G-Quadruplexes

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Tuning the Stereoselectivity of a DNA-Catalyzed Michael Addition through Covalent Modification

*Surjendu Dey and Andres Jäschke**

Abstract: *Complexes of G-quadruplex DNA and Cu^{II} ions have previously been applied as catalysts in asymmetric reactions, but the largely unspecific and noncovalent nature of the interaction has impeded understanding of the structural basis of catalysis. To better control the formation of a catalytically competent species, DNA quadruplexes were derivatized with linker-bpy-Cu^{II} complexes in a site-specific manner and applied in asymmetric aqueous Michael additions. These modified quadruplexes exhibited high rate acceleration and stereoselectivity. Different factors were found to be important for the catalytic performance of the modified G-quadruplexes, among them, the position of modification, the topology of the quadruplex, the nature of the ligand, and the length of the linker between the ligand and DNA. Moving the same ligand by just two nucleotides inverted the stereochemical outcome: quadruplexes modified at position 10 formed the (−)-enantiomer with up to 92% ee, while DNA derivatized at position 12 formed the (+)-enantiomer with up to 75% ee. This stereopreference was maintained when applied to structurally different Michael acceptors. This work demonstrates a new and simple way to tune the stereoselectivity in DNA-based asymmetric catalysis.*

DNA-based hybrid catalysis is gaining importance in aqueous-phase homogeneous asymmetric catalysis.[1] In this catalytic approach, double-stranded (ds) DNA plays a crucial role in transferring chirality from its characteristic helical structure to the product. This approach has proven highly successful for different enantioselective reactions, such as Diels–Alder reactions,[2] Friedel–Crafts reactions,[3] Michael additions,[4] fluorinations,[5] and hydrations.[6] Beside the well-known double helix structure, DNA can also be present as triplex, hairpin, G-quadruplex, and i-motif structures.[7] G-quadruplex structures are topologically more complex and offer more structural variety than dsDNA,[8] thus making them a promising platform for DNA-based hybrid catalysis. Two well-characterized G-quadruplex-forming sequences, namely the human telomeric G-quadruplex (h-Tel) and a region of the *c-kit* promoter (c-kit), have already been utilized for asymmetric Diels–Alder and Friedel–Crafts reactions in the presence of Cu^{II} ions.[9] Of these two, h-Tel showed greater versatility in terms of conversion and enantioselectivity. However, in these studies, Cu^{II} bound to the G-quadruplex sequences in an unspecific, most likely purely electrostatic fashion, thus making it impossible to localize the catalytic Cu^{II} and the substrate binding pocket in the G-quadruplex structure. Therefore, neither a detailed understanding nor prediction of the factors that determine activity and selectivity in these reactions is currently possible.

With dsDNA, there are some examples for the covalent attachment of a transition-metal ligand to DNA, which allows the placement of the metal center in a much more controlled way and sets the stage for systematic structure–function studies.[10] On the other hand, only one report has been published for a covalently modified G-quadruplex catalyst (modified with proline) for an aldol reaction, and no stereoselectivity was observed.[11] To the best of our knowledge, there are no reports on G-quadruplex structures covalently modified with metal-binding ligands and their application in asymmetric Michael addition reactions.

We reasoned that a number of different factors govern the rate acceleration and stereoselectivity of G-quadruplex-catalyzed reactions, among them the topology of the quadruplex, the attachment site of the metal-chelating ligand, the nature of the ligand, and the length and structure of the linker connecting the DNA and ligand. Herein, we report the first systematic investigation of these parameters in the context of asymmetric Michael addition reactions, which are important C–C bond forming reactions,[12] catalyzed by c-kit quadruplexes.

The 3D structure of the c-kit wild-type (wt) sequence (5′-AGGGAGGGCGCTGGGAGGAGGG-3′) has been studied by both X-ray crystallography[13] and NMR spectroscopy.[14] According to these studies, c-kit forms a unique parallel G-quadruplex structure as illustrated in Figure 1a. For our systematic investigations, we considered attaching the ligand in two different ways. In one case, ligand attachment should not affect the correct formation of the G-quadruplex stack, while it should do so in the other case. For the first case, we picked position 12, which is located in an apical loop segment, since crystallographic investigations revealed no influence of a bulky substitution at this position on the folding topology.[13] Position 10, on the other hand, was identified as important for the unique all-parallel folding topology of the c-kit quadruplex, and NMR spectra suggested a G10T mutant to favor an alternative topology.[14] However, the exact structure(s) of this mutant quadruplex c-kit-T10 are, however, unknown. A hypothetical structure is shown in Figure 1c.

