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THE SYNTHESIS OF OLIGORIBONUCLEOTIDES—VII*

THE USE OF RIBONUCLEOSIDE 2′,5′-BISKETALS AND 2′-KETAL-3′-ESTERS AS SYNTHETIC INTERMEDIATES

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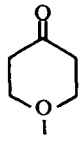
Abstract—A proposed general oligoribonucleotide synthesis, based on four suitably protected derivatives of each nucleoside is outlined. The preparations of UpU, ApA, UpUp and UpUpU, from three of the four suggested types of unit, are described. The products have been shown to contain virtually exclusively 3′ → 5′-phosphodiester linkages.

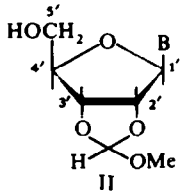
PROBABLY the most important initial decision to be made in the planning of an oligoribonucleotide synthesis[1] is the choice of a protecting group for the 2′-OH functions. This protecting group must remain intact throughout all the stages of the synthesis until the last, and it must then be removable under conditions which are mild enough to avoid any detectable isomerization or degradation of the unprotected oligomer. We previously demonstrated[1] that an acid-labile protecting group is suitable for this purpose if it can be removed within a few hours in aqueous acidic solution (pH 2) at room temperature. We also showed[1] that the commonly-used tetrahydropyranyl group has the desired hydrolytic properties, but its use leads to the introduction of new



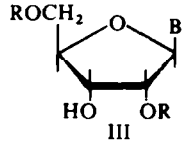
I

CHART I

Terminal nucleoside units

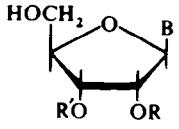


II

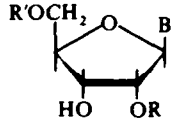


III

Non-terminal nucleoside units



IV



V

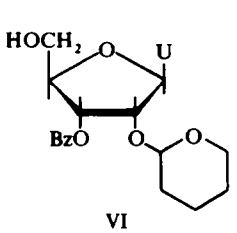
R is an acid-labile (acetal or ketal) protecting group;
R′ is a base-labile (acyl) protecting group

* For part VI of this series, see H. P. M. Fromageot, C. B. Reese and J. E. Sulston, Tetrahedron, **24**, 3533 (1968).

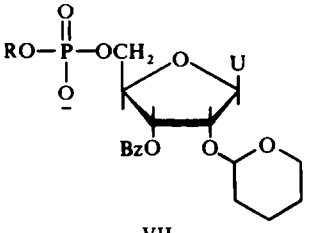
asymmetric centres, and hence to mixtures of diastereoisomers. As this was felt to be undersirable, we have developed[2] a symmetrical ketal protecting group, derived from tetrahydro-4H-pyran-4-one (I). Intermediate ketals of I have even more satisfactory hydrolytic properties[2] than corresponding tetrahydropyranyl derivatives.

For a completely general oligoribonucleotide synthesis, based on our approach, it is desirable to have four building blocks derived from each nucleoside: a terminal 2′,3′-, a terminal 2′,5′-, a non-terminal 2′,3′-, and a non-terminal 2′,5′-protected derivative. With the availability of these units, an oligonucleotide chain may be extended either from the 3′- or from the 5′-OH end. If the 2′-OH groups are to be protected with acid-labile residues, then the *terminal* 2′,3′- and 2′,5′-protected units must also have acid-labile residues blocking their 3′- and 5′-OH groups, respectively. Thus 2′,3′-O-methoxymethylidene-nucleosides[3] (II) and nucleoside 2′,5′-bisketals[2] (or bisacetals) (III) are suitable terminal units (see chart 1). On the other hand, *non-terminal* 2′,3′- and 2′,5′-protected units must have their respective 3′- and 5′-OH groups blocked by residues which are labile under non-acidic conditions. From chart 1, it can be seen that nucleoside 2′-ketal (or acetal)-3′-esters[4] (IV) and nucleoside 2′-ketal (or acetal)-5′-esters[1] (V) are suitable non-terminal units.

In the present paper, we wish to describe an approach to oligoribonucleotide synthesis which involves chain-extension from the 3′-OH end; we have previously[1] termed this the route (b) approach. This essentially requires connecting a terminal 2′,5′-protected unit (III) by a phosphodiester linkage to a non-terminal 2′,3′-protected unit (IV), and then ending the chain either by a monophosphate group or by a phosphodiester linkage to a terminal 2′,3′-protected unit (II). Although, in principle, any method of phosphorylation may be used to join the nucleoside units together, in the present work we have used the procedure developed largely by Khorana *et al.*[5]: i.e. condensation between a monoalkyl phosphate and an alcohol to give an unsymmetrical dialkyl phosphate. As will become clearer later, this phosphorylation procedure is not especially suited to the present approach to oligoribonucleotide synthesis, but it appeared to be the best available when this work was started.

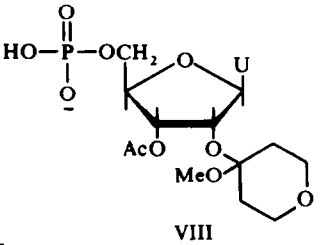


VI



VII

a; R = CH_2CH_2CN
b; R = H

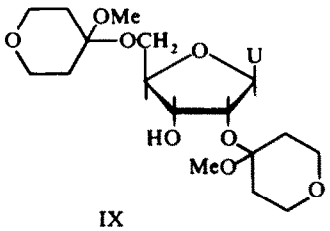


VIII

U = uracil-1

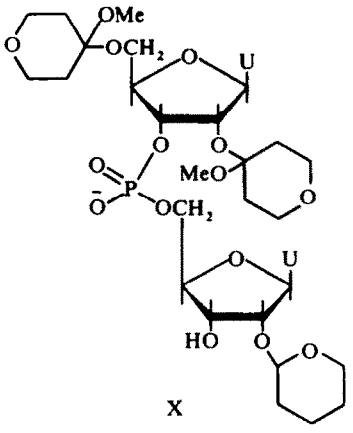
In order to follow the above procedure, it was initially necessary to convert the nucleoside 2′-ketal (or acetal)-3′-ester units (IV) into their 5′-monophosphates, In the first experiment, 2′-O-tetrahydropyranyl-3′-O-benzoyluridine[6] (VI) was converted by Tener's procedure[7] into its 5′-β-cyanoethyl phosphate (VIIa), in high yield. The latter material was purified by chromatography on DEAE-cellulose and then treated with aqueous ammonia to remove the β-cyanoethyl group. Not unexpectedly, this led to concomitant removal of the 3′-O-benzoyl group, and it was therefore necessary to re-

acylate the intermediate 2′-O-tetrahydropyranyluridine 5′-phosphate to obtain the desired product (VIIb), which was then isolated as its ammonium salt in 48% overall yield. By a similar procedure, 2′-O-methoxytetrahydropyranyl*-3′-O-acetyluridine[4] was converted into its 5′-phosphate (VIII), which was isolated in comparable yield.

