Novel 3-Hydroxy-2(1*H*)-pyridinones. Synthesis, Iron(III)-Chelating Properties, and Biological Activity

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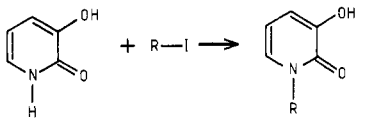
The synthesis of a range of novel bidentate and hexadentate ligands containing the chelating moiety 3-hydroxy-2(1*H*)-pyridinone is described. The pK_a values of the ligands and the stability constants of their iron(III) complexes have been determined. The stability constant of the iron(III) complex of one of the hexadentate ligands is comparable to that of desferrioxamine B. The distribution coefficients of the ligands and their iron(III) complexes were also determined. One of the novel hexadentate compounds has been shown to markedly enhance iron(III) excretion from both hepatocytes and iron-overloaded mice.

Many bacteria and fungi secrete low molecular weight iron-chelating compounds termed siderophores[1] which solubilize and transport iron to these organisms. The development of synthetic siderophore analogues with pharmaceutical application has been an area of widespread research since it was demonstrated that the naturally occurring siderophore desferrioxamine B increases the excretion of iron from iron-overloaded patients.[2] Desferrioxamine B is now in widespread clinical use for the treatment of patients with β-thalessemia. However, desferrioxamine B is orally inactive and possesses a number of side effects.[3-5] Consequently there is interest in the design of synthetic substitutes.[6,7] In principle properties such as affinity and specificity for iron, water solubility, membrane permeability, oral activity, and toxicity can be tailored to give an improved performance in chelation therapy. In this paper we report the development of some 3-hydroxy-2-pyridinone derivatives with properties suited for use as orally active iron-chelating agents.

3-Hydroxy-2(1*H*)-pyridinones. Chelators of this type have adjacent keto and hydroxyl functions, for example 1, and possess a high affinity for iron(III). These ligands have some features in common with both catechols and hydroxamates in that they form five-membered chelate rings with iron(III), using the two adjacent oxygen atoms as ligands. The delocalization in the bonding of the hydroxypyridinone anion to iron(III) presumably contributes to the stability of the complex in a similar fashion to the extremely stable iron(III) catechol complexes.[8,9] Initially we describe the synthesis of a range of bidentate, N-substituted derivatives of 2,3-dihydroxypyridine. The covalent coupling of three such moieties to form hexadentate chelators is then described. The solution properties of these ligands and their iron(III) complexes are clearly important factors contributing to the enhanced biological activity compared with those of desferrioxamine B. The pK_a values, stability constants of iron(III) complexes, and distribution coefficients of representative chelators are reported here.

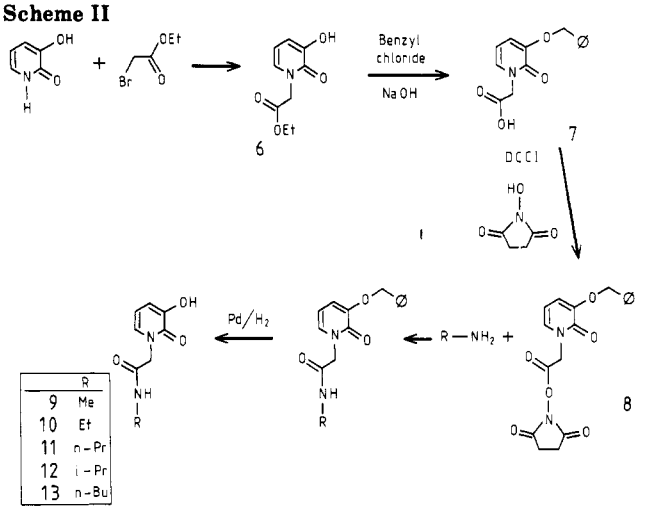
Syntheses of Bidentate 3-Hydroxy-2(1*H*)-pyridinone Ligands. The simple N-alkylation of 2,3-dihydroxypyridine with alkyl halides was first reported by Bickel and Wibaut.[10] In the present work a range of iodoalkanes gave compounds **1–5** (Scheme I) in yields which decreased as alkyl chain length increased until a practicable limit was reached with iodopentane. On this evidence the use of di- and triiodoalkanes did not appear to be a useful route to tetra- and hexadentate ligands. Instead, the introduction of an easily coupled functional

Scheme I



	R
1	Me
2	Et
3	n-Pr
4	i-Pr
5	n-Bu

Scheme II



	R
9	Me
10	Et
11	n-Pr
12	i-Pr
13	n-Bu

group was achieved with the reagent ethyl bromoacetate, which reacts with 2,3-dihydroxypyridine to give the ethyl ester **6** in good yield as shown in Scheme II. Hydrolysis of this ester and protection of the 3-hydroxyl function were carried out simultaneously by refluxing in a solution of

(1) Lankford, C. E. *Crit. Rev. Microbiol.* **1973**, *2*, 273.
(2) Sephton-Smith, R. *Brit. Med. J.* **1962**, *2*, 1577.
(3) Modell, B. *Prog. Haematol.* **1979**, *11*, 267.
(4) Lakhanpal, V.; Schocket, S. S.; Jiji, R. *Opthalmology* **1984**, *91*, 443.
(5) Olivieri, N. F.; Buncic, J. R.; Chew, E.; Gallant, T.; Harrison, R. V.; Keenan, N.; Logan, W.; Mitchell, D.; Ricci, G.; Skarf, B.; Taylor, M.; Freedman, M. H. *N. Eng. J. Med.* **1986**, *314* (*14*), 869.
(6) Scarrow, R. C.; Riley, P. E.; Abu-Dari, K.; White, D. L.; Raymond, K. N. *Inorg. Chem.* **1985**, *24*, 954.
(7) Raymond, K. N.; Muller, G.; Matzanke, B. F. Complexation of Iron by Siderophores: A Review of their Solution and Structural Chemistry and Biological Function. *Top. Curr. Chem.* **1984**, *123* (Struct. Chem.), 49–102.
(8) Sillen, L. G.; Martell, A. E. In *Stability Constants of Metal-Ion Complexes*; Chemical Society: London, 1971; Suppl. 1.
(9) Gordon, J.; Fenske, R. F. *Inorg. Chem.* **1982**, *21*, 2916.
(10) Bickel, A. F.; Wibaut, J. P. *Recl. Trav. Chim. Pays—Bas* **1946**, *65*, 65.

