Solid-phase synthesis of DNA fragments containing the modified base 7-hydro-8-oxo-2'-deoxyguanosine

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ABSTRACT

The 5'-(4,4'-dimethoxytrityl) protected 3'-(2-cyanoethoxy)-*N,N*-diisopropylphosphoramidite of 7-hydro-8-oxo-2'-deoxy-guanosine, the exocyclic amino and lactam functions of which are protected with acetyl and diphenylcarbamoyl groups, respectively, has been prepared from the 8-bromo derivatives of deoxy- and riboguanosine. This synthon, in combination with standard d-nucleoside 3'-(2-cyanoethoxy)-*N,N*-diisopropylphosphoramidites, was applied successfully to a solid-phase synthesis. Well-defined oligodeoxyribonucleotides containing a 7-hydro-8-oxo-2'-deoxy-guanosine residue at predetermined positions were obtained after deprotection with methanolic ammonia and purification by gel filtration.

INTRODUCTION

It is well established¹ that deoxyguanosine residues in DNA are prone to hydroxylation by oxygen free radicals at the C-8 position to form 7-hydro-8-oxo-2'-deoxyguanosine (dG^{8oxo}). It has also been advocated that there exists a direct correlation between the formation of dG^{8oxo} in DNA and carcinogenesis.² The latter was suggested by the finding that the presence of dG^{8oxo} in a DNA-template causes α -polymerase to miscode incorporation of nucleotides in the replicated strand.³ Recently, however, it was demonstrated^{4–6} that dG^{8oxo} directs incorporation of dC as well as dA opposite the lesion on primed templates in reactions catalysed by DNA polymerases. In addition, biophysical studies showed that the dG^{8oxo} molecule occurs predominantly as the 6,8-diketo tautomer^{7–11} and adopts the *syn* conformation as a nucleoside or when located opposite dA in duplex DNA.¹²

In order to investigate in detail the miscoding properties of dG^{8oxo} and the possible existence of a repair mechanism for removing this lesion, we here report an efficient synthesis of the dG^{8oxo} phosphoramidite 3, which proved to be a suitable building unit for the solid-phase synthesis of well-defined oligodeoxyribonucleotides containing dG^{8oxo} residues at predetermined positions.

EXPERIMENTAL

General materials and methods

Pyridine, dioxane and triethylamine were dried by refluxing with calcium hydride (CaH₂, 5 g/L) for 16 h and then distilled. Toluene and dichloromethane (CH₂Cl₂) were dried by heating under reflux with P₂O₅ (30 g/L) during 2 h and distilled. Pyridine, dioxane and dichloromethane (CH₂Cl₂) were stored over molecular sieves 4Å, 5Å and 4Å, respectively. Toluene was stored over sodium wire. Methanol (MeOH, Baker) was dried over methoxide, *in situ* prepared by iodine (catalytic) activated magnesium curls (5 g/L) in methanol (200 mL, p.a. Baker), refluxed for 1 h and distilled. Acetonitrile (MeCN, Rathburn HPLC-grade) for chemical reactions was stored over molecular sieves 3Å. For use on the Gene Assembler MeCN (Rathburn, HPLC-grade) was used and stored on molecular sieve rods (Fluka, Carbide Type 3Å, 1/8" rods). 1,2-Dichloroethane (DCE, Rathburn, HPLC-grade) was stored over molecular sieves 3Å before use. *N*-Methylimidazole (NMI, Aldrich) was dried by heating under reflux with calcium hydride (5 g/L) and distilled under reduced pressure. *N,N*-Diisopropylethylamine (DIPEA) was distilled from chlorosulfonic acid (5 g/L) and then redistilled from KOH-pellets (20 g/L). 1,3-Dichloro-1,1,3,3-tetraisopropyl-disiloxane (TIPSCl₂) and tetra-*n*-butylammonium fluoride [(*n*-Bu)₄NF] solution (1M) in dioxane were prepared as described before²⁵. 4,4'-Dimethoxytrityl chloride (DMT-Cl, Janssen), acetic acid anhydride (Ac₂O, Baker), acetic acid (AcOH, Baker), sodium acetate (NaOAc, Aldrich), diphenylcarbamoyl chloride (DPC-Cl, Aldrich), potassium *tert*-butoxide (KOtBu, Aldrich), tri-*n*-butyltin hydride [(*n*-Bu)₃SnH, Aldrich], azo bis(isobutyronitrile) (AIBN, Janssen), phenyl chlorothionocarbonate (Aldrich), dicyclohexylcarbodiimide (DCC, Merck), trichloroacetic acid (TCA, Baker), 4-(dimethylamino)pyridine (DMAP, Merck), iodine (I₂, Merck), *sym*-collidine (Fluka) and 1*H*-tetrazole (Janssen) were of pure analysis quality and used without further purification. 1-Hydroxybenzotriazole (HOBt, Aldrich) was dried *in vacuo* (P₂O₅) for 72 h at 50°C before use. Methanolic ammonia was prepared by passing ammonia gas over KOH through a cooled (ice-salt bath) solution of dry methanol (500 ml) until saturation and diluted with dry methanol (500 ml). Triethylammonium bicarbonate buffer

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(TEAB, 2 M) was prepared by passing a stream of carbon dioxide (CO₂) gas through a cooled (ice-water bath) mixture of triethylamine (825 mL) and deionized water (2175 mL) until pH 7.0–7.5.

N-Acyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxynucleoside-3'-*O*-(2-cyanoethyl)-*N,N*-diisopropylphosphoramidites were obtained from Synorchem and used as 0.1 M solutions in special dry MeCN. 2-Cyanoethyl-*N,N*-diisopropylphosphoramidic chloride (**7**) was prepared according a published procedure.¹⁴

Aminopropyl-derivatized controlled pore glass (CPG/AP) was obtained as described before²⁶ by using CPG-Silica carrier (30–45 mesh, pore size 375 Å), purchased from Fluka.

Schleicher and Schüll DC Fertigfolien F1500 LS254 were used for TLC analysis in the solvent system A: CH₂Cl₂/MeOH (92/8, v/v); B: CH₂Cl₂/MeOH (95/5, v/v); C: CH₂Cl₂/acetone (95/5, v/v); D: CH₂Cl₂/MeOH (90/10, v/v) or E: EtOAc/petroleum ether (40–60°C)/Et₃N (50/45/5, v/v), unless otherwise stated. Short column chromatography was performed on Kieselgel 60 (230–400 mesh ASTM) suspended in CH₂Cl₂. Sephadex LH-20 and G-50 were purchased from Pharmacia (Uppsala, Sweden).

³¹P NMR spectra were measured at 80.7 MHz using a JEOL JNM-FX 200 spectrometer equipped with a PG 200 computer and operating in the Fourier-transform mode. Chemical shifts are given in ppm (δ) relative to 85% H₃PO₄ as external standard. ¹H NMR spectra were measured at 200 MHz as described above for ³¹P NMR spectra or at 300 MHz using a Bruker WM-300 spectrometer, equipped with an ASPECT-2000 computer and operating in the Fourier-transform mode. ¹³C NMR spectra were measured at 50.1 MHz as described above for ³¹P NMR spectra. Chemical shifts for ¹H and ¹³C NMR are given in ppm (δ) relative to tetramethylsilane (TMS).

NMR-samples of the unprotected oligonucleotides were made by lyophilizing the purified oligonucleotide (5–10 mg, sodium salt), after adjusting the pH to 6.9–7.0 (meter reading), two times from D₂O (99.75%) and dissolved in D₂O (99.95%). Chemical shifts are given in ppm (δ) relative to sodium 3-(trimethylsilyl)-propanesulfonate.

