and the filtrate was washed with cold water and hot acetone to give 0.37 g (28%) of tan solid: mp 275–276 °C; IR (KBr) 2200 (C≡N), 1610 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.5 (3 H, s, CH_3), 7.25–8.5 (4 H, m, aromatic and C=C*H*N), 12.4 (1 H, br, N*H*).

6-Cyano-3-(trifluoroacetyl)indole (precursor to compound 12) was synthesized in the same manner: yield 39% mp 230–235 °C; IR (KBr) 3300 (NH), 2225 (C≡N), 1620 (C=O), 1200 and 1250 (CF_3) cm^{-1}.

6-Cyano-3-benzoylindole (Precursor to Compound 13). To a cold, stirring solution of 6-cyanoindole (1.0 g, 7 mmol) and benzoyl chloride (1.7 g, 14 mmol) was added dropwise stannic chloride (3.6 g, 14 mmol) in 10 mL of benzene. After stirring for 1 h, the mixture was poured onto 125 mL of ice–water and stirred for an additional 30 min. A solid was collected, washed with H_2O, dried, and recrystallized in ethanol to yield 0.4 g (23%) of buff solid: mp 263–265 °C; IR (KBr) 3100 (NH), 2210 (C≡N), 1600 (C=O) cm^{-1}; ^1H NMR δ 7.5–8.5 (9 H, m, aromatic and C=C*H*N), 12.5 (1 H, br, NH).

The precursor to compound 14 is a known compound and was synthesized according to a published method.[21]

Acknowledgment. The authors are grateful to Deborah Simpson, Sammy Shaver, and Richard Lombardy for their skillful technical assistance. This study was supported by Public Health Service Grants HL 21540 and HL 191771-06, by a grant-in-aid from the American Heart Association, and with funds contributed in part by the North Carolina Heart Association.

Registry No. 1, 74733-75-8; 2, 83783-17-9; 3, 40069-57-6; 4, 40069-58-7; 5, 40069-59-8; 6, 66639-21-2; 7, 71889-75-3; 8, 71889-74-2; 9, 83783-18-0; 9·2HCl, 83783-37-3; 10, 83783-19-1; 10·HCl, 83783-38-4; 11, 83801-88-1; 11·2HCl, 83783-39-5; 12, 83783-20-4; 12·HCl, 83783-40-8; 13, 83783-21-5; 13·HCl, 83783-41-9; 14, 83783-22-6; 14·HCl, 83783-42-0; 15, 77838-88-1; 16, 80531-08-4; 17, 80531-09-5; 18, 83783-23-7; 18·HCl, 83783-43-1; 19, 83783-24-8; 19·HCl, 83783-44-2; 20, 80531-07-3; 20·HCl, 80531-17-5; 21, 80531-10-8; 22, 83783-25-9; 22·HCl, 83783-45-3; 23, 83783-26-0; 23·HCl, 83801-89-2; 24, 83783-27-1; 24·4HCl, 83783-46-4; 5-cyano-1-ethylindole, 83783-28-2; 5-cyanoindole, 15861-24-2; ethyl iodide, 75-03-6; 5-cyano-1-(3-nitrobenzyl)indole, 83783-29-3; *m*-nitrobenzyl bromide, 3958-57-4; 1-(4-cyanobenzyl)indole, 83783-30-6; 4-cyanobenzyl bromide, 17201-43-3; indole, 120-72-9; 1-benzyl-5-cyanoindole, 80531-13-1; benzyl bromide, 100-39-0; 1-(4-cyanobenzyl)-5-nitroindole, 83783-31-7; 5-nitroindole, 6146-52-7; 1,4-bis(5-cyano-1-indolyl)butane, 83783-32-8; 1,4-diiodobutane, 628-21-7; 6-cyano-3-indolecarboxyaldehyde, 83783-33-9; 6-cyanoindole, 15861-36-6; 3-acetyl-6-cyanoindole, 83783-34-0; acetyl chloride, 75-36-5; 6-cyano-3-(trifluoroacetyl)indole, 83783-35-1; trifluoroacetyl chloride, 354-32-5; 6-cyano-3-benzoylindole, 83783-36-2; benzoyl chloride, 98-88-4; plasmin, 9001-90-5; urokinase, 9039-53-6; thrombin, 9002-04-4; trypsin, 9002-07-7.