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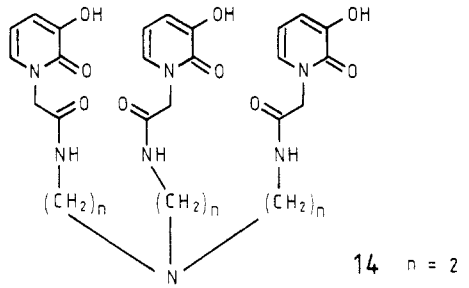
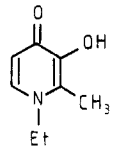
sodium hydroxide and benzyl chloride to give the benzyl-protected carboxylic acid derivative **7**. Coupling of this carboxylic function to a variety of amines by amide bond formation gives access to a wide range of new ligands. Coupling was achieved by first preparing the activated ester **8** from the carboxylic function with dicyclohexylcarbodiimide (DCCI) and *N*-hydroxysuccinimide. The activated ester was then condensed with one of a series of 1-aminoalkanes. Finally, removal of the protecting benzyl group by catalytic hydrogenolysis yielded compounds **9–13**.

Syntheses of Hexadentate 3-Hydroxy-2(1*H*)-pyridinone Ligands. Coupling of the active ester **8** with di- and triamines provides a route to the formation of tetradentate and hexadentate chelators, respectively. Although tetradentate chelators are not ideal for binding to iron(III) they may be of value for binding divalent metals such as copper(II) or zinc(II). A number of such compounds have already been synthesized and these tetradentate chelators will be discussed in a later publication. Here we describe the synthesis of hexadentate chelators which provide octahedral coordination of iron(III). The method makes use of compounds which have three primary amine functions connected in a tripodal arrangement, $[NH_2(CH_2)_n]_3N$, where n = 2 or 3. Each of the primary amine functions of these tripodal tetraamines may be condensed with one molecule of the activated ester **8** to give an amide bond. This was facilitated by maintaining the molar ratio of the activated ester in excess of 3:1 with respect to the tetraamine. Removal of the protecting benzyl group by catalytic hydrogenolysis yielded tripodal hexadentate chelators **14** and **15**. Deprotection with boron tribromide in dichloromethane also gave an acceptable yield.



14 n = 2
15 n = 3



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A variety of tripodal tetraamines may be synthesized by a method first described by Mann and Pope.[11] The first step is the synthesis of aza-linked diphthalimides, which are then converted to tripodal triphthalimides and deprotected to give the tripodal tetraamines. A modification of this method, previously described by Keypour,[12] was used to synthesize the tetraamine tris(3-aminopropyl)amine while tris(2-aminoethyl)amine was obtained from a commerical source (Aldrich).

Determination of Solution Properties

Bidentate ligands can form a number of complexes with iron(III) so that aqueous solutions equilibrate to give mixtures in which the speciation depends on the metal ion, ligand, and hydrogen ion concentrations. A method based on competition studies with ethylenediaminetetraacetic acid was used to determine log β_3 values for bidentate ligands. The experimental conditions ensured that the most associated species predominated. With hexadentate ligands only one complex is formed with iron(III) and log K_1 values for these ligands were also obtained by competition with EDTA. The fraction of the metal ion (Z) bound by each of the two competing ligands can be calculated from the change in absorbance produced by the addition of EDTA. The stability constant of the sample ligand may then be calculated from literature values for the stability constants of FeEDTA complexes. Ligand pK_a values have also been determined in an effort to describe fully the speciation of ligands and their iron(III) complexes as a function of pH.

Distribution coefficients of the ligands and their iron(III) complexes were determined in an aqueous/octanol system.

Table I. Optimized pK_a Values at 0.2 M Ionic Strength (Potassium Nitrate) and 22.5 °C[a]

	pK_a
1-ethyl-3-hydroxy-2(1*H*)-pyridinone (**2**)	8.99 (0.01)
3-hydroxy-1-[(propylcarbamoyl)methyl]-2(1*H*)-pyridinone (**11**)	8.75 (0.01)
N,*N*,*N*-tris[2-(3-hydroxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido]ethylamine (**14**)	9.26 (0.01)[b]
	8.70 (0.01)[b]
	8.18 (0.01)[b]
	5.82 (0.02)

[a] The standard error is given in brackets. [b] Spectrophotometrically determined pK_a values assuming independent absorptivities.

Biological Experiments

The rate of chelator-induced permeation of iron across erythrocyte and hepatocyte membranes was monitored with ^{59}Fe. Iron-overloaded mice were used to establish the profile of ^{59}Fe excretion with time after administration of each chelator.[13]

Results

Stability Constants of Iron(III) Complexes. For bidentate ligands the logarithm of the cumulative stability constants was determined. This value, log β_3, is obtained by summation of the logarithms of three stepwise equilibrium constants corresponding to the model shown in eq 1. This model has previously been shown to apply to the

$$Fe^{3+} + L^- \rightleftarrows FeL^{2+} \quad K_1 \qquad (1)$$
$$FeL^{2+} + L^- \rightleftarrows FeL_2^+ \quad K_2$$
$$FeL_2^+ + L^- \rightleftarrows FeL_3 \quad K_3$$

3-hydroxy-2(1*H*)-pyridinones.[14] Solutions of EDTA, the ligand **2**, and iron(III) were mixed so that the respective molar ratios varied from 0:10:1 to 125:10:1. The average value determined for log β_3 was 32.3. For hexadentate ligands the equilibrium constant corresponding to eq 2 was

$$Fe^{3+} + L^{3-} \rightleftarrows FeL \quad K_1 \qquad (2)$$

calculated. Solutions of EDTA, the hexadentate ligand **14**, and iron(III) were mixed so that the molar ratios varied from 0:1.04:1 to 100:1.04:1. The average value determined for log K_1 was 28.8.

Ligand pK_a Values. Some representative ligands have been studied by simultaneous spectrophotometric/poten-

(11) Mann, F. G.; Pope, W. J. *J. Chem. Soc.* **1926**, 489.
(12) Keypour, H. Ph.D. Thesis, University of Essex, 1978.
(13) Gyparaki, M.; Porter, J. B.; Hirani, S.; Streater, M.; Hider, R. C.; Huehns, E. R. *Acta Haematol.* **1987**, *78*, 217.
(14) Streater, M. Ph.D. Thesis, University of Essex, 1987.

