Synthesis and Biological Evaluation of New Acinetoferrin Homologues for Use as Iron Transport Probes in Mycobacteria

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Four new acinetoferrin homologues were synthesized using a modular synthetic approach. Two linear and two cyclic imide derivatives were generated and evaluated for growth stimulating behavior in *Mycobacterium avium* subsp *paratuberculosis*. The yield for the tandem coupling of a functionalized aminohydroxamic acid motif (2 equiv) to a *tert*-butyl citrate derivative was significantly improved using DCC and *N*-hydroxysuccinimide. ^{1}H NMR spectroscopy (CD_3OD) provided a convenient method for monitoring the final imidization step in TFA using the doublet patterns between 2.5 and 3.06 ppm. New protocols demonstrated that only a 20% growth enhancement was observed with *M. avium* subsp. *paratuberculosis* using the imide of acinetoferrin. Last, a siderophore from *Streptomyces pilosus*, deferrioxamine B, was shown to cross-feed *M. avium* subsp. *paratuberculosis* with the same efficiency as the more costly, native chelator, mycobactin J.

Introduction

Iron transport and storage in mammals are carried out by high molecular weight proteins such as transferrin (transport) and ferritin (storage).[1,2] In contrast, iron acquisition in microorganisms is the responsibility of siderophores, which are low molecular weight iron-specific ligands.[1,2] In the environment, iron is mainly found in its insoluble ferric (Fe^{3+}) form as ferric hydroxide polymers. Siderophores allow bacteria to solubilize and sequester iron(III) from these insoluble extracellular sources. Once bound to the siderophore, the iron (Fe^{3+}) is made available to the cell by intracellular modification, reduction to Fe^{2+}, or siderophore decomposition.[2]

Most bacteria produce distinct siderophore constructs. As shown in Figure 1, many of these have been isolated including mycobactin J **1** and carboxymycobactin **2** from *Mycobacterium tuberculosis*,[2] deferrioxamine B **3** from *Streptomyces pilosus*,[3] nannochelin A **4** from *Nannocystis exedens*,[4] acinetoferrin **5** from *Acinetobacter haemolyticus*,[5] schizokinen **6** from *Bacillus megaterium*,[6] and aerobactin **7** from *Aerobacter* strains.[7] Although the siderophores differ in their overall structure, several iron-binding motifs have been conserved. These ligands primarily include the hydroxamic acids, catechols, and/or α-hydroxycarboxylic acids.[1] Several siderophores (**4**−**7**) also share a common feature of a central citric acid component.

One antibiotic strategy could rely on the molecular recognition events involved in the internalization of the siderophore−iron complex for targeted drug delivery into specific microbes.[2] Siderophores could act as antibiotic agents through several mechanisms. First, they could act as deferration agents, which complex all

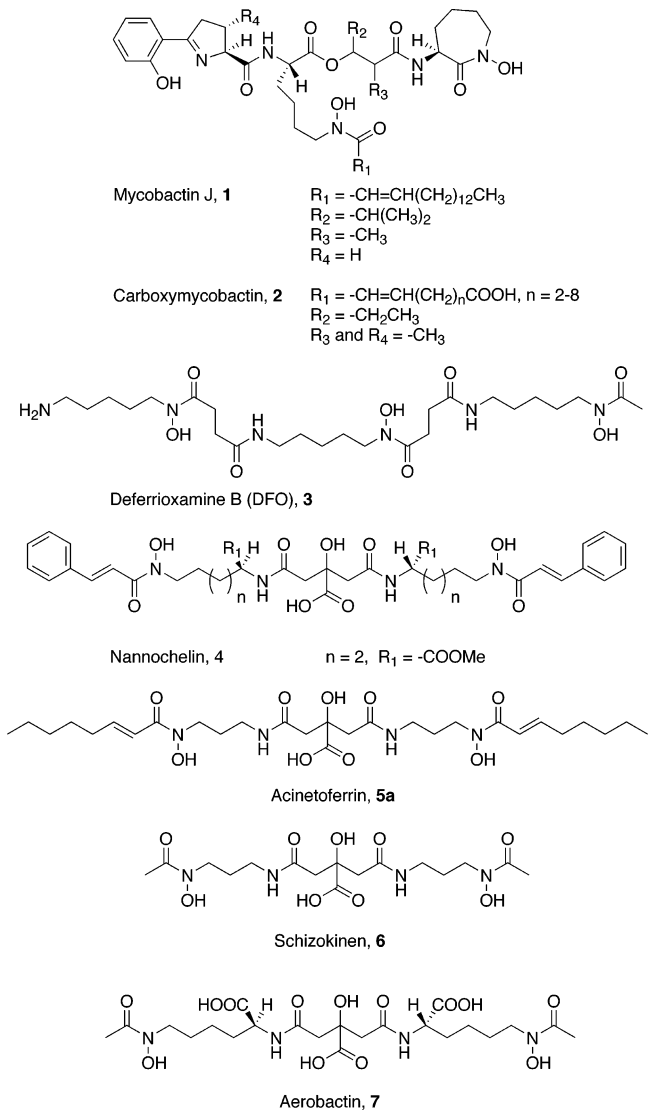

Figure 1. Structures of iron chelators.

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available iron and make it unavailable for microbial use. This action would in effect sequester this essential micronutrient and "iron-starve" the bacteria, thereby limiting its growth.[2] Second, bioconjugates between siderophores and antimicrobial agents may provide targeted delivery into microbes that have transporters for the import of siderophore−iron complexes. Alternatively these agents could inhibit microbial iron uptake by blocking cell-surface binding sites with a rogue siderophore−iron complex.[8,9] Therefore, understanding the structure−activity relationships involved in siderophore-mediated transport has real value in terms of antibiotic agent development.

The virulence of many microbes depends on their ability to compete for and obtain essential elements such as iron(III). Perhaps most pernicious is the bacterium (*Mycobacterium tuberculosis*) responsible for tuberculosis (TB) in humans, which continues to infect millions of people each year.[10] Although approximately 40% of the world's population is infected with *M. tuberculosis*, it grows slowly and the immune system can indefinitely suppress it. Still, according to the World Health Organization (WHO), the disease killed more people in 1995, when effective treatment was available, than in 1900 when no drugs were available to combat the disease.[10] The first antibiotic made available to fight tuberculosis was streptomycin, which was discovered in 1944. While other antituberculosis drugs have also been discovered, the current increase in mortality is due to the emergence of multiple drug resistant (MDR) strains of *M. tuberculosis*.[10,11] The WHO reports that the death rate of people with MDR-TB in the U.S. in 1995 was 70% and TB continues to be a public health threat to the extent that the WHO has declared the disease as a global public health emergency.[10]

