CHAPTER 1

A general overview of flavin catalyzed reactions

*Flavins are effective redox catalysts, either in enzymes or, after modification, as organocatalysts. In this chapter the advances that have been made in the field of flavin based catalysis are described.*
The oxidation of organic molecules represents a key chemical transformation in both the chemical industry and biological processes. Control over chemo-, regio-, and stereoselectivity is very important in these reactions and over the years many efficient oxidation methods have been developed for specific reactions.\(^1\)

While molecular oxygen and hydrogen peroxide can be regarded as the best oxidants compatible with current environmental concerns, these oxidants are rarely used alone and frequently require heavy metal catalysis for further activation. The application of nonmetal catalysts for homogeneous oxidations and more specifically the application of heterocyclic hydroperoxides in selective oxidation reactions has been described in detail.\(^2\) One of these possible organocatalytic oxidation methods with \(O_2\) or \(H_2O_2\) is based on flavins. In the following sections an overview is given concerning flavin based catalysis followed by the aim and outline of the work presented in this thesis.

1.1 **Flavoproteins**

Flavins were discovered in 1879\(^3\) and chemically characterized in the 1930’s. The discovery that flavins are capable of both one- and two-electron transfer processes, indicated a pivotal role in coupling the two-electron oxidation of most organic substrates to the one-electron transfers of the respiratory chain. Flavins are now also known for their function as electrophiles and nucleophiles and covalent intermediates of flavins and their substrates are frequently involved in catalysis. While flavins are thought to contribute to oxidative stress due to their ability to produce superoxide, at the same time flavins are involved in the reduction of hydroperoxides. Nowadays, flavoproteins play an important role in soil detoxification processes via the hydroxylation of many aromatic compounds,\(^4\) and flavoproteins found in liver microsomes catalyse many reactions similar to those carried out by cytochrome P450 enzymes.\(^5\) Flavins were also shown to be involved in the production of light in bioluminescent bacteria, and play an important role in light-mediated reactions such as plant phototropism and nucleic acid repair processes.\(^6\) Recent reports link flavins to programmed cell death and magnetosensing.\(^7\) The chemical versatility of flavins is clearly controlled by specific interactions with the proteins in which they are bound.

1.1.1 **Oxidoreductases**

The enzymes by which electrons are transferred from one molecule to another are called oxidoreductases, or redox enzymes\(^8\)(E.C 1.x.x.x).\(^9\) A number of cofactor-independent oxidoreductases is known, and these typically contain several aromatic residues in the active site that are catalytically active.\(^10\) However, for most biochemical reactions catalyzed by oxidoreductases, (in)organic cofactors are necessary. The most well known cofactors are flavins, metal-ions, hemes and nicotinamides (Figure 1.1).\(^11\) Binding of cofactors to the enzymes is usually tight, with affinities in the nM range.
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Figure 1.1 A selection of the most common cofactors found in oxidoreductases.

Although oxidation reactions are without doubt of great value in organic synthesis, selective oxidation can be a significant problem. If oxidations are carried out with strong oxidizing agents, a lack of chemo-, regio- and enantioselectivity is often encountered. A way to deal with this is the application of man-made catalysts or enzymes for oxidations reactions.

Enzymes mostly function under environmentally friendly conditions (e.g. in aqueous solvents, with moderate pH and temperature). A drawback of the enzymatic systems is that often a stoichiometric amount of a nicotinamide coenzyme (mostly NAD(P)H) is necessary to regenerate the catalyst, while (in)organic catalysts can often perform oxidations solely with hydrogen peroxide or oxygen. So far there has been quite some research in nicotinamide coenzyme regeneration and the regeneration can
be accomplished a chemical fashion, with electro- or photochemical methods and enzymatically.\textsuperscript{13}