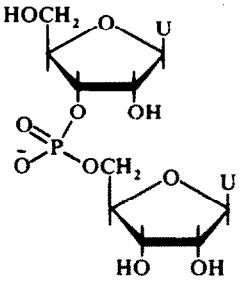


IX

U = uracil



X



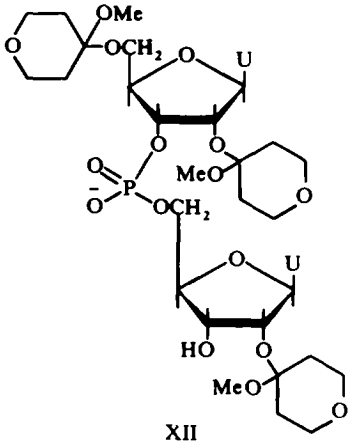
XI

The uridine 2′,5′-bisketal[2] (IX) and the pyridinium salt of VIIb were allowed to react together in the presence of DCC in pyridine solution, the crude products treated with methanolic ammonia, and then fractionated by chromatography on DEAE-cellulose to give the partially protected dinucleoside phosphate (X) in ca. 84% yield (based on VIIb). When a solution of the latter compound in 0·01N hydrochloric acid was allowed to stand at 20° for 10 hr,[1,2] uridyl-(3′ → 5′)-uridine {UpU}† (XI) was obtained in quantitative yield. The latter material had the expected paper electrophoretic and chromatographic properties and underwent virtually complete (ca. 99%) digestion to uridine 3′-phosphate and uridine in the presence of pancreatic ribonuclease.

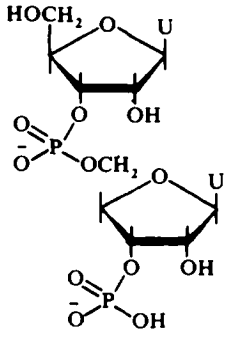
When the ammonium salt of VIII and the bisketal (IX) were allowed to react with an excess of 2,4,6-triisopropylbenzenesulphonyl chloride (TPS)[8] in anhydrous pyridine solution, the products treated with methanolic ammonia and then fractionated as before, the trisketal of UpU (XII) was obtained in ca. 57% yield (based on VIII). Thus, although the TPS-activated condensation proceeded at a faster rate than that of the above DCC reaction (see experimental section), the yield of partially-protected dinucleoside phosphate was lower, However, such a comparison of condensing agents is of doubtful significance as different 5′-nucleotide components (VIII and VIIb, respectively) were used in the two reactions. When the trisketal (XII) was allowed to stand in hydrochloric acid solution (pH 2) at 20° for 6 hr[2], UpU (XI) was again obtained in quantitative yield. The physical and enzymatic properties of this material were identical to those of the UpU described above.

* Methoxytetrahydropyranyl is used as an abbreviation for 4-methoxytetrahydropyran-4-yl.

† It is convenient to abbreviate uridylyl-(3′→5′)-uridine to UpU. In general, XpYpZp. . . . denotes an oligonucleotide in which the 3′-hydroxyl group of nucleoside X is connected through a phosphodiester linkage to the 5′-hydroxyl group of nucleoside Y, which in turn is similarly connected through its 3′-hydroxyl group to the 5′-hydroxyl group of nucleoside Z, and so on. The nucleosides adenosine, uridine, cytidine and guanosine are respectively denoted by A.U.C and G.



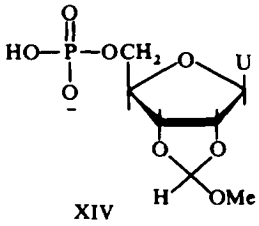
XII



XIII

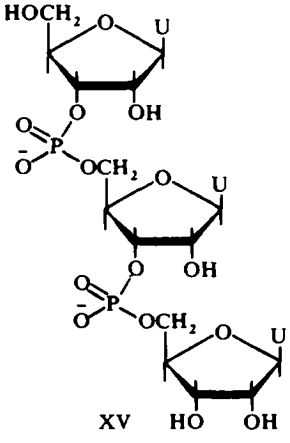
U = uracil-1

The conversion of each of the partially-protected dinucleoside phosphates (X and XII) into UpU, in quantitative yield, suggested that they were suitable intermediates for chain-extension. In the first place, both X and XII were phosphorylated on their free 3′-OH functions and the protecting groups removed to give the dinucleotide, uridylyl-(3′ → 5′)-uridine 3′-phosphate {UpUp} (XIII). The phosphorylation method chosen[7] was that used above in the preparation of VIIb and VIII. The fully-protected dinucleotide β-cyanoethyl ester, which was isolated in ca. 65% yield {based on unrecovered starting material (X)} by chromatography of the crude products on DEAE-cellulose, was first treated with 9*N*-aqueous ammonia at 50° and then with hydrochloric acid (pH 2) at 20° to give the desired product, UpUp (XIII). This material, which was isolated as its calcium salt, had the expected paper electrophoretic properties, and was virtually quantitatively digested to uridine 3′-phosphate in the presence of pancreatic ribonuclease. By the same procedure, XII was converted into UpUp in 52% yield (based on nucleotide starting material). In this experiment, the crude products were first treated with aqueous ammonia and then fractionated by chromatography on Dowex-1 (Cl^- form) anion-exchange resin. It should be noted that these experiments represent the first satisfactory and completely unambiguous



XIV

U = uracil-1

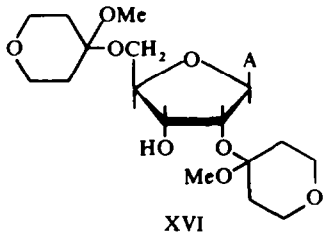


XV

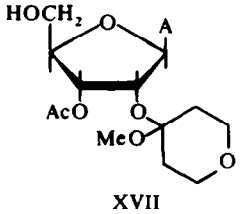
synthesis of a dinucleotide terminating in a 3′-monophosphate function, by solely chemical methods.[9]

It was then disirable to convert one or both of the partially-protected dinucleoside phosphates (X and XII) into a trinucleoside diphosphate (i.e. UpUpX). If the oligonucleotide chain was to be terminated at this stage, a 5′-monophosphate ester of a terminal 2′,3′-protected unit (II) was the most suitable intermediate to use. Such 2′,3′-O-methoxymethylidene nucleoside 5′-phosphates had been prepared previously.[3b] The pyridinium salts of X and XIV (1·5 molecular equivs) were allowed to react with DCC in pyridine solution under the usual conditions, and the products fractionated by chromatography on DEAE-cellulose. The fully protected trinucleoside diphosphate, which was obtained in 47% yield, was treated with hydrochloric acid (pH 2), and the desired UpUpU (XV) isolated as its calcium salt. This material had the expected paper electrophoretic and chromatographic properties, and was virtually completely (ca. 98%) digested to uridine 3′-phosphate and uridine in the presence of pancreatic ribonuclease.

When it was established that the present approach to oligoribonucleotide synthesis gave satisfactory results with units of types II, III and IV (chart 1), derived from uridine, the above experiments were repeated with the corresponding adenosine derivatives. The required terminal 2′,5′-protected adenosine unit (XVI) was available, and the non-terminal 2′,3′-adenosine unit was prepared from 2′,3′-O-methoxyethylideneadenosine.[10] Formylation of the latter compound followed by mild acidic hydrolysis gave 3′-O-acetyl-5′-O-formyladenosine, which was isolated in a pure crystalline state. Ketalation of this compound, followed by treatment of the product with methanolic ammonia under very mild conditions gave the desired crystalline 2′-O-methoxytetrahydropyranyl-3′-O-acetyladenosine* (XVII).

