Total Synthesis of a Mycobactin S, a Siderophore and Growth Promoter of *Mycobacterium Smegmatis*, and Determination of its Growth Inhibitory Activity against *Mycobacterium tuberculosis*

Jingdan Hu and Marvin J. Miller*

Contribution from the Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556

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Abstract: A general total synthesis of mycobactins, represented by a mycobactin S, was achieved by a convergent approach. Two hydroxamic acid residues, **28** and **40b**, were prepared from commercially available N^{α}-Cbz-L-lysine *via* dimethyldioxirane oxidations. Cyclization of hydroxylamine **34** to a seven-membered hydroxamic acid, **35**, was mediated by DCC, DMAP, and DMAP·HCl. The use of a [2-(trimethylsilyl)ethoxy]methyl group as a hydroxyl protecting group for N^{α}-Cbz-N^{ϵ}-hydroxy-N^{ϵ}-palmitoyl-L-lysine methyl ester (**28**) was critical for this synthesis. Biological tests indicated that the synthetic mycobactin S was a potent growth inhibitor of *Mycobacterium tuberculosis* H37Rv, though it differs in only one stereogenic center from mycobactin T, the siderophore growth promoter of *M. tuberculosis*.

Introduction

In 1993, the World Health Organization (WHO) declared tuberculosis (TB) a global emergency with the hope of drawing the world's attention to the growing severity of the TB epidemic. Tuberculosis is a historically chronic, debilitating, and often fatal disease that normally severely affects respiratory tracts of humans. After the discovery and utilization of antibiotics such as streptomycin and isoniazid in the 1940s and 1950s, TB was considered a controllable disease. However, in the mid-1980s, a significant resurgence of TB infections occurred, primarily because of the prevalence of drug-resistant or multiple-drug-resistant strains, which are potentially incurable.[1] The number of TB cases in the United States in 1994 increased about 10% relative to 1985, the year with the lowest number of reported TB cases since national reporting began in 1953.[2] The situation worldwide is even worse. In 1996, the WHO global TB program estimated that from 1990 to the year 2000, the total number of TB cases among adults will exceed 40 million.[3] The widespread HIV epidemic is believed to play an important role in the reemergence of TB. With its contagious nature, TB is the only major opportunistic infection which can be spread through the air among people. As expected, HIV-positive patients with compromised immune systems are especially susceptible to TB infection. In fact, TB has become the leading killer of HIV-positive people. One out of three AIDS patients dies of TB. The medical crisis associated with TB infections, especially the development of potentially incurable multiple-drug-resistant (MDR) TB strains, has raised a serious challenge for current chemotherapeutic treatment.[4−6]

One important aspect of drug resistance in strains of TB is thought to be related to the specific mycobacterial cell wall. Most cells of mycobacteria, including *Mycobacterium tuberculosis*, are covered by lipid-rich cell walls, which consist of a large amount of mycolic acids with long hydrocarbon chains. The lengths of the chains produce an exceptionally tightly packed array with extremely low fluidity, which limits penetration of antibiotics and chemotherapeutic agents into the cell walls.[7−9] On the other hand, this also causes nutrient acquisition difficulties for mycobacteria themselves. Studies have shown that almost all mycobacteria possess a serious problem of iron acquisition, because the predominant form of iron occurs in its oxidized ferric [Fe(III)] state, which is highly insoluble at physiological pH. Also, in animal tissues, most iron is chelated by host molecules such as lactoferrin and transferrin. In order to survive, microbes secrete specific low molecular weight iron chelators termed siderophores (iron carriers) to sequester and transport iron.[10−17] Siderophores usually chelate iron from extracellular media. In many mammalian infections, siderophores which are produced by microorganisms are even able to acquire the host's lactoferrin- or transferrin-bound iron.[18]

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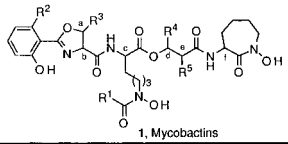
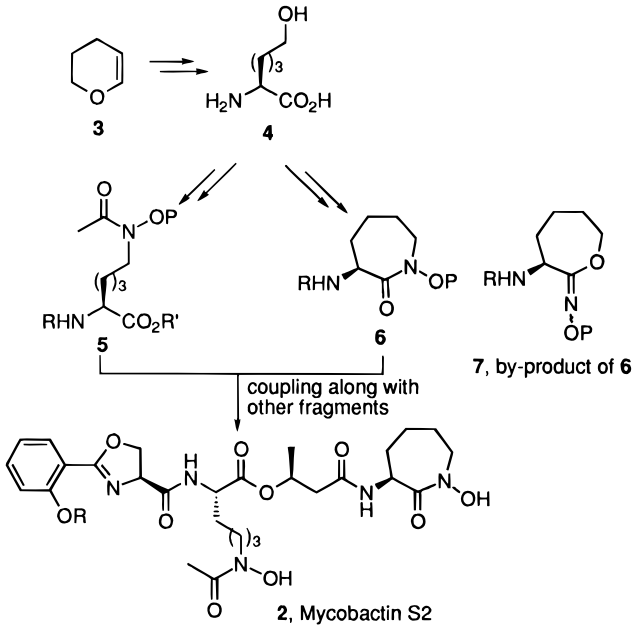
Table 1. General Structures of Mycobactins

