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A NOVEL ROUTE FOR SOLID PHASE SYNTHESIS OF POLYNUCLEOTIDES USING PHOSPHITE CHEMISTRY

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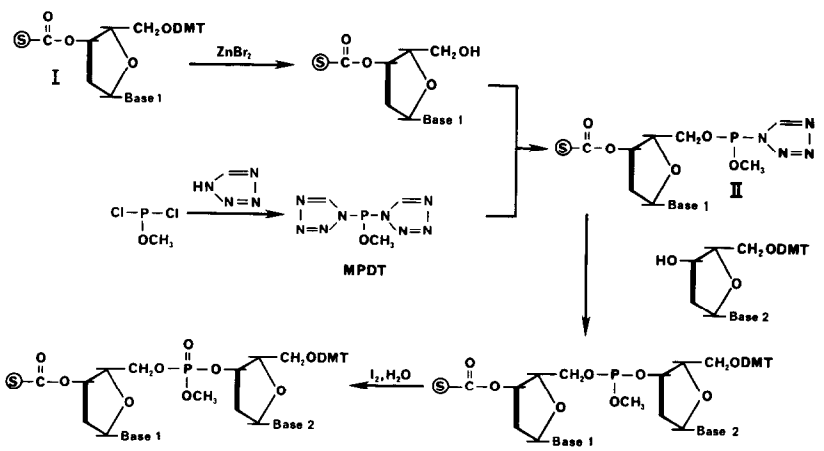
Summary - A new route leading to a simple and rapid procedure employing Methyl Phosphorditetrazolide (MPDT) as condensing agent for polynucleotide synthesis is described.

The phosphite triester procedure, introduced by Letsinger (1), has proven to be a general procedure for the synthesis of oligonucleotides (2-5) by the solid phase technique. Alkyl phosphorodichloridites react with a small excess of a 5'-O-protected nucleoside at -78°C to convert the dichloridite to a nucleosido phosphoromonochloridite. This mononucleoside phosphite is a key intermediate involved in the condensation with the 5'OH of a nucleoside primer linked to a solid phase resin. Several difficulties have been encountered in the preparation of this highly reactive reagent: it requires stringent control of laboratory environment, the procedure for its preparation is laborious, a side reaction yields an inert 3'-3' dinucleotide by-product; and during the coupling step it produces insoluble collidine hydrochloride which leads to reduction in flow in automatic polynucleotide synthesis (2).

Attempts have been made to synthesize nucleoside phosphite intermediates using an excess of phosphorylating agent to avoid the formation of dimers (4). Tetrazole (6) and triazole have been used as leaving groups for the mononucleoside phosphite to avoid clogging the column used in automatic synthesis (2). The most interesting intermediate, deoxynucleoside phosphoramidite (5), is stable upon storage and can be activated and used directly for nucleotide condensation when needed. However, the synthesis of phosphoramidite requires large scale preparation and skilled labor. Hence the method is not generally feasible for many laboratories

We report a new route for solid phase polynucleotide (either deoxy or ribonucleotide) synthesis and employing methyl phosphoroditetrazolide (MPDT) as a bifunctional prototype phosphorylating reagent. Other heterocyclic bases have also been successfully utilized.



Our method, as illustrated above, differs from the conventional phosphite procedure in

one major respect; following deblocking the nucleoside on the resin (I) is activated with an excess of MPDT in large excess to yield exclusively a nucleosido-phosphoro-monotetrazolide resin (II). Unreacted reagent is removed by filtration and, upon introduction of the incoming nucleoside, an internucleotide linkage is formed. Subsequently, the phosphite triester is oxidized to its phosphate counterpart with I_2 and the condensation cycle is repeated to extend the chain. The detailed protocol for our method is given below; procedures not described are those previously published (2).

MPDT is prepared in a one flask operation by the addition of methylphosphorodichloridite (5.3 mM), under an inert atmosphere, to a well-stirred solution of collidine (16.5 mM) and tetrazole (12.5 mM) in anhydrous tetrahydrofuran (15 ml) at room temperature. The MPDT recovered after removal of the insoluble collidine· HCL by centrifugation, is sufficient for ten condensations involving 0.01 mM of 5' deblocked deoxynucleoside. MPDT is stable for weeks when stored at -20°C; however, its preparation is so simple, that it can be conveniently prepared daily. The activation of resin nucleoside with MPDT is done using a molar ratio of MPDT to terminal deoxynucleoside of 20:1. Typically, 1.0 mL solution of MPDT prepared as described is added to 75 mg of Fractosyl resin bearing a 5' deblocked deoxynucleoside and the mixture is gently swirled for 5 minutes. After removal of excess MPDT, and flushing with argon, the 5'DMT-deoxynucleoside (0.05 mM) in 0.5 mL of a 1% collidine-dry THF (v/v) mixture is introduced. The suspension is again swirled for 5 minutes. The molar ratio of incoming nucleoside to terminal nucleoside phosphite is 5:1. As measured by the release of dimethoxytritanol after each cycle, condensations employing blocked derivatives of dA, dC, dG, and T proceed reproducibly at greater than 98% yield. In the synthesis of 3'TAGGAATATAGCGG 5', at step 13 trityl alcohol was recovered at 94% of the initial resin deoxynucleoside. This corresponds to an average yield of 99.4%, for each step. In our hands, under comparable conditions, the conventional phosphite method gave a yield of only 30% at step 13. The fundamental difference between the two methods is in the origin of the nucleoside activation. In our method, the 5'-OH of the resin immobilized nucleoside is being activated with the MPDT reagent in 20-fold molar excess, leading to a complete reaction with all available primary hydroxyl groups. In addition, a high concentration of MPDT prevents possible internucleotide crosslinks and yields exclusively a nucleosido-phosphoro-monotetrazolide resin. Our new concept of directly adding the desired nucleoside to the solid phase resin for internucleotide condensations circumvents the laborious activation of individual nucleosides at low temperature.

The ease and celerity of our polynucleotides chemistry obviates the need to prepare stable phosphorylation intermediates such as phosphoramidites. With this method, virtually any laboratory can undertake small scale polynucleotide synthesis.

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