

Occolwitz (Eli Lilly and Co.) kindly provided the mass spectral data.

Registry No. 1a, 94136-68-2; 1b, 94136-69-3; 2a, 94136-70-6; 2b, 94136-71-7; 3, 94136-53-5; 4, 94136-54-6; 5, 94136-55-7; 6, 58885-58-8; 8, 94136-56-8; 9, 58885-60-2; 10, 94136-57-9; 11, 94136-58-0; 12a, 3739-94-4; 12b, 4422-95-1; 14a, 56173-26-3; 14b, 56173-27-4; 14c, 94136-72-8; 14d, 94136-73-9; 15a, 94136-74-0; 15b,

94136-75-1; 15c, 94136-76-2; 15d, 94136-77-3; 16, 83966-23-8; 17, 94136-59-1; 18, 75178-90-4; 19, 94136-60-4; 20, 94136-61-5; 21, 94136-62-6; 22, 94136-63-7; 25, 60-32-2; 26, 6404-29-1; 27, 71925-14-9; 28, 94136-64-8; 29, 94136-65-9; 30, 94136-66-0; 31, 94136-67-1; CbzNHOCH₂Ph, 15255-86-4; Fe, 7439-89-6; *O*-benzylhydroxylamine hydrochloride, 2687-43-6; 5-[(*tert*-butoxycarbonyl)amino]pentanal, 94136-78-4; di-*tert*-butyl pyrocarbonate, 24424-99-5.

Artificial Siderophores. 2. Syntheses of Trihydroxamate Analogues of Rhodotorulic Acid and Their Biological Iron Transport Capabilities in *Escherichia coli*

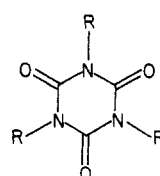
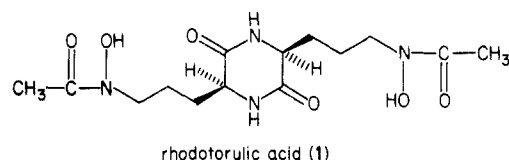
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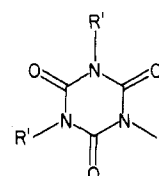
Tris[(acetylhydroxyamino)alkyl] isocyanurates **2a-c** were synthesized from α,ω -dibromoalkanes **5** in four steps. The alkylation of the bromides **5a-c** with *O*-benzyl-*N*-[(trichloroethoxy)carbonyl]hydroxylamine in the presence of DBU gave *N*-alkylation products **7a-c**. The (trichloroethoxy)carbonyl protecting group of **7a-c** was easily removed by Zn dust in acetic acid. When the reaction was performed with acetic anhydride, the desired *N*-acetylated materials **10a-c** were obtained. The alkylation of cyanuric acid with **12** in the presence of base provided the *N*-alkylated materials **13**, which were hydrogenated to provide **2a-c**. In order to determine the affect of structural modifications on biological activity, various chain lengths of the side arms were utilized and the retroanalogue **3** was prepared. Most of the compounds examined acted as ferrichrome in supporting the iron nutrition of *Escherichia coli*. However, tris[(acetylhydroxyamino)butyl] isocyanurate **2b**, and to some extent its pentyl analogue, **2c**, displayed the unique and remarkable property of supporting growth of *fhuB* mutants, the latter unresponsive to the other analogues and to all natural siderophores tested.

Microbial iron chelators (siderophores) are useful substrates for the study of iron metabolism¹⁻³ and the development of drugs for the treatment of iron-overloaded patients.⁴⁻⁶ We reported the syntheses and biological activities of several analogues of schizokinen and arthrobactin in the earlier paper.⁷ Most of the analogues prepared behaved nutritionally like ferrichrome in iron transport in *Escherichia coli*. In this paper, we report syntheses and biological activities of isocyanuric acid derivatives with structures similar to rhodotorulic acid⁸ (**1**). Rhodotorulic acid is more effective than the currently used drug Desferal in promoting urinary and fecal iron excretion in the rat screen. But the painful local reaction to this compound administered im or sc curtailed its use.⁹ These side effects may be due to the insolubility of rhodotorulic acid in water. In addition, rhodotorulic acid has only two binding sites per molecule (quadridentate); therefore the rhodotorulate-iron complex must have a minimum of a 3:2 stoichiometry.¹⁰ To improve the iron binding efficiency, molecules designed to mimic the siderophore should be reasonably soluble, coordinate iron stoichiometrically (have hexadentate coordination with Fe³⁺), and perhaps maintain a core similar to the diketopiperazine ring of rhodotorulic acid. Thus, isocyanuric acid derivatives **2-4** were chosen as target molecules.

