Crystal Structure of a Mispaired Dodecamer, d(CGAGAATTC(O^6Me)GCG)$_2$, Containing a Carcinogenic O^6-Methylguanine[†,‡]

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ABSTRACT: The crystal structure of the synthetic deoxydodecamer d(CGAGAATTC(O^6Me)GCG)$_2$ has been determined and refined to an *R*-factor of 16.9% with data up to 2.9-Å resolution. This sequence contains two mismatched base pairs between O^6-methylguanine and adenine with the arrangement A(*syn*)·(O^6-Me)G(*anti*) which differs from the geometry observed in solution by NMR. The intermolecular arrangement is equivalent to the other isomorphous deoxydodecamers. However, the weakening of some significant crystal packing contacts was observed and related to the effect of stacking between the mispaired adenine and the adjacent guanine in the sequence. The structure is highly hydrated, with a total of 49 solvent molecules located. The methyl group and the mismatched base-pair geometry locally disrupt the B-DNA-type solvent network with two solvent molecules found close to the N1 and N6 of the mispaired adenine.

Methylation, or other alkylation, of the guanine O^6 position has been shown to result in mutations both *in vitro* (Loechler *et al.*, 1984; Mitra *et al.*, 1989) and *in vivo* (Zarbl *et al.*, 1985) and has been implicated as an event in the carcinogenesis of alkylating agents (Balmain & Brown, 1988; Basu & Essigmann, 1988; Singer & Grunberger, 1983). The structural bases for the mutagenic and carcinogenic effects of alkylation have begun to be addressed in both NMR and X-ray studies (Brown & Kennard, 1992; Goswami *et al.*, 1993; Kalnik *et al.*, 1989; Leonard *et al.*, 1990; Patel *et al.*, 1986a–c; Sriram *et al.*, 1992a; Williams & Shaw, 1987). An X-ray structure of d(CGC(O^6Me)GCG)$_2$ found that this molecule crystallizes in a Z conformation and that the (O^6Me)G·C pair has normal Watson–Crick geometry (Ginell *et al.*, 1990). Watson–Crick geometry was also found in an X-ray structure of the (O^6-Me)G·T pair present in the duplex d(CGC(O^6Me)GAA-TTTGCG)$_2$, which overall had a B conformation (Leonard *et al.*, 1990). On the other hand, ^{1}H and ^{15}N NMR studies of the same sequence (Goswami *et al.*, 1993) and another B-DNA duplex, d(CGTGAATTC(O^6Me)GCG)$_2$ (Patel *et al.*, 1986c), found that the (O^6Me)G·T pair was hydrogen bonded only at the N2. Bifurcated and wobble hydrogen bonds were observed for the two independent (O^6Et)G·C pairs in an X-ray structure of a complex of d(CGC(O^6Et)GAATTCGCG)$_2$ with netropsin (Sriram *et al.*, 1992b).

There are several possible structures for unmodified A·G mismatches listed in Figure 1, some of which have been observed in crystal structures: A(*anti*)·G(*anti*) (Privé *et al.*, 1987) (Figure 1a), A(*syn*)·G(*anti*) (Brown *et al.*, 1986; Webster *et al.*, 1990) (Figure 1b), and A$^+$(*anti*)·G(*syn*) (Brown *et al.*, 1989) (Figure 1d). NMR studies have shown structures with A(*anti*)·G(*anti*) (Kan *et al.*, 1983) (Figure 1a) and A(*anti*)·G(*syn*) (Gao & Patel, 1988) (Figure 1d). In a recent NMR study, a sheared geometry was observed in a sequence with two contiguous mismatched A·G base pairs (Cheng *et al.*, 1992) (Figure 1c).

The methylation of the O^6 of the guanine alters the hydrogen-bonding possibilities because the N1 position on the guanine does not contain a hydrogen; it is thus a potential hydrogen bond acceptor rather than a donor. As shown in Figure 2, there are a number of ways in which the modified guanine can interact with adenine. This is the first crystallographic analysis of a DNA oligonucleotide containing such a mismatch. A ^{1}H NMR study of the same duplex studied here, at pH 6.9, found a B-DNA duplex with a base-pair arrangement, A(*anti*)·(O^6Me)G(*anti*) (Patel *et al.*, 1986a) (Figure 2a,f). In the present study, the effects of the O^6-methyl lesion on the conformation of the duplex and on crystal packing are described and compared with the structure in solution.

EXPERIMENTAL PROCEDURES

The synthesis and purification of d(CGAGAATTC(O^6-Me)GCG)$_2$ have been described (Gaffney *et al.*, 1984). Crystals were grown at 23 °C by the vapor diffusion technique. Drops containing 0.93 mM DNA, 30 mM sodium cacodylate buffer (pH 7.0), 15 mM $MgCl_2$, 1.0 mM spermine tetrahydrochloride, and 17.5% 2-methyl-2,4-pentanediol (MPD) equilibrated against a well concentration of 35% MPD produced rod-shaped crystals.

