DNA-Based Asymmetric Inverse Electron-Demand Hetero-Diels Alder

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Abstract: *While artificial cyclases hold great promise in chemical synthesis, we present here the first example of a DNA-catalyzed inverse electron-demand hetero-Diels-Alder (IEDHDA) between dihydrofuran and various α,β-unsaturated acyl imidazoles. The resulting fused bicyclic O,O-acetals containing three contiguous stereogenic centers are obtained in high yields (up to 99%) and excellent diastereo- (up to >99:1 dr) and enantioselectivities (up to 95% ee) using a low catalyst loading. Most importantly, these results show that the concept of DNA-based asymmetric catalysis can be expanded to new synthetic transformations offering an efficient, sustainable, and highly selective tool for the construction of chiral building blocks.*

The field of bio-hybrid catalysis has evolved over the years to become a particularly powerful tool for the construction of carbon−carbon and carbon−heteroatom bonds, with metalloenzymes playing a key role.[1] The double stranded helix of DNA has however recently integrated the bio-hybrid catalyst arsenal emerging as a valuable alternative. This new concept, which was first introduced by Roelfes and Feringa in 2005,[2] is based on a transfer of chirality of the DNA double helix to a pro-chiral substrate and relies on a subtle association between an achiral transition metal catalyst and the DNA through either a covalent or a supramolecular interaction. Since the first example, which involved a Diels-Alder cycloaddition between an α,β-unsaturated 2 acyl pyridine and cyclopentadiene using a 9-aminoacridine-derived copper-binding diamine and salmon testes DNA (st-DNA), the concept of DNA-based asymmetric catalysis has been extended to various other synthetic transformations[3] by a number of groups around the world including ours.[4] In particular, many efforts have been devoted to unveil new biomimetic DNA-based cycloaddition processes inspired by the seminal [4+2] Diels-Alder cycloaddition[2,3a-g] (Figure 1, **A**). This has resulted in the development of various key cycloaddition reactions including a [2+2] photocatalysed cycloaddition[5] using a benzophenone-modified DNA as a photosensitizer (Figure 1, **B**) and two cyclopropanation reactions using either a Cu dmbipy-DNA complex[6] or a heme-DNA artificial enzyme[7] (Figure 1, **C**). Surprisingly, despite all these efforts, there has been no example of an asymmetric DNA-catalyzed hetero-Diels-Alder cycloaddition reported in the literature so far. As a matter of fact, the number of natural or artificial biocatalysts capable of promoting a hetero-Diels-Alder cycloaddition are rather scarce.[8,9] We report here the results of our endeavor which have led to the development of a highly stereoselective DNA-catalyzed inverse electron-demand hetero-Diels-Alder cycloaddition between α,β-unsaturated acyl imidazoles and dihydrofuran leading to the corresponding bicyclic *O*,*O*-acetals in high yields and excellent enantio- and diasteroselectivities (Figure 1, **D**).

