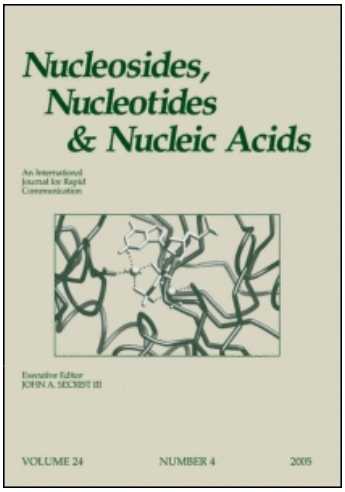

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

NMR Studies of a DNA Containing 8-Methoxydeoxyguanosine

Yasushi Oda[a]; Seiichi Uesugi[a]; Masaya Orita[a]; Hideo Inoue[b]; Yasutoshi Kawase[b]; Eiko Ohtsuka[b]; Morio Ikeharay[c]
[a] Faculty of Pharmaceutical Sciences, Osaka University, Suita, Osaka, Yamadaoka [b] Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo [c] Protein Engineering Research Institute, Suita, Osaka, Japan

To cite this Article Oda, Yasushi , Uesugi, Seiichi , Orita, Masaya , Inoue, Hideo , Kawase, Yasutoshi , Ohtsuka, Eiko and Ikeharay, Morio(1992) 'NMR Studies of a DNA Containing 8-Methoxydeoxyguanosine', Nucleosides, Nucleotides and Nucleic Acids, 11: 2, 261 — 272
To link to this Article: DOI: 10.1080/07328319208021701
URL: http://dx.doi.org/10.1080/07328319208021701

NUCLEOSIDES & NUCLEOTIDES, 11(2-4), 261-272 (1992)

NMR STUDIES OF A DNA CONTAINING 8-METHOXYDEOXYGUANOSINE

Yasushi Oda, Seiichi Uesugi*, Masaya Orita, Hideo Inoue[1], Yasutoshi Kawase[1], Eiko Ohtsuka[1], and Morio Ikehara[2]

Faculty of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565, [1]Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060 and [2]Protein Engineering Research Institute, 6-23 Furuedai, Suita, Osaka 565, Japan

Abstract: ^{1}H NMR experiments have been undertaken to elucidate the structural effects of methoxy substitution at the C8 of a deoxyguanosine residue in a self-complementary dodecadeoxyribonucleotide, d(C-G-C-mo^{8}G-A-A-T-T-C-G-C-G), duplex, which has an 8-methoxy-2'-deoxyguanosine (mo^{8}dG) residue at the 4th position. NMR data indicate that the mo^{8}dG residue takes an *anti* glycosidic conformation in a mo^{8}dG(*anti*):dC(*anti*) base-pair structure in a B-form duplex. The thermal stability of the duplex is reduced, but the overall structure is much the same as that of the unmodified d(C-G-C-G-A-A-T-T-C-G-C-G) duplex.

INTRODUCTION

Nucleosides and nucleotide monomers containing the normal bases, A, G, C, and T (U), take an *anti* glycosidic conformation (1). The normal nucleotide residues in DNA and RNA duplex also generally take an *anti* conformation (1). In contrast, it has been

---

This paper is dedicated to the memory of Professor Tohru Ueda.

shown that purine nucleoside and nucleotide monomers with a bulky substituent at the C8 position are in a *syn* conformation (2), because of steric hindrance between the bulky C8-substituent and the sugar moiety.

We have recently reported a structural study of a self-complementary dodecadeoxyribonucleotide, d(C-G-C-oh^{8}G-A-A-T-T-C-G-C-G) (designated oh^{8}GC12-mer), a duplex containing a C8-substituted deoxyguanosine, 8-hydroxy-2'-deoxyguanosine (oh^{8}dG) (3). The results indicated that the oh^{8}dG residue takes an *anti* glycosidic conformation with a oh^{8}dG(*anti*):dC(*anti*) base pair in the DNA duplex. In contrast, it has been reported that oh^{8}dG monomer favors a *syn* conformation (4), as generally observed in C8-substituted purine nucleosides. The 8-hydroxylation caused destabilization of the duplex, which may be due to steric hindrance between the 8-substituent and the sugar in oh^{8}dG(*anti*). In other words, the unfavorable monomer conformation, an *anti*, is compensated for by the favorable duplex conformation. It should be noted that the 8-hydroxy-deoxyguanosine takes a 6,8-diketo tautomeric form in the base moiety (3-5); it may be called alternatively 8-oxo-7,8-dihydro-deoxyguanosine. So the substituent at C8 is actually an oxo group.

