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Total Synthesis and Biological Evaluation of Transvalencin Z

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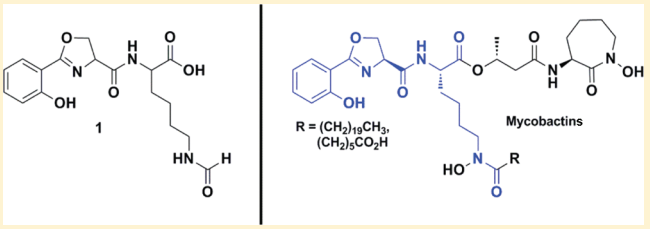
Supporting Information

ABSTRACT: The emerging global epidemic of drug-resistant tuberculosis has created an urgent need to identify novel therapeutic approaches for disease treatment. Transvalencin Z (**1**) is a natural product from *Nocardia transvalensis* with relatively potent and selective antimycobacterial activity against *Mycobacterium smegmatis*, making it an attractive target for structure−activity and mechanism of action studies. The total synthesis of the four possible diastereomers of transvalencin Z was completed (**1a**−**d**), and the absolute configurations were defined using chemical synthesis, HPLC retention times, and optical rotation measurements. Surprisingly, none of the transvalencin Z diastereomers exhibited any inhibitory activity against a panel of microbial pathogens, including several species of mycobacteria.



Tuberculosis (TB), a contagious pulmonary infection from the slow growing bacillus *Mycobacterium tuberculosis* (*Mtb*), is the leading cause of mortality globally due to bacterial infectious disease.[1] One-third of the world's population is estimated to be infected with latent *Mtb*, an asymptomatic infection that may be triggered to an active infection through stress on the immune system. A predicted 5−10% of these individuals will develop the active disease in their lifetime.[2] The current therapy recommended by the World Health Organization (WHO), termed DOTS (directly observed treatment short-course), involves a minimum of 6−9 months on a combination drug therapy of isoniazid, rifampin, ethambutol, and pyrazinamide, resulting in a global average cure rate of 85%.[2] This therapy employs antitubercular agents that were developed over 4 decades ago and has a limited ability to treat latent *Mtb*. Co-infection with HIV is especially concerning, as it can trigger latent TB into active infection. The emergence of multidrug-resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) has continued to limit the number of patients cured during DOTS treatment.[2,3] Recently, new totally drug resistant TB (TDR-TB) strains with resistance to all second-line therapeutics have been isolated in small patient populations in both Iran and India.[4,5] New, more effective antitubercular agents that operate via novel mechanisms of action are highly desirable to increase the TB cure rate, decrease the duration of treatment, target latent infection, and increase activity against drug-resistant strains.

Transvalencin Z (**1**) was isolated by Mukai and co-workers from a clinical strain of *Nocardia transvalensis* (IFM 10065) and was found to exhibit potent and specific inhibition of *Mycobacterium smegmatis* (MIC, minimum inhibitory concentration that results in >99% reduction of observable growth, of 0.125 μg/mL or 0.344 μM) and other acid-fast *Nocardia* spp., with no activity against Gram-negative bacilli, fungi, or mammalian cell lines.[6] Compound **1** also shows striking structural similarity to the mycobactin siderophores produced by *Mtb* (Figure 1).

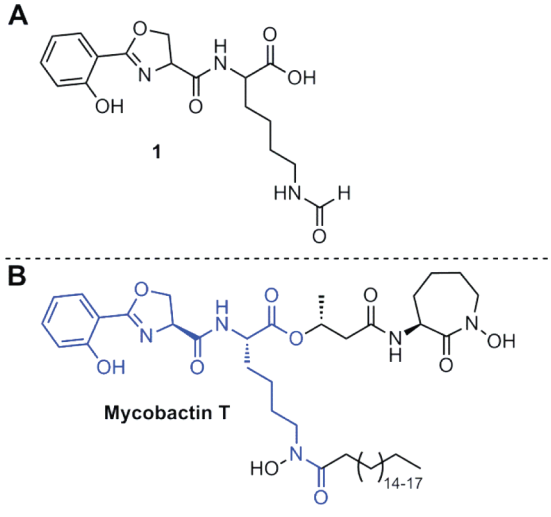


Figure 1. (A) Reported structure of transvalencin Z (**1**).[6] (B) Structure of mycobactin-T.[7]

Siderophores are small molecule iron (Fe^{3+}) chelators produced by many bacterial species.[8] Bacterial pathogens synthesize, secrete, and reimport siderophores to scavenge essential iron in limiting environments such as a human host. The biosynthetic pathway for mycobactin synthesis in *Mtb* is essential for growth and virulence in iron-limiting conditions,

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making it an attractive drug target.[9] The structural similarity of **1** to mycobactins suggests that it may interfere with the mycobactin-mediated iron-scavenging process based on the hypothesis proposed by Snow that mycobactin analogues may antagonize mycobacterial growth.[10,11] Indeed, Miller and co-workers have shown that mycobactin S, produced by *M. smegmatis*, which differs by only one stereocenter from mycobactin T, inhibits *Mtb* growth, while a mycobactin analogue wherein the beta-hydroxybutyrate was replaced with a 2,3-diaminopropionate moiety exhibited exceptionally potent antimycobacterial activity.[12,13] More recently, Miller and co-workers demonstrated that small organic molecules derived from the phenyloxazoline headgroup of the mycobactins display antimycobacterial activity, although the activity is likely unrelated to antagonism of iron acquisition.[12] Quadri and colleagues discovered a series of small molecules, which incorporate a 2-hydroxyphenyl moiety and thus bear some resemblance to the mycobactins, that elicit iron-dependent antimycobacterial activity.[13,14]

Herein we report our complete synthesis and attempt to define the stereochemistry of **1**, as well as our investigation into its antimycobacterial activity. We began by designing a total synthesis of the four possible diastereomers (**1a–d**) since the relative and absolute configurations of the chiral centers at C-2 and C-9 were not reported (Figure 2). All transvalencin Z

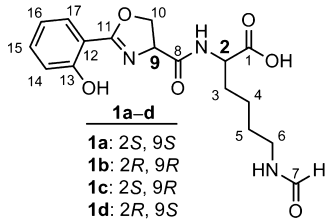


Figure 2. Diastereomers of transvalencin Z.

