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Syntheses of mycobactin analogs as potent and selective inhibitors of *Mycobacterium tuberculosis*[†]

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Three analogs of mycobactin T, the siderophore secreted by *Mycobacterium tuberculosis* (*Mtb*) were synthesized and screened for their antibiotic activity against *Mtb* H_{37} Rv and a broad panel of Grampositive and Gram-negative bacteria. The synthetic mycobactins were potent (MIC₉₀ 0.02–0.88 µM in 7H12 media) and selective *Mtb* inhibitors, with no inhibitory activity observed against any other of the microorganisms tested. The maleimide-containing analog **40** represents a versatile platform for the development of mycobactin-drug conjugates, as well as other applications.

Introduction

Tuberculosis (TB), the disease caused by *Mycobacterium tuberculosis* (*Mtb*), is one of the most critical infections burdening the world. It has been estimated that in 2009 alone, TB-related mortality reached 1.7 million people (1.3 million, being HIV-negative; 0.4 million HIV-positive). Furthermore, about one third of the planet's population is currently infected.¹ The complexity and length of TB treatment has led to poor patient compliance, and, together with the emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains,² the urgency for the development of new antibiotic agents has increased.

We are interested in exploiting the process for iron assimilation by *Mtb* to deliver compounds of interest inside the cell under the so-called "Trojan Horse" approach.³ Almost all organisms require iron, an essential element in many metabolic processes. Ensuring its acquisition is therefore, a high priority for any pathogen during the course of an infectious cycle.⁴ Although highly abundant, ferric iron forms insoluble salts in the presence of oxygen and water, rendering the effective concentration of the metal in aqueous solutions $(10^{-9}-10^{-10} \text{ M}, \text{ pH} = 7)$,⁵ lower than the optimal levels for bacterial growth $(10^{-6}-10^{-7} \text{ M})$. In biological systems, these levels are further decreased $(10^{-15}-10^{-25} \text{ M})$ due to sequestration by molecules such as transferrin and lactoferrin.⁶

The iron uptake system of *Mtb* utilizes two, structurally related, high affinity, Fe^{3+} -chelators: mycobactin T, isolated by Snow in 1965,^{7,8} and the carboxymycobactin T, isolated by



Fig. 1 General structures of the *Mtb* siderophores.⁹

Gobin in $1995^{9,10}$ (Fig. 1). A series of studies revealed the importance of mycobactin T as a growth factor and agent of virulence.¹¹

During the characterization of these molecules, Snow observed that the siderophore produced by one species of mycobacteria had an inhibitory effect on the growth of a different strain if added externally. Demonstrating this hypothesis would prove challenging because of the isolation and complexity of these compounds.

In 1983, our group reported the total synthesis of mycobactin S2 (1, Fig. 2), the first example of an iron-binding analog that was found to be inactive as a growth inhibitor of Mtb.¹² The assembly of this molecule emphasized the importance of the long acyl chain present in the natural mycobacterial siderophores, which appeared to be critical for adequate activity. Although significant work is needed to completely understand

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Fig. 2 Synthetic and structurally-related analogs of mycobactin T.

the process of iron-uptake in Mtb,^{13–18} it is hypothesized that mycobactin T effectively diffuses through the cell membrane, where it is involved in iron acquisition, whether by exchange with the excreted carboxymycobactins^{9,10} or through sequestration from the iron pools within the macrophage.^{13,14} Considering this mode of action, the advantage of a long, hydrophobic acyl substituent, became more evident, which was corroborated with the assembly of mycobactin S (**2**, Fig. 2) in 1997, when the synthetic analog was shown to effectively inhibit (>99%) *Mtb* H₃₇Rv at a concentration of 12.5 µg mL⁻¹.¹⁹

Further structural modification of the mycobactin core was reported in 2007,²⁰ with the substitution of the oxazoline moiety by a catechol group and the syntheses of two analogs (4–5, Fig. 2) that were hypothesized to be effective growth promoters for mycobacteria. The artificial siderophores were tested in a chrome azurol S (CAS) assay²¹ to confirm their iron binding capabilities, and screened for antibacterial activity against a panel of Gram-positive, and Gram-negative bacteria, as well as mycobacteria. While no antibiotic activity was observed, including *Mtb* which was not inhibited at 6.25 μ g mL⁻¹ (*via* the Tuberculosis Antimicrobial Acquisition and Coordinating Facility, www.taacf.org), both analogs had almost identical growth promoting effects as that of the naturally occurring mycobactin J,^{9,13,15} particularly with strains of *M. smegmatis* which were expected to be more receptive towards these siderophores.

In 2008, Fennell reported the syntheses of three analogs of amamistatin B, a natural product isolated from the actinomycete *Nocardia asteroides*, and screened them against MCF-7 and PC-3 human tumor cells. Because of their structural similarity with the mycobactins, these analogs were screened against *Mtb* but only analog **6** (Fig. 2) was shown to have modest inhibitory activity (MIC = 47 μ M),²² perhaps stressing the importance of

structural requirements needed for *Mtb* activity: oxazoline moiety, stereochemistry and substitution near the ester bond, and a long acyl chain.

Our group also reported the synthesis of a mycobactin-artemisinin conjugate in 2011,²³ the first approach towards the development of such Trojan Horse molecules using a mycobacterial siderophore. Because the naturally occurring mycobactins do not possess a site for chemical elaboration, the core structure was modified to include a diaminopropionate spacer, with a N-Boc protected functionality (9, Fig. 2) that allowed derivatization. Interestingly, this precursor, itself, was shown to have potent inhibitory activity against *Mtb* $H_{37}Rv$ (MIC = $<0.2 \ \mu g \ mL^{-1}$). The drug-conjugate (10, Fig. 2) had strong and specific inhibition of *Mtb* H_{37} Rv (MIC = 0.39 µg mL⁻¹), MDR *Mtb* (MIC = $0.16-1.25 \ \mu g \ mL^{-1}$), XDR *Mtb* (MIC = $0.078-0.625 \ \mu g \ mL^{-1}$), and against four strains of *Plasmodium falciparum* (IC₅₀ = $0.004-0.005 \ \mu g \ mL^{-1}$), the causative agent of malaria in humans. Because no inhibitory activity was observed at the highest concentration tested (2 mM) against a broad panel of bacteria, this synthetic analog represents a clear example of the ability to develop disease-specific agents by exploiting the ironuptake as a biological target.

One of the main difficulties in the syntheses of drug-conjugates is the tailoring of the chemistry needed to couple the drug of interest with the siderophore of choice, which can result in extensive exploration and protective group strategies to find the adequate conditions. With the objective of developing a more universal platform towards mycobactin-conjugates, as well as other applications, we decided to synthesize a maleimide-containing analog (40, Fig. 3), while maintaining the rest of the mycobactin T structure unaltered. Herein, we describe the syntheses of new mycobactin analogs suitable for eventual use in preparing novel drug conjugates and report that the new constructs themselves have inherent anti-TB activity.

Results and discussion

ing mycobactin (T) analog 40.

