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Metabolically Programmed Iron Chelators

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Abstract

Extensive structure activity relationship (SAR) studies focused on the desferrithiocin [DFT, (*S*)-4,5-dihydro-2-(3-hydroxy-2-pyridinyl)-4-methyl-4-thiazolecarboxylic acid] pharmacophore have led to three different DFT analogues being evaluated clinically for the treatment of iron overload diseases, e.g., thalassemia. The SAR work revealed that the lipophilicity of a ligand, as determined by its partition between octanol and water, $\log P_{app}$, could have a profound effect on the drug's iron clearing efficiency (ICE), organ distribution, and toxicity profile. While within a given structural family the more lipophilic a chelator the better the ICE, unfortunately, the more lipophilic ligands are often more toxic. Thus, a balance between lipophilicity, ICE, and toxicity must be achieved. In the current study, we introduce the concept of “metabolically programmed” iron chelators, i.e., highly lipophilic, orally absorbable, effective deferration agents which, once absorbed, are quickly converted to their nontoxic, hydrophilic counterparts.

Graphical Abstract

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1. Introduction

Nearly all life forms require iron as a nutrient [1]. However, the low solubility of Fe(III) hydroxide, the predominant form of the metal in the biosphere [2], necessitated the development of sophisticated iron storage and transport systems by both prokaryotes and eukaryotes. Microorganisms utilize low molecular weight, ferric iron-specific ligands, siderophores [3–7]; eukaryotes tend to employ proteins to transport and store iron [8,9]. Humans have developed a highly efficient iron management system [10] in which we absorb and excrete only about 1 mg of the metal daily; there is no mechanism for the excretion of excess iron [11]. Disruption of this homeostasis can lead to severe consequences, even lethal events.

Whether derived from transfused red blood cells [12,13], required in the treatment of hemolytic anemias, or from increased absorption of dietary iron [14], without effective treatment, iron deposition occurs in the liver, heart, pancreas, and elsewhere [15]. This can lead to liver disease [16], diabetes [17], increased risk of cancer [18], and heart disease, often the cause of death in these patients [19]. The nontransferrin-bound plasma iron (NTBI^a) [20–22] pool, a still somewhat ill defined pool of the metal, is the origin of the organ damage that develops with iron overload.

The toxicity associated with excess iron derives from its interaction with reactive oxygen species, for instance, endogenous hydrogen peroxide (H₂O₂) [23–25]. In the presence of Fe(II), H₂O₂ is reduced to the hydroxyl radical (HO•) and HO[−], a process known as the Fenton reaction. The hydroxyl radical reacts very quickly with a variety of cellular constituents and can initiate radical-mediated chain processes that damage DNA and membranes, as well as produce carcinogens [26]. The cyclic nature of the Fenton reaction adds to the potential danger. The Fe(III) liberated in this reaction is converted back to Fe(II) via a variety of biological reductants, e.g., ascorbate or glutathione.

In the majority of patients with thalassemia major or other transfusion-dependent refractory anemias, treatment with a chelating agent capable of sequestering iron and permitting its excretion from the body is the only therapeutic option available. Current choices (Figure 1) include Desferal, the mesylate salt [27] of desferrioxamine B (DFO), 1,2-dimethyl-3-hydroxypyridin-4-one (deferiprone, L1) [28,29], and 4-[3,5-bis(2-hydroxyphenyl)-1,2,4-triazol-1-yl]benzoic acid (deferasirox, ICL670A) [30,31]. Each presents with palpable shortcomings [32] and in some cases, serious toxicity issues. DFO, a hexacoordinate hydroxamate iron chelator produced by *Streptomyces pilosus* [33], a siderophore, is not orally active and is best administered subcutaneously (sc) by continuous infusion over long periods of time [34], a patient compliance issue. Deferiprone, while orally active, does not remove enough iron to keep patients in a negative iron balance and has been associated with agranulocytosis [35]. The Novartis drug deferasirox did not show noninferiority to DFO, is associated with a number of serious side effects, and, unfortunately, has a narrow therapeutic window [31,36].

Our pursuit of an efficient orally active iron chelator with an acceptable toxicity profile began with desferrithiocin, (S)-4,5-dihydro-2-(3-hydroxy-2-pyridinyl)-4-methyl-4-

thiazolecarboxylic acid (DFT, **1**, Table 1). DFT is a tridentate siderophore [37,38] excreted by *Streptomyces antibioticus* [37] that forms a stable 2:1 complex with Fe(III); the cumulative formation constant is 4×10^{29} [38]. Initial animal trials with DFT in rodents [39] and primates [40–42] revealed it to be orally active and highly efficient at removing iron. Its iron clearing efficiency (ICE) was $5.5 \pm 3.2\%$ in rodents [39], and $16.1 \pm 8.5\%$ in primates [41] (Table 1). ICE is calculated as (ligand-induced iron excretion/theoretical iron excretion) $\times 100$, expressed as a percent. However, DFT presented with unacceptable renal toxicity in rats [43,44]. Nevertheless, the ligand's remarkable oral bioavailability and ICE drove a very successful structure-activity study aimed at ameliorating DFT-induced nephrotoxicity [40,45–48]. The outcome revealed that removal of the desferrithiocin aromatic nitrogen, providing (*S*)-4,5-dihydro-2-(2-hydroxyphenyl)-4-methyl-4-thiazolecarboxylic acid (desazadesferrithiocin, DADFT), and introduction of a hydroxyl at either the 4'-aromatic to yield (*S*)-2-(2,4-dihydroxyphenyl)-4,5-dihydro-4-methyl-4-thiazolecarboxylic acid [40] [(*S*)-4'-(HO)-DADFT, deferitricin, **2**] or the 3'-aromatic to afford (*S*)-2-(2,3-dihydroxyphenyl)-4,5-dihydro-4-methyl-4-thiazolecarboxylic acid [40] [(*S*)-3'-(HO)-DADFT, **3**] led to chelators with good ICE values (Table 1) and a remarkable reduction in toxicity. Deferitricin (**2**, Figure 1), was taken into human clinical trials by Genzyme. Ligand **2** was well tolerated in patients at doses of 5, 10, or 15 mg/kg/day once daily (s.i.d) for up to 12 weeks [49], and iron clearance levels were approaching the requisite 450 $\mu\text{g/kg/d}$ [50]. However, when the drug was given twice daily (b.i.d) at a dose of 12.5 mg/kg (25 mg/kg/d), unacceptable renal toxicity, i.e., increases in blood urea nitrogen (BUN) and serum creatinine (SCr), were observed in three patients after only 4–5 weeks of treatment and the study was terminated [51]. Nevertheless, the results were attractive enough to compel us to reengineer deferitricin in an attempt to ameliorate the b.i.d. toxicity.

The redesign of **2** was predicated on the observation that both ligands **2** and **3** were orally active iron chelators in rodents and primates (Table 1), and that when the 4'-(OH) of **2** was methylated, providing (*S*)-4,5-dihydro-2-(2-hydroxy-4-methoxyphenyl)-4-methyl-4-thiazolecarboxylic acid [46] [(*S*)-4'-(CH₃O)-DADFT, **4**], there was a remarkable enhancement in both ICE and lipophilicity ($\log P_{\text{app}}$). The $\log P_{\text{app}}$ data are expressed as the log of the fraction of the chelator seen in the octanol layer; measurements were done in TRIS buffer, pH 7.4, using a “shake flask” direct method [52]. The more negative the $\log P_{\text{app}}$, the less chelator is in the octanol phase, the less lipophilic. Unfortunately, the increase in ICE of **4** came with a concomitant increase in toxicity [53]. Thus, the delicate balance between the increase in ICE and toxicity with enhanced lipophilicity needed to be understood and exploited. Ultimately, it was determined that introducing polyether fragments at the 4'-(OH) of **2** to produce (*S*)-4,5-dihydro-2-[2-hydroxy-4-(3,6,9-trioxadecyloxy)phenyl]-4-methyl-4-thiazolecarboxylic acid [53] [(*S*)-4'-(HO)-DADFT-PE, **5**] or the 3'-(OH) of **3**, yielding (*S*)-4,5-dihydro-2-[2-hydroxy-3-(3,6,9-trioxadecyloxy)phenyl]-4-methyl-4-thiazolecarboxylic acid [54] [(*S*)-3'-(HO)-DADFT-PE, **6**] led to less lipophilic, i.e., lower $\log P_{\text{app}}$, remarkably efficient iron chelators (Table 1). Unlike deferitricin, there was no renal toxicity seen with either **5** or **6**, even when the molecules were administered b.i.d. [53–55].

A magnesium salt of ligand **6** (SPD602, deferitazole magnesium, CAS # 1173092-59-5) was evaluated in clinical trials by Shire [56,57]. Since **6** is an oil, and its sodium salt is hygroscopic, dosage form issues may have driven their choice of a magnesium salt. It is interesting to speculate as to whether or not the gastrointestinal (GI) and other side effects observed with the magnesium salt of **6** [56,57] derive from the magnesium itself [58–60]. All of our studies with **6** were conducted with the monosodium salt [54,55,61]. It remains to be seen how well the magnesium salt will perform in patients.

There were, in fact, two properties of the (*S*)-3'-(HO)-DADFT-PE (**6**) that needed attention. The parent drug was an oil, and the dose response curve in rats plateaued very quickly. For example, when **6** was given po to bile duct-cannulated rats at a dose of 150 $\mu\text{mol/kg}$, the drug decorporated 0.782 ± 0.121 mg/kg of iron; the ICE was $18.7 \pm 2.9\%$ [48,61]. However, when the dose of the chelator was further increased to 300 $\mu\text{mol/kg}$, the quantity of iron excreted, 0.887 ± 0.367 mg/kg, was within error of that induced by the drug at 150 $\mu\text{mol/kg}$ ($p > 0.05$), and the ICE was $10.6 \pm 4.4\%$ [48,61]. Thus, the deferration induced by **6** was saturable over a fairly narrow dose range. Additional SAR studies were carried out to search for a chelator that had better physiochemical properties, i.e., a solid that retained its ICE over a wider range of doses. The answer would come with a simple structural modification of (*S*)-4'-(HO)-DADFT-PE (**5**): the 3,6,9-trioxadecyloxy polyether moiety was replaced with a 3,6-dioxaheptyloxy function, providing (*S*)-4,5-dihydro-2-[2-hydroxy-4-(3,6-dioxaheptyloxy)phenyl]-4-methyl-4-thiazolecarboxylic acid [61,62] [(*S*)-4'-(HO)-DADFT-norPE, **7**]. Both acid **7** (Table 1) and its ester precursor [62] were crystalline solids and could be given very effectively in capsules to both rodents and primates. Ligand **7** has excellent ICE properties (~26%) in rodents and primates. It also has a much better dose response in rodents than **6** [61].

Because of the problems with b.i.d. deferitritin, the toxicity issue of most concern with **7**, was, of course, related to renal proximal tubule damage. A series of tolerability studies focusing on (*S*)-4'-(HO)-DADFT-norPE's impact on renal function were carried out in rodents [61]. The drug was given po to rats s.i.d. for 28 d (56.9, 113.8, or 170.7 $\mu\text{mol/kg/day}$); s.i.d. for 10 d (384 $\mu\text{mol/kg/d}$), and b.i.d. at 237 $\mu\text{mol/kg/dose}$ ($474 \mu\text{mol/kg/d}$) $\times$ 7 d [61]. Blood was collected immediately prior to sacrifice and was submitted for a complete blood count (CBC) and serum chemistries, including the determination of the animals' blood urea nitrogen (BUN) and serum creatinine (SCr). No drug-related abnormalities were found, and the rats' BUN and SCr levels were within the normal range [62]. In addition, an assessment of the drug's effect on urinary kidney injury molecule-1 (Kim-1) [63,64] excretion was determined. Kim-1 is a type 1 transmembrane protein located in the epithelial cells of proximal tubules [63,64]. The ectodomain of the Kim-1 proximal tubule protein is released into the urine very early after exposure to a nephrotoxic agent or ischemia; it appears far sooner than increases in BUN or SCr are detected in the serum [65,66]. Administration of **7** to rats for up to 28 d did not elicit any increases in urinary Kim-1 excretion [61]. Extensive tissues were submitted for histopathology; no drug-related abnormalities were identified. Chelator **7** was recently licensed by Sideris Pharmaceuticals, Inc. and is moving forward in clinical trials. This SAR success set the stage for a closer look at how best to further exploit

the relationship between lipophilicity, ICE, and ligand toxicity. The design strategies would weigh heavily on how a chelator's substituents are metabolized.

