Modular assembly of novel DNA-based catalysts†

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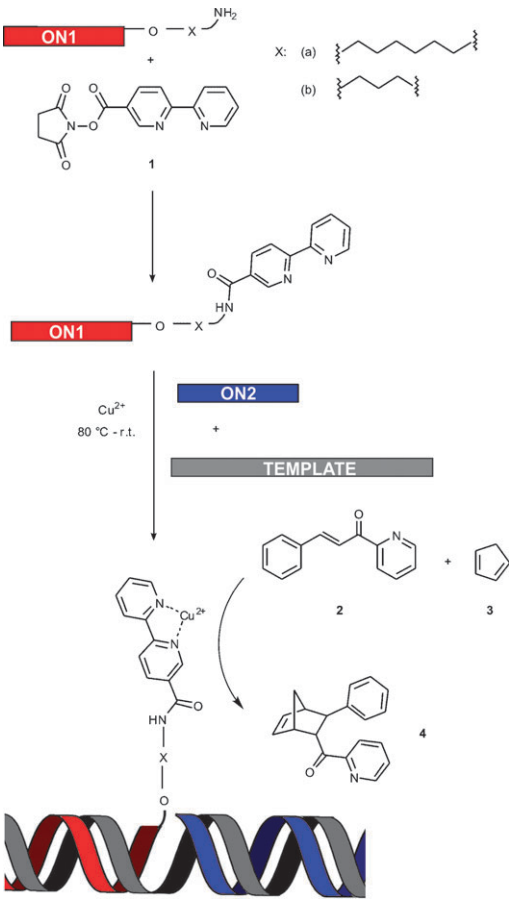
A novel modular strategy towards the assembly of DNA-based catalysts containing a covalently anchored metal complex is presented.

Hybrid catalysis has emerged as a powerful catalytic concept.[1] In hybrid catalysis, a catalytically active transition metal complex is anchored to a biopolymer scaffold, *e.g.* a protein or a polynucleotide, and the second coordination sphere that is provided by the biopolymer imparts (enantio-)selectivity and, in some cases, increased efficiency to the reaction. Three general anchoring strategies can be distinguished: supramolecular, dative and covalent anchoring. The supramolecular and dative anchoring strategies have proven to be very successful; a wide variety of highly enantioselective reactions have been reported using this approach.[2] In contrast, the enantioselectivities that have been reported to date when using the covalent anchoring strategy have been generally more modest.[3] Arguably, one of the main reasons for this is that the optimization of these hybrid catalysts is much more laborious and time-consuming. Yet, a covalent anchoring strategy potentially has many advantages since it allows for precise positioning of the metal complex and, hence, control over the structure and geometry of the catalytic site. Here we present a novel modular strategy towards DNA-based catalysts containing a covalently anchored metal complex that allows for rapid optimization.

We have introduced the concept of DNA-based asymmetric catalysis,[4] which involves the supramolecular anchoring of a metal complex to a DNA host. Using this concept good to excellent enantioselectivities have been achieved in the Cu^{2+} catalyzed Diels–Alder, Michael addition[5] and fluorination reactions.[6] Recently, several approaches towards covalent anchoring of catalytically active metal complexes to internal positions in the DNA were reported, which involve the synthesis of modified nucleotides or nucleotide analogues and their incorporation into synthetic oligonucleotides, sometimes followed by post-synthetic derivatization.[7] However, to date only low enantioselectivities have been reported for their catalyzed reactions.

Our approach to the modular assembly of a DNA-based catalyst, which allows for rapid optimization of the catalytic site, is outlined in Scheme 1.



Scheme 1 General coupling procedure between amino-functionalized DNA and bipyridine derivatives. Schematic representation of the assembly of the DNA-based catalyst and general reaction scheme.

This approach involves three oligonucleotide components: an oligonucleotide that is functionalized with a ligand, *i.e.* 2,2′-bipyridine (bipy) at the 5′ or 3′ terminal phosphate moieties (ON1), an unfunctionalized oligonucleotide (ON2) and a template oligonucleotide strand with a sequence that is complementary to both ON1 and ON2. 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 ON1 and ON2. The present approach has several advantages: terminally modified oligonucleotides are prepared routinely *via* optimized solid-phase synthesis, are commercially available and covalent attachment of a ligand is easily achieved using well-established chemistry.

Furthermore, the second coordination sphere around the metal centre is optimized readily by exchange of the

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unfunctionalized modules, *i.e.* ON2 and the template, which obviates the need for synthesis of new oligonucleotide–ligand conjugates.

The bipy–oligonucleotide conjugates (ON1) were prepared by reaction of 5′ or 3′ amino-modified oligonucleotides with the *N*-hydroxysuccinimide activated ester of bipyridine (**1**, Scheme 1). The resulting conjugates were purified by size exclusion chromatography and analyzed by rp-HPLC and MALDI-TOF. Yields were typically around 80%. The DNA-based catalyst was assembled by thermal hybridization of the bipy–oligonucleotide conjugate (ON1) and ON2 with the complementary DNA template in the presence of copper(II) (Scheme 1). The resulting duplex was studied by CD spectroscopy, which confirmed that the typical B-DNA structure is conserved despite the modification.

