Use of Fluorene-9-methanol as a Phosphate Protecting Group in the Synthesis of Deoxyribo-oligonucleotides

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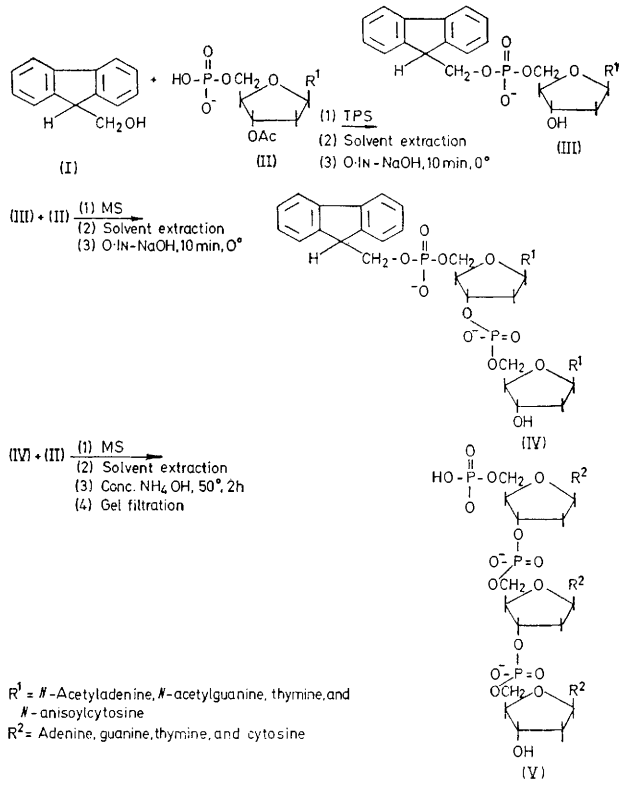
Summary The fluorene-9-methanol esters of the 5′-phosphates of oligonucleotides are soluble in organic solvents, and the protected oligonucleotides can be isolated by solvent extraction from the reaction mixture.

ONE of the major problems in oligonucleotide synthesis has been the lack of a simple method for their isolation from the chemical condensation reaction mixtures. We now report the use of fluorene-9-methanol (I) as a phosphate protecting group. Attachment of (I) to the 5′-phosphate of nucleotides makes them soluble in the commonly available organic solvents. Thus, the desired protected oligonucleotide can easily be isolated from a complex reaction mixture simply by extraction with organic solvents. This protecting group is stable under the conditions of an oligonucleotide synthesis, but it can easily be removed by treatment with conc. ammonium hydroxide (28—30%) at 50° for 2 h. A similar approach using *p*-aminophenyltriphenylmethane as the phosphate protecting group has also been reported.[1]

The protection of 3′-*O*-acetyldeoxymononucleoside 5′-phosphate (II) was completed in 4 h by treating the pyridinium salt with (I) (5 molar excess) in the presence of tri-isopropylbenzenesulphonyl chloride (TPS) [3 molar excess based on the amount of (II)]. The reaction mixture was then decomposed with aqueous pyridine and the excess of (I) recovered by extraction with ether. The fully protected mononucleotide was then extracted from the reaction mixture with chloroform–n-butanol (7 : 3 v/v). After removing the solvents by evaporation *in vacuo* and treating the residue with 0·1N-sodium hydroxide at 0° for 10 min, the esters (III) (see Scheme) were isolated in about 70% yield by precipitating their anhydrous pyridine solutions from anhydrous ether.

Each of the phosphate-protected mononucleotides (III) was condensed with (II) (1·5 molar excess) in the presence of mesitylenesulphonyl chloride (MS) [5 molar excess based on the amount of (II)] for 3 h. The reaction mixture was then decomposed with aqueous pyridine and kept overnight. Unchanged (III) was recovered from the aqueous solution (free of pyridine) by extracting it with chloroform–n-butanol (7 : 3 v/v). The fully protected dinucleotide was next



SCHEME

isolated by extracting the aqueous layer with chloroform–benzyl alcohol (7 : 3 v/v). The solvent was removed by evaporation *in vacuo*. After treating the residue with 0·1N-sodium hydroxide at 0° for 10 min, (IV) was obtained as a

white solid in 45% yield by precipitating its anhydrous pyridine solution with anhydrous ether–chloroform (5:1 v/v).

The trinucleotide condensation and work-up steps were performed as described above. The aqueous layer (free from pyridine) was extracted with chloroform–benzyl alcohol (1:5 v/v), and the organic layer concentrated *in vacuo* to remove chloroform. The residual benzyl alcohol solution was mixed with ether and was then extracted with 0·1M-triethylammonium bicarbonate buffer. The aqueous layer was concentrated *in vacuo* in the presence of excess of pyridine and the protecting groups removed by treating the pyridine solution with concentrated ammonium hydroxide (28—30%) at 50° for 2 h. Finally the unprotected trinucleotide (V) was isolated by gel filtration on Sephadex (G-25 superfine)[2] in 35% yield.

All the di- and tri-nucleotides thus prepared were fully characterized by t.l.c.[3] and from the u.v. spectra of the components after their enzymatic degradation.

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