2. Results And Discussion

2.1 Design Concept

The concept is fairly simple: fix a lipophilic fragment to a chelator that will promote its GI absorption. Once absorbed, it should be quickly converted to its hydrophilic, nontoxic counterpart. The metabolic profiles of the current chelators will thus set the structural boundary conditions for the future design strategies. Early metabolic studies with (*S*)-4'-(CH₃O)-DADFT (**4**), in which the ligand was given sc to rats at a dose of 300 μmol/kg, revealed that it was demethylated in the liver [67], producing (*S*)-4'-(HO)-DADFT (**2**, Figure 2) [45]. At 2 h post drug exposure, about 30% of the (*S*)-4'-(CH₃O)-DADFT (**4**) is demethylated to **2**, and the metabolite remains at fairly high levels through the 8-h time point (Figure 2). This observation encouraged a similar assessment of the polyethers (*S*)-4'-(HO)-DADFT-PE (**5**) and (*S*)-3'-(HO)-DADFT-PE (**6**). If, for example, **5** were converted to **2** to any great extent, this would preclude it being given b.i.d. as renal toxicity might be expected over a long-term exposure. The lack of toxicity of **5** and **6**, even when given b.i.d., suggests that cleavage to **2** was either absent or very modest. Accordingly, chelators **5** and **6** were given to rats sc at a dose of 300 μmol/kg. The tissues that were evaluated included the plasma, liver, kidney, heart, and pancreas. The only organ that presented with any 4'- or 3'-polyether cleavage was the liver (Figure 2). At 2 h, 2% of **5** was converted to **2**, and 2.6% of **6** was metabolized to the corresponding **3**. The metabolites were not detected at the 4 and 8 h time points [48]. This was not surprising. When **7** was given sc to rats under the same experimental protocol as described for **5** and **6**, there was no cleavage to **2** (Figure 2). However, based on studies with other drugs, e.g., glycodiazine, in which polyether fragments were appended [68–72], we were compelled to determine whether or not the terminal methyl of **7** was being cleaved.

With glycodiazine (Figure 3) the methyl ether was dealkylated to an alcohol (metabolite I), which was oxidized to a carboxylic acid (metabolite II) [70]. If this were the case with **7**, for example, this would lead first to the corresponding alcohol (*S*)-4,5-dihydro-2-[2-hydroxy-4-(5-hydroxy-3-oxapentyloxy)phenyl]-4-methyl-4-thiazolecarboxylic acid [(*S*)-4'-(HO)-DADFT-PEA, **8**] and then to the acid (*S*)-4,5-dihydro-2-[2-hydroxy-4-(4-carboxy-3-oxabutyloxy)phenyl]-4-methyl-4-thiazolecarboxylic acid [(*S*)-4'-(HO)-DADFT-PEAA, **9**], Figure 4. Both of these metabolic products would be expected to be very hydrophilic. This increase in hydrophilicity, based on previous studies, would further be expected to minimize ligand toxicity [40,46,48]. If indeed such a demethylation-oxidation scenario is occurring with **7**, it could support an innovative approach to “metabolically programmed” iron chelators, i.e., highly lipophilic, orally absorbable ligands that are quickly converted to hydrophilic, likely nontoxic metabolites.

The two putative metabolites of (*S*)-4'-(HO)-DADFT-norPE (**7**), the alcohol (*S*)-4'-(HO)-DADFT-PEA (**8**) and the carboxylic acid (*S*)-4'-(HO)-DADFT-PEAA (**9**), were assembled. These two synthetic chelators allowed us to develop an analytical high-pressure liquid

chromatography (HPLC) method to follow the potential conversion of **7** to its metabolites in the organs of animals treated with the parent chelator **7**. Furthermore, it provided an opportunity to evaluate the lipophilicity ($\log P_{app}$), and the ICE values of **8** and **9** when the chelators were given to the rats and primates po and/or sc.

2.2 Synthesis of **8** and **9**

Assembly of alcohol **8** and the carboxylic acid **9** (Scheme 1) began with alkylation of deferitricin ethyl ester (**15**) at the 4'-hydroxyl [55]. Specifically, reaction of **15** with 2-(2-chloroethoxy)ethanol (**16**), K_2CO_3 and KI in DMF at 100 °C provided the alcohol ester **17** in 62% yield. Treatment of **17** with 50% NaOH in CH_3OH led to the alcohol **8** in 97% yield. Alkylating **15** with ethyl 2-chloroethoxyacetate (**18**) [73] under the above conditions gave the diester **19** in 35% yield. Saponification of **19** furnished the diacid **9** as its monosodium salt in 98% yield.

2.3 Tissue Distribution/Metabolism of (S)-4'-(HO)-DADFT-norPE (**7**)

As described above, when **7** was given sc to rats at a dose of 300 $\mu\text{mol/kg}$, there was no cleavage to **2** (Figure 2). When the tissues were subjected to further analysis via HPLC for the presence of **8** or **9**, cleavage of the terminal methyl of **7** to the corresponding alcohol **8** did occur (Figure 5). However, carboxylic acid **9** was not detected, probably because the extent of the metabolism of the parent **7** to **8** was so minor. However, when *synthetic* alcohol **8** was given sc to rats, it was very efficiently converted to acid **9**.

Rodents were administered synthetic alcohol **8** sc at a dose of 300 $\mu\text{mol/kg}$. The rats were euthanized 0.5, 1, 2, 4, and 8 h post drug. The animals' plasma, liver, kidney, heart and pancreas were removed and assessed for the presence of **8** and its putative metabolite **9**. The extent and rapidity of oxidation of **8** to **9** that unfolded was surprising (Figure 5). In the plasma at 0.5 h post drug, nearly 60% of **8** had been converted to **9**. At 2 h post dosing, 88% of the drug is in the form of the metabolite. Neither the parent **8** nor the metabolite **9** was found at the 8 h time point. A similar story unfolds in the liver (Figure 5). At 0.5 h post drug, only 53% of the drug (parent + metabolite) is in the form of the parent **8**. At 1 h, the metabolite **9** comprises 59% of the total. The parent vs metabolite ratio is similar at the 2 and 4 h time points. At 8 h, very little parent alcohol **8** and no metabolite **9** remains. The kidney, heart and pancreas also demonstrated a similar and significant conversion of **8** to **9** (Figure 5). These findings set the stage for an innovative approach to metabolically programmed ligands. The question, then, became would such alcohols and acids represent useful chelators, with good ICEs and no toxicity issues?

2.4 Chelator-Induced Iron Clearance of **2** and **7–9** in Non-Iron-Overloaded, Bile Duct-Cannulated Rodents

The ICE values for compounds **2** and **7** (Table 2) are historical and included for comparative purposes. The chelators were given to the rats po at a dose of 300 $\mu\text{mol/kg}$; **9** was also given sc at the same dose. Compound **2** ($\log P_{app} = -1.05$) was the least effective ligand, with an ICE of $1.1 \pm 0.8\%$ [53]. Analogue **7** ($\log P_{app} = -0.89$) was the most effective, with an ICE of $26.7 \pm 4.7\%$ [62]. Ligands **8** ($\log P_{app} = -0.53$) and **9** ($\log P_{app} = -1.63$), the two putative

metabolites of **7**, were significantly less active than the parent drug **7** when given po. The ICE of **8** was $15.4 \pm 5.6\%$ ($p < 0.02$), while the ICE of **9** was $6.2 \pm 1.7\%$ ($p < 0.005$). As ligand **9** is very hydrophilic ($\log P_{app} = -1.63$) the lack of activity on po administration was likely due to its poor oral absorption. Indeed, when **9** was given to the rats sc at a dose of $300 \mu\text{mol/kg}$, its ICE, $11.3 \pm 3.4\%$, was nearly twice that when the drug was dosed po ($p < 0.05$).

2.5 Chelator-Induced Iron Clearance of **2** and **7–9** in Iron-Overloaded Primates

The primate iron clearance data are provided in Table 2. The ICE values for compounds **2** and **7** are historical and included for comparative purposes. The chelators were given to the monkeys po at a dose of 75 (**7–9**) or $150 \mu\text{mol/kg}$ (**2**); **9** was also given to the primates sc at a dose of $75 \mu\text{mol/kg}$. The ICE of ligand **2** was $16.8 \pm 7.2\%$ [53]. As with the rats, compound **7** was the most effective iron decorporation agent, with an ICE of $26.3 \pm 9.9\%$ when it was given po in capsules, and an ICE of $28.7 \pm 12.4\%$ when it was administered po as its monosodium salt [62]. Although **8**, the putative metabolite of **7**, is more lipophilic than **7**, $\log P_{app} = -0.53$ vs -0.89 , its ICE was lower, $9.8 \pm 3.4\%$ ($p < 0.003$, Table 2). The ICE of **9** given po was even lower, only $1.7 \pm 1.4\%$. When **9** was administered sc to the *same* group of monkeys that had been given the drug po, the ICE increased by more than 10-fold, to $17.4 \pm 9.7\%$ ($p < 0.03$). This is in keeping with the idea that highly charged ligands like **9** ($\log P_{app} = -1.63$) do not make it across the intestinal mucosa, but hydrophilic metabolic precursors do.

2.6 Metabolically Programmed Iron Chelators

The design of metabolically programmed iron chelators is thus derived from the observation that alcohol **8** is very efficiently oxidized to carboxylic acid **9** (Figure 4 and 5). The corollary to all of this is that the replacement of the 3,6-dioxaheptyl group of the parent polyether (*S*)-4'-(HO)-DADFT-norPE (**7**) with a long chain alcohol should provide a highly lipophilic chelator with good po absorption. Once absorbed, the ligand is likely to be metabolized to its less lipophilic, less toxic acid counterparts. This metabolic programming may offer an innovative way to exploit the delicate balance seen between enhanced chelator lipophilicity and ICE, and ameliorate the concomitant increase in toxicity usually associated with increases in lipophilicity.

Accordingly, five different ligands predicated on the (*S*)-4'-(HO)-DADFT platform were assembled (Schemes 2 and 3): 1) the hexamethylene analogue of **7**, (*S*)-4,5-dihydro-2-[2-hydroxy-4-(6-methoxyhexyloxy)phenyl]-4-methyl-4-thiazolecarboxylic acid [(*S*)-4'-(HO)-DADFT-HXME, **10**]; 2) the corresponding alcohol of **10**, (*S*)-4,5-dihydro-2-[2-hydroxy-4-(6-hydroxyhexyloxy)phenyl]-4-methyl-4-thiazolecarboxylic acid [(*S*)-4'-(HO)-DADFT-HXA, **11**]; 3) the putative first metabolite of **11**, (*S*)-4,5-dihydro-2-[2-hydroxy-4-(5-carboxypentyloxy)phenyl]-4-methyl-4-thiazolecarboxylic acid [(*S*)-4'-(5-carboxypentyloxy)-DADFT, **12**]; 4) the β -oxidation product of **11**, (*S*)-4,5-dihydro-2-[2-hydroxy-4-(3-carboxypropyloxy)phenyl]-4-methyl-4-thiazolecarboxylic acid [(*S*)-4'-(3-carboxypropyloxy)-DADFT, **13**], and 5) the second β -oxidation product of **11**, (*S*)-4,5-dihydro-2-[2-hydroxy-4-(carboxymethoxy)phenyl]-4-methyl-4-thiazolecarboxylic acid [(*S*)-4'-(carboxymethoxy)-DADFT, **14**]. The conversion of the parent alcohol **11** to its

putative metabolites **12–14** (Figure 6) was assessed in rats given **11** sc at a dose of 300 $\mu\text{mol/kg}$. In addition, the ligands were evaluated for their log P_{app} , and their ICE in rats and primates (Table 2).

