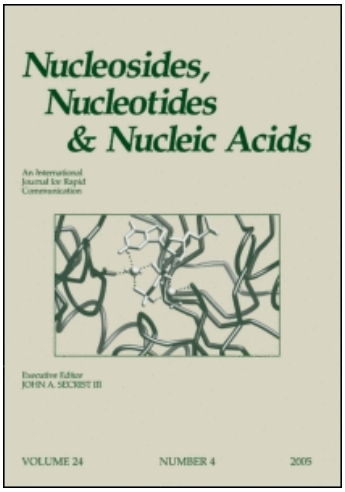

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ENGINEERING PREFERENCES OF HAIRPIN PNA BINDING TO COMPLEMENTARY DNA: EFFECT OF N7G IN *aeg*/*aep* PNA BACKBONE

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ABSTRACT

*Aeg*PNA and *aep*PNA monomeric units bearing the N7-guanine nucleobase as a substitute for C^+ have been demonstrated to bind to a GC base-pair of a duplex in a pH-independent manner when placed in the third strand. The *aep*PNA backbone exerts a preference for binding in the antiparallel Hoogsteen mode over the parallel Hoogsteen mode.

Aminoethylglycyl (*aeg*) Peptide Nucleic Acids (PNAs) are a class of DNA mimics that are neutral and achiral and bind to complementary DNA/RNA sequences with high affinity and sequence specificity (1). Homopyrimidine PNA_2:DNA triplex formation is pH-dependent due to the requirement of N3-protonation of cytosine for the Hoogsteen (HG) mode of recognition. PNA, with no configurational and conformational constraints, accommodates both Watson-Crick (WC) & HG binding modes in either antiparallel or parallel orientations (2). Aminoethylprolyl (*aep*)PNA recently reported by us (3) is a chiral positively charged PNA analogue and oligothymine *aep*PNAs show excellent binding properties with complementary DNA sequences. Chiral *aep*PNA is homomorphous with DNA and may induce orientational preferences in binding to DNA sequences. Mixed polypyrimidine sequences using this chiral backbone are therefore expected to display orientation-selective binding to DNA.

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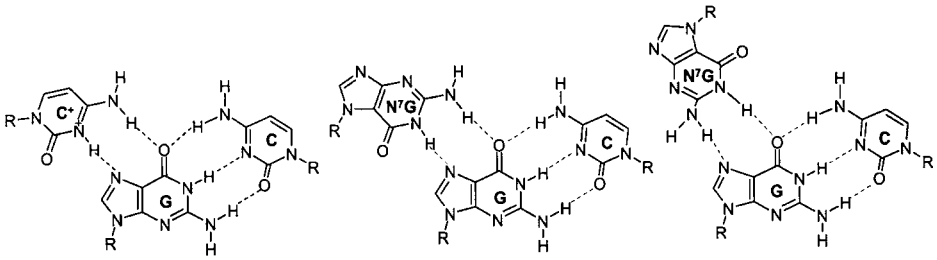

Figure 1. Hydrogen bonding schemes.

In this communication, we describe the synthesis of triplex forming mixed polypyrimidine sequences using the *aep*PNA units at predetermined positions and study of their effect on directing the orientation selectivity. N7G is known to mimic C^+ in its hydrogen bonding pattern (4) in the HG mode (Fig. 1) and hence, *aeg*/*aep*PNA-N7G monomeric units are introduced to circumvent the requirement of acidic pH for stable triplex formation.

