Chemical synthesis and properties of stereoregulated phosphorothioate RNAs

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ABSTRACT

Stereoregulated oligoribonucleoside phosphorothioates were synthesized by the use of 2'-O-TBDMS-protected ribonucleoside 3'-O-oxazaphospholidine derivatives as monomers and N-(cyanomethyl)ammonium salts as activators. Diastereoselectivity of the condensation reaction was found to be highly dependent on the substituent groups of the oxazaphospholidine ring as well as the structure of the activators. By the use of the optimized oxazaphospholidine monomers and activators, stereoregulated oligoribonucleoside phosphorothioates containing four kinds of nucleobases were synthesized in good yields. Hybridization properties of thus obtained oligomers with the complementary RNAs were evaluated by the UV melting experiments.

RESULTS AND DISCUSSION

In the previous papers, we have reported the diastereoselective synthesis of 2'-deoxyribonucleoside 3'-O-oxazaphospholidine derivatives by the use of various 2-chloro-1,3,2-oxazaphospholidines as phosphitylating reagents. In a similar manner, 2'-O-TBDMS-protected ribonucleoside were allowed to react with 2-chloro-1,3,2-oxazaphospholidines in the presence of Et3N to afford the corresponding ribonucleoside 3'-O-oxazaphospholidine derivatives (Sp)-1 and (Rp)-1 in good diastereoselectivity (Scheme 1). Notably, in the cases of bicyclic oxazaphospholidine derivatives (R1 = Ph, R2 = CH2-CH2), the monomers could be obtained in excellent diastereoselectivity (> 99:1). The eight kinds of diastereopure bicyclic monomers bearing both Sp and Rp configurations with the properly protected nucleobases (B = U, acC, acA, pacG) were synthesized in good yields.

INTRODUCTION

In recent years, non-coding RNAs (ncRNAs) have attracted a great deal of attention due to their unique and crucial biological roles in living systems. Especially, siRNAs and miRNAs are regarded as powerful tools for selective inhibition of gene expression. In order to stabilize such functional RNAs in cells, a proper modification of the internucleotide phosphodiester bond is quite effective. Phosphorothioate RNA is known as one of the stable RNA analogs possessing the RNAi activity. However, the currently used phosphorothioate RNAs are random mixture of diastereomers due to their phosphorous chirality. In order to increase the biological activity of siRNA and to elucidate the precise reaction mechanism of RNAi, stereoregulated phosphorothioate RNAs would be desirable substrates. To the best of our knowledge, the synthesis of stereoregulated phosphorothioate RNA has been reported by Stec et al. at only the dimer level. Recently, we have reported a novel approach to the stereocontrolled synthesis of phosphorothioate DNA by the use of nucleoside 3'-O-oxazaphospholidine derivatives as monomers and N-(cyanomethyl)ammonium salts as a new class of activators (oxazaphospholidine approach). In this report, we wish to describe the chemical synthesis of stereoregulated phosphorothioate RNA by the oxazaphospholidine approach.

Scheme 1

In the stereocontrolled synthesis of 2'-deoxyribonucleoside phosphorothioates, we have found the suitable oxazaphospholidine ring structure (3-methyl-5-phenyl-1,3,2-oxazaphospholidine: R1 = Ph, R2 = H, R3 = Me) and the activator, N-(cyanomethyl)pyrrolidinium triflate (CMPT) for the stereospecific condensation. The optimized method enables us to synthesize the diastereopure dinucleoside phosphate intermediates within 5 min with excellent diastereoselectivity in solution. In order to apply the oxazaphospholidine approach to the synthesis of ribonucleoside derivatives, we examined the condensation of (Sp)-1 (B = U, R1 = Ph, R2 = H, R3 = Me) and 2',3'-O-bis(phenoxycetyl)uridine 2 in the presence of CMPT in CH3CN and the reaction was monitored by 31P NMR (Scheme 2). In contrast to the 2'-deoxyribonucleotide derivatives, it required 45 min for the complete reaction to give the phosphite intermediate 3 with
considerably low diastereoselectivity ($Rp:Sp = 77:23$). When ($Rp$)-I ($B = U$, $R_1 = Ph$, $R_2 = H$, $R_3 = Me$) was used as a monomer, the condensation reaction completed within 15 min with high diastereoselectivity ($Rp:Sp = 3:97$). These results indicate that both the reactivity of the monomer and stereoselectivity of the condensation reaction are highly affected by the stereochemistry of the chiral auxiliaries as well as the steric hindrance of the $2\prime$-$O$-TBDMS group. In the case of using a bicyclic monomer ($Sp$)-I ($B = U$, $R_1 = Ph$, $R_2 = CH_2CH_2$), the condensation reaction proceeded quickly and completed within 15 min to give the phosphate intermediate ($Rp$)-3 with excellent diastereoselectivity ($Rp:Sp > 99:1$). The resulting phosphate intermediate was successively treated with Ac$_2$O-pyridine and Beaucage’s reagent to give the fully protected diribonucleoside phosphorothioate ($Rp$)-4. After removal of all the protecting groups by the conventional procedure to give UpsU with the $Rp$ configuration in good yield with excellent diastereopurity ($Rp:Sp > 99:1$). In a similar manner, the monomer ($Rp$)-2 ($B = U$, $R_1 = Ph$, $R_2 = CH_2CH_2$) gave the diastereopure UpsU with the $Sp$ configuration in good yield ($Rp:Sp > 1:99$).

Next, the present method was applied to the solid-phase synthesis. All the reactions in the chain elongation cycle as well as the deprotection reactions were optimized for the solid-phase method and consequently, the dimers, UpsU, CpsU, ApsU, and GpsU with both $Sp$ and $Rp$ configurations were obtained in good yields with excellent diastereoselectivity (> 99:1). Longer oligomers (4-10 mers) containing four kinds of nucleobases were also synthesized on the solid support in good yields in stereoselective manners. The thermal stability of the duplexes consisting of thus obtained all-$Sp$- and all-$Rp$- (Ups)$_2$U with the complementary (Ap)$_2$A was examined by the UV melting experiments. It was found that all-$Rp$- (Ups)$_2$U/(Ap)$_2$A showed the higher $T_m$ value than that of (Up)$_2$U/(Ap)$_2$A bearing the natural phosphodiester linkages, whereas all-$Sp$-(Ups)$_2$U did not form an appreciable duplex even under the high-salt conditions.

**CONCLUSION**

We have developed a new efficient method for the stereocontrolled synthesis of phosphorothioate RNA by the oxazaphospholidine approach. The method was successfully applied to the solid-phase synthesis and stereoregulated 10mers could be synthesized on the solid support. We also demonstrated that the phosphorothioate RNA bearing the $Rp$ configuration at all the phosphorous atoms has a significantly high affinity for the complementary oligoribonucleotide. Further studies on biological properties of stereoregulated phosphorothioate RNA are now under investigation.

**REFERENCES**


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