EPR and Semi-Empirical Studies as Tools to Assign the Geometric Structures of FeIII Isomer Models for Transferrins

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The reaction between Fe(ClO4)3.nH2O and N,N′-bis[(2-hydroxybenzyl)-N,N′-bis(1-methylimidazol-2-yl-methyl)]ethylenediamine (H2bbimen) affords two geometric isomers, A and B, of the complex cation [Fe(bbimen)]+. A reaction in the region of the infrared and UV-visible spectrometry, molar conductivity measurements, cyclic voltammetry, spectroelectrochemistry and EPR spectroscopy. The geometry of one of these isomers has been clearly demonstrated through X-ray crystallographic analysis. It crystallizes in the monoclinic system, space group P21/c, a = 14.104 (3), b = 15.626 (3), c = 13.291 (3) Å, β = 98.07 (3), Z = 4, R1 = 6.35% and wR2 = 20.57%. The electrochemical properties of [Fe(bbimen)]+ (-0.58 V vs. NHE) are quite similar to those of transferrins (-0.52 V vs. NHE). EPR studies revealed that isomer A is better described as a rhombically distorted high-spin FeIII complex (g1 = 4.1 with a shoulder at g2 = 9.0), while the spectrum of B has a set of lines at g1 = 3.0, g2 = 3.6 and g3 = 5.1, in addition to the line at g1 = 4.2 and a shoulder at g2 = 9.0, which have been ascribed to FeIII complexes in axial symmetry (E/D = 0.22). Semi-empirical theoretical studies, combined with EPR data, were essential to the proposition of the geometric structures of A and B.

Keywords: FeIII complex, geometric isomers, EPR, transferrins, semi-empirical studies

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Dedicated to Prof. Vincent Louis Pecoraro on the occasion of his 50th birthday.
Introduction

Transferrins (Tfs), or siderophilins, are a family of Fe-binding proteins belonging to the Fe-tyrosinate class. In vertebrates, they are found in blood (serum transferrin), eggs (ovotransferrin) and milk (lactoferrin). The main physiological role of serum transferrin is the Fe transport through the circulating blood and its release to the Fe-dependent cells. Another role is believed to be played by ovotransferrin and lactoferrin, which involves bacteriostatic effects.1,2 As the serum concentration of transferrin is about 35 μmol L⁻¹, and about 70% of it is in the apo-form (protein depleted of Fe), it is also proposed to be a blood transporter of several other metal ions, including vanadium, aluminum, gallium and cobalt.3 Of the Fe-dependent cells, tumor cells are probably the ones of most concern because of their high demand for iron and as a consequence they exhibit enhanced transferrin receptor (TfR) expression.4

During the lifetime of serum transferrin in the circulation system, it is able to perform reverse uptake and deliver Fe about 100-200 times, and thus an understanding of the kinetics and the mechanism involved in the fundamental physiological process is of interest.5 It has been proposed that the first step is the coordination of FeIII to transferrin, which is coupled to the synergistic binding of a carbonate ion. The FeIII-Tf complex is then transported through the blood to the Fe-dependent cells, where the FeIII-Tf complex binds to a membrane transferrin receptor. This system is then internalized by the cell to an ATP-driven proton-pumping endosome (pH 5.4-6.0), where the metal is released as FeII. This then leaves the endosome via a membrane metal ion transporter (DMT).6

In order to better understand this process, the full characterization of transferrins is of fundamental importance. The structures of human lactoferrin,7 serum transferrin,8,9 and duck ovotransferrin10 have all been well studied using X-ray crystallography and are quite similar. They are bilobal proteins with each domain (C- and N-lobes) binding one FeIII ion. As the FeIII uptake is carbonate-dependent, its coordination sphere is composed of the two oxygen atoms of this “synergistic” ion, two phenolate oxygen atoms from tyrosine residues, one imidazole nitrogen atom from histidine and one carboxylate oxygen from aspartic acid (Figure 1).7-10

