Chapter 6

Synthesis and characterisation of non-Heme $\text{Fe}^{\text{III}}$-OCl complexes

The conversion of $[(\text{MeN4Py})\text{Fe}^{\text{II}}(\text{Cl})](\text{Cl})$ (1) to $[(\text{MeN4Py})\text{Fe}^{\text{IV}}=\text{O}]^{2+}$ via an $[(\text{MeN4Py})\text{Fe}^{\text{III}}(\text{OCl})]^{2+}$ intermediate using 1-2 equivalents of aqueous NaOCl or Ca(OCl)$_2$ in water is reported for the first time. The intermediate $[(\text{MeN4Py})\text{Fe}^{\text{III}}(\text{OCl})]^{2+}$ is characterised by a range of spectroscopic techniques and its structure is considered using DFT methods.

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6.1 Introduction

The wide range of chemical transformations that can be achieved with metalloproteins provides a constant source of inspiration to synthetic chemistry and homogeneous catalysis. Mononuclear non-heme iron and copper proteins carry out oxidative transformations such as C-H bond hydroxylation\(^1\), epoxidation\(^2\) and hetero atom oxygenation of many organic substrates.\(^3\) Their catalytic capability is attributed to the remarkable properties of metal based species, including iron(III)-hydroperoxo, iron(III)-peroxo and iron(IV)-oxo species, formed by the reaction, for example, between an iron(II) complex and molecular oxygen.\(^4\) Functional and structural mimics for these intermediates are important in both understanding biological activity, as well as, for synthetic applications, in understanding the mechanisms by which metalloenzymes operate.\(^5\) Biomimetic metal complexes that generate similar active intermediates or provide similar coordination environments are viewed as being key to understanding enzymatic mechanisms. Spectroscopic studies of trapped intermediates have employed X-ray crystallography, X-ray absorption etc., Mössbauer, EPR, UV/Vis absorption and (resonance) Raman spectroscopies and electrochemistry.\(^6\) The spectroscopic characterisation of potential intermediates has provided insight into biological systems and in our understanding of catalytic mechanisms.\(^5\)

With notable exceptions, most oxygen activated iron intermediates have been generated in organic solvents and, hence, direct comparison with intermediates formed in biological systems is often difficult. Generation and characterisation of such intermediates in aqueous media gives rise to the possibility of direct comparison with that of active intermediates in metalloenzymes. Chloroperoxidases, for example are a class of enzyme capable of catalysing oxidation of Cl\(^-\), Br\(^-\) and I\(^-\) with H\(_2\)O\(_2\) and forming carbon-halogen bonds in the presence of the halogen acceptor. It has been proposed\(^7\) that the formation of an Fe\(^{III}\)-OCl adduct leads to halogen insertion in to substrates. To the best of our knowledge, evidence for such an Fe\(^{III}\)-OCl species has not been reported with the exception of a single example of a heme complex reported recently.\(^8\)

Fe\(^{II}\) complexes based on the pentadentate ligands N4Py and MeN4Py have served as functional and structural models for Fe\(^{II}\)-BLM (Scheme 1). As with Fe\(^{II}\)-BLM, these complexes have been shown to be capable of activating inert C-H bonds in the presence of an oxidant\(^9\) and in the oxidative cleavage of DNA with molecular oxygen.\(^10\) It has been shown that addition of oxidants such as H\(_2\)O\(_2\), PhIO and CAN
generates Fe^{III}-OOH and Fe^{IV}=O intermediates, which are kinetically competent in the reactions catalysed by these complexes. Spectroscopic evidence for Fe^{III}-OOH and Fe^{III}(OO) has been reported by Que and Feringa et al., and the crystal structure of [(N4Py)Fe^{IV}(O)]^{2+} was reported by Que et al. In this chapter the direct conversion of [(MeN4Py)Fe^{II}(Cl)](Cl) (1) to [(MeN4Py)Fe^{IV}(O)]^{2+} via an intermediate with 1-2 equivalents of aq. NaOCl or Ca(OCl)₂ in water at low pH is reported as well as the pathways involved in the reaction of NaOCl with Fe^{II} complexes to yield Fe^{IV}=O species. Similar results were obtained with the analogous complex [(N4Py)Fe^{II}(CH₃CN)](ClO₄)₂ (2). The reactions between these complexes and ClO⁻ are compared and contrasted at neutral and low pH.