The crystal data were collected using flash freezing techniques following the procedure described by Hope (1988) with an Enraf-Nonius low-temperature system operating at −140 °C. One crystal of average dimensions (0.30 × 0.10 × 0.05 mm) was placed in an oil drop (50% Paratone-N and 50% mineral oil) where precipitate, satellites, and mother liquor were removed from the crystal surface, and then the crystal was transferred onto the diffractometer. The d(CGAGAA-TTC(O^6Me)GCG)$_2$ crystallizes in orthorhombic space group $P2_12_12_1$ with cell dimensions as listed in Table 1. A total of 2924 unique reflections were collected to a resolution of 2.3 Å by the ω–θ type scan, with an Enraf-Nonius CAD4 diffractometer on an Enraf-Nonius 571 rotating anode generator equipped with a graphite monochromator. Intensity measurements were corrected for Lorentz, polarization, absorption, and decay using the program package Molen (Fair, 1990). The ratio of observed reflections [$F > 2\sigma(F)$] in

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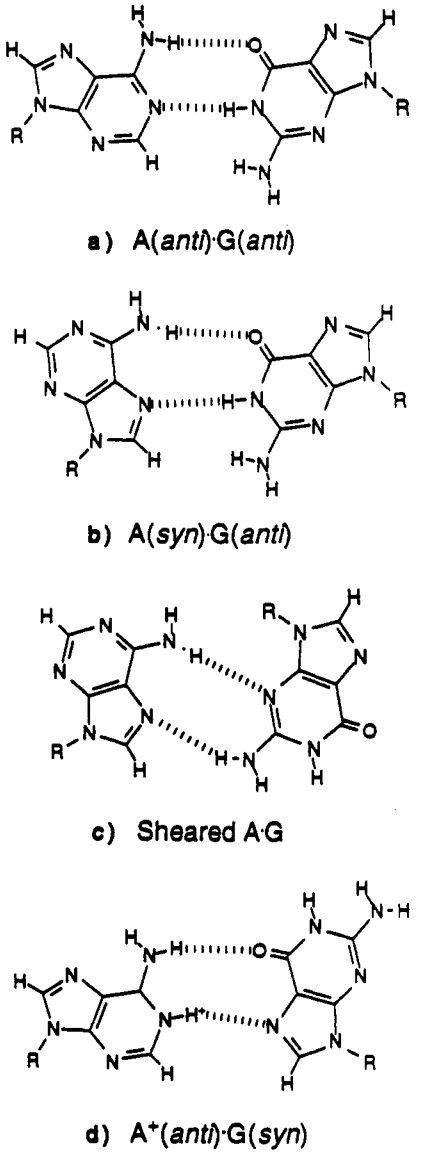

FIGURE 1: Possible and observed hydrogen-bonding schemes for A·G base pairs: (a) A(*anti*)·G(*anti*); (b) A(*syn*)·G(*anti*); (c) sheared A·G; (d) A$^+$(*anti*)·G(*syn*).

separate 0.1-Å shells dropped below 50% at a resolution of 2.9 Å. Thus, only data above 2.9 Å were used in refinement.

The cell dimensions are close to those of other B-DNA dodecamers. Initial refinement was done using the dodecamer structure d(CGCGAATTCGCG)$_2$ (Wing *et al.*, 1980) and constrained–restrained refinement procedures as implemented in the program CORELS (Sussman *et al.*, 1977). The model was first treated as a rigid double helix and finally as 34 groups, consisting of 22 phosphate groups and 12 base pairs. Fourier maps with the mispaired bases omitted indicated that both were in the A(*syn*)·(O^6Me)G(*anti*) conformation. After the replacement of the Watson–Crick C·G base pairs with those of A(*syn*)·(O^6Me)G(*anti*), CORELS refinement converged to an *R*-factor of 0.29 and a correlation coefficient of 0.86 for data in the 6.0–2.9-Å resolution range.

Further refinement was done using restraints as implemented in the program NUCLSQ (Westhof *et al.*, 1985). Although the positions of the bases and the phosphorus atoms were well-defined and stable at this resolution, the torsion angles of the sugar phosphate backbone were less well determined. Thus, it was necessary to introduce stereochemical restraints for those angles. In addition, a theoretical

Table 1: Crystal Data and Final Refinement Parameters

Crystal Data	
contents of asymmetric unit	
strands of d(CGAGAATTC(O^6Me)GCG)	2
total mol wt of DNA duplex	7342.8
space group	$P2_12_12_1$
unit cell	
a (Å)	25.13(3)
b (Å)	40.45(1)
c (Å)	64.51(2)
α, β, γ	90.00
volume (Å^3)	65 575
Data Collection Statistics	
crystal size (mm)	0.30 × 0.10 × 0.05
temperature (°C)	−140
crystal mounting	tip of fiber
data collection device	Enraf-Nonius CAD4
radiation	Cu Kα
unique data collected	2924
reflections $F > 2\sigma(F)$	1348
resolution limit (Å)	2.37
% reflections	
$F > 2\sigma(F)$: 8.0–2.9	71
$F > 2\sigma(F)$: 2.9–2.37	19
Refinement Statistics	
resolution range (Å)	8–2.9
no. of reflections [$F > 2\sigma(F)$]	1031
final *R*-factor[a] (%)	16.9
correlation coefficient	0.938
distances >2σ	66
σ on F_o map (e Å^{-3})	0.33
highest $F_o - F_c$ peak (e Å^{-3})	0.13
lowest $F_o - F_c$ peak (e Å^{-3})	−0.11
σ on $F_o - F_c$ map (e Å^{-3})	0.06
sugar–base bond distance (Å)	0.012/0.020[b]
sugar–base angle distance (Å)	0.022/0.025
phosphate bond distance (Å)	0.023/0.025
phosphate angle distance (Å)	0.024/0.030
planar groups (Å)	0.012/0.020
chiral volumes (Å^3)	0.09/0.10
single torsion contacts (Å)	0.13/0.30
multiple torsion contacts (Å)	0.18/0.30
possible hydrogen bonds (Å)	0.22/0.30
isotropic thermal factors	
sugar–base bonds (Å^2)	0.53/2.0
sugar–base angles (Å^2)	0.76/2.0
phosphate bonds (Å^2)	3.63/4.0
phosphate angles (Å^2)	2.63/8.0
weighting scheme applied to structure factors [1/SIGAPP][c]	
AFSIG	19.5
BFSIG	−80.0

