Articles

Desferrithiocin Analogue Based Hexacoordinate Iron(III) Chelators

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Traditional thinking has been that hexacoordinate Fe(III) ligands are more effective at preventing iron's interactions with reactive oxygen species, most particularly the Fe(II)-mediated reduction of hydrogen peroxide to the hydroxyl radical (i.e., Fenton chemistry), than are ligands of lower denticity. Thus, a hexacoordinate derivative of the well-characterized tricoordinate ligand (*S*)-2-(2,4-dihydroxyphenyl)-4,5-dihydro-4-thiazolecarboxylic acid [4′-(HO)-DADMDFT], (*S*,*S*)-1,11-bis[5-(4-carboxy-4,5-dihydrothiazol-2-yl)-2,4-dihydroxyphenyl]-4,8-dioxaundecane, was designed with the aid of a molecular modeling program and synthesized. Evaluations both in vitro and in vivo were carried out to determine whether there is any advantage, at the level of prevention of Fenton chemistry, radical trapping, or iron clearance, to constructing a desferrithiocin-based hexacoordinate analogue. The hexacoordinate analogue was more effective at preventing the iron-mediated oxidation of ascorbate at low ligand/metal ratios than was its tricoordinate parent and can function as an excellent radical scavenger. At equivalent iron binding doses in the bile duct cannulated rodent, oral administration of the tricoordinate ligand was 3-fold more effective than was po administration of the hexacoordinate derivative. However, sc administration of the hexacoordinate derivative resulted in an efficiency that was 3 times greater than that of the tricoordinate chelator. Unfortunately, the rodent findings were not substantiated in the primates. The hexacoordinate ligand was only about one-half as efficient as its tricoordinate parent when administered sc. Owing to these results, po dosing was not attempted. Thus, there appears to be no overall advantage to coupling two molecules of 4′-(HO)-DADMDFT to afford a hexacoordinate derivative.

Introduction

In recent years, it has become clear that manipulation of iron levels in particular biological compartments can have a profound effect on a number of disease processes. The progress of disorders as diverse as Cooley's anemia (also known as β-thalassemia major, a hereditary anemia), malaria, and reperfusion injury is dependent on iron.[1−6]

Although essentially all prokaryotes and eukaryotes have a strict requirement for this micronutrient, and iron represents 5% of the earth's crust, it is difficult for living systems to access and manage it. The low solubility of the predominant form of the metal, Fe(III) hydroxide ($K_{sp} = 1 \times 10^{-39}$),[7] in the biosphere has necessitated the development of rather sophisticated iron storage and transport systems in nature. Microorganisms utilize low molecular weight ligands, siderophores; higher eukaryotes tend to utilize proteins to transport iron (e.g., transferrin) and to store iron (e.g., ferritin).[8−10]

In primates, iron metabolism is highly efficient.[11−15] Little of the metal is absorbed, and no specific mechanism exists for its elimination. Because it cannot be effectively cleared, the introduction of "excess iron"[16−18] into this closed metabolic loop leads to chronic overload and ultimately to peroxidative tissue damage. For example, patients with severe hemolytic anemias such as β-thalassemia require continued transfusions, which increase their body iron by 200−250 mg/unit of blood. Unless these individuals receive chelation therapy, they frequently die in their third decade from complications assiciated with iron overload.

The hydroxamate desferrioxamine B (DFO, Chart 1), a bacterial siderophore that is commercially produced by large-scale fermentation of a strain of *Streptomyces pilosus*,[19] exhibits a high selectivity for iron and is the drug of choice for the treatment of transfusional iron overload. Unfortunately, treatment with DFO is cumbersome, inefficient, unpleasant, and costly. Because DFO is poorly absorbed from the gastrointestinal tract and rapidly eliminated from the circulation, prolonged parenteral [subcutaneous (sc) or intravenous (iv)] infusion, usually for 9−12 h daily, is needed.[20−23] Patient compliance with this regimen is problematic; thus, considerable interest has focused on a search for an iron chelator that does not require parenteral administration or, alternatively, a parenteral device with superior iron-clearing efficacy.

Cooley's anemia, a global iron overload disease, probably best exemplifies the hurdles that must be overcome in chelator design. The lifelong treatment necessary in

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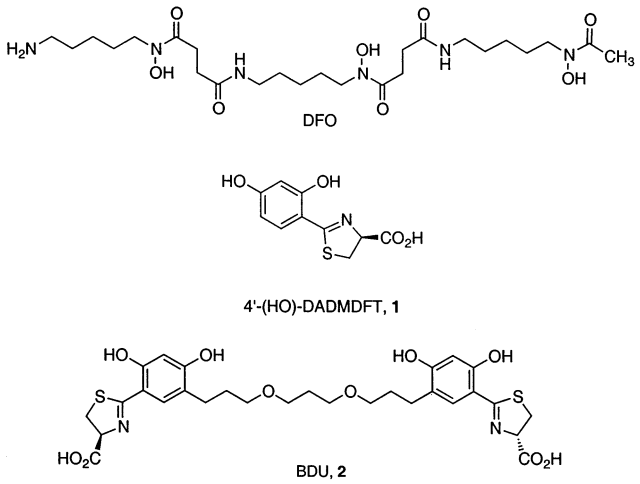
Chart 1. Compounds Evaluated in the Iron-Clearance Experiments: Desferrioxamine (DFO), 4′-(HO)-DADMDFT (**1**), and BDU (**2**)



this disorder dictates that the efficiency with which the ligand removes iron and the long-term toxicity of the drug are of paramount importance. The former issue has a tremendous impact on how the drug can be administered and thus on patient compliance. Although in other diseases the rationale of exploiting iron metabolism as a therapeutic target may also be that of chelating and removing "toxic" iron, the chelator design strategies are somewhat different. The parameters set by the nature of the disease on the toxicity profile and modes of administration of the ligands can vary significantly. For example, in malaria and reperfusion injury, somewhat more flexibility exists regarding toxicity profiles and modes of administration, inasmuch as short-term exposure to the drug is foreseen. Thus, ligands that are unacceptable for the treatment of iron overload associated with Cooley's anemia may be completely appropriate and useful in other therapeutic arenas in which the iron overload is more localized. Nevertheless, there are some considerations that are common to the design of chelators for treatment of any iron overload disorder. These considerations revolve around the capacity of iron to promote the production of free radicals.

