# The Synthesis of Oligoribonucleotides. Part XI.[1] Preparation of Ribonucleoside 2′-Acetal 3′-Esters by Selective Deacylation

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The rates of deacylation of 5′-*O*-acetyl-, -methoxyacetyl-, -phenoxyacetyl-, -formyl-, and -chloroacetyl-uridines (10a—e, respectively) in aqueous and methanolic ammonia have been measured. From these data, a procedure has been developed for the general preparation of 2′-*O*-(methoxytetrahydropyranyl)-3′-*O*-acyl ribonucleosides (9) by selective deacylation of suitably designed 2′-*O*-(methoxytetrahydropyranyl)-3′,5′-di-*O*-acyl derivatives (8).

Two series of 2′-acetal 3′-esters (9), designed as building blocks for oligoribonucleotide synthesis, have been prepared from each of the four main ribonucleosides: one series (derived from uridine, 4-*N*-benzoylcytidine, adenosine, and 2-*N*-benzoylguanosine) consists of 3′-acetates or -benzoates and the other series (derived from uridine, 4-*N*-*p*-anisoylcytidine, 6-*N*-*p*-anisoyladenosine, and 2-*N*-benzoylguanosine) consists of 3′-methoxyacetates. All these 2′-*O*-(methoxytetrahydropyranyl)-3′-*O*-acyl ribonucleosides (9) have been obtained in satisfactory yields and all except one have been isolated as pure crystalline solids.

In our approach to oligoribonucleotide [2,3] synthesis 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′-derivative [(1)—(4), respectively; see Scheme 1]. The 2′-hydroxy-functions are always blocked by acid-labile protecting groups; in the terminal building blocks (1) and (2), the 3′- and 5′-hydroxy-functions, respectively, are also protected by acid-labile groups, but in the non-terminal building blocks (3) and (4), the 3′- and 5′-hydroxy-functions, respectively, are protected by base-labile groups. We have undertaken the preparation of all four types of specifically blocked ribonucleoside derivative [2] primarily for use in oligoribonucleotide synthesis. However, such compounds will undoubtedly be useful for a number of other purposes: indeed, we have

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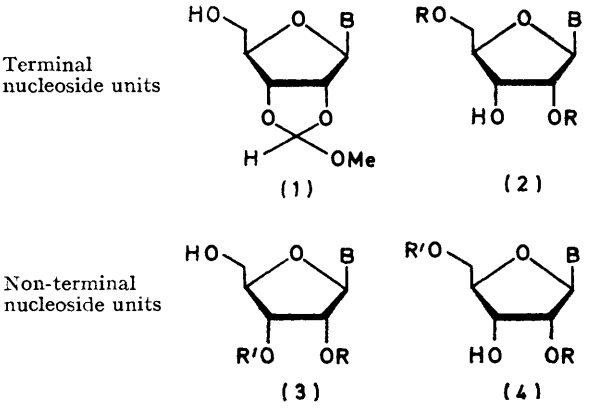
[1] Part X, J. H. van Boom, J. F. M. de Rooy, and C. B. Reese, *J.C.S. Perkin I*, 1973, 2513.

[2] C. B. Reese, Colloques Internationaux du C.N.R.S., Paris, 1970, No. 182, p. 319.

[3] J. H. van Boom, P. M. J. Burgers, G. R. Owen, C. B. Reese, and R. Saffhill, *Chem. Comm.*, 1971, 869.

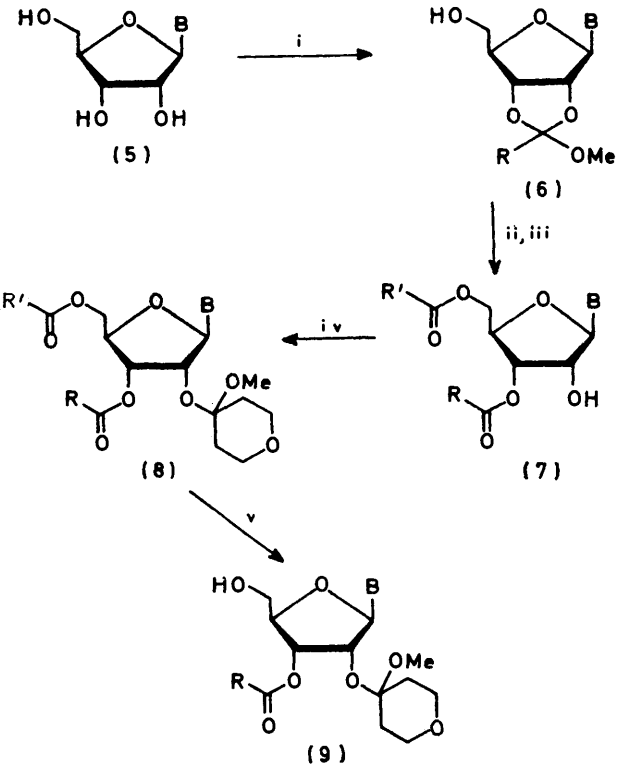
already demonstrated the value of the 2′,5′-terminal [1] and non-terminal [4] derivatives, (2) and (4), in the synthesis of ribonucleoside 2′,3′- and 3′,5′-cyclic phosphates, respectively. In previous papers we have described the preparation of building blocks of types (1),[5] (2),[6] and (4).[7] We now report, in detail, the preparation of the non-terminal 2′,3′-protected ribonucleoside building blocks (3).[8]

The general procedure which has been developed for the synthesis of 2′,3′-non-terminal building blocks (3) depends on the principle of selective deacylation; the procedure is outlined in Scheme 2. A ribonucleoside (or *N*-acyl ribonucleoside) (5) is allowed to undergo acid-catalysed exchange with a trimethyl orthoester [$RC(OMe)_3$] to give a 2′,3′-*O*-(methoxyalkylidene) derivative [9] (6). The latter is then 5′-*O*-acylated with a carboxylic acid chloride or anhydride. The carboxylic acid ($R'CO_2H$) is selected so that its esters will be appreciably more susceptible to solvolysis, under basic conditions, than corresponding esters derived from the orthoester carboxylic acid ($RCO_2H$). After acylation, the fully protected intermediate is subjected to mild acidic hydrolysis to give a mixture of the 3′,5′-di-*O*-acyl derivative (7) and its 2′,5′-isomer. It has been found [9] that satisfactory yields of pure 3′,5′-di-*O*-acyl derivatives (7) often crystallize from acid-free solutions of such mixtures of isomers. Reaction between a pure 3′,5′-di-*O*-acyl derivative and an excess of 5,6-dihydro-4-methoxy-2*H*-pyran [10] in the presence of an acid catalyst [step (iv)] gives the fully protected acetal diester (8). Treatment of the latter with ammonia, under controlled




SCHEME 1 R is an acid-labile (acetal) protecting group; R′ is a base-labile (acyl) protecting group

conditions, gives the desired 2′-acetal 3′-ester (9) often in good yield. The final reaction [step (v)] is the selective deacylation; its efficiency depends on the choice of the groups R and R′ and on steric factors. Thus the fact that this procedure involves the preferential solvolysis of an ester group derived from a primary alcohol is clearly advantageous.




SCHEME 2 Reagents: i, $RC(OMe)_3$, $H^+$; ii, R′COCl or $(R'CO)_2O$–$C_5H_5N$; iii, $H_3O^+$; iv, 5,6-dihydro-4-methoxy-2*H*-pyran, $H^+$; v, $NH_3$

Possible combinations of acyl groups (RCO and R′CO) are suggested by the data in Table 1. The

TABLE 1

Action of ammonia on 5′-*O*-acyluridine derivatives (10) at 22°

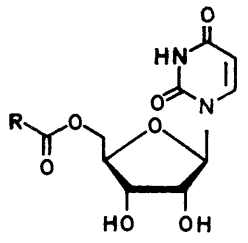
| Substrate | $t_{\frac{1}{2}}$/min Reagent I [a] | Reagent II [b] |
|---|---|---|
| (10a) | 191 | 59 |
| (10b) | 10·4 | 2·5 |
| (10c) | 3·9 | <1 [c] |
| (10d) | 0·4 | (0·22) [d] |
| (10e) | 0·28 | (0·17) [d] |

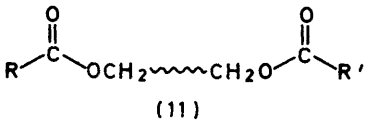
[a] Aqueous 0·155M-ammonia (pH 10·77) used in at least ten-fold excess. [b] This reagent was prepared by diluting saturated (at 0°) methanolic ammonia with an equal volume of methanol. [c] This reaction was too fast to measure. [d] Figures in parentheses represent times by which complete solvolysis of the substrate had occurred.

relative rates of solvolysis of 5′-*O*-acetyl-, 5′-*O*-methoxyacetyl-, 5′-*O*-phenoxyacetyl-, 5′-*O*-formyl-, and 5′-*O*-chloroacetyl-uridine (10a—e, respectively) in dilute aqueous ammonia (reagent I) are *ca.* 1 : 17 : 49 : 480 : 680.