Table II. Distribution Coefficients of the Ligands and Their Iron(III) Complexes between an Aqueous Phase Buffered at pH 7.4 and Octanol[a]

R	distribution coefficient: free compound	distribution coefficient: iron(III) complex
1-Alkyl-3-hydroxy-2(1*H*)-pyridinones		
methyl (**1**)	0.60	0.12
ethyl (**2**)	1.57	1.67
propyl (**3**)	4.92	24.6
isopropyl (**4**)	6.17	234
butyl (**5**)	16.00	>1000
gradient of log *D* vs alkyl chain length	0.48	1.29
3-Hydroxy-1-[(alkylcarbamoyl)methyl]-2(1*H*)-pyridinones		
methyl (**9**)	0.07	0.001
ethyl (**10**)	0.18	0.035
propyl (**11**)	0.57	0.35
isopropyl (**12**)	0.46	0.11
butyl (**13**)	2.12	3.74
gradient of log *D* vs alkyl chain length	0.49	1.17
Others		
N,N,N-tris[2-(3-hydroxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido]ethylamine (**14**)	0.025	0.008
desferrioxamine B	0.07	0.03

tiometric titrations. The optimized pK_a values obtained with the computer program NONLINGEN15[15] are shown in Table I. The pK_a values for the bidentate 3-hydroxy-2(1*H*)-pyridinones correspond to the dissociation of the hydroxyl proton. There is no evidence of any further proton equilibria down to a pH of 1, contrary to a previous report.[16] This is in contrast to 3-hydroxy-4(1*H*)-pyridinones, e.g. **16**, which have a pK_a near 3.6, corresponding to protonation of the pyridinone oxygen.[15]

Four pK_a values must be determined for hexadentate ligand **14**, one for each of the arms of the tripod and one for the central tertiary amine. The assignment of the pK_a at 5.82 to the central tertiary amine is unambiguous since this was the only buffer region for which no corresponding spectrophotometric changes were observed. This is comparable to one of the four pK_a values reported by Raymond et al.[17] (5.88) for a catecholamide ligand formed by amidification of the tripod arms of tris(2-aminoethyl)amine. However, assignment of pK_a values to specific groups in the catecholamide chelator was ambiguous because the simultaneous UV spectrophotometric titration was not reported.

The determination of the remaining three overlapping pK_a values from potentiometirc data is rendered difficult by the high pH of the buffer region. Their resolution from spectrophotometric titration requires optimization of three pK_a values and four absorptivities simultaneously. This was considered to be too many variables for the number of data points available. However, if the three hydroxypyridinone moieties are considered to absorb independently, then the absorptivities of the intermediate species can be linearly related to their proton stoichiometry. This assumption was made during the nonlinear regression optimization of the pK_a values indicated in Table I. The three pK_a values are close to those which would be predicted from the bidentate analogues when statistical and coulombic factors are considered.[18]

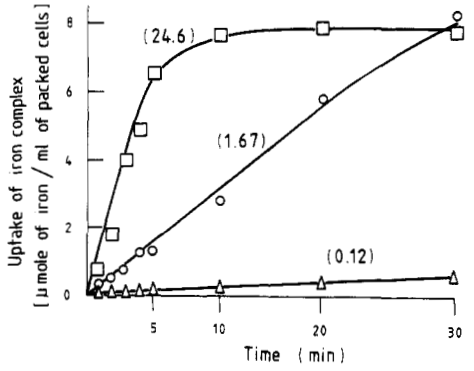

Figure 1. Uptake of tris(1-alkyl-3-hydroxy-2(1*H*)-pyridinone)-iron(III) complexes (1 mM) by human erythrocytes against time. The erythrocytes were incubated in isotonic saline, buffered with Tris·HCl (20 mM, pH 7.4): △, 1-methyl (**1**); ○, 1-ethyl (**2**); □, 1-propyl (**3**). Distribution coefficients of the iron(III) complexes are given in parentheses. The flux was determined by measuring the intracellular ^{59}Fe levels with a γ counter. Each point is the mean of six independent determinations.

Distribution Coefficients of Ligands and Their Iron(III) Complexes

Distribution coefficients between an aqueous phase buffered at pH 7.4 and octanol are presented in Table II.

In general the expected increase in distribution coefficients upon elongation of the alkyl chain was observed in both the 1-alkyl and the 1-[(alkylcarbamoyl)methyl] compounds. There is a marked difference however between the distribution coefficients of the amide derivatives (**9–13**) and the alkyl derivatives (**1–5**).

Ability of 3-Hydroxy-2(1*H*)-pyridinones To Permeate Biological Membranes

Erythrocytes. Human erythrocytes were incubated with ^{59}Fe-labeled iron–pyridinone (1:3) complexes in isotonic saline, buffered at pH 7.4. The rate of entry of the complexes was found to increase with increasing value of the distribution coefficient (*D*); for instance, 3-hydroxy-1-propyl-2(1*H*)-pyridinone (**3**, *D* for iron complex = 24.6) entered cells approximately 30 times faster than 3-hydroxy-1-methyl-2(1*H*)-pyridinone (**1**, *D* for iron complex = 0.12) (Figure 1). In the same study iron(III)–EDTA, iron(III)–desferrioxamine, and the iron(III) complex of ligand **14** did not enter cells (iron(III) uptake <200 nmol/mL of packed erythrocytes).

Hepatocytes. Rat hepatocytes were cultured overnight, preincubated with ^{59}Fe–transferrin, and then incubated with a range of chelators (Porter et al.).[19] The rate of efflux of ^{59}Fe from the hepatocytes was monitored in the presence and absence of chelator and the data are presented as the percentage of the ^{59}Fe efflux occurring in the absence of chelator. Figure 2 shows the ^{59}Fe efflux induced by the ligands 1-ethyl-3-hydroxy-2(1*H*)-pyridinone (**2**), the analogous 1-ethyl-2-methyl-3-hydroxy-4(1*H*)-pyridinone (**16**), and desferrioxamine B. 1-Ethyl-3-hydroxy-2(1*H*)-pyridinone (**2**) is less effective than either the analogous 3-hydroxy-4(1*H*)-pyridinone or desferrioxamine B at doses below 1 mg mL^{-1}. It should be noted that there was no evidence of toxicity, as judged by the release of lactic dehydrogenase (LDH), with any of the chelators even at the highest doses used. In contrast to the bidentate 3-

(15) Taylor, P. D.; Morrison, I. E.; Hider, R. C. *Talanta* **1988**, *35*, 507.
(16) Kontoghiorghes, G. J. *Inorg. Chim. Acta* **1987**, *135*, 145.
(17) Rogers, S. J.; Lee, C. W.; Ng, C. Y.; Raymond, K. N. *Inorg. Chem.* **1987**, *26*, 1622.
(18) Rossotti, H. *The Study of Ionic Equilibria*; Longman: London, 1978.
(19) Porter, J. B.; Gyparaki, M.; Huehns, E. R.; Hider, R. C. *Biochem. Soc. Trans.* **1986**, *14*, 1180.

