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3′-Amino-2′,4′-BNA: novel bridged nucleic acids having an N3′→P5′ phosphoramidate linkage

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Novel oligonucleotide analogues, containing a 3′-amino-2′,4′-BNA unit, were successfully synthesized, and they showed superior duplex and triplex forming ability as well as BNA itself, along with remarkable enzymatic stability.

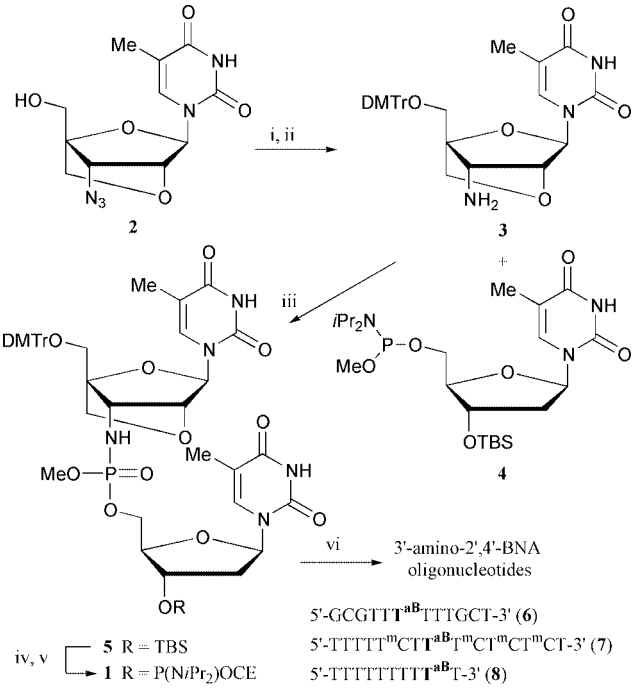
Oligonucleotides for practical antisense and/or antigene molecules should fulfil some requirements, such as a high binding affinity for the target ssRNA or dsDNA, high sequence selectivity, and sufficient enzymatic stability. During the past decade, various chemically modified oligonucleotides have been synthesized, and their properties have been investigated;[1–5] however, an ideal antisense or antigene molecule is still lacking. We recently achieved the first synthesis of a novel nucleoside with a fixed *N*-type conformation,[6] 2′-*O*,4′-*C*-methylene bridged nucleic acid (2′,4′-BNA)[7,8] (Fig. 1) and found that 2′,4′-BNA modified oligonucleotides exhibited strong hybridization ability with complementary strands of RNA and DNA.[9,10] Moreover, these 2′,4′-BNA oligonucleotides appeared to possess high affinity for dsDNA forming a stable DNA triplex.[10–15]

On the other hand, Gryaznov *et al*. reported in numerous papers that oligonucleotide analogues with an N3′→P5′ phosphoramidate linkage show superior properties of hybridization to ssDNA, ssRNA and dsDNA, along with high resistance to enzymatic degradation.[16–19]

Therefore, we became interested in a combination of a BNA structure and an N3′→P5′ phosphoramidate linkage.† Here, we report the synthesis, hybridization properties and enzymatic stability of novel oligonucleotide analogues with a 3′-amino-2′,4′-BNA monomer unit (Fig. 1).

The synthesis of 3′-amino-2′,4′-BNA heterodimer building block **1** was accomplished as shown in Scheme 1. After protection of the 5′-hydroxy group in **2**,[20] the 3′-azido group was successfully reduced to give the 3′-amino derivative **3**. Condensation of **3** with a 5′-phosphoramidite **4**[21] was performed according to the reported procedure.[17,21] The obtained heterodimer **5** was converted to the deisred compound **1** by treatment with tetrabutylammonium fluoride and subsequent phosphitylation. The 3′-amino-2′,4′-BNA oligonucleotides **6**–**8** were prepared using the phosphoramidite **1**.‡

The duplex-forming abilities of the 3′-amino-2′,4′-BNA modified oligonucleotide **6** with complementary ssDNA and ssRNA were elucidated under physiological conditions on the basis of melting temperatures (T_ms) (Table 1). Replacement of a natural 2′-deoxyribonucleotide by a 3′-amino-2′,4′-BNA monomer in the 12-mer oligonucleotide **9** resulted in significant



Scheme 1 *Reagents and conditions*: i 4,4′-dimethoxytrityl chloride, DMAP, Py, room temp., 76%; ii Ph_3P, Py, room temp., then NH_4OH aq., room temp., 97%; iii CCl_4, Et_3N, MeCN, room temp., 39%; iv TBAF, THF, room temp., 78%; v 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite, diisopropylammonium tetrazolide, MeCN–THF, room temp., 61%; vi DNA synthesizer (AB Expedite™ 8909). CE = 2-cyanoethyl. DMTr = 4,4′-dimethoxytrityl.

Table 1 T_m values for 3′-amino-2′,4′-BNA with complementary DNA and RNA[a]

	T_m (ΔT_m)/°C	
Oligonucleotides	DNA	RNA
5′-GCGTTT**T**^{aB}TTTGCT-3′ (**6**)	51 (+4)	52 (+7)
5′-GCGTTT**T**^{B}TTTGCT-3′ (**10**)	53 (+6)	52 (+7)
5′-GCGTTTTTTGCT-3′ (**9**)	47	45

[a] UV melting profiles were measured in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl at a scan rate of 0.5 °C min^{-1} at 260 nm. The oligonucleotide concentration used was 4 μM for each strand. The sequence of target DNA or RNA complements is 5′-AGCAAAAAACGC-3′.