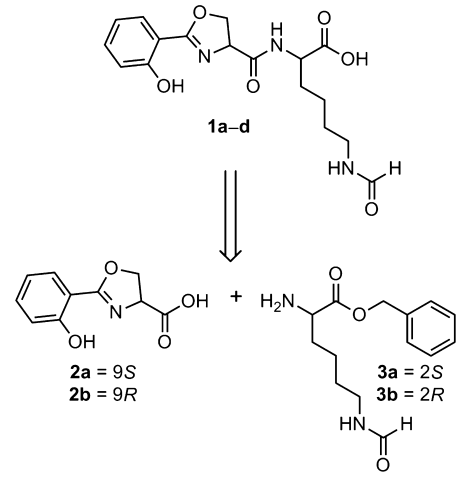
diastereomers were tested against a panel of microbial pathogens to compare the spectrum of activity of the synthetic products with that reported for the natural product.

RESULTS AND DISCUSSION

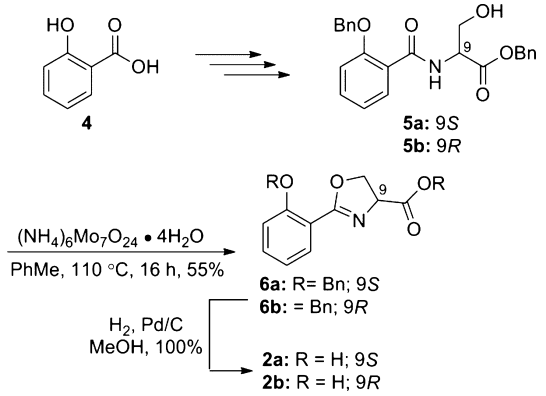
Chemistry. Retrosynthetically, we envisioned the diastereomers of transvalencin Z could be accessed through two major building blocks: a 2-hydroxyphenyl oxazoline acid **2** and a ε-formyl lysine **3**, as shown in Scheme 1. The acid **2** is a common intermediate in previously published syntheses of mycobactins and their analogues.[12−14]

The synthesis of building block **2** began with the benzylation of salicylic acid **4**,[15] followed by peptide coupling with L- or D-serine benzyl esters as previously described by Miller and co-workers to yield the serine adducts **5** in 90% average yield (Scheme 2).[12] Oxazoline cyclization utilizing the recently reported molybdenum catalyst, ammonium molybdenum tetrahydrate, afforded the benzyl protected **6**.[16] The molybdenum catalyst coordinates the amide carbonyl, thereby electrophilically activating it for intramolecular attack by the serine hydroxyl nucleophile. This biomimetic dehydrative cyclization contrasts the previous methods to effect this cyclization through electrophilic activation of the serine hydroxyl group and nucleophilic displacement by the amide carbonyl.[17,18] Global deprotection of **6** by hydrogenolysis provided acids **2**. Deprotection of the phenol ether was required for the

Scheme 1. Retrosynthetic Plan for 1a–d



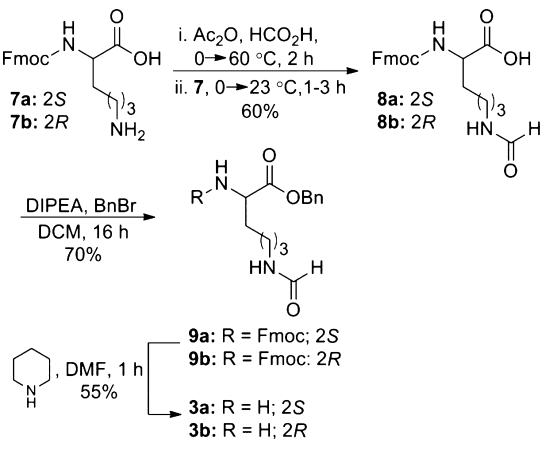
Scheme 2. Synthesis of Building Block 2



penultimate peptide coupling reaction, a phenomenon that has been documented in multiple siderophore syntheses.[13,17,19]

Formylation of N^{α}-Fmoc-L- or -D-lysine **7** was performed using the mixed anhydride method reported by Hughes and Waters to give acid **8** (Scheme 3).[20,21] Elaboration of **8** to the fully protected lysine conjugate **9** was possible using a number of common conditions for benzyl ester formation; however, conditions involving direct alkylation (DIPEA, BnBr) were ultimately chosen to prevent possible racemization.[20,22] Fmoc

Scheme 3. Synthesis of Building Block 3



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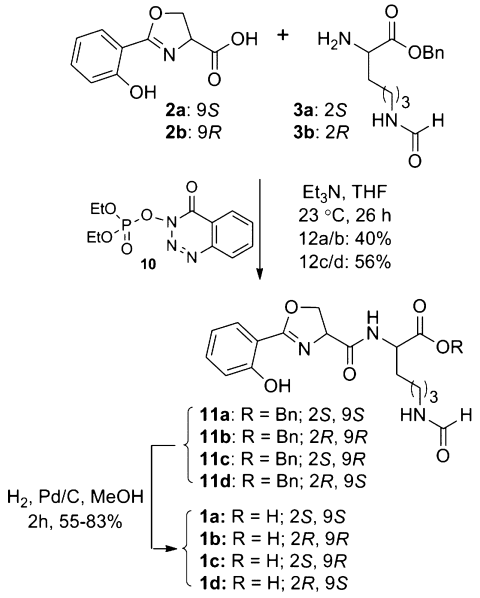
deprotection of **9** using excess piperidine afforded the free amine **3**.

