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Design, Synthesis, and Biological Evaluation of Isocyanurate-based Antifungal and Macrolide Antibiotic Conjugates: Iron Transport-mediated Drug Delivery

Manuka Ghosh and Marvin J. Miller*

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, U.S.A.

Abstract—The syntheses and preliminary biological evaluation of conjugates of a synthetic isocyanurate-based trihydroxamate siderophore with two antifungal agents, 5-FU (conjugate **9**) and norneoenactin (conjugate **12**), and a macrolide antibiotic, erythromycylamine (conjugate **18**), are described. A ^{19}F NMR study was used to determine the hydrolytic stability of conjugate **9** under assay conditions. Preliminary biological studies with ferric complexes of conjugates **9** and **12** indicated that these antifungal agents are recognized by *Candida* and perhaps are actively transported into the cell by the siderophore-transport mechanisms. While conjugate **18** did not show any significant antibacterial activity, presumably due to size restriction, the 5-FU conjugate **9** appeared to be moderately active against a variety of Gram-positive strains, and was more active than the 5-FC control against some strains of *Staphylococcus*.

Introduction

The emergence of pathogens that are resistant against existing medications is contributing to a major health care crisis especially among individuals with compromised immune systems.[1] Although our understanding of the principles involved in the delivery of antimicrobial agents into microorganisms[2] has increased through substantial advances in molecular and cellular biology, there is a continuing need for improved alternative drug delivery processes that would be selective, safe, and efficient. One attractive approach for direct transport of drugs to microbes is through conjugation to siderophores,[3] iron-sequestering agents utilized by many microbes for cellular uptake of physiologically essential iron.[4] In principal, siderophore-mediated active transport pathways involving selective recognition and delivery of the drugs to target microbes may improve the efficacy of the antimicrobial agents, reduce the chances of resistance, and perhaps, overcome permeability problems, especially where passive diffusion of the drug through porins becomes limited due to size restrictions.

We previously reported the synthesis and antimicrobial activity of several hydroxamic acid and catechol-based siderophore–antibiotic conjugates,[5] and demonstrated that their cellular entries utilize different outer membrane receptor proteins.[6] More recently, we prepared ornithine-based siderophore–antifungal conjugates **1a–c** and demonstrated the potential of iron-transport mediated drug delivery towards the development of effective antifungal agents.[7] Herein, we report the synthesis and biological evaluation of conjugates of two antifungal agents (**2** and **3**) and a macrolide antibiotic (**4**) with a synthetic trihydroxamate siderophore (**5b**).

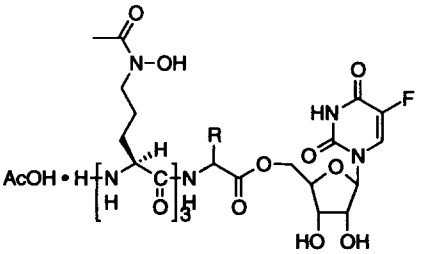
Results and Discussion

Design of conjugates

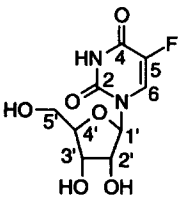
The C_3-symmetric isocyanurate-based trihydroxamate siderophore **5b**, designed in our laboratory, was employed as the iron-binding ('carrier') component for this study.[8] The ability of **5a–c** to form stoichiometric (1:1) complexes with Fe^{3+}, and to facilitate iron assimilation for microbial growth makes these compounds effective mimics of natural siderophores. Studies indicated that isocyanurates **5a–c** are nontoxic to mammals, and **5b**, in particular, is also effective in promoting iron excretion in a rat model used to simulate the consequences of transfusional iron overload.[8] Moreover, **5d**, a protected derivative of **5b** containing a succinoyl linker, was made accessible in gram quantities through improvements in the original synthetic scheme, and was used effectively to serve as a carrier for β-lactam antibiotics.[9] Thus, the inherent siderophore properties associated with **5b**, that is, its lack of toxicity and its proven ability to act as a drug carrier, prompted us to prepare new conjugates of it with different antifungal and antibacterial agents.

Our initial choice for the antifungal agent was 5-fluorouridine (5-FU, **2**), an active metabolite of the most commonly used orally active antifungal agent 5-fluorocytosine (5-FC).[10] In our earlier study with ornithine-based siderophore–5-FU conjugates **1a–c**, use of the different amino acid spacers was studied with regards to facilitating the synthesis and controlling intracellular release of the drug.[7] As demonstrated by hydrolysis studies of **1a–c** using ^{19}F NMR spectroscopy, valine-containing conjugate **1c** was less prone to ester hydrolysis (half-life ~241 h) than the glycine **1a** (half-life ~13 h) and

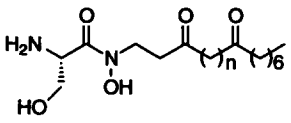
phenylglycine **1b** (half-life ~10 h) conjugates under assay conditions (pH 7.7, Lee's medium). Thus, due to the improved stability of the ester linkage between L-valine and the 5'-hydroxyl group of the ribosyl 5-FU, this amino acid was selected as the spacer for the present study.



