Chapter 2

A kinetic and structural investigation of DNA-based asymmetric catalysis using first generation ligands

The recently developed concept of DNA-based asymmetric catalysis involves the transfer of chirality from the DNA double helix within metal-catalyzed reactions using a non-covalently bound catalyst. Herein we present a study of DNA-based catalysts from ligands of the first generation which comprise a metal-binding domain linked through a spacer to a 9-aminoacridine moiety. Particular emphasis has been placed on determining the effect of DNA on the structure of the Cu$^{II}$ complex in the Cu$^{II}$-catalyzed Diels-Alder reaction in water. The most important findings are that the role of DNA is limited to being a chiral scaffold; no rate acceleration was observed in the presence of DNA. Furthermore, the optimal DNA sequence for obtaining high enantioselectivities proved to contain alternating GC nucleotides. Finally, DNA has been shown to interact with the Cu$^{II}$ complex to give a chiral structure. Comparison with the second generation of DNA-based catalysts, which bear bipyridine-type ligands, revealed marked differences, which are proposed to be related to the DNA microenvironment in which the catalyst resides and where the reaction takes place.

This chapter has been published:
2.1 Introduction

The high activities and selectivities achieved by enzymes under mild conditions continue to be a source of inspiration for the design of new catalysts. A recent approach that has proven to be quite versatile is the creation of hybrid catalysts in which the catalytic power of transition-metal complexes is combined with the chiral architecture of biopolymers.\textsuperscript{[1-5]}

This concept involves placing a metal complex in the chiral microenvironment provided by the biomolecular scaffold using covalent, supramolecular, or dative anchoring strategies. By using a protein scaffold, this method has resulted in a range of enantioselective artificial metalloenzymes that are capable of catalyzing transformations such as hydrogenation transfer,\textsuperscript{[6]} hydrogenolysis,\textsuperscript{[7]} hydrogenation,\textsuperscript{[8-10]} epoxidation,\textsuperscript{[11,12]} sulfoxidation,\textsuperscript{[13-16]} Diels-Alder,\textsuperscript{[17,18]} transamination,\textsuperscript{[19]} and allylic alkylation.\textsuperscript{[20]}

Polynucleotides such as RNA and DNA are arguably some of the most elegant chiral structures in nature and, hence, they are attractive scaffolds for the assembly of enantioselective hybrid catalysts. Prior to our studies it was demonstrated that the chirality of DNA can be transferred in a stoichiometric DNA-templated reaction.\textsuperscript{[21-23]} Furthermore, a DNAzyme capable of enantioselection of an RNA substrate\textsuperscript{[24]} and an enantioselective Diels-Alderase RNAzyme have been reported.\textsuperscript{[25]}

In our approach to DNA-based enantioselective catalysis,\textsuperscript{[26-28]} we have demonstrated that the chirality of DNA can be transferred directly in a metal catalyzed reaction. The design involves the modular assembly of a DNA-based catalyst from natural duplex DNA, usually salmon testes DNA (st-DNA), and a copper complex formed \textit{in situ} from a copper source (in our case Cu(NO\textsubscript{3})\textsubscript{2} × 3H\textsubscript{2}O) and an achiral ligand that can bind DNA in a non-covalent fashion.\textsuperscript{[26]} As a result, the active Cu\textsuperscript{II} center is brought into the proximity of the chiral environment provided by the DNA double helix, allowing the transfer of chirality and the generation of reaction products with an excess in one of their enantiomers (Scheme 1, a).

To date, two generations of DNA-based catalysts have been developed that differ in the type of ligand used (Scheme 1, b). In our first design, a metal-binding domain, that is, 2-(aminomethyl)pyridine, was linked through a short spacer to a DNA intercalator, that is, 9-aminoacridine. With this design enantiomeric excess (ee) values of up to 50% were obtained in Cu\textsuperscript{II}-catalyzed Diels-Alder reaction in water.\textsuperscript{[26]}
A kinetic and structural investigation of DNA-based asymmetric catalysis using first generation ligands

**Scheme 1.** a) Schematic representation of the DNA-based Cu\(^{II}\)-catalyzed Diels-Alder reaction in water; b) first (L1-L6) and second (dmbpy) generation of ligands used in DNA-based asymmetric catalysis.

The design of the ligand proved to have an important influence on the results of the catalysis. It was found that the ligand should preferably contain an arylmethyl group, that is 3,5-dimethoxybenzyl or 1-naphthylmethyl. This suggests that π-π stacking interactions with the substrate are important for the observed enantioselective catalysis. Moreover, both enantiomers of the product were accessible by appropriate design of the ligand; the enantiomeric preference of the reaction was demonstrated to depend both on the nature of the arylmethyl substituent and the length of the spacer.

In an alternative design, the DNA-binding moiety was integrated into the metal-binding domain; hence, a spacer was no longer required. With this second generation of ligands, a dramatic increase in enantioselectivity was observed; up to 99% ee was obtained with 4,4'-dimethyl-2,2'-bipyridine (dmbpy) as the ligand.\[27, 28\] In this case, for the Diels-Alder reaction, it was found that DNA is more than a chiral scaffold; rate accelerations of up to two orders of magnitude were observed in the presence of DNA. Moreover, both the rate acceleration and the enantioselectivity were found to be DNA-sequence dependent.\[29a\]

The scope of DNA-based catalysts has been extended beyond the Diels-Alder reaction: good-to-excellent enantioselectivities have been reported in the Michael addition reactions of malonates and nitromethane to yield α,β-unsaturated 2-acylimidazoles,\[30\] Friedel-Crafts alkylations,\[31\] fluorination reactions,\[32a\] and epoxide hydrolysis.\[32b\]
Compared with the first generation catalysts, however, the second generation catalysts are less flexible with regard to which enantiomer of the product is obtained; the product resulting from the attack of the diene or nucleophile on the Si face of the enone moiety is always obtained.

In this chapter the results of a study aimed at obtaining a deeper understanding of the mechanistic and structural aspects of the first generation of DNA-based catalysts (which comprise an acridine-based ligand) is reported. In particular, the effects of DNA on the observed catalysis and on the structure of the catalyst will be discussed.

2.2 Kinetics

The effect of DNA on the reaction rate was investigated by studying the reaction kinetics. The mechanism by which a Lewis acid, that is in our case Cu²⁺, can be expected to affect the rate of the Diels-Alder reaction of aza-chalcone (1a) with cyclopentadiene (2) in water, is depicted in Scheme 2.[33a]

Scheme 2. Proposed catalytic cycle for the Cu^{II}-catalyzed Diels-Alder reaction of 1a with 2 in water.

The first step in the cycle comprises a rapid and reversible coordination of the dienophile to the Cu²⁺ ion, which results in the activation of the dienophile. This is followed by the irreversible Diels-Alder reaction. Finally, the product dissociates from the Lewis acid, regenerating the catalyst for another cycle of the reaction.
The overall rate of the reaction is determined by the equilibrium constant for the binding of the aza-chalcone (1a) to the Cu$^{2+}$ ($K_a$), the rate of the reaction of cyclo pentadiene (2) with the Cu$^{II}$-bound aza-chalcone ($k_{cat}$), and the dissociation constant for the release of the product from the Cu$^{II}$ complex ($K_d$). In the kinetic measurements $K_d$ did not influence the overall rate because a large excess of catalyst was used. Consequently, product inhibition can be excluded.