[4] J. H. van Boom, P. M. J. Burgers, P. van Duersen, and C. B. Reese, *J.C.S. Chem. Comm.*, 1974, 618.
[5] B. E. Griffin, M. Jarman, C. B. Reese, and J. E. Sulston, *Tetrahedron*, 1967, **23**, 2301.
[6] D. P. L. Green, T. Ravindranathan, C. B. Reese, and R. Saffhill, *Tetrahedron*, 1970, **26**, 1031.
[7] J. H. van Boom, G. R. Owen, J. Preston, T. Ravindranathan, and C. B. Reese, *J. Chem. Soc.* (*C*), 1971, 3230.
[8] Preliminary report, C. B. Reese and J. C. M. Stewart, *Tetrahedron Letters*, 1968, 4273.
[9] H. P. M. Fromageot, B. E. Griffin, C. B. Reese, and J. E. Sulston, *Tetrahedron*, 1967, **23**, 2315.
[10] C. B. Reese, R. Saffhill, and J. E. Sulston, *Tetrahedron*, 1970, **26**, 1023.

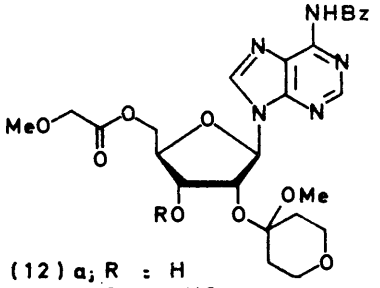
If selective deacylation of a diester such as (11), derived from a diol with its hydroxy-groups in similar environments, is to proceed in good yield, it is necessary that





(11)

(10) a; R = Me
b; R = $CH_2$·OMe
c; R = $CH_2$·OPh
d; R = H
e; R = $CH_2Cl$



(12) a; R = H
b; R = CHO

one of its ester functions should undergo solvolysis at *ca.* 50 times the rate of the other. However, as suggested above, the conversion of compounds of type (8) into type (9) is more favourable in that it involves the selective solvolysis of an ester function derived from a primary in the presence of one derived from a secondary hydroxy-group. No doubt the presence of the bulky 2′-*O*-(methoxytetrahydropyranyl) group further favours selective deacylation. The results of the present study suggest that compounds of type (8) may be converted into compounds of type (9), in satisfactory yields, if R′ and R are such that the relative rates of solvolysis of the corresponding uridine 5′-esters are about 20 : 1. We have previously reported [7] the preparation of 6-*N*-benzoyl-5′-*O*-methoxyacetyl-2′-*O*-(methoxytetrahydropyranyl)adenosine (12a), in moderate yield, from its 3′-formate ester (12b). Thus the combination of formyl and methoxyacetyl can be used with some success even in selective 3′-deacylation. The new 5′-*O*-acyl uridine derivatives (10b, c, and e) included in Table 1 were all prepared from 2′,3′-*O*-isopropylideneneuridine (see Experimental section).

The preparation of 2′-*O*-(methoxytetrahydropyranyl)-3′-*O*-acyl derivatives of each of the common ribonucleosides was undertaken (see Table 2). In the first place, 2′-*O*-(methoxytetrahydropyranyl)-3′-*O*-acetyl (or -benzoyl) derivatives [9; R = Me (or Ph)] were prepared (Table 2, experiments 1, 3, 5, and 7), but it was subsequently found [3] that a more base-labile acyl group was required if the derivatives were to be used as building blocks in the synthesis of oligoribonucleotides by the phosphotriester approach. For this reason, the preparation of the corresponding 2′-*O*-(methoxytetrahydropyranyl)-3′-*O*-methoxyacetyl ribonucleosides (9; R = $CH_2$·OMe) was undertaken (Table 2, experiments 2, 4, 6, and 8).

The first group of compounds (experiments 1, 3, 5, and 7) was synthesized by selective removal of a 5′-*O*-methoxyacetyl in the presence of a 3′-*O*-acetyl or -benzoyl group. This transformation was effected by treating the fully protected intermediates (8; R = Me or Ph, R′ = $CH_2$·OMe) with methanolic ammonia under carefully controlled conditions (see Experimental section). The base residues of the cytidine and guanosine derivatives (experiments 3 and 7) were protected by *N*-benzoylation.[11] The intermediate 3′,5′-diesters (7) all crystallized readily from mixtures containing the isomeric 2′,5′-diesters and the compounds obtained were found, by n.m.r. spectroscopy,[12] to be isomerically pure. The mixtures of 2′,5′- and 3′,5′-diesters were prepared by

TABLE 2

Preparation of 3′,5′-di-*O*-acyl ribonucleoside derivatives (7) and 2′-*O*-(methoxytetrahydropyranyl)-3′-*O*-acyl ribonucleosides (9)

| | | | | | Compound (7) | | Compound (9) | |
|---|---|---|---|---|---|---|---|---|
| Expt. no. | Nucleoside | B | R | R′ | M.p. (°C) | Yield [a] (%) | M.p. (°C) | Yield [b] (%) |
| 1 | Uridine | Uracil-1-yl | Me | MeO·$CH_2$ | 142—143 | 74 [c] | 202—203 | 78 |
| 2 | Uridine | Uracil-1-yl | MeO·$CH_2$ | H | 125 | 44 | 170 | 66 |
| 3 | Cytidine | 4-*N*-Benzoylcytosin-1-yl | Me | MeO·$CH_2$ | 99—102 | 68 [c,d] | 203—204 | 48 |
| 4 | Cytidine | 4-*N*-*p*-Anisoylcytosin-1-yl | MeO·$CH_2$ | $ClCH_2$ | 134 | 60 | | 77 [e] |
| 5 | Adenosine | Adenin-9-yl | Ph | MeO·$CH_2$ | 198—199 | 69 [c] | 233—235 | 60 |
| 6 | Adenosine | 6-*N*-*p*-Anisoyladenin-9-yl | MeO·$CH_2$ | H | 81—82 | 60 | 128—131 | 60 |
| 7 | Guanosine | 2-*N*-Benzoylguanin-9-yl | Me | MeO·$CH_2$ | 179—180 | 74 [c] | 214—215 | 67 |
| 8 | Guanosine | 2-*N*-Benzoylguanin-9-yl | MeO·$CH_2$ | H | 173 | 77 | 190 | 66 |

[a] Yields are based on the corresponding 2′,3′-*O*-(methoxyalkylidene) derivatives (6) as starting materials. [b] Overall yield based on the corresponding 3′,5′-di-*O*-acyl derivative (7). [c] These percentages represent yields of mixtures of 3′,5′-di-*O*-acyl ribonucleosides and their 2′,5′-isomers. High recoveries of pure crystalline 3′,5′-derivatives (7) were obtained from these mixtures. [d] In this case, the isomeric 2′-O-acetyl-4-*N*-benzoyl-5′-*O*-methoxyacetylcytidine was also obtained crystalline; m.p. 155—157°. [e] This compound has not yet been obtained crystalline.

[11] M. Smith, D. H. Rammler, I. H. Goldberg, and H. G. Khorana, *J. Amer. Chem. Soc.*, 1962, **84**, 430; D. H. Rammler and H. G. Khorana, *ibid.*, p. 3112.

[12] H. P. M. Fromageot, B. E. Griffin, C. B. Reese, J. E. Sulston, and D. R. Trentham, *Tetrahedron*, 1966, **22**, 705.

treating the corresponding 2′,3′-*O*-(methoxyalkylidene) derivatives (6; R = Me or Ph) with methoxyacetic anhydride in pyridine and then submitting the products to mild acidic hydrolysis. The 2′,3′-*O*-(methoxyalkylidene) derivatives themselves were prepared [9] by allowing the appropriate ribonucleosides (or *N*-benzoyl derivatives) to undergo acid-catalysed exchange with trimethyl orthoacetate or orthobenzoate. As can be seen from Table 2 (experiments 1, 3, 5, and 7), satisfactory yields of both the 3′,5′-diesters (7) and the desired 2′-acetal 3′-esters (9) were obtained by this approach.

The second group of compounds (experiments 2, 4, 6, and 8) was synthesized by selective removal of a 5′-*O*-formyl or 5′-*O*-chloroacetyl [13] (experiment 4) in the presence of a 3′-*O*-(methoxyacetyl) group. The fully protected intermediates (8; R = $CH_2$·OMe, R′ = H or $CH_2Cl$) were treated with ammonia and the products concentrated, almost immediately, under reduced pressure to give satisfactory yields of the desired 2′-*O*-(methoxytetrahydropyranyl)-3′-*O*-methoxyacetyl derivatives (9; R = $CH_2$·OMe). The base residues of the cytidine and adenosine derivatives (experiments 4 and 6) were protected by *N*-*p*-anisoylation and the base residue of the guanosine derivative (experiment 8) by *N*-benzoylation.

