Bio-inspired catalysis in water

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Chapter 1 State of the art in DNA-based asymmetric catalysis

The transfer of chirality from a DNA structure onto an organic reaction to achieve enantio-enriched products is an innovative concept in asymmetric catalysis in water. Since its initiation in 2005, the field of DNA-based asymmetric catalysis has emerged rapidly with several contributions that investigate the effects of the DNA structure, the type of the ligand and the way of anchoring into the DNA on different kinds of organic reactions. This chapter will give an elaborate literature survey about this field of asymmetric catalysis.

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1.1. DNA-based asymmetric catalysis

1.1.1. Hybrid catalysts

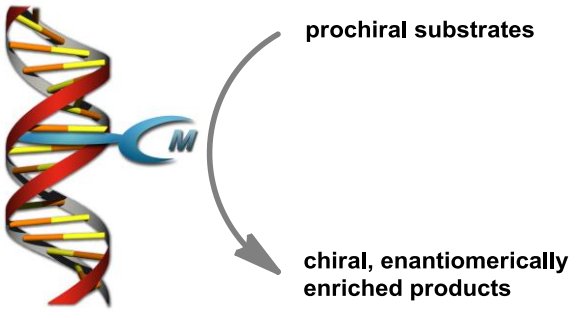
Metalloenzymes are a source of inspiration for the field of catalysis due to the high catalytic activities and selectivities achieved under mild conditions.[1] However, the catalytic repertoire of homogeneous catalysis, in particular transition metal catalysis, is much larger. This has led to efforts to combine these two fields, resulting in the design of artificial metalloenzymes, also known as hybrid catalysts.[2, 3] A hybrid catalyst comprises a catalytically active transition metal complex that is anchored to a biomolecular scaffold via a covalent linkage or by supramolecular interactions. Whereas the transition metal complex is responsible for catalysis, the second coordination sphere interactions provided by the biomolecular scaffold give rise to (enantio)selectivity and, ideally, an additional rate acceleration.[4] Using protein scaffolds a range of highly enantioselective transformations has been developed.[2, 3] The potential of using DNA as the biomolecular scaffold was demonstrated by the introduction of the concept of DNA-based asymmetric catalysis.[5]

1.1.2. Concept of DNA-based catalysis

A key aspect of the structure of DNA is its unique chirality. Built up from chiral monomers, it self-assembles into a duplex structure that possesses a higher order helical chirality. Depending on the hydration and the counter ions present, these helical structures range from the ubiquitous double right handed helix structure of B-DNA to the left handed helical structure of Z-DNA, with many variations.[6] No examples of catalytic reactions, in which DNA's chirality is used for the enantioselective synthesis of chiral molecules have been found in nature to date. This is in marked contrast with RNA; catalytic RNA has been found to catalyze a number of reactions *in vivo*.[7]

However, from a chemical perspective, DNA represents an attractive source of chirality for enantioselective catalysis; it is chemically stable and can be readily obtained from synthetic or natural sources in large quantities at reasonable prices. Moreover, using DNA implies using water as reaction solvent; replacing organic solvents by water is of particular interest for economic reasons and may give rise to more environmentally benign processes.[8]

The concept of DNA-based asymmetric catalysis involves placing a catalytically active metal complex in proximity of DNA (scheme 1). The catalyzed reaction then occurs inside or close to the chiral environment of the DNA helix, resulting in the preferential formation of one of the enantiomers of a chiral product. Anchoring of the transition metal complex can be achieved by using non-covalent interactions such as intercalation or groove-binding (section 1.1.3) or by covalently attaching the ligand to the DNA (section 1.1.5).



Scheme 1 Concept of DNA-based asymmetric catalysis.

Both attachment strategies have their own special features. Covalent attachment allows for precise control over the catalyst's location inside the DNA structure, but is inherently an approach that is synthetically more involved, thus complicating the design and optimization process. For potential applications in synthesis, a non-covalent anchoring strategy presents many advantages. These include easy assembly of the catalytic system, which allows for rapid structure variation and optimization, and the possibility to use inexpensive DNA from natural sources, such as salmon testes DNA (st-DNA).

To date, two generations of supramolecular DNA-based catalysts have been developed. The first generation involves ligands that comprise separate DNA and metal binding moieties which are connected through a spacer. In the second generation of ligands the DNA and metal binding moieties are integrated, thus obviating the need for a spacer. In principle, this allows for the catalytic metal center to be placed closer to the DNA compared to the first generation of ligands. The ligands used to date in supramolecular DNA-based asymmetric catalysis are shown in figure 1. *A priori*, it cannot be predicted which kind of ligand is most suitable for a given reaction, although some general trends were observed (see chapter 1-3 and 7 of this thesis). The DNA-based catalyst is conveniently prepared by self-assembly upon combination of the metal complex with the DNA and is used as such. Below an overview of catalyzed reactions using the supramolecular approach will be given, followed by an overview of DNA-based catalysts in which the metal complex is anchored covalently.

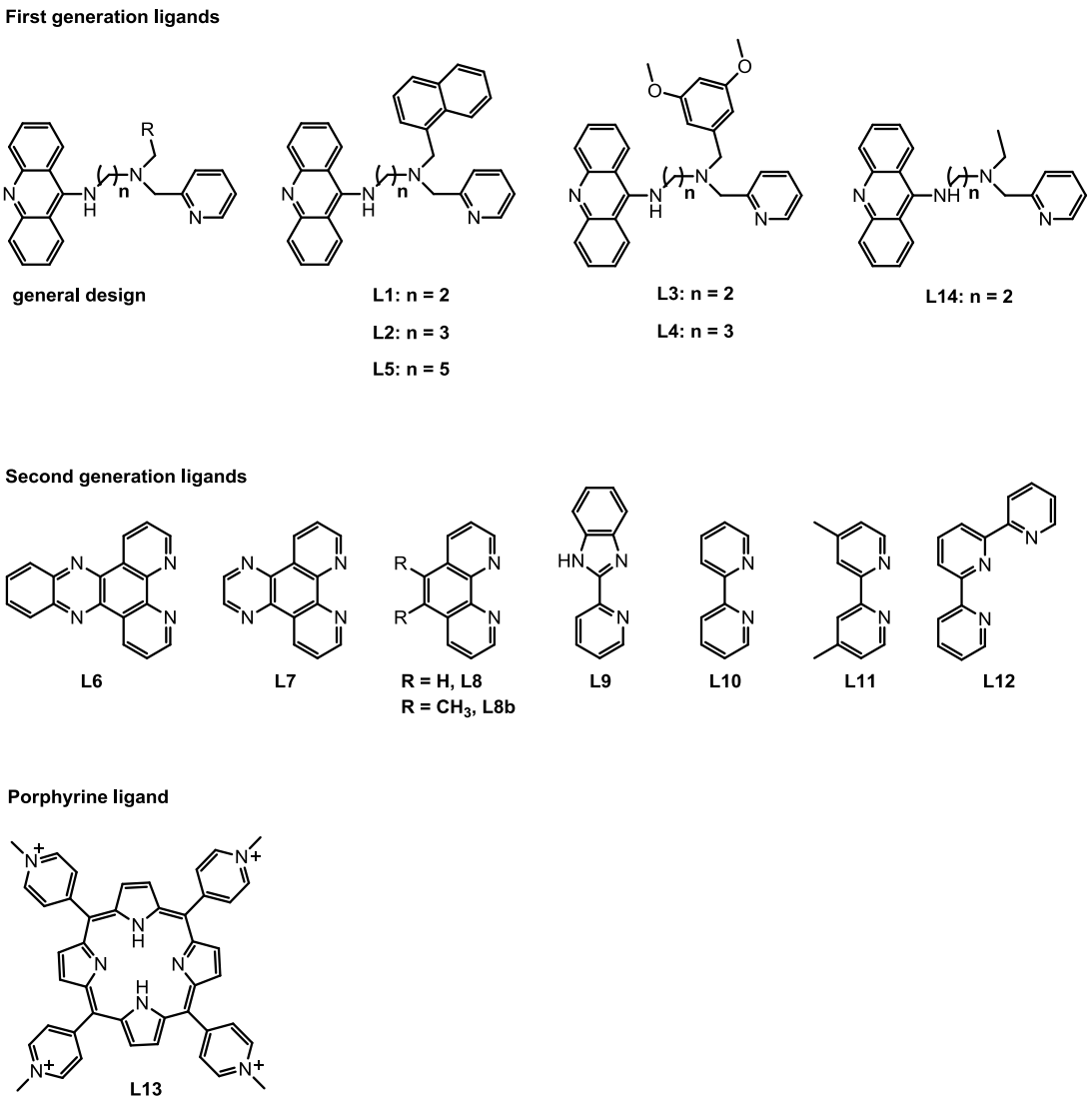


Figure 1 Ligands used in supramolecular DNA-based catalysis.

