Total Syntheses of Mycobactin Analogues as Potent Antimycobacterial Agents Using a Minimal Protecting Group Strategy

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Mycobactins are a family of iron sequestering agents (siderophores) biosynthesized as growth promoters by mycobacteria including *Mycobacterium tuberculosis*. They are important siderophores with high affinity and specificity for Fe(III) due to the chemical nature of their component chelating functional groups. The parent compounds and their synthetic analogues can be used for studies of natural iron uptake mechanisms. It was hypothesized by Snow and co-workers that alternate and modified mycobactin analogues might serve as antagonists of mycobacterial growth and be of important therapeutic value. Efficient syntheses of four different analogues are presented. Dramatic improvements on formation of amide and ester bonds were achieved using water soluble carbodiimide (EDC·HCl)-mediated couplings in the presence of 1-hydroxy-7-azabenzotriazole (HOAt) as an additive. Using HOAt over other traditional coupling additives provided significant enhancement of the reaction rate of the desired coupling reactions and minimized side reactions. Further simplifications were made possible by minimizing the use of protecting groups during the syntheses. In fact, coupling components in the presence of free hydroxamic acids and a free phenolic hydroxyl group proceeded in excellent yields. Biological studies indicated that the resulting synthetic analogues effect moderate to high inhibition of the growth of *M. tuberculosis* H37Rv.

Introduction

Among bacterial infections, tuberculosis is usually a respiratory infection, though it can damage virtually any organ in the human body. About 40% of the world's population is infected with the tuberculosis bacterium, which grows slowly and in most cases is indefinitely suppressed by the immune system. According to a new estimate by the World Health Organization, the disease killed more people in 1995, when effective treatment was available, than in 1900, when no antituberculosis drugs existed.[1] Early this decade, the incidence of tuberculosis began rising after a 33-year downward trend. In response, the federal government greatly increased spending on tuberculosis control, from about $9 million in 1986 to nearly $120 million in 1996.[1] Streptomycin, the first antibiotic capable of killing the tuberculosis organism, was discovered in 1944.[2] Many other antituberculosis agents were developed subsequently.[3] However, the recent emergence of drug resistant strains of tuberculosis is of special concern.[4] The World Health Organization recently reported that the death rate of patients with multidrug resistant tuberculosis in the U.S. was approximately 70%.[5] Between 1989 and 1992 such drug resistant strains had appeared in 17 states.[6]

Over the past decade, significant advances have been made in the discovery and development of antimicrobial agents in general. However, the need for safe, microbially selective, and effective drugs is apparent, and certainly no ideal agents have yet been developed. Continued effort must be made to promote the discovery of new antimicrobial agents, and studies of their modes of action are anticipated to facilitate the rational design of new drugs. One important aspect of natural drug resistance in TB strains is thought to be related to impermeability of the mycobacterial cell wall, which contains large amounts of lipids with an unusual structure. Like most mycobacteria, cells of *Mycobacterium tuberculosis* are covered by a lipid-rich cell wall, which consists of a large amount of mycolic acids with long hydrocarbon chains. The length and high degree of saturation of the chains produce an exceptionally tightly packed array with extremely low fluidity. Consequently, penetration of antibiotics and chemotherapeutic agents into or through the cell wall is limited.[7] Design and synthesis of an efficient drug carrier which facilitates drug delivery has become a major challenge in drug development.

Microbial iron chelators, or siderophores, are important in the study of iron metabolism[8] and in the development of drugs for the treatment of iron-overloaded patients.[9] The high affinity and specificity of siderophores for Fe(III) is due to the chemical nature of the chelating functional

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(2) Schatz, A.; Bugie, E.; Waksman, S. A. *Proc. Soc. Exp. Biol. Med.* **1944**, *55*, 66.

(3) Lemke, T. L. In *Principles of Medicinal Chemistry*, 4th ed.; Foye, W. O., Lemke, T. L., Williams, D. A., Eds.; Williams & Wilkins: Baltimore, 1995; pp 747−758.

(4) Lemonick, M. D. The Killer All Around. *Time Magazine*; Lemonick, M. D., Ed.; Time, Inc.: New York, Sept 12, 1994; p 62.

(5) Facts and Figures: Tuberculosis Today, World Health Organization's 1995 report on tuberculosis.

(6) (a) DiGiacomo, M. A Deadly Return. *Newsweek*, March 16, 1992, p 53. (b) Tuberculosis and HIV Public Health Policy: A Dual Challenge. Washington: AIDS Action Foundation, March 1992.

(7) (a) Connell, N. D.; Nikaido, H. Membrane Permeability and Transport in Mycobacterium Tuberculosis. In *Tuberculosis: Pathogenesis, Protection, and Control*; Bloom, B. R., Ed.; ASM Press: Washington, DC, 1994; p 333. (b) Liu, J.; Rosneberg, E. Y.; Nikaido, H. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 11254. (c) Besra, G. S.; Chatterjee, D. Lipid and Carbohydrates of Mycobacterium Tuberculosis. In *Tuberculosis: Pathogenesis, Protection, and Control*; Bloom, B. R., Ed.; ASM Press: Washington, DC, 1994; p 285.

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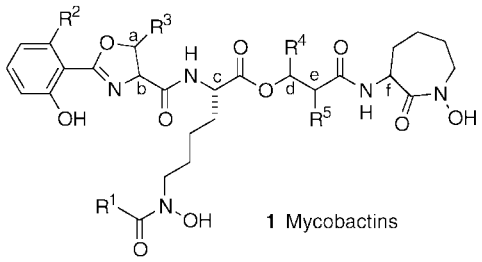


1 Mycobactins

Mycobactins	R^1	R^2	R^3	R^4	R^5	a	b	c	d	e	f
A	C_{13}	CH_3	H	CH_3	H	threo	threo	?	?	?	?
F	$C_{9\text{-}17}$	H	CH_3	CH_3	H	threo	threo	?	S	-	L
H	$C_{17,19}$	CH_3	CH_3	CH_3	H	R	L	L	S	-	L
M	CH_3	H	CH_3	$C_{15\text{-}18}$	CH_3	?	?	?	erythro	erythro	?
N	C_2H_5	H	CH_3	$C_{15\text{-}18}$	CH_3	?	?	?	?	?	?
P	$C_{15\text{-}19}$	CH_3	H	C_2H_5	CH_3	-	L	L	S	R	L
R	C_{19}	H	H	C_2H_5	CH_3	-	L	L	R	S	L
S	$C_{13\text{-}19}$	H	H	CH_3	H	-	L	L	S	-	L
T	$C_{17\text{-}20}$	H	H	CH_3	H	-	L	L	R	-	L

Figure 1.

groups, which usually consist of either catecholate or hydroxamic acid residues. The mycobactins, **1**, are among the most structurally complex of the known siderophores. They promote mycobacterial growth via iron uptake processes. This family of compounds was first isolated and characterized mainly by Snow and coworkers.[10] All members of the family of compounds possess a nearly identical main carbon skeleton with variations only in stereochemistry of chiral centers and in the peripheral groups. All the mycobactins form extremely stable hexadentate iron(III) complexes by binding the iron with two hydroxamic acids and a 2-hydroxyphenyloxazoline residue. After detailed studies, Snow[10] suggested that alternate or modified forms of mycobactins might serve as antagonists of mycobacterial growth by competitively binding iron and/or inhibiting its assimilation by targeted forms of mycobacteria and thus might be of important therapeutic value. Additionally, design and synthesis of mycobactin analogues, which contain potential "drug linkers", may provide a new class of therapeutic agents. Direct attachment of antimicrobial agents to the analogues may allow the pendant drug to be actively carried into cells by the iron transport system or at least be targeted to mycobacteria. Herein, we focus on the design of mycobactin analogues to further test the feasibility of using modified siderophores to antagonize the growth of *Mycobacterium tuberculosis*.

