Chapter 6
Conclusions and Perspective

In this chapter the results described in this thesis are summarized and a general discussion will be presented about DNA-based catalysis and what the present work adds to this. Furthermore, future perspectives are presented on the basis of ongoing projects.

6.1 Introduction

The research described in this thesis was initiated by the discovery of DNA-based asymmetric catalysis.[1] This concept is based on the use of an achiral metal complex that binds to DNA in a non-covalent fashion (Figure 6.1A). Upon binding of the complex a chiral micro-environment is created around the metal complex. This chiral environment has been used to induce enantioselectivity in a variety of reactions, like the Diels-Alder reaction,[1-5] Michael addition,[6] Friedel-Crafts alkylation[7] and the *syn*-hydration reaction[8] (Figure 6.1B).

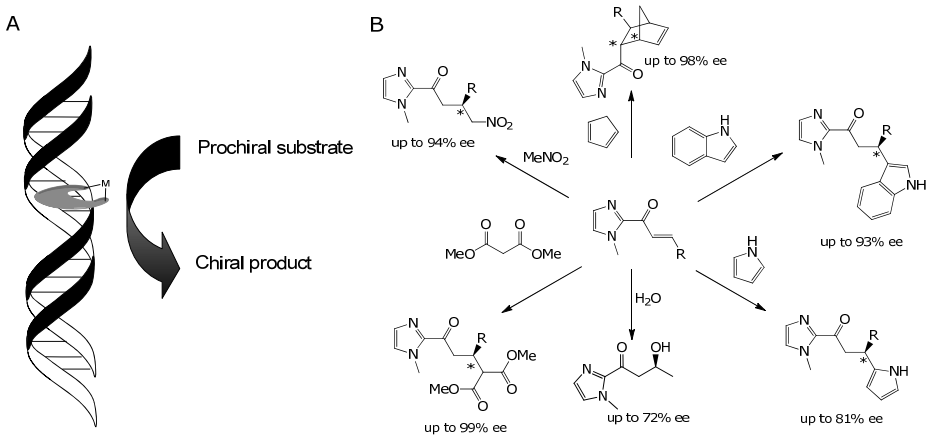


Figure 6.1. A; Concept of DNA-based catalysis, B; reaction scope.

The goal of the research described in this thesis was to develop this method into a synthetically useful technique. The specific aims were to use this approach for larger scale reactions, development of an easy recycling method of the catalyst and discovery of novel reactivities.

The main achievements reported in this thesis are:

- Water miscible organic co-solvents can be used in DNA-based catalysis. The ee was not affected and in several cases faster reactions were found, due to an increase of the rate of product dissociation. The enantioselectivity of these reactions was further enhanced since the co-solvent allowed for lowering the reaction temperature to -18 °C.
- The development of the first transition metal catalyzed enantioselective intermolecular oxa-Michael addition of alcohols.
- The development of the first catalytic enantioselective protonation of α-substituted enones in water.

In the present chapter, the reactivity of DNA-based catalysts will be discussed and combined with new insights generated in the present work. This discussion will be based on a distinction between 1st and 2nd generation catalyst and their different reactivity profile. Furthermore,

future prospects will be presented on the basis of additional experiments.

6.2 DNA-based catalysis

Over the last years, DNA-based catalysis has evolved into a promising technique for enantioselective organic synthesis. In this period, two generations of DNA-based catalysts have been developed, which differ in the type of ligands used: the acridine based ligands, which have a DNA intercalating part attached to a metal binding part (1st generation)[1] and ligands that combine the DNA binding part and the metal binding (2nd generation).[2] It has been shown that both types of ligands exhibit different characteristics and perform best in separate classes of reactions. In this chapter, I will discuss the different reactivity and present a model that gives a possible explanation for their different behavior.