The apparent second-order rate constant ($k_{app}$) was determined from the initial decrease in the absorption of aza-chalcone (1a) at various copper concentrations. From this, $K_a$ and $k_{cat}$ were determined by using the pseudophase model, as reported by Engberts and co-workers, using an iterative fitting approach. For practical reasons, the kinetic experiments were performed at a pH of 5.5 (MES buffer), which is different from the pH used in the catalytic experiments, that is, pH 6.5 (MOPS buffer).

The use of a lower pH value in the catalytic experiments resulted in only a slight decrease in the enantioselectivity (Table 1).

**Table 1.** Dependence of the enantioselectivity on the pH.$^{[a]}$

<table>
<thead>
<tr>
<th>entry</th>
<th>ligand</th>
<th>pH</th>
<th>Buffer</th>
<th>conversion(%)</th>
<th>ee endo isomer (%)$^{[b]}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L1</td>
<td>6.5</td>
<td>MOPS</td>
<td>&gt;80</td>
<td>-37 (+)</td>
</tr>
<tr>
<td>2</td>
<td>L1</td>
<td>5.5</td>
<td>MES</td>
<td>100</td>
<td>-38 (+)</td>
</tr>
<tr>
<td>3</td>
<td>L2</td>
<td>6.5</td>
<td>MOPS</td>
<td>100</td>
<td>-37 (+)</td>
</tr>
<tr>
<td>4</td>
<td>L2</td>
<td>5.5</td>
<td>MES</td>
<td>100</td>
<td>-35 (+)</td>
</tr>
<tr>
<td>5</td>
<td>L3</td>
<td>6.5</td>
<td>MOPS</td>
<td>100</td>
<td>-48 (+)</td>
</tr>
<tr>
<td>6</td>
<td>L3</td>
<td>5.5</td>
<td>MES</td>
<td>100</td>
<td>-34 (+)</td>
</tr>
<tr>
<td>7</td>
<td>L4</td>
<td>6.5</td>
<td>MOPS</td>
<td>100</td>
<td>+49(-)</td>
</tr>
<tr>
<td>8</td>
<td>L4</td>
<td>5.5</td>
<td>MES</td>
<td>100</td>
<td>+32(-)</td>
</tr>
</tbody>
</table>

$^{[a]}$ Conditions: all experiments were carried out with st-DNA (1.3 mg/mL), [Cu(L)(NO$_3$)$_2$] = 0.3 mM, [1a] = 1 mM, [Z] = 15 mM, in buffer (20 mM) for 3 days at 5 °C. Conversions were determined by $^1$H-NMR; the ee values were determined by chiral HPLC: ODH column n-heptane/i-PrOH 98:2, 0.5 mL/min; OD column n-heptane/i-PrOH 98:2, 1 mL/min. $^{[b]}$ +/- are referring to the order of elution of the two enantiomers: first and second, respectively. Retention times: 13.7 min and 16.06 min (endo isomer; ODH); 7.2 min and 8.3 min (endo isomer; OD).

A comparison of the values of $k_{app}$ obtained with ligands L1-L4 showed that the reactions were roughly equally fast, regardless of the ligand (Table 2). In the presence of DNA a slight decrease in the reaction rate was observed in all cases. A more detailed analysis revealed that the $K_a$ values did not change significantly with the different ligands used.
Table 2. Dependence of kinetic data on DNA in the Cu-L catalyzed Diels-Alder reaction of 1a with 2 in water.[a]

<table>
<thead>
<tr>
<th>entry</th>
<th>ligand</th>
<th>( k_{app} ) (M(^{-1})-s(^{-1})) \times 10^{-2}[b]</th>
<th>( k_a ) (M(^{-1}))</th>
<th>( k_{cat} ) (M(^{-1})-s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L1</td>
<td>3.1 ± 0.92</td>
<td>1.8 \times 10^4 ± 0.74</td>
<td>0.111 ± 0.027</td>
</tr>
<tr>
<td>2</td>
<td>L1[c]</td>
<td>2.6 ± 0.49</td>
<td>8.2 \times 10^3 ± 0.97</td>
<td>0.100 ± 0.008</td>
</tr>
<tr>
<td>3</td>
<td>L2</td>
<td>3.5 ± 0.008</td>
<td>1.0 \times 10^4 ± 0.40</td>
<td>0.218 ± 0.066</td>
</tr>
<tr>
<td>4</td>
<td>L2[c]</td>
<td>1.5 ± 0.49</td>
<td>1.2 \times 10^4 ± 0.45</td>
<td>0.057 ± 0.016</td>
</tr>
<tr>
<td>5</td>
<td>L3</td>
<td>4.0 ± 0.36</td>
<td>2.3 \times 10^4 ± 1.40</td>
<td>0.113 ± 0.034</td>
</tr>
<tr>
<td>6</td>
<td>L3[c]</td>
<td>1.5 ± 0.009</td>
<td>2.5 \times 10^4 ± 0.97</td>
<td>0.046 ± 0.008</td>
</tr>
<tr>
<td>7</td>
<td>L4</td>
<td>3.7 ± 0.41</td>
<td>1.9 \times 10^4 ± 0.92</td>
<td>0.124 ± 0.032</td>
</tr>
<tr>
<td>8</td>
<td>L4[c]</td>
<td>3.4 ± 0.20</td>
<td>1.2 \times 10^4 ± 0.58</td>
<td>0.064 ± 0.023</td>
</tr>
</tbody>
</table>

[a] Conditions: [Cu\(^{2+}\)-L] = 0.10 - 0.30 mM, 25 °C, MES buffer (20 mM, pH = 5.5), [1a] = 6.0 \times 10^{-3} mM, [2] = 0.5 - 2.0 mM. [b] [Cu\(^{2+}\)-L] = 0.058 mM. [c] In the presence of st-DNA.

Hence, it can be concluded that the binding of the Lewis acid to the dienophile occurs with equal efficiency in the absence and presence of DNA. The lower overall reaction rates proved to be due to a lowering of the \( k_{cat} \) with ligands L2-L4. The observed differences are not large, but significant. Notably, in the case of ligand L1 no significant decrease in \( k_{cat} \) was observed (Table 2, entries 1-2).

Attempts were made to determine the \( K_a \) values independently by a UV/Vis titration experiment\(^{[29a]}\) in order to validate the values derived from the model used. However, these measurements were complicated by the strong absorption of the acridine chromophore in the wavelength region of interest. Therefore, \( K_a \) was determined for the Cu\(^{II}\) complex of a similar ligand (ligand L5, Scheme 1), which contains the same metal-binding domain but lacks the acridine moiety. By using this Cu\(^{II}\) complex a \( K_a \) value of (2.97 ± 0.90) \times 10^4 M\(^{-1}\) was found for the binding of aza-chalcone (1a). This is similar to the values observed with the Cu\(^{II}\) complexes of ligands L1-L4, which supports the accuracy of the values derived from the iterative fitting approach.

The results of the kinetic studies are in sharp contrast to those obtained with the second generation of catalysts: with Cu-dmpby, the presence of DNA resulted in a rate acceleration with an acceleration factor of 58.\(^{[29a]}\) This suggests that the DNA-based catalysts, depending on the type of ligand, interact differently with the DNA, such that DNA has a distinct effect on the reaction rate. Most likely the reaction takes place in a different structural environment: in the case of the second-generation catalysts the reaction has been proposed to take place in the groove, which, for example, can provide favorable interactions with the activated complex.\(^{[29a]}\) In the case of the first generation catalysts, as a result of the design, the
reaction is proposed to occur further away from the DNA groove. This is consistent with the observed decrease in ee when using ligands with a longer spacer.\[^{26}\] Therefore, the reaction is more comparable to that taking place in bulk solution, that is, in the absence of DNA. Hence, only minor effects on the reactivity are observed. Thus, in the present case, DNA can be considered to act exclusively as the chiral scaffold.

