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Syntheses and Biological Activity of Amamistatin B and Analogs

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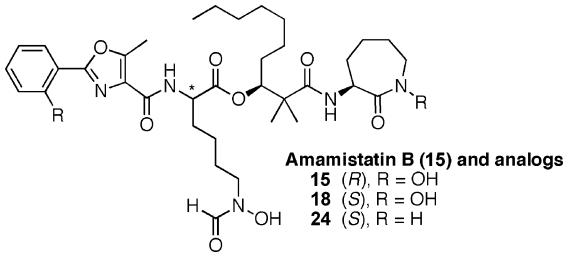

Amamistatins A and B, natural products isolated from a strain of *Nocardia*, showed growth inhibition against three human tumor cell lines (IC_{50} 0.24−0.56 μM). Structurally related mycobactins affect the growth of both mycobacterial and human cells through interference with iron chelation. To further probe the biological activity of this class of compounds, the total syntheses of amamistatin B and two analogs were completed, and the synthetic samples were screened for tumor cell growth inhibition, HDAC inhibition, and *Mycobacterium tuberculosis* growth inhibition. Amamistatin B (**15**) and diastereomer **18** were both active against MCF-7 cells (IC_{50} 0.12−0.20 μM), and less so against PC-3 cells (IC_{50} 8−13 μM). Amamistatin B only moderately inhibited the growth of *M. tuberculosis* (MIC 47 μM) but showed growth promotion of *Mycobacterium smegmatis* and other bacteria.

Introduction

Amamistatins A and B are a pair of natural products that were isolated from the actinomycete *Nocardia asteroides*.[1,2] As shown below, their structures were determined and, similar to mycobacterial siderophores, were found to contain a hydroxyphenyloxazole and both linear and cyclic forms of ϵ-*N*-hydroxylysine. Amamistatin A was reported to have antiproliferative effects against human tumor cell lines MCF-7 breast, A549 lung, and MKN45 stomach, with IC_{50} values of 0.48, 0.56, and 0.24 μM, respectively. Amamistatins A and B both showed cytotoxicity against mouse lymphocytic leukemia cells P388 (IC_{50} 15 and 16 ng/mL).[2] Related natural products formobactin,[3] nocobactin,[4] and brasilibactin[5] were also isolated from strains of *Nocardia*, with brasilibactin showing similar anticancer activity.

As indicated, the amamistatin structure is also related to that of mycobactins, intracellular siderophores produced by mycobacteria[6] for use in the acquisition and transport of iron.[7] Mycobactins are known to bind Fe^{3+} in a 1:1 ratio using three bidentate ligands: a hydroxyphenyl oxazoline and two hydroxamic acids. The addition of exogenous mycobactins can perturb the growth of mycobacteria,[6,8] and synthetic mycobactins have been investigated as anti-tuberculosis compounds based on their ability to interfere with the mycobacterial iron transport process.[9,10] Exochelin 772SM, an extracellular mycobacterial siderophore, has also shown selective cytotoxicity toward breast cancer cells versus normal cells due to effects of iron chelation.[11]

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(1) Suenaga, K.; Kokubo, S.; Shinohara, C.; Tsuji, T.; Uemura, D. *Tetrahedron Lett.* **1999**, *40*, 1945−1948.
(2) Kokubo, S.; Suenaga, K.; Shinohara, C.; Tsuji, T.; Uemura, D. *Tetrahedron* **2000**, *56*, 6435−6440.
(3) Murakami, Y.; Kato, S.; Nakajima, M.; Matsuoka, M.; Kawai, H.; Shin-Ya, K.; Seto, H. *J. Antibiot.* **1996**, *49*, 839−845.
(4) Ratledge, C.; Snow, G. A. *Biochem. J.* **1974**, *139*, 407−413.
(5) Tsuda, M.; Yamakawa, M.; Oka, S.; Tanaka, Y.; Hoshino, Y.; Mikami, Y.; Sato, A.; Fujiwara, H.; Ohizumi, Y.; Kobayashi, J. *J. Nat. Prod.* **2005**, *68*, 462−464.
(6) Snow, G. A. *Bacteriol. Rev.* **1970**, *34*, 99−125.
(7) Ratledge, C. *Tuberculosis* **2004**, *84*, 110−130.
(8) Bosne-David, S.; Bricard, L.; Ramiandrasoa, F.; Dé Roussent, A.; Kunesch, G.; Andremont, A. *Antimicrob. Agents Chemother.* **1997**, *41*, 1837−1839.
(9) Hu, J.; Miller, M. J. *J. Am. Chem. Soc.* **1997**, *119*, 3462−3468.
(10) Xu, Y.; Miller, M. J. *J. Org. Chem.* **1998**, *63*, 4314−4322.

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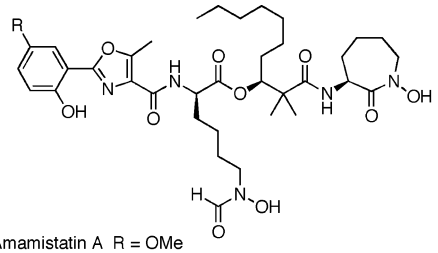
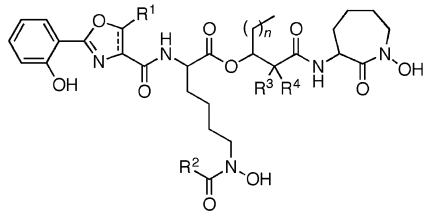


Amamistatin A R = OMe
Amamistatin B R = H



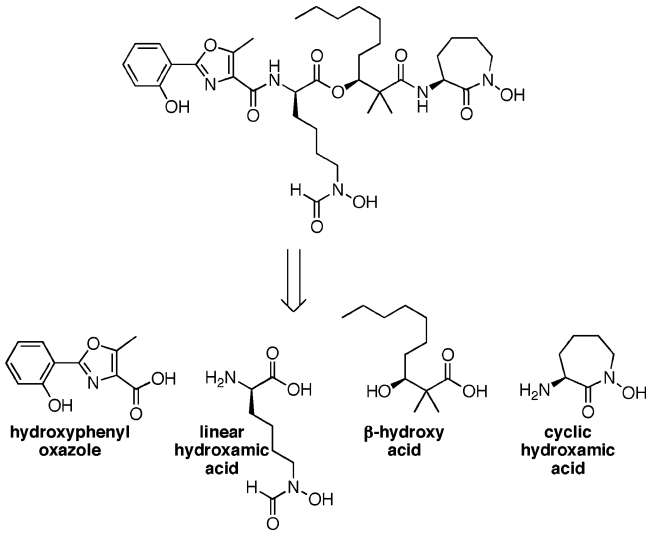
Formobactin (from *Nocardia*)
R^1 = Me, oxazole; R^2 = H; R^3 = R^4 = Me; *n* = 8
Nocobactin (from *N. asteroides*)
R^1 = Me, oxazole; R^2 = Me; R^3 = Me; R^4 = H; *n* = 8, 10
Brasilibactin (from *N. brasiliensis*)
R^1 = H, oxazoline; R^2 = H; R^3 = Me; R^4 = H; *n* = 14
Mycobactin M (from *M. marinum*)
R^1 = Me, oxazoline; R^2 = Me; R^3 = Me; R^4 = H; *n* = 16
Mycobactin T (from *M. tuberculosis*)
R^1 = H, oxazoline; R^2 = $(CH_2)_{19}CH_3$; R^3 = R^4 = H; *n* = 0
Exochelin 772SM (from *M. tuberculosis*)
R^1 = H, oxazoline; R^2 = $(CH_2)_5CO_2Me$; R^3 = R^4 = H; *n* = 0