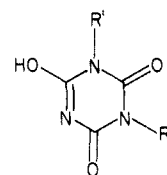
Synthesis of Artificial Siderophores. Since tri-*N*-alkylation of cyanuric acid (**12**) is preceded,¹¹ the key to the synthesis of the designated targets **2-4** was the preparation of the appropriate *N*-(ω -haloalkyl) hydroxamate side chains. Thus, as shown in Scheme I, treatment of the dihalide **5b** with *O*-benzylacetohydroxamate **8** and K₂CO₃ in acetone provided a 1:3 ratio of the *O*- and *N*-alkylated products **9** and **10** in 55% yield. The isomeric



2a, R = (CH₂)₃N(OH)C(=O)CH₃;
n = 3
b, n = 4
c, n = 5



3, R = (CH₂)₅C(=O)N(OH)CH₃



4, R' = (CH₂)₅C(=O)N(OH)CH₃

hydroximates [(*E*)- and (*Z*)-**9**] and the desired *N*-alkylated hydroxamate **10** were difficult to separate chromato-

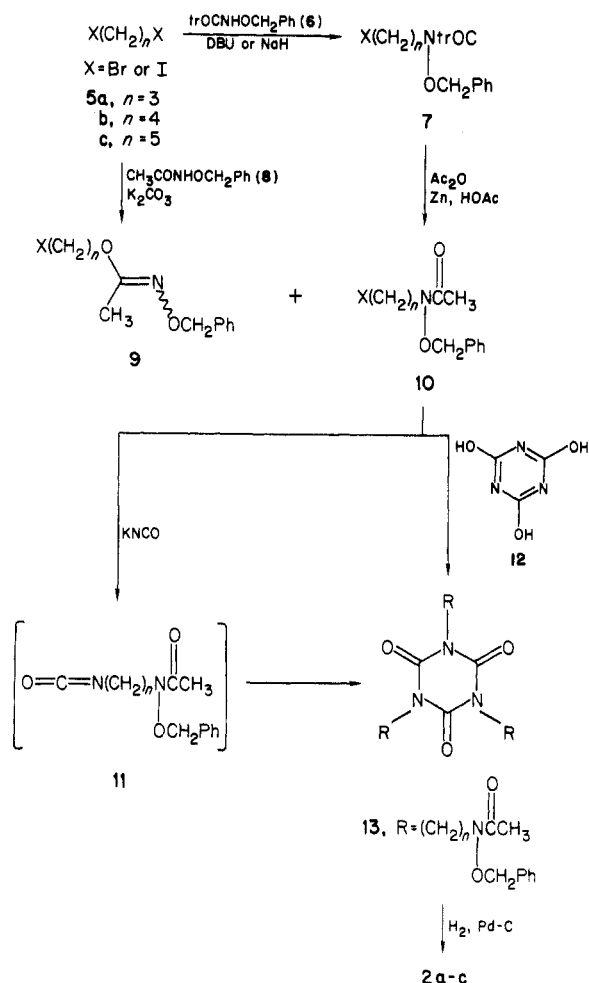
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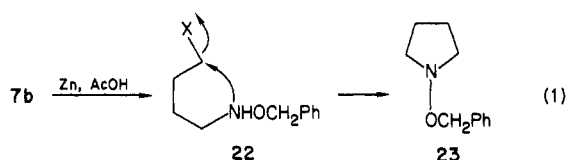
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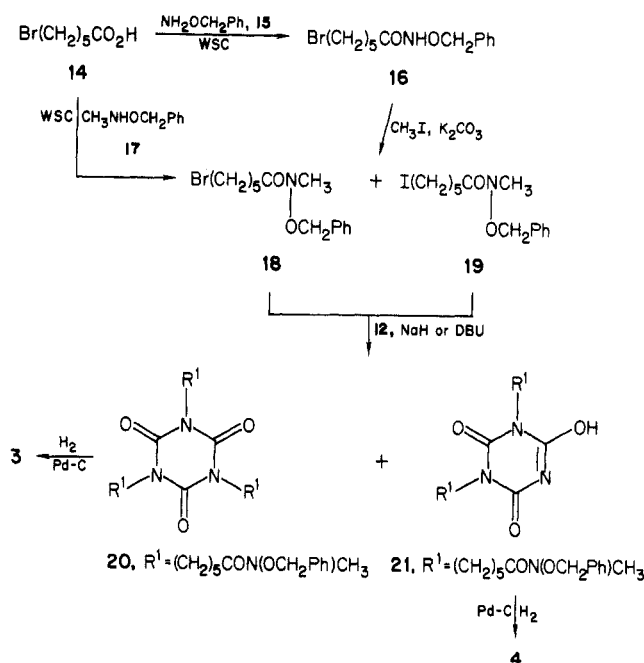
Scheme I



graphically. In order to avoid this problem, another synthetic route was considered. Treatment of the halide **5b** ($X = \text{Br}$) with *O*-benzyl-*N*-[(trichloroethoxy)carbonyl]hydroxylamine¹² (**6**) in the presence of base (NaH or DBU) provided the desired *N*-alkylated material **7b** in 40–62% yield. The (trichloroethoxy)carbonyl (trOC) protecting group of **7b** was easily removed by Zn dust in acetic acid.¹³ When the reaction was performed with acetic anhydride, the desired *N*-acetylated material **10b** was obtained in 85% yield. However, the deprotection of **7b** with Zn in acetic acid did not provide the free hydroxylamine **22**. Once the free hydroxylamine **22** was generated, it immediately cyclized to give **23** (eq 1).