The *aeg*PNA-N7G ester **2** was synthesized by alkylation of N^2-isobutyryl-guanine G^{iBu} with the ethyl *N*-Boc-aminoethyl-*N*-chloroacetyl glycinate in presence of K_2CO_3. The protected monomer units, (2*S*, 4*S*) *aep*PNA-$N7G^{iBu}$ **6b** and (2*S*, 4*S*) *aep*PNA-C^{CBz} **6a** were synthesized similarly, by alkylation of G^{iBu} and C^{CBz} with the common intermediate **5**. The compound **5** was prepared by mesylation of the known compound **4** (3) (Scheme 1). The products were purified by column chromatography and characterized (5) by ^1H, ^{13}C and mass spectrometry. The ester hydrolysis in all cases was effected with 1N NaOH in aq. MeOH to get the monomer synthons **3**, **7a & 7b**. The PNA sequences (**PNA 9–11**) incorporating these new monomers are listed in Scheme 2. The other *aeg*PNA monomers, *N*-Boc-ϵ-aminohexanoic acid and protected lysine units were synthesized according to the literature procedures (6). The PNA oligomers synthesized by standard protocols (6) were purified by FPLC and characterized by MALDI-TOF mass spectrometry (5).

The designed control *bis*PNA sequence (**PNA 8**) with *aeg*PNA-C units in both arms binds to both DNA **12** and DNA **13** sequences without discrimination as studied by UV melting experiments ($\Delta Tm = 0^\circ C$, Table 1). A higher Tm was observed at acidic pH in both the cases ($\Delta Tm \sim 7^\circ C$) indicating equally efficient HG and WC modes of recognition in either orientation (Scheme 2) of the target strand. Replacement of *aeg*PNA-C in one of the arms by *aeg*PNA-N7G (**PNA 9**) allows pH-independent triplex formation with the target DNA strands **12 & 13**, again, without any orientation selectivity ($\Delta Tm = 2^\circ C$). This may be possible, as N7G is capable of recognizing a GC base pair in HG mode with either parallel or antiparallel orientation of the third strand.

In contrast to the *aeg*PNA backbone, introduction of *aep*PNA-C units in this sequence (**PNA 10**) led to a stronger binding with DNA **13** ($Tm = 48^\circ C$) as compared to **DNA 12** ($Tm = 37.5^\circ C$) at neutral pH exhibiting a significant bias in

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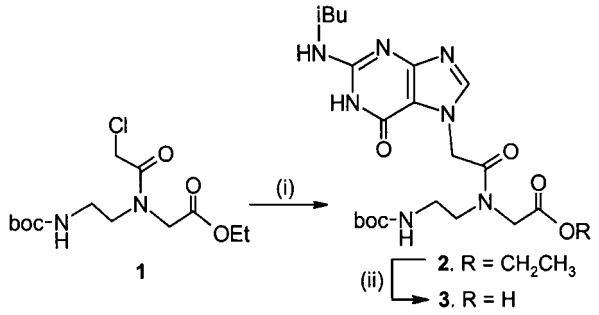
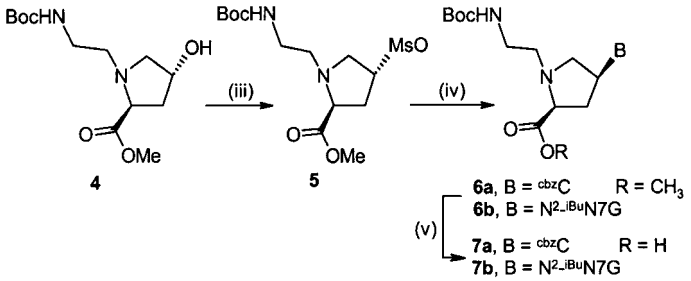


(i) N^{2}-^{iBu}G, K_2CO_3, DMF. (ii) NaOH, CH_3OH, H_2O.

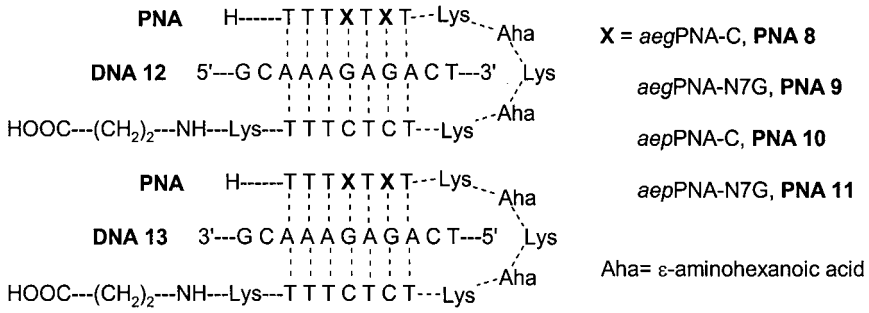
Scheme 1a.



