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Nucleosides and Nucleotides. 153. Effects of 2'-α- and 2'-β-Bromo-2'-deoxyadenosine on Oligonucleotide Hybridization and Nuclease Stability[1]

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Abstract: 1-(2-Bromo-2-deoxy-β-D-ribofuranosyl)adenine (**1**) and 1-(2-bromo-2-deoxy-β-D-arabinofuranosyl)adenine (**2**) were incorporated into DNA pentadecamers. The oligonucleotides containing **1** destabilized DNA/DNA and DNA/RNA duplex formation. However, the oligonucleotides containing **2** slightly stabilized DNA/DNA duplex formation, although they decreased the stability of DNA/RNA duplexes. Furthermore, the oligonucleotide containing **2** was more resistant to snake venom phosphodiesterase than an unmodified oligonucleotide $(dA)_{15}$ and the oligonucleotide containing **1**.

Recently, a number of oligonucleotides (ODNs) containing 2'-modified nucleoside analogues have been synthesized and used for antisense studies.[2-12] From these studies, it is apparent that affinity of ODNs to their complementary DNA or RNA strand and stability of ODNs to nucleolytic hydrolysis by nucleases, which are important factors for antisense molecules, are highly dependent on the properties of 2'-substituents. For instance, 2'-*O*-alkyl modifications such as 2'-*O*-methyl and -allyl increase the stability of DNA/RNA hybrids as well as the stability of the ODNs to nucleolytic degradation by nucleases.[2-9] However, 2'-*C*-alkyl substituents such as 2'-α-methyl, -ethyl, -allyl, and 2'-β-methyl groups increase the stability of the ODNs to nucleolytic degradation by 3'-exonuclease but reduce the stability of DNA/RNA hybrids.[10,11] On the other hand, Kawasaki *et al.* reported[12] that the 2'-α-fluoro substituent dramatically increases the stability of DNA/RNA duplexes although it does not increase stability to nucleolytic hydrolysis by nucleases. Stability of the duplex by the 2'-α-fluoro substitution could be due to the high electronegativity of the fluorine atom, which shifts the sugar conformational equilibrium to 3'-*endo*.[12-15] Shifting the conformation of the DNA strand to 3'-*endo* will put it in a more RNA-like conformation and may cause the hybridization properties to be more like those of RNA/RNA geometry. However, a fluorine atom, which has a van der Waals radius similar to that of hydrogen (1.35 Å compared to 1.20 Å), would be too small to increase the resistance to nucleases. From these findings, we envisioned that a 2'-bromo substituent may increase nuclease stability without destabilizing duplex formation.

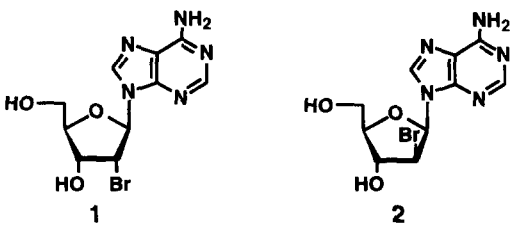
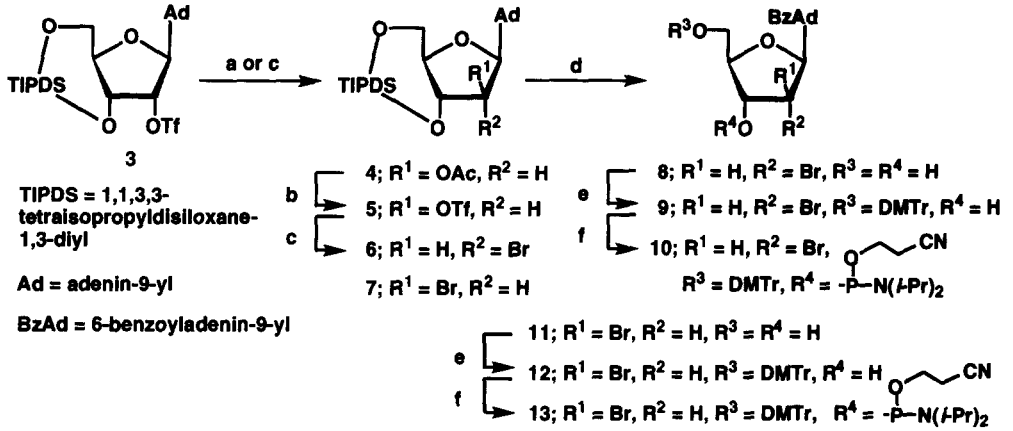


Figure 1.

