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SYNTHESIS AND PROPERTIES OF 3′-*C*-METHYLNUCLEOSIDES AND THEIR PHOSPHORIC ESTERS

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ABSTRACT

3′-*C*-Methyluridine and 3′-*C*-methylcytidine were synthesized in 11 steps starting from D-glucose. The conformation of 3′-*C*-methylnucleosides was studied in solution and in the crystal by using the techniques of c.d., ^{1}H-n.m.r. spectroscopy, and X-ray diffraction analysis. 3′-*C*-Methyluridine 2′,3′-cyclophosphate was prepared, and its hydrolysis with nucleases was studied. 3′-*C*-Methyluridine 5′-mono- and 5′-tri-phosphate were also synthesized.

INTRODUCTION

A traditional approach in preparing the analogs of nucleosides and nucleotides is to replace, or remove, functional groups. For example, hydroxyl groups in the carbohydrate moiety of a molecule are replaced by mercapto or amino groups, the naturally occurring heterocyclic bases are substituted, the *N*-glycosylic C–N bond is replaced by a C–C bond, *etc.* Although this approach has its merits, our purpose was to synthesize "complete" analogs, preserving all of the functionalities of the naturally occurring compounds in order to acquire insight into mechanisms of specific recognition of nucleotides by enzymes.

Such analogs ought to meet the following requirements: all of the functional groups, the configurations of the atoms to which they are attached, and the distances between them must remain unchanged.

Obviously, these compounds can be prepared if the hydrogen atom of a C–H group of a ribofuranosyl group or a heterocyclic base is replaced by an inert substituent, such as an alkyl group. Certain nucleoside analogs containing, on the ribosyl group, *C*-alkyl substituents, instead of the corresponding hydrogen atoms, at C-5 (refs. 1–13), C-4 (ref. 14), C-3 (refs. 15–18), C-2 (refs. 16, 19, and 20), and C-1 (refs. 21 and 22), or of those of the aglycon at C-8 and C-2 in a purine base (see, for instance, refs. 23–32), or at C-5 and C-6 in a pyrimidine base (see, for example, refs. 32–36), were synthesized earlier. However, the corresponding nucleotides were reported only in certain cases[5,8–13,27–31,36], and the properties of these compounds in enzyme-catalyzed reactions remains obscure[6,12,13,28].

RESULTS AND DISCUSSION

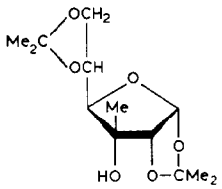
The present work is a sequel to earlier studies on the synthesis of "complete" analogs of nucleosides, nucleotides, and oligonucleotides[7-9,11,17,18,37,38]; it deals with the synthesis of 3′-*C*-methylnucleosides and their phosphoric esters. The scheme employed for the synthesis of 3′-*C*-ethyl(or butyl)uridines[17] is here extended to the synthesis of 3′-*C*-methylpyrimidine nucleosides. It is noteworthy that the compounds contain three hydroxyl groups (the primary 5′, secondary 2′, and tertiary 3′) which differ in their reactivity.

1,2:5,6-Di-*O*-isopropylidene-3-*C*-methyl-α-D-allofuranose (**1**) was used as the starting compound; it was synthesized, starting from D-glucose, in three steps by using the conventional procedures[17,39]. Selective elimination of the 5,6-*O*-isopropylidene group with 75% acetic acid yielded the dioxolane[39] **2**, whose oxidation with $NaIO_4$, followed by reduction of the product with $NaBH_4$, afforded 1,2-*O*-isopropylidene-3-*C*-methyl-α-D-ribofuranose (**3**) in high yield.

Selective benzoylation of **3** with a small excess of benzoyl chloride in pyridine produced the 5-benzoate **4**. Walton and co-workers[15] had reported the synthesis of 1,2-*O*-isopropylidene-3-*C*-methyl-5-*O*-benzoyl-α-D-ribofuranose (**4**) in five steps starting from D-xylose, and they described[15] its conversion into crude 2,3,5-tri-*O*-benzoyl-3-*C*-methyl-D-ribofuranosyl bromide in three steps, with an overall yield of 74%. The latter compound was used to synthesize 4-*O*-methyl-1-(2,3,5-tri-*O*-benzoyl-3-*C*-methyl-β-D-ribofuranosyl)uracil and 3′-*C*-methylcytidine by the Hilbert–Johnson procedure[16].

With the new glycosylation techniques now available[40,41], we decided to simplify the foregoing scheme, as it included too many steps. In order to find an optimal method, we examined possible transformations of benzoate **4** into fully acylated 3′-*C*-methyl-D-ribofuranose (**7**), a compound that had been synthesized in 73% yield[42] by acetolysis of benzoate **4** at 0°. However, in an attempt to reproduce this procedure, a complicated mixture of products was obtained. Preliminary blocking of the tertiary hydroxyl group by acetylation in the presence of 4-(dimethylamino)pyridine[43] gave acetate **5** in quantitative yield; acetolysis of **5** afforded derivative **7** in 80% yield. Other procedures for the synthesis of compound **7** were also tested. Acid hydrolysis of **4** with 90% trifluoroacetic acid for 20 min at 20° produced benzoate **6**; without isolation, this was acetylated either with acetic anhydride–pyridine in the presence of 4-(dimethylamino)pyridine, or first with acetic anhydride–pyridine (the tertiary hydroxyl group not being acetylated under these conditions[17]) and then with acetic anhydride in the presence of *p*-toluenesulfonic acid[17], producing acetate **7** in 66 and 85% yield, respectively.

Glycosylation of bis(trimethylsilyl)uracil with acetate **7** in the presence of tin tetrachloride in acetonitrile resulted in the protected nucleoside **8** in 80% yield and the corresponding N-3 isomer (**11**) in 7% yield. The cytosine nucleoside **13** was synthesized similarly. Deblocking of compounds **8**, **11**, and **13** with methanolic ammonia gave nucleosides **9**, **12**, and **14** in high yields. The u.v. spectra of these



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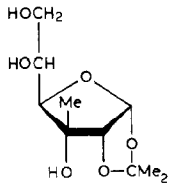

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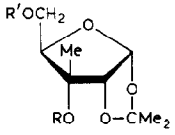
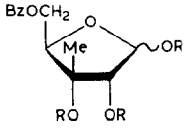


3 R = R′ = H
4 R = H, R′ = Bz
5 R = Ac, R′ = Bz



6 R = H
7 R = Ac

nucleosides are identical with the respective spectra of the D-ribofuranosyl-uracil and -cytosine derivatives[44], which confirms the site of glycosylation. A positive Cotton effect at 260 to 280 nm (B_{2u}) in the c.d. spectra is characteristic of β-nucleosides[45,46] (see Fig. 1). A large coupling-constant in the p.m.r. spectra ($J_{1',2'}$ 7.5 to 8.0 Hz) is typical of 3′-*C*-alkylnucleosides having the β configuration[15–17].

It appeared to be of interest to compare the conformational states of the resultant 3′-*C*-methylnucleosides (**9** and **14**) in solution with those of the naturally occurring compounds. It is now generally considered that *syn-anti* conformational equilibria around the glycosylic bond for pyrimidine nucleosides in solution is shifted towards *anti* states[47]. The analogs of nucleosides having a substituent at C-6 are mainly in the *syn* orientation[47]. Table I presents the coupling constants for uridine and cytidine taken from Davies' review[47], as well as the averaged constants for pyrimidine nucleotides which are mostly in *anti* and *syn* orientations, and for compounds **9** and **14**.

The conformation of the D-ribosyl group in 3′-*C*-methylnucleosides was analyzed in terms of the pseudorotation concept now generally accepted. Firstly, we proposed that introduction of a methyl group at C-3′ would not significantly affect the accuracy of calculating the ratio of "*S*" and "*N*" (South and North) conformers within the framework of this concept[47].

The $J_{1',2'}$ value for the 3′-*C*-methyl nucleosides **9** and **14** is 7.5–7.8 Hz (see Table I). In calculating the ratio of *S*:*N* conformers in the *ribo* series, the $J_{1',2'}$ value for the *S* conformer is assumed to be 9–10 Hz. We used the lower value, and found the fraction of the *S* conformer to be 83–87% for 3′-*C*-Me-pyrimidine nucleosides (see Table I). However, by analyzing molecular models of the respective nucleosides, we arrived at the conclusion that the *N* conformational state of the

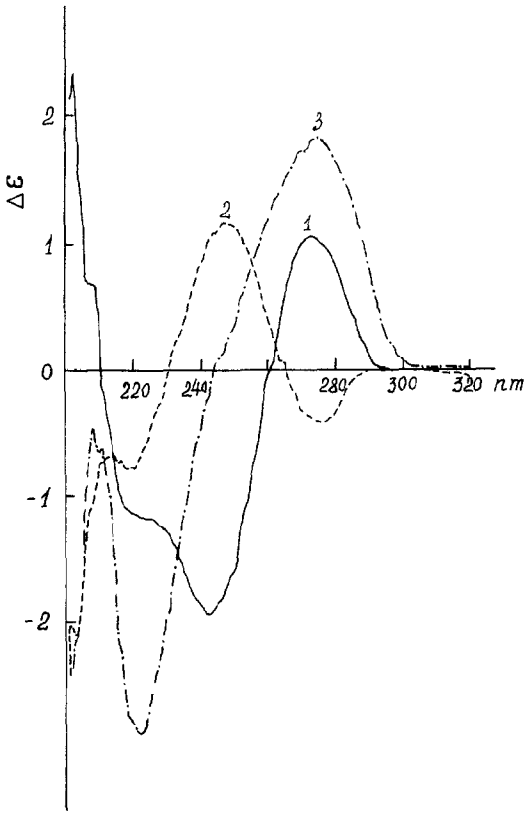
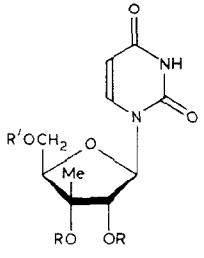
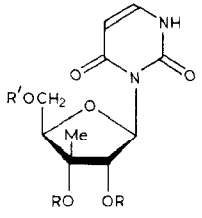


Fig. 1. Experimental c.d. spectra in water at 20°: 1, 3′-*C*-methyluridine (**9**); 2, 3-(3-*C*-methyl-β-D-ribofuranosyl)uracil(**12**); 3, 3′-*C*-methylcytidine (**14**).

