Bio-Inspired catalysis in water
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Chapter 3 Iron-porphyrins in DNA-based asymmetric catalysis: Structure dependent enantioselectivity and dramatic rate enhancement

In this chapter water soluble Iron-porphyrins in combination with double stranded DNA are described as promising catalysts for asymmetric intermolecular cyclopropanations. The influence of the metal applied, the DNA structure and the catalyst structure are investigated. The results demonstrate that DNA-based asymmetric catalysis can be achieved with iron porphyrins and that the porphyrin structure has a major effect on enantio-preference, enantioselectivity and reaction rate.

In collaboration with Ana Rioz-Martinez
3.1. Introduction

Iron-heme (C1) containing enzymes, are well known in nature (figure 1). They catalyze many different reactions essential for life. For applications in organic synthesis, especially monooxygenation reactions are reported for this class of enzymes.\(^1\), \(^2\) Recently, it was shown by Arnold et al. that engineered variants of the C1 containing Cytochrome P450 enzymes can also perform N-H bond insertion\(^3\) reactions (see chapter 5) and diastereo- and enantioselective cyclopropanations\(^4\)-\(^6\). These examples illustrate the enormous potential of engineered metallo-enzymes and hybrid catalysts for catalysis of synthetically relevant reactions. The creation of small synthetic catalysts based on iron-porphyrins is a popular strategy to mimic the catalytic properties of the active site of P450 enzymes.\(^7\)

Water soluble porphyrins, i.e. FeCl(TMe4PyP) (C2), were found to function as good analogues for the study of the catalytic activity of heme containing enzymes.\(^8\)

3.1.1. The interaction of cationic porphyrins with DNA

The first evidence for interactions between charged porphyrins, i.e. meso-tetrakis-(4-N-methylpyridyl) porphyrin (C2b), the metal free analog of C2, and DNA was found in 1979.\(^9\)

From that time on, several spectroscopic techniques were used to determine the exact binding modes of these porphyrins with different DNA structures.\(^10\) Especially the interaction of these porphyrins with G-quadruplexes was studied extensively. Spectroscopic\(^10\),\(^11\), labeling\(^12\) and crystallographic studies\(^13\) revealed a high affinity of charged porphyrins for the planar surface of G-quartets on the top of G-quadruplex structures. Often charged porphyrins show a strong selectivity towards distinct DNA sequences and G-quadruplex structures. This phenomenon was used for therapeutic drugs and the development of detection methods for human telomeric G-quadruplexes\(^10\),\(^14\).

The binding of cationic porphyrins to double stranded DNA is more complex. Several binding modes have been reported, ranging from intercalation between the base pairs of DNA\(^15\),\(^16\), to groove binding\(^17\),\(^18\) to only electro-static interactions with the phosphate backbone.\(^19\) Several factors can influence and change the binding mode of cationic porphyrins with double stranded DNA. The concentrations of porphyrin, DNA base pairs and metal ions, as well as the nature of the metal inside the porphyrin core have a big influence on the binding. While metallated analogs of C2 without an axial ligand do intercalate into the double helix of duplex DNA, C2 analogs with metals that carry an axial ligand are more likely to bind outside the DNA core in the minor groove of duplex DNA.\(^20\)

Similarly, small changes in porphyrin structure can influence the binding of cationic porphyrins to double stranded DNA. Whereas C2b with the pyridyl N-methyl groups on the para position towards the porphyrin core intercalates into calf thymus DNA\(^16\),\(^19\) C3b and C4b with the pyridyl N-methyl groups on meta and ortho position do not clearly intercalate. The sequence of the DNA also plays a crucial role in the binding of porphyrins. It was found that there is a stronger tendency for intercalation between the base pairs of poly(dG-dC) DNA\(^16\) than poly(dA-dT) DNA.\(^19\) To achieve intercalation between the base pairs of DNA, the porphyrin has to adopt a planar conformation, which means that the aromatic substituents on the porphyrin, which prefer to be perpendicular to the porphyrin core, have to rotate. The barrier for rotation is much higher for C3b and C4b than for C2b as a result of
steric hindrance of the methyl groups at the pyridine ring and the porphyrin core. This hampers the intercalative binding of these porphyrins.

![Porphyrin structures](image)

**Figure 1** Porphyrin structures.

### 3.1.2. Catalytic reactions with porphyrins and DNA

The incorporation of heme like structures into a G-quadruplex forming DNA sequence and the consequences of this on the catalytic activity of the heme structure was studied by Travascio *et al.* in the late 1990’s. It was observed that those heme/G-quadruplex assemblies accelerate the decomposition of peroxides by two orders of magnitude. This was attributed to the binding of the G-quadruplex guanines to the heme group. Since porphyrins can interact with DNA, the DNA cleavage activity of porphyrins and metallo-porphyrins has been studied extensively for biomedical applications. However, this topic is beyond the scope of this thesis.