Like most mycobacteria, the cell walls of *M. tuberculosis* are rich in lipids and consist mainly of mycolic acids containing long hydrocarbon chains. The high degree of saturation and length of these hydrocarbon chains contribute to a cell wall that is very tightly packed with extremely low fluidity.[12] The cell wall, therefore, has a low permeability to antibiotics and other chemotherapeutic agents.[12,13] A second feature of *M. tuberculosis* is the production of two kinds of siderophore: an intracellular mycobactin **1** that is associated with the cell wall and an extracellular exochelin **2**. The absorption of iron through the cell wall of *M. tuberculosis* is thought to involve an "iron handoff" between the two types of siderophores. In low-iron environments a hydrophilic exochelin is synthesized and excreted to bind exogenous ferric iron. This bound iron can then be transferred to the lipophilic mycobactin in the cell wall so that the iron can be stored or released into the cell by mycobactin reductases.[2]

Since iron is necessary for the growth and survival of *M. tuberculosis*, several approaches have been put forward for the development of antibiotics predicated upon siderophores and their analogues.[8,9] However, targeting the iron transport system of *M. tuberculosis* is very challenging because of the complex architecture of the mycobactins.[14] This structural complexity also hampers the synthesis of bioconjugates.[15]

Our initial goal was to identify non-native siderophores, which deliver iron to mycobacteria and stimulate their growth. Once a suitable surrogate siderophore was identified, then bioconjugates could be made to target this bacterial class with appended antibiotic agents. To study mycobacterial iron transport, *Mycobacterium avium* subsp. *paratuberculosis* was chosen as an alternative pathogenic model for *M. tuberculosis*. This less-pathogenic strain is responsible for Johne's disease in cattle and Crohn's disease in humans.[16] A key feature in selecting this model was that *M. avium* subsp. *paratuberculosis* requires the addition of exogenous siderophore (mycobactin J, **1**) in order to grow. In this regard, it is an excellent screen for determining how non-native siderophores influence mycobacterial growth.

Prior work involved cross-feeding *M. avium* subsp. *paratuberculosis* with several siderophores to evaluate whether non-native iron chelators could serve as surrogate siderophores and promote growth.[17] During the biological evaluation of several citrate-containing siderophores,[17] several trends became apparent. First, the number of carbons separating the bidentate ligand sites within each siderophore was vital. Indeed, longer tethers were more efficacious in cross-feeding *M. avium* subsp. *paratuberculosis*. Second, citrate derivatives that contained 2-octenoyl "tails" were better growth stimulants than those containing cinnamoyl tails. Third, derivatives that contained cyclic imides were initially shown to be 5 times more efficient in cross-feeding *M. avium* subsp. *paratuberculosis*. Collectively, these trends warranted the further investigation of these structural components. Using our earlier findings as a guide, we now report the synthesis of four new acinetoferrin analogues (**5b**, **5c**, **8b**, and **8c**) that incorporate these trends. These systems represent important new probes to study iron transport processes in *M. avium* subsp. *paratuberculosis*.

Results and Discussion

The synthetic strategy was predicated upon the modular approach developed for the synthesis of acinetoferrin **5a** and acinetoferrin imide **8a**. Four new acinetoferrin homologues were generated (**5b**, **5c**, **8b**, and **8c**). The procedure allowed for installation of a longer tether, which significantly increased the number of carbons between the bidentate ligands (e.g., from **5a** ($n = 1$) to **5b** ($n = 3$) in Figure 2). The 2-*E*-octenoyl tail was retained because of its observed enhanced cross-feeding capabilities. The approach to the cyclic imides **8b** and **8c** was via their respective precursors: the acyclic α-hydroxycarboxylic acids **5b** and **5c**. Therefore, any improvements in the methodology to access **5** directly impacted the overall yield of **8**. Moreover, an improved cyclization procedure was also developed for the direct synthesis of **8b** and **8c** from their respective *tert*-butyl esters, **20** and **21**.

The discussion will first focus on the construction of the linear analogues **5b** and **5c**. The modular synthetic approach involved the synthesis of a protected *N*-(alkylamino)-*N*-hydroxyl-*trans*-octenoyl precursor that could be deprotected and coupled to the terminal carboxylic acids of an activated citric acid derivative.[17] A series of protection and deprotection steps were needed for the synthesis of the *N*-(alkylamino)-*N*-hydroxyl-*trans*-octenoyl precursor. As shown in Scheme 1, the

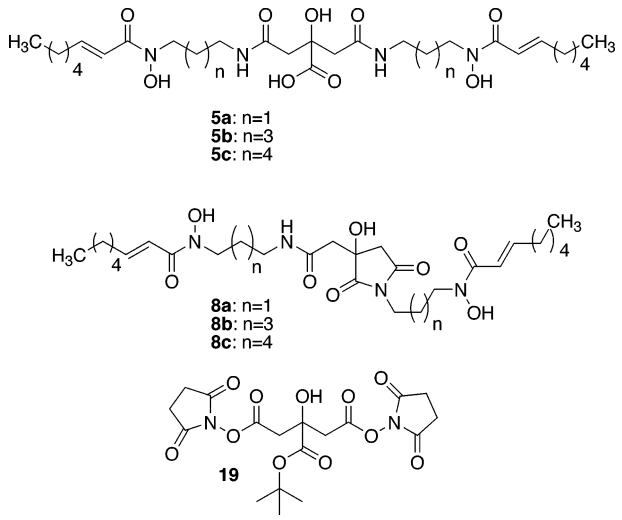
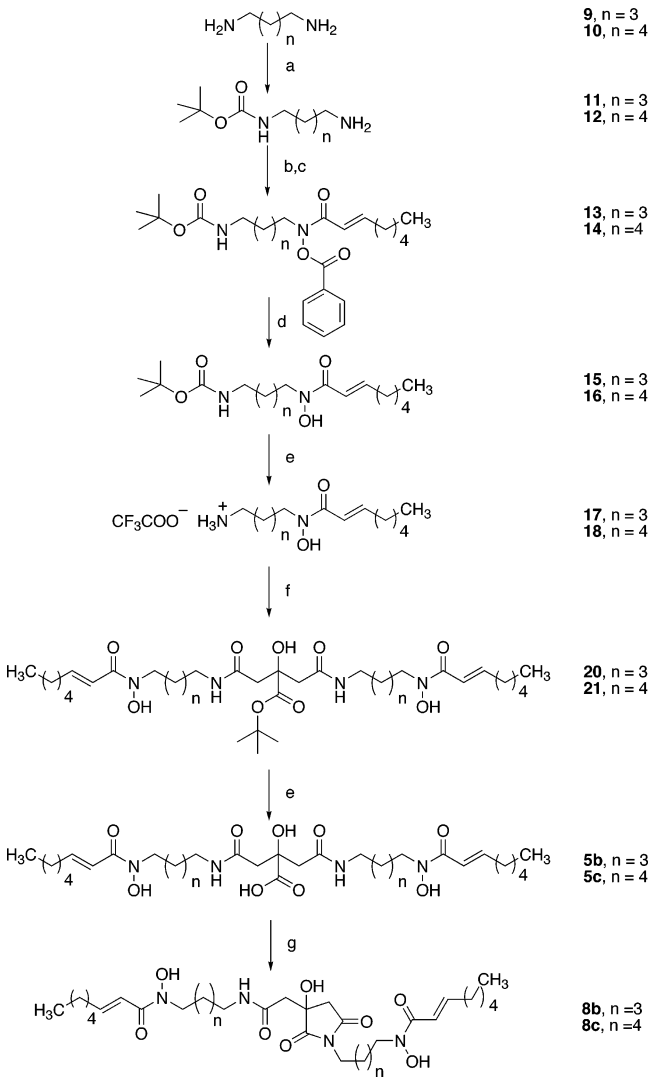


Figure 2. Acinetoferrin analogues and bis-NHS ester **19**.