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



1, Mycobactins

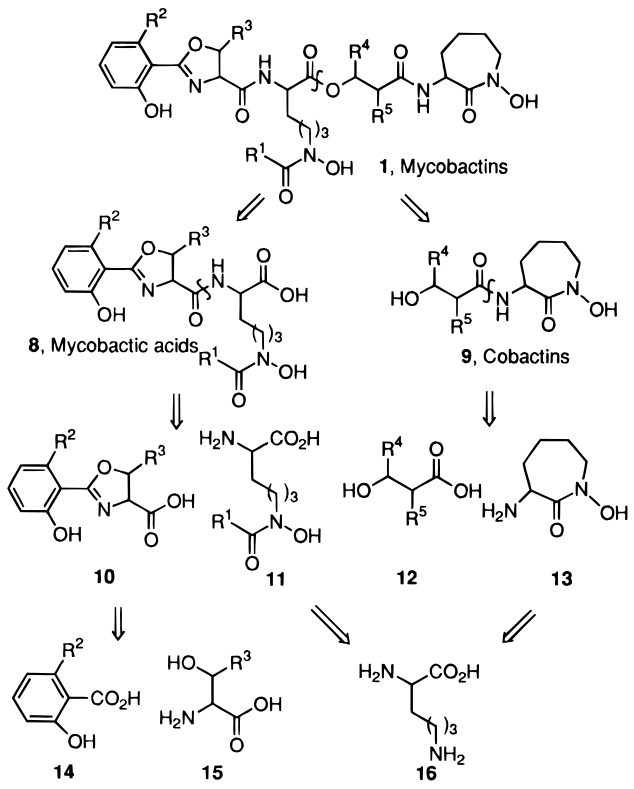
Scheme 1



Mycobactins **1** are a family of siderophores, which are isolated from mycobacteria including *M. tuberculosis* and *Mycobacterium phlei* (Table 1).[19,20] They promote mycobacterial growth *via* iron uptake processes. Mycobactins possess a nearly identical molecular nucleus, which includes two hydroxamic acids and a 2-(2-hydroxyphenyl)-Δ^2-1,3-oxazoline residue serving as iron-chelating components. They vary only in the stereochemistry of the chiral centers and in the peripheral groups. After detailed study and structural elucidation of the mycobactins, Snow[20] suggested that alternate or modified forms of mycobactins might serve as antagonists of mycobacterial growth and be of therapeutic value.

A mycobactin analog lacking the three hydroxyl groups necessary for iron chelation was synthesized[21] shortly after structural elucidation of several mycobactins. Biological tests of these analogs, incapable of chelating iron, revealed no growth inhibitory or stimulatory activity of mycobacteria, thus confirming a need for iron complexation. The first synthesis of a mycobactin analog, mycobactin S2 (**2**), which contains all iron-chelating components, but lacks a long lipophilic side chain ($R^1 = CH_3$), was accomplished in our group (Scheme 1).[22] This methodology was limited by utilizing an enzymatic resolution to synthesize intermediate L-ϵ-hydroxynorleucine (**4**) from 3,4-dihydro-2*H*-pyran (**3**). Subsequent synthesis of caprolactam **6** was complicated by *N*- vs *O*-alkylation selectivity. Biological studies of mycobactin S2 revealed that the short alkyl group ($R^1 = CH_3$) resulted in loss of lipid solubility and siderophore activity for mycobacteria.[23] This indicated that a long lipophilic side chain is essential for mycobactins to transport iron through cell membranes and therefore to be potential drug carriers. Thus, methodology directed toward syntheses of various mycobactins was explored.

Scheme 2



Preparation of an analog of mycobactin T, the siderophore and growth promoter for *M. tuberculosis*, with minimal and defined variations was anticipated to allow us to further test Snow's hypothesis that alternate forms of mycobactins might have antitubercular activity. Thus, mycobactin S (Table 1), produced by *Mycobacterium smegmatis*, was chosen to be the initial target molecule in this study. It is the closest structural relative of mycobactin T, produced by *M. tuberculosis*, differing only in variable substituent R^1 and chiral center (d of structure **1**). To facilitate the study, we also chose to use a single defined acyl group [$R^1 = CH_3(CH_2)_{14}$] that is similar to the mixture of acyl groups of both mycobactin S and T.

Retrosynthetically, disconnection of the ester bond of mycobactins **1** gives two fragments, mycobactic acids **8**, containing the acyclic hydroxamate residue, and cobactins **9**, containing the cyclic hydroxamate residue (Scheme 2). Further disconnections of the amide bonds in fragments **8** and **9** result in four corresponding components, **10**, **11**, **12**, and **13**. Oxazoline derivative **10** can be prepared from a salicylic acid, **14**, and serine or threonine derivative **15**. Hydroxamic acid **11** can be prepared from **16** by direct amine oxidation using dimethyldioxirane (DMD) oxidation methodology which we recently reported.[24] Some β-hydroxy acids, **12**, are commercially available materials. N^{ϵ}-Hydroxycyclolysine (**13**) can also be

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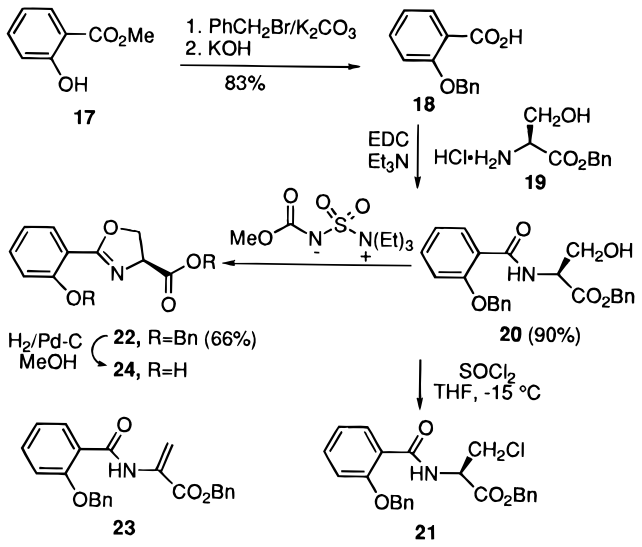
Scheme 3



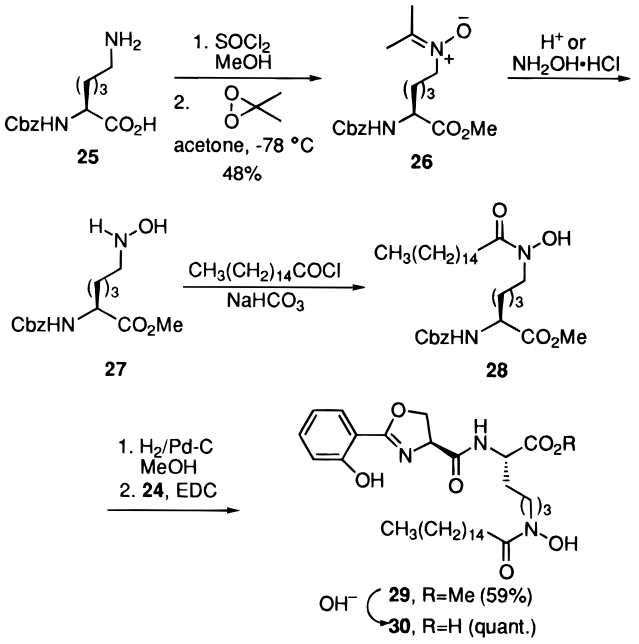
derived from **16** by DMD oxidation and subsequent further functional group manipulations.[25]