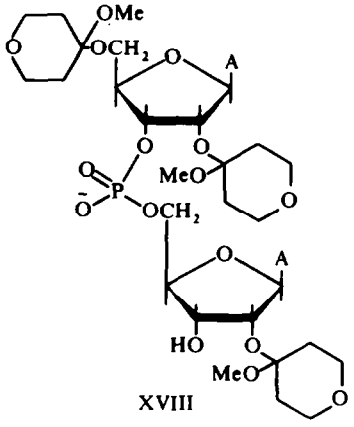


XVI



XVII

A = adenine-9



XVIII

The latter compound (XVII) was converted into its 5′-phosphate in 53% overall yield by the procedure described above for the corresponding uridine derivative. An excess of the bisketal[2] (XVI) and the pyridinium salt of the 5′-phosphate of XVII were allowed to react together in the presence of DCC, the products worked-up under the

* The corresponding 2′-O-tetrahydropyranyl-3′-O-acetyladenosine was also prepared (see Experimental). 2′-O-methoxytetrahydropyranyl-3′-O-benzoyladenosine has also been prepared[4] from its 5-O-methoxyacetyl derivative.

usual conditions, treated with methanolic ammonia and fractionated by chromatography on DEAE-cellulose. The desired trisketal of ApA (XVIII) was obtained in ca. 40% yield (based on unrecovered nucleotide starting material); treatment of this material with hydrochloric acid (pH 2) at 20° gave ApA in quantitative yield. This product had the expected paper electrophoretic and chromatographic properties, and was almost completely (ca. 98%) digested to adenosine 3′-phosphate and adenosine in the presence of calf spleen phosphodiesterase; it was also completely digested to adenosine 5′-phosphate and adenosine in the presence of *Crotalus adamanteus* snake venom phosphodiesterase.

It was thus anticipated that the trisketal of ApA (XVIII) would be a suitable intermediate for chain extension in our projected oligoribonucleotide synthesis. This did not prove to be the case. As before, the DCC-activated condensation between the pyridinium salts of β-cyanoethyl phosphate[7] and XVIII was examined first. After the usual work-up, paper electrophoretic examination of the crude products revealed mainly XVIII and only a small amount of a more mobile component, corresponding to the desired condensation product. Similarly no success was achieved with an attempted TPS-activated condensation[8] between the same reactants, or between 2′,3′-O-methoxymethylideneuridine 5′-phosphate[3b] (XIV and XVIII).

It is unlikely that the failure of the condensation between XVIII and β-cyanoethyl phosphate was due to the adenine residues being unprotected as a sufficient excess of β-cyanoethyl phosphate was used. Nor, by consideration of the comparatively successful phosphorylation of XII, can this result be attributed solely to the steric properties of the bulky ketal function adjacent to the free 3′-OH group. It therefore seems that although our general approach to oligoribonucleotide synthesis, based on four derivatives of each nucleoside (Chart 1) is perfectly feasible, further progress will not be possible until more satisfactory phosphorylation procedures have been developed. We are now concentrating our efforts on this aspect of the problem, and hope to report on our findings shortly.

EXPERIMENTAL

UV absorption spectra were measured with a Cary recording spectrophotometer, Model 14M-50. NMR spectra were measured with a Varian HA-100 spectrometer, operating at 100 Mc/s, with TMS as internal standard.

Unless otherwise stated, paper electrophorisis was conducted at ca. 4 kV on Whatman No. 4 paper in 0·1M-sodium phosphate buffer (pH 8). The following solvent systems were used for ascending paper chromatography on Whatman No. 1 paper: A, isobutyric acid–N aqueous ammonia–0·1M EDTA (100:60:1·6); B, propan-2-ol–aqueous ammonia (*d* 0·88)–water (7:1:2); C, ethanol–M aqueous ammonium acetate (5:2).

Plates coated with Merck Kieselgel GF_{254} were used for TLC in the solvent system: $CHCl_3$–MeOH (9:1, v/v). Mallinckrodt analytical grade silicic acid (100 mesh) was used for adsorption chromatography.

Pyridine was dried by heating with CaH_2, under reflux, and redistilled before use. Dioxane was first dried over molecular sieves (type 5A), and then redistilled from LAH.

3′-O-Acetyl-5′-O-formyladenosine

Formic acetic anhydride[11] (6 ml) was added to a soln of 2′,3′-O-methoxyethylideneadenosine[3] (4·0 g, 12 mmole), cooled to −40°. The reactants were kept at −15° with occasional shaking and with the exclusion of moisture. After 12 hr, the resulting soln was concentrated under reduced press (bath temp <30°) to give an oil which was dissolved in EtOH and the soln re-evaporated. TLC of the partially solid residue revealed only one component (mobility 1·75 × that of 3′-O-acetyladenosine).

When the above material was shaken with AcOH–water (9:1, v/v; 10 ml), it dissolved to give a homogeneous soln. After 1·5 hr, TLC revealed a single UV-absorbing component (mobility 1·3× that of 3′-O-acetyladenosine). The soln was concentrated under reduced press (0·1 mm) at 20° to give an oil which was dissolved in EtOH and the soln evaporated. The residue was redissolved in EtOH (40 ml) and the soln filtered. When the filtrate was kept at 0°, it slowly deposited colourless crystals (2·1 g, 50%) of 3′-O-*acetyl*-5′-O-*formyladenosine* [Found, in material recrystallized from slightly acidified (with AcOH) EtOH and dried *in vacuo* over P_2O_5 at 20°: C, 46·3; H, 4·4; N, 20·6. $C_{13}H_{15}N_5O_6$ requires: C, 46·3; H, 4·4; N, 20·8%]. UV absorption (water): λ_{max} 260 (ε 14,800), λ_{min} 225 mμ (ε 2,520); ν_{max}^{Nujol} 1720, 1740 cm^{-1}; NMR spectrum [in Me_2NCN-Me_2SO–D_2O (M with respect to AcOH) (3:1:1; v/v)] included a doublet (J = 6·0 c/s) at τ 4·10, assigned to H(1′).*

The ethanolic mother liquors (from the original crystallization), which were shown by TLC to contain approximately equivalent amounts of two components corresponding to 3′-O-acetyl-5′-O-formyl- and 3′-O-acetyl-adenosines, were treated with dilute methanolic ammonia and the products concentrated immediately to dryness under reduced press. A soln of the residue in EtOH deposited colourless crystals of 3′-O-acetyladenosine (1·2 g, 32%), m.p. 181–182°.

2′-O-*Tetrahydropyranyl*-3′-O-*acetyladenosine*

After a mixture of 3′-O-acetyl-5′-O-formyladenosine (0·265 g, 0·78 mmole), toluene-*p*-sulphonic acid, monohydrate (0·225 g, 1·2 mmole), type 4A molecular sieves (0·10 g) and anhyd dioxan (6 ml) had been stirred at 20° for 30 min, 2,3-dihydro-4*H*-pyran (1 ml) was added. The stirring was continued for a further 5 min, the products then treated with an excess of NH_3/MeOH and immediately concentrated under reduced press to yield a viscous gum. The latter was triturated with $CHCl_3$ (2 × 10 ml), the $CHCl_3$ extracts filtered and concentrated to a yellow oil which was shown by TLC to contain one main UV-absorbing component (mobility 1·5× that of 3′-O-acetyl-5′-O-formyladenosine).

