Synthesis and studies of catechol-containing mycobactin S and T analogs

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Received 1st March 2007, Accepted 29th March 2007
First published as an Advance Article on the web 19th April 2007
DOI: 10.1039/b703116e

The syntheses of catechol-containing mycobactin S and T analogs are described. These analogs incorporate a catechol-glycine moiety in place of the phenol-oxazoline of the naturally occurring mycobactins S and T. Studies indicated that the new siderophore analogs bind iron, and promote the growth of a number of microbes, especially strains of mycobacteria, as expected.

Introduction

The mycobactins are a unique class of microbial siderophores found in the cell walls of all species of mycobacteria.[1] The structures and function of these interesting compounds were described in an elegant series of studies by Snow in the 1960s that was summarized in a superb review in 1970.[2] Extended studies revealed that the mycobactins sequester and bind iron(III) with three bidentate ligands: a phenol-oxazoline and two hydroxamic acids. Besides revealing that mycobactins are important growth factors for various strains of mycobacteria, Snow also found that mycobactins from one type of mycobacteria antagonized the growth of others. Over time, the potential for the development of selective antimycobacterial agents based on altered structures of mycobactins became known as "Snow's hypothesis." Demonstrating this hypothesis became even more intriguing with further elucidation of the complex mechanisms employed by mycobacteria for assimilation of iron,[3–5] the determination of details related to biosyntheses of mycobactins[6] and demonstrations of the absolute dependence of mycobacterial growth and virulence on iron acquisition.[5,7]

Tuberculosis (TB) remains the most severe bacterial disease in the world and infects nearly one third of the world's population (over two billion people) in latent form.[8] Active forms of TB are especially prevalent in immune compromised patients and each year millions die. Treatment of infections due to *Mycobacterium tuberculosis*, the causative agent of this dreaded disease, remains complex and new chemotherapeutic approaches are needed. Since *M. tuberculosis* also depends critically on iron assimilation and metabolism for survival and virulence, it expends significant energy on maintaining proper iron balance. Under iron deficient conditions, upregulated gene expression enhances biosynthesis of mycobacterial siderophores and other iron sequestration machinery. Conditions of iron excess also result in induction of down regulatory processes since iron induced free radical processes can be degradative. Thus, the need for careful control of a critical balance of iron may provide an "Achilles heel" for the development of selective new tuberculosis and other antimicrobial agents. At least three methods of utilizing the iron assimilatory processes of mycobacteria for potential therapeutic development are being considered. Recent elegant independent studies by the groups of Aldrich[9] and Quadri[10] have shown that designed inhibitors of early steps of siderophore (mycobactin) biosyntheses by *M. tuberculosis* are potent antiTB agents. Our laboratories and others have reported that drug conjugates of siderophores can lead to the development of microbe-selective "Trojan horse" compounds that utilize microbial iron assimilation processes for active transport of antibiotics into bacteria.[11] Additional studies from our laboratories and others have shown that synthetic analogs of the mycobactins also serve as effective antimycobacterial, and antiTB, agents, presumably by interfering with iron assimilation.[12] For example, we have demonstrated that synthetic mycobactin S, with an (*S*)-methyl group configuration in the butyrate linker region, inhibits the growth of *Mycobacteria tuberculosis* H37Rv while mycobactin T, with an (*R*)-methyl group configuration, promotes growth (Fig. 1)[13] and substitution of the butyrate linker of the natural mycobactins with a α-Boc-protected α,β-diaminopropionate linker results in potent inhibition of the growth of TB.[14] Continuation of the search for compounds capable of influencing essential iron assimilation processes in microbes led us to consider syntheses of additional mycobactin analogs.

Of all the known Fe(III) binding ligands of naturally occurring siderophores, the 2,3-hydroxybenzene (catechol) ligand binds most tightly to Fe(III).[15] Replacement of the natural phenol-oxazoline iron binding component of the mycobactins with a catechol ligand was anticipated to extend structure–activity relationship (SAR) studies of mycobactins and analogs. Herein, we describe the syntheses and biological studies of catechol mycobactin analogs **1** and **2**. The butyrate linker regions of mycobactins S and T were included in the syntheses of the new analogs to probe whether or not the stereochemistry of the methyl group would affect the activity of the analogs as it does with natural mycobactins S and T.

Results and discussion

As shown below for the retrosynthesis of **1** (Scheme 1), the assembly of mycobactins typically proceeds through condensation of the corresponding mycobactic acid and cobactin fragments. These, in turn, consist of (L)-lysine-based hydroxamate iron binding ligands, present in both the linear and cyclic forms, respectively. The importance of ε-*N*-hydroxylysine and δ-*N*-hydroxyornithine derivatives for the syntheses of various siderophores has prompted considerable interest in the development of methods for generation of hydroxylamines and hydroxamic acids from amines

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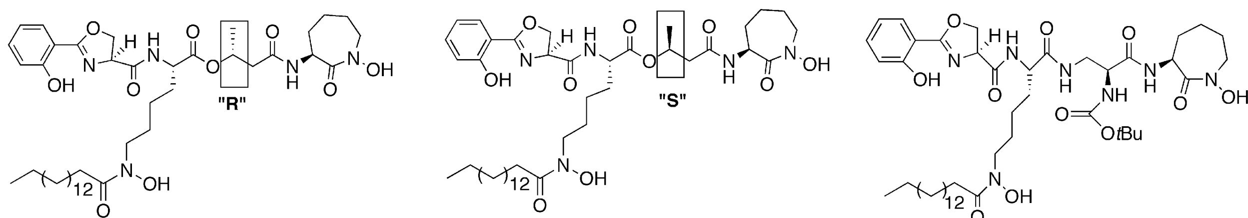


Mycobactin T, a growth promoter for *M. tuberculosis*

Mycobatin S, a growth promoter for *M.smegmatis*. Effects greater than 99% inhibition of the growth of *M. tuberculosis* H37Rv at 12.5 μg/mL