The reaction between α,β-unsaturated 2 acyl imidazole[10] **1a** and dihydrofuran **2** in the presence of [Cu(dmbipy)(NO_3)$_2$] and st-DNA was chosen as the benchmark reaction (Table 1). A thorough optimization study (see SI for the complete study) revealed that a 1:250 ratio between **1a** and the heterodienophile **2**, 3 mol% of the Cu(II) complex and a 1 mM bp concentration of st-DNA in a MOPS buffer (pH 6.5) at 4 °C for 3 days afforded the best results with the desired bicyclic cycloadduct **3a** obtained in 83% ee and a 94:6 endo/exo ratio. Control experiments confirmed that the combination of the metallic cofactor [Cu(dmbipy)(NO_3)$_2$] and DNA was necessary to achieve both high conversions and high enantioselectivities. A higher concentration of the biohybrid catalyst did not improve the result while reducing the DNA concentration to 0.5 mM affected mainly the conversion. Interestingly, the addition of a co-solvent (2% v/v) such as DMSO, DMF, THF, ACN, DCM, and dioxane increased the substrate solubility as well as the conversion without affecting the stereoselectivity, with THF standing out as the optimum co-solvent (94:6 endo/exo ratio, 83% ee) (Scheme 1). A systematic circular dichroism (CD) study confirmed that the double helical structure of DNA was maintained in the presence of all the aforementioned co solvents,[11] which is consistent with previous studies reported in the literature.[12] Moreover, varying the nature of the copper(II) complex by replacing 4,4'-dimethyl-2,2'-bipyridine (dmbpy), which binds to st-DNA through groove binding, by phenantroline (phen), which is a good DNA intercalator, or either 2,2':6',2"-terpyridine (terpy) or dipyrido[3,2-a:2',3'-c]phenazine (dppz), which are known to bind to DNA through a mix of minor groove binding and intercalation,[13] had a detrimental effect on both the conversion and the selectivity. These results are in agreement with the ones obtained for the Diels-Alder reaction; groove binding interactions allow more flexibility of the complex while maintaining the substrate in the second coordination sphere of the DNA helix.[14]

With these results in hand, we next evaluated the substrate scope by subjecting a variety of α,β-unsaturated 2-acyl imidazoles (**1b-o**) to our optimized conditions; the results are depicted in Scheme 1. As a general trend, the corresponding bicyclic adducts **3b-o** were obtained in moderate to high conversions ranging from 35% to >99%, excellent diastereoselectivities (*endo*/*exo* up to >99:1) and high enantioselectivities (ees up to 95%). Hence, the introduction of electron-donating substituents at the para position of the aromatic ring such as a methyl (**3b**, 89% ee, *endo*/*exo*>17:1), a methoxy (**3c**, 90% ee, *endo*/*exo*>9:1) or a thiomethyl (**3e**, 90% ee, *endo*/*exo*>99:1), did not alter the selectivity, however it is worth pointing out the decrease in reactivity observed in the case of the thioanisole derivative most probably due to the ability of the sulfur atom to chelate copper ions.[15] The introduction of a slightly electron-withdrawing substituent such as a fluorine atom[16] (**3f**, 88% ee, *endo*/*exo*>12:1) was not detrimental however more electron-deficient aromatic rings such as the *p*-bromo- and the *p*-nitrobenzene derivatives led to very low conversions (data not shown). β-Heteroaromatic acyl imidazoles (1g-i) were also found to be excellent substrates as showcased by the high yields and remarkable diastereo- (*endo*/*exo* up to >99:1) and enantioselectivities (ees ranging between 76% and 85%) obtained for the resulting bicyclic *O*,*O*-acetals **3g-i**. Finally, in contrast to the β-aryl- and the β-heteroaryl-substituted substrates (**1a-i**), complete conversions were observed with practically all the β-alkyl-substituted derivatives (**1j-n**) tested. Moreover, the *endo*/*exo* ratios appeared to decrease and the ees increase as the size of the alkyl chain became more bulky. The prevalence of the 2-acyl-methylimidazole motif in bio-hydrid catalysis was further confirmed by the results obtained with the analogous 2-acyl-isopropylimidazole precursor, which afforded the corresponding cycloadduct **3o** in 90% conversion albeit only 61% ee, or with the related α,β-unsaturated 2 acylpyridine, the 1,3-diphenyl-2-propenone and the 2-methyl-1-(thiazol-2-yl)prop-2-en-1-one, which failed to produce any product (data not shown).