The mixtures of 3′,5′-diesters (7; R = $CH_2$·OMe, R′ = H or $CH_2Cl$) and their 2′,5′-isomers were prepared by treating the corresponding dimethoxyethylidene derivatives (6; R = $CH_2$·OMe) with either formic acetic anhydride [14] or, in the case of the cytidine derivative (experiment 4), with chloroacetic anhydride [13] in pyridine and then submitting the products to acidic hydrolysis. As in the previous experiments (1, 3, 5, and 7), the desired 3′,5′-diesters (7; R = $CH_2$·OMe, R′ = H or $CH_2Cl$) all crystallized from the mixtures of isomers obtained. The 2′,3′-*O*-(dimethoxyethylidene) derivatives (6; R = $CH_2$·OMe) were prepared by treating the appropriate ribonucleosides (or *N*-acyl derivatives), in the presence of a slight excess of mesitylenesulphonic acid, with *ca.* 1·5 mol. equiv. of trimethyl orthomethoxyacetate [7] in anhydrous methanol. This is a particularly convenient general procedure for the preparation of 2′,3′-*O*-(alkoxyalkylidene) derivatives of ribonucleosides (6) in that it is economical in the consumption of trialkyl orthoesters and yields are high. An added advantage is that bis-orthoester derivatives [9] do not appear to be formed under these conditions. The 2′-*O*-(methoxytetrahydropyranyl)-3′-*O*-methoxyacetyl ribonucleosides (9; R = $CH_2$·OMe), prepared in this way, have been found to be useful intermediates in the synthesis of oligoribonucleotides by the phosphotriester approach.[3]

## EXPERIMENTAL

N.m.r. spectra were measured at 60 MHz with a Perkin-Elmer spectrometer and at 100 MHz with Varian HA 100 and JEOL JNM PS 100 spectrometers. Tetramethylsilane, sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate and t-butyl alcohol were used as internal standards. U.v. absorption spectra were measured with a Cary 14M-50 recording spectrometer; optical densities in the kinetic experiments were measured with a Zeiss PMQ II spectrophotometer.

Mallinckrodt silicic acid, SilicAR CC4, SilicAR CC7, and Woelm neutral alumina were used for adsorption chromatography. Plates coated with Merck Kieselgel $GF_{254}$ were used for t.l.c., with $CHCl_3$–MeOH as solvent in the following proportions (v/v): A, 75 : 25; B, 85 : 15; C, 90 : 10; D, 92 : 8; E, 80 : 20.

Pyridine, dioxan, and trimethyl orthoacetate were dried by heating with $CaH_2$, under reflux, and were then redistilled. Dimethylformamide was dried by stirring with $CaH_2$ at room temperature for 24 h; it was then distilled under reduced pressure. Mesitylenesulphonic acid was dried *in vacuo* ($P_2O_5$) at room temperature for 24 h.

*Acetic Formic Anhydride.*—Formic acid (46·0 g, 1 mol), dried by heating under reflux over phthalic anhydride for 6 h, was added dropwise during 10 min to stirred, redistilled acetic anhydride (102·0 g, 1 mol) at 0°. Care was taken to exclude moisture. The solution was then heated on a water-bath at 40—45° for 1 h. It is essential that the temperature is not allowed to rise above 50°. This experiment may be scaled either up or down but the product should be used as soon as prepared.

*Methoxyacetic Anhydride.*[14]—Methoxyacetic acid (20 g, 0·22 mol) and redistilled acetic anhydride (20 g, 0·196 mol) were heated together under gentle reflux for 24 h. The products were then fractionally distilled through a glass helices column to give methoxyacetic anhydride (12·3 g, 67%), b.p. 118—120° at 15 mmHg; τ ($CCl_4$) 5·89 (4H, s) and 6·63 (6H, s); $\nu_{max.}$ (film) 1770 and 1840 $cm^{-1}$.

*Phenoxyacetic Anhydride.*—A solution of *NN*′-dicyclohexylcarbodi-imide (11·5 g, 0·055 mol) in anhydrous tetrahydrofuran (15 ml) was added dropwise to a stirred solution of phenoxyacetic acid (15·2 g, 0·10 mol) in anhydrous tetrahydrofuran (15 ml) at room temperature. After 1 h, the precipitate of *NN*′-dicyclohexylurea was filtered off and washed with tetrahydrofuran. The combined filtrate and washings were evaporated under reduced pressure to give an oil. Crystallization from anhydrous ether gave phenoxyacetic anhydride (12·3 g, 86%), m.p. 66—68° (lit.,[15] 67—69°).

5′-O-(*Methoxyacetyl*)*uridine* (10b).—Methoxyacetic anhydride (2·1 g, 13 mmol) was added to a stirred solution of 2′,3′-*O*-isopropylideneuridine [9] (1·98 g, 6·92 mmol) in anhydrous pyridine (15 ml) at 20°. After 2 h, methanol (5 ml) was added, and after a further 30 min the products were concentrated under reduced pressure. The gum so obtained was dissolved in 80% formic acid (30 ml) and the solution set aside at 20°. After 72 h the products were concentrated under reduced pressure, the residue was dissolved in chloroform, and the solution was applied to a column of silicic acid. Elution with $CHCl_3$–MeOH (98 : 2) gave 5′-O-*methoxyacetyluridine* (0·96 g, 47%) (Found: C, 45·65; H, 4·95; N, 9·1. $C_{12}H_{16}N_2O_8$ requires C, 45·6; H, 5·1; N, 8·9%), m.p. 134—135° (from ethanol); $\lambda_{max.}$ (95% EtOH) 261 (ε 9500), $\lambda_{min.}$ 230 nm (1780).

5′-O-*Phenoxyacetyluridine* (10c).—Phenoxyacetic anhydride (0·75 g, 2·62 mmol) was added to a stirred solution

[13] A. F. Cook and D. T. Maichuk, *J. Org. Chem.*, 1970, **35**, 1940.
[14] Eastman Kodak Co., U.S.P., 2,017,182/1932.
[15] M. Koller and P. De Ruggien, *Boll. soc. ital. biol. sper.*, 1955, **31**, 1154.

of 2′,3′-*O*-isopropylideneuridine [9] (0·58 g, 2·03 mmol) in anhydrous pyridine (10 ml) at 20°. After 2 h ethanol (3 ml) was added and after a further 30 min the products were concentrated under reduced pressure. The gum so obtained was dissolved in 90% formic acid (10 ml) and the solution set aside at 20°. After 72 h the products were concentrated under reduced pressure and the residue chromatographed on silicic acid to give 5′-O-*phenoxyacetyluridine* (0·30 g, 39%) (Found: C, 54·2; H, 5·05; N, 7·9. $C_{17}H_{18}N_2O_8$ requires C, 54·0; H, 4·8; N, 7·4%), m.p. 122—123° (from ethanol); $\lambda_{max.}$ (95% EtOH) 263 (ε 10,600), $\lambda_{min.}$ 232 nm (2400).

5′-O-*Formyluridine* (10d).—This compound was prepared from 3′,5′-di-*O*-formyluridine by the previously reported procedure; [9] τ [$(D_3C)_2SO$] 1·60 (1H, s), 2·24 (1H, d, *J* 8 Hz), 4·14 (1H, d, *J* 4 Hz), and 4·20 (1H, d, *J* 8 Hz).

5′-O-*Chloroacetyluridine* (10e).—Chloroacetic anhydride (1·7 g, 10 mmol) was added to a stirred solution of 2′,3′-*O*-isopropylideneuridine [9] (1·42 g, 5 mmol) in anhydrous pyridine (50 ml) at −30° ($CCl_4$–solid $CO_2$ bath). After 3 h methanol (10 ml) was added and the products were allowed to warm to room temperature. Evaporation under reduced pressure gave a yellow glass which was dissolved in chloroform and applied to a column of SilicAR CC7 (20 g). Elution with $CHCl_3$–MeOH (98 : 2) and evaporation of appropriate fractions gave a t.l.c.-homogeneous [$R_F$ 0·64 (system A)] glass (1·4 g). This was dissolved in 95% formic acid (20 ml) and set aside at 20°. After 2 h the products were concentrated at 0·1 mmHg and the residue kept *in vacuo* over KOH for 24 h. Crystallization from ethanol gave 5′-O-*chloroacetyluridine* (1·15 g, 72%) (Found: C, 41·1; H, 3·9; N, 8·5. $C_{11}H_{13}ClN_2O_7$ requires C, 41·2; H, 4·0; N, 8·7%), m.p. 147°; $R_F$ 0·27 (system A); $\lambda_{max.}$ (95% EtOH containing 0·3% $HCO_2H$) 260 (ε 9700), $\lambda_{min.}$ 230 nm (1800); τ [$(D_3C)_2SO$] 2·26 (1H, d, *J* 8 Hz), 4·14 (1H, d, *J* 4 Hz), 4·20 (1H, d, *J* 8 Hz), and 5·54 (2H, s).