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Syntheses of Iron Bis(pyridoxal isonicotinoylhydrazone)s and the in Vivo Iron-Removal Properties of Some Pyridoxal Derivatives

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Pyridoxal isonicotinoylhydrazone (PINH; 1) and its isomeric *O*-acetates (*E* and *Z*) were synthesized and complexed with ferrous ions to afford the hitherto unisolated chelates iron(II) bis(pyridoxal isonicotinoylhydrazone)s (11) and iron(II) bis(*O*-acetylpyridoxal isonicotinoylhydrazone)s (12). The analytical and spectroscopic data of the new coordination compounds are presented. In addition, a series of imino derivatives of pyridoxal of structures 2–3 and 5–10 have been prepared and tested in vivo as chelators of storage iron, and the cumulative net excretion of radioiron in urine and in feces was estimated. This study reestablishes that PINH is a potent iron chelator in vivo comparable in efficiency with parenteral desferrioxamine (DF) and indicates that it requires further attention.

Synthetic chemicals containing the pyridoxal moiety exhibit a large variety of biological activities of great importance. One of the most interesting aspects of pyridoxal derivatives is their effect on blood diseases and, in particular, on *thalassemia* and other chronic *anemias* resulting in abnormal accumulation of iron in tissues, which causes severe damage to vital organs.

The only practical method of iron mobilization in such diseases is the use of iron-chelating drugs, of which the commercially available agent desferrioxamine (DF)[1] is distinguished by its lack of significant toxicity. However, because of its limited gastrointestinal absorption and its high cost, current interest is focused on the development of new iron-chelating agents that might be better suited for large-scale clinical use.

We report here the synthesis of some pyridoxylidenimino derivatives, comprising pyridoxal acylhydrazones (1–4), a semicarbazone (5), alkoxylcarbonylhydrazones (6–8), and thiazolylhydrazones (9 and 10), for in vivo iron-chelation studies and the isolation of crystalline iron(II) complexes 11 and 12 generated from 1 and 4, respectively. The binding constants to iron in 11 and 12 were derived from their spectrophotometric studies.

These studies aimed at shedding more light on structure–activity relationships in the new type of iron chelators related to 1. It was undertaken with a view to develop a new synthetic approach to chelation therapy.

The ability of pyridoxal isonicotinoylhydrazone (1, PINH)[2-5] to mobilize iron from both parenchymal and

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‡ Sha're Zedek Medical Center.

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(7) H. C. Beyerman, Y. S. Bontenkoe, Y. Van der Burg, and W. Le Vaer [*Recl. Trav. Chim. Pays-Bas*, **73**, 109 (1954)] gave mp 257 °C for PINH monohydrate. They did not report an analysis for chlorine.

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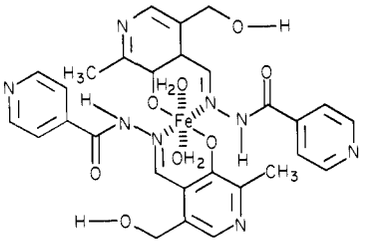
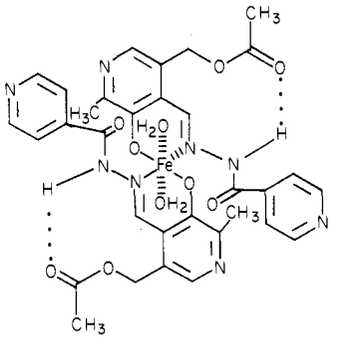


11



12

reticulocytal iron stores and to secrete the chelated metal through the gut was adequately demonstrated when given orally to normal and hypertransfused rats. The oral efficiency of the free ligand (PINH) was compared with parenteral desferrioxamine (DF).[10] We describe here the formation, isolation, structure determination, and stability constants of the iron complexes of 1 and 4.