Mössbauer spectra of human lactoferrin and serum transferrin display isomer shifts of 0.39 mm s⁻¹ and 0.38 mm s⁻¹ respectively, which are typical of high-spin FeIII centers.12 Additionally, the serum transferrin spectrum shows a large quadrupole splitting reflecting the non-symmetrical coordination field around the FeIII ion, as shown by the structural analysis.8,11 In agreement with the Mössbauer data, the EPR spectrum of serum transferrin displays a sharp signal at \( g \approx 4.3 \) and a shoulder at \( g \approx 9.0 \), both characteristics of high-spin FeIII ions.12

As the iron transport involves the uptake of FeIII and the release of FeII ions, knowledge of the redox potentials of Fe-bonded transferrins is extremely important. Redox potentials of human serum transferrin have been investigated under physiological (7.4) and endosomal (5.8) pH conditions. Spectroelectrochemical measurements13 revealed that \( E_{1/2} = -0.52 \) (8) V versus NHE at pH 7.4. At pH 5.8, the redox potentials \( (E_{1/2}) \) of the three possible FeIII-Tf forms were measured: the diferric \( (E_{1/2} = -0.526 \) V versus NHE), the monoferric C-lobe \( (E_{1/2} = -0.501 \) V versus NHE) and the monoferric N-lobe \( (E_{1/2} = -0.520 \) V versus NHE) forms.14 All of these measured redox potentials are too low for reduction of FeIII by physiological agents. However, it has recently been demonstrated that the interaction of the FeIII-Tf complex with the membrane transferrin receptor (TfR) raises the potential by more than 200 mV, explaining the FeIII→FeII reduction in physiological medium.6

Based on these studies, several articles have been published reporting FeIII complexes with phenolate- and pyridine/imidazole-containing ligands as models for Fe-
tyrosinate proteins. Very recently, we reported the synthesis and full characterization of a series of FeIII complexes, [Fe(bbpen-X)]+, using hexadentate ligands (H2bbpen-X) containing pyridine and phenol pendant arms. In this paper, we show that the reaction of the analogous H2bbimen (Scheme 1) with Fe(ClO4)3.nH2O affords a mixture of geometric isomers (A and B), which were only differentiated by EPR analysis. In order to determine the structure of the FeIII complexes, semi-empirical studies were a helpful tool when combined with EPR spectroscopic data.

Scheme 1.

**Experimental**

**Abbreviations**

H2bbpen, N,N'-bis-(2-hydroxybenzyl)-N,N'-bis-(2-pyridin-2-yl-methyl)ethylenediamine; H2bbimen, N,N'-bis-(2-hydroxybenzyl)-N,N'-bis-(1-methylimidazole-2-ylmethyl)ethylenediamine; TBAPF6, tetra-n-butylammonium hexafluorophosphate; ESI-MS, electrospray ionization mass spectrometry; CV, cyclic voltammetry; IR, infrared spectroscopy; EPR, electron paramagnetic resonance spectroscopy; SCE, standard calomel electrode; Fc, ferrocene; Fc+, ferrocenium ion; NHE, normal hydrogen electrode.

**Materials**

Electrochemical and spectroscopic data were collected in high purity solvents, and high purity argon was used when necessary to obtain inert atmosphere. All other chemicals and solvents were of reagent grade, purchased from commercial sources, and used without further purification.