Scheme 1[a]



[a] Reagents: (a) di-*tert*-butyl dicarbonate, 10% TEA/MeOH; (b) benzoyl peroxide (1.1 equiv)/CH_2Cl_2, pH 10.5 aqueous sodium carbonate buffer, R; (c) *trans*-octenoyl chloride, CH_2Cl_2; (d) 10% NH_4OH/MeOH; (e) TFA, 0 °C; (f) **19**, TEA, room temp; (g) TFA, reflux.

reaction of the diamines **9** and **10** with di-*tert*-butyl dicarbonate afforded the monoprotected diamines (**11** and **12**) in 72% and 73% yields, respectively. The tandem oxidation−acylation of a primary amine group to introduce the *O*-benzoyl-protected hydroxamate has been shown by Milewska et al.[18]

This approach led to our improved amine-oxidation method involving biphasic conditions, which was used successfully in this and many other reports.[17,19−21] The monoprotected diamines **11** and **12** were each dissolved in a biphasic mixture containing a pH 10.5 carbonate buffer and benzoyl peroxide (BPO) dissolved in CH_2Cl_2.[19,20] After the oxidation step, each mixture was acylated in situ with *trans*-octenoyl chloride to the give the desired compounds **13** (61%) and **14** (65%), respectively.

The synthesis of the *tert*-butylacinetoferrin homologues (**20** and **21**) required the coupling of the functionalized pentylamine and hexylamine fragments to the derivatized citric acid framework. Previous experience revealed that higher yields were obtained if the benzoyl group was removed prior to the amide formation.[4,5,22] Therefore, compounds **13** and **14** were treated with a solution of 10% concentrated NH_4OH in MeOH solution at −23 °C to give the "free" hydroxamic acids **15** (84%) and **16** (86%), respectively. Although the hydroxamic acids were purified for analytical purposes, they were typically used in the following steps without purification. Removal of the *tert*-butyl carbamate groups of **15** and **16** using trifluoroacetic acid (TFA) at 0 °C gave the respective TFA salts **17** and **18**. These were used without further purification in the next step, which involved coupling to the citric acid derivative, **19**. [4−6,17]

Prior methods for the selective coupling of amines to the carboxylic acid groups of the citric acid portion have involved using anhydromethylene citric acid or 2-substituted-1,3-bis-activated esters of citric acid.[6,7] However, the reported imide formation associated with the use of the anhydromethylene unit and the low overall isolated yield achieved in the synthesis of the 1,3-bis-*N*-hydroxysuccinimide esters of citric acid (20%) followed by the coupling step (80%, 16% overall) prompted the investigation for an improved method.[17]

A number of different reagent combinations were tried for the coupling step including HOBt/EDC, NHS/EDC, and NHS/DCC. In these reactions the activated 1,3-bis-*N*-hydroxy containing ester was not isolated but used directly in the coupling step. Ironically, the most efficient reagent was the NHS and DCC combination. Indeed, significantly higher yields were obtained when the bis-*N*-hydroxysuccinimide ester **19** (Figure 2) was not isolated but used directly in the subsequent coupling step. Therefore, the condensation of a solution of the respective TFA salt (**17** or **18**) and triethylamine with a solution of crude **19**[17] gave the corresponding *tert*-butylacinetoferrin homologue **20** or **21** in yields of 60% and 70%, respectively. Finally treatment of **20** and **21** with TFA gave the final homologues **5b** and **5c** in yields of 77% and 67%, respectively.

Initially, the imides **8b** and **8c** were generated in refluxing TFA for 3 h from **5b** and **5c**, respectively. The reactions were monitored by ^{1}H NMR. In particular the two doublets at δ 2.68 and δ 2.58, integrating for two protons each, were monitored. These are the citrate methylene groups, which flank the prochiral center. Upon treatment of **5b** in refluxing TFA, these two

Table 1. BACTEC Data[a]

compd	av BACTEC reading ± standard deviation	growth index (GI) relative to mycobactin J
mycobactin J, **1**	511 ± 38	1.00
DFO, **3**	555 ± 53	1.09
5C linear, **5b**	489 ± 23	0.96
6C linear, **5c**	483 ± 24	0.95
3C imide, **8a**	619 ± 58	1.21
5C imide, **8b**	600 ± 11	1.17
6C imide, **8c**	515 ± 98	1.01

[a] Siderophores were dosed at 2.4 μM and run in triplicate. All siderophores were dosed in the same media with the same cell type and uniformly cultured under the same environmental conditions (see Experimental Section). In the absence of added chelator, the bacterial growth was extremely slow and gave a relative BACTEC average reading near 100.

doublets become four distinct doublets at δ 2.99, δ 2.89, δ 2.72, and δ 2.59 (integrating for one proton each), respectively. Isolation and characterization of the product by ^{1}H NMR confirmed these doublets were derived from the cyclic imide **8b**. Although the resonances near δ 2.7 and δ 2.6 appear to overlap, the doublets at δ 2.99 and δ 2.89 are clearly resolved. Therefore, one can routinely monitor the conversion by integrating these respective doublets. Using this approach, we observed 70% conversion of the *tert*-butylacinetoferrins **5b** and **5c** to the imides **8b** and **8c**, respectively. At this point the reactions were stopped because evidence for the formation of an elimination product (an alkene) could be seen in the ^{1}H NMR at δ 5.8. Purification by flash column chromatography on silica gel gave the cyclic imides **8b** and **8c** in isolated yields of 43% and 41%, respectively. An alternative imidization method using 4 N HCl in glacial acetic acid (21 days at 25 °C) overcame alkene formation.

In summary, improvements to previous methods for the synthesis of acinetoferrin were developed during the synthesis of four new homologues of acinetoferrin (**5b, 5c**, **8b**, and **8c**). The synthesis of these homologues successfully incorporated the key features of the previous research: namely, the longer tether length, the 2-*E*-octenoyl tails, and the cyclic imide unit. Because of the previous findings with **5a** and **8a**, the four ligands reported herein were expected to be superior siderophore surrogates for mycobacteria.

Biological Evaluation. All derivatives tested were capable of forming hexadentate-binding architectures to iron(III). The respective siderophores (**5b**, **5c**, **8b**, and **8c**) were evaluated for their ability to act as growth factors for *M. avium* subsp. *paratuberculosis* at 2.4 μM (a standard concentration used routinely with **1** in *M. avium* subsp. *paratuberculosis* cultures).[14,23] Several controls (DFO **3** and **8a**) were run in tandem. Mycobactin J (the native chelator, **1**) provided the benchmark control. In this manner, systems, which provided significantly higher growth index (GI) values than the native chelator **1** (GI = 1), were identified as superior growth stimulants and more efficacious iron delivery agents (vide infra). In addition, DFO **3** probed the effect of simply providing solubilized ferric ion as the determining factor in stimulating growth. The results are listed in Table 1.

