Chapter 2

DNA-based catalysis: a covalent approach

In this chapter a novel DNA-based catalyst based on a modular approach is presented. The novelty of the system lays in the fact that the metal complex is covalently linked to a specific position on the DNA allowing for a rapid optimization. The synthesis and construction of such a catalyst is described and its performance in the Cu(II)-catalyzed Diels-Alder reaction in water is investigated. The results show to be very dependent on the design of the system. The optimization of the system is described resulting in high conversions and enantioselectivities.

Parts of this chapter have been published:

2.1. Introduction

Hybrid catalysis makes use of catalytically active transition metal complexes anchored to biopolymer scaffolds. In this way, by combining the advantageous features from both components, i.e., the (enantio-)selectivity provided by the second coordination sphere of the biopolymer and the broad catalytic and substrate scope by the metal complex, powerful new catalysts are obtained. Recently, the use of DNA as a chiral source in asymmetric catalysis was introduced in our group. The supramolecularly assembled catalysts used consist of an achiral metal complex containing a DNA interacting unit (ligand 1) (Figure 1). In the presence of DNA, the catalytic center is brought in close proximity to the chiral environment of the DNA resulting in enantiomeric excess in the product of the reaction. When no intercalation unit was used (ligand 2), so the metal complex interacts directly with the DNA, excellent enantiomeric excesses where obtained in a variety of reactions. However, the concrete position of the catalyst on the DNA is not known since the DNA presents multiple binding sites that are structurally different. This makes the optimization of the catalyst challenging.

![Figure 1. Supramolecular DNA-based catalyst. Figure reproduced and adapted from [3], with permission.](image)

A covalent anchoring strategy allows for precise positioning of the metal complex and, hence, control over the structure and geometry of the catalytic site. Recently, several approaches towards covalent anchoring of catalytically active metal complexes to internal positions in the DNA were reported (Figure 2).
These approaches involve the synthesis of modified nucleotides or nucleotide analogues and their incorporation into synthetic oligonucleotides, sometimes followed by post-synthetic derivatization.[8-11] However, to date only few of these have been applied in asymmetric catalysis leading to very low enantioselectivities.

In this chapter, the construction of a covalently modified DNA-based catalyst based on a modular approach is investigated, which allows for a rapid optimization of the system. The efficacy of the system in the copper catalyzed asymmetric Diels-Alder reaction is investigated.

2.2. Covalent DNA-based catalysts

For the creation of a DNA-based catalyst via a covalent approach, a metal complex is linked to the DNA in a specific position. The catalyst can be easily constructed by the covalent modification of one or more oligonucleotide modules and a subsequent assembly of the different modules by hybridization. Since the location of the catalyst is known, modifications in the second coordination sphere of the metal centre can be designed and implemented readily.

In the present study two different approaches were followed. Both of them involved three oligonucleotide components. In an initial design two oligonucleotides of 16 nucleobases functionalized on the 5’ (Oligo 1) and on the 3’ (Oligo 2) terminus with different isomeric pyridine moieties, i.e., substituted on the ortho, meta and para position, together with a template oligonucleotide complementary to both functionalized 16-mers were used (Figure 3a).
In the second approach an oligonucleotide of 16 nucleobases functionalized with 2, 2’-bipyridine (bipy) at the 5’ or 3’ terminal phosphate moieties (Oligo 1 or Oligo 2, respectively), an unfunctionalized oligonucleotide and a template oligonucleotide strand with a sequence that is fully complementary to both oligonucleotides were used (Figure 3b).

Compared with previous catalysts covalently anchored to DNA,[8-11] the present approaches possess several advantages: terminally modified oligonucleotides are prepared routinely via optimized solid-phase synthesis, are commercially available and covalent attachment of a ligand is readily achieved using well-established chemistry.

Furthermore, in the second approach the second coordination sphere around the metal centre is optimized readily by exchange of the unfunctionalized modules, i.e., Oligo 2 and the template, which obviates the need for synthesis of new oligonucleotide–ligand conjugates.

2.2.1. Synthesis of ligand-DNA conjugates

The pyridine- and bipy-oligonucleotide conjugates were prepared by reaction of commercially available 5’ or 3’ amino-modified oligonucleotides with an excess of the N-hydroxysuccinimide activated ester of pyridine and bipyridine, respectively (Scheme 1). Since the coupling reaction is performed in aqueous environment the reactivity of the activated ester is crucial. N-hydroxysuccinimide activated esters were selected because of their relative stability towards hydrolysis in aqueous environment and their well known chemistry with nucleophiles.