2.7 Synthesis of Deferitricin Hexamethylene Methyl Ether, (S)-4'-(HO)-DADFT-HXME (**10**), the Corresponding Alcohol Analogue, (S)-4'-(HO)-DADFT-HXA (**11**), and its Putative Metabolites **12**, **13**, and **14**

Synthesis of the methyl ether-containing chelator **10** required first generating 6-iodo-1-methoxyhexane (**21**) [74] from 1,6-diiodohexane (**20**) in 28% yield by employing 25% NaOCH_3 (1 equivalent) in DMF at 63 °C (Scheme 2). Alkylation of deferitricin ethyl ester (**15**) with **21** using K_2CO_3 in DMF at 62 °C, generated intermediate **22** in 59% yield. Alkaline cleavage of ester **22** provided (S)-4'-(HO)-DADFT-HXME (**10**) in 97% yield. The synthesis of alcohol **11** involved first alkylating the ester of deferitricin (**15**) with 6-iodohexyl acetate (**23**) [75] utilizing K_2CO_3 in DMF at 70 °C, giving diester **24** in 53% yield (Scheme 2). Alkaline hydrolysis of **24** provided the final product **11** in 91% recrystallized yield.

Synthesis of the anticipated metabolites of alcohol **11** (**12–14**) followed methodology similar to that of polyether acid **9** (Scheme 3). The DADFT ester **15** was selectively alkylated with one of three ethyl ω -bromoalkanoates (**25**, **26**, or **27**) in DMF in the presence of K_2CO_3 and catalytic iodide salt. The corresponding diesters **28**, **29**, and **30** were obtained in 61, 72, and 66% yields. Once again, these diesters were cleaved in 50% NaOH in CH_3OH , giving the required acids **12**, **13**, and **14** in 99, 90, and 98% yields, respectively.

2.8 Tissue Distribution/Metabolism of (S)-4'-(HO)-DADFT-HXA (**11**): A Metabolically Programmed Ligand

In order to verify that conversion of the alcohol **11** to its putative metabolites **12–14** (Figure 6) was occurring *in vivo*, rats were given the parent alcohol **11** sc at a dose of 300 $\mu\text{mol/kg}$. The rats were euthanized 0.5, 1, 2, 4, and 8 h post drug. The conversion of **11** $\rightarrow$ **12–14** was assessed in the animals' plasma, liver, kidney, heart and pancreas. Significant conversion of **11** $\rightarrow$ **12–14** was found in all of the tissues examined (Figure 7). The extent and rapidity of metabolism of **11** $\rightarrow$ **12–14** that unfolded was surprising. Chelator **11** and its metabolites achieve a concentration of 400 μM in the plasma at 0.5 h post drug. This tissue concentration is very similar to that seen with the 4-norpolyether **7** itself (Figure 5). However, again with **7**, the parent represented 95% of the total drug concentration, while its metabolite (**8**) only accounted for 5% of the total drug. In the case of **11** in the plasma at 0.5 h, only 22% of the total (parent + metabolites) was in the form of the parent **11** (Figure 7). The first β -oxidation product **13** comprised 62% of the total, while the second β -oxidation product **14** represented 16% of the total. The ratio of the parent **11** to its metabolites **13** and **14** was similar at the 1 and 2 h time points. By 4 h post dosing, the parent **11** was no longer detectable, and metabolites **13** and **14** each accounted for 50% of the total. At 8 h post dosing, the total plasma drug concentration had decreased to 23 μM . Metabolite **13** accounted for 65% of this quantity, while the remainder was in the form of metabolite **14**. None of the first oxidation product of alcohol **11**, the dicarboxylic acid **12**, was detected in the plasma.

Significant conversion of **11**→**12–14** also occurred in the liver (Figure 7). Interestingly, the parent **11** was not found in the liver at any of the time points. However, unlike the plasma, small quantities of first oxidation product of alcohol **11**, the dicarboxylic acid **12**, were found in the liver 0.5 and 1 h post drug. At 0.5 h, **12** represented 11% of the total drug (parent + metabolites); the first β -oxidation product **13** comprised 71% of the total, while the second β -oxidation product **14** represented 18% of the total. At 1 h post dosing, the concentration of metabolites **12** and **13** in the liver had decreased to 2% and 73% of the total, respectively, while the proportion of **14** had increased to 25% (Figure 7). Ligand **12** was not detected in the liver 2, 4, or 8 h post dosing. Metabolite **13** accounted for 66% of the total drug (parent + metabolites) at 2 h, and 70% of the total at 4 h. By 8 h post dosing, no **13** remained in the liver, and only trace amounts of **14** were detected.

The parent drug **11** was found in the kidney in trace amounts 0.5 h post drug, and the carboxylic acid **12** was found at 0.5 and 1 h post drug. Metabolite **13**, the first β -oxidation product, achieves very high levels in the kidney, ~300 nmol/g wet weight at 0.5 and 1 h, but is not detectable 8 h post dosing. Metabolite **14**, the second β -oxidation product, also reached high levels in the kidney at 0.5, 1, and 2 h, 156, 201, and 161 nmol/g wet weight, respectively, but had decreased to only 5 nmol/g wet weight at 8 h.

The parent drug **11** was found in the heart at 0.5 h post drug, 26 nmol/g wet weight. The dicarboxylic acid **12** was not found in the cardiac tissue at any of the time points. The concentration of metabolite **13** in the heart was ~46 nmol/g wet weight 0.5 and 1 h post drug, but was not found in the later time points. Metabolite **14** was found in the heart 0.5 and 1 h post dosing, 17 and 23 nmol/g wet weight, respectively, but was not found in the 2–8 h time points (Figure 7).

In the pancreas, the parent **11** was only found 0.5 h post drug, < 5 nmol/g wet weight. Metabolite **12** was not found at any of the time points. Ligand **13** was present in the 0.5 and 1 h samples, 19 and 23 nmol/g wet weight, respectively (Figure 7). Trace amounts of **14** were found at 0.5, 1, and 2 h post dosing. Ligands **11–14** were not detected in the pancreas at 4 or 8 h post drug.

2.9 Chelator-Induced Iron Clearance of **10–14** in Non-Iron-Overloaded, Bile Duct-Cannulated Rodents

Ligands **10–14** were administered to the rats po at a dose of 300 μ mol/kg; **12–14** were given to the animals sc at the same dose. The first chelator evaluated in the rats was **10**, the non-metabolizable methyl ether analogue of **11**. Ligand **10** is very lipophilic ($\log P_{\text{app}} = 0.95$), and is also profoundly toxic. When it was given po to bile duct-cannulated rats, one of animals died ~22 h post drug. The remaining rodents were euthanized 24 h post dosing due to their rapidly deteriorating condition. We had seen this same scenario with (*S*)-2-(4-butoxy-2-hydroxyphenyl)-4,5-dihydro-4-thiazolecarboxylic acid, the 4'-butoxy analogue of **2** ($\log P_{\text{app}} = 1.02$), with deaths occurring within 24 h [76]. Nonetheless, **10** was a very active decorporation agent. The baseline iron output for rats treated with **10** was 5 μ g/kg of iron. The drug-induced iron excretion peaked 6 h post drug, 300 μ g/kg of iron, and was still 80 μ g/kg of iron when the rodents were euthanized 24 h post dosing. The ICE of the drug

was $15.8 \pm 3.7\%$ (Table 2), and clearly would have been higher had the animals survived. The ICE of **10** was not assessed in the primates due to the overt toxicity seen with the rodents. The ICE and toxicity of **10** is in keeping with the molecule's lipophilicity. Next, **11**, the *O*-demethylated, metabolically labile analog of **10** was evaluated.

An alkanol, e.g., a 6-(HO) hexyl fragment, was fixed to the 4'-(HO) position of **15** leading to **11** (Scheme 2). This resulted in a less lipophilic, $\log P_{app} = 0.21$, less toxic ligand than **10**. Chelator **11** was given to the rats po at a dose of 300 $\mu\text{mol/kg}$. The drug was well absorbed, had an ICE of $9.9 \pm 0.8\%$, and did not present with any overt toxicity. As described above, when ligand **11** was given to rats sc, it was quickly converted to the corresponding hydrophilic metabolites, carboxylic acid (**12**, $\log P_{app} = -1.90$) [68,70], with β -oxidations [77] to acids **13** $\log P_{app} = -2.21$ and acid **14** $\log P_{app} = -1.98$ (Figure 6). The ICEs of **12**–**14** were determined in rodents given the drugs po and sc at a dose of 300 $\mu\text{mol/kg}$. The po ICE of the putative first metabolite, **12**, was similar to that of **11**, $8.8 \pm 1.8\%$ ($p > 0.05$). However, the po ICEs of **13** and **14** were significantly less than **11**, $3.7 \pm 1.7\%$ ($p < 0.001$) and $2.6 \pm 1.6\%$ ($p < 0.005$), respectively. When **12** and **13** were given to the rats sc, their ICEs were within error of when the drugs were dosed po (Table 2). In contrast, the ICE of **14** given sc ($6.0 \pm 1.9\%$) was more than twice that of when the drug was given po ($2.6 \pm 1.6\%$, $p < 0.05$).

2.10 Chelator-Induced Iron Clearance of **11**–**14** in Iron-Overloaded Primates

Ligand **11** was administered to the primates po at a dose of 75 $\mu\text{mol/kg}$; **12**–**14** were given po and sc at the same dose. Ligand **11**, in which a 6-(HO)-hexyl fragment was fixed to the 4'-(HO) position of **2**, was very lipophilic ($\log P_{app} = 0.21$). The ICE of this compound given to the monkeys po was $21.9 \pm 3.6\%$ (Table 2). The ICEs of the much more hydrophilic metabolites of **11**, **12**–**14**, given po to the monkeys, were all significantly less than the parent. The first oxidation product of alcohol **11**, the dicarboxylic acid **12** ($\log P_{app} = -1.90$), had an ICE of $10.6 \pm 4.0\%$ in primates when it was given po ($p < 0.01$). The first and second β -oxidation products, **13** ($\log P_{app} = -2.21$) and **14** ($\log P_{app} = -1.98$), were also significantly less effective than **11** when they were administered po, $5.4 \pm 1.5\%$ ($p < 0.005$) and $3.0 \pm 2.7\%$ ($p < 0.002$), respectively. Again, the reason for the substantial reduction in the efficacy of **12**–**14** dosed po was likely due to the poor GI absorption of the dicarboxylic acids because of their charge: they are dianions at physiological pH. In order to confirm this, ligands **12**–**14** were given to the primates sc. In each case, the *same* animals that had been given the drugs po were also given the chelators sc.

The po ICE of **12** in the monkeys was $10.6 \pm 4.0\%$; the ICE increased to $18.8 \pm 8.7\%$ when the ligand was given sc, but the increase was not significant ($p = 0.06$). The ICE of **13** increased significantly in the primates, from $5.4 \pm 1.5\%$ when it was dosed po to $18.1 \pm 7.5\%$ when it was administered sc ($p < 0.03$). Finally, the ICE of the second β -oxidation product **14** in the primates was $3.0 \pm 2.7\%$ when it was given po. The ICE increased significantly, to $15.9 \pm 4.3\%$, when it was given to the same animals sc ($p < 0.001$). Thus, the importance of ligand charge, $\log P_{app}$, takes on a much more significant role in the primate model.

2.11 ICE Observations

Several generalizations can be derived from Table 2. The performance ratio (PR), $ICE_{\text{primate}}/ICE_{\text{rodent}}$, shows that although **8** and **9** (po) were more effective at iron decorporation in the rats than in primates, the remaining ligands **11–14** are either as effective or better at iron clearance in the primates. In the rodents, **9** and **14** were approximately twice as effective sc as when they were dosed po. There was also a dramatic difference in the sc vs po ICEs of **9**, **13**, and **14** in the primates: their sc ICEs were 10.2, 3.4, and 5.3 times higher, respectively. The ICE of **12** was also increased upon sc dosing, but the increase was not significant ($p = 0.06$).

2.12 Toxicology Profile of (S)-4'-(HO)-DADFT-HXA (**11**), A Metabolically Programmed Chelator

The concept behind the development of metabolically programmed iron chelators is predicated on the administration of highly lipophilic drugs that will be well absorbed orally, present with excellent ICE properties, and, to minimize potential toxicity, must be quickly metabolized to less lipophilic but still active deferration agents. For example, deferitricin analog **10** (Table 2), with a non-metabolizable terminal methyl ether, was highly lipophilic and was an effective iron clearing agent in the bile duct-cannulated rats. Unfortunately, the chelator was profoundly toxic. Conversely, the corresponding demethylated compound, alcohol **11**, which is also lipophilic, had excellent ICE properties in rodents and primates and did not display any overt toxicity. As described above, ligand **11** was well absorbed, and, in a tissue distribution/metabolism study, was shown to be quickly converted to the corresponding very hydrophilic acids **12**, **13**, and **14** (Figure 6). However, the real litmus test for the success of the metabolically programmed chelators, e.g., **11**, is dependent upon their toxicity profile.

