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# DNA triplex stabilization by a δ-carboline derivative tethered to third strand oligonucleotides

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**Abstract**—A δ-carboline derivative was covalently coupled to a 7 mer oligonucleotide at its 5′- or 3′-end. The stability of triplexes formed from the conjugates and a double-helical target was studied by UV melting experiments. Compared to the unmodified control triple helices, triplexes with the conjugate exhibit a significantly higher stability. However, the degree of stabilization depends on the particular triplex structure formed.


In recent years, much effort has been devoted to the design of ligands that are capable of specific binding to double-helical nucleic acids. Strategies for the site-specific targeting of DNA represent the basis for numerous practical applications including the control of gene expression,[1,2] site-directed mutagenesis[3,4] or mapping of genomic DNA.[5,6] Due to the highly specific interactions involved and the ability of oligonucleotides to penetrate cell walls in either direct or indirect ways, triple helix formation through binding of a third strand oligonucleotide to a double-helical DNA target has become a promising tool for the specific recognition of an appropriate DNA sequence.

Unfortunately, applications based on the recognition of double-stranded nucleic acids by triple helix-forming oligonucleotides (TFOs) are often hampered by the low stability of the formed triple-helical complexes, especially under physiological conditions. Consequently, the stabilization of triplexes has emerged as one of the most important challenges for more general future applications. In addition to a modification of backbone, bases, and sugar residues, the affinity of oligonucleotides for their complementary sequences has been enhanced by tethering various intercalators or minor groove binders to either their 5′- or 3′-termini.[7,8] Whereas a wide variety of DNA binding ligands have been shown to stabilize double-helical structures, only a limited number of compounds have been found to specifically interact with triplexes. These include various benzopyridoindole (BPI) and benzopyridoquinoxaline (BPQ) derivatives,[9–12] dibenzophenanthrolines,[13] substituted anthraquinones[14] as well as ligands based on a quinoline moiety.[15–17] We have recently reported the synthesis of phenyl-substituted δ-carboline derivatives, that due to their low solubility in an aqueous solution caused only a modest, but noticeable, increase in the melting temperature of a triplex when added as a free drug.[18] Based on their potential triplex stabilizing effects, we report here the synthesis of a δ-carboline–TFO conjugate and present initial UV melting studies on triplexes formed by these conjugates with several target duplexes.

For its covalent attachment to the oligonucleotide, 1,7-dimethyl-5-(4-methyloxycarbonylphenyl)-6*H*-indolino[3,2-*b*]quinoline[18] was hydrolyzed under alkaline conditions and the resulting carboxylic acid **1** was activated by addition of *N*-hydroxysuccinimide (NHS) and of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (DCI) to a suspension of **1** in dry DMF (Scheme 1). The NHS ester **2**[19] was isolated in 39% yield and was coupled to 5′- or 3′-aminoalkylated oligonucleotides that were prepared using the standard *O*-β-cyanoethyl phosphoramidite method. A suspension of NHS ester **2** in DMF was added to the amino-modified oligodeoxynucleotide **3** in a K-phosphate buffer (pH 8.2) and the reaction mixture was stirred at 50 °C for 7 days. The

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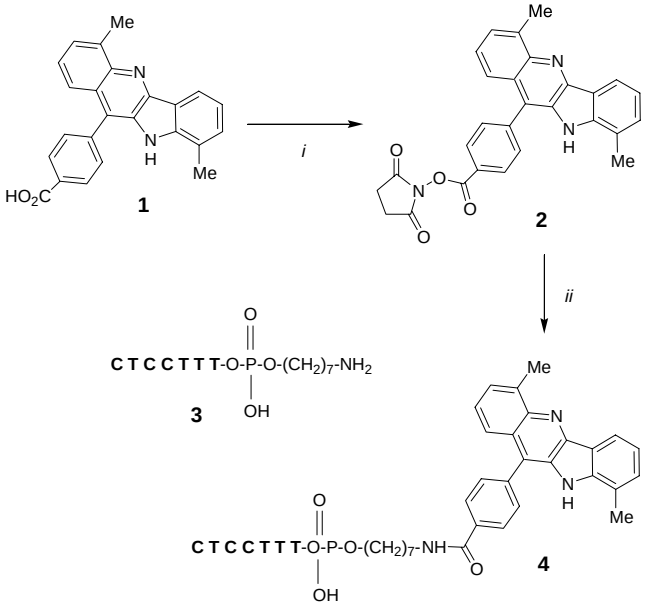


**Scheme 1.** Synthesis of the oligonucleotide–δ-carboline conjugate. Reagents and conditions: (i) NHS, DCI, DMF, rt, 39%; (ii) **3**, DMF/phosphate buffer (pH 8.2), 50 °C, 7 days, 10%.

coupling was monitored by thin-layer chromatography (*n*-propanol/water = 5:3). Despite a prolonged reaction time, the yield of coupling product **4**, which was isolated by separation, purification (HPLC), and desalting, did not exceed 10%. The low yields can be attributed to the limited solubility of **2** in the reaction mixture which could, however, not be substantially improved by minor adjustments of the solvent system. The conjugates were identified by simultaneous absorptions at 260, 281, 347, and 407 nm, and characterized through ESI mass spectrometry.

