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Influence of ligand structure on Fe(II) spin-state and redox rate in cytotoxic tripodal chelators

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Abstract

The Fe coordination chemistry of several tripodal aminopyridyl hexadentate chelators is reported along with cytotoxicity toward cultured Hela cells. The chelators are based on *cis*, *cis*-1,3,5-triaminocyclohexane (tach) with three pendant $-\text{CH}_2-2\text{-pyridyl}$ groups where 2-pyridyl is R-substituted thus are named tach-x-Rpyr where x=3, R=Me; x=3, R=MeO; x=6, R=Me. The structures of $[\text{Fe}(\text{tach-3-Mepyr})]\text{Cl}_2$ and $[\text{Fe}(\text{tach-3-MeOpyr})](\text{FeCl}_4)$ are reported and their metric parameters indicate strongly-bound, low-spin Fe(II). The structure of $[\text{Fe}(\text{tach-6-Mepyr})](\text{ClO}_4)_2$ implies steric effects of 6-Me groups push donor N_{py} 's away so one Fe- N_{py} bond is substantially longer at 2.380 (3) Å vs. 2.228(3) Å for the others, and Fe(II) in the high-spin state. Accordingly, anions $\text{X}^- = \text{Cl}$ or SCN afford $[\text{Fe}(\text{tach-6-Mepyr})(\text{X})]^{2+}$ from $[\text{Fe}(\text{tach-6-Mepyr})]^{2+}$ (UV-vis spectroscopy). Consistent with a biological cytotoxicity involving Fe chelation, chelators of low-spin Fe(II) have greater toxicity in the order $[\text{IC}_{50}(72 \text{ h})]$ is in parentheses then the spin-state SS=H (high) or L (low): tachpyr = tach-3-Mepyr (6 μM , SS=L) $\gtrsim$ tach-3-MeOpyr (12 μM , SS=L) $\gg$ tach-6-Mepyr (> 200 μM , SS=H). Iron-mediated oxidative dehydrogenation with O_2 oxidant removes hydrogens from coordinated nitrogen and the adjacent CH_2 , converting aqueous $[\text{Fe}(\text{tach-3-Rpyr})]^{2+}$ (R = H, Me and MeO) into a mix of low-spin imino- and aminopyridyl-armed complexes, but $[\text{Fe}(\text{tach-6-Mepyr})]^{2+}$ does not react (NMR and ESI-MS spectroscopies). The difference of IC_{50} for chelators at different time points ($\Delta\text{IC}_{50} = [\text{IC}_{50}(24 \text{ h}) - \text{IC}_{50}(72 \text{ h})]$) is used to compare rate of cytotoxic action to qualitative rate of oxidation in the Fe-bound chelator, giving the order: $[\text{Fe}(\text{tach-3-Mepyr})]^{2+}$ ($\Delta\text{IC}_{50} = 5 \mu\text{M}$) > $[\text{Fe}(\text{tachpyr})]^{2+}$ ($\Delta\text{IC}_{50} = 16 \mu\text{M}$) > $[\text{Fe}(\text{tach-3-MeOpyr})]^{2+}$ ($\Delta\text{IC}_{50} = 118 \mu\text{M}$). Thus, those chelators whose Fe(II) complexes undergo rapid oxidation kill cells faster, and those that bind Fe(II) as low-spin are far more cytotoxic.

Keywords

redox reaction; antitumor agent; ligand design; tripodal chelator; Fe(II)

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Introduction

Many studies now show that the *cis*, *cis*-1,3,5-triaminocyclohexane (tach) framework is well-suited to complexation of first-row dicationic transition-metals of ionic radius [1] 0.75 to 0.88 Å. [2–4] In studies of aqueous and biological coordination chemistry, we identified the *N*, *N'*, *N''*-2-pyridylmethyl pendant arm derivative, tachpyr, as a Zn(II)- and Fe(II)-complexing agent *in vitro* as well as a cytotoxic agent with potential application to cancer therapy. [5–7] Notably upon treatment of cells with tachpyr, ferritin synthesis is depressed, which is indicative of cellular iron deficiency. [6] In addition to a cytotoxic pathway involving sequestration and cellular deprivation of biometal ions by chelators, the induction of cellular oxidative damage via catalytic coordinated metal ion is also possible. [8] The cytotoxicity of tripodal aminopyridyl chelators based on tach and tren (tris(2-aminoethyl)amine) frameworks is quite sensitive to substituents on both the pyridyl rings and the framework amine nitrogens. Specifically, methylation at the pyridine-ring 6-position or the amine nitrogen completely suppresses aqueous metal ion-chelation and cytotoxicity, while methylation at the 3-ring position does not hinder metal chelation and accelerates cell death. [9–11]