Results and Discussion

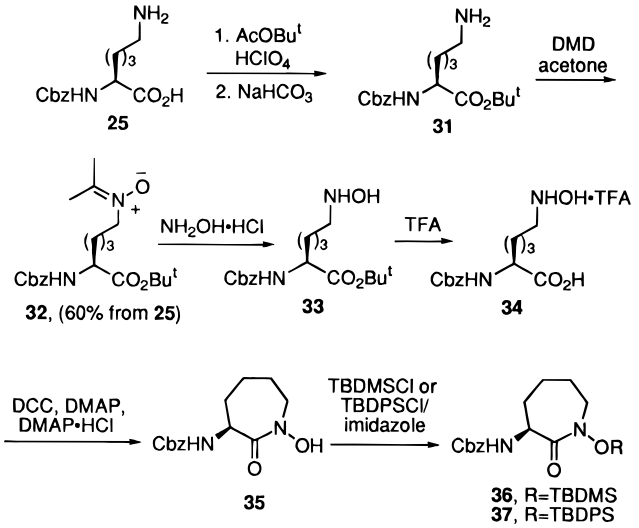
Synthesis of Mycobactic Acid. Commercially available methyl salicylate (**17**) was refluxed in a mixed solvent (methanol and chloroform) with benzyl bromide in the presence of potassium carbonate under a stream of argon (Scheme 3).[26,27] After filtration and concentration, the residue was saponified to give 2-(benzyloxy)benzoic acid (**18**) in 83% yield. Changing the solvent from chloroform to methylene chloride did not affect the reaction. However, the transformation was totally suppressed in the absence of methanol. Acid **18** was coupled with L-serine benzyl ester **19** to afford amide **20** in 90% yield. Attempted cyclization of amide **20** with thionyl chloride[22] resulted in a chloride-substituted derivative **21**. Treatment of **20** with Burgess's reagent[28] provided oxazoline **22** in 66% yield. The corresponding eliminated product **23** also was isolated in low yield and characterized by ^{1}H NMR and MS spectroscopy. Hydrogenolytic removal of the benzyl groups from compound **22** gave the corresponding acid **24**.

Hydroxylamine **27** was prepared by dimethyldioxirane oxidation of a derivative of amino acid **25** and subsequent hydrolysis or hydroxylamine exchange.[24] Acylation of hydroxylamine **27** with palmitoyl chloride in the presence of $NaHCO_3$ provided hydroxamic acid **28** (Scheme 4), a key iron-binding component of the mycobactins. Hydrogenolytic removal of the Cbz group from hydroxamic acid **28** gave the corresponding amine, which was then coupled with oxazoline acid **24** using EDC [1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride] to afford mycobactic acid methyl ester **29** in 59% yield. This smooth reaction is in contrast to our previous attempts[22] related to coupling reactions of 2-[2-(benzyloxy)phenyl]-Δ^2-1,3-oxazoline-4-carboxylic acid with different amines. Absence of a large benzyloxy group in acid **24** appeared to be the major factor in the successful reaction. As a result, this significantly improved the efficiency of the total synthesis of mycobactin S (**44**). Subsequent saponification of **29** with KOH or LiOH in aqueous THF solution at 0 °C provided mycobactic acid **30** quantitatively.

Scheme 4



Scheme 5



Synthesis of Cobactin T. N^{α}-Cbz-L-lysine (**25**) was stirred in AcOBu^t in the presence of a catalytic amount of $HClO_4$ in a sealed flask overnight to give the corresponding *tert*-butyl ester **31** (Scheme 5). Subsequent oxidation with DMD provided nitrone **32** in 60% overall yield. After treatment of nitrone **32** with NH_2OH·HCl, followed by basic workup, extraction, and concentration, the product hydroxylamine **33** was treated with TFA to yield the corresponding acid **34**. Compound **34** was cyclized using DCC (*N*,*N*-dicyclohexylcarbodiimide), DMAP [4-(dimethylamino)pyridine], and DMAP·HCl to provide the desired hydroxamic acid **35**, but still in low isolated yield. The product was perhaps chelated with Fe(III) whose presence could be traced to silica gel chromatography. The apparent Fe(III) complex was unable to be chromatographically eluted. The problem was solved by prior protection of the hydroxamate hydroxyl group with silyl groups. Treatment of hydroxamic acid **35** with TBDMSCl (*tert*-butyldimethylsilyl chloride) or TBDPSCl (*tert*-butyldiphenylsilyl chloride) in the presence of imidazole at 35 °C overnight gave the corresponding azopine **36** or **37** in 50−55% overall yield for the four steps.

With azopines **36** and **37** in hand, synthesis of cobactin T was subsequently performed. (*R*)-β-Hydroxybutanoic acid (**39**)

(25) Hu, J.; Miller, M. J. *Tetrahedron Lett.* **1995**, *36*, 6379.
(26) Farkas, L.; Vermes, B.; Nogradi, M. *Tetrahedron* **1967**, *23*, 741.
(27) Schmidhammer, H.; Brossi, A. *J. Org. Chem.* **1983**, *48*, 1469.
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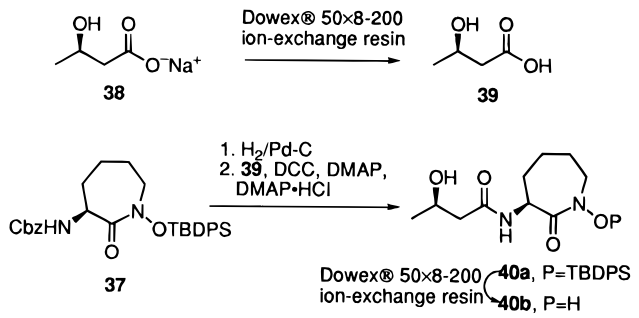
Scheme 6



was obtained by treatment of the corresponding sodium salt **38** (Aldrich) with Dowex 50 × 8−200 ion-exchange resin (Scheme 6). After hydrogenolytic removal of the Cbz group from azopine **36**, the resulting amine was coupled with (*R*)-*β*-hydroxybutanoic acid (**39**) in the presence of DCC, DMAP, and DMAP·HCl. TLC analysis indicated that the TBDMS group of compound **36** did not survive the coupling conditions, perhaps because of the acidity of butanoic acid **39**.

