tion of cyanohydrin **6** in THF at room temperature. After stirring for 2h, a standard work-up gave a mixture of erythro-7 and threo-7 in 42% yield.

threo-8-Oxa-7-cyano-1-azabicyclo[4.3.0]nonan-9-one (threo-7); ^{1}H-NMR δ 1.0-2.4 (m, 6H), 2.9 (m, 1H), 3.6-4.1 (m, 2H), 4.7 (d, J=5 Hz, 1H). **erythro-8-Oxa-7-cyano-1-azabicyclo[4.3.0]nonan-9-one (erythro-7)**; ^{1}H-NMR δ 1.0-2.3 (m, 6H), 2.8 (m, 1H), 3.5-4.2 (m, 2H), 5.2 (d, J=8 Hz, 1H).

General Procedure for the Preparation of Aminocyanide 8. A solution of cyanohydrin **3** in methanol was cooled and saturated with ammonia gas. After stirring for 2 days at room temperature, the excess ammonia was expelled by N_2, and the methanol was evaporated. **threo-*N*-Benzyl-α-amino-2-piperidineacetonitrile (threo-8b**, 35%); ^{1}H-NMR (2M DCl/D_2O) δ 1.3-2.3 (m, 6H), 2.8-3.3 (m, 2H), 3.8 (m, 1H), 4.1, 4.6 (ABq, J=12 Hz, 2H), 5.4 (d, J=3.5 Hz, 1H), 7.25 (s, 5H). **erythro-*N*-Benzyl-α-amino-2-piperidineacetonitrile (erythro-8b**, 17%); ^{1}H-NMR (2M DCl/D_2O) δ 1.3-2.3 (m, 6H), 2.8-3.3 (m, 2H), 3.85 (m, 1H), 4.15, 4.65 (ABq, J=12 Hz, 2H), 5.25 (d, J=6.5 Hz, 1H), 7.30 (s, 5H). ***N*-Benzyloxycarbonyl-α-amino-2-piperidineacetonitrile (diastereoisomer, 8b**, 29%); ^{1}H-NMR δ 1.2-2.1 (m, 8H), 2.4-3.1 (m, 1H), 3.8-4.7 (m, 3H), 5.1 (s, 2H), 7.3 (s, 5H).

NMR Studies of the Psoralen Photobinding Sites of the d(GGGTACCC) and d(GGGATCCC) Duplex in Solution

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The interactions between psoralen and DNA take place in the formation of covalent photobinding of complexed psoralens to pyrimidine bases of DNA.[1,2] There is an optimal site for these interactions. Chart I shows the sequences of the two octamers which are referred to as 5′-TA or 5′-AT where they are distinguished from each other by the difference in their sequences. Although it is generally known that both 5′-TA and 5′-AT sequences are psoralen-DNA interstrand photo-cross-linked sites,[3,4] it has been found that 5′-TA sequences are highly preferred over 5′-AT sequences.[5,6] The

5′-TA	5′-AT
GGGTACCC	GGGATCCC
12344321	12344321
CCCATGGG	CCCTAGGG

Chart 1.

