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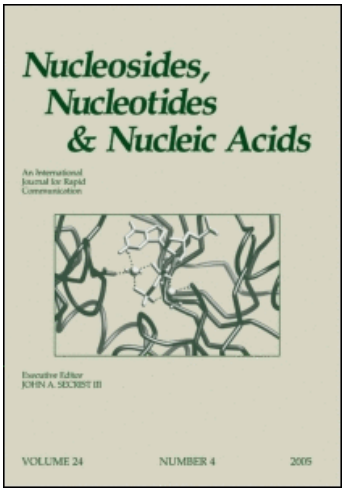

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Synthesis of Suitably-Protected Phosphoramidites of 2'-Fluoro-2'-Deoxyguanosine and 2'-Amino-2'-Deoxyguanosine for Incorporation Into Oligoribonucleotides

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SYNTHESIS OF SUITABLY-PROTECTED PHOSPHORAMIDITES OF 2'-FLUORO-2'-DEOXYGUANOSINE AND 2'-AMINO-2'-DEOXYGUANOSINE FOR INCORPORATION INTO OLIGORIBONUCLEOTIDES.

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ABSTRACT: A novel synthesis of 2'-fluoro-2'-deoxyguanosine employing DAST as the fluorinating agent is presented. The preparation of its phosphoramidite as well as that of 2'-amino-2'-deoxyguanosine is also described.

INTRODUCTION

Ribonucleosides in which the 2'-hydroxyl group has been replaced by fluorine (1, 2, 3, 4, 5) or amino groups (6, 7, 8, 9) have been known for several years. The properties of enzymatically- synthesised homopolynucleotides containing 2'-fluoro-2'-deoxyribose (10, 11, 12, 13) or 2'-amino-2'-deoxyribose (14) have also been described. In addition, 2'-fluoro-2'-deoxynucleosides have been incorporated chemically into oligodeoxynucleotides to study restriction enzyme-DNA interactions (15, 16).

Developments in automated chemical synthesis of oligoribonuceotides (17) have allowed the opportunity to

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specifically incorporate 2'-modified nucleosides into RNA. Such modifications are particularly attractive in order to gain a better understanding of the role of 2'-hydroxyl groups for RNA structure and function. The 2'-fluoro-2'-deoxynucleosides for example display a 3'-endo/2'-endo ratio of the sugar conformations greater than that of ribonucleosides while the 2'-amino-2'-deoxynucleosides favour the 2'-endo conformation even more than 2'-deoxynucleosides do (18, 19). In addition, the 2'-fluoro substituent can only serve as a hydrogen bond acceptor, whereas the 2'-amino group can act as both donor and acceptor, similar to the 2'-hydroxyl group of ribose.

There has been considerable interest in recent years in the hammerhead structure associated with the self-cleavage reaction of a number of plant viral RNA molecules. In order to investigate the importance of the 2'-hydroxyl groups in such hammerhead structures we have incorporated 2'-fluoro- and 2'-amino-pyrimidine 2'-deoxynucleosides (20) and 2'-fluoro-2'-deoxyadenosine (21) within the enzyme part of a two-stranded hammerhead ribozyme described by Uhlenbeck (22). It was found that any one of the adenosine residues or all of the cytidine and uridine residues could be replaced by the corresponding 2'-fluoro-2'-deoxynucleosides without appreciable loss of activity relative to the unmodified ribozyme, indicating that none of these 2'-hydroxyl groups are critical for activity.

In order to expand these results to determine the effect of modification of the 2'-position of guanosine we had to synthesise the corresponding phosphoramidites. We report here a novel and efficient synthesis of 2'-fluoro-2'-deoxyguanosine and the preparation of suitably-protected phosphoramidites of both 2'-fluoro- and 2'-amino-2'-deoxyguanosine for incorporation into such oligoribonucleotides via standard automated chemical synthesis.

EXPERIMENTAL

Acetonitrile and methanol (0.01 % H_2O) from Merck (Darmstadt, FRG) were stored over activated 3 Å molecular

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sieves (Merck). Dichloromethane (Merck) was passed over a column of basic alumina immediately prior to use. Toluene and THF (containing less than 0.01 % H_2O) (Merck) were stored over activated 4 Å molecular sieves. Analytical grade DMF (Merck) was distilled under reduced pressure using a dry argon bleed and a fractionating column filled with glass helices. Pyridine, acetic anhydride, 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (TPDS-dichloride), *N,N,N*-diisopropylethylamine and (diethylamino)sulphur trifluoride (DAST) were purchased from Merck. 9-Chloro-9-phenylxanthene (pixyl chloride) and S-ethyl trifluorothioacetate were purchased from Fluka AG (Neu-Ulm, FRG). Tetra-n-butylammonium fluoride (TBAF, 1.1 M in THF), β-cyanoethyl(*N,N*-diisopropylamino)chlorophosphoramidite, *N*-methylimidazole and dimethylformamide dimethylacetal were obtained from Aldrich Chemie (Steinheim, FRG). SEP-PAK C-18 cartridges were from Millipore-Waters (Boenningstedt, FRG). LiChrospher 100 RP-18, Kieselgel 60 (< 63 mm) and precoated silica gel F_{254} plates for thin-layer chromatography (TLC) were also obtained from Merck.

