Asymmetric Catalysis

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DNA vs. Mirror-Image DNA: A Universal Approach to Tune the Absolute Configuration in DNA-Based Asymmetric Catalysis**

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Since the seminal work of Roelfes and Feringa in 2005,[1] DNA-based asymmetric catalysis has drawn increasing attention to become a particularly attractive tool for synthetic organic chemists. Indeed, DNA-based catalysts have now been successfully applied to a variety of asymmetric transformations including Diels–Alder cycloadditions,[1] Michael additions,[2] Friedel–Crafts alkylations,[3] fluorinations,[4] and *syn*-hydrations.[5,6] More recently, the concept was extended to G-quadruplex DNA sequences, which induced reasonable levels of enantioselectivity in both Diels–Alder[7,8] and Friedel–Crafts reactions confirming that the G-quadruplex structure could also act as a suitable chiral template.[9]

Considering the broad scope of achievable asymmetric transformations, DNA is now morphing from a simple biological databank to a promising chiral inducer. It is therefore not surprising that a large body of work is currently being devoted to try to understand and elucidate the various elements that control the stereoinduction. In turn, all of these endeavors constitute a valuable resource for the design of new DNA-biohybrid catalysts, which will undeniably find use in synthetic organic chemistry.

Interestingly, while many efforts have been devoted to selectively access either enantiomer in the Diels–Alder or the Friedel–Crafts reactions[8] by tuning the nature of the ligand[10] and/or the topology of the DNA structure,[8,3b] no universal method has been reported so far.

As a perfect mirror image of naturally occurring D-DNA, the use of L-DNA appears as an appealing and promising option (Figure 1). Indeed, L-DNA forms duplexes with identical physical properties in terms of solubility and thermal duplex stability leading to left-handed double-stranded helices.[11] This approach is however not as trivial as it seems.

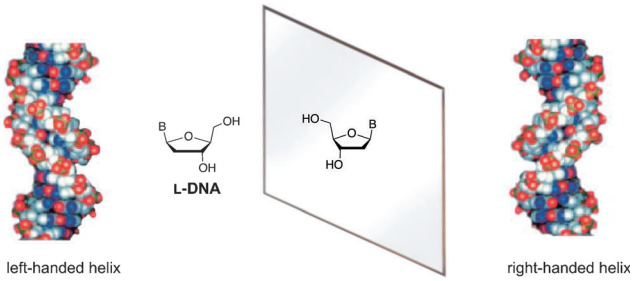

Figure 1. L-DNA (left) and natural D-DNA (right).

Erdmann and co-workers recently demonstrated the preferential stabilization of the natural D- over the non-natural L-configuration in nucleic acids and observed by X-ray spectroscopy analysis of crystals grown under microgravity that the D- and L-forms could differ not only in their chirality but also in the way they interact with divalent cations and water molecules.[12,13] A careful examination of the use of L-DNA in asymmetric catalysis is therefore necessary. In turn, this study could lead to valuable information if functional differences were to be observed.

Another interesting aspect of L-nucleic acids is their nuclease resistance; a characteristic that is notably used in a number of interesting applications as Spiegelmers and Spiegelzymes[14,15] or as specific tags in polymerase chain reactions (PCR)[16] and microarrays.[17] Moreover, while once hampered by its availability, the degree of advancement reached in carbohydrate synthesis[18,19] as well as the efficiency of enzymatic processes starting from cheap and readily available substrates,[20] have rendered the use of L-nucleic acid monomers more accessible. Ultimately, by taking advantage of these unique structural features, the use of L-DNA raises the exciting possibility to perform DNA-based asymmetric catalysis directly in biological materials.

Surprisingly, considering the various modes of ligand-binding,[21] the enantioselective outcome of L-DNA-based systems has yet to be demonstrated. We report here the first examples of mirror-image DNA-based asymmetric catalysis performed on three distinct reactions resulting, in all cases, in a complete non ligand-dependent reversal of selectivity.