Diaminoproprionate analog, Growth inhibitor of *M. tuberculosis* H37Rv, with MIC< 0.2

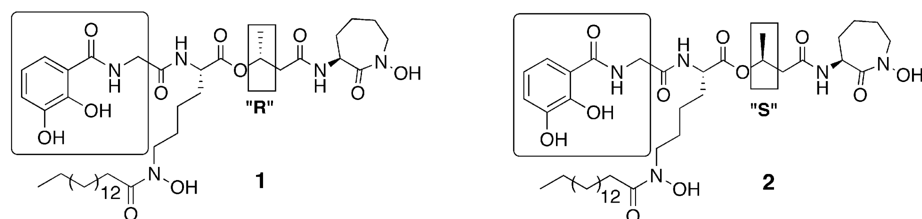
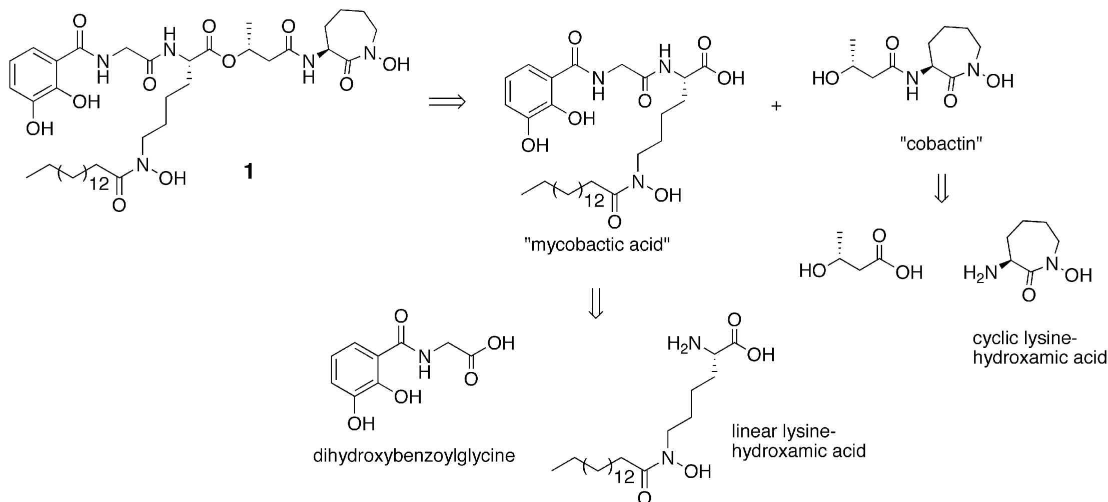


Fig. 1 Structures of representative mycobactins and analogs.



Scheme 1

(Scheme 2).[16–21] In this case we synthesized both the acyclic and cyclic lysine derived hydroxamic acids using the stable, storable and readily accessible nitrone intermediate **3** shown in Scheme 3.

Thus, condensation of *Z*-(L)-lysine with freshly distilled benzaldehyde was followed by dry *m*-CPBA-mediated oxidation of the intermediate imine to form the corresponding oxaziridine **3**. Isomerization under acidic conditions led to nitrone **4** in good overall yield from the starting protected amino acid. The reported syntheses of the azepine hydroxamic acid **6** relied upon TBDPS protection of the generated hydroxamic acid. Efforts to eliminate the need for this protecting group were elaborated. Formation of the pre-cyclization hydroxylamine HCl salt of **5** was accomplished by the action of 1.1 equivalents of $NH_2OH(HCl)$ on nitrone **4** at elevated temperatures in MeOH. Cyclization was accomplished using 1.1 and 1.2 equivalents of EDC and HOAt, respectively, under basic, high dilution conditions. After reverse phase chromatography, hydroxamic acid **6** was obtained in 55% overall yield from the nitrone **4**.

Acyclic hydroxamic acid **9** was also readily prepared from the lysine nitrone. Thus, treatment of nitrone **4** with $NH_2OH(HCl)$ resulted in the formation of hydroxylamine **5**. Reaction of **5** with $SOCl_2$ in MeOH gave methyl ester **7**. The ε-*N*-hydroxylysine methyl ester was then treated with a large excess of palmitoyl chloride and $NaHCO_3$ in CH_2Cl_2 to provide bis-acylated hydroxylamine **8** in excellent yield. According to precedent, solvolytic removal of the *O*-palmitoyl group from the hydroxamate with 6% DIPEA in MeOH resulted in the formation of the known *Z*-protected lysine hydroxamic acid **9**.[13]

Using a literature procedure,[21] 2,3 dihydroxybenzoic acid was reacted with BnBr and KOH in DMSO to give the 2,3-benzyloxybenzoic acid **10** in good yield (Scheme 4). The formation of an amide bond with this acid and glycine methyl ester has also been reported but with no experimental detail.[22] Treatment of **10** with oxalyl chloride and catalytic DMF led to the acid chloride, which, upon treatment with glycine methyl ester gave amide **11** in excellent yield. Saponification of the methyl ester of **11**