Fast Protein Liquid Chromatography (FPLC)-analysis was carried out on a Pharmacia LCC-500 liquid chromatograph equipped with a gradient mixing system, UV absorption detector (254 nm) and a photometer output recorder. Pre-packed strong anion-exchange resin Mono Q HR 5/5 (Pharmacia) was used. Gradient elution was performed at 20°C by building up a gradient starting with buffer A (0.01 M NaOH, pH 12.0) and applying buffer B (0.01 M NaOH, 1.2 M NaCl, pH 12.0) with a flow rate of 2.0 mL/min and a pressure of 3.0 MP. The used gradient for the oligomers was as follows: 2 min at 0%, 20 min from 0 to 20%, 15 min from 20 to 50%, 1 min from 50 to 100%.

Cation-exchange resin (sodium-form) was prepared by passing a 2 M solution of NaOH in water (100 mL) through a column (1.5 cm² × 10 cm) packed with resin (Dowex 50WX4, 100–200 mesh, Fluka, H⁺-form) followed by eluting of the column with water until pH 7.0. Cation-exchange resin (pyridinium-form) was prepared by treatment of resin (Dowex 50WX4, 100–200 mesh, Fluka, H⁺-form) with pyridine/water (250 mL, 1:4, v/v) followed by washing the resin with water until pH 7.0.

High Performed Liquid Chromatography (HPLC)-analysis was carried out on a Waters 990 Photodiode Array Detector equipped with a NEC APC IV Powermate 2 Computer and a Waters 990 Plotter or NEC Pinwriter PC6.

2'-Deoxy-7-hydro-2-*N*,3'-*O*,5'-*O*-triacetylguanosin-8-one (**5**)

Dry 8-bromo-2'-deoxyguanosine (**4**)¹⁷ (1.73 g, 5.0 mmol) was dissolved in pyridine (75 mL) and Ac₂O (30 mL). To this solution was added dry NaOAc (8.20 g, 100 mmol) and the mixture was refluxed for 3 h. Subsequently HOAc (75 mL) containing anhydrous NaOAc (3.28 g, 40.0 mmol) was added and the mixture again refluxed for 1 h. EtOH (50 mL) was added to quench the reaction and the mixture was concentrated under reduced pressure. The last traces of HOAc were removed by repeated evaporation with EtOH (3 × 25 mL). The residue was dissolved in EtOAc (100 mL) and washed with TEAB-buffer (1M, 30 mL) and an aqueous saturated NaCl solution (30 mL). The organic layer was dried with MgSO₄, filtered and concentrated. The residue was dissolved in CH₂Cl₂ (5 mL) and purified on a short-column by elution with a 0 to 6% gradient of MeOH in CH₂Cl₂. Yield 247 mg (12%); R_f 0.38 (A); ¹H NMR (CDCl₃): δ 9.93 (s, 1H, NH), 9.51 (s, 1H, NH), 6.38 (dd, J_{1',2'} = 7.5 Hz, J_{1',2''} = 7.2 Hz, 1H, H-1'), 5.42 (m, 1H, H-3'), 4.83 (m, 1H, H-5'), 4.44–4.36 (m, 2H, H-4' and H-5''), 2.97 (m, 1H, H-2''), 2.38 (m, 1H, H-2'), 2.32 (s, 3H, CH₃, NHAc), 2.13 and 2.12 (2 × s, 6H, 2 × CH₃, 2 × OAc); ¹³C NMR (CDCl₃): δ 172.8, 171.8, 170.4 (3 × C=O, 3 × Ac), 152.0 (C-6), 150.0 (C-2), 146.6 (C-4), 144.8 (C-8), 104.5 (C-5), 82.1 (C-1'), 81.5 (C-4'), 74.7 (C-3'), 63.9 (C-5'), 33.9 (C-2'), 23.9 (CH₃, NHAc), 20.8 (3 × CH₃, OAc).

Side product: 1'-*O*,3'-*O*,5'-*O*-triacetyl-2'-deoxyribofuranoside (α,β-mixture). Yield 0.55 g (42%); R_f 0.74 (A); ¹H NMR (CDCl₃): δ 6.40–6.36 (m, 2 × 1H, H-1'/H-1'α), 5.32–5.11 (m, 2 × 1H, 2 × H-3'(α en β), 4.42–4.06 (m, 6H, 2 × H-4', 2 × H-5' and 2 × H-5'' [α en β]), 2.55–1.96 (m, 4H, 2 × H-2' and 2 × H-2'' [α en β]) coinciding with 2.11, 2.10, 2.08, 2.06 (4 × s, 9H, 3 × CH₃, Ac); ¹³C NMR (CDCl₃): δ 169.9, 169.8, 169.7, 169.4, 169.1 (C=O, Ac), 97.7, 97.5 (C-1' [α and β]), 82.6, 82.1 (C-4' [α and β]), 73.4, 73.2 (C-3' [α and β]), 63.5, 63.0 (C-5' [α and β]), 37.6, 37.5 (C-2' [α and β]), 20.5, 20.2, 20.1, 20.0 (CH₃, OAc).

2-*N*-Acetyl-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-7-hydroguanosin-8-one (**6b**)

Compound **5** (1.02 g, 2.5 mmol) was dissolved in anhydrous MeOH (20 mL) and a fresh prepared solution of KOtBu (1M) in MeOH (3 mL) was added. The reaction mixture was neutralized after 5 min with Dowex in pyridinium-form. The resin was removed by filtration and the filtrate was concentrated to dryness. The solid was dissolved in H₂O (25 mL) and washed with CH₂Cl₂ (50 mL). The aqueous layer was concentrated affording **6a** as a white solid. Crude **6a**, dried by repeated evaporation with pyridine, was dissolved in pyridine (50 mL) and DMT-Cl (1.02 g, 3.0 mmol) was added. The mixture was stirred for 1 h at ambient temperature. MeOH (10 mL) was added and the mixture was concentrated to dryness. The residue was dissolved in CH₂Cl₂ (100 mL) and washed with a 5% aqueous NaHCO₃ solution (30 mL) and water (30 mL). The organic layer was dried with MgSO₄, filtered and concentrated to a small volume and triturated with petroleum-ether (40–60°C, 100 mL). The precipitate was dissolved in CH₂Cl₂ and purified by column chromatography. Elution with a 0 to 6% gradient of MeOH in CH₂Cl₂ afforded **6b** as a white foam. Yield 1.11 g (71%); R_f 0.30 (A); ¹H NMR (CDCl₃/CD₃OD): δ 7.45–7.14 (m, 9H, arom. H's, DMT), 6.80–6.74 (m, 4H, arom. H's,

DMT), 6.64 (dd, $J_{1',2'} = 5.8$ Hz, $J_{1',2''} = 7.3$ Hz, 1H, H-1'), 4.65 (m, 1H, H-3'), 3.99 (m, 1H, H-4'), 3.85 (s, 6H, OCH₃, DMT), 3.38–3.30 (m, 2H, H-5' and H-5''), 3.14 (m, 1H, H-2''), 2.19 (m, 1H, H-2'), 2.03 (s, 3H, CH₃, NHAc); ¹³C NMR (CDCl₃): δ 173.4 (C=O, Ac), 158.1, 144.9, 135.9 (C-arom., DMT), 151.6, 146.9, 145.1 (C6, C8, C2 and C4), 129.9, 129.8, 127.9, 126.6, 113.0 (CH-arom., DMT), 103.7 (C-5), 85.8 (C-1'), 85.4 (C-quar., DMT), 81.1 (C-4'), 71.2 (C-3'), 65.0 (C-5'), 55.1 (OCH₃, DMT), 36.2 (C-2'), 23.8 (CH₃, NHAc).

