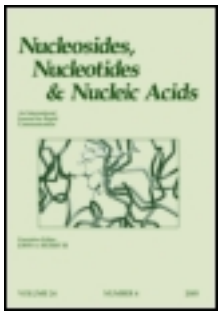

Nucleosides and Nucleotides
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn19

Simplified and Cost Effective Syntheses of Fully Protected Phosphoramidite Monomers Suitable for the Assembly of Oligo(2′-O-allylribonucleotides)

Barbro Beijer [a], Morten Grøtli [a], Mark E. Douglas [a] & Brian S. Sproat [a]
[a] European Molecular Biology Laboratory, Meyerhofstrasse 1, D-69117, Heidelberg, Germany
Published online: 24 Sep 2006.

To cite this article: Barbro Beijer, Morten Grøtli, Mark E. Douglas & Brian S. Sproat (1994) Simplified and Cost Effective Syntheses of Fully Protected Phosphoramidite Monomers Suitable for the Assembly of Oligo(2′-O-allylribonucleotides), Nucleosides and Nucleotides, 13:9, 1905-1927, DOI: 10.1080/15257779408010672

To link to this article: http://dx.doi.org/10.1080/15257779408010672

NUCLEOSIDES & NUCLEOTIDES, 13(9), 1905-1927 (1994)

SIMPLIFIED AND COST EFFECTIVE SYNTHESES OF FULLY PROTECTED PHOSPHORAMIDITE MONOMERS SUITABLE FOR THE ASSEMBLY OF OLIGO(2′-*O*-ALLYLRIBONUCLEOTIDES)

Barbro Beijer, Morten Grøtli, Mark E. Douglas and Brian S. Sproat*

European Molecular Biology Laboratory, Meyerhofstrasse 1, D-69117 Heidelberg, Germany

Abstract. Simplified, high yielding syntheses of suitably protected 2′-*O*-allylribonucleoside-3′-*O*-phosphoramidites starting from standard ribonucleosides have been elucidated. Specific 2′-*O*-allylation is readily achieved using amidine protection of the exocyclic amino groups of adenosine and cytidine and in the case of guanosine the allylation is carried out on an easily prepared intermediate bearing transient protection of the lactam function.

INTRODUCTION

Oligo(2′-*O*-allylribonucleotides) have been developed as superior antisense compounds for a variety of experiments in molecular biology,[1] and their important properties and applications have been recently reviewed.[2] These oligonucleotide analogues are particularly useful for investigations of RNA processing[3,4] and for determining the location of RNA or RNPs by microinjection.[5] 2′-*O*-Allylribonucleotides have also been used to prepare nuclease resistant primers for reverse transcription[6] and show great promise for use in stabilised hammerhead ribozymes for cleaving mRNA.[7] The observation that these analogues are poorly taken up by cells[8] limits most applications at present to *in vitro* ones, however microinjection is a useful technique to deliver such compounds into small numbers of living cells.[5,9]

The procedures originally developed by us for preparing the 2′-*O*-allylribonucleoside phosphoramidite monomers were analogous to the procedures used in the preparation of the corresponding methyl derivatives which are multistep and require the use of expensive starting materials.[10,11] The enormous price of the commercially available monomers prepared by these routes is certainly a major factor in limiting their widespread use. We reckoned that it should be possible to protect the nucleobases of the ribonucleosides in a

much simpler way since the palladium catalysed allylation procedure first described by Lakhmiri *et al.*[12] is very mild. Recently it was shown that uridine could be regioselectively allylated with allyl bromide/dibutyltin oxide/tetrabutylammonium bromide, however a 2:1 mixture of 2′-*O*-allyl to 3′-*O*-allyl isomers was obtained and these could only be separated after dimethoxytritylation.[13] Although the synthesis of the uridine phosphoramidite is only three steps the overall yield is only 34.4%. The cytidine monomer could only be obtained *via* a uridine intermediate since direct allylation always gave attack on the heterocycle, and a rather low yield of 22% was obtained for the 2′-*O*-allylcytidine monomer. The same authors also utilised the mild allylation procedure with palladium(0) catalysis and allyl ethyl carbonate and demonstrated that *N*-alkylation of the heterocycle occured when trying to allylate N^4-benzoylcytidine.[14] This article describes new, greatly simplified procedures for cost effective synthesis of the appropriate monomers.

EXPERIMENTAL

Materials and Methods

Tris(dibenzylideneacetone)dipalladium(0) and 1,4-*bis*(diphenylphosphino)butane were obtained from Aldrich (Steinheim, Germany). 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane was purchased from Ifotam (Lodz, Poland) and 2-cyanoethoxy *N,N*-diisopropylaminochlorophosphine was purchased from BioSyntech (Hamburg, Germany).

N-Methyl-2,2-dimethoxypyrrolidine was synthesised as previously described and *N,N*-diisobutylformamide dimethyl acetal was synthesised according to the procedure used to prepare *N,N*-di-n-butylformamide diethyl acetal.[15] Allyl ethyl carbonate was prepared as described previously.[10] All other reagents used were of the highest available purity. Anhydrous solvents were purchased from Romil Chemicals Ltd. (Loughborough, Leicestershire, U.K.).

Column chromatography was performed on Kieselgel 60 (Fluka, Neu-Ulm, Germany) and ascending mode t.l.c. was performed on aluminium foil supported silica gel containing a 254 nm fluor.

^{13}C and ^{31}P n.m.r. spectra were recorded on a Bruker AM250 spectrometer using tetramethylsilane and external trimethyl phosphate as the respective references. ^{31}P n.m.r. spectra were recorded using broad band proton noise decoupling. ^{13}C n.m.r. data are reported below with broad band proton decoupling, however assignments were made with the aid of the off-resonance data.

Synthesis of Monomers and Intermediates

3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-N^2*-isobutyrylguanosine (1)*

Guanosine (5.01 g, 17.7 mmol) was dried by evaporation of pyridine (3 x 30 ml) *in vacuo*, then suspended in dry DMF/pyridine (85 ml, 12:5 v/v). 1,3-Dichloro-1,1,3,3-

tetraisopropyldisiloxane (6 ml, 19.1 mmol) was added and the mixture kept at 50°C for 1 h, giving a clear yellow solution. A further portion of the siloxane (1 ml, 3.2 mmol) was added and the reaction left a further 30 min at 50°C, whereupon silica gel TLC in ethanol/dichloromethane (1:9 v/v) showed complete reaction. The reaction mixture was cooled on ice and chlorotrimethylsilane (5 ml, 39.5 mmol) was added with stirring and exclusion of moisture. TLC showed complete reaction after 30 min. Isobutyric anhydride (5 ml, 30.12 mmol) was then added and the reaction was stirred overnight. TLC showed complete reaction, the reaction mixture was quenched with methanol (10 ml) and after 10 min solvent was removed *in vacuo*. After the usual workup the 2′-*O*-trimethylsilyl group was removed by a 2 min treatment with 4-toluenesulphonic acid as previously described.[11] The reaction was quenched with triethylamine and solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate (250 ml) and washed with 1 M aqueous sodium bicarbonate (2 x 250 ml). The organic layer was dried (Na_2SO_4), filtered and evaporated to dryness *in vacuo*. The crude product was purified by chromatography on silica gel (300 g) eluting with ethanol/dichloromethane (3:97 v/v). Pure title compound was obtained as a solid white foam (8.86 g, 84%) of R_f 0.29 on TLC in ethanol/dichloromethane (1:9 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 179.94 (isobutyryl C=O), 155.81 (C-6), 148.23 (C-2), 147.89 (C-4), 136.30 (C-8), 120.82 (C-5), 88.78 (C-1′), 81.42 (C-4′), 75.06 (C-2′), 69.37 (C-3′), 60.38 (C-5′), 35.73 (isobutyryl CH), 18.81 (isobutyryl CH_3s), 17.04-16.70 (isopropyl CH_3s), 13.16, 12.69 and 12.34 p.p.m. (isopropyl CHs).

3′,5′-O-*(Tetraisopropyldisiloxane-1,3-diyl)*-N^2-*isobutyryl*-N^1,N^2-*[1,2*-bis(tert-*butyldimethylsilyloxy)ethano]guanosine (**2**)*

Compound **1** (3 g, 5.03 mmol) was dissolved in a solution of glyoxal (24 mmol) in pyridine (6 ml) under argon and the solution was evaporated to dryness *in vacuo*; further drying was achieved by evaporation of dry pyridine (5 x 20 ml) *in vacuo*. TLC in ethanol/dichloromethane (1:19 v/v) showed complete reaction. The residue was dissolved in dry DMF (60 ml) under argon, and imidazole (5 g, 73.4 mmol) was added with stirring and exclusion of moisture. After 5 min a white solid precipitated out and after 1 h at room temperature the mixture was filtered. The filtrate was concentrated *in vacuo* to about 40 ml and *tert.*-butylchlorodimethylsilane (3.8 g, 25.2 mmol) was added with stirring and exclusion of moisture. After overnight reaction TLC in ethanol/dichloromethane (1:19 v/v) showed almost complete reaction. The reaction was quenched with methanol (4 ml) and solvent was removed *in vacuo*. The residual yellow oil was dissolved in ethyl acetate (300 ml) and the solution was washed with 1 M aqueous sodium bicarbonate (300 ml) followed by saturated brine (300 ml). The separated organic phase was dried (Na_2SO_4), filtered and evaporated to dryness *in vacuo*. The crude product was purified by chromatography on

silica gel (200 g) eluting with ethyl acetate/hexane (1:4 v/v). Pure title compound was obtained as a solid white foam (3.91 g, 87.8%) of R_f 0.28 on TLC in ethyl acetate/hexane (1:3 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 176.45 (isobutyryl C=O), 154.57 (C-6), 147.85 (C-2 and C-4), 136.12 (C-8), 120.86 (C-5), 88.66 and 88.50 (C-1′), 85.63 (C-4′), 82.45, 82.31, 82.17 and 81.98 (ethano CHs), 75.21 and 75.08 (C-2′), 69.97 (C-3′), 61.20 and 61.00 (C-5′), 33.38 (isobutyryl CH), 30.63 (quaternary C of *tert*-butyl), 25.43 (CH_3s of *tert*-butyl), 18.75, 18.23, 17.79 and 17.27 (isobutyryl CH_3s), 17.11-16.76 (isopropyl CH_3s), 13.27, 13.10, 12.88. 12.78 and 12.50 (isopropyl CHs), -4.94 and -5.51 p.p.m. (Si-CH_3s).