Peptide coupling of acid **2a** and lysine **3a** with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) resulted in epimerization at position 9 to afford **11a** and **11c** in a 4.2:1 ratio, as determined by HPLC. Therefore, the penultimate step was performed using 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT), **10**, a reagent known for minimizing racemization at sensitive stereocenters (Scheme 4).[23] Hydrogenolysis of **11a** provided free acid **1a**. The remaining diastereomeric products **1b**–**d** were prepared in an analogous fashion to those described for **1a**.

Scheme 4. Peptide Coupling to Complete Synthesis of 1a–d



Microbiology. Biological evaluation of **1a**–**d** was initially performed against *M. smegmatis* MC24517. The minimum inhibitory concentration (MIC_{99}) was greater than 100 μM for all four diastereomers (Table 2, complete data Table S1). We then tested the diastereomers against the identical *M. smegmatis* strain (ATCC 607) employed by Mukai and co-workers, but again observed no activity. The four compounds were also tested against a panel of 10 additional pathogens (*Enterococcus faecalis, Staphylococcus aureus, Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans, Mycobacterium bovis* BCG, and *Mycobacterium tuberculosis*), and **1a**–**d** were inactive ($MIC_{99} \geq$ 100 μM, $\geq$ 32 μg/mL) against all strains evaluated. Five of these pathogens from our screen (*M. smegmatis, S. aureus, B. subtilis, E. coli*, and *C. albicans*) were also screened by Mukai and co-workers,[6] who reported MIC values of 0.125, 1, 16, >64, and >164 μg/mL respectively, which is inconsistent with our data (Table 2).

Discussion. Transvalencins **1a**–**d** were isolated as amorphous, white solids. Spectral data including ^{1}H and ^{13}C NMR, UV–vis, and IR absorbance were similar to those reported by Mukai and co-workers.[6] Absolute configuration can be assigned on the basis of our chiral starting materials and knowledge of the chemistry used to synthesize **1a**–**d**. The methods were chosen to reduce racemization of stereocenters in all steps. As discussed above, diastereomers of compounds **11a**–**d** and **1a**–**d** could be identified by distinct retention times on reversed-phase HPLC. We also observed nearly equal and opposite optical rotations for each set of enantiomers, giving further support to their purity. As shown in Table 1, most of the ^{1}H NMR chemical shifts for **1a**–**d** match those reported for transvalencin Z. The C-2, 2-NH, and 6-NH protons show significant differences in chemical shift for **1a**–**d** compared to the natural product. While the shift at the C-2 chiral center is significant and concerning, amide protons can be highly variable due to pH, sample concentration, and temperature of the solution, so the 2-NH and 6-NH shifts may not be diagnostic.[24] Additional NMR analysis utilizing homonuclear 2D *J* resolution spectroscopy on a 700 MHz instrument clarified the coupling constants for the C-2 proton; however the C-3 diastereotopic protons were still too complicated to resolve clearly. The optical rotation values for **1a**–**d** also do not match the reported value (+15.3°) for transvalencin Z;[6] the sets of enantiomers **1a**,**b** (+29.2°/−31.6°) and **1c**,**d** (−2.0°/+6.0°) are nearly equidistant from this value.

Analysis of the literature describing natural products produced by *Nocardia* sp. and other structurally related natural products (e.g., siderophores such as mycobactins from *M. tuberculosis*, acinetobactin from *Acinetobacter baumannii*, and structurally related amistatins, brasilibactin, formobactin, nocardichelins, and nocobactin NA from *Nocardia* sp.) does not show any trend or preference for stereochemistry. Several studies of salicyl and oxazoline/thiazoline motifs show this stereocenter (C-9 in transvalencin Z) to be *S*, resulting from naturally occurring L-serine.[17,18,25,26] However, other natural products, such as brasilibactin from *Nocardia brasiliensis*, show an *R* stereocenter, suggesting the incorporation of an epimerase in the biosynthetic machinery or the use of the less common D-serine.[27] Indeed similar natural products such as the amamistatins, formobactin, and nocobactin NA contain the fully saturated oxazole instead of the oxazoline ring system.[28] Biosynthetic feeding studies of natural products containing lysine (C-2 in transvalencin Z) motifs have demonstrated an *S* configuration by incorporation of predominantly L-lysine.[26] However, the only other transvalencin isolated to date is transvalencin A, whose absolute stereochemistry has not yet been identified.[29] These conflicting literature data, along with our lack of correlation between activity and spectral data with the original report, make the stereochemical assignment of transvalencin Z impossible without the authentic isolated sample.

In attempting to further confirm the properties of transvalencin Z, **1a** and its diastereomers were screened against a panel of representative organisms (Table 2). We were particularly interested in expanding the number of mycobacteria strains tested to give a more complete picture of the compound's selectivity displayed in the original screen.[6] In contrast to the potent *M. smegmatis* activity reported for transvalencin Z, none of the four synthetic diastereomers inhibited the growth of several species of mycobacteria, including *M. smegmatis* MC24517 and ATCC607, *M. bovis* BCG, and *M. tuberculosis* $H_{37}R_v$. These results suggest that the original natural product may have been contaminated with a small amount of a highly active compound. Alternatively, the slight discrepancies in the spectroscopic data between the four synthetic diastereomers compared to the natural product suggest that the reported structure of transvalencin Z may be incorrect. While the synthesis of **1a**–**d** has unequivocally produced the above structures, small differences in bond connections may produce this misalignment data, and chemical

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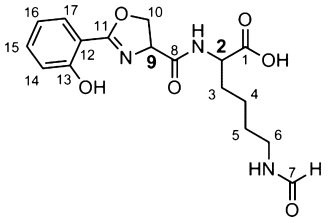
Table 1. ^{1}H NMR Data for Transvalencin Z[6] and Compounds 1a−d in d_6-DMSO at 600 MHz



