Evaluation of the Desferrithiocin Pharmacophore as a Vector for Hydroxamates

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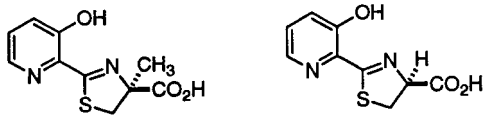
A series of (*S*)-desmethyldesferrithiocin (DMDFT, **1**) hydroxamates and a bis-salicyl polyether hydroxamate are evaluated for their iron-clearing properties in rodents; some of these are further assessed in primates. These hydroxamates include (*S*)-desmethyldesferrithiocin, *N*-methylhydroxamate (**2**); (*S*)-desmethyldesferrithiocin, *N*-[5-(acetylhydroxyamino)pentyl]-hydroxamate (**3**); desmethyldesferrithiocin, *N*-benzylhydroxamate (**4**); (*S*,*S*)-N^1,N^8-bis[4,5-dihydro-2-(3-hydroxy-2-pyridinyl)-4-thiazoyl]-N^1,N^8-dihydroxy-3,6-dioxa-1,8-octanediamine (**5**); and N^1,N^8-bis(2-hydroxybenzoyl)-N^1,N^8-dihydroxy-3,6-dioxa-1,8-octanediamine (**6**). The ligands are evaluated when given both orally (po) and subcutaneously (sc) in the bile-duct-cannulated rodent model. In iron-overloaded primates, ligands **1**−**4** are assessed when administered po and sc. The efficiencies of the hydroxamates are shown to vary considerably; giving the compounds sc consistently resulted in greater chelating efficiency in vivo. After oral administration in the primate, compound **3**, a pentacoordinate unsymmetrical dihydroxamate, produces iron excretion sufficient to warrant further preclinical evaluation both as a potential orally active iron-chelating agent and as a parenteral iron chelator. The increased iron clearance of several of these ligands when administered sc versus po also underscores the idea that parenteral administration is a reasonable alternative to a less efficient, orally active device which would require large and frequent doses.

Introduction

Desferrioxamine B (DFO) as its methanesulfonate salt, Desferal, is the drug of choice for the treatment of transfusional iron overload. This hexacoordinate iron chelator, a hydroxamate bacterial siderophore isolated from *Streptomyces pilosus*,[1] forms a very tight 1:1 complex with Fe(III). Unfortunately, desferrioxamine is not well-absorbed from the gastrointestinal tract and is rapidly eliminated from the circulation; consequently, prolonged parenteral infusion is needed.[2−4] Effective therapy usually requires subcutaneous (sc) or intravenous (iv) administration by a portable infusion pump for 9−12 h daily, 5−6 days a week; patient compliance is poor. Because transfusion-dependent patients require life-long therapy, considerable effort has been directed at a search for an iron chelator that would be easier for patients to use.

Desferrithiocin (DFT; Chart 1), a tridentate siderophore which forms 2:1 complexes with Fe(III),[5,6] is bioavailable after oral administration. Although the naturally occurring siderophore is nephrotoxic,[7] structure−activity studies have made it possible to construct DFT analogues which are substantially less toxic than the parent ligand and still orally active.[8,9] We have previously assessed two hydroxamate derivatives of the DFT analogue (*S*)-desmethyldesferrithiocin (DMDFT, **1**; Chart 1), the *N*-methylhydroxamate and a pentacoordinate dihydroxamate ligand, in the bile-duct-cannulated rat model.[9] In the current article, we describe the further evaluation of these DMDFT hydroxamates in the primate model as well as the assessment of several additional DMDFT hydroxamate ligands.

Chart 1. Naturally Occurring Siderophore (*S*)-Desferrithiocin (DFT) and Its Analogue (*S*)-Desmethyldesferrithiocin (DMDFT, **1**)



Design Concepts

Our earlier work demonstrated that conversion of the carboxylic acid group of DMDFT (**1**; Table 1) to an *N*-methylhydroxamate (**2**) or to the pentacoordinate dihydroxamate ligand (**3**) resulted in compounds that were effective iron chelators in the bile-duct-cannulated rat model when given orally (po).[9] To determine whether DMDFT hydroxamates are effective iron-clearing agents as a class of compounds when given po or sc, two additonal DMDFT hydroxamate systems were evaluated: the tricoordinate *N*-benzylhydroxamate of DMDFT, **4**, a more lipophilic version of the corresponding *N*-methylhydroxamate **2**, and (*S*,*S*)-N^1,N^8-bis[4,5-dihydro-2-(3-hydroxy-2-pyridinyl)-4-thiazoyl]-N^1,N^8-dihydroxy-3,6-dioxa-1,8-octanediamine (**5**), in which two (*S*)-DMDFTs are joined by an eight-membered dioxa tether. The latter compound, a hexacoordinate ligand, facilitates formation of a 1:1 complex with Fe(III). Thus, all of the metal's coordination sites are occupied. This offers an entropic advantage over the other Fe(III) hydrox-