Syntheses of oxazoline components

Commercially available 4-aminosalicylic acid was esterified under acidic conditions to afford intermediate 11^{24} (Scheme 1), which was then refluxed with phthalic anhydride in acetic acid. The phenol group of 12,²⁵ was protected using benzyl bromide and K₂CO₃ to afford fully protected intermediate 13 in 79% yield. Saponification of the methyl ester with KOH also effected ring opening of the phthalimide moiety. Amide 16 was then obtained in 77% yield from a two-step sequence by reacting intermediate 14 with oxalyl chloride (which induced phthalimide ring closure), followed by coupling with L-serine-OBn·HCl 15. Oxazoline cyclization was performed using DAST (diethylaminosulfur trifluoride) to afford the desired intermediate 17 in 91% yield. Removal of the phthalimide protecting group with methylhydrazine, gave the desired aniline **18** in 75% yield.

Aniline 18 was deprotected by hydrogenolysis to afford fragment 20, and because the synthesis of mycobactin 36, *vide infra*, required a re-protection strategy, intermediate 18 was reacted with di-*tert*-butyl dicarbonate to afford 19 in 57% yield, which upon hydrogenolysis gave oxazoline 21 in quantitative yield. Incorporation of the *N*-Boc protecting group at the beginning of the synthetic route proved to be less efficient and problematic.

Synthesis of Cobactin T

As described earlier, intermediate 22^{22} was deprotected using HBr in acetic acid to afford the corresponding salt 23 (Scheme 2), which was then coupled to carboxylic acid 25^{26} to afford cobactin T 26^{27} in 62% yield.

Mycobactin assembly

Protected linear lysine based hydroxamate 27,²³ was hydrolyzed using LiOH to afford carboxylic acid **28** (Scheme 3) in quantitative yield. EDC·HCl-mediated coupling of this intermediate with cobactin **26** provided the right-hand fragment of the mycobactin T core in 88% yield. Deprotection with HBr in acetic acid afforded the corresponding salt **30**, which was then coupled with oxazoline **20** to provide *O*-Bn protected intermediate **31** in 42% yield, while coupling of **30** with oxazoline **21** provided **32** in 52% yield.

After the appropriate conditions for the removal of the *O*-Bn protecting groups were determined, intermediate **31** was subjected to hydrogenolysis in THF under atmospheric pressure to afford **33** (Scheme 3), which was immediately re-protected, to facilitate purification, using acetic anhydride to afford the desired









Scheme 3 Elaboration of mycobactin analogs 34, 36 and 40.

mycobactin analog 34 in 70% yield. The acetylation of the poorly nucleophilic aniline prompted the synthesis of *N*-Boc-protected oxazoline 21, which was elaborated to intermediate 32. After hydrogenolysis and acetylation, the desired mycobactin 36 was isolated in 87% yield.

Maleimide-containing mycobactin

Treatment of **36** with TFA cleanly removed the *N*-Boc group to give **37** (Scheme 3). Maleimide-containing carboxylic acid **38** was prepared according to the literature,²⁸ and reacted with oxalyl chloride to afford the corresponding acid chloride **39**, which was then used to acylate **37**. After purification, the desired mycobactin analog **40** (Fig. 3) was obtained in 89% yield.

Biological activity

Based on our previous synthetic efforts related to the syntheses of mycobactin analogs, we can conclude that other than the fully elaborated, *O*-Bn deprotected siderophores, the hydrophobic esters of the oxazoline component possess moderate growth inhibitory activity against *Mtb*.³⁰ Therefore, oxazoline intermediates **17**, **18**, and **19** were tested against *Mtb* H₃₇Rv (Table 1); however, only minimal activity was observed with analog **19** (7H12: MIC = 23.57 μM; GAS: MIC = 5.81 μM). Mycobactin analogs **34** and **36** showed potent inhibitory activity (7H12: MIC = 0.02–0.09 μM), while **40** displayed potent but slightly decreased activity (7H12: MIC = 0.88 μM). Antibiotic activity against non-replicating *Mtb*, was determined through the Low-Oxygen-Recovery Assay (LORA)³¹ where the mycobactin analogs were found to be inactive (MIC > 50 μM, Table S2, ESI†). Interestingly, the synthetic siderophores **34** (IC₅₀ = 21.50 μM) and **36** (IC₅₀ = 22.37 μM) displayed greater cytotoxicity against Vero cells³² than the maleimide analog **40** (IC₅₀ = >50 μM).

These compounds demonstrated little to no inhibition of a broad panel of Gram-positive and Gram-negative bacteria (see Table S1, ESI \dagger).^{33,34} The fact that limited antibiotic activity can be observed for mycobactin **40** (diameters of growth inhibition 13–18 mm), might be attributed to the maleimide-linker, a Michael-acceptor, though as indicated above, the same

Table 1	Antibacterial	activity	of comp	ounds	in th	ne Micro	oplate	Alamar
Blue Assa	$(MABA)^{29}$		-				-	

	MIC_{90} (µM) determined in:			
Compound	7H12 ^{<i>a</i>}	GAS^b		
17	>128 ^c	>128		
18	$>128^{\circ}$	60.97		
19	23.57	5.81		
34	0.09	0.43		
36	0.02	2.88		
40	0.88	1.02		

^{*a*} 7H12 = 7H9 broth base media with BSA, casein hydrolysate, catalase, palmitic acid. ^{*b*} GAS = glycerol-alanine-salts media. ^{*c*} GAST = iron-deficient GAS media with added Tween 80.¹¹ Values reported are the average of three individual measurements.

maleimide derivative was not cytotoxic. It is also important to highlight that when testing the fully elaborated mycobactins, a significant level of precipitation was observed during the assay, probably due to hydrophobicity of these compounds, an effect also observed when performing chrome azurol S (CAS) assays, to determine the metal-binding properties of the siderophores.²⁰

Conclusions

We have synthesized and tested three mycobactin (T) analogs while pursuing the development of maleimide-containing mycobactin 40. This molecule represents a versatile platform for the elaboration of siderophore-drug conjugates via a thiol-maleimide reactive system. The proposed approach can simplify the synthetic route by reducing the use of protecting groups and providing a more convergent sequence. In comparison with the assembly of mycobactin analog 9, used in the synthesis of artemisinin conjugate 10^{23} we have minimized the changes in the structural identity of mycobactin T, which might be of importance for biological recognition. The coupling with antibiotics or other molecules of interest through a unique strategy may allow rapid access to conjugates, while the potentially reversible thiol addition and the use of different thiol linkers could facilitate drug delivery. Our synthetic siderophores were demonstrated to be potent and selective inhibitors against *Mtb* $H_{37}Rv$ (MIC₉₀ = 0.02–0.88 μ M in 7H12 media, MIC₉₀ = 0.43–2.88 μ M in GAS media), while displaying no inhibitory activity against a broad panel of bacteria, underlining the complexities of the iron-uptake system in Mycobacterium tuberculosis. While these mycobactins might have an inhibitory effect by interfering with the iron trafficking of Mtb, much is still to be learned about the exact process of iron delivery within the cell, and the role of the structural differences between the mycobactin analogs (natural and synthetic) and the observed biological activity. Use of the maleimide derivative for drug and bioconjugation studies will be reported in due course.

