# Mobilization of Iron by Chiral and Achiral Anionic 3-Hydroxypyrid-4-ones

John Josef Molenda,[†] Mark M. Jones,[*,†] David S. Johnston,[†] Ernest M. Walker, Jr.,[‡] and Donald J. Cannon[‡]

*Department of Chemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, Tennessee 37235, and Pathology Department, School of Medicine, Marshall University, 1542 Spring Valley Drive, Huntington, West Virginia 25755-9360*

*Received July 21, 1994*[⊗]

In the search for 3-hydroxypyrid-4-ones with enhanced iron-mobilizing ability, seven chiral, anionic amino acid derivatives of maltol (3-hydroxy-2-methyl-4-pyrone) have been synthesized, utilizing L-methionine, L-serine, L-leucine, L-phenylalanine, L-glutamic acid, and the D- and L-isomers of alanine. Two achiral, aromatic compounds were also synthesized and compared with the phenylalanine derivative. The biliary iron excretion following iv injection and the urinary iron excretion following po administration were measured using female Sprague–Dawley rats and compared to that of the standard, 1,2-dimethyl-3-hydroxypyrid-4-one (L1). While none of the compounds was as effective as L1 in enhancing the urinary excretion of iron, all monoanionic chelators increased excretion relative to the controls. All monoanionic compounds were at least equivalent to L1 in enhancing the biliary excretion of iron, with the methionine, leucine, and benzoate derivatives surpassing the standard and the other aromatic compounds also showing strong activity. The dianionic glutamate derivative showed low activity relative to the controls for both urinary and biliary iron excretion. No significant difference in iron excretion was observed due to variation in chirality; molecular weight and the number of negative charges appeared to have the greatest influence on the ability of the various derivatives to enhance iron excretion. In order to evaluate the relative purity of the stereoisomers, the alanine derivatives were analyzed by circular dichroism. Further characterization was provided by UV/vis spectroscopy for all compounds and X-ray crystallography for the novel dianionic derivative.

## Introduction

Recent studies on the role of iron in such disorders as Parkinson's disease,[1–3] ischemia reperfusion injury,[4,5] myocardial infarctions,[6] and malaria[7,8] have pointed to the urgent need for new, nontoxic iron chelators. While the current standard desferrioxamine (DFO) has been used with considerable success in thalassemia patients suffering from transfusional iron overload,[9,10] several reports have pointed not only to visual,[11–14] auditory,[11,15] pulmonary,[16] and renal[17] toxicity but to DFO's ability to contribute iron to *Yersinia entericolitica*.[18] Furthermore, since DFO is relatively ineffective when given orally, the resulting administration via subcutaneous injection has resulted in low patient compliance.[19,20] In response to such concerns, the work of Kontoghiorghes, Hider, and their collaborators has focused on obtaining chelating agents which are orally active, specifically neutral derivatives of the 3-hydroxypyrid-4-ones.[21–25] While some members of this class of chelators have been shown to be effective in enhancing the urinary and biliary excretion of iron, like DFO, concerns have been raised due to several recent toxicity studies. These findings have linked various pyridinones to effects ranging from autoimmunity and antinuclear antibodies (ANA)[26,27] to bone marrow suppression[28] to inhibition of the enzymes ribonucleotide reductase[29] and tyrosinase.[30] These toxic effects have been attributed to the lipophilicity of the tested derivatives, especially 1,2-dimethyl-3-hydroxypyrid-4-one (L1),[29] and this has been supported by the results of Kontoghiorghes, which correlate increasing lipophilicity with reduced $LD_{50}$.[21]

In previous work, we have shown that two strongly polar, monoanionic 3-hydroxypyrid-4-ones were able to enhance urinary iron excretion significantly over control values and to equal L1 in terms of biliary excretion.[31] These results prompted the examination of the effect of such factors as molecular weight, structure, and lipophilicity on the activity of these monoanionic pyridinones, resulting in a series of achiral derivatives that at least equaled L1 in either biliary or urinary iron excretion.[32] Since significant evidence has pointed to the ability of anionic chelators to remove toxic metals,[32–35] through both monoanionic[36–39] and possibly dianionic[38] transport systems, we decided to perform initial *in vivo* studies to examine whether chiral, anionic derivatives of the 3-hydroxypyrid-4-ones were also able to mobilize iron.

Thus, six monoanionic amino acid derivatives of the pyran maltol were synthesized, using D- and L-alanine, L-leucine, L-phenylalanine, L-serine, and L-methionine. Utilizing L-glutamic acid, one dianionic derivative was also synthesized to examine whether it too would be able to mobilize iron despite its highly polar nature. Two additional achiral, monoanionic pyridinones were synthesized and provided structure/activity comparisons with the phenylalanine derivative. All pyridinones were administered to female Sprague–Dawley rats and examined for their effects on the urinary and biliary excretion of iron, and these results were compared with those of the standard, 1,2-dimethyl-3-hydroxypyrid-4-one (L1).

* Address for correspondence: Mark M. Jones, Box 1583, Station B, Vanderbilt University, Nashville, TN 37235. Phone: (615)-322-2987. FAX: (615)-322-4936.
† Vanderbilt Universtiy.
‡ Marshall University.
⊗ Abstract published in *Advance ACS Abstracts*, November 1, 1994.

0022-2623/94/1837-4363$04.50/0 

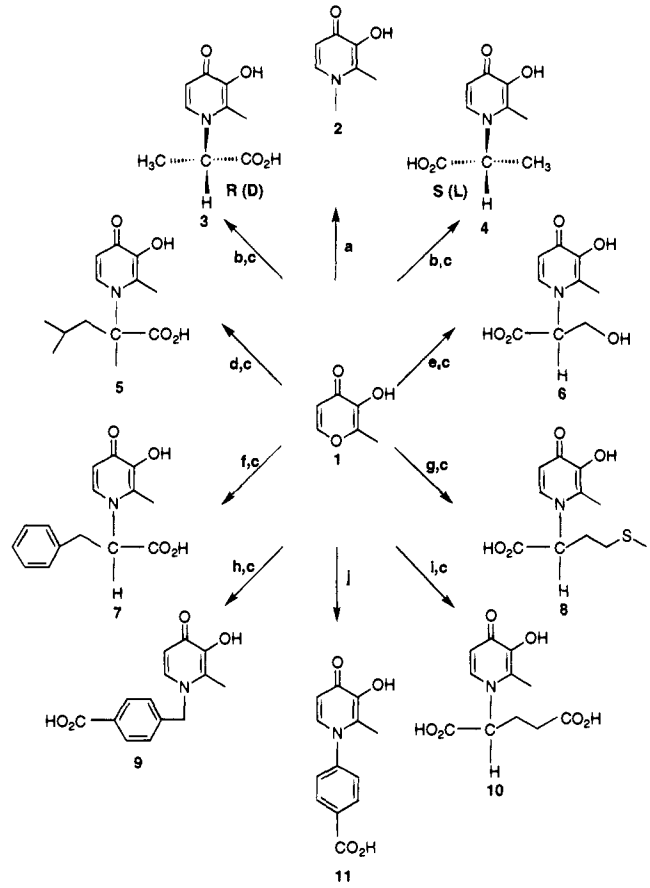
## Design and Synthesis

**Design.** During our synthesis of 3-hydroxypyrid-4-ones, it was discovered that amino acids were an untapped source of amines to generate potentially active iron chelators. As previous data had shown that the glycine derivative of maltol showed no activity,[20] we first verified its structure through X-ray diffraction and then showed that this compound significantly enhanced iron excretion in the bile. This led us to question whether other amino acid derivatives which *are* chiral would show significant iron-mobilizing ability relative to L1 (**2**). Since it has been shown that in basic, aqueous solutions amino acids have a tendency to racemize,[40] this concern prompted us to question the viability of utilizing these derivatives which may not be completely stereochemically pure.

