Some observations relating to the use of 1-aryl-4-alkoxypiperidin-4-yl groups for the protection of the 2′-hydroxy functions in the chemical synthesis of oligoribonucleotides

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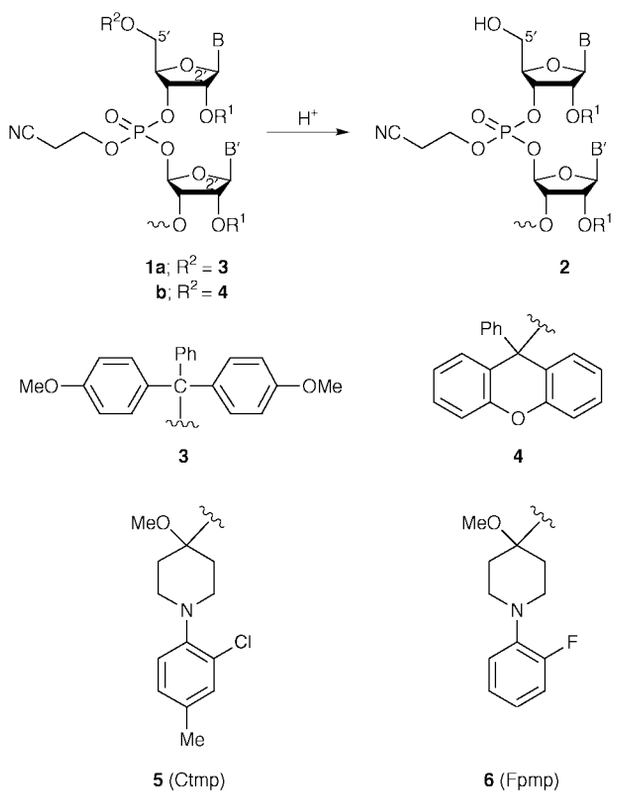
The comparative rates of acid-catalysed removal of ten 1-aryl-4-methoxypiperidin-4-yl **8** (R = Me) [including the previously reported Ctmp **5** and Fpmp **6**] protecting groups for the 2′-hydroxy functions in oligoribonucleotide synthesis are discussed. These studies have led to the development of the 1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl (Cpep) protecting group **8** (R = Et, $R^1 = R^2 = H$, $R^3 = Cl$) which is both more stable than the Ctmp and Fpmp groups at pH 0.5 and more labile at pH 3.75. The influence of the ribonucleoside aglycone on the stability of the 2′-*O*-Fpmp and 2′-*O*-Ctmp protecting groups both at low and high pH is examined.

Introduction

In the solid-phase synthesis of oligoribonucleotides (RNA sequences), it is particularly convenient to use a 4,4′-dimethoxytrityl[1] (DMTr **3**, as in **1a**) or a 9-phenylxanthen-9-yl[2] (Px **4**, as in **1b**) group to protect the 5′-terminal hydroxy function. The latter 'trityl' protecting groups have the advantage that they can be completely removed under relatively mild acidic conditions as in the conversion of structure **1** into structure **2**. Furthermore, they are easy to assay,[3] thereby enabling the efficiency of the coupling process to be monitored. However, almost certainly the most crucial decision that has to be taken in the chemical synthesis of RNA sequences is the choice of the protecting group (R^1, Scheme 1) for the 2′-hydroxy functions.[4] This protecting group must remain completely intact until the final deprotection step at the end of the synthesis and must then be removed under reaction conditions which do not promote the attack of the released 2′-hydroxy functions on the vicinal internucleotide phosphodiester linkages,[5,6] thereby leading to their cleavage or migration. As the base residues (B, B′; Scheme 1) and the internucleotide linkages are generally protected with base-labile groups,[7] we have always held the view that it is desirable to use an acid-labile group[8] to protect the 2′-hydroxy functions. Such an acid-labile protecting group needs to have very special properties in that it must be completely unaffected by the acidic conditions required to remove the 5′-*O*-DMTr **3** or 5′-*O*-Px **4** protecting group and yet be sensitive enough to acidic hydrolysis to be removable in the final deprotection step under conditions under which unprotected RNA sequences are completely stable. In order to meet these requirements, we have developed the 1-(2-chloro-4-methylphenyl)-4-methoxypiperidin-4-yl[9] (Ctmp **5**) and the 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl[10] (Fpmp **6**) protecting groups.

The main characteristic of these two 1-aryl-4-methoxypiperidin-4-yl protecting groups **5** and **6** is that, in the pH range 0.5–2.0, their susceptibility to acid-catalysed hydrolysis[11,12] is almost independent of pH. It is further noteworthy that their unblocking rates at pH 3.75 are only ≈10 times, rather than over 10^3 times (as would generally be expected for acetal systems), slower than at pH 0.5 (Table 1, entries 1 and 2). As a consequence of their unusual hydrolysis properties, the Ctmp **5** and Fpmp **6** protecting groups are stable under the [*e.g.*, CCl_3CO_2H–CH_2Cl_2 (2:98 v/v)] acidic conditions required for the removal of the DMTr **3** and Px **4** groups and yet are removable under the relatively mild conditions of acidic hydrolysis [*e.g.*, pH 3.75 buffer; 35 °C] that are desirable in the final unblocking step in RNA synthesis. Both the Ctmp[13,14] **5** and Fpmp[12,15–20] **6** protecting groups have been used successfully in solid-phase oligoribonucleotide synthesis. Recently, the Fpmp **6** group has also been used in solution-phase oligoribonucleotide synthesis.[21,22] When we carried out our original study which led to the development[9] of the Ctmp **5** protecting group, the only method then available[23] for the preparation of 1-arylpiperidin-4-ones **7** severely limited an investigation of the properties of other potentially suitable 1-aryl-4-methoxypiperidin-4-yl **8** (R = Me) protecting groups. We later developed[10] a completely

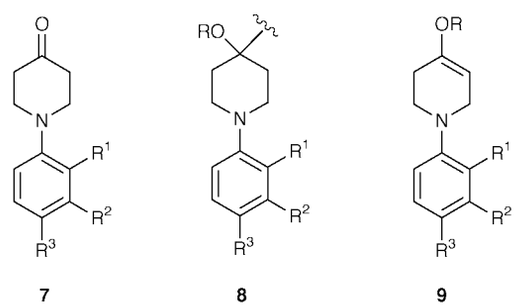


Scheme 1

Table 1 Half-lives[a] for the unblocking of 2′-protected uridine derivatives **14** as a function of pH at 30 °C

Entry	R	R^1	R^2	R^3	pH 0.5	1.0	1.5	2.0	2.5	3.0	3.25	3.5	3.75	Quotient of half-lives[b]	pK_a of corresponding primary amine[c]
1 (Fpmp)	Me	F	H	H	24.6	25.4	30.0	35.0	51.3	93.7	131	196	266	10.8	3.17
2 (Ctmp)	Me	Cl	H	Me	17.4	18.1	19.0	22.0	30.6	55.5	82.6	113	160	9.20	(3.06)
3	Me	Cl	H	H	7.1	7.1	8.2	9.6	16.2	39.7	66.9	112	171	24.1	2.66
4	Me	H	Cl	H	30.2	30.9	32.0	34.2	47.9	80.7	116	158	216	7.15	3.52
5	Me	H	H	Cl	57.1	66.0	69.7	75.5	96.2	120	149	196	267	4.68	3.98
6	Me	H	Br	H	23.5	23.9	26.5	27.7	40.7	70.3	100	138	207	8.81	3.53
7	Me	H	CF_3	H	19.4	20.3	20.4	23.7	36.8	74.5	110	166	224	11.5	3.2
8	Me	F	H	F	15.1	15.3	16.8	21.1	38.5	82.8	128	194	292	19.3	(3.00)
9	Me	H	F	F	30.1	32.2	33.4	36.8	52.6	88.9	121	170	256	8.50	(3.40)
10	Me	H	H	H	116	231	325								4.60
11 (Cpep)	Et	H	H	Cl	31.9	34.9	34.6	34.9	41.1	50.9	63.1	90.7	119	3.73	3.98
12	$AcO(CH_2)_2$	Cl	H	H	40.3									(4.89)[d]	2.66
13	$MeOCH_2CO_2$-$(CH_2)_2$	Cl	H	H	44.7									(4.41)[d]	2.66
14	$HO(CH_2)_2$	Cl	H	H									197		2.66

[a] The half-lives (min) for the unblocking of various substrates **14** between pH 0.5 and 3.75 are indicated. [b] Except for entries 13 and 14, these quotients were obtained by dividing the half-life of hydrolysis of a particular substrate at pH 3.75 by its half-life at pH 0.5. [c] Ref. 35. The pK_as in parentheses are estimated. Appropriate Hammett substituent constants were used in the calculations. [d] The ratios in parentheses were obtained by dividing the half-life of hydrolysis of the deacylated substrate at pH 3.75 (entry 14) by the half-life of hydrolysis of this substrate at pH 0.5.

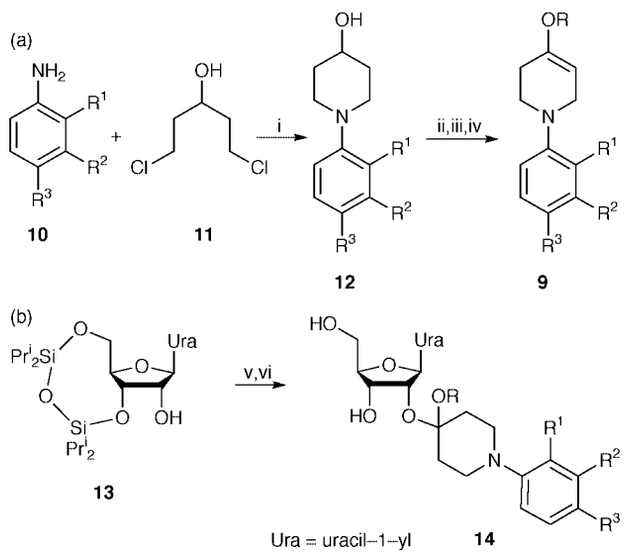


general synthesis of 1-arylpiperidin-4-ones **7**. This led to the introduction of the Fpmp group **6** which is somewhat more stable than the Ctmp protecting group **5** both at pH 0.5 and at pH 3.75 (Table 1, entries 1 and 2). We have favoured the use of the Fpmp **6** over the Ctmp **5** protecting group mainly because the required enol ether reagent[10,24] **9** (R = Me, R^1 = F, R^2 = R^3 = H) is more readily accessible. Fpmp-protected RNA phosphoramidites have now been commercially available for several years.

Results and discussion

While the Fpmp group **6** is undoubtedly suitable for the protection of 2-hydroxy functions both in solid-phase and in solution-phase RNA synthesis, we believe that the availability of really good methods for the chemical synthesis of RNA sequences is a matter of such long-term importance that a search for a 1-aryl-4-alkoxypiperidin-4-yl **8** protecting group with even better properties was well worth undertaking.

In the first part of this study, eight other 1-aryl-4-methoxypiperidin-4-yl **8** (R = Me) protecting groups were compared (Table 1, entries 3–10) with the Ctmp **5** and Fpmp **6** groups. Seven of the required 1-aryl-4-methoxy-1,2,5,6-tetrahydropyridine reagents **9** [R = Me, R^1 = Cl, R^2 = R^3 = H; R = Me, R^1 = R^2 = H, R^3 = Cl; R = Me, R^1 = R^3 = H, R^2 = Br; R = Me, R^1 = R^3 = H, R^2 = CF_3; R = Me, R^1 = R^3 = F, R^2 = H; R = Me, R^1 = H, R^2 = R^3 = F; R = Me, R^1 = R^2 = R^3 =H] were prepared from 1,5-dichloropentan-3-ol **11** *via* intermediate 1-arylpiperidin-4-ols **12** by a now superseded procedure[10] which is indicated in outline in Scheme 2a. 1-(3-Chlorophenyl)-4-methoxy-1,2,5,6-tetrahydropyridine **9** (R = Me, R^1 = R^3 = H, R^2 = Cl), like the corresponding 1-(2-fluorophenyl) and 1-(2-chloro-4-methylphenyl) derivatives **9** (R = Me, R^1 = F, R^2 = R^3 = H and R = R^3 = Me, R^1 = Cl, R^2 = H, respectively), was prepared by an improved and recently published procedure[24] that is outlined below in the preparation of 1-(4-chlorophenyl)-4-ethoxy-1,2,5,6-tetrahydropyridine **9** (R = Et, R^1 = R^2 = H, R^3 = Cl). All of the required 2′-*O*-(1-aryl-4-methoxypiperidin-4-yl)uridine derivatives **14** (R = Me) were prepared (Scheme 2b) in two-steps from 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine **13** by a reported procedure.[12]



Scheme 2 *Reagents and conditions*: i, K_2CO_3, NaI, DMF, 100 °C; ii, DCC, CF_3CO_2H, Me_2SO, C_5H_5N, C_6H_6; iii, $CH(OR)_3$, TsOH·H_2O, ROH, reflux; iv, TsOH (≈1.0 mol%), 150 °C, ≈20 mmHg); v, **9**, TFA, CH_2Cl_2, room temp., vi, Et_4NF, MeCN, room temp.