Experimental

N-Phthalimide-protected aminosalicylate, 12²⁵

A suspension of 5.01 g (29.9 mmol) of methyl ester 11,²⁴ and 5.55 g (37.5 mmol) of phthalic anhydride in 62 mL of glacial

acetic acid was refluxed at 118 °C (bath temperature) for 22 h under argon. The resulting solution was allowed to cool to room temperature and poured over ice. Upon thawing, the newly formed precipitate was filtered, air dried and rinsed with cold (0 °C) diethyl ether (20 mL), air dried again and purified by silica gel chromatography using CH₂Cl₂ as the eluent (TLC, $R_f = 0.29$) to afford 7.79 g (87%) of **12** as white crystals: mp 208 – 210 °C; ¹H NMR (600 MHz, CDCl₃) δ 10.89 (s, 1H), 8.00–7.96 (m, 3H), 7.83 (dd, J = 5.6, 2.9 Hz, 2H), 7.19 (d, J = 1.8 Hz, 1H), 7.07 (dd, J = 8.5, 2.1 Hz, 1H), 3.99 (s, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 170.2, 166.8, 162.2, 138.3, 134.9, 131.7, 130.7, 124.2, 117.0, 115.3, 111.8, 52.7; HRMS (ESI) m/z [M + Na]⁺: calcd for C₁₆H₁₁NNaO₅⁺, 320.0529; found, 320.0558; HRMS (ESI) m/z [M + H]⁺: calcd for C₁₆H₁₂NO₅⁺, 298.0710; found, 298.0722.

Benzyl ether, 13

To a suspension of 5 g (16.8 mmol) of phenol 12, in 44 mL of anhydrous DMF under argon, was added 5.87 g (42.4 mmol) of K₂CO₃ in one portion, followed by 5.40 mL (45.1 mmol) of benzyl bromide. The resulting mixture was stirred under argon for 24 h, before being concentrated under vacuum. The residue was dissolved in CH_2Cl_2 (30 mL) and washed with H_2O (30 mL). The aqueous layer was further extracted with CH₂Cl₂ $(4 \times 15 \text{ mL})$. The organic extracts were combined and washed with brine (2 \times 50 mL), dried over Na₂SO₄, filtered, concentrated and the residue was re-crystallized from CH2Cl2/hexanes to afford 5.19 g (79%) of 13 as a crystalline, off-white solid: ¹H NMR (600 MHz, CDCl₃) δ 8.01–7.96 (m, 3H), 7.83 (dd, J = 5.4, 3.1 Hz, 2H), 7.54-7.51 (m, 2H), 7.43-7.39 (m, 2H), 7.35–7.31 (m, 1H), 7.26 (d, J = 1.8 Hz, 1H), 7.20 (dd, J = 8.4, 1.9 Hz, 1H), 5.22 (s, 2H), 3.93 (s, 3H); ¹³C NMR (150 MHz, CDCl₃) *b* 167.0, 166.2, 158.8, 136.5, 136.5, 134.9, 132.6, 131.7, 128.8, 128.1, 127.1, 124.1, 119.9, 118.1, 111.7, 71.0, 52.3; HRMS (ESI) m/z [M + Na]⁺: calcd for C₂₃H₁₇NNaO₅⁺, 410.0999; found, 410.0987.

Dicarboxylic acid, 14

To a suspension of 0.98 g (2.53 mmol) of methyl ester 13 in 25 mL of MeOH was added 0.92 g (16.4 mmol) of anhydrous KOH in one portion, followed by 1 mL of H₂O. The mixture was allowed to stir at room temperature. After 18.5 h, the reaction was judged complete by TLC, dicarboxylic acid $R_{\rm f} = 0.28$ $(1:9, MeOH/CH_2Cl_2)$. The solution was concentrated under vacuum to afford a colorless oil that was dissolved in 25 mL of H_2O , and acidified to pH = 2 with 1 M HCl. The precipitate that formed was separated by filtration and used without further purification: ¹H NMR (600 MHz, CD₃OD) δ 8.04 (dd, J = 7.9, 0.9 Hz, 1H), 7.86 (d, J = 8.5 Hz, 1H), 7.81 (d, J = 1.8 Hz, 1H), 7.69–7.65 (m, 1H), 7.59 (td, J = 7.6, 1.2 Hz, 1H), 7.56–7.53 (m, 3H), 7.40–7.36 (m, 2H), 7.33–7.29 (m, 1H), 7.21 (dd, J = 8.5, 2.1 Hz, 1H), 5.25 (s, 2H); $^{13}\mathrm{C}$ NMR (150 MHz, CD₃OD) δ 171.6, 169.2, 169.2, 160.6, 145.9, 140.3, 138.0, 134.1, 133.5, 131.6, 131.0, 130.6, 129.7, 129.2, 128.9, 128.7, 116.5, 113.1, 106.4, 72.0; HRMS (ESI) m/z [M + Na]⁺: calcd for C₂₂H₁₇NNaO₆⁺, 414.0948; found, 414.0959.

Salicylserine derivative, 16

To a suspension of 1.13 g (2.89 mmol) of combined lots of dicarboxylic acid 14 in 23 mL of anhydrous CH₂Cl₂ under argon was added 1 mL (11.5 mmol) of oxalyl chloride, (COCl)₂ slowly by syringe, followed by 4 drops of anhydrous DMF. The reaction mixture bubbled profusely. The mixture was stirred under argon for 3.5 h until judged complete by TLC, N-phthalimide acid chloride $R_{\rm f} = 0.23$ (1 : 1, EtOAc/hexanes). The solution was concentrated under vacuum and sequentially coevaporated with toluene $(3 \times 10 \text{ mL})$ and CHCl₃ $(3 \times 10 \text{ mL})$ to afford an orange solid. The resulting acid chloride was dissolved in 23 mL of anhydrous CH₂Cl₂ under argon and cooled to -78 °C using a dry ice/acetone bath. Then 1.21 mL (6.95 mmol) of anhydrous DIPEA was added slowly, followed by 0.71 g (3.06 mmol) of L-serine-OBn·HCl 15, in one portion. The mixture was allowed to warm to room temperature and stir under argon overnight. After being judged complete by TLC, N-phthalimide amide $R_{\rm f} = 0.31$ (1:1, ethyl acetate/hexanes), the crude mixture was diluted with 30 mL of CH₂Cl₂ and poured over 50 mL of H₂O, the layers were separated, and the organic extract was washed with H₂O (40 mL), 10% (w/v) citric acid (3 \times 40 mL), H₂O (40 mL), satd. NaHCO₃ (3 × 40 mL), H₂O (40 mL), and brine (3 \times 40 mL), dried over Na₂SO₄, filtered and concentrated. The residue was purified by silica gel chromatography using a gradient from 35:65:2 to 100:0:2 of EtOAc/ hexanes/isopropyl alcohol as the eluent to afford 1.23 g (77% over two steps) of 16 as a white, flaky solid: mp 170 - 171 °C; ¹H NMR (600 MHz, CDCl₃) δ 8.75 (d, J = 7.3 Hz, 1H), 8.33 (d, J = 8.5 Hz, 1H), 7.98 (dd, J = 5.4, 3.1 Hz, 2H), 7.83 (dd, J =5.3, 2.9 Hz, 2H), 7.50-7.46 (m, 2H), 7.41-7.28 (m, 10H), 5.27-5.12 (m, 4H), 4.91-4.88 (m, 1H), 3.97-3.88 (m, 2H), 2.08 (t, J = 6.2 Hz, 1H); ¹³C NMR (150 MHz, CDCl₃) δ 170.3, 167.0, 165.0, 157.3, 136.2, 135.5, 135.3, 134.9, 133.1, 131.7, 129.1, 129.0, 128.8, 128.6, 128.6, 128.3, 124.2, 120.6, 119.1, 110.8, 72.0, 67.5, 63.8, 55.6; HRMS (ESI) m/z [M + Na]⁺: calcd for C₃₂H₂₆N₂NaO₇⁺, 573.1632; found, 573.1628; HRMS (ESI) m/z [M + H]⁺: calcd for C₃₂H₂₇N₂O₇⁺, 551.1813; found, 551.1784.