Accordingly, a ten-day toxicity trial of **11** was carried out in male Sprague-Dawley rats. The animals were housed in individual metabolic cages. Ligand **11** was given po by gavage once daily for ten days at a dose of 384 $\mu\text{mol/kg/day}$. Note that this dose is equivalent to 100 mg/kg/day of DFT (**1**) as its sodium salt. Urine was collected from the metabolic cages at 24-h intervals and assessed for its Kim-1 content. The studies were performed on rats with normal iron stores; each animal served as its own control. Additional age-matched rats served as untreated controls for the CBC and serum chemistry assessments and histopathology.

All of the rats treated with **11** survived the exposure to the drug. The animals were bright, alert and responsive at the beginning of the study and remained that way throughout the course of the experiment. The rodents' baseline urinary Kim-1 excretion was $< 20 \text{ ng/kg/24 h}$ and did not exceed this level at any time during the 10-day exposure to the chelator. The rats were sacrificed 24 h post drug. Blood was submitted for routine CBC and serum chemistry analysis. The BUN of the treated rats, $20 \pm 4 \text{ mg/dl}$, was within error of that of the untreated controls, $21 \pm 2 \text{ mg/dl}$ ($p > 0.05$). In addition, the SCr for both groups was $0.5 \pm 0.1 \text{ mg/dl}$ ($p > 0.05$). Note that these values are well within the normal range for this species: 9–30 mg/dl for BUN, and 0.4–1.0 mg/dl for SCr [78]. Extensive tissues (25/rat) were submitted to an outside lab for assessment of histopathology. The pathologist could not

identify any drug-related abnormalities: “There are no definitive differences in the organs of the two groups of rats.”

Taken together, these results unequivocally demonstrate that metabolically programmed ligands that are highly effective deferration agents can be successfully designed. As predicted, the parent in this case, a lipophilic alcohol, **11**, was well absorbed and was quickly metabolized to hydrophilic ligands that, collectively, have excellent ICEs with little to no discernable toxicity. Thus, the concept of developing “metabolically programmed” chelators, i.e., highly lipophilic, orally absorbable ligands that are quickly converted to hydrophilic, nontoxic metabolites (e.g., Figure 8), is indeed a credible approach.

3. Conclusion

A number of notable outcomes were derived from the metabolic studies of (*S*)-4'-(HO)-DADFT-norPE (**7**). First, ligand **7** does not sustain metabolic cleavage at the 4'-(HO) to yield **2** (Figure 2). However, what remained unclear is whether or not the terminal methyl on the polyether fragment of **7** is metabolically labile. If this were the case, it would likely be converted first to the corresponding alcohol, **8**, and then to the carboxylic acid, **9**, Figure 4. Both of these metabolic products would be expected to be very hydrophilic. This increase in hydrophilicity, based on previous studies, could further be expected to minimize ligand toxicity [40,46,48]. If indeed such a demethylation-oxidation scenario is occurring with **7**, it could support a novel approach to “metabolically programmed” iron chelators, i.e., highly lipophilic chelators that would be absorbed well orally, but would then be quickly metabolized to hydrophilic, nontoxic ligands.

The putative metabolites of **7**, **8** and **9**, were assembled. The alcohol **8** has a 3-oxa-5-hydroxypentyl fragment fixed to the 4'-(HO); the acid **9** has a 3-oxa-4-carboxybutyl group on the 4'-(HO). These two synthetic chelators allowed us to develop an analytical HPLC method to follow the potential metabolism of **7**. When the tissues of rats treated with **7** sc were subjected to further analysis via HPLC for the presence of **8** or **9**, cleavage of the terminal methyl of **7** to the corresponding alcohol **8** did occur (Figure 5). However, carboxylic acid **9** was not detected, probably because the extent of the metabolism of the parent **7** to **8** was so minor. In order to verify that conversion of the alcohol **8** to the acid **9** could occur efficiently, rodents were given the *synthetic* alcohol **8** sc at a dose of 300 μ mol/kg. Indeed, alcohol **8** was very quickly oxidized to **9** (Figure 5).

When given po to rodents and primates, neither of the synthetic metabolites of **7**, alcohol **8** nor the acid **9**, had ICE values as high as the parent ligand. The acid **9**, given po, was particularly ineffective. However, when given sc, the ICE of **9** doubled in rodents, and was 10 times higher in primates than when it was given to the same monkeys po (Table 2). This is in keeping with the idea that highly charged ligands do not make it across the intestinal mucosa. Taken collectively, the data suggested that fixing a lipophilic alcohol fragment to the 4'-(HO) of **2** would provide a chelator that should be lipophilic, orally absorbed, and quickly converted to hydrophilic acid metabolites.

Initially a non-metabolizable 6-methoxyhexyl group was appended to the 4'-(HO) of **2**, providing methyl ether **10**. This ligand was very lipophilic ($\log P_{\text{app}} = 0.95$), had an ICE of $15.8 \pm 3.7\%$ in the rats, and was very toxic. We had seen this scenario before with a 4'-butoxy analogue of **2** ($\log P_{\text{app}} = 1.02$), with deaths occurring within 24 h [76]. Nevertheless, this ligand did serve to identify the upper boundary of the lipophilicity/toxicity relationship for this structural family.

Subsequently, a *metabolizable* 6-hydroxyhexyl group was fixed to the 4'-(HO) of **2**, providing alcohol **11** (Table 2). Ligand **11** is still very lipophilic, $\log P_{\text{app}} = 0.21$, but it did not elicit any signs of overt toxicity. As predicted, each of the metabolites of **11** (**12**, **13**, and **14**) are very hydrophilic, moving from a $\log P_{\text{app}} = 0.21$ for the parent to -1.90 for acid **12**, -2.21 for acid **13**, and -1.98 for acid **14** (Figure 6). When given to rats sc, **11** is very quickly converted to the corresponding acid (**12**), and by β -oxidation to acid **13** and finally to acid **14** (Figure 7).

The po ICE of ligand **11** in the rats is $9.9 \pm 0.8\%$. The po ICE of **12** was similar, while the po ICEs of **13** and **14** were significantly less. In the primates, the po ICE of the parent **11** was $21.9 \pm 3.6\%$. The po ICEs of the hydrophilic metabolites, **12–14**, were all significantly less in the monkeys than the parent alcohol **11** (Table 2). Again, this is in keeping with the idea that highly charged ligands do not make it across the intestinal mucosa. To confirm this, ligands **12–14** were given sc to the rodents and primates. In the rats, the sc ICEs of **12** and **13** were within error of their po values, while that of **14** sc was significantly greater than when the drug was given po. In the monkeys, the sc ICE of **12** did increase vs po dosing, but the increase was not significant. In contrast, the sc ICEs of **13** and **14** were 3.4 and 5.3 times greater, respectively, than when the drugs were given to the same animals po (Table 2). The most notable finding was the complete lack of toxicity with ligand **11** when given to rodents once daily for 10 days at a dose of $384 \mu\text{mol/kg/day}$. The toxicity difference between **10** and **11** was profound. This substantiates the idea that the lipophilic parent chelator is quickly converted to hydrophilic, nontoxic deferration metabolites. Thus, the concept of developing “metabolically programmed” chelators, i.e., highly lipophilic, orally absorbable and effective molecules that are quickly converted to their hydrophilic counterparts, is indeed a credible approach in the design of highly effective iron chelators. Figure 8 probably best illustrates the concept established in this preliminary study.

4. Experimental Section

4.1 Biology

4.1.1 Materials—Male *Cebus apella* monkeys were obtained from World Wide Primates (Miami, FL). Male Sprague-Dawley rats were procured from Harlan Sprague-Dawley (Indianapolis, IN). Ultra-pure salts were obtained from Johnson Matthey Electronics (Royston, UK). All hematological and biochemical studies were performed by Antech Diagnostics (Tampa, FL). Histopathological analysis was carried out by Florida Vet Path (Bushnell, FL). Atomic absorption (AA) measurements were made on a Perkin-Elmer model 5100 PC (Norwalk, CT).

4.1.2 Biological Methods—All animal experimental treatment protocols were reviewed and approved by the University of Florida's Institutional Animal Care and Use Committee.

4.1.3 Cannulation of Bile Duct in Non Iron-Overloaded Rats—The cannulation has been described previously [41,43]. Bile samples were collected from male Sprague-Dawley rats (400–450 g) at 3 h intervals for up to 48 h. The urine sample(s) was taken at 24 h intervals. Sample collection and handling are as previously described [41,43].

4.1.4 Iron Loading of *Cebus apella* Monkeys—The monkeys (3.5–6.5 kg) were iron overloaded with intravenous iron dextran as specified in earlier publications to provide about 500 mg of iron per kg of body weight; the serum transferrin iron saturation rose to between 70 and 80% [41,43]. At least 20 half-lives, 60 days, elapsed before any of the animals were used in experiments evaluating iron-chelating agents.

4.1.5 Primate Fecal and Urine Samples—Fecal and urine samples were collected at 24 h intervals and processed as described previously [41,43,79]. 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 absorption spectroscopy as presented in other publications [41,43].

4.1.6 Drug Preparation and Administration—In the iron clearing experiments, the rats were given **8–14** orally at a dose of 300 $\mu\text{mol/kg}$. Ligand **9** and **12–14** were also given sc at the same dose. The primates were given **8–9** and **11–14** orally at a dose of 75 $\mu\text{mol/kg}$. Ligand **9** and **12–14** were also given sc at the same dose. The chelators were administered as their monosodium salts (prepared by the addition of 1 equiv of NaOH to a suspension of the free acid in distilled water).

4.1.7 Calculation of Chelator Iron Clearing Efficiency (ICE)—The term “ICE” is used as a measure of the amount of iron excretion induced by a chelator. The ICE, expressed as a percent, is calculated as (ligand- induced iron excretion/theoretical iron excretion) $\times$ 100. To illustrate, the theoretical iron excretion after administration of 1 mmol of DFO, a hexadentate chelator that forms a 1:1 complex with Fe(III), is 1 milli-g-atom of iron. The theoretical iron outputs of the chelators were generated on the basis of a 2:1 ligand:iron complex. The efficiencies in the rats and monkeys were calculated as set forth elsewhere [40]. Data are presented as the mean $\pm$ the standard error of the mean; *p*-values were generated via a one-tailed Student's *t*-test in which the inequality of variances was assumed, and a *p*-value of <0.05 was considered significant. The *p*-values for the monkeys that were given **9** and **12–14** po and sc were generated via a one-tailed, paired Student's *t*-test; a *p*-value of <0.05 was considered significant.

4.1.8 Collection of Chelator Tissue Distribution Samples from Rodents—Male Sprague-Dawley rats (250–350 g) were given a single sc injection of the monosodium salts of **8** and **11** prepared as described above at a dose of 300 $\mu\text{mol/kg}$. At times 0.5, 1, 2, 4 and 8 h after dosing ($n=3$ rats per time point), the animals were euthanized by exposure to CO₂ gas. Blood was obtained via cardiac puncture into vacutainers containing sodium citrate.

The blood was centrifuged, and the plasma was separated for analysis. The liver, kidney, heart, and pancreas were removed from the animals and frozen.

4.1.9 Tissue Analytical Methods—Tissue samples of animals treated with **8** were prepared for HPLC analysis by homogenizing them in H₂O at a ratio of 1:1 (w/v). Then, as a rinse, CH₃ OH at a ratio of 1:3 (w/v) was added, and the mixture was stored at –20°C for 30 min. This homogenate was centrifuged. The supernatant was diluted with H₂O, vortexed, and filtered with a 0.2 µm membrane. Analytical separation was performed on a Supelco Discovery RP Amide C16 HPLC system with UV detection at 310 nm as described previously [47,80]. Mobile phase and chromatographic conditions were as follows: solvent A, 5% CH₃ CN/95% buffer (25 mM KH₂ PO₄ + 2.5 mM 1-octanesulfonic acid, pH 3.0); solvent B, 60% CH₃ CN/40% buffer.