2'-Deoxy-6-O,7-N-bis(diphenylcarbamoyl)-7-hydro-2-N,3'-O,5'-O-triacetylguanosin-8-one (9)

Compound **5** (4.09 g, 10.0 mmol) was dried by evaporation with and redissolved in pyridine (50 mL). To the stirred mixture was added DPC-Cl (6.85 g, 30.0 mmol) and Et₃N (4.2 mL, 30.0 mmol). After 1 h, the mixture was concentrated to a small volume, dissolved in CH₂Cl₂ (100 mL) and washed with an aqueous solution of NaHCO₃ (1 M, 50 mL) and water (50 mL). The organic layer was dried with MgSO₄, filtered, concentrated to a small volume and triturated with petroleum-ether (40–60°C, 150 mL). The crude residue was dissolved in CH₂Cl₂ (5 mL) and purified by column chromatography. Elution of the column was performed with a 0 to 2% gradient of MeOH in CH₂Cl₂. Yield 7.21 g (84%); R_f 0.74 (B); ¹H NMR (CDCl₃): δ 8.06 (s, 1H, NH), 7.43–7.08 (m, 20H, arom. H's, phenyl, 2×DPC), 6.12 (dd, $J_{1',2'} = 6.9$ Hz, $J_{1',2''} = 7.2$ Hz, 1H, H-1'), 5.38 (m, 1H, H-3'), 4.41–4.25 (m, 1H, H-4'), 4.23–4.06 (m, 2H, H-5' and H-5''), 3.10 (m, 1H, H-2''), 2.39 (s, 3H, CH₃, NHAc), 2.11 (m, 1H, H-2'), 2.07 and 2.05 (2×s, 6H, 2×CH₃, 2×OAc); ¹³C NMR (CDCl₃): δ 170.7, 170.1, 169.6 (3×C=O, 3×Ac), 152.5, 151.5, 150.0, 148.6, 147.3, 146.6, 142.2, 141.3 (C-6, C-8, C-2, C-4, 2×C=O, DPC and 2×C-arom., DPC), 128.8, 128.0, 126.8, 125.6 (CH-arom., phenyl, 2×DPC), 106.8 (C-5), 82.1 (C-1'), 81.9 (C-4'), 74.5 (C-3'), 63.7 (C-5'), 33.0 (C-2'), 24.8 (CH₃, NHAc), 20.8, 20.7 (2×CH₃, 2×OAc).

2-N-Acetyl-6-O,7-N-bis(diphenylcarbamoyl)-2'-deoxy-7-hydro-guanosin-8-one (10)

Method A: To a solution of **9** (0.96 g, 1.20 mmol), dried by evaporation with toluene, in anhydrous MeOH (280 mL) was added KOtBu (310 mg, 2.76 mmol). The reaction was stopped after stirring for 5 min by addition of Dowex-pyridinium cation exchange resin until pH 7.0. The mixture was filtered and the filtrate was concentrated to dryness. The residue was dissolved in CH₂Cl₂ (5 mL) and purified by short-column chromatography. Elution of the column was performed with a 0 to 6% gradient of MeOH. Yield 0.74 g (86%); R_f 0.32 (A); ¹H NMR (CDCl₃): δ 10.2 (s, 1H, NH, D₂O exchangeable), 7.38–7.12 (m, 20H, arom. H's, 2×DPC), 5.93 (dd, $J_{1',2'} = 6.4$ Hz, $J_{1',2''} = 7.9$ Hz, 1H, H-1'), 5.13 (d, $J = 5.1$ Hz, 1H, 3'-OH, D₂O exchangeable), 4.52 (t, $J = 4.8$ Hz, 1H, 5'-OH, D₂O exchangeable), 4.33 (m, 1H, H-3'), 3.62 (m, 1H, H-4'), 3.48–3.29 (m, 2H, H-5' and H-5''), 2.83 (m, 1H, H-2''), 2.11 (s, 3H, CH₃, NHAc), 1.96 (m, 1H, H-2'); ¹³C NMR (CDCl₃): δ 170.8 (C=O, NHAc), 152.7, 151.4, 150.1, 148.6, 147.6, 146.5, 142.0, 141.4 (C-6, C-8, C-2, C-4, 2×C=O, DPC and 2×C-arom., DPC), 128.9, 128.6, 126.6, 126.3 (CH-arom., phenyl, 2×DPC), 107.1 (C-5), 86.6 (C-1'), 85.3 (C-4'), 71.4 (C-3'), 62.3 (C-5'), 36.5 (C-2'), 24.7 (CH₃, NHAc). Anal. Calc. for C₃₈H₃₃N₇O₈: C 63.77; H 4.65; N 13.70. Found: C 63.38; H 4.52; N 13.49. Method B: Compound **19** (2.39 g, 2.50

mmol) was evaporated with and dissolved in dioxane (6.5 mL) and treated with a stock solution of TBAF in dry dioxane (1 M, 6.5 mL). TLC analysis (A), after 5 min, showed complete removal of the silyl group. The reaction was quenched by addition of an aqueous NaH₂PO₄ solution (1M, pH = 6.0, 25 mL). The solution was diluted with CH₂Cl₂ (100 mL) and washed with aqueous NaHCO₃ solution (1 M, 50 mL) and water (50 mL). The organic layer was dried (MgSO₄), filtered, concentrated under reduced pressure to a small volume and triturated with petroleum-ether 40–60°C (100 mL). The petroleum-ether was decanted and the residue was concentrated to give a foam. Crude **10** was dissolved in CH₂Cl₂ (5 mL) and purified by column chromatography. Elution of the column was effected with a 0 to 6% gradient of MeOH in CH₂Cl₂. Yield 1.63 g (91%); the physical data were identical to those described above.

2-N-Acetyl-6-O,7-N-bis(diphenylcarbamoyl)-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-7-hydro-guanosin-8-one (11)

To a stirred solution of **10** (1.43 g, 2.00 mmol), which has been dried by evaporation with pyridine, in dry pyridine (20 mL) was added DMT-Cl (0.81 g, 2.40 mmol) and stirring was continued for 3 h. MeOH (1 mL) was added and the mixture was concentrated to dryness. The residue was dissolved in CH₂Cl₂ (75 mL) and washed with a 5% aqueous NaHCO₃ solution (30 mL) and water (30 mL). The organic layer was dried with MgSO₄, filtered and concentrated. Crude **11** was purified by short-column chromatography. Elution of the column was performed with a 0 to 3% gradient of MeOH in CH₂Cl₂. Yield 1.81 g (89%); R_f 0.60 (B); ¹H NMR (CDCl₃): δ 8.01 (s, 1H, NH), 7.69–7.11 (m, 29H, arom. H's, DMT and 2×DPC), 6.80–6.73 (m, 4H, arom. H's, DMT), 6.64 (dd, $J_{1',2'} = 5.2$ Hz, $J_{1',2''} = 7.9$ Hz), 4.67 (m, 1H, H-3'), 3.83 (m, 1H, H-4'), 3.73 (s, 6H, OCH₃, DMT), 3.35 (m, 1H, H-5'), 3.17 (m, 1H, H-5''), 2.78 (m, 1H, H-2'), 2.26 (s, 3H, CH₃, NHAc), 2.08 (m, 1H, H-2''); ¹³C NMR (CDCl₃): δ 169.8 (C=O, NHAc), 158.1, 144.7, 135.8 (C-arom., DMT), 152.5, 151.2, 150.6, 148.7, 147.3, 146.1, 142.1, 141.3 (C-6, C-8, C-2, C-4, 2×C=O, DPC and 2×C-arom., DPC), 129.8, 128.8, 127.9, 127.4, 126.6, 126.5, 126.2, 125.5, 123.6, 112.7 (CH-arom., phenyl, DMT and 2×DPC), 106.6 (C-5), 85.8 (C-quar., DMT), 85.4 (C-1'), 81.5 (C-4'), 71.7 (C-3'), 64.1 (C-5'), 54.8 (OCH₃, DMT), 43.4 (C-2'), 24.5 (CH₃, NHAc).