A soln of the products in $CHCl_3$ was applied to a column of silicic acid (7 cm × 3 cm^2, 10 g). The desired 2′-O-*tetrahydropyranyl*-3′-O-*acetyladenosine* (0·18 g, 60%) was eluted from the column with 1% MeOH/$CHCl_3$ and crystallized from EtOH [Found, in material dried *in vacuo* over P_2O_5 at 100°: C, 52·1; H, 6·1; N, 17·6. $C_{17}H_{23}N_5O_6$ requires: C, 51·9; H, 5·9; N, 17·8%]. m.p. 221–225° (initial softening at 210°); R_f 0·86 (system B).

2′-O-*Methoxytetrahydropyranyl*-3′-O-*acetyladenosine* (XVII)

3′-O-Acetyl-5′-O-formyladenosine (0·50 g, 1·8 mmole) and freshly distilled 4-methoxy-5,6-dihydro-2*H*-pyran[2] (2 ml) were added in turn to a stirred mixture of toluene-*p*-sulphonic acid, monohydrate (0·38 g, 2·0 mmole) and anhyd dioxan dioxan (6 ml) at 20°. After 10 min, the reaction mixture was treated with an excess of NH_3/MeOH (half-saturated at 0°), and then immediately concentrated under reduced press to give a yellow oil. The latter was triturated with $CHCl_3$ (2 × 20 ml) and the $CHCl_3$ extracts filtered. TLC examination of the filtrate in the system $CHCl_3$–MeOH (19:1; v/v) revealed one main UV-absorbing component (mobility 1·8× that of 3′-O-acetyl-5′-O-formyladenosine). The filtrate was concentrated (to ca. 5 ml) and applied to a column of silicic acid (22 cm × 1 cm^2, 10 g), which was washed with CH_2Cl_2 and $CHCl_3$. The desired product was eluted with 1% MeOH/$CHCl_3$ and isolated as a pale yellow glass. Crystallization from EtOAc gave 2′-O-*methoxytetrahydropyranyl*-3′-O-acetyladenosine (0·44 g, 71%) as colourless needles [Found, in material dried *in vacuo* over P_2O_5 at 90°: C, 50·9; H, 5·75; N, 16·8. $C_{18}H_{25}N_5O_7$ requires: C, 51·05; H, 5·95; N, 16·5%], m.p. 198–199°; UV absorption (95% EtOH): λ_{max} 260 (ε 15,300), λ_{min} 226 mμ (ε 1,600).

2′-O-*Tetrahydropyranyluridine* 5′-*phosphate*

To an anhyd soln of pyridinium β-cyanoethyl phosphate (0·95 mmole, obtained from 0·31 g of Ba salt by ion-exchange) in pyridine (10 ml), containing Dowex-50 (pyridinium form; 0·10 g) cation-exchange resin, was added 2′-O-tetrahydropyranyl-3′-O-benzoyluridine (0·20 g, 0·475 mmole) and N,N′-dicyclohexylcarbodiimide (DCC) (0·98 g, 4·75 mmole). The procedures for the condensation and work-up were those used previously in the preparation of 2′,3′-O-methoxymethylideneuridine 5′-phosphate.[3b]

An aqueous soln of crude β-cyanoethyl ester of 2′-O-tetrahydropyranyl-3′-O-benzoyluridine 5′-phosphate, so obtained, was applied to a column of DEAE-cellulose (HCO_3^--form, 42 cm × 5 cm^2) which

* An NMR spectrum (in the same solvent) of equilibrated material (*i.e.* a mixture of 2′- and 3′-O-acetyl-5′-O-formyladenosines showed doublets at τ 3·87 and 4·10 (J 5·0 and 6·0 c/s, respectively) with relative intensities of 1:2.

was eluted with triethylammonium bicarbonate buffer (pH 7·5, linear gradient from 0·001–0·05M over 2000 ml). 25 Ml fractions were collected: fractions 41–72 (averate buffer concentration ca. 0·026M) contained the desired β-cyanoethyl ester [5020 O.D. units, 92% (based on $\varepsilon_{262} = 11{,}200$)], which had a paper electrophoretic mobility (phosphate buffer, pH 8) 0·33× that of uridine 5′-phosphate.

Fractions 41–71 were combined and concentrated under reduced press (bath temp <30°) just to dryness, dissolved in EtOH (50 ml) and the soln re-evaporated. The latter process was repeated and the residual solid dissolved in NH_3/MeOH (half-saturated at 0°, 20 ml). After the soln had stood at 20° for 15 hr, it was concentrated under reduced press and the residue triturated with ether (2 × 50 ml).* The remaining glass was dissolved in water (10 ml) and filtered. A soln of the filtrate and 18N aqueous NH_3 (10 ml) was heated at 50° for 1·5 hr. Air was bubbled through the cooled soln to remove some of the excess ammonia, and it was then concentrated under reduced press to near dryness. Paper electrophoresis (phosphate buffer, pH 8) revealed a sole UV-absorbing anionic component with a mobility 0·85× that of uridine 5′-phosphate. It was assumed that this component [R_f 0·36 (system C)] was 2′-O-tetrahydropyranyluridine 5′-phosphate (as its NH_4^+ salt).

2′-O-*Tetrahydropyranyl*-3′-O-*benzoyluridine 5′-phosphate* (VIIb)

A solution of ammonium 2′-O-tetrahydropyranyluridine 5′-phosphate (90% of material prepared above, ca. 0·4 mmole) in aqueous pyridine (9:1, v/v; 5 ml) was passed through a column of Dowex-50 (pyridinium form; > 10-fold excess) cation-exchange resin. The column was washed with aqueous pyridine (5 bed vol) and the total eluate concentrated to small volume under reduced press (below 30°), and then re-evaporated with anhyd pyridine† (4 × 15 ml). More pyridine (10 ml) and freshly distilled benzoyl chloride (0·23 ml, 1·6 mmole) were added to the resulting concentrated soln (volume ca. 1 ml), and the reaction mixture kept at 5° with the exclusion of moisture.

After 16 hr, MeOH (3 ml) was added and then, after a further 3 hr, water (30 ml) was added. The products were extracted with ether (2 × 10 ml), the aqueous layer concentrated under reduced press to ca. 5 ml, and treated with 0·05M triethylammonium carbonate buffer (pH 9; 50 ml) at 20°. After 24 hr, when paper electrophoresis (phosphate buffer, pH 8) revealed the presence of a principal and a trace nucleotidic product, the solution was treated with solid CO_2 (to pH 7·5) and then concentrated under reduced press (below 30°) to ca. 15 ml. Dowex-50 (pyridinium form) cation-exchange resin was added until the evolution of CO_2 ceased; the resin was then removed by filtration and washed with aqueous pyridine (19:1, v/v; 20 ml).

The combined filtrate and washings were concentrated under reduced press (below 30°) to half volume, and applied to a column of DEAE-cellulose (HCO_3^- form, 45 cm × 5 cm²). The column was first washed with pH 8 triethylammonium bicarbonate buffer (0·01M, 500 ml) and then eluted with the same buffer (linear gradient from 0·01–0·15M over 2000 ml). 25 Ml fractions were collected: fractions 10–28 (average buffer concentration ca. 0·043M) contained benzoate ion; fractions 30–37 (average buffer concentration ca. 0·068M) contained 3 components (750 O.D. units at 262 mμ) with R_f's (system C) 0·37, 0·60 and 0·87‡; fractions 38–46 (average buffer concentration ca. 0·084M) contained 2 components (800 O.D. units at 262 mμ) with R_f's (system C) 0·37 and 0·61; fractions 47–61 (average buffer concentration ca. 0·105M) contained a sole component (2090 O.D. units at 262 mμ) with R_f (system C) 0·61.