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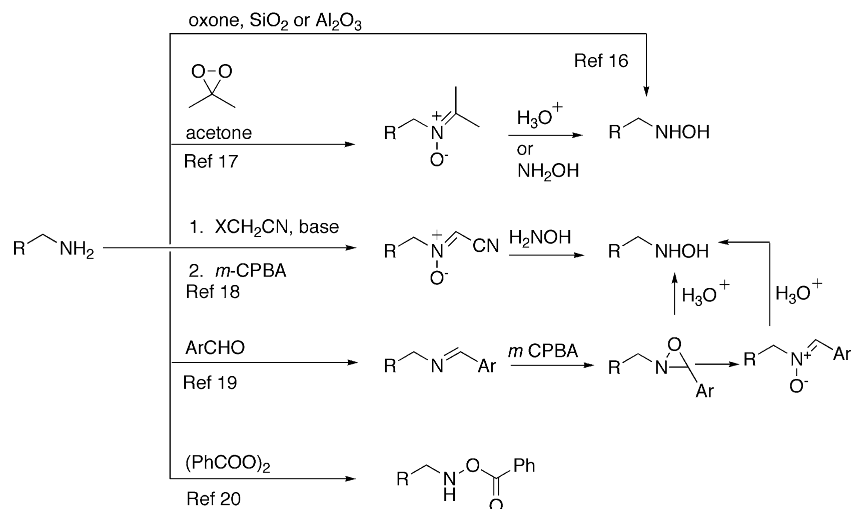
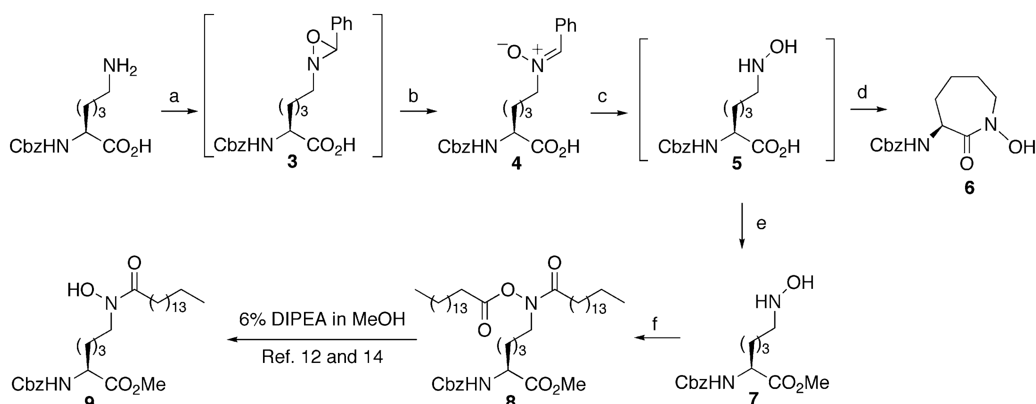
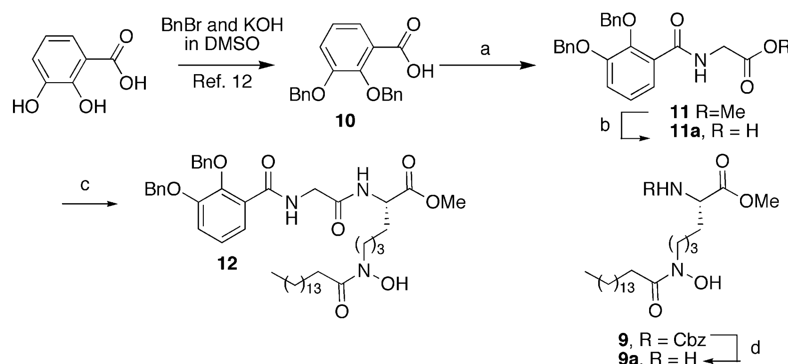


Scheme 2



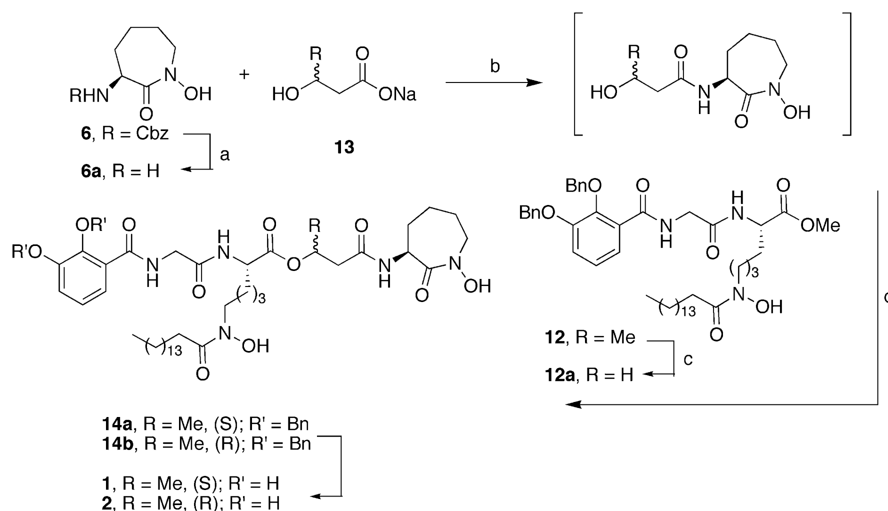
Scheme 3 *Reagents and conditions*: a. 1) PhCHO, KOH, MS, MeOH, rt, 16 h, 2) *m*-CPBA, MeOH, 0 °C to rt, 4 h. b. 1) TFA, CH_2Cl_2, rt, 1 h. 2) PhCHO, EtOAc, 0 °C to rt (66% overall). c. 1) $NH_2OH(HCl)$, MeOH, 65 °C, 20 min. d. EDC, HOAt, $NaHCO_3$, CH_3CN, DMF, rt, 48 h (55%). e. $SOCl_2$, MeOH, 0 °C to rt, 12 h. f. Palmitoyl chloride, $NaHCO_3$,CH_2Cl_2, rt, 12 h (95% overall from **5**).



Scheme 4 *Reagents and conditions*: a. 1) Oxalyl chloride, DMF (cat.), benzene, 0 °C to rt, 3 h, 2) glycine methyl ester(HCl), $NaHCO_3$, CH_3CN, rt, 12 h (91%). b. LiOH, THF, H_2O, rt, 12 h (quant.). c. 1) **9a**, EDC, HOAt, DMAP, DMF, rt, 16 h (53%). d. H_2, Pd–C, MeOH, rt and pressure, 1 h (quant.).

produced the corresponding acid which was coupled to free amine **9a**, obtained from hydrogenolysis of **9**, to give the key catechol-containing mycobactic acid methyl ester **12**.

