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Design, Synthesis, and Properties of Boat-Shaped Glucopyranosyl Nucleic Acid

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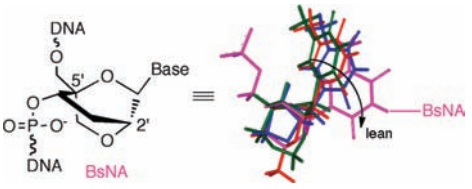
ABSTRACT



A boat-shaped glucopyranosyl nucleic acid (BsNA) was synthesized to investigate the possibility that the lean of a nucleobase is a factor affecting duplex-forming ability of oligonucleotides. From the crystal structure of a BsNA nucleoside and the thermal stability of duplex oligonucleotides, it was found that not only the lean of the base but also the rotation angle of the glycosidic bond axis were important factors in a stable duplex formation.

Much effort to develop nucleic acid therapeutics has been made to date.[1] Natural oligonucleotides (ONs) are not appropriate for therapeutic applications because they do not have enough target specificity, resistance toward nucleases, or cell membrane permeability. To improve their properties, nucleic acids have been chemically modified, and several clinical trials are currently being conducted with these artificial nucleic acids.[1] In addition to use in therapy, chemically modified ONs are also used in many other areas, such as nanotechnology,[2] diagnostics,[3] and drug target validation and gene function determination.[4] For these reasons, chemically modified ONs have attracted increasing attention.

We and other groups have developed numerous 2′,4′-bridged nucleic acid (2′,4′-BNA)[5]/locked nucleic acid (LNA)[6] analogues[7,8] whose sugar moieties are fixed in the North-type (C3′-*endo*) conformation, similar to a nucleotide in an A-type RNA duplex, by a bridge between the C2′- and C4′-positions. Because of this structural preorganization,[9] these analogues have high duplex-forming ability for complementary RNA. We have attempted to create additional artificial nucleic acids that form stable duplexes with complementary strands. However, there have been few BNA analogues[8b,c,e,11] that have affinities for RNA as high as the original 2′,4′-BNA/LNA, which remains the most promising BNA derivative even now. 2′,4′-BNA^{COC}, which has the sugar conformation closest

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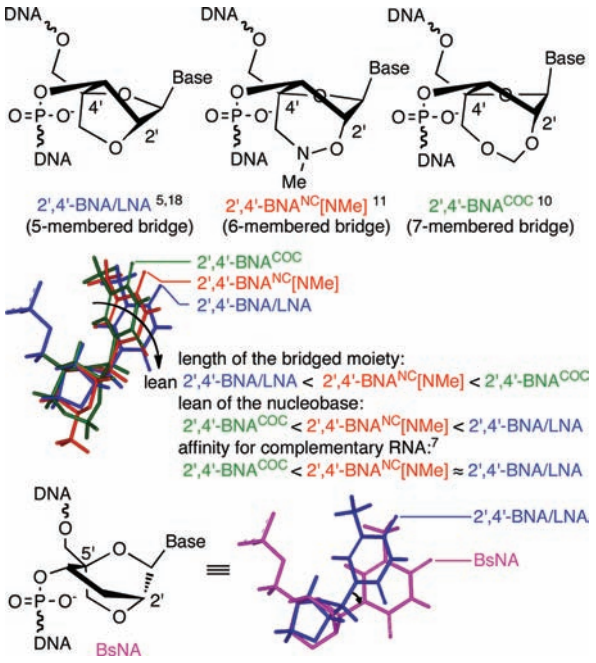
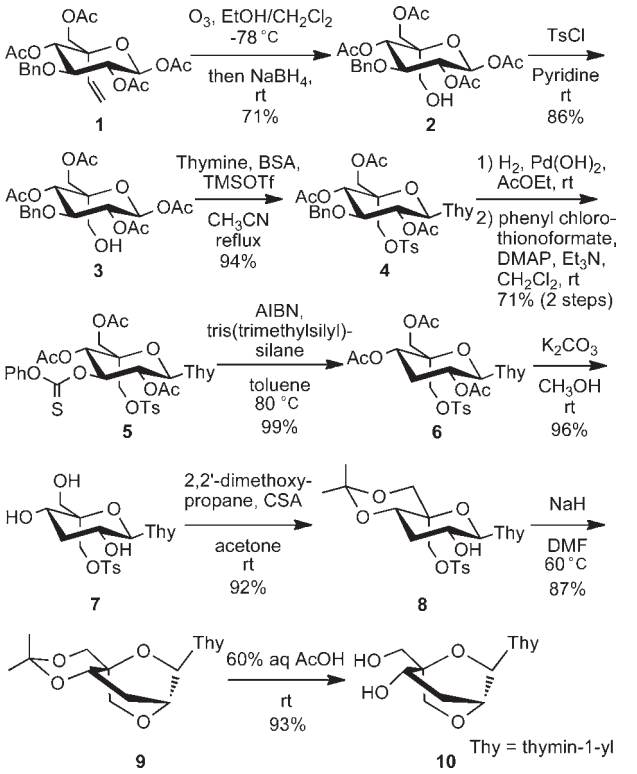