The nature of the heterodienophile was also evaluated using a selection of electron-rich alkenes, including 3,4-dihydro-2H-pyran, ethyl vinyl ether, 2-vinyloxirane or *para*-methoxystyrene, but none of them afforded the desired IEDDA product. Considering that these reactions are assumed to proceed through a concerted, but asynchronous transition state, we associate this lack of reactivity with the lower nucleophilicity of these heterodienophiles.[17]

The *endo* selectivity was confirmed by 1H NMR analysis of compounds **3a**, **3j** and **3l**, all obtained in high yields at reaction scales ranging from 0.5 to 1.2 mmol, which also demonstrates the robustness of the method (Table 2). Hence, all three compounds adopt a bicyclic *cis* junction characterized by a low coupling constant between H_{7a} and H_{3a} (^3J H_{7a}-H_{3a} ~ 4.0 Hz) and a W coupling between H_5 and H_{3a} (^4J H_5-H_{3a} ~ 1.2 Hz). In addition, the relatively low coupling constant between H4 and H3a indicates that these two protons are facing each other. Finally, a NOESY experiment established a correlation between H_4 and H_{7a} consistent with an *endo* selective cycloaddition. As for the absolute configuration, the latter was ascertained by comparing the specific optical rotation value of the endo product **3j** (3a*R*, 4*R*, 7a*S*), which was obtained in quasi-quantitative yield on a 105 mg scale, with the one reported in the literature,[18] while all other products were assigned by analogy. Compound **3l** was also engaged in a hydrogenation reaction to further support our assignment (Table 2). The reduction of the enol double bond proceeded smoothly and preserved the integrity of the *O*,*O*-acetal affording compound **4** as a single diastereoisomer in quantitative yield and with no erosion of the selectivity.[19]

In conclusion, we present here the first example of an asymmetric DNA-catalyzed inverse electron-demand hetero-Diels-Alder reaction. The reaction allows the formation of fused bicyclic *O*,*O*-acetals in high yields (up to 99%) and excellent diastereo- and enantioselectivities (up to >99:1 dr, up to 95% ee). The method was applied to a variety of α,β-unsaturated 2-acyl-imidazoles and could be easily scaled up. Most importantly, this reaction, which has no equivalent in the metalloenzyme arsenal obtained through directed evolution, emphasizes furthermore the versatility of DNA-based asymmetric catalysis and its efficacy in mimicking nature's hetero-Diels-Alderases in water. Ultimately, we hope this will trigger new developments in the field and inspire the development of other cycloaddition reactions.

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Figure 1. DNA-based asymmetric cycloadditions