[a] $R = \sum|F_o - F_d|/\sum F_o$; correlation coefficient is $\sum[(F_o - \langle F_o\rangle)(F_c - \langle F_c\rangle)]/[\sum(F_o - \langle F_o\rangle)2\sum(F_c - \langle F_c\rangle)^2]^{1/2}$. [b] The left number gives the rms deviation, and the right number is the σ value. [c] SIGAPP = AFSIG + BFSIG·(STHOL-0.1666667).

modeling program for generating stereochemically favorable backbone conformations with the known positions of the base atoms and the phosphorus atom (Srinivasan & Olson, 1987) was used to help guide the electron density fitting. The electron density maps were calculated using the program XPLOR (Brünger, 1990) and displayed on an Evans and Sutherland PS 390 using the program FRODO (Jones, 1978). After several cycles of refinement and electron density fitting, the *R*-factor dropped to 0.22 for the resolution range 8–2.9 Å.

Difference Fourier maps were used to locate 49 solvent molecules. All the solvent molecules were assigned as water oxygens and their *B*-factors and site occupancy factors were refined in alternate refinement cycles. Solvent atoms with either *B* above 40 Å^2 or occupancy below 0.5 were excluded.

F_o–F_c difference electron density maps calculated after 10 cycles of refinement with the A·(O^6Me)G pairs omitted are shown in Figure 3. The base pair A15·(O^6Me)G10 fits density

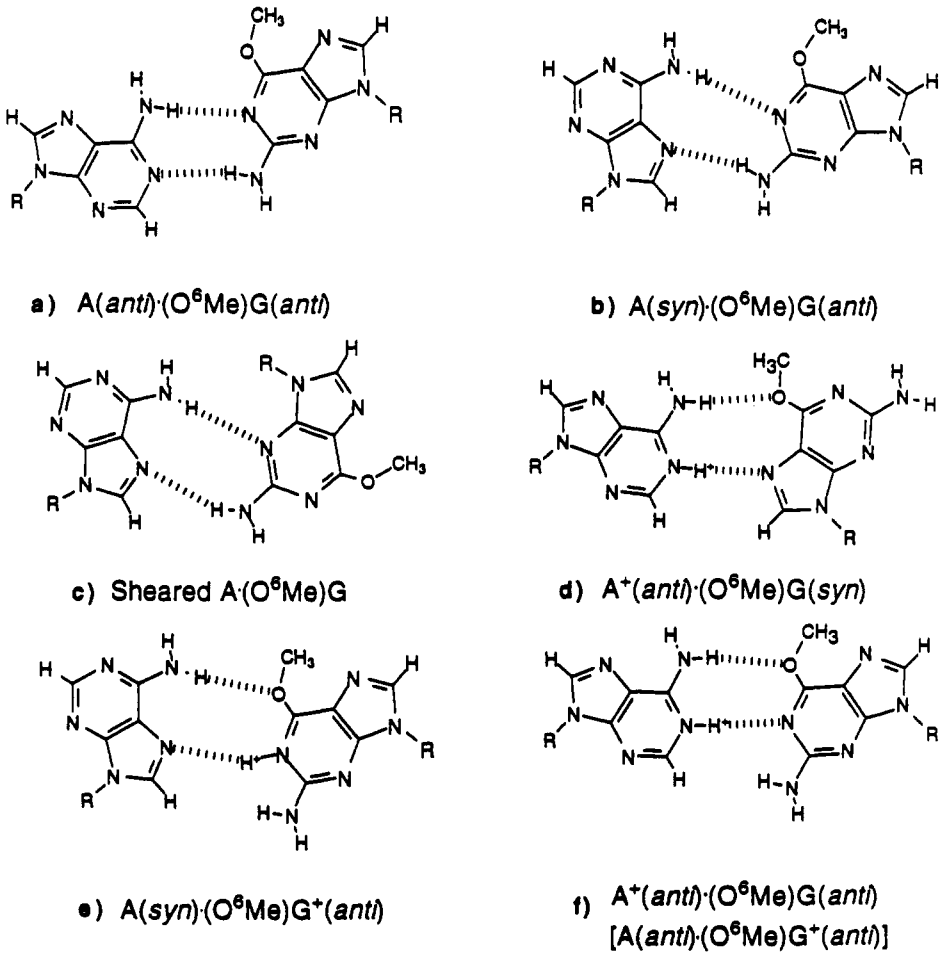

FIGURE 2: Possible hydrogen-bonding schemes for the A·(O^6Me)G base pair, assuming the major tautomers and the possibility of N1 protonation: (a) A(*anti*)·(O^6Me)G(*anti*); (b) A(*syn*)·(O^6Me)G(*anti*); (c) sheared A·(O^6Me)G; (d) A$^+$(*anti*)·(O^6Me)G(*syn*); (e) A(*syn*)·(O^6Me)G$^+$(*anti*); (f) A$^+$(*anti*)·(O^6Me)G(*anti*) or. A(*anti*)·(O^6Me)G$^+$(*anti*).

