

# Synthesis of the 3'-Terminal Half of Yeast Alanine Transfer Ribonucleic Acid (tRNA<sup>Ala</sup>) by the Phosphotriester Approach in Solution. Part 1. Preparation of the Nucleoside Building Blocks

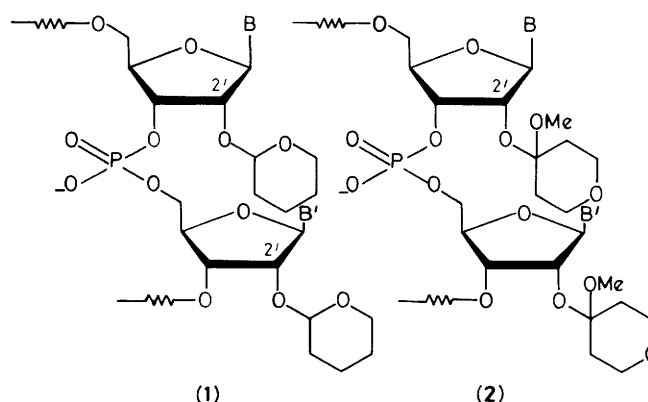
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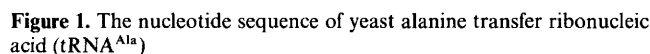
Adenosine and cytidine are converted into their 6-*N*- and 4-*N*-(4-*t*-butylbenzoyl) derivatives [(12) and (13b), respectively] which are then converted into the corresponding 2'-*O*-(4-methoxytetrahydropyran-4-yl) [(21; B = 5b) and (21; B = 6b), respectively] and 5'-*O*-[2-(dibromomethyl)benzoyl]-2'-*O*-(4-methoxytetrahydropyran-4-yl) derivatives [(35; B = 5b) and (35; B = 6b), respectively]. The conversion of (12) into its 2',3'-*O*-methoxymethylene derivative (24) is also described. Guanosine is converted, by two routes, into its 6-*O*-(3-chlorophenyl)-2-*N*-phenylacetyl derivative (16a), and the latter compound is converted into its 5'-*O*-[2-(dibromomethyl)benzoyl]-2'-*O*-(4-methoxytetrahydropyran-4-yl) and 5'-*O*-(9-phenylxanthen-9-yl)-2'-*O*-(4-methoxytetrahydropyran-4-yl) derivatives [(35; B = 9b) and (26), respectively]. The preparation of 2'-*O*-(4-methoxytetrahydropyran-4-yl)-4-*O*-(2,4-dimethylphenyl)uridine (18) from 2'-*O*-(4-methoxytetrahydropyran-4-yl)uridine (17a) and its conversion to its 5'-*O*-[2-(dibromomethyl)benzoyl] derivative (35; B = 10) are described. 5-Methyluridine (19; B = 27), pseudouridine (19; B = 28a) and inosine (19; B = 29) are converted into their 2'-*O*-(4-methoxytetrahydropyran-4-yl) derivatives (21; B = 27, 28a, and 29, respectively); (21; B = 27) is further converted into its 4-*O*-phenyl derivative (30), (21; B = 28a) is further converted into its 1-*N*-(4-bromobenzenesulphonyl) and 5'-*O*-[2-(dibromomethyl)benzoyl]-1-*N*-(4-bromobenzenesulphonyl) derivatives [(32) and (35; B = 28b), respectively], and (21; B = 29) is further converted into its 1-*N*-pivaloyloxymethyl- and 1-*N*-methyl derivatives [(33a) and (33b), respectively]. The *N*<sup>1</sup>,*N*<sup>1</sup>,*N*<sup>3</sup>,*N*<sup>3</sup>-tetramethylguanidium *E*-2-nitrobenzaldoximate-promoted removal of *O*-aryl protecting groups from the 2'-*O*-(4-methoxytetrahydropyran-4-yl) derivatives of 6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine, 4-*O*-(2,4-dimethylphenyl)uridine, 5-methyl-4-*O*-phenyluridine and 1-*N*-(4-bromobenzenesulphonyl)-5-β-D-ribofuranosyluracil [(21; B = 9b), (18), (30), and (32), respectively], and the ammonia-promoted removal of the 1-*N*-pivaloyloxymethyl group from (33a) are described. Finally, the synthesis of 2-(isopropylthiomethoxymethyl)benzoic acid [Ptmt acid, (40)], the conversion of (18) and (21; B = 5b) into their 5'-*O*-Ptmt derivatives [(41a) and (41b)], and the two-step procedure for the removal of the Ptmt protecting group are described.

In oligo- and poly-ribonucleotide synthesis, it is of crucial importance<sup>1</sup> that all the 2'-hydroxy functions should be suitably protected throughout the assembly of the desired sequences, and then released in the very last unblocking step under conditions that do not lead to cleavage or migration of the 3' → 5'-internucleotide linkages. A number of years ago, we found<sup>1</sup> that the tetrahydropyranyl [as in (1)] and more particularly the custom-designed achiral methoxytetrahydropyranyl<sup>2</sup> [Mthp, as in (2)] groups were especially suitable for 2'-protection, and indeed formed the opinion that they were the protecting groups of choice in oligoribonucleotide synthesis in solution. The results of subsequent studies have not caused us to revise this opinion.

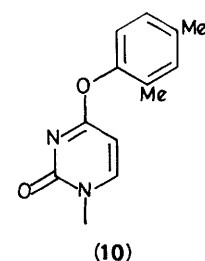
We have, for a long time,<sup>3</sup> been particularly interested in the methodology of oligoribonucleotide synthesis and, in more recent years, have evaluated the usefulness of our methods in terms of their suitability for the synthesis of sequences of yeast alanine transfer ribonucleic acid (yeast tRNA<sup>Ala</sup>, Figure 1). No really significant progress was made in our studies prior to the development of the phosphotriester approach with aryl<sup>3,4</sup> [especially 2-chlorophenyl, as in (3)] protecting groups for the internucleotide linkages. Significant progress also depended on the introduction of 'protected' protecting groups such as 2-



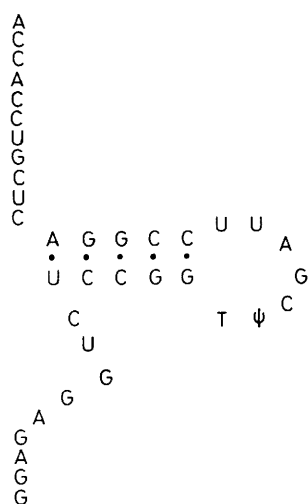
(dibromomethyl)benzoyl<sup>5</sup> [Dbmb, as in (3)]. The latter protecting group can be removed from 5'-terminal hydroxy functions of fully-protected oligoribonucleotides [as in the conversion of (3) into (4), Scheme 1] under very mildly basic conditions that do not lead to concomitant unblocking of the internucleotide linkages. Following these developments, we were able to



In the preparation<sup>6</sup> of the latter decamer, we used essentially the strategy for the protection of base-residues that was first introduced by Khorana and his coworkers<sup>7</sup> many years ago: that is, adenine, cytosine and guanine residues were protected by *N*-acylation, more precisely by *N*-benzoylation as in (5a), (6a), and (7), respectively, and uracil residues were left unprotected. As a consequence of this strategy, side-reactions involving 2-*N*-benzoylguanine (7) and uracil (8) residues were found<sup>6</sup> to occur during coupling. Although we were able to elucidate the nature of these side-reactions<sup>8</sup> and indeed found that they could be reversed<sup>8</sup> during the unblocking process, it soon became clear that it would be necessary to protect the uracil residues and to modify the protection of the guanine residues if further progress in the synthesis of yeast tRNA<sup>Ala</sup> was to be feasible. Thus attempts to prepare the next nonanucleotide sequence and to couple it with the above decaribonucleoside nonaphosphate to give the 3'-terminal nonadecaribonucleoside octadecaphosphate sequence (ApUpUpCpCpGpGpApCpUpCpGpUpCpCpApCpCpA) of yeast tRNA<sup>Ala</sup> proved to be unsuccessful.<sup>9</sup>



We therefore decided to investigate<sup>10</sup> the possibility of protecting guanine residues both on *N*-2 and on *O*-6 [as in (9a)], and uracil residues on *O*-4 [as in (10), see below]. While our main reason for doing this was to suppress side-reactions, we believed that this approach would bring additional benefit in that the protected oligonucleotides would be more lipophilic and their purification by chromatography on silica gel would thereby be facilitated. With the latter consideration in mind, we decided<sup>10</sup> to protect adenine and cytosine residues with 4-(*t*-butyl)benzoyl [as in (5b) and (6b), respectively] instead of with benzoyl protecting groups. With the combination of methoxy tetrahydropyranyl for the protection of the 2'-hydroxy functions, 2-chlorophenyl for the protection of the internucleotide linkages, 2-(dibromomethyl)benzoyl for the temporary protection of the 5'-hydroxy functions, and the adenine, cytosine, guanine and uracil residues protected as in (5b), (6b), (9a),



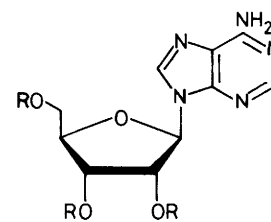
**Figure 2.** The nucleotide sequence of the 3'-terminal heptatriacontamer (37-mer) of tRNA<sup>Ala</sup>

and (10), respectively, we were able<sup>10,11</sup> successfully to complete the synthesis of the 3'-terminal nonadecaribonucleoside octadecaphosphate sequence of yeast tRNA<sup>Ala</sup>.

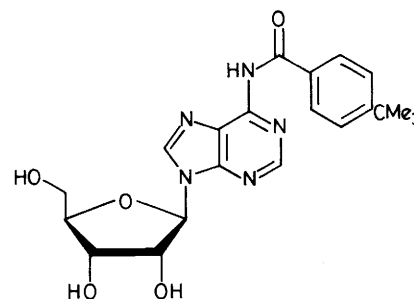
Our next synthetic target was the 3'-terminal heptatriacontanucleoside hexatriacontaphosphate (Figure 2). This 37-mer contains only one nucleotide less than one-half of the whole yeast tRNA<sup>Ala</sup> molecule. Preliminary experiments suggested that two modifications to our methodology were required if we were to complete the synthesis of this half-tRNA molecule successfully. First, although the Dbmb protecting group can readily be removed<sup>6,11</sup> from fully-protected oligonucleotides, such as (3), containing up to *ca.* 7 nucleosides, and the corresponding 5'-hydroxy compounds (4) thereby obtained in good isolated yields, the difficulty of its removal tends to increase with increasing chain length. This is most probably due to the tendency of silver(I) ions to form insoluble complexes with protected oligonucleotides. We therefore needed to develop a 'protected' protecting group that could easily be removed even from very high molecular weight fully-protected oligonucleotides. Secondly, we needed to use a temporary group for the protection of the 3'-phosphodiester ends of oligonucleotide blocks. We believed that the 2,4-dinitrobenzyl group<sup>12</sup> would be suitable for this purpose (see following paper). In this paper we describe the preparation of the nucleoside building blocks required for the synthesis of the 3'-terminal heptatriacontamer sequence of yeast tRNA<sup>Ala</sup> and, in the following paper,<sup>13</sup> we describe the actual synthesis of the heptatriacontamer itself.

The heptatriacontamer is made up from six different ribonucleosides: adenosine, cytidine, guanosine, uridine, 5-methyluridine [1-(β-D-ribofuranosyl)thymine] and pseudouridine [5-(β-D-ribofuranosyl)uracil]. As the adjacent anticodon loop (Figure 1) contains inosine and 1-*N*-methylinosine, we decided also to prepare suitable building blocks from the latter two modified ribonucleosides. It is appropriate first to consider the matter of base protection.

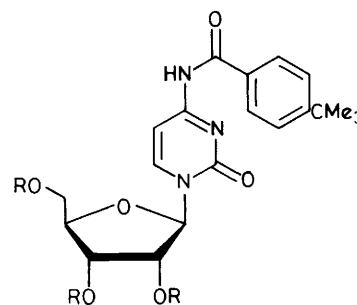
We initially undertook the relatively straightforward task of converting adenosine and cytidine into their 6-*N*- and 4-*N*-[4-(*t*-butyl)benzoyl] derivatives [(12) and (13b), respectively]. Adenosine (11a) was treated with acetic anhydride in pyridine solution to give its crystalline 2',3',5'-tri-*O*-acetate<sup>14</sup> (11b) in 91% yield. The latter compound was allowed to react with *ca.* 1.5 mol. equiv. of 4-(*t*-butyl)benzoyl chloride in pyridine solution and the products were then treated with an excess of sodium methoxide in pyridine-methanol for 10 min. at room temperature to give the required 6-*N*-[4-(*t*-butyl)benzoyl] derivative as a colourless solid in 92% isolated yield. The



(11) **a**: R = H  
**b**: R = Ac



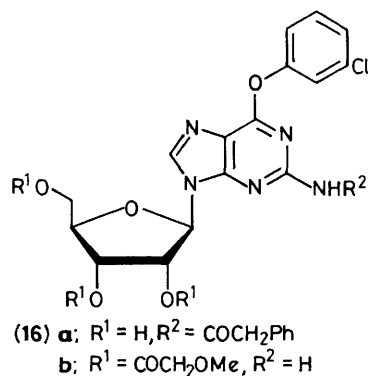
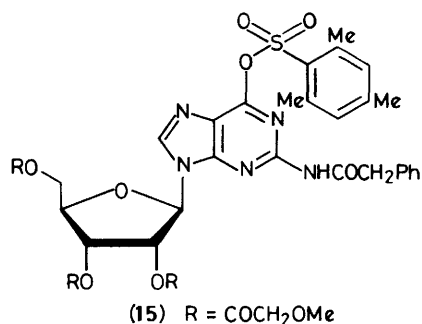
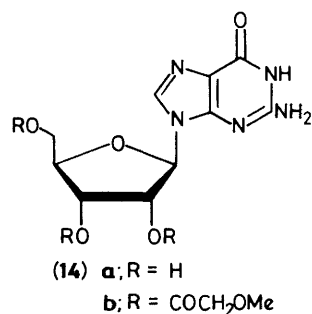
(12)



(13) **a**: R = 4-*t*-Bu<sup>1</sup>-C<sub>6</sub>H<sub>4</sub>CO  
**b**: R = H

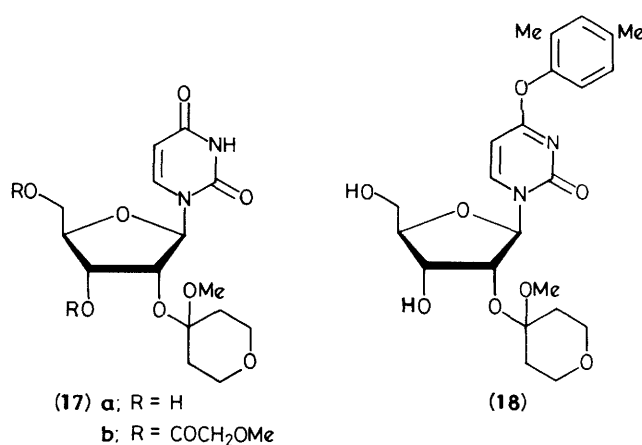
corresponding cytidine derivative (13b) was obtained, again as a colourless solid, in 74% overall yield by allowing cytidine to react with an excess of 4-(*t*-butyl)benzoyl chloride in pyridine solution and then treating the resulting tetra-acyl derivative (13a) with sodium methoxide in pyridine-methanol solution.