![Scheme 1. General coupling procedure between activated esters of pyridine or bipyridine and amino-functionalized oligonucleotides.](image)

The progress of the reaction was followed by reversed phase–HPLC (rp-HPLC). In case of the bipyridine-DNA conjugates, a precipitate appeared during the coupling. This was possibly due to the hydrophobicity of the bipyridine moiety. Nevertheless full conversion towards the coupled product was achieved. The resulting conjugates were purified by size exclusion chromatography and analyzed by reversed phase-HPLC and MALDI-TOF. Yields were typically around 80%.
2.2.2. Assembly of covalent DNA-based systems for catalysis

The covalent DNA-based systems were assembled by thermal hybridization of equimolar amounts of the oligonucleotide components in the presence of a copper (II) salt (Figure 4). In the first approach the pyridine-oligonucleotide conjugates (Oligo 1 and Oligo 2) were combined with the complementary DNA template. In the second approach the bipy–oligonucleotide conjugate (Oligo 1), the unfunctionalized Oligo 2 and the complementary DNA template were used. Hybridization in the presence of a metal ion gives rise to duplex DNA in which the catalytically active metal complex is positioned internally at the interface between Oligo 1 and Oligo 2.

Figure 4. Schematic representation of the assembly of the DNA-based catalysts.

For the bipyridine-based catalyst, circular dichroism (CD) measurements showed a positive ellipticity maximum at 275 nm and a negative minimum at around 250 nm with a crossover around 260 nm (Figure 5a). This corresponds to the typical B-DNA structure and suggests that the modification did not alter the structure of the DNA assembly. Melting curves showed that at the temperature at which the catalysis experiments are performed (5 °C) the system remained hybridized ($T_m \sim 42$ °C) (Figure 5b).
2.3. Catalysis

The catalytic properties of the novel DNA-based catalysts were investigated in the Cu$^{2+}$-catalyzed Diels–Alder reaction of aza-chalcone (1) with cyclopentadiene (2) in water (Scheme 2). Reactions were carried out using 0.13 mM oligonucleotides, 0.1 mM Cu(NO$_3$)$_2$, 1 mM aza-chalcone and 33 mM cyclopentadiene in MOPS buffer (20 mM pH 6.5). After two days in case of the pyridine-based system and three days for the bipyridine-based system at 5 °C, the endo isomer of the resulting Diels–Alder product (3), which in all reactions was the main isomer of the product (endo : exo > 4 : 1), was isolated and analyzed. Both the conversion and the enantiomeric excess of 3 proved to be dependent on the design of the catalyst.

![Scheme 2. Reaction scheme for the DNA-based Diels-Alder reaction in water.](image)

2.3.1. Pyridine-based catalysts

Initially the pyridine-based DNA system was studied. In first instance, substituted oligonucleotides of 16 nucleobases length and a template of 32 nucleobases were used (Figure 5).
DNA-based catalysis: a covalent approach

Figure 5. Schematic representation of the pyridine-DNA-based catalyst containing a 32-mer as the template.

When meta and para substituted pyridines were used, high conversions of aza-chalcone were obtained after 2 days (Table 1, entries 2 and 3). In contrast, minimal conversion was observed when ortho substituted pyridines were used (entry 1). This can be tentatively explained by the possible metal coordinating sites in the ligand, i.e., the carbonyl group and the nitrogen at the pyridine ring. Since in case of ortho substitution they are closer in space, bidentate coordination to the metal centre is possible. Binding of two ligands provided a coordination mode which results in the blocking of the coordination sites needed for substrate binding. Hence, no catalysis occurs (Figure 6).

Table 1. Results of Diels-Alder reactions of 1 with 2 catalyzed by pyridine-DNA-based catalysts.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Isomer</th>
<th>Conversion (%)</th>
<th>Ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ortho</td>
<td>10</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>2</td>
<td>Meta</td>
<td>83</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>3</td>
<td>Para</td>
<td>78</td>
<td>7 (-)</td>
</tr>
</tbody>
</table>

* All experiments were carried out with 0.13 mM oligonucleotides, 0.1 mM Cu(NO₃)₂, 1 mM aza-chalcone and 33 mM cyclopentadiene in MOPS buffer (20 mM pH 6.5) for 2 days at 5 °C. Results are based on single experiments. ‡ Determined by HPLC. § Determined by chiral HPLC. For the endo isomer.

