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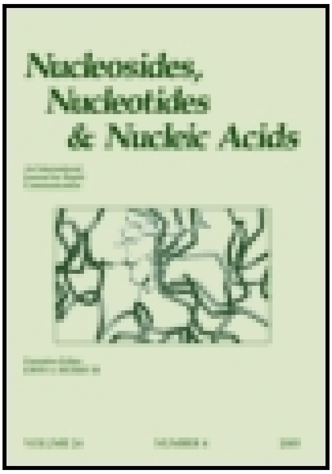

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HAIRPIN MIMICS WITH PHENANTHROLINE- AND BIPYRIDINE-DERIVED LINKERS

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□ *The synthesis and structural stabilities of modified oligonucleotide hairpins containing phenanthroline- and bipyridine-modified loops is reported. Phenanthroline (phen) and bipyridine (bipy) building blocks were synthesized, incorporated into DNA-oligonucleotides, and analyzed by thermal denaturation experiments. The so modified oligomers were found to form stable hairpin structures. Tm values were not affected by divalent transition metals.*

Keywords oligonucleotide hairpins; phenanthroline; bipyridine; thermal denaturation.

Metal-coordinating oligonucleotide conjugates combine the ability of sequence specific DNA or RNA recognition with the function of metal coordination through appropriate ligands. This class of bioconjugates is actively investigated due to their importance in the field of artificial nucleases,[1–3] the self assembly of metals arrays,[4,5] electron transfer,[6,7] and as sequence specific probes and sensors.[8–10] We have endeavored in the design, synthesis, and structural investigation of metal-coordinating DNA hairpin mimics. The hairpin is a secondary structural motif of nucleic acid which is most commonly found in all biological systems.[11] Due to its important role in the structural and functional behavior of nucleic acid, it has been the subject of numerous studies. Thus, several nonnucleosidic scaffolds such as oligo ethylene glycol linkers,[12] phenanthrene,[13] stilbene, naphthalene, and diphenylacetylene[14] have been reported as loop replacements in DNA hairpins, as well as metal-bridged[15] hairpins and metallo-salen[16] derived DNA hairpins. Metal coordinating ligands as loop replacements offer the possibility of metal-coordination in addition to serving as a structural scaffold. Previously, we have reported that the replacement of terpyridine in DNA loop shows a strong improvement of hairpin stability in the absence of metal in comparison to the dT_4- and dA_4-loop.[17] We extended this work

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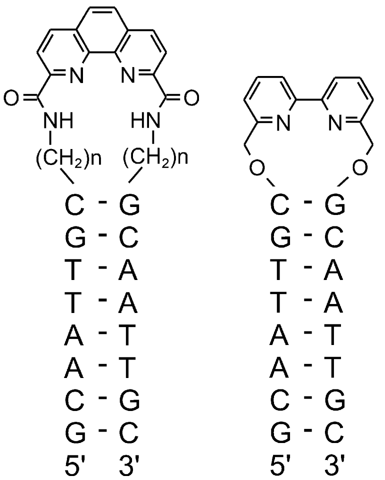
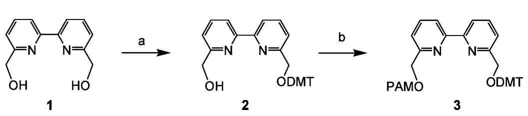


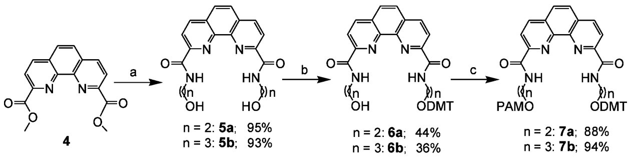
FIGURE 1 Illustration of phenanthroline- and bipyridine-modified hairpin structures.

to different oligopyridine ligands that are known to bind to transition metal ions. Here, we report the synthesis and properties of a bipyridine and a phenanthroline based metal-coordinating hairpin mimic (Figure 1).

Following the known synthesis of bipyridine derivatives,[18,19] 6,6′-bis(hydroxymethyl)-[2,2′]-bipyridine (**1**) was prepared. Mono-protected compound **2** was obtained after reaction of **1** with 4,4′-dimethoxytrityl chloride (DMT-Cl). Compound **2** was further converted into the corresponding phosphoramidite **3** by conventional phosphitylation (Scheme 1). The dimethyl ester of [1,10]phenanthroline derivative **4** was synthesized according to the described procedures.[20,21] Amides **5a** and **5b**, containing a 2- and 3-carbon linker were obtained by reaction of **4** with 2-aminoethanol



SCHEME 1 (**a**) 4,4′-Dimethoxytrityl chloride, pyridine, r.t. 24 hours; (**b**) $(iPr_2N)_2P(OCH_2CH_2CN)$, N,N-diisopropylammonium 1H-tetrazolide, CH_2Cl_2, r.t, 1 h. DMT: 4,4′-dimethoxytrityl; PAM: *N,N*′-diisopropyl-2-cyanoethyl phosphoramidite.