The L-oligonucleotide sequences L-d(TCAGGGCCCTGA)$_2$ (ODN-**1**) and L-d(CAGTCAGTACTGACTG)$_2$ (ODN-**2**), which are the mirror images of the corresponding D-DNA self-complementary sequences (ODN-**3** and ODN-**4**, respectively) previously reported to afford high levels of enantioselectivity, were chosen for our experiments.[21] These oligonucleotides were assembled on a DNA synthesizer using commercially available L- and D-nucleoside phosphorami-

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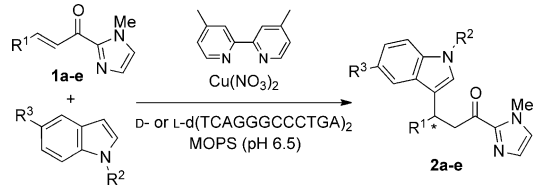
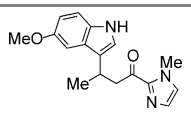
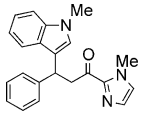
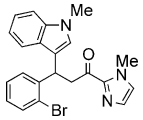
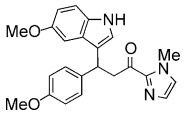
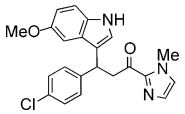
dites and purified by reverse-phase HPLC in the absence of EDTA (ethylenediaminetetraacetate) in order to prevent any potential catalyst poisoning.

With these two sequences in hand, we first investigated the Friedel–Crafts reaction between various α,β-unsaturated 2-acyl imidazoles (**1a–e**) and two commercially available indoles (*N*-methylindole and 5-methoxyindole) in the presence of a Cu^{II} complex derived from 4,4′-dimethyl-2,2′-bipyridine, [Cu(dmbpy)] (Table 1). The enantiomeric excess (*ee*) of the resulting products was determined by chiral

Table 1: Friedel–Crafts addition with D- and L-DNA.[a]



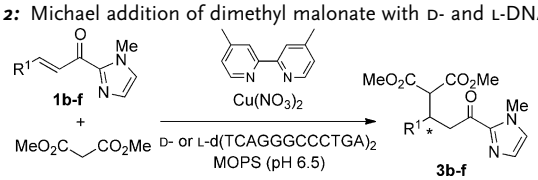
Entry	Product		*ee*[b] [%] from D-DNA	*ee*[b] [%] from L-DNA
1		**2a**	+89	−90
2		**2b**	+76	−76
3		**2c**	+76	−80
4		**2d**	+58	−58
5		**2e**	+72	−74

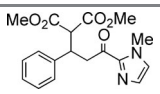
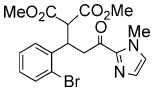
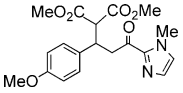
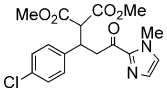
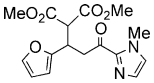
[a] MOPS = 3-Morpholinopropanesulfonic acid. [b] Enantiomeric excess determined by chiral SFC.

supercritical fluid chromatography (SFC) and compared to the values obtained using the analogous D-DNA sequences under otherwise identical conditions.

Overall, while the reactions proceeded smoothly resulting in similar *ee* values to the ones reported in the literature,[3] we were pleased to observe that in all cases the left-handed double helical structure of L-DNA produced the opposite enantiomer to the one obtained using the analogous right-handed D-DNA. An identical trend was also observed in the Michael addition of dimethyl malonate (Table 2) and nitromethane (Table 3);[2,22] in both cases, the reaction proceeded efficiently with selective formation of the corresponding (+)- or (−)-Michael addition product depending on the structure of the DNA used. Taken together, these results represent the first examples of left-helical enantioselective induction and thus expand the current toolbox of DNA-based asymmetric

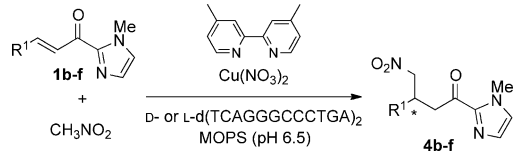
Table 2: Michael addition of dimethyl malonate with D- and L-DNA.

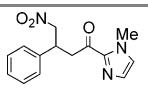
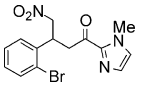
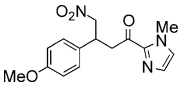
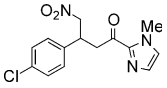
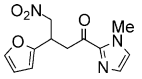


Entry	Product		*ee*[b] [%] from D-DNA	*ee*[b] [%] from L-DNA
1		**3b**	−94	+95
2		**3c**	−99	+99
3		**3d**	−89	+90
4		**3e**	−95	+94
5		**3f**	−75	+76

[a] Enantiomeric excess determined by chiral SFC.

Table 3: Michael addition of nitromethane with D- and L-DNA.



