Chapter 7 Summary, conclusions and perspectives

This chapter will provide a summary of the most important results of this thesis in combination with concluding remarks connecting the recent findings to the literature. Key parameters of useful metals and ligands in DNA-based asymmetric catalysis are described. Additionally, an overview of important characteristics of DNA-based asymmetric catalysis is added to explain accelerations of reaction in presence of DNA. At the end of this chapter, suggestions about further research in DNA-based asymmetric catalysis will be given.
7.1. Introduction

The discovery of the transfer of chirality from the DNA double helix structure to the products of catalytic reactions in water has initiated many research projects in the field of asymmetric catalysis.

As mentioned at the end of chapter 1, the main goal of the investigations described in this thesis was to develop novel organometallic DNA-based asymmetric catalysis reactions (Chapter 2 and 3). Furthermore, the research focused on the discovery of new substrates for the existing DNA-based asymmetric catalysis approaches (Chapter 4) and on the development of tools for organic synthesis in aqueous environment (Chapter 5 and 6). The main achievements are listed below. Scheme 1 shows the novel reactivities in DNA-based asymmetric catalysis. This thesis comprised:

- The first organometallic DNA-based asymmetric catalysis reaction leading to high enantioselectivity.
- The discovery of iron porphyrin / duplex DNA hybrid catalysts for the intermolecular cyclopropanation of styrenes with ethyl diazo acetate, which gave rise to large rate accelerations in presence of DNA.
- The first substrates for copper catalyzed DNA-based asymmetric catalysis that contain only oxygen atoms for coordination to copper.
- The development of synthetic methods for organic chemistry in water based on water soluble porphyrins and micellar catalysts (see below for more details).

Scheme 1 Novel DNA-based asymmetric catalysis reactions described in this thesis.
7.2. Research overview

Looking at all published reactions catalyzed by a DNA based catalyst, almost exclusively Cu\textsuperscript{II} ions in combination with ligands such as 4,4'-dimethyl-2,2'bipyridine were used before as DNA binding complex.\textsuperscript{(1)} This is most likely due to the simple reaction procedures and the very intriguing results obtained with this combination. In this thesis one of the main goals was to find novel combinations of metals and ligands for DNA-based asymmetric catalysis of new reactions. To achieve this, the characteristics of metals and ligands have to be attuned to each other, which is difficult due to several limiting factors (see paragraph 7.3). Nevertheless, due to the enormous catalytic potential, organometallic approaches, as with i.e. Ir\textsuperscript{I}, were regarded with great interest in the past, even when the enantioselectivities achieved were unsatisfactory.\textsuperscript{(2)} The first attempt to apply other metal ions other than Cu\textsuperscript{II} as catalyst in our labs first focused on Sc\textsuperscript{III} and Ir\textsuperscript{III}.

Sc\textsuperscript{III} is a versatile catalytic ion for many organic reactions, even in aqueous environment (see chapter 6). Unfortunately, due to its oxophilic character it probably binds strongly to the phosphate backbone of DNA as well and causes precipitation of DNA/catalyst assemblies (see chapter 4). Efforts to find suitable ligands that can overcome this problem gave rise to disappointing results.

An alternative catalytic metal ion for reactions in aqueous environment is Ir\textsuperscript{III}. This oxidation state of iridium allows it to be coordinated by bipyridine type ligands. The resulting catalysts are highly water soluble and can catalyze transfer hydrogenation reactions under mild reaction conditions.\textsuperscript{(3-5)} Preliminary studies with these catalysts in combinations with DNA (not discussed in detail in this thesis) showed that the assembly of Ir\textsuperscript{III}-based catalysts with DNA did not cause precipitation. Moreover, the catalytic activity of Ir\textsuperscript{III} in transfer hydrogenations was not significantly hampered by the presence of DNA. However, despite the use of several types of ligands, i.e. bipyridines, dppz and first generation like ligands, no enantioselectivity was obtained for the reactions catalyzed by Ir\textsuperscript{III}.