Reactions of TBDPS-protected azopine **37** were attempted next. The Cbz group of azopine **37** was hydrogenolytically removed, and the resulting amine was coupled with (*R*)-*β*-hydroxybutanoic acid (**39**) using DCC, DMAP, and DMAP·HCl to provide *O*-(*tert*-butyldiphenylsilyl)cobactin **40a** in 63% yield. The TBDPS group was cleaved using 49% aqueous HF solution.[29] It could also be removed by stirring with Dowex 50 × 8−200 ion-exchange resin in MeOH for a few minutes to give cobactin T (**40b**) in 62% yield.

Synthesis of Mycobactin S. Mycobactic acid **30** and cobactin **40a**, the two major components of mycobactins, were successfully synthesized by dimethyldioxirane-induced oxidation of the corresponding primary amines as the key step. Condensation of mycobactic acid **30** with cobactin **40a** remained as the last step in the construction of mycobactin S.

Our original intention was to determine if it was possible to effect direct condensation of **30** with **40a** without protection of the hydroxyl groups of **30**. Various coupling reagents (DCC, EDC, and EEDQ) and Mitsunobu conditions were utilized, but all reactions failed to effect the condensation. Subsequent protection of the hydroxyl groups of **30** did not provide a useful synthetic pathway.[30] This led to a revised synthetic strategy as shown in Scheme 7. Readily available hydroxamic acid **28** was reacted with SEMCl [[2-(trimethylsilyl)ethoxy]methyl chloride] in the presence of DIEA (*N*,*N*-diisopropylethylamine) and DMAP in toluene to give SEM-protected ester **41** in 91% yield.[31,32] Subsequent hydrogenolytic removal of the Cbz group from ester **41** gave the corresponding amine, which was then coupled with unprotected oxazoline **24** using EDC to give protected mycobactic acid methyl ester **42a** in 65% yield. Treatment of ester **42a** with LiOH provided acid **42b** quantitatively after acidification. Partially protected mycobactin S **43** was obtained in 49% yield under Mitsunobu conditions. Treatment of **43** with TFA[33,34] in methylene chloride provided mycobactin S (**44**) in 56% yield.

Biological Studies. As indicated earlier, in his excellent review of the mycobactins in 1970,[20] Snow suggested that the mycobactin structure could be used as a model for the development of drugs having specific activity against mycobacteria, but such a concept "was frustrated by the complexity of the molecule". Studies of simplified model compounds related to mycobactin but incapable of binding iron had no affect on the growth of *M. tuberculosis*, prompting the further statement "Thus, the concept of a mycobactin antagonist seems to have been sound in principle, though not yet realized in practice." We are now pleased to report that, as anticipated by Snow, biological tests showed that our synthetic mycobactin S (**44**) effects greater than 99% inhibition of the growth of *M. tuberculosis* H37Rv at the concentration of 12.5 μg/mL. This strongly indicates that the chiral center (d of structure **1**) plays a critical role in controlling TB growth since mycobactin T which stereochemically differs only at this one center is a siderophore and growth promoter of *M. tuberculosis*. Further biological studies related to the inibition of the growth of drug-resistant strains of *M. tuberculosis* are in progress.

Summary

In conclusion, a general total synthesis of mycobactins was achieved. This methodology featured applications of DMD oxidation of primary amines in natural product syntheses, DCC-, DMAP-, and DMAP·HCl-mediated cyclization to give azopine derivatives **36** and **37**, and a successful application of SEM protection for hydroxamic acid **28**. The subsequent esterification reaction accomplished the total synthesis of a mycobactin S (**44**) which was demonstrated to be an effective inhibitor of clinically important *M. tuberculosis*.

Experimental Section

Instruments and general methods used have been described earlier.[35] Optical rotations were measured on a Rudolf Research Autopol III polarimeter. The ferric chloride test was performed by treating the TLC plate with 0.3% ferric chloride in aqueous 0.5 N HCl solution. Anhydrous CH_2Cl_2 and acetonitrile were freshly distilled from CaH_2 before use. Anhydrous tetrahydrofuran was freshly distilled from sodium and benzophenone. During all hydrogenolysis reactions, the solvent was purged with nitrogen prior to addition of a catalyst.

Antimycobacterial studies were performed at the Tuberculosis Antimicrobial Acquisition and Coordinating Facility using their standard protocol.

Dimethyldioxirane. Dimethyldioxirane was generated by the method reported by Murray et al.[36] The concentration was determined by iodometric titration.[37]

2-(Benzoyloxy)benzoic Acid (18). Compound **18** was prepared by the method of Farkas et al.[26] with slight modification. Methylene chloride was used instead of chloroform. An 83% yield was obtained with mp 74−76 °C (lit.[26] 48% yield; mp 76−78 °C)

***N*-[2-(Benzyloxy)benzoyl]-L-serine Benzyl Ester (20).** To a stirred solution of 2-(benzyloxy)benzoic acid (**18**) (3.53 g, 15.5 mmol) and L-serine benzyl ester hydrochloride (**19**) (3.27 g, 14 mmol) in CH_2Cl_2 (70 mL) were added Et_3N (2.09 mL, 15 mmol) and EDC [1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; 2.96 g, 15.5 mmol]. After being stirred for 10 h at room temperature under argon, the reaction mixture was diluted with CH_2Cl_2 (200 mL), washed with H_2O, saturated $NaHCO_3$ solution, 5% citric acid aqueous solution, H_2O, and brine, dried over Na_2SO_4, filtered, and concentrated. Recrystallization from toluene afforded amide **20** (5.12 g, 90%) as white crystals: $R_f = 0.41$ ($EtOAc/CH_2Cl_2$ = 1/5); mp 116−118 °C; $[\alpha]^{23}_D = +24.1°$ (c = 1.0, CH_2Cl_2); IR (KBr) 1740, 1620 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ

