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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*-butyldimethylsilylthymidine to give the corresponding phosphite intermediates. Upon sulfurization, followed by deprotection, dithymidine phosphorothioate was obtained in good yield with good to excellent diastereoselectivity.

INTRODUCTION

Oligodeoxyribonucleoside phosphorothioates (PS-ODNs) have been recognized as antisense drugs, and further studies and clinical trials are also in progress for various diseases.[1]

These PS-ODNs are synthesized by the automated phosphoramidite method.[2] However, the resulting PS-ODNs are random mixtures of *R*p and *S*p isomers since the chirality of the phosphorous 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 phosphorous 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.[3] Quite recently, Beaucage *et al.* have reported the P-stereocontrolled synthesis of $d[(T_{PS})_{11}T]$.[4] 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 (1*R*, 2*S*)-ephedrin, as a chiral auxiliary have been reported in recent years.[5] 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 phosphorous atom.

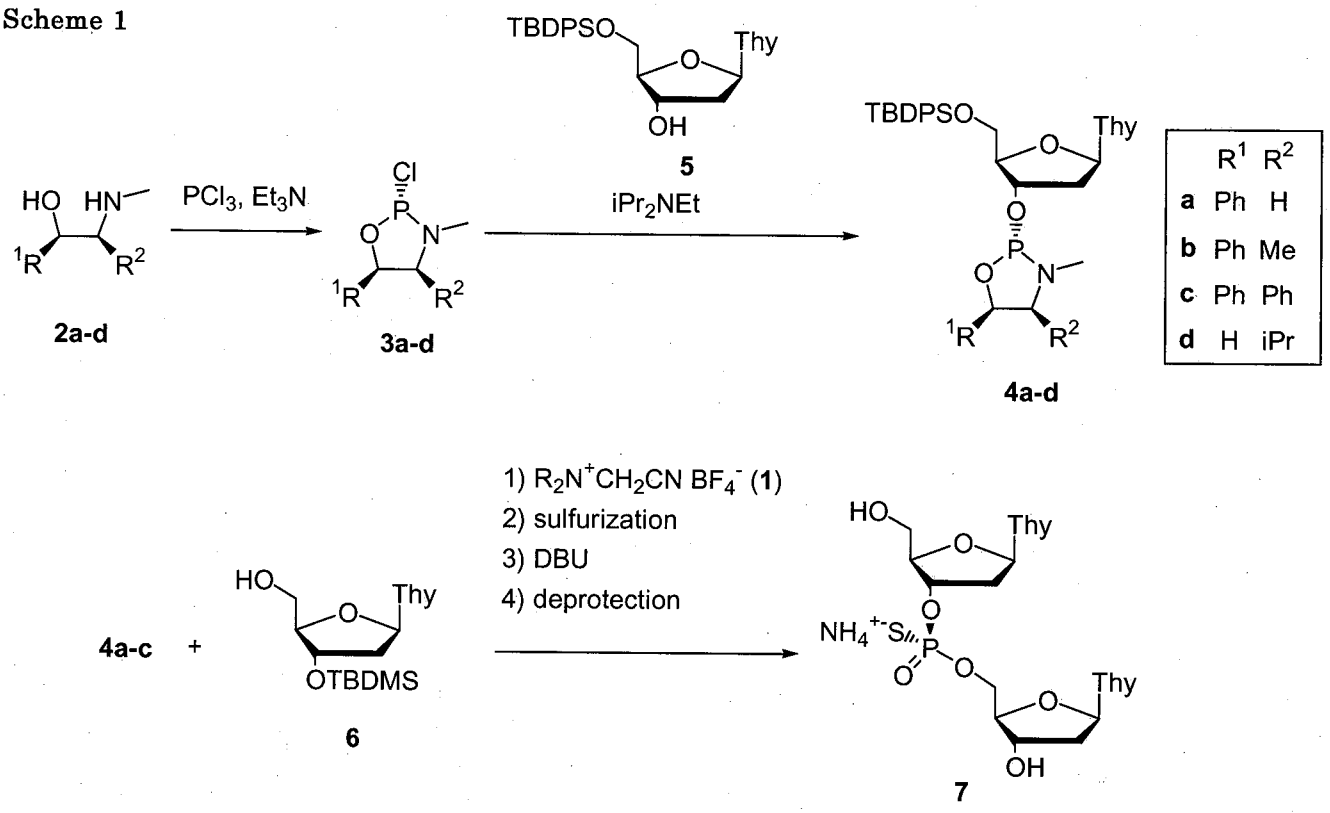
In order to solve this problem, we developed a novel class of activators, namely, dialkyl(cyanomethyl)ammonium tetrafluoroborate **1**. Since these activators do not generate nucleophilic anion species, the condensation reaction would proceed without loss of the enantiopurity of the phosphorous atom.

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*-butyldiphenylsilylthymidine **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 phosphorous 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*-butyldimethylsilylthymidine **6** in the presence of **1** in CH_3CN-CD_3CN (4:1, v/v), and the condensation reactions were monitored by ^{31}P-NMR. All of the reactions proceeded smoothly; particularly the reaction of **4a** with **6** in the presence of **1**, completed within 5 minutes and gave the corresponding phosphite with excellent diastereoselectivity. The same condensation in the presence of a conventional activator,

Scheme 1



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_3N. After reverse phase column chromatography, fully deprotected dithymidine phosphorothioate was obtained with good to excellent diastereoselectivity.

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