Entry	Product		*ee*[b] [%] from D-DNA	*ee*[b] [%] from L-DNA
1		**4b**	−68	+71
2		**4c**	−79	+79
3		**4d**	−74	+76
4		**4e**	−67	+68
5		**4f**	−71	+72

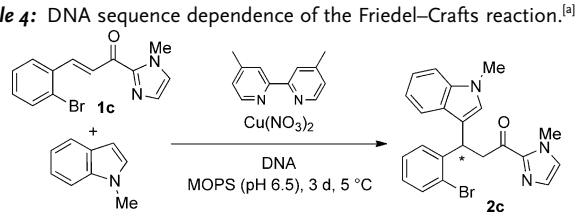
[a] Enantiomeric excess determined by chiral SFC.

catalysis. Most importantly, these results are contrasting with the ones previously reported by Sugiyama et al. pertaining to the use of a left-handed Z-DNA sequence in conjunction with a Cu^{II} complex derived from 5,6-dimethyl-1,10-phenanthroline, [Cu(5,6-dmp)], to catalyze a particularly elegant intramolecular Friedel–Crafts reaction.[3b] Indeed, in their report, the authors observed both a slight decrease in the *ee* values and, most importantly, no inversion of the selectivity as

initially expected. Interestingly, while the intercalative binding mode of [Cu(5,6-dmp)] was confirmed by viscosity studies performed on st-DNA,[3b] this unexpected stereoinduction was cautiously rationalized by the different physical properties (solubility, stability, and selectivity) between B- and Z-DNA.[23] However, this result could also be explained by the fact that intercalators are well-known allosteric effectors of the Z-to-B transition.[24,25] In this context, the use of a B-DNA hybrid catalyst (right-handed D-DNA or left-handed L-DNA) is particularly appealing as it provides a reliable and selective access to either enantiomer for any given system by simply choosing the appropriate chiral scaffold.

The capability of left-handed L-DNA to induce opposite selectivities to the corresponding D-DNA was also confirmed using a self-complementary 16 mer sequence previously reported to afford high levels of enantioselection in the Diels–Alder reaction.[21] As expected, ODN-**2** and ODN-**4** gave the opposite enantiomers in the Friedel–Crafts reaction between *N*-methylindole and the α,β-unsaturated 2-acyl imidazole **1c**, albeit with drastically lower *ee* values (entries 2 and 4, Table 4; see also Figure 2) thus confirming the sequence dependency of the selectivity for a given reaction.

Table 4: DNA sequence dependence of the Friedel–Crafts reaction.[a]



Entry	DNA Sequence[b]	Conversion[c]	*ee*[d] [%]
1	ODN-**1**	73	−80
2	ODN-**2**	66	−43
3	ODN-**3**	84	+76
4	ODN-**4**	65	+47
5	st-DNA	61	+77
6	ODN-**1**/ODN-**3** (75:25)	75	−44
7	ODN-**1**/ODN-**3** (50:50)	65	0
8	ODN-**1**/ODN-**3** (25:75)	74	+40
9	st-DNA/ODN-**1** (75:25)	65	+10
10	st-DNA/ODN-**1** (50:50)	77	−32
11	st-DNA/ODN-**1** (25:75)	76	−62
12	ODN-**1**/ODN-**4** (75:25)	89	−72
13	ODN-**1**/ODN-**4** (50:50)	66	−51
14	ODN-**1**/ODN-**4** (25:75)	61	−20
15	ODN-**2**/ODN-**3** (75:25)	74	+64
16	ODN-**2**/ODN-**3** (50:50)	62	+47
17	ODN-**2**/ODN-**3** (25:75)	87	+7

[a] Conditions: 3 mM base-pair solution of DNA in 20 mM MOPS solution (400 μL, pH 6.5), 0.9 mM of [Cu(dmbpy)$(NO_3)_2$] in 20 mM MOPS solution (200 μL), 0.5 M solution of enone in DMSO (dimethylsulfoxide), 2.5 M solution of indole in DMSO (1.2 μL), 3 d, 5 °C. [b] ODN-**1** = L-d(TCAGGGCCCTGA)$_2$, ODN-**2** = L-d(CAGTCAGTACTGACTG)$_2$, ODN-**3** = D-d(TCAGGGCCCTGA)$_2$, ODN-**4** = D-d(CAGTCAGTACTGACTG)$_2$. [c] Conversion determined by SFC. [d] Enantiomeric excess determined by chiral SFC.

