

Enantioselective Cyclization of Photochromic Dithienylethenes Bound to DNA**

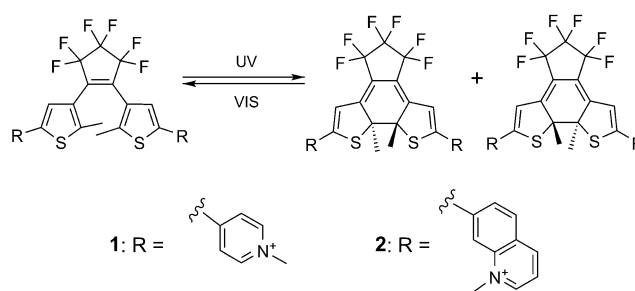
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Since the majority of the DNA-mediated processes occurring in our cells can be inhibited by molecules binding to the double helix, photoactivation of the DNA binding process would provide a dramatically improved spatiotemporal control over the action of many biochemical processes. With this idea as the inspiration, the DNA binding properties of a number of photochromic systems have recently been studied.^[1] A spiropyran,^[1a,b] a chromene,^[1c] and a benzothiazoloquinolinium^[1d] have all been shown to have only one form of the photochromic molecule which interacts with DNA, and DNA binding can therefore be modulated by irradiation. Conversely, both the isomers of a dithienylethene (DTE) derivative were shown to bind to DNA with similar binding affinities.^[1e] The typical derivative from the DTE photochromic family can be reversibly switched between the open and closed forms with high fatigue resistance and no thermal isomerization.^[2] Photocyclization to the closed form of DTE produces two enantiomers, which are usually found as a racemic mixture in solution. Enrichment of one enantiomer for a symmetrically substituted DTE has previously only been reported in single crystals, wherein two types of crystals formed, each consisting of only one conformer of the open DTE, which could be subsequently isomerized to the corresponding closed form with high enantioselectivity.^[3] Diastereoselectivity, however, has been more readily obtained for DTEs using chiral, and often bulky, substituents in solution,^[4] in gels,^[5] or in single crystals.^[6]

In solution, inclusion of achiral photoreactants in chiral supramolecular systems has been used to influence the chirality of the products for photocyclizations, photoisomerizations, photodimerizations, or photoadditions in systems including cyclodextrins,^[7] modified zeolites,^[8] and proteins.^[9] Though stereoselective binding to DNA is commonly observed, and DNA has been used to indirectly transfer chirality through an asymmetric catalyst in ground-state reactions,^[10] there is only one reported example using DNA to directly influence the chiral outcome of a photoreaction. Wada et al. observed modest enantiomeric excesses for the

photoisomerization of cyclooctene when complexed with DNA.^[11] Herein we report the binding of a methylpyridinium- and a methylquinolinium-appended DTE to DNA and the achievement of enantioselectivity for the closing reaction of the methylquinolinium DTE derivative in the presence of DNA. This is a rare example of direct transfer of chirality from DNA to a photoproduct and it is also the first reported example of enantioselectivity for a DTE derivative in solution.

DTE symmetrically substituted with methylpyridinium chloride (**1**) or methylquinolinium chloride (**2**) groups (Scheme 1) were synthesized as described in the Supporting



Scheme 1. Dithienylethenes **1** and **2** in the open form and in the closed form with enantiomers of *S,S* or *R,R* configuration.

Information. The permanent positive charges which result from methylation of the heterocyclic nitrogen atoms lead to good water solubility of both **1** and **2**. The open forms **1**_{open} and **2**_{open} absorb exclusively in the UV region (Figure 1). Isomerization to the closed form is triggered by UV light. After isomerization with $\lambda = 366$ nm UV light the photostationary states were determined from ¹H NMR spectra to be greater than 98% **1**_{closed} for **1** and approximately 92:8 **2**_{closed}/**2**_{open} for **2**. The closed forms **1**_{closed} and **2**_{closed} have significant absorption in the visible region (Figure 1), and reverse isomerization using broadband visible light ($\lambda > 530$ nm) converts both **1** and **2** into 100% of the open form. No thermal interconversion was observed after weeks at ambient temperature.