3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-N^2*-isobutyryl-*N^1,N^2*-[1,2-*bis*(tert-butyldimethylsilyloxy)ethano]-2′-O-allylguanosine (**3**)*

Compound **2** (3.17 g, 3.59 mmol) and allyl ethyl carbonate (1.2 g, 9.22 mmol) were dissolved in anhydrous tetrahydrofuran (20 ml) under argon. A mixture of *tris*(dibenzylideneacetone)dipalladium(0) (50 mg, 0.055 mmol) and 1,4-*bis*(diphenylphosphino)butane (100 mg, 0.23 mmol) in tetrahydrofuran (5 ml) was added and the mixture was heated under reflux for 30 min. TLC in hexane/ethyl acetate (2:1 v/v) showed almost complete reaction. Solvent was removed *in vacuo* and the residue was dissolved in dichloromethane (15 ml) and purified by chromatography on silica gel (100 g) eluting with hexane/ethyl acetate (5:1 to 4:1 v/v). A second column was necessary to remove coeluted allyl ethyl carbonate. Pure product was obtained as a solid white foam (2.51 g, 75.8%) of R_f 0.45 on TLC in ethyl acetate/hexane (1:3 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 176.34 and 176.18 (isobutyryl C=O), 154.52 (C-6), 147.77 (C-2), 147.39 (C-4), 136.12 (C-8), 133.97 (allyl CH), 121.08 (C-5), 116.96 and 116.71 (allyl $=CH_2$), 87.84 (C-1′), 85.77 and 85.64 (C-4′), 82.36, 82.18 and 81.95 (ethano CHs), 81.30 (C-2′), 71.89 (allyl CH_2O), 69.38 (C-3′), 59.62 (C-5′), 33.16 (isobutyryl CH), 25.41 (CH_3s of *tert*-butyl), 18.86-16.87 (isobutyryl CH_3s and isopropyl CH_3s), 13.34, 12.80 and 12.57 (isopropyl CHs), -4.95 and - 5.51 p.p.m. (Si-CH_3s).

*N^2-Isobutyryl-2′-O-allylguanosine (**4**)*

Compound **3** (2.25 g, 2.44 mmol) was dissolved in anhydrous tetrahydrofuran (20 ml) under nitrogen and 1.1 M tetrabutylammonium fluoride in tetrahydrofuran (15 ml) was added with stirring and exclusion of moisture. Silica gel TLC in ethanol/dichloromethane (1:9 v/v) showed complete reaction after 15 min. The reaction was quenched by addition of pyridine/methanol/water (20 ml, 3:1:1 by vol.), and the solution was poured into pyridinium form Dowex 50 W x 4-200 resin (40 ml) and stirred for 30 min. The resin was filtered off and the filtrate and combined washings were evaporated to dryness *in vacuo*.

To ensure cleavage of the glyoxal adduct the residue was treated with 7% triethylamine in ethanol/water (20 ml, 1:1 v/v) for 1 h. Solvent was removed *in vacuo* and the crude product was purified by chromatography on silica gel (60 g) eluting with ethanol/dichloromethane (7:93 v/v). N^2-Isobutyryl-2′-*O*-allylguanosine was isolated as a white foam (0.81 g, 84.4%) of R_f 0.21 on TLC in ethanol/dichloromethane (1:9 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 155.66 (C-6), 148.25 (C-2 and C-4), 138.99 (C-8), 133.47 (allyl CH), 121.03 (C-5), 117.93 (allyl $=CH_2$), 87.48 (C-1′), 86.16 (C-4′), 81.02 (C-2′), 71.60 (allyl CH_2O), 69.97 (C-3′), 61.92 (C-5′), 35.79 (isobutyryl CH), 18.91 p.p.m. (isobutyryl CH_3s).

5′-O-Dimethoxytrityl-N^2*-isobutyryl-2′-O-allylguanosine (**5**)*

Compound **4** (0.47 g, 1.19 mmol) was dried by evaporation of anhydrous pyridine (2 x 20 ml) *in vacuo* then dissolved in dry pyridine under argon. Triethylamine (0.3 ml, 2.14 mmol) and 4,4′-dimethoxytrityl chloride (0.51 g, 1.5 mmol) were added with stirring and exclusion of moisture. TLC showed complete reaction after 1.5 h. Solvent was removed *in vacuo* at room temperature and the residue was dissolved in ethyl acetate (100 ml) and the solution washed with 1 M aqueous sodium bicarbonate (2 x 100 ml) followed by saturated brine (100 ml). The separated organic phase was dried (Na_2SO_4), filtered and evaporated to dryness *in vacuo*. The crude product was purified by chromatography on silica gel (30 g) eluting with ethyl acetate/dichloromethane (1:1 v/v) containing 1% triethylamine followed by a gradient of ethanol from 0 to 5% in triethylamine/dichloromethane (1:99 v/v). The title compound was obtained as a solid white foam (0.69 g, 83.3%) of R_f 0.19 on TLC in triethylamine/ethanol/dichloromethane (1:5:94 by vol.). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 179.45 (isobutyryl C=O), 158.49 (C-4s of methoxyphenyls), 155.98 (C-6), 148.51 and 147.99 (C-2 and C-4), 144.62 (C-1 of phenyl), 138.10 (C-8), 135.72 and 135.54 (C-1s of methoxyphenyls), 133.47 (allyl CH), 129.87 (C-2s and C-6s of methoxyphenyls), 127.96 (phenyl C-3 and C-5), 127.73 (phenyl C-2 and C-6), 126.83 (phenyl C-4), 121.42 (C-5), 118.16 (allyl $=CH_2$), 113.05 (C-3s and C-5s of methoxyphenyls), 86.57 (C-1′), 86.27 (trityl quaternary C), 84.42 (C-4′), 80.39 (C-2′), 71.56 (allyl CH_2O), 69.70 (C-3′), 63.53 (C-5′), 55.04 (CH_3s of methoxyphenyls), 35.72 (isobutyryl CH), 18.67 p.p.m. (isobutyryl CH_3s).

5′-O-Dimethoxytrityl-N^2*-isobutyryl-2′-O-allylguanosine-3′-O-(2-cyanoethyl* N,N*-diisopropylphosphoramidite) (**6**)*

Compound **5** (0.55 g, 0.79 mmol) was dried by evaporation of anhydrous acetonitrile (2 x 20 ml) *in vacuo*, and the residual foam was dissolved in dry 1,2-dichloroethane (10 ml) under argon. The solution was cooled on ice and *N,N*-diisopropylethylamine (0.3 ml,

1.7 mmol) followed by 2-cyanoethoxy *N,N*-diisopropylaminochlorophosphine (0.27 ml, 1.22 mmol) were added. The reaction mixture was stirred for 1.5 h at room temperature whereupon TLC showed complete reaction. Dichloromethane (75 ml) was added and the solution was washed with 1 M aqueous sodium bicarbonate (75 ml) followed by saturated brine (75 ml). The separated organic phase was dried (Na_2SO_4), filtered and evaporated to dryness *in vacuo*. The crude product was purified by chromatography on silica gel (30 g) eluting with hexane/dichloromethane (2:3 v/v) containing 2.5% triethylamine followed by 2.5% triethylamine in dichloromethane. The title compound was obtained as a solid white foam (0.59 g, 83.4%) of R_f 0.32 on TLC in triethylamine/ethanol/dichloromethane (1:5:94 by vol.). ^{31}P n.m.r. spectrum (CH_2Cl_2, concentric external D_2O lock) δ: +146.63 and 146.47 p.p.m.

N^4-*(N-Methylpyrrolidin-2-ylidene)cytidine (7)*

Cytidine (2.42 g, 10 mmol) was dried by evaporation of pyridine *in vacuo* then dissolved in dry methanol (20 ml). *N*-Methyl-2,2-dimethoxypyrrolidine (2 g, 13.77 mmol) was added and the reaction mixture was stirred overnight with exclusion of moisture. Silica gel TLC showed complete reaction. Solvent was removed *in vacuo* and the residue was chromatographed on silica (70 g) eluting with a gradient of ethanol from 0 to 20% in chloroform. Pure title compound was obtained as a solid white foam (2.56 g, 78.9%) of R_f 0.31 on TLC in ethanol/dichloromethane (1:4 v/v). ^{13}C n.m.r. spectrum (DMSO-d_6) δ: 171.49 (pyrrolidine C-2), 168.25 (C-4), 155.93 (C-2), 141.66 (C-6), 102.62 (C-5), 89.75 (C-1′), 84.39 (C-4′), 74.42 (C-2′), 69.49 (C-3′), 60.64 (C-5′), 51.00 (pyrrolidine C-3), 31.52 (pyrrolidine C-5), 30.43 (N-CH_3) and 19.33 p.p.m. (pyrrolidine C-4).

3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-N^4*-(N-methylpyrrolidin-2-ylidene)cytidine (8)*

Compound **7** (2.56 g, 7.89 mmol) was silylated as described above for preparing compound **1** except that the reaction was performed in pyridine alone at room temperature. The crude product was purified by chromatography on silica (100 g) eluting with a gradient of ethanol from 0 to 5% in dichloromethane containing 0.5% triethylamine. Pure title compound was obtained as a solid white foam (4.15 g, 92.8%) of R_f 0.21 on TLC in ethanol/dichloromethane (1:19 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 171.48 (pyrrolidine C-2), 168.46 (C-4), 155.46 (C-2), 139.66 (C-6), 102.18 (C-5), 91.40 (C-1′), 80.79 (C-4′), 74.34 (C-2′), 68.17 (C-3′), 59.72 (C-5′), 50.79 (pyrrolidine C-3), 31.05 (pyrrolidine C-5), 30.10 (N-CH_3), 18.93 (pyrrolidine C-4), 16.81-16.12 (isopropyl CH_3s), 12.64, 12.47, 12.32 and 12.23 p.p.m. (isopropyl CHs).