A mycobactin analogue lacking the three hydroxyl groups necessary for iron chelation was synthesized by Carpenter and Moore in 1969.[11] Biological tests of these analogues revealed no growth inhibitory or stimulatory activity of mycobacteria, thus confirming a need for iron complexation. The first total synthesis of a mycobactin analogue, mycobactin S2, which contains all iron-chelating components but lacks a long lipophilic side chain (**1**, R^1, $R^4 = CH_3$; R^2, R^3, $R^5 = H$, Figure 1), was accomplished in our group.[12] Biological studies revealed that the short alkyl group (R^1) resulted in loss of lipid solubility and siderophore activity for mycobacteria. This indicated that a long lipophilic side chain is essential for mycobactins to transport iron through cell membranes and, therefore, to be potential drug delivery vehicles. A more recent synthesis of a mycobactin that incorporated a long acyl group (**1**, $R^1 = CH_3(CH_2)_{14}$; $R^4 = CH_3$; R^2, R^3, $R^5 = H$, Figure 1), mycobactin S, was accomplished by our group.[13] Interestingly, biological tests showed that synthetic mycobactin S, a natural growth promoter for *M. segmatis*, effects greater than 99% inhibition of the growth *M. tuberculosis* H37Rv at the concentration of 12.5 μg/mL. Subsequently, the minimum inhibitory concentration (MIC) was determined to be 3.13 μg/mL. Compared to mycobactin T, the siderophore and growth promoter for *M. tuberculosis*, mycobactin S differs only by the stereochemistry of the methyl group at R.[4] This strongly indicates that the single chiral center plays a critical role in controlling TB growth. Further biological studies showed that several mycobactin components display moderate growth promotion of mycobacteria.[14] Therefore, design and synthesis of novel mycobactin analogues would allow us not only to discover effective inhibitors of *M. tuberculosis* but also to further test Snow's hypothesis that use of modified siderophore structures may lead to the development of selective mycobacterial growth inhibitors.[10] The apparent complexity of the structures of the mycobactins makes

(8) (a) Neilands, J. B. In *Microbial Iron Metabolism. A Comprehensive Treatise*; Neilands, J. B., Ed.; Academic Press: New York, 1974; p 3. (b) Emery, T. *Met. Ions Biol. Syst.* **1978**, Chapter 3, 7. (c) Emery, T. *Biochemistry* **1971**, *10*, 1483.

(9) (a) Anderson, W. F. In *Inorganic Chemistry in Biology and Medicine*; Martell, E., Ed.; American Chemical Society: Washington, DC, 1980; Chapter 15. (b) Weatherall, D. J. In *Development of Iron Chelators for Clinical Use*; Martell, A. E., Anderson, W. F., Badman, D. G., Eds.; Elsevier/North-Holland; New York, 1981; p 3. (c) Waxman, H. S.; Brown, E. B. *Prog. Hematol.* **1969**, *6*, 338.

(10) Snow, G. A. *Bacteriol. Rev.* **1970**, *34*, 99.

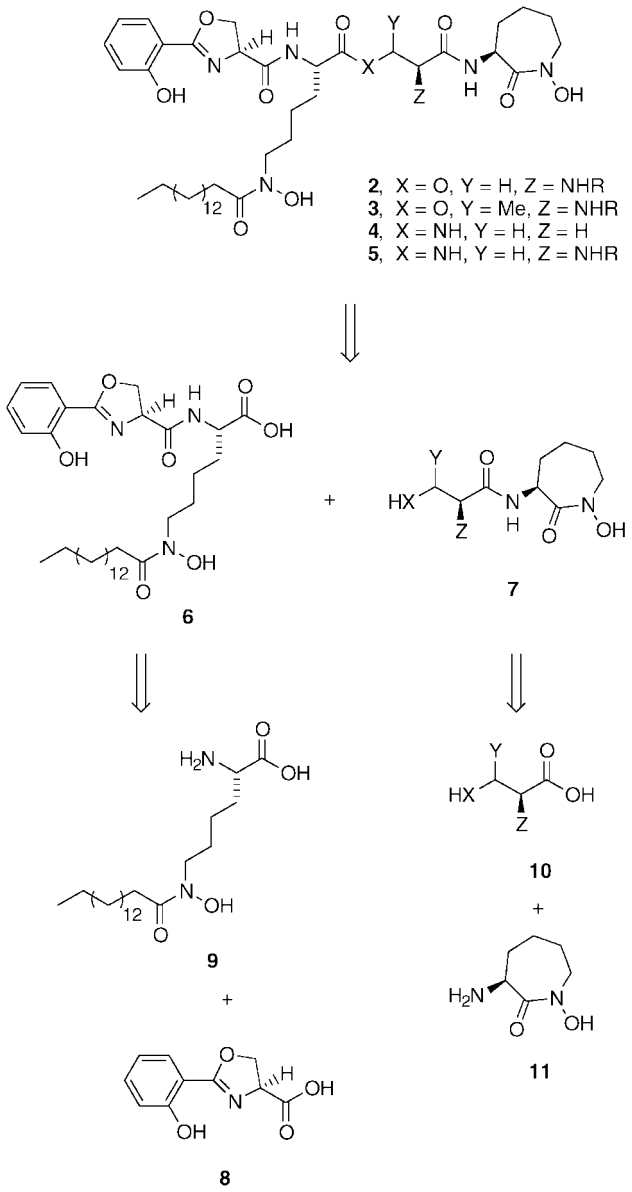
(11) Carpenter, J. G.; Moore, J. W. *J. Chem. Soc. C* **1969**, *12*, 1610.

(12) Maurer, P. J.; Miller, M. J. *J. Am. Chem. Soc.* **1983**, *105*, 240.

(13) Hu, J.; Miller, M. J. *J. Am. Chem. Soc.* **1997**, *119*, 3462.

(14) Hu, J. Ph.D. Dissertation. Total Synthesis of Mycobactins, Siderophore-Drug Conjugates, and Biological Studies. Department of Chemistry and Biochemistry, University of Notre Dame, 1996.

Scheme 1. Retrosynthetic Analysis of Mycobactin Analogs



preparation of appropriate analogues an apparently daunting task. However, as described here, substantial simplification of the syntheses will allow preparation of a number of important mycobactins and derivatives in quantities sufficient for extensive study. On the basis of dramatic differences in the biological activities of our previous synthetic mycobactin S and mycobactin T, our focus in the design of **2**−**5** was to determine the importance of the linkage (X) between the constituent iron-binding components and stereochemistry of the Y group (Scheme 1). Additionally, introduction of protected linker functionality (Z = NHR/R = Cbz or Boc) will facilitate future syntheses of drug conjugates. Efficient syntheses of four novel analogues related to **2**−**5** (Scheme 1) of mycobactins and biological studies demonstrating their antimycobacterial activities are presented.

As shown retrosynthetically in Scheme 1, the mycobactins can be constructed by coupling of the mycobactic acid portion, **6**, and the cobactin portion, **7**. Mycobactic acid **6** can be generated from coupling oxazoline **8** and amino acid **9**. The cobactins, **7**, can be easily produced by the coupling of amine **11** with acid **10**. Construction

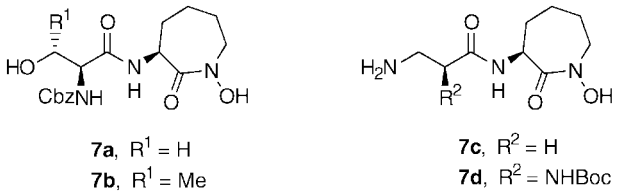


Figure 2.

of lysine derivative **9** can be achieved by dimethyldioxirane (DMD) oxidation of the ϵ-amino group of the appropriate α-protected lysine ester. Oxazoline derivative **8** can be prepared by using our previously described methodology.[13] Cyclolysine **11** also can be derived from lysine by direct DMD oxidation followed by intramolecular amidation.

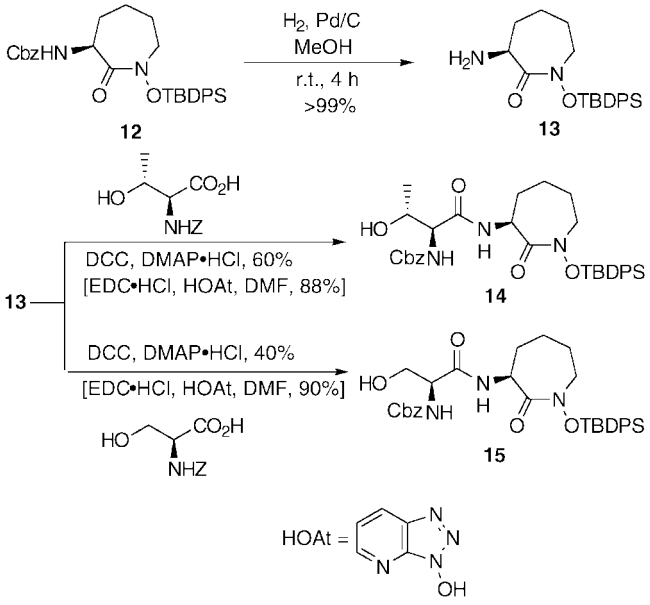
Results and Discussion

Our syntheses started by preparation of the four different cobactin constituents (**7a**−**d**, Figure 2) of the target mycobactin analogues **2**−**5**. Treatment of cyclic hydroxamate **12**[13] with 10 wt % Pd on carbon in methanol afforded free amine **13** in quantitative yield. Using the reaction conditions we reported earlier for reaction of **13** with *β*-hydroxybutyrate,[13] cross-coupling of amine **13** with Cbz-protected threonine and serine using DCC (dicyclohexylcarbodiimide)/DMAP·HCl (4-(dimethylamino)pyridine hydrochloride salt) gave only moderate yields of desired products, **14** and **15**, respectively (Scheme 2). As expected, self-coupling of the Cbz-protected *β*-hydroxy amino acids produced dimeric and oligomeric esters as the major byproducts. A number of different coupling additives were studied in attempts to circumvent the competitive esterification. 1-Hydroxy-7-azabenzotriazole (HOAt), first reported by Carpino in 1993,[15] was remarkably effective. HOAt was found to accelerate the desired amide coupling processes and provide a visual indication of the reaction endpoint. Although precise mechanistic details need yet to be established, reactions of HOAt esters appear to be enhanced relative to those of the commonly used 1-hydroxybenzotriazole (HOBt) analogues. The pronounced reactivity increase observed upon substitution of HOAt for HOBt presumably is due to assistance from internal hydrogen bonding of the incoming nucleophiles by the nitrogen atom of pyridine residue in the reagent.[15] Coupling of amine **13** with Cbz-protected threonine in the presence of HOAt afforded amide **14** in 88% yield without any detectable competitive ester formation (Scheme 2). Under similar reaction conditions, serine-based cobactin analogue **15** was formed in 90% yield.

