# SYNTHESIS OF ADENYLYL-(3′ → 5′)-URIDYLYL-(3′ → 5′)-URIDYLYL-(3′ → 5′)-CYTIDINE*

J. SMRT

*Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*

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The stability of the 2′-O-tetrahydropyranyl group on a diribonucleoside phosphate with a triester-protected internucleotidic bond has been examined under conditions when the 5′-O-dimethoxytrityl group is removed. The 2′-O-tetrahydropyranyl groups on ribooligonucleotides may be removed by the action of 1M-HCl in 7M urea. Condensation of 2′,3′-di-O-benzoyluridine with 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyluridine 3′-phosphate (*VIII*) in the presence of 2,3,5-triisopropylbenzenesulfonyl chloride affords a neutral phosphotriester, namely, 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyluridylyl-(3′ → 5′)-2′,3′-di-O-benzoyluridine[P-(2′,3′-di-O-benzoyluridine-5′)ester] (*VIa*). Adenylyl-(3′ → 5′)-uridylyl-(3′ → 5′)-uridylyl-(3′ → 5′)-cytidine was synthesised with the use of $N,O^{2'},O^{3'}$-tribenzoylcytidine (*VII*) as the nucleoside component and the phosphate *VIII* and 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyl-$N^6$-acetyladenosine 3′-phosphate (*IX*) as the prolongation units. In every step, the synthesised internucleotidic bond was protected by the 2-cyanoethyl group.

In an earlier paper of this series[1] there was discussed the protection of the $C_{(2')}$-hydroxylic function in derivatives of ribonucleoside 3′-phosphates for purposes of the triester synthesis. With the use of the 2-cyanoethyl group in protection of the internucleotidic bond and with the use of the dimethoxytrityl group in the temporary protection of the $C_{(5')}$-hydroxylic function on the growing chain, there has been taken into consideration (similarly to ref.[2]) the alkali-labile protection of the $C_{(2')}$-hydroxyl in the neighbourhood of the internucleotidic bond. Some experiments in this direction were however accompanied with rather serious difficulties consisting in the storage-instability of the starting 2′-O-acetylribonucleoside 3′-phosphates. Our attention was therefore directed to the stable 2′-O-tetrahydropyranyl derivatives[1].

In the present paper, there is examined the stability of the 2′-O-tetrahydropyranyl group in the neighbourhood of a neutral phosphotriester under those conditions when the dimethoxytrityl group is removed. The earlier reported[1] temperature of 0°C was safe with respect to the tetrahydropyranyl group, but the removal of the dimethoxytrityl group was considerably slow. It has been now found that this reaction is sufficiently selective when performed with 90% aqueous acetic acid at 20°C. At this

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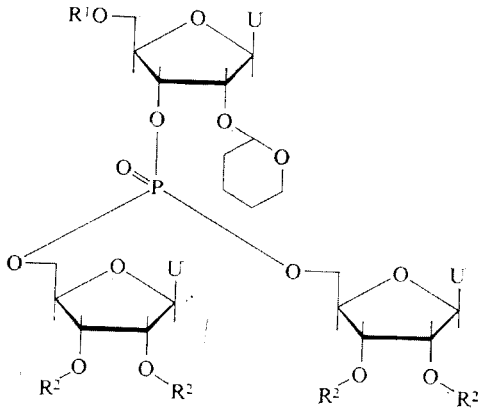
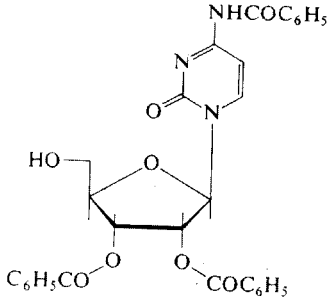
temperature, the dimethoxytrityl group of 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyluridylyl-(3′ → 5′)-2′,3′-di-O-benzoyluridine [P-(2-cyanoethyl) ester] (*I*) is lost during 1 h with the formation of compound *II*; the tetrahydropyranyl group remains intact, since compound *II* may be quantitatively transformed into 2′-O-tetrahydropyranyluridylyl-(3′ → 5′)-2′,3′-di-O-benzoyluridine (*III*) on a brief treatment with ammonia; no fission products of the internucleotidic bond were detected which would indicate the presence of the de-tetrahydropyranylated substance in compound *II*.