To study the ability of the δ-carboline derivative in stabilizing triple helices, UV thermal melting experiments were performed with the conjugates in the presence of a hairpin duplex target (Fig. 1). The design of the oligonucleotides used for the present studies allows for a

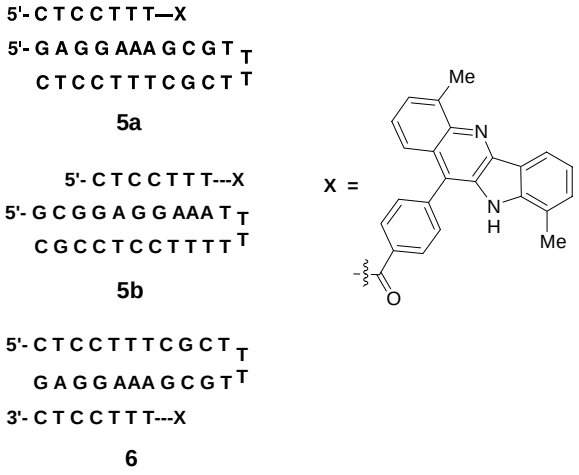


**Figure 1.** Sequence of triplexes studied.

triplex-duplex junction and is also based on previous observations showing the formation of a well-defined triplex at lower pH for the intramolecular construct.[20,21] As for the duplex melting at higher temperature, the dissociation of the third strand from the duplex is characterized by a hyperchromicity at 260 nm and the midpoint temperature of this transition $T_m$ indicates the relative stability of the triplex (Fig. 2). Melting temperatures with the δ-carboline conjugates, as determined by a first derivative plot of the temperature-dependent absorbances, are summarized together with the corresponding values from unmodified reference samples in Table 1. The results clearly show that the triple helix **5a** formed with the conjugate is significantly more stable than its unmodified control triplex with an increase in melting temperature of 22 °C and thus close to the most potent triple helix stabilizing intercalators known. Because **5a** exhibits a triplex stem with a short duplex overhang (Fig. 1), either the duplex-triplex junction or the triplex stem are possible sites for interaction. It has to be noted that we only observe a rather small stabilization of a duplex formed from the δ-carboline conjugate and its complementary strand.

To investigate the relative importance of interactions at these different sites with the conjugate, we also

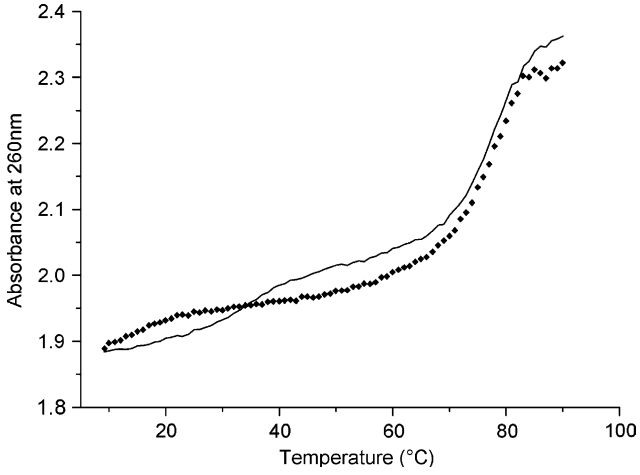


**Figure 2.** Temperature-dependent absorbance at 260 nm for triplex **5a** (solid line) and its unmodified drug-free reference (dotted line). [Triplex] = 10 μM, 50 mM NaCl, 50 mM Na-acetate, pH 5.0.