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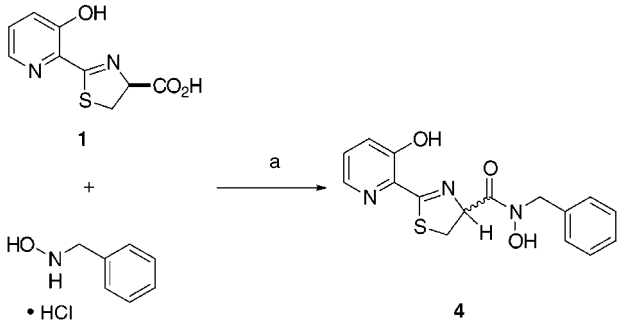
Scheme 1. Synthesis of 4,5-Dihydro-*N*-hydroxy-2-(3-hydroxy-2-pyridinyl)-*N*-(phenylmethyl)-4-thiazolecarboxamide (**4**)[a]



[a] Reagents: (a) BOP reagent/DIEA (3 equiv)/DMF.

amate complexes described. In addition, it renders reduction of Fe(III) to Fe(II) by biological reductants such as superoxide anion or ascorbate unlikely. Thus, participation of Fe(II) in the reduction of hydrogen peroxide to the highly reactive and destructive hydroxyl radical (Fenton chemistry) is minimized. N^1,N^8-Bis(2-hydroxybenzoyl)-N^1,N^8-dihydroxy-3,6-dioxa-1,8-octanediamine (**6**), a hexacoordinate ligand in which the DMDFT donor groups are substituted with salicyl donors, was synthesized in an effort to determine whether the DMDFT donors were the source of toxicity associated with the hexacoordinate ligand **5**. The iron-clearing properties and toxicities in vivo of the hydroxamate chelators in Table 1 were evaluated in comparison to the most effective, least toxic DFT derivative to date, **1**.

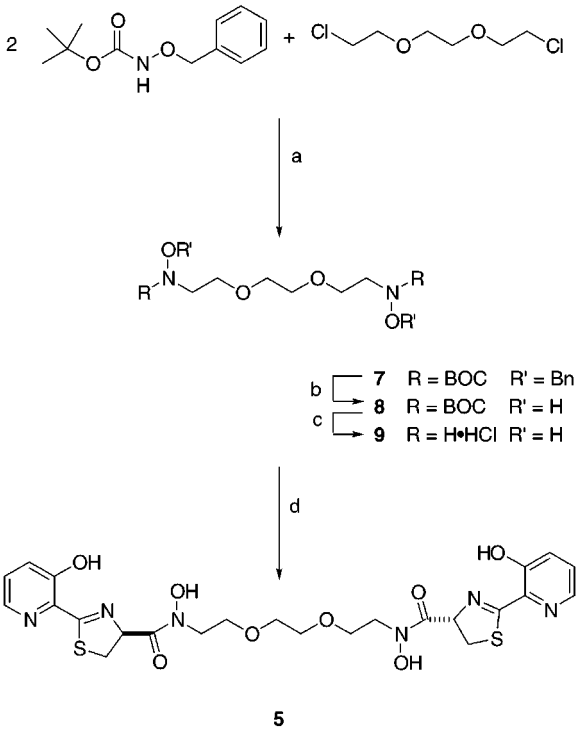
Synthetic Methods

The *N*-benzylhydroxamate of (*S*)-DMDFT (**4**) was synthesized by N-acylation of *N*-benzylhydroxylamine hydrochloride with (*S*)-DMDFT (**1**) activated by (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) in *N*,*N*-diisopropylethylamine (DIEA; 3 equiv) and DMF (Scheme 1). Although it was possible to isolate an optically active hydroxamate via Sephadex LH-20 chromatography, the compound epimerized on recrystallization. The magnitude of optical rotation decreased from $[\alpha]_D = -16.4°$ to essentially zero. Because of the spontaneous epimerization, no attempt was made to evaluate the biological properties of optically active material. The racemic product was utilized in all of the studies.