Removal of protecting groups under basic conditions

Deprotection of 11 with ammonia/water. Ammonia/water (5 mL) was added to a solution of nucleoside **11** (102 mg, 0.1 mmol) in dioxane (1 mL). The mixture was kept in a sealed flask at 50°C. Monitoring of the reaction by TLC analysis (D) showed the conversion of starting material. The mixture was concentrated, the residue dissolved in CH₂Cl₂ (5 mL) and purified by column chromatography. Elution with a 0 to 6% gradient of CH₃OH in CH₂Cl₂ followed by collection and evaporation of the appropriate fractions afforded the mono-DPC derivative **12**. Yield: 64 mg (84%); R_f (D) 0.48; ¹H NMR (CDCl₃/CD₃OD): δ 7.39–7.15 (m, 19H, arom. H's, DMT and 1×DPC), 6.81–6.77 (m, 4H, arom. H's, DMT), 5.94 (t, $J_{1',2'} = J_{1',2''} = 7.5$ Hz, 1H, H-1'), 5.81 (s, 1H, NH, D₂O exchangeable), 5.62 (bs, 2H, NH₂, D₂O exchangeable), 4.59 (m, 1H, H-3'), 3.75–3.73 (m, 7H, H-4' and 2×OCH₃, DMT), 3.38–3.14 (m, 2H, H-5' and H-5''), 2.74 (m, 1H, H-2''), 2.06 (m, 1H, H-2'); ¹³C NMR (CDCl₃): δ 158.4, 144.7, 136.0, 135.8 (C-

arom., DMT), 154.0, 152.2, 149.9, 149.8, 147.5, 142.0 (C-6, C-8, C-2, C-4, $1 \times \text{C}=\text{O}$, DPC and $1 \times \text{C}$ -arom., DPC), 129.9, 129.0, 128.1, 127.7, 127.0, 126.8, 126.7, 113.0 (CH-arom., DMT and DPC), 98.4 (C-5), 86.3 (C-quat., DMT), 84.5 (C-1'), 80.9 (C-4'), 73.1 (C-3'), 64.2 (C-5'), 55.1 (OCH₃, DMT), 35.6 (C-2').

Deprotection of 6b with ammonia/water. Similarly, ammonia/water (5 mL) was added to a solution of nucleoside **6b** (63 mg, 0.1 mmol) in dioxane (1 mL) and processed as described above to afford **13** as a white solid. Yield 52 mg (88%); *R_f* (D) 0.16; ¹H NMR (DMSO): δ 8.91 (s, 1H, 1-NH or 7-NH, D₂O exchangeable), 7.39–7.20 (m, 9H, arom. H's, DMT), 6.86–6.76 (m, 4H, arom. H's, DMT), 6.46 (s, 1H, 1-NH or 7-NH, D₂O exchangeable), 6.30 (s, 2H, NH₂, D₂O exchangeable), 6.02 (t, $J_{1',2'} = J_{1',2''} = 7.5$ Hz, 1H, H-1'), 5.10 (d, $J = 4.9$ Hz, 1H, OH, D₂O exchangeable), 4.41 (m, 1H, H-3'), 3.76–3.71 (m, 7H, H-4' and $2 \times \text{OCH}_3$, DMT), 3.36–3.12 (m, 2H, H-5' and H-5''), 3.04 (m, 1H, H-2''), 2.05 (m, 1H, H-2'); ¹³C NMR (CDCl₃/CD₃OD): δ 159.0, 145.7, 136.8 (C-arom., DMT), 153.5, 153.3, 153.1, 149.3, 145.3 (C-6), (C-2), (C-8), (C-4), 130.7, 128.8, 128.3, 127.2, 113.6 (CH-arom., DMT), 100.2 (C-5), 86.6 (C-quat., DMT), 86.4 (C-1'), 82.1 (C-4'), 72.7 (C-3'), 65.0 (C-5'), 55.5 (OCH₃), 36.7 (C-2').

Deprotection of 12 with dry ammonia/methanol. Dry ammonia/methanol (5 mL) was added to a solution of nucleoside **12** (62 mg, 0.08 mmol) in dioxane (1 mL). The mixture was heated in a sealed flask for 48 h at 50°C. TLC analysis (D) and then concentrated to dryness. The residue was redissolved in CH₂Cl₂/CH₃OH (2:1, v/v, 5 mL) and applied to a Sephadex LH-20 column, which was equilibrated and eluted with the same solvent mixture. The appropriate fractions were collected and concentrated to afford **13** as a white solid, the physical data of which were identical to those described above. Yield 38 mg (80%) based on **12**.

Deprotection of 6b and 11 with dry ammonia/methanol. Similarly, ammonia/methanol (5 mL) was added to each of the solutions of nucleoside **6b** (63 mg, 0.1 mmol) and **11** (102 mg, 0.1 mmol) in dioxane (1 mL). Further processing of the individual mixtures was performed as described above to afford in both cases **13** as a white solid. Yield 50 mg (85%) based on **6b** and 49 mg (84%) based on **11**. The analytical data (TLC-, ¹H- and ¹³C NMR analysis) were identical with those described above.

7-Hydro-2-N,2'-O,3'-O,5'-O-tetraacetylguanosin-8-one (15)

To a solution of 8-bromoguanosine (**14**)²⁰ (7.24 g, 20.0 mmol), which was dried by evaporation with pyridine, in pyridine (150 mL) and Ac₂O (50 mL) was added dry NaOAc (16.4 g, 200 mmol) and the mixture was refluxed for 2 h. Subsequently, HOAc (150 mL) containing anhydrous NaOAc (6.56 g, 80.0 mmol) was added and the mixture again refluxed for 2 h. EtOH (100 mL) was added to quench the reaction and the mixture was concentrated under reduced pressure. The last traces of HOAc were removed by repeated evaporation with EtOH (3 $\times$ 50 mL). The residue was dissolved in EtOAc (150 mL) and washed with TEAB-buffer (1M, 50 mL) and an aqueous saturated NaCl solution (50 mL). The organic layer was dried with MgSO₄, filtered, concentrated to a small volume and triturated with petroleum-ether (40–60°C, 150 mL). The precipitate was

dissolved in CH₂Cl₂ (5 mL) and purified by short-column chromatography. Elution with a 0 to 6% gradient of MeOH in CH₂Cl₂ afforded **15** as a light yellowish foam. Yield 7.81 g (84%); *R_f* 0.49 (A); ¹H NMR (CDCl₃): δ 10.0 (bs, 1H, NH), 9.47 (s, 1H, NH), 6.12 (d, $J_{1',2'} = 5.4$ Hz, 1H, H-1'), 5.96 (dd, $J_{1',2'} = 5.4$ Hz, $J_{2',3'} = 5.1$ Hz, 1H, H-2'), 5.72 (dd, $J_{2',3'} = 5.1$ Hz, $J_{3',4'} = 4.8$ Hz, 1H, H-3'), 4.67 (m, 1H, H-4'), 4.42 (m, 2H, H-5' and H-5''), 2.32 (s, 3H, CH₃, NHAc), 2.13, 2.09, 2.08 (3 $\times$ s, 9H, 3 $\times$ CH₃ OAc); ¹³C NMR (CDCl₃): δ 172.6, 171.2, 169.7, 169.5 (4 $\times$ C=O, Ac), 151.8 (C-6), 150.0 (C-2), 146.8 (C-4), 144.5 (C-8), 104.5 (C-5), 83.8 (C-1'), 78.8 (C-4'), 71.5 (C-3'), 70.6 (C-2'), 63.3 (C-5'), 23.8 (CH₃, NHAc), 20.6, 20.3, 20.2 (3 $\times$ CH₃, OAc).