When certain tachpyr-family chelators bind Fe(II) under aerobic conditions (or bind Fe(III) under N₂ or O₂, or when Fe(II)-tach-family complexes are exposed to oxidants), iron-mediated oxidative dehydrogenation occurs giving [Fe(tachpyr-monoimine)]²⁺ and [Fe(tachpyr-diimine)]²⁺ complexes, as shown in Scheme 1. For reasons that will be discussed in a separate paper, tachpyr-trisimine does not interact strongly with Fe(II) and the [Fe(tachpyr-trisimine)]²⁺ complexes do not form readily. This reaction is significant to chemistry and biology of these chelators in at least two ways. The redox process may generate reactive oxygen species that can cause cellular injury and death. [12] Second, π -backbonding is greater to the iminopyridyl group than to the aminopyridyl group, affording stronger Fe(II) binding. [13] Thus although Zn(II) competes favorably with Fe(II) for tachpyr under anaerobic conditions, under aerobic conditions tachpyr binds increasing amounts of iron relative to zinc, which we postulated is due to inertness of Fe(II)-tachpyr-mono- and bis-imine complexes relative to the reversibly-bound Zn(II) of the complex [Zn(tachpyr)]²⁺. [14] Studies of extracts of cells treated with tachpyr indicate that the mono- and bis-imine complexes are likely forms of sequestered iron or zinc in cells. [5]

It was therefore of great interest to pursue further substitutions (beyond methylation) at the 3-position of the tachpyr chelator, particularly to study oxidative dehydrogenation of Fe complexes with derivatized tachpyr. Herein, we report synthesis of the 3-methoxylated chelator, tach-3-MeOpyr, structural and solution properties of the Fe(II) complexes of tach-3-Mepyr, tach-3-MeOpyr and tach-6-Mepyr, the oxidative dehydrogenation reactivity of the Fe(II)-complexes of these three chelators relative to the parent tachpyr, and a comparison of cytotoxic action of the chelators toward cultured Hela cells.

Synthesis and spectra

The chelator¹ tach-3-MeOpyr was synthesized from the triamine and the appropriately substituted pyridine carboxaldehyde by first forming a tris-imine that was subsequently reduced to tris-amine. The ligand readily complexed Fe(II) as seen by reaction with a slight excess of FeCl₂•4H₂O in MeOH or H₂O under anaerobic conditions. The complex crystallized as [Fe(tach-3-MeOpyr)](FeCl₄)•2MeOH₂ when a greater excess of FeCl₂•4H₂O was employed. We prepared the ligands tach-3-Mepyr, tach-6-Mepyr and tachpyr plus their Fe(II) complexes **1**, **3**, and **4** as previously reported (Scheme 1). [9,15]

The ¹H NMR spectrum of [Fe(tach-3MeOpyr)]²⁺ indicates a diamagnetic, low-spin state of Fe(II), while the electronic spectrum is dominated by a metal-to-ligand charge-transfer (MLCT) band at 23000 cm⁻¹ (ϵ = 6500 M⁻¹ cm⁻¹) with a low-energy shoulder at 18800 cm⁻¹ (ϵ = 350

$\text{M}^{-1} \text{cm}^{-1}$), quite similar to that of $[\text{Fe}(\text{tach-3-Mepyr})]^{2+}$ whose MLCT band appears at 22700 cm^{-1} ($\epsilon = 7000 \text{ M}^{-1} \text{cm}^{-1}$) and shoulder at 18200 cm^{-1} ($\epsilon = 290 \text{ M}^{-1} \text{cm}^{-1}$). [9] The low-energy shoulder has previously been assigned as the $^1\text{A}_{1g} \rightarrow ^1\text{T}_{1g}$ transition. [16,17]

