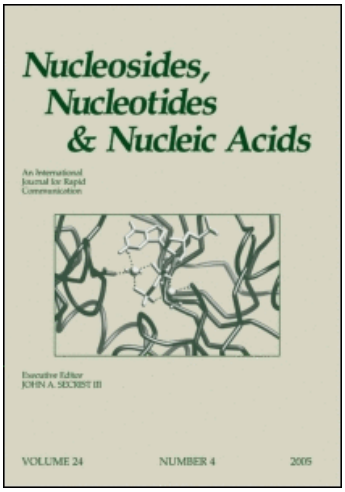

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NMR Study and Improvement of H-Phosphonate Oligonucleotide Synthesis

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NMR STUDY AND IMPROVEMENT OF H-PHOSPHONATE OLIGONUCLEOTIDE SYNTHESIS

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Abstract. The mechanism of the internucleotide condensation and side-reactions in H-phosphonate approach has been investigated. The modification of this method allowed to minimize side-reactions during the preactivation of the nucleotidic component has been developed.

Nowadays, the H-phosphonate method is widely used for chemical synthesis of oligonucleotides [1,2]. However, some side-reactions between the condensing reagent and the starting material are observed, that leads to decreasing the yield of the desired compound.

Earlier, it was shown that rapid H-phosphonate diester formation is achieved when H-phosphonate monoester is activated by the condensing reagent in the presence of a OH-component. Preactivation of nucleoside 3'-H-phosphonate, which usually takes place in the synthesis on polymer supports, followed by the addition of a OH-component, resulted in lower yields of the H-phosphonate diesters [3,4].

We investigated the reaction of H-phosphonate monoester preactivation by pivaloyl chloride (PivCl) with the use of ^{31}P-NMR spectroscopy. The treatment of H-phosphonate (I) with PivCl in pyridine (Py) resulted in the conversion of (I) to mixed anhydride (II), which reacted with the excess of PivCl and in 5-7 min converted into bis-acylphosphite (IV) [3]. The latter gave the mixed anhydride (V) during 4-5 h (Fig.1). The addition of ethanol to compounds (II), (IV) and (V) resulted in the formation of dialkylphosphite (VII), trialkylphosphite (VIII) and dialkylacylphosphonate (IX), respectively.

The examination of the compounds (IV) and (V) reactivity has shown that they are less active phosphorylating agents than mixed anhydride