2.3 DNA-sequence Dependence

The structure of the second coordination sphere provided by DNA has been demonstrated to have a strong effect on the enantioselectivity in the catalytic Diels-Alder\[^{29a}\] and Friedel-Crafts reactions\[^{31}\] in the presence of DNA-based catalysts from the second generation ligands. Salmon testes DNA (st-DNA) is a heterogeneous polymer and its sequence can be considered to be random. Loadings of one complex per six base pairs were used in the catalytic experiments. Therefore, the DNA-based catalyst is a mixture of copper-ligand complexes located in different chiral microenvironments. Consequently, the observed enantioselectivity most likely is the (weighted) average of all the contributing DNA sequences.

A series of different DNAs were tested to establish the effect of the chiral microenvironment provided by the DNA on the Cu-L1 catalyzed Diels-Alder reaction. DNA from an alternative natural source, that is, calf thymus DNA (ct-DNA), resulted in an ee comparable to that found with st-DNA (Table 3, entry 2). Next, a series of synthetic self-complementary oligonucleotides of defined sequence were tested. The sequences were selected to cover a broad range of the available sequence space. From the data acquired (Table 2), a general pattern can be discerned. First, sequences that are rich in AT base pairs generally give rise to very poor enantioselectivities (Table 3, entries 3-5). Furthermore, in sharp contrast to the results obtained with Cu-dmppy as ligand,\[^{29a}\] sequences containing stretches of guanines give ee’s that are significantly lower than those obtained with st-DNA (Table 3, entries 14-15, 17 and 21). The best results were obtained with sequences rich in GC base pairs, in particular with alternating GC sequences (Table 3, entries 6-10); up to 62% ee was obtained with poly(GC) (Table 3, entry 6), which is significantly higher than the value obtained with st-DNA. A GC dodecamer gave similar ee values, however, further truncation to an octamer led to a significantly lower ee (Table 3, entry 7). This is possibly the result of the increased chance that
the CuII complex is located near one of the termini, where the influence of the chirality of the DNA is smaller.

Table 3. Dependence of the enantioselectivity on the DNA sequence using ligand L1.[a]

<table>
<thead>
<tr>
<th>Entry</th>
<th>DNA sequence</th>
<th>conversion(%)</th>
<th>ee endo isomer (%)[b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>st-DNA</td>
<td>100</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>ct-DNA</td>
<td>100</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>d(ATATATATATAT)₂</td>
<td>100</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>d(AAAAAATTTTTT)₂</td>
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<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Poly(dA-dT)( dA-dT)</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Poly(dG-dC)( dG-dC)</td>
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<td>62</td>
</tr>
<tr>
<td>7</td>
<td>d(GCGCGCGCCCGC)₂[c]</td>
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<td>27</td>
</tr>
<tr>
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<td>d(CGCGCGCGCGCGC)₂</td>
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<td>25</td>
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<td>10</td>
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<td>26</td>
<td>d(CGCGCGCGCGCGC)₂</td>
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<td>60</td>
</tr>
</tbody>
</table>

[a] Conditions: all experiments were carried out with [oligomer] = 1.3 mg/mL, [Cu(L)(NO₃)₂] = 0.3 mM, [1a] = 1 mM, [2] = 15 mM in MOPS buffer (20 mM, pH = 6.5) for 3 days at 5 °C. [b] ee values were determined by chiral HPLC (see experimental section); ee they refer to the (+) enantiomer in all cases and they are an average of two experiments; they are reproducible within 2%. [c] 100 mM NaCl.

It was interesting to note that the results obtained with the d(GC)₆ sequence are dependent on the presence of salt; in the absence of NaCl a significantly lower ee was found (Table 3, entries 7-8). Surprisingly, the enantioselectivity could be restored by interchanging the order of the G and C nucleobases; with d(CG)₆ an ee of 60% was obtained. The origins of these effects are, at present, not well understood, but are most likely related to small differences in the structure of the DNA.
The high enantioselectivities obtained with alternating GC sequences can tentatively be explained by the acridine ligands having an increased affinity for GC-rich regions. Since aminoacridines have no strong preference for binding GC,\textsuperscript{34} in this case the substituent on the amino group of the acridine moiety (that is the spacer and the metal binding domain) within the ligand molecule, can be determinant of the specificity observed.\textsuperscript{35} Alternatively, the coordination sphere provided by GC-rich regions may provide the most favorable environment for the catalyzed reaction.

2.4 Substrates

An investigation of the substrate scope previously revealed that dienophiles able to chelate copper in a bidentate fashion are a general requirement for both catalysis and enantioselectivity.\textsuperscript{29a} The general design is amenable to variations in the metal-binding domain, however, a N,O donor set is required. For example, the pyridine moiety can be replaced by an N-methylimidazole moiety.\textsuperscript{29a}

The role of the pyridine in the interaction with DNA was probed by introducing a methyl substituent onto the pyridyl ring and systematically varying its position. It was found that, depending on the ligand used, this affected the conversion significantly (Table 4). However, no clear pattern could be discerned. The \textit{endo} isomer of the Diels-Alder product was always favored, albeit with ligand L1 a significantly lower \textit{endo}/\textit{exo} ratio was always observed (Table 4, entries 9-12). The magnitude of the ee did not change to a major extent for 1b-1d compared with the unsubstituted aza-chalcone (Table 4). Which enantiomer is formed in excess depends on the ligand used. Interestingly, ligand L4 always gives the opposite enantiomer of the Diels-Alder product compared with ligands L1-L3, except with the substrate 1d, which has the methyl substituent at the 3-position of the pyridyl ring. This particular behavior can be explained tentatively on the basis that the aza-chalcone substrate can adopt either a \textit{cisoid} or \textit{transoid} conformation. In the case of 1d, the dienophile is forced to react in the \textit{cisoid} conformation (Scheme 3, a) because the \textit{transoid} form is unfavorable for steric reasons.\textsuperscript{36} Most likely this is the reason why with 1d the same enantiomer is obtained in excess with all ligands.
Table 4. Substrate variations in the Diels-Alder reaction with cyclopentadiene [2].\[^{[a]}\]

![Diels-Alder reaction substrates](image)

<table>
<thead>
<tr>
<th>entry</th>
<th>ligand</th>
<th>dienophile</th>
<th>conversion(%)[^{[b]}]</th>
<th>endo/exo ratio</th>
<th>ee endo isomer (%)[^{[c]}]</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>L1</td>
<td>1b</td>
<td>55</td>
<td>88:12</td>
<td>-46 (+)</td>
</tr>
<tr>
<td>2</td>
<td>L2</td>
<td>1b</td>
<td>75</td>
<td>99:1</td>
<td>-40 (+)</td>
</tr>
<tr>
<td>3</td>
<td>L3</td>
<td>1b</td>
<td>100</td>
<td>97:3</td>
<td>-59 (+)</td>
</tr>
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<td>&gt;80</td>
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<td>-54 (+)</td>
</tr>
<tr>
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<td>1d</td>
<td>29</td>
<td>73:27</td>
<td>-53 (+)</td>
</tr>
<tr>
<td>12</td>
<td>L4</td>
<td>1d</td>
<td>100</td>
<td>95:5</td>
<td>-54 (+)</td>
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</tbody>
</table>

\[^{[a]}\] Conditions: all the experiments were carried out with the following reagents: [Cu(L)(NO\(_3\))\(_2\)] = 0.30 mM, st-DNA 1.3 mg/mL, [1b-1d] = 1 mM and [2] = 15 mM in MOPS buffer (20 mM, pH = 6.5) for 3 days at 5 °C. \[^{[b]}\] Determined by \(^1\)H-NMR spectroscopy. \[^{[c]}\] Determined by chiral HPLC; +/- refers to the order of elution of two enantiomers, first and second, respectively; the endo/exo ratio, ee and conversion data are reproducible to within 3%.