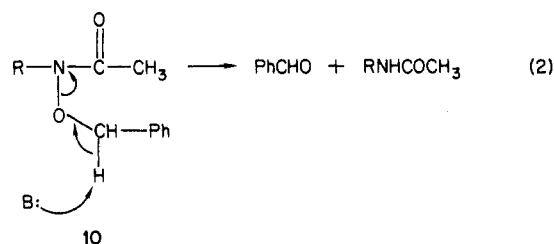
Scheme II



The trialkylation of cyanuric acid (**12**) with **10b** in the presence of NaH provided the *N*-alkylated material **13b** in 69% yield. An alternative synthesis of **13b** was carried out with use of the isocyanate intermediate **11**. In this case, the reaction of **10b** with KOCN in the presence of DMF at high temperature^{14,15} gave only the *N*-alkylated material **13b** directly in 32% yield. Both preparations provided **13b** with the same TLC R_f value and ^1H NMR spectrum. Catalytic hydrogenation of **13b** in CH_3OH with Pd-C (5%) provided **2b**.

The same sequence was used for the synthesis of homologues **2a** and **2c**. *O*-benzyl-*N*-[(trichloroethoxy)carbonyl]hydroxylamine (TOBHA) was separately alkylated with the dibromides **5a** and **5b** in the presence of DBU to provide **7a** or **7b**, respectively. The deprotection of **7a** and **7c** with Zn in the presence of acetic anhydride gave the *N*-alkylated material **10a** and **10c**. The reaction of cyanuric acid (**12**) with bromo hydroxamate **10a** and **10c** in the presence of DBU gave **13a** and **13c**. Catalytic hydrogenation with Pd-C provided **2a** and **2c**.

Interestingly when the alkylation of cyanuric acid with **10** using a slight excess of base (NaH or DBU) was performed, benzaldehyde was obtained as a byproduct (probably by the route shown in eq 2).



Synthesis of the retroanalogues of isocyanuric acid derivatives **3** and **4** is shown in Scheme II. Bromohexanoic acid (**14**) was coupled with *O*-benzylhydroxylamine (OBHA) in the presence of the water-soluble carbodiimide

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Table I. Characteristics and Sources of *E. coli* K12 Strains

strain	genotype	relevant phenotype	origin/ref
RW193	F ⁻ , <i>proC</i> , <i>leu</i> , <i>trp</i> , <i>thi</i> , <i>lacY</i> , <i>rpsL</i> , <i>galK</i> , <i>ara</i> , <i>entA</i> , <i>mtl</i> , <i>xyl</i> , <i>azi</i> , <i>tsx</i> , <i>supE44</i>	Cannot synthesize enterobactin. Is able to transport hydroxamate and catechol type siderophores	17
AN193	as RW 193, but <i>tonA</i>	Lacks the outer membrane ferrichrome receptor, and thus cannot transport ferrichrome type	I. G. Young
BN3300	as RW 193, but <i>fhuB</i>	Deficient in the transport of all natural hydroxamate type siderophores	18
BN3306	as RW 193, but <i>panB</i> , <i>leu</i> ⁺ , <i>fhuB</i>	Deficient in the transport of all natural hydroxamate type siderophores	18
RWB7	as RW 193, but <i>tonB</i>	Deficient in the transport lab stocks of all siderophores—hydroxamates and catechols, iron citrate, and B12	lab stocks

Table II. Response of Different Strains to Artificial Siderophores^{a,b}

Strain	1	2a	2b	2c	3	4	FC	DA	Ent
RW 193	+	+	++	+	+	-	++	+	+
AN 193	+	-	++	-	-	-	-	+	+
BN 3300	-	±	++	+	-	-	-	-	+
BN 3306	-	±	++	+	-	-	-	-	+
RWB7	-	-	-	-	-	-	-	-	-

^a FC = ferrichrome, DA = dimerum acid, Ent = enterobactin.^b Compounds 2a-4 were tested at 50 and 500 μ M; compound 1, ferrichrome, and dimerum acid were tested at 50 μ M; and enterobactin was tested at 25 μ M. A 10 μ L sample was applied in all cases. ++ indicates a > 25-mm halo of growth. + indicates a 10-25-mm halo. ± indicates a <10-mm halo. - indicates no zone of growth around the disc.

1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (WSC) to provide the hydroxamate 16. Subsequent treatment of 16 with CH_3I and K_2CO_3 gave a mixture of the N-methylated bromide and iodide 18 and 19 (18:19 = 1:9 by ^1H NMR). Apparently the iodide 19 was obtained by direct displacement of the bromide on 16 or 18. Alternatively, treatment of 14 with N-methyl-O-benzylhydroxylamine¹⁶ (17) in the presence of WSC provided 18 directly. The mixture of 18 and 19 or pure 18 was alkylated with cyanuric acid 12 with use of NaH to provide the tri- and dialkylated compounds 20 and 21. After chromatographic separation, each component (20 and 21) was hydrogenated separately with Pd-C (5%) to provide 3 and 4, respectively.