N-Phthalimide-protected oxazoline, 17

A solution of 3.03 g (5.5 mmol) of combined lots of 16 in 110 mL of anhydrous CH2Cl2 under argon was cooled to -78 °C in a dry-ice/acetone bath. Then 0.84 mL (6.4 mmol) of diethylaminosulfur trifluoride (DAST) was added slowly and the reaction was allowed to proceed under argon for 5 h, while being monitored every 60 min by TLC. After the reaction was judged complete by TLC $R_f = 0.38$ (1:1, EtOAc/hexanes), 2.05 g (14.8 mmol) of anhydrous K₂CO₃ were added to the reaction in one portion and the mixture was allowed to warm to room temperature. The solution was poured over saturated aqueous NaHCO₃ (80 mL) and the layers were separated. The aqueous layer was then extracted with CH_2Cl_2 (2 × 60 mL) and the organic layers were combined and washed with saturated NaHCO₃ (2 × 60 mL), H₂O (2 × 60 mL), brine (3 × 60 mL), dried over Na₂SO₄, filtered and concentrated to afford an offwhite solid that was re-crystallized from CH2Cl2/EtOAc to afford 2.67 g (91%) of 17 as a white cotton-like solid: ¹H NMR

(600 MHz, CDCl₃) δ 7.99–7.95 (m, 3H), 7.82 (dd, J = 5.4, 3.1 Hz, 2H), 7.54–7.52 (m, 2H), 7.42–7.28 (m, 8H), 7.24 (d, J = 1.8 Hz, 1H), 7.21–7.19 (m, 1H), 5.32–5.20 (m, 4H), 5.04 (dd, J = 10.6, 7.9 Hz, 1H), 4.70–4.66 (m, 1H), 4.59 (dd, J = 10.6, 8.5 Hz, 1H); ¹³C NMR (150 MHz, CDCl₃) δ 171.2, 167.0, 165.2, 158.3, 136.6, 135.8, 135.7, 134.9, 132.3, 131.7, 128.8, 128.7, 128.6, 128.5, 127.9, 127.1, 124.1, 118.4, 116.7, 111.8, 71.1, 69.5, 69.1, 67.4; HRMS (ESI) m/z [M + H]⁺: calcd for C₃₂H₂₅N₂O₆⁺, 533.1707; found, 533.1708.

4-Amino oxazoline, 18

A solution of 1.63 g (3.1 mmol) of N-phthalimide protected oxazoline 17 in a 60 mL of anhydrous THF was cooled to 0 °C (ice-H₂O bath) for 15 min, then 0.18 mL (3.4 mmol) of methylhydrazine was added dropwise and the solution was allowed to warm to room temperature. After 3 h, the reaction was judged incomplete by TLC, aniline $R_f = 0.54$ (EtOAc), so it was again cooled to 0 °C and 0.05 mL (0.75 mmol) of methylhydrazine was added slowly. The reaction was allowed to warm to room temperature and stirred for 2 h until completion. The solvent was evaporated and CHCl₃ was added to precipitated phthalimide by-product, which was then removed by filtration. The filtrate was concentrated and the residue was purified by silica gel chromatography with 2:98 isopropyl alcohol/CH₂Cl₂ as the eluent to afford 0.93 g (75%) of 18 as a light-tan oil: ¹H NMR (600 MHz, CDCl₃) δ 7.69 (d, J = 8.5 Hz, 1H), 7.53–7.50 (m, 2H), 7.41-7.26 (m, 8H), 6.26-6.22 (m, 2H), 5.30-5.19 (m, 2H), 5.15–5.09 (m, 2H), 4.97 (dd, J = 10.6, 7.9 Hz, 1H), 4.62–4.57 (m, 1H), 4.51 (dd, J = 10.6, 8.5 Hz, 1H), 3.98 (br. s., 2H); ¹³C NMR (150 MHz, CDCl₃) δ 171.7 165.9, 159.6, 151.2, 137.2, 135.8, 133.4, 128.7, 128.6, 128.5, 128.5, 127.6, 126.8, 107.4, 106.8, 100.1, 70.6, 68.9, 68.9, 67.2; HRMS (ESI) m/z $[M + Na]^+$: calcd for $C_{24}H_{22}N_2NaO_4^+$, 425.1472; found, 425.1493. HRMS (ESI) $m/z [M + H]^+$: calcd for $C_{24}H_{23}N_2O_4^+$, 403.1652; found, 403.1667.

N-Boc-Protected oxazoline, 19

To a solution of 0.937 g (2.32 mmol) of 4-amino oxazoline 18 in 10 mL of anhydrous THF under argon, was slowly added a premixed solution of 1.45 g (6.61 mmol) of di-tert-butyl dicarbonate in 10 mL of anhydrous CH₃CN. The reaction was stirred at 60 °C for 68 h while periodically monitored by TLC. N-Bocprotected oxazoline $R_{\rm f} = 0.28$ (1 : 1, ethyl EtOAc/hexanes). The reaction mixture was concentrated and purified by silica gel chromatography using 4:6 ethyl EtOAc/hexanes to afford 0.664 g (57%) of **19** as an off-white foam/residue: ¹H NMR (600 MHz, CDCl₃) δ 7.77 (d, J = 8.5 Hz, 1H), 7.56–7.53 (m, 2H), 7.42 (br. s., 1H), 7.40–7.26 (m, 8H), 6.78 (dd, J = 8.5, 2.1 Hz, 1H), 6.69 (s, 1H), 5.32–5.17 (m, 4H), 4.99 (dd, J = 10.4, 8.1 Hz, 1H), 4.63 (dd, J = 8.5, 7.9 Hz, 1H), 4.54 (dd, J = 10.6, 8.5 Hz, 1H), 1.53 (s, 9H); $^{13}\mathrm{C}$ NMR (150 MHz, CDCl₃) δ 171.5, 165.5, 159.0, 152.4, 143.0, 137.0, 135.7, 132.6, 128.8, 128.6, 128.5, 128.5, 127.7, 127.1, 111.4, 110.1, 103.3, 81.3, 70.7, 69.2, 69.0, 67.4, 28.5; HRMS (ESI) m/z [M + H]⁺: calcd for C₂₉H₃₁N₂O₆⁺, 503.2177; found, 503.2165.

