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

V. A. Kumar<sup>a</sup>; M. D'Costa<sup>a</sup>; K. N. Ganesh<sup>a</sup>

<sup>a</sup> Division of Organic Chemistry (Synthesis), National Chemical Laboratory, Pune, Maharashtra, India

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

V. A. Kumar,\* M. D'Costa, and K. N. Ganesh

Division of Organic Chemistry (Synthesis),  
National Chemical Laboratory, Pune, Maharashtra, India

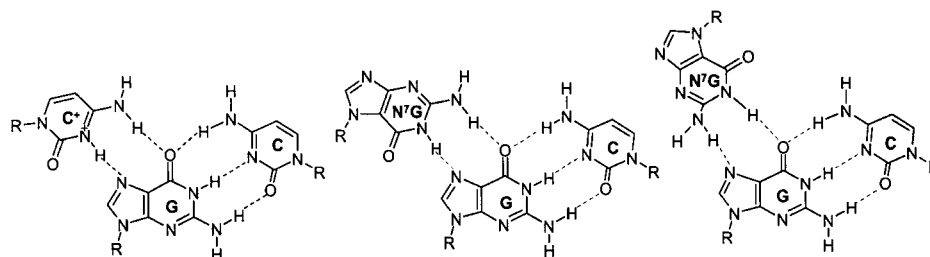
### ABSTRACT

*Aeg*PNA and *aep*PNA monomeric units bearing the N7-guanine nucleobase as a substitute for C<sup>+</sup> 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<sub>2</sub>: 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). Aminoethylpropyl (*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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\*Corresponding author.



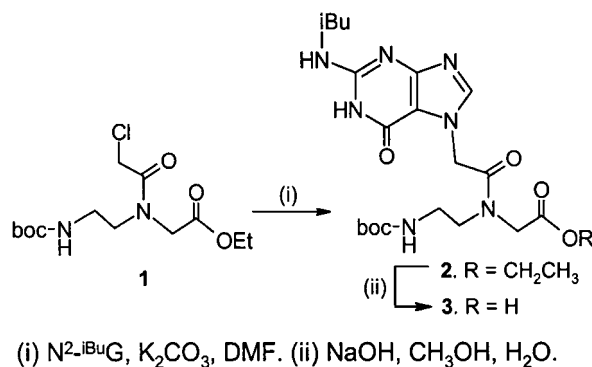
**Figure 1.** Hydrogen bonding schemes.

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<sup>+</sup> 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.

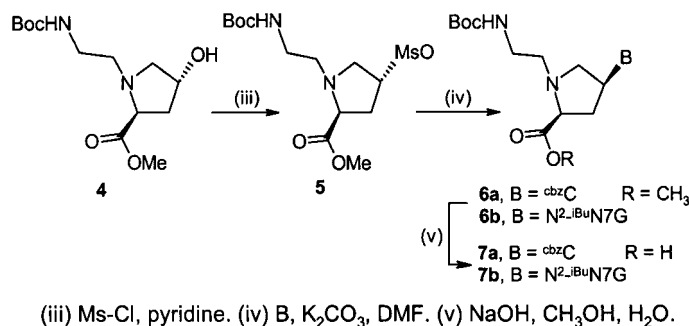
The *aeg*PNA-N7G ester **2** was synthesized by alkylation of N<sup>2</sup>-isobutyryl-guanine G<sup>iBu</sup> with the ethyl *N*-Boc-aminoethyl-*N*-chloroacetyl glycinate in presence of K<sub>2</sub>CO<sub>3</sub>. The protected monomer units, (2*S*, 4*S*) *aep*PNA-N7G<sup>iBu</sup> **6b** and (2*S*, 4*S*) *aep*PNA-C<sup>CBz</sup> **6a** were synthesized similarly, by alkylation of G<sup>iBu</sup> and C<sup>CBz</sup> 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 <sup>1</sup>H, <sup>13</sup>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- $\epsilon$ -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 ( $\Delta T_m = 0^\circ\text{C}$ , Table 1). A higher *T<sub>m</sub>* was observed at acidic pH in both the cases ( $\Delta T_m \sim 7^\circ\text{C}$ ) 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 ( $\Delta T_m = 2^\circ\text{C}$ ). 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** (*T<sub>m</sub>* = 48°C) as compared to DNA **12** (*T<sub>m</sub>* = 37.5°C) at neutral pH exhibiting a significant bias in