1.1.3. Non-covalent anchoring

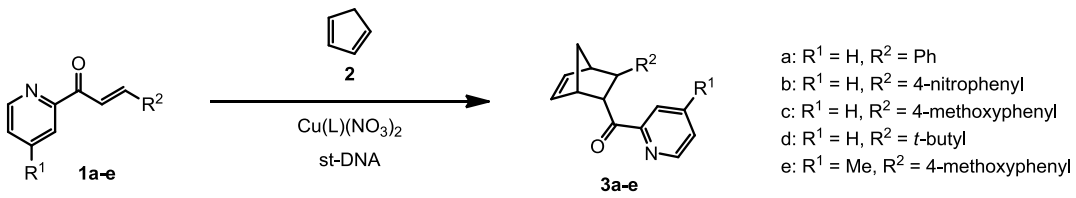
C-C Bond Forming Reactions

Early on, the choice was made to focus on Lewis acid-catalyzed enantioselective reactions in water. The reason for this was two-fold: this class of reactions is compatible with aqueous conditions, as had been demonstrated in particular by Kobayashi[9] and Engberts[10], and with the DNA scaffold since these reactions do not involve changes in the redox state of the catalytic metal, which might give rise to the formation of reactive oxygen species and, hence, cause DNA damage.[11]

Diels-Alder Reaction

The enantioselective Cu^{II}-catalyzed Diels-Alder reaction between aza-chalcone **1** and cyclopentadiene **2** was the first successful demonstration of a C-C bond forming reaction in DNA-based catalysis (scheme 1).[5] This reaction was selected, because Diels-Alder reactions benefit from an aqueous environment[12]; rate accelerations up to 700 fold were

reported by Breslow when replacing organic solvents by water. Additionally, this particular reaction was reported before by the group of Engberts.[13] Up to 74% enantiomeric excess (ee) was reached using commercially available amino acids as ligands.[10] Moreover, Diels-Alder reactions are known to be quite sensitive to interactions provided by biomolecular scaffolds, *i.e.* the so-called "second coordination sphere interactions", such as hydrogen bonding and hydrophobic effects, as was demonstrated with catalytic antibodies[14, 15] and ribozymes.[16] In going to the activated complex, bimolecular Diels-Alder reactions undergo a large structural change that makes them sensitive to interactions with the biomolecular scaffold. Therefore, it was expected that the DNA scaffold could direct the reaction towards one enantiomer of the Diels-Alder product, resulting in an enantiomeric excess.
Indeed, using DNA-based catalysts of the first generation (figure 1), *ee*'s of up to 53% were observed for the major (endo) isomer of the Diels-Alder product.[5] Both the enantiomeric excess and the enantiomeric preference, that is, which enantiomer of the product is obtained in excess, proved to be dependent on the design of the ligand and in particular the nature of the substituent R and the length of the spacer n. The preference for an aromatic R substituent, such as 1-naphthylmethyl and 3,5-dimethoxybenzyl, was consistent with the observations made previously in the enantioselective reaction catalyzed Cu^{II}-amino acid complexes, where the highest *ee*'s were obtained with amino acids containing aromatic side chains, such as tyrosine and L-abrine.[10] This was attributed to π-stacking interactions between the arylmethyl group and the substrate, although the involvement of cation-*π* interactions between the arylmethyl moiety and the Cu^{II} ion also has been claimed.[17]



Scheme 2 DNA-based asymmetric Diels-Alder reaction. Typical conditions: 1.3 mg/ml st-DNA; [catalyst] = 0.3 mM; [substrate] = 1 mM; [reactant] = 5 mM; [3-(N-morpholino)propanesulfonic acid (MOPS buffer)] = 20 mM; pH = 6.5; 5 °C; 3 days.

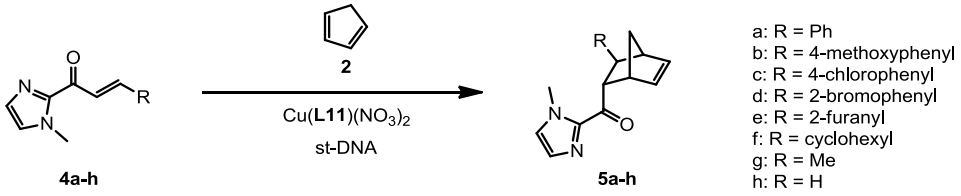
The product **3** of the Diels-Alder reaction is a mixture of endo (major) and exo (minor) isomers (scheme 2). With the R group of the ligand being 1-naphthylmethyl (**L1**, **L2)**, almost complete endo selectivity (endo:exo 98:2) and up to 49% enantioselectivity of the (-)-enantiomer was reached in case of **L2**. Interestingly, with a two carbon spacer the enantiomeric outcome of the reaction was opposite; with **L1** 48% *ee* of the (+)-enantiomer was formed. The change in enantiopreference was not observed in case of R = 3,5-dimethoxybenzyl (**L3, L4**); always the (+)-enantiomer of the Diels-Alder product was obtained in excess, regardless of the spacer length. Up to 37% *ee* was found for the endo enantiomer. Noteworthy is that in case of substrate **1c** a relatively low endo:exo ratio (91:9) was found with **L3** and the exo enantiomer was obtained in 90% *ee*. Elongation of the spacer length to n = 5 **L5** led to strong decrease in enantioselectivity, indicating that close contact between the DNA and the catalyst is required for efficient transfer of chirality.

Further optimization of the catalysis was achieved by variation of the DNA sequence and, hence, the structure of the bio-molecular scaffold. Using synthetic self-complementary oligonucleotides of defined sequence, an increase in enantioselectivity was obtained. In case of the first generation of ligands **L1**-**L5**, the *ee* could be increased from 37 to 62%, by using DNA containing alternating GC sequences.[18] With the second generation of catalysts, oligonucleotides containing tracts of three consecutive guanines proved to give the highest enantioselectivity, that is, up to 99.4%.[19] These latter sequences result in B-DNA that is distorted towards A-DNA.[20, 21] The DNA sequence did not only affect the enantioselectivity, but also the kinetics of the reaction, as will be discussed in section 1.2.

By the use of second generation ligands **L6**-**L12** both enantiomers were obtained in good to excellent *ee*'s. It was found that the ligand denticity plays a crucial role in determining which enantiomer is formed preferably. While 4,4'-dimethyl bipyridine **L11** gave rise to >99% *ee* of one enantiomer in the Diels-Alder reaction between **1e** and **2** with terpyridine **L12** the opposite enantiomer was formed in 92% *ee* with the same right handed helical conformation of st-DNA.[22]

A general approach to obtain both enantiomers of the product in DNA based catalysis was recently reported by Wang and coworkers.[23] Left handed DNA was synthesized by the use of L-DNA nucleotides and applied in various asymmetric DNA based reactions using **Cu-L11** as ligand. The results demonstrated that both mirror-image forms of DNA gave rise to the same enantioselectivity for one or the other enantiomer. Interestingly, the nuclease resistance of L-DNA could allow DNA based catalysis directly in biological systems.

With the aim of increasing the synthetic applicability of the DNA-based asymmetric catalysis concept, a new class of dienophiles was introduced.[24] Evans *et al.* demonstrated that α,β-unsaturated 2-acyl imidazoles are versatile substrates in Lewis acid-catalyzed reactions in organic solvents.[25-27] Comparable to aza-chalcones, they provide a bidentate coordination to the Cu^{II} that is strong enough to compete with coordinating solvent molecules. Additionally, α,β-unsaturated 2-acyl imidazoles are prepared in a straightforward fashion and the imidazole moiety can be removed readily after the catalytic reaction, making further synthetic transformations possible (scheme 3).

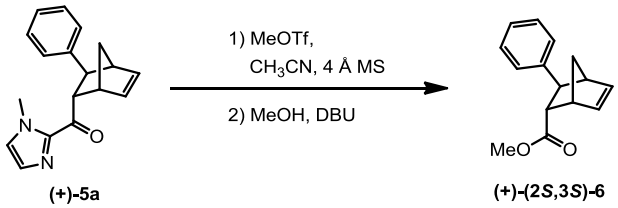


Scheme 3 DNA-based catalytic Diels-Alder reaction with α,β-unsaturated 2-acyl imidazoles.

The Lewis acid-catalyzed Diels-Alder reaction with α,β-unsaturated 2-acyl imidazoles proved to be strongly ligand accelerated; <5% conversion after 3 days was obtained with $Cu(NO_3)_2$ alone, without ligand. With first generation ligands **L1-L4** good conversions were obtained, but the enantioselectivity was only modest. The second generation ligands **L6**-**L12**, however, gave rise to excellent *ee*'s, albeit that Cu^{II} complexes based on ligands **L8** and **L10** are less reactive than when using the first generation ligands. Again, the Cu^{II} complex of **L11** was found to combine highest selectivity and activity with up to 97% *ee* and 90% conversion. With **L11** a variety of different substrates **4a-h** where converted into the Diels-Alder products **5a-h**

with endo:exo selectivities ranging from 96:4 to >99:1 and *ee*'s from 80% when R = H to 98% when R = Ph or 4-methoxyphenyl.

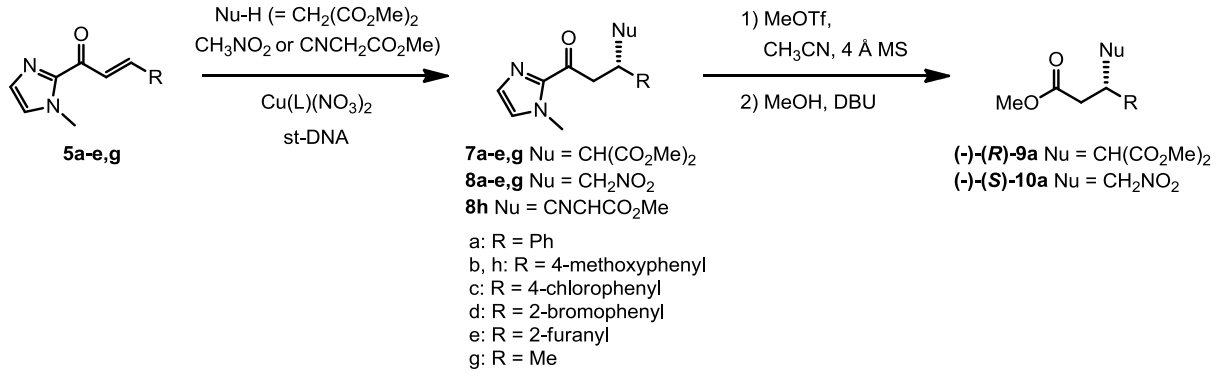
Applying the protocol of Evans,[27] the imidazole moiety of **5a** was substituted by methanol which allowed for determination of the absolute configuration (scheme 4). The product obtained by the DNA-based catalyst in the Diels-Alder reaction gave **5a** as the 2*S*,3*S*-enantiomer.