The half-life of hydrolysis of nine 2′-*O*-(1-aryl-4-methoxypiperidin-4-yl)uridine derivatives **14** [R = Me, R^1 = F, R^2 = R^3 = H (2′-*O*-Fpmp-uridine); R = R^3 = Me, R^1 = Cl, R^2 = H (2′-*O*-Ctmp-uridine); R = Me, R^1 = Cl, R^2 = R^3 = H; R = Me, R^1 = R^3 = H, R^2 = Cl; R = Me, R^1 = R^2 = H, R^3 = Cl; R = Me, R^1 = R^3 = H, R^2 = Br; R = Me, R^1 = R^3 = H, R^2 = CF_3; R = Me, R^1 = R^3 = F, R^2 = H; and R = Me, R^1 = H, R^2 = R^3 = F] were determined at pHs 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.25, 3.5 and 3.75 and at 30 °C (Table 1, entries 1–9). The rate of hydrolysis of the 4-methoxy-1-phenylpiperidin-4-yl group **8** (R = Me, R^1 = R^2 =

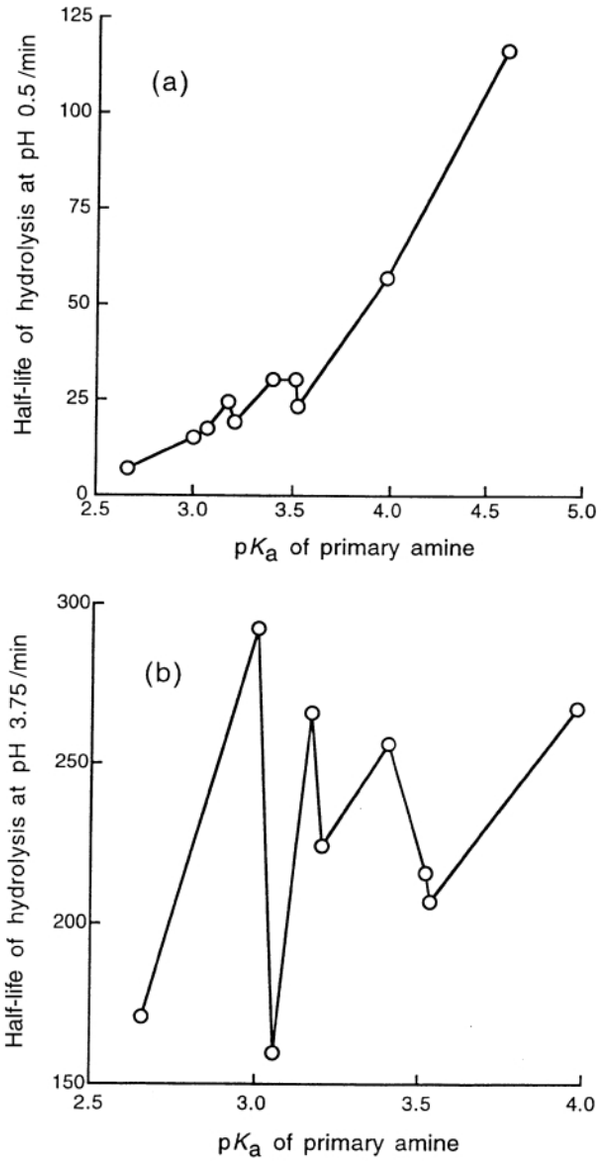
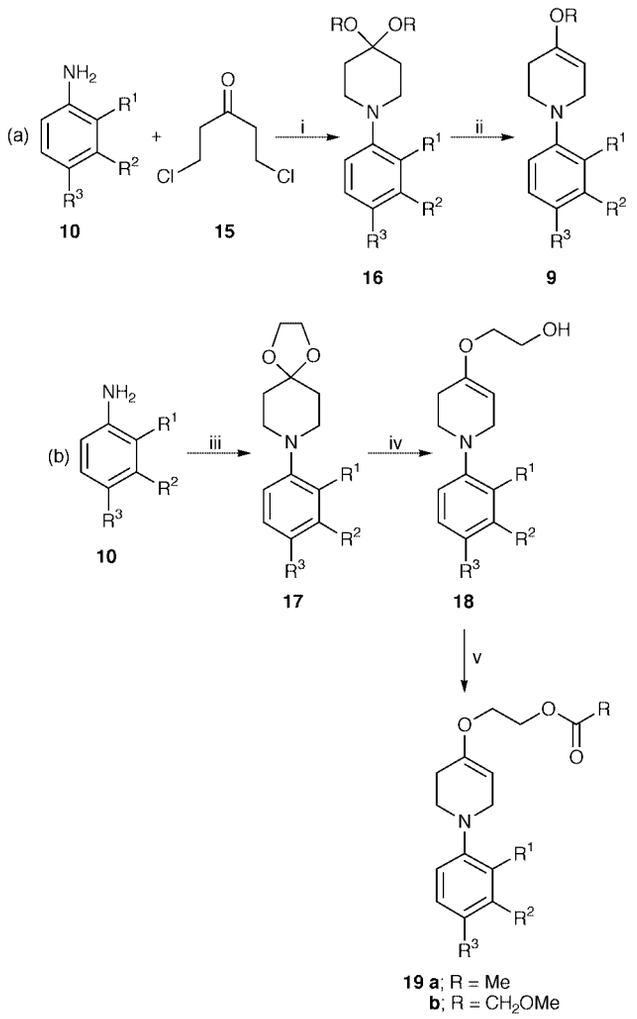


Fig. 1 Dependence of the half-lives of hydrolysis of 2′-*O*-[(1-aryl-4-methoxy)piperidin-4-yl]uridine derivatives **14** (R = Me) at 30 °C on the pK_as of the corresponding primary aromatic amines (a) at pH 0.5 and (b) at pH 3.75.

R^3 = H) (entry 10) is so slow above pH 1.5 that it cannot be measured conveniently and, in any case, this group is much too stable to acid-catalysed hydrolysis to make it useful for the protection of 2′-hydroxy functions in RNA synthesis. As would be expected, there is some correlation between the rates of hydrolysis of the 2′-*O*-(1-aryl-4-methoxypiperidin-4-yl)uridine derivatives **14** (R = Me) and the pK_as of the primary amines from which the 1-aryl-4-methoxypiperidin-4-yl groups are derived (Table 1). Thus the 1-(2-chlorophenyl)-4-methoxypiperidin-4-yl protecting group **8** (R = Me, R^1 = Cl, R^2 = R^3 = H), as in substrate **14** (R = Me, R^1 = Cl, R^2 = R^3 = H) (entry 3) which is derived from 2-chloroaniline (pK_a 2.66), is more labile over the whole pH range than is the 1-(3-chlorophenyl)-4-methoxypiperidin-4-yl protecting group **8** (R = Me, R^1 = R^3 = H, R^2 = Cl), as in substrate **14** (R = Me, R^1 = R^3 = H, R^2 = Cl) (entry 4) which is derived from 3-chloroaniline (pK_a 3.52). It can be seen from Fig. 1a that, at pH 0.5, an almost linear relationship exists between the half-lives of hydrolysis of all ten 2′-*O*-(1-aryl-4-methoxypiperidin-4-yl)uridine derivatives **14** (R = Me) (Table 1, entries 1–10) and the pK_as (or estimated pK_as) of the corresponding primary aromatic amines. However, what is most interesting and also of considerable practical significance in the present study is that this relationship appears to break down almost completely at higher pHs (*e.g.*, at pH 3.75; see Fig. 1b). Thus a non-Hammett substituent effect then appears to be operating. Assuming that it is sufficiently stable to withstand the acidic conditions [*e.g.*, CCl_3CO_2H–CH_2Cl_2 (2:98 v/v); room temp.] required for the removal of the 5′-*O*-'trityl' protecting groups (see above) and is also sufficiently labile to be completely removable under mild conditions of acidic hydrolysis [*e.g.*, pH 3.75 buffer; 35 °C; *ca.* 12 h], the 1-aryl-4-methoxypiperidin-4-yl protecting group **8** (R = Me) with the optimum properties would be the one which had the lowest possible pH 3.75 : pH 0.5 ratio of various half-lives. It can be seen from Table 1 (entries 1–9) that these half-lives quotients vary from 4.68 to 24.1 and that the 1-(4-chlorophenyl)-4-methoxypiperidin-4-yl group **8** (R = Me, R^1 = R^2 = H, R^3 = Cl) (entry no. 5) has the lowest quotient.

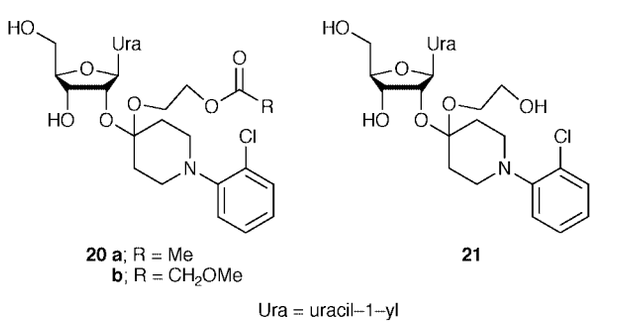
When we first investigated the properties of 1-aryl-4-methoxypiperidin-4-yl protecting groups **8**, we believed[9] that it would be possible to unblock 2′-protected RNA sequences safely at pH 2.0–2.5 and room temperature. This view was based[6] on some earlier studies on the action of acid on uridylyl-(3′→5′)-uridine. We later showed[16] that the internucleotide linkages of certain RNA sequences readily underwent cleavage and migration under the latter conditions. We now believe that, particularly if migration of internucleotide linkages is to be avoided, unblocking should be carried out at as high a pH as possible and certainly never below pH 3. We have no evidence to suggest that the Fpmp protecting group **6** is unstable under 'detritylation' conditions. We therefore concluded that, for a 1-aryl-4-alkoxypiperidin-4-yl protecting group to have even better properties, it would need to be at least as stable as the Fpmp group **6** at pH 0.5 and more labile at pH 3.75 or above. We were clearly looking for a new protecting group with a half-life quotient which was lower than 10.8 (Table 1, entry 1). From a consideration of these criteria, it is apparent that the 1-aryl-4-methoxypiperidin-4-yl protecting group **8** (R = Me) that is potentially the most superior to the Fpmp group **6** is the 1-(3-chlorophenyl)-4-methoxypiperidin-4-yl group **8** (R = Me, R^1 = R^3 = H, R^2 = Cl) (entry 4, half-life quotient 7.15). This group is both more stable than the Fpmp group at pH 0.5 and more labile at pH 3.75. The 1-(3,4-difluorophenyl)-4-methoxypiperidin-4-yl group **8** (R = Me, R^1 = H, R^2 = R^3 = F) (entry 9) meets the same criteria but to a lesser extent. The 1-(4-chlorophenyl)-4-methoxypiperidin-4-yl protecting group **8** (R = Me, R^1 = R^2 = H, R^3 = Cl) (entry 5) is particularly interesting in that it is over twice as stable as the Fpmp group **6** at pH 0.5 and yet, within experimental error, just as labile at pH 3.75. This, of course, is a consequence of its very low half-lives quotient (4.68). From a consideration of these observations, it occurred to us that the 1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl protecting group **8** (R = Et, R^1 = R^2 = H, R^3 = Cl) might very well have the hydrolysis properties that we were seeking.

The required enol ether reagent, 1-(4-chlorophenyl)-4-ethoxy-1,2,5,6-tetrahydropyridine **9** (R = Et, R^1 = R^2 = H, R^3 = Cl) was prepared from 4-chloroaniline **10** (R^1 = R^2 = H, R^3 = Cl) in two-steps and in 90% overall yield by our recently reported improved procedure[24] (Scheme 3a and Experimental section). 2′-*O*-[1-(4-Chlorophenyl)-4-ethoxypiperidin-4-yl]uridine **14** (R = Et, R^1 = R^2 = H, R^3 = Cl) was then prepared from 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine[12] **13** and the enol ether **9** (R = Et, R^1 = R^2 = H, R^3 = Cl) in the usual way (Scheme 2b and Experimental section) and obtained as a crystalline solid in 96% isolated yield. It can be seen from Table 1 (entry 11) that the 1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl (Cpep) protecting group **8** (R = Et, R^1 = R^2 = H, R^3 = Cl) has a remarkably good hydrolysis profile. Thus, it has a half-lives quotient of 3.73 which means that its rate of hydrolysis at pH 3.75 is only 3.73 times (rather than 1778 times, as calculated for a standard acetal system) slower than at pH 0.5. The Cpep group **8** (R = Et, R^1 = R^2 = H, R^3 = Cl) is more stable than the Fpmp group **6** at pH 0.5 and yet over twice



Scheme 3 *Reagents and conditions*: i, (a) $TsOH\cdot H_2O$, EtOH, reflux, 1.5 h; (b) $(EtO)_3CH$, reflux, 30 min; ii, Pr^i_2NEt, $Et_2O\cdot BF_3$, CH_2Cl_2, 0 °C, 2 h; iii, **15**, $TsOH\cdot H_2O$, $HOCH_2CH_2OH$, petroleum spirit (boiling range 80–100 °C), reflux, 3.5 h; iv, $CF_3SO_3SiMe_3$, CH_2Cl_2, 0 °C; v, Ac_2O (→**19a**) [or $(MeOCH_2CO)_2O$ (→**19b**)], C_5H_5N, room temp.

as labile at pH 3.75. The half-life of hydrolysis of 2′-*O*-Cpep-uridine **14** (R = Et, $R^1 = R^2 = H$, $R^3 = Cl$) is only 170 min at pH 4.0 and 30 °C (Fig. 2). The relative dependence of half-lives of hydrolysis on pH for 2′-*O*-Fpmp- and 2′-*O*-Cpep-uridines **14** (R = Me, $R^1 = F$, $R^2 = R^3 = H$ and R = Et, $R^1 = R^2 = H$, $R^3 = Cl$, respectively) is illustrated in Fig. 2. It can be seen from Fig. 2 that the half-life of hydrolysis of 2′-*O*-Cpep-uridine **14** (R = Et, $R^1 = R^2 = H$, $R^3 = Cl$) is almost pH independent in the range 0.5–2.5, *i.e.* over two pH units.



Other workers[25–27] have used 'protected' protecting groups[28] to block the 2′-hydroxy functions in the chemical synthesis of RNA sequences. The enol ether reagents **19a** ($R^1 = Cl$, $R^2 = R^3 = H$) and **19b** ($R^1 = Cl$, $R^2 = R^3 = H$) were prepared *via* their 2-hydroxyethyl precursor **18** in three steps and in 72 and 77% overall yield, respectively, from 2-chloroaniline **10** ($R^1 = Cl$, $R^2 = R^3 = H$) (Scheme 3b and Experimental section). The required 2′-*O*-[4-(2-acetoxyethoxy)-1-(2-chlorophenyl)piperidin-4-yl]uridine **20a** and 2′-*O*-{1-(2-chlorophenyl)-4-[2-(methoxyacetoxy)ethoxy]piperidin-4-yl}uridine **20b** were prepared in the usual way (Scheme 2b and Experimental section) from 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine[12] **13** and the respective enol ethers **19a** ($R^1 = Cl$, $R^2 = R^3 = H$) and **19b** ($R^1 = Cl$, $R^2 = R^3 = H$); these compounds were isolated as crystalline solids in 83 and 91% yield, respectively. The half-lives of hydrolysis of compounds **20a** and **20b** were found to be 40.3 and 44.7 min, respectively, at pH 0.5 and 30 °C (Table 1,† entries 12 and 13). Therefore both of these 'protected' protecting groups would be expected to withstand normal 'detritylation' conditions. These protecting groups would also both be expected to undergo quantitative deacylation to give the same 1-(2-chlorophenyl)-4-(2-hydroxyethoxy)piperidin-4-yl group (as in compound **21**) under the standard ammonolytic conditions which generally follow the complete assembly of the desired fully protected oligonucleotide sequences in solid-phase synthesis. For this reason, the acetoxy compound **20a** was treated with alcoholic methylamine to give 2′-*O*-[1-(2-chlorophenyl)-4-(2-hydroxyethoxy)piperidin-4-yl]uridine **21**, which was isolated as a crystalline solid in 88% yield. The half-life of hydrolysis of compound **21** was found to be 197 min at pH 3.75 and 30 °C (Table 1, entry 14). Although the 'protected' protecting groups in compounds **20a** and **20b** (entries 12 and 13) are somewhat more stable to acidic hydrolysis than is the Cpep group **8** (R = Et, $R^1 = R^2 = H$, $R^3 = Cl$) (entry 11) at pH

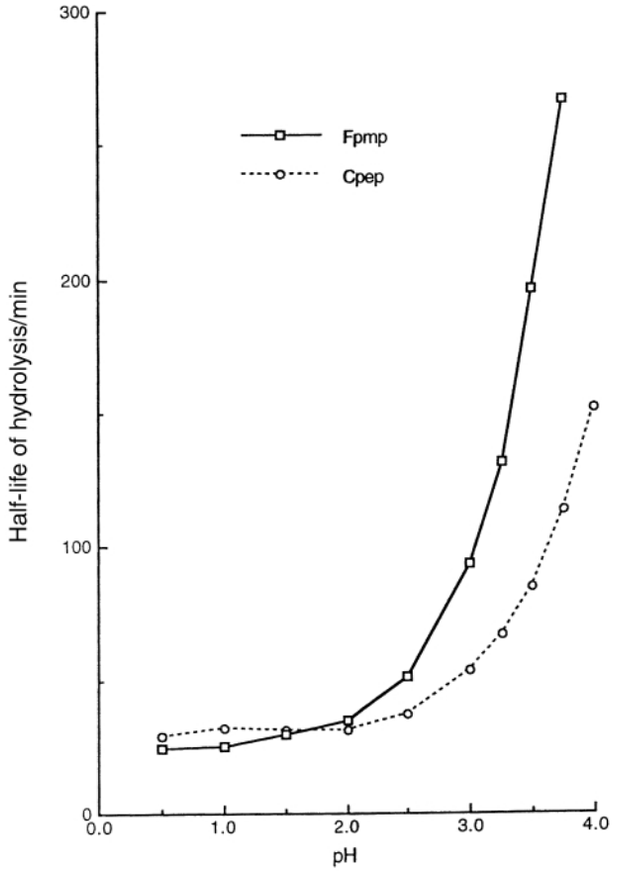


Fig. 2 Dependence of the half-lives of hydrolysis at 30 °C of the 2′-*O*-Fpmp- and 2′-*O*-Cpep-uridines **14** (R = Me, $R^1 = F$, $R^2 = R^3 = H$ and R = Et, $R^1 = R^2 = H$, $R^3 = Cl$, respectively) on pH.

