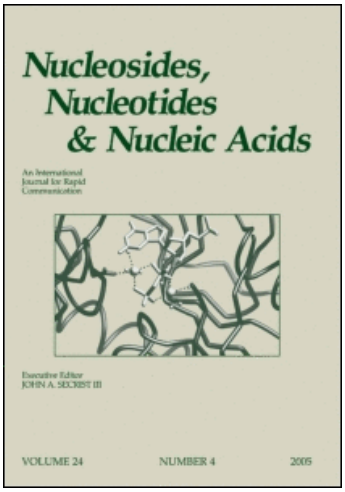


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Acyclonucleoside Iron Chelators of 1-(2-Hydroxyethoxy)methyl-2-alkyl-3-hydroxy-4-pyridinones: Potential Oral Iron Chelation Therapeutics

Gang Liu[a]; Ping Men[a]; Gerry H. Kenner[a]; Scott C. Miller[a]; Fred W. Bruenger[a]
[a] Radiobiology Division, University of Utah, Salt Lake City, Utah, USA

Online publication date: 03 December 2004

To cite this Article Liu, Gang , Men, Ping , Kenner, Gerry H. , Miller, Scott C. and Bruenger, Fred W.(2004) 'Acyclonucleoside Iron Chelators of 1-(2-Hydroxyethoxy)methyl-2-alkyl-3-hydroxy-4-pyridinones: Potential Oral Iron Chelation Therapeutics', Nucleosides, Nucleotides and Nucleic Acids, 23: 3, 599 — 611
To link to this Article: DOI: 10.1081/NCN-120030718
URL: http://dx.doi.org/10.1081/NCN-120030718

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, No. 3, pp. 599–611, 2004

Acyclonucleoside Iron Chelators of 1-(2-Hydroxyethoxy)methyl-2-alkyl-3-hydroxy-4-pyridinones: Potential Oral Iron Chelation Therapeutics

Gang Liu,* Ping Men, Gerry H. Kenner, Scott C. Miller, and Fred W. Bruenger

Radiobiology Division, University of Utah, Salt Lake City, Utah, USA

ABSTRACT

The method of synthesizing acyclonucleoside iron chelators is both convenient and cost effective compared to that of synthesizing ribonucleoside iron chelators. The X-ray crystal structural analysis shows that the 2-hydroxyethoxymethyl group does not affect the geometry of the iron chelating sites. Therefore, the iron binding and removal properties of the acyclonucleoside iron chelators should remain similar to the ribonucleoside iron chelators, which is confirmed by the titration and competition reaction of the acyclonucleoside chelators with iron and ferritin, respectively. The acyclonucleoside iron chelators are more lipophilic with measured n-octanol and Tris buffer distribution coefficients than ribonucleoside iron chelators.

Key Words: Acyclonucleoside; Pyridinones; Iron chelators; Desferrioxamine; Therapeutics.

*Correspondence: Gang Liu, Radiobiology Division, 729 Arapeen Dr. Suite 2334, University of Utah, Salt Lake City, UT 84108, USA; E-mail: gang.liu@m.cc.utah.edu.

DOI: 10.1081/NCN-120030718 1525-7770 (Print); 1532-2335 (Online)

INTRODUCTION

A milestone in iron chelation therapy was the development of desferrioxamine (DFO), a hexadentate iron chelator, to treat iron overload diseases which affect millions of people worldwide.[1] DFO results in significant iron excretion and leads to great improvement in both the quality of life and the life span of iron overload patients. Unfortunately, DFO has a number of drawbacks. It is not orally absorbed and requires extended subcutaneous administration, which leads to poor patient compliance. It is also expensive. These factors limit its widespread use.[2] Another iron chelator in clinical use outside the US is 1,2-dimethyl-3-hydroxy-4-pyridinone (L1). Although L1 is orally effective and shows high iron chelation efficacy, it has toxic side effects that have hampered its clinical application.[3] There is, moreover, some controversy regarding its efficacy in reducing tissue iron stores in iron-overload patients.[4] Therefore, there is a great need to develop new orally effective, low toxicity and inexpensive iron chelators that can be used for the clinical treatment of hereditary and transfusion iron overload diseases. Furthermore, because iron plays an important role in the regulation of many cellular functions, manipulating iron levels in particular biological compartments can have a profound effect on certain disease processes. Therefore, the search for new iron chelators with different biological properties may lead to efficient drugs for use against some other diseases,[5–7] including some cancers, malaria, and free radical-mediated injury.

Increasing the lipophilic nature of chelators is often utilized to improve oral bioavailability and other biological properties. However, this often increases the toxicity of the chelators.[8] Keeping the optimal balance of oral activity and low toxicity by adjusting the lipophilicity of chelators is very difficult in iron chelator design. Therefore, exploration of other strategies is indicated.