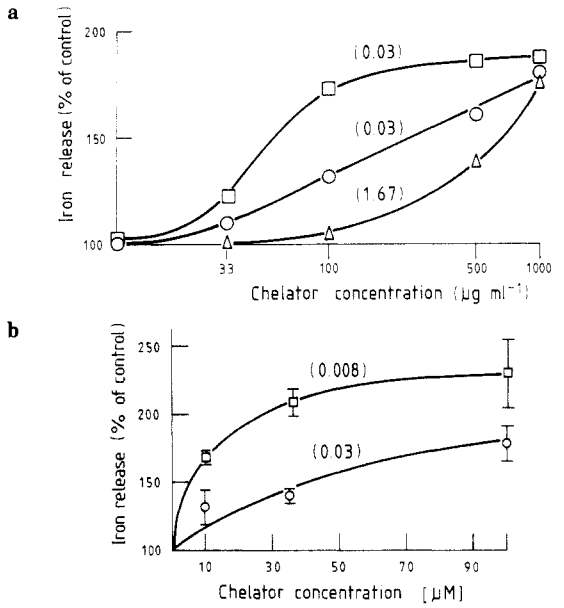

Figure 2. Hepatocyte ^{59}Fe release by hydroxypyridinone chelators. Hepatocytes derived from rat liver were placed on collagen plates and pulsed with [^{59}Fe]transferrin. After 1 h of incubation and washing, the hepatocytes were exposed for 2 h to chelators at the concentrations indicated. The quantity of ^{59}Fe released by the hepatocytes was determined with a γ counter. Distribution coefficients of the iron(III) complexes are given in parentheses. The curves within each plot are from the same batch of hepatocytes; however there was some variation between the batches used for a and b: (a) □, 1-ethyl-3-hydroxy-2-methyl-4(1*H*)-pyridinone (**16**); ○, desferrioxamine; and △, 1-ethyl-3-hydroxy-2(1*H*)-pyridinone (**2**); (logarithmic scale); and (b) □, the hexadentate ligand (**14**); ○, desferrioxamine. The mean of six independent determinations is shown with the standard error.

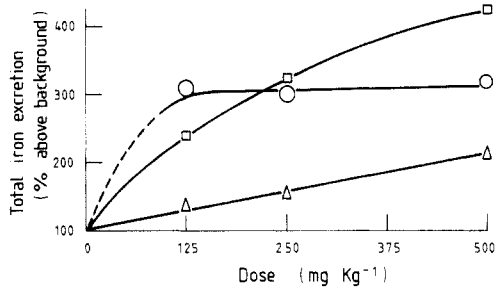

Figure 3. Removal of ^{59}Fe from iron-overloaded mice by chelators at various doses: □, Desferrioxamine; ○, the hexadentate ligand (**14**); △, the hexadentate ligand (**15**). Mice were loaded with iron dextran, labeled with [^{59}Fe]transferrin and allowed to stabilize for 2 weeks. After base-line excretion rates were recorded, chelators were added intraperitoneally. The amount of ^{59}Fe in the urine and feces over the next 24 h was measured with a γ counter. Each point is the mean of four independent readings.

hydroxy-2(1*H*)-pyridinone, the hexadentate analogue **14** proved to be more effective than desferrioxamine at all doses investigated (Figure 2b). Again no LDH release was observed.

Mobilization of Iron from Iron-Overloaded Mice. Iron-overloaded mice[13] were treated intraperitoneally with either desferrioxamine, ligand **14**, or ligand **15**, and the chelator-induced excretion of ^{59}Fe was monitored. All three chelators markedly enhanced the excretion of iron (Figure 3), with ligand **14** being marginally more active at the lower dose levels (<125 mg kg^{-1}).

Discussion

Bidentate 3-Hydroxy-2(1*H*)-pyridinones. The high value of log β_3 indicates that the neutral 3:1 complex is a highly favored species at neutral pH values. By analogy with other dioxygen bidentate ligands, for example catechol,[20] 3-hydroxy-4(1*H*)-pyridinones,[6] 1-hydroxy-(2*H*)-pyridinones,[6] and 3-hydroxy-4-pyrones,[21] the iron(III) complex is likely to have a distorted octahedral geometry. Modification of the N-substituent has a large effect on distribution coefficients and hence on the rate of permeation of biological membranes. This is exemplified by the ability of the iron complexes to permeate red blood cells. The rate of entry is strongly dependent on the distribution coefficient of the complex (Figure 1).

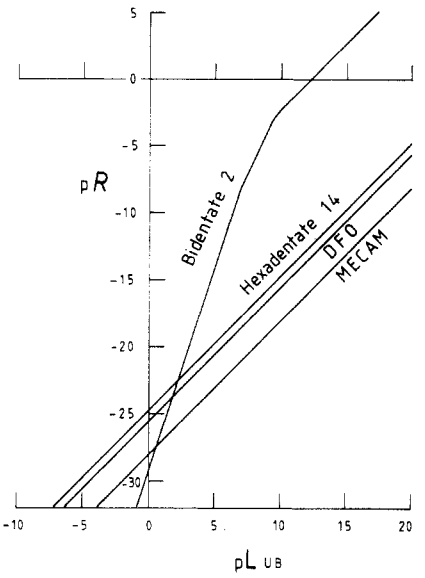

Figure 4. Plot of pR (−log [bound metal]/[unbound metal]) versus pL_{UB} (−log [unbound ligand]) at pH 7.4 for (i) the hexadentate ligand **14**, (ii) desferrioxamine (DFO), (iii) the catecholamide hexadentate ligand MECAM,[25] and (iv) the bidentate ligand **2**. The curve for the bidentate ligand **2** has been shifted to the right by the log 3 (0.477) to give the same number of coordination sites as the hexadentate ligands at any value of pL_{UB}.

The rate of iron(III) removal from iron-overloaded hepatocytes by bidentate 3-hydroxy-2(1*H*)-pyridinones was inferior to that achieved by 3-hydroxy-4(1*H*)-pyridinones (Figure 2). This parallels the relative rates of iron(III) removal from transferrin[22] and ferritin[23] induced by the two classes of pyridinones. The enhanced ability of 3-hydroxy-4(1*H*)-pyridinones over 3-hydroxy-2(1*H*)-pyridinones to scavenge iron(III) is probably related to the higher affinity constants of the iron(III) complexes of the former; 1-ethyl-3-hydroxy-2-methyl-4(1*H*)-pyridinone (**16**) has log β_3 = 37.2;[24] 1-ethyl-3-hydroxy-2(1H)-pyridinone (**2**) has log β_3 = 32.3 (this work).

