

## Expedited Articles

### Synthesis and Biological Evaluation of Naphthyldesferrithiocin Iron Chelators

Raymond J. Bergeron,\* Jan Wiegand, Markus Wollenweber, James S. McManis, Samuel E. Algee, and Katie Ratliff-Thompson

Department of Medicinal Chemistry, University of Florida, Gainesville, Florida 32610

Received November 28, 1995<sup>®</sup>

The synthesis and iron-clearing properties of the naphthyldesferrithiocins 2-(2'-hydroxynaphth-1'-yl)- $\Delta^2$ -thiazoline-(4*R*)-carboxylic acid, 2-(2'-hydroxynaphth-1'-yl)- $\Delta^2$ -thiazoline-(4*S*)-carboxylic acid, 2-(3'-hydroxynaphth-2'-yl)- $\Delta^2$ -thiazoline-(4*R*)-carboxylic acid, and 2-(3'-hydroxynaphth-2'-yl)- $\Delta^2$ -thiazoline-(4*S*)-carboxylic acid are described. While the bile duct-cannulated rat model clearly demonstrates that the 3'-hydroxynaphthyl-2'-yl compounds are orally active iron-clearing agents and the corresponding 2'-hydroxynaphthyl-1'-yl compounds are not, in the primate model none of the benz-fused desazadesferrithiocin analogues are active. Oral versus subcutaneous administration of these ligands strongly suggests that metabolism is a key issue in their iron-clearing properties and that these benz-fused desferrithiocins are not good candidates for orally active iron-clearing drugs.

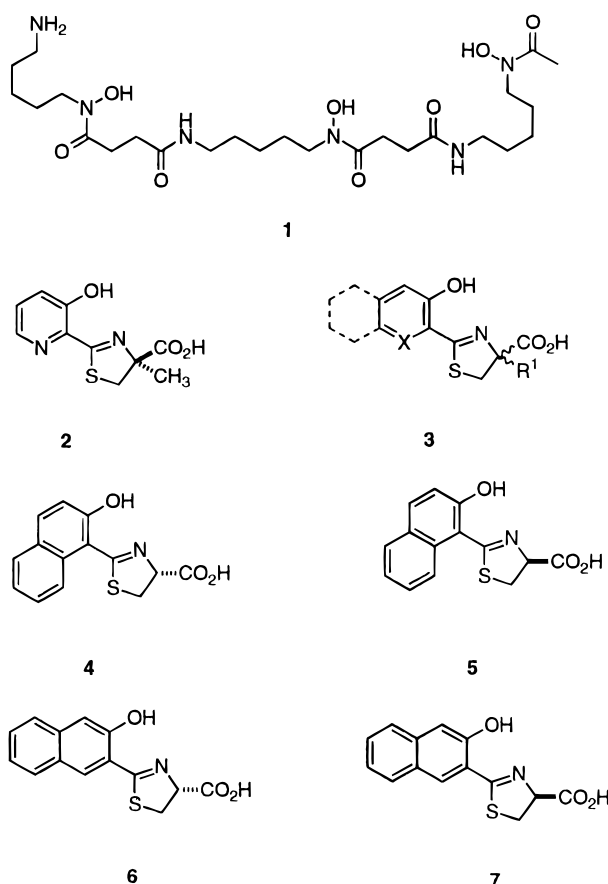
#### Introduction

Perhaps it is not too surprising that iron, a metal that comprises 5% of the earth's crust and can serve as both an electron source and an electron sink, finds such a key role in life processes. This transition metal is essential in many biologic redox reactions, serving as a prosthetic group in a variety of key enzymes, e.g., oxidases, catalase, and ribonucleotide reductase, as well as in the oxygen transport protein hemoglobin.

While many organisms are auxotrophic for Fe(III), because of the insolubility of the hydroxide ( $K_{sp} = 1 \times 10^{-38}$ ),<sup>1</sup> its predominant form in the environment, virtually all life forms have developed rather sophisticated iron storage and transport systems. Microorganisms utilize low molecular weight ligands, siderophores, while eukaryotes tend to employ proteins to transport iron, e.g., transferrin,<sup>2</sup> and store iron, e.g., ferritin, in order to overcome iron's insolubility.

Iron utilization in primates is characterized by a highly efficient recycling process,<sup>3–6</sup> with no specific mechanism for eliminating this transition metal. Because it cannot be effectively cleared, the introduction of "excess iron"<sup>7–9</sup> leads to iron overload, hemochromatosis, and ultimately peroxidative tissue damage. Although hemochromatosis has received considerable attention from a variety of scientific disciplines,<sup>10–12</sup> treatment remains essentially unchanged. Patients with primary hemochromatosis are still managed by phlebotomy, whereas those suffering from hemochromatosis secondary to blood transfusions must be maintained on chelation therapy.

Subcutaneous infusion of desferrioxamine B (DFO) (**1**) (Figure 1), a hexacoordinate hydroxamate iron chelator produced by *Streptomyces pilosus*,<sup>13</sup> is still regarded as the treatment of choice for handling transfusional iron overload.<sup>14–18</sup> DFO forms a 1:1 hexacoordinate octahedral complex in situ with Fe(III) with a formation constant<sup>19</sup> of  $3 \times 10^{30} \text{ M}^{-1}$ . If one assumes that the



**Figure 1.** Structure of the the desferrithiocin pharmacophore **3** and the compounds chosen for evaluation: DFO (**1**), (*S*)-desferrithiocin (**2**), 2-(2'-hydroxynaphth-1'-yl)- $\Delta^2$ -thiazoline-(4*R*)-carboxylic acid (**4**), 2-(2'-hydroxynaphth-1'-yl)- $\Delta^2$ -thiazoline-(4*S*)-carboxylic acid (**5**), 2-(3'-hydroxynaphth-2'-yl)- $\Delta^2$ -thiazoline-(4*R*)-carboxylic acid (**6**), and 2-(3'-hydroxynaphth-2'-yl)- $\Delta^2$ -thiazoline-(4*S*)-carboxylic acid (**7**).

same stoichiometry applies when DFO is administered to an animal, 10% or less of the theoretical iron excretion is observed.<sup>20</sup> This situation is further complicated by the fact that DFO is not orally active and,

<sup>®</sup> Abstract published in *Advance ACS Abstracts*, March 15, 1996.

when administered subcutaneously, has a very short half-life in the body and must therefore be given by continuous infusion over long periods of time. For these reasons, patient compliance is a serious problem,<sup>21</sup> a situation that could be solved with an orally active drug.

Although a substantial number of synthetic iron chelators have been studied in recent years as potential orally active therapeutics, e.g., pyridoxyl isonicotinoyl hydrazone (PIH),<sup>22</sup> hydroxypyridones,<sup>23,24</sup> and *N,N*-bis-(*o*-hydroxybenzyl)ethylenediamine-*N,N*-diacetic acid (HBED) analogues,<sup>25</sup> none have yet proven to be completely satisfactory. Interestingly, the siderophores have remained relatively untouched in this search. Their evaluation as iron-clearing agents has not at all paralleled the rate of their isolation and structural elucidation. In fact, until recently, beyond DFO, only two of some 100 siderophores identified have been studied in animal models: enterobactin<sup>26</sup> and rhodotorulic acid.<sup>27</sup> Unfortunately, both of these cyclic siderophores exhibited unacceptable toxicity, and neither possessed any oral activity. They were abandoned, as there were any number of synthetic chelators with equally unsatisfactory properties from which to choose. The siderophores thus became *persona non grata* as potential clinical deferration agents.

