Asymmetric Catalysis

DNA-Based Asymmetric Catalysis**

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The ubiquitous right-handed double helix of DNA is arguably the most elegant example of chirality in nature, yet chirality in biocatalysis is almost exclusively the domain of the enzymes encoded by DNA.[1] The direct transfer of chiral information from DNA to chemical reactions will require the use of DNA-based catalysts. In marked contrast to catalytic RNAs, which have been employed successfully in a wide range of reactions including enantioselective catalysis,[2,3] the synthetic repertoire of "DNAzymes" is still very modest.[4] The limited applicability of catalytic DNA has occasionally been attributed to the absence of the 2′-OH functional group in the sugar-phosphate backbone and the propensity for natural DNA to adopt a double-helical duplex structure, which precludes the formation of catalytically competent tertiary structures.[4] Although the catalytic power of DNA has been enhanced by the incorporation of nucleotide bases with extended functionality,[5] enantioselective catalysis based on DNA has yet to be reported. However, the reported chirality transfer from DNA in stoichiometric DNA-templated synthesis, which leads to diastereoselectivity in chemical reactions and enantioselection of chiral substrates,[6] suggests the potential of DNAzymes in asymmetric catalysis.

Herein, we demonstrate that the chirality of the DNA double helix can be transferred directly to a metal-catalyzed reaction, in the present case the copper(II)-catalyzed Diels–Alder reaction (Figure 1). This can be done by positioning a nonchiral or racemic catalyst in intimate contact with DNA and using the chiral information of the DNA double helix to generate reaction products with an excess in one of their enantiomers. These artificial DNAzymes can be generated through the propensity of small aromatic molecules to intercalate in a noncovalent, yet kinetically stable and stereoselective fashion, which enables the anchoring of metal complexes to DNA.[7] This noncovalent and modular approach allows rapid structural variation and optimization of the catalytic system.

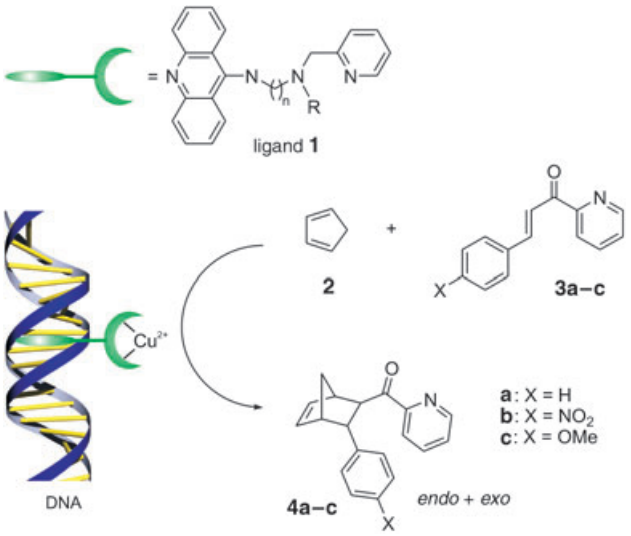

Figure 1. Schematic representation of the asymmetric Diels–Alder reaction of cyclopentadiene (**2**) with aza-chalcone **3**, catalyzed by copper complexes of ligand **1** in the presence of DNA.

The catalyst is a complex formed in situ from copper(II) with ligand **1**, which contains three key structural features: a DNA-intercalating moiety such as 9-aminoacridine, a spacer component, and a metal-binding group. The ligands were prepared in an efficient and straightforward fashion starting from monoprotected diamines (Supporting Information).[8] The copper(II) complex has a characteristic green color, with a weak UV/Vis spectroscopic absorbance at $\lambda_{max} = 620$ nm ($\varepsilon = 50\,M^{-1}cm^{-1}$) in the case of ligand **1a** (Supporting Information). This absorption, which is typical for copper di- and polyamine complexes,[9] is slightly red-shifted in the presence of DNA ($\lambda_{max} = 660$ nm). The addition of extra copper salt did not give a significant increase in this absorption. The combination of DNA with either $Cu(NO_3)_2$ or the free ligand does not have discernable features in this wavelength region, which demonstrates that DNA does not sequester the copper(II) ion from the ligand. Although complexation of these achiral ligands to copper generates chiral complexes, they are formed as a racemic mixture; thus any enantiomeric excess found in the product of the catalyzed reaction originates from DNA.[10]