Hexadentate 3-Hydroxy-2(1*H*)-pyridinones. The value of log K_1 found for ligand **14** (28.8) is comparable to that of desferrioxamine B (31). The finding that log K_1 for the hexadentate ligand is lower than log β_3 of the analogous tris-bidentate complex (log β_3 = 32.3) indicates that the stereochemistry prevents the attainment of the maximum entropic effect. However, at any given metal ion concentration, the concentration of hexadentate com-

(20) Raymond, K. N.; Isied, S. S.; Brown, L. D.; Fronczek, F. R.; Nibert, J. H. *J. Am. Chem. Soc.* **1976**, *98*, 1767.
(21) Ahmet, M. T.; Frampton, C. S.; Silver, J. *J. Chem. Soc. Dalton. Trans.* **1988**, 1159.
(22) Hider, R. C.; Kontoghiorghes, G. J.; Silver, J. U.K patent GB 2118176A, 1983. Kontoghiorghes, G. J. *Biochim. Biophys. Acta* **1986**, *869*, 141.
(23) Brady, M. C.; Lilley, K. S.; Treffry, A.; Harrison, P. M.; Hider, R. C.; Taylor, P. D. *J. Inorg. Biochem.* **1989**, *35*, 9.
(24) Taylor, P. D. Ph.D. Thesis, University of Essex, 1987.

plex in solution has first-order dependence on ligand concentration while the concentration of the tris-bidentate complex has third-order dependence. This means that as the ligand's concentration decreases below a critical level (about 10 mM in this case), the hexadentate ligand progressively competes more effectively for iron(III) than the tris-bidentate complex (Figure 4). Under in vivo conditions, the chelator will be present at a concentration well below the crossover point.

The distribution coefficients at pH 7.4 of hexadentate ligand 14 (0.025) and of its iron(III) complex (0.008) are comparable to those of desferrioxamine B (0.07 and 0.03, respectively).[19] The tertiary amino group on the hexadentate iron(III) complex of 14 contributes a charge of less than +0.1 at pH 7.4 [preliminary work suggests that the pK_a of the free ligand (5.82) is not significantly altered on chelation to iron(III). The net charge on the iron(III) complex at pH 7.4 is therefore the same as that of the free chelator.] The low distribution coefficients of both 14 and its iron(III) complex cannot then be attributed to the overall charge. Moreover the analogous tris-bidentate compounds and their iron(III) complexes, which have no net charge at pH 7.4, have comparable distribution coefficients. The membrane permeation of the free ligands and their iron(III) complexes is likely to be limited by these low distribution coefficients.

The similarities in the pR (Figure 4) and distribution coefficient D values for desferrioxamine B and ligand 14 indicate that the latter hexadentate chelator has the potential to sequester iron(III) under physiological conditions and this has been confirmed by studies with hepatocytes and iron-overloaded mice[13] (Figures 2 and 3).

3-Hydroxy-2(1H)-pyridinones can be compared with other siderophore analogues. Raymond and co-workers have synthesized a number of hexadentate catecholate analogues of the siderophore enterobactin.[7] Amide bonds were formed by the reaction between acid chlorides and primary amines, examples being the syntheses of MECAM and TRIMCAM.[25] Substitution at the 5-position of the catecholate rings with sulfonate groups increased the resistance of the compounds to air oxidation and improved their aqueous solubility. However it also gives the iron(III) complex an overall charge of up to −6 (depending on the pH), which results in poor membrane-transport properties. Thus the free chelators would be predicted to be orally inactive and the iron(III) complexes, once formed intracellularly, would tend to be trapped by their high charge density. By comparison it is feasible to synthesize hexadentate 3-hydroxy-2(1H)-pyridinone–iron(III) complexes with no net charge at physiological pH. Furthermore the 3-hydroxy-2(1H)-pyridinones described in this paper have been exposed to strongly acid conditions in the presence of oxygen and iron(III) with no apparent spectral evidence of the redox reactions which are a severe problem with catecholamide compounds.[26,27]

Below pH 8 the hexadentate catecholamides are predominantly in the form of the hexaprotonated species. This means that up to six protons are displaced by complexation with iron(III) compared to the three protons displaced from ligand 14 or from desferrioxamine B. Thus a graph of pR versus pH for catecholamides will have a gradient which is up to three times greater than that of ligand 14 or desferrioxamine B. Therefore, at some pH below 7.4, the latter two ligands will overtake the pR value that can be attained with catecholamides. Further characterization of the pK_a values of the iron(III) complexes of 14 is under way which will enable the crossover pH to be determined. The retention of high affinity for iron(III) at low pH may have great kinetic importance.[28] Thus hexadentate 3-hydroxy-2(1H)-pyridinones meet many of the requirements of an orally active iron(III)-sequestering drug. The iron(III) complexes are stable at the low pH values found in lysozomes; the uncomplexed chelator survives the strongly acid conditions of the stomach, and both the complexed and uncomplexed ligand can permeate cell membranes.

In principle the properties of ligands 14 and 15 described in this paper can be further enhanced by optimizing the stereochemical arrangement of the three coordinating aromatic rings (the maximum entropic effect that can be achieved corresponds to an increase of 6 log units above the value of log β_3 for the tris-bidentate complex, giving a log K_1 value of 38). Furthermore, in order to enhance oral activity the distribution coefficients of 14 and 15 (Table II) could be increased by alkylation of the amide nitrogens. Both these possibilities are currently under investigation.

Table III. Syntheses of 1-Alkyl-3-hydroxy-2(1H)-pyridinones[a]

R	mp, °C	% yield	formula	anal.
methyl (1)	126–127	61	$C_6H_7NO_2$	C,H,N
ethyl (2)	109–110	66	$C_7H_9NO_2$	C,H,N
propyl (3)	89–90	42	$C_8H_{11}NO_2$	C,H,N
isopropyl (4)	190 dec	3	$C_8H_{11}NO_2$	C,H,N
butyl (5)	60–61	9	$C_9H_{13}NO_2$	C,H,N

[a] See the supplementary material.

Experimental Section

General Procedures. Melting points are uncorrected. IR spectra were recorded on a Unicam SP 200. Proton NMR were recorded on a Varian EM360. Elemental analyses were performed by the microanalytical laboratory of the Department of Chemistry, University of Manchester. Full analytical data are available as supplementary material.