† In Table 1, compounds **20a**; R = Me, **20b**; R = CH_2OMe and **21** are represented by **14** [R = $AcO(CH_2)_2$, $R^1 = Cl$, $R^2 = R^3 = H$], **14** [R = $MeOCH_2CO_2(CH_2)_2$, $R^1 = Cl$, $R^2 = R^3 = H$] and **14** [R = $HO(CH_2)_2$, $R^1 = Cl$, $R^2 = R^3 = H$], respectively.

Table 2 Half-lives[a] for the unblocking of other 2′-protected nucleoside derivatives **23** as a function of pH at 30 °C

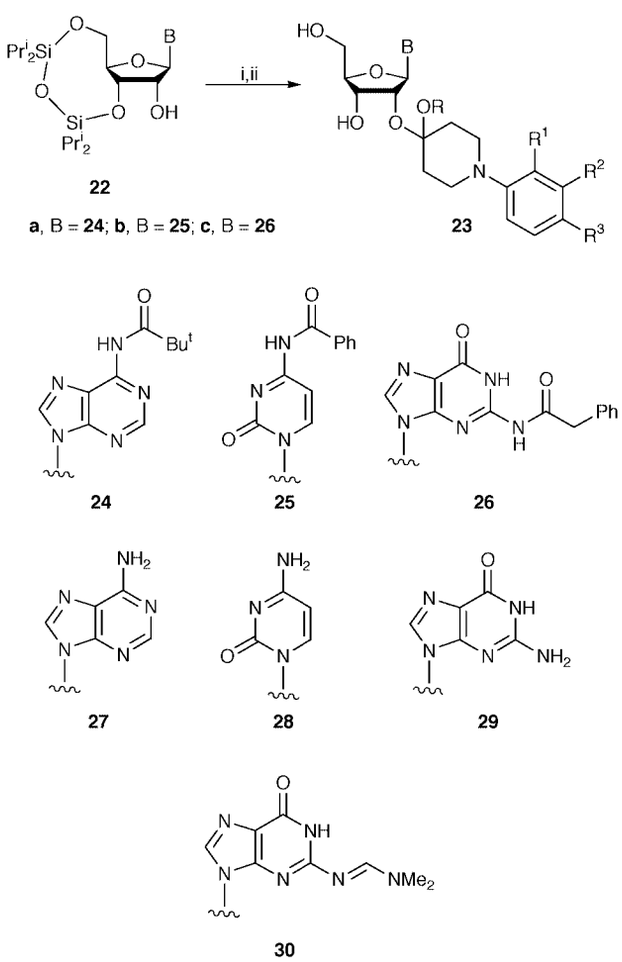
Entry	Base residue	R	R^1	R^2	R^3	pH 0.5	pH 3.75
1	**24**	Me	F	H	H	23.7	
2	**24**	Et	H	H	Cl	24.9	
3	**25**	Me	F	H	H	20.7	
4	**25**	Et	H	H	Cl	24.6	
5	**26**	Me	F	H	H	10.5	
6	**26**	Et	H	H	Cl	9.1	
7	**27**	Me	F	H	H		338
8	**28**	Et	H	H	Cl		161
9	**29**	Me	F	H	H	22.7	339
10	**29**	Et	H	H	Cl	26.8	135
11	**30**	Et	H	H	Cl	19.2	

[a] The half-lives (min) for the unblocking of various substrates at pHs 0.5 and 3.75 are indicated.

0.5, the deacylated protecting group (as in compound **21**; entry 14), obtained after ammonolysis, is markedly less labile than the Cpep group at pH 3.75. It was therefore concluded that neither of the 'protected' protecting groups offered an advantage over the Cpep group.

All of the data relating to the pH dependence of the rate of removal of 2′-protecting groups in Table 1 pertain solely to uridine derivatives. Clearly, it cannot be assumed that these unblocking rates will be the same for other nucleoside derivatives. A further complication arises because adenine, cytosine and guanine residues are generally *N*-protected (as in **24**, **25** and **26**, respectively) during the assembly of fully protected oligonucleotide sequences but are unprotected (as in **27**, **28** and **29**, respectively) during the final unblocking step [*i.e.* the removal of the 2′-*O*-(4-alkoxy-1-arylpiperidin-4-yl) protecting groups **8**]. The 2′-*O*-Fpmp derivatives of 6-*N*-pivaloyladenosine (**23a**, R = Me, R^1 = F, $R^2 = R^3$ = H), 4-*N*-benzoylcytidine (**23b**, R = Me, R^1 = F, $R^2 = R^3$ = H) and 2-*N*-(phenylacetyl)guanosine (**23c**, R = Me, R^1 = F, $R^2 = R^3$ = H) were prepared as previously reported[12] in two-steps (Scheme 4) from the appropriate 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl) derivatives (**22a**, **22b** and **22c**, respectively). The corresponding 2′-*O*-Cpep derivatives (**23a**, R = Et, $R^1 = R^2$ = H, R^3 = Cl; **23b**, R = Et, $R^1 = R^2$ = H, R^3 = Cl; **23c**, R = Et, $R^1 = R^2$ = H, R^3 = Cl) were similarly prepared from the enol ether **9** (R = Et, $R^1 = R^2$ = H, R^3 = Cl) and compounds **22a**, **22b** and **22c**, respectively (Scheme 4); they were obtained as crystalline solids in 79.5, 86 and 89% isolated yield, respectively. The 2′-*O*-Cpep and 2′-*O*-Fpmp derivatives of 6-*N*-pivaloyladenosine **23a** (R = Et, $R^1 = R^2$ = H, R^3 = Cl and R = Me, R^1 = F, $R^2 = R^3$ = H, respectively) were treated with alcoholic methylamine to give the corresponding deacylated derivatives **23** (B = **27**, R = Et, $R^1 = R^2$ = H, R^3 = Cl and B = **27**, R = Me, R^1 = F, $R^2 = R^3$ = H, respectively) in 96 and 85% isolated yield. The two corresponding 2-*N*-(phenylacetyl)guanosine derivatives **23c** (R = Et, $R^1 = R^2$ = H, R^3 = Cl and R = Me, R^1 = F, $R^2 = R^3$ = H) were similarly deacylated in 94 and 91% yields, respectively. 2′-*O*-Cpep-4-*N*-benzoylcytidine **23b** (R = Et, $R^1 = R^2$ = H, R^3 = Cl) was deacylated with conc. aq. ammonia and the product **23** (B = **28**, R = Et, $R^1 = R^2$ = H, R^3 = Cl) was isolated in 85% yield.

At pH 0.5 and 30 °C the half-lives of hydrolysis of 2′-*O*-Fpmp-6-*N*-pivaloyladenosine **23a** (R = Me, R^1 = F, $R^2 = R^3$ = H) and 2′-*O*-Fpmp-4-*N*-benzoylcytidine **23b** (R = Me, R^1 = F, $R^2 = R^3$ = H) (Table 2, entries 1 and 3, respectively) do not differ very significantly from that of 2′-*O*-Fpmp-uridine **14** (R = Me, R^1 = F, $R^2 = R^3$ = H) (Table 1, entry 1). However, at pH 0.5 the half-life of hydrolysis of 2′-*O*-Fpmp-2-*N*-(phenylacetyl)-guanosine **23c** (R = Me, R^1 = F, $R^2 = R^3$ = H) (Table 2, entry 5) is less than one-half that of 2′-*O*-Fpmp-uridine. Similarly,



Scheme 4 *Reagents and conditions*: i, **9** (R = Me, R^1 = F, $R^2 = R^3$ = H) or **9** (R = Et, $R^1 = R^2$ = H, R^3 = Cl), TFA, CH_2Cl_2, room temp.; ii, Et_4NF, MeCN, room temp.

while the half-lives of hydrolysis of 2′-*O*-Cpep-6-*N*-pivaloyl-adenosine **23a** (R = Et, $R^1 = R^2$ = H, R^3 = Cl) and 2′-*O*-Cpep-4-*N*-benzoylcytidine **23b** (R = Et, $R^1 = R^2$ = H, R^3 = Cl) (Table 2, entries 2 and 4, respectively) at pH 0.5 are not very much shorter than that of 2′-*O*-Cpep-uridine **14** (R = Et, $R^1 = R^2$ = H, R^3 = Cl) (Table 1, entry 11), the half-life of hydrolysis of 2′-*O*-Cpep-2-*N*-phenylacetylguanosine **23c** (R = Et, $R^1 = R^2$ = H, R^3 = Cl) (Table 2, entry 6) is considerably shorter. In contrast to the latter results, the half-lives of hydrolysis at pH 0.5 of 2′-*O*-Fpmp-guanosine **23** (B = **29**, R = Me, R^1 = F, $R^2 = R^3$ = H) and 2′-*O*-Cpep-guanosine **23** (B = **29**, R = Et, $R^1 = R^2$ = H, R^3 = Cl) (Table 2, entries 9 and 10, respectively) are not significantly shorter than those of 2′-*O*-Fpmp-uridine **14** (R = Me, R^1 = F, $R^2 = R^3$ = H) and 2′-*O*-Cpep-uridine **14** (R = Et, $R^1 = R^2$ = H, R^3 = Cl) (Table 1, entries 1 and 11, respectively). It is also noteworthy that the half-life of hydrolysis at pH 0.5 of the 2-*N*-(dimethylamino)methylene derivative[29] of 2′-*O*-Cpep-guanosine **23** (B = **30**, R = Et, $R^1 = R^2$ = H, R^3 = Cl) (Table 2, entry 11; see Experimental section for preparation) is more than twice that of 2′-*O*-Cpep-2-*N*-phenylguanosine **23c** (R = Et, $R^1 = R^2$ = H, R^3 = Cl) (Table 2, entry 6).

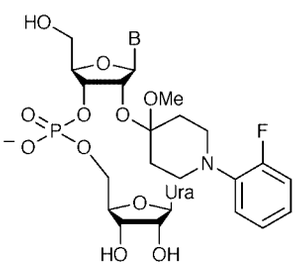
At pH 3.75, it would appear that 2′-protected uridine derivatives **14** are deblocked somewhat more rapidly than are the corresponding 2′-protected adenosine, cytidine and guanosine derivatives **23** (B = **27**, **28** and **29**, respectively). Thus the half-lives of hydrolysis of 2′-*O*-Fpmp-adenosine **23** (B = **27**, R = Me, R^1 = F, $R^2 = R^3$ = H) and 2′-*O*-Fpmp-guanosine **23** (B = **29**, R = Me, R^1 = F, $R^2 = R^3$ = H) (Table 2, entries 7 and 9, respectively) are both ≈1.27 times greater than that of 2′-*O*-Fpmp-uridine **14** (R = Me, R^1 = F, $R^2 = R^3$ = H) (Table 1, entry 1). Similarly, the half-lives of hydrolysis of 2′-*O*-Cpep-cytidine

Table 3 Half-lives[a] for the unblocking of 2′-*O*-Fpmp-protected diribonucleoside phosphates **31** as a function of pH at 30 °C

Entry	Base residue B	pH 3.25	3.50	3.75	4.0
1	**27**	64.5	119	190	266
2	**28**	59.9	103	145	202
3	**29**	48.6	86.1	149	220
4	uracil-1-yl	49.7	79.1	155	218

[a] The half-lives (min) for the unblocking of various substrates between pH 3.25 and 4.0 are indicated.

23 (B = **28**, R = Et, $R^1 = R^2 = H$, $R^3 = Cl$) and 2′-*O*-Cpep-guanosine **23** (B = **29**, R = Et, $R^1 = R^2 = H$, $R^3 = Cl$) (Table 2, entries 8 and 10, respectively) are ≈1.35 and 1.13 times greater than that of 2′-*O*-Cpep-uridine **14** (R = Et, $R^1 = R^2 = H$, $R^3 = Cl$) (Table 1, entry 11). Due to the sparing solubility of some of the substrates at higher pHs, the half-lives of hydrolysis measured at pH 3.75 may be somewhat less accurate than those measured at pH 0.5. This is perhaps suggested by hydrolysis studies carried out on four 2′-*O*-Fpmp-protected dinucleoside phosphates **31** (Table 3). These substrates, which were prepared by standard phosphoramidite-based solid-phase synthesis[12] (see below and Experimental section), were freely soluble in 0.5 mol dm^{-3} sodium acetate buffer solutions at the pHs indicated. It can be seen that at pH 3.75 (and also at pH 4.0), only 2′-*O*-Fpmp-adenylyl-(3′→5′)-uridine **31** (B = **27**) (Table 3, entry 1)



31; Ura = uracil–1–yl

has a half-life of hydrolysis that is significantly greater than that of 2′-*O*-Fpmp-uridylyl-(3′→5′)-uridine **31** (B = Ura) (entry 4). It has been noted previously[6,9] that 2′-acetal protecting groups which are vicinal to 3′→5′-internucleotide linkages are more labile to acidic hydrolysis than are the same acetal systems in 2′-protected ribonucleosides. This is certainly advantageous from the standpoint of oligonucleotide synthesis and may be due to the inductive effect of an ionized phosphodiester being less than that of a 3′-hydroxy function.