^{1}H-NMR spectra were recorded at 360.13 MHz on a Bruker WH 360 spectrometer with tetramethylsilane as the internal standard. CD_3OD was added to ^{1}H-NMR samples for the identification of exchangeable protons. ^{19}F- and ^{31}P-NMR spectra were recorded on the same instrument at 338.87 and 145.79 MHz respectively with ^{1}H-decoupling and C_6F_6 as the internal standard or 85 % H_3PO_4 as the external standard. ^{19}F and ^{31}P chemical shift values which are upfield of the respective standards possess a negative value.

Silica TLC plates were developed using one of the following solvent systems: S1, CH_2Cl_2/MeOH 9:1 (v/v); S2, CH_2Cl_2/MeOH 4:1 (v/v); S3, isopropanol/water/25 % aqueous ammonia 7:2:1 (v/v/v); S4, isopropanol/water/25 % aqueous ammonia 70:25:5 (v/v/v); S5, CH_2Cl_2/MeOH 75:25 (v/v); S6, CH_2Cl_2/MeOH 85:15 (v/v). Compounds were visualized by UV light or by spraying with 5 % aqueous sulphuric acid fol-

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lowed by heating. Flash column chromatography was performed on Kieselgel 60. Preparative HPLC for 2'-fluoro-2'-deoxyguanosine was performed on a column (21 mm x 305 mm) containing LiChrospher 100 RP-18 material using a DuPont 8800 instrument equipped with preparative pump heads (40 ml), an 8 ml injection loop and coupled to a DuPont 8800 UV detector. Preparative HPLC for 2'-amino-2'-deoxyguanosine was performed using a Waters Delta Prep 3000 instrument equipped with a Waters Delta Pak reverse-phase preparative HPLC column (57 mm x 300 mm). The following HPLC gradients were employed; Gradient I, an isocratic gradient of water for 5 min, followed by a linear gradient of acetonitrile (0 - 17 % in 50 min) in water, with a flow rate of 15 ml/min. It is preferable to use water as solvent A and an aqueous acetonitrile solution as solvent B.; gradient II, an isocratic gradient of 50 mM TEAA, pH 7.0 for 10 min, followed by an isocratic gradient of 0.9 % acetonitrile in 50 mM triethylammonium acetate (TEAA), pH 7.0 with a flow rate of 45 ml/min; gradient III, an isocratic gradient of water for 10 min, followed by an isocratic gradient of 10 % acetonitrile in water with a flow rate of 45 ml/min.

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9-(3,5-O-TPDS-β-D-ribofuranosyl)guanine (**1**) was prepared according to the literature (23).

9-(3,5-O-TPDS-β-D-arabinofuranosyl)guanine (**2**)
Compound **2** was prepared from **1** as described by Samano and Robins (24) and purified by flash column chromatography (Kieselgel 60) using a stepwise gradient of 2 %, 3 %, 4 %, and 5 % MeOH in CH_2Cl_2. After evaporation of product-containing fractions, a light brown foam was obtained. Yield 68 %, ^1H NMR: $\delta(d_6\text{-DMSO})$ 7.61 (s, 1H, H8), 6.23 (dd, 1H, $J_{1',2'}$=6.5 Hz, H1'), 4.41 (dd, 1H, H2'), 4.29 (dd, 1H, H3'); 3.97 (dd, 1H, H5'); 3.92 (dd, 1H, H5''); 3.74 (m, 1H, H4'); 1.02 (broad m, 28H, 4 isopropyl).

N^2-Pixyl-9-(3,5-O-TPDS-β-D-arabinofuranosyl)guanine (**3**)
Compound **2** (5.5 g, 10.5mmol) was dried by coevaporation twice with pyridine, dissolved in CH_2Cl_2 (150 ml) and

diisopropylethylamine (15 ml) and pixyl chloride (3.4 g, 11.5 mmol) added portionwise over 60 min. The reaction was followed by TLC (S1, Rf of **2**, 0.26; Rf of **3**, 0.49). After 3 h, MeOH (10 ml) was added and the solution washed with saturated aqueous $NaHCO_3$ (150 ml), the organic phase was dried over Na_2SO_4, the filtrate evaporated and then coevaporated twice with toluene (2 x 20 ml). The crude product was purified by flash chromatography (Kieselgel 60, 80 g) using a stepwise gradient of 0.5 % and 1 % MeOH in CH_2Cl_2 containing 0.5 % of triethylamine. Product-containing fractions were pooled and evaporated to dryness to give a yellow foam. Yield 5.23 g (64 %). The ^1H NMR spectrum was in agreement with the proposed structure.