Fractions 46–61 were combined, concentrated under reduced press (below 30°) just to dryness, then dissolved in water, and the soln lyophilized; yield of paper chromatographically (system C) and electrophoretically (phosphate buffer, pH 8) homogeneous 2′-O-tetrahydropyranyl-3′-O-benzoyluridine 5′-phosphate: 0·14 g (ca. 47%). Fractions 30–46 were combined and lyophilized to give a mixture of 2′-O-tetrahydropyranyl- and 2′-O-tetrahydropyranyl-3′-O-benzoyluridine 5′-phosphates [R_f's (system C) 0·37 and 0·61, respectively].

2′-O-*Methoxytetrahydropyranyl*-3′-O-*acetyluridine 5′-phosphate* (VIII)

To an anhydrous soln of pyridinium β-cyanoethyl phosphate (0·75 mmole, from 0·25 g of Ba salt) in

* Paper electrophoresis (phosphate buffer, pH 8) indicated that the remaining solid contained 2′-O-tetrahydropyranyluridine 5′-phosphate and its β-cyanoethyl ester in the relative proportions of 1:4.

† Great care was taken in this and similar evaporations not to concentrate pyridine or aqueous pyridine solutions of protected nucleotide derivatives to dryness.

‡ When this mixture was treated with aqueous NH_3 (pH 10), the component with R_F 0·87 disappeared rapidly and that with R_F 0·60 disappeared more slowly.

pyridine (10 ml), containing Dowex-50 (pyridinium form, 0·05 g) cation-exchange resin, was added 2′-O-methoxytetrahydropyranyl-3′-O-acetyluridine[4] (0·15 g, 0·375 mmole) and DCC (0·775 g, 3·75 mmole). The procedures for the condensation and work-up were those used previously in the preparation of 2′,3′-O-methoxymethylideneuridine 5′-phosphate.[3b]

An aqueous soln (5 ml) of the crude β-cyanoethyl ester of 2′-O-methoxytetrahydropyranyl-3′-O-acetyluridine 5′-phosphate, so obtained, was applied to a column of DEAE-cellulose (HCO_3^- form, 45 cm × 4·7 cm²) which was first washed with pH 7·5 triethylammonium bicarbonate buffer (0·005M, 500 ml) and then eluted with the same buffer (linear gradient from 0·005–0·05M over 2000 ml). 25 Ml fractions were collected: fractions 20–36 (average buffer concentration 0·02M) contained the desired β-cyanoethyl ester [3.325 O.D. units, 88% (based on ε_{260} = 10,000)], which had a paper electrophoretic mobility (phosphate buffer, pH 8) 0·45× that of uridine 5′-phosphate; this material had R_f 0·60 (system b).

Fractions 20–36 were combined and concentrated under reduced press (bath temp <30°) just to dryness. The residue was dissolved in 9N aqueous NH_3 (20 ml), the soln heated at 50° for 90 min and then concentrated under reduced press to small volume. The aqueous products, which contained a single nucleotidic component [paper electrophoretic mobility (phosphate buffer, pH 8) 0·9× that of uridine 5′-phosphate; R_f 0·26 (system B)] were re-evaporated with anhydrous pyridine (4 × 10 ml) under reduced press, dissolved in pyridine (10 ml) and treated with Ac_2O (0·6 ml, 6·3 mmole) at 20°. After 15 hr, water (10 ml) was added, the soln left for 4 hr at 20° and then concentrated under reduced press (below 30°) to small volume. The soln of products was again dried by repeated evaporation with anhyd pyridine. The final pyridine soln (1 ml) was added dropwise with stirring to dry ether (30 ml). The NH_4^+ *salt of* 2′-O-*methoxytetrahydropyranyl*-3′-O-*acetyluridine* 5′-*phosphate* (0·20 g) was precipitated as a pale-coloured solid, collected, washed with ether, and air-dried. This material contained a single UV-absorbing component with paper electrophoretic mobility (phosphate buffer, pH 8) 0·85× that of uridine 5′-phosphate; it had R_f 0·40 (system B).

2′-O-*methoxytetrahydropyranyladenosine* 5′-*phosphate*

To an anhyd soln of pyridinium β-cyanoethyl phosphate (0·48 mmole, from 0·155 g of Ba salt) in pyridine (5 ml), containing Dowex-50 (pyridinium form; 0·05 g) cation-exchange resin, XVII (0·10 g, 0·24 mmole) and DCC (0·50 g, 2·4 mmole) were added. The condensation and work-up procedures were as before.[3b]

An aqueous soln of the crude β-cyanoethyl ester of 2′-O-methoxytetrahydropyranyl-3′-O-acetyladenosine 5′-phosphate was applied to a column of DEAE-cellulose (HCO_3^- form, 50 cm × 3·8 cm²) which was first washed with pH 7·5 triethylammonium bicarbonate (0·002M, 500 ml) and then eluted with the same buffer (linear gradient from 0·002–0·06M over 2000 ml). 25 Ml fractions were collected: fractions 29–37 (average buffer concentration 0·026M) contained the desired β-cyanoethyl ester [2360 O.D. units, 66% (based on ε_{260} = 15,000)]. These fractions were combined, evaporated under reduced press just to dryness, dissolved in 9N aqueous NH_3 (10 ml), and the soln heated at 50° for 1·5 hr. After air had been bubbled through the aqueous products, they were concentrated under reduced press (below 30°) to small volume (ca. 5 ml), treated with pyridine (5 ml) and the soln re-evaporated. The latter process was repeated 3 × and the final pyridine solution (ca. 2 ml) added dropwise to dry ether (30 ml) to give the NH_4^+ *salt of* 2′-O-*methoxytetrahydropyranyladenosine* 5′-*phosphate* as a colourless precipitate; R_f (system B) 0·26, paper electrophoretic mobility (borate buffer, pH 9) 0·64× that of adenosine 5′-phosphate.

2′-O-*Methoxytetrahydropyranyl*-3′-O-*acetyladenosine* 5′-*phosphate*

A soln of the above NH_4^+ salt of 2′-O-methoxytetrahydropyranyladenosine 5′-phosphate (ca. 0·16 mmole) in pyridine (5 ml) was treated with Ac_2O (0·15 ml, 1·5 mmole) at 20°. After 16 hr, water (25 ml) was added and the soln kept at ca. 5° for 12 hr. Pyridine (5 ml) was added, the products concentrated

salt of 2′-O-*methoxytetrahydropyranyl*-3′-O-*acetyladenosine* 5′-*phosphate* was isolated as a pale yellow solid (0·075 g, ca. 80%) by the above precipitation technique.* An analytical sample was obtained by dissolving the material in dilute NH_3/MeOH and then adding the soln to ether. The resulting precipitate was collected by centrifugation, washed with ether and dried *in vacuo* over P_2O_5 at 20° for 24 hr [Found: N, 15·0; P, 5·4. $C_{18}H_{24}N_5O_{10}P.NH_4.2H_2O$ requires: N, 15·1; P, 5·6%]; UV absorption (water): λ_{max} 260

* This material was re-dissolved in pyridine and re-precipitated.