Hexacoordinate ligand **5** can be visualized as the joining of two (*S*)-DMDFT hydroxamic acids through their nitrogens by an eight-membered diether chain. *N*-(*tert*-Butoxycarbonyl)-*O*-benzylhydroxylamine[10] was alkylated (NaH/DMF) with 1,2-bis(2-chloroethoxy)-ethane (0.5 equiv) to give masked dihydroxamate **7**. Hydrogenolysis of the benzyl groups of **7** afforded tetracoordinate chelator **8**. The BOC protecting groups were removed with 3 M methanolic hydrochloric acid, providing dihydroxylamine dihydrochloride **9**. Bis-N-acylation of **9** with **1** using BOP (excess DIEA, DMF) completed the synthesis of hexadentate chelator **5** (Scheme 2).

Fully masked dihydroxamate **7** (Scheme 2) was also utilized for the preparation of N^1,N^8-bis(2-hydroxybenzoyl)-N^1,N^8-dihydroxy-3,6-dioxa-1,8-octanediamine (**6**), a bis-salicyl analogue of **5**, also hexacoordinate, in which the thiazoline groups have been removed (Scheme 3). Selective deprotection of **7** with hydrogen chloride in methanol yielded bis(benzyloxyamine) **10**. Acylation of **10** with acetylsalicyloyl chloride (DIEA/CH_2Cl_2) afforded the fully protected chelator **11**. Next, deacetylation of **11** with sodium methoxide in methanol gave the diphenol **12**, followed by hydrogenolysis (1 atm, 10% Pd/C, MeOH) to afford hexacoordinate chelator **6**.

Scheme 2. Synthesis of (*S*,*S*)-N^1,N^8-Bis[4,5-dihydro-2-(3-hydroxy-2-pyridinyl)-4-thiazoyl]-N^1,N^8-dihydroxy-3,6-dioxa-1,8-octanediamine (**5**)[a]



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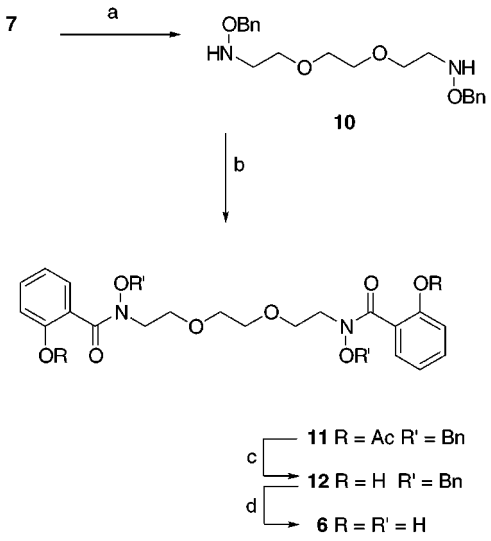
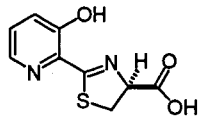
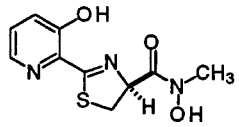
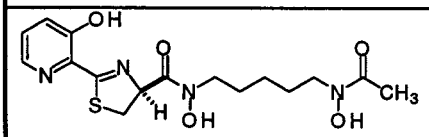
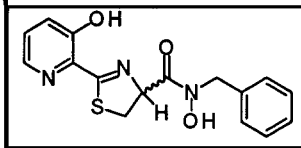
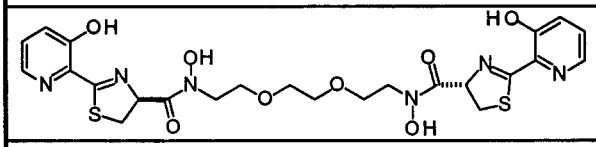
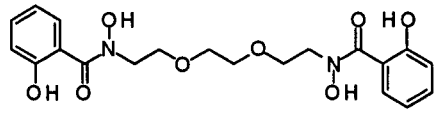
[a] Reagents: (a) NaH/DMF; (b) H_2/Pd−C/MeOH; (c) HCl/MeOH; (d) **1**/BOP reagent/DIEA/DMF.

Scheme 3. Synthesis of N^1,N^8-Bis(2-hydroxybenzoyl)-N^1,N^8-dihydroxy-3,6-dioxa-1,8-octanediamine (**6**)[a]



[a] Reagents: (a) HCl/CH_3OH; (b) acetylsalicyloyl chloride/DIEA (2.2 equiv)/CH_2Cl_2; (c) CH_3ONa/CH_3OH; (d) H_2/10% Pd−C/CH_3OH.

