Synthesis and properties of oligodeoxyribonucleotides containing the new base pairs, Im-N\textsuperscript{O}:Na-O\textsuperscript{N} and Im-O\textsuperscript{N}:Na-N\textsuperscript{O}, with the ability to form four hydrogen bonds

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ABSTRACT
Previously, we synthesized oligodeoxyribonucleotides (ODNs) containing novel base pairing motif consisting of tricyclic nucleosides, Im-N\textsuperscript{O} and Im-O\textsuperscript{N}, with the ability to form four hydrogen bonds (H-bonds). When the base pair, Im-N\textsuperscript{O}:Im-O\textsuperscript{N}, pair, was incorporated into the duplex, thermal stability of the duplex depended on the sequence context. In this time, on the basis of previous results, we designed two new bicyclic nucleosides, Na-N\textsuperscript{O} and Na-O\textsuperscript{N} as complementary nucleobase of the tricyclic nucleobases. These nucleosides were synthesized via a Pd-mediated cross coupling reaction as a key step. When the new Im-O\textsuperscript{N}:Na-N\textsuperscript{O} pair was incorporated into the duplex, it was remarkably stabilized independent of the sequence context.

INTRODUCTION
The structure of the DNA double helix is based on the Watson-Crick hydrogen bonding (H-bonding) of A:T (two H-bonds) and G:C (three H-bonds) base pairing. H-bondings between nucleobases are thought to play a critical role in thermal stability of DNA duplex. Taking these things into consideration, we previously synthesized ODNs containing base pair consisting of Im-N\textsuperscript{O} and Im-O\textsuperscript{N}, which were capable of forming four H-bonds (Fig. 1). When one residue of this base pair was incorporated into the duplex, it was less thermally stable than the duplexes consisting of natural base pairs (A:T and G:C). On the other hand, when three residues of this base pair were consecutively incorporated into the duplex, it was markedly stabilized. As an explanation of these results, the structure of DNA double helix might be also destabilized in adjacent positions of the base pair. When one residue of the Im-N\textsuperscript{O}:Im-O\textsuperscript{N} pair was incorporated into the duplex, the destabilization factor would be greater than the stabilization arising from the stable four H-bonds. On the other hand, when three residues of the base pair were consecutively incorporated into the duplex, it was thermally stabilized since the stabilization factor would be superior to the conformational destabilization around the boundary of the base pairs. On the basis of these results, we designed two new bicyclic nucleosides, Na-N\textsuperscript{O} and Na-O\textsuperscript{N}, as complementary nucleobases of Im-N\textsuperscript{O} and Im-O\textsuperscript{N} (Fig. 2). The duplexes containing tricyclic-bicyclic base pairs (Im-N\textsuperscript{O}:Na-O\textsuperscript{N} and Im-O\textsuperscript{N}:Na-N\textsuperscript{O} pairs) are expected to show higher thermal stability independent of the sequence context. In this study, we report the synthesis of Na-N\textsuperscript{O} and Na-O\textsuperscript{N} and thermal stability of the duplex containing new Im-N\textsuperscript{O}:Na-O\textsuperscript{N} and Im-O\textsuperscript{N}:Na-N\textsuperscript{O} pairs.
RESULT AND DISCUSSION

The desired Na-No was synthesized via a regio- and stereospecific Pd-mediated cross coupling reaction of monoiodonaphthyridine derivative 2 with glycal derivative 3, as shown in Scheme 1. Compound 1, which was obtained by treatment of 2,6-diaminopyridine with malic acid in H2SO4, was converted to 2 by a monoiodination with NIS, followed by protection of the exocyclic amino group. When 2 was treated with 3, which was easily prepared from thymidine, the C-nucleoside derivative 4 was obtained. Treatment of 4 with TBAF gave 3′-keto derivative 5. Stereospecific reduction of the 3′-keto group of 5 with sodium triacetoxyborohydride yielded 2′-deoxyribofuranosyl C-nucleoside 6. Deprotection of the dimethylformamidine group with methanolic ammonia gave 7-amino-2-oxo derivative Na-No. 2-Amino-7-oxo derivative Na-O was also obtained with the similar procedure. To incorporate Na-No and Na-O into ODNs, these were converted to the corresponding phosphoramidites with appropriate protecting groups. The usual phosphoramidite method was used for the synthesis of ODNs.

![Scheme 1](https://example.com/scheme1.png)

Thermal stability of the duplexes containing either one or three consecutive Im-No:Na-O or Im-O:Na-N pair in middle of 17mer were evaluated by melting temperatures (Tm) (Fig. 3). The duplex formed by ODN I and ODN II, where Im-O and Na-N was introduced at the X and Y position, was stabilized by +8.6 °C relative to the duplex containing the G:C pair, the result of which was different from the duplex containing one residue of Im-O:Im-N pair. Furthermore, when three molecules of Im-O or Na-N were consecutively incorporated into the center of each ODN (ODNs III and IV), the resulting duplex was remarkably stabilized by +26.0 °C relative to the duplex containing the G:C pair. This results indicate that Im-O:Na-N pair stabilize the DNA duplex without distorting the structure of double helix in adjacent positions where the new base pairs are incorporated.

REFERENCES