Iron Transport-Mediated Drug Delivery: Synthesis and Biological Evaluation of Cyanuric Acid-Based Siderophore Analogs and β-Lactam Conjugates

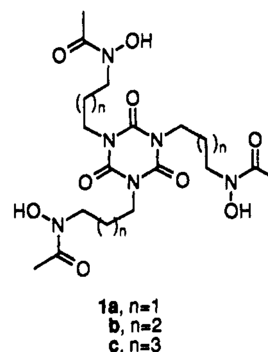
Manuka Ghosh and Marvin J. Miller*

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556

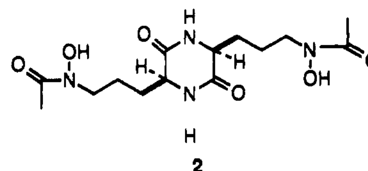
Received October 11, 1993®

Trihydroxamate-containing isocyanurates **4a–c** were synthesized and determined to be capable of substituting for natural siderophores (microbial iron sequestering agents) in limited microbiological assays. The direct coupling of protected **4a** to carbacephalosporins **16** and **17** and subsequent deprotection gave conjugates **20** and **21**. The Lorabid conjugate **21** was especially active against *E. coli* X580 in preliminary biological tests, thus demonstrating the continued potential of siderophore-mediated drug delivery.

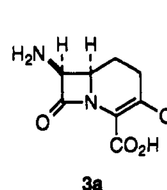
We have previously described the synthesis and study of isocyanuric acid-based trihydroxamic acids **1a–c**.[1] Initially designed to mimic the natural dihydroxamate siderophore rhodotorulic (**2**),[2] but with the ability to bind ferric ion stoichiometrically, these compounds were found to facilitate iron assimilation by a number of microorganisms. Thus, **1a–c** appeared to be effective mimics of natural siderophores (microbial iron chelators). Trihydroxamate **1b** ($n = 2$) also was especially effective for the promotion of iron excretion in a rat model of iron overload which simulated the consequences of transfusional therapy associated with the treatment of thalassemias. These studies also indicated that isocyanurate-based trihydroxamates **1a–c** were completely nontoxic. The combination of the siderophore properties and lack of mammalian toxicity suggested that antibiotic conjugates of **1a–c** would be ideal candidates for further studies of siderophore-mediated drug delivery.[3] Herein, we describe the first synthesis and preliminary biological studies of conjugates of cyanuric acid-based trihydroxamate siderophore analogs similar to **1b** and selected carbacephalosporins.



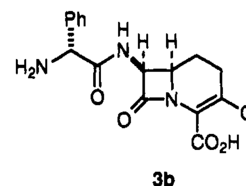
1a, n=1
b, n=2
c, n=3



2



3a



3b

Carbacephalosporin nucleus **3a** and its phenylglycyl derivative,[4] Lorabid (**3b**), were chosen as the antibiotic components of the conjugates since the mode of action of β-lactam antibiotics is well-known, the absence of sulfur relative to other β-lactam antibiotics was anticipated to facilitate syntheses, especially anticipated hydrogenolytic deprotections, and the activity of the resulting conjugates could be directly compared to previously studied siderophore–carbacephalosporin conjugates.[3] Since **1b** was the most effective iron transport agent of the isocyanurate siderophore analogs previously studied, a derivative of it suitable for conjugation to the carbacephalosporins was desired. Thus, the initial goal of this research was to modify the synthesis of **1b** to incorporate appropriate linker group functionality as in **4a–c** by simply changing the acylating agent(s) used during the synthesis of **1b**. All three siderophore analogs **4a–c** were of interest to determine if the presence of one, two, or three ionizable carboxyl groups, respectively, would have an effect on the microbial recognition and transport of the corresponding iron complexes. Furthermore, **4a–c** would provide the potential for attachment of one or more of the same or eventually different drugs to the same siderophore component.

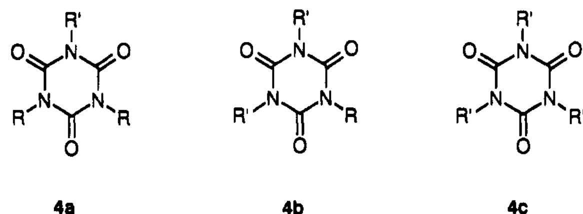
Results and Discussion

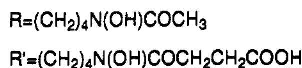
Chemical Studies and Synthesis of Siderophore Components. The first step towards the syntheses of

® Abstract published in *Advance ACS Abstracts*, February 1, 1994.

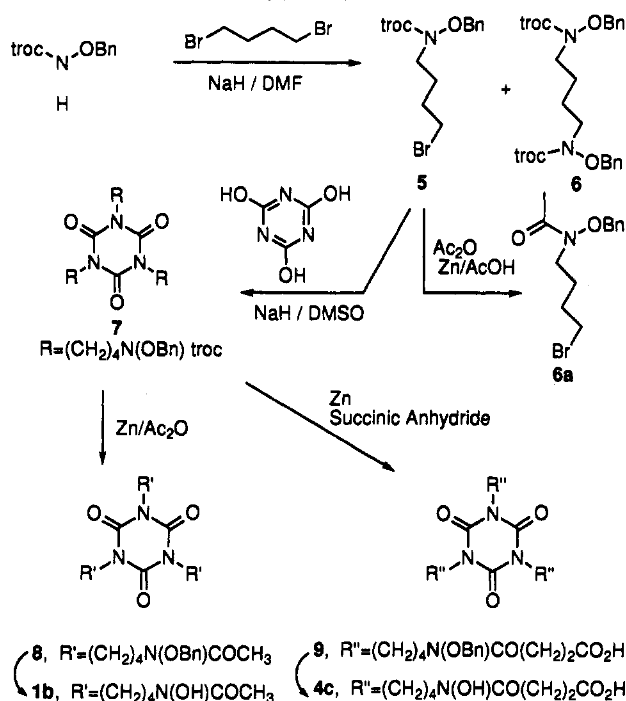
(1) Lee, B. H.; Miller, M. J.; Prody, C. A.; Neilands, J. B. *J. Med. Chem.* 1985, *28*, 323.
(2) Neilands, J. B.; Altin, C. L. *Biochemistry* 1968, *7*, 734.
(3) (a) Miller, M. J.; Malouin, F. *Acc. Chem. Res.* 1993, *26*, 241–249. (b) Miller, M. J. *Chem. Rev.* 1989, *89*, 1563–1579. (c) Miller, M. 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. Iron Transport-Mediated Drug Delivery. (Bentley, P. H., Ponsford, R., Eds.); In *Recent Advances in the Chemistry of Anti-Infective Agents*; Royal Society of Chemistry Special Publication no. 119; Royal Society of Chemistry: London, 1993; pp 135–159.
(4) (a) Jones, R. N.; Barry, A. L. *J. Antimicrob. Ther.* 1988, *22*, 315. (b) Jorgensen, J. H.; Redding, J. S.; Maher, L. A. *J. Antimicrob. Ther.* 1988, *22*, 445. (c) Bodurow, C. C; Boyer, B. D.; Brennan, J.; Bunnell, C. A.; Burks, J. E.; Carr, M. A.; Doecke, C. W.; Eckrich, T. M.; Fisher, J. W.; Gardner, J. P.; Graves, B. J.; Hines, P.; Hoying, R. C.; Jackson, B. G.; Kinnick, M. D.; Kochert, C. D.; Lewis, J. S.; Luke, W. D.; Moore, L. L.; Morin, J. M., Jr.; Nist, R. L.; Prather, D. E.; Sparks, D. L.; Vladuchick, W. C. *Tetrahedron Lett.* 1989, *30*, 2321.