6-O,7-N-Bis(diphenylcarbamoyl)-7-hydro-2-N,2'-O,3'-O,5'-O-tetraacetylguanosin-8-one (16)

Compound **15** (4.67 g, 10.0 mmol) was evaporated with and dissolved in pyridine (50 mL). To the stirred mixture was added DPC-Cl (6.85 g, 30.0 mmol) and Et₃N (4.2 mL, 30.0 mmol) and stirring was continued for 1 h at room temperature. The mixture was concentrated to a small volume, dissolved in CH₂Cl₂ (100 mL) and washed with an aqueous solution of NaHCO₃ (1 M, 50 mL) and water (50 mL). The organic layer was dried with MgSO₄, filtered, concentrated to a small volume and triturated with petroleum-ether (40–60°C, 150 mL). The crude residue was dissolved in CH₂Cl₂ (5 mL) and purified on a short-column by elution with a 0 to 2% gradient of MeOH in CH₂Cl₂. Yield 7.21 g (84%); *R_f* 0.74 (B); ¹H NMR (CDCl₃): δ 8.17 (s, 1H, NH), 7.39–7.05 (m, 20H, arom. H's, phenyl, 2 $\times$ DPC), 5.80 (m, 2H, H-1' and H-2'), 5.66 (m, 1H, H-3'), 4.40 (m, 1H, H-4'), 4.28 (m, 2H, H-5' and H-5''), 2.38 (s, 3H, CH₃, NHAc), 2.08, 2.04, 2.02 (3 $\times$ s, 9H, 3 $\times$ CH₃, 3 $\times$ OAc); ¹³C NMR (CDCl₃): δ 170.1, 169.0, 168.8, 168.7 (4 $\times$ C=O, 4 $\times$ Ac), 151.7, 151.5, 149.7, 148.2, 146.7, 146.3, 141.9, 141.1 (C-6, C-8, C-2, C-4, 2 $\times$ C=O, DPC and 2 $\times$ C-arom., DPC), 128.6, 126.4, 125.6 (CH-arom., phenyl, 2 $\times$ DPC), 106.4 (C-5), 84.2 (C-1'), 79.1 (C-4'), 71.0 (C-3'), 70.2 (C-2'), 63.1 (C-5'), 24.3 (CH₃, NHAc), 20.2, 20.0, 19.9 (3 $\times$ CH₃, 3 $\times$ OAc).

2-N-Acetyl-6-O,7-N-bis(diphenylcarbamoyl)-7-hydro-3'-O,5'-O-(tetraisopropylidisiloxane-1,3-diyl)guanosin-8-one (17)

To a solution of **16** (7.03 g, 8.20 mmol), dried by evaporation with toluene, in anhydrous MeOH (280 mL) was added a solution of KOtBu (1M) in MeOH (12.5 mL). After 5 min, the reaction was stopped by addition of Dowex-pyridinium cation exchange resin until pH 7.0. The mixture was filtered and the filtrate was concentrated to dryness. The residue was dissolved in CH₂Cl₂ (5 mL) and purified by column chromatography. Elution with a 0 to 5% gradient of MeOH in CH₂Cl₂ and concentrating of the appropriate fractions gave 2-N-acetyl-6-O,7-N-bisdiphenylcarbamoyl-7-hydro-guanosin-8-one as a white foam. Yield 5.15 g (86%); *R_f* 0.30 (B); ¹H NMR (DMSO): δ 10.72 (s, 1H, NH, D₂O exchangeable), 7.41–7.12 (m, 20H, arom. H's, phenyl, 2 $\times$ DPC), 5.49 (d, $J_{1',2'} = 5.1$ Hz, 1H, H-1'), 5.23 (d, $J = 4.1$ Hz, 1H, OH, D₂O exchangeable), 4.97 (d, $J = 4.1$ Hz, 1H, OH, D₂O exchangeable), 4.72 (m, 1H, H-2'), 4.54 (t, $J = 4.3$ Hz, 1H, 5'-OH, D₂O exchangeable), 4.14 (m, 1H, H-3'), 3.69 (m, 1H, H-4'), 3.60–3.53 (m, 2H, H-5' and H-5''), 2.11 (s, 3H, CH₃, NHAc); ¹³C NMR (CD₃OD): δ 170.9 (C=O, Ac), 152.6, 151.4, 150.1, 148.8, 147.2, 146.3, 142.0, 141.3 (C-6, C-8, C-2, C-4, 2 $\times$ C=O, DPC and 2 $\times$ C-arom., DPC), 128.9, 126.9, 126.6, 125.6 (CH-arom., phenyl, 2 $\times$ DPC), 106.9 (C-5),

87.0 (C-1'), 81.1 (C-4'), 71.7 (C-3'), 70.4 (C-2'), 64.4 (C-5'), 24.7 (CH₃, NHAc). To the triol compound (4.00 g, 5.47 mmol), which has been dried by evaporation with and redissolved in pyridine (30 mL), was added dropwise over a period of 15 min a solution of 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane²⁵ (2.22 mL, 6.94 mmol) in dry pyridine (5 mL). The reaction mixture was neutralized after 24 h with TEAB-buffer (2 M, 16 mL), concentrated under reduced pressure to a small volume, dissolved in CH₂Cl₂ (100 mL) and washed with an aqueous solution of NaHCO₃ (1 M, 50 mL) and water (50 mL). The organic layer was dried with MgSO₄, filtered and concentrated to a colourless oil. The residue was dissolved in CH₂Cl₂ (5 mL) and applied to a column of silica gel suspended in CH₂Cl₂ with a small amount of Et₃N (0.5%). Elution of the column was effected with a 0 to 2% gradient of MeOH in CH₂Cl₂. The fractions containing pure **17** were collected and concentrated to a colourless foam. Yield 4.30 g (81%), R_f 0.60 (B), 0.32 (C), ¹H NMR (CDCl₃): δ 7.87 (s, 1H, NH), 7.42–7.13 (m, 20H, arom H's, 2×DPC), 5.78 (d, J_{1',2'} = 3.1 Hz, 1H, H-1'), 4.72 (m, 1H, H-2'), 4.60 (m, 1H, H-3'), 4.34 (m, 1H, H-5'), 4.09 (m, 2H, H-4' and H-5''), 2.44 (s, 3H, CH₃, NHAc), 1.09–0.93 (m, 28H, 4×CH and 4×CH₃, TIPS); ¹³C NMR (CDCl₃): δ 170.5 (C=O, Ac), 152.5, 151.7, 150.0, 148.6, 146.7, 146.4, 142.2, 141.2 (C-6, C-8, C-2, C-4, 2×C=O, DPC and 2×C-arom., DPC), 128.7, 127.9, 126.6, 125.0 (CH arom., phenyl, 2×DPC), 106.6 (C-5), 87.5 (C-1'), 81.2 (C-4'), 73.7 (C-3'), 72.8 (C-2'), 64.2 (C-5'), 24.6 (CH₃, NHAc), 17.5, 17.3, 17.2, 17.1, 17.0, 16.9, 16.8, 13.0, 12.6, 12.5, 12.4, 12.1 (4×CH and 4×CH₃, TIPS).