However, with substrates 1b and 1c this limitation does not exist and we propose that with the ligand L4 a different substrate-bound complex is formed preferentially,\[^{[36]}\] that is, with the dienophile in the transoid conformation (Scheme 3, b).

**Scheme 3.** Possible reactive conformations of the unsubstituted dienophile: a) cisoid, b) transoid.
Assuming that the conformation of the Cu-bound dienophile is an important determinant for the enantiofacial selectivity of the reaction, this results in the formation of the opposite enantiomer of the product formed with ligands L1-L3 in which the substrate is most likely bound to the Cu$^{2+}$ center in the cisoid conformation.

2.5 Crystal structure

The complexation of L1 with [Cu-(ClO$_4$)$_2$ $\times$ 6H$_2$O] in CH$_3$CN in the presence of HClO$_4$ (1 eq.) resulted in the formation of dark-green crystals, of which the crystal structure was determined. The identification of the atoms and the configuration of the cation moieties as well as the packing of the molecules in the cell are shown in Figure 1. Each asymmetric unit contains 11 moieties: two cations of mononuclear copper complexes, six perchlorate anions, and three acetonitrile solvent molecules. The asymmetric unit contains two chemically distinct mononuclear copper complexes. The structure of cation complex 1 (Figure 1, top) shows a mononuclear Cu$^{II}$ center in a square pyramidal geometry. The ligand is bound in the equatorial plane and coordinates to the cation through the two nitrogen atoms of the aminomethylpyridine moiety. The Cu-N bond lengths range from 1.96 to 2.06 Å, which is characteristic for Cu$^{II}$ complexes.$^{[37,38]}$ The remaining three coordination sites are occupied by acetonitrile molecules, which was the solvent for crystallization. The structure of the cation complex 2 (Figure 1, bottom) is very similar, except for the fact that the three remaining coordination sites are occupied by two acetonitrile molecules and one water molecule. The latter is bound in the equatorial plane trans to the tertiary amine of the ligand. It is at these equatorial sites that the substrate is proposed to bind through the pyridyl nitrogen and carbonyl oxygen, thereby displacing the solvent molecules. An interesting aspect of this structure is that the acridine moiety of complex 1 lies in a parallel orientation above the dimethoxybenzyl moiety of complex 2, which in turn lies above the pyridyl moiety of complex 2. The distance between the planes of the aromatic rings is approximately 3.5 Å, which is characteristic of π−π stacking interactions.$^{[39,40]}$ The π−π stacking interactions are considered to be important for achieving enantioselectivity in the aqueous Diels-Alder reaction of aza-chalcone with cyclopentadiene.$^{[26]}$
Figure 1. ORTEP diagram of the Cu-L1 complex 1 and 2 in the unit cell. Bond lengths: Cu-N_{eq}: 1.96 - 2.06 Å; Cu-N_{ax}: 2.24 Å.

The present structure most likely does not reflect the structure of the complex in aqueous solution, but it does demonstrate the propensity of the ligand for π-π stacking interactions.

2.6 Circular Dichroism (CD) Spectroscopy

Because of the different behavior of the copper complexes of ligands L1 and L4 in catalysis, a CD study of the DNA/copper-ligand complexes was performed to determine whether this difference could be assigned to a different interaction with the DNA. Aminoacridine is achiral, but upon interaction with DNA, which provides a chiral environment, an induced CD is observed.\(^{[41]}\) Signals from DNA are found in the region below 300 nm. However, in the presence of 9-aminoacridine, the CD spectrum shows characteristic absorption bands around 340 and 400 nm (Figure 2, a).\(^{[42]}\) For the ligands with a propyl spacer \((n = 3)\), that is, L2 and L4 (Scheme 1), a spectrum similar to 9-aminoacridine was observed with characteristic absorption bands around 330 and 400 nm (Figure 2, a), which correspond to two \((\pi-\pi^*)\) electronic transitions polarized along the short and long axes of the acridine
molecule, respectively.\textsuperscript{[42]} By using a ligand with a shorter spacer, that is, ligands L1 and L3, a different pattern was observed. An increase in the intensity of the absorption at around 400 nm was observed as well as an exciton coupling at 330 nm, which suggests a chiral interaction between the acridine moiety and one of the two possible chromophores on the ligand: the pyridyl ring or the aromatic substituent of the ligand, that is, the 1-naphthylmethyl or 3,5-dimethoxybenzyl moiety. For the ligands with a longer spacer, such as ligands L2 and L4, these chromophores are apparently too far from the DNA cavity to have a defined orientation with respect to the acridine.

Then, to identify which chromophore is involved in this interaction, a new design of the ligand was employed in which the arylmethyl moiety on the nitrogen atom was replaced with an ethyl group (L6). By using the copper complex of this ligand with DNA, the exciton coupling was still observed (Figure 2, b).

**Figure 2.** a) CD spectra of Cu-DNA-Lx: L1 (--), L2 (---), L3 (--), and L4 (--) complexes; b) comparison of the complexes of ligands L1 (--) , L3 (--), and L6 (--).

![Figure 2](image)

From this it can be concluded that the aromatic side-chain in ligands L1 and L3 is not involved in the interaction that gives rise to the exciton coupling. Most likely, the pyridyl chromophore interacts with the acridine, possibly as a result of partial intercalation, which suggests that it is located closer to the DNA groove. Importantly, the behavior observed by CD spectroscopy with ligands L1-L4 does not correlate with the configuration observed for the product of the reaction; apparently the chiral structure of the complex is only present in the resting state of the catalyst.
2.7 Comparison with the second Generation of DNA-based Catalysts

A comparison of the two generations of DNA-based catalysts reveals marked differences, both with regard to enantioselectivity and the role DNA plays in the catalytic reactions.

1) Compared with the second generation catalysts, which give rise to up to 99% ee in the Diels-Alder reaction, the ee values obtained with the first generation of DNA-based catalysts are modest.

2) Both enantiomers of the Diels-Alder product are accessible by using the first generation of catalysts, whereas with the complexes formed between DNA and the bipyridine (bpy)-based ligands, the enantiomer resulting from the approach of the cyclopentadiene at the Si face of the enone moiety is always favored.

3) DNA plays a different role in the catalysis depending on the ligand used. In combination with the copper complex of the 4,4'-dimethylbipyridine ligand, the presence of DNA results in a significant rate acceleration of up to two orders of magnitude. In contrast, with the first generation ligands, there is a slight decrease in the reaction rate in the presence of DNA.

4) The first and second generation of DNA-based catalysts also have different requirements regarding the DNA sequence and, hence, structure. With the acridine based catalysts the best results are obtained with alternating GC sequences, whereas with the bpy-based catalysts the presence of guanine tracts is beneficial.

