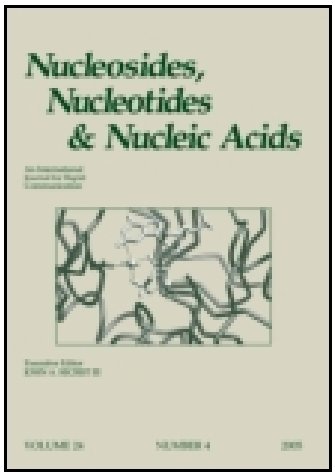


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# SYNTHESIS AND PROPERTIES OF A NOVEL BRIDGED NUCLEIC ACID ANALOGUE, 5′-AMINO-3′,5′-BNA

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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′). $^1$H 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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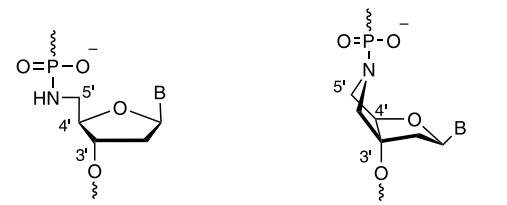
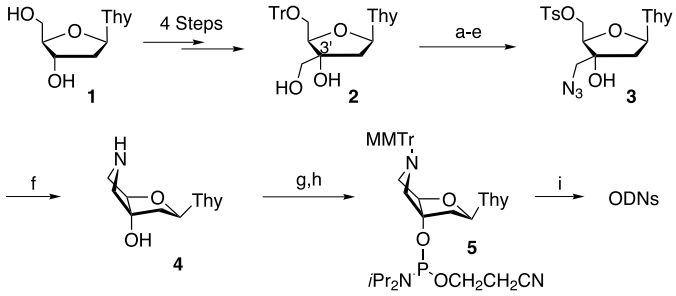



**FIGURE 1** Structure of the oligonucleotide P3′→N5′ phosphoramidate analogues used in this work.



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 $^1$H 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(GCGTTTTTTGCT)-3′]. The melting temperature ($T_m$) of the duplex




**SCHEME 1** (a) *p*TsCl, $n$Bu$_2$SnO, Et$_3$N, CH$_2$Cl$_2$, rt; (b) K$_2$CO$_3$, MeOH, rt; (c) NaN$_3$, DMF, 90°C; (d) CSA, CH$_2$Cl$_2$–MeOH, rt; (e) *p*TsCl, pyridine, 50°C, 37% for 5 steps; (f) wet.10%Pd/C, MeOH, H$_2$, rt, 52%; (g) MMTCl, pyridine, rt, 56%; (h) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, $i$Pr$_2$NEt, CH$_2$Cl$_2$, rt, 86%; (i) DNA synthesizer; Thy; thymin-1-yl.

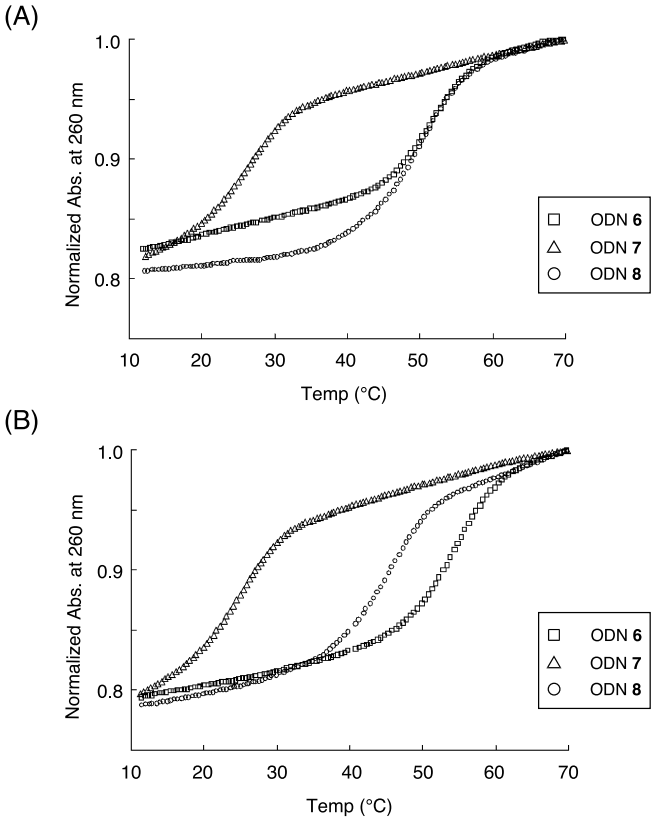



**FIGURE 2** $T_m$ profiles of the duplexes comprising ODNs **6–8** and DNA complement (A) or RNA complement (B). UV melting experiments were carried out in 10 mM $NaH_2PO_4$ (pH7.0) containing 100 mM NaCl at a scan rate of 0.5°C/min at 260 nm. The concentration of each strand was 4.0 μM. Target sequence: 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 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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