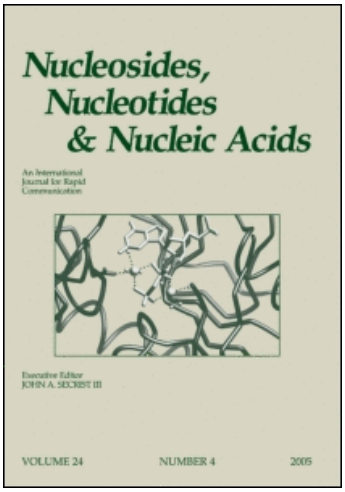

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Ivan Trkulja[a]; Alfred Stutz[a]; Robert Häner[a]
[a] Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland

To cite this Article Trkulja, Ivan , Stutz, Alfred and Häner, Robert(2007) 'A Phenanthrene Modified RNA Hairpin', Nucleosides, Nucleotides and Nucleic Acids, 26: 8, 879 — 882
To link to this Article: DOI: 10.1080/15257770701505352
URL: http://dx.doi.org/10.1080/15257770701505352

Nucleosides, Nucleotides, and Nucleic Acids, 26:879–882, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701505352

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A PHENANTHRENE MODIFIED RNA HAIRPIN

Ivan Trkulja, Alfred Stutz, and Robert Häner □ *Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland*

□ *The influence of hairpin loop replacement with the phenanthrene moiety in RNA was investigated. The stability of this novel structure was compared to a hairpin with a U_4 loop, an extra stable tetra-loop (UUCG), and an analogous phenanthrene modified DNA hairpin. Thermal denaturation experiments and CD spectra were used to study the structure and stability of the modified hairpin.*

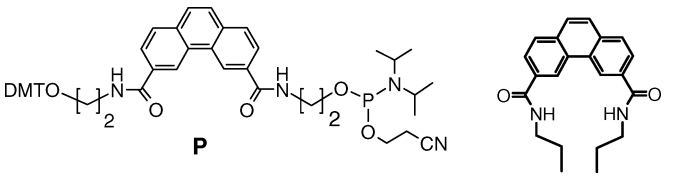
Keywords Hairpin loop; phenanthrene moiety; modified hairpin

Hairpins are one of the most common and most important secondary structural elements of RNA secondary structure.[1] In functional RNA, they are essential elements for the formation of the correct three-dimensional structure.[2–5] Hairpins containing a four-base loop (tetraloop) have emerged as unusually stable components of ribonucleic acids.[1,6,7] The importance of the hairpin motif has raised an interest in its replacement with synthetic, nonnucleosidic linkers.[8] The non-natural modifications promise higher resistance in biological media, better cellular uptake and improved hybridization properties.[9] Therefore, the hairpin loop has been replaced or modified, with flexible, oligoethylene glycol linkers in RNA.[10–12]

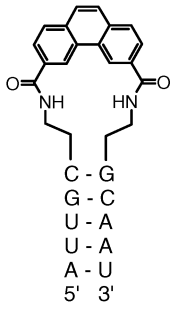
We previously have demonstrated that self-complementary oligodeoxynucleotides containing 3,6-disubstituted phenanthrenes adopt highly stable hairpin-like structures.[13] Since hairpins are predominantly an RNA secondary structure, we have aimed to replace the hairpin loop in a oligoribonucleotide with the phenanthrene moiety. Where polyethylene linkers already have been used to synthesize RNA hairpin mimic, the use of aromatic building blocks to this end has not yet been reported. Of primary interest to us was the stability of the modified RNA hairpin in comparison to its natural analogues, as well as its DNA counterpart.

The synthesis of the phenanthrene phosphoramidite **P** (Scheme 1) was performed as described.[13,14] The building block was further used for the

Address correspondence to Robert Häner, Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH 3012 Bern, Switzerland. E-mail: robert.haener@ioc.unibe.ch



1 5' r(AUUGC **P** GCAAU)
2 5' r(AUUGC **UUCG** GCAAU)
3 5' r(AUUGC **UUUU** GCAAU)
4 5' d(ATTGC **P** GCAAT)
5 5' d(ATTGC **TTTT** GCAAT)



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SCHEME 1 Phosphoramidite building block and hairpins used in this study; bold: hairpin loop; **1-3**: RNA hairpins; **4** and**5**: DNA hairpins. **P** is used as symbol for both the phenanthrene derived phosphoramidite and the incorporated building block.

preparation of the oligomer **1** and **4** by automated oligonucleotide synthesis. Incorporation of the phenanthrene monomer proceeded with coupling yields comparable to those of the unmodified nucleotide phosphoramidites. The obtained oligomers were purified by ion-exchange HPLC and characterized by mass spectrometry. Oligonucleotides **2**, **3**, and **5** served as control hairpins.