The homo-oligoribonucleotide sequence r[$(Up)_{19}$U] was prepared by solid-phase synthesis,[16] starting from (a) the commercially supplied 2′-*O*-Fpmp-protected phosphoramidite **32** and (b) the 2′-*O*-Cpep-protected phosphoramidite **33**. The latter material was prepared from 2′-*O*-Cpep-uridine **14** (R = Et, $R^1 = R^2 = H$, $R^3 = Cl$) by the standard two-step procedure.[12] Following the complete assembly of the fully protected sequences, the loaded controlled-pore glass support was submitted to ammonolysis and the 5′-*O*-(DMTr)-2′-protected material was purified by preparative reversed-phase HPLC. The purified, partially protected icosamers (20-mers) were dissolved in sterile 0.5 mol dm^{-3} aq. sodium acetate buffer (pH 4.0) which was spiked with adenosine as an HPLC marker. The solutions were incubated at 35 °C. Aliquots were removed after appropriate intervals and were analysed by reversed-phase HPLC. The reactions were continued until the ratio of the integrals (measured at 260 nm) of the fully unblocked r[$(Up)_{19}$U] and adenosine peaks reached a maximum. It was assumed that the r[$(Up)_{19}$U] was then fully unblocked. It can be seen from Fig. 3a

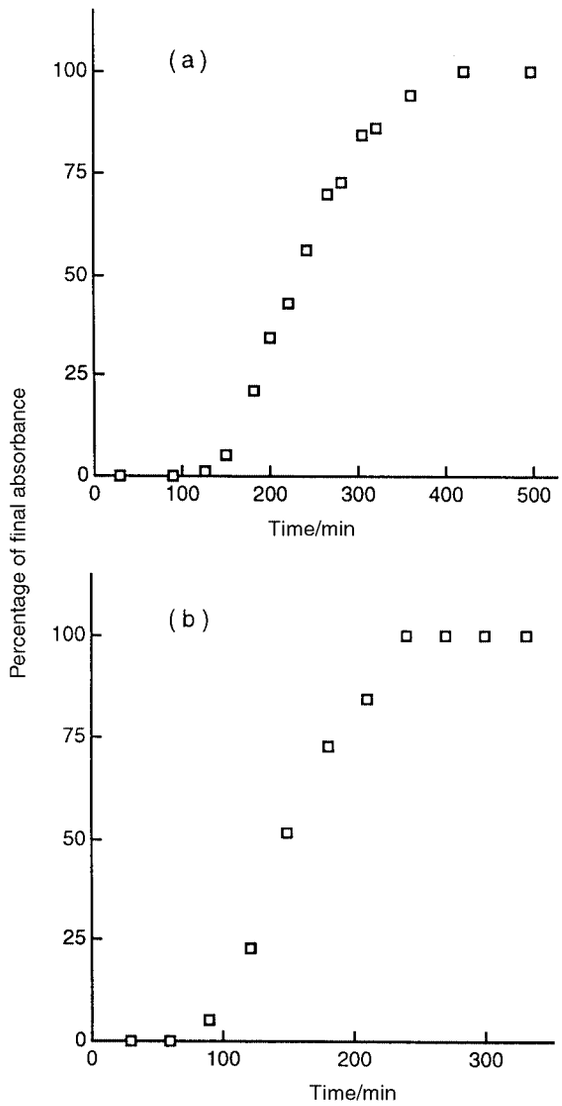
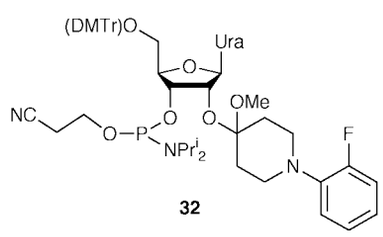
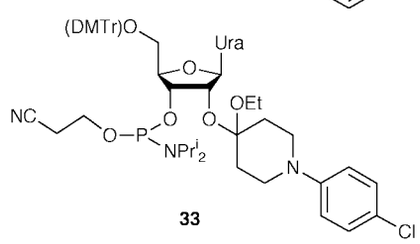


Fig. 3 Unblocking profiles in 0.5 mol dm^{-3} sodium acetate buffer (pH 4.0) at 35 °C of (a) r[$(Up)_{19}$U] protected on its 5′-terminal hydroxy function with a DMTr group and on all (except the 3′-terminal) 2′-hydroxy functions with Fpmp groups and of (b) the corresponding partially protected r[$(Up)_{19}$U] in which all of the Fpmp are replaced by Cpep groups.



32



33

Ura = uracil–1–yl

that the Fpmp-protected material was completely unblocked in *ca.* 7 h, and from Fig. 3b that the Cpep-protected material was completely unblocked in *ca.* 4 h. Digestion of material unblocked under these very mild conditions yielded no uridylyl-

(2′→5′)-uridine and hence no migration of the internucleotide linkages could be detected.[16,30]

We believe that the following conclusions may be drawn from this study. First, there is no reason to doubt that the Fpmp group **6** is suitable for the protection of 2′-hydroxy functions in the chemical synthesis of RNA sequences. While it is clear from its successful use in RNA synthesis over a number of years that the Fpmp group is able to withstand the acidic conditions required for 'detritylation', the present studies suggest that it should be possible to remove 2′-*O*-Fpmp protecting groups from relatively high molecular mass RNA sequences in pH 4.0 buffer solution overnight at 35 °C. Under these conditions, cleavage and migration of the internucleotide linkages would be expected to be negligible. Secondly, the Cpep protecting group **8** (R = Et, $R^1 = R^2 = H$, $R^3 = Cl$) has a better hydrolysis profile than the Fpmp group (See Tables 1 and 2, and Fig. 2). As well as being more stable to acidic hydrolysis at low pHs, the Cpep protecting group can be removed in approximately one-half of the time in the final unblocking step at pH 4.0 and 35 °C. Alternatively, it could presumably be removed in the same time at the same pH as the Fpmp group but at a lower temperature (perhaps at 30 °C) or at a higher pH (perhaps pH 4.25) at the same temperature. The chances of unblocking leading to hydrolysis or migration of the internucleotide linkages under any of these sets of reaction conditions would be very small indeed. A third important conclusion to be drawn from this study is that, in solid-phase synthesis, it is possibly advisable to leave guanine residues unprotected (as in **29**) and thereby make the 2′-protecting groups more stable to acidic hydrolysis at low pHs. As the 2-amino function is not the main nucleophilic centre of a guanine residue, the lack of a protecting group is unlikely[31] to lead to any adverse side reactions. Alternatively, the guanine residues can be protected with 2-*N*-(dimethylamino)methylene groups (as in **30**).

Experimental

Mps were measured with a Büchi melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured at 360 MHz with a Bruker AM 360 spectrometer. ^{13}C NMR spectra were measured at 90.6 MHz with the same spectrometer. Chemical shifts are given in ppm relative to tetramethylsilane, and *J*-values are given in Hz. UV absorption spectra were measured with a Perkin-Elmer Lambda-3 recording spectrometer. Merck silica gel 60 F_{254} plates were used for TLC. Merck Kieselgel H (Art 7729) was used for short-column chromatography. HPLC was carried out on a Jones Apex or Hypersil ODS 5μ column (25 cm × 4.6 mm) which was eluted with 0.1 mol dm^{-3} aq. triethylammonium acetate–acetonitrile mixtures. Acetonitrile, pyridine, triethylamine and diisopropylethylamine were dried by heating, under reflux, with calcium hydride for 3–5 h and were then distilled; ethanol was dried by heating, under reflux, over magnesium ethoxide (generated from magnesium turnings) and was then distilled; dichloromethane was dried by heating, under reflux, over phosphorus pentaoxide and was then distilled; toluene was dried by distillation at atmospheric pressure, the first 20% of distillate being disregarded. Light petroleum was the fraction with distillation range 40–60 °C unless stated otherwise. Kinetic studies on 2′-protected nucleoside derivatives, 2′-protected dinucleoside phosphates and 2′-protected oligoribonucleotides were carried out in 1.5 cm^3 Eppendorf tubes placed in a Digi-block heating apparatus. Some ribonucleoside intermediates and 2′-*O*-Fpmp-protected phosphoramidites were supplied by Cruachem Ltd., Glasgow. Phosphorolytic enzymes were purchased from the Sigma Chemical Company.

Preparation of 1-aryl-4-methoxy-1,2,5,6-tetrahydropyridines 9 (R = Me)

The preparation of 1-(2-fluorophenyl)-4-methoxy-1,2,5,6-tetrahydropyridine **9** (R = Me, $R^1 = F$, $R^2 = R^3 = H$), 1-([2-chloro-4-methyl]phenyl)-4-methoxy-1,2,5,6-tetrahydropyridine **9** (R = R^3 = Me, R^1 = Cl, R^2 = H) and 1-(3-chlorophenyl)-4-methoxy-1,2,5,6-tetrahydropyridine **9** (R = Me, $R^1 = R^3 = H$, R^2 = Cl) by essentially the same procedure as that described in the two-step preparation of 1-(4-chlorophenyl)-4-ethoxy-1,2,5,6-tetrahydropyridine **9** (R = Et, $R^1 = R^2 = H$, R^3 = Cl) (see below) has already been reported.[24] The other 1-aryl-4-methoxy-1,2,5,6-tetrahydropyridines used in this study were prepared by a now obsolete procedure[10] (Scheme 2a), starting from 1,5-dichloropentan-3-ol **11**. NMR spectroscopic data relating to these compounds are provided below.

4-Methoxy-1-phenyl-1,2,5,6-tetrahydropyridine 9 (R = Me, $R^1 = R^2 = R^3 = H$). δ_H[$(CD_3)_2SO$] 2.21 (2 H, m), 3.37 (2 H, t, *J* 5.8), 3.49 (3 H, s), 3.68 (2 H, m), 4.74 (1 H, m), 6.74 (1 H, m), 6.92 (2 H, m), 7.20 (2 H, m); δ_C[$(CD_3)_2SO$] 27.6, 44.7, 46.0, 53.8, 91.2, 114.8, 118.1, 128.8, 150.2, 153.3.

4-(2-Chlorophenyl)-4-methoxy-1,2,5,6-tetrahydropyridine 9 (R = Me, R^1 = Cl, $R^2 = R^3 = H$). δ_H($CDCl_3$) 2.34 (2 H, m), 3.26 (2 H, t, *J* 5.8), 3.58 (3 H, s), 3.66 (2 H, m), 4.71 (1 H, m), 6.95 (1 H, m), 7.10 (1 H, dd, *J* 1.5 and 8.1), 7.20 (1 H, m), 7.36 (1 H, dd, *J* 1.5 and 7.9); δ_C($CDCl_3$) 28.5, 48.5, 49.0, 54.2, 91.6, 120.6, 123.5, 127.5, 128.7, 130.7, 148.9, 154.4.

1-(4-Chlorophenyl)-4-methoxy-1,2,5,6-tetrahydropyridine 9 (R = Me, $R^1 = R^2 = H$, R^3 = Cl). δ_H($CDCl_3$) 2.31 (2 H, m), 3.38 (2 H, t, *J* 5.8), 3.56 (3 H, s), 3.71 (2 H, m), 4.67 (1 H, m), 6.83 (2 H, m), 7.19 (2 H, m); δ_C($CDCl_3$) 28.1, 45.9, 46.9, 54.2, 90.8, 116.6, 123.6, 128.9, 149.2, 154.1.

1-(3-Bromophenyl)-4-methoxy-1,2,5,6-tetrahydropyridine 9 (R = Me, $R^1 = R^3 = H$, R^2 = Br). δ_H[$(CD_3)_2SO$] 2.20 (2 H, m), 3.39 (2 H, t, *J* 5.8), 3.48 (3 H, s), 3.68 (2 H, m), 4.70 (1 H, m), 6.88 (2 H, m), 7.05 (1 H, m), 7.12 (1 H, m); δ_C[$(CD_3)_2SO$] 27.6, 44.3, 45.7, 54.0, 90.9, 113.5, 116.8, 120.3, 122.7, 130.7, 151.6, 153.4.

4-Methoxy-1-[3-(trifluoromethyl)phenyl]-1,2,5,6-tetrahydropyridine 9 (R = Me, $R^1 = R^3 = H$, $R^2 = CF_3$). δ_H[$(CD_3)_2SO$] 2.25 (2 H, m), 3.48 (2 H, t, *J* 5.8), 3.51 (3 H, s), 3.77 (2 H, m), 4.76 (1 H, m), 7.04 (1 H, d, *J* 7.6), 7.15 (1 H, m), 7.21 (1 H, m), 7.42 (1 H, m); δ_C[$(CD_3)_2SO$] 27.5, 44.3, 45.6, 53.9, 90.8, 110.2, 113.8, 118.1, 124.5 (quart, $J_{C,F}$ 272.5), 129.93, 129.95 (quart, $J_{C,F}$ 30.9), 150.4, 153.4.

1-(2,4-Difluorophenyl)-4-methoxy-1,2,5,6-tetrahydropyridine 9 (R = Me, $R^1 = R^3 = F$, $R^2 = H$). δ_H[$(CD_3)_2SO$] 2.18 (2 H, m), 3.14 (2 H, t, *J* 5.8), 3.48 (3 H, s), 3.51 (2 H, m), 4.71 (1 H, m), 6.95 (1 H, m), 7.00–7.20 (2 H, m); δ_C[$(CD_3)_2SO$] 27.9, 47.7, 48.0, 53.8, 91.3, 153.3 and aromatic carbon resonance signals.

1-(3,4-Difluorophenyl)-4-methoxy-1,2,5,6-tetrahydropyridine 9 (R = Me, $R^1 = H$, $R^2 = R^3 = F$). δ_H[$(CD_3)_2SO$] 2.19 (2 H, m), 3.35 (2 H, t, *J* 5.8), 3.48 (3 H, s), 3.65 (2 H, m), 4.71 (1 H, m), 6.69 (1 H, m), 6.94 (1 H, m), 7.20 (1 H, m); δ_C[$(CD_3)_2SO$] 27.5, 45.0, 46.3, 53.9, 90.9, 153.4 and aromatic carbon resonance signals.

Preparation of 2′-*O*-(1-aryl-4-methoxypiperidin-4-yl)uridines 14 (R = Me)

These compounds were prepared from uridine by the previously reported three-step procedure (i, reaction with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[32] and imidazole in acetonitrile; ii, reaction between the resulting 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine[12] **13**, the appropriate 1-aryl-4-methoxy-1,2,5,6-tetrahydropyridine reagent **9** (R = Me) and trifluoroacetic acid in dichloromethane; iii, treatment with

tetraethylammonium fluoride in acetonitrile). Data relating to the individual derivatives are provided below.

2′-*O*-[1-(2-Chlorophenyl)-4-methoxypiperidin-4-yl]uridine 14 (R = Me, R^1 = Cl, R^2 = R^3 = H). (Found, in material crystallised from absolute ethanol: C, 53.0; H, 5.6; N, 9.15. $C_{21}H_{26}ClN_3O_7$·0.5 H_2O requires C, 52.89; H, 5.71; N, 8.81%), mp 204–205 °C; δ_H[$(CD_3)_2SO$] 1.8–2.0 (4 H, m), 2.74 (2 H, m), 2.85 (5 H, m), 3.61 (2 H, m), 3.95 (1 H, m), 4.05 (1 H, m), 4.39 (1 H, m), 5.27 (2 H, m), 5.76 (1 H, d, *J* 8.1), 6.07 (1 H, d, *J* 7.7), 7.0–7.4 (4 H, m), 7.99 (1 H, d, *J* 8.1), 11.4 (1 H, br s); δ_C[$(CD_3)_2SO$] 32.7, 34.5, 47.2, 48.3, 48.7, 62.0, 71.3, 72.8, 85.3, 86.8, 99.5, 102.8, 121.3, 124.1, 128.0, 128.3, 130.6, 141.1, 149.4, 151.4, 163.4.