0022-3263/94/1959-1020$04.50/0 © 1994 American Chemical Society





Scheme 1

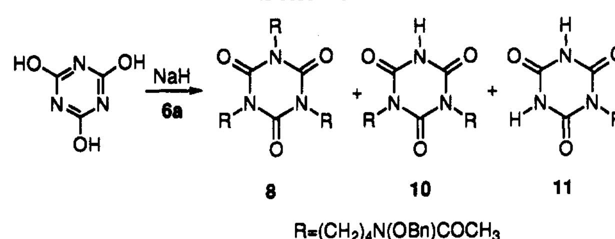


compounds **4a–c** paralleled our earlier synthesis[1] of **1b** and involved preparation of an appropriately protected N-(ω-haloalkyl)hydroxamate side chain precursor. As shown in Scheme 1, treatment of 1,4-dibromobutane with *O*-benzyl-*N*-[(trichloroethoxy)carbonyl]hydroxylamine in the presence of base (NaH) provided the desired N-alkylated material **5** in 75% reproducible yield (reported[1] 40%) along with about 10% of bisalkylated product **6**. Even though N-alkylation of cyanuric acid is precedented,[5] the proper choice of solvent for the trialkylation of the poorly soluble cyanuric acid with bromide **5** proved to be crucial. DMSO was found to be more effective than DMF as the solvent for this transformation. Thus, treatment of isocyanuric acid with >300 mol % of dimsylsodium at 0 °C to rt for 30 min, followed by addition of alkylating agent **5**, provided **7** in 85% yield after stirring for 24 h (Scheme 1). At this stage, since an efficient synthesis of compound **1b** itself is of interest, a reductive acylation of **7** in the presence of excess activated Zn and acetic anhydride was also attempted. This provided **8** in 63% overall yield (lit.[1] 24%) for the three- step transformation from dibromobutane and afforded an improved route to **1b** after hydrogenolysis.

For the preparation of **4c**, with three attached succinate groups, tris-troc-protected **7** was subjected to a similar reaction sequence with substitution of succinic anhydride as the acylating agent. This afforded a 99% yield of desired

(5) Smolin, E. M.; Rapoport, L. *In S-Triazines and Derivatives*; Interscience: New York, 1957; Chapter 7.

Scheme 2

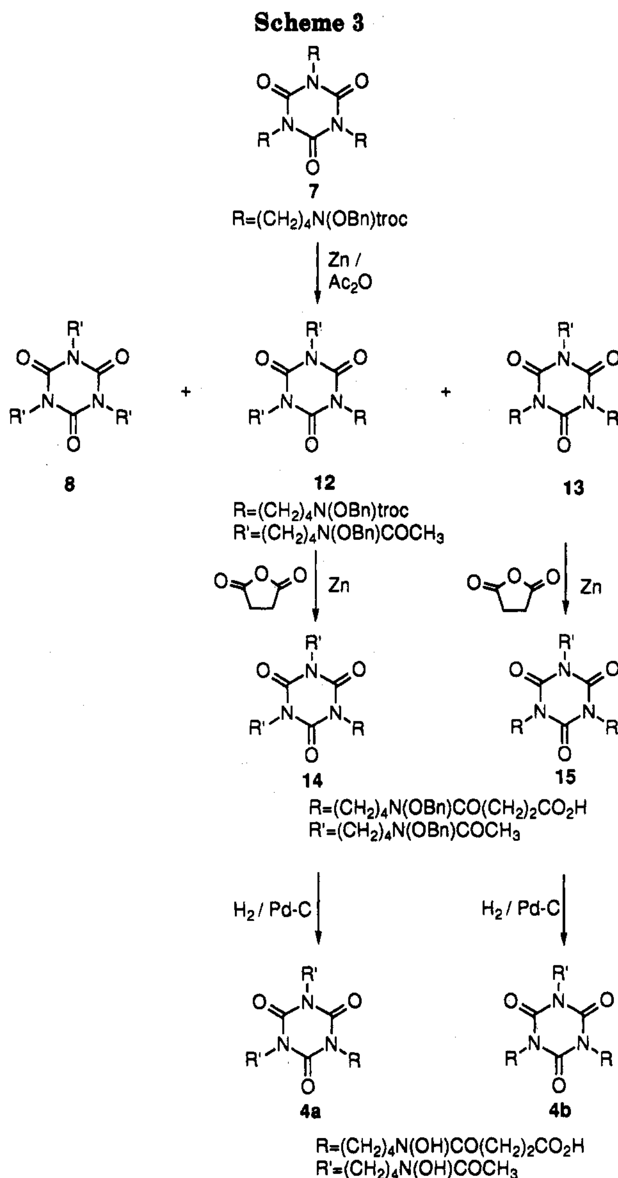


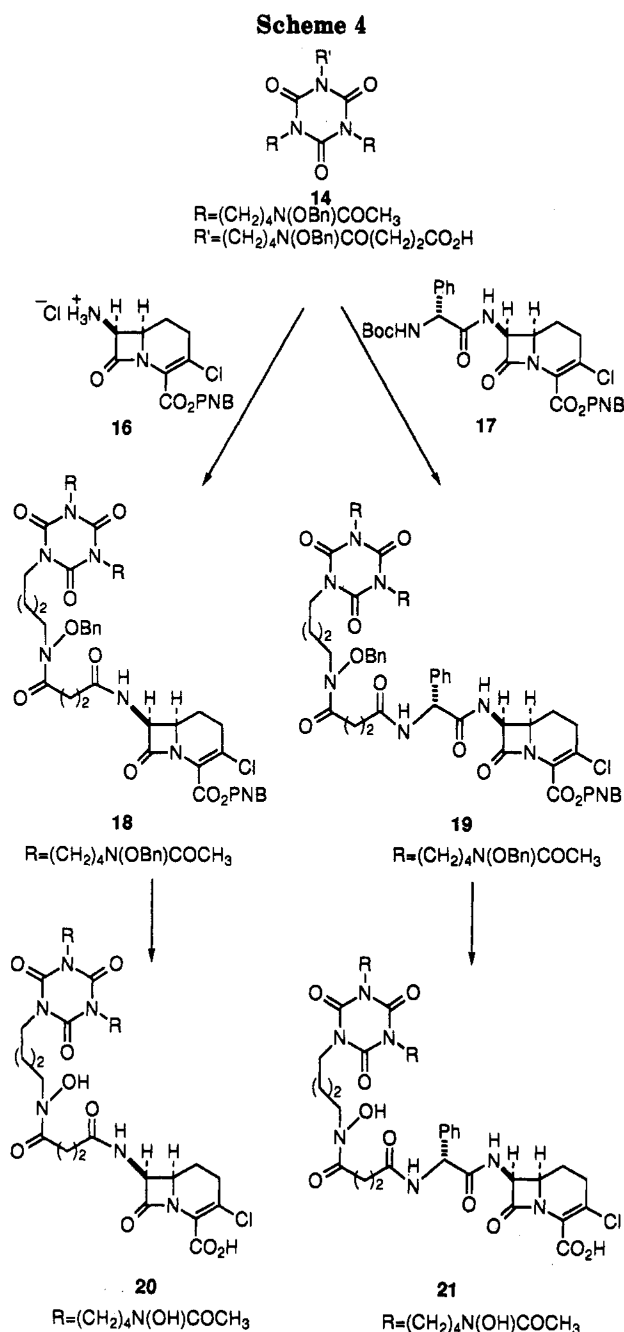
triacid **9** after purification. Hydrogenolysis of **9** with 10% Pd–C furnished compound **4c** in nearly quantitative yield.