The growth inhibition observed in breast cancer and other[12,13] cell lines upon treatment with exochelin 772SM is most likely caused by inhibition of ribonucleotide reductase,[14] an iron-containing enzyme that serves as the rate-limiting step in DNA synthesis.

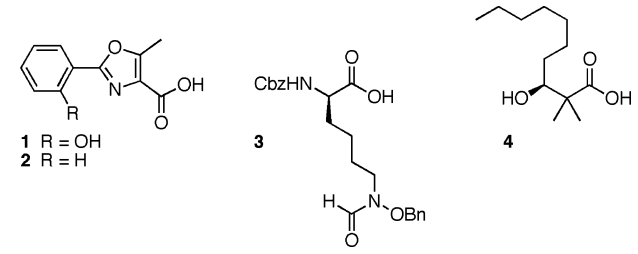
The anti-tumor activity of the amamistatins may be explained by a mode of action based on iron chelation, similar to that employed by mycobacterial compounds. The authors also proposed that the amamistatins could act as histone deacetylase (HDAC) inhibitors via their *N*-formyl hydroxylamine, or retrohydroxamate, moiety.[15] Inhibition of the HDAC enzyme leads to tumor growth suppression,[16−19] and the retrohydroxamate ligand has been previously employed in small molecule HDAC inhibitors.[20,21] In order to further probe the biological activities of this class of compounds, amamistatin B was synthesized along with two amamistatin analogs. All three compounds were screened for growth inhibition against MCF-7 and PC-3 human tumor cell lines as well as *Mycobacterium tuberculosis* $H_{37}Rv$ and other mycobacteria.

Results and Discussion

The synthesis of amamistatin B began with the preparation of its four primary components, a strategy also used for the syntheses of amamistatin A[22] and several mycobactin-type compounds.[9,10] The fragments obtained from retrosynthetic disconnection of amamistatin B include a hydroxyphenyl oxazole, linear and cyclic hydroxamic acids derived from lysine, and a β-hydroxy acid. The fragments were then coupled in a stepwise fashion to produce amamistatin B. Using this strategy, alternate fragment structures can easily be included for the synthesis of amamistatin analogs.



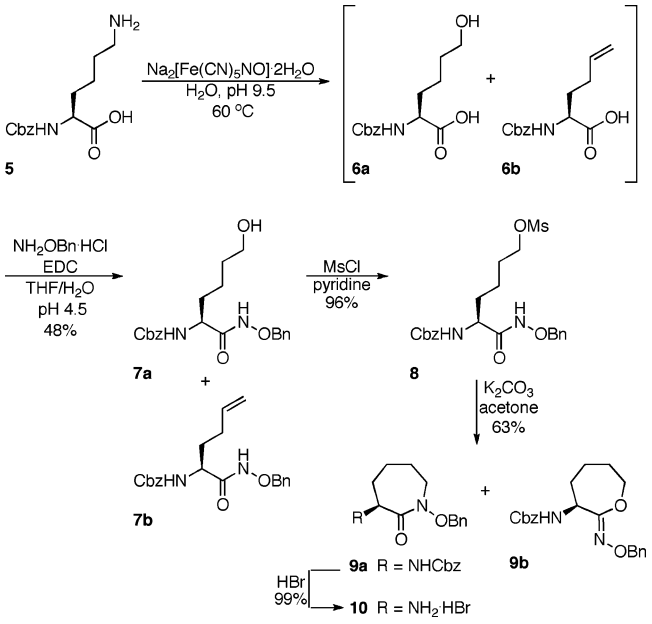
Syntheses of hydroxyphenyl oxazole fragments **1** and **2** were carried out according to a procedure described previously.[23] The synthesis of protected linear hydroxamate **3** from Cbz-D-lysine has also been reported.[23] β-Hydroxy acid **4** was synthesized using the Mukaiyama aldol method[24] reported during the synthesis of amamistatin A.[22]



The synthetic route used for cyclic hydroxamate **9** was based on earlier work on the synthesis of serine-derived β-lactams, where it was found that β-mesylated benzyl hydroxamates could be cyclized upon treatment with *t*BuOK at low temperature.[25] This same idea was applied to the larger ring system of **9** with

(11) Pahl, P. M. B.; Horwitz, M. A.; Horwitz, K. B.; Horwitz, L. D. *Breast Cancer Res. Treat.* **2001**, *69*, 69−79.
(12) Pahl, P. M. B.; Yan, X.-D.; Hodges, Y. K.; Rosenthal, E. A.; Horwitz, M. A.; Horwitz, L. D. *J. Biol. Chem.* **2000**, *275*, 17821−17826.
(13) Chong, T. W.; Horwitz, L. D.; Moore, J. W.; Sowter, H. M.; Harris, A. L. *Cancer Res.* **2002**, *62*, 6924−6927.
(14) Hodges, Y. K.; Antholine, W. E.; Horwitz, L. D. *Biochem. Biophys. Res. Commun.* **2004**, *315*, 595−598.
(15) Fennell, K. A.; Miller, M. J. *Abstracts of Papers*, 39th National Organic Chemistry Symposium, Salt Lake City, UT, June 12−16, 2005; American Chemical Society: Washington, DC, 2005; A80. Fennell, K. A.; Miller, M. J. *Abstracts of Papers*, 231st National Meeting of the American Chemical Society, Atlanta, GA, March 26, 2006; American Chemical Society: Washington, DC, 2006; ORGN 642.
(16) Meinke, P. T.; Liberator, P. *Curr. Med. Chem.* **2001**, *8*, 211−235.
(17) Johnstone, R. W. *Nat. Rev. Drug Discovery* **2002**, *1*, 287−299.
(18) Grozinger, C. M.; Schreiber, S. L. *Chem. Biol.* **2002**, *9*, 3−16.
(19) Miller, T. A.; Witter, D. J.; Belvedere, S. *J. Med. Chem.* **2003**, *46*, 5097−5116.
(20) Wu, T. Y. H.; Hassig, C.; Wu, Y.; Ding, S.; Schultz, P. G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 449−453.
(21) Nishino, N.; Yoshikawa, D.; Watanabe, L. A.; Kato, T.; Jose, B.; Komatsu, Y.; Sumida, Y.; Yoshida, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2427−2431.
(22) Yokokawa, F.; Izumi, K.; Omata, J.; Shioiri, T. *Tetrahedron* **2000**, *56*, 3027−3034.
(23) Fennell, K. A.; Miller, M. J. *Org. Lett.* **2007**, *9*, 1683−1685.
(24) Kiyooka, S.; Kaneko, Y.; Komura, M.; Matsuo, H.; Nakano, M. *J. Org. Chem.* **1991**, *56*, 2276−2278.

