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THE SYNTHESIS OF OLIGORIBONUCLEOTIDES—VI*

2′-O-ACYL RIBONUCLEOSIDE DERIVATIVES AS INTERMEDIATES IN THE SYNTHESIS OF DINUCLEOSIDE PHOSPHATES

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Abstract—N^2, $O^{2'}$, $O^{5'}$-Tribenzoylguanosine has been obtained as a pure crystalline compound. The di-ribonucleoside phosphates: guanylyl-(3′→5′)-uridine[GpU] and cytidylyl-(3′→5′)-uridine[CpU] have been prepared in moderate yields by the condensation (in pyridine solution with mesitylenesulphonyl chloride as the condensing agent) between 2′,3′-di-O-acetyluridine 5′-phosphate and, respectively, N^2,$O^{2'}$,$O^{5'}$-tribenzoylguanosine and N^4,$O^{2'}$,$O^{5'}$-triacetylcytidine. The GpU was completely digested to guanosine 3′-phosphate and uridine in the presence of ribonuclease T_1, while the CpU was ca. 98% digested to cytidine 3′-phosphate and uridine in the presence of pancreatic ribonuclease.

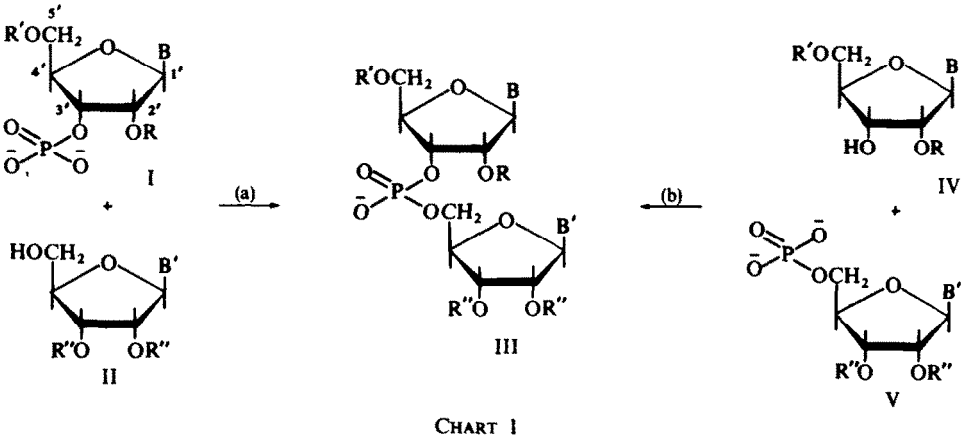

CHART 1

THE approach adopted (route (a), Chart 1) by three of the main laboratories concerned with oligoribonucleotide synthesis,[1–3] involves the condensation between a 2′,5′-protected nucleoside 3′-phosphate (I) and the free 5′-OH function of a nucleoside derivative II or partially-protected oligonucleotide. For reasons considered elsewhere,[4] we have pursued the alternative approach (route (b), Chart 1): that is, condensation between a 2′,3′-protected nucleoside 5′-phosphate (V) and the free 3′-OH function of a nucleoside derivative IV or partially-protected oligonucleotide.

The other crucial decision to be made in oligonucleotide synthesis lies in the choice of the protecting group (R) for the 2′-OH functions vicinal to the internucleotidic phosphodiester linkages. This protecting group must remain in position

* For part V of this series, see B. E. Griffin and C. B. Reese, *Tetrahedron* **24**, 2537 (1968).

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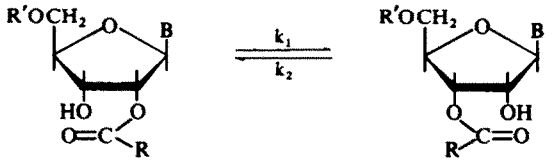
throughout the condensation and fractionation processes, but must ultimately be removable under conditions which do not lead to the degradation or isomerization of the product. The choice of R is to some extent dependent on the approach adopted, as the required synthetic intermediates must be readily available. In the route (a) approach, both acid-labile (acetal)[2,5] and base-labile (acyl)[1] groups have been used to protect the 2'-OH functions. We have so far reported on the use of acid-labile[4,6] and catalytically hydrogenolyzable[7] (benzyl ether) protecting groups in the route (b) approach, and now wish to consider the use of base-labile groups.