In 1998 Batinic-Haberle *et al.* reported that manganese porphyrins C2c, C3c and C4c are very potent superoxide dismutase (SOD) mimics and that the structure of the porphyrin is related to the activity of the porphyrin/DNA complex. The three porphyrins were studied in terms of electrochemistry, interactions with DNA, *in vitro* and *in vivo* SOD activity. Due to the different orientation of the pyridyl N-methyl group towards the porphyrin core, clearly different electrochemical properties were found, i.e. a much higher redox potential for the ortho isomer C4c compared to the metha and para isomers C2c and C3c. The difference in steric, resonance and inductive properties between the three possible isomers of substituted aromatic porphyrins was studied before and was called the *ortho-effect.* In this case the
ortho effect was proposed to lead to a 16-fold higher SOD activity of C4c compared to C2c and C3c, in vitro. Additionally, binding studies of the manganese porphyrins with double stranded DNA and RNA revealed a clear difference in binding mode of the three porphyrins. Whereas C2c clearly intercalated into the nucleic acid structures, it was found that the binding of C3c and C4c was much less well-defined, which was attributed to the effects already described in paragraph 3.1.1. It was proposed that these differences in binding mode additionally contributed to the much higher in vivo SOD activity of C4c compared to C2c.

Recently, DNA-based asymmetric catalysis with copper-porphyrins has been reported by the group of Hennecke. This report showed that the Lewis acid catalyzed Diels-Alder benchmark reaction of azachalcone with cyclopentadiene is efficiently catalyzed by CuII-(TM4PyP) (C2d). This is surprising, since bidentate coordination of the substrate to copper, which is often required for reactivity[27] is not possible in the porphyrin structure. Mostly, the interactions of C2d with G-quadruplex DNA structures were studied and it was found that the sequence of the G-quadruplex forming DNA has a big influence on the enantioselectivity of the reaction. However, the difference in enantioselectivity between the reactions catalyzed by C2d compared to the enantioselectivity of the reaction catalyzed by Cu(NO3)2 without ligand was small. With double stranded DNA no enantioselectivity was found for C2d as catalyst in the benchmark Diels-Alder reaction.[28]

### 3.1.3. Cyclopropanation reactions catalyzed by iron porphyrins

In organic solvents the development of iron porphyrins as catalysts for intermolecular cyclopropanations was driven by the search for highly diastereoselective catalysts.[29] Iron porphyrins C5, C5b and C5c were found to provide good to excellent trans selectivity (trans/cis ratio up to 21) in the cyclopropanation of styrene (1) with ethyl diazoacetate (2), which was often used as a benchmark reaction (scheme 1a).[30, 31]

**Scheme 1** a) Intermolecular cyclopropanation with iron porphyrins in organic solvents. b) In situ generation of 2 and subsequent cyclopropanation catalyzed by C5c. c) In situ generation of diazomethane and subsequent cyclopropanation in a biphasic reaction mixture.

Recently, the group of Carreira found that these reactions can be carried out in aqueous mixtures and that the highly reactive diazo compounds used for cyclopropanations in organic
solvents can be created in situ by simple and reliable reactions. This development led to a variety of different intermolecular cyclopropanations catalyzed by C5c in biphasic reaction mixtures. For example, ethyl diazoacetate 2 was formed in situ by the addition of sodium nitrite and acetic acid to glycine ethyl ester hydrochloride. Subsequent reaction with styrene catalyzed by C5c provided 3 in good yield with a trans/cis ratio of 10. The same principle was applied to trifluoroethylamine hydrochloride and styrene derivatives in a tandem diazotization/carbene generation/ cyclopropanation sequence, giving rise to trifluoromethyl substituted cyclopropanes in good to excellent yields. With the in situ generated trifluoro diazomethane it was possible to prepare vinyl- and alkynylcyclopropanes with good chemo- and diastereoselectivity using C5c as catalyst for the carbene generation and cyclopropanation step. It was additionally found that by slow addition of nitrosamide 5 to a biphasic reaction medium consisting of the organic substrates and 6M KOH solution, the in situ generation of diazomethane was possible. This is a breakthrough for the synthesis of cyclopropanes, because this procedure avoids the isolation of explosive and toxic diazomethane.