SCHEME 1. Synthesis of Cyclic Hydroxamate Fragment

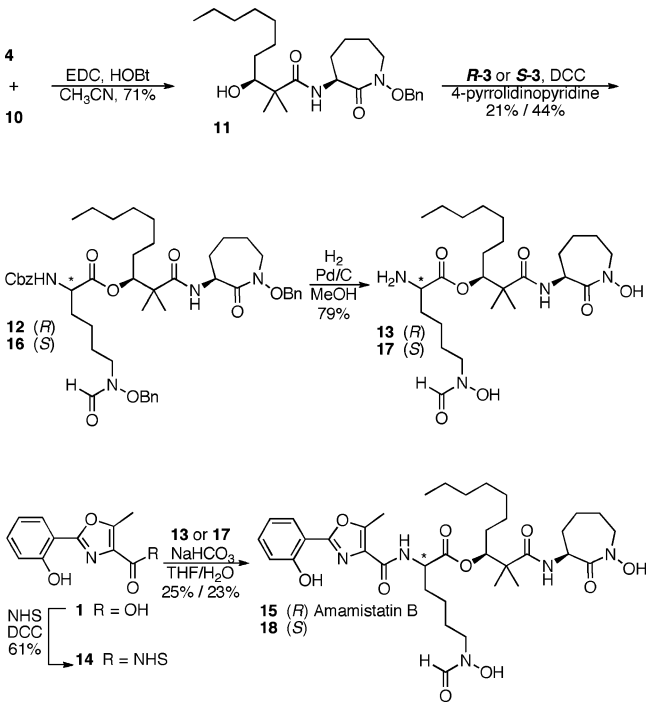


moderate success. For the first step, Cbz-lysine was converted to Cbz-hydroxynorleucine **6a** using nitroferricyanide, according to the procedure published by Baldwin[26] (Scheme 1). This reaction also resulted in the corresponding dehydration product **6b**, and the two products were used as a mixture for the following step. EDC-mediated coupling with *O*-benzyl hydroxylamine gave benzyl hydroxamates **7a** and **7b**, which were easily separated by chromatography. Conversion of **7a** to mesylate **8** proceeded cleanly in near quantitative yield. Upon treatment with *t*BuOK in DMF, mesylate **8** gave desired benzyl hydroxamate **9a** along with the O-cyclized hydroximate isomer **9b** in ~3:1 ratio and 80% combined yield. Cyclization with K_2CO_3 in refluxing acetone gave **9a** and **9b** in similar ratio and total yield, and the reaction was more consistent on a larger scale. Deprotection of the Cbz group of **9a** with HBr resulted in amine **10** as the hydrobromide salt.

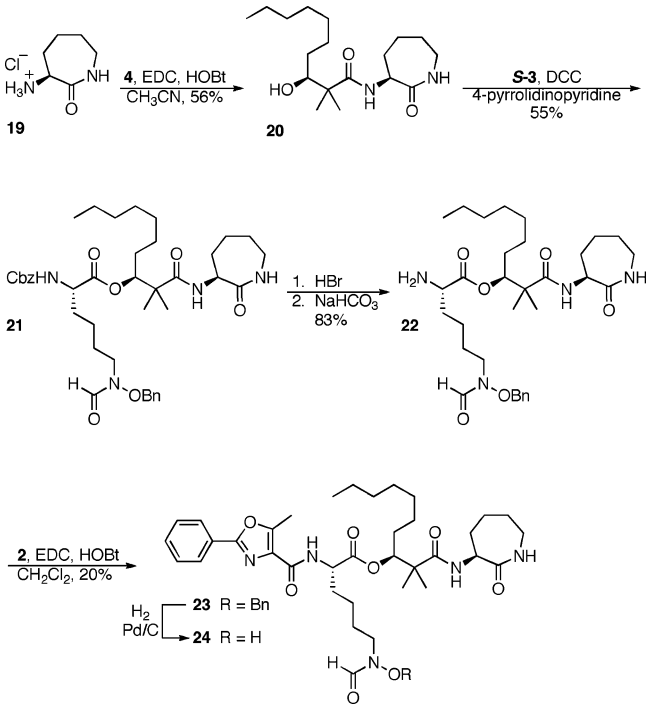
The synthesis of amamistatin B from its fragments began with EDC-mediated coupling between β-hydroxy acid **4** and cyclic hydroxamate **10** (Scheme 2). Ester formation between the resulting β-hydroxy amide **11** and linear hydroxamate **3** was carried out using DCC and catalytic 4-pyrrolidinopyridine.[27] Other esterification conditions, including acid chloride, acid fluoride, mixed anhydride, and active ester formation, as well as Mitsunobu conditions, were tried with no success. Global deprotection of ester **12** was accomplished by hydrogenolysis to give amine **13** with two free hydroxamic acids. Hydroxyphenyl oxazole **1** was converted to NHS-ester **14** and purified prior to use. Coupling of **14** with **13** led directly to amamistatin B **15**. This same synthetic sequence was repeated using the opposite enantiomer of linear hydroxamate **3** to give **18**, a diastereomer of amamistatin B.

A similar method, shown in Scheme 3, was used for the synthesis of **24**, an amamistatin analog lacking two of the metal-binding sites present in the parent compound. Commercially available L-(−)-α-amino-ϵ-caprolactam·HCl **19** was coupled to

SCHEME 2. Synthesis of Amamistatin B and a Diastereomer



SCHEME 3. Synthesis of Amamistatin Analog 24



β-hydroxy acid **4**, and the resulting β-hydroxy amide **20** was again esterified with *S*-**3** using DCC and catalytic 4-pyrrolidinopyridine. Cbz-deprotection of **21** was accomplished with 33 wt % HBr/AcOH. Coupling of amine **22** to phenyl oxazole **2** and deprotection of the benzyl hydroxamate with hydrogenolysis afforded amamistatin analog **24**.