**Physical measurements**

Infrared spectra were obtained on a FT-IR Perkin-Elmer 16PC spectrophotometer as KBr pellets or films. Elemental analyses were performed on a CHN Perkin-Elmer 2400 analyser. Molar conductivity was measured in CH3CN (10-3 mol L-1) at 298K with a Digimed CD-21 equipment. Electrospray- ionization (ESI-MS) mass spectra were recorded in acetonitrile using a Micromass LCT time-of-flight mass spectrometer with electrospray and APCI, coupled to a Waters 1525 Binary HPLC pump. UV-visible absorption spectra were measured in CH3CN on a Perkin-Elmer Lambda 19 spectrophotometer. Cyclic voltammograms were recorded at room temperature using a PAR 273 (Princeton Applied Research) potentiostat in acetonitrile solution, under argon atmosphere, with TBAPF6 (0.1 mol L-1) as supporting electrolyte. A standard three-electrode cell was used: a gold working electrode, a platinum wire auxiliary electrode and a SCE reference electrode. Ferrocene was used as internal standard (E1/2 = 0.16 V versus SCE). Spectroelectrochemical experiments were carried out at room temperature in acetonitrile, under argon, with TBAPF6 (0.1 mol L-1) as supporting electrolyte, using an optically transparent thin-layer cell constructed according a previously reported procedure. A gold minigrid and a platinum wire were used, respectively, as working and auxiliary electrodes, and a SCE was used as the reference electrode. Potentials were applied with a PAR 263 potentiostat/galvanostat and the spectra were recorded with a Perkin-Elmer Lambda 19 spectrophotometer. Spectral changes were registered after the establishment of equilibrium (120 s) and the experiment was stopped when no further changes were observed. Potentials were applied in the range of -0.84 to -1.06 V versus Fc+/Fc and the spectra recorded from 300 to 750 nm. The Fc+/Fc couple was used separately to monitor the reference electrode (E1/2 = 0.16 V versus SCE). X-band EPR spectra were recorded on a Bruker ESP 300E spectrometer at 77 K in CH2Cl2 solution.

**Synthesis of [Fe(bbimen)]ClO4**

**Mixture of isomers (1).** Fe(ClO4)3.nH2O (0.36 g, 1 mmol) was slowly added to a methanolic solution of H2bbimen (0.46 g, 1 mmol, 30 mL). This reaction mixture was heated (~ 40 °C) under stirring for ca. 30 min. After cooling the solution to room temperature, a violet microcrystalline precipitate was filtered off, washed with small amounts of cold 2-propanol and dried under vacuum. Yield 0.40 g (62%). FT-IR (KBr pellet) vmax/cm⁻¹: v(O-H) 3426; v(C=Nimidazole) 1592; v(C=Nphenol) 1514-1446; v(C=Ophenol) 1278; v(Cl-O) 1154 and 1094. ESI-MS m/z (positive mode): 512.2 (6.38%), 513.2 (2.25%), 514.2 (100%), 515.2 (32.63%) and 516.2 (5.88%). Anal. Calc. for FeC28H38N6O8Cl: C, 49.61; H, 5.65; N, 12.40. Found: C, 49.62; H, 5.11; N, 12.64%. Molar conductivity: 130 Ω⁻¹ cm² mol⁻¹ in acetonitrile at 298 K (1:1 electrolyte). Few single
crystals suitable for X-ray crystallographic analyses were obtained by slow evaporation of a solution of 1 in methanol:ethanol:water (10:1:1).

While attempting to find solvent systems to recrystallize the product, we were able to isolate two geometric isomers of [Fe(bbimen)]ClO4 by fractional crystallization. About 60% of the mixture of isomers (1) is soluble in hot acetone (isomer A) and was recrystallized in this solvent, yielding sharp purple needles that were not suitable for X-ray analysis. The remaining amount (isomer B) was then recrystallized in methanol yielding a microcrystalline sample.

Isomer A. ESI-MS m/z (positive mode): 512.2 (6.41%), 513.2 (2.25%), 514.2 (100%), 515.2 (32.73%) and 516.2 (5.89%).

Isomer B. ESI-MS m/z (positive mode): 512.2 (6.16%), 513.2 (2.26%), 514.2 (100%), 515.2 (32.49%) and 516.2 (5.87%).

Caution! Perchlorate salts of metal complexes with organic ligands are potentially explosive, and only small amounts should be carefully handled.