4-Amino oxazoline acid, 20

To a solution of 0.084 g (0.21 mmol) of 4-amino oxazoline 18 in 5 mL of anhydrous THF under argon, was added 41.4 mg of (10% wt) Pd/C (0.04 mmol) in one portion. The reaction flask was evacuated and purged with H₂ gas four times before allowing the reaction to proceed at room temperature for 6 h, at which time it was judged complete by TLC analysis, oxazoline acid $R_{\rm f} = 0.10$ (EtOAc). The mixture was purged with argon, filtered through a Whatman 0.2 µm, Puradisc 25 PP filter, attached to a 10 mL syringe. The filtered catalyst was rinsed with THF (8 mL), followed by CHCl₃ (8 mL), and the combined solution was concentrated to afford 20 in quantitative yield, as a light yellow-colored solid: ¹H NMR (600 MHz, DMSO-d₆) δ 7.25 (d, J = 8.5 Hz, 1H), 6.11 (dd, J = 8.5, 2.1 Hz, 1H), 6.05 (d, J = 2.1 Hz, 1H), 5.88 (br. s., 2H), 4.85 (dd, J = 10.0, 7.0, 1H), 4.54–4.45 (m, 2H); ¹³C NMR (150 MHz, DMSO-d₆) δ 172.3, 166.6, 160.9, 154.4, 128.9, 106.0, 98.9, 97.7, 68.7, 66.4; HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{10}H_{11}N_2O_4^+$, 223.0713; found, 223.0730.

N-Boc-Protected oxazoline acid, 21

To a solution of 0.643 g (1.28 mmol) of N-Boc oxazoline 19 in 28 mL of anhydrous THF under argon, was added 0.257 g of (10% wt) Pd/C (0.24 mmol) in one portion. The system was evacuated and purged with H2 gas four times before allowing the reaction to proceed at room temperature for 21 h, when it was judged complete by TLC analysis, oxazoline acid $R_{\rm f} = 0.10$ (1:1, EtOAc/hexanes). The mixture was evacuated and purged with argon, then filtered through a celite pad with 70 mL of THF. The filtrate was concentrated to afford 21 in quantitative yield, as a brown solid with the appearance of a dried film which was used without further purification: ¹H NMR (600 MHz, DMSO-d₆) δ 9.68 (s, 1H), 7.49 (d, J = 8.5 Hz, 1H), 7.17 (d, J =1.8 Hz, 1H), 7.04 (dd, J = 8.7, 1.9 Hz, 1H), 4.93 (dd, J = 10.0, 7.3 Hz, 1H), 4.62–4.52 (m, 2H), 1.48 (s, 9H); ¹³C NMR (150 MHz, DMSO-d₆) δ 172.0, 165.9, 159.9, 152.4, 144.8, 128.5, 109.1, 104.5, 103.8, 79.7, 69.2, 66.8, 28.0; HRMS (ESI) m/z $[M + H]^+$: calcd for C₁₅H₁₉N₂O₆⁺, 323.1238; found, 323.1250.

(R)-3-Hydroxybutanoic acid, 25²⁶

A solution of 0.453 g (3.56 mmol) of sodium (*R*)-3-hydroxybutanoate **24** in 4 mL of H₂O was acidified with 1 M HCl by dropwise addition until pH = 1 (pH paper). The mixture was partitioned between 15 mL of (5 : 95, isopropyl alcohol/EtOAc) and 20 mL of H₂O. The aqueous layer was extracted with 5 : 95, isopropyl alcohol/EtOAc (5 × 15 mL). The organic extracts were combined, washed with brine (1 × 25 mL), dried over Na₂SO₄, filtered and concentrated to afford 0.256 g (69%) of **25** as a colorless oil: ¹H NMR (600 MHz, CDCl₃) δ 4.27–4.21 (m, 1H), 2.56–2.46 (m, 2H), 1.25 (d, *J* = 6.5 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 177.5, 64.6, 42.7, 22.5.

Cobactin T, 26²⁷

To a suspension of 0.145 g (1.39 mmol) of (R)-3-hydroxybutanoic acid 25, a solution of 0.395 g (1.25 mmol) of *O*-BnProtected caprolactam, HBr salt 23,²² and 0.188 g (1.39 mmol) of HOBt in 12 mL of anhydrous CH₃CN under argon and 0.24 mL (1.38 mmol) of anhydrous DIPEA were added dropwise, followed by addition of 0.293 g (1.52 mmol) of EDC·HCl in one portion. The reaction was allowed to proceed for 25 h and monitored by TLC, cobactin T $R_{\rm f}$ = 0.16 (EtOAc, CAM stain). Then the reaction was partitioned between 25 mL of EtOAc and 25 mL of H₂O. The aqueous layer was extracted with EtOAc $(4 \times 15 \text{ mL})$, the organic extracts were combined, washed with brine (2 \times 30 mL), dried over Na₂SO₄, filtered, concentrated and purified by silica gel chromatography with EtOAc as the eluent to afford 0.249 g (62%) of 26 as a white solid: ¹H NMR (600 MHz, CDCl₃) δ 7.45–7.36 (m, 5H), 7.02 (d, J = 6.2 Hz, 1H), 5.00 (d, J = 10.3 Hz, 1H), 4.91 (d, J = 10.3 Hz, 1H), 4.50 (ddd, J = 11.4, 6.5, 1.8 Hz, 1H), 4.24–4.15 (m, 1H), 3.89 (d, J = 2.9 Hz, 1H), 3.62 (dd, J = 16.1, 11.4 Hz, 1H), 3.53-3.48 (m, 1H), 2.44 (dd, J = 15.3, 2.6 Hz, 1H), 2.33 (dd, J = 15.3, 9.1 Hz, 1H), 2.04–1.98 (m, 1H), 1.96–1.90 (m, 1H), 1.77–1.64 (m, 2H), 1.54–1.37 (m, 2H), 1.24 (d, J = 6.2 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 171.8, 170.2, 135.2, 129.9, 129.2, 128.9, 77.1, 65.0, 53.0, 51.9, 43.9, 31.6, 27.8, 26.4, 22.8; HRMS (ESI) m/z [M + Na]⁺: calcd for C₁₇H₂₄N₂NaO₄⁺, 343.1628; found, 343.1619. HRMS (ESI) m/z [M + H]⁺: calcd for C₁₇H₂₅N₂O₄⁺, 321.1809; found, 321.1801.

N-Cbz-Protected acid, 28

To a solution of 0.115 g (0.18 mmol) of N-Cbz-protected methyl ester 27,²³ in 4 mL of THF, was added a premixed solution of 14.5 mg (0.60 mmol) of anhydrous LiOH in 4 mL of H₂O and the mixture was allowed to stir at room temperature. After 2 h, the reaction was judged complete by TLC analysis, carboxylic acid $R_{\rm f} = 0.25$ (EtOAc, CAM stain). The solution was acidified to pH < 2 (pH paper) using 1 M HCl and partitioned between 20 mL of EtOAc and 20 mL of H₂O. The aqueous layer was extracted with EtOAc (3 \times 15 mL). The organic extracts were combined, washed with brine $(2 \times 25 \text{ mL})$, dried over Na₂SO₄, filtered and concentrated to afford 28 in quantitative yield, as a colorless oil: ¹H NMR (600 MHz, CDCl₃) δ 7.42–7.28 (m, 10H), 5.60 (d, J = 7.9 Hz, 1H), 5.16–5.07 (m, 2H), 4.83–4.76 (m, 2H), 4.40–4.33 (m, 1H), 3.77-3.54 (m, 2H), 2.38 (t, J =7.5 Hz, 2H), 1.94-1.19 (m, 32H), 0.89 (t, J = 7.0 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 175.9, 175.4, 156.5, 136.5, 134.5, 129.3, 129.2, 129.0, 128.7, 128.3, 128.2, 76.6, 67.2, 53.8, 44.8, 32.5, 32.1, 31.7, 29.9, 29.9, 29.9, 29.9, 29.8, 29.6, 29.6, 29.6, 26.5, 24.8, 22.9, 22.2, 14.3; HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{37}H_{57}N_2O_6^+$, 625.4211; found, 625.4205.

