Pergamon

Tetrahedron, Vol. 52, No. 6, pp. 2047-2064, 1996

0040-4020/96 $15.00 + 0.00

0040-4020(95)01043-2

Synthesis and Characterization of O^6-Modified Deoxyguanosine-Containing Oligodeoxyribonucleotides for Triple-Helix Formation

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Abstract: Two new modified deoxyguanosine derivatives linked through their C-6 position to a psoralen and an acridine derivative have been synthesized and incorporated into oligonucleotide chains. Difficulties observed during the purification of oligonucleotides containing a stretch of G and one method used to solve this problem are discussed.

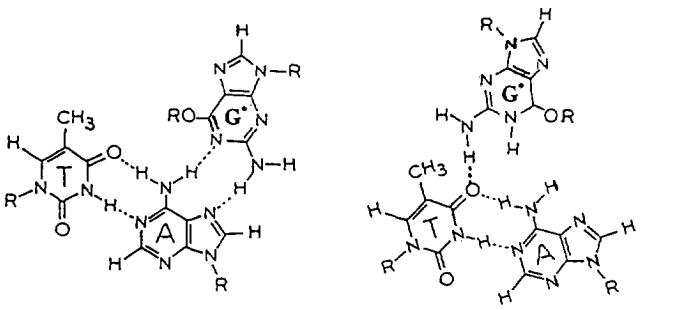
INTRODUCTION

Nucleic acid triple-helix are of current interest because of their potential pharmacological and experiment utility in molecular biology of nucleic acid "third strands"[1,2,3]. One of the major limitation to DNA recognition by oligonucleotides to form triple-helical complexes remains the requirement for polypurine.polypyrimidine target sequence. Recognition involves the formation of hydrogen bonds between the bases of the third strand oligonucleotides and the purine of the target DNA. The specific recognition of double-helical DNA was first described for pyrimidine oligonucleotides[4,5], then for the oligonucleotides containing T and G^6, G and $A^{7,8}$, T, C and G^9.

In order to increase the stability of the triplex, there is value in linking to third strands intercalating agents such as psoralen and acridine, which can strengthen their affinity to target Watson-Crick duplexes. Previously, acridine and psoralen substituents have only been linked to the termini of third strands via a phosphate[10,11] . The construction of an oligonucleotide with acridine covalently linked to nucleic bases should increase stability by intercalating within the triple-helix[10]. The presence of the psoralen in the third strand should allow induction of a crosslink between the latter and the duplex DNA target under irradiation[11].

On particular, linking an acridine or a psoralen derivative to the 6-position of G (G^*) can be especially favorable, since this position is not involved in the formation of hydrogen bonds between the third strand and the purine target sequence.

The modified G can possibly be used to recognize an A.T base pair adjacent to a stretch of G.C base pairs via the base triplex G*xA.T (Fig. 1a). G* might also be incorporated into a pyrimidine third strand in order to bind to T within a purine tract to form G*xT.A (Fig. 1b), since it has been demonstrated that G in a pyrimidine oligonucleotide could recognize T within a purine target forming a GxT.A triplet[12,13,14,15].



a) G*xA.T

OR : O-$(CH_2)_6$-OPso

b) G*xT.A

OR : O-$(CH_2)_5$-NH-Acr

Figure 1: Base triplexes formed by Watson-Crick A.T, T.A base pairs with G*.

We describe here the synthesis of G derivatives modified at the C-6 position by a psoralen and an acridine derivative, and their conversion to phosphoramidite monomers and modified support for incorporation into oligonucleotides. The acridine derivative was included between two runs of pyrimidines in order to recognize an 11-mer and 10-mer oligopurine separated by a thymidine located on the SV 40 genome, while G bearing the psoralen moiety was incorporated at the 3'-end of a G rich 16-mer destined to bind with a 16-mer oligopurine sequence twice present in the integrated proviral HIV DNA.

RESULTS AND DISCUSSION

Approach to substitution at the C-6 position of dG

O^6-[2-methoxy-6-chloro-9-(ω-pentylamino)]-acridine-2'-deoxyguanosine and O^6-(ω-hexyloxypsoralen)-2'-deoxyguanosine derivatives were synthesized following a five-step procedure involving a sulfonylation-displacement method and a four-step procedure including the Mitsunobu[16] reaction, respectively.

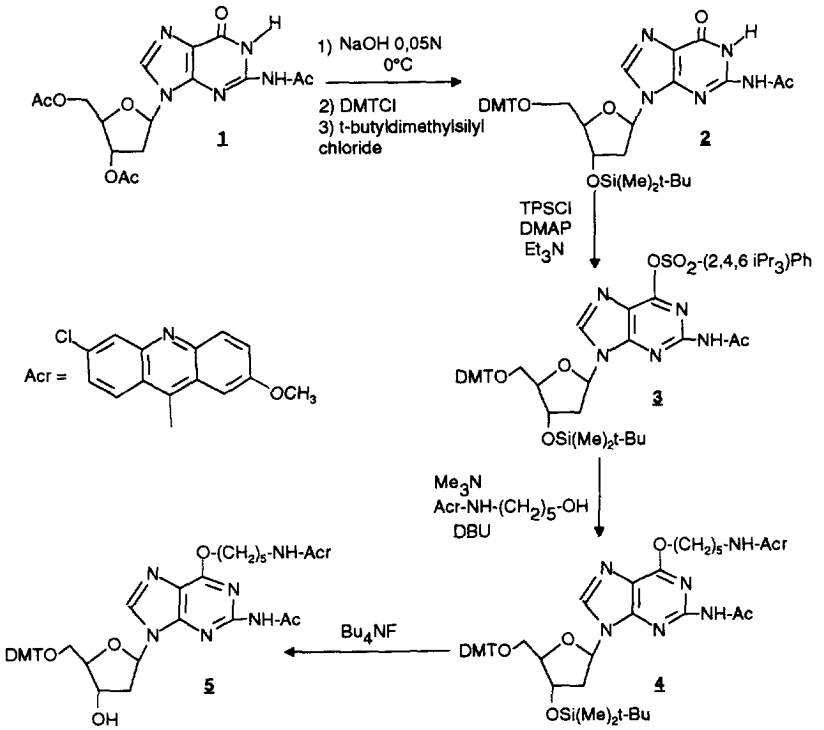
Because the modification of O^6 changes significantly the lability of the exocyclic amine protecting group, the use of certain standard reagents was not possible. For example, the traditional isobutyryl group for guanine is quite difficult to remove from the N^2 position of 6-substituted deoxyguanosine, particularly after incorporation into the oligonucleotide. This leads to the presence of incompletely deprotected units which

contaminate the final product. Moreover, a study carried out by Borowy-Borowski and Chambers[17] showed that significant amounts of side-products were produced. This has also been described by Gaffney and Jones[18]. In fact, the greatest impurity is 2,6-diaminopurine, which is generated from a nucleophilic attack on the C^6 alkylguanine by the aqueous ammonia used to remove the protecting groups from the oligonucleotides after synthesis. To avoid this inconvenience, the reaction time with aqueous ammonia must be shorter which is why it is important to choose the more labile base protecting groups. In the case of acridine containing compounds whose deprotection requires sodium hydroxide instead of the usual ammonia treatment because of the instability of the bond between the C9 atom of the acridine ring and the N-atom of the linker[19], we chose to use the acetyl group for protection of the exocyclic amine of G. For the psoralen derivative, we selected a more easily removable group for the protection of the N^2 position of G. Thus the phenoxyacetyl (Pac) group was selected, which was removed very quickly in 29% ammonia at room temperature[20] with half time (t½) of 15 min for the N-deacylation of N^2-(phenoxyacetyl)-O^6-alkyl 2'-deoxyguanosine without the formation of by-products.