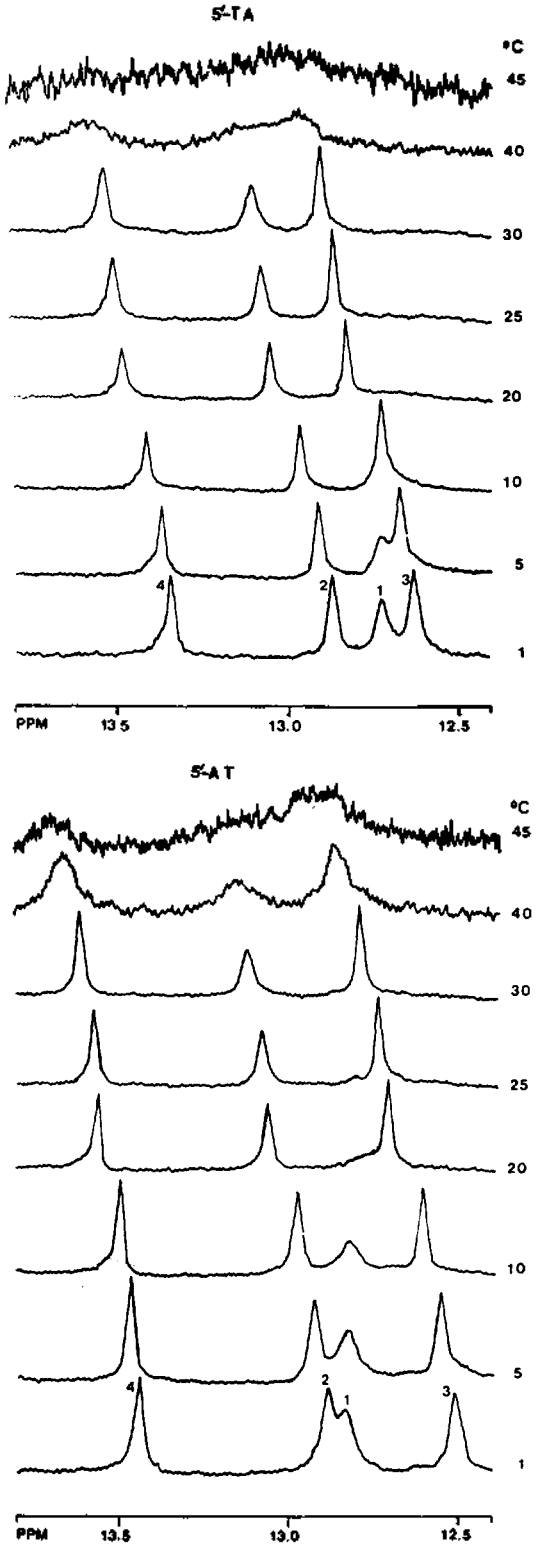

Figure 1. The 500-MHz imino proton spectra of the d(GGGTACCC)(left) and d(GGGATCCC)(right) in 2 mM phosphate, 200 mM NaCl, H_2O, pH 7 between 1 and 45℃. The imino proton assignments to specific base pairs are designated over the resonances.

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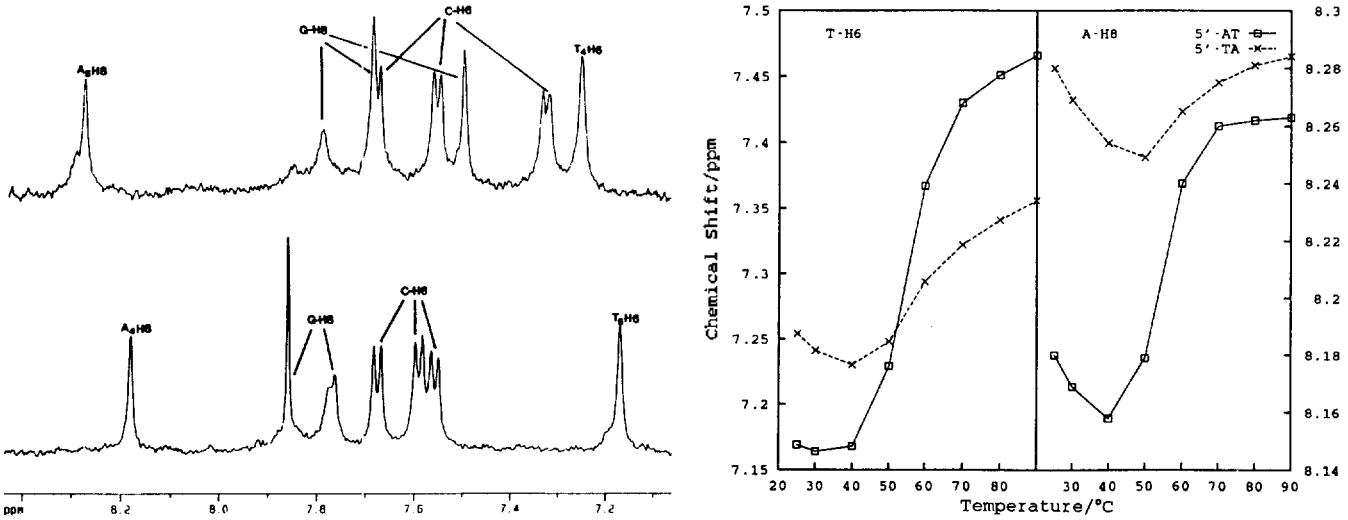