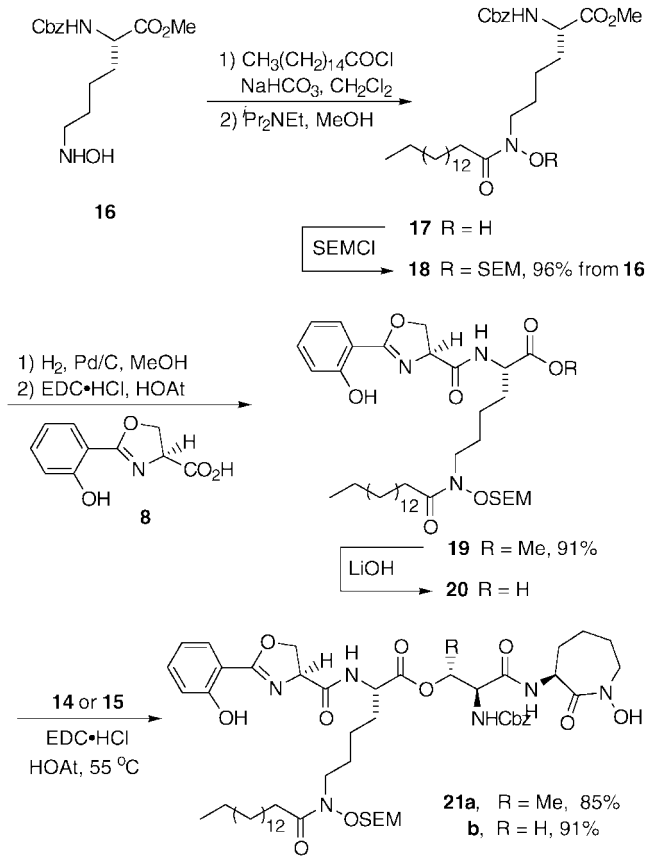
The synthesis of the mycobactic acid component commenced with preparation of hydroxamate intermediate **19** (Scheme 3). Treatment of hydroxylamine **16**[13] with excess palmitoyl chloride in the presence of $NaHCO_3$ resulted in N- and O-acylation of the hydroxylamine moiety. Subsequent removal of the *O*-palmitoyl hydroxamate group afforded hydroxamic acid **17**. Reaction of **17** with SEMCl provided O-protected hydroxamate **18** in

(15) (a) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397. (b) Carpino, L. A. *J. Org. Chem.* **1995**, *60*, 3561. (c) Musiol, H.-J. *J. Org. Chem.* **1994**, *59*, 6144. (d) Angell, Y. M. *J. Am. Chem. Soc.* **1995**, *117*, 7279. (e) Carpino, L. A. *Tetrahedron Lett.* **1994**, *35*, 2279. (f) Carpino, L. A.; EI−Faham, A. *J. Org. Chem.* **1994**, *59*, 695. (g) Carpino, L. A. *J. Chem. Soc., Chem. Commun.* **1994**, 201.

Scheme 2

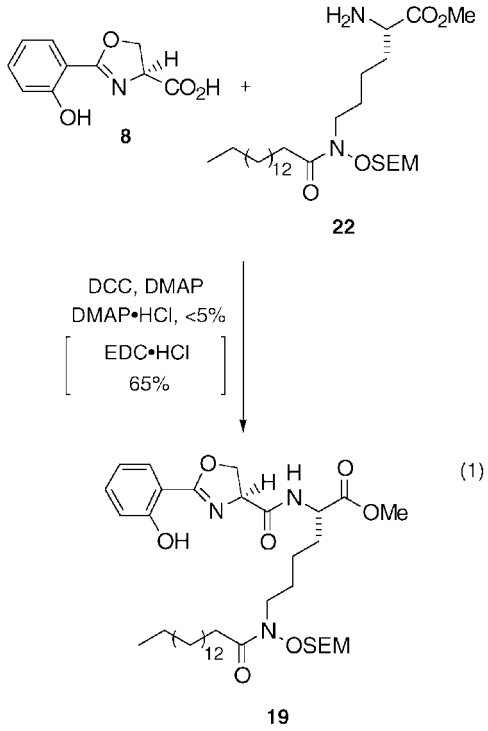


Scheme 3



96% overall yield from **16**. Desired hydroxamate **19** was produced by hydrogenation of **18** followed by EDC (1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride)-mediated coupling with oxazoline derivative **8**.[13]

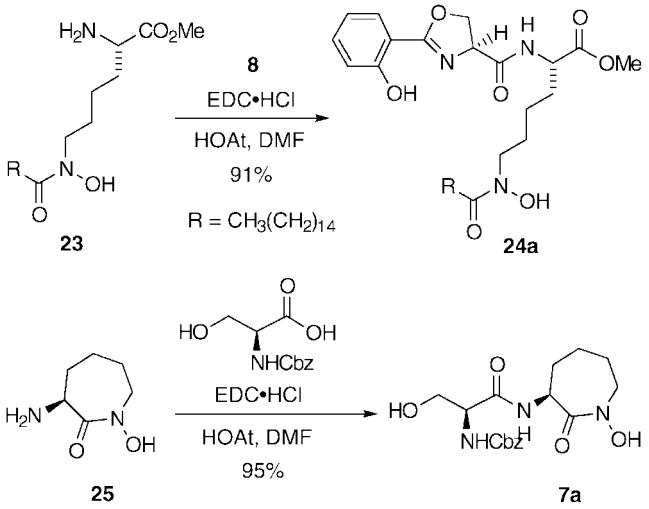
Saponification of **19** yielded the corresponding acid **20** which was subsequently coupled with modified cobactins **14** and **15** to give desired protected mycobactin analogues **21a** and **21b** in 85% and 91% yields, respectively. Unfortunately, all attempts to remove the protecting groups from **21a** and **21b** only resulted in total decomposition. This was probably due to instability of the internal ester linkage. To overcome this problem a different synthetic approach was executed. As our earlier results indicated, effective acidity of the reaction solution has a major influence on the outcome of coupling reactions.[14] On the basis of pK_a values, coupling reactions can be carried out under finely tuned conditions without masking certain functional groups. Our earlier studies showed that in the presence of 4-(dimethylamino)pyridine (DMAP) and an equal molar amount of its hydrogen chloride salt no desired coupling product (**19**) was obtained by treatment of oxazoline **8** with amine **22** (eq 1). The inefficiency of this reaction was attributed to competitive chemistry associated with partial ionization of the phenolic group of **8**. Alternative use of EDC·HCl and no added base produced mycobactin analogue **19** in 65% yield, emphasizing the importance of "acidic" coupling conditions.[13]



