2',4'-BNA derivatives bearing an unnatural nucleobase: Synthesis and application to triplex-forming oligonucleotides

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ABSTRACT
Recognition of dsDNA by a triplex-forming oligonucleotide (TFO) is limited to homopurine-homopyrimidine sequences. Therefore, it is necessary to develop novel nucleoside analogues which recognize pyrimidine-purine basepairs (C-G or T-A). We have designed and synthesized novel 2',4'-BNA/LNA monomers bearing 3-hydroxybenzene and indole as a nucleobase (3HB and InB), and these nucleoside analogues have been introduced into TFOs. On melting temperature (Tm) measurements, 3HB and InB were found to interact with T-A base pair interruption with moderate binding affinity.

INTRODUCTION
Triplex formation by dsDNA and TFO has been expected as fundamental to novel therapeutic tools to control gene expression. Native TFO can bind to a homopurine-homopyrimidine sequence in the target dsDNA through the formation of Hoogsteen hydrogen bonds. However, the natural nucleobases in TFO cannot recognize pyrimidine-purine interruption (a C-G or T-A base pair), because pyrimidine bases have only one hydrogen-bonding site. To overcome this problem, some attempts have been made to develop various nucleoside analogues bearing an unnatural nucleobase. We have recently developed a 2',4'-BNA/LNA having 2-pyridone (Pb) as a nucleobase, and revealed that it had powerful and selective binding affinity for C-G interruption. On the basis of the recognition mode of a C-G base pair by Pb, we designed a 2',4'-BNA/LNA bearing 2-hydroxybenzene (2HB) as a nucleobase to recognize T-A base pair interruption. We found that 2HB had significant binding affinity for a U-A base pair. However 2HB failed to recognize a T-A base pair. This result suggests that 2HB formed a single hydrogen bond with 4-carbonyl oxygen of T or U, but the interaction was interrupted by steric hindrance of a 5-methyl group in T. Here, we have designed and synthesized novel 2',4'-BNA/LNA monomers bearing 3-hydroxybenzene (3HB) and indole (InB) as nucleobases to form a single hydrogen bond with 4-carbonyl oxygen of T without steric hindrance of the 5-methyl group in T. The triplex-forming ability of the TFOs containing 3HB and InB is also discussed.

RESULTS AND DISCUSSION
Synthesis of 3HB and InB.
The synthetic route to the target compounds 5 and 9 is shown in Scheme 1. Treatment of the starting material 1 with 3-allyloxypyrrolidinemagnesium bromide in THF gave coupling product 2 in 86 % yield (R selectively). Mitsunobu reaction of 2 gave bicyclic nucleoside 3 in 77 %. Deallylation of 3 followed by benzoylation provided 4 in 68 % yield (2 steps from 3). After hydrogenolysis of 4, dimethoxytritylation followed by phosphorylation afforded the phosphoramidite 5 in 58 % yield (3 steps from 4). On the other hand, treatment of the starting material 1 with N-t-butyl(dimethyl)silyldimethyl-6-magnesium bromide in THF gave coupling product 6 in 63 % yield (R: S: 6: 1). Mitsunobu reaction of 6 provided bicyclic nucleoside 7 in 78 %. Treatment of 7 with TBAF followed by benzoylation gave 8 in 68 % yield (2 steps from 7). After hydrogenolysis of 8, dimethoxytritylation followed by phosphorylation provided the phosphoramidite 9 in 58 % yield (3 steps from 8). The obtained phosphoramidites 5 and 9 were introduced into TFOs by a standard phosphoramidite protocol on a DNA synthesizer.

Figure 1. Structure of 3HB-T-A and InB-T-A.
Scheme 1. Reagents and conditions: a) 3-allyloxyphenylmagnesium bromide, THF, rt, 13 h (86%); b) TMAD, Ph,P, benzene, rt, 19 h (77%); c) Pd(PPh3)4, NaBH4, rt, 8 h (76%); d) BzCl, Et3N, CH2Cl2, rt, 1 h (89%); e) 20% Pd(OH)2-C, cyclohexene, THF, reflux, 22 h (92%); f) DMTcCl, pyridine, rt, 30 min (89%); g) iPr2P(Cl)OCH2CH2CN, iPr2NET, CH2Cl2, rt, 1 h (71%); h) N-t-butylidimethylsilylidinolyl-6-magnesium bromide, THF, -78 °C, 1 h (63%); i) DEAD, Ph,P, THF, rt, 16 h (86%); j) TBAF, THF, rt, 30 min (quant.); k) BzCl, Et3N, DEAD, rt, 26 h (78%); l) 20% Pd(OH)2-C, H2, AcOEt, rt, 1 h (61%); m) DMTcCl, pyridine, rt, 4 h (89%); n) iPr2P(Cl)OCH2CH2CN, iPr2NET, CH2Cl2, rt, 3 h (73%).

Triplex-forming Ability of TFO Containing 3HB or InB with dsDNA (Tm measurements). In a pyrimidine motif triplex, G nucleobase was known to interact with T-A base pair interruption in dsDNA. Therefore, triplex-forming ability of TFO containing 3HB or InB was compared with that of TFOs containing G or 2’,4’-BNA abasic analogue (H). The UV melting experiments were carried out in 7 mM sodium phosphate buffer (pH 7.0) containing 140 mM potassium chloride and 10 mM magnesium chloride. All Tm data are summarized in Table 1. The triplex comprising the TFO (X=3HB) and the duplex (Y-Z=T-A) was more stable than those containing 2HB-T-A, G-T-A and H-T-A triads. Next, the triplexes containing 3HB-T-A and 3HB-U-A triads showed almost the same stability, while the triplex including 2HB-T-A decreased in Tm value by 17°C compared with that containing 2HB-U-A triad. This result suggests that 3HB successfully avoids a sterical repulsion of the 5-methyl group in T. The triplex comprising the TFO (X=InB) and the duplex (Y-Z=T-A) was more stable than that containing 2HB-T-A, G-T-A and H-T-A triads. It was also observed that InB formed a stable triad with a G-C base pair.

CONCLUSION

We have synthesized novel 2’,4’-BNA/LNA monomers bearing 3-hydroxybenzene and indole as a nucleobase. It was found that the TFO containing 3HB had significant binding affinity for a T-A base pair, but had a similar binding affinity for other base pairs. This result suggests that 3HB can be utilized as a universal base. On the other hand, the TFO containing InB interacted with a T-A base pair to form a stable triplex, although it also recognized a G-C base pair. These results were considered to be a clue to developing nucleoside analogues with efficient recognition ability of T-A base pair interruption.

REFERENCES