We thus substituted the natural nucleotides at positions 10 or 12 with a deoxyuridine derivative covalently modified at the C5-position with a bipyridine moiety attached via linkers of different lengths (Figure 1b and d). Modified nucleosides

[*] S. Dey, Prof. Dr. A. Jäschke
Institut für Pharmazie und Molekulare Biotechnologie
Universität Heidelberg, 69120 Heidelberg (Germany)
E-mail: jaeschke@uni-hd.de
Homepage: http://www.jaeschke.uni-hd.de

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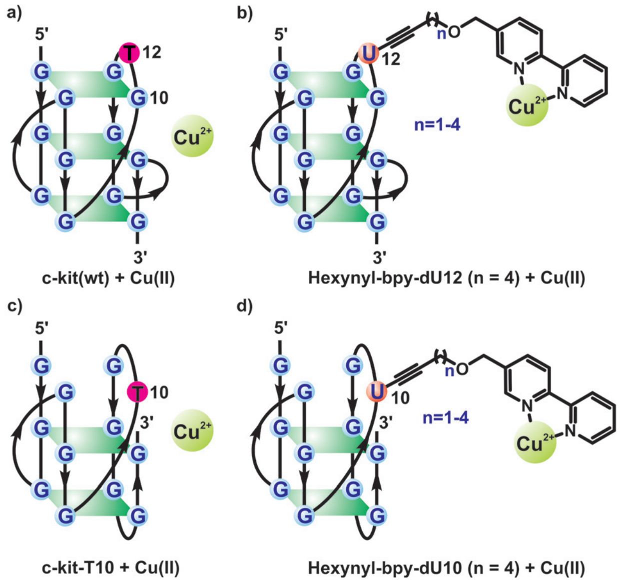


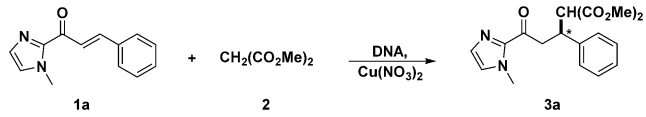
Figure 1. G-quadruplex folding of different DNA sequences in the presence of Cu^{II}. a) Observed folding of c-kit(wt) DNA.[13,14] b) Hypothetical folding and metal binding of dU12-modified DNA. c) Hypothetical folding of c-kit-T10 DNA. d) Hypothetical folding and metal binding of dU10-modified DNA.

were prepared by Sonogashira coupling of 5′-DMT-5-iodo-2′-deoxyuridine and bipyridine alkynes with different linker lengths (see the Supporting Information for details). By using standard methodology, these four modified nucleosides were protected and converted into phosphoramidites. Solid-phase synthesis was applied to synthesize dU12- and dU10-modified DNA sequences (see the Supporting Information). After purification and analytical characterization, these modified sequences were investigated along with Cu^{II} as catalysts in the asymmetric Michael addition of standard acceptor **1a** and the nucleophile dimethyl malonate (DMM, **2**).

While the buffer controls (Table 1, entries 1 and 2), and the unmodified c-kit(wt) sequence (Table 1, entries 3 and 4) showed negligible activity and stereoinduction in the absence or presence of Cu^{II}, the four different dU12-modified G-quadruplexes yielded near-quantitative conversion (92–95%) with 10 mol% of catalyst (Table 1, entries 5–8). These results indicate that bpy-coordinated Cu^{II} covalently attached to quadruplex DNA is a better catalyst than copper ions unspecifically bound to quadruplex DNA.

The stereoselectivity increased almost linearly with the linker length from 31% for propargyl to 52% *ee* for the hexynyl linker. In all cases, the (+)-enantiomer was obtained in excess. To ensure that stereoinduction is caused by the topology of the DNA scaffold and not by the chirality of the modified nucleoside, the reaction was investigated in the presence of the different linker-bpy-modified deoxyuridine nucleosides, with or without added unmodified quadruplex DNA (Table 1, entry 9, and Table S1 in the Supporting Information), and much lower *ee* (always in favour of the (+)-enantiomer) and conversion values were observed. These findings demonstrate that the ligand-modified quadruplexes provide both rate acceleration and stereoselectivity.