As shown in Table 1, most derivatives were virtually equivalent or superior to mycobactin J in stimulating *M. avium* subsp. *paratuberculosis* growth. Earlier studies had suggested that systems containing longer tethers gave higher GI values.[17] We speculated that the longer tether allowed for a more conformationally flexible ligand to properly coordinate to iron and provides an incremental increase in hydrophobicity. Indeed, such tether effects have been observed with other siderophore systems such as DFO **3**. Molecular modeling studies with DFO suggest that tethers, which are too short (<5 C), may compromise the optimum binding geometry around iron by perturbing the octahedral complex.[1b]

Caveat. Earlier studies showed a nearly 5-fold growth increase of *M. avium* subsp. *paratuberculosis* in the presence of acinetoferrin imide **8a** (GI = 4.6)[17] vs the control platform (mycobactin J). Using a revised protocol, we observed an increase in growth index for media supplemented with acinetoferrin imide compared to those with mycobactin J. However, the increase in growth index was lower than reported in an earlier trial (Table 1, **8a**: 1.2). We believe that this earlier result was correct in identifying the growth enhancement trend but incorrect in its magnitude. The earlier experiments were also run in triplicate as reported here and had both mycobactin J and DFO as separate controls run in parallel. The different observations with the same siderophore **8a** warranted further investigation.

Careful scrutiny of the bacteria-dosing protocol found that the mycobacteria tend to clump upon standing. The original bacteria-injection protocol inoculated each vial in a successive manner using a stock suspension of mycobacteria. Therefore, it was possible that more bacterial count (e.g., a rogue clump) could be introduced into one vial over another. This clumping phenomenon could then skew the results because the BACTEC instrument would detect more bacteria over time. To remove the possibility of this event, we altered our protocol to include a resuspension of the bacterial culture just before each inoculation. In this manner the bacterial clumps were broken up and resuspended just prior to injection into the respective BACTEC vial. Additionally, the new protocol was shown to be very reproducible, where repeated trials gave the same relative growth indices.

Trends. As shown in Table 1, the BACTEC readings were normalized to the growth observed with native mycobactin J, **1**. By use of the revised protocol, **8a** was only a modest growth stimulant (Table 1, **8a** GI = 1.21) for *M. avium* subsp. *paratuberculosis*. The related C5 analogue **8b** also had enhanced growth activity (GI = 1.17). However, the C6 homologue **8c** was equivalent to **1** (GI = 1.01). [Note: since the error associated with the BACTEC reading of **8c** (515 ± 98) was large, the experiment was repeated and gave the same relative activities.] Therefore, the longer pentylene tether length of **8b** did not radically improve the GI found with **8a**. However, this modest enhancement was lost upon further extension of the tether (e.g., hexylene **8c**, GI = 1.01). It is possible that this reduction in growth-stimulating activity may be due to increasing entropic considerations associated with the highly flexible ligand **8c**.

Comparison of acyclic **5b** (C5 tether, GI = 0.96) and imide **8b** (C5 tether, GI = 1.17) reaffirmed the significance of the cyclic imide architecture. However, this trend was only significant when the proper tether was

in place [e.g., acyclic **5c** (C6 tether, GI = 0.95) and imide **8c** (C6 tether, GI = 1.01)]. The fact that both the native chelator (**1**) and **8** each contain a cyclic bidentate ligand within their architectures may be more than a curious coincidence and deserves further study.

Last, the fact that all ligands tested had growth activity near the native chelator **1** is noteworthy. While the bacteria's structural preferences for the iron complex seem less important than our original estimates, the tolerances accommodated by the *M. avium* subsp. *paratuberculosis* iron transport system provide new opportunities for drug delivery by this pathway. In addition, there may be significant cost savings associated with harvesting these slow-growing organisms by using alternative chelators. Since one is able to replace ferric mycobactin J (Allied Monitor, Inc.: $6500/g) with a less expensive chelator like DFO **3** (mesylate salt, Sigma-Aldrich, Inc.: $70/g), these studies have also provided an economic alternative to the mycobacteria community.

Conclusions

In summary, several acinetoferrin homologues were synthesized and showed good activity in cross-feeding *M. avium* subsp. *paratuberculosis* with similar efficiency as the native chelator, mycobactin J, **1**. In this regard, significant cost savings can be obtained by using alternative chelators to grow mycobacteria.

Experimental Section

General. *Trans*-2-octenoic acid (97.1% pure) was purchased from Lancaster Synthesis, Inc. All solutions are expressed in vol %. Ammonia-containing solutions were prepared by measuring out concentrated aqueous NH_4OH in the listed vol %. The pH 10.5 carbonate buffer solution was prepared by combining 222 mL of 0.75 N aqueous $NaHCO_3$ and 78 mL of 1.5 N aqueous NaOH. "Iron-free" glassware was obtained by soaking the glassware in 6 N HCl bath overnight, then washing with deionized water. Deionized water was obtained from a "B-pure" filtration system by collecting the water when the "in-line" electrical resistance was 17.0 MΩ·cm. "Iron-free" silica gel was made by washing with methanol/acetone/10 M HCl (45:45:10), followed by 10% (w/w) Na_2CO_3 solution, then rinsing with deionized water until pH 7 was attained, and air-drying. Every effort was made to prepare the final acinetoferrin analogues as metal-free ligands. While it is possible that trace iron contaminants could be present, all tested materials passed elemental analysis as metal-free ligands (see Supporting Information). The respective metal complexes are formed when these ligands are dosed into a culture broth containing ferric ammonium citrate (0.04 g/L media) during the bioassay.

Acinetoferrin C5 Homologue (5b). TFA (15 mL) was added dropwise to **20** (0.287 g, 0.41 mmol) at 0 °C. After the addition was complete, the stirred solution was allowed to warm to room temperature. TLC (10% MeOH/$CHCl_3$) showed that no starting material remained after 3 h. The volatiles were removed in vacuo to give a light-brown oil that was purified by column chromatography using LH-20 Sephadex (30 g, 8% EtOH/toluene) to give the acinetoferrin analogue **5b** as a white solid (0.182 g, 77%). **5b**: R_f = 0.3 (10% MeOH/$CHCl_3$); mp 122−124 °C; ^{1}H NMR ($CDCl_3$) δ 7.15 (br s, 2H, NH), 6.85 (m, 2H, olefinic), 6.65 and 6.05 (m, 2H, olefinic), 3.67 (m, 4H, CH_2NO), 3.20 (q, 4H, CH_2NHCO), 2.67 (d, 2H, CH_2C=O), 2.52 (d, 2H, CH_2C=O), 2.21 (q, 2H, CH_2C=C), 1.68 (m, 4H, CH_2), 1.58−1.11 (m, 29H, 10 × CH_2, $C(CH_3)_3$), 0.83 (t, 6H, CH_3); ^{1}H NMR (CD_3OD) δ 6.83 (dt, 2H, olefinic), 6.61 (d, 2H, olefinic), 3.65 (t, 4H, CH_2NO), 3.17 (t, 4H, CH_2NHCO), 2.73 (d, 2H, CH_2C=O), 2.63 (d, 2H, CH_2C=O), 2.25 (ddd, 2H, CH_2C=C), 1.67 (quin, 4H, CH_2), 1.52 (m, 8H, 4 × CH_2), 1.41−1.27 (m, 12H, 6 × CH_2), 0.92 (t, 6H, CH_3); ^{13}C NMR (CD_3OD) δ 176.87, 171.96, 168.26, 148.07, 120.46, 75.20, 44.99, 40.27, 33.58, 31.69, 30.02, 29.38, 27.49, 25.04, 23.71, 14.56; HRMS (FAB) calcd for $C_{32}H_{57}N_4O_9$ (M + 1), 641.4126; found (M + 1), 641.4112.