$$\underset{I}{\text{DMTr—U(Thp)p(CNEt)—UBz}_2} \longrightarrow \underset{II}{\text{HO—U(Thp)p(CNEt)—UBz}_2} \longrightarrow$$

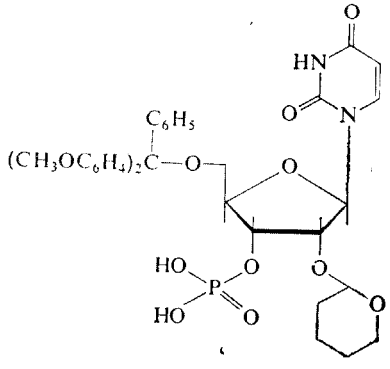
$$\longrightarrow \underset{III}{\text{HO—U(Thp)p—UBz}_2}$$



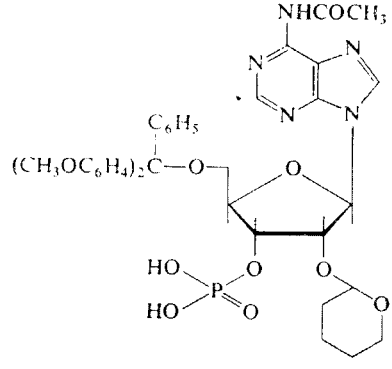
*VIa*, $R^1$ = Dimethoxytrityl, $R^2 = C_6H_5CO—$
*VIb*, $R^1$ = H, $R^2 = C_6H_5CO—$
*VIc*, $R^1 = R^2$ = H



*VII*



*VIII*



*IX*

When the treatment of compound *I* with 90% aqueous acetic acid is longer (more than 2 h), a trace amount of the 2′-O-tetrahydropyranyl group is split off. The resulting derivative with a free $C_{(2')}$-hydroxylic function undergoes intramolecular cyclisation, accompanied by removal of either the 2-cyanoethyl group or of 2′,3′-di-O-benzoyluridine, and the cyclic phosphotriester thus-obtained is opened with the formation of the phosphodiester. The acidity of the phosphodiester autocatalytically accelerates the removal of the tetrahydropyranyl group and additional above mentioned reactions. As the final products, there are obtained (after 20 h) uridylic acid, its 2-cyanoethyl ester, dibenzoyl derivatives of uridylyluridine (2′ → 5′ and 3′ → 5′), and 2′,3′-di-O-benzoyluridine.

A brief treatment with 90% aqueous acetic acid is thus satisfactorily selective when a dimethoxytrityl group has to be removed in the presence of a tetrahydropyranyl group from compounds with a phosphotriester internucleotidic bond. Consequently, the present procedure may be used in the synthesis of oligonucleotidic chains. The *cis*-diol system of the terminal nucleoside cannot be of course protected by the acidolabile ethoxymethylene group but the acidostable acyl groups must be applied. The products of the synthesis are deblocked by the action of ammonia which removes the 2-cyanoethyl group and then the alkali-labile protecting group from the *cis*-diol system and from the amino groups of the base with the formation of 2′-O-tetrahydropyranylated oligonucleotides.

The final removal of tetrahydropyranyl groups from ribooligonucleotides has been usually performed in this Laboratory by the action of 20% aqueous acetic acid at 50°C. This system shows fair solvent properties and the isomerisation of ribointernucleotidic bonds is lower than 1% as indicated by detailed investigations in the case of uridylyl-uridine[3]. As it has been observed by Griffin and coworkers[4], the tetrahydropyranyl groups may be removed from ribooligonucleotides by the action of aqueous 0·01M-HCl (pH about 2) without danger of isomerisation of the internucleotidic bond. In addition to acid media however, the substances from which the tetrahydropyranyl group is being removed, should be soluble in the reagent. Since some purine-containing oligonucleotidic derivatives[3] were not sufficiently soluble in aqueous 0·01M-HCl, another reagent of a wider applicability was looked after and discovered in 1M-HCl in 7M urea. Preliminary experiments with this reagent and 2′-O-tetrahydropyranyluridylyl-(3′ → 5′)-2′,3′-O-ethoxymethyleneuridine (*IV*) and 2′-O-tetrahydropyranyluridylyl-(3′ → 5′)-2′,3′-O-ethoxymethyleneadenosine (*V*) have shown that both the tetrahydropyranyl group and the ethoxymethylene group are quantitatively removed from compounds *IV* and *V* at 20°C in the course of 40 min. The internucleotidic bond was not cleaved even in traces and the resulting deblocked dinucleoside phosphates were then quantitatively degraded with pancreatic ribonuclease. The stability of the internucleotidic bond in uridylyl-(3′ → 5′)-uridine was lower in the case of aqueous hydrochloric acid of the corresponding pH value than in the HCl-urea reagent, probably because of a special stabilisation effect of 7M urea