3.1.4. Research goal

In Chapter 2 of this thesis, it was demonstrated that the chiral environment of double stranded DNA can serve as a source of chirality for organometallic reactions in water. Moreover, intermolecular cyclopropanations as a subclass of carbene reactions can be catalyzed by iron-porphyrins in water, as described above. Based on these results, the goal of this study is the asymmetric intermolecular cyclopropanation of styrenes with ethyl diazoacetate catalyzed by a metallo-porphyrin/DNA hybrid catalyst. The most important research questions that will be addressed are; (i) what kind of metallo-porphyrins can catalyze intermolecular cyclopropanations in water (ii) what are the best conditions for metal porphyrin/DNA hybrid catalysts and (iii) what are the effects of porphyrin and DNA structures on the results of catalysis?
3.2. Results and Discussion

3.2.1. Pyridyl N-methyl porphyrines in DNA-based catalysis

Reactions with G-quadruplex and double strand DNA as scaffold

The first experiments were performed with commercially available Fe$^{III}$ meso-tetrakis-(4-N-methylpyridyl) porphyrin (C2) as catalyst due to the catalytic similarities of C2 with P450 enzymes found in literature.[8] The catalytic properties of C2 in combination with different G-quadruplex and double strand forming DNA sequences were investigated in the intermolecular cyclopropanation of 4-methoxy styrene (6) using ethyldiazoacetate (2) as a benchmark reaction (scheme 2). The analysis of the conversion of 6 and 2 after workup is difficult due to the volatility of these compounds. The ee and trans/cis ratio of the cyclopropane products were determined by HPLC analysis. G-quadruplex formation was achieved using a procedure adapted from literature[28] (see experimental section). C2 dissolved in buffer was added to the G-quadruplex containing solutions in order to form the reported catalytic assemblies.

Scheme 2 Intermolecular cyclopropanation catalyzed by an iron porphyrine / DNA assembly

Unfortunately, with all tested sequences that form G-quadruplexes under catalytic conditions no significant transfer of chirality from the G-quadruplex DNA onto the products was obtained. (table 2, entry 1-3). However, it was found that C2 together with duplex forming DNAs lead to 13% ee in case of dTCAGGGCCCTGA and 19% ee in case of salmon testes DNA (st-DNA) (entry 4 and 5). These results showed that intermolecular cyclopropanations in water are feasible using porphyrin derived catalyst in combination with DNA.

Table 1 Results for the intermolecular cyclopropanations catalyzed by C2 in presence of G-Quadruplex and duplex forming DNAs.

<table>
<thead>
<tr>
<th>Entry</th>
<th>DNA sequence</th>
<th>ee$^b$</th>
<th>Trans/cis$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>dGGGTT</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>dGGGTAGGGTTAGGGTTAGG</td>
<td>-4</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>dAGGGAGGGCGCTGGGAGGAGG</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>4$^c$</td>
<td>d(TCAGGGCCCTGA)$_2$</td>
<td>-13</td>
<td>5</td>
</tr>
<tr>
<td>5$^d$</td>
<td>st-DNA</td>
<td>-19</td>
<td>7</td>
</tr>
</tbody>
</table>

$^a$ The experiments were carried out with 5 mM 6, 10 mM 2, 60 µM G-quadruplex DNA and 50 µM C2 in 20 mM MOPS buffer (pH 6.5) with 50 mM KCl, 5% v/v DMSO, 500 µl reaction volume, for 1 h at room temperature, unless otherwise specified, in a single experiment. $^b$ Enantioselectivities and trans/cis ratios are based on areas of HPLC peaks.$^c$ with 1.5 mM in base pairs of duplex-DNA.$^d$ with 2.25 mM in base pairs of st-DNA.
St-DNA as source of chirality

The combination of st-DNA with C2 as a catalyst provided the highest ee and hence, was investigated in more detail. Since commercially available and inexpensive (in comparison with synthetic DNA oligomers) st-DNA was used, it was possible to increase the scale of the reaction and this facilitated analysis. Together with the use of 2-methoxy styrene (8) as substrates for the catalytic reaction instead of 4-methoxy styrene, this enabled a full separation of all peaks on HPLC and a complete analysis of the products including the yield.

For the reactions with st-DNA, water soluble C2 was dissolved in 20 mM MOPS buffer pH 6.5 and added to a solution of 6 mM in base pairs of st-DNA. The reaction between 5 mM 8 and an excess of 2 was mixed by continuous inversion under aerobic conditions for the indicated time (scheme 3).