Preparation of the deoxyguanosine derivative substituted at the 6-position by an acridine derivative

This was achieved following a five-step procedure including sulfonylation and nucleophilic displacement similar to a reported method[21] (Scheme 1).

Starting from the deoxyguanosine acetylated both on the exocyclic amino function and the hydroxyl groups **1**, the 5'- and 3'- positions of the sugar were deprotected by methanolic sodium hydroxide treatment and reprotected by the dimethoxytrityl and the *tert*-butyldimethylsilyl groups, respectively. The reaction of the resultant product **2** with triisopropylbenzenesulfonylchloride in the presence of triethylamine and catalytic amounts of DMAP led to the sulfonylated compound **3** in a good yield. The latter was activated by reaction with trimethylamine and the product obtained was reacted with the hydroxyl group of the acridine-linker derivative[22] in the presence of DBU to afford the fully protected deoxyguanosine bearing the acridine ring at its 6-position via a pentamethylene linker **4**. The 3'-position of the modified nucleoside **4** was then deblocked to give **5**.

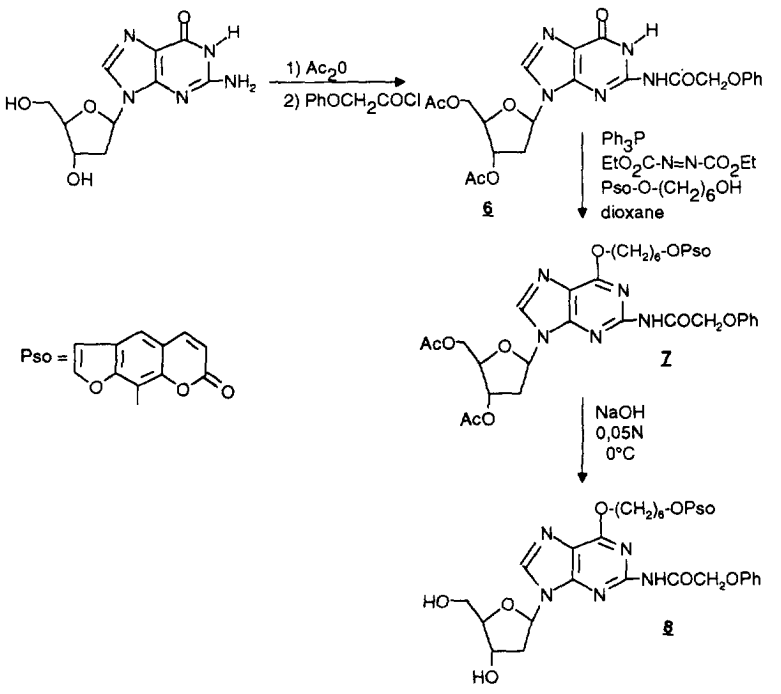


Scheme 1: Preparation of N^2-acetyl-O^6-pentylaminoacridine deoxyguanosine monomer

DMTCl: 4,4' dimethoxytrityl chloride; Ac: acetyl; DMAP: dimethylaminopyridine; Acr: 2-methoxy-6-chloroacridinyl; TPSCl: 2, 4, 6, triisopropylbenzene sulfonyl chloride.

Preparation of the deoxyguanosine derivative substituted at the 6-position by a psoralen derivative

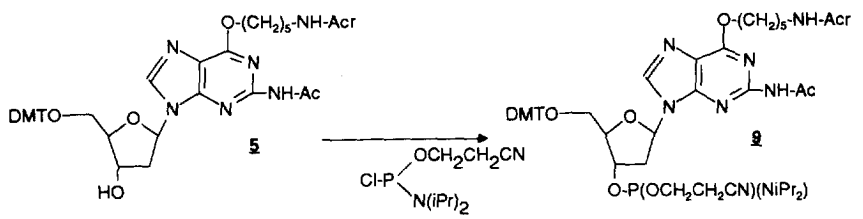
After protection of the N^2-, 5'- and 3'- OH positions of the starting compound (3'-OH had to be protected to avoid formation of the N^3-3' anhydro derivative from 3'-O unprotected deoxyguanosine[23]), treatment of 3'-, 5'-bis(O-acetyl)-N^2-phenoxyacetyl)-2' deoxyguanosine **6** respectively with diethyl azodicarboxylate (DEAD), triphenylphosphine and 8-(ω-hydroxyhexyloxy)psoralen in dioxane at room temperature, led to the desired product **7** after 18 h. This crude product was directly treated with 0.05 N aqueous sodium hydroxide at 0°C to selectively remove the acetyl groups from the 5'- and 3'- positions of the sugar. This is a tedious step since some loss of the phenoxyacetyl group from N^2 is unavoidable. The product was then purified by silica column chromatography and eluted with a gradient of MeOH in CH_2Cl_2 to give N^2-(phenoxyacetyl)-O^6-(hexyloxypsoralen) deoxyguanosine **8** (Scheme 2).



Scheme 2: Preparation of the N^2-phenoxyacetyl-O^6-hexyloxypsoralen deoxyguanosine monomer. DMTCl: 4, 4' dimethoxytrityl chloride; Ac: acetyl; Pac: phenoxyacetyl; Pso: psoralen.

Synthesis of O^6-modified deoxyguanosine-containing oligodeoxyribonucleotides

The modified acridine deoxyguanosine derivative was introduced by solid phase synthesis into two oligonucleotides **10** (22-mer) and **11** (23-mer), between two pyrimidine blocks to recognize a 22-mer SV40 target containing an 11-mer and 10-mer oligopurine separated by thymine (Fig. 2). The requiered phosphoramidite derivative **9** (Scheme 3) was synthesized by reaction of 2-cyanoethyl-N,N-diisopropylamido-chlorophosphite with the 3'-hydroxyl group of compound **5**.



Scheme 3: Synthesis of modified acridine deoxyguanosine phosphoramidite **9**.