Several factors prompted us to examine these derivatives despite this concern. We were first encouraged by the work of Avramovici-Grisaru et al.[41] with the orally active iron chelator PINH, a photosensitive compound which isomerizes in the presence of light. Throughout the substantial clinical studies, PINH was administered as a mixture of isomers.[42,43] Work in the related fields of both cadmium and arsenic chelation has shown that among the chelators that utilize monoanionic transport systems to gain access to these metals, there was no statistical difference in activity between isomers.[44,45] Furthermore, our successful synthesis of the D- and L-alanine derivatives enabled us to examine the relative difference in activity between *pyridinone* isomers. As with the various isomeric iron, cadmium, and arsenic chelators, no activity difference was observed; these results suggest that factors other than stereochemistry, primarily charge, govern the ability of monoanionic chelators to access intracellular deposits of toxic metals. This conclusion is strongly substantiated by the extensive work of Bergeron et al. with the negatively charged siderophore desferrithiocin.[35] These researchers showed that the inclusion of the carboxyl group was of intrinsic importance to the activity of the molecule, a finding consistent with Wolfe's suggestion that there is some type of hepatic circulation mechanism of the drug.[46] Our data and related work strongly suggest that this mechanism is monoanionic transport, and these systems appear to show no preference regarding stereochemistry.

**Synthesis.** In synthesizing the amino acid derivatives shown in Scheme 1, it was first desirable to obtain one pair of isomers that could be readily isolated in stereochemically pure form, and this was accomplished utilizing D- and L-alanine to generate isomers **3** and **4**. Several other structures were also chosen in order to observe the effect of other factors such as structure and molecular weight. Thus, the leucine derivative **5** which contains an isobutyl group and the serine (**8**) and methionine (**6**) derivatives which possess hydroxyl and thioether groups, respectively, as well as the aromatic phenylalanine derivative **7**, were synthesized. Attempts to generate stereochemically pure D- and L-isomers of the high molecular weight amino acids were less successful; circular dichroism (CD) data indicated that the D- and L-products of **5**, **7**, and **8** contained substantial concentrations of the respective isomer, though they were not stereochemically pure. Two achiral compounds containing benzene rings, **9** and **11**, were also synthesized, providing structural comparisons to **7**, with **11** enabling comparison of the effect of insertion of an aromatic ring directly attached to the nitrogen. Lastly, since no dianionic 3-hydroxypyrid-4-ones had been previously examined, the glutamic acid derivative **10** was also prepared to see if it was able to remove iron as effectively as the monoanionic derivatives.

**Scheme 1**[a]




[a] (a) Excess $CH_3NH_2$, $H_2O$, 100 °C; (b) D- or L-alanine, $NaHCO_3$, $H_2O$, $N_2$, 100 °C; (c) dilute HCl; (d) L-leucine, $NaHCO_3$, $H_2O$, $N_2$, 100 °C; (e) L-serine, $NaHCO_3$, $H_2O$, $N_2$, 100 °C; (f) L-phenylalanine, $NaHCO_3$, $H_2O$, $N_2$, 100 °C; (g) L-methionine, $NaHCO_3$, $H_2O$, $N_2$, 100 °C; (h) 4-(aminomethyl)benzoic acid, $NaHCO_3$, $H_2O$, $N_2$, 100 °C; (i) L-glutamic acid, $NaHCO_3$, $H_2O$, $N_2$, 100 °C; (j) 4-aminobenzoic acid, HCl, $H_2O$, $N_2$, 100 °C.

The general synthetic method for the preparation of the 3-hydroxypyrid-4-ones was that originally used by Kleipool and Wibaut,[47] and later by Kontoghiorghes and Sheppard,[48] as a considerably more direct route than that used previously.[49] All amino acids and the 4-(aminomethyl)benzoic acid were first neutralized with $NaHCO_3$ to provide the sodium salt, and these in turn were reacted with **1**. In the case of **11**, the preparation closely followed the method of Orvig et al.,[35] which accomplishes the N-substitution in *acidic* media. Hence, the 4-aminobenzoic acid was not neutralized. All reactions were closely monitored with $^1H$ NMR in order to stop the reaction shortly after maximum yield was obtained to avoid undesired racemization. This appeared to be the most difficult aspect of the syntheses, due to the fact that these one-step reactions are not high in yield.[48] The purification of the products following acidification was surprisingly facile, with derivatives **3** and **4** requiring little more than repeated washings with $H_2O$ and EtOH and the aromatic compounds **7**, **9**, and **11** requiring only exhaustive washings with MeOH preceded by washings with $H_2O$ to remove NaCl. The other products were

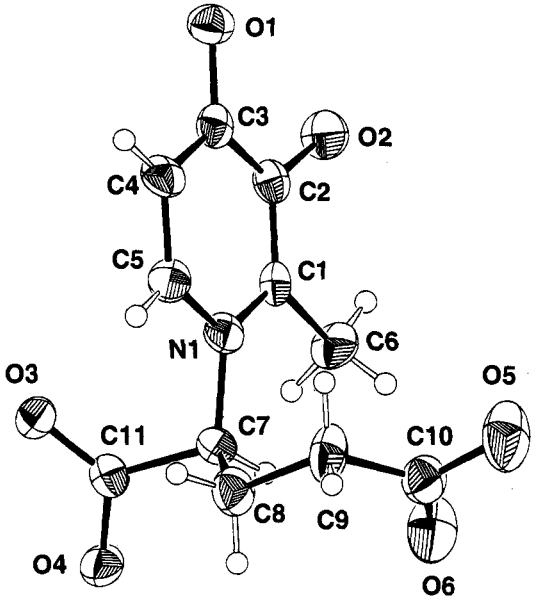



**Figure 1.** ORTEP diagram of 1-(3-hydroxy-2-methyl-4-oxopyridyl)-1,3-propanedicarboxylic acid (**10**).

readily recrystallized from either MeOH or MeOH/$H_2O$ solutions.

## Characterization

**X-ray Crystallography.** Since all reactions were performed in strictly aqueous media, previous concerns over Schiff base formation were not considered; however, characterization of the first dianionic 3-hydroxypyrid-4-one was of considerable interest to observe its chemical properties. In previous work, we and other researchers have examined several pyridinone structures based on kojic acid,[31] comenic acid,[31] and maltol.[32,50-52] The bond lengths within these various molecules imply that the position of substituents within the ring most directly affects the degree of electron delocalization, whether these substituents are electron withdrawing or releasing. Maximum aromatic character is displayed by monoanionic derivatives of kojic acid and comenic acid, where a substituent is para relative to the hydroxyl group. In maltol, with the methyl group in position 2, this phenomenon is greatly diminished, as seen by both the large differences in bond lengths within the ring and the substantially different C–O bond distances in the chelating oxygens, which all imply a more localized structure. This trend can also be observed in the chelate structures with tripositive metals. The pyridinones appear to possess the same degree of localization regardless of whether they are free or bonded to the metal, and this can be observed in complexes of the pyridinones to iron,[53,54] aluminum,[55] and gallium.[55]

Since **10**, whose ORTEP diagram is shown in Figure 1, is also a derivative of maltol, it too was expected to exist in a more localized structure, though surprisingly, the contrary was true. Within the pyridinone ring, all bond distances (Table 4) were within $2\sigma$, while the C–O bond distances of the chelating oxygens were statistically equivalent. Since these factors suggest a considerably delocalized system, this points to possible effects outside of the positioning of substituents on the ring itself. Possibly the physiochemical properties of the substituent attached to the nitrogen play a significant part as well, though this relationship is unclear. In the glycine derivative of maltol,[32] a high degree of localization was observed within the ring, despite the methylcarboxyl group attached to the nitrogen. Interestingly, even though these structural relationships are essentially the same in **10** (with the exception of the length of the alkyl chain), one observes a significantly higher degree of electron delocalization within the molecule.