As indicated above, in our first successful synthesis<sup>10,11</sup> of the 3'-terminal nonadecanucleoside octadecaphosphate sequence of yeast tRNA<sup>Ala</sup>, we protected guanine residues with 4-*t*-butylphenylacetyl and 2-nitrophenyl groups on *N*-2 and *O*-6, respectively [as in (9a)]. We have also protected guanine residues with the 2-nitrophenyl group on *O*-6 in some recent studies<sup>15,16</sup> relating to the synthesis of oligodeoxyribonucleotides. However, as the procedure for the introduction of the 2-nitrophenyl group was not particularly convenient, we decided to use the 3-chlorophenyl protecting group [as in (9b)] instead. For reasons of preparative convenience, we also decided to replace the 4-*t*-butylphenylacetyl by the phenylacetyl protecting group. The required building block (16a) was prepared from 2',3',5'-tri-*O*-methoxyacetylguanosine (14b) in two ways. In the first preparation, the latter compound (14b), which was obtained from guanosine (14a) as a crystalline solid in 90% yield, was heated with a fourfold excess of phenylacetic anhydride,<sup>17</sup> under reflux, in pyridine solution and was then allowed to react with an excess of mesitylene-2-sulphonyl chloride and triethylamine in acetonitrile solution at room temperature to give (15) as the putative product. When the latter compound (15) was treated first with 3-chlorophenol and

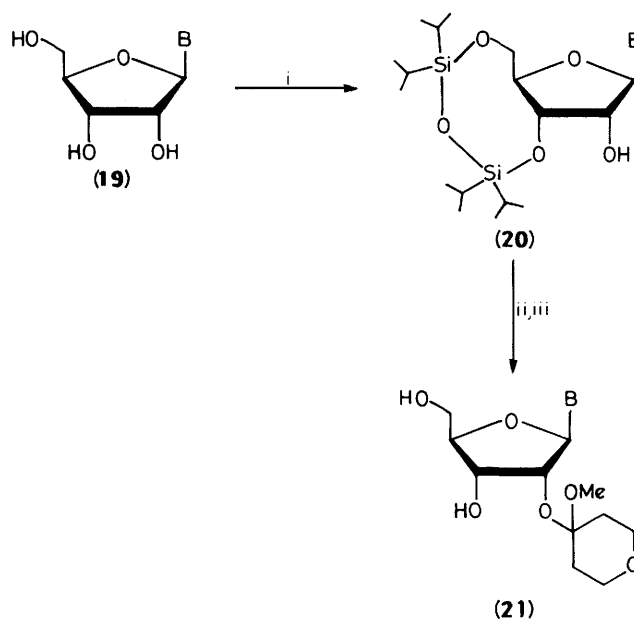


a large excess of *N*-methylpyrrolidine<sup>15</sup> in dichloromethane and the product was then treated with 4M methanolic ammonia at room temperature, the desired 6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine (**16a**) was obtained and isolated as a precipitated solid in 73% yield. In the second preparation, 2',3',5'-tri-*O*-methoxyacetylguanosine (**14b**) was treated first with a threefold excess each of mesitylene-2-sulphonyl chloride and triethylamine in acetonitrile at room temperature and the product was allowed to react with a large excess each of *N*-methylpyrrolidine and 3-chlorophenol in dichloromethane solution to give (**16b**) as the putative product. The latter material (**16b**) was treated with *ca.* 6 mol. equiv. of phenylacetyl chloride in the presence of 2,6-lutidine in acetonitrile at 0 °C, and then with methanolic ammonia at room temperature to give the desired product (**16a**), which was isolated as a colourless solid in 67% yield. Although the second preparation appears to be slightly less efficient than the first, it has the advantage that the 2-*N*-acylation step involves the use of commercially available phenylacetyl chloride instead of phenylacetic anhydride.

As indicated above, uracil residues were protected<sup>10,11</sup> with the 4-*O*-(2,4-dimethylphenyl) group [as in (**10**)] in our first successful synthesis of the 3'-terminal nonadecanucleoside octadecaphosphate sequence of yeast tRNA<sup>A1a</sup>. We decided to



retain the latter protecting group. In the preparation of the required building block, we had found that it was convenient to introduce the 2'-*O*-methoxytetrahydropyranyl (Mthp) group before the base residue was protected. Thus 2'-*O*-methoxytetrahydropyranyluridine<sup>2</sup> (**17a**) was treated with an excess of methoxyacetic anhydride in pyridine solution and the resulting 3',5'-di-*O*-methoxyacetyl derivative (**17b**) was allowed to react with 2 mol. equiv. each of 3-nitro-1,2,4-triazole<sup>6</sup> and diphenyl phosphorochloridate in pyridine for 2 h at room temperature to give the corresponding 4-(3-nitro-1,2,4-triazolo) compound.<sup>8</sup> The latter intermediate was treated with a threefold excess each of 2,4-dimethylphenol and triethylamine in acetonitrile solution for 2 h at room temperature. Treatment of the product with 8M methanolic ammonia for 15 min, also at room temperature, gave 2'-*O*-methoxytetrahydropyranyl-4-*O*-(2,4-dimethylphenyl)uridine (**18**) which was isolated as a crystalline solid in 72% overall yield, based on (**17a**) as starting material.



**Scheme 2.** Reagents: i, Pr<sub>2</sub>Si(Cl)OSi(Cl)Pr<sub>2</sub> (**22**), imidazole; ii, 4-methoxy-5,6-dihydro-2*H*-pyran (**23**), protic acid catalyst; iii, Et<sub>4</sub>NF, MeCN

The 2'-*O*-Mthp derivatives of the other three principal protected ribonucleosides were prepared by the three-step procedure indicated in Scheme 2. The substrates [(**12**), (**13b**), and (**16a**)] were each allowed to react with a slight excess of 1,3-

**Table 1.** Preparation of 2'-*O*-methoxytetrahydropyranyl ribonucleoside derivatives (31)

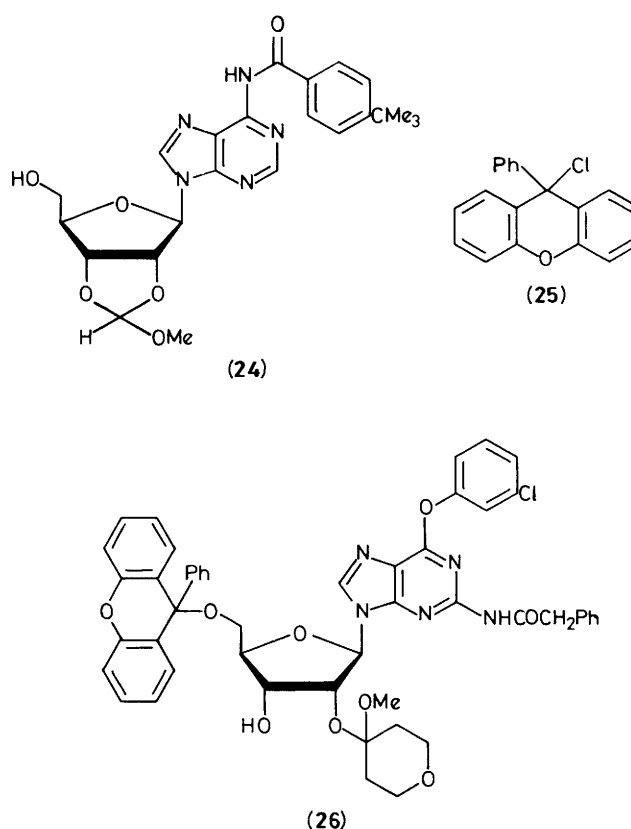
Entry no.	Substrate	Mol. equiv. of (22) <sup>a</sup>	Solvent	Reaction time (min)	Mol. equiv. of (23) <sup>a</sup>	Protic acid <sup>b</sup> (mol. equiv.)	Solvent	Reaction time (min)	% Yield of Mthp-derivative (21)
1	(12)	1.1	MeCN	20	6.75	A (0.21)	dioxane	120	67
2	(13b)	1.2	MeCN	20	5.0	A (0.20)	dioxane	120	53 (39.5) <sup>c</sup>
3	(16a)	1.2	THF	20	5.1	B (1.0)	CH <sub>2</sub> Cl <sub>2</sub>	300	59
4	(19; B = 27)	1.2	DMF	20	10.1	A (0.05)	dioxane	60	76
5	(19; B = 28a)	1.2	DMF	70	5.0	A (0.10)	dioxane	30	42 <sup>d</sup>
6	(19; B = 29)	1.5	MeCN-DMF (3:1)	60	4.3	A (0.2)	dioxane	60	53 <sup>e</sup>

<sup>a</sup> Compounds (22) and (23) are 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane and 4-methoxy-5,6-dihydro-2*H*-pyran, respectively (see Scheme 2). <sup>b</sup> A and B are toluene-4-sulphonic acid monohydrate and pyridinium toluene-4-sulphonate, respectively. <sup>c</sup> The overall yield based on cytidine is given in parentheses. <sup>d</sup> When methoxytetrahydropyranylation of (20; B = 28a) was effected with (23) (3.9 mol. equiv.) in the presence of trifluoroacetic acid (0.34 mol. equiv.) in dichloromethane solution, and the product was allowed to react with 4-bromobenzenesulphonyl chloride in the presence of triethylamine in acetonitrile solution before treatment with tetraethylammonium fluoride, 1-(4-bromobenzenesulphonyl)-2'-*O*-methoxytetrahydropyranyl-5-β-D-ribofuranosyluracil (32) (see below) was obtained in 54% overall yield for the four steps, starting from pseudouridine. <sup>e</sup> The 1,1,3,3-tetraisopropylidisiloxanyl protecting group was removed with tetrabutylammonium fluoride in THF instead of with tetraethylammonium fluoride in acetonitrile solution.

dichloro-1,1,3,3-tetraisopropylidisiloxane <sup>18</sup> [(22), Scheme 2 and Table 1, entries nos. 1, 2, and 3, respectively] in the presence of imidazole for 20 min at room temperature and the 3',5'-protected intermediates (20) were then allowed to react with an excess of 4-methoxy-5,6-dihydro-2*H*-pyran <sup>6,19</sup> [(23), Scheme 2] in the presence of a protic acid. After treatment with tetraethylammonium fluoride in acetonitrile, the desired 2'-*O*-Mthp derivatives [(21); B = (5b), (6b), and (9b), respectively] were obtained in satisfactory overall yields (Table 1, entries nos. 1, 2, and 3, respectively). Like uridine (see above), the modified nucleosides [5-methyluridine (19; B = 27), pseudouridine (19; B = 28a) and inosine (19; B = 29)] were all converted into their 2'-*O*-Mthp derivatives (21) before their base-residues were protected. The procedure indicated in Scheme 2 was again followed and the overall yields obtained (Table 1, entries nos. 4–6) were satisfactory except in the case of the 2'-*O*-Mthp derivative (19; B = 28a) of pseudouridine (entry no. 5). The rather modest yield of the latter derivative (19; B = 28) was probably partly due to difficulties in its isolation (see Table 1, footnote<sup>d</sup> and below).

In our approach to oligoribonucleotide synthesis, we have found <sup>6,11</sup> the methoxymethylene group <sup>20</sup> to be suitable for the protection of the 2'- and 3'-hydroxy functions of the 3'-terminal nucleoside residue. The adenosine building block (24) required for the synthesis of the 3'-terminal heptatriacontamer sequence of yeast tRNA<sup>Ala</sup> (Figure 2) was prepared in the usual way <sup>20</sup> by treating 6-*N*-(4-*t*-butylbenzoyl)adenosine (12) with a large excess of trimethyl orthoformate in the presence of toluene-4-sulphonic acid in dimethylformamide (DMF) solution; it was isolated as a crystalline solid in 50% yield. It is convenient at this point also to consider the preparation of 5'-*O*-(9-phenyl-xanthen-9-yl)-2'-*O*-(methoxytetrahydropyranyl)-6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine (26), the building block required for the 5'-terminal nucleoside residue of the target heptatriacontamer sequence. The latter compound (26) was readily prepared, in 78% isolated yield, by treating (21; B = 9b) with 9-chloro-9-phenylxanthene (25) in pyridine solution. <sup>21</sup>

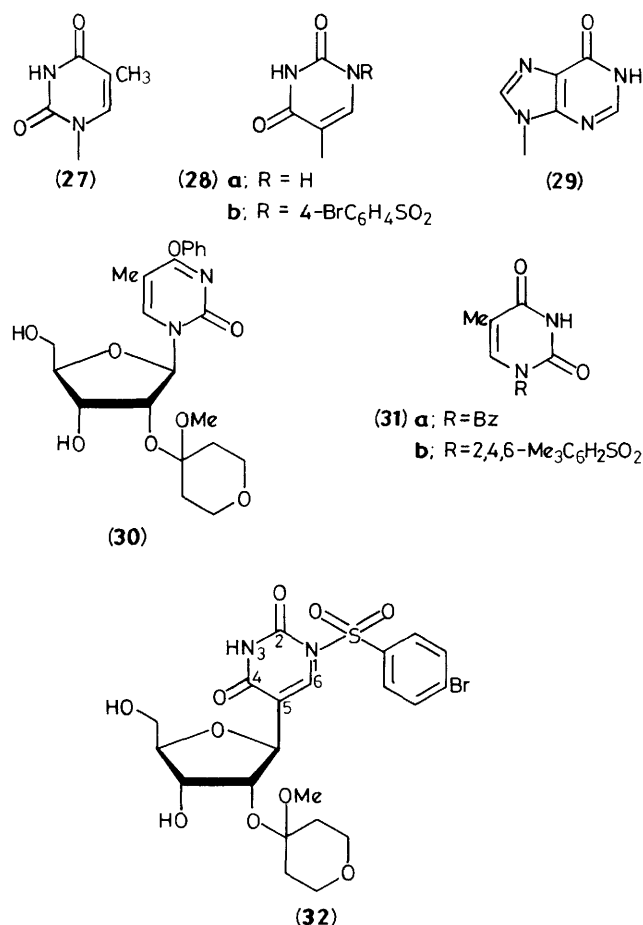
It is now appropriate to consider the matter of the protection of the base-residues of the modified nucleosides. First, following the strategy used for the protection of thymidine in oligodeoxyribonucleotide synthesis, <sup>15,16</sup> the thymine residue in 5-methyluridine (19; B = 27) was protected with a 4-*O*-phenyl group. The four-step procedure used for the conversion of 2'-*O*-methoxytetrahydropyranyl-5-methyluridine (21; B = 27) into its 4-*O*-phenyl derivative (30) corresponded (see Experimental) to that used above in the conversion of 2'-*O*-methoxytetra-



hydropyranyluridine (17a) into its 4-*O*-(2,4-dimethylphenyl) derivative (18) except that 2,4-dimethylphenol was replaced by phenol in the third step. The overall yield of (30) was 61 and 46%, based on (21; B = 27) and 5-methyluridine (19; B = 27) itself, respectively, as starting materials.

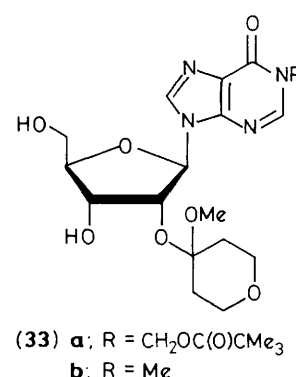
Preliminary studies involving pseudouridine (19; B = 28a) suggested that it would be desirable to protect its base-residue and, in any case, such base-protection would be expected to promote the solubility of its derivatives in organic solvents. We had previously reported <sup>22</sup> that thymine (31; R = H) undergoes benzylation first on *N*-1 [as in (31a)] and have since found that it reacts in the same way with arenesulphonyl chlorides: for example, when it is treated with mesitylene-2-sulphonyl chloride in the presence of triethylamine in acetonitrile at room





temperature, (31b) is obtained.<sup>23</sup> It therefore seemed likely that phosphorylation would also take place preferentially on *N*-1, and that it would be advisable to protect the uracil residue of pseudouridine (19; B = 28a) with a 1-*N*-acyl group in order to suppress side-reactions in oligoribonucleotide synthesis. As it seemed clear that a benzoyl group<sup>22</sup> would be much too sensitive to hydrolysis for the present purpose, it was decided to use a 1-*N*-arenesulphonyl protecting group. Stability studies (see below) suggested that the 4-bromobenzenesulphonyl (brosyl) group would be particularly suitable. The desired building block (32) was prepared first from crystalline 2'-*O*-methoxytetrahydropyranyl-pseudouridine (21; B = 28a). The latter compound was treated with a threefold excess of methoxyacetic anhydride in pyridine solution, and the putative intermediate 3',5'-dimethoxyacetate was then allowed to react with a slight excess of brosyl chloride in the presence of triethylamine in acetonitrile. After the methoxyacetyl protecting groups had been removed by treatment with 8M methanolic ammonia at room temperature, (32) was obtained as a colourless precipitated solid in 54% yield, based on (21; B = 28a). As this represents a low overall yield based on pseudouridine (19; B = 28a), which is a particularly valuable starting material, an alternative preparation of (32) was undertaken. Pseudouridine (19; B = 28a) was converted into its 3',5'-*O*-(1,1,3,3-tetraisopropylidisiloxanyl)derivative (20; B = 28a) (Table 1, entry no. 5) and the latter compound was converted, in three steps, into (32) in 54% overall yield (see Table 1, footnote *d* and Experimental).