Encouraged by the high enantioselectivities achieved with Cu\textsuperscript{II}-complexes in DNA-based asymmetric catalysis, the use of Cu\textsuperscript{I} as organometallic counterpart of the Lewis acid Cu\textsuperscript{II} was explored (chapter 2). Because Cu\textsuperscript{I} can be readily obtained by the reduction of Cu\textsuperscript{II} it was possible to start with the complexes that were already applied successfully in DNA-based asymmetric catalysis. It was observed that for the intramolecular cyclopropanation of α-diazo-β-keto sulfones, other ligand classes are preferred than for Cu\textsuperscript{II}-catalyzed reactions. For achieving product formation, strongly intercalating ligands were necessary. Three possible reasons are: (i) Intercalation locks the complexes inside the DNA and this prevents the substrate from reacting with the DNA itself, or (ii) the strong binding to DNA could change the electronic properties of the ligand and this could accelerate the cyclopropanation to such an extent that it can compete with the insertion into the O-H bond of water, which is the major side reaction, or (iii) the DNA shields the metal carbene that is formed by the combination of Cu\textsuperscript{I} and the diazo substrate from the surrounding water. The later hypothesis is supported by a study in literature with ruthenium complexes that carry a dppz ligand.\textsuperscript{(6)} There, the ruthenium complex that normally showed bright luminescence in organic solvents was quenched in water. However, in presence of duplex DNA the complex intercalated into the DNA double helix and luminescence was restored. This was attributed to the hydrophobic surrounding around the dppz ligand inside the DNA helix. The higher enantioselectivities
obtained when using intercalating ligands in asymmetric cyclopropanations, can be explained by the chiral microenvironment resulting from the close proximity of the catalyzed reaction to the DNA helix and hence, an efficient transfer of chirality occurs. Using dmdppz (figure 1) as ligand up to 84% ee could be achieved in the catalytic reaction (scheme 1).

The use of iron-based catalysts in synthesis is attractive, because iron is an inexpensive and readily available metal and is used as catalyst in many catalytic reactions in nature. In chapter 3, the first DNA-based asymmetric catalysis using water soluble iron porphyrins was described. Strikingly, small changes in the catalyst structure gave rise to dramatic differences in catalyst behavior. Whereas porphyrins bearing the methyl pyridyl group at the para-position with respect to the porphyrin gave rise to 22% ee of the trans product in a slow reaction, the porphyrin where the methyl pyridyl group is at the ortho-position with respect to the porphyrin ring gave rise to a very fast reactions resulting in 43% ee of the other enantiomer of the product (scheme 1). The possible reasons for the strong acceleration of the catalytic reaction will be discussed in more detail below.

The good results obtained for water soluble Fe$^{III}$-porphyrin catalysts in cyclopropanation reactions encouraged the use of these catalysts in other insertion reactions in water. Chapter 5 described the initial experiments for the use of soluble Fe$^{III}$-porphyrin complexes in N-H bond insertion reactions. It was found that these iron-porphyrins are able to catalyze selectively the N-H bond insertion of diazo compounds into anilines, despite the enormous excess of water (~1000 fold) and the thermal decomposition of the diazo substrate. The optimization of reaction conditions resulted in very good selectivities of N-H bond insertion over O-H bond insertion (95:5, respectively).

Based on the broad scope and excellent enantioselectivities of Cu$^{II}$-catalyzed reactions in DNA-based asymmetric catalysis it is clear that Cu$^{II}$ is still the most important catalytic metal ion for DNA-based asymmetric catalysis, to date. One drawback of Cu$^{II}$ is that it needs a strong bidentate coordination to activate the substrate and place it in the right microenvironment inside the DNA. In chapter 4, the first successful DNA-based asymmetric catalytic reaction is described that uses alkylidene malonates, which coordinates via two oxygen atoms to copper, as substrate. Up to 62% ee was achieved for the Friedel-Crafts reactions of these substrates with indoles (scheme 1). However, the conversions were found to be very low, which suggests that these substrates are less well activated by Cu$^{II}$.

In DNA-based asymmetric catalysis the synthesis of racemic reference products on a preparative scale sometimes is very challenging, especially when the catalytic reaction is accelerated by DNA. It was often observed that catalytic reactions without DNA do not proceed at all or take several days for completion. For that reason, simple and high yielding catalytic tools for the synthesis of racemic products with Lewis acids in water had to be developed. In chapter 6 the use of sodium dodecyl sulfate amphiphiles in combination with Sc$^{III}$ was described as a highly potent catalytic system to synthesize 3-functionalized indoles in water. A broad substrate scope, high yields and an easy procedure are the strong points of this catalytic system. This micellar catalysis method enabled the synthesis of the racemic products used as reference compounds in chapter 5 and is in general a useful catalytic tool for certain Lewis acid catalyzed Friedel-Crafts reactions in water.
7.3. **Important characteristics of metals and ligands**

In the following paragraph the most important characteristics for suitable metal and ligand combinations in DNA-based asymmetric catalysis based on the literature published in the corresponding field and the experience obtained during the experimental work described in this thesis will be discussed. Here, the term DNA covers all structures of DNA, e.g. duplex DNA and G-quadruplex DNA.