The total synthesis of natural product amamistatin B (**15**) was completed as described above. The syntheses of amamistatin B diastereomer **18** and structural analog **24** were also carried out using similar methodology. The synthesis of amamistatin B

(25) Krook, M. A.; Miller, M. J. *J. Org. Chem.* **1985**, *50*, 1126−1128.
(26) Baldwin, J. E.; Killin, S. J.; Adlington, R. M.; Spiegel, U. *Tetrahedron* **1988**, *44*, 2633−2636.
(27) Hassner, A.; Alexanian, V. *Tetrahedron Lett.* **1978**, *46*, 4475−4478.

TABLE 1. Cellular Growth Inhibition by Amamistatin B and Analogs

compound	IC_{50} (μM) MCF-7	PC-3	VERO
15 (amamistatin B)	0.12	8	24
18	0.20	13	26
24	14	16	40

required approximately the same number of synthetic steps as were used in Shioiri's synthesis of amamistatin A,[22] and a similar stepwise fragment coupling strategy was employed. In order to facilitate the characterization of synthetic compounds, spectral data for amamistatin B (**15**) and analogs **18** and **24** were compared with data reported for amamistatins A and B[2] and formobactin[3] upon isolation (Supporting Information).

Synthetic amamistatin B (**15**) and analogs (**18**, **24**) were tested for growth inhibitory activity against MCF-7 (breast) and PC-3 (prostate) human tumor cells and for inhibition of HDAC in vitro. All three compounds possess an *N*-formyl hydroxylamine ligand but differ in their ability to bind iron with a 1:1 stoichiometry. None of the compounds displayed HDAC activity when tested at 100 μM. This mirrors the result obtained with a synthetic sample of brasilibactin A,[28] even though smaller amamistatin fragments containing the same retrohydroxamate ligand previously showed modest HDAC inhibition.[23] Presumably, the complete amamistatin and brasilibactin structures adopt a conformation that prevents the retrohydroxamate moiety from accessing the enzyme active site, thus resulting in the observed lack of HDAC inhibition.

All three amamistatin-like compounds demonstrated significant growth-inhibitory activity in the MCF-7 and PC-3 tumor cell assays (Table 1). Amamistatin B (**15**) and its diastereomer (**18**) both reached the nanomolar range of inhibition against MCF-7 cells, which are often more sensitive than other cell lines. In both assays, **15** showed the highest level of growth inhibition, followed closely by **18**. Analog **24**, lacking two of the bidentate metal ligands, was significantly less active. These values are comparable to the 0.48 μM IC_{50} value reported for amamistatin A against MCF-7 cells at the time of its isolation.[1] Growth inhibition of VERO (monkey kidney) cells was used as a measure of general mammalian cytotoxicity. Although all three synthetic compounds exhibited a concerning level of cytotoxicity, the amount needed for activity against MCF-7 cells may be low enough to provide an acceptable therapeutic window for compounds **15** and **18**. The difference in activity between analog **24** and stronger iron chelators **15** and **18** reinforces the idea that growth inhibition (of both tumor cells and normal cells) can be affected by changes in iron availability due to chelation. These results further support the belief that amamistatins and related natural products could serve as the source or inspiration for effective therapeutic agents for the treatment of cancer.

On the basis of the results of earlier studies using natural[6,8] and synthetic[9,10] mycobactins, it was proposed that amamistatins could have a similar inhibitory effect on the growth of *M. tuberculosis*. Of the three synthetic compounds studied here (**15**, **18**, **24**), only amamistatin B (**15**) exhibited observable activity against *M. tuberculosis* $H_{37}Rv$ in iron-sufficient (GAS) media (MIC 46.7 μM). In iron-deficient media, **15** showed 84% growth inhibition at 128 μM, but the activity quickly dropped off at lower concentrations.

(28) Mitchell, J. M.; Shaw, J. T. *Org. Lett.* **2007**, *9*, 1679−1681.

TABLE 2. Bacterial Growth Promotion by Amamistatin B

compound	diameter of growth zone (mm) *M. smegmatis* mc^2 155[a]	*B. subtilis* ATCC 6633	*S. aureus* SG 511	*P. aeruginosa* K199 PAO1[b]
amamistatin B **15**	24	37	9 (inhib)	28
Fe-mycobactin J (control)	19			
Fe-ferricrocin (control)	20	35	34	45

[a] Siderophore biosynthesis and transport deletion mutants exhibit growth zones of 20−24 mm. [b] Mutants deficient for siderophore biosynthesis and uptake exhibit growth zones of 24 and 27 mm, respectively.

Further studies showed that amamistatin B (**15**) is a potent growth promoter for a different mycobacteria, *Mycobacterium smegmatis* mc^2 155, as well as other Gram-positive and Gram-negative bacteria (Table 2). Growth of wild-type *M. smegmatis* mc^2 155[29] was promoted by **15** at a level similar to that of the native siderophore, Fe-loaded mycobactin J. Growth of *M. smegmatis* mutants[30] deficient for siderophore biosynthesis and transport was also promoted to the same extent, indicating that the activity of amamistatin B (**15**) is not dependent on the presence or uptake of native siderophores. The *M. smegmatis* mutants used for this study included mc^{2}155-B1 (exochelin biosynthesis deleted), mc^{2}155-M24 (mycobactin biosynthesis deleted), mc^{2}155-M24−B3 (exochelin and mycobactin biosynthesis deleted), and mc^{2}155-M24−U3 (mycobactin biosynthesis and exochelin permease deleted).

Growth of wild-type Gram-positive bacteria *Bacillus subtilis* ATCC 6633 was also promoted by **15**, while *Staphylococcus aureus* SG 511 was inhibited relative to the control (Fe-loaded ferricrocin). Amamistatin B was also able to promote the growth of Gram-negative *Pseudomonas aeruginosa* K199 PAO1 (wild-type) as well as mutants deficient for pyoverdin and pyochelin biosynthesis (K648) and the pyoverdin receptor (K690). *Salmonella typhimurium* (enb-7, enterobactin biosynthesis mutant) and *Eschericia coli* (wild-type AB 2847 and iron transport mutants BR 158, H1876, HK9/7, MS 172, and 41/2 lacking siderophore receptors TonB, FepA, Cir, Fiu, FhuA, and FhuE alone or in combination) failed to recognize or utilize **15**, and no growth promotion was observed (data not shown). Growth promotion by amamistatin B can be attributed to either direct uptake of the iron-loaded compound or transfer of solubilized Fe^{3+} to another chelator recognized by the organism. For the organisms in which no growth promotion is observed, it appears that iron sequestered by **15** cannot be accessed by the organism through direct uptake or ligand transfer, and growth in the iron-depleted media is impaired.