Although 5-methyluridine and pseudouridine are the only modified nucleosides in the 3'-terminal heptatriacontamer sequence of yeast tRNA<sup>Ala</sup> (Figure 1) we decided, as indicated above, also to prepare building blocks derived from inosine and



1-*N*-methylinosine, the two modified nucleosides that occur in the adjacent anticodon loop of the tRNA molecule (Figure 1). It can be seen (Table 1, entry no. 6) that inosine (19; B = 29) was readily converted into its 2'-*O*-methoxytetrahydropyranyl derivative (21; B = 29) in 53% isolated yield by the standard three-step procedure (Scheme 2). It seemed very likely<sup>24</sup> that the hypoxanthine residues (29) of inosine building blocks would need to be protected in oligoribonucleotide synthesis. After some preliminary studies, we came to the conclusion that the pivaloyloxymethyl (POM) protecting group<sup>25</sup> should be suitable for this purpose. When 2'-*O*-methoxytetrahydropyranylinosine (21; B = 29) was treated with an excess both of chloromethyl pivalate and potassium carbonate in anhydrous DMF at room temperature, what was assumed to be 2'-*O*-methoxytetrahydropyranyl-1-*N*-pivaloyloxymethylinosine (33a) was obtained and isolated as a crystalline solid in 74% yield. The preparation of the required 2'-*O*-methoxytetrahydropyranyl-1-*N*-methylinosine (33b) also presented no problem. Treatment of 2'-*O*-methoxytetrahydropyranylinosine (21; B = 29) with dimethyl sulphate and potassium carbonate<sup>26</sup> in DMF at room temperature gave (33b) as a crystalline solid in 78% isolated yield.

Two main considerations were taken into account in developing the strategy for the protection of the base-residues of the above building blocks. First, it was essential that the protecting groups should be effective in suppressing the side-reactions<sup>6,8</sup> that might occur during phosphorylation and the other steps of oligoribonucleotide synthesis. Secondly, the choice of protecting groups was governed to a large extent by the unblocking conditions that it was expected would be required to remove them at the end of the synthesis. There are three unblocking steps in our approach<sup>6,11</sup> to oligo- and poly-ribonucleotide synthesis in solution. The first step, which involves treatment with the *N*<sup>1</sup>,*N*<sup>1</sup>,*N*<sup>3</sup>,*N*<sup>3</sup>-tetramethylguanidinium salt of *E*-2-nitrobenzaloxime<sup>27</sup> preferably in dioxane-acetonitrile solution,<sup>15</sup> leads to the removal of the 2-chlorophenyl protecting groups from the internucleotide linkages and the 3-chlorophenyl and 2,4-dimethylphenyl protecting groups from the guanine and uracil residues, respectively (see Table 2 and below). The second step, which involves treatment with concentrated aqueous ammonia, leads to the removal of the *N*-acyl groups from the base residues and any terminal *O*-acyl protecting groups present. The final step, which involves treatment with 0.01M hydrochloric acid, leads to the removal of the Mthp protecting groups from the 2'-hydroxy functions and the 3'-terminal methoxymethylene group.

It would appear from Table 2 that the conditions generally used<sup>11</sup> in the second unblocking step (*ca.* 0.3M *N*<sup>1</sup>,*N*<sup>1</sup>,*N*<sup>3</sup>,*N*<sup>3</sup>-tetramethylguanidinium *E*-2-nitrobenzaloxime, overnight, room temperature) would also be sufficient for the removal of the 3-chlorophenyl, 2,4-dimethylphenyl, phenyl and brosyl protecting groups from the guanosine, uridine, 5-methyluridine and pseudouridine moieties, respectively, provided that the

**Table 2.** Removal of protecting groups from base-residues of 2'-*O*-methoxytetrahydropyranyl derivatives

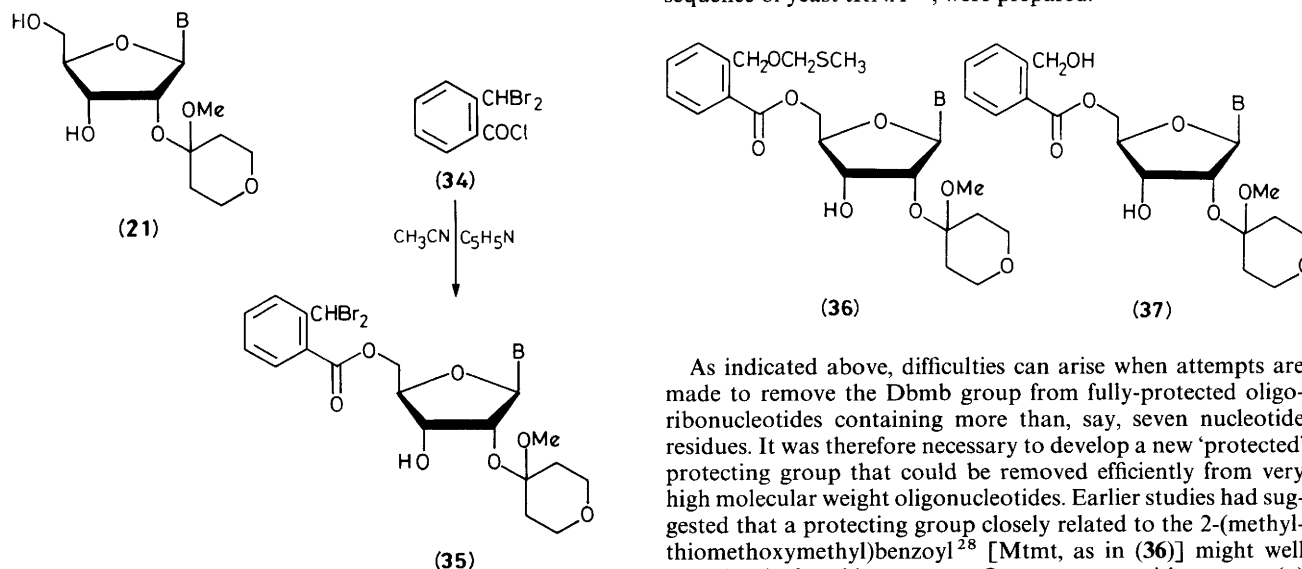
Entry no.	Substrate	Unblocking reagent <sup>a</sup>	Solvent	<i>t</i> <sub>1/2</sub> (min) <sup>b</sup>	<i>t</i> (min) <sup>b</sup>
1	(21; B = 9b)	A	dioxane	—	(~48 h) <sup>c</sup>
2	(21; B = 9b)	A	dioxane-MeCN <sup>d</sup>	20	150
3	(21; B = 9b)	A	MeCN	8	40
4	(18)	A	dioxane	20	180
5	(18)	A	dioxane-MeCN <sup>d</sup>	15	150
6	(18)	A	MeCN	15	150
7	(30)	A	dioxane-MeCN <sup>d</sup>	10	75
8	(32)	A	dioxane-MeCN <sup>d</sup>	10	90
9	(32)	B	H <sub>2</sub> O	—	— <sup>e</sup>
10	(33a)	B	H <sub>2</sub> O	7	20

<sup>a</sup> Unblocking agent A consists of *ca.* 0.45M *N*<sup>1</sup>,*N*<sup>1</sup>,*N*<sup>3</sup>,*N*<sup>3</sup>-tetramethylguanidine (*ca.* 9 mol. equiv. with respect to substrate) and *ca.* 0.5M *E*-2-nitrobenzaloxime (*ca.* 10 mol. equiv. with respect to substrate); unblocking agent B is concentrated aqueous ammonia. All reactions were carried out at room temperature. <sup>b</sup> *t*<sub>1/2</sub> and *t* are the approximate times in minutes for half and complete reactions, respectively. <sup>c</sup> This reaction was *ca.* 30% complete after 3 h. <sup>d</sup> (1:1). <sup>e</sup> No reaction could be detected after 24 h.

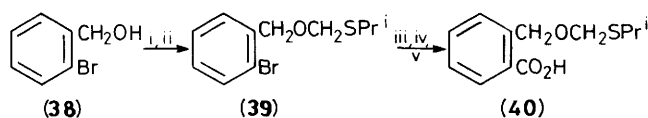
unblocking reaction is carried out in dioxane-acetonitrile (1:1) solution (Table 2, entries nos. 2, 5, 7, 8). Although the concentration of oximate ions used in the base-unblocking reactions (Table 2) was *ca.* 1.5 times greater than that normally used<sup>11</sup> in the unblocking of oligonucleotides, the slowest reactions in dioxane-acetonitrile solution were complete after 2.5 h (entries nos. 2, 5). It can be seen (entry no. 1) that the oximate ions promoted removal of the 6-*O*-(3-chlorophenyl) protecting group from the guanosine building block (16a) occurs very slowly in dioxane solution. However, this reaction proceeds rapidly in acetonitrile solution (entry no. 3), and at a convenient rate in dioxane-acetonitrile (1:1) solution (entry no. 2). It is noteworthy that this solvent effect does not appear to operate in the removal of the 4-*O*-(2,4-dimethylphenyl)-protecting group from the uridine building block (18) (entries nos. 4–6). These results correspond to those previously obtained<sup>15</sup> in the unblocking of 2'-deoxyguanosine and thymidine building blocks in the deoxy-series. It is noteworthy that oximate ions are very effective in the removal of the 1-*N*-brosyl protecting group from the pseudouridine building block (32) (entry no. 8), and that no detectable removal of the latter protecting group occurred when (32) was treated with concentrated aqueous ammonia for 24 h (entry no. 9). Finally, the conditions of ammonolysis required for the removal of the POM protecting group from (33a) (entry no. 10) are far milder

than those used [concentrated aqueous ammonia (*d* 0.88), room temperature, 72 h]<sup>11</sup> for the removal of the 4-(*t*-butyl)benzoyl protecting group from the 6- and 4-amino functions of adenine and cytosine residues [i.e. from (5b) and (6b), respectively].

The actual nucleoside building blocks required in oligoribonucleotide synthesis are 2'-*O*-methoxytetrahydropyranyl derivatives of base-protected ribonucleosides that are also protected on their 5'-hydroxy functions. The 2-(dibromomethyl)benzoyl (Dbmb)<sup>5</sup> 'protected' protecting group [as in (3), see above] is particularly suitable for the protection of the 5'-hydroxy functions as it can be selectively removed from oligoribonucleotides of moderate length under very mild conditions. The required 5'-*O*-[2-(dibromomethyl)benzoyl]-2'-*O*-methoxytetrahydropyranyl ribonucleoside derivatives (35) were readily prepared (Scheme 3) by adding a slight excess of 2-(dibromomethyl)benzoyl chloride<sup>5</sup> (34) in acetonitrile solution slowly to a stirred solution of the corresponding 2'-*O*-methoxytetrahydropyranyl derivative (21) in pyridine at room temperature. The total reaction times were *ca.* 1–2 h, and the isolated yields varied between 70 and 86%. Five such building blocks derived from adenosine [i.e. (35; B = 5b), 70%, m.p. 135–136 °C], cytidine [i.e. (35; B = 6b), 86%], guanosine [i.e. (35; B = 9b), 76%], uridine [i.e. (35; B = 10), 81%] and pseudouridine [i.e. the 5'-*O*-[2-(dibromomethyl)benzoyl] derivative of (32), 76%], required for the synthesis of the 3'-terminal heptatriacontamer sequence of yeast tRNA<sup>A1a</sup>, were prepared.

**Scheme 3.**

As indicated above, difficulties can arise when attempts are made to remove the Dbmb group from fully-protected oligoribonucleotides containing more than, say, seven nucleotide residues. It was therefore necessary to develop a new 'protected' protecting group that could be removed efficiently from very high molecular weight oligonucleotides. Earlier studies had suggested that a protecting group closely related to the 2-(methylthiomethoxymethyl)benzoyl<sup>28</sup> [Mtmmt, as in (36)] might well be suitable for this purpose. On treatment with mercury(II) perchlorate in the presence of 2,4,6-collidine in slightly wet



**Scheme 4.** Reagents: i, NaH, THF; ii,  $\text{Pr}^i\text{SCH}_2\text{Cl}$ , NaI, THF; iii, BuLi in hexane, THF; iv,  $\text{CO}_2$ ; v, dil. HCl

tetrahydrofuran (THF), the Mtmt is converted into the (2-hydroxymethyl)benzoyl group [as in (37)] which is then readily removable<sup>28</sup> under very mild conditions of alkaline hydrolysis (see below). Mercury(II) perchlorate shows less tendency than silver(I) perchlorate to form insoluble complexes with fully-protected oligoribonucleotides but, unfortunately, studies carried out with the corresponding thymidine 5'-esters suggested<sup>28</sup> that the conversion of (36) into (37) would proceed too slowly. It was therefore decided to investigate the possible use of the 2-(isopropylthiomethoxymethyl)benzoyl [Ptmt, as in (41), see below] protecting group in the hope that the mercury(II) perchlorate-promoted unblocking step [to give (37)] would occur more rapidly.

The procedure used for the preparation of 2-(isopropylthiomethoxymethyl)benzoic acid (40) is outlined in Scheme 4. Chloromethyl isopropyl sulphide was prepared<sup>29</sup> in 71% yield from propane-2-thiol, paraformaldehyde and hydrogen chloride in dichloromethane solution. When 2-bromobenzyl alcohol (38) was treated with sodium hydride and its resulting conjugate base was allowed to react with chloromethyl isopropyl sulphide and sodium iodide in THF, 1-bromo-2-isopropylthiomethoxymethylbenzene (39) was obtained as a distillable liquid in 66% yield. When (39) was treated with butyllithium in hexane-THF solution at  $-78^\circ\text{C}$ , the products allowed to react with solid carbon dioxide and then acidified with dilute hydrochloric acid, 2-(isopropylthiomethoxymethyl)benzoic acid [(40), Ptmt acid] was obtained and isolated as a crystalline solid in 73% yield.