Oligonucleotides **10** and **11** were obtained at the µmole scale, using increased coupling time for this modified phosphoramidite **9**.

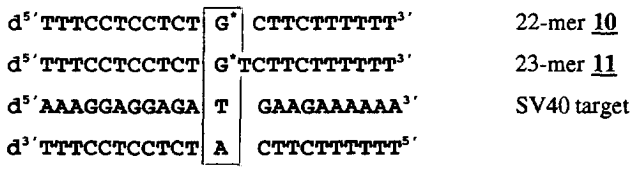


Figure 2: SV40 target and oligonucleotides **10** (22-mer) and **11** (23-mer).

We decided to add the acridine derivative to the 6-position of G located at the internal position of the sequence in order to stabilize the triplex formed with the double-stranded target. However, it is neither possible to know if the acridine derivative is able to intercalate within a triple-helix nor what to expect when oligonucleotide **10** is used. Another possibility is that the acridine moiety could intercalate within the double-stranded target. In this case, the double-stranded target will be elongated along the width of one base-pair, and in order to allow the binding of the third strand it is necessary to add one base in face of the intercalation site. For these reasons, oligomer **11** containing an additionnal T was synthesized.

After oligonucleotide assembly, deprotection was carried out by sodium hydroxide treatment (0.4 N in H_2O/MeOH, 50:50 V/V) for 48 h at room temperature. After being purified by ion exchange chromatography at pH 6.8, the oligomers were analyzed by reversed-phase chromatography (Fig. 3). Full deprotection and the base composition of the oligomers were ascertained by nuclease degradation followed by reversed-phase analysis of the hydrolysate using as standards dT, dC monomers and a sample of dG* whose structure had been confirmed by NMR (see experimental section) (Fig. 3).

The results obtained by MALDI/TOF analysis gave a mass in accordance with the proposed structure for compound **11**.

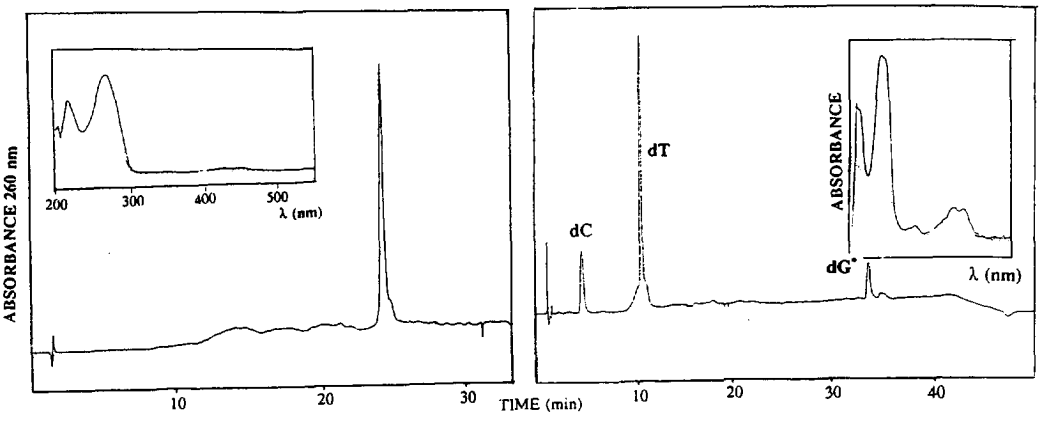
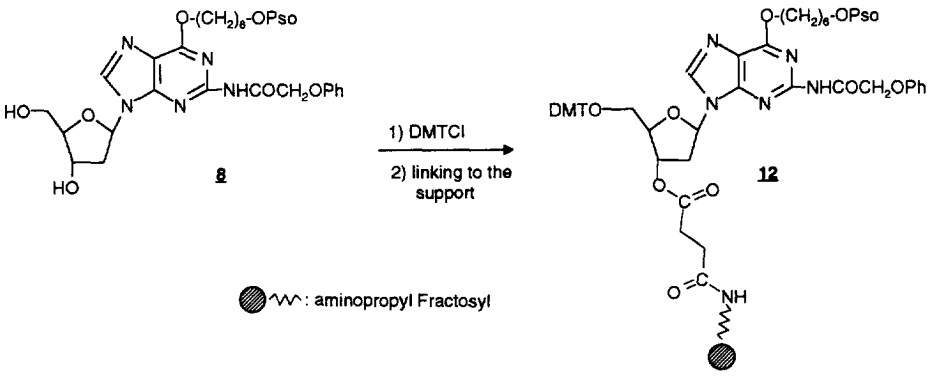


Figure 3: Reversed-phase HPLC analysis of purified compound **d(TTTCCTCCTCTG*CTTCTTTTTT)** **10** (left) and the hydrolysate of compound **10** (right) with nucleases (P1 from Penicilium citrinum and AP alkaline phosphatase) on a Lichrospher 100 RP-18 (5 μm) column (125×4 mm) using a linear gradient of CH_3CN in 0.1M aqueous triethyl ammonium acetate buffer, pH 7 with a flow rate of 1ml/min [0% CH_3CN for 5 min then 0 to 30% in 30 min (left) and 0% CH_3CN for 5 min then 0 to 20% in 20 min then 20 to 50% CH_3CN in 15 min (right)]. The insets show the UV-Visible absorption spectra between λ 200 nm and λ 550 nm of oligonucleotides **10** (left) and **dG*** (right).

The psoralen derivative was introduced at the 3'-end of the 16-mer **13**, in order to recognize a 16-mer purine HIV target DNA and to photoinduce a cross-link reaction, under irradiation, with T or C in the double helix DNA target (Fig. 4); hence the necessity to prepare the support **12** (Scheme 4). After protection of the 5'-hydroxyl function by the dimethoxytrityl, the 3'-hydroxyl was immobilized on 3-aminopropyl Fractosil 500 using the classical procedure[24], including preparation of the succinylated derivative and the corresponding *p*-nitrophenyl ester; the latter was reacted with the 3-aminopropyl Fractosil support in the presence of triethylamine. The remaining amino groups were capped by acetylation. The dimethoxytrityl cation assay indicated a loading of 12 μmol per gram of solid support **12**. This modified support was used for the preparation of the 16-mer **d(TTTTCTTTTGGGGGGG*)** **13** (with G* for modified G), at the μmol scale, via β-cyanoethyl-phosphoramidite chemistry. The coupling yield measured by release of DMT cation was satisfactory ()97%).



Scheme 4: Synthesis of modified psoralen deoxyguanosine support **12**.

