1H), 4.22 (s, 2H), 4.13 (q, J = 7.1 Hz, 2H), 3.88 (s, 1H), 3.91 (s, 1H), 1.21 (t, J = 7.1 Hz, 3H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 171.6 (Cq), 170.3 (Cq), 156.6 (Cq), 142.0 (Cq), 110.5 (Cq), 145.6 (CH), 128.9 (2CH), 128.4 (CH), 126.3 (2CH), 87.7 (CH), 87.6 (Cq), 61.5 (CH_2), 55.9 (CH_2), 46.3 (CH_2), 15.0 (CH_3); MS (DCI/NH_3) *m/z* 315 (M + H^+).

1-Hydroxy-3-oxo-1-phenyl-5-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4,5-tetrahydro-3*H*-pyrrolo[3,4-*c*]pyridine (19). A solution of crude **12** (0.53 mmol, 300 mg) in EtOH (20 mL) at 0 °C was incubated with sodium borohydride (0.53 mmol, 20 mg) for 10 min at 0 °C. Then water was added, the product was extracted with dichloromethane and dried over Na_2SO_4, and the solvent was evaporated under vacuum. The product was purified by two successive column chromatography on silica gel (dichloromethane/MeOH, 100:0 to 97:3 and ethyl acetate) to give 129 mg of **19** as a bright yellow powder (50% yield for two steps): ^{1}H NMR (300 MHz, DMSO-d_6; a splitting of some signals was observed because of the presence of two epimers) δ 8.38 (s, 1H), 7.38 (m, 5H), 6.54 (2s, 1H), 6.46 (d, J = 6.3 Hz, 1H), 5.33 (m, 1H), 5.14 (m, 2H), 4.58 (2d, J = 6.9 Hz, 1H), 4.26 and 4.16 (2m, 5H), 2.07 (m, 9H); ^{13}C NMR (75 MHz, DMSO-d_6), δ 171.3 (Cq), 170.4 (Cq), 170.3 (Cq), 170.2 (Cq), 156.0 (Cq), 143.7 (CH), 141.5 (Cq), 129.0 (CH), 128.5 (CH), 112.8 (Cq), 95.2 (CH), 90.1 (CH), 87.6 (Cq), 78.6 (CH), 71.3 (CH), 68.3 (CH), 64.2 (CH_2), 39.8 (CH_2), 21.4, 21.3 and 21.2 ($3CH_3$); MS (ESI, positive mode) *m/z* 487 (M + H^+), 509 (M + Na^+), 525 (M + K^+).

1-Hydroxy-3-oxo-1-phenyl-5-β-D-ribofuranosyl-1,2,4,5-tetrahydro-3*H*-pyrrolo[3,4-*c*]pyridine (20). To 30 mg (0.053 mmol) of **19** was added NH_3 in MeOH 7 M (3 mL). After 3 h at rt, the solvent was concentrated in a vacuum and the product was precipitated from MeOH/ether (9:1). The precipitate was chromatographed on silica gel (dichloromethane/MeOH, 90:0 to 85:20) to give 6 mg of **20** (31% yield). The product can be precipitated from methanol/ethyl acetate to give a bright yellow powder: IR (KBr, ν_{max}/cm^{-1}) 3376 (broad), 1669, 1600, 1198, 1046; UV (λ_{max}, ϵ) 398 nm, 4600 M^{-1} cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6; two epimers) δ 8.29 and 8.28 (2s, 1H), 7.37 (m, 5H), 6.51 (d, J = 7.2 Hz, 1H), 6.43 and 6.42 (2s, 1H), 5.10 (2d, J = 6.0 Hz, 1H), 4.99 (2d, J = 4.7 Hz, 1H), 4.85 (2d, J = 5.3 Hz, 1H), 4.67 (2d, J = 6.4 Hz, 1H), 4.48 (2d, J = 7.2 Hz, 1H), 4.24 (2s, 2H), 3.81 (m, 1H), 3.66 (m, 1H), 3.56 to 3.42 (m, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.6 (Cq), 156.9 and 157.0 (Cq), 144.6 and 144.7 (CH), 141.9 (Cq), 128.9 (2CH), 128.4 (CH), 126.4 (2CH), 111.6 and 111.5 (Cq), 97.6 (CH), 88.5 (CH), 87.5 (Cq), 84.4 (CH), 71.3 and 71.2 (CH), 69.5 and 69.4 (CH), 62.5 (CH_2), 39.8 (CH_2); MS (ESI, positive mode) *m/z* 361 (M + H^+), 383 (M + Na^+), 399 (M + K^+).

