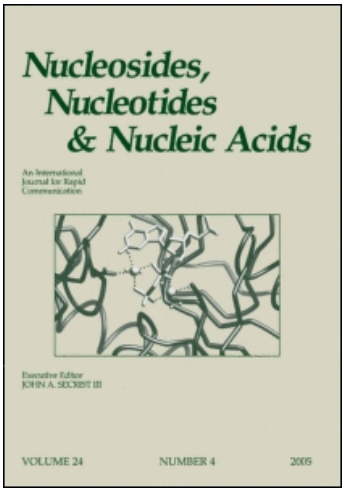

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

DNA-PHONA-PNA Chimeric Molecules: Contributions to Binding Against Complementary DNA

A. Peyman[a]; E. Uhlmann[a]; K. Wagner[a]; S. Augustin[a]; C. Weiser[a]; S. Hein[a]; D. Langner[a]; G. Breipohl[a]; D. W. Will[a]
[a] Hoechst Marion Roussel Deutschland GmbH, Frankfurt, Germany

To cite this Article Peyman, A. , Uhlmann, E. , Wagner, K. , Augustin, S. , Weiser, C. , Hein, S. , Langner, D. , Breipohl, G. and Will, D. W.(1998) 'DNA-PHONA-PNA Chimeric Molecules: Contributions to Binding Against Complementary DNA', Nucleosides, Nucleotides and Nucleic Acids, 17: 9, 1997 — 2001
To link to this Article: DOI: 10.1080/07328319808004739
URL: http://dx.doi.org/10.1080/07328319808004739

NUCLEOSIDES & NUCLEOTIDES, 17(9–11), 1997–2001 (1998)

DNA-PHONA-PNA Chimeric Molecules: Contributions to Binding against Complementary DNA

A. Peyman*, E. Uhlmann, K. Wagner, S. Augustin, C. Weiser, S. Hein, D. Langner, G. Breipohl, D. W. Will

Hoechst Marion Roussel Deutschland GmbH, D-65926 Frankfurt, Germany

Abstract: The synthesis of a DNA-PHONA-PNA chimeric molecule using the Mmt protection strategy is described. The chimeric oligomer shows duplex binding properties that are comparable to PNA. Obviously, PHONA building blocks can be incorporated into PNAs without distortion of the PNA structure

Numerous synthetic analogs of oligonucleotides have been described for their promising application in antisense chemotherapy and in DNA diagnostics.[1-4] Nielsen *et al.* have developed polyamide nucleic acid (PNA) mimics of DNA in which the entire deoxyribose-phosphate backbone has been exchanged with a structurally homomorphous uncharged polyamide backbone composed of *N*-(2-aminoethyl)glycine units.[5] Due to their excellent binding properties to complementary nucleic acids, PNAs have not only attracted much attention, they have also been widely chemically modified. [5,6] Examples for such modifications are PNA/DNA chimera[7] and PHONAs[8] which are derived from PNAs by replacement of the peptide bond by a phosphonic acid monoester group. The combination of various structural modifications leads often to molecules with interesting properties. We have recently reported on the synthesis and binding properties of alternating PNA-PHONA oligomers using the dimeric building block $\mathbf{t^{p}t}$ (Figure 1).[9] In

---

Dedicated to Professor Hartmut Seliger on the occasion of his sixtieth birthday

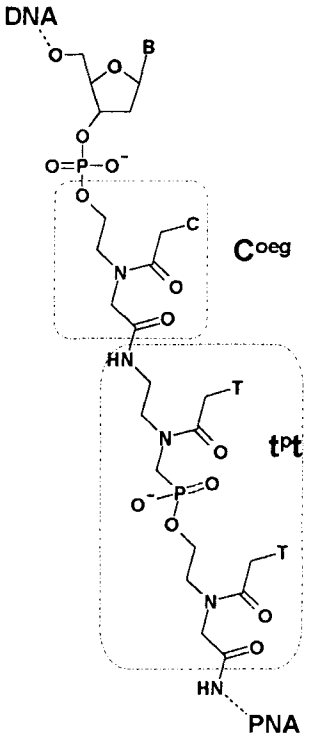

Figure 1: Structural elements of the PHONA-DNA-PNA chimeric molecule:
t^pt: dimeric building block for the synthesis of PHONA-PNA chimeric molecules.[9]
C^{oeg}: PNA-DNA linker molecule derived from *N*-(2-hydroxyethyl)glycine.[7]

the present work we used this building block to form a PHONA-DNA-PNA chimeric molecule, and we report data on its binding properties to complentary DNA.