These differences can be rationalized by considering that the catalyzed reaction can take place in different microenvironments depending on the type of ligand used. The general design of the catalysts comprises a metal-binding domain and a DNA-binding domain to anchor the catalyst to the DNA. In the second generation of catalysts, which comprises planar, symmetric polyaromatic molecules, the DNA binding moiety is integrated into the metal binding site. Therefore, the catalytic metal center is most likely very close to the DNA helix and the distance from the DNA decreases going from larger, for example, dipyrido[3,2-a:2’,3’-c]phenazine (dppz), to smaller polyaromatic moieties such as bpy and dmbpy. Initially, it has been proposed that with the catalysts based on the bpy-type ligands, the Diels-Alder reaction takes place in the DNA groove, which provides a chiral environment.
that is potentially structurally compatible with the transition state of the reaction.\textsuperscript{[29a]} However, since an acceleration effect, albeit less pronounced, was found for the Michael and Friedel-Crafts reactions,\textsuperscript{[29b]} which involve structurally different activated complexes, later it was suggested that the destabilization of the ground state, similar for all the reactions investigated, would explain the rate acceleration and in case of the Diels-Alder reaction, the 58-fold rate enhancement and the high enantioselectivity observed with Cu-dmpby. This requires a specific structure of the DNA groove, as demonstrated by the strong DNA-sequence dependence of the enantioselectivity of the reaction, which was shown to be positively affected by sequences containing guanine tracts, which give rise to B-DNA but with A-DNA characteristics.\textsuperscript{[29a]}

In the first generation of ligands, the DNA binding moiety, that is, 9-aminoacridine, is linked to a metal-binding domain through a spacer. Hence, the catalyzed reaction is in this case expected to take place at the edge of the DNA groove and, therefore, will more resemble the reaction as it occurs in solution in the absence of DNA. As a result only small differences in reaction rate are observed between the reactions with and without DNA. Furthermore, the enantioselectivity does not originate from the structural compatibility of the DNA groove with one enantiomer of the transition state, but most likely by the shielding of one face of the enone by the arylmethyl side-chain of the ligand, as has also been suggested for the enantioselective Diels-Alder reaction catalyzed by Cu\textsuperscript{II}-amino acid complexes.\textsuperscript{[33a]}

The interaction of the ligand with the substrate has been proposed to occur by π–π stacking interactions. This hypothesis implies that, in contrast to the bpy-based catalysts, with the first generation of DNA-based catalysts the chirality is not transferred directly from the DNA in the catalyzed reaction. Instead, the chirality is transferred in two steps; the DNA forces a chiral conformation on the bound copper complex, which is in turn translated into enantioselective bond formation in the catalyzed Diels-Alder reaction. Indeed, the induced CD effects, in particular the observed exciton coupling, demonstrate that the DNA can enforce a chiral structure on the copper complex, even though the observed induced CD effect proved to be unrelated to the enantiomeric preference of the reaction. Based on the results obtained by varying the substrates, we hypothesize that an important aspect in determining which enantiomer is formed in excess is whether the substrate binds in the cisoid or transoid conformation. This is in turn most likely determined by a combination of the structure of the ligand, which is supported by
the fact that both enantiomers of the product can be accessed depending on the design of the ligand, and the interaction of the DNA with the substrate-bound complex.

2.8 Conclusions

A detailed study of the first generation of DNA-based catalysts in the Cu$^{II}$-catalyzed Diels-Alder reaction in water was performed with a particular focus on establishing the role of DNA in the catalysis. A comparison with the second generation of DNA-based catalysts, which involves bpy-type ligands, revealed marked differences, which are most likely the result of different micro-environments for the catalyzed reactions. These findings emphasize the fact that the design of the ligand is an important aspect in the development of hybrid catalysts: depending on the ligand used, the catalyzed reaction can take place in very different microenvironments, which results in different reaction rates and enantioselectivities.

2.9 Experimental Section

**General remarks:**

st-DNA was obtained from Sigma, pAT and pGC were obtained from Amersham. Synthetic oligonucleotides were obtained from BioTez GmbH (Berlin, Germany). Cyclopentadiene [2] was freshly distilled from its dimer prior to use. Aza-chalcone dienophiles (1a-1d) and acridine-based ligands (L1-L4) were prepared by following published procedures.$^{[26,43,44]}$ For kinetic studies st-DNA was dialyzed extensively against MES buffer (20 mM, pH = 5.5) prior to use. $^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Varian 400 (400 and 100 MHz) in CDCl$_3$. Chemical shifts ($\delta$) are denoted in ppm using residual solvent peaks as internal standard ($\delta_c = 77.0$ and $\delta_H = 7.26$ ppm for CDCl$_3$). Mass spectra (HRMS) were recorded on a LTQ XL Orbitrap by Thermo Fisher Scientific. Flash chromatography was performed using aluminum oxide neutral, activity stage I for column chromatography, 0.063 - 0.200 mm (Merck, 70 - 230 mesh ASTM).
**Physical methods, general remarks:**

Circular Dichroism spectra were measured on a JASCO J-715 spectropolarimeter equipped with a temperature control attachment. The UV/Vis absorption spectra were measured on JASCO V-560 and JASCO V-570 spectrophotometers. Enantiomeric excess determination was performed by HPLC (LC20-AD from Shimadzu, Japan) analysis using UV-detection (Daicel-Chiralcel ODH n-heptane/i-PrOH 98:2, 0.5 mL/min or a Daicel OD column n-heptane/i-PrOH 98:2, 1 mL/min).

**Representative procedure for the [Cu(L)(NO₃)₂]/DNA-catalyzed Diels–Alder cycloaddition reaction:**

10 mL of a solution of st-DNA (2 mg/mL solution of st-DNA dissolved in 30 mM MOPS buffer, pH = 6.5, and prepared 24 h in advance) was added to a catalyst solution of [Cu(L)(NO₃)₂] (5 mL of an aqueous solution with a final concentration of 0.9 mM in Cu and 1.17 mM in ligand) to a final concentration of 1.3 mg/mL. An aliquot of a stock solution of dienophile aza-chalcone in CH₃CN (0.5 M, 30 mL) was added to obtain a final concentration of 1 mM and the mixture was subsequently cooled to 5 °C. The reaction was started by the addition of cyclopentadiene (20 μL, 0.016 M final concentration) and allowed to continue for 3 days at 5 °C, while mixing continuously. The product was isolated by extraction with Et₂O. After drying (Na₂SO₄) and after removal of the solvent, the crude product was analyzed by ¹H NMR spectroscopy and HPLC.

**Dissolution of synthetic oligonucleotides:**

The lyophilized powders were dissolved in MOPS buffer (20 mM, pH = 6.5) and the solutions were heated to 94 °C, slowly cooled to 5 °C, and left for 2 h at 0 °C prior to use. The concentration was determined by UV/Vis absorption spectrophotometry at 25 °C.

**Reactions in the presence of synthetic oligonucleotides:**

A 2 mL Eppendorf container was loaded with oligomer solution (400 μL, 2 mg/mL) in buffer (20 mM MOPS, pH = 6.5), [Cu(L)(NO₃)₂] complex (200 μL, 0.3 mM), and a stock solution of aza-chalcone (1a; 2.4 μL of a stock solution 0.024 mM (3.1 mg/60
μL CH₃CN)) at 5 °C. Freshly distilled cyclopentadiene (2; 0.8 μL, 0.016 M final concentration) was added to this mixture through a microsyringe. The reaction mixture was mixed by continuous inversion for 3 days at 5 °C. After extraction with Et₂O, drying with Na₂SO₄, and concentration under reduced pressure, the products were analyzed by HPLC (ODH column n-heptane/i-PrOH 98:2, 0.5 mL/min).

