Novel Solid-phase Synthesis of Branched Oligoribonucleotides, including a Substrate for the RNA Debranching Enzyme

Brian S. Sproat,* Barbro Beijer, Morten Grøtli, Ursula Ryder, Kenneth L. Morand and Angus I. Lamond
European Molecular Biology Laboratory, Meyerhofstrasse 1, D-69117 Heidelberg, Germany

An effective new route for synthesizing branched oligoribonucleotides in the solid phase in the 5′ to 3′ direction has been developed. This required the synthesis of reversed monomers, *viz.* protected nucleoside 5′-phosphoramidites bearing 2′-*O*-Fpmp and 3′-*O*-pixyl protecting groups as well as special branch-point monomers, *viz.* protected nucleoside 5′-phosphoramidites bearing either 2′,3′-*O*-dipixyl protection in the case of adenosine, cytidine and uridine, or 2′,3′-*O*-dilaevulinyl protection in the case of guanosine. These monomers are assembled on commercial synthesizers into branched oligoribonucleotides in high yield, the crude products are readily purified by reversed-phase HPLC whilst still partially protected, and the fully deprotected products are conveniently analysed by electrospray mass spectrometry. Moreover, the branched oligoribonucleotides can be recognised and cleaved by a specific 2′–5′ phosphodiesterase present in mammalian cell nuclei. We expect that this will prove valuable for future biochemical and biological studies on the properties of branched RNA molecules and the protein factors and enzymes that interact with branched RNA substrates.

The primary transcripts of many eukaryotic protein-coding genes, *viz.* pre-mRNAs (mRNA = messenger RNA), are accurately spliced in the nucleus by a process involving two sequential transesterification reactions.[1] In *cis*-splicing, the intervening sequences (introns) are converted into lariats,[2,3] *i.e.* single-stranded circular RNAs with a tail. In *trans*-splicing the introns are converted into Y-shaped or forked structures.[4] These so-called branched RNAs contain a branch-point adenosine which bears vicinal 2′–5′ and 3′–5′ phosphodiester linkages.

In 1985 Ruskin and Green discovered an RNA-processing activity in HeLa† cell extracts that debranches RNA lariats.[5] This activity cleaves the lariat by a specific single endonucleolytic cleavage on the 2′-side of the 2′,5′-phosphodiester linkage to generate a linear RNA molecule, and is able to function in the absence of exogenous magnesium ions. Chromatography of HeLa nuclear extracts showed that RNA-debranching activity could be separated from the essential splicing factor, SF2.[6] Arenas and Hurwitz[7] partially purified a 2′,5′-phosphodiesterase activity from the cytosolic fraction of HeLa cells, using lariat RNA substrates to assay the enzyme activity. However, the purified enzyme required magnesium ions and, moreover, was capable of debranching branched triribonucleotides in which the branch point was A or G. Chapman and Boeke recently, and serendipitously, isolated the gene called PRP26 encoding for a yeast RNA-debranching enzyme.[8] Interestingly, PRP26 mutants are not lethal, but inhibit transposition by Ty elements.‡ The mechanism responsible for the inhibition of transposition is unclear but implies that the RNA-debranching enzyme may have other roles apart from the metabolism of introns. We are interested in purifying to homogeneity the nuclear form of the RNA-debranching enzyme with a view to isolating the gene, performing measurements on substrate specificity, and obtaining a crystal structure.

In order to be able to assay conveniently for the mammalian lariat debranching enzyme during purification, but also to explore the substrate specificity and reaction mechanism of the purified enzyme, we decided to synthesize small branched, *i.e.* Y-shaped, oligoribonucleotides as substrates. A survey of the literature on the chemical synthesis of branched oligoribonucleotides revealed a variety of routes, and problems caused by protecting-group incompatibility which are discussed below. As a direct result, practically all reported syntheses were conducted in solution, a laborious process. Thus, Kierzek *et al.* were able to synthesize 5′-GA$^{2'-G}_{3'-C}$ by using phosphoramidite chemistry in solution.[9] The fully protected dimer AC was first prepared and purified bearing a 2′-*O*-*tert*-butyldimethylsilyl protecting group on the adenosine. After selective removal of the 2-cyanoethyl group at the adjacent phosphotriester, the 2′-*O*-silyl group could be safely removed without the danger of rearrangement that occurs when a 2′-*O*-protecting group vicinal to a phosphotriester linkage is removed under acidic[10–12] or basic conditions.[13,14] Subsequent coupling of the 2′-hydroxy group of the adenosine with a suitable protected guanosine 5′-*O*-phosphoramidite gave the branched trimer, which was subsequently chain extended from the 5′-hydroxy group of the adenosine to afford the branched tetramer. Fourrey *et al.* were able to prepare a branched trimer in solution involving the low-temperature 2′-*O*-desilylation of the dimer bearing a vicinal methyl phosphotriester function.[15] Subsequent phosphitylation of the 2′-hydroxy group enabled the dimer to be coupled to the 5′-hydroxy group of a suitably protected nucleoside. However, the overall yield was poor.

A combination of phosphotriester and phosphoramidite chemistry as well as the acid-labile 4-methoxytetrahydropyran-2-yl (Mthp) protecting group for the 2′-hydroxy group of the branch-point adenosine enabled Huss *et al.*[16,17] to prepare several branched trimers in solution. The Mthp group was removed by mild acid hydrolysis after the vicinal 2-chlorophenyl phosphotriester linkage had been converted into the phosphodiester. Extension from the free 2′-hydroxy group was then achieved by using a protected nucleoside 5′-*O*-phosphoramidite.

Sekine and Hata originally used a mixture of phosphotriester and phosphodiester chemistry based on phosphoranilidates and *S*,*S*-diphenyl phosphorodithioates to generate a branched trimer in low yield.[18] They then improved their methodology considerably by developing a fully protected adenosine 2′,3′-

† HeLa cells are a continuously cultured strain isolated from a human uterine cervical carcinoma in 1951.
‡ Ty elements are yeast transposons or retroviral elements.

diphosphate derivative capable of chain elongation in the 2', 3' and 5'-directions.[19,20] This enabled the synthesis of the hexamer, 5'-CUGA$A^{2'-G}_{3'-C}$; however, the overall yield was relatively poor due to the complicated chemistry and multiple purification steps.

A significant breakthrough in branched RNA synthesis came from Zhou *et al.*[21] They synthesized branched triribonucleotides, pentaribonucleotides and a heptaribonucleotide by solution methodology using novel key intermediates which circumvented the need for orthogonal protecting groups for the 2'-hydroxy groups and the internucleotidic phosphodiesters, to enable specific introduction of the second phosphoryl group on the branch-point nucleoside. This strategy enabled the branched core trimer to be extended for the first time in all three directions. The key intermediate is an adenosine bearing a 5'-acid-labile protecting group and a 2'-*O*-(2-chlorophenyl phosphate) moiety. Extension at the 3'-hydroxy group was then performed with a 5'-*O*-phosphoramidite. Thus, the heptamer 5'-CU$A^{2'-GU}_{3'-UC}$ was obtained in sufficient quantity to enable structural investigations to be carried out by ^{1}H NMR spectroscopy. Although the chemistry is very elegant it is nonetheless very laborious. Further elaboration of their techniques showed that a combination of phosphotriester, *H*-phosphonate and phosphoramidite chemistry could be used to advantage for preparing branched tetramers in solution.[22] Here the problem of unblocking a hydroxy group vicinal to a phosphotriester linkage was solved nicely by coupling a nucleoside 5'-*O*-*H*-phosphonate to the free 3'-hydroxy group of a suitably protected branch-point adenosine. After oxidation to generate the 3',5'-phosphodiester linkage, the 2'-protecting group could be safely removed. Moreover, the use of the 2'-*O*-pixyl group* on the branch-point adenosine enabled selective removal of this group when using the more acid-stable tetrahydropyran-2-yl group for protection of all other 2'-hydroxy groups, thus enabling easy regiospecific phosphorylation.[23] This even led to the synthesis of a tetrameric cyclic branched tetraribonucleotide,[24] and a cyclic branched heptaribonucleotide with 5 nucleotides in the loop.[25] Recently it has been shown that some of these small lariat RNAs are capable of self-cleavage.[26]

The early work of Damha *et al.*[12] and Damha and Ogilvie[27] is of particular importance since it laid the foundations for the later solid-phase synthesis of branched RNA. Thus, the problems associated with regiospecific phosphorylation could be avoided by simultaneous introduction of both 2',5'- and 3',5'-phosphotriester linkages. This could be achieved in two ways to prepare branched trimers, either by reaction of a 5'-*O*-monomethoxytrityl base protected adenosine with two molecules of a suitably protected ribonucleoside 5'-*O*-phosphoramidite, or by reaction of a protected adenosine 2',3'-*O*-bis(phosphoramidite) with two molecules of a suitably protected ribonucleoside bearing a free 5'-hydroxy group. Damha and Zabarylo were able to adapt this method for the first automated solid-phase synthesis of branched oligoribonucleotides, albeit with identical sequences at the 2'- and 3'-position of the branch-point adenosine.[28,29] They demonstrated that a reasonable yield of branched sequence $A^{2'-N_1N_2N_3\cdots N_n}_{3'-N_1N_2N_3\cdots N_n}$ could be formed by reaction of a low concentration of an adenosine 2',3'-*O*-bis(phosphoramidite) with the free 5'-hydroxy groups of two adjacent support-bound oligonucleotide chains present on a highly loaded support. Synthesis could then be continued in the normal fashion from the 5'-hydroxy group of the adenosine branch, using 2'-*O*-(*tert*-butyldimethylsilyl)-5'-*O*-dimethoxytrityl ribonucleoside 3'-*O*-phosphoramidites. The latest conclusions of Damha *et al.* show that for this method of synthesis a concentration of 20–30 mmol dm^{-3} of the bis(phosphoramidite) and a long-chain-alkylamine controlled-pore glass support with a loading of 25–30 μmol g^{-1} are optimal.[29] This results in ~68% efficiency in the branching reaction.

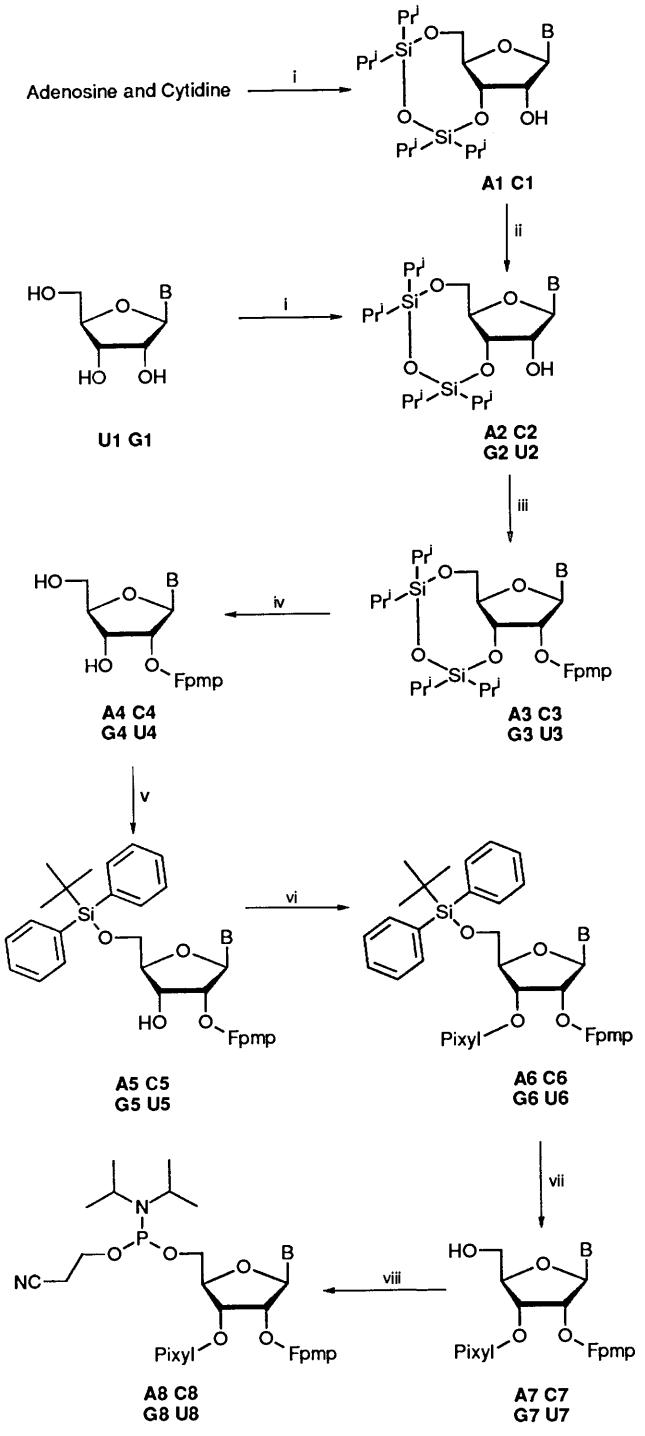
Here we describe an efficient solid-phase phosphoramidite procedure for preparing branched RNA involving synthesis from the 5' to 3' end with 5'-*O*-phosphoramidites and special branch-point nucleosides, enabling simultaneous extension from the 2'- and 3'-hydroxy groups. We reasoned that the combination of the highly acid-labile 9-phenylxanthen-9-yl (pixyl) group[30] for 3'-hydroxy-group protection and the 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl, *i.e.* Fpmp group,[31] for 2'-hydroxy-group protection would be an excellent choice for the ribonucleoside 5'-*O*-phosphoramidites (reversed monomers) and, moreover, would enable us to purify the partially protected branched oligoribonucleotides by reversed-phase HPLC.