Table 2: Hydrogen-Bonding Geometry in the Title and Related Structures[a]

sequence	base-pair geometry	donor	acceptor	d_{av}	reference
d(CGAAGATTGG)$_2$	A(*anti*)·G(*anti*) (1a)	N6 A	O6 G	3.01	Privé *et al.*, 1987
		N1 G	N1 A	2.91	
d(CGCAAGCTGGCG)$_2$	A(*syn*)·G(*anti*) (1b)	N6 A	O6 G	2.64	Webster *et al.*, 1990
		N1G	N7 A	3.10	
d(CGCGAATTAGCG)$_2$	A(*syn*)·G(*anti*) (1b)	N6 A	O6 G	2.74	Brown *et al.*, 1986
		N1 G	N7 A	2.83	
d(CGCAAATTGGCG)$_2$	A$^+$(*anti*)·G(*syn*) (1d)	N6 A	O6 G	2.56	Brown *et al.*, 1989
		N1 A	N7 G	2.82	
d(CGC(O^6Me)GAATTTGCG)$_2$	T(*anti*)·(O^6Me)G(*anti*)	N2 G	O2 T	2.69	Leonard *et al.*, 1990
		N3 T	N1 G	2.96	
d(CGAGAATTC(O^6Me)GCG)$_2$	A(*syn*)·(O^6Me)G(*anti*) (2b)	N2 G	N7 A	2.65	this work
		N6 A	N1 G	2.95	

[a] The mispaired bases are underlined in the sequence. The geometry of the bases in the A·G mispair is given. The numbers in parentheses refer to diagrams in Figures 1 and 2. Donor and acceptor atoms in the mispair are listed. The average hydrogen bond lengths (in Å) are given in column d_{av}.

better than does that of A3·(O^6Me)G22. Omit maps calculated without the methyl groups clearly showed the positions of these groups.

The final *R*-factor is 16.9% for 1031 reflections [$F > 2\sigma(F)$] in the resolution range 8–2.9 Å. Table 1 gives the refinement statistics including the restraints employed. Coordinates have been deposited with the Protein Data Bank.

RESULTS AND DISCUSSION

The title compound forms an antiparallel double helix, with overall features similar to those of the parent dodecamer structure d(CGCGAATTCGCG)$_2$ (Figure 4). The rms between the two structures is 0.78 Å with all the mispaired bases omitted from the calculation. As in the other isomorphous dodecamer structures, the helices are oriented along the *c* cell axis and the bending per 12 base pairs is 19°. The minor-groove widths, computed as the inter-phosphorus distance minus the sum of the van der Waals radii, are slightly shorter except at the (O^6Me)G10·A15 position (Figure 5).

There are two A·(O^6Me)G pairs in the third and tenth positions, respectively. The conformation of both mismatched base pairs is A(*syn*)·(O^6Me)G(*anti*) (Figure 2b). This conformation does not require any protonation to form the two hydrogen bonds. A summary of the essential features of

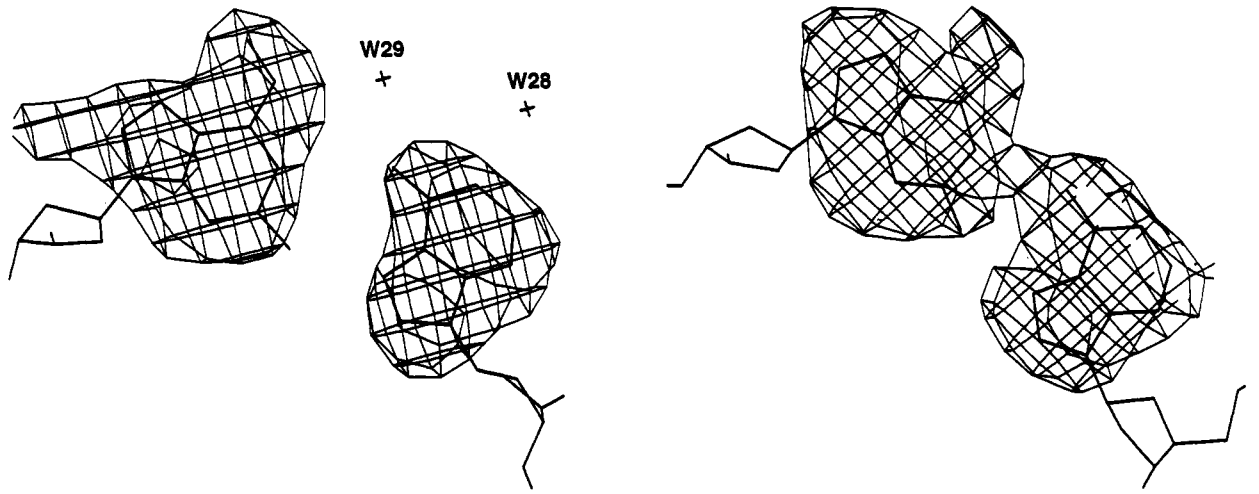

FIGURE 3: Difference $F_o - F_c$ maps in which A15 and (O^6Me)G10 (left) or A3 and (O^6Me)G22 (right) were omitted from the structure during the refinement. The water molecules around the base pairs are labeled.

