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Synthesis and hybridization property of a boat-shaped pyranosyl nucleic acid containing an exocyclic methylene group in the sugar moiety

Kazuto Mori [a], Tetsuya Kodama [b,*], Satoshi Obika [a,*]

[a] Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan
[b] Graduate School of Pharmaceutical Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8601, Japan

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ABSTRACT

A boat-shaped pyranosyl nucleic acid (BsNA) having an exocyclic methylene group in the sugar moiety was synthesized to investigate the possibility that the axial H3′ of original BsNA is the cause of its duplex destabilization. The synthesized BsNA analog was chemically stable against various nucleophiles. From the thermal stability of duplex oligonucleotides including the BsNA analog, it was found that the duplex-forming ability can be sensitive to the size of functional groups at the 3′-position.

1. Introduction

Many conformationally restricted nucleotides have been developed to date.[1] Especially, 2′,4′-bridged nucleic acid (2′,4′-BNA)[2]/locked nucleic acid (LNA)[3] developed independently by our group and Wengel's group is used in therapeutic application[4] and nanotechnology[5] because of its high affinity with complementary single stranded DNA and RNA (ssDNA, ssRNA). Its outstanding duplex-forming ability is derived from the preorganized sugar conformation that mimics a nucleotide in an A-type RNA duplex. Although various 2′,4′-BNA/LNA analogs have been developed in the past,[1] few analogs[6] have higher binding affinities for complementary strands than that of original 2′,4′-BNA/LNA. In addition, 2′,4′-BNA^{COC}, whose sugar conformation is closest to a typical A-type RNA duplex in the 2′,4′-BNA/LNA analogs, does not have the highest duplex-forming ability.[7] Hence, it is necessary to develop a novel type of artificial nucleic acid on the basis of new strategy.

Recently, we designed and synthesized a boat-shaped glucopyranosyl nucleic acid (BsNA) **1**,[8] which had a constrained pyranose as the basic skeleton (Fig. 1). Regrettably, the incorporation of BsNA **1**-T into oligonucleotides decreased the duplex-forming ability with a complementary ssDNA and ssRNA. Some factors can be attributed to this destabilization. One is the axial H3′ which can invade between neighboring nucleobases and inhibit π–π stacking interaction (Fig. 2a). Pedersen's group reported 3′-methoxy locked pyranosyl nucleic acid (LpNA)[9] (Fig. 1), which had a low binding

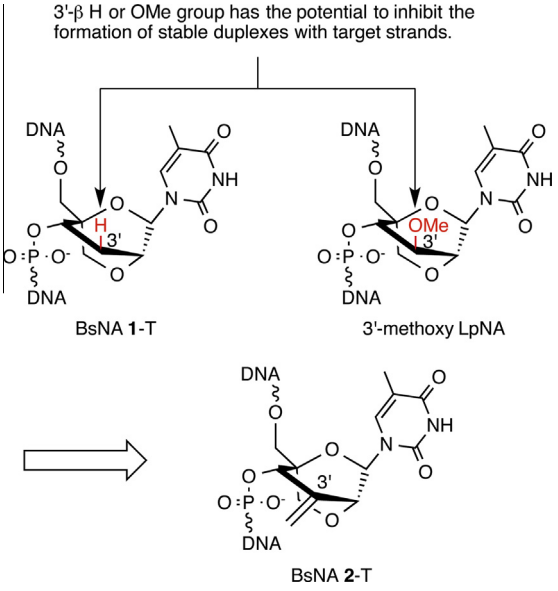

Figure 1. The structure of BsNA **1**-T and 3′-methoxy LpNA and design of BsNA **2**-T.

* Corresponding authors. Tel./fax: +81 5 2789 2971 (T.K.); tel.: +81 6 6879 8200; fax: +81 6 6879 8204 (S.O.).
E-mail addresses: kodama@ps.nagoya-u.ac.jp (T. Kodama), obika@phs.osaka-u.ac.jp (S. Obika).

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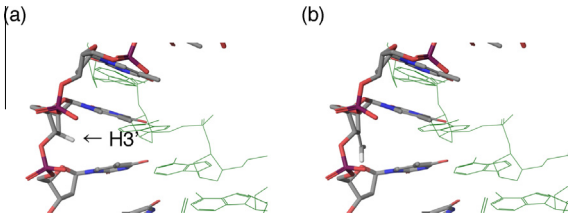


Figure 2. Representative low energy structure of an A-type DNA duplex comprising BsNA **1** (a) and the structure with an exocyclic methylene group (BsNA **2**) modeled into the sugar moiety (b).

affinity with complementary strands as with the case of BsNA **1**. They also suggested that the axial position of the C3′-OMe group can cause a destabilization of a duplex.

In this study, we newly designed and synthesized a BsNA analog that does not have the axial substituent at 3′-position. At first, replacing the C3′ with oxygen atom was occurred to us, but it takes a great deal of exertion to synthesize that analog owing to its own acetal structure.[10] Therefore, we decided to introduce an exocyclic methylene group to the C3′ position to eliminate the axial H3′ (Fig. 1). Recently, Seth's group also reported that an exocyclic methylene group could act as a bioisostere of the 2′-oxygene atom in 2′,4′-BNA/LNA.[6a] Since BsNA **2**-T has no functional groups at the axial C3′ position (Fig. 2b), the duplex forming ability will be improved if the axial H3′ is the cause of the duplex destabilization.

