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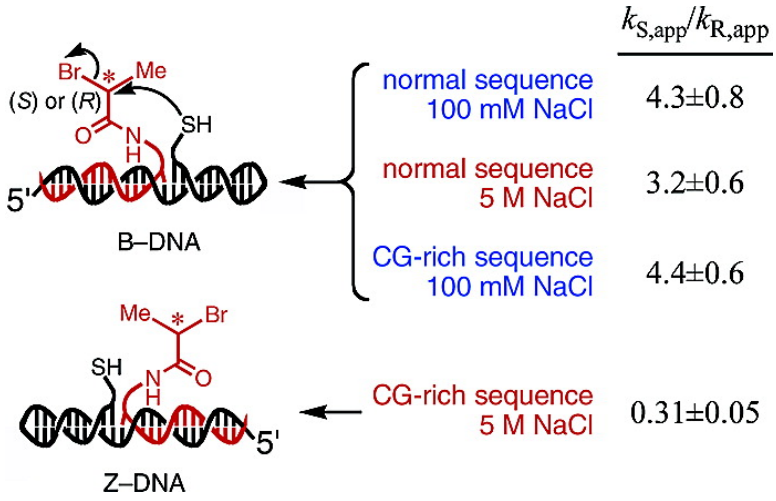

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Stereoselectivity in DNA-Templated Organic Synthesis and Its Origins

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DNA templates are capable of directing chemical reactions without obvious structural requirements or functional group adjacency.[1] The generality of DNA-templated synthesis[1,2] enables otherwise incompatible reactions to take place in the same solution,[1d] multistep small molecule syntheses programmed by DNA sequences,[1b] and the selection and amplification of synthetic molecules paralleling key aspects of biological molecule evolution in nature.[1a] The chiral nature of DNA raises the possibility that DNA-templated synthesis can proceed stereoselectively without the assistance of chiral groups beyond those present in DNA, thereby transferring not only sequence but also stereochemical information from the template to the product. Previous studies[3a,b] have demonstrated that the chirality of nucleic acid templates can induce a preference for the template-directed ligation of D-nucleotides over L-nucleotides. Stereoselectivity during the DNA-templated synthesis of structures unrelated to the DNA backbone, however, has to our knowledge not been studied. Here, we describe stereoselectivity during DNA-templated organic synthesis and provide insights into its origins.

We examined stereoselectivity in the context of DNA-templated nucleophilic substitution reactions.[1a] Hairpin architecture[1a,e] templates conjugated at their 5′ amino termini directly to (*S*)- or (*R*)-2-bromopropionamide were combined with 3′ thiol-linked reagent oligonucleotides at 25 °C (Figure 1, top). The stability of the bromides under the reaction conditions was confirmed by several independent methods (see Supporting Information). Initial rates of thioether product formation were determined by denaturing gel electrophoresis, and products were additionally characterized by MALDI-TOF mass spectrometry (see Supporting Information). Apparent rates of product formation were 4.0 ± 0.2-fold higher for (*S*)-bromide-linked templates than for (*R*)-bromide-linked templates. Because template−reagent annealing could be partially rate-determining,[1a,b] this value is a lower limit of the actual ratio of k_S/k_R, assuming annealing rates are unaffected by bromide stereochemistry. Surprisingly, similar preferences favoring the (*S*)-bromide were also observed using end-of-helix template architectures[1a,e] (Figure 1, middle), even when 12 nucleotides separated the thiol and bromide in the template−reagent complexes. Stereoselectivity also appeared independent of whether the bromide or the thiol was conjugated to the template (Figure 1, middle and bottom). Similar selectivities emerged from pseudokinetic resolutions containing both bromide stereoisomers in which thioether products arising from (*S*)- and (*R*)-bromides were distinguished using templates of two distinct lengths (k_S/k_R = 4.2 ± 0.4 to 4.9 ± 0.3). Taken together, these findings indicate that the chirality of a DNA template can be transferred to products of DNA-templated synthesis that do not resemble the DNA backbone.

