Desferrithiocin Analogues and Nephrotoxicity

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The syntheses of a series of 4′-O-alkylated (*S*)-4,5-dihydro-2-(2,4-dihydroxyphenyl)-4-methyl-4-thiazolecarboxylic acid and 5′-O-alkylated (*S*)-4,5-dihydro-2-(2,5-dihydroxyphenyl)-4-methyl-4-thiazolecarboxylic acid ligands are described. Their partition between octanol and water, log P_{app}, is determined, along with their iron-clearing efficiency (ICE) in both non-iron-overloaded, bile duct-cannulated rodents and in iron-overloaded primates. The ligand-promoted biliary ferrokinetics in rats are described for each of the chelators. Plots of log P_{app} versus ICE in a rodent model for both the 4′-O-alkylated 2,4-dihydroxy and 5′-O-alkylated 2,5-dihydroxy series produced an inverse parabola plot with r^2 values of 0.97 and 0.81, respectively. The plots indicate an optimum log P_{app}/ICE relationship. Because of the nature of the data spread in the 4′-O-alkylated 2,4-dihydroxy series, it will be used to help assess the origin of nephrotoxicity in desferrithiocin analogues: is toxicity simply related to lipophilicity, ICE, or a combination of these properties?

Introduction

Although iron comprises 5% of the earth's crust, living systems have great difficulty in accessing and managing this vital micronutrient. It is essential to redox processes in all prokaryotes and eukaryotes. The very poor solubility of Fe(III) hydroxide, the predominant form of the metal in the biosphere ($K_{sp} = 1 \times 10^{-39}$),[1] has led to the development of sophisticated iron storage and transport systems in nature. Microorganisms utilize low molecular weight, virtually ferric iron specific ligands, siderophores.[2] Higher life forms, animals, tend to employ proteins to transport and store iron (e.g., transferrin and ferritin, respectively).[3–5]

Humans have developed a highly efficient iron management system in which we absorb and excrete only about 1 mg of the metal daily; there is no mechanism for the excretion of excess iron.[6] Whether derived from transfused red blood cells[7–9] or from increased absorption of dietary iron,[10,11] without effective treatment, body iron progressively increases with deposition in the liver, heart, pancreas, and elsewhere. Iron accumulation eventually produces (i) liver disease that may progress to cirrhosis,[12–14] (ii) diabetes related both to iron-induced decreases in pancreatic β-cell secretion and to increases in hepatic insulin resistance,[15,16] and (iii) heart disease, still the leading cause of death in thalassemia major and related forms of transfusional iron overload.[6,17,18] Non-transferrin-bound plasma iron (NTBI[a]), a heterogeneous pool of iron in the circulation that is not bound to the physiological iron transporter transferrin, seems to be a principal source of the abnormal tissue distribution of iron that develops with chronic iron overload. NTBI is rapidly taken up by hepatocytes in the liver, the major iron storage organ, perhaps through the divalent metal transporter 1[19] and other pathways that remain poorly characterized. NTBI also seems to gain entry into cardiomyocytes, pancreatic β-cells, and anterior pituitary cells specifically via L-type voltage-dependent Ca^{2+} channels not found in hepatocytes or Kupffer cells.[20] These differences in modes of iron uptake also may underlie clinical observations of differences in the course and pace of iron deposition and damage to the liver, pancreas, and heart in patients with all forms of iron overload.[6]

The toxicity associated with excess iron, whether a systemic or a focal problem, derives from its interaction with reactive oxygen species, for instance, endogenous hydrogen peroxide (H_2O_2).[21–24] In the presence of Fe(II), H_2O_2 is reduced to the hydroxyl radical ($HO^{\bullet}$) and HO^-, a process known as the Fenton reaction. The hydroxyl radical reacts very quickly with a variety of cellular constituents and can initiate free radicals and radical-mediated chain processes that damage DNA and membranes, as well as produce carcinogens.[22,25,26] The Fe(III) liberated in the peroxide reduction can be converted back to Fe(II) via a variety of biological reductants, for example, ascorbate or glutathione, exacerbating the situation.

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 approach available. Some of the iron-chelating agents that are now in use or that have been clinically evaluated include desferrioxamine B mesylate (DFO), 1,2-dimethyl-3-hydroxypyridin-4-one (deferiprone, L1),[27–30] 4-[3,5-bis(2-hydroxyphenyl)-1,2,4-triazol-1-yl]benzoic acid (deferasirox, ICL670A),[31–34] and the desferrithiocin (DFT) analogue, (*S*)-2-(2,4-dihydroxyphenyl)-4,5-dihydro-4-methyl-4-thiazolecarboxylic acid [deferitrin[35] (**1**), Table 1]. Each of these agents chelates iron derived predominantly from systemic storage sites, that is, from iron released by macrophages after catabolism of senescent red blood cells, from iron in hepatic pools, or from both; each has significant shortcomings.[36]

Desferrioxamine B (DFO), a hexacoordinate hydroxamate iron chelator produced by *Streptomyces pilosus*,[37] is not orally active and is best administered subcutaneously (sc) by continuous infusion over long periods of time,[7,38] a patient compliance issue.[7,39] Deferiprone, while orally active, does not remove enough iron to keep patients in a negative iron balance.[27–30] The Novartis drug deferasirox did not show noninferiority to DFO and is associated with numerous side effects;[31–34] it has a narrow therapeutic window. The clinical trial on the desfer-

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[a] Abbreviations: b.i.d., twice a day; DFO, desferrioxamine B mesylate; DFT, desferrithiocin (*S*)-4,5-dihydro-2-(3-hydroxy-2-pyridinyl)-4-methyl-4-thiazolecarboxylic acid; DTPA, diethylenetriaminepentaacetic acid; ICE, iron-clearing efficiency; log P_{app}, octanol−water partition coefficient (lipophilicity); MIC, maximum iron clearance; NTBI, non-transferrin bound iron; s.i.d., once a day.

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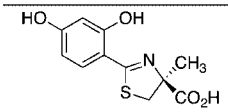
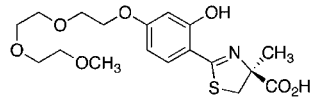
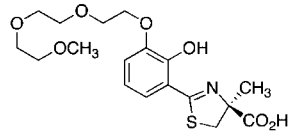
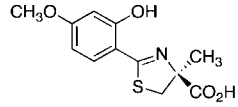
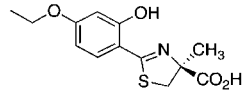
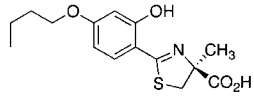
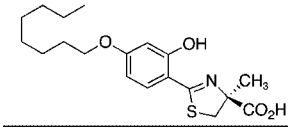
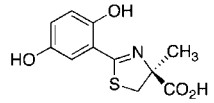
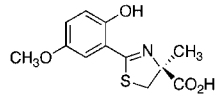
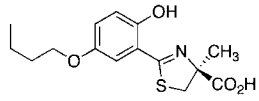
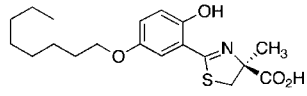
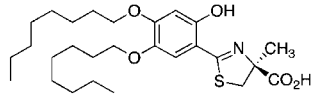
Table 1. Iron-Clearing Activity of Desferrithiocin Analogues Administered Orally to Rodents and Primates and the Partition Coefficients of the Compounds

Desferrithiocin Analogue	Comp. No.	Rodent Iron-Clearing Efficiency (%)[a]	Primate Iron-Clearing Efficiency (%)[b]	log P_{app}[c]
	**1**	1.1 ± 0.8[d] [100/0]	16.8 ± 7.2[e] [88/12]	–1.05
	**2**	5.5 ± 1.9[d] [90/10]	25.4 ± 7.4[d] [96/4]	–1.10
	**3**	10.6 ± 4.4[f] [95/5]	24.5 ± 7.6[f] [72/28]	–1.22
	**4**	6.6 ± 2.8[d] [98/2]	24.4 ± 10.8[g] [91/9]	–0.70
	**5**	18.0 ± 0.9 [100/0]	25.1 ± 6.7 [75/25]	–0.04
	**6**	13.1 ± 3.3 [98/2]	Toxic in rats	1.02
	**7**	6.4 ± 2.3 [94/6]	17.8 ± 2.1 [82/18]	1.53
	**8**	1.0 ± 0.9[h] [99/1]	12.6 ± 3.0[h] [88/12]	–1.14
	**9**	6.3 ± 1.2[h] [95/5]	18.9 ± 2.3[h] [94/6]	–0.61
	**10**	21.0 ± 4.0 [99/1]	Toxic in rats	0.94
	**11**	4.0 ± 1.2 [97/3]	12.3 ± 6.9 [83/17]	1.56
	**12**	<0.5	2.9 ± 2.5 [68/32]	2.05

[a] In the rodents [n = 3 (**6**, **10**), 4 (**3**, **4**, **7**–**9**, **11**, **12**), 5 (**2**, **5**), or 8 (**1**)]. The rats were given a single 300 μmol/kg dose of the ligands orally by gavage. The compounds were given as their sodium salts, prepared by the addition of 1 equiv of NaOH to a suspension of the free acid in distilled water. 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. The relative percentage of the iron excreted in the bile and urine are in brackets. Compound **2** was solubilized in distilled water. [b] In the primates [n = 3 (**12**), 4 (**2**, **5**, **7**–**9**, **11**), 6 (**1**), or 7 (**3**, **4**)]. The doses were [37.5 μmol/kg (**5**, **12**), 75 μmol/kg (**3**, **7**, **11**), or 150 μmol/kg (**1**, **2**, **4**, **8**, **9**)]. Ligands **6** and **10** were not given to the primates because of toxicity observed in the rodents. The compounds were prepared as for the rodents except that **4** was solubilized in 40% Cremophor RH-40. The efficiency of each compound 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 percentage of the iron excreted in the feces and urine are in brackets. [c] Data are expressed as the log of the fraction in the octanol layer (log P_{app}); measurements were done in Tris buffer, pH 7.4, using a "shake flask" direct method. The values obtained for compounds **1** and **4** are from ref 46; the value for **2** is from ref 42; the value for **3** is from ref 55; the values for **8** and **9** are from ref 47. [d] Data are from ref 42. [e] Data are from ref 44. [f] Data are from ref 55. [g] Data are from ref 46. [h] Data are from ref 47.

rithiocin analogue **1** (Table 1) has now been abandoned by Genzyme because of renal toxicity.[35]