Ethyl (1-Hydroxy-3-oxo-1-phenyl-1,2,5,7a-tetrahydro-3*H*-pyrrolo[3,4-*c*]pyridin-5-yl)acetate (21) and Ethyl (4-Benzoyl-3-carboxamido-1,4-dihydropyridin-1-yl)acetate (23). To a solution of **10** (100 mg, 0.25 mmol) in EtOH (10 mL) at 0 °C was added sodium triacetoxyborohydride (80 mg, 0.38 mmol) in solution in 3 mL of acetic acid. After 10 min at 0 °C, water was added, and the product was extracted with dichloromethane. This organic layer was washed with

saturated $NaHCO_3$ solution and dried over Na_2SO_4, and the solvent was evaporated under vacuum. The product could be precipitated from dichoromethane/hexane (9:1) to give 65 mg of a pale orange powder, Yield 80% of cyclized **21**, which was slowly and spontaneously converted in **23**. NH_3 in MeOH (1 M) could be used to catalyze this conversion. Compound **21**: IR (KBr, ν_{max}/cm^{-1}) 3247 (very broad), 1742, 1687, 1662, 1606, 1219, 1164, 1044; ^1H NMR (300 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.31 (m, 4H), 7.25 (m, 1H), 6.70 (s, 1H), 6.35 (s, 1H), 5.67 (d, J = 7.8 Hz, 1H), 4.08 (q, J = 7.1 Hz, 2H), 4.08 (s, 2H), 3.91 (dd, J = 7.8 Hz and J = 1.5 Hz, 1H), 3.79 (d, J = 1.5 Hz, 1H), 1.17 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.7 (Cq), 170.2 (Cq), 142.7 (Cq), 132.6 (CH), 131.7 (CH), 128.4 (2CH), 128.0 (CH), 127.2 (2CH), 103.7 (Cq), 98.6 (CH), 92.5 (Cq), 61.4 (CH_2), 54.0 (CH_2), 47.9 (CH), 14.9 (CH_3); MS (ESI, positive mode) *m/z* 337 (M + Na^+), 353 (M + K^+). Compound **23**: IR (KBr, ν_{max}/cm^{-1}) 3344, 1738, 1683, 1652, 1596, 1393, 1216; ^1H NMR (250 MHz, DMSO-d_6) δ 8.00 (m, 2H), 7.62 (m, 1H), 7.52 (m, 2H), 7.20 (s, 1H), 6.67 (s, 2H), 6.05 (d, J = 7.8 Hz, 1H), 5.05 (d, J = 4.6 Hz, 1H), 4.64 (dd, J = 7.8 Hz and J = 4.6 Hz, 1H), 4.12 (s, 2H), 4.12 (q, J = 7.2 Hz, 2H), 1.20 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 198.4 (Cq), 170.5 (Cq), 169.6 (Cq), 138.4 (CH), 136.4 (Cq), 133.5 (CH), 131.6 (CH), 129.5 (4CH), 103.5 (Cq), 100.7 (CH), 61.6 (CH_2), 54.0 (CH_2), 42.4 (CH), 14.9 (CH_3); MS (ESI, positive mode) *m/z* 337 (M + Na^+), 353 (M + K^+).

4-Benzoyl-3-carboxamido-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-1,4-dihydropyridine (24). To a solution of crude **12** (0.56 mmol, 317 mg) in EtOH (20 mL) at 0 °C was added sodium triacetoxyborohydride (0.73 mmol, 155 mg) in solution in 5 mL of acetic acid. After 10 min at 0 °C, water was added, and the product was extracted with dichloromethane. This organic layer was washed with saturated $NaHCO_3$ solution and dried over Na_2SO_4, and the solvent was evaporated under vacuum. The crude product was characterized by NMR as the cyclized structure **22**, but after purification by two successive column chromatography on silica gel (dichloromethane/MeOH, 100:0 to 97:3 and ethyl acetate), only pure **24** was obtained as a pale orange powder (90 mg; 35% yield): ^1H NMR (300 MHz, DMSO-d_6; two epimers) δ 7.99 (m, 2H), 7.63 (m, 1H), 7.52 (m, 2H), 7.41 and 7.38 (2s, 1H), 6.86 (bs, 2H), 6.33 (d, J = 7.8 Hz, 1H), 5.27 to 5.09 (m, 3H), 4.78 (m, 1H), 4.30 to 4.14 (m, 4H), 2.07 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 198.5 and 198.4 (Cq), 171.0 (Cq), 170.3 (Cq), 170.2 (Cq), 169.1 and 169.3 (Cq), 136.2 (Cq), 135.2 (CH), 133.8 (CH), 129.6 (2CH), 129.5 (2CH), 127.5 (CH), 106.6 and 106.2 (Cq), 102.8 and 102.2 (CH), 93.4 (CH), 78.9 (CH), 71.1 and 71.0 (CH), 70.6 and 70.4 (CH), 64.2 and 64.1 (CH_2), 47.2 (CH), 21.4, 21.2, 21.1 ($3CH_3$); MS (ESI, positive mode) *m/z* 487 (M + H^+), 509 (M + Na^+), 525 (M + K^+).