N-Cbz-Protected mycobactin T fragment, 29

To a solution of 0.420 g (0.67 mmol) of combined lots of **28** and 0.202 g (0.63 mmol) of cobactin T, **26**, in 3 mL of anhydrous CH₂Cl₂ under argon, was added 78.8 mg (0.65 mmol) of DMAP in one portion, followed by 0.640 g (3.34 mmol) of EDC·HCl. The reaction was stirred at room temperature for 24 h and monitored by TLC, mycobactin fragment $R_f = 0.47$ (EtOAc, CAM stain). Since it was observed that a significant amount of **26** was still present, 0.236 g (1.23 mmol) of EDC·HCl was added in one portion and the mixture was stirred at room temperature under argon for an additional 24 h. The crude mixture was partitioned between 50 mL of EtOAc and 50 mL of H₂O. The aqueous layer was extracted with EtOAc (3×20 mL). The organic extracts were combined, washed with brine $(2 \times 40 \text{ mL})$, dried over Na₂SO₄, filtered, concentrated and purified by silica gel chromatography with EtOAc as eluent to afford 0.514 g (88%) of 29 as a colorless, opaque oil: ¹H NMR (600 MHz, CDCl₃) δ 7.46–7.28 (m, 15H), 7.01 (d, J = 6.2 Hz, 1H), 5.64 (d, J = 8.2 Hz, 1H), 5.33 (td, J = 12.1, 6.0 Hz, 1H), 5.13–5.03 (m, 2H), 4.98-4.94 (m, 1H), 4.89-4.83 (m, 1H), 4.79 (s, 2H), 4.45 (ddd, J = 11.3, 6.5, 1.9 Hz, 1H), 4.32 (td, J = 7.7, 5.4 Hz, 1H),3.68–3.43 (m, 4H), 2.56–2.44 (m, 2H), 2.37 (t, J = 7.3 Hz, 2H), 1.99–1.20 (m, 41H), 0.89 (t, J = 7.0 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 171.6 170.3, 168.4, 156.3, 136.6, 135.2, 129.9, 129.8, 129.3, 129.1, 128.9, 128.8, 128.7, 128.7, 128.3, 128.3, 128.3, 77.0, 76.6, 69.3, 67.0, 54.2, 52.9, 51.9, 51.9, 42.8, 32.6, 32.1, 32.1, 31.7, 29.9, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 27.8, 27.7, 26.7, 26.4, 24.8, 22.9, 22.6, 19.8, 14.3; HRMS (ESI) m/z [M + Na]⁺: calcd for C₅₄H₇₈N₄NaO₉⁺, 949.5661; found, 949.5641. HRMS (ESI) m/z [M + H]⁺: calcd for C₅₄H₇₉N₄O₉⁺, 927.5842; found, 927.5833.

4-Amino, O-benzyl-protected mycobactin T, 31

Intermediate deprotection: A solution of 45.1 mg (0.05 mmol) of N-Cbz-protected mycobactin T fragment 29, in 5.5 mL of anhydrous CH₂Cl₂ under argon, was cooled to 0 °C (ice-H₂O bath) for 15 min. Then 1.5 mL of HBr/CH₃CO₂H (33% wt) were added slowly and, after 15 min, the yellow-colored solution was allowed to warm to room temperature. After 1.5 h, the reaction was judged complete by TLC, HBr salt $R_{\rm f} = 0.02$ (EtOAc, CAM stain). The crude mixture was concentrated, co-evaporated with CHCl₃ (2 \times 5 mL), toluene (2 \times 5 mL), and CHCl₃ (2 \times 5 mL) to afford 35.4 mg (83%) of the corresponding HBr salt 30 as an orange residue that was used without further characterization. Mycobactin assembly: To a mixture of 39.3 mg (0.04 mmol) of intermediate 30, 15.0 mg (0.07 mmol) of 4-amino oxazoline acid 20, and 11.6 mg (0.09 mmol) of HOBt in 3 mL of anhydrous CH2Cl2 under argon and 0.013 mL (0.07 mmol) of anhydrous DIPEA were added dropwise, followed by 19.2 mg (0.10 mmol) of EDC·HCl in one portion. The homogeneous solution was stirred at room temperature under argon for 18 h and monitored by TLC, mycobactin 31 $R_{\rm f} = 0.30$ (EtOAc, CAM stain). The crude mixture was partitioned between 25 mL of EtOAc and 30 mL of H₂O. The aqueous layer was extracted with EtOAc (3 \times 15 mL). The organic extracts were combined, washed with brine $(2 \times 40 \text{ mL})$, dried over Na₂SO₄, filtered, concentrated and purified by silica gel chromatography with EtOAc as eluent to afford 19.0 mg (42%) of **31** as a colorless oil: ¹H NMR (600 MHz, CDCl₃) δ 11.55 (br. s., 1H), 7.48–7.33 (m, 11H), 7.00 (dd, J = 11.0, 7.2 Hz, 2H), 6.26 (d, J = 2.3 Hz, 1H), 6.18 (dd, J = 8.4, 2.2 Hz, 1H), 5.39–5.33 (m, 1H), 4.99 (d, J = 10.3 Hz, 1H), 4.91–4.83 (m, 2H), 4.76 (s, 2H), 4.61–4.49 (m, 3H), 4.46 (ddd, J = 11.2, 6.5, 1.9 Hz, 1H), 4.05 (s, 2H), 3.66–3.45 (m, 4H), 2.59 (dd, J = 14.7, 7.3 Hz, 1H), 2.52–2.47 (m, 1H), 2.33 (t, J = 7.3 Hz, 2H), 1.96–1.19 (m, 41H), 0.89 (t, J = 7.0 Hz, 3H); ¹³C NMR

(150 MHz, CDCl₃) δ 171.1, 170.9, 170.3, 168.4, 168.1, 161.8, 152.4, 135.3, 130.2, 129.8, 129.3, 129.1, 129.1, 128.9, 128.8, 106.8, 101.3, 101.0, 77.0, 76.5, 69.7, 69.6, 68.0, 52.9, 52.4, 51.9, 42.6, 32.6, 32.1, 32.1, 31.6, 29.9, 29.9, 29.9, 29.8, 29.7, 29.7, 29.6, 27.8, 26.7, 26.4, 24.8, 22.9, 22.7, 20.0, 14.4; HRMS (ESI) *m*/*z* [M + H]⁺: calcd for C₅₆H₈₁N₆O₁₀⁺, 997.6009; found, 997.6006.

