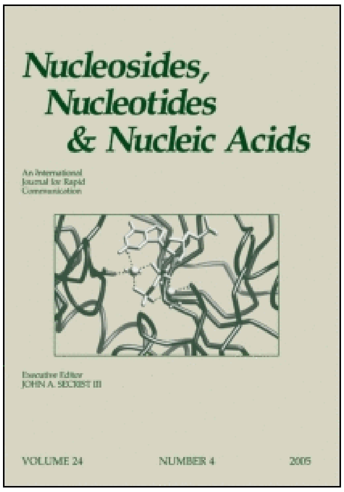

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The Use of Nucleoside H-Phosphonates in the Synthesis of Deoxyoligonucleotides

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THE USE OF NUCLEOSIDE H-PHOSPHONATES IN THE SYNTHESIS OF DEOXYOLIGONUCLEOTIDES

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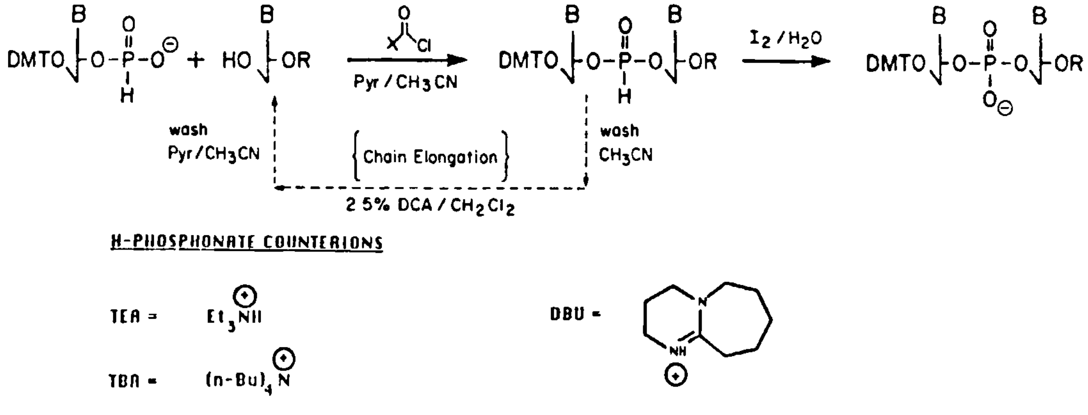
Abstract: Nucleoside H-phosphonates are valuable intermediates in the synthesis of deoxyoligonucleotides. The DBU salt form is more stable in solution and is consequently an improvement in routine synthesis of DNA.

Recent reports have shown that nucleoside H-phosphonates are valuable intermediates in the chemical synthesis of DNA (Scheme 1)[1,2]. Synthesis of DNA up to 107 bases in length has been demonstrated using a rapid, simple and reagent efficient protocol (4 min/cycle,1.5 mg/coupling)[2]. Our preliminary results have been repeated by other workers[3,4] and they supported our intial conclusions that pivaloyl chloride (Piv-Cl) is the preferred activator of nucleoside H-phosphonates[1]. Reported here are the results of experiments leading to the improved purity and yield of synthetic deoxyoligonucleotide.

Our previous results indicate that a small amount of detritylation of the nucleoside H-phosphonate takes place leading to N+1 oligomer contamination of the desired product[2]. Initially we speculated that this was taking place during the condensation reaction but have since determined that detritylation of the monomer occurs in an anhydrous pyridine (Pyr)/ acetonitile (CH_3CN) solution (appox. 2% after 4 weeks for thymidine). One possible solution to this problem would be to add stronger bases to the nucleoside solution. The



SCHEME 1

effect of bases, other then pyridine, in the synthesis of DNA was examined by the synthesis and HPLC analysis of T_5 as decribed earlier[1]. The addition of stronger bases (50 m$\underline{M}$) such as triethylamine (TEA), diisopropylethylamine (DIPEA), N-methyl imidazole (NMI) and 2,6-Lutidine to the Pyr/CH_3CN solution results in a dramatically lower yield of T_5 product relative to the synthesis without added base. A possible explanation for this effect is an increase in rate of acylating of the 5'-OH relative to phosphitylation, or formation of an internucleotide acyl phosphonate[5]. An acyl phosphonate would be unaffected by the oxidation conditions and subsequent ammonolysis would lead to a H-phosphonate linkage which is hydrolytical unstable[5]. Other experiments demonstrate that the use of Pyr is necessary in the synthesis of DNA, less than 10% Pyr as a cosolvent gives low yields of product deoxyoligonucleotide. Various cosolvents for pyridine were examined by the synthesis and polyacrylamide gel electrophoresis (PAGE) of T_{20} as described earlier[1]. The results indicate that 100% Pyr, or 50% Pyr/CH_3CN are the preferred solvent systems. Methylene chloride as a cosolvent (50%) is slightly inferior to CH_3CN and THF gives a very low yield of product T_{20}.