Figure 6. Possible tetra-coordination to the metal center when ortho-substituted pyridine ligands are used.

Although in the catalysis using the meta and para substituted pyridines high conversions were obtained, no significant enantioselectivity in the product of the reaction was observed. This would indicate that either, the binding of both pyridine moieties is not optimal to coordinate the metal in a bidentate fashion, letting the copper ions free in solution or the distance between the metal centre and the chiral environment provided by the DNA is too large.
In order to elucidate the optimum distance between both pyridines and investigate the influence on the chiral induction, two other designs were tested in which templates of 33 (Figure 7a) and 34 (Figure 7b) nucleobases were used. In this way the distance between both functionalized 16-mers was increased.

Figure 7. Schematic representation of the pyridine-DNA-based catalyst containing: a) a 33-mer and b) a 34-mer as the template.

As before, very low conversions were obtained when ortho substituted pyridines were used (Table 2, entries 1 and 4). Only for the ortho substituted system when a 34-mer was used as the template a significant ee was found (entry 4) indicating that there is no chiral induction from the DNA in the other cases either.

Table 2. Results of Diels-Alder reactions of 1 with 2 catalyzed by pyridine-DNA-based catalysts.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Isomer</th>
<th>Template</th>
<th>Conversion (%)\textsuperscript{b}</th>
<th>Ee (%)\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1\textsuperscript{d}</td>
<td>Ortho</td>
<td>(a)</td>
<td>5 ± 2</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>2\textsuperscript{d}</td>
<td>Meta</td>
<td>(a)</td>
<td>82 ± 5</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>3\textsuperscript{d}</td>
<td>Para</td>
<td>(a)</td>
<td>91 ± 4</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>4\textsuperscript{e}</td>
<td>Ortho</td>
<td>(b)</td>
<td>8</td>
<td>16 (+)</td>
</tr>
<tr>
<td>5\textsuperscript{e}</td>
<td>Meta</td>
<td>(b)</td>
<td>81</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>6\textsuperscript{e}</td>
<td>Para</td>
<td>(b)</td>
<td>60</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>7\textsuperscript{e, f}</td>
<td>-</td>
<td>(a)</td>
<td>62</td>
<td>&lt; 5</td>
</tr>
</tbody>
</table>

\textsuperscript{a} All experiments were carried out with 0.13 mM oligonucleotides, 0.1 mM Cu(NO\textsubscript{3})\textsubscript{2}, 1 mM aza-chalcone and 33 mM cyclopentadiene in MOPS buffer (20 mM pH 6.5) for 2 days at 5 ⁰C. \textsuperscript{b} Determined by HPLC. \textsuperscript{c} Determined by chiral HPLC. For the \textit{endo} isomer. \textsuperscript{d} Results correspond to the average of two experiments. \textsuperscript{e} Results are based on single experiments. \textsuperscript{f} Oligonucleotides without tethered pyridines were used.

The results obtained for the meta and para substituted pyridines are comparable to what was obtained when amino modified oligonucleotides were used, i.e., without attached pyridine moieties (entry 7). In this case the coordination from both amino groups to the copper is expected to be very weak. This would suggest that the copper is free in solution, leading to the racemic product.
Furthermore, the proximity of the two pyridine modified chains possibly impedes the metal complex to locate in the chiral environment of the DNA helix and in that way the chiral induction to take place. A different design in which the bidentate ligand was attached to just one of the oligonucleotides was investigated. Via this approach the hindrance between neighboring chains is avoided what can have a possible influence on the resulting enantiomeric excesses.

2.3.2. Bipyridine-based catalysts

Preliminary experiments performed with the bipyridine-based system depicted in Figure 7 showed good conversions and enantioselectivities. In an initial design, the bipyridine ligand was attached to the 5ʹ-terminal phosphate moiety of a G-terminated 16-mer oligonucleotide via a 1-aminoethyl linker (linker (a)) (Figure 8).