on the *ribo*-internucleotidic bond. Thus, UpU was completely resistant towards 1M-HCl in 7M urea for 48 h, while 2% of UpU were degraded by the action of hydrochloric acid (pH 1·5) in this period of time. The nucleosidic bond of adenosine 5′-phosphate is absolutely stable towards the HCl-urea reagent. On the other hand, 62% of deoxyguanosine 5′-phosphate is degraded over 40 h by this reagent while the degradation of deoxyadenosine 5′-phosphate is quantitative. Nevertheless, the HCl-urea reagent is harmless in the ribo series and should make possible the removal of acidolabile groups even from substances of a high molecular weight.

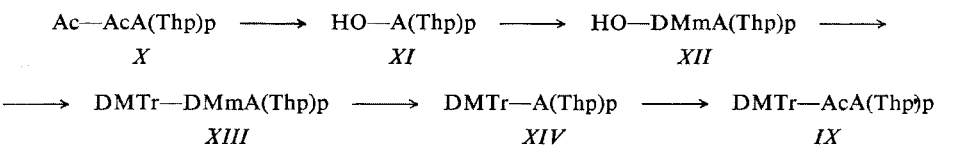
$$\text{U(Thp)p—UEm } (IV) \longrightarrow \text{UpU}$$

$$\text{U(Thp)p—AEm } (V) \longrightarrow \text{UpA}$$

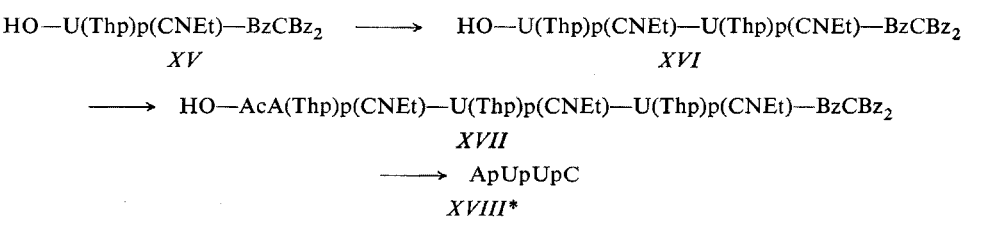
As suggested by the above observations, a combined synthesis with the use of 2′-O--tetrahydropyranyl derivatives could be advantageously applied in the praxis to the preparation of oligonucleotides. The present synthesis of adenylyl-(3′ → 5′)-uridylyl--(3′ → 5′)-uridylyl-(3′ → 5′)-cytidine (*XVIII*) may serve as an example.

The first step of the synthesis is performed from a protected ribonucleoside 3′-phosphate and a nucleoside derivative with a free $C_{(5')}$-hydroxylic function. Two equivalents of the hydroxylic component are usually applied. This ratio has also been used in the first paper on the combined synthesis[1]. Reexamination of the product obtained by condensation of 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyluridine 3′-phosphate (*VIII*) with 2 equivalents of 2′,3′-di-O-benzoyluridine in the presence of excess 2,3,5-triisopropylbenzenesulfonyl chloride and the subsequent treatment with 2-cyanoethanol shows that the seemingly homogeneous (on thin-layer chromatography) product affords two substances on treatment with ammonia. One of these substances is 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyluridylyl-(3′ → 5′)-2′,3′-di--O-benzoyluridine. The higher $R_F$ value (thin-layer chromatography in 8 : 2 chloroform–methanol) of the other substance suggested a neutral phosphotriester. As shown by additional experiments, this phosphotriester is formed in the first step of the synthesis as the by-product of the phosphodiester. Reaction (20 h) of 2 equivalents of 2′,3′-di-O-benzoyluridine with one equivalent of the phosphate *VIII* and 8 equivalents of 2,3,5-triisopropylbenzenesulfonyl chloride affords a dimethoxytrityl--containing product, chromatographically identical with the 2-cyanoethyl ester of the expected dinucleoside phosphate. As shown by elemental analysis of this substance and the product resulting after removal of the dimethoxytrityl group and the benzoyl group and as suggested by chromatographical and electrophoretical properties, the original product is a trinucleoside monophosphate derivative, namely, 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyluridylyl-(3′ → 5′)-2′,3′-di-O-benzoyl uridine [P-(2′,3′-di-O-benzoyluridine-5′) ester] (*VIa*). Compounds of this type are always formed when an excess of the hydroxylic component is used in the synthesis of the internucleotidic bond in the presence of a sulfonyl chloride which is capable