While most siderophores fall primarily into two structural classes, hydroxamates or catecholamides,<sup>28,29</sup> there are a number of compounds that do not belong to either family, e.g., pyochelin,<sup>30</sup> rhizobactin,<sup>31</sup> and 2-(3'-hydroxypyrid-2'-yl)-4-methyl- $\Delta^2$ -thiazoline-(4*S*)-carboxylic acid (desferrithiocin) (DFT) (**2**) (Figure 1). DFT, isolated from *Streptomyces antibioticus*,<sup>32</sup> was shown to form a stable 2:1 complex with iron ( $K_f = 4 \times 10^{29} \text{ M}^{-1}$ ).<sup>33</sup> Studies in rodents focused on the reduction of liver ferritin by DFT,<sup>34</sup> and a preliminary investigation in primates<sup>35</sup> suggested that it was indeed an orally active iron chelator.

A more comprehensive investigation in our laboratory carried out in a non-iron-overloaded, bile duct-cannulated rat model<sup>36</sup> as well as an iron-overloaded Cebus monkey model<sup>20,37</sup> supported these findings and identified the magnitude of the drug's effectiveness. The acid form of DFT was shown to be more efficient when given orally than DFO given sc. It was far superior to any of the numerous hydroxypyridones (including CP20 and CP94) and PIH analogues that we have investigated.<sup>20,38</sup> Also, the sodium salt of DFT (po) cleared iron more efficiently from the primates than DFO (sc). Unfortunately, animals exposed to the drug chronically presented with nephrotoxicity.<sup>37</sup> Nevertheless, the chelator's remarkable gastrointestinal (GI) absorption and iron-clearing efficiency underscore the idea that the DFT skeleton represents an excellent pharmacophore (**3**, Figure 1) on which to predicate the design of orally effective iron chelators. However, it was first necessary to identify the minimal structural components of DFT required for iron clearance in animals when the drug is given orally.

## Design Concepts

On the basis of X-ray studies of the Cr(III)-DFT complex, the chelator likely forms a pair of diastereomeric 2:1 complexes with Fe(III).<sup>39</sup> The three ligating centers in both diastereomers are the aromatic hydroxyl, the thiazoline nitrogen, and the carboxyl group. Any structural alterations that compromise the ability of

these functional groups to coordinate with iron should significantly reduce the compound's iron-clearing properties. This is in keeping with the observations from these laboratories that removal of the desferrithiocin hydroxyl or conversion of the carboxyl to the corresponding methyl ester resulted in inactive compounds.<sup>36</sup> Although modification of some of the nonchelating fragments, such as removal of the thiazoline methyl or the aromatic nitrogen, had little impact on the deferration properties of the resulting molecules, the replacement of the sulfur with oxygen or expansion of the five-membered thiazoline to a six-membered  $\Delta^2$ -thiazine resulted in compounds with little iron-clearing activity.<sup>40</sup>

These last observations somewhat complicate design concepts associated with alterations in the nonchelating fragments of the molecule. This becomes somewhat problematic if one would like to introduce functionality into the desferrithiocin molecule that would alter its membrane transport, volume of distribution, or iron clearance properties. These kinds of changes are generally associated with changing a molecule's lipophilic properties. As no evidence was available regarding how the introduction of substituents at the aromatic ring, as illustrated by general structure **3**, would affect the molecule's iron-clearing properties, we elected to synthesize naphthyl analogues of desferrithiocin (Figure 1): 2-(2'-hydroxynaphth-1'-yl)- $\Delta^2$ -thiazoline-(4*R*)-carboxylic acid (**4**), 2-(2'-hydroxynaphth-1'-yl)- $\Delta^2$ -thiazoline-(4*S*)-carboxylic acid (**5**), 2-(3'-hydroxynaphth-2'-yl)- $\Delta^2$ -thiazoline-(4*R*)-carboxylic acid (**6**), and 2-(3'-hydroxynaphth-2'-yl)- $\Delta^2$ -thiazoline-(4*S*)-carboxylic acid (**7**). Clearly, the impact of such alterations on iron chelation should be minimal.

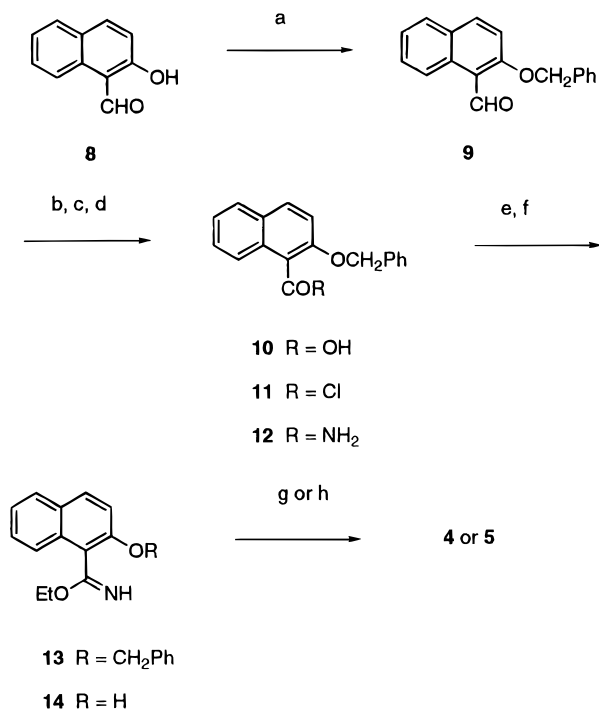
## Synthetic Methods

Since cysteine reacted poorly with 2-hydroxy-1-naphthonitrile, synthesis of the naphthyl DFTs **4** and **5** (Scheme 1) was thus dependent on accessing the intermediate ethyl imidate **14**,<sup>36,40</sup> which was then condensed with L- or D-cysteine to produce the respective thiazolines **4** or **5**. The thiazolines **6** and **7** were generated via condensation of the requisite cysteine with naphthyl nitrile **20** (Scheme 2).

Ethyl 2-hydroxy-1-naphthimidate (**14**) (Scheme 1) was assembled in six steps in an overall yield of 42% starting from 2-hydroxy-1-naphthaldehyde (**8**). The hydroxyl of aldehyde **8** was alkylated (benzyl chloride/ $\text{K}_2\text{CO}_3$ , EtOH, reflux, 39 h), providing benzyl ether **9**.<sup>41</sup> The aldehyde function of **9** was oxidized<sup>42</sup> to acid **10**<sup>41</sup> with  $\text{NaClO}_2$  and sulfamic acid in aqueous acetone at 0 °C for 50 min. Conversion of **10** to acid chloride **11** (oxalyl chloride/toluene/DMF, 25 °C, 1 h) and treatment with concentrated  $\text{NH}_4\text{OH}/\text{CH}_2\text{Cl}_2$  at room temperature for 1 day afforded amide **12**. The amide was smoothly converted to the ethyl imidate **13** with Meerwein's salt ( $\text{Et}_3\text{O}^+/\text{PF}_6^-/\text{CH}_2\text{Cl}_2$ ) for 1 day at room temperature. The benzyl protecting group of **13** was removed by hydrogenolysis ( $\text{Pd}-\text{C}/\text{ethanol}$ ) to yield the key intermediate **14**.<sup>43</sup> Cyclocondensation of **14** with L- or D-cysteine in refluxing methanol for 2 days generated iron chelator **4** or **5**, respectively, as the free acid.