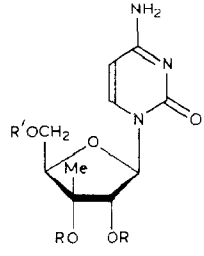
3-*C*-methylribofuranosyl ring should be destabilized due to steric repulsion between exocyclic groups. It may be seen that the 3-C-methyl group and the exocyclic 4-CH_2OH group lose freedom of rotation around the C-3–C-3[1] and C-4–C-5 bonds, respectively, in this conformational state. This gives rise to a decrease in the en-



8 R = Ac, R′ = Bz
9 R = R′ = H
10 R = H, R′ = Bz



11 R = Ac, R′ = Bz
12 R = R′ = H



13 R = Ac, R′ = Bz
14 R = R′ = H

TABLE I

SPIN–SPIN COUPLING-CONSTANTS (Hz), AND CALCULATED CONFORMATIONS OF PYRIMIDINE NUCLEOSIDES

Nucleosides	$J_{1',2'}$	$J_{4',5'a}$	$J_{4',5'b}$	$J_{4',5'a} + J_{4',5'b}$	S_X[a]	P_+[b]	P_a	P_-
Uridine[47]	4.8	2.9	4.4	7.3	0.53	0.63	0.25	0.12
Cytidine[47]	4.0	2.8	4.3	7.1	0.44	0.64	0.25	0.11
Pyrimidine nucleoside in the *anti* orientation[c]	4.0	2.9	4.4	7.3	0.44	0.63	0.25	0.12
Pyrimidine nucleoside in the *syn* orientation[c]	4.0	3.5	6.0	9.5	0.44	0.45	0.39	0.16
3′-*C*-Methyluridine (**9**)	7.8	3.8	4.9	8.7	0.87	0.53	0.26	0.21
3′-*C*-Methylcytidine (**14**)	7.5	3.6	4.9	8.5	0.83	0.54	0.27	0.19

[a] S_X is the mole fraction of the *S* conformer, determined from the equations[47] $J_{1',2'} = 9.0\,S_X$ and $1 = S_X + N_X$. [b] P_+, P_a, and P_- are the mole fractions of the *gauche-gauche*, *gauche-trans*, and *trans-gauche* rotamers, calculated by using the equations[47]: $J_{4',5'a} = 1.3\,P_+ + 2.7\,P_a + 11.7\,P_-$; $J_{4',5'b} = 1.3\,P_+ + 11.5\,P_a + 5.8\,P_-$; $1 = P_+ + P_a + P_-$. [c] Averaged data for the pyrimidine nucleosides, which are mainly in the *anti* or the *syn* conformation[47].

tropy, and the energy of the N conformer would therefore increase[48] by 13 to 21 kJ/mol (3 to 5 kcal/mol) at 25°. Therefore, it is reasonable to assume that 3′-methylnucleosides exist almost entirely in the *S* state.

As may be seen from Table I, the $J_{1',2'}$ value characterizing the $S \rightleftharpoons N$ equilibrium is much greater for 3′-*C*-methylnucleosides than for uridine and cytidine. The presence of the 3′-*C*-methyl group increases the $J_{1',2'}$ value, which is caused by an increase of the *S* population. However, this modification hardly changes the orientation around the C-4′–C-5′ bond, as follows from comparing the values of $J_{4',5'a}$ and $J_{4',5'b}$. The mean values of $J_{4',5'a}$ and $J_{4',5'b}$ for the pyrimidine nucleosides having the *syn* orientation are greater than those for the nucleosides in the *anti* orientation by 0.6 and 1.6 Hz, respectively. This reflects the decrease in the population of the *gauche-gauche* rotamer. In the case of 3′-*C*-methylnucleosides, the sum of $J_{4',5'a}$ and $J_{4',5'b}$ (8.5–8.7 Hz) is intermediate between the sums of these constants for nucleosides that are mainly in the *anti* or the *syn* orientation (7.3 and 9.5 Hz). Probably, the proportion of the *syn* population in 3′-*C*-methylnucleosides is higher compared to that for the naturally occurring nucleosides.

The c.d. spectra of the 3′-*C*-methylnucleosides **9**, **12**, and **14** and the corresponding β-D-ribofuranosyl nucleosides are similar (see Fig. 1). Besides, the Cotton effect in the B_{2u} band (260 to 280 nm) is nearly identical in the c.d. spectra of 3′-*C*-methyluridine (**9**) and of 3′-*C*-ethyluridine and 3′-*C*-butyluridine, synthesized earlier[17]; at the same time, the amplitude of the Cotton effect in the B_{1u} band (240 nm) decreases, in terms of absolute values, with an increase of the alkyl substituent: $\Delta\varepsilon$ $-2.0 \rightarrow -1.0 \rightarrow -0.7$.

The decreasing Cotton effect of the B_{2u} transition found experimentally on passing from the natural nucleosides to their 3′-*C*-substituted derivatives may be attributed to two events. (*1*) The *syn–anti* equilibrium shifts towards the *syn* orien-

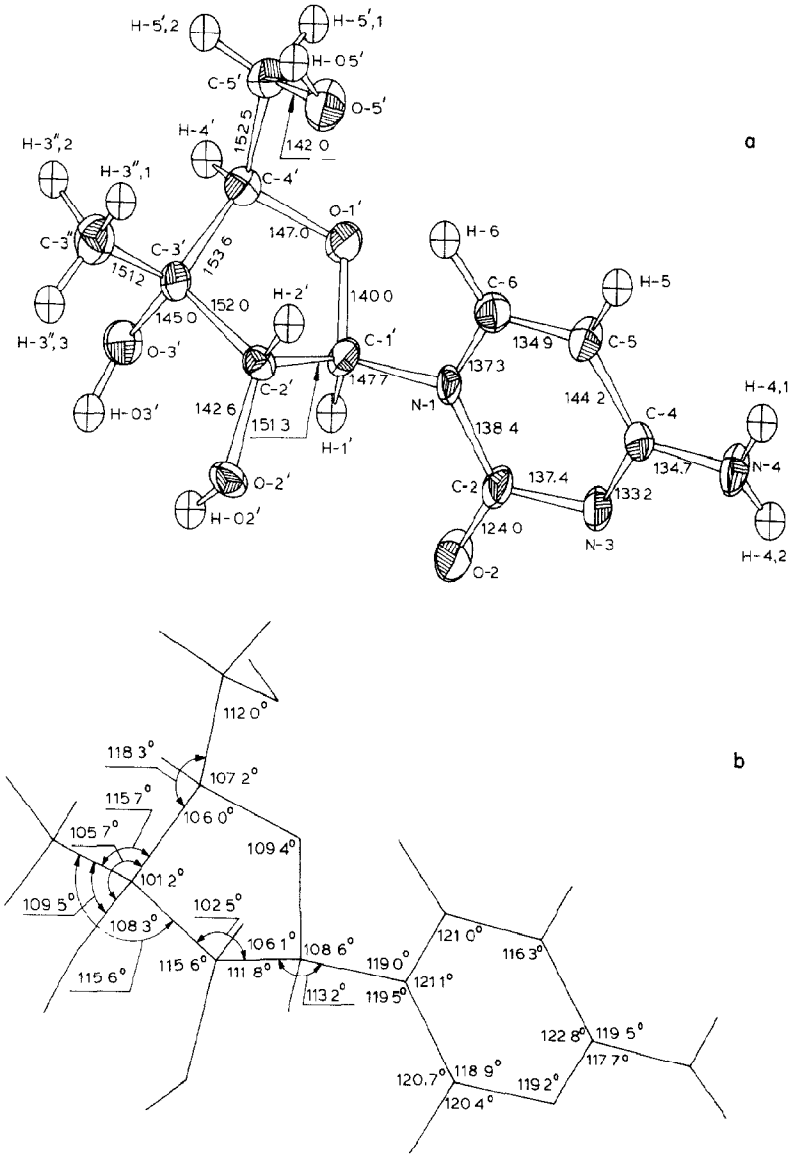


Fig. 2. (a) Numbering of atoms, and interatomic distances (in pm), and (b) bond angles in the structure of 3'-*C*-methylcytidine (**14**).

tation; if the shift yields solely the *syn* orientation, this can result in a negative Cotton-effect in the B_{2u} band[46]. (*2*) According to the calculations of Miles *et al.*[46], a shift of the $S \rightleftharpoons N$ equilibrium towards the *S* population would decrease the amplitude of the B_{2u} band. The decrease of intensity in a transition from the *N* to *S* conformation in the anti orientation of a molecule is ~2.5 for cytidine and 1.6 for uridine[46]. The fractions of the *S* conformer are, respectively, 53 and 44% in natural

uridine and cytidine. We have found that the amplitude of a Cotton effect is halved on passing from cytidine to its 3′-*C*-methyl derivative (**14**) and diminished by a factor of 1/2.7 for the uridine nucleosides. The decreased Cotton effect here cannot be interpreted merely in terms of a change in the $S \rightleftharpoons N$ equilibrium. It might also be attributable to a shift in the *syn–anti* equilibrium towards the *syn* rotamer for 3′-*C*-methylnucleosides in comparison with that of natural compounds, which is consistent with the foregoing conclusion made upon examining $J_{4',5'a}$ and $J_{4',5'b}$. Really, this shift is relatively small, as the chemical shifts for H-1′ and H-2′ in **9** and **14** differ by merely 0.06 (H-1′) and 0.16 p.p.m. (H-2′) compared to the chemical shifts for the respective protons in uridine and cytidine[49].