The deleterious effects of excess iron, whether a global or a focal problem, is derived from its interaction with reactive oxygen species such as superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2). The latter species is derived either from the spontaneous dismutation of superoxide anion or from the reaction catalyzed by superoxide dismutase. Hydrogen peroxide can, in the presence of Fe(II), be reduced to the hydroxyl radical ($HO^{\bullet}$) and HO^- . Oxidation of Fe(II) to Fe(III) occurs concomitantly; this sequence is known as the Fenton reaction:

$$Fe(II) + H_2O_2 \rightleftharpoons Fe(III) + HO^{\bullet} + HO^-$$

The hydroxyl radical is an excellent oxidizing agent, reacting at a diffusion-controlled rate with many different biomolecules. The hydroxyl radical, the resulting lower-energy radicals that it produces, or the radical chain reactions that it initiates can cause damage to cell membranes and mitochondria as well as promote the formation of carcinogens.[24−26] If this were a single event, it would be problematic, but the system could likely repair the damage in most instances. However, there are a number of physiological reductants that can reduce Fe(III) back to Fe(II), such as $O_2^{\bullet -}$ and ascorbate:

$$Fe(III) + O_2^{\bullet -} \rightleftharpoons Fe(II) + O_2$$

$$Fe(III) + \text{ascorbate} \rightleftharpoons Fe(II) + \text{ascorbyl radical anion}$$

In addition, it has been demonstrated that certain Fe(III) chelators such as ethylenediaminetetraacetic acid (EDTA),[27] nitrilotriacetic acid (NTA),[28] 5-aminosalicylic acid (5-ASA),[29,30] and 1,2-dimethyl-3-hydroxypyridin-4-one (L1)[27,31] can indeed promote the reduction of Fe(III) to Fe(II). Of course, this would exacerbate the damage caused by the Fenton reaction. Thus, one of the paramount concerns in the design of therapeutic iron chelators has revolved around the impact of a given ligand on Fenton chemistry. The traditional thinking has been that hexacoordinate Fe(III) ligands are more effective at preventing Fenton chemistry than are ligands of lower denticity.[24,27,32] Hexacoordinate chelators surround the metal, preventing its reduction to Fe(II) and thus the Fe(II)-mediated reduction of H_2O_2. A ligand that prevents the conversion of Fe(III) to Fe(II) altogether is the most desirable. However, an acceptable compromise would be a chelator that does not actively promote the reduction but still facilitates iron excretion from the animal. In the absence of iron removal, Fenton chemistry would continue.

A second desirable feature of interest is the capacity to trap free radicals. Clearly, a deferrating agent that traps these reactive species might well solve some of the problems associated with radicals, either $HO^{\bullet}$ or those generated by $HO^{\bullet}$. Other investigators have noted these properties with a variety of hexacoordinate ligands, including the naturally occurring hydroxamate DFO[33] and the synthetic polycarboxylate *N*,*N*′-bis(2-hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid (HBED).[34]

The current study focuses on the issue of whether there is any advantage, at the level of prevention of Fenton chemistry, radical trapping, or iron clearance, to constructing a desferrithiocin-based hexacoordinate analogue. The ligands evaluated were the well-characterized tricoordinate ligand (*S*)-2-(2,4-dihydroxyphenyl)-4,5-dihydro-4-thiazolecarboxylic acid [4′-(HO)-DADMDFT, **1**][35−37] and a hexacoordinate derivative (*S*,*S*)-1,11-bis[5-(4-carboxy-4,5-dihydrothiazol-2-yl)-2,4-dihydroxyphenyl]-4,8-dioxaundecane (BDU, **2**) (Chart 1).

Results and Discussion

Ligand Design and Synthesis. The tricoordinate derivative 4′-(HO)-DADMDFT (**1**) is a moderately effective iron-clearing device in both the bile duct cannulated rodent and the iron-overloaded *Cebus apella* monkey.[35,36] The compound's toxicity was profoundly less than that of its non-hydroxylated parent (*S*)-4,5-dihydro-2-(2-hydroxyphenyl)-4-thiazolecarboxylic acid.[35] On the basis of these observations, we decided to anneal two 4′-(HO)-DADMDFT fragments to generate a hexacoordinate ligand. The initial considerations were, first, which positions would best serve as tether points and, second, the length and nature (hydrocarbon or ether)

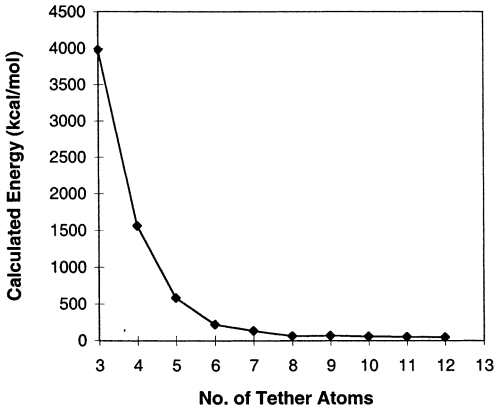


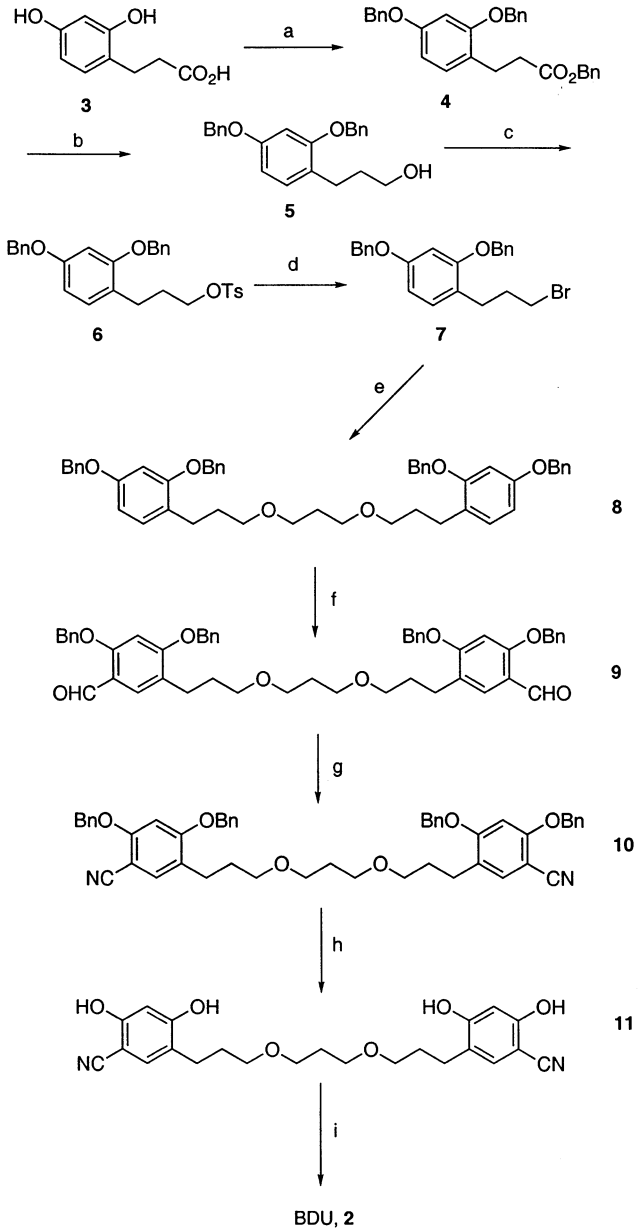
Figure 1. Calculated energies (in kcal/mol) vs tether length for 5,5′ tethered bis-DFT analogues.