*3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-N⁴-(N-methylpyrrolidin-2-ylidene)-2′-O-allylcytidine (**9**)*

Compound **8** (4.15 g, 7.32 mmol) was allylated as described above for the preparation of compound **3** and the crude reaction product was purified by chromatography on silica (150 g) eluting with a gradient of ethanol from 0 to 8% in dichloromethane containing 0.5% triethylamine. The title compound was obtained as a white solid (4 g, 90%) of R_f 0.28 on TLC in ethanol/dichloromethane (1:19 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 171.93 (pyrrolidine C-2), 168.82 (C-4), 155.83 (C-2), 139.65 (C-6), 134.46 (allyl CH), 116.49 (allyl $=CH_2$), 102.37 (C-5), 89.39 (C-1′), 81.13 (C-4′), 80.91 (C-2′), 70.68 (allyl CH_2O), 67.52 (C-3′), 59.35 (C-5′), 51.16 (pyrrolidine C-3), 31.48 (N-CH_3), 30.55 (pyrrolidine C-5), 19.38 (pyrrolidine C-4), 17.24-16.54 (isopropyl CH_3s), 13.07, 12.86, 12.61 and 12.19 p.p.m. (isopropyl CHs).

*N⁴-(N-Methylpyrrolidin-2-ylidene)-2′-O-allylcytidine (**10**)*

Compound **9** (4.0 g, 6.59 mmol) was desilylated as described above for the preparation of compound **4**. Chromatography of the crude product on silica (100 g) eluting with a gradient of ethanol from 0 to 15% in dichloromethane containing 0.5% triethylamine afforded the title compound as a white solid (2.16 g, 90%) of R_f 0.18 on TLC in ethanol/dichloromethane (1:9 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 170.76 (pyrrolidine C-2), 166.54 (C-4), 154.82 (C-2), 139.86 (C-6), 133.24 (allyl CH), 114.96 (allyl $=CH_2$), 101.20 (C-5), 87.42 (C-1′), 82.57 (C-4′), 80.66 (C-2′), 69.19 (allyl CH_2O), 65.51 (C-3′), 57.77 (C-5′), 49.91 (pyrrolidine C-3), 30.17 (N-CH_3), 28.75 (pyrrolidine C-5) and 18.07 p.p.m. (pyrrolidine C-4).

*5′-O-Dimethoxytrityl-N⁴-(N-methylpyrrolidin-2-ylidene)-2′-O-allylcytidine (**11**)*

Compound **10** (2.16 g, 5.9 mmol) was dimethoxytritylated as described above for the preparation of compound **5**. The crude product was purified by chromatography on silica (100 g) eluting with a gradient of ethanol from 0 to 4% in dichloromethane/triethylamine (199:1 v/v). Pure product was obtained as an off-white foam (3.2 g, 81.4%) of R_f 0.35 on TLC in ethanol/dichloromethane (1:19 v/v) containing 1% triethylamine. ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 172.09 (pyrrolidine C-2), 168.90 (C-4), 158.44 (C-4s of methoxyphenyls), 156.11 (C-2), 144.41 (phenyl C-1), 140.21 (C-6), 135.64 and 135.40 (C-1s of methoxyphenyls), 133.85 (allyl CH), 130.01 (C-2s and C-6s of methoxyphenyls), 128.10 (phenyl C-3 and C-5), 127.76 (phenyl C-2 and C-6), 126.78 (phenyl C-4), 117.64 (allyl $=CH_2$), 113.12 (C-3s and C-5s of methoxyphenyls), 102.96 (C-5), 88.65 (C-1′), 86.64 (quaternary C of trityl), 82.72 (C-4′), 81.56 (C-2′), 71.05 (allyl CH_2O), 68.11 (C-3′), 61.33 (C-5′), 55.09 (CH_3s of methoxyphenyls), 51.36 (pyrrolidine C-3), 31.67 (N-CH_3), 30.62 (pyrrolidine C-5) and 19.57 p.p.m. (pyrrolidine C-4).

5′-O-Dimethoxytrityl-N⁴-(N-methylpyrrolidin-2-ylidene)-2′-O-allylcytidine-3′-O-(2-cyanoethyl N,N-*diisopropylphosphoramidite) (**12**)*

Compound **11** (3.1 g, 4.65 mmol) was phosphitylated as described above for the synthesis of compound **6**, and the crude product was purified by chromatography on silica (100 g) eluting with a gradient of ethanol from 0 to 2% in dichloromethane/triethylamine (49:1 v/v). Pure title compound was obtained as a solid white foam (3.44 g, 85.3%) of R_f 0.38 on TLC in ethanol/dichloromethane (1:19 v/v) containing 1% triethylamine. ^{31}P n.m.r. spectrum (CH_2Cl_2, concentric external D_2O lock) δ: +146.56 and 146.33 p.p.m.

*N⁶-Diisobutylaminomethyleneadenosine (**13**)*

Adenosine (5.34 g, 20 mmol) and *N,N*-diisobutylformamide dimethyl acetal (8.12 g, 40 mmol) were stirred overnight under anhydrous conditions in dry methanol (25 ml). TLC showed complete reaction and the clear solution was evaporated to dryness in vacuo. Chromatographic purification of the residue on silica (100 g) eluting with a gradient of ethanol from 0 to 10% in dichloromethane afforded the title compound as a white foam (7.92 g, 97.4%) of R_f 0.32 on TLC in ethanol/dichloromethane (1:9 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 159.08 (C-6), 158.81 (amidine CH), 151.17 (C-2), 149.29 (C-4), 141.65 (C-8), 125.94 (C-5), 90.81 (C-1′), 87.04 (C-4′), 72.79 (C-2′), 71.99 (C-3′), 62.62 (C-5′), 59.79 and 52.47 (isobutyl CH_2s), 26.95 and 25.72 (isobutyl CHs) and 19.64-19.28 p.p.m. (isobutyl CH_3s).

*3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-N⁶-diisobutylaminomethyleneadenosine (**14**)*

Compound **13** (3.96 g, 9.74 mmol) was silylated as described above for the preparation of compound **8**. The crude product was purified by chromatography on silica (100 g) eluting with a gradient of ethanol from 0 to 5% in dichloromethane. Evaporation of solvent afforded pure compound **14** as a solid white foam (5.37 g, 85%) of R_f 0.43 on TLC in ethanol/dichloromethane (1:19 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 159.40 (C-6), 158.87 (amidine CH), 151.91 (C-2), 149.95 (C-4), 139.78 (C-8), 125.97 (C-5), 89.40 (C-1′), 81.15 (C-4′), 74.51 (C-2′), 69.87 (C-3′), 60.77 (C-5′), 59.36 and 52.04 (isobutyl CH_2s), 26.62 and 25.61 (isobutyl CHs), 19.47 and 19.22 (isobutyl CH_3s), 16.72-16.34 (isopropyl CH_3s), 12.65, 12.40, 12.24 and 11.90 p.p.m. (isopropyl CHs).

*3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-N⁶-diisobutylaminomethylene-2′-O-allyladenosine (**15**)*

Compound **14** (3.25 g, 5 mmol) was allylated as described above for the preparation of compound **3** and the crude product was purified by chromatography on silica (100 g) eluting with a gradient of ethanol from 0 to 4% in dichloromethane. Pure title compound was obtained as a colourless oil (3.44 g, 99%) of R_f 0.55 on TLC in ethanol/dichloro-

methane (1:19 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 159.40 (C-6), 158.78 (amidine CH), 152.04 (C-2), 150.17 (C-4), 139.19 (C-8), 133.77 (allyl CH), 125.96 (C-5), 116.56 (allyl $=CH_2$), 88.06 (C-1′), 80.74 (C-4′), 80.53 (C-2′), 71.09 (allyl CH_2O), 69.12 (C-3′), 59.57 (C-5′), 59.38 and 52.13 (isobutyl CH_2s), 26.70 and 25.64 (isobutyl CHs), 19.51 and 19.25 (isobutyl CH_3s), 16.88-16.34 (isopropyl CH_3s), 12.78, 12.37 and 12.08 p.p.m. (isopropyl CHs).

*N^6-Diisobutylaminomethylene-2′-O-allyladenosine (**16**)*

Compound **15** (3.44 g, 4.95 mmol) was desilylated as described above for the synthesis of compound **4**. Purification of the reaction product on silica (100 g) eluting with a gradient of ethanol from 0 to 10% in dichloromethane afforded the title compound as a white semi-solid (2.11 g, 95.5%) of R_f 0.6 on TLC in ethanol/dichloromethane (1:9 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 160.02 (C-6), 158.95 (amidine CH), 151.36 (C-2), 149.64 (C-4), 141.39 (C-8), 132.69 (allyl CH), 126.76 (C-5), 117.74 (allyl $=CH_2$), 88.73 (C-1′), 87.47 (C-4′), 79.76 (C-2′), 71.20 (allyl CH_2O), 70.24 (C-3′), 62.53 (C-5′), 59.52 and 52.26 (isobutyl CH_2s), 26.66 and 25.71 (isobutyl CHs), 19.55 and 19.28 p.p.m. (isobutyl CH_3s).

