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THE SYNTHESIS OF OLIGORIBONUCLEOTIDES—VIII*

THE PREPARATION OF RIBONUCLEOSIDE 2′,5′-BISKETALS

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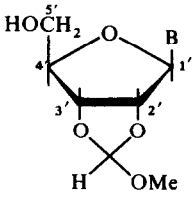
Abstract—The conversion of N^4-benzoylcytidine, N^4-anisoylcytidine, N^2-benzoylguanosine and inosine into their 3′-acetates, and N^6,N^6-dimethyladenosine into its 3′-benzoate by the orthoester exchange method, are described. All these 3′-O-acyl derivatives (IX) were obtained crystalline and in good or satisfactory yields. 2′-O-Acetyl-N^4-anisoylcytidine (XIIIb) and 2′-O-benzoyl-N^6,N^6-dimethyladenosine (XVII) were also obtained crystalline.

The above 3′-O-acyl derivatives, and also 3′-O-acetyl-uridine and -adenosine, were treated with 4-methoxy-5,6-dihydro-2*H*-pyran (X) in the presence of acid to give the corresponding 3′-O-acyl ribonucleoside 2′,5′-bisketals (XI) in good yields. Treatment of the latter with base gave the desired ribonucleoside 2′,5′-bisketals (V). Some of the bisketal esters (XI) and most of the bisketals (V) were isolated as pure crystalline solids.

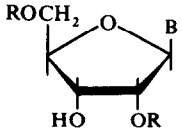
Treatment of the uridine 2′,5′-bisketal (V; B = uracil-1) with aqueous acid under controlled conditions revealed that its 5′-ketal function was ca. twice as labile to acidic hydrolysis as its 2′-ketal function.

CHART 1

Terminal nucleoside units

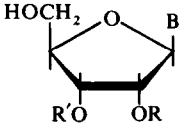


I

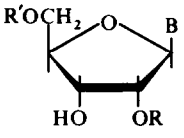


II

Non-terminal nucleoside units



III



IV

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

IN our approach to oligoribonucleotide synthesis,[1] it is desirable to have four building blocks derived from each ribonucleoside: a terminal 2′,3′-, a terminal 2′,5′-, a non-terminal 2′,3′- and a non-terminal 2′,5′-protected derivative (I, II, III and IV

* For part VII of this series, see ref 1.

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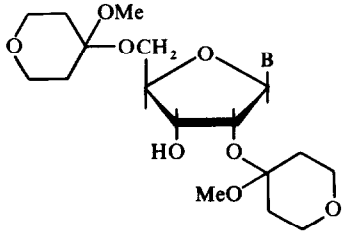
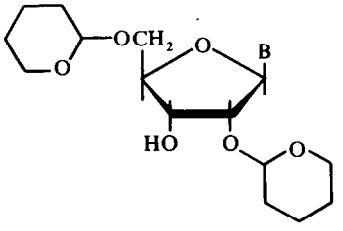
respectively; chart 1). As 2′-OH functions are invariably protected with acid-labile groups,[2] the 3′- or 5′-OH function must be protected with an acid-stable group in a non-terminal (chain-extension) unit (III or IV), and with an acid-labile group in a terminal unit (I or II). The present paper is concerned with the preparation of terminal 2′,5′-protected derivatives (II).



V



VI

We had previously shown[2] that the tetrahydropyranyl group was suitable for the protection of the 2′-OH functions of the ribose moieties in an oligoribonucleotide synthesis. This protecting group can be removed under mild conditions of acidic hydrolysis so that the extent of concomitant fission and isomerization of the internucleotidic linkages is negligible.[2] However, it is advantageous to work with pure crystalline ribonucleoside derivatives, as it is then possible to avoid contamination with position isomers and other impurities which could lead to oligomers with incorrectly orientated internucleotidic linkages.

As the use of the tetrahydropyranyl group leads to mixtures of diasteroisomeric nucleoside derivatives,[2] it seemed desirable to develop a symmetrical acid-labile group with similar hydrolysis properties. For this reason the methoxytetrahydropyranyl* group has been introduced[3,4] as a symmetrical alternative to the tetrahydropyranyl protecting group (see preceding paper), and the preparation of 2′,5′-di-O-methoxytetrahydropyranyl ribonucleosides† (V) rather than that of the corresponding tetrahydropyranyl derivatives (VI) has been undertaken. The inherent difficulty in obtaining the latter compounds in a pure crystalline state became apparent in the preparation of 2′,5′-di-O-tetrahydropyranyluridine (VI; B = uracil-1). The product obtained[5] was resolved into three components by TLC, but none of them could be crystallized.

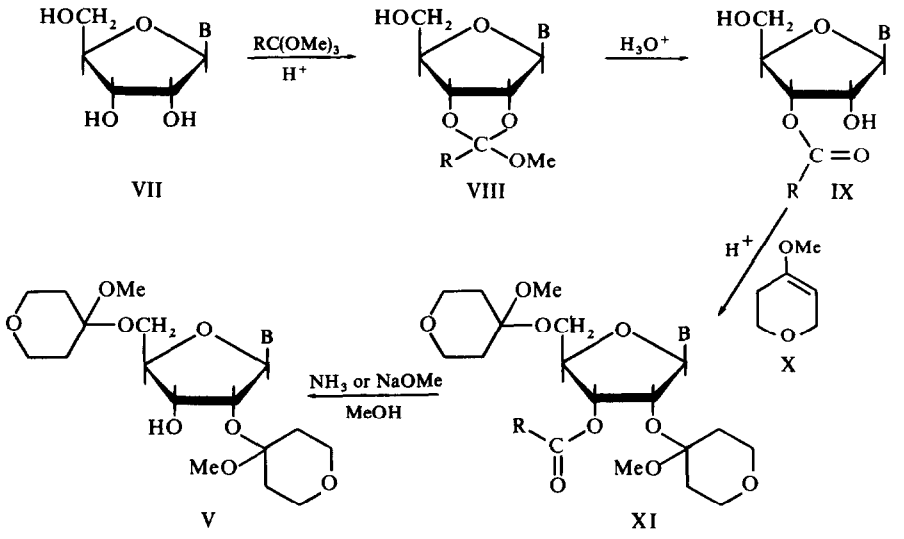
The general scheme for the preparation of ribonucleoside 2′,5′-bisketals (V) is outlined in Chart 2. Acid-catalyzed exchange between a ribonucleoside (VII) [or its N-acyl derivative, see below] and a trimethyl orthoester gives a 2′,3′-O-methoxyalkylidene derivative[6] (VIII). Such cyclic orthoesters may readily be hydrolyzed[6] to mixtures of the corresponding 2′- and 3′-O-acyl derivatives. Solutions of these mixtures in acid-free solvents often deposit the 3′-O-acyl derivatives (IX) in a pure crystalline state.[6] As the isomeric 2′- and 3′-esters generally equilibrate in solution,[7] it is sometimes possible to convert a ribonucleoside into its 3′-O-acyl derivative in

* 4-Methoxytetrahydropyran-4-yl has been abbreviated to methoxytetrahydropyranyl.