The most common toxicity seen in animals treated with iron chelators is nephrotoxicity. This has been the case with diethylenetriaminepentaacetic acid (DTPA),[40] deferasirox,[31–34] the catecholamides,[41] and **1**.[35,42] The renal toxicity has by and large been limited to the proximal tubules. The animal preclinical toxicity data have been predictive of human outcomes.[43]

The origin of **1**-induced renal toxicity remains elusive. Although **1** was profoundly less toxic than desferrithiocin itself,[44] it seemed likely that nephrotoxicity would be the dose-limiting factor.[42] In fact, nephrotoxicity was shown to be the dose-limiting issue with **1** clinically.[35] However, there was an interesting clinical caveat: the nephrotoxicity appeared to be very much dependent on chelator dosing schedule. Although **1** was well tolerated in patients at doses of 5, 10, or 15 mg/kg/d once daily (s.i.d.) for up to 12 weeks, unacceptable renal toxicity was observed in three patients after only 4−5 weeks of treatment when the drug was given twice daily (b.i.d) at a dose of 12.5 mg/kg (25 mg/kg/d).[35]

We confirmed this finding in a recent study[45] in rodents in which the rats were given **1** orally by gavage s.i.d. at a dose of 474 μmol/kg/d for 7 days. This dose is equivalent to 120 mg/kg/d of the parent **1**. An additional group of animals was given the drug b.i.d. at 237 μmol/kg/dose (474 μmol/kg/d) for 7 days.[42,45] Untreated rats served as age-matched controls. One day postdrug, the rodents were anesthetized and the kidneys were perfusion-fixed; one kidney from each rat was dissected. The differences in proximal tubule damage were profound. The kidneys of animals treated s.i.d. had mild proximal tubule vacuolization, while the kidneys of animals treated b.i.d. presented significant architectural changes. Vacuolization was now severe.[42,45]

Under an identical dosing regimen, rodents were given the desferrithiocin polyether analogues (*S*)-4,5-dihydro-2-[2-hydroxy-4-(3,6,9-trioxadecyloxy)phenyl]-4-methyl-4-thiazolecarboxylic acid[42] (**2**, Table 1) and (*S*)-4,5-dihydro-2-[2-hydroxy-3-(3,6,9-trioxadecyloxy)phenyl]-4-methyl-4-thiazolecarboxylic acid[45] (**3**, Table 1) orally by gavage at a dose of 237 μmol/kg b.i.d. (474 μmol/kg/d) for 7 days. There was little if any damage to the proximal tubules, only occasional pale, refractile inclusions in the S1 segments, which were more prevalent in the rats treated with **2**, a surprising finding. While these polyether analogues are undergoing further preclinical development, we are nevertheless compelled to look at additional desferrithiocin analogue platforms in the hopes of better understanding structure−activity issues that regulate nephrotoxicity.

Previous studies were consistent with the idea that within a family of ligands, the more lipophilic analogues are generally more efficient iron chelators.[42,46,47] However, the more lipophilic ligands are also more toxic. The reason for this was not clear. Was the increase in toxicity due to lipophilicity or ICE? In order to develop a better understanding of the relationship between ICE, lipophilicity, and chelator-induced renal toxicity, a basis set of ligands with variable log P_{app}s was synthesized (Table 1). Their iron clearing properties were evaluated in rodents and primates. With this information in hand, these ligands can now be evaluated for proximal tubule damage as a function of both ICE and log P_{app}; this will be the subject of a future manuscript.

Design Concept

Desferrithiocin is a natural product tridentate iron chelator isolated from *Streptomyces antibioticus*.[48] It forms a tight 2:1 complex with Fe(III), $\beta_2 = 4 \times 10^{29}$ M^{-1}.[49,50] Although the compound was shown to be an excellent deferration agent when given orally to rodents[51] and *Cebus apella* primates,[52,53] it caused severe nephrotoxicity in rats.[53] However, the compound's oral activity spurred a structure−activity relationship (SAR) study focused on the desferrithiocin platform aimed at identifying an analogue with similar iron clearing properties but without the attendant nephrotoxicity.[44,46,54]

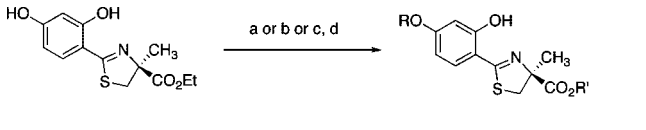
In the course of these SAR studies, we discovered that the lipophilicity of a ligand, as determined by its partition between octanol and water, log P_{app}, could have a profound effect on the ligand's iron-clearing efficiency (ICE), organ distribution, and toxicity profile.[47] The more lipophilic a chelator, the better the ICE; often, the more lipophilic the chelator, the more toxic it was.[42] This is best exemplified by (*S*)-4,5-dihydro-2-(2-hydroxy-4-methoxyphenyl)-4-methyl-4-thiazolecarboxylic acid (**4**, Table 1).[42,46] This ligand is lipophilic, log $P_{app} = -0.70$, and a very effective iron chelator when given orally to rodents or primates; the ICE values in rodents and primates were 6.6 ± 2.8%[42] and 24.4 ± 10.8%,[46] respectively. Unfortunately, the ligand was also very toxic. All of the rats given the drug po at a dose of 384 μmol/kg/d (equivalent to 100 mg/kg of the DFT sodium salt) were dead by day 6 of a planned 10-d study.[42] The rats presented with nephrotoxicity, which was acute, diffuse, severe, and characterized by proximal tubular epithelial necrosis and sloughing. Chelator **4** was indeed a worst-case scenario: lipophilic, high ICE values, and very toxic, a perfect candidate for assessing toxicity mechanisms.

Ultimately, it was demonstrated that substituting the 4′-methoxy group of **4** with a 3,6,9-trioxadecyloxy group, providing **2**, reduced the lipophilicity from −0.70 log P_{app} for **4** to −1.10 and virtually eliminated nephrotoxicity in a rodent model.[42] The ICE of the 4′-polyether **2** (5.5 ± 1.9%) was within error of the ICE for the 4′-methoxy ligand **4** (6.6 ± 2.8%) upon po administration to rats at a dose of 300 μmol/kg ($p > 0.05$).[42] The ICEs of the two chelators in the iron-loaded primates were also similar, 25.4 ± 7.4%[42] for **2** versus 24.4 ± 10.8%[46] for **4** ($p > 0.05$). This encouraged the synthesis of an additional ligand, the 3′-(3,6,9)-trioxadecyloxy analogue **3**.[55] This chelator had a higher ICE in rodents than **2**, 10.6 ± 4.4%, at a dose of 300 μmol/kg po, and did not present any nephrotoxicity.[55] These findings elicited a series of extensive comparative dose−response iron clearance studies and toxicity trials between the polyether analogues and **1**.[45]

The most important observations to come out of the toxicity studies were the profound difference in nephrotoxicity between the polyether analogues and deferitrin and how dependent **1**-induced renal damage was on dosing schedule. **1** given to rodents po s.i.d. at a dose of 474 μmol/kg/d for 7 days caused vacuolization of the renal proximal tubules.[42,45] However, **1** given b.i.d. po to rodents at 237 μmol/kg/dose (474 μmol/kg/d) for 7 days caused extensive and profound architectural changes (vacuolization) of the proximal tubules.[45] This was not the case with either of the polyether analogues in identical b.i.d. dosing experiments.[45] There were little, if any, changes in the cellular architecture of the proximal tubules of rats treated with **3** and only some minor inclusions in the proximal tubules of rodents treated with **2**.[42,45]

These findings encouraged additional toxicity and dose−response studies[45] of the 3′- and 4′-polyethers in order to identify which ligand would be best to take forward to GLP preclinical trials. Although these results further underscore the importance of lipophilicity on how a chelator performs in animals, what remains unclear is the extent to which lipophilicity can impact on ligand ICE and toxicity. In order to settle this issue, we have

Scheme 1. Synthesis of (*S*)-4′-Alkoxy Desazadesferrithiocin Analogues **5**−**7**[a]



13

14 R = R' = CH_2CH_3
15 R = $(CH_2)_3CH_3$, R' = CH_2CH_3
16 R = $(CH_2)_7CH_3$, R' = CH_2CH_3
5 R = CH_2CH_3, R' = H
6 R = $(CH_2)_3CH_3$, R' = H
7 R = $(CH_2)_7CH_3$, R' = H

[a] Reagents: (a) CH_3CH_2I (1.8 equiv), K_2CO_3 (2.1 equiv), acetone, 65 °C, 84%; (b) $CH_3(CH_2)_3I$ (1.7 equiv), K_2CO_3 (1.6 equiv), acetone, 94%; (c) $CH_3(CH_2)_7I$ (0.95 equiv), NaOEt (0.95 equiv), EtOH, 64%; (d) NaOH (aq) (12−14 equiv), MeOH, 90% (**5**), 92% (**6**), 80% (**7**).

elected to first assemble a group of alkoxydesazadesferrithiocin analogues with large differences in log P_{app} and measure their ICE values in rodents and primates (Table 1). This will then be followed by extensive ligand organ distribution and toxicity/histopathology studies. The focus of the current study is to develop a basis set of ligands to help define more precisely the relationship between log P_{app}, ICE, and toxicity. This will help to establish whether chelator-induced renal toxicity is derived from lipophilicity, ICE, or some combination thereof.

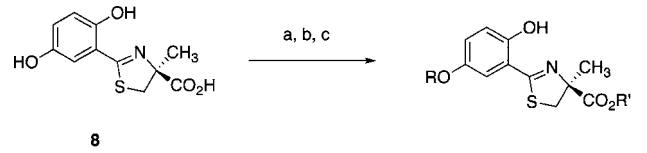
Synthetic Methods

DFT analogues **1**−**4**, **8**, and **9** were synthesized using methods published by this laboratory.[44–47] The key step in the synthesis of (*S*)-4,5-dihydro-2-(4-ethoxy-2-hydroxyphenyl)-4-methyl-4-thiazolecarboxylic acid (**5**), (*S*)-2-(4-butoxy-2-hydroxyphenyl)-4,5-dihydro-4-thiazolecarboxylic acid (**6**), and (*S*)-4,5-dihydro-2-[2-hydroxy-4-(octyloxy)phenyl]-4-methyl-4-thiazolecarboxylic acid (**7**, Table 1) was alkylation of the ethyl ester **13**[56] at the less hindered hydroxy group (Scheme 1). Specifically, resorcinol system **13** was heated with K_2CO_3 (2.1 equiv) and iodoethane (1.8 equiv) in acetone, giving ethyl ester **14** in 84% yield. Similarly, refluxing **13** with K_2CO_3 and 1-iodobutane in acetone provided masked DFT analogue **15** in 94% yield. When ethyl ester **13** was heated with sodium ethoxide and 1-iodooctane (0.95 equiv each) in EtOH, monoalkylated product **16** was obtained in 64% yield. Hydrolysis of the ethyl ester group in **14**−**16** using excess 50% NaOH (aqueous) in CH_3OH at room temperature and then acidification generated lipophilic DFT analogues **5**−**7** as solids in yields of at least 80%.