1.1.2 Monoxygenases

Monoxygenases are enzymes that catalyze the insertion of a single oxygen atom from O\textsubscript{2} into an organic substrate. In order to carry out this type of reaction, the enzymes need to activate a molecule of oxygen to overcome its spin-state. In most cases, monoxygenases utilize (in)organic cofactors to transfer electrons to molecular oxygen for its activation. Monoxygenases are dependent on a diverse range of different cofactors, most of which were depicted in figure 1.1. The most well known monoxygenases are non-heme iron-,\textsuperscript{14} heme-,\textsuperscript{15} flavin-,\textsuperscript{16} copper,\textsuperscript{17} and pterin-dependent monoxygenases.\textsuperscript{18} A good overview of these monoxygenases and their catalytic cycles is available.\textsuperscript{13}

A group of flavoproteins is capable of oxidising a diverse range of substrates via utilization of the peroxy intermediate of the flavin cofactor (figure 1.2).\textsuperscript{19} These oxidations follow a general mechanism as will be described in section 1.3. In a number of reviews flavoprotein monoxygenases are described in more detail.\textsuperscript{20}

1.1.3 Artificial enzymes

Flavin cofactors have been incorporated into non-native enzymes with the aim to obtain more active or selective catalytic systems. The group of Kaiser covalently linked a flavin cofactor in the cysteine protease papain.\textsuperscript{21} With introduction of this cofactor, a loss of more than 90\% of the original enzyme activity was observed, while modest activity in the oxidation of NADPH took place (Scheme 1.1).

\begin{figure}[h]
  \centering
  \includegraphics[width=\textwidth]{schemes.png}
  \caption{Reactions catalyzed by flavoprotein monoxygenases.} \label{fig:reactions}
\end{figure}

\begin{figure}[h]
  \centering
  \includegraphics[width=\textwidth]{schemes2.png}
  \caption{Chemically altered papain utilized in NADPH oxidation.} \label{fig:papain}
\end{figure}
The group of Reetz could likewise anchor chelating ligands and flavin derivatives into the enzyme tHisF from *Thermotoga maritima* using a Michael addition reaction.²² (Figure 1.3)

![Figure 1.3](image_url) (chelating) ligands and organocatalysts applied in covalent binding to tHisF.

As of yet, no catalytic activity with these modified proteins was reported.

### 1.2 General properties and synthesis of flavin organocatalysts

![Figure 1.4](image_url) The general structure of a flavin and its isomeric alloxazine derivative.

Flavins are composed of a heterocyclic 7,8-dimethylisoalloxazine ring system, (1.14) and are comparable to the isomeric alloxazine derivatives (1.15). The latter are usually not regarded as flavins (Figure 1.4). Flavins can exist in three oxidation states: oxidized, semiquinone radical, or a fully reduced form. Due to these different states, flavins can easily participate in electron transfer processes. The enzyme-bound 4a-hydroperoxyflavin (Enz-4a-FlHOOH, 1.18), which is formed after reductive activation of molecular oxygen with the 1,5-dihydro form of flavin (FlH₂, 1.17), is generally accepted as the key intermediate in monoxygenations (Figure 1.5).²³

![Figure 1.5](image_url) The oxidation states of flavin compounds and the related 4a-hydroperoxyflavin.

The four nitrogen atoms and two carbonyl groups in the heterocyclic rings contribute to the electronegativity of the 4a-position and the electrophilicity of the terminal peroxy oxygen atom. Artificial flavins have been designed as active site probes of flavoproteins in an effort to understand the mechanism of the enzymatic reactions.²⁴ More detailed insight into the chemistry and biochemistry of flavins, historical aspects, and biochemical significance of riboflavin and flavoproteins is available.²⁵ Furthermore, there is detailed information concerning the synthesis of...
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flavin analogues with or without substituents at the aromatic ring and/or the nitrogen atoms and stable flavininium salts. Two general approaches for the synthesis of 4a-hydroperoxyflavins were developed by Kemal and Bruice (Scheme 1.2). (i) nucleophilic attack by H$_2$O$_2$ on oxidized flavinium cations such as 1.19 and (ii) reaction of molecular oxygen with reduced flavins such as 1.21.

\begin{center}
\textbf{Scheme 1.2} Methods for obtaining artificial 4a-hydroperoxyflavins and the N(5)-ethyl-10a-spirohydantoin.
\end{center}