As discussed in chapter 2 for complexes 1 and 2, immediate ligand exchange of the Cl-/CH₃CN ligand(s) for hydroxide or aqua ligands, depending on pH, occurs in water. Complex 1 is in general more stable towards ligand dissociation at low and high pH compared to 2. In the case of 2, ligand dissociation is observed at pH below 2.5. Full dissociation of the N4Py ligand was observed pH > 10. In the pH range 5 to 8, complexes 1 and 2 show an equilibrium between two distinct species (Scheme 1).13

6.2 Results and discussions
Complexes 1 and 2 were available from earlier studies. [(MeN4Py)Fe^{IV}(O)]^{2+} and [(N4Py)Fe^{IV}(O)]^{2+} were generated by addition of 2.2 equiv of CAN to the aqueous solution of 1 or 2, respectively, and were characterised by UV/Vis absorption, (resonance)Raman and ¹H NMR spectroscopy (see chapter 4).
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6.2.1 UV/Vis absorption spectroscopy

6.2.1.1 Reaction of 1 and 2 with NaOCl in water at low pH

Addition of 0.5 equiv of NaOCl to a solution of 1 in water (pH 2.9) led to a decrease in absorbance at 490 nm (within 150 s) and the appearance of a band at 480 nm. Addition of a further 0.5 equiv of NaOCl results initially in an increase in absorbance at 480 nm, after which, a band at 670 nm appeared concomitant with a decrease in absorbance at 480 nm (Figure 1).

Figure 1 Changes in UV/Vis absorption (a) after addition of 0.5 equiv of NaOCl to an aqueous solution of 1 (0.5 mM, pH 2.9) and (b) corresponding time dependence of the absorbance at 480 nm and at 670 nm, (c) after addition of a second 0.5 equiv of NaOCl and (d) corresponding time dependence of the absorbance at 480 nm and at 670 nm. # the sharp spike is an instrumental artefact. ‘t’ is in s.
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![Graphs and figures]

Figure 2 Changes in UV/Vis absorption (a) after addition of 1 equiv of NaOCl to an aqueous solution of 1 (0.5 mM, pH 2.2) and (b) corresponding time dependence of the absorbance at 480 nm and at 670 nm, (c) after addition of a second 1 equiv of NaOCl, (d) corresponding time dependence of the absorbance at 480 nm and at 670 nm and (e) the effect of NaOH on the spectrum of Fe$^{IV}$=O species. # the sharp spike is an instrumental artefact. ‘t’ is in s.

Direct addition of 1 equiv of NaOCl to 1 at pH 2.2 led to a near complete loss in absorbance at 490 nm within 15 s, indicating a complete loss of the Fe$^{II}$ complex, after which a new band grew at 480 nm and reached a maximum absorbance within ca. 2 min. Subsequently a decrease but not complete loss in absorbance at 480 nm, concomitant with an increase in absorbance at 670 nm, was observed (Figure 2).
Upon addition of a second equivalent of NaOCl, the band at 480 nm increased initially with no change in absorbance at 670 nm. After the absorbance at 480 nm reached a maximum, the absorbance at 670 nm began to increase with a concomitant decrease in absorbance at 480 nm. The absorption band in the NIR region (i.e. at 670 nm) is typical of an Fe$^{IV}=O$ species. Under acidic conditions the absorption at 670 nm is persistent but disappears rapidly upon an increase in pH (Figure 2e).

6.2.1.2 Reaction of 1 and 2 with NaOCl at pH 7 - 8

![Figure 3](image)

**Figure 3** Changes in UV/Vis absorption (a) after addition of 0.5 equiv of NaOCl to an aqueous solution of 1 (0.5 mM, pH 7.4), (b) corresponding time dependence of the absorbance at 480 nm and at 670 nm, (c) after addition of a second 0.5 equiv of NaOCl and (d) corresponding time dependence of the absorbance at 480 nm and at 670 nm. # the sharp spike is an instrumental artefact. ‘t’ is in s.

In contrast to that observed at low pH, addition of 0.5 equiv of NaOCl to an aqueous solution of 1 (0.5 mM) at pH 7.4 resulted in an immediate (< 4 s) disappearance of a band at 490 nm and the appearance of the band at 480 nm. An absorption band at 670 nm was not observed. A second addition of 0.5 equiv NaOCl results in an increase in absorbance at 670 nm with concomitant loss in
absorbance at 480 nm (Figure 3). It is shown in Figures 2 and 3, that the rate of formation of the species that absorbs at 480 nm is slower at low pH.

6.2.1.3 Comparison with [(N4Py)FeII(CH3CN)](ClO4)2 (2)

Figure 4 Changes in UV/Vis absorption (a) after addition of 0.5 equiv of NaOCl to an aqueous solution of 2 (0.5 mM, pH 3.3) and (b) corresponding time dependence of the absorbance at 495 nm and at 675 nm, (c) after addition of a second 0.5 equiv of NaOCl and (d) corresponding time dependence of the absorbance at 495 nm and at 675 nm. # the sharp spike is an instrumental artefact. ‘t’ is in s.

Parallel experiments were carried out on the related complex [(N4Py)Fe(CH3CN)](ClO4)2 (2). In contrast to 1, addition of 0.5 equiv of NaOCl to an aqueous solution of 2 (at pH = 3.3)\textsuperscript{17,18} generates [(N4Py)FeIV(O)]\textsuperscript{2+} (\(\lambda_{\text{max}}\) at 675 nm)\textsuperscript{19} immediately with a weaker absorption at 495 nm. Addition of a second 0.5 equiv of NaOCl resulted in a further increase in absorption at 675 nm (i.e. [(N4Py)FeIV(O)]\textsuperscript{2+}, Figure 4). Interestingly, addition of 0.5 equiv of NaOCl to an aqueous solution of 2 (at pH = 6.5) generates [(N4Py)FeIV(O)]\textsuperscript{2+} (\(\lambda_{\text{max}}\) at 675 nm)\textsuperscript{19} rapidly without the intermediate appearance of an absorption band at 495 nm. Furthermore, a second batch addition of 0.5 equiv of NaOCl to the same
solution results in an increase in absorbance at 675 nm. At pH 6.5 - 7 the band at 495 nm was not observed.