8-methoxy-2'-deoxyguanosine (mo^{8}dG) has a methoxy group, which is bulkier than the oxo group of oh^{8}dG, at C8 of deoxyguanosine. For this paper, we examined the conformation of the mo^{8}dG in a self-complementary dodecadeoxyribonucleotide, d(C1-G2-C3-mo^{8}G4-A5-A6-T7-T8-C9-G10-C11-G12) (designated mo^{8}GC12-mer), duplex by ^{1}H NMR. The modification site is the dG at the 4th position opposite dC at the 9th position in the duplex, as in the oh^{8}GC12-mer. The parent oligonucleotide, d(C-G-C-G-A-A-T-T-C-G-C-G) (designated GC12-mer), has been well studied by NMR (6-8) and X-ray crystallography (9); it takes a B-form double helical structure. We also examined the effects of the 8-methoxylation of the deoxyguanosine on the base-pairing structure, the duplex structure, and the thermal stability, compared with the effects of the 8-hydroxylation.

MATERIALS AND METHODS

The oligonucleotide d(C-G-C-mo^{8}G-A-A-T-T-C-G-C-G) was synthesized by the phosphotriester method in solution using 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole as the condensing reagent (10). N^2-acetyl-8-methoxy-5'-*O*-monomethoxytrityl-2'-deoxyguanosine 3'-(*o*-chlorophenyl) phosphate was prepared by phosphorylation of the protected nucleoside (Inoue, H., *et al.*, paper in preparation) and condensed with 3'-(*o*-chlorophenyl)-β-cyanoethyl phosphate derivatives (11) of *N*-protected deoxynucleosides. The protected oligonucleotide was deblocked, purified and analyzed by essentially the same methods as previously described (12). The protected oligomer was deblocked by treatment with 1,1,3,3-tetra-methylguanidinium 2-pyridinealdoximate and with ammonium hydroxide to yield the corresponding 5'-dimethoxytritylated dodecamer, which could be separated by reversed-phase chromatography, and then was treated with acetic acid to yield the completely deblocked product. The 8-methoxy group was stable under these conditions. The oligomer was also isolated by reversed-phase chromatography. The purified oligonucleotide was desalted by gel filtration on Sephadex G-10 and converted to the sodium salt with an ion exchange column, and then lyophilized. The lyophilized oligonucleotide was dissolved in H_2O-D_2O (4:1) or in D_2O containing 0.1 M NaCl, 10 mM sodium phosphate buffer (pH 6.8). The oligonucleotide concentration of the NMR sample was 180 A_{260} units/0.4 ml (3.7 mM).

All the NMR experiments were done on a JEOL GX500 spectrometer (500 MHz for ^{1}H). ^{1}H chemical shifts were measured relative to internal 2-methyl-2-propanol (1.23 ppm). ^{1}H NMR spectra in H_2O were obtained with a 1-1 pulse sequence (13) to suppress the H_2O signal. The NOE difference spectrum represents the spectrum with an off-resonance preirradiation pulse subtracted from the spectrum with an on-resonance preirradiation pulse. A single-frequency preirradiation pulse was applied for 0.3 s, giving an irradiated signal saturation of approximately 60%. Two-dimensional NOESY and DQF-COSY spectra in D_2O were recorded with 2048 points in t_2 and 256 points in t_1 (spectral