6.2.1 1st generation vs. 2nd generation ligands

The first generation ligands comprise an acridine moiety, responsible for DNA intercalation, tethered to a metal binding domain via a small linker. The design of the ligand has an important influence on the enantioselectivity of the catalyzed reaction (Figure 6.2). Generally ligands containing a (substituted) arylmethyl group give rise to the best results. One of the characteristics of these ligands is that the outcome of the reaction can be influenced by the length of the spacer.[1,2] It has been shown in the Diels-Alder reaction that different enantiomers could be obtained by extending the linker with one extra carbon atom (48% (n=2; **1**), -49% (n=3; **2**)).

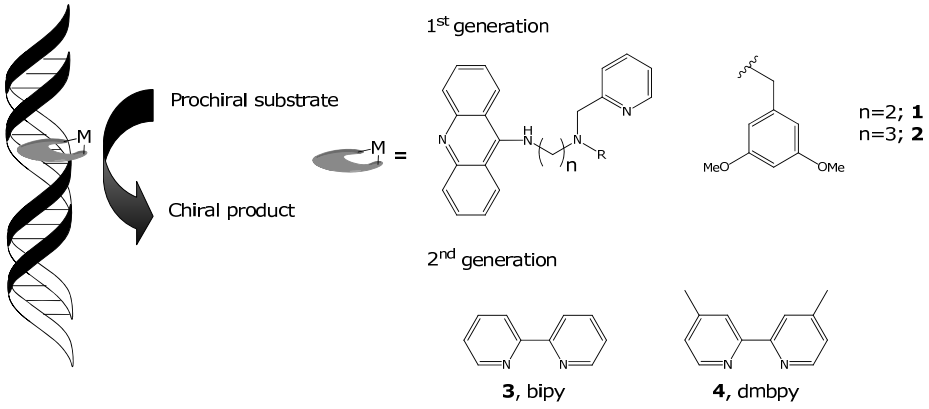


Figure 6.2. Concept of DNA-based catalysis with examples of 1st and 2nd generation ligands.

In the second generation ligands the DNA-binding moiety is combined with the metal-binding region into one structural unit, hence the linker is no longer required. With this class of ligands a dramatic

increase in enantioselectivity was observed in the Diels-Alder reaction (up to 99% ee for 4,4'-dimethyl-2,2'-bipyridine (dmbpy)), however only one enantiomer of the product can be generated.[2] Using terpyridine, instead of dmbpy, made it possible to obtain the opposite enantiomer of the product.[9]

6.2.2 Reactivity

1st and 2nd generation metal-ligand complexes are active in a wide variety of reactions. However, there is a striking difference in their reactivity and (enantio-)selectivity. The second generation copper complexes give rise to excellent ee's in C-C bond-forming reactions, like the Diels-Alder reaction,[2] the Michael addition,[6] the Friedel-Crafts alkylation[7] and the Friedel-Crafts alkylation/enantioselective protonation cascade reaction (Chapter 5). The first generation copper complexes can also catalyze these reactions albeit with much lower enantioselectivities. However, these are more successful in catalyzing reactions with oxygen based nucleophiles, like the oxa-Michael addition (Chapter 4) and the *syn* hydration reaction, resulting in good ee's.[8]

This can be explained by the difference in interaction of the metal complexes with the DNA. In the case of the 2nd generation ligands the reaction is proposed to take place in the minor and major groove of the DNA.[10] It is thought that in these cases the tighter microenvironment provided by the binding of the complex into the DNA ensures the higher enantioselectivity. Hydrophilic nucleophiles like alcohols and water are less present in the DNA core, due to the hydrophobicity of the DNA core.[11,12] Hence the low reactivity of these catalysts in the oxa-Michael addition.

The 1st generation ligands are based on the intercalating acridine which is tethered via a carbon spacer to the metal-binding domain. Due to the linker, the active copper centre is partially located in the hydration shell of the DNA. As a concequence, the conjugate addition of oxygen based nucleophiles can take place with high selectivity. This hypothesis is also in agreement with the observed decrease in enantioselectivity in the Diels-Alder reaction when using 1st generation ligands with a longer spacer.