4-Benzoyl-3-carboxamido-1-β-D-ribofuranosyl-1,4-dihydropyridine (1). To 113 mg of **24** (0.23 mmol) was added 14 mL of a solution of guanidine hydrochloride (50 mM) and sodium methanolate (11 mM) in methanol/dichloromethane (9:1). The mixture was allowed to stand at room temperature for 15 min, and Amberlite IRP 64 ion-exchange resin (100−500 wet mesh, carboxylic acid, hydrogen form) was added. After filtration and concentration by evaporation, the residue was chromatographed on silica gel (dichloromethane/MeOH, 90:10 to 85:15) to give 22 mg of **1** (25% yield) The product can be precipitated from methanol/ethyl acetate to give a pale orange powder: IR (KBr, ν_{max}/cm^{-1}) 3360 (broad), 1683, 1648, 1596, 1221, 1097; UV (λ_{max}, ϵ) 318 nm, 4600 L mol^{-1} cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6; two epimers) δ 7.98 (m, 2H), 7.63 (m, 1H), 7.53 (m, 2H), 7.37 and 7.33 (2s, 1H), 6.77 (bs, 2H), 6.33 and 6.29 (2d, J = 8.1 Hz, 1H), 5.25 (bs, 1H), 5.12 and 5.07 (2d, J = 4.5 Hz, 1H), 4.90 (bs, 1H), 4.69 (m, 2H), 4.09 (bs, 1H), 3.99 to 3.86 (m, 2H), 3.71 (m, 1H), 3.48 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 199.2 (Cq), 170.0 (Cq), 137.2 (CH), 135.7 (Cq), 134.2 (CH), 129.7 (2CH), 129.5 (2CH), 127.4 (CH), 103.5 and 103.2 (Cq), 102.3 and 101.7 (CH), 95.8 (CH), 84.7 (CH), 72.3 and 70.8 (2CH), 62.2 (CH_2), 42.9 (CH); MS (ESI, positive mode) *m/z* 361 (M + H^+), 383 (M + Na^+), 399 (M + K^+).

Activity Assays for InhA and MabA. For InhA, the preincubation reactions were performed in 80 μL (total volume) of 100 mM sodium phosphate buffer solution, pH 7.5, at 25 °C containing 19 nM InhA and 100 μM ligand compounds **1**−**24** (or 58 μM or 10 nM of the pool of INH−NAD adducts). After 5 min of preincubation, the addition of 112 μM 2-*trans*-decenoyl-CoA and 100 μM NADH initiated the reaction. Control reactions were carried out under the same conditions as those described above without ligands. For MabA, the preincubation reactions were performed in 80 μL (total volume) of 100 mM sodium phosphate buffer solution, pH 7.0, at 25 °C containing 3 μM MabA and 100 μM ligand compounds **1**-**24** (or 58 μM or 10 nM of the pool of INH−NAD adducts). After 5 min of preincubation, the addition of 350 μM acetoacyl-coenzyme A (acetoacyl-CoA) and 100 μM NADPH to a final volume of 1 mL initiated the reaction. Control reactions were carried out under the same conditions as those described above without ligands. All activity assays were performed in duplicate.

Enoyl reductase activity of InhA was assayed by monitoring the oxidation of NADH at 340 nm (25 °C), and initial velocities were determined. Results are expressed as a percentage of remaining InhA activity determined on the basis of control experiments. β-Ketoacyl reductase activity of MabA was assayed in the same way, monitoring the oxidation of NADPH at 340 nm.

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Supporting Information Available: The general experimental methods and a compound characterization checklist are provided, and copies of a ^1H NMR spectrum or a proton-decoupled ^{13}C NMR spectrum for each new compound are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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