1) DMTCl, pyridine; 2) linking to the support: i) succinic anhydride, 4-dimethylaminopyridine, pyridine; ii) *p*-nitrophenol, pyridine, dicyclohexylcarbodiimide, dioxane; iii) 3-aminopropyl Fractosil 500, Et_3N, DMF

d5'TTTTCTTTTGGGGGGG*3'	16-mer **13**
d5'TTT-AAAAGAAAAGGGGGGA-CT3'	HIV target
d3'AAA-TTTTCTTTTCCCCCCT-GA5'	

Figure 4: HIV target and oligonucleotide **13** (16-mer).

The synthesis was followed by a classical deprotection procedure whereby an oligomer is cleaved from the polymer support by hydrolysis of the succinate linker together with removal of all the protecting groups from the oligonucleotide chain by concentrated ammonia at room temperature. Liquid chromatography analysis of the crude psoralen-containing oligonucleotide on either ion exchange or reversed-phase columns exhibited complicated profiles. Thus, the ion exchange column chromatogram changed according to the product concentration. The main peak obtained by ion exchange was analyzed on a reversed-phase column and also gave a complicated chromatogram (Fig. 5). The UV spectrum shown in the inset corresponds to the main peak at 21.2 min, while the other peaks gave similar spectra.

Polyacrylamide gel electrophoresis (PAGE) of this oligonucleotide on a denaturing gel showed one main band with migration almost identical to that of a 16-mer, and three additional bands more retained on the gel (Data not shown).

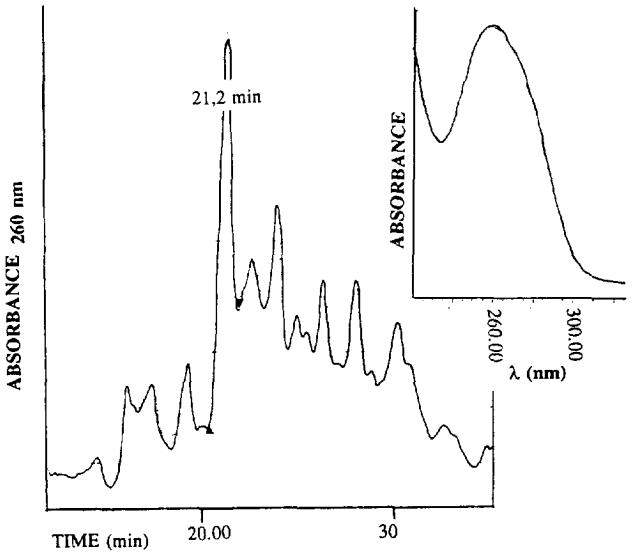


Figure 5: Reversed-phase analysis of **d(TTTTCTTTTGGGGGGG*) 13** on a Lichrospher 100 RP-18 (5 μm) column (125×4 mm) using a linear gradient of CH_3CN (buffer A 5% CH_3CN, buffer B 80% CH_3CN) in a 0.1M aqueous triethyl ammonium acetate buffer, pH 7 with a flow rate of 1ml/min. 0 to 25% of buffer B in 25 min then 25 to 40% in 10 min. The inset show the absorption spectra, recorded between λ 200 nm and λ 400 nm, of the oligonucleotide **13** .

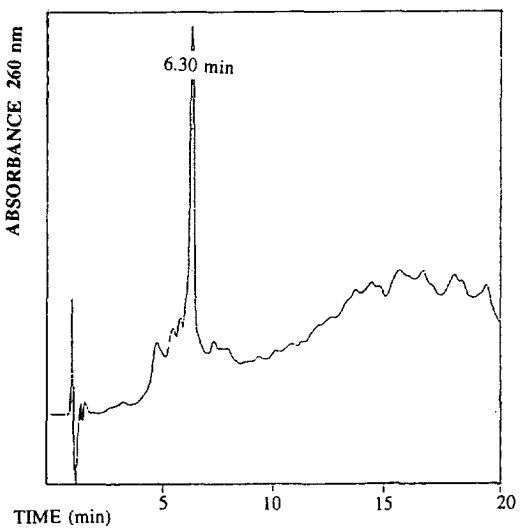
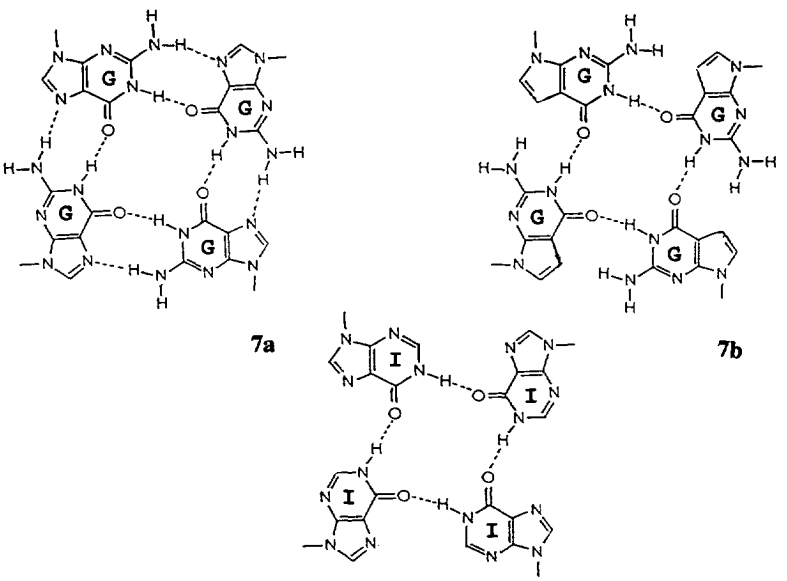


Figure 6: Reversed-phase analysis of oligomer **d(G_6) 14** on a Lichrospher 100 RP-18 (5 μm) column (125×4 mm) using a linear gradient of CH_3CN (buffer A 5% CH_3CN, buffer B 80% CH_3CN) in a 0.1M aqueous triethyl ammonium acetate buffer, pH 7 with a flow rate of 1ml/min. 0 to 25% of buffer B in 25 min then 25 to 60% in 30 min.

These observations, consistent with the literature [25,26], indicate that oligonucleotides with a run of G residues form four stranded helical aggregates of G tetrads that stack on each other (Fig.7a). In order to discern the contribution of the G_6 sequence in the chromatographic and electrophoresis behavior of the hexadecamer **d(TTTTCTTTTGGGGGGG*)** **13**, we synthesized the hexadeoxyguanylate **d(G_6)** **14** and the alternating hexadeoxynucleotide involving the 7-deazadeoxyguanosine **d(^{7C}GG^{7C}GG^{7C}GG)** **15**. Replacement of every other G with 7-deaza G is expected to decrease self-association by suppressing the Hoogsteen hydrogen bonds between the 2-amino and N(7) groups (Fig. 7b), as is the case for the four stranted helix of poly I (Fig. 7c)[27].



7a **7b**

7c

Figure 7: Models of tetrads of dG with 8 H-bonds, tetrads of ^{7C}dG with only 4 H-bonds, and tetrads of dI with only 4H-bonds.