Iron-mediated oxidative dehydrogenation

In order to investigate the process of oxidative dehydrogenation in the ring-substituted tach chelators, solutions of $[\text{Fe}(\text{tach-3-Mepyr})]^{2+}$ (**1**) or $[\text{Fe}(\text{tach-3-MeOpyr})]^{2+}$ (**2**) were exposed to air. This caused iron mediated oxidative dehydrogenation, with spectral changes matching those we reported for a solution of $[\text{Fe}(\text{tachpyr})]^{2+}$ (**4**). [11] For both **1** and **2**, a new charge-transfer band having a high-energy shoulder appears at ca. 16250 cm^{-1} and as the oxidation progresses, the MLCT band at ca. 23000 cm^{-1} shifts toward higher energy while the band at ca. 16250 cm^{-1} gains intensity as the solution changes from amber to green and ultimately blue, consistent with conversion to a mixture of two $[\text{Fe}(\text{tach-3-Rpyr-imino})]^{2+}$ species (Scheme 1). Because the monoimine complexes (left structure of Scheme 1) are oxidized by $2 \text{H}^\bullet$, they are designated $[\text{Fe}(\text{tach-3-Rpyr-ox-2})]^{2+}$ and similarly the bisimine complexes (right structure of Scheme 1) are termed $[\text{Fe}(\text{tach-3-Rpyr-ox-4})]^{2+}$ (R = Me or MeO). As expected from the low-spin, diamagnetic NMR and in analogy to other chelated σ -donor, π -acceptor ligand Fe(II) complexes such as $[\text{Fe}(\text{phen})_3]^{2+}$, the tach-3-Mepyr and tach-3-MeOpyr ligands and their mono- and diimine derivatives interact strongly with Fe(II). Oxidative dehydrogenation reactivity is not observed for the high-spin complex $[\text{Fe}(\text{tach-6-Mepyr})]^{2+}$.

Comparisons of the rates of oxidative-dehydrogenation between **1**, **2** and **4** were obtained using ^1H NMR and ESI-MS. Proton NMR proved useful for quantitation using the signals of the imino protons, $-\text{N}=\text{CH}-$, which appear as a singlet in the range of 9.2 to 9.6 ppm for the monoimine complexes derived from **1**, **2** and **4** and as a doublet in the range of 8.8 to 9.2 ppm for the diimine complexes. Figure 1 shows the ^1H NMR of **1** in the useful region for quantitation of the amount of monoimine formed, where the aromatic protons are arbitrarily set to 100 H and the integral of the monoimine species at δ 9.59 is measured at 4 h and 24 h exposure to air. Similar data was obtained for **2** and **4** and all integrals are tabulated in Table 1. This data was supported by electrospray-mass spectroscopic studies of the oxidized mixtures, which are shown in the Supplementary Data along with the ^1H NMR spectra of **2** and **4**. The qualitative order of oxidation rate of the tach-3-Rpyr family is tach-3-Mepyr > tachpyr > tach-3-MeOpyr (Table 1). Due to the similar chemical properties of the oxidized species, they could not be separated, and a rigorous kinetic analysis of the oxidation process was not successful, which

¹tach-3-MeOpyr. *N,N',N''-tris(3-methoxy-2-pyridylmethyl)-1,3,5-cis,cis-triaminocyclohexane*. Tach(HBr)₃ (0.58 g, 2.43 mmol) was dissolved in H₂O (10 mL) with NaOH (0.292 g, 7.32 mmol) to form a clear solution. Benzene (120 mL) was added and the water was removed by azeotropic distillation with a Dean-Stark trap. 3-Methoxy-pyridine-2-carboxaldehyde (FMC Lithium) (1.00 g, 7.30 mmol) was added in benzene (10 mL) during this process and continued for 12 h. After cooling, the solution was decanted and rotary evaporated to a solid. ¹³C NMR (CDCl₃) δ 156.0, 155.3, 142.9, 142.5, 128.5, 125.7, 119.0, 67.2, 55.7, 40.7; Mass. Spect. Calcd. for C₂₇H₃₁N₆O₃ 487.2458; Found 487.2468. The tris-imine (1.18 g, 2.42 mmol) was dissolved in MeOH (125 mL) and directly reduced with sodium borohydride (0.28 g, 7.3 mmol). After stirring for 18 hr, the reaction solvent was removed by rotary evaporation. The residue was taken up in CHCl₃ (100 mL) and vigorously stirred with a 1:1 mixture of sat. NaCl solution and 5% NaHCO₃ solution for 1 h. Afterwards, the organic phase was separated, dried over Na₂SO₄, filtered and solvent removed by rotary evaporation to provide the final product as a dark oil (0.97 g, 81 %). ¹H NMR (DMSO-*d*₆, s, singlet; d, doublet; dd, doublet of doublets; q, quartet; br s, broad singlet; br d, broad doublet; br t, broad triplet) δ 8.11 (dd, 1H, J = 4.65, 1.5), 7.53 (dd, 1H, J = 8.55, 1.5), 7.45 (dd, 1H, J = 8.55, 4.8), 4.23 (s, 2H), 3.92 (s, 3H), 3.07 (br. t, 1H), 2.51 (br. d, 1H, J = 11.4), 1.44 (q, 1H, J = 11.7). ¹³C NMR (d₆-DMSO) δ 153.5, 149.1, 140.6, 122.7, 116.9, 55.4, 54.2, 47.0; Mass Spect. Calcd. for C₂₇H₃₇N₆O₃ 493.2927; Found 493.2925.