Acinetoferrin C6 Homologue (5c). TFA (15 mL) was added dropwise to **21** (0.266 g, 0.37 mmol) at 0 °C. After the addition was complete, the stirred solution was allowed to warm to room temperature. TLC (10% MeOH/$CHCl_3$) showed that no starting material remained after 3 h. The volatiles were removed in vacuo to give a light-brown oil that was purified by column chromatography using LH-20 Sephadex (30 g, 8% EtOH/toluene) to give **5c** as a white solid (0.165 g, 67%). **5c**: R_f = 0.35 (10% MeOH/$CHCl_3$); mp 118−119 °C; ^{1}H NMR (CD_3OD) δ 6.83 (dt, 2H, olefinic), 6.61 (d, 2H, olefinic), 3.65 (t, 4H, CH_2NO), 3.16 (t, 4H, CH_2NHCO), 2.74 (d, 2H, CH_2C=O), 2.62 (d, 2H, CH_2C=O), 2.25 (ddd, 2H, CH_2C=C), 1.65 (quin, 4H, CH_2), 1.48 (m, 8H, 4 × CH_2), 1.42−1.21 (m, 16H, 8 × CH_2), 0.92 (t, 6H, CH_3); ^{13}C NMR (CD_3OD) δ 176.88, 171.96, 168.40, 148.03, 120.51, 75.24, 44.98, 40.38, 33.50, 32.70, 30.35, 29.40, 27.71, 27.47, 23.72, 14.57; HRMS (FAB) calcd for $C_{34}H_{61}N_4O_9$ (M + 1), 669.4439; found (M +1), 669.4417.

Imide of Acinetoferrin C5 Homologue (8b). TFA (15 mL) was added dropwise to **20** (0.12 g, 0.17 mmol) at 0 °C. After the addition was complete, the stirred solution was allowed to warm to room temperature. TLC (10% MeOH/$CHCl_3$) showed that no starting material remained after 3 h. The mixture was then refluxed for a further 3 h. TLC (10% MeOH/$CHCl_3$) showed a new spot with a higher R_f. The volatiles were removed in vacuo to give a light-brown oil that was purified by flash chromatography (5% MeOH/$CHCl_3$) using "pretreated" silica gel to give the product **8b** as a white solid (0.046 g, 43%). **8b**: R_f = 0.28 (5% MeOH/$CHCl_3$), R_f = 0.57 (10% MeOH/$CHCl_3$); mp 142−144 °C; ^{1}H NMR (CD_3OD) δ 6.83 (dt, 2H, olefinic), 6.61 (d, 2H, olefinic), 3.64 (t, 4H, CH_2NO), 3.49 (t, 2H, CH_2NHCO), 3.11 (t, 2H, CH_2NHCO), 2.99 (d, 1H, CH_2C=O), 2.89 (d, 1H, CH_2C=O), 2.72 (d, 1H, CH_2C=O), 2.59 (d, 1H, CH_2C=O), 2.24 (q, 2H, CH_2C=C), 1.63 (m, 6H, CH_2), 1.48 (m, 6H, CH_2), 1.38−1.27 (m, 12H, 6 × CH_2), 0.91 (t, 6H, CH_3); ^{13}C NMR (CD_3OD) δ 180.47, 176.83, 171.17, 168.31, 148.05, 120.53, 73.77, 40.26, 39.66, 33.71, 32.72, 30.07, 29.42, 28.35, 27.55, 25.09, 23.74, 14.58; HRMS (FAB) calcd for $C_{32}H_{55}N_4O_8$ (M + 1), 622.4020; found (M + 1), 622.4046.

Imide of Acinetoferrin C6 Homologue (8c). TFA (15 mL) was added dropwise to **21** (0.095 g, 0.13 mmol) at 0 °C. After the addition was complete, the stirred solution was allowed to warm to room temperature. TLC (10% MeOH/$CHCl_3$) showed that no starting material remained after 3 h. The mixture was then refluxed for a further 3 h. TLC (10% MeOH/$CHCl_3$) showed a new spot with a higher R_f. The volatiles were removed in vacuo to give a light-brown oil that was purified by flash chromatography (5% MeOH/$CHCl_3$) using "pretreated" silica gel to give the product **8c** as a white solid (0.035 g, 41%). **8c**: R_f = 0.33 (5% MeOH/$CHCl_3$), R_f = 0.62 (10% MeOH/$CHCl_3$); mp 154−155 °C; ^{1}H NMR (CD_3OD) δ 6.83 (dt, 2H, olefinic), 6.61 (d, 2H, olefinic), 3.63 (t, 4H, CH_2NO), 3.48 (t, 2H, CH_2NHCO), 3.09 (t, 2H, CH_2NHCO), 3.00 (d, 1H, CH_2C=O), 2.89 (d, 1H, CH_2C=O), 2.72 (d, 1H, CH_2C=O), 2.59 (d, 1H, CH_2C=O), 2.23 (q, 2H, CH_2C=C), 1.62 (m, 6H, CH_2), 1.47 (m, 6H, CH_2), 1.38−1.29 (m, 16H, 8 × CH_2), 0.91 (t, 6H, CH_3); ^{13}C NMR (CD_3OD) δ 180.39, 176.79, 171.12, 147.88, 120.63, 73.95, 43.34, 40.33, 39.83, 33.53, 32.65, 30.39, 29.37, 28.56, 27.70, 27.59, 27.47, 27.41, 23.64, 14.45; HRMS (FAB) calcd for $C_{34}H_{59}N_4O_8$ (M + 1), 651.4333; found (M +1), 651.4354.