X-ray crystallography

A violet crystal was selected and isolated for crystallographic analysis with a CAD-4 diffractometer. Cell parameters were determined from 25 carefully centered reflections in the θ range 8.79-15.25° using a standard procedure. Data were corrected for Lorentz and polarization effects and for absorption (transmission factors 0.7305 and 0.9546). The structure was solved with SIR97 and refined by full-matrix least-square methods using SHELXL97. The disorder in the perchlorate ion was modeled with two alternative positions for each oxygen atom. At C7, C8 and C2E, the H atoms were also placed using a standard disordered model. All non-H atoms were refined with anisotropic displacement parameters, except for O22. Hydrogen atoms were added at calculated positions and included in the structure factor calculations, with C-H = 0.93 Å (0.96 Å for methyl groups) and U_{iso} (H) = 1.2U_{eq} (C) or 1.5U_{eq} (methyl C). Other selected crystallographic information is shown in Table 1.

Table 1. Selected crystal data and structure refinement for [Fe(bbimen)]ClO4·EtOH

<table>
<thead>
<tr>
<th>Empirical formula</th>
<th>C28H36ClFeN6O7</th>
<th>Formula weight 659.93</th>
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<tr>
<td>Temperature</td>
<td>293(2) K</td>
<td>Wavelength 0.71073 Å</td>
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<tr>
<td>Crystal system</td>
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<td>Space group P2₁/c</td>
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<tr>
<td>Unit cell dimensions</td>
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<tr>
<td>a</td>
<td>14.104(3) Å</td>
<td>b</td>
</tr>
<tr>
<td>b</td>
<td>15.625(3) Å</td>
<td>c</td>
</tr>
<tr>
<td>c</td>
<td>13.291(3) Å</td>
<td>β</td>
</tr>
<tr>
<td>β</td>
<td>98.07(3)°</td>
<td>Volume 2900.0(11) Å³</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>1.511 Mg m⁻³</td>
<td>Absorption coefficient 0.670 mm⁻¹</td>
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<tr>
<td>F(000)</td>
<td>1380</td>
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<tr>
<td>Crystal size</td>
<td>0.50×0.17×0.07 mm³</td>
<td>Theta range for data collection 2.36 to 24.98°</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-16 ≤ h ≤ 16, 0 ≤ k ≤ 18, 0 ≤ l ≤ 15</td>
<td>Reflections collected 5280</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>5049</td>
<td>[R(int) = 0.0347]</td>
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<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F²</td>
<td>Data / restraints / parameters 5049 / 142 / 420</td>
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<tr>
<td>Goodness-of-fit on F²</td>
<td>1.104</td>
<td>R indices [I&gt;2σ(I)] R₁ = 0.0635, wR₂ = 0.1442</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>R₁ = 0.1529, wR₂ = 0.2057</td>
<td>Largest diff. peak and hole 0.686 and -0.608 e Å⁻³</td>
</tr>
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</table>

This method has been successfully used in cases involving transition metal complexes. The spin state of the FeIII isomers was stated as high-spin, ground state ⁶S, according to the EPR data. The calculations were carried out on a 2.6 GHz Athlon PC, with 1 GB RAM and 40 Gb HD, under the Windows 2000 operational system.

Results and Discussion

Synthesis

H₂bbimen was prepared in good yield as previously described. It reacts with Fe(ClO₄)₃·nH₂O in methanol to give a mixture (1) of geometric isomers (A and B) of the stable cation complex [Fe(bbimen)]⁺. The mixture was separated by fractional crystallization in hot acetone.