The iron-binding ability of amamistatin B was confirmed through the use of the chrome azurol S (CAS) assay.[31] The orange halo resulting from iron binding by a 5-μL sample of **15** had a diameter of 9 mm, a value typical for moderately lipophilic iron chelators. Mycobactins, despite their strong iron-binding ability, often display a negative result in the CAS assay, since their increased hydrophobicity prevents diffusion through the media. Although these studies show that amamistatin B (**15**) can bind iron and promote the growth of a variety of bacterial species under iron-deficient conditions, it is not yet clear whether

(29) Snapper, S. B.; Melton, R. E.; Mustafa, S.; Kieser, T.; Jacobs, W. R. *Mol. Microbiol.* **1990**, *4*, 1911−1919.

(30) Schumann, G.; Möllmann, U. *Antimicrob. Agents Chemother.* **2001**, *45*, 1317−1322.

(31) Schwynn, B.; Neilands, J. B. *Anal. Biochem.* **1987**, *160*, 47−56.

the compound is recognized as a siderophore and taken into the cell or simply serves as a source of solubilized iron that is then available for ligand exchange with the native siderophores produced by the organism.

The total syntheses of natural product amamistatin B (**15**) and two analogs (**18**, **24**) were carried out in order to further study the biological activity of this class of compounds. Amamistatin B and its analogs showed significant inhibitory activity against MCF-7 cells (**15** IC_{50} 120 nM, **18** IC_{50} 200 nM, **24** IC_{50} 14 μM) but were less potent in the PC-3 assay (IC_{50} 8−16 μM). All three compounds were inactive in the HDAC enzyme assay, and only **15** showed modest activity against *M. tuberculosis* (MIC ∼46 μM). Amamistatin B was also found to be a growth promoter for a variety of Gram-positive and Gram-negative bacteria. Insight into the structure−activity relationships of amamistatin analogs gained from these studies will help direct the future synthesis of compounds of this class targeted toward tuberculosis or tumor growth inhibition.

Experimental Section

(*S*)-*N*-(Benzyloxy)-2-(benzyloxycarbonylamino)-6-hydroxyhexanamide 7a. A solution of Cbz-Lys-OH **5** (3.0 g, 10.7 mmol) in H_2O (45 mL) at 60 °C (internal temp) was adjusted to pH 9.5 with 2 M NaOH. $Na_2[Fe(CN)_5NO]\cdot 2H_2O$ (3.71 g, 12.5 mmol) was added in portions over 30 min with vigorous stirring, and the pH was adjusted to 9.5 after each addition. The muddy brown slurry was stirred for 6 h at 60 °C and pH 9.5. The solution was cooled and filtered through Celite to remove the red-brown precipitate. The yellow-brown solution was acidified to pH 1 with 1 N HCl, and the resulting bright yellow solution was extracted with EtOAc (3 × 125 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated to give a mixture of (*S*)-2-(benzyloxycarbonylamino)-6-hydroxyhexanoic acid **6a** and (*S*)-2-(benzyloxycarbonylamino)hex-5-enoic acid **6b** as a crude orange oil (3.07 g). To a solution of **6a** and **6b** (2.91 g) in THF (95 mL) and H_2O (75 mL) was added $NH_2OBn\cdot HCl$ (2.48 g, 15.5 mmol), and the pH was adjusted to 4.5 with 4 N NaOH. EDC (11.2 g, 58.4 mmol) was added in portions, and the pH was adjusted to 4.5 with 1 N HCl after each addition. When the pH stopped rising (∼1.5 h), the solution was extracted with EtOAc (3 × 150 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The off-white solid (3.73 g) was purified by flash chromatography (SiO_2, 4:1 $CHCl_3$/EtOAc) to give **7a** as a white solid (1.99 g, 5.16 mmol, 48% from **5**): mp 115−116 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.13 (s, 1H), 7.39−7.31 (m, 4H), 7.30−7.24 (m, 6H), 5.90 (d, 1H, J = 9.0 Hz), 4.97 (2d, 2H, J = 12 Hz), 4.84 (d, 2H, J = 3.5 Hz), 4.05 (q, 1H, J = 7.5 Hz), 3.52−3.43 (m, 2H), 2.96 (br s, 1H), 1.74−1.67 (m, 1H), 1.60−1.53 (m, 1H), 1.48−1.40 (m, 2H), 1.40−1.24 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.5, 156.4, 136.0, 135.1, 129.3, 128.7, 128.6, 128.5, 128.3, 128.0, 78.2, 67.1, 62.0, 52.4, 32.3, 31.8, 21.8; HRMS (FAB) *m*/*z* calcd for $C_{21}H_{27}N_2O_5$ $[M + H]^+$ 387.1914, found 387.1917.

(*S*)-6-(Benzyloxyamino)-5-(benzyloxycarbonylamino)-6-oxohexyl methane-sulfonate 8. To a solution of **7a** (3.026 g, 7.83 mmol) in anhydrous pyridine (55 mL) in a flame-dried flask at 0 °C was added MsCl (0.73 mL, 9.43 mmol) slowly. The reaction was stirred for 25 min at 0 °C and for 2 h 20 min at room temperature. The solution was diluted with EtOAc (500 mL), washed with 1 N HCl (2 × 300 mL) and brine (300 mL), dried over Na_2SO_4, filtered, and concentrated to leave **8** as a white solid (3.49 g, 7.51 mmol, 96%) with no need for purification: mp 110−112 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.30 (s, 1H), 7.39−7.30 (m, 10H), 5.51 (d, 1H, J = 8.5 Hz), 5.08−4.96 (m, 2H), 4.87 (s, 2H), 4.19−4.11 (m, 2H), 4.06−3.99 (m, 1H), 2.95 (s, 3H), 1.81−1.58 (m, 4H), 1.44−1.36 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.1, 156.3, 135.9, 135.0, 129.3, 128.9, 128.6, 128.4, 128.1, 78.3, 69.5, 67.3, 52.2, 37.4, 31.5, 28.5, 21.4; HRMS (FAB) *m*/*z* calcd for $C_{22}H_{29}N_2O_7S$ $[M + H]^+$ 465.1690, found 465.1693.

