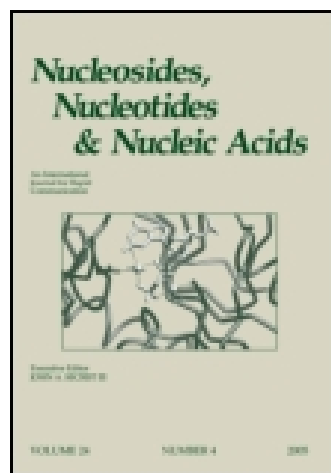


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SYNTHESIS AND PROPERTIES OF A NOVEL BRIDGED NUCLEIC ACID ANALOGUE, 5'-AMINO-3',5'-BNA

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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'→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'→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'→N5' phosphoramidate linkage (5'-amino-DNA) is of great interest in genome science, because the P3'→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.^[1,2] However, the ability of the 5'-amino-DNA to hybridize with the complementary strand is decreased, probably due to an inappropriate γ dihedral angle (N5'-C5'-C4'-C3'). ¹H NMR analysis of the 5'-amino-DNA dimer revealed that the *+ap* and *−sc* orientations for γ dihedral angle are favorable, which is quite different from the *+sc* orientation for γ in a typical DNA/DNA or RNA/RNA duplex.^[3] We supposed that restriction of the γ 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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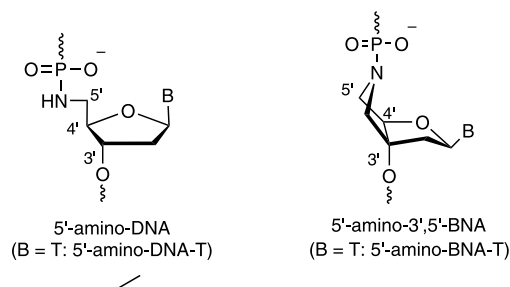
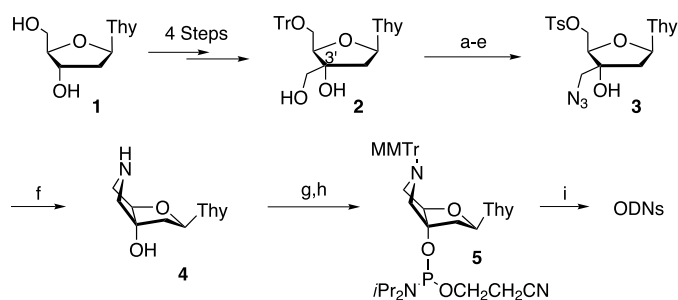


FIGURE 1 Structure of the oligonucleotide P3'→N5' phosphoramidate analogues used in this work.

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.^[4] 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 ¹H NMR and X-ray crystallographic analysis revealed that the γ dihedral angle of **4** is in the appropriate *+sc* orientation. Monomethoxytritylation of the secondary amine followed by phosphitilation 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.^[5]

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*Bu₂SnO, Et₃N, CH₂Cl₂, rt; (b) K₂CO₃, MeOH, rt; (c) NaN₃, DMF, 90°C; (d) CSA, CH₂Cl₂-MeOH, rt; (e) *p*TsCl, pyridine, 50°C, 37% for 5 steps; (f) wet.10%Pd/C, MeOH, H₂, rt, 52%; (g) MMTCl, pyridine, rt, 56%; (h) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, *i*Pr₂NEt, CH₂Cl₂, rt, 86%; (i) DNA synthesizer; Thy; thymine-1-yl.

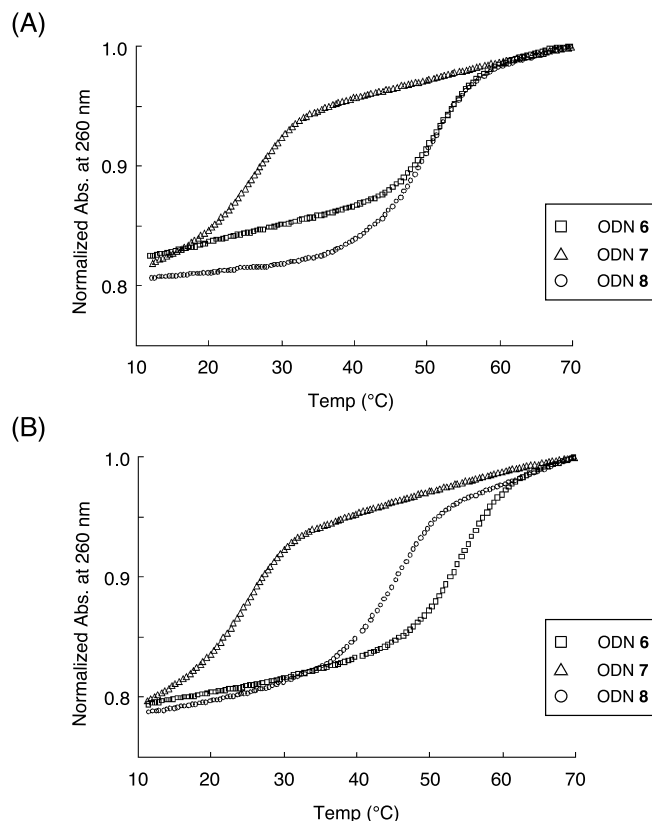


FIGURE 2 T_m profiles of the duplexes comprising ODNs **6–8** and DNA complement (A) or RNA complement (B). UV melting experiments were carried out in 10 mM NaH_2PO_4 (pH7.0) containing 100 mM NaCl at a scan rate of $0.5^\circ\text{C}/\text{min}$ at 260 nm. The concentration of each strand was $4.0\ \mu\text{M}$. Target sequence: 5'-AGCAAAAACGC-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 γ 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.

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