Figure 5 Changes in UV/Vis absorption (a) after addition of 0.5 equiv of NaOCl to an aqueous solution of 2 (0.5 mM, pH 6.5) and (b) corresponding time dependence of the absorbance at 495 nm and at 675 nm, (c) after addition of a second 0.5 equiv of NaOCl and (d) corresponding time dependence of the absorbance at 495 nm and at 675 nm. # the sharp spike is an instrumental artefact. ‘t’ is in s.

6.2.2 $^1$H NMR spectroscopy

$^1$H NMR spectroscopy was employed to characterise the species formed in the reaction of 1 and 2 with NaOCl in the neutral and low pH. Addition of 0.5 equiv of NaOCl to an aqueous solution of 1 (at pH 2.2) resulted in a loss of all signals in the $^1$H NMR spectrum. The $^1$H NMR spectrum obtained after the appearance of the blue species generated with 2 equiv of NaOCl in water matched that of $\left[(\text{MeN}_4\text{Py})\text{Fe}^{\text{IV}}(\text{O})\right]^{2+}$ generated with CAN.20 Similarly, the blue species generated in the reaction between 1 and NaOCl in water is identical to that reported for $\left[(\text{N}4\text{Py})\text{Fe}^{\text{IV}}(\text{O})\right]^{2+}$ in the literature.12
**Figure 6** $^1$H NMR spectra of [(MeN4Py)Fe$^{IV}$O(O)]$^{2+}$ generated with CAN from 1 in D$_2$O (top) and blue species generated with 1 (5 mM) in D$_2$O at pH 2.2 (bottom) with 2 equiv of NaOCl.

### 6.2.3 EPR spectroscopy

The EPR spectra obtained by flash freezing samples at 77 K, 60 s after addition of 2 equiv of NaOCl to an aqueous solution of 1 (1 mM, pH 2.2) shows signals originating from two distinct low spin Fe(III) complexes at $g = 2.42, 2.26, 2.15, 1.97$ and 1.92. The signals at $g = 2.42, 2.15$ and 1.92 are assigned to [(MeN4Py)Fe$^{III}$(OH)]$^{2+}$.

The remaining signals are assigned to a new low spin Fe(III) intermediate. Recently, Hiroshi et al assigned a complex with $g$ values of 2.256, 2.137 and 1.964 as being [(TPFP)Fe$^{III}$(OCl)$_2$]$^-$. These values match well with the signals observed in the present case. The signal at 2.13 is absent, however, which is likely to be due to overlap with the signal of [(MeN4Py)Fe$^{III}$(OH)]$^{2+}$. A minor amount of high spin Fe$^{III}$ at $g = 4.39$ was observed. Residual Fe$^{III}$ signals were observed in the EPR spectrum of the [(MeN4Py)Fe$^{IV}$(O)]$^{2+}$ (670 nm species), consistent with the consumption of the Fe$^{III}$-OH and Fe$^{III}$-OCl species. [(MeN4Py)Fe$^{IV}$(O)]$^{2+}$ is itself EPR silent at 77 K. Addition of aqueous NaOH to a solution of [(MeN4Py)Fe$^{IV}$(O)]$^{2+}$ generated with NaOCl gave rise to high spin Fe$^{III}$ signals at $g = 9.06, 5.08$ and 4.27 corresponding to a high spin Fe(III) complex.
Figure 7 EPR spectra, at 77 K, of (a) [(MeN4Py)Fe^{III}(OCl)]^{2+} and (b) [(MeN4Py)Fe^{IV}(O)]^{2+} generated by reaction of 1 (1 mM, pH 2.2) with 2 equiv of NaOCl in water at room temperature and (c) the effect of NaOH on the spectrum of [(MeN4Py)Fe^{IV}(O)]^{2+} (670 nm absorbing species).

Figure 8 (a) EPR spectra, at 77 K, of 2 after reaction with 1 equiv (bottom) and 2 equiv (top) of NaOCl in water (1 mM, pH 2.8) and (b) EPR spectra, at 77 K, of 2 after reaction with 0.5 - 2 equiv of NaOCl in water (1 mM, pH 6.5) and a solution of [(N4Py)Fe^{IV}(O)]^{2+}.