Scheme 4 Substitution of the imidazole moiety by methanol giving the known compound (2*S*,3*S*)-**6**.

Michael Addition

In addition to being suitable dienophiles, the *α*,*β*-unsaturated 2-acyl imidazole substrates are also effective Michael acceptors. The DNA-based catalytic asymmetric Michael addition reactions were investigated using dimethyl malonate and nitromethane as Michael donors in presence of a ligand-Cu^{II} complex and st-DNA (scheme 5).[28] A 100 fold excess of dimethyl malonate was required for the reaction to proceed efficiently. Whereas good *ee*'s were observed with several ligands of the second generation, again the best results were found with 4,4'-dimethyl-2,2'-bipyridine ligand **L11**, providing full conversion and 91% *ee*. The reaction has a broad substrate scope and excellent enantioselectivities up to 99% *ee* were obtained when R = aryl. As an exception, a significantly lower *ee* of 62% was found for R = Me.



Scheme 5 Asymmetric Michael addition with enolate nucleophiles catalyzed by the copper-ligand complex in the presence of st-DNA.

Using nitromethane as Michael donor, the corresponding Michael adduct was obtained in good *ee*'s (up to 94%), albeit somewhat lower than found with dimethyl malonate. However, significantly more nitromethane, up to 1000 equivalents, was needed to achieve full conversion of the substrates within the same time.

Malononitrile and cyanoesters were found to be another class of nucleophiles that react with *α*,*β*-unsaturated 2-acyl imidazole substrates in an Michael type reaction catalyzed by a DNA-

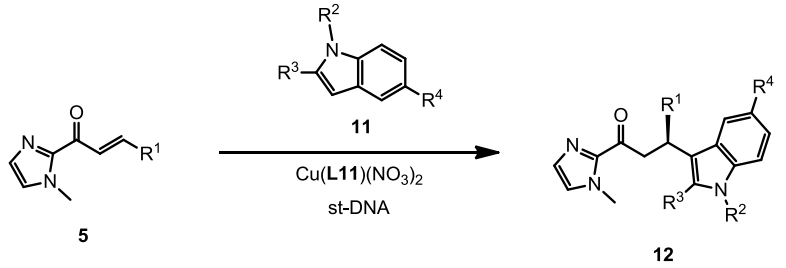
based catalyst. When Cu(**L11**)$(NO_3)_2$ was used as catalyst in presence of st-DNA, 84% *ee* was obtained for the combination of **5b** and methyl cyanoacetate.[29]

Applying the protocol of Evans, the products **7a, 8a** were converted to the corresponding methyl esters **9, 10** (scheme 5). Based on comparison with the literature, the (*R*)-stereochemistry was assigned to **7a** and the (*S*)-stereochemistry to **8a**, which corresponds to attack of the nucleophile on the *Si*-face of the Michael acceptor (Figure 4).

The catalyst solution was recycled for several times without loss of activity or enantioselectivity. The corresponding Michael adduct from the reaction of **5d** with dimethyl malonate has been isolated in 80% yield and 99% *ee* after column chromatography. Moreover, by using organic co-solvents such as acetonitrile (10% v/v), the Michael addition of dimethyl malonate to **5a** was performed on gram scale, giving rise to 85% isolated yield and 95% *ee*.[30]

Friedel-Crafts Alkylation

A related reaction is the Lewis acid-catalyzed vinologous Friedel-Crafts alkylation[31] (scheme 6). The conjugate addition of neutral π-nucleophiles such as indoles **11** and pyrrole **13** is a reaction of synthetic interest, because the resulting structural moieties are frequently found in natural products. Cu-**L11**/st-DNA was found to catalyze the reaction between α,β-unsaturated 2-acyl imidazole substrate **5g** and indole **11** (R^4 = MeO) efficiently, giving full conversions after 0.5 h. A variety of indoles **11** reacted with α,β-unsaturated 2-acyl imidazoles **5** having aryl or alkyl substituents on the *β*-position. Catalyzed by Cu-**L11**/DNA, full conversions were found after 10 h in most cases (table 1). Only when R^1 was an aryl group, the reaction was slower and needed 20 equivalents of indole for completion. The *ee* varied from 69 to 83% using st-DNA and could be increased to 93% by using the self-complementary nucleotide d$(TCAGGGCCCTGA)_2$, which also gave improved selectivities for the Diels-Alder reaction (see above).

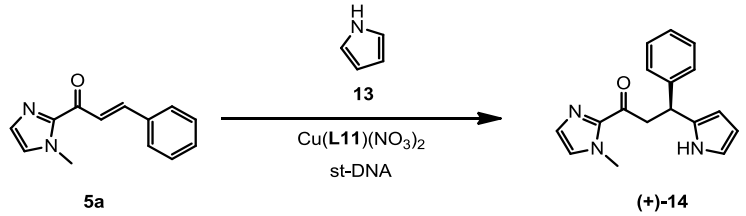


Scheme 6 Asymmetric Friedel-Crafts alkylation reaction catalyzed by Cu-**L11** in the presence of DNA.

Entry	5	11				Analytical scale[a]		Preparative scale[a, c]	
		R^1	R^2	R^3	R^4	*ee* (%)	*ee*[b] (%)	Yield (%)	*ee* (%)
1	**5g**	Me	H	H	MeO	83 (+)	93	78	82
2	**5g**	Me	H	H	H	72 (+)-R	85	79	69
3	**5g**	Me	H	H	Cl	72 (+)	82	54	69
4	**5g**	Me	Me	H	H	79 (–)-R	85	68	71
5	**5g**	Me	H	Me	H	81 (–)	76	45	77
6	**5a**	Ph	Me	H	H	75 (+)-S	74	87	77
7	**5d**	*o*-BrC_6H_4	Me	H	H	79 (+)	79	79	78
8	**5b**	*p*-$MeOC_6H_4$	H	H	MeO	69 (+)	49	71	64
9	**5c**	*p*-ClC_6H_4	H	H	MeO	79 (+)	75	77	71
10	**5i**	*n*-pentyl	Me	H	H	82 (–)	84	68	82

Table 1 Results of the Friedel-Crafts reaction catalyzed by Cu-**L11** in presence of st-DNA; [a]Results for product **12**; [b]DNA: d(TCAGGGCCCTGA)$_2$; [c]0.086 mmol of **5.**

In addition, the reaction with pyrrole **13** in the presence of Cu-**L11**/DNA was performed on a preparative scale, giving 60% isolated yield and 76% *ee*, which was improved to 81% *ee* with the oligonucleotide d(TCAGGGCCCTGA)$_2$ (scheme 7).



Scheme 7 Asymmetric Friedel-Crafts alkylation of pyrrole **13.**

The catalyst loading has been lowered to 0.3 mol%, while maintaining the same Cu/DNA ratio without causing a significant decrease in enantioselectivity. Measurements of the binding affinity K_b = (1.12 ± 0.02) x 10^4 M^{-1} of Cu-**L11** to the DNA showed that under these conditions only 16% of the complex are bound to DNA, which corresponds to an effective catalyst loading of only 0.05 mol%. Compared to the highest catalyst loadings, when 95% is bound to DNA, the enantioselectivity did not change. This suggested that the reaction is strongly accelerated by DNA. For further information about the mechanism see section 1.2.

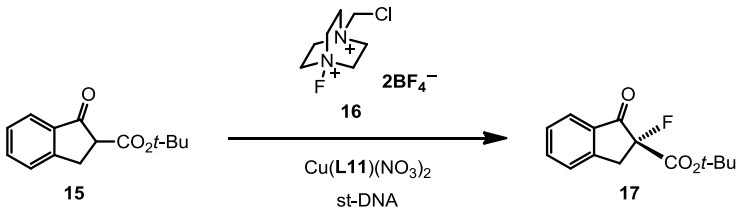
Up to 30% v/v of methanol, ethanol, dimethylformamide, dimethylsulfoxide or 1,4 dioxane were used as co-solvent in Friedel-Crafts reactions, giving enantioselectivities comparable to the reactions in pure water.[30] However, a significant effect was observed on the reaction rate; with certain substrates the reaction became significantly faster upon addition of organic co-solvents, whereas for others the reaction was slower. Moreover, addition of organic co-solvents allowed to perform the reaction at -18°C, which led to an increase in the enantioselectivity. In the particular case of reaction **5g** with **11,** which was performed on gram scale**,** the *ee* was increased from 82 to 90% performing the reaction at -18°C.

To obtain more information about the binding of ligands to DNA and the chiral induction by DNA in asymmetric Friedel-Crafts reactions, Park *et al.* investigated the intramolecular Friedel-Crafts reaction of several 3-propyl indoles bound to **5** at the R^1 position.[32] The highest *ee*'s of 82% were observed with **L8b** as ligand, which is different to all other copper catalyzed DNA based catalysis reactions reported before. For that reason, the data obtained were not generally applicable, but could be a first step towards elucidating the mechanism of stereo control.