For the synthesis of mono- and bis-succinates **4a** and **4b**, initially selective mono- and dialkylations of isocyanuric acid were attempted by limiting the amount of base (NaH) to 100 and 200 mol %, respectively, and using our previous acetylated alkylating agent **6a**[1] (Scheme 2). Despite considerable variation of conditions, the major isolated product was always trialkylated compound **8** along with minor amounts of mono- (**11**) and dialkylated product (**10**). Even use of separately preformed and isolated mono- and disodio/potassio salts of cyanuric acid[6] gave trialkylated compound **8** as the major product, probably due to the equilibration of the mono- and/or disalt to the trisalt in solution.

However, with tris-troc-protected product **7** in hand, we next focused our attention on the introduction of one or two acetyl groups by controlled removal of troc protecting groups. According to literature precedent,[7] the rate of reaction for the reductive removal of troc groups is highly dependent on the acidity of the reaction medium, and the required time for complete reduction may vary from 30 min to 12 h. When compound **7** was treated with an excess of Zn and acetic anhydride in THF containing 0.1% AcOH, the reaction was complete within 2 h with complete disappearance of **7**. However, the initiation of the reaction was slow (40 min to 1 h), and triacetylated compound **8** was always the major product even when only 200 mol % of acetic anhydride was used. In the presence of 0.05% of AcOH, the reaction was sluggish and furnished an unfavorable distribution of **8**, **12**, and **13** along with some recovered starting **7** after 24 h. In order to obtain a better product to substrate ratio with a better product distribution, fast initiation of the reaction followed by an immediate quench was attempted. Indeed, at slightly elevated temperature the reaction could be successfully initiated within 15–20 min. Thus, treatment of **7** with an excess of freshly activated Zn and acetic anhydride in THF containing 0.1% AcOH at 50–60 °C (water bath) followed by quenching of the reaction within 5–10 min after initiation provided a reasonably good distribution of **8**, **12**, and **13** (10%, 25%, and 30% respective yields). The recovery of recyclable starting material **7** (35%) further added to the utility of this modified procedure (Scheme 3). After straightforward chromatographic separation of the product mixture, compounds **12** and **13** were separately subjected to reductive acylation with succinic anhydride to incorporate one or two linker arms, respectively. Here again, reductive removal of the troc group of compounds **12** and **13** using excess zinc dust and in the presence of succinic anhydride provided **14** and **15**, respectively, in nearly quantitative yield (>99%). Hydrogenation of **14** and **15** over 10% Pd–C proceeded cleanly to afford **4a** and **4b**, respectively, in an 85% average yield.

(6) Ponomarew, J. *Chem. Ber.* **1885**, *18*, 3261.
(7) Just, G.; Grozinger, K. *Synthesis* **1976**, 457.





Synthesis of Conjugates. The syntheses of carbacephalosporin conjugates **20** and **21** are shown in Scheme 4. The hydrochloride salt of carbacephalosporin **16** and acid **14** was suspended in anhydrous methylene chloride and treated with triethylamine followed by addition of the *N*-hydroxybenzotriazole (HOBT) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC) to provide protected conjugate **18** in 57.5% yield. Similarly, Boc protected carbacephalosporin **17** was treated with trifluroacetic acid (TFA) in anhydrous methylene chloride to provide the amine salt which was suspended in anhydrous methylene chloride and treated with triethylamine to liberate the free amine. The free amine was not isolated but treated with acid **14** in the presence of HOBT and EDC to provide protected conjugate **19** in 57% yield. The problem associated with the deprotection of the above conjugates without reducing the essential double bond or removing the chlorine atom in the carbacephalosporin component was eliminated using the modified conditions described during the previous syntheses of siderophore–carbacephalosporin conjugates.[8] Thus, successful deprotection was achieved with hydrogen, 10% Pd–C (20% w/w), concentrated hydrogen chloride (3 equiv), and 5% aqueous DMF to give conjugates **20** and **21** quantitatively.

Biological Studies. Compounds **20** and **21** were initially screened for their antibacterial activity by using a standard agar dilution minimum inhibitory concentration (MIC) assay. Conjugate **20** showed only weak activity against one *S. aureus* strain, and compound **21** showed only weak activity against several Gram-positive isolates. Although these initial results using standard biological screens based on MIC studies indicated "poor" activity, our previous experience with siderophore conjugates[3a,9] suggests that MIC studies do not adequately reflect the activity or mode of action of siderophore conjugates. MIC studies tend to be evaluated after a given time frame (24–36 h). We have found that the biological action of other siderophore conjugates is missed by just looking for growth or lack of growth after such extended times. Thus, we

(8) McKee, J. A.; Sharma, S. K.; Miller, M. J. *Bioconjugate Chem.* **1991**, *2*, 281–291.

(9) (a) Minnick, A. A.; McKee, J. A.; Dolence, E. K.; Miller, M J. *Antimicrob. Agents Chemother.* **1992**, *36*, 840. (b) Brochu, A.; Brochu, N.; Nicas, T. I.; Parr, T. R., Jr.; Miller, M. J.; Malouin, F. *Antimicrob. Agents Chemother.* **1992**, *36*, 2166.

Table 1. Stimulation of Growth of *E. coli* X580[a] by Cyanuric Acid Based Trihydroxamate Siderophores

	zone diam[c] (mm)			
	MeOH (after 36 h)		H_2O (after 24 h)	
compd[b]	5 μM	10 μM	5 μM	10 μM
4a	0	0	0	0
4a–Fe^{3+}	24	30	20	25
4b	0	0	0	0
4b–Fe^{3+}	32	32	25	26
4c	0	0	0	0
4c–Fe^{3+}	34	36	26	28

[a] *E. coli* X580 overnight culture, 1:200 000 dilution into Luria agar supplemented with 100 μg/mL of EDDA. [b] Filter paper disks containing 5 μL of the siderophores at concentration indicated were placed on the surface of the Luria agar. [c] Plates were incubated at 37 °C for 24 or 36 h and examined for zones of stimulation around the compounds.

initiated more detailed studies of the siderophore analogs and conjugates synthesized and described earlier in this paper.

Growth Promotion Activities of Trihydroxamate Isocyanurate Siderophore Analogs. Petri dish bioassays were used to determine the abilities of trihydroxamate isocyanurates **4a–c** to reverse the growth inhibition effect of ethylenediamine bis(*o*-hydroxyphenylacetic acid) (EDDA), a chelator which effectively induces microbial iron deficiency in media (Luria agar, in this case). The concentration of EDDA (100 μg per mL) which was necessary to suppress the growth of, but not to kill, the seeded *E. coli* X580 test strain was determined emperically in accord with analogous studies.[9] Preformed Fe^{3+} complexes of compounds **4a–c**, which contain trihydroxamates, stimulated growth. However, deferric isocyanurates **4a–c** and a ferric chloride control exhibited no growth stimulatory activity under identical conditions (Table 1). Thus, at least for *E. coli* X580, the iron complexes of **4a–c** appear to serve as effective siderophores, despite the presence of one, two, and even three peripheral ionizable carboxyl groups!