Completion of the syntheses of the target compounds required the preparation of cobactins S and T. Cobactins S and T consist of (*S*)- or (*R*)-hydroxy butyrate linked by an amide bond to the cyclic, lysine-based hydroxamic acid derived from ester hydrolysis of mycobactins S and T, similar to the retrosynthesis scheme shown earlier. Using commercially available (*S*)- or (*R*)-3-hydroxybutyric acid sodium salts, separate EDC/HOAt-mediated coupling reactions were attempted with the amine **6a** resulting from hydrogenolytic removal of the *Z*-protecting group of **6**.



Scheme 5 *Reagents and conditions*: a. H_2, 10% Pd–C, MeOH, rt (quant.). b. EDC, HOAt, DMAP, DMF, rt, 24 h. c. LiOH, THF, H_2O, rt, 12 h (quant.). d. **7a**, EDC, 24 h (41–55%). e. H_2, 10% Pd–C, MeOH, rt (80–98%).

The reactions employed DMF as the solvent and, after the starting materials were consumed, the aqueous work-ups proved to be quite troublesome. Isolation of the cobactins from the aqueous DMF/water solutions was extremely inefficient. Since other solvents were not as effective as DMF in peptide bond formation in the presence of hydroxamic acids, an alternative one-pot, two coupling reaction protocol was found to be moderately effective.

A one flask/two EDC/HOAt-mediated coupling reaction sequence has been employed in the synthesis of a mycobactin analog.[14] For the generation of the catechol analogs, the reactions to form cobactins S and T were performed, in the same manner, for 24 h (Scheme 5). Concurrently, the methyl ester of **12** was saponified. After the 24 h reaction time, acid **12a** and another 1.1 equivalents of EDC were added. This protocol generated the benzyl protected analogs **14a**,**b** in 41% overall yield for the (*S*)-methyl group configuration and 54% for the (*R*)-methyl group configuration. Pd–C catalyzed hydrogenolysis of the benzyl protecting groups provided the target compounds **1** and **2** cleanly.

As with many peptides, the presence of rotational isomers, detected in the NMR spectra of synthetic analogs, is possible. Distinguishing them from diastereomers can be quite time consuming. This proved to be the case with compounds **14a**,**b** and **1**,**2**. Splitting of peaks in both the ^{1}H and ^{13}C NMR spectra was noted. With the bis-benzylated intermediates **14a**,**b**, the splitting of peaks was severe and extended to the peaks corresponding to the methyl and methylene groups of the butyrate linker. Upon hydrogenation of the benzyl groups, giving rise to analogs **12a**,**b**, the splitting of peaks was lessened overall and completely eliminated from the signals corresponding to the methyl and methylene groups in the ^{1}H NMR, thus indicating that peak multiplicity arose from rotation isomers and not from diastereomers.

Assays of synthetic conjugates 1 and 2

The relative iron binding capacity of the desferri- or ferri-siderophores is often determined by the chrome azurol S (CAS) assay.[23] Usually, the diameter of the resulting orange halo in mm for a 5 μL sample reflects the extent of binding. However, as indicated we have found that the test is not always positive with very hydrophobic siderophores or analogs that tend to precipitate in the assay. Also, of course, the CAS assay cannot work with iron bound siderophores. As shown in Tables 1–3 below, the synthetic mycobactin analogs, **1** and **2**, did give a positive CAS test while authentic mycobactin J, which contains iron, was negative, and the more hydrophilic trihydroxamate, desferrioxamine B (Desferal), was very responsive, as expected. With the iron binding affinity of **1** and **2** confirmed, the compounds were also tested for their ability to inhibit or promote the growth of various bacterial strains, including mycobacteria. Potential antibacterial activity of the compounds was studied by determination of minimal inhibitory

Table 1 Growth promotion of Gram-negative strains by mycobactin analogs **1** and **2**, and desferrioxamine (Desferal)[a]

		P. aeruginosa			*E. coli*	*S. typhimurium*
Compound	CAS	K799/WT	ATCC 9027	NCTC 10662	ATCC 25922	enb-7
1	++	A	A	13	14	0
2	+	0	13	A11	12	0
Desferal	++++	40	38	40	H20	14

[a] Samples (5 μg) were applied to 6 mm diameter discs and the growth zones are given in mm. Additional indicators are: H = inhibition of growth in mm, A = small indication of growth.

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Table 2 Growth promotion of mycobacterial strains by mycobactin analogs **1** and **2**, and mycobactin J[a]

Compound	CAS	*Mycobacterium smegmatis* SG 987	SG 987-M10	mc^2155	mc^2155-M24	mc^2155-B1	mc^2155-M24-B3	mc^2155-M24-U3
1	++	32	16	17	17	17	14	H7/A14
2	+	24	16	18	15	15	8	H7/A
Mycobactin J	—	25	15	16	17	15	12	15

[a] Samples (5 μg) were applied to 6 mm diameter discs and the growth zones are given in mm. Additional indicators are: H = inhibition of growth in mm, A = small indication of growth.

Table 3 Characteristics of the *M. smegmatis* strains used in these studies

Bacterial strains/characteristic	*Mycobacterium smegmatis* SG 987	SG 987-M10	mc^2155	mc^2155-M24	mc^2155-B1	mc^2155-M24-B3	mc^2155-M24-U3
Biosynthesis of:							
Exochelin	+	−	+	+	−	−	+
Mycobactin	+	+	+	−	+	−	−
Exochelin permease	+	+	+	+	+	−	−

concentrations (MIC) according to the NCCLS guidelines using the micro broth dilution method.[24] Neither **1** nor **2** displayed antibiotic activity against Gram-positive and Gram-negative bacteria or against mycobacteria under normal assay conditions (data not shown).

