CHAPTER 8

Conclusions and Perspectives

8.1 Introduction

The research described in this thesis was initiated by the discovery and characterization of the low-spin \([(N4Py)Fe^{III}O(OH)]^{2+}\) intermediate (1), which was observed in the reaction of \([(N4Py)Fe(CH_3CN)][ClO_4]_2\) (2) with \(H_2O_2\) (figure 1).\(^1\) The Fe^{III}OOH structural feature in 1 is also found in natural systems like iron bleomycin\(^2\) and cytochrome P450,\(^3\) and 1 represented the first example of such an intermediate for which this formulation could be established.

\[ \text{Figure 1. The ligand N4Py and the } [(N4Py)Fe^{III}O(OH)]^{2+} \text{ intermediate (I).} \]

As was mentioned in chapter 1, the primary goal of this research was to explore the chemistry of the N4Py ligand and the corresponding iron complexes. The research focussed on two central themes: N4Py iron complexes as enzyme models and the development of new catalysts, based on N4Py iron complexes, that are capable of selective oxidation.

In this chapter the most important achievements of this research will be surveyed and some suggestions for future research will be given. Furthermore, the prospects of these iron complexes as enzyme models and oxidation catalyst will be discussed.

8.2 Catalytic Cycle

The chemistry of N4Py iron complexes was described in chapters 2, 3 and 4. Based on the cumulative data presented in these chapters a catalytic cycle for N4Py iron catalyzed oxidations can be compiled (scheme 1). The key intermediate in the cycle is the iron(III) hydroperoxide species 1. It is formed from the low-spin iron(II) complex 2 in two steps, as was demonstrated by titration of \(H_2O_2\).\(^4\) First step an oxidation takes place to form a mixture of mono and dinuclear iron(III) species, \([(N4Py)Fe^{III}OH]^{2+}\) (3) and \[{(N4Py)Fe^{III}}_{2}(\mu-O)]^{4+}\) (4). In methanolic solution rapid ligand exchange takes place to form the methoxy complex, \([(N4Py)Fe^{III}OMe]^{2+}\) (5). These complexes were characterized spectroscopically and, in case of 4 and 5, by X-ray crystallography. The second step is the reaction of 3, 4 or 5 with \(H_2O_2\) to form the purple colored intermediate 1. Resonance Raman studies showed 1 to contain an unusually weak O-O bond, which could explain the high reactivity in catalytic oxidation.\(^5\) It was demonstrated that in case of oxidation of alkanes 1 is the precursor for the active species,
which are formed by homolysis of the O-O bond of the peroxide to give two radical type oxidants, \([(N4Py)Fe^{IV}O]^2+\) (6) and \(\cdot\text{OH}\).\(^6\) These radical type active species can affect hydrogen abstraction from a substrate to form an iron(III) species and a substrate radical. The latter reacts either with \(O_2\) to give both alcohol and ketone in a Russell termination reaction, or with 6 to afford alcohol. This homolytic pathway was proposed on the basis of chemical evidence, with the use of various mechanistic probes.

**Scheme 1.** Proposed catalytic cycle for oxidation of alkanes by 2 and \(H_2O_2\).

In the literature there are no precedents for hydrogen abstraction by Fe\(^{IV}\)O species, but in heme chemistry the Fe\(^{IV}\)O moiety has been reported to be able to perform oxidative transformations like the oxygenation of triphenylphosphine to triphenylphosphine oxide\(^7\) and the nonstereospecific epoxidation of olefins.\(^8\) Apparently, the fact that N4Py is a neutral ligand gives rise to the formation of a more electronegative Fe\(^{IV}\)O species, compared to the doubly negative charged porphyrin ligands, making hydrogen abstraction from alkanes more likely. The iron(III) species formed after hydrogen abstraction by 6, presumably 3 and 4, can
re-enter the catalytic cycle to give additional turnovers. This catalytic cycle is only valid for alkanes as substrate. Using other substrates, like alkenes, alcohols or aromatic compounds the mechanism of oxidation is probably more complex, since with these substrates other reaction pathways, e.g. electron transfer\textsuperscript{9} or radical additions to double bonds,\textsuperscript{10} are possible.