Recently, we developed a new class of ribonucleoside iron chelators[9,10] (Figure 1, Ia,b) which is potentially orally active with reduced toxicity. The riboside groups decreased the lipophilic nature that may be the cause of chelator toxicity. Moreover, this decrement may not affect the oral activity and cell membrane penetration because, as a functional group, the riboside plays a role in carrying the chelation moiety across the cell membrane through sugar-transport systems. However, when dealing with ribosides, the multiple synthetic steps and the cost of materials are a problem because efficient synthesis and affordable price are very important if a drug is to be for worldwide patient use. To overcome these drawbacks and retain the biological activities of the ribonucleoside iron chelators as well as to investigate other possible

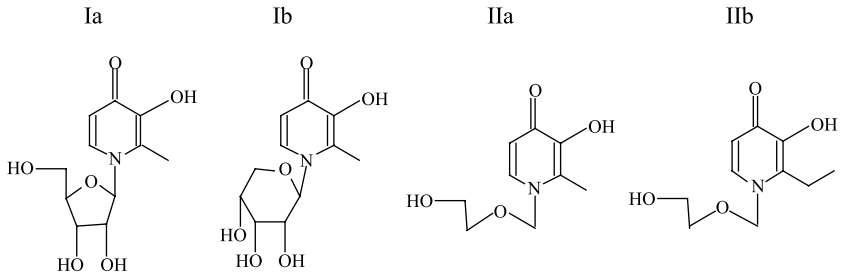


Figure 1. Chemical structures of ribonucleoside (Ia,b) and acyclonucleoside (IIa,b) iron chelators.

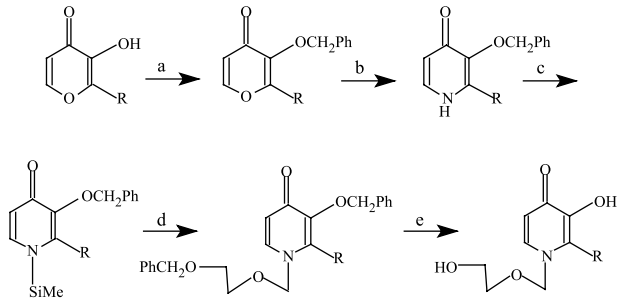
MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

functional groups for facilitating transmembrane shuttles, we have designed and synthesized so called acyclonucleoside analogues in which the ribosides were replaced by a (2-hydroxyethoxy)methyl group (Figure 1, IIa,b). Our motivation was the evidence that acyclonucleosides with the replacement of (2-hydroxyethoxy)methyl group may still retain some biological properties of the parent ribonucleosides.[11] Also, the synthetic method is simplified and the cost is reduced. Obviously, it is very desirable that the biological and pharmacological properties and the molecular structure of acyclonucleoside iron chelators are examined and compared with those of ribonucleoside iron chelators and established iron chelators. This will provide insights into acyclonucleoside iron chelators as orally active, low toxicity, and inexpensive iron chelation agents, and into the design of new iron chelators of ribonucleoside and acyclonucleoside analogues with optimal biological and pharmacological properties.

RESULTS AND DISCUSSION

The acyclonucleoside iron chelators were synthesized using the procedure as described in the experimental section and in detail as previously reported.[9,12] In Scheme 1, the alkyl-3-benzyloxyl-4-pyridinone was prepared according to the method in Ref. [13] with a minor modification; the mixture of 2-alkyl-3-benzyloxyl-4-pyranone and aqueous ammonia was stirred at room temperature for 48 h instead of refluxing for 18 h to avoid the violent evaporation of ammonia by heating. In step d of Scheme 1, benzyloxyethoxymethylchloride[11] could be replaced by (2-acetoxyethoxy)methyl bromide[14] and $SnCl_4$ could be used as catalyst instead of trimethylsilyl trifluoromethanesulfonate, but these changes resulted in separation difficulties and low yields. In the last step, both of the protection benzyl groups were simultaneously removed by hydrogenation in the presence of Pd/C in aqueous ethanol. In contrast, in the synthesis of ribonucleoside iron chelators, two deprotection steps, one by hydrogenation and another by basic hydrolysis with ammonia, have to be used to remove the different protection groups of the benzyl group on the pyridinone ring and acetyl or benzoyl groups on the riboside ring, respectively.[9]

The reason for using a (2-hydroxyethoxy)methyl group to replace the ribosides for the preparation of these kinds of chelators is that it involves fewer synthetic steps with



Scheme 1. R = Me, Et. a: $PhCH_2Cl$/NaOH/refluxing/6 h. b: NH_4OH/r.t./48 h. c: Hexamethyldisilazane, chlorotrimethylsilane. d: Benzyloxyethoxymethylchloride, trimethylsilyl trifluoromethanesulfonate in 1,2-dichloroethane. e: H_2, Pd/C, AcOH in 95% EtOH.