Figure 1. Relevance between the leans of nucleobases of BNA analogues, their binding affinities for ssRNA, and the molecular model of BsNA.

to a typical A-type RNA duplex in the BNA analogues developed by us, forms a stable duplex with complementary RNA, but its affinity is not as high as that of LNA.[10] Thus, there is a need to develop a new type of artificial nucleic acid based on a brand new concept. We investigated all aspects of the structural properties of BNAs to determine the factors that affect the duplex-forming ability of ONs.

When internucleotidic phosphodiester bonds are kept immobile, the nucleobases of BNA analogues lean in the direction of the arrow in Figure 1 with decreasing numbers of atoms forming the bridge. In BNA analogues, the binding affinity for complementary RNA tends to become higher with decreasing size of the bridge and is the highest when the bridge is composed of 5 or 6 atoms. We suspected the lean of the nucleobases could be a factor impacting the duplex-forming ability and wondered what the binding affinity would be if the nucleobase leaned at still larger angles than that of LNA. We newly designed a boat-shaped glucopyranosyl nucleic acid (BsNA[12]), which had a pyranose ring as the basic skeleton. The nucleobase will lean more than that of LNA owing to this unusual skeleton.[13] We report here the synthesis of BsNA and the thermal stability of the ONs including BsNA.

Scheme 1. Synthesis of Nucleoside **10**



BsNA was synthesized from known glucopyranoside **1**[14] as shown in Scheme 1. First, ozonolysis of glucopyranoside **1** followed by sodium borohydride reduction yielded alcohol **2**. Then, the tosylation of alcohol **2** was carried out with *p*-toluenesulfonyl chloride in pyridine, and the resulting compound **3** was coupled with a silylated thymine by Vorbrüggen's method[15] to give compound **4**. Next, **4** was subjected to hydrogenolysis conditions using palladium hydroxide and the resultant alcohol was treated with phenyl chlorothionoformate in the presence of *N*,*N*-dimethyl-4-aminopyridine to yield compound **5**. Subsequently, **5** was deoxygenated smoothly using tris(trimethylsilyl)silane and azobisisobutyronitrile,[16] and the obtained compound **6** was deacetylated to give triol **7**. The 4′- and 6′-hydroxy groups of triol **7** were protected as a isopropylidene ketal, and the resulting compound **8** was subjected to sodium hydride under moderate heating conditions to form the bridge between the C2′- and C5′-positions. Finally, removal of the isopropylidene group with aqueous acetic acid furnished desired nucleoside **10**.

Tritylation of **10** at the 6′-hydroxy group with 4, 4′-dimethoxytrityl chloride and phosphitylation at the

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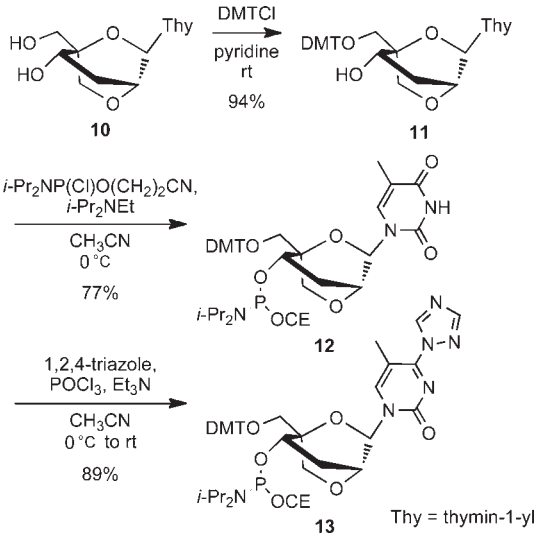
Scheme 2. Synthesis of Phosphoramidite Building Blocks



