A Novel Phosphate Protecting Group for Oligonucleotide Synthesis

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2-Phenylmercaptoethanol is found to be a very stable phosphate protecting group during oligonucleotide synthesis. However, when necessary it can be easily removed, after activation.

Le phényl-2 mercaptoéthanol apparaît comme étant un groupe phosphate protecteur très stable pendant la synthèse d'oligonucléotides. Il est cependant facile à enlever, après activation, lorsque c'est nécessaire.

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Protection of the terminal phosphomonoester group is a necessary step in any chemical synthesis of oligonucleotides. We wish to report 2-phenylmercaptoethanol (**1**) (1) as a new phosphate protecting group which is easily introduced and stable to conditions normally encountered in oligonucleotide manipulations, such as (*i*) 1 *N* aqueous sodium hydroxide at room temperature for 8 h, (*ii*) concentrated ammonium hydroxide at 50° for 2 h, (*iii*) aqueous pyridine at room temperature for 1 week, (*iv*) mesitylene sulfonyl chloride in anhydrous pyridine at room temperature for 3 h, (*v*) aqueous hydrochloric acid (p*H* 2) at room temperature for 3 days. However, when "activated" this group is readily removed. Additionally, it incorporates one benzene ring to take advantage of the absorptive property of benzoylated DEAE Sephadex for purification of products (2).

Protection of the deoxymononucleotide 5′-phosphate **2** was completed in 24 h by treating its pyridinium salt with 2-phenylmercaptoethanol and dicyclohexylcarbodiimide (both in 10 *M* excess). Unused DCC was then decomposed with aqueous pyridine, the urea filtered off, and excess reagent removed by extraction with ethyl acetate. The aqueous phase was concentrated and a final purification was achieved by gel-filtration on a Sephadex G-25 (superfine) column, using 0.1 *M* triethylammoniumbicarbonate buffer, (p*H* 7.5) as eluant.

Each of the protected mononucleotides **3** was condensed with 3′-*O*-acetyl thymidine 5′-phosphate **4**. Work-up with aqueous pyridine and 2 *N* aqueous sodium hydroxide followed by sequential chromatography on benzoylated DEAE Sephadex and Sephadex G-25 superfine (2) gave the desired protected dinucleotides **5** (see Scheme 1). The 2-phenylmercaptoethyl group was removed by treating the protected nucleotide in aqueous solution (or in 0.1 *M* triethylammoniumbicarbonate) with sodium metaperiodate (approximately 5 *M* excess, as a freshly prepared 0.1 *M* aqueous solution) at room temperature for 1 h. It was apparently oxidized to the corresponding sulfoxide derivative **6** (3). After decomposition of the excess periodate with ethylene glycol and precipitation of inorganic residues with 90% aqueous pyridine, the free dinucleotide **7** was liberated by treatment with 2 *N* aqueous sodium hydroxide at room temperature for 30 min. A similar approach has been reported (4) for peptide synthesis.

Since some *N*-acetyl groups were labile to alkali, we found it more convenient for this initial investigation to deblock any amino func-