Consistently with hairpin (stem-loop) geometries, the oligomers used in this study exhibit unimolecular (concentration independent) transitions in thermal denaturation experiments (Table 1). Hairpin **1** shows an increase of 3.5°C in Tm (melting temperature) in comparison to hairpin **3** containing a U_4 loop. The stabilization effect of the phenanthrene linker is notable in comparison to the hairpins modified with flexible linkers,[10] which were found to be less stable than the corresponding sequences comprising a nucleotide loop. Furthermore, the comparison between the hairpins **1** and **4**, both phenanthrene modified but with different stems (RNA and DNA,

TABLE 1 Tm-values of haripins **1–5**

Hairpin	Tm(°C)[a]	Δ Tm(°C)
1	61.5	—
2	71.0	9.5[b]
3	58.0	−3.5[b]
4	65.7	—
5	60.0	−5.7[c]

Conditions: oligomer concentration 1.5 μM, 100 mM NaCl, 10 mM Tris-HCl buffer, pH = 7.4; temp. gradient: 0.5°C/min.

[a]Melting temperatures were determined from the maximum of the first derivative of the melting curve (A_{260} against temperature); exptl. error: ± 0.5°C.

[b]Difference in Tm relative to hairpin **1**.

[c]Difference in Tm relative to hairpin **4.**

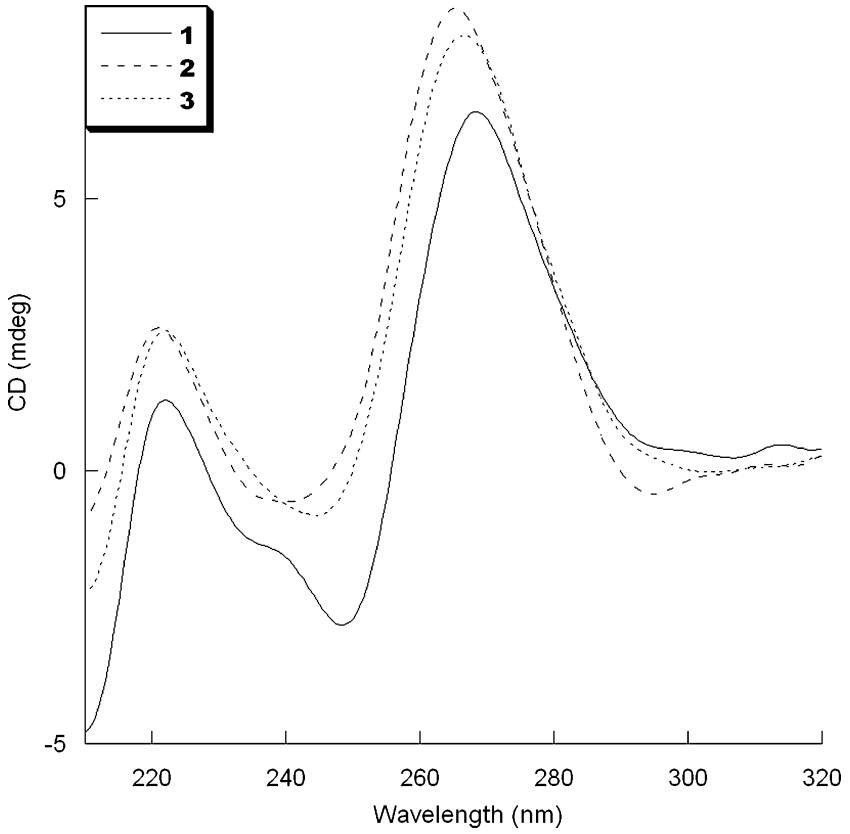

FIGURE 1 CD spectra of RNA hairpins **1–3**. Conditions: oligomer concentration 1.5 μM, 100 mM NaCl, 10 mM Tris-HCl buffer, temperature 25°C. Spectra were recorded on a Jasco J-715 spectropolarimeter equipped with a Jasco PFO-350S temperature controller.

respectively) showed that the DNA hairpin mimic is more stable. Oligomer **2** is a hairpin that contains an extra stable UUCG tetraloop and is characterised by a high Tm of 71.0°C. This specific sequence, 5′-UUCG in messenger RNA prevents reverse transcriptase from reading through.[15] The unusual stability of this structure can be explained by formation of a reverse wobble U · G base pair between the first and the last base pair of the loop and G *syn*, as well as the hydrogen bond formation between the amino group of the cytosine with a neighboring phosphate.[7]

The circular dichroism (CD) spectra of the RNA hairpins were measured to compare the oligomers with and without modification. All spectra (Figure 1) show typical A-form features.[12] The spectrum of the modified hairpin **1** shows is very similar to the ones of the two natural hairpins (**2** and **3**) over the wavelength range examined (210–320 nm).

In conclusion, we have incorporated a phenanthrene moiety into a self-complementary oligoribonucleotide. The stability of the hairpin is en-

hanced compared to the natural U_4-loop. CD spectra showed that the overall structure of the hairpin is not significantly perturbed by the introduction of this aromatic molecule. This structure represents a novel model for RNA hairpin mimics.

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