5-(*tert*-Butoxycarbonylamino)pentylamine (11). 1,5-Diaminopentane **9** (10.2 g, 0.1 mol) was dissolved in a solution of triethylamine and methanol (10% TEA in MeOH, 220 mL). A solution of di-*tert*-butyl dicarbonate (7.27 g, 0.033 mol) in methanol (20 mL) was added to this mixture with vigorous stirring. The mixture was refluxed for 2 h and left to stir at room temperature overnight. The *tert*-butoxy-carbonylation was complete as evidenced by TLC (4% NH_4OH/MeOH). The excess **9**, methanol, and TEA were removed in vacuo to yield an oily residue that was dissolved in CH_2Cl_2 and washed with a solution of 10% aqueous Na_2CO_3. The organic layer was

separated, dried over anhydrous Na_2SO_4, and filtered, and the solvent was removed in vacuo. The oily residue was purified by flash column chromatography to give the product **11** as a yellow oil (4.88 g, 73%). **11**: $R_f = 0.3$ (1:10:89 NH_4OH/MeOH/$CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.59 (br s, 1H, NH), 3.10 (q, 2H, CH_2NBOC), 2.68 (t, 2H, CH_2N), 1.55−1.27 (m, 15H, 3 × CH_2, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 156.12, 79.02, 42.12, 40.63, 33.38, 30.11, 28.64, 24.27; HRMS (FAB) calcd for $C_{10}H_{23}N_2O_2$ (M + 1), 203.1760; found (M + 1), 203.1757.

6-(*tert*-Butoxycarbonylamino)hexylamine (12). 1,6-Diaminohexane **10** (11.6 g, 0.1 mol) was dissolved in a solution of triethylamine and methanol (10% TEA in MeOH, 220 mL). A solution of di-*tert*-butyl dicarbonate (7.27 g, 0.033 mol) in methanol (20 mL) was added to this mixture with vigorous stirring. The mixture was refluxed for 2 h and left to stir at room temperature overnight. The *tert*-butoxy-carbonylation was complete as evidenced by TLC (4% NH_4OH/MeOH). The excess **10**, methanol, and TEA were removed in vacuo to yield an oily residue that was dissolved in CH_2Cl_2 and washed with a solution of 10% aqueous Na_2CO_3. The organic layer was separated, dried over anhydrous Na_2SO_4, and filtered, and the solvent was removed in vacuo. The oily residue was purified by flash column chromatography (1:10:89 NH_4OH/MeOH/$CHCl_3$) to give the product **12** as a yellow oil (5.17 g, 72%). **12**: R_f = 0.35 (1:10:89 NH_4OH/MeOH/$CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.90 (br s, 1H, NH), 3.11 (q, 2H, CH_2NBOC), 2.67 (t, 2H, CH_2N), 1.55−1.27 (m, 17H, 4xCH_2, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 156.13, 79.07, 42.29, 40.73, 33.86, 30.33, 28.72, 26.92, 26.82; HRMS (FAB) calcd for $C_{11}H_{25}N_2O_2$ (M + 1), 217.1916; found (M + 1), 217.1717.

***N*-(5-(*tert*-Butoxycarbonylamino)pentyl)-*N*-(benzoyloxy)-2(*E*)-octenamide (13).** A solution of benzoyl peroxide (BPO, 3.29 g, 0.014 mol) in CH_2Cl_2 (50 mL) was added dropwise to a vigorously stirred mixture of **11** (2.5 g, 0.0124 mol) in a carbonate buffer solution (pH 10.5, 50 mL) at room temperature. The buffer solution was prepared by combining 0.75 N aqueous $NaHCO_3$ (37 mL) and 1.5 N aqueous NaOH (13 mL). The starting material was consumed overnight as shown by TLC (4% NH_4OH/MeOH). Acylation of the oxidized intermediate was carried out in situ by the dropwise addition of a solution of *trans*-2-octenoyl chloride (1.99 g, 0.0124 mol) in CH_2Cl_2 (20 mL). Note: the acid chloride was generated from *trans*-2-octenoic acid using oxalyl chloride[24] and was freshly made prior to use. The disappearance of the *N*-benzoyloxyamine was monitored by TLC (40% EtOAc/hexane). After the acylation was complete, the organic layer was separated off and the remaining water layer was extracted with CH_2Cl_2. The organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give the crude product. The crude product was purified by flash column chromatography (20% EtOAc/hexane) to yield the product **13** as a brown oil (3.36 g, 61%). **13:** $R_f = 0.3$ (25% EtOAc/hexane); ^1H NMR ($CDCl_3$) δ 8.10 (d, 2H, phenyl), 7.67 (t, 1H, phenyl), 7.52 (t, 2H, phenyl), 7.01 (dt, 1H, olefinic), 6.05 (d, 1H, olefinic), 4.64 (br s, 1H, NH), 3.86 (t, 2H, CH_2NO), 3.10 (q, 2H, CH_2NBOC), 2.14 (q, 2H, $CH_2C{=}C$), 1.69 (quin, 2H, CH_2), 1.59−1.07 (m, 19H, 5 × CH_2, $C(CH_3)_3$), 0.83 (t, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 164.74, 156.24, 149.55, 134.70, 130.28, 129.19, 127.05, 118.32, 79.39, 48.72, 40.83, 32.88, 31.66, 30.06 28.84, 28.20, 27.25, 24.29, 22.82, 14.39; HRMS (FAB) calcd for $C_{25}H_{39}N_2O_5$ (M + 1), 447.2859; found (M +1), 447.2846.

***N*-(6-(*tert*-Butoxycarbonylamino)hexyl)-*N*-(benzoyloxy)-2(*E*)-octenamide (14).** A solution of BPO (3.29 g, 0.014 mol) in CH_2Cl_2 (50 mL) was added dropwise at room temperature to a vigorously stirred mixture of **12** (2.67 g, 0.0124 mol) in a carbonate buffer solution (pH 10.5, 50 mL). The starting material was consumed overnight as shown by TLC (4% NH_4OH/MeOH). Acylation of the oxidized intermediate was carried out in situ by the dropwise addition of a solution of *trans*-2-octenoyl chloride (1.99 g, 0.0124 mol) in CH_2Cl_2 (20 mL). The disappearance of the *N*-benzoyloxyamine was monitored by TLC (40% EtOAc/hexane). After the acylation was complete the organic layer was separated and the remaining water layer was extracted with CH_2Cl_2. The organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give the crude product. The crude product was purified by flash column chromatography (20% EtOAc/hexane) to yield the product **14** as an oil (3.69 g, 65%). **14**: $R_f = 0.35$ (25% EtOAc/hexane); ^1H NMR ($CDCl_3$) δ 8.11 (d, 2H, phenyl), 7.67 (t, 1H, phenyl), 7.52 (t, 2H, phenyl), 7.01 (dt, 1H, olefinic), 6.04 (dt, 1H, olefinic), 4.61 (br s, 1H, NH), 3.85 (t, 2H, CH_2NO), 3.08 (t, 2H, CH_2NBOC), 2.14 (ddd, 2H, $CH_2C{=}C$), 1.67 (quin, 2H, CH_2), 1.58−1.05 (m, 21H, 6 × CH_2, $C(CH_3)_3$), 0.83 (t, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 164.65, 156.14, 149.40, 134.60, 130.20, 129.10, 127.03, 118.29, 79.40, 48.70, 40.77, 32.80, 31.58, 30.26 28.78, 28.13, 27.43, 26.74, 26.67, 22.75, 14.31; HRMS (FAB) calcd for $C_{26}H_{41}N_2O_5$ (M + 1), 461; found (M + 1), 461.