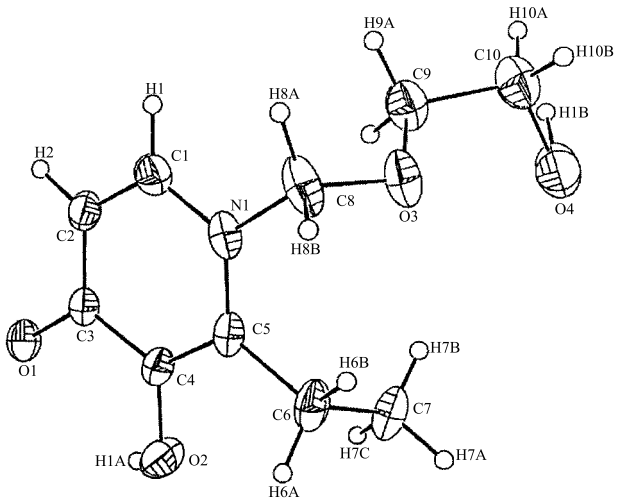


Figure 2. ORTEP stereoview of chelator IIb.

less expensive starting materials. Moreover, this substitution may retain the biological properties of the parent ribonucleoside iron chelators presumably because of the superposition of the conformations of acyclonucleosides and ribonucleosides with respect to the positions of C-1′, ether oxygen, C-4′, C-5′, and the 5′-OH.[11] Of interest are studies which, in some cases, show that this replacement decreased the toxicity of original nucleosides.[15–17] Furthermore, the terminal hydroxyl group in the (2-hydroxyethoxy)-methyl side chain plays a role in preventing the chelators from entering the brain,[18] hence lowering the neurotoxicity.

X-ray crystallographic analysis was performed in this study to determine the molecular and crystal structures of IIb. An ORTEP stereoview of the molecular structure of IIb is depicted in Figure 2. This analysis provided important information about iron binding sites and some pharmacological properties of this class of chelators.

The X-ray crystallographic analysis showed that the C3–O1, C4–O2, and C3–C4 bond distances of iron binding site in IIb are 1.302 (3) Å, 1.350 (3) Å, and 1.418 (4) Å, respectively. In comparison with the corresponding bond distances of 1.333 (2) Å, 1.346 (2) Å, and 1.406 (2) Å in CP94 (1,2-diethyl-3-hydroxyl-4-pyridinone),[13] it is apparent that the C3–O1 carbonyl bond distance is about 0.029 Å shorter. But in contrast, the corresponding bond distances in 1,2-dimethyl substituted congener, L1 are 1.271 Å, 1.364 Å, and 1.438 Å;[19] the C3–O1 carbonyl bond distance is about 0.031 Å longer. The ribonucleoside iron chelator of Ib has similar bond lengths of C3–O1, C4–O2, and C3–C4 (1.269 (6) Å, 1.361 (6) Å, and 1.428 (7) Å)[10] to the corresponding bond lengths in L1. These phenomena can be explained with electronic effects of the substitution groups on the 1 and 2 positions of the pyridinone ring. The ethyl group is a stronger electron donor group than the methyl group. There are two ethyl groups in CP94, one in IIb, and none in L1 and Ib. The methyl, riboside, and (2-hydroxyethoxy)methyl groups may have the similar electronic effects. The carbonyl group is an electron withdrawing group. The more electronic density on the pyridinone ring (CP94 > IIb > L1 and Ib), the longer the carbonyl bond length should be (1.333 (2) Å

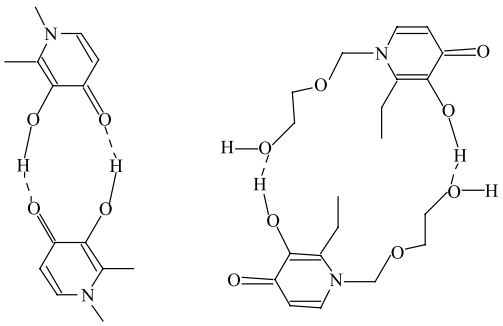


Figure 3. Centrosymmetric dimeric ring structures of L1 and IIb.

in CP94 > 1.302 (3) Å in IIb > 1.271 Å in L1 and 1.269 (6) Å in Ib). All of the four-carbonyl bond lengths are significantly longer than a pure carbonyl bond (1.210 Å),[13] which results in a partial negative charge on the oxygen atom and thus favors for chelation. Interestingly, the carbonyl bond distance of IIb is the closest one to that of the iron-L1 complex.[20] In the complex the corresponding carbonyl bond length is 1.299(8) Å. The C4–O2 and C3–C4 bond lengths also compare very well with the corresponding bond lengths of 1.342(7) Å, and 1.408(7) Å in the iron-L1 complex.[20] These observations indicated that replacement of the 2-hydroxyethoxymethyl group does not affect the iron binding geometry of the chelator and that the iron binding sites of IIb may be more favorable for iron chelation than other chelators because of the preorganization effect.[21] The bond distances in the pyridinone ring of IIb are 1.351 (4) Å for C1–C2, 1.391 (4) Å for C2–C3, 1.418 (4) Å for C3–C4, 1.376 (4) Å for C4–C5, 1.353 (4) Å for C1–N, and 1.369 (4) Å for C5–N. These are comparable with the corresponding bond lengths determined for iron-L1 complex (1.346 (10) Å, 1.423 (8) Å, 1.408 (7) Å, 1.382 (9) Å, 1.365 (7) Å, and 1.364 (7) Å).[20]

Table 1. Bond lengths (Å) and angles of IIb (°).

