Stereoselective synthesis of dinucleoside phosphorothioate using enantiopure 1,2-amino alcohols as chiral auxiliaries

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ABSTRACT
Diastereopure nucleoside 3'-cyclic phosphoramidites were synthesized stereoselectively from enantiopure 1,2-amino alcohols. In the presence of a novel activator, these phosphoramidites underwent the condensation with 3'-O-tert-butylmethylislylthymidine to give the corresponding phosphate intermediates. Upon sulfurization, followed by deprotection, dithymidine phosphorothioate was obtained in good yield with good to excellent diastereoselectivity.

INTRODUCTION
Oligodeoxynucleoside phosphorothioates (PS-ODNs) have been recognized as antisense drugs, and further studies and clinical trials are also in progress for various diseases. These PS-ODNs are synthesized by the automated phosphoramidite method. However, the resulting PS-ODNs are random mixtures of Rp and Sp isomers since the chirality of the phosphorus atom cannot be controlled by use of the current method. Because the properties of PS-ODNs, such as hybridization abilities with mRNA, affinities to proteins, and tolerances against nucleases, are considered to be affected by the chirality of the phosphorus atoms, it is an important subject to develop an efficient method for obtaining P-stereodefined PS-ODNs. Thus, stereoselective synthesis of PS-ODNs has been extensively studied, but to date, the oxathiaphospholane method, which has been developed by Stec et al., is only the way to obtain stereodefined PS-ODNs. Quite recently, Beaucage et al. have reported the P-stereocontrolled synthesis of d[(TPS)$_2$]$_1$T. However, in these methods, diastereopure monomers have to be separated from a mixture of diastereomers by troublesome column chromatography.

Phosphoramidite methods utilizing enantiopure amino alcohols, such as (1R, 2S)-ephedrin, as a chiral auxiliary have been reported in recent years. The advantage of these methods is that diastereopure monomers can be obtained stereoselectively from the enantiopure amino alcohols. In spite of this advantage, condensation reactions are more or less non-stereospecific. The reason would be attributed to the repetitive attack of tetrazole to the phosphorus atom.

In order to solve this problem, we developed a novel class of activators, namely, dialkyl(cyanomethyl)ammonium tetrafluoroborate I. Since these activators do not generate nucleophile anion species, the condensation reaction would proceed without loss of the enantiopurity of the phosphorus atom.

RESULTS AND DISCUSSION
One of the important points of the present method is to synthesize diastereopure nucleoside 3'-cyclic phosphoramidites from enantiopure amino alcohols. We examined four kinds of enantiopure 1,2-amino alcohols 2a-d as starting materials. First, chlorophosphines 3a-d were prepared from enantiopure 2a-d and phosphorous trichloride. By using these chlorophosphines as phosphitylating agents, we synthesized nucleoside 3'-cyclic phosphoramidites 4a-d. The reactions of 5'-O-tert-butyldiphenylislylthymidine 5 with 3a, b gave trans-4a, b stereoselectively, but the reactions of 5 with 3c, d gave 4c, d with diastereoselectivity of 92:8 and 44:56 (trans: cis), respectively, at room temperature. However, the 1-hour reflux of the reaction mixture of 5 with 3c let the more stable trans isomer enriched, and silica gel column chromatography gave almost diastereopure trans-4c in excellent yield. On the other hand, the diastereomeric ratio of 4d did not exceed 76:24. These reactions giving trans-4a-c proceeded with the retention of the configuration at the phosphorus atom.

Thus obtained diastereopure nucleoside 3'-cyclic phosphoramidites were used for the condensation in the presence of a novel activator. Trans-4a-c were allowed to condense with 3'-O-tert-butyldimethylislylthymidine 6 in the presence of I in CH$_3$CN-CD$_3$CN (4:1, v/v), and the condensation reactions were monitored by $^3$P-NMR. All of the reactions proceeded smoothly; particularly the reaction of 4a with 6 in the presence of I, completed within 5 minutes and gave the corresponding phosphate with excellent diastereoselectivity. The same condensation in the presence of a conventional activator,
tetrazole, proceeded very slowly with low diastereoselectivity.

After sulfurization, the chiral auxiliary was removed by DBU treatment at 50°C without racemization. Finally, the 5'- and 3'- silyl groups were removed by treatment with 3HF-Et,N. After reverse phase column chromatography, fully deprotected dithymidine phosphorothioate was obtained with good to excellent diastereoselectivity.

REFERENCES