Besides homolysis of the O-O bond the Fe\textsuperscript{III}OOH intermediate can also react with base to form a blue colored, high-spin [(N4Py)Fe-\(\eta^2\)-(OO)]\textsuperscript{+} intermediate (7), which does not show any oxidation activity.\textsuperscript{11} This reaction is reversible, as the purple intermediate can be fully reconstituted upon addition of acid. The side-on binding mode of the peroxide was established by resonance Raman spectroscopy of 7 generated with H\textsuperscript{16}O\textsuperscript{18}OH. An interesting question that arises is whether 7 is part of the pathway responsible for H\textsubscript{2}O\textsubscript{2} decomposition during the catalytic reactions. This would explain the relatively low yields of oxidized products, based on H\textsubscript{2}O\textsubscript{2}.\textsuperscript{6}

Support for this hypothesis comes from the observed kinetic solvent isotope effect (KSIE) on the decomposition of 1. Monitoring the absorption of 1 at 548 nm in time demonstrated that purple color disappeared slower in CD\textsubscript{3}OD than in CH\textsubscript{3}OH (figure 2), indicating that the intermediate is more stable in the former. If the time that the absorption has reached half of its original value is used as a measure for stability, a kinetic solvent isotope effect of 2.2 can be calculated. This increased stability is not caused by slower oxidation of CD\textsubscript{3}OD compared to CH\textsubscript{3}OH, as was indicated by the stability in CH\textsubscript{3}OD, which is approximately similar to that in CD\textsubscript{3}OD. A KSIE of 1.8 was reported for cytochrome-P450cam in the course of O\textsubscript{2} activation.\textsuperscript{12} This was ascribed to proton assisted heterolysis of the O-O bond of the FeOOH intermediate.

![Figure 2. Absorption of 1 at 548 nm monitored in time, at 25 °C. Solid line: CH\textsubscript{3}OH as solvent; dashed line: CH\textsubscript{3}OD; dotted line: CD\textsubscript{3}OD.](attachment:image)

However, this cannot be the cause for the observed KSIE since it has been demonstrated that 1 reacts via homolysis of the O-O bond,\textsuperscript{6} and this is not expected to be proton assisted or dependent. Therefore the only reasonable explanation is that the KSIE is caused by the slower formation of Fe(OO) (7) by FeOOD, which is formed by proton-deuterium exchange
with the solvent, compared to FeOOH. This will be the result of the lower acidity of the
deuterated intermediate, FeOOD, compared to the protonated form 1. Hence, with one of the
decay pathways of 1 being less favorable in CD$_3$OD and CH$_3$OD an increased stability of the
intermediate will be observed in these solvents. Further proof for this hypothesis will have to
come from a full kinetic investigation of all the steps in the catalytic cycle and the
characterization of the decomposition products of 7.

8.3 N4Py Iron Complexes as Enzyme Model

Enzyme models were defined in chapter 1 as small synthetic active-site analogs that can be
used to obtain information about fundamental aspects of structure, reactivity and mechanism,
and are very suitable for spectroscopic study of putative reactive intermediates. Evaluating
the research described in this thesis it can be concluded that, based on these criteria, N4Py
iron complexes are excellent enzyme models.