![Figure 8. Schematic representation of the bipyridine-DNA-based catalyst.](image)

The results obtained showed that the covalent linkage of the metal complex to a concrete position of the DNA helix has an influence on the results. Using non-covalently linked Cu-bipy complex in combination with the same duplex DNA, i.e., supramolecular assembly of the DNA-based catalyst, resulted in a similar conversion but significantly decreased ee (entry 3). This clearly demonstrates the advantage of covalent anchoring of Cu–bipy; whereas supramolecular anchoring results in a heterogeneous mixture of complexes that all reside in a different part of the sequence, and hence, all catalyze the reaction with different enantioselectivity, covalent anchoring allows for precise positioning of the complex in the DNA. The result is a better defined catalyst that, in the present case, gives rise to a higher enantioselectivity.
To optimize the present system, the position of the complex on the DNA helix, the template length, the DNA sequence and the distance between the DNA and the metal complex were optimized.

Table 3. Results of Diels-Alder reactions of 1 with 2 catalyzed by DNA-based catalysts.\textsuperscript{a, b, c}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst (Oligo 1, Oligo 2 3’→ 5’/template 5’→ 3’)</th>
<th>Linker</th>
<th>Conv. (%)\textsuperscript{d}</th>
<th>Ee (%)\textsuperscript{e}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>*GTTCCAGTCTGTAGAGACCATGCTTAAGCGAGCAAGGTCAGACATGTCTGGTACGAATTCGCTC</td>
<td>(a)</td>
<td>54</td>
<td>77 (+)</td>
</tr>
<tr>
<td>2</td>
<td>*GTTCCAGTCTGTAGAGACCATGCTTAAGCGAGCAAGGTCAGACATGTCTGGTACGAATTCGCTC</td>
<td>(a)</td>
<td>51</td>
<td>69 (+)</td>
</tr>
<tr>
<td>3\textsuperscript{f}</td>
<td>*GTTCCAGTCTGTAGAGACCATGCTTAAGCGAGCAAGGTCAGACATGTCTGGTACGAATTCGCTC</td>
<td>(a)</td>
<td>56</td>
<td>53 (+)</td>
</tr>
<tr>
<td>4</td>
<td>*GTTCCAGTCTGTAGAGACCATGCTTAAGCGAGCAAGGTCAGACATGTCTGGTACGAATTCGCTC</td>
<td>(a)</td>
<td>7</td>
<td>22 (+)</td>
</tr>
<tr>
<td>5</td>
<td>*GTTCCAGTCTGTAGAGACCATGCTTAAGCGAGCAAGGTCAGACATGTCTGGTACGAATTCGCTC</td>
<td>(a)</td>
<td>21</td>
<td>32 (+)</td>
</tr>
<tr>
<td>6</td>
<td>*GTTCCAGTCTGTAGAGACCATGCTTAAGCGAGCAAGGTCAGACATGTCTGGTACGAATTCGCTC</td>
<td>(a)</td>
<td>94</td>
<td>7 (-)</td>
</tr>
<tr>
<td>7\textsuperscript{d}</td>
<td>*GTTCCAGTCTGTAGAGACCATGCTTAAGCGAGCAAGGTCAGACATGTCTGGTACGAATTCGCTC</td>
<td>(a)</td>
<td>5</td>
<td>39 (+)</td>
</tr>
<tr>
<td>8</td>
<td>*GTTCCAGTCTGTAGAGACCATGCTTAAGCGAGCAAGGTCAGACATGTCTGGTACGAATTCGCTC</td>
<td>(a)</td>
<td>65</td>
<td>89 (+)</td>
</tr>
<tr>
<td>9</td>
<td>*GTTCCAGTCTGTAGAGACCATGCTTAAGCGAGCAAGGTCAGACATGTCTGGTACGAATTCGCTC</td>
<td>(b)</td>
<td>71</td>
<td>93 (+)</td>
</tr>
<tr>
<td>10\textsuperscript{f}</td>
<td>*GTTCCAGTCTGTAGAGACCATGCTTAAGCGAGCAAGGTCAGACATGTCTGGTACGAATTCGCTC</td>
<td>(a)</td>
<td>61</td>
<td>40 (+)</td>
</tr>
<tr>
<td>11\textsuperscript{h}</td>
<td>*GTTCCAGTCTGTAGAGACCATGCTTAAGCGAGCAAGGTCAGACATGTCTGGTACGAATTCGCTC</td>
<td>(a)</td>
<td>76</td>
<td>14 (+)</td>
</tr>
<tr>
<td>12</td>
<td>*GTTCCAGTCTGTAGAGACCATGCTTAAGCGAGCAAGGTCAGACATGTCTGGTACGAATTCGCTC</td>
<td>(b)</td>
<td>47</td>
<td>79 (+)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} All experiments were carried out with 0.13 mM oligonucleotides, 0.1 mM Cu(NO\textsubscript{3})\textsubscript{2}, 1 mM aza-chalcone and 33 mM cyclopentadiene in MOPS buffer (20 mM pH 6.5) for 3 days at 5 °C, unless noted otherwise. \textsuperscript{b} * indicates the position where the metal complex is attached. \textsuperscript{c} Results correspond to the average of at least two experiments. \textsuperscript{d} Determined by HPLC. Reproducible within ± 10%. \textsuperscript{e} Determined by chiral HPLC. For the endo isomer. Reproducible within ± 3%. \textsuperscript{f} 0.1 mM Cu(bipy)(NO\textsubscript{3})\textsubscript{2}. \textsuperscript{g} The sequence contains a different base pair in position 4 compared with the other sequences. This mutation is not expected to influence the results since it is too far removed for a direct interaction of the nucleotides with the catalyst. However, effects on the overall structure of the DNA cannot be excluded. \textsuperscript{h} 0.1 mM Cu(L)(NO\textsubscript{3})\textsubscript{2}. L is N-propyl-2, 2’-bipyridine-5-carboxamide.
2.3.2.2. Effect of the position on the DNA helix