Tissue samples of animals treated with **11** were prepared for HPLC analysis by homogenizing them in 0.5 N HClO₄ at a ratio of 1:3 (w/v). Then, as a rinse, CH₃ OH at a ratio of 1:3 (w/v) was added, and the mixture was stored at –20°C for 30 min. This homogenate was centrifuged. The supernatant was diluted with 95% buffer (25 mM KH₂ PO₄, pH 3.0)/5% CH₃ CN, vortexed, and filtered with a 0.2 µm membrane. Analytical separation was performed on a Supelco Ascentis Express RP-Amide HPLC system with UV detection at 310 nm as described previously [47,80]. Mobile phase and chromatographic conditions were as for **8**.

Ligand concentrations were calculated from the peak area fitted to calibration curves by nonweighted least-squares linear regression with Shimadzu Class-VP 7.4 software. The method had a detection limit of 0.25 µM and was reproducible and linear over a range of 1–1000 µM. Tissue distribution data are presented as the mean; *p*-values were generated via a one-tailed student's *t*-test, in which the inequality of variances was assumed; a *p*-value of < 0.05 was considered significant.

4.1.10 Toxicity Assessment of (S)-4'-(HO)-DADFT-HXA (11) in Rats—A 10-day toxicity trial on ligand **11** was performed in rodents. Male Sprague-Dawley rats (*n*=5, 375–400 g) were given the drug, administered as its monosodium salt, po once daily for 10 days at a dose of 384 µmol/kg/day. Note that this dose is equivalent to 100 mg/kg/day of DFT (**1**) as its sodium salt. The rats were housed in individual metabolic cages and were weighed each day. A baseline (day 0) urine sample was collected and assessed for its Kim-1 content [48,61]; each animal served as its own control. Chilled urine was collected from the metabolic cages at 24-h intervals as previously described [61] to allow for the determination of Kim-1 levels.

The rats were fasted overnight and were given the chelator first thing in the morning. The rodents were fed •3 h post-drug and had access to food for ~5 h before being fasted overnight. The animals were euthanized one day post drug (day 11). Blood was collected for the performance of a routine CBC and serum chemistries [81]. Extensive tissues [40] were collected and submitted to an outside laboratory for histopathological analysis. Additional age-matched rats served as untreated controls for the CBC and serum chemistries and

histopathology. No urine was collected from these animals. The studies were performed on rats with normal iron stores.

4.2. Chemistry

Reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI). Fisher Optima grade solvents were routinely used. DMF was dried over 4 Å molecular sieves. Potassium carbonate was flame activated and cooled in a desiccator over Drierite. Reactions were run under a nitrogen atmosphere, and organic extracts were dried with sodium sulfate and filtered. Silica gel 40–63 from SiliCycle, Inc. (Quebec City, Quebec, Canada) was used for column chromatography. Glassware that was presoaked in 3 N HCl for 15 min, washed with distilled water and distilled EtOH, and oven-dried was used during the isolation of **8–14**. Melting points are uncorrected. Optical rotations were run at 589 nm (sodium D line) and 20 °C on a Perkin-Elmer 341 polarimeter, with *c* being concentration in grams of compound per 100 mL of solution (CHCl₃ not indicated). NMR spectra were obtained at 400 MHz (¹H) or 100 MHz (¹³C). Chemical shifts (δ) for ¹H spectra are given in parts per million downfield from tetramethylsilane for organic solvents (CDCl₃ not indicated) or sodium 3-(trimethylsilyl)propionate-2,2,3,3-*d*₄ for D₂O. Chemical shifts (δ) for ¹³C spectra are given in parts per million referenced to CH₃OH (δ 49.50) in D₂O or to the residual solvent resonance in CDCl₃ (δ 77.16) (not indicated) or DMSO-*d*₆ (δ 39.52). The base peaks are reported for the ESI-FTICR mass spectra. Elemental analyses were performed by Atlantic Microlabs (Norcross, GA) and were within ± 0.4% of the calculated values. The purity of all compounds was confirmed by elemental analysis. Furthermore, the purity of **8–14** was 95% by HPLC analysis.

4.2.1. (S)-4,5-Dihydro-2-[2-hydroxy-4-(5-hydroxy-3-oxapentyloxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (8)—A solution of 50% (w/w) NaOH (3.0 mL, 57 mmol) in CH₃OH (25 mL) was added slowly to a solution of **17** (2.0 g, 5.4 mmol) in CH₃OH (50 mL) at 0 °C. The reaction mixture was stirred at room temperature for 16 h, and the bulk of the solvent was removed under reduced pressure. The residue was dissolved in dilute NaCl (50 mL) and was extracted with Et₂O (2 × 30 mL). The aqueous layer was cooled in ice, acidified with cold 6 M HCl to pH = 2, and extracted with EtOAc (8 × 30 mL). The combined EtOAc layers were concentrated in vacuo to furnish 1.78 g of **8** (97%) as a yellow oil: [α]_D²⁵ +25.3° (*c* 0.88). ¹H NMR δ 7.9 (br s, 1H), 7.21 (d, *J* = 8.8 Hz, 1H), 6.50 (d, *J* = 2.4 Hz, 1H), 6.47 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.15–4.19 (m, 2H), 3.74–3.92 (m, 5H), 3.65–3.70 (m, 2H), 3.20 (d, *J* = 10.8 Hz, 1H), 1.68 (s, 3H). ¹³C NMR δ 176.29, 171.96, 163.02, 161.32, 131.97, 109.79, 107.69, 101.49, 82.68, 72.57, 69.19, 67.56, 61.78, 39.78, 24.61. HRMS *m/z* calcd for C₁₅H₂₀NO₆S, 342.1006 (*M* + H), C₁₅H₁₉NNaO₆S, 364.0825 (*M* + Na); found, 342.1014, 364.0826. Anal. Calcd for C₁₅H₁₉NO₆S: C, 52.78; H, 5.61; N, 4.10. Found: C, 52.93; H, 5.83; N, 4.02.

4.2.2. (S)-4,5-Dihydro-2-[2-hydroxy-4-(4-carboxy-3-oxabutyloxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (9)—A solution of 50% (w/w) NaOH (2.88 mL, 55.1 mmol) in CH₃OH (30 mL) was added to a mixture of **19** (2.27 g, 5.52 mmol) in CH₃OH (62 mL) over 5 min at 0 °C. The reaction mixture was stirred at room temperature for 17 h, and the bulk of the solvent was removed by rotary evaporation. The residue was dissolve in 3 M

NaCl (70 mL) and was extracted with Et₂O (3 × 40 mL). The aqueous layer was cooled in ice, treated with cold 2 M HCl (30 mL) and extracted with EtOAc (100 mL, 4 × 50 mL). Organic layers were combined, washed with 6 M NaCl (80 mL) and concentrated by rotary evaporation. The residue was combined with H₂O (43 mL) and 0.1050 M NaOH (52.76 mL, 5.540 mmol), heated on a steam bath and hot suction filtered, washing with H₂O (18 mL). The filtrate was diluted with H₂O (34 mL) and lyophilized. Solid was dried under high vacuum at 72 °C, furnishing 2.05 g of **9** as its sodium salt (98%) as an amorphous yellow solid: [α]_D²⁰ +124.3° (c 0.73, H₂O). ¹H NMR (D₂O) δ 7.57 (d, *J* = 9.0 Hz, 1H), 6.61 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.52 (d, *J* = 2.3 Hz, 1H), 4.26–4.30 (m, 2H), 4.06 (s, 2H), 3.90–3.95 (m, 3H), 3.53 (d, *J* = 11.7 Hz, 1H), 1.74 (s, 3H). ¹³C NMR (D₂O) δ 178.94, 177.92, 177.62, 166.41, 162.21, 134.11, 109.15, 107.12, 102.09, 78.41, 70.23, 69.43, 68.45, 39.67, 23.98. HRMS *m/z* calcd for C₁₅H₁₅NNaO₇S, 376.0472 (M - H), C₁₅H₁₆NO₇S, 354.0653 (M - Na); found, 376.0479, 354.0663. A sample (1.00 g) was recrystallized from EtOH (aq) to give 0.631 g of **9** (Na salt). Anal. Calcd for C₁₅H₁₆NNaO₇S: C, 47.75; H, 4.27; N, 3.71. Found: C, 47.82; H, 4.43; N, 3.75.

4.2.3. (S)-4,5-Dihydro-2-[2-hydroxy-4-(6-methoxyhexyloxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (10)—A solution of 50% (w/w) NaOH (1.87 mL, 35.8 mmol) in CH₃OH (40 mL) was added over 4 min to a solution of **22** (1.41 g, 3.56 mmol) in CH₃OH (80 mL) at 0 °C. The reaction mixture was stirred at room temperature for 15 h, and the bulk of the solvent was removed under reduced pressure. The residue was diluted in 2 M NaCl (120 mL) and was extracted with Et₂O (3 × 50 mL). The aqueous layer was cooled in ice, acidified with cold 2 M HCl (30 mL), and extracted with EtOAc (100 mL, 3 × 50 mL). The combined EtOAc extracts were washed with 6 M NaCl (60 mL) and concentrated in vacuo to generate 1.275 g of **10** (97%) as a waxy, light tan solid: mp 68–68.5 °C, [α]_D²⁰ +48.8° (c 0.80, DMF). ¹H NMR (DMSO-*d*₆) δ 13.17 (s, 1H), 12.74 (s, 1H), 7.31 (d, *J* = 8.6 Hz, 1H), 6.52 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.50 (d, *J* = 2.3 Hz, 1H), 4.00 (t, *J* = 6.6 Hz, 2H), 3.79 (d, *J* = 11.3 Hz, 1H), 3.36 (d, *J* = 11.3 Hz, 1H), 3.30 (t, *J* = 6.4 Hz, 2H), 3.21 (s, 3H), 1.66–1.74 (m, 2H), 1.58 (s, 3H), 1.47–1.54 (m, 2H), 1.29–1.45 (m, 4H). ¹³C NMR (DMSO-*d*₆) δ 173.73, 170.03, 162.96, 160.49, 131.57, 108.97, 107.27, 101.13, 82.45, 71.82, 67.80, 57.79, 28.97, 28.47, 25.42, 25.28, 24.11. HRMS *m/z* calcd for C₁₈H₂₆NO₅S, 368.1526 (M + H); found, 368.1540. Anal. Calcd for C₁₈H₂₅NO₅S: C, 58.84; H, 6.86; N, 3.81. Found: C, 58.55; H, 6.81; N, 3.80.

4.2.4. (S)-4,5-Dihydro-2-[2-hydroxy-4-(6-hydroxyhexyloxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (11)—A solution of 50% (w/w) NaOH (3.12 mL, 59.7 mmol) in CH₃OH (95 mL) was added to a mixture of **24** (2.53 g, 5.97 mmol) in CH₃OH (100 mL) over 32 min at 0 °C. The reaction mixture was stirred at room temperature for 1 d, and the bulk of the solvent was removed under reduced pressure. The residue was treated with 2 M NaCl (150 mL) and was extracted with Et₂O (3 × 50 mL). The aqueous layer was cooled in ice, treated with cold 2 M HCl (50 mL) and extracted with EtOAc (2 × 100 mL, 50 mL). Organic layers were combined, washed 6 M NaCl (65 mL) and concentrated by rotary evaporation. The residue was recrystallized from EtOAc/hexanes. Solid was collected and dried under high vacuum at 58 °C, providing 1.917 g of **11** (91%) as pale yellow crystals: mp 116–116.5 °C, [α]_D²⁰ +50.1° (c 0.83, DMF). ¹H NMR (DMSO-*d*₆) δ 13.20 (s, 1H), 12.73 (s,

1H), 7.32 (d, $J = 9.0$ Hz, 1H), 6.52 (dd, $J = 8.6, 2.3$ Hz, 1H), 6.50 (d, $J = 2.3$ Hz, 1H), 4.35 (br s, 1H), 4.00 (t, $J = 6.4$ Hz, 2H), 3.79 (d, $J = 11.3$ Hz, 1H), 3.36–3.42 (m, 2H), 3.36 (d, $J = 11.3$ Hz, 1H), 1.66–1.75 (m, 2H), 1.58 (s, 3H), 1.29–1.48 (m, 6H). ^{13}C NMR (DMSO- d_6) δ 173.76, 170.04, 162.97, 160.50, 131.59, 108.97, 107.28, 101.13, 82.45, 67.85, 60.64, 32.48, 28.56, 25.35, 25.27, 24.12. HRMS m/z calcd for $\text{C}_{17}\text{H}_{24}\text{NO}_5\text{S}$, 354.1370 ($M + \text{H}$); found, 354.1384. Anal. Calcd for $\text{C}_{17}\text{H}_{23}\text{NO}_5\text{S}$: C, 57.77; H, 6.56; N, 3.96. Found: C, 57.94; H, 6.50; N, 3.93.