Table 1: Michael addition catalyzed by dU12-modified c-kit DNA.[a]



Entry	DNA	$Cu(NO_3)_2$ (mol%)	*n*	conv. [%][b]	*ee* [%][b,c]
1	–	–	–	< 1	0
2	–	10	–	8	0
3	c-kit(wt)	–	–	< 5	< −5
4	c-kit(wt)	10	–	6	−9
5	Propargyl-bpy-dU12	10	1	94	+31
6	Butynyl-bpy-dU12	10	2	92	+40
7	Pentynyl-bpy-dU12	10	3	94	+43
8	Hexynyl-bpy-dU12	10	4	95	+52
9	Hexynyl-bpy-dUrd	10	4	22	0

[a] See the Experimental Section for detailed reaction conditions. All experiments were performed in triplicate. [b] Both conversion and *ee* values were calculated by using chiral-phase HPLC; results are reproducible within ±5%. [c] + and − symbols refer to isomers with low and high retention time, respectively, from a chiral-phase HPLC column.

Table 2: Michael addition of **1a** with **2** catalyzed by dU10-modified c-kit DNA.[a]

Entry	DNA	$Cu(NO_3)_2$ (mol%)	*n*	conv. [%][b]	*ee* [%][b,c]
1	c-kit-T10	–	–	< 5	< −5
2	c-kit-T10	10	–	8	−11
3	Propargyl-bpy-dU10	10	1	92	−32
4	Butynyl-bpy-dU10	10	2	92	−57
5	Pentynyl-bpy-dU10	10	3	91	−69
6	Hexynyl-bpy-dU10	10	4	95	−92

[a], [b], [c] see footnotes to Table 1.

Next, we investigated the dU10-modified G-quadruplexes, which likely fold differently. Again, near-quantitative conversions were achieved (91–95%), and an increase in the stereoselectivity with the linker length was observed (Table 2, entries 3–6), reaching the practically useful 92% *ee* for hexynyl-bpy-dU10 (Table 2, entry 6). As with c-kit(wt) (Table 1, entries 3 and 4), negligible activity was measured for the c-kit-T10 sequence without the ligand (Table 2, entries 1 and 2). Surprisingly, for all dU10-modified quadruplexes, the (−)-enantiomer was obtained in excess, which is the opposite result to that obtained with the dU12-modified sequences (Table 2 vs. Table 1).

The c-kit quadruplex derivatized with the hexynyl-bpy ligand in position 12 yielded 95% conversion and 52% excess of the (+)-enantiomer, while the catalyst with the same ligand attached to position 10 gave also 95% conversion but with 95% excess of the (−)-enantiomer, which was unpredicted and not easy to rationalize (see Figure 2a,b). To get a first structural insight into the molecular basis of this phenomenon, CD spectra were recorded. Figure 2c presents the CD spectra of c-kit(wt) and hexynyl-bpy-dU12 sequences in the absence and presence of Cu^{II}. These spectra are virtually identical and show the typical features of a parallel quadruplex, namely a minimum around 240 nm and a maximum around 260 nm, thus suggesting that neither the attachment of the linker-