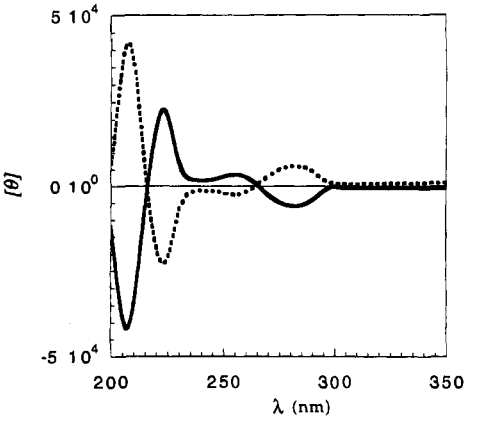



**Figure 2.** CD plots for D- and L-1-(3-hydroxy-2-methyl-4-oxopyridyl)-1-ethanecarboxylic acid (**3** and **4**, respectively). The data for the D-isomer, **3**, is presented as a solid line; that for the L-enantiomer, **4**, is presented as a dotted line.

**UV/Vis and Circular Dichroism.** While extensive work has focused on the UV/vis spectra for numerous analogs of the pyridinone class of molecules,[56,57] no work has concentrated specifically on the monoanionic derivatives of the 3-hydroxypyrid-4-ones. In order to more fully characterize the derivatives obtained in Scheme 1, the UV/vis spectra were obtained, and the $\lambda_{max}$, extinction coefficient ($\epsilon$), and respective pHs are reported in Table 5. Absorption bands centered around 220 nm were attributed to the $\pi \rightarrow \pi^*$ transition of the chromophore, and the bands centered at 280 nm were assigned to the $n \rightarrow \pi^*$ transitions. As compared to the pyridinones, the additional band centered at 318 nm for the pyran **1** arises due to a bathochromic shift of the $n \rightarrow \pi^*$ electronic transition from the nonbonding electron of the 3-hydroxy substituent.[58] In general, the values obtained were consistent with those observed for closely related, asymmetrical pyridinones.[59,60] The relatively high extinction coefficients for **11** compared to **9** may be attributed to the extended aromaticity obtained with the loss of the bridging methylene between the two chromophores. It is also interesting to note that the intensity of the absorption bands of **10** are the lowest of any of the derivatives of **1**. The significant decrease in intensity for **10**, relative to the monoanionic derivatives, may be the result of a change in the local solvent polarizability due to the presence of a dianionic species.

The circular dichroic spectra of **3** and **4** were measured under conditions similar to the UV/vis experiments. Since the concentrations were known, the CD spectra were plotted as a function of molar ellipticity, $[\theta]$, versus wavelength, $\lambda$ (Figure 2). Split Cotton curves were observed for the two enantiomers, as evidenced by the values for the D-isomer, $[\theta]_{282}$ −5980, $[\theta]_{223}$ 22 800, and $[\theta]_{207}$ −41 910, relative to those of the L-isomer, $[\theta]_{282}$ 5980, $[\theta]_{223}$ −22 800, and $[\theta]_{207}$ 41 900. These spectra lend confidence to the stereochemical purity of each isomer that was subsequently used for the animal studies. Further CD experiments revealed that attempts to generate stereochemically pure deriva-

**Table 1.** Urinary Iron Excretion Induced by the 3-Hydroxypyrid-4-ones following po Administration (μg of Fe/15 h)

| compd | N | control period | treatment period |
|---|---|---|---|
| **2** | 6 | 401 ± 110 | 17330 ± 6400[a] |
| **3** | 4 | 450 ± 72 | 4010 ± 810 |
| **4** | 4 | 297 ± 131 | 3840 ± 3065 |
| **5** | 4 | 464 ± 36 | 8550 ± 2165 |
| **6** | 4 | 318 ± 210 | 1793 ± 862 |
| **7** | 4 | 777 ± 259 | 1276 ± 146 |
| **8** | 4 | 289 ± 125 | 1738 ± 354 |
| **9** | 4 | 736 ± 204 | 1554 ± 925[b] |
| **10** | 4 | 435 ± 158 | 170 ± 95 |
| **11** | 4 | 961 ± 216 | 1113 ± 272[b] |

[a] Significantly different ($p \leq 0.05$) from all other treated groups except **5**. [b] Not significantly different from control group. Groups not so marked are significantly different ($p \leq 0.05$) from their control groups.

**Table 2.** Biliary Excretion of Iron Induced by the 3-Hydroxypyrid-4-ones following iv Injection (μg of Fe/h)

| compd | N | control period | treatment period |
|---|---|---|---|
| **2** | 5 | 407 ± 73 | 4810 ± 2130[a] |
| **3** | 3 | 439 ± 102 | 4581 ± 654[a] |
| **4** | 3 | 515 ± 145 | 6245 ± 1620[a] |
| **5** | 3 | 401 ± 86 | 11600 ± 3525[a,b] |
| **6** | 3 | 258 ± 81 | 4184 ± 1320[a] |
| **7** | 3 | 624 ± 85 | 8300 ± 251[a] |
| **8** | 4 | 387 ± 139 | 12730 ± 1860[a,b] |
| **9** | 3 | 251 ± 194 | 13680 ± 2140[a,b] |
| **10** | 4 | 561 ± 95 | 1150 ± 326[a,b] |
| **11** | 3 | 295 ± 61 | 9390 ± 2040[a] |

[a] Significantly different from control values ($p \leq 0.05$). [b] Significantly different from values for compound **2** (L1) ($p \leq 0.05$).

tives of the larger amino acid derivatives were not successful (data not shown), although the spectra did show an enrichment in the respective isomers.

## Biological Evaluation

The ability of the monoanionic pyridinones to enhance biliary and urinary iron excretion was examined using previously untreated Sprague–Dawley rats that served as their own controls. The biliary iron excretion was measured using an animal model based on those of Pippard et al.[61] and Bergeron et al.,[35] with control excretion being measured for 0.5 h and treated excretion for 2 h following a 0.2 mmol/kg iv injection. po urinary excretion was measured for 15 h following the gavage administration of either 1 mL of Millipore $H_2O$ for the control period or 1 mmol/kg chelate solution for the treated period. The results of the biliary and urinary iron excretion studies are shown in Tables **1** and 2, respectively.

**Biological Results.** As previous work has shown that monoanionic 3-hydroxypyrid-4-ones are able to mobilize iron,[31,32] it was not surprising that all of the monoanionic chelators enhanced iron excretion in either the urine, the bile, or both. Regarding urinary iron excretion (Table **1**), none of the chelators was able to equal **L1**, though the leucine derivative **5** was able to enhance iron excretion to roughly one-half that of the standard. The two isomers of D- and L-alanine showed no difference in their ability to mobilize iron into the urine, and both enhanced iron excretion to only one-quarter that of **L1**. The large standard deviations found for the D- and L-pair may be related to the stereochemistry. Derivatives **6** and **8**, which possess the methylhydroxy and thiomethyl groups, respectively, were not significantly different from the alanine derivatives. All of the aromatic derivatives showed relatively little ability to enhance iron excretion in the urine relative to the controls. Since the majority of these compounds were of substantially higher molecular weight than L1, their low activity may be explained by the fact that they preferentially target the liver due to their size. In terms of the dianionic derivative **10**, little difference was seen between the treated and control values, perhaps since this highly polar compound is filtered at the glomerulus and not reabsorbed.