Table 1. Summary of Evaluations of the Desferrithiocin-Based Hydroxamates

compound structure	number	efficiency % (rat)[a]	efficiency % (monkey)[b]
	**1**	2.4 ± 0.6 (po)[7] [82 bile, 18 urine] 1.8 ± 0.7 (sc) [77 bile, 23 urine]	150 μmol/kg 4.8 ± 2.7 (po) [48 stool, 52 urine] 300 μmol/kg 8.0 ± 2.5 (po) [42 stool, 58 urine] 300 μmol/kg 8.3 ± 2.7 (sc) [75 stool, 25 urine]
	**2**	3.1 ± 0.4 (po)[9] [98 bile, 2 urine] 5.3 ± 0.7 (sc) [64 bile, 36 urine]	150 μmol/kg 6.9 ± 3.0 (po) [58 stool, 42 urine] 300 μmol/kg 13.2 ± 7.7 (po) [83 stool, 17 urine] 150 μmol/kg 11.7 ± 5.1 (sc) [86 stool, 14 urine]
	**3**	2.8 ± 0.8 (po) [86 bile, 14 urine] 8.5 ± 0.4 (sc) [87 bile, 13 urine]	225 μmol/kg 3.2 ± 2.0 (po) [39 stool, 61 urine] 225 μmol/kg 12.8 ± 3.4 (sc) [90 stool, 10 urine]
	**4**[c]	1.0 ± 0.1 (po) [93 bile, 7 urine] 1.4 ± 0.8 (sc) [68 bile, 32 urine]	300 μmol/kg ≤ 0.5 (po) 300 μmol/kg ≤ 0.5 (sc)
	**5**	4.6 ± 2.1 (po) [87 bile, 13 urine] 20.6 ± 1.3 (sc)[d] [92 bile, 8 urine]	N.D.[e]
	**6**	≤ 0.5 (po) 0.8 ± 0.3 (sc) [62 bile, 38 urine]	N.D.[e]

[a] In the rats, doses were 150 μmol/kg via the route 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. Directly beneath the efficiency calculation is the percentage breakdown of iron excretion in the bile and urine, respectively. [b] In the monkeys, the doses and routes were as shown. 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. Directly beneath the efficiency calculation is the percentage breakdown of iron excretion in the stool and urine, respectively. [c] This compound was first dissolved in hot ethanol and then in 40% Cremophor. [d] All of the rats died during sc evaluation of this compound. [e] N.D., not determined.

Biological Analysis

In previous studies, DMDFT (**1**), which forms a 2:1 ligand:metal complex with Fe(III), had an iron-clearing efficiency of 2.4 ± 0.6% with 82% of the iron excreted in the bile and 18% in the urine when administered to rodents at a dose of 150 μmol/kg po.[7] When administered sc at the same dose, the efficiency and excretion patterns were nearly identical to what was found when the ligand was given po (Table 1). This strongly suggests that the oral bioavailability of the parent ligand is very high. The corresponding *N*-methylhydroxamate **2** was also previously shown to form a 2:1 ligand:metal complex with Fe(III). Although addition of the hydroxamate group to DMDFT results in **2** having an additional donor site, because of steric constraints, only three of the four donor atoms of **2** are actually involved in ligation.[9] When given to rodents at a dose of 150 μmol/kg po, it was about as efficient as **1**; 98% of the iron was excreted in the bile and 2% in the urine. However, in the current experiments this drug was substantially better when given sc, but much less of the iron excretion was biliary (64%, Table 1). This suggests that the oral bioavailability of the hydroxamate is less than that of **1**. The *N*-benzylhydroxamate **4** was less effective than **1** or **2** when administered either po or sc (Table 1). Once again, po dosing led to more biliary iron clearance (93%) than did administration by the sc route (68%). However, in the case of the unsymmetrical dihydroxamate **3**, the difference between the efficiencies of the drug when given po or sc became more pronounced, 2.8 ± 0.8% and 8.5 ± 0.4%, respectively (Table 1). In both instances most of the iron was excreted in the bile, ~86%. Furthermore, the difference in iron excretion after po and sc administration was even more exaggerated with the sym-

metrical dihydroxamate **5**. When bis-DMDFT ligand **5** was administered sc to the rodents, the efficiency was the highest observed in the rodents for any DFT analogue, 20.6 ± 1.3% (Table 1), almost 10-fold greater than that of the parent ligand, **1**. Interestingly, sc administration of this ligand elicited overwhelmingly biliary iron excretion, 92%. However, this standard dose of 150 μmol/kg proved to be lethal during sc evaluation of this compound. When administered po, this ligand was no more effective than administration of twice the molar equivalent of DMDFT itself, 4.6%. This suggests possible hydrolysis of this ligand to the parent compound **1** in the gut. The origin of the toxicity of **5** when administered sc remains uncertain.

In an attempt to determine whether the toxicity of hexacoordinate ligand **5** was implicit in the diether linkage, compound **6**, containing salicyl donors in place of the DMDFT moieties, was synthesized and tested. This chelator was certainly less toxic than was **5** as no lethalities were observed during evaluation. However, for compound **6**, this loss of toxicity was associated with a loss of iron-chelating activity (Table 1).

