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OLIGORIBONUCLEOTIDE SYNTHESIS FROM NUCLEOSIDE 2'-O-BENZYL ETHERS

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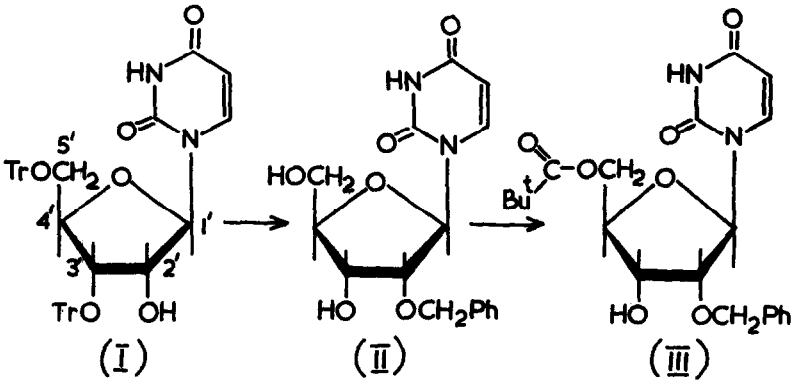
In most of the reported work on oligoribonucleotide synthesis, the first step involves the condensation of either (i) a 2',5'-protected 3'-nucleotide with a 2',3'-protected nucleoside derivative (1,2,3), or (ii) a 2',5'-protected nucleoside with a 2',3'-protected 5'-nucleotide derivative (4). So far, both acid- and base-labile functions have been used to block the 2'-hydroxyl groups in the 2',5'-protected components. This 2'-protecting function must be chosen with great care as it is necessary for it to remain in place until the final stage of the synthesis, when it must be removed under conditions which do not promote hydrolytic cleavage or migration of the internucleotidic phosphodiester linkages (5). Although we believe (4) that acid-labile groups are generally the most suitable for the protection of 2'-hydroxyl functions, we wish to report on the use of a third type of blocking group, the benzyl ether. The latter may be cleaved by catalytic hydrogenolysis under very mild conditions, which avoid the difficulties outlined above.

Yung and Fox (6) characterized 2',5'-di-O-trityluridine as one of the products of the reaction between uridine and an excess of trityl chloride in hot pyridine solution. We

have confirmed (7) that the 2',5'-isomer is the major product (44% yield) of this reaction, and have also isolated



crystalline* 3',5'-di-O-trityluridine (I), m.p. 137-140°, in 20% yield. The two isomers may readily be distinguished by thin-layer chromatography (on Merck Kieselgel GF_{254}), and by n.m.r. spectroscopy (9).

3',5'-Di-O-trityluridine (I) has been converted into 2'-O-benzyluridine (II), in 59% overall yield, by the two stage procedure reported (7) for the preparation of the isomeric 3'-benzyl ether: viz. benzylation, followed by detritylation. This compound (II), which crystallized from ethanol as colourless needles, m.p. 181-182°, was allowed to react with 1.5 molecular equivalents of pivaloyl chloride in anhydrous pyridine solution. The required 2'-O-benzyl-5'-O-pivaloyluridine (III) was isolated from the products in 36% yield†, and crystallized from aqueous ethanol. The

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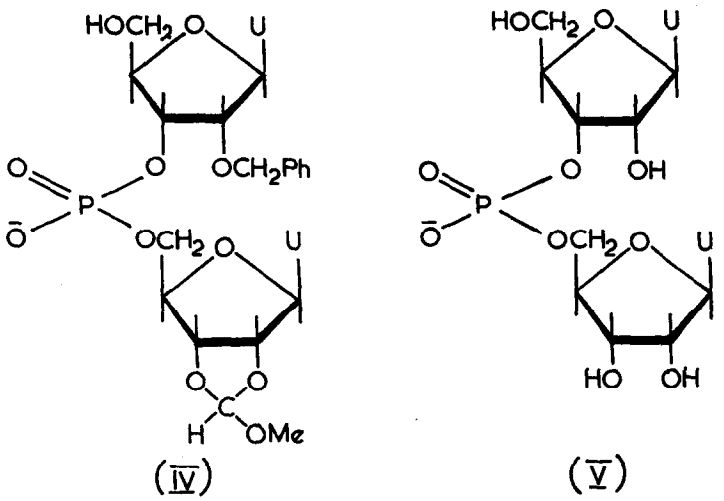
* Elemental analysis and n.m.r. spectral data indicate that 3',5'-di-O-trityluridine (I) crystallizes from ethanol with one molecule of solvent of crystallization. Satisfactory analytical data have been obtained for all new crystalline compounds described. Žemlička (8) has also reported the isolation of the 3',5'-isomer, m.p. 135-145°, in 27% yield.