† For a preliminary account of some of this work, see reference 3.

good yield by this procedure (Table 1). In certain cases (see below), the 2′-esters have also been obtained crystalline, but the 3′-esters appear to crystallize more readily.*

CHART 2

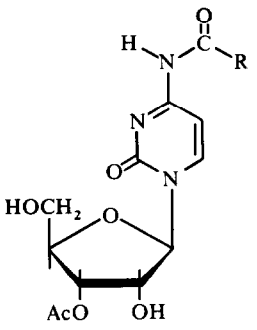


Reaction between a 3′-O-acyl derivative (IX) and an excess of 4-methoxy-5,6-dihydro-2*H*-pyran[3, 4] (X) in the presence of mesitylene- or toluene-*p*-sulphonic acid gives the bisketal ester (XI), generally in good yield (Table 2). The optimum conditions for ketal formation, including the amount of acid-catalyst required, depend on the particular substrate (IX). In some cases, the bisketal esters (XI) can be crystallized (Table 2) but this is not necessary if their saponification products, the bisketals (V), can themselves be obtained crystalline. In the remainder of this paper, preparations of the bisketals of all four common ribonucleosides [or their *N*-acyl derivatives] will be described. In order to illustrate the generality of the methods used, the preparations of the bisketals of two nucleosides which occur less widely in ribonucleic acids (RNA) will also be described.

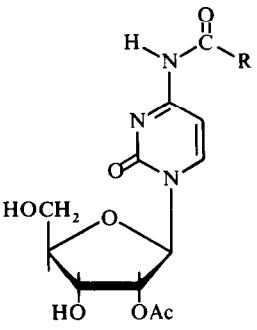
Preparation of 3′-O-acyl derivatives of ribonucleosides

The conversion of both uridine and adenosine to their 3′-O-acetyl derivatives (IX; R = Me, B = uracil-1 and adenine-9, respectively) by the procedure indicated in Chart 2 has already been described.[6] In the cases of cytidine and guanosine, it was considered advisable to prepare derivatives of the N-acyl nucleosides[9, 10] and thereby avoid the possibility of N-phosphorylation at a later stage in the projected oligoribonucleotide synthesis. As it is possible that such undesirable side-reactions might also occur with adenosine derivatives, we intend to work with N-acyl derivatives of adenosine[11] in the future. In the case of guanosine, N-acylation also facilitates crystallization of the intermediates.[6b]

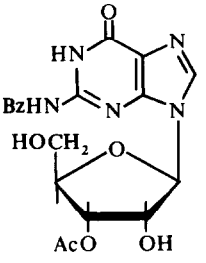
* In the majority of cases examined, the crystalline material deposited from a solution containing a mixture of 2′- and 3′-isomers has been found to be the pure 3′-isomer. However, in several instances[6b, 8], equilibrium mixtures of 2′- and 3′-isomers have deposited the less abundant 2′-ester in a pure crystalline form.



XII a; R = Ph
b; R = *p*-MeO-C_6H_4

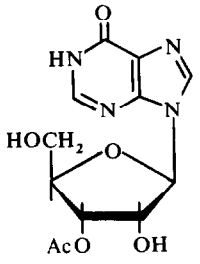


XIII

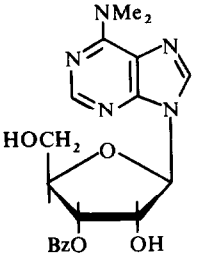


XIV

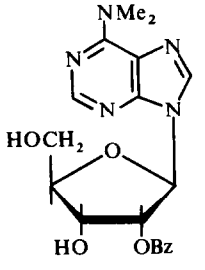
N^4-Benzoyl- and N^4-anisoyl-cytidines were prepared by treating the corresponding N^4, $O^{2'}$, $O^{3'}$, $O^{5'}$-tetraacyl-cytidines with sodium methoxide in methanol-dioxan solution.[9] The N^4-acyl-cytidines were converted into their 3'-esters (XIIa and XIIb, respectively) by the procedure indicated in Chart 2. The latter compounds (XIIa and XIIb) were both isolated crystalline, and in satisfactory yields (Table 1). Chromatography of the acid hydrolysate of the cyclic orthoester of N^4-anisoylcytidine also gave a small quantity of pure 2'-O-acetyl-N^4-anisoylcytidine (XIIIb). The 2'- and 3'-O-acyl derivatives were orientated by n.m.r. spectroscopy.[12] In the same way N^2, $O^{2'}$, $O^{3'}$, $O^{5'}$-tetrabenzoylguanosine was converted into N^2-benzoylguanosine,[13] which was in turn converted into its 3'-acetate (XIV) in good yield (Table 1).



XV



XVI



XVII

As implied above, the methods described in this paper are not restricted in their application solely to the four main ribonucleosides. The generality of the approach is illustrated by the preparation of derivatives of inosine and N^6,N^6-dimethyladenosine. The two latter nucleosides have both been isolated[14, 15] in small quantities from digests of RNA. Crystalline 3'-O-acetylinosine (XV) was readily prepared in satisfactory yield (Table 1) from the parent nucleoside by the usual procedure (Chart 2), but attempts to crystallize the mixture of 2'- and 3'-acetates, obtained by the acid-catalyzed hydrolysis of the 2',3'-cyclic orthoacetate of N^6,N^6-dimethyladenosine, were unsuccessful. However, acidic hydrolysis of the corresponding orthobenzoate (VIII; R = Ph, B = 6-dimethylaminopurine-9) gave a mixture of 2'- and 3'-O-benzoyl-N^6,N^6-dimethyladenosines (XVII and XVI, respectively), which was rich in the latter isomer (XVI). These isomeric esters were separated by chromatography on

silicic acid and were both obtained crystalline; they were identified by n.m.r. spectroscopy[12] and have been the subject of a separate study.[16]

TABLE 1. 3′-O-ACYL DERIVATIVES OF RIBONUCLEOSIDES

Nucleoside derivative	Yield (%)[a]	M.p.
3′-O-Acetyluridine[6b] (IX; R = Me, B = uracil-1)	46[b]	172–174°
3′-O-Acetyladenosine[6b] (IX; R = Me, B = adenine-9)	59	180–181°
3′-O-Acetyl-N^4-benzoylcytidine (XIIa)	59	173–174°
3′-O-Acetyl-N^4-anisoylcytidine (XIIb)	60[c]	175–176°
3′-O-Acetyl-N^2-benzoylguanosine (XIV)	65	194–196°
3′-O-Acetylinosine (XV)	55	208·5–210°
3′-O-Benzoyl-N^6,N^6-dimethyladenosine (XVI)	75[d]	176–177°

[a] Based on nucleoside or N-acyl nucleoside as starting material.
[b] 3′,5′-Di-O-acetyluridine was obtained as a by-product in 34% yield.[6b]
[c] In addition, 2′-O-acetyl-N^4-anisoylcytidine (XIIIb), m.p. 180·5–181°, was obtained in 7% yield.
[d] In addition, 2′-O-benzoyl-N^6,N^6-dimethyladenosine (XVII), m.p. 149–151°, was obtained in 7% yield.

