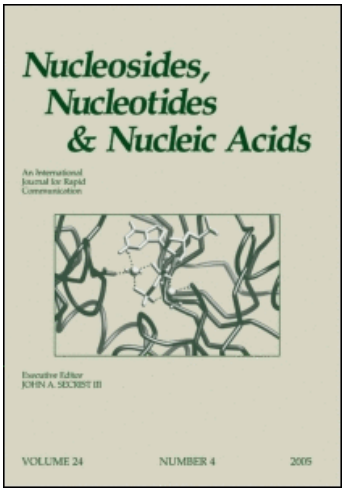

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Diastereocontrolled Synthesis of Phosphorothioate DNA via an Oxazaphospholidine Approach Using a Novel Class of Activators, Dialkyl(cyanomethyl)ammonium Salts

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ABSTRACT

Dialkyl(cyanomethyl)ammonium salts **1** were synthesized and used as a novel class of activators for the stereospecific condensations of diastereopure nucleoside 3′-*O*-oxazaphospholidines with a nucleoside. This new oxazaphospholidine method could efficiently produce both (*R*p)- and (*S*p)-dinucleoside phosphorothioates.

Oligodeoxyribonucleoside phosphorothioates (PS-ODNs) have been recognized as the most promising antisense molecules.[1] However, there still remain some problems concerning the application of PS-ODNs to antisense therapy; one of them is the polydiastereoisomerism of PS-ODN. Thus, the stereoselective syntheses of PS-ODNs have been extensively studied.[2] Among the studies, Stec et al.[2a] and Beaucage et al.[2b] have reported the synthesis of fully *P*-stereoregulated PS-ODNs. In their method, however, the diastereopure monomers had to be separated from a mixture of diastereomers by troublesome column chromatography.

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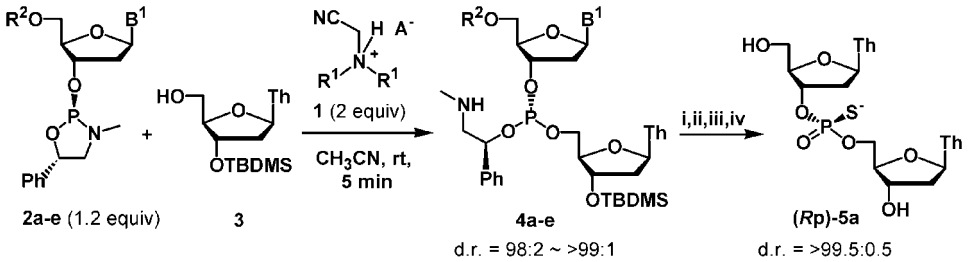

Scheme 1. [a]R^2 = TBDPS, B^1 = Th (**2a**), R^2 = DMTr, B^1 = Th (**2b**), R^2 = DMTr, $B^1 = Cy^{bz}$ (**2c**), R^2 = DMTr, $B^1 = Ad^{bz}$ (**2d**), R^2 = DMTr, $B^1 = Gu^{pa}$ (**2e**), Reagents and conditions: (i) Ac_2O, pyridine, rt, 30 s (ii) Beaucage reagent, rt, 3 min (iii) DBU, 50°C, 30 min (iv) $3HF \cdot Et_3N$, rt, 15 h.

The phosphoramidite method using chiral 1,2-amino alcohols as chiral auxiliaries has been expected as an alternative to produce *P*-stereoregulated PS-ODNs, since the nucleoside 3′-*O*-oxazaphospholidine monomers can be synthesized stereoselectively from appropriate enantiopure 1,2-amino alcohols.[3] It has been reported, however, that the condensations of diastereopure nucleoside 3′-*O*-oxazaphospholidines with a 3′-*O*-protected nucleoside in the presence of a conventional activator, 1*H*-tetrazole, proceeded with partial racemization due to the repetitive nucleophilic attacks of 1*H*-tetrazole to the chiral phosphorus atom. In order to solve this problem, we developed a new class of activators, dialkyl(cyanomethyl) ammonium salts **1** with a rather less nucleophilic counteranion. **1** were found to be effective in the highly stereoselective condensations of the diastereopure nucleoside 3′-*O*-oxazaphospholidines **2** with a 3′-*O*-protected nucleoside **3**.[4] Among a series of the activators we developed, *N*-(cyanomethyl) pyrrolidinium tetrafluoroborate (**1a**) and trifluoromethanesulfonate (**1b**) were the most effective for the highly-stereoselective condensations. We also examined the effect of the substituents on the oxazaphospholidine ring. As a result, 3-methyl-5-phenyl-1,3,2-oxazaphospholidine was found to be the best ring system.

In the presence of **1a** or **1b**, the condensations of **2a–e**, bearing a (2*S*,5*S*)-3-methyl-5-phenyl-1,3,2-oxazaphospholidine moiety, with **3** proceeded rapidly with excellent diastereoselectivity (Sch. 1). After acetylation of the secondary amino group and sulfurization of the phosphorus atom, the chiral auxiliary could be removed by treatment with DBU at 50°C for 30 min without any racemization. After removal of the 5′-*O*- and 3′-*O*-protecting groups, fully deprotected diastereopure (*R*p)-dithymidine phosphorothioate was obtained in good yield. Using (2*R*,5*R*)-**2a** as a monomer unit, diastereopure (*S*p)-dithymidine phosphorothioate was also obtained in a similar yield. The solid-phase synthesis of stereoregulated PS-ODNs by the present method will be reported in due course.

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