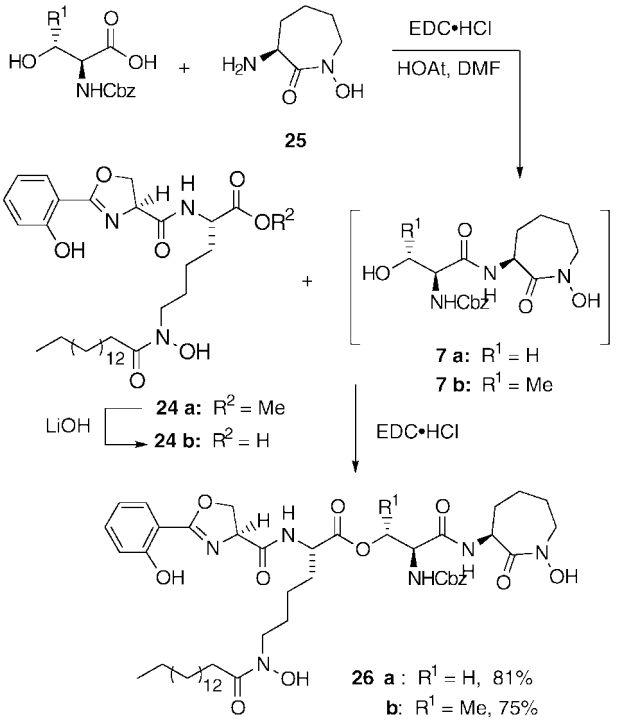
Interestingly, phenol and hydroxamic acids have amazingly close pK_a values of ca. 9. *Careful control of reaction acidity was anticipated to avoid ionization of the phenol and hydroxamate, thereby minimizing complications due to their competitive nucleophilicities.* To test this hypothesis, oxazoline derivative **8** was treated with EDC·HCl/HOAt and amine **23**, which contains a free hydroxamic acid, and indeed, the desired coupled product **24a** was formed in 91% yield (Scheme 4). Under similar conditions, free cobactin analogue **7a** was prepared in 95% yield by coupling Cbz-protected L-serine and unprotected cyclolysine **25**. Gratifyingly, under such conditions both reactions proceeded very cleanly without any detectable byproducts. These results provided opportunities to further improve our syntheses.

Thus, Cbz-protected serine was again treated with free hydroxamic acid **25** in the presence of EDC·HCl and HOAt. Upon 100% consumption of starting materials, and without any further purification of **7a**, mycobactic acid **24b** with another equivalent of EDC·HCl was introduced to the reaction mixture. The ensuing reaction

Scheme 4



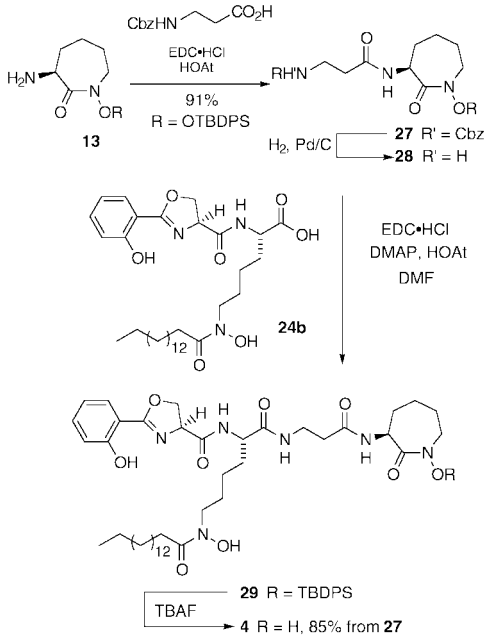
Scheme 5



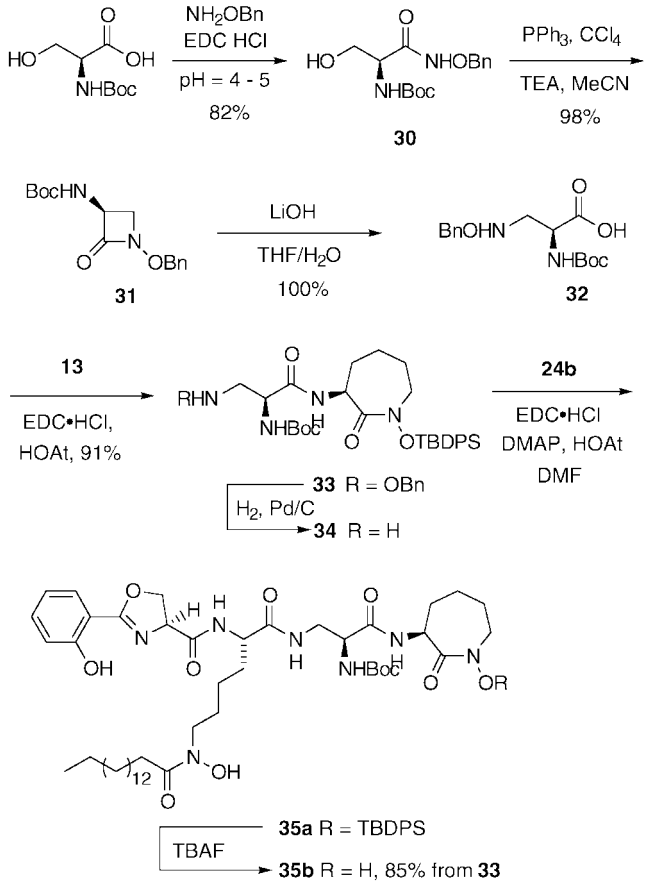
provided desired mycobactin analogue **26a** in 81% overall yield (Scheme 5). This modification not only significantly simplified our synthetic approach by circumventing unproductive protection and deprotection steps but also improved the overall chemical yield. Using similar methods, threonine analogue **26b** was produced in 75% overall yield by starting with Cbz-protected threonine (Scheme 5).

We then turned our attention to analogues with amide linkages. The synthesis of *β*-alanine analogue **4** commenced with preparation of modified analogue **28**. Amine **13** was coupled with Cbz-protected *β*-alanine in the presence of EDC·HCl and HOAt to give **27** in 91% yield. Reductive removal of the Cbz protecting group, followed by the now usual EDC·HCl/HOAt-mediated coupling with **24b**, afforded desired product **29**. Subsequent treatment of **29** with tetrabutylammonium fluoride in acetic acid gave mycobactin analogue **4** in 85% yield (Scheme 6).

Scheme 6



Scheme 7



Synthesis of **35b**, a form of mycobactin analogue **5**, started with preparation of 2,3-diaminopropionic acid derivative **32** (Scheme 7). Treatment of Boc-protected serine with *O*-benzylhydroxylamine afforded hydroxamate **30** in 82% yield.[16] Intramolecular cyclization in the presence of triphenylphosphine and carbon tetra-

(16) Miller, M. J.; Mattingly, P. G.; Morrison, M. A.; Kerwin, J. F., Jr. *J. Am. Chem. Soc.* **1980**, *102*, 7026.

Table 1. Biological Activities against *M. Tuberculosis* H37Rv

compound	% of inhibition	MIC (μg/mL) vs H37Rv
26a (**2**, R = Cbz)	44	>12.5
26b (**3**, R = Cbz)	48	>12.5
4	25	>12.5
35b (**5**, R = Boc)	98	<0.2

chloride gave β-lactam **31** in 98% yield.[16] Subsequent saponification of **31** produced the desired 2,3-diaminopropionic acid derivative **32** in quantitative yield. Cobactin analogue **33** was easily prepared in 91% yield by EDC-mediated coupling of acid **32** and cyclolysine **13**. Conversion of the benzyl-protected hydroxylamine moiety into the corresponding free amine was accomplished by simple palladium-catalyzed hydrogenation to give **34** in 92% yield by reduction of the hydroxylamine N−O bond in the presence of the less easily reduced hydroxamate N−O bond.[17] Subsequent coupling with unprotected mycobactic acid **24b**, followed by removal of the silyl protecting group on the cobactin component, provided mycobactin analogue **35b** in 85% yield (Scheme 7).

Biological Studies

The four synthetic analogues (**26a**, **26b**, **4**, **35b**) with generalized structures **2**−**5** were submitted to the Tuberculosis Antimicrobial Acquisition and Coordinating Facility of the Southern Research Institute for biological studies with *M. tuberculosis*. All four types of analogues were found to effect from moderate to high inhibitory activity against *M. tuberculosis* H37Rv at a concentration of 12.5 μg/mL (Table 1). Interestingly, the most active analogue was **35b** (MIC < 0.2 μg/mL!), the Boc-protected version of **5**. Thus, it appears that the enhanced activity of **5** relative to **4** must be attributed to the additional NHBoc group of **5**, suggesting importance of either the additional bulky functional group or additional hydrophobicity. The study of the importance of the absolute stereochemistry on the additional NHBoc group of **5** is in progress along with additional variation of X, Y, and Z in the generalized structures of **2**−**5** with special attention to their use as potential drug linkers.

Conclusion

The total syntheses of four different mycobactin analogues described here provides efficient methods for preparing this family of compounds. Using a water soluble carbodiimide (EDC·HCl) in the presence of 1-hydroxy-7-azabenzotriazole (HOAt) under acidic conditions proved to be an effective coupling process that allowed minimal use of peripheral protecting groups. Further simplification of the syntheses of analogues **2** and **3** was accomplished by applying a tandem coupling strategy. The biological studies of these synthetic analogues show from moderate to excellent inhibitorial activities against *M. tuberculosis*, further verifying Snow's assumption that alternate or modified mycobactin analogues might serve as antagonists of mycobacterial growth. Synthetic studies of different drug conjugates and structural analogues are under investigation.