4a-Hydroperoxy-N$^5$-ethyl-3-methyllumiflavin (4a-FLEt-OOH, 1.20) could be synthesized by reaction of N$^5$-ethylflavinium perchlorate (FLet$^+$ClO$_4^-$, 1.19) with H$_2$O$_2$ and isolated in 80% yield. Likewise, reaction of N$^5$-ethyl-1,5-dihydroflavin (FLetH, 1.21) with triplet oxygen ($^3$O$_2$) gave 4a-FLEt-OOH. Furthermore, it was shown that 1.20 slowly decomposes in water, tert-butyl alcohol, and dimethyl formamide leading to N$^5$-ethyl-10a-spirohydantoin 1.22. While decomposition also takes place in CHCl$_3$, in this case, the decomposition products depend on solvent purity. The group of Glusker described the pathways and intermediates involved in this decomposition. The group of Bäckvall synthesized a number of aryl-substituted 1,3-dimethyl-5-ethyl-5,10-dihydroalloxazins (Figure 1.6). Their results showed that the oxygen-donating capacity of the corresponding hydroperoxyflavins is dependent on the redox potential of the flavins and that the one-electron oxidation potentials correlate linearly with the Hammet $\sigma$ values, reflecting their efficiency as catalysts in the H$_2$O$_2$ oxidation of methyl p-tolyl sulfide. The presence of electron-withdrawing groups on the flavins increased the stability of the reduced catalyst.

\begin{center}
\textbf{Figure 1.6:} 1,3-dimethyl-5-ethyl-5,10-dihydroalloxazins
\end{center}

1.3 General mechanism of flavin catalyzed oxidations

The mechanism of flavin-catalyzed oxygen activation, be it in flavoproteins or with the flavin alone, has been investigated thoroughly and still attracts considerable attention. In the proposed cycle (Scheme 1.3) for enzymatic
monooxygenations a rate-determining single-electron transfer occurs from reduced flavin 1.17 to triplet oxygen to form a flavin semiquinone-superoxide radical pair 1.16.\(^{31}\) Subsequent spin inversion leads to collapse of the radical pair to flavin peroxide anion (4a-FlH-OO\(^-\), 1.24). Depending on the specific enzyme, 1.24 can either directly participate in nucleophilic oxidations,\(^{32}\) or be protonated to the hydroperoxide (4a-FlH-OOH, 1.18), which is an electrophilic oxidant.\(^{33}\) The positioning of the substrate relative to the peroxy group determines the selectivity of enzyme catalysed reactions. The resulting byproduct, flavin pseudobase FlHOH, 1.25, dehydrates to the oxidized flavin 1.14 that can be reduced to 1.17 by NAD(P)H. Another way of reducing a flavin cofactor is using simple sacrificial electron donors such as ethylenediaminetetraacetate (EDTA).\(^{34}\) The group of Reetz reported that this methodology could be used with a mutant of PAMO.\(^{35}\) It should be noted that species 1.18 and 1.24 spontaneously eliminate H\(_2\)O\(_2\) outside an enzyme cavity, which makes them useless as oxygen-transfer agents.

\[
\text{Scheme 1.3 General mechanism of oxygenation reactions catalyzed by external flavoprotein monoxygenases.}
\]

1.4 Stoichiometric oxidations

Bruice et al. found that by replacing the N\(^5\) hydrogen with an ethyl group, the hydroperoxide 4a-FlEt-OOH could be isolated.\(^1\) 1.23a and the analogous N\(^5\)-methylated flavin, 4a-FlMe-OOH, were applied in the stoichiometric oxidation of sulfides, amines, and iodide outside the enzyme environment. Oxidations of sulfides and amines are important transformations, since the oxidation products (sulfoxides,
sulfones, hydroxylamines, nitrones, amine oxides) are key intermediates in organic synthesis.\textsuperscript{1} Synthetic 4a-hydroperoxy-5-ethyl-3-methylumiflavin 1.21 was initially applied in the oxidation of para-substituted thioanisoles (1.26) and the oxidation of sulfide 1.28 to sulfoxide 1.29. Compound 1.29 is an intermediate in the synthesis of spirovetivane sesquiterpenes (Scheme 1.4).\textsuperscript{36}