The foregoing physicochemical data concerning the conformation of the 3′-*C*-methylnucleosides in solution allow the following conclusion to be arrived at. Introduction of a methyl group at C-3′ of the natural nucleosides shifts the conformational equilibrium: the fraction of the *S* and *syn* conformers rises, while the contribution of the *gauche-gauche* rotamer decreases.

Because of the interesting, conformational properties of the synthetic compounds in solution, and in order to confirm the structure, we were led to undertake X-ray diffraction analysis of the cytosine nucleoside **14**.

Fig. 2 presents a view of a molecule of 3′-*C*-methylcytidine (**14**) along the *b* axis, and gives the interatomic distances, the bond angles, the lengths of valence bonds, and the orientation of the 50%-probability thermal ellipsoids. The values of the bond angles are given in Fig. 2b. The accuracy of determination of the bond lengths (σ) is 0.4–0.6 pm, and that for the bond angles is 0.3–0.4°. These data have been compared with those for the structures of cytidine[50] and 1-α-D-ribofuranosylcytosine[51]; in the case of 3′-*C*-methylcytidine, the C-3–O-3, C-4–O-1, C-4–C-5, and C-5–O-5 bonds of the D-ribofuranosyl group and the C-2–N-3, C-4–N-4, and C-4–C-5 bonds of the heterocyclic base are longer by 2–3 σ, whereas the C-1′–O-1′ and C-2′–C-1′ bonds are shorter. The remaining bonds in the respective molecules coincide, within the accuracy of the measurements.

The presence of an additional methyl group in nucleoside **14** has the greatest effect on the bond angles at C-3′. The angles C-4′–C-3′–O-3′ and C-2′–C-3′–O-3′ are smaller by several degrees, while the angle C-3′–C-2′–O-2′ is larger, compared to those in cytidine. The rest of the bond angles for 3′-*C*-methylcytidine (**14**) and α- and β-cytidine are in good agreement.

The main conformational changes in the structure of nucleoside **14** compared to cytidine mainly involve the ribofuranose ring. The C-2′and C-3′ atoms in 3′-*C*-methylcytidine are arranged at the opposite sides of a plane drawn through C-1′, O-1′, and C-4′, and are spaced from it by 49.6 and 10.7 pm, respectively. Here, the C-2′ atom projects from the plane toward the N-1 and C-5′ atoms, corresponding to the $^2T_3$ conformation of the ribofuranose ring (the family of *S* conformers).

The cytosine moiety of 3′-*C*-methylcytidine (**11**) is nearly planar, just as in the other known structures of cytosine nucleosides and nucleotides. The maximum deviation of 16.4 pm from the least-squares plane drawn through the six ring-atoms

is found for O-2, which is involved in the system of intermolecular hydrogen-bonding.

The relative orientation of the base and the D-ribosyl group corresponds to an *anti* conformation of the nucleoside. The torsion angle O-1′–C-1′–N-1–C-6 is 55.5°. The orientation of the molecule with respect to the C-4′–C-5′ exocyclic bond is *gauche-gauche*. The C-3′–C-4′–C-5′–O-5′ torsion angle is 61.7°.

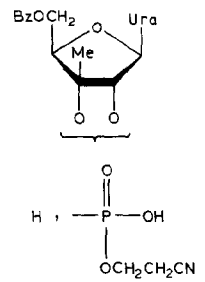
The foregoing data from X-ray diffraction analysis and physicochemical investigation of 3′-*C*-alkylnucleosides in solution indicate that the introduction of an alkyl substituent at C-3′ of the nucleosides mostly changes the conformation of the D-ribofuranosyl group; the orientations of the glycosylic and exocyclic bonds are modified only slightly.

The second part of this work is concerned with the synthesis of 3′-*C*-methyluridine phosphates. Ordinarily, the tertiary hydroxyl group exhibits low reactivity in acylation and phosphorylation reactions. As was demonstrated in the benzoylation of methyl 5-*O*-benzoyl-3-*C*-methyl-β-D-ribofuranoside[15], only the OH-2 group is benzoylated at 20°, whereas the reaction mixture must be heated to 70° in order to prepare the 3-*O*-benzoyl derivative. Furthermore, the 3-*O*-benzoyl group migrates to the adjacent OH-2 group[15], *i.e.*, the equilibrium in the system is shifted towards the 2-benzoate[15], or, in the case of 2-*C*-methylribofuranoses, towards the 3-benzoate[19]. Moffatt *et al.*[5] reported difficulties in the phosphorylation of the tertiary OH-5′ group in 5′,5′-di-*C*-methyladenosine. All of these findings indicate that the synthesis of 3′-*C*-methylnucleoside 2′,3′-cyclophosphates is a challenging problem.

As a rule, the synthesis of 2′(3′)-phosphates of nucleosides makes use of a 5′-*O*-trityl protective group, and, therefore, it includes the additional steps of blocking and deblocking of the OH-5′group. Because an *O*-benzoyl group is less susceptible to alkaline hydrolysis than an *O*-acetyl group[52], we decided to take advantage of this for synthesizing the 5′-benzoate **10** directly from the protected nucleoside **8**. Treatment of **8** with NEt_3–MeOH for 20 h at 20° produced the 5′-benzoate **10** in 61% yield and the nucleoside **9** in 16% yield. The position of the benzoyl group at O-5′ was supported by the p.m.r.-spectral data: H-5′a and H-5′b form a multiplet at 4.45 p.p.m., whereas these protons are located upfield (3.56 p.p.m.) for nucleoside **9**. The downfield shift of the protons was attributed to the anisotropic effect of the 5′-*O*-benzoyl group, whereas the chemical shifts of H-2′ in **9** and **10** hardly change.

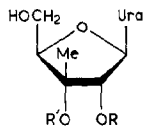
Nucleoside **10** was treated under standard conditions[53] with 2-cyanoethyl phosphate in the presence of dicyclohexylcarbodiimide (DCC) at 20°, and the products were separated on a column of DEAE-cellulose. Quite unexpectedly, the procedure gave a mixture of the respective 2-cyanoethyl esters of 5′-*O*-benzoyl-3′-*C*-methyluridine 2′(3′)-phosphate (**15**) in high yield. The ratio of 2′:3′ isomer was 2:1 according to the p.m.r.-spectral data. The mixture also contained ~25% of 5′-*O*-benzoyl-3′-*C*-methyluridine 2′,3′-cyclophosphate, apparently formed by hydrolysis of the 2-cyanoethyl esters of the nucleotides under the conditions of isolation.

The alkaline hydrolysis of esters **15** yielded nucleotides **16** and **17**; the ratio of 2′:3′ isomer was 3:2 according to the p.m.r.-spectral data. Cyclization of the 2′(3′)-nucleotides under standard conditions[54] (DCC in MeOH) afforded cyclophosphate **18** in high yield; its structure was corroborated by ^{31}P- and ^{1}H-n.m.r. spectroscopy. The decrease of $J_{1',2'}$ from 8.0 to 3.4 Hz had been shown to be typical of transformation of 2′(3′)-nucleotides into 2′,3′-cyclophosphates[47], just as is the downfield shift of the signal of the phosphorus atom in ^{31}P-n.m.r. spectra[55].



15

Ura = uracil-1-yl



16 R = PO_3H_2, R′ = H

17 R = H, R′ = PO_3H_2

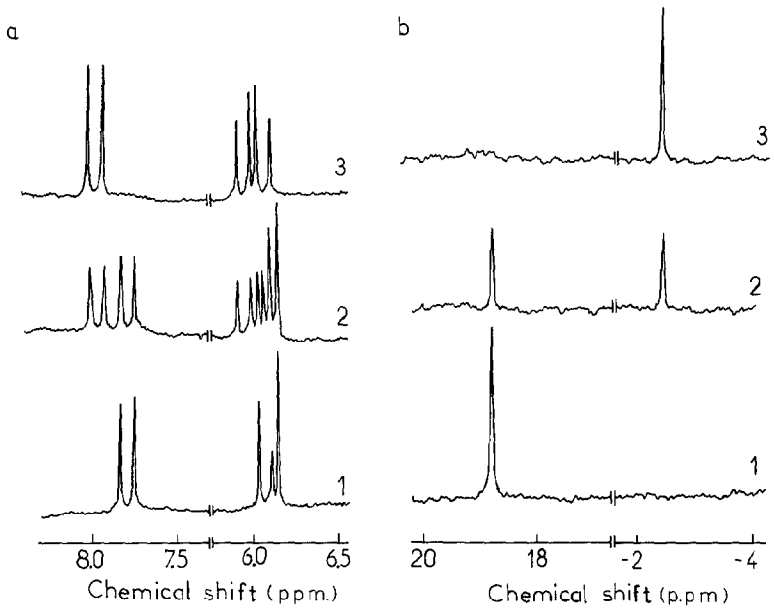


Fig. 3. Change in the intensity of signals in the (a) ^{1}H- and (b) ^{31}P-n.m.r. spectra with time in the hydrolysis of cyclophosphate (**18**) with pancreatic ribonuclease A (for conditions see Experimental section): 1, original spectrum of the cyclophosphate **18**; 2, 24 h after addition of the enzyme at 20°; 3, spectrum of the 3′-phosphate (**17**).