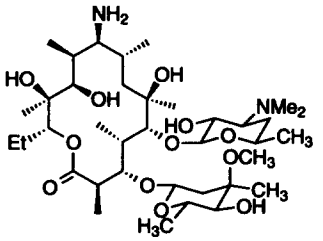
1a, R=H
1b, R=Ph
1c, R=i-Pr



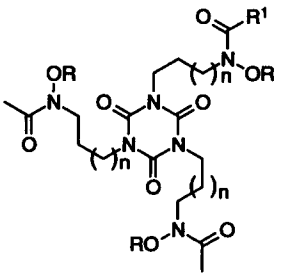
2, 5-fluorouridine



3a, norneoenactin, n=5
3b, neoenactin, n=6



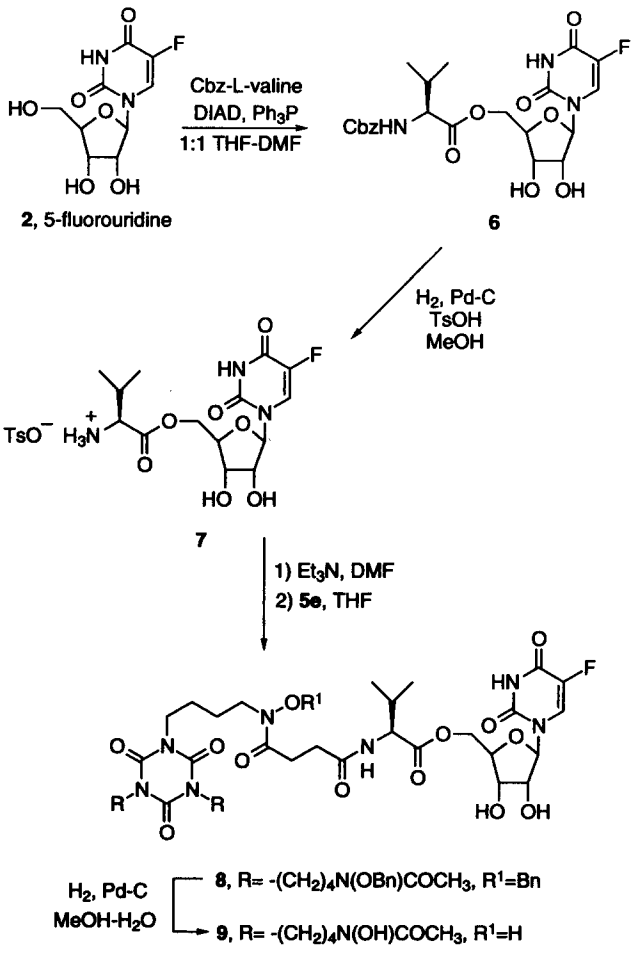
4, erythromycylamine



5a, R=H, R¹=Me, n=1
5b, R=H, R¹=Me, n=2
5c, R=H, R¹=Me, n=3
5d, R=Bn, R¹=–$(CH_2)_2CO_2H$, n=2
5e, R=Bn, R¹=–$(CH_2)_2CO_2$–succinimidyl, n=2

Another potent antifungal agent, norneoenactin A (**3a**), a synthetic analog of neoenactin A (NE A, **3b**), recently explored in our laboratory,[11] was also selected for conjugation with siderophore **5b** in an attempt to further exploit the antifungal properties of **3a**.

The macrolide antibiotic erythromycylamine (**4**), a semisynthetic derivative of erythromycin,[12] also was selected for conjugation to trihydroxamate siderophore **5b**. This drug is an ideal candidate for active transport mediated delivery considering its poor oral bioavailability, due to the inability of the drug to penetrate target cells,[13] as well as the presence of the primary amino group for convenient attachment of the siderophore component.

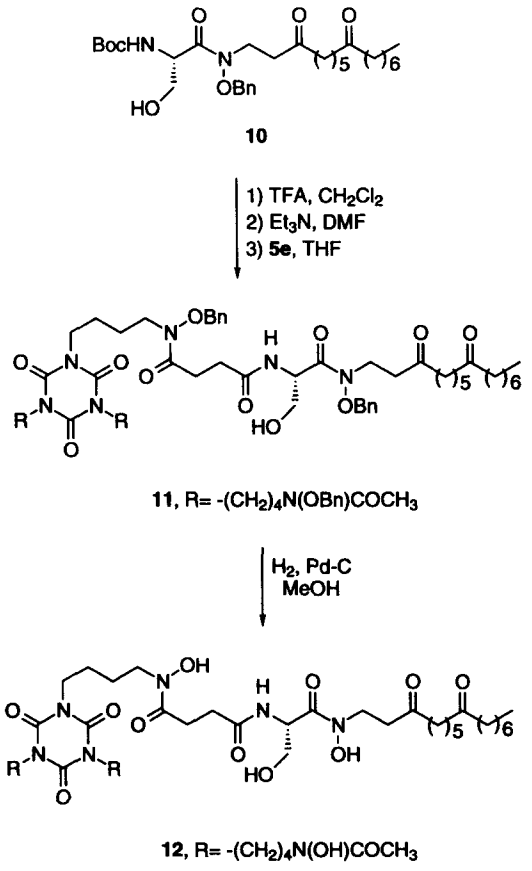


Scheme 1.