***N*-(5-(*tert*-Butoxycarbonylamino)pentyl)-*N*-(hydroxy)-2-(*E*)-octenamide (15).** A 10% NH_4OH/MeOH solution (50 mL) was added dropwise to **13** (3.12 g, 7.0 mmol) under nitrogen at −20 °C using a dry ice/MeOH bath. The reaction was monitored by TLC (40% EtOAc/hexane). After 3 h the reaction was complete. The mixture was concentrated in vacuo to give a light-brown oil that was coevaporated with benzene and $CHCl_3$. The crude product was purified by flash column chromatography (20% EtOAc/hexane) using "pretreated" silica gel to give the product **15** as a pink solid (2.02 g, 84%). **15**: R_f = 0.25 (40% EtOAc/hexane); mp 56−58 °C; ^1H NMR ($CDCl_3$) δ 6.90 (m, 1H, olefinic), 6.64 and 6.03 (m, 1H, olefinic), 4.67 (m, 1H, NH), 3.70 (t, 2H, CH_2NO), 3.11 (m, 2H, CH_2NBOC), 2.20 (m, 2H, $CH_2C{=}C$), 1.70 (m, 2H, CH_2), 1.58−1.12 (m, 19H, 5 × CH_2, $C(CH_3)_3$), 0.83 (t, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 167.52, 156.22, 147.96, 147.13, 119.61, 116.32, 79.70, 49.26, 47.85, 40.12, 39.88, 32.87, 31.74, 30.07, 28.78, 28.34, 25.80, 23.97, 22.81, 14.36; HRMS (FAB) calcd for $C_{18}H_{35}N_2O_4$ (M + 1), 343.2591; found (M + 1), 343.2619.

***N*-(6-(*tert*-Butoxycarbonylamino)hexyl)-*N*-(hydroxy)-2(*E*)-octenamide (16).** A 10% NH_4OH/MeOH solution (50 mL) was added dropwise to **14** (3.42 g, 7.4 mmol) under nitrogen at −20 °C using a dry ice/MeOH bath. The reaction was monitored by TLC (40% EtOAc/hexane). After 3 h the reaction was complete. The mixture was concentrated in vacuo to give a light-brown oil that was coevaporated with benzene and $CHCl_3$. The crude product was purified by flash column chromatography (30% EtOAc/hexane) using "pretreated" silica gel to give the product **16** as a pink solid (2.26 g, 86%). **16**: R_f = 0.3 (40% EtOAc/hexane); mp 61−63 °C; ^1H NMR ($CDCl_3$) δ 6.95 (dt, 1H, olefinic), 6.02 (d, 1H, olefinic), 4.50 (br s, 1H, NH), 3.68 (t, 2H, CH_2NO), 3.09 (t, 2H, CH_2NBOC), 2.22 (q, 2H, $CH_2C{=}C$), 1.70 (m, 2H, CH_2), 1.58−1.06 (m, 21H, 6 × CH_2, $C(CH_3)_3$), 0.83 (t, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 167.43, 156.39, 147.68, 146.98, 119.58, 116.58, 79.45, 49.04, 48.24, 41.98, 40.60, 32.86, 31.71, 30.08, 28.74, 28.47, 28.32, 26.62, 26.39, 22.79, 14.35; HRMS (FAB) calcd for $C_{19}H_{37}N_2O_4$ (M + 1), 357.2748; found (M + 1), 357.2765.

***N*-(5-Aminopentyl)-*N*-(hydroxy)-2 (*E*)-octenamide, Trifluoroacetic Acid Salt (17).** TFA (50 mL) was added dropwise over 5 min to **15** (1.95 g, 5.7 mmol) at 0 °C under a nitrogen atmosphere. The ice bath was removed, and the solution was stirred at room temperature for 15 min. The volatiles were removed in vacuo, and the residue was coevaporated with benzene and $CHCl_3$ to give the product **17** as the TFA salt (2.02 g). The oil was consumed immediately in the synthesis of the *tert*-butylacinetoferrin homologue, **20**.

***N*-(6-Aminohexyl)-*N*-(hydroxy)-2(*E*)-octenamide, Trifluoroacetic Acid Salt (18).** TFA (50 mL) was added dropwise over 5 min to **16** (2.2 g, 6.2 mmol) at 0 °C under a nitrogen atmosphere. The ice bath was removed, and the solution was stirred at room temperature for 15 min. The volatiles were removed in vacuo and the residue was coevaporated with benzene and $CHCl_3$ to give the product **18** as the TFA salt (2.28 g). The oil was consumed immediately in the synthesis of the *tert*-butyl acinetoferrin homologue, **21**.

Acinetoferrin C5 Homologue *tert*-Butyl Ester (20). In an "iron-free" round-bottom flask 3-*tert*-butyl citrate[5,17] (0.21 g, 0.85 mmol) was dissolved in dry THF (20 mL). *N*-Hydroxysuccinimide (NHS, 0.214 g, 1.86 mmol) and dicyclohexylcar-

bodiimide (DCC) (0.385 g, 1.86 mmol) were added to this solution, and the reaction mixture was stirred at room temperature for 3 h producing the activated 1, 3-bis-*N*-hydroxysuccinimide ester **19** and a white precipitate. The solution was concentrated in vacuo, giving a white solid residue that was used directly for the next coupling step. The crude white solid was dissolved in dry dioxane (20 mL). A solution of **17** (0.659 g, 0.186 mmol, 2.2 equiv) in CH_2Cl_2 (10 mL) was first neutralized with 1.4 mL of TEA at 0 °C and then added to the dioxane solution. The mixture was stirred at room temperature overnight. The dicyclohexylurea was removed by filtration, and the solvents were removed in vacuo. The oily residue was dissolved in $CHCl_3$ and washed with 0.5 N HCl solution. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$. The organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (gradient from 2% MeOH/$CHCl_3$ to 6% MeOH/$CHCl_3$) using "pretreated" silica gel to give the product **20** as a pale-yellow oil (0.36 g, 60%). **20**: R_f = 0.6 (10% MeOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.15 (br s, 2H, NH), 6.85 (m, 2H, olefinic), 6.65 and 6.05 (m, 2H, olefinic), 3.67 (m, 4H, CH_2NO), 3.20 (q, 4H, CH_2NHCO), 2.67 (d, 2H, $CH_2C{=}O$), 2.52 (d, 2H, $CH_2C{=}O$), 2.21 (q, 2H, $CH_2C{=}C$), 1.68 (m, 4H, CH_2), 1.58−1.11 (m, 29H, 10 × CH_2, $C(CH_3)_3$), 0.83 (t, 6H, CH_3); ^{1}H NMR (CD_3OD) δ 6.83 (dt, 2H, olefinic), 6.61 (d, 2H, olefinic), 3.65 (t, 4H, CH_2NO), 3.16 (t, 4H, CH_2NHCO), 2.68 (d, 2H, $CH_2C{=}O$), 2.58 (d, 2H, $CH_2C{=}O$), 2.25 (q, 2H, $CH_2C{=}C$), 1.67 (m, 4H, CH_2), 1.58−1.11 (m, 29H, 10 × CH_2, $C(CH_3)_3$), 0.83 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 173.33, 170.35, 167.42, 147.26, 119.77, 118.32, 83.10, 76.92, 48.11, 44.20, 38.93, 32.90, 31.80, 29.08, 28.42, 28.14, 25.98, 23.87, 22.84, 14.41; HRMS (FAB) calcd for $C_{36}H_{65}N_4O_9$ (M + 1), 697.4752; found (M + 1), 697.4770.