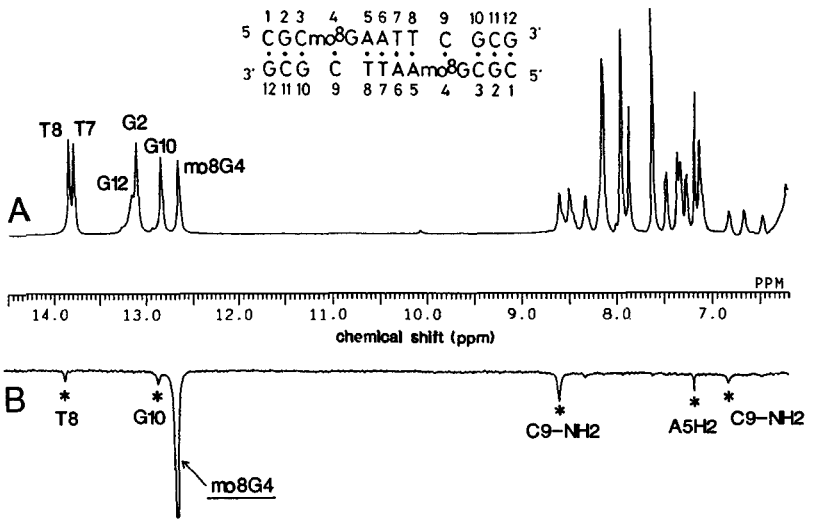

FIG. 1. ^1H NMR spectra in the imino proton region of mo^{8}GC12-mer in H_2O-D_2O (4:1) containing 0.1 M NaCl, 10 mM sodium phosphate buffer (pH 6.8) at 5°C. A: normal spectrum; B: NOE difference spectrum. The irradiated imino proton resonance is designated by an arrow while the observed NOE's are marked by asterisks. The numbering system for mo^{8}GC12-mer is also shown.

width, 5000 Hz each). The NOESY and DQF-COSY data were collected in the phase-sensitive mode by the method of States *et al.* (14). In the NOESY experiments, a mixing time of 150 ms was used. The time domain data were multiplied by an exponential window function in the t_1 direction and a Gaussian window function in the t_2 direction, and zero-filled to 1024 points in the t_1 dimension before Fourier transformation.

RESULTS AND DISCUSSION

mo^{8}dG:dC base pairing

The ^1H NMR spectrum in H_2O of mo^{8}GC12-mer duplex is shown in Fig. 1A. Six imino proton resonances are observed between 12.5 and 14.0 ppm at 5°C, suggesting that all the base residues are involved in hydrogen bonding to form a completely base-paired duplex. The imino proton resonances for mo^{8}GC12-mer were

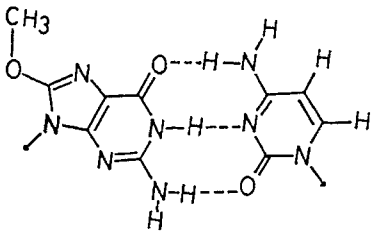

FIG. 2. Structure of the mo^{8}dG(*anti*):dC(*anti*) base pair.

assigned unambiguously by one-dimensional NOE experiments at 5°C, observing sequential interbase-pair NOEs. For instance, irradiation of the imino proton of mo^{8}G (mo^{8}G4N1H of mo^{8}G4:C9) at 12.65 ppm gives interbase-pair NOEs to the imino proton signals at 12.84 (G10N1H of C3:G10) and 13.84 (T8N1H of A5:T8) ppm and A5H2 at 7.20 ppm (Fig. 1B). A pair of intrabase-pair NOEs to the C9-NH_2 signals at 6.82 and 8.60 ppm are also observed in Fig. 1B. The observed chemical shifts and NOEs for the imino proton of mo^{8}G indicate the formation of a mo^{8}dG(*anti*):dC(*anti*) base pair in the mo^{8}GC12-mer duplex with a base-pair structure as shown in Fig. 2. In the mo^{8}dG(*anti*):dC(*anti*) structure, the imino proton of mo^{8}dG forms a hydrogen bond, and is close to the amino protons of dC. If mo^{8}dG residue takes a *syn* conformation in the duplex, mo^{8}dG should have an unpaired imino proton, which usually shows a chemical shift at around 10 ppm. Moreover, it is difficult to assume a possible base-pairing scheme for mo^{8}dG(*syn*):dC(*anti*).