Results and Discussion

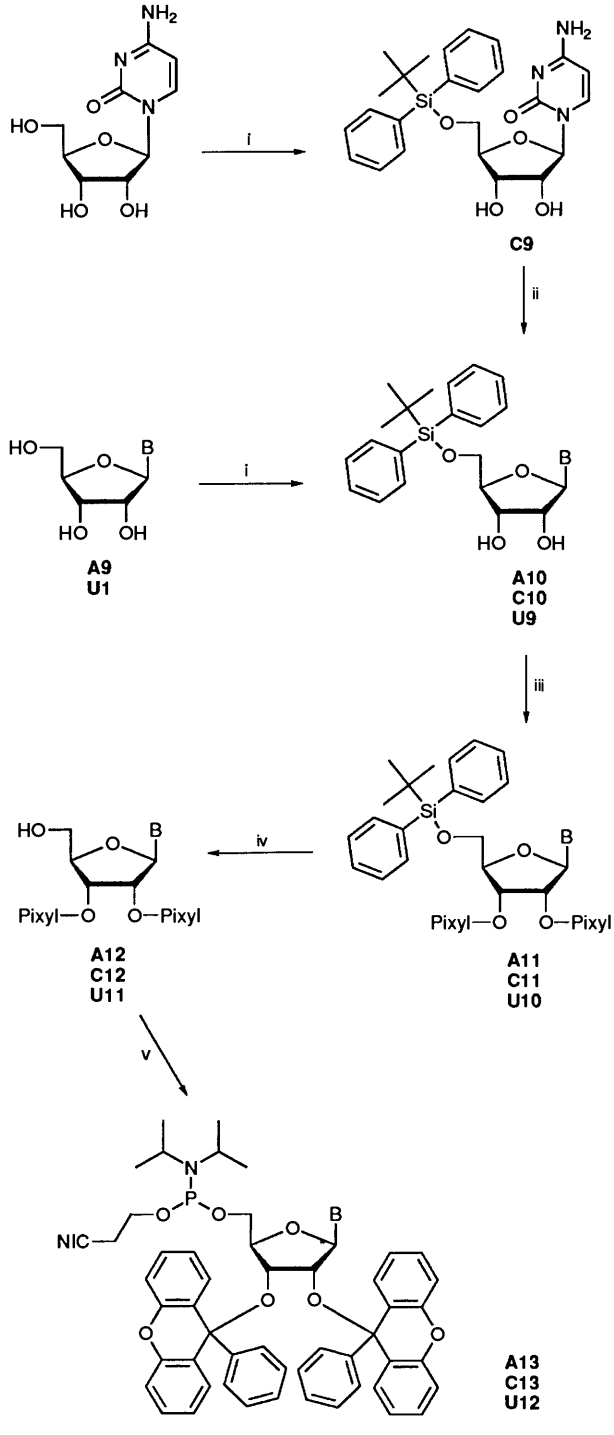
Monomer Synthesis.—The synthesis of the reversed monomers is illustrated in Scheme 1. Thus, adenosine and cytidine were first treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[32] to protect simultaneously the 3'- and 5'-hydroxy groups. The exocyclic amino protection was then introduced *via* the transient protection procedure[33] to give compounds **A2** and **C2**. Compound **U2** was silylated in quantitative yield from uridine. Guanosine was protected on the exocyclic amino group by reaction with *N*,*N*-dimethylformamide (DMF) dimethyl acetal and then treated with the Markiewicz reagent[32] to give compound **G2**. The 2'-hydroxy group in compounds **A2**, **C2**, **G2** and **U2** was then protected with the Fpmp group by reaction with 1-(2-fluorophenyl)-4-methoxy-1,2,5,6-tetrahydropyridine in the presence of mesitylenesulfonic acid to give compounds **A3**, **C3**, **G3** and **U3** in yields of between 75 and 86%. Desilylation to give compounds **A4**, **C4**, **G4** and **U4** proceeded smoothly in almost quantitative yield. In order to introduce the acid-labile pixyl group on the 3'-hydroxy moiety it was necessary first to reprotect the 5'-hydroxy function, so we chose to use the bulky lipophilic *tert*-butyldiphenylsilyl group. Thus, silylation afforded compounds **A5**, **C5**, **G5** and **U5** in excellent yield (between 84 and 98%). Overnight reaction of the free 3'-hydroxy group of the above compounds with pixyl chloride in pyridine afforded compounds **A6**, **C6** and **G6** in practically quantitative yield and **U6** in 84% yield. Removal of the *tert*-butyldiphenylsilyl protecting group with tetrabutylammonium fluoride (TBAF) was rather slow; however, compounds **A7**, **C7**, **G7** and **U7** were obtained in good isolated yield (79–94%). Finally, 5'-hydroxy-group phosphitylation with chloro-(2-cyanoethoxy)diisopropylaminophosphine during 3 h afforded the desired reversed monomers, *viz.* compounds **A8**, **C8**, **G8** and **U8** as foams in yields between 83 and 97% after column chromatography. Although the monomer syntheses are multistep the overall yields are excellent, between 37 and 48% for the seven- or eight-step syntheses from ribonucleoside to 5'-*O*-phosphoramidite. Compounds **A7**, **C7**, **G7** and **U7** were also succinylated on the 5'-hydroxy groups, enabling subsequent preparation of activated esters for coupling to aminopropyl-controlled-pore glass, giving the 'reversed supports'.

The syntheses of the branch-point monomers are illustrated in Scheme 2 (for A, C and U) and Scheme 3 (for G). Thus, uridine (**U1**) and 6-*N*-benzoyladenosine (**A9**) were protected on the 5'-hydroxy moiety with the lipophilic *tert*-butyldiphenylsilyl group to give compounds **U9** and **A10**, whilst cytidine was first silylated and then acylated to give compound **C10**. Pixylation of all three compounds (**A10**, **C10** and **U9**) with three mole equiv. of pixyl chloride afforded compounds **A11**, **C11** and **U10**, with the acid-labile pixyl group protecting both the 2'- and 3'-hydroxy groups, in almost quantitative yield. Removal of the

* Pixyl = 9-phenylxanthen-9-yl.

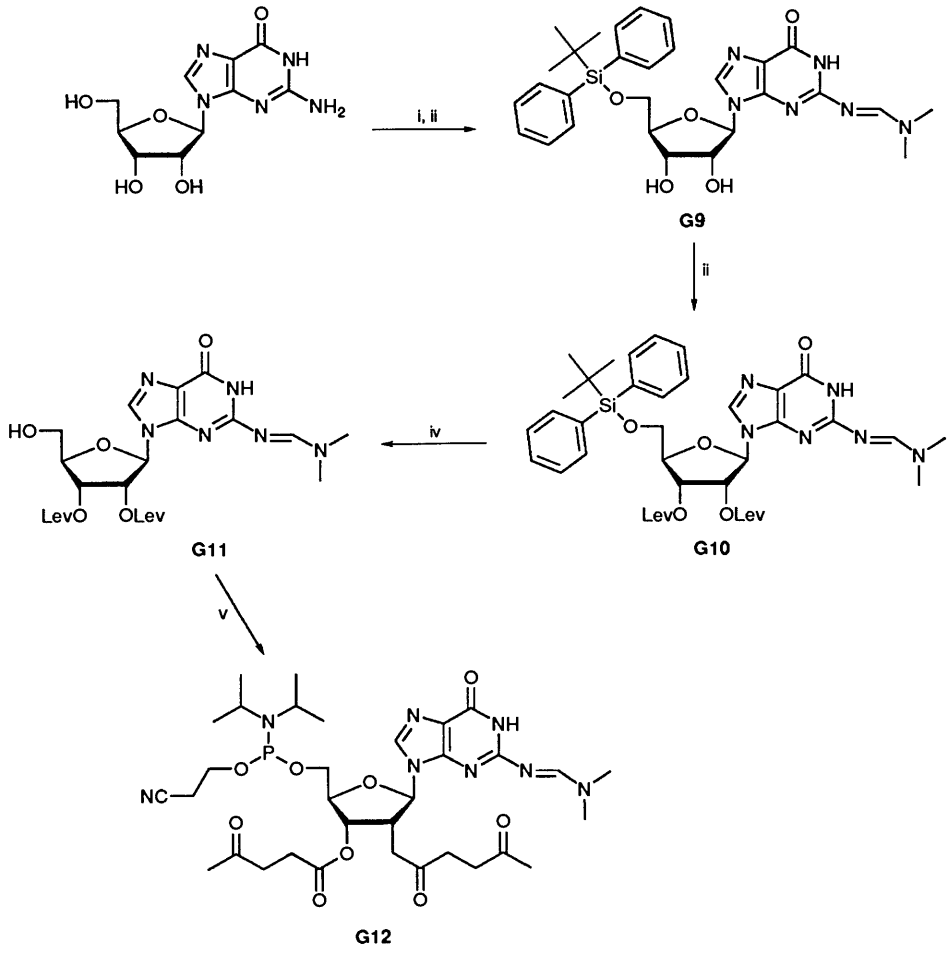


Scheme 1 Reaction scheme for the preparation of the 2′-*O*-Fpmp-protected reversed monomers: Adenosine series, compounds **A1**–**A8**: B = adenin-9-yl for compound **A1** and 6-*N*-pivaloyladenin-9-yl for compounds **A2**–**A8**; cytidine series, compounds **C1**–**C8**, B = cytosin-1-yl for compound **C1** and 4-*N*-benzoylcytosin-1-yl for compounds **C2**–**C8**; guanosine series, compounds **G1**–**G8**, B = 2-*N*-(dimethylaminomethylene)guanin-9-yl; uridine series, compounds **U1**–**U8**, B = uracil-1-yl. *Reagents:* i, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine; ii, (a) chlorotrimethylsilane in pyridine; (*b*) benzoyl chloride in pyridine; (c) dil. aq. ammonia–pyridine; (d) toluene-*p*-sulfonic acid (PTSA) in dichloromethane–1,4-dioxane; iii, 1-(2-fluorophenyl)-4-methoxy-1,2,5,6-tetrahydropyridine and 2,4,6-trimethylbenzenesulfonic acid in tetrahydrofuran (THF)–acetonitrile; iv, tetrabutylammonium fluoride (TBAF) in THF; v, *tert*-butyl(chloro)diphenylsilane and imidazole in dimethylformamide (DMF); vi, 9-chloro-9-phenylxanthene in pyridine; vii, TBAF in THF; viii, chloro-(2-cyanoethoxy)diisopropylaminophosphine and *N*,*N*-diisopropylethylamine in 1,2-dichloroethane.



Scheme 2 Reaction scheme for the preparation of the adenosine, cytidine and uridine branch-point monomers: Adenosine series, compounds **A9**–**A13**, B = 6-*N*-benzoyladenin-9-yl; cytidine series, compounds **C9**–**C13**, B = 4-*N*-benzoylcytosin-1-yl for compounds **C10**–**C13**; uridine series, compounds **U1**, and **U9**–**U12**, B = uracil-1-yl. *Reagents:* i, *tert*-butyl(chloro)diphenylsilane and imidazole in DMF; ii, (a) chlorotrimethylsilane in pyridine; (b) benzoyl chloride in pyridine; (c) dil. aq. ammonia–pyridine; iii, 9-chloro-9-phenylxanthene in pyridine; iv, TBAF in THF; v, chloro-(2-cyanoethoxy)diisopropylaminophosphine and *N*,*N*-diisopropylethylamine in 1,2-dichloroethane.

silyl group from compounds **A11**, **C11** and **U10** was rather slow, requiring 1–2 days; however, the products **A12**, **C12** and **U11** were obtained in 69–97% yield. Although the ^{13}C NMR spectra of these compounds are very complicated the presence of two pixyl groups is easily confirmed by the two characteristic



Scheme 3 Reaction scheme for the synthesis of the branch-point guanosine monomer. *Reagents:* i, *tert*-butyl(chloro)diphenylsilane and imidazole in DMF; ii, DMF dimethyl acetal in methanol; iii, laevulinic acid, *N,N'*-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) in dichloromethane–THF; iv, TBAF in THF; v, chloro-(2-cyanoethoxy)diisopropylaminophosphine and *N,N*-diisopropylethylamine in 1,2-dichloroethane.

xanthene C-9 signals, one at δ_C 76.8–76.6 and the other at δ_C 76.0–75.6. Phosphitylation afforded the desired branch monomers **A13**, **C13** and **U12** in excellent yield (87–97%). The overall yield from ribonucleoside to branch-point monomer was close to 50%. The methyl phosphoramidite analogue of compound **U12** has been described previously (prepared in a modest 30% yield from 5'-*O*-acetyluridine) and was introduced at the 5'-end of an oligodeoxyribonucleotide; after deprotection, oxidation of the ribose moiety with periodate gave a dialdehyde, which was subsequently labelled with biotin hydrazide, resulting in a 5'-biotinylated DNA probe.[34]

The branch-point guanosine monomer could not be prepared by the route described above for the other three ribonucleosides as it was not possible to obtain a 2'-*O*-pixyl moiety, probably due to steric crowding. We decided instead to protect the 2'- and 3'-hydroxy groups with the laevulinyl (4-oxopentanoyl) group[35] since this should be selectively removed under neutral conditions by brief treatment with hydrazine in buffered pyridine–acetic acid. The route to the branch-point guanosine monomer is illustrated in Scheme 3. Thus, guanosine was silylated on the 5'-hydroxy group with *tert*-butyl(chloro)diphenylsilane and was then protected on the 2-amino group to give intermediate **G9** in 90% yield. This compound was then laevulinylated on both the 2'- and 3'-hydroxy groups to give compound **G10** in 93% isolated yield. Removal of the *tert*-butyldiphenylsilyl group with fluoride ion reached completion within 90 min to afford compound **G11** in 81% yield. Finally, phosphitylation of the 5'-hydroxy group in compound **G11** gave the desired monomer, compound **G12** in 84% yield. The overall yield of branch-point guanosine monomer from guanosine was 57%.