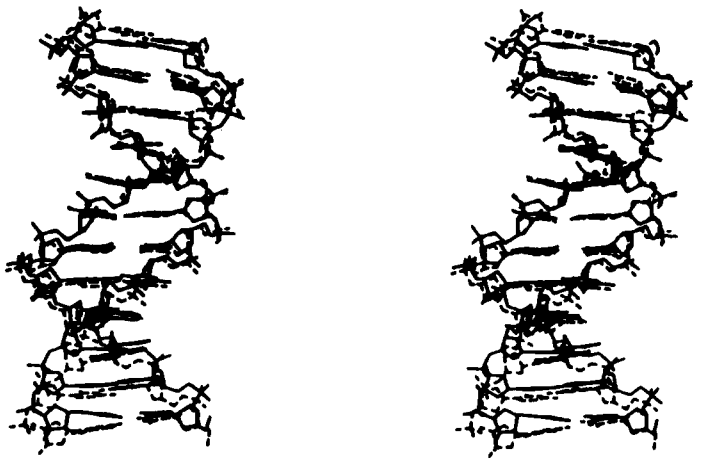

FIGURE 4: Comparison of the title structure (solid line) with the parent dodecamer (Drew *et al.*, 1981) (dashed line).

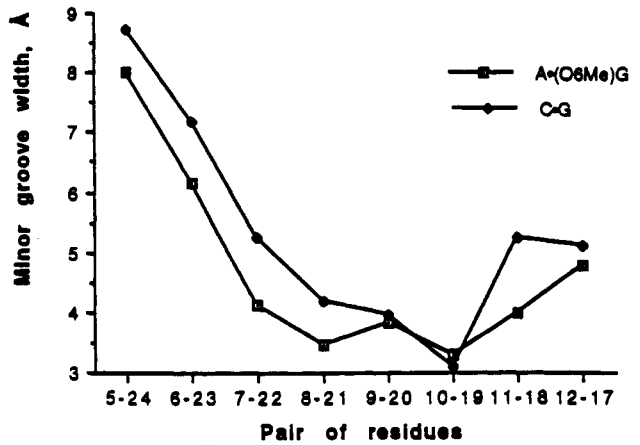

FIGURE 5: Minor groove widths (computed as a distance between phosphorus atoms less than 5.8 Å) for the title and the parent structure.

the observed conformation, as compared with other related structures with unmodified A·G mismatches, is given in Table 2. Although the conformations of the bases in this mispair are the same as in unmodified A(*syn*)·G(*anti*) pairs, the hydrogen-bonding donor and acceptor atoms are different. In the unmodified A·G pairs, the adenine sites are N7 and N6 and the guanine sites are N1 and O6. This type of hydrogen bonding is precluded in the modified pair because the N1 site of guanine is not protonated. Thus, N2 (G) donates to N7 (A), and N1 (G) accepts a hydrogen bond from N6 (A). This has the overall effect of shifting the O6 and its methyl group further into the major groove. Both methyl groups point away from the hydrogen bond and are almost coplanar with the base pair; the bonds between O6 and C6M make angles of 6°and 8° with the mean plane of guanine.

Table 3: Intermolecular Contacts (Å) in the Title Structure and the Parent Dodecamer (Drew *et al.*, 1981)[a]

atom *i*		atom *j*				
atom name	residue name	atom name	residue name	sym[b]	$d(i-j)$	$d_P(i-j)$
O3′	G24	N3	G16	i	2.71	2.99
O3′	G24	O4′	A17	i	3.26	3.15
N2	G22	O3′	G12	i	2.96	2.27
N2	G2	N3	G12	iii	3.36	2.91
N2	G12	N3	G2	iii	3.36	2.76
N2	G14	N3	G24	i	3.12	3.04
N2	G24	N3	G14	i	3.38	3.17
N2	G24	O4′	A15	i	3.08	3.47
O5′	C1	O1P	T7	ii	2.83	>4.0
O5′	C1	O2P	T7	ii	2.96	>4.0
O5′	C1	O5′	T7	ii	2.90	>4.0
O5′	C1	O1P	T8	ii	>4.0	2.73
O5′	C13	O2P	A3	iii	3.09	>4.0

[a] Distance $d(i-j)$ is the hydrogen bond length (in Å) between the possible acceptor and donor in the title structure, and $d_P(i-j)$ is the comparable distance in the parent dodecamer. Sym is the symmetry operation of atom *j*. [b] Symmetry operations: (i) $-x+1.5, -y+1.0, z-0.5$; (ii) $-x+1.0, y+0.5, -z+0.5$; (iii) $-x+1.5, -y+1.0, z+0.5$.

Both mispaired adenines exhibit high propeller twist. However, unlike one of the other structures with A(*syn*) conformations (Webster *et al.*, 1990) there are no three-center hydrogen bonds nor are there any other non-base-pairing contacts. The base-pair morphology was calculated with a program based on the algorithm developed by Babcock and Olson (1992). As shown in Figure 6, one difference between this structure and the parent dodecamer is that the mismatched base pairs open into the major groove as indicated by the values for "open" at those sites. The other significant differences in base geometry occur at the end residues.