(iii) Ms-Cl, pyridine. (iv) B, K_2CO_3, DMF. (v) NaOH, CH_3OH, H_2O.

Scheme 1b.

the directionality of binding (ΔTm $\sim$ 10°C). This selectivity was less pronounced (ΔTm $\sim$ 5°C) at acidic pH, which can be explained on the basis of the differential protonation of cytosine N3 in *aeg*PNA-C and *aep*PNA-C units in the HG strand. The *aep*PNA backbone being protonated even at physiological pH, N3-protonation of cytosine in *aep*PNA-C is probably less pronounced at pH 5.8 and is reflected



Scheme 2. PNA Oligomer Sequences.

Table 1.

DNA PNA		DNA 12 Tm (°C)	DNA 13 Tm (°C)
*aeg*PNA-C	**PNA 8**	45 (52.7)	45 (52)
*aeg*PNA-N7G	**PNA 9**	45 (47)	47 (45)
*aep*PNA-C	**PNA 10**	37.5 (47)	48 (52)
*aep*PNA-N7G	**PNA 11**	42 (43)	51 (49)

Figures in parentheses denote the Tm at 5.8 pH. Tm studies were performed in 0.01 M sodium phosphate buffer, pH 7.4 with a heating rate of 0.5°C per min. The Tm values were obtained from the peaks in the first derivative plots of % hyperchromicity at 260 nm Vs temperature and are accurate to ±1°C. Each experiment was repeated at least twice.

in the corresponding Tm values. This result indicates that the presence of chiral *aep*PNA units stabilizes the PNA:DNA complex when the HG strand containing these units is in the antiparallel orientation with respect to the DNA central strand. The *aep*PNA-N7G units in this sequence (**PNA 11**) confirm the selectivity for the antiparallel HG mode of binding of the third strand (ΔTm ∼9°C). The *aeg*PNA-C units of this sequence (**PNA 11**) are consequently in the WC mode of binding and hence, no change was observed in Tm at lower pH (ΔTm ± 1°C).

In summary, the introduction of *aep*PNA units in the backbone of triplex forming PNA oligomers strongly influences the recognition of DNA in an orientation selective manner. The triplex formation is pH-dependent when *aep*PNA-C units are used, while *aep*PNA-N7G units permit triplex formation at physiological pH.

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5. Characteristic signals: **2**: ^{1}H NMR ($CDCl_3$) δ: 10.1 (s, 1H, *G*-N*H*), 7.9 (s, 1H, *G*-*H*8). ^{13}C NMR δ: 167.2 (*G*-*C*H_2-CO), 162.9 (*G*-*C*2), 156.4 (*G*-*C*4), 148.0 (*G*-*C*6),

145.2 (*G*-*C*8), 112.2 (*G*-*C*5). **6b**: ^{1}H NMR ($CDCl_3$) δ: 12.1 (br s, 1H, *G*-N*H*), 8.4 (s, 1H, *G*-*H*8), 5.6 (m, 1H, *H*4). ^{13}C NMR δ: 156.4 (*G*-*C*2), 156.0 (*G*-*C*4), 147.5 (*G*-*C*6), 142.3 (*G*-*C*8), 111.5 (*G*-*C*5), 59.5 (*C*4). MALDI-TOF: **PNA 8:** $[M]_{obs} = 4499.75$, $[M]_{calc} = 4495.64$; **PNA 9:** $[M]_{obs} = 4580.88$, $[M]_{calc} = 4575.69$.

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