After conversion of **8**[47] to its ethyl ester **17** in 88% yield [CH_3CH_2I, *N,N*-diisopropylethylamine (DIEA), DMF], selective O-butylation of the hydroquinone system using 1-iodobutane and K_2CO_3 in acetone at reflux gave protected DFT analogue **18** in 79% yield. Ester hydrolysis generated (*S*)-2-(5-butoxy-2-hydroxyphenyl)-4,5-dihydro-4-thiazolecarboxylic acid (**10**) in 92% yield (Scheme 2). The same strategy was employed to make (*S*)-4,5-dihydro-2-[2-hydroxy-5-(octyloxy)phenyl)-4-methyl-4-thiazolecarboxylic acid (**11**, Scheme 3). The isopropyl ester **19**[55] was alkylated at the less hindered hydroxyl using 1-iodooctane and sodium isopropoxide in refluxing 2-propanol, affording masked chelator **20** in 35% yield. Base-promoted removal of the ester protecting group furnished chelator **11** in 96% yield.

The synthesis of (*S*)-2-[4,5-bis(octyloxy)-2-hydroxyphenyl]-4,5-dihydro-4-thiazolecarboxylic acid (**12**) followed the methodology of Scheme 4. Oxidative cleavage of the formyl group of 3,4-bis(octyloxy)benzaldehyde (**21**)[57] using 35% H_2O_2 and concentrated sulfuric acid in $CH_3OH/CHCl_3$ gave 3,4-bis(octyloxy)phenol (**22**) in 96% yield. Regiospecific formylation of **22** employing hexamethylenetetramine (1.0 equiv) in trifluoroacetic acid (TFA) produced substituted salicylaldehyde **23** in 39% yield. Aldehyde **23** was derivatized as oxime **24** (96% yield), which was converted to nitrile **25** under standard conditions (Ac_2O at reflux and then NaOH) in 72% yield. Cyclocondensation of **25** with (*S*)-α-methylcysteine hydrochloride (**26**, 1.4 equiv) at pH 6 followed by an esterification reaction (CH_3CH_2I, DIEA, DMF) gave ethyl (*S*)-2-[4,5-bis(octyloxy)-2-hydroxyphenyl]-4,5-dihydro-4-thiazolecarboxylate (**27**) in 90% yield. Alkaline hydrolysis of ester **27** and acidification completed the synthesis of lipophilic chelator **12** in 92% yield. The overall yield to **12** was 21%.

Scheme 2. Synthesis of (*S*)-5′-Butoxy Desazadesferrithiocin **10**[a]



8

17 R = H, R' = CH_2CH_3
18 R = $(CH_2)_3CH_3$, R' = CH_2CH_3
10 R = $(CH_2)_3CH_3$, R' = H

[a] Reagents: (a) CH_3CH_2I (1.8 equiv), DIEA (1.8 equiv), DMF, 88%; (b) $CH_3(CH_2)_3I$ (1.9 equiv), K_2CO_3 (2.1 equiv), acetone, 79%; (c) NaOH (aq) (16 equiv), MeOH, 92%.

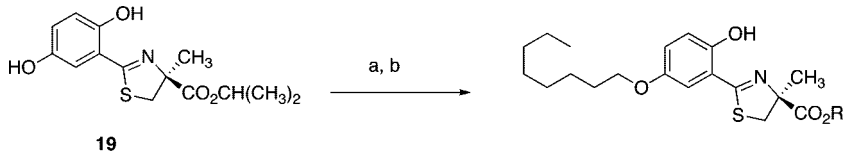
Partition Properties

The partition values between octanol and water (at pH 7.4, Tris buffer) were determined using a "shake flask" direct method of measuring log P_{app} values.[58] The fraction of drug in the octanol is then expressed as log P_{app}. These values varied widely among the basis set (Table 1) from log $P_{app} = -1.22$ for 3′-polyether **3** to log $P_{app} = 2.05$ for **12**. This represents a greater than1800-fold difference in partition. As described above, there are three structural groups: the 4′-substituted compounds **1**, **2**, **4**−**7**, the 5′-substituted compounds **8**−**11**, and the 4′,5′-disubstituted ligand **12**. Of the 4′-substituted compounds, the most lipophilic chelator is **7**, 380 times more lipophilic than the parent **1**. Of the 5′-substituted ligands, **11** is the most lipophilic, 500 times more lipophilic than the parent ligand **8**. However, the most lipophilic of all the ligands, not unexpectedly, is the dioctoxy compound **12**, over 1200 or 1500 times more lipophilic than the 4′- or 5′-hydroxy parent **1** or **8**, respectively.

Chelator-Induced Iron Clearance in Non-Iron-Overloaded Rodents

Bile duct-cannulated rodents were given a single 300 μmol/kg dose of the respective chelators orally by gavage. The data in Table 1 include the iron-clearing efficiency (ICE) and the fraction of iron excreted in the bile and urine for each ligand. The parent drug **1** performed poorly in rodents with an ICE of 1.1 ± 0.8%;[42] all of the metal was cleared in the bile. Virtually any alkylation of the 4′-hydroxy of **1** led to a more efficient iron chelator. Simple methylation of the 4′-hydroxy to produce **4** increased the ICE to 6.6 ± 2.8%,[42] while ethylation providing **5** raised the ICE to 18.0 ± 0.9%. When the alkyl group gets much longer, for example, butoxy compound **6**, the ICE decreases relative to ethyl analogue **5**, 13.1 ± 3.3%. This is also the case moving to the 4′-octyloxy analogue **7**; the ICE further decreases to 6.4 ± 2.3% (Table 1). Thus, within the series of 4′-hydroxy substituted compounds, the optimum chain length seems to be a two-carbon fragment. A plot of the rodent

Scheme 3. Synthesis of (*S*)-5′-Octoxy Desazadesferrithiocin **11**[a]

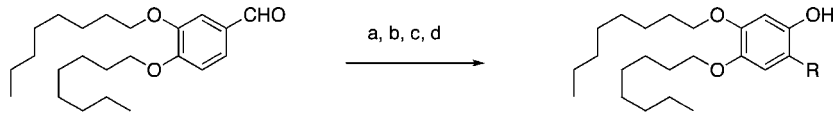


19

20 R = $CH(CH_3)_2$
11 R = H

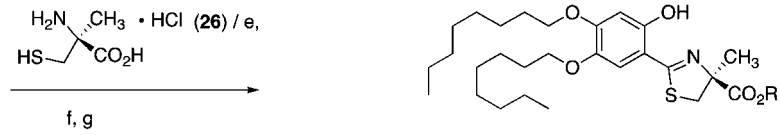
[a] Reagents: (a) $CH_3(CH_2)_7I$ (1.0 equiv), Na (1.0 equiv), *i*-PrOH, 35%; (b) NaOH (aq) (13 equiv), MeOH, 96%.

Scheme 4. Synthesis of (*S*)-4′,5′-Dioctoxy Desazadesferrithiocin **12**[a]



21

22 R = H
23 R = CHO
24 R = CH=NOH
25 R = CN



27 R = CH_2CH_3
12 R = H

[a] Reagents: (a) 35% H_2O_2, concentrated H_2SO_4, $CHCl_3$, MeOH, 96%; (b) $(CH_2)_6N_4$ (1.0 equiv), TFA, 39%; (c) $H_2NOH \cdot HCl$, NaOAc, MeOH, 96%; (d) Ac_2O, reflux then NaOH (aq), MeOH, 72%; (e) $NaHCO_3$, CH_3OH, phosphate buffer (pH 6.0), reflux; (f) CH_3CH_2I, DIEA, DMF, 90%; (g) NaOH (aq) (11 equiv), MeOH and then HCl, 92%.

ICE versus the log P_{app} for the 4′-hydroxy analogues generated a well-defined curve ($r^2 = 0.97$), clearly indicating the optimum log P_{app} value for ICE (Figure 1). Chelators with log P_{app}s above or below this value can have profoundly different iron-clearance properties. Figure 1 defines an excellent relationship between lipophilicity and ICE and will provide a strong foundation for investigating the source of nephrotoxicity in desferrithiocin-based ligands.

Alkylation of the 5′-hydroxy analogues resulted in ligands with ICE trends similar to what was seen when the 4′-hydroxy analogues were alkylated (Table 1). The parent drug **8** was the least effective, with an ICE of 1.0 ± 0.9%.[47] Fixing a methyl group to **8**, providing derivative **9**, increased the ICE to 6.3 ± 1.2%.[47] Butylation of the 5′-hydroxy of **8** to produce **10** was very effective at increasing the ICE (21.0 ± 4.0%) relative to the parent drug **8**. The 5′-octoxy analogue **11** was profoundly more lipophilic than the parent but was not as effective a deferration agent (4.0 ± 1.2% ICE) as methoxy derivative **9** or butoxy compound **10** (Table 1). A plot of the rodent ICE versus log P_{app} for the 5′-hydroxy series also suggests an optimum lipophilicity, but the curve fit ($r^2 = 0.81$, Figure 2) was not nearly as good as for the corresponding 4′-hydroxy series.

Finally, the dioctoxy ligand **12** was generated. This compound was over 1200 times more lipophilic than either the 4′- or the

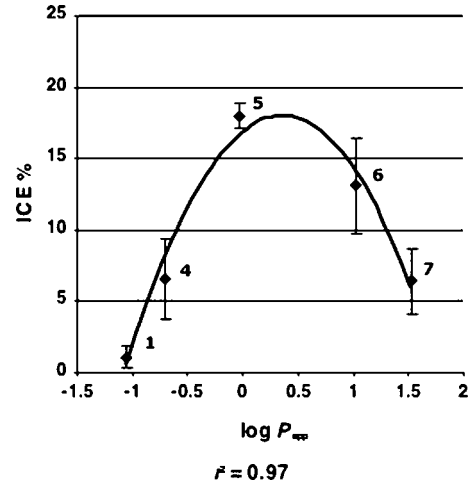


Figure 1. Iron-clearing efficiency versus log P_{app} for (*S*)-4′-hydroxy desazadesferrithiocin analogues **1**, **4**−**7** in bile duct-cannulated rats. The compounds were administered po.