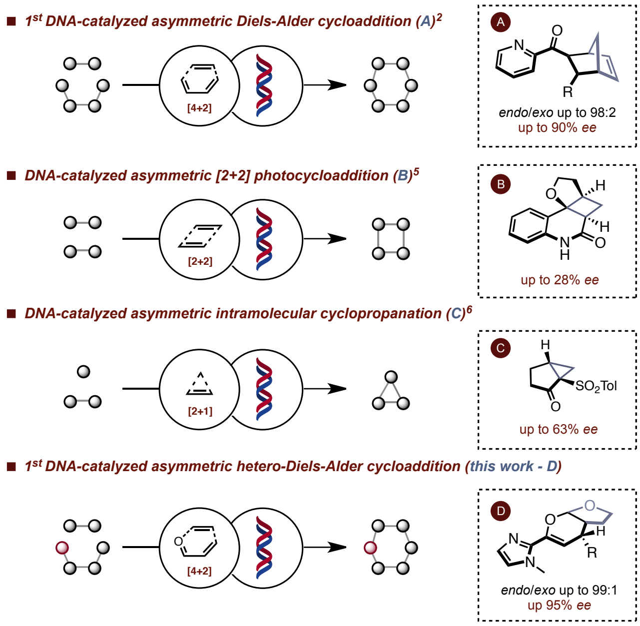
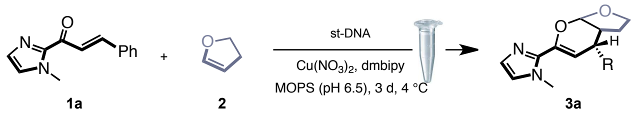
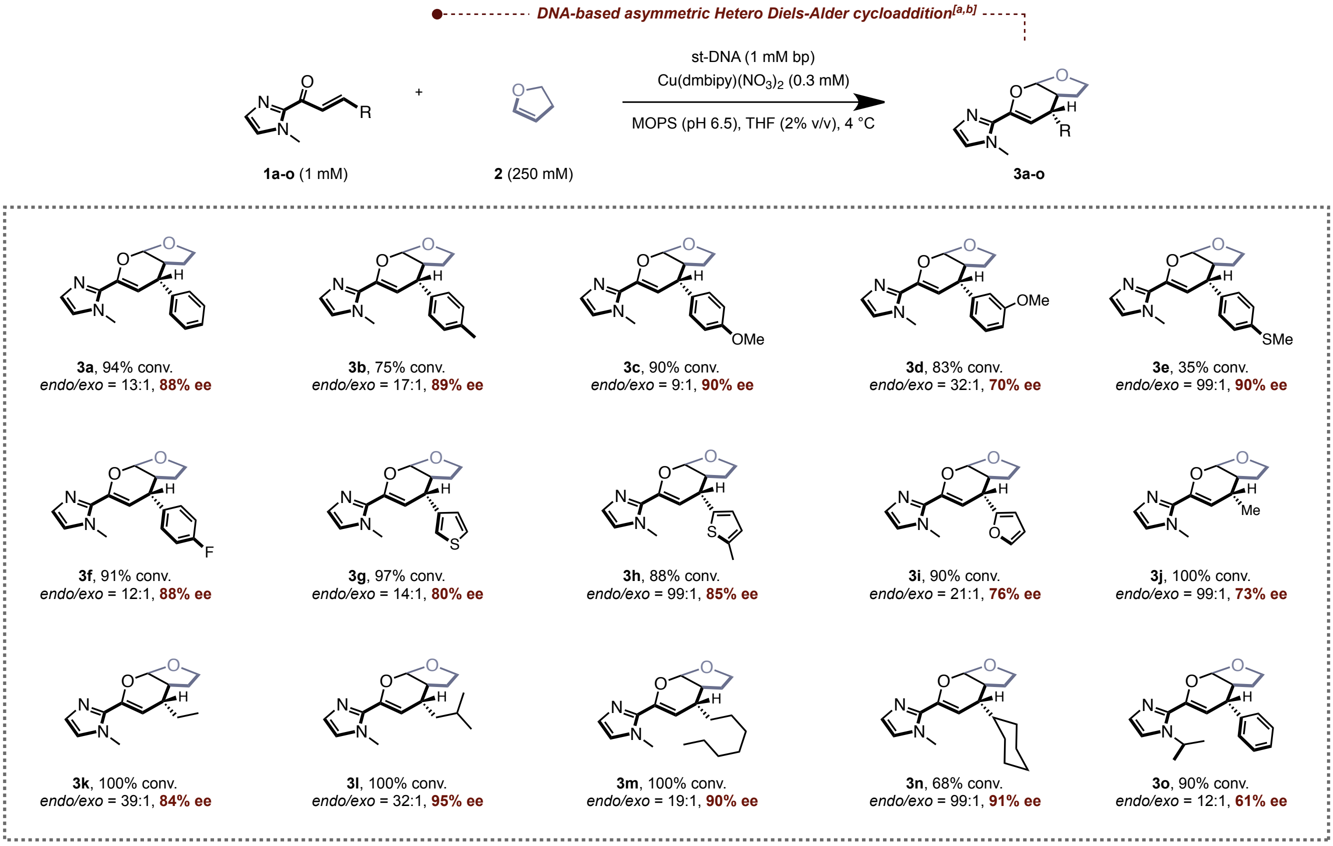


Table 1. Systematic study.[a]