2′-*O*-[1-(3-Chlorophenyl)-4-methoxypiperidin-4-yl]uridine 14 (R = Me, R^1 = R^3 = H, R^2 = Cl). (Found, in material crystallised from absolute ethanol: C, 52.8; H, 5.6; N, 8.6. $C_{21}H_{26}ClN_3O_7$·0.5 H_2O requires C, 52.89; H, 5.45; N. 8.74%), mp 194–196 °C; δ_H[$(CD_3)_2SO$] 1.65–1.90 (4 H, m), 2.90–3.10 (2 H, m), 2.98 (3 H, s), 3.34–3.47 (2 H, m), 3.58 (2 H, m), 3.92 (1 H, m), 4.00 (1 H, m), 4.37 (1 H, dd, *J* 4.9 and 7.7), 5.21 (1 H, d, *J* 4.5), 5.27 (1 H, t, *J* 4.7), 5.76 (1 H, d, *J* 8.1), 6.03 (1 H, d, *J* 7.8), 6.74 (1 H, dd, *J* 1.5 and 7.7), 6.88 (1 H, dd, *J* 2.0 and 8.4), 6.93 (1 H, m), 7.18 (1 H, d, *J* 8.1), 7.97 (1 H, d, *J* 8.2), 11.40 (1 H, br s); δ_C[$(CD_3)_2SO$] 31.2, 33.0, 45.0, 45.2, 46.9, 61.7, 71.0, 72.4, 85.0, 86.4, 99.5, 102.5, 113.9, 114.8, 117.7, 130.5, 133.9, 140.8, 151.0, 151.6, 163.0.

2′-*O*-[1-(4-Chlorophenyl)-4-methoxypiperidin-4-yl]uridine 14 (R = Me, R^1 = R^2 = H, R^3 = Cl). (Found: C, 53.7; H, 5.45; N, 8.8. $C_{21}H_{26}ClN_3O_7$ requires C, 53.90; H, 5.60; N, 8.98%), mp 196–198 °C; δ_H[$(CD_3)_2SO$] 1.65–1.90 (4 H, m), 2.85–3.05 (2 H, m), 2.97 (3 H, s), 3.20–3.40 (2 H, m), 3.57 (2 H, m), 3.90 (1 H, m), 3.98 (1 H, m), 4.34 (1 H, dd, *J* 4.9 and 7.7), 5.18 (1 H, d, *J* 4.5), 5.25 (1 H, t, *J* 4.7), 5.75 (1 H, d, *J* 8.1), 6.01 (1 H, d, *J* 7.8), 6.93 (2 H, m), 7.19 (2 H, m), 7.95 (1 H, d, *J* 8.1), 11.39 (1 H, br s); δ_C[$(CD_3)_2SO$] 31.1, 32.9, 45.2, 45.5, 46.8, 61.5, 70.9, 72.2, 84.9, 86.3, 99.3, 102.4, 117.1, 121.9, 128.6, 140.7, 149.1, 150.4, 162.9.

2′-*O*-[1-(3-Bromophenyl)-4-methoxypiperidin-4-yl]uridine 14 (R = Me, R^1 = R^3 = H, R^2 = Br). (Found, in material crystallised from ethyl acetate–hexane: C, 49.1; H, 5.1; N, 8.2. $C_{21}H_{26}BrN_3O_7$ requires C, 49.23; H, 5.11; N, 8.20%), mp 199–200 °C; δ_H[$(CD_3)_2SO$] 1.65–1.9 (4 H, m), 2.9–3.1 (2 H, m), 2.97 (3 H, s), 3.35–3.5 (2 H, m), 3.58 (2 H, m), 3.92 (1 H, m), 4.00 (1 H, m), 4.36 (1 H, dd, *J* 4.9 and 7.7), 5.20 (1 H, d, *J* 4.5), 5.26 (1 H, t, *J* 4.9), 5.75 (1 H, d, *J* 8.1), 6.02 (1 H, d, *J* 7.7), 6.89 (2 H, m), 7.05 (1 H, t, *J* 2.0), 7.12 (1 H, t, *J* 8.1), 7.96 (1 H, d, *J* 8.2), 11.39 (1 H, br s); δ_C[$(CD_3)_2SO$] 31.1, 32.9, 44.9, 45.1, 46.8, 61.5, 70.9, 72.2, 84.9, 86.3, 99.4, 102.4, 114.2, 117.5, 120.7, 122.5, 130.7, 140.7, 150.9, 151.6, 162.9.

2′-*O*-{4-Methoxy-1-[(3-trifluoromethyl)phenyl]piperidin-4-yl}uridine 14 (R = Me, R^1 = R^3 = H, R^2 = CF_3). (Found, in material crystallised from absolute ethanol: C, 53.0; H, 5.2; N, 8.25. $C_{22}H_{26}F_3N_3O_7$ requires C, 52.69; H, 5.23; N, 8.38%), mp 135–137 °C; δ_H[$(CD_3)_2SO$] 1.7–1.95 (4 H, m), 2.95–3.15 (2 H, m), 2.99 (3 H, s), 3.4–3.55 (2 H, m), 3.58 (2 H, m), 3.92 (1 H, m), 4.01 (1 H, m), 4.37 (1 H, dd, *J* 4.9 and 7.7), 5.21 (1 H, d, *J* 4.5), 5.27 (1 H, t, *J* 4.7), 5.76 (1 H, d, *J* 8.1), 6.03 (1 H, d, *J* 7.8), 7.03 (1 H, d, *J* 7.6), 7.16 (1 H, m), 7.21 (1 H, m), 7.40 (1 H, t, *J* 8.0), 7.97 (1 H, d, *J* 8.1), 11.40 (1 H, br s); δ_C[$(CD_3)_2SO$] 31.1, 32.9, 44.8, 45.1, 46.8, 61.5, 70.8, 72.2, 79.1, 84.9, 86.2, 102.4, 111.0, 114.1, 118.9, 124.4 (quart, $J_{C,F}$ 272.5), 129.8 (quart, $J_{C,F}$ 31.0), 129.9, 140.7, 150.5, 150.9, 162.9.

2′-*O*-[1-(2,4-Difluorophenyl)-4-methoxypiperidin-4-yl]uridine 14 (R = Me, R^1 = R^3 = F, R^2 = H). (Found, in material crystallised from absolute ethanol: C, 53.2; H, 5.3; N, 8.8. $C_{21}H_{25}F_2N_3O_7$·0.25 H_2O requires C, 53.22; H, 5.42; N, 8.87%), mp 199–201 °C; δ_H[$(CD_3)_2SO$] 1.75–2.05 (4 H, m), 2.73 (1 H, m), 2.83 (1 H, m), 3.00 (3 H, s), 3.01 (1 H, m), 3.09 (1 H, m), 3.59 (2 H, m), 3.93 (1 H, m), 4.03 (1 H, m), 4.36 (1 H, dd, *J* 4.9 and 7.8), 5.23 (1 H, d, *J* 4.5), 5.28 (1 H, m), 5.76 (1 H, d, *J* 8.1), 6.04 (1 H, d, *J* 7.8), 6.97 (1 H, m), 7.07 (1 H, m), 7.17 (1 H, m), 7.97 (1 H, d, *J* 8.2), 11.41 (1 H, br s); δ_C[$(CD_3)_2SO$] 32.04, 33.9, 46.8, 47.6, 48.0, 61.7, 71.0, 72.4, 84.9, 86.4, 99.0, 102.5, 140.8, 151.0, 163.0 and aromatic carbon resonance signals.

2′-*O*-[1-(3,4-Difluorophenyl)-4-methoxypiperidin-4-yl]uridine 14 (R = Me, R^1 = H, R^2 = R^3 = F). (Found, in material crystallised from ethyl acetate–hexane: C, 53.6; H, 5.35; N, 8.7. $C_{21}H_{25}F_2N_3O_7$ requires C, 53.73; H, 5.37; N, 8.95%), mp 124–125 °C; δ_H[$(CD_3)_2SO$] 1.65–1.95 (4 H, m), 2.88 (1 H, m), 2.98 (3 H, s), 2.98 (1 H, m), 3.31 (1 H, m), 3.39 (1 H, m), 3.58 (2 H, m), 3.92 (1 H, m), 4.00 (1 H, m), 4.36 (1 H, dd, *J* 4.9 and 7.6), 5.21 (1 H, d, *J* 4.4), 5.28 (1 H, m), 5.76 (1 H, d, *J* 8.1), 6.02 (1 H, d, *J* 7.7), 6.71 (1 H, m), 6.97 (1 H, m), 7.21 (1 H, dd, *J* 9.5 and 19.8), 7.96 (1 H, d, *J* 8.1), 11.40 (1 H, br s); δ_C[$(CD_3)_2SO$] 31.3, 33.0, 45.6, 45.9, 47.0, 61.7, 71.0, 72.4, 85.0, 86.4, 99.4, 102.5, 140.8, 151.0, 163.0 and aromatic carbon resonance signals.

2′-*O*-(4-Methoxy-1-phenylpiperidin-4-yl)uridine 14 (R = Me, R^1 = R^2 = R^3 = H). (Found, in material crystallised from absolute ethanol: C, 58.0; H, 6.05; N, 9.6. $C_{21}H_{27}N_3O_7$ requires C, 58.19; H, 6.28; N, 9.69%), mp 196–197 °C; δ_H[$(CD_3)_2SO$] 1.65–1.95 (4 H, m), 2.88 (1 H, m), 2.99 (3 H, s), 2.99 (1 H, m), 3.29 (1 H, m), 3.39 (1 H, m), 3.58 (2 H, m), 3.92 (1 H, m), 4.00 (1 H, t, *J* 4.6), 4.37 (1 H, dd, *J* 4.9 and 7.7), 5.20 (1 H, d, *J* 4.6), 5.26 (1 H, t, *J* 4.7), 5.76 (1 H, d, *J* 8.1), 6.03 (1 H, d, *J* 7.8), 6.75 (1 H, t, *J* 7.2), 6.92 (2 H, d, *J* 7.9), 7.19 (2 H, m), 7.97 (1 H, d, *J* 8.2), 11.39 (1 H, br s); δ_C[$(CD_3)_2SO$] 31.3, 33.0, 45.3, 45.7, 46.8, 61.5, 70.9, 72.2, 84.9, 86.3, 99.4, 102.4, 115.7, 118.4, 128.9, 140.7, 150.4, 150.9, 162.9.

1-(4-Chlorophenyl)-4,4-diethoxypiperidine 16 (R = Et, R^1 = R^2 = H, R^3 = Cl)

Toluene-4-sulfonic acid monohydrate (31.38 g, 0.165 mol) and crude 1,5-dichloropentan-3-one[33] **15** (25.59 g, ≈0.165 mol) were added to a stirred solution of 4-chloroaniline (19.77 g, 0.155 mol) in dry ethanol (120 cm^3). The reactants were then heated under gentle reflux. After 1.5 h, triethyl orthoformate (74.85 cm^3, 0.45 mol) was added and the reactants were again heated under reflux. After a further period of 30 min, the products were cooled (ice–water-bath) and triethylamine (70.8 cm^3, 0.51 mol) was added. The resulting mixture was evaporated under reduced pressure and the residue was partitioned between light petroleum (200 cm^3) and saturated aq. sodium hydrogen carbonate (200 cm^3). The organic layer was separated and the aqueous layer was back-extracted with light petroleum (2 × 50 cm^3). The combined organic layers were dried ($MgSO_4$), and evaporated under reduced pressure. The residue was dissolved in methanol (200 cm^3) at room temperature, and water (150 cm^3) was added dropwise, with stirring, over a period of 1 h to the resulting solution. Crystallisation was allowed to occur overnight at room temperature and then for a further period of 1 h at 0 °C. The crystalline precipitate was collected by filtration, washed with methanol–water (1 : 1 v/v) and dried *in vacuo* over sodium hydroxide pellets to give the *title compound* **16** (R = Et, R^1 = R^2 = H, R^3 = Cl) (Found, in colourless material recrystallised from aqueous methanol and dried *in vacuo* over sodium hydroxide pellets: C, 63.4; H, 7.7; N, 4.8. $C_{15}H_{22}ClNO_2$ requires C, 63.48; H, 7.81; N, 4.94%) (42.90 g, 97%), mp 113–114 °C; δ_H($CDCl_3$) 1.19 (6 H, t, *J* 7.1), 1.89 (4 H, t, *J* 5.7), 3.20 (4 H, t, *J* 5.7), 3.50 (4 H, quart, *J* 7.1), 6.85 (2 H, d, *J* 9.0), 7.18 (2 H, m); δ_C($CDCl_3$) 15.4, 32.9, 46.7, 55.1, 98.1, 117.5, 128.8, 149.6.

1-(4-Chlorophenyl)-4-ethoxy-1,2,5,6-tetrahydropyridine 9 (R = Et, $R^1 = R^2 = H$, $R^3 = Cl$)

1-(4-Chlorophenyl)-4,4-diethoxypiperidine **16** (R = Et, $R^1 = R^2 = H$, $R^3 = Cl$) (42.90 g, 0.151 mol) was dissolved in dry dichloromethane (250 cm^3) in an atmosphere of argon, and the solution was cooled to 0 °C (ice–water-bath). Dry *N*,*N*-diisopropylethylamine (62.9 cm^3, 0.36 mol) was added. Boron trifluoride–diethyl ether (36.9 cm^3, 0.30 mol) was then added dropwise, with stirring, over a period of 5 min. After 2 h, the products were allowed to warm up to room temperature and were then washed with saturated aq. sodium hydrogen carbonate (400 cm^3). The organic layer was separated and the aqueous layer was back-extracted with dichloromethane (2 × 50 cm^3). The combined organic layers were dried ($MgSO_4$), and evaporated under reduced pressure; light petroleum (300 cm^3) was added to the residue and the resulting suspension was filtered through a bed of silica gel (20 g). The silica gel was washed with light petroleum–ethyl acetate (98 : 2 v/v; 3 × 75 cm^3). The combined filtrate and washings were concentrated under reduced pressure. Crystallisation of the residue from methanol gave the *title compound* **9** (R = Et, $R^1 = R^2 = H$, $R^3 = Cl$) as pale yellow crystals (Found: C, 65.35; H, 6.65; N, 5.7. $C_{13}H_{16}ClNO$ requires C, 65.68; H, 6.78; N, 5.89%) (33.50 g, 93%), mp 93–94 °C; $\delta_H(CDCl_3)$ 1.31 (3 H, t, *J* 7.0), 2.32 (2 H, m), 3.39 (2 H, t, *J* 5.8), 3.70 (2 H, m), 3.75 (2 H, quart, *J* 7.0), 4.65 (1 H, m), 6.84 (2 H, m), 7.18 (2 H, m); $\delta_C(CDCl_3)$ 14.6, 28.3, 46.0, 47.0, 62.2, 91.1, 116.6, 128.9, 153.2.