*5′-O-Dimethoxytrityl-N^6-diisobutylaminomethylene-2′-O-allyladenosine (**17**)*

Compound **16** (2.11 g, 4.72 mmol) was dimethoxytritylated as described above for the preparation of compound **5** and the crude product was purified by chromatography on silica (100 g) eluting with a gradient of 0 to 2% ethanol in dichloromethane containing 0.5% triethylamine. Pure compound **17** was obtained as a solid pale yellow foam (3.25 g, 91.9%) of R_f 0.68 on TLC in ethanol/dichloromethane (1:19 v/v) containing 0.5% triethylamine. ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 159.98 (C-6), 159.15 (amidine CH), 158.46 (C-4s of methoxyphenyls), 152.72 (C-2), 151.25 (C-4), 144.55 (phenyl C-1), 139.88 (C-8), 135.91 and 135.66 (C-1s of methoxyphenyls), 133.22 (allyl CH), 130.00 (C-2s and C-6s of methoxyphenyls), 128.11 (phenyl C-3 and C-5), 127.77 (phenyl C-2 and C-6), 126.76 (phenyl C-4), 126.34 (C-5), 118.68 (allyl $=CH_2$), 113.13 (C-3s and C-5s of methoxyphenyls), 86.72 (C-1′), 86.49 (quaternary C of trityl), 83.87 (C-4′), 80.65 (C-2′), 71.73 (allyl CH_2O), 70.06 (C-3′), 63.19 (C-5′), 60.05 and 52.80 (isobutyl CH_2s), 55.12 (CH_3s of methoxyphenyls), 27.28 and 26.22 (isobutyl CHs), 20.08 and 19.83 p.p.m. (isobutyl CH_3s).

5′-O-Dimethoxytrityl-N^6-diisobutylaminomethylene-2′-O-allyladenosine-3′-O-(2-cyanoethyl N,N*-diisopropylphosphoramidite) (**18**)*

Compound **17** (3.25 g, 4.34 mmol) was phosphitylated as described above for the synthesis of compound **6** and the crude product was purified by chromatography on silica

(100 g) eluting with a gradient of ethanol from 0 to 2% in dichloromethane/triethylamine (49:1 v/v). Pure title compound was obtained as a solid off-white foam (3.08 g, 74.8%) of R_f 0.81 and 0.74 on TLC in ethanol/dichloromethane (1:19 v/v) containing 1% triethylamine. ^{31}P n.m.r. spectrum (CH_2Cl_2, concentric external D_2O lock) δ: +147.06 and 146.67 p.p.m.

3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-N³-(4-tert-butylbenzoyl)uridine (19)

Uridine (2.44 g, 10 mmol) was converted to the 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl) derivative as described for the preparation of compound **8** above. The crude product was dried by evaporation of toluene *in vacuo* and dissolved in dry dichloromethane (150 ml) and cooled in ice. Triethylamine (6.8 ml, 48.6 mmol) and chlorotrimethylsilane (3.76 ml, 29.66 mmol) were added and the mixture was stirred overnight at room temperature under anhydrous conditions. The reaction mixture was cooled in ice and poured into vigorously stirred 1 M aqueous sodium bicarbonate. The separated organic phase was dried (Na_2SO_4), filtered and evaporated to dryness *in vacuo* to give a pink foam of R_f 0.65 on TLC in ethanol/dichloromethane (1:19 v/v). The residue was dried by evaporation of pyridine *in vacuo* then redissolved in dry pyridine (100 ml) and cooled in ice. 4-*tert*-Butylbenzoyl chloride (5.9 ml, 30 mmol) and *N,N*-diisopropylethylamine (5.38 ml, 30 mmol) were added and the reaction was stirred overnight at room temperature. TLC showed complete reaction and the reaction mixture was worked up in the usual way. After removal of residual pyridine by coevaporation with toluene in vacuo, the 2′-*O*-trimethylsilyl group was cleaved with 4-toluenesulphonic acid for 2 min. After the usual workup the crude product was purified by chromatography on silica (100 g) eluting with a gradient of ethanol from 0 to 2% in dichloromethane. The title compound was obtained as an off-white foam (4.2 g, 64.9%) of R_f 0.43 on TLC in ethyl acetate/hexane (1:2 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 167.74 (benzoyl C=O), 161.70 (C-4), 158.66 (benzoyl C-4), 148.60 (C-2), 139.53 (C-6), 130.08 (benzoyl C-3 and C-5), 128.43 (benzoyl C-1), 125.77 (benzoyl C-2 and C-6), 101.26 (C-5), 90.83 (C-1′), 81.70 (C-4′), 74.63 (C-2′), 68.82 (C-3′), 60.11 (C-5′), 34.90 (quaternary C of *tert*-butyl), 30.53 (CH_3s of *tert*-butyl), 17.10-16.46 (isopropyl CH_3s), 12.98, 12.56 and 12.12 p.p.m. (isopropyl CHs).

N³-(4-tert-Butylbenzoyl)-2′-O-allyluridine (20)

Compound **19** (4.2 g, 6.49 mmol) was allylated as described above for the preparation of compound **3** and the crude product, of R_f 0.80 on TLC in ethyl acetate/hexane (1:2 v/v), was desilylated as described above for the synthesis of compound **4**. Purification on silica gel (80 g) eluting with a gradient of ethanol from 0 to 8% in dichloromethane afforded the title compound as a pale yellow foam (2.39 g, 82.9%) of R_f 0.26 on TLC in

ethanol/dichloromethane (1:19 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 168.08 (benzoyl C=O), 162.17 (C-4), 159.20 (benzoyl C-4), 148.99 (C-2), 140.62 (C-6), 133.25 (allyl CH), 130.17 (benzoyl C-3 and C-5), 125.98 (benzoyl C-1), 123.81 (benzoyl C-2 and C-6), 117.87 (allyl $=CH_2$), 101.51 (C-5), 88.09 (C-1′), 84.47 (C-4′), 80.89 (C-2′), 71.05 (allyl CH_2O), 68.02 (C-3′), 60.09 (C-5′), 35.00 (quaternary C of *tert*-butyl) and 30.54 p.p.m. (CH_3s of *tert*-butyl).

5′-O-Dimethoxytrityl-N^3*-(4-*tert*-butylbenzoyl)-2′-O-allyluridine (21)*

Compound **20** (2.39 g, 5.38 mmol) was dimethoxytritylated as described above for the synthesis of compound **5** and the crude product was purified by chromatography on silica (80 g) eluting with a gradient of ethanol from 0 to 2% in dichloromethane/triethylamine (99:1 v/v). The title compound was obtained as an off-white foam (3.55 g, 88.4%) of R_f 0.13 on TLC in ethyl acetate/hexane (1:3 v/v) containing 1% triethylamine. ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 168.04 (benzoyl C=O), 161.81 (C-4), 159.08 (benzoyl C-4), 158.47 (C-4s of methoxyphenyls), 148.95 (C-2), 144.12 (phenyl C-1), 139.60 (C-6), 135.07 and 134.80 (C-1s of methoxyphenyls), 133.18 (allyl CH), 130.30 (benzoyl C-3 and C-5), 129.94 and 129.87 (C-2s and C-6s of methoxyphenyls), 128.39 (benzoyl C-1), 127.91 (phenyl C-3 and C-5), 127.78 (phenyl C-2 and C-6), 126.92 (phenyl C-4), 126.03 (benzoyl C-2 and C-6), 118.20 (allyl $=CH_2$), 113.09 (C-3s and C-5s of methoxyphenyls), 101.69 (C-5), 87.76 (C-1′), 86.87 (quaternary C of trityl), 83.17 (C-4′), 81.19 (C-2′), 71.05 (allyl CH_2O), 68.09 (C-3′), 60.90 (C-5′), 54.97 (CH_3s of methoxyphenyls), 35.11 (quaternary C of *tert*-butyl) and 30.69 p.p.m. (CH_3s of *tert*-butyl).

5′-O-Dimethoxytrityl-N^3*-(4-*tert*-butylbenzoyl)-2′-O-allyluridine-3′-O-(2-cyanoethyl* N,N-*diisopropylphosphoramidite) (22)*

Compound **21** (3.55 g, 4.75 mmol) was phosphitylated as described above for the synthesis of compound **6** and the crude product was purified by chromatography on silica gel (100 g) eluting with a gradient of 25 to 33% ethylacetate in hexane containing 2% triethylamine. Compound **22** was obtained as a solid white foam (4.18 g, 92.9%) of R_f 0.26 on TLC in ethyl acetate/hexane (1:3 v/v) containing 2% triethylamine. ^{31}P n.m.r. spectrum (CH_2Cl_2, concentric external D_2O lock) δ: +147.09 and 146.34 p.p.m.

3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)inosine (23)

Inosine (8.04 g, 30 mmol) was dried by evaporation of dry *N,N*-dimethylformamide *in vacuo* and then treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (10.32 ml, 33 mmol) in dry DMF (200 ml) in the presence of imidazole (9 g, 132 mmol). TLC showed complete reaction after 3 h and the reaction mixture was worked up in the usual way. The

crude product was purified by chromatography on silica (250 g) eluting with a gradient of ethanol from 0 to 8% in dichloromethane. The title compound was obtained as a solid white foam (13.45 g, 87.8%) of R_f 0.18 on TLC in ethanol/dichloromethane (1:19 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 159.20 (C-6), 148.19 (C-4), 144.96 (C-2), 139.63 (C-8), 125.63 (C-5), 89.73 (C-1′), 82.22 (C-4′), 75.31 (C-2′), 70.54 (C-3′), 61.55 (C-5′), 17.43-16.96 (isopropyl CH_3s), 13.33, 13.06, 12.84 and 12.66 p.p.m. (isopropyl CHs).

*3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-N¹-pivaloyloxymethylinosine (**24**)*

Compound **23** (5.1 g, 10 mmol) was trimethylsilylated according to the procedure described above used in the preparation of compound **1** to give a solid pink foam of R_f 0.26 on TLC in ethanol/dichloromethane (1:19 v/v). This intermediate was dried by evaporation of DMF *in vacuo* and dissolved in dry DMF (60 ml). Anhydrous potassium carbonate (6.9 g, 50 mmol) and chloromethyl pivalate (1.73 ml, 12 mmol) were added and the mixture was stirred overnight under argon. The reaction mixture was filtered and the filtrate was evaporated to dryness in vacuo. The 2′-*O*-trimethylsilyl protecting group was then cleaved as described above in the synthesis of compound **1**. The crude product was purified by chromatography on silica (100 g) eluting with a gradient of ethanol from 0 to 4% in dichloromethane. The title compound was obtained as a solid white foam (3.84 g, 61.5%) of R_f 0.63 on TLC in ethanol/dichloromethane (1:19 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 177.48 (pivaloyl C=O), 155.20 (C-6), 147.87 (C-2), 146.00 (C-4), 138.46 (C-8), 124.23 (C-5), 89.13 (C-1′), 81.37 (C-4′), 74.41 (C-2′), 69.40 (C-3′), 67.50 (CH_2 of pivaloyloxymethyl), 60.43 (C-5′), 38.18 (quaternary C of *tert*-butyl), 26.26 (CH_3s of *tert*-butyl), 16.87-16.31 (isopropyl CH_3s), 12.76, 12.43, 12.26 and 11.98 p.p.m. (isopropyl CHs).