† No attempt was made to obtain the optimum yield. However, an 82% yield of 2'-O-benzyl-5'-O-trityluridine, m.p. 110-112°, was obtained (10) from the reaction between 2'-O-benzyluridine and one molecular equivalent of trityl chloride in boiling pyridine solution.

orientation of the latter compound, which had m.p. 168-168.5°, was established by its hydrogenolysis, in the presence of 10% palladized charcoal, to 5'-O-pivaloyluridine. Thus a 2',5'-protected nucleoside derivative, suitable for condensation with a 2',3'-protected 5'-nucleotide according to the above procedure (ii), was available.



(IV) (V)

[U= uracil-1]

Condensation between 2',3'-O-methoxymethylideneuridine 5'-phosphate (4) and 2'-O-benzyl-5'-O-pivaloyluridine (III) [ca. 1.4 molecular equivalents] was effected by an excess of N,N'-dicyclohexylcarbodiimide in anhydrous pyridine solution (1). After 72 hours, the reaction mixture was worked-up in the usual manner (1), and then treated with 0.85 M-tetraethylammonium hydroxide (4) in 50% aqueous methanol solution at 20°. After 6 hours, the neutralized products were examined

by paper electrophoresis*, which revealed a principal component (mobility + 3.3 cm., corresponding to the dinucleoside phosphate derivative [IV]), a trace of a material with a greater mobility (+13.2 cm., corresponding to 2',3'-O-methoxymethylideneuridine 5'-phosphate), and a third component with a negative mobility. This last component was identified as 2'-O-benzyluridine (II) [R_F 0.66 (system A)] by paper chromatography†. The yield of the required product (IV) was estimated to be ca. 95% with respect to the 5'-nucleotide starting material.

A portion of the above mixture was lyophilized, dissolved in 50% aqueous ethanol, acidified to pH 4.5, and then shaken with hydrogen at atmospheric temperature and pressure in the presence of 10% palladized charcoal. Paper chromatographic (system B) examination of the products indicated the presence of uridylyl(3'→5')uridine (V) [R_F 0.35], uridine [R_F 0.68], and a trace of uridine 5'-phosphate [R_F 0.11]. This analysis was confirmed by paper electrophoresis. The reaction solution was apparently acidic enough for the methoxymethylidene group to have been removed during hydrogenolysis.

The products were fractionated on a Dowex 1 x 2 (chloride form) anion-exchange column: uridylyl(3'→5')uridine was eluted with 0.005 N hydrochloric acid, and isolated as

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* Paper electrophoresis was conducted on Whatman No. 4 paper (45 v./cm.) in 0.1 M sodium phosphate buffer (pH 8). A positive mobility indicates that the component migrates towards the anode.

† Ascending paper chromatograms on Whatman No. 1 paper were developed in the following solvent systems: A, propan-2-ol-ammonia(d 0.88)-water (7,1,2); B, ethanol-M aqueous ammonium acetate (5,2).

a solid calcium salt. This material was completely degraded* to uridine 3'-phosphate and uridine in the presence of pancreatic ribonuclease, thus indicating the absence of the isomeric uridylyl(2'→5')uridine and un-debenzylated contaminants; it was also found (11) to be free from 5,6-dihydrouridine derivatives.

The dinucleoside phosphate derivative (IV) is a suitably protected intermediate for conversion into a dinucleotide or trinucleoside diphosphate. Subject to the availability of other ribonucleoside 2'-O-benzyl ethers, the present work suggests a general approach to the synthesis of oligoribonucleotides.

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* 1% of undegraded dinucleoside phosphate would have been detected.

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