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NEW INTERMEDIATES FOR THE SYNTHESIS OF OLIGONUCLEOTIDES BY THE PHOSPHITE TRIESTER APPROACH

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Summary - Heterocyclic bases such as triazole and tetrazole have been used to increase selectivity of phosphorylation of nucleosides in oligonucleotide synthesis via the phosphite triesters.

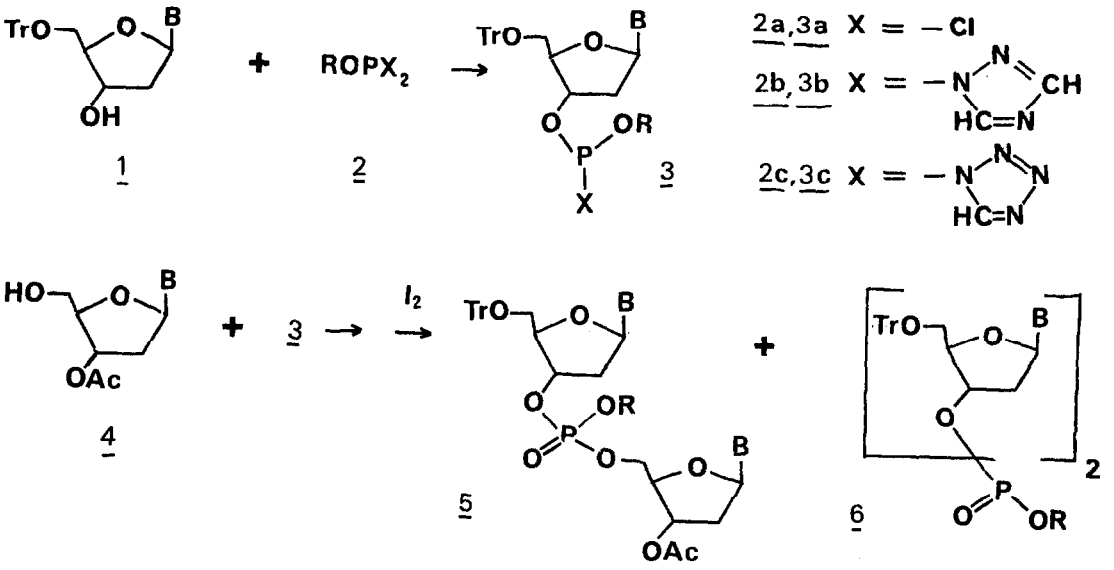
Recent interest in the chemical synthesis of oligonucleotides by the modified triester method has focussed on the development of new protecting groups and methods for generating phosphate esters [1].

Letsinger has shown that phosphite triesters can be used as intermediates for internucleotide bond formation [2] and this procedure has been exploited for syntheses in solution [3], on polymer supports [4] and for the elaboration of building blocks [5].

Phosphorodichloridites $ROPCl_2$ react rapidly at even very low temperatures with the free 3'-OH of a protected nucleoside 1 to give the intermediate 3 (X = Cl). The intermediate can then react in situ (without isolation) with a suitably protected nucleoside having a free 5'-OH group 4 to give, after simple oxidation, the dinucleoside phosphate 5.

A serious disadvantage to this method however is that, because of the high reactivity of the phosphorochloridites, poor specificity for a primary compared to a secondary alcohol is observed. In consequence, besides the desired 3',5'- dinucleoside phosphates 5, significant formation of 3',3'-dinucleoside phosphates 6 occurs. Chromatographic separation of these unwanted side-products is easy, but for the phosphite approach to become more attractive, this loss of costly material should be avoided.

In order to solve this problem we have searched for new phosphine derivatives which might exhibit a better selectivity for primary than for secondary alcohols. We anticipated that this could be achieved by replacing the chlorines in $ROPCl_2$ by heterocyclic bases, thus rendering the reagent less reactive in much the same way as for phosphorochloridates and