VI VII

CHART 2

Until recently[8,9] no pure 2'-O-acyl ribonucleoside derivative (such as VI) had been described.* It was indeed implied in the literature[10,11] that such derivatives would readily isomerize to the corresponding 3'-esters (VII), but that the reverse reaction would not occur. This misconception was due largely to there being no suitable method available for distinguishing between a pair of 2'- and 3'-isomers. We have since developed methods[8,12] for orientating 2'- and 3'-ribonucleoside derivatives, and have established that either isomer may easily be converted into an equilibrium mixture of both.[13] In all the cases so far examined,[13,14] the 3'-(VII) have been marginally more stable than the 2'-isomers (VI), with equilibrium constants (k_1/k_2, Chart 2) often less than 2.

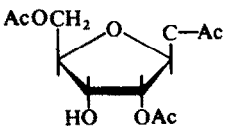
Although the equilibrium constants do not vary appreciably, the same is not true of the equilibration rates for different acyl groups. Thus[13] in anhydrous pyridine solution at 60°, the relative rates of equilibration of benzoyl, acetyl and formyl groups are 1, 18 and 670, respectively.† The ratio of the mobilities of acetyl and formyl groups is greater still in buffered (pH 7) DMSO and dimethylformamide.[14] It therefore seemed probable that the route (b) approach (Chart 1) with 2'-O-acyl protecting groups would only be feasible if the latter were comparatively non-mobile. An examination of the behaviour of 2',5'-di-O-*p*-anisoyluridine in anhydrous pyridine solution at 20° indicated[13] that only 5% had isomerized after 5 days. As phosphorylation can be effected in a few hours under these conditions, with an arenesulphonyl chloride as the condensing agent,[17] it was concluded that *p*-anisoyl and also benzoyl‡ would be satisfactory protecting groups for the 2'-OH function. It

* It now seems likely that the $N^4,O^{2'},O^{5'}$-tribenzoylcytidine, obtained by Rammler and Khorana,[10] was contaminated with $N^4,O^{3'},O^{5'}$-isomer.

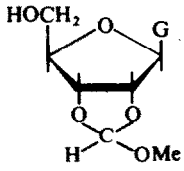
† In anhydrous pyridine solution, acyl migration is a first order equilibration reaction.[15] Equilibration rates are increased considerably by the addition of water (even in small proportions) to the pyridine solution.[16]

‡ In anhydrous pyridine solution at 20°, benzoyl is ca. 1·5 times as mobile as *p*-anisoyl.[18]

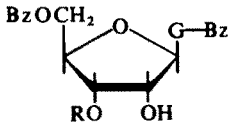
seemed likely,[13,18] however, that the use of the acetyl group would lead to a detectable proportion of 2′ → 5′-internucleotidic linkages in the product.