*3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-N¹-pivaloyloxymethyl-2′-O-allylinosine (**25**)*

Compound **24** (3.84 g, 6.15 mmol) was allylated as described above for the preparation of compound **3** and the reaction product was purified by chromatography on silica (100 g) eluting with a gradient of ethanol from 0 to 2% in dichloromethane. Slightly impure title compound was obtained as an off-white semi-solid (3.4 g, 83.1%) of R_f 0.73 on TLC in ethanol/dichloromethane (1:19 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 177.49 (pivaloyl C=O), 155.23 (C-6), 147.87 (C-2), 145.78 (C-4), 137.65 (C-8), 133.75 (allyl CH), 124.29 (C-5), 116.59 (allyl $=CH_2$), 87.93 (C-1′), 80.98 (C-4′), 80.80 (C-2′), 71.16 (allyl CH_2O), 68.67 (C-3′), 67.55 (CH_2 of pivaloyloxymethyl), 59.23 (C-5′), 38.20 (quaternary C of *tert*-butyl), 26.28 (CH_3s of *tert*-butyl), 16.92-16.34 (isopropyl CH_3s), 12.84, 12.38 and 12.08 p.p.m. (isopropyl CHs).

N^1-*Pivaloyloxymethyl-2′-O-allylinosine (**26**)*

Compound **25** (3.4 g, 5.1 mmol) was desilylated as described above for the preparation of compound **4** and the crude product was purified by chromatography on silica (100 g) eluting with a gradient of ethanol from 0 to 10% in dichloromethane. Pure compound **26** was obtained as a white solid (1.51 g, 70%) of R_f 0.24 on TLC in ethanol/dichloromethane (1:19 v/v). ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 177.82 (pivaloyl C=O), 155.42 (C-6), 148.18 (C-2), 146.24 (C-4), 139.92 (C-8), 133.13 (allyl CH), 124.49 (C-5), 117.71 (allyl $=CH_2$), 87.77 (C-1′), 86.58 (C-4′), 81.19 (C-2′), 71.26 (allyl CH_2O), 69.54 (C-3′), 67.86 (CH_2 of pivaloyloxymethyl), 61.63 (C-5′), 38.43 (quaternary C of *tert*-butyl) and 26.42 p.p.m. (CH_3s of *tert*-butyl).

5′-O-Dimethoxytrityl-N^1*-pivaloyloxymethyl-2′-O-allylinosine (**27**)*

Compound **26** (1.51 g, 3.57 mmol) was dimethoxytritylated as described above for the synthesis of compound **5** and the crude product was purified by chromatography on silica (100 g), eluting with hexane/ethyl acetate (1:1 v/v) containing 0.5% triethylamine followed by triethylamine/ethanol/ethyl acetate (1:4:195 by vol.). Pure title compound was obtained as a solid pale yellow foam (2.02 g, 78.1%) of R_f 0.14 on TLC in ethyl acetate/hexane (1:1 v/v) containing 0.5% triethylamine. ^{13}C n.m.r. spectrum ($CDCl_3$) δ: 177.63 (pivaloyl C=O), 158.05 (C-4s of methoxyphenyls), 155.36 (C-6), 147.82 (C-2), 146.76 (C-4), 144.12 (C-1 of phenyl), 138.37 (C-8), 135.20 and 135.12 (C-1s of methoxyphenyls), 133.11 (allyl CH), 129.58 (C-2s and C-6s of methoxyphenyls), 127.65 (phenyl C-3 and C-5), 127.34 (phenyl C-2 and C-6), 126.38 (phenyl C-4), 124.11 (C-5), 117.80 (allyl $=CH_2$), 112.69 (C-3s and C-5s of methoxyphenyls), 86.25 (C-1′), 86.10 (trityl quaternary C), 83.99 (C-4′), 80.65 (C-2′), 71.16 (allyl CH_2O), 69.51 (C-3′), 67.55 (CH_2 of pivaloyloxymethyl), 62.92 (C-5′), 54.64 (CH_3s of methoxyphenyls), 38.29 (quaternary C of *tert*-butyl) and 26.33 p.p.m. (CH_3s of *tert*-butyl).

5′-O-Dimethoxytrityl-N^1*-pivaloyloxymethyl-2′-O-allylinosine-3′-O-(2-cyanoethyl* N,N*-diisopropylphosphoramidite) (**28**)*

Compound **27** (2.02 g, 2.79 mmol) was phosphitylated as described above for the preparation of compound **6** and the reaction product was purified by chromatography on silica (100 g) eluting with hexane/dichloromethane/triethylamine (49:49:2 by vol.). Pure compound **28** was obtained as a solid white foam (2.4 g, 93%) of R_f 0.43 and 0.42 on TLC in ethyl acetate/hexane (1:1 v/v) containing 0.5% triethylamine. ^{31}P n.m.r. spectrum (CH_2Cl_2, concentric external D_2O lock) δ: +147.22 and 146.81 p.p.m.

RESULTS AND DISCUSSION

Our newly devised route to the 2′-*O*-allylguanosine monomer, compound **6**, is illustrated in FIG. 1. Full protection of the guanine ring is absolutely essential to prevent heterocyclic alkylation and so we based our protection of the guanine lactam function on the work described by Sekine *et al.* who established the 1,2-diisobutyryloxyethylene group as a new protecting group for the guanine moiety during phosphotriester chemistry.[16] The identification of the adduct formed between guanosine and glyoxal which was the basis for the above work had been elucidated many years previously by Shapiro and Hachmann.[17] We in any case continued to employ the Markiewicz protection[18] for simultaneous blocking of the 2′- and 3′-hydroxyl groups to ensure subsequent exclusive allylation of the ribose 2′-hydroxyl, and reckoned that the adduct formed between glyoxal and a suitably protected guanosine could be trapped by silylation such that the Markiewicz protection and a temporary 1,2-*bis*(*tert*-butyldimethylsilyloxy)ethylene group could be simultaneously removed after the allylation. Thus, guanosine was first protected with the Markiewicz disiloxane reagent, transiently protected by reaction with chlorotrimethylsilane,[19] isobutyrylated on the exocyclic amino group and the trimethylsilyl group was then cleaved by brief treatment with 4-toluenesulphonic acid giving compound **1** in 84% isolated yield. Compound **1** was then mixed with 5 mol. equiv. of anhydrous glyoxal in pyridine and formation of the adduct was driven by repeated evaporation of pyridine. This generates a 1,2-diol functionality as well as two new chiral centres, which make the ^{13}C n.m.r. spectra of compounds **2** and **3** somewhat complicated. Mixing the glyoxal adduct in DMF with imidazole removed the excess glyoxal by forming an insoluble white precipitate which was filtered off after 1 h, and subsequent reaction with a moderate excess of *tert*-butylchlorodimethylsilane afforded compound **2** in almost 88% yield. Allylation using our previously described conditions[10] gave compound **3** in 75.8% yield. Subsequent desilylation with tetrabutylammonium fluoride removed the disiloxane bridge and both *tert*-butyldimethylsilyl groups, however a brief workup under mildly basic conditions was still necessary to completely cleave the glyoxal adduct. This procedure afforded compound **4** in 84.4% isolated yield. Dimethoxytritylation and phosphitylation gave the desired 2′-*O*-allylguanosine monomer, compound **6**. The overall yield of monomer based on guanosine was 32.8% for the not yet optimised 10 step synthesis requiring 6 purifications. This is a substantial improvement over the old, labour intensive 13 step procedure which has an overall yield of about 30% starting from the very expensive 2-amino-6-chloropurine riboside and necessitated 9 chromatographic purifications. Compound **2** may well be a useful intermediate for the preparation of other guanosine analogues modified at the 2′-position. In fact this compound was readily alkylated in 68% isolated yield by reaction