Changes in the absorption spectra of both the open and closed forms of **1** and **2** on addition of calf thymus DNA (*ct*-DNA) indicated that both isomers of these DTE derivatives bind to DNA. Similarly an amine-terminated DTE has been previously shown to bind to DNA in both forms when protonated.^[1e] The changes in the absorption spectra for both isomeric forms of **1** and **2** upon addition of *ct*-DNA are similar. An initial decrease in the absorption was seen with no change in the wavelength of the absorption maxima at high

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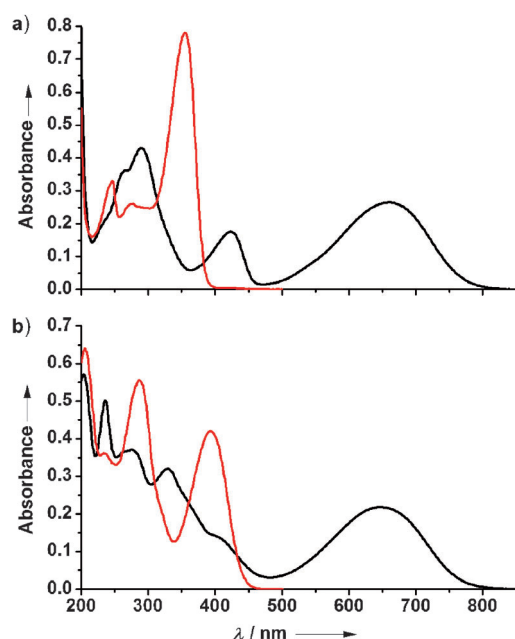


Figure 1. UV/Vis absorption spectra in 5 mM NaCl of a) 1_{open} (red) and 1_{closed} (black) and b) 2_{open} (red) and 2_{closed} (black).

DTE/DNA ratios, whereas upon further addition of DNA a red shift in the absorption maximum and an increase in the absorption was observed (see Figure S1 in the Supporting Information). This behavior is indicative of more than one type of binding interaction between each DTE and *ct*-DNA (see assignment below).

To show circular dichroism (CD) molecules must be inherently chiral or be perturbed by a chiral environment. In solution the achiral open form of symmetric DTEs or the racemic mixture (*S,S* + *R,R*) of the chiral closed form show no CD signal, however when bound to DNA induced CD signals are observed. CD titrations showed an increase in signal on addition of *ct*-DNA to DTE for both isomers of **1** and **2** (Figures 2 and 3 for 2_{open} and 2_{closed} , respectively; see Figures S2 and S3 in the Supporting Information for 1_{open} and 1_{closed} , respectively). Only specific binding interactions

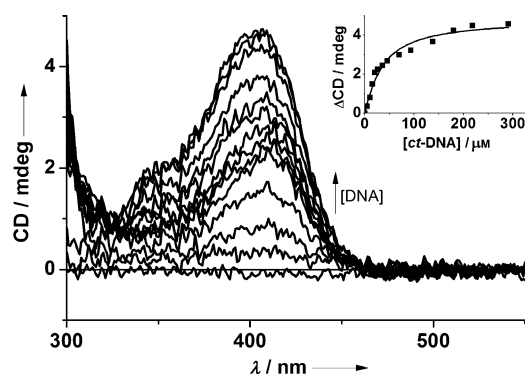


Figure 2. CD spectra of 2_{open} (15.5 μM) in 5 mM NaCl in the presence of increasing concentrations of *ct*-DNA (0–290 μM b.p.). Inset: binding isotherm at $\lambda = 405$ nm for the binding of 2_{open} to *ct*-DNA fit to Equation S1 in the Supporting Information.

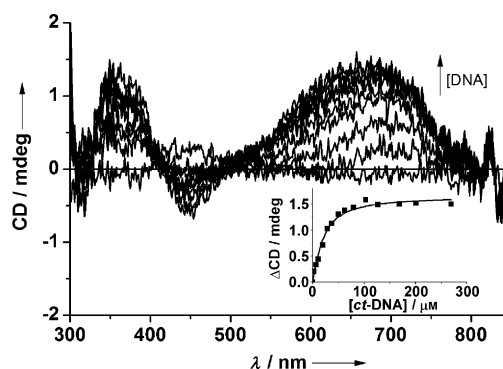


Figure 3. CD spectra of 2_{closed} (14.3 μM) in 5 mM NaCl in the presence of increasing concentrations of *ct*-DNA (0–270 μM b.p.). Inset: binding isotherm at $\lambda = 675$ nm for the binding of 2_{closed} to *ct*-DNA fit to Equation S1 in the Supporting Information.

such as groove binding or intercalation will give rise to a strong CD signal. Unlike the UV titrations above, the CD titrations showed no obvious indication of multiple binding modes between the DTEs and *ct*-DNA. We therefore assign the behavior observed at high DTE/DNA ratios in the absorption titrations to electrostatic binding of the positively charged **1** or **2** with the negative phosphate backbone of DNA, as such nonspecific interactions are likely to contribute little to the CD spectra. The second binding mode observed at low DTE/DNA ratios in the absorption titrations is the binding observed in the CD spectra, which we assign to a specific binding interaction between the DTE and DNA. Nonspecific binding is likely to have little effect on the chiral outcome of the DTE-closing reaction and we expect any observed enantioselectivity to arise from intercalative or groove binding interactions, especially at the relatively high DNA concentration used where the specific binding mode predominates.