²[Fe(tach-3-MeOpyr)](FeCl₄)•2MeOH A pale green solution of FeCl₂•4H₂O (0.0101 g, 5.10×10^{-5} mol) in MeOH (1 mL) was added to a pale yellow solution of tach-3-MeOpyr (0.0174 g, 3.53×10^{-5} mol) in MeOH (1 mL) producing a brown-red solution. Diffusion of Et₂O into the solution overnight gave clusters of red plates. The precipitate was recrystallized with MeOH (0.75 mL) and MeCN (0.75 mL) by Et₂O diffusion overnight, producing clusters of carmine red plates suitable for X-ray crystallography. Product was isolated and dried under reduced pressure affording deep red plates. ¹H NMR (400 MHz, (CD₃)₂SO, 25 °C) of a sample of [Fe(tach-3-Mepyr)]Cl₂ prepared similarly from 6.90×10^{-3} g FeCl₂•4H₂O (3.47×10^{-5} mol) and 0.0161 g tach-3-MeOpyr (3.27×10^{-5} mol): δ 7.47, 7.23, 6.65 (d, dd, d, 3H, 3-Me-C₅H₃N); 5.28 (br t, 1H, NH-CH₂); 4.23, 3.96 (dd, dd, 2H, (NH-CH₂-py), diastereotopic); 3.88 (s, 3H, (CH₃O-py)); 2.96 (br s, 1H, cyclohexyl methine H); 2.00 (br d, 1H, equatorial cyclohexyl methylene H); 1.85 (br d, 1H, axial cyclohexyl methylene H). Anal. Calcd for C₂₉H₄₄Cl₄Fe₂N₆O₅ (810.20): C, 42.99; H, 5.47; N, 10.37. Found: C, 42.91; H, 5.50; N, 10.26.

was attributed to the overlap of spectral peaks from the concurrent conversions of $[\text{FeL}]^{2+} \rightarrow [\text{Fe}(\text{L-ox-2})]^{2+}$ and $[\text{Fe}(\text{L-ox-2})]^{2+} \rightarrow [\text{Fe}(\text{L-ox-4})]^{2+}$, precluding accurate quantitation of reactants and products.

At present, an explanation for the above rate order is not apparent. Goto *et al.* reported an effect of geometry upon oxidative dehydrogenation, whereby the *mer*- isomer of $[\text{Fe}^{\text{II}}(\text{ampy})_2(\text{CN})_3]^-$ formed an Fe(II)-imine complex when oxidized with peroxodisulfate, but oxidation of the *fac*- isomer produced *fac*- $[\text{Fe}^{\text{III}}(\text{ampy})_2(\text{CN})_3]$. However in the present case, there is no significant difference in coordination geometry of **1** vs. **2**, nor is there any substantial difference in ligand-field strength of the three ligands **1**, **2** and **4**, based on interpretation of the electronic spectra of the Ni(II) and Cu(II) complexes.[9][18] Although numerous examples of iron-mediated oxidative dehydrogenation are known, more study of the mechanistic aspects is needed.[19–22] By comparison greater progress has been made understanding the oxidative dehydrogenation of Ru and Os complexes, possibly because the reaction mediated by these metals is slower and easier to study.[13,23–27].