6.2.3 Role of DNA

The role of DNA is not unambiguous. The DNA does not only act as chiral scaffold but also plays an important role for the rate of the reaction. For the Diels-Alder reaction,[4] the Michael addition[13] and the Friedel-Crafts alkylation[7] the DNA accelerates the reaction in case of the 2nd generation ligands. A rate increase of up to 58-fold was found for the Diels-Alder reaction. It is possible that this rate acceleration is caused by favorable arene-arene interactions of the substrate bound Cu^{II}-complex with the nucleobases of the DNA.[4]

The favorable arene-arene interactions might also play an important role in the product dissociation step (Chapter 3). A kinetic study of the Michael addition and the Friedel-Crafts alkylation showed a decrease in the overall rate (k_{app}) upon using water miscible organic co-solvents, while higher conversions were found under catalytic conditions. These observations were only made in the case of substrates with an aromatic or methyl substituent. Due to the fact that in the kinetic study the dissociation step is left out of consideration; the addition of water miscible co-solvents has to accelerate the dissociation step. However these observations were only made in the case of substrates with aromatic or methyl substituents. This suggests that the product bound Cu^{II}-complex is stabilized, possibly by the favorable arene-arene interaction, and thus becomes rate limiting.

Also in the Friedel-Crafts alkylation/enantioselective protonation cascade reaction the DNA is of utmost importance for enantioselectivity in this reaction (Chapter 5). The protonation needs to be the rate determining step in order to obtain high enantioselectivity. However, due to the large concentration of protonating agent and the fact that protonation is generally very fast, this can only be achieved by acceleration of the Friedel-Crafts alkylation step. Up to 60% ee was obtained in this cascade reaction demonstrating the acceleration of the Friedel-Crafts alkylation step by the DNA. Furthermore, we hypothesize that the difference in enantioselectivity obtained with different π-nucleophiles is partly caused by the difference in reactivity of these π-nucleophiles: with the less reactive indoles the DNA accelerating effect is not sufficient to make the protonation step rate limiting. The formation of products which are not formed under catalytic conditions without DNA already suggests that the rate of the Friedel-Crafts alkylation step is accelerated. However, a kinetic study is necessary in order to prove this hypothesis.

6.2.4 Importance of DNA sequence

1^{st} and 2^{nd} generation metal-ligand complexes also show a large difference in their DNA sequences dependence. The 2^{nd} generation metal-ligand complexes display a preference for sequences containing G-trimers. It has been shown that these sequences do not only induce the highest enantioselectivity but also accelerate the reaction more than sequences without this G-trimer. These results are most likely related to the structure of the DNA. The CD-spectra showed that all sequences still show a CD-signal similar to B-type DNA.[10] However, the sequences containing the G-trimer showed a CD spectrum that is indicative of a small distortion towards A-type DNA.

The sequence dependence has an important implication for the reactions catalyzed by st-DNA. As mentioned before, the sequence of st-DNA can be considered as a random sequence. Therefore, the obtained ee is a weighted average of the outcome of all sequences. However,

since sequences containing G-trimer accelerate the reaction most, they will dominate the outcome of the reaction.

Also in the case of the Friedel-Crafts alkylation/enantioselective protonation cascade, the optimal sequence contained a G-trimer (Chapter 5). However, among the tested sequences there was not a clear trend to be found. This can be attributed to the fact that this cascade reaction requires two different steps. The first step involves the addition of the neutral π-nucleophile to the α,β-unsaturated ketone and the second step involves the asymmetric protonation of the formed enolate. In order to obtain high enantioselectivity the protonation step needs to be rate-determining. Therefore, a sequence that accelerates the addition of the π-nucleophile but does not, or to a lesser extent, accelerate the protonation step, would be suitable. However, due to this conflicting argument, sequences suitable for this purpose may be limited.