The biliary excretion data (Table 2) for these compounds reveals that molecular weight and charge appear to have the most significant effect on both organ targeting and activity, for all of the monoanionic compounds at least equaled L1 in mobilizing iron into the bile. However, the dianionic **10** showed no statistical difference in activity from the controls, leading one to question whether the liver or the kidneys can efficiently transport pyridinone molecules of this ionic charge. No significant difference was observed between the D- and L-isomers of alanine. The induced biliary excretion of iron, which appears to be the major route of excretion, allows the ligands to be divided into two classes: low molecular weight ligands (**2**–**4** and **6**) with an iron excretion rate of approximately 5000 μg of Fe/h and high molecular weight ligands (**5**, **7**–**9**, and **11**) with a rate of about 11 000 μg of Fe/h.

Though all monoanionic derivatives vary regarding lipophilic character, it would appear reasonable that these variations may be of considerably less significance when compared to molecular weight and charge. In the closely related work by Dobbin et al.,[24] the partition coefficients for monoanionic derivatives were ~0.001 despite changes in the number of methylene groups. Since the distribution of negatively charged pyridinones into octanol is relatively minute, these results suggest that the ability of several of the 3-hydroxypyrid-4-ones to mobilize substantial quantities of iron into the bile may be attributed to two common factors: high molecular weight and a single negative charge. This conclusion appears particularly reasonable in light of the fact that the liver has been shown to transport high molecular weight, monoanionic molecules such as bile acids.[37,62]

## Conclusion

These studies would suggest that, in general, while amino acid derivatives of the 3-hydroxypyrid-4-ones are generally not as effective as L1 in enhancing urinary iron excretion, they have significant promise in enhancing iron excretion in the bile. Should future studies reveal that monoanionic derivatives are substantially less toxic than DFO, it would appear that the leucine, methionine, and aromatic derivatives would show the greatest promise as possible replacements, especially for parenteral administration. If these chelators show high biliary excretion when administered orally as well, they may be possible candidates for replacing the orally active **L1**. These chelators have provided further evidence that monoanionic chelators may be used to mobilize iron and that potentially active chelators may be overlooked on the basis of assumptions correlating activity with neutrality of charge. This study has also shown that, for the chiral pair selected, chirality appears to play a minor role in chelator transport. A different result may well be found with an analogous chiral pair with a larger side chain than is present in alanine.

High molecular weight and a single negative charge appear to maximize iron mobilization from the liver. Additionally, the low iron-mobilizing activity of the dianionic glutamate derivative suggests that pyridinones with more than one negative charge are likely to display low activity.

## Experimental Section

Maltol was purchased from Pfizer Inc., Doraville, GA, and the amino acids were obtained from Sigma Chem. Co., St. Louis, MO. All reagents were used without further purification. Female Sprague–Dawley rats were obtained from Sasco, Omaha, NB.

The extremely hygroscopic nature of the monoanionic pyridinones required that the target molecules be dried extensively to obtain acceptable combustion analyses. In cases where further characterization was desired, especially when fractional water was exceedingly difficult to remove, mass spectral data was also obtained for additional structural verification. For the animal studies, the molecular weights of the hydrated species were utilized in order to calculate accurate dosages for administration.

**1,2-Dimethyl-3-hydroxypyrid-4-one (L1, 2)** was prepared by a previously published procedure.[48]

**D- or L-1-(3-Hydroxy-2-methyl-4-oxopyridyl)-1-ethanecarboxylic Acid (3 or 4, respectively).** D- or L-Alanine (35.6 g, 0.40 mol) was reacted with $NaHCO_3$ (40.3 g, 0.48 mol) in $H_2O$ (350 mL). Maltol (50 g, 0.40 mol) was added to the solution, and the reaction was refluxed under $N_2$ for 25 h. The mixture was allowed to cool and then acidified with HCl (3:1 $H_2O$/HCl) to a pH of ~3. The solid which formed was subsequently filtered and washed first with $H_2O$ and then repeatedly with EtOH to yield 11.1 g (13.5%) of the L-isomer and 7.41 g (8.96%) of the D-isomer: mp (D and L) 261–262 °C; $^1$H NMR (L) ($D_2O$) $\delta$ 7.97 (d, 1H), 6.98 (d, 1H), 5.17 (q, 1H), 2.47 (s, 3H), 1.76 (d, 3H); $[\alpha]^{25}_D$ +19° (*c* 1.00, DMSO) for D, $[\alpha]^{25}_D$ −14° (*c* 1.00, DMSO) for L; MS (+LSI, *m/z*) (D, L) 198.1 (100, 100), 182.1 (5.4, 5.6), 152.1 (7.5, 7.1), 126.1 (13, 12). Anal. Calcd for $C_9H_{11}NO_4$ (D, L): C, 54.82; H, 5.62; N, 7.10. Found (D): C, 54.55; H, 5.72; N, 7.05. Found (L): C, 54.54; H, 5.56; N, 7.00.

**1-(3-Hydroxy-2-methyl-4-oxopyridyl)-3-methyl-1-butanecarboxylic Acid (5).** L-Leucine (52.48 g, 0.40 mol) was combined with $NaHCO_3$ (40.3 g, 0.48 mol) in $H_2O$ (350 mL). To this was added maltol (50 g, 0.40 mol), and the reaction was refluxed for 26 h under $N_2$. The reaction mixture was allowed to cool and then acidified to a pH of 4 with HCl (3:1 $H_2O$/HCl) in an ice bath. The resulting brown mass was then boiled in a large volume of MeOH and charcoal filtered. The liquid was decanted, and the crystals were filtered and washed with MeOH (4×). The combined volume of the decantate and the MeOH washings was reduced by rotary evaporation to yield more crystals, which were also washed with MeOH as before. The crystals were dried *in vacuo* to yield 4.05 g (5.17%) of a yellow product: mp 232–234 °C; $^1$H NMR ($D_2O$) $\delta$ 7.73 (d, 1H), 6.58 (d, 1H), 4.84 (q, 1H), 2.39 (s, 3H), 2.02 (m, 2H), 1.42 (septet, 1H), 0.88 (d, 6H); $[\alpha]^{25}_D$ −11° (*c* 1.00, DMSO). Anal. Calcd for $C_{12}H_{17}NO_4$: C, 60.22; H, 7.17; N, 5.86. Found: C, 60.30; H, 7.27; N, 5.62.

**1-(3-Hydroxy-2-methyl-4-oxopyridyl)-2-hydroxy-1-ethanecarboxylic Acid (6).** L-Serine (42.0 g, 0.40 mol) was reacted with $NaHCO_3$ (40.32 g, 0.48 mol) in $H_2O$ (350 mL). Maltol was added (50 g, 0.40 mol), and the reaction was refluxed under $N_2$ for 32.5 h. The pH was then reduced to 3 with HCl (3:1 $H_2O$/HCl) in an ice bath. The volume was reduced by rotary evaporation, and the product precipitated out of solution. Following filtration, this was washed several times with $H_2O$, EtOH, and finally a small volume of MeOH. Crystals could be obtained by recrystallization from MeOH, though they decomposed quickly due to loss of solvent. The final white product weighed 6.50 g (7.03%): mp 252–253 °C; $^1$H NMR ($D_2O$) $\delta$ 7.79 (d, 1H), 6.61 (d, 1H), 5.09 (m, 1H), 4.22 (m, 2H), 2.40 (s, 3H); $[\alpha]^{25}_D$ −1.0° (*c* 1.00, DMSO). Anal. Calcd for $C_9H_{11}O_5N\cdot H_2O$: C, 46.75; H, 5.62; N, 6.06. Found: C, 46.62; H, 5.94; N, 5.92.