Placing the Cu-bipy complex at the terminus of the duplex by using a shorter template and leaving out Oligo 2, resulted in a loss of activity and a strongly decreased selectivity (entry 4) implying that the placement of the metal complex in an internal position of the DNA helix is required to obtain high enantiomeric excesses. Once the complex is integrated in the helix it does not matter whether the complex is attached to Oligo 1 or to Oligo 2 (entry 2). This suggests that the chiral environment around the metal center is similar in both cases. Interestingly, the same effect was not observed when a different sequence was used (entries 9 and 12). Even though the same sequence is surrounding the catalytic site, the attachment of the complex to Oligo 2 led to a reduction in ee and conversion, suggesting a very different structure of the second coordination sphere.

2.3.2.3. Effect of the template length

Lower enantioselectivities and activities were obtained in case of a longer, i.e., 33 nucleotides, template. In this case one nucleobase is not involved in base-pairing near the catalytic site (entry 5). Possibly, this free adenine nucleobase competes with aza-chalcone for binding to the Cu$^{2+}$, thus reducing activity. With the supramolecularly assembled catalysts a similar effect was found when single-stranded DNA or free nucleotides were present.[12] These observations suggest that the catalyst needs to be placed in an internal position of a complete duplex in order to obtain both activity and enantioselectivity. Interestingly, a reduction in the conversion was not observed when the pyridine based system was used although in that case, there were one or two nucleobases not involved in base-pairing when templates of 33 and 34 nucleobases were used, respectively. Assuming that the copper is coordinated in a bidentated fashion by the pyridine moieties, the presence of these free nucleobases could result likewise in the competition for binding to the copper. This would lead to a decrease in the conversion. The fact that this is not observed strengthens the assumption of a hindering structure that forces the metal complex to stay away from the DNA helix and subsequently avoids the coordination to the free nucleobases. Studies in which two non-functionalized oligonucleotides and the 33-mer where used in the presence of Cu$^{2+}$ (entry 6) suggested that the interaction of the metal centre with the free adenine is probably not strong enough to bind the catalyst and perform efficient enantioselective catalysis.

2.3.2.4. Effect of the DNA sequence

The sequence dependence of the DNA-based catalyst was studied by using different DNA sequences. In the case of supramolecularly assembled DNA-based catalysts, both the activity and asymmetric induction proved to be highly dependent on the DNA sequence.[12] Therefore, the effect of the sequence surrounding the anchoring site was examined in more detail.
First, the three 5’- and 3’-terminal nucleotides of Oligo 1 and Oligo 2, respectively, and the corresponding nucleotides in the template strand were exchanged to give the sequence that was proven to be optimal in the case of supramolecularly assembled DNA-based catalysts. However, this had a negative effect on the activity and enantioselectivity of the catalyst (entry 7). Instead, changing only the three 3’-terminal nucleotides of Oligo 2 to GTA (3’ – 5’) and the corresponding nucleotides in the template to CAT (5’ – 3’) gave rise to a significantly increased conversion and enantioselectivity (entry 8).