PINH was obtained as a *E–Z* isomeric mixture. The photosensitive isomer (*Z*, colorless to bright yellow) converts (in the solid state) into the *E* isomer on exposure to daylight at room temperature. Thus, the PINH samples employed in the in vivo studies comprised, in fact, an isomeric mixture.

The acetylation of PINH (acetic anhydride–pyridine) provided the respective hitherto unknown monoacetates (*E*)- and (*Z*)-12. The presence of a free phenolic group in the latter followed from the strong red color in the $FeCl_3$ test. The colorless product (mp 186 °C) was assigned the *Z* geometry, whereas the yellow isomer (mp 164 °C) was assigned the *E* configuration.

Compounds employed in this study were either in their free-base or salt form, since it was observed that their chelating power properties were independent of the form actually employed.

The in vivo chelating abilities of PINH and a series of compounds described here have been tested, together with the distribution of chelated ^{59}Fe in organs of normal rats. The in vivo chelating properties of pyridoxylidenimino derivatives, together with their effect on organ distribution and the excretion of the radioiron label, were compared with the same parameters in untreated control animals. They were injected with [^{59}Fe]transferrin (in one series of animals) and with [^{59}Fe]ferritin (in the other series of animals). Urinary radioiron excretion was less than 0.1%, and practically all of the excretion has been fecal.

Results and Discussion

Chemical Studies. Spectrophotometric Studies of Complex Formations with Pyridoxal Isonicotinoylhydrazone and *O*-Acetylpyridoxal Isonicotinoylhydrazone. Composition of the Complex. The composition of the complex was determined by Job's method of continuous variations.[11] The experimental data clearly indicate that under these conditions a complex containing iron and the ligands in the ratio of 1:2 is formed.

Effect of pH Change. The solution of 11 shows maximum absorption at a pH around 5.0 and decreases markedly if the pH is increased or decreased.

Stability Constants. The stability constants (*K*) were determined by following the technique of Foley and Anderson.[12] Pairs of solutions having the same optical densities (containing equal concentrations of the respective coordination compounds but different total concentrations) were used. The concentration of the complexes, and thus the values of *K*, may be calculated from the relation:

$$K_{\text{stability}} = \frac{x}{(a_1 - x)(b_1 - x)} = \frac{x}{(a_2 - x)(b_2 - x)}$$

where *a*'s and *b*'s denote initial concentration of Fe^{2+}–salt and the ligand, respectively, and *x* denotes the concentration of the complex.

The log $K_{\text{stability}}$ value calculated for 11 is log *K* = 8.67, and the respective value for 12 is log *K* = 5.75. We studied the stability constants of these complexes as a function of pH values by running the measurements in 2×10^{-5} M solutions. Maximum values were obtained at pH 5.5 in methanolic solutions.

Study has shown that 11 breaks down as the pH of the solution reaches a value of ~2. On the alkaline side, complex 11 demonstrates as much greater stability than in the acidic region, and it can be detected in pH as high as ~12. Because of its tendency to undergo facile alkaline hydrolysis, 12 was not amenable to a study of binding constant against pH variations.

All substrates included in this study exhibited IR absorptions (KBr) at 1645–1690 (amide I), 1520–1560 (amide II), 1580–1610 (CH=N), and 1720–1750 (ester C=O).

The free base 1 and the two hydrochlorides (**1a**,**b**) of PINH (see Experimental Section) differ in their solubility and in their pattern of absorption in the 1700–1500 cm^{-1} region of the IR spectra. On passing from 1 to **1a**, band frequencies both of amide I and of C=N increase by quantities of 10 cm^{-1}. In addition, the absorption intensity at 1600 cm^{-1} in **1b** increases dramatically, but this trend is totally reversed on passing to the hydrate species **1a**, where this bond becomes considerably weaker. In the UV, the band at 273 nm for **1b** is shifted to 299 nm on passing to **1a**, indicating strong involvement of amide carbonyls in hydration and in hydrogen bonding. Interestingly, the patterns of both band absorptions and intensities in the corresponding region of the IR spectra of (*E*)-4 and (*Z*)-4 are essentially the same.