Acinetoferrin C6 Homologue *tert*-Butyl Ester (21). In an "iron-free" round-bottom flask 3-*tert*-butyl citrate[5,17] (0.23 g, 0.93 mmol) was dissolved in dry THF (20 mL). *N*-Hydroxysuccinimide (0.235 g, 2.0 mmol) and dicyclohexylcarbodiimide (DCC) (0.42 g, 2.0 mmol) were added to this solution, and the reaction mixture was stirred at room temperature for 3 h producing the activated 1, 3-bis-*N*-hydroxysuccinimide ester **19** and a white precipitate. The solution was concentrated in vacuo giving a white solid residue that was used directly for the next coupling step. The crude white solid was dissolved in dry dioxane (20 mL). A solution of **18** (0.75 g, 2.0 mmol, 2.2 equiv) in CH_2Cl_2 (10 mL) was first neutralized with 2.0 mL of TEA at 0 °C and then added to the dioxane solution. The mixture was stirred at room temperature overnight. The dicyclohexylurea (DCU) was removed by filtration, and the solvents were removed in vacuo. The oily residue was dissolved in $CHCl_3$ and washed with a 0.5 N HCl solution. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$. The organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (gradient from 4% MeOH/$CHCl_3$ to 6% MeOH/$CHCl_3$) using "pretreated" silica gel to give the product **21** as a pale-yellow oil (0.47 g, 70%). **21**: R_f = 0.65 (10% MeOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.10 (br s, 2H, NH), 6.81 (m, 2H, olefinic), 6.62 and 6.03 (m, 2H, olefinic), 3.64 (m, 4H, CH_2NO), 3.16 (m, 4H, CH_2NHCO), 2.69 (d, 2H, $CH_2C{=}O$), 2.55 (d, 2H, $CH_2C{=}O$), 2.19 (m, 2H, CH_2-$C{=}C$), 1.62 (m, 4H, CH_2), 1.58−1.05 (m, 33H, 12 × CH_2, $C(CH_3)_3$), 0.83 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 173.44, 170.17, 167.11, 147.79, 147.17, 119.62, 118.32, 83.06, 74.32, 49.23, 48.17, 44.04, 39.44, 32.90, 31.75, 29.44, 29.17, 28.39, 28.26, 28.14, 26.74, 26.39, 22.82, 14.40; HRMS (FAB) calcd for $C_{38}H_{69}N_4O_9$ (M + 1), 725.5065; found (M + 1), 725.5060.

Bioassay. BACTEC 12B mycobacteria, also known as Middlebrook 7H12 medium, was purchased from Becton Dickinson (Pittsburgh, PA).[14,23] It contains 7H9 broth base, casein hydrolysate, bovine serum albumin, catalase, and palmitic acid labeled with ^{14}C. It is specific for growing mycobacteria and is used in conjunction with the BACTEC brand 460 TB analyzer. The Middlebrook 7H9 broth base media consists of 4 mL of broth mixture included in a sealed bottle. The bottles therefore contain 7H9 and other ingredients such as ammonium sulfate, glutamic acid, sodium citrate, pyridoxine, biotin, disodium phosphate, ferric ammonium citrate (0.04 g/L media), magnesium sulfate, calcium chloride, zinc sulfate, and copper sulfate. The 7H9 broth was also supplemented with 100 mL/L OADC (oleic acid, bovine albumin, dextrose, catalase enrichment from the Becton Dickinson Company) and 0.05% Tween 80. Tween 80 is a detergent added to reduce clumping (Sigma-Aldrich Chemical Co.). Ferric mycobactin J (Allied Monitor, Fayette, MO) at a final concentration of 2.4 μM was added to the *M. avium* subsp. *paratuberculosis* strain ATCC 43015 culture (MAP) as a positive control. When the bacterium was challenged with other siderophores, ferric mycobactin J was not added to the culture media. Siderophores were added to the Bactec 7H12 B+ bottled liquid media using Becton Dickinson B-D "1 cm^3" sterile syringes to a final concentration of 2.4 μM. To each of these Bactec bottles, 100 μL of *freshly suspended* Bactec 7H12 B+ cultured MAP (GI reading of 100−300 with an average of 1 × 105 cfu/mL) was inoculated using Becton Dickinson B-D "1 cm^3" sterile syringes. Microbial growth activity in this culture medium is indicated by the release of $^{14}CO_2$ into the atmosphere of the sealed vial following the metabolism of ^{14}C-labeled palmitic acid by the microorganism.

The atmosphere of the sealed BACTEC 12B vial is aspirated from the vial via a sterile 1.0 cm^3 needle attached to a robotic arm inside the BACTEC 460 TB analyzer system. The BACTEC analyzer operates by initially drawing room air through a dust filter, a flush valve, and an ion chamber transferring all $^{14}CO_2$ into a CO_2 trap, where it is retained. This process cleans the electrometer and leaves it ready to start the next cycle. During the next cycle, a pair of 18G needles are heated. A pump produces a partial vacuum in the ion chamber used to lower the testing needles through the rubber septum of the vial being tested. A vacuum draws culture gas from the vial to the ion chamber. The electrometer measures the very small current that the radioactive $^{14}CO_2$ produces in the ion chamber. Following removal of the radioactive culture gas, fresh 5% CO_2 is introduced into the medium headspace every time a vial is tested, enhancing the growth of mycobacteria. The current measured by the electrometer is amplified and displayed as a numeric reading. The reading is measured on a scale of 0−999 and is an indication of microbial growth activity in the bottle. Usually, a reading of 10 or higher is an indication of definite microbial growth. The growth indices (GI values) listed in Table 1 were calculated by dividing the instrument reading obtained for the chelator by that obtained for the mycobactin J control. As is often the circumstance when looking at relative microbiological growth rates over time, the error in making these measurements can be appreciable (up to ±98 reading units). Slow-growing cultures may bias the results because their early growth is more time-sensitive, whereas faster growing cultures may give disproportionately higher values at early sample times. In this regard, one looks for significant differences in growth rates and overall trends. In the absence of any added chelator, the bacterial growth is extremely slow and well below the listed values. A typical negative control BACTEC reading is near 100 compared to the 450−620 BACTEC reading values obtained when the respective chelators are added (Table 1). Note: the acinetoferrin−iron complex was recently reported by Groves et al.[25]

Supporting Information Available: Elemental analyses for compounds **5b**, **5c**, **8b**, **8c**, **11**−**16**, **20**, and **21**. This material is available free of charge via the Internet at http://pubs.acs.org.

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