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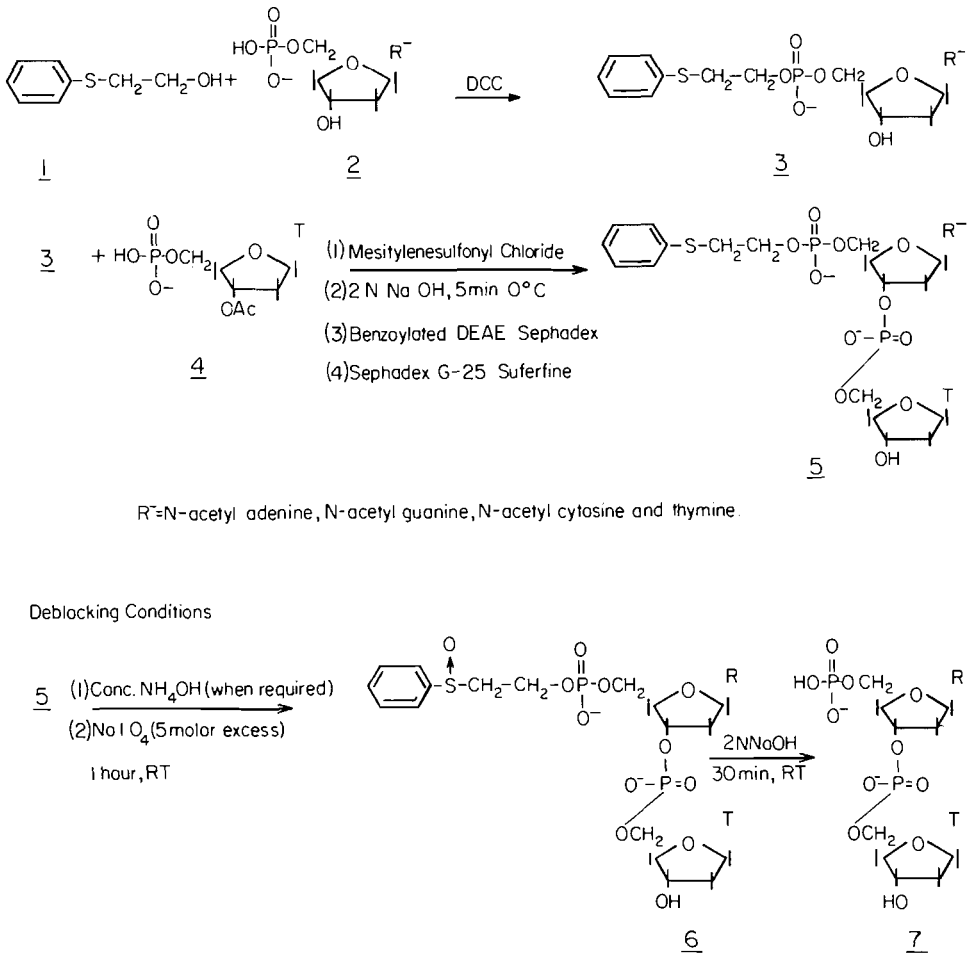

SCHEME 1

tions before activating the 2-phenylmercaptoethyl group for removal. Consequently each of the four 5′-mononucleotides (*i.e.* d-pA, d-pC, d-pG, and pT) obtained from **3** via a complete deblocking sequence were fully characterized by t.l.c. in three solvent systems (5) and u.v. spectroscopy to ensure that the periodate treatment in no way affected the heterocyclic bases. Indeed all the dinucleotides **7** were characterized by t.l.c. as well as u.v. spectra of the components obtained by enzymic degradations. We feel the properties of this protecting group, make it more generally applicable than 2-cyanoethanol (6) for many aspects of oligonucleotide synthesis.

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1. A. H. FORD-MOORE, R. A. PETERS, and R. W. WAKELIN. J. Chem. Soc. 1754 (1949).
2. (*a*) S. A. NARANG, O. S. BHANOT, J. GOODCHILD, and R. H. WIGHTMAN. Chem. Commun. 91, 516 (1970). (*b*) J. J. MICHNIEWICZ, O. S. BHANOT, J. GOODCHILD, S. K. DHEER, R. H. WIGHTMAN, and S. A. NARANG. Biochem. Biophys. Acta, **224**, 626 (1970).
3. N. J. LEONARD and C. R. JOHNSON. J. Org. Chem. **27**, 282 (1962).
4. P. M. HARDY, H. N. RYDON, and R. C. THOMPSON. Tetrahedron Lett. 2525 (1968).
5. S. A. NARANG, O. S. BHANOT, S. K. DHEER, J. GOODCHILD, and J. J. MICHNIEWICZ. Biochem. Biophys. Res. Commun. **41**, 1248 (1970).
6. G. M. TENER. J. Am. Chem. Soc. **83**, 159 (1961).