Experimental Section

TBDPS-Protected Cobactin 14. To a solution of 21 mg of amine **13** (0.055 mmol),[10] 12.5 mg of Cbz-protected threonine (0.05 mmol), 1.0 equiv of HOAt (6.8 mg, 0.05 mmol), and 7 mg of DMAP (0.03 mmol) in 0.5 mL of dry DMF was added a premixed solution of 0.055 mmol of EDC·HCl and 0.055 mmol of DMAP in 0.5 mL of dry DMF dropwise. The resulting mixture was allowed to stand at room temperature for 3 h. The reaction mixture was then diluted with 15 mL of EtOAc, washed with water (1 × 5 mL) and brine (2 × 5 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica chromatography with 1:1 ethyl acetate/hexanes as the eluent to afford 27.3 mg (88%) of **14** as a colorless oil: R_f = 0.27 (1:1 EtOAc/hexanes); IR (neat) 3315, 2950, 2865, 1735, 1530, 1456 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74−7.70 (m, 5H), 7.46−7.33 (m, 10H), 7.04 (bd, 1H), 5.63 (bd, 1H), 5.11 (s, 2H), 4.28−4.06 (m, 3H), 3.58−3.41 (m, 2H), 3.30 (bs, 1H), 1.30−1.98 (m, 6H), 1.14 (s, 9H), 1.09 (d, 3H, J = 6.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.71, 169.38, 156.30, 136.15, 136.06, 132.01, 131.52, 130.27, 130.24, 128.52, 128.15, 128.04, 127.61, 127.53, 76.51, 67.51, 67.08, 58.51, 54.16, 30.53, 27.26, 26.89, 25.29, 19.57, 18.14; HRFABMS calcd for $C_{34}H_{44}N_3O_6Si$ 618.2999, found 618.3025.

TBDPS-Protected Cobactin 15. To a solution of 17 mg of amine **13** (0.045 mmol), 9.6 mg of Cbz-protected serine (0.04 mmol), 1.0 equiv of HOAt (5.4 mg, 0.04 mmol), and 5.5 mg of DMAP (0.045 mmol) in 0.5 mL of dry DMF was added a premixed solution of 0.045 mmol of EDC·HCl and 0.045 mmol of DMAP (5.5 mg) in 0.5 mL of dry DMF dropwise. The resulting mixture was allowed to stand at the room temperature for 3 h. The reaction mixture was then diluted with 10 mL of EtOAc, washed with water (1 × 5 mL) and brine (2 × 5 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica chromatography with 3:1 ethyl acetate/hexanes as the eluent to afford 21.6 mg (90%) of **15** as a colorless oil: R_f = 0.40 (3:1 EtOAc/hexanes); IR (neat) 3315, 2940, 2862, 1725, 1535, 1455 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (m, 5H), 7.47−7.30 (m, 10H), 6.83 (bd, 1H), 5.65 (bd, 1H), 5.11 (s, 2H), 4.30 (m, 2H), 4.20 (m, 1H), 3.89 (dd, 1H, J = 6.6, 2.2 Hz), 3.56−3.51 (m, 1H), 3.49−3.42 (m, 1H), 1.81−1.74 (m, 3H), 1.59−1.51 (m, 3H), 1.45−1.41 (m, 1H), 1.13 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.82, 169.21, 155.92, 136.74, 135.40, 131.96, 131.51, 129.59, 127.90, 127.50, 126.96, 76.40, 67.09, 65.89, 64.57, 63.44, 54.96, 28.35, 27.56, 26.32, 19.56; HRFABMS calcd for $C_{33}H_{42}N_3O_6Si$ 604.2843, found 604.2823.

SEM-Protected Hydroxamate 18. To a solution of 78 mg of **16** (0.25 mmol) and 169 mg of $NaHCO_3$ (2.01 mmol) in 8 mL of dry CH_2Cl_2 was added 4 equiv of palmitoyl chloride (0.30 mL) dropwise. The resulting solution was then allowed to stand at room temperature for 14 h. The mixture was diluted with 20 mL of EtOAc, washed with 1 N HCl (3 × 5 mL) and brine (3 × 5 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was then taken up in 25 mL of 6% Hünig's base in methanol. The resulting mixture was then allowed to stand at room temperature for 12 h. The mixture was concentrated. The residue was transferred to a solution of 0.87 mL of Hünig's base (5.0 mmol) and 20 mg of DMAP in 5 mL of toluene, followed by addition of 10 equiv of SEMCl (0.44 mL, 2.5 mmol) dropwise. The resulting solution was then allowed to stand at 65 °C overnight under argon. The mixture was cooled to room temperature and diluted with 20 mL of EtOAc. Then the mixture was washed with 1 N HCl (2 × 10 mL), saturated aqueous $NaHCO_3$ (2 × 10 mL) and brine (2 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica chromatography with 1:15 ethyl acetate/hexanes as the eluent to afford 162 mg (96%) of **18** as a colorless oil: R_f = 0.21 (1:15 EtOAc/hexanes); IR (neat) 3312, 2910, 2822, 1722, 1650 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36−7.31(m, 5H), 5.36 (d, J = 7.8 Hz, 1H), 5.10 (s, 2H), 4.90 (s, 2H), 4.37−4.30 (m, 1H), 3.76−3.61 (m, 7H), 2.38 (t, J = 7.6 Hz, 2H), 1.86−1.03 (m, 32 H), 0.99−0.93 (m, 2H), 0.88 (t, J = 6.7 Hz, 3H), 0.03 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.36, 172.69, 155.82, 136.18, 128.29, 127.89, 127.85, 99.08, 67.38, 66.68, 53.64, 52.09, 47.16, 32.43, 31.75, 29.52, 29.49, 29.38, 29.30, 29.27, 29.19, 26.10, 24.33, 22.52, 22.14, 18.02, 13.96, −1.63; HRFABMS calcd for $C_{37}H_{67}N_2O_7Si$ 679.4717, found 679.4702.

(17) Arai H.; Hagmann, W. K.; Suguna, H.; Hecht, S. M. *J. Am. Chem. Soc.* **1980**, *102*, 6631.

Oxazoline Hydroxamate 19. A solution of 670 mg of **18** (0.99 mmol) in 25 mL of MeOH was purged with argon for 15 min. Then 70 mg of 10% Pd−C was added, and the system was flushed with argon for another 10 min. The resulting slurry was stirred under H_2 (1 atm) for 3 h. After the catalyst was filtered off through a pad of Celite, the solvent was removed under vacuum to provide the corresponding free amine. The amine was then treated with 202 mg of oxazoline derivative **8** (0.98 mmol) in the presence of 187 mg of EDC·HCl (0.98 mmol), 133 mg of HOAt (0.98 mmol), and 25 mg of DMAP in 30 mL of dry DMF. The resulting solution was allowed to stand at room temperature for 3 h. The mixture was diluted with 60 mL of EtOAc. The organic layer was washed with 1 N HCl (2 × 10 mL), saturated aqueous $NaHCO_3$ (2 × 10 mL), and brine (2 × 10 mL), dried over $NaSO_4$, filtered, and concentrated. The residue was purified by silica chromatography with 1:1 ethyl acetate/hexanes as the eluent to afford 660 mg (91%) of **19** as a colorless oil: R_f = 0.40 (1:1 EtOAc/hexanes); IR (neat) 3290, 2910, 2820, 1740, 1650, 1630 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.67 (dd, J = 7.9, 1.7 Hz, 1H), 7.39 (ddd, J = 8.7, 7.5, 1.7 Hz, 1H), 7.00 (dd, J = 8.5, 0.8 Hz, 1H), 6.93−6.85 (m, 1H), 4.94 (t, J = 9.6 Hz, 1H), 4.84 (s, 2H), 4.65−4.61 (m, 2H), 4.57−4.50 (m, 1H), 3.73 (s, 3H), 3.72−3.66 (m, 2H), 3.60 (t, J = 7.1 Hz, 2H), 2.33 (d, J = 7.6 Hz, 2H), 1.91−1.52 (m, 6H), 1.28−1.16 (m, 26H), 0.95−0.89 (m, 2H), 0.83 (t, J = 6.8 Hz, 3H), −0.03 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.45, 172.09, 170.27, 167.68, 159.72, 134.13, 128.47, 118.95, 116.88, 109.94, 99.13, 69.40, 67.97, 67.47, 52.37, 52.04, 47.29, 32.50, 31.82, 31.58, 29.59, 29.56, 29.44, 29.37, 29.26, 26.18, 24.42, 22.59, 22.35, 18.08, 14.02, −1.56; HRFABMS calcd for $C_{39}H_{68}N_3O_8Si$ 734.4775, found 734.4764.