Metal coordination to PINH changes more dramatically in the corresponding region of the IR spectrum. Amide I absorption at 1680 disappears in the IR spectrum of 11, and a considerably stronger but also broader band occupies

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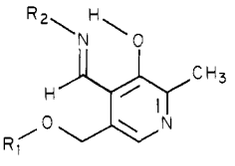
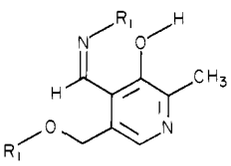
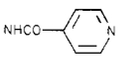
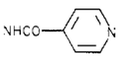
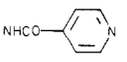
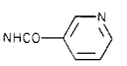
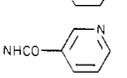
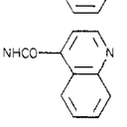
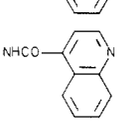
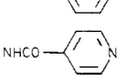
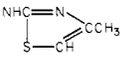
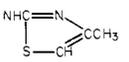
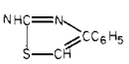
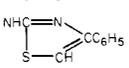
Table I. Pyridoxylidenimino Derivatives



E



Z

no.	R_1	R_2	yield, %	mp, °C	recrystn solvent	formula	anal.	MS M^+ (%)	MS base peak
1	H		78	255	MeOH	$C_{14}H_{14}N_4O_3$	C, H, N	286 (100)	286
1a	H		93	246	MeOH/ether	$C_{14}H_{17}N_4O_4Cl$	C, H, N, Cl	287 (50)	138, 150
1b	H		90	253	MeOH/ether	$C_{14}H_{14}N_4O_3$ 0.5HCl	C, H, N, Cl	287 (40)	106, 78
2	H		80	257	MeOH	$C_{14}H_{14}N_4O_3$	C, H, N	286 (35)	165, 137
2a	H		95	243	MeOH	$C_{14}H_{17}N_4O_4Cl$	C, H, N, Cl	287 (40)	165, 123
3	H		74	272	MeOH	$C_{18}H_{16}N_4O_3$	C, H, N	336 (28)	75
3a	H		97	257	MeOH	$C_{18}H_{19}N_4O_4Cl$	C, H, N, Cl	337 (35)	121, 77
(*Z*)-4	$COCH_3$		66	186	MeOH or EtOAc	$C_{16}H_{16}N\ O_4$	C, H, N	328 (55)	285, 165
6	H	$NHCOOC_2H_5$	78	202	EtOAc	$C_{11}H_{15}N_3O_4$	C, H, N	253 (100)	253
6a	H	$NHCOOC_2H_5$	68	242	MeOH	$C_{11}H_{16}N_3O_4Cl$	C, H, N, Cl	254 (100)	254
7	H	$NHCOOC(CH_3)_3$	78	254	MeOH or EtOAc	$C_{13}H_{19}N_3O_4$	C, H, N	281 (76)	207, 165, 150
7a	H	$NHCOOC(CH_3)_3$	44	180	MeOH/ether	$C_{13}H_{22}N_3O_5Cl$	C, H, N, Cl	282 (37)	225, 150
8	H	*p*-$NHCOOCH_2$·C_6H_4-OCH_3	94	207	MeOH	$C_{17}H_{20}N_3O_5Cl$	C, H, N, Cl	302 (25)	165, 122
9	H		77	272	MeOH	$C_{12}H_{14}N_4O_2S$	C, H, N	278 (76)	165
9a	H		92	289	MeOH/ether	$C_{12}H_{17}N_4O_3SCl$	C, H, N, Cl	279 (65)	165
10	H		80	239	MeOH	$C_{17}H_{20}N_4O_4S$	C, H, N, S	340 (48)	123
10a	H		91	298	MeOH/ether	$C_{17}H_{18}N_4O_4SCl$	C, H, N, S	341 (100)	341

the area between 1620 and 1580 cm^{-1}. Moreover, the amide II band was also shifted, appearing between 1520 and 1475 cm^{-1}. On passing to **12**, the IR absorption pattern of the amide bands seem to be similar to those in **11** with regard to intensities but are notably more sharper, giving absorptions at 1600–1615 and 1490–1505 cm^{-1}, respectively.