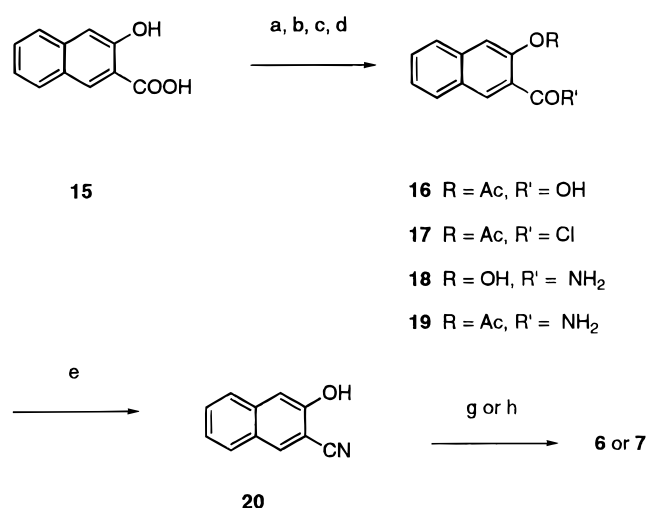
The starting material for the synthesis of the naphthyl DFTs **6** and **7** was 3-hydroxy-2-naphthoic acid (**15**) (Scheme 2). The hydroxyl group was acylated with

**Scheme 1.** Synthesis of (*R*)- and (*S*)-2-(2'-Hydroxynaphth-1'-yl)- $\Delta^2$ -thiazoline-4-carboxylic Acids (**4** and **5**)<sup>a</sup>



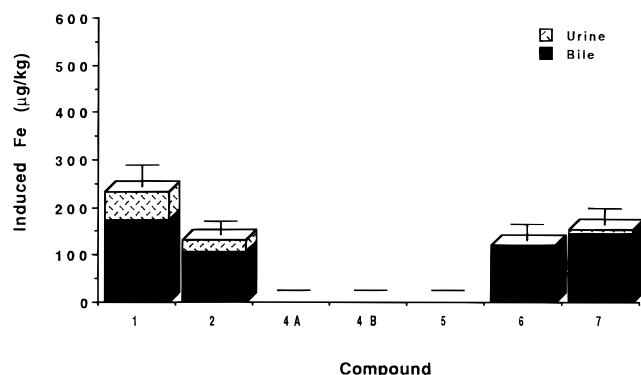
<sup>a</sup> Reagents: (a) PhCH<sub>2</sub>Cl/K<sub>2</sub>CO<sub>3</sub>/EtOH; (b) NaClO<sub>2</sub>/H<sub>2</sub>NSO<sub>3</sub>H/aqueous acetone; (c) (COCl)<sub>2</sub>/PhCH<sub>3</sub>/DMF (catalytic); (d) concentrated NH<sub>4</sub>OH/CH<sub>2</sub>Cl<sub>2</sub>; (e) Et<sub>3</sub>O<sup>+</sup>PF<sub>6</sub><sup>-</sup>/CH<sub>2</sub>Cl<sub>2</sub>; (f) H<sub>2</sub>/10% Pd-C/EtOH/1 atm; (g) L-cysteine/CH<sub>3</sub>OH/reflux/2 days; (h) D-cysteine/CH<sub>3</sub>OH/reflux/2 days.

**Scheme 2.** Synthesis of (*R*)- and (*S*)-2-(3'-Hydroxynaphth-2'-yl)- $\Delta^2$ -thiazoline-4-carboxylic Acids (**6** and **7**)<sup>a</sup>



<sup>a</sup> Reagents: (a) Ac<sub>2</sub>O/concentrated H<sub>2</sub>SO<sub>4</sub>/reflux/5 min; (b) (COCl)<sub>2</sub>/PhCH<sub>3</sub>/DMF (catalytic); (c) concentrated NH<sub>4</sub>OH/CH<sub>2</sub>Cl<sub>2</sub>; (d) Ac<sub>2</sub>O/pyr; (e) SOCl<sub>2</sub>/reflux; (g) L-cysteine/phosphate buffer (pH 5.95)/CH<sub>3</sub>OH/60 °C/1 day; (h) D-cysteine/phosphate buffer (pH 5.95)/CH<sub>3</sub>OH/60 °C/2 days.

acetic anhydride (concentrated H<sub>2</sub>SO<sub>4</sub>, reflux, 5 min) giving **16**. The acid was converted to the corresponding acid chloride **17** (oxalyl chloride/toluene/DMF, 25 °C, 1 h); reaction with concentrated NH<sub>4</sub>OH (CH<sub>2</sub>Cl<sub>2</sub>, 25 °C, 24 h) resulted in ester-cleaved amide **18**. The free hydroxyl was reacylated to **19** (Ac<sub>2</sub>O/pyridine, 25 °C, 30 min). Dehydration of amide **19** and concomitant cleavage of the acetyl group using SOCl<sub>2</sub> at reflux for 2



**Figure 2.** Chelator-induced iron excretion in the urine and bile of the non-iron-overloaded, bile duct-cannulated rats given **1** (sc), **2** (po), **4A** (po), **4B** (sc), and **5–7** (po). All of the ligands were dosed at 150 μmol/kg and administered in 40% Cremophor RH-40. The iron excretion (treated – control) is reported in μg of iron excreted/kg of rat weight.

h furnished key nitrile intermediate **20**. Compound **20** underwent cyclization with either L- or D-cysteine (methanolic 0.1 M phosphate buffer, pH 5.95, 60 °C, 1–2 days) to provide naphthyl chelator **6** or **7**, respectively.

The nature of the iron(III) complexes of the naphthyl DFTs **4** and **6** in solution was studied by Job's method<sup>44,45</sup> of continuous variation of isomolar ligand and iron(III) solutions. Job's plots of **4** and **6** revealed that both of the DFT analogues form a 2:1 complex with iron(III) at pH 7.2, as is expected for a tridentate ligand.

## Iron Clearance Studies

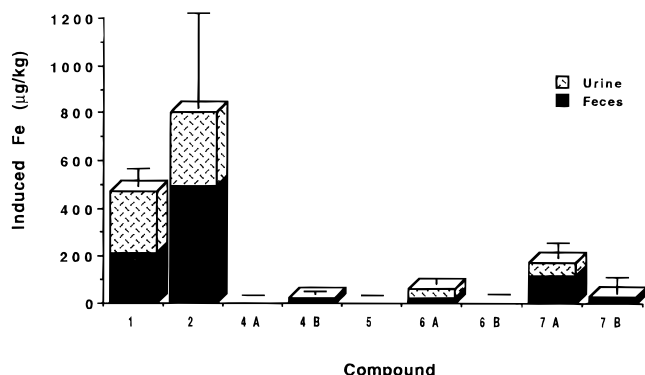
### Chelator-Induced Iron Clearance in Rodents.

The non-iron-overloaded, bile duct-cannulated rat model<sup>36–38</sup> represents a very useful and rapid initial screen of potential iron chelators. The procedures employed in this model allow us to both measure the rate at which various chelators induce iron clearance in the bile and urine as well as determine the total amount of iron cleared. While the interruption of enterohepatic circulation caused by cannulation of the bile duct may lead to an overestimation of fecal iron excretion, if the ligand of interest does not induce iron clearance in the bile or the urine, then additional investigations are unnecessary.

In previous studies,<sup>20,38</sup> we demonstrated that the iron-clearing efficiency of DFO (**1**) dosed at 150 μmol/kg sc in the rats was 2.8 ± 0.7%, while the efficiency of the sodium salt of **2** administered orally at 150 μmol/kg was 2.5 ± 1.2%. The mode of excretion for both ligands was similar, with the majority of the iron being excreted in the bile, 82% and 80%, respectively.

In the current study, only the 2-(3'-hydroxynaphth-2'-yl)- $\Delta^2$ -thiazoline-(4*R*)-carboxylic acid (**6**) and 2-(3'-hydroxynaphth-2'-yl)- $\Delta^2$ -thiazoline-(4*S*)-carboxylic acid (**7**) analogues administered orally to the rats at a dose of 150 μmol/kg were able to induce the excretion of iron above the base-line levels (Figure 2). The efficiencies of the two enantiomers were within error of each other, 2.89 ± 1.31% for naphthyl DFT **6** and 3.67 ± 1.05% for analogue **7** (*P* > 0.43). The mode of iron excretion for the two analogues was similar, with the majority of the iron being excreted in the bile, 100% and 95%, respectively.