8-(2-Chlorophenyl)-1,4-dioxa-8-azaspiro[4.5]decane 17 ($R^1 = Cl$, $R^2 = R^3 = H$)

A mixture of toluene-4-sulfonic acid monohydrate (20.92 g, 0.11 mol), crude 1,5-dichloropentan-3-one[33] **15** (16.23 g, ≈0.104 mol), 2-chloroaniline (14.03 g, 0.11 mol), ethylene glycol (100 cm^3) and light petroleum (80–100 °C; 150 cm^3) was heated in a Dean–Stark apparatus, under reflux, for 3.5 h. Triethylamine (46 cm^3, 0.33 mol) was added to the cooled products and the resulting mixture was washed with saturated aq. sodium hydrogen carbonate (3 × 50 cm^3). The dried ($MgSO_4$) organic layer was evaporated under reduced pressure to give the *title compound* **17** ($R^1 = Cl$, $R^2 = R^3 = H$) as a yellow oil which subsequently solidified (HRMS Found: M^+, 253.0872. $^{12}C_{13}{}^1H_{16}{}^{35}Cl^{14}N^{16}O_2$ requires *M*, 253.08694) (21.67 g, 81%), mp 97–98 °C; $\delta_H(CDCl_3)$ 1.91 (4 H, t, *J* 5.6), 3.12 (4 H, m), 4.00 (4 H, s), 6.95 (1 H, m), 7.06 (1 H, dd, *J* 1.5 and 8.0), 7.20 (1 H, m), 7.35 (1 H, dd, *J* 1.5 and 7.9); $\delta_C(CDCl_3)$ 35.4, 49.7, 64.4, 107.1, 120.7, 123.6, 127.5, 128.9, 130.5, 149.6.

4-(2-Acetoxyethoxy)-1-(2-chlorophenyl)-1,2,5,6-tetrahydropyridine 19a ($R^1 = Cl$, $R^2 = R^3 = H$)

Dry diisopropylethylamine (8.3 cm^3, 48 mmol) was added to a stirred solution of 8-(2-chlorophenyl)-1,4-dioxa-8-azaspiro-[4.5]decane **17** ($R^1 = Cl$, $R^2 = R^3 = H$) (5.07 g, 20 mmol) in dry dichloromethane (25 cm^3) at 0 °C (ice–water-bath). After 5 min, trimethylsilyl triflate (4.26 cm^3, 24 mmol) was added to the stirred reactants. After a further period of 30 min, the products were poured into saturated aq. sodium hydrogen carbonate (50 cm^3). The organic layer was separated, dried ($MgSO_4$), and concentrated under reduced pressure. Acetic anhydride (9.4 cm^3, 0.10 mol) was added to a stirred solution of the residue in pyridine (40 cm^3) at room temperature. After 2 h, the reaction solution was cooled to 0 °C (ice–water-bath). Methanol (10 cm^3) and then triethylamine (50 cm^3) were added. After 30 min, the products were evaporated under reduced pressure. The residue was dissolved in light petroleum (100 cm^3) and the solution was washed with saturated aq. sodium hydrogen carbonate (3 × 50 cm^3). The organic layer was dried ($MgSO_4$), and evaporated under reduced pressure to give the *title compound* **19a** ($R^1 = Cl$, $R^2 = R^3 = H$) as a pale yellow oil (HRMS Found: M^+, 295.0979. $^{12}C_{15}{}^1H_{18}{}^{35}Cl^{14}N^{16}O_3$ requires *M*, 295.0975) (5.33 g, 90%); $\delta_H(CDCl_3)$ 2.10 (3 H, s), 2.38 (2 H, m), 3.26 (2 H, t, *J* 5.7), 3.65 (2 H, m), 3.92 (2 H, t, *J* 4.7), 4.34 (2 H, t, *J* 4.7), 4.72 (1 H, m), 6.96 (1 H, m), 7.09 (1 H, dd, *J* 1.5 and 8.1), 7.21 (1 H, m), 7.36 (1 H, dd, *J* 1.5 and 7.9); $\delta_C(CDCl_3)$ 20.9, 28.5, 48.4, 48.9, 62.7, 64.6, 92.6, 120.5, 123.5, 127.4, 128.6, 130.6, 148.8, 153.3, 171.0.

1-(2-Chlorophenyl)-4-[2-(methoxyacetoxy)ethoxy]-1,2,5,6-tetrahydropyridine 19b ($R^1 = Cl$, $R^2 = R^3 = H$)

Dry diisopropylethylamine (4.15 cm^3, 24 mmol) was added to a stirred solution of 8-(2-chlorophenyl)-1,4-dioxa-8-azaspiro-[4.5]decane **17** ($R^1 = Cl$) (2.533 g, 10 mmol) in dry dichloromethane (15 cm^3) at 0 °C (ice–water-bath). After 5 min, trimethylsilyl triflate (2.13 cm^3, 10.6 mmol) was added to the stirred reactants. After a further period of 30 min, the products were poured into saturated aq. sodium hydrogen carbonate (25 cm^3). The organic layer was separated, dried ($MgSO_4$), and concentrated under reduced pressure. Methoxyacetic anhydride[34] (8.1 g, 50 mmol) was added to a stirred solution of the residue in pyridine (20 cm^3) at room temperature. After 6 h, the reaction solution was cooled to 0 °C (ice–water-bath). Methanol (5 cm^3) and then triethylamine (25 cm^3) were added. After 30 min, the products were evaporated under reduced pressure. The residue was dissolved in dichloromethane (50 cm^3) and the solution was washed with saturated aq. sodium hydrogen carbonate (3 × 25 cm^3). The organic layer was dried ($MgSO_4$), and evaporated under reduced pressure to give the *title compound* **19b** ($R^1 = Cl$, $R^2 = R^3 = H$) as a yellow oil (HRMS Found: M^+, 325.1065. $^{12}C_{16}{}^1H_{20}{}^{35}Cl^{14}N^{16}O_4$ requires *M*, 325.10806) (3.135 g, 96%); $\delta_H(CDCl_3)$ 2.36 (2 H, m), 3.26 (2 H, t, *J* 5.7), 3.46 (3 H, s), 3.64 (2 H, m), 3.94 (2 H, t, *J* 4.7), 4.08 (2 H, s), 4.41 (2 H, t, *J* 4.7), 4.71 (1 H, m), 6.96 (1 H, m), 7.09 (1 H, dd, *J* 1.6 and 8.1), 7.21 (1 H, m), 7.36 (1 H, dd, *J* 1.6 and 7.9); $\delta_C(CDCl_3)$ 28.5, 48.4, 49.0, 59.4, 63.0, 64.5, 69.7, 92.8, 120.6, 123.6, 127.5, 128.6, 130.7, 148.8, 153.3, 170.3.

2′-*O*-[1-(4-Chlorophenyl)-4-ethoxypiperidin-4-yl]uridine 14 (R = Et, $R^1 = R^2 = H$, $R^3 = Cl$)

3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)uridine **13** (5.786 g, 11.9 mmol) and 1-(4-chlorophenyl)-4-ethoxy-1,2,5,6-tetrahydropyridine **9** (R = Et, $R^1 = R^2 = H$, $R^3 = Cl$) (4.30 g, 18.1 mmol) were evaporated from dry toluene (2 × 20 cm^3) solution under reduced pressure. The residue was dissolved in dry dichloromethane (30 cm^3) at room temperature and freshly distilled trifluoroacetic acid (1.5 cm^3, 19.3 mmol) was added, in an atmosphere of argon, to the stirred solution. After 3 h, triethylamine (12.6 cm^3, 90 mmol) was added and the products were concentrated under reduced pressure. The residue was dissolved in dry acetonitrile (20 cm^3). The solution was evaporated under reduced pressure and a solution of tetraethylammonium fluoride in acetonitrile (1.0 mol dm^{-3}, 30 cm^3) was added. The resulting mixture was stirred at room temperature. After 45 min, the products were concentrated under reduced pressure and the residue was partitioned between dichloromethane (20 cm^3) and saturated aq. sodium hydrogen carbonate (3 × 20 cm^3). The dried ($MgSO_4$) organic layer was evaporated under reduced pressure and the residue was fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with CH_2Cl_2–MeOH (100 : 0 to 95 : 5 v/v), were evaporated under reduced pressure to give the *title compound* **14** (R = Et, $R^1 = R^2 = H$, $R^3 = Cl$) as a colourless solid (5.51 g, 96%) (Found, in material crystallised from aq. methanol: C, 54.7; H, 5.7; N, 8.6. $C_{22}H_{28}ClN_3O_7$ requires C, 54.83; H, 5.86; N, 8.72%), mp 152–154 °C; $\delta_H[(CD_3)_2SO]$ 0.97 (3 H, t, *J* 6.8), 1.80 (4 H, m), 2.89 (1 H, m), 3.01 (1 H, m), 3.20 (1 H, m), 3.31 (1 H, m), 3.44 (1 H, m), 3.59 (2 H, m), 3.92 (1 H, m), 3.99 (1 H, m), 4.36 (1 H, m), 5.13 (1 H, d, *J* 4.7), 5.24 (I H, t, *J* 4.7), 5.75 (1 H, d, *J* 8.1), 6.01 (1 H, d, *J* 7.5), 6.93 (2 H, d, *J* 9.1), 7.20

(2 H, d, *J* 9.0), 8.00 (1 H, d, *J* 8.1), 11.37 (1 H, br s); δ_C[$(CD_3)_2SO$] 15.0, 31.8, 33.2, 45.3, 45.6, 54.9, 61.6, 71.0, 72.5, 85.3, 86.4, 99.2, 102.6, 117.1, 121.8, 128.6, 140.7, 149.2, 150.8, 162.9.

2′-*O*-[4-(2-Acetoxyethoxy)-1-(2-chlorophenyl)piperidin-4-yl]-uridine 20a

This compound was prepared from 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine **13** (6.004 g, 12.3 mmol) and 4-(2-acetoxyethoxy)-1-(2-chlorophenyl)-1,2,5,6-tetrahydropyridine **19a** (R^1 = Cl, R^2 = R^3 = H) (5.471 g, 18.5 mmol) in the same way as for the corresponding 2′-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl] derivative **14** (R = Et, R^1 = R^2 = H, R^3 = Cl) (see above); it was isolated as a colourless solid (5.58 g, 83%) following crystallisation from aq. methanol (Found: C, 52.1; H, 5.7; N 7.5. $C_{24}H_{30}ClN_3O_9$·0.75 H_2O requires C, 52.08; H, 5.74; N, 7.59%), mp 159–161 °C; δ_H[$(CD_3)_2SO$] 1.80–2.0 (4 H, m), 2.01 (3 H, s), 2.75 (1 H, m), 2.88 (2 H, m), 3.04 (1 H, m), 3.35 (1 H, m), 3.59 (2 H, m), 3.73 (1 H, m), 3.93 (1 H, m), 4.04 (3 H, m), 4.38 (1 H, dd, *J* 5.0 and 7.2), 5.24 (2 H, m), 5.73 (1 H, d, *J* 8.1), 6.03 (1 H, d, *J* 7.3), 7.02 (1 H, m), 7.08 (1 H, dd, *J* 1.1 and 8.0), 7.27 (1 H, m), 7.39 (1 H, dd, *J* 1.4 and 7.9), 7.98 (1 H, d, *J* 8.1), 11.42 (1 H, br s); δ_C[$(CD_3)_2SO$] 20.6, 33.1, 34.2, 47.9, 48.4, 57.9, 61.5, 63.0, 70.7, 72.7, 85.5, 86.4, 99.3, 102.6, 120.8, 123.9, 127.7, 128.1, 130.3, 140.7, 149.1, 150.9, 162.9, 170.3.

2′-*O*-[1-(2-Chlorophenyl)-4-(2-hydroxyethoxy)piperidin-4-yl]-uridine 21

A solution of 2′-*O*-[4-(2-acetoxyethoxy)-1-(2-chlorophenyl)-piperidin-4-yl]uridine **20a** (0.50 g, 0.93 mmol) in ethanolic methylamine (8 mol dm^{-3}; 2.0 cm^3) was stirred at room temperature. After 2 h, the products were evaporated under reduced pressure and the residue was crystallised from methanol to give the *title compound* **21** (Found: C, 52.5; H, 5.6; N, 8.35. $C_{22}H_{28}ClN_3O_8$·0.4H_2O requires C, 52.3; H, 5.75; N, 8.32%) (0.408 g, 88%), mp 134–136 °C; δ_H[$(CD_3)_2SO$] 1.75–2.0 (4 H, m), 2.79 (1 H, m), 2.93 (2 H, m), 3.03 (1 H, m), 3.21 (1 H, m), 3.41 (2 H, m), 3.51 (1 H, m), 3.59 (2 H, m), 3.93 (1 H, m), 4.05 (1 H, m), 4.38 (1 H, m), 4.66 (1 H, m), 5.22 (2 H, m), 5.74 (1 H, d, *J* 8.1), 6.03 (1 H, d, *J* 7.4), 7.02 (1 H, m), 7.10 (1 H, m), 7.27 (1 H, m), 7.39 (1 H, dd, *J* 1.3 and 7.9), 7.97 (1 H, d, *J* 8.1), 11.38 (1 H, br); δ_C[$(CD_3)_2SO$] 33.2, 34.3, 47.9, 48.3, 60.3, 61.6, 61.8, 70.9, 72.6, 85.4, 86.4, 99.1, 102.6, 120.9, 123.8, 127.6, 128.0, 130.3, 140.7, 149.1, 150.9, 162.9.

2′-*O*-{1-(2-Chlorophenyl)-4-[2-(methoxyacetoxy)ethoxy]-piperidin-4-yl}uridine 20b

This compound was prepared from 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine **13** (0.78 g, 1.6 mmol) and 1-(2-chlorophenyl)-4-[2-(methoxyacetoxy)ethoxy]-1,2,5,6-tetrahydropyridine **19b** (R^1 = Cl, R^2 = R^3 = H) (0.782 g, 2.4 mmol) in the same way as for the corresponding 2′-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl] derivative **14** (R = Et, R^1 = R^2 = H, R^3 = Cl) (see above); it was isolated as a crystalline solid (0.832 g, 91%) following crystallisation from aq. methanol (Found: C, 52.7; H, 5.6; N, 7.2. $C_{25}H_{32}ClN_3O_{10}$ requires C, 52.68; H, 5.66, N, 7.37%), mp 171–172 °C; δ_H[$(CD_3)_2SO$] 1.80–2.0 (4 H, m), 2.75 (1 H, m), 2.88 (2 H, m), 3.03 (1 H, m), 3.30 (3 H, s), 3.39 (1 H, m), 3.60 (2 H, m), 3.76 (1 H, m), 3.93 (1 H, m), 4.06 (3 H, m), 4.12 (2 H, m), 4.38 (1 H, dd, *J* 5.1 and 7.0), 5.27 (2 H, m), 5.75 (1 H, d, *J* 8.1), 6.04 (1 H, d, *J* 7.2), 7.0–7.10 (2 H, m), 7.28 (1 H, m), 7.40 (1 H, dd, *J* 1.4 and 7.9), 8.00 (1 H, d, *J* 8.1), 11.44 (1 H br); δ_C[$(CD_3)_2SO$] 33.0, 34.0, 47.8, 48.2, 57.8, 58.4, 61.2, 63.1, 68.7, 70.4, 72.6, 85.5, 86.1, 99.2, 102.5, 120.7, 123.9, 127.6, 128.0, 130.2, 140.7, 148.9, 150.7, 162.9, 170.0.