(ε 14,300), λ_{min} 230 mμ (ε 4510); R_f 0·42 (system B), 0·65 (system C); paper electrophoretic mobility (phosphate buffer, pH 8) 0·81× that of adenosine 5′-phosphate.

A soln of this material in NH_3/MeOH (half-saturated at 0°) was kept at 20° for 16 hr, evaporated and the residue dissolved in 0·01N-HCl at 20°. After 6 hr, examination by paper chromatography (systems B and C) and electrophoresis revealed adenosine 5′-phosphate as the sole product.

2′,5′-Di-O-methoxytetrahydropyranyluridylyl-(3′→5′)-2′-O-tetrahydropyranyluridine (X)

The NH_4^+ salt of VII (0·07 g, ca. 0·09 mmole) was converted into its pyridinium salt[1], which was dried by evaporation from pyridine soln (5 × 10 ml). To a solution of the latter pyridinium salt and IX[2] (0·045 g, 0·09 mmole) in anhyd pyridine (2 ml) at 20° was added DCC (0·18 g, 0·87 mmole) and Dowex-50 (pyridinium form, 0·03 g) cation-exchange resin.[12] The stirred reactants were sealed to exclude moisture. After 24 hr, an additional quantity (0·025 g, 0·05 mmole) of IX was added and the reaction allowed to continue for a further 48 hr before the addition of water (10 ml).

After 8 hr at 20°, the products were extracted with pet ether (b.p. 40–60°, 2 × 10 ml). The material in the filtered aqueous layer was rendered anhydrous by repeated evaporations with pyridine. The resulting oil, which contained a small amount of pyridine, was treated with NH_3/MeOH (10 ml, half-saturated at 0°) at 20°. After 24 hr, the products were concentrated nearly to dryness, treated with pH 7·5 triethylammonium bicarbonate buffer (0·005M, 5 ml), and the resulting solution applied to a column of DEAE-cellulose (50 cm × 3 cm², HCO_3^- form). The column was washed first with pH 7·5 triethylammonium bicarbonate buffer (0·002M) until the eluate had negligible UV absorbance, and then eluted with the same buffer (linear gradient from 0·002–0·075M over 2000 ml). 25 Ml fractions were collected: fractions 27–39 (average buffer concentration ca. 0·03M) contained the desired product (X) (1,510 O.D. units, ca. 84% based on ε_{260} = 20,000).

Fractions 27–39 were combined and concentrated under reduced press (bath temp <30°) to near dryness and then freeze-dried to give a colourless solid. Paper electrophoresis (phosphate buffer, pH 8) revealed a major component (>95%) with the mobility expected for the protected dinucleoside phosphate; R_F (system B) 0·48.

2′,5′-Di-O-methoxytetrahydropyranyluridylyl-(3′→5′)-2′-O-methoxytetrahydropyranyluridine (XII)

A soln of the NH_4^+ salt of VIII (0·13 g, ca. 0·2 mmole; dried *in vacuo* over P_2O_5 at 50° for 24 hr), 2′,5′-di-O-methoxytetrahydropyranyluridine[2] (0·12 g, 0·25 mmole), and 2,4,6-triisopropylbenzenesulphonyl chloride[8] (0·242 g, 0·8 mmole) in anhyd pyridine (4 ml) was allowed to stand at 20°, with the exclusion of moisture. After 12 hr, the pale-red reaction soln was cooled to 0°, treated with water (5 ml) and the products left at 4° for a further 12 hr. After the addition of pH 7·5 triethylammonium bicarbonate buffer (0·1M, 1 ml), the products were evaporated just to dryness (bath temp <30°) and the residue dissolved in NH_3/MeOH (10 ml, half-saturated at 0°).

After the soln had stood at 20° for 16 hr, it was concentrated under reduced press (bath temp <30°), the resulting oil dissolved in pH 7·5 triethylammonium bicarbonate buffer (0·01M, 2 ml) and the soln applied to a column of DEAE-cellulose (HCO_3^- form, 60 cm × 3·8 cm²). The column was washed with the same buffer (0·005M, 500 ml) and then eluted with it (linear gradient from 0·005–0·05M over 2000 ml). 25 Ml fractions were collected: fractions 32–45 (average buffer concentration 0·028M), which contained the required material (2300 O.D. units, ca. 57% based on ε_{260} = 20,000), were combined, concentrated under reduced press (bath temp <30°) to small volume, and finally lyophilized to give a colourless powder. This material contained virtually (>95%) one UV-absorbing component with paper electrophoretic mobility (phosphate buffer, pH 8) 0·23× that of uridine 5′-phosphate; R_F (system B) 0·54.

Uridylyl-(3′→5′)-uridine [*UpU*]

(a) *From 2′,5′-di-O-methoxytetrahydropyranyluridylyl-(3′→5′)-2′-O-tetrahydropyranyluridine* (X). A soln of this material (225 O.D. units) in 0·01N HCl was allowed to stand at 20° for 10 hr, and then neutralized with dil aqueous NH_3. The product was identified as by its paper electrophoretic (phosphate buffer, pH 8) mobility and by paper chromatography [R_f (system C) 0·39].

When the product (45 O.D. units) was treated with pancreatic ribonuclease,[13] it underwent hydrolysis to give uridine 3′-phosphate and uridine. Paper chromatography (system C) of the products revealed ca. 1% of undigested material.

(b) *From 2′,5′-di-O-methoxytetrahydropyranyluridylyl-(3′→5′)-2′-O-methoxytetrahydropyranyluridine* (XII). A soln of this material (ca. 300 O.D. units) in HCl (pH 2, 2 ml) was allowed to stand at 20° for 6 hr, and then neutralized (to pH 6·5) with LiOH aq. The products were evaporated under reduced pressure

(bath temp <30°) just to dryness, redissolved in aqueous EtOH (1:4, v/v; 2 ml) and treated with saturated $CaCl_2$/EtOH (0·1 ml). The Ca salt of UpU was precipitated by the addition of Me_2CO-EtOH (3:1, v/v; 30 ml); it was collected by centrifugation washed with Me_2CO–EtOH (10 ml), then with ether and dried *in vacuo* over P_2O_5. The colourless solid, so obtained, contained a single UV-absorbing constituent with a paper electrophoretic mobility (phosphate buffer, pH 8) 0·43 × that of uridine 5′-phosphate; R_F 0·15 (system A), 0·39 (system C). The latter material also underwent ca. 99% digestion in the presence of pancreatic ribonuclease.

Uridylyl-(3′→5′)-uridine 3′-phosphate [UpUp]

(a) *From 2′,5′-di-O-methoxytetrahydropyranyluridylyl-(3′→5′)-2′-O-tetrahydropyranyluridine* (X). The pyridinium salt of the partially-protected dinucleoside phosphate (ca. 0·025 mmole, obtained from 500 O.D. units of the above triethylammonium salt) in aqueous pyridine (9:1; v/v; 40 ml) soln was concentrated to ca. 5 ml, and then dried by evaporation with pyridine (5 × 10 ml). To a soln of the latter material and pyridinium β-cyanoethyl phosphate (0·05 mmole, from 0·016 g of Ba salt) in pyridine (2 ml) was added DCC (0·052 g, 0·25 mmole) and Dowex-50 (pyridinium form, 0·01 g) cation-exchange resin. The reactants were stirred at 20° with the exclusion of moisture. After 72 hr, water (5 ml) was added and, after a further 6 hr, the products were extracted with pet. ether (b.p. 40–60°, 2 × 5 ml). Paper electrophoretic (phosphate buffer, pH 8) examination of the filtered aqueous layer revealed a principal component (mobility 2·7× that of the starting material) with R_F (system B) 0·48.