Bidentate Chelators. Method A. 3-Hydroxy-1-methyl-2-(1H)-pyridinone (1). The procedure is derived from that of Wibaut et al.[10] 3-Hydroxy-2(1H)-pyridinone (11.11 g, 0.1 M) and iodomethane were placed in a thick-walled glass tube (20 cm × 1 cm i.d.), which was then frozen in liquid nitrogen, evacuated, and flame sealed. After thawing, the tube was placed in a protective steel chamber and heated at 140 °C in an oven for 2 days. The cooled tube was refrozen and reopened and the excess iodomethane was decanted. The resultant dark brown oil was warmed and decanted into distilled water (100 mL) and sulfur dioxide was bubbled through the solution to yield a beige precipitate. The pH of the solution was then adjusted to a value of 6 with sodium carbonate solution (1 M), the mixture was filtered and the aqueous solution was saturated with ammonium sulfate. The solution was extracted with chloroform (3 × 50 mL) and the combined extracts were dried over anhydrous sodium sulfate, filtered, and evaporation to dryness. The crude product was recrystallized from hot petroleum ether (bp 100–120 °C) as colorless crystals (7.26 g, 61%); mp 126–127 °C (lit.[29] mp 129–131 °C). Anal. ($C_6H_7NO_2$) C, H, N.

The compounds shown in Table III were prepared by method A with a range of iodoalkanes, the reaction giving compound 5 requiring up to 10 days.

Method B. 1-[(Ethoxycarbonyl)methyl]-3-hydroxy-2-(1H)-pyridinone (6). 3-Hydroxy-2(1H)-pyridinone (11.1 g, 0.1 mol) and ethyl bromoacetate (83.5 g, 0.5 mol) were mixed; the

(25) Weitl, F. L.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 2728.
(26) Harris, W. R., Carrano, C. J., Cooper, S. R., Sofen, S. R., Avdeef, A., McArdle, J. V., Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 6097.
(27) Harris, W. R., Raymond, K. N., Weitl, F. L. *J. Am. Chem. Soc.* 1981, *103*, 2667.
(28) Baldwin, D. A.; Egan, T. J. *South Afr. J. Sci.* **1987**, *83*, 22.
(29) Mohrle, H.; Weber, H. *Tetrahedron* **1970**, *26*, 3779.

Table IV. Syntheses of 1-[(Alkylcarbamoyl)methyl]-3-hydroxy-2(1*H*)-pyridinones[a]

R	mp, °C	% yield	formula	anal.
methyl (9)	209–211	84	$C_8H_{10}N_2O_3$	C,H,N
ethyl (10)	218–220	73	$C_9H_{12}N_2O_3$	C,H,N
propyl (11)	204–205	54	$C_{10}H_{14}N_2O_3$	C,H,N
isopropyl (12)	238–241	58	$C_{10}H_{14}N_2O_3$	C,H,N
butyl (13)	199–200	49	$C_{11}H_{16}N_2O_3$	C,H,N

[a] See the supplementary material.

vessel was flushed with nitrogen for 1 h and then refluxed under nitrogen for 24 h. On cooling the resulting beige precipitate was filtered and washed with acetone. Recrystallization from 95% ethanol in the presence of activated charcoal gave colorless needlelike crystals (15.2 g, 77%); mp 150–152 °C. Anal. ($C_9H_{11}NO_4$) C, H, N.

3-(Benzyloxy)-1-(carboxymethyl)-2(1*H*)-pyridinone (7). 1-[(Ethoxycarbonyl)methyl]-3-hydroxy-2(1*H*)-pyridinone (10 g, 0.05 mol) was dissolved in aqueous methanol (300 mL of 90%) and the pH of the solution was adjusted to 12 with aqueous sodium hydroxide. Benzyl chloride (25 g, 0.2 mol) was added and the mixture was refluxed for 6 h, the pH being kept above 12 by addition of further sodium hydroxide solution as required. The resultant solution was cooled, the methanol removed by rotary evaporation, and the aqueous solution was extracted with dichloromethane (3 × 50 mL). The solution was diluted with water (100 mL) and the pH was adjusted to 1 with concentrated hydrochloric acid, whereupon the crude product precipitated. Recrystallization from 95% ethanol gave colorless needlelike crystals (10.8 g, 83%); mp 185–185.5 °C, Anal. ($C_{14}H_{13}NO_4$) C, H, N.

3-(Benzyloxy)-1-[[(succinimidyloxy)carbonyl]methyl]-2-(1*H*)-pyridinone (8). To a solution of 3-(benzyloxy)-1-(carboxymethyl)-2(1*H*)-pyridinone (5 g, 0.019 mol) in dimethylformamide (100 mL) were added solutions of *N*-hydroxysuccinimide (2.3 g, 0.02 mol) and dicyclohexylcarbodiimide (DCCI, 4.13 g, 0.02 mol) each in dimethylformamide (50 mL). The resultant mixture was stirred overnight in darkness. Glacial acetic acid (0.5 Ml) was added and after stirring for 1 h the solution was filtered. The dimethylformamide was removed by rotary evaporation, giving the crude product. Recrystallization from ethyl acetate gave colorless needles (5.55 g, 82%); mp 174.5–175 °C. Anal. ($C_{18}H_{16}N_2O_6$) C, H, N.

1-[(Ethylcarbamoyl)methyl]-3-hydroxy-2(1*H*)-pyridinone (10). To 3-(benzyloxy)-1-[[(succinimyloxy)carbonyl]methyl]-2-(1*H*)-pyridinone (1 g, 2.8×10^{-3} mol) in dichloromethane (50 mL) was added ethylamine (0.18 g, 4×10^{-3} mol) with stirring. After 1 h the mixture was filtered and the dichloromethane layer washed first with sodium bicarbonate solution (0.05 M, 3 × 25 mL) and then with water, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The resultant solid was hydrogenated over Pd/C catalyst in ethanol (95%, 50 mL) and glacial acetic acid (1 mL) for 24 h. After filtration the solution was evaporated to dryness and the crude product was desiccated over sodium hydroxide pellets. Recrystallization from hot ethanol (95%) gave colorless crystals (0.43 g, 73%); mp 218–220 °C. Anal. ($C_9H_{12}N_2O_3$) C, H, N.

The compounds shown in Table IV were synthesized by method B using a variety of amines.