*Rate Studies on the Deacylation of Uridine 5′-Esters.*—(a) *In aqueous* 0·155M-*ammonia* (pH 10·7). The substrate (0·0045 g) was dissolved in aqueous 0·155M-ammonia (1·0 ml) at 22°. After suitable intervals of time, samples (40 μl) were removed and treated with acetic acid (20 μl). The resulting acidified solutions were applied to a Whatman No. 1 paper chromatogram which was then developed [ascending in butan-1-ol–acetic acid–water (5 : 2 : 3)]. The appropriate areas of developed chromatogram containing unchanged substrate and uridine and equal areas of blank paper were then cut out. For a particular reaction time, each of the three pieces of paper was further cut into strips which were allowed to soak in 0·1M-hydrochloric acid (5 ml) for 24 h. The optical densities of the substrate- and the uridine-containing solutions were measured against the blank. The deacylations of 5′-*O*-acetyl-, 5′-*O*-methoxyacetyl-, 5′-*O*-phenoxyacetyl-, 5′-*O*-formyl-, and 5′-*O*-chloroacetyl-uridines were all found to display pseudo-first-order kinetics: the half-times ($t_{\frac{1}{2}}$) of these reactions were 191, 10·4, 3·9, 0·4, and 0·28 min, respectively (error in the last two determinations estimated to be ±0·05 min).

(b) *In methanolic ammonia.* Methanol was saturated with ammonia gas at 0° and the solution was diluted with an equal volume of methanol. Deacylation of the substrates in this solution was then conducted at 22° as described in (a). The deacylations of 5′-*O*-acetyl- and 5′-*O*-methoxyacetyl-uridines were found to follow pseudo-first-order kinetics with $t_{\frac{1}{2}}$ 59 and 2·5 min, respectively. The half-time of deacylation of 5′-*O*-phenoxyacetyluridine was <1 min and 5′-*O*-formyl- and 5′-*O*-chloroacetyl-uridines were completely deacylated after 13 and 10 s, respectively.

3′-O-*Acetyl*-5′-O-(*methoxyacetyl*)*uridine* (7; B = uracil-1-yl, R = Me, R′ = $CH_2$·OMe).—Methoxyacetic anhydride (2·26 g, 14 mmol) was added to a stirred solution of 2′,3′-*O*-(methoxyethylidene)uridine [9] (2·77 g, 6·95 mmol) in anhydrous pyridine (25 ml) at 20°. After 2 h ethanol (5 ml) was added and after a further 30 min the products were concentrated under reduced pressure. The oil so obtained was partitioned between chloroform (25 ml) and water (25 ml). The chloroform layer was concentrated and the residue dissolved in 80% acetic acid (20 ml) at 20°. After 10 min, the solvent was removed under reduced pressure and the residue chromatographed on silicic acid. Crystallization of the product (1·84 g, 74%) from ethanol gave 3′-O-*acetyl*-5′-O-(*methoxyacetyl*)*uridine* (Found: C, 47·1; H, 5·2; N, 8·1. $C_{14}H_{18}N_2O_9$ requires C, 46·8; H, 5·05; N, 7·8%), m.p. 142—143°; $\lambda_{max.}$ (95% EtOH) 259 (ε 10,100), $\lambda_{min.}$ 229 nm (2400); τ [$(D_3C)_2SO$–$D_2O$ (M with respect to AcOH) (8 : 1 v/v)] 2·13 (1H, d, *J* 8 Hz), 3·98 (1H, d, *J* 6 Hz), and 4·07 (1H, d, *J* 8 Hz).

3′-O-*Acetyl*-2′-O-(*methoxytetrahydropyranyl*)*uridine* (9; B = uracil-1-yl, R = Me).—5,6-Dihydro-4-methoxy-2*H*-pyran [10] (4·16 g, 36·5 mmol) was added to a stirred solution of 3′-*O*-acetyl-5′-*O*-(methoxyacetyl)uridine (1·60 g, 4·4 mmol) and mesitylenesulphonic acid (0·07 g, 0·35 mmol) in anhydrous dioxan (20 ml) at 20°. After 10 min, the products were neutralized carefully with methanolic M-sodium methoxide and concentrated under reduced pressure. The residue was extracted with chloroform and the extract filtered through Hyflo–Supercel. The filtrate was concentrated to a gum which was redissolved in methanolic ammonia (half-saturated at 0°) at 20°. After exactly 10 min, the products were concentrated under reduced pressure and the residue crystallized from ethanol to give 3′-O-*acetyl*-2′-O-(*methoxytetrahydropyranyl*)*uridine* (1·38 g, 78%) (two crops) (Found: C, 51·3; H, 6·1; N, 7·0. $C_{17}H_{24}N_2O_3$ requires C, 51·0; H, 6·05; N, 7·0%), m.p. 202—203°; $\lambda_{max.}$ (95% EtOH) 260 (ε 10,500), $\lambda_{min.}$ 230 nm (3050).

2′,3′-O-(*Dimethoxyethylidene*)*uridine* (6; B = uracil-1-yl, R = $CH_2$·OMe).—Trimethyl orthomethoxyacetate [7] (1·0 ml, 6·4 mmol) was added to a stirred mixture of uridine (1·5 g, 6·15 mmol), mesitylenesulphonic acid (1·30 g, 6·5 mmol), and anhydrous methanol (19 ml) at 20°. After 1·5 h more orthoester (0·5 ml, 3·2 mmol) was added and the reaction allowed to proceed for a further 2 h. The products were then neutralized with methanolic ammonia (half-saturated at 0°) and concentrated under reduced pressure to give a gum which was extracted with chloroform. The extract was filtered through Hyflo–Supercel, dried ($MgSO_4$), and concentrated to a glass. This was chromatographed on a column of SilicAR CC7 (20 g). Elution with $CHCl_3$–MeOH (98 : 2) gave 2′,3′-*O*-(dimethoxyethylidene)uridine, obtained as a glass; yield (material dried *in vacuo* over KOH) 1·8 g (90%).

5′-O-*Formyl*-3′-O-(*methoxyacetyl*)*uridine* (7; B = uracil-1-yl, R = $CH_2$·OMe, R′ = H).—Acetic formic anhydride (1·0 ml, 11·3 mmol) was added to a stirred solution of 2′,3′-*O*-(dimethoxyethylidene)uridine (1·0 g, 3·3 mmol) in anhydrous pyridine (10 ml) at −50° (acetone–solid $CO_2$ bath). After 1 h the reactants were allowed to warm to −20° and after a further 2·5 h the products were concentrated under reduced pressure below 40°. The gum so

obtained was partitioned between chloroform (50 ml) and aqueous 5% sodium hydrogen carbonate (10 ml). The dried ($MgSO_4$) chloroform layer was filtered and concentrated to a glass which was redissolved in 95% formic acid (15 ml) at 20°. After 15 min, the products were concentrated under reduced pressure, dissolved in chloroform, and applied to a column of silicic acid (10 cm × 2 $cm^2$; 15 g). Elution with $CHCl_3$–MeOH (98 : 2) gave a t.l.c. (system B) homogeneous glass. Crystallization from warm ethanol gave 5′-O-*formyl*-3′-O-(*methoxyacetyl*)*uridine* (0·50 g, 44%) (two crops) (Found: C, 45·25; H, 4·8; N, 8·1. $C_{13}H_{16}N_2O_9$ requires C, 45·35; H, 4·65; N, 8·1%), m.p. 125°; $R_F$ 0·42 (system B); $\lambda_{max.}$ (95% EtOH containing 0·1% $HCO_2H$) 262 (ε 11,100), $\lambda_{min.}$ 228 nm (2700); τ [$(D_3C)_2SO$–$D_2O$ (M with respect to acetic acid) (9 : 1 v/v)] 1·74 (1H, s), 2·74 (1H, d, *J ca.* 7·5 Hz), 4·28 (1H, d, *J ca.* 7·5 Hz), 4·29 (1H, d, *J ca.* 6 Hz), and 4·81 (1H, m).