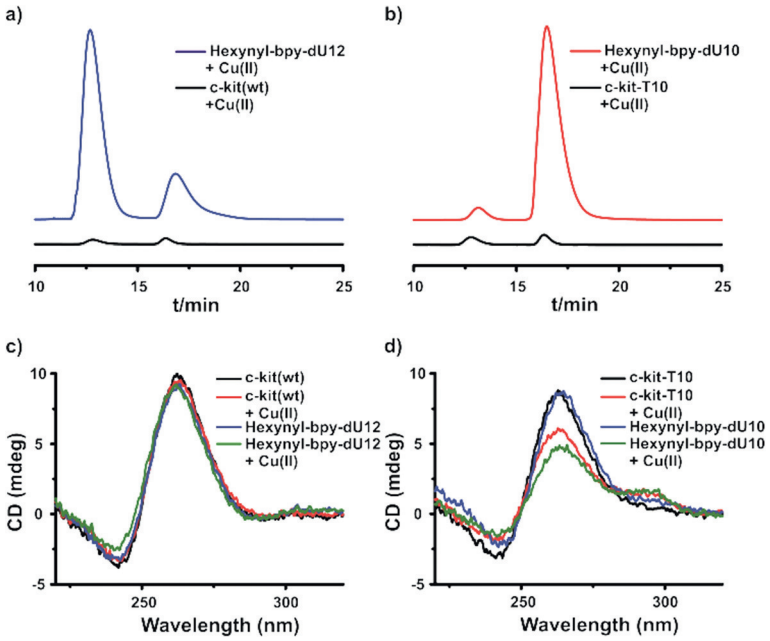


Figure 2. Correlation between quadruplex topology and stereoselectivity in the Michael addition. a) Separation of product **3a** enantiomers for the Michael addition catalyzed by c-kit(wt) and hexynyl-bpy-dU12. b) Separation of product **3a** enantiomers for the Michael addition catalyzed by c-kit-T10 and hexynyl-bpy-dU10. c) CD spectra of c-kit(wt) and hexynyl-bpy-dU12 in the absence and presence of Cu^{II}. d) CD spectra of c-kit-T10 and hexynyl-bpy-dU10 in the absence and presence of Cu^{II}.

bound bpy ligand nor the binding of copper disturbs the folding. CD spectra of other dU12-modified sequences displayed the same features, thus indicating the same type of structural folding (Figure S9). For the dU10 quadruplexes (Figure 2d), the spectra display the features of a parallel quadruplex in the absence of Cu^{II} for c-kit-T10. The addition of copper ions to c-kit-T10 leads to attenuation of both the 240 and the 260 nm bands and the appearance of a small shoulder around 290 nm, features often observed in hybrid structures with mixed parallel/anti-parallel strands.[15,16] The hexynyl-bpy-dU10 sequence shows a small shoulder around 290 nm that becomes more prominent upon the addition of Cu^{II}. Again, other dU10-modified sequences yielded very similar CD spectra (Figure S10). Thus, while not providing the same level of information as high-resolution structures, the CD spectra support our design assumption that dU10- and dU12-modified c-kit quadruplexes fold into different catalytically competent structures in the presence of Cu^{II}, which cause the opposing stereochemical outcomes of the reactions.

To further investigate the G-quadruplex-catalyzed Michael reactions, we compared the two best cases, namely the hexynyl-bpy-modified dU10 and dU12 catalysts, with respect to conversion and stereoselectivity at decreasing catalyst loadings (Table 3). Surprisingly, the dU10 catalyst, in which the regular quadruplex folding has been disturbed, turns out to be the better catalyst: even at 1 mol% catalyst, 83% conversion is measured, giving a turnover frequency of 1.2 h^{-1}. The dU12 catalyst gave 46% conversion under these conditions, which equates to a turnover frequency of 0.6 h^{-1}. In both cases the enantioselectivity was not affected by variation of the catalyst loading.

Inspired by the primary results, we chose hexynyl-bpy-dU12 and hexynyl-bpy-dU10 combined with Cu^{II} as catalysts to explore the Michael addition reaction in more depth with different acceptor substrates **1a–g** (Table 4). In all cases, we observed preference for the (+)-enantiomers of the products (**3a–g**) when using hexynyl-bpy-dU12 as the catalyst and a preference for the (−)-enantiomers when using hexynyl-bpy-dU10 (see Figures S2–S8). The two catalysts thus show defined preferences for stereoselective bond formation, which they keep when they are confronted with varying substrates. Except for substrate **1f** (which is a poor acceptor owing to the strongly electron-withdrawing CF3 group), all of the other substrates showed good to excellent conversion in the presence of both hexynyl-bpy-dU12 and hexynyl-bpy-dU10. Substrate **1g** turned out to be best for hexynyl-bpy-dU12; while substrate **1a** was most appropriate for hexynyl-bpy-dU10 in terms of enantioselectivity.

