Use of the 1-(2-Fluorophenyl)-4-methoxypiperidin-4-yl (Fpmp) Protecting Group in the Solid-Phase Synthesis of Oligo- and Poly-ribonucleotides

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An approach to the solid-phase synthesis of oligo- and poly-ribonucleotides is described. The synthetic strategy involves the use of building blocks in which two acid-labile groups, 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl (Fpmp) and 9-phenylxanthen-9-yl (Px), respectively, are used to protect the 2'- and 5'-hydroxy functions of ribonucleoside building blocks. The adenine, cytosine and guanine base residues are protected with pivaloyl, benzoyl and phenylacetyl groups, respectively. 2-Cyanoethyl *N,N*-diisopropylphosphoramidites are used in the coupling steps, and 5-(3-nitrophenyl)-1*H*-tetrazole is used as the activating agent. Following the chain-assembly process, 2'-protected oligo-and poly-ribonucleotides are released from the functionalized controlled-pore glass solid support; the latter stabilized ribonucleic acid (RNA) sequences are purified before they are fully unblocked by treatment with 0.01 mol dm^{-3} hydrochloric acid (pH 2) at room temperature for 20 h. The efficacy of this methodology is illustrated by the synthesis of the 3'-terminal decamer (r[UCGUCCACCA]), nonadecamer (r[AUUCCGGACUCGUCCACCA]), and heptatriacontamer (37-mer, r[GGAGAGGUCUCCGGUUCGAUUCCGGACUCGUCCACCA]) sequences of yeast alanine tRNA ($tRNA^{Ala}$).

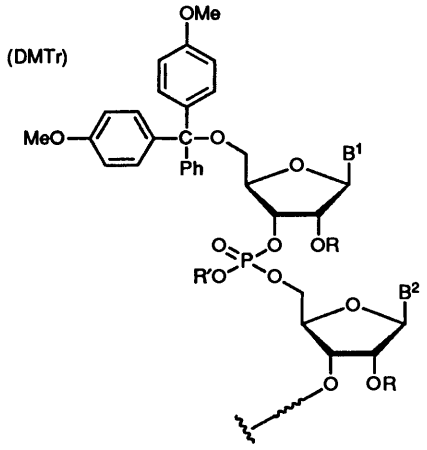
There can be little doubt that developments in the past ten years or so in the methodology of the chemical synthesis of deoxyribonucleic acid (DNA) sequences have had a profound enabling effect on research in biology. Until a few years ago, much less progress had been made in the related area of the chemical synthesis of ribonucleic acid (RNA) sequences. However, recent developments in biology and especially the discovery of ribozymes[1] have highlighted the need for synthetic RNA sequences, and have thereby stimulated work on the development of the methodology of the chemical synthesis of oligo- and poly-ribonucleotides.

Much of the earlier successful work on the chemical synthesis of oligo- and poly-ribonucleotides[2,3] was carried out by the phosphotriester approach[4] in solution with aryl (usually 2-chlorophenyl[5]) groups being used to protect the internucleotide linkages. This method proved to be effective and is particularly suitable when relatively large quantitites (say, 10–100 mg) of synthetic RNA are required, for example for NMR or X-ray crystallographic studies. On the other hand, if small quantities (say, <10 mg) of RNA are required for biological studies, synthesis in solution,[6] which is both time-consuming and experimentally demanding, is unlikely to be the most convenient approach. In order to meet the increasing need for small quantities of specific RNA sequences, much effort has recently been put into the development of a rapid synthesis of oligo- and poly-ribonucleotides,[7] corresponding to the automated solid-phase synthesis of DNA sequences.

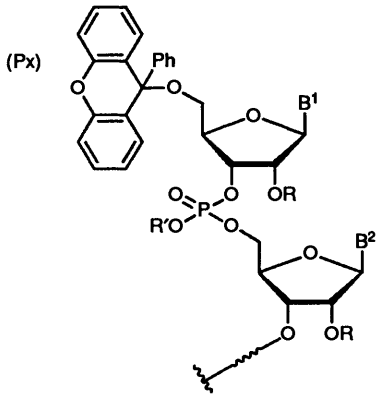
In any oligoribonucleotide synthesis, it is crucially important that all of the 2'-hydroxy functions should remain protected throughout the assembly of the desired sequence and that the protecting groups should be removable at the end of the synthesis under conditions under which RNA is completely stable. For this reason, it was clear at the outset that the success of solid-phase RNA synthesis would be largely dependent on the choice of a suitable protecting group (R in compounds **1** and **2**) for the 2'-hydroxy functions. The tetrahydropyranyl (Thp, **3**, as in **1a** and **2a**)[8–10] and more particularly the 4-methoxytetrahydropyran-4-yl (Mthp, **4**, as in **1b** and **2b**)[11] groups have been used very successfully to protect the 2'-hydroxy functions in oligoribonucleotide synthesis in solution. However, these two acetal protecting groups **3** and **4** are not particularly suitable for use in solid-phase RNA synthesis as they do not seem to be compatible (see below) with the 4,4'-dimethoxytrityl (DMTr, as in **1**)[12] and 9-phenylxanthen-9-yl (Px, as in **2**)[13] protecting groups. The latter (DMTr and Px) are also acid-labile, and only such modified trityl groups have, so far, been found to be really suitable for the protection of the 5'-terminal hydroxy functions both in solid-phase DNA and RNA synthesis.[7]

Ideally, a 2'-protecting group should be (i) easy to introduce, (ii) achiral, (iii) unable to migrate, and (iv) completely stable under the conditions required for the assembly of the fully protected oligonucleotide sequence and for its subsequent unblocking and release from the solid support. Finally, a 2'-protecting group must be removable under conditions under which RNA is completely stable. It is also important that the final unblocking step should involve as little manipulation as possible, as it must always be borne in mind that RNA readily undergoes digestion in the presence of contaminating traces of endonucleases such as ribonuclease A. It is further desirable that synthetic RNA should be purified by gel filtration, liquid chromatography (LC) or polyacrylamide gel electrophoresis (PAGE), or by a combination of these techniques while the 2'-protecting groups are still intact; it can then be stored indefinitely in the stabilized form, and the 2'-hydroxy functions need be unblocked only when the RNA is required for biological or other studies.

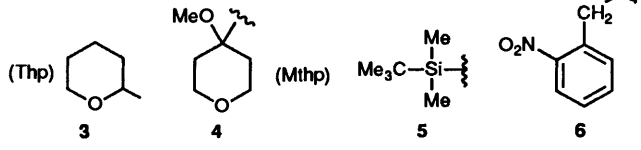
tert-Butyldimethylsilyl (TBDMS, **5**; as in **1**; R = **5** and **2**; R = **5**)[14] and 2-nitrobenzyl (**6**; as in **1**; R = **6** and **2**; R = **6**)[15] have been recommended as suitable 2'-protecting groups in oligoribonucleotide synthesis. Indeed, the TBDMS protecting group **5** is now being used fairly widely[7] in solid-phase oligo- and poly-ribonucleotide synthesis. However, its properties are by no means ideal for this purpose. While the TBDMS group meets criteria (i) and (ii) (see above), it readily migrates from 2'- to 3'-hydroxy functions, especially under mildly basic conditions.[16] Although it is almost certainly stable under the reaction conditions which obtain during the assembly of fully protected oligonucleotide sequences, cleavage of TBDMS protecting groups apparently does occur to some extent[17,18] under the ammonolytic conditions required for the release of the synthetic RNA from the solid support and for the removal of the protecting groups from the base residues and internucleo-



1a; R = 3
b; R = 4



2a; R = 3
b; R = 4



3 **4** **5** **6**

tide linkages. Thus it does not seem to be possible to isolate fully stabilized oligo- and poly-ribonucleotides in which the 2′-*O*-TBDMS protecting groups are fully intact.

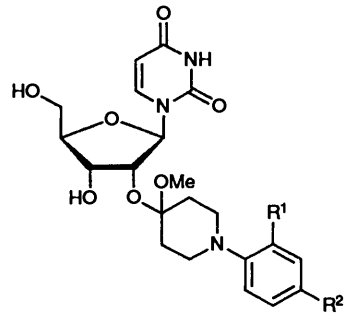
With regard to the 2-nitrobenzyl protecting group,[15] apart from the fact that it is photosensitive and therefore that care must be taken to exclude light during the preparation of the building blocks and the assembly of the desired RNA sequences, it would seem that it meets all of the above criteria (i)–(iv). However, the 2-nitrobenzyl group has not found widespread use in solid-phase RNA synthesis, and this may be due to difficulties encountered in effecting its complete removal in the final unblocking step.[19]

The Mthp protecting group (**4**; as in **1b** and **2b**) fulfils the above criteria (i)–(iii), and is also completely removable by acidic hydrolysis under mild conditions (pH 2, room temperature). It has been firmly established[4] that neither hydrolytic cleavage nor migration of the internucleotide linkages of RNA occurs under the latter conditions. However, although the Mthp group meets the second part of criterion (iv) above, that is, it is stable to the action of conc. aq. ammonia, it unfortunately does not meet this criterion fully; thus it seems that it cannot withstand repeated exposure to the relatively drastic acidic conditions[20,21] (*e.g.*, trichloroacetic acid in anhydrous dichloromethane solution) used to remove DMTr or Px groups from the 5′-terminal hydroxy functions in each synthetic cycle, even when the greatest possible care is taken to exclude traces of moisture. We therefore set out to design a protecting group for the 2′-hydroxy functions that retained all of the above advantages of the Mthp group and which was in addition stable under the latter 'detritylation' conditions.

Acetal hydrolysis is subject to specific acid catalysis; it is a second-order reaction the rate of which is dependent on the concentration of substrate and the concentration of hydrogen

7 **8** **9**

ions.[22] Mthp-protected hydroxy functions behave[23] like typical acetal systems in that their rate of unblocking increases logarithmically with decreasing pH. It is clear from our previous studies[24] that the rate of hydrolysis of acetal systems of the general structure **7** is considerably influenced by the inductive effect of the atom or group X. It occurred to us that if we were able to design an acetal system of this type the rate of hydrolysis of which was virtually independent of pH in the pH range of from ~0 to ~2.5, it would very likely prove to be an effective protecting group for the 2′-hydroxy functions in solid-phase RNA synthesis. It further occurred to us that a carefully chosen 1-arylpiperidin-4-one acetal system (**8**; Ar = aryl) with a weakly basic (pK_a ~2) tertiary amino function might well have the desired properties. Depending on the nature of the *N*-aryl substituent, it seemed probable that the inductive effect of the unprotonated *N*-aryl group (as in **8**) would not generally be greater than that of oxygen (as in Mthp derivatives: **7**; X = O) but that the inductive effect of the protonated *N*-aryl group (as in **9**) would be considerably greater. It was thought that this would result in the rate of hydrolysis of the acetal function in species **8** being several orders of magnitude faster than that of the acetal function in its conjugate acid **9**. It would therefore seem to be justifiable, as a first approximation, to ignore the term relating to species **9** in the kinetic equation representing the overall rate of hydrolysis of a mixture of substrates **8** and **9**. It would then follow, again as a first approximation, that the rate of hydrolysis of species **8** would be pH independent. Assuming second-order kinetics, if the pH were diminished by *n* units, the concentration of species **8** would *decrease* by a factor of 10^n but the rate of hydrolysis of the remaining substrate **8** would *increase* by the same factor of 10^n. This situation would be expected to obtain only within a specific pH range which would depend on the pK_a of the tertiary amino function in species **8**.