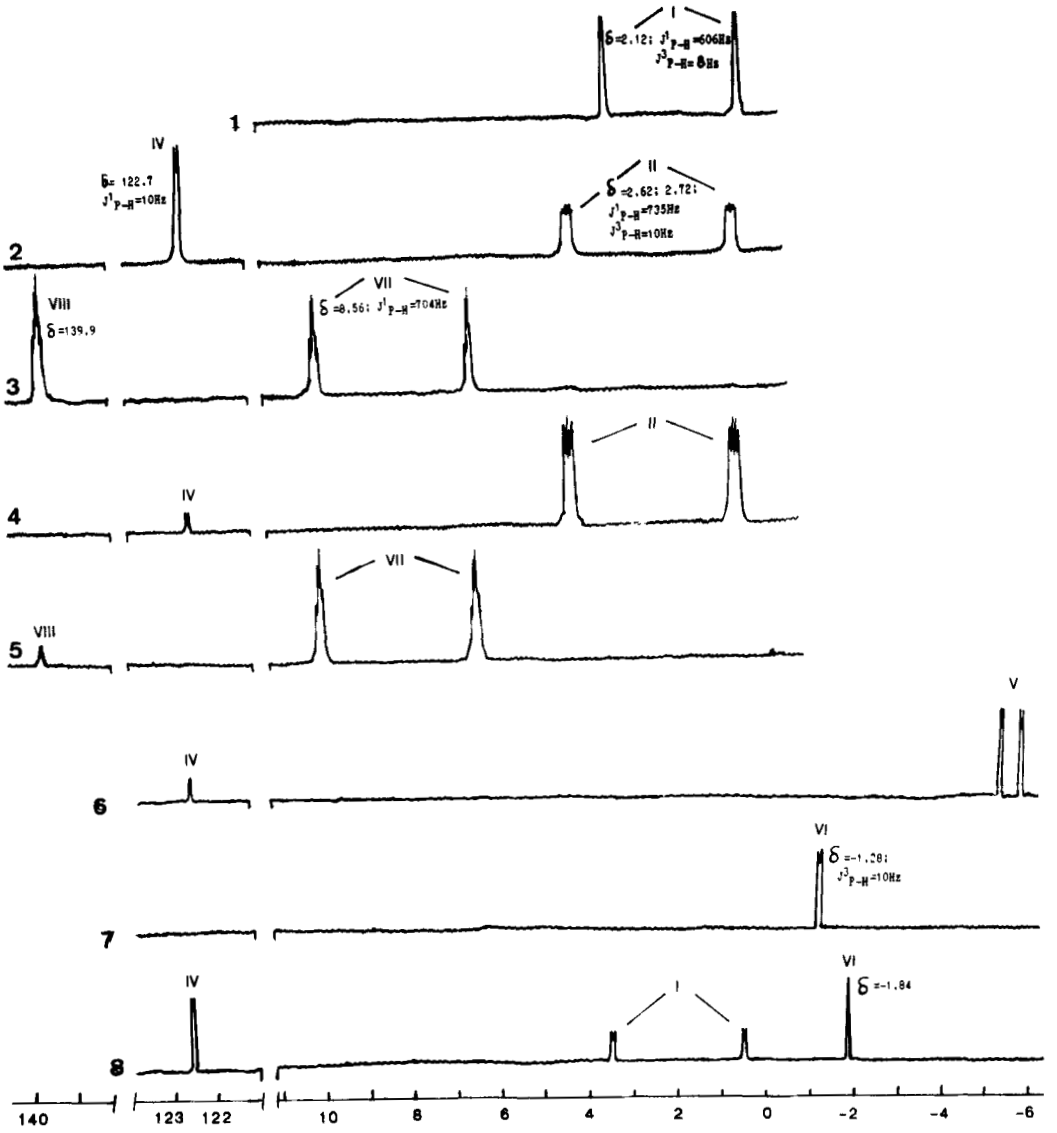
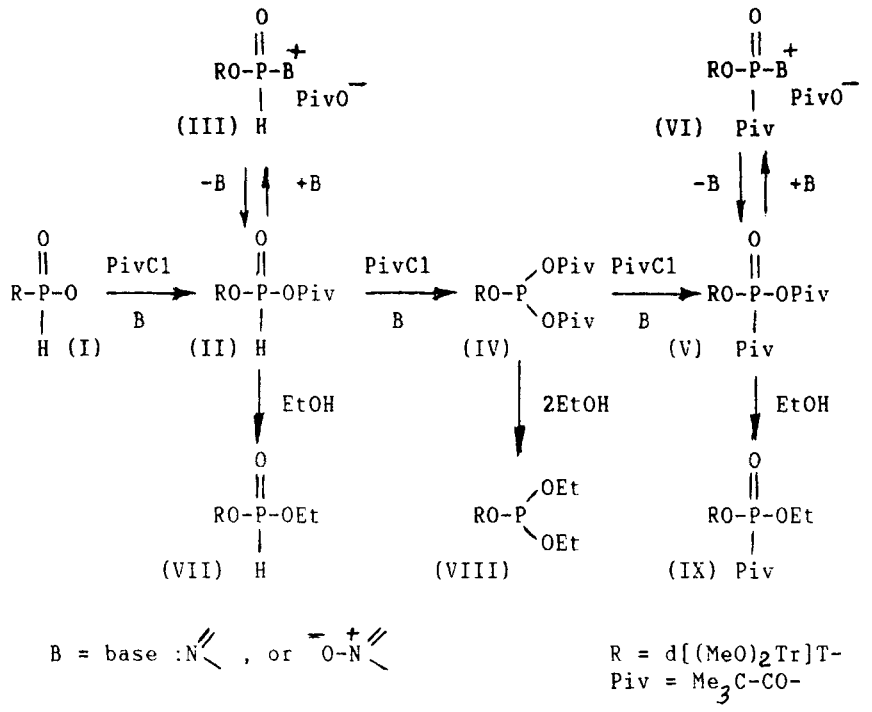