Selected Antibacterial Activity of Siderophore–Antibiotic Conjugates. Synthetic isocyanurate–β-lactam conjugates **20** and **21** were subjected to more detailed studies of antimicrobial activity against *E. coli* X580 in Luria broth cultures. Activity was observed in each case, regardless of whether the compound was added with or without 1 equiv of ferric chloride, indicating that the conjugates can effectively sequester iron from the non-deferrated media. As shown in Figure 1, 10 μM concentrations of preformed iron complexes of both of the conjugates induced considerable delay of apparent growth. The activity of conjugate **20**, derived from the parent carbacephalosporin, was intermediary between those of the two extremes of the very potent conjugate (**21**) from Lorabid and the control. Bacteria which eventually populated the culture of *E. coli* X580 after exposure to the conjugates were transferred to fresh cultures containing identical concentrations of the iron complexes of **20** and **21**. These transfers were made just as the initial culture was leaving the exponential phase of growth. In each case of reincubation with the conjugates no delay of growth was observed, indicating that the initial exposure of **20** and **21** to *E. coli* X580 probably resulted in complete inhibition of growth of the parent strain with subsequent selection of mutants resistant to the siderophore–antibiotic conjugates. Our previous studies with other conjugates gave similar results and were shown to be a consequence

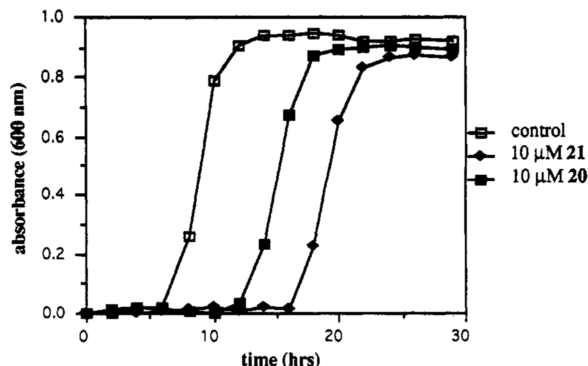


Figure 1. Growth curves of *E. coli* X580 in Luria broth in the presence of the preformed iron complexes of trihydroxamate isocyanurate conjugates **20** and **21**. Compounds were tested at 10.0 μM concentration.

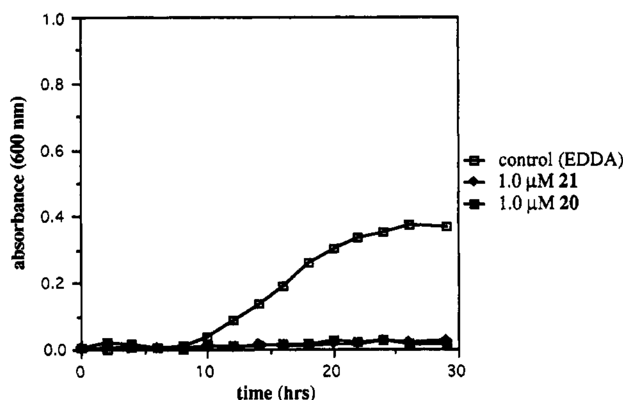


Figure 2. Growth curves demonstrating antibacterial activity of the conjugates **20** and **21** in iron-deficient culture medium deferrated with 100 μg of EDDA per mL. Compounds were tested at 1.0 μM concentration.

of mutant selection in which the mutants lacked the outer membrane receptor and/or transport protein for the siderophore bound to the drug.[3a,9] Thus, bacteria that did eventually grow after prolonged exposure to **20** and **21** may lack the ability to recognize and transport the iron bound siderophore component. Since the conjugates are too large for passive diffusion through porins, the conjugates appear to be totally ineffective against the selected "resistant" strains. We had also shown with previous siderophore conjugates that selected mutants were not pathogenic to mammals.[3a,9] Apparently, since they were missing part of their iron assimilation machinery, they were not able to sequester their essential iron under the iron-limited conditions of mammalian serum. Preliminary studies suggest the same situation with conjugates **20** and **21** and the corresponding "resistant" mutants. Thus, incubation of the conjugates with *E. coli* X580 in iron deficient culture media resulted in complete inhibition of growth even at 1.0 μM concentration (Figure 2). These results contrast those from the standard MIC assay and again emphasize that for certain classes of compounds, basing an assay on single or few time-based data points without attention to intimate details of conditions, including exogenous siderophores in standard media, may promote misinterpretation of biological activity.

In conclusion, synthetic and totally artificial iron chelators based on trihydroxamic acid derivatives of isocyanuric acid were again shown to be effective siderophores, at least in the limited studies completed. Furthermore, antibiotic conjugates of these readily available siderophore analogs are recognized by microbes, appar-

ently actively transported, at least through the outer membrane, and inhibit the growth of the parent microbial strain. Thus, the microbe recognizes each conjugate as a source of nutritional iron, but in the process of actively assimilating it also assimilates a toxic agent (the antibiotic) and in effect commits suicide. Further synthetic studies of the potential utility and details of siderophore-mediated drug delivery are being carried out in our laboratories and will be reported in due course.

Experimental Section

General Methods. Chemistry. Instruments and general methods used have been described earlier.[10] The term "dried" refers to the drying of an organic layer over anhydrous magnesium or sodium sulfate. All reactions were performed under nitrogen atmosphere.

4-[*N*-[(Trichloroethoxy)carbonyl]-*N*-(benzyloxy)amino]-1-butyl Bromide (5).[1] NaH (1.60 g, 60% dispersion in mineral oil, 40.2 mmol) was placed in a two-necked round-bottomed flask under a nitrogen atmosphere, and washed (3 × 5 mL) with dry hexanes. DMF (40 mL) was added, and the flask was cooled in an ice bath. A solution of *O*-benzyl-*N*-[(trichloroethoxy)carbonyl]-hydroxylamine (10 g, 33.5 mmol) in DMF (20 mL) was added with stirring. Evolution of hydrogen gas was apparent. The ice bath was removed, and the reaction mixture was stirred for 45 min. The ice bath was reapplied, and dibromobutane (8.68 g, 40.18 mmol) in dry DMF (20 mL) was then added dropwise at 0 °C. The ice bath was removed toward the end of the addition, and the reaction mixture was left stirring overnight at room temperature. The reaction was quenched with cold water and extracted three times with ethyl acetate. The combined organic layers were washed with water, dried, filtered, and evaporated. The crude residual oil was purified by chromatography on silica gel (ether/hexanes (1:4)) to afford 10.8 g (75%) of compound **5** which was identical to that reported earlier:[1] ^{1}H NMR ($CDCl_3$) δ 1.7–2.0 (m, 4H), 3.39 (t, 2H), 3.54 (t, 2H), 4.84 (s, 2H), 4.94 (s, 2H), 7.36–7.44 (m, 5H); IR (neat) 1720 cm^{-1}.

Tris[[*N*-[(trichloroethoxy)carbonyl]-*N*-(benzyloxy)-amino]butyl] Isocyanurate (7). NaH (0.9 g, 60% dispersion in oil, 23.8 mmol) was placed in a two-necked 250-mL round-bottomed flask equipped with a N_2 inlet and a septum. Dry hexanes were added, and the suspension was stirred and then allowed to settle. The hexanes were removed by a syringe. This process was repeated twice, and then dry DMSO (30 mL) was added. To this mixture was added cyanuric acid (0.83 g, 6.43 mmol) in dry DMSO (20 mL) while the reaction flask was cooled in an ice bath. The ice bath was then removed, and the reaction mixture was stirred at room temperature for 30 min to ensure complete anion formation. The reaction mixture was again cooled in an ice bath, and a catalytic amount of NaI was added followed by addition of bromide **5** (10 g, 23.2 mmol) in DMSO (20 mL) with a syringe. After being stirred overnight at room temperature, the reaction mixture was taken up in ethyl acetate and washed several times with water. The organic layer was dried and filtered. After removal of solvent, the residue was subjected to chromatography over silica gel eluting with ethyl acetate/hexanes (1:3) to afford the tri-*N*-alkylated material **7** as a colorless sticky liquid (6.48 g, 85%): ^{1}H NMR ($CDCl_3$) δ 1.65 (br s, 12H), 3.54 (m, 6H), 3.83 (m, 6H), 4.83 (s, 6H), 4.92 (s, 6H), 7.38 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 24.07, 24.82, 42.37, 49.04, 74.96, 77.12, 95.24, 128.44, 128.74, 129.43, 134.69, 148.69, 154.89; IR (neat) 2950, 1710, 1685, 1458 cm^{-1}; MS (CI, isobutane) *m/e* 1187 (M$^+$). Anal. Calcd for $C_{45}H_{51}N_6O_{12}Cl_9$: C, 45.53; H, 4.33; N, 7.08. Found: C, 45.32; H, 4.11; N, 6.89.