	δ_H and δ_C (ppm), mult. (J in Hz)					
	transvalencin Z[6]		1a/b		1c/d	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	173.1	11.76, br s	173.3	11.80, br s	173.8	11.81, br s
2	53.8	3.85, q-like (6.0)	52.1	4.18, dt[a] (10.5, 5.6)	51.9	4.17, dt[a] (11.9, 4.9)
2-NH		7.75, d (6.0)		7.87, ovlp m		7.85, ovlp m
3	31.7	1.54, m	30.4	1.61, m	30.8	1.65, m
3′		1.68, m		1.71, m		1.74, m
4	22.5	1.2, m	22.8	1.26, m	22.7	1.31, m
5	28.8	1.32, m	28.5	1.34, m	28.4	1.40, m
6	37.1	2.98, q-like (6.0)	36.8	3.05 m	36.7	3.05, t (6.0)
6-NH		7.94, br t (6.0)		8.50, d (6.6)		8.51, d (7.2)
7	160.7	7.92, s	160.9	7.91, s	160.8	7.98, s
8	168.6		169.7		169.5	
9	67.4	4.99, dd (8.0, 10.0)	67.1	4.98, dd[a] (7.7, 10.5)	67.0	5.01, dd[a] (7.7, 9.8)
10	69.7	4.48, t (8.0)	69.2	4.48, t (7.8)	69.2	4.50, t (7.8)
10′		4.63, dd (8.0, 10.0)		4.60, t (7.8)		4.63, t (8.4)
11	165.9		165.8		165.7	
12	159.1		159.1		158.9	
13	109.8		109.8		109.8	
14	116.5	7.0, br d (8.0)	116.6	6.95, d (8.4)	116.4	7.0, d (8.4)
15	119.1	6.94, br t (7.5)	119.1	6.89, t (7.8)	118.9	6.95, t (7.8)
16	134.0	7.46, ddd (1.5, 7.5, 8.0)	134.0	7.41, t (7.8)	133.9	7.46, t (7.2)
17	128.0	7.63, dd (1.5, 7.5)	128.0	7.59, d (7.8)	127.9	7.64, d (7.8)

[a]Determined by homonuclear 2D J spectroscopy on a Bruker 700 MHz with a 5 mm TXI 700 MHz Z-gradient probe.

derivatization of the freshly isolated natural product may aide in elucidating the true structure.

Table 2. MIC_{99} and MIC_{50} Activity Data for Reported Transvalencin Z (1) and Synthetic 1a−d

strain	reported (1)[6] (μg/mL)	1a−d (μg/mL)
M. smegmatis (ATCC 607)	0.125	>32
M. smegmatis (MC24517)	n.d.[a]	>32
M. tuberculosis ($H_{37}R_v$)[b]	n.d.	>32
M. bovis BCG strain TMC 1011 Pasteur (ATCC 35734)	n.d.	>32
E. coli (ATCC 25922)	>64	>32
A. baumannii (ATCC 19606)	n.d.	>32
K. pneumoniae (ATCC 13883)	n.d.	>32
P. aeruginosa (ATCC 27853)	n.d.	>32
B. subtilis (ATCC 6633)	16	>32
E. faecalis (ATCC 51299)	n.d.	>32
S. aureus (MSSA, clinical IDRL 8545)	n.d.	>32
S. aureus (MRSA, ATCC 43300)	1	>32
S. aureus (MRSA, clinical IDRL 6169)	n.d.	>32
C. albicans (ATCC 10231)	>64	>32
Vero (ATCC CCL-81)	n.d.	>64

[a]n.d.: not determined. [b]Testing performed by Dr. Helena I. Boshoff.

EXPERIMENTAL SECTION

General Experimental Procedures. All commercial reagents were used as provided unless otherwise indicated. An anhydrous solvent dispensing system (J. C. Meyer) using two packed columns of neutral alumina was used for drying THF and CH_2Cl_2, while two packed columns of molecular sieves were used to dry DMF, and the solvents were dispensed under argon. Anhydrous grade MeOH and toluene were purchased from Aldrich. Flash chromatography was performed using Combiflash Companion equipped with flash column silica cartridges with the indicated solvent system. All reactions were performed under an inert atmosphere of dry Ar or N_2 in oven-dried (150 °C) glassware. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 600 MHz spectrometer or a Bruker 700 MHz with a 5 mm TXI 700 MHz Z-Gradient probe. Proton chemical shifts are reported in ppm from an internal standard of residual chloroform (7.26), methanol (3.31), dichloromethane (5.32), or dimethyl sulfoxide (2.50), and carbon chemical shifts are reported using an internal standard of residual chloroform (77.23), methanol (49.15), dichloromethane (54.00), or dimethyl sulfoxide (39.51). Proton chemical data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, ovlp = overlapping), coupling constant, integration. High-resolution mass spectra were obtained on an Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface. Optical rotations were measured on a Rudolph Autopol III polarimeter. Melting points were measured on an electrothermal Mel-Temp manual melting apparatus and are uncorrected. IR spectra were obtained on a Jasco FT/IR-4100. Analytical HPLC was performed on a Phenomenex Gemini 5u C18

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110A (250 × 4.60 mm) column operating at 0.9 mL/min with detection at 254 nm.

Microbiology. *M. smegmatis* (MC24715) was a generous gift of Dr. William R. Jacobs, Jr. of the Howard Hughes Medical Institute and Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY. *S. aureus* (MRSA) clinical IDRL 6169 and *S. aureus* (MSSA) IDRL 8545 were the kind gifts of Dr. Robin Patel, Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, MN; other pathogens were obtained from the ATCC (*A. baumannii* ATCC 19606, *C. albicans* ATCC 10231, *E. faecalis* (VRE) ATCC 51299, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *P. aeruginosa* ATCC 27853, *S. aureus* (MRSA) ATCC 43300, *M. smegmatis* ATCC 607, *M. bovis* BCG strain TMC1011 Pasteur ATCC 35734) and cultured as follows. All bacterial strains were streaked from frozen glycerol stocks onto solid agar plates before being subcultured in liquid medium for assay. Agar plates consisted of the following media plus agar (Difco) to a 1.5% concentration: *E. coli*, *P. aeruginosa*, and all *S. aureus*, trypticase soy; *A. baumannii*, *K. pneumoniae*, *M. bovis* BCG, and all *M. smegmatis* nutrient broth; *E. faecalis*, brain heart infusion; and *C. albicans*, YM broth. All bacterial pathogens were grown at 37 °C, while *C. albicans* was grown at 30 °C. After 24 h incubation, single colonies were picked and grown in the respective broth above to an OD_{600} of 1.0, diluted to an OD_{600} of 0.003, and plated in 96-well sterile plates with 1% DMSO stock solutions of varying concentrations of **1a**–**d**. Plates were read at 24 and 48 h at 600 nm using a plate reader (Molecular Devices Spectramax M5e); plate background (690 nm) was subtracted and values were standardized to the DMSO controls. In initial microbiological testing against *M. smegmatis*, it was observed that the bacteria would often clump together during growth, causing large variations in optical density readings. Addition of the supplemental detergent Tyloxapol prevented clumping of the mycobacterium without the potentially toxic side effects observed when using Tween detergents with mycobacteria.[30] Mukai and co-workers did not report the use of any detergents for *M. smegmatis* growth.

