Use of Substituted Phenol as Phosphate Protecting Group in the Synthesis of Deoxyribo-oligonucleotides bearing 5′-Phosphomonoester End Group

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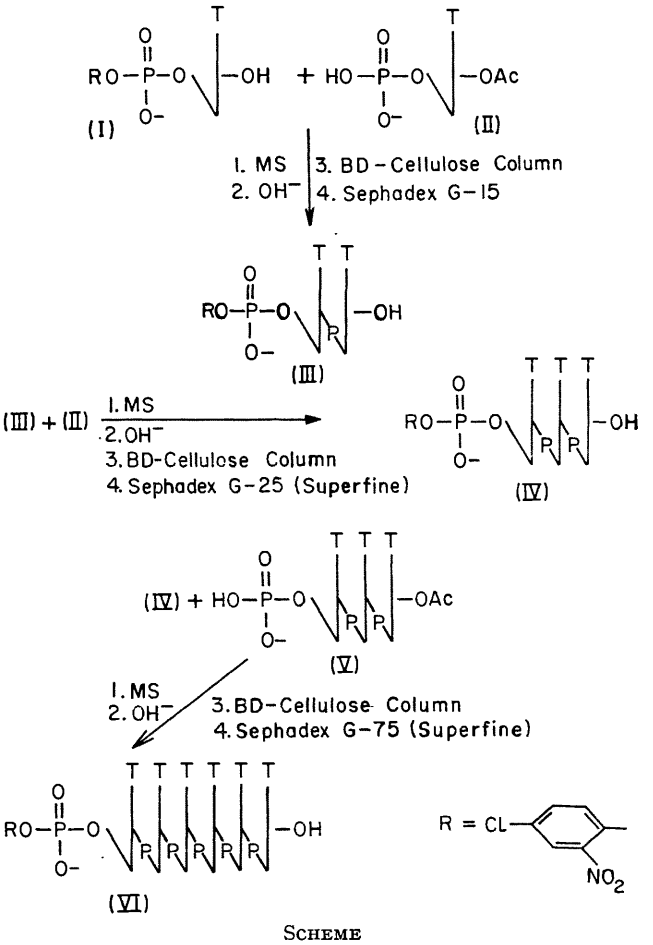
Summary A new and efficient approach, which involves the applications of substituted phenols, (i) as 5′-phosphate protecting group and (ii) to increase the binding property of the nucleotidic fragment to benzoylated DEAE-cellulose, has been developed successfully for the synthesis of deoxyribo-oligonucleotides.

WITH the main objective of synthesizing a DNA molecule for biological studies, it was considered essential to develop more efficient and rapid chemical methods for the synthesis of deoxyribo-oligonucleotides. We report a new approach towards this goal, based on the following two principles. (i) The attachment of a substituted phenol at the 5′-phosphomonoester end of the nucleotide leads to a great increase in its binding to benzoylated DEAE-cellulose.[1] Hence, after each condensation between nucleotides (see Scheme), the reaction mixture is passed through a benzoylated DEAE-cellulose column. This effects the removal of compounds lacking the phenolic residue *e.g.* pT, pyrophosphate of pT, cyclic phosphate, and other side-products. The removal of these impurities, especially pyrophosphates, is difficult by the usual techniques.[2] (ii) The substituted phenol protecting group for 5′-phosphate of the nucleotides are quite stable towards alkali. Thus, a more alkali-labile group such as acetyl on the 3′-hydroxy-end of the growing chain can be selectively removed for further elongation of the chain. This approach has been successfully applied in the synthesis of thymidine hexanucleotide $(pT)_6$.‡

Thymidine 5′-phosphate was protected by treating its pyridinium salt with a large excess (20 molar equiv.) of 4-chloro-2-nitrophenol in the presence of DCC(10 molar equiv.). The 4-chloro-2-nitrophenyl pT (I) was isolated by ether precipitation. A typical condensation step was carried out by treating an anhydrous pyridine solution of (I) with 3′-*O*-acetylthymidine 5′-phosphate (II) (3 molar equiv.) in the presence of mesitylenesulphonyl chloride [10 molar equiv. based on (II) component]. After 2 hr., the reaction was given a 2 hr. treatment with aqueous pyridine, followed by a 10 min. treatment with an equal volume of 2N-sodium hydroxide at 0° to remove the acetyl group. Next, the crude reaction mixture was rendered anhydrous by repeated evaporation of added pyridine and the salt of the mesitylenesulphonic acid was removed by precipitation of the nucleotides from a large excess of



SCHEME

chloroform–ether (40 : 60 v/v).[3] The precipitate was dissolved in 0·05M-triethylammonium hydrogen carbonate buffer pH 7·5 and applied to a benzoylated DEAE-cellulose

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‡ The system of abbreviation is essentially the same as used in *J. Biol. Chem.*

column (2 × 40 cm). The column was first eluted with 0·05M-triethylammonium hydrogen carbonate buffer pH 7·5 which immediately eluted unreacted pT and side-products lacking the phenolic residue, *e.g.* pyrophosphates. The column was subsequently washed with a stronger buffer, 0·2M-triethylammonium hydrogen carbonate containing 50% ethyl alcohol. This eluted the more strongly bound components (containing the phenolic residue), mainly the protected dinucleotide (III) and starting protected mononucleotide (I). The fractions (containing these compounds) were pooled and the desired protected dinucleotide was isolated by rapid Sephadex gel-filtration.[3] The homogeneity of the protected dinucleotide (III), trinucleotide (IV), and hexanucleotide (VI) was checked by paper chromatography in three solvent systems.§ Further characterization of products after de-blocking was checked by enzymatic degradations. The yields obtained in the case of protected di-, tri, and hexa-nucleotide were 50, 46, and 35%, respectively.

The 4-chloro-2-nitrophenyl group from the protected di-, tri-, and hexa-nucleotides was removed by heating with 2N-sodium hydroxide at 100° for 15 min. Under these conditions d-pC, d-pG were found to be quite stable whereas d-pA underwent *ca.* 5% deamination. Therefore, this approach can be used for the synthesis of long and defined sequences.

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§ Solvent A, ethyl alcohol–1M-ammonium acetate pH 7·5 (7 : 3 v/v); Solvent B, isobutyric acid–conc. ammonia–water, pH 3·7 (66 : 1 : 33 v/v); Solvent C, n-propyl alcohol–conc. ammonia–water (55 : 10 : 35 v/v); Solvent D, isopropyl alcohol–conc. ammonia–water (7 : 1 : 2 v/v).

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