Assembly, Purification and Analysis of Branched Oligoribonucleotides.—Branched oligoribonucleotides were readily synthesized by using ribonucleoside 5'-*O*-phosphoramidites with 3'-*O*-pixyl and 2'-*O*-Fpmp protection and by assembling the polymer from the 5' end to the 3' end. The special branch-point monomers bear either 2'-*O*- and 3'-*O*-pixyl groups as in the case of compounds **A13**, **C13** and **U12** or 2'-*O*- and 3'-*O*-laevulinyl groups in the case of compound **G12**. After incorporation of the branch point A, C or U the two pixyl groups are removed simultaneously in the so-called detritylation step to generate a *cis*-diol system, thus enabling simultaneous chain extension from both secondary hydroxy groups of the branch-point nucleoside with sequences of identical length and base composition. Since the branch-point guanosine is differently protected, the laevulinyl protecting groups are removed manually with concentrated buffered hydrazine,[35] conditions which do not cause loss of any of the other protecting groups. After thorough washing with acetonitrile, synthesis can be recommenced, since we now have a *cis*-diol system for further chain extension. At the end of the synthesis standard treatment with ammonia cleaves the 5'-*O*-succinate linkage, and removes both the β-cyanoethyl internucleotide protection and the heterocyclic base protection. Only the full-length branched oligoribonucleotide carries two 3'-*O*-pixyl groups and, of course,

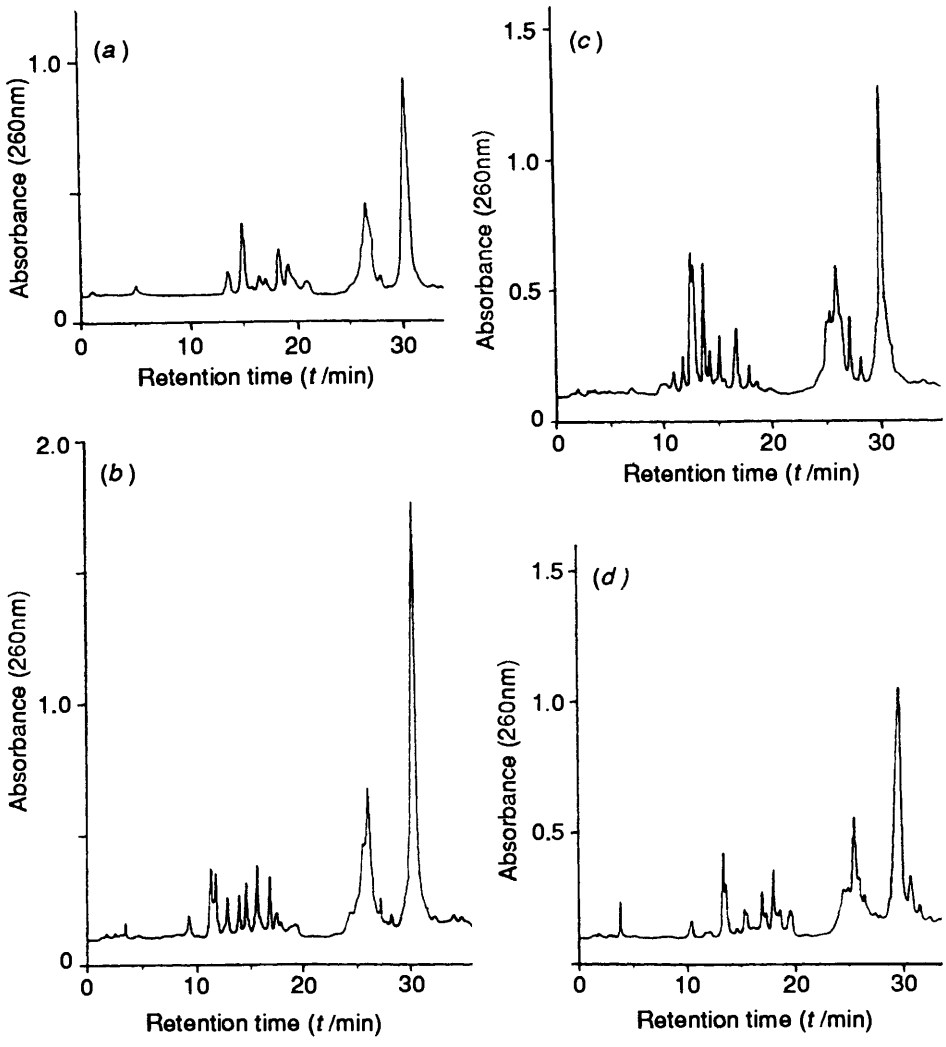


Fig. 1 Analytical reversed-phase HPLC profiles of crude 2′-*O*-Fpmp, dipixyl-protected branched oligoribonucleotides on an 8 mm × 100 mm μBondapak C_{18} cartridge (10 μ); buffer A: 95% 0.1 mol dm^{-3} triethylammonium acetate (pH 7) and 5% acetonitrile; buffer B: 30% 0.1 mol dm^{-3} triethylammonium acetate (pH 7) and 70% acetonitrile; gradient 30–100% buffer B in 40 min, flow rate 2 cm^3 min^{-1}. Panel (*a*): 5′-UGGUUA$^{2'-5'GUGUG}_{3'-5'GUGUG}$; panel (*b*): 5′-UGGUUC$^{2'-5'GUGUG}_{3'-5'GUGUG}$; panel (*c*): 5′-UGGUUG$^{2'-5'GUGUG}_{3'-5'GUGUG}$; panel (*d*): 5′-UACUUA$^{2'-5'GUGUG}_{3'-5'GUGUG}$.

every 2′-OH group is still protected with the Fpmp group. Failure sequences comprise molecules bearing a single 3′-*O*-pixyl group or no pixyl group, thus making purification by reversed-phase HPLC very straightforward. The desired product elutes at an acetonitrile concentration of ~ 60% (due to the 2 pixyl groups and 2′-*O*-Fpmp groups), whilst the failures elute in two groups, at acetonitrile concentrations of ~ 50% and 35% respectively. The reversed-phase HPLC traces of 2′-*O*-Fpmp, dipixyl-protected 5′-UACUUA$^{2'-5'GUGUG}_{3'-5'GUGUG}$ and 5′-UGGUUN$^{2'-5'GUGUG}_{3'-5'GUGUG}$ for N = A, C and G are illustrated in Fig. 1. The isolated yield of pure 5′-UGGUUA$^{2'-5'GUGUG}_{3'-5'GUGUG}$ from a 1 μmol-scale synthesis was 12.5 A_{260} units. Work is currently in progress to extend this method further to enable the synthesis of branched oligoribonucleotides with sequences of different length and base composition attached to the 2′- and 3′-hydroxy groups of the branch-point nucleoside.

Cleavage of the Branched Oligoribonucleotide by the RNA-debranching Enzyme.—The fully deprotected branched oligoribonucleotide, 5′-UGGUUA$^{2'-5'GUGUG}_{3'-5'GUGUG}$, was analysed further by denaturing polyacrylamide gel electrophoresis; see Fig. 2. First, the oligoribonucleotide was 5′-end labelled with ^{32}P, using T4 polynucleotide kinase and [γ-^{32}P]ATP. The labelled, branched oligoribonucleotide migrates as a single band on a polyacrylamide gel (Fig. 2, lane 1). This confirms that a pure major product was obtained from the solid-phase synthesis. To check that the oligoribonucleotide contained an authentic 2′–5′ branched structure, it was incubated with a HeLa cell nuclear extract containing RNA-debranching activity (Fig. 2, lane 4). This activity converted the branched oligoribonucleotide into a faster migrating, single band, which comigrates with a 5′-end-labelled linear oligoribonucleotide marker corresponding to the sequence of the expected debranched product (Fig. 2, lane 2). The position of migration of a marker oligoribonucleotide corresponding to the sequence of the short arm of the branch, *i.e.* 5′-GUGUG-3′, was also determined in parallel (Fig. 2, lane 3). This species is not detected by autoradiography after debranching (*i.e.* in lane 4) since it is not 5′-end labelled. The branched oligoribonucleotide is also cleaved efficiently by a purified RNA-debranching activity isolated from mammalian cell nuclei (data not shown). The small branched oligoribonucleotide, 5′-UUA$^{2'-5'GU}_{3'-5'GU}$, was not a substrate for the RNA-debranching activity (data not shown). A detailed study of the susceptibility of branched oligoribonucleotides of varying length and branch structures to cleavage by purified RNA-debranching enzyme will be published separately (U. Ryder *et al.*, manuscript in preparation).

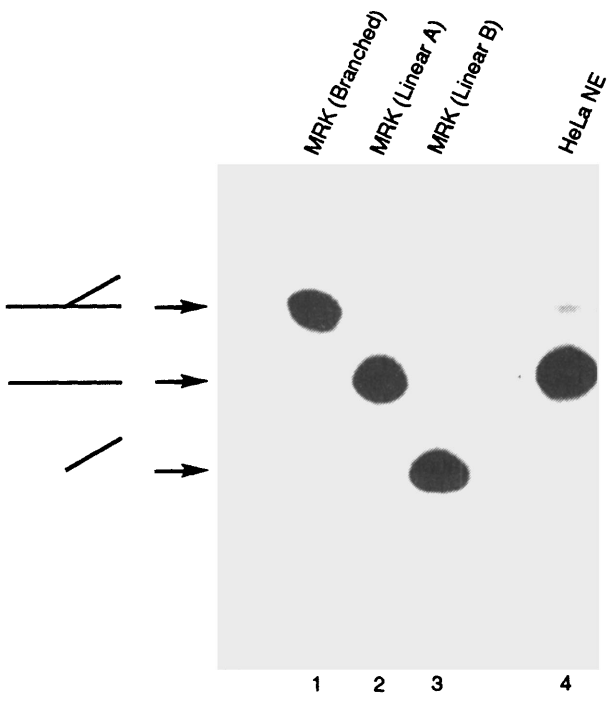


Fig. 2 The branched oligoribonucleotide, 5′-UGGUUA$^{2'-5'GUGUG}_{3'-5'GUGUG}$ (lane 1), and the linear oligoribonucleotides 5′-UGGUUAGUGUG (lane 2) and 5′-GUGUG (lane 3) were each 5′-end labelled with ^{32}P by using T4 polynucleotide kinase and [γ-^{32}P]ATP (see Experimental section). The oligonucleotides were separated by electrophoresis on a 15% polyacrylamide–8 mol dm^{-3} urea denaturing gel and were detected by autoradiography. The identity of each band is illustrated with a cartoon at the left of the figure. The branched oligoribonucleotide was also analysed in parallel following incubation in a HeLa cell nuclear extract containing RNA-debranching activity (lane 4). The debranching assay was carried out in 10 mmol dm^{-3} ethylenediaminetetraacetic acid, which inhibits most 3′-5′ RNA phosphodiesterase activity, but not mammalian 2′–5′ phosphodiesterase activity that cleaves intron lariat RNAs.[5]

Experimental

General Materials and Procedures.—Ribonucleosides were purchased from Pharma Waldhof GmbH (Düsseldorf, Germany), 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane was obtained from Ifotam (Lodz, Poland), chloro-(2-cyanoethoxy)-diisopropylaminophosphine was obtained from BioSyntech (Hamburg, Germany), and 9-chloro-9-phenylxanthene was obtained from Fluka GmbH (Neu-Ulm, Germany). All other reagents used were of the highest available purity. Anhydrous solvents were purchased from Romil Chemicals Ltd. (Loughborough, England). T4 polynucleotide kinase and RNasin were purchased from Promega (Madison, USA). HeLa cells were purchased from the Computer Cell Culture Co. (Mons, Belgium). [γ-^{32}P]ATP was purchased from Amersham Buchler (Braunschweig, Germany).

2′-*O*-[1-(2-Fluorophenyl)-4-methoxypiperidin-4-yl]uridine, compound **U4**, was prepared as described previously.[36] 1-(2-Fluorophenyl)-4-methoxy-1,2,5,6-tetrahydropyridine was prepared according to the method of Reese and Thompson.[31] 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)adenosine, compound **A1**, was prepared by a standard procedure.[37] The 5′-*O*-succinates of compounds **A7**, **C7**, **G7** and **U7** were prepared by a standard procedure and were used to derivatise aminopropyl-controlled pore glass.[36] 6-*N*-Benzoyladenosine, compound **A9**, was prepared from adenosine by using the standard transient protection procedure developed by Ti *et al.*[33]

Column chromatography was performed on Kieselgel 60H (Fluka) and ascending-mode TLC was performed on aluminium-foil-supported silica gel containing a 254 nm fluor. Light petroleum refers to the fraction boiling in the range 40–60 °C.

^{13}C and ^{31}P NMR spectra were recorded on a Bruker AM250 spectrometer, using tetramethylsilane and external trimethyl phosphate as the respective references. ^{13}C NMR spectral data are reported below with broad-band proton-noise decoupling; however, assignments were always made with the aid of the off-resonance data. ^{13}C NMR data for monomers **U8**, **C8**, **A8**, **G8**, **A13**, **U12**, **C13** and **G12** have not been included owing to their complexity, mainly due to the diastereoisomers of the phosphoramidite, but are available upon request. Likewise, the ^{13}C NMR spectral data for compounds **A11**, **U10**, **C11** and **C12** are difficult to assign absolutely, owing to the presence of many aromatic carbons (*tert*-butyldiphenylsilyl group and 2 pixyl groups and, in the case of compound **C11**, also a benzoyl group); this information is also available upon request.

The branched and linear oligoribonucleotides were synthesized on an Applied Biosystems synthesizer model 380B-02 or 394 (Foster City, California).

The HPLC-purified branched oligoribonucleotides were analysed by Electrospray Ionisation Mass Spectrometry (ESMS).[38] Experiments were performed on an API III triple quadrupole mass spectrometer (Perkin-Elmer Sciex Instruments, Ontario, Canada) equipped with an electrospray ionisation source and operated in the negative-ion mode. The RNA samples were diluted prior to analysis in methanol–water (9:1 v/v) containing 1% formic acid, to a final concentration of 10–20 pmol mm^{-3}. Sample solutions were continuously infused to the needle through a 75 μm internal diameter silica capillary by a Harvard Apparatus (South Natick, Massachusetts, USA) model 22 syringe pump at a flow rate of 3.0 mm^3 min^{-1}.

HeLa cell nuclear extracts containing RNA-debranching activity were prepared as previously described.[39,40] Oligonucleotides were 5′-end labelled in a 10 mm^3 volume containing oligoribonucleotide (300 pmol), 33 mmol dm^{-3} Tris* acetate pH 7.9, 10 mmol dm^{-3} magnesium acetate, 5 mmol dm^{-3} dithiothreitol, RNasin (3 units), T4 polynucleotide kinase (4 units), glycogen (50 μg) and [γ-^{32}P]ATP (5 μCi) (specific activity, 5000 Ci mmol^{-1}) by incubation at 37 °C for 45 min. RNA-debranching activity was assayed in a 10 mm^3 final reaction volume containing HeLa extract (3 mm^3) (15 mg cm^{-3}), 10 mmol dm^{-3} EDTA† and 5′-end-labelled branched oligoribonucleotide (0.3 pmol). The reaction was incubated at 30 °C for 60 min, then was mixed with an equal volume of formamide containing Bromophenol Blue and Xylene Cyanol, heated at 65 °C for 10 min, and loaded directly onto a 15% polyacrylamide–8 mol dm^{-3} urea gel run in standard 1 × Tris borate, EDTA buffer.