Tris[[*N*-acetyl-*N*-(benzyloxy)amino]butyl] Isocyanurate (8).[1] To a solution of compound **7** (1.0 g, 0.842 mmol) in a 1:1 mixture of THF/AcOH was added activated zinc dust (0.826 g, 12.6 mmol) and acetic anhydride (1.18 mL, 12.6 mmol). The resulting suspension was stirred overnight at room temperature. The solvent was evaporated, and the residue was taken up into ethyl acetate, filtered, washed with water, 10% $NaHCO_3$, water, and brine, dried, and filtered again. After evaporation of the solvent, the residual oil was chromatographed over silica gel. Elution with 2-propanol/EtOAc (1:4) provided compound **8** as an oil (650 mg, 98%): ^{1}H NMR ($CDCl_3$) δ 1.51–1.54 (m, 12H), 2.08 (s, 9H), 3.67 (t, J = 6.2 Hz, 6H), 3.84 (t, J = 6.3 Hz, 6H), 4.81 (s, 6H), 7.38 (s, 15 H); ^{13}C NMR ($CDCl_3$) δ 20.08, 23.67, 24.58, 42.07, 44.28, 75.83, 128.29, 128.54, 128.75, 133.97, 148.42, 171.91; IR (neat) 1460, 1640, 1680, 1700 cm^{-1}. Anal. Calcd for $C_{42}H_{54}N_6O_9$: C, 64.11; H, 6.92; N, 10.68. Found: C, 64.30; H, 6.80; N, 10.45.

Tris[(*N*-acetyl-*N*-hydroxyamino)butyl] Isocyanurate (1b).[1] A solution of compound **8** (600 mg, 0.763 mmol) in 2-propanol (10 mL) was treated with 10% Pd–C (120 mg, 20% w/w) under 1 atm of H_2 for 10 h at room temperature. The catalyst was removed by filtration, and the solvent was evaporated. Compound **1b** was obtained as a colorless sticky oil (390 mg, 99%): ^{1}H NMR (CD_3OD) δ 1.63 (br s, 12H), 2.08 (s, 9H), 3.62 (br s, 6H), 3.87 (br s, 6H), 4.88 (br s); ^{13}C NMR (CD_3OD) δ 20.25, 24.81, 25.83, 43.37, 48.28, 150.65, 173.43.

Tris[[*N*-succinyl-*N*-(benzyloxy)amino]butyl] Isocyanurate (9). Compound **7** (0.5 g, 0.421 mmol) was dissolved in 20 mL of a 1:1 mixture of acetic acid and THF. Activated zinc dust (0.413 g, 6.31 mmol) and succinic anhydride (0.421 g, 4.21 mmol) were added to the resulting solution. The reaction mixture was stirred overnight at room temperature. The solvent was evaporated, and the residue was taken up into ethyl acetate, filtered, washed with water several times, and dried (Na_2SO_4). After filtration and evaporation of the solvent, the residue was chromatographed over silica gel. Elution with a mixture of chloroform, 2-propanol, and acetic acid (90:10:1) provided **9** (0.363 g, 90%): ^{1}H NMR ($CDCl_3$) δ 1.45–1.75 (m, 12H), 2.55–2.75 (m, 12 H), 3.62–3.74 (m, 6H), 3.75–3.87 (m, 6H), 4.82 (s, 6H), 7.36 (s, 15H), 9.6 (br s, 3H); ^{13}C NMR ($CDCl_3$) δ 23.63, 24.51, 26.92, 28.31, 42.19, 44.58, 75.98, 77.19, 128.44, 128.71, 128.95, 133.98, 148.62, 173.12, 177.15; IR (neat) 1460, 1690, 1700, 1720 cm^{-1}; MS (FAB) *m/z* 961 (MH$^+$), 984 (MNa$^+$). Anal. Calcd for $C_{48}H_{60}N_6O_{15}$: C, 59.97; H, 6.29; N, 8.75. Found: C, 59.76; H, 6.53; N, 8.89.

Tris[(*N*-succinoyl-*N*-hydroxyamino)butyl] Isocyanurate (4c). Compound **9** (100 mg, 0.104 mmol) was dissolved in 2-propanol (5 mL) and treated with 10% Pd–C (20 mg) under 1 atm of H_2 for 24 h at room temperature. The catalyst was removed by filtration, and the solvent was evaporated. Compound **4c** was obtained as a colorless sticky oil (70 mg, 97.5%): ^{1}H NMR (CD_3OD) δ 1.49–1.69 (m, 12H), 2.51 (t, J = 6.6 Hz, 6H), 2.75 (t, J = 6.7 Hz, 6H), 3.48–3.68 (m, 6H), 3.73–3.93 (m, 6H), 5.01 (br s); ^{13}C NMR (CD_3OD) δ 24.83, 25.83, 28.32, 29.50, 43.42, 48.55, 150.74, 174.40, 176.60; IR (neat) 1460, 1700, 1720 (sh) cm^{-1}; MS (FAB) m/z 691 (MH$^+$), 713 (MNa$^+$); FAB HRMS calcd for $C_{27}N_6H_{43}O_{15}$ 691.2786 (MH$^+$), found 691.2775.

Controlled Exchange of Troc Groups of 7 with Acetyl Groups. Compound **7** (1.0 g, 0.84 mmol) was dissolved in anhydrous THF (6 mL). Acetic acid (7 μL) was added to the resulting solution. Freshly activated zinc dust (826 mg, 12.6 mmol) and acetic anhydride (794 μL, 8.42 mmol) were added to the above solution. The reaction mixture was slightly warmed using a water bath (50–60 °C). The initiation of the reaction took about 10 min as was indicated by TLC. The reaction mixture was then stirred for another 5 min and filtered, and the volatile components were evaporated. The residue was taken up into ethyl acetate, and the combined organic layers were washed with water, saturated $NaHCO_3$ solution, and brine, dried (Na_2SO_4), filtered, and evaporated. The crude mixture of compounds containing **8**, **12**, **13**, and starting material **7** were separated by column chromatography using ethyl acetate/hexanes (1:5, 1:2, 1:1, 2:1, 1:0 gradient).

Compound **8** was obtained as a colorless liquid (66 mg, 10%) and showed spectral characteristics identical with the sample of **8** described above.

Mono[[*N*-[(trichloroethoxy)carbonyl]-*N*-(benzyloxy)-amino]butyl] bis[[*N*-acetyl-*N*-(benzyloxy)amino]butyl] isocyanurate (12) was also separated as a colorless liquid (193 mg, 25%): ^{1}H NMR ($CDCl_3$) δ 1.54–1.74 (m, 12H), 2.08 (s, 6H), 3.50–3.57 (t, J = 6.6 Hz, 2H), 3.62–3.72 (m, 4H), 3.80–3.90 (t, J = 6.3 Hz, 6H), 4.81 (s, 4H), 4.83 (s, 2H), 4.92 (s, 2H), 7.27–7.47 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 20.49, 24.12, 24.19, 24.93, 25.04, 42.47, 42.53, 49.20, 75.11, 76.31, 77.21, 95.28, 128.51, 128.70, 128.80, 128.94, 129.12, 129.48, 134.44, 134.82, 148.84, 155.03, 171.18; IR (neat) 1460, 1660, 1685,1720 cm^{-1}; MS (FAB) *m/z* 921 (MH$^+$); FAB HRMS Calcd for $C_{43}H_{54}N_6O_{10}Cl_3$ 919.2967 (MH$^+$), found

(10) Teng, M.; Miller, M. J. *J. Am. Chem. Soc.* 1993, *115*, 548.