While the (*R*)- and (*S*)-3-hydroxynaphth-2-yl desferithiocins were found to be orally effective iron chelators,



**Figure 3.** Chelator-induced iron excretion in the urine and feces of the iron-overloaded Cebus monkeys given **1** (sc), **2** (po), **4A** (po), **4B** (sc), **5** (po), **6–7A** (po), and **6–7B** (sc). DFO (**1**) and **2** were dosed at 150  $\mu\text{mol/kg}$ , while the naphthyl-desferrithiocins **4–7** were given at a dose of 300  $\mu\text{mol/kg}$ . Sterile water for injection was used as a vehicle for DFO, while 40% Cremophor RH-40 was used as a vehicle for **2** and **4–7**. The iron excretion is reported in  $\mu\text{g}$  of iron excreted/kg of monkey weight.

the 2-hydroxynaphth-1-yl isomers **4** and **5** were ineffective when they were administered by this route (Figure 2). In addition, even when naphthyl DFT **4** was administered subcutaneously, the analogue was still unable to induce the excretion of iron above that of the control animals (Figure 2).

**Chelator-Induced Iron Clearance in Primates.** The Cebus monkeys responded differently to the ligands than did the rats. The variability in ligand-induced iron clearance was higher in the monkeys than in the rats. However, with each animal serving as its own control, the effectiveness of the chelators could be readily compared. In an earlier study,<sup>37</sup> we demonstrated that the iron-clearing efficiency of DFO (**1**) administered sc to the iron-loaded primates was  $5.5 \pm 0.9\%$ , while the efficiency of the sodium salt of **2** given orally was  $18.6 \pm 9.3\%$ . The mode of drug-induced iron excretion for the two compounds was different. For DFO, the majority of the induced iron was in the urine, 55%, while the majority of the iron induced by the sodium salt of **2** was excreted in the feces, 62%.

In the current study, none of the naphthyl desferrithiocin analogues were particularly effective iron chelators in the primate model after oral or sc administration (Figure 3). In addition, although **1** and the sodium salt of **2** were able to hold the monkeys in a significant negative iron balance, none of the naphthyl analogues tested were able to do so (Table 1).

## Discussion

In a prior study we demonstrated that, although formal reduction of the desazadesmethyldesferrithiocin thiazoline to a thiazolidine, expansion of the desmethyldesferrithiocin thiazoline to a thiazine, or substitution of the thiazoline sulfur of desazadesmethyldesferrithiocin by an oxygen led to a substantial loss of activity of the desferrithiocin pharmacophore, the conversion of (*S*)-desmethyldesferrithiocin to an *N*-methylhydroxamate or a hexacoordinate dihydroxamate ligand resulted in active compounds.<sup>40</sup> However, the impact of adding substituents to the aromatic ring of the desferrithiocins has not been investigated. This is an important issue if one intends to alter the lipophilic properties, volume of distribution, biological half-life, etc., of this ligand.

In the current study, we assessed the impact of benz fusion on desferrithiocin-induced iron clearance.

The results of this study clearly indicate that, although the naphthyl DFTs **6** and **7** were effective iron-clearing agents after oral administration to the rats, their positional isomers **4** and **5** were not. The ineffectiveness of the latter enantiomeric pair may be due to steric hindrance from the unsubstituted aromatic ring. Furthermore, since none of the benz-fused desferrithiocins investigated were effective iron chelators in the primates, the bile duct-cannulated rat model does not predict how a primate will respond to an iron chelator. On the basis of simple coordination-chemistry considerations, obviously the interspecies difference makes little sense. The compounds' lack of iron-clearing activity in the iron-loaded monkeys, even after sc administration, is most likely due to the metabolism of the ligands. We are currently preparing radiolabeled naphthyl DFT analogues to further study this concept.

## Experimental Section

Chemical reagents were purchased from Aldrich Chemical Co. Distilled solvents and glassware that had been presoaked in 3 N HCl for 15 min were employed for reactions involving chelators. Fisher Optima grade solvents were routinely used. Silica gel 32–63 (40  $\mu\text{M}$  "flash") from Selecto, Inc. (Kennesaw, GA) or silica gel 60 (70–230 mesh) from EM Science (Darmstadt, Germany) was used for column chromatography. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Proton NMR spectra were run at 300 MHz in  $\text{CDCl}_3$  or the indicated solvent with chemical shifts given in parts per million downfield from tetramethylsilane. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA. Cremophor RH-40 was obtained from BASF, Parsippany, NJ. Sprague–Dawley rats were purchased from Charles River, Wilmington, MA. Nalgene metabolic cages, rat jackets, and fluid swivels were purchased from Harvard Bioscience, South Natick, MA. Intramedic polyethylene tubing (PE 50) was obtained from Fisher Scientific, Pittsburgh, PA.

**2-(2'-Hydroxynaphth-1'-yl)- $\Delta^2$ -thiazoline-(4*R*)-carboxylic Acid (**4**).** A mixture of **14** (10.11 g, 47.0 mmol) and L-cysteine (11.39 g, 94.0 mmol) in methanol (670 mL) was heated under reflux for 46 h under nitrogen. The mixture was filtered and the filtrate concentrated. The residue was taken up in acetone (100 mL). The precipitated ammonium salt of **4** was filtered off, washed with acetone (100 mL), and then taken up in 0.5 N hydrochloric acid (200 mL). The mixture was extracted with ethyl acetate ( $3 \times 150$  mL). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated, providing 3.86 g (30%) of **4** as yellow crystals, mp 150–151  $^\circ\text{C}$ . IR (KBr): 1720 ( $\text{C=O}$ ), 1615 ( $\text{C=C}$ ), 1460 ( $\text{C-H}$ )  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  8.13 (d, 1 arom H), 7.92 (d, 1 arom H), 7.85 (d, 1 arom H), 7.50 (m, 1 arom H), 7.36 (m, 1 arom H), 7.23 (d, 1 arom H), 5.45 (dd, 1 CH), 3.82 (dd,  $1/2$   $\text{CH}_2$ ,  $J_{\text{gem}} = 12$  Hz,  $J_{\text{vic}} = 9$  Hz), 3.73 (dd,  $1/2$   $\text{CH}_2$ ,  $J_{\text{vic}} = 8$  Hz).  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  8.25 (d, 1 H,  $J = 9$  Hz), 7.78–7.87 (m, 2 H), 7.46–7.51 (m, 1 H), 7.32–7.36 (m, 1 H), 7.15 (d, 1 H,  $J = 9$  Hz), 5.45 (dd, 1 H), 3.77–3.90 (m, 2 H).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  173.9 ( $\text{C=N}^*$ ), 173.6 ( $\text{C=O}^*$ ), 157.8 (arom CO), 134.4 (arom CH), 133.4 (arom C), 129.7 (arom CH), 129.6 (arom C), 128.4 (arom CH), 124.6 (arom CH), 124.5 (arom CH), 119.7 (arom CH), 111.9 (arom C), 76.4 (CH), 36.3 ( $\text{CH}_2$ ) (\* = interchangeable). MS (CI,  $\text{NH}_3$ ):  $m/z$  (%) = 274 [ $\text{M} + 1$ ] (75), 187 (100). Anal. ( $\text{C}_{14}\text{H}_{11}\text{NO}_3\text{S}$ ) C, H, N, S.