Synthesis of Reversed Monomers.—5′-O-(tert-*Butyldiphenylsilyl*)-2′-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]*uridine* **U5**. Compound **U4** (3.3 g, 7.31 mmol) was dried by evaporation of DMF, then was dissolved in anhydrous DMF (100 cm^3). Imidazole (996 mg, 14.6 mmol) and *tert*-butyl(chloro)diphenylsilane (2.2 cm^3, 8.4 mmol) were added to the stirred mixture with exclusion of moisture, and the reaction mixture was left overnight at room temperature. Silica gel TLC showed complete reaction, so excess of reagent was quenched by addition of ethanol (1 cm^3). Solvent was removed under reduced pressure and the residue was worked up in the usual way. Chromatography of the crude product on silica gel (150 g) and elution with a gradient of 0–4% ethanol in dichloromethane, afforded pure title compound as a solid foam (4.94 g, 98%) of R_f 0.32 on TLC in ethanol–dichloromethane (1:19 v/v); δ_C-($CDCl_3$) 163.15 (C-4), 157.09 and 153.19 (fluorophenyl C-2),

* Tris = 2-amino-2-(hydroxymethyl)propane-1,3-diol.
† EDTA = ethylenediaminetetraacetic acid.

150.45 (C-2), 139.97 (C-6), 139.48 and 139.34 (fluorophenyl C-1), 135.06 and 134.84 (C-2 and -6 of SiPh), 132.42 and 131.55 (C-1 of SiPh), 129.64 (C-4 of SiPh), 127.57 and 127.52 (C-3 and -5 of SiPh), 123.93 (fluorophenyl C-5), 121.95 (fluorophenyl C-4), 119.00 (fluorophenyl C-6), 115.62 and 115.29 (fluorophenyl C-3), 102.19 (C-5), 99.83 (piperidine C-4), 85.62 (C-1'), 84.93 (C-4'), 72.75 (C-2'), 70.82 (C-3'), 64.04 (C-5'), 47.58 (OMe), 47.58 and 47.18 (piperidine C-2 and -6), 33.85 and 32.49 (piperidine C-3 and -5), 26.60 (CMe_3) and 18.88 (CMe_3).

5'-O-(tert-*Butyldiphenylsilyl*)-2'-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-3'-O-(9-*phenylxanthen*-9-*yl*)*uridine* **U6**. Compound **U5** (4.94 g, 7.16 mmol) was dried by evaporation of dry pyridine under reduced pressure. The residue was dissolved in stirred, anhydrous pyridine (80 cm^3), 9-chloro-9-phenylxanthene (4.19 g, 14.3 mmol) was added with exclusion of moisture, and the reaction mixture was left overnight at room temperature. TLC showed complete reaction, solvent was removed under reduced pressure at room temperature, and the residue was dissolved in dichloromethane (200 cm^3). The solution was washed with 1 mol dm^{-3} aq. sodium hydrogen carbonate (2 × 200 cm^3), dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. The crude product was purified by chromatography on silica gel (180 g) and eluted with a gradient of 0–5% ethanol in triethylamine–dichloromethane (1:99 v/v). The title compound was obtained as a yellow foam (5.69 g, 84%) of R_f 0.63 on TLC in triethylamine–ethanol–dichloromethane (1:5:94 by vol.); δ_C($CDCl_3$) 163.39 (C-4), 157.29 and 153.38 (fluorophenyl C-2), 151.50 (C-2), 151.31 and 151.25 (xanthene C-4a and -10a), 146.71 (C-1 of pixyl phenyl), 139.94 (C-6), 139.94 (fluorophenyl C-1), 135.17 and 134.89 (C-2 and -6 of SiPh), 132.45 and 131.52 (C-1 of SiPh), 131.80 and 131.60 (xanthene C-1 and -8), 129.71 and 129.36 (xanthene C-3 and -6), 129.64 (C-4 of SiPh), 127.75 (C-3 and -5 of pixyl phenyl), 127.57 and 127.51 (C-3 and -5 of SiPh), 127.32 (C-2 and -6 of pixyl phenyl), 126.59 (pixyl phenyl C-4), 124.46 and 123.17 (xanthene C-2 and -7), 123.96 (fluorophenyl C-5), 122.91 and 122.68 (xanthene C-8a and -9a), 121.67 (fluorophenyl C-4), 118.97 (fluorophenyl C-6), 116.18 and 115.41 (xanthene C-4 and -5), 115.75 and 115.31 (fluorophenyl C-3), 102.26 (C-5), 99.27 (piperidine C-4), 84.48 (C-1'), 84.23 (C-4'), 76.37 (xanthene C-9), 73.36 (C-2'), 71.36 (C-3'), 64.11 (C-5'), 47.53 and 47.07 (piperidine C-2 and -6), 46.79 (OMe), 33.10 and 31.40 (piperidine C-3 and -5), 26.75 (CMe_3) and 18.99 (CMe_3).

2'-O-[1-(2-*Fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-3'-O-(9-*phenylxanthen*-9-*yl*)*uridine* **U7**. Compound **U6** (5.69 g, 6 mmol) was dried by evaporation of tetrahydrofuran (THF) under reduced pressure at room temperature. The residue was dissolved in dry THF (20 cm^3) and treated with 1.1 mol dm^{-3} TBAF in THF (6 cm^3) overnight at room temperature. TLC showed more or less complete reaction. The reaction mixture was quenched with pyridine–methanol–water (20 cm^3; 3:1:1 by vol.) and pyridinium Dowex 50 W × 2 – 200 resin (20 g) suspended in pyridine–methanol–water (50 cm^3) was added. The reaction mixture was stirred for 20 min at room temperature, then the resin was filtered off. The filtrate and washings were pooled, and evaporated to dryness under reduced pressure. Residual puridine was removed by co-evaporation with toluene. The crude product was then purified by chromatography on silica gel (150 g) and eluted with a gradient of 0–2% ethanol in triethylamine–dichloromethane (1:99 v/v). The title compound was obtained as a pale yellow foam (3.35 g, 78.9%) of R_f 0.35 on TLC in triethylamine–ethanol–dichloromethane (1:5:94 by vol.); δ_C($CDCl_3$) 163.63 (C-4), 157.40 and 153.49 (fluorophenyl C-2), 151.47 and 151.38 (xanthene C-4a and -10a), 150.98 (C-2), 147.02 (C-1 of pixyl phenyl), 141.75 (C-6), 140.10 and 139.96 (fluorophenyl C-1), 131.72 and 131.61 (xanthene C-1 and -8), 129.85 and 129.58 (xanthene C-3 and -6), 127.62 (C-3 and -5 of pixyl phenyl), 127.42 (C-2 and -6 of pixyl phenyl), 126.67 (pixyl phenyl C-4), 124.52 and 123.71 (xanthene C-2 and -7), 124.09 (fluorophenyl C-5), 123.05 and 122.67 (xanthene C-8a and -9a), 121.78 (fluorophenyl C-4), 119.07 (fluorophenyl C-6), 116.27 and 115.55 (xanthene C-4 and -5), 115.89 and 115.55 (fluorophenyl C-3), 102.34 (C-5), 99.51 (piperidine C-4), 87.24 (C-1'), 84.79 (C-4'), 76.48 (xanthene C-9), 73.55 (C-2'), 70.84 (C-3'), 61.80 (C-5'), 47.68 and 47.19 (piperidine C-2 and -6), 47.00 (OMe) and 33.01 and 31.16 (piperidine C-3 and -5).

2'-O-[1-(2-*Fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-3'-O-(9-*phenylxanthen*-9-*yl*)*uridine* 5'-O-(2-*cyanoethyl* N,N-*diisopropylphosphoramidite*) **U8**. Compound **U7** (3 g, 4.24 mmol) was dissolved in dry 1,2-dichloroethane (20 cm^3) containing *N*,*N*-diisopropylethylamine (1.8 cm^3, 10 mmol) under argon, and chloro-(2-cyanoethoxy)diisopropylaminophosphine (1.12 cm^3, 5.08 mmol) was added to the stirred solution with exclusion of moisture. TLC showed complete reaction after 3 h. The reaction was quenched by addition of ethanol (1 cm^3), dichloromethane (150 cm^3) was added, and the solution was washed with 5% aq. sodium hydrogen carbonate (2 × 150 cm^3), dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure at room temperature. The crude product was purified by chromatography on silica gel (150 g) and eluted with triethylamine–dichloromethane (1:49 v/v). Pure title compound was obtained as a solid foam (3.72 g, 96.9%) of R_f 0.63 on TLC in triethylamine–ethanol–dichloromethane (2:5:93 by vol.); δ_P(CH_2Cl_2; concentric external D_2O lock) 145.59 and 144.82.

3',5'-O-(*Tetraisopropyldisiloxane*-1,3-*diyl*)*cytidine* **C1**. Cytidine (3.67 g, 15.09 mmol) was dissolved in dry, stirred pyridine (30 cm^3) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (5.3 cm^3, 16.88 mmol) was added with exclusion of moisture. The reaction was kept overnight at room temperature, quenched with methanol (3 cm^3), and worked up in the usual way. The product was purified by column chromatography on silica gel (220 g) and eluted with a gradient of ethanol 5–10% in dichloromethane. Evaporation of pure fractions under reduced pressure afforded the title compound as a solid (6.19 g, 84.4%) of R_f 0.40 on TLC in ethanol–dichloromethane (1:9 v/v); δ_C[$(CD_3)_2SO$] 165.67 (C-4), 154.77 (C-2), 139.75 (C-6), 93.25 (C-5), 90.68 (C-1'), 80.46 (C-4'), 74.11 (C-2'), 68.43 (C-3'), 60.00 (C-5'), 17.33–16.72 ($CHMe_2$) and 12.78, 12.48, 12.37 and 11.95 ($CHMe_2$).

4-N-*Benzoyl*-3',5'-O-(*tetraisopropyldisiloxane*-1,3-*diyl*)*cytidine* **C2**. Compound **C1** (5.98 g, 12.31 mmol) was dried by evaporation of pyridine under reduced pressure. The residue was dissolved in dry pyridine (30 cm^3), cooled to 0 °C and then chlorotrimethylsilane (4 cm^3, 34.46 mmol) was added. The reaction mixture was stirred for 30 min at room temperature, cooled to 0 °C, and benzoyl chloride (6.3 cm^3, 49.6 mmol) was added. The reaction mixture was kept 90 min at room temperature, cooled in ice, and water (5 cm^3) was added, followed by 25% aq. ammonia (8 cm^3). The mixture was stirred for 20 min at room temperature and then solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (200 cm^3) and the solution was washed with 1 mol dm^{-3} aq. sodium hydrogen carbonate (2 × 100 cm^3), dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. Residual pyridine was removed by coevaporation with toluene. In order to remove the 2'-*O*-TMS group the residue was dissolved in dichloromethane (100 cm^3) and treated with a solution of toluene-*p*-sulfonic acid (PTSA) monohydrate (4 g, 21 mmol) in 1,4-dioxane (50 cm^3). After 2 min the reaction was quenched by addition of triethylamine (5 cm^3, 36 mmol). Solvent was removed under reduced pressure and the residue was worked up in the usual way. The crude product was purified by column chromatography on silica gel (220 g) and eluted with light petroleum–ethyl acetate (3:1, and 2:1 v/v). Compound

C2 was obtained as a solid foam (6.59 g, 90.8%) of R_f 0.27 on TLC in light petroleum–ethyl acetate (2:1 v/v); $\delta_C(CDCl_3)$ 167.03 (C=O of benzoyl), 162.95 (C-4), 154.88 (C-2), 144.21 (C-6), 133.55 (phenyl C-1), 132.86 (phenyl C-4), 128.61 and 128.25 (phenyl C-3 and -5), 127.92 and 127.35 (phenyl C-2 and -6), 96.66 (C-5), 91.71 (C-1'), 81.84 (C-4'), 74.94 (C-2'), 68.19 (C-3'), 59.80 (C-5'), 17.32–16.70 (CH*Me*$_2$) and 14.08–12.34 (*C*HMe$_2$).

4-N-*Benzoyl*-2'-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-3',5'-O-(*tetraisopropyldisiloxane*-1,3-*diyl*)*cytidine* **C3**. Compound **C2** (6.34 g, 10.75 mmol) and 1-(2-fluorophenyl)-4-methoxy-1,2,5,6-tetrahydropyridine (13.54 g, 65.65 mmol) were dissolved in dry THF (30 cm^3). Mesitylenesulfonic acid dihydrate (0.65 g, 2.75 mmol) was dried by addition, followed by evaporation, of dry acetonitrile (2 × 50 cm^3) under reduced pressure, was redissolved in dry acetonitrile (30 cm^3), and added to the above solution. The reaction mixture was stirred overnight under anhydrous conditions whereupon TLC showed more or less complete reaction. Triethylamine (2.8 cm^3, 20 mmol) was added and the reaction mixture was worked up in the usual way. Chromatography of the residue on silica gel (240 g) and elution with light petroleum–ethyl acetate (3:1, and 2:1 v/v) afforded compound **C3** as a solid foam (6.38 g, 74.5%) of R_f 0.31 on TLC in light petroleum–ethyl acetate (1:1 v/v); $\delta_C(CDCl_3)$ 167.00 (benzoyl C=O), 162.54 (C-4), 157.31 and 153.46 (fluorophenyl C-2), 154.31 (C-2), 144.67 (C-6), 140.21 and 140.09 (fluorophenyl C-1), 133.25 (phenyl C-1), 132.73 (phenyl C-4), 128.59 (phenyl C-3 and -5), 127.62 (phenyl C-2 and -6), 124.10 (fluorophenyl C-5), 121.90 (fluorophenyl C-4), 119.11 (fluorophenyl C-6), 115.95 and 115.62 (fluorophenyl C-3), 100.08 (piperidine C-4), 96.17 (C-5), 90.59 (C-1'), 81.59 (C-4'), 73.27 (C-2'), 67.21 (C-3'), 59.3 (C-5'), 48.51 (OMe), 48.03 (piperidine C-2 and -6), 33.97 and 33.82 (piperidine C-3 and -5), 17.45–16.68 (CH*Me*$_2$) and 13.37–12.63 (*C*HMe$_2$).

