

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); $^1\text{H-NMR}$ 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)**; $^1\text{H-NMR}$ 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 Amino-cyanide 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%)**; $^1\text{H-NMR}$ (2M DCl/D₂O) 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%)**; $^1\text{H-NMR}$ (2M DCl/D₂O) 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-Benzyl-oxycarbonyl- α -amino-2-piperidineacetonitrile (diastereoisomer, 8b, 29%)**; $^1\text{H-NMR}$ 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 interst-

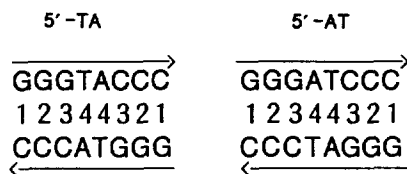


Chart 1.

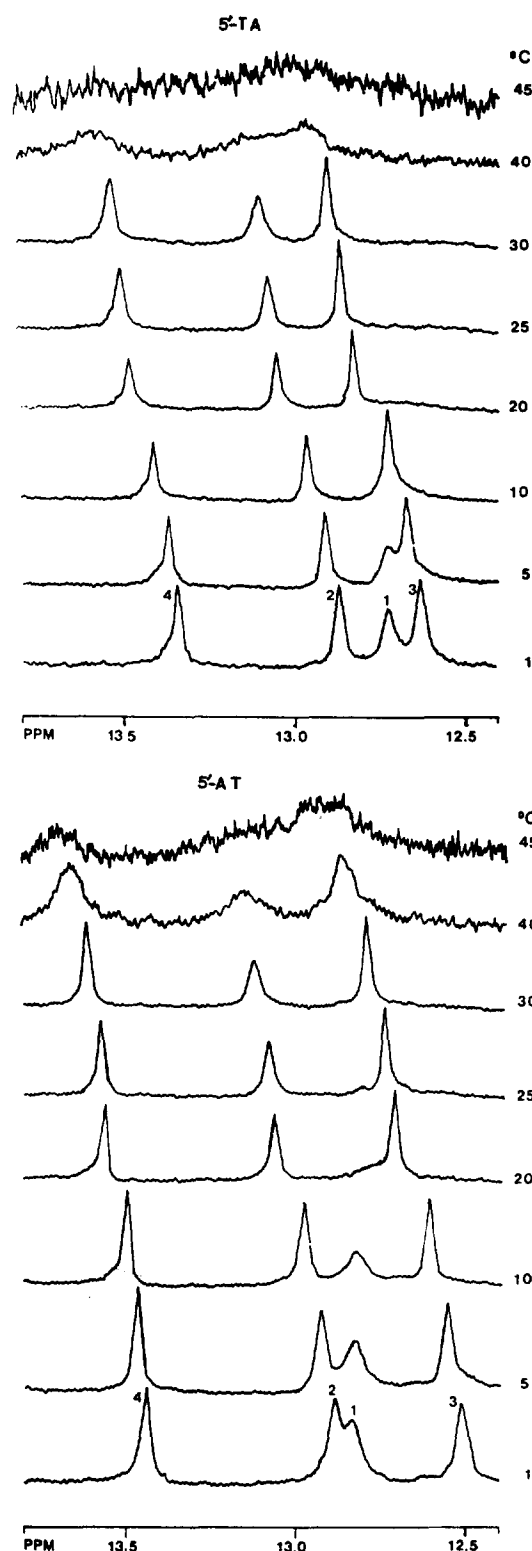


Figure 1. The 500-MHz imino proton spectra of the d(GGGTACCC)(left) and d(GGGATCCC)(right) in 2 mM phosphate, 200 mM NaCl, H₂O, pH 7 between 1 and 45°C. The imino proton assignments to specific base pairs are designated over the resonances.

rand photo-cross-linked sites,^{3,4} it has been found that 5'-TA sequences are highly preferred over 5'-AT sequences.^{5,6} The

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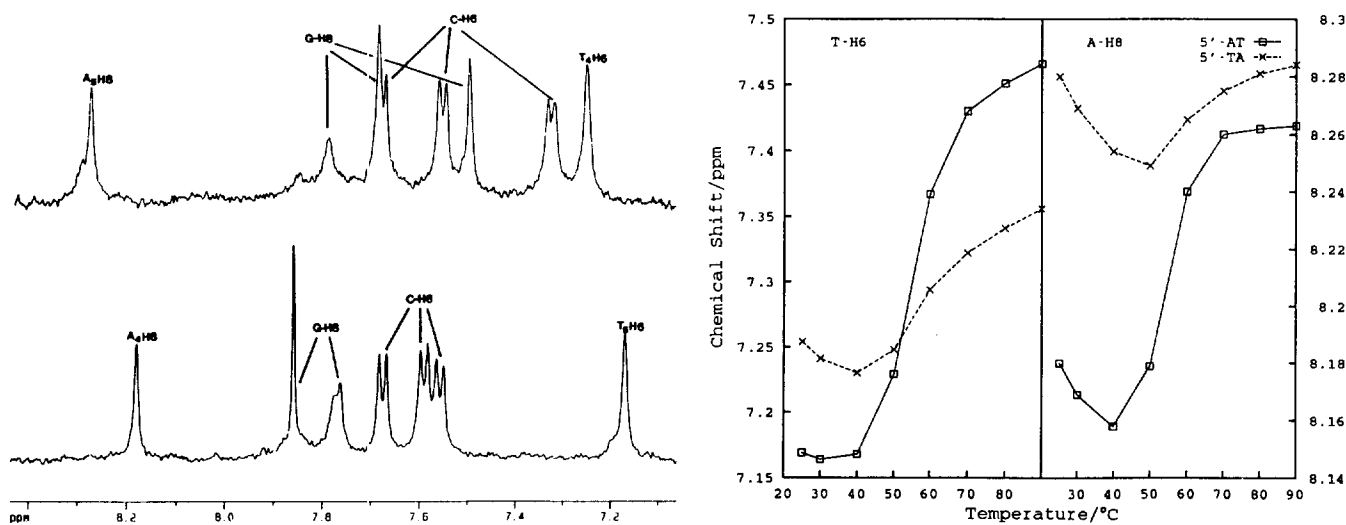


Figure 2. (A) The 500-MHz aromatic proton spectrum of the d(GGGTACCC)(top) and d(GGGATCCC)(bottom) in 20 mM phosphate, 200 mM NaCl, D₂O, pH 7 at 25°C, (B) Temperature dependence of the proton chemical shifts of thymidine H6(left) and adenosine H8(right) protons at position 4 of the two octamers in 20 mM phosphate, 200 mM NaCl, D₂O, pH 7 between 25 and 90°C.

X-ray crystallographic studies⁵ have shown that 5'-TA sequences are opened $\sim 8^\circ$ toward the minor groove while 5'-AT sequences are opened $\sim 8^\circ$ toward the major groove⁷ and suggested that the intercalation of the psoralen hydrophobic edge to DNA duplex is occurred in the minor groove⁸, 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 2 μ -mole scale. The oligonucleotides were purified by Sephadex G-25 gel filtration column chromatography. The samples were lyophilized and redissolved in 90% H₂O/10% D₂O. 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°C plotted in Figure 1. There are four unique base pairs in the 5'-TA or 5'-AT sequence, 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°C 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 disappearance 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°C 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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