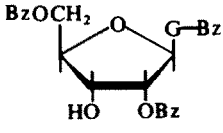
VIII



IX



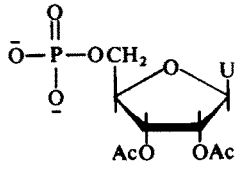
X



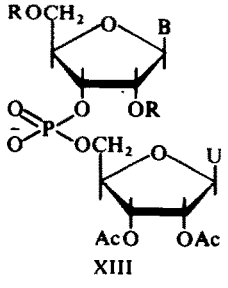
XI

C–Ac = N^4-acetylcytosine-1; G–Bz = N^2-benzoylguanine-9

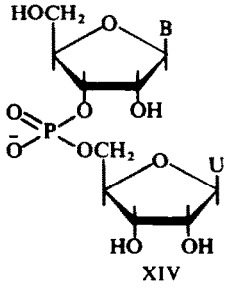
Several pure crystalline 2′-O-acyl derivatives of cytidine have been prepared by the orthoester exchange method,[9] and their orientations established by NMR spectroscopy:[12] they include $N^4,O^{2'},O^{5'}$-triacetylcytidine (VIII) and both $O^{2'}$-acetyl- and benzoyl-$N^4,O^{5'}$-dipivaloylcytidines. We now wish to describe the preparation of $N^2,O^{2'},O^{5'}$-tribenzoylguanosine (XI). Treatment of 2′,3′-O-methoxymethylideneguanosine[19] (IX) with an excess of benzoyl chloride in pyridine solution, followed by acidic hydrolysis of the products gave $N^2,O^{5'}$-dibenzoyl-$O^{3'}$-formylguanosine (X; R = CHO). This compound, which was isolated as a pure crystalline solid in 35% overall yield, was orientated by NMR spectroscopy;[12] when it was treated with dilute methanolic ammonia, it was quantitatively converted into $N^2,O^{5'}$-dibenzoylguanosine (X; R = H). In a separate experiment, the latter compound was prepared from 2′,3′-O-methoxymethylideneguanosine (IX) in 49% overall yield. A mixture of the desired $N^2,O^{2'}$-$O^{5'}$-tribenzoylguanosine (XI) and its $N^2,O^{3'},O^{5'}$-isomer (X; R = Bz) was prepared from the dibenzoyl derivative (X; R = H), by orthoester exchange,[9] in 88% yield. A modest amount of pure, crystalline $N^2,O^{2'},O^{5'}$-tribenzoylguanosine (XI) could be obtained by careful recrystallization of the mixture of isomers from ethanol. It could be seen from its NMR spectrum[12] that this material was free from its $N^2,O^{3'},O^{5'}$-isomer.



XII



XIII



XIV

U = uracil-1

$N^2,O^{2'},O^{5'}$-Tribenzoylguanosine (XI) was allowed to react with a stoicheiometric amount of pyridinium 2',3'-di-O-acetyluridine 5'-phosphate (XII)[6] and two molecular equivalents of mesitylenesulphonyl chloride,[17] in anhydrous pyridine solution at room temperature. After 6 hr, the reaction was stopped and the products [containing the fully-protected dinucleoside phosphate (XIII; B = N^2-benzoylguanine, R' = Bz)] treated with alcoholic methylamine to remove the acyl protecting groups.* The desired guanylyl-(3' → 5')-uridine [GpU] (XIV; B = guanine) was isolated in a pure state by preparative paper chromatography; its yield was estimated to be over 30% (Experimental). When this material was incubated with ribonuclease T_1, it was completely degraded† to guanosine 3'-phosphate and uridine, thus indicating[20] that the amount of contaminating guanylyl-(2' → 5')-uridine was negligible. This result establishes that the benzoyl group migrates at a sufficiently slow rate in anhydrous pyridine solution to make it suitable for the protection of 2'-OH functions in oligoribonucleotide synthesis.

A by-product was isolated in the above preparation of GpU. This material, which had virtually the same electrophoretic mobility (pH 7·5) as GpU but a higher R_f (system B, Experimental), was assigned the structure N^2-benzoylguanylyl-(3' → 5')-uridine (XIV; B = N^2-benzoylguanine). When the latter was incubated with ribonuclease T_1 under conditions which led to the quantitative digestion of GpU, no reaction occurred. Thus benzoylation of the guanine residue on N(2) inhibits the action of ribonuclease T_1.‡

Following the procedure adopted in the preparation of GpU, pyridinium 2',3'-di-O-acetyluridine 5'-phosphate (XII) and $N^4,O^{2'},O^{5'}$-triacetylcytidine[9] (VIII) [1·5 molecular equivs] were treated with mesitylenesulphonyl chloride [2 molecular equivs] in anhydrous pyridine solution at room temperature. The reaction was stopped after 1 hr, and the products [containing XIII (B = N^4-acetylcytosine, R = Ac)] treated with methanolic ammonia. The desired cytidylyl-(3' → 5')-uridine§ [CpU] (XIV; B = cytosine) was obtained in ca. 33% yield following anion-exchange chromatography of the products, and isolated as a colourless lithium salt. When this material was incubated with pancreatic ribonuclease under the usual conditions,[10] it was ca. 98% degraded to cytidine 3'-phosphate and uridine. The small amount of undigested dinucleoside phosphate (ca. 2%) was assumed to be cytidylyl-(2' → 5')-uridine, which would be an expected product if the $N^4,O^{2'},O^{5'}$-triacetylcytidine underwent isomerization during the phosphorylation process.

It is thereby established that the route (b) approach (Chart 1) to oligoribonucleotide synthesis is feasible with a base-labile protecting group (R) for the 2'-OH functions, vicinal to the internucleotidic linkages. The choice of R depends on the proportion of 2' → 5'-linkages which can be tolerated: if 2% of impurity is acceptable, then the

* $MeNH_2$ is much more effective than NH_3 for removing the N^2-benzoyl group from the guanine moiety. However, although treatment with 33% $MeNH_2$/EtOH for 5 hr. led to the quantitative removal of the O-acyl groups, the N^2-benzoyl group was not completely removed. Prolonged treatment with $MeNH_2$ resulted in the degradation of the dinucleoside phosphate.