Additional proof for the structure of the cyclophosphate **18** was obtained when it was tested as a substrate for RNAase A and nonspecific RNAase from *Penicillium brevicompactum*. Separate hydrolysis of the cyclophosphate with the two RNAases yielded the same product, which was identified as 3′-*C*-methyluridine 3′-phosphate (**17**) by using the techniques of ^{1}H- and ^{31}P-n.m.r. spectroscopy (see Fig. 3). It is noteworthy that the n.m.r. spectrum of **17** has a $J_{P,H-2'}$ value of 3.3 Hz, which has not been reported for the naturally occurring 3′-nucleotides[47].

We compared the kinetic parameters of the hydrolysis of cyclophosphate **18** with those for uridine 2′,3′-cyclophosphate. The Michaelis constant (K_M) and the catalytic rate-constant (k_{cat}) are 1.4mM and 0.04 s^{-1}, respectively, for the enzyme-catalyzed hydrolysis of cyclophosphate **18** with nonspecific *P. brevicompactum* RNAase at pH 5.7. For uridine 2′,3′-cyclophosphate, K_M is 0.34mM and k_{cat} is 64 s^{-1}.

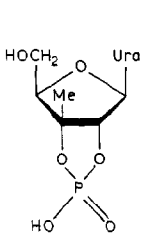
Hence, the introduction of a methyl group at the C-3′ of the substrate has only a slight effect on the affinity of cyclophosphate for the enzyme, but decreases the k_{cat} by a factor of 1/1600, *i.e.*, it increases the energy of the transition state by ~18 kJ/mol. This increase may be attributed to the fact that, in the transition state during the enzyme-catalyzed hydrolysis of the cyclophosphate, the C-3′ atom of the D-ribosyl moiety should lie above the C-1′–O-1′–C-4′ plane. In that case, as already noted, the mutually limited rotation of the 3′-*C*-Me and exocyclic 4′-CH_2OH groups makes the energy of the transition state greater by 13–21 kJ/mol. The possibility of such a transition-state conformation has been pointed out[56].

The hydrolysis of nucleotide **17** with endonuclease S_1, an enzyme having 3′-nucleotidase activity[57,58], yielded nucleoside **9** and orthophosphate, which is an independent confirmation of the structure of the synthetic nucleotide.

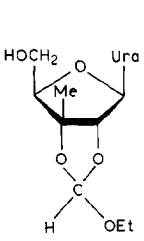
The preparation of 3′-*C*-methyluridine 5′-mono- and tri-phosphate was of interest as 3′-*C*-methylnucleosides exhibit antiviral activity[16]. It may be assumed that this effect is associated with the termination of RNA synthesis by 3′-*C*-methylnucleoside 5′-triphosphates produced in the cell from 3′-*C*-methylnucleosides[59].

The following procedure for the synthesis of nucleoside 5′-phosphates had earlier been used for the preparation of 5′-nucleotides and dinucleoside monophosphates, starting from 5′-*C*-methylnucleosides[8,9,11]. The synthesis includes phosphorylation of the readily accessible 2′,3′-*O*-(ethoxymethylidene) derivatives of nucleosides[60] with 2-cyanoethyl phosphate[53] in the presence of DCC or triisopropylbenzenesulfonyl chloride, with subsequent removal of the protecting groups.

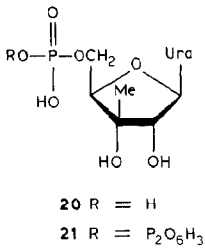
Treatment of nucleoside **9** with triethyl orthoformate in $HCONMe_2$ in the presence of HCl in $HCONMe_2$ yielded two products: a compound (**19**) with R_F 0.18, and a product with R_F 0.41 [apparently, 5′-*O*-(diethoxymethylidene)-**19**], which could be readily separated by chromatography on silica gel. Phosphorylation of the crude mixture, followed by chromatography on DEAE-cellulose and deblocking, afforded the 5′-phosphate in 32% yield (calculated on the starting nucleoside **9**). The structure of nucleotide **20** was corroborated by the u.v.-, ^{31}P-, and ^{1}H-n.m.r.-spectral data, and by chromatography.



18



19



20 R = H

21 R = $P_2O_6H_3$

Condensation of the 5′-phosphate **20** with 1,1′-carbonyldiimidazole, followed by reaction of the product with tributylammonium pyrophosphate under standard conditions[61] afforded the 5′-triphosphate **21** in 40% yield.

EXPERIMENTAL

General methods. — All melting points (uncorrected) were determined with a TP (USSR) instrument. Optical rotations were measured with a Perkin–Elmer Model 141 automatic polarimeter. U.v. spectra were determined with a Specord-UV-vis (GDR) instrument. The u.v. spectra of phosphoric esters **16–18**, **20**, and **21** were similar to those of the respective uridine derivatives in the positions of the maxima and in the millimolar extinction: $\lambda_{max}^{pH\ 1-7}$ 261 (ε_{mM} 9.00–10.00) and $\lambda_{max}^{pH\ 13}$ 261 nm (7.50–6.80). C.d. spectra were recorded with a Jobin–Yvon Dichrograph Mark V (France) spectrometer. Preparative chromatography was conducted on silica gel L, 40–100 μm (Czechoslovakia). Thin-layer chromatography was performed on Silufol UV_{254} plates (Czechoslovakia) in the following systems: *A*, $CHCl_3$; *B*, 39:1 $CHCl_3$–EtOH; *C*, 19:1 $CHCl_3$–EtOH; *D*, 9:1 $CHCl_3$–EtOH; and *E*, 7:1:2 iPrOH–conc. NH_3–water; also on PI-cellulose (Merck, FRG) in 0.15M KH_2PO_4 (*F*). Spots on the chromatograms were developed by heating to 150–200°, or detected under u.v. light. ^{1}H-N.m.r. spectra were recorded with a Varian XL-100-15 (USA) spectrometer with a working frequency of 100 MHz. The chemical shifts are given in p.p.m., with hexamethyldisiloxane (δ 0.04) as the internal standard for solutions in $CDCl_3$ and Me_2SO-d_6, and with *tert*-butanol (δ 1.27) for solutions in D_2O. The *J* values are given in Hz. The signals were assigned by using double resonance. The chemical shifts in ^{31}P-n.m.r. spectra were determined by using 85% H_3PO_4 as an external standard. ^{13}C-N.m.r. spectra were recorded with a Bruker-Physik WP-60 (FRG) spectrometer at 15.08 MHz with proton decoupling for solutions in $CDCl_3$, using tetramethylsilane as the internal standard.

Crystals for X-ray analysis were obtained from a saturated solution of 3′-*C*-methylcytidine (**14**) in 96% ethanol by slowly evaporating the solvent at 0°. The space group of the crystals is $P2_12_12_1$, with a = 1087.4(1) pm, b = 1109.0(1) pm, c = 979.9(1) pm, and Z = 4.

In the structural study, we used the intensities of 1207 independent reflexes with $I \geq 3\sigma$ (I), measured with a CAD-4 Enraf–Nonius diffractometer (Nether-

lands) with graphite monochromated CuKα radiation by the $\theta/2\theta$ method. The intensities of reflexes were corrected for the Lorentz and polarization factors. The coordinates of carbon, nitrogen, and oxygen atoms were found by direct methods, using the "Rentgen-75" program[62]; the positions of hydrogen atoms were determined from a series of electron-density difference-syntheses. The structure was refined by the full-matrix, least-squares method, with anisotropic temperature-factors for carbon, nitrogen, and oxygen atoms, and fixed positional and individual, isotropic thermal-parameters for hydrogen atoms. The final value of the R factor was 0.062.

Homogeneous preparations of pancreatic RNAase (EC 3.1.27.5), nonspecific *Penicillium brevicompactum* RNAase (EC 3.1.25.1), and *Aspergillus oryzae* endonuclease S_1 (EC 3.1.30.1) were obtained by using the procedures described in the literature[63–65]. The kinetic parameters of the cyclophosphate hydrolysis with *P. brevicompactum* RNAase were studied at pH 5.7 by the pH-stat technique, with a Radiometer instrument having an ABU-12 autoburet (Denmark), at 25°. The catalysis constants were calculated, assuming that the pK for the secondary ionization of the phosphate group in the 3′-phosphate **17** and in uridine 3′-phosphate was 5.9.