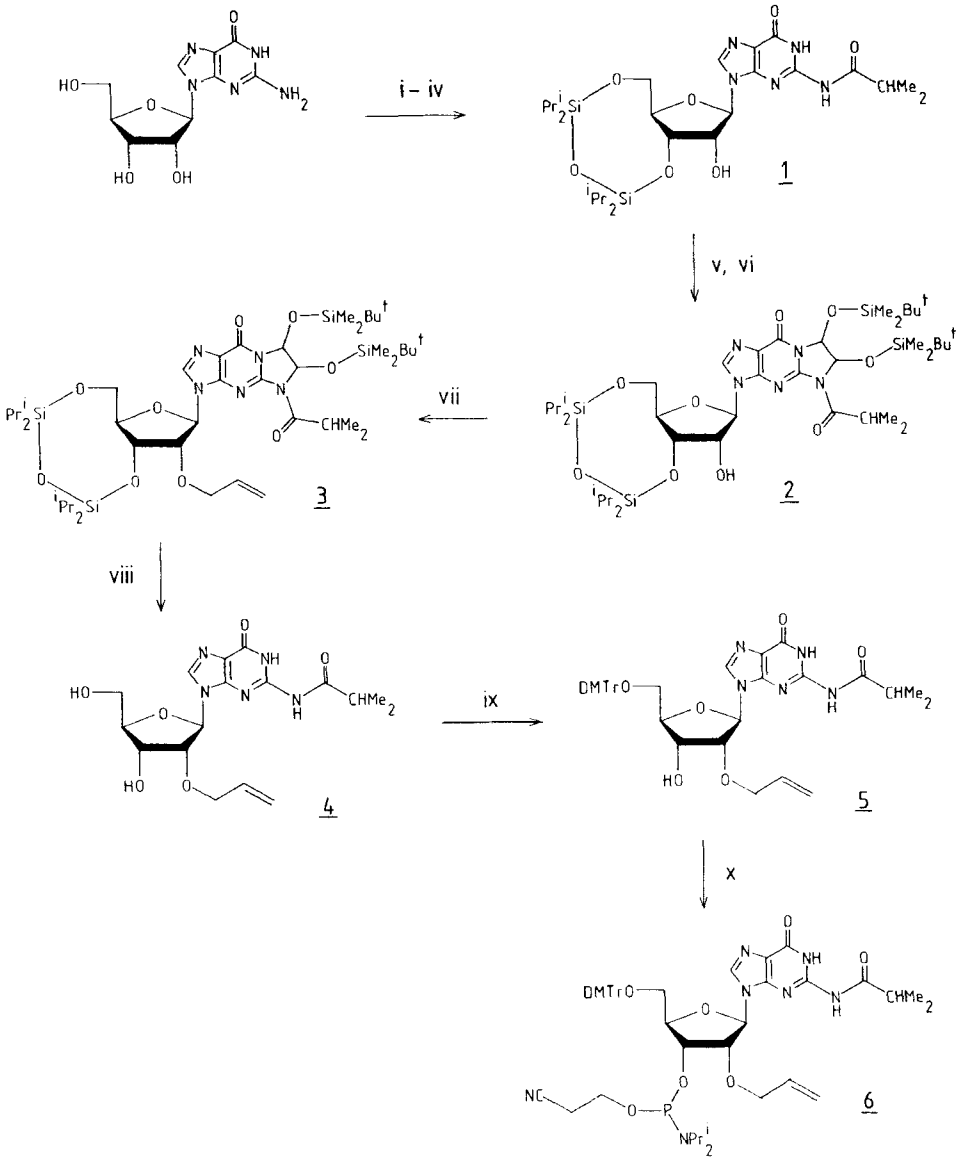

FIG. 1. Reaction scheme for the preparation of the 2′-*O*-allylguanosine monomer. Reagents: i, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in *N,N*-dimethylformamide/pyridine; ii, chlorotrimethylsilane in *N,N*-dimethylformamide/pyridine; iii, isobutyric anhydride in pyridine; iv, 4-toluene sulphonic acid in dioxan/dichloromethane; v, glyoxal in pyridine; vi, *tert*-butylchlorodimethylsilane and imidazole in *N,N*-dimethylformamide; vii, allyl ethyl carbonate, 1,4-*bis*(diphenylphosphino)butane and *tris*(dibenzylideneacetone)dipalladium(0) in tetrahydrofuran; viii, tetrabutylammonium fluoride in tetrahydrofuran followed by treatment with aqueous triethylamine; ix, 4,4′-dimethoxytrityl chloride and triethylamine in pyridine; x, 2-cyanoethoxy *N,N*-diisopropylaminochlorophosphine and *N,N*-diisopropylethylamine in 1,2-dichloroethane.

with ethyl bromoacetate in the presence of 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorin (BDDDP) using our previously described alkylation conditions.[20]

Our new route to a 2′-*O*-allylcytidine monomer is illustrated in FIG. 2. We decided to search for a direct route to the monomer without going *via* a uridine derivative as previous.[10] Attempted allylation of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-N^4-benzoylcytidine under the usual conditions[10] led to the expected exclusive N^3-allylation in 83.4% isolated yield. This observation is in agreement with others.[14] We reckoned that the problem was due to the enolisable amide proton in the acyl protected cytidine and that if we made an amidine protected cytidine instead, allylation should only occur on the ribose moiety. A search of the literature revealed that the *N*-methylpyrrolidine amidine of cytidine would be the protecting group of choice, since this is the only amidine derivative of cytidine tested that is stable to silica gel chromatography.[15] Thus cytidine was reacted with a small excess of *N*-methyl-2,2-dimethoxypyrrolidine to give compound **7** in 79% isolated yield. Subsequent Markiewicz protection[18] afforded compound **8** in 92.8% yield. Allylation of compound **8** gave a 90% isolated yield of compound **9**, which was desilylated, dimethoxytritylated and phosphitylated to give the desired 2′-*O*-allylcytidine monomer, compound **12** in excellent yield. The overall, not yet optimised yield of 2′-*O*-allylcytidine monomer was 41.2% for the 6 step synthesis, compared with 34% for our previous 12 step route.

The new route to a 2′-*O*-allyladenosine monomer, compound **18**, is illustrated in FIG. 3. In initial experiments we confirmed that allylation of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-N^6-benzoyladenosine with less than one mole equivalent of allyl ethyl carbonate led to N^1-allylation in 65% isolated yield. 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)adenosine could be allylated more rapidly on the ribose moiety than on the heterocycle however the isolated yield of desired product did not exceed 45%. We therefore decided to adopt the amidine protection strategy that proved so successful for cytidine above. Thus adenosine was reacted with *N,N*-diisobutylformamide dimethyl acetal to give compound **13** in almost quantitative isolated yield. Silylation afforded compound **14**, which was allylated almost quantitatively affording compound **15**. Subsequent desilylation, dimethoxytritylation and phosphitylation afforded an excellent yield of the 2′-*O*-allyladenosine monomer, compound **18**. The overall yield of compound **18** for the 6 step synthesis was 53.8% based on adenosine. If the *N,N*-diisobutylformamide dimethyl acetal is absolutely amine free then chromatographic purification of compound **13** can probably be omitted. The previous route was a 9 step synthesis with an overall yield of 34% starting from the expensive 6-chloropurine riboside and required the use of anhydrous ammonia at elevated temperature.

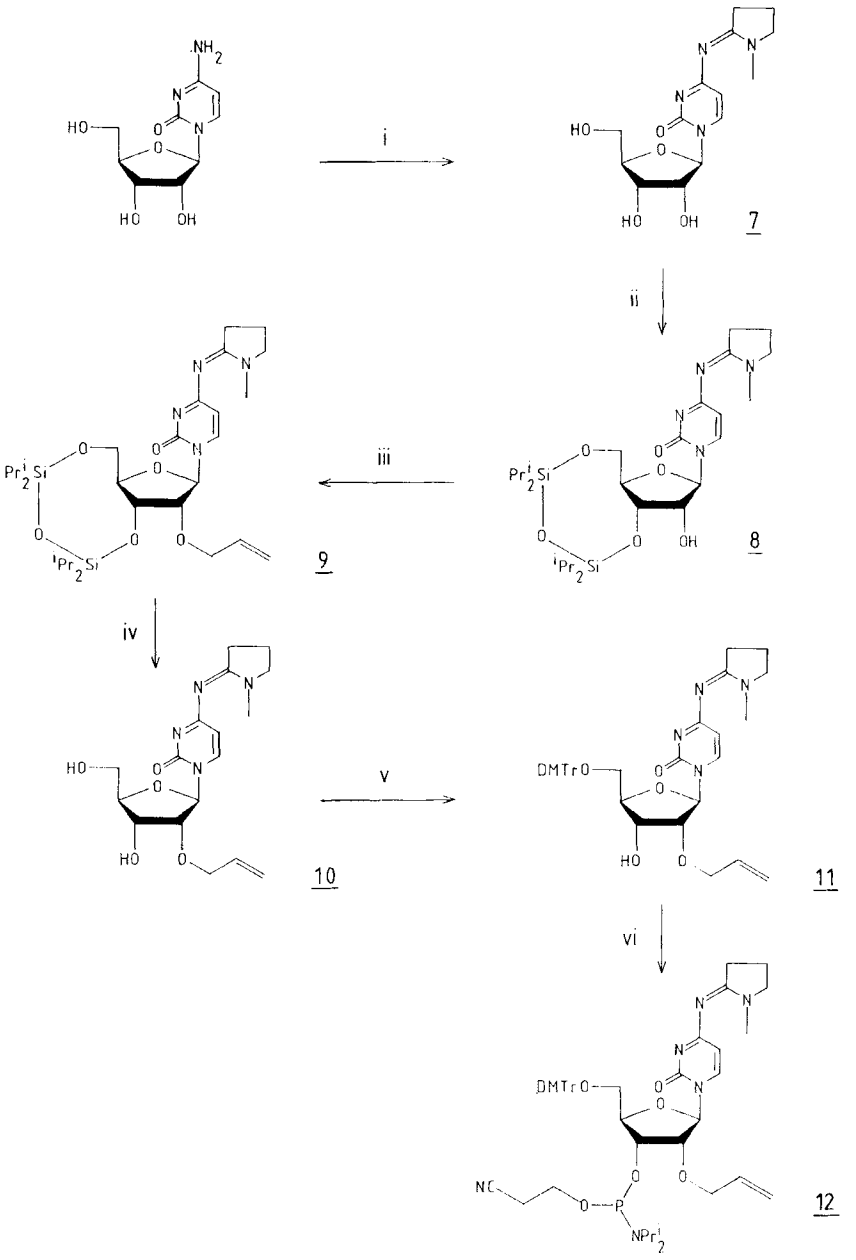

FIG. 2. Reaction scheme for the preparation of the 2′-*O*-allylcytidine monomer. Reagents: i, *N*-methyl-2,2-dimethoxypyrrolidine in methanol; ii, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine; iii, allyl ethyl carbonate, 1,4-*bis*(diphenylphosphino)butane and *tris*(dibenzylideneacetone)dipalladium(0) in tetrahyd furan; iv, tetrabutylammonium fluoride in tetrahydrofuran; v, 4,4′-dimethoxytrityl chloride and triethylamine in pyridine; vi, 2-cyanoethoxy *N,N*-diisopropylaminochlorophosphine and *N,N*-diisopropylethylamine in 1,2-dichloroethane.