919.2984. Anal. Calcd for $C_{43}H_{53}N_6O_{10}Cl_3$: C, 56.12; H, 5.8; N, 9.13. Found: C, 56.23; H, 5.96; N, 9.08.

Bis[[*N*-[(trichloroethoxy)carbonyl]-*N*-(benzyloxy)amino]butyl] mono[[*N*-acetyl-*N*-(benzyloxy)amino]butyl] isocyanurate (13) was obtained as colorless liquid (266 mg, 30%: ^{1}H NMR (CDCl$_3$) δ 1.48–1.78 (m, 12H), 2.08 (s, 3H), 3.50–3.58 (t, *J* = 5.4Hz, 4H), 3.62–3.72 (m, 2H), 3.78–3.90 (t, *J* = 6 Hz, 6H), 4.80 (s, 2H), 4.83 (s, 4H), 4.92 (s, 4H), 7.32–7.46 (m, 15H); ^{13}C NMR (CDCl$_3$) δ 20.38, 24.01, 24.08, 24.82, 24.92, 42.36, 49.08, 75.00, 76.19, 77.12, 95.21, 128.41, 128.60, 128.70, 128.84, 129.03, 129.38, 134.34, 134.72, 148.72, 154.91, 171.89; IR (neat) 1460, 1640, 1690, 1710 cm^{-1}; MS (FAB) *m/z* 1053 (MH$^+$). Anal. Calcd for $C_{44}N_6H_{52}O_{11}Cl_6$: C, 50.28; H, 4.99; N, 8.0. Found: C, 49.96; H, 5.11; N, 7.93.

Mono[[*N*-succinoyl-*N*-(benzyloxy)amino]butyl] Bis[[*N*-acetyl-*N*-(benzyloxy)amino]butyl] Isocyanurate (14). A solution of compound 12 (500 mg, 0.54 mmol) in 10 mL of acetic acid and THF (1:1) was stirred overnight at room temperature with activated zinc dust (530 mg, 8.1 mmol) and succinic anhydride (210 mg, 2.1 mmol). After the usual workup, the residue was purified by column chromatography eluting with methylene chloride/2-propanol/acetic acid (90:10:1) to furnish pure 14 as a colorless viscous liquid (454 mg, 99%): ^{1}H NMR (CDCl$_3$) δ 1.53–1.73 (m, 12 H), 2.08 (s, 6H), 2.57–2.78 (m, 4H), 3.58–3.73 (m, 6H), 3.78–3.93 (m, 6H), 4.80 (s, 4H), 4.84 (s, 2H), 7.34 (s, 15H); ^{13}C NMR (CDCl$_3$) δ 20.37, 23.94, 24.03, 24.75, 24.88, 27.24, 28.62, 42.46, 44.89 (br), 76.22, 76.27, 128.67, 128.94, 129.13, 134.25, 148.81, 148.83, 172.49, 173.51, 175.66; IR (neat) 1460, 1690–1700 (br), 1720 cm^{-1}; MS (FAB) *m/z* 845.35 (MH$^+$); FAB HRMS calcd for $C_{44}H_{57}N_6O_{11}$ 845.4085 (MH$^+$), found 845.4081.

Mono[(*N*-succinoyl-*N*-hydroxyamino)butyl] Bis[(*N*-acetyl-*N*-hydroxyamino)butyl] Isocyanurate (4a). Compound 14 (60 mg, 0.071 mmol) was dissolved in 2-propanol (5 mL) and was treated with 10% Pd–C (10 mg) at 1 atm of H_2 for 8 h. The reaction mixture was filtered, and the solvent was removed to afford compound **4a** (35 mg, 85%) as a sticky oil: ^{1}H NMR (CD$_3$OD) δ 1.53–1.73 (m, 12H), 2.08 (s, 6H), 2.51(t, *J* = 6.8 Hz, 2H), 2.75 (t, *J* = 6.7 Hz, 2H), 3.52–3.72 (m, 6H), 3.77–3.97 (m, 6H), 4.91 (br s); ^{13}C NMR (CD$_3$OD) δ 20.23, 24.79, 25.80, 28.35, 29.73, 43.39, 48.26, 150.61, 173.48, 174.34, 176.75; IR (neat) 1460, 1690, 1700 cm^{-1}; MS (FAB) *m/z* 575.30 (MH$^+$); FAB HRMS calcd for $C_{23}H_{39}N_6O_{11}$ 575.2677 (MH$^+$), found 575.2666.

Bis[[*N*-succinoyl-*N*-(benzyloxy)amino]butyl] Mono[[*N*-acetyl-*N*-(benzyloxy)amino]butyl] Isocyanurate (15). Compound 13 (500 mg, 0.475 mmol) was dissolved in 10 mL of a 1:1 mixture of acetic acid and THF. Activated zinc dust (465 mg, 7.12 mmol) and succinic anhydride (474 mg, 4.74 mmol) were added to the solution. The resulting solution was stirred overnight at room temperature. The usual workup provided crude compound 15 which was purified by column chromatography on silica gel eluting with methylene chloride/methanol/acetic acid (90:10:1). Compound 15 (423 mg, 99%) was obtained as a colorless sticky liquid which resisted crystallization: ^{1}H NMR (CDCl$_3$) δ 1.48–1.78 (m, 12H), 2.09 (s, 3H), 2.55–2.78 (m, 8H), 3.62–3.74 (m, 6H), 3.75–3.90 (m, 6H), 4.80 (s, 2H), 4.83 (s, 4H), 7.36 (s, 15H); ^{13}C NMR (CDCl$_3$) δ 20.13, 23.76, 23.85, 24.62, 24.73, 27.05, 28.43, 42.29, 44.86 (br), 76.09, 128.53, 128.80, 129.02, 134.10, 148.67, 148.70, 172.49, 173.23, 176.68; IR (neat) 1460, 1610, 1630, 1690 (br), 1720 cm^{-1}; MS (FAB) *m/z* 903 (MH$^+$); FAB HRMS calcd for $C_{46}H_{59}N_6O_{13}$ 903.4140 (MH$^+$), found 903.4136.

Bis[(*N*-succinoyl-*N*-hydroxyamino)butyl] Mono[(*N*-acetyl-*N*-hydroxyamino)butyl] Isocyanurate (4b). Compound 15 (100 mg, 0.110 mmol) was dissolved in 2-propanol (5 mL) and treated with 10% Pd–C (20 mg) under 1 atm of H_2 for 20 h at room temperature. The reaction mixture was filtered, and the solvent was evaporated to provide compound **4b** (60 mg, 85%): ^{1}H NMR (CD$_3$OD) δ 1.54–1.74 (m, 12H 2.09 (s, 3H), 2.56 (t, *J* = 6.7 Hz, 4H), 2.76 (t, *J* = 6.8 Hz, 4H), 3.53–3.73 (m, 6H), 3.78–3.98 (m, 6H), 4.89 (br s); ^{13}C NMR (CD$_3$OD) δ 20.25, 24.84, 25.83, 28.40, 29.74, 43.42, 48.55, 150.72, 173.53, 174.41, 176.77; IR (neat) 1460, 1610, 1630, 1690 (br), 1720 cm^{-1}; MS (FAB) *m/z* 633 (MH$^+$); FAB HRMS calcd for $C_{25}H_{41}N_6O_{13}$ 633.2732 (MH$^+$), found 633.2723 .