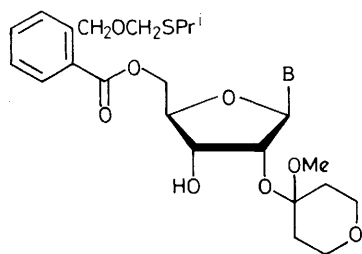
*N,N'*-Di-isopropylcarbodi-imide (2 mol equiv.) and Ptmt acid [(40), 2 mol equiv.] were added to a solution of 2'-*O*-methoxytetrahydropyranyl-4-*O*-(2,4-dimethylphenyl)uridine (18) in THF solution at room temperature. After 1 h, triethylamine (2 mol equiv.) was added and the reaction was allowed to proceed for 13 h to give the desired product (41a) which was isolated in 62% yield. The corresponding adenosine derivative (41b), which was prepared in the same way from 2'-*O*-methoxytetrahydropyranyl-6-*N*-(4-*t*-butylbenzoyl)adenosine (21; B = 5b), was obtained only in 30% yield. However, the firm assignment of the double-doublets at  $\delta$  4.54 and 4.65 (Experimental) in its  $^1\text{H}$  n.m.r. spectrum to the 5'- $\text{CH}_2$  protons clearly established that (21; B = 5b) had undergone acylation on its 5'-hydroxy function. The Ptmt protecting group was found to be rapidly removable under very mild conditions indeed. Thus when the uridine derivative (41a) was treated with mercury(II) perchlorate (2 mol equiv.) and 2,4,6-collidine (5 mol equiv.) in slightly wet tetrahydrofuran at room temperature, no starting material remained after 5 min. The putative 2-hydroxymethylbenzoyl derivative<sup>28</sup> (37; B = 10) was treated with a large excess of triethylamine in aqueous THF at room tem-

perature and, within 5 min, it was quantitatively converted back into 2'-*O*-methoxytetrahydropyranyl-4-*O*-(2,4-dimethylphenyl)uridine (18). These unblocking conditions appeared to us to be ideally suited to the requirements of oligoribonucleotide synthesis. The Ptmt protecting group was also found to be directly removable by ammonolysis. Thus when the adenosine derivative (41b) was treated with concentrated aqueous ammonia at room temperature for 24 h, it was completely converted into a product with the same t.l.c. mobility as 2'-*O*-methoxytetrahydropyranyl-adenosine (21; B = adenin-9-yl). The use of the two Ptmt-protected building blocks [(41a) and (41b)] and all the other nucleoside building blocks indicated above, except those derived from inosine, in the synthesis of the 3'-terminal heptatriacontamer sequence (Figure 2) of yeast tRNA<sup>Ala</sup> is described in the following paper.<sup>13</sup>

## Experimental

$^1\text{H}$  N.m.r. spectra were measured, unless otherwise stated, at 250 MHz with a Bruker WM250 spectrometer; tetramethylsilane was used as an internal standard. U.v. absorption spectra were measured with either a Perkin-Elmer 402 or a Cary 17 recording spectrophotometer. T.l.c. was carried out on Merck silica gel 60 F<sub>254</sub> plates which, unless otherwise stated, were developed in system A [ $\text{CHCl}_3$ -MeOH (9:1)]. Merck silica gel H was used for short column chromatography. Dioxane, acetonitrile and pyridine were dried by heating, under reflux, with calcium hydride; these solvents were then distilled at atmospheric pressure and were stored over 4 Å molecular sieves. Dimethylformamide (DMF) was stirred with calcium hydride at room temperature for 16 h, then distilled under reduced pressure and stored over 4 Å molecular sieves. All solvent ratios are v/v.

**6-*N*-(4-*t*-Butylbenzoyl)adenosine (12).**—A solution of adenosine (10.0 g, 37.4 mmol) and acetic anhydride (23.0 ml, 0.244 mol) in anhydrous pyridine (50 ml) was stirred at room temperature. After 16 h, methanol (40 ml) was added dropwise and, after a further period of 1 h, the products were evaporated to dryness under reduced pressure. Crystallization of the residue from ethanol (100 ml) gave 2',3',5'-tri-*O*-acetyladenosine (13.4 g, 91%) as a colourless solid, m.p.  $168$ – $169^\circ\text{C}$  (lit.,<sup>14</sup>  $174^\circ\text{C}$ ). A solution of the latter compound (5.0 g, 12.7 mmol) and 4-*t*-butylbenzoyl chloride (3.80 ml, 19.5 mmol) in anhydrous pyridine (25 ml) was stirred at room temperature. After 90 min, water (4 ml) was added and, after a further period of 30 min, the products were concentrated to small volume under reduced pressure and then added to saturated aqueous sodium hydrogen carbonate (150 ml). The resulting mixture was extracted with chloroform ( $3 \times 60$  ml), and the combined extracts were evaporated under reduced pressure in the presence of toluene to give a glass. The latter material was dissolved in pyridine-methanol (1:1) (30 ml) and treated with a 25% solution of sodium methoxide in methanol (8.2 g, 38 mmol) at room temperature. After 10 min, the products were neutralized with glacial acetic acid and concentrated under reduced pressure to small volume. The residual material was partitioned between saturated aqueous sodium hydrogen carbonate (80 ml) and chloroform ( $3 \times 50$  ml). The dried ( $\text{MgSO}_4$ ) chloroform extracts were evaporated to dryness under reduced pressure. The residue was dissolved in chloroform (25 ml) and the resulting solution was added dropwise to stirred light petroleum (b.p.  $30$ – $40^\circ\text{C}$ ) (500 ml) to give 6-*N*-(4-*t*-butylbenzoyl)adenosine as a colourless solid (5.01 g, 92%);  $R_F$  0.26 (system A);  $\delta_{\text{H}}[(\text{CD}_3)_2\text{SO}-d_6]$  1.34 (9 H, s), 3.55–3.8 (2 H, m), 4.04 (1 H, m), 4.22 (1 H, m), 4.65 (1 H, m), 6.05 (1 H, d,  $J$  5.9 Hz), 7.57 (2 H, d,  $J$  8.4 Hz), 8.00 (2 H, d,  $J$  8.4 Hz), 8.67 (1 H, s), and 8.71 (1 H, s).



(41) a; B = (10)

b; B = (5b)



2'-O-(4-Methoxytetrahydropyran-4-yl)-6-N-(4-*t*-butylbenzoyl)adenosine (**21**; **B** = **5b**).—1,3-Dichloro-1,1,3,3-tetraisopropylidisiloxane<sup>18</sup> (8.13 g, 25.8 mmol) was added to a stirred suspension of 6-*N*-(4-*t*-butylbenzoyl)adenosine (10.0 g, 23.4 mmol) and imidazole (7.64 g, 112 mmol) in acetonitrile (50 ml) at room temperature. After 20 min, water (10 ml) was added and, after a further period of 10 min, the products were concentrated under reduced pressure to small volume. The residue was dissolved in chloroform (200 ml) and the solution was washed with saturated aqueous sodium hydrogen carbonate (250 ml), 0.1M hydrochloric acid (300 ml) and water (200 ml). The dried (MgSO<sub>4</sub>) organic layer was separated and evaporated under reduced pressure. The residual glass obtained was fractionated by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>–EtOH (97:3), were combined and concentrated under reduced pressure to give a glass (10.6 g). The latter material (10.0 g) and 5,6-dihydro-4-methoxy-2H-pyran<sup>2,19</sup> (17.0 g, 149 mmol) were dissolved in dioxane (50 ml) at room temperature and toluene-4-sulphonic acid monohydrate (0.89 g, 4.68 mmol) was added to the stirred solution. After 2 h, the products were neutralized with 4M methanolic ammonia and were then evaporated under reduced pressure. The residual oil was dissolved in chloroform (100 ml), and the solution was filtered through Hyflo supercel and then evaporated under reduced pressure. A 1M solution of tetraethylammonium fluoride in acetonitrile (45 ml, 45 mmol) was added to the residue, and the resulting solution was stirred at room temperature for 15 min and then evaporated under reduced pressure. The material obtained was partitioned between chloroform (150 ml) and saturated aqueous sodium hydrogen carbonate (200 ml). The aqueous layer was re-extracted with chloroform (2 × 50 ml), and the dried (MgSO<sub>4</sub>) combined organic extracts were evaporated under reduced pressure. The residual oil was fractionated by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>–EtOH (97:3) were combined and concentrated under reduced pressure. The material obtained was dissolved in chloroform (50 ml), and the resulting solution was added dropwise to light petroleum (b.p. 30–40 °C) (1 000 ml) to give 2'-O-(4-methoxytetrahydropyran-4-yl)-6-*N*-(4-*t*-butylbenzoyl)adenosine as a colourless solid (8.08 g, 67%); *R*<sub>F</sub> 0.42 (system A); δ<sub>H</sub>[(CD<sub>3</sub>)<sub>2</sub>SO–D<sub>2</sub>O] 1.34 (9 H, s), 1.4–1.65 (2 H, m), 1.65–1.9 (2 H, m), 2.55 (3 H, s), 3.2–3.5 (4 H, m), 3.5–3.8 (2 H, m), 4.07 (1 H, m), 4.19 (1 H, m), 5.01 (1 H, dd, *J* 4.8, 7.1 Hz), 6.20 (1 H, d, *J* 7.3 Hz), 7.58 (2 H, d, *J* 8.3 Hz), 8.00 (2 H, d, *J* 8.3 Hz), and 8.78 (2 H, s).

5'-O-[2-(Dibromomethyl)benzoyl]-2'-O-(4-methoxytetrahydropyran-4-yl)-6-*N*-(4-*t*-butylbenzoyl)adenosine (**35**; **B** = **5b**).—A solution of 2-(dibromomethyl)benzoyl chloride<sup>5</sup> (0.95 g, 3.04 mmol) in acetonitrile (15 ml) was added dropwise to a solution of 2'-O-(4-methoxytetrahydropyran-4-yl)-6-*N*-(4-*t*-butylbenzoyl)adenosine (1.50 g, 2.77 mmol) in anhydrous pyridine (20 ml) at room temperature. After 90 min, water (2 ml) was added and, after a further period of 10 min, the products were poured into saturated aqueous sodium hydrogen carbonate (80 ml). The resulting mixture was extracted with chloroform (4 × 30 ml), and the combined organic extracts were dried (MgSO<sub>4</sub>) and concentrated under reduced pressure to give a glass. This material was fractionated by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>–EtOH (93:7) were combined and concentrated under reduced pressure to give 5'-O-[2-(dibromomethyl)benzoyl]-2'-O-(4-methoxytetrahydropyran-4-yl)-6-*N*-(4-*t*-butylbenzoyl)adenosine as a colourless solid. Crystallisation of the latter material from methanol gave colourless crystals (Found: C, 51.05; H, 4.9; N, 8.7; Br, 19.7. C<sub>35</sub>H<sub>39</sub>Br<sub>2</sub>N<sub>5</sub>O<sub>8</sub> requires: C, 51.4; H, 4.8; N, 8.6; Br, 19.5%), m.p. 135–136 °C; yield, 1.60 g (70%);

*R*<sub>F</sub> 0.50 (system A); δ<sub>H</sub>[(CD<sub>3</sub>)<sub>2</sub>SO] 1.33 (9 H, s), 1.55 (2 H, m), 1.79 (2 H, m), 2.64 (3 H, s), 3.25–3.75 (4 H, m), 4.40 (2 H, m), 4.68 (2 H, m), 5.24 (1 H, m), 5.59 (1 H, d, *J* 4.6 Hz), 6.24 (1 H, d, *J* 6.9 Hz), 7.45–7.6 (3 H, m), 7.7–7.9 (3 H, m), 7.98 (2 H, d, *J* 8.3 Hz), 8.08 (1 H, d, *J* 7.8 Hz), 8.63 (1 H, s), 8.75 (1 H, s), and 11.11 (1 H, br s).

2',3'-O-Methoxymethylene-6-*N*-(4-*t*-butylbenzoyl)adenosine (**24**).—Trimethyl orthoformate (7.5 ml, 68.6 mmol) was added to a stirred solution of 6-*N*-(4-*t*-butylbenzoyl)adenosine (3.0 g, 7.0 mmol) and toluene-4-sulphonic acid monohydrate (1.465 g, 7.7 mmol) in anhydrous DMF at room temperature. After 15 min, the products were neutralized with methanolic ammonia and evaporated under reduced pressure. The residue was dissolved in chloroform (60 ml), filtered through Celite and re-evaporated. The glass obtained was dissolved in methanol (10 ml) and formic acid (1.2 ml) was added. After 1 h, the products were neutralized with methanolic ammonia, concentrated, and partitioned between chloroform (100 ml) and aqueous sodium hydrogen carbonate (150 ml). The dried (MgSO<sub>4</sub>) organic layer was fractionated by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>–EtOH (97:3) were combined and concentrated under reduced pressure. Crystallization of the residue from ethyl acetate–di-isopropyl ether (3:2) (*ca.* 50 ml) gave 2',3'-O-methoxymethylene-6-*N*-(4-*t*-butylbenzoyl)adenosine as colourless crystals (Found: C, 58.9; H, 5.8; N, 14.75. C<sub>23</sub>H<sub>27</sub>N<sub>5</sub>O<sub>6</sub> requires: C, 58.8; H, 5.8; N, 14.9%; m.p. 131–132 °C; yield, 1.635 g (50%); *R*<sub>F</sub> 0.50 (system A); δ<sub>H</sub>[(CD<sub>3</sub>)<sub>2</sub>SO–D<sub>2</sub>O, 90 MHz] 1.34 (9 H, s), 3.28 (0.8 H, s), 3.41 (2.2 H, s), 3.64 (2 H, m), 4.2–4.5 (1 H, m), 4.9–5.2 (1 H, m), 5.4–5.6 (1 H, m), 6.10 (0.8 H, s), 6.21 (0.2 H, s), 6.31 (0.25 H, d, *J* 3.5 Hz), 6.42 (0.75 H, d, *J* 3.0 Hz), 7.58 (2 H, d, *J* 8.4 Hz), 8.01 (2 H, d, *J* 8.5 Hz), 8.67 (0.2 H, s), 8.71 (0.8 H, s), and 8.77 (1 H, s).

2'-O-(4-Methoxytetrahydropyran-4-yl)-4-*N*-(*t*-butylbenzoyl)cytidine (**21**; **B** = **6b**).—4-*t*-Butylbenzoyl chloride (42 ml, 0.215 mol) was added dropwise over a period of 30 min to a stirred suspension of cytidine (10.0 g, 41.1 mmol) in anhydrous pyridine (100 ml) at 0 °C. The reactants were then stirred at room temperature for 18 h and methanol (10 ml) was added slowly. After a further period of 30 min, the products were concentrated to *ca.* one-half volume, the resulting oil was dissolved in chloroform (80 ml) and the solution was extracted with saturated aqueous sodium hydrogen carbonate (200 ml). The aqueous layer was back-extracted with chloroform (3 × 60 ml). The dried (MgSO<sub>4</sub>) combined organic layers were evaporated under reduced pressure, and the solid residue obtained was dissolved in pyridine (50 ml) and methanol (50 ml). 25% Methanolic sodium methoxide (29.6 ml, 28 g, 0.13 mol) was added to the stirred solution at room temperature and, after 10 min, an excess of Dowex 50 cation exchange resin (pyridinium form) was added. The resin was removed by filtration, the filtrate was concentrated under reduced pressure to *ca.* one-third volume and then poured into saturated aqueous sodium hydrogen carbonate (150 ml). The resulting mixture was extracted with chloroform (4 × 40 ml), and the latter extracts were combined, dried (MgSO<sub>4</sub>) and evaporated under reduced pressure. Trituration of the residue obtained with light petroleum (b.p. 30–40 °C) (2 × 250 ml) gave a colourless solid (12.3 g).

