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## Nucleosides, Nucleotides and Nucleic Acids

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### Synthesis and Properties of a Novel Bridged Nucleic Acid Analogue, 5'-Amino-3',5'-BNA

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## SYNTHESIS AND PROPERTIES OF A NOVEL BRIDGED NUCLEIC ACID ANALOGUE, 5'-AMINO-3',5'-BNA

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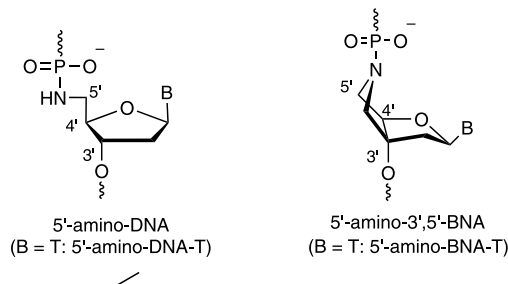
□ An oligonucleotide  $P3' \rightarrow N5'$  phosphoramidate (5'-amino-DNA) attracts much attention because of its potential for application to DNA sequencing; however, its ability to hybridize with complementary strands is low. To overcome this drawback of the 5'-amino-DNA, we have designed and successfully synthesized a novel nucleic acid analogue having a  $P3' \rightarrow N5'$  phosphoramidate linkage and a constrained sugar moiety, 5'-amino-3'-C,5'-N-methylene bridged nucleic acid (5'-amino-3',5'-BNA). The binding affinity of the 5'-amino-3',5'-BNA towards complementary DNA and RNA strands was investigated by UV melting experiments. The melting temperature ( $T_m$ ) of the duplex comprising the 5'-amino-3',5'-BNA and its complementary strand was much higher than that of the duplex containing the corresponding 5'-amino-DNA.

### INTRODUCTION

A nucleic acid analogue having a  $P3' \rightarrow N5'$  phosphoramidate linkage (5'-amino-DNA) is of great interest in genome science, because the  $P3' \rightarrow N5'$  phosphoramidate linkage is readily hydrolyzed under mild acidic conditions. In fact, several applications of the 5'-amino-DNA for a DNA sequence-determining technology have been reported to date.<sup>[1,2]</sup> However, the ability of the 5'-amino-DNA to hybridize with the complementary strand is decreased, probably due to an inappropriate  $\gamma$  dihedral angle ( $N5'-C5'-C4'-C3'$ ). <sup>1</sup>H NMR analysis of the 5'-amino-DNA dimer revealed that the  $+ap$  and  $-sc$  orientations for  $\gamma$  dihedral angle are favorable, which is quite different from the  $+sc$  orientation for  $\gamma$  in a typical DNA/DNA or RNA/RNA duplex.<sup>[3]</sup> We supposed that restriction of the  $\gamma$  dihedral angle into a suitable  $+sc$  orientation promotes stable duplex formation of the 5'-amino-DNA. We therefore designed and synthesized a novel 5'-amino-DNA analogue, 5'-amino-3'-C,5'-N-methylene bridged nucleic acid (5'-amino-3',5'-BNA) depicted in Figure 1.

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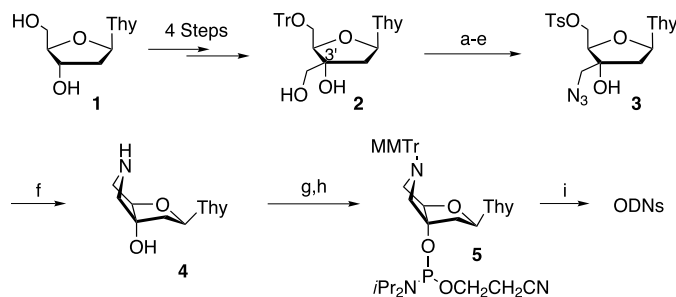
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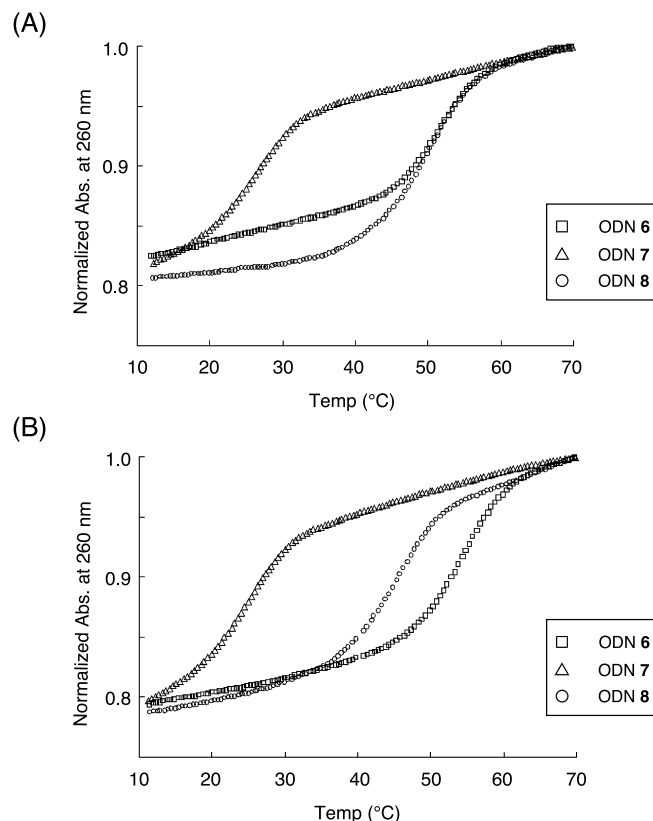
**FIGURE 1** Structure of the oligonucleotide P3'→N5' phosphoramidate analogues used in this work.

