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OLIGORIBONUCLEOTIDE SYNTHESIS VIA 2',5'-PROTECTED RIBONUCLEOSIDE DERIVATIVES

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In the past few years, much progress has been made in the synthesis of oligoribonucleotides with exclusively 3',5'-phosphodiester linkages.[1,2,3] In principle, the initial dinucleoside phosphate intermediate can be prepared either (a) by condensation of a 2',5'-protected 3'-nucleotide with a 2',3'-protected nucleoside or (b) by condensation of a 2',3'-protected 5'-nucleotide with a 2',5'-protected nucleoside. So far, the principal workers in this field[1,2,3] have concentrated on approach (a), partly because pure 3'-nucleotide derivatives could be readily prepared whereas no suitable 2',5'-protected nucleosides had been described. Rammler and Khorana[4a] have concluded that approach (a) is the superior and have shown[4b] that this approach permits the use of the base-labile

acetyl group, as an alternative to the apparently less satisfactory† tetrahydropyranyl group, to protect the 2'-hydroxyl function.

We have been investigating approach (b) and have found that the tetrahydropyranyl group may be used quite satisfactorily to protect the 2'-hydroxyl, and in future communications we hope to report that, under certain circumstances, acyl and other groups[5] may also be used. In a fuller account of this work, we shall discuss what we believe to be some of the advantages of pursuing approach (b).

When 3',5'-di-O-acetyladenosine[6] was stirred with excess 2,3-dihydro-4H-pyran (dihydropyran) in dioxane in the presence of toluene-p-sulphonic acid, it was quantitatively converted into 2'-O-tetrahydropyranyl-3'-5'-di-O-acetyladenosine (I; R = R' = Ac) (R_F, system A‡ 0.87). The latter was treated with methanolic ammonia and gave 2'-O-tetrahydropyranyladenosine (I; R = R' = H), isolated as an analytically pure crystalline solid, m.p. 199–200° (R_F, system A 0.75) in 58% yield. This in turn was treated with a slight excess of pivalyl chloride in pyridine and, after a suitable work-up, the products were chromatographed on silicic acid. A material isolated in 72%

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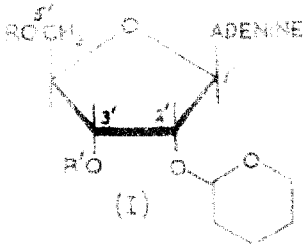
† A satisfactory protecting group is defined, in this context, as one which can be completely removed without any measurable migration of the 3',5'-internucleotidic linkages or any appreciable degradation.

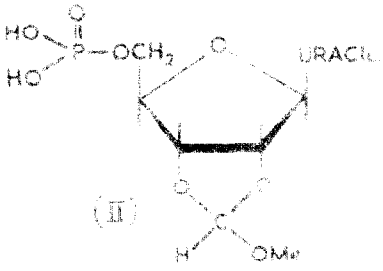
‡ Chromatographic solvent systems: A, butan-1-ol- acetic acid - water (4:1:5); B, ethanol - N aqueous ammonium acetate (5:2).

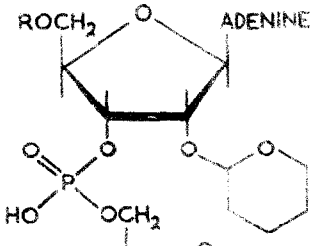
yield, corresponded to 2'-O-tetrahydropyranyl-5'-O-pivalyladenosine (I; R = t-BuCO, R' = H) (R_F, system A 0.94). Although this compound has yet failed to crystallize†, its structure rests firmly on elemental analysis and on its quantitative conversion by aqueous acid to 5'-O-pivalyladenosine (R_F, system A 0.85), identical (m.p. and mixed m.p., 162-163°) with authentic material.[8]

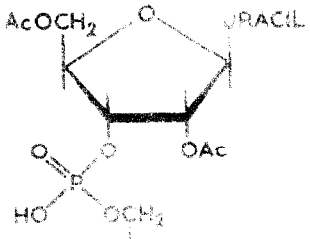
The orientation and suitability in oligoribonucleotide synthesis of the key intermediate (I; R = t-BuCO, R' = H) was readily established. The latter compound was allowed to react with 2',3'-di-O-acetyluridine-5' phosphate (2 mol.) and dicyclohexylcarbodiimide (DCC) (10 mol.) in anhydrous pyridine for 3 days and worked up in the usual manner.[1] After the acetyl and pivalyl protecting groups had been removed by respective treatment with methanolic ammonia and 0.8 M aqueous methanolic (1:1) tetraethylammonium hydroxide, the resulting 2'-O-tetrahydropyranyladenylyl (3'→5') uridine was absorbed on a Dowex 1 (Cl^-) column. The required adenylyl (3'→5') uridine (ApU) (R_F, system B 0.37) was eluted with 0.003 N hydrochloric acid (the 2'-tetrahydropyranyl group was completely removed within 24 hr. by contact with this acid, i.e. at pH 2.5). Analytically pure ApU was isolated as a calcium salt in 60% yield; it was completely

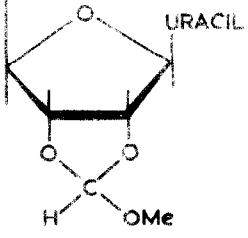
† However, the corresponding 2'-O-tetrahydropyranyl-5'-O-pivalyluridine[7] crystallizes readily; it has m.p. 170-172°.