Ribonucleoside 2′,5′-bisketals

The preparation of the desired ribonucleoside 2′,5′-bisketals (V) from the corresponding 3′-O-acyl derivatives (Table 1), according to the procedure outlined in Chart 2 is relatively straightforward. As mentioned above, the experimental conditions of the ketalation reaction, such as the nature of the solvent, quantities of acid-catalyst and of 4-methoxy-5,6-dihydro-2*H*-pyran[3,4] (X) and the reaction time, depend on the starting material (IX). Thus an excess of acid was required in the ketalation of the adenosine and cytidine derivatives. Although satisfactory yields of the intermediate bisketal esters (XI) were obtained in all cases (Table 2), it was by no

TABLE 2. PREPARATION OF 3′-O-ACYL RIBONUCLEOSIDE 2′,5′-BISKETALS (XI) AND RIBONUCLEOSIDE 2′,5′-BISKETALS (V)

Starting Material	Compound XI		Compound V	
	Yield (%)	M.p.	Yield[a] (%)	M.p.
3′-O-Acetyluridine (IX; R = Me, B = uracil-1)	74	102–104°	65	
3′-O-Acetyladenosine (IX; R = Me, B = adenine-9)			52	183–184°
3′-O-Acetyl-N^4-benzoylcytidine (XIIa)	73		52	
3′-O-Acetyl-N^4-anisoylcytidine (XIIb)	75		67	115–116°
3′-O-Acetyl-N^2-benzoyl-guanosine (XIV)	85[b]	179–180·5°	54	173–174°
3′-O-Acetylinosine (XV)	85	199–201°	80	194–195°
3′-O-Benzoyl-N^6,N^6-dimethyl-adenosine (XVI)	88		72	133–133·5°

[a] Overall yields based on 3′-O-acyl ribonucleoside as starting material.
[b] This result is due to Dr. K.-W. Lo (Experimental).

means certain that the optimum reaction conditions had been found in any instance (Experimental). Three of the bisketal esters were isolated in a pure crystalline state (Table 2).

The bisketal esters (XI) were converted into the corresponding bisketals (V) by treatment with methanolic ammonia or sodium methoxide. The latter reagent was used in the case of N-acyl nucleoside derivatives to avoid concomitant removal of the N-acyl residues.[17] In all cases except those of uridine and N^4-benzoylcytidine, the ribonucleoside 2′,5′-bisketals (V) were isolated in a pure crystalline state. However, pure 2′,5′-di-O-methoxytetrahydropyranyl derivatives of both uridine and cytidine are nevertheless available: de-acetylation of the crystalline uridine bisketal ester (XI; R = Me, B = uracil-1) gives 2′,5′-di-O-methoxytetrahydropyranyluridine (V; B = uracil-1) free from position isomers, and 2′,5′-di-O-methoxytetrahydropyranyl-N^4-anisoylcytidine (V; B = N^4-anisoylcytosine-1) may be obtained crystalline in good yield.

It can be seen from Table 2 that the use of the methoxytetrahydropyranyl protecting group leads, in most cases, to good yields of the crystalline terminal 2′,5′-protected building blocks required in oligoribonucleotide synthesis.[1] This conclusion emphasizes the superiority of the methoxytetrahydropyranyl[4] over the conventional tetrahydropyranyl group for the protection of optically-active alcohols.

Finally, a study of the relative rates of hydrolysis of the 2′- and 5′-O-methoxytetrahydropyranyl groups was undertaken. 2′,5′-Di-O-methoxytetrahydropyranyluridine (V; B = uracil-1) was treated with 0·01 N HCl at 20° for 17 min, and the monoketal fraction (a mixture of 2′- and 5′-O-methoxytetrahydropyranyluridines) then isolated by preparative TLC. The latter material was estimated by paper electrophoresis to contain the 2′- and the 5′-ketals in the proportions of 2·5:1. This result, which was confirmed qualitatively by n.m.r. spectroscopy (experimental), indicated that the rate of removal of the 5′-protecting group was ca. twice that of the 2′-protecting group. This result is in accord with the relative rates of acidic hydrolysis of 5′-O-methoxytetrahydropyranylthymidine and 2′-O-methoxytetrahydropyranyluridine.[4]

EXPERIMENTAL

UV absorption spectra were measured with a Cary recording spectrophotometer, model 14M-50. NMR spectra were measured at 100 MHz with a Varian HA 100 spectrometer and at 60 MHz with a Perkin–Elmer spectrometer. Chemical shifts are given in ppm on a τ scale. Me_4Si and Me_3COH were used as internal standards.

Paper electrophoresis on Whatman No. 4 paper was conducted in a CCl_4-cooled apparatus. Plates coated with Merck Kieselgel GF_{254} were used for TLC—the chromatograms were developed with solns of MeOH in $CHCl_3$. Mallinckrodt analytical grade silicic acid (100 mesh), silic AR CC7 (100–200 mesh), and Woelm neutral alumina were used for adsorption chromatography.

Trimethyl orthoacetate and dioxan were dried by heating under reflux with CaH_2 and LAH, respectively, and were redistilled before use. Trimethyl orthobenzoate was purified by fractional distillation under reduced pressure, but it was still contaminated with methyl benzoate.

3′-O-*Acetyl*-2′,5′-*di*-O-*methoxytetrahydropyranyluridine* (XI; R = Me, B = *uracil*-1). 4-Methoxy-5,6-dihydro-2*H*-pyran[4] (7·8 g, 68 mmole) was added to a solution of 3′-O-acetyluridine[6b] (2·5 g, 8·74 mmole) and toluene-*p*-sulphonic acid, monohydrate (0·10 g, 0·53 mmole) in anhyd dioxan (25 ml) at 20°. After 15 min, when TLC indicated that no starting material remained, the products were neutralized with NaOMe/MeOH and concentrated to a gum, which was extracted with $CHCl_3$. The extract was filtered through hyflo-supercel, the filtrate evaporated, redissolved in CH_2Cl_2 and the soln applied to a column of neutral alumina

(grade III, 150 g). After the column had been washed with CH_2Cl_2, it was eluted with $CHCl_3$-MeOH (99:1 v/v) and the eluate evaporated to give a glass. Crystallization of this glass from water (neutralized to pH 7 with aqueous NH_3) gave 3′-O-*acetyl*-2′,5′-*di*-O-*methoxytetrahydropyranyluridine*. [Found: C, 53·8; H, 6·6; N, 5·4. $C_{23}H_{34}N_2O_{11}$ requires: C, 53·6; H, 6·6; N, 5·4%] as colourless crystals (3·07 g, 74%), m.p. 102–104°. UV absorption (95% EtOH): λ_{max} 260 (ε 9,500), λ_{min} 230 nm (ε 2,630): $[\alpha]_D^{23}$ −12·2° (*c* 2, in EtOH): R_f: 0·63 [$CHCl_3$-MeOH (9:1)].