Similar experiments were carried out with 2. As for 1, the EPR spectrum obtained from flash frozen samples when the absorbance at 495 nm was at a maximum (generated by adding 2 equiv of NaOCl to the aqueous solution of 2, 1 mM, pH 2.8) shows signals at \( g = 2.42, 2.26, 2.16, 1.97 \) and 1.92 characteristic for low spin Fe(III) complexes. In contrast to 1, however the contribution of the high spin Fe(III) signal was higher (Figure 8). At medium pH (6.5), a signal related to a high spin Fe(III) species was observed (Figure 8).
6.2.4 ESI-MS analysis

Cryo spray ESI-MS has been applied recently to the analysis of first row transition metal complexes and their intermediates formed by reaction with a range of oxidants. Addition of 1 equiv of NaOCl to an aqueous solution of I (0.5 mM, pH 2.2), resulted in the appearance of several major ions $[(\text{MeN4Py})\text{Fe}^\text{III}(\text{OH})]^2^+ (m/z 227.066), [(\text{MeN4Py})\text{Fe}^\text{IV}(\text{O})]^2^+ (m/z 226.563), [(\text{MeN4Py})\text{Fe}^\text{III}(\text{OCl})(\text{ClO}_4)]^+ (m/z 587.043), [(\text{MeN4Py})\text{Fe}^\text{III}(\text{OH})(\text{ClO}_4)]^+ (m/z 553.080), [(\text{MeN4Py})\text{Fe}^\text{IV}(\text{O})(\text{ClO}_4)]^+ (m/z 552.075) and [(\text{MeN4Py})\text{Fe}^\text{III}(\text{ClO}_4)]^+ (m/z 536.079). The only ion in the Fe(II) oxidation state observed was $[(\text{MeN4Py})\text{Fe}^\text{II}(\text{ClO}_4)]^+$, which decreases in intensity over time. Due to small differences in their m/z values, the ions $[(\text{MeN4Py})\text{Fe}^\text{III}(\text{OH})]^+ & [(\text{MeN4Py})\text{Fe}^\text{IV}(\text{O})]^2^+ \text{ and } [(\text{MeN4Py})\text{Fe}^\text{III}(\text{OH})(\text{ClO}_4)]^+$ & $[(\text{MeN4Py})\text{Fe}^\text{III}(\text{O})(\text{ClO}_4)]^+ (m/z 552.075)$ overlap. Addition of a second equiv of NaOCl increases the intensity of the $[(\text{MeN4Py})\text{Fe}^\text{IV}(\text{O})(\text{ClO}_4)]^+ (m/z 552.075)$ signal concomitantly with a decrease in intensity of other mono cationic ions. Eventually $[(\text{MeN4Py})\text{Fe}^\text{III}(\text{OCl})(\text{ClO}_4)]^+$ is not observed, and only $[(\text{MeN4Py})\text{Fe}^\text{IV}(\text{O})(\text{ClO}_4)]^+ (m/z 552.075)$ was observed. With Na$^{18}$OCl instead of Na$^{16}$OCl all mono cationic ions except for $[(\text{MeN4Py})\text{Fe}^\text{II}(\text{ClO}_4)]^+ (m/z 536.079)$, were shifted by two m/z units and dicationic ions shifted by one m/z unit. This supports their assignment as $[(\text{MeN4Py})\text{Fe}^\text{III}(^{18}\text{OH})]^2^+ (m/z 228.070), [(\text{MeN4Py})\text{Fe}^\text{IV}(^{18}\text{O})]^2^+ (m/z 227.564), [(\text{MeN4Py})\text{Fe}^\text{III}(^{18}\text{OCl})(\text{ClO}_4)]^+ (m/z 589.047), [(\text{MeN4Py})\text{Fe}^\text{III}(^{18}\text{OH})(\text{ClO}_4)]^+ (m/z 555.084)$ and $[(\text{MeN4Py})\text{Fe}^\text{IV}(^{18}\text{O})(\text{ClO}_4)]^+ (m/z 554.078)$ (Figure 9).23
Figure 9 Experimental and simulated Cryo ESI-MS spectra obtained from the reaction mixture containing NaOCl and 1 in H$_2$O$^{16}$ and H$_2$O$^{18}$ at pH 2.5 (a) [(MeN$_4$Py)Fe$^{III}$(OCl)(ClO$_4$)]$^+$ (m/z 587.04) (b) [(MeN$_4$Py)Fe$^{IV}$(O)(ClO$_4$)]$^+$ (m/z 552.07) generated with Na$^{16}$OCl and (c) [(MeN$_4$Py)Fe$^{III}$(18OCl)(ClO$_4$)]$^+$ (m/z 587.04) and (d) [(MeN$_4$Py)Fe$^{IV}$(18O)(ClO$_4$)]$^+$ (m/z 552.07) generated with Na$^{18}$OCl.