O(1)–C(3)	1.302(3)	O(2)–C(4)	1.350(3)	O(3)–C(8)	1.393(4)
O(3)–C(9)	1.428(4)	O(4)–C(10)	1.388(4)	N(1)–C(1)	1.353(4)
N(1)–C(5)	1.369(4)	N(1)–C(8)	1.493(4)	C(1)–C(2)	1.351(4)
C(2)–C(3)	1.391(4)	C(3)–C(4)	1.418(4)	C(4)–C(5)	1.376(4)
C(5)–C(6)	1.500(4)	C(6)–C(7)	1.527(5)	C(9)–C(10)	1.487(4)
C(8)–O(3)–C(9)	114.1(3)	C(1)–N(1)–C(5)	120.3(2)		
C(1)–N(1)–C(8)	117.7(3)	C(5)–N(1)–C(8)	122.0(3)		
C(2)–C(1)–N(1)	122.5(3)	C(1)–C(2)–C(3)	120.1(3)		
O(1)–C(3)–C(2)	123.9(2)	O(1)–C(3)–C(4)	119.3(2)		
C(2)–C(3)–C(4)	116.7(2)	O(2)–C(4)–C(5)	117.5(2)		
O(2)–C(4)–C(3)	120.7(2)	C(5)–C(4)–C(3)	121.7(3)		
N(1)–C(5)–C(4)	118.5(2)	N(1)–C(5)–C(6)	121.7(2)		
C(4)–C(5)–C(6)	119.7(3)	C(5)–C(6)–C(7)	111.9(3)		
O(3)–C(8)–N(1)	112.5(2)	O(3)–C(9)–C(10)	110.1(3)		
O(4)–C(10)–C(9)	109.3(3)				

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

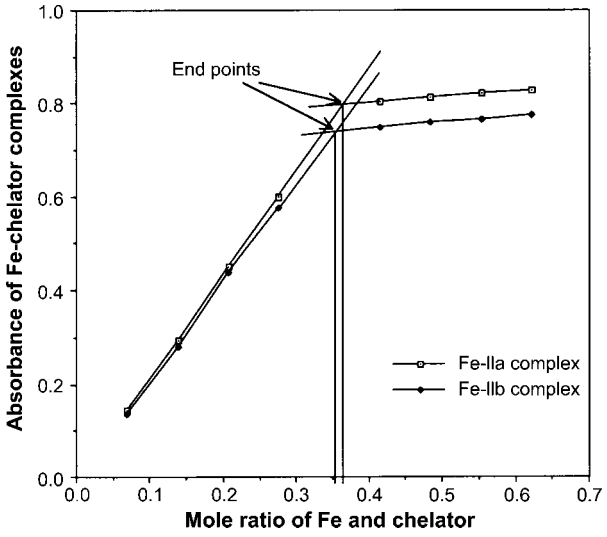


Figure 4. Titration of IIa and IIb with iron.

It is also interesting to see that the crystal structure of IIb exhibits centrosymmetric hydrogen bonded dimeric units, but it is unlike that known in most other hydroxypyridinone chelators[13] (Figure 3). The hydrogen (H1A) of the hydroxyl group on the pyridinone ring forms a hydrogen bond with the oxygen (O4) on the tail of the side chain with a 1.94 (5) Å bond length and a 164 (3) ° hydrogen bond angle, resulting in a H-bonding linked ring (Figure 3). The rings formed a cylinder with the ethyl groups inside and the free carbonyl groups and oxygen atoms outside. In each unit cell there are two IIb and one-water molecules. Studies showed that water incorporation into a solid form influenced the intermolecular interactions and the crystalline disorder, hence altering the thermodynamic activity of the solid.[22] All bond lengths and angles of IIb were presented in Table 1.

The acyclonuleoside chelators of IIa,b reacted with iron (III) forming purple complexes (λ_{max} = 456 nm with ε 4.6 × 10^3 for IIa and λ_{max} = 459 nm with ε 4.45 × 10^3 for IIb) which have the chelator:iron stoichiometry of 3:1 (Figure 4). This was demonstrated spectrophotometrically by titration of the chelators with iron in Tris buffer (25 mM, pH 7.5, 22°C).[23] Also, the titration result revealed that the oxygen atoms of hydroxyl and ether groups in the side chain are not involved with the iron complexation under physiological conditions. It is consistent with ribonucleoside iron chelators that the oxygen atoms on the riboside rings do not chelate iron. This study confirmed that both the ribonucleoside and acyclonucleoside chelators formed non-charge complexes with iron. The non-charge forms of the free chelators and iron-chelator complexes are easily absorbed and excreted through the cell membrane.[8]

The acyclonuleoside iron chelators of IIa,b, like the ribonucleoside iron chelators of Ia,b,[10] removed iron from ferritin in a slow reaction. Ferritin is an important protein for iron storage under physiological conditions. The results of the reactions of the chelators (IIa,b and DFO) with ferritin were monitored by the increase in absorbance of