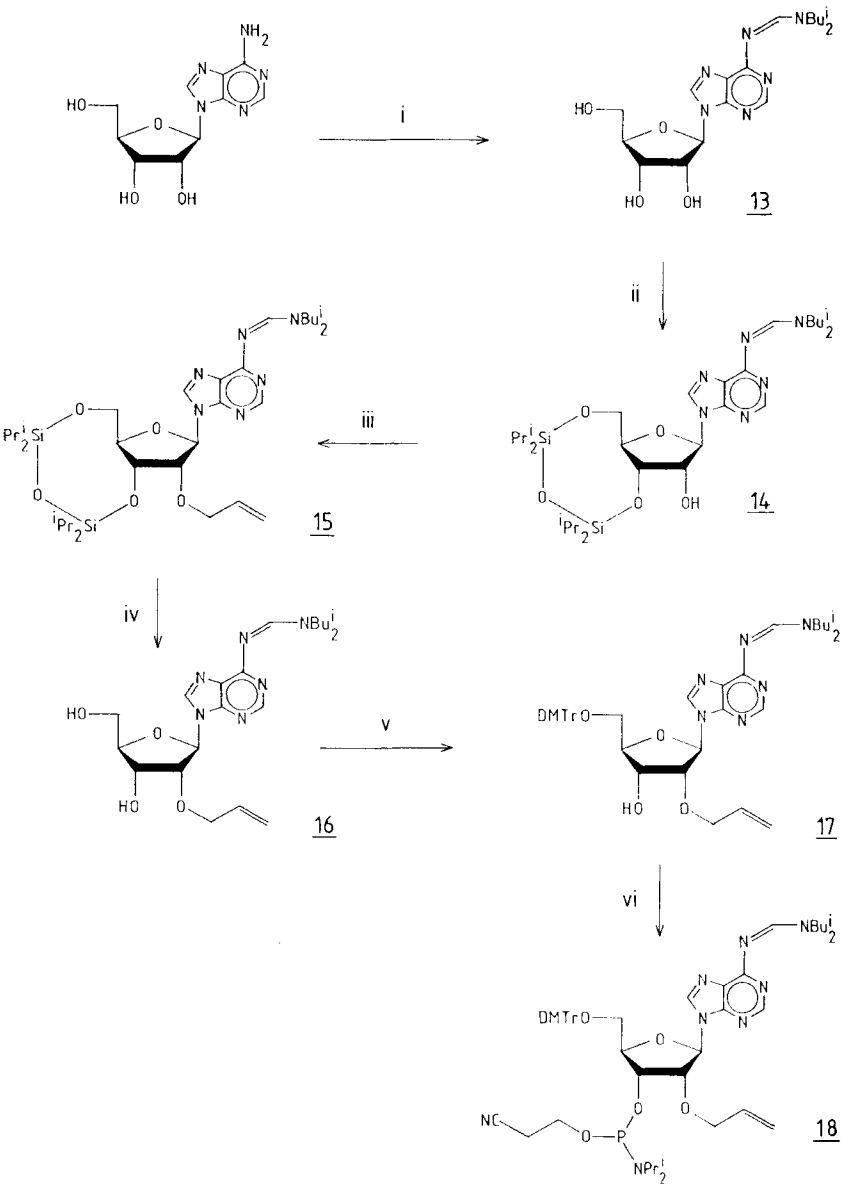

FIG. 3. Reaction scheme for the preparation of the 2′-*O*-allyladenosine monomer. Reagents: i, *N,N*-diisobutylformamide dimethyl acetal in methanol; ii, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine; iii, allyl ethyl carbonate, 1,4-*bis*(diphenylphosphino)butane and *tris*(dibenzylideneacetone)dipalladium(0) in tetrahydrofuran; iv, tetrabutylammonium fluoride in tetrahydrofuran; v, 4,4′-dimethoxytrityl chloride and triethylamine in pyridine; vi, 2-cyanoethoxy *N,N*-diisopropylaminochlorophosphine and *N,N*-diisopropylethylamine in 1,2-dichloroethane.

The modified 2′-*O*-allyluridine monomer synthesis is illustrated in FIG. 4. Thus, reaction of uridine with the Markiewicz reagent, followed by trimethylsilylation, N^3-acylation with 4-*tert*-butylbenzoyl chloride and subsequent cleavage of the trimethylsilyl ether afforded compound **19** in 64.9% isolated yield. This material was allylated and desilylated to afford compound **20** in 82.9% isolated yield. Dimethoxytritylation and subsequent phosphitylation gave an excellent yield of the desired 2′-*O*-allyluridine monomer, compound **22**. The *tert*-butylbenzoyl group serves a dual purpose, it prevents allylation of the uracil moiety and substantially increases the lipophilicity of compounds **20**, **21** and **22** thus simplifying their purification. The overall yield of the new 8 step synthesis, which requires 4 purification steps was 44.2%, compared with 28% and 6 purification steps for the previous 10 step synthesis.

The reaction scheme for the synthesis of the 2′-*O*-allylinosine monomer, compound **28** is illustrated in FIG. 5. Inosine was reacted with the Markiewicz reagent in DMF in the presence of imidazole to afford compound **23** in 87.8% isolated yield. Performing this reaction in pyridine only, gave a substantially reduced yield.[21] In order to protect the hypoxanthine ring from allylation we decided to block the N^1-position with the ammonia labile pivaloyloxymethyl (Pom) group, in addition this group increases the lipophilicity of the various intermediates and prevents undesirable N^1-dimethoxytritylation at a later stage in the synthesis. The Pom group[22] has been previously used to protect the N^1 of inosine[23] and the N^3 of uridine.[24] Thus, compound **23** was trimethylsilylated and the crude reaction product was reacted with fresh chloromethyl pivalate in DMF in the presence of anhydrous potassium carbonate. Cleavage of the trimethylsilyl group afforded compound **24** in 61.5% isolated yield. Allylation gave compound **25** in 83.1% yield and subsequent desilylation, dimethoxytritylation and phosphitylation afforded the desired 2′-*O*-allylinosine monomer, compound **28** in good yield. The overall yield of monomer, not yet optimised was 22.8% for the 8 step synthesis involving 6 purification steps. The previous route had an overall yield of 20% for the 7 step synthesis starting from the expensive 6-chloropurine riboside and generated a monomer without protection of the nucleobase.

Compounds **8**, **14** and **19** could also be rapidly and cleanly alkylated on the 2′-hydroxyl group with esters (methyl, ethyl and t-butyl) of bromoacetic acid as mentioned above for the guanosine derivative, compound **2**, in the presence of the hindered organophosphorus base, BDDDP, under the conditions previously described.[20] The esters thus obtained are the starting point for the synthesis of a variety of useful analogues. The further elaboration of the 2′-*O*-(alkyloxycarbonyl)methyl group into a variety of different residues such as lipophilic, intercalating and aminoalkyl groups has been recently described by Keller and Häner, who introduced the 2′-modification on methyl 3,5-*O*-

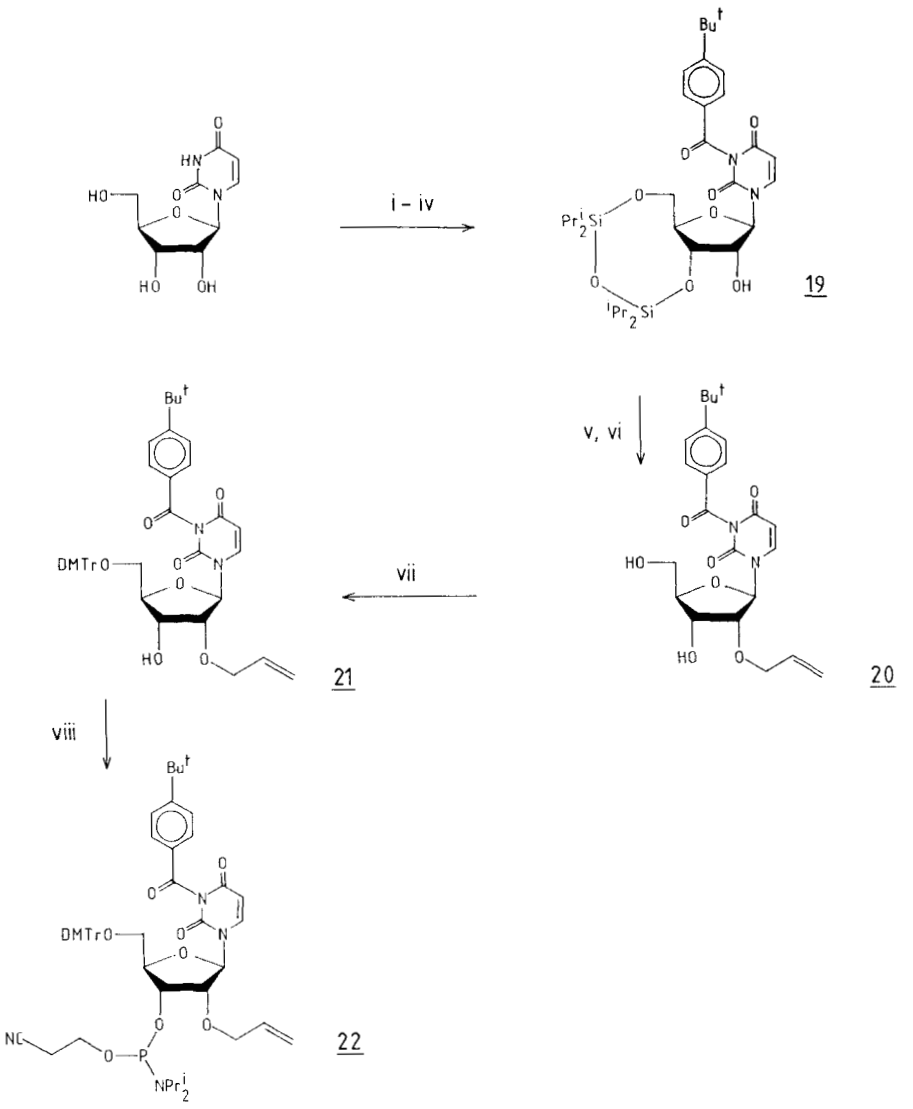

FIG. 4. Reaction scheme for the preparation of the 2′-*O*-allyluridine monomer. Reagents: i, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine; ii, chlorotrimethylsilane and triethylamine in dichloromethane; iii, 4-*tert*-butylbenzoyl chloride and *N,N*-diisopropylethylamine in pyridine; iv, 4-toluene sulphonic acid in dioxan/dichloromethane; v, allyl ethyl carbonate, 1,4-*bis*(diphenylphosphino)butane and *tris*(dibenzylideneacetone)dipalladium(0) in tetrahydrofuran; vi, tetrabutylammonium fluoride in tetrahydrofuran; vii, 4,4′-dimethoxytrityl chloride and triethylamine in pyridine; viii, 2-cyanoethoxy *N,N*-diisopropylaminochlorophosphine and *N,N*-diisopropylethylamine in 1,2-dichloroethane.