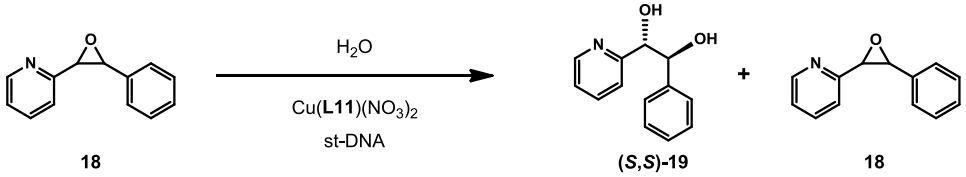
Other Reactions

In addition to the C-C bond forming reactions DNA-based asymmetric catalysis has been used in a variety of other Lewis acid-catalyzed reactions.

Applying the Cu-**L11**/DNA system, asymmetric carbon-fluorine bond formation was reported by the group of Toru [33]. Lewis acidic Cu^{II} was used to catalyze enolate formation in β-keto esters **15**. These enolates were subsequently reacted with an electrophilic fluoride source like Selectfluor® **16** (scheme 8). Structural variations in the β-keto ester had a strong effect on the enantioselectivity which ranged from negligible to 74% in the case of indanones containing a bulky ester substituent **15**.



Scheme 8 Asymmetric fluorination reaction of β-keto esters.

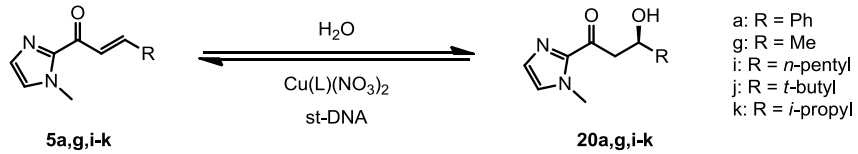


Scheme 9 Hydrolytic kinetic resolution with a DNA-based catalyst.

Another application of DNA-based asymmetric catalysis is the hydrolytic kinetic resolution of 2-pyridyloxiranes.[34] Ni^{II} and Cu^{II} salts were found to catalyze the hydrolysis of 2-pyridyloxiranes to the corresponding diols, but only Cu^{II} in combination with **L11** and st-DNA provided selectivity (scheme 9). Depending on the substituents on the oxirane, the selectivity factor varied from S = 1.1 to 2.7 in the case of trans-2-(3-phenyloxiranyl)pyridine **18**. These selectivities certainly have to be improved to lead to synthetic applications, but they show the potential of DNA-based asymmetric catalysis for a kinetic resolution.

In 2010, Roelfes and coworkers published the first enantioselective conjugate addition reaction of water in water,[35] a reaction for which is no precedent using conventional homogeneous catalysts (scheme 10). It was shown that DNA-based catalysis can overcome the apparent challenges for conjugate addition reaction with water as a nucleophile, which include the intrinsic reversibility of the hydration reaction and the poor nucleophilicity of water

under neutral conditions. This reaction illustrates the potential of hybrid catalysis and second coordination sphere interactions.



Scheme 10 Asymmetric conjugate addition of water to enones catalyzed by Cu-L in the presence of DNA.

Interestingly, for the addition of water to α,β-unsaturated 2-acyl imidazoles **5**, catalysts consisting of the first generation ligands **L1 - L4**, proved to be the most active and enantioselective. The catalyst based on st-DNA and the Cu^{II} complex of **L3** with a two carbon spacer and R = 3,5-dimethoxyphenyl showed the highest activity giving 55% conversion after 24 h with 72% *ee* for the hydration product. In contrast, only low enantioselectivity of 19% (at 14% conversion after 24 h) was observed with the Cu-**L11**/st-DNA catalyst, which performed best in all the DNA-based catalytic C-C bond formation reactions published.
The substrate scope of this reaction was investigated using **L3**. A relation was observed between the steric bulk of R and the *ee*, which ranged from 28 to 72% following the order methyl < *n*-pentyl < *i*-propyl < *t*-butyl. However, when R = phenyl, no hydration reaction was observed, which is most likely due to the reaction being thermodynamically unfavorable.
The enantioselectivity was found to decrease over time, which was attributed to the reversibility of the reaction, as will be discussed further in section 1.2.5.

1.1.4. Higher order DNA structures

Higher order DNA structures have first been explored by Moses *et al*. who have reported a G-quadruplex based catalytic system.[36] Specific DNA sequences containing three consecutive guanine-bases build G-tetrads in the presence of monovalent cations such as K^+ or Na^+.[37] Two well-studied G-quadruplex forming sequences were investigated as scaffold, namely h-Tel and c-kit. h-Tel is known to form an anti-parallel G-quadruplex structure, whereas the c-kit sequence tends to adopt a parallel quadruplex architecture. These G-quadruplexes are proposed to bind Cu^{II} complexes of ligands **L8-L11** on the terminal faces, which places them in a chiral microenvironment provided by the DNA G-quadruplex. Generally, the h-Tel structure gave higher selectivities in the Diels-Alder reaction of **1** with **2**, with ligands **L8**, **L10** and **L11**. However, when **L9** was employed, c-kit was found to give a higher enantiomeric excess compared to h-Tel in the endo (21% *ee*) and exo (30% *ee*) isomers. Interestingly, in this case the opposite enantiomer was formed in excess. Binding affinity measurements showed that only when there was an interaction between the ligand and the G-quadruplex DNA, enantioselective catalysis occurred.

Later Li *et al.* studied the combinations of catalytically active metal ions without ligands with human telomeric G-quadruplex forming DNA in more detail. They showed that for the Cu^{II}-catalyzed Diels-Alder reaction between **1a** and **2** enantioselectivities up to 74% *ee* can be achieved with the DNA sequence $G_3(TTAG_3)_3$ and no metal binding ligand present. Which enantiomer is formed was depending on whether the G-quadruplex was in the parallel or antiparallel conformation.[38] This switch in enantiopreference by a switch in G-quadruplex conformation can be induced by the use of PEG200[38] or different alkali metal ions. By

changing the metal cations from Na^+ to K^+, a switch from about +49% to -56% enantioselectivity was observed for product **5a**.[39]
G-quadruplex structures in DNA-based asymmetric catalysis were additionally studied by the group of Hennecke.[40] Water soluble porphyrins are able to bind to G-quadruplex structures in various manners and represent a promising class of catalysts in DNA-based catalysis (see Chapter 3). Hennecke *et al.* showed that Cu^{II}-**L13** can function as a catalyst for the Diels-Alder reaction between **1a** and **2**, but the results obtained with Cu^{II} bound to the porphyrin or not bound to the porphyrin were quite similar. The catalytic activity of Cu^{II}-T4-MPyP is rather surprising due to the fact that the equatorial binding sides of Cu^{II} are all occupied by the porphyrin and that means that only a monodentate binding for the substrate is possible.[40]

The DNA-based asymmetric catalysis with G-quadruplexes can also be applied to the Friedel-Crafts reaction described in 1.1.3. Here, 75% *ee* was reached for the reaction between **5g** and 4-methoxy indole. The authors noticed a strong dependence of the *ee* on the DNA sequence.[41]

1.1.5. Covalent anchoring in DNA-based catalysis

Although the supramolecular anchoring approach to DNA-based catalysis has proven quite successful, the interaction between DNA and the metal complex, and hence the structure of the catalyst, is still unclear. From this perspective, the covalent anchoring strategy potentially offers an advantage due to the precise control over the positioning of the catalyst in the DNA and, therefore, the microenvironment in which the catalyzed reaction takes place. Here we provide an overview of the approaches reported to date.

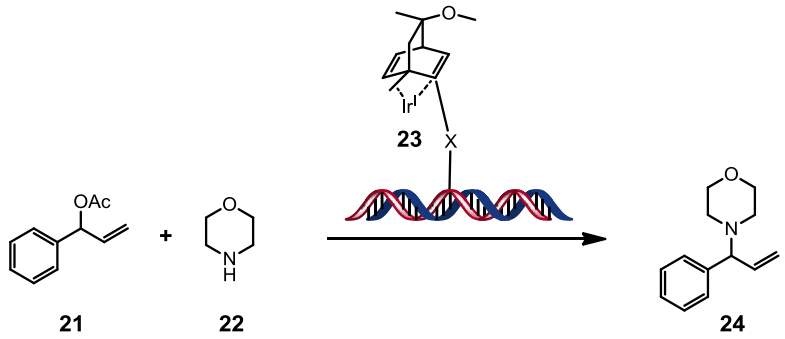
In an early design, a functionalized polyaza crown ether capable of binding Cu^{II}, was incorporated into an oligonucleotide. Only low enantioselectivities were observed with this catalyst in the Diels-Alder reaction of aza-chalcone with cyclopentadiene.[42]

Phosphine ligands are among the most powerful ligands used in asymmetric catalysis with late transition metals such as rhodium and palladium.[43] Therefore, considerable effort has been devoted to the development of DNAs incorporating phosphine ligands. Several attempts were published using either post-synthetic modifications[44, 45] or functionalization of mononucleotides and subsequent incorporation into an oligonucleotide.[46] However, with the corresponding catalysts no successful asymmetric catalysis has been reported and achieving DNA-based catalysis involving phosphine-metal complexes remains one of the challenges in this field.

The strong binding of cis-platinum complexes to DNA was used for a novel covalent anchoring DNA-based catalysis strategy.[47] 2,2'-Bipyridine ligands were equipped with diamine moieties that can bind platinum. The addition of these assemblies to st-DNA covalently linked the 2,2'-bipyridine to the double stranded DNA. Subsequent addition of Cu^{II} as catalytically active metal created a DNA-based catalyst that gave rise to moderate enantioselectivities in the Diels-Alder and Friedel-Crafts reactions described above.