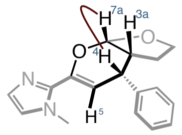
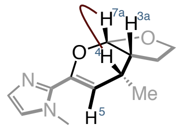
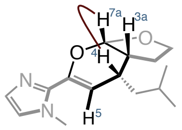
Entry	Cu^{II}/dmbipy (mol%)	st-DNA (mM)	Buffer (pH)	Co-solvent (2% v/v)	Conv[a] (%)	de[a] (%)	ee[a] (%)
1	30	-	MOPS (6.5)	DMSO	56	94	-
2	-	2	MOPS (6.5)	DMSO	-	-	-
3	30	2	MOPS (6.5)	DMSO	90	85	82
4	30	2	MOPS (7.0)	DMSO	84	85	82
5	30	2	MOPS (7.5)	DMSO	89	85	83
6	30	2	MOPS (8.0)	DMSO	84	85	83
7	15	1	MOPS (6.5)	DMF	88	85	82
8	15	1	MOPS (6.5)	ACN	96	86	82
9	15	1	MOPS (6.5)	DCM	93	87	84
10	15	1	MOPS (6.5)	THF	96	86	84
11	15	1	MOPS (6.5)	Dioxane	93	87	82
12	**3**	**1**	**MOPS (6.5)**	**THF**	**95**	**86**	**88**

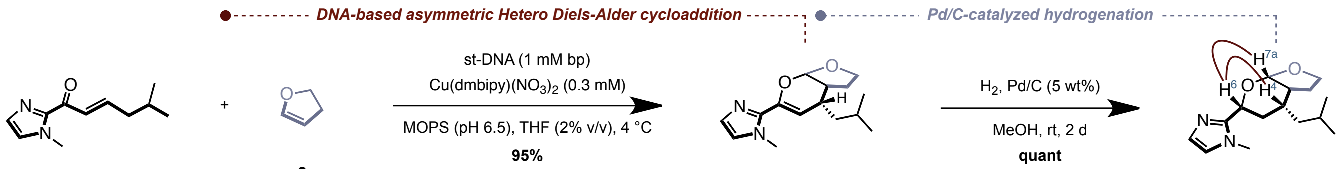
[a] Reactions conditions: **1a** (1 mM), **2** (250 equiv), in a 20 mM MOPS buffer solution pH 6.5 for 3 d at 4 °C. Conversions, *des* and *ees* were determined by High Pressure Liquid Chromatography (HPLC) analysis.



Scheme 1. Reaction scope. [a] All reactions were carried out with *st*-DNA (1 mM bp concentration), **1a-o** (1 mM), **2** (250 equiv), and [Cu(dmbipy)$(NO_3)_2$] (0.03 mM) in a 20 mM MOPS buffer solution pH 6.5 with 2% THF as co-solvent for 3 d at 4 °C. [b] Conversions and *ees* were determined by High Pressure Liquid Chromatography (HPLC) analysis.

Table 2. Scale-up of the hetero Diels-Alder reaction and structural determination by NMR.

Compound	Isolated Yield (scale)	ee (%), dr	[α]D (c, solvent)	Coupling constants (Hz) ^3J H7a-H3a	^3J H4-H3a	*NOE effect* ^4J H7a-H4
 **3a**	79% (255 mg, 1.2 mmol)	88%, 12:1	+6,0° (0.3, CH_2Cl_2)	4.0	4.5	strong
 **3j**	99% (105 mg, 0.7 mmol)	73%, >99:1	+9.7° (0.4, CH_2Cl_2)[17]	3.8	6.4	strong
 **3l**	88% (96 mg, 0.5 mmol)	95%, 32:1	+12.5° (0.6, CH_2Cl_2)	4.1	4.4	strong



1l (0.5 mmol scale) **2** **3l** (endo/exo = 32:1, 95% ee) **4** (dr > 95:5)

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19 NOESY experiments confirmed the *syn*-addition of H_2 on the *Si* face of **3l**. Strong NOE effects were observed between H_6 and both H_4 and H_{7a} in compound **4**.