Biological Activity. The ability of the artificial siderophores to support the growth of *E. coli* strains defective in different steps of hydroxamate transport (Table I) was examined (Table II). Strain RW 193, which is not mutated in any of the functions required for iron hydroxamate uptake, is able to use all but compound 4 for iron transport, possibly since it is only a dihydroxamate. Strain AN193, which lacks the outer membrane receptor for ferrichrome, but which can transport rhodotorulic acid and dimerum acid, is unable to use all but compound 2b as a source of iron. Other *tonA* strains gave the same results. These compounds thus appear to behave like ferrichrome in *E. coli*. The most surprising result is the ability of *fhuB* strains BN3300 and BN3306 to use compounds 2b and 2c. *FhuB* strains are deficient in a function that is required for the transport of natural hydroxamate type siderophores. Strain BN3300 has a point mutation in the *fhuB* gene and hence this may revert to give *fhuB*⁺ strain. Accordingly, strain BN3306, which carries a deletion in the *fhuB* gene and cannot revert, was also tested. Several other *fhuB* strains also gave the same results. The function and cellular location of the *fhuB* gene product are as yet unknown. One possibility for *fhuB* function is that it plays some role in releasing the siderophore from the outer membrane receptor to the inner membrane. Compounds 2b and 2c could bypass this function by displaying a

weaker affinity for an outer membrane receptor (as yet unknown), and so they could be released internally in the absence of an effector. Alternatively, compounds 2b and 2c may form polynuclear, ribbonlike Fe(III) complexes that transverse the outer membrane pore without need of assistance from *fhuB*. A bacteriocin, colicin M, penetrates via the *tonA* pore, and several phages, T1, T5, and Φ 80, bind to the *tonA* protein to insert their DNA. In none of these cases is the *fhuB* gene product required. The *tonB* gene product is required for the action of colicin M and phages T1 and Φ 80, but not T5, and the *tonB* gene product is also required for the utilization of compounds 2b and 2c (Table II). A third possibility may involve differences in solubility properties between the native siderophores and compounds 2b and 2c. Most of the native siderophores are highly water soluble, and so the *fhuB* gene product may be necessary for maintaining phase integrity within the outer membrane. Because compounds 2b and 2c are not very soluble in water, perhaps they are able to bypass *fhuB*. It is interesting that compound 2a did not give the same results as compounds 2b and 2c. Hence a longer chain length may be the structural feature required to bypass the *fhuB* gene product.

The isocyanuric acid derivative 2b was also subjected to an in vivo mouse bioassay for potential iron chelators by the E G & G Mason Research Institute.¹⁹ When administered ip, compound 2b was found to be grossly nontoxic. It did not decrease splenic iron, but did decrease liver iron slightly. In contrast, the compound stimulated excretion of iron via the feces (24%) and urine (119%). Compound 2a was also found to be nontoxic, and when administered ip, it also stimulated excretion of iron via the feces (18%) and urine (75%). Both 2a and 2b were inactive when administered orally.

Experimental Section

General Methods. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Perkin-Elmer 727B spectrometer. Proton NMR spectra were obtained on a Varian A-60A or EM-390 spectrometer. Chemical shifts are reported in ppm relative to tetramethylsilane (δ units). Mass spectra were recorded on an AEI Scientific Apparatus MS 92 or Du Pont DP 102 spectrometer. Field-desorption mass spectra were obtained by Dr. John L. Occolwitz (Eli Lilly and Co.). Elemental analyses were performed by Midwest Microlabs, Indianapolis, IN.

Alkylation Reaction of Dihalide 5 with Benzyl Acetohydroxamate 8. Compound 5b [X = Br; 2.8 g, 13 mmol], benzyl acetohydroxamate (8; 1.65 g, 10 mmol), and K_2CO_3 (6.91 g, 50 mmol) were placed in dry acetone (20 mL) and refluxed for 24 h. After filtration and evaporation, the residue was taken into ether. The ether was washed twice with 0.5 N NaOH and once with H_2O . After the mixture was dried (MgSO_4) and the solvent evaporated, the residue was chromatographed on silica gel (2 \times 50 cm), eluting with ethyl acetate/hexane (70:30). The desired N-alkylated

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compound **10b** (X = Br) was obtained as a colorless oil: 1.2 g (40%); ^1H NMR (CDCl_3) δ 1.6–1.9 (m, 4 H), 2.07 (s, 3 H), 3.35 (t, 2 H), 3.61 (t, 2 H), 4.80 (s, 2 H), 7.38 (s, 5 H); IR (neat) 1660 cm^{-1} .

In addition, a small amount of the hydroxamate **9b** (X = Br) was obtained from the earlier column fraction (13%): ^1H NMR (CDCl_3) δ 1.7–2.0 (m, 4 H), 1.90 (s, 3 H), 3.42 (t, 2 H), 4.05 (t, 2 H), 4.95 (s, 2 H), 7.35 (s, 5 H).

Preparation of **10b** (X = I) was the same as that of **10b** (X = Br) except dihalide **5b** (X = I) was used. Compound **10b** (X = I): 40% yield; a colorless oil; ^1H NMR (CDCl_3) δ 1.6–1.95 (m, 4 H), 2.07 (s, 3 H), 3.18 (t, 2 H), 3.67 (t, 2 H), 4.83 (s, 2 H), 7.41 (s, 5 H); mass spectrum (EI), m/e 220 (M – I); IR (neat) 1660 cm^{-1} . Anal. ($\text{C}_{13}\text{H}_{18}\text{INO}_2$) C, H, N.