Assignments for the nonexchangeable proton resonances

The assignments of the nonexchangeable proton resonances for the parent GC12-mer (7) and the oh^{8}GC12-mer (3) have been done using sequential NOE connectivities (NOESY) and scalar coupling connectivities (COSY). We have assigned the resonances for mo^{8}GC12-mer in the same way. For instance, the sequential NOE connectivities for the H8/H6-H1' cross-peaks are shown in Fig. 3. We could trace the NOE cross-peaks along the oligonucleotide chain as indicated with lines, and assigned the resonances

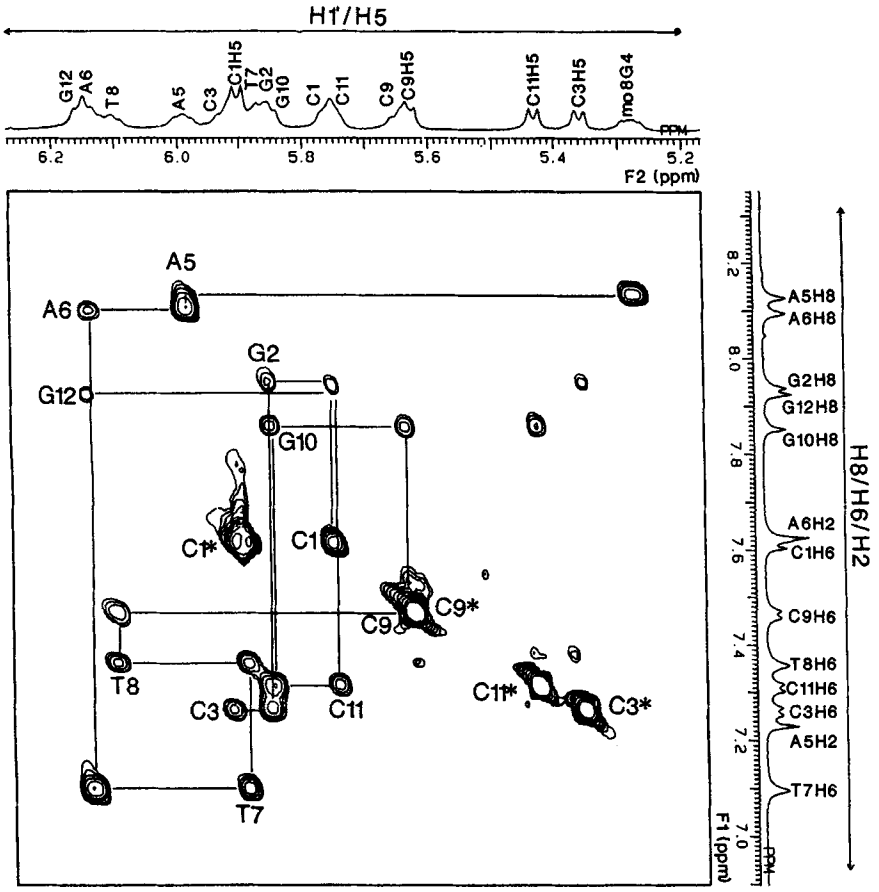

FIG. 3. Expanded contour plots of the NOESY spectrum (150-ms mixing time) of mo^{8}GC12-mer in D_2O containing 0.1 M NaCl, 10 mM sodium phosphate buffer (pH 6.8) at 25°C. The sequential connectivities from G1 to C3 and A5 to G12 through H8/H6-H1' cross-peaks are shown by continuous lines. The intraresidue cross-peaks are labeled. The deoxycytidine H5-H6 cross-peaks are designated by asterisks.