5′-hydroxy parents. However, there was no improvement in ICE relative to the parent drugs. In fact, iron excretion was virtually baseline. It is important to note that both the 4′- and 5′-butoxy

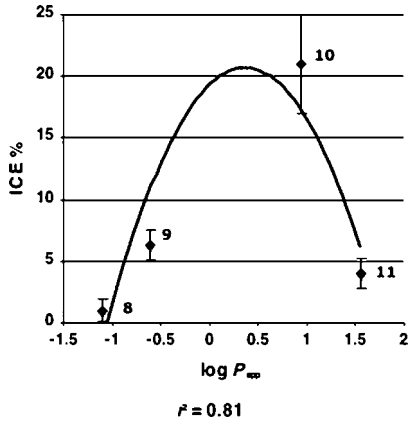


Figure 2. Iron-clearing efficiency versus log P_{app} for (*S*)-5′-hydroxy desazadesferrithiocin analogues **8**−**11** in bile duct-cannulated rats. The compounds were administered po.

analogues, **6** and **10**, were found to be toxic in the bile duct-cannulated rodents; in each instance, two of five rats given a single 300 μmol/kg po dose of the chelators either died or were sacrificed in moribund condition during the course of the 24 h sample collection period. Thus, these ligands were not given to the primates.

Iron Clearance in Iron-Loaded Primates

Iron-loaded primates were given the ligands orally by gavage. Prior to exposure to any compound, a complete hematologic and metabolic screen was run on each animal as previously described.[59] The same screen is also run after the iron clearing study is complete in order to identify any untoward effects of the chelator. The parent drug of the 4′-hydroxy series (**1**) was given po at a dose of 150 μmol/kg and had an ICE of 16.8 ± 7.2%[42] (Table 1). The corresponding 4′-methoxy ligand **4**, also given po at 150 μmol/kg, had an ICE of 24.4 ± 10.8%.[46] The 4′-ethoxy analogue **5** was given to the monkeys po at a dose of 37.5 μmol/kg and had an ICE of 25.1 ± 6.7%. This ligand was given to the primates at a lower dose because of concerns about potential toxicity associated with its lipophilicity. However, the hematologic and metabolic screens taken after exposure to the chelator were normal. As described above, the butoxy analogue **6** was not given to the primates because it was toxic in the bile duct-cannulated rodents. Finally, the octoxy ligand **7** was administered to the monkeys po at a dose of 75 μmol/kg. The ICE was 17.8 ± 2.1%, and all hematologic and metabolic screens were normal.

The relative ICE values of the 4′- and 5′-hydroxydesazadesferrithiocin analogues in the primates were similar to the rodent results; the 4′-hydroxy series was consistently more efficient (Table 1). The 5′-hydroxy parent **8** at 150 μmol/kg provided an ICE of 12.6 ± 3.0%.[47] Methylation of the 5′-hydroxy to give **9** increased the parent ICE; at 150 μmol/kg the ICE rose to 18.9 ± 2.3%.[47] Again, butoxy analogue **10** was not given to the primates because of toxicity issues in the rodents. Octoxy analogue **11** was given to the monkeys po at 75 μmol/kg. The ICE was similar to the parent drug **8**, ICE 12.6 ± 3.0% versus 12.3 ± 6.9% ($p > 0.05$) for the octoxy analogue **11**. Again, there were no untoward hematologic or metabolic screen numbers for the octoxy analogue. Finally, the dioctoxy analogue **12** was given po to the primates at a dose of 37.5 μmol/kg. It was only minimally effective, ICE 2.9 ± 2.5% (Table 1).

There is a consistent difference in ICE values seen for primates versus rodents. In every instance the ligands' ICE values were higher in the iron-overloaded primates than in the non-iron-loaded rodents (Table 1). While it is tempting to attribute these differences solely to the fact that the primates are iron-overloaded and the rodents are not, this is not likely the case. In an earlier paper,[55] we pointed out that the performance ratios of the ligands ($ICE_{rodent}/ICE_{primate}$) can be very different, even within a structurally similar family, and thus are not consistent with the idea that iron overload is the only explanation.

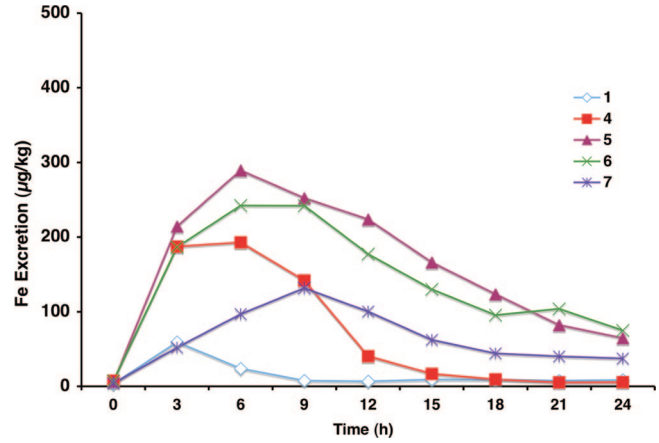


Figure 3. Biliary ferrokinetics of (*S*)-4′-hydroxy analogues **1**, **4**−**7** in bile duct-cannulated rats. The compounds were administered po.

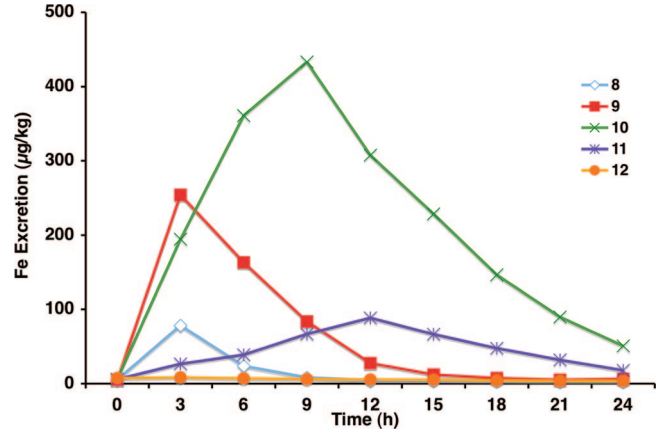


Figure 4. Biliary ferrokinetics of (*S*)-5′-hydroxy analogues **8**−**11** and 4′,5′-dioctoxy analogue **12** in bile duct-cannulated rats. The compounds were administered po.

Biliary Ferrokinetics of Desferrithiocin Analogues

Non-iron-overloaded, bile duct-cannulated rats were given a single 300 μmol/kg dose of ligands **1** and **4**−**12** orally by gavage. Bile samples were collected at 3 h intervals for 24 h. Iron content was evaluated by atomic absorption spectroscopy. The biliary ferrokinetics were followed for all of the ligands in the 4′- and 5′-hydroxydesazadesferrithiocin series (Figures 3 and 4). The areas under the curves reflect the *relative* iron-clearing efficiencies of the ligands.

The parent drug **1**, achieves maximum iron clearance (MIC) at 3 h, faster than any of the analogues in this series (Figure 3). However, the iron clearance is also over sooner than with the other ligands, complete by 9 h postdrug. The 4′-octoxy analogue **7** is next in terms of ICE, with an MIC at 9 h. It has a protracted iron clearance time and is still not back to baseline levels at 24 h. The 4′-methoxy ligand **4** is a more efficient chelator than the octoxy chelator **7** and has an MIC between 3 and 6 h; iron excretion is essentially back to baseline at 15 h. The 4′-butoxy

chelator **6** presents an MIC between 6 and 9 h, but significant quantities of iron are still being excreted 24 h postdrug. It is the second most efficient of the 4′-hydroxy analogues (Table 1). Finally, the 4′-ethoxy compound **5** is the most efficient of the 4′-substituted ligands with a clear MIC at 6 h, and like the ligands other than the parent **1** and **4**, iron clearance is not back to baseline levels at 24 h (Figure 3). The reasons for the geometry of these curves will later be correlated with pharmacokinetic and chelator organ distribution data.

With the 5′-hydroxy parent **8**, MIC is achieved at 3 h (Figure 4); like the 4′-hydroxy analogue **1**, iron clearance is completed at 9 h. The (*S*)-5′-octoxy analogue **11**, like its 4′-octoxy counterpart **7**, is slow to reach MIC, not doing so until 12 h postdrug and returning to baseline at 24 h. The 5′-methoxy analogue **9** presents a sharply defined MIC at 3 h; iron clearance is complete at 15 h. The 5′-butoxy analogue **10**, the most efficient iron chelator in both families, had an MIC at 9 h, and iron clearance is still not back to baseline at 24 h. Finally, the 4′,5′-dioctoxy compound **12** did not induce any iron excretion beyond baseline levels (Figure 4).

Conclusion

Although previous structure−activity studies led to the identification of a number of desferrithiocin analogues that were profoundly less nephrotoxic than desferrithiocin itself,[42,44,47] for example, **1**, nephrotoxicity was still a concern. Indeed, the dose-limiting property of **1** in both animal models and in patients would prove to be nephrotoxicity.[35] Furthermore, studies in both rodents and in human clinical trials identified a significant relationship between nephrotoxicity, not only at a total dose level, but more importantly as a function of dose scheduling. For example, the kidneys of rodents given **1** po s.i.d. at a dose of 474 μmol/kg/d for 7 days presented moderate damage to the proximal tubules.[45] However, when the rats were given the drug po b.i.d. at 237 μmol/kg/dose (474 μmol/kg/d) for 7 days, the architectural changes to the proximal tubules were profoundly more severe.[42] The same phenomenon occurred clinically; although the s.i.d. regimen (5, 10 or 15 mg/kg/d) was well tolerated, b.i.d. dosing (12.5 mg/kg/dose, 25 mg/kg/d) led to unacceptable nephrotoxicity, and the clinical study was terminated.[35]

Further SAR studies of **1** itself led to the discovery that the more lipophilic a ligand is, the better the ICE, but the more toxic.[42] This finding was translated into the syntheses of the 4′- and 3′- polyether analogues,[42,55] **2** and **3**, respectively, which were less lipophilic, less toxic, and had significantly greater ICE than the parent **1** in rodent models.[45] Histopathology studies revealed little, if any, damage to the kidneys of rodents treated with either of the polyether ligands.[42,45] Both short-term (10 d) and protracted toxicity (up to 30 d) trials underscore this idea. The conundrum then became "How does one reconcile the reduced lipophilicity and increased ICE of these ligands in view of previous findings?" In order to settle this issue and to gain a better understanding of the ligand structural parameters that impact a chelator's toxicity profile, a basis set of desferrithiocin analogues was constructed (Table 1).