4-N-*Benzoyl*-2'-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]*cytidine* **C4**. Compound **C3** (6.07 g, 7.62 mmol) was dissolved in dry THF (30 cm^3) and treated with a solution of 1.1 mol dm^{-3} TBAF in THF (17 cm^3) for 8 min at room temperature. Work-up was identical with the procedure used for the preparation of compound **U7** above. Chromatography of the residue on silica gel (100 g), and elution with a gradient of ethanol 0–5% in dichloromethane, afforded the title compound as a solid foam (4.0 g, 94.7%) of R_f 0.12 on TLC in ethanol–dichloromethane (1:19 v/v); $\delta_C(CDCl_3)$ 167.31 (benzoyl C=O), 163.11 (C-4), 156.64 and 152.94 (fluorophenyl C-2), 154.42 (C-2), 145.68 (C-6), 139.85 and 139.72 (fluorophenyl C-1), 133.10 (phenyl C-1), 132.65 (phenyl C-4), 128.43 (phenyl C-3 and -5), 128.32 (phenyl C-2 and -6), 124.63 (fluorophenyl C-5), 122.16 and 122.04 (fluorophenyl C-4), 119.46 (fluorophenyl C-6), 115.90 and 115.58 (fluorophenyl C-3), 99.11 (piperidine C-4), 96.98 (C-5), 87.09 (C-1'), 86.33 (C-4'), 73.42 (C-2'), 70.58 (C-3'), 61.35 (C-5'), 47.60 (OMe), 47.23 and 47.07 (piperidine C-2 and -6) and 33.71 and 32.31 (piperidine C-3 and -5).

4-N-*Benzoyl*-5'-O-(tert-*butyldiphenylsilyl*)-2'-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]*cytidine* **C5**. Compound **C4** (3.53 g, 6.37 mmol) was silylated and worked up according to the procedure used to prepare compound **U5** above. The residue was purified by chromatography on silica gel (150 g) and eluted with light petroleum–ethyl acetate (2:1, 1:1, and 1:2 v/v) to yield pure title compound as a solid foam (4.89 g, 96.8%) of R_f 0.37 on TLC in ethanol–dichloromethane (1:19 v/v); $\delta_C(CDCl_3)$ 167.25 (benzoyl C=O), 162.04 (C-4), 157.09 and 153.19 (fluorophenyl C-2), 154.21 (C-2), 144.43 (C-6), 139.60 and 139.47 (fluorophenyl C-1), 135.12 and 134.94 (C-2 and -6 of SiPh), 132.95 (phenyl C-1), 132.34 (C-1 of SiPh), 131.80 (phenyl C-4), 129.68 (C-4 of SiPh), 128.23 (phenyl C-3 and -5), 127.56 (phenyl C-2 and -6; C-3 and -5 of SiPh), 123.87 (fluorophenyl C-5), 121.73 (fluorophenyl C-4), 118.94 (fluorophenyl C-6), 115.61 and 115.28 (fluorophenyl C-3), 100.06 (piperidine C-4), 97.06 (C-5), 87.69 (C-1'), 87.69 (C-4'), 74.03 (C-2'), 69.80 (C-3'), 63.31 (C-5'), 47.86 (OMe), 47.52 and 47.26 (piperidine C-2 and -6), 33.63 and 32.64 (piperidine C-3 and -5), 26.61 (C*Me*$_3$) and 18.85 (*C*Me$_3$).

4-N-*Benzoyl*-5'-O-(tert-*butyldiphenylsilyl*)-2'-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-3'-O-(9-*phenylxanthen*-9-yl)-*cytidine* **C6**. Compound **C5** (4 g, 5.04 mmol) was pixylated according to the procedure used to prepare compound **U6** above, using 1.6 mol equiv. of pixyl chloride. The crude product was purified by column chromatography on silica gel (175 g) and eluted with ethyl acetate–light petroleum (1:2, and 1:1 v/v) containing 1% triethylamine. Pure compound **C6** was obtained as a solid foam (5.04 g, 95.2%) of R_f 0.20 on TLC in ethyl acetate–light petroleum (1:1 v/v) containing 1% triethylamine; $\delta_C(CDCl_3)$ 167.21 (benzoyl C=O), 161.63 (C-4), 157.61 and 153.70 (fluorophenyl C-2), 154.85 (C-2), 151.76 and 151.58 (xanthene C-4a and -10a), 147.03 (pixyl phenyl C-1), 144.74 (C-6), 140.32 and 140.19 (fluorophenyl C-1), 135.49 and 135.22 (C-2 and -6 of SiPh), 133.46 (benzoyl C-1), 132.94 and 132.68 (C-1 of SiPh), 132.11 (benzoyl C-4), 132.11 and 131.79 (xanthene C-1 and -8), 130.00 (C-4 of SiPh), 130.00 and 129.57 (xanthene C-3 and -6), 128.79 (benzoyl C-3 and -5), 127.96 (benzoyl C-2 and -6), 127.90 (C-3 and -5 of SiPh), 127.69 (C-3 and -5 of pixyl phenyl), 127.58 (pixyl phenyl C-2 and -6), 126.84 (pixyl phenyl C-4), 124.69 and 123.17 (xanthene C-2 and -7), 124.21 (fluorophenyl C-5), 123.54 and 122.94 (xanthene C-8a and -9a), 121.95 and 121.83 (fluorophenyl C-4), 119.24 (fluorophenyl C-6), 116.44 and 115.55 (xanthene C-4 and -5), 116.03 and 115.70 (fluorophenyl C-3), 99.56 (piperidine C-4), 97.55 (C-5), 85.70 (C-1'), 84.76 (C-4'), 76.66 (xanthene C-9), 73.75 (C-2'), 73.18 (C-3'), 64.31 (C-5'), 47.81 and 47.38 (piperidine C-2 and -6), 47.18 (OMe), 33.37 and 31.66 (piperidine C-3 and -5), 27.07 (C*Me*$_3$) and 19.23 (*C*Me$_3$).

4-N-*Benzoyl*-2'-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-3'-O-(9-*phenylxanthen*-9-*yl*)*cytidine* **C7**. Compound **C6** (4.72 g, 4.50 mmol) was desilylated according to the procedure used to prepare compound **U7** above. The crude product was purified by chromatography on silica gel (100 g) and eluted with light petroleum–ethyl acetate (2:1, 1:1, and 1:2 v/v) containing 1% triethylamine. Compound **C7** was obtained as a solid foam (3.13 g, 85.8%) of R_f 0.25 on TLC in triethylamine–ethanol–dichloromethane (1:3:96 by vol.); $\delta_C(CDCl_3)$ 167.20 (benzoyl C=O), 162.19 (C-4), 157.52 and 153.16 (fluorophenyl C-2), 155.01 (C-2), 151.67 and 151.52 (xanthene C-4a and -10a), 147.33 (C-6), 147.21 (pixyl phenyl C-1), 140.27 and 140.13 (fluorophenyl C-1), 133.00 (benzoyl C-1), 132.91 (benzoyl C-4), 131.76 and 131.55 (xanthene C-1 and -8), 129.92 and 129.65 (xanthene C-3 and -6), 128.71 (benzoyl C-3 and -5), 127.73 (pixyl phenyl C-3 and -5), 127.57 (benzoyl C-2 and -6), 127.57 (pixyl phenyl C-2 and -6), 126.78 (pixyl phenyl C-4), 124.46 and 123.08 (xanthene C-2 and -7), 124.18 (fluorophenyl C-5), 123.36 and 122.69 (xanthene C-8a and -9a), 121.88 and 121.76 (fluorophenyl C-4), 119.16 (fluorophenyl C-6), 116.45 and 115.76 (xanthene C-4 and -5), 115.98 and 115.66 (fluorophenyl C-3), 99.64 (piperidine C-4), 97.34 (C-5), 90.71 (C-1'), 85.17 (C-4'), 76.71 (xanthene C-9), 73.46 (C-2'), 73.33 (C-3'), 61.92 (C-5'), 47.78 and 47.42 (piperidine C-2 and -6), 47.26 (OMe) and 33.19 and 31.57 (piperidine C-3 and -5).

4-N-*Benzoyl*-2'-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-3'-O-(9-*phenylxanthen*-9-*yl*)*cytidine* 5'-O-(2-*cyanoethyl* N,N-*diisopropylphosphoramidite*) **C8**. Compound **C7** (2.81 g, 3.47 mmol) was phosphitylated according to the procedure used to prepare compound **U8** above. Chromatography of the crude product on silica gel (120 g) and elution with light petroleum–dichloromethane (1:2 v/v) containing 3% triethylamine afforded the title compound as a solid pale yellow foam (3.01 g, 85.8%) of R_f 0.44 on TLC in triethylamine–ethanol–dichloro-

methane (1:5:94 by vol.); $\delta_P(CH_2Cl_2$; concentric external D_2O lock) 146.16 and 145.56.

6-N-*Pivaloyl*-3',5'-O-(*tetraisopropyldisiloxane*-1,3-*diyl*)*adenosine* **A2**. 3',5'-*O*-(Tetraisopropyldisiloxane-1,3-diyl)adenosine, compound **A1** (5.06 g, 9.93 mmol) was converted into compound **A2** by the procedure given for the preparation of compound **C2** above but substituting pivaloyl chloride for benzoyl chloride. The residue was purified by chromatography on silica gel (180 g) and elution with light petroleum–ethyl acetate (1:2 v/v). Pure compound **A2** was obtained as a solid foam (4.38 g, 74.3%) of R_f 0.15 on TLC in light petroleum–ethyl acetate (1:2 v/v); $\delta_C(CDCl_3)$ 175.50 (pivaloyl C=O), 152.49 (C-2), 150.78 (C-6), 149.63 (C-4), 141.76 (C-8), 123.46 (C-5), 89.77 (C-1'), 82.21 (C-4'), 74.98 (C-2'), 70.92 (C-3'), 61.76 (C-5'), 40.44 (CMe_3), 27.33 (Me_3C), 17.27–16.90 ($CHMe_2$) and 13.21–12.58 ($CHMe_2$).

2'-O-[1-(2-*Fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-6-N-*pivaloyl*-3',5'-O-(*tetraisopropyldisiloxane*-1,3-*diyl*)*adenosine* **A3**. Compound **A2** (4.04 g, 6.8 mmol) was converted into compound **A3** by the procedure described above for compound **C3**. Column chromatography of the crude product on silica gel (240 g) and elution with light petroleum–ethyl acetate (3:1, then 2:1 v/v) afforded the title compound as a solid pale yellow foam (4.70 g, 86.3%) of R_f 0.21 on TLC in light petroleum–ethyl acetate (1:1 v/v); $\delta_C(CDCl_3)$ 175.41 (pivaloyl C=O), 157.22 and 153.31 (fluorophenyl C-2), 152.49 (C-2), 150.63 (C-6), 149.96 (C-4), 141.29 (C-8), 140.09 and 139.96 (fluorophenyl C-1), 124.16 (fluorophenyl C-5), 123.17 (C-5), 122.18 and 122.06 (fluorophenyl C-4), 119.21 (fluorophenyl C-6), 116.07 and 115.73 (fluorophenyl C-3), 100.07 (piperidine C-4), 89.36 (C-1'), 81.54 (C-4'), 73.36 (C-2'), 68.41 (C-3'), 59.80 (C-5'), 48.48 (OMe), 47.91 (piperidine C-2 and -6), 40.41 (CMe_3), 34.51 and 33.52 (piperidine C-3 and -5), 27.31 (CMe_3), 17.39–16.81 ($CHMe_2$) and 13.24–12.86 ($CHMe_2$).

2'-O-[1-(2-*Fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-6-N-*pivaloyladenosine* **A4**. Compound **A3** (4.59 g, 5.73 mmol) was desilylated according to the procedure used to prepare compound **C4** above. The crude product was purified by chromatography on silica gel (100 g) and eluted with a gradient of ethanol 0–5% in dichloromethane. This afforded the title compound as a solid foam (3.17 g, 99%) of R_f 0.52 on TLC in ethanol–dichloromethane (1:19 v/v); $\delta_C(CDCl_3)$ 175.58 (pivaloyl C=O), 157.42 and 153.52 (fluorophenyl C-2), 152.18 (C-2), 150.30 (C-4 and -6), 142.98 (C-8), 139.54 and 139.40 (fluorophenyl C-1), 124.23 (fluorophenyl C-5), 123.89 (C-5), 122.60 (fluorophenyl C-4), 119.23 (fluorophenyl C-6), 116.01 and 115.68 (fluorophenyl C-3), 100.08 (piperidine C-4), 89.31 (C-1'), 87.77 (C-4'), 72.31 (C-2'), 72.13 (C-3'), 63.11 (C-5'), 47.80 and 47.44 (piperidine C-2 and -6), 47.44 (OMe), 40.45 (CMe_3), 34.34 and 33.42 (piperidine C-3 and -5) and 27.13 (CMe_3).

5'-O-(tert-*Butyldiphenylsilyl*)-2'-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-6-N-*pivaloyladenosine* **A5**. Compound **A4** (3.16 g, 5.66 mmol) was silylated according to the procedure for compound **U5** above. Chromatography of the crude product on silica gel (100 g) and elution with light petroleum–ethyl acetate (2:1, then 1:1 v/v) afforded a solid foam (3.80 g, 84.2%) of R_f 0.20 on TLC in light petroleum–ethyl acetate (1:1 v/v); $\delta_C(CDCl_3)$ 175.24 (pivaloyl C=O), 157.21 and 153.31 (fluorophenyl C-2), 152.42 (C-2), 151.46 (C-6), 149.35 (C-4), 141.54 (C-8), 139.43 and 139.30 (fluorophenyl C-1), 135.14 (C-2 and -6 of SiPh), 132.53 and 132.34 (C-1 of SiPh), 129.54 (C-4 of SiPh), 127.50 (C-3 and -5 of SiPh), 124.03 (fluorophenyl C-5), 122.73 (C-5), 122.16 (fluorophenyl C-4), 118.98 (fluorophenyl C-6), 115.80 and 115.47 (fluorophenyl C-3), 100.04 (piperidine C-4), 86.28 (C-1'), 85.48 (C-4'), 72.66 (C-2'), 70.89 (C-3'), 63.67 (C-5'), 47.65 and 47.17 (piperidine C-2 and -6), 47.65 (OMe), 40.14 (CMe_3 of pivaloyl), 33.88 and 33.72 (piperidine C-3 and -5), 27.03 (Me_3C pivaloyl), 26.69 (Me_3C) and 18.95 (CMe_3).