2′,5′-*Di*-O-*methoxytetrahydropyranyluridine* (V; R = Me, B = *uracil*-1). A soln of 3′-O-acetyl-2′,5′-di-O-methoxytetrahydropyranyluridine (0·5 g) in NH_3/MeOH (half saturated at 0°, 10 ml) was allowed to stand at 20° for 16 hr and then evaporated. The residue was dissolved in EtOH and the soln re-evaporated. After this procedure had been repeated several times, the glass obtained was dissolved in $CHCl_3$ and the soln applied to a column of silicic acid (5 g). Elution of the column with $CHCl_3$-MeOH (99:1), followed by evaporation of the solvents gave 2′,5′-*di*-O-*methoxytetrahydropyranyluridine*. [Found: C, 52·7; H, 6·8; N, 5·9. $C_{21}H_{32}N_2O_{10}$ requires: C, 53·4; H, 6·8; N, 5·9%] as a colourless glass (0·46 g) which could not be induced to crystallize. UV absorption (95% EtOH): λ_{max} 262 (ε 9,650), λ_{min} 230 nm (ε 2,320); $[\alpha]_D^{23}$ +2·4° (*c* 1·5, in EtOH); R_f: 0·43 [$CHCl_3$-MeOH (9:1)].

2′,5′-*Di*-O-*methoxytetrahydropyranyladenosine* (V; R = Me, B = *adenine*-9). 4-Methoxy-5,6-dihydro-2*H*-pyran[4] (5·2 g, 45 mmole) was added to a stirred suspension of 3′-O-acetyladenosine[6b] (1·0 g, 3·24 mmole) and toluene-*p*-sulphonic acid, monohydrate (0·65 g, 3·42 mmole) in anhyd dioxan (10 ml) at 20°. After 10 min, when TLC indicated that no starting material remained, the products were neutralized with NaoMe/MeOH and then concentrated. The residual gum was extracted with $CHCl_3$, the extract filtered through hyflo-supercel, the filtrate evaporated and redissolved in CH_2Cl_2. The latter soln was applied to a column of neutral alumina (grade III; 50 g) which was then washed with CH_2Cl_2. Elution of the column with $CHCl_3$-MeOH (99:1) followed by evaporation of the solvents gave a glass, which was redissolved in NH_3/MeOH (half-saturated at 0°, 20 ml). After the soln had stood at 20° for 16 hr, it was evaporated and the residue crystallized from absolute EtOH to give 2′,5′-*di*-O-*methoxytetrahydropyranyladenosine* [Found: C, 53·4; H, 6·7; N, 14·0. $C_{22}H_{33}N_5O_8$ requires: C, 53·4; H, 6·7; N, 14·1%] as colourless crystals (0·84 g, 52%), m.p. 183–184°; UV absorption (95% EtOH): λ_{max} 260 (ε 15,100), λ_{min} 227 nm (ε 3,220); $[\alpha]_D^{23}$ −50° (*c* 1, in EtOH); R_f: 0·41 [$CHCl_3$-MeOH (9:1)].

N^2,$O^{2'}$,$O^{3'}$,$O^{5'}$-*Tetrabenzoylguanosine*.[13] Dry guanosine (19 g, 0·067 mole), freshly distilled PhCOCl (69 g, 0·49 mole) and pyridine (250 ml) were stirred together at 20°. After 4 hr, water (50 ml) was added and, after a further 1 hr, the products were concentrated. The gum, so obtained, was partitioned between $CHCl_3$ (200 ml) and saturated $NaHCO_3$ aq (200 ml). The dried (Na_2SO_4) organic layer was evaporated, dissolved in abs EtOH and re-evaporated. After this latter procedure had been repeated several times, the residue was recrystallized from 2-butanone to give N^2, $O^{2'}$, $O^{3'}$, $O^{5'}$-*tetrabenzoylguanosine* [Found: C, 64·8; H, 4·2; N, 10·0. Calc. for $C_{38}H_{29}N_5O_9$: C, 65·2; H, 4·2; N, 10·0%], m.p. 167–168° (lit[13] 162–165°), yield, 40·0 g (85%); UV absorption (95% EtOH): λ_{max} 233, 267, 285 (ε 54,300, 17,100, 15,200), λ_{infl} 256, 296 (ε 18,300, 14,300), λ_{min} 217, 263, 274 nm (ε 29,500, 16,900, 13,700).

3′-O-*Acetyl*-N^2-*benzoylguanosine* (XIV). Trimethyl orthoacetate (20 g, 0·167 mole) was added to a stirred soln of N^2-benzoylguanosine[13] (7·5 g, 19·4 mmole) and toluene-*p*-sulphonic acid, monohydrate (0·40 g, 2·1 mmole) in anhyd DMF (15 ml). After 2 hr, when TLC indicated that no starting material remained, the products were concentrated under reduced press. The glass, so obtained, was dissolved in aqueous AcOH (50 ml; 1:1 v/v) and the soln concentrated under reduced press. The residue was dissolved in EtOH and the soln re-evaporated. After this procedure had been repeated several times, the residue was dissolved in $CHCl_3$ and the soln applied to a column of silicic acid (80 g). The column was eluted with (a) $CHCl_3$, (b) $CHCl_3$-MeOH (99:1), and (c) $CHCl_3$-MeOH (97:3).

Eluate (b) was concentrated to give an uncharacterized product (probably 2′(3′),5′-di-O-acetyl-N^2-benzoylguanosines). Concentration of eluate (c), and recrystallization of the residue from absolute EtOH gave 3′-O-*acetyl*-N^2-*benzoylguanosine* [Found: C, 52·8; H, 4·6; N, 16·2. $C_{19}H_{19}N_5O_7$ requires: C, 53·1; H, 4·4; N, 16·3%] as colourless crystals (5·44 g, 65%), m.p. 194–196°; NMR spectrum [$(D_3C)_2SO$-D_2O (*M* with respect to AcOH) (9:1, v/v)] included the following signals: τ 1·57 (s), 1H, H(8); τ 4·00 (d, $J \sim 7$ Hz), 1H, H(1′); τ 4·63 (d-d, $J \sim 2$ and 5 Hz), 1H, H(3′); τ 5·15 (d-d, $J \sim 5$ and 7 Hz), 1H, H(2′). UV absorption (95% EtOH): λ_{max} 238, 257, 264, 296, (ε 16,900, 14,500, 14,400, 14,700), λ_{min} 222, 253, 263, 273 nm (ε 13,700, 14,000, 14,000, 10,300); R_f: 0·15 [$CHCl_3$-MeOH (9:1)].