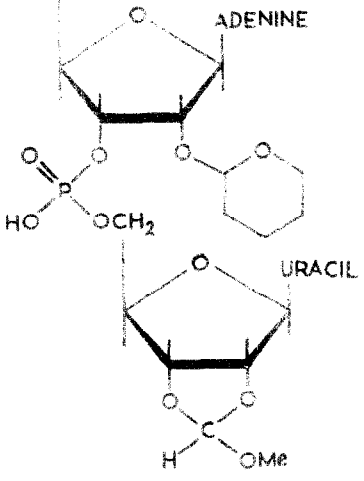

ADENINE
3'
2'
(I)

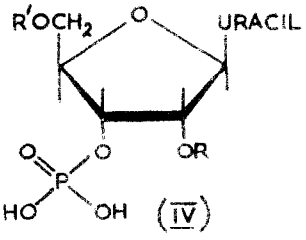

HO
HO
OCH2
URACIL
(II)
OMe


ROCH2
ADENINE
HO
OCH2


AcOCH2
OAc
HO
OCH2


URACIL
(III)
OMe


ADENINE
HO
OCH2
URACIL
OMe
(V)


R'OCH2
URACIL
OR
HO
OH
(IV)

degraded† by spleen phosphodiesterase to uridine and adenosine-3' phosphate.

If a nucleoside component with an acid-labile 2'- and a base-labile 5'-protecting group is used in an approach to synthesis, and it is required to proceed beyond the dinucleoside phosphate stage, then the 2',3'-cis-diol system of the 5'-nucleotide component should be blocked by an acid-labile protecting group. The methoxymethylidene group has been found[9] to be very suitable for this purpose. 2',3'-O-Methoxymethylideneuridine-5' phosphate (II) (R_p, system B 0.24) was prepared from the corresponding nucleoside derivative by the method of Tener[10]; it was isolated as an analytically pure diammonium salt in over 90% yield. This material was allowed to react with 2'-O-tetrahydropyranyl-5'-O-pivalyladenosine (I; R = t-BuCO, R' = H) under precisely the conditions described above for 2',3'-di-O-acetyluridine-5' phosphate. After the products had been treated with tetraethylammonium hydroxide to remove the pivalyl group from the fully protected dinucleoside phosphate (III; R = t-BuCO), they were chromatographed on DEAE cellulose (HCO_3^- form). The required 2'-O-tetrahydropyranyladenylyl (3'→5')-2',3'-O-methoxymethylidene-

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† The assay method used in this and the other enzyme-catalysed hydrolytic reactions described, was insensitive to less than 1-2% of undegraded starting material. Phosphoryl migration was not observed to accompany the removal of the 2'-O-tetrahydropyranyl group with 0.01 N hydrochloric acid. However, more labile acetals can be prepared, if necessary: preliminary experiments show that 2'-O-tetrahydrofuranyluridine is hydrolysed at ca. 20 times the rate of its tetrahydropyranyl analogue at pH2.

uridine (III; R = H) (R_F, system B 0.76) was eluted from the column with triethylammonium bicarbonate in the concentration range 0.015 to 0.02 M. When this material was treated with 0.01 N hydrochloric acid for 6 hr. at room temperature and the pH then increased to 7.5, it was quantitatively converted to ApU, which, as before, was completely degraded† by spleen phosphodiesterase.

In order to carry this synthesis to the trinucleoside diphosphate stage, we required a protected 3'-nucleotide such as (IV; R = tetrahydropyranyl, R' = acyl). Such an intermediate is in preparation from 2'-O-tetrahydropyranyl-5'-O-pivalyluridine. However, in a preliminary experiment, we allowed the dinucleoside phosphate derivative (III; R = H) to react with 2',5'-di-O-acetyluridine-3'-phosphate[1] (IV; R = R' = Ac) (2 mol.) and DCC (10 mol.) in pyridine. After 3 days, the products were worked up in the usual manner, treated with 0.01 N hydrochloric acid and then with methanolic ammonia. In this way the resulting fully protected trinucleoside diphosphate (V) was converted into uridylyl (3'→5') adenylyl (3'→5') uridine (UpApU). This material (R_p, system B 0.24) was isolated in 30% yield by chromatography on DEAE cellulose. It was completely degraded by pancreatic ribonuclease into ApU and uridine-3'-phosphate.

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†UpU, prepared[7] similarly from 2'-O-tetrahydropyranyl-5'-O-pivalyluridine, was completely degraded to uridine and its 3'-phosphate by pancreatic ribonuclease.

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