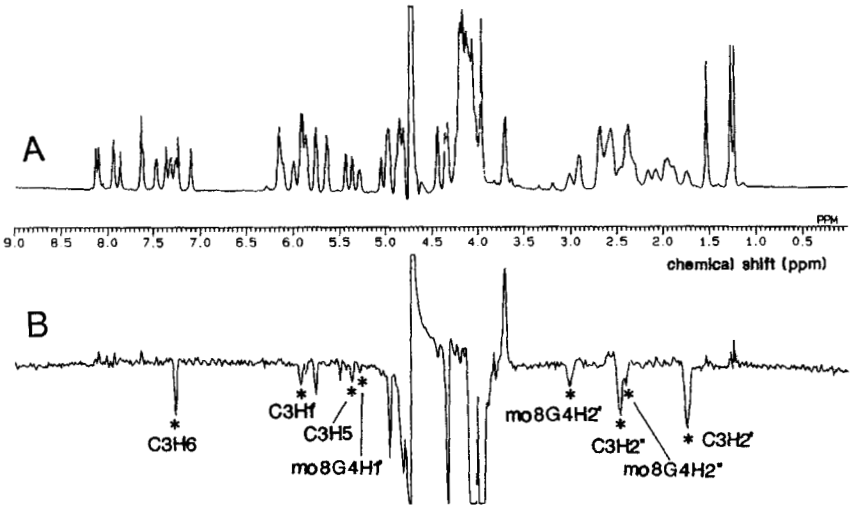

FIG. 4. A: ^{1}H NMR spectrum of mo^{8}GC12-mer. B: Slice data of the NOESY spectrum (150-ms mixing time) of mo^{8}GC12-mer at the frequency of methyl proton resonance of mo^{8}G4 (3.96 ppm). The observed NOE's for the methyl proton of mo^{8}G4 are marked by asterisks. The spectra were measured in D_2O containing 0.1 M NaCl, 10 mM sodium phosphate buffer (pH 6.8) at 25°C.

sequentially. The NOE connectivity was interrupted at the C3-mo^{8}G4 step, because the methoxy protons at the C8 position for the mo^{8}dG4 residue resonate at 3.96 ppm, which is out of the observing range in Fig. 3. One-dimensional slice data of the NOESY spectrum for the methoxy proton signal at 3.96 ppm of the mo^{8}dG4 residue is shown in Fig. 4B. We could observe the sequential NOE connectivities between the methoxy proton of the mo^{8}dG4 residue and the sugar H1'/H2'/H2" of its own and its 5'-flanking C3 residues.

All the nonexchangeable proton resonances except for H5'/H5" were unambiguously assigned by analysis of the NOESY and COSY spectra. The results are presented in Table 1. The chemical shift differences between the mo^{8}GC12-mer and the GC12-mer are also given in Table 1.

TABLE 1. Nonexchangeable proton chemical shifts for mo^{8}GC12-mer duplex in D_2O containing 0.1 M NaCl, 10 mM sodium phosphate buffer (pH 6.8) at 25°C.

	chemical shifts (ppm)								
residue	H8/H6	H2	H5	CH_3	H1'	H2'	H2"	H3'	H4'
C1	7.61 (0.02)		5.90 (0.06)		5.75 (0.04)	1.94 (0.05)	2.39 (0.04)	4.68 (0.03)	4.06 (-0.09)
G2	7.94 (0.02)				5.86 (0.02)	2.62 (0.03)	2.68 (0.00)	4.96 (0.05)	4.33 (0.02)
C3	7.31 (0.07)		5.36 (0.01)		5.92 (0.37)	1.74 (-0.07)	2.47 (0.25)	4.82 (0.04)	4.21 (0.04)
mo^{8}G4				3.96	5.28 (-0.14)	3.02 (0.39)	2.43 (-0.27)	4.85 (-0.13)	4.16 (-0.13)
A5	8.13 (0.04)	7.23 (0.00)			5.99 (0.03)	2.68 (0.03)	2.91 (0.01)	5.04 (-0.01)	4.44 (0.00)
A6	8.10 (0.01)	7.63 (0.02)			6.15 (0.02)	2.58 (0.05)	2.91 (-0.01)	4.99 (-0.01)	4.45 (0.01)
T7	7.10 (-0.01)			1.22 (-0.02)	5.89 (0.00)	1.98 (0.00)	2.55 (0.01)	4.79 (-0.03)	4.16 (-0.08)
T8	7.36 (0.01)			1.52 (0.01)	6.10 ((0.02)	2.17 (0.00)	2.56 (0.02)	4.88 (-0.01)	4.20 (-0.04)
C9	7.47 (0.03)	5.63 (0.02)			5.64 (-0.03)	2.08 (0.06)	2.40 (0.00)	4.85 (-0.02)	4.13 (-0.07)
G10	7.86 (-0.03)				5.86 (0.04)	2.62 (0.04)	2.68 (0.00)	4.97 (0.00)	4.36 (0.01)
C11	7.26 (-0.04)	5.43 (0.02)			5.75 (0.02)	1.89 (0.04)	2.32 (0.04)	4.80 (0.01)	4.13 (-0.07)
G12	7.93 (0.03)				6.15 (0.05)	2.59 (0.02)	2.37 (0.03)	4.67 (0.01)	4.14 (-0.06)