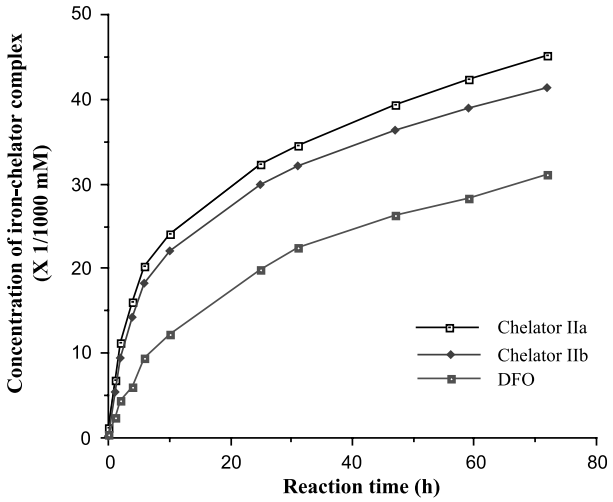


Figure 5. Removal of iron from ferritin by the acyclonucleoside iron chelators and DFO. (*View this art in color at www.dekker.com.*)

the iron-chelator complexes at the wavelength regions of the λ_{max} (Figure 5).[10,24] The formation of iron-chelator complexes in all the reactions indicated a common kinetic mobilization pattern that was characterized by an initial rapid iron removal, followed by a slower iron removal rate and finally the end of the reaction as judged from stable absorbance. Both the acyclonucleoside and ribonucleoside iron chelators[10] were faster and more effective at removing iron from ferritin than DFO. This difference may result from the different sizes of the chelators or their iron complexes. The steric hindrances due to the size of the chelators or their iron complexes compared with the size of the iron mobile channels in ferritin may affect the effectiveness of iron removal. Thus small iron chelators may be better able to remove iron from ferritin.[24]

The distribution coefficients (DC) of the acyclo and ribonucleoside iron chelators, measured in n-octanol in a Tris buffer system[25] showed that acyclonucleoside iron chelators have higher DC than ribonucleoside iron chelators (Table 2). This was expected because of the multihydroxyl groups in the ribonucleoside iron chelators. However, the acyclonucleoside iron chelators were dissolved more easily in Tris buffer than the ribonucleoside iron chelators. This is because the multiple hydroxyl groups in the ribonucleoside iron chelators form intermolecular hydrogen bonds that crosslink the molecules forming polymer clusters.[10] This formation slowed the dissolution of ribonucleoside iron chelators in aqueous solution. In contrast, the acyclonucleoside iron chelators form centrosymmetric hydrogen bonded dimeric units which shield the lipophilic ethyl group inside but leave the hydrophilic ketone part outside, resulting in

Table 2. Data of DCs for iron chelators.

Free chelator	Ia	Ib	IIa	IIb	L1	CP94 (25)
DCs	0.05	0.04	0.10	0.26	0.21	0.85

good water solubility. Comparing the DC of the acyclonucleoside iron chelators of IIa with L1 and IIb with CP94 (Table 2), the replacement of the 2-hydroxyethoxymethyl group decreased the lipophilic nature of their parent chelators, respectively, which may decrease their toxicity. Although the oral absorption and iron removal by L1 and CP94 are proportionately correlated with their DC, the good water solubility and appreciable lipid solubility of acyclonucleoside iron chelators allow them to permeate through cell membranes by both aqueous and non-aqueous routes. In this case, the (2-hydroxyethoxy)methyl group serves as a carrier[26–28] similar to the ribosides in ribonucleoside iron chelators. Furthermore, the introduction of the terminal hydroxyl group of the side chain in acyclonucleoside iron chelators may limit their penetration through the blood–brain barrier, hence decreasing their neurotoxicity.[18] Also, this hydroxyl group may limit the formation of non-chelating glucuronide metabolites (this impedes the efficacy of iron chelation in vivo[29]) because the glucuronide reaction may take place with the terminal hydroxyl group hence leaving the hydroxyl group of iron binding site active like ribonucleoside iron chelators.[10]

EXPERIMENT

Materials and Instruments. Chemicals used in the experiment were of analytical grade or better. Melting points of chelators were determined on a Fisher–Johns apparatus without correction. The analyses of X-ray crystal and molecular structures of chelators were performed on an Enraf-Nonius CAD-4 diffractometer. ^{1}H-NMR spectra were recorded at 200 MHz with an IBM NR 200 spectrometer. Mass spectra were recorded on a VG ANALYTICAL 70-SEQ instrument. UV-vis spectra were recorded on a Beckman DU-60. Thin layer chromatography was done on Sigma acid-washed silica gel with a 254 nm fluorescent indicator on polyester plates. Column chromatography was performed with Aldrich silica gel (70–230 mesh). Elemental analyses were carried out by Desert Analytic Organic Microanalysis Inc (Tucson, Arizona).