We next evaluated compounds **1**−**4** in primates. When **1** was given to iron-overloaded primates at a dose of 150 or 300 μmol/kg po, it was more efficient than it was in rodents, with about an equal distribution of the iron in stool and urine (Table 1).[7] When administered sc, the efficiency was essentially the same as when administered po. The pattern of iron excretion was somewhat different; 75% was in the stool. The *N*-methylhydroxamate **2** was also substantially more active in primates than in rodents at a po dose of 150 μmol/kg, but less of the iron was excreted through the stool, 58% (Table 1). However, at a po dose of 300 μmol/kg, the efficiency was higher, and a greater percentage of the iron, 83%, was found in the stool (Table 1). Compound **2** was well-tolerated when administered either po or sc to the rodents at a dose of 150 μmol/kg. When the drug was initially given sc to two primates at this dose, it was similarly well-tolerated. However, in a subsequent experiment in which two additional monkeys were given the drug sc at 150 μmol/kg and four primates were given it sc at 300 μmol/kg, one of the animals in the low-dose group and three of the four monkeys in the high-dose group had to be dropped from the study on day +1 due to development of clinically significant tremors and an abnormally low serum potassium level. One of the animals (300 μmol/kg) died approximately 25 h post-drug. Postmortem examination was unable to unequivocally identify a cause of death. The iron-clearing efficiency of the ligand when administered at 150 μmol/kg was about twice that seen in the rodents at the same sc dose, 11.7 ± 5.1%, with 86% of the iron excreted in the stool and 14% excreted in the urine.

The apparent toxicity of this compound in the primates was unexpected based on the absence of any deleterious effects when it was administered acutely to rodents. Thus, we were compelled to conduct a chronic toxicity evaluation in rats. Ten-day po[7] and sc dosing regimens were planned. When the rats were given **2** po at a dose of 384 μmol/kg/day for 10 days, all the animals survived the exposure to the drug; no tremors or any other behavioral abnormalities were noted. Histopathological examination of numerous tissues, including intestines, stomach, spleen, pancreas, and kidney, was performed. The only notable effects were on the kidneys; in the opinion of the pathologist the drug was a "renal tubular epithelial toxin." Due to the renal toxicity of the compound given po, the sc studies were not carried out.

When **4** was given to the primates either po or sc at a dose of 300 μmol/kg, the performance was similar to that in rodents (Table 1). Neither **5** nor **6** was taken forward into the primates. Symmetrical dihydroxamate **5** was simply too toxic in the rodents; the poor performance of **6** did not justify further studies in primates. However, unsymmetrical dihydroxamate **3** was somewhat effective when given to primates po; this ligand was very effective when given sc. Orally at 225 μmol/kg this dihydroxamate had an efficiency of 3.2 ± 2.0%. In contrast to po administration of **2**, most of the iron, 61%, was cleared in the urine and 39% was in the stool. Interestingly, its effectiveness climbed to 12.8 ± 3.4% when given sc; 90% of the iron was cleared in the stool and 10% in the urine (Table 1). Furthermore, **3** did not elicit any deleterious effects in either rodents or primates.

Discussion

The current study brings several issues to light. Of the ligands (**1**−**3** and **5**) that had good iron-clearing efficiencies in rodents when given po, **2**, **3**, and **5** were even more efficient when given sc. This is consistent with the idea that **1** has the greatest oral bioavailability of the ligands. Bis analogue **5** perfomed exceptionally well when administered sc to the rodents; however, it remains to be seen whether the toxicity of this compound is due to its astounding iron-clearing efficiency. The same scenario holds for the primate evaluations: Of the three chelators that were active in this model (**1**−**3**), only **1** was equally effective when given po or sc. Both ligands **2** and **3** performed far better when given sc. The other notable observation is that all three of these chelators were more active in primates than in rodents whether administered po or sc. Of the hydroxamate derivatives of DMDFT assembled and evaluated, the unsymmetrical dihydroxamate compound **3**, the *N*-[5-(acetylhydroxyamino)pentyl]hydroxamate of DMDFT, seems the best candidate for further preclinical evaluation, and additional studies are underway.