N^2-Pixyl-9-(2-O-acetyl-3,5-O-TPDS-β-D-arabinofuranosyl)-guanine (**4**)

Acetic anhydride (1.9 ml, 20.1 mmol) and *N*-methylimidazole (300 µL, 3.8 mmol) were added to a solution of **3** (5.2 g, 6.7 mmol) in pyridine (120 ml). After 3 h, TLC (S1, Rf of **3**, 0.49; Rf of **4**, 0.62) indicated that the reaction was complete. MeOH (5 ml) was added, the solution concentrated to dryness and the residue dissolved in CH_2Cl_2 (250 ml). This solution was washed with 5 % aqueous $NaHCO_3$ (250 ml) followed by saturated brine (250 ml) and the organic layer was dried over Na_2SO_4, and the filtrate then evaporated and coevaporated twice with toluene (2 x 20 ml). The crude product was purified by flash chromatography (Kieselgel 60, 80 g) using a stepwise gradient of 0.5 % and 1 % MeOH in CH_2Cl_2 containing 0.5 % of triethylamine. Evaporation of the pooled product-containing fractions afforded a white foam. Yield 4.5 g (82 %). The ^1H NMR spectrum was in agreement with the proposed structure.

N^2-Pixyl-9-(2-O-acetyl-β-D-arabinofuranosyl)guanine (**5**)

To a solution of **4** (4.5 g, 5.5 mmol) in THF (50 ml), was added a 1.1 M solution of TBAF (6 ml, 6.6 mmol) in THF and the reaction was followed by TLC (S1, Rf of **4**, 0.49; Rf of **5**, 0.21; S2, Rf of **5**, 0.61). To the resulting thick, white suspension was added CH_2Cl_2 (400 ml) and the solution washed

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with saturated aqueous $NaHCO_3$ (300 ml). The organic layer was dried over Na_2SO_4, the filtrate evaporated and the crude product purified by flash chromatography (Kieselgel 60, 50 g) using a stepwise gradient of 2 % and 4 % MeOH in CH_2Cl_2 containing 0.5 % of triethylamine. Evaporation of the pooled product-containing fractions afforded a white foam. Yield 2.75 g (86 %). The ^1H NMR spectrum was in agreement with the proposed structure.

N^2-Pixyl-9-(2-O-acetyl-3,5-di-O-pixyl-β-D-arabinofuranosyl)-guanine (**6**)

Compound **5** (1.92 g, 3.3 mmol) was dried by coevaporation of pyridine (2 x 50 ml), dissolved in pyridine (150 ml) and pixyl chloride (2.4 g, 8 mmol) was added. After stirring overnight, TLC indicated the reaction to be complete (S1, Rf of **5**, 0.16; Rf of monopixylated intermediate, 0.41; Rf of dipixylated product **6**, 0.47). MeOH (10 ml) was then added, the solution concentrated and the residue dissolved in CH_2Cl_2 (300 ml) and washed with saturated aqueous $NaHCO_3$ (300 ml). The organic layer was dried over Na_2SO_4, evaporated, coevaporated twice with toluene (2 x 20 ml) and the crude product purified by flash chromatography (Kieselgel 60, 40 g) using a stepwise gradient of 0.5 %, 1 %, and 2 % MeOH in CH_2Cl_2 containing 0.5 % of triethylamine. Product-containing fractions were pooled and evaporated to dryness to afford a white foam. Yield 5.25 g (62 %). The ^1H NMR spectrum was in agreement with the proposed structure.

N^2-Pixyl-9-(3,5-di-O-pixyl-β-D-arabinofuranosyl)guanine (**7**)

To a suspension of compound **6** (2.25 g, 2.1 mmol) in a mixture of ethyl acetate (10 ml) and MeOH (140 ml) was added 4 M NaOH (1.5 ml, 6 mmol) with vigourous stirring. The resulting clear solution was stirred overnight and the reaction monitored by TLC (S1, Rf of **6**, 0.47, Rf of **7**, 0.42). After completion of the reaction, CH_2Cl_2 (500 ml) was added and the solution washed with saturated aqueous $NaHCO_3$ (300 ml), followed by saturated brine (300 ml). The organic layer was dried over Na_2SO_4, the filtrate evaporated and

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then coevaporated twice with toluene (2 x 20 ml) to afford a white foam. Yield 2.18 g (62 %). The ^{1}H NMR spectrum was in agreement with the proposed structure.

9-(2-Fluoro-2-deoxy-β-D-ribofuranosyl)guanine (2'-fluoro-2'-deoxyguanosine (**8**)