† ca. 0·5% of undegraded GpU would have been detected.

‡ This contrasts with the observation[21] that acetylation of the cytosine residue on N(4) does not have an inhibitory effect on the action of pancreatic ribonuclease.

§ No attempt was made to free the CpU from any isomeric cytidylyl-(2' → 5')-uridine. However, it is likely[22] that such a separation would be possible by anion-exchange chromatography.

acetyl group is satisfactory and if not, the benzoyl or an even less mobile acyl group should be used.

In this work, the principal aim has been to obtain dinucleoside phosphates which were as free as possible from their 2′ → 5′-isomers, rather than to obtain optimum yields. It seems likely that the moderate yields described could be improved, and thus that the present approach is quite practicable. However, as the required pure crystalline 2′-O-acyl ribonucleoside derivatives are not always readily accessible, we still regard route (b) with acid-labile protecting groups for the 2′-OH functions,[4] as the most convenient approach to oligoribonucleotide synthesis.

EXPERIMENTAL

UV absorption spectra were measured with a Cary recording spectrophotometer, model 14M-50. NMR spectra were measured with a Varian H-100 spectrometer, operating at 100 Mc/s.

Paper electrophoresis on Whatman No. 4 paper was conducted in a CCl_4-cooled apparatus (at 30–40 v/cm) with 0·1M sodium phosphate buffer (pH 7·0 and 7·5).

The following solvent systems were used for paper chromatography: A, butan-1-ol–acetic acid–water (5:2:3); B, ethanol—M aqueous ammonium acetate (5:2); C, isobutyric acid–ammonia (*d* 0·88)–water (66:1:33).

Plates coated with Merck Kieselgel GF_{254} were used for TLC, and the chromatograms were developed with solns of MeOH in $CHCl_3$. Mallinckrodt analytical grade silicic acid (100 mesh) was used for adsorption chromatography.

Pyridine was dried by heating with CaH_2, under reflux, and then redistilled before use.

$N^2,O^{5'}$-Dibenzoyl-$O^{3'}$-formylguanosine (X; R = CHO)

Benzoyl chloride (6·5 ml, 56 mmole) was added slowly to a rapidly stirred soln of 2′,3′-O-methoxymethylideneguanosine[19] (3·25 g, 10 mmole) in pyridine (50 ml), maintained at 0°. The reactants were then allowed to stand in the dark for 2 hr at 20° before MeOH (10 ml) was added. After a further 15 min, the products were concentrated under reduced press (0·1 mm), and partitioned between $CHCl_3$ and water (2 × 50 ml). The combined $CHCl_3$ layers were dried (Na_2SO_4) and evaporated to a gum, which was kept *in vacuo* over P_2O_5 and KOH for 2 days. The residue was dissolved in 98% formic acid (50 ml) and the soln allowed to stand at 20° for 10 min before it was concentrated under reduced press (0·1 mm). The products were then applied to a column of silicic acid (60 g, 12 cm × 7 cm^2), which was eluted first with $CHCl_3$ and then with solns of MeOH in $CHCl_3$. The desired product was eluted with 2% MeOH/$CHCl_3$, yield, 2·0 g. (95% pure by TLC). Recrystallization from EtOH, acidified with formic acid (0·1%) gave *$N^2,O^{5'}$-dibenzoyl-$O^{3'}$-formylguanosine* (Found: C, 57·4; H, 4·3; N, 13·45. $C_{25}H_{21}N_5O_8$ requires: C, 57·8; H, 4·1; N, 13·5%) as colourless needles (1·8 g, 35%), m.p. 204–206° dec. NMR spectrum* in dimethylcyanamide-D_2O (0·1N with respect to HCl) [20:3, v/v] includes the following signals: τ 4·02, doublet ($J \sim 5{\cdot}5$ c/s), weight 1, assigned to H(1′); τ 4·32, multiplet, weight 1, assigned to H(3′); τ 4·87, triplet, weight 1, assigned to H(2′). UV (95% EtOH): λ_{max} 295, 265, 258, 233 (log ε 4·10, 4·11, 4·13, 4·39), λ_{min} 272, 262, 254, 215 mμ (log ε 4·00, 4·11, 4·12, 4·26).