10a; R^1 = Cl, R^2 = Me (Ctmp protecting group)
b; R^1 = F, R^2 = H (Fpmp protecting group)

It seemed clear that it would be necessary to choose the aryl substituent (Ar) carefully in order to ensure that the rate of

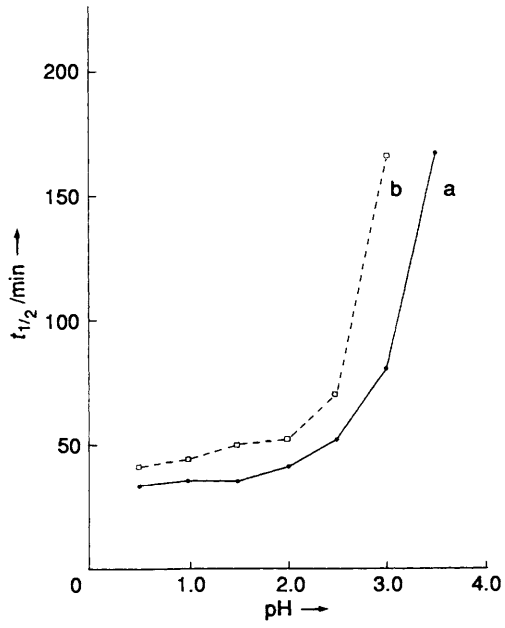
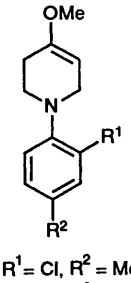


Fig. 1 Half-time ($t_{\frac{1}{2}}$) of hydrolysis of **a** 2′-*O*-(Ctmp)-uridine **10a** (●) and **b** 2′-*O*-(Fpmp)-uridine **10b** (□) as a function of pH at 25 °C

hydrolysis of the acetal system **8** would be pH independent in the desired pH range (see above) and that, like the Mthp acetal system (**7**; X = O), species **8** would undergo hydrolysis at a reasonable rate at pH ~ 2 and room temperature. Initially, we found[23] that the unsubstituted 1-phenyl-4-methoxypiperidin-4-yl group (as in **10**; $R^1 = R^2 = H$) was too stable to acidic hydrolysis at pH 2 and that the 1-(2,4-dichlorophenyl)-4-methoxypiperidin-4-yl group (as in **10**; $R^1 = R^2 = Cl$) was too labile below pH 2 to be suitable for the present purpose. We then found[23] that the 1-(2-chloro-4-methylphenyl)-4-methoxypiperidin-4-yl (Ctmp, as in **10a**) protecting group possessed the hydrolysis properties that we were seeking. It can be seen from Fig. 1a that the half-time of hydrolysis of compound **10a** at 25 °C hardly changes between pH 0.5 and 2.0, and that its rate of hydrolysis is only 1.55-times faster at pH 0.5 than at pH 2.5. Furthermore, the Ctmp protecting group undergoes hydrolysis at a convenient rate at pH 2–2.5 and room temperature, and thus would be expected to be completely removable under conditions under which RNA is completely stable. It was soon established that the Ctmp group was suitable for the protection of the 2′-hydroxy functions in the solid-phase synthesis of oligoribonucleotides using either the phosphoramidite[25] or the *H*-phosphonate[26] approach.

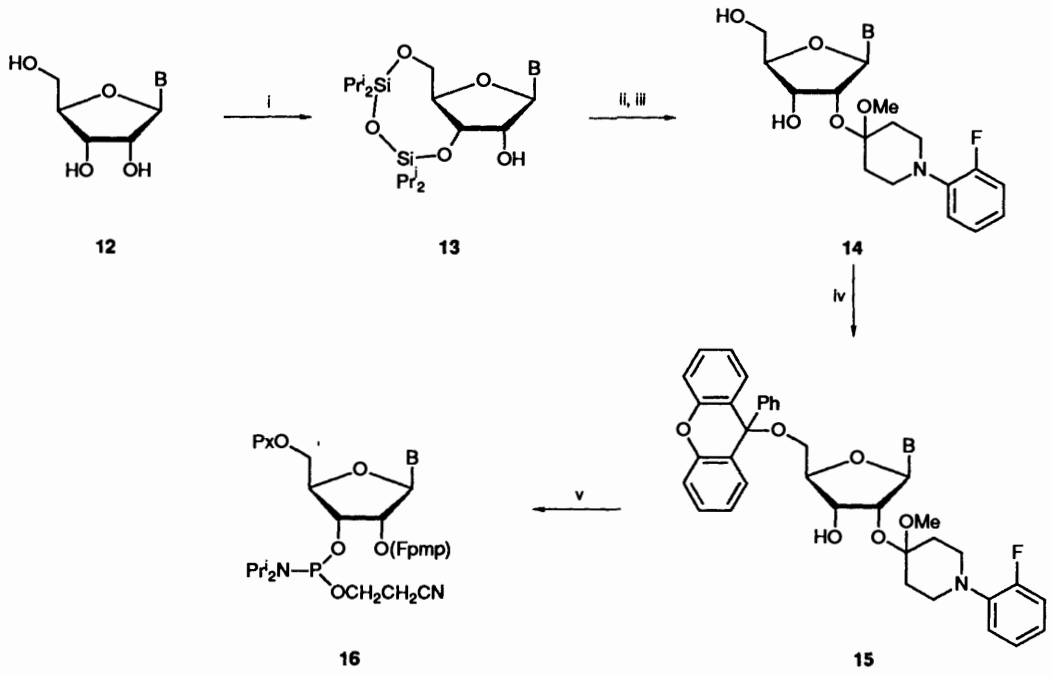


11a; R^1 = Cl, R^2 = Me
b; R^1 = F, R^2 = H

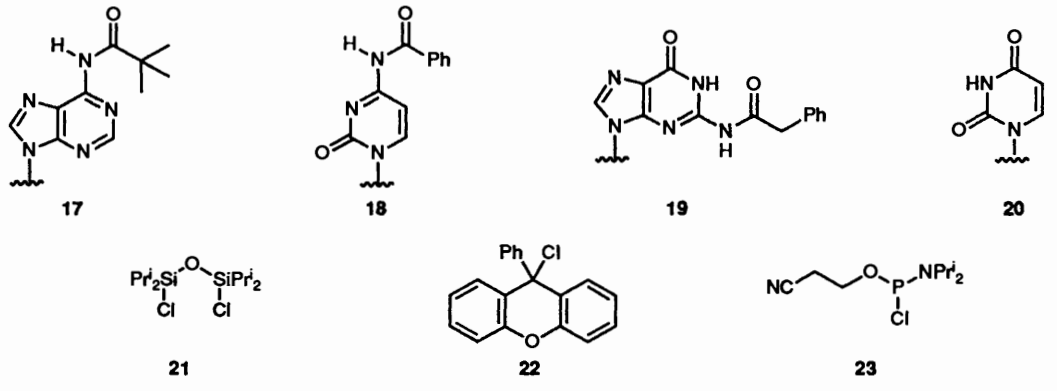
The only significant problem that arose in connection with the use of the Ctmp protecting group (as in **10a**) was that the enol ether reagent **11a** required for its introduction was relatively difficult to prepare. This led us to develop a new synthetic route[27] that has made a number of related nuclear halogenated enol ethers available for the first time. We then found[27] that 1-(2-fluorophenyl)-4-methoxy-1,2,3,6-tetrahydropyridine **11b** was easier to prepare than **11a**, and that the derived 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl (Fpmp, as in **10b**) group arguably had even better properties[28] than the Ctmp (as in **10a**) protecting group. It can be seen from Fig. 1b that, although the rate of hydrolysis of the Fpmp group at 25 °C is ~1.7-times faster at pH 0.5 than at pH 2.5 compared with 1.55-times faster for the Ctmp group (see above and Fig. 1), it is on average ~1.3-times more stable than the Ctmp group in the pH range 0.5–1.5. Therefore the removal of 5′-terminal DMTr (as in **1**) or Px (as in **2**) protecting groups would be expected to be even more selective when Fpmp rather than Ctmp groups are used to protect the 2′-hydroxy functions. We therefore now regard Fpmp as the protecting group of choice in the solid-phase synthesis of oligo- and poly-ribonucleotides. Some of our results have already been reported in a preliminary form,[28] and Sproat and his co-workers[29] have also used the Fpmp protecting group in solid-phase RNA synthesis.

We decided to base our solid-phase RNA synthesis on nucleoside phosphoramidite building blocks (see structure **16**). The procedure used for the preparation of the building blocks is indicated in outline in Scheme 1. Adenine, cytosine and guanine residues were protected[30] by 6-*N*-pivaloylation, 4-*N*-benzoylation and 2-*N*-phenylacetylation (as in formulae **17**, **18** and **19**, respectively), and uracil residues were left unprotected. The latter three acyl protecting groups were selected to ensure that the base residues would remain intact during the preparation of the building blocks and throughout the solid-phase synthesis but would nevertheless undergo quantitative deprotection during the subsequent ammonolytic unblocking step (see below). 6-*N*-Pivaloyladenosine **12** (B = **17**) and 2-*N*-(phenylacetyl)guanosine **12** (B = **19**) were prepared (see Experimental section) from the parent nucleosides in 66 and 71% isolated yield, respectively. The three *N*-acylribonucleosides **12** (B = **17**, **18** and **19**) and uridine **12** (B = **20**) were then converted by a three-step process (Scheme 1, steps i–iii and Experimental section) into the corresponding 2′-*O*-Fpmp derivatives **14** (B = **17**, **18**, **19** and **20**, respectively) [(**14**, B = **20**) ≡ **10b**]. The average overall yield for the three steps, which have not been optimized, was over 60%. On average ~1.5 mol equiv. of the enol ether **11b** was used in the acetalation step (Scheme 1, step ii). The latter 2′-*O*-Fpmp derivatives **14** (B = **17**, **18**, **19** and **20**) were treated with a slight excess of 9-chloro-9-phenylxanthene[13] **22** in pyridine solution (Scheme 1, step iv and Experimental section) to give the required nucleoside building blocks **15** (B = **17**, **18**, **19** and **20**, respectively) in an average isolated yield of 87%. These nucleoside building blocks were treated with 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite[31] **23** and diisopropylethylamine in tetrahydrofuran (THF) to give the corresponding 2-cyanoethyl *N*,*N*-diisopropylphosphoramidites[31,32] **16** (B = **17**, **18**, **19**, and **20**, respectively) in an average isolated yield of ~92%.