**Scheme 3** Intermolecular cyclopropanation of 8 in presence of DNA.

The reaction with 75 µM (1.5 mol%) of C2 and 10 mM of 2 gave disappointing 2% yield of 5 after 1 day at 5°C (table 2, entry 1). By increasing the reaction time to 3 days and the concentration of 2 to 50 mM it was possible to increase the yield to 9% (entry 3). A decrease of DNA concentration to 3.0 mM in base pairs increased the yield further to 13% (entry 5). The ee for the trans isomers was found in all cases to be around 20%. From these experiments it was concluded that a 10 fold excess of EDA is needed to obtain reasonable yields of product.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Temp.</th>
<th>Time</th>
<th>[EDA] (mM)</th>
<th>Yield b (%)</th>
<th>ee trans b (%)</th>
<th>ee cis b (%)</th>
<th>Trans/cis b</th>
</tr>
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<tbody>
<tr>
<td>1 c</td>
<td>5°C</td>
<td>1d</td>
<td>10</td>
<td>2</td>
<td>13</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>5°C</td>
<td>1d</td>
<td>50</td>
<td>5</td>
<td>20</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>r.t.</td>
<td>1d</td>
<td>50</td>
<td>7</td>
<td>19</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>5°C</td>
<td>3d</td>
<td>50</td>
<td>9</td>
<td>21</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>5 d</td>
<td>5°C</td>
<td>3d</td>
<td>50</td>
<td>13</td>
<td>22</td>
<td>12</td>
<td>5</td>
</tr>
</tbody>
</table>

a The experiments were carried out with 5 mM 8, 50 mM 2, 6.0 mM in base pairs st-DNA and 75 µM C2 in 20 mM MOPS buffer (pH 6.5), 5% v/v DMSO, unless otherwise specified, in a single experiment, b Yields, enantioselectivities and trans/cis ratios are based on areas of HPLC peaks that are compared to 4-methoxy styrene as external standard, c with 10 mM of 2, d With 3.0 mM in base pairs of st-DNA and 150 µM C2.

From previous research in DNA-based catalysis it is known that small changes in the catalyst structure can lead to significant differences in the catalytic behavior of the DNA based catalysts, i.e. the beneficial effect of methyl substituents on bipyridine-type[37] or dppz-type[36] ligands. Therefore, the position of the pyridyl N-methyl moiety with respect to the porphyrin core was investigated.

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Iron-porphyrins in DNA-based asymmetric catalysis

Fe$^{III}$ meso-tetrakis-(3-N-methylpyridyl) porphyrin (C3) and Fe$^{III}$ meso-tetrakis-(2-N-methylpyridyl) porphyrin (C4) were synthesized by insertion of iron into the commercially available porphyrins using a published procedure.[38] To study the influence of DNA on the catalytic reaction with catalysts C2, C3 and C4, a series of experiments was performed with and without st-DNA. The reactions were stopped after 5 minutes, because for the reactions with C4 instant gas formation was noticed (see below). In case of catalyst C2 almost no conversion was observed after 5 minutes, when st-DNA was present in the reaction mixture (table 3, entry 1). Without DNA the yield was slightly higher (4%), but still almost negligible. Interestingly, catalyst C3 with the pyridyl N-methyl in meta position to the porphyrin core behaved differently. Here, the reaction in presence of DNA gave rise to higher yields than the reaction in absence of DNA (entry 3 and 4). With catalyst C3 the yield of product formation was 17% (10% ee) with DNA and 7% without DNA. These results clearly showed a strong dependence of the rate and selectivity of the cyclopropanation reaction on the structure of the catalyst applied. The DNA had a positive influence on the rate of product formation, but the ee was lower than obtained with C2 as catalyst.

**Table 3** Results of the intermolecular cyclopropanation catalyzed by pyridyl N-methyl porphyrin/st-DNA hybrid catalysts.$^a$

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>[Catalyst] (µM)</th>
<th>DNA (mM)</th>
<th>Yield (%)</th>
<th>TTN</th>
<th>ee trans (%)</th>
<th>ee cis (%)</th>
<th>Trans/cis $^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C2</td>
<td>75</td>
<td>6</td>
<td>&lt;1</td>
<td>0</td>
<td>+19</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>2</td>
<td>C2</td>
<td>75</td>
<td>-</td>
<td>4</td>
<td>3</td>
<td>+1</td>
<td>+10</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>C3</td>
<td>75</td>
<td>6</td>
<td>17</td>
<td>11</td>
<td>+10</td>
<td>n.d.</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>C3</td>
<td>75</td>
<td>-</td>
<td>7</td>
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<td>C4</td>
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<td>28</td>
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<td>-27</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>C4</td>
<td>75</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>+1</td>
<td>n.d.</td>
<td>11</td>
</tr>
<tr>
<td>7$^g$</td>
<td>C4</td>
<td>75</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>-18</td>
<td>-9</td>
<td>5</td>
</tr>
<tr>
<td>8$^h$</td>
<td>C4</td>
<td>75</td>
<td>6</td>
<td>25</td>
<td>17</td>
<td>-36</td>
<td>-26</td>
<td>12</td>
</tr>
<tr>
<td>9$^i$</td>
<td>C4</td>
<td>75</td>
<td>6</td>
<td>43</td>
<td>29</td>
<td>-40</td>
<td>-27</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>C4</td>
<td>37.5</td>
<td>6</td>
<td>35</td>
<td>47</td>
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<td>3</td>
<td>34</td>
<td>45</td>
<td>-38</td>
<td>-24</td>
<td>11</td>
</tr>
<tr>
<td>12</td>
<td>C4</td>
<td>7.5</td>
<td>0.6</td>
<td>6</td>
<td>1</td>
<td>-21</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>13</td>
<td>C4</td>
<td>7.5</td>
<td>0.6</td>
<td>3</td>
<td>18</td>
<td>-26</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