Ligand substitution

Although the chelator tach-6-Mepyr has been found not cytotoxic,[9] the possibility that its iron complex possesses an open coordination site was studied. It is known that open coordination sites in iron complexes are important to the redox activity of complexes such as $[\text{Fe}^{\text{II/III}}\text{EDTA}]^{2-/-}$. [28] The complex $[\text{Fe}(\text{tach-6-Mepyr})]^{2+}$ has been assigned as high-spin (HS) based on an MLCT band at 28800 cm^{-1} (ϵ of this band is weak, $650\text{ M}^{-1}\text{ cm}^{-1}$, as is characteristic of Fe(II)-HS) along with a weak *d-d* transition at 11700 cm^{-1} ($\epsilon = 9.4\text{ M}^{-1}\text{ cm}^{-1}$) in the UV-Vis spectrum and a measured magnetic moment $\mu_{\text{eff}} = 5.46\text{ B.M.}$ (DMSO solution, Evans' method).[9] In view of this and the X-ray structural findings (*vide infra*), it was considered likely that **3** could contain an open or substitutable coordination site in solution. Therefore, ancillary ligands were added to a MeCN solution of **3** to probe the presence of such sites. With the addition of one molar equivalent of either a chloride or thiocyanate anion, a red shift of the charge-transfer band was observed. The shift is believed to be due to the formation of $[\text{Fe}(\text{tach-6-Mepyr})(\text{Cl})]^+$ and $[\text{Fe}(\text{tach-6-Mepyr})(\text{SCN})]^+$ respectively, as this is consistent with observations of Bernal and Toftlund who identified replacement of pyridine by water in another Fe^{II} complex of a ligand containing 6-methyl-2-aminomethylpyridyl donors, $[\text{Fe}(\text{N,N}'\text{-bis(6-methyl-2-pyridylmethyl)-N,N'-bis(2-pyridylmethyl)ethane-1,2-diamine})(\text{H}_2\text{O})]^{2+}$. [29] In sum, although this experiment could not be conducted in aqueous medium due to the lability of $[\text{Fe}(\text{tach-6-Mepyr})]^{2+}$, [9], the reactivity of **3** in MeCN solvent is predicted by its structure.

Structural studies

To obtain further chelator structural parameters that may underlie optimal Fe(II) binding, the X-ray structures³ of the dications **1**, **2** and **3** were determined as the salts $[\text{Fe}(\text{tach-3-Mepyr})]\text{Cl}_2 \cdot 2\text{MeOH}$, $[\text{Fe}(\text{tach-3-MeOpyr})](\text{FeCl}_4) \cdot 2\text{MeOH}$, and $[\text{Fe}(\text{tach-6-Mepyr})](\text{ClO}_4)_2 \cdot \text{H}_2\text{O} \cdot \text{MeOH}$ respectively (Figures 2 – 4). The average Fe-N_{py} and Fe-N_{amine} bond lengths for **1** and **2** are shown in Table 2. A comparison with other low-spin (LS), six-coordinate Fe(II) complexes that contain the 2-aminomethylpyridyl group such as $[\text{Fe}(\text{tptcn})]^{2+}$ and *fac*- $[\text{Fe}(\text{ampy})_3]^{2+}$ shows good agreement in the bond lengths (Table 1). As expected, the Fe-N_{py} bonds are generally shorter than Fe-N_{amine} bonds due to $d_{\pi} \rightarrow p_{\pi}^*$ backbonding of Fe(II) to the ligand.[30] Both **1** and **2** possess a trigonally distorted octahedral coordination geometry, as indicated by their trigonal twist angles (ϕ) of 54.9° and 54.6° respectively compared to a ϕ of 60° for a perfect octahedron. The average N_{py}-Fe-N_{amine} angles of the 5-membered chelate rings (α) of 83.6° in **1** and 84.0° in **2** are comparable to other LS aminomethylpyridyl iron complexes as seen in the top section of Table 2.