Figure 7 shows the distribution of conformation angles in this structure. Although at this resolution the torsion angles are not well determined, especially in the backbone region, there are some features that are well-defined. The χ angles are 35° (*syn*) and 71° (*high syn*) for the A3 and A15, respectively. In addition, there are angles which take on some values that are not within the usual ranges and may be attributed to a combination of end effects and poor resolution.

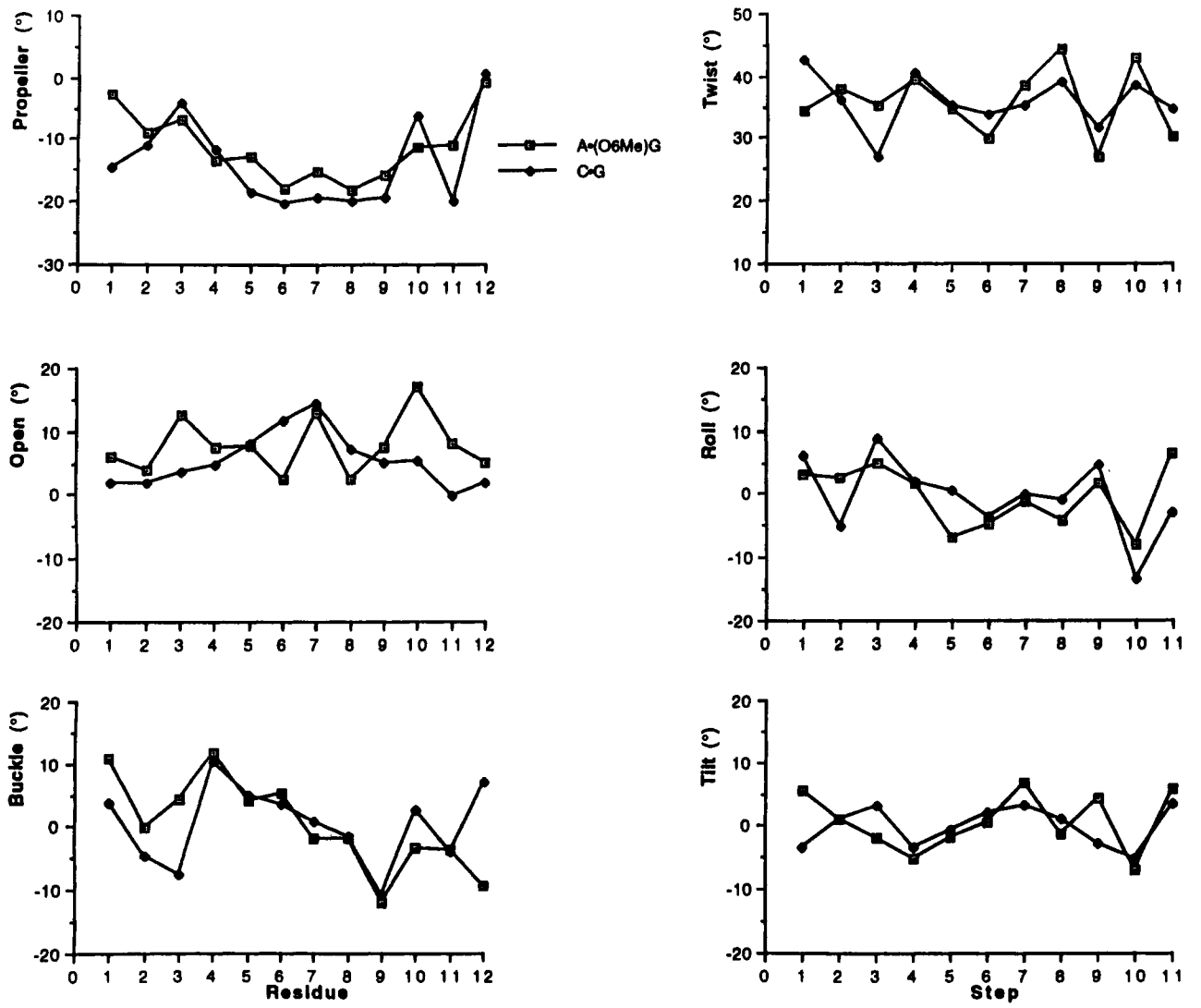

FIGURE 6: Base morphology parameters compared with the parent structure (Drew *et al.*, 1981), computed by the program of Babcock and Olson (1992). The solid points are the values for the parent structure and the open squares are for the title structure.

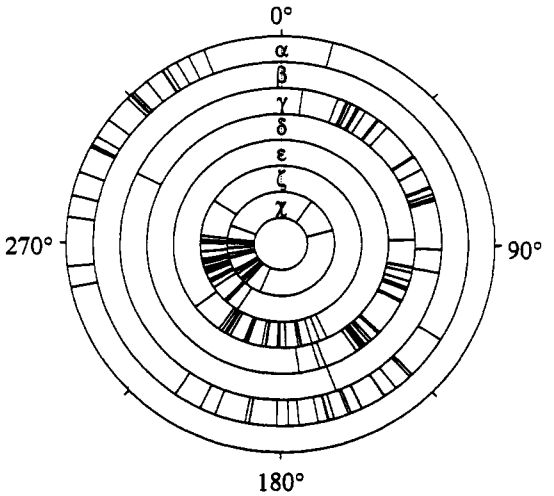

FIGURE 7: Conformational rings of torsion angles α, ..., ζ (deg) of the sugar phosphate backbone and the glycosidic torsion angles χ (deg).