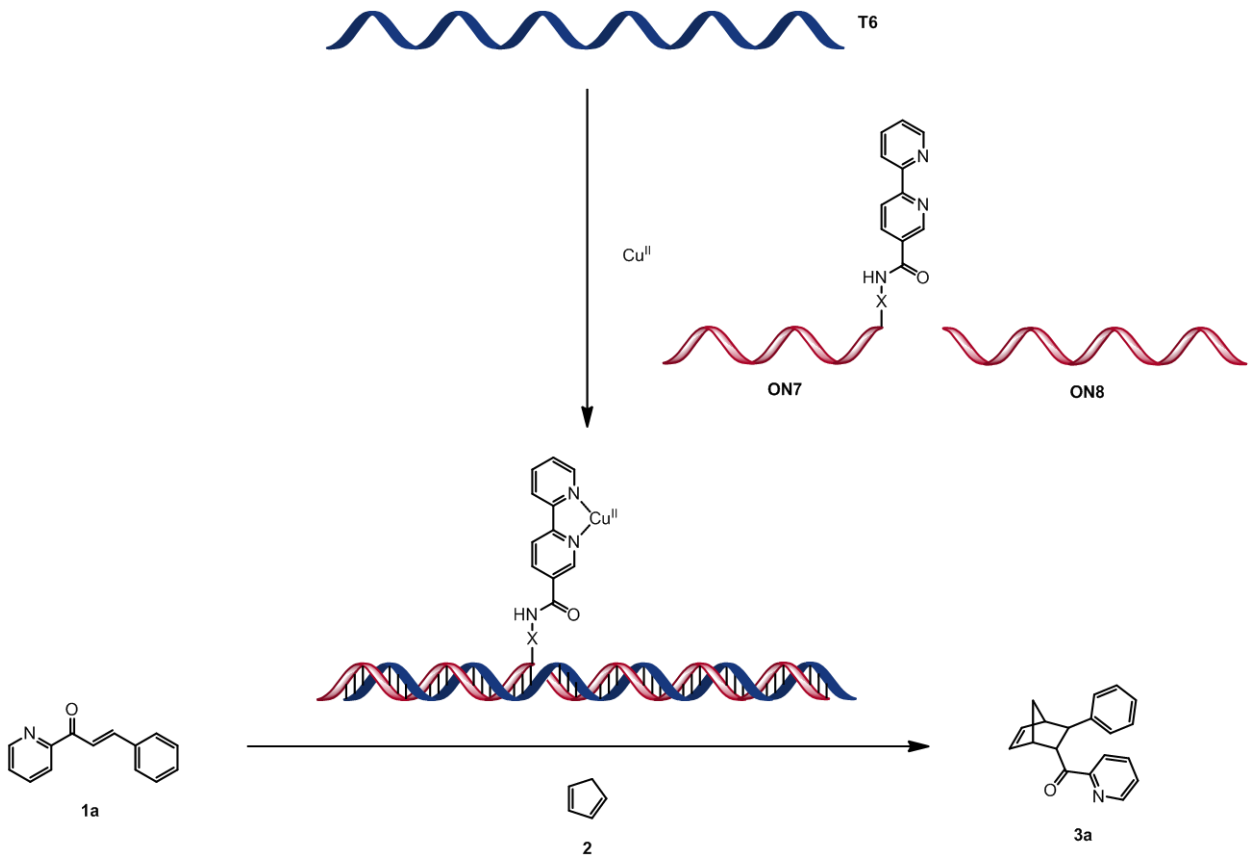
In an elegant approach, Jäschke et al. reported the first organometallic DNA-based catalyst suitable for asymmetric allylic amination reactions based on a DNA with a covalently attached diene ligand.[48] An oligonucleotide carrying a diene ligand on an internal nucleotide

was synthesized. After complexation with Ir^{I}, the catalyst showed good activity in the allylic amination of phenyl allyl acetate **21** with morpholine **22** (scheme 11). The enantioselectivity of this reaction was modest (23%) and most likely induced by the chiral diene ligand **23**. However, the enantiomeric preference was shown to be related to the nature and structure of the polynucleotide assembly. Whereas hybridization with a complementary DNA strand decreased the *ee* to 9%, the opposite enantiomer was formed in 27% *ee* when a complementary RNA strand was used.



Scheme 11 Iridium(I) catalyzed allylic amination; X = spacer

Notwithstanding the promising results obtained by the group of Jäschke, the abovementioned examples illustrate that one of the main hurdles to overcome in these covalent anchoring strategies is the synthetic complexity of preparing modified DNA's, which limits the ability to rapidly optimize a catalyst for a given reaction. For this reason, modular approaches towards the assembly of the DNA-based catalysts are desirable.



Scheme 12 Covalent approach to DNA-based asymmetric catalysis.

The first example of such a modular approach is based on hybridization of three oligonucleotides in presence of copper ions.[49] Oligonucleotide **ON7** is functionalized via a three or six carbon linker at the 5'or 3' terminus with a bipyridine moiety, which serves as a ligand for the copper catalyst. Upon hybridization of **ON7** with an unfunctionalized oligonucleotide **ON8** and the template **T6** in the presence of Cu^{II}, the system self-assembles, resulting in the catalytically active metal center being located at the interface between **ON7** and **ON8** in an internal position of the duplex DNA structure (scheme 12).
The resulting DNA-based catalyst was evaluated in the Diels-Alder reaction between **1** and **2**. Conversion and enantiomeric excess of the product proved to be dependent on the design of the catalyst, in particular the length of the spacer and the DNA sequence around the interface. The DNA sequence in the region of the metal center was readily optimized by variation of the unfunctionalized **ON8**, giving rise to an *ee* of up to 93% for the major (endo) enantiomer. Surprisingly, this enantioselectivity is higher than what was obtained with the related Cu-**L10** complex in the supramolecular approach.[50]

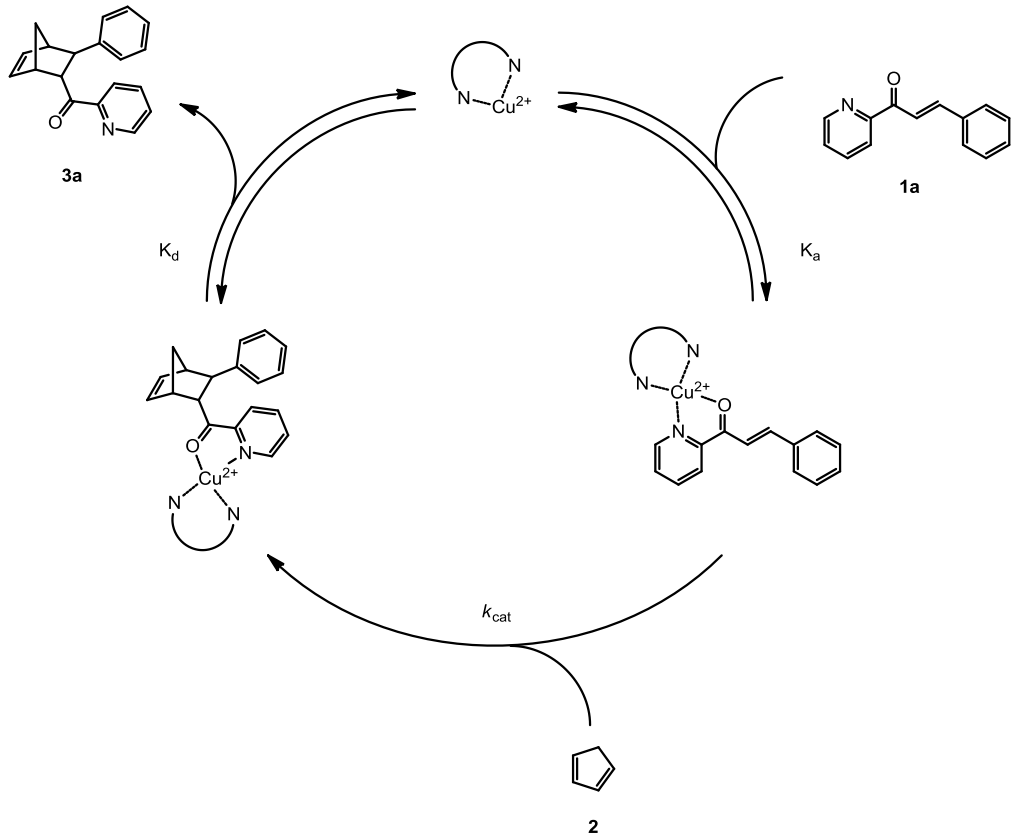
1.2. Mechanistic investigations into DNA-based catalysis

An important question, with implications for catalysis in general, is how the DNA scaffold influences the mechanism of the catalyzed reactions. Additionally, mechanistic information can help to optimize existing catalysts and to find new synthetic applications. To date, mechanistic information is only available for the supramolecular assembled DNA-based catalyst.

The use of DNA as a chiral source, presents considerable challenges for the elaboration of the mechanism. From the perspective of DNA-based catalysis, the DNA sequences of natural DNAs such as st-DNA can be considered random. Without sequence selectivity in binding of the metal complex, "the catalyst" actually is a heterogeneous mixture of metal complexes that all reside in a different microenvironment. Additionally, the supramolecular approach involves multiple binding equilibriums between metal ion, ligand, substrate and DNA, which further complicates analysis. Nevertheless, considerable effort has been devoted to elucidating the mechanism of the DNA-based asymmetric catalysis for the Diels-Alder, conjugate addition and hydration reactions. The main questions addressed in this section are: how does the DNA influence the catalytic reaction and the catalyst and what is the difference in mechanism between first and second generation catalysts?