Flavin 1.21 was also applied in the oxidation of benzyl amine (1.30) and N,N-dimethylaniline (1.33). Oxidation of N,N-dimethylaniline and N,N-dimethyl benzyl amine did not take place with tert-butylhydroperoxide and hydrogen peroxide. Together with similar flavin hydroperoxides, the substrate scope was broadened to secondary amines, tertiary amines, and hydroxylamines to yield hydroxylamines, amine oxides, and nitrones together with 4a-FlEtOH in quantitative yield.\textsuperscript{37} In the oxidation of N-methyl benzyl amine (1.35), benzylmethylhydroxylamine (1.36) was found as the major product. However, further oxidation of 1.36 gave a mixture of the nitrones N-benzylidenemethylamine-N-oxide (1.37) (53%) and N-methylenebenzylamine-N-oxide (1.38) (24%), probably due to dehydration of a common intermediate. The rates of the oxidation of aryl-substituted dimethyl anilines again implicated electrophilic reactivity of the hydroperoxide.

Nucleophilic displacement of the terminal oxygen by the incoming nucleophile (N, S, I-) was proposed (Scheme 1.6) and later confirmed by Oae et al.\textsuperscript{38} These results also showed that the hydroperoxide 4a-FlEt-OOH oxidized all types of amines including n-octylamine and other primary amines in contrast to the oxidations by the microsomal FAD-containing monooxygenases, which do not oxidize primary amines.\textsuperscript{39}
As of yet, 4a-hydroperoxyflavins have not shown any activity in epoxidation reactions, although this activity has been observed in flavoproteins.

\[
\begin{align*}
\text{Scheme 1.6} & \quad \text{Nucleophilic displacement of the terminal oxygen of the 4a-Fl-OOH species during nucleophilic oxidations.}
\end{align*}
\]

1.5 Catalytic oxidations

In 1989 Murahashi et al. reported the oxidation of thioethers and amines with H\textsubscript{2}O\textsubscript{2} using a catalytic amount of the flavinium perchlorate 1.20 (FlEt+ClO\textsubscript{4}⁻)\textsuperscript{40} Excellent yields (96-99%) were obtained in the oxidation of dibenzyl-, dibutyl-, and diphenylsulfides, which were oxidized to their respective sulfoxides with 1 equiv of H\textsubscript{2}O\textsubscript{2} and 10 mol% of catalyst 1.20 (Scheme 1.7). Interestingly, dibenzylsulfoxide gave an excellent yield (98%) of dibenzylsulfone with 1 equiv of H\textsubscript{2}O\textsubscript{2} and 10 mol% of catalyst, suggesting a high electrophilicity of the reactive hydroperoxide species.

\[
\begin{align*}
\text{Scheme 1.7} & \quad \text{Catalytic sulfoxidations with H}_2\text{O}_2 \text{ and flavinium salt 1.20.}
\end{align*}
\]

In the proposed catalytic cycle, the flavinium salt (1.49) is first oxidized with O\textsubscript{2}, after which the cycle closely resembles the general mechanism of oxygenation reactions catalyzed by external flavoprotein monoxygenases. However, spontaneous elimination of H\textsubscript{2}O\textsubscript{2} does not take place and the hydroperoxy species 1.50 can be regenerated with H\textsubscript{2}O\textsubscript{2} via a shunt pathway from iminium species 1.52 (Scheme 1.8). Although flavin 1.20 proved to be the best catalyst, other flavin sources such as 4a-
FLEtOOH, N²-alkylated reduced flavins (FlEtH), FMNHEt, and FMNHMe also showed activity. Riboflavin (1.1), and FMN (1.2) however were unreactive, which indicated the importance of N²-alkylation. The active oxidant was, as in the stoichiometric reaction, the flavin hydroperoxide 1.50. In 2006, Lindén et al. reported that a recyclable system for sulfoxidation reactions was obtained with the application of a flavin catalysts in an ionic liquid.  