Parallel experiments were carried with complex 2. As for complex 1, addition of 1 or 2 equiv of NaOCl to an aqueous solution of 2 (0.5 mM, pH 3), shows mono cationic ions [(N$_4$Py)Fe$^{III}$(OCl)(ClO$_4$)]$^+$ (m/z 573.027), [(N$_4$Py)Fe$^{III}$(OH)(ClO$_4$)]$^+$ (m/z 539.064), [(N$_4$Py)Fe$^{IV}$(O)(ClO$_4$)]$^+$ (m/z 538.058) and [(N$_4$Py)Fe$^{II}$(ClO$_4$)]$^+$ (m/z 522.064) and dicatonic ions [(N$_4$Py)Fe$^{III}$(OH)]$^{2+}$ (m/z 220.058), [(N$_4$Py)Fe$^{IV}$(O)]$^{2+}$ (m/z 219.555). The peaks related to Fe$^{IV}$=O increased in intensity over time where as other ions decrease in intensity. Again with the labelled Na$^{18}$OCl, all mono cationic, with exception of [(N$_4$Py)Fe$^{II}$(ClO$_4$)]$^+$, and increased by two m/z units and dicatonic ions were increased by one m/z unit.

To avoid interference of the water present in aqueous NaOCl, we carried out similar experiments using Ca(OCl)$_2$ (as solid). As with NaOCl, addition of 2 or 4 equiv of Ca(OCl)$_2$ to an aqueous solution of 1 (0.5 mM, pH 2.5), shows mono cationic ions [(MeN$_4$Py)Fe$^{III}$(OCl)(ClO$_4$)]$^+$ (m/z 587.042), [(MeN$_4$Py)Fe$^{III}$(OH)(ClO$_4$)]$^+$...
Synthesis and characterisation of Fe\textsuperscript{III}-OCl species

(m/z 553.080), [(MeN\textsubscript{4}Py)Fe\textsuperscript{IV}(O)(ClO\textsubscript{4})]\textsuperscript{+} (m/z 552.073) and [(MeN\textsubscript{4}Py)Fe\textsuperscript{II}(ClO\textsubscript{4})]\textsuperscript{+} (m/z 536.079) and dicaticionic ions [(MeN\textsubscript{4}Py)Fe\textsuperscript{II}(OH)]\textsuperscript{2+} (m/z 227.066), [(MeN\textsubscript{4}Py)Fe\textsuperscript{IV}(O)]\textsuperscript{2+} (m/z 226.563). With labelled Ca\textsuperscript{18OCl}\textsubscript{2} mono caticionic ions except [(MeN\textsubscript{4}Py)Fe\textsuperscript{II}(ClO\textsubscript{4})]\textsuperscript{+} (m/z 536.079), increased by two m/z units and dicaticionic ions were increased by one m/z unit (Figure 10).

Figure 10 Experimental and simulated Cryo ESI-MS spectra obtained from the reaction mixture containing Ca(OCl)\textsubscript{2} and \textbf{1} in H\textsubscript{2}O\textsuperscript{16} and H\textsubscript{2}O\textsuperscript{18} at pH 2.5 (a) [(MeN\textsubscript{4}Py)Fe\textsuperscript{III}(OCl)(ClO\textsubscript{4})]\textsuperscript{+} (m/z 587.04) (b) [(MeN\textsubscript{4}Py)Fe\textsuperscript{IV}(O)(ClO\textsubscript{4})]\textsuperscript{+} (m/z 552.07) generated with Ca\textsuperscript{16OCl}\textsubscript{2} and (c) [(MeN\textsubscript{4}Py)Fe\textsuperscript{III}(18OCl)(ClO\textsubscript{4})]\textsuperscript{+} (m/z 587.04) and (d) [(MeN\textsubscript{4}Py)Fe\textsuperscript{IV}(18O)(ClO\textsubscript{4})]\textsuperscript{+} (m/z 552.07) generated with Ca\textsuperscript{18OCl}\textsubscript{2}.

Under neutral conditions Fe\textsuperscript{III}-OCl species were not observed, and only signals assignable to [(N\textsubscript{4}Py)Fe\textsuperscript{IV}(O)(ClO\textsubscript{4})]\textsuperscript{+} (m/z 538.061) for \textbf{2} and [(MeN\textsubscript{4}Py)Fe\textsuperscript{IV}(O)]\textsuperscript{2+} (m/z 226.565) for \textbf{1} were observed upon addition with 1 or 2 equiv of NaOCl.
6.2.5 Resonance Raman spectroscopy

Resonance Raman spectroscopy was employed to characterise the Fe$^{III}$-OCl and Fe$^{IV}$=O intermediates presumed to form in solution. Although the Fe$^{IV}$=O species absorb strongly at 670 nm, $\lambda_{\text{exc}}$ 473 nm was chosen to take advantage of the potential resonance enhancement of the Raman scattering of the putative Fe$^{III}$-OCl species.

![Figure 11](image)

Figure 11 Reaction between 1 (1 mM in H$_2$O at pH 2.2) and NaOCl (two equiv followed by another two equiv in H$_2$O) followed by Raman spectroscopy at $\lambda_{\text{exc}}$ 473 nm. Spectra were normalized to the ClO$_4^-$ band at 934 cm$^{-1}$ except for the initial spectrum. The legend is time in minutes after addition of NaOCl.