Apparent binding constants for the specific binding mode were estimated from the CD titrations assuming a 1:1 binding stoichiometry per DNA base pair (see the Supporting Information for details). Binding constants of $(5.0 \pm 0.1) \times 10^5 \text{ M}^{-1}$ and $(2.3 \pm 0.1) \times 10^5 \text{ M}^{-1}$ were obtained for 1_{open} and 1_{closed} , respectively, and binding constants of $(2.8 \pm 0.5) \times 10^5 \text{ M}^{-1}$ and $(7.6 \pm 1.1) \times 10^5 \text{ M}^{-1}$ were obtained for 2_{open} and 2_{closed} , respectively.

In flow-oriented linear dichroism (LD) experiments DNA is oriented in a shear flow and any LD signal from the DTE is due to specific binding to DNA. The sign of the LD signal contains valuable information about the orientation of the DTE relative to DNA. Molecules which interact with DNA through intercalation between the base pairs exhibit only negative bands in the LD spectra, whereas molecules which bind in the DNA groove tend to have both negative and positive LD bands.^[12] Inspection of the LD spectra shows only negative bands for 1_{open} , while both negative and positive bands were seen for 1_{closed} (Figure 4a). This observation shows that the interaction of 1_{open} with *ct*-DNA is intercalative, while 1_{closed} interacts with *ct*-DNA through groove binding. Only negative bands were observed in the LD spectra of 2_{open} and 2_{closed} (Figure 4b), thus demonstrating that both isomers of **2**

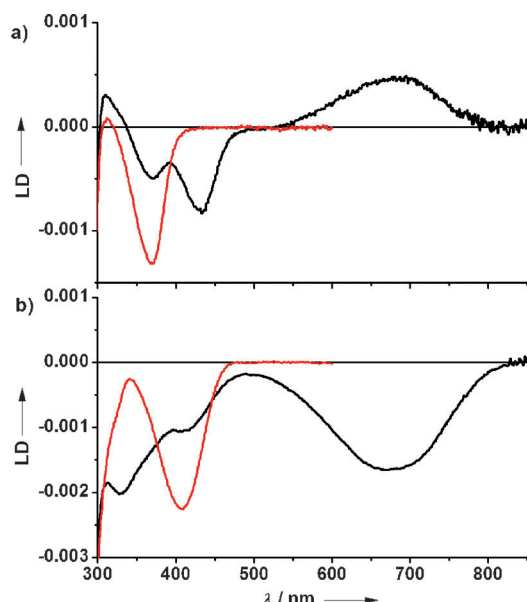


Figure 4. a) LD spectra for 1_{open} (red) and 1_{closed} (black) ($18\ \mu\text{M}$) in 5 mM NaCl with $200\ \mu\text{M}$ *ct*-DNA. b) LD spectra for 2_{open} (red) ($14\ \mu\text{M}$) and 2_{closed} (black) ($13\ \mu\text{M}$) in 5 mM NaCl with $175\ \mu\text{M}$ *ct*-DNA.

interact with *ct*-DNA through intercalation. The binding mode of the closed forms is also supported by a comparison of the intensity of the bands in the LD spectra with those in the corresponding isotropic absorption spectra (see the Supporting Information).

The observed rate of the closing reaction is not significantly changed in the presence of DNA. Thus, although binding is a dynamic process, isomerization of **1** and **2** can easily occur while bound to DNA without dissociation from the double helix. However, a remarkable difference was observed between the CD spectra obtained for a sample of **2** where racemic 2_{closed} was formed in bulk solution then added to DNA, and for a sample where the closing reaction $2_{\text{open}} \rightarrow 2_{\text{closed}}$ was carried out in the presence of DNA (Figure 5a). The sample that was isomerized in the absence of DNA displays a positive CD band centered at $\lambda = 668\ \text{nm}$ when DNA is added. This CD signature is assigned to the induced CD from the racemic solution of 2_{closed} when bound to DNA. In sharp contrast, the sample that was isomerized in the presence of DNA displays only a very weak CD signal. This observation implies that closing 2_{open} in the presence of DNA does not lead to the formation of racemic 2_{closed} , but instead to the preferential formation of one of the enantiomers of 2_{closed} . The inherent CD signal of the favored enantiomer must be of opposite sign to that of the CD signal induced by DNA binding, thus resulting in the apparent disappearance of the long-wavelength band in the CD spectra for the latter solution.