The conformational angles (α, γ, and δ) for the terminal G12 residue have values of 14°, –60°, and 151°, respectively. The values of γ that define the position of the terminal O5′ for residues C1 and C13 are different than those of the parent. These give rise to intermolecular contacts not seen in the parent duplex.

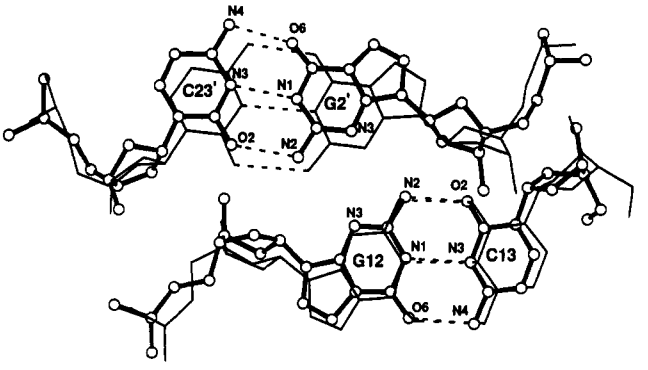

FIGURE 8: Comparison of the intermolecular contacts between G12 and G2 in the title structure (ball and stick model) and the parent structure (Drew *et al.*, 1981) (solid line).

The intermolecular contacts observed in this structure are compared with those observed in the parent structure in Table 3. For the most part they are quite similar. It is significant that the hydrogen bonds observed in the parent between the N2 and N3 of guanine G2 and the terminal G12 in a symmetry-related molecule are significantly longer as illustrated in Figure 8. A possible interpretation for this may be explained by the stacking of the mispaired bases in the helix. In the parent dodecamer, the C3 stacks on top of the G2 with the N3 (C3) centered over the six-membered ring of the G2 (see Figure

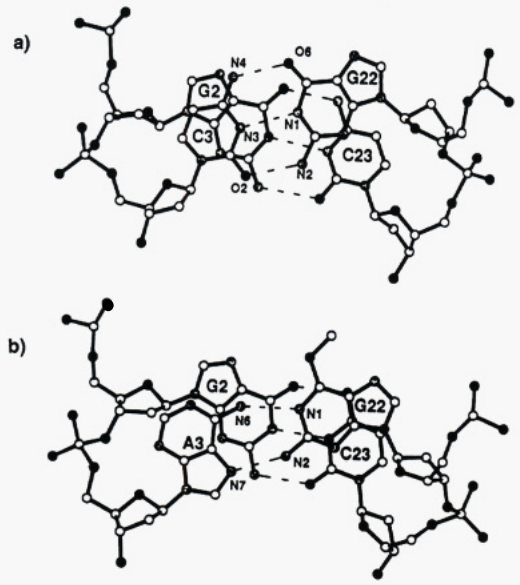

FIGURE 9: Perpendicular view of stacking between G2·C23 and C3·G22 in the parent structure (Drew *et al.*, 1981) (a) and between base pairs G2·C23 and A3·(O^6Me)G22 in the title structure (b). Oxygen atoms are shown as filled circles, and the nitrogen atoms are stippled.

9a). In the modified structure, the N6 of the A3 (*syn*) tends to be centered over the six-membered ring of the G2 (see Figure 9b). If that A3 were to have the same orientation with respect to the G2 as in the parent, this N6 (A3) would not be so favorably stacked onto the G2. The displacement of this G2 to accommodate the stacking interaction puts it in an unfavorable hydrogen-bonding position. The stacking of the other mispaired adenine A15 (*syn*) over the G14 is equivalent and has also some effect on intermolecular contacts between G14 and G24. The possible absence or weakening of these hydrogen bonds may contribute to the poor quality of the crystals.

Although the resolution of the structure is relatively low, a significant number of solvent molecules are observed in the electron density maps. Of the 49 solvent molecules, 5 are in the minor groove, 12 are in the major groove, and 32 are around the backbone. Two solvent molecules (W28 and W29) are close to the mismatched base pair A15·(O^6Me)G10 (Figure 3a). W28 is 2.59 Å from the N1 of A15. W29 is 2.56 Å from the N6 of A15 and 3.06 Å from the O6 of G10. Thus, W29 serves as a bridge between the paired bases. Both molecules are very close to the mean plane of the adenine; the deviations are 0.08 and 0.13 Å for W28 and W29, respectively. Although similar peaks were found close to the second mispair, the fit was generally worse, and these solvent molecules were not included in the final structure.