3′-O-*Acetyl*-2′,5′-*di*-O-*methoxytetrahydropyranyl*-N^2-*benzoylguanosine* (XI: R = Me, B = N^2-*benzoylguanine*-9). 4-Methoxy-5,6-dihydro-2*H*-pyran[4] (9·4 g, 82 mmole) was added to a stirred solution of 3′-O-acetyl-N^2-benzoylguanosine (1·0 g, 2·33 mmole) and anhyd mesitylenesulphonic acid (0·14 g, 0·70 mmole)

in dioxan (15 ml) at 20°. After 10 min, when TLC indicated that no starting material remained, the products were neutralized with NaOMe/MeOH and concentrated. The residue was extracted with $CHCl_3$, the extract filtered through hyflo-supercel, evaporated, and redissolved in CH_2Cl_2. The latter soln was applied to a column of neutral alumina (grade III; 80 g), which was then washed with CH_2Cl_2. When the column was eluted with $CHCl_3$-MeOH (99:1), the eluate concentrated and the residue crystallized from EtOH, 3′-O-*acetyl*-2′,5′-*di*-O-*methoxytetrahydropyranyl*-N^2-*benzoylguanosine* [Found: C, 56·8; H, 5·8; N, 10·5. $C_{31}H_{39}N_5O_{11}$ requires: C, 56·7; H, 6·0; N, 10·6%], m.p. 179–180·5°, was obtained, yield,* 0·65 g (41%); UV absorption (95% EtOH): λ_{max} 240, 257, 264, 295 (ε 16,700, 15,200, 14,800, 15,100), λ_{min} 224, 252, 261, 273 nm (ε 13,400, 14,900, 14,700, 10,900); R_f: 0·63 [$CHCl_3$-MeOH (9:1)].

2′,5′-*Di*-O-*methoxytetrahydropyranyl*-N^2-*benzoylguanosine* (V; B = N^2-*benzoylguanine*-9). NaOMe/MeOH (*M*, 0·9 ml) was added to a soln of 3′-O-acetyl-2′-5′-di-O-methoxytetrahydropyranyl-N^2-benzoylguanosine (0·3 g, 0·46 mmole) in dioxan (3 ml) and MeOH (3 ml) at 20°. After 10 min, the products were treated with an excess of Zeo-Karb 225 (pyridinium form) cation-exchange resin, and then filtered. The filtrate was concentrated to a gum, which was dissolved in $CHCl_3$ and the solution applied to a column of silicic acid (5 g). The desired material was eluted from the column with $CHCl_3$-MeOH (99:1). Concentration of this eluate, and recrystallization of the residue from water (neutralized to pH 7 with aqueous NH_3) gave 2′,5′-*di*-O-*methoxytetrahydropyranyl*-N^2-*benzoylguanosine* [Found: C, 56·5; H, 5·9; N, 11·4. $C_{29}H_{37}N_5O_{11}$ requires: C, 56·6; H, 6·0; N, 11·4%], m.p. 173–174°, yield, 0·18 g (64%); UV absorption (95% EtOH): λ_{max} 240, 257, 264, 295 (ε 16,500, 15,200, 14,900, 15,000), λ_{min} 224, 252, 261, 273 nm (ε 13,100, 14,900, 14,600, 10,600).

N^4,$O^{2'}$,$O^{3'}$,$O^{5'}$-*Tetraanisoylcytidine.* Anisoyl chloride (17·5 g, 103 mmole) was added to a stirred soln of cytidine (5·0 g, 20·6 mmole) in pyridine (50 ml) at 20°. After 4 hr, water (20 ml) was added and, after a further 1 hr, the products were concentrated. The gum, so obtained, was partitioned between $CHCl_3$ (100 ml) and saturated aqueous $NaHCO_3$ (100 ml), and the layers separated. The dried (Na_2SO_4) organic layer was evaporated, the residual gum dissolved in EtOH and the solution re-evaporated. After the latter procedure had been repeated several times, the final residue was crystallized from 2-butanone to give N^4,$O^{2'}$,$O^{3'}$,$O^{5'}$-*tetraanisoylcytidine* [Found: C, 63·0; H, 5·0; N, 5·3. $C_{41}H_{37}N_3O_{13}$ requires: C, 63·2; H, 4·8; N, 5·4%], m.p. 162–163°, yield, 11·25 g (70%); UV absorption (95% EtOH): λ_{max} 262 (ε 71,000), λ_{min} 232 nm (ε 17,500).

N^4-*Anisoylcytidine.* Freshly prepared NaOMe/MeOH (*M*, 39 ml) was added to a stirred solution of N^4, $O^{2'}$, $O^{3'}$, $O^{5'}$-tetraanisoylcytidine (5·0 g, 6·42 mmole) in MeOH (50 ml) and dioxan (50 ml) at 20°. After 15 min, the soln was added to an excess of an aqueous slurry of Zeo–Karb 225 (pyridinium form) cation-exchange resin. After they had been stirred for 10 min, the products were filtered, the filtrate concentrated, redissolved in EtOH and the soln evaporated. The solid residue was triturated with ether, and then recrystallized from water to give N^4-*anisoylcytidine* [Found: C, 54·2; H, 5·1; N, 11·1. $C_{17}H_{19}N_3O_7$ requires: C, 54·2; H, 5·1; N, 11·1%], m.p. 178–179°, yield, 2·18 g (90%); U.V. absorption (95% EtOH): λ_{max} 285 (ε 20,400), λ_{min} 236 nm (ε 8,550); R_f: 0·37 [$CHCl_3$-MeOH (9:1)].

2′- and 3′-O-*Acetyl*-N^4-*anisoylcytidines* (XIIIb *and* XIIb, respectively). Trimethyl orthoacetate (3·75 g, 31·3 mmole) was added to a stirred soln of N^4-anisoylcytidine (2·0 g, 5·31 mmole) and mesitylenesulphonic acid (0·2 g, 1·0 mmole) in DMF (5 ml) at 20°. After 15 min, when TLC indicated that no starting material remained, the products were neutralized with NaOMe/MeOH and concentrated under reduced press. The gum, so obtained, was extracted with $CHCl_3$, the extract filtered, evaporated and redissolved in aqueous AcOH (25 ml; 1:1, v/v). The latter soln was concentrated under reduced press, the residue dissolved in EtOH and re-evaporated to a glass, which was then dissolved in $CHCl_3$ and applied to a column of silicic acid (30 g). The column was eluted with (a) $CHCl_3$ and (b) $CHCl_3$-MeOH (99:1).