The effect of the H-phosphonate counterion (Scheme 1) in the synthesis of T_{20} was examined in an attempt to solve the problem of instability of the DMT group in solution. The TEA, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and tetrabutylammonium (TBA) salt forms were compared and the results indicate that the DBU salt and the TEA salt give comparable high yields of T_{20}, T_{40} and T_{80} (data not shown). It was also found that the DBU salt inhibits detritylation of the nucleoside H-phosphonate in solution (less then 0.5% after 4 weeks) and is therefore a superior counterion to be used in the synthesis of DNA. Use of the TBA salt leads to extraneous bands (low electrophoretic mobility) on PAGE, probably due to base modification or modification of the poly H-phosphonate backbone.

P^{31} NMR studies were done to examine the species formed upon activation of Ethyl H-phosphonate with Piv-Cl in Pyr/CH_3CN (1/1). Figure 1 shows the spectrum of Ethyl H-phosphonate (δ -2.2 ppm, Panel A). Addition of approx. 0.6 eq. of Piv-Cl to the solution generates a dominant resonace (δ -3.0 ppm, Panel B) and a minor resonace (δ -1.8 ppm, Panel B). As more Piv-Cl is added the equilibrium mixture changes to favor the -1.9 ppm resonance (Panel C). Addition of Ethyl H-phosphonate to this increases the amount of the δ -3.0 ppm resonance (Panel D), which is then converted back to the δ -1.9 ppm resonance with additional Piv-Cl (Panel E). These results are consistent with the δ -3.0 ppm resonance being that of the symmetrical anhydride and the δ -1.9 ppm resonance being that of the mixed anhydride. Further support for this assignment is the observation that the δ -3.0 ppm resonance is broad, suggesting two unresolved peaks. This is expected since the symmetrical anhydride exists as two diasteriomers. When 5 equivalents of Piv-Cl is used to activate the H-phosphonate monoester only the mixed anhydride is observed, which is a very reactive and selective phosphitylating agent of alcohols. We have also observed a trivalent phosphite species (δ -123 ppm) upon prolonged activation of the H-phosphonate with Piv-Cl.

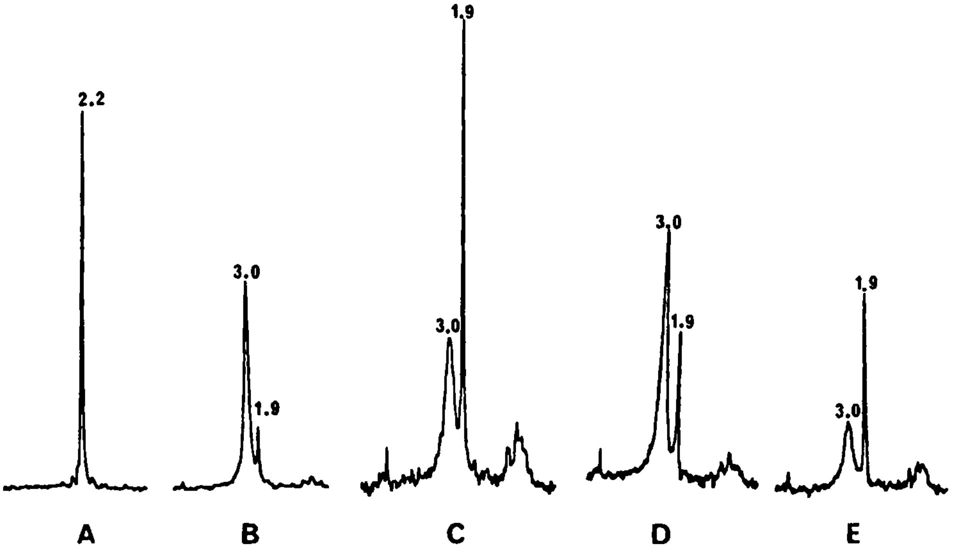


FIGURE 1
P^{31} NMR spectra of activated Ethyl H-phosphonate; Shifts are reported in negative ppm.

This activated species generates trialkyl phosphite when quenched with excess alcohol (data not shown). Complete formation of this active trivalent species requires 5 min using 100mM Ethyl H-phosphonate and 500 mM Piv-Cl.

Deoxynucleoside H-phosphonates are valuable intermediates in the synthesis of DNA. The use of the DBU salt as a counterion improves the purity of the product deoxyoligonucleotide by inhibiting detritylation of the nucleoside H-phosphonate in solution.

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