Addition of 2 equiv of NaOCl to an aqueous solution of 1 (1 mM in H$_2$O at pH 2.2) was followed by Raman spectroscopy at $\lambda_{\text{exc}}$ 473 nm. Before addition the intense (resonantly enhanced) Raman scattering of the low spin Fe$^{II}$-OH species was observed.$^{13}$ Immediately, after addition of 2 equiv of NaOCl to an aqueous solution of 1 bands at 580, 653 and 673 cm$^{-1}$ appeared (within one minute total acquisition time). These bands are in the region typical for Fe$^{III}$-O vibrational modes.$^{24}$ Over time these bands decrease in intensity and a new band at 843 cm$^{-1}$ increases concomitantly. Addition of another two equiv of NaOCl regenerates the bands in the region 570 to 750 cm$^{-1}$, which again decrease over time with only the band at
Figure 12 Reaction between 1 (1 mM in $^{18}$OH$_2$ at pH 2.2) and Na$^{18}$OCl (two additions of two equiv in $^{18}$OH$_2$) followed by Raman spectroscopy at $\lambda_{exc}$ 473 nm. Spectra were normalized to the ClO$_4^-$ band at 934 cm$^{-1}$ except for the initial spectrum. The legend is time in minutes after addition of Na$^{18}$OCl.

Addition of 2 equiv of Na$^{18}$OCl to a solution of 1 (1 mM in $^{18}$OH$_2$ at pH 2.2) results in the appearance of the bands at 673, 628 and 562 cm$^{-1}$. Again, these bands decrease in intensity over time with a concomitant appearance of a new band at 807 cm$^{-1}$ (Figures 12 and 13). Three bands are shifted when compared with the non-$^{18}$O-labelled samples. The band at 673 cm$^{-1}$ was not sensitive to $^{18}$O labelling. The bands at 843, 653 and 580 cm$^{-1}$ shift to 807, 628 and 562 cm$^{-1}$. The observed shift of 36 cm$^{-1}$ of the band at 843 cm$^{-1}$ is in good agreement with the calculated shift (for a two atom approximation) for a Fe-O bond (37 cm$^{-1}$). This is in agreement with the assignment of the species formed later in the reaction with an absorption at 670 nm as the [(MeN4Py)Fe$^{IV}$(O)]$^{2+}$ complex.$^{19}$ The bands 653 and 580 cm$^{-1}$ were shifted by 25 and 18 cm$^{-1}$, respectively, these shifts are close to those expected for an Fe-O bond (29 cm$^{-1}$ for the band at 653 cm$^{-1}$ and 26 cm$^{-1}$ for the band at 580 cm$^{-1}$) and O-Cl (26 cm$^{-1}$ for the band at 653 cm$^{-1}$ and 23 cm$^{-1}$ for the band at 580 cm$^{-1}$) modes. Hence definitive assignment of the mode cannot be made on the basis of isotope shift.
Figure 13 Intermediates generated upon the reaction of 1 (1 mM at pH 2.2) with (a) Na$^{16}$OCl in $^{16}$OH$_2$ and (b) Na$^{18}$OCl in $^{18}$OH$_2$ followed by Raman spectroscopy at $\lambda_{exc}$ 473 nm.

Figure 14 Raman Spectra of aqueous NaOCl before and after addition of NaBr at $\lambda_{exc}$ 785 nm. Spectra were normalized to the water band at ca. 1650 cm$^{-1}$.

NaOBr was employed to facilitate band assignments. Addition of 2 equiv of NaOBr$^{25}$ to an aqueous solution of 1 (1 mM in H$_2$O at pH 2.2), results in substantial interference from fluorescence and hence it is not possible to obtain Raman spectra under the conditions employed. The solvent system was changed to water/acetonitrile (1:1) to circumvent this problem. As with water, addition of 2 equiv of NaOCl to a solution of 1 (1:1 water/acetonitrile at pH 2.2) shows bands at 580, 656, 676 and 843 cm$^{-1}$. Addition of 2 equiv of NaOBr instead of NaOCl
shows the bands 843, 673 and 629 cm\(^{-1}\). The band at 843 cm\(^{-1}\) was not affected. The band at 676 was moderately shifted (3 cm\(^{-1}\)) by replacement of Cl with Br, this band is also oxygen insensitive indicating that it is a Fe-N mode. The 656 cm\(^{-1}\) band was shifted to 629 cm\(^{-1}\), the observed shift of 27 cm\(^{-1}\) is too small to be assign to be due to an O-Br vibrational mode. Interestingly, the band at 580 cm\(^{-1}\) was not observed. Even though Hiroshi\(^8\) assigned the 786 cm\(^{-1}\) band in the heme (Fe\(^{III}\)-OCl) system as an O-Cl vibrational mode, \(^{18}\)O labelling and bromine labelling data support the assignment of the 580 cm\(^{-1}\) band as the O-Cl vibrational mode. The other oxygen sensitive mode at 656 cm\(^{-1}\) was tentatively assigned to an Fe\(^{III}\)-O stretch. The shift of 27 cm\(^{-1}\) might be due to the effect of bromine on the Fe-O stretch (i.e. a change in force constant).

![Figure 15](image)

**Figure 15** Reaction of 1 (1 mM in H\(_2\)O at pH 2.2) with (a) NaOCl and (b) NaOBr followed by Raman spectroscopy at \(\lambda_{exc}\) 473 nm.