2.3.2.5. Effect of the spacer length

The proximity of the metal complex to the DNA helix was expected to have an influence on the chiral induction from the DNA to the product of the reaction. Therefore, different linker lengths were tested, using the catalyst with the optimized sequence. By decreasing the linker length from six to three carbons a further increase in ee to 93% was found (entry 9), indicating that a close proximity of the active complex to the DNA is required to achieve the most efficient transfer of chirality. Again, this is significantly higher than for the free Cu–bipy complex and [Cu(L)(NO$_3$)$_2$],$^{[13]}$ in combination with the same duplex DNA (entries 10 and 11). Interestingly, using only Cu(NO$_3$)$_2$ with this DNA sequence gave rise to 32% ee of the opposite enantiomer. This suggests that the substrates by themselves interact in some way with the DNA helix but the coordination to the metal complex that is covalently attached to the DNA is necessary to obtain selectively the (+) enantiomer in higher ee.

Most gratifying, however, is the fact the enantioselectivity is also higher than the best results found with the related supramolecularly assembled Cu–bipy–DNA catalyst.$^{[14]}$ This clearly illustrates the potential of the covalent anchoring strategy in hybrid catalysis.

2.4. Summary and conclusions

A novel modular approach to DNA-based catalysis using covalently attached metal catalysts to DNA has been presented. Two different approaches were followed for the copper (II) catalyzed Diels–Alder reaction in water which involved the use of two monodentate ligands, i.e., pyridine, or one bidentated ligand, i.e., bipyridine. The results were very dependent on the design of the catalyst. The best results were obtained for the bipyridine-based catalyst when the metal complex was integrated in the middle of a fully complementary DNA helix using a short three carbon linker between the DNA and the catalyst. The sequence used also affected the results.

It was demonstrated that this covalent approach allows the control over the position of the catalyst in the DNA helix and enables the easy optimization of the resulting catalyst to obtain high conversions and ee’s.
2.5. Experimental section

General remarks

Picolinic acid, nicotinic acid and isonicotinic acid were purchased from Acros Organics. Thionyl chloride and N-hydroxysuccinimide were purchased from Sigma-Aldrich. THF was distilled over sodium/benzophenone. Dichloromethane was distilled over CaH₂. Reversed phase-HPLC analysis were performed on a Shimadzu LC-10AD VP, Waters Xterra MS C18 column (3.0 x 150 mm, particle size 3.5 μm) using a gradient of CH₂CN/TEAA buffer 50 mM pH 7; gradient: 05/95 0 to 10 min, to 35/65 at 60 min, to 70/30 at 65 min. Flow: 0.5mL/min. MALDI-TOF measurements were done on a Voyager-DE Pro apparatus. (Matrix: 20 μL of a solution of 2, 4, 6- trihydroxyacetophenone 0.5 M in ethanol + 10 μL of a solution of ammonium citrate dibasic 0.1 M in Milli Q water + 2 μL sample solution in Milli Q water) in negative mode. Aza-chalcone (1) was prepared following published procedures.[15] N-Hydroxsuccinimide nicotinate and N-hydroxsuccinimide isonicotinate were synthesized following the same procedure as for N-hydroxysuccinimide picolinate starting from nicotinic acid and isonicotinic acid, respectively. 2,2’-Bipyridine-5-carboxylic acid was synthesized following published procedures.[16, 17]

N-hydroxysuccinimide picolinate (4).

Picolinic acid (1 g, 8.12 mmol) was heated under reflux in 2 mL of thionyl chloride and 1 drop of dimethylformamide for 1 h under a nitrogen atmosphere. The thionyl chloride was then evaporated. The product obtained was stirred with 20 mL of dichloromethane and 0.94 g (8.17 mmol) of N-hydroxysuccinimide under nitrogen. The flask was placed in an ice bath and 2 mL (14.34 mmol) of triethylamine were added dropwise. The mixture was stirred overnight at room temperature. The ammonium salts formed were filtered off and the filtrate was evaporated. The crude product was dissolved in 20 mL of dichloromethane and extracted three times with a saturated aq. NaHCO₃ solution. The organic layer was dried over anhydrous Na₂SO₄, filtered and evaporated to yield a white off crystalline solid (1.07 g, 4.86 mmol, 60%).