Mono[[*N*-[[[1-carba-3-chloro-4-(*p*-nitrobenzyloxycarbonyl)-3-cephem-7-yl]amino]succinyl]-*N*-(benzyloxy)amino]butyl] Bis[[*N*-acetyl-*N*-(benzyloxy)amino]butyl] Isocyanurate (18). Compound 14 (100 mg, 0.118 mmol), carbacephalosporin 16 (50.4 mg, 0.130 mmol), and *N*-hydroxybenzotriazole hydrate (HOBT, 63 mg, 0.472 mmol) were suspended in 1.0 mL of anhydrous methylene chloride under nitrogen. The reaction was cooled to 0 °C in an ice bath. 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide·HCl (EDC, 90 mg, 0.472 mmol) was added to the reaction mixture followed by triethylamine (82 μL, 0.59 mmol). The solution was stirred for 30 min, allowed to warm to room temperature, and stirred overnight. The solution was diluted with ethyl acetate and washed with a 5% aqueous citric acid solution, $NaHCO_3$ solution (10%), water, and brine. Drying (Na_2SO_4), filtration, and removal of solvent afforded an oily residue which was purified by radial chromatography eluting with 3% methanol in ethyl acetate to obtain 80 mg (57.5%) of 18 as a yellow oil: ^{1}H NMR (CDCl$_3$) δ 1.53–2.05 (m, 14H), 2.08 (s, 6H), 2.45–2.95 (m, 6H), 3.55–3.75 (m, 6H), 3.76–3.95 (m, 7H), 4.80 (s, 4H), 4.84 (s, 2H), 5.28–5.47 (m, 3H), 7.30–7.52 (m, 16H), 7.59 (d, *J* = 8.7 Hz, 2H), 8.19 (d, *J* = 8.7 Hz, 2H); ^{13}C NMR (CDCl$_3$) δ 20.38, 21.81, 23.78, 23.97, 24.70, 24.89, 27.72, 30.06, 31.68 (allylic), 42.39, 44.64, 52.48, 58.61, 66.07, 76.15, 76.21, 122.97, 123.60, 123.78, 128.61, 128.66, 128.87, 129.06, 130.92, 134.13, 134.22, 142.13, 147.62, 148.76, 160.01, 165.40, 172.25, 172.80, 173.40; IR (neat) 1460, 1520, 1695, 1725, 1760 cm^{-1}; MS (FAB) *m/z* 1178.5 (MH$^+$); FAB HRMS calcd for $C_{59}H_{69}N_9O_{15}Cl_1$, 1178.4601 (MH$^+$), found 1178.4598. Anal. Calcd for $C_{59}H_{68}N_9O_{15}Cl_1 \cdot H_2O$: C, 59.22; H, 5.9; N, 10.53. Found: C, 59.26; H, 5.91; N, 10.38.

Mono[[*N*-[[(1-carba-4-carboxy-3-chloro-3-cephem-7-yl)-amino]succinyl]-*N*-hydroxyamino]butyl] Bis[(*N*-acetyl-*N*-hydroxyamino)butyl] Isocyanurate (20). Conjugate 20 was prepared as a $FeCl_3$-positive, amber oil from 18 using the acidic hydrogenolysis conditions described for the conversion of 19 to 21: ^{1}H NMR (CD$_3$OD) δ 1.45–1.80 (m, 14 H), 2.08 (s, 6H), 2.45–3.00 (m, 6H), 3.55–3.75 (m, 6H), 3.75–3.95 (m, 7H), 5.35–5.45 (m, 1H), 7.00–8.25 (br m); ^{13}C NMR (CD$_3$OD) δ 20.32, 21.00, 23.10, 24.87, 24.79, 25.88, 29.55, 30.68 32.34 (allylic), 33.71, 39.49, 43.41, 53.78, 59.48, 129.65, 131.61, 150.73, 163.75, 165.36, 166.12 (m), 173.49, 174.27, 175.33; IR (neat) 3360–2800 (br), 1780, 1690, 1460 cm^{-1}; MS (FAB) *m/z* 773.23 (MH$^+$); FAB HRMS calcd for $C_{31}H_{45}N_9O_{13}Cl_1Na$, 795.2692, (MNa$^+$) found 795.2666.

Mono[[*N*-[[[[[1-carba-3-chloro-4-(*p*-nitrobenzyloxycarbonyl)-3-cephem-7-yl]carbamoyl]phenylmethyl]amino]-succinyl]-*N*-(benzyloxy)amino]butyl] Bis[[*N*-acetyl-*N*-(benzyloxy)amino]butyl] Isocyanurate (19). *N*-Boc protected Lorabid 17 (152 mg, 0.260 mmol) was dissolved in dry methylene chloride (1 mL) and cooled in an ice bath, and trifluoroacetic acid (TFA, 1 mL) was slowly added. The reaction mixture was stirred for an additional 30 min while being allowed to warm to room temperature. The solvent and excess TFA were removed under a vacuum. Traces of TFA were removed by azeotropic evaporation with benzene to provide the TFA salt as a pale yellow oil.

The TFA salt, compound 14 (200 mg, 0.236 mmol), and HOBT (128 mg, 0.947 mmol) were dissolved in dry methylene chloride (5 mL), and the reaction mixture was cooled to 0 °C. Dry triethylamine (164 μL, 1.18 mmol) and EDC (181 mg, 0.944 mmol) were added sequentially to the resulting solution. The reaction mixture was slowly allowed to warm to room temperature and stirred overnight under nitrogen. The solvent was evaporated, and the residue was extracted with ethyl acetate (3 × 15 mL). The ethyl acetate extract was washed with citric acid solution (5%, 3 × 10 mL), $NaHCO_3$ solution (10%, 2 × 10 mL), deionized and distilled H_2O, and brine. The organic layer was dried (Na_2SO_4), filtered, and evaporated. The crude product was purified by radial chromatography using a 2-mm chromatotron plate eluting with ethyl acetate/methanol (97:3) to provide protected conjugate 19 (176 mg, 56.7%) as a foamy solid; mp 65–69 °C; ^{1}H NMR (CDCl$_3$) δ 1.40–1.95 (m, 14H), 2.01 (s, 6H), 2.25–3.35 (m, 6H), 3.55–4.05 (m, 13H), 4.65–4.90 (m containing s at 4.78, 6H), 5.25–5.45 (m, 3H), 5.49 (d, *J* = 6.6 Hz, 1H), 7.05 (d, *J* = 6.6 Hz, 1H), 7.30–7.45 (m, 15H), 7.62 (d, *J* = 8.7 Hz, 2H), 8.23 (d, *J* = 8.7 Hz, 2H), 8.54 (d, *J* = 7.5 Hz, 1H); ^{13}C NMR (CDCl$_3$) δ 20.26, 21.11, 23.52, 23.89, 24.28, 24.78, 27.79, 30.59, 31.72 (allylic), 42.26, 42.33, 43.74, 44.36, 52.65, 57.94, 58.86, 66.09, 76.06, 123.06, 123.56, 123.71, 127.78, 128.26, 128.31, 128.56, 128.71, 128.82, 129.01, 130.17, 133.78, 136.77, 142.12, 147.58, 148.71, 159.98, 164.80, 171.10, 172.15, 172.34, 173.88; IR (neat) 1460, 1520, 1690, 1730, 1770 cm^{-1}; MS (FAB) m/z 1311 (MH$^+$). Anal. Calcd for

$C_{67}H_{75}N_{10}O_{18}Cl$: C, 61.35, H, 5.77; N, 10.69. Found: C, 61.41; H, 5.71; N, 10.69.

