Use of New Protecting Groups in the Synthesis of Deoxyribo-oligonucleotides of Defined Sequence

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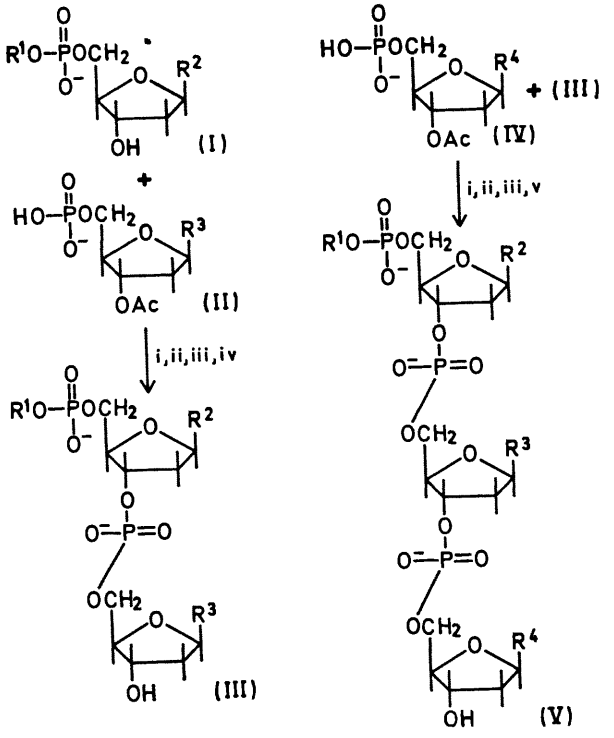
Summary Four new easily removable phosphate-protecting groups containing aromatic rings have been exploited in combination with benzoylated DEAE-Cellulose in the synthesis of deoxyribo-oligonucleotides of defined sequence.

WE reported[1] a new method involving the applications of 4-chloro-2-nitrophenol as a phosphate-protecting group and benzoylated DEAE-Cellulose[2] chromatography for the synthesis of homo-deoxyribo-oligonucleotides. Under conditions for the removal of the above protecting group (2N-sodium hydroxide 100°/15 min) from the protected oligonucleotides, deoxyriboadenosine-5′-phosphate suffered about 5% deamination. We now report the use of four new phosphate-protecting groups containing an aromatic ring, *i.e.* *N*-(*p*-methoxyphenyl)hydracrylamide, *N*-phenylhydracrylamide, *N*-benzylhydracrylamide, and benzaldoxime in the synthesis of deoxyribo-oligonucleotides of defined sequence as shown in the Scheme. These phosphate-protecting groups can easily be removed under mild alkali treatment at room temperature without causing deamination.



(i) Mesitylenesulphonyl chloride; (ii) 3·0N-NaOH (0°, 5 min); (iii) BD-Cellulose column; (iv) Sephadex G-15; (v) Sephadex G-25 (Superfine).
(Ia) R^1 = *p*-MeO·C_6H_4·NH·CO·CH_2·CH_2·
(Ib) R^1 = PhNH·CO·CH_2·CH_2·
(Ic) R^1 = PhCH : N·
(Id) R^1 = $PhCH_2$·NH·CO·CH_2·CH_2·
R^2 = Thymine
R^3,R^4 = *N*-Acetyladenine, or *N*-isobutyloxycarbonyl-cytosine, or *N*-acetylguanine

SCHEME

Thymidine-5′-phosphate was quantitatively protected by treating its pyridinium salt with a large excess (15 molar equiv.) of *N*-(*p*-methoxyphenyl)hydracrylamide or *N*-phenylhydracrylamide or benzylhydracrylamide in the presence of dicyclohexylcarbodi-imide (10 molar equiv.) for 4 hr. The benzaldoxime derivative was prepared by overnight treatment of thymidine-5′-phosphomorpholidate with benzaldoxime in the presence of a catalytic amount of hydrochloric acid. It was isolated in 60% yield by chromatography on a benzoylated DEAE-Cellulose column. Condensation of these protected compounds with appropriate 3′-*O*-acetyl-*N*-protected nucleoside-5′-phosphate (II) (*e.g.*, *N*-acetyldeoxyadenosine-5′-phosphate,[3] or *N*-isobutyloxycarbonyldeoxycytidine-5′-phosphate, or *N*-acetyldeoxyguanosine-5′-phosphate[4]) was carried out in the presence of mesitylenesulphonyl chloride for 2 hr. The protected dinucleotides (III) and trinucleotides (V) were isolated by chromatography through benzoylated DEAE-Cellulose and Sephadex gel-filtration[5] as described previously.[1]

Characterization of the various protected oligonucleotides was accomplished by paper and thin-layer chromatography. The phosphate-protecting groups from the protected oligonucleotides were removed by 2N-sodium hydroxide treatment at room temperature, the *N*-phenylhydracrylamide derivative requiring 40 min, the *N*-(*p*-methoxyphenyl) hydracrylamide derivative 60 min, *N*-benzylhydracrylamide derivative 6 hr, and the benzaldoxime derivative 8 hr. The *N*-protecting groups of the protected oligonucleotides were removed by concentrated ammonia treatment at 50° for 2 hr or 2 days at room temperature. Final characterization of the unprotected compounds were carried out by paper and thin-layer chromatography and also their enzymatic degradation.

(*Received, February 23rd*, 1970; *Com.* 272.)

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