of activating the phosphodiester to undergo reaction with the hydroxyl-containing substance to afford a phosphotriester. When the ratio of reactants is reversed, *i.e.*, when an excess of the phosphate component is used, the above trinucleoside monophosphate is not formed. On the basis of these observations and considerations, we have now used an excess of the nucleotide component also in the first step of the oligonucleotidic chain synthesis.




In the synthesis of the oligonucleotide, the following components were used: 2′,3′-di-O-benzoyl-$N^4$-benzoylcytidine[5] (*VII*), 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyluridine 3′-phosphate[1] (*VIII*), and 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyl-$N^6$-acetyladenosine 3′-phosphate (*IX*). Compound *IX* was prepared from the calcium salt of 5′-O-acetyl-2′-O-tetrahydropyranyl-$N^6$-acetyladenosine 3′-phosphate[6] (*X*) by a sequence of reactions including removal of the two acetyl groups by the action of ammonia with the formation of 2′-O-tetrahydropyranyladenosine 3′-phosphate (*XI*), conversion of compound *XI* into the $N^6$-dimethylaminomethylene derivative *XII* by the action of dimethylformamide dimethylacetal, replacement of the dimethylaminomethylene group in the 5′-O-dimethoxytrityl derivative *XIII* by the acetyl group, and isolation of the final product *IX* in the form of a triethylammonium salt by extraction into chlorofom and precipitation from ether.




The tetranucleotide *XVIII* was prepared by a stepwise combined synthesis with the use of repetitions of the standard procedures. In every step, the hydroxylic component was condensed with an excess of the phosphate component by the action of 2,3,5-triisopropylbenzenesulfonyl chloride and the resulting diester internucleotidic

* Abbreviations in this and the preceding schemes correspond to recommendations of the IUPAC—IUB committee for the biochemical nomenclature. In a ribonucleotide unit X—B(Y)p(Z), the symbol X is a substituent at position 5′, Y (in parentheses) is a substituent at position 2′, and Z (in parentheses) is placed on the phosphate residue.

bond was converted *in situ* by the action of 2-cyanoethanol into the neutral triester. The dimethoxytrityl group-containing intermediates were isolated by preparative chromatography on a loose layer of silica gel. The products were identified by means of the colour test with perchloric acid. The eluates were evaporated and the residues purified by precipitation from chloroform with ether to remove excess 2-cyanoethanol and the bis(2-cyanoethyl) esters of the nucleotides used in the condensation. The dimethoxytrityl group was removed by the action of 90% aqueous acetic acid. The $C_{(5')}$-hydroxyl-containing intermediates (*XV*, 65%; *XVI*, 74%) were isolated by precipitation with ether and used in the next condensation. As determined by deblocking experiments, compound *XVI* contained 12% of the unreacted dinucleotide fraction[7]. It was not possible to separate the product from the one-step shorter starting material by means of a simple thin-layer chromatography. On the other hand, the column chromatography on DEAE-cellulose (as used for the final purification of the tetranucleotidic product *XVII*) was successful in separation of shorter chains from the tetranucleotide *XVIII*.

The thus-obtained tetranucleotide *XVIII* was characterised by the pancreatic ribonuclease degradation affording quantitatively cytidine, uridine 3'-phosphate and a dinucleotide which was identified as adenylyl(-3' → 5')-uridine 3'-phosphate by means of the bacterial phosphatase degradation.