$^a$ The experiments were carried out with 5 mM 8, 50 mM 2, the indicated concentration catalyst and st-DNA in 20 mM MOPS buffer (pH 6.5), 5% v/v DMSO, for 5 minutes at 5°C, unless otherwise specified, in a single experiments. $^b$ In DNA base pairs, $^c$ Yields, enantioselectivities and trans/cis ratios are based on areas of HPLC peaks that are compared to 4-methoxy styrene as external standard.$^5$ TTN (total turnover number) = [8]$^*$ Yield/100/[catalyst]$^{[4]}$, $^d$ + and – referring to the elution order on HPLC, $^f$ n.d. = not determined, $^g$ with 37.5 mM Na$_2$S$_2$O$_4$, $^h$ at room temperature, $^i$ adding 10 times equivalent every 30 sec., $^j$ equivalent of 2.

When C4 was used in combination with st-DNA in the cyclopropanation reaction an instantaneous formation of gas bubbles was observed upon addition of 2 to the reaction mixture. This was most likely due to the formation of N$_2$, indicating a fast decomposition of 2. After only 5 minutes with 75 µM (1.5 mol%) C4, at 5°C, 2 was consumed completely and 28% yield of product 9 was obtained (entry 5). This was a significant increase in yield, but still most of 2 was converted into side products. These side products were expected to be the products of the O-H bond insertion of 2 into water that could not be detected by HPLC analysis and products of the dimerization of 2. Strikingly, with C4 the opposite enantiomer of
the trans and cis isomers compared to the reaction with C2 was formed, in 43 and 27% ee, respectively. The trans/cis ratio was 13 and much higher than the ratios observed with C2.

In order to increase the yield of 5, the conditions for the reaction with C4 and st-DNA were optimized. Performing the reaction at room temperature did not result in a significant change in the yield (entry 8). When 2 was not added at once at the start of the reaction, but in 10 portions of 1 equivalent every 30 seconds the yield increased to 43% (entry 9). Also lowering the catalyst concentration to 37.5 µM (0.75 mol%) led to an increase in yield to 35% (entry 10). The calculated Total Turnover Number (TTN) under these conditions was 47, which is very high for a DNA based catalyst, when compared to all other reactions that have been published so far. At 7.5 µM catalyst loading the yield dropped significantly.

3.2.2. Water insoluble metallo-porphyrins as catalyst

The interaction of metallo-porphyrins with oligonucleotides enables the use of usually highly water insoluble molecules, i.e. metallo-porphyrins and metallo-phthalocyanides (figure 2), as catalyst in aqueous environment. To prove if the activity of FeIII meso-tetrakis-phenyl porphyrin C5d, that is a known catalyst for the intermolecular cyclopropanation in organic solvents[29] (see above), is retained under DNA-based catalysis conditions in water, the metal-porphyrin was dissolved in DMSO and added slowly to an aqueous st-DNA containing solution. It proved possible to form a homogeneous reaction mixture of the catalysts through the interactions of the normally insoluble catalysts due to st-DNA. HPLC analysis showed 8% yield and 5% e.e. of the trans isomer of the cyclopropanation product 9, when C5d was applied as the catalyst (table 4, entry 1). Interestingly, the trans/cis ratio of 16 was quite high.

![Figure 2 Water insoluble metallo-porphyrins and metallo-phthalocyanine used in this study.](image)

**Table 4** Results of intermolecular cyclopropanation of 4 catalyzed by water insoluble porphyrins and phthalocyanide in the presence of st-DNA.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Yield (%)</th>
<th>ee (%)</th>
<th>Trans/cis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C5d</td>
<td>8</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>C6</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>C7</td>
<td>68</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>C8</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>C9</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>FeCl3</td>
<td>&lt;3</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

The experiments were carried out with 5 mM 8, 50 mM 2, 6.0 mM in base pairs st-DNA and 75 µM catalyst in 20 mM MOPS buffer (pH 6.5), 10% v/v DMSO, for 3 days at 5°C, unless otherwise specified, in a single experiments. b Yields, enantioselectivities and trans/cis ratios are based on areas of HPLC peaks that are compared to 4-methoxy styrene as external standard. c n.d. = not determined.
Other commercially available metallo-porphyrins and metallo-phthalocyanine that are similar to reported catalysts for cyclopropanation reactions\textsuperscript{39-41} were investigated under the same reaction conditions. The choice of metal and the structure of the ligand were found to be important for achieving high catalytic activities. Whereas Zn\textsuperscript{II} meso-tetrakis-phenyl porphyrin (C6), Co\textsuperscript{II} meso-tetrakis-(4-pyridyl) porphyrin (C8), Cu\textsuperscript{II} phthalocyanide (C9) and FeCl\textsubscript{3} alone gave rise to no or negligible formation of cyclopropanation products, with the Ru\textsuperscript{II} meso-tetrakis-phenyl porphyrin (C7) 68\% yield of 9 was obtained. Unfortunately, no transfer of chirality from the DNA to the products was achieved in the reaction with C7 (table 4, entry 3).