The aqueous layer was applied to a column of DEAE-cellulose (HCO_3^- form, 40 cm × 3 cm^2) which was washed with pH 7·5 triethylammonium bicarbonate buffer (0·01M) until the eluate showed negligible UV absorption. The column was then eluted with the same buffer (linear gradient from 0·01–0·15M over 2000 ml). 25 Ml fractions were collected: fractions 14–20 (140 O.D. units at 260 mμ) contained mainly unchanged starting material; fractions 38–44 (260 O.D. units at 260 mμ; ca. 65% based on unrecovered partially protected UpU) contained the desired product. These fractions (38–44) were combined, concentrated under reduced pressure (bath temperature <30°) and then heated with 9N aqueous NH_3 (10 ml) at 50° for 90 min.

The reaction soln, which was found by paper electrophoresis (phosphate buffer, pH 8) to contain a sole UV-absorbing component (with mobility 0·72× that of uridine 5′-phosphate), was concentrated (bath temp <30°) just to dryness and the residue dissolved in 0·01N HCl (2 ml) at 20°. After 5 hr, LiOH aq was added until pH 6, and the soln concentrated to ca. 1 ml. A soln of $CaCl_2$ (0·02 g) in EtOH (1 ml) was then added, followed by EtOH-Me_2CO (1:1, v/v; 20 ml). The precipitated *Ca salt of uridylyl-(3′→5′)-uridine 3′-phosphate* (0·015 g), which was collected by centrifugation, was washed first with EtOH-Me_2CO, then with ether, and finally dried *in vacuo*; this material had a paper electrophoretic mobility (phosphate buffer, pH 8) 1·03× that of uridine 5′-phosphate; R_F 0·20 (system A), 0·05 (system B).

When the Ca salt (0·001 g), in 0·05M tris hydrochloride buffer (pH 7·5) soln, was incubated with pancreatic ribonuclease for 1 hr at 37°, it was virtually quantitatively converted into one product. The latter was identified as uridine 3′-phosphate by paper electrophoresis (0·2M sodium acetate buffer, pH 4·6) and chromatography (R_F (system A) 0·35).

(b) *From 2′,5′-di-O-methoxytetrahydropyranyluridylyl-(3′→5′)-2′-O-methoxytetrahydropyranyluridine* (XII). The pyridinium salt of the partially-protected dinucleoside phosphate (0·033 mmole, obtained from 670 O.D. units of the above triethylammonium salt) was dried by evaporation from pyridine (4 × 10 ml) soln, and then redissolved in pyridine (4 ml). To this soln was added a soln of pyridinium β-cyanoethyl phosphate (0·16 mmole, from 0·053 g of Ba salt) in pyridine (4 ml). The combined solutions were concentrated almost to dryness, redissolved in pyridine (3 ml), and DCC (0·103 g, 0·5 mmole) and Dowex-50 (pyridinium form, 0·02 g) cation-exchange resin added.

The reactants were allowed to stand at 20° for 3 days with the exclusion of moisture, and then worked up as above. However, the DEAE-cellulose chromatography was omitted; the crude products were heated at 55° aqueous NH_3 (ca. 9N, 20 ml) for 90 min, and then concentrated under reduced press just to dryness. The residue was dissolved in HCl (pH 2, 5 ml), the soln allowed to stand at 20° for 6 hr, and then neutralized with 0·5N LiOH. The mixture of Li salts was then applied to a column of Dowex 1 × 2 (Cl^- form, 8 cm × 1 cm^2) anion-exchange resin. The column was washed with water and then eluted with HCl/LiCl (linear gradient from 0·001N HCl to 0·001N HCl + 0·2M LiCl over 2000 ml). 25 Ml fractions were collected: fractions 12–15 (46 O.D. units at 260 mμ) contained UpU; fractions 32–42 (352 O.D. units at 260 mμ; 52% based on nucleotidic starting material) contained the desired UpUp; fractions 65–75 contained an unidentified product.

Fractions 32–42 were combined, neutralized (to pH 6·5) with 0·5N LiOH, and concentrated under reduced press (bath temp <30°) just to dryness. The Ca salt of UpUp (0·025 g), which was isolated by the procedure described above, had paper electrophoretic and chromatographic properties identical to those of the material obtained previously.

This product underwent almost quantitative (>98%) digestion to uridine 3′-phosphate in the presence of pancreatic ribonuclease.

Uridylyl-(3′→5′)-uridylyl-(3′→5′)-uridine [UpUpU]

To a soln of the anhydrous pyridinium salts of partially-protected UpU (X) (ca. 0·038 mmole, obtained from 760 O.D. units of the above triethylammonium salt) and 2′,3′-O-methoxymethylideneuridine 5′-phosphate[36] (XIV) (ca. 0·057 mmole, from 0·024 g of diammonium salt) in pyridine (4 ml) was added DCC (0·072 g, 0·35 mmole) and Dowex-50 (pyridinium form, 0·02 g) cation-exchange resin. The reactants were stirred at 20° for 72 hr, with the exclusion of moisture, and then worked up as above.

The aqueous products were applied to a column of DEAE-cellulose (HCO_3^- form, 50 cm × 3 cm²), which was washed with pH 7·5 triethylammonium bicarbonate buffer (0·002M) until the eluate showed negligible UV absorption. The column was then eluted with the same buffer (linear gradient from 0·002–0·15M over 2000 ml). 25 Ml fractions were collected: fractions 8–18 (average buffer concentration 0·025M) contained mainly unchanged partially-protected UpU (X) (190 O.D. units at 260 mμ); fractions 41–45 (average buffer concentration 0·08M) contained 40 O.D. units of material; fractions 48–58 (average buffer concentration 0·10M) contained the desired fully protected UpUpU (400 O.D. units at 260 mμ; ca. 47% based on unrecovered dinucleoside phosphate starting material).

The latter fractions (48–58) were combined, concentrated under reduced pressure (bath temperature <30°) to small volume, and then freeze-dried to give a colourless solid. Paper electrophoresis (phosphate buffer, pH 8) of this material revealed a sole UV-absorbing component (mobility 0·43 × that of uridine 5′-phosphate); R_F 0·50 (system B).

A soln (3 ml) of the above solid triethylammonium salt (ca. 300 O.D. units) was treated with 0·1N HCl to pH 2, allowed to stand at 20° for 8 hr, and then neutralized (to pH 6·5) with LiOH aq. The *Ca salt of uridylyl-(3′→5′)-uridylyl-(3′→5′)-uridine* (0·014 g) was then isolated by the procedure described above; this material had a paper electrophoretic mobility (phosphate buffer, pH 8) 0·55× that of uridine 5′-phosphate; R_F 0·14 (system A), 0·18 (system C).

