Design and synthesis of the novel cross-linking reagents triggered by the triple helix formation

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ABSTRACT
In our attempt to new nucleobase analogs capable of interstrand cross-linking, we developed 2-amino-6-vinyl purine analog (1). The oligonucleotides incorporating 1 showed efficient interstrand cross-linking with selectivity toward cytidine at a target site. In this paper, we describe the design of the new cross-linking reagents (2) bearing 2-amino-6-vinyl purine motif, and triplex-directed alkylation with 2 to double-stranded DNA.

INTRODUCTION
Sequence-specific triple helix formation is an attractive method for controlling gene expression by oligonucleotides. However, parallel-type triplexes formed with homopyrimidine oligonucleotides (TFOs) are not tight enough to strongly inhibit gene expression. In order to enhance the efficacy of TFOs, a number of improvements have been investigated. Interstrand covalent bond formation within triplex is expected to effect stability of triplexes, and a variety of reactive groups have been conjugated to TFOs. Recently, interesting applications of covalent modification to DNA have been reported, in which point mutation of the gene may be resulted at the site of reaction. Thus, alkylating agents will be useful for manipulation of gene expression, but existing methods still need further improvement in reactivity, selectivity as well as stability for possible application in living system.

We have previously reported that 2-amino-6-vinylpurine nucleotide (1) exhibited efficient and selective cross-linking to cytidine within duplex. Further remarkable point of 1 is that the alkylation activity can be auto-generated within duplex from its stable precursors, phenylsulfide or phenylsulfoxide derivatives. In this study, 2-amino-6-vinylpurine motif was applied for cross-linking within triplexes, and the new nucleoside derivative (2) was designed. In the previous model study in organic solvents, 2-amino-6-vinylpurine motif reacted with N-7 of guanosine derivative, bringing an idea that 1 would react with guanosine in a purine strand within the parallel triplex. On the other hand, the new nucleoside derivative 2 having an alkyl spacer between the sugar part and the 2-amino-6-vinylpurine motif would react with guanosine in pyrimidine strand at a far side of the TFO (Fig 1).

Scheme 1

Fig. 1.
RESULTS AND DISCUSSION
Synthesis of the ODNs incorporating functionalized nucleoside analog 2 is summarized in Scheme 1. The sugar part was synthesized via β-selective Wittig-Honor reaction with 5-O-trityl deoxy-D-ribose, following several transformations produced the tosylate 3. 9-N-Alkylation of 2-amino-6-chloroprine with 3 yielded the desired product as a major isomer, which was used for the Pd(II)-catalyzed cross-coupling reaction with nBu3SnCH=CH2 to afford 2-amino-6-vinylpurine derivative 4. The phosphoramidite precursor (6) was transformed from 4 through 5 by conventional method, which was applied to an automated DNA synthesizer. The TFO was then converted to 8 by oxidation with magnesium monoperphthalate (MMPP), followed by elimination under an alkaline condition. The TFO incorporating 1 was obtained in a similar manner as described previously.7 Structures were confirmed by measurement of UV and MALDI-TOF spectroscopy.

The cross-linking was investigated with the following conditions: the TFO was allowed to react with guanosine at 30°C for 20 hours after 1 was added to the reaction mixture at the 5'-terminus of the TFO (8, Z=2). The reaction site of guanosine should be away from the complementary position, we next investigated the cross-linking reaction with another target sites 1-3 bp far from the reactive terminus of the TFO (8, Z=2). In these combinations, selective reaction to adenosine was observed (Fig 2C, lane 7), and the reactivity was decreased in the order of A>G>C>T. The reaction site of the adenosine is not yet clear whether 6-amino group or 7-nitrogen. These results indicated that I showed selectivity to guanosine at the target site of the pyrimidine strand, and that the long-vinyl derivative 2 exhibited selectivity to adenine at the target sites of the pyrimidine strand 1-3 bp away from the complementary position.

CONCLUSION
In summary, we have successfully demonstrated the strand selective cross-linking reaction within triplexes using TFO bearing 2-amino-6-vinylpurine derivatives 1 and 2. Although strand selectivity should be useful in future work for site-directed chemical reaction, neither base selectivity nor cross-linking efficiency achieved by this study might be satisfactory. The reactive moiety might not be located at the ideal position in the triplexes. Further work is now ongoing in the search for the appropriate structure having higher reactivity in triplex forming cross-linking reaction.

REFERENCES