HPLC analysis of the crude **d(G_6)** (Fig. 6) showed a complicated chromatogram as did the hexadecamer **13** (Fig. 5). On the contrary, the alternating hexamer **15** gave an expected main peak (Fig. 8a).

Having obtained these results, we replaced three of the guanines of the hexadecamer **13** with the 7-deaza deoxyguanosine by synthesizing the sequence **d(TTTTCTTTT^{7C}GG^{7C}GG^{7C}GGG*)** **16**, which gave one main peak on reversed-phase chromatography (Fig. 8b) and only one band on gel electrophoresis corresponding to the main one obtained with the oligomer **13**.

MALDI/TOF analysis, carried out on oligonucleotides **13** and **16**, confirmed the calculated mass (see Experimental Part).

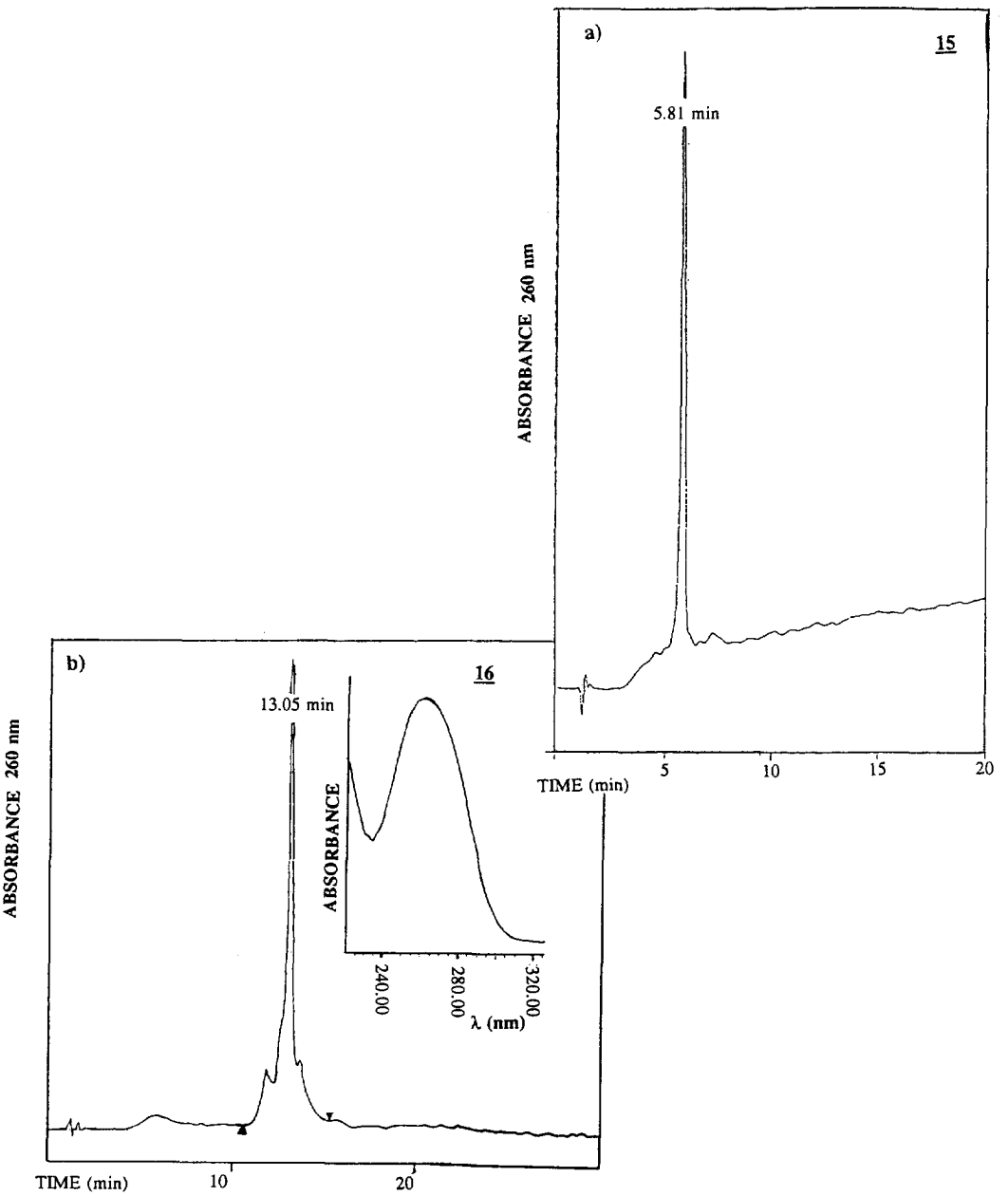


Figure 8: Reversed-phase chromatography of oligomers **d(^{7C}GG^{7C}GG^{7C}GG)** **15** and **d(TTTTCTTTT^{7C}GG^{7C}GG^{7C}GGG*)** **16** on a Lichrospher 100 RP-18 (5 μm) column (125×4 mm) in a 0.1M aqueous triethylammonium acetate buffer, pH 7 with a flow rate of 1ml/min using a linear gradient of CH_3CN. 0 to 25% in 25 min then 25 to 60% in 30 min for **15**; 25 to 40 % in 10 min for **16**. The inset shows the absorption spectrum, recorded between λ 200 nm and λ 320 nm, of the oligonucleotide **16** .

CONCLUSION

In this work, we have described the preparation of two new deoxyguanosine derivatives modified at their O^6 position and their incorporation into triple-helix forming oligonucleotides. The first compound, a modified monomer derivatized with a psoralen moiety incorporated at the 3'-end of a G-rich containing sequence **d(TTTTCTTTTGGGGGGG*)** **13** to recognize A.T base pairs adjacent to G.C base pairs and to enable induction of a photo-cross-link to the target double-helix. The second modified monomer, substituted by an acridine moiety, was incorporated into an oligonucleotide between two pyrimidine stretches **[d(TTTCCTCCTCTG*CTTCTTTTTT)** **10** and **d(TTTCCTCCTCTG*TCTTCTTTTTT)** **11]** to recognize T.A base pairs in the duplex DNA target. The sequences with several contiguous G residues gave self-associated products. This problem was solved by substituting some of the Gs by ^{C7}G. Studies on the hybridization properties and crosslinking abilities of these modified oligonucleotides are presently under investigation in collaboration with an another group.

