Synthesis and properties of oligonucleotides containing carbocyclic L-nucleoside analogues with a restricted glycosyl conformation

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ABSTRACT
To construct nuclease-resistant oligonucleotides, we designed novel carbocyclic L-nucleoside analogues (1-4) whose glycosyl conformation is fixed at \( \chi = 180^\circ \) by an oxygen-bridge between the base and the cyclopentane ring. We have already achieved the racemic synthesis of these analogues. In this study, we succeeded in synthesizing an optically active form of these analogues. The properties of oligonucleotides containing them will be shown.

INTRODUCTION
Recently, gene therapy for serious diseases such as AIDS is rapidly progressing. Antisense methodology is one of the strategies for selective regulation of gene expression by introducing antisense oligonucleotides into cells.\(^1\) However, natural oligonucleotides are rapidly decomposed by certain nucleases in serum and in cells.\(^2\) Therefore, we noted L-oligonucleotides, which have an unnatural chirality of the sugar moiety and have resistance to nucleases. However, it was reported that L-oligonucleotides are not able to hybridize with natural DNA and RNA.\(^3\)

We have investigated the structure of the heterochiral oligonucleotide, which contains an unnatural L-nucleotide\(^4\) residue in the natural sequence, and have found that the L-nucleotide residue adopts an unusual low anti glycosyl conformation (\( \chi = 180^\circ \)) to form stable base-pairing.\(^5\) This result suggests that the low anti glycosyl conformation might be a critical conformational feature for the L-nucleotide to form Watson-Crick base-pairing in the double helix. To confirm this hypothesis, we designed novel carbocyclic L-nucleoside analogues (1-4) whose glycosyl conformation is fixed at the low anti. The racemic synthesis of the analogues has already been achieved.\(^6,7\) Here, we report the synthesis of an optically active form of the carbocyclic nucleoside analogues (1-4) and the properties of oligonucleotides containing these analogues.

RESULTS AND DISCUSSION

As shown in Scheme 1, the (-)-epoxide 5 was used as a key material, which is prepared from cyclopentadiene in three steps via asymmetric hydroboration.\(^8\) Ring opening of the (-)-epoxide 5

![Figure 1 Carbocyclic L-nucleoside analogues](https://academic.oup.com/nass/article-abstract/42/1/45/1077786/1077786)
by the adenine sodium salt proceeded regioselectively to give the carbocyclic adenosine derivative 6 in 66% yield. Compound 6 was treated with bromine and then tosyl chloride to give the 8-bromo-6'-tosylate. The following cyclization was performed by two step reactions according to the Ikehara's method.9 First, acetylation of the 8-bromo group with sodium acetate in acetic acid-acetic anhydride afforded the 8-keto derivative. Secondly, treatment of this derivative with methanolic ammonia afforded the 8,6'-O-anhydro derivative 9. Finally, debenzylation with 20%Pd(OH)$_2$/C-cyclohexene furnished (−)-carbocyclic 8,6'-O-anhydro-8,6'-dihydroxy-2'-deoxyadenosine 1. The overall yield from the (−)-epoxide 5 was 31% in six steps.

Synthesis of the guanosine analogue (2)$^6$

The (−)-epoxide 5 was treated with 2,6-diaminopurine in the presence of sodium hydride and 15-crown-5 to give the 2,6-diaminopurine nucleoside derivative 7 in 73% yield. This reaction was highly regioselective for N-9.$^10$ Compound 7 was mesylated at the 6'-hydroxy group by mesyl chloride, and then acetylated selectively at the 2-amino group to give the N-2-acetyl-6'-O-mesylate. After the 2,6-diaminopurine moiety was converted into the guanine base by diazotization and hydrolysis at 6-amino group, treatment of this guanosine derivative with NBS afforded the 8-bromoguanosine derivative. Acetolysis of the 8-bromo group with acetic acid in the presence of acetic anhydride and silver carbonate afforded the 8-keto derivative, and then cyclization was performed by sodium hydrogen carbonate in DMF to give the 8,6'-O-anhydro derivative 10. Finally, debenzylation furnished (−)-carbocyclic 8,6'-O-anhydro-8,6'-dihydroxy-2'-deoxyguanosine 2. The overall yield from the (−)-epoxide 5 was 30% in eight steps.

Synthesis of the uridine analogue (3)$^7$

The uridine derivative 11 was converted to the 4-triazolide by treatment with tri(1H-1,2,4-triazol-1-yl) phosphine oxide prepared by phosphoryl chloride and 1,2,4-triazole in the presence of triethylamine$^{11}$, and then treatment of this triazolide with aqueous NH$_2$OH in dioxane afforded the cytidine derivative 12. Finally, debenzylation furnished (−)-carbocyclic 6,6'-O-anhydro-6,6'-dihydroxy-2'-deoxyuridine 4. The yield from the uridine derivative 11 was 63%.

We will also discuss some properties of oligonucleotides containing these analogues.

REFERENCES