4′-hydroxy group with 2-cyanoethyl *N*,*N*-diisopropylaminochlorophosphoramidite afforded the desired phosphoramidite building block **12** (Scheme 2). A portion of the thymine phosphoramidite **12** was converted to the triazolyl derivative **13**, which is a convertible phosphoramidite, by the treatment of 1,2,4-triazole in the presence of triethylamine and phosphoryl chloride.[17]

The conformation of the sugar in BsNA was determined from the crystal structure of compound **10**[19] (Figure 2). Compared to the X-ray structure of 2′,4′-BNA analogues,[10a,11,18] the thymine base of **10** leans more, as we designed; C5′/C6′−C1′−N1 angles, e.g., of 2′,4′-BNA^{COC}, 2′,4′-BNA^{NC}[NMe], 2′,4′-BNA/LNA, and **10** were 106°, 111°, 112°, and 125°, respectively.

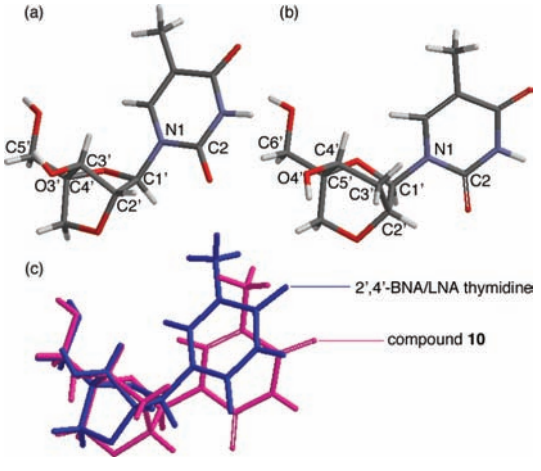


Figure 2. X-ray structures of 2′,4′-BNA/LNA thymidine (a)[18] and **10** (b),[19] and their superimposed images (c).

BsNA-phosphoramidites **12** and **13** were introduced into ONs using an automated DNA synthesizer (Table 1).

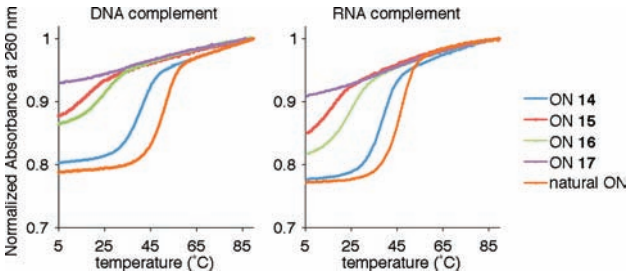
Table 1. Yields and MALDI-TOF-MS Data for the Oligonucleotides

oligonucleotide[a]	yield (%)[b]	calcd [M-H]$^-$	found [M-H]$^-$
5′-d(GCGTTT**T**TTTGCT)-3′ (**14**)	46	3675.4	3676.2
5′-d(GCG**T**T**T**T**T**TGCT)-3′ (**15**)	55	3759.5	3755.6
5′-d(GCGT**TTT**TTGCT)-3′ (**16**)	29	3759.5	3756.7
5′-d(GCG**TTTTTT**GCT)-3′ (**17**)	34	3885.6	3883.9
5′-d(TTTTT^{m}CT**T**T^{m}CT^{m}CT^{m}CT)-3′ (**18**)	14	4539.0	4536.9
5′-d(TTTTT^{m}CTTT**^{m}C**T^{m}CT^{m}CT)-3′ (**19**)	6	4539.0	4539.0

[a] Underlined bold characters indicate the modified residues. Superscript m shows that the following C is a 5-methylcytidine derivative. [b] The isolation yields for ON **14**–**19** were calculated from the UV absorbance at 260 nm.

The sequences were the same as those in our previous studies, and cytidines of ONs **18** and **19** were replaced by 5-methylcytidines for the stable triplex formation.[7] Each coupling reaction of modified monomers was accomplished using 5-[3,5-bis(trifluoromethyl)phenyl]-1*H*-tetrazole as an activator over 6 min. Coupling yields were checked by trityl monitoring and were estimated to be over 95%. Synthesized ONs were cleaved from the solid supports and deprotected by treatment with concentrated ammonium hydroxide solution. Simultaneously, the triazole group of ON **19** was converted to an amino group to give a BsNA-5-methylcytosine-modified oligonucleotide.