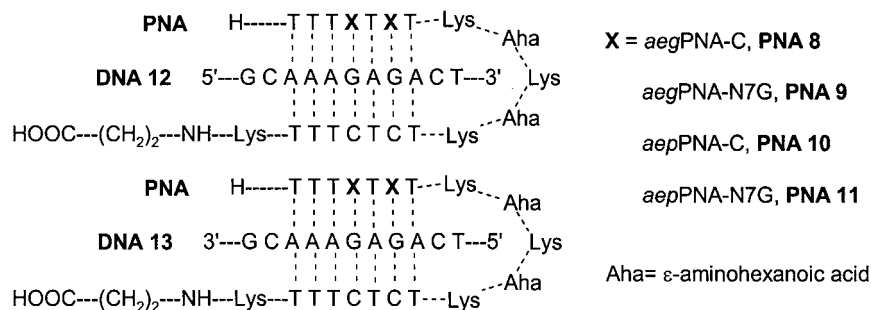


Scheme 1a.



Scheme 1b.

the directionality of binding ( $\Delta T_m \sim 10^\circ C$ ). This selectivity was less pronounced ( $\Delta T_m \sim 5^\circ C$ ) 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 T <sub>m</sub> (°C)	DNA 13 T <sub>m</sub> (°C)
<i>aeg</i> PNA-C	<b>PNA 8</b>	45 (52.7)	45 (52)
<i>aeg</i> PNA-N7G	<b>PNA 9</b>	45 (47)	47 (45)
<i>aep</i> PNA-C	<b>PNA 10</b>	37.5 (47)	48 (52)
<i>aep</i> PNA-N7G	<b>PNA 11</b>	42 (43)	51 (49)

Figures in parentheses denote the T<sub>m</sub> at 5.8 pH. T<sub>m</sub> studies were performed in 0.01 M sodium phosphate buffer, pH 7.4 with a heating rate of 0.5°C per min. The T<sub>m</sub> 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 T<sub>m</sub> 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 ( $\Delta T_m \sim 9^\circ\text{C}$ ). 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 T<sub>m</sub> at lower pH ( $\Delta T_m \pm 1^\circ\text{C}$ ).

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.

## ACKNOWLEDGMENTS

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5. Characteristic signals: **2**: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ : 10.1 (s, 1H, *G*-NH), 7.9 (s, 1H, *G*-H8). <sup>13</sup>C NMR  $\delta$ : 167.2 (*G*-CH<sub>2</sub>-CO), 162.9 (*G*-C2), 156.4 (*G*-C4), 148.0 (*G*-C6),



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- 145.2 (*G*-C8), 112.2 (*G*-C5). **6b**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$ : 12.1 (br s, 1H, *G*-NH), 8.4 (s, 1H, *G*-H8), 5.6 (m, 1H, *H*4),  $^{13}\text{C}$  NMR  $\delta$ : 156.4 (*G*-C2), 156.0 (*G*-C4), 147.5 (*G*-C6), 142.3 (*G*-C8), 111.5 (*G*-C5), 59.5 (*C*4). MALDI-TOF: **PNA 8**:  $[\text{M}]_{\text{obs}} = 4499.75$ ,  $[\text{M}]_{\text{calc}} = 4495.64$ ; **PNA 9**:  $[\text{M}]_{\text{obs}} = 4580.88$ ,  $[\text{M}]_{\text{calc}} = 4575.69$ .
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