In recent years, we have generally tested the efficacy of the methods that we have developed for the synthesis of oligo- and poly-ribonucleotides by undertaking the synthesis of specific sequences of yeast alanine transfer RNA ($tRNA^{Ala}$, see Fig. 2a).[6,25,33] Thus we successfully carried out[33] the preparation of the 3′-terminal heptatriacontamer (37-mer) sequence of yeast $tRNA^{Ala}$ by the phosphotriester approach in solution using the Mthp group **4** for the protection of the 2′-hydroxy functions. We therefore set out to prepare 3′-terminal sequences of unmodified yeast $tRNA^{Ala}$ (see Fig. 2b and below) by solid-phase synthesis. Solid-phase synthesis[34] is carried out first by attaching the 3′-terminal nucleoside residue to a solid support. Long-chain alkylamine functionalized controlled-pore glass (CPG)[35] has been found to be a suitable solid support both for oligodeoxyribo- and oligoribo-nucleotide synthesis. The procedure used for attaching the 3′-terminal nucleoside residue to the solid

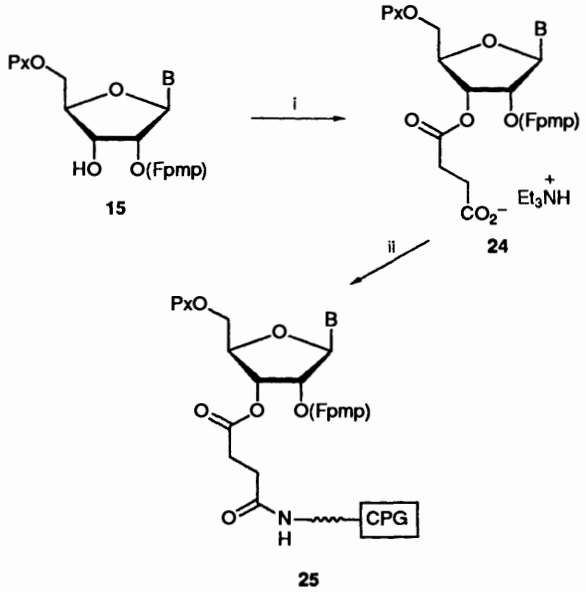


Scheme 1 *Reagents:* i, **21**, imidazole, MeCN; ii, **11b**, CF_3CO_2H, CH_2Cl_2; iii, Et_4NF, MeCN; iv, **22**, C_5H_5N; v, **23**, Pr^i_2NEt, THF



support[36] is indicated in outline in Scheme 2. The nucleoside building block **15** (B = **17**), an adenosine derivative as the synthesis of a 3′-terminal tRNA sequence was being undertaken, was treated with an excess both of succinic anhydride and of 4-(dimethylamino)pyridine (DMAP) to give its 3′-succinate, which was isolated as its triethylammonium salt **24** (B = **17**). The latter material was shaken together with long-chain alkylamine functionalized 500 Å CPG, N^1,N^3-dicyclohexylcarbodiimide (DCC), triethylamine and DMAP in anhydrous dimethylformamide (DMF) at room temperature to give loaded CPG **25** (B = **17**). The nucleoside loading of this material was estimated by 'trityl' assay[34] (see below) to be ~29 μmol g^{-1}.

The actual process of solid-phase RNA synthesis which was carried out automatically on a ~1 μmolar scale [*i.e.* with 30–35 mg of loaded CPG **25** (B = **17**)] in an Applied Biosystems 381A synthesizer is illustrated in Scheme 3 and Table 1. Each synthetic cycle[34] involved four chemical reactions (Table 1, steps nos. 1, 3–5): step no. 1, the 'detritylation' step, involved the acid-promoted removal of the 5′-terminal 9-phenylxanthen-9-yl (Px) protecting group from the CPG-supported nucleoside **25** or oligonucleotide (such as **27**); step no. 3, the coupling step, involved the phosphitylation of the released 5′-terminal hydroxy function with a 10- to 12-fold excess of the appropriate phosphoramidite **16** activated by a ~30-fold excess of 5-(3-nitrophenyl)-1*H*-tetrazole **26**;[37,38] step no. 4, the capping



Scheme 2 *Reagents:* i, succinic anhydride, 4-(dimethylamino)pyridine (DMAP), CH_2Cl_2; ii, N^1,N^3-dicyclohexylcarbodiimide (DCC), triethylamine, DMAP, functionalized controlled-pore glass (CPG), dimethylformamide (DMF)

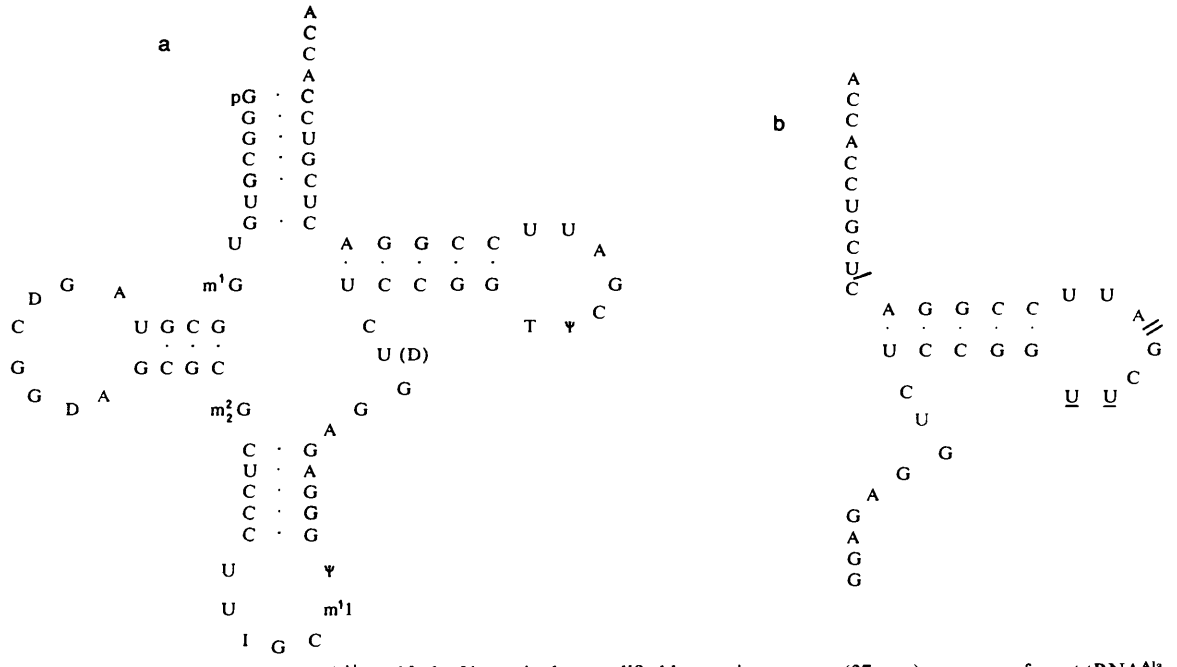
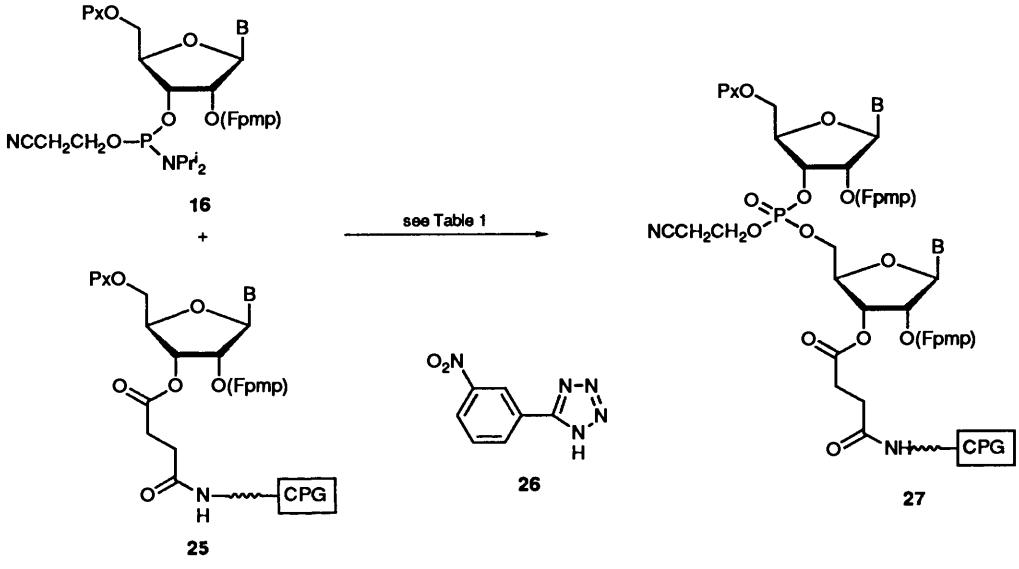


Fig. 2 Nucleotide sequences of **a** yeast $tRNA^{Ala}$ and **b** the 3′-terminal unmodified heptatriacontamer (37-mer) sequence of yeast $tRNA^{Ala}$



Scheme 3

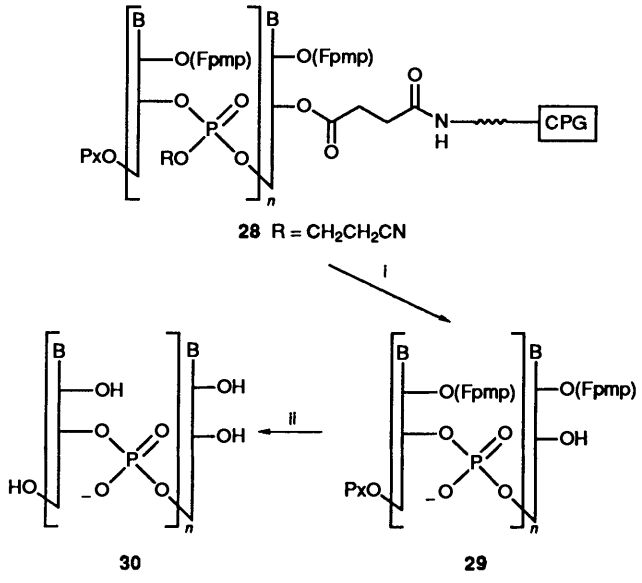
Table 1 Protocol for solid-phase RNA synthesis

Step no.	Process	Reagent and/or solvent[a]	Time (t/min)
1	Detritylation	Cl_3CCO_2H–CH_2Cl_2 (2:98 w/v)	0.92
2	Washing	MeCN	3.5
3	Coupling	0.1 mol dm^{-3} phosphoramidite **16** and 0.1 mol dm^{-3} **26** in MeCN	6.0
4	Capping	Ac_2O, AcOH, 2,6-lutidine, 1-methylimidazole, THF	1.75
5	Oxidation	Bu^tO_2H, $Me_2CHCH_2CMe_3$, CH_2Cl_2	1.42
6	Washing	MeCN	3.8

[a] See Experimental section for details relating to the purification of the solvents and the preparation of the reagents.

step, involved the acetylation of any remaining unphosphitylated 5′-terminal hydroxy functions; step no. 5, the oxidation step, involved the conversion of the phosphite triester into the phosphate triester internucleotide linkage. Oxidation is usually carried out by treatment with iodine–water[39] rather than with *tert*-butyl hydroperoxide.[40] However, we decided to take every possible precaution to exclude moisture from the system. The duration of the cycle time (20–25 min) was greater than that of the usual cycle time of solid-phase DNA synthesis. This was due largely to the greater time allowed for the coupling step (Table 1, step no. 3).

After all of the synthetic cycles had been completed, the



Scheme 4 *Reagents and conditions:* i, conc. aq. ammonia (*d* 0.88), 55 °C, 8–10 h; ii, 0.01 mol dm^{-3} hydrochloric acid, room temp., 20 h

loaded CPG **28** was treated with conc. aq. ammonia at 55 °C for 8–10 h (Scheme 4, step i). Under these conditions, the products were released from the solid support, the *N*-acyl protecting groups [as in moieties **17**, **18** and **19**] were removed from the base residues, the 2-cyanoethyl protecting groups were removed from the internucleotide linkages, and the crude 2′-protected RNA sequence **29** was obtained. The Fpmp protecting groups are completely stable under the ammonolysis conditions. As indicated above, this is advantageous as it allows the synthetic oligoribonucleotides to be purified by, if necessary, a combination of techniques without any risk of their being digested by contaminating traces of endonucleases. After the stabilized RNA sequence **29** had been purified, the 2′-*O*-(Fpmp) groups and the 5′-terminal Px group were removed (see below) by treatment with 0.01 mol dm^{-3} hydrochloric acid at room temperature (Scheme 4, step ii). The unprotected RNA sequence **30** was then, if necessary, further purified and isolated by precipitation.