## EXPERIMENTAL

Paper chromatography was performed by the descending technique on Paper Whatman No 1. Thin-layer chromatography was carried out on ready-for-use Silufol $UV_{254}$ (Kavalier Glassworks, Votice, Czechoslovakia) silica gel foils. Preparative layer chromatography was performed on 18 × 32 × 0·6 cm layers of loose fluorescent indicator-containing silica gel (produced by Service Laboratories of this Institute, Prague - Suchdol). Solvent systems: $S_1$, chloroform–pyridine (9 : 1); $S_2$, 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2); $S_3$, chloroform–methanol (7 : 3); $S_4$, chloroform–pyridine–methanol (90 : 5 : 5); $S_5$, chloroform–methanol (9 : 1); $S_6$, chloroform–pyridine–methanol (8 : 1 : 1). Unless stated otherwise, the $R_F$ values refer to thin-layer chromatography. The preparative layers were eluted with the solvent system $S_E$, chloroform–methanol (1 : 1), unless stated otherwise. Electrophoresis was performed on paper Whatman No 1 dipped in tetrachloromethane, in 0·05M triethylammonium hydrogen carbonate (pH 7·5). Solutions were taken down on a rotatory evaporator equipped with a Dry Ice condenser, unless stated otherwise. In evaporations of the condensation mixtures with pyridine, the atmospheric pressure was restored through a Blue Gel column.

### Reaction of DMTr—U(Thp)p(CNEt)—$UBz_2$ (*I*) with 90% Acetic Acid

A solution of compound *I* (2 mg) in 90% aqueous acetic acid (0·05 ml) was stored at 20°C. Samples were withdrawn in 30 min intervals and chromatographed on Silufol in the solvent system $S_5$ as well as on paper (in $S_2$). In both systems, the dimethoxytrityl group was quantitatively removed after 1 h with the formation of a homogeneous product. $R_F$ values: 0·30 (Silufol in $S_5$; compound *II*); 0·77 (paper in $S_2$; compound *III*).

Reaction of HO—U(Thp)p—UEm (*IV*) and HO—U(Thp)p—AEm (*V*) with 1M-HCl in 7M Urea

Solutions of compounds *IV* and *V* (2 mg each) in the reagent (0·1 ml) were stored at 20°C. Samples were withdrawn in 10 min intervals and chromatographed on paper in the solvent system $S_2$. In the course of 40 min, both the compounds afforded quantitatively the corresponding dinucleoside phosphates, the degradation of which with pancreatic ribonuclease was again quantitative.

Stability of UpU in (*a*) 1M-HCl in 7M Urea, (*b*) Aqueous Hydrochloric Acid of the Same pH Value (1·5)

The ammonium salt of UpU (1·5 mg) was dissolved in reagents *a* or *b* (0·05 ml each) and the resulting solutions were kept at 20°C. Samples were withdrawn after 24 and 48 h and chromatographed in $S_2$ (paper). In reagent *a* no degradation of UpU has been observed after 48 h as indicated by spectrophotometry or viewing. In reagent *b*, 1% (after 24 h) and 2% (after 48 h) of the internucleotidic bond was cleaved with the formation of Up and U.

Treatment of Adenosine 5′-Phosphate, Deoxyadenosine 5′-Phosphate, and Deoxyguanosine 5′-Phosphate with 1M-HCl in 7M Urea

Solutions of the title compounds (1·0—1·5 mg) in the title reagent (0·05 ml) were kept at room temperature for 48 h and chromatographed in $S_2$ (paper). Adenosine 5′-phosphate was recovered without any change; deoxyguanosine 5′-phosphate afforded 62% of guanine; and deoxyadenosine 5′-phosphate was quantitatively converted into adenine.

5′-O-Dimethoxytrityl-2′-O-tetrahydropyranyluridylyl-(3′ → 5′)-2′,3′-di-O-benzoyluridine [P-(2′,3′-Di-O-benzoyluridine-5′) Ester] (*VIa*)

A mixture of the triethylammonium salt of 5′-O-dimethoxytrityl-2′-O-tetrahydropyranyluridine 3′-phosphate (*VIII*; 1·6 g; 2 mmol) and 2′,3′-di-O-benzoyluridine (1·8 g; 4 mmol) is coevaporated with two portions of pyridine, the residue is treated with 2,3,5-triisopropylbenzenesulfonyl chloride (2·4 g; 8 mmol) and pyridine (20 ml), the whole mixture is shaken for several minutes, and evaporated to the consistence of a sirup. After 20 h, the sirup is diluted with chloroform (3 ml) and chromatographed on two layers of silica gel in the solvent system $S_1$. The dimethoxytrityl--positive bands ($R_F$ 0·75) are eluted with acetone, and the eluate is evaporated first at 15°C/15 Torr and then at 1 Torr. The residue is coevaporated with toluene, dissolved in chloroform (3 ml), and the solution is precipitated with ether (150 ml). This solid is collected with suction, washed with 1 : 9 chloroform–ether and ether, and dried under diminished pressure to afford 826 mg (26%) of compound *VIa*, $R_F$ value 0·55 (in $S_5$). For $C_{81}H_{75}N_6O_{26}P$ (1 579) calculated: 61·50% C, 4·75% H, 5·32% N, 1·98% P; found: 61·11% C, 4·88% H, 5·35% N, 1·53% P.