1,2-O-Isopropylidene-3-C-methyl-α-D-allofuranose (**2**). — A solution of compound **1** (12.6 g, 46 mmol) in 75% AcOH (100 mL) was kept for 24 h at 20°, evaporated to dryness, and then evaporated with 1-butanol (3 × 50 mL); the residue crystallized from ethanol; yield 9.6 g (89%); m.p. 133–134°, $[\alpha]_D^{20}$ +23° (*c* 1, chloroform), $[\alpha]_D^{20}$ +40.1° (*c* 1, methanol); lit.[39] m.p. 132.5–133.5°, $[\alpha]_D^{20}$ +23° (*c* 0.4, chloroform); p.m.r.: δ 5.71 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 4.16 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-2), 3.85–3.55 (m, 4 H, H-4,5,6,6′), 1.60 (s, 3 H, Me), 1.37 (s, 3 H, Me), and 1.35 (s, 3 H, *C*-Me-3); ^{13}C-n.m.r. ($CDCl_3$): δ 103.7 (C-1), 85.3 (C-2), 79.1 (C-4), 78.1 (C-3), 70.9 (C-5), 64.9 (C-6), 113.1, 26.8, 26.6 (Me_2C), and 19.4 (*C*-Me-3).

1,2-O-Isopropylidene-3-C-methyl-α-D-ribofuranose (**3**). — $NaIO_4$ (8.3 g, 38.8 mmol) was added in portions to a stirred solution of compound **2** (9.0 g, 38.4 mmol) in water (70 mL), and the mixture was kept for 1 h at 20°. Ethanol (300 mL) was added; the solid was removed by filtration, and washed with ethanol (30 mL), $NaBH_4$ (3 g, 80 mmol) was added in portions to the combined filtrates, and the mixture was kept for 16 h at 20°. The solution was made neutral with acetic acid (to pH 7.0), water (100 mL) was added, the mixture was extracted with chloroform (2 × 300 mL), and the extracts were combined, evaporated to dryness, and then evaporated with methanol (5 × 20 mL); the residue was dissolved in the minimal volume of chloroform, hexane was added to incipient opalescence, and the mixture was kept for 16 h at 0°. Filtration yielded 7.0 g (89%) of compound **3**; m.p. 90–92°, $[\alpha]_D^{20}$ +40° (*c* 1, methanol; lit.[66] m.p. 92.5–93.5°, $[\alpha]_D^{26}$ +24.8° (*c* 0.5, chloroform); p.m.r. ($CDCl_3$): δ 5.89 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 4.15 (d, 1 H, $J_{2,1}$ 4.0 Hz, H-2), 4.04–3.68 (m, 3 H, H-4,5,5′), 1.62 (s, 3 H, Me), 1.38 (s, 3 H, Me), and 1.20 (s, 3 H, *C*-Me-3); ^{13}C-n.m.r. ($CDCl_3$): δ 105.0 (C-1), 86.1 (C-2), 83.2 (C-4), 77.9 (C-3), 61.8 (C-5), 113.6, 27.0, 26.8 (CMe_2), and 19.2 (*C*-Me-3).

5-O-Benzoyl-1,2-O-isopropylidene-3-C-methyl-α-D-ribofuranose (**4**). — A solution of compound **3** (6 g, 29.5 mmol) in abs. pyridine (50 mL) was dried by evaporation; the residue was dissolved in abs. pyridine (60 mL), and the solution cooled to 0°. Benzoyl chloride (3.7 mL, 32 mmol) was added, and the mixture was kept for 16 h at 0°. A saturated solution of $NaHCO_3$ (30 mL) was added, and the mixture was extracted with chloroform (2 × 100 mL). The extracts were combined, washed successively with saturated $NaHCO_3$ solution (50 mL) and water (2 × 50 mL), dried (Na_2SO_4), filtered, evaporated to dryness, evaporated with toluene (3 × 30 mL), and the residue dissolved in the minimal volume of chloroform; hexane was added to faint turbidity, and the mixture was allowed to crystallize at 0°; yield 7.7 g (85%); m.p. 109–110°, $[\alpha]_D^{20}$ +13.5° (*c* 1, chloroform); lit.[15] m.p. 109–111°, $[\alpha]_D^{20}$ +12.6° (*c* 2.4, chloroform); p.m.r. ($CDCl_3$): δ 8.10–7.30 (m, 5 H, Bz), 5.77 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 4.60–4.00 (m, 3 H, H-4,5,5'), 4.11 (d, 1 H, $J_{2,1}$ 4.0 Hz, H-2), 2.70 (s, 1 H, OH, exchanged with D_2O), 1.57 (s, 3 H, Me), 1.34 (s, 3 H, Me), and 1.23 (s, 3 H, *C*-Me-3); ^{13}C-n.m.r. ($CDCl_3$): δ 103.7 (C-1), 84.1 (C-2), 79.5 (C-4), 77.0 (C-3), 63.1 (C-5), 112.6, 26.6, 26.5 (CMe_2), 18.4 (*C*-Me-3), and 166.9, 133.4, 130.0, 128.6 (Bz).

3-O-Acetyl-5-O-benzoyl-1,2-O-isopropylidene-3-C-methyl-α-D-ribofuranose (**5**). — To a solution of compound **4** (1.23 g, 4 mmol) in dry pyridine (10 mL) were added acetic anhydride (4 mL) and 4-(dimethylamino)pyridine (10 mg), and the mixture was kept for 16 h at 20°. Cold water (20 mL) was added, the mixture was extracted with chloroform (2 × 50 mL), the extracts were combined, and washed successively with saturated $NaHCO_3$ solution (2 × 30 mL) and water (50 mL), dried (Na_2SO_4), filtered, and evaporated to dryness, and the residue was evaporated with toluene (3 × 10 mL). The residue was chromatographed on a column of silica gel (100 g) with system *A*. Fractions containing the product were evaporated, and a syrup was obtained in quantitative yield (1.4 g); R_F 0.69 (*A*); p.m.r. ($CDCl_3$): δ 8.11–7.30 (m, 5 H, Bz), 5.78 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 4.80 (d, 1 H, $J_{2,1}$ 4.0 Hz, H-2), 4.59–4.31 (m, 3 H, H-4,5,5'), 2.02 (s, 3 H, Ac), 1.53 (s, 3 H, Me), 1.45 (s, 3 H, *C*-Me-3), and 1.32 (s, 3 H, Me); ^{13}C-n.m.r. ($CDCl_3$): δ 104.4 (C-1), 82.7 (C-2), 82.9 (C-3), 78.0 (C-4), 62.7 (C-5), 113.0, 26.5, 26.6 (CMe_2), 15.9 (*C*-Me-3), 166.7, 133.5, 130.0, 128.6 (Bz), and 170.0, 21.2 (Ac).

Anal. Calc. for $C_{18}H_{22}O_7$: C, 61.71; H, 6.33. Found: C, 61.48; H, 6.01.

Preparation of 1,2,3-tri-O-acetyl-5-O-benzoyl-3-C-methyl-α,β-D-ribofuranose (**7**). — *Method A.* Conc. H_2SO_4 (0.63 mL) was added to a solution of compound **5** (1 g, 2.86 mmol) in a mixture of acetic acid (12.5 mL) and acetic anhydride (1.9 mL). The mixture was kept for 4 days at 20°, diluted with chloroform (50 mL), washed successively with saturated $NaHCO_3$ solution (2 × 10 mL) and water (30 mL), dried (Na_2SO_4), filtered, evaporated to dryness, and the residue chromatographed on silica gel with system *A*. Fractions containing the product were evaporated to dryness, and a syrup (0.9 g) was obtained; yield 80%; R_F 0.49 (*A*); p.m.r. ($CDCl_3$): δ 8.15–7.40 (m, 5 H, Bz), 6.43 (d, 0.33 H, $J_{1,2}$ 4.5 Hz, H-1α), 6.10 (d, 0.67 H, $J_{1,2}$ 1.5 Hz, H-1β), 5.46 (d, 0.67 H, $J_{2,1}$ 1.5 Hz, H-2β), 5.41 (d, 0.33 H, $J_{2,1}$

4.5 Hz, H-2α), 4.80–4.39 (m, 3 H, H-4,5,5'), 2.10 (s, 1 H, Acα), 2.09 (s, 2 H, Acβ), 2.06 (s, 1 H, Acα), 2.03 (s, 5 H, Acα, Ac-2β), 1.70 (s, 2 H, *C*-Me-3β), and 1.63 (s, 1 H, *C*-Me-3α). The ratio of α:β anomer (determined by p.m.r.) was 1:2.

Anal. Calc. for $C_{19}H_{22}O_9$: C, 57.87; H, 5.62. Found: C, 57.72; H, 5.40.

Method B. A solution of compound **4** (1.85 g, 6 mmol) in 90% trifluoroacetic acid (15 mL) was kept for 20 min at 20° (t.l.c. in system *C* then demonstrated quantitative R_F 1.0 → 0.33 transformation), toluene (25 mL) was added to the solution, which was dried by evaporation, and the residue was evaporated with toluene (2 × 20 mL), to afford 5-*O*-benzoyl-3-*C*-methyl-α,β-D-ribofuranose (**6**) as a syrup in quantitative yield; p.m.r. (D_2O–Me_2SO-d_6): δ 8.10–7.40 (m, 5 H, Bz), 5.32 (d, 0.5 H, $J_{1,2}$ 4.0 Hz, H-1), 5.21 (d, 0.5 H, $J_{1,2}$ 4.0 Hz, H-1), 4.46–4.10 (m, 3 H, H-4,5,5'), 3.82 (d, 0.5 H, $J_{2,1}$ 4.0 Hz, H-2), 3.70 (d, 0.5 H, $J_{2,1}$ 4.0 Hz, H-2), 1.30 (s, 1.5 H, *C*-Me-3), and 1.27 (s, 1.5 H, *C*-Me-3). The ratio of α:β anomer (determined by p.m.r. spectroscopy) was 1:1.