3′-O-(*Methoxyacetyl*)-2′-O-(*methoxytetrahydropyranyl*)-*uridine* (9; B = uracil-1-yl, R = $CH_2$·OMe).—5,6-Dihydro-4-methoxy-2*H*-pyran [10] (5·0 ml, 43·5 mmol) was added to a stirred solution of 5′-*O*-formyl-3′-*O*-(methoxyacetyl)uridine * (1·72 g, 5·0 mmol) and mesitylenesulphonic acid (0·1 g, 0·5 mmol) in anhydrous dioxan (25 ml) at 20°. After 20 min, the products were carefully neutralized (to pH 7·5) with twenty-fold diluted saturated (at 0°) methanolic ammonia and concentrated under reduced pressure. The gum so obtained was dissolved in chloroform (50 ml) and methanolic ammonia (half-saturated at 0°; 50 ml) was added. After 8 s, the products were flash-evaporated under reduced pressure, dissolved in chloroform, and applied to a column of SilicAR CC7 (16 cm × 2·3 $cm^2$; 20 g). Elution with $CHCl_3$–MeOH (98 : 2—97 : 3) gave 3′-O-(*methoxyacetyl*)-2′-O-(*methoxytetrahydropyranyl*)*uridine* (1·4 g, 66%) (two crops) (Found: C, 50·2; H, 6·2; N, 6·5. $C_{18}H_{26}N_2O_{10}$ requires C, 50·1; H, 6·0; N, 6·5%), m.p. 170° (from ethyl acetate); $R_F$ 0·36 (system C); $\lambda_{max.}$ (95% EtOH) 261 (ε 10,600), $\lambda_{min.}$ 228 nm (2100); τ [$(D_3C)_2SO$–$D_2O$ (9 : 1 v/v)] 2·19 (1H, d, *J* 8 Hz), 4·03 (1H, d, *J* 7 Hz), 4·20 (1H, d, *J* 8 Hz), 4·60 (1H, d, *J* 5 Hz), 5·63 (1H, m), 6·50 (3H, s), and 6·95 (3H, s).

4-N-*Benzoyl*-2′,3′-O-(*methoxyethylidene*)*cytidine* (6; B = 4-*N*-benzoylcytosin-1-yl, R = Me).—Trimethyl orthoacetate [7] (25 ml) was added to a stirred anhydrous solution of 4-*N*-benzoylcytidine [16] (5·37 g, 15 mmol) and mesitylenesulphonic acid (2·53 g, 12 mmol) in dimethylformamide (30 ml) at 20°. After 10 min, the mixture was neutralized (to *ca.* pH 8) with methanolic M-sodium methoxide and then concentrated under reduced pressure to an oil. The latter was extracted with warm chloroform and the extracts were filtered and concentrated. The products were chromatographed on neutral alumina (grade III; 150 g). Elution with $CHCl_3$ to $CHCl_3$–MeOH (99 : 1) gave 4-*N*-benzoyl-2′,3′-*O*-(methoxyethylidene)cytidine as a glass (3·48 g, 55%).

2′(*and* 3′)-O-*Acetyl*-4-N-*benzoyl*-5′-O-(*methoxyacetyl*)-*cytidines*.—Methoxyacetic anhydride (2·00 g, 12·3 mmol) was added to a stirred solution of 4-*N*-benzoyl-2′,3′-*O*-(methoxyethylidene)cytidine (3·50 g, 8·5 mmol) in anhydrous pyridine (25 ml) at 20°. After 2 h ethanol (5 ml) was added and after a further 30 min the products were concentrated under reduced pressure. The oil obtained was partitioned between chloroform (50 ml) and water (50 ml). The chloroform layer was dried ($MgSO_4$), filtered (Hyflo–Supercel), and concentrated under reduced pressure, and the residue redissolved in 80% acetic acid (20 ml) at 20°. After 10 min, the solvents were removed under reduced pressure to give a gum which was triturated with ether. The material so obtained was chromatographed on a column of silicic acid (25 g). Elution with $CHCl_3$ to $CHCl_3$–MeOH (99 : 1), gave a mixture of 2′(and 3′)-*O*-acetyl-4-*N*-benzoyl-5′-*O*-(methoxyacetyl)cytidines as a glass (2·67 g, 68%).

Crystallization of this material from ethanol gave 3′-O-*acetyl*-4-N-*benzoyl*-5′-O-(*methoxyacetyl*)*cytidine* (Found: C, 54·7; H, 5·05; N, 8·3. $C_{21}H_{24}N_3O_9$ requires C, 54·7, H, 5·25; N, 8·1%), m.p. 99—102°: $\lambda_{max.}$ (95% EtOH) 262 and 304 (ε 25,300 and 10,200), $\lambda_{min.}$ 232 and 288 nm (9800 and 8900); τ [$(D_3C)_2SO$–$D_2O$ (M with respect to AcOH) (9 : 1 v/v)] 4·02 (1H, d, *J* 4·5 Hz) and 4·84 (1H, m). The mother liquors deposited crystals of 2′-O-*acetyl*-4-N-*benzoyl*-5′-O-(*methoxyacetyl*)*cytidine* (Found: C, 54·8; H, 5·3; N, 8·6%), m.p. 150—151°; $\lambda_{max.}$ (95% EtOH) 262 and 302 (ε 24,200 and 9800), $\lambda_{min.}$ 230 and 287 nm (8600 and 8400); τ [$(D_3C)_2SO$–$D_2O$ (M with respect to AcOH) (9 : 1 v/v)] 3·96 (1H, d, *J* 3 Hz) and 4·60 (1H, m).

3′-O-*Acetyl*-4-N-*benzoyl*-2′-O-(*methoxytetrahydropyranyl*)-*cytidine* (9; B = 4-*N*-benzoylcytosin-1-yl, R = Me).—5,6-Dihydro-4-methoxy-2*H*-pyran [10] (4·10 g, 35 mmol) was added to a stirred anhydrous solution of 3′-*O*-acetyl-4-*N*-benzoyl-5′-*O*-(methoxyacetyl)cytidine (0·92 g, 2·0 mmol) and mesitylenesulphonic acid (0·57 g, 2·8 mmol) in dioxan (10 ml) at 20°. After 15 min, the solution was carefully neutralized with methanolic M-sodium methoxide and concentrated under reduced pressure. The oil obtained was extracted with chloroform and the extracts were filtered (Hyflo–Supercel) and concentrated. The residue was redissolved in methanolic ammonia (half-saturated at 0°) at 20°. After 10 min, the solution was concentrated under reduced pressure and the residue crystallized from ethanol to give 3′-O-*acetyl*-4-N-*benzoyl*-2′-O-(*methoxytetrahydropyranyl*)*cytidine* (0·48 g, 48%) (Found: C, 56·7; H, 5·9; N, 8·3. $C_{24}H_{30}N_3O_9$ requires C, 57·2; H, 6·0; N, 8·35%), m.p. 203—204°; $\lambda_{max.}$ (95% EtOH) 263 and 307 (ε 25,100 and 8500), $\lambda_{min.}$ 231 and 292 nm (8400 and 8500).

4-N-p-*Anisoyl*-2′,3′-O-(*dimethoxyethylidene*)*cytidine* (6; B = 4-*N*-*p*-anisoylcytosin-1-yl, R = $CH_2$·OMe).—Trimethyl orthomethoxyacetate [7] (11·0 ml, 60·4 mmol) was added to a stirred mixture of 4-*N*-*p*-anisoylcytidine [6] (9·5 g, 25·0 mmol), mesitylenesulphonic acid (5·25 g, 26·2 mmol), and anhydrous methanol at 20°. After 2 h more orthoester (4·0 ml, 25·6 mmol) was added and after a further 2 h the products were neutralized with methanolic ammonia (half-saturated at 0°). Evaporation left an oil which was partitioned between chloroform (200 ml) and aqueous 10% sodium hydrogen carbonate (50 ml). The dried ($MgSO_4$) chloroform layer was concentrated under reduced pressure to give 4-*N*-*p*-anisoyl-2′,3′-*O*-(dimethoxyethylidene)cytidine as a yellow glass (10·0 g, 87%), $R_F$ 0·85 (system C).

4-N-p-*Anisoyl*-5′-O-*chloroacetyl*-3′-O-(*methoxyacetyl*)-*cytidine* (7; B = 4-*N*-*p*-anisoylcytosin-1-yl, R = $CH_2$·OMe, R′ = $CH_2Cl$).—Chloroacetic anhydride (11·4 g, 66·6 mmol)

* It is possible to start with a mixture of 5′-*O*-formyl-3′ (and 2′)-*O*-(methoxyacetyl)uridines. Pure 3′-*O*-(methoxyacetyl)-2′-*O*-(methoxytetrahydropyranyl)uridine crystallized from a mixture which contained 10—15% of 2′-*O*-(methoxyacetyl)-3′-*O*-(methoxytetrahydropyranyl)uridine. The latter isomer can readily be distinguished from the desired product by the OMe resonances (at τ 6·54 and 6·74) in its n.m.r. spectrum [$(D_3C)_2SO$–$D_2O$].