**2-(2'-Hydroxynaphth-1'-yl)- $\Delta^2$ -thiazoline-(4*S*)-carboxylic Acid (**5**).** Compound **5** was prepared from D-cysteine and **14** using the method of **4**. The  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ) of **5** is identical with that of **4**. HRMS (FAB, *m*-nitrobenzyl alcohol): calcd for  $\text{C}_{14}\text{H}_{11}\text{NO}_3\text{S}$ , 274.0538 ( $\text{M} + 1$ ); found, 274.0538 ( $\text{M} + 1$ ). Anal. ( $\text{C}_{14}\text{H}_{11}\text{NO}_3\text{S}$ ) C, H, N.

**2-(3'-Hydroxynaphth-2'-yl)- $\Delta^2$ -thiazoline-(4*R*)-carboxylic Acid (**6**).** A mixture of **20** (3.77 g, 22.3 mmol) and

**Table 1.** Net Iron Balance in Cebus Monkeys<sup>a</sup>

drug	dosage ( $\mu\text{mol/kg}$ )	route	vehicle	predrug ( $\mu\text{g/kg}$ )	postdrug ( $\mu\text{g/kg}$ )	significance of <i>t</i> -test
<b>1</b>	150	sc	dH <sub>2</sub> O	217 $\pm$ 128	-245 $\pm$ 142	<i>p</i> < 0.001
<b>2</b>	150	po	40% Cremophor	230 $\pm$ 50	-500 $\pm$ 225	<i>p</i> < 0.001
<b>4</b>	300	po	40% Cremophor	54 $\pm$ 60	63 $\pm$ 30	NS
<b>4</b>	300	sc	40% Cremophor	26 $\pm$ 25	-9 $\pm$ 19	NS
<b>5</b>	300	po	40% Cremophor	358 $\pm$ 49	381 $\pm$ 37	NS
<b>6</b>	300	po	40% Cremophor	118 $\pm$ 84	49 $\pm$ 92	NS
<b>6</b>	300	sc	40% Cremophor	80 $\pm$ 22	120 $\pm$ 21	NS
<b>7</b>	300	po	40% Cremophor	25 $\pm$ 68	-162 $\pm$ 120	NS
<b>7</b>	300	sc	40% Cremophor	164 $\pm$ 63	156 $\pm$ 126	NS

<sup>a</sup> The amount of iron absorbed by the untreated animals over a 3-day period is compared with the amount of iron absorbed by the treated animals over a 3-day period. Net iron balance = dietary iron intake - (urinary iron + fecal iron). Animals in a negative iron balance are excreting more iron than they are absorbing. NS = not significant.

L-cysteine (5.40 g, 44.6 mmol) in methanol (133 mL) and 0.1 M phosphate buffer (pH 5.95, 66 mL) was stirred at 60 °C for 5 h under nitrogen. L-Cysteine (2.70 g, 22.3 mmol) was added, and the mixture was stirred at 60 °C for an additional 18 h. After concentration to about 70 mL, 2% KHCO<sub>3</sub> (156 mL) was added, and the mixture was extracted with ether (3  $\times$  100 mL). The aqueous layer was acidified to a pH of 3 with 1 N hydrochloric acid (75 mL) and extracted with ethyl acetate (3  $\times$  150 mL). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to furnish 5.34 g (88%) of **6** as yellow crystals, mp 226–227 °C. IR (KBr): 3040 (O–H), 1710 (C=O), 1635 (C=C), 1230 (C–O) cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  13.28 (s, 1 CO<sub>2</sub>H), 12.15 (s, 1 OH), 8.20 (s, 1 arom H), 8.02 (d, 1 arom H), 7.78 (d, 1 arom H), 7.54 (dd, 1 arom H), 7.37 (s, 1 arom H), 7.36 (dd, 1 arom H), 5.57 (dd, 1 H), 3.80 (dd,  $\frac{1}{2}$  CH<sub>2</sub>, *J*<sub>gem</sub> = 12 Hz, *J*<sub>vic</sub> = 9 Hz), 3.71 (dd,  $\frac{1}{2}$  CH<sub>2</sub>, *J*<sub>vic</sub> = 8 Hz). <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.09 (s, 1 H), 7.84 (d, 1 H, *J* = 8 Hz), 7.69 (d, 1 H, *J* = 8 Hz), 7.45–7.50 (m, 1 H), 7.27–7.34 (m, 2 H), 5.49 (t, 1 H, *J* = 9 Hz), 3.74 (d, 2 H, *J* = 8 Hz). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta$  172.6 (C=N)\*, 171.2 (C=O)\*, 154.3 (arom CO), 135.9 (arom C), 132.1 (arom CH), 128.7 (arom CH), 128.6 (arom CH), 126.8 (arom C), 125.9 (arom CH), 123.8 (arom CH), 117.9 (arom C), 110.6 (arom CH), 76.7 (CH), 33.5 (CH<sub>2</sub>) (\* = interchangeable). Anal. (C<sub>14</sub>H<sub>11</sub>NO<sub>3</sub>S) C, H, N, S.

**2-(3'-Hydroxynaphth-2'-yl)- $\Delta^2$ -thiazoline-(4S)-carboxylic Acid (7).** Compound **7** was prepared from D-cysteine and **20** using the method of **6**. The <sup>1</sup>H NMR (CD<sub>3</sub>OD) of **7** is identical with that of **6**. Anal. (C<sub>14</sub>H<sub>11</sub>NO<sub>3</sub>S) C, H, N.

**2-(Benzyloxy)-1-naphthaldehyde (9).** A mixture of **8** (20.00 g, 116 mmol), K<sub>2</sub>CO<sub>3</sub> (16.03 g, 116 mmol), and benzyl chloride (19.11 g, 151 mmol, 17.4 mL) in ethanol (200 mL) was heated under reflux for 39 h. Water (200 mL) was added, and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (1  $\times$  200 mL, 2  $\times$  100 mL). The organic layer was dried (MgSO<sub>4</sub>) and concentrated. The residue was crystallized from 10:1 cyclohexane/ethyl acetate (400 mL), giving 22.14 g (73%) of **9** as yellow crystals, mp 123–124 °C (lit.<sup>41</sup> mp 120–121 °C).

**2-(Benzyloxy)-1-naphthoic Acid (10).** Sulfamic acid (17.28 g, 178 mmol) was added to a solution of **9** (21.11 g, 80.5 mmol) in acetone (420 mL) and water (210 mL) at 0 °C. Over a period of 20 min, 80% NaClO<sub>2</sub> (10.42 g, 92.2 mmol) was added at 0 °C. The solution was stirred at 0 °C for 30 min and then concentrated to about 200 mL. After dilution with water (200 mL), the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (1  $\times$  200 mL, 2  $\times$  100 mL). The organic layer was dried (MgSO<sub>4</sub>) and concentrated. Crystallization from cyclohexane/ethyl acetate (1:1) generated 19.12 g (85%) of **10** as pale yellow crystals, mp 127–128 °C (lit.<sup>41</sup> mp 128–130 °C).

**2-(Benzyloxy)-1-naphthamide (12).** Oxalyl chloride (25.9 mL, 137 mmol) was added to a mixture of **10** (19.12 g, 68.7 mmol) and DMF (4.34 mL) in dry toluene (325 mL). The solution was stirred at room temperature for 1 h. The toluene phase was separated from the DMF phase and concentrated. The residue was taken up in toluene (300 mL) and concentrated again, affording 20.21 g (99%) of **11** as yellow crystals, which were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (300 mL) and added dropwise to a mixture of concentrated NH<sub>4</sub>OH (200 mL) and CH<sub>2</sub>Cl<sub>2</sub> (120 mL). The mixture was vigorously stirred at room temperature for 24 h. Water (200 mL) was added, the phases were

separated, and the aqueous layer was extracted with ethyl acetate (3  $\times$  200 mL). The combined organic layers were dried (MgSO<sub>4</sub>) and concentrated. Crystallization from CH<sub>2</sub>Cl<sub>2</sub> gave 16.00 g (85%) of **12** as colorless crystals, mp 153–154 °C. IR (KBr): 3400 (N–H), 3180 (N–H), 1510 (C=C) cm<sup>-1</sup>. <sup>1</sup>H NMR:  $\delta$  8.11 (d, 1 arom H), 7.85 (d, 1 arom H), 7.78 (d, 1 arom H), 7.54–7.26 (8 arom H), 6.18 (s, 1 NH), 6.09 (s, 1 NH), 5.26 (s, 1 CH<sub>2</sub>). Anal. (C<sub>18</sub>H<sub>15</sub>NO<sub>2</sub>) C, H, N.