Experimental Section

Chelators **1**,[8] **2**,[9] and **3**[9] were previously prepared in these laboratories. All other reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI). Fisher Optima grade solvents were routinely used, and DMF was distilled. Organic extracts were dried with sodium sulfate. Glassware that was presoaked in 3 N HCl for 15 min and distilled solvents were employed for reactions involving chelators. Silica gel 32−63 (40 μm "flash") from Selecto, Inc. (Kennesaw, GA) or Lipophilic Sephadex LH-20 from Sigma Chemical Co. (St. Louis, MO) was used for column chromatography. Melting points are uncorrected. Proton NMR spectra were run at 300 MHz in deuterated organic solvents ($CDCl_3$ not indicated) or in D_2O with chemical shifts in parts per million downfield from tetramethylsilane or sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$, respectively. Coupling constants (*J*) are in hertz (Hz). Optical rotations were determined at 589 nm (sodium D line) with *c* as g of compound/100 mL of solution. 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 obtained from Harvard Bioscience (South Natick, MA). Intramedic polyethylene tubing PE 50 was obtained from Fisher Scientific (Pittsburgh, PA). *Cebus apella* monkeys were purchased from World Wide Primates (Miami, FL). Ultrapure salts were obtained from Johnson Matthey Electronics (Royston, U.K.). Imferon, an iron dextran solution, was obtained from Fisons (Bedford, MA).

(*S*)-4,5-Dihydro-*N*-hydroxy-2-(3-hydroxy-2-pyridinyl)-*N*-(phenylmethyl)-4-thiazolecarboxamide (4). BOP reagent (6.13 g, 13.9 mmol) was added to a solution of **1** (3.16 g, 14.1 mmol) and *N*-benzylhydroxylamine hydrochloride (2.01 g, 12.6 mmol) in DMF (130 mL). Diisopropylethylamine (DIEA; 7.0 mL, 40 mmol) in DMF (17 mL) was added dropwise at 0 °C to the reaction solution, which was stirred as it warmed to room temperature for 1 day. Solvents were removed under high vacuum, and the residue was treated with 0.4 M citric acid (200 mL) and extracted with EtOAc (3 × 100 mL). The organic extracts were washed with 100 mL: 1:1 saturated $NaHCO_3$/brine (2×), H_2O (3×), and brine, and solvent was removed by rotary evaporation. Purification of the residue on a Sephadex LH-20 column, eluting with 4% EtOH/toluene, produced 1.72 g (41%) of **4** as a yellow solid: mp 171−173 °C; $[\alpha]^{25}_D$ −16.4° (*c* 0.52, CH_3OH). Anal. ($C_{16}H_{15}N_3O_3S$) C, H, N.

(±)-4. Reaction of **1** (7.03 g, 31.4 mmol) and *N*-benzylhydroxylamine hydrochloride (4.55 g, 28.5 mmol) under the above conditions and recrystallization from aqueous EtOH gave 4.83 g (51%) of (±)-**4** as light-green needles: mp 175−177 °C; $[\alpha]^{21}_D$ −0.39° (*c* 0.52, CH_3OH); NMR (CD_3OD) δ 3.47 (dd, 1 H, J = 11, 9), 3.59−3.71 (m, 1 H), 4.84 (d, 1 H, J = 2, second N-C*H*$_2$-Ph under O*H*), 6.01 (t, 1 H, J = 9), 7.26−7.42 (m, 7 H), 8.13 (t, 1 H, J = 3).

(*S*,*S*)-N^1,N^8-Bis[4,5-dihydro-2-(3-hydroxy-2-pyridinyl)-4-thiazoyl]-N^1,N^8-dihydroxy-3,6-dioxa-1,8-octanediamine (5). BOP reagent (8.59 g, 19.4 mmol) was added to a solution of **1** (4.26 g, 19.0 mmol) and **9** (2.23 g, 8.81 mmol) in DMF (130 mL). DIEA (9.6 mL, 55 mmol) in DMF (23 mL) was added dropwise at 0 °C to the reaction solution, which was stirred as it warmed to room temperature for 1 day. Solvents were removed under high vacuum, and the residue was treated with 0.5 M citric acid (150 mL) and brine (50 mL) and was extracted with EtOAc (4 × 100 mL). The organic extracts were washed with 100 mL: 1:1 saturated $NaHCO_3$/brine (2×), H_2O (4×), and brine; solvent was removed by rotary evaporation. Purification of the residue on a Sephadex LH-20 column, eluting with 4% EtOH/toluene, furnished 0.59 g (11%) of **5** as a pale-green foam: $[\alpha]^{22}_D$ −8.7° (*c* 0.28, CH_3OH); NMR (CD_3OD) δ 3.45−3.98 (m, 16 H), 6.00 (t, 2 H, J = 9), 7.38 (d, 4 H, J = 3), 8.09−8.15 (m, 2 H). Anal. ($C_{24}H_{28}N_6O_8S_2$) C, H, N.