The UV spectrum of synthetic **11** in methanol is essentially identical with that resulting from complexation of ferrous ions with PINH in water at pH 7.4, reported earlier by Ponka and co-workers.[13,14]

The acylhydrazones (**1** and **4**) appear to function as tridentate ligands. The phenolic group, the double-bonded nitrogen, and the oxygen atom of the carbonyl group participate in metal binding.[15] They should be noted for their enhanced affinity for ferrous ions.

The stability constant of the PINH–Fe complex (log K = 8.67) is not as strong as the desferrioxamine–Fe complex, but, as will be shown under Biological Studies, its efficiency in chelating iron in vivo is quite comparable. Significantly, the in vivo iron-chelating efficiency of **4** decreases by a factor of 3, whereas the binding constant to the iron decreases only by a factor of 32 (see in the sequence).

Biological Studies. Table II shows the organ distribution and excretion of radioiron following intravenous injection of transferrin-bound ^{59}Fe for the control group and transferrin-bound ^{59}Fe preincubated with 10 mg of chelating agent.[10] The control group shows the typical distribution pattern of transferrin iron in normal rats with 28% radioactivity incorporated into newly formed erythrocytes and 12% into the hepatic parenchyma. Sponta-

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Table II. Organ Distribution and Excretion of Radioiron in Normal Rats Following Intravenous Injection of [^{59}Fe]Transferrin Preincubated with 10 mg of Chelating Agent

substrate	n	organ distribution				excretion	
		blood	liver	spleen	kidney	urine	feces
desferrioxamine	4	7.4 ± 1.6[a]	3.0 ± 0.4	0.2 ± 0.1	0.6 ± 0.1	62.9 ± 3.2	18.7 ± 2.4
1	4	32.0 ± 3.1	9.6 ± 1.3	0.4 ± 1	0.6 ± 0.3	4.3 ± 0.4	32.8 ± 5.9
2	4	39.6 ± 14.0	16.2 ± 5.3	0.9 ± 0.3	1.0 ± 0.1	0.3 ± 0.1	6.0 ± 0.3
3	4	27.9 ± 5.1	12.6 ± 1.4	0.6 ± 0.1	1.0 ± 0.1	0.4 ± 0	6.8 ± 0.3
8	2	27.0 ± 5.4	14.7 ± 3.6	0.6 ± 0.2	0.8 ± 0.2	0.02	3.3
4	4	31.7 ± 1.6	15.7 ± 2.7	0.6 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	10.4 ± 0.1
untreated controls	4	28.2 ± 8.3	12.4 ± 2.8	0.5 ± 0	0.9 ± 0.2	0.5	4.6 ± 0.5

[a] Plus or minus SD (percent of injected ^{59}Fe).

Table III. [^{59}Fe]Ferritin Excretion and Organ Distribution of Radioiron in Normal Rats Following Intravenous Injection of Chelating Compounds

substrate	n	organ distribution				excretion	
		blood	liver	spleen	kidney	urine	feces
desferrioxamine	2	1.8 ± 0.3[a]	71.3 ± 5.2	0.4 ± 0.0	0.3 ± 0.1	0.08	15.5
1	8	2.3 ± 0.6	78.7 ± 6.2	0.5 ± 0.1	0.3 ± 0.1	0.05	12.0 ± 2.8
5	2	12.3 ± 1.9	82.4 ± 0.9	1.0 ± 0.0	0.3 ± 0.0	0.09	4.1 ± 0.2
6	8	19.0 ± 4.6	63.8 ± 6.8	0.7 ± 0.4	0.4 ± 0.0	0.03	8.6 ± 2.3
7	2	19.4 ± 1.2	63.2 ± 0.3	0.55 ± 0.0	0.4 ± 0.0	0.08	7.3
9	2	19.1 ± 3.3	60.2 ± 3.6	0.8 ± 0.0	0.4 ± 0.0	0.01	6.5
10	6	19.6 ± 2.4	66.7 ± 6.0	0.6 ± 0.1	0.5 ± 0.1	0.04	8.2 ± 1.5
untreated controls	6	13.9 ± 4.4	70.9 ± 2.9	0.54 ± 0.15	0.5 ± 0.1	0.04	4.7 ± 1.5

[a] Plus or minus SD (percent of injected ^{59}Fe).

neous excretion of radioiron was limited and confined mainly to the feces.