The 1st generation metal-ligand complexes showed a preference for AT-rich sequences in the *syn* hydration reaction.[8] However, in the case of the oxa-Michael addition of alcohols, lower enantioselectivities were found upon using specific DNA sequences (Chapter 4). The presence of the alcohol destabilizes the duplex formation of the oligonucleotides and thereby also influences the outcome of the reaction. However, sequences containing a central ATAT segment tend to show the highest enantioselectivities, although, these are considerably lower compared to the results obtained with st-DNA.

6.2.5 Recycling of the DNA-based catalyst

In order to optimize the recyclability of the catalyst after use a DNA-functionalized gold nanoparticles (DNA-Au np's) have been synthesized and tested in catalysis. However, when using these DNA-Au np's in the DNA-based catalyzed Diels-Alder reaction and Michael addition, no enantioselectivity could be obtained. This is attributed to the blocking of the active copper centre by neighbouring DNA strands, since unfunctionalized Au np's did not influence the catalysis. Alternative approaches for such a recyclable catalyst should be based on mixed monolayers or the use of a different solid support, such as glass.

6.2.6 Water-miscible organic co-solvents

Water-miscible organic co-solvents can be used in DNA-based catalysis and can even be beneficial for it. Chapter 3 describes the use of up to 33 v/v% of water-miscible organic co-solvents without a loss in conversion and enantioselectivity. In the case of substrates with an aromatic or methyl substituent even higher conversions were found compared to the reactions in water alone. However, a kinetic study revealed that the use of water miscible organic co-solvents result in a decrease in the overall rate (k_{app}). This can only be explained by the product dissociation step, which is not taken into consideration under kinetic conditions. Organic co-solvents accelerate the product

dissociation and thereby the overall reaction rate. Moreover, the use of water-miscible organic co-solvents has made it possible to reduce the catalyst loading down to 0.75 mol% and has as additional advantage that it allows for lower reaction temperatures. This has been used in the Friedel-Crafts alkylation. By performing the reaction at -18 °C the enantioselectivity was increased from 83% to 93%. However, this was achieved at the expense of the reactivity.

The use of water-miscible organic co-solvents has also led to the development of the oxa-Michael addition of alcohols (Chapter 4). The combination of the water and alcohol is crucial for this reaction to take place even though it also gives rise to the *syn*-hydration product as side product. The alcohol is required as nucleophile whereas water seems to be important for reverting unwanted side reactions such as 1,2-additions, resulting in higher overall yields. The formation of the *syn*-hydration product could be reduced by performing the reaction at -18 °C. Apparently, the rate of the hydration reaction depends much stronger on the temperature than the oxa-Michael reaction. Hence, even though the requirement for aqueous conditions causes the formation of a side product resulting from hydration of the enone, the reaction can be made chemoselective by lowering the reaction temperature.

6.3 Future prospects

6.3.1 DNA-based catalysis as synthetic tool

DNA-based catalysis has made an impressive progress since its discovery in 2005. However, as a tool for organic synthesis it has not made its entrance yet. This is probably due to the fact that DNA is not commonly used in organic synthesis and presents a mental barrier for the synthetic chemist. DNA-based catalysis has however a great potential and shows several advantages which make it suitable as a synthetic tool.

- DNA is, compared to other chiral ligands, relatively inexpensive and can be obtained from natural sources.
- DNA-based catalysis is performed in water. Water is a "green" solvent and is one of the most inexpensive and safe solvents imaginable. Moreover, whenever water is problematic, due to solubility issues, water miscible organic co-solvents can be added in order to improve the solubility.
- DNA-based catalysis shows interesting reactivities. Using DNA-based catalysis unusual enantioselective reactions, like the oxa-Michael addition of alcohols and the *syn*-hydration reaction, can be performed. These reactions are problematic using conventional catalysis.

- DNA-based catalysis is performed under ambient conditions. Generally, the reactions are performed at 5 °C without a protective atmosphere. However, by the addition of a water miscible organic co-solvent the temperature can be lowered to -18 °C. which can be beneficial for either the enantioselectivity or chemoselectivity.