of the tether. Although substitution at two positions, either 3′ or 5′, seemed reasonable, 5′ substitution was chosen because of the facility of access. A polyether tether was selected; the rationale was that this chain would impart more water solubility to the molecule than would a simple hydrocarbon backbone. This is in keeping with our earlier experience with DFO analogues.[38] The length of the tethers connecting the tridentate fragments was based on a series of molecular models prepared with the SYBYL modeling program, which utilized the X-ray coordinates for the 2:1 DFT/Cr(III) complex.[39] Tethers of various lengths were modeled onto the complex, and the energy was minimized with the atoms of the complex artificially constrained, essentially "freezing" their coordinates. No additional constraints were applied to the atoms comprising the tethers. This methodology preserved the structure of the chelate about the metal ion but permitted the atoms of the chain to reorient to a lower energy conformation. The energy minimization profiles clearly illustrate that small increases in length beyond the optimum have far less impact than do small decreases in the length on the stability of the complex (Figure 1). Specifically, the addition of methylene segments is less detrimental to the stability of the chelate than reduction in backbone length to a dimension too small to accommodate the preferred octahedral geometry for Fe(III)-hexacoordinate ligand complexes. The target ligand chosen was (*S*,*S*)-1,11-bis[5-(4-carboxy-4,5-dihydrothiazol-2-yl)-2,4-dihydroxyphenyl]-4,8-dioxaundecane (BDU, **2**).

The synthesis of **2** began with the conversion of 3-(2,4-dihydroxyphenyl)propionic acid (**3**)[40] to its tri-*O*-benzylated derivative **4** in 87% yield using benzyl bromide and K_2CO_3 in refluxing acetone (Scheme 1). Carboxylic ester **4** was reduced with $LiAlH_4$ in refluxing THF, generating 3-(2,4-dibenzyloxyphenyl)propanol (**5**) in 82% yield.

At this point, several attempts were made to couple the sodium salt of primary alcohol **5** with 1,3-dihalopropanes (chloro-, bromo-, iodo-) and with 1,3-propanediol di-*p*-tosylate. Unfortunately, dialkylation did not take place; only the allyl ether of **5** was formed. Also, attempts to alkylate both termini of the disodium salt of 1,3-propanediol with the iodide, bromide, chloride, or tosylate of carbinol **5** in a variety of solvents (e.g., DMF, DMSO, HMPA) and temperatures led to 1-allyl-2,4-dibenzyloxybenzene from an elimination reaction. However, after **5** was converted to its tosylate **6** (TsCl, pyridine) in 77% yield and subsequently to primary

Scheme 1. Synthesis of a Hexacoordinate DFT Analogue (BDU, **2**)[a]



[a] Reagents: (a) K_2CO_3, BnBr, acetone, reflux, 87%; (b) $LiAlH_4$, THF, 82%; (c) TsCl, pyridine, CH_2Cl_2, 77%; (d) LiBr, acetone, reflux, 89%; (e) $HOCH_2CH_2CH_2OH$, KOH, DMSO, 58%; (f) $POCl_3$, DMF, CH_3CN, reflux, then aqueous 1,4-dioxane, 61%; (g) $NH_2OH\cdot HCl$, Et_3N, CH_3CN, then phthalic anhydride, reflux, 77%; (h) H_2, 10% Pd/C, EtOAc, EtOH, 90%; (i) D-cysteine·HCl·H_2O, $NaHCO_3$, CH_3OH, phosphate buffer (pH 6.0), reflux, 55%.

bromide **7** (LiBr, acetone, reflux) in 89% yield, its dialkylation of 1,3-propanediol (0.5 equiv) using powdered KOH in DMSO at 50 °C resulted in 1,11-bis(2,4-dibenzyloxyphenyl)-4,8-dioxaundecane (**8**) in 58% yield.

Vilsmeier−Haack reaction of **8** with phosphorus oxychloride and DMF in CH_3CN and hydrolysis of the complex[41] provided 1,11-bis(2,4-dibenzyloxy-5-formylphenyl)-4,8-dioxaundecane (**9**) in 61% yield. Dialdehyde **9** was converted in situ to the dioxime, which was dehydrated using phthalic anhydride in refluxing CH_3CN[42] to give dicyano compound **10** in 77% yield. Catalytic removal of the benzyl protecting groups from **10** over a palladium catalyst afforded 1,11-bis(5-cyano-

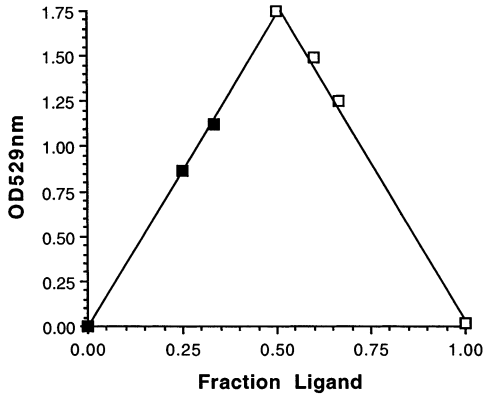


Figure 2. Job's plot of **2**. Solutions containing different ligand/Fe(III) ratios were prepared so that [ligand] + [Fe(III)] = 1.0 mM. The data points, each the mean of three experiments with a maximum standard deviation of ±0.012, were fitted to the mole fractions (1) from 0 to 0.50 (filled symbols) and (2) 0.50−1.00 (open symbols); r^2 = 1.000 and 0.995, respectively. Theoretical mole fraction for a 1:1 ligand/Fe complex is 0.50. The observed maximum is 0.52.

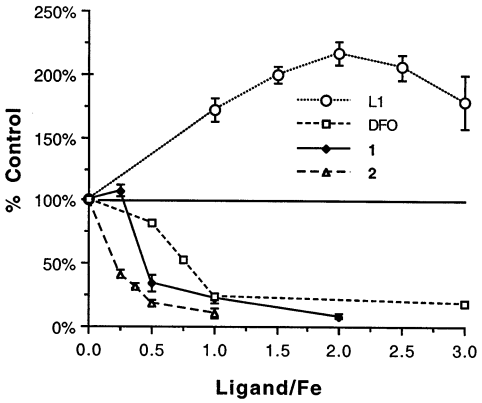


Figure 3. Effect of various chelators on the iron-mediated oxidation of ascorbate (percent of control, *y* axis): 1,2-dimethyl-3-hydroxypyridin-4-one (L1, $n = 3$), desferrioxamine (DFO, $n = 3$), 4′-(HO)-DADMDFT (**1**, $n = 4$), and BDU (**2**, $n = 4$) at several ligand/metal ratios (*x* axis). Typically, each assay contained three controls with only ascorbate and $FeCl_3$. These varied less than 5% (±SD). Each assay also usually included a "negative control" containing ascorbate, $FeCl_3$, and L1 at a ligand/iron ratio of 2:1. This value was 213% ± 12%.