Determination of \( k_{\text{app}} \):

The procedure to determine \( k_{\text{app}}, K_a, \) and \( k_{\text{cat}} \) were adapted from the procedure of Engberts and co-workers.\(^{[33,43]}\)

A fresh solution of aza-chalcone (1a; 2.0 μL, 0.95 mM in CH₃CN) was added to the appropriate catalyst in buffer (20 mM MES, pH = 5.5) in a quartz cuvette. After the absorption had stabilized, a freshly prepared cyclopentadiene (2) solution in CH₃CN (1 - 10 μL, 0.5 M) was added, with a final concentration 0.5 - 2.0 mM in the reaction mixture. The cuvette was closed immediately to prevent evaporation of cyclopentadiene and sealed. The reaction was monitored at the appropriate temperature at 326 nm on a JASCO V-560 or JASCO V-570 spectrophotometer. The decrease in absorption of 1a was followed for the first 15% of the reaction, and Equation (1) was used to calculate \( k_{\text{app}} \), in which \( \varepsilon_1 \) and \( \varepsilon_2 \) are the extinction coefficients of dienophile (1a) and product (3a), respectively, and \( d \) is the path length of the cuvette. The observed rate constants were determined at different concentrations of cyclopentadiene (2) and the value of \( k_{\text{app}} \) was extracted from the slope of the resulting plot.

\[
k_{\text{app}} = \frac{dA1}{dt} \cdot \frac{1}{d(\varepsilon_1 - \varepsilon_2) \cdot [1]_0 \cdot [2]_0}
\]

(1)

Determination of \( K_a \) and \( k_{\text{cat}} \):

The rate of the reaction of the dienophile with the diene can be described by the sum of the rate of the catalyzed and uncatalyzed reactions (Eq. (2)).

\[
\frac{d[\text{aza}]}{dt} = k_{\text{uncat}} \cdot [\text{Cp}]_{\text{tot}} \cdot [\text{aza}]_t + k_{\text{cat}} \cdot [\text{Cp}]_{\text{tot}} \cdot [\text{aza}]_b
\]

(2)
In this equation $k_{\text{uncat}}$ and $k_{\text{cat}}$ are the uncatalyzed and catalyzed rate constants, respectively. The dienophile is either bound to the catalyst (Lewis acid) or free in solution, whereas the diene is distributed homogeneously through the solution. The concentrations of diene and dienophile are given by $[\text{Cp}]_{\text{tot}}, [\text{aza}]_{\text{tot}}, [\text{aza}]_{b},$ and $[\text{aza}]_{b},$ which represent the total concentration of the diene, the total concentration of the dienophile, the concentration of the free dienophile, and the concentration of the bound dienophile, respectively. The total concentration of the dienophile is given by the sum of the free and bound concentrations. Considering that a large excess of catalyst is used, instead of using the concentration of the free catalyst ($[\text{M}^{n+}])$, the total concentration of the catalyst ($[\text{M}^{n+}]_{\text{tot}}$) can be used to calculate the binding constant ($K_a$) of the dienophile to the catalyst (Eq. (3)). An expression for the concentration of the coordinated dienophile (Eq. (5)) can be obtained by combining the expression for the binding constant (Eq. (3)) with the mass balance for the dienophile (Eq. (4)).

\[
K_a = \frac{[\text{aza}]_b}{[\text{aza}]_f \cdot [\text{M}^{n+}]_{\text{tot}}} \quad (3)
\]

\[
[\text{aza}]_{\text{tot}} = [\text{aza}]_f + [\text{aza}]_b \quad (4)
\]

\[
[\text{aza}]_b = \frac{K_a \cdot [\text{M}^{n+}]_{\text{tot}}}{1 + K_a \cdot [\text{M}^{n+}]_{\text{tot}}} \cdot [\text{aza}]_{\text{tot}} \quad (5)
\]

Substitution of Equation (5) into Equation (2) leads to Equation (6).

\[
\frac{d[\text{aza}]_{\text{tot}}}{dt} = k_{\text{uncat}} \cdot [\text{Cp}]_{\text{tot}} \cdot [\text{aza}]_f + k_{\text{cat}} \cdot [\text{aza}]_{\text{tot}} \cdot [\text{Cp}]_{\text{tot}} \cdot \frac{K_a \cdot [\text{M}^{n+}]_{\text{tot}}}{l + K_a \cdot [\text{M}^{n+}]_{\text{tot}}} \quad (6)
\]

Under pseudo first-order conditions (excess diene) $k_{\text{app}}$ is given by:

\[
k_{\text{app}} = k_{\text{uncat}} \cdot \frac{[\text{aza}]_f}{[\text{aza}]_{\text{tot}}} + k_{\text{cat}} \cdot \frac{K_a \cdot [\text{M}^{n+}]_{\text{tot}}}{l + K_a \cdot [\text{M}^{n+}]_{\text{tot}}} \quad (7)
\]

Substitution of Equation (6) in Equation (7) yields:

\[
k_{\text{app}} = \frac{K_a \cdot [\text{M}^{n+}]_{\text{tot}}}{l + K_a \cdot [\text{M}^{n+}]_{\text{tot}}} \quad (8)
\]
Since the conditions are such that the uncatalyzed reaction is negligible, and the concentration of free dienophile ([aza]_i) is small, the Equation (8) simplifies to:

$$k_{app} = k_{cat} \cdot \frac{K_a \cdot [M^{n+}]_{tot}}{1 + K_a \cdot [M^{n+}]_{tot}}$$

Equation (9) can be re-written as Equation (10) so that a straight line is obtained by plotting 1/k_{app} versus 1/[M^{n+}].

$$\frac{1}{k_{app}} = \frac{1}{k_{cat} \cdot K_a \cdot [M^{n+}]_{tot}} + \frac{1}{k_{cat}}$$

From the values of the slope and the intercept, values of $K_a$ and $k_{cat}$ can be obtained. Because $k_{app}$ can only be calculated if $K_a$ is known, the data have to be fitted iteratively until the value of $K_a$ inserted into Equation (5) converges to the value obtained from the ratio of the intercept to the slope.

**Determination $K_a$:**\[^{[33a, 43]}\]

The $K_a$ values were determined also independently by titration experiments using ligand L5 which contains the same binding site as the other ligands tested, but depleted of the acridine moiety. The catalyst solution (without DNA) was prepared as previously described.\[^{[26]}\] The equilibrium constant $K_a$ was determined by measuring the extinction coefficient of the partially complexed aza-chalcone as a function of the concentration of Cu\(^{II}\) complex. The temperature was stabilized at 25 °C. The following equation was then applied:

$$\frac{[Cu^{2+}]}{\varepsilon_1 - \varepsilon_{obs}} = \frac{1}{(\varepsilon_1 - \varepsilon_{complex}) \cdot K_a} + \frac{[Cu^{2+}]}{\varepsilon_1 - \varepsilon_{complex}}$$

The concentration of Cu\(^{II}\) complex was varied between 0.1 - 0.6 mM in the absence of DNA, and 0.1 - 0.3 mM of Cu\(^{II}\) complex in the presence of DNA. In all the measurements the ratio of Cu\(^{II}\) complex to base pairs DNA was kept at 1:6. Because of the low concentration of copper complex, the $K_a$ was determined from three independent measurements. The concentration of aza-chalcone (1a) was $6.0 \times 10^{-3}$ mM.
Crystal structure determination:

Data were collected on a Bruker SMART APEX CCD diffractometer (Platform with full three-circle goniometer).