(*S*)-1-(Benzyloxy)-3-(benzyloxycarbonylamino)azepan-2-one 9a. To a solution of **8** (1.94 g, 4.18 mmol) in acetone (100 mL) was added K_2CO_3 (1.73 g, 12.5 mmol), and the reaction was heated at reflux in a 75 °C oil bath for 16 h. The opaque white solution was cooled to room temperature, diluted with EtOAc (400 mL), washed with H_2O (2 × 250 mL) and brine (250 mL), dried over Na_2SO_4, filtered, and concentrated to a light yellow oil (1.57 g). The crude material was purified by flash chromatography (SiO_2, 98:2−90:10 CH_2Cl_2/EtOAc) to give **9a** as a light colored oil that solidified to a waxy off-white solid upon standing (962 mg, 2.61 mmol, 63%): mp 58−61 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43−7.31 (m, 10H), 6.15 (d, 1H, J = 6.0 Hz), 5.11 (s, 2H), 4.93 (2d, 2H, J = 10.5 Hz), 4.29−4.23 (m, 1H), 3.59−3.54 (m, 1H), 3.49−3.43 (m, 1H), 2.03−1.99 (m, 1H), 1.94−1.83 (m, 1H), 1.73−1.61 (m, 2H), 1.58−1.43 (m, 1H), 1.41−1.34 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.0, 155.4, 136.4, 135.0, 129.6, 128.8, 128.5, 128.4, 128.0, 127.9, 76.7, 66.6, 53.2, 52.6, 31.8, 27.5, 26.1; HRMS (FAB) *m*/*z* calcd for $C_{21}H_{25}N_2O_4$ $[M + H]^+$ 369.1809, found 369.1834.

(*S*)-3-Amino-1-(benzyloxy)azepan-2-one Hydrobromide 10. To a solution of **9a** (817 mg, 2.22 mmol) in CH_2Cl_2 (20 mL) was added 33 wt % HBr in AcOH (20 mL), and the reaction was stirred for 30 min. Volatiles were removed under reduced pressure. The orange residue was left under vacuum for 5 h to give **10** as an off-white solid (695 mg, 2.20 mmol, 99%): mp 217−219 °C (d); ^{1}H NMR (500 MHz, CD_3OD) δ 7.48−7.45 (m, 2H), 7.40−7.36 (m, 3H), 4.97 (2d, 2H, J = 10.5 Hz), 4.25−4.23 (m, 1H), 3.84−3.78 (m, 1H), 3.67−3.62 (m, 1H), 2.01−1.97 (m, 2H), 1.80−1.64 (m, 3H), 1.42−1.37 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 169.0, 136.6, 131.0, 130.1, 129.7, 77.8, 53.8, 53.5, 29.9, 28.0, 27.4; HRMS (FAB) *m*/*z* calcd for $C_{13}H_{19}N_2O_2$ $[M + H]^+$ 235.1441, found 235.1459.

(*S*)-*N*-((*S*)-1-(Benzyloxy)-2-oxoazepan-3-yl)-3-hydroxy-2,2-dimethyl-decanamide 11. To a suspension of (3*S*)-3-hydroxy-2,2-dimethyldecanoic acid **4** (456 mg, 2.11 mmol) and **10** (654 mg, 2.08 mmol) in CH_3CN (15 mL) were added Et_3N (0.293 mL, 2.09 mmol), HOBt (279 mg, 2.07 mmol), and EDC (435 mg, 2.27 mmol) sequentially. The off-white suspension was stirred at room temperature for 2 days. The reaction mixture was diluted with EtOAc (150 mL), washed with H_2O (75 mL), saturated $NaHCO_3$ (2 × 75 mL), H_2O (50 mL), 5% citric acid (2 × 75 mL), and brine (75 mL), dried over Na_2O_4, filtered, and concentrated to a white solid. Purification by flash chromatography (SiO_2, 5:1 CH_2Cl_2/EtOAc) gave **11** as a white solid (637 mg, 1.47 mmol, 71%): mp 153.5−154.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41−7.38 (m, 2H), 7.37−7.30 (m, 3H), 7.29 (d, 1H, J = 6.5 Hz), 4.92 (2d, 2H, J = 10.5 Hz), 4.42−4.39 (m, 1H), 3.64−3.57 (m, 1H), 3.54 (br s, 1H), 3.51−3.42 (m, 2H), 1.96−1.84 (m, 2H), 1.73−1.52 (m, 2H), 1.48−1.40 (m, 2H), 1.35−1.20 (m, s, 15H), 1.16 (s, 3H), 0.86−0.82 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.6, 170.8, 135.1, 129.6, 128.9, 128.6, 77.4, 76.8, 52.8, 51.9, 46.1, 31.9, 31.7, 31.0, 29.6, 29.3, 27.6, 26.7, 26.3, 23.6, 22.7, 20.7, 14.1; HRMS (FAB) *m*/*z* calcd for $C_{25}H_{41}N_2O_4$ $[M + H]^+$ 433.3061, found 433.3050; LC/MS (5%−80% CH_3CN/10 mM ammonium acetate) t_R 9.97, *m*/*z* for $[M + H]^+$ 433.4; $[\alpha]^{20}_D$ +13.7 (*c* 7.94, $CHCl_3$).

((*S*)-1-((*S*)-1-(Benzyloxy)-2-oxoazepan-3-ylamino)-2,2-dimethyl-1-oxodecan-3-yl) (2*R*)-2-(Benzyloxycarbonylamino)-6-(*N*-(benzyloxy)formamido)hexanoate 12. A solution of (2*R*)-2-(benzyloxycarbonylamino)-6-(*N*-(benzyloxy)formamido) hexanoic acid *R*-**3** (176 mg, 0.43 mmol) and **11** (150 mg, 0.35 mmol) in anhydrous CH_2Cl_2 (3 mL) in a flame-dried flask was stirred over 4 Å MS for 15 min. A solution of dried DCC (113 mg, 0.55 mmol) in CH_2Cl_2 (1 mL) was added, followed by a solution of dried 4-pyrrolidinopyridine (51 mg, 0.34 mmol) in CH_2Cl_2 (1 mL). The reaction was stirred overnight at room temperature. The solution was filtered to remove sieves and white precipitate. The filtrate was diluted with

EtOAc (30 mL), washed with H_2O (15 mL) and brine (20 mL), dried over Na_2SO_4, filtered, and concentrated to a white residue (305 mg). Purification by flash chromatography (SiO_2, 9:1 CH_2Cl_2/EtOAc) gave **12** as a colorless oil (61 mg, 0.074 mmol, 21%) along with recovered **11** (73 mg, 49%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.55−7.51 (m, 1H), 7.46−7.25 (m, 15H), 6.28−6.21 (m, 1H), 5.17−5.00 (m, 2H), 4.88−4.79 (m, 2H), 4.78−4.75 (m, 2H), 4.71−4.69 (m, 1H), 4.41−4.31 (m, 2H), 3.62−3.46 (m, 4H), 2.06−1.35 (m, 12H), 1.33−1.14 (m, 14H), 1.17 (s, 3H), 0.86−0.81 (m, 3H); ^{13}C NMR (125 MHz, CDCl) δ 174.2, 171.7, 170.3, 163.1, 156.4, 136.5, 135.0, 134.3, 129.6, 129.5, 129.2, 129.0, 128.8, 128.6, 128.5, 128.2, 128.1, 79.7, 77.7, 66.8, 54.6, 52.9, 52.1, 46.2, 46.1, 43.9, 31.9, 31.7, 31.0, 30.2, 29.3, 29.1, 27.6, 26.3, 26.2, 25.9, 24.9, 23.5, 23.0, 14.1; HRMS (FAB) *m*/*z* calcd for $C_{47}H_{65}N_4O_9$ $[M + H]^+$ 829.4746, found 829.4745; $[\alpha]^{20}_D$ +0.9 (*c* 2.95, $CHCl_3$).