The synthetic route to the 5'-amino-3',5'-BNA thymidine monomer (5'-amino-BNA-T) and its amidite derivative is outlined in Scheme 1. Starting from thymidine **1**, 3'-*C*-hydroxymethylthymidine derivative **2** was obtained in 26% overall yield according to the literature.<sup>[4]</sup> Introduction of the azido group to the epoxide, and the following conversion of the 5'-*O*-trityl group to a *p*-toluenesulfonyl group, afforded **3** in 38% yield. Next, the desired compound, 5'-amino-BNA-T **4** was successfully synthesized by Pd-mediated hydrogenation. Both <sup>1</sup>H NMR and X-ray crystallographic analysis revealed that the  $\gamma$  dihedral angle of **4** is in the appropriate *+sc* orientation. Monomethoxytritylation of the secondary amine followed by phosphitulation gave phosphoramidite building block **5**. The obtained **5** was incorporated into 12-mer ODN **6** [5'-d(GCG**TTTTTT**GCT)-3', **T** = 5'-amino-BNA-T] using an automated DNA synthesizer. The corresponding 5'-amino-DNA modified ODN **7** [5'-d(GCG**tttttt**GCT)-3', **t** = 5'-amino-DNA-T] was also prepared according to the literature.<sup>[5]</sup>

To evaluate the hybridization property of the 5'-amino-3',5'-BNA, we have carried out UV melting experiments by using the 5'-amino-3',5'-BNA ODN **6**, the 5'-amino-DNA ODN **7**, and the corresponding natural DNA ODN **8** [5'-d(GCGTTTTTTTGCT)-3']. The melting temperature ( $T_m$ ) of the duplex



**SCHEME 1** (a) *p*TsCl, *n*Bu<sub>2</sub>SnO, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, rt; (b) K<sub>2</sub>CO<sub>3</sub>, MeOH, rt; (c) NaN<sub>3</sub>, DMF, 90°C; (d) CSA, CH<sub>2</sub>Cl<sub>2</sub>-MeOH, rt; (e) *p*TsCl, pyridine, 50°C, 37% for 5 steps; (f) wet.10%Pd/C, MeOH, H<sub>2</sub>, rt, 52%; (g) MMTCl, pyridine, rt, 56%; (h) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, *i*Pr<sub>2</sub>NEt, CH<sub>2</sub>Cl<sub>2</sub>, rt, 86%; (i) DNA synthesizer; Thy; thymine-1-yl.



**FIGURE 2**  $T_m$  profiles of the duplexes comprising ODNs **6–8** and DNA complement (A) or RNA complement (B). UV melting experiments were carried out in 10 mM  $\text{NaH}_2\text{PO}_4$  (pH7.0) containing 100 mM NaCl at a scan rate of  $0.5^\circ\text{C}/\text{min}$  at 260 nm. The concentration of each strand was  $4.0 \mu\text{M}$ . Target sequence: 5'-AGCAAAAAACGC-3'.

ODN **6**/DNA was similar to that of the ODN **8**/DNA and much higher than that of the ODN **7**/DNA (Figure 2A). Furthermore, it is noteworthy that the 5'-amino-3',5'-BNA ODN **6** formed a significantly stable duplex with its RNA complement (Figure 2B). The  $T_m$  value of the ODN **6**/RNA was higher than those of the ODN **7**/RNA and ODN **8**/RNA. These results clearly indicate that an adjustment of the  $\gamma$  dihedral angle to the  $+sc$  orientation by a methylene bridge between the N5' and C3' atoms enhances the duplex-forming ability of the 5'-amino-DNA.

## REFERENCES

- Shchepinov, M.S.; Denissenko, M.F.; Smylie, K.J.; Wörl, R.J.; Leppin, A.L.; Cantor, C.R.; Rodi, C.P. Matrix-induced fragmentation of P3'-N5' phosphoramidate-containing DNA: high-throughput MALDI-TOF analysis of genomic sequence polymorphisms. *Nucleic Acid Res.* **2001**, *29*(18), 3864–3872.
- Wolfe, J.L.; Wang, B.H.; Kawate, T.; Stanton, V.P., Jr. Sequence-specific dinucleotide cleavage promoted by synergetic interaction between neighboring modified nucleotides in DNA. *J. Am. Chem. Soc.* **2003**, *125*(35), 10500–10501.

3. Nottoli, E.M.; Lambert, J.B.; Letsinger, R.L. Study of conformational changes induced on substituting NH for O at C(5') of thymidine nucleosides and nucleotides. *J. Am. Chem. Soc.* **1977**, *99*(10), 3486–3491.
4. Jørgensen, P.N.; Svendsen, M.L.; Scheuer-Larsen, C.; Wengel, J. Incorporation of 3'-C-(hydroxymethyl) thymidine into novel oligodeoxynucleotide analogues. *Tetrahedron* **1995**, *51*(7), 2155–2164.
5. Bannwarth, W. Solid-phase synthesis of oligodeoxynucleotides containing phosphoramidate internucleotide linkages and their specific chemical cleavage. *Helv. Chim. Acta* **1988**, *71*(6), 1517–1527.