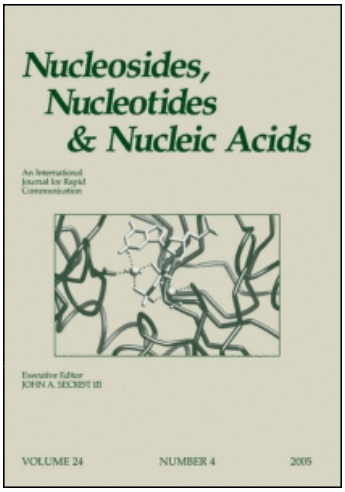

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Stereochemical Problems in Oligonucleotide Synthesis

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STEREOCHEMICAL PROBLEMS IN OLIGONUCLEOTIDE SYNTHESIS

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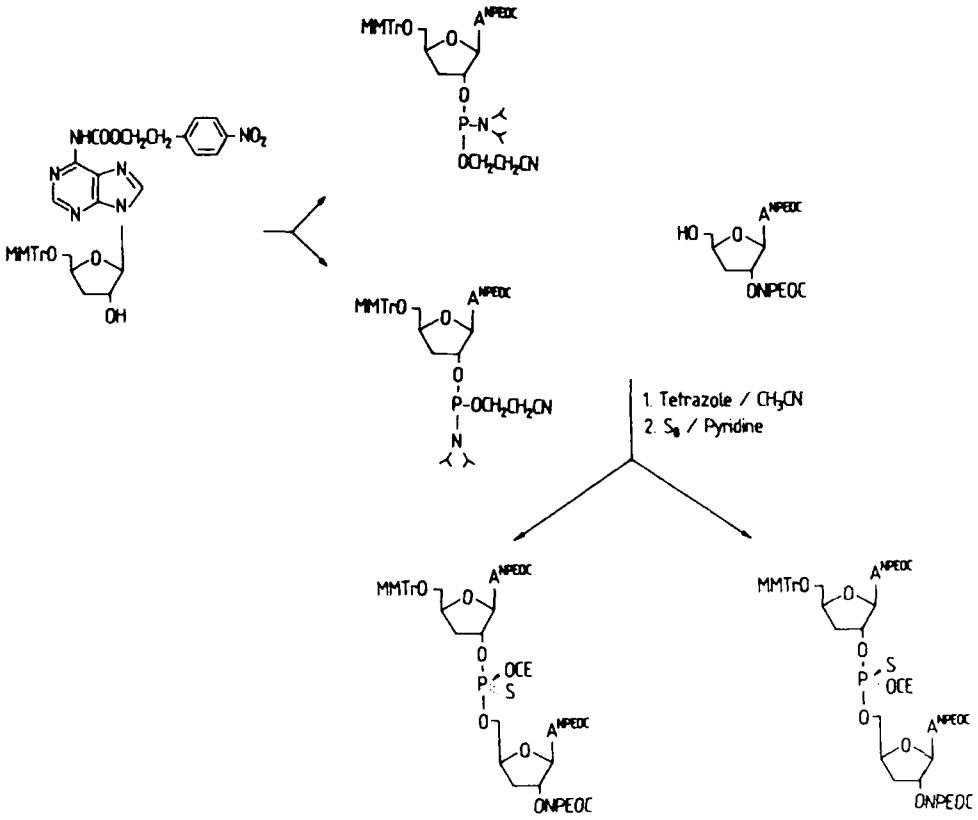
Abstract. The phosphoramidites of protected cordycepin, thymidine and deoxyadenosine were prepared and the two isomers were well separated by medium-pressure chromatography. Condensations were performed in solution to dimers and on solid support to oligomers. The stereochemical consequences will be discussed.

Oligonucleotides containing diastereomerically pure phosphorothioate linkages have been extensively used to study enzymatic cleavage of phosphodiester bonds[1,2] and conformational properties of DNA[3,4].

The phosphorothioate function can be introduced into oligonucleotides through routes based on phosphotriester[5,6] and phosphoramidite methods[7,8] respectively. We have recently reported the synthesis, separation and characterization of the Rp and Sp diastereoisomers of the dimer 2'-5'-A, the four diastereomeric trimers (RR, RS, SR, SS) and four of the eight possible tetramers (RRR, SRR and SSS, RSS) in connection with enzymatic investigations[9,10]. Although separation of the protected compounds was achieved quantitatively, it is tedious to do the chromatographic separations.

Recently Hata et al.[11] reported the stereospecific syntheses of Rp and Sp dimer phosphorothioates by separating the 2,2,2-trichloroethoxycarbonyl phosphonate isomers, which were transformed separately to the Rp and Sp product respectively in 90 % yield.

Cosstick and Williams[12] applied the phosphotriester approach and got with 1-mesitylensulfonyl-5-(pyridin-2-yl)-tetrazole as a condensing agent in a stereoselective reaction over 70 % of the Sp dimer phosphorothioates.



During the synthesis of cordycepin dimer phosphorothioate we separated the two diastereoisomeric phosphoramidites by medium-pressure chromatography. Then each of these isomers was condensed with a second nucleoside in presence of tetrazole as activating agent. After oxidation with sulfur and work-up both the isomers gave a mixture of Rp and Sp dimers in a ratio of 54:46. Similar results are obtained using 3-nitro-1,2,4-triazole as activating agent. These findings indicate that the epimerisation takes place during the coupling reaction, as reported already in the literature[13].

Furthermore we also separated the phosphoramidite isomers in the case of thymidine and N^6-benzoyl-2'-deoxyadenosine quantitatively by medium-pressure chromatography. From the proton NMR spectra it can be seen that the methyl signals of the diisopropylamino group in the 2'-deoxyadenosine series fall together in the case of the higher Rf isomer, whereas resolved signals are noticed in the lower Rf isomer. Also the CH signals of the diisopropyl groups reveal corresponding patterns.

When we used these isomers for synthesizing oligomers in a DNA synthesizer, according to the general procedure, we noticed a striking difference in the coupling yields giving about 98 % with the higher Rf isomer, whereas the lower Rf isomer progresses only to a yield of 80 % in 120 secs. Extension of the coupling time to 500 secs. improved the condensation yields of higher Rf isomer almost to 100 % and those of the lower Rf isomer up to 98 %.

These large differences in coupling yields shown by the two diastereoisomeric phosphoramidites of both the thymidine and deoxyadenosine series suggest that steric factors influence significantly phosphitester formation.

Future investigations will be concerned with this matter.

R E F E R E N C E S

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