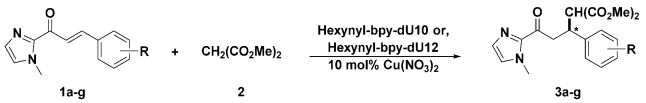
In conclusion, we found that G-quadruplex DNA sequences covalently modified with linker-bpy-Cu^{II} complexes are efficient catalysts for asymmetric Michael addition reactions in water. Quadruplex topology, the ligand and its attachment site, as well as the length of the linker

Table 3: Dependence of the DNA-catalyzed Michael addition of **1a** with **2** on catalyst loading.[a]

Entry	DNA[d]	$Cu(NO_3)_2$ (mol%)	conv. [%][b]	*ee* [%][b,c]
1	Hexynyl-bpy-dU12	10	95	+52
2	Hexynyl-bpy-dU12	5	81	+52
3	Hexynyl-bpy-dU12	2.5	72	+52
4	Hexynyl-bpy-dU12	1	46	+52
5	Hexynyl-bpy-dU10	10	95	−92
6	Hexynyl-bpy-dU10	5	95	−92
7	Hexynyl-bpy-dU10	2.5	94	−92
8	Hexynyl-bpy-dU10	1	83	−92

[a], [b], [c] see footnotes to Table 1. [d] DNA concentration was varied in proportion to that of $Cu(NO_3)_2$.

Table 4: Substrate variation for the Michael addition reaction.[a]



1a-g 2 3a-g

Entry	Substrate, R	Hexynyl-bpy-dU12 conv. [%][b]	*ee* [%][b,c]	Hexynyl-bpy-dU10 conv. [%][b]	*ee* [%][b,c]
1	H (**1a**)	95	+52	95	−92
2	*p*-Me (**1b**)	92	+55	90	−85
3	*p*-OMe (**1c**)	96	+42	97	−71
4	*p*-Br (**1d**)	65	+62	75	−83
5	*o*-OMe (**1e**)	96	+51	92	−61
6	*o*-CF_3 (**1f**)	13	+46	10	−45
7	*o*-Br (**1g**)	99	+75	98	−66

[a], [b], [c] see footnotes to Table 1.

connecting the DNA and ligand were all found to have an effect on the reaction outcome. The finding that longer linkers yield higher *ee* values is counterintuitive. This result might be due to an optimal stacking orientation of the bpy ligand upon the G-quadruplex for stereoselective catalysis, which is more easily achieved with longer linkers. Importantly, the stereoselectivity of the reaction can be easily tuned by selecting different attachment positions on the DNA sequence. Moving the same linker-derivatized bpy ligand by just two nucleotides completely changed the stereochemical outcome of the reaction: while quadruplexes modified at position 10 preferentially formed the (−)-enantiomer with high *ee*, those derivatized at position 12 preferentially formed the (+)-enantiomer. For each of these catalysts, the stereopreference was conserved across a panel of differently substituted Michael acceptors. This first example of covalently modified quadruplex DNA catalysts and the reported dependence of the catalytic performance on structural variations expands the scope of DNA-based hybrid catalysis.

Experimental Section

Standard Michael addition procedure: An aqueous stock solution containing 40 nmol of the oligonucleotide was lyophilized. The lyophilized DNA was then dissolved in a 297 μL solution of 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (20 mM, pH 7) containing KCl (100 mM) then 3 μL $Cu(NO_3)_2$ (10 mM) solution was added. The final DNA and Cu^{II} concentrations were 133 μM and 100 μM, respectively. The solution was heated for 5 min at 90 °C, and slowly cooled to room temperature. The solution was kept at 5 °C overnight before use. To the catalyst solution, 3 μL of a fresh stock solution of Michael acceptor (100 mM) in acetonitrile was added, followed by the addition of 3.42 μL dimethyl malonate. The reaction mixture was stirred for 3 days at 5 °C. Extraction was performed with Et_2O (3 × 500 μL). After drying the reaction mixture with anhydrous Na_2SO_4, the solvent was removed to obtain the crude product. Chiral-phase HPLC was performed directly with this crude product to determine the conversion and enantiomeric excess.

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[16] Alternatively, these spectral changes might be explained by a mixed population of parallel quadruplexes and antiparallel quadruplexes. This hypothesis, however, is difficult to reconcile with the high and opposing stereoselectivity of the reaction.

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