Of the various compounds reported in Table II, only 1 and 4 induced iron excretion at substantially higher than control levels. The highest excretion was observed with PINH with 4.3 ± 0.4% urinary and 32.8 ± 5.9% fecal excretion.

Table III shows the organ distribution and excretion of radioiron in rats labeled with [^{59}Fe]ferritin. In contrast to [^{59}Fe]transferrin shown in Table II, [^{59}Fe]ferritin is located entirely in the hepatic parenchyma; therefore, drug-induced ^{59}Fe excretion in Table III represents hepatic storage iron excretion. Only compounds 1, **6**, and **10** elicited storage iron excretion substantially higher than in control rats. In contrast to Table II where drugs were preincubated with free radioiron, in Table III all drugs were given separately at a dose of 40 mg im following labeling of storage iron with ^{59}Fe.

A careful search for laboratory evidence of toxicity, including complete blood counts, renal function tests, serum proteins, and liver enzymes, revealed no abnormalities at any of the dose levels used.

Structural Effects. From Table II it can be seen that the conversion of PINH into its *O*-acetate (**4**) causes a 3-fold reduction in iron transfer from [^{59}Fe]transferrin to the iron mobilizer via excretion into feces, while the decrease in the complex to Fe(II) stability is 32-fold (Δ log $K_{stability} \approx 1.5078$).

We observed about a 6-fold decrease in the iron-chelating power of the acylhydrazone on going from **1** to **2**. On the other hand, Ponka and Wilczyska,[16] while utilizing reticulocytes with a high level of nonheme radioiron, observed a 2.9-fold reduction on going from 1 to **2**. Of particular interest are the carbonate hydrazones **6** and **7**, which are considerably more effective than **2** as an iron mobilizer and as effective as **4**.

Significant stimulation of iron release is evidently induced by compounds with an OH group adjacent to an aldehyde group as in pyridoxal-containing hydrazides of either isonicotinic, benzoic, or carbonic acids. The aldimine nitrogen appears also to play a role in iron binding.[15]

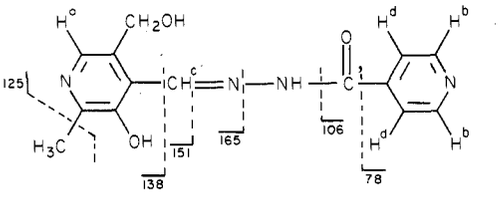
In conclusion, the most effective iron mobilizer found so far is pyridoxal isonicotinoylhydrazone (1). It compares with desferrioxamine (DF) as an iron mobilizer when given parenterally or orally.[10] Therefore, PINH may be much more convenient for clinical use than DF, which at present is the only commercially available iron-chelating drug and which is poorly absorbed from the gastrointestinal tract. These encouraging results merit further attention both on the structure–activity relationship side and on the toxicological side in anticipation toward clinically useful substitutes for desferrioxamine.

Experimental Section

Chemical Studies. Infrared spectra were obtained with a Perkin-Elmer Model 457 Grating infrared spectrophotometer. Ultraviolet spectra were run on a Varian Techtron Model 635 UV–Vis spectrophotometer. Mass spectra were recorded with an LKB 90 21 spectrometer. ^{1}H NMR spectra were obtained on a WH-300 Bruker spectrophotometer with tetramethylsilane as an internal standard and methanol-d_3 or Me_2SO-d_6 (the following abbreviations are used: t, triplet; q, quartet; m, multiplet).