The groups of Murahashi and Imada developed an aerobic version of the same reactions, utilizing flavinium perchlorates such as 1.20 as the catalyst.  

The use of H₂O₂ could be avoided by in situ reduction of FLEt⁺ back to the flavin (FLEtH) with hydrazine hydrate (NH₂NH₂•H₂O) which serves both as electron and proton source, releasing dinitrogen (Scheme 1.9). The reduced flavin FLEtH reacts with oxygen, resulting in the active oxidant 4a-FLEt-OOH. The generation of FLEtH is proposed to take place via two routes. Initially the hydrazine forms the adduct flavin hydrazide (4a-FLEt-NHNH₂), which releases diimide and the reduced flavin FLEtH. On reaction with another molecule of FLEt⁺ the diimide forms the adduct 4a-FLEt-N=NH, which also decomposes to FLEtH and dinitrogen. In this system 2,2,2-trifluoroethanol was used as the solvent due to the high solubility of O₂ in this solvent. In this way high yields of sulfoxides were obtained using molecular oxygen (1 atm) or air.
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Scheme 1.9 Proposed mechanism for aerobic oxidation reactions catalyzed 5-Et-Fl catalysts with hydrazine hydrate and oxygen.

The method developed by Imada et al. was applied successfully in aerobic oxidation of several secondary and tertiary amines and a hydroxylamine with good yields.\textsuperscript{43} Chemoselective oxidation of sulfides to sulfoxides\textsuperscript{44} and oxidation of tertiary amines to amine oxides\textsuperscript{45} was obtained with 30% aq H\textsubscript{2}O\textsubscript{2} and 1,5-dihydroalloxazine (1.56 - Figure 1.6) as catalyst. Bäckvall et al. likewise described selective sulfoxidation of thioethers in the presence of many potentially reactive electron-rich functional groups.\textsuperscript{44} Further oxidation to sulfones was not reported, even with a prolonged reaction time and when a large excess of H\textsubscript{2}O\textsubscript{2} was used, suggesting high chemoselectivity of the catalysts. The oxidation of vinylic sulfides required more catalyst and extended reaction times as well as a large excess of H\textsubscript{2}O\textsubscript{2} compared to allylic sulfides, aryl methyl sulfides, and dialkyl sulfides due to conjugation.

In 2007, Baxová et al. reported the introduction of an amphiphilic flavinium salt in micellar systems and the application of this system for organocatalytic sulfoxidations with hydrogen peroxide as terminal oxidant.\textsuperscript{46} Although the rate of sulfoxidation was only 1.5 times higher in non-ionic micelles and 3 times faster in anionic micelles compared to a homogeneous solution, the authors suggest that this is still interesting with respect to potential practical applications. For example, it could help to increase enantiomeric excess in the case of stereoselective oxidations mediated by chiral flavinium salts. However, chiral flavinium salts that so far have
been applied successfully in oxidation reactions, have their chirality introduced at the N^10 position, the same location as the lipophilic group has in the micellar systems, which might hinder combining both systems.

Next to electrophilic oxidations, 4a-flavinhydroperoxides can undergo nucleophilic reactions with appropriate electrophilic substrates. The Baeyer-Villiger oxidation of ketones to lactones or esters is an example of such a shift in the reactivity of hydroperoxyflavins. In nature this transformation is carried out by Baeyer-Villiger monooxygenases (BVMOs), using molecular oxygen and a stoichiometric amount of NAD(P)H to catalyze smooth and mild conversion of ketones to lactones or esters, usually with synthetically interesting stereoselectivities. After molecular redesign, the biocatalytic properties of certain BVMOs could be improved. More detailed information can be obtained from reviews concerning mechanistic aspects and modern synthetic advances.