2-N-Acetyl-6-O,7-N-bis(diphenylcarbamoyl)-2'-deoxy-7-hydro-3'-O5'-O-(tetraisopropyl-disiloxane-1,3-diyl)guanosin-8-one (19)

To a stirred solution of compound **17** (4.00 g, 4.11 mmol), dried by evaporation with toluene, in CH₂Cl₂ (50 mL) was added a solution of DMAP (1.00 g, 8.24 mmol) in CH₃CN (5 mL) and the mixture was kept under an atmosphere of dry N₂. Phenyl chlorothionocarbonate (712 μL, 5.15 mmol) was added. After 3 h at room temperature, TLC analysis indicated the formation of a product with a higher mobility (R_f 0.72 in system B and 0.48 in system C). The reaction mixture was concentrated to dryness and the residue was dissolved in CH₂Cl₂ (100 mL) and washed with a cold aqueous NaH₂PO₄ solution (1 M, 50 mL) and water (50 mL). The organic layer was dried (MgSO₄), filtered and concentrated to a light yellow foam. The crude product **18** was sufficiently pure for further reaction and was evaporated with and dissolved in toluene (40 mL). After being degassed with oxygen-free argon for 15 min tri-*n*-butyltinhydride (1.67 mL, 6.20 mmol) and a catalytic amount of AIBN (105 mg, 0.64 mmol) were added. Then the mixture was heated under reflux for 1 h. The solvent was evaporated and the residue was dissolved in CH₂Cl₂ (5 mL) and purified by column chromatography. Elution was performed with a 0 to 3% gradient of MeOH in CH₂Cl₂ containing a small amount of Et₃N (0.5%). Evaporation of the appropriate fractions gave compound **19** as a white foam. Yield 2.39 g (59%); R_f 0.67 (B), 0.35 (C); ¹H NMR (CDCl₃): δ 7.84 (s, 1H, NH), 7.34–7.16 (m, 20H, arom. H's, 2×DPC), 6.04 (dd, J_{1',2'} = 6.0 Hz, J_{1',2''} = 7.5 Hz, 1H, H-1'), 4.88 (m, 1H, H-3'), 3.90–3.65 (m, 3H, H-4', H-5' and H-5''), 2.45 (s, 3H, NHAc), 2.43–2.21 (m, 2H, H-2' and H-2''), 1.29–0.93 (m, 28H, 4×CH and 4×CH₃, TIPS); ¹³C NMR (CDCl₃): δ 170.4 (C=O, NHAc), 152.7, 151.6, 150.1, 148.8, 146.6, 146.3,

142.4, 141.2 (C-6, C-8, C-2, C-4, 2×C=O, DPC and 2×C-arom., DPC), 128.9, 128.0, 126.8 (CH-arom., phenyl, 2×DPC), 106.5 (C-5), 85.2 (C-1' and C-4'), 79.9 (C-3'), 63.5 (C-5'), 37.8 (C-2'), 24.8 (CH₃, NHAc), 17.4, 17.3, 17.1, 16.9, 13.1, 12.8, 12.5, 12.4 (4×CH and 4×CH₃, TIPS).

2-N-Acetyl-6-O,7-N-bis(diphenylcarbamoyl)-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-7-hydro-guanosin-8-one 3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] (3)

To a stirred solution of **11** (730 mg, 0.72 mmol), which was dried by evaporation with toluene, in dry CH₂Cl₂ (5 mL) containing DIPEA (0.51 mL, 2.92 mmol) was added 2-cyanoethyl-*N,N*-diisopropylphosphoramidic chloride (190 mg, 0.80 mmol). After 15 min TLC (E) indicated that phosphitylation was complete. The reaction mixture was diluted with CH₂Cl₂ (50 mL) and washed with 1M TEAB (25 mL) and brine (25 mL). The organic layer was dried (MgSO₄), filtered and concentrated to an oil, which was purified by flash chromatography on silica gel. Elution with EtOAc/petroleum ether (40–60°C)/Et₃N (50:45:5, v/v) gave pure amidite as a mixture of diastereoisomers. Yield: 788 mg (0.65 mmol, 90%); R_f 0.32 and 0.25 (E); ³¹P NMR (CH₂Cl₂): δ 148.80 and 148.74 ppm.

Functionalisation of solid support CPG

To a solution of 5'-*O*-dimethoxytrityl-2'-deoxynucleoside-3'-*O*-succinate²⁷ (1.0 mmol) in dioxane (10 mL) was added HOBt (202 mg, 1.5 mmol) and DCC (309 mg, 1.5 mmol). The mixture was stirred for 5 h at 20°C. After removing the precipitate by filtration, the solution was added to the amino-support (2 g, CPG-AP) and the mixture was shaken overnight at 20°C in the presence of NMI (0.4 mL, 5.0 mmol). The support was isolated by filtration and washed successively with dioxane (5 mL), MeCN (5 mL), methanol (2×5 mL) and CH₂Cl₂ (2×5 mL). Unreacted NH₂-groups were capped by a treatment with a 5% solution of NMI in Ac₂O/*sym*-collidine/dioxane (3:3:13, v/v/v) for 30 min at 20°C followed by extensive washing of the support with dioxane, methanol and finally with CH₂Cl₂. The extent of loading of the first nucleoside on the solid support was established according to a described procedure²⁸.

Synthesis of oligodeoxynucleotides on CPG

The polymer-supported synthesis of oligodeoxynucleotides was performed on a fully automated synthesizer (Pharmacia, Gene Assembler) using as well the conventional protected commercially available deoxynucleoside 2-cyanoethylphosphoramidites as the above synthesized amidite **3**. Controlled pore glass (CPG-AP, 200 mg), covalently linked to the appropriate nucleoside (loading 50 μmol/g), was used as solid phase. The individual steps of one complete elongation cycle are depicted in Table 1 and, after each step, the column was washed to remove impurities and excess of reagents.

Cleavage from resin, deprotection and purification

To the resin bound oligodeoxynucleotide was added a saturated dry ammonia/MeOH solution (20 mL) and kept in a sealed flask for 48 h at 50°C. The support was removed by filtration and the filtrate was evaporated under reduced pressure. The crude unprotected DNA-fragments were purified on Sephadex G-50 (2 cm²×150 cm) suspended and eluted in TEAB-buffer (0.05 M). The appropriate fractions, checked by FPLC analysis, were pooled, concentrated to a small volume, again checked by FPLC analysis and lyophilized. The oligonucleotides were converted

into the Na^+ -form by passing them through a column of Dowex 50W $\times$ 4 cation-exchange resin (100–200 mesh, Na^+ -form). The resulting UV-positive fractions were pooled, concentrated to a small volume, checked by FPLC analysis and again lyophilized. Octamer d-[GpCpG^{8oxo}pTpTpGpCpG] (20). Yield: 16.2 mg (62%) based on 10 μmol of resin; retention time: (FPLC conditions as described in materials and methods) R_t = 13.3 min. ^1H NMR (D_2O , pD = 7.0): δ 7.89 (s, 1H, G-H8), 7.87 (s, 1H, G-H8), 7.86 (s, 1H, G-H8), 7.56 (d, $J_{5,6}$ = 7.55 Hz, 1H, C-H6), 7.51 (d, $J_{5,6}$ = 7.51 Hz, 1H, C-H6), 7.46 (s, 1H, T-H6), 7.36 (s, 1H, T-H6), 6.15–5.92 (m, 10H, 8 $\times$ H-1' and 2 $\times$ C-H5), 5.75 (s, 1H, T-H5), 5.72 (s, 1H, T-H5), 3.06 (m, 1H, 8-oxo-7H-G-H2'), 1.74 (s, 6 H, 2 $\times$ 5- CH_3T). Hexadecamer d-[ApApTpTpCpApTpCpG^{8oxo}pApTpApTpCpTpA] (21). Yield: 22.6 mg (44%) based on 10 μmol of resin; retention time: (FPLC conditions as described in materials and methods) R_t = 11.8 min.

Analysis of the nucleoside composition of oligonucleotides 20 and 21

The composition of the oligonucleotide was verified by hydrolyzing 10 μg oligonucleotide to nucleosides using a modification of a known procedure²⁴. In addition to DNase I, alkaline phosphatase and nuclease P1 12 mU Venom phosphodiesterase were added for each reaction. Nucleosides were separated on a Supelco LE-18C reversed phase HPLC column. Elution was carried out with a buffer of 40 mM ammonium formate (pH 3.5) with a linear gradient of 0–50% MeCN (see Figure 2). The dG^{8oxo} adduct was identified both by its UV absorbance spectrum and by comigration with the synthetically prepared reference dG^{8oxo} nucleoside. The molar ratios of the nucleosides from the digestion of 20 and 21 were as follows: dC: dT: dG^{8oxo}: dG = 2.06: 2.10: 1.00: 0.95 and dC: dT: dG^{8oxo}: dA = 3.13: 6.14: 1.00: 5.91.