4.2.5. (S)-4,5-Dihydro-2-[2-hydroxy-4-(5-carboxypentyloxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (12)—A solution of 50% (w/w) NaOH (5.00 mL, 95.6 mmol) in CH_3OH (50 mL) was added to a mixture of **28** (4.434 g, 10.47 mmol) in CH_3OH (120 mL) over 7 min at 0 °C. The reaction mixture was stirred at room temperature for 2 d, and the bulk of the solvent was removed under reduced pressure. The residue was dissolved in 3 M NaCl (110 mL) and was extracted with Et_2O (2×100 mL). The aqueous layer was cooled in ice, treated with cold 2 M HCl (54 mL) and extracted with EtOAc (150 mL, 2×60 mL). Organic layers were combined, washed with 6 M NaCl (100 mL), and concentrated by rotary evaporation. The residue was dried under high vacuum at 57 °C for 16 h to afford 3.80 g of **12** (99%) as light colored crystals: mp 153.5–155 °C, $[\alpha] +47.5^\circ$ (c 0.76, DMF). ^1H NMR (DMSO- d_6) δ 12.72 (s, 2H), 7.31 (d, $J = 8.6$ Hz, 1H), 6.52 (dd, $J = 8.6, 2.3$ Hz, 1H), 6.49 (d, $J = 2.3$ Hz, 1H), 4.00 (t, $J = 6.4$ Hz, 2H), 3.79 (d, $J = 11.3$ Hz, 1H), 3.36 (d, $J = 11.3$ Hz, 1H), 2.23 (t, $J = 7.2$ Hz, 2H), 1.66–1.75 (m, 2H), 1.58 (s, 3H), 1.51–1.60 (m, 2H), 1.36–1.45 (m, 2H). ^{13}C NMR (DMSO- d_6) δ 174.46, 173.76, 170.05, 162.96, 160.50, 131.60, 108.98, 107.29, 101.16, 82.46, 67.76, 33.61, 28.26, 25.08, 24.25, 24.12. HRMS m/z calcd for $\text{C}_{17}\text{H}_{22}\text{NO}_6\text{S}$, 368.1162 ($M + \text{H}$); found, 368.1169. Anal. Calcd for $\text{C}_{17}\text{H}_{21}\text{NO}_6\text{S}$: C, 55.57; H, 5.76; N, 3.81. Found: C, 55.58; H, 5.79; N, 3.78.

4.2.6. (S)-4,5-Dihydro-2-[2-hydroxy-4-(3-carboxypropyloxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (13)—A solution of 50% (w/w) NaOH (6.34 mL, 0.121 mol) in CH_3OH (100 mL) was added dropwise to a solution of **29** (6.56 g, 16.6 mmol) in CH_3OH (50 mL) at 0 °C. The reaction mixture was stirred at room temperature for 36 h, and the bulk of the solvent was removed under reduced pressure. The residue was dissolved in dilute NaCl (150 mL) and was washed with Et_2O (2×100 mL). The aqueous layer was cooled at 0 °C, acidified with 6 M HCl to pH = 2. Solid was filtered and washed with cold water. Crystallization in hot CH_3OH and EtOAc afforded 5.06 g of **13** (90%) as a white solid: mp 202–204 °C, $[\alpha] +22.1^\circ$ (c 0.086, CH_3OH). ^1H NMR (DMSO- d_6) δ 7.32 (d, $J = 8.4$ Hz, 1H), 6.53 (dd, $J = 8.4, 2.0$ Hz, 1H), 6.50 (d, $J = 2.4$ Hz, 1H), 4.03 (t, $J = 6.4$ Hz, 2H), 3.79 (d, $J = 11.2$ Hz, 1H), 3.35 (d, $J = 11.2$ Hz, 1H), 2.38 (t, $J = 7.2$ Hz, 2H), 1.93 (quintet, $J = 7.2$ Hz, 2H), 1.57 (s, 3H). ^{13}C NMR (DMSO- d_6) δ 177.42, 177.11, 173.34, 166.13, 163.85, 135.00, 112.48, 110.60, 104.58, 85.86, 70.38, 33.42, 27.51, 27.43. HRMS m/z calcd for $\text{C}_{15}\text{H}_{18}\text{NO}_6\text{S}$, 340.0849 ($M + \text{H}$); found, 340.0849. Anal. Calcd for $\text{C}_{15}\text{H}_{17}\text{NO}_6\text{S}$: C, 53.09; H, 5.05; N, 4.13. Found: C, 52.81; H, 5.17; N, 4.09.

4.2.7. (S)-4,5-Dihydro-2-[2-hydroxy-4-(carboxymethoxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (14)—A solution of 50% (w/w) NaOH (5.60 mL, 0.107 mol) in CH_3OH (75 mL) was added to a solution of **30** (4.32 g, 11.76 mmol) in CH_3OH (120 mL)

over 10 min at 0 °C. The reaction mixture was stirred at room temperature for 2 d, and the bulk of the solvent was removed under reduced pressure. The residue was dissolved in H₂O (120 mL) and was extracted with Et₂O (2 × 100 mL). The aqueous layer was cooled in ice, treated with 2 M HCl (60 mL) and extracted with EtOAc (150 mL, 2 × 100 mL). Organic layers were combined, washed with 6 M NaCl (100 mL), and concentrated by rotary evaporation. The residue was dried under high vacuum to afford 3.589 g of **14** (98%) as a pale yellow solid: mp 206–206.5 °C (dec), [α]_D²⁵ +56.3° (c 0.76, DMF). ¹H NMR (DMSO-*d*₆) δ 13.14 (s, 2H), 12.75 (s, 1H), 7.34 (d, *J* = 8.6 Hz, 1H), 6.53 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.47 (d, *J* = 2.3 Hz, 1H), 4.75 (s, 2H), 3.79 (d, *J* = 11.3 Hz, 1H), 3.37 (d, *J* = 11.3 Hz, 1H), 1.58 (s, 3H). ¹³C NMR (DMSO-*d*₆) δ 173.71, 170.04, 169.72, 161.94, 160.33, 131.62, 109.52, 107.17, 101.46, 82.47, 64.57, 39.34, 24.09. HRMS *m/z* calcd for C₁₃H₁₄NO₆S, 312.0536 (*M* + *H*); found, 312.0546. Anal. Calcd for C₁₃H₁₃NO₆S: C, 50.16; H, 4.21; N, 4.50. Found: C, 50.06; H, 4.38; N, 4.41.

4.2.8. Ethyl (S)-4,5-Dihydro-2-[2-hydroxy-4-(5-hydroxy-3-oxapentyloxy)phenyl]-4-methyl-4-thiazolecarboxylate (17)—Potassium carbonate (2.76 g, 20.0 mmol) and KI (200 mg, 1.2 mmol) were added to a mixture of **15** [82] (2.81 g, 10 mmol) in DMF (100 mL). A solution of **16** (1.24 g, 10 mmol) in DMF (10 mL) was added to the reaction mixture, which was heated at 100 °C for 24 h. After cooling to room temperature, H₂O (100 mL) was added followed by extraction with EtOAc (2 × 100 mL). Organic layers were combined, washed with H₂O (100 mL) and 6 M NaCl (100 mL), and solvent was removed in vacuo. Column chromatography using 30% EtOAc/CH₂Cl₂ furnished 2.30 g of **17** (62%) as a viscous oil: [α]_D²⁵ +48.0° (c 0.15). ¹H NMR δ 12.70 (br s, 1H), 7.30 (d, *J* = 8.8 Hz, 1H), 6.50 (d, *J* = 2.4 Hz, 1H), 6.47 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.24 (dq, *J* = 7.6, 1.6 Hz, 2H), 4.15–4.17 (m, 2H), 3.83–3.88 (m, 3H), 3.76–3.79 (m, 2H), 3.66–3.69 (m, 2H), 3.20 (d, *J* = 10.8 Hz, 1H), 1.66 (s, 3H), 1.3 (t, *J* = 7.2 Hz, 3H). ¹³C NMR δ 172.96, 170.92, 162.95, 161.32, 131.87, 110.14, 107.39, 101.51, 83.25, 72.73, 69.54, 67.61, 62.05, 61.89, 39.98, 24.61, 14.23. HRMS *m/z* calcd for C₁₇H₂₄NO₆S, 370.1319 (*M* + *H*); found, 370.1323. Anal. Calcd for C₁₇H₂₃NO₆S: C, 55.27; H, 6.28; N, 3.79. Found: C, 54.99; H, 6.24; N, 3.75.

4.2.9. Ethyl (S)-4,5-Dihydro-2-[2-hydroxy-4-[(4-ethoxycarbonyl)-3-oxabutyloxy]phenyl]-4-methyl-4-thiazolecarboxylate (19)—Sodium iodide (0.553 g, 3.69 mmol) and K₂CO₃ (5.13 g, 37.1 mmol) were added to **15** (5.08 g, 18.1 mmol) and **18** [73] (3.31 g, 19.9 mmol) in DMF (46 mL), and the reaction mixture was heated at 95 °C for 22 h. The mixture was cooled, filtered, washing the solids with acetone (100 mL, 2 × 50 mL). Solvents were removed in vacuo, and the concentrate was treated with 1:1 0.5 N HCl/6 M NaCl (120 mL) followed by extraction with EtOAc (100 mL, 2 × 50 mL). Organic layers were combined, washed with 1% NaHSO₃ (75 mL), H₂O (75 mL) and 6 M NaCl (55 mL), and solvent was removed by rotary evaporation. Flash column chromatography using 8.4:25:66.5 EtOAc/petroleum ether/CH₂Cl₂ gave 2.574 g of **19** (35%) as a yellow oil: [α]_D²⁵ +41.0° (c 0.68). ¹H NMR δ 12.70 (s, 1H), 7.29 (d, *J* = 8.6 Hz, 1H), 6.50 (d, *J* = 2.3 Hz, 1H), 6.47 (dd, *J* = 8.8, 2.5 Hz, 1H), 4.17–4.28 (m + s, 8H), 3.92–3.97 (m, 2H), 3.84 (d, *J* = 11.3 Hz, 1H), 3.20 (d, *J* = 11.3 Hz, 1H), 1.66 (s, 3H), 1.298 (t, *J* = 7.2 Hz, 3H), 1.290 (t, *J* = 7.0 Hz, 3H). ¹³C NMR δ 172.96, 170.92, 170.42, 162.88, 161.29, 131.84, 110.13, 107.34,

101.52, 83.25, 69.94, 69.02, 67.71, 62.05, 61.10, 39.97, 24.60, 14.33, 14.22. HRMS m/z calcd for $C_{19}H_{26}NO_7S$, 412.1424 ($M + H$); found, 412.1440. Anal. Calcd for $C_{19}H_{25}NO_7S$: C, 55.46; H, 6.12; N, 3.40. Found: C, 55.66; H, 6.21; N, 3.44.