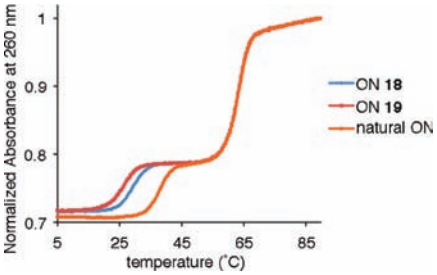
Table 2. Evaluation of Thermal Denaturation Temperatures (T_m Values) of Duplexes[a]



	T_m (°C)[b]	
oligonucleotide	DNA complement	RNA complement
Natural	51	48
14	41	39
15	ND[c]	ND[c]
16	25	24
17	ND[c]	ND[c]

[a] UV melting curves for the duplexes formed by ONs and the target strand, 5′-AGCAAAAAACGC-3′, were measured under the following conditions: 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl; each strand concentration = 4 μM; scan rate of 0.5 °C min^{-1} at 260 nm. [b] T_m was determined by taking the first derivative of the melting curve. The number is the average of three independent measurements. [c] ND = not detected.

Table 3. Evaluation of Thermal Denaturation Temperatures (T_m Values) of Triplexes[a]



oligonucleotide	T_m (°C)[b]
Natural	37
18	29
19	26

[a] UV melting curves for the triplexes formed by ONs and the target strand, 5′-d(GCT*AAAAAGAAAGAGAGA*TCG)-3′/3′-d(CGA*TTTTTC-TT-TCTCTCT*AGC)-5′, were measured under the following conditions: 7 mM sodium cacodylate buffer (pH 7.0) containing 140 mM KCl and 10 mM $MgCl_2$; each strand concentration = 1.5 μM; scan rate of 0.5 °C min^{-1} at 260 nm. The italic portions indicate the target site for triplex formation. [b] T_m was determined by taking the first derivative of the melting curve. The number is the average of three independent measurements.

We evaluated the affinity of the synthesized ONs with complementary single-stranded RNA (ssRNA) and DNA (ssDNA) and double-stranded DNA (dsDNA) through UV melting experiments. The UV melting profiles and thermal denaturation temperatures (T_m values) are summarized in Tables 2 and 3. BsNA formed unstable duplexes with ssRNA and ssDNA and a triplex with dsDNA. When a larger number of BsNA monomers were introduced into ONs, a smaller hyperchromicity was observed. This is perhaps explained by steric repulsion or destabilization of the hydrogen bonds between the base pairs due to a too large lean of the BsNA nucleobase. This indicates that the lean of the nucleobase may be an important factor in the duplex-forming ability.

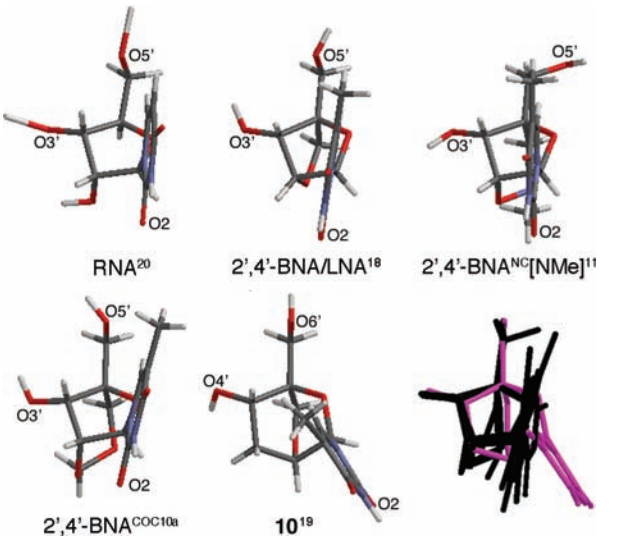


Figure 3. Nucleobase orientations of a typical A-type RNA duplex, BNA analogues, and **10**, and their superimposed images. Hydrogen atoms are omitted in the superimposed images.

However, other factors can be attributed to the destabilization of the duplex and triplex. As shown in Figure 3, the nucleobase orientation of **10** (magenta) differs from those of a typical A-type RNA duplex or other BNA analogues (black), and moreover, the rotation of the C1′−N1 bond axis in **10** may be restricted. Therefore, in the hybridization, the nucleobase orientation of the target strand perhaps needs to be altered to form hydrogen bonds, which is an unfavorable process. In addition, the axial H3′ has the potential to inhibit π-stacking between neighboring bases. Investigation of these possibilities is currently underway in our laboratory.

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Supporting Information Available. Experimental details and characterization data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) Crystallographic data for **10** can be found in the Supporting Information. Deposition no. CCDC 840597.

(20) RNA structure was obtained using Spartan '06, Wavefunction, Inc., Irvine, CA.