Yeast $tRNA^{Ala}$ (Fig. 2a) is derived from a number of modified nucleosides [pseudouridine (ψ), 5-methyluridine (T), 5,6-dihydrouridine (D), 1-methylinosine (m^1I), inosine (I), 2-*N*,2-*N*-dimethylguanosine ($m^2{}_2G$) and 1-*N*-methylguanosine (m^1G)] in addition to the four principal ribonucleosides [adenosine (A), cytidine (C), guanosine (G) and uridine (U)]. Although it is clearly important to be able to incorporate these modified nucleosides into RNA sequences, we are concerned in this study solely with the development of a general automated solid-phase RNA synthesis. We have therefore undertaken the synthesis of unmodified sequences of yeast $tRNA^{Ala}$ in which, for example, pseudouridine (ψ) and 5-methyluridine (T) are both replaced by uridine (U) (see Fig. 2b; the replacement unmodified nucleosides are underlined). We now report the solid-phase synthesis of the 3′-terminal decamer (r[UCGUCCACCA]), nonadecamer (r[AUUCCGGACUCGUCCACCA]) and heptatriacontamer (37-mer, r[GGAGAGGUCUCCGGUUCGAUUCCGGACUCGUCCACCA] sequences of unmodified yeast $tRNA^{Ala}$, using the methodology described above. The last sequence contains only one nucleotide residue less than one-half of the whole tRNA nolecule. In Fig. 2b, the unmodified heptatriacontamer sequence is divided by two lines (//) into the 3′-terminal nonadecamer and an octadecamer, and the nonadecamer is further divided by one line (/) into the 3′-terminal decamer and a nonamer.

The 3′-terminal decamer, nonadecamer and heptatriacontamer sequences were prepared on ~0.94, 0.89 and 0.92 μmolar scales, respectively. The average coupling yields, as determined by 'trityl' (*i.e.*, 9-phenylxanthen-9-yl) cation assays,[34] were found to be 97, 98 and 98%, respectively. Following a system of abbreviations proposed earlier,[41] A′, C′, G′ and U′ represent 2′-*O*-(Fpmp) derivatives of adenosine, cytidine, guanosine and uridine, respectively. After ammonolysis of the loaded CPG (Scheme 4, step i), the crude partially protected 3′-terminal decamer **31**, nonadecamer **32** and heptatriacontamer **33** sequences were isolated in spectrophotometrically estimated yields of 49, 69 and 185 A_{260} units, respectively. The reverse-phase LC elution profiles of crude oligomers **31**, **32** and **33** are illustrated in Figs. 3a, 3c and 3e, respectively, and their 20% polyacrylamide gel electrophoretic (PAGE) properties are revealed in Fig. 4 (lanes 1 and 2, lanes 3 and 4, and lanes 5 and 6, respectively). It is at this stage that the real advantage of using the 2′-*O*-Fpmp protecting group and thereby obtaining stabilized RNA sequences becomes clearly apparent. The crude products can then be purified by a number of techniques without any risk of digestion by contaminating enzymes. In the present study, the above crude stabilized RNA sequences **31**, **32** and **33** were then chromatographed on Sephadex G-75 and isolated in yields of 34, 35 and 65 A_{260} units, respectively. Each sequence was then further purified by preparative LC on a reversed-phase column. The LC elution profiles of purified compounds **31**, **32** and **33** are illustrated in Figs. 3b, 3d and 3f, respectively. The PAGE properties of purified **31** are revealed in Fig. 6 (lane 2), those of purified **32** in Fig. 6 (lane 5) and Fig. 7 (lane 1), and those of purified **33** in Fig. 7 (lane 3).

While such stabilized RNA sequences can safely be stored for long periods of time, they may readily be unblocked to give free RNA. Hence, compounds **31**, **32** and **33** were kept in ~0.01 mol dm^{-3} hydrochloric acid (pH ~2) solution at room temperature for 20 h to give the corresponding unprotected RNA sequences r[UCGUCCACCA], r[AUUCCGGACUCGUCCACCA] and r[GGAGAGGUCUCCGGUUCGAUUCCGGACUCGUCCACCA], respectively. The latter free RNA molecules were isolated by means of the butan-1-ol precipitation procedure.[42] The heptatriacontamer sequence, which was ~80–85% pure, was further purified by preparative LC. The reversed-phase LC elution profiles of the purified free decamer, nonadecamer and heptatriacontamer sequences are illustrated in Figs. 5a, 5b and 5c, respectively; the PAGE properties of the decamer are revealed in Fig. 6 (lane 3), those of the nonadecamer in Fig. 6 (lane 6) and Fig. 7 (lane 2), and those of the heptatriacontamer in Fig. 7 (lane 4).

It would appear from Figs. 5, 6 and 7 that high quality, relatively high molecular mass (*i.e.*, up to ~12000 daltons) RNA can be obtained by automated solid-phase synthesis using ribonucleoside phosphoramidite building blocks **16** in which the 2′-hydroxy functions are protected with Fpmp groups. The 3′-terminal decamer and nonadecamer sequences of yeast $tRNA^{Ala}$ were found to have LC retention times and PAGE mobilities identical with those of authentic decamer and nonadecamer sequences prepared[33] by the phosphotriester approach in solution using a block condensation strategy. The

Px-U′pC′pG′pU′pC′pC′pA′pC′pC′pA′

31

Px-A′pU′pU′pC′pC′pG′pG′pA′pC′pU′pC′pG′pU′pC′pC′pA′pC′pC′pA′

32

Px-G′pG′pA′pG′pA′pG′pG′pU′pC′pU′pC′pC′pG′pG′pU′pU′pC′pG′pA′pU′pU′pC′pC′pG′pG′pA′pC′pU′pC′pG′pU′pC′pC′pA′pC′pC′pA′

33

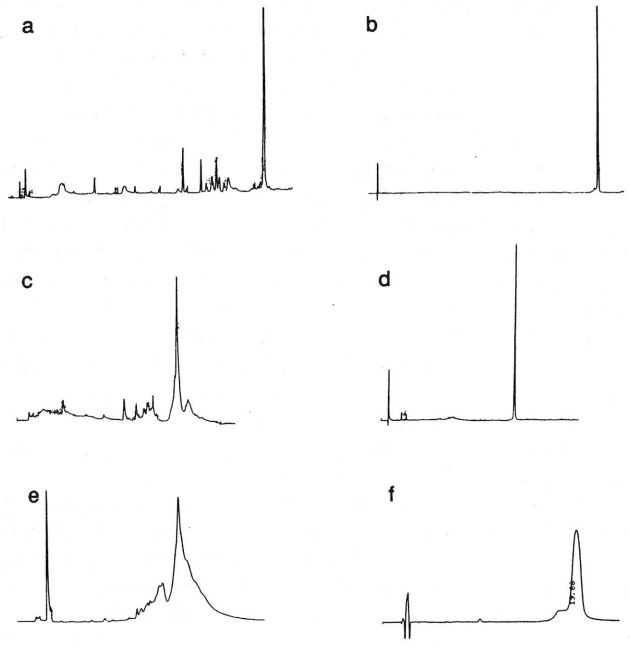


Fig. 3 LC profiles (on a Jones APEX ODS 5μ column, eluted with 0.1 mol dm^{-3} triethylammonium acetate–acetonitrile) of crude and purified Px-U′pC′pG′pU′pC′pC′pA′pC′pC′pA′ **31** [**a** and **b**, respectively], crude and purified Px-A′pU′pU′pC′pC′pG′pG′pA′pC′pU′pC′pG′pU′-pC′pC′pA′pC′pC′pA′ **32** [**c** and **d**, respectively], and crude and purified Px-G′pG′pA′pG′pA′pG′pG′pU′pC′pU′pC′pC′pG′pG′pU′pU′pC′-pG′pA′pU′pU′pC′pC′pG′pG′pA′pC′pU′pC′pG′pU′pC′pC′pA′pC′-pC′pA′ **33** [**e** and **f**, respectively]

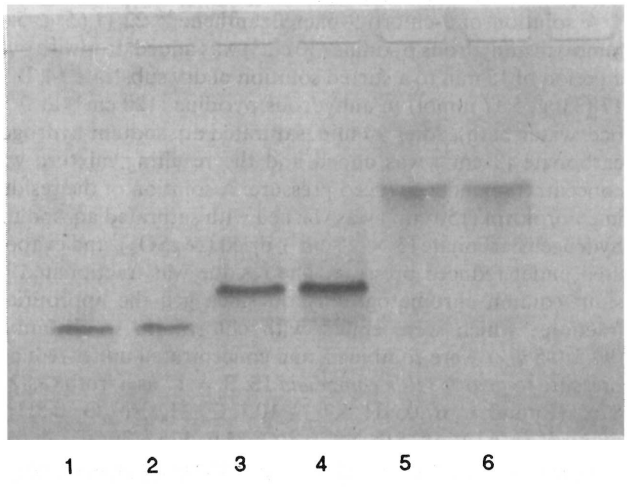


Fig. 4 Photograph of UV-illuminated 20% polyacrylamide gel electrophoretogram: lanes 1 and 2, crude Px-U′pC′pG′pU′pC′pC′pA′ pC′pC′pA′ **31**; lanes 3 and 4, crude Px-A′pU′pU′pC′pC′pG′pG′pA′-pC′pU′pC′pG′pU′pC′pC′pA′pC′pC′pA′ **32**; lanes 5 and 6, crude Px-G′pG′pA′pG′pA′pG′pG′pU′pC′pU′pC′pC′pG′pG′pU′pU′pC′pG′-pA′pU′pU′pC′pC′pG′pG′pA′pC′pU′pC′pG′pU′pC′pC′pA′pC′pC′-pA′ **33**

latter two sequences were further characterized by enzymatic digestion; thus in the presence of snake venom phosphodiesterase and bacterial alkaline phosphatase, they were converted into a mixture of their constituent ribonucleosides in the expected proportions. The 3′-terminal heptatriacontamer sequence was similarly converted by enzymatic digestion into a mixture of its constituent ribonucleosides which were obtained, within the limits of experimental error, in the expected proportions.

In conclusion, we believe that we have clearly demonstrated that relatively high molecular mass oligo- and indeed polyribonucleotides of high purity are readily available by our approach to solid-phase RNA synthesis. However, we are still vigorously pursuing our studies in this area with the intention of

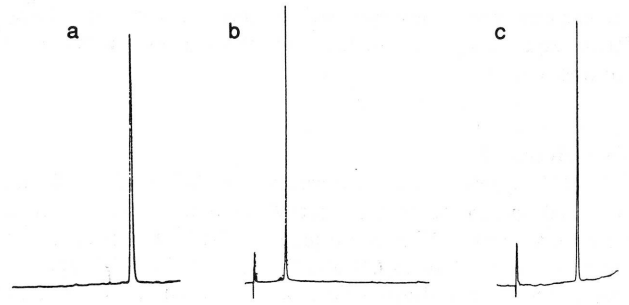


Fig. 5 LC profiles (on a Jones APEX ODS 5μ column, eluted with 0.1 mol dm^{-3} triethylammonium acetate–acetonitrile) of **a** purified r[UCGUCCACCA], **b** purified r[AUUCCGGACUCGUCCACCA], and **c** purified r[GGAGAGGUCUCCGGUUCGAUUCCGGACUC-GUCCACCA]

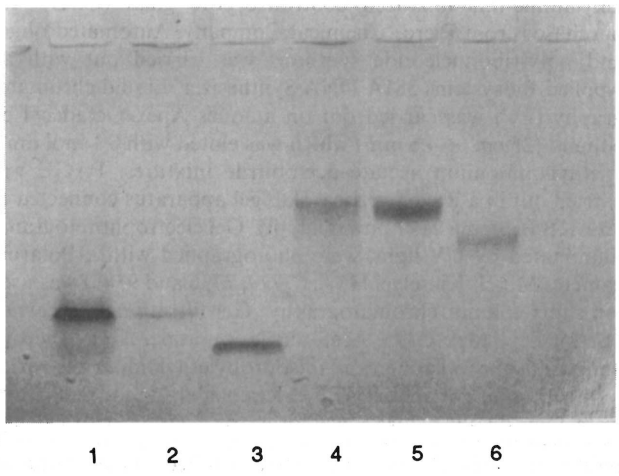


Fig. 6 Photograph of UV-illuminated 20% polyacrylamide gel electrophoretogram: lanes 1 and 2, respectively, crude and purified Px-U′pC′pG′pU′pC′pC′pA′pC′pC′pA′ **31**; lane 3, purified r[UCGUCCACCA]; lanes 4 and 5, respectively, crude and purified Px-A′pU′pU′pC′pC′pG′pG′pA′pC′pU′pC′pG′pU′pC′pC′pA′pC′pC′pA′ **32**; lane 6, purified r[AUUCCGGACUCGUCCACCA]