It was demonstrated that N4Py iron complexes serve as structural models for iron bleomycin,
which also contains a pentadentate nitrogen ligand. Using the N4Py ligand several iron
complexes could be synthesized, including low-spin [(N4Py)Fe$^{II}$](CH$_3$CN)](ClO$_4$)$_2$ (2), high-
spin [(N4Py)Fe$^{III}$Cl](ClO$_4$)$_2$ (8), high-spin [(N4Py)Fe$^{III}$OMe](ClO$_4$)$_2$ (5) and the dinuclear
complex [{(N4Py)Fe$^{III}$}$_2$(µ-O)](ClO$_4$)$_2$ (4), and their relation could be established (vide supra).4

More importantly, using N4Py iron complexes two iron-peroxide intermediates with
biological significance, e.g. a purple low-spin Fe$^{III}$-η$^1$-OOH and a blue high-spin Fe$^{III}$-η$^2$-(OO) intermediate, could be characterized using UV/Vis, EPR, ES/MS and resonance Raman spectroscopy.1,5,11 The spectroscopic signatures of these intermediates will facilitate future characterization of such intermediates in biological systems.

Finally, some important mechanistic insights have been obtained by use of N4Py iron
complex as enzyme model. First of all, it was demonstrated that protonation of an iron-
peroxide intermediate is required for catalytic activity.11 This was already proposed on
theoretical grounds for cytochrome-P450$^3$ and Fe-EDTA$^4$ catalyzed oxidations, but the
results obtained with the Fe$^{III}$-η$^1$-OOH, which is active in oxidation of cyclohexane, and Fe$^{III}$-
η$^2$-(OO), which is not active, represent the first experimental proof for this hypothesis.
Furthermore, mechanistic study of oxidations catalyzed by 2, which involve a Fe$^{III}$-η$^1$-OOH
intermediate, revealed that the intermediate reacts through homolysis of the O-O bond of the
peroxide.6 This produces two radical oxidants: N4PyFe$^{IV}$O and ·OH. These findings are in
contrast with the current proposal for iron bleomycin catalyzed oxidations, which involves
heterolysis of the O-O bond to produce a formally Fe$^V$O species.15 In view of the similar
reactivity of 2 and iron bleomycin, combined with the fact that they both form a low-spin
Fe$^{III}$-η$^1$-OOH intermediate, it can be hypothesized that they would also react via the same
mechanism. Therefore it is recommended that the mechanism of iron bleomycin catalyzed
oxidations should be carefully re-examined.
Conclusions and Perspectives

8.4 Catalytic Oxidation with [(N4Py)Fe(CH3CN)](ClO4)2

One of the advantages of enzyme models is that they can serve as the starting point for the development of new generations of catalyst with improved reactivity, selectivity and stability.16 Although 2 is an excellent enzyme model and is very reactive in catalytic oxidation with H2O2 as sacrificial oxidant, it must be concluded that 2 cannot be of use in organic synthesis. Important requirements for a catalyst that can be used in synthetic organic chemistry are besides activity also chemo-, regio- and stereoselectivity. Due to the involvement of oxygen radicals in the oxidation reaction catalyzed by 2 the latter condition cannot be fulfilled.

Two approaches can be followed to overcome this. The first approach is ligand variation. A series of penta and tetradentate ligands based on N4Py have been prepared and their corresponding iron complexes tested in catalytic oxidation.17 This revealed that tetradentate ligands like N3Py derivatives are very promising ligands for stereoselective oxidation of alkanes and alkenes. The second possibility is to follow the supramolecular approach,18 which entails attachment of a recognition unit to the N4Py ligand. The N4Py ligand with a covalently attached DNA binding moiety, e.g. an acridine, represents a successful example of this approach.19 A considerable enhancement in efficiency in oxidative DNA cleavage compared to the “basic” complex 2 could be observed (vide infra). It can be envisioned that this approach also will work for catalytic oxidation of organic substrates, where substrate specificity, chemo- and regioselectivity can be introduced by proper choice of the binding and recognition unit. An example would be the covalent attachment of crown ethers, which can recognize and bind ammonium compounds and carboxylic acids.20 After binding regioselective oxidation of an alkyl or alkenyl side chain can possibly be achieved. Both ligand variations and DNA cleavage will be discussed in more detail below.