[16] K. A. Watanabe and J. J. Fox, *Angew. Chem. Internat. Edn.*, 1966, **5**, 579.

was added to a stirred anhydrous solution of 4-*N*-*p*-anisoyl-2′,3′-*O*-(dimethoxyethylidene)cytidine (10·25 g, 22·1 mmol) in pyridine (150 ml) at −30° ($CCl_4$–solid $CO_2$ bath). After 2 h methanol (20 ml) was added, the products were concentrated under reduced pressure, and the residue was partitioned between chloroform (250 ml) and aqueous 10% sodium hydrogen carbonate (50 ml). The chloroform layer was separated, washed with aqueous 10% sodium hydrogen carbonate (50 ml), dried ($MgSO_4$), filtered, and concentrated to a gum. The latter was dissolved in ethanol and the solution evaporated. This process was repeated and the residual glass then dissolved in 95% formic acid (150 ml) at 20°. After 15 min, the products were concentrated to a gum which was dissolved in chloroform and the solution was evaporated. After this process had been repeated several times, the residual material was chromatographed on a column of SilicAR CC4 (130 g). Elution with $CHCl_3$–MeOH (98 : 2) gave 4-N-p-*anisoyl*-5′-O-*chloroacetyl*-3′-O-(*methoxyacetyl*)*cytidine* (7·0 g, 60%) (Found: C, 50·8; H, 4·9; N, 8·0. $C_{22}H_{24}ClN_3O_{11}$ requires C, 50·45; H, 4·7; N, 8·0%), m.p. 134° (from ethyl acetate); $R_F$ 0·60 (system C); $\lambda_{max.}$ (95% EtOH containing 0·1% $HCO_2H$) 288 (ε 26,300), $\lambda_{min.}$ 235 nm (7100); τ [$(D_3C)_2SO$–$D_2O$ (M with respect to AcOH) (9 : 1 v/v)] 1·89 (1H, d, *J ca.* 8 Hz), 2·05 (2H, d, *J ca.* 8 Hz), 2·55 (1H, d, *J ca.* 8 Hz), 2·95 (2H, d, *J ca.* 8 Hz), 4·13 (1H, d, *J* 4 Hz), 4·79 (1H, m), 6·11 (3H, s), and 6·59 (3H, s).

4-N-p-*Anisoyl*-3′-O-(*methoxyacetyl*)-2′-O-(*methoxytetrahydropyranyl*)*cytidine* (9; B = 4-*N*-*p*-anisoylcytosin-1-yl, R = $CH_2$·OMe).—5,6-Dihydro-4-methoxy-2*H*-pyran [10] (10·0 ml, 87 mmol) was added to a stirred anhydrous solution of 4-*N*-*p*-anisoyl-5′-*O*-chloroacetyl-3′-*O*-(methoxyacetyl)-cytidine (5·2 g, 10 mmol) and mesitylenesulphonic acid (0·57 g, 2·86 mmol) in dioxan (37 ml) and acetonitrile (40 ml) at 20°. After 2 h, more 5,6-dihydro-4-methoxy-2*H*-pyran (7·0 ml, 61 mmol) was added and after a further 2·5 h the products were neutralized with methanolic ammonia (half-saturated at 0°) and concentrated under reduced pressure. The oil obtained was dissolved in methanol (75 ml) and treated with methanolic ammonia (75 ml; saturated at 0°). After the resulting solution had been swirled for a few seconds, it was flash-evaporated under reduced pressure (oil-pump) at 30° to give an oil which was dissolved in chloroform, and the solution was re-evaporated. After this process had been repeated several times, the residual material was chromatographed on a column of SilicAR CC7 (80 g). Elution with $CHCl_3$–MeOH (98 : 2) gave 4-N-p-*anisoyl*-3′-*O*-(*methoxyacetyl*)-2′-O-(*methoxytetrahydropyranyl*)*cytidine* (Found: C, 55·3; H, 5·8. $C_{26}H_{33}N_3O_{11}$ requires C, 55·4; H, 5·9%) as a glass which could not be induced to crystallize (4·3 g, 77%); $R_F$ 0·43 (system D); $\lambda_{max.}$ (95% EtOH) 289 (ε 26,100), $\lambda_{min.}$ 235 nm (7000); τ ($CDCl_3$) 1·88 (1H, d, *J ca.* 8 Hz), 2·16 (2H, d, *J ca.* 8 Hz), 2·46 (1H, d, *J ca.* 8 Hz), 3·08 (2H, d, *J ca.* 8 Hz), 4·00 (1H, d, *J* 4 Hz), 4·50 (1H, d, *J* 4 Hz), 4·92 (1H, m), 6·11 (3H, s), 6·50 (3H, s), and 7·02 (3H, s).

2′,3′-O-(*Methoxybenzylidene*)*adenosine* (6; B = adenin-9-yl, R = Ph).—Trimethyl orthobenzoate (25 ml) was added to a stirred solution of adenosine (4·50 g, 16·3 mmol) and toluene-*p*-sulphonic acid monohydrate (3·82 g, 21·2 mmol) in dimethylformamide (30 ml) at 20°. After 8 h the products were neutralized with methanolic M-sodium methoxide and concentrated under reduced pressure (oil-pump). The oil obtained was extracted with chloroform and the extract filtered (Hyflo–Supercel) and concentrated under reduced pressure (oil-pump). The residual oil was chromatographed on a column of neutral alumina (grade III; 200 g). Elution with $CHCl_3$ to $CHCl_3$–MeOH (99 : 1) gave 2′,3′-*O*-(methoxybenzylidene)adenosine as a glass (3·40 g, 55%).

3′-O-*Benzoyl*-5′-O-(*methoxyacetyl*)*adenosine* (7; B = adenin-9-yl, R = Ph, R′ = $CH_2$·OMe).—Methoxyacetic anhydride (2·12 g, 13·0 mmol) was added to a stirred anhydrous solution of 2′,3′-*O*-(methoxybenzylidene)adenosine (3·40 g, 8·8 mmol) in pyridine (30 ml) at 20°. After 16 h ethanol (5 ml) was added and after a further 1 h the products were concentrated under reduced pressure and the residue was partitioned between chloroform (25 ml) and water (25 ml). The chloroform layer was washed with water (25 ml) and evaporated and the resultant gum dissolved in 80% acetic acid (30 ml) at 20°. After 10 min, the solvents were removed under reduced pressure and the residue was chromatographed on a column of silicic acid. Elution with $CHCl_3$ to $CHCl_3$–MeOH (9 : 1) gave a glass (2·69 g, 69%). Crystallization from ethanol gave 3′-O-*benzoyl*-5′-O-(*methoxyacetyl*)*adenosine* (Found: C, 54·0; H, 4·8; N, 15·8. $C_{20}H_{21}N_5O_7$ requires C, 54·3; H, 4·8; N, 15·85%), m.p. 198—199°; $\lambda_{max.}$ (95% EtOH) 234 and 259 (ε 16,400 and 15,800), $\lambda_{min.}$ 223 and 246 nm (12,400 and 12,500); τ [$Me_2NCN$–$D_2O$ (M with respect to AcOH) (9 : 1 v/v)] 3·76 (1H, d, *J ca.* 6 Hz) and 4·32 (1H, m).

3′-O-*Benzoyl*-2′-O-(*methoxytetrahydropyranyl*)*adenosine* (9; B = adenin-9-yl, R′ = Ph).—5,6-Dihydro-4-methoxy-2*H*-pyran [10] (11·3 g, 100 mmol) was added to a stirred solution of 3′-*O*-benzoyl-5′-*O*-(methoxyacetyl)adenosine (0·60 g, 1·35 mmol) and toluene-*p*-sulphonic acid monohydrate (0·32 g, 1·78 mmol) in anhydrous dioxan (30 ml) at 20°. After 15 min, the solution was carefully neutralized (to pH 7·2) with methanolic M-sodium methoxide and then concentrated under reduced pressure. The oil obtained was partitioned between chloroform and water. The separated chloroform layer was dried ($MgSO_4$) and evaporated and the residue redissolved in methanolic ammonia (half-saturated at 0°) at 20°. After 10 min, the solution was concentrated rapidly under reduced pressure. Crystallization of the resultant glass from ethanol gave 3′-O-*benzoyl*-2′-O-(*methoxytetrahydropyranyl*)*adenosine* (0·40 g, 60%) (two crops) (Found: C, 56·5; H, 5·7; N, 14·6. $C_{23}H_{27}N_5O_7$ requires C, 56·9; H, 5·6; N, 14·5%), m.p. 233—235°; $\lambda_{max.}$ (95% EtOH) 234 and 259 (ε 16,300 and 17,000), $\lambda_{min.}$ 222 and 246 (12,600 and 12,600).

6-N-p-*Anisoyladenosine* (5; B = 6-*N*-*p*-anisoyladenin-9-yl).—An anhydrous solution of 2′,3′,5′-tri-*O*-acetyladenosine (27·5 g, 70 mmol) and freshly distilled *p*-anisoyl chloride (18 g, 106 mmol) in pyridine (140 ml) was stirred at 20°. After 24 h water (10 ml) was added and after 1 h the products were concentrated under reduced pressure. The gum so obtained was partitioned between chloroform (180 ml) and saturated aqueous sodium hydrogen carbonate (180 ml). The chloroform layer was separated, dried ($MgSO_4$), and concentrated under reduced pressure. The glassy residue was dissolved in methanol (350 ml) and dioxan (350 ml), and freshly prepared methanolic M-sodium methoxide (420 ml) was added to the stirred solution at 20°. After 15 min, the products were neutralized by adding them to an excess of Dowex 50 × 8 (pyridinium form) cation-exchange resin. The mixture was stirred for 10 min and then filtered. When the filtrate was concentrated (to *ca.* 250 ml), crystals of 6-*N*-*p*-anisoyladenosine (21·0 g, 75%) (Found: C, 53·7; H, 4·7; N, 17·2. Calc. for

$C_{18}H_{19}N_5O_6$: C, 53·9; H, 4·7; N, 17·45%) separated, m.p. 155—156°; $R_F$ 0·44 (system E); $\lambda_{max.}$ (95% EtOH) 292 (ε 28,400), $\lambda_{min.}$ 242 nm (5900); τ [$(D_3C)_2SO$–$D_2O$ (9 : 1 v/v)] 1·15 (1H, s), 1·20 (1H, s), 1·86 (2H, d, *J ca.* 9 Hz), 2·84 (2H, d, *J ca.* 9 Hz), 3·86 (1H, d, *J ca.* 6 Hz), and 5·90 (3H, s).