The major characteristics of a suitable metal for DNA-based asymmetric catalysis are:

- The metal ion has to be compatible with the conditions necessary for DNA-based asymmetric catalysis. This means that the metal needs to form complexes that are soluble and stable in aqueous mixtures within a defined pH range (often pH 5-7).
- The metal ion should have bio-orthogonal activity, which means it should preferably not catalyze reactions between substrates and DNA or water, or at least only to a small extent (see O-H bond insertion in chapter 2 and 3).
- The metal ion should not require other sterically demanding ligands, since these could hinder a good interaction of the DNA binding ligand with the DNA.
- The binding affinities of the metal ion towards the functional groups of DNA should be smaller than the binding affinities of the metal to the ligand where it is bound on. This guarantees that the metal complexes are stable throughout the catalysis (see problems with Sc$^{III}$ in chapter 4).
- The metal ion should have a relatively fast exchange rate with the substrate and product, otherwise the metal would be poisoned, which slows down the reaction rate.

Additionally there are several requirements for suitable ligands in DNA-based asymmetric catalysis:

- The ligand should form complexes with the applied metal that are soluble and stable under the conditions of DNA-based asymmetric catalysis (sometimes the interaction between ligand and DNA, i.e. intercalation, helps to obtain homogeneous reaction mixtures) (see chapter 2 and 3).
- The ligand has to enable binding of the complex to DNA. In cases where the catalyzed reaction is highly accelerated when the complex is bound to the DNA, the binding to DNA does not need to be strong (see reference$^7$ and chapter 3).
- The ligand should place the metal in close proximity to the DNA, so it will provide a chiral microenvironment that can direct the catalyzed reaction towards selective formation of one of the enantiomers of the product.
- For later applications it is desirable that the ligand is commercially available or relatively easy to be synthesized. The ligand itself should not be chiral, since the chirality is supplied by DNA. Moreover, using a non-chiral ligand avoids possible matched/mismatched combinations of complex and DNA.$^2$
7.4. Rate acceleration by DNA

In DNA-based asymmetric catalysis the affect of DNA on the rate of the catalytic reaction plays a crucial role.\[7, 8\] Looking at all reactions in DNA-based asymmetric catalysis it can be concluded that DNA can either decelerate\[8, 9\], not affect (chapter 4) or accelerate\[7, 8\] (chapter 3) the catalyzed reaction. When the rate of the catalytic reactions is decelerated or not affected, DNA has the function of a chiral auxiliary group that only provides the chiral environment for the catalyzed reaction. From a practical point of view acceleration is highly desirable. In the case of rate acceleration, the DNA provides favorable interactions with the metal ion, the ligand and/or the substrates and/or the transition state that lead to a faster reaction. In these cases the DNA can be seen as a biomolecular catalyst, a so called DNAzyme. The second coordination sphere can provide intermolecular interactions that give rise to rate accelerations. In the following paragraph several possible pathways of rate acceleration by DNA will be described.

- Since the inner core of the DNA contains less water, DNA could potentially shield the reagents of an organic reaction from water (desolvation, see chapter 2).
- The hydrophobic interior of the DNA structure could concentrate organic reagents similar to the hydrophobic effect in micelles.
- The DNA could activate a metal catalyst by additional coordination to the metal ion.
- The second coordination sphere of DNA could contribute to stabilizing the transition state of a reaction or destabilize the ground state and by this causes an accelerated reaction.\[10\]
- DNA could potentially assist reactions by positioning of reagents. Through intermolecular interactions, reagents can be placed in a favorable position for a faster reaction, the so called shuttle effect.\[11\]
- DNA may provide interactions with the ligands, e.g. π-π interactions that increase the electronic density on the ligand and by this increase the catalytic activity of the metal.

For Cu\[^{II}\] catalyzed reactions a detailed analysis of possible modes of rate acceleration by DNA was provided by Arnold Boersma in his doctoral thesis.\[11\] A tentative structural and stereochemical model of dimethyl bipyridine Cu\[^{II}\] complex with a short oligonucleotide was used to explain possible reasons for the rate acceleration by DNA. Boersma proposed that ground state destabilization by dehydration and the stabilization of the transition state by DNA as two possible contributions to Cu\[^{II}\]-dmbpy catalyzed reactions in DNA-based asymmetric catalysis.