The catalytic properties of the novel DNA-based catalysts were investigated in the Cu^{2+}-catalyzed Diels–Alder reaction of aza-chalcone (**2**) with cyclopentadiene (**3**) in water.[8] After three days at 5 °C, the *endo* isomer of the resulting Diels–Alder product (**4**), 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 **4** proved to be very dependent on the design of the catalyst (Table 1).

In an initial design, the bipyridine ligand was attached to the 5′-terminal phosphate moiety of a G-terminated 16-mer oligonucleotide *via* a 1-aminohexyl linker (linker (a)).‡ The corresponding DNA-based catalyst gave rise to 54% conversion and a good enantioselectivity of **4** (entry 1).

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 2). 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 selectivity,[9] 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. Placing the Cu-bipy complex at the terminus of the duplex by using a shorter template and leaving out ON2, resulted in a loss of activity and a strongly decreased selectivity (entry 3). Similar results were obtained in case of a longer, *i.e.* 33 nucleotides, template, which causes the presence of a nucleobase that is not involved in base-pairing near the catalytic site (entry 4). Possibly, this free adenine nucleobase

Table 1 Results of Diels–Alder reactions of **2** with **3** catalyzed by DNA-based catalysts[abc]

Entry	Catalyst (ON1, ON2 3'→ 5'/ template 5'→ 3')	Linker	Conversion (%)[d]	Ee (%)[e]
1	GTTCCAGTCTGTACA**G**ACCATGCTTAAGCGAG CAAGGTCAGACATGTCTGGTACGAATTCGCTC	(a)	54	77 (+)
2[f]	GTTCCAGTCTGTACAGACCATGCTTAAGCGAG CAAGGTCAGACATGTCTGGTACGAATTCGCTC		56	53 (+)
3	GTTCCAGTCTGTACA**G** CAAGGTCAGACATGTC	(a)	7	22 (+)
4	GTTCCAGTCTGTACA**G** ACCATGCTTAAGCGAG CAAGGTCAGACATGTCATGGTACGAATTCGCTC	(a)	21	32 (+)
5[g]	GTTGCAGTCTGTAGG**G**CCCATGCTTAAGCGAG CAACGTCAGACATCCCGGGTACGAATTCGCTC	(a)	5	39 (+)
6	GTTCCAGTCTGTACA**G**GTAATGCTTAAGCGAG CAAGGTCAGACATGTCCATTACGAATTCGCTC	(a)	65	89 (+)
7	GTTCCAGTCTGTACA**G**GTAATGCTTAAGCGAG CAAGGTCAGACATGTCCATTACGAATTCGCTC	(b)	71	93 (+)
8[f]	GTTCCAGTCTGTACAGGTAATGCTTAAGCGAG CAAGGTCAGACATGTCCATTACGAATTCGCTC		61	40 (+)
9[h]	GTTCCAGTCTGTACAGGTAATGCTTAAGCGAG CAAGGTCAGACATGTCCATTACGAATTCGCTC		76	14 (+)
10	GTTCCAGTCTGTACAG**G**TAATGCTTAAGCGAG CAAGGTCAGACATGTCCATTACGAATTCGCTC	(b)	47	79 (+)

[a] All experiments were carried out with 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) for 3 days at 5 °C, unless noted otherwise. [b] Bold letters indicate the position where the metal complex is attached. [c] Results correspond to the average of at least two experiments. [d]Determined by HPLC. Reproducible within ±10%. [e]Determined by chiral HPLC. For the *endo* isomer. Reproducible within ±3%. [f]0.1 mM $Cu(bipy)(NO_3)_2$. [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. [h]0.1 mM $Cu(L)(NO_3)_2$. L is *N*-propyl-2, 2'-bipyridine-5-carboxamide.

competes with azachalcone 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.[9] 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.

Next, the DNA-based catalyst was optimized by varying the length of the linker, the DNA sequence and the relative position of the complex.

In the case of supramolecularly assembled DNA-based catalysts, both the activity and asymmetric induction proved to be highly dependent on the DNA sequence.[9] Therefore, the effect of the sequence surrounding the anchoring site was examined in more detail. First, the three 5′- and 3′-terminal nucleotides of ON1 and ON2, 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 5). Instead, changing only the three 3′-terminal nucleotides of ON2 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 6).

By decreasing the linker length to 3 carbons a further increase in ee to 93% was found (entry 7), 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 $[Cu(L)(NO_3)_2]$,§ which is structurally closer related to the DNA-based catalyst, in combination with the same duplex DNA (entries 8 and 9). Interestingly, using only $Cu(NO_3)_2$ with this DNA sequence gave rise to 32% ee of the opposite enantiomer.¶

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.[4] This clearly illustrates the potential of the covalent anchoring strategy in hybrid catalysis.