1,3-Dichloro-1,1,3,3-tetraisopropylidisiloxane<sup>18</sup> (11.26 g, 35.7 mmol) was added to a stirred suspension of the latter solid (12.0 g) and imidazole (9.72 g, 0.143 mol) in acetonitrile (100 ml) at room temperature. After 20 min, water (5 ml) was added to the clear solution obtained and, after a further period of 10 min, the products were concentrated under reduced pressure to *ca.* one-fifth volume. Chloroform (100 ml) was added and the resulting solution was extracted first with saturated aqueous

sodium hydrogen carbonate (150 ml) and then with 0.1M hydrochloric acid (300 ml). Both aqueous extracts were back-extracted with chloroform (3 × 50 ml). The combined chloroform extracts were washed with water (150 ml), dried (MgSO<sub>4</sub>) and evaporated under reduced pressure to give a colourless solid. The latter material was fractionated by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>–EtOH (95:5), were combined and concentrated under reduced pressure to give a colourless glass (16.0 g). The latter material, 5,6-dihydro-4-methoxy-2H-pyran<sup>2,19</sup> (17.0 g, 0.15 mol) and toluene-4-sulphonic acid, monohydrate (1.14 g, 6.0 mmol) were stirred together in anhydrous dioxane (100 ml) at room temperature. After 2 h, the products were neutralized with 4M methanolic ammonia, and were then concentrated under reduced pressure. A solution of the residue in chloroform (80 ml) was filtered through Hyflo supercel and was then concentrated under reduced pressure. A 1M solution of tetraethylammonium fluoride in acetonitrile (90 ml, 90 mmol) was added with stirring to the residual oil at room temperature. After 10 min, the products were concentrated under reduced pressure, redissolved in chloroform (80 ml) and the solution was washed with saturated aqueous sodium hydrogen carbonate (100 ml). The dried (MgSO<sub>4</sub>) chloroform layer was evaporated under reduced pressure and the material obtained was fractionated by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>–EtOH (97:3), were combined and concentrated under reduced pressure. Crystallization of the residue from aqueous ethanol gave 2'-O-(4-methoxytetrahydropyran-4-yl)-4-N-(*t*-butylbenzoyl)cytidine as colourless microcrystals (Found: C, 58.3; H, 6.9; N, 7.7. C<sub>26</sub>H<sub>35</sub>N<sub>3</sub>O<sub>8</sub>·H<sub>2</sub>O requires: C, 58.3; H, 7.0; N, 7.85%; m.p. 196–197 °C; yield, 8.50 g (39.5%, based on cytidine); R<sub>F</sub> 0.36 (system A); δ<sub>H</sub>[(CD<sub>3</sub>)<sub>2</sub>SO] 1.31 (9 H, s), 1.65–1.9 (4 H, m), 2.93 (3 H, s), 3.35–3.75 (6 H, m), 3.97 (1 H, m), 4.03 (1 H, m), 4.41 (1 H, m), 5.19 (1 H, d, *J* 5.0 Hz), 5.25 (1 H, m), 6.15 (1 H, d, *J* 6.9 Hz), 7.37 (1 H, m), 7.53 (2 H, d, *J* 8.7 Hz), 7.98 (2 H, d, *J* 8.7 Hz), 8.43 (1 H, d, *J* 7.3 Hz), and 11.21 (1 H, br s).

5'-O-[2-(Dibromomethyl)benzoyl]-2'-O-(4-methoxytetrahydropyran-4-yl)-4-N-(*t*-butylbenzoyl)cytidine (**35**; B = **6b**).—A solution of 2-(dibromomethyl)benzoyl chloride<sup>5</sup> (1.31 g, 4.19 mmol) in acetonitrile (23 ml) was added to a stirred solution of 2'-O-(4-methoxytetrahydropyran-4-yl)-4-N-(*t*-butylbenzoyl)cytidine (1.81 g, 3.38 mmol) in anhydrous pyridine (35 ml) at room temperature. After 1 h, water (3 ml) was added and, after a further period of 10 min, the products were poured into saturated aqueous sodium hydrogen carbonate (120 ml). The resulting mixture was extracted with chloroform (3 × 50 ml), and the combined organic extracts were dried (MgSO<sub>4</sub>) and evaporated under reduced pressure. The glass obtained was fractionated by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>–EtOH (96:4), were combined and evaporated under reduced pressure. A solution of the residue in chloroform (10 ml) was added dropwise with stirring to light petroleum (b.p. 30–40 °C, 200 ml) to give the desired 5'-O-[2-(dibromomethyl)benzoyl]-derivative as a colourless precipitated solid; yield, 2.3 g (86%), R<sub>F</sub> 0.48 (system A); δ<sub>H</sub>[(CD<sub>3</sub>)<sub>2</sub>SO] 1.31 (9 H, s), 1.6–1.9 (4 H, m), 3.00 (3 H, s), 3.3–3.75 (4 H, m), 4.19 (1 H, m), 4.28 (1 H, m), 4.60 (2 H, m), 6.11 (1 H, d, *J* 6.0 Hz), 7.33 (1 H, m), 7.53 (3 H, m), 7.75–7.9 (3 H, m), 7.97 (2 H, d, *J* 8.3 Hz), 8.11 (1 H, d, *J* 7.8 Hz), 8.20 (1 H, d, *J* 7.8 Hz), and 11.75 (1 H, br s).

2',3',5'-Tri-O-methoxyacetylguanosine (**14b**).—Methoxyacetic anhydride (57.19 g, 0.35 mol) was added in one portion to a stirred suspension of guanosine (20 g, 70.6 mmol) in anhydrous DMF (170 ml) and pyridine (240 ml) at room temperature. The reactants were heated at 100 °C for 30 min

and were then allowed to cool to room temperature. Methanol (100 ml) was then added to the resulting solution and, after a further period of 30 min, the products were concentrated under reduced pressure (water-pump followed by oil-pump). Crystallization of the thick residual oil obtained from ethanol gave 2',3',5'-tri-O-methoxyacetylguanosine as colourless crystals (Found: C, 44.5; H, 5.0; N, 13.8. C<sub>19</sub>H<sub>25</sub>N<sub>5</sub>O<sub>11</sub>·0.5 H<sub>2</sub>O requires: C, 44.9; H, 5.15; N, 13.8%; m.p. 168–170 °C; yield, 32.4 g (90%) in two crops, R<sub>F</sub> 0.26 (system A); δ<sub>H</sub>[(CD<sub>3</sub>)<sub>2</sub>SO] 3.27 (3 H, s), 3.32 (3 H, s), 3.35 (3 H, s), 4.0–4.25 (6 H, m), 4.35–4.55 (3 H, m), 5.67 (1 H, m), 5.92 (1 H, t, *J* 6.0 Hz), 6.02 (1 H, d, *J* 6.4 Hz), 6.59 (2 H, br s), 7.97 (1 H, s), and 10.81 (1 H, br s).

6-O-(3-Chlorophenyl)-2-N-phenylacetylguanosine (**16a**).—(a) Phenylacetic anhydride<sup>17</sup> (10.19 g, 40.0 mmol) was added to a stirred solution of 2',3',5'-tri-O-methoxyacetylguanosine (5.0 g, 10.0 mmol) in anhydrous pyridine (50 ml) at room temperature, and the reactants were placed in an oil-bath preheated to 120 °C. After 30 min, the products were cooled (ice-bath) and poured with care into saturated aqueous sodium hydrogen carbonate (200 ml) at room temperature. The resulting mixture was extracted with dichloromethane (2 × 100 ml). The dried (MgSO<sub>4</sub>) organic extracts were concentrated under reduced pressure and the residual oil obtained was fractionated by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>–EtOH (95:5), were combined and evaporated under reduced pressure to give a glass. The latter material was dissolved in acetonitrile (33.4 ml) at room temperature and mesitylene-2-sulphonyl chloride (6.28 g, 28.7 mmol), followed by triethylamine (4.0 ml, 28.7 mmol; dropwise over 5 min) were added to the resulting solution. After 30 min, more triethylamine (4.0 ml, 28.7 mmol) and water (2.0 ml) were added, and the products were poured into ice-cold saturated aqueous sodium hydrogen carbonate (200 ml). After stirring for 15 min, the mixture obtained was extracted with dichloromethane (2 × 100 ml). The dried (MgSO<sub>4</sub>) organic extracts were evaporated under reduced pressure and the oil obtained was triturated with hot light petroleum (b.p. 40–60 °C) (5 × 100 ml) and then with light petroleum (b.p. 30–40 °C) (2 × 100 ml).

The residual gum obtained was dissolved in dichloromethane (9.55 ml) and 3-chlorophenol (6.14 g, 47.8 mmol) and *N*-methylpyrrolidine (9.55 ml, 92 mmol) were added to the cooled (ice-bath), stirred solution. After 210 min, by which time the reaction vessel had warmed up to room temperature, the products were concentrated under reduced pressure. The residue was redissolved in methanol (25 ml) at room temperature, and 8M methanolic ammonia (25 ml) was added. After 100 min, the products were concentrated under reduced pressure, the residue was dissolved in dichloromethane (100 ml), and the resulting solution was washed with saturated aqueous sodium hydrogen carbonate (100 ml). The aqueous layer was back extracted with dichloromethane (100 ml), and the dried (MgSO<sub>4</sub>) combined organic layers were evaporated under reduced pressure. The oil obtained was thoroughly triturated with hot light petroleum (b.p. 40–60 °C), and the residue was purified by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>–EtOH (94:6), were combined and concentrated under reduced pressure to give a glass. When a solution of the latter material in dichloromethane (40 ml) was added dropwise to light petroleum (b.p. 30–40 °C) (2 000 ml), 6-O-(3-chlorophenyl)-2-N-phenylacetylguanosine was obtained as a precipitated solid; yield, 3.73 g (73%), R<sub>F</sub> 0.31 (system A); δ<sub>H</sub>[(CD<sub>3</sub>)<sub>2</sub>SO] 3.5–3.75 (2 H, m), 3.72 (2 H, s), 3.95 (1 H, m), 4.20 (1 H, m), 4.62 (1 H, m), 4.98 (1 H, m), 5.21 (1 H, d, *J* 4.7 Hz), 5.53 (1 H, d, *J* 5.8 Hz), 5.93 (1 H, d, *J* 5.9 Hz), 7.2–7.6 (9 H, m), 8.59 (1 H, s), and 10.65 (1 H, s).

(b) Mesitylene-2-sulphonyl chloride (6.56 g, 30 mmol) and

triethylamine (4.18 ml, 30 mmol) were added to a stirred solution of 2',3',5'-tri-*O*-methoxyacetylguanosine (5.00 g, 10.0 mmol) in anhydrous acetonitrile (50 ml) at room temperature. After 20 min, water (2 ml) was added and, after a further period of 10 min, the products were concentrated to small volume under reduced pressure and were then redissolved in chloroform (100 ml). The resulting solution was washed with saturated aqueous sodium hydrogen carbonate (150 ml), dried (MgSO<sub>4</sub>), and evaporated under reduced pressure. The glass obtained was dissolved in dichloromethane (50 ml) and *N*-methylpyrrolidine (8.5 ml, 82 mmol) was added to the cooled (ice-bath), stirred solution. After 30 min, 3-chlorophenol (12.85 g, 0.10 mol) was added to the reaction solution and, after a further period of 2 h, the products were concentrated under reduced pressure. The residue was redissolved in chloroform (100 ml) and the solution was washed with saturated aqueous sodium hydrogen carbonate (150 ml). The aqueous layer was back-extracted with chloroform (50 ml). The combined organic layers were dried (MgSO<sub>4</sub>) and evaporated under reduced pressure. The residual oil was triturated with hot light petroleum (b.p. 60–80 °C) (3 × 100 ml), and was then redissolved in acetonitrile (100 ml). 2,6-Lutidine (14.0 ml, 0.12 mol) was added to the stirred, cooled (ice-bath) solution and then phenylacetyl chloride (7.9 ml, 59.7 mmol) was added dropwise over a period of 15 min. After a further period of 15 min, water (2 ml) was added, and the products were concentrated under reduced pressure. The residue was redissolved in chloroform (150 ml), and the solution was washed with saturated aqueous sodium hydrogen carbonate (180 ml). Evaporation of the dried (MgSO<sub>4</sub>) organic layer gave an oil which was triturated with hot light petroleum (b.p. 60–80 °C) (3 × 100 ml). The residue was dissolved in methanol (25 ml) and 8M methanolic ammonia (25 ml) was added to the solution. After 90 min, the products were concentrated under reduced pressure, the residue was redissolved in chloroform (100 ml) and the solution was washed with saturated aqueous sodium hydrogen carbonate (150 ml). The aqueous layer was back extracted with chloroform (2 × 50 ml). The combined organic layers were dried (MgSO<sub>4</sub>) and evaporated under reduced pressure to give a glass which was purified by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>–EtOH (93:7) were combined and evaporated under reduced pressure. The material obtained was dissolved in chloroform (15 ml) and the resulting solution was added dropwise to light petroleum (b.p. 30–40 °C) (300 ml) to give 6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine as a colourless precipitated solid, identical [t.l.c. (system A); <sup>1</sup>H-n.m.r.] to the material obtained by procedure (a) above; yield, 3.47 g (67%).

2'-*O*-(4-Methoxytetrahydropyran-4-yl)-6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine (**21**; B = **9b**).—1,3-Dichloro-1,1,3,3-tetraisopropylidisiloxane<sup>18</sup> (2.65 g, 8.4 mmol) was added to a stirred solution of 6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine (3.58 g, 7.0 mmol) and imidazole (2.29 g, 33.6 mmol) in THF (35 ml) at room temperature. After 20 min, methanol (5 ml) was added and, after a further period of 10 min, the products were concentrated under reduced pressure to *ca.* one-third volume. Dichloromethane (100 ml) was added and the resulting solution was washed with 0.1M hydrochloric acid (2 × 100 ml), followed by saturated aqueous sodium hydrogen carbonate (2 × 100 ml). The dried (MgSO<sub>4</sub>) organic layer was concentrated under reduced pressure, and the residual glass was purified by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>–EtOH (97:3) were combined and evaporated under reduced pressure to give a glass (4.5 g).

The latter material was dissolved in dichloromethane (30 ml) at room temperature, and 5,6-dihydro-4-methoxy-2*H*-pyran<sup>2,19</sup>

(4.08 g, 35.7 mmol) followed by pyridinium toluene-4-sulphonate (1.77 g, 7.0 mmol) were added to the stirred solution. After 5 h, more dichloromethane (100 ml) was added and the resulting solution was washed with saturated aqueous sodium hydrogen carbonate (100 ml). The dried (MgSO<sub>4</sub>) organic layer was concentrated under reduced pressure and 1M tetraethylammonium fluoride in acetonitrile (24 ml, 24 mmol) was added to the residue at room temperature. After 30 min, the solution obtained was evaporated under reduced pressure, the residue was dissolved in dichloromethane (100 ml) and the solution was washed with saturated aqueous sodium hydrogen carbonate (200 ml). The aqueous layer was back-extracted with dichloromethane (100 ml). The organic layers were combined, dried (MgSO<sub>4</sub>) and then evaporated under reduced pressure to give an oil which was purified by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>–EtOH (95:5), were combined and concentrated under reduced pressure to give a glass. When a solution of the latter material in dichloromethane (15 ml) was added dropwise to light petroleum (b.p. 30–40 °C) (300 ml), 2'-*O*-(4-methoxytetrahydropyran-4-yl)-6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine was precipitated as a colourless solid; yield, 2.6 g (59%); *R*<sub>F</sub> 0.33 (system A); δ<sub>H</sub>[(CD<sub>3</sub>)<sub>2</sub>SO] 1.45–1.85 (4 H, m), 2.63 (3 H, s), 3.2–3.5 (4 H, m), 3.55–3.8 (2 H, m), 3.71 (2 H, s), 3.99 (1 H, m), 4.17 (1 H, m), 4.96 (1 H, dd, *J* 4.8, 7.3 Hz), 5.09 (1 H, m), 5.27 (1 H, d, *J* 4.2 Hz), 6.09 (1 H, d, *J* 7.3 Hz), 7.2–7.6 (9 H, m), 8.66 (1 H, s), and 10.65 (1 H, s).

5'-*O*-[2-(Dibromomethyl)benzoyl]-2'-*O*-(4-methoxytetrahydropyran-4-yl)-6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine (**35**; B = **9b**).—2'-*O*-(4-Methoxytetrahydropyran-4-yl)-6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine (1.06 g, 1.7 mmol) was thoroughly dried by evaporation from pyridine (2 × 10 ml) solution and was then redissolved in pyridine (17 ml) at room temperature. A solution of 2-(dibromomethyl)benzoyl chloride<sup>5</sup> (0.637 g, 2.04 mmol) in acetonitrile (10 ml) was then added dropwise with stirring over a period of 30 min to the latter solution. After a further period of 30 min, saturated aqueous sodium hydrogen carbonate (1.0 ml) was added, the products were concentrated to *ca.* one-third volume and were then poured into saturated aqueous sodium hydrogen carbonate (60 ml). The resulting mixture was extracted with chloroform (2 × 30 ml), and the combined extracts were dried (MgSO<sub>4</sub>) and evaporated under reduced pressure. The glass obtained was purified by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>–EtOH (98:2), were combined and evaporated under reduced pressure. When a solution of the residual glass in chloroform (5 ml) was added dropwise to light petroleum (b.p. 30–40 °C) (100 ml), 5'-*O*-[2-(dibromomethyl)benzoyl]-2'-*O*-(4-methoxytetrahydropyran-4-yl)-6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine was precipitated as a colourless solid; yield, 1.16 g (76%); *R*<sub>F</sub> 0.44 (system A); δ<sub>H</sub>[(CD<sub>3</sub>)<sub>2</sub>SO] 1.58 (2 H, m), 1.76 (2 H, m), 2.72 (3 H, s), 3.15–3.45 (4 H, m), 3.69 (2 H, s), 4.3–4.45 (2 H, m), 4.70 (2 H, m), 5.33 (1 H, m), 5.50 (1 H, d, *J* 5.0 Hz), 6.14 (1 H, d, *J* 6.7 Hz), 7.21 (4 H, m), 7.34 (2 H, m), 7.45–7.6 (3 H, m), 7.76 (3 H, m), 8.07 (1 H, d, *J* 7.5 Hz), 8.62 (1 H, s), and 10.62 (1 H, s).