To a suspension of **7** (560 mg, 0.53 mmol) in CH_3CN (15 ml), and DMF (750 µl), was added (diethylamino)sulphur trifluoride (350 µl, 2.6 mmol). After a few minutes, a clear solution had formed, which was stirred at 35°C for 10 h, after which time a new suspension had formed. TLC indicated the reaction to be complete [S1, Rf of **7**, 0.63; Rf of **8**, 0.80; S3 (after removal of the pixyl groups by treatment of the applied material with a solution of 10 % dichloroacetic acid in CH_2Cl_2 and pre-running the TLC plate in S2 to remove deblocked, pixyl-positive material), Rf of **7**, 0.50; Rf of **8**, 0.59] and the suspension was placed on ice for 15 min, then methanol (1 ml) and triethylamine (500 ml) were added. The mixture was evaporated almost to dryness and the residue partitioned between CH_2Cl_2 (25 ml) and water (20 ml). The organic phase was evaporated to dryness, then residual triethylamine removed by coevaporation with ethyl acetate/ethanol (5 ml; 1:1, v/v). The resulting material was dissolved in 0.1 M HCl in methanol and stirred for 1 hr at room temperature. The solution was then neutralized with 1 M aqueous NaOH solution, evaporated almost to dryness and the residue was redissolved in water (20 ml) and extracted twice with CH_2Cl_2 (20 ml). The aqueous phase was concentrated to about 5 ml and remaining pixyl-positive material was removed by SEP-PAK C-18 filtration. The product was eluted with 5 % methanol in water and the solution evaporated. The residue was redissolved in water (2 ml) and then purified by preparative HPLC using gradient I (retention time, 27 min). The product-containing fractions were evaporated and re-evaporated twice with water. Yield 42 mg (28 %); ^{1}H NMR: δ(d_6-DMSO) 7.95 (s, 1H, H8); 6.01 (dd, 1H, J_{HH}=2.7 Hz, J_{HF}=16.5 Hz, H1'); 5.23 (ddd, 1H, J_{HF}=52.1 Hz, H2'); 4.37 (ddd, 1H, J_{HF}=18.5 Hz, H3'); 3.93 (m, 1H, H4'); 3.73 (dd, 1H, H5'); 3.58 (dd, 1H, H5'); ^{19}F NMR: δ(d_6-DMSO) - 203.6 (s).

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N^2-Isobutyryl-2'-fluoro-2'-deoxyguanosine (**9**)
2'-Fluoro-2'-deoxyguanosine (**8**) was protected as its N^2-isobutyryl derivative using standard conditions, with the exception that isobutyric anhydride was replaced by isobutyryl chloride (25). Yield 72 %; Rf value (S2), 0.24. The ^1H NMR spectrum was in agreement with the proposed structure.

5'-O-(4,4'-Dimethoxytrityl)-N^2-isobutyryl-2'-fluoro-2'-deoxyguanosine (**10**)
5'-O-Dimethoxytritylation of **9** was performed according to the literature procedure (26) and the crude product was purified by flash chromatography (Kieselgel 60). Yield 89 %; Rf value (S1), **9**, 0.12; **10**, 0.40. The ^1H NMR spectrum was in agreement with the proposed structure.

5'-O-(4,4'-Dimethoxytrityl)-N^2-isobutyryl-2'-fluoro-2'-deoxyguanosine-3'-O-(β-cyanoethyl N,N-diisopropylphosphoramidite) (**11**)
Compound **11** was prepared following the literature procedure (27) with the modifications previously described (16). Yield 65 %; ^{31}P NMR: $\delta(CDCl_3)$ 150.81 (d, J_{PF} = 8.4 Hz); 151.51 (d, J_{PF} = 9.2 Hz); 8.37 (s, 12 % phosphonate impurity); ^{19}F NMR: $\delta(CDCl_3)$ - 200.47 (d, J_{PF} = 9.3 Hz),- 201.93 (d, J_{PF} = 8.6 Hz); - 203.50 (s, phosphonate impurity).

2'-Amino-2'-deoxyguanosine (**12**)
Compound **12** was prepared according to the literature procedure (9), with the exception that crude product was purified by preparative HPLC using gradient II (retention time approx. 55-77 min). After evaporation of pooled product-containing fractions, residual TEAA was removed by using gradient III after reinjection of the sample onto the HPLC column. The material obtained (yield 60%) was identical to that previously described (9).

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2'-(Trifluoroacetamido)-2'-deoxyguanosine (**13**)
2'-Amino-2'-deoxyguanosine (**12**) (282 mg, 1 mmol) was suspended in dry methanol (20 ml) and S-ethyl trifluorothioacetate (191 µL, 1.5 mmol) was added and the reaction monitored by TLC. After stirring at room temperature for 12 hr the clear solution which had formed was evaporated to dryness. Yield 334 mg (88 %); Rf value (S4), **13**; 0.76, **12**, 0.56; ^{19}F NMR: $\delta(d_6\text{-DMSO})$ -73.40 (77 %); ^{1}H NMR: $\delta(d_6\text{-DMSO})$ 10.68 (broad s, 1H, N1-H); 9.60 (d, 1H, J=7.7Hz, 2'-NH); 7.84 (s, 1H, H8); 6.45 (s, 2H, NH_2); 5.99 (d, 1H, J=8.3 Hz, H1'); 5.79 (d, 1H, OH); 5.13 (t, 1H, OH); 4.96 (q, 1H, H2'); 4.30 (broad s, 1H, H3'); 3.96 (broad s, 1H, H4'); 3.62-3.54 (m, 2H, H5' & 5'').