Preparation of Ribonucleoside and Acyclonucleoside Iron Chelators. The ribonucleoside and acyclonucleoside iron chelators were synthesized using the procedure described in Refs. [9,12]. Briefly, to protect the hydroxyl group, 2-alkyl-3-hydroxy-4-pyranone reacted with benzyl chloride in aqueous methanol solution containing sodium hydroxide under refluxing for six hours to obtain 2-alkyl-3-benzyloxyl-4-pyranone in 80% yield after recrystalization.[13] Then, the mixture of 2-alkyl-3-benzyloxyl-4-pyranone and aqueous ammonia was stirred at room temperature for 48 h and yielded 2-alkyl-3-benzyloxyl-4-pyridinone (93% yield). A Hilbert–Johnson type reaction modified by Niedballa and Vorbrueggen[30] was used to link the 2-alkyl-3-benzyloxyl-4-pyridinone moiety and the (2-hydroxyethoxy)methyl group as well as ribosides. First, 2-alkyl-3-benzyloxyl-4-pyridinone was silylated in hexamethyldisilazane under refluxing for 2h and then alkylated by using benzyloxyethoxymethylchloride[11] or ribosides at room temperature for 4h with trimethylsilyl trifluoromethanesulfonate as a catalyst. The yields were 95% and 89% for benzyloxyethoxymethyl and riboside alkylation, respectively.[9,12] $SnCl_4$ was also used as a catalyst instead of trimethylsilyl trifluoromethanesulfonate in the alkylation reaction but this resulted in separation difficulties and low yields, about 50 to 60%.[12] Both of the protection benzyl groups were simultaneously removed by hydrogenation in the

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

presence of Pd/C in aqueous ethanol for the preparation of IIa, b, with 95% yields. For the synthesis of Ia, b, in contrast, two deprotection steps, one by hydrogenation and another one by basic hydrolysis with ammonia, have to be employed to remove the different protection groups of the benzyl group on the pyridinone ring and acetyl or benzoyl groups on the riboside ring, respectively.[9] The total yield was larger than 90%. The chelators were characterized by ^{1}H-NMR, MS, IR, UV and elemental analyses.

Single Crystal X-Ray Diffraction Analysis of IIb. The IIb was crystallized from a solution of MeOH and ethyl acetate and formed colorless crystals. A piece of crystal (0.33 × 0.33 × 0.11 mm) was used for X-ray measurement. All diffraction data were taken on an Enraf-Nonius CAD-4 diffractometer equipped with a graphite monochromator of Mo Kα (0.71073 Å). The intensities were collected in the ω-2θ ($2.36° < 2\theta < 24.98°$) scan mode at 291 K. The structure of IIb was determined by the direct method using SHELXS and refined by full-matrix least-squares minimization on F^2. A summary of the crystal data and final refinement results of IIb was presented in Table 3.

Determination of the Stoichiometry of Iron-Chelator Complexes. The stoichiometry of iron-IIa and iron-IIb Complexes was determined spectrophotometrically by titration of the chelators in 2.3 mL (0.474 mM) of Tris buffer (25 mM, pH 7.5) solution

Table 3. Crystal data and final refinement results for IIb.

Chemical formula	$C_{10}H_{17}NO_5$
Formula weight	231.25
Crystal system	Triclinic
Space group	P-1
Unit cell dimensions	
a, Å	7.626 (2)
b, Å	8.607 (3)
c, Å	8.802 (3)
Alpha, °	88.36 (3)
Beta, °	101.46 (3)
Gamma, °	93.55 (3)
Volume, A^3	565.0 (3)
Z	2
Density, g/cm^3	1.359
Absorption coeff, mm^{-1}	0.109
F (000)	248
Index ranges	*h*0 to 9
	K−10 to 10
	l−10 to 10
Data/Restraints/Parameters	1978/0/149
Reflections collected	2144
Independent reflections	1978 [R(int) = 0.0534]
Goodness-of-fit on F^2	1.052
Final R indices [I > 2sigma (I)]	R_1 = 0.0585
	wR_2 = 0.1584
R indices [all data]	R_1 = 0.0732
	wR_2 = 0.1725

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

with freshly prepared iron solution (15.1 mM) at room temperature according to Ref. [23]. The iron solution was added gradually (5 μL increments). The change in absorbance of the iron-chelator complex was monitored by UV-vis spectrophotometer at the wavelength of the λ_{max} of the iron-chelator complex (λ_{max} of IIa: 456 nm, λ_{max} of IIb: 459 nm) after the chelating reaction reached equilibrium. The effect of dilution in titration[23] was considered in constructing the graph in Figure 4.

Studies of Iron Mobilization from Ferritin by Chelators. The removal of iron from ferritin was studied by incubation of horse spleen ferritin (Sigma, 100 mg/mL) with chelators in Tris buffer (25 mM, pH 7.5). Each chelator in 2.3 mL Tris buffer (0.474 mM for IIa,b and 0.187mM for DFO) was mixed with 9.2 μL ferritin, respectively. The mixtures were incubated at 37°C. The changes in absorbance of the iron-chelator complexes were measured spectrophotometrically at different time intervals. The concentration of the iron-chelator complex was estimated from ε_{max} values (iron-IIa: ε 456 nm = 4.6 $\times$ 10^3, iron-IIb: ε 459 nm = 4.5 $\times$ 10^3, iron-DFO: ε 428 nm = 2.8 $\times$ 10^3) at the wavelength of the λ_{max} of the iron-chelator complex according to Ref. [24]. The kinetics of iron release were investigated for periods up to 72 h.