**1-(3-Hydroxy-2-methyl-4-oxopyridyl)-2-phenyl-1-ethanecarboxylic acid (7).** L-Phenylalanine (66.10 g, 0.40 mol) was combined with $NaHCO_3$ (40.42 g, 0.48 mol) in $H_2O$ (350 mL). Maltol was added to the mixture (50 g, 0.40 mol), and the solution was refluxed under $N_2$ for 26 h. The pH was reduced to ~4.5 with HCl (3:1 $H_2O$/HCl), and the brown product was filtered. The impure crystals were washed with $H_2O$ (2×) followed by boiling in MeOH. Further MeOH washings were performed until no more color was removed, and the pure, light brown solid was washed with ether to dry, yielding 5.82 g (5.33%): mp 257–258 °C; $^1$H NMR ($D_2O$) $\delta$ 7.75 (d, 1H), 7.23 (m, 5H), 6.55 (d, 1H), 5.15 (m, 1H), 3.53 (m, 2H), 2.10 (s, 3H); $[\alpha]^{25}_D$ −25° (*c* 1.00, DMSO). Anal. Calcd for $C_{15}H_{15}NO_4$: C, 65.92; H, 5.53; N, 5.13. Found: C, 65.69; H, 5.60; N, 4.86.

**2-(3-Hydroxy-2-methyl-4-oxopyridyl)-4-(methythio)-1-butanoic Acid (8).** L-Methionine (10 g, 67 mmol) was reacted with $NaHCO_3$ (6.78 g, 80.4 mmol) in $H_2O$ (75 mL). Maltol (8.46 g, 67 mmol) was then added, and the reaction was refluxed under $N_2$ for 22 h. Despite cold acidification with HCl (3:1 $H_2O$/HCl), the product precipitated out of solution as a thick mass. The water was decanted, and the product was washed with $H_2O$ and then repeatedly with MeOH. Final washings with $H_2O$ and acetone were performed to yield 0.80 g (4.6%) of a pure yellow solid following drying *in vacuo*: mp 219–220 °C; $^1$H NMR ($D_2O$) $\delta$ 7.72 (d, 1H), 6.60 (d, 1H), 5.05 (m, 1H), 2.46 (m, 7H), 2.08 (s, 3H); $[\alpha]^{25}_D$ −7.0° (*c* 1.00, DMSO). Anal. Calcd for $C_{11}H_{15}NO_4S$: C, 51.35; H, 5.88; N, 5.44. Found: C, 50.94; H, 5.89; N, 5.19.

**1-(4-Carboxybenzyl)-3-hydroxy-2-methyl-4-oxopyridine (9)** was prepared according to a method published previously.[32]

**1-(3-Hydroxy-2-methyl-4-oxopyridyl)-1,3-propanedicarboxylic Acid (10).** L-Glutamic acid (58.84 g, 0.40 mol) was reacted with $NaHCO_3$ (73.92, 88 mmol) in $H_2O$ (350 mL). Maltol (50 g, 0.40 mol) was added, and the reaction was refluxed under $N_2$ for 31 h. The solution was allowed to cool and then acidified with HCl (3:1 $H_2O$/HCl) in an ice bath. The volume was reduced by rotary evaporation, and the subsequent product was filtered and washed with water, yielding a white compound. The volume of the mother liquor was reduced, and the resulting crystals were collected. These were combined with the first batch and then transferred to a beaker for repeated MeOH washings. The resulting product weighed 4.62 g (4.45%). Colorless crystals were obtained by recrystallization from a 1:2 $H_2O$/MeOH solution, though the initial solid was analytically pure: mp 245–247 °C; $^1$H NMR ($D_2O$) $\delta$ 7.70 (d, 1H), 6.62 (d, 1H), 4.89 (m, 1H), 2.31 (m, 7H); $[\alpha]^{25}_D$ −7.0° (*c* 1.00, DMSO). Anal. Calcd for $C_{11}H_{13}NO_6$: C, 51.77; H, 5.13; N, 5.49. Found: C, 51.92; H, 5.08; N, 5.50.

**1-(4-Carboxyphenyl)-3-hydroxy-2-methyl-4-oxopyridine (11).** The preparation of 11 was with minor modification of a method published previously.[63] Maltol (20 g, 0.16 mol) was combined with 4-aminobenzoic acid (21.94 g, 0.16 mol) in an acidic $H_2O$ solution (7 mL of concentrated HCl, 250 mL of $H_2O$). The reaction was refluxed under $N_2$ for 24 h, after which the product that had formed was filtered. The collected product was readily purified by washing with $H_2O$ and then with MeOH. Refluxing was stopped after 74 h, and purification was attempted unsuccessfully on the second batch of product which contained black particles, possibly due to decomposition. The pure solid weighed 3.08 g (7.31%) and was of a beige coloration: mp >300 °C (lit.[63] mp 222 °C); $^1$H NMR ($D_2O$) $\delta$ 8.02 (d, 2H), 7.70 (d, 1H), 7.44 (d, 2H), 6.62 (d, 1H), 2.11 (s, 3H). Anal. Calcd for $C_{13}H_{11}NO_4\cdot H_2O$: C, 59.34; H, 4.94; N, 5.32. Found: C, 59.58; H, 4.94; N, 5.38.

**Animal Studies.** All animal studies were performed on female Sprague–Dawley Rats (200–240 g) obtained from Sasco, Omaha, NB. The animals were provided food and water *ad libitum* in between experimental periods and housed in an AAALAC approved facility. Chelate solutions of the monoanionic derivatives were first neutralized with $NaHCO_3$ which facilitated their dissolution into Millipore water. iv solutions were prepared to provide a dosage of 0.2 mmol/kg, such that the injection for a 200 g rat was 1 mL. po solutions were prepared on a 1 mmol/kg basis, with each dose again equaling 1 mL for the 200 g rat. iv administration of the chelating

**Table 3.** Summary of Crystal Data and Intensity Collection for 1-(3-Hydroxy-2-methyl-4-oxopyridyl)-1,3-propanedicarboxylic Acid (**10**)

| | |
|---|---|
| formula | $C_{11}H_{13}NO_6$ |
| formula weight | 223.1 |
| color of crystal | golden |
| crystal system | monoclinic |
| $a$ (Å) | 8.884 (5) |
| $b$ (Å) | 12.937 (3) |
| $c$ (Å) | 9.638 (2) |
| $\alpha$ (deg) | 90 |
| $\beta$ (deg) | 93.75 |
| $\gamma$ (deg) | 90 |
| volume (Å) | 1105.4 (7) |
| $D$ (g/cm$^3$) | 1.533 |
| $F$(000) | 536 |
| $\mu$ (cm$^{-1}$) | 1.150 |
| absorption correction | none |
| space group | $P2_1/a$ |
| $Z$ | 4 |
| crystal dimensions (mm) | 0.40 × 0.075 × 0.25 |
| temperature (°C) | 20 ± 1 |
| radiation | Mo Kα |
| data collection mode | $\omega$-scan |
| scan width | 1.37 + 0.30 tan $\theta$ |
| scan speed (deg/min) | 8.0 |
| background counts | stationary counts; peak/bkgd counting time = 2:1 |
| $2\theta$ limits (deg) | $6 \leq 2\theta \leq 50.1$ |
| total reflections collected | 2199 |
| no. of unique intensities | 2063 |
| no. of intensities with $F > 3.00(F)$ | 997 |
| $R, R_w$ | 0.062, 0.099 |

agents was performed by tail vein injection; passage of the tail under warm water before the injection facilitated observation of the vein and hence the injection process.

**Biliary Cannulation.** All biliary cannulations were performed while the rats were under ether anesthesia. Following anesthesia, two insertions were made under the xiphoid process along the *linea alba* to minimize bleeding. The bile duct was isolated from the pancreas using cotton applicators, and a small incision was made to allow insertion of the PE-10 tubing. The tubing was inserted and then tied firmly to the bile duct with suture. The first incision was sewn closed, and a needle was passed through the back, just behind the neck to allow for passage of the tubing. The second incision was then sutured closed, and a Velcro jacket was placed on the animal to hold the 10 × 75 poly(styrene) collecting tube. The animals were allowed to recover from anesthesia and, after the control period, reanesthetized for injection. During the collection periods, the animals were kept in metabolic cages and given free access to tap water. The rats were sacrificed shortly after the final collection.