5'-*O*-(9-Phenylxanthen-9-yl)-2'-*O*-(4-methoxytetrahydropyran-4-yl)-6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine (**26**).—2'-*O*-(4-Methoxytetrahydropyran-4-yl)-6-*O*-(3-chlorophenyl)-2-*N*-phenylacetylguanosine (0.626 g, 1.0 mmol) was thoroughly dried by evaporation from pyridine (2 × 9 ml) solution and was then redissolved in pyridine (9 ml) at room temperature. A solution of 9-chloro-9-phenylxanthene<sup>21</sup> (0.35 g, 1.2 mmol) in pyridine (9 ml) was added dropwise with stirring over a period of 30 min to the latter solution. After a further



period of 30 min, saturated aqueous sodium hydrogen carbonate (2.0 ml) was added, and the products were partitioned between chloroform and saturated aqueous sodium hydrogen carbonate (60 ml). The aqueous layer was back extracted with chloroform (30 ml). The combined organic layers were dried ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. The residue was purified by short-column chromatography on silica gel: the appropriate fractions, eluted with  $\text{CHCl}_3$ -EtOH (98:2) were combined and evaporated under reduced pressure. When a solution of the residual glass in chloroform (5 ml) was added dropwise to light petroleum (b.p. 30–40 °C) (100 ml), 5'-O-(9-phenylxanthen-9-yl)-2'-O-(4-methoxytetrahydropyran-4-yl)-6-O-(3-chlorophenyl)-2-N-phenylacetylguanosine was precipitated as a colourless solid; yield, 0.688 g (78%);  $R_F$  0.48 (system A);  $\delta_H[(\text{CD}_3)_2\text{SO}]$  1.45–1.84 (4 H, m), 2.72 (3 H, s), 3.2–3.55 (4 H, m), 3.6–3.8 (4 H, m), 4.1–4.25 (2 H, m), 5.03 (1 H, m), 5.30 (1 H, d,  $J$  5.2 Hz), 6.09 (1 H, d,  $J$  6.5 Hz), 6.9–7.6 (22 H, m), 8.45 (1 H, s), and 10.54 (1 H, s).

**Reaction between 2'-O-(4-Methoxytetrahydropyran-4-yl)-6-O-(3-chlorophenyl)-2-N-phenylacetylguanosine (21; B = 9b) and  $N^1,N^1,N^3,N^3$ -Tetramethylguanidinium 2-Nitrobenzaldehyde Oximate.**—(a)  $N^1,N^1,N^3,N^3$ -Tetramethylguanidine (0.056 ml, 0.45 mmol) was added to a stirred solution of the substrate [(21, B = 9b), 0.031 g, 0.05 mmol] and *E*-2-nitrobenzaldehyde oxime (0.083 g, 0.5 mmol) in dioxane (1.1 ml) at room temperature. After 180 min, t.l.c. (system A) revealed the presence of starting material [(21; B = 9b),  $R_F$  0.48, ca. 70%] and 2'-O-(4-methoxytetrahydropyran-4-yl)-2-N-phenylacetylguanosine [(21; B = 7),  $R_F$  0.34, ca. 30%]. No substrate (21; B = 9b) could be detected after 48 h.

(b) The experiment was repeated under the conditions described in (a) above except that dioxane (1.1 ml) was replaced by dioxane-acetonitrile (1:1) (1.1 ml). After 20 min, ca. 50% of substrate (21; B = 9b) remained, and after 150 min, the reaction was complete.

(c) The experiment was repeated under the conditions described in (a) above except that dioxane (1.1 ml) was replaced by acetonitrile (1.1 ml). After 8 min, ca. 50% of substrate (21; B = 9b) remained, and after 40 min, the reaction was complete.

**2'-O-(4-Methoxytetrahydropyran-4-yl)-4-O-(2,4-dimethylphenyl)uridine (18).**—Methoxyacetic anhydride (7.24 g, 44.7 mmol) was added to a stirred solution of 2'-O-(4-methoxytetrahydropyran-4-yl)uridine<sup>2</sup> (4.00 g, 11.16 mmol) in anhydrous pyridine (32 ml) at room temperature. After 1 h, water (2 ml) was added and, after a further period of 30 min, the products were concentrated to small volume under reduced pressure. The residue was dissolved in chloroform (60 ml), and the solution was extracted with saturated aqueous sodium hydrogen carbonate (200 ml). The aqueous layer was re-extracted with chloroform (3 × 80 ml) and the organic layers were combined, dried ( $\text{MgSO}_4$ ) and evaporated under reduced pressure. 3-Nitro-1,2,4-triazole<sup>6</sup> (2.55 g, 22.36 mmol) and diphenyl phosphorochloridate (6.07 g, 4.68 ml, 22.6 mmol) were added to a stirred solution of the residue in anhydrous pyridine (64 ml) at room temperature. After 2 h, solid potassium bicarbonate (5.6 g, 55.9 mmol) was added, followed by water (2 ml). After a further period of 10 min, the products were concentrated to small volume under reduced pressure, dissolved in chloroform (80 ml) and the solution was washed with saturated aqueous sodium hydrogen carbonate (120 ml), followed by water (100 ml). The dried ( $\text{MgSO}_4$ ) chloroform layer was concentrated under reduced pressure to give a glass.

2,4-Dimethylphenol (4.09 g, 33.5 mmol) and triethylamine (4.67 ml, 33.5 mmol) were added to a stirred solution of the latter glass in acetonitrile (65 ml) at room temperature. After 2 h, the reaction mixture was concentrated to small volume

under reduced pressure, redissolved in chloroform (80 ml) and the solution was washed with saturated aqueous sodium hydrogen carbonate (2 × 80 ml). The dried ( $\text{MgSO}_4$ ) chloroform layer was concentrated under reduced pressure and the residue was dissolved in 8M methanolic ammonia (400 ml) at room temperature. After 15 min, the products were evaporated under reduced pressure and the residue was fractionated by short column chromatography on silica gel: the appropriate fractions, eluted with  $\text{CHCl}_3$ -EtOH (93:7) were combined and evaporated under reduced pressure. Crystallization of the residue from aqueous ethanol gave 2'-O-(4-methoxytetrahydropyran-4-yl)-4-O-(2,4-dimethylphenyl)uridine as colourless crystals (Found: C, 55.1; H, 6.7; N, 5.6.  $\text{C}_{23}\text{H}_{30}\text{N}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$  requires: C, 55.4; H, 6.9; N, 5.6%; m.p. 115 °C; yield 4.01 g [72%, based on 2'-O-(4-methoxytetrahydropyran-4-yl)uridine];  $R_F$  0.38 (system A);  $\delta_H[(\text{CD}_3)_2\text{SO}]$  1.55–1.85 (4 H, m), 2.00 (3 H, s), 2.29 (3 H, s), 2.89 (3 H, s), 3.25–3.7 (6 H, m), 3.95 (1 H, m), 4.00 (1 H, m), 4.34 (1 H, dd,  $J$  4.8, 7.4 Hz), 5.19 (1 H, d,  $J$  4.8 Hz), 5.27 (1 H, m), 6.11 (1 H, d,  $J$  7.4 Hz), 6.40 (1 H, d,  $J$  7.4 Hz), 6.94 (1 H, d,  $J$  8.1 Hz), 7.0–7.2 (2 H, m), and 8.43 (1 H, d,  $J$  7.4 Hz).

**5'-O-[2-(Dibromomethyl)benzoyl]-2'-O-(4-methoxytetrahydropyran-4-yl)-4-O-(2,4-dimethylphenyl)uridine (35; B = 10).**—A solution of 2-(dibromomethyl)benzoyl chloride<sup>5</sup> (0.937 g, 3.0 mmol) in acetonitrile (15 ml) was added dropwise over a period of 90 min to a stirred solution of 2'-O-(4-methoxytetrahydropyran-4-yl)-4-O-(2,4-dimethylphenyl)uridine (1.156 g, 2.32 mmol) in anhydrous pyridine (30 ml) at room temperature. After a further period of 30 min, water (2 ml) was added and stirring was continued for 5 min. The products were then poured into saturated aqueous sodium hydrogen carbonate (80 ml), and the resulting mixture was extracted with chloroform (2 × 50 ml). The chloroform extracts were combined, dried ( $\text{MgSO}_4$ ) and evaporated under reduced pressure. The glass obtained was fractionated by short column chromatography on silica gel: the appropriate fractions, eluted with  $\text{CHCl}_3$ -EtOH (96:4), were combined and evaporated under reduced pressure. A solution of the residue in chloroform (10 ml) was added dropwise with stirring to light petroleum (b.p. 30–40 °C) (200 ml) to give the desired 5'-O-[2-(dibromomethyl)benzoyl]-derivative as a colourless precipitated solid; yield 1.39 g (81%);  $R_F$  0.48 (system A);  $\delta_H[(\text{CD}_3)_2\text{SO}]$  1.55–1.85 (4 H, m), 2.00 (3 H, s), 2.28 (3 H, s), 2.95 (3 H, s), 3.3–3.75 (4 H, m), 4.13 (1 H, m), 4.25 (1 H, m), 4.56 (3 H, m), 5.43 (1 H, d,  $J$  5.5 Hz), 6.07 (1 H, d,  $J$  6.9 Hz), 6.27 (1 H, d,  $J$  7.3 Hz), 6.93 (1 H, d,  $J$  8.3 Hz), 7.05 (1 H, m), 7.11 (1 H, m), 7.51 (1 H, m), 7.8 (3 H, m), 8.10 (1 H, d,  $J$  7.8 Hz), and 8.20 (1 H, d,  $J$  7.3 Hz).

**Reaction Between 2'-O-(4-Methoxytetrahydropyran-4-yl)-4-O-(2,4-dimethylphenyl)uridine (18) and  $N^1,N^1,N^3,N^3$ -Tetramethylguanidinium 2-Nitrobenzaldehyde Oximate.**—(a)  $N^1,N^1,N^3,N^3$ -Tetramethylguanidine (0.029 ml, 0.23 mmol) was added to a solution of 2'-O-(4-methoxytetrahydropyran-4-yl)-4-O-(2,4-dimethylphenyl)uridine (0.12 g, 0.024 mmol) and *E*-2-nitrobenzaldehyde oxime (0.042 g, 0.25 mmol) in dioxane (0.5 ml) at room temperature. After 20 min, t.l.c. (system A) revealed the presence of starting material [(18), ca. 50%] and 2'-O-(4-methoxytetrahydropyran-4-yl)uridine (ca. 50%). No starting material could be detected after 180 min.

(b) The experiment described in (a) above was repeated except that dioxane (0.5 ml) was replaced by acetonitrile (0.5 ml). After 15 min, t.l.c. (system A) revealed the presence of starting material [(18), ca. 50%] and 2'-O-(4-methoxytetrahydropyran-4-yl)uridine (ca. 50%). No starting material could be detected after 150 min.

(c) The experiment described in (a) above was repeated except that dioxane (0.5 ml) was replaced by dioxane-acetonitrile (1:1)



(0.5 ml). After 15 min, t.l.c. (system A) revealed the presence of starting material [(18), *ca.* 50%] and 2'-*O*-(4-methoxytetrahydropyran-4-yl)uridine (*ca.* 50%). No starting material could be detected after 150 min.

(d)  $N^1, N^1, N^3, N^3$ -Tetramethylguanidine (0.62 ml, 4.9 mmol) was added to a stirred solution of 2'-*O*-(4-methoxytetrahydropyran-4-yl)-4-*O*-(2,4-dimethylphenyl)uridine (0.25 g, 0.50 mmol) and *E*-2-nitrobenzaldehyde oxime (0.898 g, 5.4 mmol) in anhydrous dioxane (10.8 ml) at room temperature. After 2 h, the products were concentrated under reduced pressure to *ca.* one quarter volume, chloroform (10 ml) was added and the resulting solution was extracted with saturated aqueous sodium hydrogen carbonate (50 ml). The aqueous layer was re-extracted with chloroform (6 × 10 ml). The combined organic layers were dried ( $MgSO_4$ ) and concentrated under reduced pressure. The residue was purified by short column chromatography on silica gel: the appropriate fractions, eluted with  $CHCl_3$ -EtOH (95:5), were combined and evaporated under reduced pressure. Crystallization of the residue from ethyl acetate (5 ml) gave 2'-*O*-(4-methoxytetrahydropyran-4-yl)uridine (0.16 g, 89%) as colourless crystals. This material was identical [m.p., mixed m.p.,  $^1H$  n.m.r., t.l.c. (system A)] to authentic 2'-*O*-(4-methoxytetrahydropyran-4-yl)uridine.<sup>2</sup>

2'-*O*-(4-Methoxytetrahydropyran-4-yl)-4-*O*-phenyl-5-methyluridine (30).—5-Methyluridine<sup>30</sup> (1.314 g, 5.09 mmol), 1,3-dichloro-1,1,3,3-tetraisopropylsiloxane<sup>18</sup> (1.92 g, 6.09 mmol), imidazole (1.66 g, 24.4 mmol) and DMF (15 ml) were stirred together at room temperature. After 20 min, water (2 ml) was added, and the products were concentrated under reduced pressure and worked-up as in the corresponding conversion of inosine to its 2'-*O*-(4-methoxytetrahydropyran-4-yl) derivative (see below). 5,6-Dihydro-4-methoxy-2*H*-pyran<sup>2,19</sup> (5.8 g, 51.5 mmol), followed by toluene-4-sulphonic acid, monohydrate (0.048 g, 0.25 mmol) were added, at room temperature, to a stirred solution of the products obtained in dioxane (15 ml). After 1 h, the products were again worked-up as in the preparation of 2'-*O*-(4-methoxytetrahydropyran-4-yl)inosine, and then treated with 1*M* tetraethylammonium fluoride in acetonitrile (15.3 ml, 15.3 mmol). After 15 min, the products were concentrated under reduced pressure and fractionated by short column chromatography on silica gel. When a solution of the purified material in chloroform was added to light petroleum (b.p. 30–40 °C), 2'-*O*-(4-methoxytetrahydropyran-4-yl)-5-methyluridine was obtained as a colourless precipitate (1.445 g). The latter material was dissolved in anhydrous pyridine (10 ml) at room temperature and methoxyacetic anhydride (1.44 g, 8.9 mmol) was added to the stirred solution. After 45 min, methanol (1.0 ml) was added and, after a further period of 10 min, the products were concentrated under reduced pressure. The residue was dissolved in chloroform (40 ml) and the solution was extracted with saturated aqueous sodium hydrogen carbonate (2 × 20 ml). The aqueous extracts were back extracted with chloroform (40 ml). The combined organic layers were dried ( $MgSO_4$ ) and evaporated under reduced pressure. The residue was dissolved in anhydrous acetonitrile (10 ml) and 3-nitro-1,2,4-triazole<sup>6</sup> (0.885 g, 7.76 mmol), diphenyl phosphorochloridate (2.08 g, 7.74 mmol) and triethylamine (2.16 ml, 15.5 mmol) were added to the stirred solution at room temperature. After 30 min, water (1.0 ml) was added and the products were concentrated under reduced pressure. The residue was partitioned between chloroform and aqueous sodium hydrogen carbonate and then worked up as above to give an oil. This material was dissolved in acetonitrile (10 ml), and phenol (0.547 g, 5.8 mmol) and triethylamine (0.81 ml, 5.8 mmol) were added to the stirred solution at room temperature. After 25 min, the solution was evaporated under reduced pressure and the residue was redissolved in 8*M* methanolic

ammonia (30 ml) at room temperature. After 25 min, the products were concentrated under reduced pressure and the residue was crystallized from water (15 ml) containing a few drops of methanol to give 2'-*O*-(4-methoxytetrahydropyran-4-yl)-4-*O*-phenyl-5-methyluridine as colourless crystals (Found: C, 58.35; H, 6.3; N, 6.1.  $C_{22}H_{28}N_2O_8 \cdot 0.25H_2O$  requires: C, 58.3; H, 6.3; N, 6.2%; m.p. 146.5–147 °C; yield, 1.076 g (46.6%, based on 5-methyluridine);  $R_F$  0.36 (system A);  $\delta_H[(CD_3)_2SO]$  1.55–1.9 (4 H, m), 2.08 (3 H, s), 2.90 (3 H, s), 3.25–3.75 (6 H, m), 3.93 (1 H, m), 4.01 (1 H, m), 4.34 (1 H, dd,  $J$  5.0, 6.9 Hz), 5.15 (1 H, d,  $J$  5.0 Hz), 5.27 (1 H, m), 6.07 (1 H, d,  $J$  6.9 Hz), 7.18 (2 H, m), 7.29 (1 H, m), 7.45 (2 H, m), and 8.25 (1 H, s).