6-N-p-*Anisoyl*-2′,3′-O-(*dimethoxyethylidene*)*adenosine* (6; B = 6-*N*-*p*-anisoyladenin-9-yl, R = $CH_2$·OMe).—Trimethyl orthomethoxyacetate [7] (3·0 ml, 19·2 mmol) was added to a stirred mixture of dry, finely divided 6-*N*-*p*-anisoyladenosine (4·01 g, 10 mmol), mesitylenesulphonic acid (2·20 g, 11 mmol), and anhydrous methanol (30 ml) at 20°. After 1·5 h more orthoester (2·0 ml, 12·8 mmol) was added and after a further 2 h the products were neutralized with methanolic ammonia (half-saturated at 0°) and concentrated under reduced pressure. The resultant solid was dissolved in chloroform (260 ml) and the solution washed with aqueous 10% sodium hydrogen carbonate (60 ml). The chloroform layer was separated, dried ($MgSO_4$), and concentrated under reduced pressure to a solid mass (4·6 g). This was purified by chromatography on a column of SilicAR CC7 (60 g). Elution with $CHCl_3$–MeOH (97 : 3) gave 6-*N*-*p*-anisoyl-2′,3′-*O*-(dimethoxyethylidene)adenosine as a solid (4·38 g, 90%). This material was separated by t.l.c. (system D) into two components ($R_F$ 0·54 and 0·61) which are believed to be diastereoisomers. The higher $R_F$ component was isolated in a pure state after careful chromatography on SilicAR CC7; τ [$(D_3C)_2SO$–$D_2O$ (9 : 1 v/v)] 1·21 (1H, s), 1·29 (1H, s), 1·94 (2H, d, *J ca.* 8 Hz), 2·89 (2H, d, *J ca.* 8 Hz), 3·60 (1H, d, *J* 3 Hz), 4·34 (1H, dd, *J* 3 and 6·5 Hz), 4·77 (1H, dd, *J* 3 and 6·5 Hz), 5·66 (1H, m), 6·15 (3H, s), 6·56 (3H, s), and 6·77 (3H, s).

6-N-p-*Anisoyl*-5′-O-*formyl*-3′-O-(*methoxyacetyl*)*adenosine* (7; B = 6-*N*-*p*-anisoyladenin-9-yl, R = $CH_2$·OMe, R′ = H).—Formic acetic anhydride (5·0 ml, 56·5 mmol) was added to a stirred anhydrous solution of unchromatographed (see above) 6-*N*-*p*-anisoyl-2′,3′-*O*-(dimethoxyethylidene)-adenosine (4·8 g, 10 mmol) in anhydrous pyridine (50 ml) at −50° (acetone–solid $CO_2$ bath). After 1 h the reactants were allowed to warm to −20° and after a further 2·5 h the products were concentrated under reduced pressure below 40°. The gum so obtained was partitioned between chloroform (100 ml) and aqueous 5% sodium hydrogen carbonate (25 ml). The dried ($MgSO_4$) chloroform layer was filtered and concentrated to a glass which was re-dissolved in 98% formic acid (50 ml) at 20°. After 15 s the products were concentrated under reduced pressure, dissolved in chloroform, and applied to a column of silicic acid (60 g). Elution with $CHCl_3$–MeOH (98 : 2) gave a glass (3·9 g). When warm ethanol (40 ml) was added to a solution of the latter in chloroform (10 ml), crystals of 6-N-p-*anisoyl*-5′-O-*formyl*-3′-O-(*methoxyacetyl*)*adenosine* (3·1 g, 60%) separated (Found: C, 52·6; H, 4·3; N, 13·9. $C_{22}H_{23}N_5O_9$ requires C, 52·7; H, 4·6; N, 13·95%), m.p. 81—82°; $R_F$ 0·49 (system D); $\lambda_{max.}$ (95% EtOH containing 0·1% $HCO_2H$) 287 (ε 29,900), $\lambda_{min.}$ 238 nm (5600); τ [$(D_3C)_2SO$–$D_2O$ (M with respect to AcOH) (9 : 1 v/v)] 1·26 (1H, s), 1·33 (1H, s), 1·73 (1H, s), 1·96 (2H, d, *J ca.* 8 Hz), 2·90 (2H, d, *J ca.* 8 Hz), 3·90 (1H, d, *J* 6·5 Hz), 4·49 (1H, dd, *J* 2 and 5·25 Hz), 4·83 (1H, dd, *J* 5·25 and 6·5 Hz), 6·09 (3H, s), and 6·56 (3H, s).

6-N-p-*Anisoyl*-3′-O-(*methoxyacetyl*)-2′-O-(*methoxytetrahydropyranyl*)*adenosine* (9; B = 6-*N*-*p*-anisoyladenin-9-yl, R = $CH_2$·OMe).—5,6-Dihydro-4-methoxy-2*H*-pyran [10] (7·0 ml, 61 mmol) was added to a stirred anhydrous solution of 6-*N*-*p*-anisoyl-5′-*O*-formyl-3′-*O*-(methoxyacetyl)adenosine (3·5 g, 7·0 mmol) and mesitylenesulphonic acid (0·32 g, 1·6 mmol) in dioxan (35 ml) at 20°. After 1 h more 5,6-dihydro-4-methoxy-2*H*-pyran (7·0 ml, 61 mmol) was added and after a further 3 h the products were neutralized (to pH 7) with sixteen-fold diluted saturated (at 0°) methanolic ammonia and concentrated under reduced pressure. The residual gum was dissolved in chloroform (75 ml) and methanolic ammonia (half-saturated at 0°; 75 ml) was added with swirling. The solution was immediately flash-evaporated under reduced pressure (oil-pump) at 20° and the residue chromatographed on a column of SilicAR CC7 (35 g). Elution with $CHCl_3$–MeOH (99 : 1) gave a glass which crystallized from ethyl acetate (45 ml) to give 6-N-p-*anisoyl*-3′-O-(*methoxyacetyl*)-2′-O-(*methoxytetrahydropyranyl*)*adenosine* (2·5 g, 60%) (Found: C, 53·2; H, 5·45; N, 11·6. $C_{27}H_{33}N_5O_{10}$ requires C, 53·5; H, 5·5; N, 11·9%), m.p. 128—131°; $R_F$ 0·56 (system D); $\lambda_{max.}$ (95% EtOH) 287 (ε 34,700), $\lambda_{min.}$ 238 nm (6600); τ [$(D_3C)_2SO$–$D_2O$ (9 : 1 v/v)] 1·25 (1H, s), 1·91 (1H, s), 1·96 (2H, d, *J ca.* 9 Hz), 2·90 (2H, d, *J ca.* 9 Hz), 3·78 (1H, d, *J ca.* 8 Hz), 4·47 (1H, dd, *J* 1 and 5 Hz), 4·67 (1H, dd, *J* 5 and 8 Hz), 6·11 (3H, s), 6·55 (3H, s), and 7·45 (3H, s).

2-N-*Benzoyl*-2′,3′-O-(*methoxyethylidene*)*guanosine* (6; B = 2-*N*-benzoylguanin-9-yl, R = Me).—2-*N*-Benzoylguanosine [17] (3·22 g, 8·32 mmol), toluene-*p*-sulphonic acid monohydrate (0·34 g, 1·9 mmol), and trimethyl orthoacetate (15 ml) were stirred together at 20°. After 15 min the products were basified (to pH 8) with methanolic M-sodium methoxide and then concentrated under reduced pressure. The oil obtained was extracted with chloroform and the extracts were filtered (Hyflo–Supercel) and concentrated under reduced pressure to a gum. This was chromatographed on a column of SilicAR CC7; elution with $CHCl_3$–MeOH (98 : 2) gave 2-*N*-benzoyl-2′,3′-*O*-(methoxyethylidene)guanosine as a glass (2·48 g, 67%).