**Urinary Iron Excretion.** Following ether anesthesia, the rats were administered either 1 mL of Millipore $H_2O$ for the control period or the appropriate amount of chelator solution for the treated period at roughly 6:00 p.m. The animals were allowed to recover and placed in specially designed metabolic cages for urine collection which allowed for the separation of the feces from the urine. Urine was collected for 15 h until the following morning due to the nocturnal nature of the animals. The animals were allowed access to tap water *ad libitum* and returned to their food after collection. The rats were sacrificed following the treatment period.

**Iron Analysis.** All iron analyses were performed on Perkin-Elmer atomic absorption spectrometers, models 4000 or 5100ZL, using a wavelength of 248.3 nm. The analyzers were run using standard conditions.

**X-Ray Crystallography.** All measurements were performed on a Rigaku AFC6S diffractometer at Vanderbilt University with graphite monochromated Mo Kα radiation. The structure of **10** was solved by direct methods using SHELXS-86. All non-hydrogen atoms were refined anisotropically. The ORTEP diagram for **10** is shown in Figure 1, a summary of crystal data and intensity collection is shown in Table 3, and bond lengths and angles are shown in Table 4.

**Table 4.** Intramolecular Distances and Bond Angles Involving the Non-hydrogen Atoms for 1-(3-Hydroxy-2-methyl-4-oxopyridyl)-1,3-propanedicarboxylic Acid (**10**)

| atom | atom | distance | atom | atom | distance |
|---|---|---|---|---|---|
| O(1) | C(3) | 1.343(9) | C(1) | C(2) | 1.36(1) |
| O(2) | C(2) | 1.355(9) | C(1) | C(6) | 1.50(1) |
| O(3) | C(11) | 1.233(9) | C(2) | C(3) | 1.40(1) |
| O(4) | C(11) | 1.268(9) | C(3) | C(4) | 1.39(1) |
| O(5) | C(10) | 1.35(1) | C(4) | C(5) | 1.39(1) |
| O(6) | C(10) | 1.20(1) | C(7) | C(8) | 1.56(1) |
| N(1) | C(1) | 1.38(1) | C(7) | C(11) | 1.55(1) |
| N(1) | C(5) | 1.369(9) | C(8) | C(9) | 1.53(1) |
| N(1) | C(7) | 1.492(9) | C(9) | C(10) | 1.50(1) |

| atom | atom | atom | angle | atom | atom | atom | angle |
|---|---|---|---|---|---|---|---|
| C(1) | N(1) | C(5) | 122.0(7) | N(1) | C(5) | C(4) | 119.7(7) |
| C(1) | N(1) | C(7) | 120.8(6) | N(1) | C(7) | C(8) | 113.1(6) |
| C(5) | N(1) | C(7) | 116.9(6) | N(1) | C(7) | C(11) | 108.4(6) |
| N(1) | C(1) | C(2) | 118.5(7) | C(11) | C(7) | C(8) | 108.8(6) |
| N(1) | C(1) | C(6) | 119.4(7) | C(7) | C(8) | C(9) | 113.9(7) |
| C(2) | C(1) | C(6) | 122.1(7) | C(10) | C(9) | C(8) | 113.6(7) |
| O(2) | C(2) | C(1) | 118.0(7) | O(5) | C(10) | O(6) | 121.1(8) |
| O(2) | C(2) | C(3) | 120.3(7) | O(5) | C(10) | C(9) | 111.2(8) |
| C(3) | C(2) | C(1) | 121.6(8) | O(6) | C(10) | C(9) | 127.7(8) |
| O(1) | C(3) | C(2) | 118.6(7) | O(3) | C(11) | C(7) | 120.1(7) |
| O(1) | C(3) | C(4) | 122.6(7) | O(4) | C(11) | O(3) | 124.5(7) |
| C(2) | C(3) | C(4) | 118.8(8) | O(4) | C(11) | C(7) | 115.1(7) |
| C(3) | C(4) | C(5) | 119.4(8) | | | | |

**Table 5.** Ultraviolet $\lambda_{max}$ and Respective Extinction Coefficients

| compd | $\lambda_{max}$ (nm) | $\epsilon$($\times 10^4$ M$^{-1}$ cm$^{-1}$) |
|---|---|---|
| **1**[a] | 215, 274, 318 | 2.50, 1.40, 0.56 |
| **2**[b] | 216, 278 | 3.13, 2.26 |
| **3**[c] | 219, 281 | 1.85, 1.44 |
| **4**[c] | 219, 281 | 1.85, 1.44 |
| **5**[a] | 223, 282 | 1.77, 1.32 |
| **6**[c] | 219, 282 | 1.28, 1.17 |
| **7**[d] | 216 (s), 284 | 1.78, 1.30 |
| **8**[b] | 219, 283 | 1.66, 1.38 |
| **9**[b] | 220, 280 | 1.30, 0.87 |
| **10**[a] | 218, 282 | 0.55, 0.45 |
| **11**[e] | 223 (s), 287 | 3.68, 3.25 |

[a] pH = 8.3. [b] pH = 7.8. [c] pH = 8.0. [d] pH = 8.2. [e] pH = 8.6. (s) = shoulder.

**UV Analysis.** UV/vis spectra for each compound was performed on a Shimadzu UV spectrophotometer, model UV-2101PC. Concentrations of samples were carried out by carefully measuring milligram quantities of material and then converting to moles using the formula weights determined from elemental analyses. A 50 $\mu$M solution of each compound was placed in a solution with an equimolar concentration of $NaHCO_3$ to facilitate dissolution, except for compounds **6**, **9**, and 11, in which a 3:1 excess of $NaHCO_3$ was required. The pH of all samples was measured on a Fisher pH meter, model 915, fitted with an Ingold model 6030 electrode. Samples were then placed in a 1 cm quartz cell for UV/vis measurements at ambient temperature. Spectra were obtained over the wavelengths from 200 to 500 nm. The pH, $\lambda_{max}$, and extinction coefficient ($\epsilon$) for all compounds are shown in Table 5.

**CD Analysis.** The circular dichroic spectra of **3** and **4** were measured on a JASCO model J-720 instrument. The solutions and concentrations were identical to those used for the UV/vis spectroscopy. Measurements were made in a 1 cm path length, circular quartz cell at ambient temperature. Since the concentration was known, the CD spectra are shown in Figure 2 with molar ellipticity, [$\theta$], versus wavelength, $\lambda$.

**Acknowledgment.** We wish to acknowledge the support received for this study from the Center in Molecular Toxicology at Vanderbilt University through Grant NIEHS 0268 and the National Institute of Environmental Health Sciences via Grant 02638. The CD instrument was provided via NIH Grant RR06381.

We thank Dr. Kim Cecil for her help in the elemental analyses, as well as Christina M. Sheidler and Deborah L. Kitchen for their help with the iron analyses. We also thank Mrs. Jacqueline L. Johnston for her assistance with the manuscript.

**Supplementary Material Available:** Tables 6 and 7 for the positional and $U_{ij}$ parameters, respectively, for compound **10** (2 pages). Ordering information is given on any current masthead page.

## References


(1) Youdim, M. B. H.; Ben-Shachar, D.; Yehuda, S.; Riederer, P. The role of iron in the basal ganglion. *Adv. Neurol.* **1990**, *53*, 155–162.