1.2.1. General mechanistic aspects

The proposed catalytic cycle of the Cu^{II}-catalyzed Diels-Alder reaction in water between aza-chalcone **1a** and cyclopentadiene **2** is shown in scheme 13.[13] The catalytic cycle starts with a reversible bidentate coordination of the dienophile **1a** to the Lewis acidic Cu^{II} ion. The activated dienophile **1a** subsequently reacts in an irreversible Diels-Alder reaction with cyclopentadiene **2**. Finally, the Diels-Alder product **3a** dissociates from the Cu^{II} ion, and allows the catalyst to enter a new cycle. Three parameters determine the overall rate of the reaction: the equilibrium constant of binding dienophile to Cu^{II} (K_a), the rate of the catalytic reaction between the activated dienophile and cyclopentadiene (k_{cat}), and the equilibrium constant for dissociation of the product from the copper complex (K_d). Generally, the kinetic experiments are performed with an excess of catalyst to exclude product inhibition by slow dissociation of the product from copper.[13]



Scheme 13 Proposed catalytic cycle of Diels-Alder reaction catalyzed by Cu^{II}-L.

1.2.2. First generation DNA-based catalysts

Kinetics

With catalyst based on the Cu^{II} complex of first generation ligands **L1-L4** a slight deceleration of the reaction was observed in the presence of DNA (table 2).[18] Further analysis showed that this could be attributed to a small decrease in the k_{cat} of the actual Diels-Alder reaction; the K_a was found not to change significantly. Since only minor effects on the reaction rates

were observed, it was concluded that, in the first generation catalytic systems, DNA exclusively acts as a chiral scaffold.

Table 2 Dependence of kinetic data on DNA in **Cu-L** catalyzed Diels-Alder reaction of **1a** with **2** in water.

Entry	Ligand	k_{app} ($10^{-2}M^{-1}s^{-1}$)	K_a (M^{-1})	k_{cat} ($M^{-1}s^{-1}$)
			Without DNA	
1	**L1**	3.1 ± 0.92	1.8 ± 0.74 x 10^4	0.111 ± 0.027
2	**L2**	3.50 ± 0.008	1.0 ± 0.40 x 10^4	0.218 ± 0.066
3	**L3**	4.0 ± 0.36	2.3 ± 1.40 x 10^4	0.113 ± 0.034
4	**L4**	3.7 ± 0.41	1.9 ± 0.92 x 10^4	0.124 ± 0.032
			In the presence of st-DNA	
5	**L1**	2.6 ± 0.49	8.2 ± 0.97 x 10^3	0.100 ± 0.008
6	**L2**	1.5 ± 0.49	1.2 ± 0.45 x 10^4	0.057 ± 0.016
7	**L3**	1.50 ± 0.009	2.5 ± 0.97 x 10^4	0.046 ± 0.008
8	**L4**	3.4 ± 0.20	1.2 ± 0.58 x 10^4	0.064 ± 0.023

DNA sequence dependence

The source of DNA generally used in the catalytic reactions is st-DNA which can be considered as an oligonucleotide polymer with high molecular weight and, for the purpose of these studies, a random sequence. In an effort to elucidate the effect of the DNA sequence on the observed catalysis with the first generation ligands, a series of self-complementary synthetic oligonucleotides were tested.[18] It was observed that oligonucleotides with a high AT content gave rise to low enantioselectivities. In contrast, sequences containing alternating GC bases had a positive influence on the enantioselectivity. Indeed, poly(GC) was found to give the highest enantioselectivity in this reaction when combined with Cu-**L3**, that is, an *ee* of 62%. This is tentatively explained by the increased affinity of acridine for GC-rich regions in DNA or alternatively by the micro environment in those regions inducing a higher enantioselectivity.[51-53]

Structure

In section 1.1.3 the relation between the structure of the first generation ligands and enantioselectivity was discussed already. Here, the structure and interaction with DNA of the corresponding Cu^{II} complexes will be described.

A crystal structure analysis of **L1** complexed with $[Cu(ClO_4)_2 \bullet 6H_2O]$ in CH_3CN showed that two distinct mononuclear copper complexes are present in one unit cell (figure 2).[18] Both show a square pyramidal arrangement with two equatorial coordination sites occupied by nitrogen atoms from the ligand. The remaining three coordination sites are occupied by solvent molecules. In complex 1 three CH_3CN molecules are coordinated to Cu^{II} whereas two CH_3CN and one H_2O molecule are bound in complex 2. Upon binding of the substrate, the two solvent molecules in the equatorial positions have to be replaced by the pyridyl nitrogen and the carbonyl oxygen of the azachalcone. Noteworthy is the arrangement between the aromatic moieties of the two complexes. The acridine group of complex 1 is oriented parallel to the 3,5-dimethoxybenzyl group of complex 2, which in turn lies above the pyridyl moiety of the same complex. The distance between the aromatic moieties is approximately 3.5 Å. This

illustrates the ability of the ligand to engage π-π staking interactions, which are expected to be of importance in the observed enantioselective catalysis.[10, 18]

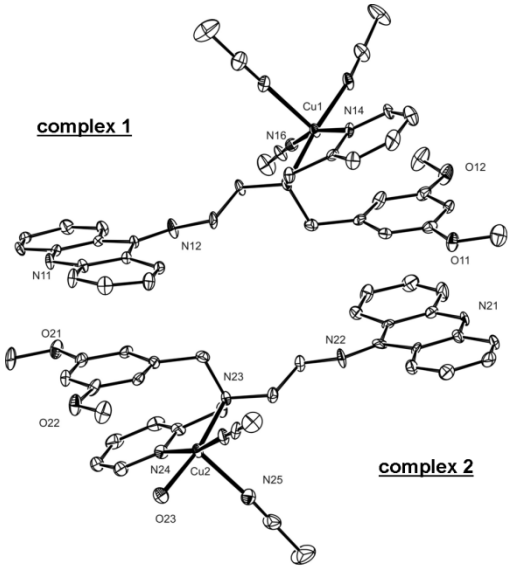


Figure 2 ORTEP diagram of the Cu-**L3** complexes **1** and **2** in the unit cell. Bond length: Cu-N_{eq}: 1.96-2.06 Å; Cu-N_{ax}: 2.24 Å;[18]

Although, the first generation ligands **L1-L5** are not chiral themselves, the complexes formed with copper are chiral. Therefore, a two step transfer of chirality was proposed. First, one enantiomer of the ligand-Cu^{II} complex is formed preferentially when bound to DNA and this subsequently results in selective formation of one enantiomer of the product of the catalyzed reaction.

Circular dichroism spectroscopy (CD) has been used to study the interactions between the Cu^{II} complexes and st-DNA. Upon binding to DNA, an induced CD effect for the acridine moiety is observed at 400 and 330 nm. The CD spectra of **L2** and **L4** are similar to those of 9-aminoacridine. Using ligands with a spacer length n=2, *i.e.* **L1** and **L3**, an increased absorption at 400 nm and exciton coupling at 330 nm was observed. This suggests a chiral interaction between the acridine and one of the two possible chromophores on the ligand, the pyridyl moiety or the aromatic substituent. By using **L14,** which has no aromatic substituent (figure 1), the exciton coupling was still observed. This indicates that the pyridyl chromophore interacts with the acridine inside the chiral environment of DNA. However, it is important to note that these differences in the CD spectra observed between ligands **L1**, **L3** and **L2**, **L4** do not correlate to the enantiomeric outcome of the catalysis and, hence, most likely reflect differences in structure of the resting state of the catalyst.

1.2.3. Second generation DNA-based catalysts

Kinetics

Using Cu-**L10** as catalyst, a modest 2-fold rate acceleration was found in the presence of DNA. Surprisingly, Cu-**L11**, was found to accelerate the reaction 58 fold in the presence of DNA compared to the reaction without DNA.[19] The only difference between **L10** and **L11** are the methyl substituents on the 4 and 4'-position, which are unlikely to interact with the

substrates during catalysis. Yet, these two methyl groups appear to be the crucial factor to achieving high rate accelerations and enantioselectivities.

A more detailed study showed that the binding of the dienophile to the catalytically active copper ion (K_a) is not significantly influenced by DNA (table 3); the observed rate acceleration is almost exclusively due to an increase in the k_{cat} of the actual Diels-Alder reaction. Notably, only the rate of the formation of the preferred enantiomer ($k_{(+)}$) is increased, whereas the rate of formation of the second enantiomer ($k_{(-)}$) stays almost unchanged compared to the reaction without DNA.

Table 3 Kinetic parameters for the Diels-Alder reaction of **1a** with **2** for Cu-**L11** in absence and presence of st-DNA at 298 K.

	Cu-**L11**	Cu-**L11**/st-DNA
K_a (M^{-1})	(4.0 ± 0.8) x 10^2	(5.0 ± 01.4) x 10^2
k_{cat} ($M^{-1}s^{-1}$)	(4.5 ± 1.2) x 10^{-2}	3.8 ± 0.8
$k_{(-)}$ ($M^{-1}s^{-1}$)	(2.2 ± 0.6) x 10^{-2}	(5.8 ± 1.2) x 10^{-2}
$k_{(+)}$ ($M^{-1}s^{-1}$)	(2.2 ± 0.6) x 10^{-2}	3.8 ± 0.8

Sequence dependence

A broad range of single and double stranded DNAs were evaluated in the Cu-**L11** catalyzed Diels-Alder reaction.[19] The K_b values for binding of Cu-**L11** to these oligonucleotides range from 7.26 x 10^{-3} to 1.35 x 10^{-4} M^{-1}, which indicates the complex does not have a sequence dependent binding specificity, but rather binds the DNA at random positions.
Cu-**L11** was found to have different sequence requirements than the first generation catalysts. Poly d(AT):poly d(AT) sequence gave a poor *ee* (15%) of the opposite enantiomer compared to st-DNA. Also alternating GC sequences, which gave the best *ee*'s for the first generation ligands, gave rise to lower *ee*'s. However, using sequences containing a G-tract of three G's, excellent *ee*'s were obtained. Remarkably, also the largest rate accelerations were found with these oligomers; $d(TCAGGGCCCTGA)_2$ which gave the highest enantioselectivity of 99.4% accelerates the reaction by a factor of 10^2. Interestingly, a clear correlation was observed between the *ee* and the k_{app} (figure 3a). This becomes particularly clear when log k_{app} is plotted versus $\Delta\Delta G^{\ddagger}$; the difference in activation energy going towards either enantiomer, which results in a linear correlation (figure 3b). This clearly shows that the micro environment provided by the oligonucleotide significantly influences the enantioselectivity and the rate of the reaction.