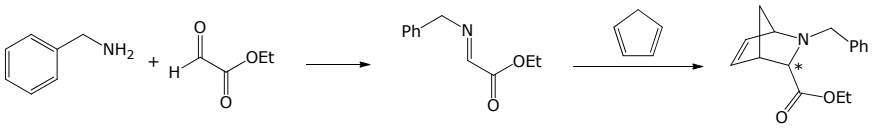
- Although an auxiliary group is required, for the coordination to the copper, it can be readily replaced by a variety of functional groups, such as carboxylic acids, esters, aldehydes and ketones.[14,15] Still, the use of substrates without the auxiliary group would be desirable.

Of course, there is still room for improvement. Challenges remain, they will be discussed in combination with some insights obtained from ongoing projects.

6.3.2 Challenges

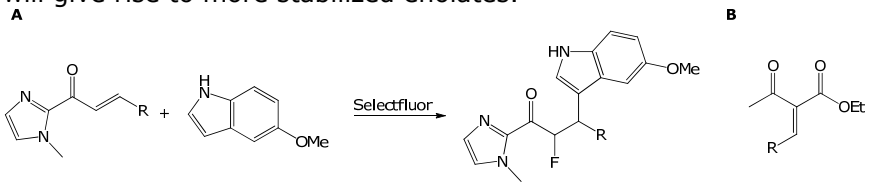
- *Recycling*
  One of the challenges mentioned before is still to improve the recyclability of the catalyst after use. The most suitable method is the covalent attachment of a short duplex of DNA to a solid support. However, the problem here is the blocking of the catalyst by the dense coverage of DNA on the solid support. This could be solved in two possible ways. Firstly, by making a mixed monolayer of alkanethiols and thiol modified DNA on gold nanoparticles. One possible problem could be that the cooperative binding of DNA on the gold support results in domains of DNA and thus not in reducing the packing of the DNA. The second solution would be to switch to a gold support with a mixed monolayer of alkanethiols and alkanethiol with a functional group and attach the DNA to these functionalized alkane thiols in a second step. This will result in separated duplexes of DNA on the solid support, however at the expense of the duplex stabilization induced by the cooperativity.

- *Expanding the scope of reactions*
  Although DNA-based catalysis has shown its power in several reactions, there is still plenty of room for the development of reactions. The asymmetric addition of thiols and amines are obvious but one could also think of cascade reactions like an aza-Diels-Alder reaction in which the dienophile is *in situ* formed from an amine and an aldehyde. Such a reaction would benefit from the addition of an organic co-solvent since the formed imine would readily hydrolyse in water. An aza-Diels-Alder reaction of cyclopentadiene with an *in situ* formed imine from benzylamine and ethylglyoxylate was performed (Scheme 6.1) with 33 v/v% of dioxane.[16] Unfortunately, even though the product could be isolated, no separation could be found on chiral HPLC.



Scheme 6.1. Aza-Diels-Alder reaction of cyclopentadiene with an *in situ* formed imine from benzylamine and ethylglyoxylate.

An alternative cascade reaction could make use of two previously developed reactions for DNA-based catalysis. In this cascade a conjugate addition is followed by the trapping of the enolate with an electrophile, such as for example an electrophillic fluorine source.[17] Such a cascade reaction was attempted by perfoming a Friedel-Crafts alkylation followed by the trapping with selectfluor (Scheme 6.2A). However, after the reaction only the Friedel-Crafts alkylation product could be obtained. Probably the lifetime of the enolate is too short to allow for an efficient fluorination of the intermediate enolate. A β-ketoester substrate (Scheme 6.2B) could be an interesting alternative substrate for this cascade reaction, since this will give rise to more stabilized enolates.



Scheme 6.2. A; Friedel-Crafts alkylation followed by the trapping with selectfluor, B; Alternative substrate, β-ketoester.