Compound **6** was acetylated by either of two procedures.

1. A solution of the residue in abs. pyridine (10 mL) was evaporated to dryness, pyridine (15 mL) and acetic anhydride (12 mL) were added to the residue, and the mixture was kept for 16 h at 20°. Cold water (30 mL) was added, with stirring for 30 min, the mixture was extracted with chloroform (2 × 50 mL), the extracts were combined, washed successively with water (2 × 30 mL), saturated $NaHCO_3$ solution (50 mL), and water (30 mL), dried (Na_2SO_4), filtered, evaporated to dryness, the residue evaporated with toluene (3 × 20 mL), and the residue dried *in vacuo* (133.3 Pa) for 1 h at 30°. To the resultant syrup were added acetic anhydride (15 mL) and *p*-toluenesulfonic acid monohydrate (0.57 g, 3 mmol), and the mixture was allowed to react for 3 days at 20°. Chloroform (100 mL) was added, the organic layer was washed successively with saturated $NaHCO_3$ solution (2 × 50 mL) and water, dried (Na_2SO_4), filtered, evaporated to dryness, and the residue azeotropically dried with toluene (2 × 20 mL), and chromatographed on silica gel (200 g) in system *A*. Fractions containing the product were dried by evaporation, to afford a syrup (2.0 g, 85% yield).

2. The residue was dissolved in abs. pyridine (10 mL) and dried by evaporation; then, abs. pyridine (12 mL), acetic anhydride (9.5 mL), and 4-(dimethylamino)pyridine (20 mg) were added to the residue, and the mixture was kept for 3 days at 20°. Chloroform (100 mL) was added, and the organic layer was successively washed with water (50 mL), saturated $NaHCO_3$ solution (2 × 20 mL), and water (50 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was azeotropically dried with toluene (2 × 30 mL), and isolated on silica gel just as in Procedure 1; yield, 66%.

*1-(2,3-Di-*O*-acetyl-5-*O*-benzoyl-3-*C*-methyl-β-*D*-ribofuranosyl)uracil* (**8**) *and 3-(2,3-di-*O*-acetyl-5-*O*-benzoyl-3-*C*-methyl-β-*D*-ribofuranosyl)uracil* (**11**). — A suspension of dry uracil (1.12 g, 10 mmol) in a mixture of hexamethyldisilazane (10 mL) and chlorotrimethylsilane (0.3 mL) was boiled under reflux, in the absence of moisture, until completely dissolved (~4 h), and evaporated to dryness. A solution

of compound **7** (2.6 g, 6.6 mmol) in dry acetonitrile (60 mL) and tin tetrachloride (0.84 mL, 7 mmol) was added to the residue, and the mixture was kept for 16 h at 20°. Chloroform (100 mL) and saturated $NaHCO_3$ solution (40 mL) were added, and the mixture was stirred for 30 min at 20°, and filtered through Hyflo Supercel; the organic layer was separated, washed consecutively with saturated $NaHCO_3$ solution (30 mL) and water (2 × 30 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was chromatographed on silica gel (100 g) in system *B*. Fractions containing the product were evaporated to dryness, to afford compound **8** (2.35 g) as a foam; yield 80%; R_F 0.35 (*C*); p.m.r. ($CDCl_3$): δ 8.67 (br s, 1 H, NH; exchanged with D_2O), 8.10–7.40 (m, 5 H, Bz), 7.41 (d, 1 H, $J_{6,5}$ 8.0 Hz, H-6), 6.20 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1′), 5.46 (dd, 1 H, $J_{5,6}$ 8.0, $J_{NH,5}$ 2.0 Hz, H-5; converted into doublet, J 8.0, on addition of D_2O), 5.39 (d, 1 H, $J_{2',1'}$ 7.5 Hz, H-2′), 4.90–4.40 (m, 3 H, H-4′,5′a,5′b), 2.14 (s, 6 H, 2 Ac), and 1.70 (s, 3 H, *C*-Me-3′).

Anal. Calc. for $C_{21}H_{22}N_2O_9$: C, 56.50; H, 4.97; N, 6.28. Found: C, 56.38; H, 4.81; N, 6.18.

The second product eluted by system *B* was compound **11**; yield 0.21 g (7%); a syrup; R_F 0.29 (*C*); p.m.r. ($CDCl_3$): δ 9.42 (br s, 1 H, NH; exchanged with D_2O), 8.14–7.15 (m, 5 H, Bz), 7.45 (d, 1 H, $J_{6,5}$ 8.0 Hz, H-6), 6.38 (d, 1 H, $J_{1',2'}$ 6.5 Hz, H-1′), 6.09 (d, 1 H, $J_{2',1'}$ 6.5 Hz, H-2′), 5.72 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-5), 4.73–4.50 (m, 3 H, H-4′,5′a,5′b), 2.12 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), and 1.80 (3 H, *C*-Me-3′).

Anal. Calc. for $C_{21}H_{22}N_2O_9$: C, 56.50; H, 4.97; N, 6.28. Found: C, 56.40; H, 4.72; N, 6.39.

*1-(2,3-Di-*O*-acetyl-5-*O*-benzoyl-3-*C*-methyl-β-*D*-ribofuranosyl)cytosine* (**13**). — A suspension of dry cytosine (333 mg, 3 mol) in hexamethyldisilazane (3 mL) and chlorotrimethylsilane (0.2 mL) was boiled under reflux, in the absence of moisture, to complete dissolution (~2 h), evaporated to dryness, and **7** (0.8 g, 2 mmol) in acetonitrile (20 mL) was added. To the resultant solution was added tin tetrachloride (0.3 mL, 2.5 mmol), and the homogeneous mixture was kept for 16 h at 20°. Chloroform (50 mL) and saturated $NaHCO_3$ solution (20 mL) were added, and the mixture was stirred for 20 min at 20°, and filtered through Hyflo Super-Cel. The organic layer was separated, washed successively with saturated $NaHCO_3$ solution (20 mL) and water (20 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was chromatographed on silica gel (50 g) in system *C*. Fractions containing the product were evaporated, to afford compound **13** (0.6 g) as a foam; yield 68%; R_F 0.42 (*D*); p.m.r. ($CDCl_3$): δ 8.14–7.44 (m, 5 H, Bz), 7.50 (d, 1 H, $J_{6,5}$ 7.5 Hz, H-6), 6.35 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1′), 5.55 (d, 1 H, $J_{5,6}$ 7.5 Hz, H-5), 5.38 (d, 1 H, $J_{2',1'}$ 7.5 Hz, H-2′), 4.90–4.53 (m, 3 H, H-4′,5′a,5′b), 2.15 (s, 6 H, 2 Ac), and 1.70 (s, 3 H, *C*-Me-3′).

Anal. Calc. for $C_{21}H_{23}N_3O_8$: C, 56.63; H, 5.20; N, 9.43. Found: C, 56.50; H, 5.11; N, 9.32.

1-(3-C-methyl-β-D-ribofuranosyl)uracil (**9**). — A solution of compound **8** (1 g, 2.24 mmol) in methanol (15 mL) semisaturated with ammonia at 0° was kept for

16 h at 20°, evaporated, and the residue crystallized from ethanol–water–ether; yield 0.43 g (75%); m.p. 213–214°; $\lambda_{max}^{pH\ 1-7}$ 262 nm (ε_{mM} 10.50), $\lambda_{max}^{pH\ 13}$ 262 nm (ε_{mM} 7.70); p.m.r. (D_2O): δ 7.90 (d, 1 H, $J_{6,5}$ 8.0 Hz, H-6), 5.96 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 5.90 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-5), 4.18 (d, 1 H, $J_{2',1'}$ 7.8 Hz, H-2'), 4.09 (dd, 1 H, $J_{4',5'a}$ 3.8, $J_{4',5'b}$ 4.9 Hz, H-4'), 3.79 (m, 2 H, H-5'a,5'b), and 1.40 (s, 3 H, *C*-Me-3').

Anal. Calc. for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46; N, 10.85. Found: C, 46.67; H, 5.62; N, 10.71.

The foregoing procedure was also used to prepare the following compounds.

3-(3-C-Methyl-β-D-ribofuranosyl)uracil (**12**); yield 72%; m.p. 220–223°; $\lambda_{max}^{pH\ 1-7}$ 264 nm (ε_{mM} 8.30); $\lambda_{max}^{pH\ 13}$ 292 nm (ε_{mM} 11.40).

Anal. Calc. for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46; N, 10.85. Found: C, 46.42; H, 5.31; N, 10.64.

1-(3-C-Methyl-β-D-ribofuranosyl)cytosine (**14**); yield 78%; m.p. 222–225°; lit.[16] m.p. 235–238°; $\lambda_{max}^{pH\ 1}$ 280 nm (ε_{mM} 12.70); $\lambda_{max}^{pH\ 7-13}$ 271 nm (ε_{mM} 8.80); p.m.r. (D_2O): δ 7.82 (d, 1 H, $J_{6,5}$ 7.5 Hz, H-6), 6.04 (d, 1 H, $J_{5,6}$ 7.5 Hz, H-5), 5.94 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'), 4.16 (d, 1 H, $J_{2',1'}$ 7.5 Hz, H-2'), 4.10 (dd, 1 H, $J_{4',5'a}$ 3.6, $J_{4',5'b}$ 4.9 Hz, H-4'), 3.78 (m, 2 H, H-5'a,5'b), and 1.38 (s, 3 H, *C*-Me-3').