The first fractions of eluate (b) contained an uncharacterized material (probably 2′(3′),5′-di-O-acetyl-N^4-anisoylcytidines). The middle fractions of eluate (b) were concentrated to a $CHCl_3$-insoluble solid, which was recrystallized from abs EtOH to give 3′-O-*acetyl*-N^4-*anisoylcytidine* [Found: C, 54·2; H, 5·1; N, 10·0. $C_{19}H_{21}N_3O_8$ requires: C, 54·4; H, 5·1; N, 10·0%], m.p. 175–176°, yield, 1·61 g (60%); NMR spectrum [$(D_3C)_2SO$-D_2O (*M* with respect to AcOH) (9:1)] included the following signals: τ 1·47 (d, $J \sim 8$ Hz), 1H, H(6); τ 2·53 (d, $J \sim 8$ Hz), 1H,H(5); τ 4·01 (d, $J \sim 5$ Hz), 1H,H(1′); UV absorption (95% EtOH): λ_{max} 287 (ε 25,400), λ_{min} 237 nm (ε 8,120); R_f: 0·56 [$CHCl_3$-MeOH (9:1)].

The latter fractions of eluate (b) were concentrated to a $CHCl_3$-soluble solid, which was recrystallized

* Recently, Dr. K-W. Lo obtained this product in 85% yield by allowing 3′-O-acetyl-N^2-benzoylguanosine to react with ca. 25 molecular equiv of 4-methoxy-5,6-dihydro-2*H*-pyran for 24 hr at 1–2°.

from absolute EtOH to give the isomeric 2′-O-*acetyl*-N⁴-*anisoylcytidine* [Found: C, 54·3; H, 5·0; N, 9·9%], m.p. 180·5–181°, yield, 0·19 g (7%); NMR spectrum [$(D_3C)_2SO$-D_2O (*M* with respect to AcOH) (9:1)] included the following signals: τ 1·46 (d, *J* ~ 8Hz), 1H,H(6); τ 2·56 (d, *J* ~ 8Hz), 1H,H(5); τ 3·93 (d, *J* ~ 4Hz), 1H,H(1′); R_f: 0·54 [$CHCl_3$-MeOH (9:1)].

2′,5′-Di-O-*methoxytetrahydropyranyl*-N⁴-*anisoylcytidine* (V; B = N⁴-*anisoylcytosine*-1). 4-Methoxy-5,6-dihydro-2*H*-pyran[4] (8·3 g, 73 mmole) was added to a stirred soln of 3′-O-acetyl-N⁴-anisoylcytidine (1·0g, 2·39 mmole) and mesitylenesulphonic acid (0·50 g, 2·5 mmole) in dioxan (15 ml) at 20°. After 10 min, when TLC indicated that no starting material remained, the products were neutralized with NaOMe/MeOH and then concentrated. The gum obtained was extracted with $CHCl_3$, the extract filtered through hyflo-supercel, evaporated and redissolved in CH_2Cl_2. The latter soln was applied to a column of neutral alumina (grade III; 50 g), which was then washed with CH_2Cl_2. Elution of the column with $CHCl_3$ and evaporation of the eluate gave a TLC homogeneous glass (1·16 g, 75%), which was assumed to be 3′-O-acetyl-2′,5′-di-O-methoxytetrahydropyranyl-N⁴-anisoylcytidine (XI; R = Me, B = N⁴-anisoylcytosine-1).

NaOMe/MeOH (*M*, 3·1 ml) was added to a stirred soln of the above glass (1·0 g, 1·55 mmole) in MeOH (5 ml) and dioxan (5 ml) at 20°. After 10 min, the products were treated with an excess of wet Zeo–Karb 225 (pyridinium form) cation-exchange resin. After 10 min, the resin was removed by filtration and the filtrate concentrated. The residue was dissolved in $CHCl_3$ and applied to a column of silicic acid (15 g). The column was eluted with (a) $CHCl_3$ and (b) $CHCl_3$-MeOH (99:1). Evaporation of eluate (b) and crystallization of the residue from water (neutralized to pH 7 with aqueous NH_3) gave 2′,5′-*di*-O-*methoxytetrahydropyranyl*-N⁴-*anisoylcytidine* [Found: C, 57·4; H, 6·5; N, 6·9. $C_{29}H_{39}N_3O_{11}$ requires: C, 57·5; H, 6·5; N, 6·9%], m.p. 115–116°, yield 0·84 g (67%, based on 3′-O-acetyl-N⁴-anisoylcytidine); UV absorption (95% EtOH): λ_{max} 289 (ε 26,200), λ_{min} 238 nm (ε 7,860); R_f: 0·72 [$CHCl_3$-MeOH (9:1)].

3′-O-*Acetyl*-N⁴-*benzoylcytidine* (XIIa). Trimethyl orthoacetate (37·7 g, 314 mmole) was added to a stirred soln of N⁴-benzoylcytidine[9] (12·0 g, 34·6 mmole) and toluene-*p*-sulphonic acid, monohydrate (1·2 g, 6·32 mmole) in dimethylformamide (15 ml) at 20°. After 2 hr, when TLC indicated that no starting material remained, the soln was concentrated under reduced press. The gum obtained was dissolved in aqueous AcOH (75 ml; 1:1, v/v), the soln concentrated under reduced press, the residue dissolved in EtOH and re-evaporated. After the latter procedure had been repeated several times, the residue was dissolved in $CHCl_3$ and applied to a column of silicic acid (150 g). The column was eluted with (a) $CHCl_3$, (b) $CHCl_3$-MeOH (99:1), and (c) $CHCl_3$-MeOH (97:3).

Eluate (b) contained an uncharacterized material (probably 2′(3′),5′-di-O-acetyl-N⁴-benzoylcytidines); eluate (c) was concentrated and the residue recrystallized from EtOH to give 3′-O-*acetyl*-N⁴-*benzoylcytidine* [Found: C, 55·3; H, 5·1; N, 10·6. $C_{18}H_{19}N_3O_7$ requires: C, 55·5; H, 5·1; N, 10·6%], m.p. 173–174°, yield 7·96 g (59%); NMR spectrum [$(D_3C)_2SO$-D_2O (*M* with respect to AcOH) (9:1)] included the following signals: τ 1·46 (d, *J* ~ 8 Hz), 1H,H(6); τ 4·01 (d, *J* ~ 5·5 Hz), 1H,H(1′); UV absorption (95% EtOH): λ_{max} 262, 305 (ε 26,700, 11,100), λ_{min} 230, 287 nm (ε 10,100, 9,050); R_f: 0·49 [$CHCl_3$-MeOH (9:1)].