RESULTS AND DISCUSSION

Up to now, preliminary information dealing with the synthesis of two dG^{8oxo} derivatives (*i.e.* 1 and 2) suitable for the preparation of modified oligonucleotides has been reported in the literature.

Thus compound 1, the 3'-(2-chlorophenyl)phosphate function of which can be coupled in the presence of an activating agent with the 5'-hydroxyl of a properly protected d-nucleoside, has been applied by Oda *et al.*¹³ for the preparation in solution and by Kuchino *et al.*³ on a solid support of dG^{8oxo} containing DNA fragments. On the other hand, the 3'-phosphoramidite derivative 2 proved to be very convenient, as reported by Shibutani *et al.*⁴ and also Kouchakdjian *et al.*¹², for the solid-phase synthesis of defined dG^{8oxo} modified DNA fragments by a well-established

phosphite triester approach.¹⁴ The attractive features of compound 2, the synthesis of which has been published recently¹⁵, urged us to prepare in the first place the analogous *N*²-acetyl derivative 8 (see Scheme 1).

The synthetic route to target compound 8 commences with the preparation, according to a published procedure¹⁶, of the fully acetylated 8-oxo-7H-deoxyguanosine derivative 5. Thus, acetylation of 8-bromo-2'-deoxyguanosine (4)¹⁷ followed by the conversion of the 8-bromo function with sodium acetate in acetic acid gave 5 in a very low yield (12%) for the two steps. Selective de-*O*-acetylation of 5 with potassium *tert*-butoxide in methanol, and subsequent tritylation of diol 6a with 4,4'-dimethoxytrityl chloride, afforded 6b in 71% overall yield. Unfortunately, several attempts to prepare the required phosphoramidite 8 by phosphitylation of 6b with the commonly used agent 7¹⁴ were met with little success. The failure to prepare 8 may be due to the presence in 6b of unprotected lactam functions which are presumably very prone to phosphitylation: a well-established side reaction¹⁸ observed earlier for the deoxynucleosides T and G. In order to prevent this possible side reaction, it was decided to protect the lactam functions with the attractive diphenylcarbamoyl group according to a procedure originally devised by Kamimura *et al.*¹⁹. Accordingly, treatment of 5 with diphenylcarbamoyl chloride (DPC-Cl) in pyridine containing triethylamine gave, after silica gel chromatography, a homogeneous compound in an excellent yield. ^1H - and ^{13}C -NMR analysis of the thus obtained product revealed the presence of two diphenylcarbamoyl groups. On the basis of the available NMR data it was impossible to corroborate which of the four possible isomers [*i.e.*, the *N*¹,*N*⁷-; *N*¹,*O*⁸-; *N*⁷,*O*⁶- or the *O*⁶,*O*⁸-bis(diphenylcarbamoyl) derivative] had been formed. However, additional chemical evidence (see later) showed that one of the DPC-groups could be removed selectively under basic conditions. The latter finding indicates that the structure of the bis-DPC derivative can be tentatively assigned structure 9 or its *N*¹,*O*⁸-positional isomer. Selective de-*O*-acetylation of 9 followed by tritylation of 10 gave homogeneous 11 in 77% overall yield (based on 9). Interestingly, treatment of 11 with aqueous ammonia for 3 h at 20°C afforded a mono-DPC derivative, the ^1H - and ^{13}C -NMR data of which were in accordance with structure 12 or its *N*¹-DPC isomer. Furthermore, ammonolysis (NH_3/MeOH) of 11 or 12 under dry conditions furnished homogeneous 13, which was in every aspect identical with the *N*²-deacetylated product obtained after subjecting 6b to the same reaction conditions. Finally, phosphitylation of 11 with agent 7 gave, after short-column flash chromatography, the required phosphoramidite 3 in an excellent yield.

Despite the efficient phosphitylation of 11 with 7, it is evident that the low yielding two-step conversion of 8-bromo-2'-deoxyguanosine (4) into the key intermediate 5 has a dramatic effect on the overall yield of the crucial compound 10 (*i.e.*, the precursor of 11). The low recovery of 5 is mainly due to the acetic acid mediated depurination of the fully acetylated 8-bromo-derivative of 4. In order to nullify this unwanted side-reaction, we used the more acid stable 8-bromo-riboguanosine (14)²⁰ (see Scheme 2) as the starting compound for the preparation of key intermediate 10. Thus, acetylation of 14 followed by treatment of the fully acetylated 8-bromo derivative with acetic acid in the presence of sodium acetate gave, after purification, the fully acetylated 8-oxo-7H-guanosine derivative 15 in 84% yield. In this respect, it is of interest to note that the acetylation of 14 in the presence of pyridine had a beneficial effect

on the overall yield of **15**. In comparison, the omission of pyridine resulted, as reported earlier²¹ for the synthesis of **15**, in an overall decrease in yield of 31%. Treatment of **15** with DPC-Cl, as mentioned before (*i.e.*, conversion of **4** into **9**), afforded the homogeneous bis(diphenylcarbamoyl) derivative **16** in 84% yield. Selective de-*O*-acetylation of **16**, and subsequent regioselective protection of the 2',3',5'-triol intermediate with

1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane²², gave **17** in 68% yield for the two steps. Deoxygenation of the 2'-hydroxyl in **17** could easily be realized following the procedure of Barton *et al.*²³ Thus, reaction of **17** with phenyl chlorothionocarbonate in the presence of 4-(dimethylamino)pyridine and subsequent treatment of **18** with tri-*n*-butyltin hydride and catalytic azobis(isobutyronitrile) furnished the silyl derivative **19** in 59% for

Scheme 1

Scheme 2

the two steps. Finally, quantitative removal of the 3',5'-disiloxane group from **19** with fluoride ions afforded **10**, which was in every aspect identical with the same compound prepared in Scheme 1 starting from **4**.

The application of phosphoramidite **3** was nicely illustrated in the solid-phase synthesis of the dG^{8oxo}-containing octamer d(G-CG^{8oxo}TTGCG) (**20**) and hexadecamer d(AATTCATCG^{8oxo}A-TATCTA) (**21**) using an automated Gene Assembler (Pharmacia),

Table 1: Chemical steps involved in each elongation cycle.

Step	Manipulation	Solvents and reagents ^a	Time (min)
1	deprotection	2% TCA in DCE.	2.5
2	coupling	^b 3 and commercial available amidites ^b , 1 <i>H</i> -tetrazole ^c , MeCN.	3.0
3	oxidation	0.02 M I ₂ in MeCN/sym-collidine/H ₂ O, 11:1:5 (v/v/v).	1.0
4	capping	0.25 M DMAP in Ac ₂ O/sym-collidine/MeCN, 1:1:8 (v/v/v).	1.2

^aReactions were performed on 200 mg (10 μmol) of resin. ^b0.1M amidite (0.4 ml) in MeCN. ^c0.5 M 1*H*-tetrazole (0.6 ml) in MeCN. Abbreviations: TCA = trichloroacetic acid. DCE = 1,2-dichloroethane. MeCN = acetonitrile. DMAP = 4-dimethyl-aminopyridine. Ac₂O = acetic anhydride.