¹H-NMR (CDCl₃, 400 MHz) δ 8.83 (d, J = 3.2 Hz, 1H), 8.21 (d, J = 8 Hz, 1H), 7.90 (t, J = 6.4 Hz, 1H), 7.59 (t, J = 7.1 Hz, 1H), 2.92 (d, 4H) ppm.

N-hydroxysuccinimide 2,2’-bipyridine-5-carboxylate (7).

2, 2’- Bipyridine-5-carboxylic acid (314 mg, 1.57 mmol) was dissolved in 4 mL of thionyl chloride and a catalytic amount of dimethylformamide under N₂ atmosphere and the mixture was heated under reflux for 4 h. The thionyl chloride was evaporated under reduced pressure and the residue was dried under vacuum for several hours. Subsequently, 15 mL of dry CH₂Cl₂ and 181 mg (1.57 mmol) of
N-hydroxysuccinimide were added and the reaction mixture was cooled in an ice bath. While stirring, 0.4 mL (2.87 mmol) of triethylamine was added dropwise. The mixture was stirred overnight at room temperature under a nitrogen atmosphere. The solvent was evaporated and the product was purified by column chromatography (silica, EtOAc/CH₂Cl₂ 1:1 v/v) to yield the product (286 mg, 61%) as a white solid. m.p. = 180.0-181.8 °C.

$^1$H-NMR (CDCl₃, 300 MHz) δ 9.37 (s, 1H), 8.75 (d, $J = 4.39$ Hz, 1H), 8.64 (d, $J = 8.42$ Hz, 1H), 8.56-8.49 (m, 2H), 7.91 (t, $J = 7.8$, 1H), 7.43 (t, $J = 7.5$, 1H), 2.94 (s, 4H) ppm; $^{13}$C-NMR (101 MHz, CDCl₃) δ 169.2, 161.1, 154.5, 151.3, 149.4, 139.0, 137.7, 125.3, 122.6, 121.4, 121.1, 25.9 ppm (a signal of carbonyl is missing due to low intensity); MS (Cl): 298 (M+1).

N-propyl-2,2′-bipyridine-5-carboxamide (8).

N-hydroxysuccinimide 2,2′-bipyridine-5-carboxylate (7) (18 mg, 0.06 mmol) was dissolved in dry CH₂Cl₂. Two drops of propylamine were added and the mixture was stirred for 1.5 h at room temperature. The solvent was evaporated and the product was purified by column chromatography (silica, EtOAc/CH₂Cl₂ 1:1 v/v) to yield the product as a white solid (11.5 mg, 79%). m.p. = 139.6-140.4 °C.

$^1$H-NMR (CDCl₃, 400 MHz) δ 9.04 (s, 1H), 8.71 (d, $J = 4.03$ Hz, 1H), 8.46 (m, 2H), 8.20 (dd, $J = 8.3$, $J = 2.4$ Hz, 1H), 7.85 (t, $J = 7.9$, 1H), 7.35 (t, $J = 6.2$ Hz, 1H), 6.17 (bs, 1H), 3.48 (q, $J = 7.0$, 2H), 1.68 (m, 2H), 1.02 (t, $J = 7.5$, 3H) ppm; $^{13}$C-NMR (50 MHz, CDCl₃) δ 158.3, 151.0, 147.8, 142.0, 140.3, 129.8, 128.4, 122.6, 117.0, 114.3, 113.4, 15.6, 4.1 ppm.

[Cu(N-propyl-2,2′-bipyridine-5-carboxamide) (NO₃)₂] (9).

N-propyl-2,2′-bipyridine-5-carboxamide (8) (9.7 mg, 0.04 mmol) was added to a Cu(NO₃)₂·3H₂O (16.51 mg, 0.068 mmol) solution in ethanol (1.5 mL). The complex was crystallized by vapour diffusion of ethylacetate. After standing for 3 nights, the resulting blue needles were filtered, washed with ethanol and dried under vacuum at 40 °C. Yield: 10 mg (0.023 mmol, 58%). Anal. Calcd. for C₁₄H₁₅CuN₅O₇: C, 39.21 H, 3.53 N, 16.33. Found: C, 38.99 H, 3.48 N, 16.06.