X-ray structural characterization

A single crystal suitable for X-ray analysis was isolated from 1, i.e., from the mixture of isomers. The structure of the complex consists of a discrete mononuclear cation, [Fe(bbimen)]⁺, and a ClO₄⁻ counterion, in general positions. The elemental cell also possesses an ethanol molecule as crystallization solvent. Crystallographic data and main bond distances and angles are presented in Tables...
1 and 2, respectively. X-ray crystallographic analysis shows that the Fe\textsuperscript{III} center is in a pseudo-octahedral environment, with the two halves of the bbimen\textsuperscript{2−} ligand coordinated in a facial mode (Figure 2). This same arrangement (fac-N\textsubscript{2}O) is observed in other complexes with the analogous hexadentate bbpen\textsuperscript{2−} ligand: [Fe\textsuperscript{III}(bbpen)]\textsuperscript{4+},16,20 [V\textsuperscript{III}(bbpen)]\textsuperscript{3+},34 and [Mn\textsuperscript{III}(bbpen)]\textsuperscript{2+}.23 The equatorial plane is geometrically defined by the O1-O2-N1-N2 atoms, from which the Fe\textsuperscript{III} deviates only 0.001 Å. The equatorial plane is then composed of two nitrogen and two oxygen atoms from the ethylenediamine backbone and the phenolate groups, respectively. They are coordinated in such a way that the nitrogen atoms are cis to each other, and trans to the oxygen atoms. Completing the coordination sphere of the metal center are the two nitrogen atoms from the 1-methylimidazole group. The octahedral geometric distortion can be observed by the angles of the Fe\textsuperscript{III} coordination sphere that deviate from 90° (Table 2). The coordination of the ethylenediamine group results in a distorted five-membered ring (FeN\textsubscript{2}C\textsubscript{5}C\textsubscript{6}N\textsubscript{1}), in which the C\textsubscript{5} and C\textsubscript{6} atoms lie at opposite sides of the equatorial plane, with deviations of -0.358 and 0.202 Å, respectively.

Table 2. Selected bond lengths and angles, Å or (°), for [Fe(bbimen)]ClO\textsubscript{4}.EtOH

<table>
<thead>
<tr>
<th>Bond</th>
<th>Length</th>
<th>Angle</th>
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<tr>
<td>Fe-O1</td>
<td>1.877(5)</td>
<td></td>
</tr>
<tr>
<td>Fe-N41</td>
<td>2.114(6)</td>
<td></td>
</tr>
<tr>
<td>Fe-N31</td>
<td>2.116(6)</td>
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<tr>
<td>Fe-N1</td>
<td>2.269(5)</td>
<td></td>
</tr>
<tr>
<td>Fe-N2</td>
<td>2.272(6)</td>
<td></td>
</tr>
<tr>
<td>O1-Fe-O2</td>
<td>101.9(2)</td>
<td></td>
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<tr>
<td>O1-Fe-N41</td>
<td>99.6(2)</td>
<td></td>
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<tr>
<td>O2-Fe-N41</td>
<td>92.1(2)</td>
<td></td>
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<tr>
<td>O1-Fe-N31</td>
<td>92.1(2)</td>
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<tr>
<td>O2-Fe-N31</td>
<td>97.4(2)</td>
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<td>N41-Fe-N31</td>
<td>163.0(2)</td>
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<td>O1-Fe-N1</td>
<td>88.9(2)</td>
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<tr>
<td>O2-Fe-N1</td>
<td>165.2(2)</td>
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<tr>
<td>N41-Fe-N1</td>
<td>76.0(2)</td>
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<tr>
<td>N31-Fe-N1</td>
<td>92.1(2)</td>
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<tr>
<td>O1-Fe-N2</td>
<td>163.3(2)</td>
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<tr>
<td>O2-Fe-N2</td>
<td>91.3(2)</td>
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<tr>
<td>N41-Fe-N2</td>
<td>90.0(2)</td>
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<tr>
<td>N31-Fe-N2</td>
<td>75.9(2)</td>
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<tr>
<td>N1-Fe-N2</td>
<td>80.1(2)</td>
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<tr>
<td>C11-O1-Fe</td>
<td>134.0(4)</td>
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<tr>
<td>C21-O2-Fe</td>
<td>128.9(4)</td>
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</table>