Cytotoxicity. Cytotoxicity of each compound was determined with a standard tetrazolium assay using Vero green monkey kidney cells (ATCC CCL-81).[31,32] All tissue culture reagents were purchased from Gibco, Invitrogen (Carlsbad, CA, USA). Cells were grown in MEM media supplemented with 10% fetal bovine serum (FBS), 1% Penn-Strep, and 1% Glutamax. Cells were seeded at 3.0×10^4 cells per well in a 96-well microtiter plate (Corning) and allowed to adhere overnight. Medium was carefully aspirated and replaced with 195 μL of fresh medium, and compound solutions in DMSO (5 μL) were added to give a final concentration ranging from 100 to 0.16 μM. All concentrations were tested in triplicate. Plates were incubated for 72 h at 37 °C and 4.5% CO_2 in a humidified chamber. The solutions were carefully removed, and RPMI without phenol red containing 1.0 mg/mL 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) was added (200 μL). The mixture was incubated for 3 h, after which the MTT medium was carefully removed. Isopropyl alcohol (200 μL) was added to dissolve the precipitated purple formazan crystals, and the plates were read at 570 nm using a plate reader (Molecular Devices Spectramax M5e); plate background (690 nm) was subtracted and cell viability was estimated as the percentage absorbance relative to the DMSO control. Dose–response curves were generated using GraphPad Prism 5 software and used to determine the MIC_{50} concentrations (minimal concentration that inhibits 50% of growth).

Chemistry. Compounds **5a** and **5b** were prepared according to literature procedures.[12]

*(4S)-Benzyl-2-[2-(benzyloxy)phenyl]-Δ²-1,3-oxazoline-4-carboxylate (**6a**).* To a stirring solution of *N*-[2-(benzyloxy)benzoyl]-L-serine benzyl ester (**5a**) (2.0 g, 4.9 mmol, 1.0 equiv) in toluene (50 mL) was added ammonium molybdate(VI) tetrahydrate ($(NH_4)_6MoO_{24}{\cdot}4H_2O$) (2.4 g, 1.9 mmol, 0.39 equiv), and the solution was refluxed for 18 h using a Dean–Stark trap to remove the generated water. The reaction was concentrated to a dark, green oil and partitioned between EtOAc and 5% aqueous $NaHCO_3$. The organic layer was washed with saturated aqueous NaCl (100 mL), dried ($MgSO_4$), and concentrated to an off-white, amorphous solid. Purification by flash chromatography (linear gradient 0–50% EtOAc–hexanes) on silica gel afforded the title compound (1.00 g, 55%) as an off-white solid: mp 68–71 °C; $[\alpha]_D^{23}$ +79.1 (*c* 1.0, $CHCl_3$); R_f 0.33 (30% EtOAc–hexanes); ^1H NMR, ^{13}C NMR, and HRMS identical to reported values.[12]

*(4R)-Benzyl-2-[2-(benzyloxy)phenyl]-Δ²-1,3-oxazoline-4-carboxylate (**6b**).* Reaction conditions were identical to those for **6a**, except for the use of *N*-[2-(benzyloxy)benzoyl]-D-serine benzyl ester (**5b**): $[\alpha]_D^{23}$ −81.7 (*c* 1.0, $CHCl_3$).

*(4S)-2-[2-Hydroxyphenyl]-Δ²-1,3-oxazoline-4-carboxylic Acid (**2a**).* (4*S*)-Benzyl-2-[2-(benzyloxy)phenyl]-Δ²-1,3-oxazoline-4-carboxylate (**6a**) (0.385 g, 1.00 mmol) was dissolved in anhydrous methanol (10 mL) and added to a Parr flask containing 10% by weight Pd/C (0.039 g) under Ar. The reaction vessel was evacuated, then backfilled with hydrogen gas to 3 atm, and the mixture was shaken at 25 °C for 1 h. The reaction vessel was opened, and the reaction mixture was filtered through Celite. The filtrate was concentrated to a dark red-orange oil (190 mg, 91%) and used directly in the next step without further purification. However, for analytical characterization, purification by flash chromatography (isocratic 50% EtOAc–hexanes with 1% formic acid) afforded the title compound as a clear, colorless oil: $[\alpha]_D^{23}$ +39.2 (*c* 1.0, MeOH); R_f 0.22 (1:1 hexanes–EtOAc with 1% formic acid); ^1H NMR (CD_3OD, 600 MHz) δ 4.54–4.59 (m, 2H), 4.92 (apparent br t, *J* value not discernible due to broadening, 1H), 6.81 (t, *J* = 7.8 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 7.33 (t, *J* = 7.2 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 1H); ^{13}C NMR (CD_3OD, 150 MHz) δ 68.4, 70.7, 111.3, 117.7, 120.1, 129.5, 135.2, 161.1, 168.5, 174.1; HRMS (ESI−) calculated for $C_{10}H_8NO_4$ $[M - H]^-$ 206.0459, found 206.0435 (error 11.6 ppm) and $C_9H_8NO_2$ $[M - CO_2]^-$ 162.0561, found 162.0552 (error 5.6 ppm).