5'-O-(4,4'-Dimethoxytrityl)-N^2-[(dimethylamino)methylene]-2'-(trifluoroacetamido)-2'-deoxyguanosine (**14**)
2'-Amino-2'-deoxyguanosine (**12**) (1 mmol) was treated with S-ethyl trifluorothioacetate as described above and the solution was evaporated to dryness. The residue was dried by re-evaporating with dry methanol (3 x 20 ml) and was then suspended in dry methanol (2.5 ml). To this solution was added dimethylformamide dimethylacetal (480 µl, 4 mmol). The solution was stirred at room temperature for 8 h after which time TLC (S5, Rf of **13**; 0.60, Rf of amidine, 0.73) indicated the reaction to be essentially complete. The solution was then evaporated to dryness and the residue dried overnight in vacuo. The material was then redissolved in dry pyridine (10 ml) and 4,4'-dimethoxytrityl chloride (407 mg, 1.2 mmol) added portionwise to the stirred solution over 2 h. The reaction was monitored by TLC (S6, Rf of amidine, 0.28; Rf of **14**, 0.71) and after 24 h methanol (500 ml) was added. The solution was stirred for a further 5 min, then evaporated. The residue was dissolved in CH_2Cl_2 (300 ml), washed with water (100 ml) and the organic layer dried over Na_2SO_4 and the filtrate evaporated. The crude product was purified on two preparative silica TLC plates eluted with CH_2Cl_2 / MeOH 9:1 (v/v) containing 1 % triethylamine. The purified product

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was then dissolved in CH_2Cl_2 and precipitated in petroleum ether (40°C-60°C). The precipitate was collected by centrifugation. Yield 311 mg (42 %); λ_{max} (H_2O) 232 nm, 295 nm; λ_{min} (H_2O) 249 nm; ^{19}F NMR: d($CDCl_3$) - 75.91 (s); ^1H NMR: δ(d_6-DMSO) 11.30 (broad s, 1H, NH), 8.48 (s, 1 H, N=C-H); 7.90 (s, 1 H, H8), 7.38-6.81 (m, 13 H, DMT), 6.08 (d, 1 H, H1', J= 6.7Hz), 5.09 (broad pseudotriplet, 1 H, H2'), 4.35 (pseudotriplet, 1 H, H3'), 4.14 (broad m, 1 H, H4'), 3.73 (s, 6 H, 2 DMT-MeO), 3.25-3.16 (m, 2 H, H5', H5''), 3.07 (s, 3 H, amidine CH_3), 3.02 (s, 3 H, amidine CH_3).

5'-O-(4,4'-Dimethoxytrityl)-N^2-[(dimethylamino) methylene]-2'-(trifluoroacetamido)-2'-deoxyguanosine-3'-O-(β-cyanoethyl N,N-diisopropylphosphoramidite) **(15)**

Compound **14** (155 mg, 0.21 mmol) was dissolved in dry CH_2Cl_2 (5 ml) and treated with *N,N*-diisopropylethylamine (147 µl, 0.84 mmol) and β-cyanoethyl(*N,N*-diisopropylamino)chlorophosphoramidite (94 µl, 0.42 mmol) and stirred at room temp. under argon for 1 h. MeOH (100 µl) was then added, and after 1 min the solution was taken up in ethyl acetate (100 ml) then washed with 5 % aqueous sodium carbonate solution (20 ml), followed by saturated brine (20 ml). The organic phase was dried over Na_2SO_4 and the filtrate then evaporated. The crude product was purified by flash chromatography (Kieselgel 60, 12 g) employing CH_2Cl_2/ethyl acetate/triethylamine 45:45:10 (v/v/v). The residue was redissolved in ethyl acetate (5 ml) and precipitated by adding the solution dropwise into stirred pentane (200 ml). The white precipitate was collected by centrifugation and dried. Yield, 91 mg (46 %); ^{31}P NMR: δ($CDCl_3$) 152.4 (s); 149.10 (s); 14.75 (s, 8 % phosphonate impurity); ^{19}F NMR: δ($CDCl_3$) -76.09 (s),- 75.96 (s).

RESULTS AND DISCUSSION

2'-Fluoro-2'-deoxyguanosine has previously been synthesised by Ikehara and Imura (4, 5). However, this

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procedure requires the initial preparation of 8,2'-O-cyclo-guanosine (28) in 4 steps from 8-bromoguanosine, followed by a six-step conversion to the desired 2'-fluoro analogue employing tetra-n-butyl ammonium fluoride as the fluorinating agent. The desired compound is obtained in a yield of approximately 1% from guanosine. We have developed an alternative synthetic route to 2'-fluoro-2'-deoxyguanosine as shown in Figure 1, which
starts with guanosine and employs the fluorinating agent (diethylamino)sulphur trifluoride (DAST) (29, 30, for a review see 31). DAST has previously facilitated access to a wide variety of sugar-fluorinated nucleosides (32) and has also previously been used by us for the preparation of several other 2'-fluoro-2'-deoxynucleosides (20, 21).

We intitially protected both the 3'- and 5'-hydroxyl groups of guanosine using the Markiewicz disiloxane reagent, as described (23) to afford **1**. The *arabino* configuration at C2' required for the fluorination reaction with DAST was obtained via the procedure of Samano and Robins employing the Dess-Martin 12-I-5-periodinane reagent followed by reduction of the intermediate 2'-ketonucleoside with sodium triacetoxyborohydride (24). In agreement with these authors, this procedure appeared to be preferable to using CrO_3/pyridine/acetic anhydride (33) for the oxidation of **1** to the 2'-ketonucleoside, which after subsequent reduction to give the *arabino* nucleoside **2** often required extensive column chromatography to remove coloured, tar-like chromium byproducts. Compound **2** was then pixylated on the N^2-amino group resulting in **3** which was obtained in 64 % yield after silica-gel chromatography. We have found that addition of the lipophilic pixyl group at this stage facilitated the removal of small amounts of impurities which could not be removed from the non-pixylated material **2**.