5'-O-(tert-*Butyldiphenylsilyl*)-2'-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-3'-O-(9-*phenylxanthen*-9-*yl*)-6-N-*pivaloyladenosine* **A6**. Compound **A5** (3.77 g, 4.73 mmol) was pixylated as described for the synthesis of compound **U6** above. The crude product was purified by chromatography on silica gel (150 g) and elution with light petroleum–ethyl acetate (3:1, then 2:1 v/v) containing 1% triethylamine. The title compound was obtained as a solid foam (4.85 g, 97.4%) of R_f 0.23 on TLC in light petroleum–ethyl acetate (1:1 v/v) containing 1% triethylamine; $\delta_C(CDCl_3)$ 175.17 (pivaloyl C=O), 157.21 and 153.30 (fluorophenyl C-2), 152.43 (C-2), 152.05 (C-6), 151.40 and 151.16 (xanthene C-4a and -10a), 149.21 (C-4), 146.97 (pixyl phenyl C-1), 141.14 (C-8), 139.83 and 139.70 (fluorophenyl C-1), 135.15 and 134.92 (C-2 and -6 of SiPh), 132.38 and 132.10 (C-1 of SiPh), 131.62 and 131.30 (xanthene C-1 and -8), 129.60 and 129.30 (xanthene C-3 and -6), 129.50 (C-4 of SiPh), 127.47 (pixyl phenyl C-3 and -5; C-3 and -5 of SiPh), 127.27 (pixyl phenyl C-2 and -6), 126.55 (pixyl phenyl C-4), 124.26 and 123.09 (xanthene C-2 and -7), 123.91 (fluorophenyl C-5), 123.09 (C-5), 122.57 and 122.43 (xanthene C-8a and -9a), 121.63 (fluorophenyl C-4), 118.87 (fluorophenyl C-6), 116.21 and 115.40 (xanthene C-4 and -5), 115.71 and 115.40 (fluorophenyl C-3), 99.29 (piperidine C-4), 84.67 (C-1'), 84.54 (C-4'), 76.39 (xanthene C-9), 72.96 (C-2'), 72.37 (C-3'), 63.76 (C-5'), 47.49 and 46.49 (piperidine C-2 and -6), 46.68 (OMe), 40.03 (CMe_3 of pivaloyl), 33.21 and 31.24 (piperidine C-3 and -5), 27.03 (Me_3C of pivaloyl), 26.72 (CMe_3) and 18.85 (CMe_3).

2'-O-[1-(2-*Fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-3'-O-(9-*phenylxanthen*-9-*yl*)-6-N-*pivaloyladenosine* **A7**. Compound **A6** (4.8 g, 4.56 mmol) was desilylated according to the procedure used to prepare compound **U7** above. Chromatography of the reaction product on silica gel (120 g) and elution with a gradient of 0–3% ethanol in dichloromethane afforded the title compound as a solid foam (3.45 g, 92.9%) of R_f 0.17 on TLC in ethyl acetate–light petroleum (2:1 v/v); $\delta_C(CDCl_3)$ 175.06 (pivaloyl C=O), 157.02 and 153.11 (fluorophenyl C-2), 151.45 (C-2), 150.30 (C-6), 151.25 and 151.20 (xanthene C-4a and -10a), 149.70 (C-4), 146.78 (pixyl phenyl C-1), 142.94 (C-8), 139.67 and 139.53 (fluorophenyl C-1), 131.25 and 131.11 (xanthene C-1 and -8), 129.52 and 129.32 (xanthene C-3 and -6), 127.30 (pixyl phenyl C-3 and -5), 127.09 (pixyl phenyl C-2 and -6), 126.41 (pixyl phenyl C-4), 124.11 and 122.75 (xanthene C-2 and -7), 123.87 (C-5), 123.75 (fluorophenyl C-5), 122.75 and 122.27 (xanthene C-8a and -9a), 121.39 (fluorophenyl C-4), 118.70 (fluorophenyl C-6), 116.16 and 115.42 (xanthene C-4 and -5), 115.50 and 115.18 (fluorophenyl C-3), 99.14 (piperidine C-4), 88.41 (C-1'), 86.15 (C-4'), 76.26 (xanthene C-9), 73.76 (C-2'), 70.76 (C-3'), 62.11 (C-5'), 47.15 and 46.92 (piperidine C-2 and -6), 46.13 (OMe), 39.84 (CMe_3 of pivaloyl), 32.87 and 30.81 (piperidine C-3 and -5) and 26.77 (Me_3C of pivaloyl).

2'-O-[1-(2-*Fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-3'-O-(9-*phenylxanthen*-9-*yl*)-6-N-*pivaloyladenosine* 5'-O-(2-*cyanoethyl* N,N-*diisopropylphosphoramidite*) **A8**. Compound **A7** (3.36 g, 4.12 mmol) was phosphitylated according to the procedure used to synthesize compound **U8** above. Chromatography of the crude product on silica gel (180 g) and elution with light petroleum–dichloromethane (1:2, then 1:3 v/v) containing 3% triethylamine afforded pure compound **A8** as a solid foam (3.83 g, 91.6%) of R_f 0.64 on TLC in triethylamine–ethanol–dichloromethane (1:5:95 by vol.); $\delta_P(CH_2Cl_2$; concentric external D_2O lock) 145.87 and 144.60.

2-N-(*Dimethylaminomethylene*)*guanosine* **G1**. Guanosine (3.96 g, 14 mmol) was suspended in dry methanol (100 cm^3) and DMF dimethyl acetal (10 cm^3, 76 mmol) was added. The mixture was stirred overnight at room temperature and the desired product was removed by filtration. The solid was washed with methanol and dried over P_2O_5 *in vacuo*. The title compound was obtained as a powder (4.74 g, 100%);

δ_C[$(CD_3)_2SO$] 157.97 (amidine CH), 157.80 (C-6), 157.39 (C-2), 150.07 (C-4), 137.08 (C-8), 119.87 (C-5), 87.03 (C-1'), 85.56 (C-4'), 73.97 (C-2'), 70.60 (C-3'), 61.60 (C-5') and 40.67 and 34.65 (amidine Me_2N).

2-N-*Dimethylaminomethylene*-3',5'-O-(*tetraisopropyldisiloxane*-1,3-*diyl*)*guanosine* **G2**. Compound **G1** (4.74 g, 14 mmol) was silylated according to the procedure used to prepare compound **C1** above. The crude product was chromatographed on silica gel (180 g) and eluted with a gradient of 0–10% ethanol in dichloromethane. This afforded pure title compound as a solid foam (6.66 g, 81.9%) of R_f 0.26 on TLC in ethanol–dichloromethane (1:19 v/v); $\delta_C(CDCl_3)$ 158.19 (C-6), 157.91 (amidine CH), 156.73 (C-2), 149.44 (C-4), 135.14 (C-8), 119.80 (C-5), 88.05 (C-1'), 81.23 (C-4'), 74.77 (C-2'), 69.54 (C-3'), 57.38 (C-5'), 41.02 and 34.71 (amidine Me_2N), 16.94–16.49 ($CHMe_2$) and 13.03, 12.54 and 12.13 ($CHMe_2$).

2-N-*Dimethylaminomethylene*-2'-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-3',5'-O-(*tetraisopropyldisiloxane*-1,3-*diyl*)*guanosine* **G3**. Compound **G2** (6.60 g, 11.36 mmol) was converted into compound **G3** by the procedure described above for compound **C3**. Chromatography of the crude product on silica gel (170 g) and elution with a gradient of ethanol 0–5% in dichloromethane, afforded pure compound **G3** as a solid foam (7.72 g, 86.2%) of R_f 0.38 on TLC in ethanol–dichloromethane (1:19 v/v); $\delta_C(CDCl_3)$ 158.01 (C-6 and amidine CH), 157.14 and 153.25 (fluorophenyl C-2), 156.60 (C-2), 148.64 (C-4), 139.73 and 139.59 (fluorophenyl C-1), 135.27 (C-8), 123.98 (fluorophenyl C-5), 122.01 and 121.89 (fluorophenyl C-4), 120.33 (C-5), 118.90 (fluorophenyl C-6), 115.70 and 115.37 (fluorophenyl C-3), 99.53 (piperidine C-4), 87.99 (C-1'), 81.12 (C-4'), 73.61 (C-2'), 67.72 (C-3'), 57.38 (C-5'), 47.68 (OMe), 47.51 and 47.04 (piperidine C-2 and -6), 40.76 and 34.75 (amidine Me_2N), 34.45 and 33.12 (piperidine C-3 and -5), 17.18–16.55 ($CHMe_2$) and 12.94, 12.78 and 12.51 ($CHMe_2$).

2-N-*Dimethylaminomethylene*-2'-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]*guanosine* **G4**. Compound **G3** (7.72 g, 9.8 mmol) was desilylated according to the procedure used to prepare compound **C4** above. The crude product was purified by chromatography on silica gel (150 g) and eluted with a gradient of ethanol 0–20% in dichloromethane to afford a solid, off-white foam (5.06 g, 94.6%) of R_f 0.2 on TLC in ethanol–dichloromethane (1:9 v/v); $\delta_C(CDCl_3)$ 158.19 (C-6), 157.97 (amidine CH), 157.31 and 153.41 (fluorophenyl C-2), 157.11 (C-2), 149.60 (C-4), 139.64 and 139.50 (fluorophenyl C-1), 138.51 (C-8), 124.16 (fluorophenyl C-5), 122.37 (fluorophenyl C-4), 121.03 (C-5), 119.19 (fluorophenyl C-6), 115.87 and 115.53 (fluorophenyl C-3), 99.88 (piperidine C-4), 88.11 (C-1'), 86.73 (C-4'), 72.24 (C-2'), 71.68 (C-3'), 62.45 (C-5'), 47.83 and 47.38 (piperidine C-2 and -6), 47.38 (OMe), 41.08 and 34.84 (amidine Me_2N) and 34.28 and 33.53 (piperidine C-3 and -5).

5'-O-(tert-*Butyldiphenylsilyl*)-2-N-*dimethylaminomethylene*-2'-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]*guanosine* **G5**. Compound **G4** (5.06 g, 9.27 mmol) was silylated according to the procedure used for compound **U5** above. Chromatography of the crude product on silica gel (120 g) and elution with a gradient of ethanol 0–5% in dichloromethane afforded the title compound as a solid foam (6.99 g, 96.2%) of R_f 0.26 on TLC in ethanol–dichloromethane (1:19 v/v); $\delta_C(CDCl_3)$ 157.70 (C-6), 157.51 (amidine CH), 156.84 and 152.94 (fluorophenyl C-2), 156.52 (C-2), 150.26 (C-4), 139.29 and 139.16 (fluorophenyl C-1), 135.53 (C-8), 134.89 and 134.76 (C-2 and -6 of SiPh), 132.12 and 131.92 (C-1 of SiPh), 129.28 (C-4 of SiPh), 127.23 (C-3 and -5 of SiPh), 123.77 (fluorophenyl C-5), 121.77 (fluorophenyl C-4), 119.20 (fluorophenyl C-6), 118.72 (C-5), 115.36 and 115.03 (fluorophenyl C-3), 99.55 (piperidine C-4), 85.16 (C-1'), 84.15 (C-4'), 73.29 (C-2'), 70.27 (C-3'), 63.78 (C-5'), 47.36 and 46.98 (piperidine C-2 and -6), 47.36 (OMe), 40.45 and 34.29 (amidine Me_2N), 33.49 and 32.44 (piperidine C-3 and -5), 26.33 (CMe_3) and 18.59 (CMe_3).

5'-O-(tert-*Butyldiphenylsilyl*)-2-N-*dimethylaminomethylene*-2'-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-3'-O-(9-*phenylxanthen*-9-*yl*)*guanosine* **G6**. Compound **G5** (6.99 g, 8.92 mmol) was pixylated according to the procedure used to prepare compound **U6** above. The crude product was purified by chromatography on silica gel (150 g) and eluted with a gradient of ethanol 0–5% in dichloromethane containing 0.5% triethylamine. This yielded a solid, pale yellow foam (8.87 g, 95.6%) of R_f 0.30 on TLC in triethylamine–ethanol–dichloromethane (1:5:94 by vol.); $\delta_C(CDCl_3)$ 158.32 (C-6 and amidine CH), 157.57 and 153.66 (fluorophenyl C-2), 156.94 (C-2), 151.78 and 151.59 (xanthene C-4a and -10a), 151.19 (C-4), 147.32 (pixyl phenyl C-1), 140.31 and 140.17 (fluorophenyl C-1), 137.70 (C-8), 135.52 and 135.24 (C-2 and -6 of SiPh), 132.49 and 132.41 (C-1 of SiPh), 131.87 and 131.67 (xanthene C-1 and -8), 130.02 and 129.64 (xanthene C-3 and -6), 129.80 (C-4 of SiPh), 127.81 (C-3 and -5 of SiPh, and pixyl phenyl C-3 and -5), 127.48 (pixyl phenyl C-2 and -6), 126.86 (pixyl phenyl C-4), 124.68 and 123.29 (xanthene C-2 and -7), 124.20 (fluorophenyl C-5), 123.09 and 122.76 (xanthene C-8a and -9a), 121.97 and 121.86 (fluorophenyl C-4), 119.24 (fluorophenyl C-6), 120.00 (C-5), 116.58 and 115.71 (xanthene C-4 and -5), 115.99 and 115.71 (fluorophenyl C-3), 99.51 (piperidine C-4), 84.79 (C-1'), 83.55 (C-4'), 76.70 (xanthene C-9), 73.32 (C-2'), 72.61 (C-3'), 64.16 (C-5'), 47.83 (OMe), 47.30 and 47.13 (piperidine C-2 and -6), 41.23 and 35.16 (amidine Me_2N), 33.47 and 31.51 (piperidine C-3 and -5), 26.98 (CMe_3) and 19.15 (CMe_3).