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(33) Schlessinger, R. H.; Poss, M. A.; Richardson, S. *J. Am. Chem. Soc.* **1986**, *108*, 3112.
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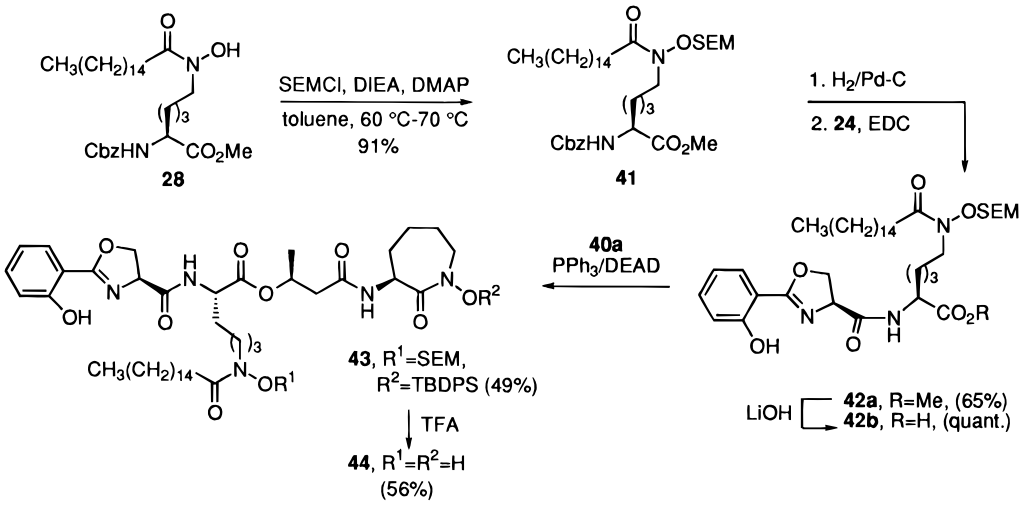
(37) Adam, W.; Chan, Y.-Y.; Cremer, D.; Gauss, J.; Scheutzow, D.; Schindler, M. *J. Org. Chem.* **1987**, *52*, 2800.

Scheme 7



28 41

8.81 (d, J = 6.9 Hz, 1H), 8.19 (dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz, 1H), 7.46−7.25 (m, 11H), 7.10−7.04 (m, 2H), 5.21−5.11 (m, apparently 4 overlapping doublets, 4H), 4.90−4.85 (m, 1H), 3.93−3.89 (m, 2H), 2.32 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.13, 165.56, 156.91, 135.50, 135.26, 133.17, 132.31, 128.74, 128.53, 128.33, 128.06, 127.99, 121.48, 121.09, 112.79, 71.23, 67.17, 63.61, 55.38; FABMS *m/z* 406 (M + 1). Anal. Calcd for $C_{23}H_{21}NO_5$: C, 70.58; H, 5.41; N, 3.58. Found: C, 70.43; H, 5.60; N, 3.37.

(*S*)-Benzyl 2-[2-(Benzyloxy)phenyl]-Δ^2-1,3-oxazoline-4-carboxylate (22).[28] To a stirred solution of *N*-[2-(benzyloxy)benzoyl]-L-serine benzyl ester (**20**) (545 mg, 1.35 mmol) in THF (10 mL) was added Burgess's reagent [(methoxycarbonylsulfamoyl)triethylammonium hydroxide, inner salt, 360 mg, 1.55 mmol, 1.1 equiv]. After being refluxed for 30 min at room temperature under argon, the reaction mixture was diluted with EtOAc (100 mL), washed with H_2O and brine, dried over Na_2SO_4, filtered, concentrated, and chromatographed on silica gel eluting with EtOAc/CH_2Cl_2 (1/15) to yield oxazoline **22** (343 mg, 66%) as a white, amorphous solid: R_f = 0.58 (EtOAc/CH_2Cl_2 = 1/15); mp 69−71 °C (recrystallized from EtOAc and hexanes); IR (KBr) 1732, 1630 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz, 1H), 7.50−7.47 (m, 2H), 7.42−7.26 (m, 9H), 7.00−6.96 (m, 2H), 5.28 (d, J = 12.3 Hz, 1H), 5.20 (d, J = 11.7 Hz, 1H), 5.18 (s, 2H), 5.00 (dd, J_1 = 10.5 Hz, J_2 = 8.1 Hz, 1H), 4.68−4.52 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.03, 165.65, 157.56, 136.79, 135.40, 132.64, 131.62, 128.50, 128.36, 128.29, 128.25, 127.53, 126.69, 120.67, 117.12, 113.75, 70.55, 69.16, 68.77, 67.12; FABMS *m/z* 388 (M + 1); HREIMS *m/z* calcd for $C_{24}H_{21}NO_4$ 387.1471, found 387.1458.

N^{α}-Cbz-N^{δ}-hydroxy-N^{δ}-palmitoyl-L-lysine Methyl Ester (28).[38] To a stirred solution of nitrone **26**[24] (680 mg, 1.94 mmol) in MeOH (10 mL) at 40 °C was added hydroxylamine hydrochloride salt (203 mg, 2.91 mmol, 1.5 equiv). After the solution was stirred for 10 min at 40 °C, the solvent was removed. The residue was dissolved in saturated $NaHCO_3$ solution (15 mL) and then extracted with CH_2Cl_2. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated to give the corresponding hydroxylamine **27**. Compound **27** was dissolved in CH_2Cl_2 (20 mL) at 0 °C. To this mixture were added $NaHCO_3$ (326 mg, 3.88 mmol, 2 equiv) and palmitoyl chloride (0.706 mL, 2.33 mmol, 1.2 equiv). After being stirred for 3 h at room temperature, the mixture was filtered, concentrated, and chromatographed on silica gel eluting with CH_2Cl_2/EtOAc (5/2) to give hydroxamic acid **28** (749 mg, 71%) as a white solid: R_f = 0.33 (CH_2Cl_2/EtOAc = 5/2); mp 66−68 °C (recrystallized from EtOAc/Skelly B); ^{1}H NMR (300 MHz, CD_3OD) δ 7.35−7.28 (m, 5H), 5.08 (s, 2H), 4.18−4.13 (m, 1H), 3.70 (s, 3H), 3.62−3.56 (m, 2H), 2.44 (t, J = 7.6 Hz, 2H), 1.83−1.28 (m, 32H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.09, 174.58, 158.61, 138.13, 129.43, 128.95, 128.72, 67.60, 55.34, 52.63, 33.26, 33.07, 32.08, 30.79, 30.64, 30.50, 27.09, 25.96, 23.81, 23.73, 14.47; HRFABMS *m/z* calcd for $C_{31}H_{53}N_2O_6$ 549.3904, found 549.3897. Anal. Calcd for $C_{31}H_{52}N_2O_6$: C, 67.85; H, 9.55; N, 5.10. Found: C, 67.83; H, 9.60; N, 5.16.