Fig.1. ^{31}P NMR spectra of activated thymidine H-phosphonate. Shifts are reported in negative ppm. 1) [$(MeO)_2$Tr]TpH in CH_3CN-Py(Qu) (4:1, v/v); 2) [$(MeO)_2$Tr]TpH (1 eqv.) + PivCl (3 eqv.) in CH_3CN-Py (4:1, v/v) after 3 min.; 3) mixture from (2) + EtOH (2 eqv.); 4) [$(MeO)_2$Tr]TpH + (1 eqv.) PivCl (5 eqv.) in CH_3CN-Qu (4:1, v/v) after 3 min.; 5) mixture from (4) + EtOH (2 eqv.); 6) mixture from (2) after 4 hours; 7) [$(MeO)_2$Tr]TpH (1 eqv.) + PivCl (3 eqv.) in CH_3CN-MeIm (5:1, v/v) after 5 min.; 8) [$(MeO)_2$Tr]TpH (1 eqv.) + PivCl (3 eqv.) in CH_3CN-4-methoxypyridine (5:1,v/v) after 5 min.



(II). Thus, the reaction of (II) with EtOH is complete in 20-30 sec, whereas the reactions of (IV) and (V) with EtOH were complete only within 4-5 min and 1-1.5 h, respectively. As a consequence, the coupling reaction is not complete in standard reaction time (about 2 min) after short preactivation of P-component, and the increase of preactivation time led to the reduction of coupling yield. The latter stabilized after 7 min preactivation when practically only compound (IV) was present in the reaction mixture.

The dependence of the rate of the bis-acylphosphite (IV) formation upon basicity of solvent used has been examined. It was revealed that the conversion of (II) into (IV) speeded up in more basic conditions. Thus, this reaction proceeded very slowly in the presence of quinoline (Qu) (pKa 4.87) or N,N-dimethylaniline (DMA)(pKa=5.07). At the same time, it was complete in 5-7 min in Py (pKa=5.25) and less than in 2 min - in 4-methoxypyridine (MeOPy)(pKa=6.62) [5].

We investigated also the existence of a nucleophilic catalysis in H-phosphonate coupling reactions. It was shown that the conversion of compound (II) into diester (VII) was complete in 4-5 min in the presence

of DMA, which is not a nucleophilic catalyst. On the other hand, the rate of phosphorylation reaction increased in 8-10 times when 5-10 eqv. of Py, or Qu, were added to the compound (II) preliminary obtained in the presence of DMA. The basicity of DMA differs only slightly from that of Py, or Qu, but the latter two compounds can promote nucleophilic catalysis via the formation of a highly reactive intermediate (III). The signal of (III) was not observed in the ^{31}P NMR spectra, presumably due to shift of the equilibrium towards the mixed anhydride (II).

The 2-4 times acceleration of compound(II) conversion into (VII) was also observed in the presence of some other nucleophilic catalysts, namely pyridine N-oxide (pKa=0.79) and its 4-chloro- and 4-nitro-derivatives. The use of more powerful catalists, such as 1-methylimidazole (MeIm) (pKa=7.3), MeOPy, 4-N,N-dimethylaminopyridine (DMAP), 4-N,N-dimethylaminopyridine N-oxide (DMAPO)(pKa=3.88), led to rapid conversion of reactive intermediates (II) and (IV) into compound (VI), which provided low phosphorylating activity. As a result, the condensation reaction in the presence of MeIm, or MeOPy, was not complete, and in the presence of DMAP, or DMAPO, the reaction did not practically proceeded. At the same time, Py and Qu are not able to form intermediate (VI), and they provide rapid conversion of compound (II) into H-phosphonate diester (VII).

The best results in the H-phosphonate synthesis were obtained by us with Qu. In the reaction of (I) with PivCl, the formation almost only mixed anhydride (II) was observed with the use of acetonitrile - Qu mixture as a solvent. On the other hand, Qu like Py provided almost quantitative yields on the second step of condensation reaction, when compound (II) reacts with OH-component. The coupling reaction in these conditions was complete in 30 sec in solution and in 1.5-2 min on solid support.

On the base of the results obtained, we have modified the H-phosphonate approach by the use of acetonitrile - quinoline (4:1,v/v) as a solvent for internucleotide condensations. The reactions were carried out on polymer support on the base of porous glass beads in automatic conditions. At each step the 10-fold excess of P-component over the resin capacity and 5-6-fold excess of PivCl over H-phosphonate monoester were used. The time needed to perform one cycle of elongation was 5-6 min. The average yield per step was about 99%. Using this

method, a set of oligodeoxyribonucleotides with the chain length up to 40-mer has been synthesized.

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