Table 5. Crystal data and details of the structure determination.

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The diffractometer was equipped with a 4K CCD detector set 60.0 mm from the crystal. The crystal was cooled to 100 K using the Bruker KRYOFLEX low-
temperature device. Intensity measurements were performed using graphite monochromated MoKα radiation from a sealed ceramic diffraction tube (Siemens). Generator settings were 50 kV/40 mA. SMART was used for preliminary determination of the unit cell constants and data collection control. Data integration and global cell refinement were performed with the program SAINT. Intensity data were corrected for Lorentz and polarization effects, scale variation, for decay and absorption: a multi-scan absorption correction was applied, based on the intensities of symmetry-related reflections measured at different angular settings (SADABS), and reduced to F₀^2. The program suite SHELXTL was used for space group determination (XPREP). The structure was solved by Patterson methods and extension of the model was accomplished by direct methods applied to difference structure factors using the program DIRDIF. Details of the data collection and refinement are given in Table 5. CCD 719143 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge.

Synthesis of Ligand L6:

\[ \text{Boc-} \text{H} - \text{N} \text{NH}_2 \xrightarrow{a} \text{Boc-} \text{H} - \text{NNH} \xrightarrow{b} \text{Boc-} \text{H} - \text{N} \text{N} \]

\[ \text{H}_2 \text{N} - \text{N} \text{NH} \xrightarrow{c} \text{L6} \]

Reductive aminations: *tert*-butyl 3-[(2-pyridinylmethyl)amino]ethylcarbamate (a).[^26]

Starting from mono-Boc protected ethylenediamine (1.22 g, 7.68 mmol) the pure product a was obtained (1.5 g, 5.97 mmol, yield 78%) as slightly yellow oil.
**Alkylations: tert-butyl-3-[(1-ethyl)(2-pyridinylmethyl)amino]-ethylcarbamate (b).**

A solution of the product tert-butyl 3-[(2-pyridinylmethyl)amino]ethylcarbamate (a) (2.01 g, 8.01 mmol), ethylbromide (0.78 mL, 1.13 g, 1.3 eq.), K₂CO₃ (1.10 g, 8.06 mmol) in CH₃CN was placed under nitrogen. After heating under reflux for 16 h the solvent was evaporated and the crude material redissolved in water (30 mL). Extraction with ethyl acetate (3 × 20 mL) was followed by washing the combined organic layers with brine. After drying (Na₂SO₄) the ethyl acetate was evaporated and the crude material subjected to column chromatography (Alox, akt III, neutral, n-heptane/ethyl acetate/triethyl amine 20:10:1). The pure product b was obtained (1.77 g, 6.3 mmol, 79% yield) as a yellow oil.

¹H-NMR (CDCl₃, 400 MHz) δ = 8.59 (m, 1H), 7.70 (m, 1H), 7.45 (m, 1H), 7.21 (m, 1H), 5.32 (s, 1H, NH), 3.78 (s, 2H), 3.20 (m, 2H), 2.69 (m, 4H), 1.51 (s, 9H), 1.11 (t, J = 7.03 Hz, 3H); ¹³C-NMR (CDCl₃, 100 MHz) δ = 12.41, 40.33, 48.82, 57.34, 60.78, 122.29, 123.24, 136.83, 149.47; HRMS calcd for [M-H]⁺ C₁₅H₂₅N₃O₂ = 279.1947, found = 279.1963 (ESI⁺).

**Deprotection: N¹-(ethyl)-N¹-(2-pyridinylmethyl)-1,2-ethanediamine (c).**

To a solution of tert-butyl 3-[(1-ethyl)(2-pyridinylmethyl)amino]ethylcarbamate (b) (1.36 g, 4.84 mmol) in CH₂Cl₂ (27 mL) was added thiophenol (2.95 mL, 0.028 mmol) and trifluoroacetic acid (2.95 mL, 0.04 mmol). After stirring for 6 h at room temperature aq. 1 M HCl solution (30 mL) was added. The CH₂Cl₂ was evaporated and the aqueous layer was extracted with diethyl ether (3 × 70 mL). The pH of the aqueous phase was brought to > 10 by addition of aq. 2 M NaOH. Extraction with CH₂Cl₂ (3 × 30 mL) was followed by washing of the combined organic layers with brine. After drying the solvent was removed in vacuo to give the pure product c (0.55 g, 3.06 mmol, yield 63%) as yellow oil.

¹H-NMR (CDCl₃, 400 MHz) δ = 8.62 (d, J = 4.83 Hz, 1H), 7.66 (m, 1H), 7.47 (s, 1H), 7.16 (m, 1H), 3.84 (s, 2H), 2.82 (m, 4H), 2.64 (m, 2H), 1.41 (s, 2H), 1.12 (t, J = 7.10 Hz, 3H); HRMS calcd for [M-H]⁺ C₁₀H₁₇N₃ = 179.1422, found = 179.1426 (ESI⁺).

**Coupling to acridine: N¹-(9-acridinyl)-N¹-(2-pyridinylmethyl)-1,3-ethane-diamine (d).**

A mixture of N¹-(ethyl)-N¹-(2-pyridinylmethyl)-1,2-ethanediamine (c) (0.28 g, 1.6 mmol), 9-chloroacridine ( 0.34 g, 1 eq.) and phenol (1.71 g, 0.018 mmol) was
placed under nitrogen and heated at 100 °C for 2 h. After cooling to room temperature diethyl ether (50 mL) was added and the mixture was stirred for 1 h. The diethyl ether was decanted from the dark oil, and fresh diethyl ether (50 mL) was added. After stirring for 2 days the hydrochloride salt of d (0.26, 0.66 mmol, yield 41%) as isolated as a yellow solid. Dissolving a small amount in aq. 1 M NaOH, followed by extraction with CH₂Cl₂ gave the free base as a dark yellow oil, which was used for characterization.

\[ ^1H-NMR \ (CDCl_3, \ 400 \ MHz) \ \delta = 8.56 \ (m, \ 1H), \ 8.25 \ (d, \ J = 8.56 \ Hz, \ 2H), \ 8.08 \ (d, \ J = 8.20 \ Hz, \ 2H), \ 7.68 \ (m, \ 3H), \ 7.40 \ (m, \ 2H), \ 7.17 \ (m, \ 2H), \ 5.32 \ (s, \ 1H, \ NH), \ 3.91 \ (m, \ 2H), \ 2.95 \ (m, \ 2H), \ 2.80 \ (m, \ 2H), \ 1.26 \ (s, \ 2H), \ 1.17 \ (m, \ 3H); \ \text{HRMS \ calcd \ for} \ [M-H]^+ \ C_{23}H_{24}N_4 = 356.2001, \ \text{found} \ = 356.1999 \ (ESI^{+}). \]

**Synthesis Substrates:**

[Images of substrates 1b, 1c, and 1d]

2-acetyl-5-methylpyridine and 2-acetyl-3-methylpyridine were prepared following literature procedures.[44] 2-acetyl-4-methylpyridine was obtained from Sigma. Aza-chalcone dienophiles were obtained following published procedures.[43]

**(E)-1-(5-methyl-2-pyridinyl)-3-phenyl-2-propen-1-one (1b).**

Starting from 2-acetyl-5-methylpyridine (0.4 g, 2.95 mmol), 0.54 g (0.5 g, 2.24 mmol, yield 76%) of product 1b were obtained, after chromatography on silica gel (pentane/ethyl acetate 10:1) as yellow solid.