N-Boc, O-Benzyl-protected mycobactin T, 32

A solution of 95.8 mg (0.11 mmol) of 30 in 5 mL of anhydrous CH₂Cl₂ under argon was cooled to 0 °C (ice-H₂O bath) and stirred for 15 min. Then 0.028 mL (0.16 mmol) of anhydrous DIPEA was added dropwise. To the stirring mixture, a premixed solution of 35.0 mg (0.11 mmol) of N-Boc oxazoline acid 21 in 1 mL of anhydrous THF was added slowly, followed by 15.4 mg (0.11 mmol) of HOBt and 21.0 mg (0.11 mmol) of EDC·HCl in one portion. The homogeneous solution was stirred at room temperature under argon for 48 h and monitored by TLC, mycobactin 32 $R_{\rm f} = 0.38$ (EtOAc, CAM stain). Since it was determined that a significant amount of the of intermediate 30 was still present, the mixture was cooled to 0 °C, and 0.01 mL (0.06 mmol) of anhydrous DIPEA was added, followed by 18.0 mg (0.06 mmol) of N-Boc oxazoline acid 21, and 10.0 mg (0.05 mmol) of EDC·HCl in one portion. The homogeneous solution was stirred at room temperature, under argon for 24 h and was then partitioned between 25 mL of EtOAc and 30 mL of H₂O. The aqueous layer was extracted with EtOAc (3 \times 15 mL). The organic extracts were combined, washed with brine $(2 \times 40 \text{ mL})$, dried over Na₂SO₄, filtered, concentrated and purified by silica gel chromatography with EtOAc as the eluent to afford 62.3 mg (52%) of 32, as a colorless, opaque residue: ¹H NMR (600 MHz, CDCl₃) δ 11.49 (br. s., 1H), 7.58 (d, J = 8.5 Hz, 1H), 7.43-7.32 (m, 10H), 7.05-6.97 (m, 4H), 6.69 (s, 1H), 5.39–5.32 (m, 1H), 4.98 (d, J = 10.6 Hz, 1H), 4.93–4.87 (m, 2H), 4.75 (s, 2H), 4.62–4.56 (m, 2H), 4.53 (td, J = 7.6, 5.6 Hz, 1H), 4.46 (ddd, J = 11.3, 6.5, 1.9 Hz, 1H), 3.67–3.52 (m, 3H), 3.48 (dd, J = 15.8, 5.0 Hz, 1H), 2.58 (dd, J = 14.8, 7.2 Hz, 1H), 2.49 (dd, J = 14.7, 5.3 Hz, 1H), 2.33 (t, J = 7.5 Hz, 2H), 2.00–1.21 (m, 50H), 0.88 (t, J = 7.0 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 171.0, 170.7, 170.3, 168.4, 167.6, 161.0, 152.2, 144.1, 135.3, 135.2, 129.8, 129.8, 129.6, 129.3, 129.1, 129.0, 128.9, 128.8, 109.3, 105.5, 105.1, 81.3, 76.5, 69.6, 69.6, 68.1, 52.9, 52.4, 51.9, 42.6, 32.6, 32.1, 31.9, 31.6, 29.9, 29.9, 29.9, 29.7, 29.7, 29.6, 29.6, 29.6, 28.5, 27.7, 26.6, 26.4, 24.8, 22.9, 22.7, 20.0, 14.3; HRMS (ESI) m/z [M + H]⁺: calcd for $C_{61}H_{89}N_6O_{12}^+$, 1097.6533; found, 1097.6516.

Acetylated 4-amino mycobactin T, 34

Mycobactin deprotection: To remove traces of iron, all the glassware involved was washed with 6 M HCl, rinsed thoroughly with deionized H₂O, until pH > 6 (pH paper), washed with acetone and oven-dried. To a mixture of 7.7 mg (0.008 mmol) of **31** in 5 mL of anhydrous THF under argon, was added 3.2 mg of (10% wt) Pd/C (0.003 mmol) in one portion. The reaction flask was evacuated and purged with H₂ gas four times before allowing the reaction to proceed at room temperature for 24 h. The mixture was evacuated and purged with argon, then filtered

through a Whatman 0.2 µm, Puradisc 25 PP filter, attached to a 10 mL syringe. The filtered catalyst was rinsed with CHCl₃ (3 \times 10 mL), and the combined crude filtrate was concentrated to afford mycobactin 33 as a white, opaque residue that was used without further characterization. Acetvlation: To a suspension of the above intermediate 33 in 3 mL of anhydrous CH₃CN under argon, was added 0.13 mL (1.2 mmol) of distilled acetic anhydride dropwise. The resulting suspension was allowed to stir under argon for 50 h. The reaction was monitored by LCMS and after 24 h, a mixture of tri and tetra-acetylated product was observed. The crude mixture was then concentrated, co-evaporated with $CHCl_3$ (5 × 3 mL) and purified over iron-free silica gel³⁵ with 1:9, isopropyl alcohol/EtOAc as eluent, to afford 5.3 mg (70% over two steps) of the acetylated mycobactin 34 as a light, pink-colored residue: ¹H NMR (600 MHz, CDCl₃) δ 7.93 (d, J = 8.5 Hz, 1H), 7.59–7.51 (m, 2H), 7.33 (d, J =7.9 Hz, 1H), 7.03 (d, J = 7.9 Hz, 1H), 6.93 (d, J = 6.5 Hz, 1H), 5.35 (dq, J = 12.5, 6.3 Hz, 1H), 4.85 (dd, J = 10.9, 7.6 Hz, 1H), 4.60 (dd, J = 10.4, 6.3 Hz, 1H), 4.54 (dd, J = 10.9, 8.8 Hz, 1H), 4.51–4.46 (m, 2H), 3.97 (dd, J = 16.1, 12.0 Hz, 1H), 3.71–3.50 (m, 3H), 2.58 (dd, J = 14.7, 6.7 Hz, 1H), 2.48 (dd, J = 14.7, 5.6 Hz, 1H), 2.41 (s, 3H), 2.25-2.12 (m, 9H), 2.02 (m, 2H), 1.86–1.20 (m, 41H), 0.89 (t, J = 7.0 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 171.9, 171.2, 170.5, 168.6, 168.4, 167.7, 163.0, 151.1, 142.5, 131.8, 116.4, 115.3, 114.4, 69.8, 69.6, 69.5, 53.5, 52.1, 42.7, 32.1, 32.1, 31.6, 29.9, 29.9, 29.9, 29.9, 29.7, 29.6, 29.6, 29.5, 27.9, 26.3, 25.0, 22.9, 22.6, 21.7, 19.9, 18.7, 18.3, 14.4; HRMS (ESI) m/z [M + H]⁺: calcd for C₅₀H₇₇N₆O₁₄⁺, 985.5492; found, 985.5502.