Protected Mycobactin 21a. To a solution of 66 mg of amine **13** (0.173 mmol), 41 mg of Cbz-protected threonine (0.173 mmol), 1.2 equiv of HOAt (21 mg, 0.210 mmol), and 21 mg of DMAP (0.173 mmol) in 1.0 mL of dry DMF was added a premixed solution of 0.21 mmol of EDC (40 mg) and 0.173 mmol of DMAP (21 mg) in 0.5 mL of dry DMF dropwise. After being allowed to stand at room temperature for 3 h, the mixture was charged with 119 mg of acid **20** (0.165 mmol) and 25 mg of EDC·HCl (0.13 mmol). The resulting solution was heated at 55 °C for 42 h before it was cooled to room temperature. The reaction mixture was then diluted with 10 mL of EtOAc, washed with water (3 × 5 mL) and brine (3 × 5 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica chromatography with 3:1 ethyl acetate/hexanes as the solvent system to afford 151.5 mg (85%) of **21a** as a colorless oil: IR (neat) 3405, 2960, 2925, 1790, 1725, 1705, 1660 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 11.23 (bs, 1H), 7.68 (td, 1H, J = 8.1, 1.5 Hz), 7.52 (m, 1H), 7.44−7.13 (m, 6H), 7.01 (d, 1H, J = 8.1 Hz), 6.91−6.86 (m, 1H), 5.77−5.75 (m, 1H), 5.12, 5.09 (ABq, 2H, J = 9.9 Hz), 5.01−4.82 (m, 1H), 4.92 (s, 1H), 4.87 (s, 1H), 4.68−4.54 (m, 4H), 4.25−4.19 (m, 2H), 3.92−3.46 (m, 6H), 2.43−2.34 (m, 2H), 2.05−1.24 (m, 41H), 1.13 (t, 2H, J = 6.9 Hz), 0.99−0.91 (m, 2H), 0.87 (t, 3H, J = 6.3 Hz), 0.02 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 175.81, 170.72, 169.82, 169.13, 168.96, 167.55, 159.62, 156.53, 136.17, 134.11, 128.47, 128.40, 128.01, 127.98, 127.91, 118.92, 116.87, 109.95, 99.10, 69.06, 67.83, 67.54, 67.29, 67.01, 58.66, 53.00, 52.00, 50.97, 32.51, 31.83, 30.89, 30.77, 30.65, 29.61, 29.60, 29.58, 29.56, 29.47, 29.43, 29.38, 27.67, 27.48, 26.09, 25.82, 24.45, 22.60, 22.37, 22.16, 18.33, 18.08, 14.04, −1.47; HRFABMS calcd for $C_{56}H_{89}N_6O_{13}Si$ 1081.6257, found 1081.6227.

Protected Mycobactin 21b. To a solution of 33 mg of amine **13** (0.086 mmol), 21 mg of Cbz-protected serine (0.086 mmol), 1.0 equiv of HOAt (12 mg, 0.086 mmol), and 13 mg of DMAP (0.045 mmol) in 1.0 mL of dry DMF was added a premixed solution of 0.10 mmol of EDC·HCl and 0.10 mmol of DMAP (12.2 mg) in 0.5 mL of dry DMF dropwise. After being allowed to stand at room temperature for 3 h, the mixture was charged with 62 mg of acid **20** (0.086 mmol) and 19 mg of EDC·HCl (0.10 mmol). The resulting solution was heated at 55 °C for 30 h before it was cooled to room temperature. The reaction mixture was then diluted with 10 mL of EtOAc, washed with water (3 × 5 mL) and brine (3 × 5 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica chromatography with 3:1 ethyl acetate/hexanes as the eluent to afford 83.3 mg (91%) of **21b** as a colorless oil: IR (neat) 3430, 2975, 2940, 1740, 1730, 1655 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 11.31 (bs, 1H), 7.68 (d, 1H, J = 8.0 Hz), 7.54 (dd, 1H, J = 12.5, 6.5 Hz), 7.42−7.29 (m, 5H), 7.24−7.22 (m, 1H), 7.16 (bd, 1H), 7.00 (d, 1H, J = 8.5 Hz), 6.89 (dt, 1H, J = 7.5, 2.5 Hz), 5.78 (m, 1H), 5.12, 5.09 (ABq, 2H, J = 16.0 Hz), 4.96 (m, 1H), 4.92 (s, 1H), 4.87 (s, 1H), 4.65−4.55 (m, 4H), 4.28−4.25 (m, 1H), 4.22 (dd, 1H, J = 16.5, 6.5 Hz), 3.96−3.81 (m, 1H), 3.76−3.64 (m, 4H), 3.57−3.54 (m, 1H), 2.41 (t, 1H, J = 7.5 Hz), 2.37 (t, 1H, J = 7.5 Hz), 2.03−1.23 (m, 38H), 1.13 (dd, 2H, J = 12.0, 6.5 Hz), 0.98−0.92 (m, 2H), 0.87 (t, 3H, J = 6.5 Hz), 0.02 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.78, 170.88, 170.73, 167.88, 167.62, 166.84, 159.48, 155.55, 136.07, 134.05, 128.57, 128.52, 128.20, 128.15, 128.08, 119.09, 116.93, 110.32, 99.21, 69.26, 67.66, 67.15, 64.54, 53.44, 52.78, 52.35, 51.24, 50.75, 32.62, 31.91, 31.89, 31.23, 30.87, 29.66, 29.56, 29.48, 29.45, 29.35, 29.33, 27.60, 26.12, 25.70, 25.54, 24.61, 22.68, 22.65, 22.59, 22.36, 18.18, 14.12, −1.47; HRFABMS calcd for $C_{55}H_{86}N_6O_{13}Si$ 1067.6100, found 1067.6104.

Mycobactic Methyl Ester 24a. A slurry of 880 mg of protected amine **17** (1.61 mmol) with 200 mg of 10% Pd−C in 20 mL of MeOH was purged with an N_2 stream for 10 min and subsequently with H_2 for an additional 15 min. The reaction mixture was stirred under H_2 (1 atm) at room temperature for 3 h. Then the palladium catalyst was filtered off through a pad of Celite. The solution was concentrated to yield the corresponding free amine **23**. Without further purification, the crude amine **23** was dissolved in 20 mL of a freshly distilled DMF solution containing 330 mg of oxazoline **8** (1.59 mmol) and 217 mg of HOAt (1.59 mmol). To above solution was added 336 mg of EDC·HCl (1.75 mmol). The resulting solution was allowed to stand at room temperature for 3 h before it is diluted with 60 mL of EtOAc and 10 mL of 1 N HCl. The organic layer was washed with 0.5 N HCl (2 × 10 mL), 5% ascorbic acid (1 × 10 mL), saturated aqueous $NaHCO_3$ (1 × 10 mL) and brine (2 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified silica chromatography with 1:1 ethyl acetate/dichloromethane as the eluent to afford 872 mg (91%) of **24a** as a white solid: mp 81−82.5 °C; IR (neat) 3230, 2920, 2850, 1740, 1650 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$/CD_3OD) δ 7.69 (dd, J = 8.0, 1.5 Hz, 1H), 7.41 (dt, J = 8.0, 1.5 Hz, 1H), 6.96 (d, J = 8.1 Hz, 1H), 6.91 (t, J = 7.4 Hz, 1H), 5.02 (t, J = 9.0 Hz, 1H), 4.65−4.60 (m, 2H), 4.43 (dd, J = 8.8, 4.9 Hz, 1H), 3.73 (s, 3H), 3.62−3.55 (m, 2H), 2.43 (t, J = 7.6 Hz, 2H), 1.95−1.26 (m, 32 H), 0.91 (t, J = 6.6 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.18, 173.87, 172.95, 168.55, 161.02, 135.02, 129.50, 119.97, 117.70, 111.45, 70.43, 69.20, 53.85, 52.80, 33.26, 33.06, 31.82, 30.75, 30.62, 30.49, 30.46, 27.10, 25.95, 23.80, 23.72, 14.45; HRFABMS calcd for $C_{33}H_{53}N_3O_7$ 604.3961, found 604.3975.