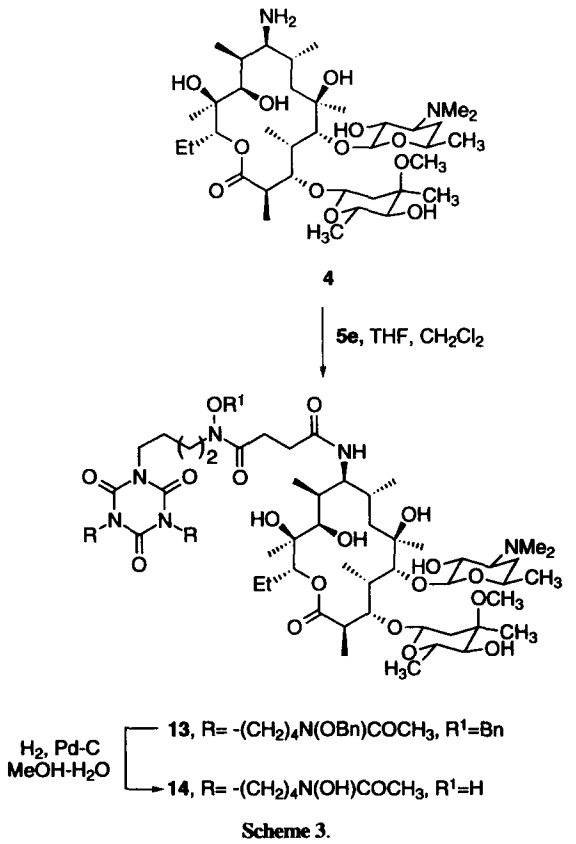
Synthesis of conjugates

The synthesis of 5-FU conjugate **9** is shown in Scheme 1. Direct coupling of Cbz-Val with 5-fluorouridine (**2**) under Mitsunobu conditions furnished *N*-protected valine ester **6**. Hydrogenolytic removal of the Cbz protecting group of **6** in the presence of one equivalent of *p*-TsOH provided amine salt **7**. ^1H and ^{13}C NMR analysis of **7** indicated that the 5-FU carbon–carbon double bond had not been reduced under the hydrogenolytic conditions. Salt **7** was treated with Et_3N in DMF to generate the free amine which was immediately acylated with *N*-hydroxysuccinimide active ester **5e**[9] to afford **8** in 80% yield. The benzyl protecting groups of **8** were removed by hydrogenolysis to obtain conjugate **9** in 70% yield.

For the synthesis of the norneoenactin conjugate **12** (Scheme 2), Boc protected amine **10**[11] was briefly exposed to trifluoroacetic acid in anhydrous CH_2Cl_2. The TFA salt, after removal of the volatiles, was treated with Et_3N to generate the free amine, which was immediately acylated with a solution of preformed *N*-hydroxysuccinimide ester **5e** in THF. The crude acylated product, after purification by radial chromatography, furnished conjugate **11** in 69% yield. Removal of the benzyl protecting groups by hydrogenation in the presence of Pd–C in methanol provided conjugate **12** in near quantitative yield.



Scheme 2.



Scheme 3.

The synthesis of the erythromycylamine conjugate **14** is shown in Scheme 3. 9-(*S*)-Erythromycylamine (**4**) upon acylation with active ester **5e** in a mixture of anhydrous CH_2Cl_2–THF afforded protected conjugate **13** in 75% yield after silica gel chromatography. The benzyl protecting groups were removed by hydrogenolysis to obtain deprotected conjugate **14** in 99% yield.

^{19}F NMR

As in related studies with conjugates **1a–c**,[7] a hydrolysis study employing ^{19}F NMR spectroscopy was used to determine the relative stability of conjugate **9** under bioassay conditions. A stack plot of the NMR recorded each hour is shown in Figure 1. Conjugate **9** showed less than 5% hydrolysis even after 19 h, corresponding to a half-life on the order of 296 h. Thus, this study further

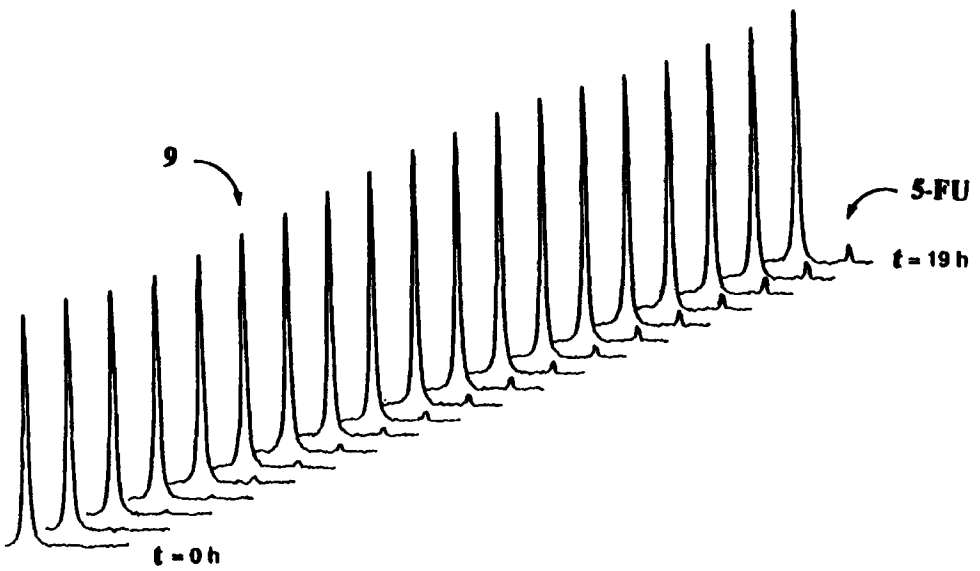


Figure 1. Stack plot showing the degradation of conjugate **9** (**9**, 29.2 ppm; 5-FU, 28.9 ppm) in Lee's medium at 37 °C from 0–19 h as measured by 470 MHz ^{19}F NMR referenced to NaF (0 ppm) in D_2O.

established the superior stability of valinyl conjugates over tripeptide-containing glycyl (**1a**) and phenylglycyl (**1b**) counter parts under assay conditions.

Biological studies

An assay used to determine the ability of the conjugates **9** and **12** either to stimulate (siderophore activity) or to inhibit (antifungal activity) *Candida* growth was performed in Lee's medium (pH 7.7, Table 1).[14] Both ferri (with stoichiometric Fe^{3+} added) and deferri forms of **9** and **12** were used for this study. As expected, in the presence of ethylenediamine bis(*o*-hydroxyphenylacetic acid) (EDDA), that is, in an iron-deficient medium, conjugates **9** and **12** did not show any growth promotion of *Candida* both in the ferri and deferri forms (zone diameters were 0 after 24 and 48 h). Likewise, the control experiment with 5-FU also showed no growth promoting effect. In Lee's agar alone (without EDDA), zones of inhibition against *Candida* for both the ferri and deferri forms of **9** and **12** were observed after 24 h. In both cases, the zones of inhibition were comparable to the 5-FU control. The deferri forms showed larger inhibition zones than the ferri forms. However, after an additional 24 h, all zones of inhibitions had decreased somewhat, presumably due to the selection of resistant strains.[7] This trend was even more prominent at lower test concentrations.