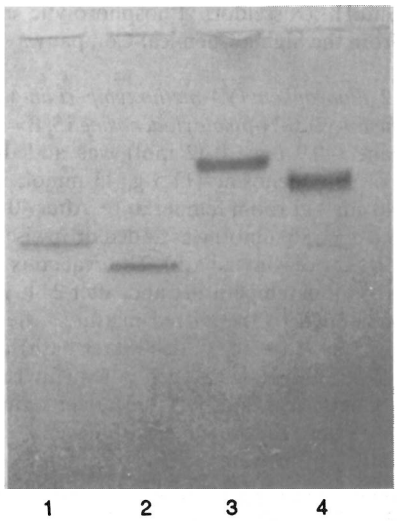


Fig. 7 Photograph of UV-illuminated 20% polyacrylamide gel electrophoretogram: lane 1, purified Px-A′pU′pU′pC′pC′pG′pG′pA′pC′pU′-pC′pG′pU′pC′pC′pA′pC′pC′pA′ **32**; lane 2, purified r[AUUCCG-GACUCGUCCACCA]; lane 3, purified Px-G′pG′pA′pG′pA′pG′pG′-pU′pC′pU′pC′pC′pG′pG′pU′pU′pC′pG′pA′pU′pU′pC′pC′pG′pG′-pA′pC′pU′pC′pG′pU′pC′pC′pA′pC′pC′pA′ **33**; lane 4, purified r[GGA-GAGGUCUCCGGUUCGAUUCCGGACUCGUCCACCA]

further developing this approach so that the synthesis of pure RNA sequences of even higher molecular mass will become a routine matter.

Experimental

^{1}H NMR spectra were measured at 360 MHz with a Bruker AM 360 spectrometer; tetramethylsilane was used as an internal standard. *J*-Values are given in Hz. ^{31}P NMR spectra were measured at 101.3 MHz with a Bruker WM 250 spectrometer; 85% orthophosphoric acid was used as an external standard. UV absorption spectra were measured with a Perkin-Elmer Lambda-3 recording spectrophotometer. Merck silica gel 60 F_{254} pre-coated plates (Art 5715 and 5642), which were developed in solvent systems A [$CHCl_3$–MeOH (9:1 v/v)] and B [$CHCl_3$–MeOH (19:1 v/v)], were used for TLC. Long-chain alkylamine-functionalized controlled-pore glass (CPG) was purchased from Pierce Chemical Company. Automated oligo- and poly-ribonucleotide synthesis was carried out with an Applied Biosystems 381A DNA Synthesizer. Liquid chromatography (LC) was carried out on a Jones Apex Octadecyl 5μ column (25 cm × 4.6 mm) which was eluted with 0.1 mol dm^{-3} triethylammonium acetate–acetonitrile mixtures. PAGE was carried out in a Raven vertical slab gel apparatus connected to an LKB Bromma 2197 power supply. Gel electrophoretograms, illuminated by UV light, were photographed with a Polaroid camera. Merck Kieselgel H (Art 7729, 7736 and 9385) was used for short-column chromatography. Gel filtration was carried out on Sephadex G-75. Acetonitrile, pyridine, THF, triethylamine, diisopropylamine and diisopropylethylamine were dried by heating, under reflux, with calcium hydride for 3–5 h and were then distilled; DMF was dried by heating with calcium hydride at 100 °C, and was then distilled under reduced pressure; dichloromethane was dried by heating, under reflux, over phosphorus pentaoxide and was then distilled. All solvents were stored over no. 4 Å molecular sieves in sealed containers. Additional precautions taken in the preparation of solvents for solid-phase oligo- and poly-ribonucleotide synthesis are indicated below. Ribonucleosides were purchased from Pharma-Waldhof GmbH, Düsseldorf. Phosphorolytic enzymes were purchased from the Sigma Chemical Company.

2′-O-[1-(2-*Fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-5′-O-(9-*phenylxanthen*-9-*yl*)-6-N-*pivaloyladenosine* **15**; B = **17**.—Chlorotrimethylsilane (40.9 cm^3, 0.32 mol) was added to a stirred suspension of dry adenosine (11.5 g, 43 mmol) in anhydrous pyridine (240 cm^3) at room temperature. After 40 min, pivaloyl chloride (10.6 cm^3, 86 mmol) was added dropwise to the stirred products at 0 °C (ice–water-bath). The reactants were allowed to warm up to room temperature and, after 24 h, ice-cold water (40 cm^3) was added to the stirred mixture. After 10 min, the products were cooled to 0 °C (ice–water-bath) and conc. aq. ammonia (*d* 0.88; 80 cm^3) was added. After a further period of 15 min, the products were concentrated under reduced pressure, and the residue was evaporated with toluene (4 × 30 cm^3) and then crystallized from water (250 cm^3) to give 6-*N*-pivaloyladenosine **12**; B = **17** (10.0 g, 66%) as crystals, m.p. 160–162 °C; R_f 0.20 (system A).

1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane[43] **21** (8.9 g, 28.2 mmol) was added to a stirred suspension of dry 6-*N*-pivaloyladenosine (9.0 g, 25.6 mmol) and imidazole (8.7 g, 0.128 mol) in anhydrous acetonitrile (200 cm^3) at room temperature. After 40 min, water (20 cm^3) was added and the resulting solution was stirred for a further period of 10 min and then concentrated under reduced pressure. The residue was dissolved in dichloromethane (150 cm^3), and the solution was washed first with 0.1 mol dm^{-3} hydrochloric acid (2 × 50 cm^3) and then with water (3 × 100 cm^3). The dried ($MgSO_4$) solution was concentrated under reduced pressure and the residue was fractionated by short-column chromatography on silica gel. The appropriate fractions, eluted with chloroform, were evaporated under reduced pressure (water-pump followed by oil-pump) to give compound **13**; B = **17** as a solid (10.6 g); R_f 0.42 (system B).

Trifluoroacetic acid (TFA) (0.57 cm^3, 7.4 mmol) was added to a stirred solution of the latter material (3.96 g) and 1-(2-fluorophenyl)-4-methoxy-1,2,3,6-tetrahydropyridine[27] **11b** (4.15 g, 20.0 mmol) in dry dichloromethane (45 cm^3) at room temperature. After 20 h, triethylamine (2.0 cm^3, 14 mmol) was added and the products were concentrated under reduced pressure. The residue was dissolved in 1 mol dm^{-3} tetraethylammonium fluoride in acetonitrile (14.6 cm^3, 14.6 mmol) at room temperature. After 20 min, the products were evaporated under reduced pressure and the residue was partitioned between chloroform (100 cm^3) and saturated aq. sodium hydrogen carbonate (3 × 50 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 chloroform–ethanol (98:2 to 93:7 v/v), were combined and concentrated under reduced pressure to give 2′-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-6-N-*pivaloyladenosine* **14**; B = **17** as a froth (2.87 g, 54% based on 6-*N*-pivaloyladenosine **12**; B = **17**) (Found: C, 56.7; H, 6.2; N, 14.4. $C_{27}H_{35}FN_6O_6 \cdot 0.7H_2O$ requires C, 56.8; H, 6.4; N, 14.7%), m.p. 113–115 °C; R_f 0.34 (system B); $\delta_H(CDCl_3–D_2O)$ 1.41 (9 H, s), 1.62 (1 H, m), 1.78 (1 H, m), 1.88 (1 H, m), 2.01 (1 H, m), 2.65 (3 H, s), 2.69 (1 H, m), 2.86 (1 H, m), 2.95 (1 H, m), 3.14 (1 H, m), 3.77 (1 H, dd, *J* 1.5 and 13.1), 3.97 (1 H, dd, *J* 1.5 and 13.1), 4.39 (1 H, s), 4.44 (1 H, d, *J* 4.6), 5.36 (1 H, dd, *J* 4.6 and 7.9), 5.96 (1 H, d, *J* 7.9), 6.8–7.05 (4 H, m), 8.08 (1 H, s) and 8.79 (1 H, s).

A solution of 9-chloro-9-phenylxanthene[44] **22** (1.65 g, 5.64 mmol) in anhydrous pyridine (30 cm^3) was added dropwise over a period of 15 min to a stirred solution of dry substrate **14**; B = **17** (3.0 g, 5.37 mmol) in anhydrous pyridine (120 cm^3) at 0 °C (ice–water-bath). After 90 min, saturated aq. sodium hydrogen carbonate (3 cm^3) was added and the resulting mixture was concentrated under reduced pressure. A solution of the residue in chloroform (150 cm^3) was washed with saturated aq. sodium hydrogen carbonate (3 × 75 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was fractionated by short-column chromatography on silica gel; the appropriate fractions, which were eluted with chloroform–triethylamine (99.5:0.5 v/v), were combined, and concentrated under reduced pressure to give the *title compound* **15**; B = **17** as a froth (3.82 g, 87%) (Found: C, 67.05; H, 5.7; N, 10.1. $C_{46}H_{47}FN_6O_7 \cdot 0.5H_2O$ requires C, 67.1; H, 5.9; N, 10.2%), m.p. 118–120 °C; R_f 0.45 (system B); $\delta_H(CDCl_3)$ 1.41 (9 H, s), 1.8–2.05 (4 H, m), 2.80 (5 H, m), 2.95 (2 H, m), 3.15 (1 H, m), 3.39 (1 H, dd, *J* 4.2 and 10.3), 4.30 (1 H, m), 5.33 (1 H, dd, *J* 4.7 and 6.9), 6.17 (1 H, d, *J* 6.9), 6.8–7.45 (17 H, m), 8.22 (1 H, s), 8.51 (1 H, br s) and 8.68 (1 H, s).

4-N-*Benzoyl*-2′-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-5′-O-(9-*phenylxanthen*-9-*yl*)*cytidine* **15**; B = **18**.—1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane[43] **21** (22.4 cm^3, 70 mmol) was added to a stirred mixture of dry 4-*N*-benzoylcytidine **12**; B = **18** (22.8 g, 65.6 mmol), imidazole (22.1 g, 0.325 mol) and anhydrous DMF (500 cm^3) at room temperature. After 30 min, water (10 cm^3) was added and, after a further period of 10 min, the products were concentrated under reduced pressure (oil-pump). A solution of the residue in chloroform (500 cm^3) was washed first with 0.1 mol dm^{-3} hydrochloric acid (3 × 400 cm^3) and then with water (2 × 400 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, eluted with chloroform, were concentrated under reduced pressure (water-pump fol-

lowed by oil-pump) to give compound **13**; B = **18** as a froth (32.0 g); R_f 0.38 (system B).

