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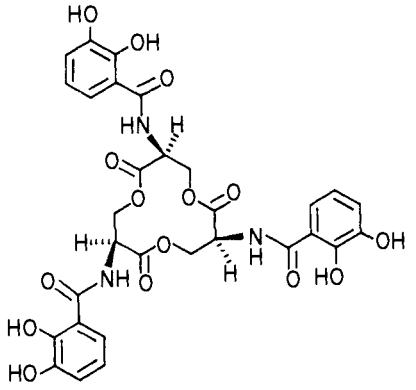
1,3,5-Tris(*N*,*N*′,*N*″-2,3-dihydroxybenzoyl)aminomethylbenzene, a Synthetic Iron Chelator Related to Enterobactin

Sir:

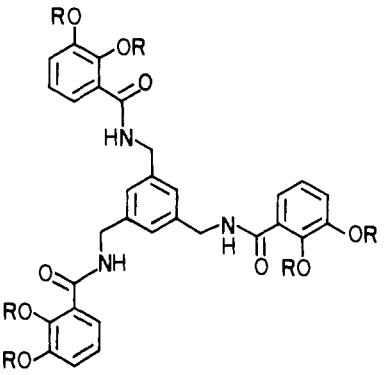
Secondary hemochromatosis, the iron-overload disease induced by a prolonged regimen of transfusion therapy for the genetic disease β-thalassemia major (Cooley's anemia), accounts for the eventual death of most patients by early adulthood. The search for an effective replacement for desferrioxamine, an iron chelator which has found limited use, is currently the subject of intense effort.[1] Of the many compounds tested as iron-chelating agents, those containing the 2,3-dihydroxybenzoyl (DHB) moiety, the active chelation site of the bacterial siderophore enterobactin, 1,[2] have shown greatest potential as orally effective drugs.[3]



1

While the natural product 1 has been demonstrated to be one of the most powerful iron chelators known ($K_s \simeq 10^{51}$)[4] and has undergone preliminary studies in test animals,[5] there now exists no plentiful source of this compound.[6] As part of a program directed toward the total synthesis of 1 and closely related, possibly useful analogues, we have designed and prepared the title compound **2**.

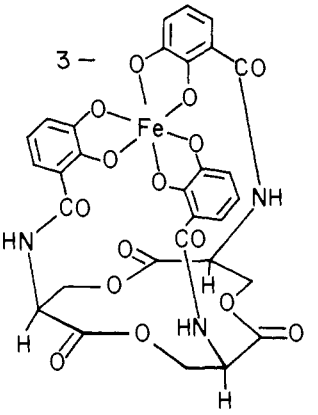
The synthesis of **2** proceeds straightforwardly and in high yield from known materials. Catalytic reduction of 1,3,5-benzenetrialdoxime[7] in ethanol–THF over 10% Pd



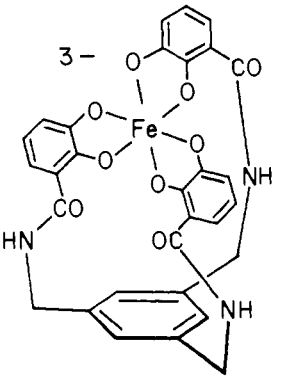
2, R = H
3, R = $CH_2C_6H_5$

on C in the presence of dry HCl gave 1,3,5-tris(aminomethyl)benzene trihydrochloride, mp >350 °C (EtOH–H_2O), in 97% yield. Acylation with 2,3-dibenzyloxybenzoyl chloride[8] under Schotten–Baumann conditions (aqueous NaOH) afforded protected **3** in 81% yield after silica gel chromatography. Removal of the benzyl protection by catalytic hydrogenation in ethanol–acetic acid (20:1) gave **2** as a white powder in 90–95% yield.[9]

The design of **2** as an isosteric equivalent of enterobactin (1) was governed by the geometrical feature of 1 responsible for its high affinity for iron(III), i.e., the incorporation of three DHB units into one ligand providing a chelation site of proper size. Comparison of the Dreiding models of the iron complexes of enterobactin, **4**, and the synthetic analogue, **5**, clearly indicates that the rigidity



4



5

conferred upon **5** by the planar 1,3,5-benzylic carbon system effectively mimics that imparted to **4** by the 12-membered cyclic triester. Moreover, the hydrolytically

Table I. Growth Response Displayed by Ferric Catecholates in Enterobactin Requiring and Ferric Enterobactin Utilization Deficient Strains of Enteric Bacteria