N^1,N^8-Bis(2-hydroxybenzoyl)-N^1,N^8-dihydroxy-3,6-dioxa-1,8-octanediamine (6). To a solution of **12** (455 mg, 0.76 mmol) in CH_3OH (30 mL) was added 10% Pd−C (160 mg). The mixture was stirred under H_2 (1 atm) at room temperature for 1 h. The catalyst was filtered, and the solvent was removed in vacuo. Purification of the residue on a Sephadex LH-20 column, eluting with 4% EtOH/toluene, followed by lyophilization gave 254 mg (81%) of **6** as a pale-yellow oil: NMR (CD_3OD) δ 3.57 (s, 4 H), 3.73 (m, 4 H), 3.81 (m, 4 H), 6.86 (m, 4 H), 7.27 (m, 2 H), 7.44 (m, 2 H). Anal. ($C_{20}H_{24}N_2O_8$) C, H, N.

N^1,N^8-Bis(benzyloxy)-N^1,N^8-bis(*tert*-butoxycarbonyl)-3,6-dioxa-1,8-octanediamine (7). NaH (60%, 3.46 g, 86.5 mmol) was added to *N*-(*tert*-butoxycarbonyl)-*O*-benzylhydroxylamine (15.08 g, 67.5 mmol) in DMF (160 mL) at 0 °C. The reaction mixture was stirred for 40 min at room temperature, and 1,2-bis(2-chloroethoxy)ethane (5.0 mL, 32 mmol) was introduced by syringe. After the flask was heated for 21 h at 53 °C, it was cooled in ice water; the reaction was quenched with H_2O (20 mL). Solvents were removed in vacuo, and the residue was treated with dilute brine (200 mL) and EtOAc (250 mL). The aqueous portion was further extracted with EtOAc (2 × 100 mL). The organic extracts were washed with H_2O (2 × 100 mL) and brine (100 mL). After solvent removal, the solid was purified by silica gel flash column chromatography using 24% EtOAc/petroleum ether providing 13.16 g (73%) of **7** as an oil: NMR δ 1.49 (s, 18 H), 3.57−3.64 (m, 12 H), 4.84 (s, 4 H), 7.30−7.42 (m, 10 H). Anal. ($C_{30}H_{44}N_2O_8$) C, H, N.

N^1,N^8-Bis(*tert*-butoxycarbonyl)-N^1,N^8-dihydroxy-3,6-dioxa-1,8-octanediamine (8). Degassed CH_3OH (400 mL) was added to **7** (10.2 g, 18.1 mmol), and 10% Pd−C (1.44 g) was carefully introduced. The mixture was stirred under H_2 at 1 atm for 7.5 h. Solids were filtered using Celite, which was washed with CH_3OH (400 mL). Solvent was removed in vacuo. Purification of the concentrate on a Sephadex LH-20 column, eluting with 5% EtOH/toluene, produced 3.67 g (53%) of **8** as a colorless oil: NMR δ 1.50 (s, 18 H), 3.63 (s, 4 H), 3.70 (s, 8 H), 7.70 (s, 2 H). Anal. ($C_{16}H_{32}N_2O_8$) C, H, N.

N^1,N^8-Dihydroxy-3,6-dioxa-1,8-octanediamine Dihydrochloride (9). Methanolic HCl (3 N, from concentrated HCl, 100 mL) was added to **8** (3.66 g, 9.62 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 1 day. Solvent was removed under high vacuum. The residue was dissolved in EtOH and concentrated. Toluene was added and removed in vacuo to give 2.34 g (96%) of **9** as a hygroscopic white solid: NMR (D_2O) δ 3.47−3.52 (m, 4 H), 3.75 (s, 4 H), 3.85−3.90 (m, 4 H). Anal. ($C_6H_{18}Cl_2N_2O_4$) C, H, N.

N^1,N^8-Bis(benzyloxy)-3,6-dioxa-1,8-octanediamine (10). A solution of **7** (6.00 g, 10.7 mmol) in CH_3OH (144 mL) and concentrated HCl (56 mL) was stirred at room temperature for 20 h. The mixture was concentrated to dryness, H_2O (50 mL) was added to the residue, the pH was adjusted to 9 with saturated Na_2CO_3, and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with H_2O (2 × 50 mL), and the solvent was removed in vacuo. Silica gel flash chromatography (1:1 cyclohexane/EtOAc) afforded 3.25 g (84%) of **10** as a colorless oil: NMR δ 3.09 (t, 4 H, J = 5.1), 3.58 (s, 4 H), 3.60 (t, 4 H, J = 5.1), 4.70 (s, 4 H), 5.93 (br s, 2 H), 7.26−7.37 (m, 10 H). Anal. ($C_{20}H_{28}N_2O_4$) C, H, N.