8.5 Towards Selective Catalytic Oxidation

In chapter 5 a series of complexes of N4Py based ligands other pentadentate ligands were discussed. Since all these complexes display the same reactivity pattern as observed for 2, e.g. radical type chemistry,6 it can be concluded that iron complexes with pentadentate ligands are not the catalysts of choice to achieve selectivity in catalytic oxidation.

Changing the denticity of the ligand from pentadentate to tetradentate by replacing one of the pyridine groups by a non-coordinating group, i.e. the N3Py ligands, resulted in stereoselective oxidation reactions.21 This included stereoselective hydroxylation of alkanes and stereoselective epoxidation and dihydroxylation of alkenes. Especially surprising is the fact that the N3Py-iron catalysts can be either a cis-dihydroxylation or a trans-dihydroxylation catalyst dependent on the solvent that is used for the reaction. In acetonitrile cis-dihydroxylation is observed as the major reaction whereas in acetone this is trans-dihydroxylation, although the scope of the latter reaction remains to be established.

Taken together these results, combined with those obtained by other groups,22 allow the formulation of a set of empirical guidelines for ligand design and selection of conditions for catalytic oxidation with iron complexes using H2O2.
(i) Activity. If the most important requirement for a catalyst is activity rather than chemo or stereoselectivity, complexes with pentadentate ligands are preferable since these complexes generally give an efficient reaction without the need of precautions like slow addition of peroxide. Applications where this may be important are for example stain bleaching, for use in detergents, pulping bleaching for use in the paper industry or wastewater treatment. When desired, substrate specificity or regiospecificity might be obtained by introducing recognition moieties into the ligand (vide infra).

(ii) Hydroxylation. Stereoselective hydroxylation of alkanes complexes can be accomplished with iron complexes containing tetradentate ligands, in CH₃CN as solvent. At this point it is not clear if the location of the two open coordination sites on the metal is important, i.e. whether it matters if they are cis or trans with respect to each other. If the solvent is changed to acetone the preference for hydroxylation at tertiary carbon atoms is increased.

(iii) Epoxidation. Complexes containing two open coordination sites trans to each other are the best choice for epoxidation. Acetonitrile is the solvent of choice to obtain the product with good stereoselectivity.

(iv) Cis-dihydroxylation. Complexes containing two cis open coordination sites on the metal are capable of stereoselective cis-dihydroxylation of olefins, especially when acetonitrile is used as solvent. Epoxidation is usually found as side reaction with these catalysts, although it appears that the ratio between these competing pathways can be controlled by proper choice of the ligand. However, the precise requirements for ligand to achieve cis-dihydroxylation exclusively are not clear to date.

(v) Trans-dihydroxylation. The catalysts used for cis-dihydroxylation of olefins can also be used to obtain trans-dihydroxylation. Only in the latter case acetone is needed as solvent. Again epoxidation is found as competing reaction. Preliminary results indicate that trans-dihydroxylation is limited to cis-alkenes. However, more substrates should be tested to assess the validity of this hypothesis. At this point much is not clear about this trans-dihydroxylation reaction. An unanswered question is whether the open coordination sites on the metal have to be cis with respect to each other, or that trans location is also allowed. Furthermore, it should be investigated whether this reaction is unique for acetone, or that more solvents can be used.

These guidelines are only a rough indication how to obtain a certain type of chemistry. The activity or stereoselectivity of the catalyst is usually controlled by the precise geometry of the ligand, where small structural changes can have a profound effect.