The desferrimycobactin analogs **1** and **2** were also studied for their ability to promote the growth of a series of Gram-negative bacteria and a larger set of mycobacteria since the latter were anticipated to be most responsive. Growth zones surrounding the discs were read after 1 day for *P. aeruginosa*, *E. coli* and *S. typhimurium* strains and after two days for *M. smegmatis* strains. The results are summarized in Tables 1 and 2. Table 3 provides characteristics of the mycobacterial strains used. Indeed, as expected, mycobacteria were more responsive to **1** and **2** as growth promoters than were the Gram-negative bacteria. In fact, with mycobacteria, analogs **1** and **2** were generally as effective as natural mycobactin J. While in most cases normal zones of growth promotion were observed, in a few instances additional affects were noted as designated by the descriptor "H" (inhibition) in Tables 1 and 2. This less common phenomenon is not yet well understood, but might be due to the ability of the applied desferrisiderophore or analog to scavenge the trace of iron that remains in the specially prepared iron depleted media used for the assays. Thus, at higher concentration of the siderophore and depending on the individual mode of iron uptake for a specific strain, the microbes are additionally stressed for the essential nutrient. This type of growth inhibition is reflected by an inhibition-like zone near the compound loaded paper disc where the concentration of the siderophore is highest and before the growth zone appears. The two analogs were also tested for possible anti-tuberculosis activity at the Tuberculosis Acquisition And Coordinating Facility (TAACF) and found to not inhibit the growth of *M. tuberculosis* (2% and 0% inhibition for **1** and **2**, respectively, at 6.25 μg mL^{-1}). Thus, syntheses and studies of **1** and **2** demonstrate that novel analogs of natural mycobactins can be used by mycobacteria for microbial growth.

Conclusions

The synthesis of catechol containing mycobactin S and T analogs has been accomplished. The novel catechol analogs were demonstrated to bind iron as reflected by the standard CAS assay. Consistent with their similarity to the natural mycobactins, they preferentially promoted the growth of the mycobacterial strains studied relative to other bacteria. The potential use of these compounds as mycobacterial selective drug delivery agents is under consideration.

Experimental

Lysine nitrone 4

To a solution of KOH (1.05 g, 19.0 mmol) and 5 g of 3 Å molecular sieves in 50 mL of MeOH at room temperature was added *Z*-(L)-lysine (5.00 g, 17.8 mmol). When the stirred solution became clear, benzaldehyde (2.08 g, 19.6 mmol) was added and the mixture was stirred for an additional 16 h at room temperature. The mixture was filtered and the solvents removed under reduced pressure to give the crude imine product. The residue was taken up in 40 mL of MeOH and cooled to 0 °C. Dry *m*-CPBA (3.70 g, 21.4 mmol), dissolved in 50 mL of MeOH, was added dropwise over 20 min and the resulting solution was stirred for an additional 2 h at 0 °C. The reaction mixture was filtered and the solvents removed under reduced pressure. Isomerization of the crude oxaziridine was accomplished by the addition of 25 mL of TFA followed by 25 mL of CH_2Cl_2 to the residue under a nitrogen atmosphere. The solution was stirred for 1 h at room temperature and the solvents removed under reduced pressure. The residue was taken up in 60 mL of EtOAc and cooled to 0 °C under a nitrogen atmosphere. Benzaldehyde (1 mL) was added and the solution was stirred overnight while coming to room temperature. The solvents were removed under reduced pressure and the residue purified by flash chromatography (80% CH_2Cl_2 : 20% acetone to remove

m-CPBA and PhCHO, then gradient elution to 15% MeOH : 65% CH_2Cl_2 : 20% acetone) to give 4.50 g (66% yield) of the product nitrone as a pale yellow oil. ^{1}H NMR (300 MHz, CD_3CN) δ 8.24–8.21 (m, 2H), 7.60 (s, 1H), 7.43–7.26 (m, 8H), 6.14 (d, 1H, J = 8.06 Hz), 5.35–4.85 (br s, 1H), 5.02 (s, 2H), 4.14–4.08 (m, 1H), 3.87 (t, 2H, J = 6.84 Hz), 1.94–1.34 (m, 6H); ^{13}C NMR (75 MHz, CD_3CN) δ 174.6, 157.3, 138.1, 137.1, 131.7, 131.5, 129.9, 129.5, 129.4, 128.9, 128.7, 67.1, 66.9, 58.9, 31.9, 27.8, 23.3; IR (neat) 3323, 3064, 2953, 1713, 1531 cm^{-1}; HRFABMS for $C_{21}H_{25}N_2O_5$ (MH^+) calcd 385.1763, found 385.1750.

Azepinone hydroxamic acid 6

Lysine nitrone **4** (1.00 g, 2.87 mmol) and hydroxylamine hydrochloride (0.22 g, 3.16 mmol) were dissolved in 10 mL of MeOH and heated in a 60 °C oil bath for 20 min and allowed to cool. The solution was concentrated, taken up in distilled H_2O and extracted with diethyl ether until there was no more by-product in the organic phase as detected by TLC. The aqueous solvent was removed under reduced pressure. Residual water was removed azeotropically under reduced pressure with benzene and methanol. The crude hydroxylamine in 90 mL of 7 : 2 CH_3CN : DMF was added dropwise over 2 h to a stirred solution of EDC (546 mg, 2.84 mmol), HOAt (355 mg, 2.61 mmol), and $NaHCO_3$ (996 mg, 11.9 mmol) in 50 mL of 7 : 2 CH_3CN : DMF. The solution was stirred for 48 h at room temperature. The CH_3CN was removed under reduced pressure and EtOAc and H_2O were added. The aqueous layer was acidified to approximately pH 2 with 1 N HCl and extracted with EtOAc until no more product could be detected by TLC. The combined organic extracts were washed twice with 5% $NaHCO_3$ and once with brine. The organic layer was dried with Na_2SO_4, filtered and concentrated. The residue was purified by C_{18} reverse phase silica gel chromatography (3 : 2 H_2O : THF) to give 363 mg (55% yield) of the product as an amorphous white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 7.37–7.28 (m, 5H), 5.08 (s, 2H), 4.35 (d, 1H, J = 11.48 Hz), 3.91 (dd, 1H, J = 11.23, 16.11 Hz), 3.66 (d, 1H, J = 15.87 Hz), 2.00–1.50 (m, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ 171.3, 157.9, 138.2, 129.4, 129.0, 128.8, 67.6, 54.3, 53.9, 32.0, 28.6, 26.9; IR (neat) 3333, 3125, 2917, 1688, 1650, 1622, 1529 cm^{-1}; HRFABMS for $C_{14}H_{19}N_2O_4$ (MH^+) calcd 279.1345, found 279.1352.