Table 1. Inhibition of growth of *Candida albicans* (ATCC 48130) without EDDA in Lee's agar containing conjugates **9** and **12**[a]

Compound	Without EDDA	
	24 h	48 h
2 (5-FU)	48	30
9	48(28)	38(18)
9 + Fe^{3+} (1:1)	30(12)	20(10)
12	45(28)	35(18)
12 + Fe^{3+} (1:1)	30(22)	22(21)

[a]Diameters (in mm) of zones of inhibition of *C. albicans* (ATCC 48130) around filter paper disks containing 25 nmol and 5 nmol (within parentheses) of the test compounds were measured. The petri dishes were incubated upside down at 37 °C.

The *in vitro* antifungal activity of conjugates **9** and **12** against *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* was also determined in RPMI and MOPS (3-[*N*-morpholino]propanesulfonic acid) broth. While both conjugates **9** and **12** showed some activity against *C. albicans* (8 μg mL^{-1}), the 5-FU conjugate **9** also showed mild activity against *C. neoformans* (64 μg mL^{-1}). The decreased activity of norneoenactin conjugate **12**, when compared to norneoenactin[11b] itself (2.7 μg mL^{-1}, *C. albicans*; 1.5 μg mL^{-1}, *C. neoformans*), may reflect several controlling factors: (1) recognition problems associated with conformational changes due to conjugation; (2) insufficient release of the drug at or near the target, if release is necessary; or (3) limitations of the assay which could not distinguish selected resistant strains from the parent strain.[7]

A broad screen for antibacterial activity of **7**, **9** and **14** against a variety of bacteria is shown in Table 2. Though conjugate **12**, of the antifungal agent norneoenactin, as expected, did not show any significant activity, conjugate **9** was moderately active against a number of Gram-positive bacteria. Trihydroxamate conjugate **9** was more active than the 5-FC control against strains of *Staphylococcus* and *Enterococcus*. However, these conjugates showed little activity against Gram-negative strains. Interestingly, the tosyl salt of valinyl-5-FU (**7**) also showed considerable activity against a number of strains.

The preliminary broad screen assay indicated that erythromycylamine conjugate **14** was not active against erythromycin-sensitive organisms (Table 2). While detailed growth studies might be helpful to conclusively determine the effectiveness of **14** as an antibacterial agent, the apparent lack of activity is not surprising since the drug is larger than the delivery agent and probably acts as a barrier to the overall transport process. Alternatively, if the conjugate is transported, a mechanism to release the erythromycylamine may be necessary. Detailed related studies and growth-curve generation and analysis are under way, and will be published in due course.

In summary, these results further demonstrate the potential of siderophore-based iron transport systems towards the development of effective microbially selective drug delivery agents.

Experimental

General methods and instruments used have been described previously.[15]

5'-O-(N-(Benzyloxycarbonyl)-L-valinyl)-5-fluorouridine (6). To a solution of 5-fluorouridine (**2**, 5-FU, 521 mg, 1.98 mmol) in 6 mL of anhydrous THF:DMF (1:1) under N_2 was added *N*-(benzyloxycarbonyl)-L-valine (500 mg, 1.99 mmol), Ph_3P (521 mg, 1.98 mmol), and diisopropyl azodicarboxylate (402 mg, 1.98 mmol). The resulting mixture was stirred overnight at 25 °C. The volatile components were removed under reduced pressure, and the residue chromatographed on silica gel eluting with MeOH:$CHCl_3$ (1:5) to provide compound **6** (413 mg, 40%) as a white solid: mp 64–67 °C; IR (KBr) 3450 (br), 1700 (br), 1580 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.91 (*d*, *J* = 6.6 Hz, 3H), 0.97 (*d*, *J* = 6.6 Hz, 3H), 2.03–2.23 (*m*, 1H), 3.85–4.55 (*m*, 7H), 5.01–5.15 (*m*, 2H), 5.46 (*d*, *J* = 8.1 Hz, 1H), 5.79 (*d*, *J* = 2.7 Hz, 1H), 7.28–7.38 (*m*, 5H), 7.64 (*d*, *J* = 6.0 Hz, 1H), 10.27 (*br s*, 1H); ^{13}C NMR ($CDCl_3$): δ 172.11, 157.41 (*d*, *J* = 104 Hz, for C-4), 156.55, 149.58, 140.51 (*d*, *J* = 944 Hz, for C-5), 124.39 (*d*, *J* = 134 Hz, for C-6), 135.91, 128.51, 128.23, 128.08, 89.85, 81.69, 77.20, 74.39, 69.88, 67.22, 63.94, 59.48, 30.81, 18.95, 17.58; Anal. Calcd for $C_{22}H_{25}N_3O_9F \cdot H_2O$: C, 51.56; H, 5.31; N, 8.2. Found: C, 51.62; H, 5.33; N, 7.92.

Mono[[N-succinoyl[(valinyl)-5-fluorouridine](benzyloxy)-amino]butyl]-bis[[N-acetyl(benzyloxy)amino]butyl] isocyanurate (8). To a solution of compound **6** (85 mg, 0.17 mmol) in 3.0 mL of MeOH (spectral grade) was added *p*-toluenesulfonic acid (33 mg, 0.17 mmol). The solution

Table 2. MIC data from broad screen antibacterial testing of compounds **7**, **9** and **14** compared with Ampicillin and 5-FC[a]