2-N-*Dimethylaminomethylene*-2'-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-3'-O-(9-*phenylxanthen*-9-*yl*)*guanosine* **G7**. Compound **G6** (8.7 g, 8.4 mmol) was desilylated according to the procedure used to prepare compound **U7** above. The crude product was purified by chromatography on silica gel (150 g) and eluted with a gradient of ethanol 0–5% in dichloromethane containing 0.5% triethylamine. Pure compound **G7** was obtained as a solid, pale yellow foam (6.34 g, 94.1%) of R_f 0.20 on TLC in triethylamine–ethanol–dichloromethane (1:5:94 by vol.); $\delta_C(CDCl_3)$ 157.92 (C-6), 157.71 (amidine CH), 157.22 and 153.32 (fluorophenyl C-2), 157.08 (C-2), 151.42 and 151.34 (xanthene C-4a and -10a), 149.31 (C-4), 147.00 (pixyl phenyl C-1), 139.97 and 139.83 (fluorophenyl C-1), 138.54 (C-8), 131.52 and 131.43 (xanthene C-1 and -8), 129.74 and 129.52 (xanthene C-3 and -6), 127.53 (pixyl phenyl C-3 and -5), 127.29 (pixyl phenyl C-2 and -6), 126.60 (pixyl phenyl C-4), 124.39 and 123.34 (xanthene C-2 and -7), 123.93 (fluorophenyl C-5), 123.34 and 122.88 (xanthene C-8a and -9a), 122.88 and 122.59 (fluorophenyl C-4), 121.58 (C-5), 118.90 (fluorophenyl C-6), 116.33 and 115.52 (xanthene C-4 and -5), 115.70 and 115.37 (fluorophenyl C-3), 99.27 (piperidine C-4), 88.18 (C-1'), 85.70 (C-4'), 76.36 (xanthene C-9), 74.15 (C-2'), 70.58 (C-3'), 62.09 (C-5'), 47.46 and 47.09 (piperidine C-2 and -6), 46.27 (OMe), 40.82 and 34.61 (amidine Me_2N) and 33.16 and 30.91 (piperidine C-3 and -5).

2-N-*Dimethylaminomethylene*-2'-O-[1-(2-*fluorophenyl*)-4-*methoxypiperidin*-4-*yl*]-3'-O-(9-*phenylxanthen*-9-*yl*)*guanosine* 5'-O-(2-*cyanoethyl* N,N-*diisopropylphosphoramidite*) **G8**. Compound **G7** (5.53 g, 6.9 mmol) was phosphitylated according to the procedure used to prepare compound **U8** above. Chromatography of the crude product on silica gel (150 g) and elution with light petroleum–dichloromethane (2:3 v/v) containing 2% triethylamine afforded pure title compound as a solid foam (5.73 g, 82.9%) of R_f 0.51 on TLC in triethylamine–ethanol–dichloromethane (1:5:94 by vol.); $\delta_P(CH_2Cl_2$; concentric external D_2O lock) 146.35 and 144.76.

Synthesis of Branch-point Monomers.—6-N-*Benzoyl*-5'-O-(tert-*butyldiphenylsilyl*)*adenosine* **A10**. 6-*N*-Benzoyladenosine,

compound **A9** (6.68 g, 18 mmol), was silylated with *tert*-butyl(chloro)diphenylsilane (18 mmol) according to the procedure used to prepare compound **U5** above. However, the reaction was complete after 1 h. The crude product was purified by chromatography on silica gel (170 g) and elution with a gradient of ethanol 0–5% in dichloromethane. Pure title compound was obtained as a solid foam (7.63 g, 69.5%) of R_f 0.42 on TLC in ethanol–dichloromethane (1:19 v/v); δ_C-($CDCl_3$) 165.36 (benzoyl C=O), 152.02 (C-2), 151.85 (C-6), 149.51 (C-4), 141.08 (C-8), 135.19 and 135.03 (C-2 and -6 of SiPh), 133.36 (benzoyl C-1), 132.47 and 132.21 (C-1 of SiPh), 132.29 (benzoyl C-4), 129.56 and 129.51 (C-4 of SiPh), 128.19 (C-3 and -5 of phenyls), 127.89 (benzoyl C-2 and -6), 122.36 (C-5), 88.81 (C-1'), 85.01 (C-4'), 74.74 (C-2'), 69.89 (C-3'), 63.10 (C-5'), 26.50 (CMe_3) and 18.83 (CMe_3).

6-N-*Benzoyl*-5'-O-(tert-*butyldiphenylsilyl*)-2',3'-*bis*-O-(9-*phenylxanthen*-9-*yl*)*adenosine* **A11**. Compound **A10** (3.65 g, 6 mmol) was pixylated with 3 mol equiv. of pixyl chloride according to the procedure used to prepare compound **U6** above. Purification of the crude product on silica gel (180 g) and elution with light petroleum–ethyl acetate (2:1, then 1:1 v/v) containing 1% triethylamine afforded pure title compound as a solid foam (6.6 g, 98%) of R_f 0.36 on TLC in light petroleum–ethyl acetate (1:1 v/v) containing 1% triethylamine.

6-N-*Benzoyl*-2',3'-*bis*-O-(9-*phenylxanthen*-9-*yl*)*adenosine* **A12**. Compound **A11** (6.6 g, 5.88 mmol) was desilylated according to the procedure used to prepare compound **U7** above. The reaction required two days to reach completion. The residue was purified by chromatography on silica gel (180 g) and eluted with light petroleum–ethyl acetate (3:1 v/v) containing 1% triethylamine, followed by light petroleum–ethyl acetate (1:1 v/v) containing 5% ethanol and 1% triethylamine. Pure product was obtained as a solid foam (5.05 g, 97%) of R_f 0.78 on TLC in triethylamine–ethanol–dichloromethane (1:5:94 by vol.); δ_C($CDCl_3$) 164.22 (benzoyl C=O), 151.12 (C-2), 150.78, 150.62, 147.66 and 147.52 (xanthene C-4a and -10a), 150.09 (C-6), 149.38 (C-4), 149.11 and 148.58 (pixyl phenyl C-1), 142.77 (C-8), 130.66–126.52 (xanthene C-1, -8, -3 and -6; pixyl phenyl C-2, -3, -5, -6; and benzoyl C-2, -3, -5, -6), 126.25 and 125.79 (pixyl phenyl C-4), 124.15, 123.43, 122.94 and 122.61 (xanthene C-2 and -7), 122.61 (C-5), 122.80, 122.14, 121.95 and 120.70 (xanthene C-8a and -9a), 115.76, 115.52, 115.40 and 114.03 (xanthene C-4 and -5), 87.74 (C-1'), 83.75 (C-4'), 76.57 and 75.63 (xanthene C-9), 74.11 (C-2'), 73.46 (C-3') and 61.75 (C-5').

6-N-*Benzoyl*-2',3'-*bis*-O-(9-*phenylxanthen*-9-*yl*)*adenosine* 5'-O-(2-*cyanoethyl* N,N-*diisopropylphosphoramidite*) **A13**. Compound **A12** (5.05 g, 5.71 mmol) was phosphitylated according to the procedure used to prepare compound **U8**. Chromatography of the crude product on silica gel (150 g) and elution with triethylamine–dichloromethane (1:49 v/v) afforded a solid foam (6.02 g, 97.2%) of R_f 0.86 on TLC in triethylamine–ethanol–dichloromethane (1:5:94 by vol.); δ_P(CH_2Cl_2; concentric external D_2O lock) 144.57 and 143.22.

5'-O-(tert-*Butyldiphenylsilyl*)*uridine* **U9**. Uridine, compound **U1** (2.44 g, 10 mmol), was silylated according to the procedure used to prepare compound **U5**. The crude product was purified by chromatography on silica gel (100 g) and eluted with a gradient of ethanol 0–5% in dichloromethane. Pure product was obtained as a solid foam (3.64 g, 75.4%) of R_f 0.10 on TLC in ethanol–dichloromethane (1:19 v/v); δ_C($CDCl_3$) 163.63 (C-4), 150.96 (C-2), 139.92 (C-6), 135.24 and 134.99 (C-2 and -6 of SiPh), 132.58 and 131.91 (C-1 of SiPh), 129.67 (C-4 of SiPh), 127.59 (C-3 and -5 of SiPh), 101.91 (C-5), 89.30 (C-1'), 84.05 (C-4'), 74.95 (C-2'), 69.03 (C-3'), 62.57 (C-5'), 26.67 (CMe_3) and 18.96 (CMe_3).

5'-O-(tert-*Butyldiphenylsilyl*)-2',3'-*bis*-O-(9-*phenylxanthen*-9-*yl*)*uridine* **U10**. Compound **U9** (3.64 g, 7.54 mmol) was pixylated with 3 mol equiv. of pixyl chloride by using the procedure described for the preparation of compound **U6**. Purification of the crude product by chromatography on silica gel (100 g) and elution with a gradient of ethanol 0–5% in triethylamine–dichloromethane (1:199 v/v) afforded pure material as a solid, off-white foam (7.5 g, 100%) of R_f 0.55 on TLC in triethylamine–ethanol–dichloromethane (1:5:94 by vol.).

2',3'-O-*Bis*-(9-*phenylxanthen*-9-*yl*)*uridine* **U11**. Compound **U10** (7.5 g, 7.54 mmol) was desilylated according to the procedure used to prepare compound **U7**, with a reaction time of 24 h. The crude product was purified by chromatography on silica gel (150 g) and eluted with a gradient of ethanol 0–10% in triethylamine–dichloromethane (1:199 v/v). Pure product was obtained as a solid foam (3.92 g, 68.7%) of R_f 0.38 on TLC in triethylamine–ethanol–dichloromethane (1:5:94 by vol.); δ_C($CDCl_3$) 163.47 (C-4), 151.59, 151.38, 151.10 and 150.38 (xanthene C-4a and -10a), 149.03 (C-2), 148.33 and 147.68 (pixyl phenyl C-1), 139.86 (C-6), 132.36–126.10 (xanthene C-1, -8, -3 and -6; and pixyl phenyl C-2, -6, -3, -5 and -4), 123.75, 123.08, 122.66 and 121.48 (xanthene C-2 and -7), 115.96, 115.75 and 115.51 (xanthene C-4 and -5), 102.43 (C-5), 86.21 (C-1'), 82.86 (C-4'), 76.80 and 76.03 (xanthene C-9), 74.68 (C-2'), 74.25 (C-3') and 61.54 (C-5').

2',3'-O-*Bis*-(9-*phenylxanthen*-9-*yl*)*uridine* 5'-O-(2-*cyanoethyl* N,N-*diisopropylphosphoramidite*) **U12**. Compound **U11** (3.92 g, 5.18 mmol) was phosphitylated according to the procedure used to prepare compound **U8**. Chromatography of the crude product on silica gel (100 g) and elution with triethylamine–dichloromethane (1:49 v/v) afforded the title compound as a solid foam (4.40 g, 88.8%) of R_f 0.47 on TLC in triethylamine–ethanol–dichloromethane (1:5:94 by vol.); δ_P(CH_2Cl_2; concentric external D_2O lock) 143.40 and 142.26.

5'-O-(tert-*Butyldiphenylsilyl*)*cytidine* **C9**. Cytidine (2.43 g, 10 mmol) was silylated according to the procedure used to synthesize compound **U5** above. The crude residue, a solid, was triturated with diethyl ether, then dried over P_2O_5 *in vacuo*. Pure title compound was obtained as a solid (3.14 g, 65.2%) of R_f 0.1 on TLC in ethanol–dichloromethane (1:9 v/v); δ_C[$(CD_3)_2SO$] 165.50 (C-4), 155.12 (C-2), 140.40 (C-6), 135.06 and 134.87 (C-2 and -6 of SiPh), 132.62 and 132.25 (C-1 of SiPh), 129.77 (C-4 of SiPh), 127.75 (C-3 and -5 of SiPh), 93.70 (C-5), 89.49 (C-1'), 82.95 (C-4'), 74.33 (C-2'), 68.86 (C-3'), 63.13 (C-5'), 26.51 (CMe_3) and 18.71 (CMe_3).

4-N-*Benzoyl*-5'-O-(tert-*butyldiphenylsilyl*)*cytidine* **C10**. Compound **C9** (3.14 g, 6.52 mmol) was benzoylated according to the procedure used to prepare compound **C2** except that treatment with PTSA was unnecessary. Work-up of the reaction mixture gave a foam (3.8 g, 99%), pure by NMR spectroscopy, with R_f 0.23 on TLC in ethanol–dichloromethane (1.19 v/v); δ_C-($CDCl_3$) 166.82 (benzoyl C=O), 162.50 (C-4), 155.30 (C-2), 144.09 (C-6), 135.23 and 135.04 (C-2 and -6 of SiPh), 132.89 and 132.03 (C-1 of SiPh), 132.46 (phenyl C-1 and -4), 129.67 (C-4 of SiPh), 128.31 and 127.58 (C-3 and -5 of SiPh, and C-2, -6, -3 and -5 of phenyl), 96.79 (C-5), 91.62 (C-1'), 84.36 (C-4'), 75.43 (C-2'), 68.62 (C-3'), 62.22 (C-5'), 26.61 (CMe_3) and 18.91 (CMe_3).

4-N-*Benzoyl*-5'-O-(tert-*butyldiphenylsilyl*)-2',3'-*bis*-O-(9-*phenylxanthen*-9-*yl*)*cytidine* **C11**. Compound **C10** (3.8 g, 6.49 mmol) was pixylated according to the procedure used to prepare compound **U6**, but using 3 mol equiv. of pixyl chloride. The crude product was purified by chromatography on silica gel (150 g) and eluted with a gradient of ethanol 0–2% in triethylamine–dichloromethane (1:199 v/v). This afforded a solid foam (7.06 g, 99%) of R_f 0.54 on TLC in triethylamine–ethanol–dichloromethane (1:5:94 by vol.).

4-N-*Benzoyl*-2',3'-*bis*-O-(9-*phenylxanthen*-9-*yl*)*cytidine* **C12**. Compound **C11** (7.06 g, 6.42 mmol) was desilylated during 2 days according to the procedure used to prepare compound **U7**.

Chromatography of the crude product on silica gel (150 g) and elution with a gradient of ethanol 0–5% in triethylamine–dichloromethane (1:199 v/v) afforded a solid foam (4.6 g, 83.3%) of R_f 0.43 on TLC in triethylamine–ethanol–dichloromethane (1:5:94 by vol.).

4-N-*Benzoyl*-2′,3′-O-*bis*-(9-*phenylxanthen*-9-*yl*)*cytidine* 5′-O-(2-*cyanoethyl* N,N-*diisopropylphosphoramidite*) **C13**. Compound **C12** (4.6 g, 5.34 mmol) was phosphitylated according to the procedure used to prepare compound **U8**. Chromatography of the residue on silica gel (100 g) and elution with light petroleum–dichloromethane (1:2 v/v) containing 2% triethylamine afforded a solid, pale yellow foam (4.91 g, 86.7%) of R_f 0.43 on TLC in triethylamine–ethanol–dichloromethane (1:5:94 by vol.); $\delta_P(CH_2Cl_2$; concentric external D_2O lock) 143.52 and 142.43.