### 6.2.6 DFT calculations

DFT optimised geometries of the complexes \([(\text{MeN4Py})\text{Fe}^{\text{III}}(\text{OCl})]^{2+}\) (S = 1/2) and \([(\text{MeN4Py})\text{Fe}^{\text{IV}}(\text{O})]^{2+}\) (S = 1) are shown in Figure 16. Both are in distorted octahedral geometries. Bond distances of 2.000 Å and 2.026 Å are observed for Fe-N\(_{\text{py}}\) and Fe-N\(_{\text{amine}}\) respectively for \([(\text{MeN4Py})\text{Fe}^{\text{III}}(\text{OCl})]^{2+}\), which are comparable to low spin Fe(III) polypyridyl complexes in agreement with the EPR data. The Fe\(^{III}\)-O and O-Cl bond distances are 1.807 and 1.712 Å, respectively. Nearly linear geometry for N\(_{\text{amine}}\)-Fe-O (175.09\(^{\circ}\)) was observed, whereas a bent geometry with an angle of 123.32\(^{\circ}\) was observed for Fe-O-Cl. In the case of \([(\text{MeN4Py})\text{Fe}^{\text{IV}}(\text{O})]^{2+}\), distances of 1.991 Å and 1.994 Å are observed for the Fe-N\(_{\text{py}}\) bonds. The Fe-O bond (1.624 Å) is comparable to the X-ray structural Fe-O bond distance for
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\[(\text{N4Py})\text{Fe}^{IV}(O)\]\(^{2+}\) (1.639(5) Å).\(^{12}\) The Fe-O bond is shorter in \([(\text{MeN4Py})\text{Fe}^{IV}(O)\]\(^{2+}\) compared to \([(\text{MeN4Py})\text{Fe}^{III}(\text{OCl})\]\(^{2+}\) as expected due to its double bond character. The Fe-O bond of \([(\text{MeN4Py})\text{Fe}^{IV}(O)\]\(^{2+}\) pulls the iron center out of the plane of the four pyridyl nitrogen atoms compared to \([(\text{MeN4Py})\text{Fe}^{III}(\text{OCl})\]\(^{2+}\) (0.2642 Å vs. 0.2394 Å).

![Figure 16 DFT optimised geometries of \([(\text{MeN4Py})\text{Fe}^{III}(\text{OCl})\]\(^{2+}\) and \([(\text{MeN4Py})\text{Fe}^{IV}(O)\]\(^{2+}\).](image)

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<th></th>
<th>([(\text{MeN4Py})\text{Fe}^{III}(\text{OCl})](^{2+})</th>
<th>([(\text{MeN4Py})\text{Fe}^{IV}(O)](^{2+})</th>
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</thead>
<tbody>
<tr>
<td>Fe-NPy (Å)</td>
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</tr>
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<td></td>
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<tr>
<td></td>
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<tr>
<td>Fe-Namine (Å)</td>
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<td>2.077</td>
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<td>Fe-O (Å)</td>
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<td>O-Cl (Å)</td>
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<td>O-Fe-Namine (°)</td>
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<td>Fe-mean eq. plane (Å)</td>
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<td>0.2642</td>
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### 6.3 Summary and conclusions

In summary, through a combination of UV/Vis absorption, \(^1\)H NMR, EPR and Raman spectroscopy and Cryo ESI-MS, a non-Heme intermediate Fe\(^{III}\)-OCl species that is able to convert to an Fe\(^{IV}\)=O species is observed for the first time. Initially,
the chlorido ligand in 1 and CH₃CN in 2 exchanges with H₂O/OH⁻, depending on the pH of the solution. Addition of 0.5 equiv of NaOCl to an aqueous solution of 1 (pH ~ 2 to 2.5) leads to the formation of an Fe³⁺-OH intermediate, identified by ESI-MS and EPR spectroscopies (appear with m/z 227.066 assigned to [(MeN₄Py)Fe³⁺(OH)]²⁺ and EPR spectrum with g = 2.42, 2.15 and 1.92 characteristic for [(MeN₄Py)Fe³⁺(OH)]²⁺). The initial oxidation however may be due to comproportionation of Fe⁴⁺=O and Fe²⁺-OH which is rapid in aqueous solutions (see chapter 4).

Scheme 2 Reaction between Fe²⁺-OH and Fe⁴⁺=O complexes in water.

Addition of a 2nd equivalent of NaOCl generates the intermediate Fe³⁺-OCl species as confirmed by an absorption band at ca. 480 nm, which shows bands at 580 cm⁻¹ (O-Cl), 656 cm⁻¹ (Fe-O) and 673 cm⁻¹ (Fe-N) in its resonance Raman spectrum at λₑxc 473 nm and a peak at m/z 587.043 assignable to [(MeN₄Py)Fe³⁺(OCl)(ClO₄)]⁺. Over time this intermediate converts to an Fe⁴⁺=O species. Addition of a further equivalent of NaOCl generates more of the Fe³⁺-OCl intermediate, which then converts to additional [(MeN₄Py)Fe⁴⁺(O)]²⁺ again, identified by its characteristic NIR absorption band at 670 nm, EPR silence at 77 K, Raman band at 843 cm⁻¹ (807 cm⁻¹ upon ¹⁸O labelling) assignable to Fe⁴⁺=O, ¹H NMR spectrum and peak at m/z 552.075 assigned to [(MeN₄Py)Fe⁴⁺(O)(ClO₄)]⁺ (Scheme 3). Similar results were obtained when complex 2 was used.