(2) Youdim, M. B. H.; Ben-Shachar, D.; Riederer, P. Iron in brain function and dysfunction with emphasis on Parkinson's disease. *Eur. Neurol.* **1991**, *31* (Suppl. 1), 34–40.

(3) Ben-Shachar, D.; Eshel, G.; Riederer, P.; Youdim, M. B. H. Role of iron and iron chelation in dopaminergic-induced neurodegeneration: Implication for Parkinson's disease. *Ann. Neurol.* **1992**, *32*, S105–S110.

(4) DeBoer, D. A.; Clark, R. E. Iron chelation in myocardial preservation after ischemia-reperfusion injury: the importance of pretreatment and toxicity. *Ann. Thorac. Surg.* **1992**, *53*, 412–418.

(5) Katoh, S.; Toyama, J.; Kodama, I.; Akita, T.; Abe, T. Deferoxamine, an iron chelator, reduces myocardial injury and free radical generation in isolated neonatal rabbit hearts subjected to global ischaemia-reperfusion. *J. Mol. Cell. Cardiol.* **1992**, *24*, 1267–1275.

(6) Salonen, J. T.; Nyssönen, K.; Korpela, H.; Tuomilehto, J.; Seppänen, R.; Salonen, R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation* **1992**, *86*, 803–811.

(7) Shanzer, A.; Libman, J.; Lytton, S. D.; Glickstein, H.; Cabantchik, Z. I. Reversed siderophores act as antimalarial agents. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6585–6589.

(8) VanZyl, R. L.; Havlik, I.; Monteagudo, F. S. E. The combined effect of iron chelators and classical antimalarials on the in-vitro growth of *Plasmodium falciparum*. *J. Antimicrob. Chemother.* **1992**, *30*, 273–278.

(9) Cohen, A. Current status of iron chelation therapy with deferoxamine. *Semin. Hematol.* **1990**, *27*, 86–90.

(10) Olivieri, N. F.; Berriman, A. M.; Tyler, B. J.; Davis, S. A.; Francombe, W. H.; Liu, P. P. Reduction in tissue stores with a new regimen of continuous ambulatory intravenous deferoxamine. *Am. J. Hematol.* **1992**, *41*, 61–63.

(11) Olivieri, N. F.; Buncie, J. R.; Chew, E.; Gallant, T.; Harrison, R. V.; Keenan, N.; Logan, W.; Mitchell, D.; Ricci, G.; Skarf, R.; Taylor, M.; Freedman, M. H. Visual and auditory neurotoxicity in patients receiving subcutaneous deferoxamine infusions. *N. Engl. J. Med.* **1986**, *314*, 869–873.

(12) Davies, S. C.; Hungerford, J. L.; Arden, G. B.; Marcus, R. E.; Miller, M. H.; Huehns, E. R. Ocular toxicity of high-dose intravenous desferrioxamine. *Lancet* **1983**, *2*, 181–184.

(13) Borgna-Pignatti, C.; Stefano, P. D.; Broglia, A. M. Visual loss in patient on high-dose subcutaneous desferrioxamine. *Lancet* **1984**, *1*, 681.

(14) Lakhanpal, V.; Schocket, S. S.; Jiji, R. Deferoxamine (Desferal®)-induced toxic retinal pigmentary degeneration and presumed optic neuropathy. *Ophthalmology* **1984**, *91*, 443–451.

(15) Albera, R.; Pia, F.; Morra, B.; Lacilla, M.; Bianco, L.; Gabutti, V.; Piga, A. Hearing loss and desferrioxamine in homozygous beta-thalassemia. *Audiology* **1988**, *27*, 207–214.

(16) Adamson, I. Y. R.; Sienko, A.; Tenenbein, M. Pulmonary toxicity of deferoxamine in iron-poisoned mice. *Toxicol. Appl. Pharmacol.* **1993**, *120*, 13–19.

(17) Koren, G.; Bentur, Y.; Strong, D.; Harvey, E.; Klein, J.; Baumal, R.; Speilberg, S. P.; Freedman, M. H. Acute changes in renal function associated with deferoxamine therapy. *Am. J. Dis. Child.* **1989**, *143*, 1077–1080.

(18) Porter, J. B.; Hider, R. C.; Huehns, E. R. Update on the hydroxypyridinone oral iron-chelating agents. *Semin. Hematol.* **1990**, *27*, 95–100.

(19) Al-Refaie, F. N.; Wonke, B.; Hoffbrand, A. V.; Wickens, D. G.; Nortey, P.; Kontoghiorghes, G. J. Efficacy and possible adverse effects of the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L1) in thalassemia major. *Blood* **1992**, *80*, 593–599.

(20) Kontoghiorghes, G. J. Orally active α-ketohydroxypyridine iron chelators: studies in mice. *Mol. Pharmacol.* **1986**, *30*, 670–673.

(21) Kontoghiorghes, G. J.; Barr, J.; Nortey, P.; Sheppard, L. Selection of a new generation of orally active α-ketohydroxypyridine iron chelators intended for use in the treatment of iron overload. *Am. J. Hematol.* **1993**, *42*, 340–349.

(22) Kontoghiorghes, G. J.; Bartlett, A. N.; Hoffbrand, A. V.; Goddard, J. G.; Sheppard, L.; Barr, J.; Nortey, P. Long-term trial with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L1). *Br. J. Haematol.* **1990**, *76*, 295–300.

(23) Hider, R. C.; Hall, A. D. Clinically useful chelators of tripositive elements. *Prog. Med. Chem.* **1991**, *28*, 42–173.

(24) Dobbin, P. S.; Hider, R. C.; Hall, A. D.; Taylor, P. D.; Sarpong, P.; Porter, J. B.; Xiao, G.; Helm, D. v. d. Synthesis, physiochemical properties, and biological evaluation of N-substituted 2-alkyl-3-hydroxy-4(1H)-pyridinones: orally active iron chelators with clinical potential. *J. Med. Chem.* **1993**, *36*, 2448–2458.

(25) Gyparaki, M.; Porter, J. B.; Hirani, S.; Streater, M.; Hider, R. C.; Huehns, E. R. In vivo evaluation of hydroxypyridinone iron chelators in a mouse model. *Acta Haematol.* **1987**, *78*, 217–221.

(26) Mehta, J.; Singhal, S.; Mehta, B. C. Oral iron chelator L1 and autoimmunity. *Blood* **1993**, *81*, 1970–1971.

(27) Berdoukas, V. Antinuclear antibodies in patients taking L1. *Lancet* **1991**, *337*, 672.

(28) Alter, B. P. Agranulocytosis and thromobocytopenia, Blackfan-Diamond anaemia, and oral chelation. *Lancet* **1990**, *335*, 970.

(29) Berdoukas, V.; Bentley, P.; Frost, H.; Schnebli, H. P. Toxicity of oral chelator L1. *Lancet* **1993**, *341*, 1088.

(30) Hider, R. C.; Lerch, K. The inhibition of tyrosinase by pyridinones. *Biochem. J.* **1989**, *257*, 289–290.

(31) Molenda, J. J.; Jones, M. M.; Basinger, M. A. Enhancement of iron excretion via monoanionic 3-hydroxypyrid-4-ones. *J. Med. Chem.* **1994**, *37*, 93–98.

(32) Molenda, J. J.; Jones, M. M.; Cecil, K. M.; Basinger, M. A. *Chem. Res. Toxicol.*, in press.

(33) Tasende, M. S. G.; Gale, G. R.; Smith, A. B.; Jones, M. M.; Singh, P. K. Monoisoamyl *meso* 2,3-dimercaptosuccinate: Interaction with metallothionein-bound cadmium in vitro and evidence of active transport into renal and hepatic cells in vivo. *Res. Commun. Chem. Pathol. Pharmacol.* **1992**, *76*, 323–339.