5′-O-(tert-*Butyldiphenylsilyl*)-2-N-(*dimethylaminomethylene*)*guanosine* **G9**. Guanosine (2.82 g, 10 mmol) was silylated according to the procedure used to prepare compound **U5** but using, however, exactly 1 mol equiv. of silylating reagent. After removal of solvent under reduced pressure, the crude 5′-O-(*tert*-butyldiphenylsilyl)guanosine, an oil of R_f 0.32 on TLC in ethanol–dichloromethane (1:4 v/v), was dissolved in dry methanol (150 cm^3), DMF dimethyl acetal (20 cm^3) was added, and the reaction mixture was stirred overnight. The thick precipitate that had formed was filtered off, washed with a small amount of methanol, and dried over P_2O_5 *in vacuo* to leave a solid (2.6 g), pure by TLC. The filtrate was diluted with dichloromethane (300 cm^3), washed with 0.5 mol dm^{-3} aq. sodium hydrogen carbonate (2 × 300 cm^3), dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. The residue was triturated with diethyl ether and dried over P_2O_5 *in vacuo* to give a further crop (2.59 g) of product. Pure title compound was obtained as a solid (5.19 g, 90%) of R_f 0.58 on TLC in ethanol–dichloromethane (1:4 v/v); $\delta_C[(CD_3)_2SO]$ 157.72 (amidine CH), 157.53 (C-2), 157.17 (C-6), 149.94 (C-4), 136.31 (C-8), 134.90 (C-2 and -6 of SiPh), 132.76 and 132.59 (C-1 of SiPh), 129.74 (C-4 of SiPh), 127.73 (C-3 and -5 of SiPh), 119.70 (C-5), 86.79 (C-1′), 84.22 (C-4′), 73.61 (C-2′), 69.88 (C-3′), 64.08 (C-5′), 40.51 and 34.59 (amidine Me_2N), 26.57 (CMe_3) and 18.71 (CMe_3).

5′-O-(tert-*Butyldiphenylsilyl*)-2-N-*dimethylaminomethylene*-2′,3′-*di*-O-*laevulinylguanosine* **G10**. Compound **G9** (2.78 g, 4.82 mmol) was suspended in dry THF–dichloromethane (300 cm^3; 1:1 v/v). Laevulinic acid (2.8 g, 24 mmol), dicyclohexylcarbodiimide (DCC) (4.95 g, 24 mmol) and 4-(dimethylamino)pyridine (DMAP) (50 mg) were added and the reaction mixture was stirred overnight under anhydrous conditions. TLC showed complete reaction. The reaction mixture was filtered to remove dicyclohexylurea and the filtrate plus washings were evaporated to dryness under reduced pressure. The residue was dissolved in dichloromethane (100 cm^3) and the solution was washed with 1 mol dm^{-3} aq. sodium hydrogen carbonate (2 × 100 cm^3), dried (Na_2SO_4), filtered, and then evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (120 g) and eluted with a gradient of ethanol 0–8% in dichloromethane. Pure title compound was obtained as a solid, almost colourless foam (3.45 g, 92.6%) of R_f 0.38 on TLC in ethanol–dichloromethane (1:19 v/v); $\delta_C[(CD_3)_2SO]$ 205.94 and 205.79 (ketone C=O of laevulinyls), 171.36 and 171.03 (ester C=O of laevulinyls), 158.06 (amidine CH), 157.66 (C-6), 156.63 (C-2), 149.46 (C-4), 137.00 (C-8), 134.84 and 134.76 (C-2 and -6 of SiPh), 132.01 (C-1 of SiPh), 129.17 (C-4 of SiPh), 127.08 (C-3 and -5 of SiPh), 119.97 (C-5), 86.44 (C-1′), 80.95 (C-4′), 72.63 (C-2′), 69.20 (C-3′), 61.93 (C-5′), 40.37 and 34.2 (Me_2N of amidine), 37.02 (C-2 of laevulinyls), 28.97 (C-5 of laevulinyls), 27.00 (C-3 of laevulinyls), 26.03 (CMe_3) and 18.42 (CMe_3).

2-N-*Dimethylaminomethylene*-2′,3′-*di*-O-*laevulinylguanosine* **G11**. Compound **G10** (1.54 g, 2 mmol) was desilylated according to the procedure used to prepare compound **U7**. TLC showed complete reaction after 90 min. The crude product was purified by chromatography on silica gel (100 g) and eluted with a gradient of ethanol 0–10% in dichloromethane. Pure title compound was obtained as a solid foam (870 mg, 81.4%) of R_f 0.42 on TLC in ethanol–dichloromethane (1:9 v/v); $\delta_C(CDCl_3)$ 206.08 and 205.85 (ketone C=O of laevulinyls), 171.35 and 170.93 (ester C=O of laevulinyls) 158.09 (amidine CH), 157.50 (C-6), 156.85 (C-2), 149.19 (C-4), 137.54 (C-8), 120.47 (C-5), 86.86 (C-1′), 83.10 (C-4′), 72.62 (C-2′), 70.47 (C-3′), 60.91 (C-5′), 40.73 and 34.45 (amidine Me_2N), 37.19 and 37.11 (C-2 of laevulinyls), 29.16 (C-5 of laevulinyls) and 27.15 and 27.01 (C-3 of laevulinyl).

2-N-*Dimethylaminomethylene*-2′,3′-*di*-O-*laevulinylguanosine* 5′-O-(2-*cyanoethyl* N,N-*diisopropylphosphoramidite*) **G12**. Compound **G11** (870 mg, 1.63 mmol) was phosphitylated according to the procedure used to prepare compound **U8**. Chromatography of the crude product on silica gel (50 g) and elution with a gradient of ethanol 0–3% in triethylamine–dichloromethane (1:49 v/v) afforded the title compound as a solid foam (1.0 g, 83.5%) of R_f 0.25 on TLC in triethylamine–ethanol–dichloromethane (1:5:94 by vol.); $\delta_P(CH_2Cl_2$; concentric external D_2O lock) 145.80 and 145.66.

Synthesis of Branched Oligoribonucleotides.—The following branched oligoribonucleotides were synthesized from the 5′ to 3′ end on a 1 μmol scale by using the reversed support derived from the 5′-*O*-succinate of compound **U7** and the monomers described above and the trityl on manual ending procedure:

branch 1A—5′-UUA$^{2'\text{–}5'GU}_{3'\text{–}5'GU}$

branch 2N—5′-UGGUUN$^{2'\text{–}5'GUGUG}_{3'\text{–}5'GUGUG}$ (where N = A, C, U or G)

branch 3A—5′-UACUUA$^{2'\text{–}5'GUGUG}_{3'\text{–}5'GUGUG}$

The carefully dried monomers were prepared as 0.1 mol dm^{-3} solutions in anhydrous acetonitrile. The reversed monomers, *viz.* compounds **A7**, **G7**, **C7** and **U7**, were placed on the Applied Biosystems 394 synthesizer in positions 1–4 respectively. The branch-point monomers, *viz.* compounds **A13**, **G12**, **C13** and **U12** were placed in amidite positions 5–8 respectively. A 1 μmol β-cyanoethyl phosphoramidite DNA cycle was used with the condensation wait time increased to 15 min and 0.5 mol dm^{-3} 1*H*-tetrazole in acetonitrile as activator. In the case of oligomers carrying a G at the branch point the synthesis must be interrupted directly after the incorporation of the branch G monomer, *viz.* compound **G12**; cap and oxidation steps, plus washing, were then performed and the synthesis column was removed from the synthesizer. A fresh solution of 0.5 mol dm^{-3} hydrazine hydrate in pyridine–acetic acid (4:1 v/v) was slowly passed backwards and forwards for 5 min through the column, using two plastic syringes to remove the 2′-*O*- and 3′-*O*-laevulinyl protecting groups. The column was washed thoroughly with anhydrous acetonitrile and was then put back on the synthesizer so that the 2′- and 3′-hydroxy groups could be simultaneously extended.

Deprotection and Purification of the Branched Oligoribonucleotides.—At the end of the assembly, the carrier-bound oligoribonucleotide was treated with ethanol–30% aq. ammonia (1:3 v/v; 2 cm^3) for 12 h at 60 °C in a sealed, sterile vial. When cool the sample was lyophilised in a sterile Eppendorf tube and was then purified by reversed-phase HPLC on a μBondapak C_{18} column with a gradient of acetonitrile in 0.1 mol dm^{-3} aq. triethylammonium acetate, pH 7, as eluent. The dipixyl, 2′-*O*-Fpmp-protected branched RNA containing peak

was eluted at an acetonitrile concentration of ~60% and was collected and lyophilised. The acid-labile pixyl and Fpmp groups were then removed under sterile conditions as described previously.[36] The relative molecular mass of pure branch 2A was found to be 5172.0 by ESMS (calc. M, for $C_{153}H_{186}N_{59}O_{116}P_{15}$ is 5172.08).

Acknowledgements

We thank Julia Pickles for typing the manuscript and Samantha O'Loughlin and Viviane Adam for synthesizing the oligonucleotides.

References

1 A. I. Lamond, *BioEssays*, 1993, **15**, 595.
2 B. Ruskin, A. R. Krainer, T. Maniatis and M. R. Green, *Cell*, 1984, **38**, 317.
3 R. A. Padgett, M. M. Konarska, P. J. Grabowski, S. F. Hardy and P. A. Sharp, *Science*, 1984, **225**, 898.
4 P. W. Laird, *Trends Genet.*, 1989, **5**, 204.
5 B. Ruskin and M. R. Green, *Science*, 1985, **229**, 135.
6 A. Krämer and W. Keller, *EMBO J.*, 1985, **4**, 3571.
7 J. Arenas and J. Hurwitz, *J. Biol. Chem.*, 1987, **262**, 4274.
8 K. B. Chapman and J. D. Boeke, *Cell*, 1991, **65**, 483.
9 R. Kierzek, D. W. Kapp, M. Edmonds and M. H. Caruthers, *Nucleic Acids Res.*, 1986, **14**, 4751.
10 D. M. Brown, D. I. Magrath and A. R. Todd, *J. Chem. Soc.*, 1955, 4396.
11 J. F. M. De Rooij, G. Wille-Hazeleger, P. M. J. Burgers and J. H. van Boom, *Nucleic Acids Res.*, 1979, **6**, 2237.
12 M. J. Damha, R. T. Pon and K. K. Ogilvie, *Tetrahedron Lett.*, 1985, **26**, 4839.
13 C. B. Reese and P. A. Skone, *Nucleic Acids Res.*, 1985, **13**, 5216.
14 T. Pathak and J. Chattopadhyaya, *Acta Chem. Scand., Ser. B*, 1985, **39**, 799.
15 J. L. Fourrey, J. Varenne, C. Fontaine, E. Guittet and Z. W. Yang, *Tetrahedron Lett.*, 1987, **28**, 1769.
16 S. Huss, G. Gosselin and J.-L. Imbach, *Tetrahedron Lett.*, 1987, **28**, 415.
17 S. Huss, G. Gosselin and J.-L. Imbach, *J. Org. Chem.*, 1988, **53**, 499.
18 M. Sekine and T. Hata, *J. Am. Chem. Soc.*, 1985, **107**, 5813.
19 M. Sekine, J. Heikkilä and T. Hata, *Tetrahedron Lett.*, 1987, **28**, 5691.
20 M. Sekine, J. Heikkilä and T. Hata, *Bull. Chem. Soc. Jpn.*, 1991, **64**, 588.
21 X.-X. Zhou, G. Remaud and J. Chattopadhyaya, *Tetrahedron*, 1988, **44**, 6471.
22 N. Balgobin, A. Földesi, G. Remaud and J. Chattopadhyaya, *Tetrahedron*, 1988, **44**, 6929.
23 C. Sund, A. Földesi, S. Yamakage, P. Agback and J. Chattopadhyaya, *Tetrahedron*, 1991, **47**, 6305.
24 C. Sund, P. Agback and J. Chattopadhyaya, *Tetrahedron*, 1991, **47**, 9659.
25 C. Sund, P. Agback and J. Chattopadhyaya, *Tetrahedron*, 1993, **49**, 649.
26 P. Agback, C. Glemarec, L. Yin, A. Sandström, J. Plavec, C. Sund, S. Yamakage, G. Viswanadham, B. Rousse, N. Puri and J. Chattopadhyaya, *Tetrahedron Lett.*, 1993, **34**, 3929.
27 M. J. Damha and K. K. Ogilvie, *J. Org. Chem.*, 1988, **53**, 3710.
28 M. J. Damha and S. Zabarylo, *Tetrahedron Lett.*, 1989, **30**, 6295.
29 M. J. Damha, K. Ganeshan, R. H. E. Hudson and S. V. Zabarylo, *Nucleic Acids Res.*, 1992, **20**, 6565.
30 J. B. Chattopadhyaya and C. B. Reese, *J. Chem. Soc., Chem. Commun.*, 1978, 639.
31 C. B. Reese and E. A. Thompson, *J. Chem. Soc., Perkin Trans. 1*, 1988, 2881.
32 W. T. Markiewicz, *J. Chem. Res. (S)*, 1979, 24.
33 G. S. Ti, B. L. Gaffney and R. A. Jones, *J. Am. Chem. Soc.*, 1982, **104**, 1316.
34 S. Agrawal, C. Christodoulou and M. J. Gait, *Nucleic Acids Res.*, 1986, **14**, 6227.
35 J. H. van Boom and P. M. J. Burgers, *Tetrahedron Lett.*, 1976, 4875.
36 B. Beijer, I. Sulston, B. S. Sproat, P. Rider, A. I. Lamond and P. Neuner, *Nucleic Acids Res.*, 1990, **18**, 5143.
37 J. H. van Boom and C. T. J. Wreesmann in *Oligonucleotide Synthesis: a Practical Approach*, ed. M. J. Gait, IRL Press, Oxford, 1984, p. 163.
38 M. Mann, *Org. Mass Spectrom.*, 1990, **25**, 575.
39 J. D. Dignam, R. M. Lebowitz and R. G. Roeder, *Nucleic Acids Res.*, 1983, **11**, 1475.
40 S. Barabino, B. J. Blencowe, U. Ryder, B. S. Sproat and A. I. Lamond, *Cell*, 1990, **63**, 293.

Paper 3/04931K
Received 16*th August* 1993
Accepted 22*nd September* 1993