The 2'-hydroxyl group of **3** was then acetylated to give **4** which was isolated in 82 % yield after silica-gel column

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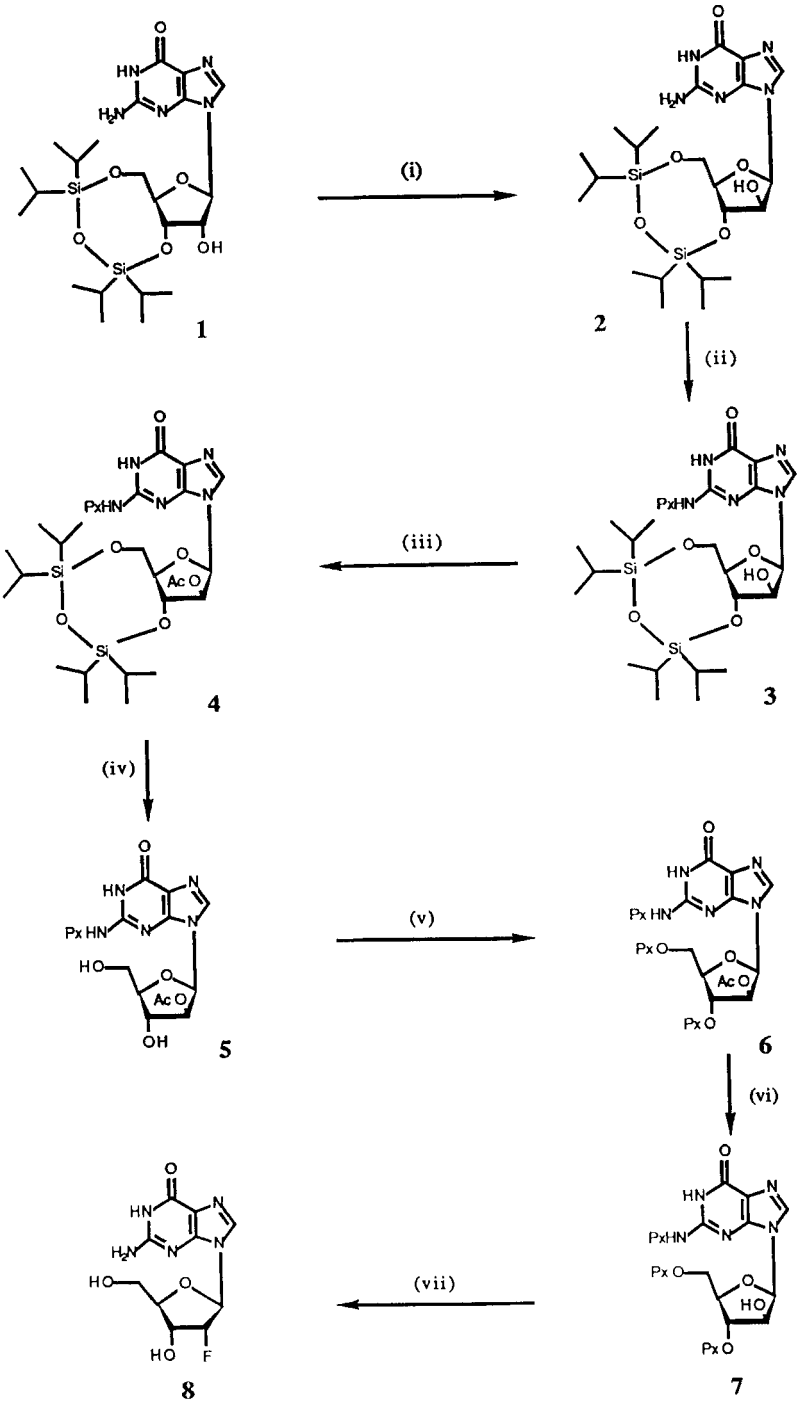

FIG. 1. Reaction scheme for the preparation of 2'-fluoro-2'-deoxyguanosine. Reagents: i, 1,1,1,-tris(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one in dichloromethane, followed by sodium triacetoxyborohydride; ii, pixyl chloride in pyridine; iii, acetic anhydride in pyridine; iv, TBAF in THF; v, pixyl chloride in pyridine; vi, 4 M NaOH in methanol/ethyl acetate; vii, DAST, DMF in acetonitrile, followed by 0.1 N HCl in methanol.

chromatography. The silyl protection was then removed by treatment with TBAF in THF, the resulting product **5** being purified by silica-gel column chromatography. During the desilylation we sometimes observed some concommitant loss of the 2'-O-acetyl group.