The first flavin-catalyzed Baeyer-Villiger oxidation of ketones was described by Mazzini et al. Using flavinium salt 1.57 and H_2O_2 as the oxygen source, ketones 1.58a-c were converted to the expected lactones 1.59a-c in good yields under mild conditions (Scheme 1.10). The presence of a double bond and an alkoxy group alpha to the carbonyl function were tolerated with negligible background reaction, showing both good chemo- and regioselectivity. The catalytic cycle was proposed to be similar to the cycle described for the catalytic oxidation of sulfides and amines using catalyst 1.20.

![Scheme 1.10 Catalytic Baeyer-Villiger oxidations with a flavinium perchlorate salt 1.57 and H_2O_2.](image-url)

Again, the non N^5-alkylated neutral catalysts proved to be inactive while utilization of molecular oxygen gave only <10% yields of products. While reduced activity was mentioned for the oxidation of substrates like cyclohexanones, cyclopentanones, and linear ketones, it was not mentioned how much was actually converted.
More recently, Imada et al. prepared catalyst 1.60 in five steps from riboflavin 1.1 (Scheme 1.11), which was also used in highly chemoselective aerobic Baeyer-Villiger oxidation with 1.60 under air or O₂ (1 atm) at 60 °C in acetonitrile/EtOAc/H₂O (8:1:1 v/v). Intriguingly, with their method the aerobic Baeyer-Villiger oxidation of 1.61 gave mixtures of the expected (1.62a and 1.62b) and the unexpected (1.63a and 1.63b) lactones, while with a system that uses the catalyst 1.64 only the expected lactones were reported as products (Scheme 1.12). The group of Imada also proved that their system was highly chemoselective. Under the aerobic conditions, with a 1:1 mixture of a ketone and cis-cyclooctene, only the lactone was obtained (97%). With a mixture of the ketone and methyl p-tolyl sulfide, both the lactone (88%) and traces of sulfoxide (3%) were found. With the application of H₂O₂ as terminal oxidant instead of O₂, only 32% conversion to the lactone was detected, while the sulfoxide was obtained in 87%.

Imada et al. proposed that zinc dust reduces the flavin cation to its reduced state, FlEt−, which is known to react with triplet oxygen to give the 4a-FlEt-OO− intermediate. This was suggested to react with ketones via a nucleophilic pathway similar to that described by the catalytic cycle of flavoproteins (Scheme 1.3). The greater nucleophilicity of the flavin peroxyanion intermediate compared to the corresponding hydroperoxide 4a-FlEt-OOH is probably the reason for the high chemoselectivity to ketones compared to the softer thioethers.
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1.6 Catalytic asymmetric oxidations

A number of methodologies has been developed for the enantioselective oxidation of sulfides to enantiomerically pure sulfoxides which are important intermediates in many transformations. Shinkai et al. reported the first attempt of biomimetic catalytic asymmetric sulfoxidation with the planar chiral flavophane (+)-1.65 (Scheme 1.13). In the sulfoxidation of aryl methylsulfides 1.66a and 1.66b with 35% aqueous H$_2$O$_2$ and 10 mol % (+)-1.65 in a solvent mixture of H$_2$O/MeOH at -20 °C, 65% ee and turnover numbers of up to 8 were obtained after 5 days. The strongly electronwithdrawing CN group in the 4-position of the substrate 1.66c was shown to be detrimental to both the yield and the e.e. of the product 1.67c, again suggesting the electrophilic nature of the hydroperoxide.

![Scheme 1.13 Asymmetric sulfoxidations with a chiral flavin catalyst 1.65.](image)

Murahashi et al. claimed that the capped flavin perchlorate catalyst 1.68 can be utilized in the asymmetric sulfoxidation of methyl naphthylsulfide 1.69 to sulfoxide 1.70 with H$_2$O$_2$ in 94% yield and 72% ee (Scheme 1.14). Details of this work, however are not available.