Cobactin 7a. To a mixture of 50 mg of unprotected cyclolysine **25** (0.35 mmol), 83 mg of Cbz-protected serine (0.35 mmol), and 47.3 mg of HOAt (0.35 mmol) in 6 mL of freshly distilled DMF was added 73.2 mg of EDC·HCl (0.38 mmol). The reaction mixture was allowed to stand at room temperature for 2 h. The reaction mixture was then diluted with 20 mL of EtOAc. The organic layer was washed with 5% aqueous ascorbic acid (2 × 5 mL), saturated aqueous $NaHCO_3$ (1 × 5 mL), and brine (2 × 5 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica chromatography with 10% of methanol in dichloromethane as the eluent to afford 121 mg (95%) of **7a** as a white solid: R_f = 0.40 (10% methanol in dichloromethane); mp 140−142 °C; IR (neat) 3306, 2930, 2846, 1710, 1644, 1060 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$/CD_3OD) δ 7.46 (s, 5H), 6.41 (bd, 1H), 5.24 (s, 2H), 4.59 (m, 1H), 4.51−3.27 (m, 7H), 2.19−1.41 (m, 6H); 13C NMR (75 MHz, $CDCl_3$/CD_3OD) δ 170.30, 168.39, 156.29, 128.92, 128.35, 128.00, 127.82, 66.94, 62.74, 55.77, 52.00, 51.59, 30.01, 27.45, 25.55; HRFABMS calcd for $C_{17}H_{24}N_3O_6$ 366.1665, found 366.1689.

***N*-Cbz-Protected Mycobactin Analogue 26a.** To a solution of 6.7 mg of Cbz-protected serine (0.028 mmol) and 4.0

mg of cyclolysine (0.028 mmol) **25** with 1 equiv of HOAt (4.0 mg) in 1 mL of freshly distilled DMF was added 5.4 mg of EDC·HCl (0.028 mmol). The resulting solution was allowed to stand at room temperature for 2.5 h. Upon disappearance of starting material based on TLC analysis, 16.5 mg of mycobactic acid **24b** (0.028 mmol) with 0.056 mmol of EDC·HCl[18] was introduced. After being allowed to stand at room temperature for 12 h, the reaction mixture was diluted with 10 mL of EtOAc. The organic portion was washed with water (2 × 5 mL), 0.5 N HCl (1 × 5 mL) and brine (2 × 5 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by reverse phase C_{18} silica gel chromatography with 1:1 methanol/acetonitrile as the eluent to afford 21.2 mg (81%) of **26a** as a white solid: $R_f = 0.40$ on reverse phase C_{18} silica gel TLC (methanol/acetonitrile = 1/1); mp 73.5−74.5 °C; IR (neat) 3300, 2905, 2856, 1775, 1660, 1635 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3/CD_3OD$) δ 7.64 (d, 1H, $J = 6.6$ Hz), 7.37 (d, 1H, $J = 7.2$ Hz), 7.28−7.25 (m, 5H), 7.07 (bd, 1H), 6.98 (m, 1H), 6.89−6.84 (m, 1H), 6.03 (bs, 1H), 5.06 (s, 1H), 4.92−4.89 (m, 2H), 4.65−4.48 (m, 4H), 4.33−4.25 (m, 2H), 4.85 (bs, 1H), 3.72−3.53 (m, 3H), 3.18 (bd, 1H), 2.60−2.49 (m, 1H), 2.40−2.38 (m, 1H), 1.97−1.41 (m, 40H), 0.83 (t, 3H, $J = 7.2$ Hz); ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$) δ 179.12, 176.73, 176.47, 170.47, 168.27, 167.71, 159.36, 156.18, 136.01, 134.25, 128.58, 128.47, 128.38, 128.03, 127.86, 119.12, 116.75, 109.86, 77.19, 69.36, 69.20, 69.06, 67.65, 66.99, 62.70, 55.60, 53.17, 50.21, 49.87, 49.59, 49.31, 49.11, 49.02, 48.73, 47.14, 33.91, 33.07, 31.79, 29.57, 29.36, 29.31, 29.23, 29.00, 24.65, 23.62, 23.44, 22.55, 13.96; HRFABMS calcd for $C_{49}H_{73}N_6O_{12}$ 937.5286, found 937.5309.

***N*-Cbz-Protected Mycobactin Analogue 26b.** To a solution of 17.5 mg of Cbz-protected threonine (0.069 mmol) and 10 mg of cyclolysine (0.069 mmol) **25** with 1 equiv of HOAt (9.4 mg) in 6 mL of freshly distilled DMF was added 13.3 mg of EDC·HCl (0.069 mmol). The resulting solution was allowed to stand at room temperature for 2.5 h. Upon disappearance of starting material based on TLC analysis, 40 mg of mycobactic acid **24b** (0.069 mmol) with 33.3 mg of EDC·HCl (0.173 mmol)[18] was introduced. After being allowed to stand at room temperature for 12 h, the reaction mixture was diluted with 25 mL of EtOAc. The organic portion was washed with water (2 × 5 mL), 0.5 N HCl (1 × 5 mL), 5% ascorbic acid (2 × 5 mL), and brine (2 × 5 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by reverse phase C_{18} silica gel chromatography with 1:3 methanol/acetonitrile as the eluent to afford 49 mg (75%) of **26b** as a white solid: $R_f =$ 0.50 (10% methanol in EtOAc); mp 77.0−79.0 °C; IR (neat) 3300, 2925, 2835, 1780,1655 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 8.58 (bs, 1H), 7.66−7.62 (m, 2H), 7.41−7.35 (m, 1H), 7.30 (s, 5H), 7.00−6.93 (m, 1H), 6.89−6.84 (m, 1H), 6.08 (bs, 1H), 6.03 (bs, 1H), 5.07 (s, 2H), 4.95−4.91 (m, 1H), 4.67−4.52 (m, 4H), 4.31−4.25 (m, 2H), 3.91−3.89 (m, 1H), 3.63−3.52 (m, 3H), 2.44−2.40 (m, 2H), 2.18 (bs, 1H), 1.95−1.23 (m, 39H), 1.12 (bs, 3H), 0.86 (t, 3H, $J = 6.6$ Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.98, 170.32, 169.82, 169.21, 168.34, 167.53, 159.48, 156.52, 136.03, 134.18, 128.41, 128.32, 127.94, 127.77, 119.01, 116.77, 116.71, 109.79, 69.10, 67.60, 67.36, 66.94, 58.83, 53.09, 52.07, 50.82, 50.11, 49.05, 47.08, 33.01, 32.36, 31.76, 30.78, 29.56, 29.34, 29.29, 29.20, 28.96, 27.60, 25.57, 25.19, 24.58, 23.58, 23.38, 22.53, 21.79, 18.09, 13.98; HRFABMS calcd for $C_{50}H_{75}N_6O_{12}$ 951.5443, found 951.5453.

Protected Cobactin 27. To a mixture of 93 mg of *N*-Cbz-β-alanine (0.42 mmol), 160 mg of amine **13** (0.42 mmol), and 1 equiv of HOAt (57 mg) in 3 mL of DMF was added 1.1 equiv of EDC·HCl (88.3 mg). After being allowed to stand at room temperature for 2 h, the mixture was diluted with 20 mL of EtOAc and 5 mL of saturated aqueous $NaHCO_3$. The organic layer was then washed with water (2 × 5 mL) and brine (2 × 5 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica chromatography with 1.5:1 ethyl acetate/hexanes as the eluent to afford 224 mg (91%) of **27** as a colorless oil: $R_f = 0.40$ (3:1 EtOAc/hexanes); IR (neat) 3320, 3080, 2950, 2865, 1720, 1660 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.75−7.70 (m, 5H), 7.45−7.34 (m, 10H), 6.72 (d, 1H, $J = 6.0$ Hz), 5.46 (bs, 1H), 5.08 (s, 2H), 4.18 (dd, 1H, $J = 10.5$, 6.0 Hz), 3.55−3.42 (m, 4H), 2.36 (t, 2H, $J = 5.7$ Hz), 1.95−1.12 (m, 6H), 1.14 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.32, 169.31, 156.29, 136.61, 136.13, 135.99, 132.01, 131.55, 130.27, 130.23, 128.44, 127.98, 127.56, 127.49, 77.2, 66.55, 54.21, 51.62, 33.92, 31.03, 27.30, 26.87, 25.31, 19.54; HRFABMS calcd for $C_{33}H_{41}N_3O_5Si$ 588.2894, found 588.2874.