It was apparent from the spectroscopic data that at low pH the generation of the intermediates is slower and the Fe³⁺-OCl species is stabilised compared to neutral pH. The Fe³⁺-OCl intermediate generated from 1 was more stable than the intermediate generated from 2, which may reflect the steric hindrance caused by the methyl group, which pushes the pyridyl groups more towards the iron center. A non-heme M-OCl (i.e. Fe³⁺-OCl) species has been spectroscopically characterised in this study. We believe that this intermediate is capable of inserting the halogen into substrates in a catalytic fashion. Preliminary results indicate that this is the case. Exploring the catalytic activity of this intermediate is expected to improve our understanding of the biological function of haloperoxidases and to support the proposed involvement of such species in the catalytic pathway of vanadium-dependent and heme-dependent haloperoxidases.
Scheme 3 Intermediates formed during the reaction between 1 and 2 in water (pH 2 to 3) and NaOCl.

6.4 Experimental section
The ligands 1,1-di(pyridin-2-yl)-N,N-bis(pyridin-2-ylmethyl)methanamine (N4Py) and 1,1-di(pyridin-2-yl)-N,N-bis(pyridin-2-ylmethyl)ethanamine (MeN4Py) and [(N4Py)Fe(CH3CN)](ClO4)2 (2) were prepared by literature methods.14a For the synthesis of [(MeN4Py)Fe(Cl)](Cl) (1) see chapter 4. Commercially available chemicals were purchased and used without further purification. Ca(OCl)2 and aqueous NaOCl (10 - 15 %) were purchased from Sigma Aldrich. Na18OCl was prepared by dissolving aqueous Na16OCl in 18OH2 with 1:7 v/v. Similarly Ca(18OCl)2 was prepared by dissolving solid Ca(16OCl)2 in 18OH2. All the experiments were carried out at room temperature. Samples were prepared using MilliQ water and pH was adjusted using dilute aqueous HClO4 or H2SO4 and NaOH solutions. Buffers such as phosphate changes the chemistry of these complexes in water, hence, buffers are not employed to control the pH of the reaction mixture in this study.
6.5.1 Physical Methods
For details of UV/Vis absorption, Raman, resonance Raman and $^1$H NMR spectroscopy see the chapter 2. For details of EPR spectroscopy see the chapter 3. High resolution mass spectra (HRMS) were recorded on a Bruker MicrOTOF-Q II™ Instrument at Serveis Tècnics of the University of Girona. A cryospray attachment was used for CSI-MS (cryospray mass spectrometry). Temperature of the nebulizing and drying gases was set at 5 and 0 ºC, respectively. Samples were introduced into the mass spectrometer ion source by direct infusion using a syringe pump and were externally calibrated using sodium formate. The instrument was operated in the positive ion mode. DFT Calculations were performed using unrestricted hybrid density functional level B3LYP$^{26,27}$ in Gaussian-09 program package. All the geometries reported were result of full optimization without geometric constraints and using analytical frequency to validate the absence of imaginary frequency. The basis sets used are LANL2DZ and LANL2TZ+(f) with ECP core potential for iron and from 6-311+G* to 6-311+G(d,p) for the other elements (H, C, N, O and Cl).

6.5 Acknowledgements
S. K. Padamati is kindly acknowledged for assistance in obtaining the data on 2. Dr. L. Gómez is thanked for assistance with Cryo ESI-MS experiments. D. Angelone and M. G. Quesne are acknowledged for DFT calculations.

6.6 References
(15) The pH of the solution changed from 2.9 to 2.4 and 2.5 for first and second batch additions of 0.5 equiv of NaOCl, respectively.
(16) The initial pH of the solution 7.4 changed to 8.4 after the addition of first 0.5 equiv of NaOCl and persistent after addition of second 0.5 equiv of NaOCl.
(17) The pH of the solution changed from 3.3 to 3.5 and 3.7 for first and second batch additions of 0.5 equiv of NaOCl, respectively.
(18) Due to the instability of 2 in water towards ligand dissociation at pH < 3, experiments were performed at ca. pH 3.
(20) Chapter 4
(23) Assignment of the origin of the oxygen in [([MeN4Py]FeIV(O)]2+ is not possible due to the rapid exchange of NaOCl with H2O.
(25) Aqueous NaOBr was prepared by adding solid NaBr to an aqueous solution of NaOCl. The O-Cl stretching mode in the NaOCl (711 cm⁻¹) was replaced by a new band at 618 cm⁻¹ assigned to O-Br stretch after addition of NaBr (Figure 14).