After the Ca salt (0·002 g) in 0·05M tris hydrochloride buffer (pH 7·5) soln had been incubated with pancreatic ribonuclease for 1 hr at 37°, paper electrophoresis (phosphate buffer, pH 8) and chromatography (systems A and C) indicated that it had been almost completely (ca. 98%) degraded to uridine 3′-phosphate and uridine. Trace amount of materials corresponding to UpUpU, UpUp and UpU were also detected.

2′,5′-Di-O-methoxytetrahydropyranyladenylyl-(3′→5′)-2′-O-methoxytetrahydropyranyladenosine (XVIII)

The NH_4^+ salt of 2′-O-methoxytetrahydropyranyl-3′-O-acetyladenosine 5′-phosphate (0·05 g, ca. 0·087 mmole) was converted into its pyridinium salt, which was dried by evaporation from pyridine soln (4 × 10 ml). To a solution of the latter and XVI[2] (0·052 g, 0·105 mmole) in anhyd pyridine (4 ml) at 20° was added DCC (0·18 g, 0·87 mmole) and Dowex-50 (pyridinium form, 0·01 g) cation-exchange resin. The stirred reactants were sealed to exclude moisture. After 24 hr, an additional quantity (0·021 g, 0·042 mmole) of XVI was added and the reaction allowed to continue for a further 72 hr before being worked up in the usual manner.

The aqueous soln of products, so obtained, was rendered anhydrous by repeated evaporations with pyridine. The resulting oil, which contained a small amount of pyridine, was treated with NH_3/MeOH (half-saturated at 0°) at 20°. After 16 hr, the products were concentrated under reduced press to dryness, redissolved in pH 7·5 triethylammonium bicarbonate buffer (0·002M, 5 ml), and the aqueous soln applied to a column of DEAE-cellulose (HCO_3^- form, 60 cm × 3 cm²). The column was washed with pH 7·5 triethylammonium bicarbonate buffer (0·002M) until the eluate showed negligible UV absorption; it was then eluted with the same buffer (linear gradient from 0·005–0·05M over 2000 ml, followed by linear gradient from 0·05–0·10M over 1000 ml). 25 Ml fractions were collected: fractions 21–28 (average buffer concentration ca. 0·02M) contained the desired partially-protected ApA (XVIII) (700 O.D. units at 260 mμ; ca. 40% based on unrecovered nucleotide starting material; fractions 34–36 (average buffer concentration ca. 0·025M) contained an unidentified product (72 O.D. units); fractions 60–70 (average buffer concentration ca. 0·04M) contained a material (140 O.D. units) with a paper electrophoretic

mobility (phosphate buffer, pH 8) corresponding to a partially-protected P^1, P^2-diadenosine 5'-pyrophosphate; fractions 80–90 (average buffer concentration ca. 0·055M) contained 2'-O-methoxytetrahydropyranyladenosine 5'phosphate (220 O.D. units).

Fractions 21–28 were combined, concentrated under reduced press (bath temp <30°) to small volume, and then freeze-dried to give the triethylammonium salt of XVIII as a colourless solid; UV absorption (water, pH 7): λ_{max} 258, λ_{min} 230 mμ; paper electrophoretic mobility (phosphate buffer, pH 8) 0·16 × that of adenosine 5'-phosphate; R_f 0·73 (system B), 0·58 (system C).

Adenylyl-(3'→5')-adenosine [ApA]

A soln (1 ml) of the above triethylammonium salt of XVIII (50 O.D. units) was treated with 0·1N HCl to pH 2, allowed to stand at 20° for 6 hr, and then neutralized with dil aqueous NH_3. The NH_4^+ salt of adenylyl-(3' → 5')-adenosine was obtained by lyophilization of the products; this material had a paper electrophoretic mobility (phosphate buffer, pH 8) 0·30× that of adenosine 5'-phosphate; it had R_f 0·54 (system A), 0·16 (system B).

The ApA, so obtained, underwent quantitative digestion in the presence *Crotalus adamanteus* snake venom phosphodiesterase to give adenosine 5'-phosphate and adenosine [identified by paper electrophoresis (phosphate buffer, pH 8) and chromatography (systems A and B)]; it was also virtually completely (ca. 98%) digested[14] to adenosine 3'-phosphate and adenosine in the presence of calf spleen phosphodiesterase (generously provided by Dr. G. Weimann, C. F. Boehringer & Sochne, Mannheim, Germany).

Attempted preparation of adenylyl-(3'→5')-adenosine 3'-phosphate [ApAp]

An anhydrous solution of pyridinium 2',5'-*di*-O-methoxytetrahydropyranyladenylyl-(3' → 5')-2'-O-methoxytetrahydropyranyladenosine (ca. 0·023 mmole, from 700 O.D. units of the above triethylammonium salt of XVIII), pyridinium β-cyanoethyl phosphate (0·058 mmole, from 0·019 g of the Ba salt) and DCC (0·047 g, 0·23 mmole) in pyridine (3 ml), containing Dowex-50 (pyridinium form, 0·01 g) cation-exchange resin, was allowed to stand at 20° for 3 days, and the products then worked up as above in preparation of UpUp.

The products were then examined by paper electrophoresis (phosphate buffer, pH 8), which revealed unchanged partially-protected ApA as the principal constituent (confirmed by paper chromatography (system B)). A small amount of material (ca. 10%) with the paper electrophoretic mobility expected for the desired product was detected.

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REFERENCES

[1] B. E. Griffin, M. Jarman and C. B. Reese, *Tetrahedron* **24**, 639 (1968).
[2] C. B. Reese, R. Saffhill and J. E. Sulston, *J. Am. Chem. Soc.* **89**, 3366 (1967).
[3] [a] M. Jarman and C. B. Reese, *Chem. & Ind.* 581 (1964);
[b] B. E. Griffin, M, Jarman, C. B. Reese and J. E. Sulston, *Tetrahedron* **23**, 2301 (1967).
[4] C. B. Reese and J. C. M. Stewart, *Tetrahedron Letters* 4273 (1968).
[5] H. G. Khorana, *Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest* p. 99. Wiley, New York (1961).
[6] J. E. Sulston, Ph.D. Thesis, Cambridge University, p. 85 (1966).
[7] G. M. Tener, *J. Am. Chem. Soc.* **83**, 159 (1961).
[8] R. Lohrmann and H. G. Khorana, *Ibid.* **88**, 829 (1966).
[9] For other procedures for the synthesis of dinucleotides terminating in 3'-phosphate residues see: J. Smrt and F. Sorm, *Coll. Czech. Chem. Comm.* **28**, 2415 (1963); D. Söll and H. G. Khorana, *J. Am. Chem. Soc.* **87**, 350, 360 (1965); H.-J. Rhaese, W. Siehr and F. Cramer, *Liebigs Ann. Chem.* **703**, 214 (1967); F. Cramer and G. Schneider, *Ibid.* **717**, 193 (1968).
[10] H. P. M. Fromageot, B. E. Griffin, C. B. Reese and J. E. Sulston, *Tetrahedron* **23**, 2315 (1967).
[11] A. Béhal, *Chem. Zentr.* **2**, 750 (1900).
[12] Y. Lapidot and H. G. Khorana, *J. Am. Chem. Soc.* **85**, 3852 (1963).
[13] D. H. Rammler and H. G. Khorana, *Ibid.* **84**, 3112 (1962).
[14] L. A. Heppel and R. J. Hilmoe in *Methods in Enzymology* Vol. 2; p. 565. Academic Press, New York (1965).