The average Fe-N_{py} and Fe-N_{amine} bond lengths for **3** are 2.279(3) and 2.173(3) Å respectively. The elongation of the Fe-N bonds in **3** compared to **1** and **2** is attributed to the HS nature of the complex where electrons now populate the e_g* orbitals and the six-coordinate ionic radius of Fe(II) is 0.92 Å. The steric interactions between the 6-methyl groups are most evident when examining the Fe-N_{py} bonds. For each of the two independent molecules in the asymmetric unit there is one Fe-N_{py} bond that is ~0.15 Å longer than the other two. The lengthening of one bond to compensate for steric interactions between 6-methyl groups was also reported in the Zn(II) and Mn(II) complexes of the same ligand, which are isostructural with [Fe(tach-6-Mepyr)](ClO₄)₂ (Table 1, bottom section).[9] Even with these steric interactions the complex is able to obtain a geometry close to octahedral with an average twist angle of 53.0°. The bite angle of 77.9° is consistent with the elongated Fe-N bonds and is in good agreement with other HS aminopyridyl iron complexes as seen in Table 2. Taken together the structural findings indicate that tach-3-Mepyr and tach-3-MeOpyr interact strongly with Fe(II) in an ideal geometry for this metal ion, whereas steric hindrance prevents a good interaction of tach-6-Mepyr with Fe(II).

Cytotoxicity

The cytotoxicities of tach-3-Mepyr and tach-3-MeOpyr toward cultured Hela cancer cells⁴ were determined against a tachpyr control (Table 3). The chelator tach-6-Mepyr displayed no cytotoxicity and was not studied further. At 24 h tach-3-Mepyr is more toxic than tachpyr and much more toxic than tach-3-MeOpyr. However, the toxicity of tach-3-MeOpyr increases dramatically from 24 h to 72 h, as expressed by the value ΔIC₅₀ (24h – 72h). After 72 h, it only takes twice as much tach-3-MeOpyr as either tach-3-Mepyr or tachpyr to kill half the cells. This finding might be explained by the need for a greater degree of imine formation in order to retain bound iron. Thus, a chelator with slower iron-mediated oxidative dehydrogenation such as tach-3-MeOpyr does not convert to the inert [Fe(L-monoimine)]²⁺ form as quickly, so it takes longer to sequester iron inertly. An alternative explanation which cannot be distinguished at this time is that the oxidative dehydrogenation activity forms reactive oxygen species which cause cell death. It is also possible that tach-3-MeOpyr does not as readily diffuse to the regions of the cell where iron may be bound.

³**Crystal data.** Suitable crystals of [Fe(tach-3-Mepyr)]Cl₂•2MeOH were grown from a MeOH/MeCN (1:1 v/v) solution of Fe(tach-3-Mepyr)Cl₂, those of [Fe(tach-3-MeOpyr)](FeCl₄)•2MeOH from a MeOH/MeCN solution by vapor-phase diffusion of Et₂O and those of [Fe(tach-6-Mepyr)](ClO₄)₂•H₂O•MeOH from MeOH at 5°C. **Crystal data for [Fe(tach-3-Mepyr)]Cl₂•2MeOH** (Figure 2): Dark red fragments, crystal dimensions 0.20 × 0.25 × 0.25 mm, C₂₉H₄₆Cl₂FeN₆O₂.50, *M* = 651.47, monoclinic, space group *P*2₁/n, *a* = 10.9853(7), *b* = 13.5083(8), *c* = 21.1005(13) Å, β = 91.1240(10)°, *V* = 3130.5(3) Å³, *Z* = 4, *D_c* = 1.382 g cm⁻³, μ(Mo Kα) = 0.692 mm⁻¹. A total of 19145 reflections were used, 1.79° < θ < 28.19°. A total of 7043 independent reflections having *I* > 2σ(*I*); 337 parameters. Final results: *R*(*F*) = 0.0368, *R_w*(*F*) = 0.0970, GOF = 1.101, maximum residual electronic density = 0.661 eÅ⁻³. **Crystal data for [Fe(tach-3-MeOpyr)](FeCl₄)•Et₂O** (Figure 3): Brown fragments, crystal dimensions 0.15 × 0.15 × 0.03 mm, C₃₁H₄₆Cl₄Fe₂N₆O₄, *M* = 820.24, orthorhombic, space group *P*2₁2₁2₁, *a* = 12.3590(9), *b* = 14.2250(11), *c* = 20.4170(15) Å, α = β = γ = 90°, *V* = 3589.4(5) Å³, *Z* = 4, *D_c* = 1.518 g cm⁻³, μ(Mo Kα) = 1.151 mm⁻¹. A total of 8113 reflections were used, 2.18° < θ < 28.19°. A total of 8113 independent reflections having *I* > 2σ(*I*); 382 parameters. Final results: *R*(*F*) = 0.0413, *R_w*(*F*) = 0.0746, GOF = 0.959, maximum residual electronic density = 0.569 eÅ⁻³. **Crystal data for [Fe(tach-6-Mepyr)](ClO₄)₂•H₂O•MeOH** (Figure 4). Dichroic/red plates, crystal dimensions 0.40 × 0.30 × 0.08 mm, C₅₅H₇₅Cl₄Fe₂N₁₂O₁₈, *M* = 1445.77, triclinic, space group *P*-1, *a* = 9.5245(9), *b* = 17.4908(17), *c* = 19.0395(19) Å, α = 96.239(2)°, β = 95.334(2)°, γ = 95.093(2)°, *V* = 3123.7(5) Å³, *Z* = 4, *D_c* = 1.924 g cm⁻³, μ(Mo Kα) = 1.383 mm⁻¹. A total of 20964 reflections were used, 1.69° < θ < 26.00°. A total of 12159 independent reflections having *I* > 2σ(*I*); 821 parameters. Final results: *R*(*F*) = 0.0607, *R_w*(*F*) = 0.1648, GOF = 1.202, maximum residual electronic density = 1.051 eÅ⁻³.