TFA (0.98 cm^3, 12.7 mmol) was added to a stirred solution of the latter material (6.35 g) and 1-(2-fluorophenyl)-4-methoxy-1,2,3,6-tetrahydropyridine[27] **11b** (3.0 g, 14.5 mmol) in dry dichloromethane (42 cm^3) at room temperature. After 24 h, an additional quantity of compound **11b** (1.0 g, 4.8 mmol) was added and, after a further period of 10 h, the reaction was quenched with triethylamine (3.0 cm^3, 21.5 mmol) and the products were then concentrated under reduced pressure. The residue was dissolved in 1 mol dm^{-3} tetraethylammonium fluoride in acetonitrile (30 cm^3, 30 mmol). After 20 min, the products were evaporated under reduced pressure and the residue was dissolved in chloroform (150 cm^3). The solution obtained was shaken vigorously with saturated aq. sodium hydrogen carbonate (2 × 100 cm^3). The resulting precipitate was collected by filtration and washed first with water (10 cm^3) and then with diethyl ether (10 cm^3) to give 4-N-*benzoyl*-2′-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]*cytidine* **14**; B = **18** as a solid (5.52 g, 76% based on 4-*N*-benzoylcytidine **12**; B = **18**) (Found: C, 57.3; H, 5.8; N, 9.4. $C_{28}H_{31}FN_4O_7 \cdot 1.8H_2O$ requires C, 57.3; H, 5.8; N, 9.4%), m.p. 180–182 °C; R_f 0.25 (system B); $\delta_H[(CD_3)_2SO]$ 1.80–2.05 (4 H, m), 2.74 (1 H, m), 2.86 (1 H, m), 2.95 (3 H, s), 3.02 (1 H, m), 3.12 (1 H, m), 3.62 (2 H, m), 3.96 (1 H, m), 4.06 (1 H, m), 4.43 (1 H, dd, *J* 4.9 and 6.8), 5.21 (1 H, d, *J* 4.8), 5.25 (1 H, t, *J* 5.1), 6.15 (1 H, d, *J* 7.0), 6.9–7.15 (4 H, m), 7.37 (1 H, m), 7.50 (2 H, m), 7.62 (1 H, m), 8.0 (2 H, m), 8.44 (1 H, m) and 11.30 (1 H, br).

The above compound **14**; B = **18** (4.56 g, 8.22 mmol) was dried by evaporation from pyridine (3 × 16 cm^3) solution, dissolved in pyridine (100 cm^3), and allowed to react with 9-chloro-9-phenylxanthene[44] **22** (2.66 g, 9.1 mmol) in anhydrous pyridine (30 cm^3) according to the procedure described above in the preparation of compound **15**; B = **17**. The products were worked up, and fractionated by short-column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–light petroleum (boiling range 60–80 °C)–triethylamine (80:20:0.5 to 90:10:0.5 v/v) were concentrated to give the *title compound* as a froth (6.1 g, 91%) (Found: C, 69.15; H, 5.3; N, 6.6. $C_{47}H_{43}FN_4O_8 \cdot 0.3H_2O$ requires C, 69.2; H, 5.4; N, 6.9%), m.p. 132–134 °C (after crystallization from aq. ethanol); R_f 0.45 (system B); $\delta_H[(CD_3)_2SO]$ 1.85–2.05 (4 H, m), 2.82 (1 H, m), 2.92 (1 H, m), 3.0–3.3 (4 H, m), 3.09 (3 H, s), 4.08 (2 H, m), 4.53 (1 H, m), 5.31 (1 H, d, *J* 5.3), 6.15 (1 H, d, *J* 6.0), 6.9–7.5 (18 H, m), 7.52 (2 H, m), 7.63 (1 H, m), 8.02 (2 H, m), 8.25 (1 H, m) and 11.34 (1 H, br s).

2′-O-[1-(2-*Fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-2-N-(*phenylacetyl*)-5′-O-(9-*phenylxanthen*-9-*yl*)*guanosine* **15**; B = **19** (carried out by Dr. J. H. Marriott).—Phenylacetyl chloride (5.9 cm^3, 44.6 mmol) was added to a stirred suspension of dry 1-hydroxybenzotriazole (6.40 g, 47 mmol) in anhydrous acetonitrile (14 cm^3) at room temperature. After 1 min, anhydrous pyridine (7.0 cm^3) was added, with external cooling, to give the phenylacetylating agent (see below). Chlorotrimethylsilane (30.5 cm^3, 0.24 mol) was added dropwise over a period of 30 min to a rapidly stirred suspension of dry guanosine (8.498 g, 30.0 mmol) in anhydrous pyridine (165 cm^3). After the mixture had been stirred for a further 90 min at room temperature, the phenylacetylating agent (see above) was added dropwise over a period of 2 min to the stirred products. After 18 h, an additional quantity of phenylacetylating agent [prepared from phenylacetyl chloride (1.2 cm^3, 9.1 mmol), 1-hydroxybenzotriazole (1.30 g, 9.5 mmol), pyridine (1.4 cm^3) and acetonitrile (2.85 cm^3)] was added. After a further period of 24 h, the products were filtered and the residue was washed with pyridine (50 cm^3). The combined filtrate and washings were cooled to 5 °C, and treated with water (20 cm^3). After 1 h, the products were evaporated under reduced pressure, dissolved in water (300 cm^3) and the solution was extracted with ethyl acetate (200 cm^3). The organic layer was back-extracted with water (50 cm^3), and the combined aqueous layers were washed with ethyl acetate (100 cm^3) and then stored overnight at ~4 °C. Crystals of 2-N-(*phenylacetyl*)*guanosine* **12**; B = **19** (8.60 g, 71%) (Found: C, 53.0; H, 4.7; N, 17.2. $C_{18}H_{19}N_5O_6 \cdot 0.3H_2O$ requires C, 53.15; H, 4.9; N, 17.2%), m.p. 206–209 °C, were collected by filtration; R_f 0.15 (system A); $\delta_H[(CD_3)_2SO]$ 3.5–3.7 (2 H, m), 3.81 (2 H, s), 3.91 (1 H, m), 4.13 (1 H, m), 4.43 (1 H, m), 5.06 (1 H, m), 5.20 (1 H, m), 5.51 (1 H, m), 5.81 (1 H, d, *J* 5.7), 7.25–7.45 (5 H, m), 8.28 (1 H, s) and 11.97 (2 H, br).

1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane[43] **21** (4.0 cm^3, 12.5 mmol) was added to a stirred solution of dry 2-*N*-(phenylacetyl)guanosine (4.625 g, 11.5 mmol) and imidazole (3.13 g, 46 mmol) in anhydrous DMF (125 cm^3) at room temperature. After 40 min, water (5 cm^3) was added and the products were concentrated under reduced pressure (water-pump, followed by oil-pump). A solution of the residue in chloroform (100 cm^3) was washed successively with 0.1 mol dm^{-3} hydrochloric acid (2 × 50 cm^3) and water (5 × 75 cm^3), and was then dried ($MgSO_4$), and evaporated under reduced pressure. The residue was fractionated by short-column chromatography on silica gel: the appropriate fractions, which were eluted with dichloromethane–ethanol (97:3 v/v), were evaporated under reduced pressure to give compound **13**; B = **19** as a pale yellow froth (6.1 g); R_f 0.55 (system A).

TFA (0.575 cm^3, 7.5 mmol) was added to a stirred solution of the latter material (3.4 g) and 1-(2-fluorophenyl)-4-methoxy-1,2,3,6-tetrahydropyridine[27] **11b** (2.0 g, 9.65 mmol) in anhydrous dichloromethane (25 cm^3) at room temperature. After 24 h, triethylamine (2.0 cm^3, 14.3 mmol) was added, the products were concentrated under reduced pressure, and the residue was dissolved in a 1 mol dm^{-3} solution of tetraethylammonium fluoride in acetonitrile (15.8 cm^3, 15.8 mmol). After 30 min, the products were evaporated under reduced pressure and the residue was dissolved in chloroform (50 cm^3). The solution obtained was washed with saturated aq. sodium hydrogen carbonate (2 × 50 cm^3), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was fractionated by short-column chromatography on silica gel: the appropriate fractions were eluted with chloroform–ethanol (95:5 v/v) and concentrated under reduced pressure to give 2′-*O*-[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]-2-*N*-(phenylacetyl)guanosine **14**; B = **19** as a pale yellow froth [2.84 g, 72% based on 2-*N*-(phenylacetyl)guanosine] (Found: C, 56.55; H, 5.1; N, 13.2. $C_{30}H_{33}FN_6O_7 \cdot 1.5H_2O$ requires C, 56.7; H, 5.7; N, 13.2%), m.p. 131–134 °C; R_f 0.45 (system A); $\delta_H[(CD_3)_2SO]$ 1.72 (2 H, m), 1.87 (1 H, m), 1.96 (1 H, m), 2.62 (1 H, m), 2.65 (3 H, s), 2.7–2.9 (2 H, m), 3.09 (1 H, m), 3.64 (2 H, m), 3.80 (2 H, s), 4.01 (1 H, m), 4.16 (1 H, m), 4.76 (1 H, dd, *J* 4.5 and 7.8), 5.23 (2 H, m), 6.01 (1 H, d, *J* 7.8), 6.90 (2 H, m), 6.95–7.1 (2 H, m), 7.25–7.4 (5 H, m), 8.35 (1 H, s) and 11.9–12.05 (2 H, br).

The above compound **14**; B = **19** (2.84 g, 4.67 mmol) was dried by evaporation with pyridine (3 × 30 cm^3), dissolved in anhydrous pyridine (180 cm^3), and allowed to react with 9-chloro-9-phenylxanthene[44] **22** (1.43 g, 4.9 mmol) in anhydrous pyridine (20 cm^3) according to the procedure described above in the preparation of compound **15**; B = **17**. The products were worked up, and fractionated by short-column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–triethylamine (99.5:0.5 v/v), were evaporated under reduced pressure to give the *title compound* as a solid (3.48 g, 86%) (Found: C, 67.8; H, 5.2; N, 9.4. $C_{49}H_{45}FN_6O_8$ requires C, 68.0; H, 5.2; N, 9.7%), m.p. 146–149 °C; R_f 0.40 (system B); $\delta_H(CDCl_3)$ 1.75–2.2 (4 H, m), 2.73 (1 H, m), 2.85 (3 H, s), 2.90 (2 H, m), 3.05–3.25 (4 H, m), 3.75 (2 H, m), 4.19 (1 H, m), 4.22 (1 H, m), 5.05 (1 H, dd, *J* 4.8 and 6.9), 5.91 (1 H, d, *J* 6.9), 6.81 (1 H, m),

6.85–7.1 (5 H, m), 7.1–7.35 (14 H, m), 7.41 (2 H, m), 8.01 (1 H, s), 9.34 (1 H, br) and 12.07 (1 H, br).

2′-O-[1-(2-Fluorophenyl)-4-methoxypiperidin-4-yl]-5′-O-(9-phenylxanthen-9-yl)uridine **15**; B = **20**.—1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane[43] **21** (23.1 cm^3, 72 mmol) was added to a stirred suspension of dry uridine **12**; B = **20** (15.75 g, 64.5 mmol) and imidazole (22.35 g, 0.33 mol) in anhydrous acetonitrile (300 cm^3) at room temperature. After 30 min, water (18 cm^3) was added and, after a further period of 10 min, the products were concentrated under reduced pressure. A solution of the residue in chloroform (300 cm^3) was washed successively with 0.1 mol dm^{-3} hydrochloric acid (2 × 90 cm^3) and water (2 × 300 cm^3). The dried ($MgSO_4$) organic layer was evaporated under reduced pressure. The residue obtained was fractionated by short-column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol (98:2 v/v), were evaporated under reduced pressure to give compound **13**; B = **20** as a glassy solid (17.73 g); R_f 0.50 (system B).