The Diels–Alder reaction between cyclopentadiene (**2**) and the aza chalcone **3** in water[11] was catalyzed by copper(II) complexes of ligand **1** in the presence of salmon testes or calf thymus DNA, both of which are readily available and inexpensive. The reaction was allowed to proceed until $>80\,\%$ conversion. Product **4** was obtained as a mixture of the *endo* (major) and *exo* (minor) isomers, both with a significant enantiomeric excess, depending on the ligand used (Table 1). A series of control experiments established that the combination of ligand, copper salt, and DNA is required to obtain both efficient catalysis and enantioselectivity (Supporting Information).

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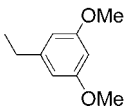
Table 1: Results of the catalytic Diels–Alder reaction with 1-naphthylmethyl- and 3,5-dimethoxybenzyl-substituted ligand **1**.[a]

Entry	Ligand	Ligand **1** R	*n*	Dienophile	Diels–Alder Product **4** *endo*/*exo*	*endo* [% *ee*]	*exo* [% *ee*]
1	**1a**		3	**3a**	98:2	49	18
2[b]	**1a**		3	**3a**	97:3	49	23
3[c]	**1a**		3	**3a**	98:2	47	23
4	**1a**		3	**3b**	96:4	37	16
5	**1a**		3	**3c**	98:2	48	24
6	**1b**		4	**3a**	98:2	33	19
7	**1c**		5	**3a**	97:3	<5	<5
8	**1d**		2	**3a**	96:4	−48	−37
9	**1e**		3	**3a**	98:2	−37	−7
10	**1f**		2	**3a**	92:8	−37	−78
11[b]	**1f**		2	**3a**	92:8	−34	−74
12[c]	**1f**		2	**3a**	92:8	−35	−82
13[d]	**1f**		2	**3a**	82:18	−34	−80
14	**1f**		2	**3b**	88:12	−47	−78
15	**1f**		2	**3c**	91:9	−53	−90

[a] All experiments were carried out with salmon testes DNA under the standard conditions (see Experimental Section) unless noted otherwise. [b] Conditions: catalyst (0.18 mM), dienophile (4 mM), cyclopentadiene (34 mM). [c] Calf thymus DNA. [d] DNA = synthetic duplex $d(GACT)_2$-$(AGTC)_2$ (0.39 mM), cyclopentadiene (21 mM), buffer contained NaCl (75 mM).

The substituent R and the spacer length *n* of the ligand are crucial for both the observed enantioselectivity and the enantiopreference (that is, which enantiomer is formed in excess). A screen of ligands with a fixed spacer length ($n = 3$) revealed the importance of the R group and specifically, the requirement for it to contain an aromatic (arylmethyl) group (Supporting Information). This suggests the involvement of π–π interactions between the substituent and the dienophile, as was previously described in the case of catalysts based on amino acids.[11] The best results in the series examined were obtained for ligands with R = 1-naphthylmethyl (**1a**), for which an *endo*/*exo* ratio of 98:2 and 49% *ee* for the *endo* isomer were found (Table 1, entry 1). Comparison of these results with those from ligand with R = 2-naphthylmethyl, which did not produce any significant enantiomeric excess, demonstrates the subtlety of the interaction of the substituent of the ligand with the dienophile.

Elongation of the spacer in **1a** resulted in a rapid decrease of the enantioselectivity; for $n = 5$ (**1c**) no significant enantiomeric excess was observed (Table 1, entry 7). In contrast, a decrease in spacer length to $n = 2$ (**1d**) gave a value similar to **1a** (48% *ee*), but surprisingly of the opposite enantiomer (entry 8). These findings demonstrate that intimate contact between the DNA double helix and the catalyst is required for efficient chirality transfer. They therefore offer compelling evidence for DNA as the source of chirality in these reactions.