Synthesis of pyridine-DNA conjugates, representative procedure. 295 µL of a stock solution of amino-modified oligonucleotide (200 µM in H₂O) was mixed with 236 µL of phosphate buffer (200 mM pH 7.2). To this solution, 89 µL of a stock solution of the N-hydroxysuccinimide of the corresponding pyridine derivative (20 mg/mL in dimethylformamide) was added. The mixture was shaken and allowed to stand for 1 h. The coupled product was purified by size exclusion chromatography (SephadexTM G-25 DNA Grade, Triethylammonium acetate 50 mM pH 7). The products were analyzed by reversed phase-HPLC (RP-HPLC) and MALDI-TOF.
RP-HPLC – retention time = 37 min  
MALDI-TOF (m/z) = 5177 (calcd. 5174)

RP-HPLC – retention time = 36 min  
MALDI-TOF (m/z) = 5178 (calcd. 5174)

RP-HPLC – retention time = 39 min  
MALDI-TOF (m/z) = 5174 (calcd. 5174)

RP-HPLC – retention time = 38 min  
MALDI-TOF (m/z) = 5161 (calcd. 5156)

RP-HPLC – retention time = 37 min  
MALDI-TOF (m/z) = 5158 (calcd. 5156)

RP-HPLC – retention time = 37 min  
MALDI-TOF (m/z) = 5160 (calcd. 5156)

**Synthesis of bipyridine-DNA conjugates, representative procedure.** 250 μL of a stock solution of amino-modified oligonucleotide (200 μM in H₂O) was mixed with 200 μL of Phosphate buffer (200 mM pH 7.2) and 50 μL of dimethylformamide. To this solution, 100 μL of a stock solution of N-hydroxysuccinimide 2,2'-bipyridine-5-carboxylate (20 mg/mL in
dimethylformamide) was added in fractions of 25 μL over a period of 4 h with continuous shaking. The mixture was shaken overnight and the coupled product was purified by size exclusion chromatography (Sephadex™ G-25 DNA Grade, Triethylammonium acetate 50 mM pH 7). The products were analyzed by RP-HPLC and MALDI-TOF.

RP-HPLC – retention time = 36 min
MALDI-TOF (m/z) = 5191 (calcd. 5191)

RP-HPLC – retention time = 43 min
MALDI-TOF (m/z) = 5229 (calcd. 5233)

RP-HPLC – retention time = 44 min
MALDI-TOF (m/z) = 5337 (calcd. 5330)

RP-HPLC – retention time = 36 min
MALDI-TOF (m/z) = 5270 (calcd. 5265)

General procedure for the assembly of the catalysts and the catalytic Diels-Alder reactions.

The DNA-based catalysts were assembled by mixing of aqueous solutions containing 0.04 μmol of the oligonucleotides Oligo 1, Oligo 2 and the template oligonucleotide. After
freeze-drying the mixture of oligonucleotides 300 μL of a 0.1 mM solution of Cu(NO₃)₂·3H₂O in MOPS buffer (20 mM pH 6.5) was added and the solution was warmed to 80 °C for 3 min and then cooled down slowly to room temperature. Prior to use the solution was kept at 5 °C for 1.5 h. To the catalyst solution was added 3 μL (0.296 μmol) of a stock solution of 100 mM of aza-chalcone in acetonitrile. The reaction was started by the addition of 0.8 μL (9.8 μmol) of cyclopentadiene. After mixing by continuous inversion for 3 d at 5 °C the mixture was extracted with diethyl ether (3 x 200 μL). The conversion and enantiomeric excess were determined by chiral HPLC. HPLC conditions: Daicel chiralcel-ODH column, heptane/iPrOH 99:1, 0.5 mL/min. Retention times: 14.1, 15.9 (exo isomer), 18.5, 22.9 (endo isomer), 29.78 (aza-chalcone).

Conversions were calculated using the formula:

\[
\text{conv.} (%) = \frac{\text{area } P}{\text{area } S + \text{area } P} \cdot 100\%
\]

Where \(\text{area } P\) is the total peak area of the product of the reaction, \(\text{area } S\) is the peak area of the starting material and \(c\) is a correction factor of 1.21, which was determined from a calibration curve.

2.6. References