Mono[[*N*-[[[[(1-carba-4-carboxy-3-chloro-3-cephem-7-yl)-carbamoyl]phenylmethyl]amino]succinyl]-*N*-hydroxy-amino]butyl] Bis[(N-acetyl-*N*-hydroxyamino)butyl] Isocyanurate (21). To a solution of 19 (100 mg, 0.076 mmol) in 1.0 mL of 5% aqueous DMF (made from deionized distilled water and HPLC grade DMF) was added 19.7 μL (0.229 mmol, 300 mol %) of concentrated HCl and 20 mg of 10% Pd–C. This mixture was exposed to H_2 at atmospheric pressure for 48 h. The catalyst was removed by filtration. The DMF/water mixture was removed by evaporation under high vacuum. The residue was redissolved in methanol and was evaporated repeatedly in an attempt to remove the residual DMF. ^{1}H NMR showed traces of DMF despite this attempt to remove it. Evidence that other siderophore conjugates have a relatively high affinity for DMF has been reported.[8] Product 21 was obtained in essentially quantitative yield as an amber oil: $FeCl_3$ positive (deep violet); ^{1}H NMR (CD_3OD) δ 1.45–1.95 (m, 14H), 2.01 (s, 6H), 2.25–3.35 (m, 6H), 3.55–4.05 (m, 13H), 5.25–5.45 (m, 2H), 7.25–8.25 (br m); ^{13}C NMR (CD_3OD) δ 20.35, 21.01, 22.87, 24.84, 25.85, 28.72, 29.17, 30.68, 31.66 (allylic), 37.47, 39.47, 43.40, 53.80, 59.27, 59.53, 70.99, 129.08, 129.47, 129.85, 131.58, 138.15, 140.43, 150.70, 164.39, 164.81, 165.53, 166.06, 173.43, 174.42; IR (neat) 3590–2600 (br), 1770, 1690, 1460 cm^{-1}; MS (FAB) *m/z* 906.5 (MH$^+$); FAB HRMS Calcd for $C_{39}H_{52}N_9O_{14}Cl_1Li$ 912.3482 (MLi$^+$), found 912.3478.

Bioassays of Siderophores 4a–c and Conjugates 20 and 21. Materials and Methods. Bacterial Strains. The parent β-lactam-hypersusceptible strain, *E. coli* X580, was the generous gift of Jan Turner, Eli Lilly & Co., Indianapolis, IN. The feature of this strain that is responsible for this hypersusceptibility is unknown. Individual mutants that were resistant to the test compounds were isolated from 1:25 000 000 dilutions of test cultures approximately 24 h postinoculation. Parent strains were maintained at −70 °C in a mixture containing equal volumes of sterile α-D-lactose–glycerol[11] and an overnight Luria broth culture.

Culture Media. Dehydrated tryptone, yeast extract, and agar were all purchased from Fisher. Luria broth and agar (1.5% Bacto agar) were prepared by the procedure of Miller.[12] All media were stored at 4 °C and were used within 36 h. The ferric iron chelator ethylenediamine bis(*o*-hydroxyphenylacetic acid) (EDDA) was added by sterile filtration through an Acrodisc 4192 sterile assembly (pore size, 0.2 μm; Fisher) to a slightly concentrated culture broth. The final culture volume was typically 100 mL. All shake flasks containing test compounds were either inoculated immediately or subjected to an 8- or 24-h preincubation at 37 °C and 300 rpm to determine compound stability.

Iron-deficient Luria broth was prepared by adding aliquots of a 50 mg/mL stock solution of the ferric iron chelator EDDA to the appropriate cultures. The EDDA concentration necessary to slow down but not completely inhibit the growth of the *E. coli* X580 parent strain in Luria broth was empirically determined to be 100 μg/mL. With Luria agar, the supplemented EDDA was added to the freshly sterilized and melted agar just before solidification. All liquid and solid culture media containing EDDA were stored at 4 °C for at least 30 h before use.

Chemicals and Substrates. All cyanuric acid based siderophores and siderophore–drug conjugates were prepared in our laboratory as described in this paper. For petri dish bioassays, a stock solution (20 mM) of the trihydroxamate isocyanurates (4a–c) was prepared in methanol from which two test solutions at 10 and 5 mM concentrations were prepared by proper dilution. Dark brown solutions (10 mM) of preformed iron complexes of these siderophores were prepared by mixing equal volumes of sterile 20 mM aqueous $FeCl_3 \cdot 6H_2O$ and the siderophore stock solution (20 mM). From this 10 mM solution, another solution (5.0 mM) was also prepared by dilution with an equal volume of methanol. The aqueous 10 and 5 mM working solutions were prepared by mixing equal volumes of the above methanolic (10 or 5 mM) siderophore solutions (after evaporation of the methanol) and sterile distilled and deionized water.

For liquid cultures, stock solutions (20 mM) of conjugates 20 and 21 were prepared in HPLC-grade DMF from which two test solutions 10 and 1 mM were made by dilution. Preformed Fe^{3+} complexes of 20 and 21 (10 mM) were made by addition of an equal volume of test stock solutions (20 mM) and aqueous $FeCl_3 \cdot 6H_2O$ (20 mM) and from those two test solutions (1 mM) were made by proper dilution with DMF. Solutions of the test compounds (100 μL of 10 mM and 1 mM solutions) were added to sterile Luria broth (100 mL) or sterile Luria broth (100 mL) containing EDDA (100 μg/mL) to give 10 and 1.0 μM final concentration of the conjugates, respectively.

All inorganic compounds were obtained from Fisher. EDDA, ferric iron chelator used to deferrate the culture medium was obtained from Sigma and was deferrated using Rogers' procedure,[13] and a stock solution (50 mg/mL)[14] was prepared immediately prior to use.

Growth Curves. Seed cultures were prepared by adding a 0.20-mL aliquot of thawed bacteria in a culture tube containing sterile lactose–glycerol to 100 mL of sterile Luria broth. After overnight incubation at 37 °C and 300 rpm, two 10-μL aliquots of the turbid seed culture were transferred to corresponding control and test cultures. All flasks were incubated at 37 °C and 300 rpm for up to 28 h. Aliquots (approximately 0.80 mL) were removed every 2 h to determine the culture turbity (A600) with a Beckman DU-40 spectrophotometer.

All studies were performed in duplicate, and the individual sets of data were averaged to provide the growth curves.

Petri Dish Growth Promotion Studies. Melted Luria agar supplemented with 100 μg of EDDA per mL was seeded with an overall 1:200 000 dilution of an overnight culture of the *E. coli* X580 parent strain and was then poured into sterile petri dishes. After solidification, aliquots (5 μL) of 5 and 10 mM sterile aqueous solutions of the performed iron complexes of **4a**, **4b**, and **4c** were added to pairs of sterile Whatmann no. 1 filter paper disks (6.0-mm diameter) that had already been placed on the agar surface. Methanolic solutions (5 and 10 mM) of the above compounds or their preformed iron complexes were tested by adding an aliquot (5 mL) to the dry sterile filter paper disk. After evaporation of the solvent, the impregnated disk was placed onto the agar surface. Sterile water (5 μL) was then added to the disk to help move the compound into the surrounding agar. Following an incubation at 37 °C for 24–36 h, the petri dishes were examined for the presence of a circular and symmetrical zone of stimulation around the disk. This general procedure was patterned after other siderophore growth promotion studies.[15]

Acknowledgment. Support of this research by the NIH is gratefully acknowledged. We sincerely appreciate the standard broad spectrum antimicrobial assays (MIC analyses) performed at Eli Lilly and Co.

Supplementary Material Available: ^{1}H and ^{13}C NMR spectra of 4a–c, 14, 15, 20, and 21 (14 pages). This material is contained in libraries on microfiche, immediately follows this article in microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(11) Turner, J. (Eli Lilly & Co., Indianapolis, IN) personal communication.

(12) Miller, J. H. Appendix 1. Formulas and Recipes. In *Experiments in Molecular Genetics*; Cold Spring Harbor Laboratories: Cold Spring Harbor, NY, 1972; pp 431–433.

(13) Rogers, H. J. *Infect. Immun.* **1973**, *7*, 445–456.

(14) Ong, S. A.; Peterson, T.; Nielands, J. B. *J. Biol. Chem.* **1979**, *254*, 1860–1865.

(15) Reference 9a and refs 32, 41, and 50 therein.