3.2.3. The influence of the porphyrin structure

The quantitative consumption of 50 mM 2 by 75 µM Fe\textsuperscript{III} meso-tetrakis-(2-N-methylpyridyl) porphyrin (C4) in only 5 minutes corresponds to a turnover frequency (TOF) of >2 \textit{s}\textsuperscript{-1}, which makes this the fastest DNA-based catalytic reaction to date. Unfortunately, also in case of C4 the ratio between the side reactions and the formation of 9 favors for the side reactions, similar to the case of C2 and C3. The TOF of product formation is ~5 \cdot 10\textsuperscript{-2} \textit{s}\textsuperscript{-1} (table 3, entry 5). For the reaction without st-DNA only 1\% yield was found after 5 minutes (table 3, entry 6). This indicates an enormous rate acceleration for the consumption of 2 and the formation of 9 in presence of st-DNA.

Compared to C2 the iron porphyrin catalyst with the pyridyl N-methyl moiety in ortho position of the porphyrin core C4 most likely binds in different position inside the double helix structure of st-DNA. Similarly, this was reported for metal free analogues of C2 and C4 in literature, as described in the introduction. There it was found that C2b intercalates into the DNA double helix and C4b does not intercalate, but most likely has some interactions in the groove of the double helix. Here, this not only leads to the formation of the other enantiomer in the catalytic cyclopropanation reaction, but also gives rise to an enormous acceleration of the rate of the consumption of 2 and formation of 9. The acceleration of the reaction with C4 could be explained by the combination of steric, resonance and inductive effects of the pyridyl N-methyl in ortho position, the so called “ortho effect” (see 3.1.2). However, an increase in yield was not observed for C4 in absence of st-DNA. This means that the DNA plays a crucial role in the acceleration of the catalytic cyclopropanation reaction with C4. A much higher catalytic activity of the ortho-isomer compared to the para-isomer was observed before for in vivo SOD studies with the manganes analogs of C2 and C4 as described in paragraph 3.1.2.\textsuperscript{29} In that case the acceleration in catalysis was attributed to two major effects, i.e. the change in redox potential as a result of the positive charge being closer to the porphyrin core and the hindered intercalation into duplex DNA for the catalyst with pyridyl N-methyl at the ortho position (For a more detailed discussion see chapter 7).

3.2.4. The oxidation state of iron during the reaction

For all reactions shown, the oxidation state of the applied iron catalyst was Fe\textsuperscript{III}. For catalyst C5 it was found by Woo \textit{et al}.\textsuperscript{30} that the reduction of Fe\textsuperscript{III} to Fe\textsuperscript{II} by EDA can take place at elevated temperatures >40°C under anaerobic conditions in organic solvents. Without reduction to Fe\textsuperscript{II} Woo \textit{et al}. did not observe any formation of cyclopropanation product and even at higher temperatures in ambient atmosphere the reaction proceeded very sluggishly. Arnold \textit{et al}. reported similar findings in water.\textsuperscript{4} Their iron heme containing Cytochrome P450 enzyme needed high amounts of reducing agent (Na\textsubscript{2}S\textsubscript{2}O\textsubscript{4}) and anaerobic conditions.
to achieve the best catalysis results. The catalyst showed 50% inhibition by air suggesting that dioxygen and EDA competed for the reduced Fe$^{ll}$.

In contrast, the reaction with catalyst C4 in presence of DNA proceeded very fast even in water, open to air and at 5°C. To elucidate if indeed Fe$^{ll}$ is the active catalyst in the DNA-based asymmetric catalysis reactions, 37.5 mM Na$_2$S$_2$O$_4$ (500 equivalents to C4) was added to the reaction mixture. A small color change from greenish to brownish was noticed when the reducing agent was added, which could indicate a reduction of the catalyst. The color change was not noticed during the catalytic reactions without Na$_2$S$_2$O$_4$. Surprisingly, the yield of the cyclopropanation product from the reaction with Na$_2$S$_2$O$_4$ was only 2% and also the ee's dropped significantly (table 4, entry 7). This indicates that Fe$^{ll}$ most likely is not the active form of the catalyst during the DNA-based asymmetric catalysis reactions.