Oxazoline−Hydroxamic Acid 29. To a stirred solution of hydroxamic acid **28** (44.5 mg, 0.08 mmol) in MeOH (1 mL) at room temperature was added Pd/C (5 mg, 10%). After being stirred for 1 h under H_2 (1 atm), the mixture was filtered and concentrated to give the corresponding amine. To another stirred solution of oxazoline **22** (31 mg, 0.08 mmol) in MeOH (1 mL) at room temperature was added Pd/C (5 mg, 10%). After being stirred for 1 h at room temperature under H_2 (1 atm), the mixture was filtered and concentrated to give the corresponding acid **24**.[39] The free amine and acid **24** were then dissolved in CH_2Cl_2 (2 mL). To this mixture was added EDC (23 mg, 1.2 mmol, 1.5 equiv). After being stirred for 3 h at room temperature, the reaction mixture was diluted with EtOAc (5 mL) and then washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated to give oxazoline−hydroxamic acid **29** (crude product, 43 mg, 92%), as a light-yellow solid: R_f = 0.28 (EtOAc/CH_2Cl_2 = 1/5); mp 80.0−82.0 °C (recrystallized from CH_2Cl_2 and hexanes, 59% yield after recrystallization); IR (KBr) 1740, 1650 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 7.69 (dd, J_1 = 7.9 Hz, J_2 = 1.5 Hz, 1H), 7.41 (dt, J_1 = 7.8 Hz, J_2 = 1.6 Hz, 1H), 6.96 (d, J = 8.3 Hz, 1H), 6.90 (t, J = 7.6 Hz, 1H), 5.02 (t, J = 9.0 Hz, 1H), 4.67−4.60 (m, 2H), 4.44 (dd, J_1 = 8.9 Hz, J_2 = 4.9 Hz, 1H), 3.73 (s, 3H), 3.62−2.56 (m, 2H), 2.43 (t, J = 7.5 Hz, 2H), 1.93−1.27 (m, 32H), 0.90 (t, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.18, 173.87, 172.96, 168.55, 161.00, 135.02, 129.51, 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, 25.96, 23.80, 23.72, 14.44; HRFABMS *m/z* calcd for $C_{33}H_{53}N_3O_7$ 604.3961, found 604.3983. Anal. Calcd for $C_{33}H_{52}N_3O_7$: C, 65.64; H, 8.85; N, 6.96. Found: C, 65.58; H, 8.81; N, 6.75.

Nitrone 32. To a stirred solution of N^{α}-Cbz-L-lysine (**25**) (1 g, 3.6 mmol) in AcOBu^t (50 mL) was added $HClO_4$ (70%, 0.46 mL, 5.3 mmol, 1.5 equiv). The mixture was sealed with a septum and covered with parafilm. After being stirred overnight, the reaction was extracted with H_2O and 0.5 N HCl solution. The combined aqueous solution was basified with K_2CO_3 (10% aqueous solution) to pH 8 and then extracted with CH_2Cl_2. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated to give the corresponding *tert*-butyl ester, as an oil. The oil was dissolved in acetone (20 mL) and cooled to −78 °C. To the acetone solution was added an excess of dimethyldioxirane (50 mL).[36] After being stirred for another 10 min at −78 °C, the mixture was concentrated and chromatographed on silica gel eluting with EtOAc/MeOH (8/3) to give nitrone **32** (847 mg, 60%) as a clear oil: R_f = 0.29 (EtOAc/MeOH = 8/3); IR (neat) 1715 (br) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37−7.31 (m, 5H), 5.41 (d, J = 7.9 Hz, 1H), 5.10 (s, 2H), 4.28−4.21 (m, 1H), 3.83 (t, J = 7.2 Hz, 2H), 2.14 (s, 3H), 2.09 (s, 3H), 2.01−1.31 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.29, 155.83, 143.50,

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136.28, 128.43, 128.07, 128.02, 82.13, 66.77, 58.51, 54.05, 32.48, 27.92, 26.85, 22.37, 20.36, 19.90; HRFABMS *m/z* calcd for $C_{21}H_{33}N_2O_5$ 393.2389, found 393.2400.

TBDPS-Protected Azopine Derivative 37. To a stirred solution of nitrone **32** (481 mg, 1.23 mmol) in MeOH (5 mL) was added NH_2-OH·HCl (423 mg, 6.14 mmol, 5 equiv). The solution was stirred at 40 °C for 10 min. After removal of the solvent, the residue was taken up in saturated $NaHCO_3$ (10 mL). The aqueous solution was extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to afford hydroxylamine **33**. Hydroxylamine **33** was then treated with TFA/CH_2Cl_2 (3 mL/3 mL) for 1.5 h at room temperature and concentrated to give acid **34**. To a refluxing solution of DCC (1.26 g, 6.14 mmol, 5 equiv), DMAP (749 mg, 6.14 mmol, 5 equiv), and DMAP·HCl (976 mg, 6.14 mmol, 5 equiv) in $CHCl_3$ (70 mL) was added acid **34** in $CHCl_3$ (70 mL) dropwise over 2 h. The reaction was refluxed for 1 h after addition. The mixture was concentrated, taken up in DMF (5 mL), and treated with TBDPSCl (800 μL, 3.08 mmol, 2.5 equiv) and imidazole (418 mg, 6.14 mmol, 5 equiv). After being stirred at 35 °C under argon overnight, the reaction was diluted with EtOAc. The EtOAc solution was washed with H_2O and brine, dried over Na_2SO_4, filtered, concentrated, and chromatographed on silica gel eluting with EtOAc/Skelly B (1/5) to give azopine **37** (339 mg, 54%) as a clear oil: $R_f = 0.19$ (EtOAc/Skelly B = 1/5); IR (neat) 3410, 3330, 1720, 1675 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75−7.71 (m, 4H), 7.45−7.33 (m, 11H), 6.03 (d, $J = 6.3$ Hz, 1H), 5.05 (ab q, J_1 =17.9 Hz, J_2 =12.4 Hz, 2H), 4.05−3.99 (m, 2H), 3.52−3.45 (m, 2H), 1.89−1.05 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.49, 155.26, 136.50, 136.09, 135.99, 132.04, 131.57, 130.19, 130.14, 128.36, 127.90, 127.76, 127.49, 127.44, 66.46, 54.17, 53.06, 31.54, 27.30, 26.85, 25.25, 19.48; HRFABMS *m/z* calcd for $C_{30}H_{37}N_2O_4Si$ 517.2523, found 517.2568.