Two basis sets were assembled, one predicated on 4′-hydroxy analogue **1** and a second based on the corresponding 5′-hydroxy compound **8**. One additional derivative was generated, the 4′,5′ dioctoxy analogue **12**, a very lipophilic chelator. In the 4′-hydroxy series, the log P_{app} varied from −1.05 for the parent **1** to 1.53 for the octoxy derivative **7**. In rodents the ICE was as low as 1.1 ± 0.8%[42] for **1** and as high as 18.0 ± 0.9% for 4′-ethoxy analogue **5** (Figure 1). In the 5′-hydroxy series, the log P_{app} varied from −1.14 for the parent **8** to 1.56 for octoxy compound **11**, an array similar in magnitude to the 4′-hydroxy series. The rodent ICE varied from 1.0 ± 0.9%[47] for **8** to 21.0 ± 4.0% for 5′-butoxy **10**, a range similar to the 4′-hydroxy series (Figure 2).

The biliary ferrokinetics of the 4′-hydroxy analogues presented a number of notable features (Figure 3). The parent **1** iron clearance was over by 9 h, with an MIC at 3 h. Methoxy compound **4**-induced iron clearance was also complete relatively early, 15 h, with an MIC at 6 h. All of the other 4′-hydroxy analogues (**5**−**7**) presented protracted iron clearance times; deferration was ongoing even at 24 h postdrug.

The biliary ferrokinetics of 5′-hydroxy analogue **8** are similar to 4′-hydroxy analogue **1**, complete by 9 h with an MIC at 3 h. The similarities between the two families end here. The MIC of 5′-methoxylated analogue **9** occurs at 3 h, much earlier than that of the corresponding 4′-methoxylated derivative **4**; however, the ICEs of the two ligands are within error of each other ($p > 0.05$). The 5′-butoxy ligand **10** induces the excretion of significantly more iron than its 4′-counterpart **6**, although its MIC also occurs at 9 h. The 5′-octoxy analogue **11** has an MIC slightly later than its 4′-counterpart **7**, 12 h versus 9 h; overall iron excretion is also lower. Finally, the ferrokinetics "curve" of the 4′,5′-dioctoxy analogue **12** is almost a flat line, which is in agreement with its poor ICE, <0.5%.

As in previous studies, the ligands in general performed very well in the iron-overloaded primate model. The relationship between log P_{app} and ICE is not nearly as obvious with the monkeys as it is with the rodents. Again, while it is tempting to ascribe the difference in ICE between the rodents and the primates to iron overload, this may not be the whole story. The performance ratios of the ligands ($ICE_{rodent}/ICE_{primate}$) can be very different,[55] even within a structurally similar family, and thus are not consistent with the idea that iron overload is the only explanation.

While there are two families of chelators to choose from in explaining the relationship between ligand ICE, ferrokinetics, organ distribution, lipophilicity, and nephrotoxicity, the 4′-hydroxy analogues **1** and **4**−**7** (Table 1) are more attractive. The curve of the 4′-hydroxy ligands relating rodent ICE and log P_{app} is smoother, $r^2 = 0.97$ (Figure 1), than for the corresponding 5′-hydroxy analogues **8**−**11**, $r^2 = 0.81$ (Figure 2). The discrepancy is largely due to the ICE of the 5′-butoxy ligand **10**; its ICE is much greater than would have been predicted based on its log P_{app} and is out of sync with the curve. We thus have defined a set of desferrithiocin analogues from which to launch a study focused on the origin of ligand-induced renal toxicity. It will now be possible to evaluate the relationship between ICE and log P_{app} on architectural changes in the proximal tubules. This will be the subject of a future manuscript.

Experimental Section

C. apella monkeys were obtained from World Wide Primates (Miami, FL). Male Sprague−Dawley rats were procured from Harlan Sprague−Dawley (Indianapolis, IN). Cremophor RH-40 was acquired from BASF (Parsippany, NJ). Ultrapure salts were purchased from Johnson Matthey Electronics (Royston, U.K.). All hematological and biochemical studies[53] were performed by Antech Diagnostics (Tampa, FL). Atomic absorption (AA) measurements were made on a Perkin-Elmer model 5100 PC (Norwalk, CT). Histopathological analysis was carried out by Florida Vet Path (Bushnell, FL).

Cannulation of Bile Duct in Non-Iron-Overloaded Rats. The cannulation has been described previously.[52,53] Bile samples were collected from male Sprague−Dawley rats (400−450 g) at 3 h

intervals for 24 h. The urine sample was taken at 24 h. Sample collection and handling are as previously described.[52,53]

Iron Loading of *C. apella* Monkeys. The monkeys (3.5−4 kg) were iron overloaded with intravenous iron dextran as specified in an earlier publication to provide about 500 mg of iron per kg of body weight;[59] the serum transferrin iron saturation rose to between 70% and 80%. At least 20 half-lives, 60 d,[60] elapsed before any of the animals were used in experiments evaluating iron-chelating agents.

Primate Fecal and Urine Samples. Fecal and urine samples were collected at 24 h intervals and processed as described previously.[52,53,61] Briefly, the collections began 4 d prior to the administration of the test drug and continued for an additional 5 d after the drug was given. Iron concentrations were determined by flame atomic absorption spectroscopy as presented in other publications.[52,62]

Drug Preparation and Administration. In the iron-clearing experiments, the rats were given a single 300 μmol/kg dose of drugs **1**−**12** orally (po). The compounds were administered as the monosodium salt of the compound of interest (prepared by the addition of 1 equiv of NaOH to a suspension of the free acid in distilled water). Compound **2** was solubilized in distilled water.

The drugs were given to the monkeys po at a dose of 37.5 μmol/kg (**5**, **12**), 75 μmol/kg (**3**, **7**, **11**), or 150 μmol/kg (**1**, **2**, **4**, **8**, **9**). The drugs were prepared as for the rats except that **4** was solubilized in 40% Cremophor RH-40/distilled water. Note that **6** and **10** were not given to the monkeys because of toxicity seen with the drugs in the bile duct-cannulated rats.

Calculation of Iron Chelator Efficiency. The theoretical iron outputs of the chelators were generated on the basis of a 2:1 complex. The efficiencies in the rats and monkeys were calculated as set forth elsewhere.[44] Data are presented as the mean ± 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.

Synthetic Methods. DFT analogues **1**−**4**, **8**, and **9** were synthesized using methods published by this laboratory.[44−47] Reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI). Fisher Optima grade solvents were routinely used, and DMF was distilled. Reactions were run under a nitrogen atmosphere, and organic extracts were dried with sodium sulfate. Silica gel 40−63 from SiliCycle, Inc. (Quebec City, Quebec, Canada) was used for column chromatography, and Sephadex LH-20 was obtained from Amersham Biosciences (Piscataway, NJ). Melting points are uncorrected. 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 **5**−**7** and **10**−**12**. Optical rotations were run at 589 nm (sodium D line) utilizing a Perkin-Elmer 341 polarimeter, with *c* being concentration in grams of compound per 100 mL. Chemical shifts (δ) for ^{1}H NMR spectra at 400 MHz are given in parts per million downfield from tetramethylsilane ($CDCl_3$ not indicated). Chemical shifts (δ) for ^{13}C NMR spectra at 100 MHz are given in parts per million referenced to the residual solvent resonance: $CDCl_3$ (δ 77.16), CD_3OD (δ 49.00), or DMSO-d_6 (δ 39.52). Coupling constants (*J*) are in hertz. Elemental analyses were performed by Atlantic Microlabs (Norcross, GA).

(*S*)-4,5-Dihydro-2-(4-ethoxy-2-hydroxyphenyl)-4-methyl-4-thiazolecarboxylic Acid (5). A solution of 50% (w/w) NaOH (17.7 mL, 339 mmol) in CH_3OH (215 mL) was added over 12 min to **14** (7.66 g, 24.76 mmol) in CH_3OH (250 mL) with ice bath cooling. The reaction mixture was stirred at room temperature for 18 h, and the bulk of the solvent was removed by rotary evaporation. The residue was treated with dilute NaCl (350 mL) and was extracted with ether (3 × 100 mL). The basic aqueous phase was cooled in ice, acidified with cold 2 N HCl (225 mL), and was extracted with EtOAc (250 mL, 2 × 100 mL). The EtOAc extracts were washed with saturated NaCl (120 mL) and were concentrated in vacuo. Recrystallization from EtOAc/hexanes furnished 6.289 g of **5** (90%), as small yellow crystals, mp 191.5-192.5 °C: $[\alpha]^{23}$ +61.6° (*c* 1.20, DMF); ^{1}H NMR (DMSO-d_6) δ 1.32 (t, 3 H, J = 6.8), 1.58 (s, 3 H), 3.36 (d, 1 H, J = 11.2), 3.79 (d, 1 H, J = 11.6), 4.07 (q, 2 H, J = 6.8), 6.49 (d, 1 H, J = 2.4), 6.52 (dd, 1 H, J = 8.4, 2.4), 7.32 (d, 1 H, J = 8.4), 12.74 (s, 1 H), 13.18 (br s, 1 H); ^{13}C NMR (DMSO-d_6) δ 14.48, 24.13, 63.56, 82.45, 101.12, 107.24, 108.98, 131.62, 160.51, 162.83, 170.08, 173.77; HRMS *m/z* calcd for $C_{13}H_{16}NO_4S$, 282.0800 (M + H); found, 282.0763. Anal. ($C_{13}H_{15}NO_4S$) C, H, N.