SCHEME 2 (**a**) 2-Aminoethanol (or 3-aminopropanol), reflux, 1 hour; (**b**)) 4,4′-dimethoxytrityl chloride, pyridine, r.t. overnight; (**c**) $(iPr_2N)_2P(OCH_2CH_2CN)$, N,N-diisopropylammonium 1H-tetrazolide, CH_2Cl_2, r.t. 90 minutes.

TABLE 1 Phenanthroline- and bipyridine-containing hairpins **8–10** and control oligonucleotides **11-13** used in this study and Tm values obtained by thermal denaturation experiments. Conditions: 1.5 μM oligomer concentration, 10 mM phosphate buffer, 100 mM NaCl, pH = 7.5; temperature gradient: 0.5°C/minute. Melting temperatures were determined from the maximum of the first derivative of the melting curve (A_{260} against temperature); exptl. error ±0.5°C. Abbreviations (C2) and (C3) refer to $(CH_2)_2$- and $(CH_2)_3$-linkers, respectively.

		Tm(°C)			
Oligomers	Sequences	(−)	Co(II)	Zn(II)	Cu(II)
8	5′-GCAATTGC-phen(C2)-GCAATTGC-3′	64.0	65.4	64.5	—[a]
9	5′- GCAATTGC-phen(C3)-GCAATTGC-3′	63.0	62.7	63.5	—[a]
10	5′- GCAATTGC-bipy-GCAATTGC-3′	62.1	—[a]	—[a]	63.0
11	5′- GCAATTGC- 3′	29.0	—[a]	—[a]	—[a]
12	5′- GCAATTGC-AAAA-GCAATTGC-3′	73.6	—[a]	—[a]	—[a]
13	5′- GCAATTGC-TTTT-GCAATTGC-3′	74.6	74.4	74.6	74.8

[a] Values not determined.

and 3-aminopropanol, respectively. Mono-protection of **5a** and **5b** with DMT-Cl afforded the corresponding compounds **6a** and **6b** which were further transformed into desired phenanthroline phosphoramidites **7a** and **7b** (Scheme 2).

The syntheses of the phenanthroline- and bipyridine-modified oligomers **8**, **9**, and **10** were performed by automated oligonucleotide synthesis (Table 1). Oligonucleotides **11**, **12**, and **13** were prepared for control purposes.

The stability of the oligonucleotides in the presence and absence of different metals was analysed by thermal denaturation experiments. The Tm (melting temperature) values obtained are shown in Table 1. Tm values were independent of the oligomer concentration (0.5–5 μM) demonstrating that the observed process is monomolecular (data not shown). The phenanthroline-modified hairpins **8** and **9** as well as the bipyridine-modified

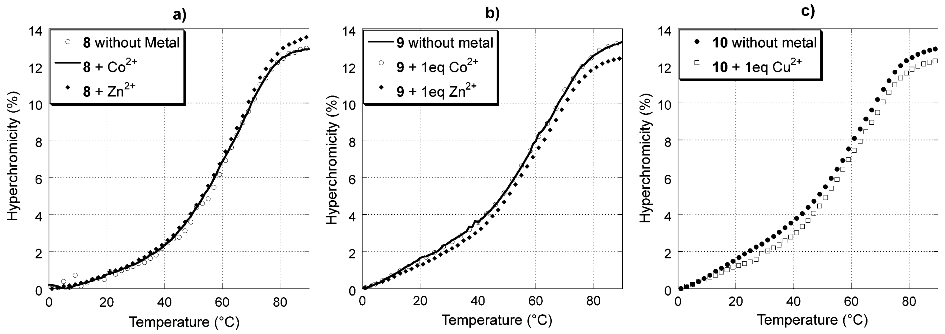


FIGURE 2 Influence of different metals on the thermal denaturation curves of the phenanthroline-modified hairpins **8** (a) and **9** (b) as well as the bipyridine-modified hairpin **10** (c). Conditions: 1.5 μM oligomer concentration, 10 mM NaH_2PO_4, 100 mM NaCl, pH = 7.5.

hairpin **10** had lower Tm's (64.0, 63.0, and 62.1°C, respectively) than the corresponding dA_4- or dT_4-hairpins in the absence of polyvalent cations. This is in contrast to previous reports,[12–14] for which generally a substantial increase in the Tm was observed. Most importantly, no change in the Tm-values was observed with phenanthroline- and bipyridine-modified hairpins upon addition of metals, such as Co(II), Zn(II) or Cu(II) as shown in Figure 2.[22]

CONCLUSIONS

Phenanthroline and bipyridine phosphoramidite building blocks were synthesized and incorporated into self complementary oligonucleotides. The so modified oligomers were found to form stable hairpin structures. In comparison to the analogous control hairpins containing either a dA_4- or dT_4-hairpin loop, all modified structures were found to be less stable. Moreover, the hairpin mimics were not sensitive to the presence of divalent metals, i.e., Co(II) or Zn(II) in the case of the phenanthroline- and Cu(II) in the case of the bipyridine-modified hairpin.

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