Organism	MIC (μg mL^{-1})				
	Ampicillin	5-FC	**7**	**9**	**14**
Staphylococcus aureus X 1.1	2	8	0.5	8	128
Staphylococcus aureus V 41	4	8	0.25	4	128
Staphylococcus aureus X 400	128	8	0.25	4	128
Staphylococcus aureus S13E	128	2	0.125	1	128
Staphylococcus epidermis 70	8	16	0.03	4	128
Staphylococcus epidermis 222	2	16	1	8	128
Streptococcus A C203	NT[b]	NT[b]	NG[c]	128	64
Streptococcus pn PARK	NT[b]	NT[b]	128	128	64
Enterococcus D X66	128	0.25	32	128	64
Enterococcus D 2041	1	4	2	16	128
E. Coli EC14	0.06	>128	128	128	128
E. Coli TEM	0.03	>128	16	128	128
Klebsiella X26	0.008	>128	32	128	128
Enterobacter aerogenes C32	0.5	>128	64	128	128
Enterobacter aerogenes EB17	0.06	>128	64	128	128
Enterobacter cloacae EB5	0.25	>128	32	128	128
Salmonella X514	0.06	>128	32	128	128
Salmonella 1335	0.125	>128	32	128	128
Serratia X99	0.25	>128	16	64	128
Serratia SE3	0.5	>128	16	128	128
Shig. sonn. N9	0.6	>128	64	128	128
Morgnella morganii PR15	1	>128	8	64	128
Proteus stu PR33	0.06	>128	32	128	128
Proteus rett C24	0.06	>128	16	128	128
Pseudomonas PS19	32	>128	128	128	128

[a]These MIC data were generously provided by Eli Lilly and Company and were determined by the standard agar dilution method.
[b]Not tested.
[c]No growth.

was deoxygenated with nitrogen, and then 10% Pd–C (17 mg, 20% w/w) was added. The resulting suspension was stirred under 1 atm H_2 for 45 min after which TLC indicated that the reaction was complete. The catalyst was removed by filtration through a short pad of Celite and the solution was concentrated to afford **7** (98%) as a white solid.

The crude *p*-TsOH salt **7** was dissolved in 1 mL of anhydrous DMF under N_2. Et_3N (48 μL, 0.34 mmol) was added to the solution, quickly followed by active ester **5e** in THF [**5e** was obtained from overnight stirring of acid **5d**[9] (145 mg, 0.17 mmol), dicyclohexylcarbodiimide (DCC, 43 mg, 0.21 mmol), and *N*-hydroxysuccinimide (HOSu, 24 mg, 0.21 mmol) in anhydrous THF at 25 °C]. The reaction mixture was stirred at room temperature overnight, concentrated under reduced pressure, diluted with EtOAc (20 mL), then washed with water and brine, then dried, filtered, and concentrated. Radial silica gel chromatography of the residue eluting with MeOH:$CHCl_3$ (1:9) afforded compound **8** (163 mg, 81%) as a foamy solid: mp 50–55 °C; IR (KBr) 3450 (br), 1700 (br), 1580, 1460 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.92 (*d*, *J* = 6.6 Hz, 3H), 0.93 (*d*, *J* = 6.6 Hz, 3H), 1.55–1.72 (*m*, 12H), 2.08 (s, 6H), 2.12–2.20 (*m*, 1H), 2.51–3.02 (*m*, 6H), 3.57–3.72 (*m*, 6H), 3.76–3.89 (*m*, 6H), 4.15–4.22 (*m*, 1H), 4.35–4.50 (*m*, 5H), 4.81 (s, 4H), 4.85 (*m*, 2H), 5.60 (*d*, *J* = 3.0 Hz, 1H), 6.97 (*d*, *J* = 7.5 Hz, 1H), 7.32–7.42 (*m*, 15H), 7.55 (*d*, *J* = 6.3 Hz, 1H), 9.95 (*br s*, 1H); ^{13}C NMR ($CDCl_3$): δ 173.82, 172.83, 172.43, 171.20, 157.05 (*d*, *J* = 105 Hz, for C-4), 149.13, 148.90, 148.83, 140.32 (*d*, *J* = 937 Hz, for C-5), 134.21, 134.09, 129.11, 128.93, 128.65, 125.3 (*d*, *J* = 132 Hz, for C-6), 91.50, 81.63, 77.20, 76.30, 76.19, 74.15, 69.42, 63.41, 58.35, 44.73 (*m*), 42.44, 30.69, 29.92, 27.64, 24.87, 24.76, 23.99, 23.86, 20.38, 18.97, 17.98; HRMS (FAB) calcd for $C_{58}H_{75}N_9O_{17}F$ (MH^+) 1188.5265, found 1188.5251.

*Mono[[*N*-succinoyl[(valinyl)-5-fluorouridine](hydroxy)-amino]butyl]-bis[[*N*-acetyl(hydroxy)amino]butyl] isocyanurate (9).* A solution of compound **8** (130 mg, 0.11 mmol) in MeOH (3 mL, spectral grade) and water (1 mL, distilled, deionized) was degassed under Ar, and 10% Pd–C (26 mg, 20% w/w) was added. The resulting suspension was stirred under hydrogen at atmospheric pressure for 3 h. The catalyst was removed by filtration through a short pad of celite, and the solution was concentrated to afford **9** (77 mg, 70%) as a gummy oil: $FeCl_3$ positive (red-purple); IR (neat) 3450 (br), 1700 (br), 1465 cm^{-1}; ^{1}H NMR (CD_3OD): δ 0.96 (*d*, *J* = 6.9 Hz, 6H), 1.57–1.68 (*m*, 12H), 2.08 (s, 6H), 2.11–2.20 (*m*, 1H), 2.51–2.60 (*m*, 2H), 2.72–2.81 (*m*, 2H), 3.56–3.67 (*m*, 6H), 3.80–3.92 (*m*, 6H), 4.03–4.41 (*m*, 6H), 5.80 (*d*, *J* = 3.3 Hz, 1H), 7.86 (*d*, *J* = 6.6 Hz, 1H); ^{13}C NMR (CD_3OD): δ 175.37, 174.43, 173.54, 173.23, 172.87, 159.53 (*d*, *J* = 103 Hz, for C-4), 151.01, 150.74, 141.85 (*d*, *J* = 928 Hz, for C-5), 126.27 (*d*, *J* = 137 Hz, for C-6), 91.67, 82.73, 74.93, 70.89, 64.99, 59.63, 48.56, 48.33, 48.15, 43.44, 31.65, 30.98, 28.70, 25.87, 24.86, 20.25, 19.50, 18.62; HRMS (FAB) calcd for $C_{37}H_{57}N_9O_{17}F$ (MH^+) 918.3856, found 918.3858.