**Table 1.** UV melting temperatures of triplexes[a,b]

| Sample | $T_{m1}$ (°C) triplex-duplex | $T_{m2}$ (°C) duplex-single strand | $\Delta T_{m1}$ (°C) triplex-duplex |
|---|---|---|---|
| **5a** | 36.5 | 78 | 22 |
| Ref. | 14.5 | 78 | |
| **5b** | 32 | 75 | 15 |
| Ref. | 17 | 75 | |
| **6** | 27.5 | 79 | 7.5 |
| Ref. | 20[c] | 79 | |

[a] 50 mM NaCl, 50 mM Na-acetate, pH 5.0, and 10, 8, and 4 μM triplex concentrations for **5a**, **5b**, and **6**, respectively.
[b] Average of three independent measurements; uncertainty ± 1 °C.
[c] Very broad transition; uncertainty ± 4 °C.

performed melting temperature studies of triplex **5b** (Fig. 1) with a modified target duplex where the duplex overhang is shifted to the opposite end of the drug attachment (Fig. 3). For interactions within the triplex region, $\Delta T_m$ is expected to not significantly be affected when the overhang duplex sequence is removed for direct interactions with the drug. However, while still highly stabilizing, a decrease in $\Delta T_m$ of about 7 °C may be attributed to some stabilizing interactions at the triplex-duplex junction.

We have also studied triplex **6** with a 5′-attachment of the drug to the third strand oligonucleotide and reversed polarities in all strands as compared to triplex **5a** (Fig. 1). The strand polarity may profoundly affect the formation of intramolecular DNA triple helices and triplexes with a purine tract at the 3′-end have often been found to exhibit some structural heterogeneity.[20] Indeed, the thermal melting curve of the unmodified reference for **6** with the purine tract at the 3′-end of the hairpin duplex only displays a very broad melting below the duplex dissociation at 79 °C (Fig. 4). However, with the 5′-conjugate as a third strand a sharper transition centered at 27.5 °C is observed for **6**. Although much lower in melting temperature than those of triplexes **5a** and **5b**, these results may provide an indication that the drug specifically stabilizes a triplex conformation.

Without any third strand base modifications or the presence of other triplex stabilizers like $Mg^{2+}$ cations or polyamines, the short triplexes of the pyrimidine motif as used here only exhibit melting temperatures above 10 °C at lower pH. However, the significant triplex stabilization by the δ-carboline moiety also indicates its ability in promoting the formation of stable complexes in a cellular system under appropriate conditions. It is also interesting to note that recent studies using oligonucleotide conjugates with a δ-carboline moiety lacking methyl and phenyl substituents showed no or only weak triplex stabilization.[22] It has to be determined, whether substituent or sequence effects are responsible for this very different strength of interactions. Work is in progress, to study the sequence-dependence of binding in more detail and also to obtain more structural information on drug binding.

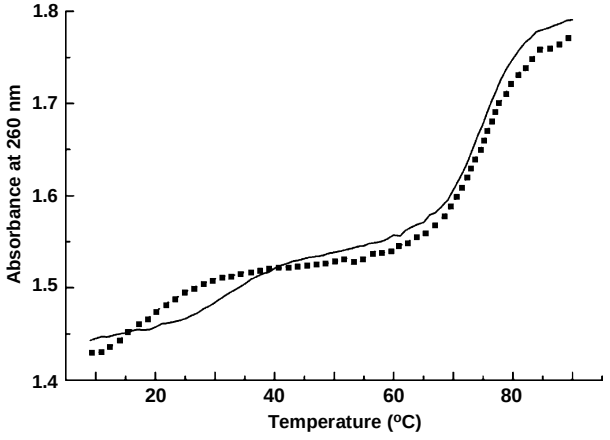


**Figure 3.** Temperature-dependent absorbance at 260 nm for triplex **5b** (solid line) and its unmodified drug-free reference (dotted line). [Triplex] = 8 μM, 50 mM NaCl, 50 mM Na-acetate, pH 5.0.

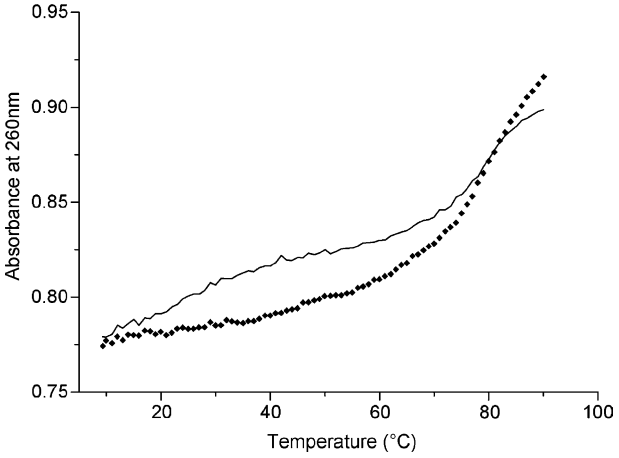


**Figure 4.** Temperature-dependent absorbance at 260 nm for triplex **6** (solid line) and its unmodified drug-free reference (dotted line). [Triplex] = 4 μM, 50 mM NaCl, 50 mM Na-acetate, pH 5.0.

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