Bis-palmitoyl lysine hydroxylamine 8

Lysine nitrone **4** (1.48 g, 3.86 mmol) and $NH_2OH(HCl)$ (0.295 g, 4.25 mmol) were dissolved in 25 mL of MeOH and heated in a 65 °C oil bath for 17 min. The solution was allowed to cool to room temperature and was placed in an ice water bath. $SOCl_2$ (3.26 g, 27.4 mmol) was added and the solution was stirred for 16 h while coming to room temperature. The solvents were removed under reduced pressure and saturated $NaHCO_3$ and CH_2Cl_2 were added. The layers were separated and the aqueous layer was extracted three times with CH_2Cl_2. The combined organic extracts were dried with NaS_2O_4, filtered and concentrated. This residue was purified by silica gel chromatography (9 : 1 CH_2Cl_2 : EtOAc to remove impurities, then EtOAc to 95 : 5 EtOAc : MeOH). The resulting hydroxylamine was then dissolved in 120 mL of CH_2Cl_2 and $NaHCO_3$ (2.59 g, 31.0 mmol) and palmitoyl chloride (4.24 g, 15.4 mmol) were added and the mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure and the residue was taken up in EtOAc. The organic layer was washed with water, twice with 1 N HCl, and twice with brine. The organic solution was dried with NaS_2O_4, filtered and concentrated. The residue was purified by silica gel chromatography (CH_2Cl_2 to 16 : 1 CH_2Cl_2 : EtOAc) to give 2.89 g (95% yield) of the product as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34–7.25 (m, 5H), 5.66 (br d, 1H, J = 8.55 Hz), 5.10 (s, 2H), 4.36–4.31 (m, 1H), 3.37 (s, 3H), 3.69–3.58 (m, 2H), 2.42 (t, 2H, J = 7.45 Hz), 2.17 (br t, 2H, J = 7.32 Hz), 1.82–1.18 (m, 58H), 0.87 (t, 6H, J = 6.32 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.1, 173.0, 171.6, 156.1, 136.4, 128.7, 128.4, 128.3, 67.2, 53.9, 52.6, 34.0, 32.5, 32.1, 32.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 26.7, 24.9, 24.5, 22.9, 22.4, 14.3; IR (neat) 3332, 2924, 2853, 1790, 1725, 1674 cm^{-1}; HRFABMS for $C_{47}H_{83}N_2O_7$ (MH^+) calcd 787.6200, found 787.6218.

Dibenzyloxybenzoylglycine methyl ester 11

2,3-Benzyloxybenzoic acid **10** (0.500 g, 1.50 mmol) was added to 5 mL of benzene and cooled to 0 °C. Oxalyl chloride (0.762 g, 6.00 mmol) was added followed by one drop of DMF. The reaction was stirred for 12 h while coming to room temperature. The solution was concentrated and dissolved in 10 mL of CH_3CN. At room temperature, $NaHCO_3$ (1.26 g, 15.00 mmol) was added followed by the HCl salt of glycine methyl ester (0.208 g, 1.65 mmol). The reaction was stirred for 24 h and the solvents removed under reduced pressure. The residue was taken up in EtOAc and washed with 1 N HCl, water, and brine. The organic layer was dried with NaS_2O_4, filtered and concentrated. The residue was purified by silica gel chromatography (4 : 1 hexanes : EtOAc) to give 0.553 g (91% yield) of the product as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52–8.44 (m, 1H), 7.73 (dd, 1H, J = 3.59, 6.05 Hz), 7.49–7.15 (m, 12 H), 5.16 (s, 2H), 5.14 (s, 2H), 4.08 (d, 2H, J = 5.46 Hz), 3.73 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 165.5, 152.0, 147.2, 136.5, 129.2, 128.9, 128.7, 128.6, 128.5, 128.0, 126.8, 124.6, 123.5, 117.5, 76.6, 71.5, 52.4, 41.7; IR (neat) 3380, 3032, 2951, 1748, 1660 cm^{-1}; HRFABMS for $C_{24}H_{24}NO_5$ (MH^+) calcd 406.1654, found 406.1641.

Catechol-containing mycobactic acid methyl ester analog 12

To methyl ester **11** (0.468 g, 1.15 mmol) in 10 mL of 1 : 1 THF : water was added LiOH (0.138 g, 5.75 mmol) at room temperature. The reaction was stirred for 12 h, diluted with water, and acidified to pH = 2 with 1 N HCl. The aqueous layer was extracted three times with EtOAc. The combined organic extracts were washed with water and brine. The organic solution was dried with NaS_2O_4, filtered and concentrated to give acid **11a** which was used immediately in the next reaction. In a separate flask, a solution of **9** (0.760 g, 1.39 mmol) in 10 mL of MeOH was purged with nitrogen. Pd–C (10%, 10% weight) was added and a hydrogen atmosphere was established with a balloon. The solution was stirred for 45 min at room temperature and was filtered through Celite. Acid **11a** (0.400 g, 1.02 mmol), amine **9a** (0.421 g, 1.02 mmol), HOAt (0.139 g, 1.02 mmol), and catalytic DMAP were dissolved in 5 mL of DMF at room temperature. EDC (0.215 g, 1.12 mmol) was added and the solution was stirred for 16 h and diluted with water. The solution was extracted three times with EtOAc. The