N^1,N^8-Bis(2-acetoxybenzoyl)-N^1,N^8-bis(benzyloxy)-3,6-dioxa-1,8-octanediamine (11). A solution of acetylsalicyloyl chloride (1.10 g, 5.54 mmol) in CH_2Cl_2 (7 mL) was added dropwise under Ar to a solution of **10** (0.91 g, 2.52 mmol) and DIEA (0.72 g, 5.57 mmol) in CH_2Cl_2 (30 mL). The mixture was stirred at room temperature under Ar for 4 h, and CH_2Cl_2 (100 mL) was added. The organic layer was washed with 10% citric acid (3 × 30 mL), saturated $NaHCO_3$ (3 × 30 mL), H_2O (2 × 30 mL), and brine (30 mL), and the solvent was removed in vacuo. Silica gel flash chromatography (5% then 10% acetone in CH_2Cl_2) gave 1.54 g (89%) of **11** as a colorless oil: NMR δ 2.24 (s, 6 H), 3.62 (s, 4 H), 3.71 (m, 4 H), 3.84 (br s, 4 H), 4.69 (br s, 4 H), 6.95−7.29 (m, 14 H), 7.37−7.46 (m, 4 H). Anal. ($C_{38}H_{40}N_2O_{10}$) C, H, N.

N^1,N^8-Bis(benzyloxy)-N^1,N^8-bis(2-hydroxybenzoyl)-3,6-dioxa-1,8-octanediamine (12). Sodium methoxide (0.5 M, 18.0 mL, 9.0 mmol) was added at 0 °C to a solution of **11** (1.45 g, 2.12 mmol) in CH_3OH (50 mL). The mixture was stirred at 0 °C for 1 h. A solution of concentrated HCl (0.8 mL) in EtOH (19.2 mL) was added, and the solvent was removed in vacuo. Water (100 mL) was added to the residue, and the aqueous layer was extracted with Et_2O (4 × 80 mL). The combined organic extracts were washed with H_2O (2 × 50 mL) and brine (50 mL), and the solvent was removed by rotary evaporation. Silica gel flash chromatography (5% acetone in CH_2Cl_2) yielded 1.15 g (91%) of **12** as a colorless oil: NMR δ 3.61 (s, 4 H), 3.73 (t, 4 H, J = 5.3), 3.89 (t, 4 H, J = 5.3), 4.76 (s, 4 H), 6.80 (m, 2 H), 6.95 (m, 2 H), 7.18−7.38 (m, 12 H), 7.83 (m, 2 H). Anal. ($C_{34}H_{36}N_2O_8$) C, H, N.

Cannulation of Bile Duct in Rats. The cannulation has been described previously.[8,11,12] Briefly, male Sprague−Dawley rats averaging 400 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) given 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 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. Urine samples were taken every 24 h. Sample collection and handling were as previously described.[8]

Iron Loading of *C. apella* Monkeys. *C. apella* monkeys were given iron dextran iv as presented in earlier publications.[7,11,13] Briefly, the iron dextran was administered to the animals by slow infusion at a dose of 200−300 mg of iron/kg of body weight over 45−60 min. Infusions were performed as necessary to provide about 500 mg of iron/kg of body weight. After administration of iron dextran, we waited at least 60 days before using any of the animals in experiments evaluating iron-chelating agents.

Primate Fecal and Urine Samples. Fecal and urine samples were collected and processed as previously described.[7,11,13] 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 described in earlier publications.[12,13]

Drug Preparation and Administration. The iron chelators were solubilized in 40% Cremophor RH-40/H_2O (v/v) and given po or sc to the rats at a dose of 150 μmol/kg. In the primates, the compounds were solubilized in 40% Cremophor RH-40/H_2O (v/v) and given po or sc at a dose of 150, 225, or 300 μmol/kg as indicated in Table 1.

Calculation of Iron Chelator Efficiency. The efficiency of each chelator was calculated on the basis of a 2:1 (compounds **1**, **2**,[9] and **4**), 3:2 (**3**),[9] or 1:1 (**5** and **6**) ligand:iron complex. The efficiencies in the rodent model were calculated by subtracting the iron excretion of control animals from the iron excretion of treated animals. This number was then divided by the theoretical output; the result is expressed as a percentage. 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 result is expressed as a percentage.

Toxicity in Rodents. Compound **2** was evaluated in a chronic toxicity study in rodents.[7] The compound was given po at a dose of 384 μmol/kg/day for 10 days; 24 h after the final dose, histopathological analysis of tissues (spleen, kidney, liver, lung, esophagus, small intestine, stomach, large intestine) was carried out by an outside pathologist.

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