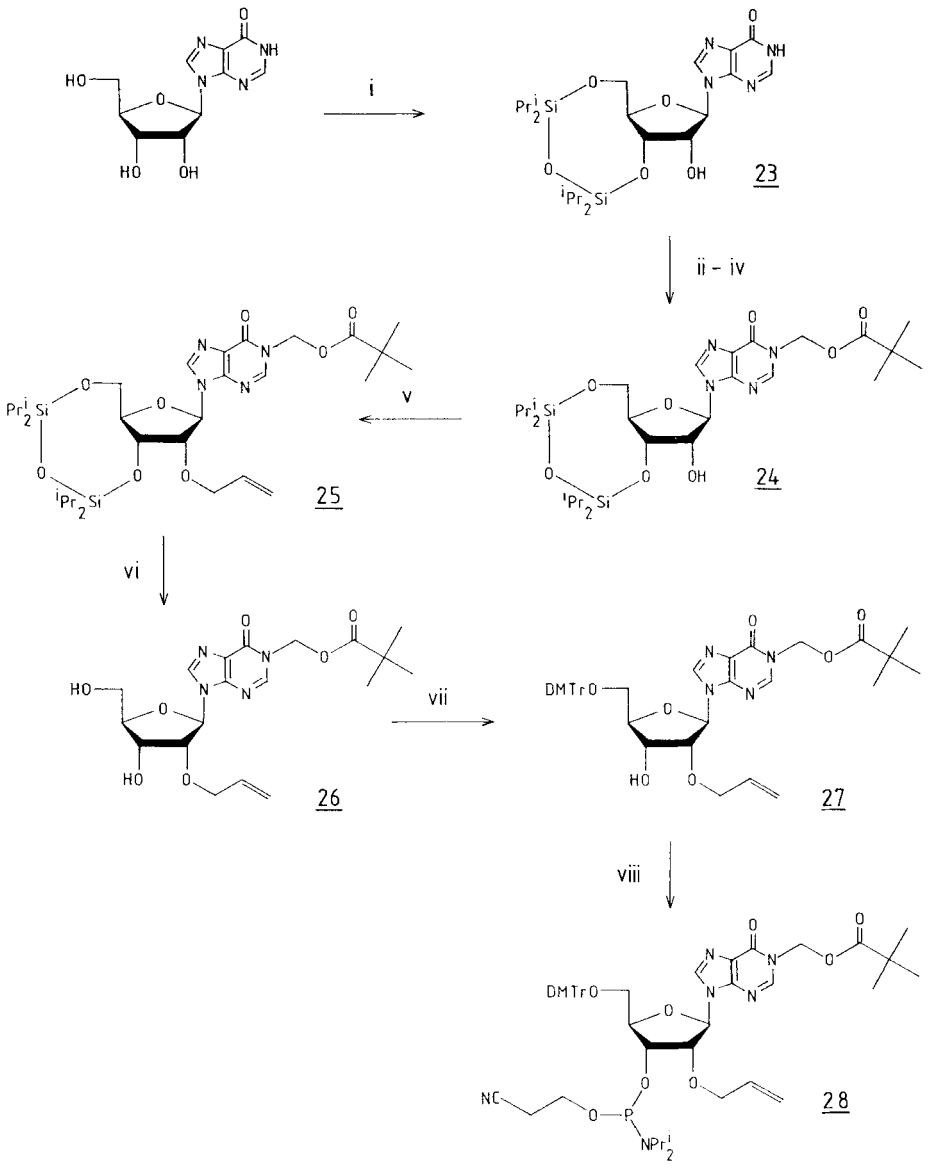

FIG. 5. Reaction scheme for the preparation of the 2′-*O*-allylinosine monomer. Reagents: i, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane and imidazole in *N,N*-dimethylformamide; ii, chlorotrimethylsilane and triethylamine in dichloromethane; iii, chloromethyl pivalate and potassium carbonate in *N,N*-dimethylformamide; iv, 4-toluene sulphonic acid in dioxan/dichloromethane; v, allyl ethyl carbonate, 1,4-*bis*(diphenylphosphino)butane and *tris*(dibenzylideneacetone)dipalladium(0) in tetrahydrofuran; vi, tetrabutylammonium fluoride in tetrahydrofuran; vii, 4,4′-dimethoxytrityl chloride and triethylamine in pyridine; viii, 2-cyanoethoxy *N,N*-diisopropylaminochlorophosphine and *N,N*-diisopropylethylamine in 1,2-dichloroethane.

(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranoside and subsequently obtained the modified nucleosides by glycosylation.[25]

As this article was almost completed Chanteloup and Thuong published a convenient general synthesis of 2′-*O*-alkyl ribonucleosides employing 2-*O*-alkyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl trichloroacetimidates as ribosyl donors for glycosylation of silylated nucleobases.[26] The 2-*O*-propyl sugar was obtained in 46% yield in 5 steps starting from D-ribose. Conversion of this common intermediate to a 2′-*O*-propylribonucleoside-3′-*O*-phosphoramidite required a further 4 steps and gave isolated yields of monomer between 30 and 33% based on D-ribose. Nonetheless, the new routes that we described above involving the direct allylation (alkylation with esters of bromoacetic acid was also achieved; data not shown) of suitably protected ribonucleosides are substantially higher yielding and require less synthetic steps.

The phosphoramidite monomers, compounds **6**, **12**, **18**, **22** and **28** have been used to prepare oligo(2′-*O*-allylribonucleotides) according to the published procedure[11] with the exception that we added ammonium acetate to the aqueous ammonia deblock solution to accelerate removal of the amidine protection on adenosine following the recommendation of McBride *et al.*[15]

REFERENCES

1. Iribarren, A. M.; Sproat, B. S.; Neuner, P.; Sulston, I.; Ryder, U.; Lamond, A. I. *Proc. Natl. Acad. Sci. USA*, **1990**, *87*, 7747.
2. Lamond, A. I.; Sproat, B. S. *FEBS Lett.*, **1993**, *325*, 123.
3. Lamm, G. M.; Blencowe, B. J.; Sproat, B. S.; Iribarren, A. M.; Ryder, U.; Lamond, A. I. *Nucleic Acids Res.*, **1991**, *19*, 3193.
4. Barabino, S. M. L.; Sproat, B. S.; Lamond, A. I. *Nucleic Acids Res.*, **1992**, *20*, 4457.
5. Carmo-Fonseca, M.; Pepperkok, R.; Sproat, B. S.; Ansorge, W.; Swanson, M. S.; Lamond, A. I. *EMBO J.*, **1991**, *10*, 1863.
6. Johansson, H. E.; Sproat, B. S.; Melefors, Ö. *Nucleic Acids Res.*, **1993**, *21*, 2275.
7. Paolella, G.; Sproat, B. S.; Lamond, A. I. *EMBO J.*, **1992**, *11*, 1913.
8. Wagner, R. W.; Matteucci, M. D.; Lewis, J. G.; Gutierrez, A. J.; Moulds, C.; Froehler, B. C. *Science*, **1993**, *260*, 1510.
9. Fisher, T. L.; Terhorst, T.; Cao, X.; Wagner, R. W. *Nucleic Acids Res.*, **1993**, *21*, 3857.
10. Sproat, B. S.; Iribarren, A. M.; Guimil Garcia, R.; Beijer, B. *Nucleic Acids Res.*, **1991**, *19*, 733.

11. Sproat, B. S. In *Methods in Molecular Biology, Vol.20: Protocols for Oligonucleotides and Analogs*; Agrawal, S., Ed.; Humana Press Inc.: Totowa, NJ, **1993**, pp.115-141.
12. Lakhmiri, R.; Lhoste, P.; Sinou, D. *Tetrahedron Lett.*, **1989**, *30*, 4669.
13. Gopalakrishnan, V.; Kumar, V.; Ganesh, K. N. *Nucleosides & Nucleotides*, **1992**, *11*, 1263.
14. Kumar, V.; Gopalakrishnan, V.; Ganesh, K. N. *Bull. Chem. Soc. Jpn.*, **1992**, *65*, 1665.
15. McBride, L. J.; Kierzek, R.; Beaucage, S. L.; Caruthers, M. H. *J. Am. Chem. Soc.*, **1986**, *108*, 2040.
16. Sekine, M.; Matsuzaki, J.; Hata, T. *Tetrahedron Lett.*, **1982**, *23*, 5287.
17. Shapiro, R.; Hachmann, J. *Biochemistry*, **1966**, *5*, 2799.
18. Markiewicz, W. T. *J. Chem. Res. (S)*, **1979**, 24.
19. Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.*, **1982**, *104*, 1316.
20. Sproat, B. S.; Beijer, B.; Iribarren, A. *Nucleic Acids Res.*, **1990**, *18*, 41.
21. Beijer, B.; Sulston, I.; Sproat, B. S.; Rider, P.; Lamond, A. I.; Neuner, P. *Nucleic Acids Res.*, **1990**, *18*, 5143.
22. Rasmussen, M.; Leonard, N. J. *J. Am. Chem. Soc.*, **1967**, *89*, 5439.
23. Brown, J. M.; Christodoulou, C.; Jones, S. S.; Modak, A. S.; Reese, C. B.; Sibanda, S.; Ubasawa, A. *J. Chem. Soc. Perkin Trans 1*, **1989**, 1735.
24. Sekine, M.; Hata, T. *J. Am. Chem. Soc.*, **1986**, *108*, 4581.
25. Keller, T. H.; Häner, R. *Helv. Chim. Acta*, **1993**, *76*, 884.
26. Chanteloup, L.; Thuong, N. T. *Tetrahedron Lett.*, **1994**, *35*, 877.

Received March 25, 1994
Accepted May 2, 1994