Hexadentate Chelators. *N,N,N*-Tris[2-(3-hydroxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido]ethylamine (14). Tris(2-aminoethyl)amine (Fluka, 0.73 g, 5 mmol) was added with stirring to a solution of 3-(benzyloxy)-1-[[(succinimyloxy)-carbonyl]methyl]-2(1*H*)-pyridinone (5.0 g, 0.014 mol) in DMF (100 mL). After 30 min the solution was evaporated to dryness, redissolved in dichloromethane (100 mL), and washed first with sodium bicarbonate solution (0.05 M, 3 × 25 mL) and then with water. The organic phase was then dried over anhydrous sodium sulfate, filtered, and evaporated to dryness and the resultant crude product was crystallized from hot 95% ethanol as colorless crystals. The benzyl protecting group was then removed by hydrogenation over Pd/C catalyst in ethanol/glacial acetic acid (10:1, 100 mL). The solution was filtered, evaporated to dryness, and desiccated over sodium hydroxide pellets. Recrystallization from absolute ethanol gave colorless crystals (2.1 g, 72%): mp 178–180 °C dec; ν_{max} 1590 (ring C=C), 1655 (pyridinone C=O), 3250 (amide NH); ^{1}H NMR (DMSO-d_6) δ 9.1 (3 H, s br, OH), 8.0 (3 H, s br, NH), 7.0 (3 H, d, 6-H), 6.7 (3 H, d, 4-H), 6.05 (3 H, t, 5-H), 4.55 (6 H, s, NCH_2CO), 3.2 (6 H, s br, $NHCH_2$), 2.5 (6 H, s br, CH_2N). Found: C, 53.0; H, 5.6; N, 16.1. $C_{27}H_{33}O_9N_7 \cdot {}^1/_2H_2O$ requires C, 53.2; H, 5.6; N, 16.1.

***N,N,N*-Tris[3-(3-hydroxy-2-oxo-1,2-dihydropyridin-1-yl)acetamido]propylamine Hydrobromide (15).** The method used was as described above except that tris(3-aminopropyl)amine (0.94 g, 0.5 mmol) replaced tris(2-aminoethyl)amine. Evaporation of the dried dichloromethane solution gave a red oil, which was redissolved in dichloromethane (5 mL) and eluted on a silica gel column (60–120 mesh) with dichloromethane. Evaporation of the solvent gave the benzyl-protected product, which was deprotected by hydrogenation over Pd/C catalyst in ethanol/glacial acetic acid (100:1) to give **15** in the form of a pale purple solid (1.4 g, 62%); mp 103–107 °C; ν_{max} 1590 (ring C=C), 1650 (pyridinone C=O), 3300 (amide NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.1 (3 H, s br, NH), 7.0 (3 H, d, 6-H), 6.6 (3 H, d, 4-H), 6.1 (3 H, t, 5-H), 4.4 (6 H, s, CH_2CO), 3.0 (6 H, s br, $CONHCH_2$), 2.3 (6 H, s br, CH_2N), 1.5 (6 H, s br, CCH_2C).

Slightly higher yields were obtained by deprotection with boron tribromide. The protected compound (1 g, 1.1 mmol) in dichloromethane (50 mL) was added dropwise with vigorous stirring to boron tribromide (11 mL of a 1M solution in dichloromethane, 10 molar excess) in dichloromethane (50 mL) in a dry nitrogen flushed flask fitted with a calcium chloride drying tube. The mixture was stirred overnight and then water (100 mL) was added and the mixture was stirred for 2 h. The aqueous layer was evaporated to dryness and the resultant solid was dissolved in hot ethanol (20 mL). This solution was poured into warm diethyl ether (200 mL) to precipitate the product. Most of the solvent was decanted and the remaining suspension was repeatedly washed with ether and recovered by filtration. Finally, drying under vacuum gave the hydrobromide salt of **15** as a colorless solid (0.66 g, 75%): mp 200–203 °C; ν_{max} 1605 (ring C=C), 1650 (pyridinone C=O), 1680 (amide C=O), 3250 (amide NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.3 (3 H, br, amide NH), 7.1 (3 H d, 6-H), 6.8 (3 H, d, 4-H), 6.1 (3 H, t, 5-H), 4.6 (6 H, s, CH_2CO), 3.2 (12 H, br, $CONHCH_2$ and CH_2N), 1.9 (6 H, br, CCH_2C). Found: C, 45.4; H, 5.9; N, 12.3. $C_{30}H_{39}O_9N_7 \cdot HBr \cdot 4H_2O$ requires C, 45.3; H, 6.1; N, 12.3.

Tris(3-aminopropyl)amine was synthesized by the procedures described by Mann and Pope.[11] Phthalic anhydride (59.3 g, 0.40 mol) was melted at 180 °C with an oil bath. 3,3′-Iminobispropylamine (26.3 g, 0.20 mol) was added dropwise with vigorous stirring. The glassy solid which formed on cooling was extracted with hot absolute ethanol. **3,3′-Diphthalimidodipropylamine** precipitated on cooling (61.1 g, 78%); mp 136–137 °C.

3,3′-Diphthalimidodipropylamine (20 g, 0.05 mol) and 3-bromo-1-phthalamidopropane (13.7 g, 0.05 mol) were stirred together at 150 °C for 45 min and allowed to cool. The resultant glassy solid was ground to a powder and extracted twice with hot absolute ethanol. The solid which precipitated on cooling was recrystallized from glacial acetic acid and dried over sodium hydroxide pellets at 110 °C under vacuum to give **3,3′,3″-triphthalimidotripropylamine hydrobromide** (17.5 g, 52%); mp 240–242 °C (lit.[11] mp 243–244 °C). 3,3′,3″-triphthalimidotripropylamine hydrobromide (33 g, 0.05 mol) was refluxed in aqueous 25% hydrochloric acid (300 mL) for 8 h. On cooling the colorless precipitate of phthalic acid was removed by filtration and the filtrate was evaporated to an orange solid. This was washed for 15 min with aqueous sodium hydroxide (5 M, 200 mL) and the solution was extracted with dichloromethane (3 × 200 mL). Evaporation gave **tris(3-aminopropyl)amine** as a pale orange oil (6.2 g, 66%).

Determination of Distribution Coefficients. These were determined spectrophotometrically for both free ligands and metal–ligand complexes. Ligands were prepared at approximately 0.1 mM in 20 mM Tris·HCl buffer (pH 7.4) and equilibrated with a volumes of octan-1-ol by shaking overnight in a thermostated bath at 25 °C. The volume of octan-1-ol was chosen by trial to give a decrease of between 30 and 70% in the absorbance of the aqueous layer after equilibration. Three preparations within this range were used to calculate the distribution coefficients. The full spectrum of the aqueous phase was measured over the range

250–350 nm for free ligand and 350–600 for the complexes. The distribution coefficients of iron(III) complexes were determined under conditions of greater than 10-fold excess ligand over iron(III).