MATERIALS AND METHODS

All reagents and solvents, except for the twice distilled pyridine stored over molecular sieves 4 Å, were of reagent-grade quality and were used without further purification. The following were obtained from commercial sources: acetic anhydride, acetic chloride, phenoxyacetyl chloride, hydroxybenzotriazole hydrate, 4-dimethylaminopyridine, triphenylphosphine, diethylazodicarboxylate , 8-methoxypsoralen, anhydrous potassium carbonate, pyridine hydrochloride, 6-bromo-1-hexanol, succinic anhydride, p-nitrophenol, dicyclohexylcarbodiimide, Fractosil 500, 6,9-dichloro-2-methoxy-acridine, 5-amino-1-hexanol, phenol, 2,4,6 triisopropylbenzenesulfonyl chloride, trimethylamine, DBU, diisopropylethylamine, 2-cyanoethyl-N,N(diisopropyl)chlorophosphoramidite, dimethylformamide, dioxane (Aldrich), triethylamine, sodium sulfate (Merck), pyridine, dichloromethane, dichloroethane (SDS), 7-deaza dG-CE phosphoramidite (Glen Research).

3-aminopropyl-derivatized fractosil 500 was prepared by the standard method[24]. Oligonucleotides were synthesized on a Pharmacia synthesizer using the phosphoramidite method[28]. Analytical TLC was carried out on Merck 5554 kieselgel 60 F 254 plates and eluted with the following solvents systems: (A) methylene chloride/methanol (90:10 V/V); (B) methylene chloride/methanol (90:5 V/V); (C) methylene chloride/methanol (80:20 V/V); (D) methylene chloride/methanol (85:15 V/V). Merck 9385 Kieselgel 60 was used for column chromatography. HPLC was performed on a Waters 626E (system controller) equipped with a Waters 996 photodiode array detector.

Analysis and purification by ion exchange chromatography were carried out with a Pharmacia FPLC with a DEAE (8 μm, 100 mm×10 mm) column from Waters (with a linear gradient of NaCl in Tris/HCl 25 mM buffer pH 6.8 containing 10% CH_3CN) and a QMA (8 μm, 100 mm×10 mm) column from Waters (using a

linear gradient of NaCl in NaOH 0.01 M buffer pH 12 with 10% CH_3CN), with a flow rate of 1 ml/min. Reversed-phase chromatography analysis was performed on a lichrospher 100 RP (5 μm) column (125 mm×4 mm) from Merck using a linear gradient of CH_3CN in a 0.1 M aqueous triethylammonium acetate, pH 7, with a flow rate of 1 ml/min.

NMR spectra were obtained on a Bruker AM 300 WB spectrometer.

Mass spectrometry analysis : Ion-molecular weights of the oligonucleotides were confirmed by mass spectrometry using a Lasermat Time-of-flight instrument (LD-TOF). The average power of the nitrogen laser (337 nm) was about $10^7 w/cm^2$. To improve the signal to noise ratio, 10 to 20 single shot spectra were accumulated and averaged. All mesurements were performed using the negative detection mode. The spectrometer was calibrated with the $[M-H]^-$ and $[2M-H]^-$ mass peaks of dT_{12} and dT_{12}-Acr as references. For sample preparation, 10 μl of a 0.5 M solution of 2,4,6-trihydroxy acetophenone in ethanol, 5 μl of a 0.1 M aqueous solution of diammonium-L-Tartrate were mixed, 1μl of the conjugate-containing solution (10 OD/ml) was added and the mixture was briefly vortexed. 1 μl of this solution was applied to the probe tip and the solvents were removed.

Synthesis of 2-methoxy-6-chloro-9-(ω-hydroxypentylamino)acridine

2-methoxy-6-chloro-9-(ω-hydroxypentylamino)acridine was obtained as previously described[22].

Rf = 0.45 in system C.

Synthesis of 3'-, 5'-, N^2-triacetyldeoxyguanosine **1**

3'-, 5'-, N^2-triacetyldeoxyguanosine was obtained following a procedure similar to that described in reference 29. Rf = 0.5 in system A.

Synthesis of N^2-acetyldeoxyguanosine

3'-, 5'-, N^2-triacetyldeoxyguanosine (3.5 g, 8.90 mmol) was stirred in a 0.05 M solution of MeONa for 5 min at 0°C. The solution was neutralized with a Dowex 50 pyridinium form resin. The solution was concentrated and the gum was purified on a silica gel column. 70% yield. Rf = 0.18 in system A.

Synthesis of 5'-dimethoxytrityl-N^2-acetyldeoxyguanosine

Dimethoxytritylchloride (2.15 g, 6.30 mmol) was added to a magnetically stirred solution of N^2-acetyldeoxyguanosine (1.85 g, 6 mmol) in anhydrous pyridine (30 ml) at 0°C. After reacting for 1 h at room temperature, the excess dimethoxytritylchloride was quenched by the addition of methanol (1 ml). The mixture was concentrated under vacuum, the residue was dissolved in methylene chloride and the organic solution was washed with a 5% sodium hydrogen carbonate solution. After being dried over Na_2SO_4, the organic solution was concentrated to dryness and the residue was purified on silica gel using a methylene chloride/methanol mixture as eluent. 95% yield; Rf = 0.48 in system A.

Synthesis of 5'-O-dimethoxytrityl-3'-O-(*tert*-butyldimethylsilyl)-N^2-acetyldeoxyguanosine **2**

5'-O-Dimethoxytrityl-N^2-acetyldeoxyguanosine (3.48 g, 5.7 mmol) and imidazole (1.08 g, 15 mmol) were dried and solubilized in anhydrous pyridine (50 ml). *Tert*-butyldimethylchlorosilane (1.11 g, 7.43 mmol) was added

and the reaction monitored by TLC with methylene chloride/methanol, (95:5 V/V) as eluent.

After 10 h of reaction at room temperature, the mixture was diluted with water and extracted with dichloromethane. The organic phase was washed with 5% sodium hydrogen carbonate solution, dried over sodium sulfate and concentrated under reduced pressure. The residue was purified on a silica gel column using a methylene chloride/methanol mixture. 90% yield; Rf = 0.60 with system B.

Synthesis of O^6-(2, 4, 6 triisopropylbenzenesulfonyl)-5'-O-Dimethoxytrityl-3'-O-(*tert*-butyldimethylsilyl) N^2-acetyldeoxyguanosine **3**

Compound **2** (2.26 g, 3 mmol) was dried by coevaporation (three times) with anhydrous CH_3CN and solubilized in dry CH_2Cl_2 (75 ml). Triethylamine (1.51 g, 2.1 ml, 15 mmol), dimethylaminopyridine (18.3 mg, 0.15 mmol) and then 2,4,6 triisopropylbenzenesulfonylchloride (2.27 g, 7.5 mmol) were added with rapid stirring at 0°C. The reaction was monitored by TLC with methylene chloride/methanol (97:3 V/V) as eluent. When the reaction was completed, the mixture was diluted with CH_2Cl_2 and washed with a 5% sodium hydrogen carbonate solution, dried over sodium sulfate and concentrated under reduced pressure. The residue was purified on a silica gel column using a methylene chloride/methanol mixture. 90% yield; Rf = 0.9 in system B.