![Figure 1](Overview of catalysts.)
To answer questions about the differences between Lewis acid catalysis and organometallic catalysis in DNA-based asymmetric catalysis, the mode of binding of the complexes to DNA as well as the coordination geometry around the catalytically active metal should be taken into account. Whereas little is known about the geometry of the CuI-dmdppz during the carbene reaction described in chapter 2, the coordination geometry of CuII-dmbpy bound to substrate was studied in more detail. The complex with substrate is expected to be planar with all equatorial positions occupied (see chapter 1.2 for more detail).

The binding with DNA is strongly depending on the ligand applied. For CuII-dmbpy it was found that the binding to DNA takes place via the minor groove and that intercalation is possible, but most likely not very prominent. In contrast, for dppz ligands that were used in the asymmetric cyclopropanation with CuI it was found that when those ligands are bound in ruthenium complexes, strong intercalation takes place via the major groove of the DNA double helix. This strong intercalation might activate the complex by \(\pi-\pi\) interactions and additionally the hydrophobic core could shield the metal-carbene species from water resulting in a higher degree of cyclopropanation (chapter 2).

Likewise, some charged porphyrins, as i.e. FeIII-(TM4PyP) (figure 1) bind via intercalation in the major groove of duplex DNA and via groove binding. Interestingly, when FeIII-(TM4PyP) was applied in catalysis, the reaction was slow in presence of st-DNA, but with FeIII-(TM2PyP) the reaction was considerably faster in presence of DNA. For the corresponding manganese porphyrins the same catalytic effect was noticed in superoxide dismutase activity. The authors correlated this effect to a difference in the tendency for intercalation. The strongly intercalating MnII-(TM4PyP) was hidden inside the DNA and could not be reached by the reactants. A lower degree of intercalation was noticed for MnII-(TM2PyP) which gave a considerably faster reaction. Similarly, for the FeIII-porphyrins this could mean that FeIII-(TM2PyP) is more accessible for the reactants in the cyclopropanation reaction but is still in close enough proximity to the DNA to profit from the other characteristics of DNA, such as the hydrophobic effect and other interactions that can give rise to rate accelerations (see above).

It can be concluded that in those cases were the DNA accelerated the catalytic reaction, i.e. Lewis acid catalysis with CuII-dmbpy and iron porphyrin catalyzed reactions with FeIII-(TM2PyP), binding to DNA via the grooves of DNA is favored over a stronger binding by intercalation, compared to CuII-dppz and FeIII-(TM4PyP), respectively. This was not the case when the presence of DNA decelerated the reaction as in the intramolecular cyclopropanation. There, a strong intercalation of the catalyst was needed for an efficient intermolecular cyclopropanation reaction in the presence of DNA.

### 7.5. Mechanistic considerations

As mentioned in chapter 5 the mechanism of iron porphyrin catalyzed N-H bond insertion reaction is still under debate in the literature. Whereas FeIII-porphyrins can catalyze the insertion reaction of aniline and EDA in organic solvents without the need of a reducing agent, iron heme containing P450 enzymes need to be kept under anaerobic conditions in presence of a reducing agent to reduce the FeIII resting state back to the catalytically active FeII (scheme 2). Also the two proposed intermediates are different. In the case of
Fe\textsuperscript{III}-porphyrins most likely an ylide intermediate is formed as whereas with P450 enzymes a carbenoid intermediate is favored.

In the case of Fe\textsuperscript{III}-((TM4PyP), which was used for N-H bond insertions in chapter 5, no reducing agent and anaerobic conditions were needed. Additionally, the catalyst was not poisoned by an excess of amine as observed with Fe\textsuperscript{II}-porphyrins.

**Scheme 2** N-H bond insertion reactions in literature and from this thesis.

Thus, it seems that iron porphyrins and iron heme-containing enzymes prefer different reaction pathways. Whereas Fe\textsuperscript{III}-porphyrins are active enough to catalyze the N-H bond insertion reactions, the Fe\textsuperscript{III} in heme-like structures is not active enough. It needs to be reduced to Fe\textsuperscript{II}, which then can be activated by an additional axial ligand in the enzyme structure\textsuperscript{[17]} The axial ligand at the same time maybe provides protection against poisoning by an excess of amine.