SDS micelles were added to the above solutions to extract **2** from DNA. After addition of SDS the solution containing racemic 2_{closed} had no CD activity (red line in Figure 5b), as expected for complete extraction of **2** from DNA. In contrast, the solution where 2_{closed} was formed in the presence of DNA showed a significant negative CD signal following extraction

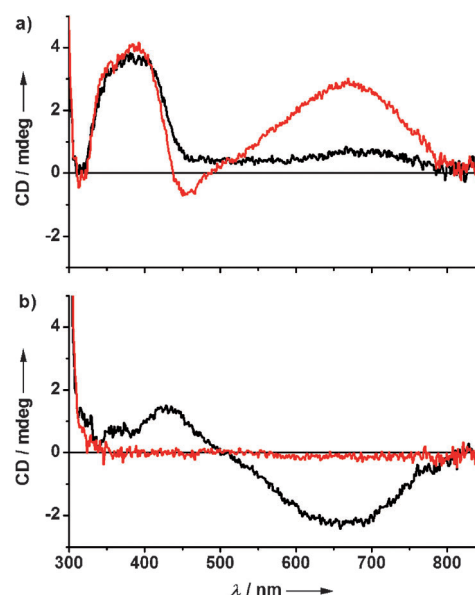


Figure 5. a) CD spectra of **2** ($50\ \mu\text{M}$) closed with $\lambda = 366\ \text{nm}$ light in bulk solution then added to $480\ \mu\text{M}$ *ct*-DNA (red) and closed with $\lambda = 366\ \text{nm}$ light in the presence of $480\ \mu\text{M}$ *ct*-DNA (black). b) CD spectra of above solutions on addition of 50 mM SDS.

from DNA (black line in Figure 5b). This inherent CD signal confirms that this solution has an excess of one of the enantiomers of 2_{closed} . Further irradiation of this extracted solution with visible light to form 2_{open} and subsequent UV irradiation to reform 2_{closed} led to the disappearance of the CD signal, that is, the enantioselectivity achieved when **2** is bound to DNA is lost following isomerization in bulk solution. Unfortunately, to date, attempts to separate the enantiomers and quantify the enantiomeric excess achieved have not been successful.

For **1** no difference was observed for the CD spectra of 1_{closed} bound to DNA when the closing reaction was carried out in the absence or presence of DNA (see Figure S4 in the Supporting Information), thereby indicating that no significant enantioselectivity is achieved for the $1_{\text{open}} \rightarrow 1_{\text{closed}}$ reaction in the presence of DNA. Previously, a similar experiment with a DTE that underwent photoisomerization to the closed form while included in a cyclodextrin cavity showed no indication of enantiomer enrichment.^[13]

The conformers of the DTE open form undergo fast exchange in solution, thus resulting in the formation of a racemic mixture of the closed form on photocyclization. The observed enantioselectivity of the photocyclization of **2** in the presence of DNA indicates either that one conformer of 2_{open} is preferentially bound to DNA or that when bound one conformer is more easily able to undergo isomerization to 2_{closed} . The binding affinities for both forms of **1** and **2** with *ct*-DNA are of a similar magnitude and the main difference in the interaction of **1** and **2** with DNA is that the binding modes of 2_{open} and 2_{closed} are the same (intercalation), while 1_{open} and 1_{closed} have different binding modes (intercalation and groove binding, respectively). The fact that enantioselectivity is achieved for the closing reaction of **2** but not for **1** could be explained by this difference in binding modes. Since the

binding mode of **1** changes during isomerization it seems likely that this would lead to a disruption of the interaction of **1** with the chiral environment of DNA when moving along the reaction coordinate from **1**_{open} to **1**_{closed}. This would not be the case for **2** where isomerization may occur freely within the chiral environment of the double helix. Consequently, chirality transfer from DNA occurs in the case of **2**, but not in the case of **1**.

In summary, we report two water-soluble, positively charged DTEs, which bind to *ct*-DNA in both the open and closed forms. Significant enantioselectivity was observed for the DTE closing reaction for the DTE that interacts with DNA through intercalation in both forms. The observed enantiomeric enhancement in this system is a rare realization of direct chirality transfer from DNA. Moreover, using this approach to affect the chiral outcome of the DTE photocyclization allowed us to obtain enantioselectivity for symmetric DTE derivatives in solution for the first time.

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