PINH (1) was prepared according to literature procedures.[2-5] The composition of the hydrochlorides **1a,b** previously described has been revised.[6,7]

Under electron impact, 1 and **1a,b** exhibit similar fragmentation patterns, commencing with fission of the weak nitrogen–nitrogen bond to yield ionic fragments m/e 165 and 106, followed by losses of N, CO, HCN, and CH_3 as indicated in the following formula:



The UV spectrum of **1a** shows bands (MeOH) at λ_{max} 215 nm (ϵ 19360), 305 (14320), 340 (7280). The corresponding bands for **1b** (MeOH) were 217 nm (19800), 293 (16800), 341 (9520). The ^{1}H NMR of **1a** (Me_2SO-d_6) exhibited resonances at δ 9.16 (1 H, s, H^a), 8.91 (2 H, d, H^b), 8.23 (1 H, s, H^c), 8.02 (2 H, d, H^d), 4.79 (2 H, s, CH_2O), 3.68 (2 H, m, OH), 2.64 (3 H, s, CH_3). The

(16) P. Ponka and A. Wilczyska in ref 10b, p 287.

corresponding proton resonances in D_2O for **1b** appeared at δ 8.88, 8.60, 7.47, 7.80, 4.50, 4.65, and 2.35.

Synthesis of 4. At room temperature, PINH (1 g) (in either form 1 or **1b**) was added to a stirred solution containing acetic anhydride (10 mL) and pyridine (20 mL), and allowed to stand for 24 h. After the usual workup, the crude (yellow) monoacetate was dispersed in water and filtered, leaving behind a colorless product: mp 186 °C [(*Z*)-4]; UV (EtOH) λ_{max} 215 nm (ϵ 19760), 296 (16200), 340 (8240). IR (KBr) 1745, 1682, 1615, 1615, 1535–1565, 1495–1512 cm^{-1}; From the filtrate, yellow product crystallized: mp 164 °C (*E*)-4; uV (EtOH) λ_{max} 215 nm (ϵ 15840), 295 (13360), 340 (5920); IR (KBr) 1740, 1688, 1605, 1570, 1560, 1520, 1495 cm^{-1} In CD_3OD, the ^1H NMR spectrum of (*Z*)-4 exhibited resonances at δ 8.862 (1 H, s, H^a), 8.826 and 8.807 (2 H, d, H^b), 8.036 (1 H, s, H^c), 7.96 and 7.94 (2 H, dd, H^d), 5.315 (2 H, s, CH_2O), 2.54 (3 H, s, CH_3), 2.13 (3 H, s, CH_3CO). The resonances of (*E*)-4 ($CDCl_3$) appeared at δ 9.116 (1 H, s, H^a), 8.840 (2 H, d, H^b), 8.492 (1 H, br s, NH), 8.179 (1 H, s, H^c), 7.775 (2 H, d, H^d), 5.289 and 5.272 (2 H, d, CH_2O), 2.611 (3 H, s, CH_3), 2.116 (3 H, s, CH_3CO).

Synthesis of 11. A solution of 700 mg of **1a** or **1b** in 80 mL of H_2O was brought to pH 6.0 by addition of NaOH pellets; a solution of 300 mg of $FeSO_4 \cdot 7H_2O$ in 20 mL of H_2O was then added with stirring and allowed to stand for 30 min. Brown-red iron-containing crystals (11) precipitated, showing no tendency to melt up to 400 °C. Its UV spectrum exhibits a new band at 465 nm (ϵ 6200) attributable to iron chelate and absorptions at 212 (ϵ 13200), 308 (33500), 368 (22600), 465 nm (6200).

Under electron impact, 11 does not show the molecular ion peak at *m/e* 626, probably because of its instability, but it does exhibit peaks of low abundance at *m/e* 502, 488, 446, and 341, arising from losses of mass units of 124 ($C_6H_8N_2O$), 138 ($C_7H_8NO_2$), 180 ($C_8H_{10}N_3O_2$), and 286 (one molecule of PINH) with retention of the iron atom in the ionic fragment *m/e* 341. More prevalent peaks appear at *m/e* 284 (20%), 178 (30), 160–164 (50), 123 (100%, base peak), 106 (75) and 78 (80), attributable to ionic fragments arising from cracking of the free ligand. Anal. Calcd for $C_{28}H_{26}N_8O_6Fe \cdot 2H_2O$: C, 50.72; H, 4.83; N, 16.91. Found: C, 50.81; H, 4.86; N, 16.34.