Anal. Calc. for $C_{10}H_{15}N_3O_5$: C, 46.69; H, 5.88; N, 16.33. Found: C, 46.63; H, 5.83; N, 16.28.

1-(5-O-Benzoyl-3-C-methyl-β-D-ribofuranosyl)uracil (**10**) *and 1-(3-C-methyl-β-D-ribofuranosyl)uracil* (**9**). — A solution of **8** (0.45 g, 1 mmol) in abs. MeOH (45 mL) and dry NEt_3 (5 mL) was kept for 20 h at 20°; t.l.c. then showed the absence of the starting compound. The mixture was evaporated *in vacuo*, water (20 mL) and ethyl acetate (20 mL) were added to the residue, and the layers were separated; the aqueous layer was extracted with ethyl acetate (20 mL), and the organic layer, with water (20 mL). The organic layers were combined and evaporated, ether (20 mL) was added to the residue, and the solution was kept for 16 h at 0°. The resulting solid was filtered off, washed with ether, and dried. The yield of compound **10** was 220 mg (61%); m.p. 217–218°; R_F 0.13 (*C*); $\lambda_{max}^{pH\ 7}$ 262, 235 nm; p.m.r. (Me_2SO-d_6): δ 11.20 (br s, 1 H, NH, exchanged with D_2O), 8.06–7.58 (m, 5 H, Bz), 7.58 (1 H, $J_{6,5}$ 8.0 Hz, H-6), 5.87 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 5.51 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-5), 5.48 (d, 1 H, $J_{2',OH}$ 6.3 Hz, OH-2', exchanged with D_2O), 5.01 (s, 1 H, OH-3', exchanged with D_2O), 4.45 (m, 2 H, H-5'a,5'b), 4.15 (t, 1 H, $J_{4',5'a} = J_{4',5'b}$ = 5.0 Hz, H-4'), 3.97 (dd, 1 H, $J_{2',1'}$ 8.0, $J_{2',OH}$ 6.3 Hz, H-2'; was converted into a doublet, *J* 8.0 Hz, on addition of D_2O), and 1.28 (s, 3 H, *C*-Me-3').

Anal. Calc. for $C_{17}H_{18}N_2O_7$: C, 56.35; H, 5.01; N, 7.73. Found: C, 56.17; H, 4.95; N, 7.67.

The aqueous layers were evaporated, acetone (5 mL) was added to the residue, and the solution was kept for 16 h at 0°. The resulting solid was filtered off, successively washed with acetone and ether, and dried. The yield of compound **9** was 42 mg (16%); m.p. 213–214°; R_F 0.02 (*C*); p.m.r. (Me_2SO-d_6): δ 11.22 (br s,

1 H, NH, exchanged with D_2O), 8.04 (d, 1 H, $J_{6,5}$ 8.0 Hz, H-6), 5.89 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 5.65 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-5), 5.27 (d, 1 H, $J_{OH,2'}$ 6.5 Hz, OH-2′, exchanged with D_2O), 5.06 (t, 1 H, *J* 4.0 Hz, OH-5′, exchanged with D_2O), 4.65 (s, 1 H, OH-3′, exhanged with D_2O), 3.86 (dd, 1 H, $J_{2',1'}$ 8.0, $J_{2',OH}$ 6.5 Hz, H-2′, was converted into a doublet, *J* 8.0 Hz, on addition of D_2O), 3.81 (t, 1 H, $J_{4',5'a} = J_{4',5'b}$ = 3.0 Hz, H-4′), 3.56 (m, 2 H, H-5′,5′b), and 1.24 (s, 3 H, *C*-Me-3′).

*1-(5-O-Benzoyl-3-C-methyl-β-*D*-ribofuranosyl)uracil 2′(3′)-(2-cyanoethyl phosphate)* (**15**). — A solution of nucleoside **10** (174 mg, 0.48 mmol) and M 2-cyanoethyl phosphate solution in pyridine (1 mL) in abs. pyridine (5 mL) was dried by evaporation *in vacuo,* the residue was azeotropically dried by evaporation with abs. pyridine (3 × 10 mL), and dissolved in abs. pyridine (5 mL); dicyclohexylcarbodiimide (630 mg, 3 mmol) was added, the mixture was stirred for 4 days at 20°, and the dicyclohexylurea was filtered off, and washed with 20% aqueous pyridine (30 mL). The combined filtrates were washed with ether (2 × 20 mL), diluted with water to 200 mL, and applied to a column of DEAE-cellulose (HCO_3^-) (200 mL). The column was washed with water (1 L) and eluted with a concentration gradient (0.0–0.2M) of NH_4HCO_3 (total vol. 6 L). Fractions absorbing in the u.v. and containing the product were pooled, evaporated *in vacuo* to dryness, evaporated with water (5 × 20 mL), and freeze-dried; yield 220 mg (93%). The eluting concentration of NH_4HCO_3 was 0.05–0.06M; R_F 0.55 (*E*); $\lambda_{max}^{pH\,7}$ 262, 235 nm. According to the p.m.r.-spectral data recorded for a solution in D_2O, the product contained, along with the esters of the 2′-phosphate [δ 6.20 (d, 1/2 H, $J_{1',2'}$ 8.0 Hz, H-1′)] and 3′-phosphate [δ 6.08 (d, 1/4 H, $J_{1',2'}$ 8.0 Hz, H-1′)] in the ratio of 2:1, an admixture (25%) of 1-(5-*O*-benzoyl-3-*C*-methyl-β-D-ribofuranosyl)uracil 2′,3′-cyclophosphate [δ 5.85 (d, 1/4 H, $J_{1',2'}$ 3.3 Hz, H-1′).

*1-(3-C-Methyl-β-*D*-ribofuranosyl)uracil 2′(3′)phosphate* (**16**) *and* (**17**). — A solution of compound **15** (200 mg, 0.38 mmol) in M NaOH (15 mL) was kept for 20 min at 20°, loaded onto a column of Dowex-50 (H^+) resin (20 mL), and eluted with water. The first (u.v.-absorbing) fraction contained phosphates **16** and **17**, and the second fraction contained benzoic acid. The first fraction was made neutral with M NH_4OH (to pH 7.5), diluted with water to 100 mL, applied to a column of DEAE-cellulose (HCO_3^-) (200 mL), and eluted with a concentration gradient (0.0–0.3M) of NH_4HCO_3 (total vol. 6 L). Fractions absorbing in the u.v. and containing the product were combined, evaporated *in vacuo,* evaporated with water (5 × 10 mL), and freeze-dried. The yield of the diammonium salts of the nucleoside 2′(3′)-phosphates was 90 mg. The overall yield (calculated on starting nucleoside) was 55%; R_F 0.16 (*E*); for uridine 2′(3′)-phosphate, R_F 0.14 (*E*). The u.v. spectra were identical with those of uridine 2′(3′)-phosphate; p.m.r. (D_2O): δ 7.98 (d, 1 H, $J_{6,5}$ 8.0 Hz, H-6), 6.12 (d, 0.6 H, $J_{1,2'}$ 8.0 Hz, H-1′, 2′-phosphate), 6.08 (d, 0.4 H, $J_{1',2'}$ 8.0 Hz, H-1′, 3′-phosphate), 5.96 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-5), 4.90–3.90 (m, 4 H, H-2′,4′,5′a,5′b), 1.65 (s, 1.2 H, *C*-Me-3′), and 1.45 (s, 1.8 H, *C*-Me-3′, 2′-phosphate). The ratio of 2′ to 3′ isomer (determined by p.m.r. spectroscopy) was 3:2.

*1-(3-C-Methyl-β-*D*-ribofuranosyl)uracil 2′,3′-cyclophosphate* (**18**). — A solu-

tion of nucleotides **16** and **17** (50 mg, 134 μmol) in abs. MeOH (5 mL) was evaporated to dryness *in vacuo*, and azeotropically dried with abs. MeOH (2 × 5 mL); the residue was dissolved in abs. MeOH (3 mL), dicyclohexylcarbodiimide (206 mg, 1 mmol) was added, and the mixture was stirred for 16 h at 20°. Water (100 mL) was added to the mixture, the dicyclohexylurea was filtered off, and washed with water (20 mL), the combined filtrates were washed with ether (2 × 20 mL), and concentrated to 100 mL, and the concentrate loaded onto a column of DEAE-cellulose (HCO_3^-) (200 mL). The column was washed with water, and eluted with a concentration gradient (0.0–0.2M) of NH_4HCO_3 (total vol. 6 L). Fractions absorbing in the u.v. and eluted over a concentration range of 0.05 to 0.06M NH_4HCO_3 were combined, evaporated *in vacuo*, evaporated with water (5 × 10 mL), and freeze-dried. The yield of the ammonium salt of cyclophosphate **18** was 39 mg (86%); R_F 0.53 (*E*). For uridine 2′,3′-cyclophosphate, R_F 0.52 (*E*). The u.v. spectra of the product were identical with those of uridine 2′,3′-cyclophosphate; p.m.r. (D_2O): δ 7.81 (d, 1 H, $J_{6,5}$ 8.0 Hz, H-6), 5.94 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-5), 5.91 (d, 1 H, $J_{1',2'}$ 3.4 Hz, H-1′), 4.30 (dd, 1 H, $J_{4',5'a}$ 4.2, $J_{4',5'b}$ 6.8 Hz, H-4′), 3.94 (m, 2 H, H-5′a,5′b), 1.57 (s, 3 H, *C*-Me-3′); H-2′ signal is under that of HOD; ^{31}P-n.m.r. (D_2O, pH 7.15): δ +18.87 (s).