2,4-dihydroxyphenyl)-4,8-dioxaundecane (**11**) in 90% yield. Cyclocondensation of tetrahydroxydinitrile **11** with excess D-cysteine in aqueous CH_3OH buffered at pH 6 at reflux for 2 days[35] furnished hexadentate ligand **2** (BDU) in 55% yield.

Stoichiometry of the Iron Complex. The stoichiometry of the complex was determined spectrophotometrically for **2** at the λ_{max} (529 nm) of the visible absorption band of the ferric complex. The Job's plot for mixtures containing various ratios of ligand to Fe(III) NTA ([ligand] + [Fe] = 1.00 mM constant) (Figure 2) suggests a 1:1 hexacoordinate complex.

Ascorbate Reduction. Although this assay has been interpreted as a measure of how effectively a ligand can prevent iron from participating in Fenton chemistry,[27] the measurement is indirect; it examines the disappearance of ascorbate. Recall that ascorbate is a physiological reductant that can cycle Fe(III) back to Fe(II), the key player in the Fenton reaction. When ascorbate reduces Fe(III) to Fe(II), the ascorbyl radical anion is produced; this species has been postulated to disproportionate to dehydroascorbic acid and ascorbate.[43] Clearly, if a chelator could prevent reduction of Fe(III) by ascorbate, the pool of Fe(II) available for peroxide reduction would be diminished. It is known that DFO, a hydroxamate chelator that forms a 1:1 complex with Fe(III) at a formation constant of approximately 10^{31} M^{-1},[44−46] prevents ascorbate-mediated reduction of Fe(III);[27] it serves as a positive control in the present study. Both NTA[28] and L1[27,31] promote ascorbate-mediated reduction of Fe(III) and serve as negative controls.

Consistent with others' findings, NTA exerted a profoundly stimulatory effect on reduction of Fe(III), 485% at a 0.5:1 ligand/metal ratio that further increased to 551% at a 1.5:1 ratio. L1 also promoted the reaction, but not as dramatically, at ligand/metal ratios of up to 3:1 (Figure 3). Iron(III) reduction was inhibited by DFO at ligand/metal ratios of less than 1:1, although the optimum effect was seen at greater than or equal to 1:1. Both desferrithiocin analogues provided clear protection at ligand/metal ratios of between 0.5:1 and 1:1, and there is no significant difference between the two compounds when the ligands are compared at equivalent iron-binding ratios (e.g., 2:1 for **1** vs 1:1 for **2**). Nevertheless, the tricoordinate chelator (**1**) was significantly ($P < 0.005$) less inhibitory than its hexacoordinate analogue (**2**) at ratios of 0.25:1, 0.5:1, and 1:1 (Figure 3).

Table 1. ABTS Radical Cation Quenching Activity of Selected Compounds

compound	slope × 10^3 OD units/μM[a]
Trolox	−37[b]
L1	−53[b]
1	−102[b]
2	−136
DFO	−137[b]

[a] The slope was derived from A_{734} vs concentration data after a 6 min reaction period between the chelator of interest and the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$), which was formed from the reaction between ABTS and persulfate. A negative slope represents a decrease in the amount of highly colored radical cation over the time interval from an initial OD_{470} of 1.000. Trolox, a water-soluble analogue of vitamin E, served as a positive control. [b] Bergeron, R. J.; Wiegand, J.; Weimar, W. R.; Nguyen, J. N.; Sninsky, C. A. *Dig. Dis. Sci.*, in press.

Radical Scavenging. This radical cation decolorization assay utilizes the preformed radical monocation of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) and has been used to evaluate the antioxidant capacity of a large number of compounds and mixtures.[34,47] Briefly, the change in absorbance of the blue-green chromophore was recorded after the addition of the chelator of interest at each of the different concentrations, and the slope of the ΔA_{734} vs ligand concentration line was calculated. The positive control for this reaction was Trolox, a water-soluble analogue of vitamin E.[47] The decrease in A_{734} as a function of ligand concentration is the comparitor among the five compounds evaluated, Trolox, L1, DFO, **1**, and **2** (Table 1). All four of the iron chelators performed better than Trolox: DFO ∼ **2** > **1** > L1 > Trolox. Thus, all of these ligands could be expected to serve as excellent radical scavengers.

Table 2. Iron-Clearing Efficacy of Desferrithiocin Analogues in Rodents and Primates

compound	dose (μmol/kg)	route	efficiency in rodents (%)[a] [% urine/% bile]	efficiency in monkeys (%)[b] [% urine/% stool]
DFO	150	po	1.1 ± 0.6[c] [13/87]	0.1 ± 0.4[d] [45/55]
DFO[e]	150	sc	2.8 ± 0.7 [25/75]	5.5 ± 0.9 [55/45]
4′-(HO)-DADMDFT (**1**)	300	po	2.9 ± 2.8 [0/100]	5.3 ± 1.7[f] [10/90]
4′-(HO)-DADMDFT (**1**)	150	sc		5.6 ± 0.9[f] [8/92]
4′-(HO)-DADMDFT (**1**)	300	sc	2.1 ± 0.9 [10/90]	5.3 ± 1.7[g] [25/75]
BDU (**2**)	150	po	0.9 ± 0.4 [20/80]	
BDU (**2**)	75	sc		3.2 ± 1.8[g] [2/98]
BDU (**2**)	150	sc	6.5 ± 2.0 [5/95]	2.1 ± 1.4[g,h] [0/100]

[a] In the rats ($n = 4$, unless otherwise indicated), the net iron excretion was calculated by subtracting the iron excretion of control animals from the iron excretion of treated animals. Efficiency of chelation is defined as net iron excretion/total iron-binding capacity of chelator administered, expressed as a percent. [b] In the monkeys ($n = 4$, unless otherwise indicated), the efficiency of each compound was calculated by averaging the iron output for 4 days before the administration of the drug, subtracting these numbers from the 2-day iron clearance after the administration of the drug, and then dividing by the theoretical output. The result is expressed as a percent. [c] These results are from ref 48 ($n = 3$) and included for comparison. [d] These results are from ref 50 (dose administered, 300 μmol/kg) and included for comparison. [e] These results are from ref 49 ($n = 6$ in the rodents, $n = 5$ in the primates) and included for comparison. [f] These results are from ref 36 and included for comparison. In the po experiment, **1** was administered in 40% Cremophor. In the sc experiment, the compound was given as a suspension in water, $n = 3$. [g] The compound was administered as its sodium salt. [h] $n = 3$ in this experiment.