Finally, the Cu–bipy complex was anchored to the 3′ end of ON2. Even though the same sequence is surrounding the catalytic site, this led to a reduction in ee (entry 10), suggesting a very different structure of the second coordination sphere.

In conclusion, we have developed a novel modular approach to the design of enantioselective DNA-based catalysts. This strategy allows for rapid optimization of the activity and enantioselectivity of DNA-based catalysts containing a covalently anchored transition metal complex, as was demonstrated in the Cu^{2+}-catalyzed Diels–Alder reaction in water.

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Notes and references

‡ The length of ON1 and ON2 is 16 nucleotides to ensure total hybridization with the template at the reaction temperature, *i.e.* 5 °C. The sequence was designed to have a GC content of 40–60%. See ESI† for the melting curve of the DNA-duplex.
§ L is *N*-propyl-2, 2′-bipyridine-5-carboxamide.
¶ Surprisingly, some conversion was also observed with this sequence without Cu^{2+}, albeit that only a very low ee of the (−) enantiomer of **4** was obtained. See Table S1, ESI†.

1 (*a*) J. Steinreiber and T. R. Ward, *Coord. Chem. Rev.*, 2008, **252**, 751–766; (*b*) M. D. Mihovilovic, *J. Chem. Technol. Biotechnol.*, 2007, **82**, 1067–1071.
2 (*a*) C. Letondor, N. Humbert and T. R. Ward, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 4683–4687; (*b*) J. Pierron, C. Malan, M. Creus, J. Gradinaru, I. Hafner, A. Ivanova, A. Sardo and T. R. Ward, *Angew. Chem., Int. Ed.*, 2008, **47**, 701–705; (*c*) A. Pordea, M. Creus, J. Panek, C. Duboc, D. Mathis, M. Novic and T. R. Ward, *J. Am. Chem. Soc.*, 2008, **130**, 8085–8088; (*d*) J. Collot, J. Gradinaru, N. Humbert, M. Skander, A. Zocchi and T. R. Ward, *J. Am. Chem. Soc.*, 2003, **125**, 9030–9031; (*e*) M. E. Wilson and G. M. Whitesides, *J. Am. Chem. Soc.*, 1978, **100**, 306–307; (*f*) M. T. Reetz and N. Jiao, *Angew. Chem., Int. Ed.*, 2006, **45**, 2416–2419; (*g*) M. Ohashi, T. Koshiyama, T. Ueno, M. Yanase, H. Fujii and Y. Watanabe, *Angew. Chem., Int. Ed.*, 2003, **42**, 1005–1008; (*h*) A. Mahammed and Z. Gross, *J. Am. Chem. Soc.*, 2005, **127**, 2883–2887.
3 (*a*) J. R. Carey, S. K. Ma, T. D. Pfister, D. K. Garner, H. K. Kim, J. A. Abramite, Z. Wang, Z. Guo and Y. Lu, *J. Am. Chem. Soc.*, 2004, **126**, 10812–10813; (*b*) R. R. Davies and M. D. Distefano, *J. Am. Chem. Soc.*, 1997, **119**, 11643–11652; (*c*) M. T. Reetz, M. Rentzsch, A. Pletsch, M. Maywald, P. Maiwald, J. J.-P. Peyralans, A. Maichele, Y. Fu, N. Jiao, F. Hollmann, R. Mondière and A. Taglieber, *Tetrahedron*, 2007, **63**, 6404–6414.
4 (*a*) G. Roelfes and B. L. Feringa, *Angew. Chem., Int. Ed.*, 2005, **44**, 3230–3232; (*b*) G. Roelfes, A. J. Boersma and B. L. Feringa, *Chem. Commun.*, 2006, 635–637.
5 D. Coquière, B. L. Feringa and G. Roelfes, *Angew. Chem., Int. Ed.*, 2007, **46**, 9308–9311.
6 N. Shibata, H. Yasui, S. Nakamura and T. Toru, *Synlett*, 2007, 1153–1157.
7 (*a*) L. Ropartz, N. J. Meeuwenoord, G. A. van der Marel, P. W. N. M. van Leeuwen, A. M. Z. Slawin and P. C. J. Kamer, *Chem. Commun.*, 2007, 1556–1558; (*b*) M. Caprioara, R. Fiammengo, M. Engeser and A. Jäschke, *Chem.–Eur. J.*, 2007, **13**, 2089–2095; (*c*) U. Jakobsen, K. Rohr and S. Vogel, *Nucleosides, Nucleotides Nucleic Acids*, 2007, **26**, 1419–1422.
8 S. Otto, F. Bertoncin and J. B. F. N. Engberts, *J. Am. Chem. Soc.*, 1996, **118**, 7702–7707.
9 A. J. Boersma, J. E. Klijn, B. L. Feringa and G. Roelfes, *J. Am. Chem. Soc.*, 2008, **130**, 11783–11790.