We had previously found that the fluorination reaction with DAST was most efficient when pixyl protection of the nucleoside was used (16, 21). Thus **5** was subsequently converted to the tripixyl derivative **6** which was obtained in 62 % yield after silica-gel chromatography. The pixyl groups were introduced at this stage of the synthesis since acetic acid liberated during the oxidation of the 2'-hydroxyl group with the iodinane reagent (34) leads to partial deprotection. Deacylation of **6** in ethyl acetate-methanol with aqueous sodium hydroxide produced the desired tripixylated *arabino*-guanosine derivative **7**. Fluorination with DAST as previously described (16, 21), followed by removal of the pixyl groups afforded the 2'-fluoro-2'-deoxyguanosine in 28 % yield, which was obtained after purification by preparative reverse-phase HPLC. The ^{1}H NMR of this material was identical to that of 2'-fluoro-2'-deoxyguanosine described in the literature (4, 5). Thus, H2' was shifted downfield to 5.23 ppm and proton-fluorine coupling constants of 16.5, 52.1 and 18.5 Hz for H1', H2' and H3' were determined, respectively. The ^{19}F NMR spectrum was also consistent with the proposed structure. The overall yield from guanosine is 2.5%.

The 2'-fluoro-2'-deoxyguanosine was then converted to the fully-protected 3'-phosphoramidite as shown in Figure 2. Hence, it was first protected in 72 % yield as its N^2-isobutyryl derivative using the transient protection procedure (25), followed by 5'-O-dimethoxytritylation, **10** being obtained in 89 % yield after silica-gel column chromatography. The 3'-phosphoramidite derivative **11** was obtained using the procedure of Sinha et al. (27) in 65 % yield. Each phosphoramidite diastereomer displayed phosphorus-fluorine coupling constants of about 9 Hz in both the ^{31}P and ^{19}F nmr spectra.

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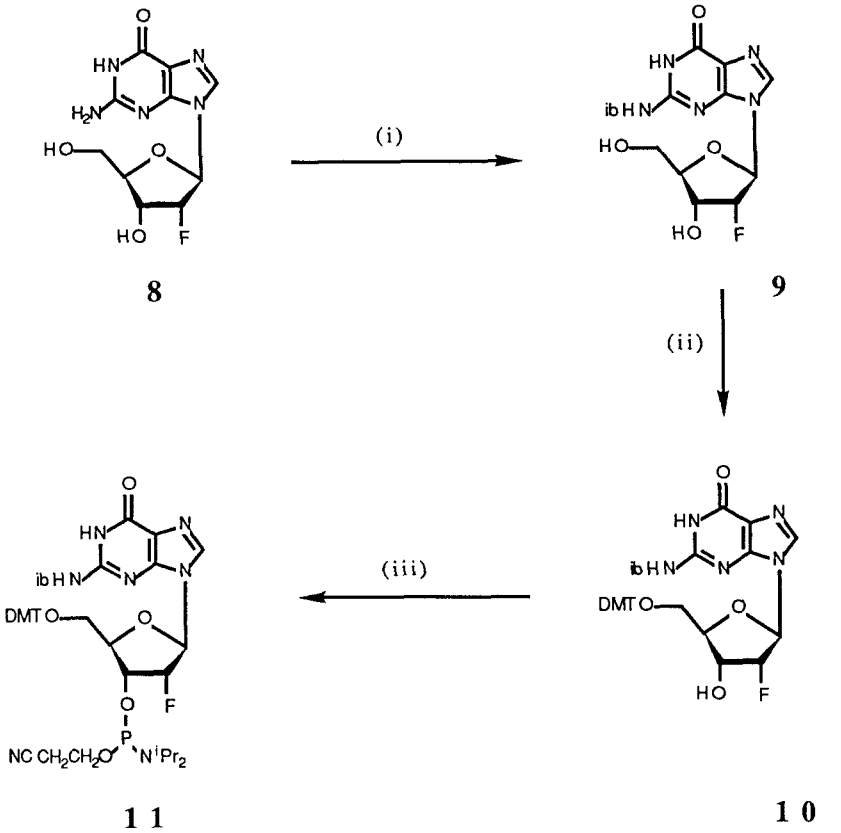

FIG. 2. Reaction scheme for the preparation of protected 2'-fluoro-2'-deoxyguanosine phosphoramidite. Reagents: i, chloro trimethylsilane in pyridine, followed by isobutyryl chloride, followed by dilute aq. ammonia solution; ii, dimethoxytrityl chloride, triethylamine in pyridine; iii, β-cyanoethyl(*N,N*-diisopropylamino)chlorophosphoramidite and *N,N*-diisopropylethylamine in dichloromethane.