The special case of R = 3,5-dimethoxybenzyl gave the same enantiomer of the product in excess regardless of spacer length ($n = 2$ or 3). In the case of ligand **1f** ($n = 2$) relatively more of the *exo* isomer was formed (*endo*/*exo* = 92:8), with 37% *ee* for the *endo* and 78% *ee* for the *exo* isomer (Table 1, entry 10).

The difference in behavior of the catalyst based on ligands **1a** and **1f** is also evident from the reactions with substrates **3b** and **3c**, which contain a nitro and a methoxy group, respectively, on position 4 of the phenyl ring. Although the conversions observed with these substrates were generally lower (≈50%) than those obtained with **3a** (a possible result of their lower solubility), similar results with **3c** and slightly lower *ee* values with **3b** were obtained with ligand **1a** (Table 1, entries 4 and 5). In contrast, the complex with ligand **1f** gave a much improved enantiomeric excess for both substrates; in the case of the methoxy-substituted substrate **3c**, up to 53 and 90% *ee* were observed for the *endo* and *exo* isomers, respectively (entry 15). These values represent the highest *ee* values obtained thus far with this system. These results provide strong evidence that the interaction of the substituent R with the dienophile is important for the stereochemical outcome of the reaction. However, the exact nature of this interaction and in particular, the differences between R = 1-naphthylmethyl and R = 3,5-dimethoxybenzyl are the subject of further study.

Neither the substrate/catalyst ratio, which could be increased to 4 mM:0.18 mM dienophile/catalyst (that is, catalyst at 4.5 mol% with respect to substrate, giving up to 22 turnover events; Table 1, entries 2 and 11) nor the source of the DNA used (salmon testes versus calf thymus DNA; entries 3 and 12) had a significant effect on the results. A small synthetic dsDNA (the self-complementary 16-mer $d(GACT)_2(AGTC)_2$) also gave a similar enantioselectivity for **1f** (entry 13), which rules out any possible residual impurity in the DNA from natural sources as influencing the catalytic reaction. Interestingly, in this case the *exo* product was favored even more.

The results presented herein demonstrate that the chirality of DNA can be transferred directly to a catalytic reaction. Despite the invariance of the chirality of the DNA employed, both enantiomers of the Diels–Alder product are accessible by a judicious choice of ligand. The key strengths of the present system are its modular nature, which together with the noncovalent binding of the catalytic moiety to DNA and the use of achiral ligands, allows rapid structural variation and optimization of catalysts for new reactions. An additional advantage of the present approach is the isolation of the product from the reaction mixture. The use of a DNA intercalator in the catalytic system creates a very tightly bound Cu–ligand–DNA complex, which remains in the aqueous phase during extraction of the products. The possibility to address specific DNA sequences in both natural and synthetic DNA, for example, by using a selective DNA binding moiety tethered to the catalyst, is particularly appealing for the future design of DNA-based catalysts.

Experimental Section

Catalytic Diels–Alder reactions: DNA-bound catalyst in buffered solution (salmon testes DNA (1.3 mg mL^{-1}), catalyst (0.3 mM, ligand/Cu^{2+} = 1.3), and MOPS (20 mM, pH 6.5))[12] was prepared by mixing salmon testes DNA (2 mg mL^{-1}) in solution with MOPS (30 mM) in a

volume of 10 mL (prepared 24 h in advance) with a solution of the preformed catalyst: $Cu(NO_3)_2$ (0.9 mM) and ligand **1a** (1.17 mM) in a volume of 5 mL. An aliquot of a stock solution of dienophile **3a** in CH_3CN (0.5 M, 30 μL) was added to a final concentration of 1 mM and the mixture was cooled to 5 °C. The reaction was started with the addition of cyclopentadiene (5 mM, 7 μL) and mixed by continuous inversion for 3 days, followed by extraction of the product with diethyl ether. After ^1H NMR spectroscopic analysis the percent *ee* value was determined by chiral HPLC (Daicel chiralcel-ODH column, elution with heptane/*i*PrOH 98:2). Selected products were purified by column chromatography and analyzed on an Daicel chiralpak-AD column to confirm the results obtained from analysis of the crude product.

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