To probe the origins of the observed stereoselectivity, we synthesized a series of template and reagent analogues in which nucleotides near the thiol or bromide were replaced with flexible achiral linkers (see Supporting Information). Replacing the 12 template nucleotides separating the bromide and thiol in either of

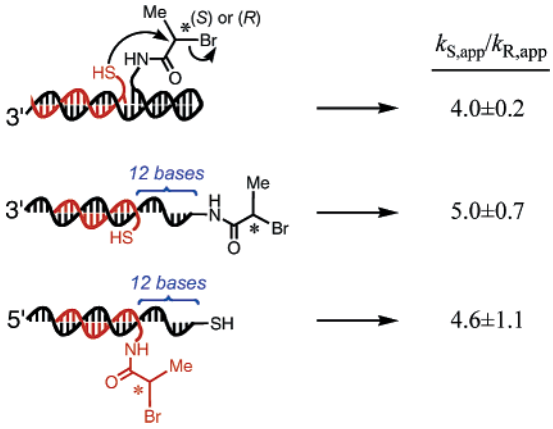

Figure 1. Stereoselective DNA-templated substitution reactions. Relative rates of product formation from the (*S*)- and (*R*)-bromides were determined by denaturing polyacrylamide gel electrophoresis followed by densitometry. Values reflect the mean and standard deviation of at least three independent experiments.

the end-of-helix reactions with an achiral poly(ethylene glycol) linker of similar length (72 bonds) resulted in the loss of stereoselectivity. Stereoselectivity was also abolished when flexible achiral linkers consisting of three or five consecutive methylene or ether oxygens were inserted between the 5′ end of the template oligonucleotide and the thiol or bromide groups, or between the 3′ end of the reagent oligonucleotide and the thiol or bromide. Chiral linkers between reactants are therefore required for stereoselectivity in this DNA-templated reaction. These results also suggest that both the thiol and the bromide participate in the rate-determining step of the reaction, consistent with an S_N2 mechanism.

The known sensitivity of single- and double-stranded DNA conformations on distal base stacking or base pairing interactions[4] suggests that groups distal from the bromide or thiol could play important roles in inducing stereoselectivity. To test these possibilities, we replaced 11 of the 12 template nucleotides closest to the 5′ bromide in the end-of-helix reaction with chiral abasic phosphoribose linkers in which the aromatic base was replaced with a proton (Figure 2, left). Even though the 5′ thymidine nucleotide closest to the bromide was unchanged, the resulting reactions were not stereoselective, indicating that the nucleotide closest to the bromide was not sufficient to induce the observed stereoselectivity. We restored each of the 11 missing aromatic bases from the 5′ end (Figure 2, left) and measured rates of the (*S*)-bromide and (*R*)-bromide reaction for each resulting template. Surprisingly, we observed no stereoselectivity when up to five bases were restored, and we observed steadily increasing stereoselectivity up to k_S/k_R = 4.3 when 6−11 bases were restored (Figure 2, top right). Restoration of the missing aromatic bases from the 3′ end of the abasic region instead of from the 5′ end also induced stereoselectivity only after several bases were restored (5−11 bases, in this case) (Figure 2, bottom right). Collectively, these findings suggest that stereoselectivity arises from the conformation of nucleotides

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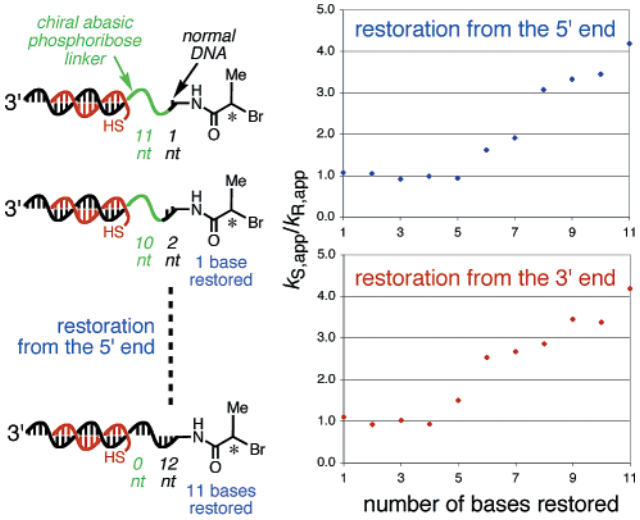