Mycobactin Analogue 4. A slurry of 50 mg of cobactin **27** (0.082 mmol) with 15 mg of 10% Pd−C in 8 mL of MeOH was purged with a stream of N_2 over 10 min and subsequently with H_2 for an additional 15 min. The reaction mixture was stirred under H_2 (1 atm) at room temperature for 3 h. Then palladium catalyst was filtered, off through a pad of Celite. The solution was concentrated to yield the corresponding free amine **28**. Without further purification, amine **28** was dissolved in a 2 mL of a DMF solution containing 42 mg of mycobactic acid **24b** (0.073 mmol) and 10 mg of HOAt (0.073 mmol). To the above solution was added 14 mg of EDC·HCl (0.073 mmol). The resulting mixture was allowed to stand at room temperature for 3.5 h. The reaction was quenched by addition of 15 mL of EtOAc and 5 mL of H_2O. The organic mixture was then washed with water (2 × 5 mL) and brine (2 × 5 mL), dried over Na_2SO_4, filtered, and concentrated to give 60 mg of desired coupling product **29**. To a solution of 60 mg of **29** (0.06 mmol) with 6.9 μL of HOAc (0.12 mmol) in 2 mL of dry THF was added 0.12 mL of 1 M TBAF (0.12 mmol) in THF dropwise at 0 °C. Then the reaction mixture was allowed to warm to room temperature and stand for 1 h. After removal of solvents, the residue was purified by reverse phase C_{18} silica gel chromatography with 3:2 methanol/acetonitrile as the eluent to afford 48.6 mg (85%) of **4** as a white solid: $R_f = 0.55$ on reverse phase C_{18} silica gel TLC (3:1 methanol/ acetonitrile); mp 173.5−175.0 °C; IR (neat) 3300, 2935, 2850, 1680, 1500 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3/CD_3OD$) δ 7.65 (dd, 1H, $J =$ 7.8, 1.0 Hz), 7.41−7.35 (m, 1H), 6.99−6.96 (m, 1H), 6.88 (t, 1H, $J = 7.8$ Hz), 5.00−4.95 (m, 1H), 4.66−4.49 (m, 3H), 4.33−4.30 (m, 1H), 3.89−3.51 (m, 5H), 3.22−3.15 (m, 1H), 2.48−0.95 (m, 42H), 0.85 (t, 3H, $J = 6.5$ Hz); ^{13}C NMR (125 MHz, $CDCl_3/CD_3OD$) δ 175.16, 172.12, 170.76, 168.52, 167.33, 159.21, 155.32, 134.11, 128.51, 119.16, 116.72, 110.34, 77.20, 68.95, 67.79, 58.68, 53.58, 51.78, 51.51, 47.24, 36.40, 36.14, 32.34, 31.86, 31.21, 29.63, 29.59, 29.51, 29.46, 29.30, 29.11, 27.73, 25.74, 24.63, 23.84, 22.62, 21.82, 19.63, 14.05, 13.54; HRFABMS calcd for $C_{41}H_{67}N_6O_9$ 787.4969, found 787.4969.

Protected Diaminopropionic Acid 32. To the solution of 1.02 g of β-lactam **31**[13] (3.49 mmol) in 18 mL of 50% H_2O in THF was added 125 mg of LiOH (5.22 mmol). The resulting mixture was allowed to stand at room temperature for 3 h. Then the solution was acidified by addition of 8 mL of 2 N HCl. The solution was then extracted with EtOAc (3 × 10 mL). The combined organic mixture was washed with H_2O (2 × 10 mL) and brine (2 × 15 mL), dried over Na_2SO_4, filtered, and then concentrated to afford 1.08 g of acid **32** (100%) as a white solid: mp 132.0−134.0 °C; IR (neat) 3400, 3250, 2960, 2860, 1770, 1690 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3/CD_3OD$) δ 7.29−7.17 (m, 5H), 4.58 (s, 2H), 4.08 (bt, 1H, $J = 5.1$ Hz), 3.11 (d, 2H, $J = 5.1$ Hz), 1.32 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3/CD_3OD$) δ 176.80, 156.15, 137.20, 128.13, 128.09, 127.60, 79.45, 75.41, 53.53, 53.00, 28.03; HRFABMS calcd for $C_{15}H_{23}N_2O_5$ 311.1607, found 311.1617.

Protected Cobactin 33. To a solution of 23 mg of amine **13** (0.061 mmol), 17 mg of Boc-protected diaminopropionic acid (0.055 mmol) **32**, 1.0 equiv of HOAt (7.5 mg, 0.055 mmol), and 7.4 mg of DMAP (0.055 mmol) in 0.5 mL of dry DMF was added a premixed solution of 0.055 mmol of EDC and 0.055 mmol of DMAP in 0.5 mL of dry DMF dropwise. The resulting mixture was allowed to stand at the room temperature for 6 h. The reaction mixture was then diluted with 15 mL of EtOAc, washed with water (2 × 5 mL) and brine (2 × 5 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica chromatography with 1:1 ethyl acetate/hexanes as the eluent to afford 33.8 mg (91%) of **33** as a colorless oil: IR (neat) 3350, 2990, 2895, 1720, 1695 cm^{-1}; ^1H

(18) Additional EDC·HCl was used to accelerate the reaction.

NMR (300 MHz, $CDCl_3$) δ 7.75−7.70 (m, 4H), 7.54 (bd, 1H), 7.42−7.27 (m, 11H), 5.95 (s, 1H), 5.48 (bd, 1H), 4.68 (s, 2H), 4.31 (bs, 1H), 4.24−4.16 (m, 1H), 3.51−3.40 (m, 1H), 3.35 (bs, 1H), 3.30 (d, 1H, J = 5.1 Hz), 3.51 (dd, 1H, J = 13.8, 6.3 Hz), 1.82−1.18 (m, 6H), 1.44 (s, 9H), 1.14 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.86, 169.53, 155.71, 137.42, 136.14, 136.06, 132.10, 130.22, 130.18, 129.61, 129.46, 128.54, 128.39, 127.89, 127.56, 127.48, 76.63, 76.50, 76.04, 54.15, 53.36, 51.81, 30.85, 28.29, 27.29, 26.90, 25.36, 19.58; HRFABMS calcd for $C_{37}H_{51}N_4O_6Si$ 675.3578, found 675.3551.

***N*-Boc-Protected Mycobactin Analogue 35b.** A slurry of 56 mg of cobactin **33** (0.083 mmol) with 15 mg of 10% Pd−C was purged with an N_2 stream for 10 min and subsequently with H_2 for an additional 15 min. The reaction mixture was stirred under H_2 (1 atm) at 56 °C for 2 h. Then the palladium catalyst was filtered, off through a pad of Celite. The solution was concentrated to yield 45 mg of the corresponding free amine **34**. Without further purification, amine **34** was dissolved in 2 mL of a DMF solution containing 49 mg of mycobactic acid **24b** (0.084 mmol) and 11.4 mg of HOAt (0.084 mmol). To the above solution was added 16 mg of EDC·HCl (0.083 mmol). The resulting mixture was allowed to stand at room temperature for 3 h. The reaction was quenched by addition of 20 mL of EtOAc and 5 mL of H_2O. The organic mixture then washed with water (2 × 5 mL) and brine (2 × 5 mL), dried over Na_2SO_4, filtered, and concentrated to give 84 mg of desired coupled product **35a** in 89% yield. To a solution of 37 mg of **35a** (0.032 mmol) with 5.6 μL of HOAc (0.097 mmol) in 2 mL of dry THF was added 0.1 mL of 1 M TBAF (0.1 mmol) in THF dropwise at 0 °C. Then the reaction mixture was allowed to warm to room temperature and stand for 1 h. After removal of solvents, the residue was purified by reverse phase C_{18} silica gel chromatography with 1:8 methanol/acetonitrile as the eluent to afford 27.6 mg (85% from **33**) of **35b** as a white solid: R_f = 0.30 on reverse phase C_{18} silica gel TLC(1:8 methanol/acetonitrile); mp 164.5−165.5 °C; IR (neat) 3390, 3090, 2990, 2850, 1690, 1670 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD) δ 7.62 (d, 1H, J = 7.5 Hz), 7.35 (t, 1H, J = 8.0 Hz), 6.94 (d, 1H, J = 8.0 Hz), 6.84 (t, 1H, J = 7.5 Hz), 4.94 (t, 1H, J = 9.0 Hz), 4.91−4.58 (m, 1H), 4.55−4.51 (m, 1H), 4.50−4.47(m, 1H), 4.12 (bs, 2H), 3.85−3.81 (m, 1H), 3.78−3.76 (m, 1H), 3.69−3.66 (m, 1H), 3.57−3.51 (m, 3H), 3.37 (t, 2H, J = 7.5 Hz), 1.91−1.83 (m, 40 H), 1.38 (s, 9H), 0.82 (t, 3H, J = 6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD) δ 175.15, 172.60, 171.02, 170.68, 168.81, 167.17, 159.20, 155.21, 133.95, 128.39, 119.02, 116.60, 109.99, 80.00, 77.20, 68.97, 67.65, 54.15, 53.35, 51.96, 51.88, 47.21, 41.06, 32.25, 31.78, 30.62, 29.56, 29.52, 29.44, 29.39, 29.33, 29.22, 28.78, 28.12, 27.59, 25.68, 24.61, 22.55, 22.06, 13.95; HRFABMS calcd for $C_{46}H_{76}N_7O_{11}$ 902.5603, found 902.5595.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of new compounds (30 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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