2′,5′-Di-O-*Methoxytetrahydropyranyl*-N⁴-*benzoylcytidine* (V; B = N⁴-*benzoylcytosine*-1). 4-Methoxy-5,6-dihydro-2*H*-pyran[4] (12·5 g, 0·109 mole) was added to a stirred solution of 3′-O-acetyl-N⁴-benzoylcytidine (1·5 g, 3·86 mmole) and mesitylenesulphonic acid (0·81 g, 4·05 mmole) in dioxan (24 ml) at 20°. After 10 min, when TLC indicated that no starting material remained, the products were neutralized with NaOMe/MeOH and concentrated. The gum obtained was extracted with $CHCl_3$, the extract filtered through hyflo-supercel and concentrated. The residue was dissolved in CH_2Cl_2 and applied to a column of neutral alumina (grade III; 75 g), which was then washed with CH_2Cl_2. Elution of the column with $CHCl_3$, followed by evaporation of the solvent gave a glass (1·74 g, 73%), which was assumed to be 3′-O-acetyl-2′,5′-di-O-methoxytetrahydropyranyl-N⁴-benzoylcytidine (XI; R = Me, B = N⁴-benzoylcytosine-1). Attempts to crystallize this compound failed.

NaOMe/MeOH (*M*, 1·6 ml) was added to a soln of the above glass (0·5 g, 0·81 mmole) in MeOH (5 ml) and dioxan (5 ml) at 20°. After 10 min, the reactants were added to an excess of wet Zeo–Karb 225 (pyridinium form) cation-exchange resin. After a further 10 min, the resin was collected by filtration, the filtrate concentrated and the residue re-dissolved in $CHCl_3$. This soln was applied to a column of silicic acid (5 g). The column was eluted with (a) $CHCl_3$ and (b) $CHCl_3$-MeOH (9:1). Eluate (b) was evaporated to give 2′,5′-di-O-methoxytetrahydropyranyl-N⁴-benzoylcytidine as a TLC homogeneous glass (0·33 g, 52% based on 3′-O-acetyl-N⁴-benzoylcytidine). Attempts to crystallize this compound failed.

3′-O-*Acetylinosine* (XV). Trimethyl orthoacetate (23·6 g, 0·196 mole) was added to a stirred suspension of inosine (10·72 g, 40 mmole) and toluene-*p*-sulphonic acid, monohydrate (0·76 g, 4 mmole) in anhydrous DMF (100 ml) at 20°. After 40 min, the clear soln obtained was found by TLC to contain little unreacted

inosine. The products were neutralized with NaOMe/MeOH, concentrated under reduced press (below 45°) and the residue dissolved in AcOH-H_2O (100 ml; 3:2, v/v). After the soln had been allowed to stand at 20° for 30 min, it was concentrated under reduced press. The residue, so obtained, was dissolved in EtOH and the soln evaporated. After the latter process had been repeated several times, the resultant amorphous solid was dissolved in the minimum quantity of $CHCl_3$-MeOH (10:1), and the soln applied to a column (15 cm × 18 cm^2) of silicic acid. The desired product (8·2 g, 66%) was eluated from the column with $CHCl_3$-MeOH (92:8) and crystallized from MeOH to give 3′-O-*acetylinosine* [Found: C, 46·3; H, 4·5; N, 17·75. $C_{12}H_{14}N_4O_6$ requires: C, 46·45; H, 4·5; N, 18·1%], m.p. 208·5–210°; yield 6·8 g (55%), NMR spectrum [$(D_3C)_2SO$-D_2O (*M* with respect to AcOH) (9:1, v/v)] included the following signals: τ 1·50 (s) and τ 1·75 (s), each 1H,H(2) and H(8); τ 3·94 (d, J ~ 7Hz), 1H,H(1′); τ 4·60 (d-d, J ~ 3 and 5·5Hz), 1H,H(3′); UV absorption (95% EtOH): λ_{max} 243, 250 (ε 10,900, 10,900), λ_{infl} 270 (ε 4,480) λ_{min} 222, 247 nm (ε 3,650, 10,700).

3′-O-*Acetyl*-2′,5′-*di*-O-*methoxytetrahydropyranylinosine* (XI; R = Me, B = *hypoxanthine*-9). 4-Methoxy-5,6-dihydro-2*H*-pyran[4] (5·7 g, 50 mmole) was added to a stirred soln of 3′-O-acetylinosine (0·93 g, 3·0 mmole) and mesitylenesulphonic acid (0·30 g, 1·50 mmole) in DMF (15 ml) at 0°. After 20 hr, when TLC indicated that no starting material remained, the products were carefully neutralized with NaOMe/MeOH and concentrated under reduced press to a gum. The latter was extracted with $CHCl_3$, the extract filtered through hyflo-supercel, and then evaporated. The residue was dissolved in EtOH and the solution re-evaporated. After this process had been repeated several times, the glass obtained was dissolved in $CHCl_3$ and the solution applied to a column (10 cm × 3 cm^2) of SilicAR CC-7. The desired material (1·37 g, 85%) was eluted from the column with $CHCl_3$-MeOH (96:4) and crystallized from MeOH to give 3′-O-*acetyl*-2′,5′-*di*-O-*methoxytetrahydropyranylinosine* [Found: C, 53·3; H, 6·2; N, 10·4. $C_{24}H_{34}N_4O_{10}$ requires: C, 53·5; H, 6·3; N, 10·4%], m.p. 199–201°, UV absorption (95% EtOH): λ_{max} 245 (ε 12,500), λ_{infl} 250, 267 (ε 12,200, 5,430), λ_{min} 223 nm (ε 4,920).

2′,5′-*Di*-O-*methoxytetrahydropyranylinosine* (V; B = *hypoxanthine*-9). NH_3/MeOH (half-saturated at 0°, 12 ml) was added to a soln of 3′-O-acetyl-2′,5′-di-O-methoxytetrahydropyranylinosine (0·93 g) in MeOH at 20°. The reactants were allowed to stand for 16 hr and then concentrated under reduced press to give a homogeneous (TLC) powder (0·81 g, 94%). Recrystallization from MeOH gave 2′,5′-*di*-O-*methoxytetrahydropyranylinosine* [Found: C, 53·6; H, 6·6; N, 11·2. $C_{22}H_{32}N_4O_9$ requires: C, 53·2; H, 6·45; N, 11·3%], m.p. 194–195°; UV absorption (EtOH): λ_{max} 244, 251 (ε 11,300, 11,200), λ_{infl} 270 (ε 4,730), λ_{min} 223, 248 nm (ε 3,560, 11,100).