TBDMS-Protected Azopine Derivative 36. Compound **36** was prepared in 54% yield as a clear oil using the procedure described for the synthesis of **37**: $R_f = 0.44$ (EtOAc/CH_2Cl_2 = 1/15); IR (neat) 1720, 1675 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44−7.28 (m, 5H), 6.11 (d, $J = 6.5$ Hz, 1H), 5.10 (s, 2H), 4.34 (ddd, $J_1 = 11.3$ Hz, $J_2 = 6.6$ Hz, $J_3 = 1.9$ Hz, 1H), 3.83−3.74 (m, 1H), 3.59−3.52 (m, 1H), 2.07−1.46 (m, 6H), 0.96 (s, 9H), 0.22 (s, 3H), 0.15 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.18, 155.43, 136.50, 128.47, 128.01, 127.90, 66.66, 54.36, 53.23, 31.90, 27.58, 25.73, 25.64, 18.04, −4.66, −5.24; HRFABMS *m/z* calcd for $C_{20}H_{33}N_2O_4Si$ 393.2210, found 393.2223.

TBDPS-Protected Cobactin 40a. To a stirred solution of compound **37** (103 mg, 0.199 mmol) in MeOH (4 mL) was added Pd/C (10 mg, 10%). After being stirred for 2 h at room temperature under H_2 (1 atm), the reaction mixture was filtered and concentrated to give the corresponding amine. To a beaker with Dowex 50 × 8−200 resin in MeOH was added sodium (*R*)-β-hydroxybutyrate (**38**; Aldrich, 25 mg). Acid **39** was obtained after filtration and concentration. To the mixture of the amine, DCC (103 mg, 0.498 mmol, 2.5 equiv), DMAP (61 mg, 0.498 mmol, 2.5 equiv), and DMAP·HCl (79 mg, 0.498 mmol, 2.5 equiv) in $CHCl_3$ (10 mL) at 40 °C was added acid **39** in $CHCl_3$ (2 mL) slowly over 10 min. After being stirred further for 20 min at 40 °C, the reaction mixture was concentrated and then taken up in EtOAc. The EtOAc solution was washed with H_2O and brine, dried over Na_2SO_4, filtered, concentrated, and chromatographed on silica gel eluting with EtOAc to give TBDPS-protected cobactin **40a** (58 mg 63%) as a clear oil: $R_f = 0.32$ (EtOAc); IR (neat) 3360 (br), 1645 (br) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (t, $J = 7$ Hz, 4H), 7.47−7.35 (m, 6H), 6.78 (d, $J = 6$ Hz, 1H), 4.22 (dd, $J_1 = 10$ Hz, $J_2 = 6$ Hz, 1H), 4.20−4.10 (m, 1H), 3.90 (br, 1H), 3.56−3.40 (m, 2H), 2.32 (dd, $J_1 = 15$ Hz, $J_2 = 3$ Hz, 1H), 2.21 (dd, $J_1 = 15$ Hz, $J_2 = 8$ Hz, 1H), 1.86−1.35 (m, 9H), 1.15 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.37, 169.29, 136.08, 135.94, 131.94, 131.49, 130.25, 130.21, 127.54, 127.47, 64.69, 54.18, 51.42, 43.48, 30.92, 27.26, 26.84, 25.25, 22.46, 19.50; HRFABMS *m/z* calcd for $C_{26}H_{37}N_2O_4Si$ 469.2523, found 469.2519.

Cobactin T (40b). To a stirred mixture of Dowex resin (50 × 8−200) in MeOH (1 mL) was added compound **40a** (54 mg). After being stirred for 1 h at room temperature, the reaction mixture was filtered and concentrated to give a white solid. The solid was recrystallized from EtOAc/hexanes to give colorless crystals (17 mg, 62%): $R_f = 0.50$ (EtOAc/MeOH = 8/3); mp 137−138 °C; IR (KBr) 1630 cm$^{-1;}$ ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.97 (br, 1H), 4.64−4.58 (m, 1H), 4.24−4.14 (m, 1H), 3.86−3.69 (m, 2H), 2.45 (dd, $J_1 = 15.3$ Hz, $J_2 = 3.0$ Hz, 1H), 2.32 (dd, $J_1 = 15.3$ Hz, $J_2 = 8.6$ Hz, 1H), 2.08−1.42 (m, 6H), 1.23 (d, $J = 6.3$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.61, 167.06, 64.80, 50.84, 50.31, 43.78, 31.12, 27.72, 25.70, 22.61; HRFABMS *m/z* calcd for $C_{10}H_{19}N_2O_4$ 231.1345, found 231.1329.

N^α-Cbz-N^ϵ-palmitoyl-N^ϵ-[[2-(trimethylsilyl)ethoxy]methoxy]-L-lysine Methyl Ester (41). To a stirred solution of N^α-Cbz-N^ϵ-hydroxy-N^ϵ-palmitoyl-L-lysine methyl ester (**28**) (3.65 g, 6.67 mmol) in toluene (100 mL) were added *N*,*N*-diisopropylethylamine (23.2 mL, 133 mmol, 20 equiv), DMAP (catalytic amount), and SEMCl (11.8 mL, 66.7 mmol, 10 equiv) under argon. After being stirred for 48 h at 60−70 °C, the reaction mixture was concentrated and then dissolved in EtOAc (200 mL). The solid in EtOAc was filtered off, and then the organic layer was washed with H_2O and brine, dried over Na_2SO_4, filtered, concentrated, and chromatographed on silica gel eluting with EtOAc/CH_2Cl_2 (1/15) to give product **41** (4.31 g, 91%) as a clear oil: R_f = 0.19 (EtOAc/CH_2Cl_2 = 1/15); IR (neat) 3310 (w), 1720, 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), 3.76−3.62 (m, 7H), 2.38 (t, $J = 7.6$ Hz, 2H), 1.86−1.03 (m, 32H), 0.99−0.93 (m, 2H), 0.88 (t, $J = 6.7$ Hz, 3H), 0.03 (s, 9H); ^{13}C NMR (75 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 *m/z* calcd for $C_{37}H_{67}N_2O_7Si$ 679.4718, found 679.4702.