![Scheme 1.14 Asymmetric sulfoxidations with a chiral flavin catalyst.](image)

As is the case in asymmetric sulfoxidation reactions, a number of synthetic methods using metal catalyzed asymmetric reactions and biocatalytic processes have
been developed for the synthesis of optically active lactones.\textsuperscript{50} Organocatalytic methods are rare. Murahashi \textit{et al.} reported the first flavin catalyzed asymmetric Baeyer-Villiger reaction in 2002.\textsuperscript{55} In five steps, starting from (S,S)-1,2-diaminocyclohexane (or (R,R)-1,2-diaminocyclohexane), the C2-symmetric chiral bisflavin perchlorate (S,S,pR,pR)-1.71 and its antipode were synthesized. With 10 mol\% of catalyst 1.71 and 30\% \(\text{H}_2\text{O}_2\), 3-aryl-cyclobutanones 1.72 were converted to the corresponding lactones 1.73 with up to 74\% ee (Scheme 1.15). The \textit{in situ} formed flavinhydroperoxide is expected to be the chiral catalyst responsible for asymmetric induction into the products. Interestingly, with product 1.73a both yield and ee went down if dichloromethane was used as the solvent. It was presumed that \(\pi-\pi\) interactions between the substrate and the phenyl rings of the catalyst determine the preferred side of nucleophilic attack by the hydroperoxy and thus the magnitude of asymmetric induction. As such, the substrate scope was expected to be limited to ketones capable of undergoing \(\pi-\pi\) interactions.

![Scheme 1.15 Asymmetric Baeyer-Villiger oxidations with a chiral diflavin catalyst.](image)

### 1.7 Tandem reactions in which flavin catalysts play a role

As was shown in section 1.4, catalyst 1.52 could be regenerated with hydrazine hydrate. During this regeneration, diimide is formed, which was proposed to associate with the catalyst. The groups of Murahashi and Imada showed that the formed diimide could be used for \textit{in situ} aerobic hydrogenation of a series of alkenes in acetonitrile (Scheme 1.16).\textsuperscript{56} It was proposed that diimide was formed in two ways: (1) [FlEt\(\text{H=NH}\)] is produced from the reaction of FlEt\(+\) with hydrazine (2) 4a-FlEt-OOH selectively and aerobically oxidizes hydrazine, generating a reactive [4a-FlEtOH \(\text{NH=NH}\)] species. Both species were deemed capable of reducing the substrates with release of \(\text{N}_2\). While the reaction in acetonitrile proved to be quite chemoselective, a large shift in chemoselectivity was observed in different solvents, which was proposed to be due to reduced nucleophilicity of hydrazine in more acidic media, to the extent that 4a-FlEt-OOH preferentially transferred oxygen to sulfur.
The group of Bäckvall investigated the potential of using flavin catalyst 1.21 as an electron transfer mediator in the osmium-catalyzed dihydroxylation of alkenes, a reaction that is chemo- and stereoselective and one of the most important oxidative chemical transformations of alkenes. Potassium ferricyanide (K$_3$[Fe(CN)$_6$]), N-methylmorpholin-N-oxide (NMO), and more recently molecular oxygen have been used to reoxidize the Os(VI) back to Os(VIII). The group of Bäckvall performed the oxidation of N-methylmorpholin (NMM) to NMO with catalyst 1.21 and the OsO$_4^-$ catalyzed dihydroxylation of alkenes in one pot (Scheme 1.17). Presumably, 1.21 functions as was described before for amine oxidations. The amine oxide in turn reoxidizes the OsO$_3$ produced from the reaction of alkenes to diols with the OsO$_4$ catalyst. In this way a series of alkenes was oxidized to diols with up to 95% isolated yield with low catalyst loading. To obtain a higher yield, 2 equivalents of tetraethylammonium acetate (TEAA) were needed.

Interestingly, Bäckvall et al. also showed that the system could be applied for asymmetric dihydroxylations. They showed that this was possible by applying catalyst 1.21 in combination with either a chiral ligand and NMM, or with only a chiral ligand containing an oxidizable tertiary nitrogen as part of its structure. (Scheme 1.18)
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Scheme 1.18 Osmium-catalyzed enantioselective dihydroxylation of alkenes with flavin ETM and a chiral catalyst.

