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### Sequence-Specific Recognition of Double-Stranded DNA by Synthetic Minor Groove Binder Conjugates. toward the Construction of Artificial Site-Specific Deoxyribonucleases

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## SEQUENCE-SPECIFIC RECOGNITION OF DOUBLE-STRANDED DNA BY SYNTHETIC MINOR GROOVE BINDER CONJUGATES. TOWARD THE CONSTRUCTION OF ARTIFICIAL SITE-SPECIFIC DEOXYRIBONUCLEASES

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□ *Bis-conjugates of hairpin N-methylpyrrole/N-methylimidazole oligocarboxamide minor groove binders (MGB) possessing enhanced affinity and sequence-specificity for dsDNA were synthesized. Two hairpin MGBs were connected by their N-termini via an aminodiacetate linker. The binding of bis-MGB conjugates to the target DNA was studied by gel mobility retardation, footprinting, and circular dichroism; their affinity and binding mode in the DNA minor groove were determined. In order to functionalize the bis-MGB conjugates, DNA-cleaving agents such as phenanthroline or bipyridine were attached. Effective site-specific cleavage of target DNA in the presence of Cu<sup>2+</sup> ions was observed.*

**Keywords** Minor groove binder; double-stranded DNA; DNA recognition; sequence specificity; conjugate; DNA cleavage

### INTRODUCTION

Antiparallel hairpin *N*-methylpyrrole and *N*-methylimidazole oligocarboxamides are considered as potential gene-directed agents.<sup>[1]</sup> They are able to recognize specifically double-stranded DNA sequences and bind them in the DNA minor groove (minor groove binders: MGB) according to Dervan's rules.<sup>[2]</sup> The DNA recognition area for non-modified hairpin MGB is about 4–6 base pairs. In a previous work, we conjugated two MGBs by a

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bifunctional linker, thus synthesizing bis-MGB conjugates linked together by their N-termini. The length of the DNA recognition sequence enhanced to 8–10 base pairs and the affinity was comparable to that of DNA-binding proteins ( $K_d = 3\text{--}5\text{ nM}$ ).<sup>[3]</sup>

In this work we present further studies of interaction between DNA and bis-MGB conjugates. Using gel mobility retardation, circular dichroism, and DNase footprinting we demonstrate that both parts of the conjugate interact with DNA in a sequence-specific manner forming two short antiparallel hairpins rather than one long parallel hairpin. Introduction of 1, 9-phenanthroline or 2, 2'-bipyridine between MGB components permits the construction of potential metal-chelating DNA cleaving agents.

## RESULTS AND DISCUSSION

Three different molecules of bis-conjugates were synthesized (Figure 1). The molecule **1** has been used for DNase I footprinting studies using a 492 base pair DNA fragment. The DNA fragment was prepared by digestion with restriction endonucleases NcoI and ApaI of a modified plasmid pGEM7(f+) containing an insert of synthetic oligonucleotides within the polylinker.<sup>[4,5]</sup> Results of footprinting studies (not shown) demonstrate protection by bis-conjugate **1** of all the T:A/A:T regions with a length of at least 7 base pairs. In addition, when two sequences contained at least three T:A/A:T base pairs separated by one or two G:C/C:G pairs, such regions also were protected, as, for example, the sequences AAAAGAAAA or TTTTGCAAAA, which is in agreement with our previous results from gel mobility retardation experiments.<sup>[3]</sup> These results indicated that two components of bis-conjugate interact with two sequences of three-four A:T/T:A base pairs

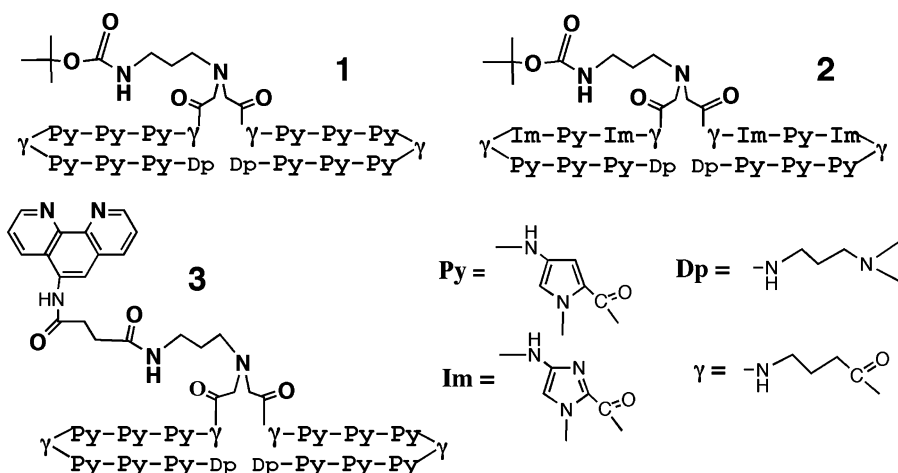
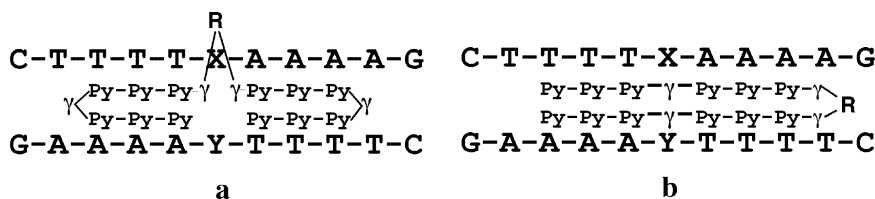


FIGURE 1 Bis-conjugates of hexa(N-methylpyrrole/N-methylimidazole) carboxamides.