*(4R)-2-[2-Hydroxyphenyl]-Δ²-1,3-oxazoline-4-carboxylic Acid (**2b**).* Reaction conditions were identical to those for **2a**, except for the use of (4*R*)-benzyl-2-[2-(benzyloxy)phenyl]-Δ²-1,3-oxazoline-4-carboxylate (**6b**): $[\alpha]_D^{23}$ −27.5 (*c* 1.0, MeOH).

*(2S)-({[(9H-Fluoren-9-yl)methyloxy]carbonyl}amino)-6-formamidohexanoic Acid (**8a**).* Formic acid (1.5 mL, 39 mmol) was added dropwise to acetic anhydride (4.5 mL, 48 mmol) at 0 °C followed by heating to 60 °C for 2 h. The mixture was cooled to 0 °C, and a slurry of 2-*N*-fluorenylmethyloxycarbonyl-L-lysine (**7a**) (2.5 g, 6.79 mmol) in CH_2Cl_2 (25 mL) was added. The reaction was warmed to 25 °C and stirred for 3 h. The reaction mixture was partitioned between EtOAc (75 mL) and H_2O (75 mL), and the organic layer was washed successively with H_2O (75 mL) and saturated aqueous NaCl (75 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography (linear gradient 0–7% MeOH–CH_2Cl_2 with 1% formic acid) afforded the title compound (1.61 g, 60%) as a foamy, yellow oil: ^1H NMR, ^{13}C NMR, and HRMS identical to reported values.[20]

*(2R)-({[(9H-Fluoren-9-yl)methyloxy]carbonyl}amino)-6-formamidohexanoic Acid (**8b**).* Reaction conditions were identical to those for **8a**, except for the use of 2-*N*-fluorenylmethyloxycarbonyl-D-lysine (**7b**): ^1H NMR, ^{13}C NMR, and HRMS identical to reported values.[20]

*(2S)-Benzyl-2-({[(9H-fluoren-9-yl)methyloxy]carbonyl}amino)-6-formamidohexanoate (**9a**).* To a stirring slurry of 2-(*N*-fluorenylmethyloxycarbonyl)-6-*N*-formyl-L-lysine (**8a**) (1.00 g, 2.52 mmol, 1.0 equiv) in CH_2Cl_2 (25 mL) was added diisopropylethylamine (0.66 mL, 3.78 mmol, 1.5 equiv) followed by benzyl bromide (0.45 mL, 3.78 mmol, 1.5 equiv) to afford a homogeneous clear solution. The reaction was stirred for 20 h at 25 °C, then partitioned between EtOAc (100 mL) and H_2O (100 mL), and the organic layer was washed successively with saturated aqueous $NaHCO_3$ (100 mL) and saturated aqueous NaCl (100 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography (linear gradient 10–100% EtOAc–hexanes) on silica gel provided the title compound (740 mg, 60%) as a yellow oil: $[\alpha]_D^{23}$ −6.2 (*c* 1.0, $CHCl_3$); R_f 0.38 (1:1 CH_2Cl_2–EtOAc with 1% formic acid); ^1H NMR (CD_2Cl_2, 600 MHz) δ 1.17–1.30 (m, 2H), 1.32–1.42 (m, 2H), 1.57–1.63 (m, 1H), 1.71–1.78 (m, 1H), 3.05–3.12 (m, 2H), 4.12 (t, *J* = 6.6 Hz, 1H), 4.22–4.32 (m, 3H), 5.03 (d, *J* = 12.6 Hz, 1H), 5.08 (d, *J* = 12.6 Hz, 1H), 7.19–7.27 (m, 7H), 7.30 (t, *J* = 7.8 Hz, 2H), 7.52 (t, *J* = 6.6 Hz, 2H), 7.67

(d, J = 7.2 Hz, 2H), 7.95 (s, 1H); ^{13}C NMR (CD_2Cl_2, 150 MHz) δ 22.9, 29.4, 32.4, 37.8, 47.8, 54.4, 67.3, 67.5, 120.5, 125.6, 127.6, 128.2, 128.6, 128.8, 129.1, 136.2, 141.8, 144.4, 156.5, 161.6, 172.8; HRMS (APCI+) calcd for $C_{29}H_{31}N_2O_5^+$ $[M + H]^+$ 487.2227, found 487.2228 (error 0.2 ppm).

*(2R)-Benzyl-2-({[(9H-fluoren-9-yl)methyloxy]carbonyl}amino)-6-formamidohexanoate (**9b**).* Reaction conditions were identical to those for **9a**, except for the use of 2-(*N*-fluorenylmethyloxycarbonyl)-6-*N*-formyl-D-lysine (**8b**): $[\alpha]_D^{23}$ +7.9 (c 1.0, $CHCl_3$).

*(2S)-Benzyl-2-amino-6-formamidohexanoate (**3a**).* To a stirring solution of (2*S*)-benzyl-2-[([(9*H*-fluoren-9-yl)methyloxy]carbonyl)-amino]-6-formamidohexanoate (**9a**) (0.600 g, 1.23 mmol, 1.0 equiv) in DMF (12 mL) was added piperidine (0.61 mL, 6.17 mmol, 5.0 equiv), and the mixture stirred for 1 h at 25 °C. The reaction was concentrated by rotary evaporation under high vacuum to remove DMF, and the crude residue was partitioned between H_2O (40 mL) and hexane (40 mL). The hexane layer was discarded, and the aqueous layer was basified to a pH 10 with saturated aqueous $NaHCO_3$, then extracted with 6:1 EtOAc–MeOH (3 × 40 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography (linear gradient 0–10% MeOH–EtOAc) over basic alumina yielded the title compound (140 mg, 50%) as a yellow oil: $[\alpha]_D^{23}$ +3.9 (c 1.0, CH_3OH); R_f 0.13 (10% MeOH–EtOAc with 1% Et_3N); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.31–1.40 (m 2H), 1.46–1.51 (m, 2H), 1.65–1.67 (m, 1H), 1.72–1.77 (m, 1H), 3.17 (t, J = 7.2 Hz, 2H), 3.52 (t, J = 6.6 Hz, 1H), 5.15 (d, J = 12.6 Hz, 1H), 5.21 (d, J = 12.6 Hz, 1H), 7.32–7.39 (m, 5H), 8.00 (s, 1H); ^{13}C NMR (CD_3OD, 150 MHz) δ 23.8, 30.2, 35.0, 38.8, 55.1, 67.9, 129.5, 129.6, 129.7, 137.5, 163.9, 176.1; HRMS (ESI+) calcd for $C_{14}H_{21}N_2O_3$ $[M + H]^+$ 265.1547, found 265.1546 (error 0.4 ppm).