Iron Clearance. Iron clearance studies were carried out both in the non-iron-overloaded, bile duct cannulated rodent model and in the iron-overloaded *Cebus apella* monkey, and the results are reported as iron-clearing efficiency (Table 2). This number is generated by dividing the net iron clearance [total iron excretion (bile or stool plus urine) minus background] by the theoretical iron clearance and multiplying by 100. The theoretical iron clearance is based on a 2:1 metal complex stoichiometry for **1**,[35] a 1:1 stoichiometry for DFO, and a 1:1 stoichiometry for **2**, as shown in the Job's plot (Figure 2). The drugs were administered both orally (po) and sc to the rodents and primates; the positive control was DFO.

When DFO was administered at a dose of 150 μmol/kg (100 mg/kg) to rodents, although the proportions of iron excreted in the bile and urine were comparable, po dosing was considerably less effective than was sc dosing (Table 2, $P < 0.006$).[48,49] The iron-clearing efficiency of **1** was similar whether given po or sc at a dose of 300 μmol/kg, 2.9 ± 2.8% vs 2.1 ± 0.9%, respectively ($P > 0.05$). Again, the percentages of iron excreted in the bile were close to each other, 100% when the ligand was administered po, and 90% when administered sc, and higher than that observed with DFO. When hexacoordinate ligand **2** was given po at a dose of 150 μmol/kg (the iron-binding equivalent of 300 μmol/kg of **1**), the efficiency was considerably lower than, though still within experimental error of, that of the tricoordinate chelator and not unlike that of DFO given orally, 0.9 ± 0.4% ($P > 0.05$ vs **1** or DFO); 20% of the iron excretion was urinary, and 80% was biliary. However, when **2** was administered sc at a dose of 150 μmol/kg, the iron-clearing efficiency was 3 times as great as that of 300 μmol/kg of **1** given by this route 6.5 ± 2.0% ($P < 0.008$); 95% of the iron was in the bile and 5% in the urine. This mean efficiency is also more than twice that of an equivalent iron-binding dose of DFO ($P < 0.02$).

In the primates, the situation was somewhat different (Table 2). Again, DFO served as the benchmark; when given orally, the efficiency, 0.1 ± 0.4%, was less than that observed in the rodent ($P < 0.05$).[50] However, when administered sc, DFO was more efficient in the primate, 5.5 ± 0.9%, than in the rodent ($P < 0.002$).[49] When 4′-(HO)-DADMDFT (**1**) was given po to the primates at a dose of 300 μmol/kg, the efficiency, 5.3 ± 1.7%, was similar to that in the rodent ($P > 0.05$).[36] Adminstration of this same dose sc was equally efficient, 5.3 ± 1.7% ($P > 0.05$ vs po dosing); however, less of the iron excretion was fecal, 75% vs 90%, when this method of administration was employed. Subcutaneous administration of **2** to the primates was carried out at two different doses, 75 and 150 μmol/kg, equivalent in iron-binding capacity to 150 and 300 μmol/kg, respectively, of 4′-(HO)-DADMDFT. On the basis of the rodent data (Table 2) and previous results with **1** at the equivalent iron-binding dose in primates,[36] it was surprising that the efficiency of **2** at a dose of 75 μmol/kg, 3.2 ± 1.8%, was lower than that of **1** when administered sc at the equivalent iron-binding dose, 5.6 ± 0.9% ($P < 0.05$); the fecal-to-urinary ratio of the excreted iron was 98:2. When the dose of **2** was increased to 150 μmol/kg, the ligand's efficiency was 2.1 ± 1.4%; the iron excretion was completely accounted for in the feces. This figure is consistent with the 75 μmol/kg dose and about half of that of an equivalent dose of DFO or **1** administered sc ($P < 0.05$ vs **1**, $P < 0.02$ vs DFO). A po study was not pursued in the monkeys, owing to the poor performance of **2** when given po to the rats and sc to the primates.

Conclusion

It is quite clear that both 4′-(HO)-DADMDFT and its hexacoordinate analogue are both very effective at preventing Fenton chemistry and are both excellent radical scavengers. The hexacoordinate compound was more efficacious as a free radical quencher. A comparison of the iron-clearing efficiency of both ligands given po in the bile duct-cannulated rodent revealed that 4′-(HO)-DADMDFT is more efficient than BDU when given by this route. Subcutaneous dosing demonstrated

the hexacoordinate ligand to be roughly 3 times more effective than its tridentate component. Owing to the poor po activity of the hexacoordinate chelator in the rodent, the comparison between the two ligands in the primates was made only when the compounds were given sc. In this model, the results were disappointing. When the three ligands were administered sc, not only was the hexacoordinate DFT less efficient than DFO, it was even only about one-half as efficient as one of its tricoordinate components.

Thus, there appears to be no advantage to coupling two molecules of 4′-(HO)-DADMDFT to afford a hexacoordinate derivative. However, we are currently evaluating whether applying this approach to the 4-methyl analogue of **1** [i.e., (*S*)-2-(2,4-dihydroxyphenyl)-4,5-dihydro-4-methyl-4-thiazolecarboxylic acid], which was 3 times as efficient as 4′-(HO)-DADMDFT in primates,[36] would generate a hexacoordinate iron chelator more effective in vivo.

Experimental Section

All reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) and were used without further purification. Fisher Optima grade solvents were routinely used, and reactions were run under nitrogen. DMF and THF were distilled, the latter from sodium and benzophenone. Organic extracts were dried with anhydrous sodium sulfate. Silica gel 32-63 from Selecto Scientific, Inc. (Suwanee, GA) was used for flash column chromatography. NMR spectra were recorded at 300 MHz (^{1}H) or at 75 MHz (^{13}C) on a Varian Unity 300. Unless otherwise indicated, the spectra were run in $CDCl_3$ with tetramethylsilane (δ 0.0 ppm) for ^{1}H or the solvent (δ 77.0 ppm) for ^{13}C as standards. Coupling constants (J) are in hertz. Elemental analyses were performed by Atlantic Microlabs (Norcross, GA). Computer-based molecular modeling and energy minimizations were accomplished using SYBYL (version 6.5, Tripos, St. Louis, MO) on a Silicon Graphics Indigo-2 workstation and visualized with Chem 3D (CambridgeSoft, Cambridge, MA) on a model 6400/200 Power Macintosh computer.

Desferrioxamine B in the form of the methanesulfonate salt, Desferal (Novartis Pharma AG, Basel, Switzerland), was obtained from a hospital pharmacy. 1,2-Dimethyl-3-hydroxypyridin-4-one (L1) was a generous gift from Dr. H. H. Peter (Ciba-Geigy, Basel). Compound **1** was accessed by the method published from this laboratory.[35]

Spectrophotometric readings (A_λ) for the ascorbate and radical cation assays were taken on a Perkin-Elmer Lambda 3B spectrophotometer (Norwalk, CT).