3′-O-*Acetyl*-2-N-*benzoyl*-5′-O-(*methoxyacetyl*)*guanosine* (7; B = 2-*N*-benzoylguanin-9-yl, R = Me, R′ = $CH_2$·OMe).—Methoxyacetic anhydride (0·95 g, 6·3 mmol) was added to a stirred anhydrous solution of 2-*N*-benzoyl-2′,3′-*O*-(methoxyethylidene)guanosine (2·48 g, 5·6 mmol) in pyridine (20 ml) at 20°. After 2 h ethanol (1 ml) was added and after a further 30 min the products were concentrated under reduced pressure. The oil so obtained was partitioned between chloroform (25 ml) and water (25 ml). The chloroform layer was separated, filtered (Hyflo–Supercel), and concentrated under reduced pressure to a gum. This was dissolved in 80% acetic acid (25 ml); the solution was set aside at 20° for 10 min and then concentrated under reduced pressure. The residue was chromatographed on a column of silicic acid to give a glass (2·08 g, 74%), which crystallized from ethanol to afford 3′-O-*acetyl*-2-N-*benzoyl*-5′-O-*methoxyacetylguanosine* (Found: C, 52·9; H, 4·9; N, 13·9. $C_{22}H_{23}N_5O_9$ requires C, 52·8; H, 4·6; N, 14·0%), m.p. 179—180°; $\lambda_{max.}$ (95% EtOH) 240, 257, 265, and 297 (ε 17,500, 15,300, 15,100, and 15,600), $\lambda_{min.}$ 222, 253, 262, and 273 nm (12,800, 14,500, 14,300, and 10,700); τ [$(D_3C)_2SO$–$D_2O$ (M with respect to AcOH)] 3·99 (1H, d, *J ca.* 7 Hz) and 4·63 (1H, m).

3′-O-*Acetyl*-2-N-*benzoyl*-2′-O-(*methoxytetrahydropyranyl*)-*guanosine* (9; B = 2-*N*-benzoylguanin-9-yl, R = Me).—5,6-Dihydro-4-methoxy-2*H*-pyran [10] (0·32 g, 2·8 mmol) was added to a stirred anhydrous suspension of 3′-*O*-acetyl-2-*N*-benzoyl-5′-*O*-(methoxyacetyl)guanosine (0·11 g,

[17] S. Chládek and J. Smrt, *Coll. Czech. Chem. Comm.*, 1964, **29**, 214.

0·22 mmol) and mesitylenesulphonic acid (0·012 g, 0·06 mmol) in acetonitrile (3 ml) at 20°. After 15 min, the products were neutralized with methanolic M-sodium methoxide and concentrated under reduced pressure to a gum. This was dissolved in methanolic ammonia (half-saturated at 0°) at 20°; the solution was set aside for 12 min and then rapidly concentrated under reduced pressure. The residual gum was chromatographed on a column of silicic acid to give 3′-O-*acetyl*-2-N-*benzoyl*-2′-O-(*methoxytetrahydropyranyl*)*guanosine* as a glass (0·088 g, 67%) which crystallized readily from ethanol; m.p. 214—215° (Found: C, 55·7; H, 5·5; N, 13·25. $C_{25}H_{28}N_5O_9$ requires C, 55·3; H, 5·35; N, 12·8%); $\lambda_{max.}$ (95% EtOH) 238, 257, 264, and 296 (ε 16,600, 14,700, 14,400, and 14,900), $\lambda_{min.}$ 223, 252, 261, and 273 nm (12,900, 14,300, 14,200, and 10,800).

2-N-*Benzoyl*-2′,3′-O-(*dimethoxyethylidene*)*guanosine* (6; B = 2-*N*-benzoylguanin-9-yl, R = $CH_2$·OMe).—Trimethyl orthomethoxyacetate [7] (3·0 ml, 19·2 mmol) was added to a stirred anhydrous suspension of 2-*N*-benzoylguanosine [17] (3·87 g, 10·0 mmol) and mesitylenesulphonic acid (2·20 g, 11·0 mmol) in methanol (30 ml) at 20°. After 1·5 h more orthoester (1·5 ml, 9·6 mmol) was added, and after a further 1·5 h the products were neutralized with methanolic ammonia (half-saturated at 0°) and concentrated under reduced pressure. The oil obtained was partitioned between chloroform (80 ml) and aqueous 10% sodium hydrogen carbonate (30 ml). The dried ($MgSO_4$) chloroform layer was evaporated under reduced pressure to a yellow glass (5·0 g). This was chromatographed on a column of SilicAR CC7 (60 g); elution with $CHCl_3$–MeOH (97·5 : 2·5 to 97 : 3) gave a glass (4·2 g, 90%).

2-N-*Benzoyl*-5′-O-*formyl*-3′-O-(*methoxyacetyl*)*guanosine* (7; B = 2-*N*-benzoylguanin-9-yl, R = $CH_2$·OMe, R′ = H).—Acetic formic anhydride (8·6 ml, 97 mmol) was added to a stirred solution of 2-*N*-benzoyl-2′,3′-*O*-(dimethoxyethylidene)guanosine (8·42 g, 17·8 mmol) in anhydrous pyridine at −50° (acetone–solid $CO_2$ bath). After 1 h the reactants were allowed to warm to −20° and after a further 2·5 h the products were concentrated under reduced pressure below 40°. The gum so obtained was partitioned between chloroform (250 ml) and aqueous 5% sodium hydrogen carbonate (50 ml). The dried ($MgSO_4$) chloroform layer was filtered and concentrated to a glass which was redissolved in 95% formic acid (60 ml) at 20°. After 15 min, the products were concentrated under reduced pressure and the residue was chromatographed on a column of silicic acid (70 g); elution with $CHCl_3$–MeOH (97 : 3 to 96 : 4) gave a glass (8·06 g). When hot ethanol (50 ml) was added slowly to a warm, filtered solution of this glass (4·7 g) in $CHCl_3$–EtOH (3 : 7 v/v; 100 ml), crystals of 2-N-*benzoyl*-5′-O-*formyl*-3′-O-(*methoxyacetyl*)*guanosine* (Found: C, 51·1; H, 4·5; N, 14·0. $C_{21}H_{21}N_5O_9$ requires C, 51·75; H, 4·3; N, 14·4%); m.p. 173°; yield 3·9 g [6·69 g (77%) would be expected from 8·06 g of glass]; $R_F$ 0·38 (system B); $\lambda_{max.}$ (95% EtOH containing 0·1% $HCO_2H$) 295, 264, 258, and 238 (ε 13,000, 13,100, 13,400, and 15,600), $\lambda_{min.}$ 273, 262, 254, and 223 nm (9400, 13,000, 13,000, and 11,100); τ [$(D_3C)_2SO$–$D_2O$ (M with respect to AcOH) (9 : 1 v/v)] 1·79 (2H, s), 4·16 (1H, d, *J ca.* 7 Hz), and 6·68 (3H, s).

2-N-*Benzoyl*-3′-O-(*methoxyacetyl*)-2′-O-(*methoxytetrahydropyranyl*)*guanosine* (9; B = 2-*N*-benzoylguanin-9-yl, R = $CH_2$·OMe).—5,6-Dihydro-4-methoxy-2*H*-pyran [10] (2·8 ml, 24·3 mmol) was added to a stirred anhydrous suspension of 2-*N*-benzoyl-5′-*O*-formyl-3′-*O*-(methoxyacetyl)guanosine (1·35 g, 2·77 mmol) and mesitylenesulphonic acid (0·075 g, 0·375 mmol) in dioxan (12 ml) and acetonitrile (12 ml) at 20°. After 1·5 h, more 5,6-dihydro-4-methoxy-2*H*-pyran (2·0 ml, 17·4 mmol) was added and after a further 1·5 h the products were carefully neutralized to (pH 7·5) with twenty-fold diluted, saturated (at 0°) methanolic ammonia and then concentrated under reduced pressure. The oil obtained was triturated with ether, then dissolved in chloroform (15 ml) and treated with methanolic ammonia (half-saturated at 0°; 15 ml). After 8 s the solution was flash-evaporated under reduced pressure (oil-pump) at 20° and the residue chromatographed on a column of SilicAR CC7 (15 g); elution with $CHCl_3$–MeOH (98 : 2) afforded a glass (1·2 g). Crystallization from ethyl acetate (15 ml) gave 2-N-*benzoyl*-3′-O-(*methoxyacetyl*)-2′-O-(*methoxytetrahydropyranyl*)*guanosine* (1·05 g, 66%) (Found: C, 54·35; H, 5·1; N, 12·1. $C_{26}H_{31}N_5O_{10}$ requires C, 54·4; H, 5·4; N, 12·1%); m.p. 190°; $R_F$ 0·49 (system B); $\lambda_{max.}$ (95% EtOH) 293, 262, 254, and 231 (ε 11,200, 11,500, 11,900, and 16,800), $\lambda_{min.}$ 272, 258, 251, and 222 nm; τ ($CDCl_3$) 1·91 (1H, s), 4·11 (1H, d, *J ca.* 8 Hz), 4·44 (1H, d, *J ca.* 4 Hz), 4·76 (1H, dd, *J* 4 and 8 Hz), 6·49 (3H, s), and 7·21 (3H, s).

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