(34) Gale, G. R.; Smith, A. B.; Jones, M. M.; Singh, P. K. Evidence of active transport of cadmium complexing dithiocarbamates into renal and hepatic cells in vivo. *Pharmacol. Toxicol.* **1992**, *71*, 452–456.

(35) Bergeron, R. J.; Wiegand, J.; Dionis, J. B.; Egli-Karmakka, M.; Frei, J.; Huxley-Tencer, A.; Peter, H. H. Evaluation of desferrithiocin and its synthetic analogues as orally effective iron chelators. *J. Med. Chem.* **1991**, *34*, 2072–2078.

(36) Fritzsch, G.; Rumrich, G.; Ullrich, K. J. Anion transport through the contraluminal cell membrane of renal proximal tubule. The influence of hydrophobicity and molecular charge distribution on the inhibitory activity of organic anions. *Biochim. Biophys. Acta* **1989**, *978*, 249–256.

(37) Boyer, J. L.; Graf, J.; Meier, P. J. Hepatic transport systems regulating pH, cell volume and bile secretion. *Annu. Rev. Physiol.* **1992**, *54*, 415–438.

(38) Pritchard, J. B. Luminal and peritubular steps in renal transport of *p*-aminohippurate. *Biochim. Biophys. Acta* **1987**, *906*, 295–308.

(39) Pritchard, J. B.; Miller, D. S. Proximal tubular transport of organic anions and cations. In *The Kidney: Physiology and Pathophysiology*; Seldin, D. W., Giebisch, G., Eds.; Raven Press, Ltd.: New York, 1992; Vol. 2, pp 2921–2945.

(40) Jakubke, H. K.; Jeschkeit, H. *Animo Acids, Peptides and Proteins: An Introduction*; John Wiley & Sons: New York, 1977; pp 163–175.

(41) Avramovici-Grissaru, S.; Sarel, S.; Link, G.; Hershko, C. Synthesis of iron bis(pyridoxal isonicotinoylhydrazone)s and the in vivo iron-removal properties of some pyridoxal derivatives. *J. Med. Chem.* **1983**, *26*, 298–302.

(42) Hershko, C.; Avramovici-Grisaru, S.; Link, G.; Gelfand, L.; Sarel, S. Mechanism of in vivo iron chelation by pyridoxal isonicotinoyl hydrazone and other imino derivatives of pyridoxal. *J. Lab. Clin. Med.* **1981**, *98*, 99–108.

(43) Martell, A. E. The Design and Synthesis of Chelating Agents. In *Development of Iron Chelators for Clinical Use*; Martell, A. E., Anderson, W. F., Badman, D. G., Eds.; Elsevier/North Holland: New York, 1981; pp 67–104.

(44) Jones, M. M.; Singh, P. K.; Jones, S. G.; Mukundan, C. R.; Banton, J. A.; Gale, G. G.; Atkins, L. M.; Smith, A. B. Chirality, charge, and chain branching effects on dithiocarbamate-induced mobilization of cadmium from intracellular deposits in mice. *Chem. Res. Toxicol.* **1991**, *4*, 27–34.

(45) Burkitt, M. J.; Kadiiska, M. B.; Hanna, P. M.; Jordan, S. J.; Mason, R. P. Electron spin resonance spin-trapping investigation into the effects of paraquat and desferrioxamine on hydroxyl radical generation during acute iron poisoning. *Mol. Pharmacol.* **1993**, *43*, 257–263.

(46) Wolfe, L. C. Desferrithiocin. *Semin. Hematol.* **1990**, *27*, 117–120.

(47) Kleipool, R. J. C.; Wibaut, J. P. The Preparation of some 3-hydroxy-4-pyridones substituted on the nitrogen atom. *Recl. Trav. Chim. Pays-Bas* **1950**, *69*, 1041–1047.

(48) Kontoghiorghes, G. J.; Sheppard, L. Simple synthesis of the potent iron chelators 1-alkyl-3-hydroxy-2-methylpyrid-4-ones. *Inorg. Chim. Acta* **1987**, *136*, L11–L12.

(49) Hider, R. C.; Kontoghiorghes, G.; Silver, J. Pharmaceutical compositions. U.K. Patent Application GB 2 136 807A, September 26, 1984.

(50) Ghosh, S.; Adsmond, D. A.; Huotari, J.; Grant, D. J. W. Hydrogen-bond patterns of dialkylpyridone iron chelators and their 1:1 formic acid solvates: description, prediction, and role in crystal packing. *J. Pharm. Sci.* **1993**, *82*, 901–911.
(51) Chan, H.; Ghosh, S.; Venkataram, S.; Rahman, Y.; Grant, D. J. W. Crystal structures of a new oral iron chelator, 1,2-dimethyl-3-hydroxy-4-pyridone, and its solvates with acetic acid and formic acid. *J. Pharm. Sci.* **1992**, *81*, 353–358.
(52) Nelson, W. O.; Karpishin, T. B.; Rettig, S. J.; Orvig, C. Physical and structural studies of N-substituted-3-hydroxy-2-methyl-4(1H)-pyridinones. *Can. J. Chem.* **1988**, *66*, 123–131.
(53) Molenda, J. J.; Basinger, M. A.; Hanusa, T. P.; Jones, M. M. Synthesis and iron (III) binding properties of 3-hydroxypyrid-4-ones derived from kojic acid. *J. Inorg. Biochem.* **1994**, *55*, 131–146.
(54) Charalambous, J.; Dodd, A.; McPartlin, M.; Matondo, S. O. C.; Pathirana, N. D.; Powell, H. R. Synthesis and X-ray crystal structure of tris(1,2-dimethyl-3-hydroxypyrid-4-onato)iron(III). *Polyhedron* **1988**, *7*, 2235–2237.
(55) Nelson, W. O.; Karpishin, T. B.; Rettig, S. J.; Orvig, C. Aluminum and gallium compounds of 3-hydroxy-4-pyridinones: Synthesis, characterization, and crystallography of biologically active complexes with unusual hydrogen bonding. *Inorg. Chem.* **1988**, *27*, 1045–1051.
(56) Scott, A. I. *Interpretation of the ultraviolet spectra of natural products*; Pergamon Press: Oxford, 1964; Vol. 7, p 443.
(57) Beak, P. F. S.; Fry, J.; Lee, J.; Steele, F. Equilibration studies. Protomeric equilibria of 2- and 4-hydroxypyridines, 2- and 4-hydroxypyrimidines, 2- and 4-mercaptopyridines, and structurally related compounds in the gas phase. *J. Am. Chem. Soc.* **1976**, *98*, 171–179.
(58) Choux, G.; Benoit, R. L. Substituent effects on 6-substituted 3-hydroxy-4-pyrones. *J. Org. Chem.* **1967**, *32*, 3974–3977.
(59) Batts, B. D.; Madeley, A. J. Dipole moment studies of some derivatives of 4-pyridone. *Aust. J. Chem.* **1972**, *25*, 2605–2613.
(60) Cignitti, M.; Paoloni, L. The electronic structure of groups of isomeric heteroaromatic systems. *Theoret. Chim. Acta* **1972**, *25*, 277–288.
(61) Pippard, M. J.; Johnson, D. K.; Finch, C. A. A rapid assay for evaluation of iron-chelating agents in rats. *Blood* **1981**, *58*, 685–692.
(62) Klassen, C. D.; Watkins, J. B., III. Mechanisms of bile formation, hepatic uptake and biliary excretion. *Pharmacol. Rev.* **1984**, *36*, 1–67.
(63) Zhang, Z.; Rettig, S. J.; Orvig, C. Lipophilic coordination compounds: aluminum, gallium, and indium complexes of 1-aryl-3-hydroxy-2-methyl-4-pyridinones. *Inorg. Chem.* **1991**, *30*, 509–515.