⁴HeLa cells were obtained from the American Type Culture Collection and grown in a humidified 5% CO₂ atmosphere at 37°C in Dulbecco's modified Eagle's medium (Gibco BRL) supplemented with 10% fetal bovine serum and penicillin/streptomycin. Cells (2 × 10³) were plated in 96 well tissue culture dishes and allowed to attach overnight before test compounds were added at various concentrations. Six replicate cultures were used for each point. After 72 h, viability was assessed using an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, in which MTT is added to the medium and the formation of a reduced product is assayed by measuring the optical density at 560/650 nm after 3 hours. Color formation is proportional to viable cell number[34] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.. IC₅₀ is the concentration of chelator required to inhibit proliferation by 50%.

Conclusions

The chelators tach-3-Mepyr, tach-3-MeOpyr and tachpyr bind Fe(II) strongly and equally well based on ground-state structure, however their rates of iron-mediated oxidative dehydrogenation differ in the order, fast to slow, of tach-3-Mepyr > tachpyr > tach-3-MeOpyr. They are equally cytotoxic to cultured Hela cells, but their rates of cell-killing are also in the order fast to slow of tach-3-Mepyr > tachpyr > tach-3-MeOpyr. The underlying biological and chemical reasons for these relationships are not yet understood. Introduction of steric bulk produces an elongated Fe(II)-N_{py} bond in the structure of [Fe(tach-6-Mepyr)]²⁺ and accordingly this complex has a substitutable coordination site in MeCN solution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

The ^1H NMR spectra of the oxidation process of **2** and **4** and ESI-MS spectra of **1** and **2** are contained in the supplementary data, which can be found in the online version, at _____. The CIF files for the structural analyses of $[\text{Fe}(\text{tach-3-Mepyr})]\text{Cl}_2 \cdot 2\text{MeOH}$, $[\text{Fe}(\text{tach-3-MeOpyr})](\text{FeCl}_4) \cdot \text{Et}_2\text{O}$ and $[\text{Fe}(\text{tach-6-Mepyr})](\text{ClO}_4)_2 \cdot \text{H}_2\text{O} \cdot \text{MeOH}$ have been deposited with the Cambridge Crystallographic Data Centre, CCDC nos. 621772, 620255, and 620253. Copies of this information may be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1233-336033; e-mail deposit@ccdc.cam.ac.uk).

Figure 1. Proton NMR spectrum (D₂O, 25 °C) of the progressing oxidation of [Fe(tach-3-Mepyr)]²⁺ (24 h, upper; 4 h, lower) showing the monimine species as a singlet at δ 9.56 ppm and diimine as a doublet centered at δ 9.25 ppm. The integration set-up used to quantitate the monimine oxidation product is directly under the spectral trace.

Figure 2.
Structure of the complex cation of [Fe(tach-3-Mepyr)]Cl₂ (ORTEP; 50% probability ellipsoids) showing the atom-numbering scheme.

Figure 3. Structure of the complex cation of [Fe(tach-3-MeOpyr)](FeCl₄) (ORTEP; 50% probability ellipsoids) showing the atom-numbering scheme.

Figure 4. Structure of the complex cation of [Fe(tach-6-Mepyr)](ClO₄)₂ (ORTEP; 50% probability ellipsoids) showing the atom-numbering scheme.