In this paper, we demonstrate the synthesis of DNA pentadecamers containing 1-(2-bromo-2-deoxy-β-D-ribofuranosyl)adenine (**1**) and 1-(2-

bromo-2-deoxy-β-D-arabinofuranosyl)adenine (**2**). Thermal stability of these ODNs with their complementary DNA and RNA strands, and stability of these ODNs to nucleolytic digestion were also studied.



Scheme 1. a; NaOAc, HMPA, r.t. 56% (**4**). b; 1) Et_3N, MeOH, r.t. 2) TfCl, Et_3N, DMAP, CH_2Cl_2, r.t. 65% (**5**). c; LiBr, HMPA, r.t. 92% (**6**). 80% (**7**). d; 1) BzCl, DMAP, pyridine, r.t. 2) concd. NH_4OH / MeOH (2 : 3, v/v), 0 °C. 3) Bu_4NF, THF, 0 °C. 94% (**8**). 98% (**11**). e; DMTrCl, DMAP, pyridine. 84% (**9**). 80%(**12**). f; 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite, *N,N*-diisopropylethylamine, CH_2Cl_2. 69% (**10**). 80% (**13**).

The synthesis of 2'-bromo-2'-deoxyadenosine analogues and their amidite units is shown in Scheme 1. 2'-*O*-Triflates **3** and **5**, which were obtained from adenosine according to the reference procedures,[16] were treated with LiBr in HMPA to afford 2'-bromo substituted adenosines **6** and **7** in 92% and 80% yield, respectively. Then, **6** and **7** were converted into the nucleoside 3'-phosphoramidites **9** and **10** by the reported methods.[17] Pentadecamers (**14** - **20**) were synthesized on a DNA synthesizer by the standard phosphoramidite method.[17] Coupling yields of **10** and **13** were 96% and 98%, respectively.

Table 1. Thermal denaturation of the oligonucleotides.[18]

ODNs		Tm (°C)	
		DNA/DNA[a]	DNA/RNA[b]
AAAAAAAAAAAAAAA	(**14**)	35.5	35.5
AAAAAAA**1**AAAAAAA	(**15**)	32.0	36.0
AAA**1**AAA**1**AAA**1**AAA	(**16**)	28.0	34.5
1AA**1**AAA**1**AAA**1**AA**1**	(**17**)	24.0	31.0
AAAAAAA**2**AAAAAAA	(**18**)	36.5	35.0
AAA**2**AAA**2**AAA**2**AAA	(**19**)	36.0	32.0
2AA**2**AAA**2**AAA**2**AA**2**	(**20**)	35.5	25.5

a) Complementary DNA sequence: $(T)_{15}$.
b) Complementary RNA sequence: poly U.

Stability of the duplexes formed by the ODNs and their complementary strands, $(T)_{15}$ and poly U, was studied by thermal denaturation. Melting temperatures are listed in Table 1. With $(T)_{15}$ as the complementary strand, the Tms of the duplexes containing **1** were lower than that of the control duplex $(dA)_{15}$-$(T)_{15}$. The stability of the duplexes is dependent on the numbers of the modified nucleoside **1**. On the other hand, Tms of the duplexes containing **2** were slightly higher than that of the control duplex even when the numbers of **2** were increased. With poly U as the complementary strand, Tms of the duplexes containing **1** and **2** were lower than that of the control duplex $(dA)_{15}$-poly U. The duplexes became less stable as the numbers of the modified nucleosides increased. Furthermore, the modified nucleoside **2** appeared to strongly reduce the stability of the duplexes relative to the modified nucleoside **1**. Although no detailed NMR analysis

was done on **2**, the coupling constant $J_{1',2'} = 6.5$ Hz and $J_{3',4'} = 7.6$ Hz is suggestive of a 3'-*endo* sugar conformation (N-type conformer),[19] while a conformational feature of dA and **1** is the high population density of the S-type conformer.[13-15] However, we cannot explain the stability of these duplexes only by the sugar conformation. These results seemed to indicate that the stability of the duplexes containg **1** and **2** is more affected by steric interactions between the 2'-bromo substituents and the adjacent nucleotide units in the duplexes than the sugar conformation.