2′-O-Tetrahydropyranyluridylyl-(3′ → 5′)-2′,3′-di-O-benzoyluridine [P-(2′,3′-Di-O-benzoyluridine-5′) Ester] (*VIb*)

A solution of compound *VIa* (580 mg) in 90% aqueous acetic acid is kept at room temperature for 80 min and evaporated under diminished pressure. The residue is coevaporated with 1-butanol, dissolved in chloroform (5 ml), and the solution precipitated with ether (95 ml). The solid is collected with suction, washed with ether, and dried under diminished pressure to afford 417 mg

of compound *VIb*, $R_F$ value 0·20 (in $S_5$). For $C_{60}H_{57}N_6O_{24}P$ (1277) calculated: 56·55% C, 4·47% H, 6·58% N, 2·43% P; found: 56·35% C, 4·61% H, 6·22% N, 1·98% P.

*Degradation.* A solution of compound *VIb* (120 mg) in 4M methanolic ammonia was heated at 50°C for 2 h and evaporated to afford a mixture of the benzamide and an UV-absorbing substance, $R_F$ value 0·25 (in $S_2$, paper) and 0·23; $E_{Up}$ value 0·10. On treatment with 20% aqueous acetic acid (50°C, 30 min), the substance affords a mixture of uridylyl-uridine and uridine while a mixture of uridylic acid and uridine (1 : 1·96) is obtained by the action of 1M-HCl (50°C, 1 h). Consequently, the reaction product of *VIb* and ammonia is 2′-O-tetrahydropyranyluridylyl-(3′→5′)-uridine[P-(uridine-5′) ester] (*VIc*).

2′-O-Tetrahydropyranyluridylyl-(3′→5′)-2′,3′-di-O-benzoyl-$N^4$-benzoylcytidine [P-(2-Cyanoethyl)Ester] (*XV*)

A solution of the triethylammonium salt of compound *VIII* (2·9 g; 3 mmol) and $O^{2'},O^{3'}$,N-tribenzoylcytidine (*VII*; 833 mg; 1·5 mmol) in pyridine (20 ml) is evaporated and to the residue there is added 2,3,5-triisopropylbenzenesulfonyl chloride (3·0 g) and pyridine (30 ml). The whole mixture is shaken for 5 min, evaporated, and the residual sirup kept at room temperature for 6 h. 2-Cyanoethanol (1·6 ml) is added, the mixture kept for 20 h, diluted with chloroform (5 ml), and chromatographed on 3 layers (18 × 35 × 0·6 cm) of silica gel in the solvent system $S_4$. The dimethoxytrityl-positive bands (19—30 cm) are eluted with $S_E$, the eluate is evaporated, the residue coevaporated with toluene (10 ml), and the final residue triturated with ether (150 ml). The solid is collected with suction, washed with ether, and dried under diminished pressure. Yield, 2·45 g of the crude dimethoxytrityl derivative, $R_F$ value 0·80 (in $S_5$). This derivative is dissolved in 90% aqueous acetic acid (50 ml), the solution kept at 20°C for 90 min, evaporated, the residue coevaporated with two 10 ml portions of 1-butanol, and the final residue dissolved in chloroform (5 ml). The solution is precipitated with ether (100 ml), the solid collected with suction, and dried under diminished pressure to afford 975 mg (65%) of compound *XV*. For $C_{47}H_{47}N_6O_{17}P$ (998·7) calculated: 8·41% N, 3·11% P; found: 8·32% N, 2·92% P. $R_F$ value: 0·40 (in $S_5$).