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combined organic extracts were washed with 1 N HCl, water, 5% $NaHCO_3$, and brine. The organic solution was dried with NaS_2O_4, filtered and concentrated. The residue was purified by silica gel chromatography (4 : 1 to 2 : 1 hexanes : EtOAc) to give 0.425 g (53% yield) of the product as an amorphous white solid. ^1H NMR (600 MHz, CD_3OD) δ 7.49 (br d, 1H, J = 7.56 Hz), 7.43 (dd, 1H, J = 1.50, 7.92 Hz), 7.39–7.22 (m, 10H), 7.13 (t, 1H, J = 8.01 Hz), 5.17 (s, 2H), 5.13 (s, 2H), 4.42 (dd, 1H, J = 4.95, 8.88 Hz), 4.00 (d, 1H, J = 16.80 Hz), 3.94 (d, 1H, J = 16.90 Hz), 3.70 (s, 3H), 3.63–3.54 (m, 5H), 2.44–2.42 (m, 2H), 1.88–1.82 (m, 1H), 1.73–1.54 (m, 5H), 1.39–1.26 (m, 28H), 0.88 (t, 3h, J = 7.10 Hz); ^{13}C NMR (150 MHz, CD_3OD) δ 175.8, 173.6, 170.7, 167.8, 153.1, 147.4, 137.7, 137.4, 130.0, 129.3, 129.1, 129.0, 128.9, 128.7, 128.5, 125.1, 122.7, 118.2, 76.7, 71.9, 53.3, 52.4, 43.3, 32.9, 32.7, 31.7, 30.4, 30.3, 30.2, 30.1, 26.8, 25.6, 23.4, 14.1; IR (neat) 3283, 2924, 2854, 1746, 1643 cm^{-1}; HRFABMS for $C_{46}H_{66}N_3O_8$ (MH^+) calcd 788.4850, found 788.4870.

Bis-benzylated catechol-containing mycobactin S analog 14a

A solution of azepine hydroxamic acid **6** (0.053 g, 0.188 mmol) in 5 mL of MeOH was purged with nitrogen. Pd–C (10 mol% of 10% by weight) was added and a hydrogen atmosphere was established with a balloon. The solution was stirred for 45 min at room temperature and was filtered through Celite to give a solution of **6a** that was used immediately in the next reaction. The filtrate was concentrated and (*R*)-3-hydroxybutyric acid sodium salt (0.024 g, 0.188 mmol), HOAt (0.026 g, 0.188 mmol), catalytic DMAP, and 4 mL of DMF were added. EDC (0.040 g, 0.207 mmol) was added and the solution was stirred at room temperature for 24 h. Concurrently, **12** (0.150 g, 0.188 mmol) was dissolved in 5 mL of 1 : 1 THF : water. LiOH (0.014 g, 0.565 mmol) was added and the mixture was stirred at room temperature for 12 h. The solution was diluted with water and acidified to pH = 2 with 1 N HCl. The aqueous solution was extracted three times with EtOAc. The combined organic extracts were washed with water and brine. The organic solution was dried with NaS_2O_4, filtered and concentrated. The resulting acid, **12a**, was added to the above reaction mixture after 24 h followed by 1.1 equivalents of EDC. The solution then was stirred for 24 h at room temperature and diluted with EtOAc. The organic solution was washed with 1 N HCl, water, 5% $NaHCO_3$, and brine. The organic solution was dried with NaS_2O_4, filtered and concentrated. The residue was purified by C_{18} reverse phase silica gel chromatography (1 : 1 to 85 : 15 MeOH : H_2O) to give 0.100 g of the product as a clear solid in 41% yield. ^1H NMR (rotational isomers present, major peaks reported when possible): (600 MHz, CD_3OD) δ 7.51–7.49 (m, 1H), 7.44–7.42 (m, 1H), 7.40–7.23 (m, 10H), 7.16–7.13 (m, 1H), 5.19 (s, 2H), 5.14 (s, 2H), 4.64–4.58 (m, 1H), 4.40 (dd, 1H, J = 4.65, 8.70 Hz), 4.13–4.10 (m, 1H), 4.03–3.90 (m, 3H), 3.67–3.55 (m, 3H), 2.43 (dd, 2H, J = 6.63, 14.64 Hz), 2.39 (dd, 1H, J = 7.85, 14.04 Hz), 2.33 (dd, 1H, J = 4.97, 14.05 Hz), 1.97–1.87 (m, 2H), 1.81–1.54 (m, 8H), 1.40–1.23 (m, 28H), 1.20 (d, 3H, J = 6.30 Hz), 0.88 (t, 3H, J = 7.05 Hz); ^{13}C NMR (rotational isomers present, major peaks reported when possible): (150 MHz, CD_3OD) δ 176.3, 173.6, 171.5, 171.2, 171.1, 168.4, 153.7, 148.0, 138.3, 138.0, 130.5, 129.8, 129.6, 129.5, 129.4, 129.2, 129.1, 125.6, 123.2, 118.7, 77.2, 72.4, 66.2, 54.2, 53.9, 53.4, 53.0, 46.4, 46.2, 43.9, 43.7, 33.5, 33.2, 32.5, 31.8, 31.0, 30.9, 30.8, 30.7, 30.6, 28.8, 27.4, 27.3, 27.1, 26.1, 24.0, 23.9, 23.3, 14.6; IR (neat) 3306, 2925, 1634 cm^{-1}; HRFABMS for $C_{55}H_{80}N_5O_{11}$ (MH^+) calcd 986.5854, found 986.5884.