4-[(Trichloroethoxy)carbonyl](benzyloxy)amino]-1-butyl Bromide (7b, X = Br). Compounds **5b** (2.16 g, 10 mmol) and **6** (1.5 g, 5 mmol) were dissolved in DMF (25 mL) and treated with NaH (0.312 g, 6.5 mmol). The reaction mixture was stirred at room temperature for 30 min. It was taken into ethyl acetate and washed several times with H_2O . The mixture was dried (Na_2SO_4) and the solvent evaporated, and the residue was chromatographed on a silica plate (2 mm, Chromatotron), eluting with ether/hexane (1:4). The desired product was obtained as a colorless oil: 0.86 g (40%); ^1H NMR (CDCl_3) δ 1.6–2.0 (m, 2 H), 3.37 (t, 2 H), 3.53 (t, 2 H), 4.72 (s, 2 H), 4.93 (s, 2 H), 7.40 (s, 5 H); IR (neat) 1720 cm^{-1} ; mass spectrum (EI), m/e calcd M = 430.946, found M = 430.945.

Exchange of the trOC Group with an Acetyl Group. Compound **7b** (X = Br) (0.431 g, 1 mmol) and acetic anhydride (0.5 g, 5 mmol) were dissolved in acetic acid (4 mL) and treated with Zn dust (0.4 g). The mixture was allowed to stir for 1 h at room temperature. It was taken into ether (100 mL), filtered, and washed twice with 0.5 N NaOH and once with H_2O and brine. After the mixture was dried (Na_2SO_4) and the solvent evaporated, the desired product **10b** (X = Br) was obtained as a colorless oil: 0.26 g (85%); mass spectrum (EI), m/e 170, 172 (M – Br – CH_3CO^+). This product had identical spectral and TLC properties when compared with those of previously prepared **10b** (X = Br).

Alkylation Reaction of the Bromide of Previously Prepared 10b (X = Br) with Cyanuric Acid (12). NaH (100 mg, 2 mmol) was placed in a two-neck 100-mL round-bottomed flask equipped with a N_2 line and a septum cap. Dry hexane was added and the suspension stirred and then allowed to settle. The hexane was removed by a syringe. This process was repeated twice more, and then DMF (20 mL) was added. To this mixture was added cyanuric acid (80 mg, 0.6 mmol) in DMF (15 mL). The solution was treated with **10b** (X = Br; 0.7 g, 2 mmol) and allowed to stir for 24 h at room temperature. This mixture was taken into ethyl acetate and washed several times with H_2O . After the mixture was dried (MgSO_4) and the solvent evaporated, the residue was chromatographed on silica gel (2 \times 50 cm), eluting with ethyl acetate to provide the N-alkylated material **13b** as a colorless oil: 0.326 g (69%); ^1H NMR (CDCl_3) δ 1.7–2.0 (m, 12 H), 2.07 (s, 9 H), 3.65 (t, 6 H), 3.88 (t, 6 H), 4.80 (s, 6 H), 7.38 (s, 15 H); IR (neat) 1460, 1640, 1680, 1700 (sh) cm^{-1} .

Compound 13b via the Isocyanate Intermediate 11. Compound **10b** (X = Br) (0.45 g, 1.5 mmol) and KOCN (0.16 g, 1.95 mmol) were dissolved in DMF (0.22 g, 4.5 mmol). The reaction mixture was heated to 130 $^\circ\text{C}$ (external) for 3 h and raised to 150 $^\circ\text{C}$ (external) for 2 h. The residue was chromatographed on silica gel (2 \times 50 cm), eluting with ethyl acetate. The desired N-alkylated material **13b** was obtained as a colorless oil: 126 mg (32%). This product had identical spectral and TLC properties when compared with those for the previous preparation of **13b**.

Tris[(acetylhydroxyamino)butyl] Isocyanurate (2b). Compound **13b** (400 mg, 0.5 mmol) was dissolved in methanol (50 mL) and treated with Pd on carbon (5%, 100 mg) under 1 atm of H_2 for 3 h at room temperature. The mixture was filtered and evaporated, and the desired product **2b** was isolated as a colorless oil: 155 mg (60%); ^1H NMR ($\text{Me}_2\text{SO}-d_6$) δ 1.3–1.8 (m, 12 H), 2.0 (s, 9 H), 3.3–4.0 (m, 12 H); mass spectrum (FD), m/e 516 (M^+), 517 (M + 1), 559 (M + CH_3CO^+); IR (neat) 1640, 1680 cm^{-1} ; R_f 0.86 for reverse phase (RP-2) in 2-PrOH/ H_2O (70:30); R_f 0.73 for reverse phase (RP-2) in 2-PrOH/ H_2O (30:70).

Compound 23 from 7b (X = Br). Compound **7b** (0.1 g, 0.232 mmol) was dissolved in acetic acid (2 mL) and treated with Zn

dust (100 mg). The suspension was allowed to stir for 45 min at room temperature. The mixture was taken into ethyl acetate and filtered. This solution was washed three times with saturated Na_2CO_3 and once with H_2O and brine. After the mixture was dried (Na_2CO_3) and the solvent evaporated, the product **23** was obtained as a colorless oil: 35 mg (85%); ^1H NMR (CDCl_3) δ 1.8–2.1 (m, 4 H), 3.43 (t, 4 H), 5.17 (s, 2 H), 7.42 (s, 5 H); IR (neat) 1490, 1440, 1380, 1200 cm^{-1} ; mass spectrum (EI), m/e 177 (M^+).