2. Results and discussion

2.1. Synthesis of BsNA 2

BsNA **2**-T was synthesized from known glucopyranoside **3**[8a] as shown in Scheme 1. First, glucopyranoside **3** was deacetylated to give triol **4**. When glucopyranoside **3** was reacted with K_2CO_3 and MeOH, the reaction yield was very low because of the elimination of the tosylate group. This problem was negligible when **3** was treated with methylamine at 0 °C, and desired triol **4** was obtained at high yield. Next, the 4′- and 6′-hydroxy groups of triol **4** were protected as a isopropylidene acetal, and the resulting compound **5** was subjected to sodium hydride under moderate heating conditions to form the bridge between the C2′- and C5′-positions.

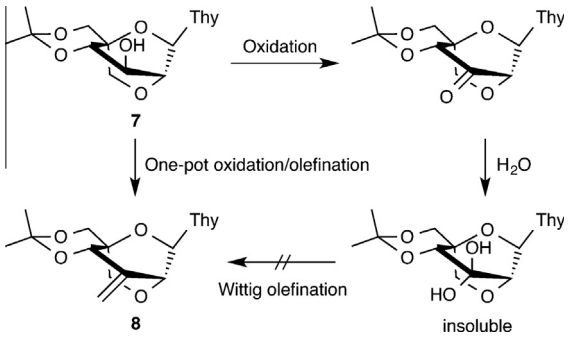
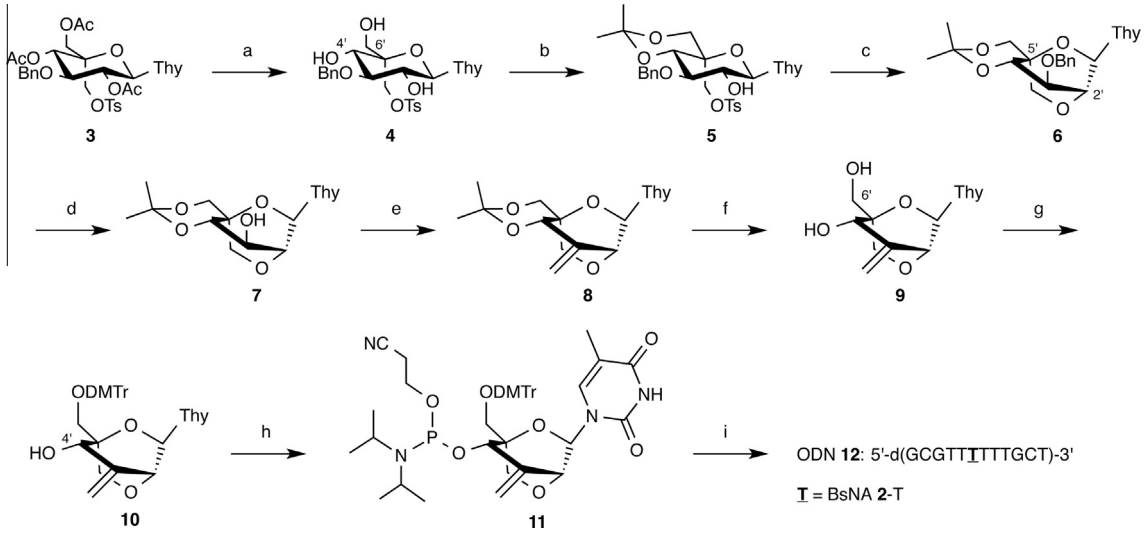


Figure 3. The reason for adopting one-pot oxidation/olefination procedure to obtain alkene **8**.

Subsequently, the resultant compound **6** was exposed to hydrogenolysis conditions using palladium hydroxide to remove benzyl group. When alcohol **7** was oxidized, the resultant ketone was easily hydrated under an air atmosphere. The desired alkene **8** was not yielded from the hydrated compound using Wittig reagent (Fig. 3). Once the hydrate generated, it exhibited an insoluble property and the removal of water from the hydrate was difficult by typical dehydration procedures. Therefore, one-pot oxidation/olefination procedure was adopted. Alcohol **7** was firstly oxidized using PDC, and then methyl triphenyl phosphonium ylide was added to the reaction mixture to afford alkene **8**. Next, removal of the isopropylidene group with aqueous acetic acid furnished nucleoside **9**. Finally, tritylation of **9** at the 6′-hydroxy group with 4,4′-dimethoxytrityl chloride (DMTrCl) and phosphitylation at the 4′-hydroxy group of **10** with 2-cyanoethyl *N*,*N*-diisopropylaminochlorophosphoramidite afforded the desired phosphoramidite building block **11**.

Phosphoramidite **11** was introduced into oligodeoxynucleotide (ODN) using an automated DNA synthesizer. The sequence was the same as that of our previous work.[8a] The concentration of phosphoramidite **11** was 0.1 M and the coupling time was prolonged to 8 min. 5-[3,5-Bis(trifluoromethyl)phenyl]-1*H*-tetrazole was used as an activator. Coupling yields were checked by trityl monitoring and were estimated to be over 95%. Synthesized ODN was cleaved from the solid support and deprotected by treatment with ammonium hydroxide solution. The obtained ODN **12** was purified by



Scheme 1. Reagents and conditions: (a) 40% aq CH_3NH_2, THF, 0 °C, 95%; (b) 2,2-dimethoxypropane, CSA, DMF, rt, 88%; (c) NaH, DMF, 60 °C, 97%; (d) H_2, $Pd(OH)_2$/C, AcOEt, rt, 94%; (e) PDC, MS4A, CH_2Cl_2, rt, then Ph_3PCH_3Br, *n*-BuLi, THF, rt, 61%; (f) 60% aq AcOH, rt, 93%; (g) DMTrCl, pyridine, rt, 96%; (h) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, *N*,*N*-diisopropylethylamine, CH_3CN, 0 °C, 79%; (i) DNA synthesis. Thy = thymin-1-yl.