Synthesis of O^6[2-methoxy-6-chloro-9-(ω-pentylamino)]-acridine-5'-O-dimethoxytrityl-3'-O-(*tert*-butyldimetylsilyl)-N^2-acetyldeoxyguanosine **4**

Compound **3** (0.99 g, 1 mmol) and 2-methoxy-6-chloro-9-(ω-hydroxypentylamino)-acridine (0.69 g, 2 mmol) were dried by coevaporation with anhydrous pyridine and solubilized in a 50:50 V/V mixture of pyridine and CH_3CN with magnetic stirring. Excess trimethylamine (17 eq g in CH_3CN solution) was added. Following this, DBU (0.23 g, 1.5 mmol) was added and the reaction was allowed to react at room temperature. The reaction was monitored by TLC analysis with system D. After 3 days the mixture was concentrated to dryness under reduced pressure and the residue was twice purified on glass-backed plates of silica gel 60 (ref. 9385) using methylene chloride/methanol (88:12 V/V) as eluent. 65% yield; Rf =0.28 in system D.

O^6[2-methoxy-6-chloro-9-(ω-pentylamino)]-acridine -5'-O-dimethoxytrityl-N^2-acetyldeoxyguanosine **5**

The compound obtained above (0.63 g, 0. 6mmol) was treated with a 1M tetrabutylammonium fluoride (10 mmol) in THF at room temperature. The reaction monitored by TLC using system D, was completed after 1 h and the reaction mixture was dissolved in CH_2Cl_2. The organic solution was washed with water, dried over sodium sulfate, concentrated under reduced pressure, and the residue was purified on glass-backed plates of silica gel 60 (Ref. 9385) using system D as eluent. 85% Yield; Rf = 0.30 with system D; Rf = 0.56 with system C.

^1H NMR (DMSO) δ: 10.22 (s, 1H, H-N- C=O); 8.35 (d, 1H, H-8 Acr); 7.79-7.85 (m, 1H, H-5 Acr); 7.69 (s, 1H, H-8 base); 7.41-7.13 (m, 17H, trityl ar. and H1-3-4-7 Acr); 6.34 (m, 1H, H-1'); 5.35 (m, 1H, C_3-OH); 4.52 (m, 1H, H-4'); 4.44 (t, 2H, CH_2O-base); 4.12 (m, 1H, H-3'); 3.96 (m, 2H, H-5' H-5"); 3.84 (s, 3H, CH_3O Acr); 3.79 (t, 2H, CH_2-NH) ; 3.67 (d, 6H, CH_3O trityl); 2.84 (m, 1H, H-2'); 2.35 (m, 1H, H-2"); 2.15 (s,

3H,Ac); 1.80 (m, 6H, O-$CH_2\underline{CH_2CH_2CH_2}CH_2N$).

Synthesis of O^6-[2-methoxy-6-chloro-9-(ω-pentylamino)]-acridine-5'-O-dimethoxytrityl-3'-O-(2-cyano-ethyl N,N diisopropylamidophosphite)-N^2-acetyldeoxyguanosine **9**

2-cyanoethyl-N,N-diisopropylamidochlorophosphite (0.075 g, 0.32 mmol) was added dropwise under argon atmosphere to a magnetically stirred mixture of compound **5** (0.2 g, 0.21 mmol) and diisopropylethylamine (0.1 g, 0.8 mmol) in methylene chloride (3 ml) at room temperature. The phosphitylation reaction was monitored by TLC using system D as eluent. After 25 min, the reaction mixture was diluted with ethyl acetate and washed with a 10% sodium hydrogen carbonate solution, then with saturated sodium chloride. The organic phase was dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by precipitation with cold hexane and then with cold pentane, giving the amidite **9** as a yellow powder. 85% yield; Rf = 0.36 in system D.

Synthesis of 3'-, 5'-bis-(acetyl)-2'-deoxyguanosine

This compound was obtained using Matsuda's procedure[30].

Reaction time = 3 h; Rf = 0.28 (system A); yield 83%

Synthesis of 3'-, 5'-bis-(O-acetyl)-N^2-(phenoxyacetyl)-2'-deoxyguanosine **6**

Compound **6** was synthesized according to a method similar to that of Téoule[20].

3'-, 5'-bis-(O-acetyl)-2'-deoxyguanosine (1 g, 2.85 mmol) was dried by coevaporation twice and solubilized in anhydrous pyridine (15 ml). In a second flask, 1-hydroxybenzotriazole (0.63 g, 4.66 mmol) was dried by coevaporation with CH_3CN and solubilized in anhydrous acetonitrile (3 ml) and pyridine (3 ml). Phenoxyacetyl chloride (0.48 g, 2.85 mmol) was then added. The two flasks were cooled and the first solution was added dropwise to the second through a rubber septum. After 8 h of reaction at 100°C, the reaction mixture was cooled at 5°C and water (2 ml) was added to hydrolyze the excess reagents, and the solution was left for 30 min. Solvents were evaporated under reduced pressure, the reaction mixture was diluted with chloroform, and the organic phase was washed with a 5% sodium hydrogen carbonate solution, dried over sodium sulfate and evaporated to dryness. The residue was purified on a silica gel column with a methylene chloride/methanol mixture as eluent. 76% yield; Rf = 0.52 in system A.

^{1}H-NMR ($CDCl_3$): δ 1.96 (s, 3H, OAc); 2.11 (s, 3H, OAc); 2.56 (m, 1H, H-2"); 2.9 (m, 1H, H-2'); 4.19 (m, 1H, H-5"); 4.27 (m, 1H, H-5'); 4.41 (m, 1H, H-4'); 4.7 (s, 2H, $\underline{CH_2}$OPh); 5.47 (m, 1H, H-3'); 6.24 (t, 1H, H-1'); 6.92-7.41 (m, 5H, Ph); 7.82 (s, 1H, H-8).

Synthesis of 8-(ω-hydroxyhexyloxy)psoralen [31]

To a solution of 8-hydroxypsoralen (1 g, 4.95 mmol) in anhydrous DMF (15 ml), were added successively 6-bromohexanol (1.94 ml, 14.8 mmol) and anhydrous potassium carbonate (1.7 g, 12.37 mmol) and the mixture was stirred in the dark under nitrogen atmosphere overnight at room temperature. The insoluble mineral salts were then removed by filtration. The filtrate was concentrated to dryness under reduced pressure and the residue was chromatographed on silica gel with a methylene chloride/methanol mixture as eluent. The pure

compound was obtained as white solid after washing with pentane. 80% yield; Rf = 0.64 in system A. ^{1}H-NMR ($CDCl_3$): δ 1.16 (m, 4H, CH_2); 1.44 (m, 4H, CH_2); 3.83 (t, 2H, CH_2-O-Pso); 3.94 (t, 2H, CH_2-O-base); 6.26 (d, 1H, J = 9.6 Hz, H-3 pso); 6.78 (d, 1H, J = 2.2 Hz, H-4' pso); 7.23 (s, 1H, H-5 pso); 7.62 (d, 1H, J = 2.2 Hz, H-5' pso); 8.09 (d, 1H, J = 9.6 Hz, H-4 pso).