Competition Studies with EDTA. For the competition studies reported here it is safe to assume that uncomplexed metal ion is practically absent. Absorbance at 510 nm was monitored for several hours until no further change was observed in order to establish that equilibrium was achieved. The value of Z was measured from eq 3, where A = absorbance of the competing

$$Z = (A - A_E)/(A_L - A_E) \quad (3)$$

system at equilibrium, A_E = absorbance of FeEDTA in the absence of the sample ligand, and A_L = absorbance of FeLn in the absence of EDTA. The overall stability constant of the equilibrium (eq 4, charges omitted for clarity) is given by eq 5, where n is the

$$Fe + nLH \rightleftarrows FeL_n + nH \quad (4)$$

$$K_L = [Z/(1 - Z)][(E_t - (1 - Z)M_t)/(L_t - nZM_t)^n](\alpha_L{}^n/\alpha_Y)K_E \quad (5)$$

number of ligands in the metal complex, α_L and α_Y have the form $1 + \sum_{i=1}^{m} (H^+)^i/K_{a_i}$, and K_{a_i} are the three acid dissociation constants (m = 3) of the pyridinone moieties (this work) or the four acid dissociation constants (m = 4) of EDTA.[8] E_t, L_t, and M_t are total analytical concentrations of EDTA, ligand, and metal ion, respectively, and K_E is a collection of terms including the stability constants of the various complexes of EDTA with iron(III) (see the Supplementary Material). Experiments were performed at pH 7.2, necessitating the inclusion of a number of FeEDTA complexes, the stability constants of which were obtained from literature sources.[8]

Biological Methods. Erythrocyte Uptake Studies. Whole blood was centrifuged at 3000 rpm for 5 min at 4 °C on a Beckman Model J-21 centrifuge. The plasma was removed by aspiration and discarded. The packed erythrocytes were then washed (2×) with approximately twice their volume of Tris·HCl (20 mM, pH 7.4; NaCl 130 mM). The packed erythrocytes (0.5 mL) were added to 2 mL of iron(III) complex in a 25-mL conical flask and the flask was incubated at 37 °C for the desired length of time in a shaking water bath. After incubation, 0.1-mL aliquots of the cell suspension were added to 0.4-mL MCC plastic tubes containing 0.3 mL of silicone fluid (ρ = 1.12). After centrifugation in a Beckman Microfuge B for 30 s the erythrocytes separated from the supernatent. The fractions were then placed in separate scintillation vial inserts by use of a hot scalpel. The ^{59}Fe content was determined by γ counting. The dry weight of the packed erythrocytes was determined by drying two 0.2-mL samples at 100 °C to constant weight.

Iron Removal from Rat Hepatocytes. Hepatocyte isolation and oxygenation were essentially as described by Berry and Friend[30] with the modifications of Young[31] and Porter et al.[32] Cell viability following centrifugation was estimated by fluorescence microscopy using ethidium bromide and acridine orange to mark dead and live cells, respectively.[33] Suspensions containing less than 80% viable cells were not used as these resulted in insufficient cells after primary adherence. Plates were cultured at 37 °C in an atmosphere of 5% CO_2/95% air. Cell viability could generally be maintained at >95% for over 48 h. After removal of nonadherent cells, 2 mL of culture medium containing ^{59}Fe human diferric transferrin[34] at 100 μg/mL final concentration was added. In order to measure the time course of ^{59}Fe release in the presence of chelators, medium was removed for ^{59}Fe counting and replaced with fresh medium (2 mL) at regular intervals. Cell damage was monitored by measuring lactate dehydrogenase (LDH)[35] in the incubation media, while cell viability was determined at the end of the experiment by fluorescence microscopy.

Iron Removal from Iron-Overloaded Mice. A modification of the mouse model developed by Huehns and co-workers was used.[36] The mice were overloaded with iron dextran given intraperitoneally at weekly intervals for 4 weeks. The iron stores were then radiolabeled with [^{59}Fe]lactoferrin. This treatment preferentially delivers iron to the liver.[37] Iron excretion was allowed to equilibrate for 3 weeks when a plateau was reached. At this stage the mice were used to measure the ^{59}Fe excretion induced by the test compounds given intraperitoneally. Both feces and urine were collected separately over defined time periods for a number of days and the ^{59}Fe excretion was monitored. The excretion following the administration of each compound was expressed as a percentage of the counts excreted during the previous 24-h period.

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Registry No. 1, 19365-01-6; 1 (Fe complex), 89952-78-3; 2, 90037-19-7; 2 (Fe complex), 89952-77-2; 3, 90037-20-0; 3 (Fe complex), 89952-79-4; 4, 90037-21-1; 4 (Fe complex), 89973-54-6; 5, 116407-52-4; 5 (Fe complex), 126453-02-9; 6, 95215-70-6; 7, 95215-72-8; 8, 95215-73-9; 9, 95215-77-3; 10, 95215-75-1; 11, 95215-79-5; 12, 95215-81-9; 13, 95215-83-1; 14, 99110-76-6; 14 (Fe complex), 99030-87-2; 14 (benzylated), 99110-77-7; 15, 126328-32-3; 15 (benzylated), 126328-33-4; 3-hydroxy-2(1*H*)-pyridinone, 16867-04-2; ethyl bromoacetate, 105-36-2; *N*-hydroxysuccinimide, 6066-82-6; tris(2-aminoethyl)amine, 4097-89-6; tris(3-aminopropyl)amine, 4963-47-7; phthalic anhydride, 85-44-9; 3,3′-iminobispropylamine, 56-18-8; 3,3′-diphthalimidodipropylamine, 102202-87-9; 3-bromopropylphthalimide, 5460-29-7; 3,3′,3″-triphthalimidotripropylamine hydrobromide, 126328-34-5; iron, 7439-89-6.

Supplementary Material Available: Full elemental and spectral analyses of compounds 1–15 and details of the EDTA competition studies are available (6 pages). Ordering information is given on any current masthead page.

(30) Berry, M. N.; Friends, D. S. *J. Cell. Biol.* **1969**, *43*, 506.
(31) Young, S. P., Aisen, P. *Biochim. Biophys. Acta* **1980**, *633*, 145.
(32) Porter, J. B.; Gyparaki, M.; Burke, L. C.; Huehns, E. R.; Sarpong, P.; Saez, V.; Hider, R. C. *Blood.* **1988**, *72*, 1497.
(33) Mills, K.; Armitage, R.; Worman, C. *Immunol. Lett.* **1983**, *6*, 241.
(34) Huebers, H.; Josephson, B.; Huebers, E.; Csiba, E.; Finch, C. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2572.
(35) Reeves, W., Fimognari, G. In *Methods in Enzymology*; Wood, W. Ed.; Academic Press: New York, 1966; Vol. 9, p 228, Method 54.
(36) Gyparaki, M.; Porter, J. B.; Hirani, S.; Streater, M.; Hider, R. C.; Huehns, E. R. *Acta Haematol.* **1987**, *78*, 217.
(37) Van Snick, J.; Mason, P. L.; Hermans, J. F. In *Proteins of Iron Storage and Transport in Biochemistry and Medicine*; North-Holland/Elsevier: Amsterdam, 1975; pp 33–438.