**Ethyl 2-(Benzyloxy)-1-naphthimidate (13).** Triethylxonium hexafluorophosphate (19.20 g, 58.1 mmol) was added to a solution of **12** (16.00 g, 57.7 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (710 mL). The solution was stirred at room temperature for 24 h and then poured into ice-cold 0.5 M K<sub>2</sub>CO<sub>3</sub> (700 mL). The phases were separated, and the aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2  $\times$  100 mL). The organic layers were dried and concentrated. Chromatography with CH<sub>2</sub>Cl<sub>2</sub> as the eluant gave 15.21 g (86%) of **13** as colorless crystals, mp 55–56 °C. IR (KBr): 3325 (N–H), 2975 (C–H), 1640 (C=C) cm<sup>-1</sup>. <sup>1</sup>H NMR:  $\delta$  7.80–7.72 (3 arom H), 7.50–7.21 (8 arom H), 6.77 (s, 1 NH), 5.20 (s, 1 CH<sub>2</sub>), 4.48 (q, 1 CH<sub>2</sub>, *J* = 7 Hz), 1.40 (t, 1 CH<sub>3</sub>). Anal. (C<sub>20</sub>H<sub>19</sub>NO<sub>2</sub>) C, H, N.

**Ethyl 2-Hydroxy-1-naphthimidate (14).** Palladium on carbon (10%, 2.57 g) was introduced into a solution of **13** (15.21 g, 49.8 mmol) in ethanol (430 mL). The suspension was stirred under a hydrogen atmosphere for 6 h. After filtration through Celite, the filtrate was concentrated giving 10.11 g (94%) of **14** as yellow crystals, mp 145–146 °C. IR (KBr): 2980 (C–H), 1620 (C=C), 1085 (C–O) cm<sup>-1</sup>. <sup>1</sup>H NMR:  $\delta$  8.65 (d, 1 arom H), 7.76 (d, 1 arom H), 7.71 (d, 1 arom H), 7.47 (m, 1 arom H), 7.29 (m, 1 arom H), 7.15 (d, 1 arom H), 4.29 (q, 1 CH<sub>2</sub>, *J* = 7 Hz), 1.42 (t, 1 CH<sub>3</sub>). Anal. (C<sub>13</sub>H<sub>13</sub>NO<sub>2</sub>) C, H, N.

**3-Acetoxy-2-naphthoic Acid (16).** Concentrated sulfuric acid (8 drops) was added to a refluxing mixture of **15** (40.00 g, 213 mmol) in acetic anhydride (38 mL, 426 mmol). The mixture was maintained at reflux for 5 min. After cooling to room temperature, the solid was filtered off, washed with acetic acid and ethanol, and dried under high vacuum to yield 40.31 g (82%) of **16** as pale yellow crystals, mp 185–186 °C (lit.<sup>46</sup> mp 184–186 °C).

**3-Hydroxy-2-naphthamide (18).** Oxalyl chloride (65 mL, 350 mmol) was added dropwise to a solution of **16** (40.31 g, 175 mmol) in DMF (17.2 mL) and dry toluene (850 mL). The mixture was stirred at room temperature for 60 min. The toluene layer was separated from DMF and concentrated. The residue was taken up in toluene (500 mL) and concentrated again providing 42.02 g (97%) of **17** as yellow crystals, which were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (500 mL) and added dropwise to a mixture of concentrated NH<sub>4</sub>OH (420 mL) and CH<sub>2</sub>Cl<sub>2</sub> (250 mL). The mixture was vigorously stirred at room temperature for 24 h and then acidified to a pH of 3 with 9% HCl (1.60 L). Precipitate was filtered off, washed with water (4  $\times$  150 mL), and dried under high vacuum, providing 29.95 g (95%) of **18** as yellow crystals, mp 216–217 °C (lit.<sup>46</sup> mp 217–218 °C).

**3-Acetoxy-2-naphthamide (19).** Acetic anhydride (19.2 mL, 200 mmol) was added to a solution of **18** (29.95 g, 160 mmol) in pyridine (32 mL, 400 mmol), and the mixture was maintained at room temperature for 30 min. The solid was filtered off, washed with acetone, and dried (MgSO<sub>4</sub>) generat-

ing 27.06 g (74%) of **19** as pale yellow crystals, mp 201–202 °C (lit.<sup>46</sup> mp 203–205 °C).

**3-Hydroxy-2-naphthonitrile (20).** A mixture of **19** (4.96 g, 21.6 mmol) and thionyl chloride (6.32 mL, 86.4 mmol) was heated under reflux for 2 h. The solution was concentrated *in vacuo* to dryness. The residue was taken up in water (10 mL), methanol (200 mL), and 1 N NaOH solution (75 mL). The solution was stirred at room temperature for 19 h and then neutralized with 1 N HCl. The mixture was extracted with ether (3 × 150 mL). The organic layer was dried and concentrated. Chromatography, eluting with 1:1 cyclohexane/ethyl acetate, provided 2.41 g (66%) of **20** as yellow crystals, mp 188–189 °C (lit.<sup>46</sup> mp 188–189 °C).

**Job's Plots of 4 and 6.** Tris(hydroxymethyl)aminomethane (TRIS) buffer (100 mM, pH 7.2) was prepared as follows: 1 N Hydrochloric acid was added to TRIS (12.11 g, 100 mmol) in water (800 mL) until the pH was 7.2, and water was added to a volume of 1000 mL. A solution 0.5 mM in iron(III) and 1.0 mM in nitrilotriacetic acid (NTA) was also made up: NTA trisodium salt monohydrate (68.78 mg, 0.250 mmol) and ammonium iron(III) sulfate dodecahydrate (60.27 mg, 0.125 mmol) were dissolved in 3 mM hydrochloric acid (25 mL). The solution was diluted with TRIS buffer until a volume of 250 mL was obtained. Separate 0.5 mM solutions of **4** and **6** in TRIS buffer were prepared from the chelator (13.67 mg, 0.05 mmol) and TRIS buffer (100 mL).

Test solutions with 0, 0.4, 0.525, 0.6, 0.7, 0.8, 1.05, 1.3, 1.4, 1.5, 1.575, 1.8, and 2.1 mL of 0.5 mM chelator solution were brought to a final volume ( $V_T$ ) of 2.1 mL by the addition of 0.5 mM Fe(III) solution. The extinction at 550 nm was measured.

**Bile Duct Cannulation.** Male Sprague–Dawley rats averaging 400 g were housed in Nalgene plastic metabolic cages and given free access to water. The animals were anesthetized using sodium pentobarbital (50 mg/kg), given ip. The bile duct was cannulated using 22 gauge PE 50 tubing, which was inserted ca. 2 cm into the duct and tied firmly in place.

A skin-tunneling needle was inserted from the shoulder area around to the abdominal incision. The cannula was threaded through the needle until it emerged from the shoulder opening. The cannula was then passed from the animal to the swivel inside a metal torque transmitting tether, which was attached to a rodent jacket. The cannula was directed from the animal to a Gilson microfraction collector by a fluid swivel mounted above the metabolic cage. This system allowed the animal to move freely in the cage while continuous bile samples were being collected. Sample collection and processing were as previously described.<sup>36,38</sup>

**Primate Hematological Screens.** For all untreated animals, complete blood counts (CBC) and kidney and liver profiles, except ferritin levels, were within the accepted normal range of the human values. Monkey ferritin could not be measured using the commercially available human ferritin antibody assay. Under the conditions of the experiments, there were no significant changes in the CBC or kidney and liver profiles.