The sequence of the DNA-PHONA-PNA chimeric oligomer **1** is shown in Table 1 and its synthesis is outlined in Figure 2. First, the PNA portion of the molecule is assembled according to previously reported procedures using the monomethoxytrityl protecting group strategy.[10] The dimeric PHONA-PNA building block $\mathbf{t^pt}$ is then coupled onto the amino-terminus by activation with HATU (0.1M solution in DMF) in the presence of *N*-ethylmorpholine (0.1M solution in DMF). The coupling yield is > 90%. After deprotection of the Mmt group, the PNA-DNA linker molecule $\mathbf{c^{oeg}}$ derived from *N*-(2-

Table 1: Sequence of the DNA-PHONA-PNA chimeric oligomer **1**, and its corresponding DNA-PNA (**2**) and DNA (**3**) analogs. DNA: capital letters; PNA: small letters, $\mathbf{t^pt}$: dimeric PHONA-PNA building block; $\underline{c}$: PNA-DNA linker molecule derived from *N*-(2-hydroxyethyl)glycine.[7]

1	(DNA-PHONA-PNA)	5'-A C A T $\underline{c}$ t^pt g g t c g -3'
2	(DNA-PNA)	5'-A C A T $\underline{c}$ t t g g t c g -3'
3	(DNA)	5'-A C A T C T T G G T C G -3'

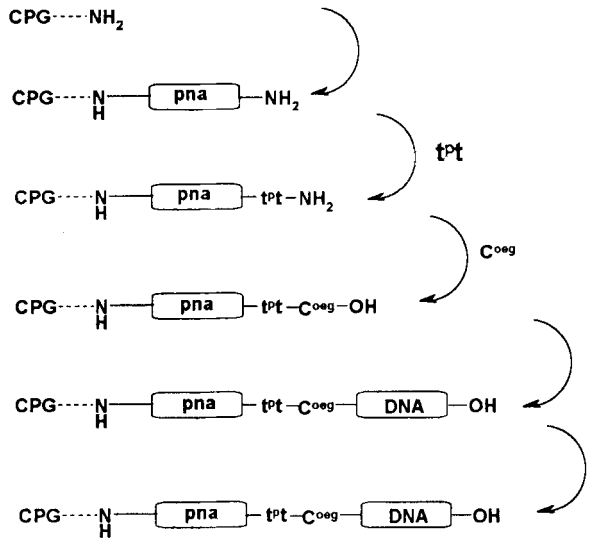

Figure 2: Outline of the synthesis of the PHONA-DNA-PNA chimeric oligomer **1**. For details see text.

hydroxyethyl)glycine[7], is introduced by coupling with HATU.[7] In the final step the DNA portion of the molecule is assembled by standard phosphoramidite chemistry and the chimeric oligomer is cleaved from the solid support and deprotected by treatment with conc. NH_4OH at 50 °C for 8 h. The crude product is then purified by PAGE, it appears as a single peak on HPLC (Dionex) and the ES-MS spectrum shows a mass of 3549.9 (M_{calc} for $C_{130}H_{170}N_{57}O_{54}P_5$: 3550.0). The corresponding DNA-PNA chimeric (**2**) and DNA (**3**) analogs were synthesized by standard procedures.

Table 2: Comparison of T_m values of the oligomers **1-3** when hybridized to complementary or partially mismatched DNA. The T_m values were measured under physiologically relevant conditions (140 mM KCl, 10 mM NaH_2PO_4, 0.1 mM Na-EDTA pH 7.4) by cooling from 85 °C to 15 °C at 1 $Kmin^{-1}$ and measuring the UV absorbance at 260 mM.