Next, stability of the ODNs to nucleolytic digestion was studied. Several studies have demonstrated that 3'-exonuclease activities are the major cause of degradation of unmodified ODNs in serum.[20] Therefore, snake venom phosphodiesterase (a 3'-exonuclease) was used in this study. The ODNs labeled at the 5'-end with ^{32}P were incubated with the nuclease and the reactions were analyzed by polyacrylamide gel electrophoresis under denaturing conditions (Figure 2).[21] Although the control ODN **14** and the ODN **16** containing **1** were hydrolyzed randomly by snake venom phosphodiesterase after 30 min (Figure 2, lanes 3 and 7), ODN **19** was hydrolyzed only at the 3'-side from the nucleoside analogue **2** (lane 11). The phosphodiester linkage at the 5'-side of **2** was more resistant to the nuclease than the phosphodiester linkages at the 5'-side of **1** and dA.

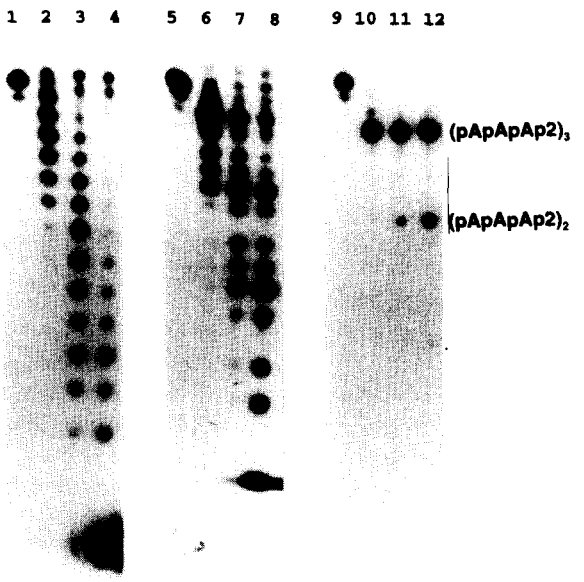


Figure 2. Polyacrylamide gel electrophoresis of oligonucleotides hydrolyzed by snake venom phosphodiesterase: **14** (lanes 1-4), **16** (lanes 5-8) and **19** (lanes 9-12) were incubated with snake venom phosphodiesterase at 37 °C for 0 min (lanes 1, 5, and 9), 10 min (lanes 2, 6, and 10), 30 min (lanes 3, 7, and 11), 60 min (lanes 4, 8, and 12). See the References and Notes for conditions.[21]

In conclusion, we demonstrated the synthesis of DNA pentadecamers containing 1-(2-bromo-2-deoxy-β-D-ribofuranosyl)adenine (**1**) and 1-(2-bromo-2-deoxy-β-D-arabinofuranosyl)adenine (**2**). The ODNs containing **1** destabilized DNA/DNA and DNA/RNA duplex formation. On the other hand, the ODNs containing **2** slightly stabilized DNA/DNA duplex formation although they decreased the stability of DNA/RNA duplexes. Furthermore, the ODN containing **2** was more resistant to snake venom

phosphodiesterase than an unmodified ODN $(dA)_{15}$ and the ODN containing **1**. Therefore, the modified nucleoside **2** may be suitable tools for the stabilization of ODNs to degradation by 3'-exonucleases.

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17. Each ODN was prepared on an ABI 391 DNA synthesizer using standard phosphoramidite chemistry according to M. J. Gait, Oligonucleotides Synthesis: A Practical approach, IRL Press, Oxford 1984. The purity of the pentadecamers were higher than 99% according to HPLC analysis with a C-18 silica gel column (Inertsil ODS-2, GL Science).
18. The solution containing each ODN was heated at 70 °C for 20 min, then cooled gradually to an appropriate temperature and used for the thermal denaturation study. Thermally induced transitions of each mixture of ODNs were monitored at 260 nm by a Gilford Response II. Sample temperature was increased one degree per min. Each sample contained ODNs (3 μM) and its complementary DNA or RNA (3 μM) in a buffer of 0.01 M sodium cacodylate (pH 7.0) containing 0.1 M NaCl.
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21. Each ODN labeled with ^{32}P at the 5' end (15 pmol)[22] was incubated with snake venom phosphodiesterase (0.4 μg) in the presence of Tolula RNA (0.26 OD units at 260 nm) in a buffer containing 50 mM Tris-HCl (pH 8.0) and 10 mM $MgCl_2$ (total 20 μL) at 37 °C. Samples of the reaction mixture were separated and added to a solution of EDTA (5 mM, 20 μL) at appropriate times, and then the mixtures were heated at 100 °C for 3 min. The solutions were analyzed by electrophoresis on 20% polyacrylamide gel containing 8 M urea.[22]
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