Reaction Between 2'-*O*-(4-Methoxytetrahydropyran-4-yl)-4-*O*-phenyl-5-methyluridine (30) and  $N^1, N^1, N^3, N^3$ -Tetramethylguanidinium 2-Nitrobenzaldehyde Oximate.— $N^1, N^1, N^3, N^3$ -Tetramethylguanidine (0.56 ml, 0.45 mmol) was added to a stirred solution of 2'-*O*-(4-methoxytetrahydropyran-4-yl)-4-*O*-phenyl-5-methyluridine (0.022 g, 0.05 mmol) and *E*-2-nitrobenzaldehyde oxime (0.083 g, 0.5 mmol) in acetonitrile-dioxane (1:1) (1.1 ml) at room temperature. After 10 min, t.l.c. (system A) revealed the presence of starting material [(30), *ca.* 50%] and 2'-*O*-(4-methoxytetrahydropyran-4-yl)-5-methyluridine [(21; B = 27), *ca.* 50%]. No starting material could be detected after 75 min.

2'-*O*-(4-Methoxytetrahydropyran-4-yl)-5-β-D-ribofuranosyluracil (21; B = 28a).—1,3-Dichloro-1,1,3,3-tetraisopropylsiloxane<sup>18</sup> (1.51 g, 4.8 mmol) and imidazole (1.307 g, 19.2 mmol) were added to a stirred suspension of 5-β-D-ribofuranosyluracil (0.977 g, 4.0 mmol) in anhydrous DMF (10 ml) at room temperature. After 70 min, methanol (4 ml) was added and, after a further period of 10 min, the products were concentrated under reduced pressure. The residue was redissolved in chloroform (40 ml) and the solution was washed with saturated aqueous sodium hydrogen carbonate (40 ml), 0.1*M* hydrochloric acid (40 ml), and water (40 ml). The dried ( $MgSO_4$ ) organic layer was concentrated under reduced pressure. The glass obtained was dissolved in dioxane (7 ml) and toluene-4-sulphonic acid, monohydrate (0.076 g, 0.40 mmol) and 5,6-dihydro-4-methoxy-2*H*-pyran<sup>2,19</sup> (2.3 g, 20 mmol) were added to the stirred solution at room temperature. After 30 min, an excess of methanolic ammonia was added, and the products were evaporated under reduced pressure. The residual oil was dissolved in chloroform (50 ml), and the solution was filtered through Celite and then evaporated under reduced pressure. The oil obtained was dissolved in a 1*M* solution of tetraethylammonium fluoride in acetonitrile (12 ml, 12 mmol) at room temperature. After 30 min, the products were concentrated under reduced pressure and the oil obtained was purified by short column chromatography on silica gel: the appropriate fractions, eluted with  $CHCl_3$ -EtOH (90:10–80:20), were combined and concentrated under reduced pressure. Crystallization of the residue from ethanol gave 2'-*O*-(4-methoxytetrahydropyran-4-yl)-5-β-D-ribofuranosyluracil as colourless crystals (Found: C, 50.3; H, 6.2; N, 7.9.  $C_{15}H_{22}N_2O_8$  requires: C, 50.3; H, 6.2; N, 7.8%) as colourless crystals, m.p. 194–197 °C; yield, 0.598 g (42%);  $R_F$  0.32 (system A);  $\delta_H[(CD_3)_2SO]$  1.6–1.9 (4 H, m), 3.02 (3 H, s), 3.3–3.75 (6 H, m), 3.75 (1 H, m), 3.93 (1 H, m), 4.35 (1 H, dd,  $J$  5.0, 6.9 Hz), 4.54 (1 H, d,  $J$  7.3 Hz), 4.71 (1 H, m), 4.84 (1 H, m), 7.64 (1 H, s), and 11.10 (1 H, br s).

1-*N*-(4-Bromobenzenesulphonyl)-2'-*O*-(4-methoxytetrahydropyran-4-yl)-5-β-D-ribofuranosyluracil (32).—(a) Methoxyacetic anhydride (0.49 g, 3.0 mmol) was added to a stirred solution of 2'-*O*-(4-methoxytetrahydropyran-4-yl)-5-β-D-ribofuranosyluracil (0.358 g, 1.0 mmol) in anhydrous pyridine (3 ml) at room temperature. After 30 min, methanol (2 ml) was added

and, after a further period of 5 min, the products were concentrated under reduced pressure and redissolved in chloroform (100 ml). The solution obtained was washed with saturated aqueous sodium hydrogen carbonate (50 ml), and the aqueous layer was back extracted with chloroform-pyridine (19:1) (2 × 50 ml). The combined organic layers were dried (MgSO<sub>4</sub>), evaporated under reduced pressure, and redissolved in acetonitrile (3 ml). 4-Bromobenzenesulphonyl chloride (0.319 g, 1.25 mmol) and triethylamine (0.174 ml, 1.25 mmol) were added to the stirred solution at room temperature. After 10 min, more triethylamine (0.174 ml, 1.25 mmol) and water (*ca.* 1 ml) were added and, after a further period of 10 min, the products were concentrated under reduced pressure. The residue was dissolved in 8M methanolic ammonia (30 ml) at room temperature. After 18 h, the products were evaporated under reduced pressure and the residue was purified by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>-EtOH (96:4) were combined and evaporated under reduced pressure. When a solution of the residue in chloroform (2 ml) was added dropwise to light petroleum (b.p. 30–40 °C) (50 ml), the desired 4-bromobenzenesulphonyl derivative (**32**) was obtained as a colourless precipitate; yield, 0.314 g (54%); *R*<sub>F</sub> 0.55 (system A),  $\delta_{\text{H}}[(\text{CD}_3)_2\text{SO}]$ , 1.65–1.9 (4 H, m), 3.10 (3 H, s), 3.35–3.75 (6 H, m), 3.81 (1 H, m), 4.02 (1 H, m), 4.27 (1 H, t, *J* 4.5 Hz), 4.71 (1 H, d, *J* 4.1 Hz), 4.76 (1 H, d, *J* 6.6 Hz), 4.90 (1 H, m), 7.93 (4 H, m), 8.48 (1 H, s), and 11.86 (1 H, s).

(b) 5- $\beta$ -D-Ribofuranosyluracil (0.488 g, 2.0 mmol), 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane<sup>18</sup> (0.75 g, 2.4 mmol), imidazole (0.652 g, 9.6 mmol) and DMF (10 ml) were allowed to react together at room temperature. After 20 min, methanol (3 ml) was added and, after a further period of 10 min, the products were worked up as described in procedure (a) above, and then purified by short column chromatography on silica gel. The product obtained was allowed to react with 5,6-dihydro-4-methoxy-2H-pyran<sup>2,19</sup> (0.89 g, 7.8 mmol) and trifluoroacetic acid (0.052 ml, 0.67 mmol) in dichloromethane (5 ml). After 150 min, the products were neutralized with methanolic ammonia and concentrated under reduced pressure. The residue obtained was fractionated by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>-EtOH (97:3) were combined and evaporated under reduced pressure. The residue was dissolved in acetonitrile (5 ml) and triethylamine (0.28 ml, 2.0 mmol), and 4-bromobenzenesulphonyl chloride (0.514 g, 2.0 mmol) were added to the stirred solution at room temperature. After 20 mins, the products were evaporated under reduced pressure and the residue was dissolved in 1M tetraethylammonium fluoride in acetonitrile (4.5 ml, 4.5 mmol) at room temperature. The solution was stirred for 20 min and was then evaporated under reduced pressure. The residue obtained was fractionated by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>-EtOH (97:3) were combined and evaporated under reduced pressure. When a solution of the residual glass in chloroform (5 ml) was added dropwise with stirring to light petroleum (b.p. 30–40 °C) (100 ml), 1-(4-bromobenzenesulphonyl)-2'-O-(4-methoxytetrahydropyran-4-yl)-5- $\beta$ -D-ribofuranosyluracil was obtained as a colourless precipitate; yield, 0.623 g (54%, based on 5- $\beta$ -D-ribofuranosyluracil). This material was identical [t.l.c. (system A), <sup>1</sup>H-n.m.r.] to that obtained by procedure (a) above.

1-N-(4-Bromobenzenesulphonyl)-5'-O-[2-(dibromomethyl)-benzoyl]-2'-O-(4-methoxytetrahydropyran-4-yl)-5- $\beta$ -D-ribofuranosyluracil (**35**; B = **28b**).—A solution of 2-(dibromomethyl)benzoyl chloride<sup>5</sup> (0.562 g, 1.8 mmol) in acetonitrile (10 ml) was added dropwise over a period of 1 h to a stirred solution of 1-N-(4-bromobenzenesulphonyl)-2'-O-(4-methoxytetrahydropyran-4-yl)-5- $\beta$ -D-ribofuranosyluracil (0.866 g, 1.50

mmol) in pyridine (15 ml) at room temperature. Saturated aqueous sodium hydrogen carbonate (*ca.* 4 ml) was then added and, after 5 min, the products were concentrated under reduced pressure. The residue was partitioned between chloroform (50 ml) and saturated aqueous sodium hydrogen carbonate (50 ml). The aqueous layer was back-extracted with chloroform (3 × 50 ml). The combined organic layers were dried (MgSO<sub>4</sub>) and evaporated under reduced pressure. The residue was purified by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>-EtOH (98:2–96:4), were combined and evaporated under reduced pressure. When a solution of the residue in chloroform (5 ml) was added dropwise to light petroleum (b.p. 30–40 °C) (100 ml), the desired 2-(dibromomethyl)benzoyl derivative (**35**; B = **28b**) was obtained as a colourless precipitate; yield, 0.975 g (76%); *R*<sub>F</sub> 0.72 (system A);  $\delta_{\text{H}}[(\text{CD}_3)_2\text{SO}]$ , 1.65–1.9 (4 H, m), 3.09 (3 H, s), 3.3–3.55 (2 H, m), 3.55–3.75 (2 H, m), 4.15 (2 H, m), 4.35–4.6 (3 H, m), 4.75 (1 H, d, *J* 5.3 Hz), 5.12 (1 H, d, *J* 5.9 Hz), 7.51 (2 H, m), 7.77 (2 H, m), 7.89 (4 H, m), 8.10 (1 H, m), 8.18 (1 H, s), and 11.89 (1 H, br s).

*Removal of the 4-Bromobenzenesulphonyl Group from 1-N-(4-Bromobenzenesulphonyl)-2'-O-(4-methoxytetrahydropyran-4-yl)-5- $\beta$ -D-ribofuranosyluracil (**32**).—(a)* N<sup>1</sup>,N<sup>1</sup>,N<sup>3</sup>,N<sup>3</sup>-Tetramethylguanidine (0.056 ml, 0.45 mmol) was added to a stirred solution of 1-N-(4-bromobenzenesulphonyl)-2'-O-(4-methoxytetrahydropyran-4-yl)-5- $\beta$ -D-ribofuranosyluracil (0.029 g, 0.05 mmol) and *E*-2-nitrobenzaldehyde oxime (0.083 g, 0.5 mmol) in acetonitrile-dioxane (1:1) (1.1 ml) at room temperature. After 10 min, t.l.c. (system A) revealed the presence of starting material [(**32**), *ca.* 50%] and 2'-O-(4-methoxytetrahydropyran-4-yl)-5- $\beta$ -D-ribofuranosyluracil [(**21**; B = **28a**), *ca.* 50%]. No starting material could be detected after 90 min.

(b) A solution of 1-N-(4-bromobenzenesulphonyl)-2'-O-(4-methoxytetrahydropyran-4-yl)-5- $\beta$ -D-ribofuranosyluracil (0.014 g, 0.025 mmol) in concentrated aqueous ammonia (*d* 0.88, 1.0 ml) was allowed to stand at room temperature. T.l.c. (system A) revealed that the starting material (**32**) was completely unreacted after 24 h.

2'-O-(4-Methoxytetrahydropyran-4-yl)inosine (**21**; B = **29**).—Inosine (2.68 g, 10.0 mmol), 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane<sup>18</sup> (4.73 g, 15.0 mmol) and imidazole (4.21 g, 61.8 mmol) were stirred together in acetonitrile (30 ml) and DMF (10 ml) at room temperature. After 1 h, water (2 ml) was added, the products were concentrated under reduced pressure, and the residue was dissolved in chloroform (150 ml). The resulting solution was washed with saturated aqueous sodium hydrogen carbonate (100 ml), 0.1M hydrochloric acid (100 ml) and water (100 ml). The dried (MgSO<sub>4</sub>) chloroform layer was evaporated under reduced pressure (oil-pump). The oily residue obtained was dissolved in dioxane (30 ml), and toluene-4-sulphonic acid monohydrate (0.38 g, 2.0 mmol) and 5,6-dihydro-4-methoxy-2H-pyran<sup>2,19</sup> (5.0 g, *ca.* 43 mmol) was added to the stirred solution at room temperature. After 1 h, methanolic ammonia (8M; 1.0 ml) was added and the resulting solution was concentrated under reduced pressure. Chloroform (100 ml) was added and, after filtration, the filtrate was evaporated under reduced pressure to give a glass. A solution of tetrabutylammonium fluoride in THF (1M; 30 ml, 30 mmol) was added to the latter material and the solution obtained was stirred at room temperature. After 30 min, the products were concentrated under reduced pressure and purified by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>-EtOH (9:1) were combined and evaporated under reduced pressure. Crystallization of the residue from ethanol gave 2'-O-(4-methoxytetrahydropyran-4-yl)inosine as colourless crystals (Found: C, 50.2; H, 6.0; N, 14.1. C<sub>16</sub>H<sub>22</sub>N<sub>4</sub>O<sub>7</sub>·

0.2C<sub>2</sub>H<sub>5</sub>OH requires: C, 50.3; H, 6.0; N, 14.3%; m.p. 192—193 °C; yield, 2.08 g (53%); *R<sub>F</sub>* 0.18 (system A);  $\delta_{\text{H}}[(\text{CD}_3)_2\text{SO}-\text{D}_2\text{O}]$  1.4—1.9 (4 H, m), 2.60 (3 H, s), 3.2—3.75 (6 H, m), 4.07 (1 H, m), 4.16 (1 H, m), 4.84 (1 H, dd, *J* 4.8, 7.6 Hz), 6.04 (1 H, d, *J* 7.3 Hz), 8.13 (1 H, s), and 8.41 (1 H, s).