*(2R)-Benzyl-2-amino-6-formamidohexanoate (**3b**).* Reaction conditions were identical to those for **3a**, except for the use of **9b**: $[\alpha]_D^{23}$ −1.2 (c 1.0, CH_3OH).

*(2S,9S)-Benzyl-6-formamido-2-[(2-hydroxyphenyl)-Δ^2-1,3-oxazoline-4-carboxamido]hexanoate (**11a**).* To a stirring solution of (4*S*)-2-[2-hydroxyphenyl]-Δ^2-1,3-oxazoline-4-carboxylic acid (**2a**) (31 mg, 0.15 mmol, 1.0 equiv), (2*S*)-benzyl-2-amino-6-formamidohexanoate (**3a**) (40 mg, 0.15 mmol, 1.0 equiv), and DEPBT (49 mg, 0.16 mmol, 1.06 equiv) in THF (2.5 mL) was added Et_3N (40 μL, 0.30 mmol, 2.0 equiv) at 25 °C. After 48 h, the reaction mixture was concentrated, and the residue was partitioned between EtOAc (50 mL) and H_2O (50 mL). The organic layer was washed with saturated aqueous NaCl (50 mL), dried ($MgSO_4$), and concentrated to a yellow oil. Purification by flash chromatography (linear gradient 0–10% MeOH–CH_2Cl_2) afforded the title compound (16.6 mg, 24%) as an amorphous, colorless solid: $[\alpha]_D^{23}$ +6.0 (c 1.0, CH_3OH); R_f 0.65 (10% MeOH–CH_2Cl_2); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.37–1.42 (m, 2H), 1.47–1.54 (m, 2H), 1.75–1.82 (m, 1H), 1.87–1.93 (m, 1H), 3.16 (t, J = 7.2 Hz, 2H), 4.48 (dd, J = 9.0, 4.8 Hz, 1H), 4.58 (d, J = 9.0 Hz, 2H), 5.00 (t, J = 9.0 Hz, 1H), 5.15 (d, J = 12.6 Hz, 2H), 5.21 (d, J = 12.6 Hz, 1H), 6.90 (t, J = 7.8 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H), 7.30–7.33 (m, 1H), 7.34–7.38 (m, 4H), 7.41 (td, J = 7.2, 1.2 Hz, 1H), 7.67 (d, J = 7.8, 1.2 Hz, 1H), 7.97 (s, 1H); ^{13}C NMR (CD_3OD, 150 MHz) δ 24.2, 29.9, 32.0, 38.7, 54.1, 68.2, 69.3, 70.5, 111.6, 117.8, 120.1, 129.46, 129.52, 129.6, 129.7, 135.2, 137.3, 161.1, 163.9, 168.6, 173.1, 173.3; HRMS (ESI+) calcd for $C_{24}H_{28}N_3O_6$ $[M + H]^+$ 454.1973, found 454.1994 (4.6 ppm error).

*(2R,9R)-Benzyl-6-formamido-2-[(2-hydroxyphenyl)-Δ^2-1,3-oxazoline-4-carboxamido]hexanoate (**11b**).* Reaction conditions were identical to those for **11a**, except for the use of **2b** and **3b**: $[\alpha]_D^{23}$ −6.0 (c 1.0, CH_3OH).

*(2S,9R)-Benzyl-6-formamido-2-[(2-hydroxyphenyl)-Δ^2-1,3-oxazoline-4-carboxamido]hexanoate (**11c**).* Reaction conditions were identical to those for **11a**, except for the use of (4*R*)-2-[2-hydroxyphenyl]-Δ^2-1,3-oxazoline-4-carboxylic acid (**2b**). Purification by flash chromatography (linear gradient 0–10% MeOH–CH_2Cl_2) afforded the title compound (49.9 mg, 58%) as an amorphous, colorless solid: $[\alpha]_D^{23}$ −6.7 (c 1.0, CH_3OH); R_f 0.61 (10% MeOH–CH_2Cl_2); ^{1}H NMR (CD_3OD, 600 MHz) δ 1.38–1.42 (m, 2H), 1.50–1.55 (m, 2H), 1.78–1.82 (m, 1H), 1.89–1.92 (m, 1H), 3.20 (t, J = 7.2 Hz, 2H), 4.85 (dd, J = 9.0, 5.4 Hz, 1H), 4.56–4.64 (m, 2H), 5.01 (dd, J = 10.6, 7.8 Hz, 1H), 5.09 (d, J = 12 Hz, H), 5.17 (d, J = 12 Hz, H), 6.91 (t, J = 7.2 Hz, 1H), 6.98 (d, J = 9.0 Hz, 1H), 7.27–7.32 (m, 5H), 7.42 (t, J = 7.2 Hz, 1H), 7.69 (d, J = 7.8 Hz, 1H), 8.01 (s, 1H); ^{13}C NMR (CD_3OD, 150 MHz) δ 24.2, 29.9, 32.0, 38.7, 54.1, 68.1, 69.3, 70.5, 111.7, 117.8, 120.1, 129.3, 129.4, 129.6, 129.7, 133.6, 135.1, 137.2, 161.0, 163.9, 168.6, 173.1, 173.2; HRMS (ESI+) calcd for $C_{24}H_{28}N_3O_6$ $[M + H]^+$ 454.1973, found 454.2004 (error 6.8 ppm).