Mycobactic Acid Methyl Ester Analog 42a. Protected hydroxamic acid **41** (200 mg, 0.295 mmol) was stirred in MeOH (5 mL) in the presence of Pd/C (10%, 20 mg) under H_2 (1 atm) for 1 h. After filtration, the solvent was removed *in vacuo* to provide the corresponding free amine. The free amine was then treated with acid **24** [derived from ester **22** (137 mg) by hydrogenolysis] and EDC (85 mg, 0.442 mmol, 1.5 equiv) in CH_2Cl_2 (5 mL). After being stirred for 30 min, the reaction mixture was concentrated and chromatographed on silica gel eluting with EtOAc/CH_2Cl_2 (1/15) to afford compound **42a** (140 mg, 65%) as a clear oil: $R_f = 0.19$ (EtOAc/CH_2Cl_2 = 1/15); IR (neat) 1740, 1650 (br), 1630 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 (dd, $J_1 = 7.9$ Hz, $J_2 = 1.7$ Hz, 1H), 7.39 (ddd, $J_1 = 8.7$ Hz, $J_2 = 7.5$, $J_3 = 1.7$ Hz, 1H), 7.00 (dd, $J_1 = 8.5$ Hz, $J_2 = 0.8$ 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), −0.003 (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 *m/z* calcd for $C_{39}H_{68}N_3O_8Si$ 734.4776, found 734.4764.

Protected Mycobactin 43. To a stirred solution of protected mycobactic acid **42b** (obtained from **42a** by saponification with LiOH; 72 mg, 0.1 mmol), protected cobactin **40a** (48 mg, 0.1 mmol, 1 equiv), and PPh_3 (131 mg, 0.5 mmol, 5 equiv) in freshly distilled THF (4 mL) was added DEAD (79 μL, 0.5 mmol, 5 equiv)/THF (0.5 mL). After being stirred for 1 h at room temperature, the reaction was concentrated. The residue was recrystallized from EtOAc/Skelly B to remove reduced DEAD and triphenylphosphine oxide. The mother liquor was concentrated and chromatographed on silica gel eluting with EtOAc/CH_2Cl_2 (1/5) to afford protected mycobactin S **43** (57 mg, 49%) as a clear oil: $R_f = 0.71$ (EtOAc/CH_2Cl_2 = 1/1); IR (neat) 3320, 1740, 1650 (br), 1640 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.44 (s, 1H), 7.74−7.68 (m, 5H), 7.46−7.35 (m, 7H), 7.02 (dd, $J_1 = 8.3$ Hz, $J_2 = 1.0$ Hz, 1H), 6.98 (d, $J = 7.8$ Hz, 1H), 6.91−6.87 (m, 2H), 5.26−5.23 (m, 1H), 4.95 (t, $J = 10$ Hz, 1H), 4.86 (s, 2H), 4.63 (d, $J = 9.3$ Hz, 2H), 4.45−4.41 (m, 1H), 4.23−4.18 (m, 1H), 3.73−3.69 (m, 2H), 3.64−3.57 (m, 2H), 3.50−3.48 (m, 2H), 2.50 (dd, $J_1 = 14.1$ Hz, $J_2 = 6.8$ Hz, 1H), 2.37−2.33 (m, 3H), 1.85−1.51 (m, 12H), 1.28 (d, $J = 6.4$ Hz, 3H), 1.26−1.24 (m, 26H), 1.13 (s, 9H), 0.96−0.92 (m, 2H), 0.87 (t, $J = 6.8$ Hz, 3H), 0.017 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.48, 170.24, 169.31, 167.82, 167.48, 159.73, 136.04, 135.91, 134.02, 131.88, 131.49, 130.22, 130.15, 128.43, 127.45, 118.87, 116.88, 109.99, 98.99, 69.36, 69.25, 67.95, 67.50, 54.11, 52.49, 51.58, 42.27, 32.50, 31.82, 31.35, 30.84, 29.58, 29.55, 29.45, 29.39, 29.24, 27.14, 26.80, 26.23,

25.18, 24.42, 22.59, 22.39, 19.58, 19.46, 18.07, 14.02, −1.54; HR-FABMS *m/z* calcd for $C_{64}H_{100}N_5O_{11}Si_2$ 1170.6958, found 1170.6941.

Mycobactin S (44). Protected mycobactin **43** (180 mg, 0.15 mmol) was stirred in TFA/CH_2Cl_2 (2 mL, 1/1) at room temperature for 1 h. The mixture was concentrated *in vacuo* and then taken up in EtOAc. The solution was washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated to give a colored solid. The solid was recrystallized from $CHCl_3$/EtOAc to give mycobactin S (**44**) (83 mg, 68%) as a white, amorphous solid: $R_f = 0.27$ (EtOAc/MeOH = 20/1); mp 178−180 °C; IR (KBr) 3305 (br), 1735, 1630 (br) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$ with CD_3OD) δ 7.70 (dd, $J_1 = 8.0$ Hz, $J_2 = 1.7$ Hz, 1H), 7.44−7.40 (m, 1H), 7.01 (d, $J = 8.3$ Hz, 1H), 6.92 (t, $J = 7.5$ Hz, 1H), 5.36−5.30 (m, 1H), 5.00 (dd, $J_1 = 10.0$ Hz, $J_2 = 8.7$ Hz, 1H), 4.68−4.61 (m, 2H), 4.50 (d, $J = 10.7$ Hz, 1H), 4.45 (dd, $J_1 = 8.0$ Hz, $J_2 = 5.0$ Hz, 1H), 3.81 (dd, $J_1 = 16.0$ Hz, $J_2 = 11.3$ Hz, 1H), 3.70 (dd, $J_1 = 16.0$ Hz, $J_2 = 5.0$ Hz, 1H), 3.63−3.50 (m, 2H), 2.59 (dd, $J_1 = 15.0$ Hz, $J_2 = 8.7$ Hz, 1H), 2.50 (dd, $J_1 = 15.0$ Hz, $J_2 = 4.0$ Hz, 1H), 2.43 (dd, $J_1 = 8.3$ Hz, $J_2 = 5.7$ Hz, 2H), 2.00−1.48 (m, 12H), 1.34 (d, $J = 6.3$ Hz, 3H), 1.30−1.25 (m, 26H), 0.88 (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$ with CD_3OD) δ 174.96, 170.93, 169.05, 168.44, 167.35, 159.11, 133.94, 128.34, 118.96, 116.50, 109.85, 69.33, 69.07, 67.76, 52.33, 52.18, 51.05, 47.15, 42.13, 32.16, 31.67, 30.96, 30.83, 29.44, 29.31, 29.26, 29.19, 29.10, 27.28, 25.65, 25.42, 24.53, 22.41, 22.00, 19.40, 13.75; HRFABMS *m/z* calcd for $C_{42}H_{68}N_5O_{10}$ 802.4966, found 802.4927. Anal. Calcd for $C_{42}H_{67}N_5O_{10}$ C, 62.90; H, 8.42; N, 8.73. Found: C, 62.70; H, 8.26; N, 8.66.

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