	Duplex	T_m (°C)		
		1•DNA	2•DNA	3•DNA
complementary sequence	5′-A C A T c̲ t^{p}t g g t c g -3′ 3′-T G T A G A A C C A G C -5′	48.1	50.5	48.8
mismatch 1	5′-A C A T c̲ t^{p}t g g t c g -3′ 3′-T G T A G A T̲ C C A G C -5′	36.1 *(-12.0 K)*	42.4 *(-8.1 K)*	38.3 *(-10.5 K)*
mismatch 2	5′-A C A T c̲ t^{p}t g g t c g -3′ 3′-T G T A G T̲ A C C A G C -5′	41.1 *(-7.0 K)*	44.4 *(-6.1 K)*	39.8 *(-9.0 K)*
mismatch 3	5′-A C A T c̲ t^{p}t g g t c g -3′ 3′-T G T A G T̲ T̲ C C A G C -5′	36.1 *(-12.0 K)*	42.3 *(-8.2 K)*	31.8 *(-10.0 K)*

To assess the binding affinities of such chimeric molecules the melting points of the duplexes of **1-3** with complementary DNA were determined (Table 2). The stability of the DNA-PNA•DNA duplex (T_m 50.5 °C) is only slightly increased as compared to the DNA•DNA duplex (T_m 48.8 °C). The DNA-PHONA-PNA chimeric oligomer **1** binds with almost equal affinity (T_m 48.1 °C) indicating that the introduction of a PHONA moiety into the PNA part of a DNA-PNA chimera does not result in a strong deviation from the PNA structure.

To determine the different contributions to binding of the two bases in the PHONA-PNA dimer we introduced mismatches in the complementary DNA, mismatch 1 opposite to the "PNA"-base, mismatch 2 opposite to the "PHONA"-base and mismatch 3 opposite two both bases (see Table 2). The drop in T_m for mismatch 1 is stronger than for mismatch 2 for all three sequences **1-3**. The most pronounced difference between the two mismatches as well as the largest absolute decrease of T_m is obtained with the chimeric oligomer **1**.

This suggests that the contribution to binding of the PHONA base is smaller than that of the PNA base. Interestingly, while there is a second drop in T_m for the DNA•DNA duplex when a second mismatch (mismatch 3) is introduced, there is no change in T_m for the DNA-PNA **2** or the chimeric DNA-PHONA-PNA oligomer **1** as compared to mismatch 1. Mismatch 1 also seems to eliminate the contribution of the base between the mismatch and C^{oeg}-DNA linkage of the molecules **1** and **2**, which also represents a structural distortion, so that the introduction of a mismatch opposite to this position has no further effect on duplex stability.

In conclusion it seems that PHONA building blocks can be incorporated into PNAs without distortion of the PNA structure.

REFERENCES

1) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, ***90***, 543-584.

2) Uhlmann, E., Peyman, A., Will, D. W. in *Enzyclopedia of Cancer*, Bertino, J. R., Ed., Academic Press, New York, 1997, p. 64-81.

3) De Mesmaker, A., Häner, R., Martin, P., Moser, H. E. *Acc. Chem. Res.* **1995**, ***28***,366-374.

4) Englisch, U., Gauss, D. *Angew. Chem. Int. Ed. Engl.* **1991**, ***30***, 613-630.

5) Hyrup, B., Nielsen, P. E. *Bioorganic & Medicinal Chemistry* **1996**, ***4***, 5-23.

6) Uhlmann, E., Peyman, A. Will, D. W., Breipohl, G., *Angew. Chem.*, submitted.

7) Uhlmann, E., Will, D. W., Breipohl, G., Langner, D., Ryte, A. *Angew. Chem. Int. Ed. Engl.* **1996**, ***35***, 2632-2635.

8) Peyman, A., Uhlmann, E., Wagner, K., Augustin, S., Breipohl, G., Will, D. W., Schäfer, A., Wallmeier, H. *Angew. Chem. Int. Ed. Engl.* **1996**, ***35***, 2636-2638.

9) Peyman, A., Uhlmann, E., Wagner, K., Augustin, Weiser, C., S., Breipohl, G., Will, D. W. *Angew. Chem.*, in the press.

10) Will, D. W., Breipohl, G., Langner, D., Knolle, J., Uhlmann, E. *Tetrahedron* **1995**, ***51***, 12069-12082.