**Primate Iron Balance Studies.** Seven days before the administration of the drug, the animals were placed in metabolic cages<sup>20</sup> and started on the low-iron liquid diet.<sup>37</sup> The animals were given food according to their body weight, and their intake was very carefully monitored.

Three days before drug administration (day –2 to day 0), base-line iron intake and output values were measured. This same measurement was made for days 1–3. The total amount of iron intake was compared with the total iron output.

**Primate Fecal and Urine Samples.** Fecal and urine samples were collected at 24-h intervals. The collections began 4 days before the administration of the test drug. Fecal samples were assayed for the presence of occult blood, weighed, mixed with distilled deionized water, and autoclaved for 30 min. The mixture was then freeze-dried, and a known portion of the powder was mixed with low-iron nitric acid and refluxed for 24 h. Once any particulate matter in the digested samples was removed by centrifugation, iron concentrations were determined by flame atomic absorption (AA). Monkey urine

samples were acidified and reconstituted to initial volume after sterilization, if necessary.

**Atomic Absorption Iron Determinations.** Urine and bile/feces samples were analyzed on a Perkin Elmer 5100 PC atomic absorption spectrophotometer fitted with a model AS-51 autosampler using a quartz sampling probe as previously described.<sup>20</sup>

**Drug Preparation and Administration.** DFO was administered sc to the rats in 40% Cremophor RH-40. The sodium salt of **2** and the naphthyl DFTs **4–7** were prepared in 40% Cremophor and administered orally. Analogue **4** was also given sc. All of the ligands were administered to the rats at a dose of 150 µmol/kg.

DFO was given to the monkeys sc in sterile water for injection at 150 µmol/kg. The sodium salt of **2** was given orally at a dose of 150 µmol/kg, while the naphthyl analogues **4–7** were administered via this route at a dose of 300 µmol/kg. In addition, the primates were also given the naphthyl analogues **4**, **6**, and **7** sc at the same dose, 300 µmol/kg. The DFT analogues **2** and **4–7** were dissolved in 40% Cremophor RH-40. Prior to drug administration, the monkeys were sedated with ketamine (7–10 mg/kg, im) and given scopolamine (0.04–0.07 mg/kg, im) to prevent ketamine-related salivation and vomiting.

Both the monkeys and the rats were fasted for 24 h before dosing.

**Efficiency Calculations.** The efficiency of DFO was calculated on the basis of a 1:1 ligand–iron complex, while the efficiency of DFT and the naphthyl compounds was calculated on the basis of a 2:1 ligand–iron complex. In the monkeys, the numbers were generated 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 efficiencies in the rodent model were calculated by subtracting the iron excretion of control animals from the iron excretion of the treated animals. This number was then divided by the theoretical output to obtain the efficiency.

**Acknowledgment.** Financial support was provided by National Institutes of Health Grant No. RO1DK49108.

## References

- (1) Raymond, K. N.; Carrano, C. J. Coordination Chemistry and Microbial Iron Transport. *Acc. Chem. Res.* **1979**, *12*, 183–190.
- (2) Huebers, H. A.; Finch, C. A. Transferrin: Physiologic Behavior and Clinical Implications. *Blood* **1984**, *64*, 763–767.
- (3) Finch, C. A.; Huebers, H. A. Iron metabolism. *Clin. Physiol. Biochem.* **1986**, *4*, 5–10.
- (4) Hallberg, L. Bioavailability of Dietary Iron in Man. *Ann. Rev. Nutr.* **1981**, *1*, 123–147.
- (5) Finch, C. A.; Huebers, H. Perspectives in Iron Metabolism. *N. Engl. J. Med.* **1982**, *306*, 1520–1528.
- (6) Finch, C. A.; Deubelbeiss, K.; Cook, J. D.; Eschbach, J. W.; Harker, L. A.; Funk, D. D.; Marsaglia, G.; Hillman, R. S.; Slichter, S.; Adamson, J. W.; Ganzoni, A.; Giblett, E. R. Ferrokinetics in Man. *Medicine (Baltimore)* **1970**, *49*, 17–53.
- (7) Seligman, P. A.; Klausner, R. D.; Huebers, H. A. Molecular Mechanisms of Iron Metabolism. In *The Molecular Basis of Blood Diseases*; Stamatoyannopoulos, G., Nienhuis, A. W., Leder, P., Majerus, P. W., Eds.; W. B. Saunders: Philadelphia, **1987**; p 219.
- (8) O'Connell, M. J.; Ward, R. J.; Baum, H.; Peters, T. J. The Role of Iron in Ferritin- and Haemosiderin-Mediated Lipid Peroxidation in Liposomes. *Biochem. J.* **1985**, *229*, 135–139.
- (9) Thomas, C. E.; Morehouse, L. A.; Aust, S. D. Ferritin and Superoxide-Dependent Lipid Peroxidation. *J. Biol. Chem.* **1985**, *260*, 3275–3280.
- (10) Olynyk, J. K.; Bacon, B. R. Hereditary Hemochromatosis. Detecting and Correcting Iron Overload. *Postgrad. Med.* **1994**, *96*, 151–165.
- (11) Erer, B.; Galimberti, M.; Lucarelli, G.; Polchi, P.; Angelucci, E.; Giardini, C.; Baronciani, D.; Tomasucci, M. Bone Marrow Transplantation in Adult Thalassemia. *Bone Marrow Transplant.* **1993**, *12* (S1), 65–66.
- (12) Keller, J. F.; Blausey, L. A. Diagnosis, Treatment, and Nursing Management of the Patient with Hemochromatosis. *Oncol. Nurs. Forum* **1992**, *19*, 1359–1365.