Catechol-containing mycobactin S analog 1

A solution of **14a** (0.050 g, 0.051 mmol) in 3 mL of MeOH was purged with nitrogen. Pd–C (10 mol% of 10% by weight) was added and a hydrogen atmosphere was established with a balloon. The solution was stirred for 3 h at room temperature. The mixture was filtered through a KimWipe plug/pipette and the filtrate was concentrated. The procedure was repeated until no catalyst was visible in the filtrate. The residue was purified by C_{18} reverse phase silica gel chromatography (1 : 1 to 85 : 15 MeOH : H_2O) to give 0.033 g (98% yield) of the product as a clear glassy solid. ^1H NMR (rotational isomers present, major peaks reported when possible): (600 MHz, CD_3OD) δ 7.25 (dd, 1H, J = 1.19, 8.06 Hz), 6.93 (dd, 1H, J = 1.46, 7.80 Hz), 6.72 (t, 1H, J = 8.00 Hz), 4.63 (q, 1H, J = 11.24 Hz), 4.53–4.50 (m, 1H), 4.14–4.00 (m, 4H), 3.70–3.57 (m, 3H), 2.45 (br t, 2H, J = 7.63 Hz), 2.39 (dd, 1H, J = 7.69, 14.04 Hz), 2.33 (dd, 1H, J = 5.00, 14.16 Hz), 2.01–1.23 (m, 38H), 1.19 (d, 3H, J = 6.23 Hz), 0.89 (t, 3H, J = 7.08 Hz); ^{13}C NMR (rotational isomers present, major peaks reported when possible): (150 MHz, CD_3OD) δ 176.3, 174.2, 173.5, 172.0, 171.8, 171.2, 170.7, 150.4, 147.5, 129.9, 119.8, 116.8, 66.2, 66.1, 54.2, 54.1, 53.8, 53.4, 53.3, 53.0, 52.7, 52.6, 46.3, 43.5, 33.4, 33.2, 31.9, 31.8, 30.9, 30.8, 30.7, 30.6, 28.8, 27.4, 26.1, 23.9, 23.8, 23.3, 14.6; IR (neat) 3307, 2924, 2853, 1636 cm^{-1}; HRFABMS for $C_{41}H_{68}N_5O_{11}$ (MH^+) calcd 806.4915, found 806.4896.

Catechol-containing mycobactin T analog 2

A solution of **14b** was hydrogenated and purified as the catechol containing mycobactin S analog to provide 0.040 g (98% yield) of a clear glassy solid. ^1H NMR (rotational isomers present, major peaks reported when possible): (600 MHz, CD_3OD) δ 7.25 (dd, 1H, J = 0.85, 8.06 Hz), 6.93 (dd, 1H, J = 1.41, 7.88 Hz), 6.72 (t, 1H, J = 8.00 Hz), 4.65–4.60 (m, 1H), 4.53–4.51 (m, 1H), 4.16–4.00 (m, 4H), 3.69–3.56 (m, 3H), 2.45 (br t, 2H, J = 7.63 Hz), 2.36 (d, 1H, J = 7.45 Hz), 2.35 (d, 1H, J = 5.50 Hz), 2.00–1.27 (m, 38H), 1.20 (d, 3H, J = 6.23 Hz), 0.89 (t, 3H, J = 7.08 Hz); ^{13}C NMR (rotational isomers present, major peaks reported when possible): (150 MHz, CD_3OD) δ 176.3, 173.4, 172.0, 171.9, 170.8, 170.7, 150.4, 147.5, 120.0, 119.8, 119.2, 116.7, 66.1, 54.1, 53.3, 53.2, 52.7, 52.6, 46.1, 43.5, 33.5, 33.2, 32.0, 31.9, 31.8, 31.0, 30.9, 30.8, 30.7, 30.6, 28.9, 28.8, 27.4, 27.3, 27.1, 23.9, 23.8, 23.6, 14.6; IR (neat) 3298, 2924, 2854, 1640 cm^{-1}; HRFABMS for $C_{41}H_{68}N_5O_{11}$ (MH^+) calcd 806.4915, found 806.4952.

Bacterial strains

Test organisms from Culture Collections (ATCC, NCTC) and from the stock of the Hans Knoell Institute (SG) included: *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (SG 511), *Mycobacterium smegmatis* (SG 987, mc^{2}155[25] and mutants thereof[26,27]), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATTC 10031), *Serratia marcescens* (SG 621), *Stenotrophomonas maltophilia* GN 12853 (kindly provided by the Episome Institute, Gunma (Japan)),[28] *Pseudomonas aeruginosa* (ATCC 27853, ATCC 9027, NCTC 10662), (SG 137), K799/WT, K799/61 (wild type

and permeability mutant),[29] *E. coli* DC 0, DC 2 (wild type and permeability mutant).[30]

MIC determination

The bacteria described were grown overnight at 37 °C in Mueller–Hinton broth (MHB) (Difco). 50 µL of a compound solution of 400 µg mL^{-1} were serially diluted by a factor of two with MHB in microtiter plates. Then the wells were inoculated with 50 µL of the bacterial solution to give a final concentration of 5×10^5 CFU mL^{-1}. After the microtiter plates were incubated at 37 °C for 24 h, the MIC values were read with a Nepheloscan Ascent 1.4 automatic plate reader (Labsystems, Vantaa, Finland) as the lowest dilution of antibiotic allowing no visible growth.

Siderophore assays

Utilization of compounds **1** and **2** as siderophores was determined by a growth promotion assay as described.[26,27] Strains were suspended in the iron depleted agar media hindering normal bacterial growth. The inoculated media were poured into Petri dishes. Siderophore solutions (2 mM, 5 µL) were applied on paper discs of 6 mm in diameter on the surface of the agar plates. Growth zones surrounding the discs were read after 1 day for *P. aeruginosa*, *E. coli* and *S. typhimurium* strains and after 2 days for *M. smegmatis* strains.

Acknowledgements

Support from the NIH (GM25845 and AI054193) is gratefully acknowledged. Anti-tuberculosis testing of **1** and **2** was kindly performed by the Tuberculosis Acquisition and Coordinating Facility (TAACF). We also appreciate the use of the NMR facilities provided by the Lizzadro Magnetic Resonance Center and the mass spectrometry services (Dr W. Boggess and Ms N. Sevova) provided by the University of Notre Dame. The excellent technical assistance of Irmgard Heinemann with the assays at the HKI is gratefully appreciated. MJM gratefully acknowledges the kind hospitality of the HKI and the University of Notre Dame for the sabbatical opportunity during which time this manuscript was prepared.

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