Determination of Distribution Coefficients. DCs were determined as the ratio of the concentration of chelators between n-octanol and Tris buffer (25 mM, pH 7.5) at room temperature using a shake-flask method similar to that described in Ref. [25]. The n-octanol and Tris buffer were preequilibrated together overnight before use. The systems were mixed by gently shaking for 2 h to reach equilibrium and then permitted to stand at room temperature for 6 h. After separation of the two phases, the concentrations of the chelators were measured spectrophotometrically from ε_{max} values (see above and Ref. [10]) of the chelators. Each sample was assayed in duplicate.

CONCLUSION

Compared to ribonucleoside iron chelators, the synthesis of acyclonucleoside iron chelators is convenient and cost effective. The replacement of the ribosides by the (2-hydroxyethoxy)methyl group did not change the iron binding and iron removal properties, which were demonstrated by single crystal X-ray diffraction analysis and the reactions of acyclonucleoside iron chelators with iron and an iron-containing protein. Both of the ribonucleoside and acyclonucleoside iron chelators possessed lower lipophilic nature than their parent chelators of L1 and CP94 did and hence, potentially lowered the toxicity. Although the lipophilicity proportionately correlated with the oral absorption and iron removal abilities of the parent chelators, these abilities of the ribonucleoside and acyclonucleoside iron chelators may not be affected by their decreased lipophilicity. This is because the ribonucleoside iron chelators may use the sugar-transport systems across the cell membrane while the acyclonucleoside iron chelators with good water solubility and appreciable lipid solubility allow them to permeate through cell membranes by both aqueous and non-aqueous routes. Moreover, the introduced hydroxyl groups in the side chain of ribonucleoside and acyclonucleoside iron chelators may prevent the chelators from converting to non-chelating metabolites. From these advantages the ribonucleoside and acyclonucleoside iron

chelators have good therapeutic potential for iron overload disease and perhaps other iron mediated diseases.

ACKNOWLEDGMENT

We are grateful for support of this research by Grants, AG21300-01 and NS044064-01A1, awarded by the National Institutes of Health.