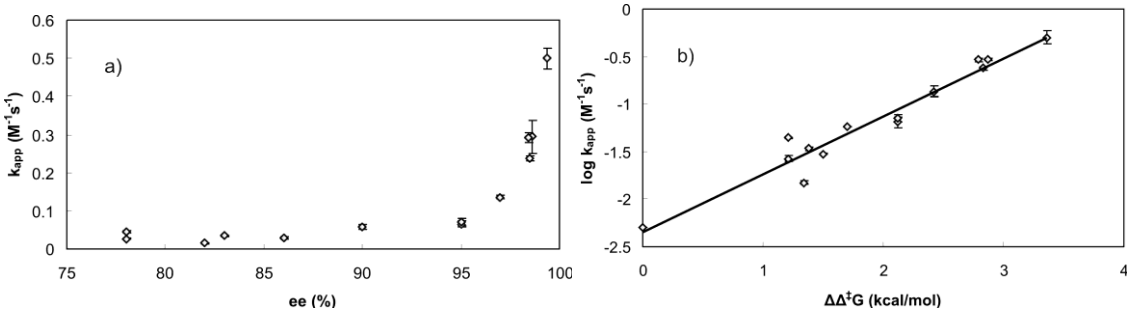


Figure 3 a) Relationship between the k_{app} and the *ee* obtained with different double stranded oligonucleotides and st-DNA. b) Relationship between log k_{app} and $\Delta\Delta G^{\ddagger}$.[19]

CD spectroscopy of the synthetic duplexes showed a significant distortion to the A-DNA type structure for DNA's containing the G-triplets compared to a normal B-DNA structure that was found for many other synthetic duplexes. This structural difference may provide a better micro environment for the catalytic reaction leading to higher reactivity and *ee*'s.
Significantly lower *ee*'s and reaction rates were observed with single stranded DNA, which shows that the duplex structures are important.

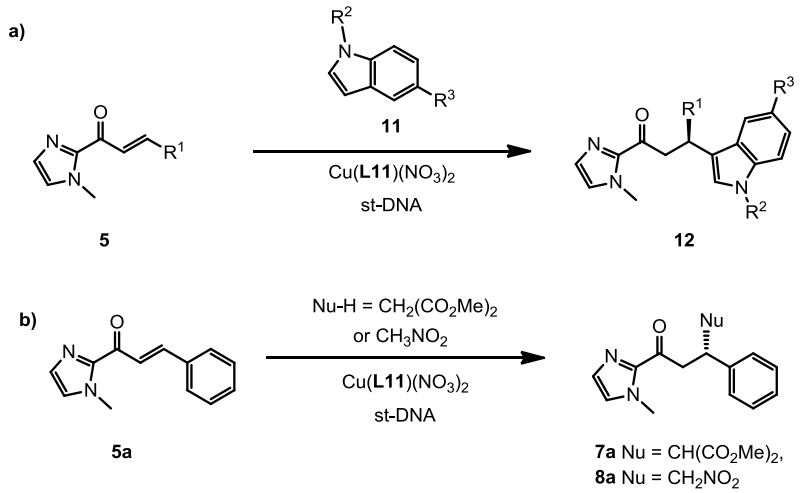
Mechanistic conclusions

Compared with the second generation of catalysts, first generation catalysts gave only moderate *ee*'s for the Diels-Alder reaction. The role of DNA is different for both generations of catalyst. In case of the first generation ligands, DNA almost exclusively acts as a chiral scaffold; it is not affecting the rate of the catalyzed reaction. In contrast, reactions catalyzed by Cu-**L11** are accelerated remarkably in the presence of DNA. Moreover, both the rate acceleration and the enantioselectivity are sequence dependent. Combining these results explains why the second generation DNA-based catalysis works so well despite the low DNA-binding affinity of Cu-**L11** and the fact that the actual catalysts is a heterogeneous mixture of Cu-**L11** complexes that are all located in a different micro environment: the DNA accelerates the reaction and those complexes bound in the DNA sequences that give rise to the highest enantioselectivity dominate the outcome of the reaction, because they also cause the largest rate accelerations. This means that the overall *ee* obtained is the weighted average of all the contributing DNA sequences.

The different behavior of the two generations of catalysts can be rationalized by considering that the catalyzed reaction can take place in different micro environments inside the DNA helix. Looking at the design of the first generation ligands it is most likely that the reaction proceeds at the edge of the DNA groove and therefore resembles more the reaction in solution. In contrast, the second generation ligands have the DNA binding moiety integrated into the metal binding site and therefore the reaction is expected to occur very close to the DNA helix which is presumably the reason for the rate accelerating effect observed. However, the binding modes of Cu^{II} complexes of **L10, L11** with DNA, *i.e.* whether they are intercalating or groove binding, is unclear at present and therefore the micro environment in which the catalytic reaction takes place cannot be identified with certainty yet.

1.2.4. Conjugate addition reactions

The DNA-based catalytic enantioselective Michael addition and Friedel-Crafts reaction both involve a conjugate addition of a anionic enolate-type or a neutral π-nucleophiles, respectively, to an α,β-unsaturated 2-acyl imidazole (scheme 14).[34] For the mechanistic investigations the Cu-**L11** complex was used, since also for these reactions it provided the highest enantioselectivities.



Scheme 14 a) Benchmark Friedel-Crafts reaction and b) benchmark Michael addition reaction for kinetic studies.

Kinetics

In the Friedel-Crafts alkylation reaction the presence of DNA has a significant positive effect on the rate of the reaction ($k_{DNA}/k_{no\ DNA}$ >10). In contrast to the observations with the Diels-Alder reaction catalyzed by the Cu-**L11**-DNA complex, here no clear trend between rate acceleration and *ee* was found.

The presence of DNA has a much smaller effect on the rate of the Michael addition reaction. In case of dimethyl malonate as the Michael donor, a 3 - 6 fold increase in k_{app} was observed. In contrast, a small 2 - 4 fold deceleration was found with nitromethane. Surprisingly, the addition of nitromethane to **5a** still results in up to 85% *ee*. Considering the presence of ~5% unbound Cu^{II}-complex, which catalyze the reaction to afford racemic product significantly faster than the bound complex to DNA, the catalytic reaction including the DNA must almost be completely enantioselective.

Sequence dependence

A significant influence of the DNA sequence was found for the Friedel-Crafts reaction with substrates **5b** and **5g**. Comparable to the Diels-Alder reaction with Cu-**L11**-DNA, the 12-mer oligonucleotide $d(TCAGGGCCCTGA)_2$ was found to give the highest enantioselectivity for substrate **5g**; an increase from 83% *ee* with st-DNA to 93% *ee* with this oligonucleotide was obtained. However, in case of substrate **5b** a considerable lower enantioselectivity was found, *i.e.* 49% compared to 69% with st-DNA. This implies that the sequence dependence varies with the structure of the substrates which suggests the structure of the micro environment needed to achieve high enantioselectivity differs per substrate. In contrast to the Diels-Alder reaction, no significant increase in reaction rate was observed for those sequences giving the highest *ee*'s.

In the case of the Michael addition, the oligonucleotide $d(TCAGGGCCCTGA)_2$ was also found to give a slight increase in enantioselectivity from 90% (st-DNA) to 93% in the reaction between **5a** and dimethyl malonate. However, it is likely that the best DNA sequence for this

reaction has not yet been found since the reaction with st-DNA is 2.5 times faster than with the tested oligonucleotides.

Conclusions

The results obtained in mechanistic studies into the DNA-based catalytic conjugate addition reaction using Cu-**L11**/DNA reveal some striking similarities with those found for the Diels-Alder reaction discussed before. In both cases the enantiomeric excess is sequence dependent. With the second generation ligands in both types of reactions stretches of guanine bases gave the highest selectivities. A noteworthy observation is that one sequence, $d(TCAGGGCCCTGA)_2$, proved to give the highest *ee*'s for several different reactions and reaction types.

Remarkably, a similar enantio-discriminating mechanism applies to both the conjugate addition and cycloaddition reaction catalyzed by Cu-**L11**/DNA. The nucleophile or diene, respectively, attacks preferentially from the same *π*-face of the enone, that is, the *Si*-face of, when R = aryl and the *Re*-face when R = alkyl (figure 4). Additionally, DNA influences the reaction rate in all cases, even if the rate accelerating effect was less pronounced in the conjugate addition reactions. The observed deceleration in the Michael addition of nitromethane is the exception; further study is needed to shed light on this phenomenon.