Examination of other mispaired structures shows that water molecules are often found at sites normally occupied by Watson–Crick base-paired mates. For example, in the crystal structure of d(CGCGAATTAGCG)$_2$ which contains an unmodified A(*syn*)·G(*anti*) mismatch (Brown *et al.*, 1986) (Figure 1*b*), there are water molecules hydrogen bonded to both N1 and N6 positions. In B-DNA and Z-DNA structures containing G·T mismatches (Ho *et al.*, 1985; Hunter *et al.*, 1987) and an A-DNA structure containing an I·T mismatch (Cruse *et al.*, 1989), there are water bridges on the major-groove side interconnecting the O4 (T) and the O6 and N7 of the mispaired guanine. In the G·T structures, there is another bridge on the minor-groove side connecting the N2 of guanine and the O2 of the thymine. Although there are too few examples of mispaired structures to make a systematic

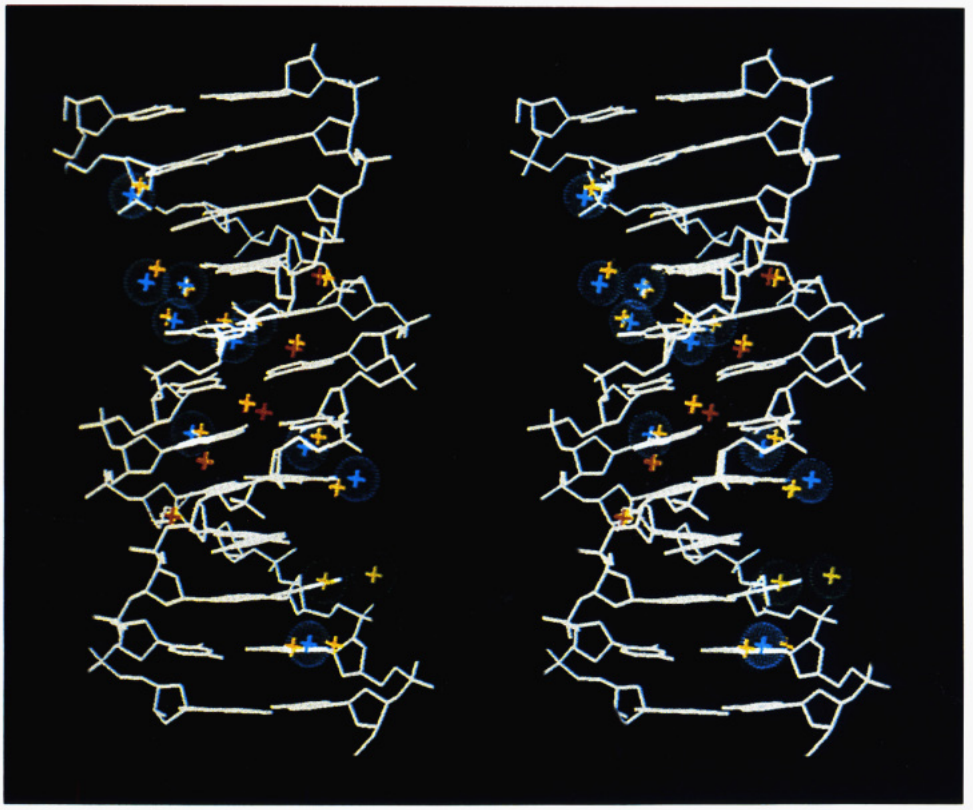

FIGURE 10: Experimentally determined solvent molecules in the minor groove (red spheres) and in the major groove (blue and green spheres) compared to those predicted (crosses) (Schneider *et al.*, 1993). The major-groove solvent molecules close to the A15(*syn*) with no predicted equivalent are displayed as green spheres.

study of their hydration structures, it is clear that a trend is emerging that may have important implications with regard to recognition of these sites.

In this structure, the O^6-methyl group disrupts the solvent network normally found on the major-groove side of guanines whereby two water molecules are hydrogen bonded to N7 and O6. On the other hand, all the solvent molecules observed around those bases not involved in mispairing agree with those predicted in a systematic study of nucleic acid hydration (Schneider *et al.*, 1993) (see Figure 10). These observations of the solvent structure around both the mispaired and normal Watson–Crick sites serve to give further credence to the concept that the water molecules around the bases are an integral part of DNA structures.

The structure of this duplex has also been studied by ^{1}H and ^{31}P NMR (Patel *et al.*, 1986a). The ^{1}H–^{1}H NOEs observed demonstrated that both bases of the A·(O^6Me)G pair were stacked in the helix and that each maintained an *anti* conformation, in contrast to the A(*syn*)·(O^6Me)G(*anti*) conformation found in this crystal structure. Further, although the individual ^{31}P resonances were not assigned, the unusually large dispersion of the ^{31}P resonances was noted. Such a large spectral range would be consistent with the distortion of the phosphodiester backbone that would be expected to result from an A(*anti*)·(O^6Me)G(*anti*) base pair, i.e., either scheme a or scheme f from Figure 2, although there was no direct evidence from the NMR study on specific details of the base pairing. Such an A(*anti*)·(O^6Me)G(*anti*) base pair is isoelectronic with a C(*anti*)·(O^6Me)G(*anti*) pair, one example of which we have shown exists in a Watson–Crick orientation, analogous to that of Figure 2, scheme f. Moreover, there is evidence from an ongoing ^{15}N NMR study of a pH-dependent conformational transition between these two types of C·(O^6-Me)G pairs (Gaffney *et al.*, unpublished results).

The results found in this X-ray analysis may indicate that A·(O^6Me)G pairs, like A·G pairs, have a variety of available base-pair geometries. It should be noted that there is no apparent reason why the A(*anti*)·(O^6Me)G(*anti*) conformation of this duplex that is present in solution could not exist in the crystal. However, given that the mispairs are adjacent to bases that are involved in intermolecular contacts, it is possible that the *syn–anti* conformation may be related to crystal packing. Experiments in which the context of this mispair as well as the solution and crystalline environments are varied would be necessary to resolve this.

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