	Diameter of Exhibition of Growth (mm)[a]					
	S. typhimurium[b]			*E. coli*[c]		
siderophore	concn, μM	*enb-1*	*enb-7*	concn, μM	RW193	RWB18
ferric enterobactin	30	26	26	3	18	8
				15	22	12
complex 5	5	20	20	2.5	10	7
				5	13	7
	50	28	29	10	17	11
dihydroxybenzoic acid	100	9	18			

[a] In each case, a 10-μL volume was pipetted onto a 6-mm filter disk; e.g., a response of 7-mm means an observed growth of 0.5 mm in radius. [b] Assays for siderophore activity were carried out according to ref 11b. [c] Assays for receptor site activity were carried out according to ref 12a; the "Tris" medium described in ref 12b was used, supplemented with 40 μg/mL each of L-Leu, L-Pro, and L-Trp, and 25 μg/mL thiamin chloride hydrochloride.

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labile *O*-seryl ester linkages of **1** and **4** have been eliminated, obviating the decomposition problem associated with **1**.[2b]

As a preliminary test of the utility of **2** as an iron chelator, the stability of the iron(III) complex **5** was examined via a competition experiment with EDTA. Equal volumes of 0.1 mM **5** in 0.1 M phosphate buffer (1:1 EtOH–H_2O, pH 7.2) and varying amounts of EDTA in 0.1 M phosphate buffer (in H_2O, pH 7.2) were mixed and equilibrated, and the resulting absorption was measured at 495 nm.[10] The absorption of **5** is unchanged at an EDTA/**5** ratio of 10:1, is 70% diminished at 100:1, and is completely quenched at 1000:1. Using these data and a $p\bar{K}_a$ of 10.1 ± 0.2 for **2**,[4] a stability constant, log K_s = 44.6 ± 1.8, can be calculated [EDTA–iron(III) complex = 25].[10]

Biological testing of **2** confirmed the calculated high affinity for iron(III). Complex **5** was found to possess the same order of activity as ferric enterobactin in supplying iron to mutants *enb-1* and *enb-7* of *S. typhimurium*,[11] which are blocked in enterobactin synthesis before and after 2,3-dihydroxybenzoic acid, respectively, suggesting that **5** acts as a siderophore in this test system without degradation to 2,3-dihydroxybenzoic acid and resynthesis to enterobactin. Complex **5** was also shown to utilize the ferric enterobactin receptor site by promoting growth in *Escherichia coli* strain RW193 (enterobactin$^+$, receptor$^+$) and not in strain RWB18 (enterobactin$^+$, receptor$^-$).[12] These data, summarized in Table I, indicate that **2** is indeed a suitable isosteric equivalent of enterobactin and may be of use in the iron-chelation treatment of secondary hemochromatosis. The criteria of nontoxicity, the ability to cross membrane barriers, and the completeness of excretion of iron complex **5** in an iron-overloaded system must be determined by in vivo and clinical trials.[13]

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(9) ^1H NMR (acetone-d_6, 60 MHz) δ (Me_4Si) 8.50 (t, J = 6 Hz, 3 H, amide NH), 7.25 (s, 3 H, mesityl aromatic H), 7.25 (q, J_{56} = 7 Hz, J_{46} = 2 Hz, 3 H, benzoyl position 6), 7.20 (br, 6 H, phenolic OH), 6.95 (q, J_{45} = 7 Hz, J_{46}, 3 H, benzoyl position 4), 6.60 (t, J_{45} = J_{56}, 3 H, benzoyl position 5), 4.55 (d, J = 6 Hz, 6 H, benzylic methylene H); ^{13}C NMR (acetone-d_6, 15.015 MHz) δ^C (Me_4Si) 170.1 (amide CO), 149.4, 146.0, 139.4, 125.7, 118.9 (double intensity), 117.4, 115.3 (aromatic), 42.9 (benzylic); IR (KBr pellet) 3380, 2950, 1640, 1590, 1460, 1325, 1265 cm^{-1}; UV (EtOAc) λ_{max} 313 nm (ϵ 9900); MS (exact) m/e ($C_{30}H_{27}N_3O_9$) calcd 573.17473, obsd 573.17726; mp 128–132 °C, after vacuum drying at 80 °C.

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(13) A sample of compound **2** has been submitted to Drs. Anthony Cerami and R. W. Grady of Rockefeller University, New York, N.Y., for testing in iron-overloaded rats.

(14) An independent synthesis of **2** was announced by Dr. K. N. Raymond (Berkeley) at the American Chemical Society National Meeting, Anaheim, Calif., March, 1978, under abstract INOR 228. Our value of K_s is in accord with a more precise determination performed by Raymond and coworkers. Particulars of their synthesis and iron-affinity study have been submitted for publication. We thank Dr. Raymond for communicating his results to us prior to journal publication.

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