5′-O-Dimethoxytrityl-2′-O-tetrahydropyranyl-$N^6$-acetyladenosine 3′-Phosphate (*IX*) Triethylammonium Salt

A suspension containing the calcium salt of 5′-O-acetyl-2′-O-tetrahydropyranyl-$N^6$-acetyladenosine 3′-phosphate[6] (15 g), Dowex 50 (ammonium cycle) ion exchange resin (60 ml), pyridine (60 ml), and conc. aqueous ammonia (60 ml) is stirred at room temperature for 20 h, diluted with pyridine (100 ml), and evaporated at 35°C/15 Torr until the pH value is 7·0—7·5. Dowex 50 (pyridinium cycle) ion exchange resin (200 ml) is then added to the residue, the mixture stirred for 10 min, and filtered through a layer of Dowex 50 (pyridinium cycle) resin (400 ml). The resin is washed with 50% aqueous pyridine (500 ml). The filtrate and washings are combined, diluted with dimethylformamide (100 ml), and evaporated at 20°C/1 Torr, a mixture of pyridine and dimethylformamide (9 : 1) being occasionally added. The resulting sirup is coevaporated with five 100 ml portions of pyridine. The final residue is diluted with dimethylformamide (50 ml) and treated with dimethylformamide dimethylacetal (30 ml). The whole mixture is kept at room temperature overnight, diluted with pyridine (100 ml), concentrated under diminished pressure to about half of the original volume, and the concentrate is treated with pyridine (100 ml) and dimethoxytrityl chloride (18 g). The mixture is kept at room temperature for 20 h and then ethanol (50 ml) and triethylamine (20 ml) are added with cooling (tap water). After 10 min, the mixture is diluted with water (100 ml), kept at room temperature for 5 h, evaporated, the residue coevapor-

ated with three portions of pyridine, and the final residue dissolved in a mixture of pyridine (100 ml) and acetic anhydride (80 ml). After 20 h, the solution is evaporated, the residue dissolved in 50% aqueous pyridine and, after additional 2 h, extracted with chloroform (250 ml). The aqueous layer is diluted with pyridine and extracted with chloroform (250 ml) again. The chloroform extracts are combined, dried over anhydrous magnesium sulfate, filtered through a layer of Cellite, and the filtrate evaporated at 35°C/15 Torr to remove chloroform. The residual pyridine solution is concentrated at 1 Torr to the volume of about 100 ml and the concentrate is added dropwise into a mixture of ether (2000 ml) and triethylamine (10 ml). The precipitate is collected with suction, washed with ether, and dried over phosphorus pentoxide under diminished pressure. The solid is then dissolved in pyridine (100 ml) and the solution is added dropwise with stirring into ether (2000 ml). The precipitate is collected with suction, washed with ether, and dried over phosphorus pentoxide under diminished pressure to afford 15·5 g of the triethylammonium salt of compound *IX*. For $C_{38}H_{42}N_5O_{11}P.C_6H_{15}N$ (876·9) calculated: 9·57% N, 3·54% P; found: 9·11% N, 3·22% P. Molecular weight as calculated from the phosphorus content, 965. $R_F$ value: 0·48 (in $S_2$).

2′-O-Tetrahydropyranyluridylyl-(3′→5′)-2′-O-tetrahydropyranyluridylyl-(3′→5′)-2′,3′-di-O-benzoyl-$N^4$-benzoylcytidine [$P^1$,$P^2$-bis(2-Cyanoethyl) Ester] (*XVI*)

A solution of compound *XV* (1·4 g; 1·4 mmol) and the triethylammonium salt of the phosphate *II* (2·7 g) in pyridine (20 ml) is evaporated to the consistence of a sirup which is dissolved in the same amount of pyridine and evaporated again. The final residue is then shaken for several hours with 2,3,5-triisopropylbenzenesulfonyl chloride (1·35 g) and pyridine (20 ml) and the mixture is evaporated to incipient crystallisation. After 20 h, another portion of 2,3,5-triisopropylbenzenesulfonyl chloride (2·25 g) and pyridine (20 ml) is added, the whole mixture shaken for 10 min, concentrated to incipient crystallisation, and the concentrate treated with 2-cyanoethanol (1·6 ml). After additional 20 h, the mixture is diluted with chloroform and chromatographed on 3 layers of silica gel in the solvent system $S_4$. The dimethoxytrityl-positive bands (20—31 cm) are eluted with $S_E$, the eluate evaporated first at 15 Torr, finally at 1 Torr, and the residue coevaporated with toluene. The final residue is dissolved in chloroform (3 ml) and the solution is precipitated with ether (100 ml). After 2 h at 0°C, the precipitate is collected with suction, and washed with ether. Drying under diminished pressure affords 2·2 g of the dimethoxytrityl derivative of compound *X* which is dissolved in 90% aqueous acetic acid (50 ml). The solution is kept at room temperature for 90 min, evaporated, the residue coevaporated with two portions of 1-butanol, the final residue dissolved in chloroform (5 ml), and the solution precipitated with ether (150 ml). After 2 h at 0°C, the precipitate is collected with suction, washed with ether, and dried under diminished pressure. Yield, 1·5 g (74%) of compound *XVII*, $R_F$ value 0·29 (in $S_5$). Successive treatment of compound *XVII* with methanol–conc. aqueous ammonia (20 h) and 1M hydrochloric acid in 7M urea (2 h) affords UpUpC (82%) and UpC (12%).