3-[(Trichloroethoxy)carbonyl](benzyloxy)amino]-1-propyl Bromide (7a). Compound **6** (1.3 g, 4.35 mmol) and **5a** (X = Br; 0.91 mL, 8.70 mmol) were dissolved in acetonitrile (20 mL) and treated with DBU (0.65 mL, 4.35 mmol). The reaction mixture was refluxed for 5 h. It was then taken into ethyl acetate and washed twice with 1 N HCl and once with H_2O and brine. After the mixture was dried (Na_2SO_4) and the solvent evaporated, the residue was chromatographed on a silica plate (2 mm, Chromatotron), eluting with ethyl acetate/hexane (20:80). Compound **7a** (X = Br) was obtained as a colorless oil: 1.09 g (60%); ^1H NMR (CDCl_3) δ 1.9–2.25 (m, 2 H), 3.38 (t, 2 H), 3.65 (t, 2 H), 4.83 (s, 2 H), 4.92 (s, 2 H); IR (neat) 1720 cm^{-1} . Anal. ($\text{C}_{13}\text{H}_{18}\text{BrCl}_3\text{NO}_3$) C, H, N.

5-[(Trichloroethoxy)carbonyl](benzyloxy)amino]-1-pentyl Bromide (7c). Compounds **6** (1.5 g, 5.02 mmol) and **5c** (X = Br; 1.4 mL, 10.04 mmol) were dissolved in acetonitrile (25 mL) and treated with DBU (0.75 mL, 5.02 mmol). The reaction mixture was refluxed for 5 h. It was then taken into ethyl acetate and washed twice with 1 N HCl and once with H_2O and brine. After the mixture was dried (Na_2SO_4) and the solvent evaporated, the residue was chromatographed on a silica plate (4 mm, Chromatotron), eluting with ethyl acetate/hexane (15:85). The desired product **7c** (X = Br) was obtained as a colorless oil: 1.39 g (62%); ^1H NMR (CDCl_3) δ 1.2–2.2 (m, 6 H), 3.35 (t, 2 H), 3.63 (t, 2 H), 4.81 (s, 2 H), 4.90 (s, 2 H), 7.38 (m, 5 H); IR (neat) 1720 cm^{-1} .

3-[Acetyl(benzyloxy)amino]-1-propyl Bromide (10a). Compound **7a** (X = Br; 0.419 g, 1 mmol) and acetic anhydride (0.2 mL, 2 mmol) were dissolved in acetic acid (4 mL) and treated with Zn dust (400 mg). The suspension was allowed to stir for 40 min at room temperature. The mixture was taken into ether and filtered. This solution was washed twice with 1 N NaOH and once with H_2O and brine. After the mixture was dried (Na_2SO_4) and the solvent evaporated, the desired N-acetylated material **10a** (X = Br) was obtained as a colorless oil: 0.24 g (85%); ^1H NMR (CDCl_3) δ 1.95–2.25 (m, 5 H), 3.40 (t, 2 H), 3.77 (t, 2 H), 4.81 (s, 2 H), 7.42 (s, 5 H); IR (neat) 1660 cm^{-1} .

5-[Acetyl(benzyloxy)amino]-1-pentyl bromide (10c) was prepared in a manner similar to that for **10a** (X = Br) and also obtained as a colorless oil (90% yield): ^1H NMR (CDCl_3) δ 1.2–2.0 (m, 6 H), 2.07 (s, 3 H), 3.37 (t, 2 H), 3.63 (t, 2 H), 4.81 (s, 2 H), 7.42 (s, 5 H); IR (neat) 1660 cm^{-1} . Anal. ($\text{C}_{14}\text{H}_{20}\text{BrNO}_2$) C, H, N.

Alkylation Reaction of Bromide 10a (X = Br) with Cyanuric Acid (12). Compound **10a** (X = Br; 0.572 g, 2 mmol) and cyanuric acid (84.4 mg, 0.65 mol) were suspended in acetonitrile (20 mL) and treated with DBU (0.331 mL, 2.21 mmol). The reaction mixture was refluxed for 16–20 h. It was taken into ethyl acetate (100 mL) and washed twice with 0.5 M citric acid and once with H_2O and brine. After the mixture was dried (Na_2SO_4) and the solvent evaporated, the residue was chromatographed on a silica plate (2 mm, Chromatotron), eluting with ethyl acetate [or ethyl acetate/2-propanol (95:5)]. The desired product **13a** was obtained as a colorless oil: 274 mg (55%); ^1H NMR (CDCl_3) δ 1.8–2.25 (m, 15 H), 3.67 (t, 6 H), 3.92 (t, 6 H), 4.83 (s, 6 H), 7.40 (s, 15 H); IR (neat), 1460, 1640, 1680, 1720 (sh) cm^{-1} . Anal. ($\text{C}_{39}\text{H}_{48}\text{N}_6\text{O}_9\cdot 3\text{H}_2\text{O}$) C, H, N.

In addition, a small amount (39 mg) of benzaldehyde was obtained from the earlier column fraction.

Alkylation reaction of 10c (X = Br) with cyanuric acid was performed in a manner similar to that for the preparation of **13a** and also was obtained as a colorless oil (60%): ^1H NMR (CDCl_3) δ 1.1–1.8 (m, 18 H), 2.07 (s, 9 H), 3.63 (t, 6 H), 3.85 (t, 6 H), 4.82 (s, 6 H), 7.42 (s, 15 H); IR (CDCl_3) 1460, 1640, 1680, 1720 cm^{-1} .