Synthesis of 12. The complexation of *O*-acetylpyridoxal isonicotinoylhydrazone (4) with Fe(II) was effected in a similar manner described above. The ligand **4** (450 mg) was dissolved in warm water (30 mL), and a solution of $FeSO_4 \cdot 7H_2O$ (200 mg) in water (3 mL) was added, followed by a pellet of NaOH to adjust the pH to 6.0, yielding a deep-brown crystalline coordination product having no melting point, shown to be structure **12**. Its structure followed from its elementary analysis, its UV (MeOH) spectrum, [λ_{max} 226 nm (ϵ 47600), 315 (21750), 365 (14600), 460 (3200)], and its IR spectrum (KBr) [3500–3150 (bonded OH), wide bands in the regions 2700–2400 and 2150–2050, 1740 (acetate), 1600 (C=N) cm^{-1}]. The mass spectrum of the complex **12** does not show the molecular ion peak at *m/e* 710 or peaks attributable to iron-containing ionic fragments. However, it exhibits peaks at *m/e* 354, 326, 293, 283, 270, 256, 210, 177, 164, 161, 149 (48%), 137 (45), 122 (50), 106 (100%), and 78 (100%) base peaks. Anal. ($C_{32}H_{30}N_8O_8Fe \cdot 4H_2O$) C, H, N.

Compound **5** was prepared according to literature.[8,9] The procedure described earlier for the preparation of compounds **6**, **7**, **9**, and **10**[10] was found useful also for the synthesis of **2**, **3**, and **8**. The physical properties of the new compounds are given in Table I.

Biological Studies. For biological testing, all chelating agents were prepared in aqueous solutions at concentrations ranging from 10 to 40 mg/mL and administered in 1-mL aliquots intramuscularly.

The radioactivity of spleen, weighed portions of the liver, and 1-mL samples of blood were determined in an automatic well type scintillation counter (Packard Auto-Gamma Model 5360 Scintillation spectrometer). Spleen and kidney were counted as whole organs.

Whole body counts were performed in a small animal counter (Packard Model 446 Armac liquid scintillation detector).

The excretion on radioactivity following ^{59}Fe labeling was measured after the animals were confined in solitary metabolic cages with stainless-steel grid bottoms, and stool and urine were collected separately.

Soluble Ferritin. In vivo ^{59}Fe-labeled ferritin was prepared by injection of 100–200 μCi of [^{59}Fe]citrate into rats given 12 mg of iron dextran the preceding week. The animals were killed 24 h later, and purified radioactive ferritin was prepared by the method of Bjorklid and Helgeland.[15] Acrylamide gel electrophoresis of the ferritin preparation at pH 8.5 revealed a single protein band, and a single precipitin line was obtained on immunodiffusion against anti-ferritin serum. The specific activity of [^{59}Fe]ferritin was 5–10 μCi/mg of iron. Shortly before injection, the purified ferritin concentrate was dissolved in sterile normal saline to a final concentration of 0.2–0.4 μCi of ^{59}Fe/mL, representing 40 μg of ferritin iron, and injected intramuscularly in aliquots of 1 mL per animal.

Transferrin. Freshly drawn rat plasma was incubated with $^{59}FeCl_3$ (specific activity 10–15 μCi/μg; Amersham Radiochemical Centre) diluted in 0.005 N HCl and mixed with sufficient sterile 4% sodium citrate to ensure a molar ratio of citrate to iron in excess of 50:1. The final concentation of ^{59}Fe was 0.5 μCi/mL plasma, and 1-mL aliquots per animal were injected intravenously.

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Registry No. (*E*)-1, 83706-03-0; (*Z*)-1, 83706-04-1; **1a**, 83706-05-2; **1b**, 83706-06-3; **2**, 2681-77-8; **2a**, 83706-07-4; **3**, 83706-08-5; **3a**, 83706-09-6; (*E*)-4, 83706-10-9; (*Z*)-4, 83706-11-0; **6**, 78886-41-6; **6a**, 83706-12-1; **7**, 78886-42-7; **7a**, 83706-13-2; **8**·HCl, 83706-14-3; **9**, 78886-43-8; **9a**, 83706-15-4; **10**, 78886-44-9; **10a**, 83706-16-5; **11**, 83719-43-1; **12**, 83710-16-1; Fe, 7439-89-6.