A Novel Chemical Synthesis for Oligoribonucleotides

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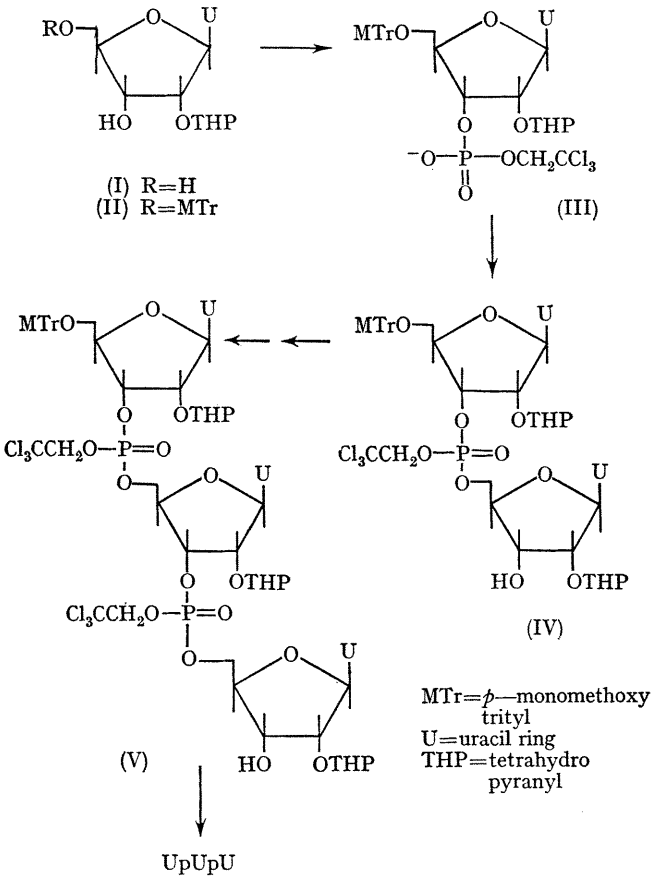
Summary A synthesis of uridyl(3′→5′)uridyl(3′→5′)-uridine illustrates a convenient general synthesis for oligoribonucleotides of defined length and sequence.

POLYDEOXYRIBONUCLEOTIDES have been conveniently prepared by coupling preformed protected oligonucleotides, themselves products of stepwise condensation of suitably protected nucleotide units.[1,2] As yet, no satisfactory parallel oligoribonucleotide synthesis has been established. I report a convenient synthesis for uridyl(3′→5′)uridyl-(3′→5′)uridine (UpUpU) from a 2′-*O*-substituted uridine. This approach promises to be generally applicable to the preparation of mixed oligonucleotides by appropriate substitution of the uridine derivative by the corresponding *N*-acyl-adenosine, -guanosine, or -cytidine analogues. Coupling of those protected oligonucleotide fragments and subsequent de-blocking of the product should provide a block synthesis for polyribonucleotides of defined length and sequence.

A protected oligoribonucleotide with 3′→5′ phosphotriester linkages is obtained by the stepwise condensation of suitably blocked ribonucleosides with a 5′-*p*-monomethoxytrityl nucleoside derivative destined to become the 5′-terminal nucleoside unit of the oligonucleotide.

Gentle removal of the protecting groups furnishes an oligoribonucleotide of defined sequence and length.

Convenient synthesis of protected oligoribonucleotides has been delayed by the lack of specific protection of the 2′-hydroxy-group of ribose as well as by the fact that considerable isomerisation of 3′→5′ phosphodiester bond during the subsequent de-blocking step, may occur.



Recently the insertion and removal of a tetrahydropyranyl grouping has been used[3] successfully to solve this problem. A phosphotriester approach[4] is chosen in preference to a phosphodiester method since rapid isolation and purification of the various intermediates can be accomplished by simple silica gel column chromatography in organic solvents.

The chemistry is illustrated by the synthesis of an uridyl(3′→5′)uridyl(3′→5′)uridine derivative (V) as shown in the accompanying scheme. 5′-*O*-Monomethoxytrityl-2′-*O*-tetrahydropyranyluridine (II) prepared from 2′-*O*-tetrahydropyranyluridine[3] (I), gave phosphorylated product (III) on treatment with the pyridinium salt of 2,2,2-trichloroethylphosphate[5] and tri-isopropylsulphonyl chloride (TPS). Reaction of (III) with TPS and (I) gave a dinucleotide derivative (IV) (62% yield over two steps) which was purified by silica gel column chromatography, being eluted as a single species by 2% methanol in methylene chloride. Repetition of this phosphorylation procedure on (IV) and subsequent coupling with (I) gave trinucleotide derivative (V) in 67% yield. No condensation of (III), or of the 3′-phosphorylated derivative of (IV), with the 3′-hydroxy-group of (I) to give products containing 3′→3′ phosphoester linkages, was detected. Apparently the steric inference between the neighbouring 2′-*O*-tetrahydropyranyl group and the bulky arylsulphonic-phosphoric anhydride, together with the secondary nature of the 3′-hydroxy-group, forbids reaction. Consequently no special 3′-protection was necessary.

De-blocking of (V) was effected by (1) treatment with Cu–Zn couple in *NN*-dimethylformamide[5] and then (2) treatment with 0·01N-hydrochloric acid solution[3] to give UpUpU in 59% yield. UpUpU (R_{Up} 1·02 in solvent A† and R_{Up} 0·60 in solvent B) was completely degraded with pancreatic RNase to give uridine phosphate (Up) (R_F 0·29) and uridine (R_F 0·70) in the ratio of 2·0 : 1·0 in solvent A. Extension to syntheses of mixed oligonucleotides containing defined sequences of all four ribonucleotides is currently under way in this laboratory.

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† Paper chromatography was performed using the descending technique. The solvent systems used were: solvent A, ethanol–M-ammonium acetate, pH 7·5 (7 : 3, v/v); solvent B, n-propanol–conc. ammonia–water (55 : 10 : 35 v/v).

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