Figure 2. Deletion and restoration of aromatic bases between reactive groups in DNA-templated reactions. Stereoselectivity as a result of restoring aromatic DNA bases from the 5′ end (left and top right) or from the 3′ end (bottom right) of the 12-base intervening region is shown.

adjacent to either reactant and that the conformation(s) leading to stereoselectivity require ≥5−6 consecutive aromatic bases. We speculate that this requirement arises from the role of base stacking in stabilizing single-stranded helical conformations. Indeed, single-stranded DNA of mixed sequence has been reported to retain helical structure even when approximately one-half of its aromatic bases are removed.[5]

This model of stereoselectivity predicts that global conformational changes in the template−reagent complex could alter stereoselectivity even if the covalent structure and absolute stereochemistry of all reactants were preserved. Double-stranded DNA sequences rich in (5-Me-C)G repeats can adopt a left-handed helix (Z-form) rather than the usual right-handed helix (B-form) at high salt concentrations.[6−8] We prepared bromide-linked (5-Me-C)G-rich hairpin templates and complementary thiol-linked reagents protected as unreactive disulfides. When combined in equimolar ratios, the circular dichroism (CD) spectra of the resulting template−reagent complexes in low salt (100 mM NaCl) were characteristic of B-form DNA (see Supporting Information). In the presence of high salt concentrations (5 M NaCl or 2.5 M Na_2SO_4), the same template−reagent complexes exhibited CD spectra representative of Z-form DNA. In contrast, the CD spectra of template−reagent complexes of normal sequence were representative of B-form DNA under both low salt and high salt conditions, as expected.

We then compared the stereoselectivity of DNA-templated reactions between bromide-linked templates and thiol-linked reagents using either the mixed or the (5-Me-C)G-rich sequences in the presence of low or high salt concentrations. Consistent with our initial observations, the mixed sequence templates and reagents (B-form DNA) in the presence of low or high salt concentrations favored the (*S*)-bromide by 4.3- or 3.2-fold, respectively (Figure 3). The (5-Me-C)G-rich template and reagent in low salt concentrations (B-form DNA) exhibited a 4.4-fold preference for reaction of the (*S*)-bromide. Remarkably, repeating this reaction in the presence of high salt concentrations that induce Z-form DNA resulted in a 14-fold change in stereoselectivity, now favoring the (*R*)-bromide by 3.2-fold ($k_S/k_R = 0.31$) (Figure 3). This inversion of stereoselectivity as a result of changing the handedness of the DNA double helix is consistent with our model implicating the conformation of the template and reagent in determining the stereoselectivity of this DNA-templated reaction.

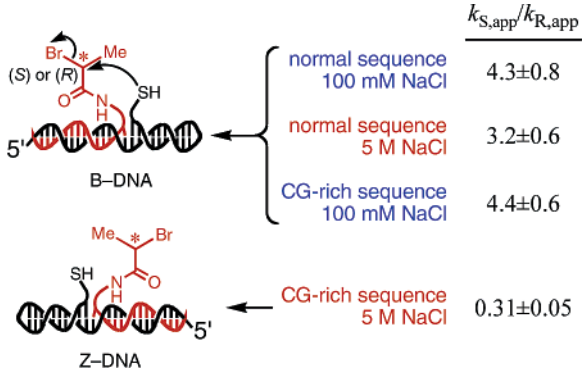

Figure 3. Stereoselectivities of DNA-templated reactions mediated by right-handed helix (B-form) or left-handed helix (Z-form) hairpin architectures. Values reflect the mean and standard deviation of at least three independent experiments.

This work is the first example to our knowledge of stereoselectivity during general nucleic acid-templated organic synthesis. Conformations of DNA dependent on base stacking together with a partially constrained presentation of reactants are responsible for the observed stereoselectivity. During the course of these studies, we discovered that a single structure with one absolute stereochemistry can induce opposite stereoselectivities when its macromolecular conformation is altered. Understanding the conditions under which templated reactions are stereoselective is required to perform DNA-templated synthesis in a manner that is general for the widest possible range of substrate structures and stereochemistries. In addition, our results reveal a unique way in which the nature of a nucleic acid template can influence the outcome of a laboratory-designed (or a prebiotic) chemical reaction.

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Supporting Information Available: Experimental details, additional data, and template and reagent oligonucleotide sequences and structures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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