Mp = 72.5 - 81.4 °C; \[ ^1H-NMR \ (CDCl_3, \ 400 \ MHz) \ \delta = 8.56 \ (s, \ 1H), \ 8.32 \ (d, \ J = 16.04 \ Hz, \ 1H), \ 8.11 \ (d, \ J = 7.98 \ Hz, \ 1H), \ 7.94 \ (d, \ J = 16.07 \ Hz, \ 1H), \ 7.74 \ (m, \ 2H), \ 7.67 \ (m, \ 1H), \ 7.42 \ (m, \ 3H), \ 2.48 \ (m, \ 3H); \ \text{13C-NMR \ (CDCl}_3, \ 100 \ MHz) \ \delta = 190.80, \ 152.75, \ 150.84, \ 145.29, \ 139.17, \ 136.31, \ 131.91, \ 130.00, \ 134.07, \ 122.16, \ 19.87. \ \text{HRMS \ calcd \ for} \ [M-H]^+ \ C_{15}H_{13}NO = \ 223.09970, \ \text{found} = \ 223.0999 \ (ESI^{+}); \ \text{elemental \ analysis \ calcd} \ (%) \ \text{for} \ C_{15}H_{13}ON: \ C, \ 80.69; \ H, \ 5.87; \ N, \ 6.27; \ \text{found} = \ C, \ 80.69; \ H, \ 5.85; \ N, \ 6.32.**
(E)-1-(4-methyl-2-pyridinyl)-3-phenyl-2-propen-1-one (1c).

Starting from 1 g (7.4 mmol) of 2-acetyl-4-methylpyridine, 1.32 g (5.92 mmol, yield 80%) of product 1c were obtained, after purification on silica gel (pentane/ethyl acetate 10:1) as yellow solid.

Mp = 100.9 - 101.0 °C; $^1$H-NMR (CDCl$_3$, 400 MHz) $\delta$ = 8.61 (d, 1H, $J = 4.88$ Hz), 8.32 (d, 1H, $J = 16.05$), 8.03 (m, 1H), 7.96 (d, 1H, $J = 16.05$), 7.74 (m, 2H), 7.42 (m, 3H), 7.32 (m, 1H), 2.35 (s, 3H); $^{13}$C-NMR (CDCl$_3$, 100 MHz) $\delta$ = 190.62, 155.21, 149.62, 146.08, 136.57, 131.35, 130.23, 128.55, 124.46, 122.04, 23.26. HRMS calcd for [M-H]$^+$ C$_{15}$H$_{13}$NO = 223.1069, found = 223.1069 (ESI$^+$); elemental analysis calcd (%) for C$_{15}$H$_{13}$ON: C, 80.69; H, 5.87; N, 6.27; found: C, 80.56; H, 5.86; N, 6.21.

(E)-1-(3-methyl-2-pyridinyl)-3-phenyl-2-propen-1-one (1d).

Starting from 0.41 g (3.03 mmol) of 2-acetyl-3-methylpyridine, 0.5 g (2.23 mmol, yield 74%) of product 1d were obtained, after chromatography on silica gel (pentane/ethyl acetate 10:1) and recrystallisation as yellow solid.

Mp = 78.7 - 79.0 °C; $^1$H-NMR (CDCl$_3$, 400 MHz) $\delta$ = 8.55 (s, 1H), 8.00 (d, $J = 16.09$ Hz, 1H), 7.79 (d, $J = 16.01$ Hz, 1H), 7.67 (m, 2H), 7.60 (m, 1H), 7.40 (m, 3H), 7.33 (m, 1H), 2.69 (s, 3H); $^{13}$C-NMR (CDCl$_3$, 100 MHz) $\delta$ = 192.28, 153.19, 146.36, 144.49, 140.16, 135.31, 130.61, 129.01, 125.90, 124.20, 20.20; HRMS calcd for [M-H]$^+$ C$_{15}$H$_{13}$NO = 223.09970; found 223.10017 (ESI$^+$); elemental analysis calcd (%) for C$_{15}$H$_{13}$ON: C, 80.69; H, 5.87; N, 6.27; found: C, 80.48; H, 5.87; N, 6.31.

Diels-Alder products (major, endo isomer):


$^1$H-NMR (CDCl$_3$, 400 MHz) $\delta$ = 8.52 (s, 1H), 7.97 (d, $J = 7.85$ Hz, 1H), 7.65 (d, $J = 7.82$ Hz, 1H), 7.38 (m, 5H), 6.50 (s, 1H), 4.56 (m, 1H), 3.57 (m, 1H), 3.10 (s, 1H), 2.49 (s, 3H), 2.08 (d,
(4-methyl-2-pyridinyl)(3-phenylbicyclo[2.2.1]hept-5-en-2-yl)methanone (3c).

$^1$H-NMR (CDCl$_3$, 400 MHz) $\delta$ = 8.56 (d, $J = 4.86$ Hz, 1H), 7.85 (s, 1H), 7.35 (m, 5H), 6.51 (m,1H), 5.84 (m, 1H), 4.56 (m,1H), 3.57 (s, 1H), 3.44 (d, $J = 4.25$ Hz, 1H), 3.11 (s, 1H), 2.44 (s, 3H), 2.10 (d, $J = 8.10$, 1H), 1.63 (d, $J = 9.94$ Hz, 1H); HRMS calcd for [M-H$^+$] $^{\text{C}_{20}\text{H}_{19}\text{NO}}$ = 289.1467, found = 289.1461 (ESI$^{\text{pos}}$).


$^1$H-NMR (CDCl$_3$, 400 MHz) $\delta$ = 8.53 (d, $J = 4.59$ Hz, 1H), 7.58 (d, $J = 7.75$ Hz, 1H), 7.34 (m, 6H), 5.94 (m, 1H), 4.55 (m, 1H), 3.35 (d, $J = 3.81$ Hz, 2H), 3.42 (s, 1H), 3.08 (s, 1H), 2.49 (s, 3H), 1.98 (d, $J = 8.44$ Hz, 1H), 1.55 (dd, $J = 1.71$, $J = 8.48$ Hz, 1H); HRMS calcd for [M-H$^+$] $^{\text{C}_{20}\text{H}_{19}\text{NO}}$ = 289.1467, found = 289.1463.

Synthesis and crystallisation Cu-L1 complex for X-ray study:

Crystallisation from a mixture acetonitrile and acetyl acetate. (0.14 mmol, 0.067 g) $N^1$-(9-acridinyl)-N$^3$-(3,5-dimethoxybenzyl)-N$^3$-2-pyridinylmethyl)-1,3-ethanediamine (L1), was added to 20 mg (0.2 mmol) of HClO$_4$ in MeOH (1.5 mL) and then 1.1 eq. (57 mg) of Cu(ClO$_4$)$_2 \times$ 6H$_2$O in CH$_3$CN (1.5 mL) was added. The resulting solution was placed in an ethyl acetate bath. After 3 days, the supernatant was removed and the solid was redissolved in 7 mL of CH$_3$CN and placed in an AcOEt bath. Fragile platelet shaped green colored crystals were obtained suitable for X-Ray analysis.

2.10 References


A kinetic and structural investigation of DNA-based asymmetric catalysis using first generation ligands


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