N-Boc-Protected, acetylated mycobactin T, 36

Mycobactin deprotection: To remove traces of iron, all the glassware involved was washed with 6 M HCl, rinsed thoroughly with deionized H_2O , until pH > 6 (pH paper), washed with acetone and oven-dried. To a mixture of 62.3 mg (0.056 mmol) of 32 in 11 mL of anhydrous THF under argon, was added 36.2 mg of (10% wt) Pd/C (0.034 mmol) in one portion. The reaction flask was evacuated and purged with H₂ gas four times before allowing the reaction to proceed at room temperature for 30 h. The reaction flask was evacuated and purged with argon. The reaction mixture was filtered through a Whatman 0.2 µm, Puradisc 25 PP filter, attached to a 10 mL syringe. The filtered catalyst was rinsed with CHCl₃ (3×10 mL), and the combined solution was concentrated to afford mycobactin 35 as a light, tan-colored residue that was used without further characterization. Acetylation: To a solution of 35 in 1 mL of anhydrous THF under argon, was added 1 mL of anhydrous CH₃CN, followed by dropwise addition of 1 mL (10.6 mmol) of distilled acetic anhydride. The homogeneous solution was stirred for 48 h under argon. The reaction mixture was then concentrated, coevaporated with $CHCl_3$ (8 × 3 mL) and purified over iron-free silica gel³⁵ with 2:8, CH₂Cl₂/EtOAc as the eluent to afford 51.5 mg (87%) of 36 as a light-pink residue: ¹H NMR (600 MHz, CDCl₃) δ 7.89 (d, J = 8.8 Hz, 1H), 7.44 (br. s., 1H), 7.15 (dd, J = 8.8, 2.1 Hz, 1H), 7.03 (d, J = 7.9 Hz, 1H), 6.94 (d, J = 6.5 Hz, 1H), 6.73 (s, 1H), 5.35 (dq, J = 12.5, 6.3 Hz, 1H), 4.84 (dd, J = 11.0, 7.5 Hz, 1H), 4.60 (dd, J = 10.3, 6.5 Hz, 1H),

4.54 (dd, J = 11.0, 8.7 Hz, 1H), 4.51–4.43 (m, 2H), 3.97 (dd, J = 16.1, 12.3 Hz, 1H), 3.69–3.52 (m, 3H), 2.58 (dd, J = 14.7, 7.0 Hz, 1H), 2.48 (dd, J = 14.7, 5.6 Hz, 1H), 2.40 (s, 3H), 2.21 (s, 3H), 2.15 (s, 3H), 2.07–1.97 (m, 2H), 1.87–1.20 (m, 50H), 0.88 (t, J = 7.0 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 172.0, 171.3, 170.5, 168.4, 167.7, 163.1, 152.1, 151.3, 143.2, 131.8, 115.2, 114.1, 113.0, 81.8, 69.7, 69.6, 69.4, 53.5, 52.1, 52.1, 42.7, 32.1, 32.1, 31.6, 29.9, 29.9, 29.9, 29.9, 29.9, 29.7, 29.6, 29.6, 29.5, 28.5, 28.4, 27.9, 26.3, 22.9, 22.6, 21.7, 19.9, 18.7, 18.3, 14.4; HRMS (ESI) *m*/*z* [M + H]⁺: calcd for C₅₃H₈₃N₆O₁₅⁺, 1043.5911; found, 1043.5918.

N-Maleimide acetylated mycobactin T derivative, 40

N-Boc Deprotection: To a solution of 10.0 mg (0.01 mmol) of intermediate 36 in 3 mL of anhydrous CH₂Cl₂ under argon, was added 1 mL of TFA slowly. After 5 min, the argon inlet was removed and the reaction was allowed to proceed at room temperature for 2 h until judged complete by TLC, 37 $R_{\rm f} = 0.28$ (1:9, isopropyl alcohol/EtOAc). The crude mixture was concentrated and co-evaporated with $CHCl_3$ (5 × 3 mL) to afford a vellow residue that was used without further characterization. Acid chloride preparation: To a solution of 32.5 mg (0.16 mmol) of maleimide-containing acid 38,²⁸ in 3 mL of anhydrous CH₂Cl₂ under argon, was added 0.032 mL (0.37 mmol) of oxalyl chloride, (COCl)₂ slowly followed by 0.002 mL (0.03 mmol) of anhydrous DMF. The reaction was allowed to proceed for 4 h until judged complete by TLC, 39 $R_{\rm f} = 0.21$ (1 : 1, EtOAc/hexanes, KMnO₄ stain), the mixture was then concentrated, co-evaporated with toluene $(3 \times 5 \text{ mL})$, and $CHCl_3$ (3 × 5 mL) to afford a yellow residue that was used immediately without further characterization. Acylation: A solution of (0.01 mmol) of intermediate 37 in 2 mL of anhydrous CH₂Cl₂ under argon, was cooled to -78 °C (dry-ice/acetone bath) and stirred for 15 min. Then 0.06 mL of anhydrous DIPEA (0.34 mmol) was added dropwise, followed by (0.16 mmol) of a premixed solution of intermediate 39 in 1.5 mL of anhydrous CH₂Cl₂. After 30 min, the mixture was allowed to warm to room temperature where it was stirred under argon for 16 h. The crude reaction mixture was then partitioned between EtOAc (20 mL) and H_2O (20 mL). The organic layer was washed with brine (4 \times 15 mL), dried over Na₂SO₄, filtered, concentrated and the residue was purified by iron-free silica gel³⁵ chromatography, TLC, 40 $R_{\rm f} = 0.11$ (EtOAc, CAM stain, plate developed twice) with 1:9, isopropyl alcohol/EtOAc as the eluent to afford 9.6 mg (89%) of a light-pink oil: ¹H NMR (600 MHz, CDCl₃) δ 7.92 (d, J = 8.8 Hz, 1H), 7.78 (br. s., 1H), 7.60 (br. s., 1H), 7.38 (d, J = 8.2 Hz, 1H), 7.04 (d, J = 7.9 Hz, 1H), 6.96 (d, J =6.5 Hz, 1H), 6.71 (d, J = 15.8 Hz, 2H), 5.34 (dq, J = 12.5, 6.4 Hz, 1H), 4.85 (dd, J = 11.0, 7.5 Hz, 1H), 4.60 (dd, J = 10.9, 5.9 Hz, 1H), 4.54 (dd, J = 11.0, 8.7 Hz, 1H), 4.51–4.45 (m, 2H), 3.97 (dd, J = 15.8, 11.7 Hz, 1H), 3.69-3.52 (m, 5H), 2.58 (dd, J = 14.7, 6.7 Hz, 1H), 2.48 (dd, J = 14.7, 5.6 Hz, 1H), 2.45-2.36 (m, 5H), 2.35-2.33 (m, 1H), 2.25-2.11 (m, 7H), 2.02 (m, 2H), 1.85-1.19 (m, 43H), 0.88 (t, J = 7.0 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 176.6, 171.9, 171.3, 171.2, 171.2, 171.2, 171.0, 170.5, 168.5, 167.7, 163.1, 151.1, 142.7, 134.4, 134.4, 134.3, 131.8, 116.4, 115.1, 114.4, 69.8, 69.5, 69.5,

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53.5, 52.1, 42.7, 37.5, 36.9, 36.8, 33.2, 32.1, 32.1, 31.6, 29.9, 29.9, 29.9, 29.9, 29.7, 29.6, 29.6, 29.5, 28.1, 27.9, 26.3, 22.9, 22.6, 22.6, 22.0, 21.7, 19.9, 19.8, 18.7, 18.3, 14.3; HRMS (ESI) $m/z \ [M + H]^+$: calcd for $C_{57}H_{84}N_7O_{16}^+$, 1122.5969; found, 1122.5959.

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