Mono[[N-succinoyl[benzyl-S-norneoenactin A](benzyloxy)amino]butyl]-bis[[N-acetyl(benzyloxy)amino]butyl] isocyanurate (11). To a solution of **10** (70 mg, 0.12 mmol) in 1.0 mL of anhydrous CH_2Cl_2 under N_2 was added 1.0 mL of trifluoroacetic acid (TFA). The solution was stirred at 25 °C for 20 min under Ar. TLC indicated that the starting material had been consumed. The solution was concentrated, and excess TFA was removed by repeated evaporation with benzene. The solid residue was dissolved in 1.0 mL of anhydrous DMF under N_2. Et_3N (80 μL, 0.62 mmol) was added quickly followed by active ester **5e** [**5e** was obtained from overnight reaction of **5d** (105 mg, 0.12 mmol) with *N*-hydroxysuccinimide (17 mg, 0.15 mmol) and DCC (30 mg, 0.15 mmol) in anhydrous THF (3 mL) at 25 °C]. The resulting reaction mixture was stirred at room temperature for 24 h, concentrated, and the residue was rediluted with EtOAc (25 mL). The organic layer was separated, washed with water, brine, dried, and concentrated. Radial silica gel chromatography of the residue eluting with MeOH:$CHCl_3$ (1:20) provided compound **11** (110 mg, 69%) as a gummy oil: IR (neat) 3450 (br), 2925, 1685, 1650, 1460 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (t, J = 6.8 Hz, 3H), 1.21–1.32 (*m*, 10H), 1.45–1.70 (*m*, 18H), 2.08 (*s*, 6H), 2.32–2.42 (*m*, 6H), 2.44–2.61 (*m*, 2H), 2.67 (*t*, J = 6.6 Hz, 2H), 2.70–2.96 (*m*, 3H), 3.55–3.73 (*m*, 7H), 3.75–3.90 (*m*, 8H), 4.17–4.30 (*m*, 1H), 4.81 (*s*, 4H), 4.85 (*s*, 2H), 4.89 (*s*, 2H), 5.02–5.10 (*m*, 1H), 6.89 (*d*, J = 7.5 Hz, 1H), 7.32–7.45 (*m*, 20H); ^{13}C NMR ($CDCl_3$): δ 210.02, 209.07, 173.58, 172.39, 172.33, 170.93, 148.79, 134.29, 133.56, 129.49, 129.10, 128.90, 128.65, 77.20, 76.28, 62.74, 52.39, 44.79 (*m*), 42.95, 42.76, 42.52, 42.42, 40.52, 39.31, 31.52, 30.59, 28.75, 27.89, 24.95, 24.81, 24.02, 13.97; HRMS (FAB) calcd for $C_{70}H_{97}N_8O_{15}$ 1289.7073 (MH^+), found 1289.7103.

Mono[[N-succinoyl[norneoenactin A](hydroxy)amino]butyl]-bis[[N-acetyl(hydroxy)amino]butyl] isocyanurate (12). To a solution of protected **11** (83 mg, 0.06 mmol) in MeOH (2.0 mL, spectra grade) purged with N_2 was added 10% Pd–C (16 mg, 20% w/w). The resulting suspension was placed under hydrogen at atmospheric pressure for 2.5 h. TLC indicated that the reaction was complete. The reaction mixture was filtered (Celite) and then concentrated to afford **12** (61 mg, 100%) as a colorless oil: $FeCl_3$ positive (purple); IR (neat) 3300 (br), 2925, 1685, 1625 cm^{-1}; ^1H NMR (CD_3OD): δ 0.79 (*t*, J = 6.6 Hz, 3H), 0.96–1.35 (*m*, 10H), 1.36–1.65 (*m*, 18H), 1.98 (*s*, 6H), 2.1–2.5 (*m*, 10H), 2.62–2.72 (*m*, 2H), 3.17–3.25 (*m*, 2H), 3.45–3.57 (*m*, 6H), 3.62–3.85 (*m*, 8H), 4.86–4.96 (*m*, 1H); ^{13}C NMR (CD_3OD): δ 214.19, 214.12, 174.94, 174.52, 174.45, 150.77, 63.00, 62.52, 62.46, 54.51, 54.06, 53.77, 45.00 (*m*), 43.47, 43.43, 40.20, 37.45, 36.99, 35.24, 34.71, 32.79, 31.67, 31.37, 30.45, 30.03, 29.97, 28.79, 25.88, 25.21, 24.85, 24.66, 23.57, 20.24, 14.40; HRMS (FAB) calcd for $C_{42}H_{72}N_8O_{15}Na$ (MNa^+) 951.5015, found 951.5000.