$N^2,O^{5'}$-Dibenzoylguanosine (X; R = H)

2′,3′-O-Methoxymethylideneguanosine[19] (3·25 g, 10 mmole) was benzoylated as in the above preparation of $N^2,O^{5'}$-dibenzoyl-$O^{3'}$-formylguanosine. The products were worked up, treated with formic acid, and then evaporated as before. The gum, so obtained, was dissolved in ca. 0·3M-methanolic ammonia (100 ml). After the soln had stood at 20° for 15 min, it was evaporated under reduced press, dissolved in $CHCl_3$ (50 ml) and then re-evaporated. The residue was shaken with $CHCl_3$ (50 ml) and silicic acid (30 g), and the resultant material applied to a column of silicic acid (60 g, 12 cm × 7 cm^2). The desired product (2·4 g, 49%), which was eluted with 4% MeOH/$CHCl_3$, crystallized as fine colourless needles. Recrystallization from EtOH gave pure *$N^2,O^{5'}$-dibenzoylguanosine*. (Found, in material dried over P_2O_5 at 100°:

* The NMR spectrum of a mixture of $N^2,O^{5'}$-dibenzoyl-$O^{2'}(O^{3'})$-formylguanosines exhibits a doublet at τ 3·85 ($J \sim 4$ c/s), assigned to H(1′) of the $O^{2'}$-formyl derivative.[12]

C, 58·0; H, 4·5; N, 14·3. $C_{24}H_{21}N_5O_7$ requires: C, 58·65; H, 4·31; N, 14·25%), m.p. 231–232°. UV (95% EtOH): λ_{max} 296, 265, 258, 232 (log ε 4·15, 4·17, 4·17, 4·42), λ_{min} 273, 262, 253, 217 mμ (log ε 4·06, 4·16, 4·16, 4·29). R_f: 0·90 (system A), 0·80 (system B).

$N^2,O^{2'},O^{5'}$-*Tribenzoylguanosine* (XI)

$N^2,O^{5'}$-Dibenzoylguanosine (1·5 g, 3·05 mmole), toluene-*p*-sulphonic acid monohydrate (0·65 g, 3·4 mmole) and trimethyl orthobenzoate (15 ml) were stirred together at 20° for 45 min.* The reaction mixture was then neutralized with methanolic MeONa, and treated with 60% AcOH (15 ml) at 20° for 15 min. The products were concentrated under reduced press (0·1 mm) at 30°, and an equal volume of CH_2Cl_2 was added to the oil so obtained. The resultant mixture was filtered and the filtrate applied to a column of silicic acid (50 g, 8 cm × 7 cm^2). The desired mixture of $N^2,O^{2'}(O^{3'}),O^{5'}$-tribenzoylguanosines (1·6 g, 88%), which was eluted with 1% MeOH/$CHCl_3$, was dissolved in hot EtOH (100 ml) and the soln allowed to cool to 20°. The ppt, which was collected as soon as the liquors became turbid, was twice recrystallized from EtOH (50 ml) to give $N^2,O^{2'},O^{5'}$-*tribenzoylguanosine* (0·3 g, 16%) as colourless plates. (Found: C, 62·55; H, 4·4; N, 11·5. $C_{31}H_{25}N_5O_8$ requires: C, 62·5; H, 4·2; N, 11·8%), m.p. 146–147°. NMR spectrum in dimethylcyanamide-D_2O (N with respect to AcOH) [8:1; v/v] included the following signals: τ 3·67, doublet ($J \sim 3·5$ c/s), weight 1, assigned to H(1'); τ 3·97, quartet, weight 1, assigned to H(2'); τ 4·90, triplet, weight 1, assigned to H(3'). UV (95% EtOH): λ_{max} 297, 285, 265, 257, 233 (log ε 4·14, 4·14, 4·18, 4·19, 4·60), λ_{min} 290, 273, 262, 255, 216 mμ (log ε 4·14, 4·08, 4·18, 4·19, 4·38). R_f: 0·95 (system A), 0·90 (system B).