(*S*)-2-(4-Butoxy-2-hydroxyphenyl)-4,5-dihydro-4-methyl-4-thiazolecarboxylic Acid (6). A solution of 50% (w/w) NaOH (19.11 g, 239 mmol) in CH_3OH (150 mL) was added over 17 min to **15** (5.472 g, 16.22 mmol) in CH_3OH (150 mL) with ice bath cooling. The reaction mixture was stirred at room temperature for 1 d, and the bulk of the solvent was removed by rotary evaporation. The residue was treated with dilute NaCl (200 mL), cooled in ice, acidified with cold 2 N HCl (150 mL), and extracted with EtOAc (200 mL, 2 × 100 mL). The EtOAc extracts were washed with H_2O (150 mL) and saturated NaCl (80 mL) and were concentrated in vacuo. Recrystallization from EtOAc/hexanes generated 4.62 g of **6** (92%) as a yellow solid, mp 138−140 °C: $[\alpha]^{24}$ +57.8° (*c* 0.95, DMF); 500 MHz ^{1}H NMR (DMSO-d_6) δ 0.93 (t, 3 H, J = 7.4), 1.42 (sextet, 2 H, J = 7.5), 1.58 (s, 3 H), 1.65−1.72 (m, 2 H), 3.36 (d, 1 H, J = 11.5), 3.79 (d, 1 H, J = 11.5), 4.01 (t, 2 H, J = 6.5), 6.50 (d, 1 H, J = 2.3), 6.52 (dd, 1 H, J = 8.7, 2.4), 7.32 (d, 1 H, J = 8.6), 12.7 (s, 1 H), 13.2 (br s, 1 H); 125 MHz ^{13}C NMR (DMSO-d_6) δ 13.55, 18.55, 24.01, 30.44, 67.47, 82.35, 101.15, 107.16, 108.88, 131.46, 160.38, 162.87, 169.91, 173.60; HRMS *m/z* calcd for $C_{15}H_{20}NO_4S$, 310.1113 (M + H); found, 310.1112. Anal. ($C_{15}H_{19}NO_4S$) C, H, N.

(*S*)-4,5-Dihydro-2-[2-hydroxy-4-(octyloxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (7). A solution of 50% (w/w) NaOH (7.0 mL, 134 mmol) in CH_3OH (70 mL) was added to **16** (4.28 g, 10.88 mmol) in CH_3OH (100 mL) with ice bath cooling. The reaction mixture was stirred at room temperature for 16 h, and the bulk of the solvent was removed by rotary evaporation. The residue was treated with cold dilute NaCl (50 mL), acidified with 1 N HCl to pH ∼2, and extracted with EtOAc (4 × 80 mL). The EtOAc layers were washed with saturated NaCl (100 mL) and were concentrated by rotary evaporation. The residue was subjected to a Sephadex LH-20 column, eluting with 10% EtOH/toluene, to furnish 3.2 g of **7** (80%) as an off-white solid: $[\alpha]^{20}$ +65.3 (*c* 0.294, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 0.90 (t, 3 H, J = 6.8), 1.25−1.52 (m, 10 H), 1.67 (s, 3 H), 1.72−1.82 (m, 2 H), 3.34 (d, 1 H, J = 11.6), 3.86 (d, 1 H, J = 11.2), 4.0 (t, 2 H, J = 6.6), 6.45 (d, 1 H, J = 2.4), 6.49 (dd, 1 H, J = 8.8, 2.4), 7.38 (d, 1 H, J = 9.2); ^{13}C NMR (CD_3OD) δ 14.49, 23.75, 24.77, 27.15, 30.26, 30.43, 30.50, 33.02, 40.56, 69.29, 84.18, 102.29, 108.08, 110.71, 132.83, 162.31, 165.03, 172.31, 176.08; HRMS *m/z* calcd. for $C_{19}H_{28}NO_4S$, 366.1734 (M + H); found, 366.1728. Anal. ($C_{19}H_{27}NO_4S$) C, H, N.

(*S*)-2-(5-Butoxy-2-hydroxyphenyl)-4,5-dihydro-4-methyl-4-thiazolecarboxylic Acid (10). A solution of 50% (w/w) NaOH (17.96 g, 224 mmol) in CH_3OH (125 mL) was added to **18** (4.81 g, 14.25 mmol) in CH_3OH (125 mL) with ice bath cooling. The reaction mixture was stirred at room temperature for 1 d, and the bulk of the solvent was removed by rotary evaporation. The concentrate was treated with dilute NaCl (165 mL) and was extracted with ether (3 × 60 mL). The basic aqueous phase was cooled in ice, acidified with 2 N HCl (140 mL), and was extracted with EtOAc (150 mL, 2 × 80 mL). The EtOAc extracts were washed with H_2O (120 mL) and saturated NaCl (75 mL) and were concentrated in vacuo. Drying under high vacuum at 69 °C gave 4.40 g of a green, viscous oil, 3.94 g of which was chromatographed on Sephadex LH-20 with 6% EtOH/toluene, affording 3.63 (92%) of **10:** $[\alpha]^{24}$ +15.6 (*c* 1.29, DMF); ^{1}H NMR (DMSO-d_6) δ 0.93 (t, 3 H, J = 7.4), 1.38−1.48 (m, 2 H), 1.59 (s, 3 H), 1.63−1.71 (m, 2 H), 3.41 (d, 1 H, J = 11.3), 3.83 (d, 1 H, J = 11.7), 3.93 (t, 2 H, J = 6.4), 6.87 (d, 1 H, J = 2.7), 6.92 (d, 1 H, J = 9.0), 7.09 (dd, 1 H, J = 9.0, 2.7), 12.0 (s, 1 H), 13.3 (br s, 1 H); ^{13}C NMR (DMSO-d_6) δ 13.75, 18.76, 24.01, 30.82, 39.64, 67.92, 83.01, 114.10, 115.34, 117.84, 121.27, 151.08, 152.56, 170.39, 173.63; HRMS *m/z* calcd for $C_{15}H_{20}NO_4S$ (M + H), 310.1108; found, 310.1108. Anal. ($C_{15}H_{19}NO_4S$) C, H, N.

(*S*)-4,5-Dihydro-2-[2-hydroxy-5-(octyloxy)phenyl]-4-methyl-4-thiazolecarboxylic Acid (11). A solution of 50% (w/w) NaOH (0.70 mL, 13 mmol) in CH_3OH (10 mL) was added to **20** (0.41 g,

1.0 mmol) in CH_3OH (20 mL) with ice bath cooling. The reaction mixture was stirred at room temperature for 20 h and was concentrated by rotary evaporation. The residue was treated with dilute NaCl (150 mL) and was extracted with ether (3 × 25 mL). The basic aqueous phase was cooled in ice, acidified with 2 N HCl (10 mL), and extracted with EtOAc (50 mL, 2 × 25 mL). The EtOAc layers were washed with saturated NaCl (25 mL), and solvent was removed in vacuo. Lyophilization furnished 0.35 g of **11** (96%) as a light-yellow oil: $[\alpha]^{20}$ +17.7° (*c* 0.192, CH_3OH); ^{1}H NMR (DMSO-d_6) δ 0.86 (t, 3 H, J = 6.8), 1.24−1.44 (m, 10 H), 1.59 (s, 3 H), 1.65−1.72 (m, 2 H), 3.41 (d, 1 H, J = 11.2), 3.82 (d, 1 H, J = 11.6), 3.92 (t, 2 H, J = 6.8), 6.86−6.93 (m, 2 H), 7.08 (dd, 1 H J = 8.8, 2.4), 11.97 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 14.13, 22.27, 24.14, 25.65, 28.82, 28.90, 31.40, 68.37, 83.13, 114.25, 115.45, 117.99, 121.44, 151.19, 152.64, 170.53, 173.78; HRMS *m/z* calcd for $C_{19}H_{27}NO_4S$, 366.1734 (M + H); found, 366.1723. Anal. ($C_{19}H_{27}NO_4S$) C, H, N.

(*S*)-2-[4,5-Bis(octyloxy)-2-hydroxyphenyl]-4,5-dihydro-4-methyl-4-thiazolecarboxylic Acid (12). A solution of 50% (w/w) NaOH (1 mL, 19 mmol) in CH_3OH (10 mL) was added to a solution of **27** (0.92 g, 1.76 mmol) in CH_3OH (10 mL). The reaction mixture was stirred at room temperature for 16 h, and the solvent was removed by rotary evaporation. The residue was treated with dilute NaCl (5 mL) and was acidified with 2 N HCl to pH ∼1. Extraction with EtOAc (3 × 20 mL) and solvent removal in vacuo provided 0.80 g of **12** (92%) as a glassy yellow solid: $[\alpha]^{20}$ +36.2° (*c* 0.235, $CHCl_3$); ^{1}H NMR δ 0.89 (2 t, 6 H, J = 7.2), 1.24−1.38 (m, 16 H), 1.42−1.52 (m, 4 H), 1.72 (s, 3 H), 1.74−1.87 (m, 4 H), 3.24 (d, 1 H, J = 11.2), 3.88 (d, 1 H, J = 11.6), 3.91 (t, 2 H, J = 6.8), 4.01 (t, 2 H, J = 6.8), 6.56 (s, 1 H), 6.83 (s, 1 H), 9.38 (br s, 1 H); ^{13}C NMR δ 14.24, 22.80, 22.82, 24.57, 26.11, 26.14, 29.02, 29.38, 29.42, 29.45, 29.52, 29.54, 31.93, 31.96, 39.78, 69.07, 71.06, 82.13, 101.38, 106.83, 116.07, 141.81, 155.67, 156.55, 172.58, 177.06; HRMS *m/z* calcd for $C_{27}H_{44}NO_5S$, 494.2935 (M + H); found, 494.2991. Anal. ($C_{27}H_{43}NO_5S$) C, H, N.