((*S*)-1-((*S*)-1-(Hydroxy)-2-oxoazepan-3-ylamino)-2,2-dimethyl-1-oxodecan-3-yl) 6-(*N*-(Hydroxy)formamido)-(2*R*)-2-(2-(2-hydroxyphenyl)-5-methyloxazole-4-carboxamido)hexanoate 15 (amamistatin B). A solution of **12** (20.5 mg, 0.025 mmol) in MeOH (3 mL) was purged with Ar for 5 min, 10 wt % Pd on carbon (7.8 mg) was added, and the solution was purged again for 3 min. The reaction was bubbled with H_2 for 30 s and stirred under 1 atm H_2 for 1.25 h. The catalyst was removed by gravity filtration and rinsed with MeOH (15 mL). The solution was concentrated to give **13** as a colorless residue (13.2 mg). 2,5-Dioxopyrrolidin-1-yl 2-(2-hydroxyphenyl)-5-methyl oxazole-4-carboxylate **14** (9.6 mg, 0.030 mmol), **13**, and $NaHCO_3$ (17.1 mg, 0.204 mmol) were dissolved in H_2O (0.6 mL) and THF (0.6 mL). More $NaHCO_3$ (5.5 mg) was added to bring the pH to 8. The reaction was stirred overnight at room temperature and then concentrated under reduced pressure to a clumpy white residue. The crude material was dissolved in CH_3CN/H_2O and purified by reversed-phase prep TLC (25:75 H_2O/CH_3CN, 1.5 plates used) to give **15** as a white residue (4.5 mg, 0.006 mmol, 25%): (many peaks doubled due to formamide rotamers) ^{1}H NMR (600 MHz, DMSO) δ 10.29 (s, 1H), 9.88 (s, 0.5H), 9.71 (s, 1H), 9.44 (s, 0.5H), 8.88−8.81 (m, 1H), 8.20 (s, 0.5H), 7.87 (s, 0.5H), 7.78 (dd, 1H, J = 1.8 Hz, 6 Hz), 7.46−7.43 (m, 1H), 7.41−7.38 (m, 1H), 7.04 (d, 1H, J = 8.4 Hz), 6.99−6.96 (m, 1H), 5.08−5.06 (m, 1H), 4.49−4.42 (m, 1H), 4.41−4.36 (m, 1H), 3.87−3.82 (m, 1H), 3.46−3.36 (m, 3H), 2.63 (s, 3H), 1.87−1.84 (m, 3H), 1.76−1.67 (m, 1H), 1.62−1.46 (m, 5H), 1.41−1.05 (m, 15H), 1.04 (s, 3H), 1.03 (s, 3H), 0.81−0.75 (m, 3H); ^{13}C NMR (150 MHz, DMSO) δ 173.3, 171.7, 168.6, 160.8, 157.2, 157.0, 155.9, 152.4, 150.6, 137.0(imp.), 134.3(imp.), 132.6, 128.5, 126.8-(imp.), 126.5, 123.7(imp.), 119.7, 117.2, 110.4, 78.0, 52.3, 52.0, 50.9, 45.6, 34.3(imp.), 31.2, 31.0, 30.8, 30.0, 29.7, 28.9, 28.6, 28.4, 27.7, 27.0, 25.6, 25.5, 24.4, 22.8, 22.0, 15.8, 13.8, 11.3; HRMS (FAB) *m*/*z* calcd for $C_{36}H_{54}N_5O_{10}$ $[M + H]^+$ 716.3865, found 716.3866; LC/MS (5−80% CH_3CN/10 mM ammonium acetate) t_R 10.63, *m*/*z* for $[M + H]^+$ 716.5, 769.5 (+Fe).

(*S*)-3-Hydroxy-2,2-dimethyl-*N*-((*S*)-2-oxoazepan-3-yl)decanamide 20. To a solution of **4** (150 mg, 0.69 mmol) and (*S*)-3-aminoazepan-2-one hydrochloride **19** (113 mg, 0.69 mmol) in CH_3CN (4.5 mL) were added Et_3N (97 μL, 0.69 mmol), HOBt (93 mg, 0.69 mmol) and EDC (145 mg, 0.76 mmol) sequentially. The solution was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc (75 mL), washed with H_2O (30 mL), saturated $NaHCO_3$ (2 × 40 mL), H_2O (30 mL), 5% citric acid (2 × 40 mL), and brine (40 mL), dried over Na_2O_4, filtered, and concentrated to a colorless oil. Purification by flash chromatography (SiO_2, 1:1−1:2 hexanes/EtOAc) gave **20** as a colorless oil (126 mg, 0.39 mmol, 56%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 (d, 1H, J = 6.9 Hz), 6.73 (t, 1H, J = 6.0 Hz), 4.53−4.43 (m, 1H), 3.62 (br s, 1H), 3.48−3.41 (m, 1H), 3.27−3.21 (m, 2H), 2.04−1.96 (m, 2H), 1.86−1.69 (m, 2H), 1.58−1.37 (m, 2H), 1.34−1.21 (m, 12H), 1.20 (s, 3H), 1.14 (s, 3H), 0.86−0.81 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.6, 175.9, 77.4, 52.2, 46.0, 42.1, 31.9, 31.6, 31.0, 29.6, 29.3, 28.9, 28.0, 26.7, 23.5, 22.7, 20.4, 14.1; HRMS (FAB) *m*/*z* calcd for $C_{18}H_{35}N_2O_5$ $[M + H]^+$ 327.2642, found 327.2621. $[\alpha]^{20}_D$ +9.6 (*c* 0.70, $CHCl_3$).

((*S*)-2,2-Dimethyl-1-oxo-1-((*S*)-2-oxoazepan-3-ylamino)decan-3-yl) (2*S*)-2-(Benzyloxycarbonylamino)-6-(*N*-(benzyloxy)formamido)hexanoate 21. To a solution of ***S*-3** (90 mg, 0.22 mmol) and **20** (60 mg, 0.18 mmol) in dry CH_2Cl_2 (2 mL) was added DCC (61 mg, 0.29 mmol) followed by 4-pyrrolidinopyridine (5 mg, 0.04 mmol). The reaction was stirred at room temperature for 2 days. The solution was diluted with CH_2Cl_2 (5 mL) and filtered to remove the white solid. The filtrate was diluted with CH_2Cl_2 (50 mL), washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography (SiO_2, 2:1 EtOAc/CH_2Cl_2) to give **21** as a colorless oil (73 mg, 0.10 mmol, 55%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.56−7.51 (m, 1H), 7.37−7.28 (m, 10H), 6.47−6.41 (m, 1H), 5.98 (d, 1H, J = 8.7 Hz), 5.11−4.97 (m, 2H), 4.80 (s, 2H), 4.51−4.45 (m, 1H), 4.39−4.31 (m, 1H), 3.57−3.40 (m, 1H), 3.25−3.07 (m, 4H), 2.09−1.40 (m, 12H), 1.31−1.09 (m, 12H), 1.22 (s, 3H), 1.15 (s, 3H), 0.90−0.81 (m, 3H); HRMS (FAB) *m*/*z* calcd for $C_{40}H_{59}N_4O_8$ $[M + H]^+$ 723.4327, found 723.4348.