Mono[[N-succinoyl[N^9-S-erythromycylamine](benzyloxy)amino]butyl]-bis[[N-acetyl(benzyloxy)amino]butyl] isocyanurate (13). To a solution of the trihydroxamate cyanurate **5d** (110 mg, 0.13 mmol) and *N*-hydroxy succinimide (16 mg, 0.14 mmol) in THF (3 mL) under N_2 was added DCC (30 mg, 0.14 mmol). After stirring overnight at 25 °C, the reaction mixture containing cyanurate active ester **5e** was filtered (to remove DCU) into a solution of erythromycylamine (95 mg, 0.13 mmol) in anhydrous CH_2Cl_2 (1 mL). The resulting solution was stirred at 25 °C for 3 h under Ar. The volatiles were removed under reduced pressure, and the residue was dissolved in EtOAc (20 mL), washed with water and brine, and then dried. Flash chromatography on a silica gel column eluting with MeOH:$CHCl_3$:NH_4OH (10:90:1) afforded **13** (150 mg, 75%) as a foamy solid: mp 70–74 °C; IR (KBr) 3500 (br), 2980, 2960, 1730, 1690, 1460 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (*t*, J = 7.2 Hz, 3H), 1.03 (*d*, J = 6.6 Hz, 3H), 1.06–1.36 (*m*, 25H), 1.45–2.05 (*m*, 22H), 2.08 (s, 6H), 2.12–2.22 (*m*, 1H), 2.34–2.72 (*m*, 5H), 2.38 (*s*, 6H), 2.73–2.98 (*m*, 4H), 3.07 (*d*, J = 7.8 Hz, 1H), 3.25–3.40 (*m*, 1H), 3.33 (*s*, 3H), 3.45–3.52 (*m*, 1H), 3.55–3.72 (*m*, 8H), 3.75–3.92 (*m*, 8H), 3.95–4.06 (*m*, 1H), 4.11 (*d*, J = 3.9 Hz, 1H), 4.63 (*d*, J = 7.2 Hz, 1H), 4.73–4.82 (*m*, 1H), 4.80 (*s*, 4H), 4.85 (*s*, 2H), 5.03 (*d*, J = 2.7 Hz, 1H), 7.01 (*d*, J = 9.6 Hz, 1H), 7.32–7.51 (*m*, 15H); ^{13}C NMR ($CDCl_3$): δ 178.07, 173.59, 172.24, 172.21, 148.76, 134.33, 134.27, 129.08, 128.87, 128.74, 128.62, 128.56, 102.14, 96.01, 82.10, 79.58, 77.20, 76.28, 76.17, 75.22, 74.56, 72.62, 70.67, 69.82, 69.12, 66.57, 65.13, 60.28, 49.16, 44.89, 44.83, 44.72, 42.41, 40.31, 38.55, 34.80, 33.24, 31.85, 30.92, 29.25, 28.12, 26.68, 24.91, 21.47, 21.13, 21.01, 20.42, 18.74, 18.16, 16.28, 14.19, 12.77, 11.03, 8.97; HRMS (FAB) calcd for $C_{81}H_{125}N_8O_{22}$ (MH^+) 1561.8908, found 1561.8919.

Mono[[N-succinoyl[N^9-S-erythromycylamine](hydroxy)amino]butyl]-bis[[N-acetyl(hydroxy)amino]butyl] isocyanurate (14). A solution of protected **13** (100 mg, 64 mmol) in MeOH (3.0 mL, spectral grade) was purged with N_2, and 10% Pd–C (20 mg, 20% w/w) was added. The resulting suspension was placed under a hydrogen atmosphere for 4 h. TLC indicated that the reaction was complete. The reaction mixture was filtered (celite) and the volatiles were removed to afford **14** (82 mg, 99%) as a white solid: mp 145–147 °C; IR (KBr) 3450 (br), 2990, 1730, 1690 cm^{-1}; ^1H NMR (CD_3OD): δ 0.79 (*t*, J = 7.2 Hz, 3H), 0.90 (*d*, J = 6.6 Hz, 3H), 0.95–1.21 (*m*, 27H), 1.31–1.60 (*m*, 20H), 1.81–2.10 (*m*, 5H), 1.83 (*s*, 6H), 2.35–2.45 (*m*, 4H), 2.6–2.85 (*m*, 8H), 2.97–3.14 (*m*, 5H), 3.18–3.25 (*m*, 1H), 3.26 (*s*, 3H), 3.35–3.58 (*m*, 5H), 3.73–3.88 (*m*, 6H), 3.92–4.00 (*m*, 1H), 4.86–5.01 (*m*, 2H), 7.40 (*d*, J = 9 Hz, 1H), 7.95 (*t*, J = 4.8 Hz, 1H); ^{13}C NMR (CD_3OD): δ 174.58, 174.28, 173.53, 173.19, 150.72, 102.10, 97.23, 78.98, 77.76, 76.48, 75.77, 74.35, 70.97, 70.54, 68.68, 67.05, 66.18, 62.48, 58.28, 45.89, 43.45, 40.02, 35.75, 33.45, 32.27, 31.07, 29.17, 27.48, 26.20, 25.89, 24.87, 22.71, 22.62, 21.67, 21.42, 20.27, 19.75, 19.16, 18.38, 17.45, 13.29, 11.51, 9.81; MS (FAB) calcd for $C_{60}H_{106}N_8O_{22}Li(Na)$ 1297.75 (1313.73), found 1297.59 (1313.56).

Biological procedure for antifungal testing

The assay used to determine the antifungal activity of conjugates **9** and **12** was performed in Lee's medium.[14] The yeast strain *Candida albicans* (ATCC 48130) was

obtained from the American Type Culture Collection, Rockville, MD, and was stored at –78 °C in culture tubes in a 1:1 mixture of YM broth and sterile lactose–glycerol solution.

To test for siderophore activity (i.e. growth-promotion), Lee's agar containing deferrated EDDA (25 mg mL^{-1}) was used. Lee's agar with no EDDA was used to test for antifungal activity. Stock solutions (10, 5, 1 mM) of the compounds were prepared in distilled, deionized water and were then sterilized. Ferric complexes of the conjugates were prepared by mixing equal portions of sterile 10 mM stock solutions and sterile 10 mM $FeCl_3 \cdot 6H_2O$ solutions and were used immediately. The petri dishes were incubated upside down at 37 °C and were examined after 24 and 48 h for zones of stimulation or inhibition. All tests were performed in duplicate.

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References and Notes

1. (a) Koshland, Jr D. E. *Science* **1994,** *254,* 327 (Editorial); (b) Travis, J. *Science* **1994,** *254,* 360; (c) Davies, J. *Science* **1994,** *254,* 375; (d) Nikaido, H. *Science* **1994,** *254,* 382; (e) Spratt, B. G. *Science* **1994,** *254,* 388.