Ethyl (*S*)-4,5-Dihydro-2-(4-ethoxy-2-hydroxyphenyl)-4-methyl-4-thiazolecarboxylate (14). Flame activated K_2CO_3 (1.21 g, 8.76 mmol) and iodoethane (0.60 mL, 7.50 mmol) were successively added to **13** (1.16 g, 4.12 mmol) in acetone (50 mL), and the mixture was heated at 65 °C for 16 h. After the mixture was cooled to room temperature, solids were filtered and washed with acetone (100 mL), and the filtrate was concentrated by rotary evaporation. The residue was dissolved in 1:1 0.5 M citric acid/saturated NaCl (150 mL) followed by extraction with EtOAc (150 mL, 2 × 70 mL). The combined organic extracts were washed with 1% $NaHSO_3$ (100 mL), H_2O (100 mL), and saturated NaCl (70 mL) and were concentrated in vacuo. Purification using flash column chromatography eluting with 9% EtOAc/petroleum ether generated 1.07 g of **14** (84%) as a yellow oil: $[\alpha]^{25}$ +54.8 (*c* 1.16, $CHCl_3$); ^{1}H NMR δ 1.30 (t, 3 H, J = 7.2), 1.42 (t, 3 H, J = 7.0), 1.66 (s, 3 H), 3.19 (d, 1 H, J = 10.9), 3.84 (d, 1 H, J = 11.3), 4.05 (q, 2 H, J = 7.0), 4.20−4.28 (m, 2 H), 6.43 (dd, 1 H, J = 8.8, 2.5), 6.48 (d, 1 H, J = 2.7), 7.29 (d, 1 H, J = 8.6), 12.69 (s, 1 H); ^{13}C NMR δ 14.22, 14.76, 24.60, 39.94, 62.02, 63.81, 83.21, 101.30, 107.38, 109.72, 131.80, 161.34, 163.33, 170.93, 173.00; HRMS *m/z* calcd for $C_{15}H_{20}NO_4S$, 310.1113 (M + H); found, 310.1109. Anal. ($C_{15}H_{19}NO_4S$) C, H, N.

Ethyl (*S*)-4,5-Dihydro-2-(4-butoxy-2-hydroxyphenyl)-4-methyl-4-thiazolecarboxylate (15). Flame activated K_2CO_3 (1.541 g, 11.15 mmol) and 1-iodobutane (1.4 mL, 12.3 mmol) were successively added to **13** (2.831 g, 10.06 mmol) in acetone (95 mL), and the mixture was heated at 65 °C for 16 h. Additional K_2CO_3 (0.620 g, 4.48 mmol) and 1-iodobutane (0.6 mL, 5.3 mmol) were added, and the reaction mixture was heated at 70 °C for 20 h. After the mixture was cooled to room temperature, solids were filtered and washed with acetone (140 mL), and the filtrate was concentrated by rotary evaporation. The residue was dissolved in 1:1 0.5 M citric acid/saturated NaCl (150 mL) followed by extraction with EtOAc (150 mL, 2 × 80 mL). The combined organic extracts were washed with 1% $NaHSO_3$ (100 mL), H_2O (100 mL), and saturated NaCl (50 mL) and were concentrated in vacuo. Purification using flash column chromatography eluting with CH_2Cl_2 gave 3.18 g of **15** (94%) as a yellow oil: $[\alpha]^{25}$ +50.9 (*c* 1.02, $CHCl_3$); ^{1}H NMR δ 0.97 (t, 3 H, J = 7.2), 1.30 (t, 3 H, J = 7.2), 1.43−1.53 (m, 2 H), 1.66 (s, 3 H), 1.73−1.81 (m, 2 H), 3.19 (d, 1 H, J = 11.3), 3.84 (d, 1 H, J = 11.3), 3.98 (q, 2 H, J = 6.4), 4.18−4.30 (m, 2 H), 6.43 (dd, 1 H, J = 8.6, 2.7), 6.48 (d, 1 H, J = 2.7), 7.28 (d, 1 H, J = 8.6), 12.68 (s, 1 H); ^{13}C NMR δ 13.94, 14.23, 19.30, 24.61, 31.20, 39.95, 62.01, 68.01, 83.21, 101.33, 107.42, 109.67, 131.75, 161.34, 163.54, 170.91, 173.01; HRMS *m/z* calcd for $C_{17}H_{24}NO_4S$, 338.1426 (M + H); found, 338.1447. Anal. ($C_{17}H_{23}NO_4S$) C, H, N.

Ethyl (*S*)-4,5-Dihydro-2-[2-hydroxy-4-(octyloxy)phenyl]-4-methyl-4-thiazolecarboxylate (16). Freshly prepared 0.51 M sodium ethoxide (40 mL, 20.26 mmol) was added to a mixture of **13** (6.00 g, 21.33 mmol) and 1-iodooctane (4.14 g, 20.26 mmol) in ethanol (15 mL). The reaction mixture was heated to 70 °C for 24 h and was concentrated in vacuo. The residue was taken up in 1:1 0.5 M citric acid/saturated NaCl (50 mL) and was extracted with EtOAc (2 × 50 mL). The organic layers were washed with 100 mL portions of 0.5 M citric acid, 1% $NaHSO_3$, H_2O, and saturated NaCl. After solvent removal by rotary evaporation, flash column chromatography on silica gel eluting with 2% EtOAc/CH_2Cl_2 furnished 5.10 g (64%) of **16** as light-yellow oil: $[\alpha]^{20}$ +49.3° (*c* 0.27, $CHCl_3$); ^{1}H NMR δ 0.89 (t, 3 H, J = 7.2), 1.24−1.39 (m, 9 H), 1.40−1.48 (m, 4 H), 1.65 (s, 3 H), 1.74−1.82 (m, 2 H), 3.19 (d, 1 H, J = 11.2), 3.83 (d, 1 H, J = 11.6), 3.97 (t, 2 H, J = 6.6), 4.24 (q, 2 H, J = 6.8), 6.43 (dd, 1 H, J = 8.8, 2.4), 6.48 (d, 1 H, J = 2.4), 7.28 (d, 1 H, J = 8.4), 12.7 (s, 1 H); ^{13}C NMR δ 14.24, 22.79, 24.62, 26.10, 29.18, 29.35, 29.45, 31.93, 39.96, 62.01, 68.35, 83.27, 101.35, 107.43, 109.68, 131.76, 161.35, 163.56, 170.92, 173.01; HRMS *m/z* calcd for $C_{21}H_{32}NO_4S$, 394.2047 (M + H); found, 394.2050. Anal. ($C_{21}H_{31}NO_4S$) C, H, N.

Ethyl (*S*)-4,5-Dihydro-2-(2,5-dihydroxyphenyl)-4-methyl-4-thiazolecarboxylate (17). Iodoethane (2.8 mL, 35 mmol) and DIEA (6.2 mL, 35.6 mmol) were successively added to **8** (5.05 g, 19.9 mmol) in DMF (100 mL), and the solution was stirred at room temperature for 1 d. After solvent removal under high vacuum, the residue was treated with 1:1 0.5 M citric acid/saturated NaCl (200 mL) and was extracted with EtOAc (200 mL, 2 × 80 mL). The combined extracts were washed with 0.25 M citric acid (100 mL), 1% $NaHSO_3$ (100 mL), H_2O (100 mL), and saturated NaCl (75 mL), and solvent was evaporated. Purification by flash column chromatography using 8% EtOAc/CH_2Cl_2 furnished 4.96 g (88%) of **17** as an orange oil: $[\alpha]^{24}$ +43.6 (*c* 1.06, $CHCl_3$); ^{1}H NMR δ 1.30 (t, 3 H, J = 7.0), 1.67 (s, 3 H), 3.23 (d, 1 H, J = 11.3), 3.87 (d, 1 H, J = 11.3), 4.20−4.31 (m, 2 H), 4.76 (s, 1 H), 6.88−6.92 (m, 3 H), 11.96 (s, 1 H); ^{13}C NMR δ 14.16, 24.48, 40.13, 62.34, 83.70, 115.91, 118.05, 121.64, 148.07, 153.04, 171.43, 173.14; HRMS *m/z* calcd for $C_{13}H_{16}NO_4S$, 282.0800 (M + H); found, 282.0837. Anal. ($C_{13}H_{15}NO_4S$) C, H, N.

Ethyl (*S*)-2-(5-Butoxy-2-hydroxyphenyl)-4,5-dihydro-4-methyl-4-thiazolecarboxylate (18). Flame activated K_2CO_3 (1.888 g, 13.66 mmol) and 1-iodobutane (1.4 mL, 12.3 mmol) were successively added to **17** (1.85 g, 6.58 mmol) in acetone (35 mL), and the mixture was heated at 69 °C for 16 h. After the mixture was cooled to room temperature, solids were filtered and washed with acetone (100 mL), and the filtrate was concentrated by rotary evaporation. The residue was dissolved in 1:1 0.5 M citric acid/saturated NaCl (100 mL) followed by extraction with EtOAc (100 mL, 2 × 60 mL). The combined organic extracts were washed with 1% $NaHSO_3$ (80 mL), H_2O (80 mL), and saturated NaCl (50 mL) and were concentrated in vacuo. Purification using flash column chromatography eluting with CH_2Cl_2 gave 4.86 g of **18** (79%): $[\alpha]^{24}$ +28.8° (*c* 1.16, $CHCl_3$); ^{1}H NMR δ 0.98 (t, 3 H, J = 7.4), 1.30 (t, 3 H, J = 7.2), 1.44−1.55 (m, 2 H), 1.67 (s, 3 H), 1.71−1.79 (m, 2 H), 3.23 (d, 1 H, J = 11.3), 3.88 (d, 1 H, J = 11.3), 3.92 (t, 2 H, J = 6.4), 4.20−4.29 (m, 2 H), 6.90 (d, 1 H, J = 3.1), 6.93 (d, 1 H, J = 9.0), 6.99 (dd, 1 H, J = 9.0, 2.7), 11.97 (s, 1 H); 125 MHz ^{13}C NMR δ 14.00, 14.24, 19.39, 24.56, 31.54, 40.19, 62.13, 68.85, 83.85, 115.01, 115.88, 118.08, 121.48, 151.62, 153.60, 171.42, 172.79; HRMS *m/z* calcd for $C_{17}H_{24}NO_4S$, 338.1426 (M + H); found, 338.1436. Anal. ($C_{17}H_{23}NO_4S$) C, H, N.

Isopropyl (*S*)-4,5-Dihydro-2-[2-hydroxy-5-(octyloxy)phenyl]-4-methyl-4-thiazolecarboxylate (20). Sodium (0.084 g, 3.65 mmol) was introduced to *i*-PrOH (10 mL) with ice bath cooling, and the solution was added to 1-iodooctane (0.88 g, 3.66 mmol) and **19** (1.10 g, 3.72 mmol). The reaction mixture was heated to 70 °C for 24 h and was concentrated in vacuo. The residue was taken up in EtOAc (50 mL), which was washed with 25 mL portions of 0.1 M citric acid, 1% $NaHSO_3$, H_2O, and saturated NaCl. After solvent was removed by rotary evaporation, column chromatography on silica gel, eluting with 1% EtOAc/CH_2Cl_2, afforded 0.53 g (35%) of **20** as a light-yellow oil: $[\alpha]^{20}$ +24.1° (*c* 0.32, $CHCl_3$); ^{1}H NMR δ 0.89 (t, 3 H, J = 6.8), 1.24−1.39 (m, 14 H), 1.40−1.49 (m, 2 H), 1.65 (s, 3 H), 1.73−1.80 (m, 2 H), 3.21 (d, 1 H, J = 11.6), 3.86 (d, 1 H, J = 11.2), 3.91 (t, 2 H, J = 6.8), 5.08 (m, 1 H), 6.89−6.96 (m, 3 H), 11.98 (s, 1 H); ^{13}C NMR δ 14.23, 21.71, 22.77, 24.37, 26.14, 29.35, 29.43, 29.49, 31.93, 39.99, 69.05, 69.67, 83.83, 114.86, 115.83, 117.99, 121.30, 151.50, 153.51, 171.18, 172.16; HRMS *m*/*z* calcd for $C_{22}H_{33}NNaO_4S$, 430.2028 (M + Na); found, 430.2072. Anal. ($C_{22}H_{33}NO_4S$) C, H, N.