2′- *and* 3′-O-*Benzoyl*-N^6,N^6-*dimethyladenosines* (XVII *and* XVI, *respectively*). A soln of N^6,N^6-dimethyladenosine[18] (1·366 g, 4·66 mmole) and toluene-*p*-sulphonic acid, monohydrate (0·966 g, 5·08 mmole) in trimethyl orthobenzoate (10 ml) was stirred at 20°. After 3 hr, the products were neutralized with NaOMe/MeOH and then concentrated under reduced press. The resultant syrup was extracted with $CHCl_3$, the extract filtered through hyflo-supercel and then evaporated. The residue was dissolved in AcOH-H_2O (20 ml; 4:1, v/v) and the soln allowed to stand at 20°. After 3 hr, the products were concentrated to dryness, dissolved in CH_2Cl_2 and the soln applied to a column (15 cm × 3·5 cm^2) of silicic acid.

Elution with $CHCl_3$-MeOH (98:2) yielded a material with R_f [$CHCl_3$-MeOH (37:3, v/v)] 0·75, which crystallized from EtOH to give 3′-O-*benzoyl*-N^6,N^6-*dimethyladenosine* [Found: C, 57·4; H, 5·3; N, 17·4. $C_{19}H_{21}N_5O_5$ requires: C, 57·1; H, 5·3; N, 17·5%], m.p. 176–177°; yield 1·394 g (75%). NMR spectrum [$(D_3C)_2SO$-D_2O (0·1 *N* with respect to HCl) (20:3, v/v)] included the following signals: τ 1·53 (s) and τ 1·71 (s), each 1H,H(2) and H(8); τ 3·86 (d, J = 7Hz), 1H,H(1′); τ 4·40 (d-d, J ~ 2 and 5·5Hz), 1H,H(3′); τ 4·95 (d-d, J ~ 5·5 and 7Hz), 1H,H(2′); τ 5·59 (m), 1H,H(4′); UV absorption (95% EtOH): λ_{max} 219, 275 (ε 22,400, 19,400), λ_{min} 249 nm (ε 6,300).

Elution with $CHCl_3$-MeOH (97:3) yielded a material with R_f [$CHCl_3$-MeOH (37:3, v/v)] 0·63 which crystallized from EtOH to give 2′-O-*benzoyl*-N^6,N^6-*dimethyladenosine* [Found: C, 57·1; H, 5·3; N, 17·2%], m.p. 149–151°; yield 0·128 g (7%); NMR spectrum [$(D_3C)_2SO$-D_2O (0·1 *N* with respect to HCl) (20:3; v/v)] included the following signals: τ 1·48 (s) and 1·74 (s), each 1H,H(2) and H(8); τ 3·58 (d, J = 6Hz), 1H,H(1′); τ 4·14 (t); 1H,H(2′); τ 5·34 (d-d), 1H,H(3′); τ 5·76 (m), 1H,H(4′), UV absorption (95% EtOH): λ_{max} 219, 275 (ε 22,000, 19,000), λ_{min} 249 nm (ε 6,250).

3′-O-*Benzoyl*-2′,5′-*di*-O-*methoxytetrahydropyranyl*-N^6,N^6-*dimethyladenosine* (XI; R = Ph, B = 6-*dimethylaminopurine*-9). Freshly prepared 4-methoxy-5,6-dihydro-2*H*-pyran[4] (0·32 g, 2·8 mmole) was added to a stirred soln of 3′-O-benzoyl-N^6,N^6-dimethyladenosine (0·093 g, 0·23 mmole) and toluene-*p*-sulphonic acid, monohydrate (0·047 g, 0·25 mmole) in anhydrous dioxan (9 ml) at 20°. After 10 min, the products were carefully neutralized with NaOMe/MeOH, concentrated under reduced press and the residual oil extracted with CH_2Cl_2. The extract was filtered through hyflo-supercel, concentrated and applied to a column

(10 cm × 0·6 cm²) of Woelm neutral alumina (grade III). Elution with CH_2Cl_2 and concentration of the eluate gave the desired product (0·119 g, 88%).

2′,5′-Di-O-methoxytetrahydropyranyl-N⁶,N⁶-dimethyladenosine (V; B = *6-dimethylaminopurine-9*). The above bisketal benzoate (0·218 g) was dissolved in NH_3/MeOH (half-saturated at 0°; 5 ml), and the soln allowed to stand at 20°. After 48 hr, the products were concentrated to dryness, dissolved in CH_2Cl_2 and the soln applied to a column (15 cm × 1 cm²) of Woelm neutral alumina (grade III). Elution with $CHCl_3$ and concentration of the eluate gave the desired product (0·147 g, 82%). This material was crystallized from EtOAc to give *2′,5′-di-O-methoxytetrahydropyranyl-N⁶,N⁶-dimethyladenosine* [Found: C, 54·8; H, 7·0; N, 13·0. $C_{24}H_{37}N_5O_8$ requires: C, 55·0; H, 7·1; N, 13·4%], m.p. 133–133·5°; UV absorption (95% EtOH): λ_{max} 214, 275 (ε 21,500, 22,100), λ_{min} 235 nm (ε 1,760).

Partial acidic hydrolysis of 2′,5′-di-O-methoxytetrahydropyranyluridine. 2′,5′-Di-O-methoxytetrahydropyranyluridine (0·15 g) was dissolved in 0·01 *N* HCl (15 ml) at 20°. After 17 min, the reactants were neutralized with 0·01 *N* KOH. The neutral soln was evaporated to dryness under reduced press, and the residue subjected to preparative TLC [$CHCl_3$-MeOH (92:8)]. The band containing an unresolved mixture of 2′- and 5′-O-methoxytetrahydropyranyluridines (the mono-ketal fraction) was eluted with MeOH, and the eluate concentrated. The residue was examined by paper electrophoresis and NMR spectroscopy.

Paper electrophoresis [0·1 *M* sodium borate buffer (pH 7·5)] resolved the mixture into (a) a non-mobile component, and (b) a component which migrated towards the anode. Equal areas of paper containing these two components and a blank were cut out; each piece of paper was then soaked in 0·1 *N* HCl (5 ml) for 16 hr. The optical densities of the eluates derived from components (a) and (b) were measured (with a Zeiss model PMQ II spectrophotometer) against the blank. The ratio (a)/(b) was found to be 2·5.

The NMR spectrum (D_2O) of 2′-O-methoxytetrahydropyranyluridine[3,4] included the following signals: τ 2·09 (d, J ~ 8Hz), H(6); τ 3·92 (d, J ~ 7Hz), H(1′); τ 4·06 (d, J ~ 8Hz), H(5). The NMR spectrum of the above mono-ketal fraction included all the above signals; it also included a doublet (J ~ 8Hz) at τ 2·04 with an intensity ca. 0·5 times that of the doublet at τ 2·09. The τ4 region of the spectrum of the mono-ketal fraction was too complicated to allow an estimate of the composition of the mixture to be made.

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