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The preparation of 2'-amino-2'-deoxyguanosine was performed via the previously-described transglycosylation reaction between N^2-palmitoylguanine and 2'-(trifluoroacetamido)-2'-deoxyuridine (9). The crude product was purified by preparative reverse-phase HPLC and was identical to that previously described. The trifluoroacetyl group was chosen for protection of the sugar amino group of the 2'-amino-2'-deoxyguanosine phosphoramidite since we had previously successfully used such protection for the chemical synthesis of oligoribonucleotides containing 2'-amino-2'-deoxycytidine and 2'-amino-2'-deoxyuridine (20). Furthermore, the benzoyl group proved to be too difficult to remove during the standard deblocking of the 2'-silyl-protected oligoribonucleotides with ethanol/ammonia solution. Thus, treatment of 2'-amino-2'-deoxyguanosine with S-ethyl trifluorothioacetate in methanol afforded the 2'-trifluoroacetamido derivative **13** in 88 % yield (Fig. 3). Initially it seemed convenient to protect both the sugar and base amino groups simultaneously with this reagent. However, after treating 2'-amino-2'-deoxyguanosine with 2.5 equivalents of S-ethyl trifluorothioacetate for 5 days, the ^{19}F NMR spectrum indicated only a small amount of base acylation (δ -77.0), in addition to the expected signal (δ -73.4) for the N2'-acylated compound. However, the amount of N^2-acylation was too small to be of synthetic value presumably due to the lability of the resulting amide. Additionally it was unclear whether an electron-withdrawing protecting group at this position might undesirably increase the reactivity of the guanine O6 carbonyl group to reagents used in the oligonucleotide synthesis. Thus we chose to use the dimethylformamidine protecting group (35) which may be introduced in high yield under mild conditions. After the trifluoroacetylation reaction, the crude product **13** was converted to the amidine derivative by treatment with dimethylformamide dimethylacetal in methanol. A UV spectrum of the resulting compound displayed maxima at 232 and 295 nm and a minimum at 249 nm as expected for an N^2-amidine-protected guanosine derivative (36). Subsequent 5'-O-dimethoxytritylation,

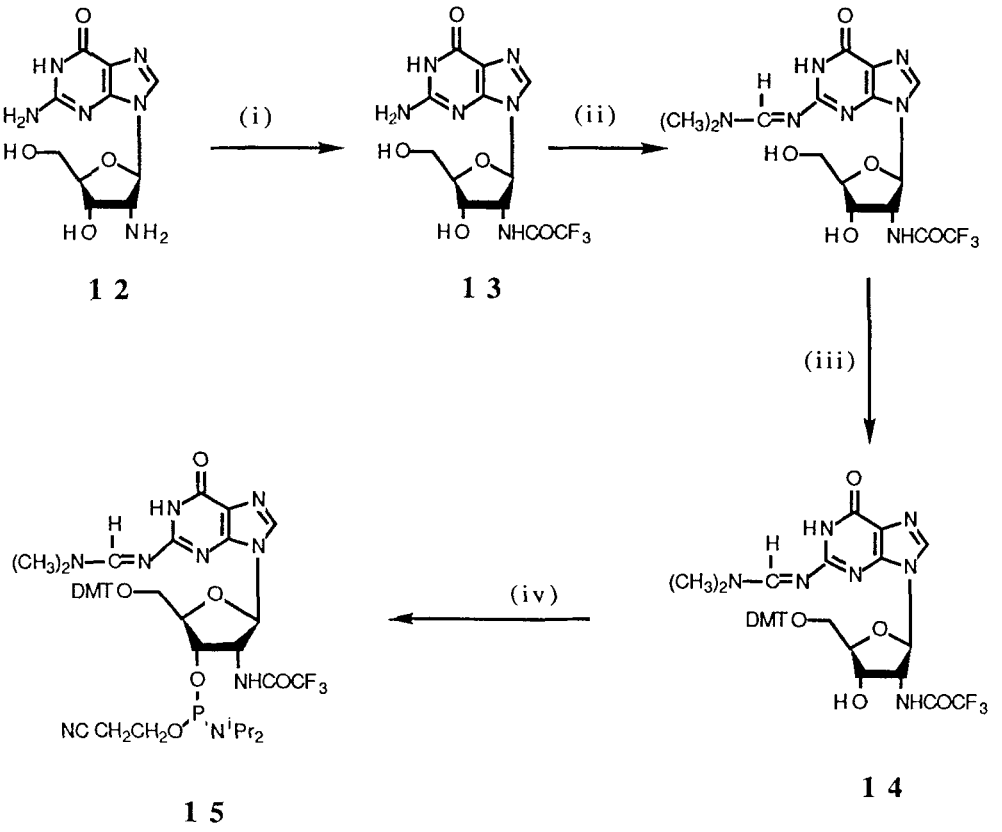

FIG. 3. Reaction scheme for the preparation of protected 2'-amino-2'-deoxyguanosine phosphoramidite. Reagents: i, *S*-ethyl-trifluorothioacetate in methanol; ii, dimethylformamide dimethylacetal in methanol; iii, dimethoxytrityl chloride, triethylamine in pyridine; iv, β-cyanoethyl(*N,N*-diisopropylamino)chlorophosphoramidite and *N,N*-diisopropylethylamine in dichloromethane.

followed by silica-gel column chromatography afforded the fully-protected nucleoside **14** in 46 % yield from 2'-amino-2'-deoxyguanosine. Phosphitylation with β-cyanoethyl(*N,N*-diisopropylamino)chlorophosphoramidite afforded the corresponding nucleoside 3'-O-phosphoramidite **15** in 46 % yield. The two diastereomers could be clearly distinguished by both ^{19}F and ^{31}P NMR.

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In summary, we have developed a novel synthetic route to 2'-fluoro-2'-deoxyguanosine and have prepared suitably protected phosphoramidite monomers of both 2'-fluoro- and 2'-amino-modified 2'-deoxyguanosine. The incorporation of these analogues into chemically-synthesised hammerhead ribozymes and their kinetic characterisation will be reported shortly (37).

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