Male Sprague−Dawley rats (averaging 450 g) were procured from Harlan Sprague−Dawley (Indianapolis, IN). Cremophor RH-40 was obtained from BASF (Parsippany, NJ). Nalgene metabolic cages, rat jackets, and fluid swivels were purchased from Harvard Bioscience (South Natick, MA). Intramedic polyethylene tubing (PE 50) and surgical supplies were obtained from Fisher Scientific (Pittsburgh, PA). *Cebus apella* monkeys were obtained from World Wide Primates (Miami, FL). Ultrapure salts were obtained from Johnson Matthey Electronics (Royston, U.K.). All hematological and biochemical studies[51] were performed by Antech Diagnostics (Tampa, FL). Atomic absorption (AA) measurements were made on a Perkin-Elmer model 5100 PC (Norwalk, CT).

3-(2,4-Dihydroxyphenyl)propionic Acid (3). The title compound (**3**) was prepared according to a literature method.[40] ^{1}H NMR (DMSO-d_6, 2.49) δ 2.37 (t, 2 H, J = 7.8), 2.60 (t, 2 H, J = 7.8), 6.09 (dd, 1 H, J = 8.4, 2.4), 6.24 (d, 1 H, J = 2.4), 6.78 (d, 1 H, J = 8.4), 8.96 (s, 1 H), 9.14 (br s, 1 H), 11.98 (br s, 1 H); ^{13}C NMR (DMSO-d_6, 39.50) δ 24.89, 34.15, 102.34, 105.84, 117.28, 129.94, 155.75, 156.53, 174.24.

Benzyl 3-(2,4-Dibenzyloxyphenyl)propionate (4). Activated K_2CO_3 (391 g, 2.83 mol) was added to a solution of **3** (128.8 g, 0.71 mol) and benzyl bromide (336 mL, 2.83 mol) in acetone (3 L), and the mixture was heated at reflux overnight. After the reaction mixture was cooled and filtered, the solid was rinsed with acetone. The filtrate was concentrated under reduced pressure; chromatography (hexanes, then 8:1 hexanes/EtOAc) furnished **4** (278.7 g, 87%) as a white solid: ^{1}H NMR δ 2.67 (t, 2 H, J = 7.5), 2.96 (t, 2 H, J = 7.5), 5.00 (s, 2 H), 5.03 (s, 2 H), 5.08 (s, 2 H), 6.47 (dd, 1 H, J = 8.4, 2.4), 6.57 (d, 1 H, J = 2.4), 7.04 (d, 1 H, J = 8.4), 7.34 (m, 15 H); ^{13}C NMR δ 25.66, 34.47, 66.04, 69.77, 70.15, 100.53, 105.25, 106.76, 121.67, 127.00, 127.52, 127.76, 127.96, 128.06, 128.09, 128.47, 128.53, 128.57, 130.31, 136.08, 137.00, 157.36, 158.63, 173.21; HRMS m/z calcd for $C_{30}H_{29}O_4$ 453.2066 (M + H), found 453.2054. Anal. ($C_{30}H_{28}O_4$) C, H.

3-(2,4-Dibenzyloxyphenyl)propanol (5). A solution of **4** (16.64 g, 36.77 mmol) in THF (150 mL) was added dropwise to $LiAlH_4$ (1.0 M in THF, 40.5 mL, 40.5 mmol) in THF (150 mL). After the reaction mixture was stirred overnight, H_2O (20 mL) was cautiously added. After the mixture was concentrated in vacuo, the residue was treated with 1 M HCl (150 mL) and was extracted with CH_2Cl_2 (3 × 150 mL). The organic extracts were washed with aqueous $NaHCO_3$ and brine; solvent was removed by rotary evaporation. Recrystallization from aqueous EtOH gave **5** (10.44 g, 82%) as a waxy white solid: ^{1}H NMR δ 1.59 (t, 1 H, J = 6.3), 1.83 (m, 2 H), 2.70 (t, 2 H, J = 7.5), 3.58 (q, 2 H, J = 6.3), 5.02 (s, 2 H), 5.03 (s, 2 H), 6.53 (dd, 1 H, J = 8.4, 2.4), 6.61 (d, 1 H, J = 2.4), 7.06 (d, 1 H, J = 8.4), 7.39 (m, 10 H); ^{13}C NMR δ 25.42, 33.18, 61.91, 70.16, 70.19, 100.58, 105.67, 122.93, 127.30, 127.54, 127.98, 128.00, 128.58, 128.63, 130.38, 136.84, 137.02, 157.36, 158.30; HRMS m/z calcd for $C_{23}H_{25}O_3$ 349.1804 (M + H), found 349.1872. Anal. ($C_{23}H_{24}O_3$) C, H.

3-(2,4-Dibenzyloxyphenyl)propyl *p*-Tosylate (6). *p*-Tosyl chloride (1.14 g, 6.00 mmol) in CH_2Cl_2 (20 mL) was added dropwise to **5** (1.74 g, 5.00 mmol) and pyridine (8.0 mL) in CH_2Cl_2 (40 mL). The mixture was cooled in an ice bath and was stirred at room temperature overnight. The mixture was poured into 1 N HCl (200 mL) in an ice slurry and was extracted with $CHCl_3$ (200 mL). The organic layer was washed with H_2O, aqueous $NaHCO_3$, and brine; solvent was removed in vacuo. Purification by chromatography ($CHCl_3$) provided **6** (1.93 g, 77%) as a white solid: ^{1}H NMR δ 1.92 (m, 2 H), 2.43 (s, 3 H), 2.62 (t, 2 H, J = 7.2), 4.01 (t, 2 H, J = 6.3), 4.99 (s, 2 H), 5.00 (s, 2 H), 6.44 (dd, 1 H, J = 8.4, 2.4), 6.56 (d, 1 H, J = 2.4), 6.89 (d, 1 H, J = 8.4), 7.37 (m, 12 H), 7.76 (m, 2 H); ^{13}C NMR δ 21.60, 25.80, 28.98, 69.76, 70.15, 70.20, 100.54, 105.24, 121.63, 127.00, 127.51, 127.82, 127.86, 127.97, 128.57, 129.75, 130.36, 133.21, 136.97, 144.51, 157.29, 158.51; HRMS m/z calcd for $C_{30}H_{31}O_5S$ 503.1892 (M + H), found 503.1885. Anal. ($C_{30}H_{30}O_5S$) C, H.

1-(3-Bromopropyl)-2,4-dibenzyloxybenzene (7). A mixture of **6** (4.52 g, 9.00 mmol) and LiBr (3.15 g, 36.0 mmol) in acetone (300 mL) was heated at reflux overnight. The solvent was removed under reduced pressure, and the residue was taken up in Et_2O. Treatment with H_2O and brine, solvent removal under reduced pressure, and chromatography (4:1 hexanes/EtOAc) furnished **7** (3.29 g, 89%) as a white solid: ^{1}H NMR δ 2.13 (m, 2 H), 2.76 (t, 2 H, J = 7.2), 3.38 (t, 2 H, J = 6.6), 5.01 (s, 2 H), 5.02 (s, 2 H), 6.51 (dd, 1 H, J = 8.1, 2.4), 6.59 (d, 1 H, J = 2.4), 7.07 (d, 1 H, J = 8.1), 7.40 (m, 10 H); ^{13}C NMR δ 28.36, 32.84, 33.75, 69.83, 70.17, 100.61, 105.28, 121.83, 127.08, 127.53, 127.82, 127.96, 128.53, 128.57, 130.51, 137.00, 137.04, 157.37, 158.53; HRMS m/z calcd for $C_{23}H_{23}{}^{79}BrO_2$ 410.0882 (M), found 410.0884.