For each proton, the number in parenthesis refers to the chemical shift differences ($\Delta\delta$) between mo^{8}GC12-mer duplex (a) and GC12-mer duplex (b), defined as $\Delta\delta = \delta(a) - \delta(b)$.

Glycosidic conformation for the mo^{8}dG residue

The glycosidic conformation, *syn* or *anti*, can be identified by the NOESY experiment in D_2O for nonexchangeable protons (15). The methoxy proton of mo^{8}dG4 had larger intraresidue NOE with H2' than those with the other sugar protons (Fig. 4B), indicating that the mo^{8}dG4 takes an *anti* glycosidic conformation. It has now been confirmed that a mo^{8}dG(*anti*):dC(*anti*) base pair is indeed formed in the mo^{8}GC12-mer duplex.

We have reported that 8-hydroxy-2'-deoxyguanosine takes an *anti* glycosidic conformation in the oh^{8}dG(*anti*):dC(*anti*) base pair in a DNA duplex (3). In contrast, a *syn* glycosidic conformation is generally favorable in 8-substituted purine nucleosides, including 8-methoxy- and 8-hydroxy-guanosine (2). oh^{8}dG and even mo^{8}dG with a bulkier substituent positioned opposite dC take an *anti* conformation in the duplex.

Effects on the duplex structure

The imino protons resonated between 12-14 ppm (see Fig. 1A) had intrabase-pair NOEs between GN1H and C-NH_2 or between TN3H and AH2 (data not shown), indicating that all the dG:dC and dA:dT base pair structures are normal Watson-Crick type in the mo^{8}GC12-mer duplex (17). The duplex structure was further examined by analysis of the ^{1}H NMR spectra in D_2O for nonexchangeable protons. It has been reported that the parent GC12-mer takes a B-form double helical structure both in solution (6-8) and crystal (9). The pattern of the NOE connectivities for the H8/H6-H1' region for the mo^{8}GC12-mer duplex (Fig. 3) was similar to that for the parent GC12-mer duplex (7) and the oh^{8}GC12-mer duplex (3), suggesting similar B-form structures for these three duplexes.

Between mo^{8}GC12-mer and GC12-mer (Table 1), significant chemical shift differences (> 0.1 ppm) are only observed for some sugar protons of C3 and mo^{8}G4 residues, such as H1' and H2" for the C3 residue and H1', H2', H2", H3', and H4' for the mo^{8}G4 residue. We observe the same phenomena between oh^{8}GC12-mer and GC12-mer (3). The chemical shift differences are caused not only by conformational changes but also by changes caused by the

substitution at C8 of the deoxyguanosine. The observed chemical shift differences for mo^{8}GC12-mer and oh^{8}GC12-mer suggest that the structural changes, if any, are localized near the modified site and the global structures are much the same for these three duplexes.

The downfield shift of mo^{8}G4H2' (0.39 ppm) is remarkable. We also observe such a downfield shift on the H2' of oh^{8}G in oh^{8}GC12-mer duplex (3). A downfield shift of H2' is generally observed when purine nucleosides or nucleotides take a *syn* glycosidic conformation, where the lone pair electrons on N3 come close to the C2'-H2' bond of the C2'-*endo* sugar moiety (16). For the oh^{8}dG and mo^{8}dG taking an *anti* conformation, the lone pair electrons on oxygen of the C8-substituents may cause the downfield shift of H2'. The upfield shifts observed for some sugar protons of C3 and mo^{8}G4 residues may also be caused by the diamagnetic anisotropic effect of the C8-substituents.