Scheme 1.
 Fe(II) complexes studied herein.

Scheme 2.
Chelators for structural comparisons to **1**, **2**, and **3**.

Table 1

Ratio of the area under the monoimine peak of $[\text{Fe}(\text{L-ox-2})]^{2+}$ to the area under the aromatic peaks (^1H NMR, D_2O , 25°C).

Time (h)	1^a	2^b	$[\text{Fe}(\text{tachpyr})]^{2+\text{c}}$	theoretical maximum^d
0	0	0	0	0
4	0.066	0.047	0.048	0.111
24	0.071	0.049	0.056	

^a Diimine species was seen at both 4 h and 24 h for **1**, thus, the degree of oxidation for **1** at 4h and 24 h is greater than indicated by these data.

^b Diimine species was not observed for **2** during this study.

^c Diimine species was seen at 24 h for $[\text{Fe}(\text{tachpyr})]^{2+}$, thus, the degree of oxidation for $[\text{Fe}(\text{tachpyr})]^{2+}$ at 24 h is greater than indicated by these figures.

^d maximum for total conversion to $[\text{Fe}(\text{L-ox-2})]^{2+}$ without formation of $[\text{Fe}(\text{L-ox-4})]^{2+}$.

Table 2

Selected structural parameters of [Fe(tach-3-Mepyr)]²⁺ (1), [Fe(tach-3-MeOpyr)]²⁺ (2), [Fe(tach-6-Mepyr)]²⁺ (3), and reference compounds.

	Spin – State	Fe-N _(amine) distance (Å)	Fe-N _(py) distance (Å)	Bite angle, α(°)	Twist angle, φ (°)
[Fe(tach-3-Mepyr)] ²⁺	LS	2.0078 (13)	1.9708 (13)	83.16 (5)	54.9
		2.0118 (14)	1.9725 (13)	83.77 (6)	
		2.0127 (14)	1.9734 (14)	83.91 (5)	
[Fe(tach-3-MeOpyr)] ²⁺	LS	2.016 (2)	1.974 (3)	84.01 (10)	54.6
		2.009 (2)	1.983 (3)	84.04 (10)	
		2.010 (3)	1.969 (2)	84.00 (11)	
	LS	2.001 (6)	1.979 (6)	83.1 (2)	48.9
	LS	2.030 (1)	1.979 (1)	82.92 (4)	53.7
		2.021 (1)	1.987 (1)	83.08 (4)	
[Fe(tach-6-Mepyr)] ²⁺ [a]	HS	2.026 (1)	1.991 (1)	80.75 (4)	52.1
		2.169 (3)	2.234 (3)	76.85 (11)	
		2.173 (3)	2.238 (3)	78.42 (12)	
		2.181 (3)	2.384 (3)	78.48 (12)	
		2.151 (3)	2.219 (3)	77.50 (13)	
	HS	2.173 (3)	2.223 (3)	77.70 (13)	53.9
		2.190 (3)	2.376 (3)	77.99 (13)	
		2.257 (5)	2.226 (5)	77.04 (19)	
		2.178 (4)	2.188 (4)	76.9 (2)	
		2.192 (4)	2.218 (4)	75.2 (2)	
[Fe(tpcd)]2+[33] mer- [Fe(ampy) ₃]2+[32]	HS	2.189 (4)	2.223 (4)	75.6 (2)	41.8 41.1
		2.128 (4)	2.203 (4)	80.1 (2)	
		2.133 (4)	2.216 (4)	80.3 (2)	
		2.135 (4)	2.566 (5)	74.89 (15)	
		2.115 (5)	2.213 (5)	79.9 (2)	
[Zn(tach-6-Mepyr)]2+[a] ₁ [9]	-	2.133 (5)	2.218 (5)	79.5 (2)	49.5
		2.151 (5)	2.527 (5)	76.3 (2)	

[a] Structure has two independent molecules in the asymmetric unit and both corresponding sets of data are given.

Table 3

IC₅₀ values (μM) of tachpyr, tach-3-Mepyr, and tach-3-MeOpyr after 24, 48, and 72 h.

	24 h	48 h	72 h	ΔIC ₅₀ (24h – 72h)
tachpyr	22	6	6	16
tach-3-Mepyr	11	6	6	5
tach-3-MeOpyr	130	22	12	118