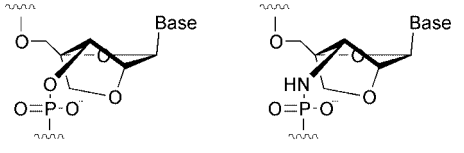


Fig. 1 Structure and conformation of 2′,4′-BNA and 3′-amino-2′,4′-BNA.

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Table 2 T_m values of 3′-amino-2′,4′-BNA with dsDNA[a]

Oligonucleotides	T_m (ΔT_m)/°C	
	+10 mM $MgCl_2$	−$MgCl_2$
5′-TTTTT^{m}CT**T^{aB}**T^{m}CT^{m}CT^{m}CT-3′ (**7**)	55 (+11)	44 (+11)
5′-TTTTT^{m}CT**T^B**T^{m}CT^{m}CT^{m}CT-3′ (**12**)	57 (+13)	44 (+11)
5′-TTTTT^{m}CTTT^{m}CT^{m}CT^{m}CT-3′ (**11**)	44	33

[a] UV melting profiles were measured in 7 mM sodium phosphate buffer (pH 7.0) containing 140 mM KCl without or with additional 10 mM $MgCl_2$. The oligonucleotide concentration used was 1.5 μM for each strand. The sequence of target dsDNA is 5′-GCTAAAAAGAAAGAGAGATCG-3′/3′-CGATTTTTCTTTCTCTCTAGC-5′. ^{m}C means 5-methylcytidine.

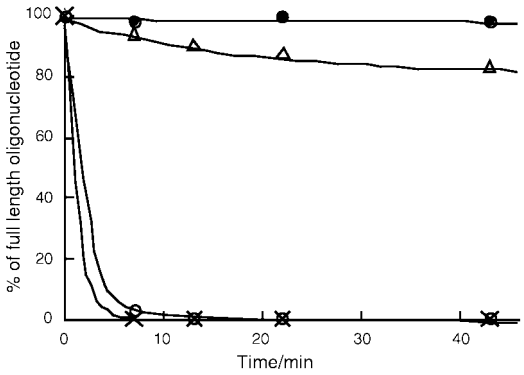

Fig. 2 Enzymatic stability of T_8**T^{aB}**T (**8**) (closed circle), T_{10} (**13**) (cross), T_8**T^B**T (**14**) (open circle) and T_8T_sT (**15**) (triangle). Hydrolysis of the oligonucleotides (10 μg) was carried out at 37 °C in a buffer (320 μl) containing 50 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$ and SVPDE (0.2 μg). '$_S$' means phosporthioate linkage.

stabilization of the duplexes formed with both complementary DNA and RNA. The increases in T_ms were up to 4 and 7 °C for the duplexes formed with the DNA and RNA strands, respectively. Next, the binding affinity of the oligonucleotide **7** to a homopurine•homopyrimidine dsDNA was also studied and shown to be much higher than that of the natural oligonucleotide **11**. The T_m values of triplex formation for **7** were 55 and 44 °C under neutral conditions with or without 10 mM $MgCl_2$, respectively, which is over 10 °C higher than the corresponding value for **11** as summarized in Table 2.

Thus, the oligonucleotides with one-point modification by 3′-amino-2′,4′-BNA, a combination of the 2′,4′-BNA backbone and N3′→P5′ phosphoramidate linkage, exhibited enhanced hybridizing properties towards complementary ssDNA, ssRNA and dsDNA, compared to the corresponding natural oligonucleotides. These ΔT_m values were almost comparable to those for 2′,4′-BNA singly modified congeners **10** and **12**, and seem to reach the utmost level of hybridization.

The nuclease-resistance of the 3′-amino-2′,4′-BNA modified oligonucleotide **8** was investigated by using snake venom phosphodiesterase (SVPDE), compared with natural and one point modified (2′,4′-BNA or phosphorthioate[22]) oligothymidilate 10-mers **13**, **14** and **15**, respectively. The reaction mixtures were analyzed at several time points by reversed-phase HPLC to monitor the percentage of full length oligonucleotides (Fig. 2). Under the conditions used, the natural T 10-mer control **13** and the 2′,4′-BNA **14** were immediately digested. In both cases, no full length oligomer was detected after 10 min, although the 2′,4′-BNA modification of oligonucleotides was found to be sufficiently resistant to enzymatic hydrolysis in other cases.[10] In contrast, more than 98% of full length 3′-amino-2′,4′-BNA oligonucleotide **8** was unchanged after 40 min, while 83% of **15** was intact after the same time period. It is noteworthy that enzymatic stability of the 3′-amino-2′,4′-BNA **8** was superior to that of the phosphorthioate modified oligonucleotide **15**.[22]

The results presented here clearly demonstrate that the novel nucleic acid analogue, 3′-amino-2′,4′-BNA, was a good candidate for a practical antisense and antigene molecule, due to its potent hybridization ability with DNA and RNA complements and homopurine•homopyrimidine dsDNA, and remarkable enzymatic stability surpassing that of phosphorthioate oligonucleotides. Further investigation of properties of the 3′-amino-2′,4′-BNA is currently under way.

Notes and references

† Recently Wang and Stoisavljevic reported a combination of a phosphoramidate linkage and another type of bicyclic nucleoside, and their modification of oligonucleotides decreased the duplex-forming ability. See ref. 21.

‡ The obtained 3′-amino-2′,4′-BNA oligonucleotides were purified by reversed-phase HPLC, and the compositions were determined by MALDI-TOF-MS. MALDI-TOF-MS data: **6** [M − H]$^-$ 3024.58 (calc. 3024.05); **7** [M − H]$^-$ 4523.32 (calc. 4523.13); **8** [M − H]$^-$ 3615.11 (calc. 3614.47).

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