The combined mother liquors containing $N^2,O^{2'}(O^{3'}),O^{5'}$-tribenzoylguanosines were concentrated to a glass, and kept *in vacuo* over KOH at 20° for 2 days. Crystallization of this material from EtOH gave a product† with a complex NMR spectrum in the region τ 3·5–5·0: a notable signal was a doublet ($J \sim 5·5$ c/s) at τ 3·91, assignable[12] to H(1') of $N^2,O^{3'},O^{5'}$-tribenzoylguanosine. The latter signal was considerably more intense than the doublet at τ 3·67, assignable to H(1') of the $O^{2'}$-isomer (see above).

Guanylyl-(3'→5')-*uridine*[GpU] (XIV; B = *guanine*)

Pyridinium 2',3'-di-O-acetyluridine 5'-phosphate[6] (0·045 g, 0·1 mmole) was dried by evaporation from anhyd pyridine soln (3 × 1 ml), and then dissolved in pyridine (0·5 ml). $N^2,O^{2'},O^{5'}$-Tribenzoylguanosine (0·06 g, 0·1 mmole) and mesitylenesulphonyl chloride (0·044 g, 0·2 mmole) were added in turn to this soln. The reactants were sealed to exclude moisture, and allowed to stand at 20°. After 6 hr water (0·1 ml) was added, followed after a further 30 min by ethanolic $MeNH_2$ (33%, 0·5 ml). The resultant mixture was concentrated under reduced press to an oil, which was dissolved in ethanolic $MeNH_2$ (33%, 5 ml) at 20°. After 5 hr, the products were filtered. The filtrate (a) and ppt (b) were examined separately.

(a) The filtrate was concentrated under reduced press (0·1 mm) and dissolved in water (0·4 ml). Paper chromatography (system B) of this soln revealed two major components [R_f's 0·21 and 0·50] and two minor components [R_f's 0·13 and 0·40 (fluorescent)]. Marker spots of uridine 5'-phosphate and guanosine had R_f's (system B) 0·13 and 0·50, respectively. Paper electrophoresis (phosphate buffer, pH 7·5) indicated the presence of two main anionic components, corresponding to uridine 5'-phosphate and dinucleoside phosphate, and a neutral component.

A portion (0·25 ml) of the aqueous soln was applied as a band to a sheet of Whatman No. 31 ET paper, which was then developed in system B.

(i) The band with R_f 0·2 was eluted with water (67 ml), and the eluate (195 O.D. units at 257 mμ) concentrated to 5 ml. Paper chromatography (systems B and C) revealed the presence of a single component (R_f 0·21 and 0·24, respectively); paper electrophoresis (phosphate buffer, pH 7·5) confirmed the presence of a single anionic component, corresponding to a dinucleoside phosphate. UV absorption in water (pH 7): λ_{max} 257, λ_{min} 230 mμ. The action of ribonuclease T_1 on this material is described below.

(ii) The band with R_f 0·4 was also eluted with water (53 ml), and the eluate (53 O.D. units at 255 mμ) concentrated to 5 ml. Paper chromatography (system B) revealed the presence of two main components [R_f's 0·40 (fluorescent), 0·50]; paper electrophoresis (phosphate buffer, pH 7·5) resolved the eluate into a neutral component and a fluorescent anionic component, which corresponded to a dinucleoside phosphate. The action of ribonuclease T_1 on this mixture is described below.

(b) The ppt from the above ethanolic $MeNH_2$ soln was washed with EtOH and dissolved in water (2 ml).

* This is the optimum reaction time.

† This product was TLC [$CHCl_3$-MeOH (85:15, v/v)] homogeneous, with the same R_f as pure $N^2,O^{2'},O^{5'}$-tribenzoylguanosine.

Paper chromatography (system B) revealed two major (R_f's 0·13, 0·21) and a minor constituent (R_f 0·50). A portion of this material (20 μl of soln) was resolved by paper electrophoresis (phosphate buffer, pH 7·5) into a neutral (corresponding to guanosine) and two anionic components (corresponding to dinucleoside phosphate and uridine 5'-phosphate). Each component was eluted with water (10 ml) and estimated spectrophotometrically. It was calculated that the ppt (b) was composed of guanosine (60 O.D. units at 256 mμ), GpU (330 O.D. units at 257 mμ) and uridine 5'-phosphate (210 O.D. units at 261 mμ).

The total yield* of GpU (640 O.D. units at 257 mμ) was calculated to be 46% on the basis of the amount of guanosine remaining in the products, but only 32% if ε_{257} of GpU was assumed to be 20,000.