1-(3-C-Methyl-β-D-ribofuranosyl)uracil 3′-phosphate (**17**). — Pancreatic ribonuclease A (1.2 mg) was added at pH 7.15 to a solution of the ammonium salt of cyclophosphate **18** (5.2 mg) in D_2O (0.5 mL) in an ampoule for a n.m.r. spectrometer. The reaction was monitored by recording the ^{1}H- and ^{31}P-n.m.r. spectra. The half-time of hydrolysis was 24 h at 20° (see Fig. 3). The mixture was kept for 5 days at 20°, applied to a column (1.5 × 60 cm) of Sephadex G-25, and eluted with water. Fractions (absorbing in the u.v.) containing the product were loaded onto a column of DEAE-celulose (HCO_3^-) (30 mL). The procedure of isolation was similar to that for the nucleoside 2′(3′)-phosphates **16** and **17**; yield 3.8 mg (64%); R_F 0.16 (*E*). The u.v. spectrum was identical with that of uridine 3′-phosphate; p.m.r. (D_2O): δ 7.98 (d, 1 H, $J_{6,5}$ 8.0 Hz, H-6), 6.08 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 5.96 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-5), 4.21 (dd, 1 H, $J_{2',1'}$ 8.0, $J_{2',P}$ 3.3 Hz, H-2′), 3.85 (m, 3 H, H-4′,5′a,5′b), and 1.65 (s, 3 H, *C*-Me-3′); ^{31}P-n.m.r. (D_2O, pH 7.15): δ −2.48 (s).

Hydrolysis of 1-(3-C-methyl-β-D-ribofuranosyl)uracil 3′-phosphate (**17**) *with nuclease* S_1. Nuclease S_1 solution (3 μM; 20 μL) in 0.03M acetate buffer, pH 4.7, containing 0.10mM $ZnCl_2$, was added to a solution of nucleoside 3′-phosphate **17** (0.3 mg) in 0.1 mL of 0.1M sodium acetate buffer, pH 5.2. The mixture was allowed to react for 2 days at 20°; t.l.c. then demonstrated the complete conversion of nucleoside 3′-phosphate **17** into 3′-*C*-methyluridine (**9**), identical with the nucleoside previously obtained.

1-[2,3-O-(Ethoxymethylidene)-3-C-methyl-β-D-ribofuranosyl]uracil (**19**). — 6M HCl (0.1 mL) in DMF was added to a solution of 1-(3-*C*-methyl-β-D-ribofuranosyl)uracil (174 mg, 0.65 mmol) in DMF (1.2 mL) and triethyl orthoformate (0.5 mL), and the mixture was kept for 16 h at 20°. NEt_3 (0.1 mL) and water (10 mL) were added to the mixture, which was then extracted with ether (10 mL).

The organic layer was washed with water (10 mL), the aqueous layers were combined and extracted with ethyl acetate (5 × 20 mL), and the extracts were combined, dried (Na_2SO_4), filtered, and evaporated to dryness *in vacuo,* to yield 170 mg of oil; R_F 0.18 (*C*). As found by chromatography and p.m.r. spectroscopy, the product contained ~10% of a bis(orthoester) derivative of 3′-*C*-methyluridine; R_F 0.41 (*C*).

Pure compound **19** may be isolated by chromatography on silica gel in system *B*; yield 142 mg (69%) of a syrup; p.m.r. ($CDCl_3$): δ 9.66 (br s, 1 H, NH, exchanged with D_2O), 7.63 (0.5 H, $J_{6,5}$ 8.0 Hz, H-6), 7.55 (d, 0.5 H, $J_{6,5}$ 8.0 Hz, H-6), 6.03 (s, 0.5 H, orthoformate proton), 6.01 (s, 0.5 H, orthoformate proton), 5.92 (d, 0.5 H, $J_{1',2'}$ 4.0 Hz, H-1′), 5.72 (d, 0.5 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.70 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-5), 4.62 (d, 0.5 H, $J_{2',1'}$ 3.5 Hz, H-2′), 4.60 (d, 0.5 H, $J_{2',1'}$ 4.0 Hz, H-2′), 4.20–3.86 (m, 3 H, H-4′,5′a,5′b), 3.68 (q, 1 H, *J* 7.0 Hz, OCH_2CH_3), 3.62 (q, 1 H, *J* 7.0 Hz, OCH_2CH_3), 1.62 (s, 1.5 H, *C*-Me-3′), 1.52 (s, 1.5 H, *C*-Me-3′), 1.24 (t, 1.5 H, *J* 7.0 Hz, OCH_2CH_3), and 1.20 (t, 1.5 H, *J* 7.0 Hz, OCH_2CH_3). The ratio of diastereoisomers (determined by p.m.r. spectroscopy) was 1:1.

Anal. Calc. for $C_{13}H_{18}N_2O_7$: C, 49.68; H, 5.77; N, 8.92. Found: C, 49.51; H, 5.68; N, 8.76.

1-(3-C-Methyl-β-D-ribofuranosyl)uracil 5′-phosphate (**20**). — A solution of crude **19** (140 mg) in M 2-cyanoethyl pyridinium phosphate solution in abs. pyridine (1 mL) and abs. pyridine (3 mL) was evaporated to dryness *in vacuo*; the residue was azeotropically dried with abs. pyridine (3 × 5 mL), dissolved in abs. pyridine (6 mL), 2,4,6-triisopropylbenzenesulfonyl chloride (0.6 g, 2 mmol) was added, and the mixture was kept for 3 h at 20°. Water (5 mL) was added, and the mixture was stirred for 1 h at 20°, washed with ether (2 × 10 mL), diluted with water to 100 mL, and loaded onto a column of DEAE-cellulose (HCO_3^-) (200 mL). The column was washed with water (500 mL), and eluted in a concentration gradient (0.0–0.1M) of NH_4HCO_3 (total vol. 6 L). 2-Cyanoethyl ester was eluted at 0.03M NH_4HCO_3. Fractions containing the product were evaporated with water (5 × 10 mL). The residue was dissolved in M NaOH (13 mL) and, after 20 min, applied to a column of Dowex-50 (H^+) resin (45 mL), and eluted with water. Fractions absorbing in the u.v. and containing the product were pooled, made neutral with M NH_4OH to pH 8, loaded onto a column of DEAE-cellulose (HCO_3^-) (200 mL), and chromatographed with a concentration gradient (0.05–0.2M) of NH_4HCO_3. Fractions absorbing in the u.v. and eluted at concentrations of 0.12 to 0.13M were combined, evaporated *in vacuo,* evaporated with water (5 × 10 mL), and freeze-dried. The yield of diammonium salt of nucleoside 5′-phosphate **20** was 64 mg (32%); R_F 0.51 (*F*) and 0.15 (*E*). The u.v. spectrum was identical with that of uridine 5′-phosphate; p.m.r. (D_2O): δ 8.22 (d, 1 H, $J_{6,5}$ 8.0 Hz, H-6), 6.13 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 6.06 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-5), 4.26 (d, 1 H, $J_{2',1'}$ 8.0 Hz, H-2′), 4.24–3.98 (m, 3 H, H-4′,5′a,5′b), and 1.46 (s, 3 H, *C*-Me-3′); ^{31}P-n.m.r. (D_2O, pH 5): δ +1.17 (s).

1-(3-C-Methyl-β-D-ribofuranosyl)uracil 5′-triphosphate (**21**). — Tributylamine (0.04 mL) was added to a solution of compound **20**, diammonium salt

(50 mg, 134 μmol), in water (2 mL). The mixture was stirred for 2 h at 20°, evaporated to dryness *in vacuo*, azeotropically dried with abs. DMF (3 × 3 mL), the residue dissolved in abs. DMF (3 mL), and 1,1′-carbonyldiimidazole (65.7 mg, 402 μmol) was added to the solution. The mixture was stirred for 16 h at 20°, and M MeOH in DMF (0.7 mL) was added. After 20 min at 20°, a solution of tributylammonium pyrophosphate (0.67 mmol) in DMF (3.5 mL) was added, and the mixture was stirred for 16 h at 20°. The solid was filtered off, and washed with DMF; EtOH (5 mL) was added to the combined filtrates, and the mixture was evaporated to dryness *in vacuo*. A solution of the residue in water (100 mL) was applied to a column of DEAE-cellulose (HCO_3^-) (200 mL), and chromatographed in a concentration gradient (0.0–0.4M) of NH_4HCO_3 (total vol. 6 L). Fractions absorbing in the u.v., and eluted at 0.2 to 0.22M, were pooled, evaporated to dryness *in vacuo*, evaporated with water (5 × 10 mL), and freeze-dried. The yield of ammonium salt of triphosphate **21** was 30 mg (40%); R_F 0.12 (*F*). For uridine 5′-triphosphate, R_F 0.11 (*F*). The u.v. spectrum was identical with that of uridine 5′-triphosphate; p.m.r. (D_2O): δ 8.10 (d. 1 H, $J_{6,5}$ 8.0 Hz, H-6), 6.12 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 6.06 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-5), 4.24 (d, 1 H, $J_{2',1'}$ 8.0 Hz, H-2′), 4.25 (m, 3 H, H-4′,5′a,5′b), and 1.48 (s, 3 H, *C*-Me-3′).

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