((*S*)-2,2-Dimethyl-1-oxo-1-((*S*)-2-oxoazepan-3-ylamino)decan-3-yl) 6-(*N*-(Benzyloxy)formamido)-(2*S*)-2-(5-methyl-2-phenyloxazole-4-carboxamido)hexanoate 23. To a solution of **21** (67 mg, 0.093 mmol) in CH_2Cl_2 (0.7 mL) was added 33 wt % HBr in acetic acid (0.7 mL), and the reaction was stirred for 30 min at room temperature. Volatiles were removed under reduced pressure. The resulting orange oil was dissolved in saturated $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 × 7 mL). The organic solution was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to a colorless residue (45 mg, 0.77 mmol, 83%). The residue was dissolved in dry CH_2Cl_2 (1.0 mL) along with 5-methyl-2-phenyloxazole-4-carboxylic acid **2** (17 mg, 0.084 mmol). HOBt (10 mg, 0.076 mmol) was added, and the solution was cooled in an ice bath. EDC (18 mg, 0.092 mmol) was added, and the reaction mixture was stirred in an ice bath for 20 min and at room temperature for 40 h. The solution was diluted with EtOAc (15 mL), washed with H_2O (10 mL), saturated $NaHCO_3$, 5% citric acid, and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography (SiO_2, 5:1 EtOAc/CH_2Cl_2) to give **23** as a colorless oil (12 mg, 0.016 mmol, 20%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.16 (s, 1H), 8.01 (s, 1H), 7.46−7.44 (m, 4H), 7.38−7.29 (m, 6H), 6.28−6.21 (m, 1H), 4.80 (s, 2H), 4.51−4.49 (m, 1H), 4.44−4.40 (m, 1H), 3.58−3.52 (m, 1H), 3.33−3.12 (m, 4H), 2.68 (s, 3H), 2.13−1.94 (m, 4H), 1.84−1.71 (m, 4H), 1.51−1.38 (m, 4H), 1.36−1.11 (m, 12H), 1.18 (s, 3H), 1.16 (s, 3H), 0.86−0.80 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.9, 175.6, 174.4, 171.8, 163.1, 162.1, 153.6, 130.8, 130.1, 129.7, 129.3, 129.0, 128.8, 128.6, 127.0, 126.6, 78.0, 77.7, 52.5, 52.4, 46.4, 46.1, 42.4, 32.4, 32.0, 31.5, 31.3, 29.8, 29.5, 29.3, 29.1, 28.1, 26.9, 26.4, 23.9, 22.8, 14.3, 12.0; HRMS (FAB) *m*/*z* calcd for $C_{43}H_{60}N_5O_8$ $[M + H]^+$ 774.4436, found 774.4426; LC/MS (5%−80% CH_3CN/10 mM ammonium acetate) t_R 11.55, *m*/*z* for $[M + H]^+$ 774.5.

((*S*)-2,2-Dimethyl-1-oxo-1-((*S*)-2-oxoazepan-3-ylamino)decan-3-yl) 6-(*N*-(Hydroxy)formamido)-(2*S*)-2-(5-methyl-2-phenyloxazole-4-carboxamido)hexanoate 24. A solution of **23** (6.8 mg, 0.009 mmol) in MeOH (1 mL) was purged with Ar for 5 min, 10 wt % Pd on carbon (1.5 mg) was added, and the solution was purged again for 2 min. The reaction was bubbled with H_2 for 30 s, stirred under 1 atm H_2 for 5.5 h, and bubbled with H_2 again for 15 min. The catalyst was removed by gravity filtration and rinsed well with MeOH. LCMS showed starting material remaining in the filtrate. The solution was concentrated under reduced pressure to a volume of 1 mL and purged with Ar. Pd on carbon (1 mg, 10 wt %) was added, and the solution was purged again. The reaction was stirred under 1 atm H_2 for 2.5 h, filtered, and concentrated. Purification by reversed phase prep TLC (75% CH_3CN/H_2O, 1/2 plate) to remove residual starting material gave **24** as a white residue (2 mg, 0.003 mmol, 33%): (many peaks doubled due to formamide

rotamers) ^{1}H NMR (600 MHz, DMSO) δ 9.91 (s, 0.5H), 9.46 (s, 0.5H), 8.24 (s, 1H), 8.02 −8.01 (m, 2H), 7.96−7.90 (m, 1H), 7.58−7.54 (m, 3H), 7.48−7.36 (m, 2H), 5.10−5.08 (m, 1H), 5.02−4.98 (m, 1H), 4.48−4.42 (m, 1H), 4.36−4.28 (m, 1H), 3.44−3.38 (m, 1H), 3.19−3.11 (m, 1H), 3.08−3.02 (m, 1H), 2.64 (s, 3H), 1.93−1.80 (m, 3H), 1.79−1.69 (m, 1H), 1.66−1.53 (m, 5H), 1.42−1.11 (m, 15H), 1.08−1.03 (m, 6H), 0.87−0.79 (m, 3H); ^{13}C NMR (150 MHz, DMSO) δ 161.3, 150.6, 137.0, 134.4, 130.9, 129.7, 129.1, 128.5, 126.8, 126.2, 126.0, 123.7, 52.2, 51.5, 45.4, 34.4, 31.1, 30.8, 29.7, 29.5, 28.9, 28.6, 28.3, 27.7, 25.3, 22.4, 22.0, 18.8, 15.8, 13.8, 11.4; HRMS (FAB) *m*/*z* calcd for $C_{36}H_{54}N_5O_8$ $[M + H]^+$ 684.3967, found 684.3961; LC/MS (5%−80% CH_3CN/10 mM ammonium acetate) t_R 9.63, *m*/*z* for $[M + H]^+$ 684.5.

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Supporting Information Available: Additional experimental procedures, ^{1}H and ^{13}C NMR spectra for synthetic compounds, and NMR data comparison tables for **15**, **18**, and **24** with natural products. This material is available free of charge via the Internet at http://pubs.acs.org.

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