Action of ribonuclease T_1 on GpU and its assumed N^2-benzoyl derivative

A stock soln of ribonuclease T_1† (0·5 mg., ca. 2500 units) in 0·04M NH_4HCO_3 aq (pH 7·8, 0·5 ml) was prepared. The action of the enzyme on the two fractions [(i) and (ii)], obtained by preparative paper chromatography (system B) of the above filtrate (a) [see preparation of GpU], was examined.

(i) 0·5 ml of the R_f 0·2 fraction (ca. 20 O.D. units) was concentrated to 20 μl, and treated with enzyme soln (20 μl) and 0·04M NH_4HCO_3 aq (20 μl). The reactants were incubated at 37° for 2 hr, and then applied *in toto* to a paper electrophoretogram. Examination of the latter after development (phosphate buffer, pH 7·5) revealed two components corresponding to uridine and guanosine 3'-phosphate, *and no undegraded GpU*.‡ This was confirmed by paper chromatography (systems B, C).

(ii) 1 ml of the R_f 0·4 fraction (ca. 10 O.D. units) was concentrated to 20 μl, and incubated with enzyme as above. Apart from the digestion of some contaminating GpU (within 3 hr), the main dinucleoside phosphate constituent (which was fluorescent, and was assumed to be the N^2-benzoyl derivative of GpU) remained unaffected after 20 hr.

Cytidylyl-(3'→5')-uridine[CpU] (XIV; B = cytosine)

Pyridinium 2',3'-di-O-acetyluridine 5'-phosphate[6] (0·15 g, 0·33 mmole) was dried by evaporation from anhyd pyridine soln (3 × 2 ml), and then dissolved in pyridine (1·5 ml). Mesitylenesulphonyl chloride (0·146 g, 0·66 mmole) and N^4,$O^{2'}$,$O^{5'}$-triacetylcytidine[9] (0·185 g, 0·5 mmole of twice recrystallized material) were added in turn to this soln. The reactants were sealed and allowed to stand at 21–22°. After 1 hr water (1 ml) was added, followed after a further 2 hr by saturated methanolic ammonia (1 ml). The mixture was then evaporated, under reduced press, to an oil which was re-dissolved in methanolic ammonia (5 ml). After it had stood for 6 hr at 20°, the soln was evaporated and the residue dissolved in water (5 ml). Paper chromatography (system B) of the products revealed three main components (R_f's 0·16, 0·33, 0·61) and two trace components (R_f's 0·70, 0·85). In system B, uridine 5'-phosphate and cytidine have R_f's 0·15 and 0·63, respectively. Paper electrophoresis (phosphate buffer, pH 7) revealed three components corresponding to cytidine, dinucleoside phosphate and uridine 5'-phosphate.

The aqueous soln of products was applied to a column (7 cm × 0·8 cm^2) of Dowex-1 (Cl^- form) anion-exchange resin, which was washed thoroughly with water and 0·0001N HCl, and then eluted with 0·001N HCl. The fractions (515 ml) containing the desired dinucleoside phosphate [2110 O.D. units at 270 mμ, 33·5% (based on ε_{270} = 20,600)] were neutralized with 0·1N LiOH, and concentrated under reduced press to small volume (< 1 ml). The Li salt of cytidylyl-(3'→5')-uridine was precipitated by the addition of acetone-EtOH (3:1, v/v; 10 ml). The ppt (0·078 g) was collected by centrifugation, washed with the same solvent mixture (5 × 10 ml), and dried over P_2O_5 *in vacuo*. This material was paper chromatographically (system B, R_f 0·38) and electrophoretically (phosphate buffer, pH 7) homogeneous.

Action of pancreatic ribonuclease on CpU

A soln of CpU (20 O.D. units at 265 mμ) and pancreatic ribonuclease (100 μg) in 0·05M tris hydrochloride buffer (pH 7·5, 0·1 ml) was incubated[10] at 37° for 2 hr. The total digest was applied to a paper electrophoretogram which was then developed (phosphate buffer, pH 7). The amount of undegraded CpU was estimated[4] to be ca. 2%. Both paper electrophoresis and paper chromatography (system B) indicated that uridine and cytidine 3' (or 2')-phosphate were the sole digestion products.

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* 330 O.D. units in the ppt (b), and 195 × 1·6 O.D. units in the filtrate (a).

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‡ At least 0·5% of undegraded GpU would have been detected.

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