1,11-Bis(2,4-dibenzyloxyphenyl)-4,8-dioxaundecane (8). Powdered KOH (86.1%, 3.01 g, 46.2 mmol) was added to 1,3-propanediol (1.02 g, 13.4 mmol) in DMSO (50 mL). After the mixture was stirred vigorously for 0.5 h, **7** (11.0 g, 26.8 mmol) was added. The reaction mixture was heated at 50 °C for 0.5 h and then was stirred at room temperature overnight. The mixture was poured into ice-cold brine (500 mL) and extracted with toluene (3 × 200 mL). The organic portion was washed with brine (2 × 500 mL) and was concentrated under reduced pressure. Chromatography (4:1 hexanes/EtOAc) afforded **8**

(5.78 g, 58%) as a yellow oil: ^{1}H NMR δ 1.84 (m, 6 H), 2.67 (t, 4 H, J = 7.5), 3.41 (t, 4 H, J = 6.6), 3.46 (t, 4 H, J = 6.3), 4.99 (s, 4 H), 5.01 (s, 4 H), 6.49 (dd, 2 H, J = 8.1, 2.4), 6.58 (d, 2 H, J = 2.4), 7.04 (d, 2 H, J = 8.1), 7.39 (m, 20 H); ^{13}C NMR δ 26.31, 29.85, 30.20, 67.71, 69.75, 70.13, 70.42, 100.51, 105.16, 123.35, 127.00, 127.54, 127.69, 127.91, 128.48, 128.54, 130.18, 137.09, 137.23, 157.35, 158.18; HRMS *m/z* calcd for $C_{49}H_{53}O_6$ 737.3842 (M + H), found 737.3819.

1,11-Bis(2,4-dibenzyloxy-5-formylphenyl)-4,8-dioxaundecane (9). Phosphorus oxychloride (5.808 g, 37.88 mmol) in CH_3CN (80 mL) was added dropwise to DMF (3.251 g, 44.47 mmol) and CH_3CN (16 mL), and the mixture was stirred at room temperature for 1 h. Compound **8** (12.14 g, 16.47 mmol) in CH_3CN (80 mL) was slowly added. The reaction mixture was stirred at room temperature for 1 h, refluxed overnight, and concentrated under reduced pressure. The residue was treated with H_2O (100 mL) and 1,4-dioxane (100 mL), heated at 50 °C for 2 h, and concentrated in vacuo. The residue was dissolved in EtOAc (500 mL), washed with brine (500 mL), and concentrated by rotary evaporation. Chromatography (2:1 hexanes/EtOAc) gave **9** (7.96 g, 61%) as a white solid: ^{1}H NMR δ 1.82 (m, 6 H), 2.65 (t, 4 H, J = 7.2), 3.40 (t, 4 H, J = 6.3), 3.44 (t, 4 H, J = 6.3), 5.09 (s, 4 H), 5.10 (s, 4 H), 6.49 (s, 2 H), 7.38 (m, 20 H), 7.65 (s, 2 H), 10.36 (s, 2 H); ^{13}C NMR δ 26.16, 29.46, 30.18, 67.75, 70.18, 70.32, 70.79, 97.20, 118.56, 124.08, 126.98, 127.21, 128.14, 128.24, 128.71, 129.48, 136.14, 161.59, 162.78, 188.23; HRMS *m/z* calcd for $C_{51}H_{53}O_8$ 793.3740 (M + H), found 793.3815.

1,11-Bis(5-cyano-2,4-dibenzyloxyphenyl)-4,8-dioxaundecane (10). A solution of **9** (20.42 g, 25.8 mmol), hydroxylamine hydrochloride (3.95 g, 56.8 mmol), and Et_3N (6.26 g, 61.9 mmol) in CH_3CN (500 mL) was stirred at 45 °C overnight. Phthalic anhydride (11.5 g, 77.4 mmol) was added, and the mixture was heated at reflux overnight. After the solution was concentrated under reduced pressure, the residue was diluted with CH_2Cl_2 (600 mL) and washed with aqueous $NaHCO_3$ (600 mL) and brine (600 mL). Solvent removal and chromatography (3:1 hexanes/EtOAc) afforded **10** (15.64 g, 77%) as a white solid: ^{1}H NMR δ 1.80 (m, 6 H), 2.62 (t, 4 H, J = 7.5), 3.38 (t, 4 H, J = 6.3), 3.45 (t, 4 H, J = 6.3), 5.03 (s, 4 H), 5.12 (s, 4 H), 6.47 (s, 2 H), 7.28 (s, 2 H), 7.36 (m, 20 H); ^{13}C NMR δ 26.04, 29.24, 30.12, 67.71, 70.01, 70.14, 70.87, 93.46, 97.96, 117.09, 124.18, 126.95, 128.17, 128.20, 128.71, 133.98, 135.80, 135.88, 160.58, 160.93; HRMS *m/z* calcd for $C_{51}H_{51}N_2O_6$ 787.3747 (M + H), found 787.3745.

1,11-Bis(5-cyano-2,4-dihydroxyphenyl)-4,8-dioxaundecane (11). Palladium on activated carbon (10%, 3.14 g) was added to a solution of **10** (5.23 g, 6.65 mmol) in EtOAc (500 mL) and iron-free EtOH (100 mL), and the suspension was stirred under H_2 (1 atm) at room temperature for 5.5 h. The reaction mixture was heated on a steam bath and was filtered through Celite. The filtrate was concentrated in vacuo; chromatography (20:3 $CHCl_3/CH_3OH$) gave **11** (2.55 g, 90%) as a white solid: ^{1}H NMR (DMSO-d_6, 2.49) δ 1.69 (m, 6 H), 2.42 (t, 4 H, J = 7.5), 3.30 (t, 4 H, J = 6.6), 3.38 (t, 4 H, J = 6.6), 6.47 (s, 2 H), 7.17 (s, 2 H), 10.30 (s, 2 H), 10.54 (s, 2 H); ^{13}C NMR (DMSO-d_6, 39.50): δ 25.34, 28.99, 29.71, 67.06, 69.50, 88.82, 102.11, 117.97, 120.72, 133.40, 159.94, 160.60; HRMS *m/z* calcd for $C_{23}H_{27}N_2O_6$ 427.1869 (M + H), found 427.1845.