The coordination of the phenolate groups in the equatorial plane has been observed in several other Fe\textsuperscript{III} complexes: [Fe(bbpen)]\textsuperscript{4+},16,20 [Fe(ephpg)]\textsuperscript{4+},36 [Fe(hbed)]\textsuperscript{2+},36 [Fe(ehgs) (CH\textsubscript{3}OH)]\textsuperscript{2+},37 [Fe(salen)(Im)]\textsuperscript{3+},38 [Fe(sal(trien)]\textsuperscript{2+},39 [Fe(salen) (4-mim)]\textsuperscript{2+},38 and [Fe(salen)(1-mim)Cl].38 The Fe-O\textsubscript{ph} average bond distance (1.884 Å) is very similar to that reported for [Fe(bbpen)]\textsuperscript{4+} (1.87 Å). Also, these distances are the shortest of the coordination sphere and, as a consequence of the trans influence, make the Fe-N\textsubscript{ph} the longest ones (av. 2.270 Å). A comparison of the average Fe-N bond distances in the axial positions shows a decrease from 2.15 Å in [Fe(bbpen)]\textsuperscript{4+} to 2.115 Å in [Fe(bbimen)]\textsuperscript{4+}, as a consequence of the greater basicity of the imidazole groups. A comparison of the Fe\textsuperscript{III}, O\textsubscript{ph} and Fe\textsuperscript{III}-N\textsubscript{His} bond distances reported\textsuperscript{11} for human lactoferrin (Fe-O\textsubscript{435}: 1.92 Å and Fe-N\textsubscript{597}: 2.13 Å) and those presented here for 1 (Fe-O1: 1.877 (5) Å and Fe-N31: 2.269 (5) Å) shows a good agreement. This indicates that using phenol and 1-methylimidazole groups as pendant arms in the design of ligands for model complexes, good structural approximations are obtained to mimic tyrosine and histidine amino acids.

Physical-chemical properties

IR spectroscopy and ESI-MS. The IR spectra of 1, A and B are quite similar and characterized by typical bands of the ligand skeletal, in addition to a band at 1094 cm\textsuperscript{-1} attributed to the Cl–O stretching of the perchlorate anion (Figure S1).\textsuperscript{40} A comparison with the IR spectrum of the free H\textsubscript{2}bbimen reveals a decrease in the band at 1372 cm\textsuperscript{-1}, which indicates the coordination of the phenol group in its deprotonated form. The ESI-MS (positive mode) spectra of 1, A and B were recorded from freshly prepared solutions in acetonitrile (Figure S2). In fact, the spectra of A and B are identical, with the base peak (100%) corresponding to [Fe(bbimen)]\textsuperscript{4+} at m/z = 514.2. The other four observed peaks agree quite well with the predicted isotopic distribution for a Fe center (predicted: 514.18 (100%); 515.18 (33.1%); 512.18 (6.4%); 516.18 (5.9%)}
UV-visible spectra. The UV-Visible spectra of 1, A and B were recorded in acetonitrile and show the same behavior with bands at identical wavelengths. The spectrum of 1 (Figure 3) is characterized by transitions at $\lambda_{\text{max}}/\text{nm (}\text{e/mol L}^{-1}\text{ cm}^{-1})$: 236 (13500-shoulder); 278 (11300); 321 (7700) and 542 (4700). As the H$_2$bbimen spectrum (Figure 3-inset) has bands at $\lambda_{\text{max}}/\text{nm (}\text{e/mol L}^{-1}\text{ cm}^{-1})$: 213 (29000) and 276 (6100), the two bands observed for 1 at higher energy are attributed to $\pi \rightarrow \pi^* $ internal transitions of the aromatic rings. The bands at lower energy (321 and 542 nm) can be ascribed to LMCT transitions from the $\pi^*$ orbital on the phenolate oxygen to the half-filled d$_\pi$($t_{2g}$) and d$_\sigma$($e_g$) on Fe$^{III}$. Comparing the UV-Visible data of 1 with those reported for the analogous [Fe(bbpen)]$^+$, it is observed that there is a hypsochromic shift of the $\pi \rightarrow d_{\pi}^*$ LMCT band from 574 to 542 nm when pyridine groups in H$_2$bbpen are replaced by 1-methylimidazole groups in H$_2$bbimen. These results are interpreted as a consequence of the greater basicity of the 1-methylimidazole groups in H$_2$bbimen. This effect has also been observed in several other complexes with the same number of phenolate groups and a variety of other pendant arms with different Lewis basicity. In order to investigate the spectral changes during the Fe$^{III} \rightarrow$ Fe$^{II}$ redox process, spectroelectrochemical measurements were carried out under the same experimental conditions as those employed in the CV studies. A decrease in the LMCT phenolate $\rightarrow$ Fe$^{III}$ band at 542 nm, with a simultaneous increase in a new band at 420 nm was observed (Figure 5). This band (420 nm) is tentatively attributed to an Fe$^{II} \rightarrow$ Fe internal standard (left) and Fe$^{III} \rightarrow$ Fe$^{II}$ (right).