8.6 DNA Cleavage

Complex 2 and derivatives were demonstrated to be very efficient catalysts for oxidative DNA cleavage using O₂, without the need of adding external reducing agents. Especially if a DNA binding moiety, e.g. an acridine, was attached to the complex, instantaneous DNA cleavage was observed. This concept of a DNA binding unit that can deliver a complex to the DNA where it reacts to cause DNA cleavage can be further extended to get sequence selective DNA cleavage. For this purpose the acridine unit has to be replaced by a moiety than can recognize and bind to base pairs or a specific sequence of base pairs. That this
approach is indeed viable was demonstrated by Dervan et al. who used distamycin\textsuperscript{24} and hairpin polyamides\textsuperscript{25} to get selectivity in DNA cleavage. In view of the enormous amount of information available on DNA recognition the possibilities for choosing the recognition moiety are seemingly endless.\textsuperscript{26} Some obvious choices include small peptide chains,\textsuperscript{27} oligonucleotides,\textsuperscript{28} and metal complexes like cis-platinum,\textsuperscript{29} rhodium\textsuperscript{30} or zinc-cyclen complexes.\textsuperscript{31} An extension of this approach would be the recognition of DNA bases or sequences at a defined distance from each other. This can be accomplished by attaching two DNA recognition groups to the N4Py ligand. The complex would then bind in between DNA sequences at a distance equal to that of the combined spacer lengths. The advantage of this approach is that smaller DNA recognition units can be used to achieve cleavage at a defined position, compared to the case with one recognition moiety.

8.7 Prospects

In this final paragraph the prospects for non-heme iron complex as enzyme model and oxidation catalyst are discussed. Non-heme iron complexes, and in particular N4Py-iron complexes, have already proven their value as enzyme model.\textsuperscript{32} Continued study of these complexes could lead to a better understanding of enzyme mechanisms and may lead to the discovery and characterization of new iron-peroxo intermediates.

An exciting new area of research involves non-heme iron complexes as oxidation catalyst. The first examples of complexes capable of stereoselective oxidation catalysts were described in this thesis and in recent literature.\textsuperscript{22} Especially interesting are the stereoselective hydroxylation of alkanes\textsuperscript{33} and cis-dihydroxylation of alkenes\textsuperscript{34} with H\textsubscript{2}O\textsubscript{2}, for which there are not many alternatives. Epoxidation is also very useful in synthetic organic chemistry, but it will be very difficult to rival the very efficient and highly enantioselective catalysts known to date.\textsuperscript{35}

Although this field of non-heme iron catalyzed stereoselective oxidations is still in its infancy and these catalysts cannot be used on a preparative scale so far, these are very promising developments. Undoubtedly the coming years will see the advent of a new generation of non-heme iron catalysts that can be used as tool in synthetic organic chemistry. A closely related topic is the design and synthesis of chiral catalysts capable of asymmetric hydroxylation, epoxidation and cis and trans-dihydroxylation. Ligand design and screening can possibly be done using combinatorial methods, as was demonstrated by Jacobsen and coworkers, who have designed the first non-heme iron complex capable of enantioselective epoxidation, albeit with a low ee.\textsuperscript{36}

Finally, a promising new area of research will be O\textsubscript{2} activation with non-heme iron complexes. Non-heme iron oxygenases are capable of highly selective oxidation with the use O\textsubscript{2} as terminal oxidant.\textsuperscript{32} The challenge will be to achieve the same with model complexes, with the ultimate goal to obtain iron catalysts that can perform selective oxidations of organic substrates with O\textsubscript{2} on a preparative scale. That model complexes are capable of O\textsubscript{2} activation was demonstrated in the DNA cleavage experiments (\textit{vide supra}).

Looking at all these possibilities it is obvious that non-heme iron based oxidation catalysts have a very bright future in front of them. Especially in view of their versatility, stability and
relatively easy synthesis, an attractive conclusion is that non-heme iron complexes are the oxidation catalysts of the future.

### 8.8 References and Notes


16. See chapter 1.

17. Chapters 5 and 6.


   (b) Roelfes, J.G.; Branum, M.E.; Wang, L.; Que, L., Jr.; Feringa, B.L. *submitted for publication*.


   (b) Beers, O.C.P.; Gribnau, M.C.M.; Hage, R.; Hermant, R.M.