- *Transition metal catalysis other than copper catalysis*
  Untill now, all examples of DNA-based catalysis are based on Cu^{II}-complexes. However, the use of additional types of transition metal complexes should be possible. One of the major challenges in finding new metal complexes for DNA-based catalysis is the DNA itself. DNA has a large amount of free amines and alcohol groups capable of binding to the metal and thereby blocking its activity. In my opinion, especially hard metals pose problems in combination with DNA, since the large amount of free alcohols on the sugar moieties and phosphates can chelate the metal strongly. However, with this in mind, new metal complexes can be envisioned. A Pd-Bpy complex would be a suitable candidate to start with. They form, like Cu^{II}, square planar complexes,[18] bind to DNA,[18] and can be used in water. Furthermore, they already showed activity in a boronic acid addition to cyclohexenone. Under unoptimized conditions, Pd-Bipy showed 25% ee; however, due to hydrolysis of the boronic acid the conversion was only 5%. Altogether this would be a promising start for a new catalyst in DNA-based catalysis.

6.4 Concluding remarks

All in all, DNA-based catalysis displays interesting reactivities and high enantioselectivities, which make it interesting for organic synthesis. The present work represents a major step towards its application in organic synthesis.

6.5 References

1. G. Roelfes, B. L. Feringa, *Angew. Chem. Int. Ed.* **2005**, *44*, 3230.
2. G. Roelfes, A. J. Boersma, B. L. Feringa, *Chem. Commun.* **2006**, 635.
3. A. J. Boersma, B. L. Feringa, G. Roelfes, *Org. Lett.* **2007**, *9*, 3647.
4. A. J. Boersma, J. E. Klijn, B. L. Feringa, G. Roelfes, *J. Am. Chem. Soc.* **2008**, *130*, 11783.
5. F. Rosati, A. J. Boersma, J. E. Klijn, A. Meetsma, B. L. Feringa, G. Roelfes, *Chem. Eur. J.* **2009**, *15*, 9596.
6. D. Coquière, B. L. Feringa, G. Roelfes, *Angew. Chem. Int. Ed.* **2007**, *46*, 9308.
7. A. J. Boersma, B. L. Feringa, G. Roelfes, *Angew. Chem. Int. Ed.* **2009**, *48*, 3346.
8. A. J. Boersma, D. Coquière, D. Geerdink, F. Rosati, B. L. Feringa, G. Roelfes, *Nature Chem.* **2010**, *2*, 991.
9. A. J. Boersma, B. de Bruin, B. L. Feringa, G. Roelfes, *Chem. Commun.* **2012**, *48*, 2394.
10. A. J. Boersma, **2009**, DNA-based asymmetric catalysis, Thesis, University of Groningen.
11. K. Guckian, B. Schweitzer, R. Ren, C. Sheils, D. Tahmassebi, E. Kool, *J. Am. Chem. Soc.* **2000**, *122*, 2213.
12. E. Kool, J. Morales, K. Guckian, *Angew. Chem. Int. Ed.* **2000**, *39*, 990.
13. E. W. Dijk, A. J. Boersma, B. L. Feringa, G. Roelfes, *Org. Biomol. Chem.* **2010**, *8*, 3868.
14. D. H. Davies, J. Hall, E. H. Smith, *J. Chem. Soc. Perkin Trans. 1* **1991**, 2691.
15. D. A. Evans, K. R. Fandrick, H. J. Song, K. A. Scheidt, R. Xu, *J. Am. Chem. Soc.* **2007**, *129*, 10029.
16. P. Bailey, R. Wilson, G. Brown, *Tetrahedron Lett.* **1989**, *30*, 6781.
17. N. Shibata, H. Yasui, S. Nakamura, T. Toru, *Synlett* **2007**, 1153.
18. M. Yodoshi, N. Okabe, *Chem. Pharm. Bull.* **2008**, *56*, 908.