REFERENCES

1. Martell, A.E.; Motekaitis, R.J.; Sun, Y.; Ma, R.; Welch, M.J.; Pajeau, T. New chelating agents suitable for the treatment of iron overload. Inorg. Chim. Acta **1999**, *291* (1–2), 238–246.
2. Gabutti, V.; Piga, A. Results of long-term iron-chelating therapy. Acta Haematol. **1996**, *95* (1), 26–36.
3. Hershko, C.; Konijn, A.M.; Link, G. Iron chelators for thalassaemia. Br. J. Haematol. **1998**, *101* (3), 399–406.
4. Olivieri, N.F.; Brittenham, G.M. Iron-chelating therapy and the treatment of thalassemia. Blood **1997**, *89* (3), 739–761.
5. Hershko, C. Control of disease by selective iron depletion: a novel therapeutic strategy utilizing iron chelators. Bailliere's Clin. Haematol. **1994**, *7* (4), 965–1000.
6. Kontoghiorghes, G.J.; Weinberg, E.D. Iron: mammalian defense systems, mechanisms of disease, and chelation therapy approaches. Blood Rev. **1995**, *9* (1), 33–45.
7. Richardson, D.R.; Ponka, P. Development of iron chelators to treat iron overload disease and their use as experimental tools to probe intracellular iron metabolism. Am. J. Hematol. **1998**, *58* (4), 299–305.
8. Porter, J.B.; Hider, R.C.; Huehns, E.R. Update on the hydroxypyridinone oral iron-chelating agents. Semin. Hematol. **1990**, *27* (2), 95–100.
9. Liu, G.; Bruenger, F.W.; Barrios, A.M.; Miller, S.C. Synthesis of 2-alkyl-3-hydroxy-4-pyridinone-ribonucleosides, potential oral iron chelators. Nucleosides Nucleotides **1995**, *14* (9&10), 1901–1904.
10. Liu, G.; Bruenger, F.W.; Miller, S.C.; Arif, A.M. Molecular structure and biological and pharmacological properties of 3-hydroxy-2-methyl-1-(β-D-ribofuranosyl or pyranosyl)-4-pyridinone: potential iron overload drugs for oral administration. Bioorg. Med. Chem. Lett. **1998**, *8* (21), 3077–3080.
11. Schaeffer, H.J.; Gurwara, S.; Vince, R.; Bittner, S. Novel substrate of adenosine deaminase. J. Med. Chem. **1971**, *14* (4), 367–369.
12. Liu, G.; Miller, S.C.; Bruenger, F.W. Efficient synthesis of n-[(2-hydroxyethoxy)methyl]-2-alkyl-3-hydroxy-4-pyridinone by a modified Hilbert–Johnson reaction. Synth. Commun. **1996**, *26* (14), 2681–2686.
13. Dobbin, P.S.; Hider, R.C.; Hall, A.D.; Taylor, P.D.; Sarpong, P.; Porter, J.B.; Xiao, G.; van der Helm, D. Synthesis, physicochemical 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* (17), 2448–2458.
14. Robins, M.J.; Hatfield, P.W. Nucleic acid related compounds. 37. Convenient and high-yield syntheses of N-[(2-hydroxyethoxy)methyl] heterocycles as ''acyclic nucleoside'' analogues. Can. J. Chem. **1982**, *60* (5), 547–553.
15. Miyasaka, T.; Tanaka, H.; Baba, M.; Hayakawa, H.; Walker, R.T.; Balzarini, J.; De Clercq, E. A novel lead for specific anti-HIV-1 agents: 1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)thymine. J. Med. Chem. **1989**, *32* (12), 2507–2509.
16. Baba, M.; Tanaka, H.; De Clercq, E.; Pauwels, R.; Balzarini, J.; Schols, D.; Nakashima, H.; Perno, C.F.; Walker, R.T.; Miyasaka, T. Highly specific inhibition of human immunodeficiency virus type 1 by a novel 6-substituted acyclouridine derivative. Biochem. Biophys. Res. Commun. **1989**, *165* (3), 1375–1381.
17. Elion, G.B.; Furman, P.A.; Fyfe, J.A.; de Miranda, P.; Beauchamp, L.; Schaeffer, H.J. Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl) guanine. Proc. Natl. Acad. Sci. U. S. A. **1977**, *74* (12), 5716–5720.
18. Habgood, M.D.; Liu, Z.D.; Dehkordi, L.S.; Khodr, H.H.; Abbott, J.; Hider, R.C. Investigation into the correlation between the structure of hydroxypyridinones and blood–brain barrier permeability. Biochem. Pharmacol. **1999**, *57* (11), 1305–1310.
19. Hider, R.C.; Taylor, P.D.; Walkinshaw, M.; Wang, J.L.; van der Helm, D. Crystal structure of 3-hydroxy-1,2-dimethyl-pyridin-4(1H)-one: an iron (III) chelator study. J. Chem. Res., Synop. **1990** (10), 316–317.
20. 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* (21), 2235–2237.
21. Xiao, G.Y.; van der Helm, D.; Hider, R.C.; Rai, B.L. Molecular modeling studies of a ferric hexadentate 3-hydroxy-2(1H)-pyridinone complex and an analogue by molecular mechanics, molecular dynamics, and free energy perturbation simulations. J. Phys. Chem. **1996**, *100* (6), 2345–2352.
22. Ledwidge, M.T.; Draper, S.M.; Wilcock, D.J.; Corrigan, O.I. Physicochemical characterization of diclofenac N-(2-hydroxyethyl)pyrrolidine: anhydrate and dihydrate crystalline forms. J. Pharm. Sci. **1996**, *85* (1), 16–21.
23. Harris, D.C. *Quantitative Chemical Analysis*; W.H. Freeman and Company: New York, 1991; 523–524.
24. Kontoghiorghes, G.J. Iron mobilization from ferritin using α-oxohydroxy hetero-aromatic chelators. Biochem. J. **1986**, *233* (1), 299–302.
25. Porter, J.B.; Gyparaki, M.; Burke, L.C.; Huehns, E.R.; Sarpong, P.; Saez, V.; Hider, R.C. Iron mobilization from hepatocyte monolayer cultures by chelators: the importance of membrane permeability and the iron-binding constant. Blood **1988**, *72* (5), 1497–1503.
26. Hider, R.C.; Porter, J.B.; Singh, S. The design of therapeutically useful iron chelator. In *The Development of Iron Chelators for Clinical Use*; Bergeron, R.J., Brittenham, G.M., Eds.; CRC: Boca Raton, 1994; 354–371.
27. Maxton, D.G.; Bjarnason, I.; Reynolds, A.P.; Catt, S.D.; Peters, T.J.; Menzies, I.S. Lactulose, ^{51}Cr-labelled ethylenediaminetetra-acetate, L-rhamnose and polyethyleneglycol 400 [corrected] as probe markers for assessment in vivo of human intestinal permeability. Clin. Sci. **1986**, *71* (1), 71–80.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

28. Travis, S.; Menzies, I. Intestinal permeability: functional assessment and significance. Clin. Sci. **1992**, *82* (5), 471–488.
29. Hider, R.C.; Choudhury, R.; Rai, B.L.; Dehkordi, L.S.; Singh, S. Design of orally active iron chelators. Acta Haematol. **1996**, *95* (1), 6–12.
30. Niedballa, U.; Vorbrueggen, H. A general synthesis of pyrimidine nucleosides. Angew. Chem., Int. Ed. **1970**, *9* (6), 461–462.

Received July 3, 2003
Accepted November 26, 2003

Request Permission or Order Reprints Instantly!