*(2R,9S)-Benzyl-6-formamido-2-[(2-hydroxyphenyl)-Δ^2-1,3-oxazoline-4-carboxamido]hexanoate (**11d**).* Reaction conditions were the same as those for **11a** except for the use of **3b**: $[\alpha]_D^{23}$ +10.8 (c 1.0, MeOH).

*(2S,9S)-6-Formamido-2-[(2-hydroxyphenyl)-Δ^2-1,3-oxazoline-4-carboxamido]hexanoic Acid (**1a**).* Solid Pd/C (10% by weight, 1.7 mg) was added to a solution of (2*S*,9*S*)-benzyl-6-formamido-2-([2-hydroxyphenyl]-Δ^2-1,3-oxazoline-4-carboxamido)hexanoate (**11a**) (16.6 mg, 0.037 mmol) in MeOH (10 mL) under Ar. The reaction vessel was evacuated then backfilled with hydrogen gas to 3 atm, and the mixture was shaken at 25 °C for 2 h. The reaction vessel was opened, the reaction mixture was filtered through Celite, and the filtrate was concentrated. Purification by flash chromatography (linear gradient 0–10% EtOH–CH_2Cl_2 plus 1% formic acid) afforded the title compound (13 mg, 100%) as an amorphous, colorless solid. Purity was determined by analytical HPLC using a Phenomenex Gemini 5u C18 110A (250 × 4.6 mm) column operating at 0.9 mL/min and a gradient of 10–100% MeOH–0.05% aqueous formic acid over 20 min followed by 100% MeOH for 5 min. The retention time of the product was 19.4 min, purity >96%: $[\alpha]_D^{23}$ +29.2 (c 0.1, MeOH); R_f 0.59 (10% MeOH–CH_2Cl_2 with 1% formic acid); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.26–1.35 (m, 2H), 1.35–1.44 (m, 2H), 1.64–1.67 (m, 1H), 1.75–1.79 (m, 1H), 3.00–3.10 (m, 2H), 4.16–4.20 (m, 1H), 4.48 (t, J = 7.8 Hz, 1H), 4.60 (t, J = 7.8, 1H), 4.98 (t, J = 7.8 Hz, 1H), 6.89 (t, J = 7.8 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H), 7.41 (t, J = 8.4 Hz, 1H), 7.59 (d, J = 7.8 Hz, 1H), 7.83–7.90 (ovlp m, 1H), 7.91 (ovlp s, 1H), 8.50 (d, J = 6.6 Hz, 1H), 11.80 (br s, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 22.8, 28.5, 30.4, 36.8, 52.1, 67.1, 69.2, 109.8, 116.6, 119.1, 128.0, 134.0, 159.1, 160.9, 165.8, 169.7, 173.3; HRMS (ESI–) calcd for $C_{17}H_{20}N_3O_6^-$ $[M - H]^-$ 362.1358, found 362.1331 (7.5 ppm error).

*(2R,9R)-6-Formamido-2-[(2-hydroxyphenyl)-Δ^2-1,3-oxazoline-4-carboxamido]hexanoic Acid (**1b**).* Reaction conditions were identical to those for **1a**, except for the use of **11b**: $[\alpha]_D^{23}$ −31.6 (c 0.1, MeOH).

*(2S,9R)-6-Formamido-2-[(2-hydroxyphenyl)-Δ^2-1,3-oxazoline-4-carboxamido]hexanoic Acid (**1c**).* Reaction conditions were identical to those for **1a**, except for the use of **11c** (15.5 mg, 0.03 mmol). Purification by flash chromatography (linear gradient 0–10% EtOH–CH_2Cl_2 with 1% formic acid) afforded the title compound (5.8 mg, 53%) as an amorphous solid. Purity was determined by analytical HPLC using a Phenomenex Gemini 5u C18 110A (250 × 4.6 mm) column operating at 0.9 mL/min and a gradient of 10–100% MeOH–0.05% aqueous formic acid over 20 min followed by 100% MeOH for 5 min. The retention time of the product was 18.8 min, purity >97%: $[\alpha]_D^{23}$ −2.0 (c 0.1, MeOH); R_f 0.59 (10% MeOH–CH_2Cl_2 with 1% formic acid); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 1.31–1.34 (m, 2H), 1.37–1.41 (m, 2H), 1.64–1.68 (m, 1H), 1.74–1.78 (m, 1H), 3.05 (t, J = 6.0 Hz, 2H), 4.15–4.21 (m, 1H), 4.50 (t, J = 7.8, 1H), 4.63 (t, J = 8.4 Hz, 1H), 5.01 (t, J = 8.4 Hz, 1H), 6.95 (t, J = 7.8 Hz, 1H), 7.00 (d, J = 8.4 Hz, 1H), 7.46 (t, J = 7.2 Hz, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.85 (ovlp m, 1H), 7.98 (ovlp s, 1H), 8.51 (d, J = 7.2 Hz, 1H), 11.81 (br s, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 23.0, 28.9, 31.0, 37.2, 53.0, 67.5, 69.6, 110.2, 116.8, 119.2, 128.3, 134.2, 159.4, 161.3, 166.3, 169.9, 173.8; HRMS (ESI–) calcd for $C_{17}H_{20}N_3O_6^-$ $[M - H]^-$ 362.1358, found 362.1360 (0.55 ppm error).

*(2R,9S)-6-Formamido-2-[(2-hydroxyphenyl)-Δ^2-1,3-oxazoline-4-carboxamido]hexanoic acid (**1d**).* Reaction conditions were identical to those for **1a**, except for the use of **11d**: $[\alpha]_D^{23}$ +6.0 (c 0.1, MeOH).

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **1a−d**, **2a,b**, **3a,b**, **6a,b**, **8a,b**, **9a,b**, **11a−d**. COSY and HMBC correlations in **1a−d**. Complete data table for bacterial and mammalian cell activity screens. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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