Figure 2. (A) The 500-MHz aromatic proton spectrum of the d(GGGTACCC)(top) and d(GGGATCCC)(bottom) in 20 mM phosphate, 200 mM NaCl, D_2O, pH 7 at 25℃, (B) Temperature dependence of the proton chemical shifts of thymidine H6(left) and adenosine H8(right) protons at position 4 of the two octamers in 20 mM phosphate, 200 mM NaCl, D_2O, pH 7 between 25 and 90℃.

X-ray crystallographic studies[5] have shown that 5'-TA sequences are opened ~8° toward the minor groove while 5'-AT sequences are opened ~8° toward the major groove[7] and suggested that the intercalation of the psoralen hydrophobic edge to DNA duplex is occurred in the minor groove[8], indicating 5'-TA sites are considerably better oriented for thymine-psoralen-thymine cross linking. In this paper, we compare the dynamic behaviours of two synthetic DNA oligomers, d(GGGTACCC) and d(GGGATCCC), to characterize localized sequence-specific conformations of 5'-TA and 5'-AT sequences in solution using NMR technique.

The octamers d(GGGTACCC) and d(GGGATCCC) were synthesized on an Applied Biosystems DNA synthesizer using β-cyanoethyl phosphoramidite chemistry on a 2μ-mole scale. The oligonucleotides were purified by Sephadex G-25 gel filtration column chromatography. The samples were lyophilized and redissolved in 90% H_2O/10% D_2O. All NMR experiments were done on a Bruker AMX-500 spectrometer. The chemical shifts were referenced relative to the internal standard sodium 3-(Trimethylsilyl)-1-propanesulfonate(TSP).

The imino proton spectra of the 5'-TA and 5'-AT have been investigated as a function of temperature with the spectra between 1 and 45℃ plotted in Figure 1. There are four unique base pairs in the 5'-TA or 5'-AT sequene, and these are designated G·C base pairs 1-3 and A·T base pair 4 from the ends to the center of the duplex(see chart 1). These imino protons have been assigned from a combination of NOE measurements and observing temperature dependence of resonances. As shown in Figure 1, the imino protons of 5'-TA duplex broaden simultaneously above 40℃ while the imino protons of 5'-AT duplex is still maintained at that temperature, indicating that the hydrogen exchange rates of the imino protons of 5'-TA increase dramatically. In addition to this observation, early broadening and disappearence of the terminal base pair imino proton resonances of 5'-TA compared to the corresponding imino proton resonances of 5'-AT duplex strongly suggest that the 5'-TA duplex has more unstable hydrogen-bonded base pairs than those of 5'-AT duplex.

Spectra of the nonexchangeable base protons in the two octamers obtained at 25℃ are given in Figure 2(A). The resonances of G-H8, C-H6, and T-H6 protons in the two octamers were assigned by the sequential assignment procedure.[9] A plot of the chemical shift of these resonances as a function of temperature is given in Figure 2(B). The resonances of T-H6 and A-H8 protons in the 5'-TA duplex exhibit a large upfield shift with a temperature variation. By contrast, the same resonances of 5'-AT duplex have relatively a small variation in this temperature range. It is known that the magnetic shielding through space is both sequence and conformation dependent.[10] Therefore, the base protons of 5'-AT duplex are located close proximity to strong magnetic shielding centers, while those of 5'-TA duplex are not shielded by the ring current magnetic field.

In summary, we have observed different behaviours of the imino and aromatic ring protons of the two octamers with a small variation in their sequences and found them to be sequence specific. These experimental results support the crystallographic observations which result in subtle differences between 5'-TA and 5'-AT sequences. More detailed structural studies of the two octamers are under progress.

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