**2'-O-(4-Methoxytetrahydropyran-4-yl)-1-N-methylinosine (33b).**—Anhydrous potassium carbonate (0.414 g, 3.0 mmol) and dimethyl sulphate (0.19 ml, 2.0 mmol) were added to a stirred solution of 2'-O-(4-methoxytetrahydropyran-4-yl)-inosine (0.765 g, 2.0 mmol) in DMF (10 ml) at room temperature. After 10 min, the products were filtered and the filtrate was concentrated under reduced pressure. The residual oil was purified by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>–EtOH (92:8) were combined and evaporated under reduced pressure. Crystallization of the residue from ethanol gave 2'-O-(4-methoxytetrahydropyran-4-yl)-1-N-methylinosine (Found: C, 51.1; H, 6.3; N, 13.95. C<sub>17</sub>H<sub>24</sub>N<sub>2</sub>O<sub>7</sub> requires: C, 51.5; H, 6.1; N, 14.1%, m.p. 184—185 °C; yield, 0.619 g (78%); *R<sub>F</sub>* 0.24 (system A);  $\delta_{\text{H}}[(\text{CD}_3)_2\text{SO}]$  1.4—1.85 (4 H, m), 2.59 (3 H, s), 3.15—3.7 (6 H, m), 3.51 (3 H, s), 4.01 (1 H, m), 4.13 (1 H, m), 4.84 (1 H, dd, *J* 4.8, 7.3 Hz), 5.23 (1 H, m), 5.29 (1 H, d, *J* 4.4 Hz), 6.01 (1 H, d, *J* 7.6 Hz), 8.41 (1 H, s), and 8.45 (1 H, s).

**2'-O-(4-Methoxytetrahydropyran-4-yl)-1-N-pivaloyloxymethylinosine (33a).**—Potassium carbonate (0.414 g, 3.0 mmol) and chloromethyl pivalate (0.29 ml, 2.0 mmol) were added to a stirred solution of 2'-O-(4-methoxytetrahydropyran-4-yl)-inosine (0.382 g, 1.0 mmol) in DMF (5 ml) at room temperature. After 4.5 h, the products were filtered and the filtrate was concentrated under reduced pressure. The residual oil was purified by short column chromatography on silica gel: the appropriate fractions, eluted with CHCl<sub>3</sub>–EtOH (92:8), were combined and evaporated under reduced pressure. Crystallization of the residue from ethyl acetate gave 2'-O-(4-methoxytetrahydropyran-4-yl)-1-N-pivaloyloxymethylinosine as colourless crystals (Found: C, 53.2; H, 6.7; N, 11.25. C<sub>22</sub>H<sub>32</sub>N<sub>2</sub>O<sub>9</sub> requires: C, 53.2; H, 6.5; N, 11.3%; m.p. 195 °C; yield 0.367 g (74%); *R<sub>F</sub>* 0.36 (system A);  $\delta_{\text{H}}[(\text{CD}_3)_2\text{SO}]$  1.11 (9 H, s), 1.4—1.9 (4 H, m), 2.60 (3 H, s), 3.15—3.75 (6 H, m), 4.02 (1 H, m), 4.13 (1 H, m), 4.82 (1 H, dd, *J* 4.7, 7.3 Hz), 5.17 (1 H, m), 5.30 (1 H, d, *J* 4.5 Hz), 5.95 (2 H, s), 6.02 (1 H, d, *J* 7.4 Hz), 8.46 (1 H, s), and 8.56 (1 H, s).

**Reaction Between 2'-O-(4-Methoxytetrahydropyran-4-yl)-N-pivaloyloxymethylinosine (33a) and Concentrated Aqueous Ammonia.**—The substrate [(33a), 0.049 g, 0.1 mmol] was dissolved in concentrated aqueous ammonia (*d* 0.88, 2.0 ml) at room temperature. After 7 min, t.l.c. (system A) revealed that the substrate (*R<sub>F</sub>* 0.36) was ca. 50% converted into a single component with the same *R<sub>F</sub>* (0.18) as that of 2'-O-(4-methoxytetrahydropyran-4-yl)inosine. After 20 min, no substrate (33a) remained, and confirmation was obtained that the product was indeed 2'-O-(4-methoxytetrahydropyran-4-yl)-inosine by <sup>1</sup>H-n.m.r. spectroscopy.

**2-(Isopropylthiomethoxymethyl)benzoic Acid (Ptmt Acid) (40).**—Dry hydrogen chloride gas was bubbled through a mechanically stirred mixture of propane-2-thiol (106 ml, 1.14 mol) and paraformaldehyde (68.5 g, 2.28 mol) at room temperature for 5 h. Dichloromethane (300 ml) was then added and the reaction mixture was allowed to stand for 16 h. The products were poured onto crushed ice (200 g). The organic layer was separated, dried (MgSO<sub>4</sub>) and the dichloromethane was removed by evaporation. Distillation of the residue gave chloromethyl isopropyl sulphide, b.p. 52 °C/40 mmHg (lit.<sup>29</sup> 56—58 °C/44 mmHg); yield, 101 g (71%);  $\delta_{\text{H}}(\text{CDCl}_3)$ , 60 MHz) 1.35 (6 H, d, *J* 7 Hz), 3.25 (1 H, hept, *J* 7 Hz), and 4.80 (2 H, s).

Sodium hydride (80% suspension in mineral oil; 3.0 g, 0.1 mol) was placed in a 250 ml 3-necked round-bottomed flask, fitted with a dropping funnel, a double-walled condenser and a mechanical stirrer, and was washed by decantation with light petroleum (b.p. 30—40 °C) (3 × 50 ml). After the remaining light petroleum had been removed by evaporation in a stream of nitrogen, THF (20 ml) was added to the flask. The stirred suspension was then cooled to ca. –10 °C (industrial methylated spirit–dry ice bath), and a solution of 2-bromobenzyl alcohol (9.35 g, 50.0 mmol) in THF (20 ml) was added dropwise over 5 min. After a further period of 30 min, the flask was allowed to warm up to room temperature and, after a while, the reactants were observed to effervesce. When the effervescence ceased, the flask was again cooled to –10 °C. Sodium iodide (7.49 g, 50.0 mmol) was added, and then a solution of 2-chloromethyl isopropyl sulphide (7.06 ml, 60.0 mmol) in THF (20 ml) was added dropwise over a period of 5 min. After a further period of 30 min, the flask was allowed to warm up to room temperature and, after ca. 1 h, a strongly exothermic reaction occurred. The flask was then cooled in order to maintain the reaction mixture at ca. 20 °C. After 2 h, the products were cooled to –10 °C, and water was added dropwise until a homogeneous solution was obtained. The latter solution was extracted with dichloromethane (3 × 100 ml), and the combined extracts were dried (MgSO<sub>4</sub>) and evaporated under reduced pressure. Approximately three-quarters of the residual liquid was purified by distillation to give 1-bromo-2-isopropylthiomethoxymethylbenzene, b.p. 114 °C/0.5 mmHg; yield, 7.0 g (estimated total yield, 9.1 g, 66%);  $\delta_{\text{H}}(\text{CDCl}_3)$ , 60 MHz) 1.3 (6 H, d, *J* 7 Hz), 3.20 (1 H, hept, *J* 7 Hz), 4.77 (2 H, s), 4.91 (2 H, s), and 7.1—7.75 (4 H, m).

Butyl-lithium (2.1M solution in hexane; 5.6 ml, 11.8 mmol) was added to a stirred solution of 1-bromo-2-isopropylthiomethoxymethylbenzene (2.75 g, 10.0 mmol) in THF (5 ml) in an atmosphere of nitrogen at –78 °C (acetone–dry ice bath). After 10 min, a heavy white precipitate was obtained and, after 90 min, the reactants were allowed to warm up to ca. –30 °C. The clear solution obtained was added to a suspension of solid carbon dioxide in ether (200 ml) at ca. –100 °C. The reaction mixture was then allowed to warm up to room temperature while dry carbon dioxide gas was bubbled into it. This was continued for 16 h, and then the products were partitioned between ether (100 ml) and water (75 + 50 ml). The combined aqueous layers were acidified to ca. pH 2.5 with dilute hydrochloric acid, and the resulting suspension was extracted with dichloromethane (3 × 50 ml). The organic layers were combined, dried (MgSO<sub>4</sub>) and evaporated under reduced pressure. Crystallization of the residue from dichloromethane–light petroleum (b.p. 40—60 °C) gave 2-(isopropylthiomethoxymethyl)benzoic acid as colourless needles (Found: C, 60.2; H, 6.8. C<sub>12</sub>H<sub>16</sub>O<sub>3</sub>S requires: C, 60.0; H, 6.7%), m.p. 68 °C; yield, 1.76 g (73% based on 1-bromo-2-isopropylthiomethoxymethylbenzene);  $\delta_{\text{H}}[(\text{CD}_3)_2\text{SO}-\text{D}_2\text{O}]$  1.26 (6 H, d, *J* 6.7 Hz), 3.08 (1 H, m), 4.84 (2 H, s), 4.90 (2 H, s), 7.42 (1 H, m), 7.58 (2 H, m), and 7.87 (1 H, d, *J* 7.7 Hz);  $\delta_{\text{C}}(\text{CDCl}_3)$  23.76, 34.84, 68.10, 72.65, 127.24, 128.03, 131.61, 133.32, 141.05, and 172.60.

**5'-O-[2-(Isopropylthiomethoxymethyl)benzoyl]-2'-O-(4-methoxytetrahydropyran-4-yl)-4-O-(2,4-dimethylphenyl)uridine (41a).**—2'-O-(4-Methoxytetrahydropyran-4-yl)-4-O-(2,4-dimethylphenyl)uridine (0.925 g, 2.0 mmol), 2-(isopropylthiomethoxymethyl)benzoic acid (0.961 g, 4.0 mmol), di-isopropylcarbodi-imide (0.626 ml, 4.0 mmol) and THF (1.5 ml) were stirred together at room temperature. After 1 h, triethylamine (0.56 ml, 4.0 mmol) was added. After 13 h, the products were partitioned between chloroform (100 ml) and saturated aqueous sodium hydrogen carbonate (100 ml). The aqueous layer was back-extracted with chloroform (2 × 50 ml). The combined



organic layers were dried ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. The crude material obtained was purified by short column chromatography on silica gel: the appropriate fractions, eluted with  $\text{CHCl}_3$ -EtOH (98:2), were combined and evaporated under reduced pressure. When a solution of the residue in chloroform (2.5 ml) was added to light petroleum (b.p. 30–40 °C) (150 ml), the desired 5'-O-[2-(isopropylthiomethoxymethyl)benzoyl]-derivative was obtained as a colourless solid precipitate; yield, 0.86 g (62%);  $R_F$  0.49 (system A);  $\delta_{\text{H}}[(\text{CD}_3)_2\text{SO}]$  1.26 (6 H, d,  $J$  6.8 Hz), 1.6–1.9 (4 H, m), 2.01 (3 H, s), 2.29 (3 H, s), 2.97 (3 H, s), 3.06 (1 H, m), 3.2–3.75 (4 H, m), 4.11 (1 H, m), 4.23 (1 H, m), 4.4–4.6 (3 H, m), 4.83 (2 H, s), 4.89 (2 H, s), 5.40 (1 H, d,  $J$  5.8 Hz), 6.08 (1 H, d,  $J$  6.5 Hz), 6.19 (1 H, d,  $J$  7.4 Hz), 6.91 (1 H, d,  $J$  8.1 Hz), 7.04 (1 H, m), 7.10 (1 H, m), 7.42 (1 H, m), 7.61 (1 H, d,  $J$  3.9 Hz), 7.90 (1 H, d,  $J$  7.7 Hz), and 8.16 (1 H, d,  $J$  7.4 Hz).

5'-O-[2-(Isopropylthiomethoxymethyl)benzoyl]-2'-O-(4-methoxytetrahydropyran-4-yl)-6-N-(4-*t*-butylbenzoyl)-adenosine (**41b**).—2'-O-(4-Methoxytetrahydropyran-4-yl)-6-N-(4-*t*-butylbenzoyl)adenosine (1.083 g, 2.0 mmol), 2-(isopropylthiomethoxymethyl)benzoic acid (0.961 g, 4.0 mmol), di-isopropylcarbodi-imide (0.626 ml, 4.0 mmol) and THF (1.5 ml) were stirred together at room temperature. After 1 h, triethylamine (0.56 ml, 4.0 mmol) was added. After 18 h, the products were partitioned between chloroform (100 ml) and saturated aqueous sodium hydrogen carbonate (100 ml). The combined organic layers were dried ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. The crude material obtained was purified by short-column chromatography on silica gel: the appropriate fractions, eluted with  $\text{CHCl}_3$ -EtOH (98:2), were combined and evaporated under reduced pressure. When a solution of the residue in chloroform (4 ml) was added to light petroleum (b.p. 30–40 °C) (100 ml), the desired 5'-O-[2-(isopropylthiomethoxymethyl)benzoyl]-derivative was obtained as a colourless solid; yield, 0.467 g (30%);  $R_F$  0.53 (system A);  $\delta_{\text{H}}[(\text{CD}_3)_2\text{SO}]$  1.23 (6 H, d,  $J$  6.7 Hz), 1.34 (9 H, s), 1.56 (2 H, m), 1.79 (2 H, m), 2.69 (3 H, m), 3.04 (1 H, hept,  $J$  6.7 Hz), 3.26 (1 H, m), 3.44 (2 H, m), 3.67 (1 H, m), 4.3–4.45 (2 H, m), 4.54 (1 H, dd,  $J$  6.0, 11.8 Hz), 4.65 (1 H, dd,  $J$  4.4, 11.7 Hz), 4.80 (2 H, s), 4.87 (2 H, s), 5.19 (1 H, m), 5.53 (1 H, d,  $J$  5.1 Hz), 6.24 (1 H, d,  $J$  6.6 Hz), 7.41 (1 H, m), 7.55 (1 H, d,  $J$  8.4 Hz), 7.60 (2 H, d,  $J$  4.0 Hz), 7.90 (1 H, d,  $J$  7.7 Hz), 8.00 (2 H, d,  $J$  8.4 Hz), 8.65 (1 H, s), 8.69 (1 H, s), and 11.05 (1 H, br s).

**Removal of the 2-(Isopropylthiomethoxymethyl)benzoyl Protecting Group.**—(a) 2,4,6-Collidine (0.033 ml, 0.25 mmol) and then a solution of mercury(II) perchlorate trihydrate (0.045 g, 0.10 mmol) in THF (98:2) (0.5 ml) were added to a stirred solution of the above uridine derivative [(**41a**), 0.034 g, 0.05 mmol] in THF-water (98:2) (0.5 ml) at room temperature. After 1 min, t.l.c. (system A) revealed that only ca. 10% of starting material (**41a**) remained. After 5 min, 5% aqueous thioacetamide (0.15 ml, 0.1 mmol) was added and, after a further period of 15 min, the resulting black precipitate was removed by centrifugation. Triethylamine (0.18 ml, 1.3 mmol) and THF (0.15 ml) were added to the stirred supernatant at room temperature. After 5 min, t.l.c. (system A) revealed virtually quantitative conversion to a product with the same  $R_F$  (0.38) as 2'-O-(4-methoxytetrahydropyran-4-yl)-4-O-(2,4-dimethylphenyl)uridine (**18**).

(b) A solution of the above adenosine derivative [(**41b**); 0.005 g, 0.006 mmol] in concentrated aqueous ammonia ( $d$  0.88; 0.5

ml) was allowed to stand at room temperature. After 6 h, t.l.c. (system A) revealed starting material ( $R_F$  0.53, ca. 10%), a component with  $R_F$  0.44 (ca. 10%), a component (ca. 20%) with the same  $R_F$  (0.42) as 2'-O-(4-methoxytetrahydropyran-4-yl)-6-N-(4-*t*-butylbenzoyl)adenosine (**21**; **B** = **5b**) and a fourth component (ca. 60%) with the same  $R_F$  (0.26) as 2'-O-(4-methoxytetrahydropyran-4-yl)adenosine; after 24 h, t.l.c. revealed the presence only of one component with  $R_F$  = 0.26.

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