(*S*,*S*)-1,11-Bis[5-(4-carboxy-4,5-dihydrothiazol-2-yl)-2,4-dihydroxyphenyl]-4,8-dioxaundecane (2). Distilled solvents and glassware that had been presoaked in 3 N HCl for 15 min were employed. D-Cysteine hydrochloride monohydrate (1.23 g, 7.02 mmol) was added to **11** (1.00 g, 2.34 mmol) in degassed CH_3OH (20 mL) and 0.1 M phosphate buffer at pH 6.0[52] (15 mL). Sodium bicarbonate (0.590 g, 7.02 mmol) was carefully added, and the mixture was stirred at reflux for 2 days. The reaction mixture was concentrated under reduced pressure, H_2O was added, and the pH was adjusted to 2 by addition of 10% citric acid solution. Solid was filtered and recrystallized from aqueous EtOH to furnish **2** (0.81 g, 55%) as a beige powder: $[\alpha]^{24}_D$ +3.1 (*c* 1.06, DMF); ^{1}H NMR (DMSO-d_6, 2.49) δ 1.72 (m, 6 H), 2.48 (t, 4 H, J = 7.2), 3.32 (t, 4 H, J = 6.3), 3.41 (t, 4 H, J = 6.3), 3.54 (dd, 1 H, J = 7.2, 11.1), 3.61 (dd, 1 H, J = 9.3, 11.1), 5.34 (dd, 2 H, J = 7.2, 9.3), 6.36 (s, 2 H), 7.03 (s, 2 H), 10.24 (br s, 2 H), 12.45 (br s, 2 H), 13.04 (br s, 2 H); ^{13}C NMR (DMSO-d_6, 39.50) δ 25.50, 29.19, 29.78, 33.15, 67.17, 69.25, 75.91, 102.00, 107.64, 120.11, 131.49, 158.55, 160.17, 171.57, 171.95; HRMS *m/z* calcd for $C_{29}H_{35}N_2O_{10}S_2$ 635.1733 (M + H), found 635.1696. Anal. ($C_{29}H_{34}N_2O_{10}S_2$) H, N. C: calcd, 54.88; found, 54.17.

Prevention of Iron-Mediated Oxidation of Ascorbate. The iron chelators (NTA, L1, DFO, **1**, and **2**) were tested for their ability to diminish the iron-mediated oxidation of ascorbate by the method of Dean and Nicholson.[27] Briefly, a solution of freshly prepared ascorbate (100 μM) in sodium phosphate buffer (5 mM, pH 7.4) was incubated in the presence of $FeCl_3$ (30 μM) and chelator (ligand/Fe ratios varied from 0 to 3) for 40 min. The A_{265} was read at 10 and 40 min; the ΔA_{265} in the presence of ligand was compared to that in its absence.

Quenching of the ABTS Radical Cation. The iron chelators were tested for their ability to quench the radical cation formed from 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) by a published method.[47] Briefly, a stock solution of ABTS radical cation was generated by mixing ABTS (10 mM, 2.10 mL) with $K_2S_2O_8$ (8.17 mM, 0.90 mL) in H_2O and allowing the solution to sit in the dark at room temperature for 18 h. This stock solution of deep blue-green ABTS radical cation was diluted in sufficient sodium phosphate (10 mM, pH 7.4) to give an A_{734} of about 0.900. Test compounds were added to a final concentration ranging from 1.25 to 15 μM, and the decrease in A_{734} was read after 1, 2, 4, and 6 min. Assays were performed in triplicate at each concentration. The reaction was largely complete by 1 min, but the data presented are based on a 6 min reaction time.

Stoichiometry of the Ligand/Fe(III) Complex. The stoichiometry of the complex was determined spectrophotometrically for **2** at the λ_{max} (529 nm) of the visible absorption band of the ferric complex by the method given in detail in an earlier publication.[35] The Job's plot for mixtures containing various ratios of ligand to Fe(III) NTA ([ligand] + [Fe] = 1.00 mM constant) was then derived.

Cannulation of Bile Duct in Rats. The cannulation has been described previously.[48,53,54] Briefly, male Sprague−Dawley rats averaging 450 g were housed in Nalgene plastic metabolic cages during the experimental period and given free access to water. The animals were anesthetized using sodium pentobarbital (55 mg/kg) administered ip. The bile duct was cannulated using 22 gauge polyethylene tubing. The cannula was inserted into the duct about 1 cm from the duodenum and tied snugly in place. After threading through the shoulder, the cannula was passed from the rat to the swivel inside a metal torque-transmitting tether, which was attached to a rodent jacket around the animal's chest. The cannula was directed from the rat to a Gilson microfraction collector (Middleton, WI) by a fluid swivel mounted above the metabolic cage. Bile samples were collected at 3 h intervals for 24 h. The urine sample was taken at 24 h. Sample collection and handling were as previously described.[48]

Iron Loading of *C. apella* Monkeys. The monkeys were iron-overloaded with iv iron dextran as previously described to provide about 500 mg of iron per kg of body weight;[49] the serum transferrin iron saturation rose to 70−80%. We waited at least 20 half-lives, 60 days,[55] before using any of the animals in experiments evaluating iron-chelating agents.

Primate Fecal and Urine Samples. Fecal and urine samples were collected at 24 h intervals and processed as given in detail in earlier publications.[51,53,56] Briefly, the collections began 4 days prior to the administration of the test drug and continued for an additional 5 days after the drug was given. Iron concentrations were determined by flame atomic absorption spectroscopy as accounted previously.[54,56]

Drug Preparation and Administration. The iron chelators were solubilized in 40% Cremophor RH-40/water (v/v) and given po and sc to the rats at the doses shown in Table 2. In the primates, DFO was dissolved in sterile H_2O at a concentration of 50 or 100 mg/mL and given po or sc at a volume of 1 mL/kg. The desferrithiocin analogues were solubilized in 40%

Cremophor RH-40/water (v/v) and given po to the monkeys at the doses shown in Table 2. However, because the Cremophor solution could not be sterilized prior to parenteral administration, the desferrithiocin analogues were administered sc either as a suspension in distilled H_2O or as their sodium salts in solution as indicated in Table 2.

Calculation of Iron Chelator Efficiency. The theoretical outputs of the chelators were generated on the basis of a 1:1 ligand/iron complex for DFO and **2** and on the basis of a 2:1 complex for **1**.[35] The efficiencies in the rodents and monkeys were calculated as set forth previously.[35,36,50] Data are presented as the mean ± the standard error of the mean.

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Supporting Information Available: Scheme of failed attempts to (1) couple an alkoxide-generated anion of **5** with 1,3-dihalopropanes and a ditosylate and to (2) couple the iodide, bromide, chloride, or tosylate of **5** with a dialkoxide of 1,3-dihydroxypropane. This material is available free of charge via the Internet at http://pubs.acs.org.

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