In later work, the racemic version of this catalyst system was immobilized in an ionic liquid (1.84). In this system, the immobilized catalyst could be reused by adding additional substrate and oxidant without loss of activity for at least 5 runs, while the products could be separated by extraction with diethyl ether (Scheme 1.19).

Scheme 1.19 Osmium-catalyzed dihydroxylation of alkenes in an ionic liquid.

1.8 *Investigation into an artificial riboflavin receptor*

In 2005 the group of Sellergren reported to have succeeded in preparing an artificial riboflavin receptor using a template analogue imprinting strategy. Due to the low solubility of riboflavin itself, the receptor was imprinted using a selection of riboflavin tetraesters. Tetraacetyl protected riboflavin was found to be the best analogue for obtaining a receptor for riboflavin. Although it was not possible to perform a thorough investigation of the binding constants, measurements that could be performed implied sites with binding affinities larger than 10^6 M^-1. The next step the group is working on, is to demonstrate that it is possible use the receptors for removal of riboflavin and other “harmful” chemicals from food. This is important for the brewery industry and food industry.
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1.9 Aim and outline of this thesis

While the application of enzymes for oxidations reactions has certain distinct advantages over “normal” organic synthesis, there are similarly a number of drawbacks. Often, a stoichiometric amount of a reductant like nicotinamide coenzyme is necessary to complete the catalytic cycle of flavoenzymes. While there are ways to regenerated the nicotinamide coenzymes in situ, organic flavin catalysts perform quite well in oxidation reactions and can be regenerated with a shunt pathway utilizing hydrogen peroxide.

The aim of the study was to modification of the flavin cofactor in enzymes could to the extent that the enzymes would function without needing a reductant, but rather utilizing a shunt pathway with hydrogen peroxide.

In Chapter 2, the synthesis of a range of flavin catalysts is described. The synthesis of a catalyst based on the common cofactor riboflavin is described, including a comparison of its UV-spectra to the spectra of active flavoproteins.

An investigation of the activity of the catalysts described in chapter 2, is presented in Chapter 3. Oxidation reactions that were investigated are sulfoxidations, tertiary amine oxidation and the Baeyer-Villiger reaction using hydrogen peroxide as the terminal oxidant.
The possible application of flavin catalysts as electron transfer mediators for the in situ regeneration of osmium tetroxide, TEMPO, IBX and seleno-oxides with hydrogen peroxide was investigated and the results are detailed in Chapter 4.

In Chapter 5 a system for the reduction of carbon-carbon double bonds with diimide, catalytically generated in situ from hydrazine hydrate using the riboflavin based catalyst described in chapter 2 is presented.

Results of the incorporation of flavin based catalysts into the proteins dodecin and riboflavin binding protein are described in Chapter 6. An initial investigation into the application of protein bound modified flavins is presented.

1.10 References and notes


9 a) The Enzyme Commission number (EC number) is a numerical classification scheme for enzymes, based on the chemical reactions they catalyze. As a system of enzyme nomenclature, every EC number is associated with a recommended name for the respective enzyme. Every enzyme code consists of the letters “EC” followed by four numbers separated by periods. Those numbers represent a progressively finer classification of the enzyme. b) Enzyme nomenclature 1992: recommendations of the Nomenclature
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12 Omura, T.; Sato, R. J. Biol. Chem. 1964, 239, 2370-2378.


14 Non-heme iron-monoxygenases do not have a specific E.C. code. Some examples are: the lipoxygenases (EC 1.13.11.12), tyrosine 3-monoxygenase (EC 1.14.16.2), catechol 2,3-dioxygenase (EC 1.13.11.2), mandelate 4-monoxygenase (EC 1.14.16.6), methane monoxygenase (EC 1.14.13.25) and anthranilate 3-monoxygenase (deaminating); from Aspergillus niger; (EC 1.14.13.35).


18 E.C. 1.14.16.x.


A general overview of flavin catalyzed reactions


Although riboflavin is not harmful for people, when exposed to light passing through a bottle it catalyzes photooxidation reactions and changes the flavor of beer.