2′-*O*-[1-(4-Chlorophenyl)-4-ethoxypiperidin-4-yl]-6-*N*-pivaloyl-adenosine 23a (R = Et, R^1 = R^2 = H, R^3 = Cl)

This compound was prepared from 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-6-*N*-pivaloyladenosine[12] **22a** (11.85 g, 20 mmol) and 1-(4-chlorophenyl)-4-ethoxy-1,2,5,6-tetrahydropyridine **9** (R = Et, R^1 = R^2 = H, R^3 = Cl) (6.94 g, 29.2 mmol) in the same way as for the corresponding uridine derivative **14** (R = Et, R^1 = R^2 = H, R^3 = Cl); it was isolated as a colourless solid (9.34 g, 79.5%) and crystallised from aq. methanol (Found: C, 57.0; H, 6.3; N, 14.2. $C_{28}H_{37}ClN_6O_6$ requires C, 57.09; H, 6.33; N, 14.27%), mp 148–149 °C; δ_H[$(CD_3)_2SO$] 0.53 (3 H, t, *J* 7.0), 1.29 (9 H, s), 1.62 (2 H, m), 1.81 (1 H, m), 1.91 (1 H, m), 2.55–2.75 (2 H, m), 2.99 (2 H, m), 3.25 (1 H, m), 3.36 (1 H, m), 3.64 (1 H, m), 3.77 (1 H, m), 4.07 (1 H, m), 4.21 (1 H, m), 5.01 (1 H, dd, *J* 4.8 and 7.1), 5.31 (1 H, d, *J* 4.6), 5.39 (1 H, dd, *J* 4.9 and 6.5), 6.17 (1 H, d, *J* 7.2), 6.84 (2 H, d, *J* 9.1), 7.17 (2 H, d, *J* 9.0), 8.73 (1 H, s), 8.78 (1 H, s), 10.25 (1 H, s); δ_C[$(CD_3)_2SO$] 14.4, 26.8, 31.6, 32.2, 45.0, 45.7, 54.7, 61.7, 71.0, 72.6, 86.4, 87.1, 99.2, 117.1, 121.8, 126.2, 128.5, 143.4, 149.1, 150.8, 151.5, 151.8, 176.2.

2′-*O*-[1-(4-Chlorophenyl)-4-ethoxypiperidin-4-yl]adenosine 23 (B = 27, R = Et, R^1 = R^2 = H, R^3 = Cl)

2′-*O*-[1-(4-Chlorophenyl)-4-ethoxypiperidin-4-yl]-6-*N*-pivaloyl-adenosine **23a** (R = Et, R^1 = R^2 = H, R^3 = Cl) (1.004 g, 1.7 mmol) was dissolved in ethanolic methylamine (8 mol dm^{-3}; 2 cm^3) and the solution was stirred at room temperature. After 2 h, the products were evaporated under reduced pressure. Crystallisation of the residue from methanol gave the *title compound* **23** (B = **27**, R = Et, R^1 = R^2 = H, R^3 = Cl) (Found: C, 53.8; H, 5.8; N 16.3. $C_{23}H_{29}ClN_6O_5$·0.5 H_2O requires C, 53.75; H, 5.88; N, 16.35%) (0.832 g, 96%), mp 163–164 °C; δ_H[$(CD_3)_2SO$] 0.48 (3 H, t, *J* 7.0), 1.60 (2 H, m), 1.75 (1 H, m), 1.88 (1 H, m), 2.52 (1 H, m), 2.69 (1 H, m), 2.95 (1 H, m), 3.09 (1 H, m), 3.20 (1 H, m), 3.38 (1 H, m), 3.59 (1 H, m), 3.72 (1 H, m), 4.05 (1 H, s), 4.15 (1 H, t, *J* 4.3), 5.00 (1 H, dd, *J* 4.7 and 7.6), 5.23 (1 H, d, *J* 4.1), 5.90 (1 H, dd, *J* 3.8 and 8.5), 6.02 (1 H, d, *J* 7.6), 6.86 (2 H, d, *J* 9.1), 7.18 (2 H, d, *J* 9.0), 7.44 (2 H, br s), 8.16 (1 H, s), 8.42 (1 H, s); δ_C[$(CD_3)_2SO$] 14.2, 31.4, 33.4, 45.0, 45.6, 54.4, 62.1, 71.3, 72.3, 86.8, 87.4, 99.0, 117.0, 119.6, 121.8, 128.5, 140.3, 148.6, 149.1, 152.2, 156.2.

2′-*O*-[1-(2-Fluorophenyl)-4-methoxypiperidin-4-yl]adenosine 23 (B = 27, R = Me, R^1 = F, R^2 = R^3 = H)

2′-*O*-[1-(2-Fluorophenyl)-4-methoxypiperidin-4-yl]-6-*N*-pivaloyladenosine[12] **23a** (R = Me, R^1 = F, R^2 = R^3 = H) (0.50 g, 0.9 mmol) was converted into the *title compound* **23** (B = **27**, R = Me, R^1 = F, R^2 = R^3 = H) (Found: C, 54.6; H, 5.5; N,17.25. $C_{22}H_{27}FN_6O_5$·0.5 H_2O requires C, 54.65; H, 5.84; N, 17.38%) (0.363 g, 85%) by the deacylation procedure described above in the preparation of the corresponding 1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl derivative **23** (B = **27**, R = Et, R^1 = R^2 = H, R^3 = Cl); mp 156–157 °C; δ_H[$(CD_3)_2SO$] 1.65 (2 H, m), 1.84 (1 H, m), 1.97 (1 H, m), 2.55 (1 H, m), 2.57 (3 H, s), 2.81 (2 H, m), 3.09 (1 H, m), 3.60 (1 H, m), 3.72 (1 H, m), 4.06 (1 H, m), 4.19 (1 H, t, *J* 4.2), 5.01 (1 H, dd, *J* 4.6 and 7.7), 5.29 (1 H, d, *J* 4.1), 5.71 (1 H, m), 6.06 (1 H, d, *J* 7.7), 6.92 (2 H, m), 7.08 (2 H, m), 7.43 (2 H, brs), 8.17 (1 H, s), 8.44 (1 H, s); δ_C[$(CD_3)_2SO$] 31.9, 33.8, 46.4, 47.0, 47.6, 54.9, 62.0, 71.2, 72.4, 86.2, 87.3, 99.0, 115.7, 115.9, 119.4, 122.2, 124.7, 139.7, 139.8, 140.2, 149.0, 152.5, 153.5, 156.2.

4-*N*-Benzoyl-2′-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl]-cytidine 23b (R = Et, R^1 = R^2 = H, R^3 = Cl)

This compound was prepared from 4-*N*-benzoyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)cytidine[12] **22b** (3.45 g, 5.85 mmol) and 1-(4-chlorophenyl)-4-ethoxy-1,2,5,6-tetrahydropyridine **9** (R = Et, R^1 = R^2 = H, R^3 = Cl) (2.086 g, 8.8 mmol) in the same way as for the corresponding uridine derivative **14** (R = Et, R^1 = R^2 = H, R^3 = Cl); it was isolated as a colourless solid (2.97 g, 86%) and crystallised from aq. methanol

(Found: C, 58.2; H, 5.7; N, 9.3. $C_{29}H_{33}ClN_4O_7 \cdot 0.8H_2O$ requires C, 58.10; H, 5.82; N, 9.35%), mp 147–149 °C; $\delta_H[(CD_3)_2SO]$ 0.93 (3 H, t, *J* 6.9), 1.75–1.94 (4 H, m), 2.93 (1 H, m), 3.04 (1 H, m), 3.18 (1 H, m), 3.28 (1 H, m), 3.44 (2 H, m), 3.67 (2 H, m), 4.00 (1 H, m), 4.07 (1 H, m), 4.46 (1 H, m), 5.19 (1 H, d, *J* 5.0), 5.31 (1 H, t, *J* 4.8), 6.16 (1 H, d, *J* 6.7), 6.92 (2 H, d, *J* 9.0), 7.19 (2 H, d, *J* 8.9), 7.41 (1 H, m), 7.53 (2 H, m), 7.64 (1 H, m), 8.03 (2 H, d, *J* 7.6), 8.50 (1 H, d, *J* 7.1), 11.35 (1 H, br); $\delta_C[(CD_3)_2SO]$ 15.0, 32.0, 32.2, 45.4, 45.7, 55.0, 61.4, 70.8, 73.7, 86.5, 87.3, 99.3, 117.2, 121.9, 128.48, 128.54, 128.6, 132.8, 145.9, 149.3.

2′-*O*-[1-(4-Chlorophenyl)-4-ethoxypiperidin-4-yl]cytidine 23 (B = 28, R = Et, $R^1 = R^2 = H$, R^3 = Cl)

4-*N*-Benzoyl-2′-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl]-cytidine **23b** (R = Et, $R^1 = R^2 = H$, R^3 = Cl) (1.03 g, 1.76 mmol) and conc. aq. ammonia (*d* 0.88; 3.0 cm^3) were heated together at 55 °C for 16 h. The products were then concentrated under reduced pressure and the residue was fractionated by short-column chromatography on silica gel. The appropriate fractions, which were eluted with CH_2Cl_2–MeOH (100 : 0 to 95 : 5 v/v), were combined, and evaporated under reduced pressure. Crystallisation from aq. methanol gave the *title compound* **23** (B = **28**, R = Et, $R^1 = R^2 = H$, R^3 = Cl) (Found: C, 54.2; H, 6.1; N, 11.3. $C_{22}H_{29}ClN_4O_6 \cdot 0.4\ H_2O$ requires C, 54.13; H, 6.15; N, 11.48%) (0.72 g, 85%), mp 198–199 °C; $\delta_H[(CD_3)_2SO]$ 0.93 (3 H, t, *J* 6.8), 1.65–2.0 (4 H, m), 2.85 (1 H, m), 3.00 (1 H, m), 3.16 (1 H, m), 3.26 (1 H, m), 3.41 (2 H, m), 3.58 (2 H, m), 3.87 (1 H, m), 3.98 (1 H, m), 4.35 (1 H, m), 5.03 (1 H, d, *J* 4.6), 5.19 (1 H, m), 5.79 (1 H, d, *J* 7.4), 6.04 (1 H, d, *J* 7.2), 6.92 (2 H, d, *J* 8.8), 7.20 (2 H, d, *J* 8.7), 7.25 (2 H, br s), 7.88 (1 H, d, *J* 7.4); $\delta_C[(CD_3)_2SO]$ 15.0, 31.8, 33.2, 45.3, 45.6, 54.8, 61.7, 70.9, 72.6, 85.8, 86.6, 94.7, 99.0, 117.1, 121.8, 128.5, 141.9, 149.2, 155.4, 165.7.

2′-*O*-[1-(4-Chlorophenyl)-4-ethoxypiperidin-4-yl]-2-*N*-(phenylacetyl)guanosine 23c (R = Et, $R^1 = R^2 = H$, R^3 = Cl)

This compound was prepared from 2-*N*-(phenylacetyl)-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)guanosine[12] **22c** (12.63 g, 19.6 mmol) and 1-(4-chlorophenyl)-4-ethoxy-1,2,5,6-tetrahydropyridine **9** (R = Et, $R^1 = R^2 = H$, R^3 = Cl) (6.995 g, 29.4 mmol) in the same way as for the corresponding uridine derivative **14** (R = Et, $R^1 = R^2 = H$, R^3 = Cl); it was isolated as a colourless solid (11.15 g, 89%) and crystallised from aq. methanol (Found: C, 56.7; H, 5.6; N, 12.8. $C_{31}H_{35}ClN_6O_7 \cdot H_2O$ requires C, 56.66; H, 5.68; N, 12.79%), mp 153–154 °C; $\delta_H[(CD_3)_2SO]$ 0.76 (3 H, t, *J* 7.0), 1.60 (2 H, m), 1.84 (2 H, m), 2.79 (2 H, m), 2.98 (2 H, m), 3.35 (2 H, m), 3.66 (2 H, m), 3.80 (2 H, s), 4.03 (1 H, m), 4.15 (1 H, m), 4.71 (1 H, dd, *J* 4.6 and 7.6), 5.19 (1 H, m), 5.26 (1 H, m), 5.99 (1 H, d, *J* 7.6), 6.82 (2 H, d, *J* 9.1), 7.13 (2 H, m, *J* 9.0), 7.25–7.40 (5 H, m), 8.36 (1 H, s), 11.97 (2 H, br); $\delta_C[(CD_3)_2SO]$ 14.7, 31.6, 33.2, 42.6, 45.1, 45.6, 55.0, 61.7, 71.4, 74.3, 84.9, 87.1, 99.2, 117.0, 120.0, 121.9, 127.0, 128.4, 128.5, 129.4, 134.2, 137.7, 148.2, 148.9, 149.1, 154.7, 174.1.

2′-*O*-[1-(4-Chlorophenyl)-4-ethoxypiperidin-4-yl]guanosine 23 (B = 29, R = Et, $R^1 = R^2 = H$, R^3 = Cl)

A solution of 2′-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl]-2-*N*-(phenylacetyl)guanosine **23c** (R = Et, $R^1 = R^2 = H$, R^3 = Cl) (4.003 g, 6.26 mmol) in ethanolic methylamine (8 mol dm^{-3}; 5 cm^3) was stirred at room temperature. After 2 h, the products were evaporated under reduced pressure and the residue was crystallised from methanol to give the *title compound* **23** (B = **29**, R = Et, $R^1 = R^2 = H$, R^3 = Cl) (Found: C, 51.1; H, 5.7; N, 15.5. $C_{23}H_{29}ClN_6O_6 \cdot H_2O$ requires C, 51.25; H, 5.80; N, 15.59%) (3.07 g, 94%), mp 180–181 °C; $\delta_H[(CD_3)_2SO]$ 0.76 (3 H, t, *J* 7.0), 1.64 (2 H, m), 1.83 (2 H, m), 2.78 (2 H, m), 2.99 (1 H, m), 3.08 (1 H, m), 3.32 (2 H, m), 3.58 (1 H, m), 3.67 (1 H, m), 3.97 (1 H, m), 4.10 (1 H, m), 4.75 (1 H, dd, *J* 4.8 and 7.4), 5.15 (1 H, d, *J* 4.2), 5.30 (1 H, t, *J* 5.4), 5.87 (1 H, d, *J* 7.5), 6.50 (2 H, br), 6.87 (2 H, d, *J* 9.1), 7.19 (2 H, d, *J* 9.0), 8.04 (1 H, s), 10.71 (1 H, br s); $\delta_C[(CD_3)_2SO]$ 14.6, 31.6, 33.2, 45.1, 45.7, 54.7, 61.7, 71.2, 73.3, 84.9, 86.6, 99.1, 116.6, 117.1, 121.9, 128.5, 135.8, 149.2, 151.1, 153.6, 156.6.