- (13) Bickel, H.; Hall, G. E.; Keller-Schierlein, W.; Prelog, V.; Vischer, E.; Wettstein, A. Metabolic Products of Actinomycetes. XXVII. Constitutional Formula of Ferrioxamine B. *Helv. Chim. Acta* **1960**, *43*, 2129–2138.
- (14) Aksoy, M.; Seyithanoglu, B. Y.; Bozbora, A. Thalassemia and Desferrioxamine (Desferal) - Introductory Remarks, and Clinical Laboratory Observations. In *Hypertension and Iron Chelation in Thalassemia*; Aksoy, M., Birdwood, G. F. B., Eds.; Berne: Huber, **1984**; p 11.
- (15) Graziano, J. H.; Markenson, A.; Miller, D. R.; Chang, H.; Bestak, M.; Meyers, P.; Pisciotto, P.; Rifkind, A. Chelation Therapy in  $\beta$ -Thalassemia Major. I. Intravenous and Subcutaneous Deferoxamine. *J. Pediatr.* **1978**, *92*, 648–652.
- (16) Pippard, M. J.; Callender, S. T. The Management of Iron Chelation Therapy. *Br. J. Haematol.* **1983**, *54*, 503–507.
- (17) Propper, R. D.; Cooper, B.; Rufo, R. R.; Nienhuis, A. W.; Anderson, W. F.; Bunn, H. F.; Rosenthal, A.; Nathan, D. G. Continuous Subcutaneous Administration of Deferoxamine in Patients with Iron Overload. *N. Engl. J. Med.* **1977**, *297*, 418–423.
- (18) Peter, H. H.; Industrial Aspects of Iron Chelators: Pharmaceutical Applications. In *Proteins Iron Storage Transp., Proc. Inter. Conf. Proteins Iron Metab., 7th*; Spik, G., Ed.; Elsevier: Amsterdam, The Netherlands, **1985**; p 293.
- (19) Anderegg, G.; L'Eplattenier, F.; Schwarzenbach, G. Hydroxamate Complexes. III. Iron (III) Exchange between Sideramines and Complexones. A Discussion of the Formation Constants of the Hydroxamate Complexes. *Helv. Chim. Acta* **1963**, *46*, 1409–1422.
- (20) Bergeron, R. J.; Streiff, R. R.; Wiegand, J.; Vinson, J. R. T.; Luchetta, G.; Evans, K. M.; Peter, H.; Jenny, H.-B. A Comparative Evaluation of Iron Clearance Models. *Ann. N. Y. Acad. Sci.* **1990**, *612*, 378–393.
- (21) Kirking, M. H. Treatment of Chronic Iron Overload. *Clin. Pharmacol.* **1991**, *10*, 775–783.
- (22) Ponka, P.; Borova, J.; Neuwirt, J.; Fuchs, O. Mobilization of Iron from Reticulocytes. Identification of Pyridoxal Isonicotinoyl Hydrazone as a New Iron Chelating Agent. *FEBS Lett.* **1979**, *97*, 317–321.
- (23) Uhler, L. C.; Durbin, P. W.; Jeung, N.; Raymond, K. N. Specific Sequestering Agents for the Actinides. 21. Synthesis and Initial Biological Testing of Octadentate Mixed Catecholate-Hydroxypyridinonate Ligands. *J. Med. Chem.* **1993**, *36*, 504–509.
- (24) Kontoghiorghes, G. J.; Aldouri, M. A.; Sheppard, L.; Hoffbrand, A. V. 1,2-Dimethyl-3-hydroxypyrid-4-one, an Orally Active Chelator for Treatment of Iron Overload. *Lancet* **1987**, *1*, 1294–1295.
- (25) Grady, R. W.; Hershko, C. HBED: A Potential Oral Iron Chelator. *Ann. N. Y. Acad. Sci.* **1990**, *612*, 361–368.
- (26) Guterman, S. K.; Morris, P. M.; Tannenber, W. J. K. Feasibility of Enterochelin as an Iron-Chelating Drug: Studies with Human Serum and a Mouse Model System. *Gen. Pharmacol.* **1978**, *9*, 123–127.
- (27) Grady, R. W.; Peterson, C. M.; Jones, R. L.; Graziano, J. H.; Bhargava, K. K.; Berdoukas, V. A.; Kokkini, G.; Loukopoulou, D.; Cerami, A. Rhodotorulic Acid-Investigation of its Potential as an Iron-Chelating Drug. *J. Pharmacol. Exp. Ther.* **1979**, *209*, 342–348.
- (28) Bergeron, R. J. Synthesis and Solution Structure of Microbial Siderophores. *Chem. Rev.* **1984**, *84*, 587–602.
- (29) Bergeron, R. J.; McManis, J. S. Synthesis of Catecholamide and Hydroxamate Siderophores. In *Handbook of Microbial Iron Chelates*; Winkelman, G., Ed.; CRC Press: Boca Raton, FL, **1991**; pp 271–307.
- (30) Cox, C. D.; Rinehart, K. L., Jr.; Moore, M. L.; Cook, J. C., Jr. Pyochelin: Novel Structure of an Iron-Chelating Growth Promoter for *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4256–4260.
- (31) Smith, M. J. Total Synthesis and Absolute Configuration of Rhizobactin, a Structurally Novel Siderophore. *Tetrahedron Lett.* **1989**, *30*, 313–316.
- (32) Naegeli, H.-U.; Zaehner, H. Metabolites of Microorganisms. Part 193. Ferrithiocin. *Helv. Chim. Acta* **1980**, *63*, 1400–1406.
- (33) Anderegg, G.; Raeber, M. Metal Complex Formation of a New Siderophore Desferrithiocin and of Three Related Ligands. *J. Chem. Soc., Chem. Commun.* **1990**, 1194–1196.
- (34) Longueville, A.; Crichton, R. R. An Animal Model of Iron Overload and its Application to Study Hepatic Ferritin Iron Mobilization by Chelators. *Biochem. Pharmacol.* **1986**, *35*, 3669–3678.
- (35) Wolfe, L. C.; Nicolosi, R. J.; Renaud, M. M.; Finger, J.; Hegsted, M.; Peter, H.; Nathan, D. G. A Non-Human Primate Model for the Study of Oral Iron Chelators. *Br. J. Haematol.* **1989**, *72*, 456–461.
- (36) Bergeron, R. J.; Wiegand, J.; Dionis, J. B.; Egli-Karmakka, M.; Frei, J.; Huxley-Tencer, A.; Peter, H. H. Evaluation of Desferrithiocin and its Synthetic Analogues as Orally Effective Iron Chelators. *J. Med. Chem.* **1991**, *34*, 2072–2078.
- (37) Bergeron, R. J.; Streiff, R. R.; Creary, E. A.; Daniels, R. D., Jr.; King, W.; Luchetta, G.; Wiegand, J.; Moerker, T.; Peter, H. H. A Comparative Study of the Iron-Clearing Properties of Desferrithiocin Analogues with Desferrioxamine B in a *Cebus* Monkey Model. *Blood* **1993**, *81*, 2166–2173.
- (38) Bergeron, R. J.; Streiff, R. R.; Wiegand, J.; Luchetta, G.; Creary, E. A.; Peter, H. H. A Comparison of the Iron-Clearing Properties of 1,2-Dimethyl-3-hydroxypyrid-4-one, 1,2-Diethyl-3-hydroxypyrid-4-one and Deferoxamine. *Blood* **1992**, *79*, 1882–1890.
- (39) Hahn, F. E.; McMurphy, T. J.; Hugi, A.; Raymond, K. N. Coordination Chemistry of Microbial Iron Transport. 42. Structural and Spectroscopic Characterization of Diastereomeric Cr(III) and Co(III) Complexes of Desferrithiocin. *J. Am. Chem. Soc.* **1990**, *112*, 1854–1860.
- (40) Bergeron, R. J.; Liu, C. Z.; McManis, J. S.; Xia, M. X. B.; Algee, S. E.; Wiegand, J. The Desferrithiocin Pharmacophore. *J. Med. Chem.* **1994**, *37*, 1411–1417.
- (41) Brain, E. G.; Doyle, F. P.; Mehta, M. D.; Miller, D.; Nayler, J. H. C.; Stove, E. R. Derivatives of 6-Aminopenicillanic Acid. Part IV. Analogues of 2,6-Dimethoxyphenylpenicillin in the Naphthalene and Quinoline Series. *J. Chem. Soc.* **1963**, 491–497.
- (42) Lindgren, B. O.; Nilsson, T. Preparation of Carboxylic Acids from Aldehydes (Including Hydroxylated Benzaldehydes) by Oxidation with Chlorite. *Acta Chem. Scand.* **1973**, *27*, 888–890.
- (43) Bergeron, R. J.; McManis, J. S.; Dionis, J. B.; Garlich, J. R. An Efficient Total Synthesis of Agrobactin and Its Gallium(III) Chelate. *J. Org. Chem.* **1985**, *50*, 2780–2782.
- (44) Job, P. Formation and Stability of Inorganic Complexes in Solution. *Ann. Chim. (Paris)* **1928**, *9*, 113–203.
- (45) Bukata, S.; Marinsky, J. A. Job's Method of Continuous Variations with Ion Exchange for the Study of Complexes in Solution. *J. Phys. Chem.* **1964**, *68*, 258–260.
- (46) Lesser, R.; Kranepuhl, E.; Gad, G. The Constitution of Naphthalenes and their Derivatives. *Chem. Ber.* **1925**, *58*, 2109–2124.

JM9508752