Tris[(acetylhydroxyamino)propyl] Isocyanurate (2a). Compound **13a** (150 mg, 0.202 mmol) was dissolved in 2-propanol (30 mL) and treated with Pd on carbon (150 mg) under 1 atm

of H₂ for 4 h at room temperature. The mixture was filtered and evaporated. Compound **2a** was obtained as a colorless oil: 74 mg (77%); ¹H NMR (CD₃OD) δ 1.8–2.2 (m, 15 H), 3.65 (t, 6 H), 3.93 (t, 6 H), 4.80 (s, OH, exchange with CD₃OD); IR (neat) 1460, 1620–1640, 1680, 1700 (sh), 1720 (sh) cm⁻¹; mass spectrum (FD), *m/e* 474 (M⁺), 475 (M + 1), 517 (M + CH₃CO⁺); *R_f* 0.84 for reverse phase (RP-2) in 2-PrOH/H₂O (70:30); *R_f* 0.65 for reverse phase (RP-2) in 2-PrOH/H₂O (30:70).

Tris[acetylhydroxyamino]pentyl isocyanurate (2c) was prepared in a manner similar to that for **2a** and also obtained as a colorless oil (96 mg, 82%): ¹H NMR (CD₃OD) δ 1.1–1.8 (m, 18 H), 2.10 (s, 9 H), 3.60 (t, 6 H), 3.87 (t, 6 H), 4.78 (s, OH, exchange with CD₃OD); IR (neat) 1460, 1620, 1680, 1700 (sh), 1720 (sh) cm⁻¹; mass spectrum (FD), *m/e* 558 (M⁺), 559 (M + 1), 601 (M + CH₃CO⁺); *R_f* 0.85 for reverse phase (RP-2) in 2-PrOH/H₂O (70:30); *R_f* 0.45 for reverse phase (RP-2) in 2-PrOH/H₂O (30:70).

Benzyl 6-Bromocaprohydroxamate (16). Bromo acid **14** (3.9 g, 0.02 mol) and *O*-benzylhydroxylamine hydrochloride (4.8 g, 0.03 mol) were dissolved in H₂O/THF (70 mL), and the pH was adjusted to 4.5 with 2 N NaOH. WSC (7.6 g, 0.04 mol) was slowly added over 5 min. The pH was maintained at 4.5 with 1 N HCl while the solution was stirred at room temperature for 1 h. The mixture was extracted with two portions of ethyl acetate (100 mL) and washed once with 5% NaCHO₃, once with H₂O, once with 0.5 M citric acid, and once with saturated NaCl. The solvent was dried (Na₂SO₄) and evaporated, and the desired hydroxamate **16** was obtained as a colorless oil: 5.08 g (84.5%), which slowly solidified; mp 48–49 °C; ¹H NMR (CDCl₃) δ 1.2–2.2 (m, 8 H), 3.33 (t, 2 H), 4.82 (s, 2 H), 7.40 (s, 5 H), 9.1 (br s, 1 H, NH); IR (neat) 1660 cm⁻¹; mass spectrum (EI), *m/e* 220, 222 (M – Br). Anal. (C₁₃H₁₈BrNO₂) C, H, N.

Benzyl 6-bromo-*N*-methylcaprohydroxamate (18) was prepared from 6-bromohexanoic acid (**14**) and *N*-methyl-*O*-benzylhydroxylamine hydrochloride in a manner similar to that for **16** and also obtained as a colorless oil (1.58 g, 82.5%): ¹H NMR (CDCl₃) δ 1.2–2.0 (m, 6 H), 2.33 (t, 2 H), 3.20 (s, 3 H), 3.35 (t, 2 H), 4.82 (s, 2 H), 7.43 (s, 5 H); IR (neat) 1660 cm⁻¹; mass spectrum (EI), *m/e* 313, 315 (M⁺).

Reaction of Methyl Iodide with 16. Compound **16** (3 g, 10 mmol) and CH₃I (4.23 g, 30 mmol) and K₂CO₃ (6.9 g, 50 mmol) were placed in dry acetone (50 mL). The mixture was allowed to stir for 24 h at room temperature. After filtration and evaporation, the residue was taken into ether (100 mL). This was washed twice with 0.5 N NaOH and once with H₂O. After the mixture was dried (Na₂CO₃) and the solvent evaporated, compounds **18** and **19** were isolated (18:19 = 1:9) as oils: 2.5 g (75–80%). Compound **19**: ¹H NMR (CDCl₃) δ 1.1–1.9 (m, 6 H), 2.33 (t, 2 H), 3.18 (t, 2 H), 3.20 (s, 3 H), 4.83 (s, 2 H), 7.43 (s, 5 H).

Alkylation of Mixture 18 and 19 with Cyanuric Acid. NaH (173 mg, 3.78 mmol) was suspended in DMF (30 mL) and treated with cyanuric acid (129 mg, 1 mmol). To this solution was added the mixture of **18** and **19** (1.25 g). The combined solution was allowed to stir for 2 h at 90–100 °C and for 24 h at room temperature. The mixture was taken into ethyl acetate (80 mL) and washed several times with H₂O. After the mixture was dried (Na₂SO₄) and the solvent evaporated, the residue was chromatographed on a silica plate (2 mm, Chromatotron), eluting with ethyl acetate/hexane (1:1). Compound **20** was isolated as a colorless oil: 415 mg (50%); ¹H NMR (CDCl₃) δ 1.1–1.8 (m, 18 H), 2.37 (t, 6 H), 3.20 (s, 9 H), 3.83 (t, 6 H), 4.83 (s, 6 H), 7.43 (s, 15 H); IR (neat) 1660, 1690 cm⁻¹. Anal. (C₄₅H₆₀N₆O₉·H₂O) C, H, N.