4.2.10. 1-Iodo-6-methoxyhexane (21)—Sodium methoxide (25 weight %, 5.5 mL, 24.1 mmol) was added by syringe to **20** (4.0 mL, 24.3 mmol) in DMF (10 mL) over 20 min. The reaction solution was heated at 63 °C for 17 h. After cooling to 0 °C, the reaction solution was treated with 3:1 cold 0.5 M HCl/6 M NaCl (200 mL) and was extracted with EtOAc (2 × 150 mL, 50 mL). The organic extracts were washed with 1% $NaHSO_3$ (150 mL), H_2O (2 × 150 mL) and 6 M NaCl (100 mL), and solvent was removed by rotary evaporation. Flash column chromatography using 4% then 6% EtOAc/petroleum ether furnished 1.61 g of **21** [74] (28%) as a liquid: 1H NMR δ 3.37 (t, $J = 6.4$ Hz, 2H), 3.33 (s, 3H), 3.19 (t, $J = 7.0$ Hz, 2H), 1.83 (quintet, $J = 7.1$ Hz, 2H), 1.54–1.62 (m, 2H), 1.33–1.46 (m, 4H). ^{13}C NMR δ 72.78, 58.72, 33.58, 30.46, 29.57, 25.26, 7.25. HRMS m/z calcd for $C_7H_{19}INO$, 260.0506 ($M + NH_4$); found, 260.0515. Anal. Calcd for $C_7H_{15}IO$: C, 34.73; H, 6.25. Found: C, 34.44; H, 6.19.

4.2.11. Ethyl (S)-4,5-Dihydro-2-[2-hydroxy-4-(6-methoxyhexyloxy)phenyl]-4-methyl-4-thiazolecarboxylate (22)—Potassium carbonate (1.81 g, 13.1 mmol) was added to **15** (1.77 g, 6.29 mmol) and **21** (1.56 g, 6.44 mmol) in DMF (32 mL), and the reaction mixture was heated at 62 °C for 22 h. After cooling in an ice bath, cold 0.5 M HCl (100 mL) was added followed by extraction with EtOAc (120 mL, 2 × 50 mL). Organic layers were combined and washed with 1% $NaHSO_3$ (100 mL), H_2O (3 × 100 mL) and 6 M NaCl (80 mL), and solvent was removed by rotary evaporation. Flash column chromatography using 10% EtOAc/petroleum ether then 1:3:6 EtOAc/petroleum ether/ CH_2Cl_2 gave 1.47 g of **22** (59%) as a viscous yellow oil: $[\alpha]_D^{25} +43.3^\circ$ (c 0.72). 1H NMR δ 12.68 (s, 1H), 7.28 (d, $J = 8.6$ Hz, 1H), 6.47 (d, $J = 2.3$ Hz, 1H), 6.43 (dd, $J = 8.8, 2.5$ Hz, 1H), 4.18–4.29 (m, 2H), 3.97 (t, $J = 6.6$ Hz, 2H), 3.83 (d, $J = 11.3$ Hz, 1H), 3.38 (t, $J = 6.4$ Hz, 2H), 3.34 (s, 3H), 3.19 (d, $J = 11.3$ Hz, 1H), 1.75–1.83 (m, 2H), 1.65 (s, 3H), 1.56–1.64 (m, 2H), 1.37–1.53 (m, 4H), 1.30 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR δ 173.02, 170.92, 163.50, 161.34, 131.77, 109.71, 107.40, 101.36, 83.23, 72.86, 68.19, 62.02, 58.70, 39.96, 29.69, 29.14, 26.03, 25.99, 24.61, 14.22. HRMS m/z calcd for $C_{20}H_{30}NO_5S$, 396.1839 ($M + H$); found, 396.1858. Anal. Calcd for $C_{20}H_{29}NO_5S$: C, 60.74; H, 7.39; N, 3.54. Found: C, 60.59; H, 7.29; N, 3.60.

4.2.12. Ethyl (S)-4,5-Dihydro-2-[2-hydroxy-4-(6-acetoxihexyloxy)phenyl]-4-methyl-4-thiazolecarboxylate (24)—Potassium carbonate (3.42 g, 24.8 mmol) was added to **15** (3.255 g, 11.57 mmol) and **23** [75] (3.44 g, 12.7 mmol) in DMF (60 mL), and the mixture was heated at 70 °C for 21 h. After cooling to 0 °C, cold 0.5 M HCl (150 mL) was added followed by extraction with EtOAc (150 mL, 2 × 80 mL). Organic layers were combined, washed with 1% $NaHSO_3$ (150 mL), H_2O (3 × 150 mL) and 6 M NaCl (100 mL), and solvent was removed by rotary evaporation. Flash column chromatography using 1:3:6 EtOAc/petroleum ether/ CH_2Cl_2 gave 2.60 g of **24** (53%) as a white solid: mp 58.5–60 °C, $[\alpha]_D^{25} +40.1^\circ$ (c 0.98). 1H NMR δ 12.69 (s, 1H), 7.29 (d, $J = 9.0$ Hz, 1H), 6.47 (d, $J = 2.3$ Hz, 1H), 6.43 (dd, $J = 8.8, 2.5$ Hz, 1H), 4.20–4.28 (m, 2H), 4.07 (t, $J = 6.6$ Hz, 2H), 3.97 (t, $J =$

6.4 Hz, 2H), 3.84 (d, J = 11.3 Hz, 1H), 3.19 (d, J = 11.3 Hz, 1H), 2.05 (s, 3H), 1.76–1.84 (m, 2H), 1.62–1.70 (m, 2H), 1.66 (s, 3H), 1.38–1.54 (m, 4H), 1.30 (t, J = 7.0 Hz, 3H). ^{13}C NMR δ 173.01, 171.39, 170.93, 163.45, 161.36, 131.79, 109.76, 107.40, 101.35, 83.23, 68.10, 64.58, 62.03, 39.98, 29.07, 28.66, 25.84, 25.83, 24.62, 21.16, 14.23. HRMS m/z calcd for $\text{C}_{21}\text{H}_{30}\text{NO}_6\text{S}$, 424.1788 ($M + \text{H}$); found, 424.1798. Anal. Calcd for $\text{C}_{21}\text{H}_{29}\text{NO}_6\text{S}$: C, 59.56; H, 6.90; N, 3.31. Found: C, 59.71; H, 6.81; N, 3.33.

4.2.13. Ethyl (S)-4,5-Dihydro-2-[2-hydroxy-4-[5-(ethoxycarbonyl)pentyloxy]phenyl]-4-methyl-4-thiazolecarboxylate (28)—

Potassium carbonate (3.19 g, 23.1 mmol), NaI (0.351 g, 2.34 mmol), and a solution of **25** (4.46 g, 20.0 mmol) in DMF (25 mL) were added to a solution of **15** (5.0 g, 17.8 mmol) in DMF (100 mL). The reaction mixture was heated at 65 °C for 5 d. After cooling to room temperature, the solvent was removed by rotary evaporation. The residue was treated with cold 0.5 M HCl (200 mL) and was extracted with EtOAc (150 mL, 2 $\times$ 50 mL). The organic extracts were washed with 1% NaHSO_3 (100 mL), H_2O (100 mL), 6 M NaCl (50 mL), and solvent was removed in vacuo. Column chromatography using 30% EtOAc/petroleum ether furnished 4.586 g of **28** (61%) as an off-white solid: mp 61–62 °C, $[\alpha]_D^{25} +40.94^\circ$ (c 0.171). ^1H NMR δ 12.68 (s, 1H), 7.28 (d, J = 8.4 Hz, 1H), 6.47 (d, J = 2.4 Hz, 1H), 6.42 (dd, J = 8.8, 2.4 Hz, 1H), 4.24 (dq, J = 7.2, 2.0 Hz, 2H), 4.13 (q, J = 7.2 Hz, 2H), 3.97 (t, J = 6.4 Hz, 2H), 3.84 (d, J = 11.2 Hz, 1H), 3.19 (d, J = 11.2 Hz, 1H), 2.33 (t, J = 7.6 Hz, 2H), 1.77–1.84 (m, 2H), 1.67–1.73 (m, 2H), 1.66 (s, 3H), 1.46–1.54 (m, 2H), 1.30 (t, J = 7.2 Hz, 3H), 1.26 (t, J = 7.2 Hz, 3H). ^{13}C NMR δ 173.70, 172.96, 170.88, 163.38, 161.31, 131.75, 109.73, 107.32, 101.33, 83.20, 67.93, 61.99, 60.37, 39.93, 34.31, 28.85, 25.68, 24.78, 24.58, 14.36, 14.20. HRMS m/z calcd for $\text{C}_{21}\text{H}_{30}\text{NO}_6\text{S}$, 424.1788 ($M + \text{H}$); found, 424.1784. Anal. Calcd for $\text{C}_{21}\text{H}_{29}\text{NO}_6\text{S}$: C, 59.56; H, 6.90; N, 3.31. Found: C, 59.69; H, 6.78; N, 3.24.

4.2.14. Ethyl (S)-4,5-Dihydro-2-[2-hydroxy-4-[3-(ethoxycarbonyl)propyloxy]phenyl]-4-methyl-4-thiazolecarboxylate (29)—

Potassium carbonate (4.41 g, 32.0 mmol) and NaI (182 mg, 1.2 mmol) were added to a mixture of **15** (6.90 g, 24.5 mmol) and **26** (5.27 g, 27.0 mmol) in DMF (150 mL). The reaction mixture was stirred at room temperature for 6 h and then heated at 100 °C for 48 h. After cooling to room temperature, the solvent was removed by rotary evaporation under high vacuum, and the residue was treated with 0.2 M HCl/6 M NaCl (50 mL) followed by extraction with EtOAc (4 $\times$ 30 mL). The organic extracts were washed with 1% NaHSO_3 (150 mL), H_2O (150 mL) and 6 M NaCl (150 mL), and solvent was removed in vacuo. Column chromatography using 30% EtOAc/ CH_2Cl_2 gave 6.94 g of **29** (72%) as a light yellow viscous oil: $[\alpha]_D^{25} +44.35^\circ$ (c 0.372). ^1H NMR δ 12.65 (s, 1H), 7.28 (d, J = 8.4 Hz, 1H), 6.47 (d, J = 2.4 Hz, 1H), 6.42 (dd, J = 8.8, 2.0 Hz, 1H), 4.24 (dq, J = 7.2, 1.6 Hz, 2H), 4.15 (q, J = 7.6 Hz, 2H), 4.03 (t, J = 6.4 Hz, 2H), 3.84 (d, J = 11.2 Hz, 1H), 3.19 (d, J = 11.2 Hz, 1H), 2.51 (t, J = 7.6 Hz, 2H), 2.11 (quintet, J = 6.2 Hz, 2H), 1.66 (s, 3H), 1.30 (t, J = 7.6 Hz, 3H), 1.26 (t, J = 7.2 Hz, 3H). ^{13}C NMR δ 173.21, 172.98, 170.90, 163.15, 161.32, 131.81, 109.91, 107.24, 101.44, 83.23, 67.02, 62.02, 60.63, 39.96, 30.84, 24.60, 24.55, 14.35, 14.22. HRMS m/z calcd for $\text{C}_{19}\text{H}_{26}\text{NO}_6\text{S}$, 396.1475 ($M + \text{H}$); found, 396.1475. Anal. Calcd for $\text{C}_{19}\text{H}_{25}\text{NO}_6\text{S}$: C, 57.71; H, 6.37; N, 3.54. Found: C, 57.72; H, 6.23; N, 3.52.

4.2.15. Ethyl (S)-4,5-Dihydro-2-[2-hydroxy-4-**(ethoxycarbonylmethoxy)phenyl]-4-methyl-4-thiazolecarboxylate (30)—**

Potassium carbonate (5.29 g, 38.3 mmol) and NaI (0.498 g, 3.32 mmol) were added to **15** (5.035 g, 17.90 mmol) in DMF (75 mL). The mixture was stirred for several minutes, **27** (2.2 mL, 19.8 mmol) was introduced, and the contents were heated at 70 °C for 3.5 d. After cooling to 0 °C, cold 0.5 M HCl (200 mL) was added followed by extraction with EtOAc (200 mL, 2 × 100 mL). Organic layers were combined, washed with 1% NaHSO₃ (200 mL), H₂O (2 × 200 mL) and 6 M NaCl (130 mL) and solvent was removed in vacuo. Flash column chromatography using 1% EtOAc/CH₂Cl₂ then 6% acetone/CH₂Cl₂ generated 4.37 g of **30** (66%) as a viscous yellow oil: [α]_D²⁵ +45.1° (c 0.88). ¹H NMR δ 12.73 (s, 1H), 7.32 (d, *J* = 8.6 Hz, 1H), 6.50 (d, *J* = 2.7 Hz, 1H), 6.47 (dd, *J* = 6.8, 2.5 Hz, 1H), 4.63 (s, 2H), 4.20–4.31 (m, 4H), 3.84 (d, *J* = 11.3 Hz, 1H), 3.20 (d, *J* = 11.3 Hz, 1H), 1.66 (s, 3H), 1.305 (t, *J* = 7.2 Hz, 3H), 1.298 (t, *J* = 7.0 Hz, 3H). ¹³C NMR δ 172.89, 170.92, 168.39, 161.91, 161.29, 132.00, 110.74, 107.18, 101.71, 83.28, 65.27, 62.07, 61.68, 40.00, 24.59, 14.28, 14.22. HRMS *m/z* calcd for C₁₇H₂₂NO₆S, 368.1162 (*M* + *H*); found, 368.1172. Anal. Calcd for C₁₇H₂₁NO₆S: C, 55.57; H, 5.76; N, 3.81. Found: C, 55.72; H, 5.72; N, 3.82.