TFA (3.13 cm^3, 40.6 mmol) was added to a stirred solution of the latter material (16.7 g) and 1-(2-fluorophenyl)-4-methoxy-1,2,3,6-tetrahydropyridine[27] **11b** (9.5 g, 45.8 mmol) in anhydrous dichloromethane (135 cm^3) at room temperature. After 24 h, an additional quantity of compound **11b** (2.9 g, 14.0 mmol) was added and, after a further period of 8 h, triethylamine (4.0 cm^3, 28.7 mmol) was added and the products were evaporated under reduced pressure. The dark red oily residue was dissolved in 1 mol dm^{-3} tetraethylammonium fluoride in acetonitrile (102 cm^3, 0.10 mol). After 1 h, the residue was dissolved in chloroform (250 cm^3) and the solution was shaken with saturated aq. sodium hydrogen carbonate (2 × 100 cm^3). The resulting suspension was filtered and the residue was washed successively with water (50 cm^3) followed by diethyl ether (50 cm^3) to give *2′-O-[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]uridine* **14**; B = **20** (≡**10b**) as a solid (12.5 g, 45.5% based on uridine) (Found: C, 55.0; H, 5.6; N, 9.0. $C_{21}H_{26}FN_3O_7 \cdot 0.4H_2O$ requires C, 55.0; H, 5.9; N, 9.2%), m.p. 207–208 °C; R_f 0.42 (system B); $\delta_H[(CD_3)_2SO]$ 1.75–2.05 (4 H, m), 2.75 (1 H, m), 2.86 (1 H, m), 2.99 (3 H, s), 3.06 (1 H, m), 3.14 (1 H, m), 3.57 (2 H, m), 3.91 (1 H, m), 4.01 (1 H, m), 4.36 (1 H, dd, *J* 4.8 and 7.7), 5.74 (1 H, d, *J* 8.1), 6.03 (1 H, d, *J* 7.7), 6.9–7.15 (4 H, m) and 7.94 (1 H, d, *J* 8.1).

The above compound **14**; B = **20** (10.14 g, 22.5 mmol) was dried by evaporation from pyridine (3 × 30 cm^3) and was then dissolved in pyridine (550 cm^3). A solution of 9-chloro-9-phenylxanthene[44] **22** (7.54 g, 25.75 mmol) was added dropwise during 20 min to the cooled (ice–water-bath), stirred solution. After 2 h, saturated aq. sodium hydrogen carbonate (10 cm^3) was added, and the products were then evaporated under reduced pressure. A solution of the residual oil in dichloromethane (250 cm^3) was washed successively with saturated aq. sodium hydrogen carbonate (2 × 200 cm^3) and water (200 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was fractionated by short-column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–light petroleum (60–80 °C)–triethylamine (40:60:0.5 to 60:40:0.5 v/v) were concentrated to give the *title compound* as a froth (13.86 g, 87%) (Found: C, 67.45; H, 5.6; N, 5.6. $C_{40}H_{38}FN_3O_8 \cdot 0.25H_2O$ requires: C, 67.45; H, 5.45; N, 5.9%), m.p. 118–120 °C; R_f 0.55 (system B); $\delta_H(CDCl_3)$ 1.9–2.15 (4 H, m), 2.9–3.35 (7 H, m), 3.23 (3 H, s), 4.14 (2 H, m), 4.70 (1 H, dd, *J* 4.8 and 6.3), 5.57 (1 H, d, *J* 8.2), 6.20 (1 H, d, *J* 6.5), 6.9–7.45 (17 H, m), 7.93 (1 H, d, *J* 8.2) and 8.94 (1 H, br s).

2-Cyanoethyl N,N-Diisopropylphosphoramidochloridite[31] **23**.—3-Hydroxypropionitrile (84.4 cm^3, 1.24 mol) and 1,1,1,3,3,3-hexamethyldisilazane (131 cm^3, 0.62 mol) were heated together under reflux (bath temperature 135 °C) for 2 h to give crude 3-(trimethylsiloxy)propionitrile (167 g). A solution of this material (87.5 g) in anhydrous acetonitrile (750 cm^3) was added dropwise to a stirred solution of phosphorus trichloride (58.8 cm^3, 0.67 mol) in anhydrous acetonitrile (750 cm^3) at −50 °C (acetone–solid CO_2-bath). The reaction mixture was allowed to warm up to room temperature and, after a further period of 2 h, the products were concentrated under reduced pressure and were then distilled to give 2-cyanoethyl phosphorodichloridite (92.5 g, 82% based on 3-hydroxypropionitrile), b.p. 68–70 °C/0.03–0.04 mmHg, as a liquid, $\delta_P(CDCl_3)$ 179.8.

A solution of dry diisopropylamine (138.5 cm^3, 0.99 mol) in anhydrous diethyl ether (300 cm^3) was added dropwise over a period of 2 h to a stirred, cooled (−20 °C) solution of 2-cyanoethyl phosphorodichloridite (85.1 g, 0.49 mol) in anhydrous diethyl ether (1.3 dm^3) under nitrogen. The stirred reaction mixture was allowed to warm up slowly to room temperature. After 20 h, the products were filtered, and the filtrate was concentrated under reduced pressure. Distillation of the residual liquid gave the title compound (104 g, 88%), b.p. 104–106 °C/0.02–0.03 mmHg; $\delta_P(CDCl_3)$ 180.7.

Preparation of 2′-O-[1-(2-Fluorophenyl)-4-methoxypiperidin-4-yl]-5′-O-(9-phenylxanthen-9-yl)ribonucleoside 3′-[(2-Cyanoethyl) N,N-Diisopropylphosphoramidites] **16**.—*N,N*-Diisopropylethylamine (0.70 cm^3, 4.0 mmol) and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite **23** (0.72 g, 3.0 mmol) were added to a stirred solution of dry *2′-O-*[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]-*5′-O-*(9-phenylxanthen-9-yl)ribonucleoside derivative **15** (2.0 mmol) in anhydrous THF (10 cm^3) under nitrogen at room temperature. After 18 h, the products were concentrated under reduced pressure and partitioned between ethyl acetate (50 cm^3) and ice-cold saturated aq. sodium hydrogen carbonate (4 × 15 cm^3). The dried ($MgSO_4$) organic layer was evaporated under reduced pressure. The residue was purified by short-column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–light petroleum (60–80 °C) containing 0.5% triethylamine, were evaporated under reduced pressure to give the title product as an off-white solid.

(*a*) *2′-O-*[1-(2-Fluorophenyl)-4-methoxypiperidin-4-yl]-*2-N-*(phenylacetyl)-*5′-O-*(9-phenylxanthen-9-yl)guanosine **15**; B = **19** (1.74 g, 2.0 mmol) was allowed to react with 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite **23** (0.72 g, 3.0 mmol) and *N,N*-diisopropylethylamine (0.70 cm^3, 4.0 mmol) in THF (10 cm^3). The corresponding *N,N*-diisopropylphosphoramidite **16**; B = **19** (1.99 g, 93%) was isolated as above; $\delta_P(CDCl_3)$ 149.33 and 150.48.

(*b*) *2′-O-*[1-(2-Fluorophenyl)-4-methoxypiperidin-4-yl]-*5′-O-*(9-phenylxanthen-9-yl)-*6-N-*pivaloyladenosine **15**; B = **17** (1.12 g, 1.37 mmol) was allowed to react with 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite **23** (0.49 g, 2.1 mmol) and *N,N*-diisopropylethylamine (0.48 cm^3, 2.75 mmol) in THF (7 cm^3). The corresponding *N,N*-diisopropylphosphoramidite **16**; B = **17** (1.30 g, 93%) was isolated as above; $\delta_P(CDCl_3)$ 150.20 and 152.36.

(*c*) 4-*N*-Benzoyl-*2′-O-*[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]-*5′-O-*(9-phenylxanthen-9-yl)cytidine **15**; B = **18** (1.10 g, 1.36 mmol) was allowed to react with 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite **23** (0.32 g, 1.35 mmol) and *N,N*-diisopropylethylamine (0.47 cm^3, 2.7 mmol) in THF (7 cm^3). The corresponding *N,N*-diisopropylphosphoramidite **16**; B = **18** (1.28 g, 93%) was isolated as above; $\delta_P(CDCl_3)$ 150.01 and 152.13.

(*d*) *2′-O-*[1-(2-Fluorophenyl)-4-methoxypiperidin-4-yl]-*5′-O-*(9-phenylxanthen-9-yl)uridine **15**; B = **20** (1.74 g, 2.46 mmol) was allowed to react with 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite **23** (0.87 g, 3.7 mmol) and *N,N*-

diisopropylethylamine (0.86 cm^3, 4.9 mmol) in THF (10 cm^3). The corresponding *N,N*-diisopropylphosphoramidite **16**; B = **20** (2.0 g, 90%) was isolated as above; $\delta_P(CDCl_3)$ 149.95 and 152.14.

5-(3-*Nitrophenyl*)-1H-*tetrazole* **26**.—3-Nitrobenzonitrile (9.0 g, 60.8 mmol), sodium azide (5.13 g, 78.9 mmol), ammonium chloride (0.32 g, 6.0 mmol) and DMF (300 cm^3) were stirred and heated together at 100 °C for 4 h. The products were then concentrated under reduced pressure. A solution of the residue in water (300 cm^3) was acidified to pH ~ 2 by the addition of conc. hydrochloric acid (~5 cm^3). The title compound was collected by filtration, washed with ice-cold water, and dried *in vacuo* over P_2O_5 (yield 10.2 g, 87%) [Found, after crystallization from ethyl acetate–light petroleum (60–80 °C): C, 43.9; H, 2.4; N, 36.6. Calc. for $C_7H_5N_5O_2$: C, 44.0; H, 2.6; N, 36.6%), m.p. 149–149.5 °C (lit.,[38] 150–151 °C); $\delta_C[(CD_3)_2SO]$ 121.50, 125.57, 126.15, 131.21, 133.05, 148.26 and 154.97.

Triethylammonium Salt of 2′-O-[1-(2-*Fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-5′-O-(9-*phenylxanthen*-9-*yl*)-6-N-*pivaloyl*-3′-O-*succinoyladenosine* **24**; B = **17**.—A solution of 2′-*O*-[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]-5′-*O*-(9-phenylxanthen-9-yl)-6-*N*-pivaloyladenosine **15**; B = **17** (0.22 g, 0.27 mmol), succinic anhydride (0.081 g, 0.81 mmol) and DMAP (0.066 g, 0.54 mmol) in anhydrous dichloromethane (4 cm^3) was stirred at room temperature for 2 h. More dichloromethane (20 cm^3) was added and the solution was washed successively with 0.1 mol dm^{-3} triethylammonium hydrogen carbonate (pH 7.5; 2 × 25 cm^3) and water (2 × 25 cm^3). The dried ($MgSO_4$) organic layer was evaporated under reduced pressure. The residue was fractionated by short-column chromatography on silica gel: the appropriate fractions, which were eluted with chloroform–ethanol–triethylamine (90:10:0.5 v/v), were evaporated under reduced pressure to give the title compound as a froth (0.216 g).

Derivatization of Functionalized Controlled-pore Glass.—Long-chain alkylamine-functionalized 500 Å controlled-pore glass (0.90 g), the triethylammonium salt of 2′-*O*-[1-(2-fluorophenyl)-4-methoxypiperidin-4-yl]-5′-*O*-(9-phenylxanthen-9-yl)-6-*N*-pivaloyl-3′-*O*-succinoyladenosine **24**; B = **17** (0.23 g, ~0.22 mmol), DCC (0.234 g, 1.13 mmol), triethylamine (32 mm^3, 0.23 mmol), DMAP (0.02 g, 0.16 mmol) and anhydrous DMF (3 cm^3) were shaken together at room temperature for 3 days. The support was filtered, and washed with DMF–triethylamine (99:1 v/v; 2 × 10 cm^3), pyridine–triethylamine (99:1 v/v; 2 × 10 cm^3), methanol–triethylamine (99:1 v/v; 5 × 20 cm^3) and diethyl ether–triethylamine (99:1 v/v; 3 × 20 cm^3). The support was then suspended in pyridine (9 cm^3) and DMAP (0.02 g, 0.16 mmol) and acetic anhydride (1.0 cm^3, 10.6 mmol) were added. After 30 min, the support was filtered, and washed with pyridine–triethylamine (99:1 v/v; 3 × 20 cm^3), methanol–triethylamine (99:1 v/v; 5 × 20 cm^3) and diethyl ether–triethylamine (99:1 v/v; 3 × 20 cm^3). The derivatized controlled-pore glass was then dried *in vacuo* over anhydrous potassium carbonate. The loading was found by 9-phenylxanthen-9-yl cation assay (see below) to be ~29 μmol g^{-1}.