Figure 1. Expanded low-field regions of the ¹H NMR spectra (300 MHz) of (A) native octamer d-[GCGTTGCG] and (B) modified octamer d-[GCG^{8oxo}TTGCG] (**20**).

and commercially available *N*-acyl protected (*i.e.* *N*⁴-benzoyl-cytidin-1-yl; *N*⁶-benzoyladenine-9-yl; *N*²-isobutyrylguanin-9-yl) 5'-*O*-(4,4'-dimethoxytrityl)-nucleotidyl-[3'-*O*-(2-cyanoethoxy)-*N,N*-diisopropyl-phosphoramidites] as the incoming d-nucleoside synthons. Stepwise elongation of immobilized d-nucleoside, linked by a 3'-*O*-succinyl bond to controlled pore glass, was carried out according to the standard protocol summarized in Table 1. Repetition of the individual coupling steps proceeded, as gauged by spectrophotometric determination of the released DMT-cation, with a coupling efficiency of 98%. After completion of the elongation process, the resulting fully protected and immobilized fragments were completely deblocked by the following two-step procedure. Acidolysis of the 5'-*O*-DMT group (step 1 in Table 1), followed by treatment with dry methanolic ammonia for 48 h at 50°C, afforded the crude fragments **20** and **21**, the purification of which could easily be effected by gel filtration (Sephadex G-50). The presence of one 8-oxo-7H-deoxyguanosine unit in fragment **20** could be inferred from the following observation. The expanded low-field region of the ¹H-NMR spectrum of **20** showed (Fig. 1), apart from the H-6 resonances of the thymidine and cytidine bases, three H-8 resonances of the guanosine moieties. On the other hand, four distinct H-8 guanosine resonances are observed for the corresponding native octamer.

A more direct prove for the presence of dG^{8oxo} in the modified fragments was obtained by quantitative analysis of the d-nucleosides released after enzymatic hydrolysis.²³ For example, quantitation of the separate digestion products (see Fig. 2) from fragment **21** showed the presence of the d-nucleosides A, T, G and G^{8oxo} in the expected molar ratios.

In conclusion, the data presented in this paper clearly illustrate that fully protected and readily accessible amidite **3** can be used successfully for the solid-support synthesis of high quality oligonucleotides containing dG^{8oxo} residues at predetermined positions via a well-established phosphite triester approach.

Figure 2. HPLC elution profiles of the d-nucleosides resulting after the enzymatic digestions of A) the native hexadecamer d-[AATTCATCGATATCTA] and B) the modified oligonucleotide d-[AATTCATCG^{8oxo}ATATCTA] (**21**).

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ABBREVIATIONS

Ac = acetyl, CNE = 2-cyanoethyl, DMT = 4,4'-dimethoxytrityl, DPC = diphenylcarbamoyl, iBu = isobutyryl, iPr = isopropyl, MMT = 4-monomethoxytrityl.

REFERENCES

- Ames, B.N. (1979) *Science* **204**, 587–593; Ames, B.N. (1983) *Science* **221**, 1256–1263; Floyd, R.A. (1990) *Carcinogenesis* **11**, 1447–1450.
- Floyd, R.A. (1990) *FASEB J.* **4**, 2587–2597.
- Kuchino, Y., Mori, F., Kasai, H., Inoue, H., Iwai, S., Miura, K., Ohtsuka, E. and Nishimura, S. (1987) *Nature* **327**, 77–79.
- Shibutani, S., Takeshita, M. and Grollman, A.P. (1991) *Nature* **349**, 431–434.
- Moriya, M., Ou, C., Bodepudi, V., Johnson, F., Takeshita, M. and Grollman, A.P. (1991) *Mutation Res.* **254**, 289–298.
- Wood, M.L., Dizdaroglu, M., Gajewski, E. and Essigmann, J.M. (1990) *Biochemistry* **29**, 7024–7032.
- Kasai, H., Nishimura, S., Toriumi, Y., Itai, A. and Iitaka, Y. (1987) *Bull. Chem. Soc. Jpn.* **60**, 3799–3800.
- Aida, M. and Nishimura, S. (1987) *Mutation Res.* **192**, 83–89.
- Culp, S.J., Cho, B.P., Kadlubar, F.F. and Evans, F.E. (1989) *Chem. Res. Toxicol.* **2**, 416–422.
- Uesugi, S. and Ikehara, M. (1977) *J. Am. Chem. Soc.* **99**, 3250–3253.
- Cho, B.P., Culp, S.J., Evans, F.E. and Kadlubar, F.F. (1989) in *Proc. Am. Assoc. Cancer Res.* **30**, p. 201, no. 797a.
- Kouchakdjian, M., Bodepudi, M., Shibutani, S., Eisenberg, M., Johnson, F., Grollman, A.P. and Patel, D.J. (1991) *Biochemistry* **30**, 1403–1412.
- Oda, Y., Uesugi, S., Ikehara, M., Nishimura, S., Kawase, Y., Ishikawa, H., Inoue, H. and Ohtsuka, E. (1991) *Nucleic Acids Res.* **19**, 1407–1412.
- Sinha, N.D., Biernat, J. and Köster, H. (1983) *Tetrahedron Lett.* **24**, 5843–5846; Sinha, N.D., Biernat, J., McManus, J. and Köster, H. (1984) *Nucleic Acids Res.* **12**, 4539–4557.
- Bodepudi, V., Iden C.R. and Johnson, F. (1991) *Nucleosides & Nucleotides*, **10**, 755–761.
- Guy, A., Duplaa, A.-M., Harel, P. and Téoule, R. (1988) *Helv. Chim. Acta* **71**, 1566–1572.
- Lin, T.-S., Cheng, J.-C., Ishiguro, K. and Sartorelli, A.C. (1985) *J. Med. Chem.* **28**, 1194–1198.
- Pon, R.T., Damha, M.J. and Ogilvie, K.K. (1985) *Nucleic Acids Res.* **13**, 6447–6465; Pon, R.T., Damha, M.J. and Ogilvie, K.K. (1985) *Tetrahedron Lett.* **26**, 2525–2528; Pon, R.T., Usman, N., Damha, M.J. and Ogilvie, K.K. (1986) *Nucleic Acids Res.* **14**, 6453–6470; Nielsen, J., Taagaard, M., Marugg, J.E., van Boom, J.H. and Dahl, O. (1986) *Nucleic Acids Res.* **14**, 7391–7403; Nielsen, J., Dahl, O., Remaud, G. and Chattopadhyaya, J. (1987) *Acta Chem. Scand.* **B41**, 633–639; Marugg, J.E., Nielsen, J., Dahl, O., Burik, A., van der Marel, G.A. and van Boom, J.H. (1987) *Recl. trav. Chim. Pays-Bas* **106**, 71–73.
- Kamimura, T., Tsuchiya, M., Koura, K., Sekine, M. and Hata, T. (1983) *Tetrahedron Lett.* **24**, 2775–2778.
- Long, R.A., Robins, R.K. and Townsend, L.B. (1968) In Zorbach, W.W. and Tipson, R.S. (eds.) *Synthetic Procedures in Nucleic Acid Chemistry*, John Wiley & Sons, Inc., New York, Vol.1, p.228–229.
- Ogilvie, K.K. and Slotin, L. (1971) *J. Org. Chem.* **36**, 2556–2558.
- Markiewicz, W.T. and Wiewiorowski, M. (1978) *Nucleic Acids Res. Spec. Publ.* **4**, s185–188; Markiewicz, W.T. (1979) *J. Chem. Res. Synop.* 24–25.
- Barton, D.H.R. and McCombie, S.W. (1975) *J. Chem. Soc. Perkin Trans I* 1574–1585; Barton, D.H.R. and Subramanian, R. (1977) *Ibid.* 1718–1723.
- Klein, J.C., Bleeker, M.J., Lutgerink, J.T., van Dijk, W.J., Brugghe, H.F., van den Elst, H., van der Marel, G.A., van Boom, J.H., Westra, J.G., Berns, A.J.M. and Kriek, E. (1990) *Nucleic Acids Res.* **18**, 4131–4137.
- Van Boom, J.H. and Wreesmann, C.T.J. (1984) In Gait, M.J. (ed.), *Oligonucleotide Synthesis: A Practical Approach*, IRL Press, Oxford (UK), p. 153.
- Tundo, P. and Venturello, P. (1979) *J. Am. Chem. Soc.* **101**, 6606–6613.
- Chow, F., Wempe, T. and Palm, G. (1981) *Nucleic Acids Res.* **9**, 2807–2817.
- Gait, M.J., Matthes, H.W.D., Singh, M., Sproat, B.S. and Titmas, R.C. (1982) *Nucleic Acids Res.* **10**, 6243–6254.