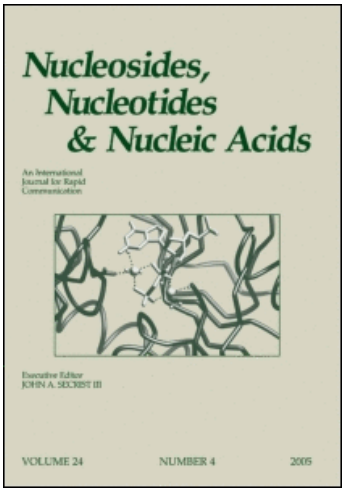

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CHEMOSELECTIVE SYNTHESIS OF DITHYMIDINE PHOSPHOROTHIOATE IN SOLUTION USING *O*-PROTECTED THIOPHOSPHATE MONOMERS

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Abstract: Diastereomerically pure *O*-protected thymine monothioate nucleotide (**I**) is efficiently coupled to protected thymidine (**II**) in a chemoselective, but not stereoselective manner, to give dithymidine phosphorothioates (**III**).

Introduction

Solution phase synthesis of oligodeoxyribonucleoside phosphorothioates (POS-ODNs) by the phosphotriester method[1] is presently performed using *S*-protected phosphorothioates.[2-4] Deprotection of the *S*-protected POS-ODNs was until recently accompanied by 5'-attack at the 3'-nucleoside. Using different protecting groups the side reactions were reduced to 0.3%[5] and very recently to none.[2] *O*-protecting groups are easier to remove than the corresponding *S*-protecting groups.[6] However the commonly used condensing agents together with *O*-protection of the phosphorothioates could result in loss of sulphur, and might slow down the coupling reaction allowing concurrent capping of the 5'-hydroxy nucleosides by the condensing agent.[7]

Phosphorothioates with sulphur in a non-bridging position have chiral phosphorus atoms leading to diastereomeric products. Stereochemically pure POS-ODNs show differences in hydrophobicity, hybridisation parameters and charge density[8] and the literature reveals several approaches to stereospecific chemical synthesis of POS-ODNs - most notably the oxathiaphospholane approach by Stec and coworkes.[8;9]

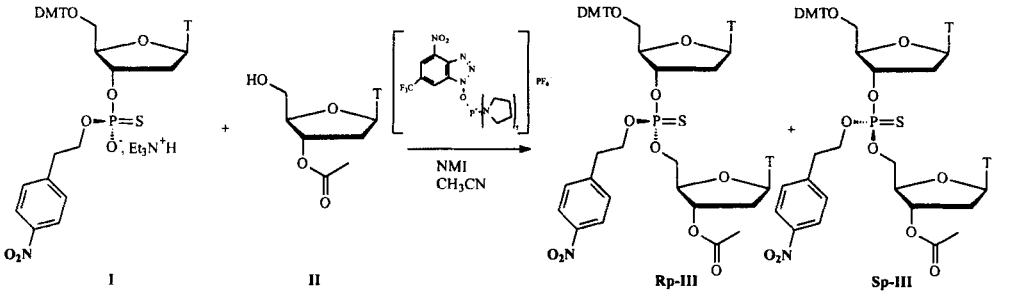

FIG. 1 Chemoselective of coupling of *O*-protected phosphorothioates.

Chemoselective coupling using PyFNOP as the condensing agent in the preparation of phosphorodithioates[10] prompted us to investigate the chemo- and stereoselectivity in the preparation of phosphoromonothioates from a pure diastereomer of **I** (**FIG. 1**).

Discussion

Pure diastereomers of **I** were prepared from pure 2-cyanoethyl triesters, which were resolved in diastereomers by flash chromatography, using MeOH/EtOAc/CH_2Cl_2 (0.5/49.75/49.75) as eluent. R_f on TLC: 0.74 and 0.76. ^{1}H- and ^{31}P-NMR verified the purity.

In a typical experiment 3 eq. of PyFNOP, and then 6 eq. NMI, was added to a solution of the phosphorothioate nucleoside **I** and 1.2 eq. of the 3'-protected nucleoside **II** in dry CH_3CN under N_2. An epimeric mixture of **III** (67.8 + 67.7 ppm) was formed, without any products near 0 ppm as would be expected in the case of activation of the sulphur atom. Within 2 minutes all **I** vanished and an intermediate at 68.5 ppm (broad peak) was transformed into **III** in 2.5 hours. After purification of **III**, *p*-nitrophenylethyl was cleaved off with DBU to give the monothioate. The choice of *O*-protecting group was crucial. Although coupling went smoothly using cyanoethyl as *O*-protecting group, it might be too labile for oligomer synthesis. 2-Nitrobenzyl and 2,4-dichlorobenzyl were also tried as *O*-protecting groups, but they were too labile and were cleaved immediately from their activated esters, thus preventing the formation of triesters.

We propose the following mechanism for the PyFNOP activated coupling reaction: An active ester of **I** and 4-nitro-6-trifluoromethyl-benzotriazole is formed before an alcohol, e.g. a nucleoside, attacks the phosphorus atom and replaces the 4-nitro-6-trifluoromethyl-HOBT moiety. The non-stereoselective coupling is explained by the existence of a fast equilibrium between the intermediate activated ester and free 4-nitro-6-trifluoromethyl-HOBT anions, which competes with the attacking alcohol. This fast equilibrium leads to racemization at phosphorus.

This mechanism is likely from a model experiment, where the phosphorothioate and PyFNOP were dissolved in dry CH_3CN. ^{31}P-NMR did not show any **I** after 2 minutes and two new peaks were formed at 68.7 ppm and 68.8 ppm. After addition of NMI and MeOH a new product was formed at 69.6 ppm.

In conclusion it has been shown that phosphorothioate thymidine dimers can be prepared by chemoselective coupling of *O*-protected phosphorothioates with protected thymidine by the phosphotriester method using PyFNOP as the condensing agent. The coupling reaction is non-stereoselective, which can be explained by the proposed mechanism.

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