In order to investigate the spectral changes during the Fe$^{III} \rightarrow$ Fe$^{II}$ redox process, spectroelectrochemical measurements were carried out under the same experimental conditions as those employed in the CV studies. A decrease in the LMCT phenolate $\rightarrow$ Fe$^{III}$ band at 542 nm, with a simultaneous increase in a new band at 420 nm was observed (Figure 5). This band (420 nm) is tentatively attributed to an Fe$^{II} \rightarrow$ 1-methylimidazole MLCT transition. This kind of transition has been observed in other systems employing pyridine and pyrimidine groups as ligands, and corroborates this assignment. During the whole process an isosbestic point was clearly observed at 340 nm. The presence of isosbestic points provides strong evidence for only two species present in solution during the redox process. Figure 5 also presents the Nernst plot, which is in agreement with the cyclic voltammetric data, and provides a redox potential of -0.94 V versus Fe$^+/F_c$ for the transference of one electron in the process.
Proposed geometric isomers for A and B

Since EPR is very sensitive to small distortions in the coordination sphere of the metal center, it was the only spectroscopic technique able to distinguish between the two isolated geometric isomers (A and B) of [Fe(bbimen)]⁺. X-band EPR spectra were recorded from frozen solutions of 1, A and B in CH₂Cl₂ at liquid nitrogen temperature, and are shown in Figure 6.

The spectrum of 1 exhibits a sharp signal at \( g_1 \approx 4.3 \), a shoulder at \( g_2 \approx 9.2 \) and another \( g \) set of absorption lines at \( g_1' \approx 3.7 \), \( g_2' \approx 4.0 \) and \( g_3' \approx 5.2 \). It is best described as a combination of the A and B spectra. The spectrum of A presents the sharp signal at \( g_1 \approx 4.1 \) and a shoulder at \( g_2 \approx 9.1 \), as expected for the middle and lower Kramer’s doublet transitions of a rhombically distorted high-spin Fe³⁺ complex (E/D ≈ 0.33), ground state \( ^4S.45,46 \) The spectrum of B, in addition to the peak at \( g_1 \approx 4.2 \) and the shoulder at \( g_2 \approx 9.0 \), also shows a set of peaks at \( g_1' \approx 3.0 \), \( g_2' \approx 3.6 \) and \( g_3' \approx 5.1 \), which has been assigned to weak resonances of the ground Kramer’s doublets in an axial symmetry (E/D ≈ 0.22).11,17,47,48

The same behavior has been observed for other octahedral Fe³⁺ complexes18 and for transferrins.11 In transferrins this may be due to the slight distortions in the Fe³⁺ coordination spheres of the N- and C-lobes. Considering the similarities in the EPR spectra of transferrins and [Fe(bbimen)]⁺, one would conclude that the complex represents a good model for the EPR properties of these metalloenzymes.

The coordination of bbimen²⁻ to Fe³⁺ could theoretically afford the three geometric isomers represented in Figure 7. As shown in our previous discussion, the geometry of one of these structures has been clearly demonstrated through the X-ray crystallographic analysis, while distinct EPR properties were found for the other isolated isomers.