3,4-Bis(octyloxy)phenol (22). Methanol (100 mL), 35% hydrogen peroxide (6.6 g, 68 mmol), and concentrated H_2SO_4 (0.6 mL, 11 mmol) were added to **21**[57] (10.0 g, 27.58 mmol) in $CHCl_3$ (250 mL), and the reaction mixture was stirred at room temperature for 6 h. Water was added until two layers formed. The organic layer was washed with H_2O (100 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. Recrystallizion from hexane/CH_2Cl_2 gave 9.3 g (96%) of **22** as a solid: 600 MHz ^{1}H NMR δ 0.88 (2 t, 6 H, J = 7.1), 1.23−1.37 (m, 16 H), 1.40−1.48 (m, 4 H), 1.71−1.83 (m, 4 H), 3.91 (t, 2 H, J = 6.6), 3.93 (t, 2 H, J = 6.5), 4.66 (s, 1 H), 6.30 (dd, 1 H, J = 8.5, 2.8), 6.44 (d, 1 H, J = 2.8), 6.75 (d, 1 H, J = 8.5); 150 MHz ^{13}C NMR δ14.10, 22.68, 26.06, 29.22, 29.29, 29.30, 29.38, 29.43, 29.55, 31.84, 31.85, 69.04, 70.83, 102.32, 106.14, 116.43, 143.08, 150.43, 150.61; HRMS *m*/*z* calcd for $C_{22}H_{39}O_3$, 351.2894 (M + H); found, 351.2884. Anal. ($C_{22}H_{38}O_3$) C, H.

4,5-Bis(octyloxy)-2-hydroxybenzaldehyde (23). Hexamethylenetetramine (3.60 g, 25.72 mmol) was added to a solution of **22** (9.0 g, 25.67 mmol) in anhydrous TFA (25 mL). The reaction mixture was heated at reflux using an oil bath at 100 °C for 16 h and then cooled to room temperature. Cold 1 N HCl (50 mL) was added, and stirring was continued for 15 min. The aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with 1 N HCl (50 mL) and then saturated NaCl (50 mL) and were concentrated in vacuo. Column chromatography eluting with 50% CH_2Cl_2/hexanes generated 3.80 g of **23** (39%) as a yellow solid: 600 MHz ^{1}H NMR δ 0.89 (2 t, 6 H, J = 6.9), 1.25−1.39 (m, 16 H), 1.44−1.50 (m, 4 H), 1.79 (quintet, 2 H, J = 7.7), 1.85 (quintet, 2 H, J = 7.8), 3.95 (t, 2 H, J = 6.6), 4.03 (t, 2 H, J = 6.6), 6.42 (s, 1 H), 6.93 (s, 1 H), 9.65 (s, 1 H), 11.36 (s, 1 H); 150 MHz ^{13}C NMR δ 14.09, 14.10, 22.66, 22.68, 25.94, 26.03, 28.80, 29.23, 29.27, 29.29, 29.35, 29.39, 31.80, 31.84, 69.17, 70.55, 100.80, 112.89, 116.93, 142.51, 158.03, 159.56, 193.92; HRMS *m*/*z* calcd for $C_{23}H_{38}NaO_4$, 401.2668 (M + Na); found, 401.2627. Anal. ($C_{23}H_{38}O_4$) C, H.

4,5-Bis(octyloxy)-2-hydroxybenzaldoxime (24). Hydroxylamine hydrochloride (0.52 g, 7.5 mmol) and NaOAc (0.62 g, 7.5 mmol) were added to a solution of **23** (1.89 g, 4.99 mmol) in CH_3OH (10 mL), and the reaction mixture was heated at reflux for 1 h. Solvent was removed by rotary evaporation; the residue was treated with 0.05 M citric acid/saturated NaCl (60 mL) and was extracted with EtOAc (4 × 50 mL). The organic phase was washed with H_2O (50 mL) and saturated NaCl (50 mL) and concentrated in vacuo to generate 1.88 g (96%) of **24**, as a pale yellow solid: ^{1}H NMR δ 0.88 (2 t, 6 H, J = 6.7), 1.25−1.50 (m, 20 H), 1.79−1.85 (m, 4 H), 3.93 (t, 2 H, J = 6.6), 3.99 (t, 2 H, J = 6.6), 6.51 (s, 1 H), 6.68 (s, 1 H), 8.09 (s, 1 H); ^{13}C NMR δ 14.15, 14.19, 22.77, 26.09, 26.10, 29.08, 29.36, 29.38, 29.43, 29.50, 29.52, 31.91, 31.93, 68.94, 71.12, 101.74, 107.79, 116.94, 141.97, 152.45, 153.34, 168.16; HRMS *m*/*z* calcd for $C_{22}H_{39}O_3$, 394.2952 (M + H); found, 394.2935.

4,5-Bis(octyloxy)-2-hydroxybenzonitrile (25). Compound **24** (1.88 g, 4.78 mmol) was treated with acetic anhydride (4.1 mL) and heated at reflux for 16 h. The reaction mixture was concentrated under high vacuum and was partitioned between $CHCl_3$ (30 mL) and 8% $NaHCO_3$ (25 mL). The aqueous layer was further extracted with $CHCl_3$ (2 × 20 mL), and the combined organic phase was washed with 4% $NaHCO_3$ (50 mL) and saturated NaCl (50 mL) followed by solvent removal. The residue was treated with a 50% NaOH (1.5 mL, 29 mmol) in CH_3OH (30 mL). After the mixture was stirred for 16 h at room temperature, solvents were removed under reduced pressure. The concentrate was treated with 2 N HCl (10 mL) and was extracted with EtOAc (3 × 50 mL). The EtOAc layers were washed with saturated NaCl (100 mL) and were concentrated in vacuo, providing 1.29 g (72%) of **25**, as a white solid: ^{1}H NMR δ 0.88 (2 t, 6 H, J = 7.2), 1.22−1.39 (m, 16 H), 1.40−1.50 (m, 4 H), 1.74−1.86 (m, 4 H), 3.91 (t, 2 H, J = 6.6), 3.96 (t, 2 H, J = 6.6), 6.48 (s, 1 H), 6.87 (s, 1 H); ^{13}C NMR δ 14.21, 22.77, 26.03, 26.06, 28.91, 29.29, 29.34, 29.37, 29.40, 29.45, 31.90, 31.92, 69.21, 70.54, 88.78, 101.70, 116.18, 117.38, 142.99, 155.34, 155.50; HRMS *m*/*z* calcd for $C_{23}H_{37}NNaO_3$, 398.2671 (M + Na); found, 398.2645. Anal. ($C_{23}H_{37}NO_3$) C, H, N.

Ethyl (*S*)-2-[4,5-Bis(octyloxy)-2-hydroxyphenyl]-4,5-dihydro-4-methyl-4-thiazolecarboxylate (27). Compound **26** (0.767 g, 4.47 mmol), pH 6 phosphate buffer (30 mL), and $NaHCO_3$ (0.482 g, 5.74 mmol) were successively added to a solution of **25** (1.2 g, 3.19 mmol) in degassed CH_3OH (30 mL). The reaction mixture was heated at 70 °C for 48 h with stirring, cooled to room temperature, and concentrated by rotary evaporation. The residue was dissolved in 8% $NaHCO_3$ (20 mL) and was extracted with ether (2 × 20 mL). The aqueous layer was acidified with cold 2 N HCl to pH ∼2 followed by extraction with EtOAc (4 × 30 mL). The latter organic layers were washed with saturated NaCl (50 mL) and concentrated in vacuo, resulting in 1.45 g of **12**. Iodoethane (0.569 g, 3.65 mmol) and DIEA (0.471 g, 3.65 mmol) were successively added to **12** (1.0 g, 2.03 mmol) in DMF (20 mL), and the solution was stirred at room temperature for 48 h. After solvent removal under high vacuum, the residue was treated with 1:1 0.5 M citric acid/saturated NaCl (25 mL) followed by extraction with EtOAc (4 × 20 mL). The EtOAc layers were washed with 50 mL portions of 1% $NaHSO_3$, H_2O, and saturated NaCl, and the solvent was evaporated. Purification by flash column chromatography using 10% EtOAc/hexane gave 0.93 g of **27** (90%) as a yellow oil: $[\alpha]^{20}$ +32.0° (*c* 0.24, $CHCl_3$); ^{1}H NMR δ 0.89 (2 t, 6 H, J = 6.8), 1.24−1.38 (m, 16 H), 1.42−1.52 (m, 4 H), 1.65 (s, 3 H), 1.74−1.87 (m, 4 H), 3.19 (d, 1 H, J = 11.2), 3.84 (d, 1 H, J = 11.6), 3.93 (t, 2 H, J = 6.6), 4.00 (t, 2 H, J = 6.8), 4.24 (dq, 2 H, J = 6.8, 1.2), 6.49 (s, 1 H), 6.86 (s, 1 H), 12.41 (br s, 1 H); ^{13}C NMR δ 14.22, 14.24, 22.79, 22.80, 24.62, 26.10, 26.14, 29.05, 29.38, 29.42, 29.44, 29.54, 29.57, 31.93, 31.96, 40.07, 61.99, 68.91, 71.14, 83.31, 101.27, 107.57, 116.45, 141.58, 154.78, 156.00, 170.75, 173.02; HRMS *m*/*z* calcd for $C_{29}H_{48}NO_5S$, 522.3248 (M + H); found, 522.3231. Anal. ($C_{29}H_{47}NO_5S$) C, H, N.

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Supporting Information Available: Elemental analytical data for synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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