3.3. Conclusion

The results presented in this chapter showed that asymmetric iron catalyzed intermolecular cyclopropanation reactions are feasible in water using the DNA-based asymmetric catalysis approach. Water soluble porphyrins formed hybrid catalysts with st-DNA that provide enantioenriched products of the intermolecular cyclopropanations between 6 or 8 and 2. Depending on the relative position of the pyridyl N-methyl group with respect to the porphyrin, both enantiomers of the products could be obtained in excess. Additionally it was found, that the reaction rate of the cyclopropanation reactions is also dramatically influenced by the structure of the catalyst and the presence of DNA. For iron-porphyrin C2 the presence of st-DNA gave rise to a decrease in yield, whereas with C4 a dramatic increase in yield was observed when st-DNA was added. The turn over frequency (TOF) of the conversion of ethyldiazoacetate 2 by the iron-porphyrin C4 in presence of st-DNA was found to be >2·s$^{-1}$. This represents the fastest DNA based catalytic reaction reported so far.

3.4. Experimental Section

3.4.1. General remarks

High resolution mass spectra (HRMS) were recorded on an Orbitrap XL (Thermo Fisher Scientific; ESI pos. mode). Heptafluorobutyric acid was added for mass analysis of metalloporphyrins. This enabled the detection of the porphyrin ion -2H$^+$. Enantiomeric excess determinations were performed by HPLC analysis (Chiracel-ADH or Chiralpak-ASH) using UV-detection (Shimadzu SCL-10Avp). Flash chromatography was performed using silica gel 60 Å (Merck, 200-400 mesh) or a Grace Reveleris$^\text{®}$ Flash System (40 µm silica column). Catalyst C5-C7 and C9 were purchased from Sigma-Aldrich. Co(TPyP) C8 was kindly provided by the research group of Prof. B. de Bruin (University of Amsterdam). FeCl(TM$\text{e}_4$PyP) C2 was purchased from Frontier Scientific. All catalysts were used without further purifications. Catalysts C3 and C4$^{[38]}$ and the reference products 7 and 9$^{[43]}$ were synthesized following published procedures. The purity (water content) of all hydroscopic catalysts C2, C3 and C4 was analyzed by comparing the UV/VIS absorption of the porphyrin in 0.01M HCl solution with the expected adsorptions calculated from the published$^{[38]}$ molar absorptivity at a given wavelength.
3.4.2. Representative procedures for the catalytic reactions

Catalytic reactions of C2 in combination with G-quadruplex forming DNA sequences.

To obtain a reaction mixture containing the indicated concentrations, 250 µl of a 0.5 mM solution of oligonucleotide in MilliQ water was added to 100 µl of 100 mM MOPS buffer pH 6.5 containing 250 mM KCl. This solution was warmed to 95°C for 5 min and allowed to cool to room temperature over 2 hours. Then 125 µl water and 15 µl of a 1.6 mM solution of C2 in water were added subsequently. The reaction mixture was rotated for 15 min and the reaction was started by first adding 6 dissolved in 5 µl of DMSO and then 2 dissolved in 20 µl of DMSO. After rotating the reaction mixture for 1 hour at room temperature, the products were isolated by extraction with diethyl ether (3 x 0.5 ml). After drying (Na₂SO₄) and evaporation of the solvent the crude product was analyzed by HPLC (details see below).

Catalytic reactions of water soluble catalysts with st-DNA

Salmon testes DNA (6.0 mg/ml) was dissolved in a 20 mM MOPS buffer pH 6.5 (9.0 mM in base pairs) 2 days before use. To reach a final concentration of 5 mM of substrate 8, 50 mM of 2, 75 µM of catalyst and 6 mM of DNA base pairs; 500 µl of a 1.13 mM solution of the iron porphyrin was added to 1.75 ml of buffer in a 15 ml plastic tube. Then, 5 ml of st-DNA solution was slowly added and the solution was mixed by continuous inversion at 5°C. After incubation for 30 min, 50 µl of a 750 mM solution of 8 in DMSO and 200 µl of a 1.88 mM solution of 2 in DMSO were added to start the catalytic reaction. After the indicated time, the products were extracted with diethyl ether (3 x 7.5 mL). After drying (Na₂SO₄) and evaporation of the solvent, the crude product was analyzed by HPLC, using 4-methoxy styrene or 2-methoxy styrene as external standard.