Effects on the thermal stability

The melting temperature (Tm) of the mo^{8}GC12-mer duplex, estimated from the chemical shift-temperature profile of the T7H6 resonance, is 63°C (data not shown). We have already reported that the Tm of the GC12-mer duplex is 72°C and that of the oh^{8}GC12-mer duplex is 67°C under the same experimental conditions (3). Because of steric hindrance between the C8-substituent and the sugar moiety, the 8-substituted guanosines favor a *syn* conformation (2). In oh^{8}GC12-mer and mo^{8}GC12-mer, the 8-substituted guanosine residues take an unfavorable *anti* conformation to form a stable base pair. This can explain the lower duplex stability for oh^{8}GC12-mer and mo^{8}GC12-mer relative to GC12-mer. An *anti* conformation for a guanosine with a bulkier substituent may be less stable. This situation can explain the lower stability of mo^{8}GC12-mer with respect to that of oh^{8}GC12-mer. The present result confirms the notion that the unfavorable monomer conformation is compensated for by the favorable duplex conformation.

ACKNOWLEDGEMENT

This work was supported in part by a Grant-in-Aid for Specially Promoted Research from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

1. Saenger, W. (1984) *Principles of Nucleic Acid Structure*, Springer-Verlag, New York.
2. Uesugi, S. and Ikehara, M. (1977) *J. Am. Chem. Soc.* **99**, 3250-3253.
3. Oda, Y., Uesugi, S., Ikehara, M., Nishimura, S., Kawase, Y., Ishikawa, H., Inoue, H., and Ohtsuka, E. (1991) *Nucleic Acids Res.* **19**, 1407-1412.
4. Culp, S. J., Cho, B. P., Kadlubar, F. F., and Evans, F. E. (1989) *Chem. Res. Toxicol.* **2**, 416-422.
5. Kouchakdjian, M., Bodepudi, V., Shibutani, S., Eisenberg, M., Johnson, F., Grollman, A. P., and Patel, D. J. (1991) *Biochemistry* **30**, 1403-1412.
6. Patel, D. J., Kozlowski, S. A., Marky, L. A., Broka, C., Rice, J. A., Itakura, K., and Breslauer, K. J. (1982) *Biochemistry* **21**, 428-436.
7. Hare, D. R., Wemmer, D. E., Chou S.-H., Drobny, G. and Reid, B. R. (1983) *J. Mol. Biol.* **171**, 319-336.
8. Ott, J. and Eckstein, F. (1985) *Biochemistry* **24**, 2530-2535.
9. Dickerson, R. E. and Drew, H. R. (1981) *J. Mol. Biol.* **149**, 761-786.
10. Jones, S. S., Rayner, B., Reese, C. B., Ubasawa, A., and Ubasawa, M. (1980) *Tetrahedron* **36**, 3075-3085.
11. Broka, C., Hozumi, T., Arentzen, R., and Itakura, K. (1980) *Nucleic Acids Res.* **8**, 5461-5471.
12. Kawase, Y., Iwai, S., and Ohtsuka, E. (1989) *Chem. Pharm. Bull.* **37**, 599-601.
13. Clore, G. M., Kimber, B. J. and Gronenborn, A. M. (1983) *J. Magn. Reson.* **54**, 170-173.
14. States, D. J., Haberkorn, R. A. and Ruben, D. J. (1982) *J. Magn. Reson.* **48**, 286-292.

15. Patel, D. J., Kozolowski, S. A., Nordheim, A. and Rich, A. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1413-1417.
16. Tavale, S. S. and Sobell, H. M. (1970) *J. Mol. Biol.* **48**, 109-123.
17. Chou, S.-H., Hare, D. R., Wemmer, D. E., and Reid, B. R. (1983) *Biochemistry* **22**, 3037-3041.

Received 7/20/91
Accepted 11/7/91