Aiming to investigate the formation of these possible geometric isomers, semi-empirical studies were performed. These studies revealed that only two of the three structures (Figure 7) were confirmed as being true minimalis. This was inferred by means of the vibrational frequency calculations, that showed all frequencies to be real. Calculated energies of the two optimized configurations show that (1) is favored by ca. 12 kcal mol⁻¹ in relation to (2), and in spite of a relative energy difference, the results are in good agreement with the trends observed experimentally. It is also observed that only configuration (1) shows a rhombic behavior, which is in agreement with the structure shown by X-ray analysis (Figure 2), and gives a theoretical vibrational spectrum in good agreement with the experimental data (Figure S2). As the EPR studies revealed that isomer A has rhombic symmetry, it seems reasonable to assign it as being configuration (1). On the other hand, the geometry of isomer B can be assigned to configuration (2) (Figure 7). The graphical representation of the SOMO molecular orbitals for the geometric isomers A and B (Figure 8) shows that both have the main contribution of the \( p_\pi \) orbital from the phenolate groups, which corroborates the fact that similar results were observed in the UV-Visible data. As the electronic transitions (321 and 542 nm) have been assigned to LMCT transitions from the \( p_\pi \) orbital on the phenolate oxygen to the half-filled \( d_\pi^\ast \) (t₂g) and \( d_\sigma^\ast \) (e_g) on Fe³⁺,11,15-20,41 and both isomers A and B have the SOMO orbital with the same participation of the phenolate groups, the two complexes should have similar electronic spectra. Therefore, we have demonstrated here that the theoretical calculations describe the electronic properties of these metalloenzymes.
behavior of both isomers, and are in good agreement with the experimental data.

In spite of different calculation methods being used to obtain the data presented here for [Fe(bbimen)]⁺ and those reported¹⁶ for [Fe(bbbpen)]⁺, a comparison between these results showed that, in both complexes, the SOMO participation is predominantly from the phenolate groups. In addition, the LUMO of [Fe(bbbpen)]⁺ and [Fe(bbimen)]⁺ are mainly of the pyridine and the imidazole groups, respectively.

**Conclusions**

In summary, we have synthesized, isolated and characterized two geometric Fe³⁺ isomers employing the ligand H₂bbimen. We have demonstrated that the substitution of the two pyridines in H₂bbpen by two 1-methylimidazole groups in H₂bbimen was successful for tuning the redox properties of [Fe(bbimen)]⁺, making it a good model for the redox properties of transferrins. In addition, we established that EPR was the only spectroscopic technique able to differentiate the isolated isomers A and B, and, when combined with semi-empirical calculations, was essential to describe their structures. It was also possible to demonstrate that the main contributions for the SOMO and the LUMO are from the phenolate and the imidazole groups, respectively, as previously observed for the analogous [Fe(bbpen)]⁺.

**Supplementary Information**

Crystallographic data (atomic coordinates, equivalent isotropic displacement parameters, calculated hydrogen

![Figure 7. Representation of the three theoretically possible geometric isomers of the [Fe(bbimen)]⁺ cation complex.](image1)

![Figure 8. Graphical representation of the SOMO orbitals for the geometric isomers A and B.](image2)
atom parameters, anisotropic thermal parameters and bond lengths and angles) have been deposited at the Cambridge Crystallographic Data Center (deposition number CCDC 609269). Copies of this information may be obtained free of charge from: CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk). Additional Figures are also available at http://jbcs.sbq.or.br, as PDF files.

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To Luiz Sérgio Gonçalves da Cunha (in memoriam).
To Arlindo Scarpellini (in memoriam).

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EPR and Semi-Empirical Studies as Tools to Assign the Geometric Structures of Fe$^{III}$ Isomer Models for Transferrins

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Figure S1. IR spectra of 1 and A recorded as KBr pellets.

Figure S2. Theoretical IR spectrum of A.

Figure S3. ESI-MS (positive mode) of A (bottom) and B (middle) recorded in acetonitrile solution, and predicted isotopic distribution (top).

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Dedicated to Prof. Vincent Louis Pecoraro on the occasion of his 50th birthday.
Figure S4. UV-visible spectrum of A in acetonitrile solution.

Figure S5. Cyclic voltammograms of A in CH$_3$CN, TBA$\text{PF}_6$ (0.1 mol L$^{-1}$) at 50 (internal), 100, 150, 200 and 250 mV s$^{-1}$ scan rates, Fe$^{3+}$/Fe internal standard (left) and Fe$^{3+}$/Fe$^{2+}$ (right).