2′-*O*-[1-(2-Fluorophenyl)-4-methoxypiperidin-4-yl]guanosine 23 (B = 29, R = Me, R^1 = F, $R^2 = R^3 = H$)

2′-*O*-[1-(2-Fluorophenyl)-4-methoxypiperidin-4-yl]-2-*N*-(phenylacetyl)guanosine[12] **23c** (R = Et, R^1 = F, $R^2 = R^3 = H$) (0.406 g, 0.67 mmol) was converted into the *title compound* **23** (B = **29**, R = Me, R^1 = F, $R^2 = R^3 = H$) (Found: C, 51.0; H, 5.6; N, 16.2. $C_{22}H_{27}FN_6O_6 \cdot 1.5H_2O$ requires C, 51.06; H, 5.84; N, 16.24%) (0.298 g, 91%), mp 178–179 °C; $\delta_H[(CD_3)_2SO]$ 1.74 (2 H, m), 1.86 (1 H, m), 1.97 (1 H, m), 2.62 (1 H, m), 2.71 (3 H, s), 2.86 (2 H, m), 3.09 (1 H, m), 3.62 (2 H, m), 3.98 (1 H, m), 4.13 (1 H, m), 4.75 (1 H, dd, *J* 4.7 and 7.6), 5.19 (1 H, m), 5.25 (1 H, m), 5.90 (1 H, d, *J* 7.8), 6.54 (2 H, br s), 6.93 (2 H, m), 7.08 (2 H, m), 8.03 (1 H, s), 10.69 (1 H, br); $\delta_C[(CD_3)_2SO]$ 31.9, 33.8, 46.8, 47.0, 47.7, 61.8, 71.1, 73.3, 84.3, 86.7, 99.0 and aromatic resonance signals.

2′-*O*-[1-(4-Chlorophenyl)-4-ethoxypiperidin-4-yl]-2-*N*-[(dimethylamino)methylene]guanosine 23 (B = 30, R = Et, $R^1 = R^2 = H$, R^3 = Cl)

N,*N*-Dimethylformamide dimethyl acetal (0.55 cm^3, 4.1 mmol) was added to a stirred suspension of 2′-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl]guanosine **23** (B = **29**, R = Et, $R^1 = R^2 =$ H, R^3 = Cl) (0.503 g, 0.96 mmol) in methanol (15 cm^3) at room temperature. After 2.5 h, the products were concentrated under reduced pressure. The residue was fractionated by short-column chromatography on silica gel: the appropriate fractions, which were eluted with CH_2Cl_2–MeOH (100 : 0 to 95 : 5 v/v), were evaporated under reduced pressure to give the *title compound* **23** (B = **30**, R = Et, $R^1 = R^2 = H$, R^3 = Cl) as a colourless solid (0.535 g, 96%) (Found: C, 52.3; H, 6.0, N, 16.1. $C_{26}H_{34}ClN_7O_6 \cdot H_2O$ requires C, 52.57; H, 6.11; N, 16.50%); $\delta_H[(CD_3)_2SO]$ 0.76 (3 H, t, *J* 7.0), 1.55–1.95 (4 H, m), 2.78 (2 H, m), 3.00 (1 H, m), 3.04 (3 H, s), 3.09 (1 H, m), 3.14 (3 H, s), 3.35 (2 H, m), 3.61 (1 H, m), 3.70 (1 H, m), 4.00 (1 H, m), 4.14 (1 H, m), 4.83 (1 H, dd, *J* 4.9 and 6.9), 5.20 (1 H, d, *J* 4.5), 5.25 (1 H, m), 5.94 (1 H, d, *J* 7.0), 6.86 (2 H, d, *J* 9.1), 7.18 (2 H, d, *J* 9.0), 8.15 (1 H, s), 8.49 (1 H, s), 11.40 (1 H, br s); $\delta_C[(CD_3)_2SO]$ 14.7, 31.9, 33.2, 34.6, 40.7, 45.3, 45.8, 54.8, 61.8, 71.0, 73.1, 85.6, 86.6, 99.2, 117.1, 120.0, 121.9, 128.6, 137.3, 149.2, 149.8, 157.4, 157.5, 157.8.

Preparation of 2′-*O*-[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl] (Fpmp)-protected diribonucleoside phosphates 31

These substrates were prepared on a 1 μmolar scale by solid-phase synthesis (see below) following the published procedure.[12] The 2′-*O*-(Fpmp) derivatives of ApU, CpU, GpU and UpU were purified by preparative HPLC on a reversed-phase column.

Hydrolysis studies on 2′-protected ribonucleosides and 2′-protected diribonucleoside phosphates

Hydrolysis studies were carried out at 30 °C in a Digi-block heating apparatus. The following acid and buffer solutions were used: 0.316 mol dm^{-3} hydrochloric acid (pH 0.5), 0.10 mol dm^{-3} hydrochloric acid (pH 1.0), 0.032 mol dm^{-3} hydrochloric acid (pH 1.5), 0.01 mol dm^{-3} hydrochloric acid (pH 2.0), 0.33 mol dm^{-3} acetic acid (pH 2.5), and 0.5 mol dm^{-3} sodium acetate buffers (pHs 3.0, 3.25, 3.5, 3.75 and 4.0). The sodium acetate buffer solutions were prepared by mixing 0.5 mol dm^{-3} acetic acid and 0.5 mol dm^{-3} sodium acetate solutions in the

appropriate proportions. The pHs of all of these solutions were checked with a pH meter.

(a) 2′-Protected ribonucleosides. A solution of adenosine (≈0.001 g) in water (1.0 cm^3) was prepared. This solution (10 mm^3) was placed in a 1.5 cm^3 Eppendorf tube. A solution of the substrate (≈0.001 g) in the appropriate acid or buffer solution (10 cm^3) was prepared if necessary by sonification. This solution (0.5 cm^3) was rapidly transferred to the Eppendorf tube containing the adenosine solution. The Eppendorf tube was sealed and was immediately placed in the heating apparatus, which was maintained at 30 (±0.1) °C. After appropriate intervals of time, aliquots (25 mm^3) of the reaction solution were removed from the Eppendorf tube, neutralised with aq. Tris solution, and the products were analyzed by reversed-phase HPLC. The percentage of substrate remaining was determined by measuring the ratio of the integrals of the substrate and adenosine peaks. The ratio and half-lives of removal of the 2′-protecting groups were obtained by plotting $\log_{10}$ [percentage of remaining substrate] against time. Good straight-line plots were generally obtained.

(b) 2′-Protected diribonucleoside phosphates. A sealed 1.5 cm^3 Eppendorf tube containing the stock adenosine solution (10 mm^3) [see (a) above] and a freshly prepared solution of the substrate (≈4 A_{260} units) in the appropriate buffer solution (0.5 cm^3) was placed in a Digi-block heating apparatus, maintained at 30 (±0.1) °C. After appropriate intervals of time, aliquots (25 mm^3) of the reaction solution were removed from the Eppendorf tube and neutralised with aq. Tris solution. The products were analysed by reversed-phase HPLC, and the data obtained were processed as in (a) above.

Preparation of 2′-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl]-5′-*O*-(4,4′-dimethoxytrityl)uridine 3′-[(2-cyanoethyl) *N*,*N*-diisopropylphosphoramidite] 33

2′-*O*-[1-(4-Chlorophenyl)-4-ethoxypiperidin-4-yl]uridine **14** (R = Et, $R^1 = R^2 = H$, $R^3 = Cl$) was converted into the title phosphoramidite **33** by the previously reported[12] two-step procedure.

Solid-phase oligoribonucleotide synthesis

Automated oligoribonucleotide synthesis was carried out on a 1.0 μmolar scale in an Applied Biosystems 381A DNA synthesizer using the above phosphoramidite **33**, Cruachem 5′-*O*-(DMTr)-2′-*O*-(Fpmp)uridine 3′-[(2-cyanoethyl) *N*,*N*-diisopropylphosphoramidite] **32** and Cruachem 1.0 μmol [2′(3′)-*O*-benzoyl]uridine controlled-pore glass disposable columns. Solvents and other reagents were purified as previously described.[12] 1*H*-Tetrazole was used as the activator in coupling reactions which were allowed to proceed for 10–15 min. The crude 5′-*O*-(DMTr)-2′-protected oligoribonucleotides, which were released from the solid support by heating with conc. aq. ammonia (*d* 0.88; 2.0 cm^3) at 55 °C for 14 h, were purified by preparative HPLC on a reversed-phase column.

Determination of the conditions required for the complete unblocking of 2′-protected-5′-*O*-(DMTr) oligoribonucleotides

A solution of adenosine (≈0.001 g) in 0.5 mol dm^{-3} sterile sodium acetate buffer (pH 4.00; 1.0 cm^3) was prepared. This solution (10 mm^3) and the partially protected oligoribonucleotide (≈4 A_{260} units) were dissolved in the same 0.5 mol dm^{-3} sodium acetate buffer (pH 4.00; 0.5 cm^3), contained in a 1.5 cm^3 Eppendorf tube. The Eppendorf tube was sealed and placed immediately in a Digi-block heating apparatus, maintained at 35 (±0.1) °C. After appropriate intervals of time, aliquots (25 mm^3) of the reaction solution were removed, neutralised with aq. Tris and analysed by reversed-phase HPLC. Ratios of the integrals (at 260 nm) of the fully unblocked oligoribonucleotides and adenosine were plotted against time.

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References

1 H. Schaller, G. Weimann, B. Lerch and H. G. Khorana, *J. Am. Chem. Soc.*, 1963, **85**, 3821.
2 J. B. Chattopadhyaya and C. B. Reese, *J. Chem. Soc.*, *Chem. Commun.*, 1978, 639.
3 T. Brown and D. J. S. Brown, in *Oligonucleotides and Analogues. A Practical Approach*, ed. F. Eckstein, IRL Press, Oxford, 1991, pp. 12–14.
4 C. B. Reese, *Tetrahedron*, 1978, **34**, 3143.
5 D. M. Brown, D. I. Magrath, A. H. Neilson and A. Todd, *Nature*, 1956, **177**, 1124.
6 B. E. Griffin, M. Jarman and C. B. Reese, *Tetrahedron*, 1968, **24**, 639.
7 S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1992, **48**, 2223.
8 C. B. Reese, in *Nucleic Acids and Molecular Biology*, ed. F. Eckstein and D. M. J. Lilley, Springer, Berlin, 1989, vol. 3, pp. 164–181.
9 C. B. Reese, H. T. Serafinowska and G. Zappia, *Tetrahedron Lett.*, 1986, **27**, 2291.
10 C. B. Reese and E. A. Thompson, *J. Chem. Soc.*, *Perkin Trans. 1*, 1988, 2881.
11 C. B. Reese, M. V. Rao, H. T. Serafinowska, E. A. Thompson and P. S. Yu, *Nucleosides*, *Nucleotides*, 1991, **10**, 81.
12 M. V. Rao, C. B. Reese, V. Schehlmann and P. S. Yu, *J. Chem. Soc.*, *Perkin Trans. 1*, 1993, 43.
13 T. S. Rao, C. B. Reese, H. T. Serafinowska, H. Takaku and G. Zappia, *Tetrahedron Lett.*, 1987, **28**, 4897.
14 O. Sakatsume, M. Ohtsuki, H. Takaku and C. B. Reese, *Nucleic Acids Res.*, 1989, **17**, 3689.
15 B. Beijer, I. Sulston, B. S. Sproat, P. Rider, A. I. Lamond and P. Neuner, *Nucleic Acids Res.*, 1990, **18**, 5143.
16 D. C. Capaldi and C. B. Reese, *Nucleic Acids Res.*, 1994, **22**, 2209.
17 B. S. Sproat, B. Beijer, M. Groetli, U. Ryder, K. L. Morand and A. I. Lamond, *J. Chem. Soc.*, *Perkin Trans. 1*, 1994, 419.
18 U. Pieles, B. Beijer, K. Bohmann, S. Weston, S. O'Laughlin, V. Adam and B. S. Sproat, *J. Chem. Soc.*, *Perkin Trans. 1*, 1994, 3423.
19 M. V. Rao and K. Macfarlane, *Nucleosides*, *Nucleotides*, 1995, **14**, 911.
20 A. McGregor, M. V. Rao, G. Duckworth, P. G. Stockley and B. A. Connolly, *Nucleic Acids Res.*, 1996, **24**, 3173.
21 C. B. Reese and Q. Song, *Nucleic Acids Res.*, 1999, **27**, 963.
22 C. B. Reese and Q. Song, *Nucleic Acids Res.*, 1999, **27**, 2672.
23 M. J. Gallagher and F. G. Mann, *J. Chem. Soc.*, 1962, 5110.
24 M. Faja, C. B. Reese, Q. Song and P.-Z. Zhang, *J. Chem. Soc.*, *Perkin Trans. 1*, 1997, 191.
25 A. Sandström, M. Kwiatkowski and J. Chattopadhyaya, *Acta Chem. Scand.*, *Ser. B*, 1985, **39**, 273.
26 H. Rastogi and D. A. Usher, *Nucleic Acids Res.*, 1995, **23**, 4872.
27 S. A. Scaringe, F. E. Wincott and M. H. Caruthers, *J. Am. Chem. Soc.*, 1998, **120**, 11820.
28 S. S. Jones, C. B. Reese and S. Sibanda, in *Current Trends in Organic Synthesis*, ed. H. Nozaki, Pergamon Press, Oxford and New York, 1983, pp. 71–81.
29 L. J. McBride, R. Kierzek, S. L. Beaucage and M. H. Caruthers, *J. Am. Chem. Soc.*, 1986, **108**, 2040.
30 M. A. Morgan, S. A. Kazakov and S. M. Hecht, *Nucleic Acids Res.*, 1995, **23**, 3949.
31 Y. Hayakawa and M. Kataoka, *J. Am. Chem. Soc.*, 1998, **120**, 12395.
32 W. T. Markiewicz, *J. Chem. Res.*, 1979, (*S*), 24; (*M*), 181.
33 G. R. Owen and C. B. Reese, *J. Chem. Soc. C*, 1970, 2401.
34 C. B. Reese, J. C. M. Stewart, J. H. van Boom, H. P. M. de Leeuw, J. Nagel and J. F. M. de Rooy, *J. Chem. Soc.*, *Perkin Trans. 1*, 1975, 934.
35 D. D. Perrin, *Dissociation Constants of Organic Bases in Aqueous Solution: Supplement 1972*, ed. Butterworths, London, 1972.

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