In addition a small amount (50 mg) of the dialkylated product **21** was obtained as a colorless oil from the later column fraction: ¹H NMR (CDCl₃) δ 1.2–1.8 (m, 12 H), 2.38 (t, 4 H), 2.60 (br, s, 1 H), 3.20 (s, 6 H), 3.65 (t, 4 H), 4.80 (s, 4 H), 7.42 (s, 10 H); IR (neat) 3450, 1640–1690 cm⁻¹.

Retroanalogue of the Cyanuric Acid Derivative 3. Compound **20** (0.33 g, 0.40 mmol) was dissolved in THF/H₂O (2:1, 30 mL) and treated with Pd on carbon (5%, 200 mg) under 1 atm of H₂ for 4 h at room temperature. The mixture was filtered and evaporated, compound **3** was obtained as a colorless oil: 130 mg (58.6%). It slowly solidified; mp > 250 °C; ¹H NMR (CD₃OD) δ 1.2–1.9 (m, 18 H), 2.50 (t, 6 H), 3.25 (s, 9 H), 3.90 (t, 6 H), 4.8 (s, exchange with CD₃OD); IR (neat) 1660, 1690 cm⁻¹; mass spectrum (FD), *m/e* 559 (M + 1); *R_f* 0.87 for reverse phase (RP-2) in 2-PrOH/H₂O (70:30); *R_f* 0.35 for reverse phase (RP-2) in 2-PrOH/H₂O (30:70).

Dialkylated Retroanalogue of the Cyanuric Acid Derivative 4. Compound **21** (40 mg, 0.07 mmol) was dissolved in ethanol/H₂O (8:2, 10 mL) and treated with Pd on carbon (5%, 40 mg) under 1 atm of H₂ for 3 h at room temperature. The mixture was filtered and evaporated, and compound **4** was obtained as a colorless oil: 18 mg (64.5%); ¹H NMR (CD₃OD) δ 1.2–1.8 (m, 12 H), 2.47 (t, 4 H), 3.20 (s, 6 H), 3.57 (t, 4 H), 4.8 (br s, OH's, exchange with CD₃OD); IR (neat) 1640–1690 cm⁻¹; *R_f* 0.84 for reverse phase (RP-2) in 2-PrOH/H₂O; *R_f* 0.75 for reverse phase (RP-2) in 2-PrOH/H₂O (30:70).

Biological Activity. The ability of artificial siderophores to support growth of *Escherichia coli* K12 strains was examined by placing filter paper discs on nutrient agar plates seeded with an overlay of the strain in soft nutrient agar containing 0.1 mM deferriferrichrome A. Because *E. coli* is unable to use ferrichrome A, addition of this chelator makes the nutrient plate iron deficient. Each disc was then impregnated with 10 μL of 50–500 μM siderophore solution. The siderophores were dissolved in water, and the hydroxamic acid functionality was measured by the ferric perchlorate reagent with use of *a*_{nm} = 1.0 at 480 nm. The diameter of the growth response zone was scored after 12 and 24 h of incubation at 37 °C. Growth response assays were done with both the ferri and deferri forms of the artificial siderophores.

The in vivo mouse bioassays were performed by established procedures at E G & G Mason Research Institute.

Acknowledgment. We gratefully acknowledge support of this research at Notre Dame by NIH Grant GM 25845 and at Berkeley by NIH Grant AI 04156. Dr. John L. Occolowitz (Eli Lilly and Co.) kindly provided the mass spectral data. We are also grateful to Dr. H. Rosenkrantz and J. J. Metterville (E G & G Mason Research Institute) for performing the in vivo mouse bioassays.

Registry No. **2a**, 94136-50-2; **2b**, 94136-44-4; **2c**, 94136-52-4; **3**, 94136-33-1; **4**, 94136-34-2; **5a** (X = Br), 109-64-8; **5b** (X = Br), 110-52-1; **5b** (X = I), 628-21-7; **5c** (X = Br), 111-24-0; **6**, 90195-00-9; **7a** (X = Br), 94136-45-5; **7b** (X = Br), 94136-42-2; **7c** (X = Br), 94136-46-6; **8**, 4797-81-3; **9b** (X = Br), 94136-40-0; **10a** (X = Br), 94136-47-7; **10b** (X = Br), 94136-39-7; **10b** (X = I), 94136-41-1; **10c** (X = Br), 94136-48-8; **12**, 108-80-5; **13a**, 94136-49-9; **13b**, 94136-43-3; **13c**, 94136-51-3; **14**, 4224-70-8; **16**, 94136-35-3; **18**, 94136-36-4; **19**, 94136-37-5; **20**, 94136-38-6; **21**, 94161-24-7; **23**, 46235-83-0; *O*-benzylhydroxylamine hydrochloride, 2687-43-6; *N*-methyl-*O*-benzylhydroxylamine hydrochloride, 71925-14-9; Fe, 7439-89-6.