Preparation of Solvents and Reagents for Solid-phase Synthesis.—(a) *Acetonitrile.* HPLC grade acetonitrile was heated, under reflux, with calcium hydride under nitrogen for 8 h and was then distilled; it was stored over activated no. 4 Å molecular sieves and filtered through a Millipore Millex-FGS 0.2 μ filter under dry argon before use. Water content (Karl Fischer titration): < 15 ppm.

(b) *Dichloromethane.* This solvent was dried by heating, under reflux, under nitrogen over phosphorus pentaoxide for 3 h and was then distilled; it was also stored over no. 4 Å molecular sieves and filtered as above before use. Water content: < 10 ppm.

(c) *THF.* This solvent was heated, under reflux, with benzophenone and sodium and potassium metals, under nitrogen for 4 h and was then distilled. Water content: < 10 ppm.

(d) *Acetic acid.* Acetic acid–acetic anhydride (9:1 v/v) was heated, under reflux, for 2 h and was then fractionated.

(e) 1-*Methylimidazole.* This reagent was heated, under reflux, over calcium hydride under nitrogen for 2 h; it was then distilled under reduced pressure and stored over activated no. 4 Å molecular sieves.

(f) 2,6-*Lutidine.* Dry 2,6-lutidine was obtained in the same way as dry 1-methylimidazole except that it was distilled at atmospheric pressure.

(g) *Acetic anhydride.* This reagent was heated, under reflux, over magnesium turnings (~10 g dm^{-3}), and was then distilled.

(h) *Capping solutions.* Solution A was prepared by dissolving acetic anhydride (16 cm^3), acetic acid (4 cm^3) and 2,6-lutidine (32 cm^3) in THF (108 cm^3). Solution B was prepared by dissolving 1-methylimidazole (16 cm^3) in THF (144 cm^3). Both solutions were filtered through Millipore Millex-FGS 0.2 μ filters before use.

(i) *Trichloroacetic acid solution.* Trichloroacetic acid was distilled under reduced pressure, under nitrogen, into preweighed flasks. Dichloromethane (49 cm^3 g^{-1}) was added and the resulting solution was filtered through a Millipore Millex-FGS 0.2 μ filter.

(j) 5-(3-*Nitrophenyl*)-1H-*tetrazole solution.* 5-(3-Nitrophenyl)-1*H*-tetrazole **26** (0.865 g, 4.5 mmol) was dissolved in anhydrous acetonitrile (45 cm^3). The resulting solution was then filtered through a Millipore Millex-FGS 0.2 μ filter.

(k) *Oxidizing solution. tert*-Butyl hydroperoxide [5.5 mol dm^{-3} solution in isooctane (2,2,4-trimethylpentane), 18.5 cm^3] was diluted to 100 cm^3 with dichloromethane, and the resulting solution was filtered through a Millipore Millex-FGS 0.2 μ filter.

(l) *Phosphoramidite solutions.* All glassware was dried at 150 °C/10 mmHg for 4–6 h before use. Phosphoramidites were dissolved in anhydrous dichloromethane (20 cm^3 g^{-1}) and the resulting solutions were evaporated under reduced pressure. The residual glasses obtained were dried *in vacuo* over phosphorus pentaoxide and potassium hydroxide for 12–14 h. 0.1 Mol dm^{-3} solutions were prepared by dissolving the phosphoramidite (0.50 g) in the appropriate volume of acetonitrile (4.93 cm^3 for **16**; B = **17**; 4.70 cm^3 for **16**; B = **18**; 4.95 cm^3 for **16**; B = **19**; and 5.51 cm^3 for **16**; B = **20**). The resulting solutions were further dried over activated no. 4 Å molecular sieves for 4–6 h, and were then filtered through Millipore Millex-FGS 0.2 μ filters.

Solid-phase Synthesis.—Automated oligoribonucleotide synthesis was carried out in an Applied Biosystems 381A synthesizer on a ~1.0 μmolar scale. The protocol followed is indicated in outline in Table 1. In each synthetic cycle, the 9-phenylxanthen-9-yl cation (ε_{375} is estimated to be ~32 000), released in the course of each step involving treatment with trichloroacetic acid, was assayed spectrophotometrically. The 3′-terminal 10-mer, 19-mer and 37-mer sequences of yeast $tRNA^{Ala}$ were prepared from 0.0324 g (~0.94 μmol), 0.0308 g (~0.89 μmol) and 0.0322 g (~0.92 μmol), respectively, of adenosine-derivatized controlled-pore glass. The average coupling yields (as determined by 9-phenylxanthen-9-yl cation assays) were 97, 98 and 98%, respectively.

Deprotection and Partial Purification of 2′-*Protected RNA Sequences.*— After assembly of the desired sequence, the controlled-pore glass with attached fully protected oligoribonucleotide was transferred to a 5 cm^3 Pierce Reacti-vial and conc. aq. ammonia (*d* 0.88; 1.5 cm^3) was added. The vial was

capped and the cap was well covered with Parafilm. The vial was then heated at 55 °C for 8–10 h. The cooled products were then evaporated to dryness and the residue was redissolved in 0.01 mol dm^{-3} triethylammonium hydrogen carbonate buffer (pH 7.5; 2–4 cm^3). The crude yields of 2′-protected [5′-*O*-(9-phenylxanthen-9-yl)] 10-mer, 19-mer and 37-mer were estimated spectrophotometrically to be 49, 69 and 185 A_{260} units. These crude protected RNA sequences were further purified by chromatography on a column (46 g; 75 cm × 3 cm diameter) of Sephadex G-75. The column was eluted with 0.01 mol dm^{-3} triethylammonium hydrogen carbonate buffer (pH 7.5). The yields after Sephadex G-75 chromatography of partially purified 10-mer, 19-mer and 37-mer were 34, 35 and 65 A_{260} units, respectively. Further purification was effected by preparative reversed-phase liquid chromatography (see below).

Preparative Liquid Chromatography of 2′-Protected RNA Sequences.—The above partially purified 2′-protected [5′-*O*-(9-phenylxanthen-9-yl)] 10-mer, 19-mer and 37-mer RNA sequences were purified by liquid chromatography on a Jones APEX ODS 5μ (25 cm × 4.6 mm) column. In the case of the 10-mer and the 19-mer, the eluent was 0.1 mol dm^{-3} triethylammonium acetate (pH 7.0)–acetonitrile (95:5 to 55:45 v/v) and, in the case of the 37-mer, the eluent was 0.1 mol dm^{-3} triethylammonium acetate (pH 7.0)–acetonitrile (75:25 to 35:65 v/v). The eluate containing the desired component was collected and transferred to an Eppendorf tube; it was then evaporated under reduced pressure in a vacuum centrifuge. The residue was redissolved in de-ionized water (2 × 1 cm^3) and re-evaporated.

Preparation and Precipitation[42] *of Pure Unprotected RNA Sequences.*—The above purified 2′-protected [5′-*O*-(9-phenylxanthen-9-yl)] RNA sequence (10-mer, 19-mer or 37-mer; ~10 A_{260} units) was dissolved in 0.01 mol dm^{-3} hydrochloric acid (prepared from sterile water, 0.6 cm^3) and the pH was adjusted to 2.0–2.3 by the addition of similarly prepared 0.1 mol dm^{-3} hydrochloric acid. The slightly turbid solution was kept at 20 °C for 20 h and ~0.5 mol dm^{-3} aq. ammonia (also prepared from sterile water) was added until the pH increased to 7.0–8.0. 0.7 Mol dm^{-3} aq. ammonium acetate [0.001 mol dm^{-3} with respect to ethylenediaminetetraacetic acid (EDTA) and containing 0.1% sodium dodecyl sulfate, 0.20 cm^3] was added. Pre-cooled (to −70 °C) butan-1-ol (0.6 cm^3) was then added with thorough mixing (vortex mixer). The resulting mixture was cooled to −70 °C for 2–3 min and was then centrifuged. The supernatant was carefully removed. The remaining solid pellet was resuspended in cold butan-1-ol, cooled to −70 °C, and centrifuged several times more until a very small pellet remained. The latter was suspended in sterile water (0.20 cm^3) and the butan-1-ol treatment was repeated once more. The residue was evaporated to dryness under reduced pressure, redissolved in analytical-grade absolute ethanol (0.40 cm^3) and the solution was re-evaporated. The residual unprotected RNA sequence was stored at −20 °C. The unprotected 37-mer, which was found by LC (Jones APEX ODS 5μ column) to be ~80–85% pure, was further purified by preparative LC on the same column.

Enzymatic Digestion of Synthetic RNA Sequences.—(a) *10-mer and 19-mer.* A stock solution of *Crotalus adamanteus* snake venom phosphodiesterase was prepared by dissolution of ~1.0 mg (0.2–0.4 unit) in 0.1 mol dm^{-3} Tris hydrochloride buffer (pH 8.3; 0.5 cm^3) that was 0.01 molar with respect to $MgCl_2$. A stock solution of *E. coli* alkaline phosphatase was prepared by dissolution of ~1 unit in the same buffer. Phosphodiesterase solution (14 mm^3) was added to a solution of substrate (~1.0 A_{260} unit) in sterile water (10 mm^3), and the resulting solution was incubated at 37 °C for 20 h. Alkaline phosphatase solution (14 mm^3) was added and, after a further period of 14 h, the digest was analysed by LC on a Jones APEX ODS 5μ (25 cm × 4.6 mm) column. The column was eluted isocratically and the digest was found to contain adenosine (A), cytidine (C), guanosine (G) and uridine (U) as the sole nucleoside or nucleotide products. The A:C:G:U proportions observed for the 10-mer [calc. 2.0:5.0:1.0:2.0] and the 19-mer [calc. 4.0:8.0:3.0:4.0] were 2.0:5.0:1.1:2.0 and 3.7:8.0:2.6:3.7, respectively.

(b) *37-mer.* Stock solutions of ribonuclease T_2 [~50 units in 0.1 mol dm^{-3} ammonium acetate (pH 4.5, 0.4 cm^3)], ribonuclease A [~1 mg in 0.1 mol dm^{-3} Tris hydrochloride buffer (pH 8.3, 1.0 cm^3)] and ribonuclease T_1 [100 000 units in 0.1 mol dm^{-3} Tris hydrochloride buffer (pH 8.3, 1.0 cm^3] were prepared.

Ribonuclease T_2 solution (40 mm^3) was added to a solution of substrate (~1.0 A_{260} unit) in sterile water (10 mm^3) and the resulting solution was incubated at 37 °C for 20 h. 0.01 Mol dm^{-3} Tris (~5 mm^3) was added until the pH rose to ~8.0 and then ribonuclease A (14 mm^3), ribonuclease T_1 (14 mm^3) and snake venom phosphodiesterase solutions (14 mm^3) were added. After 14 h, alkaline phosphatase solution (14 mm^3) was added and, after a further period of 20 h, the digest was analysed by LC. The relative proportions of A:C:G:U observed were 6.0:11.8:11.0:9.0 (calc. 6.0:12.0:11.0:8.0).

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