Synthesis of 3'-,5'-bis(O-acetyl)-N^2-(phenoxyacetyl)-O^6-(ω-hexyloxypsoralen)-2'-deoxyguanosine **7** and N^2 -(phenoxyacetyl)-O^6-(ω-hexyloxypsoralen)-2'-deoxyguanosine **8**

Compound **6** (0.53 g, 1.09 mmol) was dried twice by coevaporation with dioxane and then dissolved in 5 ml of dry dioxane. Ph_3P (0.43 g, 1.64 mmol), 8-(ω-hydroxyhexyloxy)psoralen (0.49 g, 1.64 mmol) were added to the mixture, which was stirred for a few minutes until all solids completely dissolved. A solution of diethylazodicarboxylate (0.26 ml, 1.64 mmol) in 2 ml of dry dioxane was added dropwise, and the mixture was kept at room temperature overnight with magnetic stirring. Before purification, the 3'- and 5'-hydroxyl functions of **7** were deprotected to give the N^2-(phenoxyacetyl)-O^6-(ω-hexyloxypsoralen)-2'-deoxyguanosine **8**, by stirring **7** with a 0.05M solution of MeONa for 5 min at 0°C. The solution was neutralized with Dowex 50 pyridinium form resin, then concentrated under reduced pressure. The residue was purified on a silica gel column with a methylene chloride/methanol mixture as eluent. 45% yield; Rf = 0.30 in system A. ^{1}H-NMR (DMSO-d6): δ 1.53 (m, 4H, CH_2); 1.76 (m, 4H, CH_2); 2.28 (m, 1H, H-2"); 2.7 (m, 1H, H-2'); 3.54 (m, 2H, H-5' H-5"); 3.7 (m, 1H, H-4'); 3.85 (m, 1H, H-3'); 4.37 (t, 2H, J = 6,31 Hz, CH_2-O-Pso); 4.54 (t, 2H, J = 6.57 Hz, CH_2-O-base); 4.85 (t, 1H, J = 5.39 Hz, C_5'-OH); 5.03 (s, 2H, CH_2-O-Ph); 5.28 (d, 1H, J = 3.98 Hz, C_3'-OH); 6.31 (t, 1H, J = 6.75 Hz, H-1'); 6.4 (d, 1H, J = 9.93 Hz, H-3 pso); 6.7 (d, 1H, J = 3.10 Hz, H-4' pso); 7.07 -6.93 (m, 5H, CH_2-O-Ph); 7.26 (s, 1H, H-5 pso); 8.05 (d, 1H, J = 3.1 Hz, H-5' pso); 8.1 (d, 1H, J = 9.93 Hz, H-4 pso); 8.4 (s, 1H, H-8); 10.53 (s, 1H, NH).

Synthesis of 5'-O-dimethoxytrityl- N^2-(phenoxyacetyl)-O^6-(ω-hexyloxypsoralen)-2'-deoxyguanosine

To a magnetically stirred solution of **8** (0.2 g, 0.29 mmol) in anhydrous pyridine (5 ml), dimethoxytritylchloride (0.108 g, 0.319 mmol) was added at 0°C. The reaction was monitored by TLC on silica gel plates using system A as eluent. When the reaction was complete, the excess dimethoxytritylchloride was quenched by adding methanol (0.5 ml). The solution was concentrated under reduced pressure and the residue was dissolved in methylene chloride. The organic layer was washed with a 5% aqueous solution of $NaHCO_3$, and then with H_2O. The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness, and the residue was purified by flash chromatography. 64% yield; Rf = 0.83 in system A.

Synthesis of modified support **12**

This support was obtained following a procedure similar to that described in Ref. 28. The succinylated derivative was obtained with a 75% yield after flash chromatography purification (Rf = 0.64 system A) and the *p*-nitrophenylester with a 70% yield (Rf = 0.87 system A).

-Loading of the aminopropyl derivatized support

Reaction of the *p*-nitrophenyl ester with the aminopropyl derivatized support was achieved as described in Ref.

24 with a loading of 12 μmol per gram of solid support. Following this, the unreacted amino groups of the support were capped by acetylation.

Synthesis of oligodeoxynucleotides **10**, **11**, **13** and **16**

Preparation of the 22-mer and 23-mer involving Acridinyldeoxyguanosine:

d(TTTCCTCCTCTG*CTTCTTTTTT) 10 and **d(TTTCCTCCTCTG*TCTTCTTTTTT) 11**

Synthesis was by the classical phosphoramidite method including an increased coupling time (8 min) for the modified phosphoramidite **9**. Deprotection was carried out with a 0.4 M NaOH solution in H_2O/MeOH 50:50 V/V mixture at room temperature for 48 h, the pH was adjusted to 7, and the MeOH was removed by evaporation. The crude oligonucleotides were purified by liquid phase chromatography.

Mass spectra: mass calculated $[M-H]^-$ = 7182.20, mass observed $[M-H]^-$ = 7183.6 with dT_{12}-Acr as reference.

Preparation of the two 16-mer involving Psoralenyldeoxyguanosine: **d(TTTTCTTTTGGGGGGG*) 13** and **d(TTTTCTTTT^{7C}GG^{7C}GG^{7C}GGG*) 16**. Solid phase synthesis using the classical phosphoramidite procedure was performed using the modified support **12** at a 1 μmol scale. The different treatments are explained above. Deprotection was carried out by treatment with NH_4OH at 60°C during 40 h. The crude oligonucleotide was purified by liquid phase chromatography.

These compounds were also characterized by Mass spectrometry:

oligonucleotide **13** mass calculated $[M-H]^-$ = 5249.64, mass observed $[M-H]^-$ = 5247.8 with dT_{12} as reference,

oligonucleotide **16** mass calculated $[M-H]^-$ = 5246.64, mass observed $[M-H]^-$ = 5164.0 with dT_{12} as reference.

14 and **15** were synthesized using commercial phosphoramidite of dG and $d^{7C}G$. The assembly, deprotection and purification of the oligonucleotides **14** and **15** were performed using classical procedures except that the deprotection of compound **15** containing $d^{7C}G$ used concentrated ammonia at 60°C for 40 h.

ACKNOWLEDGEMENTS

We wish to thank the *"Association pour le Développement des Sciences Biophysiques"*, the *"Agence Nationale de Recherche contre le SIDA (ANRS)"* and Rhône Poulenc Rorer for financial support. We thank Professor R. L. Inglebert for use of the mass spectrometer.

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(*Received in Belgium* 6 *June* 1995; *accepted* 17 *November* 1995)