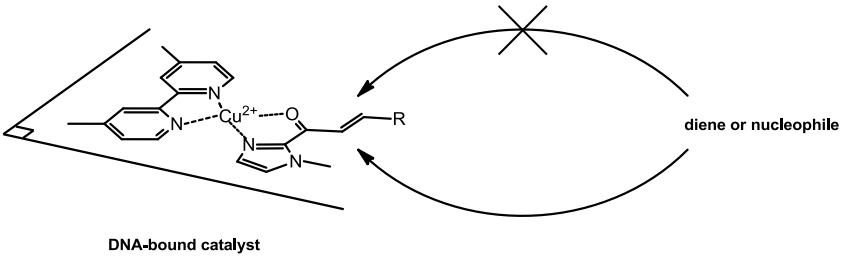
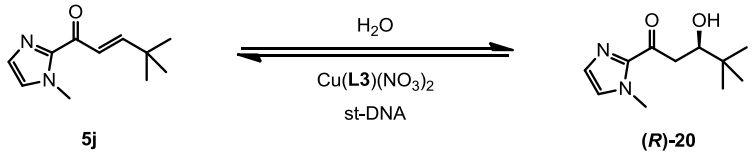


Figure 4 Stereochemistry of the approach of the diene or nucleophile.

Summarizing the observations, it can be concluded that DNA plays a similar role in Diels-Alder reactions, Michael additions and Friedel-Crafts alkylations. Due to the structural differences in the transition state of these different reactions, a stabilizing effect of DNA on the transition state is unlikely to be the reason for the observed rate acceleration. Instead it is hypothesized that the effect of DNA in these reactions is maybe predominantly exerted on the ground state, which involves structurally similar substrate bound Cu^{II} complexes.

1.2.5. Enantioselective hydration of enones

Whereas the catalyzed C-C bond forming reactions showed quite a similar pattern, the enantioselective hydration proved to be different. The highest *ee* in the catalytic enantioselective hydration of enones was observed with substrate **5j** using Cu-**L3**/DNA; up to 72% in case of st-DNA and 82% *ee* using defined sequences (see below).[30]



Scheme 15 Benchmark hydration of enone **5j** for kinetic studies

General observations

The catalytic hydration of **5j** catalyzed by Cu-**L3**/st-DNA was followed in time and it was found that the highest *ee*'s were achieved in the earlier stages of the reaction: 72% *ee* of the *R*-enantiomer after 24 h. Prolonged reaction times gave rise to a decrease in *ee* until after 360 h 23% *ee* for the S enantiomer was obtained, which is the opposite enantiomer compared to the early stages of the reaction. This is explained by considering that the hydration is reversible. Initially, the *R*-enantiomer of the hydration product **20** is formed preferentially, but due to the microscopic reversibility, this product will be the favored substrate for the back reaction as well. Thus, once the dehydration reaction becomes significant, the *ee* starts decreasing. Indeed, control experiments starting from pure racemic product and pure *R*-enantioenriched product resulted in 23% *ee* of the *S*-enantiomer of the hydrated product, which showed that indeed the *R*-enantiomer is dehydrated preferentially. This reverse reaction is a kinetic resolution for which a selectivity factor of 4.0 has been calculated. An intriguing question is why eventually the *S*-enantiomer is obtained in excess; this suggests that somehow a difference in free energy is induced between *R*-enantiomer and *S*-enantiomer. This can be tentatively explained by the formation of diastereomeric complexes of the hydration product with Cu-**L3**/DNA.

When the reaction was performed in D_2O a higher conversion was achieved and the *ee* increased to 79% albeit that the reaction was considerably slower. Apparently, D_2O causes the position of the equilibrium to shift, suggesting an equilibrium isotope effect. As a result, the relative contribution of the dehydration reaction relative to the hydration reaction is smaller, resulting in a higher enantioselectivity.

Interestingly, this reaction is also enantioselective in absence of a ligand. When only Cu^{II} and st-DNA are present, the *S*-enantiomer of the hydration product, which is the opposite enantiomer compared to in the presence of ligand **L3**, is formed in 42% *ee.* Moreover, the reaction in the absence of ligand is slower. This allows the conclusion that the ligand is important in that it modulates the activity, enantioselectivity and stereochemical outcome of the reaction.

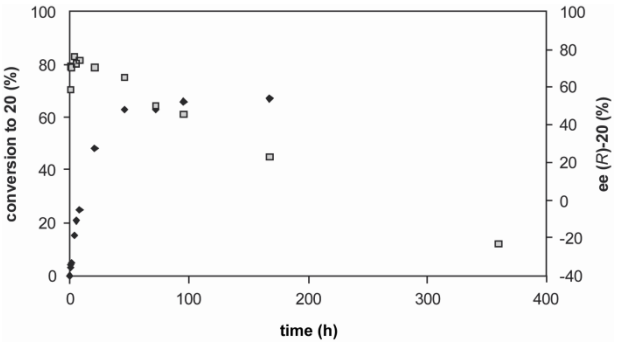


Figure 5 a) The hydration of **5j** to **20** followed in time. The enantiomeric excess of (*R*)-**20** is depicted as open squares, the conversion of **5j** to **20** as closed diamonds.

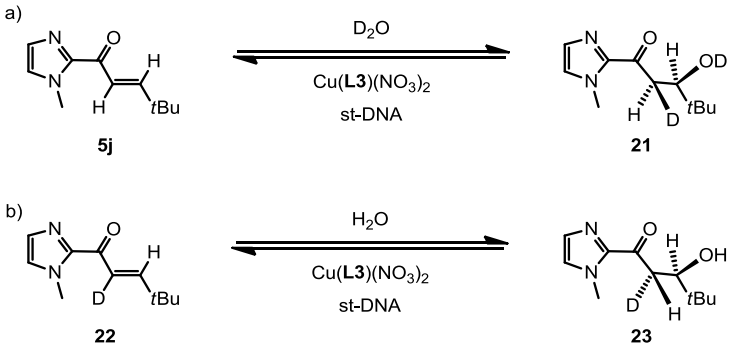
Sequence dependence

The enantiomeric excess in the hydration reaction with Cu-**L3**/DNA was found to be sequence dependent, as well. From a broad range of oligonucleotides, those containing central AT base pairs gave the best results. Up to 82% *ee* was found in case of the complementary sequence $d(GCGCTATAGCGC)_2$ in D_2O. This is surprising, since in the

Diels-Alder reaction catalyzed by the same system, alternating GC sequences gave the highest enantioselectivities and AT rich sequences generally gave rise to lower *ee*'s. Later, several ligands and DNA sequences were compared with respect to activity and *ee* in a more comprehensive study. The maximum *e*e of the hydration reaction was increased from 72 to 83% and a model was proposed in which the H_2O nucleophile is directed by the DNA to one preferred π-face of the enone, which is opposite to the pathway of the attack of diene in Diels-Alder reactions.[54]

Labeling studies

When the addition reaction was performed in D_2O (scheme 16a), the formation of a single diastereomer **21** was observed by ^{1}H-NMR.



Scheme 16 Conformational analysis of the diastereoselective formation of **21** and **23**.

Based on the vicinal coupling constants of the α-proton signal and subsequent conformational analysis, which has been well-developed for aldol products,[55] it was concluded that the product derived from *syn* addition of water to the alkene was formed. *Syn* addition of water has been reported for enzymes such as Enoyl CoA hydratase; in that case it is proposed that two active site glutamate residues bind and direct the water nucleophile towards the alkene.[56] In the present study, however, it was found that the reaction using only $Cu(NO_3)_2$ in D_2O also provides product **21** as a single diastereomer. That means that neither DNA nor the ligand are required to achieve diastereospecificity. Apparently, the Cu^{II} is responsible for directing the hydroxyl group and the proton to the same π-face of the alkene.

Conclusions

Comparison of the results for the hydration reaction with those obtained for the Diels-Alder reaction using the same catalyst reveals some marked differences. The DNA sequence dependence is different for both reaction types, suggesting a different micro environment is preferred. Remarkable is that the water nucleophile attacks from the *Re*-face of the enone, whereas the diene in the Diels-Alder reaction approaches from the *Si*-face. Based on these observations and assuming that the coordination between substrate and copper in both reactions is the same, a selective shielding of one *π*-face of the enone can be excluded. A possible explanation for the observed enantioselectivity in the hydration reaction might be found in the spine of hydration formed in the DNA groove, containing highly localized water molecules.[6] This complex network of water molecules is proposed to be involved in assisting and directing the attack of the water nucleophile on the enone.

1.3. Goals and outline of this thesis

The goal of the research described in this thesis is to overcome the current limitations in DNA-based asymmetric catalysis, that are, the dependence on Cu^{II} as catalytically active metal ion, the restriction to Lewis acid catalysis and the use of bidentate coordinating substrates. This thesis describes the first examples of organometallic asymmetric reactions with DNA as the only source of chirality and shows applications of new substrate classes. The results will broaden the fundamental understanding of DNA-based asymmetric catalysis and will lead to further developments in the field of asymmetric catalysis is water.

In Chapter 2 the first organometallic DNA-based asymmetric catalysis reaction giving rise to high enantioselectivities is described. Up to 84% *ee* is achieved in the Cu^{I}-catalyzed intramolecular cyclopropanation of α-diazo-β-keto sulfones. Iron-porphyrin-DNA hybrid catalysts for asymmetric cyclopropanations are developed during the research described in chapter 3. The main findings are the dependence of enantiopreference and reaction rate on the structure of the Iron-porphyrin and the fastest DNA based catalysis reaction observed so far. Chapter 4 shows the first examples of Cu^{II} catalyzed DNA-based asymmetric catalysis with substrates that lack the usually required nitrogen containing auxiliary group. Two projects that evolved from interesting findings for the research shown in chapter 2-4 are further described in chapter 5 and 6. Chapter 5 focuses on the functionalization of aromatic amines with diazo compounds in water using Iron-porphyrin catalysts and chapter 6 shows that micellar catalysis is a versatile tool for the synthesis of 3-functionalized indoles by the vinylogous Friedel-Crafts alkylations in aqueous media. An overview of the most important findings and a discussion of the future perspectives in DNA-based asymmetric catalysis will be provided in chapter 7.

1.4. References

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