**Scheme 3** Concerted and stepwise mechanism for the X-H bond insertion by metal carbenes. Adapted from Zhu et al.\textsuperscript{[20]}

For asymmetric catalysis it is important that the reaction follows a pathway in which the catalyst is bound to the substrate during the step in which the stereocenter is formed (scheme 3). This can either be the case in a concerted mechanism (step a) or during a stepwise mechanism involving ylide I (step b), where catalyst dissociation and proton
transfer are simultaneously (step c). A mechanism including a free ylide II would give rise to racemization during the reaction (step d and e).

In the case of Fe\textsuperscript{II}-containing P450 enzymes this means that stereoselectivity in N-H bond insertion reactions via a concerted mechanism, containing Fe\textsuperscript{II}-carbenoids as proposed by Arnold et al., is possible. Unfortunately, despite the formation of potential chiral products no stereoselectivity has been reported, yet.\textsuperscript{[17]}

![Structure of iron porphyrin carbene complexes](image)

Scheme 4 Two possible structures of iron porphyrin carbene complexes that are under debate in literature. Adapted from Khade et al.\textsuperscript{[21]}

For cyclopropanations with iron porphyrins the concerted mechanistic pathway via a carbenoid intermediate involving the carbene coordinated to Fe\textsuperscript{II} (scheme 4), is favored in literature.\textsuperscript{[21]} However, for the cyclopropanation with Fe\textsuperscript{III}-porphyrin no reducing agent and/or inert atmosphere were required to produce the product with a reasonable enantioselectivity (see chapter 3). The reduction of Fe\textsuperscript{III} to Fe\textsuperscript{II} by EDA could be a reason for this observation, but often elevated temperatures are needed for the reduction of Fe\textsuperscript{III} to Fe\textsuperscript{II} by EDA\textsuperscript{[19]} and a control experiment with reducing agent and under anaerobic conditions gave rise to lower yield and ee’s (see chapter 3). The active catalyst for DNA-based asymmetric catalysis is most likely in Fe\textsuperscript{III}-state. Woo et al.\textsuperscript{[19]} proposed the amine assisted formation of a Fe\textsuperscript{III}-carbenoid structure for the N-H bond insertion reaction. Likewise, this could be the explanation for the rate acceleration of the cyclopropanation in presence of DNA. As mentioned in paragraph 7.4, the DNA could provide a favorable axial coordination to the Fe\textsuperscript{III}-porphyrin that gives rise to the enormous rate accelerations.

7.6. Perspectives for DNA-based asymmetric catalysis

Prior to this thesis the catalytic scope of DNA-based asymmetric catalysis was limited to Lewis acid catalysis. The results described here are the first examples of novel reactivities in DNA-based asymmetric catalysis. Organometallic reactions should be the focus of further investigations. Especially the use of iron containing catalysts for asymmetric reactions in water is highly desirable, because iron is cheap and readily available. Fe\textsuperscript{III}-based catalysis can be performed under aerobic conditions, which makes the study of Fe\textsuperscript{III}-containing reactions relatively straightforward. Additionally, iron porphyrins as catalysts provide a very useful connection to other biomolecules, i.e. heme containing enzymes. As there are many studies about heme containing enzymes in literature, the connection to this class of biomolecular catalysts could potentially provide a more detailed understanding of the characteristics that are important for rate accelerations in presence of biomolecules and the transfer of chirality from the biomolecule, i.e. DNA or enzyme to small molecular substrates. The binding of porphyrins to DNA is studied in literature (see chapter 3) and will enable a better model of this kind of DNA-based asymmetric catalysis. Especially the observation of a dramatic rate acceleration of cyclopropanations catalyzed by Fe\textsuperscript{II}-(TM2PyP) in presence of DNA should be studied spectroscopically in more detail. It should be investigated which factors contribute to the observed rate accelerations in the case of iron porphyrins. The enormous rate acceleration could also be a step forward towards the application of DNA-based asymmetric catalysis in industry, because carbene reactions are widely used as a tool.
in organic synthesis, while asymmetric variants of this class of reactions in water are rare. Additionally, detailed understanding of iron porphyrins in DNA-based asymmetric catalysis could open a new field of *in vivo* catalysis, because the bio-orthogonal character of iron porphyrins could be the bridge between traditional catalysis and biomolecular catalysis. This means that catalysis in more complex systems like cells could be feasible.

Altogether in this project, DNA-based asymmetric catalysis has made the step from only Lewis acid catalysis to organometallic catalysis, which potentially paves the way for a wider use of this approach in synthesis. Moreover, this contributes to the study of asymmetric catalysis in more complex catalytic systems like cells.

### 7.7. Reference