Adenylyl-(3′→5′)-uridylyl-(3′→5′)-uridylyl-(3′→5′)-cytidine (*XVIII*)

A solution of compound *XVI* (150 mg; about 0·1 mmol) and the triethylammonium salt of the phosphate *IX* (290 mg) in pyridine (10 ml) is evaporated, the residue dissolved in the same amount of pyridine, and the solution evaporated again. 2,3,5-Triisopropylbenzenesulfonyl chloride (180 mg) and pyridine (3 ml) are then added, the mixture shaken for several minutes, and concentrated to a sirup. After 20 h at room temperature, the sirup is treated with additional 2,3,5-triisopropylbenzenesulfonyl chloride (270 mg) and pyridine (5 ml), the whole shaken, and the resulting solution concentrated under diminished pressure. 2-Cyanoethanol is added to the con-

centrate, the mixture kept at room temperature for 20 h, diluted with chloroform (2 ml), and chromatographed on one layer of silica gel in the solvent system $S_6$. The dimethoxytrityl-positive band (23—30 cm) is eluted with the solvent system $S_E$, the eluate evaporated (first at 15 Torr, then at 1 Torr), the residue coevaporated with toluene (10 ml), and finally dissolved in 90% acetic acid (10 ml). The solution is kept at room temperature for 90 min, evaporated, the residue coevaporated with 1-butanol, dissolved in chloroform (1 ml), and the solution precipitated with ether (20 ml). After 3 h at 0°C, the precipitate is collected with suction, washed with ether, and dried under diminished pressure. The thus-obtained crude compound *XVII* (170 mg) is dissolved in pyridine (3 ml), the solution treated with conc. aqueous ammonia (3 ml), the whole kept at room temperature for 20 h, and evaporated. The residue is coevaporated with toluene and the final residue is dissolved in 1M hydrochloric acid in 7M urea (5 ml). After 2 h, triethylamine (0·7 ml) is added and the whole is applied to a column (800 ml) of DEAE-cellulose (bicarbonate). The column is washed with water (800 ml) and then eluted with a linear gradient of triethylammonium hydrogen carbonate (pH 7·5) using 3 l of water in the mixing chamber and 3 l of 0·5M buffer solution in the reservoir. There are obtained three UV-absorbing peaks. The first peak (eluted by 0·15M buffer at the top of the column) contains dinucleoside phosphates ($A_{260}$ 320), the second one (0·28M) contains trinucleoside diphosphates ($A_{260}$ 1 200), and the third one (0·4M) corresponds to compound *XVIII* ($A_{260}$ 1 130; 27%). The product-containing fractions were evaporated, the residue coevaporated with five portions of ethanol, the final residue taken up into a little water, and kept at −10°C. $R_{Up}$ value: 0·40 (in $S_2$; paper); $E_{Up}$: 0·69.

*Enzymatic degradation.* An aqueous solution of compound *XVIII* (an aliquot corresponding to $20A_{260}$) was adjusted to the concentration of 0·05M by the addition of 0·5M-Tris-HCl (pH 8) and treated with a solution of pancreatic ribonuclease (5 µl; 5 mg in 1 ml). The whole mixture was incubated at 37°C for 3 h and then chromatographed on paper Whatman No 1 in the solvent system $S_2$ for 40 h to show a quantitative disappearance of compound *XVIII* with the formation of cytidine, uridine 3′-phosphate, and a substance of the $R_{Up}$ value equal to 0·65. The bacterial alkaline phosphatase degradation (Tris-HCl, pH 9·5) of the last mentioned substance afforded ApU which was identified on comparison with an authentic sample.

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