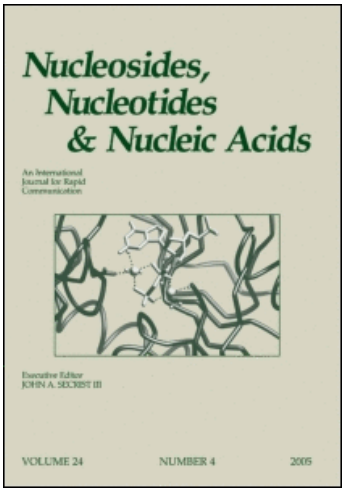


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# $^{31}$P NMR Studies on the Putative Chemoselective Activation of Nucleoside-3'-thiophosphate-O-esters

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# $^{31}$P NMR Studies on the Putative Chemoselective Activation of Nucleoside-3′-thiophosphate-O-esters

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## ABSTRACT

P-diastereomerically pure O-esters of $N^{Bz}$-5′-DMT-dA-3′-monothiophosphate, having charged S=P–O$^-$ moiety, have been synthesized. Chemoselectivity of their activations by formation of different mixed anhydrides, followed by couplings with $N^{Bz}$-3′-levulinyl-dA, were studied by $^{31}$P NMR spectroscopy.

*Key Words:* Nucleoside-3-thiophosphates; Fluorenylmethyl- and p-nitrophenylethyl-esters; Mixed anhydrides; Chemoselectivity; Dynamic $^{31}$P NMR spectroscopy.

The pure $R_P$- and $S_P$-isomers of $N^{Bz}$-5′-DMT-dA-3′-monothiophosphate-O-esters (2-p.nitrophenylethyl /NPE/ and 9-fluorenylmethyl /Fm/, respectively), having charged S=P–O$^-$ moiety, were synthesized starting from $N^{Bz}$-5′-DMT-dA-3′-H-phosphonate (**1**), according to the Sch. 1. By the analogy of highly chemospecific O-activation of 5′-DMT-dT-3′-dithiophosphate-S-esters,[1] $S_P$-isomers of NPE- and Fm-esters (**2a** and **2b**) were selected and reacted with pivaloyl chloride, diphenylphosphoryl chloride or 2,4,6-triisopropylbenzenesulfonyl chloride (TIPS-Cl) to

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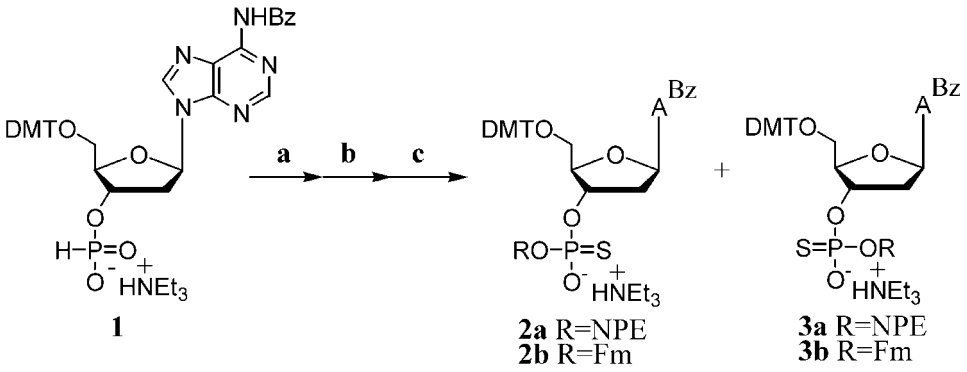


***Scheme 1.*** a, R-OH, pivaloyl chloride, pyridine **b**, $S_8$, pyridine.$CS_2$ **c**, silica gel chromatography

investigate the chemoselectivity of mixed anhydride formations. The in situ formed intermediates were then reacted with $N^{Bz}$-3′-levulinyl-dA (**4**) in each case, in the hope of gaining P-stereochemically pure dA-dA-phosphorothioate derivatives. The progress of activation and putative dimer coupling reactions was followed by $^{31}P$ NMR spectroscopy.

Reaction of **2b** with pivaloyl chloride in $CDCl_3$, using $Et_3N$ (8 equiv.) as base, was sluggish at ambient temperature and led to formation of both O-pivaloyl (34%) and S-pivaloyl (27%) mixed anhydrides while 39% of **2b** remained unreacted. However, neither the O- nor the S-activated intermediate was able to react with **4** even after 2 days, only formation of 2 by-products ($\delta = 50.9$ and 51.5 ppm) was observed.

Contrary to the previous case, activation of **2b** via phosphorylation by $(PhO)_2POCl$, under the same conditions, resulted in the exclusive formation of O-phosphorylated intermediate, i.e., the activation was chemospecific. However, the rate of phosphorylation was similar to that of pivaloylation in addition, the mixed anhydride obtained was also unable to react with **4**. On the basis of its $^{31}P$ chemical shifts ($\delta = 47.1$–47.7 ppm for the thiophosphate and $-23.9$ – $-23.7$ ppm for the diphenylphosphate P-atom, respectively) this intermediate must have one negative charge on the thiophosphate moiety, consequently the Fm group was likely eliminated.

Reaction of the less base sensitive $S_P$-NPE-ester (**2a**) with TIPS-Cl in $CDCl_3$, using larger excess (20 equiv.) of $Et_3N$, was also found to be slow since the proportion of unreacted **2a** was about 58% in the mixture even after 2 days. The required P-diastereomerically pure dinucleoside-phosphorothioate-O-NPE ester ($\delta = 68.2$ ppm) formed only as a very minor product (its proportion was 2%). The remaining mixed anhydride intermediates amounted to 40% in the mixture.

Reactivity of TIPS-Cl drastically increased in the presence of pyridine, tetrazole or N-methyl-imidazole (NMI) due to in situ formation of the more reactive pyridinium- or azolium-sulfonates.[2] However, all of these reactions gave dinucleoside-monothiophosphates, as diastereomeric mixtures due to isomerization of the chiral center on direct nucleophilic attack of the azoles. Because of the higher reactivity





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of P-azolium sulfonates the chemoselectivity of activation steps was much lower compared to that of the O-specific azole- and pyridine-free sulfonylation, e.g., in the case of TIPS-Cl–NMI reagent combination the proportion of sulfur-free final products was about 20% in the mixture. However, due to the weak basicity of NMI the ß-elimination of NPE ester was negligable compared to the other reactions, thus in this case the required dA-dA P-thioate-O-NPE esters ($\delta = 67.9$ and 68.2 ppm) were the main products.

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