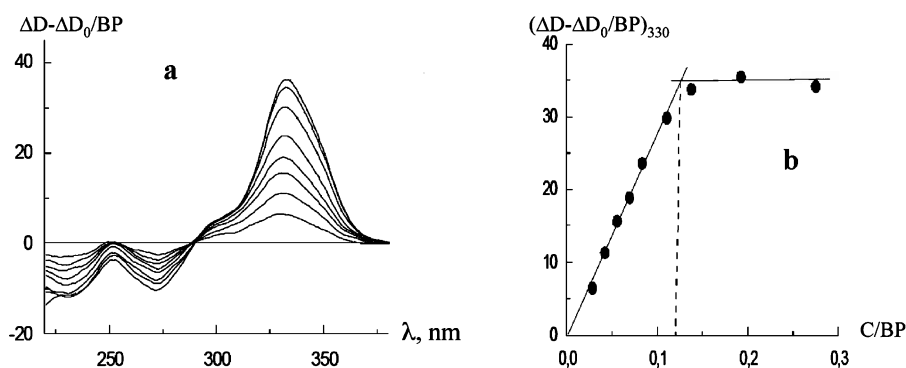


**FIGURE 2** Possible orientations of bis-MGB conjugate **1** in the DNA minor groove: (a) two short antiparallel hairpins; (b) one long parallel hairpin.

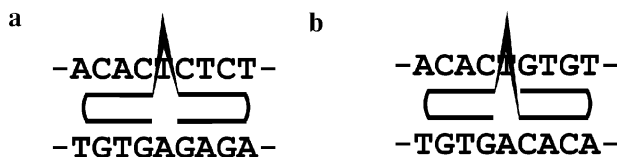
in the form of two short antiparallel hairpins leaving one or two central base pairs under the linker, as it is shown in Figure 2a, and not in a form of one long head-to-head parallel hairpin (Figure 2b).

In order to prove this binding mode, we studied the interaction of bis-MGB conjugate **1** with poly[d(AT)]:poly[d(TA)] duplex by circular dichroism.<sup>[6,7]</sup> Comparison of the CD spectra shown in Figure 3a to those obtained using models with fixed parallel, antiparallel and linear orientations<sup>[6]</sup> clearly indicated an antiparallel hairpin orientation of the minor groove binder in complex with the target DNA. CD titration of the target DNA with bis-MGB conjugate **1** revealed a binding stoichiometry of 0.12 bis-MGB molecules per base pair. This means that each bis-MGB molecule occupies a 8 base pair region of the DNA (Figure 3b).

The sequence specificity of bis-hairpin oligocarboxamides was demonstrated using the *N*-methylimidazole-containing molecules **2** (Figure 1). Two target oligonucleotide duplexes were synthesized in order to bind this conjugate in “direct” and “inversed” orientations as it is shown in Figure 4, according to Dervan’s rules. Gel mobility retardation was applied and apparent dissociation constants were calculated as described before<sup>[8]</sup>. The affini-



**FIGURE 3** (a) Differential circular dichroism spectra of poly[d(AT)]:poly[d(AT)] complexed with hexa(*N*-methylpyrrole) carboxamide bis-conjugate **1** (Figure 1). Duplex concentration was  $1.56 \cdot 10^{-4}$  M (b.p.), bis-MGB concentration varied from  $0.55 \cdot 10^{-5}$  to  $0.4 \cdot 10^{-4}$  M. (b) CD titration of DNA by bis-conjugate **1** at 330 nm ( $\Delta D$ —circular dichroism of the complex,  $\Delta D_0$ —circular dichroism of the duplex. BP—concentration of DNA in base pairs, C—concentration of bis-conjugate **1**).



**FIGURE 4** “Direct” (a) and “inversed” (b) orientations of bis-hairpin conjugate **2** in the minor groove of DNA.

ties of the ligand **2** to both cognate sequences ( $K_d = 4.1 \pm 0.8$  and  $3.6 \pm 0.5$  nM, respectively) are as high as those of bis-MGB conjugate **1** to HIV polypurine tract ( $K_d = 4.8$  nM) and the (A:T/T:A)<sub>n</sub> sequences ( $n > 7$ ).<sup>[3]</sup> This result confirms an antiparallel hairpin conformation of bis-MGB conjugates: in parallel orientation, two Im/Im pairs would form. Such a pair is not a DNA recognition unit, according to Dervan’s rules.

We also functionalized bis-MGB conjugate **1** by removing BOC-protection and attaching 5'-aminophenanthroline moiety (Figure 1, **3**) via a short linker in order to chelate Cu<sup>2+</sup> ions by phenanthroline and try to cleave the target DNA in the presence of reducing agents as in case of DNA-phenanthroline conjugates.<sup>[9]</sup> However, compound **3** was almost inactive. In order to shorten the distance between the cleaving moiety and the DNA backbone, we used phenanthroline and bipyridine to connect the two oligocarboxamides. The resulting bis-ligands in the presence of Cu<sup>2+</sup> ions revealed a site-specific deoxyribonuclease activity cleaving the target DNA in the binding region. Preliminary results demonstrated that sequence-specific nuclease activity (up to 45% cleavage at 2–3 adjacent bonds) may be achieved when chelating residues are deeply buried in the DNA minor groove. Detailed studies of new sequence-specific DNA cleaving agents are in progress in our laboratory and will be published soon.

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