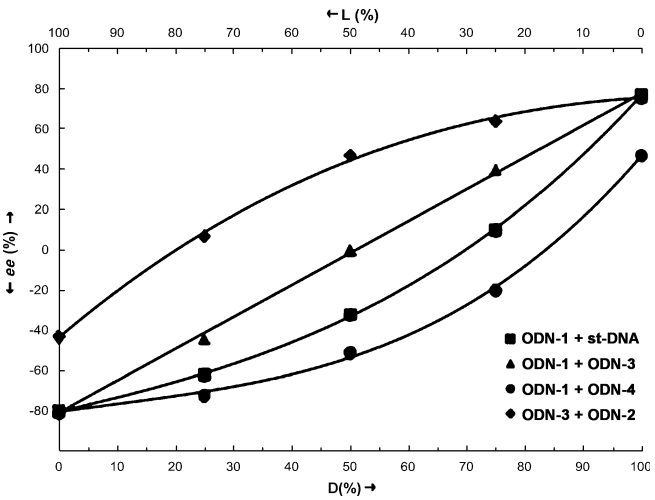


Figure 2. Enantioselectivity of the Friedel–Crafts addition at different ratios of L- over D-DNA.

In view of these results and in order to evaluate the impact of the sequence (nature and length) on the selectivity, we next performed a series of reactions mixing various concentrations of ODN-**1** and ODN-**3** which resulted in no chiral bias, thus suggesting that both strands associate independently from each other (entries 6–8, Table 4). Interestingly, when various concentrations of a random st-DNA sequence were mixed to the self-complementary left-handed oligonucleotide ODN-**1**, we were able to observe an unprecedented non-linear selectivity outcome as illustrated by the −32% *ee* obtained at equimolar base-pair concentration (entries 9–11, Table 4). This chiral bias was even more marked when using ODN-**1**/ODN-**4** (entries 12–14, Table 4) and ODN-**2**/ODN-**3** (entries 15–17, Table 4) mixtures. As chiral information is transferred through non-covalent interactions, a dynamic equilibrium of one of the helical sequence with the catalyst must therefore be preferred. These experimentally striking results corroborate an aptameric role of the defined over the random sequences. On a broader perspective, as the abundance of left- and right-handed structures are strictly equal, these observations suggest that symmetry breaking could have potentially arised from spontaneous generation of particularly selective sequences from a prebiotic pseudo-racemic pool. Moreover, the production and in vitro selection by standard processes of chimeric L-DNA hybrid catalysts with enhanced efficiencies could lead to the development of new DNA microarrays for asymmetric catalysis purposes.

In summary, we describe for the first time the use of L-DNA as a chiral scaffold in asymmetric catalysis. The power of this method lies in its simplicity and reliability. Indeed, our proposed system of D- and L-DNA is unambiguously superior in predictability to the ones presented so far thus opening new avenues in the field. In addition, the use of a combination of both D- and L-DNA sequences suggests a particularly attractive mirror symmetry-breaking opportunity and chiral amplification in biological environments. Finally, on a broader perspective, these results raise many mechanistic and prebiotic questions. In particular, mechanistic investigations are needed to explore if small energy differences between D- and

L-DNA-induced chirality might be amplified.[27] Furthermore, considering the recent results by Abe, Ito et al. pertaining to polyethylene glycol (PEG)-modified DNA,[28] our results open new avenues in the field of DNA-based asymetric catalysis in organic solvents.

Experimental Section

General procedure for the Friedel–Crafts and the Michael addition reactions: To a 3 mM base-pair solution of DNA in 20 mM MOPS (400 μL) was added a 0.9 mM solution of $[Cu(dmbpy)(NO_3)_2]$ in 20 mM MOPS (200 μL). The resulting DNA solution (2 mM base-pair, 600 μL) was cooled to 5 °C. To the cold mixture was added a 0.5 M solution of enone in DMSO (1.2 μL), followed by the appropriate nucleophile [substituted indole (2.5 M solution in DMSO, 1.2 μL), dimethyl malonate (6.9 μL), or nitromethane (32 μL)]. The reaction was mixed by inversion at 5 °C in a cold room. After 1–3 days, the solution was warmed to room temperature and extracted with Et_2O (3 × 2 mL). The combined organic layers were washed with brine (2 mL), dried over Na_2SO_4, filtered through a plug of silica gel and concentrated under reduced pressure. The crude product was subjected to SFC analysis without further purification.

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Keywords: asymmetric catalysis · biohybrid catalyst · Friedel–Crafts reaction · L-DNA · Michael addition

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