2. For a review on the general principles involved in drug delivery, see: *The Pharmacological Basis of Therapeutics;* Gilman, A. G.; Goodman, L. S.; Gilman, A., Eds; MacMillan: New York, 1980; pp. 1–55.

3. (a) Neilands, J. B.; Krystyna, K.; Schwyn, B.; Coy, M.; Francis, R. T.; Paw, B. H.; Bagg, A. In *Iron Transport in Microbes, Plants and Animals;* Winkelmann, G.; van der Helm, D.; Neilands, J. B., Eds; VCH: Weinheim, 1987; Chapter 1; (b) Payne, S. M. *CRC Crit. Rev. Microbiol.* **1988,** *16,* 81; (c) *Handbook of Microbial Iron Chelates*; Winkelman, G., Ed.; CRC: Boca Raton, 1991; (d) Hider, R. C. *Struct. Bonding* **1984,** *58,* 1.

4. (a) For naturally occurring examples of antibiotic-containing siderophores (e.g. albomycins and ferrimycins) that are ingested by bacteria by the use of their active iron transport systems, see: Benz, G.; Schmidt, D. *Liebigs Ann. Chem.* **1984,** 1434 and references cited; (b) for a detailed discussion on iron chelator-mediated drug delivery including a comprehensive list of references, see: Miller, M. J.; Malouin, F.; Dolence, E. K.; Gasparski, C. M.; Ghosh, M.; Guzzo, P. R.; Lotz, B. T.; McKee, J. A.; Minnick, A. A.; Teng, M. In *Recent Advances in the Chemistry of Antiinfective Agents;* Bentley, P. H., Ponsford, R., Eds; Royal Society of Chemistry: London, 1993; Special Publication No. 119, pp. 135–159.

5. (a) Dolence, E. K.; Minnick, A. A.; Miller, M. J. *J. Med. Chem.* **1990**, *33,* 464; (b) Dolence, E. K.; Minnick, A. A.; Lin, C.-E.; Miller, M. J. *J. Med. Chem.* **1991**, 34, 956; (c) McKee, J. A.; Sharma, S. K.; Miller, M. J. *Bioconjugate Chem.* **1991**, 2, 281; (d) Miller, M. J. *Chem. Rev.* **1989**, *89,* 1563.

6. (a) Dolence, E. K.; Minnick, A. A.; Lin, C.-E.; Miller, M. J. *J. Med. Chem.* **1991**, 34, 968; (b) Minnick, A. A.; McKee, J. A.; Dolence, E. K.; Miller, M. J. *J. Antimicrob. Agents Chemother.* **1992,** *36,* 840; (c) Brochu, A; Brochu, N.; Nicas, T. I.; Parr, Jr T. R.; Miller, M. J.; Malouin, F. *Antimicrob. Agents Chemother.* **1992,** *36*, 2166.

7. Ghosh, M.; Dolence, J. A.; Miller, M. J. manuscript to be submitted.

8. Lee, B. H.; Miller, M. J.; Prody, C. A.; Neilands, J. B. *J. Med. Chem.* **1985,** *28*, 323.

9. Ghosh, M.; Miller, M. J. *J. Org. Chem.* **1994,** *59*, 1020.

10. For reviews of various antifungal agents and their modes of action, see: (a) Saksena, A. K.; Girijavallabhan, V. M.; Cooper, A. B.; Loenberg, D. *Ann. Reports Med. Chem.* **1989,** *24,* 111; (b) *CRC Handbook of Chemotherapeutic Agents;* Verderame, M., Ed.; CRC: Boca Raton, 1986; Vol. 1, pp. 219–260; (c) Bossche, H. V.; Willemsens, G.; Marichal, P. *CRC Crit. Rev. Microbiol.* **1987,** *15*, 57.

11. (a) Darwish, I. S.; Patel, C.; Miller, M. J. *J. Org. Chem.* **1993,** 58, 6072; (b) some hydroxamate-based and catechol-based siderophore-**3a**,**b** conjugates have been recently found effective against several human and plant pathogenic fungi, Darwish, I. S.; Miller, M. J. manuscript in preparation; (c) for related compounds (lipoxamycins and sphingofungins) see: (i) Mandala, S. M.; Frommer, B. R.; Thornton, R. A.; Kurtz, M. B.; Young, N. M.; Cabello, M. A.; Genilloud, O.; Liesch, J. M.; Smith, J. L.; Horn, W. S. *J. Antibiot.* **1994,** *47,* 376; (ii) VanMiddlesworth, F.; Giacobbe, R. A.; Lopez, M.; Garrity, G.; Bland, J. A.; Bartizal, K.; Fromtling, R. A.; Polishook, J.; Zweerink, M.; Edison, A. M.; Rozdilsky, W.; Wilson, K. E.; Monaghan, R. L. *J. Antibiot.* **1992,** *45*, 861; (iii) Horn, W. S.; Smith, J. L.; Bills, G. F.; Raghoobar, S. L.; Helms, G. L.; Kurtz, M. B.; Marrinan, J. A.; Frommer, B. R.; Thornton, R. A.; Mandala, S. M. *J. Antibiot.* **1992,** *45*, 1692.

12. Cannon, M. In *Comprehensive Medicinal Chemistry* ; Hansch, C.; Sammes, P. G.; Taylor, J. B., Eds; Pergamon Press: Oxford, 1990; Vol. 2, Chapter 10.5.

13. Mitscher, L. A.; Martin, F. G. In *CRC Handbook of Chemotherapeutic Agents*; Verderame, M., Ed.; CRC: Boca Raton, 1986; pp. 99–194.

14. For details of the preparation of the Lee's media and the testing procedures, see: Minnick, A. A.; Eizember, L. E.; McKee, J. A.; Dolence, E. K.; Miller, M. J. *Anal. Biochem.* **1991,** *194,* 223.

15. Teng, M.; Miller, M. J. *J. Am. Chem. Soc.* **1993,** *115*, 548.

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