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Abbreviations

NTBI	nontransferrin-bound iron
DFO	Desferrioxamine
sc	subcutaneously
DFT	desferrithiocin
DADFT	desazadesferrithiocin
s.i.d.	once daily
b.i.d.	twice daily
BUN	blood urea nitrogen
SCr	serum creatinine
GI	gastrointestinal
ICE	iron clearing efficiency
Kim-1	kidney injury molecule-1
po	orally
PR	performance ratio

HPLC	high-pressure liquid chromatography
SAR	structure activity relationship
log P_{app}	lipophilicity

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Desferal

Deferiprone

Exjade

Deferitritin

Figure 1.
Structures of some of the iron chelators evaluated clinically in humans.

Figure 2. Tissue metabolism/distribution of DADFT analogues **2** and **4–7** in rat liver. The rats ($n=3$ per group) were given the drugs sc at a dose of $300 \mu\text{mol/kg}$. The chelator concentration data (y-axis) are expressed as nmol/g wet weight.

Figure 3. Dealkylation of the methyl ether of glycodiazine, first to an alcohol (metabolite I), which is then oxidized to a carboxylic acid (metabolite II).

Figure 4.
The structures of the putative metabolites of **7**, **8** and **9**, and the lipophilicity (log P_{app}) of the ligands.

Figure 5.

Tissue metabolism/distribution of DADFT analogues **7** and synthetic **8** in rat plasma, liver, kidney, heart and pancreas. The rats ($n=3$ per group) were given the drugs sc at a dose of 300 μmol/kg. The chelator concentration data (y-axis) are expressed as nmol/g wet weight, or μM (plasma).

Figure 6.

The structures of the putative metabolites of **11**, **12–14**, and the lipophilicity ($\log P_{\text{app}}$) of the ligands.

Figure 7.

Tissue metabolism/distribution of metabolically programmed DADFT analogue **11** in rat plasma, liver, kidney, heart and pancreas. The rats ($n=3$ per group) were given the drug sc at a dose of 300 $\mu\text{mol/kg}$. The chelator concentration data (y-axis) are expressed as nmol/g wet weight, or μM (plasma).

Figure 8.

Schematic representation of the oral absorption of the highly lipophilic ligand **11**, and its rapid conversion to hydrophilic metabolites **12–14**.

Scheme 1.

Synthesis of (*S*)-4,5-Dihydro-2-[2-hydroxy-4-(5-hydroxy-3-oxapentyloxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (**8**) and (*S*)-4,5-Dihydro-2-[2-hydroxy-4-(4-carboxy-3-oxabutyloxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (**9**)^a

^aReagents and conditions: (a) K_2CO_3 (2.0 equiv), KI, DMF, 100 °C, 1 d, 62%; (b) K_2CO_3 (2.1 equiv), NaI, DMF, 95 °C, 22 h, 35%; (c) 50% NaOH (aq), CH_3OH , 97% (**8**), 98% (**9** as its monosodium salt).

Scheme 2.

Synthesis of (S)-4,5-Dihydro-2-[2-hydroxy-4-(6-methoxyhexyloxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (**10**) and (S)-4,5-Dihydro-2-[2-hydroxy-4-(6-hydroxyhexyloxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (**11**)

^aReagents and conditions: (a) 25% NaOCH₃ (1.0 equiv), DMF, 63 °C, 17 h, 28%; (b) K₂CO₃ (2.0 equiv), DMF, 62 °C, 22 h, 59%; (**22**); K₂CO₃ (1.9 equiv), DMF, 70 °C, 18 h, 53% (**24**); (c) 50% NaOH (aq), CH₃OH, 97% (**10**) 91% (**11**).

Scheme 3.

Synthesis of (*S*)-4,5-Dihydro-2-[2-hydroxy-4-(5-carboxypentyloxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (**12**), (*S*)-4,5-Dihydro-2-[2-hydroxy-4-(3-carboxypropyloxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (**13**), and (*S*)-4,5-Dihydro-2-[2-hydroxy-4-(carboxymethoxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (**14**)^a

^aReagents and conditions: (a) K_2CO_3 (1.3 equiv), NaI, DMF, 65 °C, 5 d, 61% (**28**); K_2CO_3 (1.3 equiv), NaI, DMF, 100 °C, 2 d, 72% (**29**); K_2CO_3 (2.1 equiv), NaI, DMF, 70 °C, 20 h, 66% (**30**); (b) 50% NaOH (aq), CH_3OH , 99% (**12**), 90% (**13**), 98% (**14**).

Table 1

Iron Clearing Efficiency of Iron Chelators given to Rats and *Cebus apella* Primates, and the Log P_{app} of the Compounds

Compound	Route	$^a\text{Log } P_{app}$	$^b\text{Rodent ICE (\%)}$	$^d\text{Primate ICE (\%)}$
Desferrioxamine (DFO)	sc	< -3.2	2.5 ± 0.7 [74/26]	5.5 ± 0.9 [45/55]
(S)-Desferrithiocin (1)	po	-1.77	5.5 ± 3.2 [93/7]	16.1 ± 8.5 [78/22]
(S)-4'-(HO)-DADFT (2)	po	-1.05	1.1 ± 0.8 [100/0]	16.8 ± 7.2 [88/12]
(S)-3'-(HO)-DADFT (3)	po	-1.17	4.6 ± 0.9 [98/2]	23.1 ± 5.9 [83/17]
(S)-4'-(CH ₃ O)-DADFT (4)	po	-0.70	6.6 ± 2.8 [98/2]	24.4 ± 10.8 [91/9]
(S)-4'-(HO)-DADFT-PE (5)	po	-1.10	5.5 ± 1.9 [90/10]	25.4 ± 7.4 [96/4]

Compound	Route	^a Log <i>P</i> _{app}	^b Rodent ICE (%)	^d Primate ICE (%)
(<i>S</i>)-3'-(HO)-DADFT-PE (6)	po	-1.22	10.6 ± 4.4 ^c [95/5]	23.0 ± 4.1 [95/5]
(<i>S</i>)-4'-(HO)-DADFT-norPE (7)	po	-0.89	26.7 ± 4.7 ^c [97/3]	26.3 ± 9.9 ^e [93/7] 28.7 ± 12.4 ^f [83/17]

^aData are expressed as the log of the fraction of the chelator seen in the octanol layer (log *P*_{app}); measurements were done in TRIS buffer, pH 7.4, using a "shake flask" direct method.

^bIn the rodents *n* = 3 (**7**), 4 (**3–6**), 5 (**1**), 6 (DFO) or 8 (**2**). The drugs were given orally (po) or subcutaneously (sc) as indicated in the table at a dose of 150 (DFO and **1**) or 300 μmol/kg (**2–7**). The drugs were solubilized in 40% Cremophor RH-40/water (DFO and **1**), distilled water (**5**), administered in capsules (**7**), or were given as their monosodium salts, prepared by the addition of 1 equiv of NaOH to a suspension of the free acid in distilled water (**2–4**, **6**). The efficiency of each compound was calculated by subtracting the iron excretion of control animals from the iron excretion of the treated animals. The number was then divided by the theoretical output; the result is expressed as a percent.

^cICE is based on a 48 h sample collection period. The relative percentages of the iron excreted in the bile and urine are in brackets.

^dIn the primates *n* = 3 (**6**), 4 (**1**, **3–5**, **7** in capsules^d), 5 (DFO), or 7 (**2**, **7** as its monosodium salt^e). The chelators were given po or sc at a dose of 75 (**7**) or 150 μmol/kg (DFO, **1–6**). The ligands were solubilized in 40% Cremophor RH-40/water (**1**, **3**, **4**), distilled water (DFO, **5**), administered in capsules (**7**^d), or were given as their monosodium salts, prepared by the addition of 1 equiv of NaOH to a suspension of the free acid in distilled water (**2**, **6**, **7**^f). The ICE was calculated by averaging the iron output for 4 days before 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. The relative percentages of the iron excreted in the feces and urine are in brackets.

Iron Clearing Efficiency of Iron Chelators given to Rats and *Cebus apella* Primates, and the Log P_{app} of the Compounds

Table 2

Compound	Log P_{app}	b_{Rat} ICE (%) [bile/urine]	Rat n	d_{Cebus} ICE (%) [bile/urine]	Primate n	$g_{Performance}$ Ratio (PR)
2	-1.05	1.1 ± 0.8 [100/0]	8	16.8 ± 7.2 [88/12]	7	15.3
7	-0.89	26.7 ± 4.7 ^c [97/3]	3	26.3 ± 9.9 ^e [93/7] 28.7 ± 12.4 ^f [83/17]	4 6	1.0 1.1
8	-0.53	15.4 ± 5.6 [98/2]	8	9.8 ± 3.4 [54/46]	4	0.6
9	-1.63	6.2 ± 1.7 (po) [100/0] 11.3 ± 3.4 (sc) [99/1]	3 3	1.7 ± 1.4 (po) [51/49] 17.4 ± 9.7 (sc) [84/16]	4 4	0.3 1.5

Compound	Log P_{app}	b_{Rat} ICE (%) [bile/urine]	Rat n	d_{Cebus} ICE (%) [bile/urine]	Primate n	$g_{Performance}$ Ratio (PR)
10	0.95	15.8 ± 3.7 (po) [99/1]	4	Toxic in Rats
11	0.21	9.9 ± 0.8 (po) [96/4]	4	21.9 ± 3.6 (po) [90/10]	3	2.2
12	-1.90	8.8 ± 1.8 (po) [94/6]	5	10.6 ± 4.0 (po) [82/18]	4	1.2
			4	18.8 ± 8.7 (sc) [69/31]	4	4.4
13	-2.21	3.7 ± 1.7 (po) [89/11]	5	5.4 ± 1.5 (po) [97/3]	4	1.5
			4	18.1 ± 7.5 (sc) [78/22]	4	4.2
14	-1.98	2.6 ± 1.6 (po) [89/11]	3	3.0 ± 2.7 (po) [60/40]	4	1.2
			3	15.9 ± 4.3 (sc) [53/47]	4	2.7

^dData are expressed as the log of the fraction of the chelator seen in the octanol layer (log P_{app}); measurements were done in TRIS buffer, pH 7.4, using a "shake flask" direct method.

^bIn the rodents the drugs were given po or sc at a dose of 300 $\mu\text{mol/kg}$. The drugs were administered in capsules (7), or were given as their monosodium salts, prepared by the addition of 1 equiv of NaOH to a suspension of the free acid in distilled water (2, 8–14). The efficiency of each compound was calculated by subtracting the iron excretion of control animals from the iron excretion of the treated animals. The number was then divided by the theoretical output; the result is expressed as a percent.

^cICE is based on a 48 h sample collection period. The relative percentages of the iron excreted in the bile and urine are in brackets.

^dIn the primates the chelators were given po or sc at a dose of 75 $\mu\text{mol/kg}$ (7–9, 11–14) or 150 $\mu\text{mol/kg}$ (2). The drugs were administered in capsules (7^e), or were given as their monosodium salts, prepared by the addition of 1 equiv of NaOH to a suspension of the free acid in distilled water (2, 7^f–9, 11–14). The efficiency was calculated by averaging the iron output for 4 days before 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. The relative percentages of the iron excreted in the feces and urine are in brackets.

^gPerformance ratio (PR) is defined as the mean $\text{ICE}_{\text{primates}}/\text{ICE}_{\text{rodents}}$.