Catalytic reactions of water insoluble catalysts with st-DNA

Salmon testes DNA (6.0 mg/ml) was dissolved in a 20 mM solution of MOPS buffer pH 6.5 (9.0 mM in base pairs) 2 days before use. To reach a final concentration of 5 mM of substrate 8, 50 mM of 2, 75 µM of catalyst and 6 mM of DNA base pairs; 5 ml of st-DNA solution was added to 1.75 ml of buffer in a 15 ml plastic tube. Then, 500 µl of a 1.13 mM solution of the catalysts dissolved in DMSO was slowly added and the solution was mixed by continuous inversion at 5°C. After incubation for 30 min, 50 µl of a 750 mM solution of 8 in DMSO and 200 µl of a 1.88 M solution of 2 in DMSO were added to start the catalytic reaction. After 3 days, the products were extracted with diethyl ether (3 x 7.5 mL). After drying (Na₂SO₄) and evaporation of the solvent, the crude product was analyzed by HPLC, using 4-methoxy styrene as external standard.

3.4.3. HPLC analysis

To determine the yield of the catalytic reaction, pure reference compound was analyzed together with an external standard. The observed peak areas for products (P) and external standard (S) $\frac{area_P}{area_S}$ were plotted against the concentrations of both compounds in the analytic sample $\frac{[P]}{[S]}$. The slope of the linear fit gave the correction factor c.
The concentration of products in the catalysis reaction samples was calculated using the following formula:

\[
[P] = \frac{\text{area } P}{\text{area } S \cdot c} \times [S]
\]

Where \([P]\) is the concentration of product in the catalysis reaction sample, \(\text{area } P\) is the addition of all products peak areas, \(\text{area } S\) is the peak area of the external standard, \(c\) is the correction factor and \([S]\) is the concentration of the standard solution.

The yield of the reaction was calculated as follows:

\[
\text{Yield (\%)} = \frac{[P]}{[P]_{\text{max}}} \times 100
\]

Where \([P]\) is the concentration of product in the catalysis reaction samples and \([P]_{\text{max}}\) is the maximal theoretical concentration of product in the catalysis reaction samples depending on the amount of substrate added to the catalytic reaction.

### 3.4.4. Reference compounds and catalysts

**Reference compounds**

**Ethyl 2-(4-methoxyphenyl)cyclopropanecarboxylate 7**[^43]

![Ethyl 2-(4-methoxyphenyl)cyclopropanecarboxylate](image)

Enantiomeric excess was determined by HPLC analysis (Chiralcel-OBH, n-heptane/iPrOH 95:05, 0.5 ml/min. Retention times \(\text{trans}\) isomers: 16.6 and 17.6 min. Retention times \(\text{cis}\) isomers: 19.0 and 32.5 min.)

**Ethyl 2-(2-methoxyphenyl)cyclopropanecarboxylate 9**[^43]

![Ethyl 2-(2-methoxyphenyl)cyclopropanecarboxylate](image)

Enantiomeric excess was determined by HPLC analysis (Chiralcel-ODH, n-heptane/iPrOH 98:02, 0.5 ml/min. Retention times \(\text{trans}\) isomers: 17.8 and 35.9 min. Retention times \(\text{cis}\) isomers: 13.3 and 14.0 min.)

**Catalysts**

**Fe\(^{III}\)-meso-tetrakis-4-N-methylpyridylporphyrin C2**

( Frontier Scientific)

![Fe\(^{III}\)-meso-tetrakis-4-N-methylpyridylporphyrin C2](image)

Elemental analysis (calcd %) for C\(_{44}\)H\(_{38}\)Cl\(_2\)FeN\(_8\)·11H\(_2\)O:

C, 47.69; H, 5.28; N, 10.11; found: C, 47.59; H, 4.62; N, 10.12.
Iron(III)-meso-tetrakis-3-N-methylpyridylporphyrin C3[38]

Elemental analysis (calcd %) for C$_{44}$H$_{36}$Cl$_{5}$FeN$_{8}$$\cdot$11H$_{2}$O:
C, 47.69; H, 5.28; N, 10.11; found: C, 47.63; H, 4.55; N, 10.82.

HRMS calcd for C$_{48}$H$_{34}$F$_{7}$FeN$_{8}$O$_{2}$$^{2+}$ [M$^{5+}$+HFBA$^{-}$2H$^{+}$]/2: 471.602, found 471.604 HRMS calcd for C$_{44}$H$_{34}$FeN$_{8}$$^{3+}$ [M$^{5+}$-2H$^{+}$]/3: 243.408, found 243.410

Fe$^{III}$-meso-tetrakis-2-N-methylpyridylporphyrin C4[38]

Elemental analysis (calcd %) for C$_{44}$H$_{36}$Cl$_{5}$FeN$_{8}$$\cdot$12H$_{2}$O:
C, 46.96; H, 5.37; N, 9.95; found: C, 46.46; H, 4.80; N, 10.61.

HRMS calcd for C$_{48}$H$_{34}$F$_{7}$FeN$_{8}$O$_{2}$$^{2+}$ [M$^{5+}$+HFBA$^{-}$2H$^{+}$]/2: 471.602, found 471.605

3.5. References