B = thymine, R = methyl, Tr = trityl, Ac = acetyl

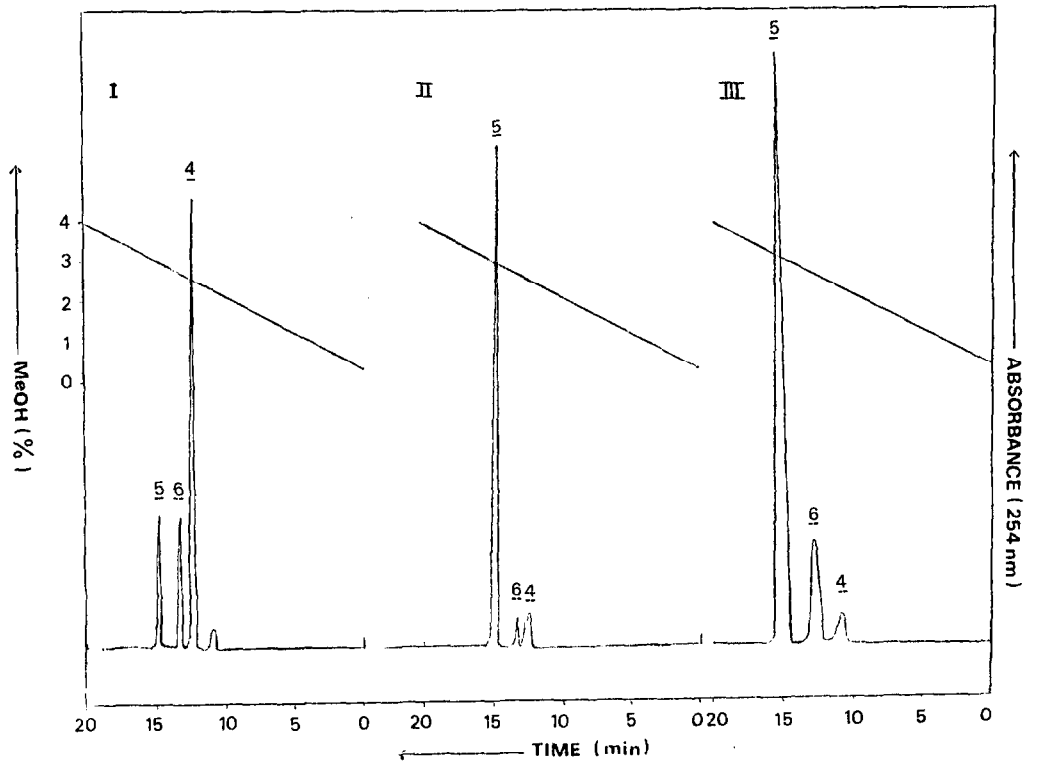


Figure - HPLC profiles of crude reaction mixtures : I : Pyridine, II : Triazole, III : Tetrazole. Elution 5-80 % B (A is CH_2Cl_2/0.05 % acetic acid ; B is CH_2Cl_2/5 % MeOH/ 0.05 % acetic acid). Flow 2 ml min -1 through a 250 x 4 mm Lichrosorb Si60 column. The numbers above the peaks correspond to the compounds in the text.

sulfonyl chlorides [6]

The following experiments show that, compared to the corresponding chlorophosphine $ROPCl_2$, the bis-triazolyl and bis-tetrazolyl derivatives exhibit enhanced selectivity and can be used as phosphorylating agents. To have a valid comparison equimolar quantities of nucleosides were treated under the following experimental conditions : low temperature (-70°C) addition of one equivalent of 5'-O-trityl thymidine 1 (0.25 mM in 2 ml THF) to a solution of 1 equivalent of $MeOPCl_2$ [7] 2a in THF-pyridine (2:0,5 ; 2.5 ml) followed by addition of one equivalent of 3'-O-acetyl thymidine 4 (0.25 mM in 2 ml THF) gave, after iodine oxidation the dinucleoside phosphates 5 and 6 isolated by silica gel short column chromatography. Structures 5 and 6 are consistent with the proton NMR spectra of both compounds. In the case of phosphate 5 this spectrum exhibits three methyl singlets due to the acetyl and two C-5 methyl groups. The OMe signal appears as two doublets (J = 11 Hz) indicating the presence of two diastereoisomeric phosphates. In agreement with the proposed structure the spectrum of 6 is devoid of an acetyl group signal and shows the OMe signal as a doublet.

As shown in the figure HPLC can be used for the detection of reaction products and recovered reactants and for the determination of their relative proportions.

In the above experiment, where both protected nucleosides 1 and 4 were reacted in equimolecular amounts, the isolated yields of phosphates 5 and 6, were 38 and 15 %, respectively. These yields, based on 5'-O-tritylthymidine 1 are consistent with those previously reported [2] which call for an excess of 3'-OH unprotected deoxynucleoside to ensure complete reaction of the second 5'-OH unprotected unit. It is noteworthy that a significant amount of polar material, presumably methyl 5'-O-trityl thymidinyl phosphate, was isolated [8].

We have established the advantage of using triazole or tetrazole containing intermediates 2b, 2c by repeating this phosphorylation reaction in the following manner. To four equivalents of triazole or tetrazole in THF-pyridine (2:0.5) kept at - 20°C was added one equivalent of $MeOPCl_2$ 2a. After 10 minutes this solution was cooled to - 70°C and sequentially treated with one equivalent of 5'-O-trityl thymidine 1 (10 min.) and with one equivalent of 3'-O-acetyl thymidine 4. When the temperature reached - 20°C the reaction mixture was oxidized. Based on 5'-O-trityl thymidine 1 the isolated yields of 3',5'-phosphate 5 were 70 and 75 % for triazole and tetrazole, respectively.

It is clear from the examination of the HPLC chromatograms that under these reaction conditions the formation of 3',3'-phosphate 6 is dramatically decreased. It has been

virtually avoided in the case of triazole (Figure).

Compound 5 was deprotected (MeOH, NH_3) yielding the free 3'-OH dinucleoside phosphate 7 which was subsequently treated with the tetrazole substituted phosphine and 3'-O-acetylthymidine 4 (one equivalent) to give after oxidation the fully protected TpTpT in 70 % isolated yield.

The above results clearly demonstrate the interest of triazole and tetrazole substituted phosphines of structure 2b or 2c in oligonucleotide synthesis [9]. They can be generated in situ and can serve as selective phosphorylating agents, leading to satisfactory yields of the desired 3',5'-phosphates even when equimolecular amounts of protected 5'- and 3'-OH nucleoside reactants are used. We anticipate that further work in progress will show that these new reaction conditions will be very useful for the synthesis of oligonucleotides.

Acknowledgement

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8. This compound is not eluted from the HPLC column under the conditions indicated in the figure.
9. The use of imidazole leads to lower yields of compound 5 (Dr. E. GUIBE, unpublished observations).

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